Synthesis and Preliminary Biological Evaluation of Arimetamycin A Disaccharide Decorated Anthracyclines

By

Eric Douglas Huseman

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Approved:

Steven D. Townsend, Ph.D.

Jeffrey N. Johnston, Ph.D.

Nathan D. Schley, Ph.D.

Neil Osheroff, Ph.D.

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Note: For chemicals, CAS number given in square brackets when available.

$(COBr)_2$	oxalyl bromide [15219-34-8]
$(COCl)_2$	oxalyl chloride [79-37-8]
$(H_3C)_2SO_4$	dimethyl sulfate [77-78-1]
+SC	positively supercoiled
−SC	negatively supercoiled
1,2-DCE	1,2-dichloroethane [107-602-2]
Å	angstrom (10^{-10} meter)
Ac_2O	acetic anhydride [108-24-7]
AcCl	acetyl chloride [75-36-5]
AcOH	acetic acid [64-19-7]
$AgPF_6$	silver hexafluorophosphate [26042-63-7]
AIBN	2,2′-azobis(2-methylpropionitrile) [78-67-1]
AMA	arimetamycin A [1477521-85-9 for free base]
AMB	arimetamycin B [1477521-86-0]
AMC	arimetamycin C [1477521-87-1]
aq.	aqueous
ATP	adenosine 5′-triphosphate [56-65-5 for neutral compound]
$BF_3{\bullet}OEt_2$	boron trifluoride diethyl etherate [109-63-7]
BnBr	benzyl bromide [100-39-0]
$BnONH_2{\bullet}HCl$	*O*-benzylhydroxylamine hydrochloride [2687-43-6]
Boc	*tert*-butyloxycarbonyl
Boc_2O	di-*tert*-butyl decarbonate [24424-99-5]
bp	base pair
BRSM	based on recovered starting material
c (italicized)	concentration (g/mL)
ca.	circa
CAM	ceric ammonium molybdate (TLC stain)
$CaSO_4$	calcium sulfate [7778-18-9]
cat.	catalytic
CATCH-IT	covalent attachment to tags to capture histone and identify turnover
Cbz-OSu	*N*-(benzyloxycarbonyloxy)succinimide [13139-17-8]
CbzCl	benzyl chloroformate [501-53-1]
CCl_4	carbon tetrachloride [56-23-5]
CD_3OD	methanol-d_4 [811-98-3]
$CDCl_3$	chloroform-*d* [865-49-6]
CF_3CO_2H	trifluoroacetic acid [76-05-1]
CH_2Cl_2	methylene chloride [75-09-2]
CH_3CN	acetonitrile [75-05-8]
CH_3Li	methyllithium [917-54-4]
CH_3OH	methanol [67-56-1]
CO_2	carbon dioxide [124-38-9]
COSY	homonuclear correlation spectroscopy
CsF	cesium fluoride [13400-13-0]
D-Thr	D-threonine [632-20-2]
DAST	(diethylamino)sulfur trifluoride [38078-09-0]
DBU	1,8-diazobicyclo[5.4.0]undec-7-ene [51376-18-2]
de	diastereomeric excess
DEIPS	*iso*-propyldiethylsilyl
DEIPSCl	*iso*-propyldiethylsilyl chloride [107149-56-4]

DIBAL-H	diisobutylaluminum hydride [1191-15-7]
DIPEA	*N,N,*-diisopropylethylamine [7087-68-5]
DMAP	4-(dimethylamino)pyridine [1122-58-3]
DME	1,2-dimethoxyethane [110-71-4]
DMSO	dimethyl sulfoxide [67-68-5]
DNA	deoxyribonucleic acid
DNR	daunorubicin [23541-50-6 for hydrochloride salt]
DNR-AMA	daunorubicin-arimetamycin A
DOX	doxorubicin [25316-40-9 for hydrochloride salt]
DOX-AMA	doxorubicin-arimetamycin A
dr	diastereomeric ratio
DSB	double strand break
EDC•HCl	*N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride [25952-53-8]
eDNA	environmental DNA
ee	enantiomeric excess
ELS	Evaporative light scattering
Eq	equivalent
Et_2O	diethyl ether [60-29-7]
EtOAc	ethyl acetate [141-78-6]
ETP-AMA	etoposide-arimetamycin A
EXSY	exchange spectroscopy
FCC	flash column chromatography
g	gram
H_2O	water [7732-18-5]
H_2SO_4	sulfuric acid [7664-93-9]
HCHO	formaldehyde [50-00-0 for 37 wt. % solution in water]
HCl	hydrochloric acid [7647-01-0]
$HCl•NH_2OCH_3$	*O*-methylhydroxylamine hydrochloride [593-56-6]
Hex	hexane, mixture of isomers
HF•pyr.	Olah's reagent = hydrogen fluoride pyridine [62778-11-4]
$Hg(OAc)_2$	mercury (II) acetate [1600-27-7]
HMBC	heteronuclear multiple-bond correlation spectroscopy
$HN(i\text{-}Pr)_2$	diisopropylamine [108-18-9]
hr	hour
HRMS	high resolution mass spectrometry
HSQC	heteronuclear single quantum coherence
hTopIIa	human topoisomerase IIa
IBX	2-iodoxybenzoic acid [61717-82-6]
IDCP	iodonium di-collidine perchlorate [69417-67-0]
Im_2CS	1,1'-thiocarbonyldiimidazole [6160-65-2]
ImH	imidazole [288-32-4]
IR	infrared
K_2CO_3	potassium carbonate [584-08-7]
KCl	potassium chloride [7447-40-7]
L	liter
L-Aha	L-azidohomoalanine
L-Met	L-cysteine
LC/MS	liquid chromatography/mass spectrometry
LDA	lithium diisopropylamide [4111-54-0]
$LiAlH_4$	lithium aluminum hydride [16853-85-3]
$LiBH_4$	lithium borohydride [16949-15-8]
LiHMDS	lithium bis(trimethylsilyl)amide [4039-32-1]
LLS	longest linear sequence (step counting metric)
M	molarity (moles per liter)

m-CPBA	3-chloroperbenzoic acid [937-14-4]
mg	milligram (10^{-3} grams)
$Mg(OCH_3)_2$	magnesium methoxide [109-88-6]
$MgCl_2$	magnesium chloride [7786-30-3]
$MgSO_4$	magnesium sulfate [7487-88-9]
MHz	megahertz (10^6 hertz)
min	minute
mL	milliliter (10^{-3} liters)
mmol	millimole (10^{-3} moles)
mmol	Millimole (10^{-3} mole)
MP	melting point
MPLC	medium pressure liquid chromatography
MRP1	multidrug resistance protein 1
MS	molecular sieves
MsOH	methanesulfonic acid [75-75-2]
N	normality (equivalents per liter)
$Na_2EDTA•H_2O$	disodium ethylenediaminetetraacetate dihydrate [6381-92-6]
Na_2SO4	sodium sulfate [7757-82-6]
$NaBH_3CN$	sodium cyanoborohydride [25895-60-7]
$NaBH_4$	sodium borohydride [16940-66-2]
NaH	sodium hydride [7646-69-7]
$NaHCO_3$	sodium bicarbonate [144-55-8]
NaOAc	sodium acetate [127-09-3]
NaOH	sodium hydroxide [1310-73-2]
NBS	*N*-bromosuccinimide [128-08-5]
NDMBA	*N,N*-dimethylbarbituric acid [769-42-6]
NEt_3	triethylamine [121-44-8]
NH_3	ammonia [7664-41-7]
NH_4HCO_2	ammonium formate [540-69-2]
NIS	*N*-iodosuccinimide [516-12-1]
NMM	*N*-methyl morpholine [109-02-4]
NMR	nuclear magnetic resonance
NOESY	nuclear Overhauser effect spectroscopy
NsCl	2-nitrobenzenesulfonyl chloride [1694-92-4]
°C	degrees Celsius
OR	optical rotation
ORTEP	Oak Ridge thermal ellipsoid plot
p-TolSH	4-methylbenzenethiol [106-45-6]
P_2O_5	phosphorus (V) pentoxide [1314-56-3]
Pd/C	palladium on carbon [7440-05-3]
PDC	pyridinium dichromate [20039-37-6]
Ph_2SO	diphenyl sulfoxide [945-51-7]
$PhCH_3$	toluene [108-88-3]
$PhCH_3$	toluene [108-88-3]
PhH	benzene [71-43-2]
PhSeOTf	benzene selenyl triflate [112238-64-9]
PhSH	thiophenol [108-98-5]
PivOH	pivalic acid [75-98-9]
PSF	pear shaped flask
pyr	pyridine [110-86-1]
$pyr•BH_3$	borane pyridine complex [110-51-0]
RBF	round bottom flask
Red-Al	sodium bis(2-methoxyethyoxy)aluminum hydride [22722-98-1]
R_f	retention factor (TLC)

$RhCl(PPh_3)_3$	Wilkinson's catalyst = chlorodotris(triphenyphosphine)rhodium(I) [14694-95-2]
ROESY	rotating-frame Overhauser effect spectroscopy
RP	reverse phase
rt	room temperature
sat.	saturated
SCC	squamous cell carcinoma
SDS	sodium dodecyl sulfate [151-21-3]
SiO_2	silicon dioxide
SM	starting material
SmI_2	samarium (II) iodide [32248-43-4)
$SOCl_2$	thionyl chloride [7719-09-7]
TASF(Me)	tris(dimethylamino)sulfonium difluorotrimethylsilicate [59218-87-0]
TBAF	tetrabutylammonium fluoride [429-41-4]
TBAI	tetrabutylammonium iodide [311-28-4]
$TBAN_3$	tetrabutylammonium azide [993-22-6]
TBDPSCl	*tert*-butyldiphenylsilyl chloride [58479-61-1]
TBS	*tert*-butyldimethylsilyl
TBSCl	*tert*-butyldimethylsilyl chloride [18162-48-6]
TBSOTf	*tert*-butyldimethylsilyl trifluoromethanesulfonate [69739-34-0]
^{t}BuOK	potassium *tert*-butoxide [865-47-4]
TCE	trichloroethylene [79-01-6]
Tf_2O	trifluoromethane sulfonic anhydride [358-23-6]
TFA	trifluoroacetic acid [76-05-1]
TFA_2O	trifluoroacetic anhydride [407-25-0]
TFE	2,2,2-trifluoroethanol [75-89-8]
TfN_3	trifluoromethanesulfonyl azide [3855-45-6]
THF	tetrahydrofuran [109-99-9]
TIPSCl	triisopropylsilyl chloride [13154-24-0]
TMS-SPh	trimethyl(phenylthiol)silane [4551-15-9]
TMSOTf	trimethylsilyl trifluoromethanesulfonate [27607-77-8]
t_R	retention time (LC/MS)
Tris•HCl	tris(hydroxymethyl)aminomethane hydrochloride [1185-53-1]
TsOH	*p*-toluenesulfonic acid [6192-52-5 for monohydrate]
TTBP	2,4,6-tri-*tert*-butylpyrimidine [67490-21-5]
XRD	X-ray diffraction
ZPS	zwitterionic polysaccharide
μL	microliter (10^{-6} liter)
μmol	micromole (10^{-6} mole)

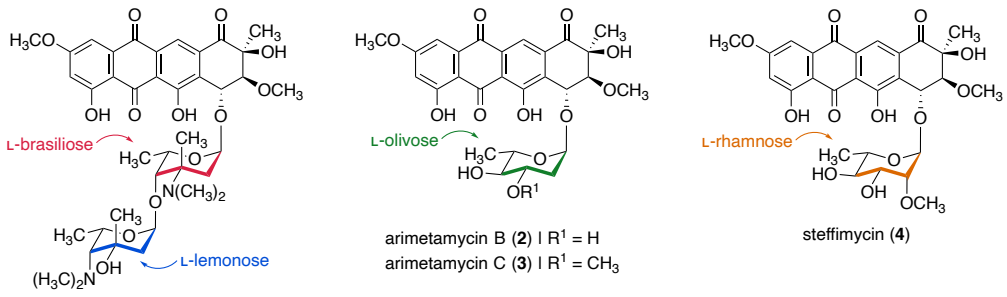
CHAPTER 1

Introduction: Biological Background and Synthetic Inspirations

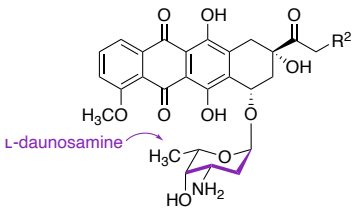
1.1 *Introduction to the Arimetamycins (**1**-**3**)*

The arimetamycins are a family of anthracycline natural products isolated following heterologous expression of environmental DNA (eDNA) gathered from soil (Figure 1.1a).[1-2] They demonstrated varying levels of antiproliferative activity across a panel of four cancer cell lines with arimetamycin A (AMA, **1**) emerging with nanomolar cytotoxicity (IC_{50}) while arimetamycin B-C (AMB-AMC, **2**-**3**) exhibited micromolar activity (Figure 1.1c). Furthermore, AMA proved more cytotoxic than the clinical anthracycline chemotherapeutics daunorubicin (DNR, **5**)[3] and doxorubicin (DOX, **6**)[4] and maintained its nanomolar activity against the multidrug resistant (MDR) lung cancer line H69AR,[5] a line with known anthracycline resistance due to its overexpression of the multidrug resistance protein 1 (MRP1) efflux pump[6-8] (Figure 1.1b-c).

a. Structures of the Arimetamycins (**1**-**3**) and Parent Compound Steffimycin (**4**).



b. Structures of Two Clinically Relevant Anthracyclines.



c. Cytotoxicity Assays of Arimetamycins A-C (**1**-**3**).[a]

Compound	HCT116[a-b] (colon)	WiDR[a-b] (colon)	MDAMB231[a-b] (breast)	H69AR[a-b] (lung/MDR)
Arimetamycin A (**1**)	2.5	0.5	164	67
Arimetamycin B (**2**)	1800	7500	29,600	47,100
Arimetamycin C (**3**)	1400	5200	27,700	18,800
Daunorubicin (**5**)	6.8	5.5	330	4800
Doxorubicin (**6**)	22.5	30.9	970	18,400

[a]Cell line (cancer type). [b]IC_{50} values reported in nM.

Figure 1.1 Structure and Biological Evaluation of the Arimetamycins (**1**-**3**).[a]Data from Brady, S. F. et al.[1-2]

Structurally, the arimetamycins possess a common steffimycinone aglycone, indicating that their glycans serve as the activity determining domain. The less potent AMB and AMC feature neutral L-olivose based sugars and thus prove similar to the L-rhamnose decorated steffimycin (**4**) (Figure 1.1a).[9-11] In contrast, AMA bears a

disaccharide composed of the rare branched deoxy-amino sugars L-brasiliose and L-lemonose, making it one of the few steffimycin anthracyclines to incorporate an amino sugar (Figure A.1)[12-15] and the only known anthracycline with two branched *N*,*N*-dimethylamino sugars (Figure A.2).

As most clinically approved anthracyclines feature a deoxy-amino sugar (usually L-daunosamine or a derivative thereof), we hypothesized that merging the AMA disaccharide with the aglycone of a clinically useful anthracycline would yield a novel anthracycline hybrid more potent than either of the parent compounds. We envisioned that the synthesis of a suitable AMA disaccharide donor would enable us to divergently access the hybrid anthracyclines required to test this hypothesis as well as arimetamycin A itself. In anticipation of this synthetic work and subsequent biological evaluation, we took stock of the anthracycline literature, focusing in particular on the role of the glycan in determining activity.

1.2 *Anthracyclines: Proposed Mechanisms of Action*

Despite the clinical effectiveness of daunorubicin (**5**), doxorubicin (**6**), and their synthetically derived derivatives idarubicin (**7**) and epirubicin (**8**), a complete understanding of the mechanism(s) responsible for their anticancer activity remains elusive (Figure 1.2).[16] Proposed mechanisms include interference with macromolecular biosynthesis, production of free radical species through quinone redox cyclizing, and the formation of covalent DNA adducts via an orthoquinone methide intermediate or formaldehyde mediated cross linking with the carbohydrate's amine moiety.[16-17] Historically, the most accepted mechanism is topoisomerase II (TopII)[18-21] poisoning while new mechanistic work in the last decade also implicates chromatin damage as key to the activity of the anthracyclines.[16, 22-27] Below, we discuss these latter two mechanisms before examining recent SAR studies with implications for divorcing the desired cytotoxicity from the dose limiting cardiotoxicity based on an understanding of these two mechanisms.

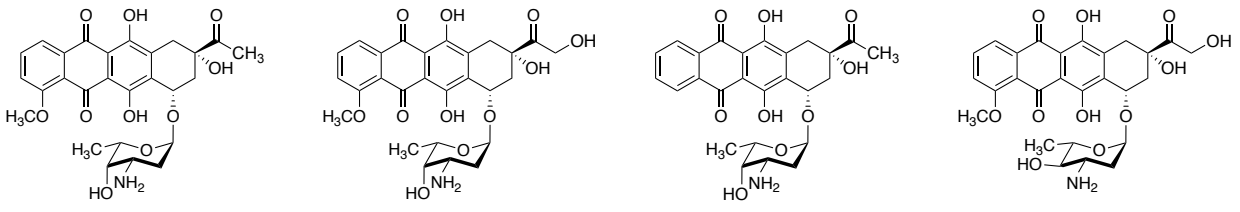


Figure 1.2 Structures of Several Clinically Relevant Anthracycline Chemotherapeutics.

1.2.1 *Anthracyclines as Topoisomerase II Poisons*

The topoisomerases comprise a family of enzymes that maintain DNA topology during cellular processes such as transcription, replication, and chromosome condensation.[28-29] They accomplish this through a three-step mechanism that involves (1) temporary strand breakage, (2) topological reorganization, and (3) strand religation. Type 1 topoisomerases (in humans: TopI, $TopI_{mt}$, TopIIIα, TopIIIβ) utilize a single strand breakage mechanism while type 2 topoisomerases (in humans: TopIIα, TopIIβ) employ a double strand breakage. Importantly, this catalytic cycle involves the intermediacy of covalent DNA-enzyme complexes as strand breakage occurs through reversible transesterification of the DNA phosphate backbone with an active site tyrosine (Figure 1.3).[29-36]

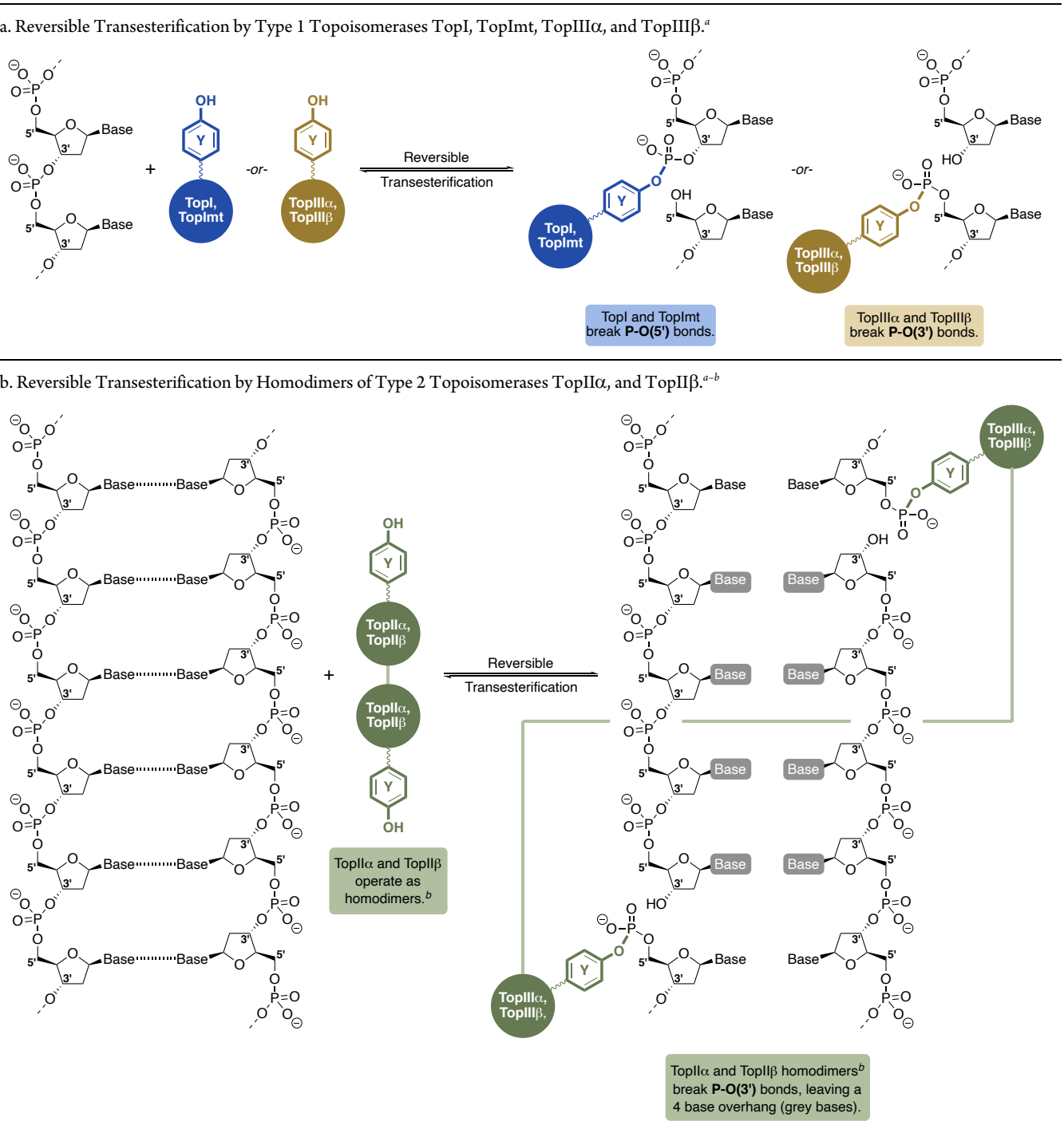


Figure 1.3 A Chemical Perspective on the Reversible Transesterification by the Human Topoisomerases. [a]Adapted from Pommier, Y. and Nitiss, J. L. et al.[29] [b]Linker between monomer units is not meant to represent any specific structural feature linking the monomers; it is only provided as a reminder that TopIIα and TopIIβ operate as homodimers.

Anthracycline chemotherapeutics such as daunorubicin and doxorubicin target both isoforms of TopII. Inhibition of TopIIα is hypothesized to account for anthracycline's toxicity toward rapidly dividing cancer cells while TopIIβ inhibition is implicated in the drug's dose limiting cardiotoxicity.[37-40] Both TopII isoforms regulate DNA topology through double strand break mechanism in which a homodimeric enzyme complex passes one

segment of DNA (T segment, *t*ransport) through a second, transiently broken segment (G segment, *g*ate) (Figure 1.4a).[41-52] Following T segment passage, reversal of the initial transesterification restores the phosphodiester backbone of G segment to its original state. This mechanism allows TopII to relax both positively and negatively supercoiled DNA; the enzyme can also unknot/knot and decatenate/catenate DNA (Figure 1.4b).

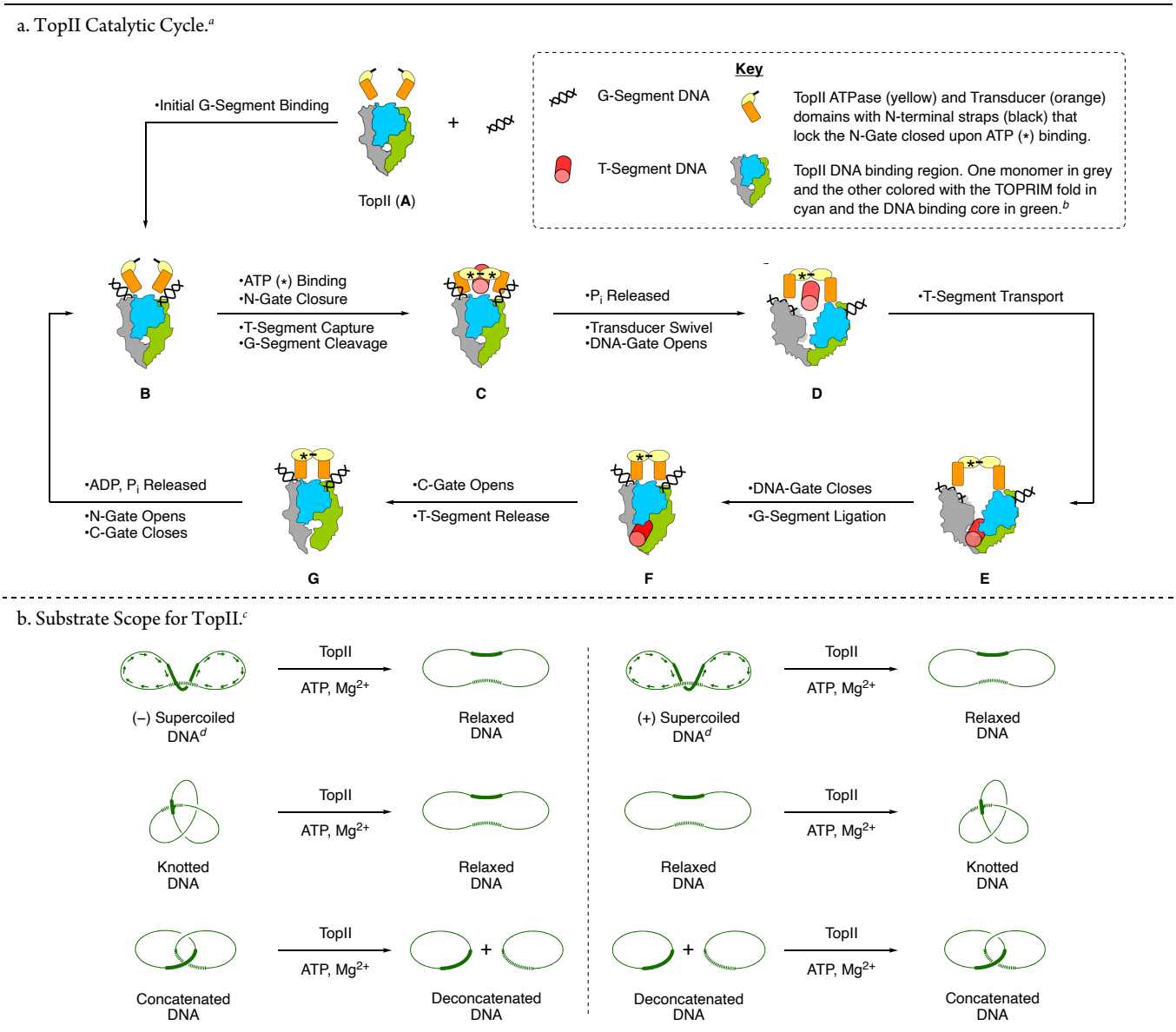


Figure 1.4 Enzymology of Topoisomerase II (TopII).[a]Adapted from Osheroff, N. and Clarke, D. J. et al.[51] [b]Osheroff, N. and Clarke, D. J. et al[51] based their illustration of the TopII DNA binding region on the structure described by Dong, K. C. and Berger, J. M.[46] [c]Adapted from Pommier, Y. et al.[49] [d]Arrows indicated DNA directionality. +/− supercoiling assigned based on Bates and Maxwell.[53]

In general, pharmacologic inhibition of TopII can occur through two mechanisms.[47] In the first, the drug traps the covalently linked TopII-DNA cleavage complex, leading to toxic double strand breaks; these drugs are

referred to a TopII poisons. In the second, the drug inhibits the enzyme's catalytic cycle without increasing the amount of breakage complexes; these drugs are referred to as TopII catalytic inhibitors. Interestingly, the anthracyclines daunorubicin and doxorubicin can function as both TopII poison and inhibitors depending on the concentration of the drug.[18-19, 54-56] At low concentrations, they function as TopII poisons that form drug-TopII-DNA ternary complexes that prevent the religation of the G segment. Cellular processing of these complexes leads to toxic double strand breaks. At high concentrations, these anthracyclines serve as TopII inhibitors that attenuate the enzyme's activity.

1.2.2 *Anthracyclines as Modifiers of Chromatin Dynamics*

Though often depicted as an unencumbered double helix, the majority of a eukaryote's DNA finds itself wrapped around histone proteins (Figure 1.5). These assemblies, termed nucleosomes, involve approximately 146 DNA base pairs wrapped around an octameric histone protein rich in positively charged lysine and arginine amino acids. The condensation of long DNA molecules results in the formation of a polymeric collection of nucleosomes connected by short strands of linker DNA, collectively referred to as chromatin.

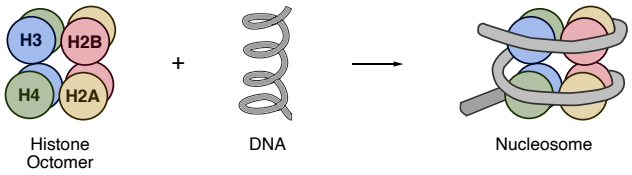
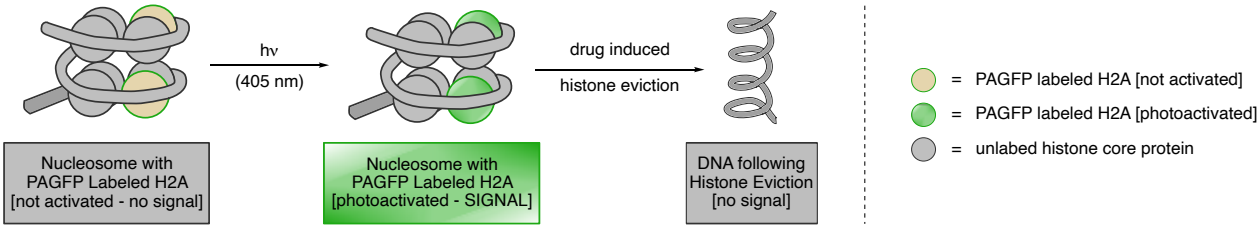


Figure 1.5 Cartoon Depiction of Nucleosome Formation from a Histone Octomer and DNA.

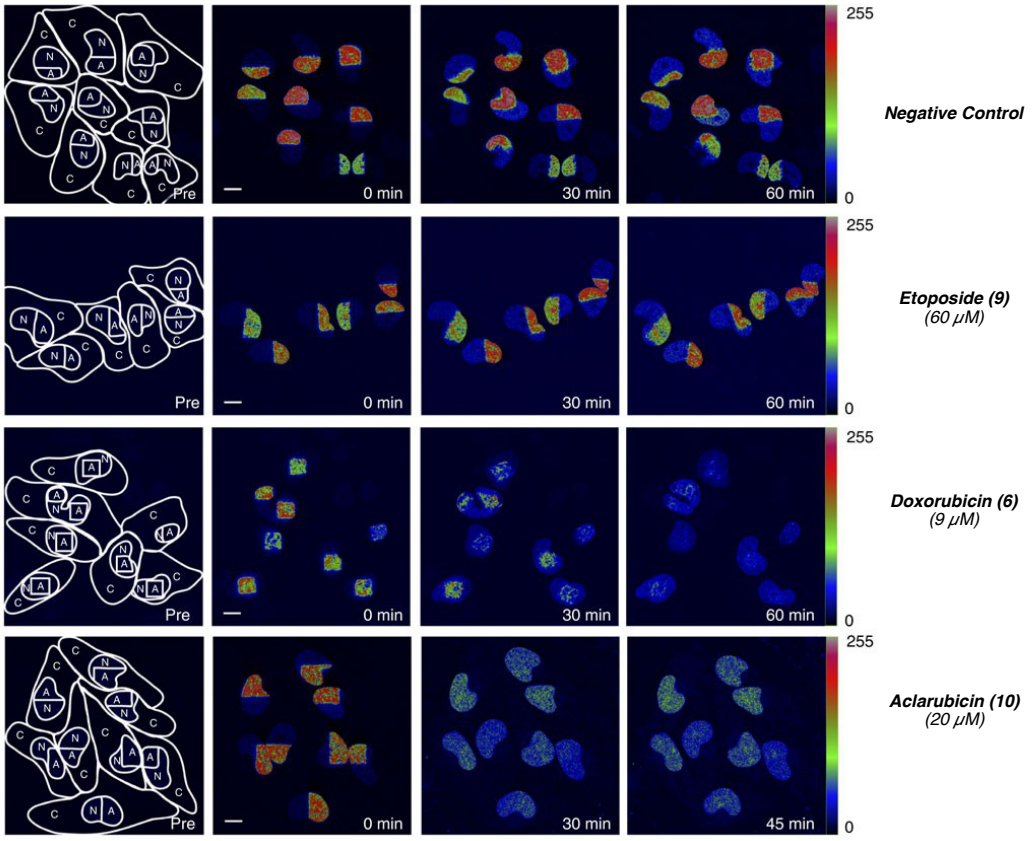
In 2013, two teams of researchers separately reported that anthracyclines increased the rate of nucleosome turnover by evicting the core histones from their DNA loops.[22-23] The Dutch team visualized this phenomenon using photo-activatable fluorescent protein (PAGFP) coupled histones.[22] In this assay, the team photoactivated (405 nm) portions of the nuclei of MelJuSo human melanoma cells line expressing PAGFP labeled histone protein H2A and used time lapse focal microscopy to follow the change in fluorescence (500-530 nM) resulting from treatment with various TopII inhibitors (Figure 1.6a). They found that control and etoposide (**9**) treatments caused no significant decrease in fluorescence over 1 hr, indicating that etoposide does not evict histones (Figure 1.6b). In contrast, the addition of the anthracyclines doxorubicin (**6**) and aclarubicin (**10**) resulted in considerable decreases in fluorescence, indicating that these compounds evict histones; they observed similar results with daunorubicin (**5**) and idarubicin (**7**) (see Figure 1.6c for compound structures). Moving forward, they noted that this histone eviction phenotype persisted in an *in vitro* assay in which they exposed reconstituted single

nucleosomes to etoposide, aclarubicin, doxorubicin, or the doxorubicin aglycone (doxorubicinone). Analysis by native polyacrylamide gel electrophoresis (PAGE) indicated that only aclarubicin and doxorubicin evicted the reconstituted single nucleosomes, validating the results of the cell-based assay and indicating that the doxorubicin glycan plays a role in histone eviction.

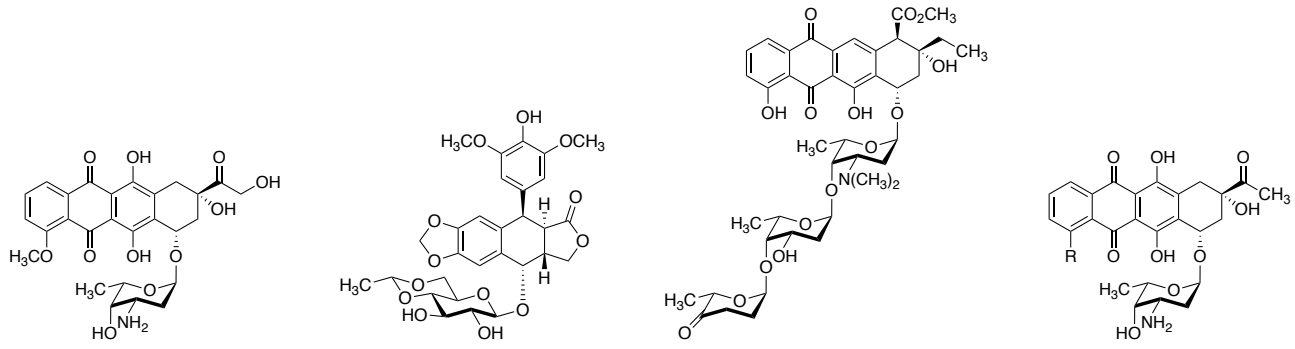
a. Overview of the Fluorescence Based Histone Eviction Assay.



b. Results of the Fluorescence Based Histone Eviction Assay.



c. Compounds Studied with the Fluorescence Based Histone Eviction Assay.



doxorubicin (**6**) | etoposide (**9**) | aclarubicin (**10**) | daunorubicin (**5**) I R = OCH_3 idarubicin (**7**) I R = H

Figure 1.6 Neefjes' 2013 Study of Histone Eviction with a Fluorescence Based Assay.

Concurrently, an American team published work showing that doxorubicin and aclarubicin treatment increased the rate of nucleosome turnover around gene promoters[23] as determined using their previously developed CATCH-IT (*c*ovalent *a*ttachment to *t*ags to *c*apture *h*istone and *i*dentify *t*urnover) methodology.[57-58] This technique capitalizes on the cell's ability to incorporate the click chemistry precursor L-azidohomoalanine (L-Aha, **12**) in place of L-methionine (L-Met, **11**) (Figure 1.7). In brief, following the treatment of L-Met starved cells with L-Aha rich media, cell nuclei are harvested and digested to give mostly mononucleosomes. Subsequent biotin click ligation, streptavidin affinity purification, washing, and DNA analysis provide a read out of nucleosome turnover. The team found that doxorubicin (**6**) increased nucleosome turnover in a time dependent manner independent of the kinase ATM and transcription factor p53 in mouse squamous cell carcinoma (SCC) cell lines. They observed a similar response with aclarubicin (**10**), a catalytic TopII inhibitor that does not induce DNA double strand breaks, indicating that enhanced nucleosome tumor does not depend on doxorubicin's double strand breakage activity.

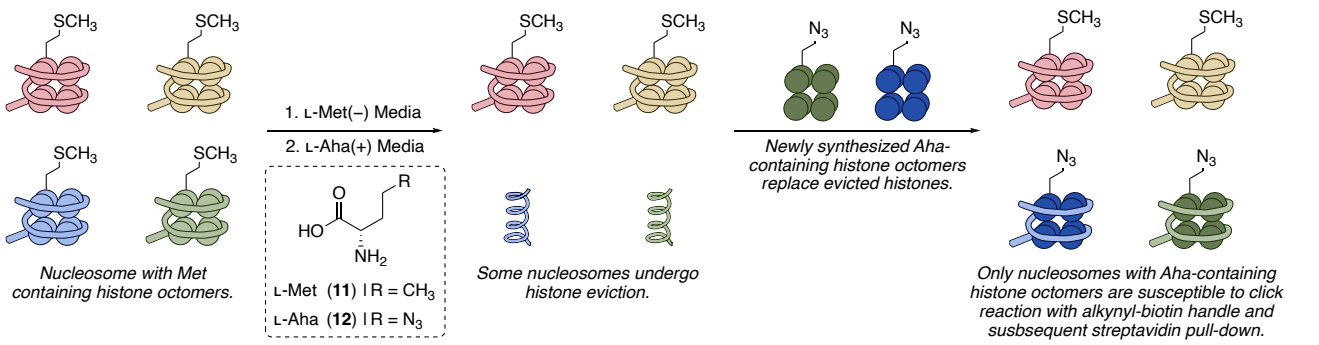


Figure 1.7 Overview of Henikoff's CATCH-IT Methodology as Applied to a 2013 Study of Doxorubicin and Aclarubicin.

Regarding the mechanism of histone eviction, the teams developed two different hypotheses. Based on the inability of the doxorubicin aglycone to evict histones, the Dutch team postulated that the daunosamine glycan may play an important role in histone eviction.[22] Interestingly, they found that docking doxorubicin (based on DNA/doxorubicin co-crystal PDB: 1D12[59]) into a previously reported nucleosome crystal structure (PDB:1AOI[60]) showed a competition for the DNA minor groove between the drug's positively charged amino sugar and a key nucleosome stabilizing H4-arginine residue (Figure 1.8). In contrast, the American team proposed a topologically based mechanism for histone eviction in which positive supercoiling resulting from drug intercalation and/or the accumulation of positive supercoiling due to TopII inhibition results in nucleosome destabilization.[23]

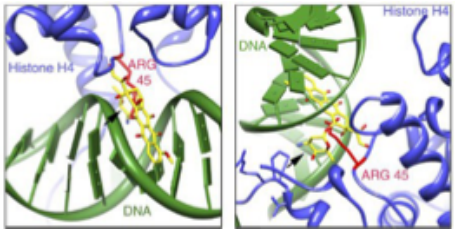


Figure 1.8 Two Views of Neefjes' 2013 Docking of Doxorubicin (**5**) into a Nucleosome Crystal Structure.

1.2.3 Probing the Roles of DNA and Chromatin Damage in Anthracycline Toxicity

In 2020, follow up work from the Netherlands team provided insight into the interconnected roles of double strand damage and histone eviction in determining the toxicity of anthracycline chemotherapeutics.[26] They noted that etoposide (**9**), a TopII poison that induces double strand breaks (DSBs)[61] but does not evict histones (D+/H−), exhibits decreased cytotoxicity[22, 62] and cardiotoxicity[63] as compared to doxorubicin (**6**), a TopII poison that induces DSBS and evicts histones (D+/H+). To dissect the roles of DSB induction and histone eviction, the authors performed an orienting experiment in which they treated spontaneous tumor forming *Trp53*$^{+/-}$ SVB mice with etoposide (D+/H−), doxorubicin (D+/H+), aclarubicin (**10**, D−/H+), or saline control. Histopathological heart examination revealed that only the doxorubicin treated mice showed cardiac damage, indicating that cardiotoxicity required both DNA strand breakage and histone eviction (D+/H+ phenotype).

Next, the group established preliminary structure activity relationships (SARs) for these phenotypes. They found that amrubicin (**13**), an anthracycline derivative in which the amine group is located on the aglycone rather than on the sugar[64], exhibited a D+/H− phenotype; accordingly, it is not surprising that amrubicin shares both this phenotype and a profile of reduced cardiotoxicity[65] with etoposide (**9**). From an SAR perspective, this data indicates the necessity of an amino sugar for histone eviction. Interestingly, examination of the amino sugar portion of daunorubicin (**5**), doxorubicin (**6**), idarubicin (**7**), epirubicin (**8**), and aclarubicin (**10**) reveals a structural trend: anthracyclines with an unsubstituted amino sugar (i.e., NH_2) cause both DSBs and histone eviction (D+/H+) while aclarubicin, the only drug featuring an *N*,*N*-dimethylated amino sugar, only evicts histones (D−/H+) (Figure 1.9a). To determine if this trend extends beyond aclarubicin, the team synthesized *N*,*N*-dimethyldoxorubicin (diMeDOX, **14**) (Figure 1.9b). Mechanistic profiling showed that this compound, like aclarubicin, only evicted histones (D−/H+). Furthermore, treatment of wild type SVB mice with doxorubicin, aclarubicin, and diMeDOX resulted in cardiotoxicity only in the doxorubicin treated mice. Regarding cytotoxicity, the authors observed similar if not superior toxicity for diMeDOX as compared to the unmethylated parent compound in 70% of the twenty tested cell lines. In contrast, amrubicin (D+/H−) showed decreased cytotoxocity as compared to doxorubicin, aclarubicin, and diMeDOX in K562 cells.

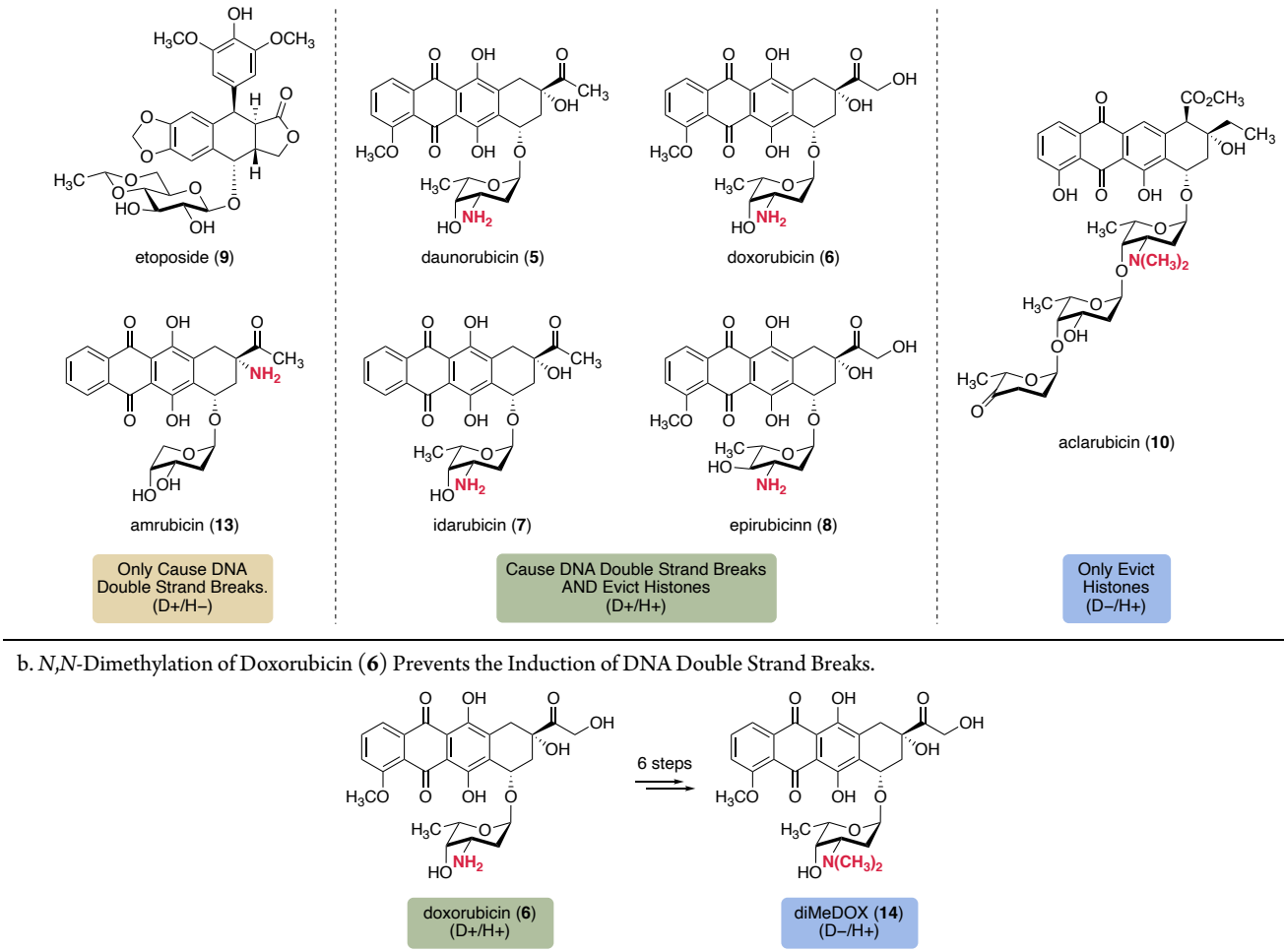
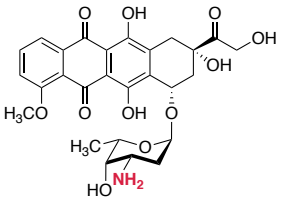
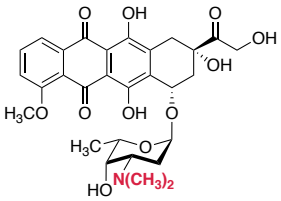
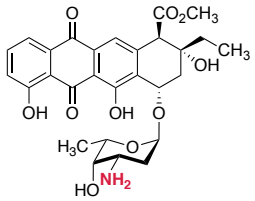
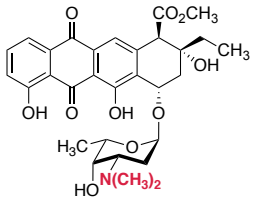
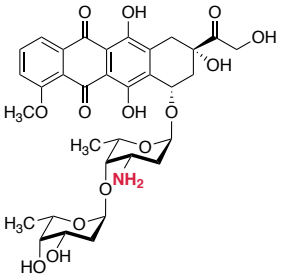
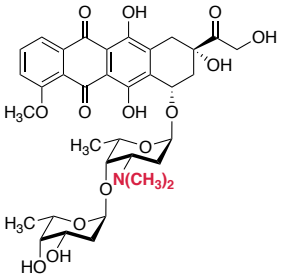
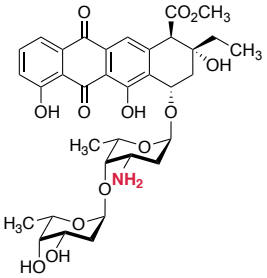
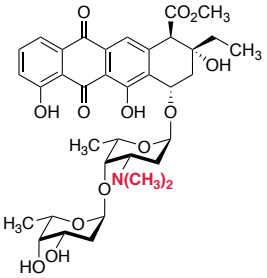
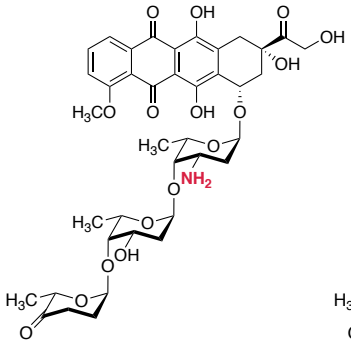
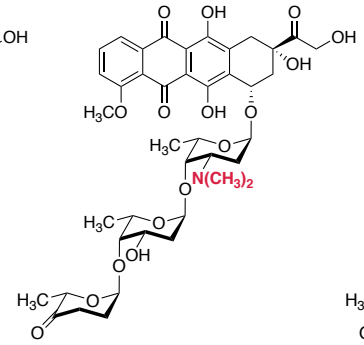
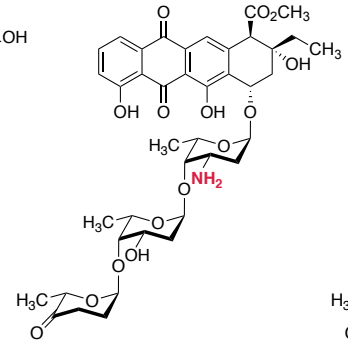
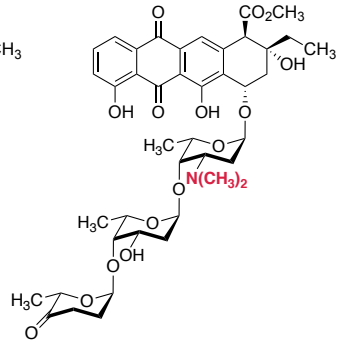


Figure 1.9 Compounds Assayed in Qiao, Li, and Neefjes' 2020 Work on the Relationship between Mechanism of Action and Anthracycline Toxicity.

In a separate study, the Dutch team fully explored the SAR of the doxorubicin and aclarubicin glycans through the synthesis of anthracyclines **14**–**23** (Table 1.1).[27] Six of the ten synthetic derivatized exhibited the desired D–/H+ phenotype, with three of these compounds (**14**, **20**, and **22**) demonstrating equipotent histone eviction as compared to their parent compounds doxorubicin (**6**) and aclarubicin (**10**). Cytotoxicity evaluation revealed that *N,N*-dimethylation increased potency (IC_{50}) with hybrid derivative **22** featuring the doxorubicin aglycone and the aclarubicin trisaccharide emerging as the most cytotoxic compound.

Table 1.1 Neefjes and Codée's 2020 SAR Study of the Doxorubicin and Aclarubicin Glycans. [a–b]

			
doxorubicin (**6**) Phenotype : D+/H++ IC_{50} Range : 0.234 – 1.145 μM	diMeDOX (**14**) Phenotype : D–/H++ IC_{50} Range : 0.034 – 0.186 μM	**15** Phenotype : D–/H– IC_{50} Range : n/d[c]	**16** Phenotype : D–/H+ IC_{50} Range : 4.475 – 8.847 μM
			
17 Phenotype : D–/H– IC_{50} Range : n/d[c]	**18** Phenotype : D–/H+ IC_{50} Range : 0.603 – 3.204 μM	**19** Phenotype : D–/H+ IC_{50} Range : 9.711 – 9.782 μM[d]	**20** Phenotype : D–/H++ IC_{50} Range : 0.211 – 0.509 μM
			
21 Phenotype : D+/H– IC_{50} Range : 1.365 – 2.213 μM	**22** Phenotype : D–/H++ IC_{50} Range : 0.007 – 0.034 μM	**23** Phenotype : D–/H– IC_{50} Range : 3.035 μM[e]	aclarubicin (**10**) Phenotype : D–/H++ IC_{50} Range : 0.040 – 0.137 μM

[a]The "D" component of a compound's phenotype indicates its ability to induce DNA double strand breaks (dsbs): "D+" indicates dsb formation; "D–" indicates no dsb. The "H" component of a compound's phenotype indicates its ability to evict histones: : "H++" indicates drug eviction equipotent with the parent compounds (doxorubicin (**6**) and aclarubicin (**10**); "+" indicates drug eviction less potent than the parent compounds (doxorubicin (**6**) and aclarubicin (**10**)); "–" indicates no significant histone eviction. [b]Unless indicated otherwise, the IC_{50} ranges represent the highest and lowest IC_{50} obtained across the following panel of six human cancer cell lines: K562 (acute myeloid leukemia), MelJuSo (melanoma), HCT116 (colorectal carcinoma), PC3 (prostate cancer), DU145 (prostate cancer), and U87 (glioblastoma). [c]No IC_{50} values determined for this compound. [d]IC_{50} values only determined for MeIJuSO and HCT116 cell lines. [e]IC_{50} only determined for K562 cell line.

1.2.4 Mechanistic Conclusions

Based on the preceding data, particularly that from the Dutch team[22, 26-27], we present the following conclusions:

(1) Anthracycline cardiotoxicity results from simultaneous generation of DNA DSBs and histone eviction (D+/H+).

(2) Anthracyclines that only evict histones (D−/H+) prove more cytotoxic than anthracyclines that only cause DNA DSBs (D+/H−).

(3) Histone eviction requires an amino sugar, preferably with *N*,*N*-dimethyl functionalization.

(4) *N*,*N*-dimethylation of the amino sugar often improves cytotoxicity as compared to the unmethylated compound.

(5) Anthracyclines that share the same sugar but bear different aglycone may demonstrate substantially different cytotoxicities.

1.3 *Synthetic Planning*

With a better understanding of mechanism of action, we decided to pursue the synthesis of three types of compounds (Figure 1.10). First, we targeted Arimetamycin A (AMA, **1**) as synthesis of the parent compound would allow us to recapitulate the results of Brady; additionally, **1** would serve as a point of biological comparison for evaluating the hybrid compounds. Second, we designed daunorubicin-AMA (DNR-AMA) and doxorubicin-AMA (DOX-AMA) anthracycline hybrids **24** and **25**, respectively; synthesis of these compounds would allow us to test our hypothesis that combining the aglycone of a clinically relevant anthracycline with the AMA disaccharide will yield a compound more potent than either parent compound. Finally, we envisioned that synthesis of etoposide-AMA (ETP-AMA) hybrid **26** would allow us to determine if the cytotoxicity associated with the AMA disaccharide is transferable to a non-anthracycline aglycone.

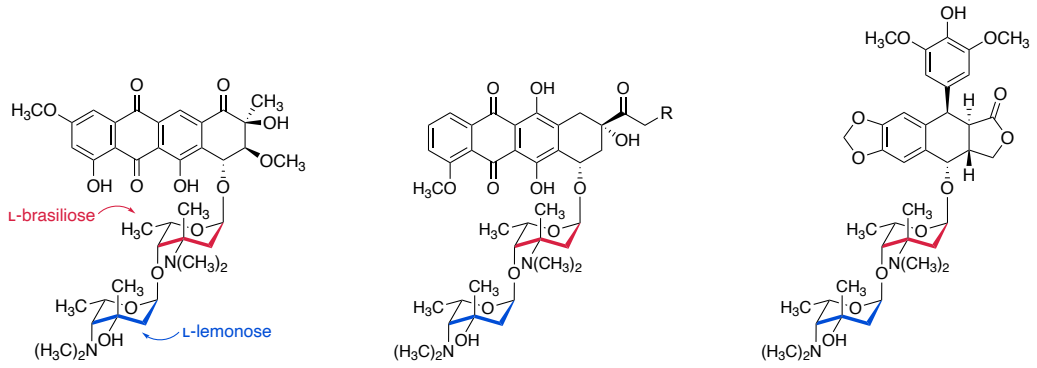
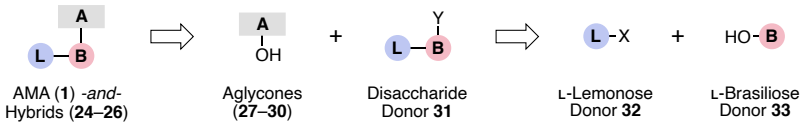


Figure 1.10 Synthetic Targets Pursued in this Dissertation.

As we set out to synthesize arimetamycin A (**1**) and hybrids **24**–**26**, we elected to pursue a divergent strategy in which we would assemble one AMA disaccharide glycosyl donor (**31**) that could undergo subsequent glycosidation with each of the necessary aglycone acceptors (**27**–**30**) (Scheme 1.1). Continuing this conceptual

retrosynthesis, we imagined that disaccharide donor **31** would result from the glycosylation of protected L-lemonose donor **32** with protected L-brasiliose acceptor **33**. In total, this approach would require two glycosylation events and the synthesis of aglycone, L-lemonose, and L-brasiliose building blocks. In preparation for this synthetic endeavor, we surveyed the literature or guidance and inspiration.



Scheme 1.1 Our Conceptual Retrosynthetic Analysis for the Synthesis of Arimetamycin A (AMA, **1**) and AMA Hybrids **24–26**.

1.3.1 Synthetic Precedent: Glycosylation

Due to their lack of C2 functionality, achieving high α or β selectivity in the glycosylations of 2-deoxy donors, such as the targeted disaccharide (**31**) and L-lemonose donors (**32**), proves challenging. This has inspired the development of countless approaches for glycosylating 2-deoxy glycosidic donors.[66] Historically, investigators have accomplished the union of sugar donor and anthracyclinone acceptor using glycosyl chlorides (**34**, promoters: HgO/$HgCl_2$[67]; HgO/$HgBr_2$[68]; AgOTf[69-70]), bromides (**35**, promoter: $Hg(CN)_2$/$HgBr_2$[71]), acetates (**36**, promoter: TMSOTf[72-73]), and *p*-nitrobenzoates (**37**, promoter: TMSOTf[73-75]) as well as glycal donors (**38**, promoter: TsOH[76-77]; IDCP[78]). More recent studies have utilized phenyl thioglycosides (**39**, promoter: NIS[79], $AgPF_6$/TTBP[80-81]) and *o*-alkynylbenzoates (**40**, promoter: PPh_3AuNTf_2[26-27]).

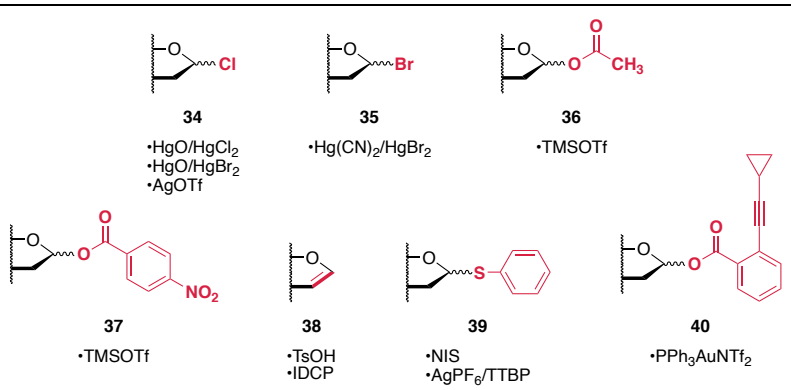
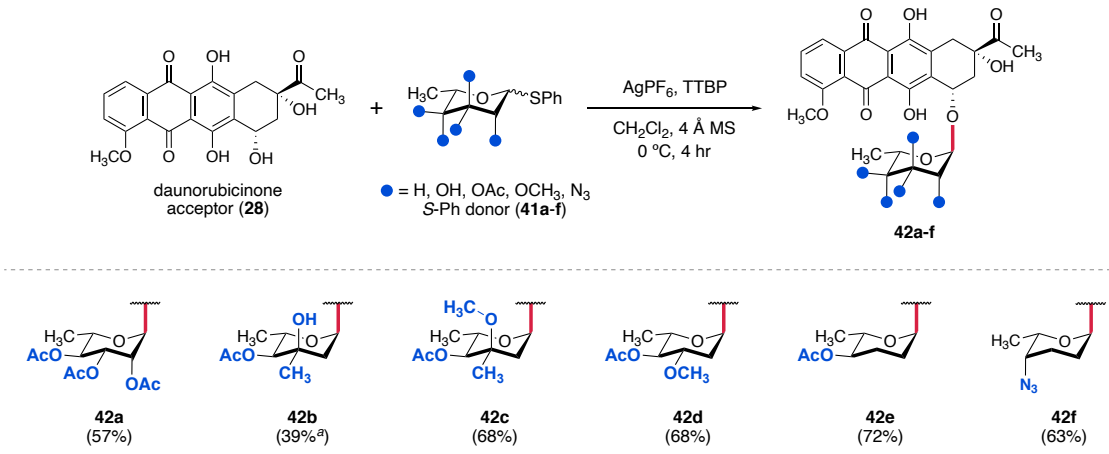


Figure 1.11 Examples of Glycosyl Donors Used in Glycosidations with Anthracycline Aglycones.

We elected to pursue the synthesis of phenyl thioglycoside donors based upon Zhu and Wang's successful use of Hirama's α-selective, $AgPF_6$ promoted thioglycoside activating conditions[82] to synthesize a library of daunorubicin derivatives (**42a–f**) with good α-selectivity, including two (**42b–c**) with C3

branching (Table 1.2).[80] Furthermore, Hirama[83] and Myers[84-85] have also demonstrated the proficiency of this methodology for establishing α-linkages with C3 branched donors in the context of complex natural product synthesis.

Table 1.2 Sun and Wang's 2005 Synthesis of Daunorubicin Derivatives (**42a**–**f**) Via the Activation of Phenylthioglycosides (**41a**–**f**) with $AgPF_6$.



[a]Yield obtained over 2 steps following deacetylation (0.1 M NaOH, THF).

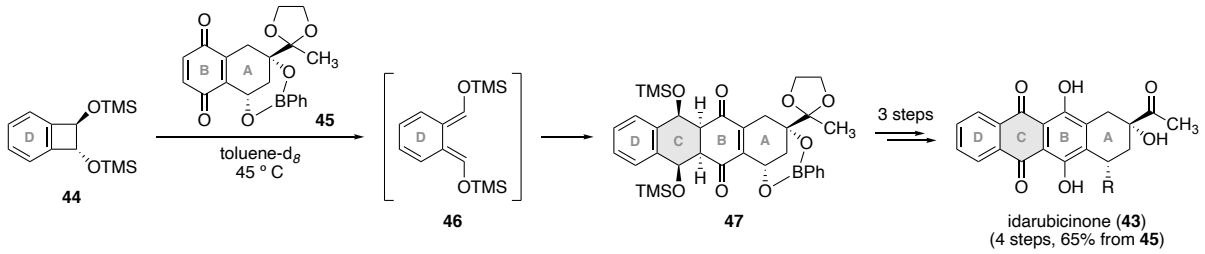
1.3.2 Synthetic Precedent: Aglycone Building Blocks

Our survey of the literature revealed that medicinal chemistry efforts focusing on glycan variation most often acquire the requisite aglycone moieties via the semi-synthetic degradation of commercially available anthracyclines such as daunorubicin (**5**) and doxorubicin (**6**). We elected to adopt this approach for obtaining the steffimycinone (**27**), daunorubicinone (**28**), and 14-*O*-TBS-doxorubicinone (**29**) aglycones as our synthetic and biological interests lay in the unique AMA disaccharide. Pleasingly, for the etoposide hybrid, we could synthesize the necessary aglycone **30** in one step from commercially available 4′-demethylepipodophyllotoxin (< \$20/gram). For the sake of completeness, we provide below a non-comprehensive overview of *de novo* strategies for accessing various anthracycline aglycones.[86-90] We begin with a brief discussion of various annulation approaches as organized by Sarlah before examining his lab's 2019 annulation-free synthesis of (±)-idarubicinone (**43**).[91]

1.3.2.1 Aglycone Synthesis: C-Ring Annulations

As noted by Sarlah, syntheses based around C-ring annulation have implemented neutral, cationic, and anionic D ring fragments to forge the C-ring. Our brief overview of these chemistries begins with Danishefsky's 2000 use of neutral D ring fragment **44**.[92] In this Diels-Alder based strategy, heating a mixture of **44** and **45**

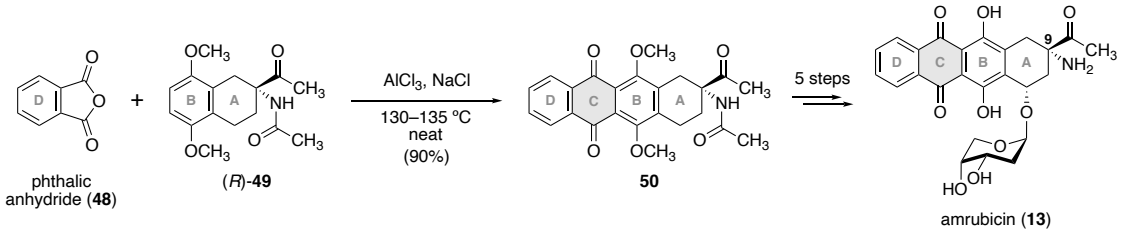
afforded tetracycle **47** through initial conrotatory ring opening to give **46** (Scheme 1.2). This intermediate then underwent an *endo*-selective Diels-Alder cycloaddition with diene **45** to give **47**. Three subsequent steps yielded idarubicinone **43**.



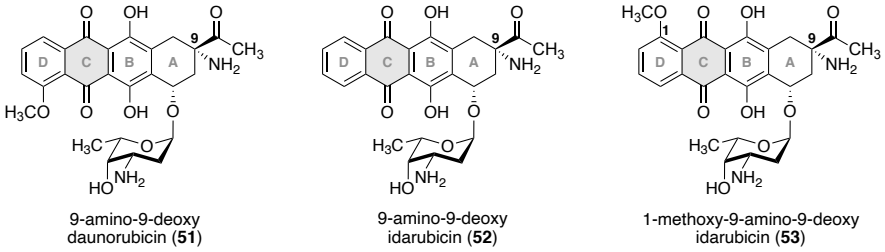
Scheme 1.2 Danishefsky's 2000 Synthesis of Idarubicinone **43** Via a Neutral C-Ring Annulation.

For examples of ionic approaches, we begin with Ishizumi's 1987 synthesis of C9-amino anthracycline derivatives.[93] Using the synthesis of amrubicin (**13**) as an example, heating a neat mixture of phthalic anhydride (**48**) and enantiopure (*R*)-**49** with $AlCl_3$ and NaCl at 130–135 °C afforded tetracycle **50** through a double Friedel-Crafts acylation in 90% yield (Scheme 1.3a). Five additional steps converted **50** to amrubicin **13**. The authors also used this cationic C-ring annulation approach to access 9-amino-9-deoxy derivatives of daunorubicin (**51**), idarubicin (**52**), and 1-methoxyidarubicin (**53**) (Scheme 1.3b).

a. Synthesis of Amrubicin (**13**).

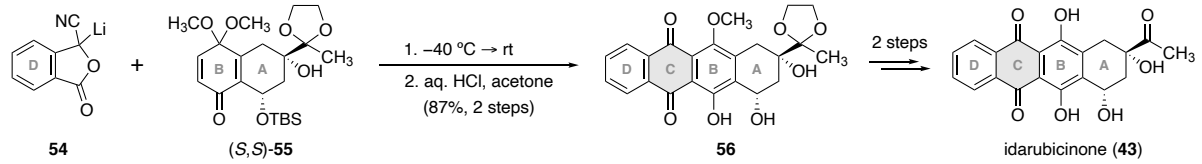


b. Other Anthracycline Derivatives Synthesized Using This Approach.



Scheme 1.3 Ishizumi's 1987 Synthesis of 9-Amino-9-Deoxy Anthracycline Derivatives **13** and **51**–**53** Via a Cationic C-Ring Annulation.

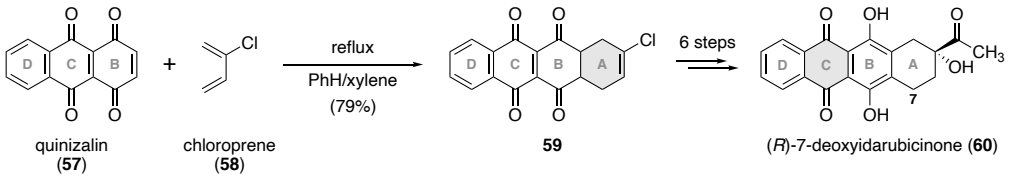
In contrast, the Swenton lab utilized an anionic C-ring annulation strategy in their 1984 synthesis of idarubicinone (**43**).[94] They found that addition of (*S*,*S*)-**55** to preformed lithiate **54** brought about a Kraus annulation that yielded **56** following acid mediated silyl deprotection (Scheme 1.4). A two-step deprotection sequence afforded idarubicinone (**43**).



Scheme 1.4 Swenton's 1984 Synthesis of Idarubicinone **43** Via an Anionic C-Ring Annulation.

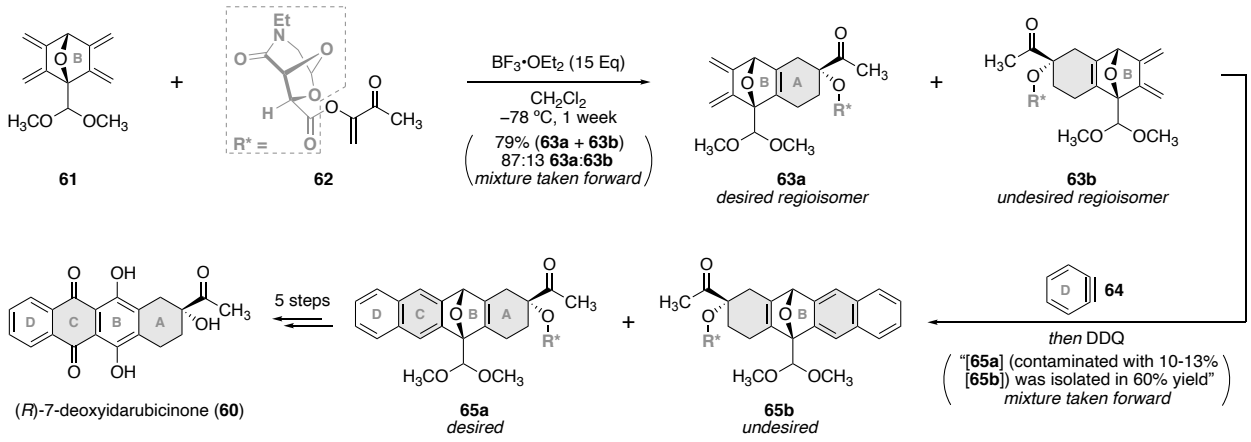
1.3.2.2 *Aglycone Synthesis: Non-C-Ring Annulations*

Some labs have adopted alternative annulation strategies. For example, in 1986, the Terashima lab utilized an A-ring annulation approach that began with the Diels-Alder reaction between quinizalin (**57**) and chloroprene (**58**) to give vinyl chloride **59** (Scheme 1.5).[95] Six additional steps, including a diastereomeric resolution of a carboxylic acid intermediate with (–)-*N*-methylephedrine, yielded (*R*)-7-deoxyidarubicinone (**60**).



Scheme 1.5 Terashima's 1986 Synthesis of (*R*)-7-deoxyidarubicinone (**60**) Via a Diels-Alder A-Ring Annulation Approach.

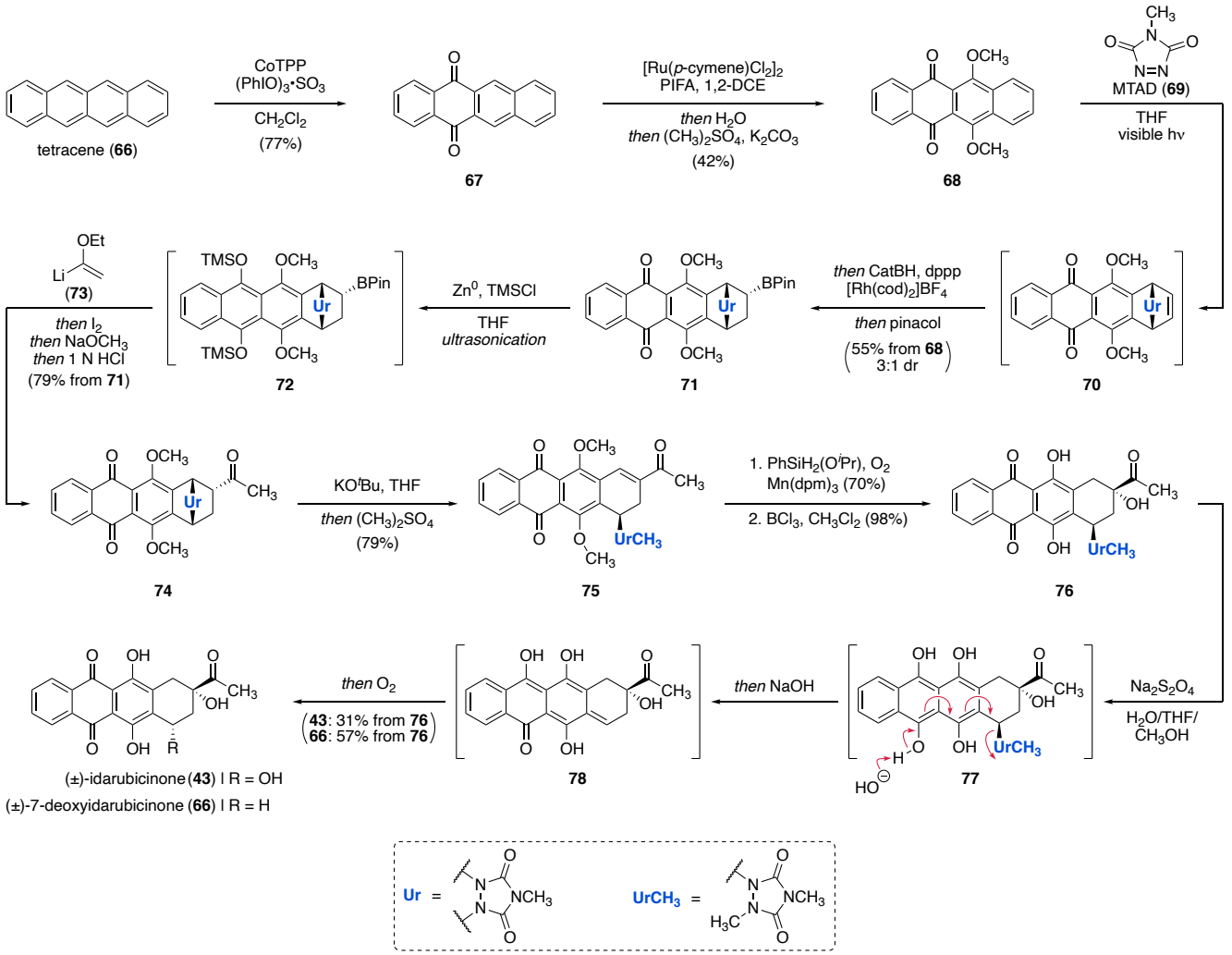
In 1993, Vogel reported an alternative Diels-Alder approach to (*R*)-7-deoxyidarubicinone (**60**) that employed two consecutive [4+2] cycloadditions.[96] Beginning with bis-diene **61**, reaction with chiral axillary[97] appended dienophile **62** in the presence of excess $BF_3 \bullet OEt_2$ (15 Eq) for one week at −78 °C afforded an 87:13 mixture of desired **63a** and regioisomer **63b** in 79% yield (Scheme 1.6); the authors noted that ^1H NMR indicated the production of less than 10% of isomeric products. Next, treatment of the **63a**–**b** mixture with benzyne **80** followed by DDQ oxidation gave a mixture of tetracyclic products **65a**–**b** which yielded the target (*R*)-7-deoxyidarubicinone (**60**) after five additional steps.



Scheme 1.6 Vogel's 1993 Synthesis of (*R*)-7-deoxyidarubicinone (**60**) Via a Double Diels-Alder Annulation Approach.

1.3.2.3 *Aglycone Synthesis: Sarlah's 2019 Annulation Free Synthesis of (±)-Idarubicinone (**43**).*

Sarlah's annulation free synthesis of (±)-idarubicinone (**43**) began with the oxidation of tetracene (**66**) using catalytic cobalt(II) tetraphenylprophyrin (CoTPP) and stoichiometric $(PhIO)_3 \bullet SO_3$ to give quinone **67** (Scheme 1.7).[91] Next, oxidation via Ru-catalyzed C-H activation[98] followed by *in situ* methylation afforded cyclization precursor **68**. Exposure of this compound to visible light in the presence of arenophile MTAD (**69**) brought about a regioselective *para*-photocycloaddition in accordance with the Sarlah group's established methodology.[99] *In situ* Rh-catalyzed hydroboration of presumed urazole intermediate **70** with catecholborane (CatBH) followed by transesterification with pinacol gave boronic ester **71**, bringing the one pot conversion of **68** to **71** to its conclusion. As initial attempts to leverage a *B*-alkyl Suzuki coupling to install the C13-C14 carbons proved ineffective, the authors elected to implement a Zweifel olefination to install this two-carbon unit.[100-101] In the event, *in situ* quinone reduction and silyl protection gave **72**[102-103] which yielded desired ketone **74** upon the sequential addition of lithiate **73**, I_2 (which also served to reoxidize the C-ring to the quinone), $NaOCH_3$, and 1 N HCl. Subsequent ^{t}BuOK mediated E1cb elimination followed by methylation of thr liberated urazole nitrogen using $(CH_3)_2SO_4$ yielded **75** in a one pot process. Next, a two-step Mukaiyama hydration[104-105]/BCl_3 deprotection sequence afforded penultimate intermediate **76**. Inspired by Moore's bioreduction alkylation hypothesis[106-107], the authors excised the final urazole bond through quinone oxidation to presumed intermediate **77** and subsequent NaOH mediated elimination to give likely quinone methide intermediate **78**. *In situ* quenching with O_2 gave desired (±)-idarubicinone (**43**) in a modest 31% yield along with 7-deoxy congener (**60**, 57%) resulting from electrophilic quenching of **78** with a proton.



Scheme 1.7 Sarlah's 2019 Synthesis of (±)–Idarubicinone (**43**).

1.3.3 Synthetic Precedent: L-Brasiliose Donor

The 3-amino-2,3,6-trideoxysugar L-brasiliose (**79**) serves as the aglycone-linked sugar in the anthracycline natural products arimetamycin A (**1**)[1-2], norcardicyclin A (**80**)[108], and norcardicyclin B (**81**)[108]. Additionally, various pluramycin natural products[109-110] feature *C*-linked L-brasiliose residues such as saptomycin B (**82**)[111-113] (Figure 1.12a). This sugar falls into the wider category of vancosamine like sugars as replacement of L-brasiliose's $N(CH_3)_2$ moiety with NH_2 yields L-vancosamine (**83**), the non-reducing sugar of the important antibiotic vancomycin (**84**) (Figure 1.12b). The synthetic complexity of these various natural products along with their biological activity, particularly that of vancomycin, has inspired various syntheses **79** and **83**. Due to the ready availability of vancomycin, its degradation is currently the most efficient route to these sugars.[114-117] As we elected to follow this approach, we begin with a discussion of various semi-synthetic strategies before providing a few examples of non-degrative approaches.

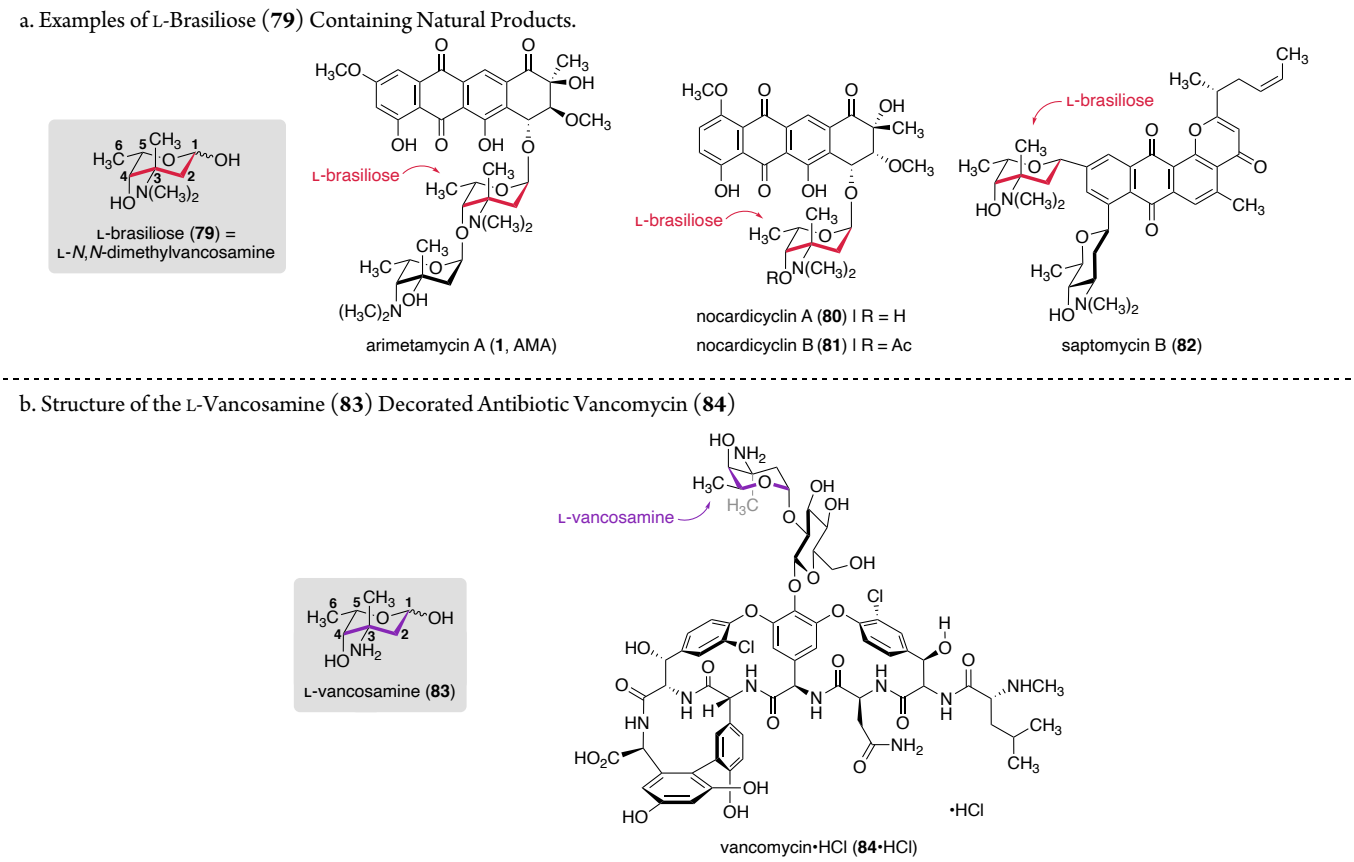
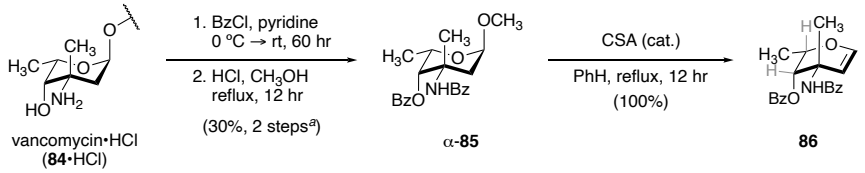


Figure 1.12 Examples of Natural Products Featuring L-Brasiliose and Its Parent Sugar L-Vancosamine.

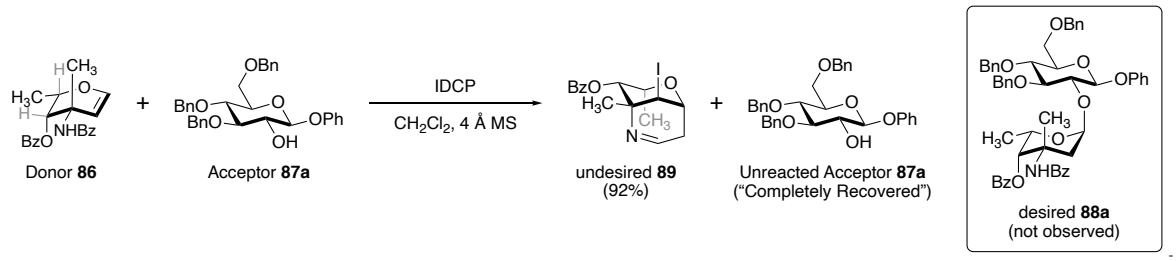
*1.3.3.1 Vancosamine Semisynthesis: Danishefsky's 1992 Synthesis of Model Vancomycin Disaccharide **88b***

Danishefsky's degradative semisynthesis of L-vancosamine glycal **86** began with benzoylation and subsequent acidic methanolysis of vancomycin•HCl (**84**•HCl) as described by Johnson (Scheme 1.8a).[118-119] Refluxing the resulting α-methylglycoside **85** with catalytic CSA in benzene yielded glycal donor **86**. However, an iodonium mediated glycal glycosylation[120-126] proved unsuccessful as IDCP activation in the presence of acceptor **87a** yielded undesired **89** rather than the desired disaccharide **88a** (Scheme 1.8b). Switching tactics, the authors found that CSA mediated glycosylation of acceptor **87a** with excess glycal **86** (1.5 Eq) provided the desired disaccharide **88a** in 64% yield (100% BRSM, Scheme 1.8c). Furthermore, these conditions proved successful when applied to 2,6-dimethoxyphenyl glycoside **87b**, an acceptor that better approximates the theoretical aglycone-glucoside acceptor **90** required to synthesize vancomycin (**84**).

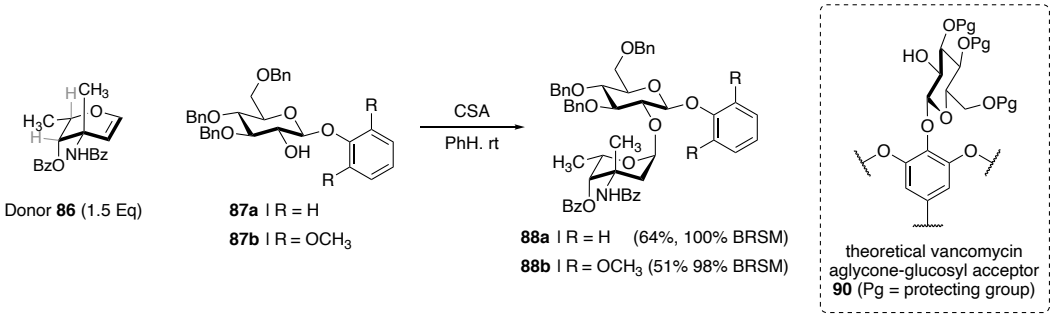
a. Semisynthesis of L-Vancosamine Glycal Donor **86** from Vancomycin•HCl (**84**).



b. Unsuccessful IDCP Mediated Glycosylation of L-Vancosamine Glycal Donor **86** with D-Glucose Acceptor **87a**.



c. Successful CSA Mediated Glycosylation of L-Vancosamine Glycal Donor **86** with D-Glucose Acceptors **87a–b**.

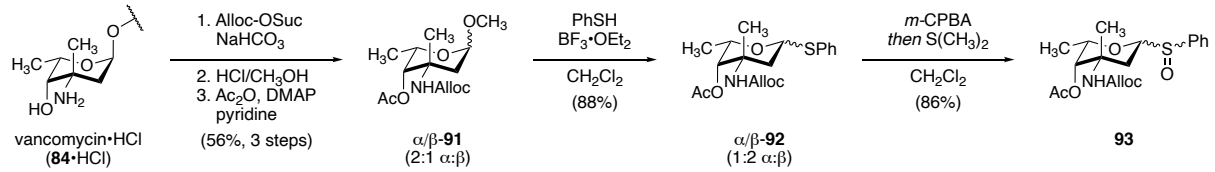


Scheme 1.8 Danishefsky's 1992 Synthesis and Glycosylation of L-Vancosamine Glycal **86**. [a]Hydrolysis of undesired β-**85** followed by Fisher glycosylation with CH_3OH yielded additional α-**85** (22% based on **84**•HCl).

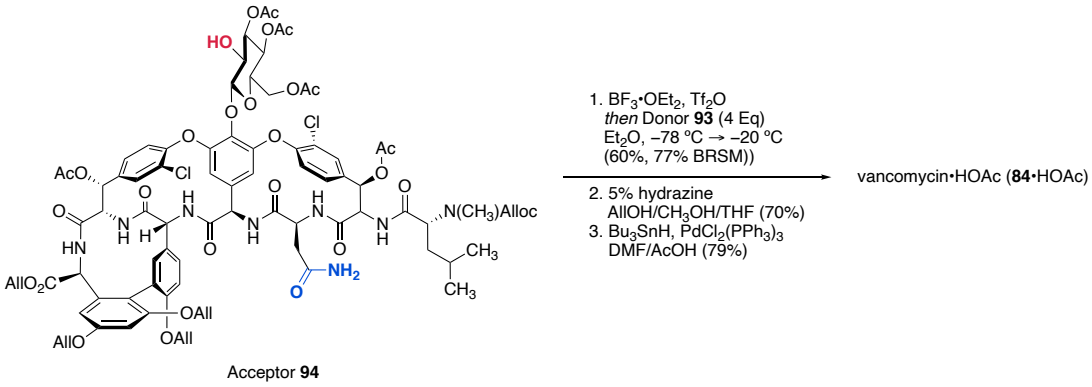
*1.3.3.2 Vancosamine Semisynthesis: Kahne's 1999 Semisynthesis of Vancomycin (**84**)*

Kahne's degradative semisynthesis of L-vancosamine sulfoxide donor **92** began with Alloc protection of vancomycin•HCl (**84**•HCl) (Scheme 1.9a).[114-115] Next, acidic methanolysis followed by C4-OH acetate protection gave methyl glycoside α/β-**91** in 56% yield over three steps. Exposure to PhSH and BF_3•OEt_2 afforded thioglycoside α/β-**92** which underwent subsequent oxidation with *m*-CPBA to give sulfoxide donor **93**, presumably as a mixture of anomers and sulfoxide epimers; Kahne also reported the synthesis of the corresponding *N*-Cbz analog of **93** (i.e, **95** Scheme 1.10a).[114] Next, BF_3•OEt_2/Tf_2O mediated glycosylation of **93** with acceptor **94** afforded the corresponding α-linked glycoside in good yield (60%, 77% BRSM) (Scheme 1.9b); the addition of BF_3•OEt_2 prevented the Tf_2O mediated dehydration of the aglycone's asparagine residue (highlighted in blue). Two subsequent deprotection steps yielded the acetate salt of vancomycin (**84**•HOAc).

a. Semisynthesis of L-Vancosamine Donor **93** from Vancomycin•HCl (**84**•HCl).



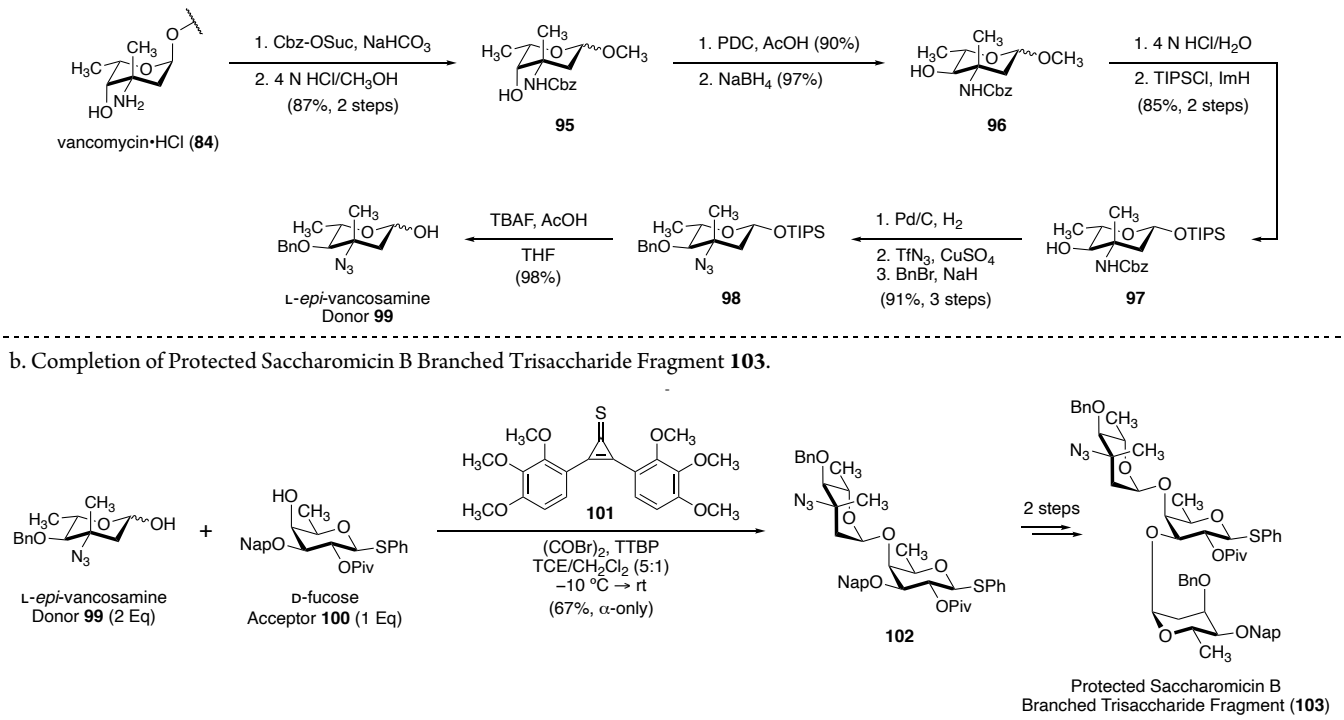
b. Completion of the Synthesis of Vancomycin•OAc (**84**•OAc).



Scheme 1.9 Kahne's 1999 Semisynthesis of Vancomycin•OAc (**84**•OAc).

1.3.3.3 *4-Epi-Vancosamine Semisynthesis: Bennett's 2018 Synthesis of Protected Saccharomicin B Branched Trisaccharide Fragment* ***103***

Bennett's semisynthesis of L-*epi*-vancosamine donor **99** began with *N*-Cbz protection and subsequent acidic methanolysis of vancomycin•HCl (**84**•HCl) to give **95** as described by Kahne (Scheme 1.10a).[114-115, 117] Next, C4 epimizeration was accomplished according to the two step oxidation/reduction protocol of Kahne and Walsh.[127] Ensuing anomeric hydrolysis followed by chemoselective anomeric silyl reprotection exclusively yielded β-*O*TIPS glycoside **97** which then underwent *N*-Cbz hydrogenolysis, TfN_3 mediated diazotransfer[128-131], and *O*-benzylation to give azide **98**. Finally, desilylation using TBAF and AcOH afforded L-*epi*-vancosamine donor **99**. Using methodology developed previously in the Bennett lab[132-134], glycosylation of **99** with D-fucose acceptor **100** in the presence of thione **101**, oxalyl bromide, and the bulky base 2,4,6-*tri*-tertbuytlpyrimidine (TTBP) provided disaccharide **102** in 67% yield with exclusive α-selectivity (Scheme 1.10b). An additional 2 steps transformed this intermediate to targeted protected saccharomicin B branched trisaccharide fragment **103** (see Figure A.3 for full structure of saccharomicin B, **104**).



Scheme 1.10 Bennett's 2018 Synthesis of Protected Saccharomicin B Branched Trisaccharide Fragment **103**.

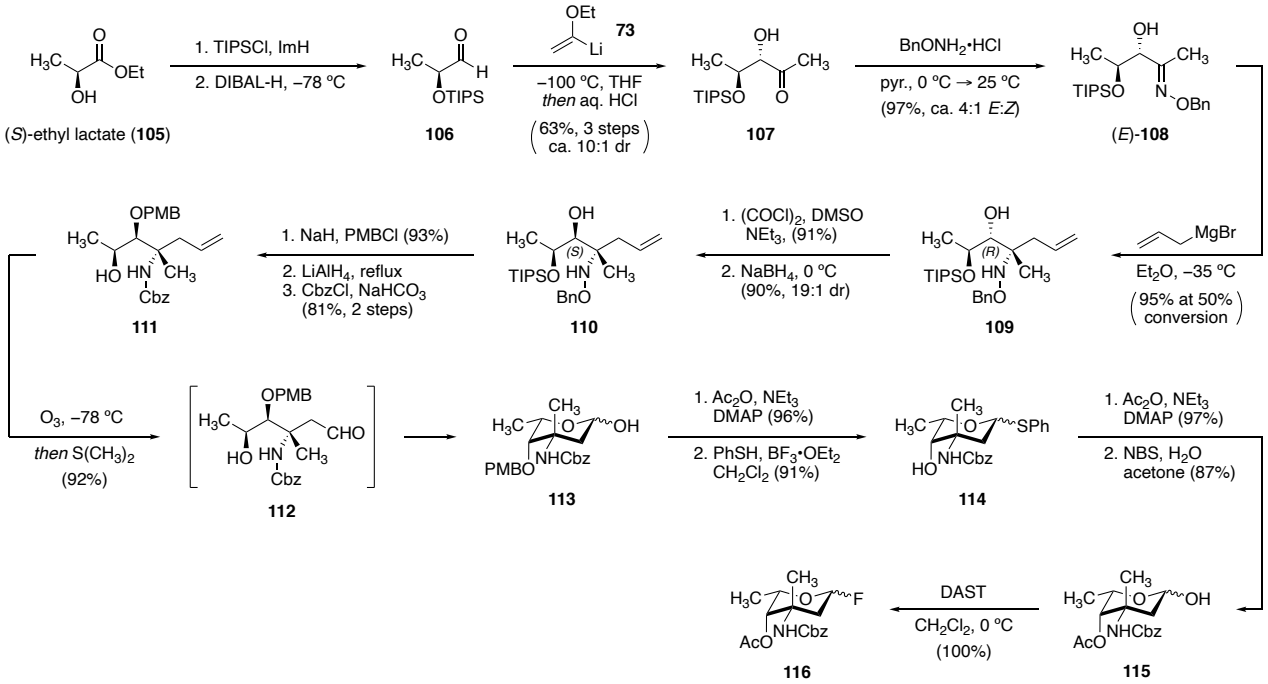
1.3.3.4 De Novo Vancosamine Synthesis: Nicolaou's 1998 Total Synthesis of Vancomycin

Nicolaou's *de novo* synthesis of L-vancosamine glycosyl fluoride **115** began with (*S*)-ethyl lactate (**105**) (Scheme 1.11a).[135-137] Silyl protection (TIPSCl) followed by partial reduction with DIBAL-H yielded aldehyde **106**. Ensuing addition of lithiate **73** occurred from the *re* face with good selectivity (ca. 10:1 dr) to give ketone **107** following acidic workup. Next, conversion to oxime **108** and subsequent treatment with vinylmagnesium bromide established the tetrasubstituted C3 center to give **109**. At this stage, two-step inversion of the neighboring (4*R*)-alcohol via Swern oxidation and $NaBH_4$ reduction (19:1 dr) afforded the required (4*S*)-epimer **110**.

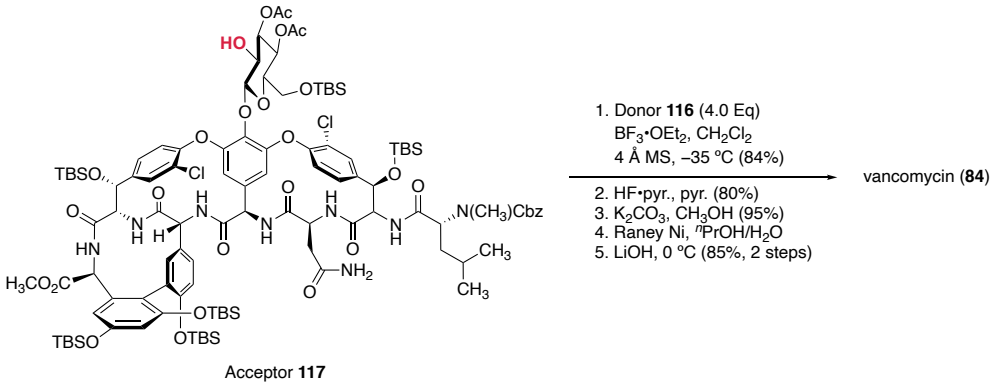
With the skeleton of **115** in place, PMB protection of alcohol **110**, $LiAlH_4$ mediated fissure the *N*-*O* bond and silyl ether bonds, and *N*-Cbz protection yielded **112**, setting the stage for pyranose ring closure (Scheme 1.11a).[136-137] In the event, alkene ozonolysis and decomposition of the resulting ozonide with $S(CH_3)_2$ yielded presumed intermediate **112** which underwent hemiacetalization to give **113**. Following anomeric acetylation, treatment with PhSH and BF_3•OEt_2 resulted in thioglycosylation and concurrent PMB deprotection to afford **114**. Reprotection of the C4 alcohol, this time as its acetate, and subsequent NBS mediated thioglycoside hydrolysis provided protected L-vancosamine hemiacetal **115**. Finally, treatment with DAST yielded the targeted glycosyl fluoride donor **116**. BF_3•OEt_2 promoted glycosylation of acceptor **117** with excess donor **116** (4.0 Eq)

yielded the corresponding glycoside in excellent yield (84%) and α-selectivity, as the authors reported only trace amount of another compound assumed to be the β-linked epimer (Scheme 1.11b). Finally, a four step deprotection sequence completed the Nicolaou group's total synthesis of vancomycin, a true synthetic tour de force.[135-143]

a. *De Novo* Synthesis of L-Vancosamine Donor **116** from (*S*)-Ethyl Lactate (**105**).



b. Completion of Vancomycin **84**.

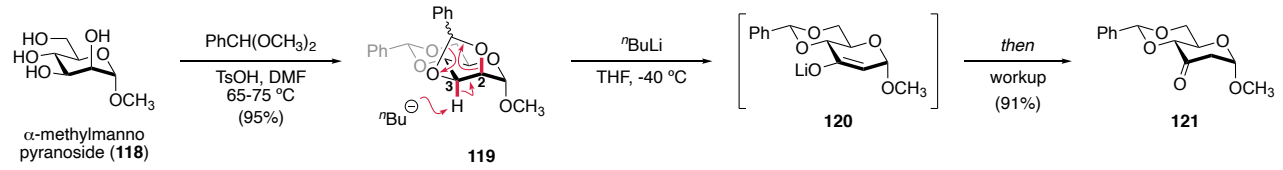


Scheme 1.11 Nicolaou's 1998 Total Synthesis of Vancomycin (**84**).

1.3.3.5 *Carbohydrate-Based Synthesis of Vancosamine: Matsumoto and Suzuki's 2013 Synthesis of Various L-Vancosamine Related Glycosyl Donors*

Matsumoto and Suzuki's 2013 carbohydrate-based synthesis of various L-vancosamine related glycosyl donors began from known D-mannose derived ketone **121** and constituted an improvement on Suzuki's 2006

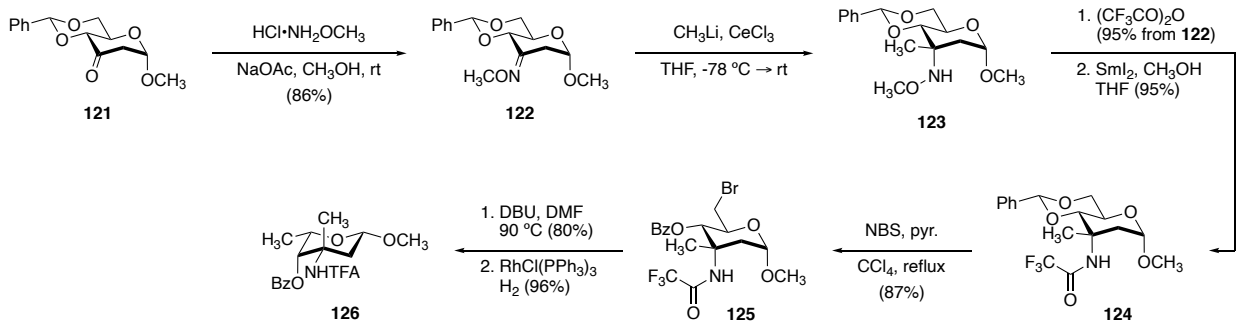
route based on earlier chemistry from Thang.[77, 144-146] Ketone **121** is available in two steps from α-D-methylmannopyranoside (**118**) as synthesized by Horton and Weckerie (Scheme 1.12).[147] Its synthesis begins with the dibenzlidenation of **118** to yield **119** as an inconsequential mixture of epimers. Next, treatment with excess *n*BuLi selectively abstracts the C3 proton with concomitant ejection of an equivalent of benzaldehyde to give lithium enolate **120** which yields ketone **121** upon aqueous workup.[148] Presumably, the regioselectivity of this reaction results from the antiperiplanar alignment of the abstracted C3 proton and the severed C2-O bond.



Scheme 1.12 Horton and Weckerie's 1975 Synthesis of Ketone **121**.

Returning to Matsumoto and Suzuki[146], condensation of ketone **121** with *O*-methyl hydroxylamine hydrochloride yielded an oxime (**122**) that underwent a subsequent diastereoselective methyl addition to give **123** upon introduction to a preformed methyl cerium species resulting from the mixing of CH_3Li and anhydrous $CeCl_3$ (Scheme 1.17a).[149-150] *N*-trifluoroacetylation followed by reductive *N*-*O* bond cleavage with SmI_2 gave trifluoroacetamide **124**. Next, Hanessian-Hullar ring opening[151-152] of the benzylidene acetal in the presence of NBS and pyridine afforded bromide **125**; in their 2006 paper, the Suzuki group noted that use of the more traditional base $BaCO_3$ led to a poor reproducibility (22-71% yields).[145] Finally, DBU mediated elimitation followed by hydrogenation over Wilkinson's catalyst ($RhCl(PPh_3)_3$) yielded diversifiable L-vancosamine derivative **126**. Between their 2006 and 2013 papers, the authors demonstrated the conversion of **126** to L-vancosamine donors **127**–**129** and to L-brasiliose derivative (**130**).[145-146]

a. Synthesis of Diversifiable L-Vancosamine Derivative **126**.



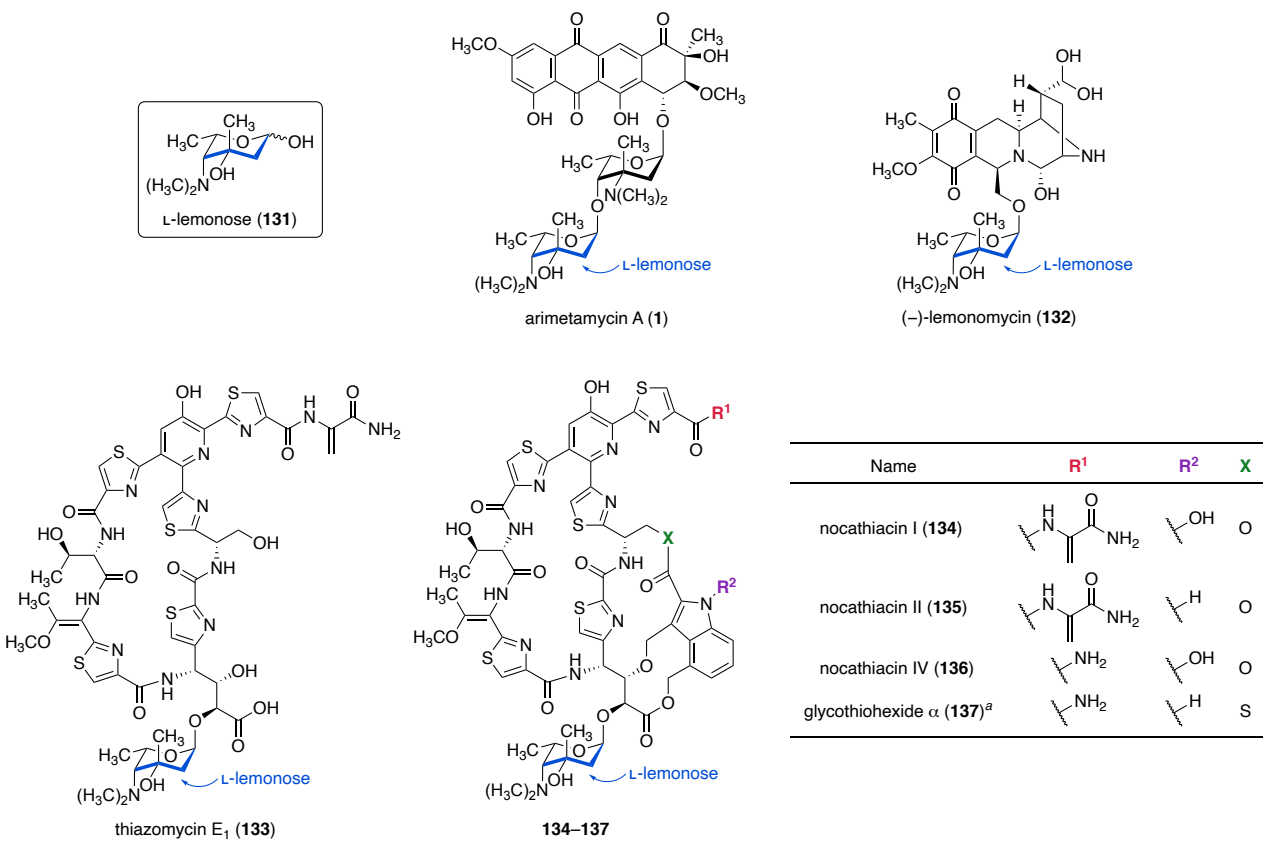
b. Diversification of L-Vancosamine Derivative **126**.

127 2 steps from **126**[a]; **128** 4 steps from **126**; **129** 6 steps from **126**; **130** 4 steps from **126**

Scheme 1.13 Matsumoto and Suzuki's 2013 Synthesis and Diversification of L-Vancosamine Derivative **126**. [a]From Suzuki's 2006 synthesis of **126**.

1.3.4 Synthetic Precedent: L-Lemonose Donor

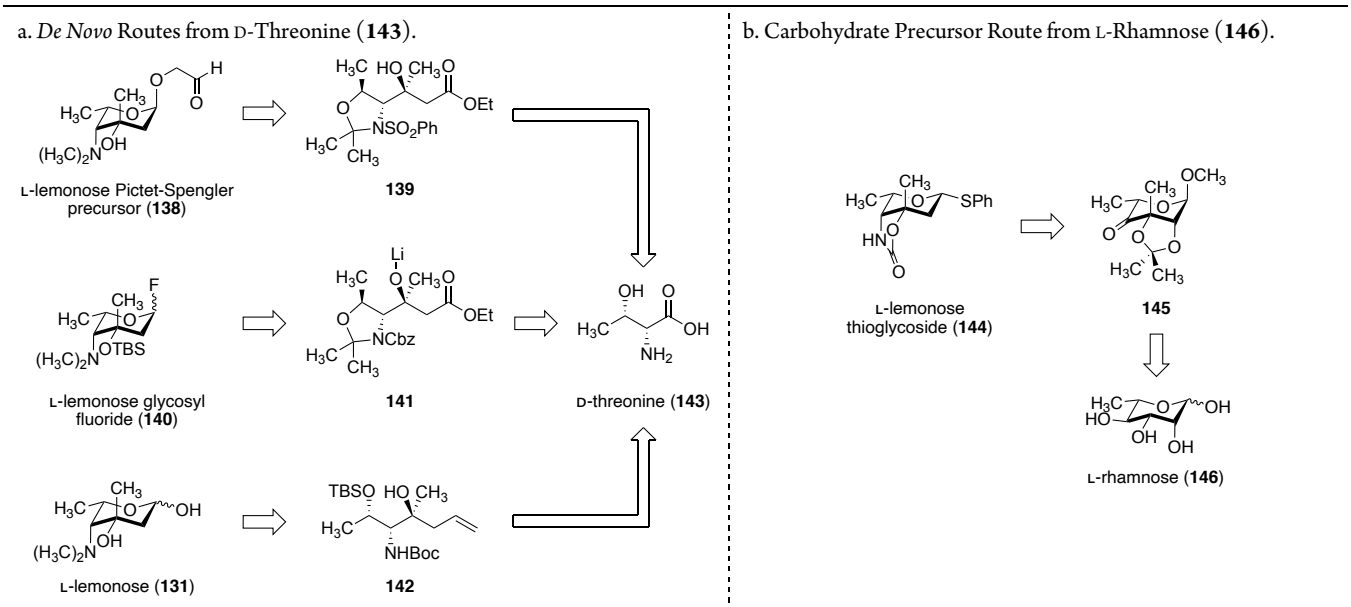
In designing our synthesis of a suitable L-lemonose thioglycoside donor (**32**), we surveyed the available literature concerning this relatively rare 4-amino-2,4,6-trideoxysugar. We found that L-lemonose (**131**) occupies the non-reducing position of the arimetamycin A (**1**) glycan[1-2] and serves as the sole glycosidic residue of the tetrahydroisoquinoline natural product (–)-lemonomycin (**132**)[153-154] (Figure 1.13). Additionally, it is featured by various members of the thiazolyl peptide class of natural products (e.g., **133**-**137**).[155-162]



Name	R^1	R^2	X
nocathiacin I (**134**)			O
nocathiacin II (**135**)			O
nocathiacin IV (**136**)			O
glycothiohexide α (**137**)[a]			S

Figure 1.13 Structures of L-Lemonose and L-Lemonose Containing Natural Products. [a]Isolation report does not define the stereochemistry of the aglycone.[155]

Four groups have achieved the synthesis of either an L-lemonose based synthetic intermediate (**138**)[163], a protected L-lemonose glycosyl donor (**140**[164], **144**[165]), or the L-lemonose hemiacetal itself (**131**)[166]. Retrosynthetically, these syntheses have adopted one of two strategies: (1) a *de novo* approach from D-threonine (**143**) involving a C1-O5 cyclization of a linear precursor (Scheme 1.14a) and (2) a carbohydrate precursor route involving the manipulation of L-rhamnose (**146**) (Scheme 1.14b). Below, we discuss each route in detail, beginning with Stolz's 2003 synthesis of L-lemonose Pictet-Spengler precursor **138**.[163] We then discuss the syntheses of related deoxyamino sugars that influenced our retrosynthetic analysis.

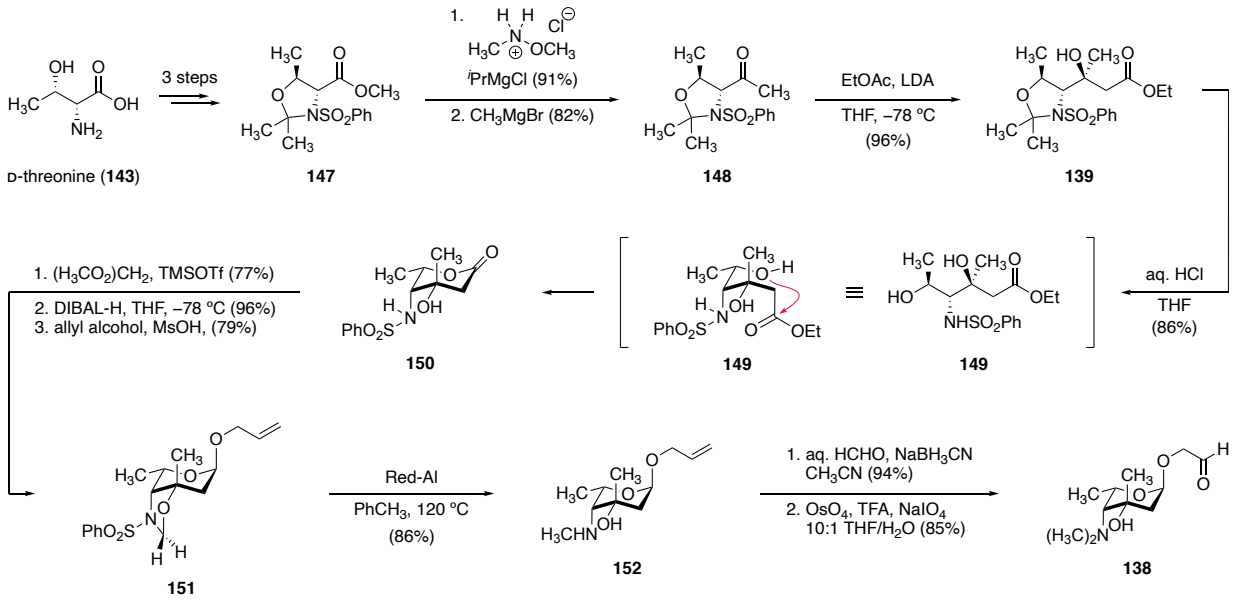


Scheme 1.14 Summary of Retrosynthetic Analyses from Known Syntheses of L-Lemonose (**131**) and Its Derivatives **138**, **140**, and **144**.

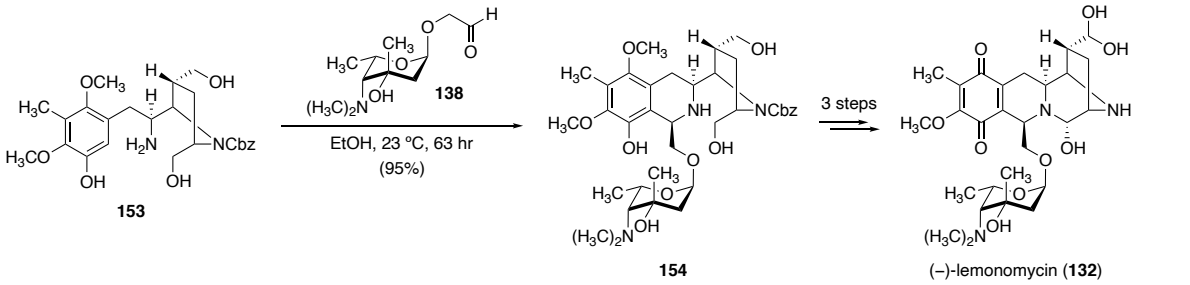
1.3.4.1 Lemonose Syntheses: Stoltz's 2003 Synthesis of (−)-Lemonomycin (132)

Stoltz's synthesis of L-lemonose Pictet-Spengler precursor **138** began with known ester **147**, available in three steps from D-threonine (**143**, Scheme 1.15a).[167] Two step conversion of **147** to methyl ketone **148** occurred via transformation to the Weinreb amide and subsequent displacement with CH_3MgBr. Next, reverse addition of ketone **148** to a solution of ethyl acetate's lithium enolate delivered aldol product **139** as a single observable diastereomer, presumably via a Felkin-Ahn transition state (discussed *vide infra*, see Scheme 1.16c). Next, acid mediated acetonide deprotection gave presumed diol **149** which underwent *in situ* transesterification to yield lactone **150**. Subsequent oxazolidine formation (dimethoxymethane, TMSOTf), lactone reduction (DIBAL-H), and Fisher esterification (allyl alcohol, MsOH) gave **151**. Ensuing Red-Al reduction at elevated temperature affected both benzenesulfonyl deprotection and oxazolidine opening to yield disubstituted amine **152**. Finally, Stoltz and co-workers obtained desired L-lemonose based Pictet-Spengler precursor **138** upon installation of the second *N*-methyl substituent by reductive amination (aq. HCHO, $NaBH_3CN$) and oxidative cleavage of the terminal alkene (OsO_4, $NaIO_4$) to reveal the necessary aldehyde. The key Pictet-Spengler cyclization between aldehyde **138** and late stage aglycone intermediate **153**•TFA occurred with remarkable efficiency, yielding **154** as a single diastereomer in 95% yield (Scheme 1.15b). Three subsequent transformations completed the first total synthesis of (−)-lemonomycin (**132**).

a. Synthesis of L-Lemonose Derived Pictet-Spengler Precursor **138**.



b. Completion of (–)-Lemonomycin (**132**).

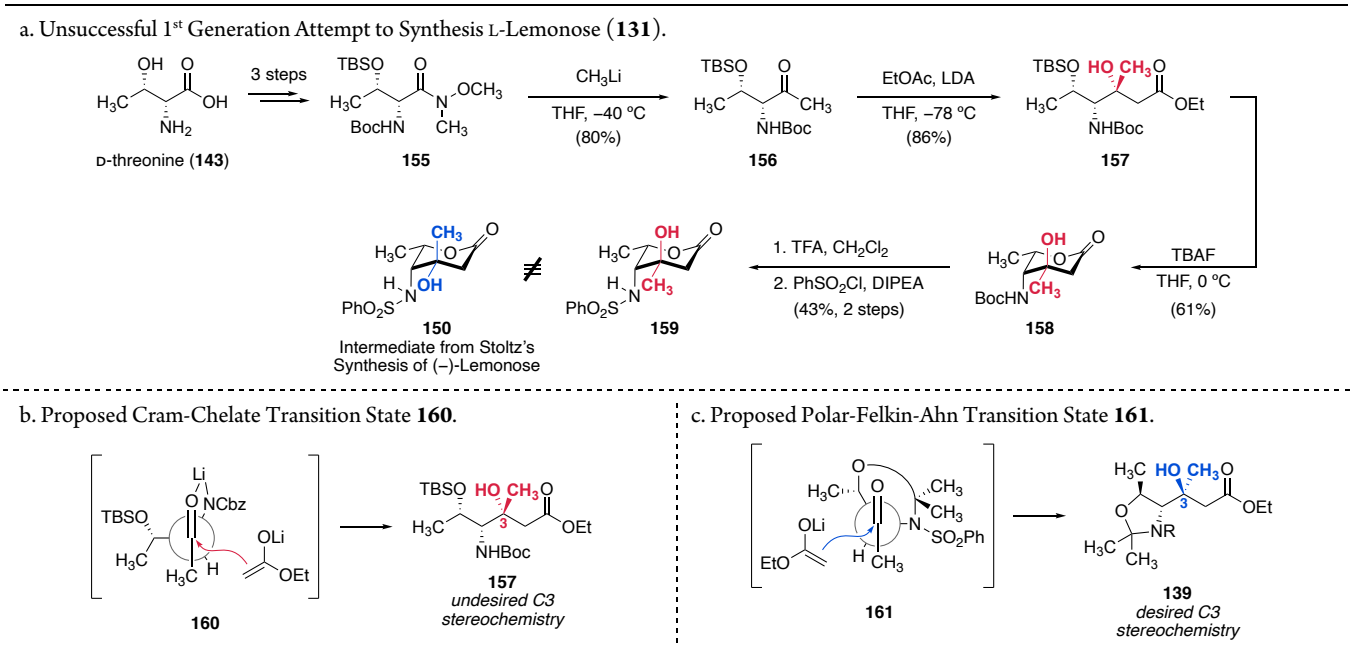


Scheme 1.15 Stolz's 2003 Synthesis of (–)-Lemonomycin (**132**).

*1.3.4.2 Lemonose Syntheses: Masson and Zhu's 2011 Synthesis of L-Lemonose (**131**)*

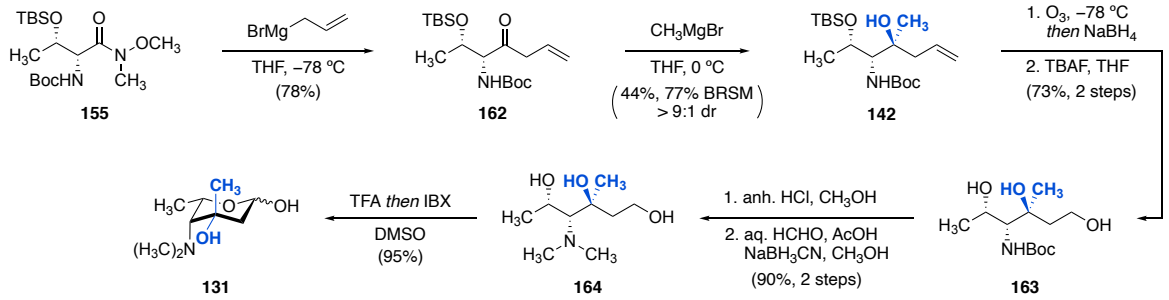
Masson and Zhu's 2011 synthesis of the L-lemonose monosaccharide (**131**) began with the three-step conversion of D-threonine (**143**) to Weinreb amide **155** (Scheme 1.16a).[166] Subsequent CH_3Li addition afforded ketone **156**. Addition of the lithium enolate of ethyl acetate yielded 3° alcohol **157**. Unsure at this point of the stereochemistry of the newly formed tetrasubstituted center, the authors pushed this material forward to a one pot TBAF mediated silyl deprotection/lactonization to give lactone **158**. *N*-Boc to *N*-SO_2Ph exchange yielded **159**, allowing for direct comparison with **150** from Stolz's (–)-lemonomycin synthesis.[163] Discrepancies in the NMR data indicated that **159** was the C3 epimer of **150**, meaning that the nucleophilic addition to ketone **156** had occurred from the undesired face of the carbonyl. The authors postulated that the observed stereochemistry for **157** resulted from the intermediacy of a chelated transition state such as **160** (Scheme 1.16b); in contrast, the use of a doubly protected amine moiety in Stoltz's L-lemonose synthesis (Scheme 1.15a) precluded chelate

formation and forced the reaction to proceed through a polar Felkin-Ahn transition state such as **161** (Scheme 1.16c).[163, 168]



Scheme 1.16 Masson and Zhu's Unintended Synthesis of 3-*epi*-L-Lemonose Derivative (**159**).

The authors reasoned that they could take advantage of this chelate driven selectivity by inverting the order in which they installed the C3 substituents. In practice, addition of vinyl magnesium bromide to Weinreb amide **155** yielded ketone **162** (Scheme 1.17). Subsequent addition of CH_3MgBr proved more challenging than anticipated; however, optimization efforts allowed for the synthesis of 3° alcohol **142** with the desired C3 stereochemistry (> 9:1 dr) with moderate efficiency (44% yield, 77% BRSM). Reductive ozonolysis followed silyl deprotection yielded triol **163** which underwent *N*-Boc deprotection and reductive amination to give penultimate intermediate **164**. Finally, *in situ* amine protection with TFA followed by the addition of IBX resulted in chemoselective 1° alcohol oxidation to give L-lemonose hemiacetal **131**.

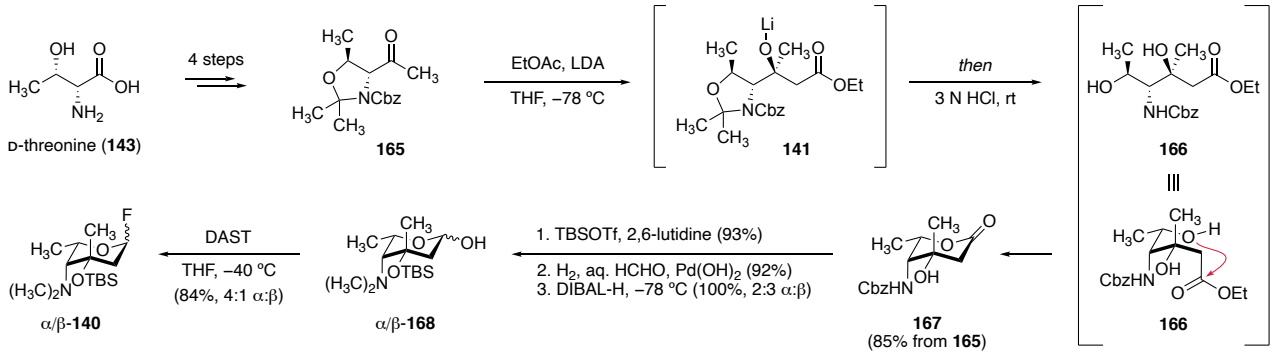


Scheme 1.17 Masson and Zhu's Successful 2011 Synthesis of L-Lemonose (**131**).

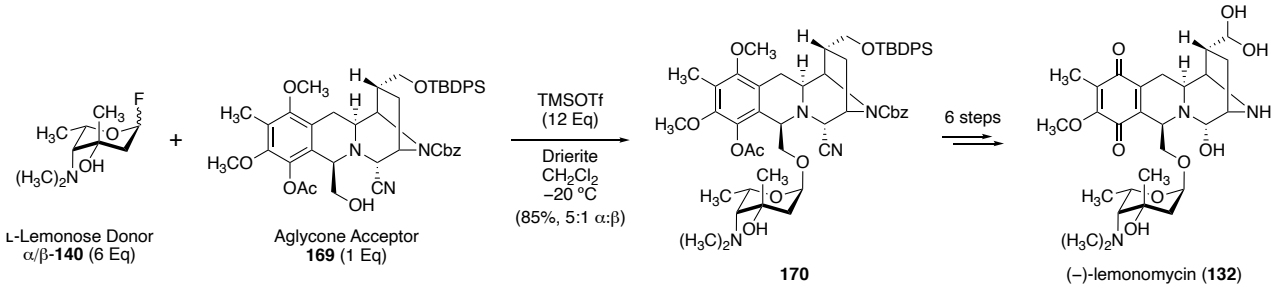
1.3.4.3 Lemonose Syntheses: Fukuyama and Kan's 2012 Synthesis of (−)-Lemonomycin (132)

In their 2012 total synthesis of (−)-lemonomycin (**132**)[164], Fukuyama and Kan constructed the framework of the L-lemonose sugar in a manner reminiscent of Stoltz's 2003 work.[163] They began from known D-threonine (**143**) derived ketone **165**[168] which was identical to ketone **148** from Stolz's synthesis except for the *N*-Cbz protecting group (Scheme 1.18a). Stereoselective addition of the lithium enolate of ethyl acetate to **165** give intermediate lithiate **141** (see Scheme 1.16c for an applicable transition state model). Upon quenching with 3 N HCl, this presumed intermediate underwent oxazolidine deprotection and subsequent ring closing transesterification to give lactone **167** in 85% yield from **165** in a one pot, three transformation procedure. At this point, Fukuyama and Kan's strategy deviated from that of Stoltz as they envisioned installing the L-lemonose glycan via a traditional glycosylation (as opposed to the Pictet-Spengler cyclization utilized by Stolz, see Scheme 1.15b). To this end, 3° alcohol protection (TBSOTf, 2,6-lutidine), one pot amine deprotection/*N*,*N*-dimethylation (H_2, aq. HCHO, $Pd(OH)_2$), and lactone reduction (DIBAL-H) provided α/β-**168**. Exposure of this reducing sugar to DAST yielded targeted glycosyl fluoride donor α/β-**140**. Taking this donor forward, the authors found that aglycone acceptor **169** underwent high yielding glycosidation in the presence of excess donor α/β-**140** (6 Eq) and TMSOTF promoter (12 Eq) to give **170** (Scheme 1.18b). Six final steps completed the synthesis of (−)-lemonomycin.

a. Synthesis of L-Lemonose Glycosyl Fluoride Donor α/β-**140**.



b. Completion of (−)-Lemonomycin (**132**).

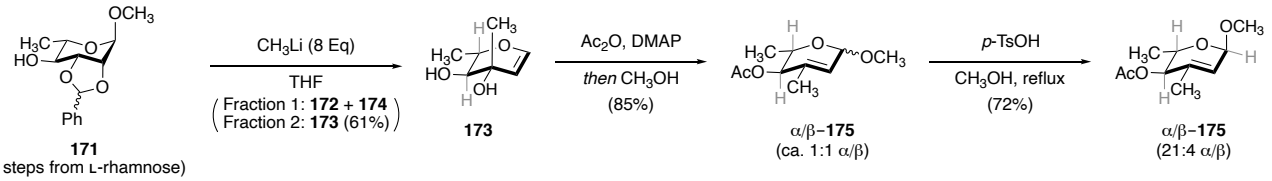


Scheme 1.18 Fukuyama and Kan's 2012 Synthesis of (−)-Lemonomycin (**132**).

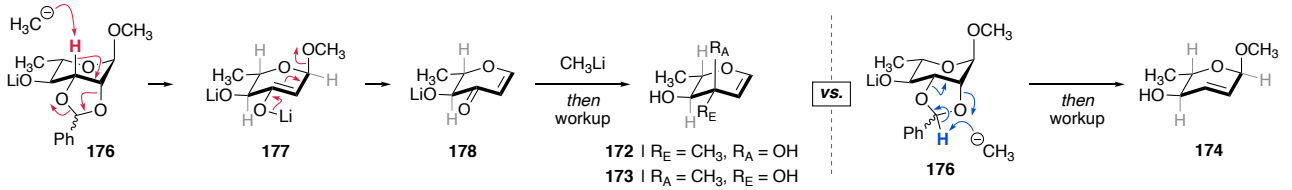
1.3.4.4 Lemonose Syntheses: Giuliano's 2015 Synthesis of L-Lemonose (131)

In 2015, Giuliano reported the first synthesis of L-lemonose that started from a carbohydrate precursor rather than from D-threonine (**143**).[165] They began from glycal **179**, available in 1 step from **175** as synthesized by Parker.[169] The synthesis of precursor **175** begins with exposure of rhamnose derived benzylidene acetal **171** to excess CH_3Li (8 Eq) (Scheme 1.19a).[170] Chromatography yields pure **173** as well as a co-eluting mixture of **172** and **174**. Mechanistically, products **172** and **173** arise from elimination of the antiperiplanar C3 hydrogen from lithiate **176** (Scheme 1.19b, left). E1cb elimination of the anomeric OCH_3 group gives vinylogous ester **178** which then undergoes 1,2-addition with remaining CH_3Li to yield a mixture of **172** and **173**. In contrast, elimination of the benzylic hydrogen results in the formation of glycal **174** (Scheme 1.19b, right). Returning to the synthesis, subjection of pure **173** to a type I Ferrier rearrangement followed by thermodynamically driven anomerization yields an α-enriched mixture of **175** (21:4 α:β).

a. Synthesis of α/β-**175** from L-Rhamnose.



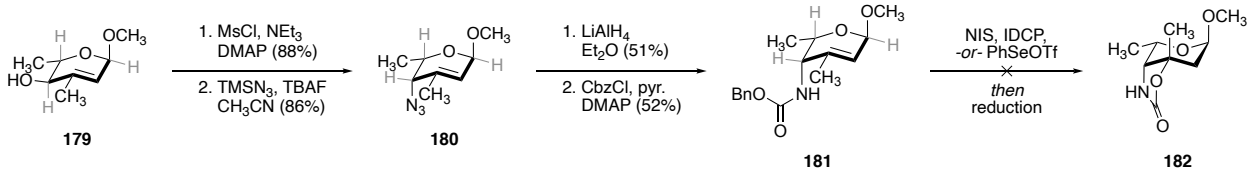
b. Proposed Mechanisms for the Formation for **172**–**174**.



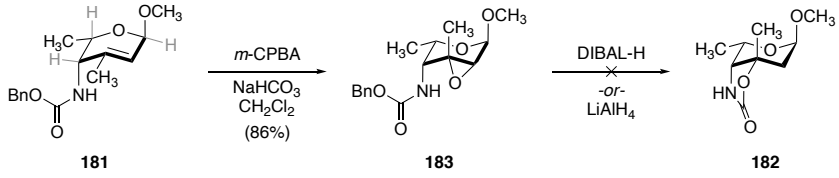
Scheme 1.19 Parker's 1988 Synthesis of Glycal **175**.

Following deacetylation of **175**, the Giuliano group subjected the resulting allylic alcohol **179** to a two-step mesylation/azide addition sequence to obtain **180** (Scheme 1.20a).[165] $LiAlH_4$ reduction of the azide followed by carbamate formation gave cyclization precursor **181**. Unfortunately, attempts to induce cyclization using NIS, IDCP, and PhSeOTf proved ineffective. Reasoning that epoxidation followed by reductive opening could trigger the formation of desired oxazolidinone **182**, Giuliano stereospecifically transformed glycal **181** to epoxide **183** (Scheme 1.20b). However, treatment of **183** with DIBAL-H resulted in no observable reaction while the use of $LiAlH_4$ led to a complex mixture of products.

a. Unsuccessful 1st Generation Approach to Synthesizing **182**.



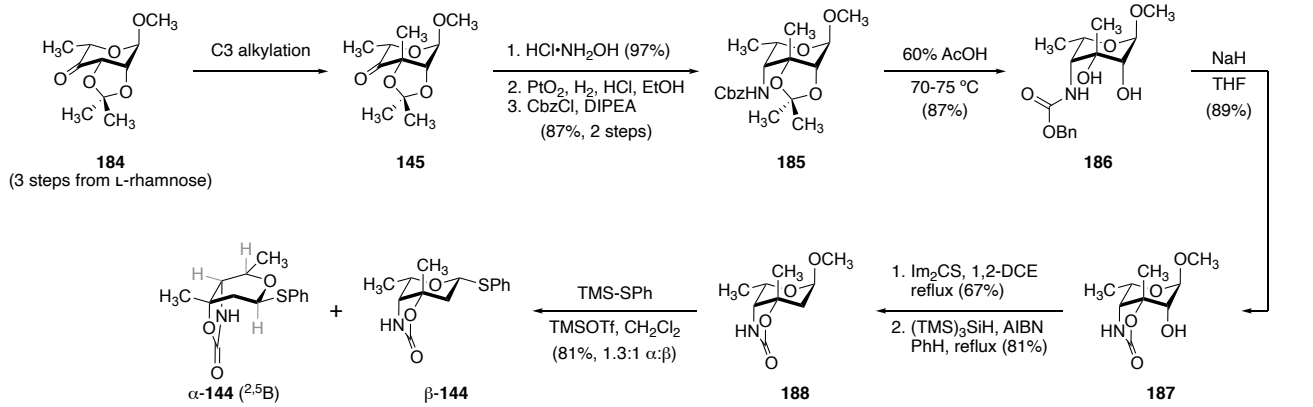
b. Unsuccessful 2nd Generation Approach to Synthesizing **182**.



Scheme 1.20 Giuliano's Unsuccessful Attempts at Synthesizing Oxazolidinone **182**.

At this point the Giuliano group opted to alter their strategy, realizing that deoxygenation of intermediate **187** from their 1998 synthesis of L-callipeltose[171] would provide access to L-lemonose sugars. They initiated their callipeltose synthesis with the C3 alkylation of L-rhamnose derived ketone **184** to give **145** as described by Kiemer

and Beermann (Scheme 1.21).[172] Next, oxime formation[173], reduction over Adam's catalyst, and *N*-Cbz protection yielded carbamate **185**. Ensuing acetonide deprotection set the stage for cyclization which occurred upon treatment of diol **186** with NaH to yield D-callipeltose precursor **187**. Acylation of the C2 alcohol with Im_2CS[174] followed by $(TMS)_3SiH$ mediated deoxygenation yielded methyl glycoside **188**. Finally, exposure of **188** to TMS-SPh in the presence of TMSOTf gave thioglycosides α/β-**144** (1.3:1 α:β). Interestingly, X-ray crystallography showed that α-**144** assumed a boat conformation ($^{2,5}B$) that placed the anomeric hydrogen in an axial position; anomalously large anomeric coupling constants ($^3J_{H1\text{-}H2}$ = 10.4, 6.4 Hz)[175] indicate that α-**144** likely assumes a similar conformation in solution.

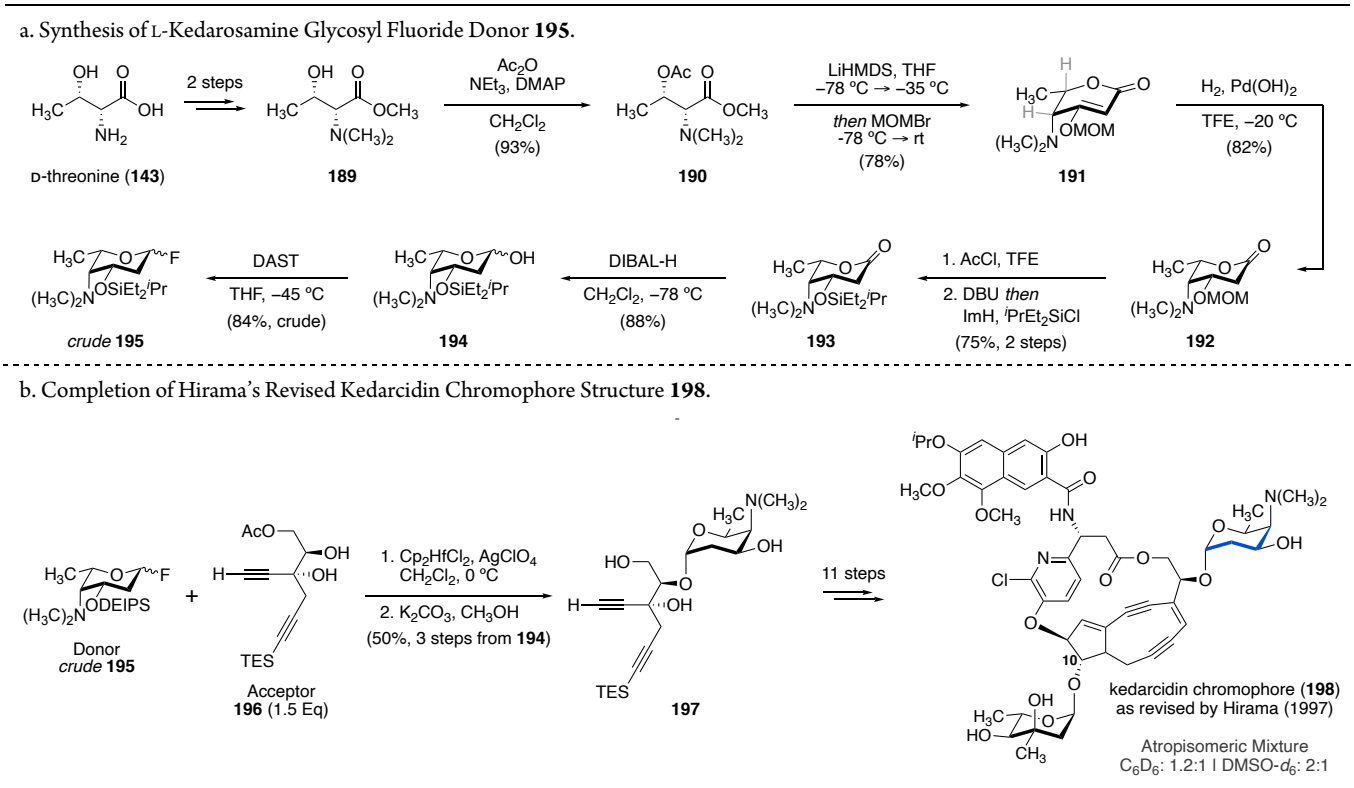


Scheme 1.21 Giuliano's Successful 2015 Synthesis of L-Lemonose Thioglycosides α/β-**144**.

1.3.4.5 *Non-Lemonose Syntheses: Myers' 2007 Synthesis of Hirama's Revised Kedarcidin Chromophore Structure (**195**)*

Myers' synthesis of L-kedarosamine glycosyl fluoride **195** began from known D-threonine derivative **189**[176], available in two steps from D-threonine (**143**) (Scheme 1.22a).[177] Routine alcohol acetylation yielded **190** which underwent Dieckmann condensation upon inversion addition to LiHMDS. *In situ* trapping of the resulting vinylogous lithium carboxylate with MOMBr afforded **191** in a one pot, two step transformation. Next, chemo- and stereoselective alkene hydrogenation over Pearlman's catalyst ($Pd(OH)_2$) yielded lactone **192**; the authors noted that careful optimization of solvent and catalyst proved necessary to avoid reductive MOM ether cleavage. Treatment with AcCl in TFE brought about the desired acid mediated MOM deprotection, yielding an unexpected linear TFE ester. Solvent removal followed by the addition of DBU reestablished the cyclic structure, and subsequent addition of imidazole and $^i PrEt_2SiCl$ furnished silyl ether **193**. Penultimate DIBAL-H mediated lactone reduction and subsequent treatment of the resulting hemiacetal **194** with DAST yielded L-kedarosamine glycosyl fluoride donor **195**.

Pressing forward to the glycosidation, reaction of crude donor **195** with acceptor **196** under Suzuki conditions ($CpHfCl_2$, $AgClO_4$) followed by deacetylation gave glycosyl diyne **197** (Scheme 1.22b).[177] Eleven further steps transformed this intermediate into kedarcidin chromophore **198**, obtained as an atropisomeric mixture.[84] Comparison of the ^1H NMR spectrum of **198** to that of the isolated compound showed considerable differences. Examination of these data revealed that neither Bristol-Myer Squibbs' originally proposed structure[178-179] nor Hirama's revised structure **198**[180] (which had served as the Myers group's synthetic target) were consistent with the original NMR data. Based on their reexamination of data and contact with the lead author from the isolation reports, Myers and co-workers proposed *epi*-10-**198** as the likely structure for the kedarcidin chromophore.

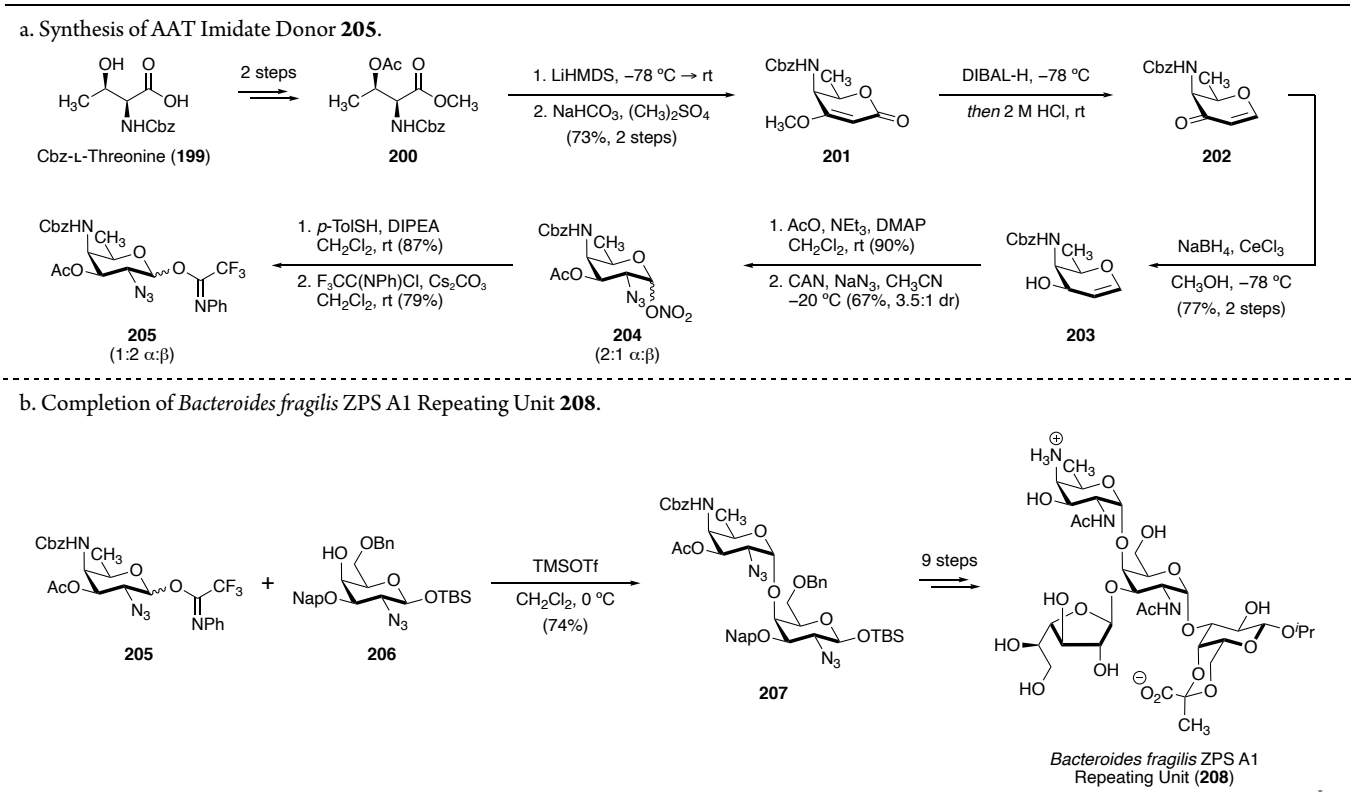


Scheme 1.22 Myers' 2007 Synthesis of Hirama's Revised Kedarcidin Chromophore Structure (**198**).

1.3.4.6 Non-Lemonose Syntheses: Seeberger's 2011 Synthesis of Bacteroides fragilis ZPS A1 Repeating Unit **208**

Seeberger's synthesis of D-AAT donor **205** began from commercially available Cbz-L-threonine (**199**, Scheme 1.23a).[181] Routine esterification and alcohol acetylation afforded cyclization precursor **200** which underwent Dieckmann condensation upon exposure to LiHMDS; subsequent trapping of the β-keto ester with dimethyl sulfate gave vinylogous carbonate **201**. Next, DIBAL-H mediated lactone reduction followed by 2 N HCl quench and warming to room temperature brought about a Stork-Danheiser like transposition to provide

202. Taken on crude, Luche diastereoselective reduction of this vinylogous ester yielded allylic alcohol **203**. Protection of the alcohol as its acetate followed by azidonitration installed the C2 amine moiety to give anomeric nitrate **204**. Finally, *p*-TolSH mediated anomeric denitration followed by acylation with $F_3CC(NPh)Cl$ provided L-AAT donor α/β-**142**. Glycosylation of α/β-**142** with acceptor **206** under the influence of TMSOTf gave α-linked disaccharide **207** in 74% yield.[182] An additional nine transformations converted **207** into PS A1 repeating unit **208**.



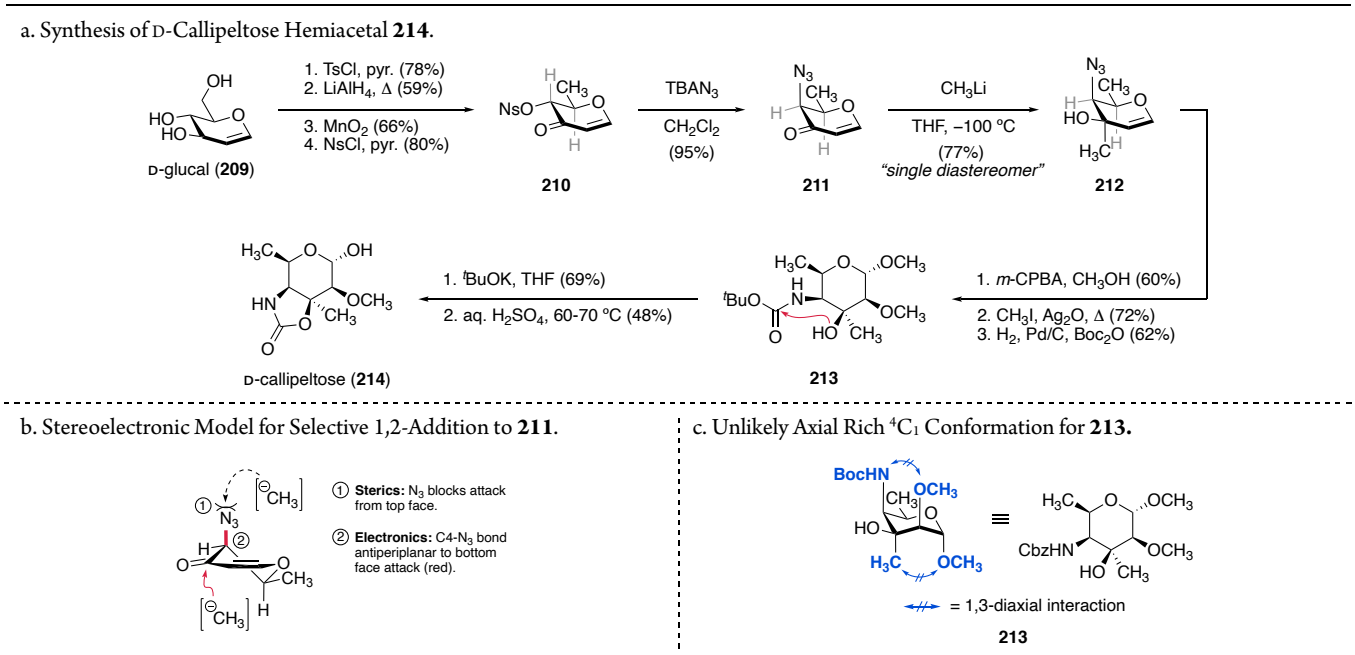
Scheme 1.23 Seeberger's 2011 Synthesis of *Bacteroides fragilis* ZPS A1 Repeating Unit **208**.

*1.3.4.7 Non-Lemonose Syntheses: Nicolaou and Koskinen's 2001 Synthesis of D-Callipeltose (**214**)*

Nicolaou and Koskinen's synthesis of D-callipetlose hemiacetal **214** began from commercially available D-glucal (**209**) (Scheme 1.24a).[183] Regioselective selective C6 tosylation followed by $LiAlH_4$ mediated deoxygenation establish the require C6 deoxygenation. Ensuing allylic oxidation with MnO_2 followed by nosylation gave vinylogous ester **210** (along with ca. 3-5% C4 epimer), setting the stage for installation of the C4 amine. In the event, S_N2 inversion using $TBAN_3$ yielded azide **211** in 95% yield (along with 5-10% C4 epimer). The authors noted that mesylate, triflate, and imidazolyl sulfonate groups proved ineffective for this transformation, and that the overnight reaction of nosylate **210** with NaN_3 at room temperature did not go to

completion and gave a ca. 1:1 mixture of **211** and its C4 epimer. From a safety perspective, the use of $TBAN_3$ in CH_2Cl_2 requires comment, as the authors note that the formation of explosive materials (presumably diazidomethane) upon storage of CH_2Cl_2 solutions of $TBAN_3$ has been reported.[184] We furthermore caution against any use of CH_2Cl_2 or $CHCl_3$ in reactions involving the azide ion in accordance with similar recommendations in the literature.[185-190]

Returning to the synthesis, 1,2-addition of CH_3Li to vinylogous ester **211** afforded desired epimer **212** with complete stereocontrol (Scheme 1.24a).[183] The authors posit that the steric and electronic influences of the angular C4-N_3 directed methyl addition to the opposite face of the carbonyl (Scheme 1.24b). Next, *m*-CBPA oxidation in the presence of CH_3OH followed by C2-OH methylation and transformation of the azido moiety to an *N*-Boc protected amine yielded **213**. Interestingly, if drawn in the $^4C_1$ conformation typically assumed for D-sugars, compound **213** has more axial substituents than equatorial. As such, we posit that this compound does not sit in the $^4C_1$ conformation and thus present it in a flat representation. The synthesis concluded with ^{t}BuOK mediated intramolecular oxazolidinone formation followed by acidic hydrolysis of the methyl glycoside to give α-D-callipeltose hemiacetal **214**. Surprisingly, the authors describe **214** as a single anomer and do not comment on its apparent lack of mutarotation.



Scheme 1.24 Nicolaou and Koskinen's 2001 Synthesis of α-D-Callipeltose Hemiacetal **214**.

1.4 *References for Chapter 1*

(1) Kang, H. S.; Brady, S. F. Arimetamycin A: Improving Clinically Relevant Families of Natural Products through Sequence-Guided Screening of Soil Metagenomes. *Angew. Chem. Int. Ed.* **2013**, *52*, 11063-11067.
(2) Brady, S.; Kang, H.-S. Anticancer Agents of the Anthracycline Family. US9217008B1, **2015**.
(3) Older literature often refers to daunorubicin as "daunomycin".
(4) Older literature often refers to doxorubicin as "adriamycin".
(5) Mirski, S. E. L.; Gerlach, J. H.; Cole, S. P. C. Multidrug Resistance in a Human Small Cell Lung Cancer Cell Line Selected in Adriamycin. *Cancer Res.* **1987**, *47*, 2594-2598.
(6) Cole, S. P.; Bhardwaj, G.; Gerlach, J. H.; Mackie, J. E.; Grant, C. E.; Almquist, K. C.; Stewart, A. J.; Kurz, E. U.; Duncan, A. M.; Deeley, R. G. Overexpression of a Transporter Gene in a Multidrug-Resistant Human Lung Cancer Cell Line. *Science* **1992**, *258*, 1650-1654.
(7) Cole, S. P. Multidrug Resistance Protein 1 (Mrp1, Abcc1), a "Multitasking" Atp-Binding Cassette (Abc) Transporter. *J. Biol. Chem.* **2014**, *289*, 30880-30888.
(8) Cole, S. P. Targeting Multidrug Resistance Protein 1 (Mrp1, Abcc1): Past, Present, and Future. *Annu. Rev. Pharmacol. Toxicol.* **2014**, *54*, 95-117.
(9) Bergy, M. E.; Coats, J. H.; Fritz, R. Antibiotic Steffisburgensimycin and Method of Producing. US3309273A, **1967**.
(10) Bergy, M. E.; Reusser, F. A New Antibacterial Agent (U-20,661) Isolated from a Streptomycete Strain. *Experientia* **1967**, *23*, 254-255.
(11) Kelly, R. C.; Schletter, I.; Koert, J. M.; MacKellar, F. A.; Wiley, P. F. Structures of Steffimycin and Steffimycin B. *J. Org. Chem.* **1977**, *42*, 3591-3596.
(12) Krohn, K.; Broser, E. Synthetic Anthracyclinones. 25. An Improved Route to 8-Demethoxyaranciamycinone and Synthesis of the A-L-Daunosamine Glycosides. *J. Org. Chem.* **1984**, *49*, 3766-3771.
(13) Renous, B.; Gaudin, N.; Gesson, J.-P.; Jacquesy, J.-C. New Process for the Preparation of Steffimycinone Analogues, New Analogues of Steffimycinone Thus Obtained, New Intermediates Useful for the Synthesis of These Compounds, Use of These Compounds as Antibacterial Agents and for the Synthesis of Antitumour Agents. FR2543545A1, **1985**.
(14) Steffimycinone Analogs. JP61047439A, **1986**.
(15) Krohn, K.; Carlson, G.; Köhle, H.; Hussain, H.; Green, I. R. Synthesis of Daunosamyl Anthraquinone and Anthracyclinone Analogues. *Arkivoc* **2008**, *2008*, 216-233.
(16) Yang, F.; Teves, S. S.; Kemp, C. J.; Henikoff, S. Doxorubicin, DNA Torsion, and Chromatin Dynamics. *Biochim. Biophys. Acta* **2014**, *1845*, 84-89.
(17) Gewirtz, D. A. A Critical Evaluation of the Mechanisms of Action Proposed for the Antitumor Effects of the Anthracycline Antibiotics Adriamycin and Daunorubicin. *Biochem. Pharmacol.* **1999**, *57*, 727-741.
(18) Tewey, K. M.; Rowe, T. C.; Yang, L.; Halligan, B. D.; Liu, L. F. Adriamycin-Induced DNA Damage Mediated by Mammalian DNA Topoisomerase Ii. *Science* **1984**, *226*, 466-468.
(19) Capranico, G.; Zunino, F.; Kohn, K. W.; Pommier, Y. Sequence-Selective Topoisomerase Ii Inhibition by Anthracycline Derivatives in Sv40 DNA: Relationship with DNA Binding Affinity and Cytotoxicity. *Biochemistry* **1990**, *29*, 562-9.
(20) Binaschi, M.; Farinosi, R.; Austin, C. A.; Fisher, L. M.; Zunino, F.; Capranico, G. Human DNA Topoisomerase Iiα-Dependent DNA Cleavage and Yeast Cell Killing by Anthracycline Analogues. *Cancer Res.* **1998**, *58*, 1886-92.
(21) Marinello, J.; Delcuratolo, M.; Capranico, G. Anthracyclines as Topoisomerase Ii Poisons: From Early Studies to New Perspectives. *Int J Mol Sci* **2018**, *19*.
(22) Pang, B.; Qiao, X.; Janssen, L.; Velds, A.; Groothuis, T.; Kerkhoven, R.; Nieuwland, M.; Ovaa, H.; Rottenberg, S.; van Tellingen, O.; Janssen, J.; Huijgens, P.; Zwart, W.; Neefjes, J. Drug-Induced Histone Eviction from Open Chromatin Contributes to the Chemotherapeutic Effects of Doxorubicin. *Nat. Commun.* **2013**, *4*, 1908.
(23) Yang, F.; Kemp, C. J.; Henikoff, S. Doxorubicin Enhances Nucleosome Turnover around Promoters. *Curr. Biol.* **2013**, *23*, 782-787.
(24) Pang, B.; de Jong, J.; Qiao, X.; Wessels, L. F.; Neefjes, J. Chemical Profiling of the Genome with Anti-Cancer Drugs Defines Target Specificities. *Nat. Chem. Biol* **2015**, *11*, 472-480.
(25) Nesher, E.; Safina, A.; Aljahdali, I.; Portwood, S.; Wang, E. S.; Koman, I.; Wang, J.; Gurova, K. V. Role of Chromatin Damage and Chromatin Trapping of Fact in Mediating the Anticancer Cytotoxicity of DNA-Binding Small-Molecule Drugs. *Cancer Res.* **2018**, *78*, 1431-1443.
(26) Qiao, X.; van der Zanden, S. Y.; Wander, D. P. A.; Borras, D. M.; Song, J. Y.; Li, X.; van Duikeren, S.; van Gils, N.; Rutten, A.; van Herwaarden, T.; van Tellingen, O.; Giacomelli, E.; Bellin, M.; Orlova, V.; Tertoolen, L. G. J.; Gerhardt, S.; Akkermans, J. J.; Bakker, J. M.; Zuur, C. L.; Pang, B.; Smits, A. M.; Mummery, C. L.; Smit, L.; Arens, R.; Li, J.; Overkleeft, H. S.; Neefjes, J. Uncoupling DNA Damage from Chromatin Damage to Detoxify Doxorubicin. *Proc. Natl. Acad. Sci. U.S.A.* **2020**, *117*, 15182-15192.
(27) Wander, D. P. A.; van der Zanden, S. Y.; van der Marel, G. A.; Overkleeft, H. S.; Neefjes, J.; Codee, J. D. C. Doxorubicin and Aclarubicin: Shuffling Anthracycline Glycans for Improved Anticancer Agents. *J. Med. Chem.* **2020**, *63*, 12814-12829.

(28) Wang, J. C. Cellular Roles of DNA Topoisomerases: A Molecular Perspective. *Nat. Rev. Mol. Cell Biol.* **2002**, *3*, 430-40.
(29) Pommier, Y.; Sun, Y.; Huang, S. N.; Nitiss, J. L. Roles of Eukaryotic Topoisomerases in Transcription, Replication and Genomic Stability. *Nat. Rev. Mol. Cell Biol.* **2016**, *17*, 703-721.
(30) Liu, L. F.; Rowe, T. C.; Yang, L.; Tewey, K. M.; L., C. G. Cleavage of DNA by Mammalian DNA Topoisomerase Ii. *J. Biol. Chem.* **1983**, *258*, 15365-15370.
(31) Sander, M.; Hsieh, T. Double Strand DNA Cleavage by Type Ii DNA Topoisomerase from Drosophila Melanogaster. *J. Biol. Chem.* **1983**, *258*, 8421-8428.
(32) Horowitz, D. S.; Wang, J. C. Mapping the Active Site Tyrosine of Escherichia Coli DNA Gyrase. *J. Biol. Chem.* **1987**, *262*, 5339-5344.
(33) Tsai-Pflugfelder, M.; Liu, L. F.; Liu, A. A.; Tewey, K. M.; Whang-Peng, J.; Knutsen, T.; Huebner, K.; Croce, C. M.; Wang, J. C. Cloning and Sequencing of Cdna Encoding Human DNA Topoisomerase Ii and Localization of the Gene to Chromosome Region 17q21-22. *Proc Natl Acad Sci U S A* **1988**, *85*, 7177-81.
(34) Worland, S. T.; Wang, J. C. Inducible Overexpression, Purification, and Active Site Mapping of DNA Topoisomerase Ii from the Yeast Saccharomyces Cerevisiae. *J. Biol. Chem.* **1989**, *264*, 4412-4416.
(35) Okada, Y.; Ito, Y.; Kikuchi, A.; Nimura, Y.; Yoshida, S.; Suzuki, M. Assignment of Functional Amino Acids around the Active Site of Human DNA Topoisomerase Iialpha. *J. Biol. Chem.* **2000**, *275*, 24630-8.
(36) Deweese, J. E.; Osheroff, N. The DNA Cleavage Reaction of Topoisomerase Ii: Wolf in Sheep's Clothing. *Nucleic Acids Res.* **2009**, *37*, 738-48.
(37) Lyu, Y. L.; Kerrigan, J. E.; Lin, C. P.; Azarova, A. M.; Tsai, Y. C.; Ban, Y.; Liu, L. F. Topoisomerase Iibeta Mediated DNA Double-Strand Breaks: Implications in Doxorubicin Cardiotoxicity and Prevention by Dexrazoxane. *Cancer Res.* **2007**, *67*, 8839-46.
(38) Zhang, S.; Liu, X.; Bawa-Khalfe, T.; Lu, L. S.; Lyu, Y. L.; Liu, L. F.; Yeh, E. T. Identification of the Molecular Basis of Doxorubicin-Induced Cardiotoxicity. *Nat. Med.* **2012**, *18*, 1639-42.
(39) Jiang, J.; Mohan, N.; Endo, Y.; Shen, Y.; Wu, W. J. Type Iib DNA Topoisomerase Is Downregulated by Trastuzumab and Doxorubicin to Synergize Cardiotoxicity. *Oncotarget* **2018**, *9*, 6095-6108.
(40) Cornarottie, M.; Tinelli, S.; WIllmore, E.; Zunino, F.; Fisher, L. M.; Austin, C. A.; Capranico, G. Drug Sensitivity and Sequence Specificity of Human Recombinant DNA Topoisomerases Iialpha (P170) and Iibeta (P180). *Mol. Pharmacol.* **1996**, *50*, 1463-1471.
(41) Liu, L.; Liu, C.; Alberts, B. Type Ii DNA Topoisomerases: Enzymes That Can Unknot a Topologically Knotted DNA Molecule Via a Reversible Double-Strand Break. *Cell* **1980**, *19*, 697-707.
(42) Roca, J.; Wang, J. C. The Capture of a DNA Double Helix by an Atp-Dependent Protein Clamp: A Key Step in DNA Transport by Type Ii DNA Topoisomerases. *Cell* **1992**, *71*, 833-840.
(43) Roca, J.; Wang, J. C. DNA Transport by a Type Ii DNA Topoisomerase: Evidence in Favor of a Two-Gate Mechanism. *Cell* **1994**, *77*, 609-616.
(44) Berger, J. M.; Gamblin, S. J.; Harrison, S. C.; Wang, J. C. Structure and Mechanism of DNA Topoisomerase Ii. *Nature* **1996**, *379*, 225-32.
(45) Fortune, J. M.; Osheroff, N. Topoisomerase Ii as a Target for Anticancer Drugs: When Enzymes Stop Being Nice. In *Progress in Nucleic Acid Research and Molecular Biology Volume 64*; **2000**; pp 221-253.
(46) Dong, K. C.; Berger, J. M. Structural Basis for Gate-DNA Recognition and Bending by Type Iia Topoisomerases. *Nature* **2007**, *450*, 1201-5.
(47) Nitiss, J. L. Targeting DNA Topoisomerase Ii in Cancer Chemotherapy. *Nat. Rev. Cancer* **2009**, *9*, 338-50.
(48) Wang, J. C. A Journey in the World of DNA Rings and Beyond. *Annual Review of Biochemistry* **2009**, *78*, 31-54.
(49) Pommier, Y.; Leo, E.; Zhang, H.; Marchand, C. DNA Topoisomerases and Their Poisoning by Anticancer and Antibacterial Drugs. *Chem. Biol.* **2010**, *17*, 421-33.
(50) Chen, S. H.; Chan, N. L.; Hsieh, T. S. New Mechanistic and Functional Insights into DNA Topoisomerases. *Annu Rev Biochem* **2013**, *82*, 139-70.
(51) Furniss, K. L.; Tsai, H. J.; Byl, J. A.; Lane, A. B.; Vas, A. C.; Hsu, W. S.; Osheroff, N.; Clarke, D. J. Direct Monitoring of the Strand Passage Reaction of DNA Topoisomerase Ii Triggers Checkpoint Activation. *PLoS Genet.* **2013**, *9*, e1003832.
(52) Lindsey, R. H., Jr.; Pendleton, M.; Ashley, R. E.; Mercer, S. L.; Deweese, J. E.; Osheroff, N. Catalytic Core of Human Topoisomerase Iialpha: Insights into Enzyme-DNA Interactions and Drug Mechanism. *Biochemistry* **2014**, *53*, 6595-602.
(53) Bates, A. D.; Maxwell, A. *DNA Topology*. 2nd ed.; Oxoford University Press: Oxford; New York, **2005**.
(54) Capranico, G.; Soranzo, C.; Zunino, F. Single-Strand DNA Breaks Induced by Chromophore-Modified Anthracyclines in P388 Leukemia Cells. *Cancer Res.* **1986**, *46*, 5499-503.
(55) Capranico, G.; Tinelli, S.; Zunino, F. Formation, Resealing and Persistence of DNA Breaks Produced by 4-Demethoxydaunorubicin in P388 Leukemia Cells. *Chemico-Biological Interactions* **1989**, *72*, 113-123.
(56) Binaschi, M.; Capranico, G.; Dal Bo, L.; Zunino, F. Relationship between Lethal Effects and Topoisomerase Ii-Mediated Double-Stranded DNA Breaks Produced by Anthracyclines with Different Sequence Specificity. *Mol. Pharmacol.* **1997**, *51*, 1053-9.
(57) Deal, R. B.; Henikoff, J. G.; Henikoff, S. Genome-Wide Kinetics of Nucleosome Turnover Determined by Metabolic Labeling of Histones. *Science* **2010**, *328*, 1161-4.

(58) Teves, S. S.; Deal, R. B.; Henikoff, S. Measuring Genome-Wide Nucleosome Turnover Using Catch-It. *Methods Enzymol.* **2012**, *513*, 169-84.
(59) Frederick, C. A.; Williams, L. D.; Ughetto, G.; Van der Marel, G. A.; Van Boom, J. H.; Rich, A.; Wang, A. H. J. Structural Comparison of Anticancer Drug-DNA Complexes: Adriamycin and Daunomycin. *Biochemistry* **1990**, *29*, 2538-2549.
(60) Luger, K.; Mader, A. W.; Richmond, R. K.; Sargent, D. F.; Richmond, T. J. Crystal Structure of the Nucleosome Core Particle at 2.8 a Resolution. *Nature* **1997**, *389*, 251-60.
(61) Tewey, K. M.; Chen, G. L.; Nelson, E. M.; Liu, L. F. Intercalative Antitumor Drugs Interferece with the Breakage-Reunion Reaction of Mammalian DNA Topoisomerase Ii. *The Journal of Biological Chemistry* **1984**, *259*, 9182-9187.
(62) Girling, D. J. Comparison of Oral Etoposide and Standard Intravenous Multidrug Chemotherapy for Small-Cell Lung Cancer: A Stopped Multicentre Randomised Trial. *The Lancet* **1996**, *348*, 563-566.
(63) Hong, W. K.; Nicaise, C.; Lawson, R.; Maroun, J. A.; Comis, R.; Speer, J.; Luedke, D.; Hurtubise, M.; Lanzotti, V.; Goodlow, J.; et al. Etoposide Combined with Cyclophosphamide Plus Vincristine Compared with Doxorubicin Plus Cyclophosphamide Plus Vincristine and with High-Dose Cyclophosphamide Plus Vincristine in the Treatment of Small-Cell Carcinoma of the Lung: A Randomized Trial of the Bristol Lung Cancer Study Group. *J Clin Oncol* **1989**, *7*, 450-6.
(64) Ishizumi, K.; Ohashi, N.; Tanno, N. Stereospecific Total Synthesis of 9-Aminoanthracyclines: (+)-9-Amino-9-Deoxydaunomycin and Related Compounds. *J. Org. Chem.* **2002**, *52*, 4477-4485.
(65) Ogawara, D.; Fukuda, M.; Nakamura, Y.; Kohno, S. Efficacy and Safety of Amrubicin Hydrochloride for Treatment of Relapsed Small Cell Lung Cancer. *Cancer Manag. Res.* **2010**, *2*, 191-195.
(66) Bennett, C. S.; Galan, M. C. Methods for 2-Deoxyglycoside Synthesis. *Chem Rev* **2018**, *118*, 7931-7985.
(67) Arcamone, F.; Penco, S.; Vigevani, A.; Redaelli, S.; Franchi, G.; DiMarco, A.; Casazza, A. M.; Dasdia, T.; Formelli, F.; Necco, A.; Soranzo, C. Synthesis and Antitumor Properties of New Glycosides of Daunomycinone and Adriamycinone. *J. Med. Chem.* **1975**, *18*, 703-707.
(68) Arcamone, F.; Bernardi, L.; Giardino, P.; Patelli, B.; Di Marco, A.; Casazza, A. M.; Pratesi, G.; Reggiani, P. Synthesis and Antitumor Activity of 4-Demethoxydaunorubicin, 4-Demethody-7,9-Diepidaunorubicin, and Their B Anomers. *Cancer Treatment Reports* **1976**, *60*, 829-834.
(69) Arcamone, F.; Bernardi, L.; Patelli, B.; Giardino, P.; Di Marco, A.; Casazza, A. M.; Soranzo, C.; Pratesi, G. Synthesis and Antitumour Activity of New Daunorubicin and Adriamycin Analogues. *Experientia* **1978**, *34*, 1255-7.
(70) Broadhurst, M. J.; Hassall, C. H.; Thomas, G. J. Anthracyclines. Part 3. The Total Synthesis of 4-Demethoxydaunomycin. *J. Chem. Soc., Perkin Trans. 1* **1982**.
(71) Acton, E. M.; Fujiwara, A. N.; Henry, D. W. Total Synthesis of the Antitumor Antibiotic Danuorubicin. Coupling of the Sugar and Aglycone. *J. Med. Chem.* **1974**, *17*, 659-60.
(72) Kimura, Y.; Suzuki, M.; Matsumoto, T.; Abe, R.; Terashima, S. Trimethylsilyl Trifluoromethanesulfonate (Trimethylsilyl Triflate) as an Excellent Glycosidation Reagent for Anthracycline Synthesis. Simple and Efficient Synthesis of Optically Pure 4-Demethoxydaunorubicin. *Chem. Lett.* **1984**, *13*, 501-504.
(73) Kolar, C.; Dehmel, K.; Knoedler, U.; Paal, M.; Hermentin, P.; Gerken, M. Semisynthetic ∈-Isorhodomycins: Glycosylation and Modification Reactions1. *J. Carbohydr. Chem.* **1989**, *8*, 295-305.
(74) Cipollone, A.; Berettoni, M.; Bigioni, M.; Binaschi, M.; Cermele, C.; Monteagudo, E.; Olivieri, L.; Palomba, D.; Animati, F.; Goso, C.; Maggi, C. A. Novel Anthracycline Oligosaccharides: Influence of Chemical Modifications of the Carbohydrate Moiety on Biological Activity. *Biorg. Med. Chem.* **2002**, *10*, 1459-1470.
(75) Animati, F.; Arcamone, F.; Berettoni, M.; Cipollone, A.; Franciotti, M.; Lombardi, P. New Anthracycline Disaccharides. Synthesis of L-Daunosaminyl- A (1→4)-2-Deoxy-L-Rhamnosyl and of L-Daunosaminyl- A (1→4)-2-Deoxy-L-Fucosyl Daunorubicin Analogues. *J. Chem. Soc., Perkin Trans. 1* **1996**, 1327-1329.
(76) Umezawa, H.; Takahashi, Y.; Konoshita, M.; Naganawa, H.; Tatsuta, K.; Takeuchi, T. Synthesis of 4-Demethoxy-11-Deoxy-Analogs of Daunomycin and Adriamycin. *J Antibiot (Tokyo)* **1980**, *33*, 1581-5.
(77) Thang, T. T.; Imbach, J. L.; Fizames, C.; Lavelle, F.; Ponsinet, G.; Olesker, A.; Lukacs, G. Synthesis and Antitumor Activity of 3′ -C-Methyl-Daunorubicin. *Carbohydr. Res.* **1985**, *135*, 241-247.
(78) Suzuki, K.; Sulikowski, G. A.; Friesen, R. W.; Danishefsky, S. J. Application of Substituent-Controlled Oxidative Coupling of Glycals in a Synthesis and Structural Corroboration of Ciclamycin 0: New Possibilities for the Construction of Hybrid Anthracyclines. *J. Am. Chem. Soc.* **1990**, *112*, 8895-8902.
(79) Yu, S.; Zhang, G.; Zhang, W.; Luo, H.; Qiu, L.; Liu, Q.; Sun, D.; Wang, P.-G.; Wang, F. Synthesis and Biological Activities of a 3'-Azido Analogue of Doxorubicin against Drug-Resistant Cancer Cells. *Int. J. Mol. Sci.* **2012**, *13*, 3671-3684.
(80) Zhu, L.; Cao, X.; Chen, W.; Zhang, G.; Sun, D.; Wang, P. G. Syntheses and Biological Activities of Daunorubicin Analogs with Uncommon Sugars. *Bioorg. Med. Chem.* **2005**, *13*, 6381-7.
(81) Zhang, G.; Shi, L.; Liu, Q.; Liu, X.; Li, L.; Wang, J. A Divergent Approach to 3-Azido-2,3,6-Trideoxy-L-Hexoses from Rhamnal. *Tetrahedron Lett.* **2007**, *48*, 3413-3416.

(82) Lear, M. J.; Yoshimura, F.; Hirama, M. A Direct and Efficient A-Selective Glycosylation Protocol for the Kedarcidin Sugar, L-Mycarose: $Agpf_6$ as a Remarkable Activator of 2-Deoxythioglycosides. *Angew. Chem. Int. Ed.* **2001**, *40*, 946-949.
(83) Lear, M. J.; Hirai, K.; Ogawa, K.; Yamashita, S.; Hirama, M. A Convergent Total Synthesis of the Kedarcidin Chromophore: 20-Years in the Making. *J Antibiot (Tokyo)* **2019**, *72*, 350-363.
(84) Ren, F.; Hogan, P. C.; Anderson, A. J.; Myers, A. G. Kedarcidin Chromophore: Synthesis of Its Proposed Structure and Evidence for a Stereochemical Revision. *J. Am. Chem. Soc.* **2007**, *129*, 5381-3.
(85) Magauer, T.; Smaltz, D. J.; Myers, A. G. Component-Based Syntheses of Trioxacarcin a, Dc-45-A1 and Structural Analogues. *Nat Chem* **2013**, *5*, 886-93.
(86) Krohn, K. Building Blocks for the Total Synthesis of Anthracyclinones. In *Progress in the Chemistry of Natural Products*; Hertz, W.; Tamm, C., Eds.; Spring: Berlin
Heidelberg
New York, **1989**; pp 37-88.
(87) Krohn, K. Synthesis of Anthracyclinones by Electrophilic and Nucleophilic Addition to Anthraquinones. *Tetrahedron* **1990**, *46*, 219-318.
(88) Thomas, G. J. Synthesis of Anthracyclines Related to Daunomycine. In *Recent Progress in the Chemical Synthesis of Antibiotics*; Lukacs, G.; Ohno, M., Eds.; Spring-Verlag: Berlin
Heidelberg
New York, **1990**; pp 467-496.
(89) Wheeler, D. M. S.; Wheeler, M. M. Stereoselective Synthesis of Doxorubicine and Re- Lated Compounds. In *Studies in Natural Product Chemistry*; Rahman, A., Ed.; Elsevier: Amsterdam, **1994**; pp 3-46.
(90) Achmatowicz, O.; Szechner, B. Synthesis of Enantiomerically Pure Anthracyclinones. In *Anthracycline Chemistry and Biology I: Biological Occurence and Biosynthesis, Synthesis and Chemistry*; Krohn, K., Ed.; Spring-Verlag: Bernin
Heidelberg, **2008**; pp 143-186.
(91) Dennis, D. G.; Okumura, M.; Sarlah, D. Synthesis of (+/-)-Idarubicinone Via Global Functionalization of Tetracene. *J. Am. Chem. Soc.* **2019**, *141*, 10193-10198.
(92) Allen, J. G.; Hentemann, M. F.; Danishefsky, S. J. A Powerfulo-Quinone Dimethide Strategy for Intermolecular Diels–Alder Cycloadditions. *J. Am. Chem. Soc.* **2000**, *122*, 571-575.
(93) Ishizumi, K.; Ohashi, N.; Tanno, N. Stereospecific Total Synthesis of 9-Aminoanthracyclines: (+)-9-Amino-9-Deoxydaunomycin and Related Compounds. *J. Org. Chem.* **1987**, *52*, 4477-4485.
(94) Swenton, J. S.; Freskos, J. N.; Morrow, G. W.; Sercel, A. D. A Convergent Synthesis of (+)-4-Demethoxydaunomycinone and (+)-Daunomycinone. *Tetrahedron* **1984**, *40*, 4625-4632.
(95) Kimura, Y.; Suzuki, M.; Matsumoto, T.; Abe, R.; Terashima, S. A Simple and Efficient Synthesis of Optically Active (+)-4-Demethoxydaunomycinone. *Bull. Chem. Soc. Jpn.* **1986**, *59*, 415-421.
(96) Dienes, Z.; Antonsson, T.; Vogel, P. Enantioselective Synthesis of (R)-(−)-2-Acetyl-2,5,12-Trihydro-1,2,3,4-Tetrahydro-6,11-Naphthacenequinone Via Diastereoselective Diels-Alder Cycloaddition. *Tetrahedron Lett.* **1993**, *34*, 1013-1016.
(97) Reymond, J.-L.; Vogel, P. New Chiral Auxiliaries and New Optically Pure Ketene Equivalents Derived from Tartaric Acids. Improved Synthesis of (−)-7-Oxabicyclo[2.2.1]Hept-5-En-2-One. *Tetrahedron: Asymmetry* **1990**, *1*, 729-736.
(98) Thirunavukkarasu, V. S.; Ackermann, L. Ruthenium-Catalyzed C-H Bond Oxygenations with Weakly Coordinating Ketones. *Org. Lett.* **2012**, *14*, 6206-9.
(99) Southgate, E. H.; Pospech, J.; Fu, J.; Holycross, D. R.; Sarlah, D. Dearomative Dihydroxylation with Arenophiles. *Nat Chem* **2016**, *8*, 922-8.
(100) Zweifel, G.; Arzoumanian, H.; Whitney, C. C. A Convenient Stereoselective Synthesis of Substituted Alkenes Via Hydroboration-Iodination of Alkynes. *J. Am. Chem. Soc.* **1967**, *89*, 3652-3653.
(101) Armstrong, R.; Aggarwal, V. 50 Years of Zweifel Olefination: A Transition-Metal-Free Coupling. *Synthesis* **2017**, *49*, 3323-3336.
(102) Rasmussen, J. K.; Krepski, L. R.; Heilmann, S. M.; Smith, I. H. K.; Tumey, M. L. A Convenient Synthesis of 1,2-Bis[Trimethylsiloxy]Alkenes from A-Diketones. *Synthesis* **1983**, *1983*, 457-459.
(103) Boudjouk, P.; So, J. H. Organic Sonochemistry. Ultrasonic Acceleration of the Reaction of Dicarbonyls with Trimethylchlorosilane in the Presence of Zinc. *Synth. Commun.* **1986**, *16*, 775-778.
(104) Isayama, S.; Mukaiyama, T. A New Method for Preparation of Alcohols from Olefins with Molecular Oxygen and Phenylsilane by the Use of Bis(Acetylacetonato)Cobalt(Ii). *Chem. Lett.* **1989**, *18*, 1071-1074.
(105) Obradors, C.; Martinez, R. M.; Shenvi, R. A. Ph(I-Pro)Sih2: An Exceptional Reductant for Metal-Catalyzed Hydrogen Atom Transfers. *J. Am. Chem. Soc.* **2016**, *138*, 4962-71.
(106) Moore, H. W. Bioactivation as a Model for Drug Design Bioreductive Alkylation. *Science* **1977**, *197*, 527-532.
(107) Moore, H. W.; Czerniak, R. Naturally Occurring Quinones as Potential Bioreductive Alkylating Agents. *Med Res Rev* **1981**, *1*, 249-80.
(108) Tanaka, Y.; Grafe, U.; Yazawa, K.; Mikami, Y.; Ritzau, M. Nocardicyclins a and B: New Anthracycline Antibiotics Produced by Nocardia Pseudobrasiliensis. *J Antibiot (Tokyo)* **1997**, *50*, 822-7.

(109) Maeda, K.; Takeuchi, T.; Nitta, K.; Yagishita, K.; Utahara, R.; Ōsato, T.; Ueda, M.; Kondō, S.; Okami, Y.; Umezawa, H. A New Antitumor Substance, Pluramycin. *The Journal of Antibiotics, Ser. A* **1966**, *9*, 75-81.
(110) Bililign, T.; Griffith, B. R.; Thorson, J. S. Structure, Activity, Synthesis and Biosynthesis of Aryl-C-Glycosides. *Nat. Prod. Rep.* **2005**, *22*, 742-60.
(111) Abe, N.; Nakakita, Y.; Nakamura, T.; Enoki, N.; Uchida, H.; Munekata, M. Novel Antitumor Antibiotics, Saptomycins. I. Taxonomy of the Producing Organism, Fermentation, Hplc Analysis and Biological Activities. *J Antibiot (Tokyo)* **1993**, *46*, 1530-5.
(112) Abe, N.; Enoki, N.; Nakakita, Y.; Uchida, H.; Nakamura, T.; Munekata, M. Novel Antitumor Antibiotics, Saptomycins. Ii. Isolation, Physico-Chemical Properties and Structure Elucidation. *J Antibiot (Tokyo)* **1993**, *46*, 1536-49.
(113) Kitamura, K.; Maezawa, Y.; Ando, Y.; Kusumi, T.; Matsumoto, T.; Suzuki, K. Synthesis of the Pluramycins 2: Total Synthesis and Structure Assignment of Saptomycin B. *Angew. Chem. Int. Ed. Engl.* **2014**, *53*, 1262-5.
(114) Ge, M.; Thompson, C.; Kahne, D. Reconstruction of Vancomycin by Chemical Glycosylation of the Pseudoaglycon. *J. Am. Chem. Soc.* **1998**, *120*, 11014-11015.
(115) Thompson, C.; Ge, M.; Kahne, D. Synthesis of Vancomycin from the Aglycon. *J. Am. Chem. Soc.* **1999**, *121*, 1237-1244.
(116) O'Keefe, B. M.; Mans, D. M.; Kaelin, D. E., Jr.; Martin, S. F. Studies toward the Syntheses of Pluramycin Natural Products. The First Total Synthesis of Isokidamycin. *Tetrahedron* **2011**, *67*, 6524-6538.
(117) Soliman, S. E.; Bennett, C. S. Reagent-Controlled Synthesis of the Branched Trisaccharide Fragment of the Antibiotic Saccharomicin B. *Org. Lett.* **2018**, *20*, 3413-3417.
(118) Johnson, A. W.; Smith, R. M.; Guthrie, R. D. Vancosamine: The Structure and Configuration of a Novel Amino-Sugar from Vancomycin. *J Chem Soc Perkin 1* **1972**, *17*, 2153-9.
(119) Dushin, R. G.; Danishefsky, S. J. Stereospecific Synthesis of Aryl B-Glucosides: An Application to the Synthesis of a Prototype Corresponding to the Aryloxy Carbohydrate Domain of Vancomycin. *J. Am. Chem. Soc.* **1992**, *114*, 3471-3475.
(120) Lemieux, R. U.; Levine, S. Synthesis of Alkyl 2-Deoxy-A-D-Glycopyranosides and Their 2-Deuterio Derivatives. *Can. J. Chem.* **1964**, *42*, 1473-1480.
(121) Lemieux, R. U.; Morgan, A. R. The Synthesis of B-D-Glucopyranosyl 2-Deoxy-A-D-Arabino-Hexopyranoside. *Can. J. Chem.* **1965**, *43*, 2190-2197.
(122) Thiem, J.; Karl, H. Synthesen Von Methyl-3-0-(A-D-Olivosyl)-A-D-Olivosid. *Tetrahedron Lett.* **1978**, *19*, 4999-5002.
(123) Thiem, J.; Karl, H.; Schwentner, J. Synthese Ａ-Verknüpfter 2′ -Deoxy-2′ -Iododisaccharide. *Synthesis* **1978**, *1978*, 696-698.
(124) Thiem, J.; Prahst, A.; Lundt, I. Untersuchungen Zur B-Glycosylierung Nach Demn-Iodsuccinimid-Verfahren: Synthese Der Terminalen Disaccharideinheit Von Orthosomycinen. *Liebigs Ann. Chem.* **1986**, *1986*, 1044-1056.
(125) Thiem, J.; Klaffke, W. Facile Stereospecific Synthesis of Deoxyfucosyl Disaccharide Units of Anthracyclines. *J. Org. Chem.* **1989**, *54*, 2006-2009.
(126) Danishefsky, S. J.; Bilodeau, M. T. Glycals in Organic Synthesis: The Evolution of Comprehensive Strategies for the Assembly of Oligosaccharides and Glycoconjugates of Biological Consequence. *Angewandte Chemie International Edition in English* **1996**, *35*, 1380-1419.
(127) Losey, H. C.; Peczuh, M. W.; Chen, Z.; Eggert, U. S.; Dong, S. D.; Pelczer, I.; Kahne, D.; Walsh, C. T. Tandem Action of Glycosyltransferases in the Maturation of Vancomycin and Teicoplanin Aglycones: Novel Glycopeptides. *Biochemistry* **2001**, *40*, 4745-55.
(128) Cavender, C. J.; Shiner, V. J. Trifluoromethanesulfonyl Azide. Its Reaction with Alkyl Amines to Form Alkyl Azides. *J. Org. Chem.* **1972**, *37*, 3567-3569.
(129) Alper, P. B.; Hung, S.-C.; Wong, C.-H. Metal Catalyzed Diazo Transfer for the Synthesis of Azides from Amines. *Tetrahedron Lett.* **1996**, *37*, 6029-6032.
(130) Nyffeler, P. T.; Liang, C. H.; Koeller, K. M.; Wong, C. H. The Chemistry of Amine-Azide Interconversion: Catalytic Diazotransfer and Regioselective Azide Reduction. *J. Am. Chem. Soc.* **2002**, *124*, 10773-8.
(131) Titz, A.; Radic, Z.; Schwardt, O.; Ernst, B. A Safe and Convenient Method for the Preparation of Triflyl Azide, and Its Use in Diazo Transfer Reactions to Primary Amines. *Tetrahedron Lett.* **2006**, *47*, 2383-2385.
(132) Nogueira, J. M.; Nguyen, S. H.; Bennett, C. S. Cyclopropenium Cation Promoted Dehydrative Glycosylations Using 2-Deoxy- and 2,6-Dideoxy-Sugar Donors. *Org. Lett.* **2011**, *13*, 2814-7.
(133) Nogueira, J. M.; Issa, J. P.; Chu, A.-H. A.; Sisel, J. A.; Schum, R. S.; Bennett, C. S. Halide Effects on Cyclopropenium Cation Promoted Glycosylation with Deoxy Sugars: Highly A-Selective Glycosylations Using a 3,3-Dibromo-1,2-Diphenylcyclopropene Promoter. *Eur. J. Org. Chem.* **2012**, *2012*, 4927-4930.
(134) Nogueira, J. M.; Bylsma, M.; Bright, D. K.; Bennett, C. S. Reagent-Controlled Alpha-Selective Dehydrative Glycosylation of 2,6-Dideoxy- and 2,3,6-Trideoxy Sugars. *Angew. Chem. Int. Ed. Engl.* **2016**, *55*, 10088-92.
(135) Nicolaou, K. C.; Mitchell, H. J.; van Delft, F. L.; Rübsam, F.; Rodríguez, R. M. Expeditious Routes to Evernitrose and Vancosamine Derivatives and Synthesis of a Model Vancomycin Aryl Glycoside. *Angew. Chem. Int. Ed.* **1998**, *37*, 1871-1874.
(136) Nicolaou, K. C.; Mitchell, H. J.; Jain, N. F.; Winssinger, N.; Hughes, R.; Bando, T. Total Synthesis of Vancomycin. *Angew. Chem. Int. Ed.* **1999**, *38*, 240-244.

(137) Nicolaou, K. C.; Mitchell, H. J.; Jain, N. F.; Bando, T.; Hughes, R.; Winssinger, N.; Natarajan, S.; Koumbis, A. E. Total Synthesis of Vancomycin—Part 4: Attachment of the Sugar Moieties and Completion of the Synthesis. *Chem. Eur. J.* **1999**, *5*, 2648-2667.
(138) Nicolaou, K. C.; Natarajan, S.; Li, H.; Jain, N. F.; Hughes, R.; Solomon, M. E.; Ramanjulu, J. M.; Boddy, C. N. C.; Takayanagi, M. Total Synthesis of Vancomycin Aglycon—Part 1: Synthesis of Amino Acids 4-7 and Construction of the Ab-Cod Ring Skeleton. *Angew. Chem. Int. Ed.* **1998**, *37*, 2708-2714.
(139) Nicolaou, K. C.; Jain, N. F.; Natarajan, S.; Hughes, R.; Solomon, M. E.; Li, H.; Ramanjulu, J. M.; Takayanga, M.; Koumbis, A.; Bando, T. Total Synthesis of Vancomycin Aglycon–Part 2: Synthesis of Amino Acids 1‐3 and Construction of the Ab ‐ Cod ‐ Doe Ring Skeleton. *Angew. Chem. Int. Ed.* **1998**, *37*, 2714-2716.
(140) Nicolaou, K. C.; Takayanagi, M.; Jain, N. F.; Natarajan, S.; Koumbis, A. E.; Bando, T.; Ramanjulu, J. M. Total Synthesis of Vancomycin Aglycon—Part 3: Final Stages. *Angew. Chem. Int. Ed.* **1998**, *37*, 2717-2719.
(141) Nicolaou, K. C.; Li, H.; Boddy, C. N. C.; Ramanjulu, J. M.; Yue, T.-Y.; Natarajan, S.; Chu, X.-J.; Bräse, S.; Rübsam, F. Total Synthesis of Vancomycin—Part 1: Design and Development of Methodology. *Chem. Eur. J.* **1999**, *5*, 2584-2601.
(142) Nicolaou, K. C.; Boddy, C. N. C.; Li, H.; Koumbis, A. E.; Hughes, R.; Natarajan, S.; Jain, N. F.; Ramanjulu, J. M.; Bräse, S.; Solomon, M. E. Total Synthesis of Vancomycin—Part 2: Retrosynthetic Analysis, Synthesis of Amino Acid Building Blocks and Strategy Evaluations. *Chem. Eur. J.* **1999**, *5*, 2602-2621.
(143) Nicolaou, K. C.; Koumbis, A. E.; Takayanagi, M.; Natarajan, S.; Jain, N. F.; Bando, T.; Li, H.; Hughes, R. Total Synthesis of Vancomycin—Part 3: Synthesis of the Aglycon. *Chem. Eur. J.* **1999**, *5*, 2622-2647.
(144) Thang, T. T.; Winternitz, F.; Olesker, A.; Lagrange, A.; Lukacs, G. Synthesis of a Derivative of Vancosamine, a Component of the Glycopeptide Antibiotic Vancomycin. *J. Chem. Soc., Chem. Commun.* **1979**, 153-154.
(145) Suzuki, K.; Hsu, D.-S.; Matsumoto, T. Synthesis of L-Vancosamine Derivatives from Methyl A-D-Mannopyranoside. *Synlett* **2006**, *2006*, 0469-0471.
(146) Kitamura, K.; Shigeta, M.; Maezawa, Y.; Watanabe, Y.; Hsu, D. S.; Ando, Y.; Matsumoto, T.; Suzuki, K. Preparation of L-Vancosamine-Related Glycosyl Donors. *J Antibiot (Tokyo)* **2013**, *66*, 131-9.
(147) Horton, D.; Weckerle, W. A Preparative Synthesis of 3-Amino-2,3,6-Trideoxy-L-Lyxo-Hexose (Daunosamine) Hydrochloride from D-Mannose. *Carbohydr. Res.* **1975**, *44*, 227-240.
(148) Klemer, A.; Rodemeyer, G. Eine Einfache Synthese Von Methyl-4,6-O-Benzyliden-2-Desoxy-A-D-Erythro-Hexopyranosid-3-Ulose. *Chem. Ber.* **1974**, *107*, 2612-2614.
(149) Use Ofcerium(Iii) Chloride in the Reactions of Carbonyl Compounds with Organolithiums or Grignard Reagents for the Suppression of Abnormal Reactions:1-Butyl-1,2,3,4-Tetrahydro-1-Naphthol. *Organic Syntheses* **1999**, *76*.
(150) Imamoto, T.; Kusumoto, T.; Tawarayama, Y.; Sugiura, Y.; Mita, T.; Hatanaka, Y.; Yokoyama, M. Carbon-Carbon Bond-Forming Reactions Using Cerium Metal or Organocerium(Iii) Reagents. *J. Org. Chem.* **2002**, *49*, 3904-3912.
(151) Hanessian, S. The Reaction of O-Benzylidene Sugars with N-Bromosuccinimide. *Carbohydr. Res.* **1966**, *2*, 86-88.
(152) Failla, D. L.; Hullar, T. L.; Siskin, S. B. Selective Transformation of O-Benzylidene Acetals into Ω-Bromo-Substituted Benzoate Esters. *Chem. Commun. (London)* **1966**, *0*, 716-717.
(153) Whaley, H. A.; Patterson, E. L.; Dann, M.; Shay, A. J.; Porter, J. N. Isolation and Characterization of Lemonomycin, a New Antibiotic. *Antimicrob. Agents Chemother.* **1964**, *8*, 83-86.
(154) He, H.; Shen, B.; Carter, G. T. Structural Elucidation of Lemonomycin, a Potent Antibiotic from Streptomyces Candidus. *Tetrahedron Lett.* **2000**, *41*, 2067-2071.
(155) Northcote, P. T.; Siegel, M.; Borders, D. B.; Lee, M. D. Glycothiohexide Alpha, a Novel Antibiotic Produced by "Sebekia" Sp., Ll-14e605. Iii. Structural Elucidation. *J Antibiot (Tokyo)* **1994**, *47*, 901-8.
(156) Steinberg, D. A.; Bernan, V. S.; Montenegro, D. A.; Abbanat, D. R.; Pearce, C. J.; Korshalla, J. D.; Jacobus, N. V.; Petersen, P. J.; Mroczenski-Wildey, M. J.; Maiese, W. M.; et al. Glycothiohexides, Novel Antibiotics Produced by "Sebekia" Sp. Ll-14e605. I. Taxonomy, Fermentation and Biological Evaluation. *J Antibiot (Tokyo)* **1994**, *47*, 887-93.
(157) Sasaki, T.; Otani, T.; Matsumoto, H.; Unemi, N.; Hamada, M.; Takeuchi, T.; Hori, M. Mj347-81f4 a & B, Novel Antibiotics from Amycolatopsis Sp.: Taxonomic Characteristics, Fermentation, and Antimicrobial Activity. *J Antibiot (Tokyo)* **1998**, *51*, 715-21.
(158) Constantine, K. L.; Mueller, L.; Huang, S.; Abid, S.; Lam, K. S.; Li, W.; Leet, J. E. Conformation and Absolute Configuration of Nocathiacin I Determined by Nmr Spectroscopy and Chiral Capillary Electrophoresis. *J. Am. Chem. Soc.* **2002**, *124*, 7284-5.
(159) Constantine, K. L.; Mueller, L.; Huang, S.; Abid, S.; Lam, K. S.; Li, W.; Leet, J. E. Conformation and Absolute Configuration of Nocathiacin I Determined by Nmr Spectroscopy and Chiral Capillary Electrophoresis [J. Am. Chem. Soc.2002,124, 7284–7285]. . *J. Am. Chem. Soc.* **2002**, *124*, 14810-14810.
(160) Leet, J. E.; Li, W.; Ax, H. A.; Matson, J. A.; Huang, S.; Huang, R.; Cantone, J. L.; Drexler, D.; Dalterio, R. A.; Lam, K. S. Nocathiacins, New Thiazolyl Peptide Antibiotics from Nocardia Sp. Ii. Isolation, Characterization, and Structure Determination. *J Antibiot (Tokyo)* **2003**, *56*, 232-42.
(161) Li, W.; Leet, J. E.; Ax, H. A.; Gustavson, D. R.; Brown, D. M.; Turner, L.; Brown, K.; Clark, J.; Yang, H.; Fung-Tomc, J.; Lam, K. S. Nocathiacins, New Thiazolyl Peptide Antibiotics from Nocardia Sp. I. Taxonomy, Fermentation and Biological Activities. *J Antibiot (Tokyo)* **2003**, *56*, 226-31.

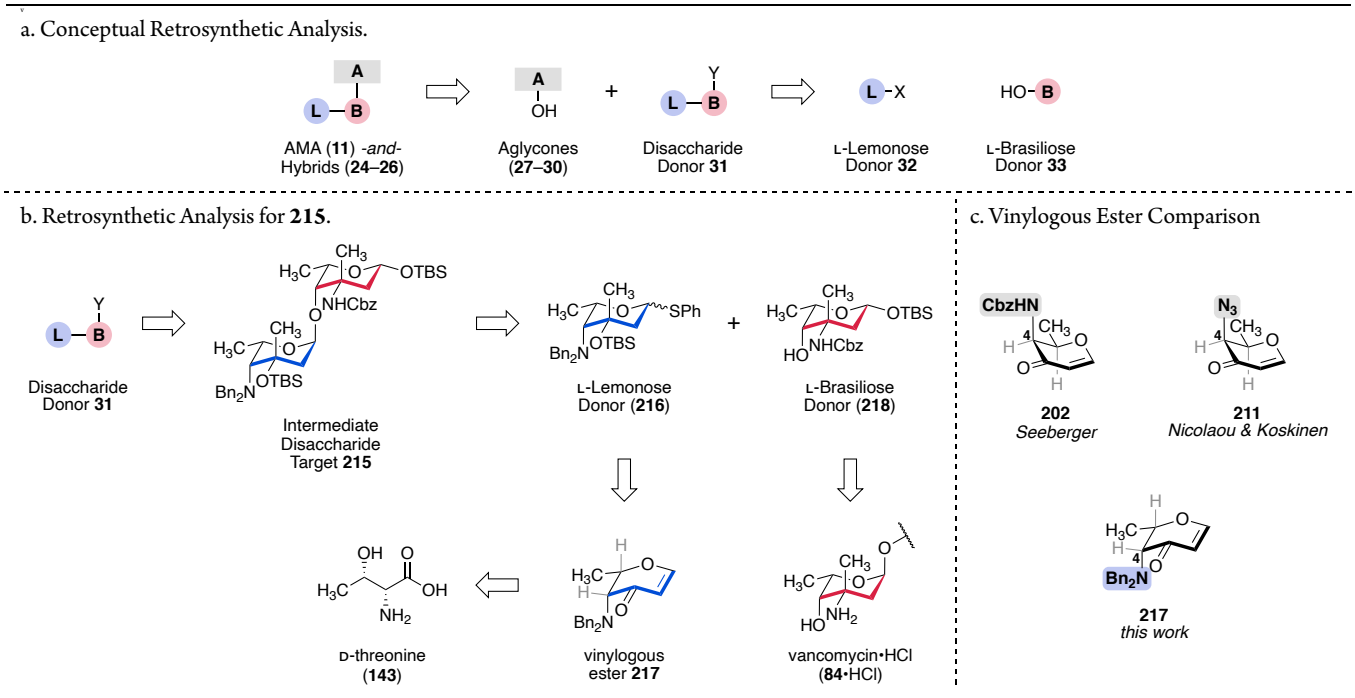
(162) Zhang, C.; Herath, K.; Jayasuriya, H.; Ondeyka, J. G.; Zink, D. L.; Occi, J.; Birdsall, G.; Venugopal, J.; Ushio, M.; Burgess, B.; Masurekar, P.; Barrett, J. F.; Singh, S. B. Thiazomycins, Thiazolyl Peptide Antibiotics from Amycolatopsis Fastidiosa. *J. Nat. Prod.* **2009**, *72*, 841-7.
(163) Ashley, E. R.; Cruz, E. G.; Stoltz, B. M. The Total Synthesis of (−)-Lemonomycin. *J. Am. Chem. Soc.* **2003**, *125*, 15000-15001.
(164) Yoshida, A.; Akaiwa, M.; Asakawa, T.; Hamashima, Y.; Yokoshima, S.; Fukuyama, T.; Kan, T. Total Synthesis of (−)-Lemonomycin. *Chem. Eur. J.* **2012**, *18*, 11192-11195.
(165) Briegel, A. C.; Cummings, A. K.; Smith, G. R.; Doroski, M. D.; Boyko, W. J.; Piro, N. A.; Kassel, W. S.; Giuliano, R. M. Synthesis of Lemonose Derivatives: Methyl 4-Amino-3-*O*,4-*N*-Carbonyl-2,4,6-Trideoxy-3-*C*-Methyl-A-L-Lyxo-Pyranoside and Its Phenyl Thioglycoside. *Carbohydr. Res.* **2015**, *409*, 63-68.
(166) Zhu, J.; Masson, G.; Bernadat, G.; George, N.; Couturier, C.; Schlama, T. Asymmetric Synthesis of 2,4,6-Trideoxy-4-(Dimethylamino)-3-*C*-Methyl-L-Lyxohexopyranose (Lemonose). *Synlett* **2011**, *2011*, 576-578.
(167) Szechner, B.; Achmatowicz, O.; Gałdecki, Z.; Fruziński, A. Synthesis and Absolute Configuration of Four Diastereoisomeric 1-(2-Furyl)-2-Aminobutane-1,3-Diols. *Tetrahedron* **1994**, *50*, 7611-7624.
(168) Evans, D. A.; Hu, E.; Tedrow, J. S. An Aldol-Based Approach to the Asymmetric Synthesis of L-Callipeltose, the Deoxyamino Sugar of L-Callipeltoside A. *Org. Lett.* **2001**, *3*, 3133-6.
(169) Parker, K. A.; Meschwitz, S. M. Short Stereoselective Synthesis of L-Nogalose. *Carbohydr. Res.* **1988**, *172*, 319-326.
(170) Jung, G.; Klemer, A. Reaktionen Von Acetalen Derl-Rhamnose Mit Lithiumorganylen Neue Synthesen Von Mycaral, Olivomycal Und Methyl-2,3,6-Tridesoxy-A-L-Erythro-Hex-2-Enopyranosid. *Chem. Ber.* **1981**, *114*, 740-745.
(171) Smith, G. R.; Finley Iv, J. J.; Giuliano, R. M. Synthesis of Methyl A-L-Callipeltoside. *Carbohydr. Res.* **1998**, *308*, 223-227.
(172) Kiemer, A.; Beermann, H. Eine Neue Methode Zur Synthese C - Verzweigter Kohlenhydrate. *J. Carbohydr. Chem.* **1983**, *2*, 457-459.
(173) Yoshimura, J.; Aqeel, A.; Sato, K.-I.; Bir Singh, R.; Hashimoto, H. Synthesis of Novel Branched-Chain Amino Sugars: Methyl L-Sibirosaminide and N-Acylkansosamine. *Carbohydr. Res.* **1987**, *166*, 253-262.
(174) Rasmussen, J. R.; Slinger, C. J.; Kordish, R. J.; Newman-Evans, D. D. Synthesis of Deoxy Sugars. Deoxygenation by Treatment with N,N'-Thiocarbonyldiimidazole/Tri-N-Butylstannane. *J. Org. Chem.* **2002**, *46*, 4843-4846.
(175) Altona, C.; Haasnoot, C. A. G. Prediction of *Anti* and *Gauche* Vicinal Proton-Proton Coupling Constants in Carbohydrates: A Simple Additivity Rule for Pyranose Rings. *Org. Magn. Reson.* **1980**, *13*, 417-429.
(176) Cohen, A.; Bergmann, E. D. Organic Fluorine Compounds—Xxxviii. *Tetrahedron* **1966**, *22*, 3545-3547.
(177) Ren, F.; Hogan, P. C.; Anderson, A. J.; Myers, A. G. Synthesis of L-Kedarosamine in Protected Form and Its Efficient Incorporation into an Advanced Intermediate to Kedarcidin Chromophore. *Org. Lett.* **2007**, *9*, 1923-1925.
(178) Leet, J. E.; Schroeder, D. R.; Hofstead, S. J.; Golik, J.; Colson, K. L.; Huang, S.; Klohr, S. E.; Doyle, T. W.; Matson, J. A. Kedarcidin, a New Chromoprotein Antitumor Antibiotic: Structure Elucidation of Kedarcidin Chromophore. *J. Am. Chem. Soc.* **1992**, *114*, 7946-7948.
(179) Leet, J. E.; Schroeder, D. R.; Langley, D. R.; Colson, K. L.; Huang, S.; Klohr, S. E.; Lee, M. S.; Golik, J.; Hofstead, S. J. Chemistry and Structure Elucidation of the Kedarcidin Chromophore. *J. Am. Chem. Soc.* **1993**, *115*, 8432-8443.
(180) Kawata, S.; Ashizawa, S.; Hirama, M. Synthetic Study of Kedarcidin Chromophore: Revised Structure. *J. Am. Chem. Soc.* **1997**, *119*, 12012-12013.
(181) Pragani, R.; Stallforth, P.; Seeberger, P. H. De Novo Synthesis of a 2-Acetamido-4-Amino-2,4,6-Trideoxy-D-Galactose (Aat) Building Block for the Preparation of a *Bacteroides Fragilis* A1 Polysaccharide Fragment. *Org. Lett.* **2010**, *12*, 1624-1627.
(182) Pragani, R.; Seeberger, P. H. Total Synthesis of the Bacteroides Fragilis Zwitterionic Polysaccharide A1 Repeating Unit. *J. Am. Chem. Soc.* **2011**, *133*, 102-7.
(183) Pihko, A. J.; Nicolaou, K. C.; Koskinen, A. M. P. An Expedient Synthesis of D-Callipeltose. *Tetrahedron Asymmetry* **2001**, *12*, 937-942.
(184) Hansson, T. G.; Kihlberg, J. O. Synthesis Of .Beta.-Benzyl N-(Tert-Butoxycarbonyl)-L-Erythro-.Beta.-(Benzyloxy)Aspartate from (R,R)-(+)-Tartaric Acid. *J. Org. Chem.* **2002**, *51*, 4490-4492.
(185) Bretherick, L. Letters: Chemical Safety - Azide-Halosolvent Hazards. *Chemical & Engineering News* **1986**, *64*, 2.
(186) Hruby, V. J.; Boteju, L.; Li, G. Letters: Chemical Safety - Explosion with Sodium Azide. *Chemical & Engineering News* **1993**, *71*, 2.
(187) Peet, N. P. Letters: Chemical Safety - Explosion with Sodium Azide in Dmso-Ch_2cl_2. *Chemical & Engineering News* **1993**, *71*, 4.
(188) am Ende, D. J.; Vogt, P. F. Safety Notables: Information from the Literature. *Organic Process Research & Development* **2004**, *8*, 1045-1048.
(189) Conrow, R. E.; Dean, W. D. Diazidomethane Explosion. *Organic Process Research & Development* **2008**, *12*, 1285-1286.
(190) Jordan, A.; Stoy, P.; Sneddon, H. F. Chlorinated Solvents: Their Advantages, Disadvantages, and Alternatives in Organic and Medicinal Chemistry. *Chem Rev* **2021**, *121*, 1582-1622.

CHAPTER 2

Gram Scale Synthesis of a Protected Arimetamycin A Disaccharide

2.1 *Retrosynthetic Analysis for Disaccharide* ***215***

As noted above (see Section 1.3), our conceptual plan to accomplish the synthesis of arimetamycin A (**1**) and hybrids **24**–**26** involved the synthesis of a disaccharide donor **31** that we could divergently glycosidate with aglycones **27**–**30** (Scheme 2.1a). To this end, we envisioned that disaccharide **215** would serve as an ideal intermediate target as it possesses the entire disaccharide framework, an easily modifiable reducing end that we could transform into a variety of glycosidic donors, and protected amines suitable for the late stage diversification necessary to probe the role of *N*,*N*-dimethylation in cytotoxicity (Scheme 2.1b). We planned to forge the disaccharide linkage using a thioglycoside donor under Hirama's $AgPF_6$ mediated α-selective methodology[1] as work from Wong, Sun, and co-workers demonstrated that these conditions are effective for coupling C3 branched donors with deoxy sugar acceptors.[2-3]



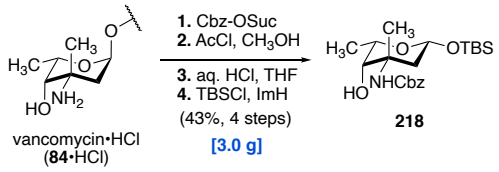
Scheme 2.1 Retrosynthetic Analysis for Intermediate Disaccharide Target **215.**

To implement this strategy, we would derive L-brasiliose acceptor **218** semi-synthetically from vancomycin•HCl (**84**•HCl) in a manner based on the work of Kahne[4-5] and Bennett[6] (Scheme 2.1b). For L-

lemonose thioglycoside donor **216**, we envisioned a *de novo* synthesis of L-lemonose thioglycoside donor **216** from D-threonine (**143**) via vinylogous ester **217**. We drew inspiration for our *de novo* approach from previous syntheses of L-lemonose[7-10] as well as syntheses of related deoxy-amino sugars D-AAT,[11] L-kedarosamine,[12] and D-callipeltose[13] as discussed above in Chapter 1. We note that the targeted vinylogous ester **217** differs only in its nitrogen protecting group from the vinylogous esters employed in the syntheses of D-AAT donor (Seeberger)[11] and D-callipeltose (Nicolaou and Koskinen)[13] (Scheme 2.1c). This strategic choice arose from (1) concerns that Cbz carbamate would not remain intact during the anticipated CH_3Li mediated installation of the C3 methyl group and (2) safety concerns regarding the large scale diazotransfer necessary to convert the amine of D-threonine (**143**) to its azide.

*2.2 Synthesis of L-Brasiliose Acceptor **218***

Synthesis of L-brasiliose acceptor **218** began from commercially available vancomycin•HCl (**84**•HCl). *N*-Cbz protection (Cbz-OSuc) under Schotten-Baumann like conditions followed by treatment with methanolic HCl provided an inconsequential mixture of methyl glycosides according to known procedures (Scheme 2.2).[4-6, 14] Next, hydrolysis and chemoselective silyl protection with TBSCl afforded β-pyranose **218**.[6]



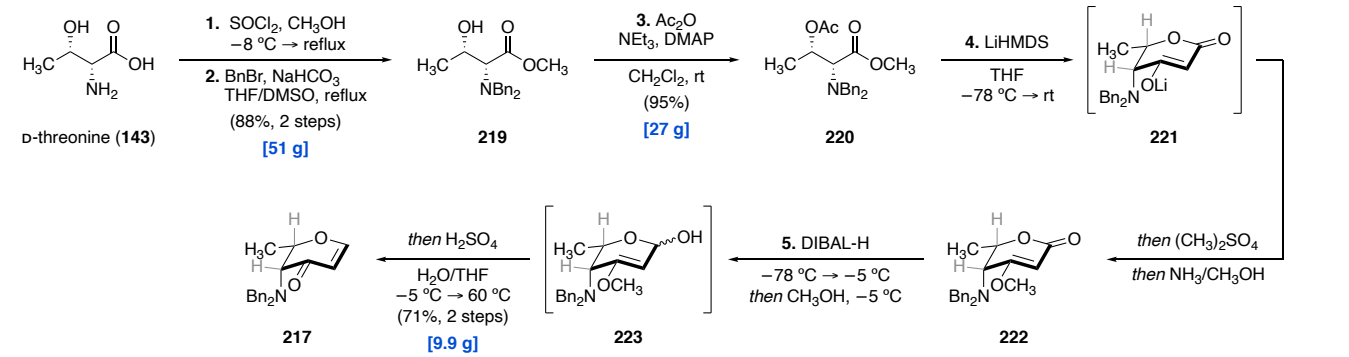
Scheme 2.2 Gram Scale Synthesis of l-Brasiliose Donor **218** from Vancomycin•HCl (**84**•HCl).

*2.3 Synthesis of L-Lemonose Thioglycoside **216***

*2.3.1 Synthesis of Vinylogous Ester **217***

Synthesis of vinylogous ester **217** started from D-threonine (**143**). Fisher esterification followed by amine dibenzylation yielded alcohol **219** (Scheme 2.3). Ensuing acylation gave cyclization precursor **220** on decagram scale. Exposure of **220** to LiHMDS facilitated the desired Dieckmann condensation.[11-12] Quenching of the intermediate vinylogous carboxylate (**221**) with dimethyl sulfate gave vinylogous methyl ester **222**[12]. We terminated the reaction by the addition of methanolic NH_3 to destroy excess dimethyl sulfate as orienting studies showed that this toxic reagent persisted through aqueous workup and column chromatography. Next, DIBAL-H reduction of the crude lactone[11] followed by protonation (aq. H_2SO_4)[15] and warming of the solution facilitated a

Stork-Danheiser like transposition that produced vinylogous ester **217** in 71% yield over the two-pot, four-transformation sequence.

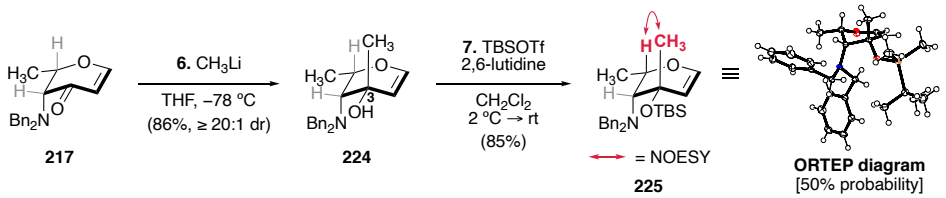


Scheme 2.3 Synthesis of Vinylogous Ester **217**.

2.3.2 *1st Generation Conversion of Vinylogous Ester **217** to Lemonose Thioglycoside **216***

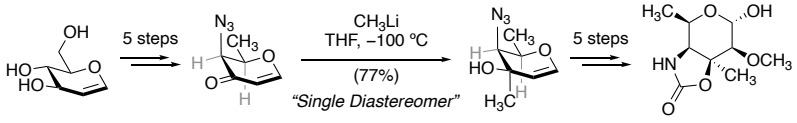
Inverse addition of vinylogous ester **217** to a solution of CH_3Li yielded glycal **224** with high diastereoselectivity (≥20:1) as crude ^1H NMR indicated the absence of the epimeric addition product (Scheme 2.4a).[13] Addition occurred from the desired face of the carbonyl as confirmed by NOESY and single crystal X-ray diffraction (XRD) analyses of derivative **225**.[16] Telescoping these steps (i.e., quenching the intermediate lithiate with TBSOTf) gave **225** in lower and less reproducible yields (34%-66%), demonstrating the superiority of the two step method. The observed selectively is analogous to that reported by Nicolaou and Koskinen for the transformation of **211** to **212** in their synthesis of D-callipeltose (**214**) from D-glucal (**209**) (Scheme 2.4a; also Scheme 1.24a).[13] In both cases, an axially oriented C4 amine substituent directs methyl addition to the opposite face of the ring (Scheme 1.24b).

a. Diastereoselective Installation of the C3 Tetrasubstituted Center.



217 224 225

b. Nicolaou and Koskinen's 2001 Synthesis of D-Callipeltose (**214**).



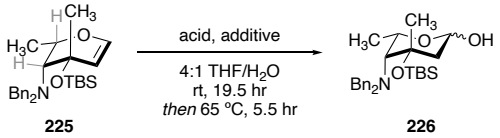
D-glucal (**209**) **211** **212** D-callipeltose (**214**)

Scheme 2.4 Successful Installation of the C3 Tetrasubstituted Center and Comparison with Literature Precedent.

Having established the full carbon skeleton of lemonose donor **216**, our next task was hydration of glycal **225** to provide a handle for further C1 functionalization. Unexpectedly, **225** resisted treatment with various strong acids as TLC indicated only the presence of starting material after 18 hr at room temperature and a mixture of starting material and desired hydrolysis product **226** after an additional 5.5 hr at 60 °C (Table 2.1a). In contrast, treatment of **225** with catalytic PPh_3•HBr (30 mol%) yielded a mixture of starting material and product after only 4.2 hr at room temperature.[17-19] However, additional time and excess reagents were not able to push this reaction to completion (Table 2.1b, Entry 1). Combining heat with excess PPh_3•HBr and H_2O as well as a Lewis acid system employing $CeCl_3$•$7H_2O$ and NaI[20] gave reducing sugar **226** in low to moderate yield (Table 2.1b, Entries 2-5). As extended heating at 40 °C in THF gave product with the best impurity profile, these conditions were scaled to mmol scale (Table 2.1b, Entry 6).

Table 2.1 Optimization of the Hydrolysis of Glycal **225**.

a. Hydrolysis of Glycal **225** with Strong Acids.

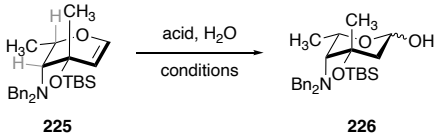


225 **226**

Entry[a]	Acid (equiv)	t = 18 hr[b] **225 | 226**	t = 25 hr[c] **225 | 226**
1	HCl (2.6)	P I NP	P I P
2	H_2SO_4 (2.9)	P I NP	P I FP
3	TsOH•H_2O (2.6)	P I NP	P I FP
4	TsOH•H_2O (2.8)[d]	P I NP	P I P

[a]Conditions: 11-13 μmol (4.8-5.7 mg) **225** in H_2O (100 μL, > 400 eq) and THF (400 μL) and acid/additive as indicated. [b]Reaction progress after 18 hr at room temperature as judged qualitatively by TLC; P = spot present after CAM staining; NP = spot not present after CAM staining. [c]Reaction progress after an additional 1.5 hr at room temperature followed by 5.5 hr at 65 °C as judged qualitatively by TLC; P = spot present after CAM staining; FP = faint spot present after CAM staining. [d]Reaction ran with LiBr (8.1 Eq) as an additive.

b. Hydrolysis of Glycal **225** with Alternative Acids.

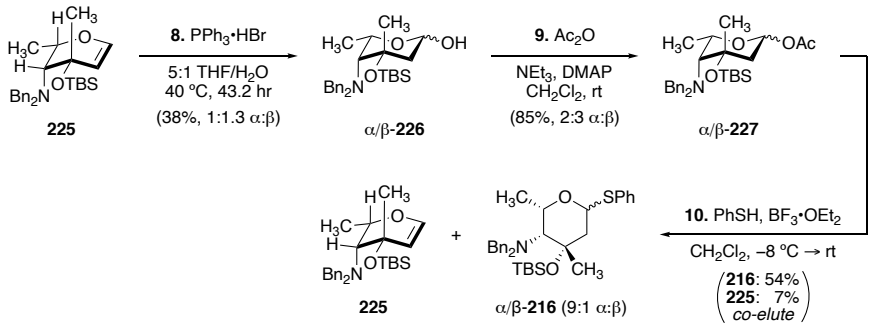


225 **226**

Entry	Scale (**225**)	Conditions	Acid (Eq)	Equiv H_2O	Yield[a]
1	57 μmol	THF, rt, 90 hr	PPh_3•HBr (1.9)[b]	98[c]	44%
2	0.24 mmol	THF, 40 °C, 40 hr	PPh_3•HBr (2.1)	99	38%
3	39 μmol	CH_3CN, 80 °C, 2 hr	PPh_3•HBr (2.3)	71	36%[d]
4	0.20 mmol	CH_3CN, 80 °C, 9.5 hr	PPh_3•HBr (2.3)	75	27%[e]
5	98 μmol	CH_3CN, 85 °C, 9 hr	NaI (1.4), $CeCl_3$•H_2O (1.6)	11	23%[f]
6	1.17 mmol	THF, 40 °C, 43.5 hr	PPh_3•HBr (2.1)	105	38%[g]

[a]Isolated yield after chromatographic purification (FCC). [b]Reaction began with 0.30 equiv PPh_3•HBr; additional 1.6 equiv added after 26.75 hr. [c]Reaction began with 10 equiv H_2O; additional 88 equiv added after 22 hr. [d]Material impure after preperatory TLC. [e]Material impure after FCC. [f]Material isolated as a brown oil after FCC; color may be result of residual iodine or cerium species. [g]Material (201 mg) isolated as white foam contaminated with some TBS containing impurity after FCC.

At this early stage in the synthetic campaign, we elected to push forward with the moderate yields obtained thus far, reasoning that we could devote more time to optimizing the hydration after proving that we could elaborate thioglycoside **216** to disaccharide **215**. Accordingly, we converted reducing sugar **226** to acetate **227** under standard conditions in 85% yield as an inconsequential mixture of anomers (Scheme 2.5). Next, BF_3•OEt_2 activation of the anomeric ester in the presence of PhSH gave the desired thioglycoside **216** in 54% yield (9:1 α:β) contaminated with elimination product **225** (7%). Hypothesizing that the presence of glycal **225** would not interfere with the ensuing glycosylation between **216** and **218**, we successfully took this material on without purification to a proof of concept glycosylation that yielded **215** (*vide infra*).[21] We note that a small-scale experiment (62 μmol α/β-**227**) showed that increasing the amount of the BF_3•OEt_2 and adding powdered 4 Å molecular sieves increased the yield of α/β-**216** to 78% and improved the ratio of α/β-**216**:**225** from 5:1 to 18:1. However, we elected not to further pursue this approach due to the success of our 2nd generation route to **216** as described below.



Scheme 2.5 Completion of the 1st Generation Synthesis of Thioglycoside **216**.

Structurally, major anomer α-**216** exhibited larger anomeric coupling constants ($^3J_{H1\text{-}H2}$ = 6.0, 5.0 Hz) than anticipated (Figure 2.1a).[22] NOESY analysis showed correlations corresponding both to the expected $^1C_4$ conformation (C3Me ↔ H5) and the chair flipped $^4C_1$ conformation (H1 ↔ TBS$_{t\text{-Bu}}$, H1 ↔ H5, TBS$_{t\text{-Bu}}$ ↔ H5, and H2$_{pro\text{-}S}$ ↔ H4). As such, we concluded that α-**216** exists either in conformational equilibrium or in an intermediate conformation. In contrast, anomeric coupling constant ($^3J_{H1\text{-}H2}$ = 12.5, 2.2 Hz) and NOESY analyses indicate that β-**216** occupies the canonical $^1C_4$ conformation (Figure 2.1b)

Interestingly, Giuliano and co-workers observed a related phenomenon in their synthesis of L-lemonose thioglycoside α/β-**144**.[10] They found that α-**144** exhibited even larger coupling constants ($^3J_{H1\text{-}H2}$ = 10.4, 6.4 Hz) than we observed for α-**216** (Figure 2.1c). An H2$_{pro\text{-}S}$ ↔ H5 NOESY correlation indicated that α-**144** assumed a boat conformation (^{2,5}B). A lack of NOESY correlations for the anomeric proton (only the H1 ↔ H2$_{pro\text{-}R}$ correlation was observed) as well as an X-ray crystal structure support this conformational assignment.

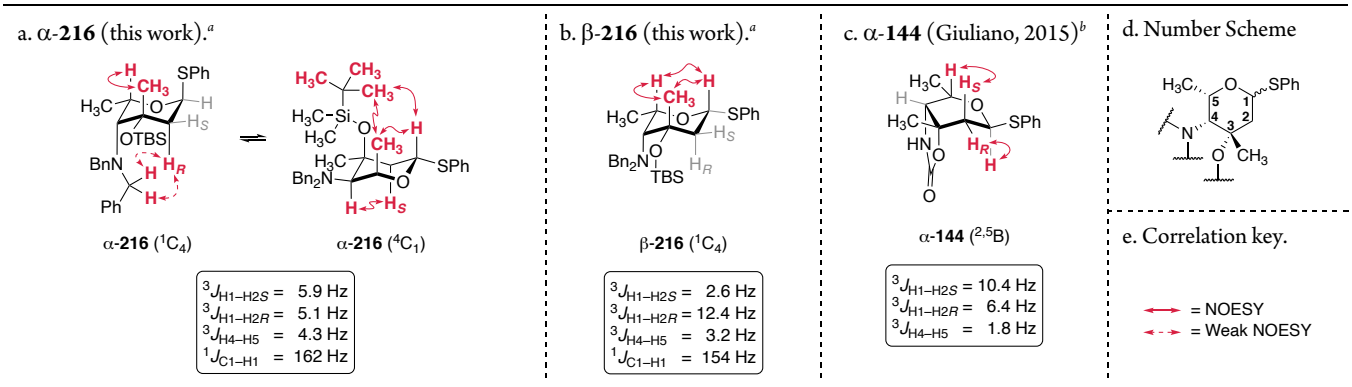


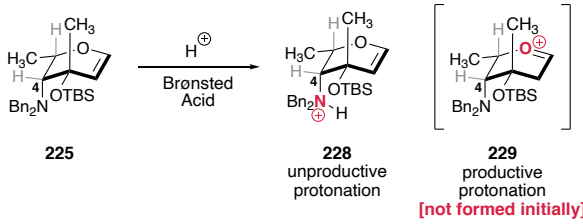
Figure 2.1 Conformational Analysis of α-**216** and β-**216** with Comparison to α-**144** from Giuliano's 2015 Synthesis of L-Lemonose Thioglycosides α/β-**144**. [a]Data collected on α/β-**216** (ca. 9:1) in $CDCl_3$ (^{1}H, 600 MHz; ^{13}C, 151 MHz) synthesized from TBS glycoside α/β-**235** as first described in **Scheme 2.9**. [b]Data collected by Giuliano et al.[10] on an inseparable mixture of α/β-**144** in $CDCl_3$ (^{1}H, 300 MHz).

2.3.3 *2nd Generation Conversion of Vinylogous Ester **217** to Lemonose Thioglycoside **216***

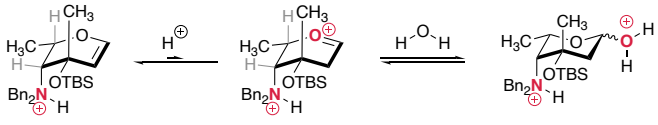
Having demonstrated that thioglycoside **216** could serve as a competent donor in the construction of disaccharide **215**, we dedicated further efforts to optimizing the synthesis of **216**. Examination of our 1st generation approach revealed two weak points: (1) the hydration of glycal **225** to give **226** (plagued by low yield) and (2) the conversion of acetate **227** to **216** (plagued by moderate yield and concomitant formation of undesired **225**). With these in mind, we began our 2nd generation approach to **216**.

Focusing first on the glycal hydration, we hypothesized that **225** had proven recalcitrant to hydrolysis due to the basicity of its C4 amine moiety. We reasoned that protonation of the C4 amine would happen faster than would the desired protonation of the glycal (Scheme 2.6a). Hydration of the resulting ammonium species **228** would then require the intermediacy of high energy, doubly charged intermediates **230** and **231** (Scheme 2.6b). Theoretically, the hydration could proceed without doubly charged intermediates via intramolecular proton transfer from the protonated nitrogen to the glycal and subsequent nucleophilic attack by water (Scheme 2.6c). However, intramolecular amine re-protonation would likely quench the intermediate oxonium ion much faster that would the desired intermolecular addition of water, making this reaction pathway unlikely.

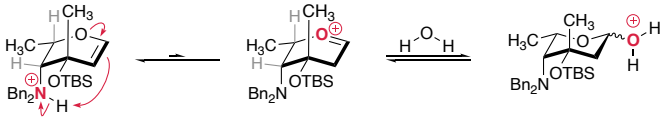
a. Initial Protonation of **225** by Brønsted Acids to Give **228** Instead of Desired **229.**



b. Glycal Hydration Proceeding through Proposed Doubly Charged Intermediates **230** and **231**.



c. Unlikely Glycal Hydration Proceeding through Singly Charged Intermediates **229** and **232**.

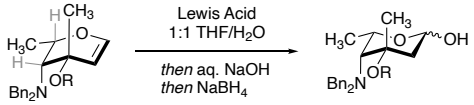


Scheme 2.6 Mechanistic Hypothesis for the Hydration of Recalcitrant Glycal **225** by Brønsted Acids.

Based on this mechanistic framework, we posited that replacing the activating Brønsted acid with a Lewis acid would ameliorate this issue. In practice, this proved correct as oxymercuration-demercuration using Hg(II)

afforded **226** in increased yield (Scheme 2.7a, Entries 1-2).[23-26] Furthermore, we found that treatment of *sans* TBS glycal **224** gave the corresponding diol (**233**) in even higher yield. The reaction scaled well as hydrolysis of 9.8 mmol of **224** afforded 2.35 g of **233** while maintaining the same yield. Further work showed that the reaction worked equally well on crude **224**, allowing us transform vinylogous ester **217** into **233** in a two-step, one purification process.

a. Hydrolysis of Lemonose Glycals **225** and **224** with Hg(II) Lewis Acids.



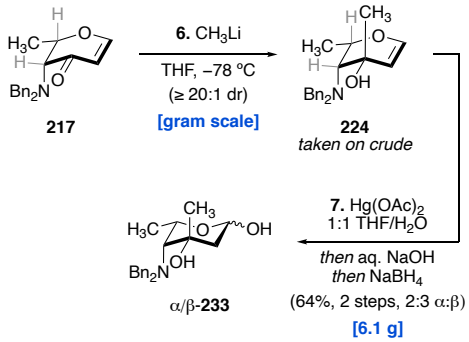
225 | R = TBS
224 | R = H

α/β-**226** | R = TBS
α/β-**233** | R = H

Entry	SM (R)[a]	Lewis Acid (Eq)	Yield[b]
1	**225** (TBS)	$Hg(OAc)_2$ (1.5)	58%
2	**225** (TBS)	$Hg(OTFA)_2$ (1.2)	60%
3	**224** (H)	$Hg(OAc)_2$ (1.2)	70% (70%)[c]

[a]SM = starting material. [b]Isolated yield after chromatographic purification (FCC) for reactions run with 0.2-0.3 mmol SM. [c]Number in parentheses is isolated yield for a reaction run with 9.8 mmol SM that yielded 2.35 g product after FCC.

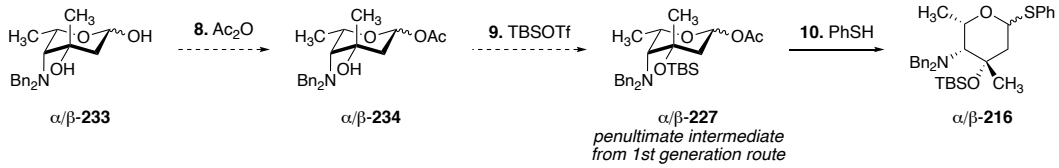
b. Optimized Gram Scale Conversion of **217** to **233**.



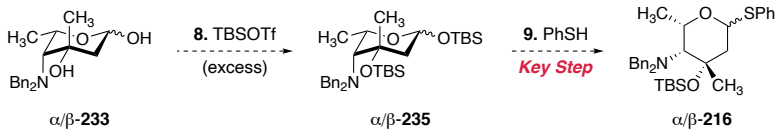
Scheme 2.7 Oxymercuration-Demercuration of **225** and **224**.

Having improved the efficiency of the glycal hydration, we turned our attention to elaborating diol **233** to thioglycoside **216**. We envisioned that chemoselective anomeric acetylation followed by tertiary alcohol silylation would yield previously synthesized acetate **227**, allowing us to converge with our 1st generation approach (Scheme 2.8a). At the same time, we realized that we could save a step by simultaneously silylating both free alcohols provided that we could convert the resulting silyl glycoside into **216** (Scheme 2.8b). While certainly a nontraditional carbohydrate transformation, we noted relevant literature precedents from the Priebe[27] (Scheme 2.8c) and Marino[28] (Scheme 2.8d) laboratories and elected to pursue the disilylation approach.

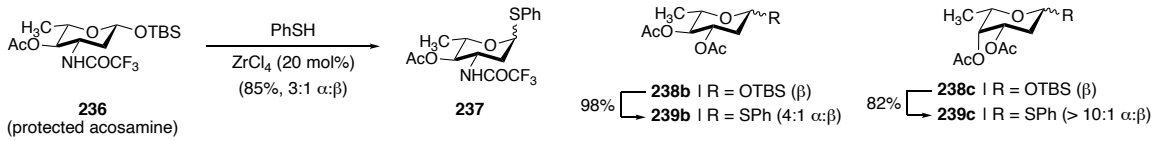
a. Proposed Three Step Conversion of α/β-**233** to α/β-**216** via Interception of Intermediate α/β-**227** from the 1st Generation Route to α/β-**216**.



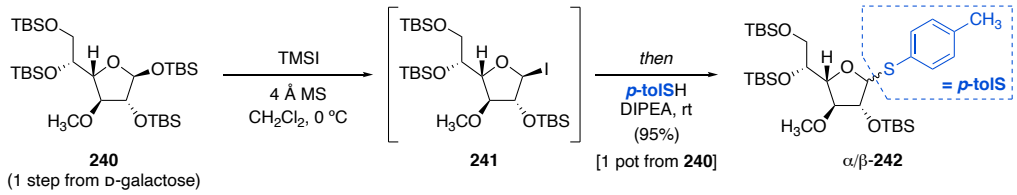
b. Proposed Two Step Conversion of α/β-**233** to α/β-**216** via Thioglycosylation of *O*-TBS Glycoside α/β-**235**.



c. Priebe's 1991 Thioglycosylation of *O*-TBS Glycosides **236** and **238a–b**.[27]

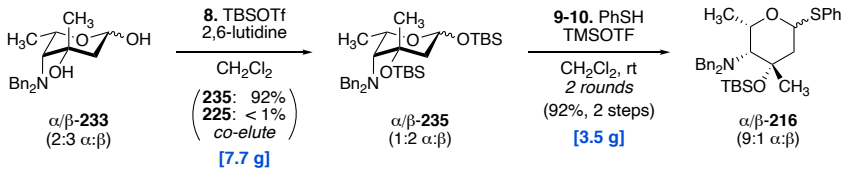


d. Marinoe's 2012 Thioglycosylation of *O*-TBS Galactofuranoside **240**.[28]



Scheme 2.8 Proposed Transformations of **233** to **216** and Relevant Literature Precedents.

In practice, treatment of diol **233** with excess TBSOTf in the presence of 2,6-lutidine afforded **235** in high yield (92%) as an inconsequential mixture of anomers (Scheme 2.9). Careful ^{1}H NMR analysis indicated the presence of trace amounts of elimination product **225** (>50:1 molar ratio of α/β-**235**:**225**). Moving forward, an orienting thioglycosylation of **235** employing $ZrCl_4$ proved disappointing as ^{1}H NMR indicated the absence of desired **216** even after the addition of excess Lewis acid (2 Eq total).[27] In contrast, we found that two-fold exposure to excess PhSH and TMSOTf gave thioglycoside **216** in excellent yield (92% over 2 steps) on gram scale; ^{1}H NMR analysis indicated the absence of glycal **225**.

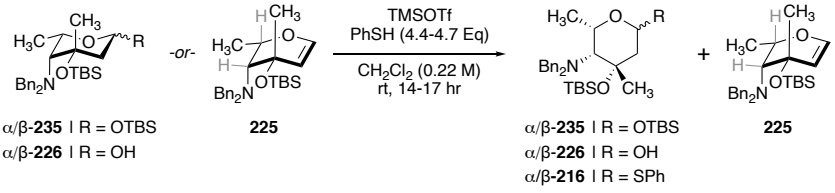


Scheme 2.9 Completion of the 2nd Generation Synthesis of Thioglycoside **216**.

Regarding the two-step nature of the thioglycosylation, crude ^{1}H NMR indicates that the first round of PhSH/TMSOTf treatment yields a complex mixture consisting of hydrolysis product α/β-**226** (52%) and thioglycoside α/β-**216** (43%) along with trace amounts of starting material α/β-**235** (4%) and elimination product **225** (1%) (Table 2.2a–b, Entry 1)[29]. This mixture completely converges to thioglycoside α/β-**216** upon resubjection to the reaction conditions, suggesting that reducing sugar α/β-**226** and glycal **225** are competent substrates for this reaction. (Table 2.2a–b, Entry 2) Independent treatment of pure α/β-**226** with PhSH/TMSOTf under similar conditions resulted in full conversion to thioglycoside α/β-**216**, thus verifying the reducing sugar as an able substrate (Table 2.2a–b, Entry 3). In contrast, exposure of pure glycal **225** to PhSH/TMSOTf gave only 20% conversion to α/β-**216**, providing inconclusive evidence as to whether the complete disappearance of **225** upon resubjection is a result of its conversion to the thioglycoside or simply the result of material losses during workup or the limits of NMR detection (Table 2.2a–b, Entry 4).

Table 2.2 Mechanistic Analysis of the 2-Step Conversion of α/β-**235** to Thioglycoside α/β-**216**.

a. Relative Composition of Crude Reaction Mixtures.



α/β-**235** I R = OTBS
α/β-**226** I R = OH

225

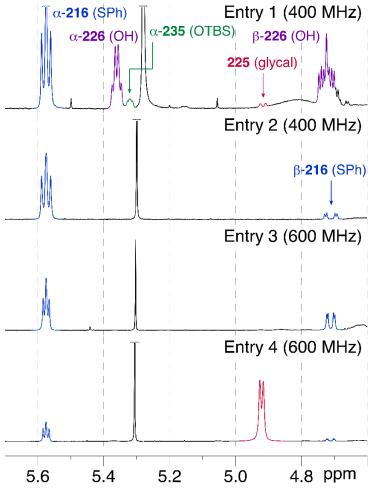
α/β-**235** I R = OTBS
α/β-**226** I R = OH
α/β-**216** I R = SPh

225

Entry	SM[a]	Eq TMSOTf[b]	Relative Composition of Crude Product [α:β][c] **235** (OTBS)	**226** (OH)	**216** (SPh)	**225** (glycal)	Yield
1[d]	**235** (OTBS)	3.1	4% [1:0][e]	52% [1:1]	43% [9:1]	1%	- - -[f]
2[f]	Crude Entry 1	3.1	- - -	- - -	100% [9:1]	- - -	92%[g]
3[h]	**226** (OH)	4.1	- - -	- - -	100% [2.2:1]	- - -	72%[i]
4[j]	**225** (glycal)	3.0	- - -	- - -	20% [4:1]	80%	79%[i]

[a]SM = starting material. [b]Eq = equivalents. [c]Determined by ^{1}H NMR. [d]Reaction run with 4.0 g (7.1 mmol) α/β-**235**. [e]Due to spectral overlap, the presence of β-**235** (OTBS) cannot be ruled out. [f]Crude product of Entry 1 resubjected as detailed in Entry 2. [g]Isolated yield over 2 steps. [h]Run with 27 mg (59 μmol) α/β-**226**. [i]Combined NMR yield vs. mesitylene. [j]Run with 40 mg (44 μmol) of **225**.

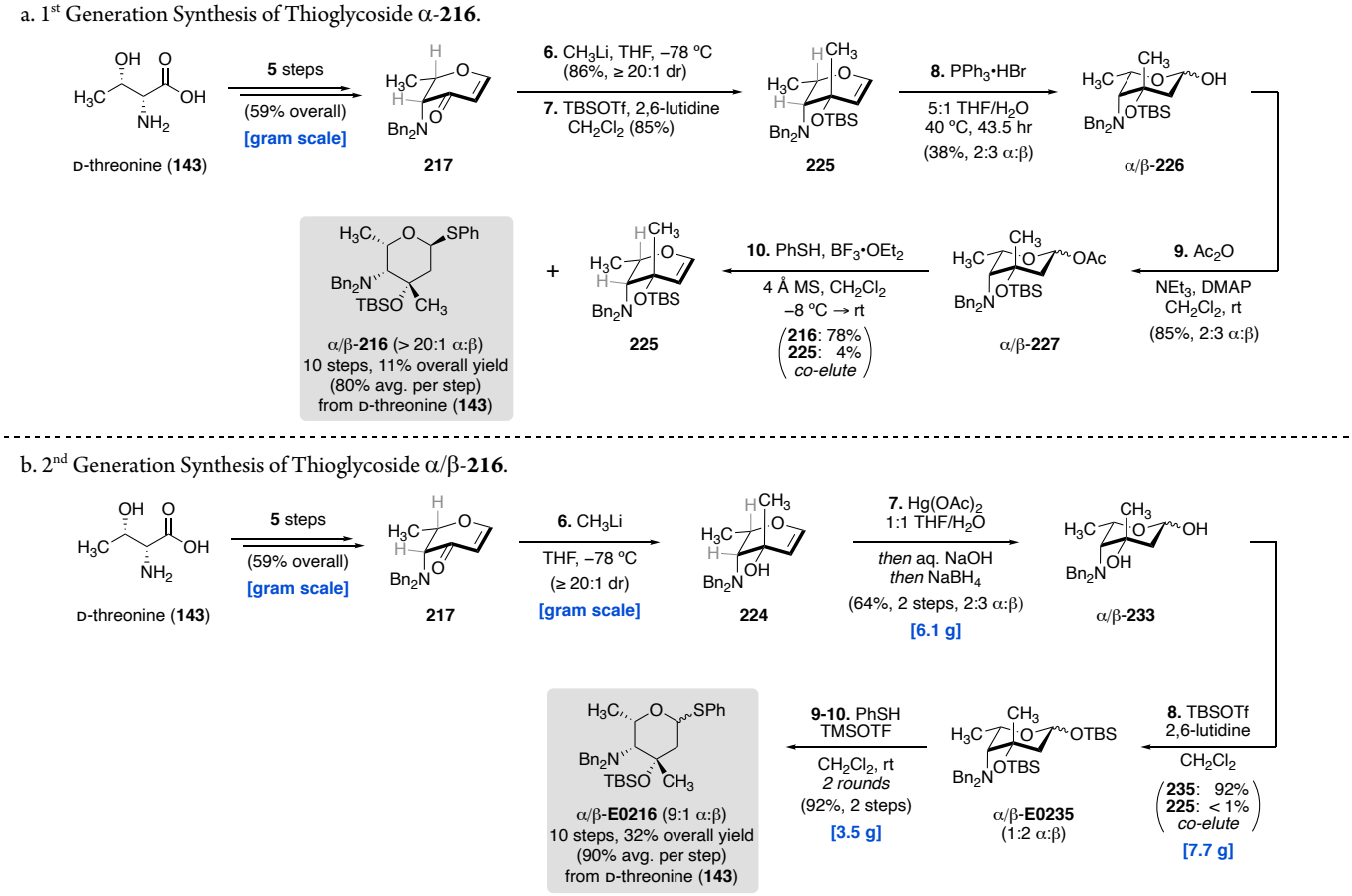
b. Crude NMR (^{1}H, $CDCl_3$) from Entries 1-4.



*2.3.4 Comparison of 1st and 2nd Generation Routes for Transforming Vinylogous Ester **217** to Lemonose Thioglycoside **216***

Our synthesis of L-lemonose thioglycoside **216** began with the gram scale synthesis of vinylogous ester **217** from D-threonine (**143**) in five steps in an overall yield of 59% (average yield per step of 90%) (Scheme 2.3). Our 1st generation route for transforming **217** into L-thioglycoside donor **216** required an additional five steps, providing **216** from D-threonine (**143**) in a total of ten steps (11% yield overall, average yield per step of 80%) (Scheme 2.10a). Optimization of the glycal hydration and thioglycosylation led to our 2nd generation route to **216**

from common intermediate **217** (Scheme 2.10b). While simultaneous silylation of the C1 and C3 alcohols did not allow us reduce the step count as envisioned (Scheme 2.8a–b) due to the necessity of subjecting the resulting *O*-TBS glycoside (**235**) to two rounds of PhSH/TMSOTf (Scheme 2.9, Table 2.2), our optimization efforts nevertheless improved the efficiency of the synthesis and allowed us to produce grams of α/β-**216** in ten steps from D-threonine (**143**) in an improved overall yield of 32% (average yield per step of 90%).



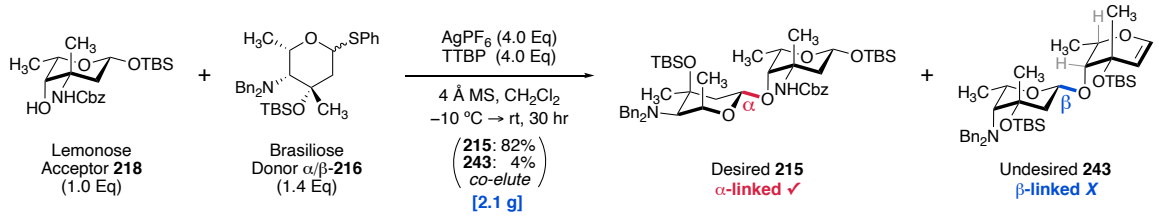
Scheme 2.10 Comparison of 1st and 2nd Generation Routes for Transforming Vinylogous Ester **217** to L-Lemonose Thioglycoside α/β-**216**.

*2.4 Glycosylation of Brasiliose Acceptor **218** with Lemonose Thioglycoside **216** to Yield Disaccharide **215***

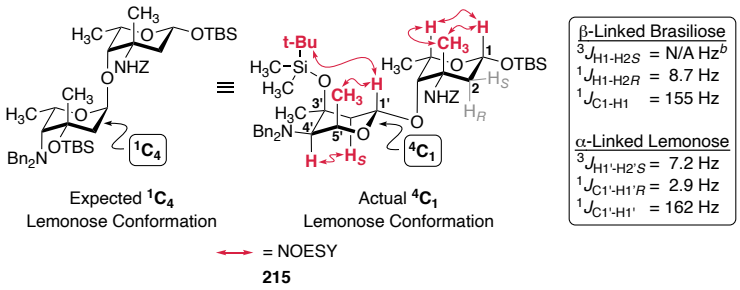
Having established gram scale routes to L-brasiliose acceptor **218** (Scheme 2.2) and L-lemonose donor **216** (Scheme 2.10), we proceeded to study their glycosylation. Subjection of acceptor **218** and excess donor α/β-**216** to Hirama's conditions ($AgPF_6$) gave a ≥20:1 mixture of co-eluting disaccharides **215** and **243** after column chromatography (Scheme 2.11a, Table A.1, and Table A.2).[1] Initial inspection of major product **215**'s $^3J_{H1'\text{-}H2'}$ coupling constants (J = 7.2, 2.9 Hz) indicated the formation of the undesired β-anomer (Scheme 2.11b).[22]

Measurement of the $^{1}J_{C1'\text{-}H1'}$ coupling constant (J = 162 Hz) provided further evidence for a β-linkage (α: $^{1}J_{CH}$ ~ 170 Hz | β: $^{1}J_{CH}$ ~ 160 Hz).[30-31] However, recalling our experience with the conformationally deviant α-**216** (Figure 2.1), we acquired a NOESY spectrum of **215**. This data revealed correlations between H1′↔ C3′Me, H1′ ↔ C3′TBS$_{t\text{-Bu}}$, and H2′$_{pro\text{-}S}$ ↔ H4′, allowing us to deduce that the non-reducing lemonose sugar of **215** had undergone a chair flip to the unexpected $^{4}C_{1}$ conformation. Furthermore, the axial position of H1′ allowed us to reconcile the NOESY data with the $^{1}J_{H1'\text{-}H2'}$ and $^{1}J_{C1'\text{-}H1'}$ values and conclude that we had in fact synthesized the desired α-linked **215** on gram scale. Synthesis and single crystal XRD of crystalline derivative **244** unequivocally confirmed this NMR based assertion (Scheme 2.11d).

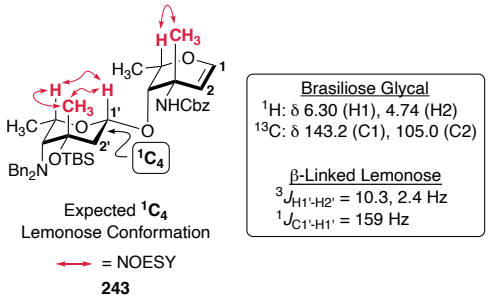
a. Gram Scale Synthesis of α-Linked Disaccharide **215**.



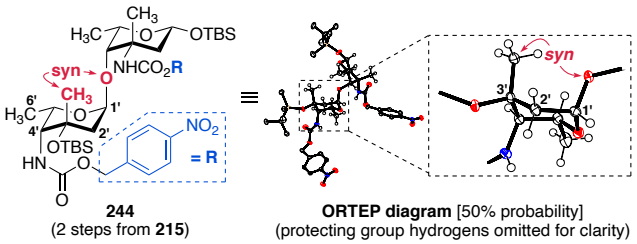
b. Conformational Analysis of α-Linked Disaccharide **215**.[a]



c. Conformational Analysis of β-Linked Side Product **243**.



d. Verification of Disaccharide **215**'s α-Linkage by Single Crystal XRD.

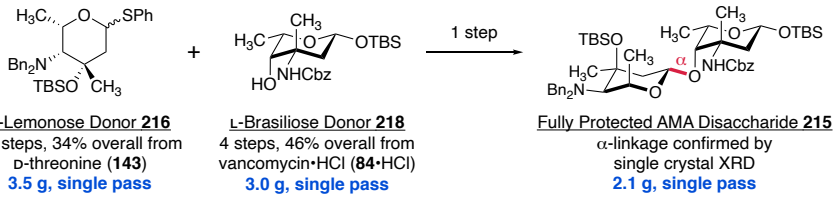


Scheme 2.11 Synthesis and Conformation Analysis of α-Linked Disaccharide **215** and Side Product **243**. [a]For clarity, the Cbz protecting group is abbreviated as "Z". [b]Not possible to determine this coupling constant; however, broadening was observed for the H1 (br d) and H2$_{pro\text{-}S}$ (br d) resonances.

Regarding the selectivity of the glycosylation event, NMR analysis of the $CDCl_3$ soluble crude materials coupled with the high isolated yield (82%) for the desired α-linked **215** led us to conservatively conclude that **215** was formed in a ≥4:1 ratio relative to any other disaccharide component (Figure A.5). NMR analysis of minor, co-eluting disaccahride **243** revealed the presence of a β-linkage (as confirmed by $^3J_{H1'\text{-}H2'}$, $^1J_{C1'\text{-}H1'}$, and NOESY analyses) and, to our surprise, a glycal resulting from formal elimination of the anomeric silyl ether (Scheme 2.11c). As **243** constituted such a minor component of the bulk material (≥20:1 **215**:**243**), we pushed the mixture forward without further purification.

2.5 *Summary of Chapter 2*

In this chapter, we described the gram scale synthesis of protected arimetamycin A disaccharide **215** via the $AgPF_6$ mediated glycosylation of L-lemonose thioglycoside donor **216** and L-brasiliose acceptor **218** (Scheme 2.12). The synthesis of acceptor **218** proved straightforward starting from vancomycin•HCl (**84**•HCl). In contrast, developing a gram scale route to donor **216** from D-threonine (**143**) involved extensive optimization of the glycal hydration and an unusual thioglycosidation of an *O*-TBS glycoside (**235**). Finally, with **216** and **218** in hand, glycosylation yielded what initially appeared to be the undesired β-linked disaccharide based on the observed $^1J_{CH}$ and $^3J_{HH}$ coupling constants. However, NOESY analysis revealed that the non-reducing lemonose moiety occupied the "chair-flipped" $^4C_1$ conformation rather than the expected $^1C_4$ conformation, allowing us to conclude that we had in fact made the desired α-linked disaccharide (**215**). Single crystal XRD analysis of a crystalline derivative (**244**) confirmed this NMR based assertion.



Scheme 2.12 Summary of Chapter 2.

2.6 *References for Chapter 2.*

(1) Lear, M. J.; Yoshimura, F.; Hirama, M. A Direct and Efficient A-Selective Glycosylation Protocol for the Kedarcidin Sugar, L-Mycarose: $Agpf_6$ as a Remarkable Activator of 2-Deoxythioglycosides. *Angew. Chem. Int. Ed.* **2001**, *40*, 946-949.
(2) Zhang, G.; Fang, L.; Zhu, L.; Aimiuwu, J. E.; Shen, J.; Cheng, H.; Muller, M. T.; Lee, G. E.; Sun, D.; Wang, P. G. Syntheses and Biological Activities of Disaccharide Daunorubicins. *J. Med. Chem.* **2005**, *48*, 5269-5278.
(3) Zhang, G.; Fang, L.; Zhu, L.; Zhong, Y.; Wang, P. G.; Sun, D. Syntheses and Biological Activities of 3'-Azido Disaccharide Analogues of Daunorubicin against Drug-Resistant Leukemia. *J. Med. Chem.* **2006**, *49*, 1792-1799.
(4) Ge, M.; Thompson, C.; Kahne, D. Reconstruction of Vancomycin by Chemical Glycosylation of the Pseudoaglycon. *J. Am. Chem. Soc.* **1998**, *120*, 11014-11015.
(5) Thompson, C.; Ge, M.; Kahne, D. Synthesis of Vancomycin from the Aglycon. *J. Am. Chem. Soc.* **1999**, *121*, 1237-1244.
(6) Soliman, S. E.; Bennett, C. S. Reagent-Controlled Synthesis of the Branched Trisaccharide Fragment of the Antibiotic Saccharomicin B. *Org. Lett.* **2018**, *20*, 3413-3417.
(7) Ashley, E. R.; Cruz, E. G.; Stoltz, B. M. The Total Synthesis of (−)-Lemonomycin. *J. Am. Chem. Soc.* **2003**, *125*, 15000-15001.
(8) Zhu, J.; Masson, G.; Bernadat, G.; George, N.; Couturier, C.; Schlama, T. Asymmetric Synthesis of 2,4,6-Trideoxy-4-(Dimethylamino)-3-*C*-Methyl-L-Lyxohexopyranose (Lemonose). *Synlett* **2011**, *2011*, 576-578.
(9) Yoshida, A.; Akaiwa, M.; Asakawa, T.; Hamashima, Y.; Yokoshima, S.; Fukuyama, T.; Kan, T. Total Synthesis of (−)-Lemonomycin. *Chem. Eur. J.* **2012**, *18*, 11192-11195.
(10) Briegel, A. C.; Cummings, A. K.; Smith, G. R.; Doroski, M. D.; Boyko, W. J.; Piro, N. A.; Kassel, W. S.; Giuliano, R. M. Synthesis of Lemonose Derivatives: Methyl 4-Amino-3-*O*,4-*N*-Carbonyl-2,4,6-Trideoxy-3-*C*-Methyl-A-L-Lyxo-Pyranoside and Its Phenyl Thioglycoside. *Carbohydr. Res.* **2015**, *409*, 63-68.
(11) Pragani, R.; Stallforth, P.; Seeberger, P. H. De Novo Synthesis of a 2-Acetamido-4-Amino-2,4,6-Trideoxy-D-Galactose (Aat) Building Block for the Preparation of a *Bacteroides Fragilis* A1 Polysaccharide Fragment. *Org. Lett.* **2010**, *12*, 1624-1627.
(12) Ren, F.; Hogan, P. C.; Anderson, A. J.; Myers, A. G. Synthesis of L-Kedarosamine in Protected Form and Its Efficient Incorporation into an Advanced Intermediate to Kedarcidin Chromophore. *Org. Lett.* **2007**, *9*, 1923-1925.
(13) Pihko, A. J.; Nicolaou, K. C.; Koskinen, A. M. P. An Expedient Synthesis of D-Callipeltose. *Tetrahedron Asymmetry* **2001**, *12*, 937-942.
(14) O'Keefe, B. M.; Mans, D. M.; Kaelin, D. E., Jr.; Martin, S. F. Studies toward the Syntheses of Pluramycin Natural Products. The First Total Synthesis of Isokidamycin. *Tetrahedron* **2011**, *67*, 6524-6538.
(15) Woodward, R. B.; Sondheimer, F.; Taub, D.; Heusler, K.; McLamore, W. M. The Total Synthesis of Steroids. *J. Am. Chem. Soc.* **1952**, *74*, 4223-4251.
(16) Deposited in the Cambridge Structural Database under deposition number CCDC 2043035.
(17) Bolitt, V.; Mioskowski, C.; Lee, S. G.; Falck, J. R. Direct Preparation of 2-Deoxy-D-Glucopyranosides from Glucals without Ferrier Rearrangement. *J. Org. Chem.* **1990**, *55*, 5812-5813.
(18) Bucher, C.; Gilmour, R. Fluorine-Directed Glycosylation. *Angew. Chem. Int. Ed. Engl.* **2010**, *49*, 8724-8.
(19) Yamada, H.; Adachi, M.; Isobe, M.; Nishikawa, T. Stereocontrolled Total Synthesis of Polygalolide A. *Chem.: Asian J.* **2013**, *8*, 1428-1435.
(20) Yadav, J. S.; Reddy, B. V. S.; Reddy, K. B.; Satyanarayana, M. $Cecl_3{\cdot}7h_2o$: A Novel Reagent for the Synthesis of 2-Deoxysugars from D-Glycals. *Tetrahedron Lett.* **2002**, *43*, 7009-7012.
(21) The 5:1 molar mixture of thioglycoside (E460):glycal (E426) made in EDH-e-0586 was taken on to a glycosylation with brasiliose acceptor E496 in EDH-e-0590.
(22) Altona, C.; Haasnoot, C. A. G. Prediction of *Anti* and *Gauche* Vicinal Proton-Proton Coupling Constants in Carbohydrates: A Simple Additivity Rule for Pyranose Rings. *Org. Magn. Reson.* **1980**, *13*, 417-429.
(23) Brown, H. C.; Geoghegan, P. The Oxymercuration-Demercuration of Representative Olefins. A Convenient, Mild Procedure for the Markovnikov Hydration of the Carbon-Carbon Double Bond. *J. Am. Chem. Soc.* **1967**, *89*, 1522-1524.
(24) Oishi, T.; Ando, K.; Chida, N. Stereoselective Total Synthesis of (+)-Myriocin from D-Mannose. *Chem Commun (Camb)* **2001**, 1932-3.
(25) Pal, P.; Shaw, A. K. Stereoselective Total Syntheses of (+)-Exo- and (−)-Exo-Brevicomins, (+)-Endo- and (−)-Endo-Brevicomins, (+)- and (−)-Cardiobutanolides, (+)-Goniofufurone. *Tetrahedron* **2011**, *67*, 4036-4047.
(26) Hernandez-Guerra, D.; Kennedy, A. R.; Leon, E. I.; Martin, A.; Perez-Martin, I.; Rodriguez, M. S.; Suarez, E. Synthetic Approaches to Phosphasugars (2-Oxo-1,2-Oxaphosphacyclanes) Using the Anomeric Alkoxyl Radical Beta-Fragmentation Reaction as the Key Step. *J. Org. Chem.* **2020**, *85*, 4861-4880.
(27) Priebe, W.; Grynkiewicz, G.; Neamati, N. 2-Deoxy-1-O-Silylated-B-Hexopyranoses. Useful Glycosyl Donors and Synthetic Intermediates. *Tetrahedron Lett.* **1991**, *32*, 2079-2082.
(28) Baldoni, L.; Marino, C. Synthesis of S- and C-Galactofuranosides Via a Galactofuranosyl Iodide. Isolable 1-Galactofuranosylthiol Derivative as a New Glycosyl Donor. *Carbohydr. Res.* **2012**, *362*, 70-8.
(29) See Figure S3 for detailed analyses of the crude reaction mixtures described in Scheme 2d-e, Entries 1-4.

(30) Bock, K.; Lundt, I.; Pedersen, C. Assignment of Anomeric Structure to Carbohydrates through Geminal ^{13}c-H Coupling Constants. *Tetrahedron Lett.* **1973**, *14*, 1037-1040.
(31) Bock, K.; Pedersen, C. A Study of ^{13}ch Coupling Constants in Hexopyranoses. *J. Chem. Soc., Perkin Trans. 2* **1974**, 293-297.

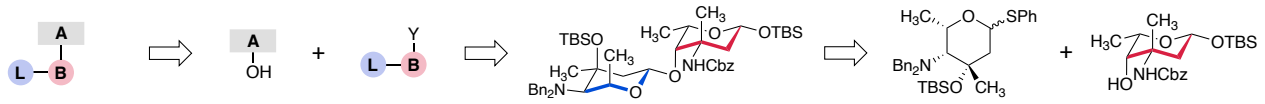
CHAPTER 3

Synthesis and Biological Evaluation of a DNR-AMA Hybrid Anthracycline

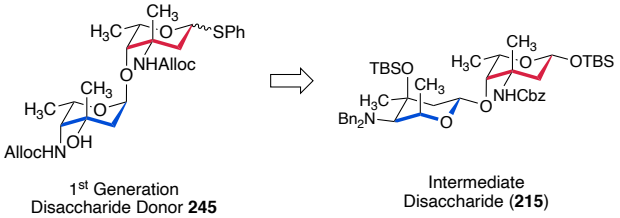
3.1 *Synthetic Strategy*

Having achieved the scalable synthesis of protected arimetamycin A disaccharide **215**, we set our sights on transforming this compound into a suitable disaccharide donor for glycosidation with aglycone acceptors (**27**–**30**) to yield arimetamycin A (**1**) and hybrid **24**–**26** (Scheme 3.1a). Success would require a strategic choice of anomeric leaving group as well as careful consideration of the C3 amine, C3′ alcohol, and C4′ amine substitution patterns. Beginning with the leaving group, we chose to pursue a phenylthioglycoside donor based on Wang's successful activation of such donors under Hirama-like conditions ($AgPF_6$)[1] to synthesize daunorubicin hybrids **42a–f** with good α-selectivity[2] (Table 1.2). Regarding the C3 and C4′ amines, we decided to delay their dimethylation until after the glycosidation for two reasons: (1) this would enable us to synthesize various *N*,*N*-substituted derivatives (i.e., NH_2, N_3) without optimizing multiple glycosidations and (2) we hypothesized that a bis-*N*,*N*-protected disaccharide would have favorable physical properties as compared to the bis-*N*,*N*-dimethyl disaccharide. We chose to mask both amines as Alloc carbamates as the deprotection of this group has been demonstrated on glycosidated anthracyclines.[3-4] Finally, as various literature precedents report successful glycosylations in the presence of unprotected 3º alcohols[2, 5-8], we elected to deprotect the C3′ alcohol prior to glycosidation to decrease the number of post-glycosidation transformations. With these considerations in mind, we identified thioglycoside **245** as our ideal 1st generation disaccharide donor (Scheme 3.1b).

a. Updated Conceptual Retrosynthetic Analysis.

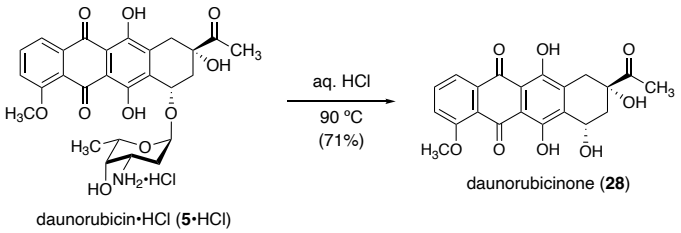


b. Retrosynthetic Analysis for **245**.



Scheme 3.1 Retrosynthetic Analysis for Disaccharide Donor **245**.

To evaluate **245** as a donor, we chose to use the daunorubicin aglycone (daunorubicinone, **28**) as our initial acceptor due to the reduced cost of daunorubicin (**5**) as compared to doxorubicin (**6**) and steffimycin B (**312**). Accordingly we prepared daunorubicinone (**28**) via the known hydrolysis of daunorubicin•HCl (**5**•HCl) in anticipation of future glycosidations (Scheme 3.2).[9]

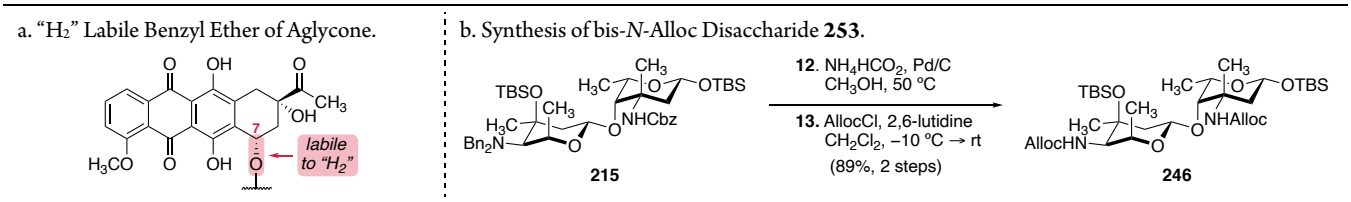


Scheme 3.2 Acid Mediated Hydrolysis of Daunorubicin (**5**) to Obtain Daunorubicinone (**28**).

3.2 *1st–3rd Generation Donors: A Post-Glycosidation Functionalization Strategy*

3.2.1 *Synthesis of Thioglycoside **245** and Intermediates **249** and **246***

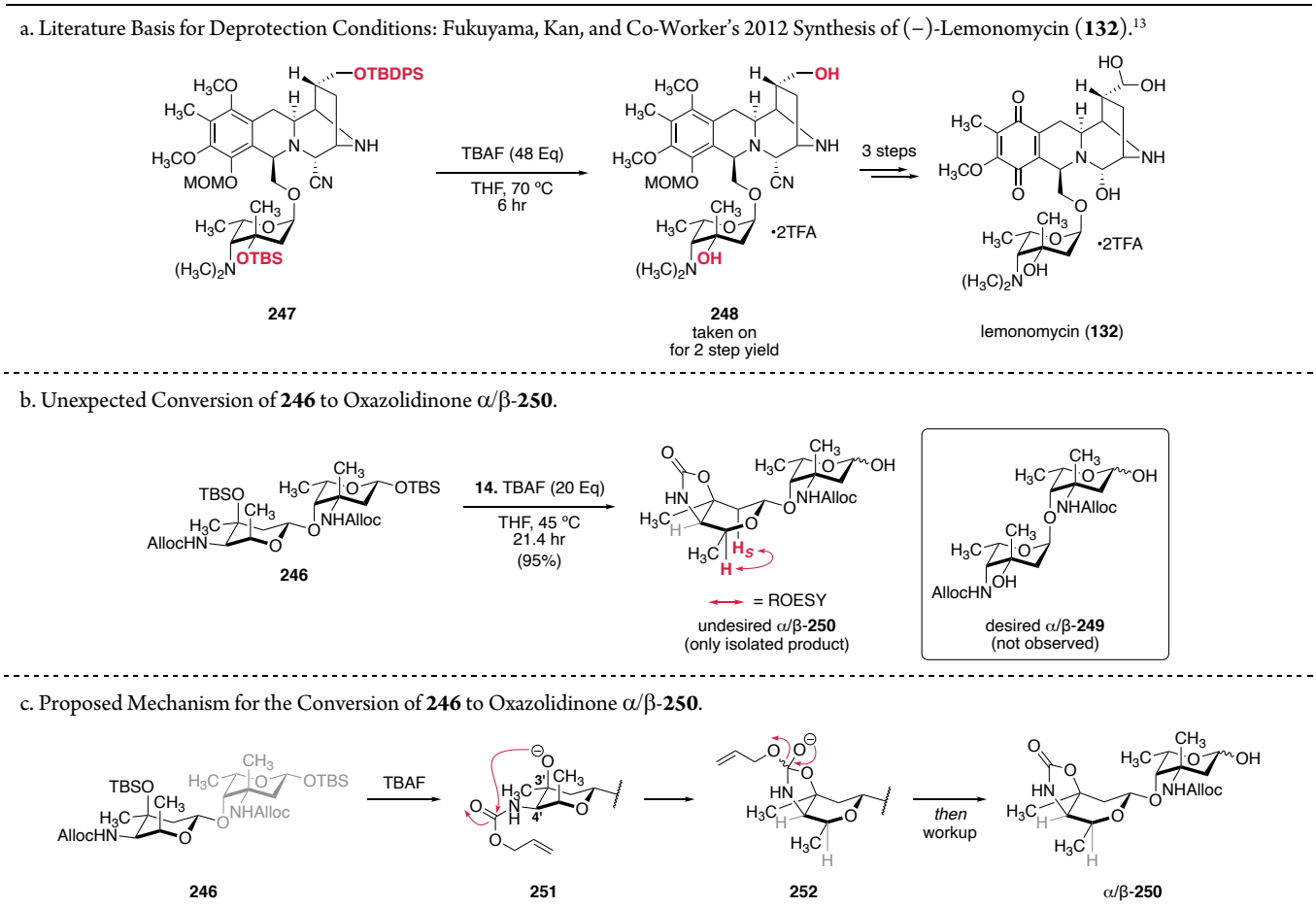
Transformation of **215** into a suitable thioglycoside donor for glycosidation with daunorubicinone acceptor **28** required swapping out the *N*-Cbz and *N*-Bn protecting groups due to the susceptibility of the aglycone's C7 benzyl ether to hydrogenolysis (Scheme 3.3a).[10-11] Strategically, we posited that performing this protecting group exchange prior to thioglycoside formation would eliminate the possibility of sulfur mediated catalyst poisoning. In the forward direction, NH_4HCO_2 mediated transfer hydrogenolysis of **215** over Pd/C yielded the presumed diamine intermediate (Scheme 3.3b).[12] Reprotection with AllocCl in the presence of 2,6-lutidine gave bis-*N*-Alloc disaccharide **246** in 89% yield over 2 steps.



Scheme 3.3 Strategic Exchange of Nitrogen Protecting Groups.

Next, we undertook the removal of both silyl protecting groups, reasoning that the resulting deprotected tertiary C3′ alcohol would not interfere in the ensuing glycosidation.[2, 5-8] Taking note of the harsh conditions employed by Fukuyama, Kan, and co-workers to effect global silyl deprotection of intermediate **247** in their synthesis of lemonomycin (**132**, Scheme 3.4a),[13] we heated disaccharide **246** at 45 °C with excess TBAF (20 Eq, Scheme 3.4b). ^{1}H NMR analysis of the resulting product showed the expected absence of silyl protecting group

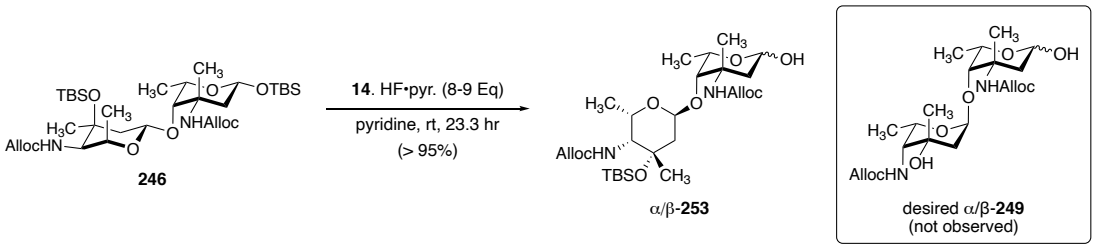
signals; however, integration of the allyl protons from the Alloc protecting groups indicated the presence of only one (rather than two) allyl. Further characterization allowed us to assign this product as oxazolidinone **250**. We hypothesize that this unexpected product arises from intramolecular attack of the liberated C3′ alkoxide at the neighboring C4′ carbamate (**251**, Scheme 3.4c). Collapse of the resulting tetrahedral intermediate **252** would yield **250** and an equivalent of allyl alcohol upon workup.



Scheme 3.4 Unexpected Synthesis of Oxazolidinone **250** from **246**.

We posited that decreasing the basicity of the reaction medium would decrease the nucleophilicity of the C3′ oxygen and thus discourage oxazolidinone formation. As such, we changed our fluoride source from TBAF (basic) to HF•pyridine (acidic); additionally, we opted to decrease the reaction temperature (45 °C → room temperature). In the event, we exposed **246** to HF•pyridine at room temperature for 23.3 hr (Scheme 3.5a). ^{1}H NMR of the purified product indicated the proper number of Alloc groups; however, the presence of remaining TBS peaks allowed us to assign the product as mono-TBS **253**. Subjection of this material to HF•pyridine, this

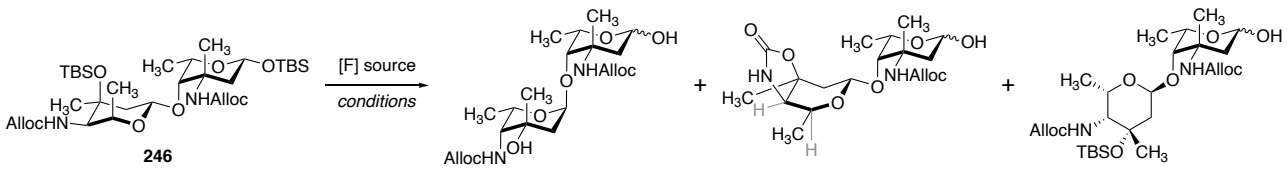
time at 50 °C, did not prove productive as TLC indicated the **253** starting material as the major spot with no substantial formation of other spots after 26 hr.[14]



Scheme 3.5 Partial De-Silylation of **246** to Give Undesired Hemiacetal **253**.

Next, we turned to AcOH buffered TBAF, hoping that the AcOH would temper the basicity of the TBAF enough to avoid oxazolidinone formation. Encouragingly, a small-scale experiment indicated that heating **246** in a 1:1 v/v solution of AcOH:TBAF (1 M in THF) at 45 °C effected bis-silyl deprotection without inducing cyclization (Table 3.1, Entry 3). However, a preparative scale up of these conditions resulted only in the isolation of mono-TBS **253** as TLC did not indicate the formation of any **249** (Table 3.1, Entry 4). While initially puzzling, we realized that order of addition was the likely cause for the discrepancy in our results. In the initial screening scale reaction, TBAF was added to the reaction flask followed 4 min later by the addition of AcOH while the scale up employed a premixed solution of AcOH buffered TBAF. As such, we hypothesized that most if not all of the reactivity responsible for removing the C3′ silyl ether could be attributed to unbuffered TBAF and that this reaction took place rapidly. When taken together with the fact that treatment with unbuffered TBAF yielded a 84:16 mixture of desired **249** to oxazolidinone **250** after 3.5 hours at room temperature (Table 3.1, Entry 5), we reasoned that exposure of **246** to unbuffered TBAF at reduced temperature might selectively give the desired **249**. This was validated as addition of chilled (−5 °C) 1 M TBAF in THF to a likewise chilled solution of **246** in THF brought about the desired bis-silyl deprotection after which the reaction was quenched at −5 °C with 1 N HCl to give desired α/β-**249** (Table 3.1, Entries 6-7).

Table 3.1 Optimization of the Bis-Silyl Deprotection of **246** to Yield α/β-**249**.



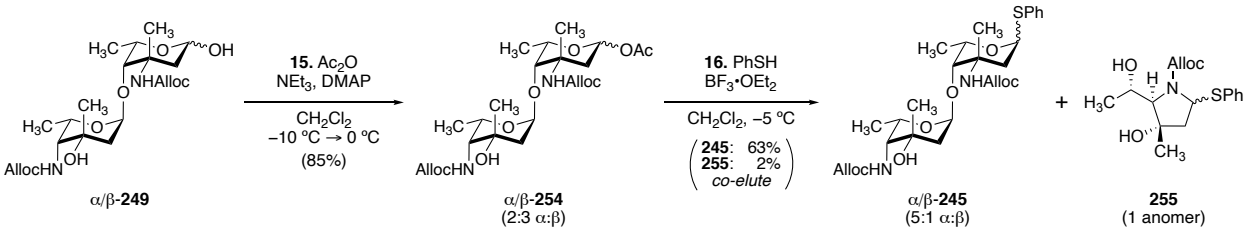
246 α/β-**249** *desired* α/β-**250** α/β-**253**

Entry	"F" Source (Eq)[a]	Solvent	Temp.	Time	Relative Composition[b] 249	250	253	Isolated Yield
1[c]	TBAF (20)	THF	45 °C	21.4 hr[d]	- - -	100%	- - -	95%
2[c]	HF•pyr. (8-9)	pyridine	rt	23.3 hr	- - -	- - -	100%	> 95%
3[e]	TBAF (20)	THF/AcOH[f]	45 °C	2.6 hr[d]	~90%[g]	~10%[g]	- - -	- - -[h]
4[c]	TBAF (20)	THF/AcOH[i]	45 °C	15 hr[d]	- - -	- - -	100%	> 95%
5[e]	TBAF (20)	THF	rt	3.5 hr	84%	16%	- - -	- - -
6[c]	TBAF (20)[j]	THF	–5 °C	30 min[k]	100%	- - -	- - -	92%
7[l]	TBAF (10)[j]	THF	–5 °C	45 min[k]	100%	- - -	- - -	84%

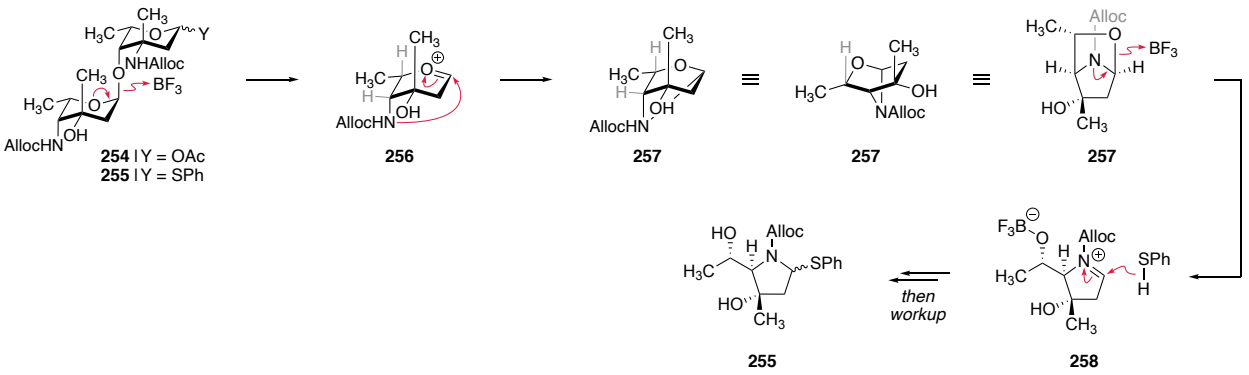
[a]Eq = equivalents; "TBAF" = commercially available solution of 1 M TBAF in THF; HF•pyr. commercially available solution of 65-70 HF in pyridine (wt/wt %). [b]Determined by ^{1}H NMR unless indicated otherwise. [c]Reaction run with 21-33 mg (30-47 μmol) **246**. [d]Reaction heated at 45 °C for indicated amount of time; then reaction allowed to cool at room temperature for 0.8-1.4 hr prior to quenching. [e]Reaction run with 5-8 mg (8-11 μmol) **246**. [f]TBAF (1 M in THF) added to **246**; AcOH added 4 min later. [g]Relative composition estimated from TLC; for this entry, only the identity of **249** was verified by isolation and NMR. [h]Yield not reported due to the small scale of the reaction. [i]Premixed solution of TBAF (1 M in THF) added to **246**. [j]TBAF chilled at –5 °C prior to addition to a chilled (–5 °C) solution of **246** in THF. [k]Reaction quenched at ca. –5 °C after indicated amount of time. [l]Reaction run with 0.22 g (0.32 mmol) **246**.

Having addressed the issue of silyl deprotection, we pressed forward toward the desired thioglycoside. Chemoselective acetylation proceeded without incident to give acyl glycoside **254** (Scheme 3.6a). Subsequent treatment of acetate **254** with PhSH and catalytic BF_3•OEt_2 yielded a co-eluting mixture of desired thioglycoside **245** (63%, 5:1 α:β) and unexpected pyrrolidine **255** (2%). This byproduct likely arises from rearrangement of the lemonose residue following Lewis acid mediated cleavage of the inter-sugar glycosidic linkage (Scheme 3.6b). As **255** constituted such a minor component of the bulk material (≥20:1 **245**:**255**), we pushed the mixture forward without further purification.

a. Synthesis of α/β-**245** from α/β-**249**.



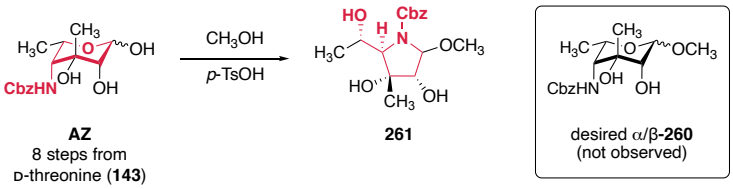
b. Plausible Mechanism for the Formation of Pyrrolidine **255**.



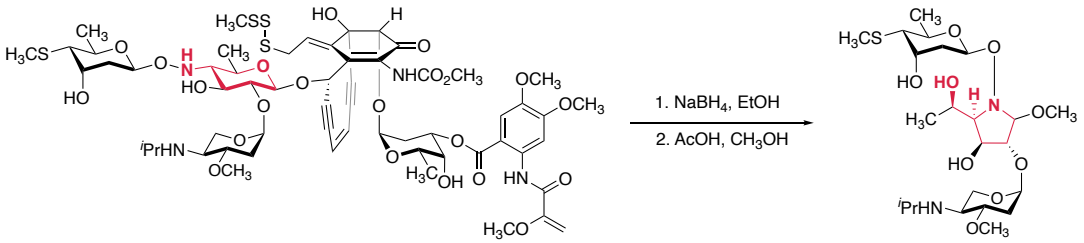
Scheme 3.6 Completion of the Synthesis of Thioglycoside **245.**

An examination of the literature reveals other examples of the pyranose to pyrrolidine rearrangement observed in the synthesis of **245** (Scheme 3.6). Evans and co-workers observed an analogous, undesired rearrangement in their attempt to convert hemiacetal **259** to methyl glycoside α/β-**260** during their synthesis of L-callipeltose (Scheme 3.7a).[15] In the context of natural products, a team at Bristol-Myer Squibb observed a pyranose to pyrrolidine rearrangement following reduction and acidic methanolysis of esperamicin A_1 (**262**) to give **263** (Scheme 3.7b).[16-17] Mellet, Fernández, and co-workers used the rearrangement to their advantage in their syntheses of pyrrolidine glycosidase inhibitors **267** and **268** from D-fructose (Scheme 3.7c).[18]

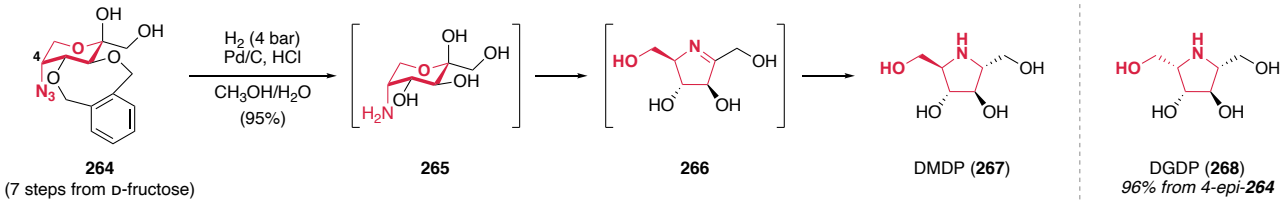
a. Inadvertent Synthesis of Pyrrolidine **261** during Evan's 2001 Synthesis of L-Callipetose.



b. Bristol-Myer Squibb's 1991 Determination of the Absolution Configuration of the Hydroxyamino Sugar Fragment of Esperamicin A_1 (**262**).

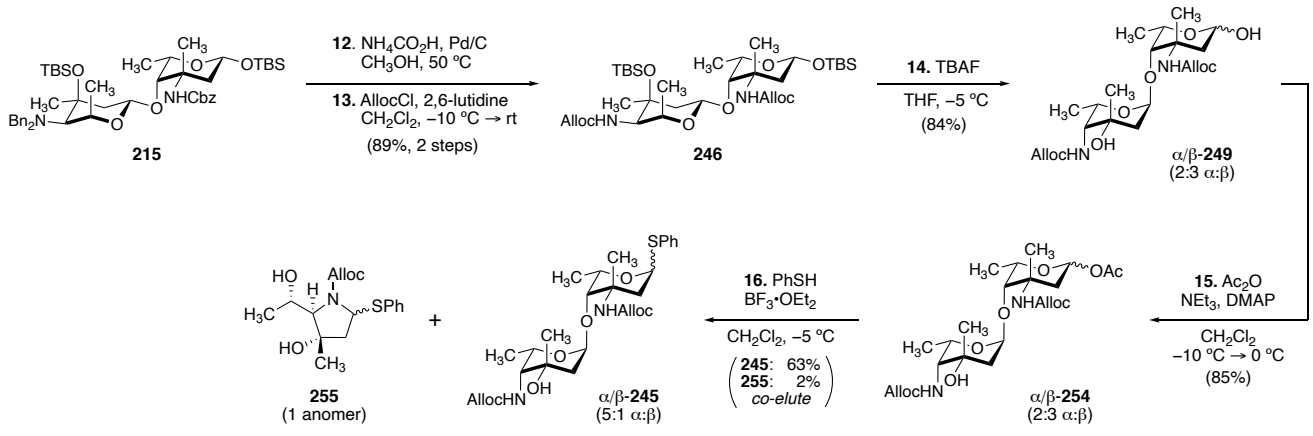


c. Mellet and Fernández's 2006 Synthesis of Glycosidase Inhibitors DMDP (**267**) and DGDP (**268**).



Scheme 3.7 Literature Examples of the 4-Aminopyranose to Pyrrolidine Rearrangement.

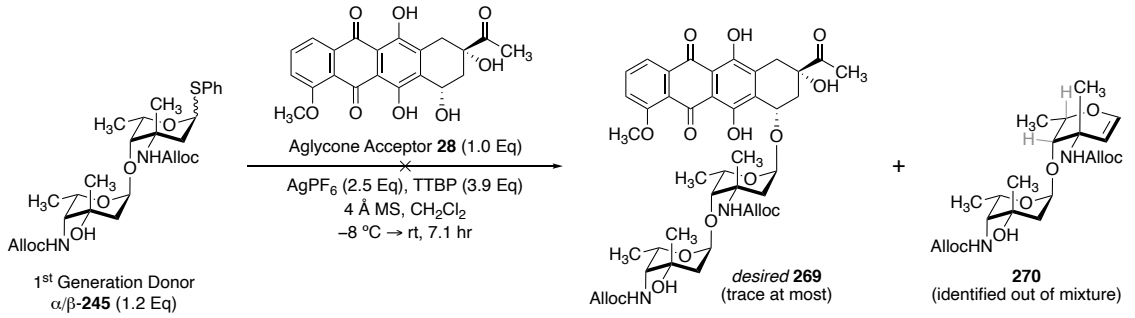
Overall, we synthesized thioglycoside donor α/β-**245** in five steps from previously described disaccharide **215** (Scheme 3.8). This involved an exchange of *N*-Cbz and *N*-Bn_2 protected groups for anthracycline-compatible *N*-Alloc groups, a *bis*-TBS ether deprotection that occurred under surprisingly mild conditions, and, following anomeric acetylation, a thioglycosidation that yielded a mixture of desired thioglycoside **245** and unexpected pyrrolidine by-product **255** (>20:1 **245**:**255**).



Scheme 3.8 Summary of the Synthesis of Thioglycoside **245** from Disaccharide **215**.

*3.2.2 1st Generation Donor (SPh): Unsuccessful Glycosidation of **245** with Daunorubicinone (**28**)*

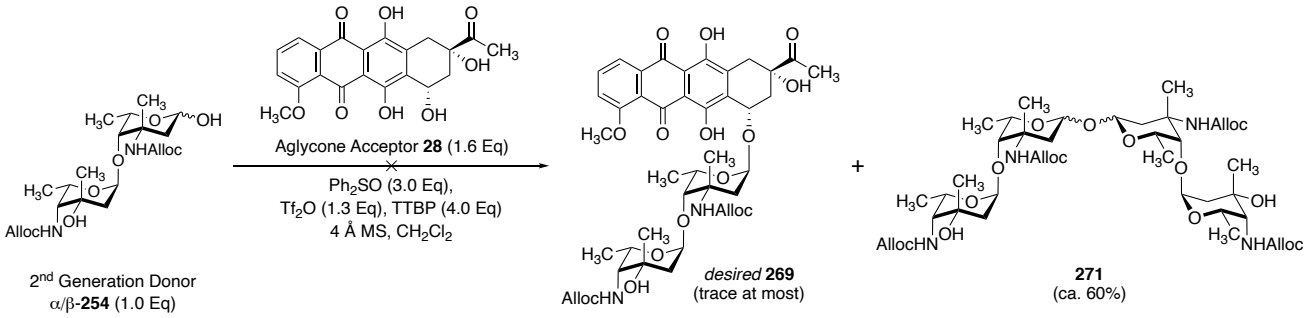
We initiated our study of anthracycline glycosidations by subjecting a mixture of daunorubicinone aglycone acceptor **28** and excess disaccharide thioglycoside donor α/β-**245** to Hirama's $AgPF_6$ medated[1] glycosidation conditions (Scheme 3.9). This reaction proved unsuccessful as we observed, at most, trace amounts of the desired product **269**. Following chromatography, the only carbohydrate component that we could confidently assign was elimination product **270**.



Scheme 3.9 Failed Glycosidation between Daunorubicinone (**28**) and 1st Generation Donor **245**.

*3.2.3 2nd Generation Donor (OH): Unsuccessful Glycosidation of **254** with Daunorubicinone (**28**)*

We next explored the use of disaccharide reducing sugar α/β-**254** as a glycosyl donor as we already had access to this intermediate from our synthesis of thioglycoside α/β-**245** (Scheme 3.8). Unfortunately, subjecting this 2nd generation donor and acceptor **28** to a modification of Gin's dehydrative methodology[19-21] provided, at most, trace amounts of desired product **269**. The major carbohydrate product that we isolated was the trehalose-like tetrasaccharide **271** resulting from dimerization of disaccharide donor **254**.

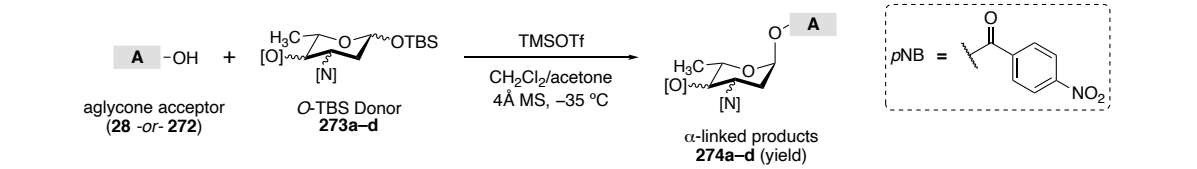


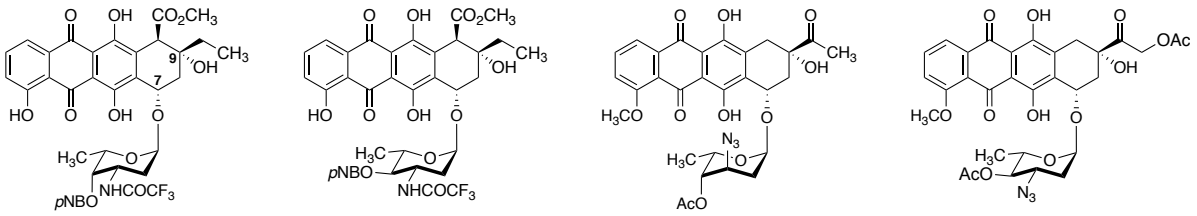
Scheme 3.10 Failed Glycosidation between Daunorubicinone (**28**) and 2nd Generation Donor **254**.

3.2.4 *3rd Generation Donor (OTBS): Successful Glycosidation of* **249** *with Daunorubicinone (*28*)*

Moving one step further back in our synthesis of thioglycoside α/β-**245** (Scheme 3.8), we decided to investigate the use of *O*-TBS glycoside **246** as a glycosyl donor. Typically, carbohydrate chemists view the anomeric silyl ether as a temporary protecting group rather than as an activating group for glycosylation. However, we noted various literature precedents that demonstrated the successful glycosidation of *O*-TBS 3-amino-2,3,6-trideoxy glycosides (**273a-d**) with anthracycline aglycones (**28**, **272a-c**) to yield the corresponding α-linked products (**274a-d**) (Table 3.2).[22-25] As such, we began our evaluation of **246** as a glycosyl donor.

Table 3.2 Literature Examples of Successful Glycosidation of *O*-TBS Donors 273**a-d** with Various Anthracycline Aglycones (**28, 272a–c**) to Yield **274a**[22], **274b**[23], **274c**[24], and **274d**[25].



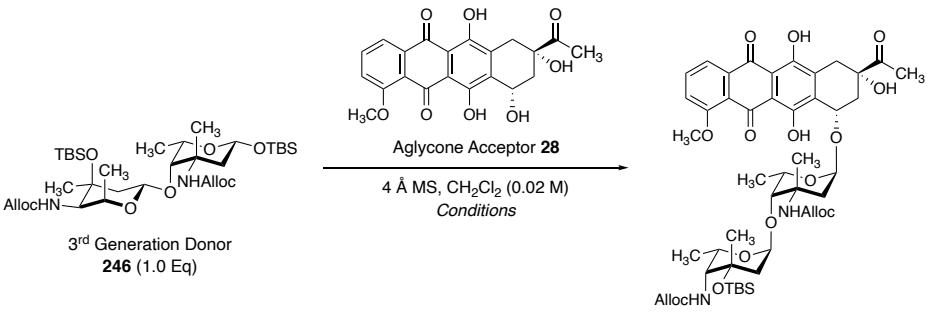


[a]5% bis-*O*-7,9-glycoside also isolated. [b]Co-eluted with ca. 12% bis-glycoside. [c]Yield obtained over 2 steps following deacetylation (K_2CO_3/CH_3OH).

In an orienting experiment, we treated a mixture of daunorubicinone (**28**), donor **246**, and 4 Å MS in CH_2Cl_2 with TMSOTf (Table 3.3, Entry 1). TLC indicated the formation of a red spot with decreased polarity as compared to aglycone **28**. This was promising as the red color suggested the presence of the anthracycline aglycone while the change in polarity implied an alteration in the aglycone's structure. However, NMR analysis of the isolated material proved challenging and did not allow us to make any structural conclusions regarding the newly synthesized product(s). Gratifyingly, we found that changing the Lewis acid to $BF_3 \bullet OEt_2$ and lowering the temperature to −78 °C allowed us to isolate desired α-linked product **275** for the first time (Table 3.3, Entry 2). Though the reaction yielded only 12% of **275**, we found the 81% recovery of unreacted donor **246** encouraging as this indicated that **28** efficiently captured any donor able to undergo activation (88% yield of **275** BRSM). To

increase the proportion of activated donor, we raised the temperature of subsequent reactions following initial addition of $BF_3 \cdot OEt_2$ at −78 °C (Table 3.3, Entries 3–4). These efforts allowed us to increase the yield of **275** to 59% at −50 °C while maintaining good efficiency for the capture of activated donor by acceptor **28** (90% yield of **275** BRSM).

Table 3.3 Optimization of the Glycosidation of Daunorubicinone (**28**) with 3rd Generation Donor **246.**



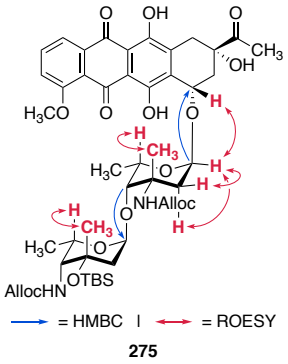
275

Entry	Promoter (Eq)[a]	Eq **28** (Acceptor)[a]	Temp.	Time	Quench	Isolated Yield **246** (Donor)[b]	Isolated Yield **275** (Product)[c]
1[d]	TMSOTf (2.8)	1.0	-40 °C → -30 °C	5.2 hr	CH_3OH	0%[e]	- - -[f]
2[g]	$BF_3 \cdot OEt_2$ (1.5)	2.2	-78 °C	2.8 hr	pyridine	81%	12% [88%]
3[g]	$BF_3 \cdot OEt_2$ (1.6)	2.0	-78 °C → -60 °C[h]	6.5 hr	pyridine	63%	35% [95%]
4[g]	$BF_3 \cdot OEt_2$ (1.5)	2.0	-78 °C → -50 °C[h]	6.5 hr	pyridine	35%	59% [90%]

[a]Eq = equivalents. [b]Recovered, unreacted donor **246**. [c]Yield of product (**275**) based on recovered donor (**246**) given in square brackets. [d]Run with 15 mg (21 μmol) **246**. [e]Estimated based on TLC of crude reaction mixture. [f]A red spot less polar than **28** was isolated; however, NMR indicated that material was a complex mixture. [g]Reaction run with ca. 50 mg (71 μmol) **246**.

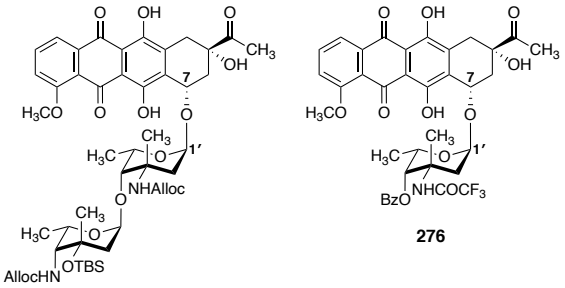
Structurally, 2-D NMR verified that glycosidation had occurred at the benzylic C7OH of aglycone **28** (HMBC: H1′ → C7; ROESY: H7 ↔ H1′) and indicated that both carbohydrate moieties adopted the canonical $^1C_4$ conformation (ROESY: C3′Me ↔ H5′ and C3″Me ↔ H5″) (Figure 3.1a). We assigned C7O-C1′ as an α-linkage based on ^{1}H multiplicity (absence of a large $^3J_{H1'\text{-}H2'}$ coupling constant indicative of an axial-axial coupling pathway)[26] and ROESY (presence of H1′ ↔ $H2'_{pro\text{-}R}$ and H1′ ↔ $H2'_{pro\text{-}S}$ correlations; absence of H1′ ↔ C3′Me and H1′ ↔ H5′ correlations) data. Comparison of ^{1}H (H7, H1′) and ^{13}C (C7, C1′) chemical shifts to those of previously described daunorubicin analog **276** further corroborated this anomeric assignment (Figure 3.1b).[27]

a. Key NMR Data for **275**.



275

b. ^{1}H and ^{13}C Chemical Shift Comparison between **275** and Known Compound **276**.



275 **276**

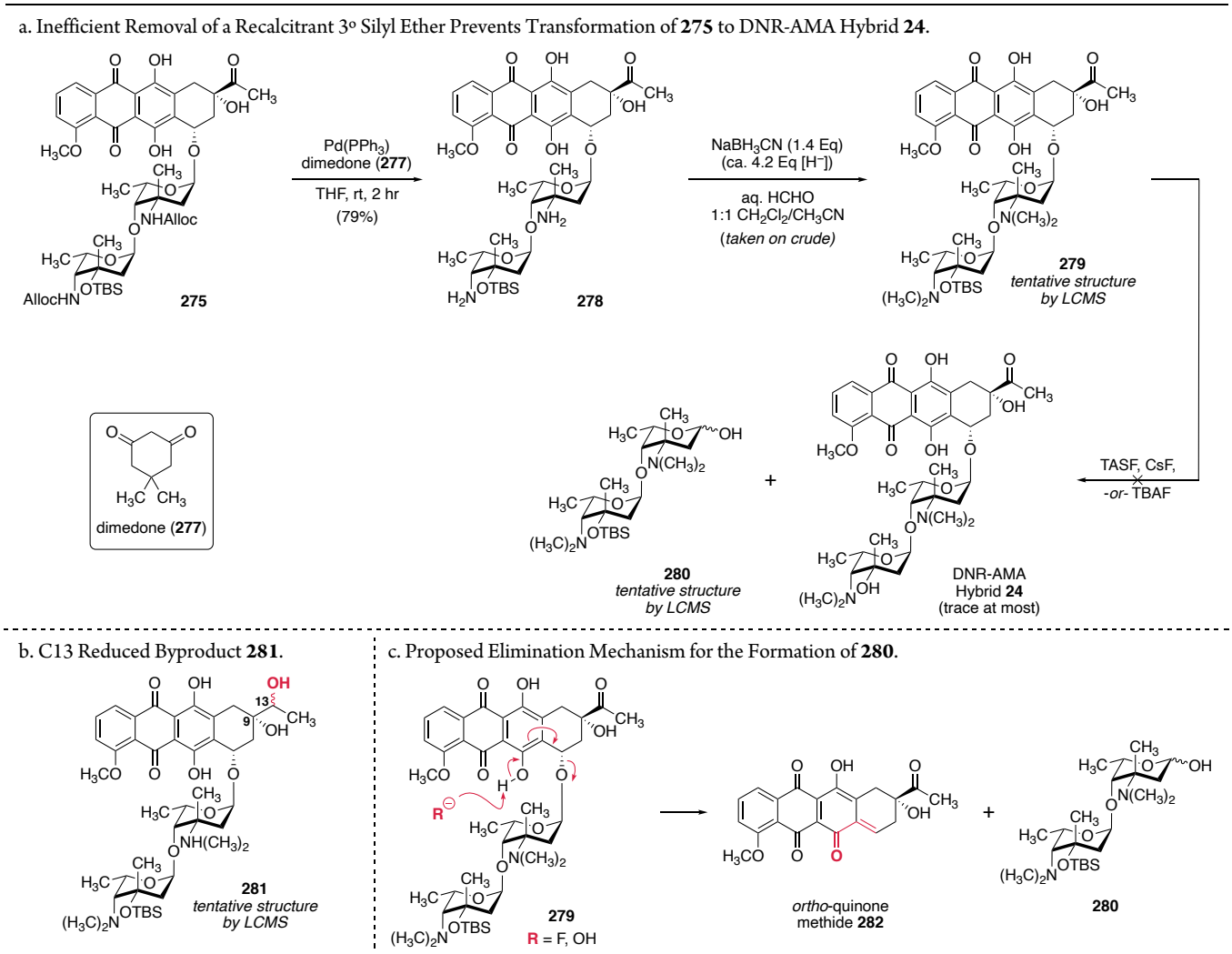
Compound	^{1}H NMR (δ)		^{13}C NMR (δ)	
	H7	H1′	C7	C1′
275[a]	5.23 (ovlp)[b-c]	5.52 (br d)[d]	69.4[b]	100.6[b]
276[e]	5.25 (br s)	5.50 (br s)	69.4	100.3

Figure 3.1 Key NMR Data Demonstrating the Synthesis of α-Linked **275**. [a]Data acquired in $CDCl_3$ at 600 MHz for ^{1}H (calibrated to δ 7.26) and 151 MHz for ^{13}C (calibrated to δ 77.16). [b]Shift obtained from ^{1}H-^{13}C HSQC {^{1}H}{^{13}C}. [c]Ovlp = overlapping. [d]J = 4.9 Hz. [e]Data acquired in $CDCl_3$ at 60 MHz or 90 MHz for ^{1}H (calibrated to $Si(CH_3)_4$) and 23 MHz for ^{13}C (calibrated to $Si(CH_3)_4$) by Thang, T. T. et al.[27]

3.2.5 *End Game: Unsuccessful Transformation of* **275** *to DNR-AMA Hybrid* **24**

At this point, three transformations stood between **275** and DNR-AMA hybrid **24**: (1) *N,N*-Alloc deprotection, (2) *N,N*-bis-dimethylation, and (3) TBS deprotection. Electing to reserve silyl deprotection for the final step as to take advantage of the protecting group's solubilizing lipophilicity for as long as possible, $Pd(PPh_3)_4$ catalyzed Alloc deprotection in the presence of dimedone (**277**) as an allyl scavenger afforded diamine **278** (Scheme 3.11a).[3] Subjecting this material to an orienting reductive amination (aq. HCHO, $NaBH_3CN$) demonstrated the liability of the C13 α-hydroxy ketone to reduction as LCMS showed the formation of masses corresponding to desired product **279** and C13 reduced **281** (Scheme 3.11b). This aberration from the typical resistance of ketones to $NaBH_3CN$ reduction under non-acidic conditions[28] likely results from coordination of a boronate species to the C9-hydoxyl and subsequent intramolecular hydride delivery. Klyosov[29] and Sjöberg[30] each observed similar C13 reduction during the reductive amination of daunorubicin with glucose/galactose and benzylic aldehydes, respectively. As Borch and co-workers' seminal work with $NaBH_3CN$ established that all three hydrides of $NaBH_3CN$ are viable reductants (i.e., 1 Eq of $NaBH_3CN$ yields ca. 3 Eq of $[H^-]$)[28], we reran the reaction using just 1.4 Eq $NaBH_3CN$ (ca. 4.2 Eq $[H^-]$ to effect 4 imminium reductions). Pleasingly, we obtained **279** with minimal amounts of **277** as judged by LCMS. Taking this material on crude, we subjected **279** to various desilylating conditions (TASF(Me), TBAF, CsF) but found that formal hydrolysis of the anthracycline-disaccharide linkage to give **280** occurred more readily than deprotection of the tertiary TBS ether as indicated by LCMS analysis. We posit that this bond breaking event could occur via traditional H_2O mediated glycosidic

hydrolysis or through an elimination pathway involving the formation of orthoquinone methide **282** (Scheme 3.11c).



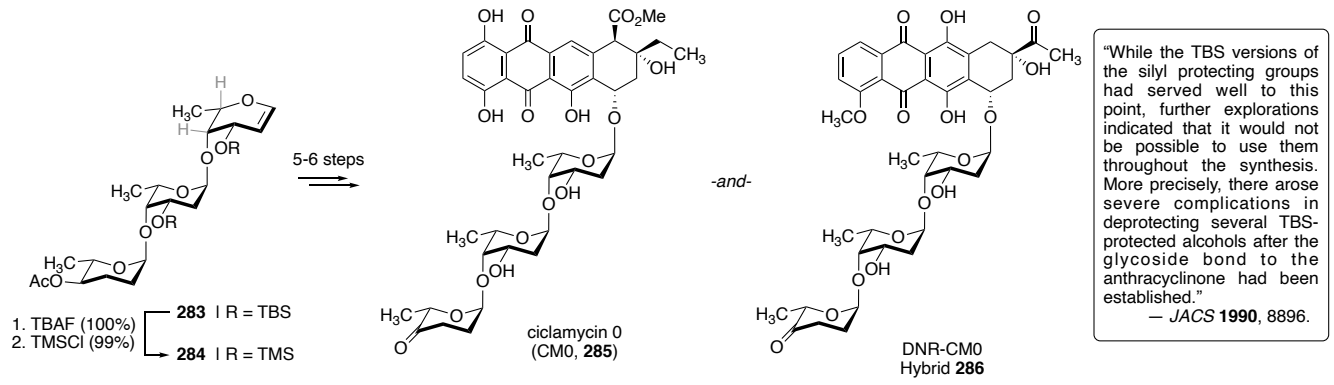
Scheme 3.11 Failed Conversion of Protected Anthracycline **275** to DNR-AMA Hybrid **24**.

3.3 4th–5th Generation Donors: A Pre-Glycosidation Functionalization Strategy

*3.3.1 4th Generation Donor (OTBS): Rational Design, Synthesis, and Successful Glycosidation to Yield DNR-AMA Hybrid **24***

Efforts to overcome the problematic TBS deprotection of **279** (Scheme 3.11a) by removing the silyl ether prior to the amine deprotection/methylation sequence or employing a donor analogous to **246** with an unprotected 3º alcohol proved unsuccessful. Interestingly, Danishefsky, Sulikowski, and co-workers also experienced difficulties when trying to unmask 2º TBS ethers on anthracycline bound sugars (Scheme 3.12).[31] They overcame this issue by swapping the TBS groups for more labile TMS ethers prior to glycosidation.

However, we had misgivings regarding our ability to deprotect a presumably more resistant 3° TMS ether in an anthracycline compatible manner.



Scheme 3.12 TBS Deprotection Difficulties Encountered by Danishefsky, Sulikowski, and Co-Workers in their Synthesis of Ciclamycin 0 (**285**) and Hybrid Anthracycline **286**.

In designing our 4th generation donor, we decided to sidestep any post-glycosidation manipulations. As such, we designed fully functionalized donor **287** featuring an unprotected 3° alcohol and two dimethylamines (Figure 3.2). For the anomeric leaving group, we retained the TBS ether from the 3rd generation donor due to its previously demonstrated success. Furthermore, we posited that the lipophilic silyl group would counteract the anticipated polarity of the two dimethyl amino sugars, easing purification and allowing us to conduct the glycosidation in standard organic solvents.

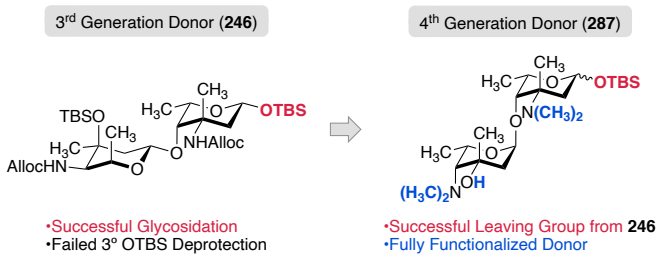
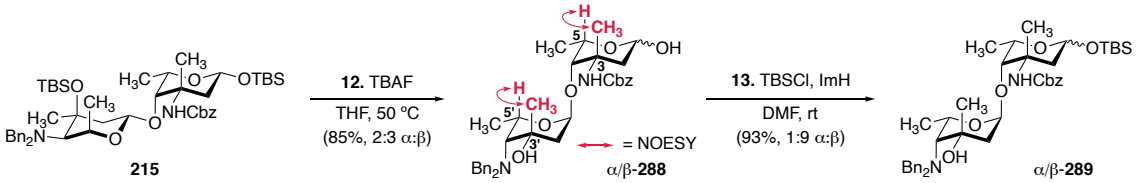


Figure 3.2 Rational Design of 4th Generation Donor **287**.

Synthesis of 4th generation donor **287** began with the bis-silyl deprotection (TBAF, 50 °C)[13] of disaccharide **215** followed by selective anomeric re-silylation (TBSCl) to give α/β-**289** (1:9 α:β, Scheme 3.13). Conformationally, we observed that removal of **215**'s silyl ether protecting groups allowed the lemonose residue of α/β-**288** to assume the canonical $^1C_4$ conformation. It maintained this conformation upon anomeric re-

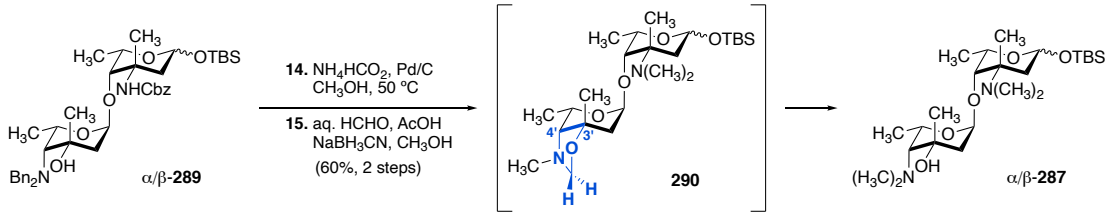
silylation, indicating that the bulky C3′-*O*-TBS group was responsible for the $^{1}C_{4}$ to $^{4}C_{1}$ chair flip observed in **215**.



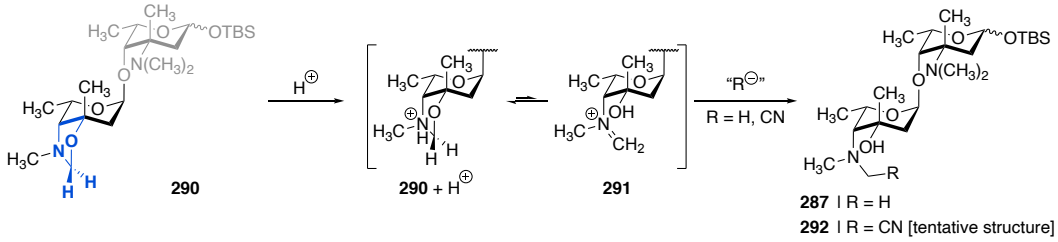
Scheme 3.13 Synthesis of *O*-TBS Glycoside **289** from Disaccharide **215**.

Next, transfer hydrogenolysis (NH_4HCO_2, Pd/C) of α/β-**289** unmasked both primary amines,[12] setting the stage for bis-dimethylation (Scheme 3.14a). To our surprise, reductive amination (aq. HCHO, $NaBH_3CN$)[28] gave a mixture featuring oxazolidine **290** as its major component (Figure A.6). Presumably, ring closure occurs via intramolecular capture of a C4′N-imine(iminium) species by C3′OH. This oxazolidine proved resistant to further reduction, opening fully only with the use of acid (AcOH) and excess reductant to give donor β-**287**. Under these forcing conditions we also observed formation of a side product resulting from cyanide addition (tentatively assigned as **292**) that could be separated from the desired β-**287** by preparatory HPLC (Scheme 3.14b, Figure A.7).[32]

a. Synthesis of β-**287** from α/β-**289**.



b. Proposed Formation of Desired of **287** and Tentatively Assigned Side-Product **292** from Oxazolidine **290**.



Scheme 3.14 Completion of 4th Generation Donor β-**287**.

We are the not the first to inadvertently synthesize oxazolidines such as **290** while trying to effect the *N*,*N*-dimethylation of a *syn*-1,2-amino alcohol. For example, Nelson noted that methylation of **293** under Eschweiler-

Clarke conditions yielded oxazolidine **295** rather than the desired *N,N*-dimethylamino **294** (Figure 3.3a).[33] Subsequent treatment of the **295** with $LiAlH_4$ opened the oxazolidine ring to give **294**. Work by Tomita and co-workers demonstrated the necessity of a *syn*-1,2-amino alcohol for oxazolidine formation (Figure 3.3b).[34] They found that Eschweiler-Clarke reductive methylation of *anti*-1,2-amino alcohol **296** yielded *N,N*-dimethylamino **297** while exposure of the epimeric *syn*-1,2-amino alcohol (4-*epi*-**296**) to the same conditions produced oxazolidine **298**. In similar fashion to Nelson, $LiAlH_4$ reduction of **298** gave *N,N*-dimethylamino **299**.

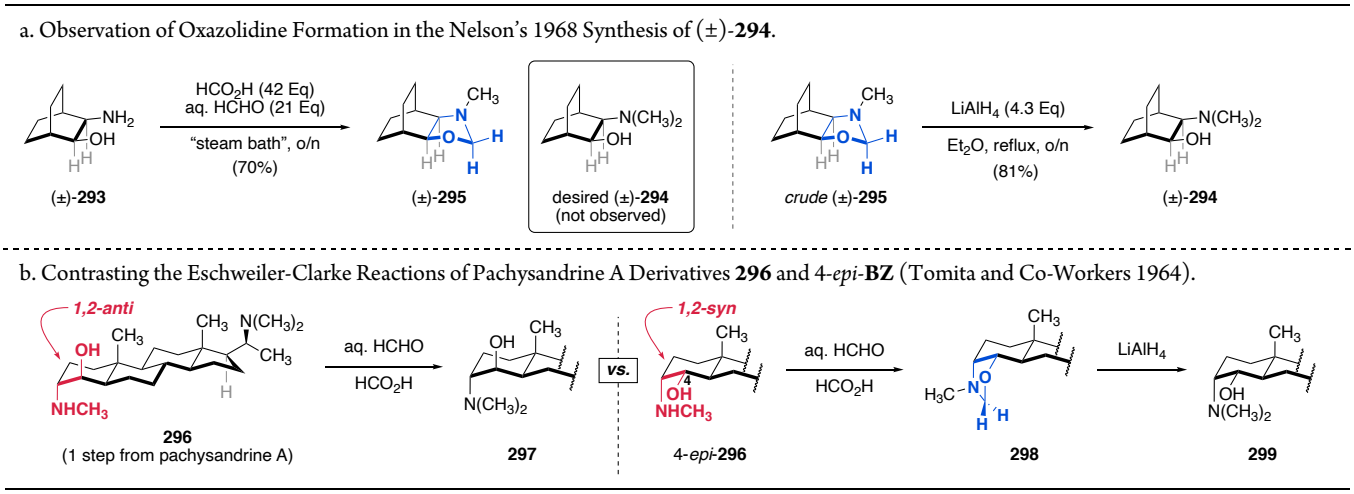
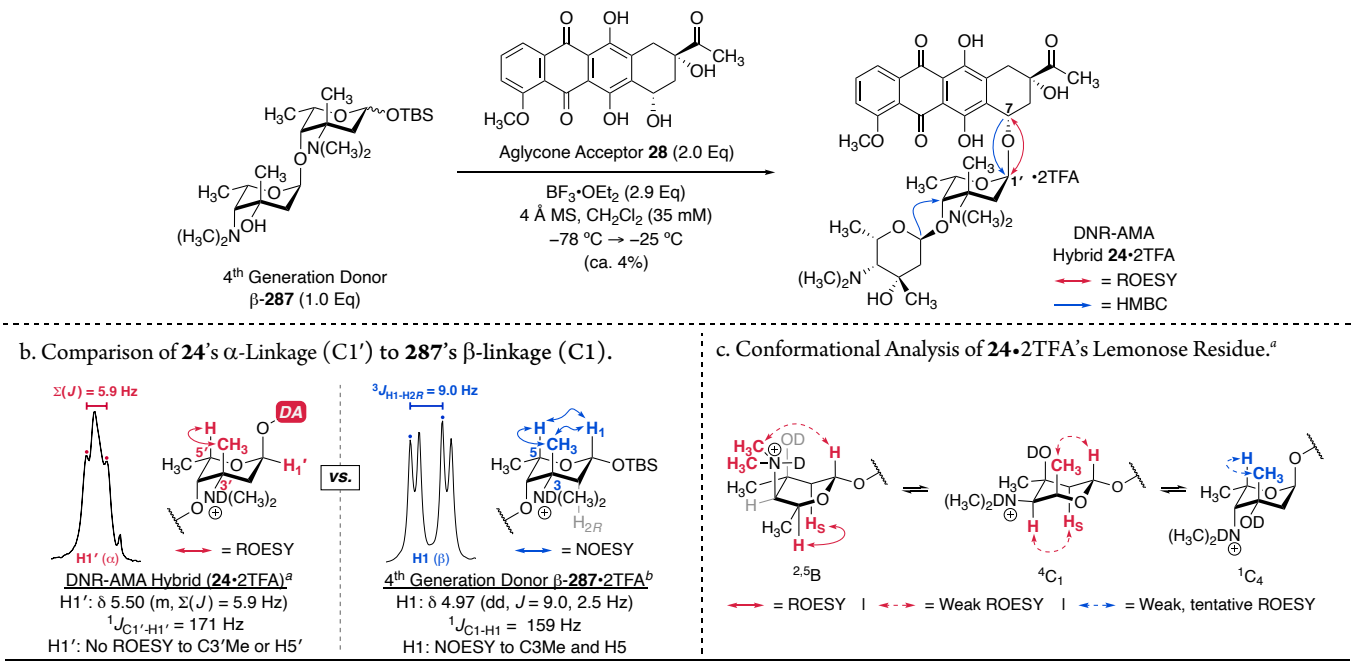


Figure 3.3 Precedent for Oxazolidine Formation with *syn*-1,2-Amino Alcohols.

Moving forward to the key event, $BF_3{\bullet}OEt_2$ mediated glycosidation of 4th generation donor β-**287** with excess **28** provided a mixture of the desired DNR-AMA hybrid **24** and unactivated **287** (Scheme 3.15a). Normal- and reverse-phase separation proved ineffective at resolving this mixture. Gratifyingly, we could leverage the phenols unique to **24** to separate the compounds by anion exchange chromatography. Following RP-HPLC purification to remove trace impurities, we isolated pure DNR-AMA hybrid (**24**•2TFA) with the desired α-C7O-C1′ linkage as verified by ^{1}H multiplicity (absence of a large $^3J_{H1'\text{-}H2'}$ coupling constant indicative of an axial-axial coupling pathway)[26], $^1J_{C1'\text{-}H1'}$ (170 Hz)[35-36], and ROESY (absence of H1′ ↔ C3′Me and H1′ ↔ H5′ correlations) (Scheme 3.15b, Table A.4, Table A.5, and Table A.6). Interestingly, ROESY analysis revealed that the lemonose residue of **24**•2TFA does not solely occupy the $^4C_1$ conformation (Scheme 3.15c); rather it exists in conformational equilibrium and/or an intermediate conformation not depicted in Scheme 3.15c.



Scheme 3.15 Synthesis, α-Linkage Verification, and Configurational Analysis of DNR-AMA Hybrid **24**•2TFA. [a]Recorded in CD_3OD (^{1}H-600 MHz, ^{13}C-151 MHz). [b]Recorded in 4:1 $CD_3OD/CDCl_3$ (w/w) + CF_3CO_2H (^{1}H-600 MHz, ^{13}C-151 MHz).

3.3.2 *5th Generation Donor (OAcyl): Rational Design, Synthesis, and Successful Glycosidation to Yield DNR-AMA Hybrid* **24** *in Improved Yield*

At this point, we had isolated a sufficient quantity of **24**•2TFA (1.8 mg) for initial biological evaluation (*vide infra*). Nevertheless, we found ourselves synthetically unsatisfied with the low yielding (ca. 4%) final transformation (Scheme 3.15a). We hypothesized that increasing the reactivity and lipophilicity of the disaccharide donor would, respectively, enable greater conversion of donor to product and ease purification, both of which would increase the isolated yield of **24**•2TFA. To this end we designed 5th generation donor **300** featuring a novel anomeric leaving group (Scheme 5e). We hypothesized that Lewis acid mediated activation would occur more readily for an acyl donor[37-40] than it would for the corresponding silyl ether. However, we also reasoned that the silyl group of **287** had lent lipophilicity to an otherwise polar disaccharide and thereby imparted it with solubility in organic solvent. Joint consideration of these factors led to the design of a novel acyl glycoside leaving group functionalized with a highly lipophilic, UV active TBDPS protected phenol.

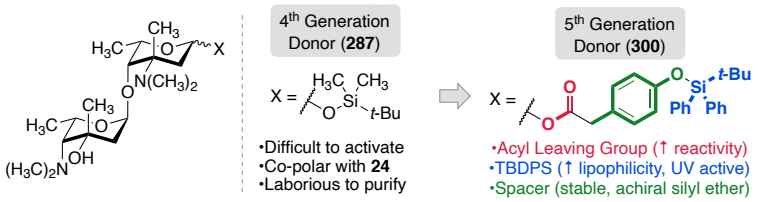
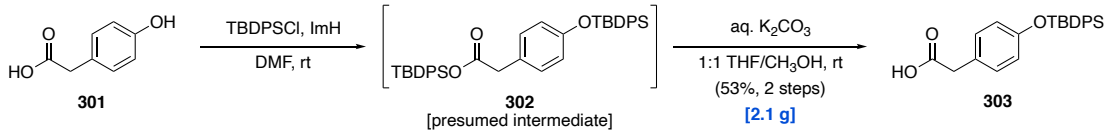


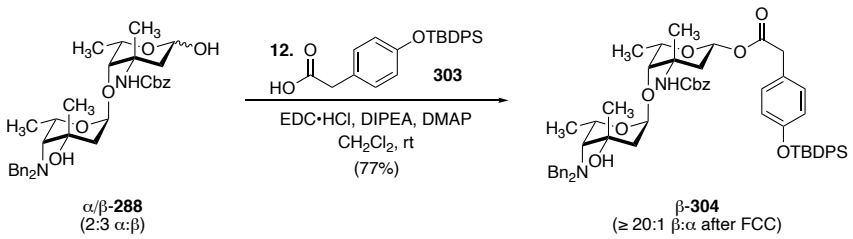
Figure 3.4 Rational Design of 5th Generation Donor **300**.

Synthesis of 5th generation donor **300** began with the synthesis of the requisite carboxylic acid **303**. Treatment of commercially available 4-hydroxyphenylacetic acid (**301**) with excess TBDPSCl (2.2 Eq) yielded the presumed *bis*-TBDPS intermediate **302** which underwent chemoselective TBDPS ester hydrolysis (aq. K_2CO_3) to give **303** (Scheme 3.16a).[41] Next, Steglich esterification (EDC•HCl, DMAP)[42] of previously synthesized diol α/β-**246** with **303** yielded acyl glycoside **304** (Scheme 3.16b). Though the reaction yielded both anomers, we elected to isolate the major β-anomer (77% yield) and move forward with anomerically pure material (≥ 20:1 β:α). Presumably, the preferential formation of the β-anomer results from its increased nucleophilicity as compared to the corresponding α-anomer due to the kinetic anomeric effect.[43-47]

a. Synthesis of Carboxylic Acid **303**.



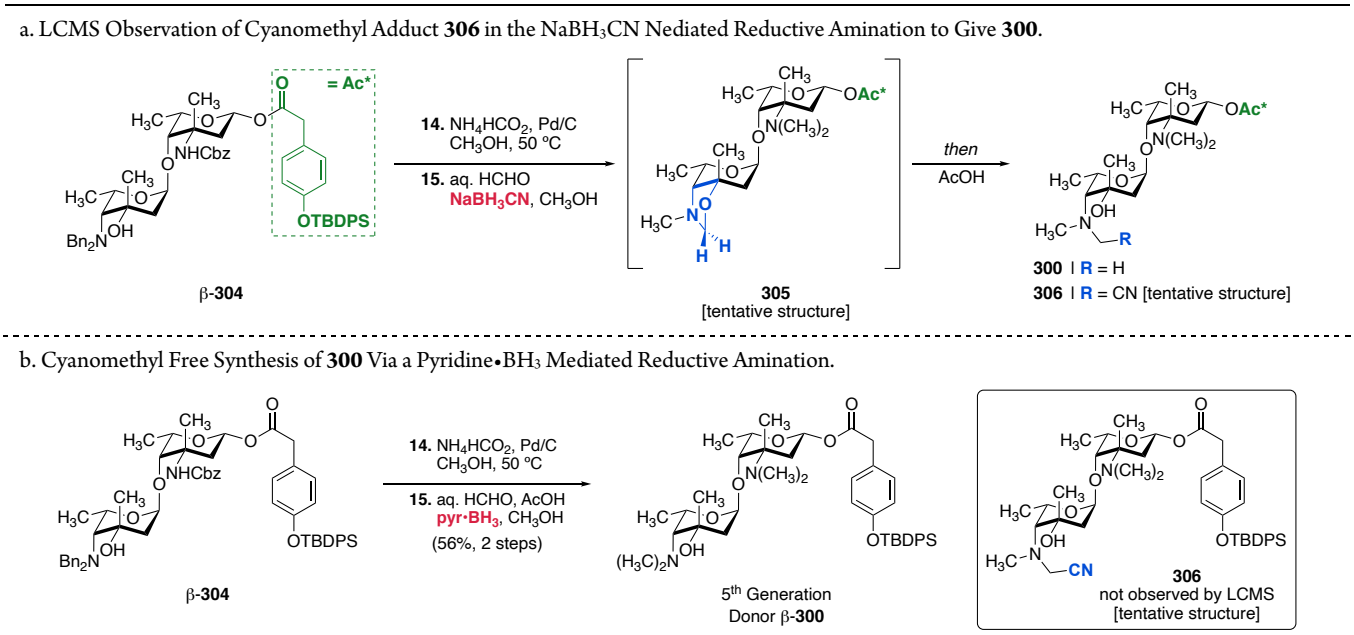
b. Acylation of α/β-**288** with **303**.



Scheme 3.16 Installation of a Novel Lipophilic, UV Active Acyl Group.

Transfer hydrogenolysis (NH_4HCO_2, Pd/C)[12] of **304** unmasked the protected amines (Scheme 3.17a). Subsequent reductive amination (aq. HCHO, $NaBH_3CN$, AcOH) proceeded in a similar manner as that detailed for the synthesis of β-**287**. Specifically, we once again observed the formation of a tentatively assigned oxazolidine (**305**) that opened to give a mixture of the desired bis-dimethylamino compound (**300**) and a tentatively assigned methylcyano adduct (**306**). Hoping to avoid cyanide addition, we took note of work from the Feeney lab

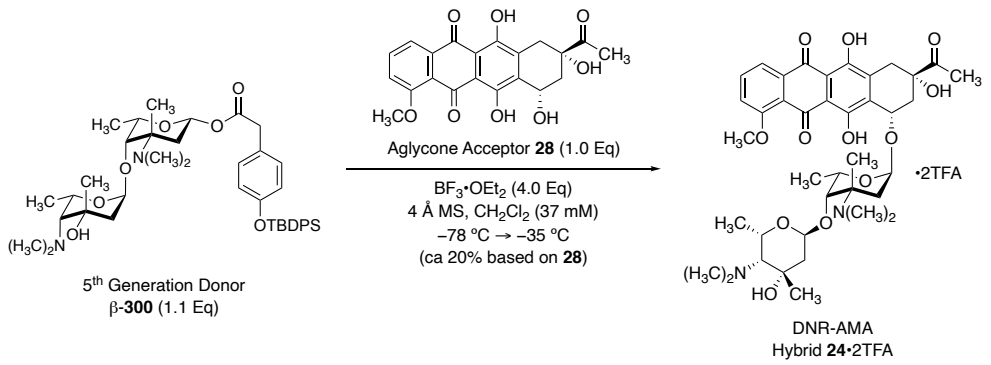
demonstrating the use of pyridine-borane to affect the *N,N*-dimethylation of proteins.[48-49] Gratifyingly, use of this reducing agent afforded desired **300** free of **306** (Scheme 3.17b).



Scheme 3.17 Completion of the Synthesis of 5th Generation Donor **300**.

Overall, we synthesized 5th generation donor β-**300** in 3 steps from previously described diol α/β-**288** and carboxylic acid **303** (2 steps from commercially available **301**). By changing the reducing agent for the reductive amination from $NaBH_3CN$ to pyr.•BH_3 we avoided the formation of cyanomethyl adduct **306** (Scheme 3.17). Importantly, comparison of LCMS retention times confirmed our hypothesis that β-**300** would exhibit increased lipophilicity as compared to 4th generation donor **287** and DNR-AMA hybrid **24** (Figure A.8 vs. Figure A.9).

Turning our attention to the glycosidation between 5th generation donor β-**300** and aglycone acceptor **28**, we began with a series of small-scale reactions (5–9 μmol donor β-**300** as limiting reagent). In the key orienting experiment, LCMS monitoring indicated that addition of 3.0 Eq of BF_3•OEt_2 produced only trace amounts of desired product **24**, even after 2 hr at room temperature. However, upon the addition of an extra 1.1 Eq of BF_3•OEt_2, we observed significant amounts of **24** within 25 min. Transferring these conditions to a preparative scale reaction (73 μmol β-**300**) yielded DNR-AMA hybrid **24**•2TFA in a modest ca. 20% isolated yield, a five-fold increase as compared to 4th generation donor β-**287** (Scheme 3.18, Table A.6).



Scheme 3.18 Glycosidation of 5th Generation Donor β-**300** with Daunorubicinone (**28**) to yield DNR-AMA Hybrid **24**•2TFA.

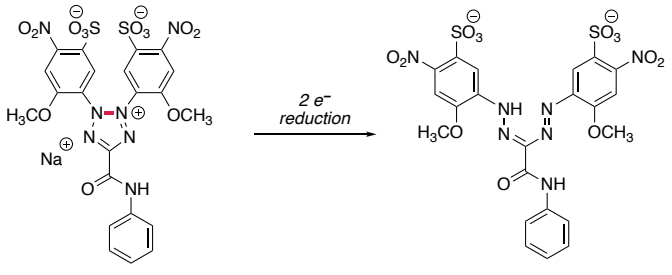
3.4 *Biological Evaluation of DNR-AMA Hybrid **24***

Having achieved the synthesis of DNR-AMA hybrid **24**, we next undertook its preliminary biological characterization. We began with an evaluation of its cytotoxicity using proliferation assays performed by ImQuest Biosciences (CRO) (Section 3.4.1). We followed this up with preliminary mechanistic experiments in collaboration with Vanderbilt Biochemistry Professor Neil Osheroff, an expert in the study of topoisomerase II enzymes; Jo Ann Byl, a senior scientist in Professor Osheroff's lab, performed all of the assays described in Section 3.4.2.

3.4.1 *Cytotoxicity Evaluation*

Cytotoxicity was evaluated using the XTT assay (Scheme 3.19).[50-54] In brief, this colorimetric assay capitalizes on the reduction of colorless/faint yellow tetrazolium XTT (**312**) to bright orange tetrazine **27** by cell-based reductants (Scheme 3.19a). Due to the molecule's net negative charge, it is hypothesized that it does not enter cells. As such, the addition of an electron-coupling agent such as phenazine methosulfate (PMS, **311**) allows for efficient electron shuttling from the intracellularly produced reductant to the extracellular XTT (Scheme 3.19b–c). As only live cells generate the reducing power necessary to reduce **312** to the bright orange **27**, spectrophotometric quantification allows for determination of viability as compared to a negative control.

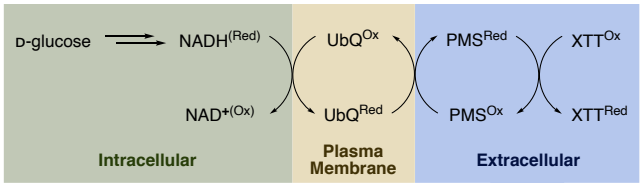
a. Color Generating Reduction of XTT.



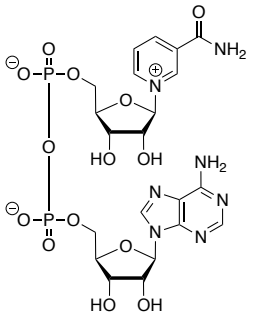
XTT^{Ox} (**307**)
Colorless/Faint Yellow

XTT^{Red} (**308**)
Bright Orange

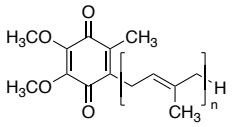
b. Redox Cascade Leading to XTT Reduction.[a–b]



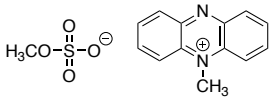
c. Structure of Oxidized Components of Redox Cascade.



NAD$^{+(Ox)}$ (**309**)



UbQ^{Ox} (**310**)



PMS^{Ox} (**311**)

Scheme 3.19 Chemical Background to the XTT Cell Viability Assay. [a]Adapted from Berridge, M. V. et al.[52] and ATCC's XTT Cell Proliferation Assay Kit Instruction Manual[53]. [b]"Ox" = oxidized; "Red" = reduced.

We evaluated DNR-AMA hybrid **24** against three of the four cancer cell lines used by Brady to determine the cytotoxicity of arimetamycin A (AMA, **1**).[55-56] We found that the synthesized hybrid demonstrated similar potency (TC_{50}) to the therapeutically useful daunorubicin (DNR, **5**) and doxorubicin (DOX, **6**) against anthracycline sensitive cell lines HCT116 (colon cancer) and MDA-MB231 (breast cancer) (Table 3.4). Furthermore, in similar fashion to arimetamycin A, DNR-AMA hybrid **24** maintained its sub-micromolar activity versus MDR cell line H69AR (lung cancer).[57-58]

Table 3.4 Cytotoxicity of DNR-AMA Hybrid **24**.

Study	Compound	HCT116 (colon)[a]	MDA-MB231 (breast)[a]	H69AR (lung/MDR)[a-b]
This Work [TC_{50} (nM)]	DNR-AMA (**7**)	90	90	200
	DNR (**5**)	210	80	1250
	DOX (**6**)	90	470	14,900
Isolation Report[c] [IC_{50} (nM)]	AMA (**1**)	2.5	164	67
	DNR (**5**)	6.8	330	4800
	DOX (**6**)	22.5	970	18,400

[a]Cell line (cancer type). [b]MDR = multidrug resistant. [c]Data from Brady, S. F. et al.[55-56]

3.4.2 Mechanism of Action Studies: Inhibition of Human TopIIα Mediated Relaxation of Supercoiled DNA

As discussed in Chapter 1, the most widely accepted mechanism of action for the anthracycline chemotherapeutics is topoisomerase II (TopII) poisoning.[59-62] Specifically, the inhibition of the 170 kDa TopIIα isoform is associated with the desired cytotoxic activity while inhibition of the 180 kDa TopIIβ isoform is hypothesized to contributed to the drugs' dose limiting cardiotoxicity. [63-70] To determine if DNR-AMA hybrid **24** inhibited TopIIα's ability to inhibit relax supercoiled DNA, we used the DNA relaxation assay developed by Osheroff, Shelton, and Brutlag.[71] This assay takes advantage of the fact that supercoiled DNA migrates faster than relaxed DNA on an agarose gel. As such, a theoretical gel of individual topoisomers in various states of relaxation ranging from fully supercoiled (Lane 1A) to fully relaxed (Lane 5A) would give the banding pattern seen in Figure 3.5a. Taking this forward to the TopII inhibition assay, a theoretical experiment with a TopII inhibitor would look like Figure 3.5b. In this gel, we see that inclusion of TopII enzyme results in full conversion of the fully supercoiled DNA to relaxed DNA topoisomers (compare Lanes 1B and 2B). Addition of increasing amounts of TopII inhibitor decreases the enzyme's ability to relax the supercoiled DNA, resulting in a dose dependent decrease in relaxed topoisomers and an increase in fully supercoiled DNA (Lanes 3B → 4B → 5B). Finally, we note that all lanes in Figure 3.5b contain nicked DNA, a single strand break byproduct from the synthesis of supercoiled DNA.

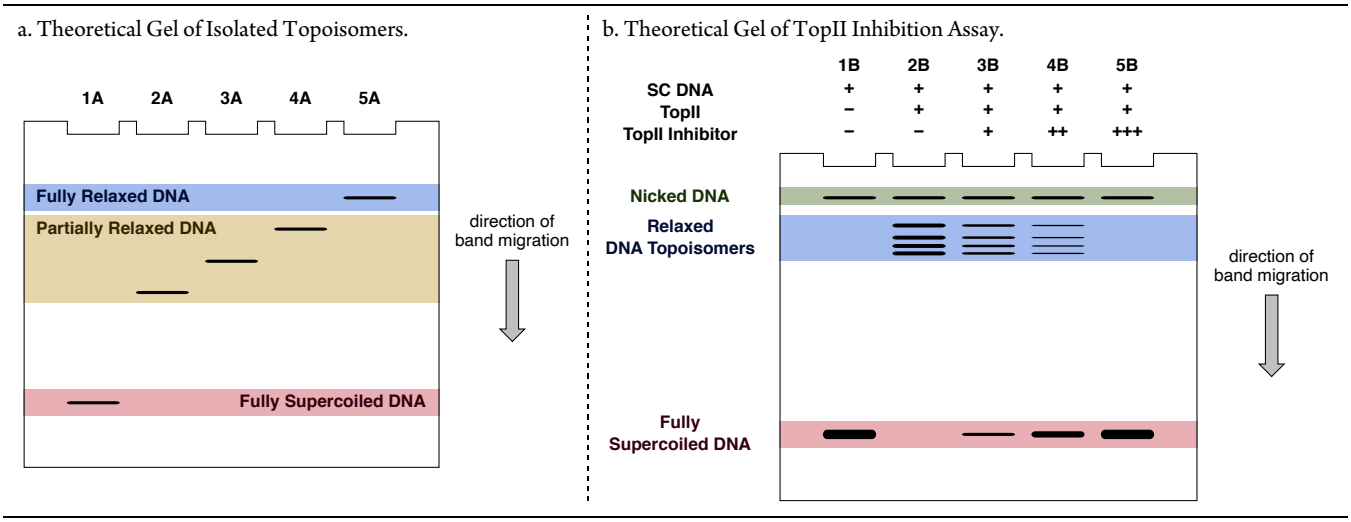


Figure 3.5 Explanation of Osheroff, Shelton, and Brutlag's TopII Inhibition Assay Using Theoretical Gels.

With this background in mind, we now turn our attention to the experimental data. We found that the addition of increasing amounts of DNR-AMA Hybrid **24** to a reaction mixture containing negatively supercoiled (–SC) DNA and human TopIIα (hTopIIα) led to the loss of relaxed DNA topoisomers (Figure 3.6ai, Lanes 2 vs. 10-13), indicating an inhibition of hTopIIα–catalyzed relaxation. However, **24** does not restore electrophoretic mobility to that of the untreated –SC DNA (Figure 3.6ai, Lanes 1 vs. 10-13). We hypothesize that this discrepancy

results from DNA intercalation[72-73] as intercalation is known to induce positive supercoiling which, in this case, would result in an overall decrease in negative supercoiling (i.e., relaxation) and a corresponding decrease in electrophoretic mobility. As expected, we observed a decrease in electrophoretic mobility upon addition of **24** to –SC DNA in the absence of enzyme (Figure 3.8aii). Repetition of these assays with daunorubicin (**5**) in place of DNR-AMA hybrid **24** indicate that these compounds inhibit hTopIIα mediated relaxation of –SC DNA to a similar extent (Figure 3.6b).

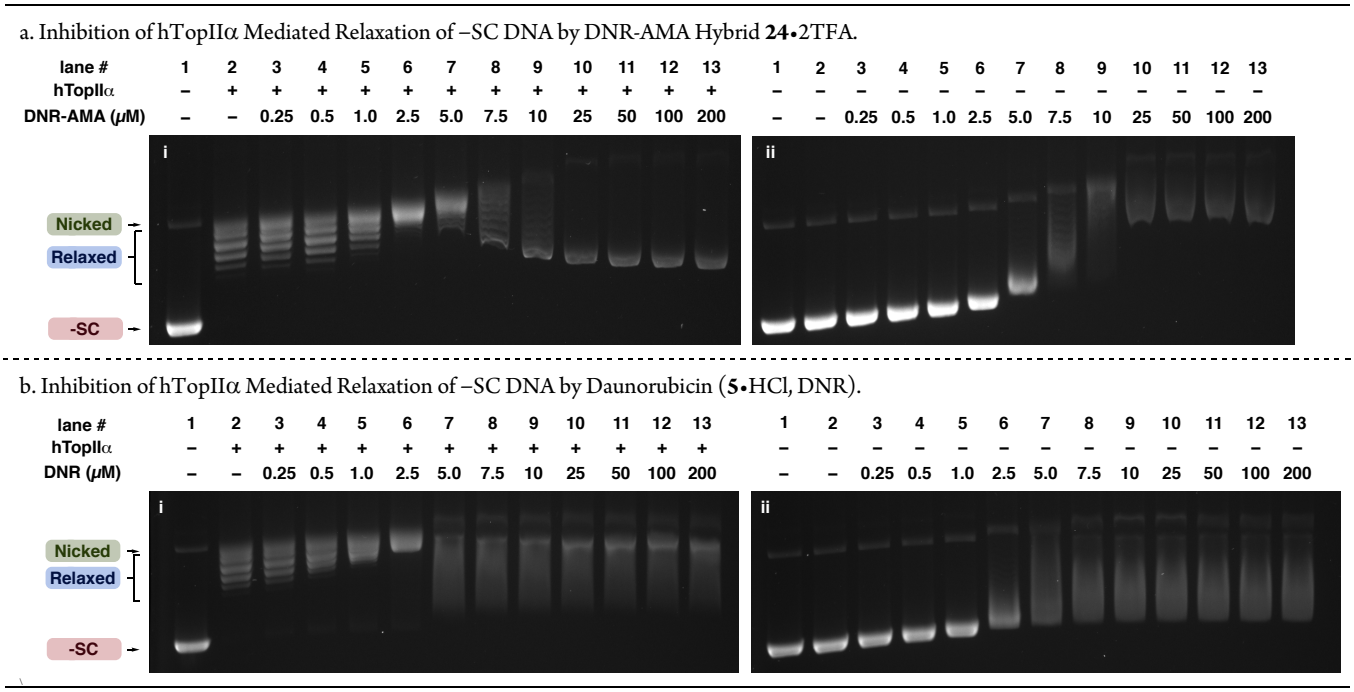


Figure 3.6 Inhibition of hTopIIα Mediated Relaxation of –SC DNA by DNR-AMA Hybrid **24**•2TFA and Daunorubicin (**5**•HCl).

In hopes of limiting the effect of intercalation on the relaxation assay, we next performed the same relaxation assays using +SC DNA rather than –SC DNA as DNA intercalators such as ethidium bromide intercalate +SC DNA to a lesser extent than they do –SC DNA;[74] conceptually, +SC DNA proves more resistant to intercalation because the positive supercoiling caused by intercalation results in a net increase in supercoiling which is energetically unfavorable. We found that addition of DNR-AMA hybrid **24** to a reaction mixture containing +SC DNA and hTopIIα led to the loss of relaxed DNA topoisomers as expected (Figure 3.7ai, Lanes 2 vs. 10-13). However, we once again noted that addition of **24** did not restore electrophoretic mobility to that of untreated +SC DNA (Figure 3.7ai, Lanes 1 vs. 10-13), even in the absence of enzyme (Figure 3.7aii). Repetition of these assays with daunorubicin (**5**) in place of DNR-AMA hybrid **24** indicate that these compounds inhibit hTopIIα mediated relaxation of +SC DNA to a similar extent (Figure 3.7b).

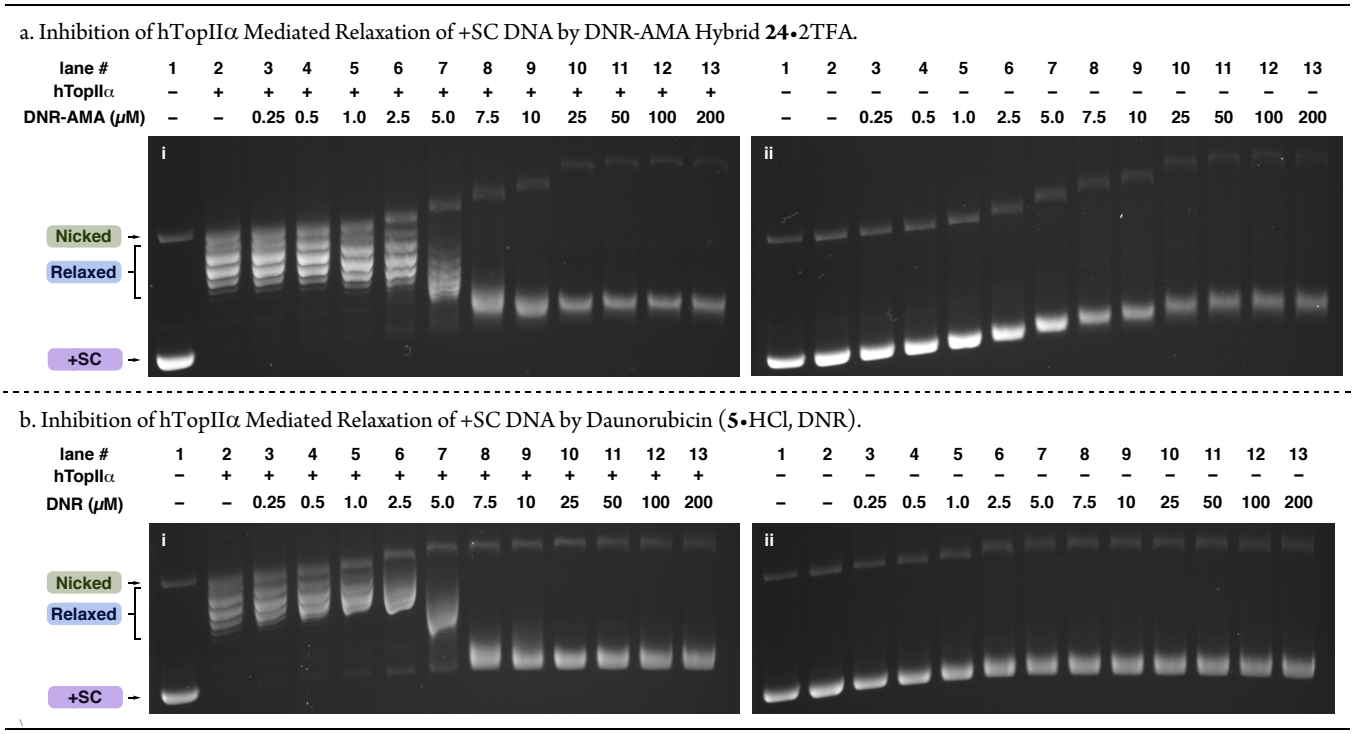


Figure 3.7 Inhibition of hTopIIα Mediated Relaxation of +SC DNA DNA by DNR-AMA Hybrid **24**•2TFA and Daunorubicin (**5**•HCl).

The ability of DNR-AMA hybrid **24** and daunorubicin (**5**) to decrease the electrophoretic mobility of +SC DNA deserves further comment and analysis. Returning to our previous theoretical discussion of the impact of DNA topology on gel mobility, we noted that an increase in supercoiling results in an increase in electrophoretic mobility (Figure 3.5a). As such, a theoretical gel of nine pure topoisomers with relative supercoiling ranging from −4 (negative SC) to +4 (positive SC) would give the banding pattern seen in Figure 3.8a; note that +SC travels further than its correspondingly supercoiled −SC isomer as +SC DNA is more compact than −SC DNA.[75-76] Because intercalators cause a net increase in relative supercoiling, this means that, in the presence of an intercalator such as chloroquine one should observe the following[77]:

(1) A *decrease* in the electrophoretic mobility of *−SC DNA* (Figure 3.8b, Lanes 1–4).

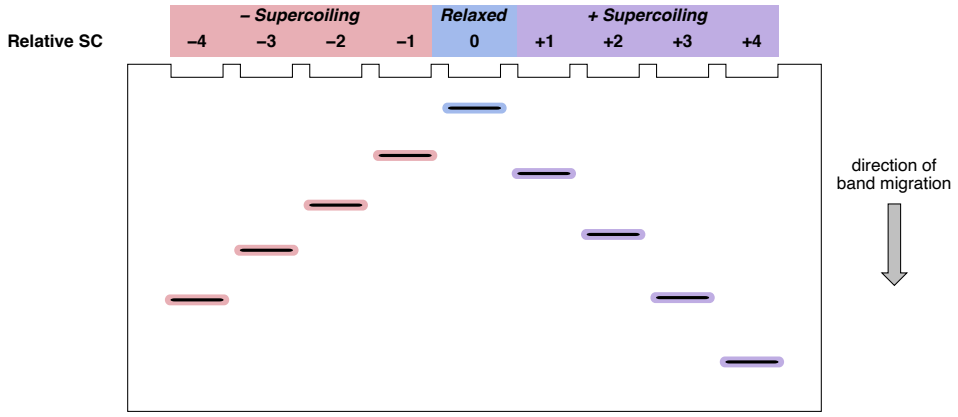
(2) An *increase* in the electrophoretic mobility of *relaxed DNA* (Figure 3.8b, Lane 5).

(3) An *increase* in the electrophoretic mobility of *+SC DNA* (Figure 3.8b, Lanes 6–8)

One can imagine an exception to this trend:

(1) The electrophoretic mobility of fully +SC DNA *should not change* as it cannot handle the additional supercoiling associated with intercalation; thus, no mobility altering intercalation should occur (Figure 3.8b, Lane 9).[74,76]

a. Theoretical Gel of Isolated Topoisomers with Relative Supercoiling from −4 (−SC) to +4 (+SC).



b. Theoretical Gel of Isolated Topoisomers with Relative Supercoiling from −4 (−SC) to +4 (+SC) Run in the Absence and Presence of the Intercalator Chloroquine.

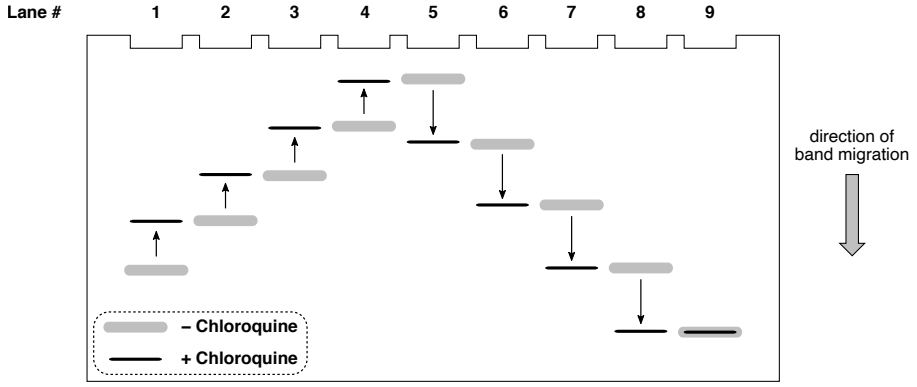


Figure 3.8 Explanation of the Relationship between DNA Supercoiling and Electrophoretic Mobility Using Theoretical Gels.

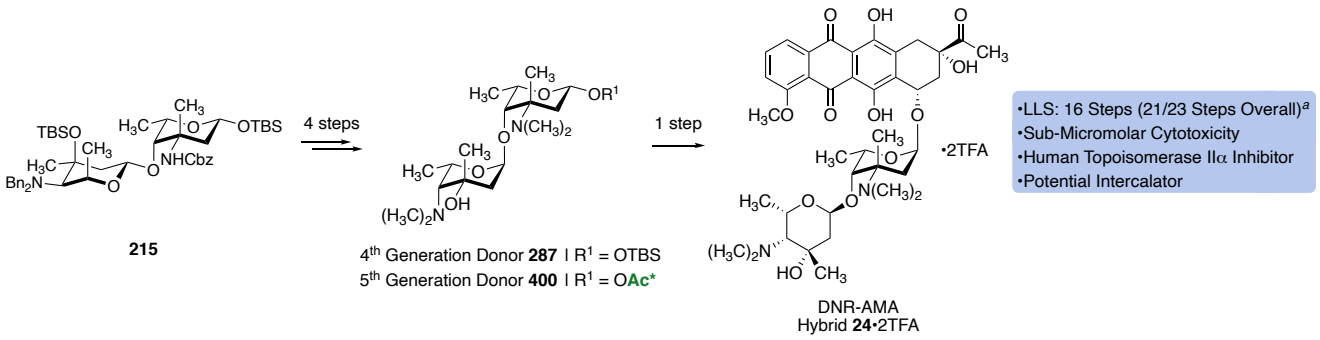
With this in mind, we would expect that intercalation of DNR-AMA hybrid **24** or daunorubicin (**5**) into −SC DNA would decrease electrophoretic mobility; examination of Figure 3.6aii (particularly Lanes 1–6) and Figure 3.6bii (particularly Lanes 1–5), confirm this expectation. In contrast, one would predict that the corresponding intercalation into +SC DNA would increase electrophoretic mobility; however, treatment of +SC DNA with either DNR-AMA hybrid **24** or daunorubicin (**5**) in the absence of hTopIIα results in a decrease in band mobility (Figure 3.7aii and Figure 3.7bii, respectively). The cationic nature of the tested anthracyclines may account for this anomalous behavior, as decreasing the net negative charge of a DNA molecule would decrease its

electrophoretic mobility as bands migrate toward the anode (positive terminal) in response to an applied electric field.

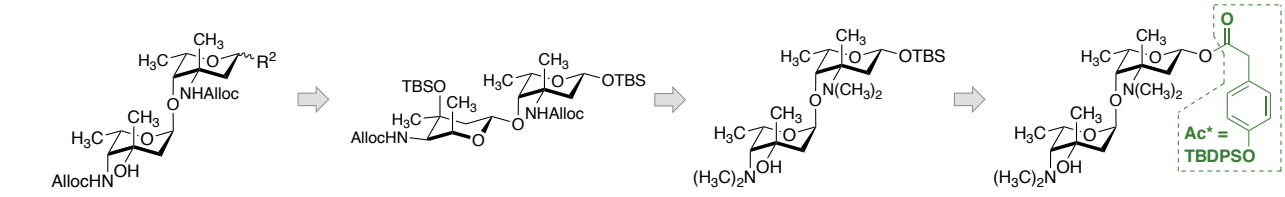
3.5 *Summary of Chapter 3*

In this chapter, we described the transformation of fully protected Arimetamycin A disaccharide **215** into DNR-AMA hybrid **24** (Scheme 3.20a). The realization of this goal required the rational iteration of disaccharide donors in a process that began with 1st generation donor **245** and culminated in novel, 5th generation donor **300** (Scheme 3.20b). Cytotoxicity studies revealed that the hybrid exhibited sub-micromolar activity (TC_{50}) against a panel of three cancer cell lines that included the MDR H69AR cell line while initial mechanistic assays demonstrated that hybrid **24** inhibits hTopIIα-catalyzed DNA relaxation in a manner comparable to DNR (**5**) and is hypothesized to be a DNA intercalator

a. Summary of the Synthesis of DNR-AMA Hybrid **24**•2TFA from Disaccharide **215**.



b. Summary of 1st–5th Generation Donors.



Scheme 3.20 Summary of Chapter 3. [a]21 steps overall using 4th generation donor **287**; 23 steps overall using 5th generation donor **300.**

3.6 *References for Chapter 3.*

(1) Lear, M. J.; Yoshimura, F.; Hirama, M. A Direct and Efficient A-Selective Glycosylation Protocol for the Kedarcidin Sugar, L-Mycarose: $AgPf_6$ as a Remarkable Activator of 2-Deoxythioglycosides. *Angew. Chem. Int. Ed.* **2001**, *40*, 946-949.
(2) Zhu, L.; Cao, X.; Chen, W.; Zhang, G.; Sun, D.; Wang, P. G. Syntheses and Biological Activities of Daunorubicin Analogs with Uncommon Sugars. *Bioorg. Med. Chem.* **2005**, *13*, 6381-7.
(3) Cotterill, I. C.; Rich, J. O.; Scholten, M. D.; Mozhaeva, L.; Michels, P. C. Reversible Derivatization to Enhance Enzymatic Synthesis: Chemoenzymatic Synthesis of Doxorubicin-14-O-Esters. *Biotechnol. Bioeng.* **2008**, *101*, 435-440.
(4) Wander, D. P. A.; van der Zanden, S. Y.; van der Marel, G. A.; Overkleeft, H. S.; Neefjes, J.; Codee, J. D. C. Doxorubicin and Aclarubicin: Shuffling Anthracycline Glycans for Improved Anticancer Agents. *J. Med. Chem.* **2020**, *63*, 12814-12829.
(5) Zhang, G.; Fang, L.; Zhu, L.; Aimiuwu, J. E.; Shen, J.; Cheng, H.; Muller, M. T.; Lee, G. E.; Sun, D.; Wang, P. G. Syntheses and Biological Activities of Disaccharide Daunorubicins. *J. Med. Chem.* **2005**, *48*, 5269-5278.
(6) Ren, F.; Hogan, P. C.; Anderson, A. J.; Myers, A. G. Kedarcidin Chromophore: Synthesis of Its Proposed Structure and Evidence for a Stereochemical Revision. *J. Am. Chem. Soc.* **2007**, *129*, 5381-3.
(7) Magauer, T.; Smaltz, D. J.; Myers, A. G. Component-Based Syntheses of Trioxacarcin a, Dc-45-A1 and Structural Analogues. *Nat Chem* **2013**, *5*, 886-93.
(8) Lear, M. J.; Hirai, K.; Ogawa, K.; Yamashita, S.; Hirama, M. A Convergent Total Synthesis of the Kedarcidin Chromophore: 20-Years in the Making. *J Antibiot (Tokyo)* **2019**, *72*, 350-363.
(9) Rho, Y. S.; Kim, S. Y.; Kim, W.-J.; Yun, Y. K.; Sin, H. S.; Yoo, D. J. Convenient Syntheses of Daunomycinone-7-D-Glucuronides and Doxorubicinone-7-D-Glucuronides. *Synth. Commun.* **2004**, *34*, 3497-3511.
(10) Arcamone, F.; Franceschi, G.; Orezzi, P.; Cassinelli, G.; Barbieri, W.; Mondelli, R. Daunomycin. I. The Structure of Daunomycinone. *J. Am. Chem. Soc.* **1964**, *86*, 5334-5335.
(11) Arcamone, F.; Franceschi, G.; Penco, S.; Selva, A. Adriamycin (14-Hydroxydaunomycin), a Novel Antitumor Antibiotic. *Tetrahedron Lett.* **1969**, *10*, 1007-1010.
(12) DeGoey, D. A.; Grampovnik, D. J.; Chen, H. J.; Flosi, W. J.; Klein, L. L.; Dekhtyar, T.; Stoll, V.; Mamo, M.; Molla, A.; Kempf, D. J. P1-Substituted Symmetry-Based Human Immunodeficiency Virus Protease Inhibitors with Potent Antiviral Activity against Drug-Resistant Viruses. *J. Med. Chem.* **2011**, *54*, 7094-7104.
(13) Yoshida, A.; Akaiwa, M.; Asakawa, T.; Hamashima, Y.; Yokoshima, S.; Fukuyama, T.; Kan, T. Total Synthesis of (−)-Lemonomycin. *Chem. Eur. J.* **2012**, *18*, 11192-11195.
(14) EDH-e-0695. Note that stir ball was used so stirring was likely not that good.
(15) Evans, D. A.; Hu, E.; Tedrow, J. S. An Aldol-Based Approach to the Asymmetric Synthesis of L-Callipeltose, the Deoxyamino Sugar of L-Callipeltoside A. *Org. Lett.* **2001**, *3*, 3133-6.
(16) Golik, J.; Wong, H.; Krishnan, B.; Vyas, D. M.; Doyle, T. W. Stereochemical Studies on Esperamicins: Determination of the Absolute Configuration of Hydroxyamino Sugar Fragment. *Tetrahedron Lett.* **1991**, *32*, 1851-1854.
(17) Halcomb, R. L.; Wittman, M. D.; Olson, S. H.; Danishefsky, S. J.; Golik, J.; Wong, H.; Vyas, D. The Synthesis of the Core Trisaccharide of Esperamicin: Corroboration of the Proposed Structure for Its Rearrangement Product and Stabilization of the Core Trisaccharide Domain. *J. Am. Chem. Soc.* **2002**, *113*, 5080-5082.
(18) Garcia-Moreno, M. I.; Aguilar, M.; Ortiz Mellet, C.; Garcia Fernandez, J. M. Intramolecular Benzyl Protection Delivery: A Practical Synthesis of Dmdp and Dgdp from D-Fructose. *Org. Lett.* **2006**, *8*, 297-9.
(19) Garcia, B. A.; Poole, J. L.; Gin, D. Y. Direct Glycosylations with 1-Hydroxy Glycosyl Donors Using Trifluoromethanesulfonic Anhydride and Diphenyl Sulfoxide. *J. Am. Chem. Soc.* **1997**, *119*, 7597-7598.
(20) Garcia, B. A.; Gin, D. Y. Dehydrative Glycosylation with Activated Diphenyl Sulfonium Reagents. Scope, Mode of *C*(1)-Hemiacetal Activation, and Detection of Reactive Glycosyl Intermediates. *J. Am. Chem. Soc.* **2000**, *122*, 4269-4279.
(21) Crich, D.; Smith, M.; Yao, Q.; Picione, J. 2,4,6-Tri-*Tert*-Butylpyrimidine (Ttbp): A Cost Effective, Readily Available Alternative to the Hindered Base 2,6-Di-*Tert*-Butylpyridine and Its 4-Substituted Derivatives in Glycosylation and Other Reactions. *Synthesis* **2001**, *2001*, 323-326.
(22) Kolar, C.; Kneissl, G.; Knödler, U.; Dehmel, K. Semisynthetic E-(Iso)Rhodomycins: A New Glycosylation Variant and Modification Reactions. *Carbohydr. Res.* **1991**, *209*, 89-100.
(23) Renneberg, B.; Li, Y.-M.; Laatsch, H.; Fiebig, H.-H. A Short and Efficient Transformation of Rhamnose into Activated Daunosamine, Acosamine, Ristosamine and *Epi*-Daunosamine Derivatives, and Synthesis of an Anthracycline Antibiotic Acosaminyl-E-*Iso*-Rhodomycinone. *Carbohydr. Res.* **2000**, *329*, 861-872.
(24) LI, L.; ZHU, J.; ZHAO, W.-x. A Divergent Approach to Synthesize Daunorubicin Analog. *Chemical Research* **2011**, *22*, 70-73.
(25) 张贵生; 刘青锋; 张志国 Synthesis of 3'-Triazoepirubicin with Anticancer Activity. CN102146108B, **2014**.
(26) Altona, C.; Haasnoot, C. A. G. Prediction of *Anti* and *Gauche* Vicinal Proton-Proton Coupling Constants in Carbohydrates: A Simple Additivity Rule for Pyranose Rings. *Org. Magn. Reson.* **1980**, *13*, 417-429.

(27) Thang, T. T.; Imbach, J. L.; Fizames, C.; Lavelle, F.; Ponsinet, G.; Olesker, A.; Lukacs, G. Synthesis and Antitumor Activity of 3′ -C-Methyl-Daunorubicin. *Carbohydr. Res.* **1985**, *135*, 241-247.
(28) Borch, R. F.; Bernstein, M. D.; Durst, H. D. Cyanohydridoborate Anion as a Selective Reducing Agent. *J. Am. Chem. Soc.* **1971**, *93*, 2897-2904.
(29) Olsufyeva, E. N.; Tevyashova, A. N.; Trestchalin, I. D.; Preobrazhenskaya, M. N.; Platt, D.; Klyosov, A. Synthesis and Antitumor Activity of New D-Galactose-Containing Derivatives of Doxorubicin. *Carbohydr. Res.* **2003**, *338*, 1359-1367.
(30) Ghirmai, S.; Mume, E.; Tolmachev, V.; Sjoberg, S. Synthesis and Radioiodination of Some Daunorubicin and Doxorubicin Derivatives. *Carbohydr. Res.* **2005**, *340*, 15-24.
(31) Suzuki, K.; Sulikowski, G. A.; Friesen, R. W.; Danishefsky, S. J. Application of Substituent-Controlled Oxidative Coupling of Glycals in a Synthesis and Structural Corroboration of Ciclamycin 0: New Possibilities for the Construction of Hybrid Anthracyclines. *J. Am. Chem. Soc.* **1990**, *112*, 8895-8902.
(32) Gidley, M. J.; Sanders, J. K. Reductive Methylation of Proteins with Sodium Cyanoborohydride Identification, Suppression and Possible Uses of *N*-Cyanomethyl by-Products. *Biochem. J.* **1982**, *203*, 331-334.
(33) Nelson, W. L. Oxazolidine Formation in the Attempted Eschweiler-Clarke Reductive Methylation of *Cis*-3-Aminobicyclo[2.2.2] Octan-2-Ol. *J. Heterocycl. Chem.* **1968**, *5*, 231-233.
(34) Tomita, M.; Uyeo, S.; Kikuchi, T. Studies on the Alkaloids of *Pachysandra Terminalis* Sieb. Et Zucc.: Structure of Pachysandrine a and B. *Tetrahedron Lett.* **1964**, *5*, 1053-1061.
(35) Bock, K.; Lundt, I.; Pedersen, C. Assignment of Anomeric Structure to Carbohydrates through Geminal ^{13}c-H Coupling Constants. *Tetrahedron Lett.* **1973**, *14*, 1037-1040.
(36) Bock, K.; Pedersen, C. A Study of ^{13}ch Coupling Constants in Hexopyranoses. *J. Chem. Soc., Perkin Trans. 2* **1974**, 293-297.
(37) Boivin, J.; Montagnac, A.; Monneret, C.; Païs, M. Hémisynthèse De Nouveaux Glycosides Analogues De La Daunorubicine. *Carbohydr. Res.* **1980**, *85*, 223-242.
(38) Kimura, Y.; Suzuki, M.; Matsumoto, T.; Abe, R.; Terashima, S. Trimethylsilyl Trifluoromethanesulfonate (Trimethylsilyl Triflate) as an Excellent Glycosidation Reagent for Anthracycline Synthesis. Simple and Efficient Synthesis of Optically Pure 4-Demethoxydaunorubicin. *Chem. Lett.* **1984**, *13*, 501-504.
(39) Kimura, Y.; Suzuki, M.; Matsumoto, T.; Abe, R.; Terashima, S. Novel Glycosidation of 4-Demethoxyanthracyclinones by the Use of Trimethylsilyl Triflate. Syntheses of Optically Active 4-Demethoxydaunorubicin and 4-Demethoxyadriamycin. *Bull. Chem. Soc. Jpn.* **1986**, *59*, 423-431.
(40) Cipollone, A.; Berettoni, M.; Bigioni, M.; Binaschi, M.; Cermele, C.; Monteagudo, E.; Olivieri, L.; Palomba, D.; Animati, F.; Goso, C.; Maggi, C. A. Novel Anthracycline Oligosaccharides: Influence of Chemical Modifications of the Carbohydrate Moiety on Biological Activity. *Biorg. Med. Chem.* **2002**, *10*, 1459-1470.
(41) Sebesta, R.; Seebach, D. Preparation of (S,S)-Fmoc-B2hile-Oh, (S)-Fmoc-B2hmet-Oh, and (S)-Fmoc-B2htyr(Tbu)-Oh for Solid-Phase Syntheses Ofβ2- Andβ2/B3-Peptides. *Helv. Chim. Acta* **2003**, *86*, 4061-4072.
(42) Zeng, J.; Wang, R.; Zhang, S.; Fang, J.; Liu, S.; Sun, G.; Xu, B.; Xiao, Y.; Fu, D.; Zhang, W.; Hu, Y.; Wan, Q. Hydrogen-Bonding-Assisted Exogenous Nucleophilic Reagent Effect for B-Selective Glycosylation of Rare 3-Amino Sugars. *J. Am. Chem. Soc.* **2019**, *141*, 8509-8515.
(43) Schmidt, R. R.; Michel, J. Direct O-Glycosyl Trichloroacetimidate Formation, Nucleophilicity of the Anomeric Oxygen Atom. *Tetrahedron Lett.* **1984**, *25*, 821-824.
(44) Schmidt, R. R. New Methods for the Synthesis of Glycosides and Oligosaccharides?Are There Alternatives to the Koenigs-Knorr Method? [New Synthetic Methods (56)]. *Angewandte Chemie International Edition in English* **1986**, *25*, 212-235.
(45) Schmidt, R. R.; Klotz, W. Glycoside Bond Formation Via Anomerico-Alkylation: How Many Protective Groups Are Required? *Synlett* **1991**, *1991*, 168-170.
(46) Schmidt, R. R. Anomeric-Oxygen Activation for Glycoside Synthesis: The Trichloroacetimidate Methods. *Adv. Carbohydr. Chem. Biochem.* **1994**, *50*, 21-123.
(47) Binkley, R. C.; Ziepfel, J. C.; Himmeldirk, K. B. Anomeric Selectivity in the Synthesis of Galloyl Esters of D-Glucose. *Carbohydr. Res.* **2009**, *344*, 237-9.
(48) Cabacungan, J. C.; Ahmed, A. I.; Feeney, R. E. Amine Boranes as Alternative Reducing Agents for Reductive Alkylation of Proteins. *Anal. Biochem.* **1982**, *124*, 272-278.
(49) Wong, W. S. D.; Osuga, D. T.; Feeney, R. E. Pyridine Borane as a Reducing Agent for Proteins. *Anal. Biochem.* **1984**, *139*, 58-67.
(50) Scudiero, D. A.; Shoemaker, R. H.; Paull, K. D.; Monks, A.; Tierney, S.; Nofziger, T. H.; Currens, M. J.; Seniff, D.; Boyd, M. R. Evaluation of a Soluble Tetrazolium/Formazan Assay for Cell Growth and Drug Sensitivity in Culture Using Human and Other Tumor Cell Lines. *Cancer Res.* **1988**, *48*, 4827-4833.
(51) Roehm, N. W.; Rodgers, G. H.; Hatfield, S. M.; Glasebrook, A. L. An Improved Colorimetric Assay for Cell Proliferation and Viability Utilizing the Tetrazolium Salt Xtt. *J. Immunol. Methods* **1991**, *142*, 257-265.
(52) Berridge, M. V.; Herst, P. M.; Tan, A. S. Tetrazolium Dyes as Tools in Cell Biology: New Insights into Their Cellular Reduction. *Biotechnol. Ann. Rev.* **2005**, *11*, 127-152.

(53) Xtt Cell Proliferation Assay Kit: Instruction Manual (Catalog Number 30-1011k). Collection, A. T. C., Ed. 2011.
(54) Gordon, J. L.; Brown, M. A.; Reynolds, M. M. Cell-Based Methods for Determination of Efficacy for Candidate Therapeutics in the Clinical Management of Cancer. *Diseases* **2018**, *6*.
(55) Kang, H. S.; Brady, S. F. Arimetamycin A: Improving Clinically Relevant Families of Natural Products through Sequence-Guided Screening of Soil Metagenomes. *Angew. Chem. Int. Ed.* **2013**, *52*, 11063-11067.
(56) Brady, S.; Kang, H.-S. Anticancer Agents of the Anthracycline Family. US9217008B1, **2015**.
(57) Mirski, S. E. L.; Gerlach, J. H.; Cole, S. P. C. Multidrug Resistance in a Human Small Cell Lung Cancer Cell Line Selected in Adriamycin. *Cancer Res.* **1987**, *47*, 2594-2598.
(58) Hipfner, D. R.; Gauldie, S. D.; Deeley, R. G.; Cole, S. P. C. Detection of the M_r 190,000 Multidrug Resistance Protein, Mrp, with Monoclonal Antibodies. *Cancer Res.* **1994**, *54*, 5788-5792.
(59) Tewey, K. M.; Rowe, T. C.; Yang, L.; Halligan, B. D.; Liu, L. F. Adriamycin-Induced DNA Damage Mediated by Mammalian DNA Topoisomerase Ii. *Science* **1984**, *226*, 466-468.
(60) Capranico, G.; Zunino, F.; Kohn, K. W.; Pommier, Y. Sequence-Selective Topoisomerase Ii Inhibition by Anthracycline Derivatives in Sv40 DNA: Relationship with DNA Binding Affinity and Cytotoxicity. *Biochemistry* **1990**, *29*, 562-9.
(61) Binaschi, M.; Farinosi, R.; Austin, C. A.; Fisher, L. M.; Zunino, F.; Capranico, G. Human DNA Topoisomerase Iiα-Dependent DNA Cleavage and Yeast Cell Killing by Anthracycline Analogues. *Cancer Res.* **1998**, *58*, 1886-92.
(62) Marinello, J.; Delcuratolo, M.; Capranico, G. Anthracyclines as Topoisomerase Ii Poisons: From Early Studies to New Perspectives. *Int J Mol Sci* **2018**, *19*.
(63) Woessner, R. D.; Mattern, M. R.; Mirabelli, C. K.; Johnson, F. H.; Drake, F. H. Proliferation- and Cell Cycle-Dependent Differences in Expression of the 170 Kilodalton and 180 Kilodalton Forms of Topoisomerase Ii in Nih-3t3 Cells. *Cell Growth Differ.* **1991**, *2*, 209-214.
(64) Capranico, G.; Tinelli, S.; Austin, C. A.; Fisher, M. L.; Zunino, F. Different Patterns of Gene Expression of Topoisomerase Ii Isoforms in Differentiated Tissues During Murine Development. *Biochim. Biophys. Acta* **1992**, *1132*, 43-48.
(65) Sandri, M. I.; Hochhauser, D.; Ayton, P.; Camplejohn, R. C.; Whitehouse, R.; Turley, H.; Gatter, K.; Hickson, I. D.; Harris, A. L. Differential Expression of the Topoisomerase Ii Alpha and Beta Genes in Human Breast Cancers. *Br. J. Cancer* **1996**, *73*, 1518-24.
(66) Turley, H.; Comley, M.; Houlbrook, S.; Nozaki, N.; Kikuchi, A.; Hickson, I. D.; Gatter, K.; Harris, A. L. The Distribution and Expression of the Two Isoforms of DNA Topoisomerase Ii in Normal and Neoplastic Human Tissues. *Br. J. Cancer* **1997**, *75*, 1340-6.
(67) Lyu, Y. L.; Kerrigan, J. E.; Lin, C. P.; Azarova, A. M.; Tsai, Y. C.; Ban, Y.; Liu, L. F. Topoisomerase Iibeta Mediated DNA Double-Strand Breaks: Implications in Doxorubicin Cardiotoxicity and Prevention by Dexrazoxane. *Cancer Res.* **2007**, *67*, 8839-46.
(68) Zhang, S.; Liu, X.; Bawa-Khalfe, T.; Lu, L. S.; Lyu, Y. L.; Liu, L. F.; Yeh, E. T. Identification of the Molecular Basis of Doxorubicin-Induced Cardiotoxicity. *Nat. Med.* **2012**, *18*, 1639-42.
(69) Jiang, J.; Mohan, N.; Endo, Y.; Shen, Y.; Wu, W. J. Type Iib DNA Topoisomerase Is Downregulated by Trastuzumab and Doxorubicin to Synergize Cardiotoxicity. *Oncotarget* **2018**, *9*, 6095-6108.
(70) Cornarottie, M.; Tinelli, S.; WIllmore, E.; Zunino, F.; Fisher, L. M.; Austin, C. A.; Capranico, G. Drug Sensitivity and Sequence Specificity of Human Recombinant DNA Topoisomerases Iialpha (P170) and Iibeta (P180). *Mol. Pharmacol.* **1996**, *50*, 1463-1471.
(71) Osheroff, N.; Shelton, E. R.; Brutlag, D. L. DNA Topoisomerase Ii from *Drosophila Melanogaster*. *The Journal of Biological Chemistry* **1983**, *258*, 9536-9543.
(72) Sigmon, J.; Larcom, L. L. The Effect of Ethidium Bromide on Mobility of DNA Fragments in Agarose Gel Electrophoresis. *Electrophoresis* **1996**, *17*, 1524-1527.
(73) Furlan, R. L. A.; Garrido, L. M.; Brumatti, G.; Amarante-Mendes, G. P.; Martins, R. A.; Facciotti, M. C. R.; Padilla, G. A Rapid and Sensitive Method for the Screening of DNA Intercalating Antibiotics. *Biotechnol. Lett.* **2002**, *24*, 1807-1813.
(74) McClendon, A. K.; Rodriguez, A. C.; Osheroff, N. Human Topoisomerase Iialpha Rapidly Relaxes Positively Supercoiled DNA: Implications for Enzyme Action Ahead of Replication Forks. *J. Biol. Chem.* **2005**, *280*, 39337-45.
(75) Bates, A. D.; Maxwell, A. *DNA Topology*. 2nd ed.; Oxoford University Press: Oxford; New York, **2005**.
(76) Gibson, E. G.; Oviatt, A. A.; Osheroff, N. Two-Dimensional Gel Electrophoresis to Resolve DNA Topoisomers. *Methods Mol Biol* **2020**, *2119*, 15-24.
(77) Linking Number and the Unwinding Assay: The DNA Intercalation Assay. Inspiralis, Ed.

CHAPTER 4

Future Directions

4.1 *Synthesis of Arimetamycin A (**1**), DOX-AMA Hybrid (**25**), and ETP-AMA (**26**)*

Having achieved the synthesis of DNR-AMA hybrid **24**, our next task is completing the syntheses of arimetamycin A (**1**), DOX-AMA hybrid (**25**), and ETP-AMA hybrid (**26**) as outlined in Chapter 1 (Figure 4.1). Below, we describe our current progress toward these targets as well as our proposed strategies for their completion.

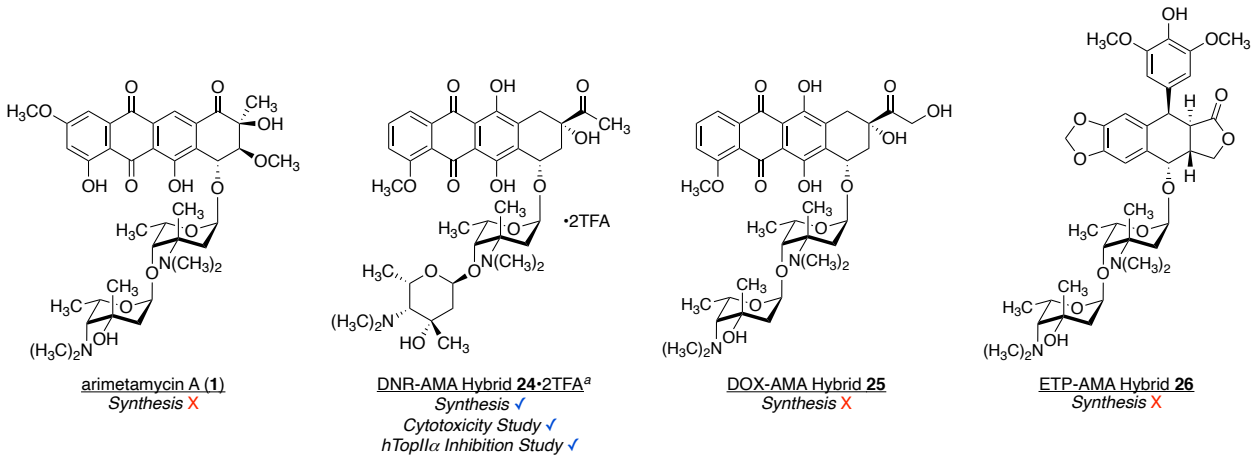


Figure 4.1 Current State of Our Efforts toward the Synthetic Targets Pursued in this Dissertation. [a]Non-reducing lemonose sugar depicted in a flat representation as ROESY studies indicate that this sugar residue exists in conformational equilibrium and/or an intermediate conformation not depicted in Scheme 3.15c.

4.1.1 *Proposed Synthesis of Arimetamycin A (**1**)*

Arimetamycin A (**1**) shares an aglycone with steffimycin (**4**) and steffimycin B (**312**).[1] As such, we reasoned that degradation of either of these natural products would provide ready access to steffimycinone (**27**). However, as neither steffimycin nor steffimycin B have demonstrated potent biological activity, commercial access to these compounds is limited. Taking matters into our own hands, efforts to isolate steffimycin by culturing its producing organism *Streptomyces steffisburgensis* proved futile.[2-4] Gratifyingly, we found a commercial supplier willing to isolate steffimycin B from cell culture, providing us with ca. 40 mg of **312**.

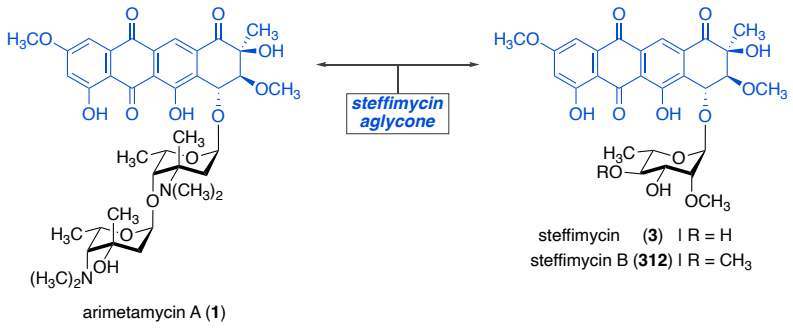
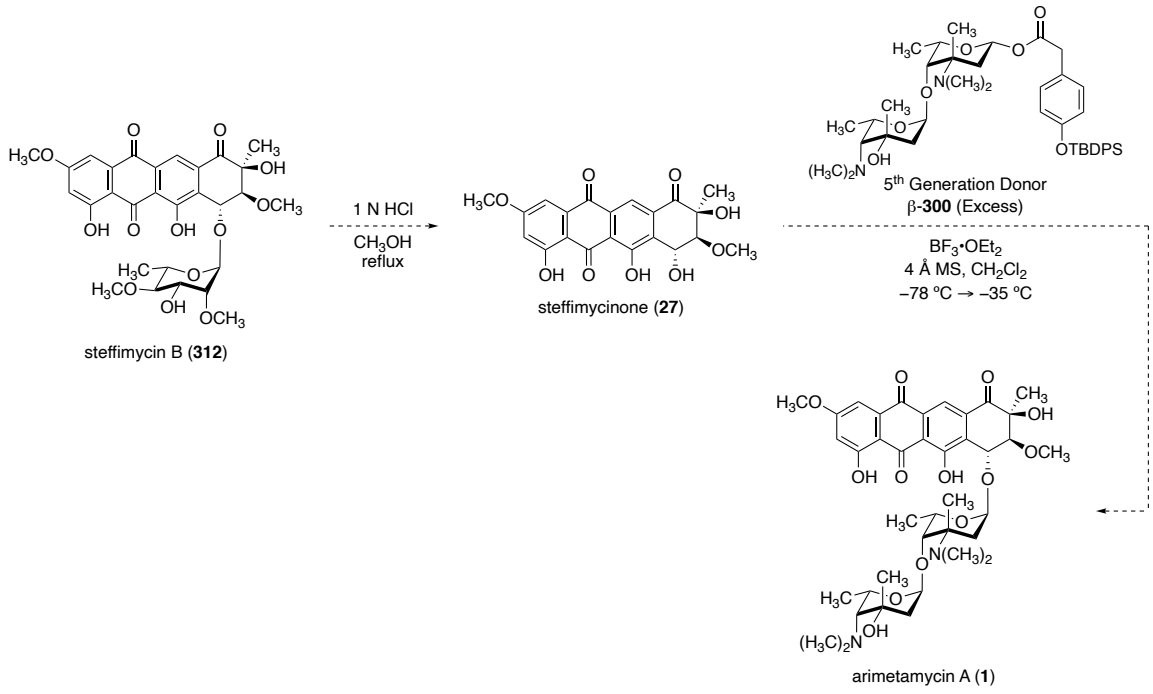


Figure 4.2 Arimetamycin A (**1**), Steffimycin (**4**), and Steffimycin B (**312**) Share the Same Aglycone.

We envision that conversion of steffimycin B to arimetamycin A (**1**) will require two steps. First, we will subject **312** to acidic methanolysis to obtained steffimycinone (**27**) as described by Kelly and Wiley (Scheme 4.1).[1] Next, we will submit **27** to BF_3•OEt_2 mediated glycosidation with 5th generation AMA disaccharide donor **300** to give arimetamycin A (**1**).

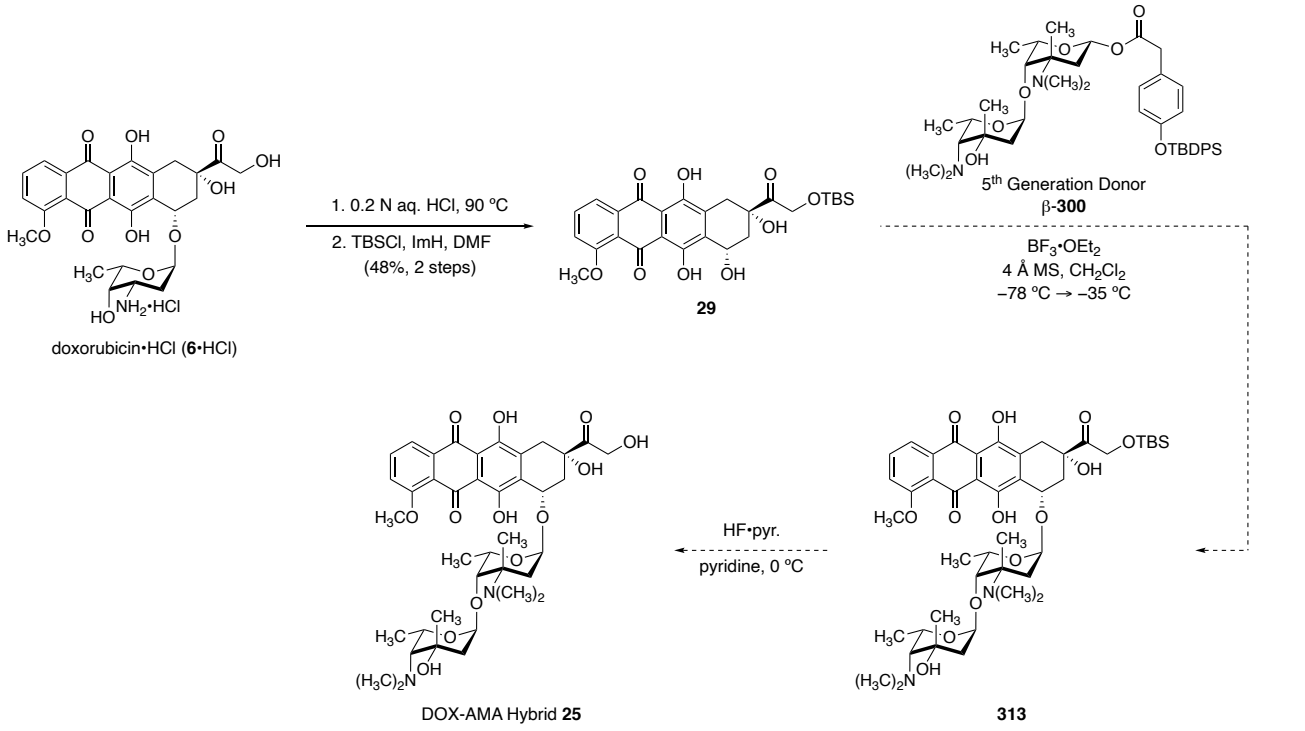


Scheme 4.1 Proposed Synthesis of Arimetamycin A (**1**).

4.1.2 Proposed Synthesis of DOX-AMA Hybrid ***25***

Our efforts toward the synthesis of DOX-AMA hybrid **25** began with the hydrolysis of commercially available doxorubicin•HCl (**6**•HCl) (Scheme 4.2). Subsequent chemoselective TBS protection of the primary alcohol gave known aglycone acceptor **29**.[5-6] Next, we envision that glycosidation with 5th generation AMA

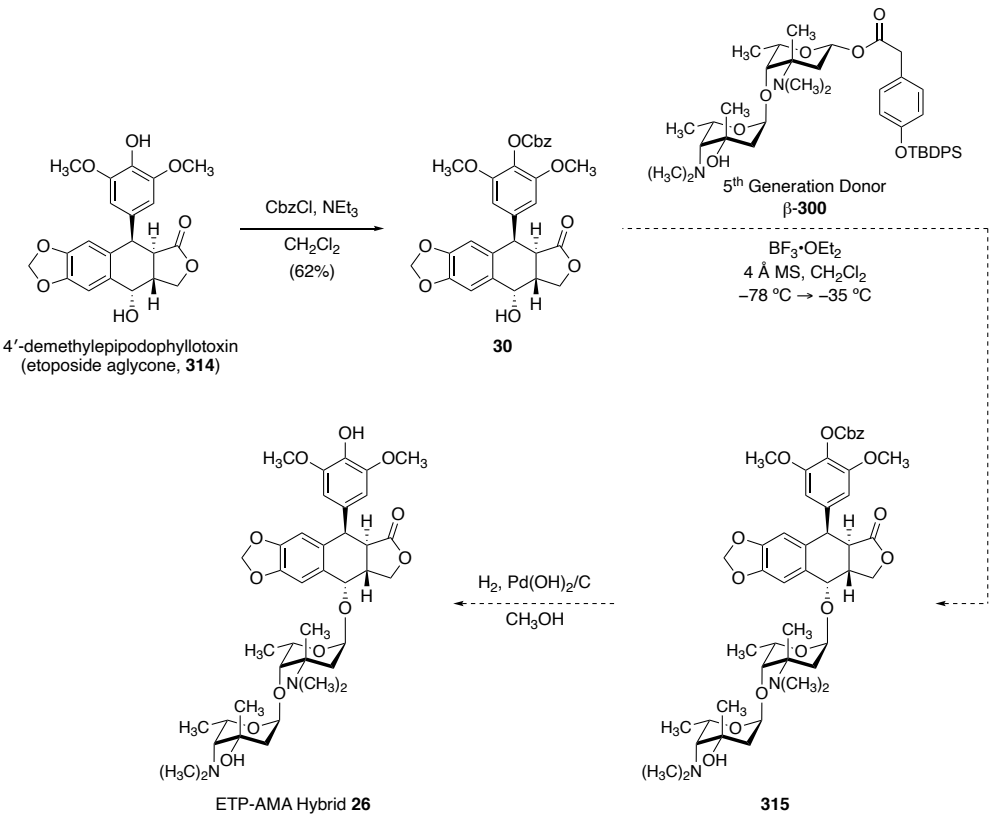
disaccharide **300** will yield protected anthracycline **313** which should afford desired DOX-AMA hybrid **25** upon desilylation with HF•pyridine.[6]



Scheme 4.2 Proposed Synthesis of DOX-AMA Hybrid **25**.

4.1.3 *Proposed Synthesis of ETP-AMA Hybrid (**26**)*

Our work toward the synthesis of ETP-AMA hybrid **26** began with the chemoselective protection of commercially available 4′-demethylepipodophyllotoxin (**314**) to give known Cbz carbonate **30** (Scheme 4.3).[7-9] Next, we anticipate that glycosidation with 5th generation AMA disaccharide **300** will give **315** which should yield desired ETP-AMA hybrid **26** upon hydrogenolysis.



Scheme 4.3 Proposed Synthesis of ETP-AMA Hybrid **26**.

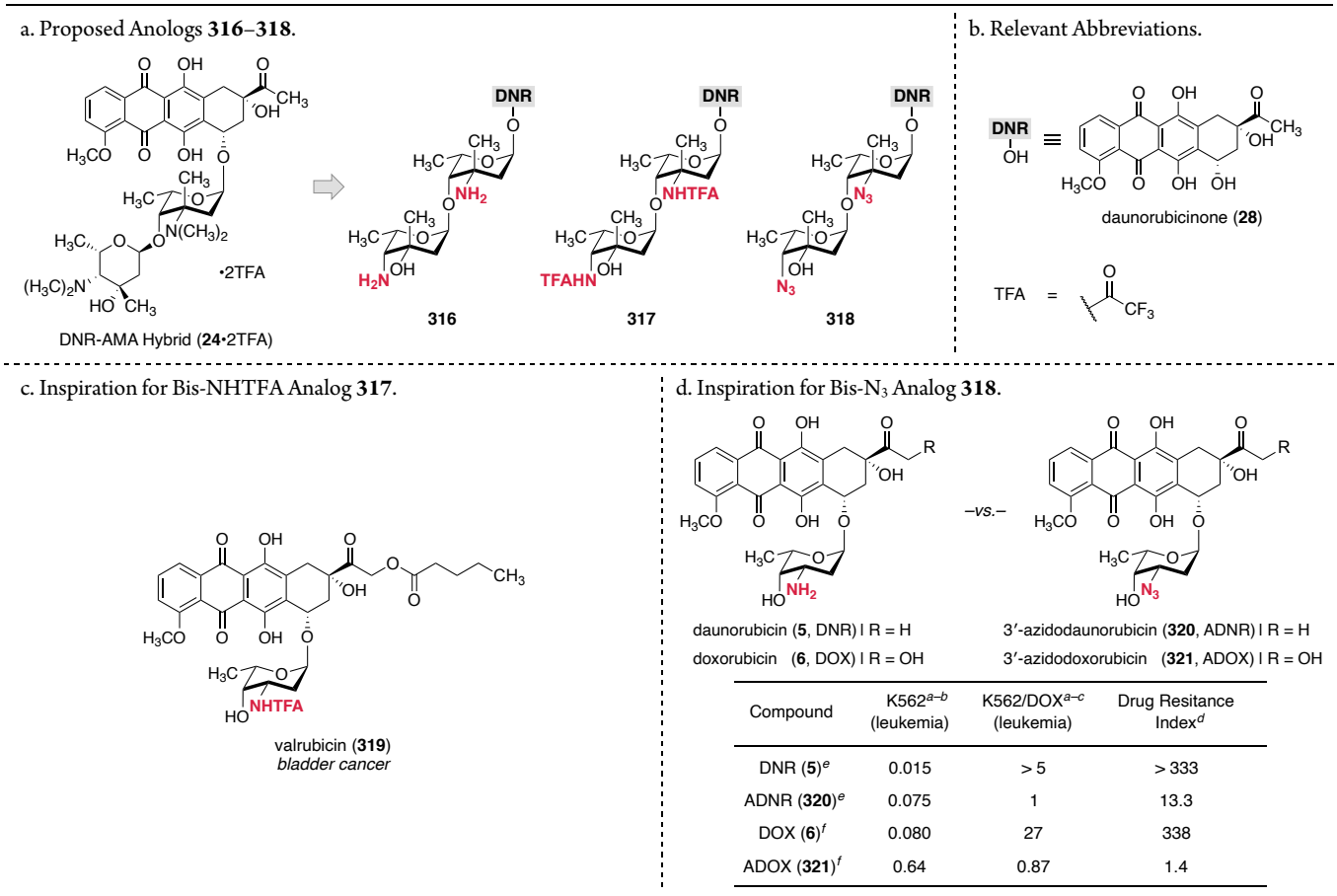
4.2 *Synthesis of Disaccharide Analogs to Develop Structure Activity Relationships*

As discussed in Chapter 1, arimetamycin A (**1**) bears a disaccharide composed of the rare branched deoxy-amino sugars L-brasiliose and L-lemonose, making it one of the few steffimycin anthracyclines to incorporate an amino sugar (Figure A.1)[10-13] and the only known anthracycline with two branched *N*,*N*-dimethylamino sugars (Figure A.2). To probe these unique aspects of the AMA disaccharide and develop its structure activity relationships (SARs), we propose below the synthesis of several derivatives of DNR-AMA hybrid **24**.

4.2.1 *Analog Proposal 1: DNR-AMA Hybrid (**24**) Analogs with Identically Substituted Amines*

To begin our investigation of the relationship between amine substitution and activity for DNR-AMA hybrid **24**, we propose the synthesis of derivatives **316**-**318** that possess identically substituted amines (Figure 4.3a). Synthesis of bis-NH_2 derivative **316** will provide a point of comparison to **24** that possesses similarly basic amines. In contrast, transformation of the amines to the corresponding trifluoroacetamides (**317**) and azides (**318**) will provide derivatives with no basic amines. We drew inspiration for bis-NHTFA derivative **317** from valrubicin (**319**), a synthetic doxorubicin derivative used to treat bladder cancer (Figure 4.3b).[14-17] Our interest in

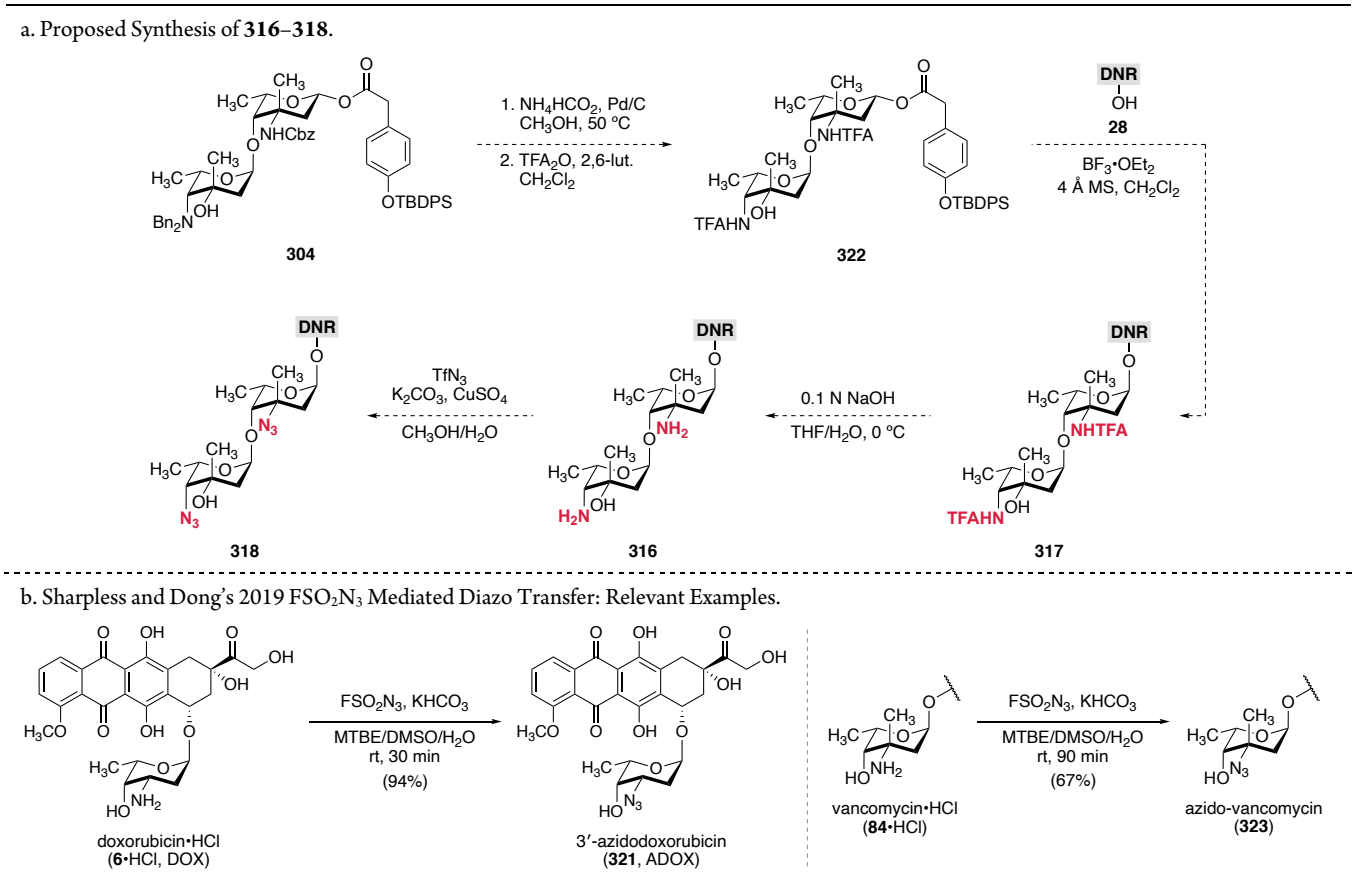
bis-N_3 derivative **318** stemmed from the ability of 3′-azidodaunorubucin (ADNR, **320**) and 3′-azidodoxorubicin (ADOX, **321**) to overcome resistance encountered by their parent compounds (Figure 4.3c).[18-19]



Compound	K562[a–b] (leukemia)	K562/DOX[a–c] (leukemia)	Drug Resitance Index[d]
DNR (**5**)[e]	0.015	> 5	> 333
ADNR (**320**)[e]	0.075	1	13.3
DOX (**6**)[f]	0.080	27	338
ADOX (**321**)[f]	0.64	0.87	1.4

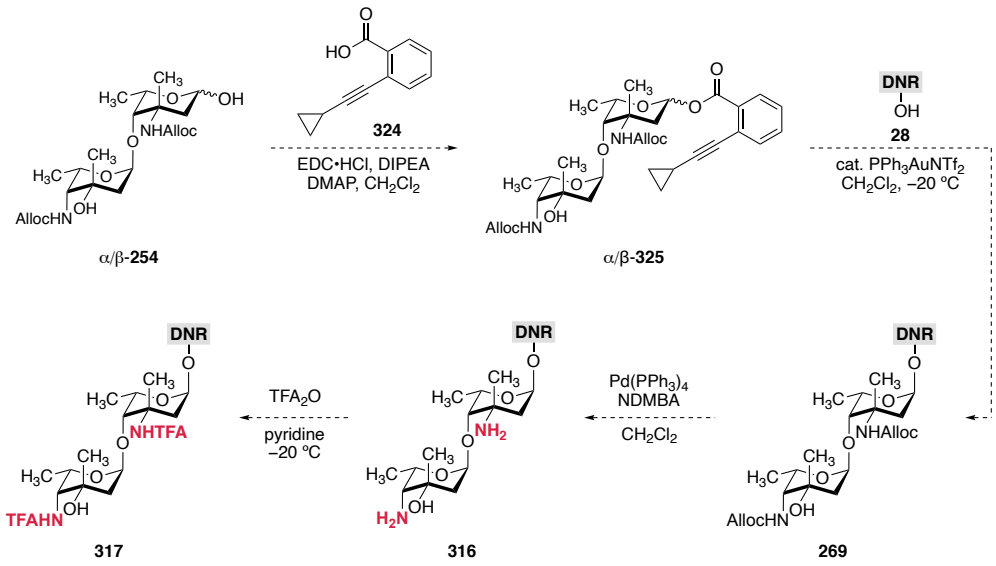
Figure 4.3 Proposed DNR-AMA Hybrid (**24**) Analogs with Identically Substituted Amines.[a]Cell line (cancer type). [b]IC_{50} values reported in μM. [c]Doxorubicin (**5**) resistant cell line. [d](IC_{50} for K562/DOX)/(IC_{50} for K562). [e]Data from Wang, P. G.; Sun, D. et al.[18] [f]Data from Wang, P. G.; Wang, F. et al.[19]

Our proposed route to these derivatives begins with previously described acyl glycoside **304**. Following deprotection of both amines using transfer hydrogenolysis, reprotection of the amines as their trifluoroacetamides would yield **322** (Scheme 4.4a). Subsequent BF_3•OEt_2 mediated glycosidation with daunorubicinone (**28**) would give targeted bis-NHTFA analog **317**. Trifluoroacetamide hydrolysis under basic conditions would reveal bis-NH_2 analog **316** which should yield bis-azido analog **318** upon subjection to Wong's diazo transfer conditions (TfN_3, $CuSO_4$)[20-21] in analogy to work from Wang and Sun.[18] If the diazo transfer proves problematic, Sharpless and Dong's recently published procedure employing FSO_2N_3 may prove more successful due to its increased reactivity[22]; notably, the authors report diazo transfer to doxorubicin•HCl (**6**•HCl) and vancomycin (**84**•HCl), demonstrating that their conditions are compatible with an anthracycline aglycone and effective even with sterically hindered amines (Scheme 4.4b).



Scheme 4.4 Proposed Synthesis of Analogs **316–318** Starting from Previously Described **304**.

Alternatively, if the glycosidation proves inefficient, we could employ an alternative strategy that takes advantage of Biao Yu's Au(I) catalyzed activation of ethynylbenzoate donors[23] as demonstrated by Neefjes and Codée in their synthesis of various doxorubicin and aclarubicin derivatives.[6] Starting from previously described hemiacetal α/β-**254**, anomeric esterification with benzoic acid derivative **324** would give α/β-**325** (Scheme 4.5). Subsequent Au(I) catalyzed glycosidation with daunorubicinone (**28**) would yield bis-Alloc species **269** which could be transformed to **316–317** upon manipulation of the amine substitution patterns.



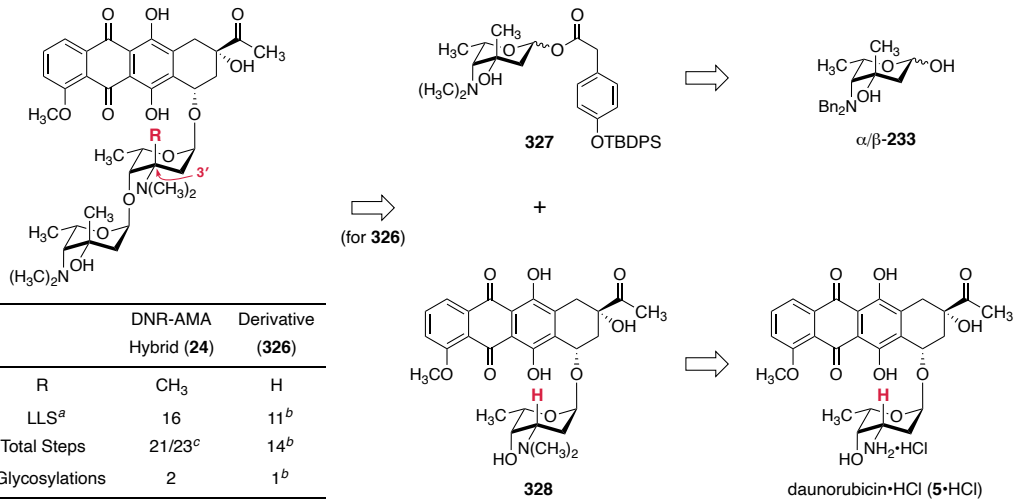
Scheme 4.5 Alternative Proposal for the Synthesis of **316–318** Starting from Previously Described α/β-**254**.

*4.2.2 Analog Proposal 2: DNR-AMA Hybrid **24** Analogs with No C3′ Branching*

From a medicinal chemistry perspective, the synthesis of analogs via the aforementioned approach is not ideal for introducing patterns of differing amine substituents (i.e., C3N′ ≠ C4N″) due to the similarity of the amine protecting groups and the fact that their differentiation would require individual deprotection and re-functionalization. Likewise, applying this approach to the synthesis of analogs with differing patterns of C3 branching would prove arduous, as each derivative would likely require the synthesis of a new disaccharide followed by protecting group adjustment and aglycone glycosidation. To circumvent these issues, we propose an alternative strategy involving the glycosylation of various L-lemonose donors with an intact, protected daunorubicin acceptor. Adopting this approach will allow for independent variation of the C3′ and C4″ amines in a convergent manner and will decrease the synthetic burden for each analog. However, this approach would only prove viable if C3′ branching is not critical for cytotoxicity. As such, the first task is the synthesis and biological evaluation of derivative **326**.

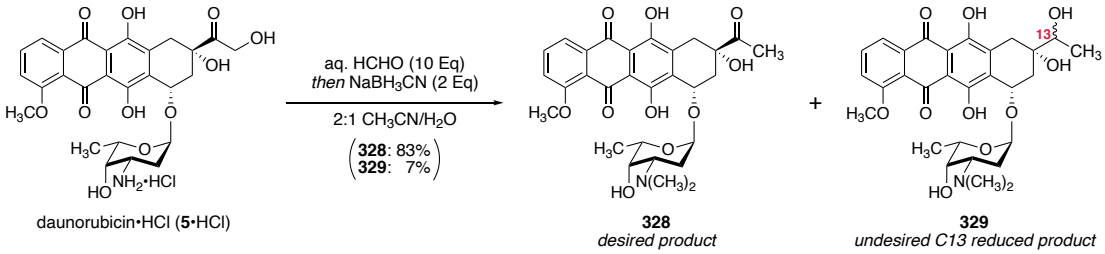
Retrosynthetically, we envisioned that **326** would result from the glycosylation of known *N*,*N*-dimethyl daunorubicin (**328**) with L-lemonose donor **327** (Scheme 4.6a). For the building blocks, we would derive **328** in one step from daunorubicin•HCl as described by Smith (Scheme 4.6b).[24] Finally, that we reasoned that we could achieve the synthesis of donor **327** from previously described diol **233.**

a. Retrosynthetic Analysis for Analog **326**.



	DNR-AMA Hybrid (**24**)	Derivative (**326**)
R	CH_3	H
LLS[a]	16	11[b]
Total Steps	21/23[c]	14[b]
# Glycosylations	2	1[b]

b. Smith's 1979 Synthesis of **328**.[d]

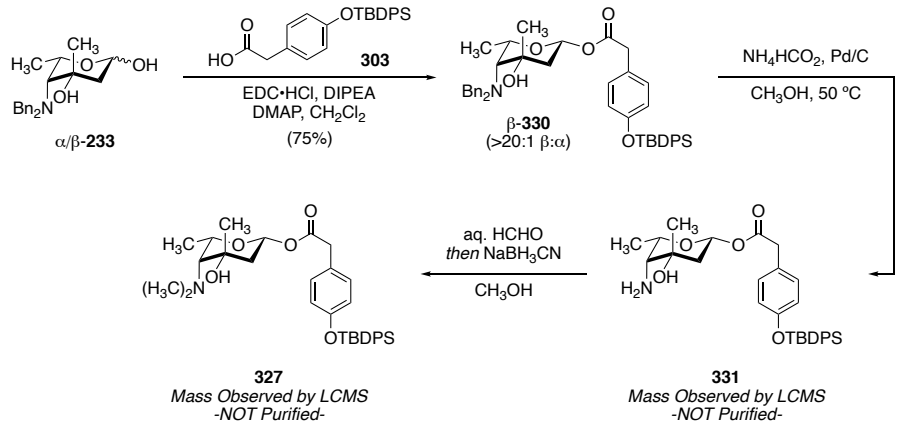


Scheme 4.6 Synthetic Planning for the Synthesis of Analog **326**.[a]LLS = longest linear sequence. [b]Based on route proposed herein. [c]21 steps using 4th generation disaccharide donor **287**; 23 steps using 5th generation disaccharide donor **300**. [d]Yields of free bases.

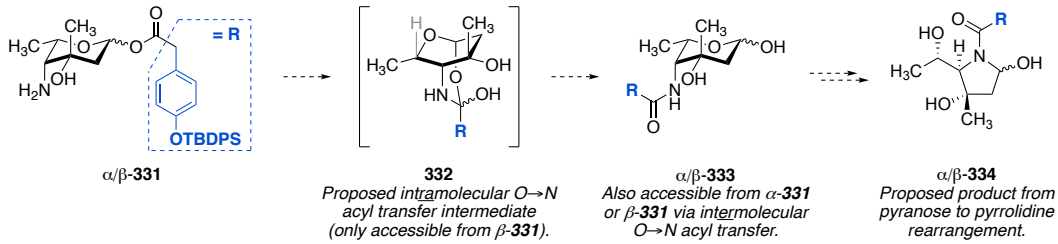
Although not discussed in Chapter 3, we used the synthesis **327** as a model system in optimizing our synthesis of 5th generation donor **300**. As such, we found that esterification diol α/β-**233** with previously described carboxylic acid **303** under Steglich-like conditions provided acyl glycoside α/β-**330** in good yield (Scheme 4.7a). Subsequent orienting studies with this material showed that hydrogenolysis afforded a compound with a mass corresponding to desired product α/β-**331**. However, we could not rule out the possibility of inter- or intramolecular *O* → *N* acyl transfer to give α/β-**333**; we further reasoned that α/β-**333** could undergo a subsequent pyranose to pyrrolidine rearrangement to yield α/β-**334** (Scheme 4.7b). As only desired product α/β-**331** has an amine that can condense with formaldehyde, we stirred the crude hydrogenolysis product(s) with excess formaldehyde (> 100 Eq) for 24 min prior to the addition of $NaBH_3CN$ in hopes that amine-formaldehyde condensation would drive the isomerization of any **333** or **334** to **331**. Gratifyingly, following the addition of reductant, LCMS indicated the conversion of starting material to a compound with a mass corresponding to desired product α/β-**327**. As this served as a proof of concept for the synthesis of **300**, we did not purify crude

α/β-**327**. However, we believe that the experiments performed to this point provide a solid starting point for preparation of donor α/β-**327**.

a. Progress toward the Synthesis of α/β-**327**.

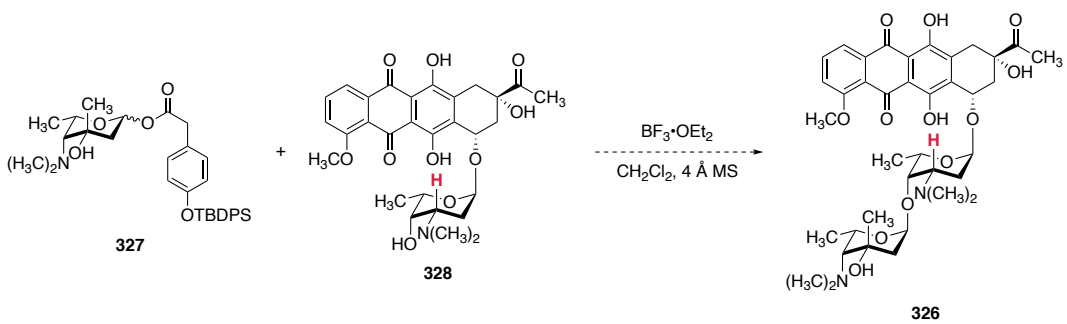


b. Potential Rearrangement of α/β-**331** into Isobaric Species α/β-**333** and α/β-**334**.



Scheme 4.7 Preliminary Work toward the Synthesis of L-Lemonose Monosaccharide Donor α/β-**327**.

Once we have donor α/β-**327** in hand, we envision that $BF_3 \bullet OEt_2$ mediated glycosylation with acceptor **328** will yield targeted analog **326** (Scheme 4.8). Assuming that the cytotoxicity of this simplified derivative proves comparable to doubly branched DNR-AMA hybrid **24**, we can proceed to the synthesis of other derivatives using this simplified synthetic strategy.



Scheme 4.8 Proposed Synthesis of Simplified Derivatives **326**.

We will first target **335–336** (Figure 4.4a) as informed by co-crystallization studies of DNA hexamer d(CGATCG) with disaccharide anthracyclines MEN10755 (**337**) and MAR70 (**338**) (Figure 4.4b) by the Ughetto lab. They found that co-crystallization of d(CGATCG) with MEN10755 afforded a non-symmetric ternary structure involving one DNA molecule and two MEN10755 molecules.[25] The lack of symmetry resulted from two distinct disaccharide binding modes. In one, both sugars occupy the minor groove; in the other, the non-reducing sugar protrudes out of the minor groove. On the other hand, co-crystallization of d(CGATCG) with MAR70 yielded a symmetric ternary structure in which the disaccharide of both bound MAR70 molecules lies completely in the minor groove.[26] Ughetto hypothesized that this discrepancy results from the presence of the positively charged 3′-amine of MAR70. As none of the compounds we have proposed to this point feature a non-basic C3′ amine and a basic C4″ amine, we hypothesize that the synthesis of derivatives **335** and **336** will allow us to further explore the SAR of the arimetamycin A disaccharide (Figure 4.4c).

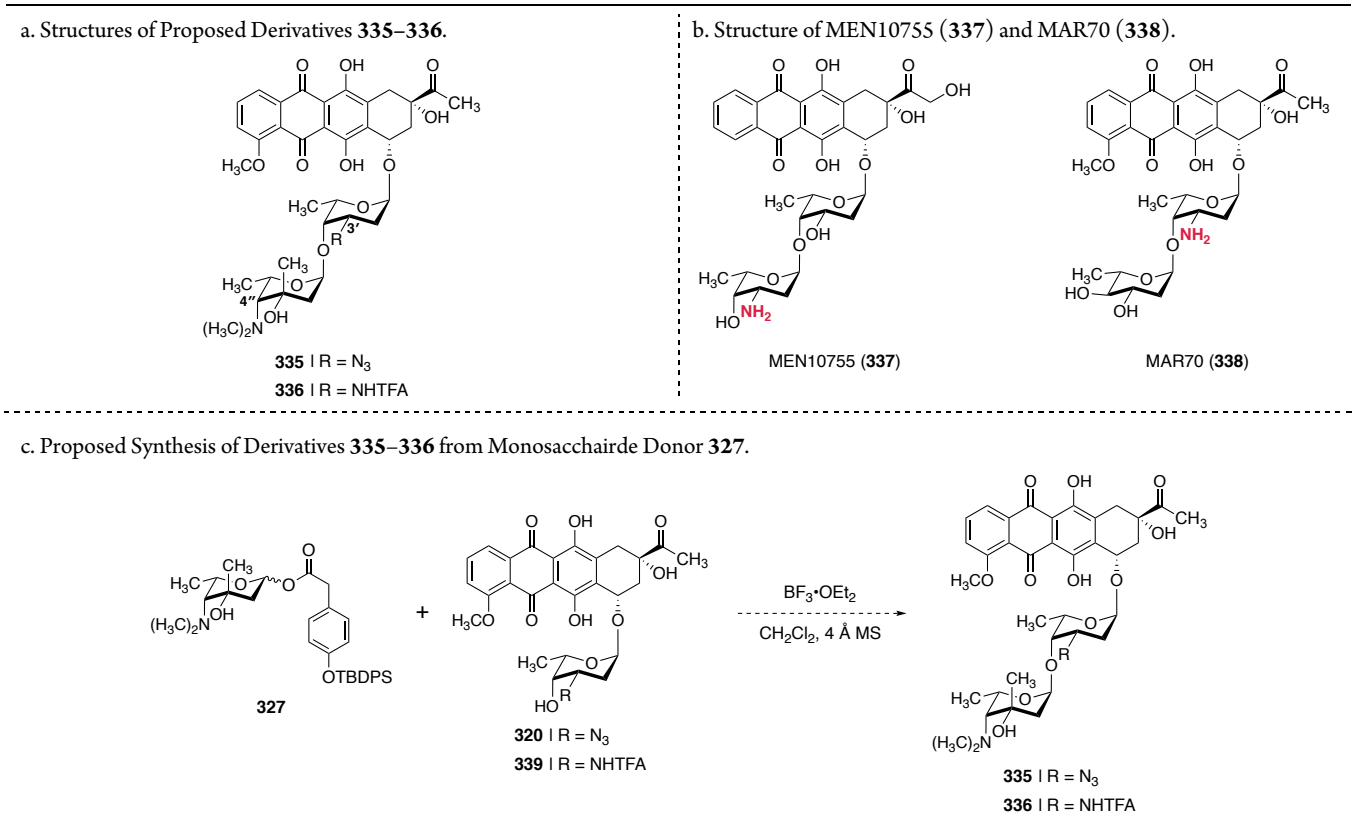


Figure 4.4 Proposed Derivatives **335–336** Featuring Non-Basic 3′-Amine and Basic 4″-Amine Substituents.

4.3 *Synthesis of Novel DNA-Alkylating Anthracyclines*

Work in the 1990s from various research groups established that various daunosamine containing anthracyclines form covalent complexes with DNA in the presence of formaldehyde via aminal formation between 3′ amino group of the anthracycline and an exocyclic N2 guanine moiety (Figure 4.5).[27-34] Hydrogen bonding between a neighboring guanine residue (N2 and N3) on the complementary strand and the anthracycline's C9 alcohol further stabilize this complex. Together, this collection of interactions constitutes a "virtual crosslink" as it functions as a crosslink but only involves one covalent interaction.

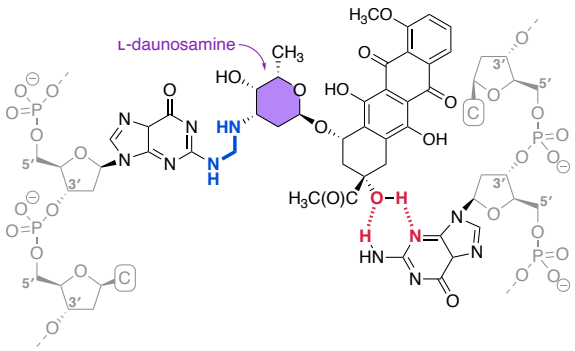
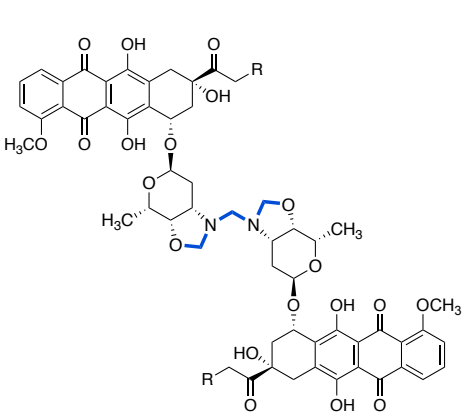


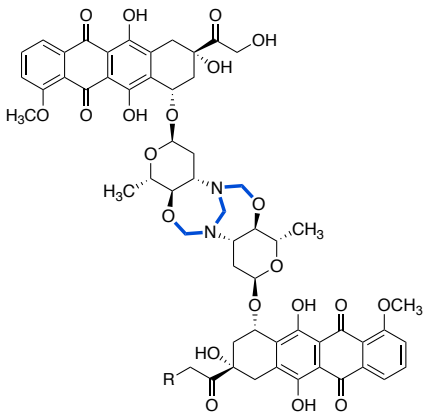
Figure 4.5 Virtual DNA Crosslinking by Daunorubicin (**5**).

Initial efforts to therapeutically exploit this crosslinking led to the synthesis of daunoform (**340**), doxoform (**341**), and epidoxoform (**342**) via the treatment of daunorubicin (**5**), doxorubicin (**6**), and epirubicin (**8**), respectively, with formaldehyde (Figure 4.6a–b).[35-36] These formaldehyde adducts proved more potent than their parent compounds against the MCF-7 breast cancer cell line and maintained their activity against the corresponding doxorubicin resistant cell line MCF-7/ADR (Figure 4.6c). Though *in vivo* investigation of doxoform, the most potent compound, is hindered by its rapid hydrolysis to doxorubicin ($t_{1/2}$ ~ 3 min at 37 °C in pooled human serum), the more stable epidoxoform proved more effective than epidoxorubicin in an *in vivo* mouse study. Furthermore, *N*-carbamate derivatives (**343**) of doxazolidine (**345**) have received attention as prodrugs due to their increased stability and their potential for selective activation (Figure 4.6d).[37-39]

a. Structures of Daunoform (**340**), Doxoform (**341**), and Epidoxoform (**342**).

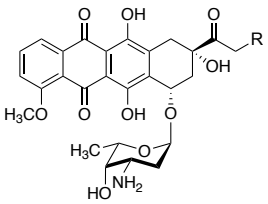


daunoform (**340**, DNR-F) | R = H
doxoform (**341**, DOX-F) | R = OH

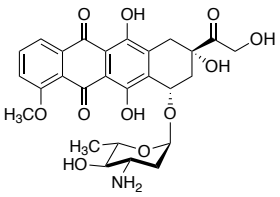


epidoxoform (**342**, EPI-F)

b. Structures of Parent Compounds.



daunorubicin (**5**, DNR) | R = H
doxorubicin (**6**, DOX) | R = OH

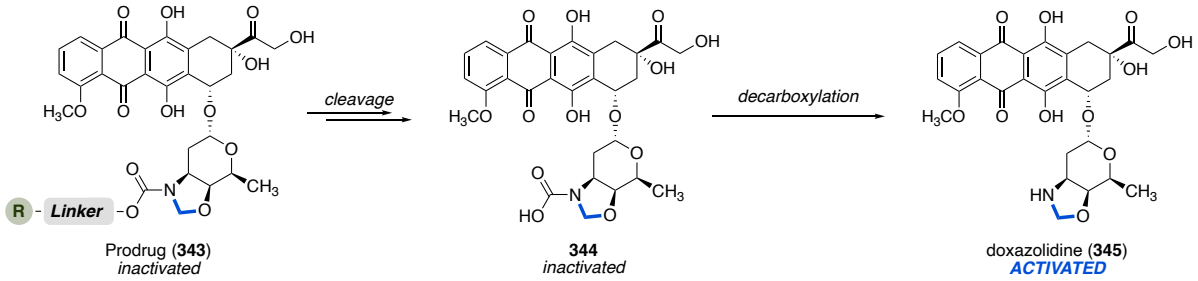


epirubicin (**8**, EPI)

c. Cytotoxicity Parent Compounds vs. Formaldehyde Adducts.[a]

Compound	MCF-7[b–c] (breast)	MCF-7/DOX[b–d] (breast)	Drug Resitance Index[e]
DNR (**5**)	60	2000	33.3
DNR-F (**340**)	8	10	1.25
DOX (**6**)	300	10,000	33.3
DOX-F (**341**)	2	1	0.5
EPI (**8**)	200	>10,000	>50
EPI-F (**342**)	65	70	1.1

d. General Scheme for the Activation of Doxazolidine Prodrugs (**343**) to Doxazolidine (**345**).



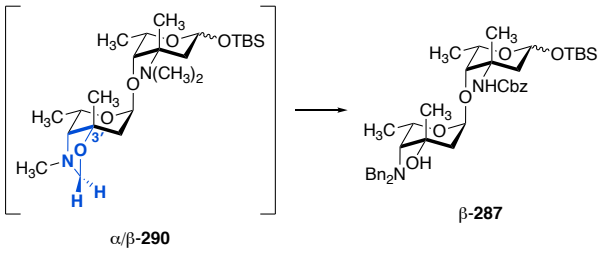
Prodrug (**343**) *inactivated*
344 *inactivated*
doxazolidine (**345**) ***ACTIVATED***

Figure 4.6 Non-Comprehensive Overview of Cytotoxic Formaldehyde-Anthracycline Adducts.
[a]Data from Koch, T. H. et al.[36] [b]Cell line (cancter type). [c]IC_{50} values reported in nmolar equiv./L as daunoform (**340**), doxoform (**341**), and epidoxoform (**342**) each contain two equivalents of anthracycline. [d]Doxorubicin (**5**) resistant cell line. [e](IC_{50} for K562/DOX)/(IC_{50} for K562).

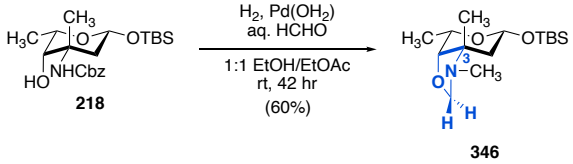
In a contrasting observation, we noted that the synthesis of 4th generation donor β-**287** involved the intermediacy of oxazolidine **290** which only opened under the influence of excess reagents (Scheme 4.9a). In a similar vein, we isolated oxazolidine **346** in 60% yield in an attempted one pot hydrogenolysis/reductive amination of **218**, further demonstrating the stability of these oxazolidines (Scheme 4.9b). We posit that the surprising stability of these oxazolidines results from steric shielding by the neighboring tetrasubstituted C3(′) centers. We hypothesize that incorporation of similar steric hindrance into DNA alkylating anthracyclines will

extend their half-life in aqueous solution and thus increase their suitability for clinical applications. With this in mind, we propose below the synthesis of several novel, sterically hindered alkylating anthracycline oxazolidines.

a. Synthesis of **287** Via Oxazolidine Intermediate **290**.



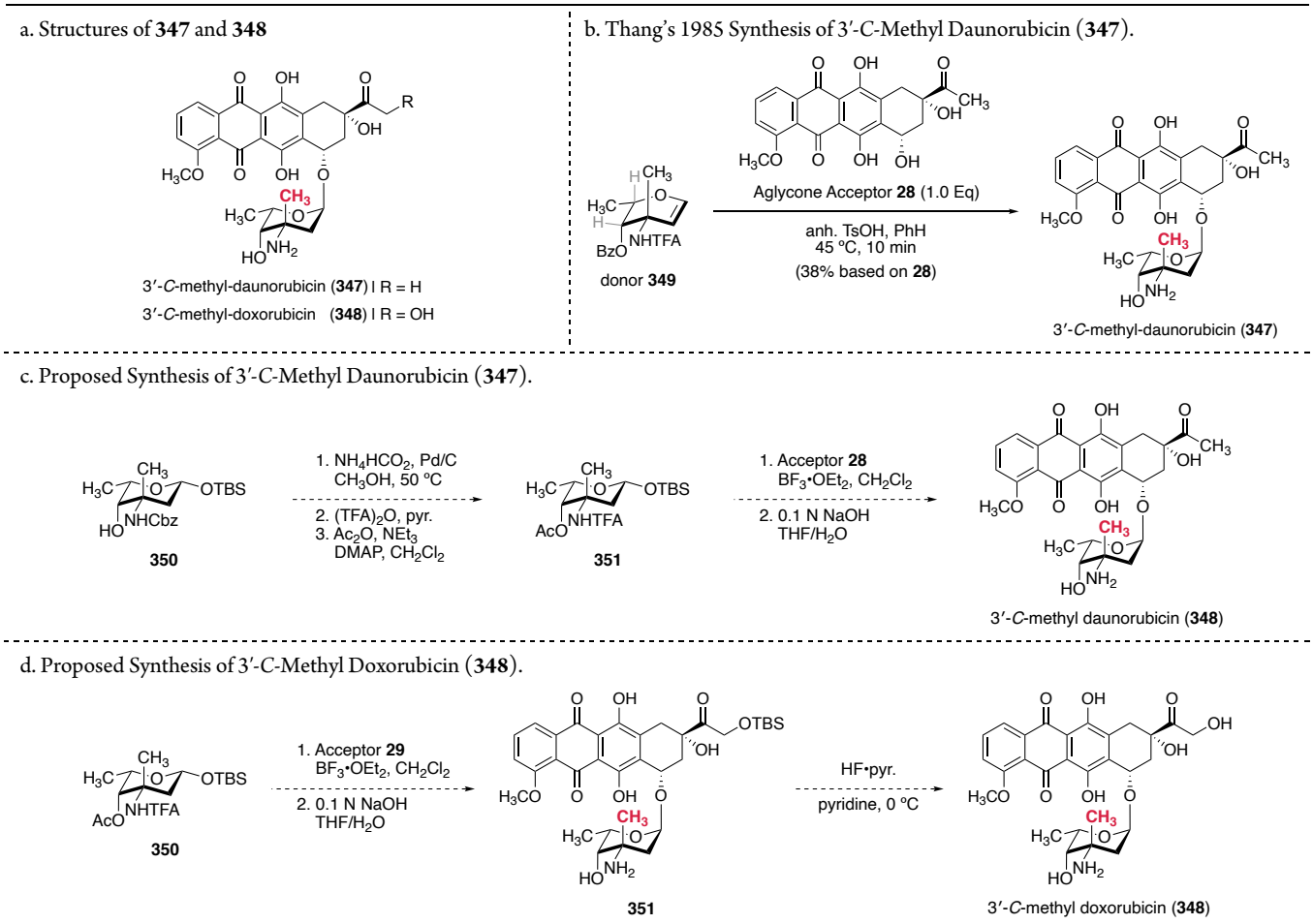
b. Synthesis of Oxazolidine from **346**.



Scheme 4.9 Inadvertent Formation of Oxazolidines **290** and **346**.

4.3.1 *Proposal 1: Alkylating Anthracyclines Based on 3′-C-Methyl Daunorubicin and Doxorubicin*

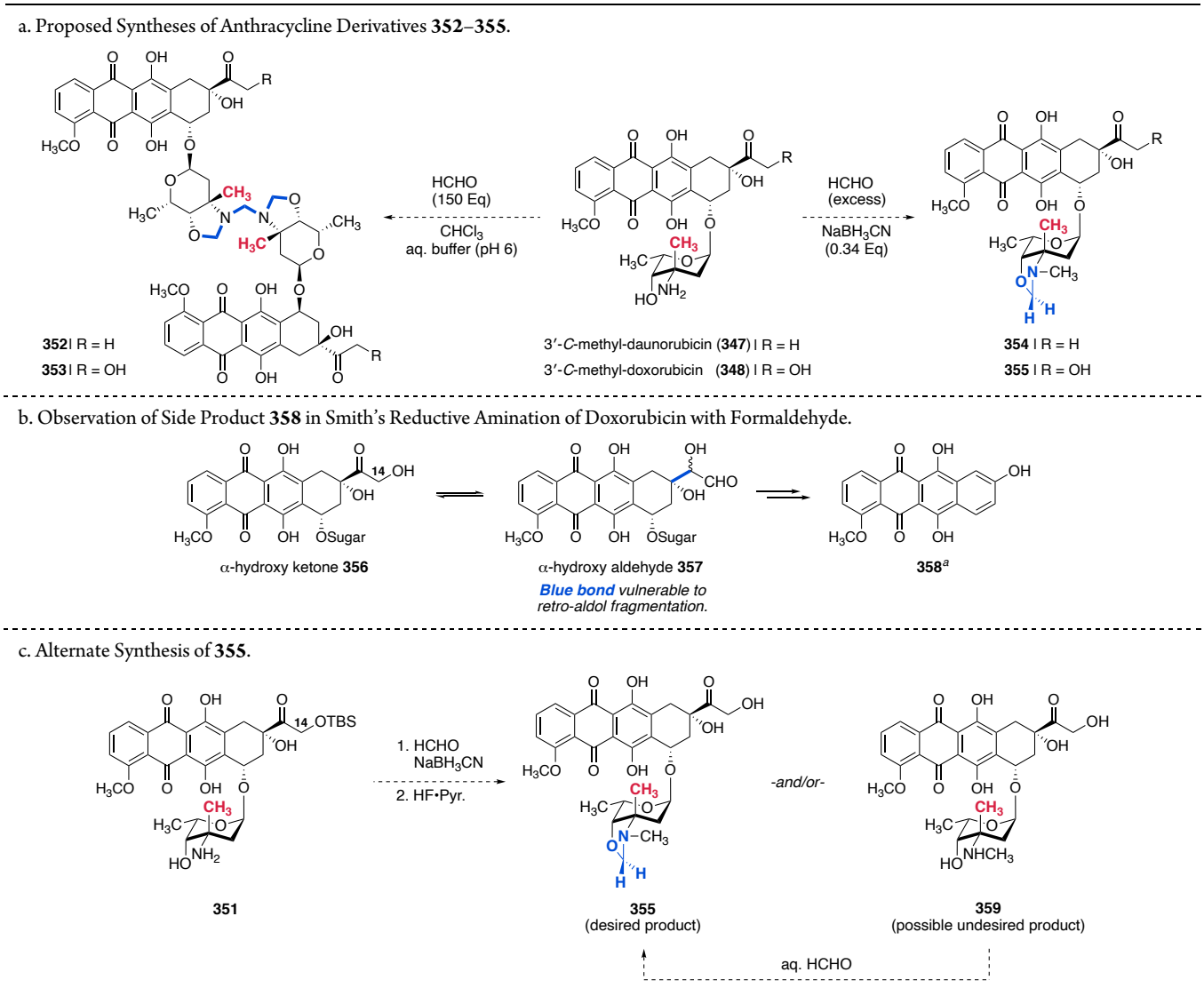
In our first series of derivatives, we propose the synthesis of derivatives based on 3′-*C*-methyl daunorubicin (**347**) and 3′-*C*-methyl doxorubicin (**348**) (Scheme 4.11a). As such, our first task is the synthesis of **347** and **348**. Though Thang demonstrated the synthesis of **347** using glycal donor **349** and daunorubicinone (Scheme 4.11b), we hypothesize that the use of an *O*-TBS glycoside donor will prove more efficient since we already have access to L-brasiliose donor **218** and have demonstrated the successful glycosidation of protected *O*-TBS glycoside donor **246** with **28**. With this in mind, our synthesis will begin with the transformation of **218** to fully protected donor **350** (Scheme 4.11c). Next, we envision that $BF_3 \bullet OEt_2$ glycosidation with daunorubicinone (**28**) followed by LiOH mediated deprotection will yield **347**. We will prepare 3′-*C*-methyl doxorubicin (**348**) in a similar manner as we propose that glycosidation of donor **350** with previously described protected doxorubicinone acceptor **29** followed by treatment with LiOH will give silyl ether **351** (Scheme 4.11d). Finally, desilylation with HF•pyridine will yield **348**.



Scheme 4.10 Proposed Synthesis of 3′-*C*-Methyl Daunorubicin (**347**) and 3′-*C*-Methyl Doxorubicin (**349**).

With **347** and **348** in hand, we will use Chaires' protocol[40] to verify that the addition of the 3′-*C*-methyl group does not inhibit formaldehyde mediated DNA alkylation. Assuming that **347** and **348** prove capable of alkylating DNA in the presence of formaldehyde, we will then synthesize two sets of derivatives. First, we will treat **347** and **349** with excess formaldehyde as described by Koch[35] with the goal of making **352** and **353**, the 3′-*C*-methyl analogs of daunoform (**340**) and doxoform (**341**), respectively (Scheme 4.11a). For the second set of derivatives, we will subject **347** and **348** to formaldehyde and one equivalent of hydride (i.e., ca. 0.34 Eq of $NaBH_3CN$) in order to arrive at monomeric oxazolidines **354** and **355**, respectively. We anticipate that the reductive amination of 3′-*C*-methyl doxorubicin (**348**) may prove problematic as Smith observed reduced yields for the reductive amination of doxorubicin (**6**) in comparison to daunorubicin (**5**). The authors hypothesized that this resulted from tautomerization of α-hydroxy ketone **356** to α-hydroxy aldehyde **357** as the later compound can fragment via a retro-aldol reaction (Scheme 4.11b); they based this hypothesis on the isolation of aromatized aglycone **358** during column chromatography.[24] As such, if the synthesis of **355** proves difficult from 3′-*C*-methyl doxorubicin (**348**), we will perform the reductive amination prior to removal of the C14-OTBS protecting group

as we anticipate that the protecting group will prevent **351** from undergoing α-hydroxy ketone to α-hydroxy aldehyde tautomerization (Scheme 4.11c). If the oxazolidine ring proves unstable to the silyl ether deprotection, we will treat the resulting disubstituted amine **359** with formaldehyde to reincorporate this one carbon unit.



Scheme 4.11 Proposed Synthesis Alkylating Anthracycline Derivatives **352–355**. [a]Smith notes that **358** likely formed during chromatography as it was not observed in the crude reaction mixture prior to purification.[24]

4.3.2 Proposal 2: Alkylating Anthracyclines Based on the Arimetamycin A Disaccharide

In 1996, the Chaires lab determined that DNA-anthracycline cross linking is highly dependent on the presence of a 3′-NH_2 group as both hydroxyrubicin (**361**, no amine) and WP608 (**362**, 4′-NH_2) did not alkylate DNA in the presence of formaldehyde (Figure 4.7a).[40] The authors rationalized this on structural grounds as intercalation brings 3′-NH_2 close to its N2-guanine formaldehyde binding partner; in contrast, the 4′-NH_2 of **362** is expected to point out of the minor groove, away from the relevant N2-guanine. With this in mind, we reasoned

that attaching an L-lemonose based *N*-methyl oxazolidine directly to an anthracycline aglycone (e.g., **363**) would yield an ineffective alkylator (Figure 4.7b). However, we wondered if an anthracycline decorated with a disaccharide terminating in such a residue would prove effective. With this in mind, we propose the synthesis of L-lemonose terminated derivatives **364**–**366** and L-daunosamine terminated derivatives **367**–**369** (Figure 4.7c).

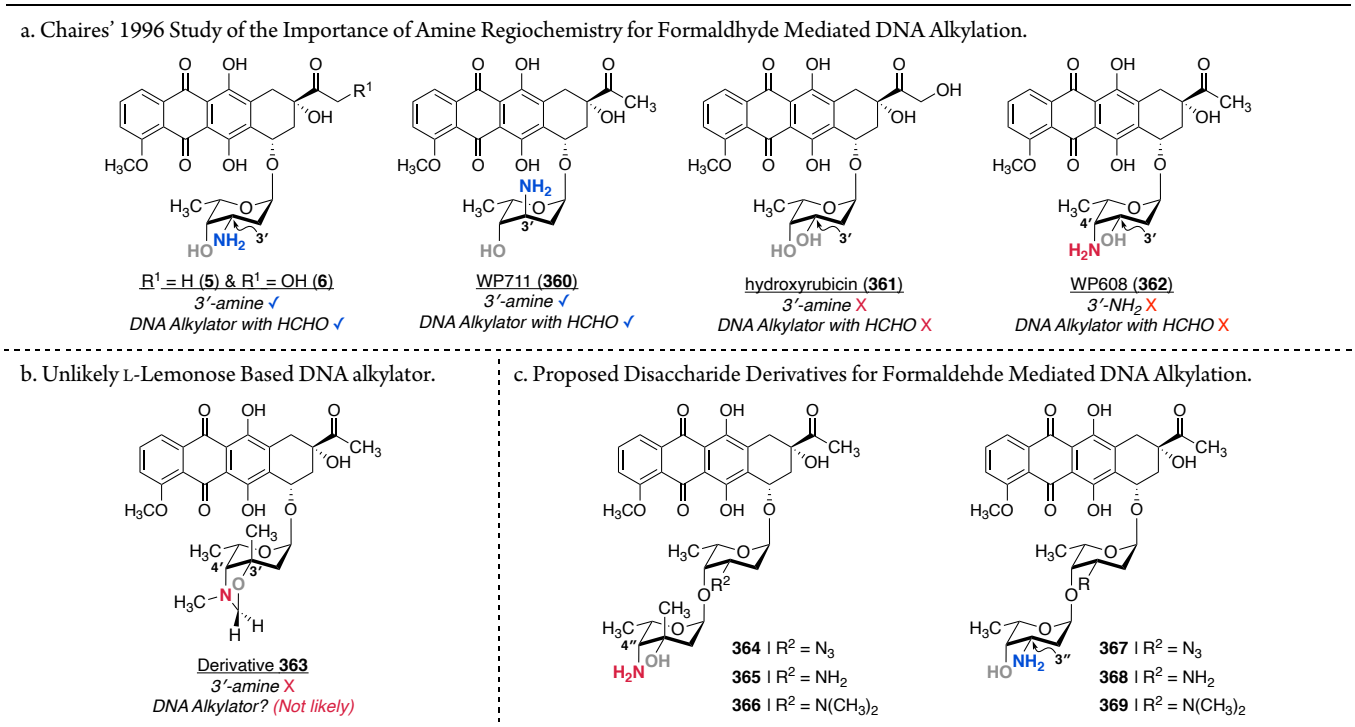
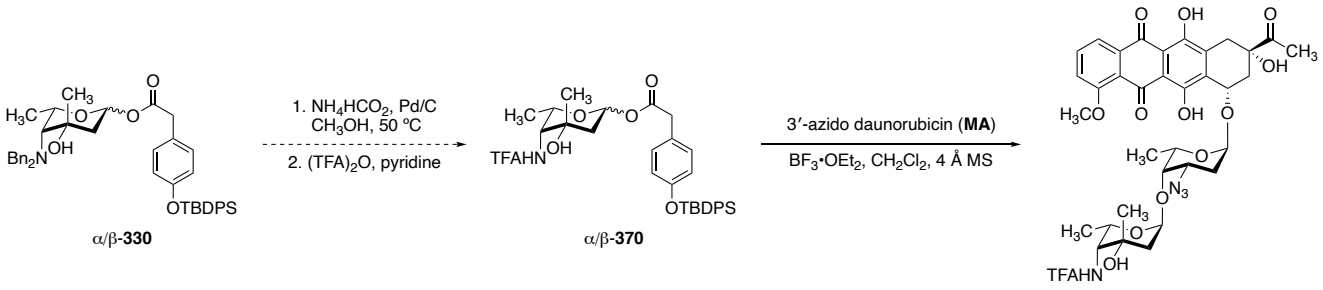


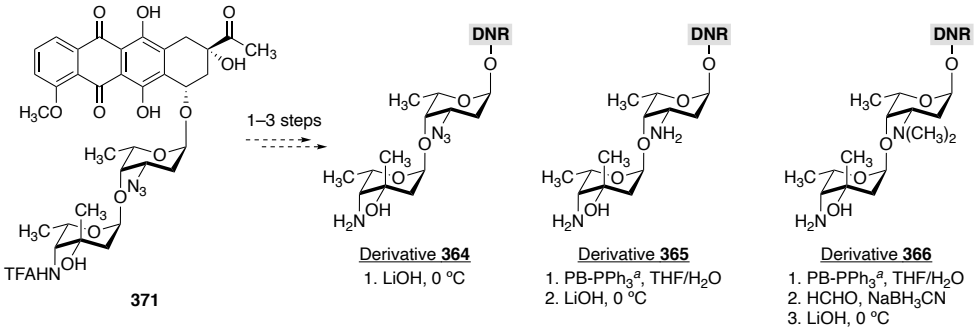
Figure 4.7 Rational Design of Proposed Derivatives **364**–**369**.

We will commence synthesis of **364**–**366** from previously described L-lemonose acyl glycoside α/β-**330**. Following a proposed conversion of the debenzylated amine to its anthracycline compatible trifluoroacetamide, we envision that glycosidation of **370** with known 3′-azido daunorubicin **320** will yield **371** (Scheme 4.12a). Subsequent combinations of trifluoroacetamide deprotection, Staudinger reduction, and reductive amination will afford **364**–**366** from **371** (Scheme 4.12b). We propose a similar synthesis of **367**–**369** starting from known L-daunosamine donor **372** (Scheme 4.12c). Once the derivatives are in hand, we will use Charies' procedure[40] to determine which, if any, of these analogs alkylate DNA in the presence of formaldehyde. Any positive results will serve as a starting point for the development of novel disaccharide-functionalized, alkylating anthracyclines.

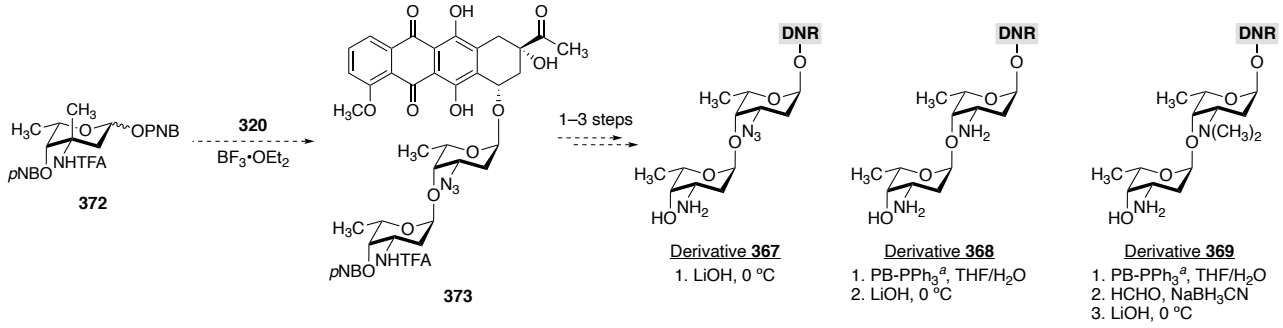
a. Proposed Synthesis of Intermediate **371**.



b. Proposed Transformation of Intermediate **371** to Derivatives **364**–**366**.



c. Proposed Synthesis of Derivatives **367**–**369**.[b]



Scheme 4.12 Proposed Synthesis of Potential Formaldehyde Mediated DNA Alkylators **364**–**369**. [a]PB-PPh_3 = polymer bound triphenylphosphine. [b]*p*NB = *para*-nitro benzoyl.

4.4 *References for Chapter 4*

(1) Kelly, R. C.; Schletter, I.; Koert, J. M.; MacKellar, F. A.; Wiley, P. F. Structures of Steffimycin and Steffimycin B. *J. Org. Chem.* **1977**, *42*, 3591-3596.
(2) Bergy, M. E.; Coats, J. H.; Fritz, R. Antibiotic Steffisburgensimycin and Method of Producing. US3309273A, **1967**.
(3) Bergy, M. E.; Reusser, F. A New Antibacterial Agent (U-20,661) Isolated from a Streptomycete Strain. *Experientia* **1967**, *23*, 254-255.
(4) Dietz, A. *Streptomyces Steffisburgensis* Sp.N. *J. Bacteriol.* **1967**, *94*, 2022-2026.
(5) Horton, D.; Priebe, W.; Varela, O. Synthesis and Antitumor Activity of 3'-Deamino-3'-Hydroxydoxorubicin. A Facile Procedure for the Preparation of Doxorubicin Analogs. *J Antibiot (Tokyo)* **1984**, *37*, 853-858.
(6) Wander, D. P. A.; van der Zanden, S. Y.; van der Marel, G. A.; Overkleeft, H. S.; Neefjes, J.; Codee, J. D. C. Doxorubicin and Aclarubicin: Shuffling Anthracycline Glycans for Improved Anticancer Agents. *J. Med. Chem.* **2020**, *63*, 12814-12829.
(7) Kuhn, M.; von Wartburg, A. Uber Ein Neues Glykosidierungsverfahren. Ii. Glykoside Des 4'-Demethylpipodophyllotoxins. *Helv. Chim. Acta* **1969**, *52*, 948-57.
(8) Lee, K. H.; Imakura, Y.; Haruna, M.; Beers, S. A.; Thurston, L. S.; Dai, H. J.; Chen, C. H.; Liu, S. Y.; Cheng, Y. C. Antitumor Agents, 107. New Cytotoxic 4-Alkylamino Analogues of 4'-Demethyl-Epipodophyllotoxin as Inhibitors of Human DNA Topoisomerase Ii. *J. Nat. Prod.* **1989**, *52*, 606-13.
(9) Li, T.-K.; Chan, N.-L.; Chein, T.-C. Human Topoisomerase Ii-Targeting Organoplatinum Compounds. WO 2020/037652 A1, **2020**.
(10) Krohn, K.; Broser, E. Synthetic Anthracyclinones. 25. An Improved Route to 8-Demethoxyaranciamycinone and Synthesis of the A-L-Daunosamine Glycosides. *J. Org. Chem.* **1984**, *49*, 3766-3771.
(11) Renous, B.; Gaudin, N.; Gesson, J.-P.; Jacquesy, J.-C. New Process for the Preparation of Steffimycinone Analogues, New Analogues of Steffimycinone Thus Obtained, New Intermediates Useful for the Synthesis of These Compounds, Use of These Compounds as Antibacterial Agents and for the Synthesis of Antitumour Agents. FR2543545A1, **1985**.
(12) Steffimycinone Analogs. JP61047439A, **1986**.
(13) Krohn, K.; Carlson, G.; Köhle, H.; Hussain, H.; Green, I. R. Synthesis of Daunosamyl Anthraquinone and Anthracyclinone Analogues. *Arkivoc* **2008**, *2008*, 216-233.
(14) Israel, M.; Tinter, S. K.; Lazarus, H.; Brown, B.; Modest, E. J. Adriamycin Derivatives: Preparation and Antitumor Evaluation. Abstracts, Eleventh International Cancer Congress, Florence, Italy, Florence, Italy, **October 1974**; pp 752-753.
(15) Israel, M.; Modest, E. J.; Frei, E. I. *N*-Trifluoroacetyladriamycin-14-Valerate, an Analog with Greater Experimental Antitumor Activity and Less Toxicity Than Adriamycin. *Cancer* **1975**, *35*, 1365-1368.
(16) Israel, M.; Modest, E. J. *N*-Trifluoroacetyladriamycin-14-Alkanoates and Therapeutic Compositions Containing Same. US4035566A, **1977**.
(17) Onrust, S. V.; Lamb, H. M. Valrubicin. *Drugs Aging* **1999**, *15*, 69-75; discussion 76.
(18) Zhang, G.; Fang, L.; Zhu, L.; Zhong, Y.; Wang, P. G.; Sun, D. Syntheses and Biological Activities of 3'-Azido Disaccharide Analogues of Daunorubicin against Drug-Resistant Leukemia. *J. Med. Chem.* **2006**, *49*, 1792-1799.
(19) Yu, S.; Zhang, G.; Zhang, W.; Luo, H.; Qiu, L.; Liu, Q.; Sun, D.; Wang, P.-G.; Wang, F. Synthesis and Biological Activities of a 3'-Azido Analogue of Doxorubicin against Drug-Resistant Cancer Cells. *Int. J. Mol. Sci.* **2012**, *13*, 3671-3684.
(20) Alper, P. B.; Hung, S.-C.; Wong, C.-H. Metal Catalyzed Diazo Transfer for the Synthesis of Azides from Amines. *Tetrahedron Lett.* **1996**, *37*, 6029-6032.
(21) Nyffeler, P. T.; Liang, C. H.; Koeller, K. M.; Wong, C. H. The Chemistry of Amine-Azide Interconversion: Catalytic Diazotransfer and Regioselective Azide Reduction. *J. Am. Chem. Soc.* **2002**, *124*, 10773-8.
(22) Meng, G.; Guo, T.; Ma, T.; Zhang, J.; Shen, Y.; Sharpless, K. B.; Dong, J. Modular Click Chemistry Libraries for Functional Screens Using a Diazotizing Reagent. *Nature* **2019**, *574*, 86-89.
(23) Yu, B. Gold(I)-Catalyzed Glycosylation with Glycosyl O-Alkynylbenzoates as Donors. *Acc. Chem. Res.* **2018**, *51*, 507-516.
(24) Tong, G. L.; Wu, H. Y.; Smith, T. H.; Henry, D. W. Adriamycin Analogues. 3. Synthesis of *N*-Alkylated Anthracyclines with Enhanced Efficacy and Reduced Cardiotoxicity. *J. Med. Chem.* **1979**, *22*, 912-918.
(25) Temperini, C.; Messori, L.; Orioli, P.; Di Bugno, C.; Animati, F.; Ughetto, G. The Crystal Structure of the Complex between a Disaccharide Anthracycline and the DNA Hexamer D(Cgatcg) Reveals Two Different Binding Sites Involving Two DNA Duplexes. *Nucleic Acids Res.* **2003**, *31*, 1464-9.
(26) Temperini, C.; Cirilli, M.; Aschi, M.; Ughetto, G. Role of the Amino Sugar in the DNA Binding of Disaccharide Anthracyclines: Crystal Structure of the Complex Mar70/D(Cgatcg). *Bioorg. Med. Chem.* **2005**, *13*, 1673-9.
(27) Gao, Y. G.; Liaw, Y. C.; Li, Y. K.; van der Marel, G. A.; van Boom, J. H.; Wang, A. H. Facile Formation of a Crosslinked Adduct between DNA and the Daunorubicin Derivative Mar70 Mediated by Formaldehyde: Molecular Structure of the Mar70-D(Cgtnacg) Covalent Adduct. *Proc Natl Acad Sci U S A* **1991**, *88*, 4845-9.
(28) Wang, A. H.; Gao, Y. G.; Liaw, Y. C.; Li, Y. K. Formaldehyde Cross-Links Daunorubicin and DNA Efficiently: Hplc and X-Ray Diffraction Studies. *Biochemistry* **1991**, *30*, 3812-5.

(29) Zhang, H.; Gao, Y. G.; van der Marel, G. A.; van Boom, J. H.; Wang, A. H. Simultaneous Incorporations of Two Anticancer Drugs into DNA. The Structures of Formaldehyde-Cross-Linked Adducts of Daunorubicin-D(Cg(Arac)Gcg) and Doxorubicin-D(Ca(Arac)Gtg) Complexes at High Resolution. *J. Biol. Chem.* **1993**, *268*, 10095-10101.
(30) Cullinane, C.; van Rosmalen, A.; Phillips, D. R. Does Adriamycin Induce Interstrand Cross-Links in DNA? *Biochemistry* **1994**, *33*, 4632-8.
(31) Taatjes, D. J.; Gaudiano, G.; Resing, K.; Koch, T. H. Alkylation of DNA by the Anthracycline, Antitumor Drugs Adriamycin and Daunomycin. *J. Med. Chem.* **1996**, *39*, 4135-8.
(32) Taatjes, D. J.; Gaudiano, G.; Koch, T. H. Production of Formaldehyde and DNA-Adriamycin or DNA-Daunomycin Adducts, Initiated through Redox Chemistry of Dithiothreitol/Iron, Xanthine Oxidase/Nadh/Iron, or Glutathione/Iron. *Chem. Res. Toxicol.* **1997**, *10*, 953-61.
(33) Koch, T. H.; Barthel, B. L.; Kalet, B. T.; Rudnicki, D. L.; Post, G. C.; Burkhart, D. J. Anthracycline-Formaldehyde Conjugates and Their Targeted Prodrugs. *Top. Curr. Chem.* **2008**, *283*, 141-70.
(34) Cutts, S.; Nudelman, A.; Rephaeli, A.; Phillips, D. The Power and Potential of Doxorubicin-DNA Adducts. *IUBMB Life* **2005**, *57*, 73-81.
(35) Fenick, D. J.; Taatjes, D. J.; Koch, T. H. Doxoform and Daunoform: Anthracycline-Formaldehyde Conjugates Toxic to Resistant Tumor Cells. *J. Med. Chem.* **1997**, *40*, 2452-61.
(36) Taatjes, D. J.; Fenick, D. J.; Koch, T. H. Epidoxoform: A Hydrolytically More Stable Anthracycline-Formaldehyde Conjugate Toxic to Resistant Tumor Cells. *J. Med. Chem.* **1998**, *41*, 1306-14.
(37) Burkhart, D. J.; Barthel, B. L.; Post, G. C.; Kalet, B. T.; Nafie, J. W.; Shoemaker, R. K.; Koch, T. H. Design, Synthesis, and Preliminary Evaluation of Doxazolidine Carbamates as Prodrugs Activated by Carboxylesterases. *J. Med. Chem.* **2006**, *49*, 7002-12.
(38) Barthel, B. L.; Rudnicki, D. L.; Kirby, T. P.; Colvin, S. M.; Burkhart, D. J.; Koch, T. H. Synthesis and Biological Characterization of Protease-Activated Prodrugs of Doxazolidine. *J. Med. Chem.* **2012**, *55*, 6595-607.
(39) Tamura, R.; Balabanova, A.; Frakes, S. A.; Bargmann, A.; Grimm, J.; Koch, T. H.; Yin, H. Photoactivatable Prodrug of Doxazolidine Targeting Exosomes. *J. Med. Chem.* **2019**, *62*, 1959-1970.
(40) Leng, F.; Savkur, R.; Fokt, I.; Przewloka, T.; Priebe, W.; Chaires, J. B. Base Specific and Regioselective Chemical Cross-Linking of Daunorubicin to DNA. *J. Am. Chem. Soc.* **1996**, *118*, 4731-4738.

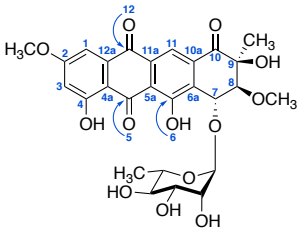
APPENDIX A

Supplementary Figures and Tables

A.1 *Supplementary Figures*

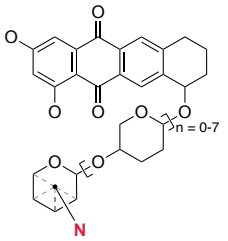
Figure A.1 Scifinder Survey of Steffimycins that Feature Amino Sugars.

a. Structure of Steffimycin (**4**).



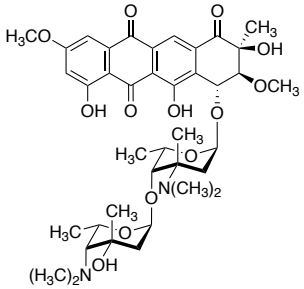
steffimycin (**4**)

b. Survey of Amino Sugar Containing Steffimycins (Search 1).[a-b]

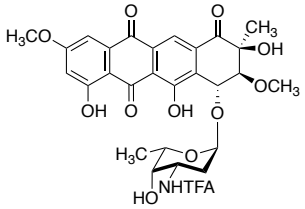


Search 1
13 substances
[*arimetamycin A* ✓]

c. Results from Search 1 Containing Full Steffimycin Aglycone.[c]

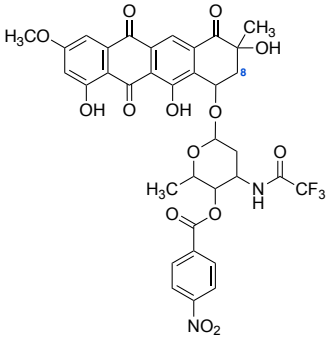


CAS No. 1477521-85-9
[arimetamycin A, **1**]

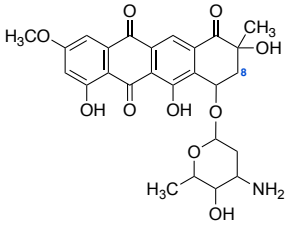


CAS No. 91310-99-5

d. Results from Search 1 Containing 8-Demethoxy Steffimycin Aglycone.[d]



CAS No. 95245-03-7

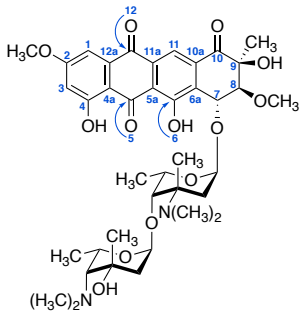


CAS No. 95256-25-0

[a]Scifinder search performed on 15-December-2020. Substructure search performed with one limiter ("single component"); results given as number of substances returned by SciFinder. Answer sets containing arimetamycin A (**1**) are designated with checkmarks; answer sets not containing arimetamycin A (**1**) are designated by strikethrough. [b]Search 1 was performed with n = 0-7 (as opposed to n = 0-8 for Searches 2-21, see Figure S2) because the query with n = 0-8 was too complex for Scifinder (as verified on 18-December-2020). [c]For CAS No. 91310-99-5, see Krohn, K. et al.[1-2]; in Krohn et al.[1], the authors refer to the corresponding free amine of CAS No. 91310-99-5 but do not explicitly provide its structure. [d]For CAS No. 95245-03-7 and 95256-25-0, see patents FR2543545A1 [3] and JP61047439A[4].

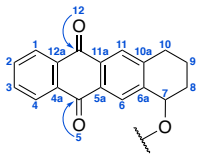
Figure A.2 Scifinder Survey of Anthracyclines that Feature Amino Sugars.

a. Structure of Arimetamycin A (**1**).



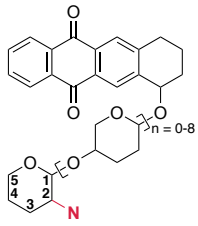
arimetamycin A (**1**)
CAS No. 1477521-85-9

b. Caveat.

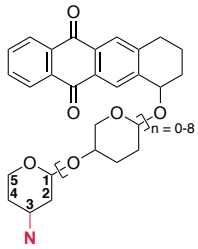


Note: The searches described in this table were only designed to provide a comprehensive examination of C7 (or by symmetry C10) glycosidated anthracyclines. As such, sugars attached to any other carbons were not accounted for in these searches.

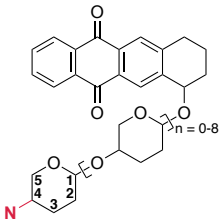
c. Survey of Anthracyclines with at Least 1 Amino Sugar.[a-b]



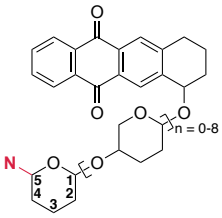
Search 2
18 substances
[~~*arimetamycin A*~~]



Search 3
9059 substances
[*arimetamycin A* ✓]

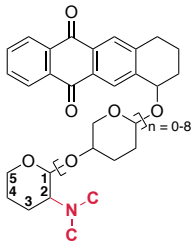


Search 4
238 substances
[*arimetamycin A* ✓]

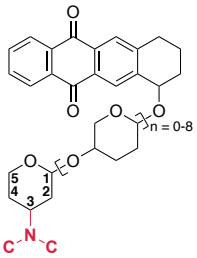


Search 5
0 substances
[~~*arimetamycin A*~~]

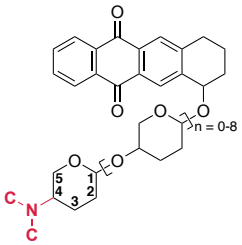
d. Survey of Anthracyclines with at Least 1 *N,N*-Substituted Amino Sugar.[a-b]



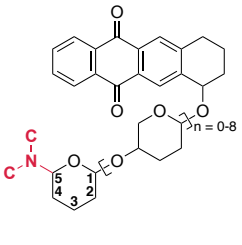
Search 6
2 substances
[~~*arimetamycin A*~~]



Search 7
1588 substances
[*arimetamycin A* ✓]

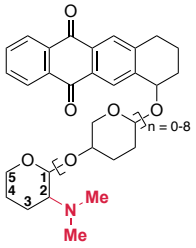


Search 8
23 substances
[*arimetamycin A* ✓]

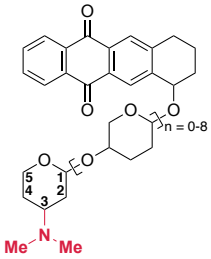


Search 9
0 substances
[~~*arimetamycin A*~~]

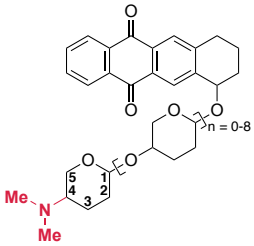
e. Survey of Anthracyclines with at Least 1 *N,N*-Dimethyl Amino Sugar.[a-b]



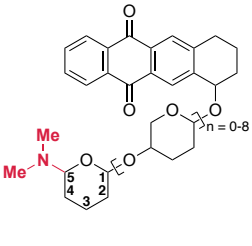
Search 10
2 substances
[*arimetamycin A*]



Search 11
434 substances
[*arimetamycin A* ✓]



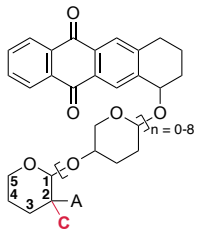
Search 12
12 substances
[*arimetamycin A* ✓]



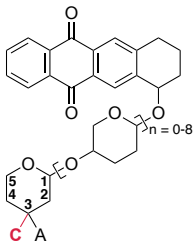
Search 13
0 substances
[*arimetamycin A*]

Figure A.2 Continued

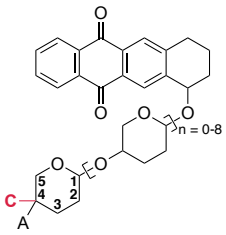
f. Survey of Anthracyclines with at Least 1 Branched Amino Sugar.[a-c]



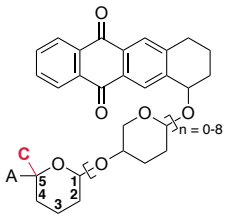
Search 14
1 substance
[~~*arimetamycin A*~~]



Search 15
82 substances
[*arimetamycin A*✓]

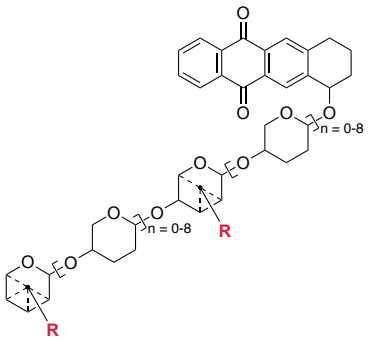


Search 16
31 substances
[~~*arimetamycin A*~~]

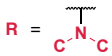


Search 17
0 substances
[~~*arimetamycin A*~~]

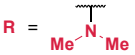
g. Survey of Anthracyclines with 2 Amino Sugars.[a-b]



Search 18
59 substances
[*arimetamycin A* ✓]

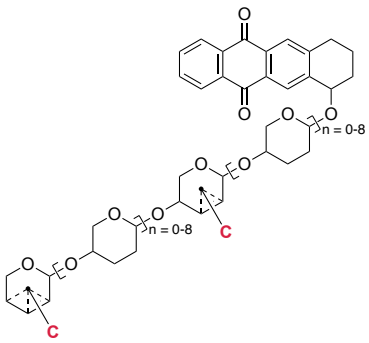


Search 19
8 substances
[*arimetamycin A* ✓]



Search 20
5 substances
[*arimetamycin A* ✓]

h. Survey of Anthracyclines with 2 Branched Sugars.[a-b]



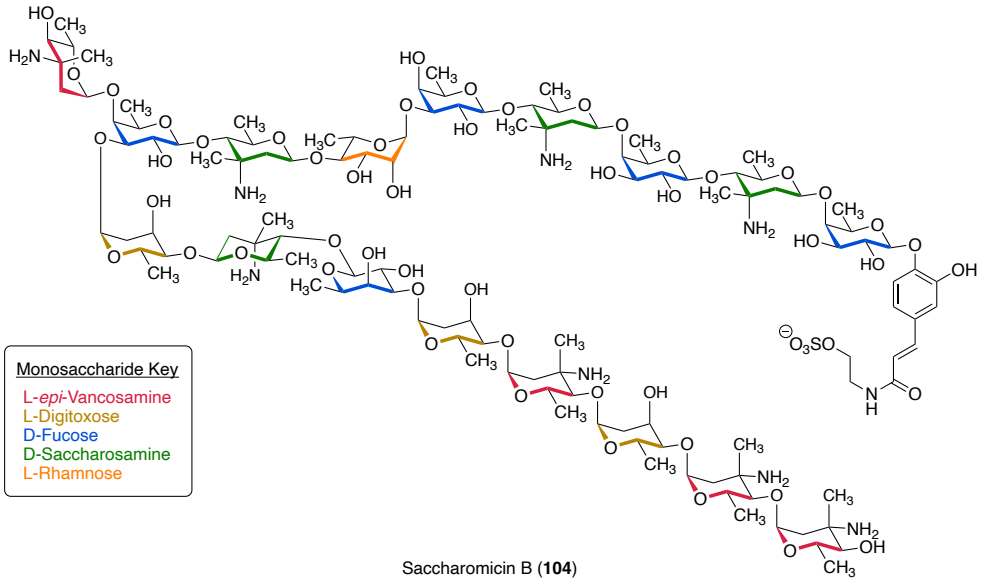
Search 21
3 substances
[*arimetamycin A* ✓]

i. Arimetamycin A (**1**) is the Only Known Anthracylcine with 2 Branched *N,N*-Dimethylamino Sugars.[a-b]

CAS Number	Search 19	Search 20	Search 21
1477521-85-9 (Arimetamycin A, **1**)	Yes	Yes	Yes
1346005-82-0	No	No	Yes
1339939-19-3	No	No	Yes
1071160-73-0	Yes	No	No
79391-08-5	Yes	Yes	No
79366-07-7	Yes	No	No
79366-02-2	Yes	No	No
79366-01-1	Yes	Yes	No
70559-01-2	Yes	Yes	No
70559-00-1	Yes	Yes	No

[a]Scifinder searches performed on 15-December-2020. Substructure searches performed with one limiter ("single component"); results given as number of substances returned by SciFinder. Answer sets containing arimetamycin A (**1**) are designated with checkmarks; answer sets not containing arimetamycin A (**1**) are designated by strikethrough. [b]Evaluate search results in light of "Note" in Figure S1b. [c]"A" = any atom other than hydrogen.

Figure A.3 Full Structure of Saccharomicin B (**104**).

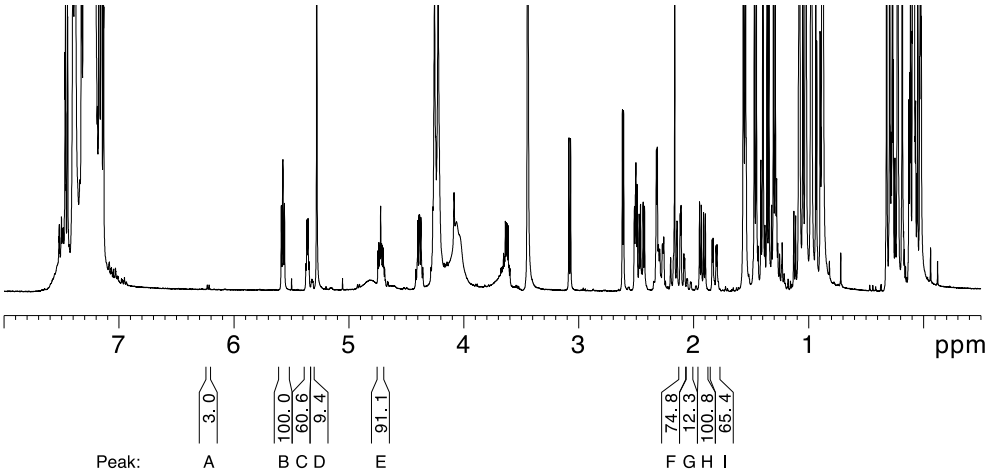


Saccharomicin B (**104**)

Figure A.4 Data and Analysis for Table 2.2.

a. Structures of Relevant Compounds.

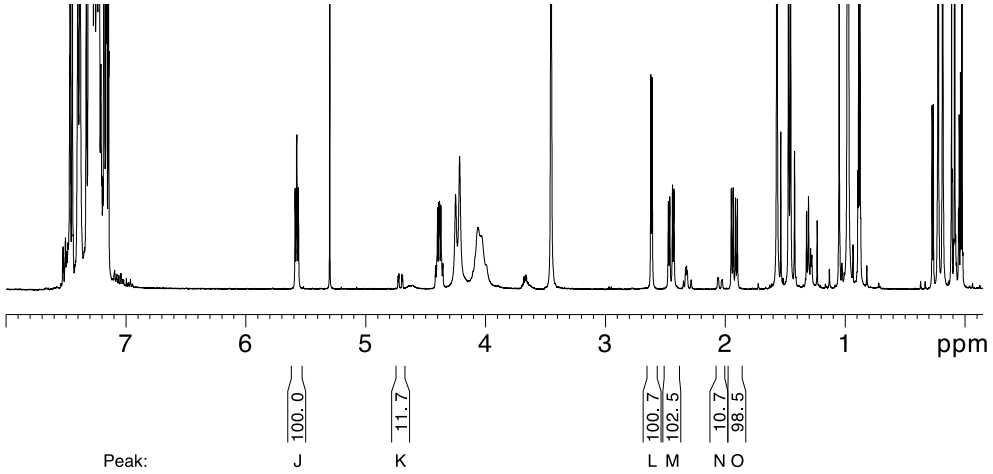
b. Crude ^{1}H NMR (400 MHz, $CDCl_3$) Data for Table 2.1, Entry 1.[a]



Peak	Compound (Assignment)	Data from Crude Mixture (Entry 1)[b]			Purified Compounds[c]	
		δ ^{1}H	^{1}H Mult. (Hz)	^{1}H Int.	δ ^{1}H	^{1}H Mult. (Hz)
A	**225** (H1)	6.22	d (6.3)	3.0	6.21	d (6.3)
B	α-**216** (H1)	5.57	dd (5.8, 5.1)	100.0	5.57	dd (5.9, 5.1)
C	α-**226** (H1)	5.36	app q (3.7)	60.6	5.36	app q (3.7)
D	α-**235** (H1)	5.32	dd (3.8, 2.5)	9.4	5.31	dd (3.7, 2.3)
E	β-**226** (H1)	4.72	app ddd (9.3, 6.1, 3.2)	91.1	4.73	ddd (9.7, 5.8, 2.8)
F	β-**226** ($H2_{pro\text{-}S}$)	2.10	ABX dd (12.7, 2.7)	74.8	2.10	dd (12.8, 2.3)
G	β- **216** ($H2_{pro\text{-}S}$)	2.04	dd (13.2, 2.1)	12.3	2.04	dd (13.1, 1.8)
H	α- **216** ($H2_{pro\text{-}S}$)	1.92	dd (13.9, 6.2)	100.8	1.92	dd (13.9, 6.2)
I	α-**226** ($H2_{pro\text{-}S}$)	1.82	dd (13.7, 3.1)	65.4	1.81	dd (13.7, 3.2)

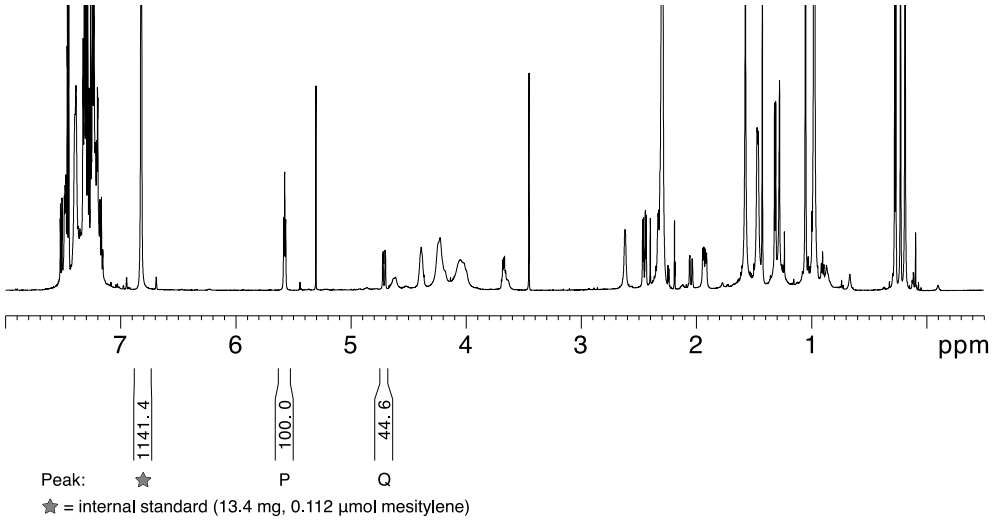
Figure A.4 Continued

c. Crude ^{1}H NMR (400 MHz, $CDCl_3$) Data for Table 2.1, Entry 2.[d]



Peak	Compound (Assignment)	Data from Crude Mixture (Entry 2)[b]			Purified Compounds[c]	
		δ ^{1}H	^{1}H Mult. (Hz)	^{1}H Int.	δ ^{1}H	^{1}H Mult. (Hz)
J	α-**216** (H1)	5.57	dd (5.8, 5.2)	100.0	5.57	dd (5.9, 5.1)
K	β-**216** (H1)	4.71	dd (12.4, 2.6)	11.7	4.71	dd (12.4, 2.6)
L	α-**216** (H4)	2.61	d (4.3)	100.7	2.61	d (4.3)
M	α-**216** ($H2_{pro\text{-}R}$)	2.45	dd (13.9, 4.8)	102.5	2.45	dd (13.9, 4.8)
N	β-**216** ($H2_{pro\text{-}S}$)	2.04	app dd (15.5, 2.2)	10.7	2.04	dd (13.1, 1.8)
O	α-**216** ($H2_{pro\text{-}S}$)	1.92	dd (13.9, 6.2)	98.5	1.92	dd (13.9, 6.2)

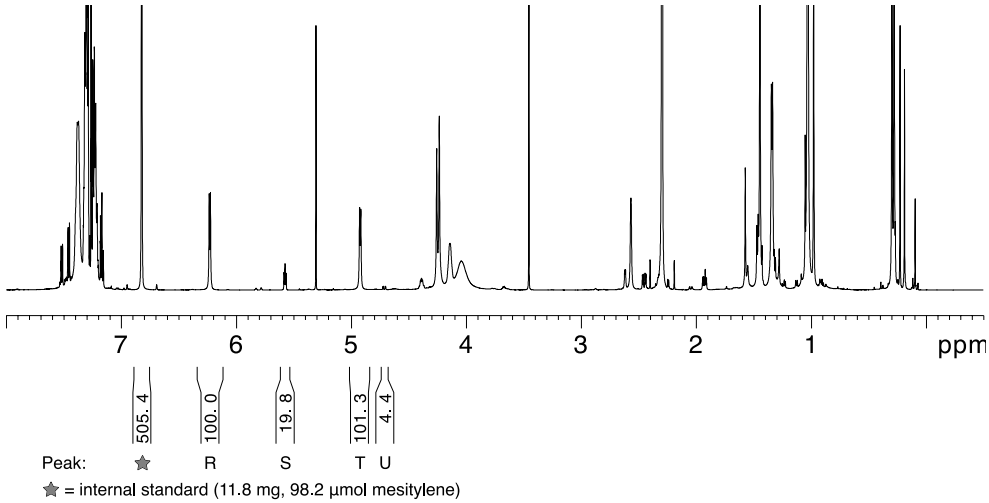
d. Crude ^{1}H NMR (600 MHz, $CDCl_3$) Data for Table 2.1, Entry 3.



Peak	Compound (Assignment)	Data from Crude Mixture (Entry 3)[c]			Purified Compound[c]	
		δ ^{1}H	^{1}H Mult. (Hz)	^{1}H Int.	δ ^{1}H[a]	^{1}H Mult. (Hz)
P	α-**216** (H1)	5.57	app "t" (5.4)	100.0	5.57	dd (5.9, 5.1)
Q	β-**216** (H1)	4.71	dd (12.4, 2.5)	44.6	4.71	dd (12.4, 2.6)

Figure A.4 Continued

d. Crude ^{1}H NMR (600 MHz, $CDCl_3$) Data for Table 2.1, Entry 4.

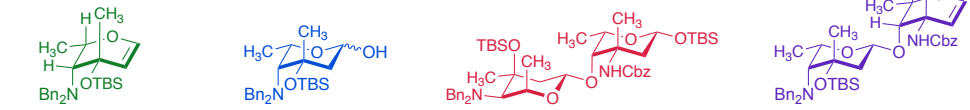


Peak	Compound (Assignment)	Data from Crude Mixture (Entry 3)[c]			Purified Compound(s)[c]	
		δ ^{1}H	^{1}H Mult. (Hz)	^{1}H Int.	δ ^{1}H	^{1}H Mult. (Hz)
R	**225** (H1)	6.24	d (6.2)	100.0	6.21	d (6.3)
S	α-**216** (H1)	5.58	app dd (5.8, 5.1)	19.9	5.57	dd (5.9, 5.1)
T	**225** (H2)	4.92	d (6.1)	101.3	4.90	d (6.2)
U	β-**216** (H1)	4.71	dd (12.4, 2.6)	4.4	4.71	dd (12.4, 2.6)

[a]See page 157. "Procedure (Step 1)" for synthetic procedure. [b] ^{1}H, 400 MHz, $CDCl_3$ – referenced to δ 7.26. [c] ^{1}H, 600 MHz, $CDCl_3$ – referenced to δ 7.26.

Figure A.5 Analysis of $CDCl_3$ Soluble Crude Material from Gram Scale Glycosylation of Donor **216** and Acceptor **218** to Yield α-Linked Disaccharide **215.**[a]

a. Compounds Identified from $CDCl_3$ Soluble Crude Material.



b. Table of Peaks from Crude NMR of $CDCl_3$ Soluble Crude Material.

Peak	Compound (Assignment)	Data from Crude Post-Glycosylation Mixture					Data from Purified Compounds		
		δ ^{1}H[b]	δ ^{13}C[c]	$^1J_{CH}$ (Hz)[d]	^{1}H Mult. (Hz)	^{1}H Int.[e]	δ ^{1}H[b]	δ ^{13}C[c]	$^1J_{CH}$ (Hz)[d]
A	Unassigned	6.32	97.4	---	d (5.9)	3.9	---	---	---
B	**243** (C1)	6.31	143.2	187	d (6.1)	6.6	6.30	143.2	---[j]
C	**225** (C1)	6.22	143.0	195	d (6.3)	10.3	6.21	143.1	---[j]
D	Unassigned[f]	6.18	141.5	188	d (6.3)	1.2	---	---	---
E	Unassigned	6.11	88.9	162	dd (10.5, 1.9)	---	---	---	---
F	Unassigned	5.89	93.0	165	dd (11.2, 2.3)	---	---	---	---
G	Unassigned[f]	5.76	99.8	183	d (4.6)	8.4	---	---	---
H	Unassigned	5.69	---	---	d (2.3)	0.4	---	---	---
I	α-**226** (C1)	5.37	91.1	165	app t (3.6)	5.3	5.36	91.1	166
J	Unassigned	5.31	91.7	163	br dd (4, 2)[g]	5.7	---	---	---
K	**225** (C2)	4.91	110.4	162	---	---	4.90	110.4	---[j]
L	**215** (C1′)	4.90	98.0	162	dd (7.2, 2.8)	105.5	4.89	98.0	162
M	**215** (C1)	4.80	94.2	155	br d (9.0)	110.7	4.79	94.2	155
N	**243** (C2)	4.75	105.0	166	d (6)[h]	22.5[i]	4.74	105.0	166
O	β-**226** (C1)	4.74	99.4	159	dd (10, 3)[h]		4.73	94.5	159
P	**243** (C1′)	4.47	102.0	158	dd (11, 3)[h]	---	4.46	101.3	159
Q	Unassigned	4.10	90.5	165	br s	15.6	---	---	---

c. Annotated Crude ^{1}H NMR (600 MHz, $CDCl_3$).[k]

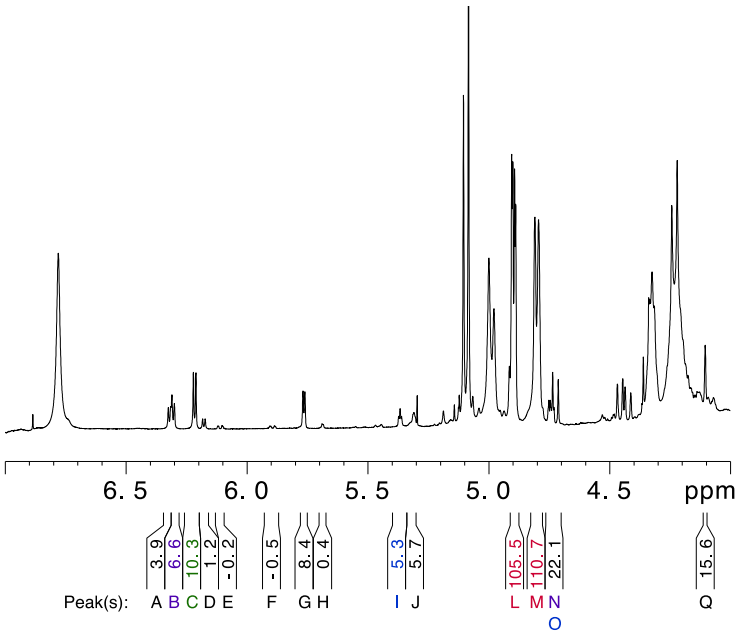
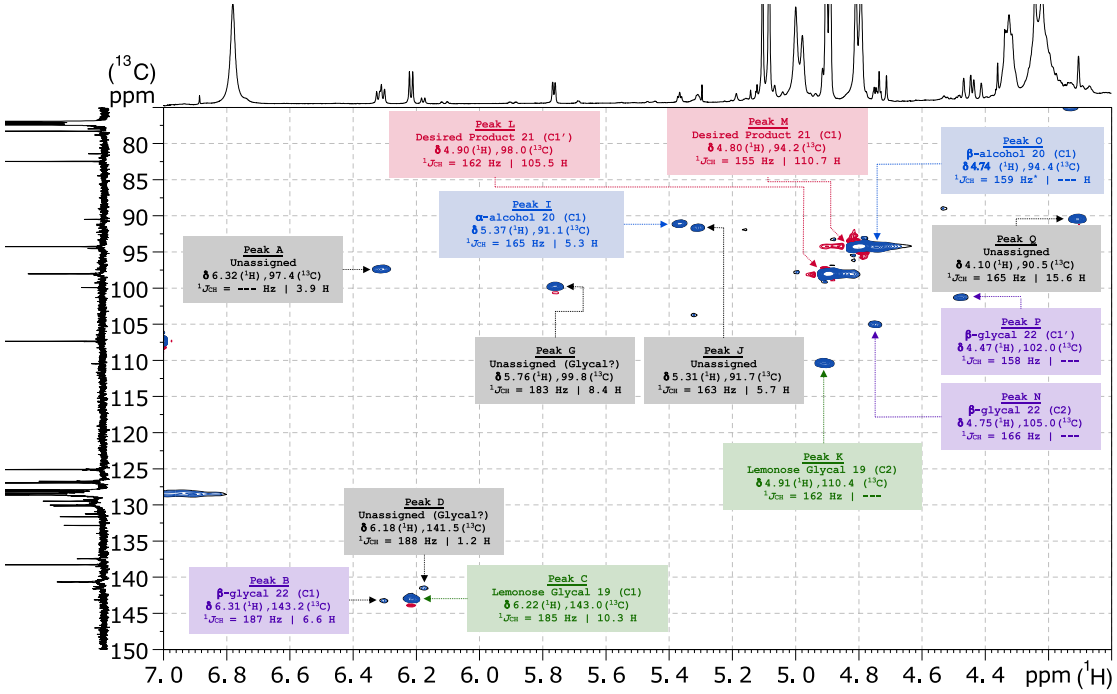


Figure A.5 Continued

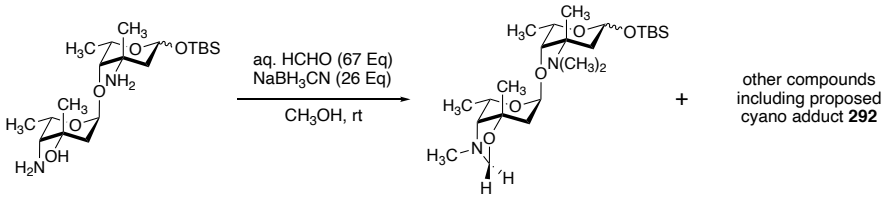
d. Annotated Crude ^{1}H-^{13}C HSQC {^{1}H}{^{13}C} (^{1}H – 600 MHz, ^{13}C –151 MHz, $CDCl_3$).[l–m]



[a]See page 159 for synthetic procedure. [b] ^{1}H, 600 MHz, $CDCl_3$ – referenced to δ 7.26; some ^{1}H shifts for crude post-glycosylation material were obtained from ^{1}H-^{13}C HSQC{^{1}H}{^{13}C}. [c] ^{13}C, 151 MHz, $CDCl_3$ – referenced to δ 77.16; some ^{13}C shifts for crude post-glycosylation material were obtained from ^{1}H-^{13}C HSQC{^{1}H}{^{13}C}. [d]Values obtained from ^{1}H-^{13}C HSQC acquired without ^{1}H or ^{13}C decoupling. [e]Relative integration to peak at 2.63 ppm (br d, J = 2.6 Hz, 100.0 H, H4′ of **215**). [f]Possibly some sort of glycal. [g]Coupling constants estimated from manually picked peaks. [h]Multiplicity and J_{HH} determined by ^{1}H-^{13}C HSQC acquired without ^{1}H or ^{13}C decoupling. [i]Combined integration for Peaks N and O. [j] $^{1}J_{CH}$ not determined for pure compound. [k]Annotations for Peaks K and P absent because clean integrations of these peaks was not possible due to spectral overlap. [l]Annotations for Peaks E and F absent due to their low intensity. [m]Annotation for Peak K absent because no HSQC correlation was observed.

Figure A.6 Evidence for the Formation of Proposed Oxazolidine **290**.

a. Procedure for Initial Attempt to Bis-*N*,*N*-Dimethylate Presumed Diamine Intermediate **374**.

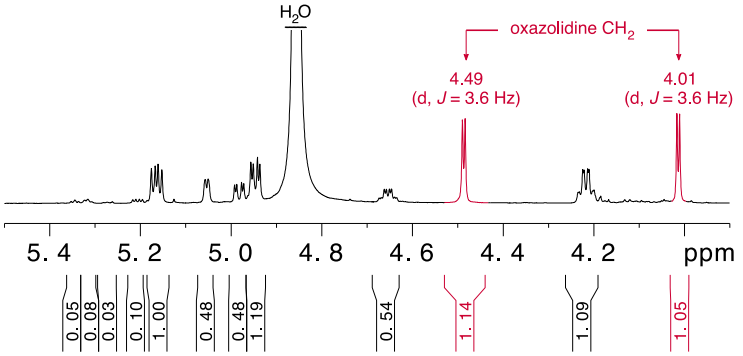


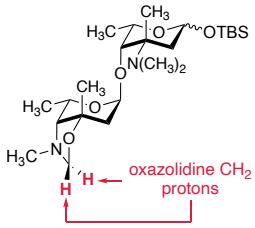
374
[tentative structure]

290
[tentative structure]

A 10 mL PSF containing crude **374** (theoretically 13 μmol)[a] was charged with a stir bar and dry CH_2OH (200 μL, 65 mM)[b]. Then aq. HCHO (65 μL, 0.87 mmol, 67 Eq | 37 wt% in H_2O)[c] was added in one portion at room temperature. 5 min later, $NaBH_3CN$ (21 mg, 0.33 mmol, 26 Eq) was added at room temperature. Finally, the PSF was lightly capped and left to stir at room temperature for 6.3 hr. At this point, the reaction was acidified by the addition of CF_3CO_2H (10 μL, 0.13 mmol, 10 Eq)[d], and purified using a strong cation exchange (SCX) cartridge[e-g]. The resulting mixture was analyzed by NMR and LCMS as detailed below.

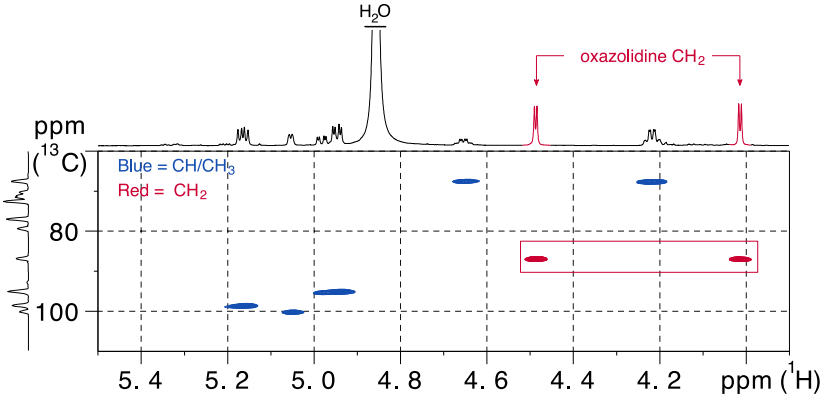
b. ^1H NMR (600 MHz, CD_3OD) after SCX Cartridge.[h]

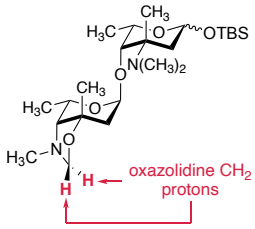




290
[Tentative Structure]

c. ^1H-^{13}C HSQC{^1H}{^{13}C} NMR (^1H – 600 MHz, ^{13}C – 151 MHz, CD_3OD) after SCX Cartridge.[i]



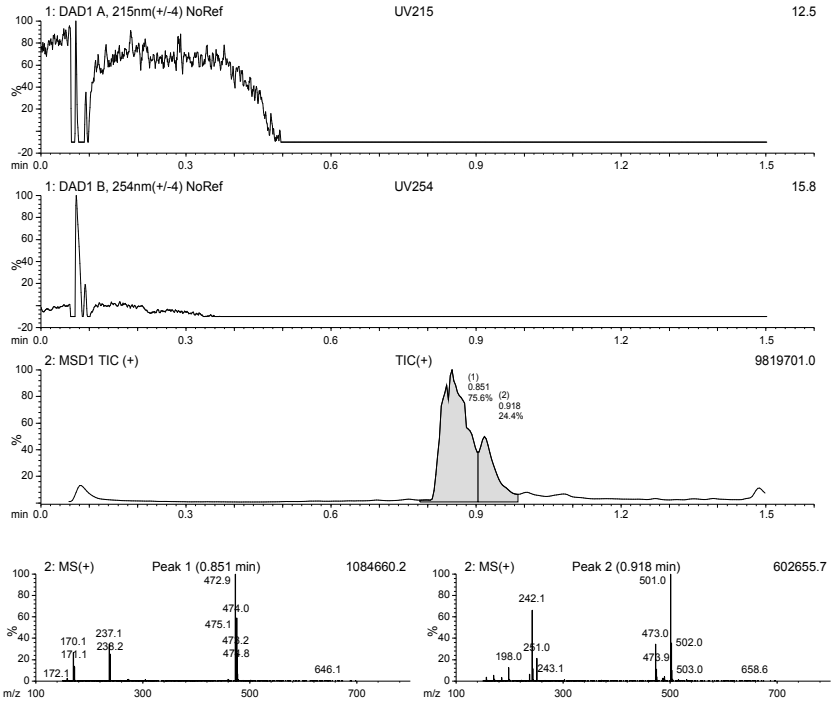


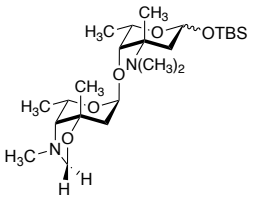
290
[Tentative Structure]

Figure A.6 Continued

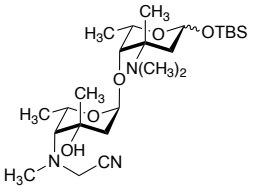
d. LCMS Report after SCX Cartridge.[j]

Peak #	Time	Area % UV215	Area % UV254	Area % TIC(+)	BPM	Area Abs UV215	Area Abs UV254	Area Abs TIC(+)
1	0.851	0.0	0.0	75.6	472.9	0	0	3.54E07
2	0.918	0.0	0.0	24.4	501.0	0	0	1.14E07





290
Exact Mass [M]: 472.3332
Exact Mass $[M+H]^+$: 473.3405
[Tentative Structure]

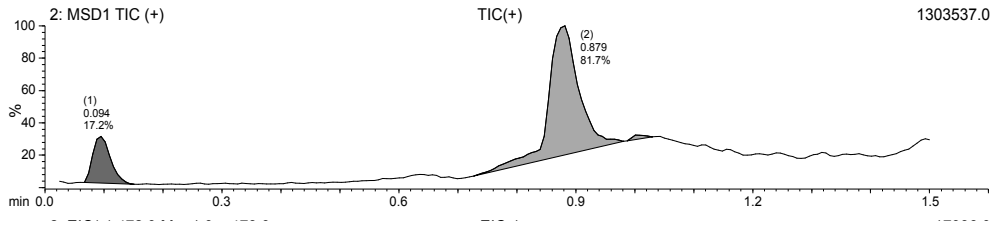


292
Exact Mass [M]: 499.3441
Exact Mass $[M+H]^+$: 500.3514
[Tentative Structure]

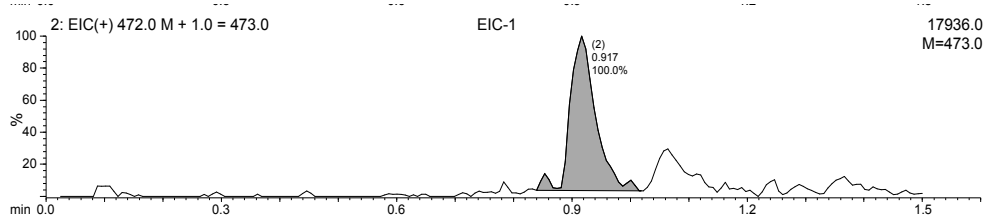
[a]Prepared from α/β-**289** (9.8 mg, 13 μmol, 1.0 Eq), NH_4HCO_2 (22.9 mg, 353 μmol, 27 Eq), and Pd/C (10.5 mg) in a similar manner as that described on page 175. [b]Dry CH_3OH measured out and dispensed with Gilson P1000 pipet. [c]Aq. HCHO measured out and dispended with Gilson P200 pipet. [d]CF_3CO_2H measured out and dispensed with Gilson P10 pipet. [e]2.75 hr after the $NaBH_3CN$ addition, a reaction aliquot was removed, diluted with CH_3OH, and analyzed by LCMS. [f]Agilent 500 mg SCX cartridge (catalog number 12113039). [g]SCX purification occurred in the following manner: (1) SCX cartridge conditioned with CH_3OH (9 mL); (2) crude mixture (as well as the reaction aliquot taken for LCMS analysis[f]) loaded onto SCX; (3) SCX washed with CH_3OH (6 mL); (4) product eluted from SCX with methanolic NH_3 (7 N NH_3 in CH_3OH | 6 mL) with the aid of a gentle vacuum. [h]Referenced to 3 δ 3.31. [i]Internal projection on ^{13}C axis. [j]Data acquired using Agilent B.

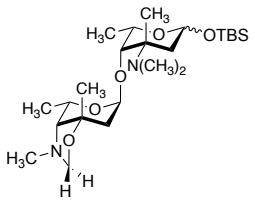
Figure A.7 Synthesis of **287**: LC/MS Data from a Reaction Aliquot taken 1.5 hr after Completion of AcOH Addition.[a–b]

a. Total Ion Chromatogram.



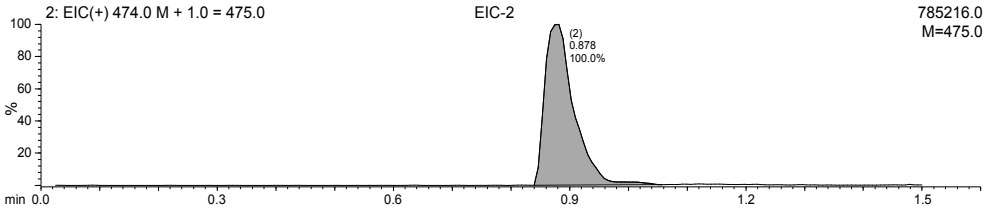
b. Extracted Ion Chromatogram for 473.0 m/z (Oxazolidine **290** $[M+H]^+$).

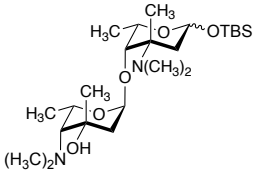




290
Exact Mass [M]: 472.3332
Exact Mass $[M+H]^+$: 473.3405
[Tentative Structure]

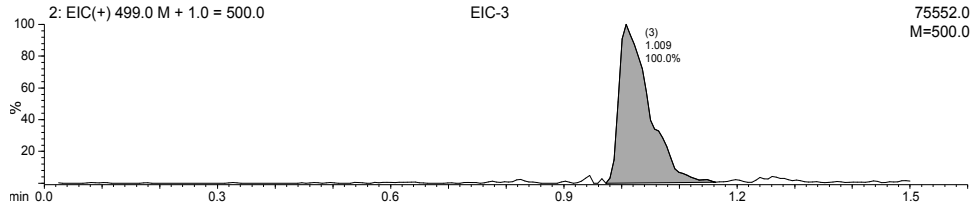
c. Extracted Ion Chromatogram for 475.0 m/z (Product **287** $[M+H]^+$).

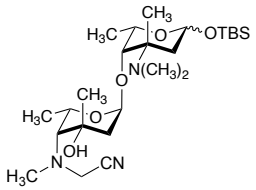




287
Exact Mass [M]: 474.3489
Exact Mass $[M+H]^+$: 475.3567

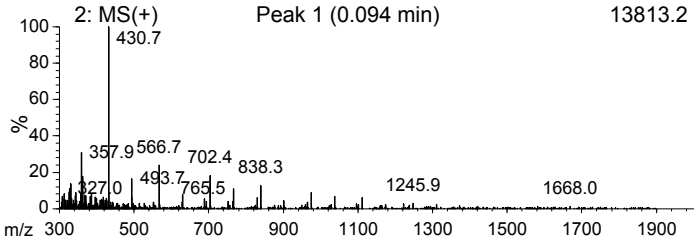
d. Extracted Ion Chromatogram for 501.0 m/z (Cyano Adduct **292** $[M+H]^+$).





292
Exact Mass [M]: 499.3441
Exact Mass $[M+H]^+$: 500.3514
[Tentative Structure]

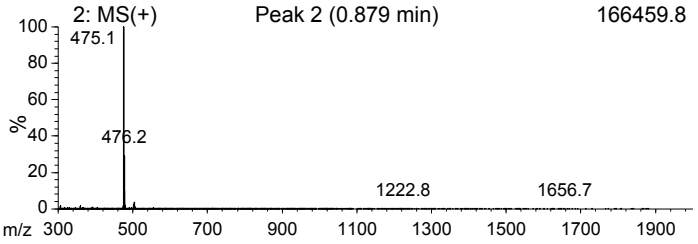
e. Mass Spectrum for Peak 1.

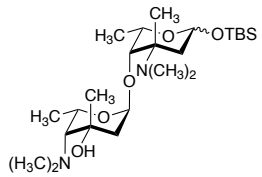


Unassigned Mass(es)

Figure A.7 Continued

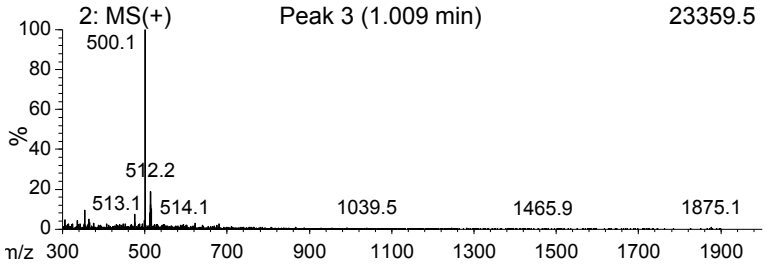
f. Mass Spectrum for Peak 2.

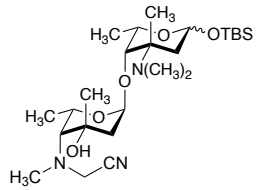




287
Exact Mass [M]: 474.3489
Exact Mass $[M+H]^+$: 475.3567

f. Mass Spectrum for Peak 3.



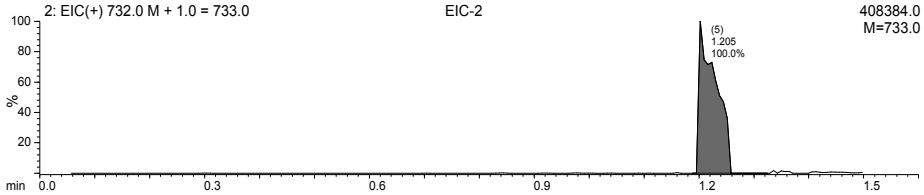


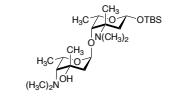
292
Exact Mass [M]: 499.3441
Exact Mass $[M+H]^+$: 500.3514
[Tentative Structure]

[a]See page 175 for synthetic procedure. [b]Data acquired using Agilent B.

Figure A.8 Synthesis of **24**•2TFA from 4th Generation Donor **287**: LC/MS Data from a Reaction Aliquot taken 2.9 hr after BF_3•OEt_2 Addition.[a–b]

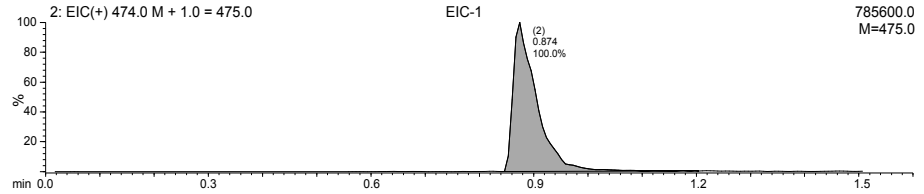
a. Extracted Ion Chromatogram for 475.0 m/z (Donor β-**287** [M+H]).

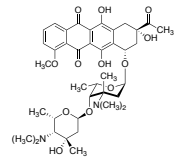




4th Generation Donor β-**287**
Exact Mass [M]: 474.3489
Exact Mass $[M+H]^+$ = 475.3567

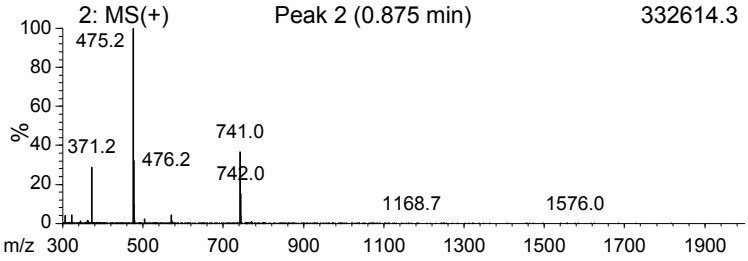
b. Extracted Ion Chromatogram for 741.0 m/z (Product **24** [M+H]).

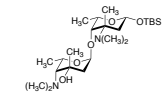




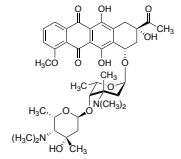
DNR-AMA Hybrid (**24**)
Exact Mass [M]: 740.3520
Exact Mass $[M+H]^+$: 741.3593

c. Mass Spectrum for Peak 2.





4th Generation Donor β-**287**
Exact Mass [M]: 474.3489
Exact Mass $[M+H]^+$ = 475.3567

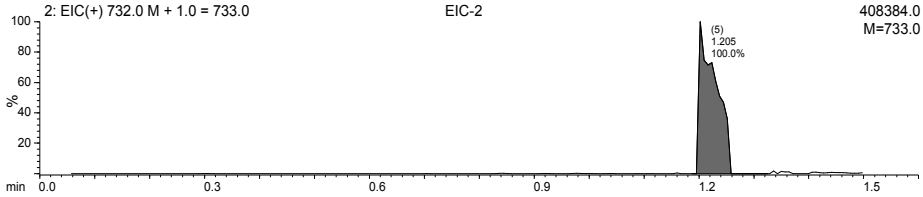


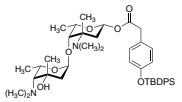
DNR-AMA Hybrid (**24**)
Exact Mass [M]: 740.3520
Exact Mass $[M+H]^+$: 741.3593

[a]See page 193 for synthetic procedure. [b]Data acquired using Agilent B.

Figure A.9 Synthesis of **24**•2TFA from 5th Generation Donor **300**: LC/MS Data from a Reaction Aliquot taken 2.9 hr after BF_3•OEt_2 Addition.[a–b]

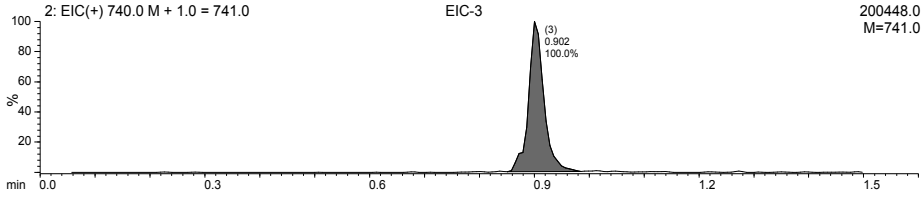
a. Extracted Ion Chromatogram for 733.0 m/z (Donor β-**300** [M+H]).

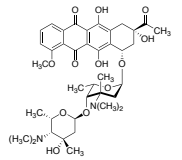




5th Generation Donor β-**300**
Exact Mass: 732.4170
Exact Mass [M+H]+: 733.4243

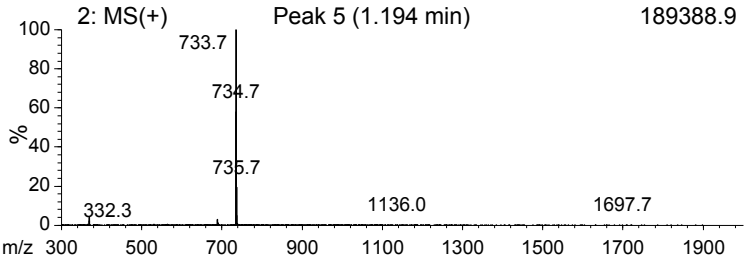
b. Extracted Ion Chromatogram for 741.0 m/z (Product **24** [M+H]).

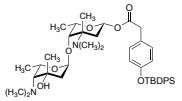




DNR-AMA Hybrid (**24**)
Exact Mass [M]: 740.3520
Exact Mass [M+H]+: 741.3593

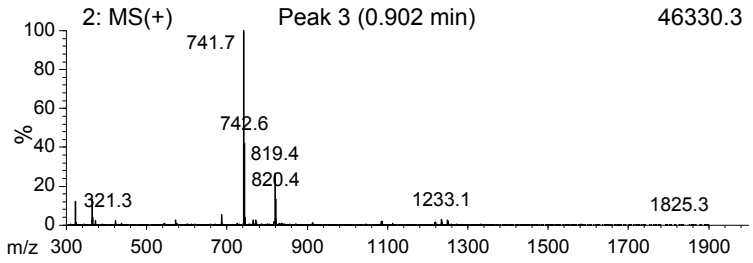
c. Mass Spectrum for Peak 5.

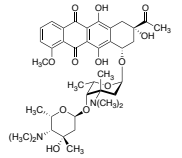




5th Generation Donor β-**300**
Exact Mass: 732.4170
Exact Mass [M+H]+: 733.4243

d. Mass Spectrum for Peak 3.[c]



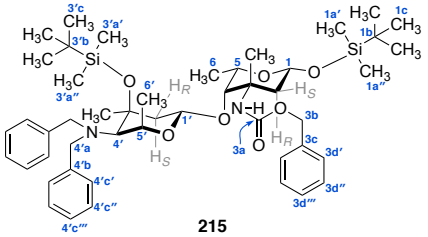


DNR-AMA Hybrid (**24**)
Exact Mass [M]: 740.3520
Exact Mass [M+H]+: 741.3593

[a]See page 195 for synthetic procedure. [b]Data acquired using Agilent A. [c]Peak at 819.4 m/z likely corresponds to $[2M+H]^+$ from acceptor **28** (see page 184 for LC/MS data from pure **28**).

A.2 *Supplementary Tables*

Table A.1 NMR Assignment Table for α-Linked Compound **215**.

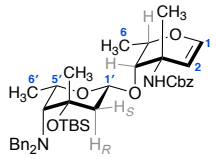


215

Assignment	^{1}H (δ)[a]	^{13}C (δ)[b]	^{1}H Mult. (Hz)	^{1}H-^{1}H COSY	^{1}H-^{13}C HMBC	^{1}H-^{1}H NOESY
C1	4.79	94.2 {155}	br d (8.7)	2*R*, 2*S*	2	1c, 2*S*, 3Me, 5
C2 (^{1}H: pro-*R*)	1.56	39.7	dd (12.9, 9.9)	1, 2*S*[c]	---	2*S*
C2 (^{1}H: pro-*S*)	2.44		br d (12.4)	1, 2*R*[c], 4	---	1, 2*R*, 3Me[c]
C3	---	54.0	---	---	---	---
C4	2.97	82.4	s	2*S*, 5[c]	2, 3, 3Me, 5[c], 1′	3Me, 3NH, 5, 6, 1′
C5	3.80	68.5	q (6.3)	4[c], 6	1, 4, 6	1, 3Me, 4, 6
C6	1.20	17.9	d (6.4)	5	4, 5	4, 5, 1′, 3′c
C1a′	0.07	[d]	ovlp s	---	---	---
C1a″	0.09	[d]	s	---	---	1c
C1b	---	[e]	---	---	---	---
C1c	0.87	26.0	br s	---	---	1, 1a″
C3Me	1.50	21.9	br s	---	2, 3, 4	1, 2*S*[c], 4, 5
C3NH	6.77	---	br s		---	4, 1′[c], 5′[c]
C3a	---	155.1	---	---	---	---
C3b′	5.09	65.7	ABq (12.4)[g]	3b″	3a, 3c	---
C3b″	4.98		ABq (12.4)[g]	3b′	3a, 3c	---
C3c	---	137.4	---	---	---	---
C1′	4.89	98.0 {162}	dd (7.2, 2.9)	2′*R*, 2′*S*	4, 3′, 5′	3NH[c], 4, 6, 2′*R*, 3′c, 6′
C2′ (^{1}H: pro-*R*)	2.20	45.4	dd (13.6, 2.7)	1′, 2′*S*	1′, 3′, 3′Me, 4′	1′, 2′*S*, 3′a″
C2′ (^{1}H: pro-S)	1.70		dd (13.6, 7.2)	1′, 2′*R*	1′	2′*R*, 3′Me, 4′
C3′	---	78.3	---	---	---	---
C4′	2.62	62.2	d (4.7)	5′	3′, 4′a, 5′	2′*S*, 3′Me, 5′, 6′, Ar
C5′	4.36-4.28	70.0	m	4′, 6′	1′, 3′	3NH[c], 3′Me[c], 4′, 6′
C6′	1.53	[f]	ovlp d (6.9)	5′	4′, 5′	1′, 3′c, 4′, 4′a′[c], 5′, Ar
C3′Me	1.47	30.5	br s	---	2′, 3′, 4′	2′*S*[c], 3′a″, 4′, 5′[c], Ar
C3′a′	0.07	[d]	ovlp s	---	---	---
C3′a″	0.18	[d]	s	---	---	2′*R*, 3′Me, 3′c
C3′b	---	[e]	---	---	---	---
C3′c	0.91	26.3	s	---	---	6, 1′, 3′a″, 6′
C4′a′	3.88	56.2	br s	---	---	4′a″, 6′[c], Ar
C4′a″	4.21		br s	---	---	4′a′, Ar
C4′b	---	140.6	---	---	---	---
Ar = 3d + 4c′	7.29-7.49	126.9, 127.9, 128.0, 128.4, 128.5, 128.6	m	---	---	---

[a] ^{1}H, 600 MHz, $CDCl_3$ – referenced to δ 7.26. [b] ^{13}C, 151 MHz, $CDCl_3$ – referenced to δ 77.16; $^{1}J_{CH}$ coupling constants (Hz) given in braces ({}) for anomeric carbons. [c] Relatively weak 2-D correlation. [d] δ -4.6, -3.9, -1.39, or -1.37. [e] δ 18.3, 18.49, or 18.52. [f] δ 18.49 or 18.52. [g] Δν = 62.8 Hz.

Table A.2 NMR Assignment Table for β-Linked Compound **243**.



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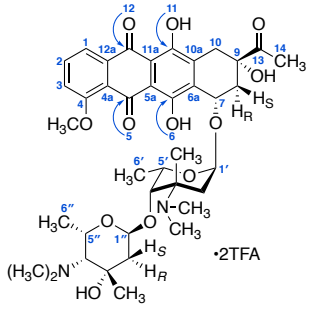
Assignment	^{1}H (δ)[a]	^{13}C (δ)[b]	^{1}H Mult. (Hz)	^{1}H-^{1}H COSY	^{1}H-^{13}C HMBC	^{1}H-^{1}H NOESY
C1	6.30	143.2	d (6.2)	2	2, 3, 5	2
C2	4.74	105.0 {166}	dd (6.2, 1.3)	1, 4	1, 4	1, 3NH
C3	---	52.4	---	---	---	---
C4	3.83	77.8	---	2	2, 3, 3Me, 1′	6, 1′
C5	4.16	71.7	---	6	---	3Me, 6
C6	1.32	17.3	d (6.5)	5	4, 5	4, 5
C3Me	1.59	27.5	---	---	1, 2, 3	5
C3NH	5.17	---	br s	---	2	2
C1′	4.46	101.3 {159}	dd (10.3, 2.4)	2′*R*, 2′*S*	4[c]	4, 2′*S*′, 3′Me, 5′
C2′ (^{1}H: pro-*R*)	2.27	45.0	---	1′, 2′*S*	---	2′*S*
C2′ (^{1}H: pro-S)	1.95		br d (~13)	1′, 2′*R*	3′	1′, 2′*R*, 3′Me[c]
C3′	---	78.8	---	---	---	---
C4′	2.25	62.4	---	5′[c]	---	3′Me, 5′
C5′	3.50	72.6	---	4′[c]	---	1′, 3′Me, 4′, 6′
C6′	1.23	---	---	5′	5′	5′
C3′Me	1.29	31.0	s	---	2′, 3′, 4′	1′, 2′S[c], 4′, 5′

[a] ^{1}H, 600 MHz, $CDCl_3$ – referenced to δ 7.26. [b] ^{13}C, 151 MHz, $CDCl_3$ – referenced to δ 77.16; $^{1}J_{CH}$ coupling constants (Hz) given in braces ({}) for select carbons. [c] Relatively weak 2-D correlations.

Table A.3 NMR Assignment Table for DNR-AMA Hybrid (**24**•2TFA).

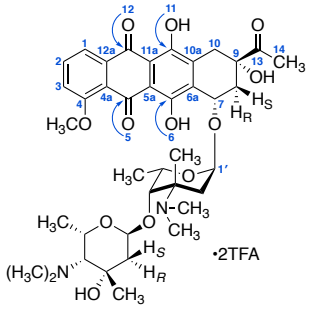


DNR-AMA Hybrid (**24**•2TFA)

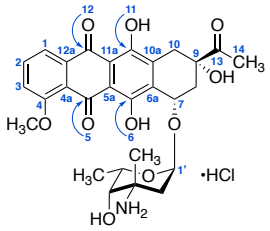
Assignment	^{1}H (δ)[a]	^{13}C (δ)[b]	^{1}H Mult. (Hz)	^{1}H-^{1}H COSY	^{1}H-^{13}C HMBC	^{1}H-^{1}H ROESY
C1	7.99	120.6	br d (7.4)	2	12	---
C2	7.86	137.3	app t (8.1)	1, 3	4, 12a	---
C3	7.59	120.4	br d (8.3)	2	4, 4a	4OMe[m]
C4	---	162.6	---	---	---	---
C4OMe	4.04	57.1	ovlp s	---	4[m]	3[m]
C4a	---	121.8	---	---	---	---
C5	---	188.5[e]	---	---	---	---
C5a	---	112.X[f,g]	---	---	---	---
C6	---	157.5	---	---	---	---
C6a	---	136.1	---	---	---	---
C7	5.01	71.6 {145}	dd (~ 5, 2)	8*R*, 8*S*	6, 6a, 9, 1′	8*S*, 1′
C8 (^{1}H: pro-*R*)	2.27[d]	37.1	ABq (14.5)[j]	7, 8*S*, 10	6a, 7, 9, 10	5′
C8 (^{1}H: pro-S)	2.13[d]		ABXdd (14.5, 5.2)[j]	7, 8*R*	---	7
C9	---	76.7	---	---	---	---
C10 (2H)	3.03, 3.07	32.9	ABq (18.8)[k]	8*R*	9, 11	---
C10a	---	135.7[g]	---	---	---	---
C11	---	156.4	---	---	---	---
C11a	---	112.X[f,g]	---	---	---	---
C12	---	188.1	---	---	---	---
C12a	---	136.6	---	---	---	---
C13	---	213.9	---	---	---	---
C14	2.35	24.4	s	---	9, 13	----
C1′	5.50	100.7 {171}	m	2′	7[c]	7, 2′
C2′ (2H)	2.02-2.06	34.5	m	1′	1′, 3′, 3′Me	1′, 3′Me, 3′NMe′
C3′	---	66.7	---	---	---	---
C4′	4.04	78.4	ovlp s	---	2′[m], 3′[m], 3′Me[m], 1″[m]	3′Me[m], 3′NMe″[m], 6′[m], 1″[m]
C5′	4.45	65.9	q (6.6)	6′	1′, 4′, 6′	8*R*, 3′Me, 6′
C6′	1.38	18.2	d (6.7)	5′	4′, 5′	4′[m], 5′, 1″
C3′Me	1.57	14.8	s	---	2′, 4′[m]	2′, 3′NMe′, 3′NMe″, 4′[m], 5′
C3′NMe′	2.77	38.2	s	3′NMe″	3′, 3′NMe″	2′, 3′Me
C3′NMe″	2.87	38.0	s	3′NMe′	3′, 3′NMe′	3′Me, 4′[m]
C1″	5.37	100.1 {170}	dd (*J* = 8.0, 4.4. Hz)	2″*R*, 2″*S*	4′, 5″	4′[m], 6′, 3″NMe$_2$[c], 6″[c]
C2″ (^{1}H: pro-*R*)	2.22	42.2	ABXdd (14.7, 4.4)[l]	1″	---	3″Me[c]
C2″ (^{1}H: pro-S)	2.16		ABXdd (14.7, 8.1)[l]	1″	1″	3″Me[c], 4″[c], 5″
C3″	---	70.1	---	---	---	---
C4″	3.42	70.5	d (3.6)	5″[c]	3″[c], 3″Me[c]	2*S*[c], 3″Me, 4″NMe$_2$, 5″
C5″	4.67	67.4	qd (7.0, 3.6)	4″[c], 6″	6″[c]	2″*S*, 3″Me[c,n], 4″NMe$_2$[c], 4″
C6″	1.59	17.6	d (7.0)	5″	---	1″[c], 4″NMe$_2$
C3″Me	1.56	31.0	s	---	2″	2″*R*[c], 2″*S*[c], 4″, 5″[c,n]
C4″NMe$_2$	3.13	44.0[i] & 44.5[i]	br s	---	---	1″[c], 4″, 5″[c], 6″

[a] ^{1}H, 600 MHz, CD_3OD – referenced to δ 3.31. [b] ^{13}C, 151 MHz, CD_3OD – referenced to δ 49.00; $^{1}J_{CH}$ coupling constants (Hz) given in braces ({}) for select carbons. [c] Relatively weak 2-D correlation. [d] Pro-*R*/*S* assignments for C8 made tentatively with the aid of You, D. J. et al.[5] and Arcamone, F.[6] [e] No ^{1}H-^{13}C HMBC correlations to this quaternary ^{13}C nucleus; assignment made based on functional group analysis (carbonyl), process of elimination (not δ 188.1 – C12), and comparison with the assignment for daunorubicin•HCl (**5**•HCl) (151 MHz, DMSO-d_6) as detailed by Stancyzk, W. et al.[7]. See Table S6 for full comparison of DNR-AMA Hybrid **24**•2TFA to daunorubicin•HCl (**5** •HCl). [f] Either δ 112.2 or δ 112.5. [g] No ^{1}H-^{13}C HMBC correlations to these quaternary ^{13}C nuclei; assignments made based on process of elimination and comparison with the assignments for daunorubicin•HCl (**5**•HCl) (151 MHz, DMSO-d_6) as detailed by Stancyzk, W. et al.[7]. See Table A.4 for full comparison of DNR-AMA Hybrid **24**•2TFA to daunorubicin•HCl (**5** •HCl). [i] Peak not visible in 1-D ^{13}C NMR; shift obtained from ^{1}H-^{13}C HSQC{^{1}H}{^{13}C}. [j] Δν = 88.2 Hz. [k] Δν = 22.9 Hz. [l] Δν = 36.8 Hz. [m] Due to overlap in the ^{1}H dimension (δ 4.04), unequivocal attribution of these ^{1}H-^{13}C HMBC and ^{1}H-^{1}H ROESY correlations to either C4OMe or H4″ was not possible; as such, these correlations were tentatively attributed as denoted in the table above based on the structure of **24**•2TFA. [n] Tentative ROESY correlation in which only 1 of 2 expected cross peaks was observed.

Table A.4 NMR Comparison of DNR-AMA Hybrid (**24**•2TFA) to Daunorubicin (**5**•HCl).



DNR-AMA Hybrid (**24**•2TFA)

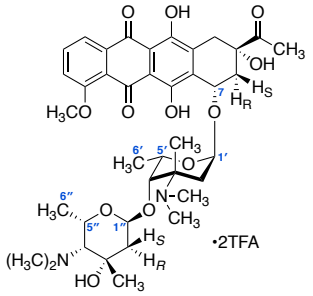


daunorubicin (**5**•HCl)

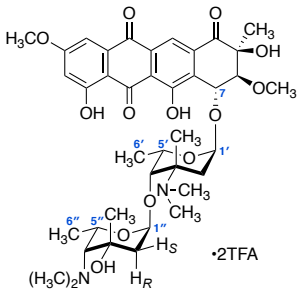
DNR-AMA	Hybrid•2TFA (24•2TFA)		Daunorubicin•HCl (5•HCl)	
	^{1}H (δ)[a]	^{13}C (δ)[b]	^{1}H (δ)[c]	^{13}C (δ)[d]
C1	7.99	120.6	7.89	120.2
C2	7.86	137.3	7.89	136.8
C3	7.59	120.4	7.64	119.5
C4	---	162.6	---	161.3
C4OMe	4.04	57.1	3.98	57.1
C4a	---	121.8	---	120.4
C5	---	188.5[fg]	---	187.0
C5a	---	112.X[h]	---	111.2
C6	---	157.5	---	156.5
C6OH	---[e]	---	13.27	---
C6a	---	136.1	---	135.8
C7	5.01	71.6	4.92	70.5
C8 (^{1}H: pro-*R*)	2.27[f]	37.1	2.12	36.4
C8 (^{1}H: pro-S)	2.13[f]			
C9	---	76.7	---	75.4
C9OH	---[e]	---	5.53	---
C10 (2H)	3.03, 3.07	32.9	2.91	32.0
C10a	---	135.7	---	135.0
C11	---	156.4	---	154.9
C11OH	---[e]	---	14.04	---
C11a	---	112.X[h]	---	111.1
C12	---	188.1	---	186.9
C12a	---	136.6	---	135.2
C13	---	213.9	---	212.3
C14	2.35	24.4	2.27	24.6
C1′	5.50	100.7	5.28	99.6

[a] ^{1}H, 600 MHz, CD_3OD – referenced to δ 3.31. [b] ^{13}C, 151 MHz, CD_3OD – referenced to δ 49.00. ^{1}H, 600 MHz, DMSO-d_6 – reference not explicitly defined (DMSO peak = δ 2.495); data from Stancyzk, et al.[7] [d] ^{1}H, 150 MHz, DMSO-d_6 – reference not explicitly defined (average of DMSO peaks = δ 40.087); data from Stancyzk, et al.[7] [e] Not observed due to exchange with NMR solvent (CD_3OD). [f] Pro-*R*/*S* assignments for C8 made tentatively in consideration of data in Table A.3 with the aid of You, D. J. et al.[5] and Arcamone, F.[6]

Table A.5 NMR Comparison of DNR-AMA Hybrid (**24**•2TFA) to Arimetamycin (**1**•2TFA).



DNR-AMA Hybrid (**24**•2TFA)

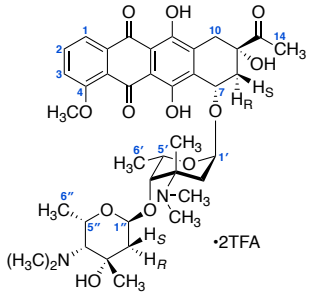


Arimetamycin A (**1**•**2TFA**)

Assignment	DNR-AMA Hybrid•2TFA (24•2TFA)			Arimetamycin A•2TFA (1)		
	^{1}H (δ)[a]	^{13}C (δ)[b]	^{1}H Mult. (Hz)	^{1}H (δ)[c]	^{13}C (δ)[d]	^{1}H Mult. (Hz)[c]
C7	5.01	71.6	dd (~ 5, 2)	5.03	71.8	d (2.1)
C1′	5.50	100.7	m	5.69	99.6	dd (3.5, 1.2)
C2′ (2H)	2.02-2.06	34.5	m	2.00	32.4	br m
C3′	---	66.7	---	---	63.6	---
C4′	4.04	78.4	ovlp s	4.05	75.9	m
C5′	4.45	65.9	q (6.6)	4.13	64.8	qd (7.1, 0.9)
C6′	1.38	18.2	d (6.7)	1.35	17.8	d (7.3)
C3′Me	1.57	14.8	s	1.36	14.5	s
C3′NH	---	---	---	8.15	---	br s
C3′NMe′	2.27	38.2	s	2.70	37.8	d (4.4)
C3′NMe″	2.87	38.0	s	2.77	36.2	d (4.7)
C1″	5.37	100.1	dd (*J* = 8.0, 4.4. Hz)	5.26	98.2	t (5.3)
C2″ (^{1}H: pro-*R*)	2.22	42.2	ABXdd (14.7, 4.4)[e]	1.96	40.1	dd (14.5, 5.3)
C2″ (^{1}H: pro-S)	2.16		ABXdd (14.7, 8.1)[e]	2.16		dd (14.5, 5.3)
C3″	---	70.1	---	---	68.3	---
C4″	3.42	70.5	d (3.6)	3.37	68.1	br d (2.2)
C5″	4.67	67.4	qd (7.0, 3.6)	4.67	64.7	qd (7.1, 2.2)
C6″	1.59	17.6	d (7.0)	1.45	17.1	d (7.1)
C3″Me	1.56	31.0	s	1.49	30.5	s
C4″NH	---	---	---	8.99	---	br s
C4″NMe$_2$	3.13	44.0 & 44.5	br s	2.99	42.9 & 45.1	br s

[a] ^{1}H, 600 MHz, CD_3OD – referenced to δ 3.31. [b] ^{13}C, 151 MHz, CD_3OD – referenced to δ 49.00. [c] ^{1}H, DMSO-*d*$_6$, 600 MHz – referenced to δ 2.50; data from Kang, H. S. et al.[8] [d] ^{13}C, DMSO-*d*$_6$, 150 MHz – referenced to δ 39.51; data from Kang, H. S. et al.[8] [e] Δν = 36.8 Hz.

Table A.6 NMR Comparison of DNR-AMA Hybrid (**24**•2TFA) as Synthesized from *O*-TBS Donor **287** vs. Acyl Donor **300**.



DNR-AMA Hybrid (**24**•2TFA)

Assignment	24•2TFA from *O*-TBS Donor 287		24•2TFA from Acyl Donor 300		Absolute Difference	
	^{1}H (δ)[a]	^{13}C (δ)[a]	^{1}H (δ)[a]	^{13}C (δ)[a]	^{1}H (Δδ)[b]	^{13}C (Δδ)[b]
C1	8.00	120.5	8.00	120.5	0.00	0.0
C2	7.86	137.3	7.86	137.3	0.00	0.0
C3	7.60	120.3	7.60	120.3	0.00	0.0
C4OMe	4.04	57.1	4.04	57.1	0.00	0.0
C7	5.01	71.6	5.02	71.6	0.01	0.0
C8 (^{1}H: pro-*R*)	2.26	37.1	2.28	37.1	0.01	0.0
C8 (^{1}H: pro-*S*)	2.12	37.1	2.12	37.1	0.00	0.0
C10 (2H)	3.04	32.9	3.06	32.9	0.01	0.0
C14	2.35	24.4	2.35	24.4	0.00	0.0
C1′	5.50	100.6	5.50	100.8	0.00	0.2
C2′ (2H)	2.04	34.5	2.05	34.5	0.01	0.0
C4′	4.04	78.4	4.04	78.2	0.00	0.2
C5′	4.46	65.9	4.45	65.9	0.01	0.0
C6′	1.37	18.3	1.37	18.3	0.00	0.0
C3′Me	1.58	14.8	1.58	14.9	0.00	0.2
C3′NMe$_2$	2.86	38.0	2.86	38.0	0.00	0.0
C3′NMe$_2$	2.77	38.3	2.77	38.3	0.00	0.0
C1″	5.38	100.1	5.38	99.9	0.00	0.2
C2″ (^{1}H: pro-*R*)	2.21	42.2	2.20	42.4	0.01	0.2
C2″ (^{1}H: pro-*S*)	2.16	42.2	2.18	42.2	0.01	0.0
C4″	3.41	70.5	3.41	70.5	0.00	0.0
C5″	4.67	67.3	4.69	67.3	0.01	0.0
C6″	1.59	17.6	1.59	17.6	0.00	0.0
C3″Me	1.55	31.1	1.55	30.9	0.00	0.2
C3″NMe$_2$	3.13	44.1	3.13	44.1	0.00	0.0
C3″NMe$_2$	3.13	45.5	3.12	45.5	0.01	0.0

[a] Data from ^{1}H-^{13}C HSQC {^{1}H}{^{13}C} experiments (^{1}H - 600 MHz, ^{13}C - 151MHz, CD_3OD); peaks picked using TopSpin 4.0.9. [b] Difference calculations performed on raw data from TopSpin 4.0.9 prior to rounding.

A.3 *References for Appendix A.*

(1) Krohn, K.; Broser, E. Synthetic Anthracyclinones. 25. An Improved Route to 8-Demethoxyaranciamycinone and Synthesis of the A-L-Daunosamine Glycosides. *J. Org. Chem.* **1984**, *49*, 3766-3771.
(2) Krohn, K.; Carlson, G.; Köhle, H.; Hussain, H.; Green, I. R. Synthesis of Daunosamyl Anthraquinone and Anthracyclinone Analogues. *Arkivoc* **2008**, *2008*, 216-233.
(3) Renous, B.; Gaudin, N.; Gesson, J.-P.; Jacquesy, J.-C. New Process for the Preparation of Steffimycinone Analogues, New Analogues of Steffimycinone Thus Obtained, New Intermediates Useful for the Synthesis of These Compounds, Use of These Compounds as Antibacterial Agents and for the Synthesis of Antitumour Agents. FR2543545A1, **1985**.
(4) Steffimycinone Analogs. JP61047439A, **1986**.
(5) Rho, Y. S.; Kim, S. Y.; Kim, W.-J.; Yun, Y. K.; Sin, H. S.; Yoo, D. J. Convenient Syntheses of Daunomycinone-7-D-Glucuronides and Doxorubicinone-7-D-Glucuronides. *Synth. Commun.* **2004**, *34*, 3497-3511.
(6) Arcamone, F. *Doxorubicin Anticancer Antibiotics*. Academic Press, Inc.: **1981**.
(7) Piorecka, K.; Stanczyk, W.; Florczak, M. Nmr Analysis of Antitumor Drugs: Doxorubicin, Daunorubicin and Their Functionalized Derivatives. *Tetrahedron Lett.* **2017**, *58*, 152-155.
(8) Kang, H. S.; Brady, S. F. Arimetamycin A: Improving Clinically Relevant Families of Natural Products through Sequence-Guided Screening of Soil Metagenomes. *Angew. Chem. Int. Ed.* **2013**, *52*, 11063-11067.

APPENDIX B

Experimental Procedures (Chemistry)

B.1 *General Experimental*

B.1.1 *Reaction Setup, Monitoring, and Purification*

Reaction vessels were dried in one of two manners: (1) flame drying under active vacuum on a Schlenk line and cooling under a steady stream of nitrogen/argon gas or (2) oven drying and cooling in a desiccator at ambient pressure over anhydrous $CaSO_4$ (Drierite brand: mixture of pure and $CoCl_2$ impregnated desiccant). Stirring was accomplished magnetically using PTFE coated magnetic stir bars and spin vanes. Heating was accomplished by immersing the reaction vessel in an oil bath heated by a stirring hot plate as regulated by a temperature probe; oil baths were preheated prior to the introduction of the reaction vessel. Inert atmospheres were maintained using double balloons filled with argon. Unless noted otherwise, all reagents and solvents were added in one portion. Dropwise additions via syringe pump were performed using a Razel Scientific Insruements R-100E Syringe Pump. Reactions were monitored by thin-layer chromatography (TLC) using EMD/Merck KGaA silica gel 60 F_{254} precoated plates and were visualized by UV, I_2 (iodine mixed with silica gel), ammonium molybdate (CAM) staining, and/or anisaldehyde staining. Normal phase flash column chromatography was performed using Silia*Flash*® silica gel (P60, 40-63 mL, 60 Å) purchased from SiliCycle.

B.1.2 *Materials*

Unless indicated otherwise, reagents and solvents were purchased from commercial sources and were used as received. Sources for key starting materials, transition metal containing reagents, and filter aids are noted below. Tetrahydrofuran (THF), methylene chloride (CH_2Cl_2), methanol (CH_3OH), and *N*,*N*-dimethylformamide (DMF) were dried through a Braun MB-SPS solvent system and used immediately or stored over activated beaded 3 Å or 4 Å molecular sieves. Molecular sieves were activated as detailed below. Commercial borane•pyridine complex (pyr•BH_3) was purified prior to its use as detailed below. 2,4,6-tri-*tert*-butylpyrimidine was synthesized according to the procedure of Crich and co-workers.[1]

Sources for Key Starting Materials, Transition Metal Containing Reagents, and Filter Aids

Category	Chemical	CAS Number	Source	Catalog Number
Starting Materials	D-threonine	632-20-2	Combi Blocks	AM 2173
	vancomycin hydrochloride	1404-93-9	Carbosynth	AV161115
	4-hydroxyphenylacetic acid	156-38-7	Sigma Aldrich	H50004
	daunorubicin hydrochloride	23541-50-6	---[a]	---[a]
Transition Metal Containing Reagents	mercury (II) acetate ($Hg(OAc)_2$)	1600-27-7	Sigma-Aldrich	176109
	silver hexafluorophosphate ($AgPF_6$)[b]	26042-63-7	Oakwood Chemical	007272
	palladium on carbon (Pd/C)[c]	7440-05-3	Acros Organics	195030500
Molecular Sieves	4 Å powdered molecular sieves	70955-01-0	Sigma-Aldrich	688363
	3 Å beaded molecular sieves	308080-99	Sigma-Aldrich	208574
	4 Å beaded molecular sieves	70955-01-0	Sigma-Aldrich	208590
Filter Aids	Celite®[d]	68855-54-9	Sigma-Aldrich	22140
	sand[e]	14808-60-7	Fisher Chemical	S25

[a] Generous gift from Prof. Dr. Samuel J. Danishefsky. [b] Stored and weighed out in a nitrogen filled glovebox. [c] 10% palladium on unreduced carbon. [d] Celite® 545 – treated with sodium carbonate, flux calcined. [e] Sand, sea (washed).

B.1.3 *Activation of Molecular Sieves (MS)*

Powdered 4 Å MS, beaded 3 Å MS, and beaded 4 Å MS were activated in the following manner. Sieves were removed from the manufacturer's container, heated in a conventional microwave[a] until reaching a temperature ≥ 350 °C[b], and stored in an oven. Immediately before use, the sieves were heated again in a conventional microwave[a] until reaching a temperature ≥ 350 °C[b] and cooled in a desiccator at ambient pressure over anhydrous $CaSO_4$.[c]

Notes:

[a] Powdered 4 Å MS heated 6-8 times for 20-30 seconds; beaded 3 Å MS and beaded 4 Å MS heated 6-8 times for 40-50 seconds.

[b] Measured with a thermocouple.

[c] Drierite brand: mixture of mixture of pure and $CoCl_2$ impregnated desiccant.

B.1.4 *Purification of Pyridine•BH_3 (pyr.•BH_3)*

A separatory funnel (60 mL) was charged with Et_2O (30 mL) and commercial pyr•BH_3 (ca. 3 mL, 85:15 pyr•BH_3:pyridine w/w by ^1H NMR). The resulting mixture was washed with DI H_2O (5 x 8 mL) to remove pyridine. The washed organics were dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo* on a rotovap to yield "purified pyr•BH_3" (90:7:3 pyr•BH_3:pyridine:Et_2O by ^1H NMR)

B.1.5 *Instrumentation*

NMR spectra were obtained on a Bruker 400 MHz or 600 MHz spectrometer in deuterated solvents and are reported relative to residual solvent peaks ($CDCl_3$: ^1H = δ 7.26, ^{13}C = δ 77.16; CD_3OD: ^1H = δ 3.31, ^{13}C = δ 49.00) unless indicated otherwise. NMR spectra acquired in a mixture of deuterated solvents were calibrated to the residual solvent peak of the more abundant solvent. NMR data were analyzed using the TopSpin software available from Bruker. ^1H NMR data are reported as follows: chemical shift in ppm (multiplicity, coupling

constants, integration, assignment). For multiplets encompassing more than one ^{1}H, the chemical shift for each ^{1}H is given immediately following the assignment enclosed in braces ({} = braces) if deemed vital to the interpretation of the spectrum. Multiplicity abbreviations are as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad, app = apparent, dis = distorted, inf = inferred, ovlp = overlapping, or combinations thereof. AB quartets (ABq) are reported as functions of J and $\Delta\nu$. ^{13}C spectra were acquired with ^{1}H decoupling. $^{1}J_{CH}$ values are reported in the $^{13}C\{^{1}H\}$ line listings for select resonances and were determined by coupled ^{1}H-^{13}C HSQC experiments. ^{1}H and ^{13}C assignments were made with aid of ^{1}H-^{1}H COSY, ^{1}H-^{13}C HSQC$\{^{1}H\}\{^{13}C\}$, coupled ^{1}H-^{13}C HSQC, ^{1}H-^{13}C HMBC, ^{1}H-^{1}H NOESY, and/or ^{1}H-^{1}H ROESY 2-D experiments as appropriate.

IR spectra were acquired as thin films on NaCl plates using a Thermo Electron IR100 series instrument and are reported in terms of frequency of absorbance (cm^{-1}). Spectra were acquired with 64 scans over a spectral window of 500-4000 cm^{-1}.

Optical rotation (OR) data were obtained using either a Jasco P-2000 Polarimeter with a Jasco cylindrical glass cell (10 mm x 10 mm) or a Rudolph Research Analytical Autopol III with a 50 mm cell. Concentrations are reported in units of g per 100 mL.

Melting point (MP) data were obtained using a Standard Research Systems MPA100 automated melting point apparatus with MeltView software with a heating rate of 5.0 °C min^{-1}. Melting points are reported as ranges from the onset point (threshold = 70%) to the clear point (threshold = 10%) with the single point (threshold = 50%) including in brackets ({}).

Reverse phase MPLC was performed on a Teledyne Isco Combiflash® Rf+ Lumen system with UV detection and ELS detection. Separation was achieved using RepiSep Rf Gold® reversed phase C18 columns (5 g, Teledyne ISCO catalog number 69-2203-328).

LC/MS data were acquired on two Agilent LC/MS systems denoted as "Agilent A" and "Agilent B" as described below:

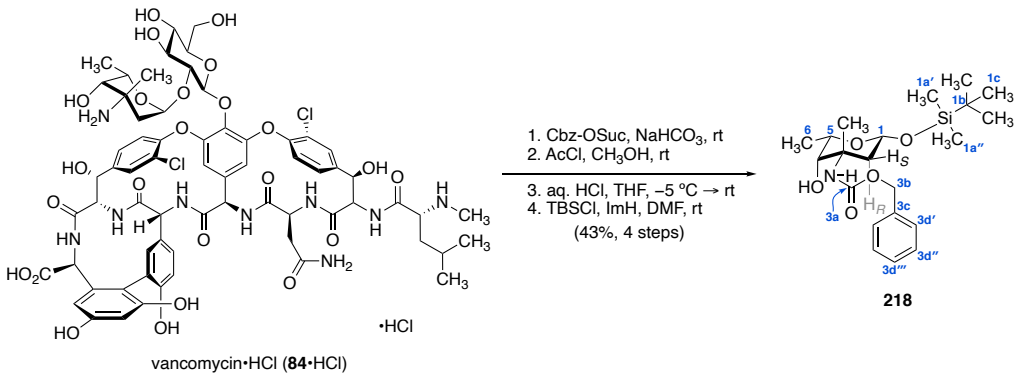
Agilent A: Analytical HPLC was performed on an Agilent 1200 series system with UV detection at 214 nm and 254 nm. Low-resolution mass spectra were obtained on an Agilent 6130 single quad mass spectrometer with electrospray ionization (ESI) in positive mode. LC-MS experiments were performed with the following parameters: column: Accucore C18 column (2.6 μm, 2.1 mm × 30 mm column) at 40 °C; gradient: CH_3CN/H_2O with 0.1% trifluoroacetic acid, 7-95 % CH_3CN over 1.1 min; temperature: 40 °C.

Agilent B: Analytical HPLC was performed on an Agilent 1200/1260 series system with UV detection at 214 nm and 254 nm. Low-resolution mass spectra were obtained on an Agilent 6130 single quad mass spectrometer with electrospray ionization (ESI) in positive mode. LC-MS experiments were performed with the following parameters: column: Waters Acquity BEH C18 (1.7 μm, 1.0 x 50 mm); gradient: 5% to 95% CH_3CN in H_2O (0.1% trifluoroacetic acid) over 1.4 min then hold at 95% CH_3CN for 0.1 min; temperature: 55 °C.

HRMS data were obtained either in the Vanderbilt University Mass Spectrometry Service Laboratory or in the Indiana University Mass Spectroscopy Facility (Bloomington). At Vanderbilt University, data were obtained using a Thermo-Finnigan Orbitrap mass spectrometer equipped with an Ion-Max source housing and a standard electrospray ionization (ESI) probe. Samples were directly injected using an isocratic gradient of H_2O (+ 0.1% formic acid) and CH_3CN (+ 0.1% formic acid) in a 1:1 (v/v) ratio. At Indiana University, data were obtained using a Thermo LTQ-Orbitrap XL with an electrospray (ESI) source.

Single crystal XRD data were acquired using with a Rigaku Oxford Diffraction Supernova EosS2 CCD with filtered Cu-Kα radiation at a temperature of 100.00(10) K.

B.2 *Preparation of L-Lemonose Brasiliose Acceptor* ***218***



vancomycin•HCl (**84**•HCl)

Name (218): benzyl ((2*S*,3*S*,4*S*,6*R*)-6-((*tert*-butyldimethylsilyl)oxy)-3-hydroxy-2,4-dimethyltetrahydro-2*H*-pyran-4-yl)carbamate

Procedure (Step 1)[a]**:** An RBF (2 L) equipped with a stir bar was charged with vancomycin•HCl (**84**, 25.1697 g, 16.9411 mmol, 1.00000 Eq), $NaHCO_3$ (5.0258 g, 59.82 mmol, 3.531 Eq), 1,4-dioxane (150 mL, 0.11 M), and DI H_2O (150 mL, 0.11 M). After stirring the resulting mixture for 23 min, Cbz-OSu (16.9359 g, 67.956 mmol, 4.0113 Eq) was added in two portions at room temperature.[a] Then the reaction was lightly capped with a plastic cap and left to stir for 15.5 hr. Next, acetone (2 x 550 mL) was added in two portions to the orange solution, causing solids to precipitate. The stir rate was increased, and the resulting opaque, pink-tinted white suspension was left to stir open to the air at room temperature for an additional 6.5 hr. Next, the reaction mixture was filtered through a 600 mL fritted funnel equipped with filter paper. The resulting solids were transferred to an RBF (2 L). Then the fritted funnel was rinsed with CH3OH (3 x 100 mL), and the filtrates were added to the collected solids. Next, the mixture was concentrated in vacuo and co-evaporated with toluene (3 x 250 mL). The resulting tan solids were dried under high vacuum for ca. 2.5 days and taken on without further purification to **Step 2**.

Procedure (Step 2)[a]**:** The RBF (2L) containing the crude product of **Step 1** (theoretically 16.9411 mmol) was equipped with a stir bar, charged with dry CH_3OH (300 mL), fitted with an addition funnel (250 mL, graduated), and placed under a balloon of argon. Freshly prepared methanolic HCl (theoretically 0.20 mol, 12 Eq HCl in 46 mL dry CH_3OH)[b] was transferred to the addition funnel and added dropwise to the opaque grapefruit colored reaction at room temperature. The resulting opaque white suspension was left to stir vigorously at room temperature for 6.6 hr during which time it became a yellow solution. Then the addition funnel was removed, and the reaction was quenched by the addition of solid $NaHCO_3$ (47.7713 g, 568.6 mmol, 33.57 Eq) in portions over ca. 15 min. After stirring for an additional 20 min, the stir bar was removed, and the reaction was concentrated *in vacuo*. To the resulting residue was added a stir bar and acetone (850 mL). The resulting mixture was stirred vigorously at room temperature for 48 hr. Then the reaction was filtered through a silica plug topped with sand (600 mL fritted funnel) with the aid of additional acetone (3 x 150 mL). The resulting opaque white filtrate was concentrated onto silica gel (SiO_2, ca. 56 g) and purified by FCC (SiO_2: solid loading, 10% → 50% EtOAc/Hex)[c] to afford a mixture of methyl glycosides as a pale yellow oil that was co-evaporated with THF (2 x 50 mL) and taken on to **Step 3** without further purification.

*Preparation of L-Brasiliose Acceptor **218** Continued*

Procedure (Step 3)[a]**:** The RBF (2 L) containing the product of **Step 2** (theoretically 16.9411 mmol) was equipped with a stir bar, charged with dry THF (90 mL), fitted with an addition funnel (250 mL, graduated), and lowered into an ice/water bath (1 °C). Meanwhile, a solution of aq. HCl was prepared as follows: a 1L collection flask equipped with a stir bar was charged with DI H_2O (360 mL) and chilled in a separate ice/water bath (0 °C) prior to the addition of concentrated HCl (12.178 N, 170 mL, 2.07 mol, 122 Eq). This chilled aq. HCl was charged to the addition funnel in portions and added dropwise to the reaction over ca. 1 hr.[d] After stirring for an additional 30 min, the reaction was removed from the cooling bath and stirred at room temperature for 5 hr. Then the reaction was quenched by the addition of solid $NaHCO_3$ (207.37 g, 2.468 mol, 145.7 Eq) in portions over 15 min. After stirring for an additional 2.5 hr, the quenched reaction mixture was transferred to a separatory funnel (2L) using Et_2O (450 mL) and DI H_2O (450 mL). The layers were separated, and the resulting aqueous layer was extracted with CH_2Cl_2 (5 x 150 mL). Then the combined organics (Et_2O and CH_2Cl_2) were dried over anhydrous $MgSO_4$, filtered through a pad of Celite® (600 mL fritted funnel), and concentrated *in vacuo*. The crude product was transferred to an oven dried 500 mL collection flask using dry CH_2Cl_2, and the solvent was removed *in vacuo* to give an orange-yellow oil. The collection flask was then equipped with an oven dried stir bar, charged with freshly activated powdered 4 Å MS (7.2150 g) followed by dry CH_2Cl_2 (90 mL), and placed under a double balloon of argon. The resulting mixture was stirred vigorously at room temperature for ca. 3.5 hr. Then the mixture was filtered through an oven dried fritted funnel (150 mL) into an oven dried 250 mL RBF. The solvent was removed *in vacuo* to give an orange oil that was taken on to **Step 4** without further purification.

Procedure (Step 4)[a]**:** The RBF (250 mL) containing the crude product of **Step 3** (theoretically 16.9411 mmol) was equipped with an oven dried stir bar and charged with dry DMF (60 mL, 0.28 M) followed by imidazole (3.1781 g, 46.68 mmol, 2.756 Eq). The reaction was placed under a balloon of argon and stirred at room temperature for 34 min. Then TBSCl (4.0738 g, 27.029 mmol, 1.5955 Eq) was added in one portion at room temperature, and the reaction was stirred at room temperature for an additional 25.6 hr. Then the reaction was transferred to a separatory funnel (1L) using EtOAc (400-500 mL) and washed with 1N HCl (5 x 60 mL) followed by brine (1 x 60 mL). The washed organics were dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. Purification by FCC (SiO_2: 5% → 25% EtOAc/Hex) afforded TBS glycoside **218** (2.998 g, 7.319 mmol, 43% yield over 4 steps) as a yellow oil.

^{1}H NMR (600 MHz, $CDCl_3$, 218): δ 7.39-7.33 (m, 4H, H3d), 7.33-7.29 (m, 3H, H3d), 5.53 (s, 1H, C3NH), 5.06 (ABq, *J* = 13.1 Hz, Δν = 0.0 Hz, 2H, H3b), 4.79 (dd, *J* = 9.3, 2.1 Hz, 1H, H1), 3.82 (br q, *J* = 6.4 Hz, 1H, H5), 3.18 (d, *J* = 10.4 Hz, 1H, H4), 2.26 (br d, *J* = 13.3 Hz, 1H, $H2_{pro\text{-}R}$), 2.11 (d, *J* = 10.4 Hz, 1H, C4OH), 1.57 (br inf dd, *J* = 13.4, 9.4 Hz, 1H, $H2_{pro\text{-}S}$), 1.52 (s, 3H, C3Me), 1.28 (d, *J* = 6.5 Hz, 3H, H6), 0.89 (s, 9H, H1c), 0.11 (s, 3H, H1a), 0.10 (s, 3H, H1a).

^{13}C{^{1}H} NMR (151 MHz, $CDCl_3$, 218): δ 155.2 (C3a), 136.7 (C3c), 128.7 (C3d), 128.19 (C3d), 128.17 (C3d), 94.1 (C1), 72.8 (C4), 69.0 (C5), 66.4 (C3d), 55.1 (C3), 40.4 (C2), 25.9 (C1c), 21.8 (C3Me), 18.2 (C1b), 17.4 (C6), -4.1 (C1a), -5.1 (C1a).

IR (thin film, cm^{-1}, 218): 3410, 2940, 2862, 1720, 1504, 1065, 843.

OR (218): $\alpha_D^{24} = -1.06^{o}$ (*c* = 1.00, $CHCl_3$)

R_f (218): 0.29 (20% EtOAc/Hex, CAM stain)

Notes:

[a] Procedure adapted from Bennett, C. S. et al.[2]

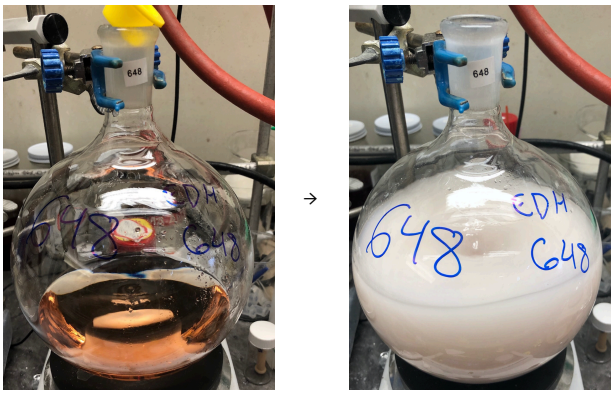
[b] Methanolic HCl was prepared as follows: a dried 100 mL RBF equipped with a stir was fitted with a dried addition funnel (50 mL, graduated). The entire apparatus was placed under a double balloon off argon. Then the RBF was charged with dry CH_3OH (46 mL) and lowered into an ice/brine bath. Next, acetyl chloride (AcCl, 14 mL, 0.20 mol, 12 Eq) was charged to the addition funnel and added dropwise over ca. 15 min. After stirring for another 15 min, the resulting solution was transferred to addition funnel (250 mL, graduated) that had been fitted to the main reaction flask.

[c] Detailed gradient: 10% (1.2 L) → 20% (1.5 L) → 40% (2 L) → 50% (2 L) EtOAc/Hex; crude product of **Step 2** eluted in 40% → 50% EtOAc/Hex.

[d] Aq. HCl was transferred in portions to the addition funnel to minimize the amount of warming that would happen in the addition funnel.

[e] Detailed gradient: 5% (1.8 L) → 10% (1 L) → 12% (0.5 L) → 14% (0.5 L) → 17% (0.8 L) → 20% (0.8 L) → 25% (0.6 L) EtOAc/Hex; **218** eluted in 12% → 20% EtOAc/Hex.

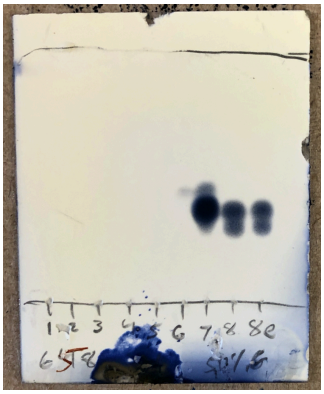
Visual Procedure:



A. Step 1: ca. 15 hr adding 2nd portion of Cbz-OSu

B. Step 1: 3 min after adding 2nd portion of acetone

TLCs

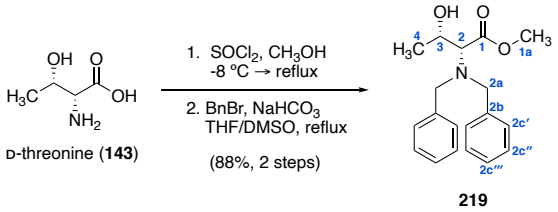


TLC #1: FCC Fractions (Step 2)
Gradient: 50% EtOAc/Hex
Visualization: CAM Stain
Lanes 6, 7, 8, and 8e: Product of **Step 2**

TLC #2: Reaction Monitoring (Step 3)
Gradient: 50% EtOAc/Hex
Visualization: CAM Stain
Left Lane: Starting material (product of **Step 2**)
Middle Lane: co-spot
Right Lane: Reaction 5.1 hr after aq. HCl addition completed

B.3 *Synthesis of Vinylogous Ester **217** from D-Threonine (**143**)*

B.3.1 *Preparation of Compound **219***



Name : methyl dibenzyl-D-threoninate

Procedure (Step 1)[a]**:** An oven dried collection flask (1 L) equipped with a stir bar and fitted with an addition funnel (50 mL, graduated) was cooled under a steady stream of nitrogen, evacuated, and placed under a double balloon of argon. The flask was charged with dry CH_3OH (370 mL, 0.498 M) and lowered into an ice/brine bath (-8 °C). $SOCl_2$ (41 mL, 0.56 mol, 3.1 Eq) was added to the additional funnel and added dropwise to the reaction over 13 min. 35 min later, D-threonine (**143**, 21.93 g, 184.1 mmol, 1.000 Eq) was added to the reaction.[b] 10 min later, the flask was removed from the cooling bath, the addition funnel was exchange for a reflux condenser, and the flask was lowered into a preheated oil bath (set point = 85 °C). After refluxing for 6 hr, the reaction was removed from the oil bath. The stir bar was removed, and the reaction was concentrated *in vacuo*. The resulting residue was co-evaporated with toluene (3 x 150 mL) to give a crude yellow oil that was taken on to **Step 2** without further purification.

Procedure (Step 2)[c]**:** The collection flask (1 L) containing the crude product of **Step 1** (theoretically 184.1 mmol) was equipped with a stir bar and charged with dry DMSO (116 mL, 1.59 M), dry THF (470 mL, 0.392 M), solid $NaHCO_3$ (63.1 g 751 mmol, 4.08 Eq),[d] and benzyl bromide (66 mL, 0.55 mol, 3.0 Eq). The flask was fitted with a reflux condenser, placed under a double balloon of argon, and lowered into a preheated oil bath (set point = 70 °C). The reaction was refluxed for 18 hr during which time it changed from a white suspension to a suspension of white solids in a yellow liquid. After cooling, the reaction was filtered through a pad of Celite® (600 mL fritted funnel) using EtOAc and H_2O.[e] The filtrate was transferred to a separatory funnel (2 L). The layers were separated, and the resulting aqueous layer was extracted with EtOAc (1 x 200 mL). The combined organics were then washed with sat. aq. $NaHCO_3$ (3 x 150 mL) and brine (1 x 150 mL), in that order. Next, the washed organics were dried over anhydrous Na_2SO_4, filtered through a pad of Celite® (600 mL fritted funnel), concentrated *in vacuo*, co-evaporated with CH_2Cl_2 (1x), and concentrated onto a mixture of Celite® and SiO_2. Purification by FCC (SiO_2: two batches, solid loading, 2 → 9% EtOAc/Hex)[f] afforded NBn_2-D-Thr(OH)-OCH_3 (**219**, 51.3 g, 164 mmol, 89% yield over 2 steps) as a pale yellow oil.

^{1}H NMR (600 MHz, $CDCl_3$): δ 7.38-7.33 (m, 4H, H2c), 7.33-7.27 (m, 6H, H2c), 4.07 (qd, *J* = 9.6, 6.0 Hz, 1H, H3), 4.04 (d, *J* = 13.3 Hz, H2a), 3.84 (s, 3H, H1a), 3.51 (br s, 1H, OH), 3.44 (d, *J* = 13.4 Hz, H2a), 3.10 (d, *J* = 9.6 Hz, 1H, H2), 1.10 (d, *J* = 6.1 Hz, 3H, H4).[g-h]

^{13}C{^{1}H} NMR (151 MHz, $CDCl_3$): δ 170.9 (C1), 138.3 (C2b), 129.3 (C2c), 128.7 (C2c), 127.7 (C2c), 67.5 (C2), 63.3 (C3), 55.0 (C2a), 51.4 (C1a), 19.3 (C4).

IR (thin film, cm^{-1}): 3457, 3029, 2946, 2896, 2847, 1731, 1599, 1494, 1451, 1371, 1278, 1243, 1191, 1106, 997, 744, 699.

HRMS (ESI+, m/z): $[M+H]^+$ calculated for $[C_{24}H_{50}N_2O_5Si + H]^+$ 475.3562; found 475.3566 (error 0.8 ppm).

LC/MS (Agilent A, ESI +): t_R = 1.04 min| $[M+H]^+$ calculated for $[C_{19}H_{23}NO_3 + H]^+$ 314.2 m/z; found 314.3 m/z.

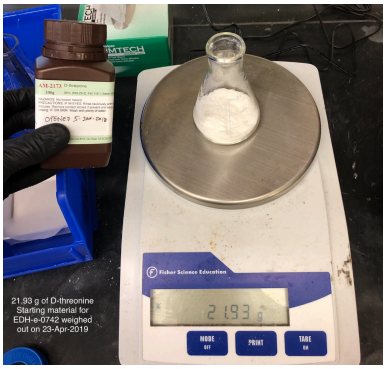
OR: α_D = +171° (*c* = 1.02, CH_2Cl_2)

R_f: 0.22 (15% EtOAc/Hex, UV/CAM)

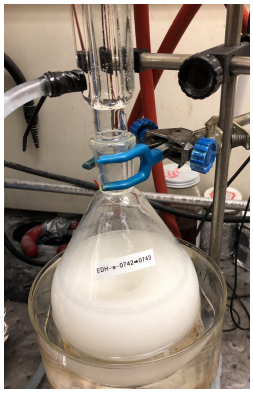
Notes

[a] Procedure for **Step 1** adapted from Zhou, P.; Toone, E. J. et al.[3] and Mal, D. et al.[4].
[b] Then the Erlenmeyer flask (125 mL) into which D-threonine (**143**) had been weighed as well as the funnel used to add **143** were rinsed into the reaction with dry CH_3OH (2 x 10 mL).
[c] Procedure for **Step 2** adapted from Zhu, J. et al.[5].
[d] Bubbling occurred upon $NaHCO_3$ addition, presumably due to residual acid from **Step 1**.
[e] The filtration was an attempt to remove solids prior to aqueous workup. However, due to concerns about losing product in the solids by only washing with EtOAc, H_2O was used to dissolve the solids.
[f] Detailed gradient for column #1: 2% (5 L) → 4% (1 L) → 7% (2 L) → 8 % (2 L); detailed gradient for column #2: 2% (5 L) → 4% (1.2 L) → 7% (2 L) → 8 % (2 L) → 9 % (< 1 L); compound **219** eluted in 4 → 9% EtOAc/Hex.
[g] Calibrated to residual CH_2Cl_2 (5.30 ppm).
[h] Agrees with ^1H spectral data for (±)-**219** from Guanti, G. et al.[5].

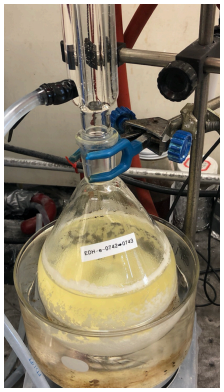
Visual Procedure:



A. Step 1:
Weighing out D-threonine (**143**) starting material.



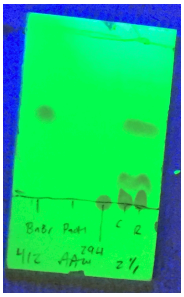
B. Step 2:
45 min after reaction lowered into oil bath.



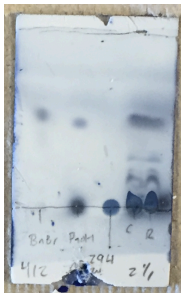
C. Step 2:
18 hr after reaction lowered into oil bath.

D. Step 2:
51.3 g of **219** after drying on high vac.

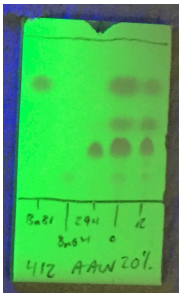
TLCs:*



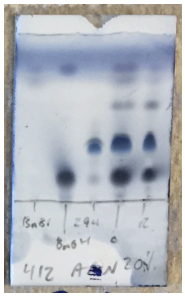
TLC #1a: Crude Step 2
Gradient: 2% EtOAc/Hex
Visualization: UV



TLC #1b: Crude Step 2
Gradient: 2% EtOAc/Hex
Visualization: CAM Stain



TLC #1c: Crude Step 2
Gradient: 20% EtOAc/Hex
Visualization: UV

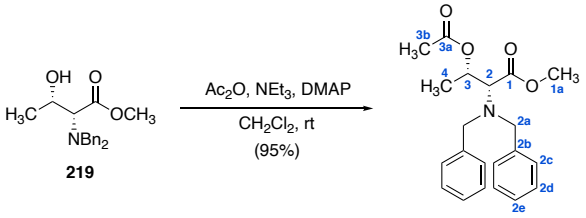


TLC #1d: Crude Step 2
Gradient: 20% EtOAc/Hex
Visualization: CAM Stain

For all 4 plates, lanes from left to right are as follows:
benzyl bromide | benzyl alcohol | *ent*-**219** | co-spot | crude reaction mixture

*TLCs from smaller scale reaction that yielded 25.4 g of **219**.

B.3.2 *Preparation of Compound* ***220***



Name: methyl O-acetyl-*N*,*N*-dibenzyl-D-threoninate

Procedure[a]**:** An RBF (1 L) equipped with an oven dried stir bar was flame dried and placed under a balloon of argon. Dry CH_2Cl_2 (200 mL, 0.40 M) was then used to transfer NBn_2-D-Thr(OH)-OCH_3 (**219**, 25.2 g, 80.4 mmol, 1.00 Eq) into the reaction flask. Then DMAP (988.3 mg, 8.090 mmol, 0.101 eq), NEt_3 (23 mL, 0.17 mol, 2.1 Eq), and Ac_2O (16 mL, 0.17 mol, 2.1 Eq) were added, in that order, and the reaction was left to stir at room temperature for 1.5 hr. A second portion of NEt_3 (11.5 mL, 82.5 mmol, 1.03 Eq) was then added followed by the addition of a second portion of Ac_2O (8 mL, 85 mmol, 1.1 eq). After stirring at room temperature for an additional 4 hr, the reaction was transferred to a separatory funnel (1 L) using CH_2Cl_2 (150 mL) and H_2O (50 mL).[b] Sat. aq. $NaHCO_3$ (200 mL) and crushed ice (ca. 150 mL) were added, the separatory funnel was shaken[c], and the layers were separated. The resulting aqueous layer was extracted with CH_2Cl_2 (3 x 100 mL). The combined organics were dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo to give a yellow oil of low viscosity. Purification by FCC (SiO_2: 15% EtOAc/Hex) afforded the desired product NBn_2-D-Thr(OAc)-OCH_3 (**220**, 27.1649 g, 76.428 mmol, 95% yield) as a very pale yellow, nearly colorless oil.

^{1}H NMR (600 MHz, $CDCl_3$): δ 7.35 (d, J = 7.1 Hz, 4H, H2c), 7.31 (dd, J = 8.5, 6.7 Hz, 4H, H2d), 7.24 (t, J = 7.2 Hz, 2H, H2e), 5.42 (dq, J = 7.5, 6.3 Hz, 1H, H3), 4.05 (d, J = 13.8 Hz, 2H, H2a), 3.77 (s, 3H, H1a), 3.57 (d, J = 13.8 Hz, 2H, H2a), 3.42 (d, J = 7.5 Hz, 1H, H2), 2.07 (s, 3H, H3b), 1.18 (d, J = 6.3 Hz, 3H, H4).

^{13}C{^{1}H} NMR (151 MHz, $CDCl_3$): δ 170.8 (C1), 170.2 (C3a), 139.5 (C2b), 129.0 (C2c), 128.4 (C2d), 127.2 (C2e), 68.7 (C3), 65.0 (C2), 55.4 (C2a), 51.4 (C1a), 21.5 (C3b), 17.8 (C4).

IR (thin film, cm^{-1}): 3029, 2990, 2947, 2842, 1738, 1598, 1494, 1449, 1371, 1240.9, 1154, 1068, 1022, 913, 787, 740, 698, 601.

HRMS (ESI+, m/z): $[M+H]^+$ calculated for $[C_{19}H_{23}NO_3 + H]^+$ 314.1751; found 314.1752 (error 0.3 ppm).

OR: α_D^{24} = +69.6° (c = 1.00, $CHCl_3$)

Rf: 0.27 (15% EtOAc/Hex, UV/CAM/I_2)

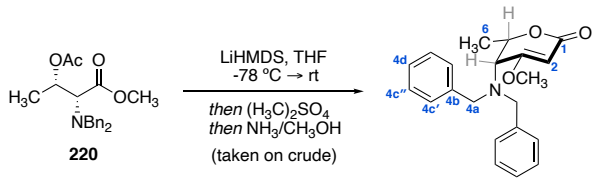
Notes:

[a] Procedure adapted from Myers, A. G. et al.[6].

[b] Prior to transferring the reaction to the separatory funnel, a reaction aliquot was removed, diluted with CH_2Cl_2, and analyzed by TLC developed in 10% EtOAc/Hex (UV/CAM).

[c] Caution! Evolution of CO_2, likely generated through the following reaction:
$Ac_2O + 2\ NaHCO_3 \rightarrow 2NaOAc + H_2O + \mathbf{2CO_2}$

B.3.3 *Preparation of Compound* ***222***



Name: (5*R*,6*S*)-5-(dibenzylamino)-4-methoxy-6-methyl-5,6-dihydro-2*H*-pyran-2-one

Procedure[a]**:** An oven dried two-neck flask (1 L) fitted with an oven dried addition funnel (center neck: 250 mL, graduated, stoppered with a septum) and a septum (side neck) was placed under a balloon of argon and charged with LiHMDS (81 mL, 0.11 mol, 2.3 Eq | ca. 1.3 M solution in THF) followed by dry THF (180 mL, 0.252 M). The resulting solution was cooled in an acetone/dry ice bath (-78 °C). Then a solution of NBn_2-D-Thr(OAc)-OCH_3 (**220**, 16.1 g, 45.3 mmol, 1.00 Eq) in dry THF (94 mL, 0.48 M)[b] was charged to the addition funnel and subsequently added dropwise to the reaction over 1.6 hr.[c] After stirring at -78 °C for an additional 1.2 hr, the orange solution was removed from the acetone/dry ice bath and left to stir at room temperature for 3.5 hr. Next, dimethyl sulfate (14 mL, 0.15 mol, 3.2 Eq) was added dropwise via syringe pump over 20 min.[d] After stirring for an additional 15 hr at room temperature, the reaction was quenched by the addition of methanolic NH_3 (27 mL, 0.19 mol, 4.2 Eq | 7 N NH_3 in CH_3OH) in one portion at room temperature.[e] The stir rate was increased, and the quenched reaction mixture was stirred for 2 hr. Then it was transferred to a separatory funnel (2 L) using 5:2 Et_2O/DI H_2O (560 mL). The layers were separated, and the resulting aqueous layers was extracted with Et_2O (1 x 150 mL). The combined organics were then washed with 1 N HCl (4 x 80 mL), sat aq. $NaHCO_3$ (1 x 80 mL), and brine (1 x 80 mL), in that order. Next, the washed organics were dried over anhydrous Mg_2SO_4, filtered through a Celite® pad topped with sand (600 mL fritted funnel) into an RBF (2L), and concentrated *in vacuo* to give a yellow oil. The RBF was charged with a dry stir bar, dry CH_2Cl_2 (ca. 350 mL), and freshly activated powdered 4 Å MS (15.1 g). The resulting mixture was placed under a balloon of argon and stirred for 3.25 hr at room temperature. Then the mixture was filtered through a pad of Celite® topped with sand (oven dried fritted funnel) into an oven dried collection flask (1 L). The filtrate was concentrated *in vacuo* to give crude **222** as an orange oil that was dried under high vacuum and then taken on to the preparation of **217** without further purification.

A purified sample was prepared in largely the same manner using NBn_2-D-Thr(OAc)-OCH_3 (**220**, 177.2 mg, 498.6 µmol, 1.000 Eq), LiHMDS (0.88 mL, 1.1 mmol, 2.3 Eq | ca. 1.3 M in THF), dimethyl sulfate (0.17 mL, 1.8 mmol, 3.6 Eq), and methanolic NH_3 (0.30 mL, 2.1 mmol, 4.2 Eq | 7 N NH_3 in CH_3OH) in the stated amounts. After aqueous workup, the washed organics were dried over anhydrous Na_2SO_4, filtered, concentrated *in vacuo*, and co-evaporated with CH_2Cl_2. Purification by FCC (SiO_2: 15% → 40% EtOAc/Hex)[f] afforded purified **222** (141.0 mg, 417.9 µmol, 84% yield) as a light orange foam.

^{1}H NMR (600 MHz, $CDCl_3$): δ 7.38 (d, *J* = 7.5 Hz, 4H, H4c), 7.32 (t, *J* = 7.5 Hz, 4H, H4c), 7.25 (inf. t, *J* = 7.2 Hz, 2H, H4d) 5.40 (s, 1H, H2), 4.45 (qd, *J* = 6.6, 4.6 Hz, 1H, H5), 3.93 (d, *J* = 13.1 Hz, 2H, H4a), 3.88 (s, 3H, C3OMe), 3.46 (d, *J* = 13.2 Hz, 2H, H4a), 3.17 (d, *J* = 4.6 Hz, 1H, H4), 1.48 (d, *J* = 6.6 Hz, 3H, H6).

^{13}C{^{1}H} NMR (151 MHz, $CDCl_3$): δ 172.9 (C3), 167.2 (C1), 139.0 (C4b), 129.2 (C4c), 128.5 (C4c), 127.4 (C4d), 93.7 (C2), 76.8 (C5), 55.9 (C3OMe/C4a), 55.8 (C3OMe/C4a), 55.2 (C4), 16.9 (C6).

IR (thin film, cm^{-1}): 3030, 2987, 2933, 2850, 1708, 1623, 1493, 1453, 1377, 1284, 1220, 1166, 1088, 1042, 975, 920, 833, 744, 702, 630.

HRMS (ESI+, m/z): $[M+H]^+$ calculated for $[C_{21}H_{23}NO_3 + H]^+$ 338.1751; found 338.1760 (error 2.7 ppm).

OR: α_D^{24} = +326° (*c* = 1.00, $CHCl_3$)

R_f: 0.12 (25% EtOAc/Hex, UV/I_2/CAM); 0.45 (50% EtOAc/Hex, UV/I_2/CAM)

*Preparation of Compound **222** Continued*

Notes:

[a] Procedure adapted from Myers, A. G. et al.[6] and Seeberger, P. H. et al.[7].

[b] NBn_2-D-Thr(OAc)-OCH3 (14) was transferred using dry CH_2Cl_2 to an oven dried, tared collection flask (200 mL). The solvent was removed in vacuo (rotovap), and then the residue was dried overnight under high vacuum before being transferred into the addition funnel using a total of 94 mL dry THF.

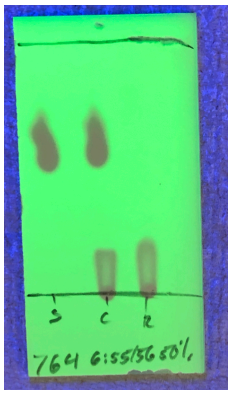
[c] When the dropwise addition of NBn2-D-Thr(OAc)-OCH3 (14) in dry THF was complete, the addition funnel was rinsed with additional dry THF (3 x 10 mL), and the rinses were added to the reaction.

[d] Prior to the addition of dimethyl sulfate, a reaction aliquot was taken, quenched into CH_3OH, and analyzed by TLC using 50% EtOAc/Hex (UV).

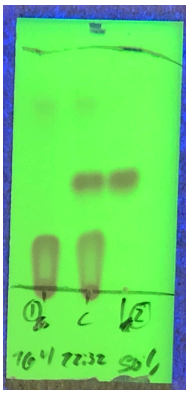
[e] Prior to the addition of methanolic NH_3, a reaction aliquot was taken, quenched into CH_3OH, and analyzed by TLC using 50% and 90% EtOAc/Hex (UV).

[f] Detailed gradient: 15% (100 mL) → 20% (50 mL) → 25% (52 mL) → 30% (50 mL) → 35% (100 mL) → 40 % (100 mL) EtOAc/Hex; compound **222** eluted in 35% → 40% EtOAc/Hex.

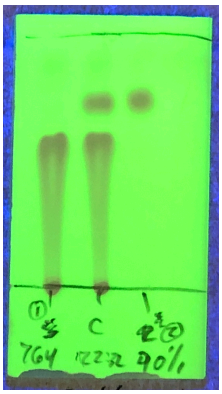
TLCs:*



TLC #1: Reaction Monitoring
Gradient: 50% EtOAc/Hex
Visualization: UV
Left Lane: **220** (SM)
Middle Lane: co-spot
Right Lane: Reaction 25 min before dimethyl sulfate addition began.



TLC #2a: Reaction Monitoring
Gradient: 50% EtOAc/Hex
Visualization: UV

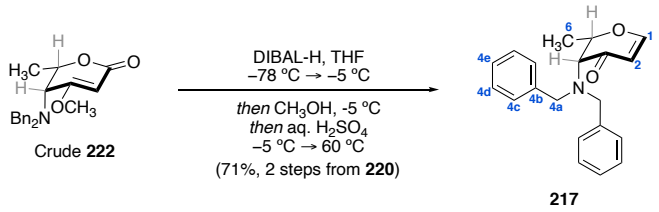


TLC #2b: Reaction Monitoring
Gradient: 90% EtOAc/Hex
Visualization: UV

For both TLC #2 plates:
Left Lane: Reaction 25 min before dimethyl sulfate addition began.
Middle Lane: co-spot
Right Lane: Reaction 15 hr after dimethyl sulfate addition completed

*TLCs from large scale reaction noted above that was taken on to the synthesis of compound **217.**

B.3.4 *Preparation of Compound* ***217***



Crude **222**

217

Name: (2*S*,3*R*)-3-(dibenzylamino)-2-methyl-2,3-dihydro-4*H*-pyran-4-one

Procedure[a]**:** The oven dried collection flask (1 L) into which crude **222** (theoretically 45.3 mmol) had been concentrated was equipped with a large oven dried stir bar, charged with dry THF (182 mL, 0.249 M), and placed under a balloon of argon. The resulting mixture was cooled in an acetone/dry ice bath (-78 °C), and then DIBAL-H (57 mL, 68 mmol, 1.5 Eq | 1.2 M solution in $PhCH_3$) was added dropwise via syringe pump over 1 hr.[b] After stirring at -78 °C for an additional 2 hr, the reaction was removed from the acetone/dry ice bath and placed into an ice/brine bath (-5 °C) and stirred for 1 hr. Then excess DIBAL-H was quenched by the portionwise addition of CH_3OH (16 mL) over 10 min.[c] Next, the septum was removed, and a freshly prepared solution of concentrated H_2SO_4 (13 mL, 0.24 mol, 5.4 Eq) in DI H_2O (94 ml) was added.[d] The reaction was removed from the ice/brine bath, stirred at room temperature for 5 min, lowered into a preheated oil bath (set point = 60 °C), fitted with a reflux condenser, and left to stir vigorously open to the air for ca. 17 hr.[e] Then the reaction mixture was cooled in an ice/water bath and quenched by the portionwise addition of solid $NaHCO_3$ (250 g, 2.98 mol, 65.7 Eq) over 35 min.[f] The quenched reaction mixture was removed from the ice/water bath and stirred for an additional 1 hr at room temperature before it was transferred to a separatory funnel (2 L) using 1:1 EtOAc/DI H_2O (560 mL, v/v). Additional DI H_2O (500 mL) was added to the separatory funnel, and the layers were separated. The resulting aqueous layer was diluted with additional DI H_2O (500 mL) and extracted with CH_2Cl_2 (4 x 150 mL). The combined organics (EtOAc + CH_2Cl_2) were dried over anhydrous $MgSO_4$, filtered through a Celite® pad topped with sand (600 mL fritted funnel), and concentrated *in vacuo* to give a crude orange-red material. Purification by FCC (SiO_2: 5% → 15% EtOAc/Hex)[g] afforded vinylogous ester **12** (9.90 g, 32.2 mmol, 71% yield over 2 steps from **220**) as a yellow solid.[h]

^{1}H NMR (600 MHz, $CDCl_3$): δ 7.44 (d, *J* = 7.7 Hz, 4H, H4c), 7.35 (d, *J* = 6.5 Hz, 1H, H1), 7.33 (t, *J* = 7.6 Hz, 4H, H4d), 7.24 (app t, *J* = 7.6 Hz, 2H, H4e), 5.58 (d, *J* = 6.1 Hz, 1H, H2), 4.51-4.43 (m, 1H, H5), 3.93 (d, *J* = 13.2 Hz, 1H, H4a), 3.59 (d, *J* = 13.2 Hz, 2H, H4a), 3.00 (d, *J* = 5.1 Hz, 1H, H4), 1.55 (d, *J* = 6.7 Hz, 3H, H6).

13C{1H} NMR (151 MHz, CDCl3): δ 193.3 (C3), 163.3 (C1), 139.4 (C4b), 129.2 (C4c), 128.4 (C4d), 127.3 (C4e), 109.4 (C2), 81.0 (C5), 61.0 (C4), 55.3 (C4a), 16.3 (C6).

IR (thin film, cm^{-1}): 3032, 2990, 2931, 2851, 1658, 1595, 1494, 1450, 1406, 1378, 1269, 1228, 1165, 1107, 1045, 1017, 979, 918, 822, 747, 700, 615.

HRMS (ESI+, m/z): $[M+H]^+$ calculated for $[C_{20}H_{21}NO_2+H]^+$ 308.1645; found 308.1648 (error 1.0 ppm).

OR: $\alpha_D^{24} = -117^o$ (*c* = 1.00, $CHCl_3$)

Rf: 0.19 (15% EtOAc/Hex, UV/CAM)

*Preparation of Compound **217** Continued*

Notes:

[a] Procedure adapted from Seeberger, P. H. et al.[7] and Woodward, R. B. et al.[8].

[b] DIBAL-H (1.2 M in $PhCH_3$) was taken up into two syringes (#1: 29 mL; #2: 28 mL). These portions were added simultaneously in a dropwise fashion using two syringe pumps.

[c] CH_3OH was added cautiously at first and then more liberally.

[d] The aq. H_2SO_4 solution was cooled to room temperature prior to use.

[e] The biphasic mixture was stirred as vigorously as possible without decoupling the stir bar and stir plate.

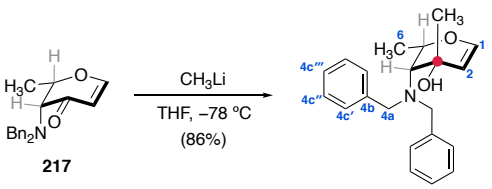
[f] During this quench procedure, additional DI H_2O (300 mL total) was added.

[g] Detailed gradient: 5% (2.6 L) → 10% (1.6 L) → 15% (3.6 L) EtOAc/Hex; compound **217** eluted in 10% →15 % EtOAc/Hex (primarily in 15%).

[h] To isolate product **217** as a solid rather than a thick oil, the purified material was concentrated *in vacuo* (rotovap), placed into an acetone/dry ice bath (-78 °C), and dried under high vacuum.

B.4 *1st Generation Synthesis of Lemonose Thioglycoside **216** from Vinylogous Ester **217***

B.4.1 *Synthesis of Compound **224***



217

224
(dr ≥ 20:1)

Name: (2S,3R,4S)-3-(dibenzylamino)-2,4-dimethyl-3,4-dihydro-2H-pyran-4-ol

Procedure[a]**:** A dried PSF (25 mL) equipped with a stir bar was placed under a double balloon of argon and charged with dry THF (1.3 mL, 0.50 M) followed by methyllithium (0.59 mL, 0.94 mmol, 1.4 Eq | 1.6 M solution in Et_2O). The resulting mixture was chilled in an acetone/dry ice bath (−78 °C). Then a solution of vinylogous ester **217** (201.3 mg, 654.9 μmol, 1.000 Eq) in dry THF (1.0 mL, 0.65 M) was added dropwise via syringe pump over 10 min.[b] The reaction was left to stir at −78 °C for an additional 3 hr at which point it was quenched by the portionwise addition of CH_3OH (0.64 mL) at −78 °C.[c] The quenched reaction was removed from the cooling bath and stirred for 10 min at room temperature. Then the stir bar was removed, and the yellow reaction mixture was concentrated *in vacuo*. The resulting residue was transferred to a separatory funnel (30 mL) using 2:1 Et_2O/DI H_2O (24 mL, v/v). The layers were separated, and the organics were washed with sat. aq NH_4Cl (1 x 3 mL) followed by DI H_2O (1 x 3 mL). The washed organics were dried over anhydrous Na_2SO_4, filtered, concentrated *in vacuo*, co-evaporated with CH_2Cl_2 (3x), and dried overnight under high vacuum to give a crude yellow oil that contained **224** in ≥ 20:1 dr as determined by ^{1}H NMR. Purification of the crude material by FCC (SiO_2: 2% → 15% EtOAc/Hex)[d] afforded tertiary alcohol **224** (182.6 mg, 565.2 μmol, ≥ 20:1 dr, 86% yield) as a pale yellow oil.

^{1}H NMR (600 MHz, $CDCl_3$): δ 7.39-7.21 (m, 10H, H4c), 6.24 (d, *J* = 6.3 Hz, 1H, H1), 5.20 (br s, 1H, OH), 5.00 (dd, *J* = 6.3, 0.7 Hz, 1H, H2), 4.56-4.05 (br s, 2H, H4a), 4.30 (qd, *J* = 6.9, 2.5 Hz, 1H, H5), 3.91 (br d, *J* = 10.2 Hz, 2H, H4a), 2.67 (br s, 1H, H4), 1.60 (d, *J* = 7.0 Hz, 3H, H6), 1.13 (s, 3H, C3Me).

^{13}C{^{1}H} NMR (151 MHz, $CDCl_3$): δ 142.4 (C1), 139.2 (br, C4b)[e], 129.3 (C4c), 128.7 (C4c), 127.5 (C4c), 111.9 (C2), 74.1 (C5), 64.2 (C3), 61.3 (C4), 32.5 (C3Me), 19.6 (C6).[f]

IR (thin film, cm^{-1}): 3420, 3287, 3059, 3031, 2975, 2927, 2862.9, 1647, 1601, 1452, 1383, 1238, 1120, 1156, 750, 701.

HRMS (ESI+, m/z): $[M+H]^+$ calculated for $[C_{21}H_{25}NO_2 + H]^+$ 312.1958 ; found 312.1954 (error 1.2 ppm).

R_f: 0.36 (15% EtOAc/Hex, UV/CAM).

OR: $\alpha_D^{24} = -116^o$ (c = 1.00, $CHCl_3$)

Notes:

[a] Procedure adapted from Nicolaou, K. C.; Koskinen, A. M. P. et al.[9].

[b] The solution of vinylogous ester **217** in dry THF was made in a dry 2 dram vial. After the dropwise addition was complete, the vial was rinsed with additional dry THF (3 x 0.3 mL), and the rinses were added to the reaction.

[c] Before quenching the reaction with CH_3OH, a reaction aliquot was removed, quenched into CH_3OH, and analyzed by TLC developed with 15% EtOAc/Hex (UV/CAM).

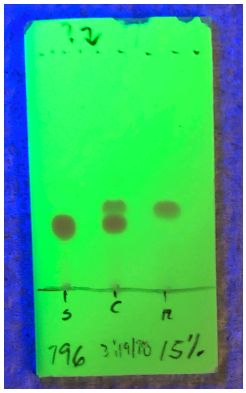
[d] Detailed gradient: 2% (80 mL) → 5% (60 mL) → 15% (180 mL) EtOAc/Hex; **224** eluted in 15% EtOAc/Hex.

[e] Broad, low intensity peak in the 1-D ^{13}C NMR spectrum. Shift obtained from ^{1}H-^{13}C HMBC correlation.

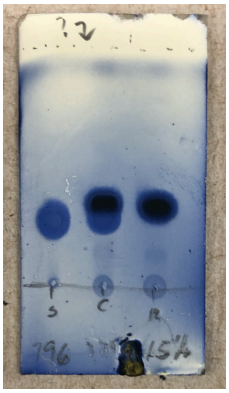
[f] A peak assignable to C4a was not observed in the 1-D ^{13}C NMR spectrum.

*Preparation of Compound **224** Continued*

TLCs:



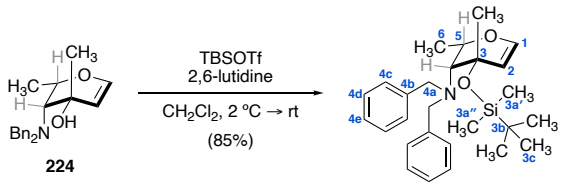
TLC #1a: Reaction Monitoring
Gradient: 15% EtOAc/Hex
Visualization: UV



TLC #1b: Reaction Monitoring
Gradient: 15% EtOAc/Hex
Visualization: CAM Stain

For both plates, lanes from left to right are as follows:
starting material (**217**) | co-spot| reaction ca. 3 hr after end of addition of **217**

B.4.2 *Preparation of Compound* ***225***



224 **225**

Name: (2*S*,3*R*,4*S*)-*N*,*N*-dibenzyl-4-((*tert*-butyldimethylsilyl)oxy)-2,4-dimethyl-3,4-dihydro-2*H*-pyran-3-amine

Procedure[a]**:** In an oven dried vial equipped with a stir bar was prepared a solution of tertiary alcohol **224** (345.8 mg, 1.059 mmol, 1.000 Eq) and 2,6-lutidine (0.50 mL, 4.3 mmol, 4.0 Eq) in dry CH_2Cl_2 (2.2 mL, 0.48 M). The solution was placed under a balloon of argon and cooled in an ice/water bath (ca. 2 °C). TBSOTf (0.50 mL, 2.2 mmol, 2.0 Eq) was added in portions by hand over 5 min. After stirring at 2 °C for another 3 min, the reaction was removed from the ice/water bath and stirred at room temperature for 75 min. Then the reaction was transferred using CH_2Cl_2 (24 mL) to a separatory funnel (60 mL) already charged with sat. aq. $NaHCO_3$ (6 mL). The layers were separated, and the organics were washed with 1 N HCl (2 x 6 mL) followed by DI H_2O (1 x 6 mL). The washed organics were dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. Purification by FCC (SiO_2: 100% Hex → 6% EtOAc/Hex)[a] afforded silyl ether **225** (399.5 mg, 912.7 μmol, 85% yield) as an off-white solid.[c]

^{1}H NMR (600 MHz, $CDCl_3$): δ 7.41-7.32 (m, 4H, H4c), 7.29 (t, *J* = 7.5 Hz, 4H, H4d), 7.20 (t, *J* = 7.3 Hz, 2H, H4e), 6.21 (d, *J* = 6.3 Hz, 1H, H1), 4.90 (d, *J* = 6.2 Hz, 1H, H2), 4.22 (d, *J* = 13.5 Hz, 2H, H4a), 4.18-4.09 (m, 1H, H5), 4.01 (s, 2H, H4a), 2.55 (br d, *J* = 2.1 Hz, 1H, H4), 1.42 (s, 3H, C3Me), 1.32 (d, *J* = 6.5 Hz, H6), 1.01 (s, 9H, H3c), 0.28 (s, 3H, H3a), 0.26 (s, 3H, H3a).

^{13}C NMR (151 MHz, $CDCl_3$): δ 143.1 (C1), 141.6 (C4b), 129.5 (C4c), 128.2 (C4d), 126.7 (C4e), 110.4 (C2), 75.0 (C5), 74.4 (C3), 61.9 (C4), 56.5 (C4a), 34.4 (C3Me), 26.5 (C3c), 18.5 (C3b), 18.3 (C6), -1.1 (C3a), -1.4 (C3a).

IR (thin film, cm^{-1}): 3061, 3027, 2934, 2860, 1646, 1456, 1253, 1220, 1122, 1065, 994, 839, 749, 698.

HRMS (ESI+/m/z): $[M+H]^+$ calculated for $[C_{27}H_{39}NO_2Si + H]^+$ 438.2823; found 438.2823 (error 0.0 ppm).

LC/MS (Agilent A, ESI +): t_R = 1.43 min | $[M+H]^+$ calculated for $[C_{27}H_{39}NO_2Si+H]^+$ 438.3 m/z; found 438.5 m/z.

OR: α_D^{24} = +27.2° (*c* = 1.00, $CHCl_3$)

R_f: 0.76 (15% EtOAc/Hex, UV/CAM)

MP: 107.3-110.9 °C {single point = 109.3 °C}

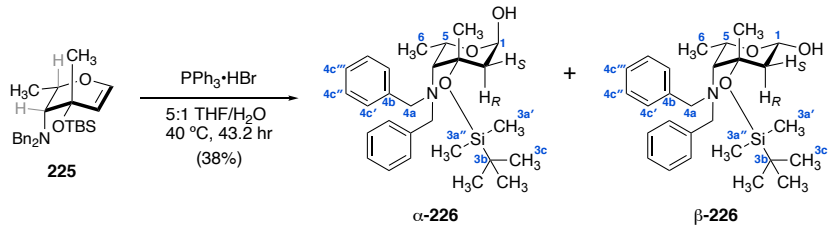
Notes

[a] Procedure adapted from Corey, E. J. et al.10 and Fukuyma, T.; Kan, T. et al.11

[b] Detailed gradient: 100% Hex (300 mL) → 1% (200 mL) → 3% (160 mL) → 6% (200 mL) EtOAc/Hex; **225** eluted in 3% → 6% EtOAc/Hex (primarily in 6%).

[c] A crystal of sufficient quality for XRD analysis was obtained by vapor diffusion (CH2Cl2 = solubilizing solvent; CH3OH = precipitating solvent). The material used to grow this XRD quality crystal was not made by the procedure outlined above; rather, it was made through a one pot sequence involving treatment of vinylogous ester **217** with CH_3Li followed by the additional of TBSOTf to yield silyl ether **225** (93.8 mg, 214 μmol, 66% yield) with 1H and 13C NMR data matching those given above.

B.4.3 *Preparation of Compound* ***226***



Name (α-226): (2*R*,4*S*,5*R*,6*S*)-4-((*tert*-butyldimethylsilyl)oxy)-5-(dibenzylamino)-4,6-dimethyltetrahydro-2*H*-pyran-2-ol

Name (β-226): (2*S*,4*S*,5*R*,6*S*)-4-((*tert*-butyldimethylsilyl)oxy)-5-(dibenzylamino)-4,6-dimethyltetrahydro-2*H*-pyran-2-ol

Procedure: An RBF (25 mL)[a] equipped with a stir bar[a] was charged with glycal **225** (510.1 mg, 1.165 mmol, 1.000 Eq) followed by dry THF (11 mL, 0.11 M). Then PPh_3•HBr (853.7 mg, 2.487 mmol, 2.134 Eq) was added in one portion at room temperature followed 6 min later by the addition of DI H_2O (2.2 mL, 0.12 mmol, $1.1*10^2$ Eq) in one portion at room temperature. 5 min later, the flask was flushed with argon, stoppered with a hard yellow plastic cap, and lowered into a preheated oil bath (set point = 40 °C). The reaction was stirred at 40 °C for 43.2 hr at which point it was removed from the oil bath. After cooling at room temperature for 1 hr, the reaction was transferred to a separatory funnel (250 mL) with Et_2O (45 mL) and H_2O (30 mL).[c] Sat. aq. $NaHCO_3$ (24 mL) was added, the funnel was shaken, and the layers were separated. The resulting aqueous layer (pH ~8–9) was extracted with CH_2Cl_2 (4 x 25 mL).[d] The combined organics (Et_2O + CH_2Cl_2) were dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. Purification by FCC (SiO_2: 2% → 15% EtOAc/Hex) afforded the desired hemiacetal α/β-**226** (201.3 mg, 1.165 mmol, 38% yield, ca. 1:1.3 α/β).[f]

^{1}H NMR (600 MHz, $CDCl_3$, α-226): δ 7.68-7.05 (m, 10H, H4c), 5.36 (app q, *J* = 3.7 Hz, 1H, H1), 4.25 (app qd, *J* = 6.5, 4.0 Hz, 1H, H5), 4.23 (app d, *J* = 4.23, 2H, H4a), 4.08 (br s, 2H, H4a), 2.57 (d, *J* = 3.3 Hz, 1H, OH), 2.49 (d, *J* = 3.8 Hz, 1H, H4), 2.27 (dd, *J* = 13.7, 4.0 Hz, 1H, $H2_{pro\text{-}R}$), 1.81 (dd, *J* = 13.7, 3.2 Hz, $H2_{pro\text{-}S}$), 1.54 (s, 3H, C3Me), 1.34 (d, *J* = 6.6 Hz, 3H, H6), 1.01 (s, 9H, H3c), 0.26 (s, 3H, H3a), 0.22 (s, 3H, H3a).

^{1}H NMR (600 MHz, $CDCl_3$, β-226): δ 7.68-7.05 (m, 10H, H4c), 4.80 (br s, 1H, EXSY with δ 3.67, H4a), 4.73 (ddd, *J* = 9.7, 5.8, 2.8 Hz, 1H, H1), 4.23 (app d, *J* = 4.23, 2H, H4a), 3.67 (br s, 1H, EXSY with δ 4.80, H4a), 3.63 (qd, *J* = 6.3, 3.4 Hz, 1H, H5), 3.14 (d, *J* = 5.9 Hz, 1H, OH), 2.31 (d, *J* = 3.2 Hz, 1H, H4), 2.16 (ABXdd, $^2J_{AB}$ = 12.7 Hz, $^3J_{AX}$ = 9.9 Hz, Δν = 38.2 Hz, 1H, $H2_{pro\text{-}R}$), 2.10 (ABXdd, $^2J_{AB}$ = 12.7 Hz, $^3J_{AX}$ = 2.3 Hz, Δν = 38.2 Hz, 1H, $H2_{pro\text{-}S}$), 1.40 (s, 3H, C3Me), 1.28 (d, *J* = 6.3 Hz, 3H, H6), 1.07 (s, 9H, H3c), 0.31 (s, 3H, H3a), 0.29 (s, 3H, H3a).

^{13}C NMR (151 MHz, $CDCl_3$, α/β-226): δ 141.5 (C4b), 141.3 (C4b), 129.2 (C4c), 128.2 (C4c), 126.8 (C4c), 126.7 (C4c), 94.5 ($^1J_{CH}$ = 159 Hz, β-C1), 91.1 ($^1J_{CH}$ = 166 Hz, α-C1), 78.7 (β-C3), 77.5 (α-C3), 72.8 (β-C5), 68.0 (α-C5), 62.6 (α-C4), 62.1 (β-C4), 56.4 (α-C4a & β-C4a), 46.4 (β-C2), 44.3 (α-C2), 32.1 (α-C3Me), 30.9 (β-C3Me), 26.7 (β-C3c), 26.5 (α-C3c), 18.9 (β-C6), 18.7 (α-C6), 18.58 (C3b), 18.56 (C3b), −0.8 (C3a), −0.9 (C3a), −0.99 (C3a), −1.03 (C3a).[d]

IR (thin film, cm^{-1}, α/β-226): 3395, 3061, 3027, 2935, 2862, 1600, 1456, 1367, 1257, 1117, 1070, 1020, 834, 742, 700.

HRMS (ESI+) m/z (α/β-226): $[M+H]^+$ Calcd for $C_{27}H_{42}NO_3Si$ 456.2928; Found 456.2922 (−1.31 ppm).

R_f (α/β-226): 0.35 & 0.41 (25% EtOAc/Hex, CAM).

Notes

[a] Neither the RBF nor the stir bar were heat dried in any fashion.

[b] Addition of DI H_2O instantaneously changed the physical appearance of the reaction from a white suspension to a colorless liquid with a few white solids.

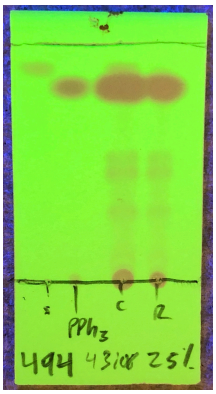
[c] Before transferring the reaction to the separatory funnel (250 mL), the reaction was spotted directly onto a TLC plate subsequently developed with 25% EtOAc/Hex (UV/CAM).

[d] During the first two CH_2Cl_2 extractions, a bright yellow color was observed after the separatory funnel was shaken; this color somewhat faded for the final two CH_2Cl_2 extractions.

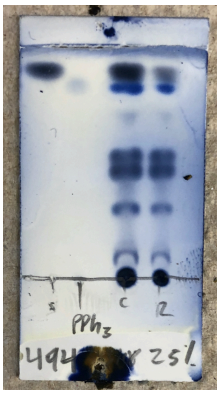
[e] Detailed gradient: 2% (300 mL) → 5% (200 mL) → 10% (180 mL) → 12.5% (200 mL) → 15% (200 mL) EtOAc/Hex; α/β-**226** eluted in 5% → 10% EtOAc/Hex.

[f] ^{1}H NMR indicates the presence of a TBS containing byproduct. As the methyl protons of this impurity were not diastereotopic, we concluded that this was not a carbohydrate bound TBS group. We tentatively assigned the impurity as *tert*-butyldimethylsilanol (TBSOH) so that we could, to some extent, account for this impurity in determining the yield of α/β -**226**.

TLCs:



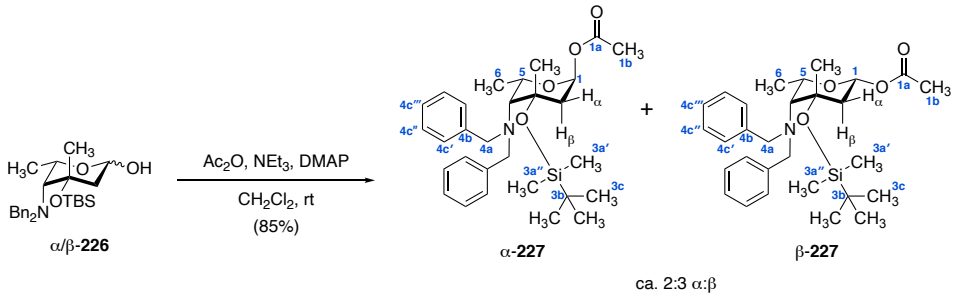
TLC #1a: Reaction Monitoring
Gradient: 25% EtOAc/Hex
Visualization: UV



TLC #1b: Reaction Monitoring
Gradient: 25% EtOAc/Hex
Visualization: CAM Stain

For both plates, lanes from left to right are as follows:
starting material (**225**) | PPh_3 | co-spot| reaction after stirring at 40 °C for ca. 43 hr.

B.4.4 *Preparation of Compound* ***227***



Procedure[a]**:** In an oven dried collection flask (100 mL) equipped with a stir bar was prepared a solution of alcohol α/β-**226** (690.2 mg, 1.515 mmol, 1.000 Eq), triethylamine (NEt_3, 0.65 mL, 4.7 mmol, 3.1 Eq), and DMAP (14.6 mg, 120 μmol, 7.89 mol %) under a balloon of argon. Acetic anhydride (Ac_2O, 0.44 mL, 4.7 mmol, 3.1 Eq) was added in one portion at room temperature, and the reaction was stirred at that temperature for 8.5 hr. Then it was transferred to a separatory funnel (60 mL) using CH_2Cl_2 and washed with sat. aq. $NaHCO_3$ (1 x 15 mL), DI H_2O (1 x 15 mL), and 1 N HCl (2 x 15 mL). The washed organics were dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. Purification by FCC (SiO_2: 3% → 15% **EtOAc/Hex**)[b] afforded the desired acetate α/β-**227** (639.6 mg, 1.285 mmol, 85% yield, ca. 2:3 α/β) as a white foam.[c]

^{1}H NMR (600 MHz, $CDCl_3$, α-227): δ (7.79-6.89, m, 10H, H_{4c}), 6.22 (dd, *J* = 4.3, 2.1 Hz, 1H, H_1), 4.23 (d, *J* = 12.7, 2H, H_{4a}), 4.12 (qd, *J* = 6.5, 3.7 Hz, H_5), 2.50 (d, *J* = 3.5 Hz, 1H, H_4), 2.45 (dd, *J* = 14.3, 4.6 Hz, 1H, $H_2\beta$), 2.05 (s, 3H, H_{1b}), 1.92 (dd, *J* = 14.2, 1.6 Hz, $H_2\alpha$), 1.53 (s, 3H, C_3Me), 1.31 (d, *J* = 6.5 Hz, 3H, H_6), 1.04 (s, 9H, H_{3c}), 0.28 (s, 3H, H_{3a}), 0.25 (s, 3H, H_{3a}).[d]

^{1}H NMR (600 MHz, $CDCl_3$, β-227): δ (7.79-6.89, m, 10H, H_{4c}), 5.65 (dd, *J* = 10.3, 2.9 Hz, H_1), 4.23 (d, *J* = 12.7, 2H, H_{4a}), 3.72 (dq, *J* = 6.3, 3.4 Hz, 1H, H_5), 2.35 (d, *J* = 3.2 Hz, 1H, H_4), 2.35-2.27 (m, 1H, 2.10 (s, 3H, H_{1b}), 1.99 (dd, *J* = 12.6, 2.4 Hz, 1H, $H_2\alpha$), 1.45 (s, 3H, C_3Me), 1.29 (d, *J* = 6.3 Hz, 3H, H_6), 1.08 (s, 9H, H_{3c}), 0.31 (s, 3H, H_{3a}), 0.29 (s, 3H, H_{3a}).[d]

^{13}C NMR (151 MHz, $CDCl_3$, α/β-227): δ 169.7 (α-C_{1a}), 169.6 (β-C_{1a}), 141.4 (C_{4b}), 141.2 (C_{4b}), 129.6 (C_{4c}), 129.4 (C_{4c}), 128.2 (C_{4c}), 126.82 (C_{4c}), 126.81 (C_{4c}), 92.8 ($^1J_{CH}$ = 164 Hz, β-C_1), 92.0 ($^1J_{CH}$ = 171 Hz, α-C_1), 78.6 (α-C_3), 76.9 (β-C_3)[e], 73.8 (β-C_5), 70.0 (α-C_5), 62.5 (α-C_4), 62.2 (β-C_4), 56.5 (β-C_{4a}), 56.3 (α-C_{4a}), 43.4 (β-C_2), 42.2 (α-C_2), 32.0 (α-C_3Me), 30.8 (β-C_3Me), 26.7 (C_{3c}), 26.6 (C_{3c}), 21.43 (C_{1b}), 21.40 (C_{1b}), 18.76 (C_{3b}/C_6), 18.68 (C_{3b}/C_6), 18.58 (C_{3b}/C_6), 18.56 (C_{3b}/C_6), -0.8 (C_{3a}), -0.87 (C_{3a}), -0.91 (C_{3a}), -1.0 (C_{3a}).[f]

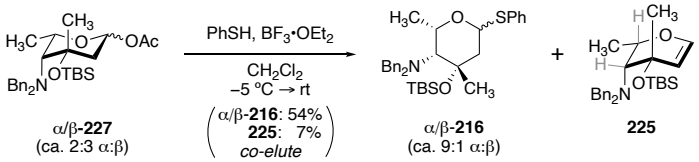
IR (thin film, cm^{-1}): 2937, 2861, 1752, 1598, 1455, 1370, 1236, 1034, 835.

HRMS (ESI+) m/z: $[M+H]^+$ Calcd for $C_{29}H_{44}NO_4Si$ 498.3034; found 498.3045 (error = 2.21 ppm).

Notes:

[a] Procedure adapted from 6) Org. Lett. 2007, 9 (10), 1923-5.
[b] Detailed gradient: 3% (200 mL) → 10% (200 mL) → 15% (200 mL) EtOAc/Hex; α/β-**227** eluted in 10% EtOAc/Hex.
[c] Foaming on high vac controlled using acetone/dry ice bath.
[d] Only 2 out of the 4 anticipated C4a resonances were included in the line listing for both anomers. EXSY indicates that broad resonances centered at 4.98 and 3.70 ppm undergo chemical exchange; we hypothesize that these broad peaks account for the missing C4a resonances.
[e] Obscured by $CDCl_3$ in 1-D ^{13}C NMR; shift determined by HMBC.
[f] Only 4 out of the 6 anticipated C4b carbon resonances were observed, likely due to signal overlap.

B.4.5 *Preparation of Compound* ***216*** *from Acetate* ***227*** *(ca. 7:1 α/β-**216**:**225**)*



Name (α-216): (2*S*,3*R*,4*S*,6*S*)-*N*,*N*-dibenzyl-4-((*tert*-butyldimethylsilyl)oxy)-2,4-dimethyl-6-(phenylthio) tetrahydro-2*H*-pyran-3-amine

Name (β-216): (2*S*,3*R*,4*S*,6*R*)-*N*,*N*-dibenzyl-4-((*tert*-butyldimethylsilyl)oxy)-2,4-dimethyl-6 (phenylthio) tetrahydro-2*H*-pyran-3-amine

Procedure[a]**:** An oven dried test tube (16 x 125 mm) equipped with a stir bar was fitted with a 24/40 septum, placed under a balloon of argon, and charged with a solution of acetate α/β-**227** (321.9 mg, 646.7 mol, 1.000 Eq) in dry CH_2Cl_2 (4.2 mL, 0.15 M). PhSH (0.09 mL, 0.9 mmol, 1 Eq)[b] was added, and the resulting mixture was lowered into an ice/brine bath (ca. −5 °C). Then a solution of BF_3•OEt_2 (0.10 mL, 0.79 mmol, 1.2 Eq)[c] in dry CH_2Cl_2 (0.20 mL, 3.2 M) was added in portions by hand over 6 min. After stirring in the ice/brine bath for an additional 1.4 hr, the reaction was removed from the cooling bath[d] and stirred vigorously for 18.4 hr. Then the reaction was quenched by the addition of sat. aq. $NaHCO_3$ (6 mL)[e–f] and transferred to a separatory funnel (60 mL) using CH_2Cl_2 (21 mL) and DI H_2O (7 mL). The layers were separated, and the resulting organic layer was washed with ca. 0.1 N NaOH (3 x 10 mL), dried over anhydrous Na_2SO_4, filtered, concentrated *in vacuo*, and co-evaporated with hexanes (2x). Purification by FCC (SiO_2: 100% Hex → 3% Et_2O/Hex)[g–h] afforded a white foam consisting of a mixture of desired thioglycoside α/β-**216** (190.3 mg, 347.3 μmol, 54% yield, ca. 9:1 α:β) and undesired glycal **225** (20.4 mg, 46.6 μmol, 7% yield).[i]

Notes:

[a] Procedure adapted from Wang, P. G. et al.[12]

[b] Assuming that the volume of the added PhSH was 0.09 ± 0.01 mL, between 1.2 Eq (0.080 mL, 0.78 mmol) and 1.5 Eq (0.10 mL, 0.97 mmol) of PhSH were added.

[c] The solution of BF_3•OEt_2 in dry CH_2Cl_2 was prepared in an oven dried/desiccator cooled vial; the BF_3•OEt_2 was a pale orange in color.

[d] At this point, the ice/brine bath had warmed to +10 °C, and the reaction was colorless to extremely faint yellow solution.

[e] Before quenching the reaction with sat. aq. $NaHCO_3$, a reaction aliquot was removed, quenched into CH_3OH, and analyzed by TLC developed with 5% EtOAc/Hex (UV/Anisaldehyde stain). TLC indicated the presence of a small amount of starting material α/β-**227**.

[f] At this point, the reaction was a pale orange solution.

[g] Crude material loaded onto column using 100% hexanes due to the low polarity of α/β-**216**.

[h] Detailed gradient: 100% Hex (180 mL) → 1% (140 mL) → 2% (130 mL) → 3% (120 mL) Et_2O/Hex; the co-eluting mixture of α/β-**216** and **225** eluted in 2% → 3% Et_2O/Hex.

[i] See pages 157–158 for the characterization of thioglycoside α/β-**216**.

Preparation of Compound ***226*** *Continued* ***216*** *from Acetate* ***227*** *(ca. 7:1 α/β-**216**:**225**)*

TLCs:

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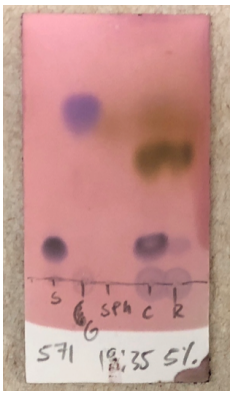
<td> 
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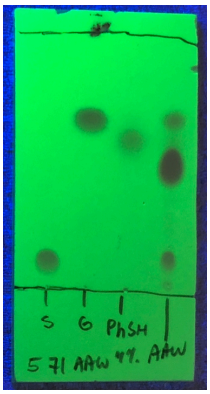
<td> 
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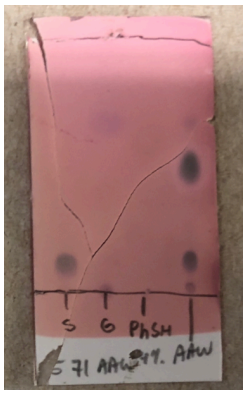
<td> 
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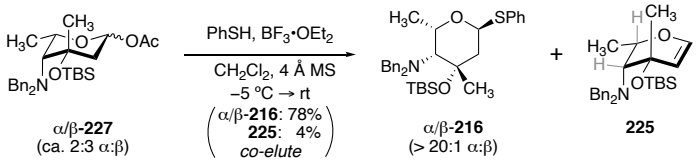
<tr>
<td>TLC #1: Reaction Monitoring
Gradient: 5% EtOAc/Hex
Visualization: Anisaldehyde Stain
Lanes from left to right are as follows:
starting material (α/β-227) | glycal 225 | thiophenol (PhSH) | co-spot | reaction 19 hr after conclusion of $BF_3 \bullet OEt_2$ addition</td>
<td>TLC #2a: Crude
Gradient: 4% Et_2O/Hex
Visualization: UV</td>
<td>TLC #2b: Crude
Gradient: 4% Et_2O/Hex
Visualization: Anisaldehyde Stain</td>
</tr>
<tr>
<td></td>
<td colspan="2">For both plates, lanes from left to right are as follows:
starting material (α/β-227) | glycal 225 | thiophenol | crude reaction mixture</td>
</tr>
</table>

*Plate was broken during staining and development; it was reassembled for this picture.

B.4.6 *Preparation of Compound* ***216*** *from Acetate* ***227*** *(ca. 18:1 α/β-**216**:**225**)*



Name (α-216): (2*S*,3*R*,4*S*,6*S*)-*N*,*N*-dibenzyl-4-((*tert*-butyldimethylsilyl)oxy)-2,4-dimethyl-6-(phenylthio) tetrahydro-2*H*-pyran-3-amine

Name (β-216): (2*S*,3*R*,4*S*,6*R*)-*N*,*N*-dibenzyl-4-((*tert*-butyldimethylsilyl)oxy)-2,4-dimethyl-6 (phenylthio) tetrahydro-2*H*-pyran-3-amine

Procedure[a]**:** A PSF (10 mL)[b] into which acetate α/β-**227** (31.1 mg, 62.5 μmol, 1.00 Eq) had been concentrated was charged with powdered 4 Å MS (40 mg), a dried stir bar, and dry CH_2Cl_2 (0.90 mL, 69 μM). PhSH (25 μL, 0.24 mmol, 3.9 Eq) was added, and the reaction was stirred at room temperature under a double balloon of argon for 30 min. Then the reaction was lowered into an ice/brine bath (ca. −5 °C). After the reaction had been chilled for 15 min, BF_3•OEt_2 (0.08 mL, 0.6 mmol, 10 Eq)[c] was added in one portion. After stirring for an addition 20 min, the reaction was removed from the cooling bath[d] and stirred at room temperature for an additional 17.5 hr. At this point, crushed ice was added followed by 1 N aq. NaOH (4.6 mL).[e] After stirring for 20 min, the quenched reaction mixture was transferred to a separatory funnel (60 mL) using CH_2Cl_2 (30 mL) and H_2O (10 mL). The funnel was shaken, and the layers were separated. The resulting organic layer was washed with 1 N aq. NaOH (1 x 8 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. Purification (SiO_2, 2% → 6% EtOAc/Hex)[f] afforded a co-eluting mixture of desired thioglycoside α/β-**216** (26.7 mg, 48.7 μmol, 78% yield, > 20:1 α:β) and undesired glycal **225** (1.2 mg, 2.7 μmol, 4% yield).[g]

Notes:

[a] Procedure adapted from Wang, P. G. et al.[12]

[b] PSF (10 mL) was neither flame nor oven dried.

[c] Assuming that the volume of the added BF_3•OEt_2 was 0.08 ± 0.01 mL, between 8.8 Eq (0.070 mL, 0.55 mmol) and 11 Eq (0.090 mL, 0.71 mmol) of BF_3•OEt_2 were added.

[d] At this point, the ice/brine bath had warmed to −2 °C.

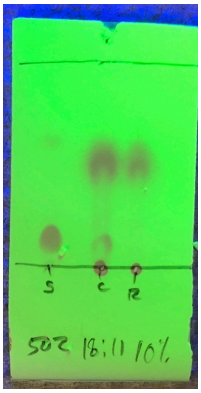
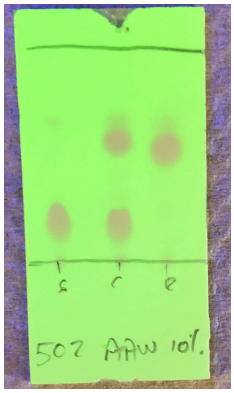
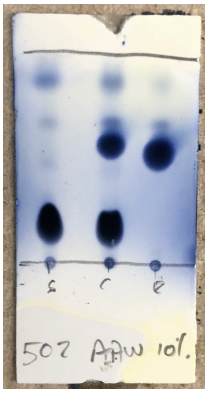
[e] Before addition the crushed ice3, a reaction aliquot was spotted directly onto a TLCs plates developed with 2%, 10%, and 25% EtOAc/Hex (UV/CAM stain).

[f] Detailed gradient: 2% (50 mL) → 3% (40 mL) → 4% (30 mL) → 5% (32 mL) → 6% (30 mL) EtOAc/Hex; mixture of α/β-**216** and **225** eluted in 2% →3% EtOAc/Hex. This mixture was so nonpolar that it eluted while loading the column (liquid loading with CH_2Cl_2 + 2% EtOAc/Hex). As the eluate from column loading was assumed to be free of reaction components, it was recycled. As such, the α/β-**216** and **225** that eluted during column loading was inadvertently poured back on top of the column and eluted a second time. As such, we note that the 100% Hex → 3% Et_2O/Hex (liquid loading with 100% Hex) used in the procedure on page 149 is more effective for this particular purification than the 2% → 6% EtOAc/Hex used for this reaction.

[g] See pages 157–158 for the characterization of thioglycoside α/β-**216**.

Preparation of Compound ***226*** *Continued* ***216*** *from Acetate* ***227*** *(ca. 18:1 α/β-****216****:****225****)*

TLCs:

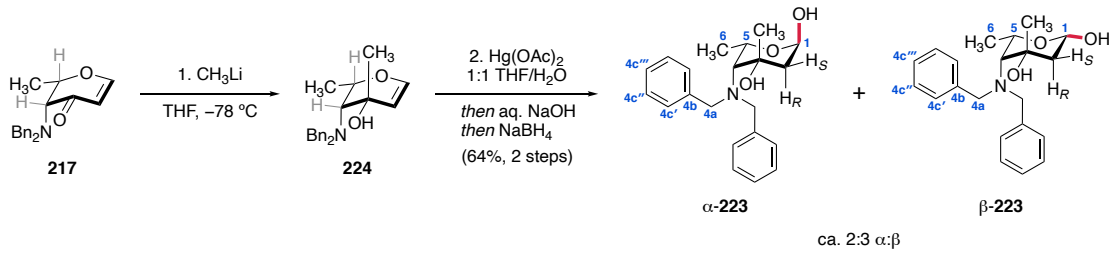
<table>
<tr>
<td>  </td>
<td> </td>
<td>  </td>
<td>  </td>
</tr>
<tr>
<td>TLC #1a: Reaction Monitoring
Gradient: 10% EtOAc/Hex
Visualization: UV</td>
<td>TLC #1b: Reaction Monitoring
Gradient: 10% EtOAc/Hex
Visualization: CAM Stain</td>
<td>TLC #2a: Crude

Gradient: 10% EtOAc/Hex
Visualization: UV</td>
<td>TLC #2b: Crude

Gradient: 10% EtOAc/Hex
Visualization: CAM Stain</td>
</tr>
<tr>
<td colspan="2">For plates 1a–1b, lanes from left to right are as follows:
starting material (α/β-227) | co-spot| reaction 17.4 hr after $BF_3{\bullet}OEt_2$ addition</td>
<td colspan="2">For both plates 2a–2b, lanes from left to right are as follows:
starting material (α/β-227) | co-spot| crude reaction mixture</td>
</tr>
</table>

B.5 *2nd Generation Synthesis of Lemonose Thioglycoside **216** from Vinylogous Ester **217***

B.5.1 *Preparation of Compound **233***



Name (α-233): (2R,4S,5R,6S)-5-(dibenzylamino)-4,6-dimethyltetrahydro-2H-pyran-2,4-diol

Name (β-233): (2S,4S,5R,6S)-5-(dibenzylamino)-4,6-dimethyltetrahydro-2H-pyran-2,4-diol

Procedure (Step 1)[a]**:** A dried collection flask (500 mL) equipped with a stir bar was placed under a balloon of argon and charged with dry THF (75 mL, 0.39 M) followed by methyllithium (25 mL, 40 mmol, 1.4 Eq | 1.6 M solution in Et_2O). The resulting mixture was chilled in an acetone/dry ice bath (−78 °C). Then a solution of vinylogous ester **217** (8.5061 g, 27.672 mmol, 1.0000 Eq) in dry THF (20 mL, 1.4 M) was added dropwise via syringe pump (60 cc/hr).[b] The reaction was stirred for an additional 3.5 hr at -78 °C and then was quenched by the portionwise addition of CH_3OH (25 mL) at −78 °C.[c] The quenched reaction was removed from the cooling bath and stirred for 30 min at room temperature. Next, the stir bar was removed, and the reaction was concentrated *in vacuo* to give a yellow residue that was transferred to a separatory funnel (1 L) using 2:1 Et_2O/DI H_2O (525 mL, v/v). The layers were separated, and the organics were washed with aq. NH_4Cl (2 x 50 mL) followed by DI H_2O (1 x 50 mL). The washed organics were dried over anhydrous Na_2SO_4, filtered, concentrated *in vacuo* (rotovap), and dried on a high vacuum line to give crude **16** which was taken on to **Step 2** without further purification.

Procedure (Step 2)[d]**:** The RBF (2 L) into which crude **16** (theoretically 27.672 mmol) had been concentrated was equipped with a large stir bar and charged with dry THF (162 mL, 0.16 M) and DI H_2O (162 mL, 0.16 M). To the resulting pale yellow mixture was added $Hg(OAc)_2$ (10.5921 g, 33.237 mmol, 1.2011 Eq) in one portion at room temperature.[e] Then the reaction was stoppered with a plastic cap and stirred vigorously at room temperature. After 5.5 hr,[f] the plastic cap was replaced with a Claisen adapter equipped with an addition funnel (500 mL, center neck).[g-h] A freshly prepared solution of aq. NaOH (230 mL, 27.7 g, 693 mmol | 120 mg/mL)[i] was added dropwise via the addition funnel over 10 min. 15 min later, a freshly made solution of $NaBH_4$ (1.9936 g, 52.70 mmol, 1.904 Eq) in aq. NaOH (230 mL, 27.7 g, 693 mmol | 120 mg/mL)[i] was added dropwise via the addition funnel over 15 min. The resulting dark grey mixture was stirred for another 60 min before it was filtered through a pad of SiO_2 topped with sand (ca. 1:3 SiO_2/sand; 600 mL fritted funnel), eluting with EtOAc (4 x 150 mL). The filtrate was transferred to a separatory funnel (2 L) using EtOAc (180 mL) and DI H_2O (100 mL). The layers were separated, and the resulting aqueous layer was extracted with EtOAc (1 x 150 mL). The combined organics were washed with brine (1 x 200 mL), dried over anhydrous Na_2SO_4, filtered through a pad of Celite® topped with sand (600 mL fritted funnel), concentrated *in vacuo*, and co-evaporated with CH_2Cl_2. Purification by FCC (SiO_2: 15% → 50% EtOAc/Hex)[j] afforded reducing sugar α/β-**17** (6.06 g, 17.7 mmol, 64% yield over 2 steps, ca. 2:3 α:β) as an off white foam.

^{1}H NMR (600 MHz, $CDCl_3$, α-233): δ 7.68-6.82 (m, 10H, H4c), 5.36 (d, *J* = 4.5 Hz, 1H, H1), 4.97 (s, 1H, C3OH), 4.51 (dq, *J* = 6.9, 2.7 Hz, 1H, H5), 3.89 (d, *J* = 13.8 Hz, 2H, H4a), 2.57 (d, *J* = 2.6 Hz, 1H, H4), 1.98 (dd, *J* = 14.4, 4.7 Hz, 1H, $H2_{pro\text{-}R}$), 1.90 (d, *J* = 14.3 Hz, 1H, $H2_{pro\text{-}S}$), 1.45 (d, *J* = 7.0 Hz, 3H, H6), 1.28 (s, 3H, C3Me).[k]

^{1}H NMR (600 MHz, $CDCl_3$, β-233): δ 7.68-6.82 (m, 10H, H4c), 5.05 (s, 1H, C3OH), 4.79 (dd, *J* = 10.0, 2.5 Hz, 1H, H1), 3.93 (qd, *J* = 6.9, 2.9 Hz, 1H, H5), 3.89 (d, *J* = 13.8 Hz, 2H, H4a), 2.48 (d, *J* = 2.7 Hz, 1H, H4), 2.00 (dd, *J* = 13.2, 2.3 Hz, 1H, $H2_{pro\text{-}S}$), 1.80 (dd, *J* = 13.3, 10.1 Hz, 1H, $H2_{pro\text{-}S}$), 1.51 (d, *J* = 6.8 Hz, 3H, H6), 1.11 (s, 3H, C3Me).[k]

^{13}C{^{1}H} NMR (151 MHz, $CDCl_3$, α/β-233): δ 139.5 (C4b), 129.2 (C4c), 128.7 (C4c), 127.5 (C4c), 95.2 ($^1J_{CH}$ = 159 Hz, β-C1), 92.4 ($^1J_{CH}$ = 167 Hz, α-C1), 71.5 (β-C5), 68.8 (β-C3), 66.7 (α-C3), 65.8 (α-C5), 63.6 (α-C4), 62.9 (β-C4), 57.0 (br, C4a)[l], 45.2 (β-C2), 41.2 (α-C2), 30.0 (α-C3Me), 28.1 (β-C3Me), 19.5 (α-C6), 19.4 (β-C6).

*Preparation of Compound **233** Continued*

IR (thin film, cm^{-1}, α/β-233): 3361, 3061, 3029, 2972, 2933, 2859, 1599, 1451, 1366, 1172, 1109, 1062, 743, 700.

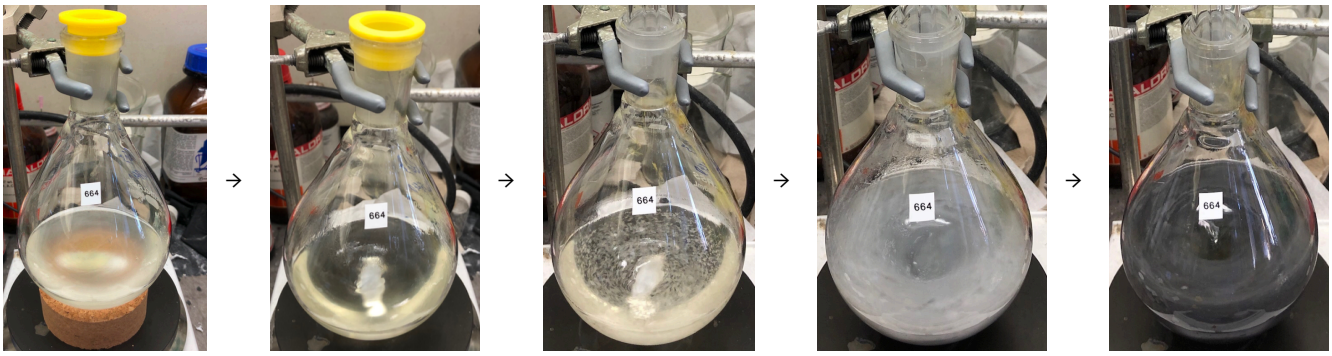
R_f: (α/β-233): 0.37 & 0.45 (50% EtOAc/Hex, CAM)

HRMS (ESI+, m/z, α/β-233): $[M+H]^+$ calculated for $[C_{21}H_{27}NO_3 + H]^+$ 342.2064 ; found 342.2064 (error 0.0 ppm).

Notes:

[a] Procedure for **Step 1** adapted from Nicolaou, K. C.; Koskinen, A. M. P. et al.[9].
[b] The solution of vinylogous ester **217** in dry THF was made in a dried 6 dram vial. After the dropwise addition was complete, the vial was rinsed with additional dry THF (3 x 10 mL), and the rinses were added to the reaction.
[c] Before the reaction was quenched with CH_3OH, the septum was briefly removed and a reaction aliquot was removed. The aliquot was quenched into CH_3OH and analyzed by TLC developed in 15% EtOAc/Hex (UV/CAM).
[d] Procedure for **Step 2** adapted from Brown, H. C. et al.[13].
[e] Additional DI H_2O (2 mL) was used to rinse $Hg(OAc)_2$ stuck on the neck of the flask into the reaction.
[f] Solids were observed 80 min after the addition of $Hg(OAc)_2$.
[g] Before the RBF was fitted with a Claisen adapter, the reaction was spotted directly onto a TLC plate developed in 25% EtOAc/Hex (CAM).
[h] The side neck of the Claisen adapter was left open to the hood to facilitate gas venting.
[i] The aq. NaOH solution was prepared as follows: an Erlenmeyer flask (1L) equipped with a stir bar was charged with solid NaOH (60.13 g, 1.503 mol) and DI H_2O (500 mL). Stirring gave a homogenous solution that was allowed to cool to room temperature prior to use.
[j] Detailed gradient: 15% (1.2 L) → 25% (1.2 L) → 30% (0.5 L) → 37% (0.5 L) → 40% (0.5 L) → 50% (1.4 L) EtOAc/Hex; α/β-**233** eluted in 40% → 50% EtOAc/Hex.
[k] Two of the H4a protons were not observed in the ^1H NMR spectrum.
[l] By ^1H-^{13}C HSQC{^1H}{^{13}C}, we observed 3 correlations with CH_2 phasing in the region expected for the benzyl C4a resonances. Of these three ^{13}C shifts (δ 57.0, 55.4, 53.1), we only included the ^{13}C shift (δ 57.0) in the line listing with a correlation to the assigned H4a protons (^1H: δ 3.89).

Visual Procedure for Step 2:*



A. Step 2: Crude SM (16) in THF/DI H_2O.

B. Step 2: ca. 9 min after $Hg(OAc)_2$ addition.

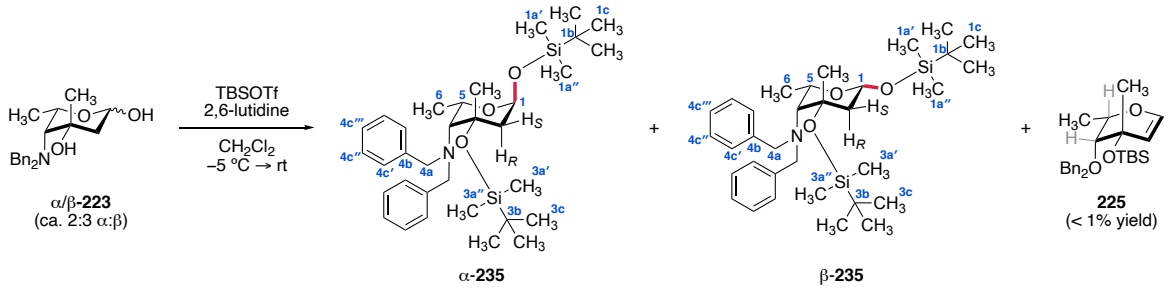
C. Step 2: ca. 3 hr after $Hg(OAc)_2$ addition.

D. Step 2: After addition of initial aq. NaOH.

E. Step 2: After addition of $NaBH_4$ + aq. NaOH.

*Visual procedure from a smaller scale reaction that yielded 2.35 g of α/β-**233**.

B.5.2 *Preparation of Compound* ***235***



Name (α-235): (2*S*,3*R*,4*S*,6*S*)-*N*,*N*-dibenzyl-4,6-bis((*tert*-butyldimethylsilyl)oxy)-2,4-dimethyltetrahydro-2*H*-pyran-3-amine

Name (β-235): (2*S*,3*R*,4*S*,6*R*)-*N*,*N*-dibenzyl-4,6-bis((*tert*-butyldimethylsilyl)oxy)-2,4-dimethyltetrahydro-2*H*-pyran-3-amine

Name (225): (2*S*,3*R*,4*S*)-*N*,*N*-dibenzyl-4-((*tert*-butyldimethylsilyl)oxy)-2,4-dimethyl-3,4-dihydro-2*H*-pyran-3-amine

Procedure[a]**:** A dried collection flask (200 mL) equipped with a stir bar was charged with reducing sugar α/β-**223** (5.0079 g, 14.667 mmol, 1.0000 Eq, ca. 2:3 α:β), dry CH_2Cl_2 (30 mL, 0.49 M), and 2,6-lutidine (10 mL, 86 mmol, 5.9 Eq). The resulting yellow solution was placed under a balloon of argon and chilled in an ice/brine bath (ca. –5 °C). TBSOTf (12 mL, 52 mmol, 3.6 Eq) was added dropwise via syringe pump (18 cc/hr) to give an orange solution. 2.9 hr after the start of the TBSOTf addition, the reaction was removed from the cooling bath and stirred at room temperature for an additional 1.3 hr. Next, the dark orange solution was quenched by the portionwise addition of sat. aq. $NaHCO_3$ (50 mL) over 7 min at rt.[b-d] The quenched reaction mixture was transferred to a separatory funnel (500 mL) using CH_2Cl_2 (90 mL) and DI H_2O (30 mL). The layers were separated, and the organics were washed with 1 N HCl (3 x 40 mL) followed by sat. aq. $NaHCO_3$ (50 mL). The washed organics were dried over anhydrous Na_2SO_4, filtered, concentrated *in vacuo*, and co-evaporated with hexanes (2x). Purification by FCC (SiO_2: 100% Hex → 5% Et_2O/Hex)[e] afforded silyl ether glycoside α/β-**235** (7.66 g, 13.4 mmol, 92% yield, ca. 1:2 α:β) as a sticky colorless foam.[f] ^{1}H NMR indicated the presence of trace amounts of elimination product **225** (35.5 mg, 81.1 μmol, 0.6% yield).[g]

^{1}H NMR (600 MHz, $CDCl_3$, α-235): δ 7.69-6.97 (m, 10H, C4c), 5.31 (dd, *J* = 3.7, 2.3 Hz, 1H, H1), 4.15 (app qd, *J* = 6.5, 3.8 Hz, 1H, H5), 2.45 (d, *J* = 3.6 Hz, 1H, H4), 2.31 (dd, *J* = 13.4, 4.1 Hz, 1H, $H2_{pro\text{-}R}$), 1.80 (dd, *J* = 13.4, 1.8 Hz, 1H, $H2_{pro\text{-}S}$), 1.54 (s, 3H, C3Me), 1.28 (d, *J* = 6.5 Hz, 3H, H6), 1.04 (s, 9H, H3a), 0.88 (s, 9H, H3c), 0.275 (s, 3H, H3a), 0.24 (s, 3H, H3a), 0.08 (s, 3H, H1a), 0.06 (s, 3H, H1a).[i]

^{1}H NMR (600 MHz, $CDCl_3$, β-235): δ 7.69-6.97 (m, 10H, C4c), 4.66 (dd, *J* = 9.7, 2.6 Hz, 1H, H1), 3.53 (qd, *J* = 6.3, 3.4 Hz, 1H, H5), 2.26 (d, *J* = 3.2 Hz, 1H, H4), 2.18 (dd, *J* = 12.7, 9.8 Hz, 1H, $H2_{pro\text{-}R}$), 1.95 (dd, = 12.8, 2.1 Hz, $H2_{pro\text{-}S}$), 1.38 (s, 3H, C3Me), 1.25 (d, *J* = 6.3 Hz, 3H, H6), 1.07 (s, 9H, H3c), 0.89 (s, 9H, H1c), 0.31 (s, 3H, H3a), 0.284 (s, 3H, H3a), 0.09 (s, 6H, H1a).[h]

^{13}C{^{1}H} NMR (151 MHz, $CDCl_3$, α/β-235): δ 142.0 (C4b), 141.6 (C4b), 129.8 (br, C4c), 129.5 (br, C4c), 129.4 (C4c), 128.3 (br, C4c), 128.3 (C4c), 127.9 (br, C4c), 126.7 (C4c), 126.6 (C4c), 95.3 ($^1J_{CH}$ = 159 Hz, β-C1), 91.7 ($^1J_{CH}$ = 170 Hz, α-C1), 79.0 (β-C3), 77.8 (α-C3), 72.5 (β-C5), 67.2 (α-C5), 62.7 (α-C4), 62.3 (β-C4), 56.7 (br, C4a), 56.4 (C4a), 56.3 (br, C4a), 47.7 (β-C2), 45.6 (α-C2), 32.6 (α-C3Me), 31.1 (β-C3Me), 26.7 (β-C3c), 26.6 (α-C3c), 26.1 (β-C1c), 25.8 (α-C1c), 18.9 (C1a/C3a/C6), 18.8 (C1a/3a/6), 18.6 (C1a/C3a/C6), 18.4 (C1a/C3a/C6), 18.0 (C1a/3a) -0.7 (β-C3a), -0.80 (β-C3a), -0.85 (α-C3a), -0.90 (α-C3a), –3.9 (β-C1a), –4.3 (α-C1a), –4.6 (β-C1a), –5.6 (α-C1a).[i]

*Preparation of Compound **235** Continued*

IR (thin film, cm^{-1}, α/β-235): 3062, 3027, 2939, 2860, 1600, 1461, 1377, 1254, 1124, 1078, 1020, 835.

HRMS (ESI+, m/z, α/β-235): $[M+H]^+$ calculated for $[C_{33}H_{55}NO_3Si_2 + H]^+$ 570.3793; found 570.3806 (error 2.3 ppm).

R_f(α/β-235): 0.39 (4% Et_2O/Hex, CAM stain)

Notes:

[a] Procedure adapted from Corey, E. J. et al.[10] and Fukuyama, T.; Kan, T. et al.[11].

[b] Before the reaction was quenched with sat. aq. $NaHCO_3$, the septum was briefly removed, and an aliquot was removed from the reaction. The aliquot was quenched into EtOAc/sat. aq. $NaHCO_3$ and analyzed by TLC developed in 5% and 50% EtOAc/Hex (UV/CAM).

[c] The reaction bubbled during the quench.

[d] The stir rate was increased during the quench.

[e] Detailed gradient: 100% Hex → 0.5% (0.5 L) → 1% (0.5 L) → 2% (1 L) → 5% (2.4 L) Et_2O/Hex; α/β-**235** eluted in 5% Et_2O/Hex.

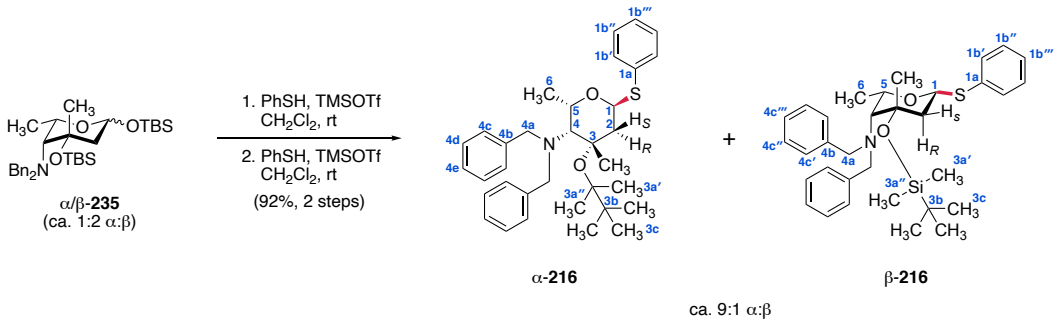
[f] Foaming on a high vac line was controlled using an acetone/dry ice bath.

[g] See page 145 for characterization of **225**.

[h] ^1H resonances for the benzyl protection group hydrogens H4a are absent from the line listing due to their complexity. Based on ^1H-^{13}C HSQC{^1H}{^{13}C}, we observed the following four resonances consistent with H4a protons: δ 4.85 (br d, *J* = 8.0 Hz), 4.30-4.18 (m), 4.07 (s), and 3.67 (br d, *J* = 8.2 Hz). ^1H-^1H NOESY/EXSY indicates that δ 4.85 and δ 3.67 are related by chemical exchange; the same experiment also shows that δ 4.30-4.18 has NOESY correlations with both of δ 4.85 and δ 3.67. Setting the integration of β-H1 to 1H, the combined integration of δ 4.85, δ 4.30-4.18, and δ 3.67 is 5.1H. This is in decent agreement with the expected total integration of 6H for a ca. 1:2 α/β mixture of **235**.

[i] For benzyl protecting group carbons C4a and C4c, we observed more signals than expected (C4a: expected 2, observed 3; C4c: expected 6, observed 8; expected resonances determined with the assumption that none of the resonances are degenerate between the α- and β-anomers). We attribute this to hindered rotation as further evidenced by the broadness (denoted as "br") of some of these resonances.

B.5.3 *Preparation of Compound* ***216*** *from TBS Glycoside* ***235***



Name (α-216): (2*S*,3*R*,4*S*,6*S*)-*N*,*N*-dibenzyl-4-((*tert*-butyldimethylsilyl)oxy)-2,4-dimethyl-6-(phenylthio) tetrahydro-2*H*-pyran-3-amine

Name (β-216): (2*S*,3*R*,4*S*,6*R*)-*N*,*N*-dibenzyl-4-((*tert*-butyldimethylsilyl)oxy)-2,4-dimethyl-6 (phenylthio) tetrahydro-2*H*-pyran-3-amine

Procedure (Step 1)[a]**:** In an oven dried collection flask (200 mL) equipped with a stir bar was made a colorless solution of α/β-**235** (4.0244 g, 7.0606 mmol, 1.0000 Eq, ca. 1:2 α:β) and PhSH (3.2 mL, 31 mmol, 4.4 Eq) in dry CH_2Cl_2 (32 mL, 0.22 M). TMSOTf (4.0 mL, 22 mmol, 3.1 Eq) was added in one portion at room temperature. Then the reaction flask was flushed with argon and stoppered with a hard plastic cap. The reaction was stirred at room temperature for 14.5 hr. Then it was transferred using CH_2Cl_2 (120 mL) to a separatory funnel (500 mL) already charged with sat. aq. $NaHCO_3$ (120 mL). The layers were separated, and then the organics were washed with additional sat. aq. $NaHCO_3$ (2 x 120 mL).[b] The washed organics were dried over anhydrous $MgSO_4$, filtered through a Celite® pad (150 mL fritted funnel), and concentrated *in vacuo*. Crude NMR analysis revealed that the reaction was not complete.[c] As such, the crude material was transferred to a flame dried collection flask (200 mL) using dry CH_2Cl_2. The majority of the solvent was removed to give a yellow-orange liquid that re-subjected to the reaction conditions as detailed in Step 2.

Procedure (Step 2)[a]**:** The flame dried collection flask (200 mL) into which the crude product (theoretically 7.0606 mmol) of Step 1 had been concentrated was equipped with an oven dried stir bar and charged with dry CH_2Cl_2 (32 mL, 0.22 M) followed by PhSH (3.2 mL, 31 mmol, 4.4 Eq). TMSOTf (4.0 mL, 22 mmol, 3.1 Eq) was added in one portion at room temperature. Then the reaction flask was flushed with argon and stoppered with a hard plastic cap. The reaction was stirred at room temperature for 17 hr. Then it was transferred using CH_2Cl_2 (120 mL) to a separatory funnel (500 mL) already charged with sat. aq. $NaHCO_3$ (120 mL). The layers were separated, and then the organics were washed with additional sat. aq. $NaHCO_3$ (2 x 120 mL). The washed organics were dried over anhydrous Na_2SO_4, filtered, concentrated *in vacuo*, and co-evaporated with hexanes (3x). Purification by FCC (SiO_2: 100% → 4% Et_2O/Hex)[d] afforded the desired thioglycoside α/β-**216** (3.5404 g, 6.4621 mmol, 92% yield over 2 steps, ca. 9:1 α:β) as a white foam.[e]

^{1}H NMR (600 MHz, $CDCl_3$, α-216): δ 7.52-7.13 (m, 15H, HAr)[e], 5.57 (dd, *J* = 5.9, 5.1 Hz, 1H, H1), 4.38 (qd, *J* = 6.7, 4.4 Hz, 1H, H5), 4.23 (dis br d, *J* = 12.5 Hz, 2H, H4a), 4.05 (br s, 2H, H4a), 2.61 (d, *J* = 4.3 Hz, 1H, H4), 2.45 (dd, *J* = 13.9, 4.8 Hz, 1H, $H2_{pro\text{-}R}$), 1.92 (dd, *J* = 13.9, 6.2 Hz, 1H, $H2_{pro\text{-}S}$), 1.57 (s, 3H, C3Me), 1.46 (d, *J* = 6.7 Hz, 3H, H6), 0.97 (s, 9H, H3c), 0.22 (s, 3H, H3a), 0.18 (s, 3H, H3a).

^{1}H NMR (600 MHz, $CDCl_3$, β-216): δ 7.52-7.13 (m, 15H, HAr)[e], 4.71 (dd, *J* = 12.4, 2.6 Hz, 1H, H1), 4.62 (br d, *J* = 10.8 Hz, 2H, H4a) 3.67 (qd, *J* = 6.3, 3.3 Hz, 1H, H5), 3.64 (app br d,[g] 2H, H4a), 2.32 (d, *J* = 3.1 Hz, 1H, H4), 2.32 (app t, *J* = 12.7 Hz, 1H, $H2_{pro\text{-}R}$), 2.04 (dd, *J* = 13.1, 1.8 Hz, 1H, $H2_{pro\text{-}S}$), 1.42 (s, 3H, C3Me), 1.31 (d, *J* = 6.3 Hz, H6), 1.05 (s, 9H, H3c), 0.27 (s, 3H, H3a), 0.26 (s, 3H, H3a).

*Preparation of Compound **216** from TBS Glycoside **235** Continued*

$^{13}C\{^1H\}$ NMR (151 MHz, $CDCl_3$, α/β-216): δ 141.0 (α-C4b), 135.5 (α-C1a), 134.4 (β-C1a), 132.5 (CAr), 131.2 (α-C1b), 129.0 (α-C4c), 128.8 (CAr), 128.3 (α-C4d), 127.7 (CAr), 127.1 (α-C1b), 126.9 (α-C4e), 126.7 (CAr), 82.1 ($^1J_{CH}$ = 154 Hz, β-C1), 80.2 ($^1J_{CH}$ = 162 Hz, α-C1), 78.9 ((β-C3), 78.2 (α-C5) 76.6 (β-C5), 70.0 (α-C5), 62.5 (α-C4), 62.1 (β-C4), 56.3 (α-C4a & β-C4a)[g], 45.7 (α-C2), 44.7 (β-C2), 31.2 (α-C3Me), 30.3 (β-C3Me), 26.6 (β-C3c), 26.4 (α-C3c), 19.3 (β-C6), 18.5 (α-C3b & β-C3b)[g], 18.2 (α-C6), −0.7 (β-C3a), −0.9 (β-C3a), −1.2 (2 x α-C3a)[h].

IR (thin film, cm^{-1}, ca. 9:1 α:β-216): 3062, 3026, 2936, 2858, 1585, 1457, 1256, 1111, 991, 836, 744, 696.

HRMS (ESI+, m/z, ca. 9:1 α:β-216): $[M+H]^+$ calculated for $[C_{33}H_{45}NO_2SSi + H]^+$ 548.3013; found 548.3012 (error 0.2 ppm).

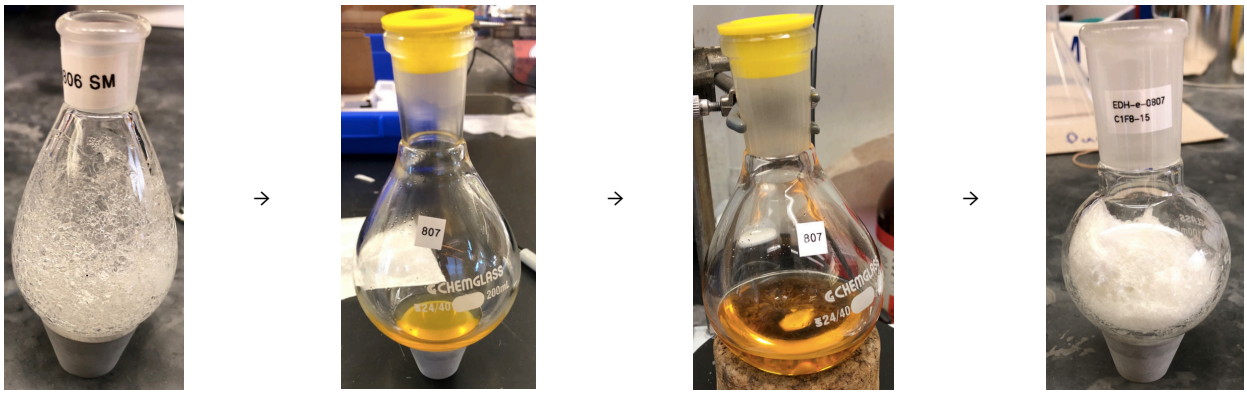
OR (ca. 9:1 α:β-216): α_D^{24} = −91.8° (*c* = 1.00, $CHCl_3$)

R_f (ca. 9:1 α:β-216): 0.23 (4% Et_2O/Hex, CAM)

Notes:

[a] Procedure adapted from Szeja, W. et al.[14].
[b] Throughout the aqueous workup, there was a fluffy yellow solid present that was always taken with the organic layer.
[c] This crude ^1H NMR spectrum is analyzed in Figure A.4b and presented in Table 2.2a-b (Entry 1).
[d] Detailed gradient: 100% Hex (2.4 L) → 1% (1 L) → 2% (1.2 L) → 3% (1.2 L) → 4% (1.2 L) Et_2O/Hex; α/β-**216** eluted in 3% → 4% Et_2O/Hex.
[e] After concentrating the purified product *in vacuo* (rotovap), a dry ice/acetone bath was used to chill the resulting residue and thus control it as it foamed under high vacuum.
[f] Due to the complexity of the ^1H NMR aromatic region, the entire region was integrated as one entity. A combination of various 2-D NMR techniques allowed us to make the following assignments in which ^1H resonances were determined from 2-D correlations/cross-peaks: δ 7.35 (H4c), 7.31 (H4d), 7.23 (H4e).
[g] Based on the ^1H-^{13}C HSQC$\{^1H\}\{^{13}C\}$, it appears that this ^1H resonance is a broad doublet centered at 3.64 ppm that is obscured by a neighboring qd (3.67 ppm).
[h] Based on the ^1H-^{13}C HSQC$\{^1H\}\{^{13}C\}$ spectrum, the indicated carbons share the same ^{13}C shift.
[i] 12 of the theoretical 16 aromatic ^{13}C resonances were observed. Presumably, incidental peak overlap and/or low intensity peaks (particularly for the less abundant β-**216**) can account for these unobserved resonances.

Visual Procedure:



A. Step 1: Starting Material (α/β-**235**)

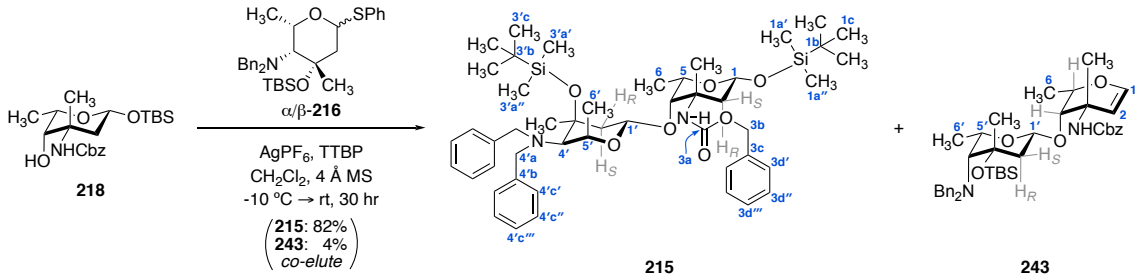
B. Step 1: Crude product (starting material for Step 2)

C. Step 2: 17 hr after TMSOTf addition

D. Step 2: Purified thioglycoside α/β-**216**.

B.6 *Synthesis and Structural Verification of Fully Protected, α-Linked Arimetamycin A Disaccharide* ***215***

B.6.1 *Preparation of Compound* ***215***



Name (215): benzyl ((2*S*,3*S*,4*S*,6*R*)-6-((*tert*-butyldimethylsilyl)oxy)-3-(((2*S*,4*S*,5*R*,6*S*)-4-((*tert*-butyldimethylsilyl)oxy)-5-(dibenzylamino)-4,6-dimethyltetrahydro-2*H*-pyran-2-yl)oxy)-2,4-dimethyltetrahydro-2*H*-pyran-4-yl)carbamate

Name (243): benzyl ((2*S*,3*S*,4*S*)-3-(((2*R*,4*S*,5*R*,6*S*)-4-((*tert*-butyldimethylsilyl)oxy)-5-(dibenzylamino)-4,6-dimethyltetrahydro-2*H*-pyran-2-yl)oxy)-2,4-dimethyl-3,4-dihydro-2*H*-pyran-4-yl)carbamate

Procedure[a]**:** In a flame dried collection flask (100 mL, 24/40 neck) equipped with a stir bar was made a mixture of acceptor β-**218** (1.2157 g, 2.9680 mmol, 1.0000 Eq), donor α/β-**216** (2.2904 g, 4.1806 mmol, 1.4085 Eq), 2,4,6-tri-*tert*-butyl pyrimidine (TTBP, 2.9513 g, 11.881 mmol, 4.0029 Eq), and freshly activated powdered 4 Å MS (ca. 3.11 g) in dry CH_2Cl_2 (30 mL, 0.10 M). The flask was flushed with argon and stoppered with a septum (secured with electrical tape and then parafilm). The reaction mixture was left to stir vigorously for 16.4 hr. Then the reaction was placed under a balloon of argon, wrapped in foil,[b] and cooled in an ice/brine bath (ca. -10 °C). A solution of $AgPF_6$ (3.0142 g, 4.0168 Eq, 11.922 mmol) in dry CH_2Cl_2 (4.0 mL, 0.74 M) was added in one continuous portion.[c] The reaction was stirred in the ice/brine bath for 3 hr, during which time the bath warmed to 10 °C, before it was removed from the cooling bath and left to vigorously at room temperature for 27 hr. The reaction was quenched by the addition of pyridine (12 mL, 0.15 mol, 50 Eq) in one continuous portion at room temperature. The reaction was stirred for an additional 20 min at room temperature before it was filtered through a Celite® pad topped with sand (150 fritted funnel, eluting with CH_2Cl_2). The white opaque filtrate was concentrated *in vacuo* and co-evaporated with $PhCH_3$ (3 x 40 mL).[d] This crude material was concentrated onto ca. 4:1 Celite®/SiO_2 (20 g, w/w). Purification by FCC (SiO_2: solid loading, 2% → 10% EtOAc/Hex)[e] afforded a white foam[f] containing a mixture of the desired disaccharide **215** (2.0707 g, 2.4439 mmol, 82% yield) and undesired glycal **243** (86.4 mg, 121 μmol, 4% yield). This ≥ 20:1 (mol:mol) mixture of **215**: **243** was taken on without further purification for characterization and further synthetic manipulation.

^{1}H NMR (600 MHz, $CDCl_3$, Compound 215): δ 7.41-7.29 (m, 10H, H3d/4′c), 7.41-7.29 (m, 3H, H3d/4′c), 7.19 (app t, J = 7.2 Hz, 2H, H3d/4′c), 6.77 (br s, 1H, C3NH), 5.09 (ABq, *J* = 12.4 Hz, Δν = 62.8 Hz, 1H, H3b), 4.98 (ABq, *J* = 12.4 Hz, Δν = 62.8 Hz, 1H, H3b), 4.89 (dd, *J* = 7.2, 2.9 Hz, 1H, H1′), 4.79 (br d, *J* = 8.7 Hz, 1H, H1), 4.36-4.28 (m, 1H, H5′), 4.21 (br s, 2H, H4′a), 3.88 (br s, 2H, H4′a), 3.80 (q, *J* = 6.3 Hz, 1H, H5), 2.97 (s, 1H, H4), 2.62 (d, *J* = 4.7 Hz, 1H, H4′), 2.44 (br d, *J* = 12.4 Hz, 1H, $H2_{pro\text{-}S}$), 2.20 (dd, *J* = 13.6, 2.7 Hz, 1H, $H2'_{pro\text{-}R}$), 1.70 (dd, *J* = 13.6, 7.2 Hz, 1H, $H2'_{pro\text{-}S}$), 1.56 (dd, *J* = 12.9, 9.9 Hz, 1H, $H2_{pro\text{-}R}$), 1.53 (ovlp d, *J* = 6.9 Hz, 3H, H6′), 1.50 (br s, 3H, C3Me), 1.47 (br s, 3H, C3′Me), 1.20 (d, *J* = 6.4 Hz, 3H, H6), 0.91 (s, 9H, H3′c), 0.87 (s, 9H, H1c), 0.18 (s, 3H, H3′a), 0.09 (s, 3H, H1a), 0.07 (s, 6H, H1a & H3′a).

^{13}C NMR (151 MHz, $CDCl_3$, Compound 215): δ 155.1 (C3a), 140.6 (C4′b), 137.4 (C3c), 128.6 (C3d/4′c), 128.5 (C3d/4′c), 128.4 (C3d/4′c), 128.0 (C3d/4′c), 127.9 (C3d/4′c), 126.9 (C3d/4′c), 98.0 ($^1J_{CH}$ = 162 Hz, C1′), 94.2 ($^1J_{CH}$ = 155 Hz, C1), 82.4 (C4), 78.3 (C3′), 70.0 (C5′), 68.6 (C5), 65.7 (C3b), 62.2 (C4′), 56.2 (C4′a), 54.0 (C3), 45.4 (C2′), 39.7 (C2), 30.5 (C3′Me), 26.3 (C3′c), 26.0 (C1c), 21.9 (C3Me), 18.52 (C1b/3′b/6′), 18.49 (C1b/3′b/6′), 18.3 (C1b/3′b) , 17.9 (C6), −1.37 (C1a/3′a), −1.39 (C1a/3′a), −3.9 (C1a/3′a), −4.6 (C1a/3′a).

*Preparation of Compound **215** Continued*

^{1}H NMR (600 MHz, $CDCl_3$, Compound 243): δ 6.30 (d, *J* = 6.2 Hz, 1H, H1), 4.74 (dd, *J* = 6.2, 1.3 Hz, 1H, H2), 4.46 (dd, *J* = 10.3, 2.4 Hz, 1H, H1′), 5.17 (br s, 1H, C3NH), 4.16 (1H, H5)[g], 3.83 (1H, H4)[g], 3.50 (1H, H5′)[g], 2.27 (1H, H2′$_{pro\text{-}R}$)[g], 2.25 (1H, H4′)[g], 1.95 (br d, *J* ~ 13 Hz, 1H, H2′$_{pro\text{-}S}$), 1.59 (3H, C3Me)[g],1.32 (d, *J* = 6.5 Hz, 3H, H5), 1.29 (s, 3H, C3′Me), 1.23 (3H, H6′)[g].[h]

^{13}C NMR (151 MHz, $CDCl_3$, Compound 243): δ 143.2 (C1), 105.0 ($^1J_{CH}$ = 166 Hz, C2), 101.3 (^1J = 159 Hz, C1′), 78.8 (C3′), 77.8 (C4), 72.6 (C5′), 71.7 (C5), 62.4 (C4′), 52.4 (C3), 45.0 (C2′), 31.0 (C3′Me), 27.5 (C3Me), 17.3 (C6).[i]

IR (thin film, cm^{-1}, ≥ 20:1 215: 243): 3340, 3028, 2940, 2892, 2861, 1724, 1513, 1457, 1380, 1249, 1072, 839.

HRMS (ESI+, m/z, Compound 215): $[M+H]^+$ calculated for $[C_{48}H_{74}N_2O_7Si_2 + H]^+$ 847.5107; found 847.5107 (error 0.0 ppm).[j]

HRMS (ESI+, m/z, Compound 243): $[M+H]^+$ calculated for $[C_{42}H_{58}N_2O_6Si + H]^+$ 715.4137; found 715.4131 (error 0.8 ppm).[j]

OR (≥ 20:1 215: 243): $\alpha_D^{24} = -20.960^o$ (*c* = 1.00, $CHCl_3$)

R_f: 0.20 (10% EtOAc/Hex, CAM stain)

Notes:

[a] Procedure adapted from Lear, M. J. et al[15]

[b] Hood light was also turned off.

[c] The solution of $AgPF_6$ in dry CH_2Cl_2 was prepared as follows: $AgPF_6$ (3.0142 g) was weighed into a 20 mL vial in a N_2 filled glovebox. The vial was capped with a septum cap, wrapped in foil, and brought out of the glovebox. The cap was secured with parafilm, the vial was placed under a balloon of argon, and dry CH_2Cl_2 (4.0 mL) was added to give the solution described above. After the addition was complete, the vial was rinsed with additional dry CH_2Cl_2 (3 x 1.4 mL), and the rinses were added to the reaction.

[d] After the $PhCH_3$ co-evaporations (3x), CH_2Cl_2 was added to give a suspension of yellow solids in a pale-yellow liquid. In an attempt to obtain a homogenous crude NMR sample, the CH_2Cl_2 suspension was swirled and an aliquot was removed without delay. This crude aliquot was concentrated *in vacuo* (rotovap), further dried under high vacuum, and analyzed by NMR (see Figure A.5 on page 114 for analysis). Due to the presence of a significant amount of solid material in the initially prepared crude NMR sample ($CDCl_3$), the $CDCl_3$ soluble supernatant from that sample was transferred to a fresh NMR tube prior to analysis. As such, the crude analysis only accounts for $CDCl_3$ soluble compounds.

[e] Detailed gradient: 2% (1 L) → 4% (0.6 L) → 6% (0.6 L) → 8% (0.6 L) → 10% (0.6 L) EtOAc/Hex; ≥ 20:1 **215**:**243** eluted in 6 → 8% EtOAc/Hex.

[f] Foaming on high vac controlled using acetone/dry ice bath.

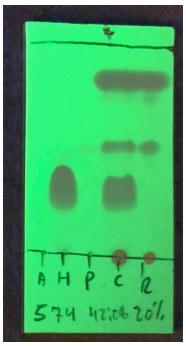
[g] ^{1}H shift determined by 2-D NMR techniques. As such, multiplicity data was unavailable. Integrations for these peaks were assumed based on their assignment.

[h] For minor component **243**, the only peaks reported are those that lent themselves to confident assignment. Resonances for both anomeric peaks as well the carbohydrate backbone are given. The integration for each resonance was assumed based on its assignment.

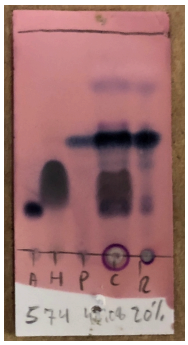
[i] For minor component **243**, the ^{13}C shifts were determined by ^{1}H-^{13}C HSQC{^{1}H}{^{13}C} and ^{1}H-^{13}C HMBC due to their low abundance. Only those shifts that lent themselves to confident assignment are reported.

[j] HRMS data acquired on a sample of ≥ 20:1 **215**:**243**; as such, both [M+H] adducts were observed in the same mass spectrum.

TLCs:*



TLC #1a: Reaction Monitoring
Gradient: 15% EtOAc/Hex
Visualization: UV



TLC #1b: Reaction Monitoring
Gradient: 15% EtOAc/Hex
Visualization: Anisaldehyde Stain

For plates 1a–1b, lanes from left to right are as follows: acceptor (**218**) | hydrolyzed donor | desired product (**215**) | co-spot | reaction 24.5 hr after $AgPF_6$ addition

*TLCs from smaller scale reaction that yielded 145 mg of disaccharide **215**.

*Preparation of Compound **215** Continued*

Visual Procedure:

A. Donor (**216**), acceptor (**218**), and base (TTBP) stirring vigorously over powdered 4 Å MS in dry CH_2Cl_2.

→

B. 12 min after $AgPF_6$ addition.

→

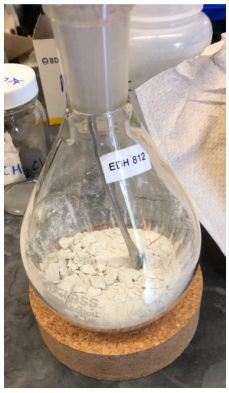
C. Quenched reaction mixture prior to filtration.

→

D. After filtration of quenched reaction mixture through Celite® pad topped with sand.

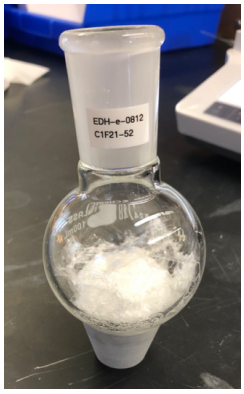
E. Aliquot for crude NMR analysis after drying overnight on high vac.

→



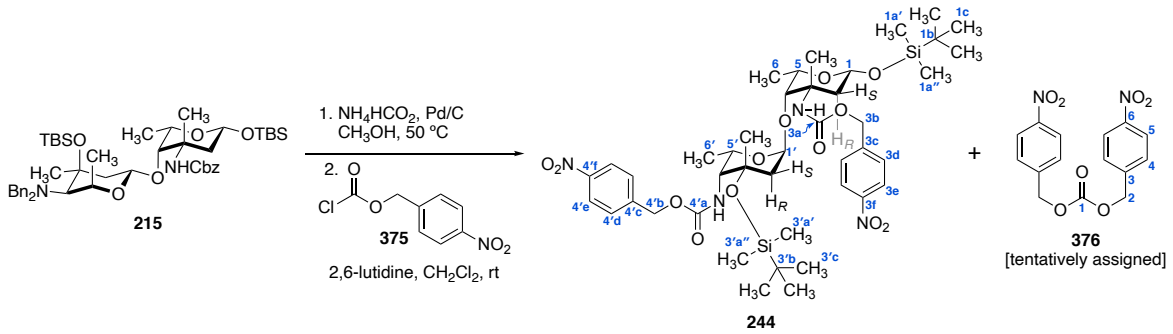
F. Crude reaction mixture loaded onto ca. 4:1 Celite®/SiO2 (20 g, w/w).

→



G. Purified disaccharide **215** (≥ 20:1 **215**:**243**).

B.6.2 *Preparation of Compound* ***244***



Name (244): 4-nitrobenzyl ((2S,3S,4S,6R)-6-((tert-butyldimethylsilyl)oxy)-3-(((2S,4S,5R,6S)-4-((tert-butyldimethylsilyl)oxy)-4,6-dimethyl-5-((((4-nitrobenzyl)oxy)carbonyl)amino)tetrahydro-2H-pyran-2-yl)oxy)-2,4-dimethyltetrahydro-2H-pyran-4-yl)carbamate

Name (376): bis(4-nitrobenzyl) carbonate

Procedure (Step 1)[a]**:** An oven dried vial (2 dram) equipped with a stir bar was charged with disaccharide **215** (20.5 mg, 24.2 μmol, 1.00 Eq), NH_4HCO_2 (34.4 mg, 546 μmol, 22.5 Eq), and Pd/C (12.5 mg)[b], in that order. The vial was stoppered with a 14/20 septum[c] and flushed with argon from a double balloon for 2 min. Leaving the vial under argon, dry CH_3OH (0.80 mL, 30 mM) was then added cautiously through the septum. Finally, the reaction was lowered into a preheated oil bath (set point = 50 °C). After stirring at 50 °C for 3.1 hr, the reaction was removed from the oil bath. 1 hr later, the reaction was filtered through a 0.2 μm syringe filter (eluting with 20% CH_3OH/CH_2Cl_2)[d]. The filtrate was concentrated *in vacuo* and co-evaporated with toluene (2x). The resulting crude yellow product was further dried under high vacuum in vacuum desiccator over P_2O_5 for 13 hr and used immediately in **Step 2**.

Procedure (Step 2): The pear-shaped flask (50 mL) into which the crude product of **Step 1** (theoretically 24.2 μmol) had been concentrated was equipped with an oven dried spin vane. The flask was charged with 4-nitrobenzyl chloroformate (**375**, 26.2 mg, 133 μmol, 5.02 Eq) followed by a solution of 2,6-lutidine in dry CH_2Cl_2 (0.40 mL, 14 mg, 0.13 mmol, 5.4 Eq | 34.4 mg/mL)[e]. The flask was lightly stoppered with a hard yellow plastic cap, and the reaction was stirred at room temperature for 1.5 hr. Then the reaction was transferred using CH_2Cl_2 to a 16 x 150 mm test tube already charged with 1 N HCl (3 mL)[f]. The layers were separated, and the organics were washed with 1 N HCl (3 x 3 mL) followed by sat aq. $NaHCO_3$ (2 x 3 mL). The washed organics were dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. Purification by FCC (SiO_2: 2% → 50% EtOAc/Hex)[g] afforded an off-white solid (18.8 mg) containing a mixture of desired **244**, tentatively assigned impurity **376**, and other unknown impurity(ies). As **23** was the only carbohydrate containing component of the isolated solid, we moved the material forward into recrystallization experiments. Slow evaporation from a mixture of CH_2Cl_2 (solubilizing solvent) and CH_3OH (precipitating solvent) gave crystals of sufficient quality for XRD analysis which revealed that disaccharide **244** had preferentially crystallized out of the mixture.

^{1}H NMR (600 MHz, $CDCl_3$, Compound 244): δ 8.23-8.19 (m, 4H, H3e & H4′e), 7.51 (d, *J* = 8.6 Hz, 2H, H4′d), 7.48 (d, *J* = 8.5 Hz, 2H, H3d), 6.57 (br s, 1H, C3NH), 5.22 (ABq, *J* = 13.5 Hz, Δν = 21.0 Hz, 1H, H4′b), 5.19 (ABq, *J* = 13.5 Hz, Δν = 21.0 Hz, 1H, H4′b), 5.16 (ABq, *J* = 13.6 Hz, Δν = 45.1 Hz, 1H, H3b), 5.09 (ABq, *J* = 13.6 Hz, Δν = 45.1 Hz, 1H, H3b), 5.03 (d, *J* = 9.9 Hz, 1H, C4′NH), 4.85 (dd, *J* = 6.5, 3.5 Hz, 1H, H1′), 4.81 (dd, *J* = 9.4, 1.5 Hz, 1H, H1), 4.35-4.26 (m, 1H, H5′), 3.82 (q, *J* = 6.3 Hz, 1H, H5), 3.66 (dd, *J* = 9.9, 4.6 Hz, 1H, H4′), 3.04 (s, 1H, H4), 2.43 (br d, *J* = 12.5 Hz, 1H, $H2_{pro\text{-}S}$), 2.11 (dd, *J* = 14.2, 3.2 Hz, 1H, $H2'_{pro\text{-}R}$), 1.89 (dd, *J* = 14.2, 6.7 Hz, 1H, $H2'_{pro\text{-}S}$), 1.61 (inf. dd, *J* ~ 13, 10 Hz, 1H, $H2_{pro\text{-}R}$)[h], 1.51 (s, 3H, C3Me), 1.43 (3H, s, C3′Me), 1.25 (3H, H6′)[i], 1.22 (d, *J* = 6.3 Hz, 3H, H6), 0.88 (s, 9H, H1c), 0.87 (s, 9H, H3′c), 0.133 (s, 3H, H3′a), 0.128 (s, 3H, H3′a), 0.10 (s, 3H, H1a), 0.09 (s, 3H, H1a).

*Preparation of Compound **244** Continued*

^{13}C NMR (151 MHz, $CDCl_3$, Compound 244): δ 156.1 (C4′a), 154.4 (C3a), 147.8 (C3f/C4′f), 147.7 (C3f/C4′f), 144.6 (C3c), 144.0 (C4′c), 128.3 (C3d/C4′d), 128.2 (C3d/C4′d), 98.3 ($^1J_{CH}$ = 164 Hz, C1′), 94.1 ($^1J_{CH}$ = 156 Hz), 82.5 (C4), 73.5 (C3′), 68.9 (C5′), 65.5 (C4′b), 64.5 (C3b), 57.4 (C4′), 54.3 (C3), 41.8 (C2′), 39.8 (C2), 28.7 (C3′Me), 26.01 (C1c/C3′c), 25.9 (C1c/C3′c), 21.9 (C3Me), 18.3 (C1b & C3b)[j], 17.9 (C6), 15.8 (C6′), −1.8 (C3′a), −2.0 (C3′a), −3.9 (C1a), −4.7 (C1a).[k]

^{1}H NMR (600 MHz, $CDCl_3$, Compound 376): δ 8.24 (d, *J* = 8.7 Hz, 4H, H4), 7.55 (d, *J* = 8.6 Hz, 4H, H5), 5.28 (s, 4H, H2).[l]

^{13}C NMR (151 MHz, $CDCl_3$, Compound 376): δ 154.7 (C1), 148.2 (C6), 142.1 (C3), 128.6 (C4).[k–l]

Notes:

[a] Procedure adapted from DeGoey, D. A. et al.[16]

[b] Pd/C = 10% unreduced Pd on activated carbon.

[c] 14/20 septum was used "right side up" and was secured to the vial with parafilm.

[d] Before filtering the reaction, the reaction was spotted directly on TLC plates developed in 25% EtOAc/Hex and 20% CH_3OH/CH_2Cl_2 (CAM stain).

[e] A stock solution 2,6-lutidine in dry CH_2Cl_2 was prepared as follows: a vial (1 dram) was charged with 2,6-lutidine (28.6 mg) and dry CH_2Cl_2 (0.80 mL). As the density of 2,6-lutidine is 0.92 g/mL, this gives a combined volume (0.031 mL 2,6-lutidine + 0.80 mL CH_2Cl_2) of 0.831 mL and a concentration of 34.4 mg 2,6 lutidine per 1 mL of stock solution.

[f] Before transferring the reaction to the test tube, the reaction was spotted directly onto TLC plates developed in 25% EtOAc/Hex, 50% EtOAc/Hex, and 20% CH_3OH/CH_2Cl_2 (UV and CAM stain).

[g] Detailed gradient: 2% (20 mL) → 5% (15 mL) → 10% (15 mL) → 15% (15 mL) → 25% (15 ml) → 30% (15 mL) → 40% (15 mL) → 50% (35 mL) EtOAc/Hex; co-eluting mixture of **244**, tentatively assigned impurity **376**, and other unkown impurity(ies) eluted in 25% → 30% EtOAc/Hex.

[h] Due to overlap with the H_2O peak (δ 1.59), this resonance was inferred to be a doublet of doublets based on the visible peaks (3 out of 4); as such, the *J* values are given as estimates.

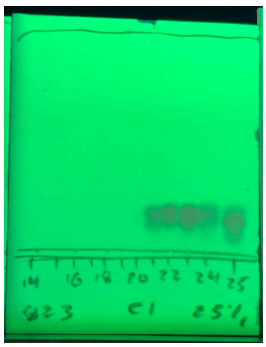
[i] Due to overlap with the "grease" peak (δ 1.25), the peak appears to be a singlet in the ^{1}H NMR. However, inspection of the ^{1}H-^{13}C HSQC{^{1}H}{^{13}C} indicates that the resonance is centered at δ 1.25. Considering the assignment of this peak to H6′, we hypothesize that this resonance is actually a doublet centered at δ 1.25.

[j] ^{1}H-^{13}C HMBC indicates that the^{13}C shift for C1b and C3′b are both δ 18.3.

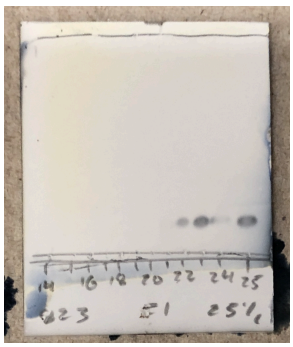
[k] Due to the combined presence of **244** and **S3**, the following ^{13}C nuclei escaped confident assignment: **244** – C3e, C5, C4′e and **S3** – C2, C5. These nuclei were not included in the line listings above and are assigned as follows: ^{13}C (151 MHz, $CDCl_3$) δ 124.1 (**244**: C3e/C4′e or **376**:C5), 123.91 (**244**: C3e/C4′e or **376**:C5), 123.89 (**244**: C3e/C4′e or **376**:C5), 68.54 (**244**: C5 or **376**:C2), 68.49 (**244**: C5 or **376**:C2).

[l] Structure of **376** was tentatively assigned.

TLCs:



TLC #1a: FCC Fractions
Gradient: 25% EtOAc/Hex
Visualization: UV

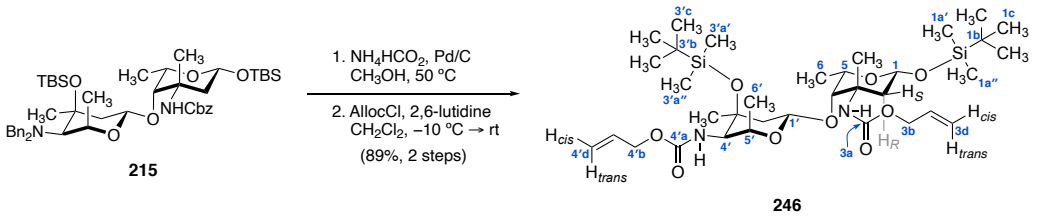


TLC #1b: FCC Fractions
Gradient: 25% EtOAc/Hex
Visualization: CAM Stain

*The fractions represented by lanes 21-25 were combined and concentrated in vacuo to give the mixture described above consisting of disaccharide **244**, tentatively assigned impurity **376**, and other unknown impurity(ies).*

B.7 *Synthesis of 1st–3rd Generation Disaccharide Donors*

B.7.1 *Preparation of Compound **246***



215 246

Name: allyl ((2*S*,3*S*,4*S*,6*R*)-3-(((2*S*,4*S*,5*R*,6*S*)-5-(((allyloxy)carbonyl)amino)-4-((*tert*-butyldimethylsilyl)oxy)-4,6-dimethyl tetrahydro-2*H*-pyran-2-yl)oxy)-6-((*tert*-butyldimethylsilyl)oxy)-2,4-dimethyltetrahydro-2*H*-pyran-4-yl)carbamate

Procedure (Step 1)[a]**:** A dried RBF (50 mL, 24/40 neck) equipped with a stir bar was charged with **215** (330 mg, 389 μmol, 1.00 Eq), NH_4HCO_2 (512 mg, 8.12 mmol, 20.8 Eq), and Pd/C (167.3 mg, ~ 2:1 w/w 215:Pd/C).[b] The RBF was stoppered with a septum, flushed with argon for 5 min, and left under a balloon of argon. Next, dry CH_3OH (6.5 mL, 60 mM) was cautiously added through the septum. The resulting mixture was lowered into a preheated oil bath (50 °C) and stirred for 2.8 hr. The cooled mixture was "double filtered," first through a pad of Celite (topped with sand) and then through a 0.2 μm syringe filter, eluting with 20% CH_3OH/CH_2Cl_2 in both cases.[c] The filtrate was concentrated *in vacuo* into a collection flask (50 mL), co-evaporated with toluene (2x), and placed under high vacuum overnight.

Procedure (Step 2): The next day, the collection flask (50 mL) containing the crude product of Step 1 (theoretically 389 μmol) was equipped with a dried stir bar and charged with dry CH_2Cl_2 (6.5 mL, 60 mM) followed by 2,6-lutidine (0.25 mL, 2.1 mmol, 5.5 Eq). The resulting colorless solution was placed under a balloon of argon and chilled in an ice/H_2O/brine bath (–10 °C). AllocCl (0.21 mL, 2.0 mmol, 5.1 Eq) was added in one portion, causing the solution to turn yellow. After stirring at –10 °C for 5 min, the now pale pink solution was removed from the cooling bath and stirred at room temperature for 1 hr. The resulting purple solution was transferred to a separatory funnel (60 mL) using CH_2Cl_2 (24 mL) and washed with 1 N HCl (3 x 6 mL) followed by sat. aq. $NaHCO_3$ (1 x 6 mL). The washed organics were dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. Without delay,[c] the crude dark purple was purified by FCC (SiO_2: 2% → 30% EtOAc/Hex)[e] to afford desired bis-Alloc product **246** (243.6 mg, 347.5 μmol, 89% yield over 2 steps) as a crunchy off-white foam.

^{1}H NMR (600 MHz, $CDCl_3$): δ 6.33 (s, 1H, C3NH), 5.98-5.83 (m, 2H, H3c + H4′c), 5.30 (br app dd, *J* = 17.3, 1.3 Hz, 1H, H4′d-*trans*), 5.26 (br app d, *J* ~ 17.3 Hz, 1H, H3d-*trans*), 5.20 (dd, *J* = 10.4, 1.0 Hz, 1H, H4′d-*cis*), 5.17 (dd, *J* = 10.4, 0.8 Hz, H3d-*cis*), 4.93 (d, *J* = 10.0 Hz, 1H, C4′NH), 4.85–4.78 (m, 2H, H1′{4.82}, H1{4.80}), 4.56 (br d, *J* = 5.6 Hz, 2H, H4′b), 4.48 (br d, *J* = 4.4 Hz, H3b), 4.31 (qd, *J* = 6.5, 4.5 Hz, 1H, H5′), 3.81 (q, *J* = 6.3 Hz, 1H, H5), 3.65 (dd, *J* = 10.0, 4.3 Hz, 1H, H4′), 3.00 (s, 1H, H4), 2.44 (d, *J* = 12.5 Hz, $H2_{pro\text{-}S}$), 2.06 (dd, *J* = 14.2, 3.6 Hz, 1H, H2′), 1.89 (dd, *J* = 14.2, 6.2 Hz, H2′), 1.59 (dd, *J* = 12.7, 9.8 Hz, 1H, $H2_{pro\text{-}R}$), 1.50 (s, 3H, C3Me), 1.44 (s, 3H, C3′Me), 1.28 (d, *J* = 6.6 Hz, 3H, H6′), 1.20 (d, *J* = 6.4 Hz, 3H, H6), 0.88 (s, 9H, H1c/3′c), 0.87 (s, 9H, H1c/3′c), 0.13 (s, 3H, H1a/3′a), 0.12 (s, 3H, H1a/3′a), 0.09 (s, 3H, H1a/3′a), 0.08 (s, 3H, H1a/3′a).

^{13}C{^{1}H} NMR (151 MHz, $CDCl_3$): δ 156.5 (C4′a), 154.9 (C3a), 133.3 (C3c/4′c), 133.1 (C3c/4′c), 117.63 (C3d/4′d), 117.60 (C3d/4′d), 98.8 (C1′), 94.2 (C1), 82.8 (C4), 73.3 (C3′), 68.7 (C5′), 68.5 (C5), 65.7 (C4′b), 65.1 (C3b), 57.4 (C4′), 54.1 (C3), 41.5 (C2′), 39.9 (C2), 28.7 (C3′Me), 26.0 C1c/3′c), 25.9 (C1c/3′c), 21.9 (C3Me), 18.3 (C1b/3′b), 17.9 (C6), 15.9 (C6′), –1.9 (C1a/3′a), –2.0 (C1a/3′a), –3.9 (C1a/3′a), –4.6 (C1a/3′a).[f]

IR (thin film, cm^{-1}): 3327, 3083, 2942, 2891, 2863, 1713, 1532, 1458, 1388, 1324, 1246, 1162, 1066, 1018, 842, 777.

OR: $\alpha_D^{24} = -20.560^{o}$ (*c* = 1.00, $CHCl_3$)

R_f: 0.41 (25% EtOAc/Hex, CAM)

Notes:

[a] Procedure adapted from DeGoey, D. A. et al.[16]

[b] 10% unreduced Pd on activated carbon from Acros (Lot A0341351).

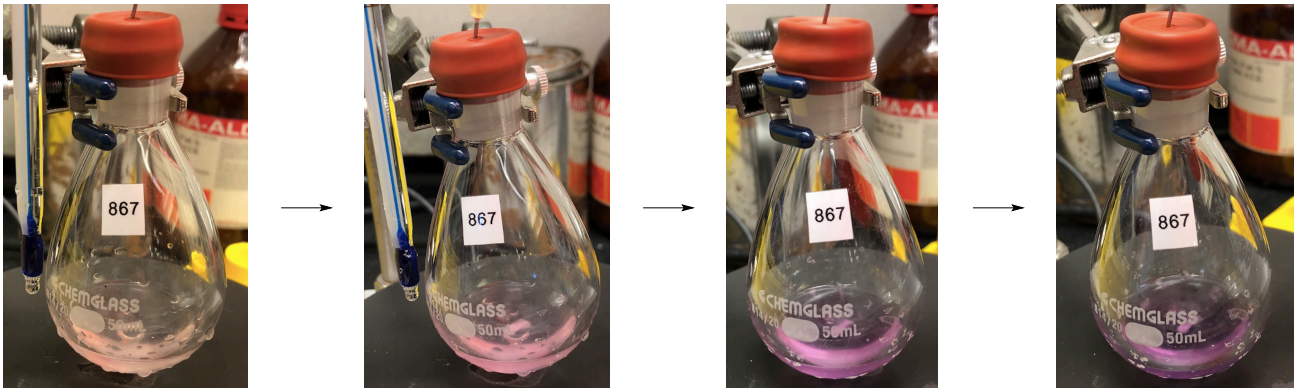
[c] On a smaller scale (197 µmol **215**; 87 mg Pd/C), single filtration through a 0.2 µm syringe filter was sufficient to remove the Pd/C. However, upon scaling up the reaction (356 µmol **215**; 148 mg Pd/C), the increased amount of Pd/C clogged the 0.2 µm syringe filter. As such, a double filtration protocol was adapted in which the first filtration (Celite pad topped with sand) removes the bulk of the Pd/C and the second filtration (0.2 µm syringe filter) removes residual Pd/C to give a particulate free filtrate.

[d] On one occasion, there was a 12-day delay between workup and purification. In this case, TLC during the reaction indicated starting material consumption and a relatively clean reaction. However, TLC of the column fractions did not indicate the presence of any **246**. Combining and concentrating all of these fractions gave sample that showed the presence of many spots by TLC but the absence of a spot co-polar with **246**.

[e] Detailed gradient: 2% (150 mL flush) → 5% (100 mL) → 10% (100 mL)) → 20% (150 mL)) → 30% (200 mL) EtOAc/Hex; **246** eluted in 20% EtOAc/Hex.

[f] Only 1 out of 2 expected ^{13}C resonance assignable to C1b/3'b was observed in the 1-D $^{13}C\{^{1}H\}$ spectrum. This is likely the result of coincidental overlap as the ^{1}H NMR indicates the presence of 2 TBS groups and resonances assignable to 4 C1a/3'a and 2 C1c/C3'c carbons were observed in the 1-D $^{13}C\{^{1}H\}$ spectrum

Visual Procedure:



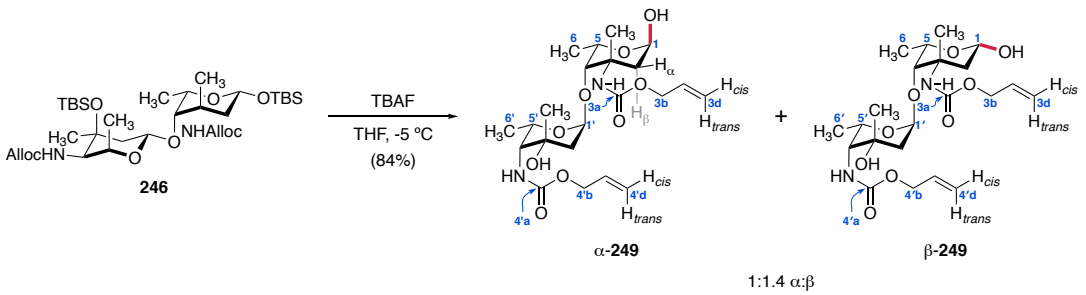
A. Step 2: 8 min after AllocCl addition.

B. Step 2: 19 min after AllocCl addition.

C. Step 2: 46 min after AllocCl addition.

D. Step 2: 61 min after AllocCl addition.

B.7.2 *Preparation of Compound* ***249***



Name (α-249): allyl ((2*S*,3*S*,4*S*,6*R*)-3-(((2*S*,4*S*,5*R*,6*S*)-5-(((allyloxy)carbonyl)amino)-4-hydroxy-4,6-dimethyltetrahydro-2H-pyran-2-yl)oxy)-6-hydroxy-2,4-dimethyltetrahydro-2*H*-pyran-4-yl)carbamate

Name (β-249): allyl ((2*S*,3*S*,4*S*,6*S*)-3-(((2*S*,4*S*,5*R*,6*S*)-5-(((allyloxy)carbonyl)amino)-4-hydroxy-4,6-dimethyltetrahydro-2*H*-pyran-2-yl)oxy)-6-hydroxy-2,4-dimethyltetrahydro-2*H*-pyran-4-yl)carbamate

Procedure: A dried RBF (25 mL) equipped with a stir bar was charged with TBS glycoside **246** (221.8 mg, 316.4 μmol, 1.000 Eq) and dry THF (3.2 mL, 99 mM). The reaction was placed under a balloon of argon and lowered into an ice/brine bath (−5 °C). Next, chilled[a] TBAF (1 M in THF | 3.2 mL, 3.2 mmol, 10 Eq) was added in portions by hand over 2 min. After stirring for an additional 45 min at −5 °C, the reaction was quenched by the addition of 1 N HCl (10 mL). The quenched reaction mixture was stirred for 5 min at −5 °C before it was removed from the cooling bath. After stirring for an additional 5 min at rt, the reaction mixture was transferred to a separatory funnel (60 mL) using EtOAc (30 mL) and DI H_2O (6 mL). The layers were separated, and the organics were washed with 1 N HCl (3 x 8 mL), sat. aq. $NaHCO_3$ (1 x 8 mL), and brine (1 x 8 mL), in that order. The washed organics were dried over anhydrous Na_2SO_4, filtered, concentrated *in vacuo*, and co-evaporated with CH_2Cl_2 (1x). Purification by FCC (SiO_2: 25% EtOAc/Hex → 100% EtOAc → 10% CH_3OH/EtOAc) gave desired hemiacetal α/β-**249** (125.0 mg, 264.5 μmol, 84% yield, 1:1.4 α/β) as a white to colorless foamy looking solid.

^{1}H NMR (600 MHz, $CDCl_3$, α-249): δ 5.93-5.82 (m, 3H, H4′c{5.93}, C3NH{5.92}, H3c{5.88}), 5.32 (br d, *J* = 17.5 Hz, 1H, H4′d-*trans*), 5.30[d] (buried, 1H, H1), 5.27 (br d, *J* = 17.4 Hz, 1H, H3d-*trans*), 5.23 (br d, *J* = 10.3 Hz, 1H, H4d-*cis*), 5.21-5.15 (m, 1H, H3d-*cis*), 5.11 (br d, *J* = 7.7 Hz, 1H, C4′NH), 4.90-4.80 (m, 1H, H1′{4.855}), 4.65-4.55 (m, 2H, H4′b), 4.50 (br s, 2H, H3b), 4.38-4.26 (m, 2H, H5{4.32}, H5′{4.31}), 3.59 (app br t, *J* = 8.6 Hz, 1H, H4′), 3.18 (s, 1H, H4), 2.84–2.66 (m, 1H, C1OH), 2.49-2.25 (m, 1H, H2,{2.40}), 2.04-1.94 (m, 1H, H2′), 1.94-1.82 (m, 2H, H2′{1.90}, H2{1.87}), 1.69 (s, 3H, C3Me), 1.49 (br s, 3H, C3′Me), 1.32-1.20 (m, 3H, H6′{1.27}), 1.19 (d, *J* = 6.4 Hz, 3H, H6).

^{1}H NMR (600 MHz, $CDCl_3$, β-249): δ 6.02 (br s, 1H, C_3NH), 5.93-5.82 (m, 2H, $H_{4'c}${5.93}, H_{3c}{5.88}), 5.27 (br d, *J* = 17.4 Hz, 1H, H3d-*trans*), 5.23 (br d, *J* = 10.3 Hz, 1H, H4d-*cis*), 5.21-5.15 (m, 1H, H3d-*cis*), 5.11 (br d, *J* = 7.7 Hz, 1H, C4′NH), 4.90-4.80 (m, 2H, H1′{4.86}), H1{4.850}), 4.65-4.55 (m, 2H, H4′b), 4.50 (br s, 2H, H3b), 4.38-4.26 (m, 1H, H5′{4.33}), 3.89 (q, *J* = 6.2 Hz, 1H, H5), 3.59 (app br t, *J* = 8.6 Hz, 1H, H4′), 3.34–3.20 (m, 1H, C1OH), 3.08 (s, 1H, H4), 2.57 (br d, *J* = 11.9 Hz, H2), 2.04-1.94 (m, 1H, H2′), 1.94-1.82 (m, 1H, H2′{1.90}), 1.57-1.43 (m, 1H, H2{1.53}), 1.52 (br s, 3H, C3Me), 1.51 (br s, 3H, C3′Me), 1.32-1.20 (m, 6H, H6′{1.27}, H6{1.23}).

^{13}C NMR (151 MHz, $CDCl_3$, α/β-249): δ 157.8 (α/β-C4′a), 157.7 (α/β-C4′a), 154.8 (α-C3a and β-C3a), 133.1 (α/β-C3c), 133.0 (α/β-C3c), 132.7 (α-C4′c and β-C4′c), 118.2 (α-C4′d and β-C4′d), 118.0 (α/β-C3d), 117.9 (α/β-C3d), 101.1 (β-C1′)[e], 100.9 (α-C1′)[e], 93.3 (β-C1, $^1J_{CH}$ = 159 Hz), 91.7 (α-C1, $^1J_{CH}$ = 168 Hz), 85.2 (α-C4), 84.4 (β-C4), 69.83 (α/β-C3′), 69.79 (α/β-C3′), 68.9 (β-C5), 67.0 (α/β-C5′), 66.8 (α/β-C5′), 66.2 (α-C4′b and β-C4′b), 65.3 (α/β-C3b), 65.2 (α/β-C3b), 63.9 (α-C5), 58.6 (α/β-C4′), 58.4 (α/β-C4′), 54.3 (β-C3), 52.7 (α-C3), 39.3 (β-C3), 39.0 (α/β-C2′), 38.9 (α/β-C2′), 35.7 (α-C2), 27.9 (α/β-C3′Me), 27.8 (α/β-C3′Me), 24.0 (α-C3Me), 21.9 (β-C3Me), 18.0 (β-C6), 17.9 (α-C6), 17.0 (α/β-C6′), 16.9 (α/β-C6′).

IR (thin film, cm^{-1}): 3351, 3078, 2930, 1710, 1525, 1452, 1382, 1325, 1273, 1237, 1163, 1106, 1067, 1001.

HRMS (ESI+) m/z: $[M+Na]^+$ Calculated for $C_{22}H_{36}N_2NaO_9$ 495.2313; found 495.2320 (error 1.41 ppm).

R_f: 0.26 (100% EtOAc, anisaldehyde stain)

*Preparation of Compound **249** Continued*

Notes:

[a] Chilled TBAF (1 M in THF) was prepared as follows: a dried 16 x 150 mm test tube was fitted with a septum, placed under a balloon of argon, charged with TBAF solution (1 M in THF), and lowered into the same ice/brine bath (−5 °C) used to chill the reaction flask.

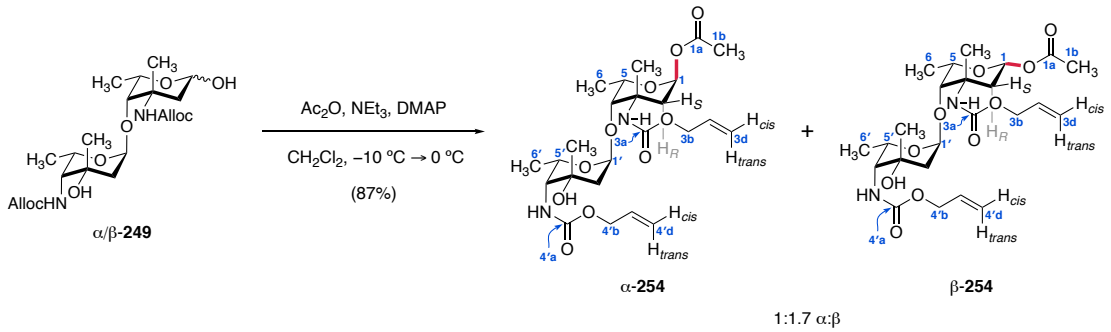
[b] Before quenching the reaction with 1 N HCl, a reaction aliquot was removed, quenched into a mixture of EtOAc/1 N HCl, and analyzed by TLCs developed in 25% EtOAc/Hex and 100% EtOAc (anisaldehyde stain).

[c] Detailed gradient: 25% (100 mL) → 50% (50 mL) → 75% EtOAc/Hex (60 mL) → 100% EtOAc (50 mL) → 10% CH_3OH/EtOAc (100 mL); α/β-**249** began to elute in last fraction of 100% EtOAc and primarily eluted in 10% CH_3OH/EtOAc.

[d] Signal buried in ^1H NMR; observed by 2-D NMR.

[e] Due to significant overlap in both the ^1H and ^{13}C dimensions, $^1J_{CH}$ could not be assigned for α-C1′ and β-C1′.

B.7.3 *Preparation of Compound* ***254***



Name (α-254): (2*S*,4*S*,5*S*,6*S*)-4-(((allyloxy)carbonyl)amino)-5-(((2*S*,4*S*,5*R*,6*S*)-5-(((allyloxy)carbonyl)amino)-4-hydroxy-4,6-dimethyltetrahydro-2*H*-pyran-2-yl)oxy)-4,6-dimethyltetrahydro-2H-pyran-2-yl acetate

Name (β-254): (2*R*,4*S*,5*S*,6*S*)-4-(((allyloxy)carbonyl)amino)-5-(((2*S*,4*S*,5*R*,6*S*)-5-(((allyloxy)carbonyl)amino)-4-hydroxy-4,6-dimethyltetrahydro-2*H*-pyran-2-yl)oxy)-4,6-dimethyltetrahydro-2H-pyran-2-yl acetate

Procedure: In a vial (2 dram) equipped with a stir bar was made a solution of diol α/β-**249** (61.3 mg, 130 μmol, 1.00 Eq) in dry CH_2Cl_2 (1.2 mL, 0.11 M). The vial was stoppered with a septum, placed under a double balloon of argon, and chilled in an ice/brine bath (ca. –10 °C). Next, NEt_3 (0.06 mL, 0.4 mmol, 3 Eq), Ac_2O (0.04 mL, 0.4 mmol, 3 Eq), and a freshly made stock solution of DMAP in dry CH_2Cl_2 (15.3 mg/mL | 0.11 mL stock solution ∴1.7 mg, 14 μmol, 0.11 Eq DMAP) were each added in one portion, in that order. After 1.5 hr, the reaction was quenched by the addition of CH_3OH (0.38 mL, 9.4 mmol, 72 Eq)[b-c]. 20 min later, aq. 1 N HCl (1000 μL) was added. Next, the quenched reaction mixture was diluted with CH_2Cl_2 and the layers were separated. Then the organics were washed with aq. 1 N HCl (2 x 1000 μL) followed by sat. aq. $NaHCO_3$ (1 x 1000 μL). The washed organics were dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. Purification by FCC (SiO_2: 25% EtOAc/Hex → 100% EtOAc) gave desired anomeric acetate α/β-**254** (58.2 mg, 113 μmol, 87% yield, 1.0:1.7 α/β) as a white foam.

^{1}H NMR (600 MHz, $CDCl_3$, α-254): δ 6.13 (br d, *J* = 4.0 Hz, 1H, H1), 6.03 (br s, 1H, C3NH), 5.97-5.82 (m, 2H, H4′c{5.89}, H3c{5.87}), 5.31 (d, *J* = 17.2 Hz, 1H, H4′d-*trans*), 5.26 (d, *J* = 17.3 Hz, 1H, H3d-*trans*), 5.22 (d, *J* = 10.5 Hz, 1H, H4′d-*cis*), 5.18 (d, *J* = 10.4 Hz, 1H, H3d-*cis*), 5.16–5.08 (m, 1H, C4′NH{5.13}), 4.89–4.81 (m, 1H, H1′{4.84}), 4.64–4.54 (m, 2H, H4′b{4.58}), 4.54–4.42 (m, 2H, H3b{4.49}), 4.39–4.24 (m, 1H, H5′{4.30}), 4.18 (q, *J* = 6.3 Hz, 1H, H5), 3.67–3.53 (m, 1H, H4′{3.60}), 3.18 (s, 1H, H4), 2.57-2.34 (m, 1H, $H2_{pro\text{-}S}${2.47})[e], 2.06 (s, 3H, H1b), 2.02–1.86 (m, 3H, H2′{1.96, 1.91}, $H2_{pro\text{-}R}${1.95}), 1.66 (s, 3H, C3Me), 1.47 (s, 3H, C3′Me), 1.30–1.25 (m, 3H, H6′{1.26}), 1.19 (d, *J* = 6.4 Hz, 3H, H6).[e]

^{1}H NMR (600 MHz, $CDCl_3$, β-254): δ 6.10 (br s, 1H, C3NH), 5.97-5.82 (m, 2H, H4′c{5.89}, H3c{5.87}), 5.76 (dd, *J* = 10.3, 2.2 Hz), 5.31 (d, *J* = 17.2 Hz, 1H, H4′d-*trans*), 5.26 (d, *J* = 17.3 Hz, 1H, H3d-*trans*), 5.22 (d, *J* = 10.5 Hz, 1H, H4′d-*cis*), 5.18 (d, *J* = 10.4 Hz, 1H, H3d-*cis*), 5.16-5.08 (m, 1H, C4′NH{5.13}), 4.84 (m, 1H, H1′), 4.64–4.54 (m, 2H, H4′b{4.58}), 4.54–4.42 (m, H3b{4.49}), 4.39–4.24 (m, 1H, H5′{4.33}), 3.97 (q, *J* = 6.3 Hz, H5), 3.67–3.53 (m, 1H, H4′{3.60}), 3.08 (s, 1H, H4), 2.57–2.34 (m, 1H, $H2_{pro\text{-}S}${2.49})[e], 2.05 (s, 3H, H1b), 1.74 (app dd, *J* = 12.0, 10.9 Hz, $H2_{pro\text{-}R}$), 1.55 (s, 3H, C3Me), 1.49 (s, 3H, C3′Me), 1.30–1.25(m, 3H, H6′{1.27}), 1.23 (grease obscured d, 3H, H6)[f].

^{13}C NMR (151 MHz, $CDCl_3$, α/β-254): δ 169.6 (α-C1a), 169.4 (β-C1a), 157.65 (C4′a), 157.58 (C4′a), 154.8 (α-C3a & β-C3a), 133.0 (α-C3c/4′c & β-C3c/4′c), 132.7 (α-C3c/4′c & β-C3c/4′c), 118.1 (α-C4′d & β-C4′d), 118.0 (α-C3d & β-C3d), 101.1 (α-C1′), 100.9 (β-C1′), 91.4 (α-C1{$^1J_{CH}$ = 173 Hz} & β-C1{$^1J_{CH}$ = 164 Hz}), 84.8 (α-C4), 84.0 (β-C4), 69.8 (α-C5/α-C3′/β-C3′), 69.7 (α-C5/α-C3′/β-C3′), 69.6 (α-C5/α-C3′/β-C3′), 67.2 (C5′), 67.0 (C5′), 66.2 (α-C4′d & β-C4′d), 66.0 (α-C5), 65.3 (α-C3b & β-C3b), 58.4 (α-C4′/β-C4′), 58.2 (α-C4′/β-C4′), 54.1 (β-C3), 52.2 (α-C3), 39.2 (C2′), 39.0 (C2′), 35.9 (β-C2), 34.4 (α-C2), 27.8 (β-C3′Me), 27.7 (α-C3′Me), 23.3 (α-C3Me), 21.7 (β-C3Me), 21.4 (C1b), 21.2 (C1b), 17.8 (C6), 17.7 (C6), 16.9 (C6′), 16.7 (C6′).

*Preparation of Compound **254** Continued*

IR (thin film, cm^{-1}): 3346, 3080, 2931, 1719, 1524, 1452, 1376, 1322, 1233, 1165, 1111, 1043, 1003, 924, 731.

HRMS (ESI+) m/z: $[M+Na]^+$ Calculated $C_{24}H_{38}N_2NaO_{10}$ for 537.2419; found 537.2427 (error 1.49 ppm).

Notes:

[a] The stock solution of DMAP in dry CH_2Cl_2 was prepared as follows: DMAP (15.3 mg) and dry CH_2Cl_2 (1.00 mL) were combined in an oven dried/desiccator cooled vial (1 dram).

[b] Before quenching the reaction with CH_3OH, the reaction was spotted directly onto a TLC plate developed in 100% EtOAc (anisaldehyde stain).

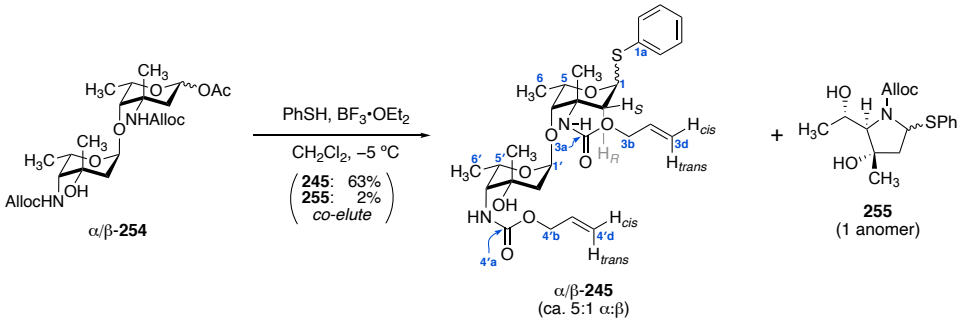
[c] The ice/brine bath had warmed to ca. 0 °C by the time the reaction was quenched with CH_3OH.

[d] Detailed gradient: 25% (32 mL) → 40% (20 mL) → 60% (20 mL) → 80% EtOAc/Hex (30 mL) → 100% EtOAc (32 mL); α/β-**254** eluted in 80% EtOAc/Hex and in 100% EtOAc.

[e] In total, the multiplet from δ 2.57–2.34 included four peaks: β-$H2_{pro\text{-}S}${2.49}, α-$H2_{pro\text{-}S}${2.47}, C3′OH {2.44}, and C3′OH {2.41}; as we could not determine which anomer gave rise to which C3′OH resonance, we did not the C3′OH resonances include these in the line listings above.

[f] As grease obscured the doublet at δ 1.23, the integration of the peak was assumed to be 3H based on the peak's assignment.

B.7.4 *Preparation of Compound* ***245***



Name (α-245): allyl ((2*S*,3*S*,4*S*,6*S*)-3-(((2*S*,4*S*,5*R*,6*S*)-5-(((allyloxy)carbonyl)amino)-4-hydroxy-4,6-dimethyltetrahydro-2*H*-pyran-2-yl)oxy)-2,4-dimethyl-6-(phenylthio)tetrahydro-2*H*-pyran-4-yl)carbamate

Name (β-245): allyl ((2*S*,3*S*,4*S*,6*R*)-3-(((2*S*,4*S*,5*R*,6*S*)-5-(((allyloxy)carbonyl)amino)-4-hydroxy-4,6-dimethyltetrahydro-2*H*-pyran-2-yl)oxy)-2,4-dimethyl-6-(phenylthio)tetrahydro-2*H*-pyran-4-yl)carbamate

Name (255): allyl (2*R*,3*S*)-3-hydroxy-2-((*S*)-1-hydroxyethyl)-3-methyl-5-(phenylthio)pyrrolidine-1-carboxylate

Procedure: A vial (2 dram) containing α/β-**254** (63.7 mg, 124 μmol, 1.00 Eq) was equipped with a dried stir bar and charged with dry CH_2Cl_2 (1.1 mL, 0.11 M) followed by thiophenol (33 μL, 0.32 mmol, 2.6 Eq)[a]. The vial was stoppered with a 14/20 septum, placed under a balloon of argon, and lowered into an ice/brine bath (ca. –5 °C). Next, a freshly prepared stock solution of BF_3•OEt_2 in dry CH_2Cl_2 (0.10 mL, 3.4 mg, 24 μmol, 0.19 Eq | 34 mg/mL)[c] was added in portions by hand over 8 minutes. After stirring for an additional 1.75 hr, the reaction was quenched by the addition of sat. aq. $NaHCO_3$ (1 mL). The quenched reaction mixture was diluted with CH_2Cl_2, and the layers were separated. The organics were washed with sat. aq. $NaHCO_3$ (3 x 1000 μL), dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. Purification by FCC (SiO_2: 12% → 80% EtOAc/Hex)[d] afforded an opaque white oil containing a mixture of the desired thioglycoside α/β-**245** (43.9 mg, 77.7 μmol, 63% yield, 5.1:1 α/β) and rearrangement product **255** (1.0 mg, 3.0 μmol, 2% yield) in > 20:1 mol:mol ratio as determined by ^{1}H NMR. This mixture was taken on without further purification for characterization and further synthetic manipulation.

^{1}H NMR (600 MHz, $CDCl_3$, α-245): δ 5.97–5.83 (m, 2H, H3c and H4′c) 5.50 (br dd, *J* = 6.1, 3.6 Hz, 1H, H1), 5.32 (br dd, *J* = 17.2, 0.7 Hz, 1H, H3d$_{trans}$/H4′d$_{trans}$), 5.26 (br d, *J* = 17.4 Hz, 1H, H3d$_{trans}$/H4′d$_{trans}$), 5.23 (br dd, *J* = 10.5, 1.0 Hz, 1H, H3d$_{cis}$/H4′d$_{cis}$), 5.18 (br d, *J* = 10.3 Hz, 1H, H3d$_{cis}$/H4′d$_{cis}$), 4.87 (app br dd, *J* = 4.1, 3.0 Hz, H1′), 4.63–4.55 (m, 2H, H4′b), 4.44 (br app qd, *J* = 6.5, 1.4 Hz, 1H, H5), 4.30 (br dis qd, *J* ~ 6.3, 1.7 Hz, H5′), 3.59 (d, *J* = 8.9 Hz, 1H, H4′), 3.27 (s, 1H, H4), 2.50 (ABX, dd, *J* = 13.5, 5.8 Hz, H2$_{pro\text{-}R}$)[e], 2.46–2.30 (m, 1H, H2$_{pro\text{-}S}${2.40}), 1.95 (ABX dd, ^2J = 14.2 Hz, ^3J = 2.1 Hz, Δν = 39.1 Hz, 1H, H2′$_{pro\text{-}S}$), 1.89 (ABX dd, ^2J = 14.2 Hz, ^3J = 4.7 Hz, Δν = 39.1 Hz, 1H, H2′$_{pro\text{-}R}$), 1.70 (s, 3H, C3Me), 1.49 (br s, 3H, C3′Me), 1.29–1.24 (m, 3H, H6′{1.26}), 1.22 (d, *J* = 6.5 Hz, 3H, H6).

^{13}C NMR (151 MHz, $CDCl_3$, α-245): δ 157.8 (C4′a) 136.0 (C1a), 100.4 (α-C1′), 84.3 (α-C4), 82.8 (α-C1), 69.7 (C3′), 66.64 (α-C5′), 66.2 (α-C4′b), 65.2 (α-C5), 58.5 (α-C4′), 53.3 (C3), 38.8 (α-C2′), 37.0 (α-C2), 27.8 (α-C3′Me), 24.2 (α-C3Me), 17.2 (α-C6/α-C6′), 17.0 (α-C6/α-C6′).

IR (thin film, cm^{-1}): 3343, 3067, 2977, 2932, 1714, 1521, 1450, 1384, 1322, 1235, 1073, 990.

HRMS (ESI–) m/z: [M+FA–H]$^-$ Calculation for $C_{29}H_{41}N_2O_{10}S$ 609.2487; found 609.2497 (error 1.64 ppm).

Notes:

[a] The thiophenol was measured out and dispensed using a Gilson P200 pipet.

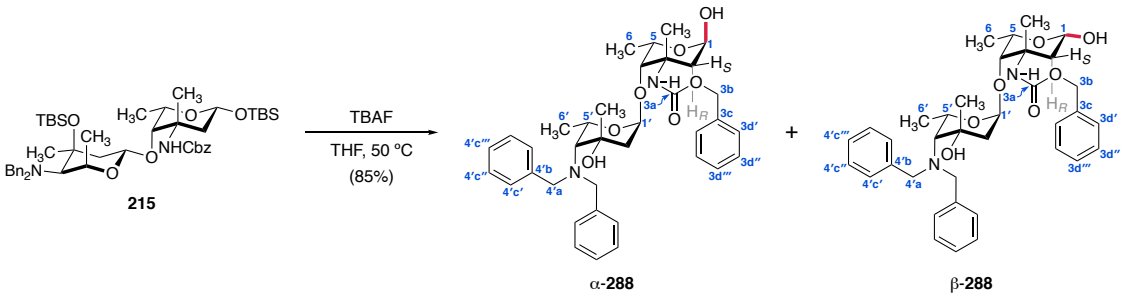
[b] The stock solution BF_3•OEt_2 in dry CH_2Cl_2 was prepared as follows: a dry vial (1/2 dram) was charged with BF_3•OEt_2 (23.1 mg) and dry CH_2Cl_2 (0.66 mL). As the density of BF_3•OEt_2 is 1.15 g/mL, this gives a combined volume of 0.6801 mL (0.0201 mL BF_3•OEt_2 + 0.66 mL CH_2Cl_2) and a concentration of 34 mg BF_3•OEt_2 per 1 mL of stock solution.

[c] Before quenching the reaction with sat. aq. $NaHCO_3$, a reaction aliquot was removed, quenched into a mixture of EtOAc/sat. aq. $NaHCO_3$, and analyzed by TLC developed in 75% EtOAc/Hex (CAM stain).

[d] Detailed gradient: 12% (50 mL) → 25% (28 mL) → 35% (40 mL) → 63% (41 ml) → 80% (40 mL) EtOAc/Hex.

B.8 *Synthesis of 4th Generation Disaccharide Donor* ***287***

B.8.1 *Preparation of Compound* ***288***



Name (α-288): benzyl ((2*S*,3*S*,4*S*,6*R*)-3-(((2*S*,4*S*,5*R*,6*S*)-5-(dibenzylamino)-4-hydroxy-4,6-dimethyltetrahydro-2*H*-pyran-2-yl)oxy)-6-hydroxy-2,4-dimethyltetrahydro-2*H*-pyran-4-yl)carbamate

Name (β-288): benzyl ((2*S*,3*S*,4*S*,6*S*)-3-(((2*S*,4*S*,5*R*,6*S*)-5-(dibenzylamino)-4-hydroxy-4,6-dimethyltetrahydro-2*H*-pyran-2-yl)oxy)-6-hydroxy-2,4-dimethyltetrahydro-2*H*-pyran-4-yl)carbamate

Procedure[a]**:** A dried vial (6 dram) equipped with a stir bar was charged with bis-TBS **215** (405.9 mg, 479.1 μmol, 1.000 Eq) followed by TBAF (9.5 mL, 9.5 mmol, 20 Eq | 1 M in THF) to give a yellow solution. Then the vial was flushed with argon, capped[b] and lowered into a preheated oil bath (set point 50 °C). After stirring at 50 °C for 17.3 hr, the now orange solution was removed from the oil bath. 30 min later, it was transferred to a separatory funnel (125 mL) using EtOAc (75 mL).[c-d] The reaction was washed with 1 N HCl (3 x 10 mL), sat. aq. $NaHCO_3$ (1 x 10 mL), and brine (1 x 10 mL), in that order. Next, the washed organics were dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo* to give a crude orange oil that was co-evaporated with CH_2Cl_2 (2x). Purification by FCC (SiO_2: 15% EtOAc/Hex → 100% EtOAc)[e] afforded diol **288** (252.4 mg, 407.9 μmol, 85% yield, ca. 2:3 α:β) as an off-white foam.

^{1}H NMR (600 MHz, $CDCl_3$, α-288): δ 7.41-7.18 (m, 15H, H3d/4′c), 6.03 (s, 1H, C3NH), 5.28 (br d, *J* = 3.5 Hz, 1H, H1), 5.15-5.00 (m, 2H, H3b), 4.92-4.86 (m, 1H, H1′), 4.39 (br s, 1H, H5′), 4.30 (dis q, *J* ~ 6.5 Hz, 1H, H5), 4.26 (br s, 2H, H4′a), 3.95-3.78 (m, 2H, H4′a [3.85])[f], 3.17 (s, 1H, H4), 2.59 (br s, 1H, H4′), 2.42 (br d, *J* = 13.2 Hz, 1H, $H2_{pro\text{-}S}$), 2.07 (ABXdd, $^2J_{AB}$ = 14.6 Hz, $^3J_{AX}$ ~ 4.4 Hz, Δν = 22.7 Hz, 1H, H2′), 2.03 (ABX_2td, $^2J_{AB}$ = 14.6 Hz, $^3J_{AX}$ ~ 3.2 Hz, Δν = 22.7 Hz, 1H, H2′), 1.88 (dd, *J* = 13.7, 4.1 Hz, 1H, $H2_{pro\text{-}R}$), 1.70 (s, 3H, C3Me), 1.36 (br s, 3H, H6′), 1.18-1.12 (m, 6H, H6 & C3′Me).

^{1}H NMR (600 MHz, $CDCl_3$, β-288): δ 7.41-7.18 (m, 15H, H3d/4′c), 6.15 (s, 1H, C3NH), 5.15-5.00 (m, 2H, H3b), 4.92-4.86 (m, 1H, H1′), 4.83 (br d, *J* = 9.2 Hz, 1H, H1), 4.39 (br s, 1H, H5′), 4.26 (br s, 2H, H4′a), 3.95-3.78 (m, 3H, H5 [3.87]. H4′a[3.85])[f], 3.05 (s, 1H, H4), 2.62 (br d, *J* ~ 11 Hz, 1H, $H2_{pro\text{-}S}$), 2.59 (br s, 1H, H4′), 2.07 (ABXdd, $^2J_{AB}$ = 14.6 Hz, $^3J_{AX}$ ~ 4.4 Hz, Δν = 22.7 Hz, 1H, H2′), 2.03 (ABX_2td, $^2J_{AB}$ = 14.6 Hz, $^3J_{AX}$ ~ 3.2 Hz, Δν = 22.7 Hz, 1H, H2′), 1.53 (s, 3H, C3Me), 1.52 (dd, *J* = 12.8, 10.5 Hz, 1H, $H2_{pro\text{-}R}$), 1.36 (br s, 3H, H6′), 1.21 (d, *J* = 6.3 Hz, 3H, H6), 1.15 (s, 3H, C3′Me).

^{13}C NMR (151 MHz, $CDCl_3$, α/β-288): δ 154.9 (C3a), 139.4 (C4′b) , 136.8 (C3c), 136.7 (C3c), 129.1 (C3d/4′c), 128.8 (C3d/4′c), 128.6 (C3d/4′c), 128.5 (C3d/4′c), 128.5 (C3d/4′c), 128.4 (C3d/4′c), 128.2 (C3d/4′c), 128.1 (C3d/4′c), 127.6 (C3d/4′c), 102.0 (β-C1′)[g], 101.6 (α-C1′)[g], 93.3 (β-C1, $^1J_{CH}$ = 159 Hz), 91.7 (α-C1, $^1J_{CH}$ =168 Hz), 84.7 (α-C4), 84.1 (β-C4), 68.9 (β-C5), 68.4 (C5′), 68.1 (C5′), 67.1 (C3′), 67.0 (C3′), 66.3 (C3b), 63.9 (α-C5), 63.4 (C4′), 57.1 (C4′a), 54.3 (β-C3), 52.7 (α-C3), 40.7 (C2′), 40.6 (C2′), 39.2 (β-C2) , 35.6 (α-C2), 29.54 (C3′Me) , 29.46 (C3′Me), 24.0 (α-C3Me), 21.9 (β-C3Me), 18.8 (C6′), 18.7 (C6′), 17.9 (β-C6), 17.8 (α-C6).

*Preparation of Compound **288** Continued*

HRMS (ESI+, m/z, α/β-288): $[M+H]^+$ calculated for $[C_{36}H_{46}N_2O_7 + H]^+$ 619.3378; found 619.3400 (error 3.55 ppm).

R_f (α/β-288): 0.37 & 0.50 (75% EtOAc/Hex, Anisaldehyde stain).

Notes:

[a] Procedure adapted from Fukuyama, T.; Kan, T. et al.[11].

[b] The hard urea cap lined with polyethylene was secured with parafilm.

[c] Before transferring the reaction to the separatory funnel, a reaction aliquot was removed, quenched into a mixture of EtOAc/1 N HCl, and analyzed by TLCs developed in 15% & 75% EtOAc/Hex (anisaldehyde stain).

[d] At this point, the remaining material from the aliquot quenched for TLC analysis[b] was also added to the 125 mL separatory funnel.

[e] Detailed gradient: 15% (160 mL) → 30% (70 mL) → 50% (70 mL) EtOAc/Hex → 100% (300 mL) EtOAc; α/β-**288** began to elute in last fraction of 50% EtOAc/Hex and primarily eluted in 100% EtOAc.

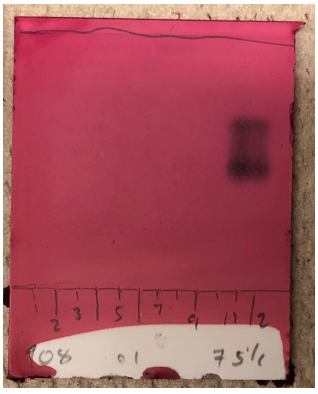
[f] Shift in square brackets determined by ^{1}H-^{13}C HSQC{^{1}H}{^{13}C}.

[g] Due to signal overlap, we could not confidently determine the value of $^1J_{CH}$ for this anomeric resonance.

TLCs:

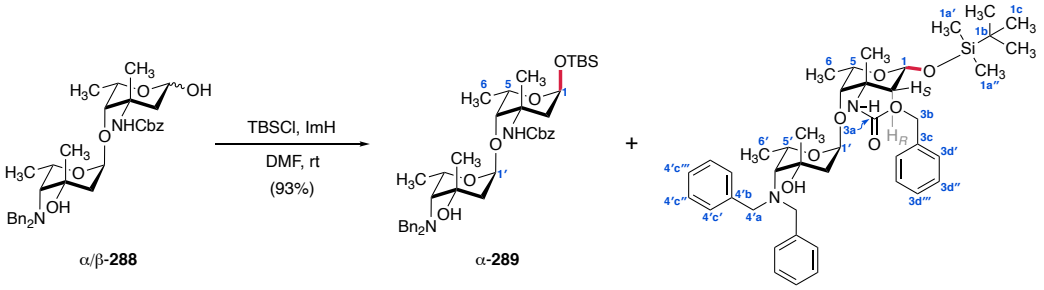
TLC #1a: Reaction Monitoring
Gradient: 15% EtOAc/Hex
Visualization: Ansialdehyde Stain
Left Lane: Starting material (**215**)
Middle Lane: co-spot
Right Lane: Reaction 17 min after being removed from oil bath

TLC #1b: Reaction Monitoring*
Gradient: 75% EtOAc/Hex
Visualization: Ansialdehyde Stain
Left Lane: Starting material (**215**)
Middle Lane: co-spot
Right Lane: Reaction 17 min after being removed from oil bath
Note how poorly the product* (α/β-288**) *stains in the middle and right lanes.*



TLC #2: FCC Fractions
Solvent(s): 75% EtOAc/Hex
Visualization: Anisaldehyde Stain
Lanes 11-12: α/β-**288**

B.8.2 *Preparation of Compound* ***289***



Name (α-289): benzyl ((2*S*,3*S*,4*S*,6*S*)-6-((*tert*-butyldimethylsilyl)oxy)-3-(((2*S*,4*S*,5*R*,6*S*)-5-(dibenzylamino)-4-hydroxy-4,6-dimethyltetrahydro-2*H*-pyran-2-yl)oxy)-2,4-dimethyltetrahydro-2*H*-pyran-4-yl)carbamate

Name (β-289): benzyl ((2*S*,3*S*,4*S*,6*R*)-6-((*tert*-butyldimethylsilyl)oxy)-3-(((2*S*,4*S*,5*R*,6*S*)-5-(dibenzylamino)-4-hydroxy-4,6-dimethyltetrahydro-2*H*-pyran-2-yl)oxy)-2,4-dimethyltetrahydro-2*H*-pyran-4-yl)carbamate

Procedure: A solution of diol α/β-**288** (245.0 mg, 395.9 μmol, 1.000 Eq) was transferred to a dried vial (3 dram) and concentrated *in vacuo*. Then the vial was equipped with a dried stir bar, charged with dry DMF (1.8 mL, 0.22 M) followed by imidazole (ImH, 90.9 mg, 1.34 mmol, 3.37 Eq), flushed with argon, and capped. The reaction was stirred for 30 min at room temperature. Then TBSCl (131.1 mg, 869.8 μmol, 2.197 Eq) was added in one portion, and the vial was flushed with argon and recapped.[a] The reaction, a pale yellow solution, was stirred at room temperature for 48.5 hr.[b] Next, the reaction was transferred to a separatory funnel (125 mL) using EtOAc (90 mL) and DI H_2O (10 mL).[c] The reaction was washed with 0.5 N HCl/brine (9:1, v/v | 4 x 20 mL), sat. aq. $NaHCO_3$/brine (9:1, v/v | 1 x 20 mL), and brine (1 x 20 mL), in that order. The washed organics were dried over anhydrous Na_2SO_4, filtered through a pad of Celite®, concentrated *in vacuo*, and co-evaporated with CH_2Cl_2 (1x). Purification by FCC (SiO_2: 5% → 35% EtOAc/Hex)[d] afforded mono-TBS product α/β-**289** (271.0 mg, 369.7 μmol, 93% yield, ca. 1:9 α:β) as a crunchy white foam.

^{1}H NMR (600 MHz, $CDCl_3$, α-289): δ 6.07 (s, 1H, C3NH), 5.21 (d, *J* = 3.3 Hz, 1H, H1), 4.89 (dd, *J* = 4.8, 3.0 Hz, 1H, H1′), 4.20 (obs q, *J* ~ 6-7 Hz, 1H, H5)[e], 3.11 (s, 1H, H4), 2.40 (1H, H2)[f], 1.80 (dd, *J* = 13.5, 4.0 Hz, 1H, H2), 1.70 (s, 3H, C3Me), 1.13 (d, *J* = 6.5 Hz, 3H, H6).[g]

^{1}H NMR (600 MHz, $CDCl_3$, β-289): δ 7.41-7.29 (m, 10H, H3d/4′c), 7.27 (br s, 1H, H3d/4′c), 7.26-7.20 (m, 4H, H3d/4′c), 6.14 (s, 1H, C3NH), 5.07 (app ABq, 2H, H3b), 4.87 (app t, *J* = 3.7 Hz, 1H, H1′), 4.81 (d, *J* = 8.8 Hz, 1H, H1), 4.53 (s, 1H, C3′OH), 4.40 (br s, 1H, H5′), 4.26 (br s, 2H, H4′a), 3.85 (br d, *J* = 13.7 Hz, 2H, H4′a), 3.80 (q, *J* = 6.3 Hz, 1H, H5), 3.01 (s, 1H, H4), 2.59 (s, 1H, H4′), 2.46 (br d, *J* = 11.4 Hz, 1H, $H2_{pro\text{-}S}$), 2.05 (m, 2H, H2′), 1.56 (dd, *J* = 12.7, 9.7 Hz, 1H, $H2_{pro\text{-}R}$), 1.52 (s, 3H, C3Me), 1.35 (br s, 3H, H6′), 1.176 (obs d, 3H, H6)[h], 1.173 (s, 3H, C3′Me), 0.87 (s, 9H, H1c), 0.08 (s, 3H, H1a), 0.07 (s, 3H, H1a).

^{13}C NMR (151 MHz, $CDCl_3$, α-289): δ 101.8 (C1′)[i], 91.9 (C1, $^1J_{CH}$ = 165 Hz), 85.1 (C4), 63.5 (C5), 52.8 (C3), 37.4[j] (C2), 24.1[j] (C3Me), 17.9 (C6).[g]

^{13}C NMR 51 MHz, $CDCl_3$, β-289): δ 154.9 (C3a), 139.4 (C4′b), 136.8 (C3c), 129.2 (C3d/4′c), 128.7 (C3d/4′c), 128.6 (C3d/4′c), 128.5 (C3d/4′c), 128.2 (C3d/4′c), 127.6 (C3d/4′c), 102.1 (C1′, $^1J_{CH}$ = 165 Hz), 94.1 (C1, $^1J_{CH}$ = 156 Hz), 84.6 (C4), 68.7 (C5), 68.2 (C5′), 67.1 (C3′), 66.2 (C3b), 63.6 (C4′), 57.2 (C4′a), 54.4 (C3), 40.7 (C2′), 40.0 (C2), 29.55 (C3′Me), 26.0 (C1c), 22.0 (C3Me), 18.8 (C6′), 18.3 (C1b), 18.0 (C6), −3.8 (C1a), −4.4 (C1a).

IR (thin film, cm^{-1}): 3342, 3029, 2937, 2860, 1721, 1514, 1455, 1385, 1228, 1071, 1014, 841.

*Preparation of Compound **289** Continued*

HRMS (ESI+, m/z): $[M+H]^+$ calculated for $[C_{42}H_{60}N_2O_7Si+H]^+$ 733.4243; found 733.4249 (error 0.8 ppm).

OR: $\alpha_D = -59.9^o$ ($c = 0.887$, CH_2Cl_2)

R_f: 0.37 (25% EtOAc/Hex, anisaldehyde)

Notes:

[g] This time, the cap was secured with parafilm.

[h] Before transferring the reaction to the separatory funnel, a reaction aliquot was removed, quenched into a mixture of EtOAc/1 N HCl, and analyzed by TLC developed in 75% EtOAc/Hex (anisaldehyde).

[i] At this point, the remaining material from the aliquot quenched for TLC analysis[b] was also added to the 125 mL separatory funnel.

[j] Detailed gradient: 5% (240 mL) → 10% (120 mL) → 15% (120 mL) → 25% (160 mL) → 35% (160 mL) EtOAc/Hex; α/β-**289** eluted in 15% EtOAc/Hex.

[k] A relatively large neighboring peak at 4.26 ppm from the major anomer (β-H4′a) obscures this minor anomer peak (4.20 ppm, α-H5), making the coupling constant and the integration difficult of this peak to determine with great accuracy. Integration was assumed to be 1H based on the peak's assignment.

[l] A relatively large neighboring peak at 2.46 ppm from the major anomer (β-$H2_{pro\text{-}S}$) obscures this minor anomer peak (2.40 ppm, α-H_2), making shift, multiplicity, and resonance difficult of this peak to determine with any great accuracy. As such, the shift was obtained from the ^{1}H-^{13}C HSQC{^{1}H}{^{13}C}, and the integration was assumed to be 1H based on the peak's assignment.

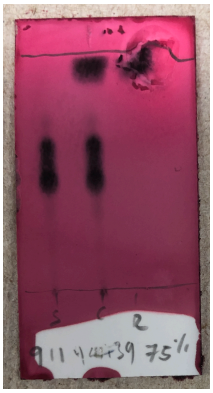
[m] For minor anomer α-**289**, the only peaks reported are those that lent themselves to confident assignment. Resonances for both anomeric peaks as well the carbohydrate backbone of the reducing sugar are given.

[n] Based on the peak assignment and the centering of the corresponding ^{1}H-^{13}C HSQC{^{1}H}{^{13}C} correlation, we hypothesize that the multiplicity of this resonance (1.176 ppm) is a doublet that is obscured by the neighboring singlet at 1.173 ppm.

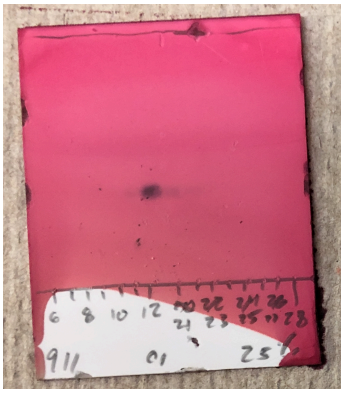
[o] $^1J_{CH}$ could not be confidently determined by coupled ^{1}H-^{13}C HSQC.

[p] Peak not observed in 1-D ^{13}C NMR; shift obtained from ^{1}H-^{13}C HSQC{^{1}H}{^{13}C}.

TLCs

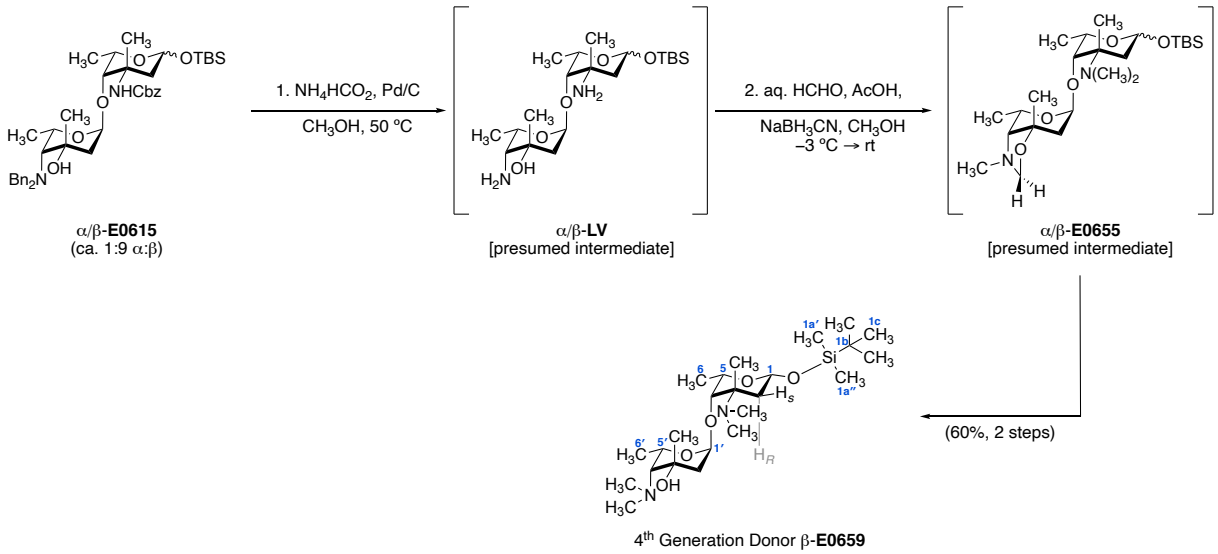


TLC #1: Reaction Monitoring
Gradient: 75% EtOAc/Hex
Visualization: Anisaldehyde Stain
Left Lane: Starting material (α/β-**288**)
Middle Lane: co-spot
Right Lane: Reaction 48 hr after TBSCl added



TLC #2: FCC Fractions
Solvent(s): 25% EtOAc/Hex
Visualization: Anisaldehyde Stain
Lane 12: α/β-**289**

B.8.3 *Preparation of Compound β-287*



Name (α-374): (2S,3R,4S,6S)-3-amino-6-(((2S,3S,4S,6S)-4-amino-6-((tert-butyldimethylsilyl)oxy)-2,4-dimethyltetrahydro-2H-pyran-3-yl)oxy)-2,4-dimethyltetrahydro-2H-pyran-4-ol

Name (β-374): (2S,3R,4S,6S)-3-amino-6-(((2S,3S,4S,6R)-4-amino-6-((tert-butyldimethylsilyl)oxy)-2,4-dimethyltetrahydro-2H-pyran-3-yl)oxy)-2,4-dimethyltetrahydro-2H-pyran-4-ol

Name (α-290): (2S,3S,4S,6S)-6-((tert-butyldimethylsilyl)oxy)-N,N,2,4-tetramethyl-3-(((3aR,4S,6S,7aS)-3,4,7a-trimethylhexahydro-4H-pyrano[3,4-d]oxazol-6-yl)oxy)tetrahydro-2H-pyran-4-amine

Name (β-290): (2S,3S,4S,6R)-6-((tert-butyldimethylsilyl)oxy)-N,N,2,4-tetramethyl-3-(((3aR,4S,6S,7aS)-3,4,7a-trimethylhexahydro-4H-pyrano[3,4-d]oxazol-6-yl)oxy)tetrahydro-2H-pyran-4-amine

Name (β-287): (2S,3R,4S,6S)-6-(((2S,3S,4S,6R)-6-((tert-butyldimethylsilyl)oxy)-4-(dimethylamino)-2,4-dimethyltetrahydro-2H-pyran-3-yl)oxy)-3-(dimethylamino)-2,4-dimethyltetrahydro-2H-pyran-4-ol

Procedure (Step 1)[a]**:** A vial (20 mL) containing α/β-**289** (125.5 mg, 171.2 μmol, 1.000 Eq) was equipped with an oven dried stir bar and charged with NH_4HCO_2 (258.9 mg, 4.106 mmol, 23.98 Eq) followed by Pd/C (135.7 mg)[b]. The vial was stoppered with an upside-down 24/40 septum secured with parafilm, flushed with argon from a double balloon for 5 min, and left under the argon balloon. Next, dry CH_3OH (4.2 mL, 41 mM) was added cautiously through the septum. Finally, the reaction was lowered into a preheated oil bath (set point = 50 °C). After stirring at 50 °C for 3.4 hr, the reaction was removed from the oil bath. 20 min later, the cooled reaction was "double filtered," first through a pad of Celite® and then through a 0.2 μm syringe filter. The final filtrate was collected in a vial (6 dram). The solvent was removed first under a stream of nitrogen (50 °C water bath) and then *in vacuo*. The resulting crude material was taken onto **Step 2** without further purification.

*Preparation of Compound β-**287** Continued*

Procedure (Step 2): The vial (6 dram) containing the crude product of **Step 1** (theoretically 171.2 μmol) was equipped with a stir bar and charged with dry CH_3OH (1.00 mL, 171 mM). Aq. HCHO (2 x 640 μL, 17.2 mmol total, 100 Eq total | 37% by weight in H_2O) was added in two portions at room temperature. Then the reaction was lowered into an ice/water bath (ca. -3 °C). After chilling for 10 min, $NaBH_3CN$ (714 mg, 11.4 mmol, 66.4 Eq) was added in one portion.[c]. Then the reaction vial was fitted with a septum cap pierced with a venting needle (18 gauge). 10 min later, neat CH_3CO_2H (0.80 mL, 14 mmol, 82 Eq) was added dropwise via syringe pump over 16 min. The reaction was stirred in the cooling bath for an additional 10 min before it was removed from the cooling bath and left to stir at room temperature for an additional 3.4 hr. Then the colorless solution was quenched by the addition of aq. 1 N NaOH (18 mL) in one portion at room temperature, causing the reaction to immediately become white and cloudy. The quenched reaction mixture was stirred for 20 min and then transferred to a separatory funnel (60 mL) using CH_2Cl_2 (20 mL) and DI H_2O (8 mL). The layers were separated, and the resulting aqueous layer (pH > 10) was extracted with CH_2Cl_2 (7 x 7 mL). The combined organics were passed through a pad of anhydrous Na_2SO_4 and concentrated *in vacuo*. Purification by RP-HPLC (25% → 55% B/A where A= 1000:1 H_2O:CF_3CO_2H v/v, B = 100% CH_3CN) afforded a mixture (53.9 mg) of β-**287** (48.7 mg, 103 μmol, 60% yield over 2 steps)[d] and two disaccharides denoted ***d*A** and ***d*B** in a molar ratio of ca. 91:6:3 (β-**287**:***d*A**:***d*B**) as estimated by ^{1}H NMR.

^{1}H NMR (600 MHz, 4:1 CD_3OD:$CDCl_3$ (w/w) + ca. 3 Equivalents CF_3CO_2H, β-287): δ 5.32 (dd, *J* = 7.2, 5.5 Hz, 1H, H1′), 4.97 (dd, *J* = 9.0, 2.5 Hz, 1H, H1), 4.70 (qd, *J* = 6.9, 3.8 Hz, 1H, H5′), 3.90 (s, 1H, H4), 3.84 (q, *J* = 6.6 Hz, H5), 3.40 (d, *J* = 3.4 Hz, 1H, H4′), 3.12 (br s, 3H, C3′NMe), 3.11 (br s, 3H, C3′NMe), 2.92 (s, 3H, C3NMe), 2.83 (s, 3H, C3NMe), 2.19-2.16 (m, 2H, H2′$_{pro\text{-}S}$ {2.174}, H2′$_{pro\text{-}R}$ {2.168}), 2.01-1.96 (m, 1H, H2$_{pro\text{-}S}$), 1.74 (br dd, *J* = 11.9, 9.2 Hz, 1H, H2$_{pro\text{-}R}$), 1.57 (d, *J* = 7.0 Hz, 3H, H6′), 1.53 (s, 3H, C3′Me), 1.43 (s, 3H, C3Me), 1.35 (d, *J* = 3.5 Hz, 3H, H6), 0.91 (s, 9H, H1c), 0.133 (s, 3H, H1a), 0.128 (s, 3H, H1a).[e]

^{13}C NMR (151 MHz, 4:1 CD_3OD:$CDCl_3$ (w/w) + ca. 3 Equivalents CF_3CO_2H, β-287): δ 99.7 (C1′, $^1J_{CH}$ = 170 Hz), 94.3 (C1, $^1J_{CH}$ = 159 Hz), 77.0 (C4), 70.3 (C4/C3′/C4′), 70.2 (C4/C3′/C4′), 70.1 (C4/C3′/C4′), 67.8 (C3), 67.3 (C5′), 45.3 (C3′NMe), 44.0 (C3′NMe), 42.2 (C2′), 38.8 (C2), 38.44 (C3NMe), 38.36 (C3NMe), 30.9 (C3′Me), 26.2 (C1c), 18.7 (C1b), 18.2 (C6), 17.5 (C6′), 14.0 (C3Me), −4.0 (C1a), −4.8 (C1a).[e]

LC/MS (Agilent B, ESI +, 374): t_R: 0.85 min | $[M+H]^+$ calculated for $[C_{20}H_{42}N_2O_5Si + H]^+$ 419.3 m/z; found 419.1 m/z.

LC/MS (Agilent B, ESI+, 290): t_R: 0.88 min | $[M+H]^+$ calculated for $[C_{24}H_{48}N_2O_5Si + H]^+$ 473.3 m/z; found 473.0 m/z.

LC/MS (Agilent B, ESI+, 287): t_R: 0.88 min | $[M+H]^+$ calculated for $[C_{24}H_{50}N_2O_5Si + H]^+$ 475.4 m/z; found 475.1 m/z.

HRMS (ESI+, m/z, 287): $[M+H]^+$ calculated for $[C_{24}H_{50}N_2O_5Si + H]^+$ 475.3562; found 475.3566 (error 0.8 ppm).

OR (≥9:1 β-287): α_D = −60.0° (*c* = 0.837, CH_3OH)

Notes:

[a] Procedure for **Step 1** adapted from DeGoey, D. A. et al.[16]

[b] Pd/C = 10% unreduced Pd on activated carbon.

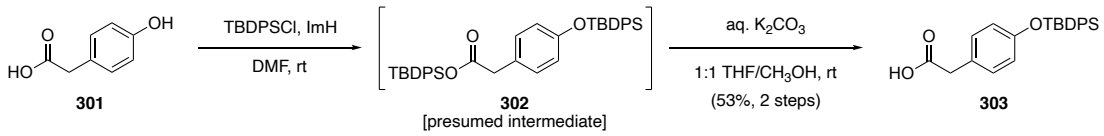
[c] $NaBH_3CN$ was weighed into a vial (2 dram). After the $NaBH_3CN$ was added in one portion from this vial, the vial was rinsed with dry CH_3OH (0.67 mL), and the rinse was added to the reaction.

[d] Conservative subtraction of ***d*A** and ***d*B** from the mass of the entire mixture (53.9 mg) gave 44.7 mg of desired disaccharide β-**287**.

[e] The NMR sample was prepared as follows: a dried vial (1 dram) was charged with CF_3CO_2H (10.5 mg) and CD_3OD (641.0 mg) to give a stock solution with 16.1 mg CF_3CO_2H per 1.00 g of solution. A portion of this CF_3CO_2H/CD_3OD stock solution (266.9 mg solution: 4.30 mg, 37.7 μmol, ca. 3.0 Eq CF_3CO_2H and 262.6 mg CD_3OD) was added to another vial containing ca. 91:6:3 β-**287**:***d*A**:***d*B** (6.0 mg, ca. 12.6 μmol disaccharides, 1.0 Eq) give a colorless solution with some white solids. $CDCl_3$ (58.5 mg) was added in an attempt to solubilize the solids. As solids remained after $CDCl_3$ addition, the mixture was filtered (Kimwipe in an 18 gauge needle) into a 3 mm NMR tube and analyzed.

B.9 *Preparation of 5th Generation Disaccharide Donor **300***

B.9.1 *Preparation of Compound **303***



Name (S6): tert-butyldiphenylsilyl 2-(4-((tert-butyldiphenylsilyl)oxy)phenyl)acetate

Name (37): 2-(4-((*tert*-butyldiphenylsilyl)oxy)phenyl)acetic acid

Procedure (Step 1)[a]**:** In a dried RBF (50 mL, 24/40 neck) equipped with a stir bar was prepared a colorless solution of commercially available 4-hydroxyphenylacetic acid (**301**, 1.533 g, 10.08 mmol, 1.000 Eq) in dry DMF (20 mL, 0.50 M). Imidazole (ImH, 3.011 g, 44.23 mmol, 4.390 Eq) was added in one portion at room temperature, and the reaction was placed under a double balloon of argon. 10 min later, TBDPSCl (5.8 mL, 22 mmol, 2.2 Eq) was added dropwise via syringe pump over 18 min to the now pale-yellow reaction solution. After stirring for an additional 45 hr at room temperature, the pale yellow solution was transferred using Et_2O (60 mL) to a separatory funnel (500 mL) already charged with brine (250 mL). The layers were separated, and the aqueous layer was extracted with Et_2O (2 x 50 mL). Next, the combined organics were washed with 1N HCl/ice (2:1 v/v | 3 x 60 mL), transferred to a collection flask (500 mL)[b], concentrated *in vacuo*, and taken on to **Step 2** without further purification.

Procedure (Step 2)[a]**:** The collection flask (500 mL) containing the crude product from **Step 1** (theoretically 10.08 mmol) was charged with a stir bar, dry THF (40 mL, 0.25 M), and CH_3OH (40 mL, 0.25 M). Next, a solution of K_2CO_3 (1.0013 g, 7.2453, 0.7191 Eq) in DI H_2O (10 mL, 1.0 M) was added in one portion at room temperature, and the reaction was left to stir vigorously at room temperature open to the air. 1.3 hr later, additional K_2CO_3 (1.997 g, 14.45 mmol, 1.434 Eq) and DI H_2O (20 mL, 0.50 M) were added in turn.[c] After stirring for another 2.5 hr[d], the K_2CO_3 was partially neutralized by the addition of acetic acid (2.0 mL, 35 mmol, 3.5 Eq).[e] Then the stir bar was removed, and the reaction was concentrated *in vacuo*. Brine (100 mL) and 1 N HCl (21 mL) were added to the resulting residue which was transferred to a separatory funnel (500 mL) using Et_2O (120 mL), 1 N HCl (20 mL), and DI H_2O (30 mL). The layers were separated, and the aqueous layer was extracted with Et_2O (2 x 50 mL). The combined organics were washed with brine (1 x 50 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. Purification by FCC (SiO_2: 80:20:0 → 30:70:0.5 Hex/Et_2O/AcOH (v/v/v))[f] afforded the desired TBDPS protected carboxylic acid product **303** (2.0668 g, 5.2920 mmol, 53% yield over 2 steps)[g] as a light brown semi-solid that further solidified upon standing in a refrigerator (2-8 °C).

^{1}H NMR (600 MHz, $CDCl_3$, 303): δ 7.80-7.70 (m, 4H), 7.48-7.42 (m, 2H), 7.42-7.36 (m, 4H), 7.03-6.98 (m, 2H), 6.78-6.72 (m, 2H), 3.52 (s, 2H), 1.13 (s, 2H).

^{13}C NMR (151 MHz, $CDCl_3$, 303): δ 178.4 (quat.), 155.0 (quat.), 135.6 (4 x CH), 133.0 (quat.), 130.3 (2 x CH), 130.0 (2 x CH), 127.9 (4 x CH), 125.7 (quat.), 119.9 (CH), 40.3 (CH_2), 26.6 (3 x CH_3), 19.6 (quat.).

LC/MS (Agilent A, ESI +, 303): t_R: 1.39 min| $[M+NH_4]^+$ calculated for $[C_{24}H_{26}O_3Si + NH_4]^+$ 408.2 m/z; found 408.4 m/z. $[M + Na]^+$ calculated for $[C_{24}H_{26}O_3Si + Na]^+$ 413.2 m/z; found 413.4 m/z.

HRMS (ESI+, m/z, 303): $[M+Na]^+$ calculated for $[C_{24}H_{26}O_3Si + Na]^+$ 413.1543; found 413.1546 (error 0.7 ppm).

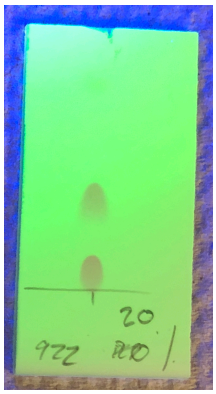
R_f (303): 0.04 (20% Et_2O/Hex + ca. 0.5% AcOH, UV)

MP (303): 133.6-139.1 °C {Single Point = 137.2 °C}

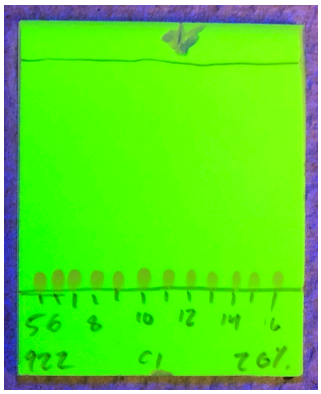
Notes:

[a] The procedures for **Steps 1-2** were adapted from Sebesta, R. et al.[17]
[b] The combined organics from **Step 1** were not dried as water was added to the reaction in **Step 2**.
[c] Addition of water immediately caused the reaction to transform from a colorless liquid with white solid to an opaque white suspension.
[d] At this point, the reaction was yellow in color.
[e] Addition of AcOH caused a color change from yellow to colorless.
[f] Detailed gradient: 80:20:0 (600 mL) → 80:20:0.5 (301.5 mL) → 30:70:0.5 (502.5 mL) Hex/Et_2O/AcOH (v/v/v); **303** eluted in 80:20:0.5 → 30:70:0.5 Hex/Et_2O/AcOH (v/v/v).
[g] This yield accounts for the residual toluene ($PhCH_3$) and CH_2Cl_2 noted in the provided ^1H NMR spectrum.

TLCs:

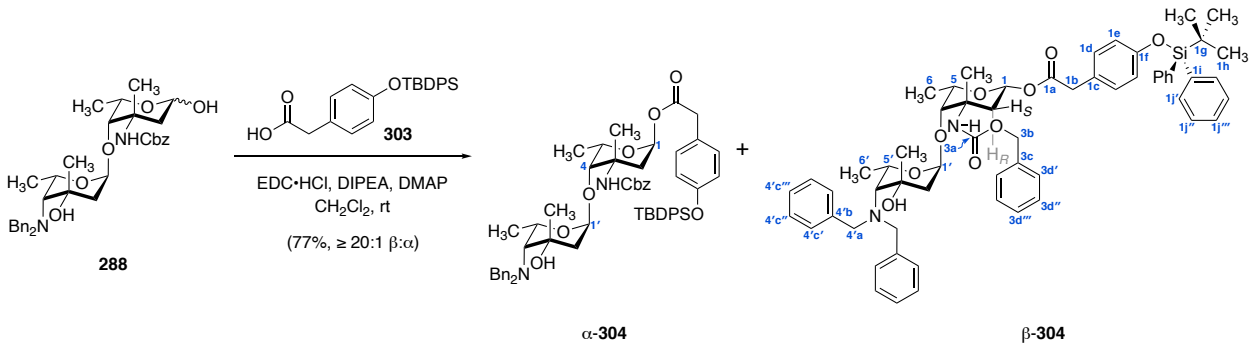


TLC #1: Crude (Step 2)
Gradient: 20% Et_2O/Hex + ca. 0.5% AcOH
Visualization: UV
Middle Lane: Crude product of Step 2 after aqueous workup



TLC #2: FCC Fractions (Step 2)
Solvent(s): 20% Et_2O/Hex + ca. 0.5% AcOH
Visualization: UV
Lanes 5-16: Compound **303**

B.9.2 *Preparation of Compound* ***304***



Name (α-304): (2S,4S,5S,6S)-4-(((benzyloxy)carbonyl)amino)-5-(((2S,4S,5R,6S)-5-(dibenzylamino)-4-hydroxy-4,6-dimethyltetrahydro-2H-pyran-2-yl)oxy)-4,6-dimethyltetrahydro-2H-pyran-2-yl 2-(4-((tert-butyldiphenylsilyl)oxy) phenyl)acetate

Name (β-304): (2R,4S,5S,6S)-4-(((benzyloxy)carbonyl)amino)-5-(((2S,4S,5R,6S)-5-(dibenzylamino)-4-hydroxy-4,6-dimethyltetrahydro-2H-pyran-2-yl)oxy)-4,6-dimethyltetrahydro-2H-pyran-2-yl 2-(4-((tert-butyldiphenylsilyl)oxy) phenyl)acetate

Procedure[a]**:** A vial (2 dram) containing diol **288** (198.4 mg, 320.6 μmol, 1.000 Eq, ca. 2:3 α:β) was charged with 2-(4-((t-butyldiphenylsilyl)oxy)phenyl)acetic acid (**303**, 231.0 mg, 591.5 μmol, 1.845 Eq), EDC•HCl (157.0 mg, 819.0 μmol, 2.554 Eq), and dry CH_2Cl_2 (1.6 mL, 0.20 M), in that order. After equipping the vial with a dried stir bar, DIPEA (0.17 mL, 0.98 mmol, 3.0 Eq) was added in one portion at room temperature. 4 min later, DMAP (77.1 mg, 631 μmol, 1.97 Eq) was added at room temperature. The vial was flushed with argon, fitted with a parafilm secured cap, vortexed, and left to stir at room temperature. After 20.4 hr, the yellow solution was transferred to a separatory funnel (125 mL) using Et_2O (25 mL) and DI H_2O (10 mL).[b] Additional Et_2O (20 mL) was added, the layers were separated, and the resulting aqueous layer was extracted with Et_2O (2 x 10 mL). Then the combined organics were washed with 1 N HCl/brine (2:1 v/v | 3 x 21 mL), DI H_2O/sat. aq. $NaHCO_3$/brine (3:2:1 v/v/v/ | 1 x 42 mL), and DI H_2O/brine (1:1 v/v | 1 x 42 mL), in that order.[c] The combined organics were passed through a plug of anhydrous Na_2SO_4 (eluting with EtOAc), concentrated in vacuo, and co-evaporated with CH_2Cl_2 (1x). Purification by FCC (SiO_2: 10% → 35% EtOAc/Hex)[d] afforded the desired product β-**304** (245.4 mg, 247.6 μmol, 77% yield, ≥20:1 β:α) as a white foam.

^{1}H NMR (600 MHz, $CDCl_3$, α-304): δ 6.12-6.07 (m, 1H, H1{6.10}), 4.86 (app dd, J ~ 4.3, 2.8 Hz, H1′), 3.04 (s, H4).[e-f]

^{1}H NMR (600 MHz, $CDCl_3$, β-304): δ 7.77-7.67 (m, 4H, H1j), 7.46-7.21 (m, 21H, H1j, H1c, & H4′c), 6.98 (d, J = 8.5 Hz, 2H, H1d), 6.71 (d, J = 8.5 Hz, 2H, H1e), 6.19 (s, 1H, C3NH), 5.76 (br d, J = 9.3 Hz, 1H, H1), 5.08 (s, 2H, H3b), 4.89 (app t, J ~ 3.8 Hz, 1H, H1′), 4.49 (br s, 1H, C3′OH)[g], 4.41 (br app d, J = 3.7 Hz, 1H, H5′), 4.27 (br d, J = 10.4 Hz, 2H, H4′a), 3.95 (q, J = 6.2 Hz, 1H, H5), 3.86 (d, J = 13.7 Hz, 2H, H4′a), 3.50 (s, 2H, H1b), 3.08 (s, 1H, H4), 2.61 (br s, 1H, H4′), 2.49 (br d, J = 10.6 Hz, 1H, $H2_{pro\text{-}S}$), 2.08 (ABX dd, ^{2}J = 14.4 Hz, Δν = 24.4 Hz, ^{3}J = 4.6 Hz, H2′), 2.04 (ABX dd, ^{2}J = 14.4 Hz, Δν = 24.4 Hz, ^{3}J = 2.9 Hz, H2′), 1.75 (dd, J = 12.2, 10.7 Hz, 1H, $H2_{pro\text{-}R}$), 1.56 (s, 3H, C3Me), 1.38 (br s, 3H, H6′), 1.21 (d, J = 6.3 Hz, H6), 1.17 (s, 3H, C3′Me), 1.10 (s, 9H, H1h).[f]

^{13}C NMR (151 MHz, $CDCl_3$, α-304): δ 102.0 (C1′, $^1J_{CH}$ = 166 Hz)[h], 91.5 (C1, $^1J_{CH}$ = 174 Hz)[i].[e-f]

^{13}C NMR (151 MHz, $CDCl_3$, β-304): δ 170.4 (C1a), 154.8 (C1f & C3a), 139.4 (C4′b), 136.7 (C3c), 135.6 (Ar), 133.0 (Ar), 130.3 (C1d), 130.0 (Ar), 129.1 (Ar), 128.8 (Ar), 128.6 (Ar), 128.5 (Ar), 128.2 (Ar), 127.9 (Ar), 127.6 (Ar), 125.9 (C1c), 119.8 (C1e), 101.9 (C1′, $^1J_{CH}$ = 166 Hz), 91.7 (C1, $^1J_{CH}$ = 164 Hz), 83.8 (C4), 69.7 (C5), 68.4 (C5′), 67.1 (C3′), 66.4 (C3b), 63.4 (C4′), 57.1 (C4′a), 54.2 (C3), 40.6 (C2′), 40.3 (C1b), 35.8 (C2), 29.6 (C3′Me), 21.8 (C3Me), 19.6 (C1g), 18.7 (C6′), 17.7 (C6).[f,j]

*Preparation of Compound **304** Continued*

HRMS (ESI+, m/z): $[M+H]^+$ calculated for $[C_{60}H_{70}N_2O_9Si + H]^+$ 991.4923; found 991.4931 (error 0.8 ppm).

OR (β-304, ≥ 20:1 β:α): $\alpha_D = -46.0^o$ ($c = 0.572$, CH_2Cl_2)

R_f (α-304): Tentatively assigned to 0.60 (40% EtOAc/Hex, CAM Stain)

R_f (β-304): 0.51 (40% EtOAc/Hex, CAM Stain)

Notes:

[a] Procedure adapted from Zeng, J. et al.[18]

[b] Before transferring the reaction to the separatory funnel, the reaction was directly spotted onto TLC plates developed in 75% EtOAc/Hex (UV/CAM stain).

[c] During the aqueous washes, any emulsions that formed were kept with the organics. EtOAc and DI H_2O were added to the separatory funnel to resolve the final emulsion after the wash with DI H_2O/brine (1:1, v/v).

[d] Detailed gradient: 10% (300 mL) → 15% (200 mL) 20% → (100 mL) → 25% (280 mL) → 30% (280 mL) → 35% (120 mL) EtOAc/Hex; β-**304** eluted in last fraction of 25% EtOAc/Hex and mainly in 30% → 35% EtOAc/Hex.

[e] For the minor anomer α-**304,** the only peaks reported are those that lent themselves to confident assignment.

[f] For both anomers of **304**, ≥10:1 β/α-**304** material from a separate reaction sequence that yielded 143.3 mg of α/β-**304** (61% yield over 2 steps from **215**, ≥10:1 β/α) was used for the line listings and peak assignments. A ^{1}H NMR spectrum of the ≥20:1 β/α-**304** obtained in the procedure detailed above is provided to demonstrate purity.

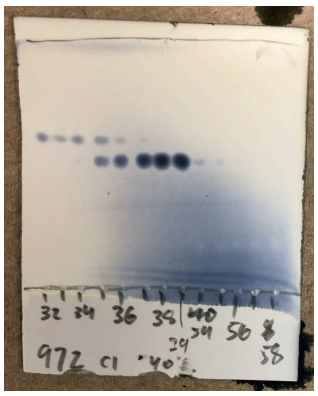
[g] Tentatively assigned based on process of elimination, the absence of an HSQC correlation to this proton, and a weak exchange cross peak in the ROESY spectrum with 1.63 ppm (H_2O).

[h] ^{13}C resonance obtained from coupled ^{1}H-^{13}C HSQC.

[i] ^{13}C resonance obtained from ^{1}H-^{13}C HSQC{^{1}H}{^{13}C}.

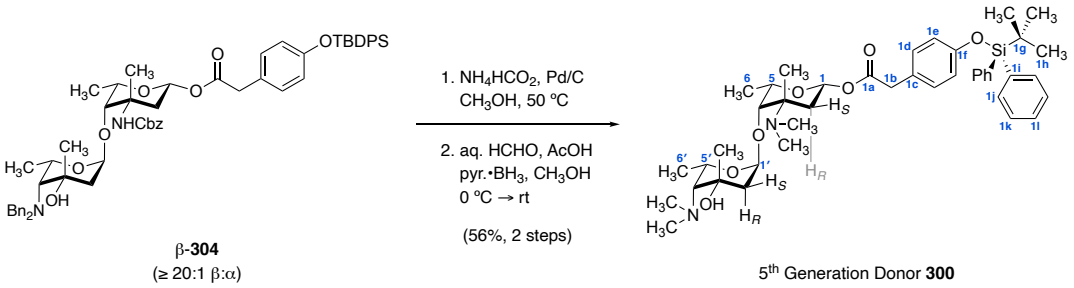
[j] Ar = C1i, C1j, C3d, and/or C4′c.

TLC:



TLC #1: FCC Fractions
Solvent(s): 40% EtOAc/Hex
Visualization: CAM Stain
Lanes 32-36: α-**304** (top spot; tentatively assigned)
Lanes 35-39, 54-55: β- **304** (bottom spot)

B.9.3 *Preparation of Compound **300***



β-**304**
(≥ 20:1 β:α)

5th Generation Donor **300**

Name: (2*R*,4*S*,5*S*,6*S*)-4-(dimethylamino)-5-(((2*S*,4*S*,5*R*,6*S*)-5-(dimethylamino)-4-hydroxy-4,6-dimethyltetrahydro-2*H*-pyran-2-yl)oxy)-4,6-dimethyltetrahydro-2*H*-pyran-2-yl 2-(4-((*tert*-butyldiphenylsilyl)oxy)phenyl)acetate

Procedure (Step 1)[a]**:** A dried 20 mL vial equipped with a stir bar was charged with β-**304** (141.0 mg, 142.2 μmol, 1.000 Eq, ≥ 20:1 β:α), NH_4HCO_2 (228.9 mg, 3.630 mmol, 25.52 Eq), and Pd/C (157.4 mg)[b], in that order. Then the reaction vial was fitted with a septum cap, flushed with argon from a balloon for 8 min, and left under the argon balloon. Next, dry CH_3OH (4.8 mL, 30 mM) was carefully added through the septum at room temperature. Then the vial was lowered into a preheated oil bath (set point 50 °C) and stirred at 50 °C for 30 min. After cooling for 6 min at room temperature, the reaction mixture was "double filtered," first through a pad of cotton and then through a 0.2 μm syringe filter[c], eluting liberally with CH_3OH in both cases. The filtrate was concentrated *in vacuo*, transferred to a 4 dram vial, and concentrated *in vacuo* to afford a crude white solid that was taken on to **Step 2** without further purification.

Procedure (Step 2)[c]**:** The 4 dram vial containing the crude product of **Step 1** (theoretically 142.2 μmol) was equipped with a dried stir bar and charged with dry CH_3OH (1.00 mL, 0.142 M). Next, aq. HCHO (446 μL, 5.99 mmol, 42.1 Eq | 37 wt% in H_2O)[d] and AcOH (326 μL, 5.69 mmol, 40.0 Eq)[d] were each added at room temperature. The reaction vial was vortexed, shaken, and then fitted with a 24/40 septum pierced with an 18 gauge vent needle. After stirring at room temperature for 20 min, the reaction was chilled in an ice/H_2O bath (ca. 0 °C). Then a mixture of purified pyr.•BH_3 (481.5 mg, 5.181, mmol, 36.43 Eq)[e] and dry CH_3OH (0.20 mL) was added dropwise by hand over 5 min.[f] After stirring for an additional 15 min at 0 °C, the colorless, bubbling solution was removed from the cooling bath and stirred 3.9 hr at room temperature. At this point, LC/MS indicated the completion of the reaction. Next, the reaction was chilled in a fresh ice/H_2O bath (ca. 0 °C). Then HN(*i*-Pr)$_2$ (0.85 mL, 6.0 mmol, 42 Eq) was added dropwise by hand over 5 min to quench excess HCHO and pyridine•BH_3. After stirring at 0 °C for an additional 5 min, the colorless, bubbling solution was removed from the cooling bath and stirred at room temperature for 25 min before the stir bar was removed and the majority of the liquid was removed *in vacuo*. The crude mixture was then purified on a Teledyne Isco Combiflash system (RP-C18: 20% → 100% B/A where A= 1000:1 H_2O:CF_3CO_2H v/v, B = 100% CH_3CN). Fractions containing **300** as indicated by LC/MS were transferred to a separatory funnel (250 mL) using sat. aq. $NaHCO_3$ (20 mL), CH_2Cl_2, and DI H_2O. The layers were separated, and the aqueous layer was extracted with CH_2Cl_2 (8 x 15 mL). The combined organics were passed through a pad of anhydrous $MgSO_4$ and concentrated *in vacuo* to yield 5th generation donor **300** (58.2 mg, 79.4 μmol, 56% yield over 2 steps) as a white foam.

^{1}H NMR (600 MHz, $CDCl_3$, 300): δ 7.70 (br d, *J* = 6.8 Hz, 4H, H1j), 7.45-7.39 (m, 2H, H1l), 7.39-7.31 (m, 4H, H1k), 7.00 (d, *J* = 8.5 Hz, 1H, H1d), 6.70 (d, *J* = 8.5 Hz, 2H, H1e), 5.86 (dd, *J* = 9.9, 2.8 Hz, 1H, H1), 5.01 (d, *J* = 4.4 Hz, 1H, H1′), 4.67 (qd, *J* = 6.7, 2.4 Hz, 1H, H5′), 3.77 (q, *J* = 6.4 Hz, 1H, H5), 3.54 (AB q, *J* ~16.5 Hz, 2H, H1b), 3.30 (s, 1H, H4), 2.69 (s, 6H, C4′NMe2), 2.22 (br s, 1H, H4′), 2.14 (s, 6H, C3NMe2), 2.06 (d, *J* = 13.8 Hz, 1H, H2′$_{pro\text{-}S}$), 1.85 (dd, *J* = 13.8, 4.5 Hz, 1H, H2′$_{pro\text{-}R}$), 1.70 (ovlp dd, *J* ~ 11.6, 10.4 Hz, 1H, H2$_{pro\text{-}R}$), 1.62 (br dd, *J* ~ 11.9, 1.6 Hz, 1H, H2$_{pro\text{-}S}$), 1.50 (s, 3H, C3′Me), 1.36 (d, *J* = 6.9 Hz), 3H, H6′), 1.24 (d, *J* = 6.5 Hz, 3H, H6), 1.08 (s, 9H, H1h), 0.94 (3H, C3Me).

^{13}C NMR (151 MHz, $CDCl_3$, 300): δ 170.8 (C1a), 154.8 (C1f), 135.6 (C1j), 133.0 (C1i), 130.3 (C1d), 130.0 (C1l), 127.9 (C1k), 126.1 (C1c), 119.8 (C1e), 99.7 (C1′, $^1J_{CH}$ = 164 Hz), 93.1 (C1, $^1J_{CH}$ = 164 Hz), 78.2 (C4), 71.0 (C5), 69.8 (C4′), 66.7 (C3′/C5′), 66.5 (C3′/C5′), 58.8 (C3), 45.0 (br, C4′NMe2), 41.1 (C2), 40.4 (C1b), 37.9 (C3NMe2), 35.9 (C2), 29.0 (C3′Me), 26.7 (C1h), 19.6 (C1g), 19.1 (C6′), 18.6 (C6), 12.2 (C3′Me).

*Preparation of Compound **300** Continued*

LC/MS (Agilent A, ESI+): t_R: 1.17 min | $[M+H]^+$ calculated for $[C_{42}H_{60}N_2O_7Si + H]^+$ 733.4 m/z; found 733.7 m/z.

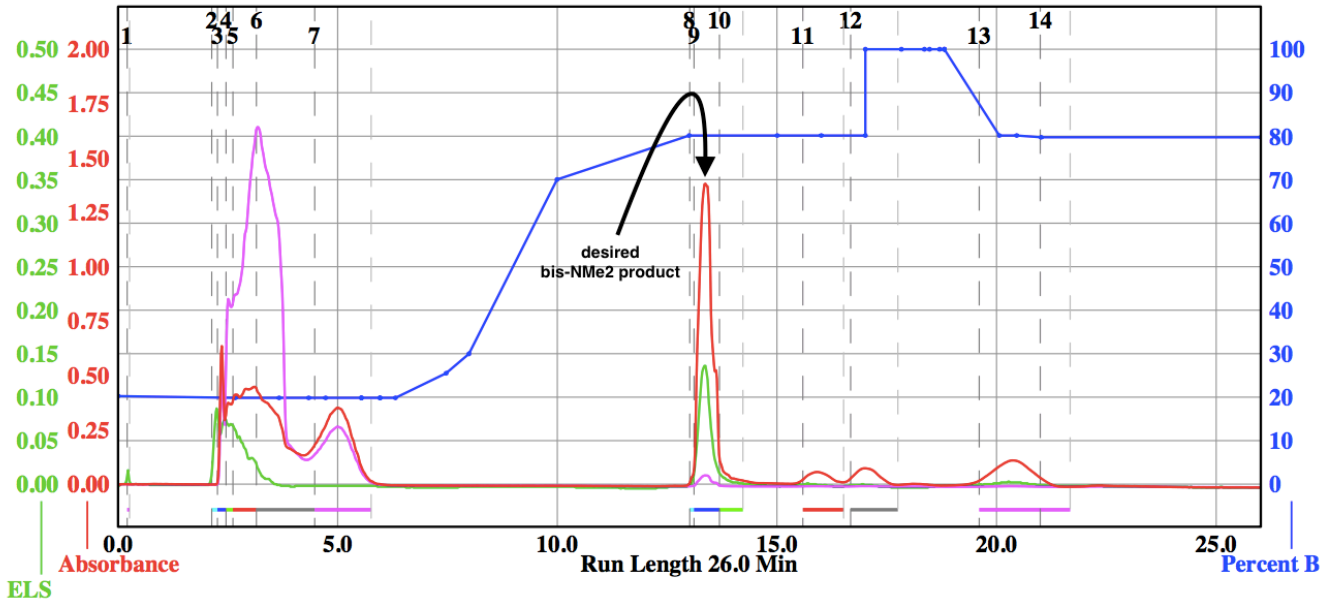
HRMS (ESI+, m/z): $[M+H]^+$ calculated for $[C_{42}H_{60}N_2O_7Si + H]^+$ 733.4243; found 733.4245 (error 0.3 ppm).

OR: $\alpha_D = -58^o$ ($c = 0.27$, CH_2Cl_2)

Notes:

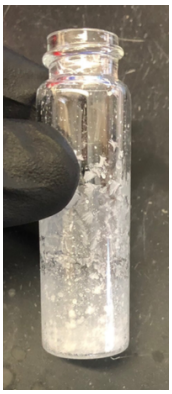
[a] Procedure for **Step 1** adapted from DeGoey, D. A. et al.[16]
[b] Pd/C = 10% unreduced Pd on activated carbon.
[c] Procedure for **Step 2** adapted from Wong, W. S. D. et al.[19]
[d] Aq. HCHO and AcOH measured and dispensed using Gilson P1000 pipet.
[e] Pyr.•BH_3 was purified as detailed in **Materials** section. 504.7 mg of the purified reagent (95.4:4.5:01 pyr.•BH_3:pyridine:Et_2O w/w/w as indicated by ^1H NMR of the purified reagent on the day that it was used in this reaction) and therefore ca. 481.5 mg pyr.•BH_3 were used in this reaction.
[f] Mixture of pyr.•BH_3/CH_3OH mixture was prepared in a vial (0.5 dram). After the addition was complete, the 0.5 dram vial was rinsed with additional dry CH_3OH (3 x 0.20 mL) and the rinses were added to the reaction.

Representative RP-C18 Injection:



*Preparation of Compound **300** Continued*

Visual Procedure:



A. Step 1:
Crude product of **Step 1** in reaction vial for **Step 2**.

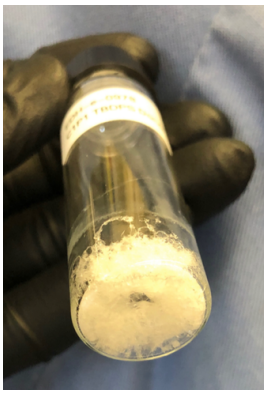
→

B. Step 2:
After the reaction was removed from the cooling bath following pyr•BH_3 addition.

→

Ci. Step 2:
Compound **300** after drying under high vacuum (side view).

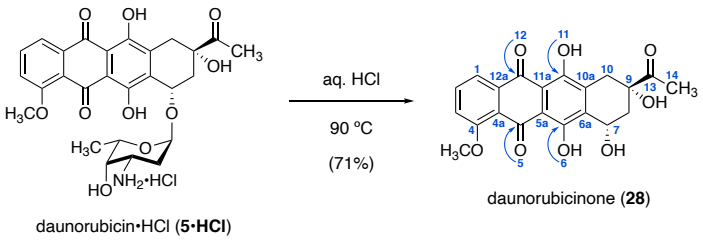
≡



Cii. Step 2:
Compound **300** after drying under high vacuum (bottom view).

B.10 *Synthesis of Aglycone Acceptors*

B.10.1 *Preparation of Daunorubicinone (**28**)*



daunorubicin•HCl (**5•HCl**) → daunorubicinone (**28**)

Name (common): daunorubicinone

Name (IUPAC): (7*S*,9*S*)-9-acetyl-6,7,9,11,12-pentahydroxy-4-methoxy-7,9,10,12-tetrahydrotetracen-5(8H)-one

Procedure[a-b]**:** An RBF (100 mL, 24/40 neck) equipped with a large stir bar was charged with daunorubicin•HCl (**5**•HCl, 565.9 mg, 1.003 mmol, 1.000 Eq) and DI H_2O (44 mL, 23 mM). Next, aq. 1 N HCl (11 mL) was added in one portion at room temperature. Then the flask was stoppered with a hard plastic cap and lowed into a preheated oil bath (set point = 90 °C). The reaction was stirred at 90 °C for 1.5 hr[c], during which time solids crashed out of solution. After cooling at room temperature for 30 min, the red solids were collected on a Büchner funnel, rinsed with DI H_2O, suction dried for several minutes, and then transferred to a 250 mL Erlenmeyer flask (24/40 ground glass joint). Then the Büchner funnel, filter paper, reaction flask, and stir bar were rinsed with CH_2Cl_2 and CH_3OH, and the rinses were added to the 250 mL Erlenmeyer flask containing the collected solids. The solvent was removed *in vacuo*, and the resulting crude material was recrystallized as follows: the crude material was dissolved in CH_2Cl_2 and acetone with the aid of a ca. 60 °C H_2O bath, hot filtered into a new 250 mL Erlenmeyer flask, allowed to cool to room temperature, and then chilled in a freezer (ca. −35 °C) for ca. 2 days. The resulting red solids were collected on a Büchner funnel, rinsed with chilled Et_2O[d], and dried under vacuum to afford daunorubicinone (**28**, 283.1 mg, 710.6 μmol, 71% yield) as a red solid.

^{1}H NMR (600 MHz, $CDCl_3$): δ 13.85 (s, 1H, C6OH/C11OH), 13.17 (s, 1H, C6OH/C11OH), 7.96 (d, *J* = 7.6 Hz, 1H, H1), 7.76 (app t, *J* = 8.1 Hz, 1H, H2), 7.37 (d, *J* = 8.4 Hz, 1H, H3), 5.28 (br s, 1H, H7), 4.64 (s, 1H, C9OH), 4.07 (s, 3H, C4OMe), 3.81 (br s, 1H, C7OH), 3.13 (dd, *J* = 18.4, 2.1 Hz, 1H, H10), 2.88 (d, *J* = 18.4 Hz, 1H, H10), 2.43 (s, 3H, H14), 2.34 (app dt, *J* = 14.5, 1.9 Hz, 1H, H8), 2.13 (dd, *J* = 14.5, 4.8 Hz, 1H, H8).[f]

^{13}C NMR (151 MHz, $CDCl_3$): δ 212.2 (C13), 187.1 (C5/C12), 186.7 (C5/C12), 161.2 (C4), 156.1 (C6/C11), 155.9 (C6/C11), 136.1 (C6a/C10a), 135.9 (C2), 135.6 (C12a), 133.8 (C6a/C10a), 120.9 (C4a), 119.9 (C1), 118.5 (C3), 111.6 (C5a/C11a), 111.2 (C5a/C11a), 77.0 (C9)[d], 62.0 (C7), 56.8 (C4OMe), 35.4 (C8), 33.3 (C10), 24.8 (C14).[g]

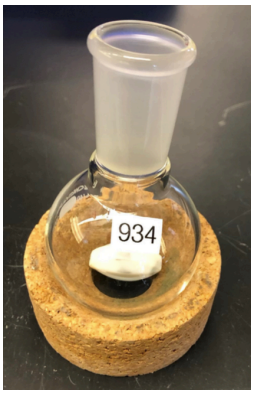
LC/MS (Agilent A, ESI+): t_R = 0.94 min | $[2M+Na]^+$ calculated for $[2x(C_{21}H_{18}O_8) + Na]^+$ 819.2 m/z; found 819.6 m/z.

Notes

[a] Procedure adapted from Yoo, D. J. et al.[20] with slight modification.
[b] No precautions were taken to protect the reaction from light.
[c] After 25 min at 90 °C, the hard plastic cap popped off the flask due to a buildup of pressure. As such, the reaction was stoppered with a new hard plastic cap pierced with an 18 gauge needle to prevent this from happening again.
[d] Et_2O was chilled in a 2–8 °C refrigerator.
[e] This peak (77.0 ppm) was not observed in the 1-D ^{13}C NMR spectrum; presumably, it was masked by the $CDCl_3$ triplet. As such, it was determined via HMBC correlations from H7, H8, H10, H14, and C9OH to C9.
[f] ^{1}H NMR data in agreement with Yoo, D. J. et al.[20]
[g] ^{13}C NMR data in decent agreement with Chan, J. A. et al.[21]; the largest discrepancy comes when comparing the C5/C10a resonances (this work: δ 136.1, 133.8; Chan, J. A. et al: δ 133.5, 133.3).

*Preparation of Daunorubicinone (**28**) Continued*

Visual Procedure:



A. Flask and stir bar used in this reaction.

B. After heating at 90 °C for 1 min.

C. After heating at 90 °C for 20 min.

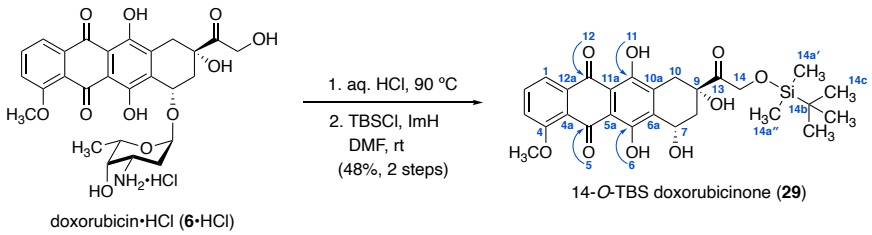
D. After reaction removed from oil bath (1.5 hr at 90 °C).

E. Crude aglycone after washing with DI H_2O.

F. Crude aglycone (solids + CH_2Cl_2/ CH_3OH rinses) before concentration.

G. Recrystallized **28** on Büchner funnel.

B.10.2 *Preparation of Compound 14-O-TBS Doxorubicinone (**29**)*



doxorubicin•HCl (**6**•HCl)

14-*O*-TBS doxorubicinone (**29**)

Name (common): 14-*O*-TBS doxorubicinone

Name (IUPAC): (8*S*,10*S*)-8-(2-((*tert*-butyldimethylsilyl)oxy)acetyl)-6,8,10,11-tetrahydroxy-1-methoxy-7,8,9,10-tetrahydrotetracene-5,12-dione

Procedure (Step 1)[a]**:** An RBF (50 mL, 24/40 neck) was charged with doxorubicin•HCl (**6**•HCl, 256 mg, 441 μmol, 1.00 Eq), a stir bar, and DI H_2O (19 mL, 23 mM). Next, aq. 1 N HCl (4 mL) was added in one portion at room temperature. The flask was placed under a double balloon of argon, wrapped in foil[b], and lowered into a preheated oil bath (90 °C). The reaction was stirred at 90 °C for 1.5 hr and then allowed to cool with stirring at room temperature for 3.6 hr. The solids were collected on a Büchner funnel, washed with water, and suction dried (water aspirator) for ca. 15 min. A small portion of the partially dried solids were transferred to a dried vial (4 dram). The majority of the solid were rinsed from the filter paper and Büchner funnel into a collection flask (200 mL) using a combination of CH_2Cl_2, CH_3OH, and acetone.[c] The solvent was removed *in vacuo*, and the resulting material was transferred to the aforementioned vial (4 dram) already containing some of the crude solids. After removing the transfer solvents *in vacuo*, the material was dried in a vacuum desiccator over P_2O_5 under high vacuum for ca. 16 hr. The resulting red solids were taken on without purification to **Step 2**.

Procedure (Step 2)[d]**:** The vial (4 dram) containing the crude product from **Step 1** (theoretically 441 μmol) was equipped with a dried stir bar and charged with DMF (1.2 mL, 0.37 M) to give a blood red solution. Next, imidazole (78.3 mg, 1.15 mmol, 2.61 Eq) was added followed 3 min later by the addition of TBSCl (71.0 mg, 471 μmol, 1.07 Eq), both added in one portion at room temperature. Then the vial was flushed with argon, capped (secured with parafim), vortexed for ca. 10 sec, inverted (10x), wrapped in foil[b], and left to stir at room temperature for 2 hr. At this point, additional portions of imidazole (91.9 mg, 1.35 mmol, 3.06 Eq) and TBSCl (73.9 mg, 490 μmol, 1.11 Eq) were each added in one portion at room temperature.[e] Then the vial was flushed with argon, capped (secured with parafim), vortexed for ca. 10 sec, inverted (10x), wrapped in foil[b], and left to stir at room temperature for an additional 2.6 hr. At this point, the blood red solution was transferred using CH_2Cl_2 (100 mL) to a separatory funnel (250 mL) already charged with 0.5 N HCl (20 mL).[f] The funnel was shaken to give two layers separated by an emulsion. The lower organic layer was collected before additional DI H_2O (20 mL) and CH_2Cl_2 (20 mL) were added to the flask in failed attempt to resolve the emulsion. As such, the organics and emulsion were combined with the initial organic portion. These combined organics were washed with 0.5 N HCl (1 x 20 mL) and DI H_2O (2 x 20 mL). The washed organics were dried over a mixture of anhydrous Na_2SO_4 (10.3 g) and $NaHCO_3$ (328 mg), filtered, and concentrated *in vacuo*. Purification by FCC (SiO_2: 100% $PhCH_3$ → 20% acetone/$PhCH_3$)[g] yielded semi-pure material by TLC that was further purified via recrystallization as follows: the semi-pure material was dissolved in CH_2Cl_2 (solubilizing solvent) and 200 proof EtOH (precipitating solvent) with the aid of a ca. 70 °C H_2O bath, allowed to cool to room temperature, chilled in a fridge (2–8 °C) for a short time, and then chilled in a freezer (ca. – 35 °C) overnight. The resulting red solids were collected on a Büchner funnel, rinsed sparingly with chilled[h] 200 proof EtOH, rinsed generously with chilled[h] pentanes, and dried under high vacuum to afford 14-*O*-TBS doxorubicinone (**29**, 112.0 mg, 221.9 μmol, 48% yield over 2 steps).

^{1}H NMR (600 MHz, $CDCl_3$): δ 7.94 (d, *J* = 7.7 Hz, 1H), 7.76 (app t, *J* = 8.0 Hz, 1H, H2), 7.37 (d, *J* = 8.2 Hz, 1H), 5.26 (dd, *J* ~ 4.7, 1.4 Hz, 1H, H7), 4.92 (ABq, *J* = 19.7 Hz, Δν = 45.0 Hz, 1H, H14), 4.85 (ABq, *J* = 19.7 Hz, Δν = 45.0 Hz, 1H, H14), 4.07 (s, 3H, C4OMe), 3.13 (ABX dd, ^2J = 18.5 Hz, ^3J = 2.0 Hz, Δν = 158.2 Hz, 1H, H10), 2.87 (AB d, ^2J = 18.5 Hz, Δν = 158.2 Hz, 1H, H10), 2.35 (ABX app dt, *J* = 14.5 Hz, 1.8 Hz, Δν = 131.2 Hz, 1H, H8), 2.13 (ABX dd, ^2J = 14.6 Hz, ^3J = 2.0 Hz, Δν = 131.2 Hz, 1H, H8), 0.95 (s, 9H, C14c), 0.14 (s, 6H, C14a).

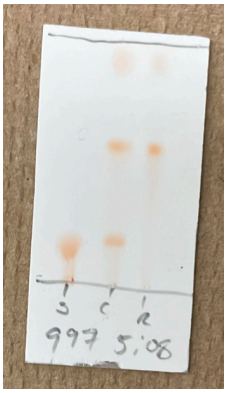
^{13}C NMR (151 MHz, $CDCl_3$): δ 211.8, 187.0, 186.5, 161.1, 156.1, 155.6, 136.0, 135.53, 135.48, 133.9, 120.7, 119.9, 118.6, 111.5, 111.2, 67.0 (C14), 62.2 (C7), 56.8 (C4OMe), 35.8 (C8), 33.8 (C10), 26.0 (C14c), 18.7 (C14b), –5.21 (C14a), –5.24 (C14a).

Notes

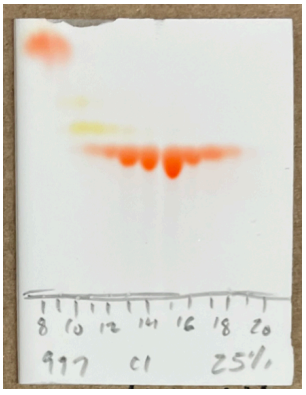
[a] Procedure for Step 1 adapted from Yoo, D. J. et al.[20] with slight modification.
[b] Reaction run in a hood with the hood light turned off.
[c] As the product of Step 1 was difficult to solubilize, much solvent was required to rinse the filter paper and Büchner funnel. In hindsight, after suction drying the collected solids on the Büchner funnel, the entire Büchner funnel and weigh paper assembly should have been dried in a vacuum desiccator over P_2O_5 without any further manipulation. Presumably, the resulting dried solid would then be easily transferred to vial for Step 2.
[d] Procedure for Step 2 adapted from Horton, D. et al.[22]
[e] Before added the 2nd portions of imidazole and TBSCl, a reaction aliquot was removed, quenched into EtOAc/H_2O, and analyzed by TLC developed in 25% acetone/toluene (red spots visualized without aid of UV lamp or stain).
[f] Before transferring the reaction to the separatory funnel, a reaction aliquot was removed, quenched into EtOAc/H_2O, and analyzed by TLC developed in 25% acetone/toluene (red spots visualized without aid of UV lamp or stain).
[g] Detailed gradient: 100% (150 mL) $PhCH_3$ → 2% (60 mL) → 5% (100 mL) → 10% (200 mL) → 20% (100 mL) acetone/$PhCH_3$; semi-pure **29** eluted in the last two fractions of 5% acetone/$PhCH_3$ and mainly in 10% acetone/$PhCH_3$.
[h] 200 proof EtOH and pentanes for washing solids collected from recrystallization were chilled in a 2–8 °C fridge.

TLCs:

TLC #1: Reaction Monitoring (Step 2)
Gradient: 25% Acetone/Toluene
Visualization: No UV or Stain
Left Lane: Crude product of **Step 1**.
Middle Lane: Co-Spot
Right Lane: Reaction (**Step 2**) 2.3 hr after 1st TBSCl addition



TLC #2: Reaction Monitoring (Step 2)
Gradient: 25% Acetone/Toluene
Visualization: No UV or Stain
Left Lane: Crude product of **Step 1**.
Middle Lane: Co-Spot
Right Lane: Reaction (**Step 2**) 2.4 hr after 2nd TBSCl addition

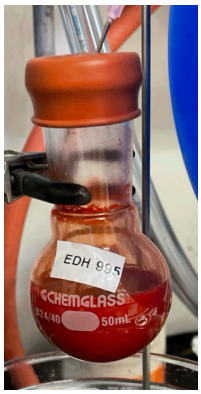


TLC #3a: FCC Fractions (Step 2)
Gradient: 25% Acetone/Toluene
Visualization: No UV or Stain
Lanes 11-20: Combined to give semi-pure **29** that was further purified by recrystallization.

TLC #3b: FCC Fractions (Step 2)
Gradient: 25% Acetone/Toluene
Visualization: CAM Stain
Lanes 11-20: Combined to give semi-pure **29** that was further purified by recrystallization.

*Preparation of 14-O-TBS Doxorubicinone (**29**) Continued*

Visual Procedure:



→

→

→

A. Step 1:
Reaction immediately before collecting red solids on Büchner funnel

B. Step 1:
DI H_2O washed red solids suction drying on Büchner funnel.

C. Step 1:
Crude product of **Step 1** in reaction vial for **Step 2**.

→

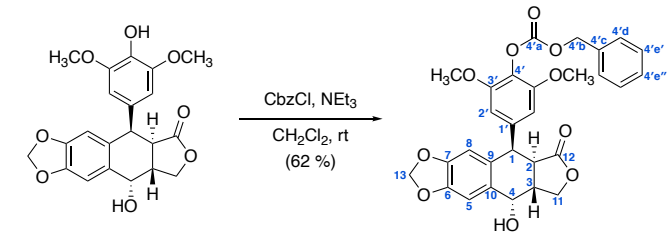
→

D. Step 2:
Crude product of **Step 1** in DMF prior to addition of 1st portions of ImH and TBSCl.

E. Step 2:
Recrystallized **29** on Büchner funnel.

F. Step 2:
Recrystallized **29** after drying under high vacuum..

B.10.3 Preparation of Compound ***30***



4′-demethylepipodophyllotoxin
(etoposide aglycone, **314**)

4′-*O*-Cbz-epipodophyllotoxin
(**30**)

Name (common): 4′-*O*-Cbz-epipodophyllotoxin

Name (IUPAC): benzyl (4-((5*R*,5a*R*,8a*R*,9*S*)-9-hydroxy-6-oxo-5,5a,6,8,8a,9-hexahydrofuro[3′,4′:6,7]naphtho [2,3-d][1,3]dioxol-5-yl)-2,6-dimethoxyphenyl) carbonate

Procedure[a]**:** In a dried collection flask (50 mL) equipped with a stir bar was made a white suspension of 4′-demethylepipodophyllotoxin (**314**, 1.03 g, 2.57 mmol, 1.00 Eq) in dry CH_2Cl_2 (17 mL, 0.15 M). The flask was placed under a double balloon of argon. Then NEt_3 (1.1 mL, 7.9 mmol, 3.1 Eq) was added in one portion at room temperature, and the reaction was stirred at room temperature for 13 min. Next, a solution of CbzCl (902 mg, 5.29 mmol, 2.05 Eq) in dry CH_2Cl_2 (1.5 mL) was added in portions by hand over 4 min at room temperature, causing the reaction to change from a white suspension to a colorless solution (CAUTION: gas evolution observed during addition of CbzCl solution).[c–e] The reaction was stirred room temperature for an additional 13.6 hr. Then reaction was transferred using CH_2Cl_2 (30 mL) to a separatory funnel (125 mL) already charged with DI H_2O (20 mL).[f] Sat. aq. NH_4Cl (10 mL) was added, and the layers were separated.[f] The resulting organic layer was washed with DI H_2O (1 x 20 mL). The washed organics were dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. Purification by FCC (SiO_2: 15% → 80% EtOAc/Hex → 20% CH_2Cl_2/EtOAc)[g] afforded desired 4′-*O*-Cbz-epipodophyllotoxin (**30**, 860.2 mg, 1.609 mmol, 63% yield) as a dense, crunchy white foam.

^{1}H NMR (600 MHz, $CDCl_3$): δ 7.45–7.40 (m, 2H, H4′d), 7.40–7.31 (m, 3H, H4′e), 6.86 (s, 1H, H5), 6.53 (s, 1H, H8), 6.31 (s, 2H, H2′), 5.98 (ABq, *J* = 1.2 Hz, Δν = 13.4 Hz, 1H, H13), 5.96 (ABq, *J* = 1.2 Hz, Δν = 13.4 Hz, 1H, H13), 5.25 (s, 2H, H4′b), 4.84 (app t , *J* = 3.7 Hz, 1H, H4), 4.62 (d, *J* = 5.1 Hz, 1H, H1), 4.40–4.29 (m, 2H, H11), 3.68 (s, 6H, C3′OMe), 3.28 (dd, *J* = 14.2, 5.1 Hz, 1H, H2), 2.79 (dddd, *J* = 14.1, 11.0, 7.9, 3.4 Hz, 1H, H3), 1.96 (d, *J* = 4.3 Hz, 1H, C4OH).[h]

^{13}C NMR (151 MHz, $CDCl_3$): δ 175.1 (C12), 153.2 (C4′a), 151.6 (C3′), 148.7 (C6/C7), 147.7 (C6/C7), 138.3 (C1′), 135.2 (C4′c), 132.1 (C9/C10), 131.7 (C9/C10), 128.63 (C4′/C4′d/C4′e), 128.60 (C4′/C4′d/C4′e), 128.5 (C4′/C4′d/C4′e), 128.4 (C4′/C4′d/C4′e), 110.7 (C8), 109.2 (C5), 107.8 (C2′), 101.8 (C13), 70.5 (C4′b), 67.7 (C11), 66.8 (C4), 56.4 (C3′OMe), 44.1 (C1), 40.6 (C2), 38.4 (C3).[i]

Notes

[a] Procedure adapted from Tsai-Kun et al.[23] with minimal modifications.

[b] Solution was prepared as follows: a dried vial (1 dram) was charged with 90.4:9.6 wt/wt CbzCl:BnCl[c] (997.5 mg total, ca. 902 mg CbzCl, ca. 96 mg BnCl) followed by dry CH_2Cl_2 (1.5 mL).

[c] ^{1}H NMR indicated that the bottle of CbzCl used in this reaction consisted of a 90.4:9.6 wt/wt mixture of CbzCl and BnCl.

[d] When the dropwise addition of CbzCl in dry CH_2Cl_2 was complete, the dried vial (1 dram) in which the solution had been prepared[c] was rinsed with additional dry CH_2Cl_2 (3 x 0.5 mL), and the rinses were added to the reaction.

[e] Before transferring the reaction to the separatory funnel, a reaction aliquot was removed, diluted into CH_2Cl_2, and analyzed by TLC developed in 60% EtOAc/Hex (UV/CAM).

[f] When the funnel was shaken prior to the addition of sat. aq. NH_4Cl, the resulting organic layer was a white suspension. After adding sat. aq. NH_4Cl (10 mL) and shaking the funnel again, the resulting organic layer was much clearer than before.

[g] Detailed gradient: 15% (320 mL) → 25% (240 mL) → 35% (240 mL) → 40% (240 mL) → 45% (240 mL) → 50% (240 mL) → 60% (240 mL) → 70% (240 mL) → 80% (240 mL) EtOAc/Hex → 20% (300 mL) CH_2Cl_2/EtOAc; pure 4′-*O*-Cbz-epipodophyllotoxin (**30**) eluted in 50% → 60% EtOAc/Hex. Fractions were cut very conservatively.

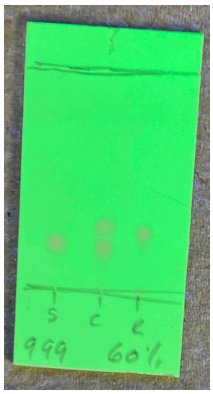
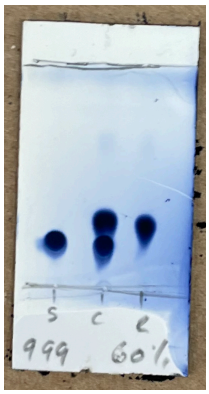
[h] ^{1}H NMR data in decent agreement with Tsai-Kun et al.[23] with the following three exceptions: (1) integration of region from ca. 7.30–7.45 ppm [this work: δ 7.45–7.40 (m, 2H, H4′d), 7.40–7.31 (m, 3H, H4′e); Tsai-Kun et al.: δ 7.43–7.35 (m, 4H)] and (2–3) integration and coupling constant for the resonance at 3.28 ppm [this work: δ 3.28 (dd, *J* = 14.2, 5.1 Hz, 1H, H2); Tsai-Kun et al.: δ 3.28 (dd, *J* = 4.4, 4.0 Hz, 2H)].[j]

[i] ^{13}C NMR data in decent agreement with Tsai-Kun et al.[23]; the only discrepancy comes in the number of resonances from 128–129 ppm [this work: δ 128.63, 128.60, 128.5, 128.4; Tsai-Kun et al.: δ 128.73, 128.50].[j]

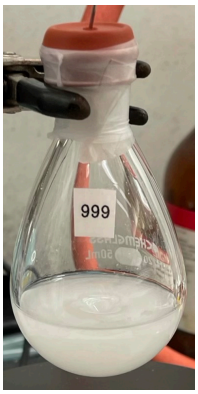
[j] Tsai-Kun et al.[23] acquired their NMR data for 4′-*O*-Cbz-epipodophyllotoxin (**30**) in $CDCl_3$ (^{1}H – 400 MHz; ^{13}C – 100 MHz).

*Preparation of 14-O-TBS Doxorubicinone (**29**) Continued*

TLCs:

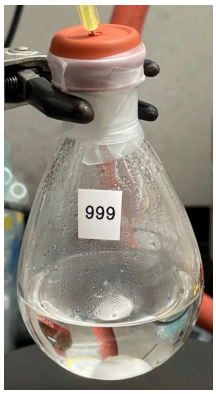
		*For plates 1a–1b:* *Left Lane:* Starting Material (**314**) *Middle Lane:* Co-Spot *Right Lane:* Reaction 13.3 hr after the addition of CbzCl addition ended.
TLC #1a: Reaction Monitoring *Gradient:* 60% EtOAc/Hex *Visualization:* UV	**TLC #1b: Reaction Monitoring** *Gradient:* 60% EtOAc/Hex *Visualization:* CAM Stain	

Visual Procedure:



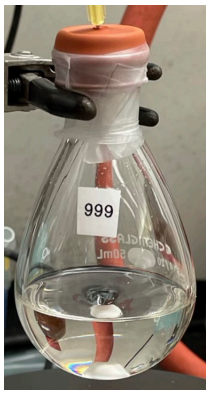
A. Reaction 3 min before the addition of CbzCl solution started.

→



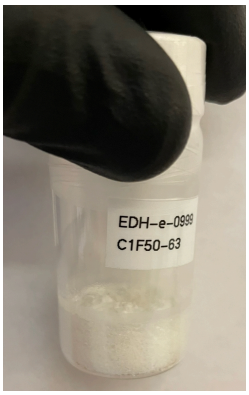
B. Reaction 5 min after the addition of CbzCl solution ended.

→



C. Reaction 13.1 hr after the addition of CbzCl solution ended.

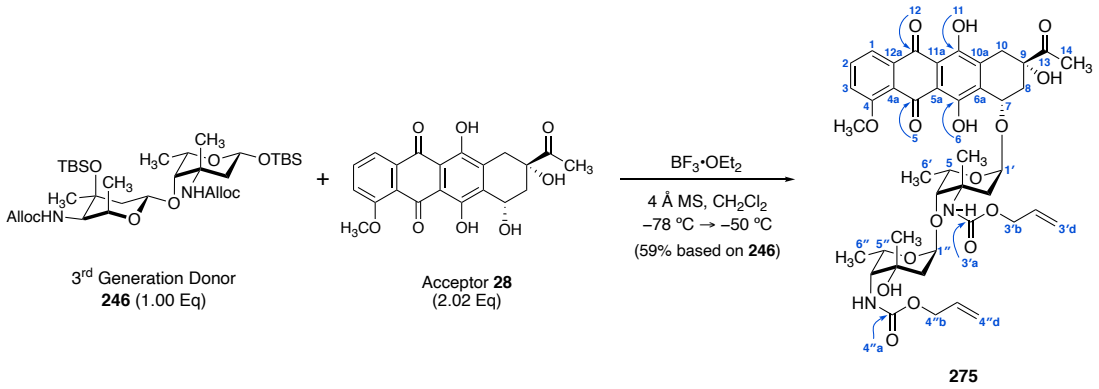
→



D. Purified **30** after drying under high vacuum.

B.11 *Unsuccessful Synthesis of DNR-AMA Hybrid* ***24*** *from 3rd Generation Disaccharide Donor* ***246***

B.11.1 *Preparation of Compound* ***275***



3rd Generation Donor **246** (1.00 Eq)

Acceptor **28** (2.02 Eq)

275

Name: allyl ((2*S*,3*S*,4*S*,6*R*)-6-(((1*S*,3*S*)-3-acetyl-3,5,12-trihydroxy-10-methoxy-6,11-dioxo-1,2,3,4,6,11-hexahydrotetracen-1-yl)oxy)-3-(((2*S*,4*S*,5*R*,6*S*)-5-(((allyloxy)carbonyl)amino)-4-hydroxy-4,6-dimethyltetrahydro-2*H*-pyran-2-yl)oxy)-2,4-dimethyltetrahydro-2*H*-pyran-4-yl)carbamate

Procedure: A dried Anton Parr G10 microwave vial (referred to hereafter as the "reaction vial") charged with a stir bar, 3rd generation disaccharide donor **246** (51.9 mg, 74.0 mmol, 1.00 Eq) and aglycone acceptor **28** (59.7 mg, 150 mol, 2.02 Eq) was dried over P_2O_5 in a vacuum desiccator on a high vacuum line for ca. 13.6 hr. Then the reaction vial was charged with freshly activated powdered 4 Å molecular sieves (181.3 mg) followed by dry CH_2Cl_2 (3.2 mL, 23 mM). The reaction vial was flushed with argon, stoppered with an upside down 14/20 septum (secured with electrical tape then parafilm), and wrapped in foil.[b] The reaction was stirred at room temperature for 2.2 hr. Then it was placed under a double balloon of argon and chilled in an acetone/dry ice bath (–78 °C). A freshly prepared stock solution of BF_3•OEt_2 in dry CH_2Cl_2 (0.31 mL, 16 mg, 0.11 mmol, 1.5Eq |52 mg/mL)[c] was added in portions by hand over 2 minutes at –78 °C. The reaction was stirred for 30 min at –78 °C. Then it was removed from the acetone/dry ice bath, lowered into an ethylene glycol/EtOH/H_2O/dry ice bath (ca. –50 °C)[d], and stirred for an additional 6 hr before the reaction was quenched by the addition of a solution of pyridine (27 μL, 0.33 mmol, 4.5 Eq) in dry CH_2Cl_2 (1.00 mL). The quenched reaction was stirred for an addition 30 min at the same temperature. Then it was removed from the cooling bath and stirred for an additional 23 min at room temperature. At this point, the quenched reaction mixture was filtered through a pad of Celite (60 mL fritted funnel; eluting with CH_2Cl_2 and CH_3OH). The bright red filtrate was concentrated *in vacuo*, and co-evaporated with toluene (2x). Purification by FCC (SiO_2: 10% → 60% EtOAc/Hex → 20% CH_3OH/CH_2Cl_2)[e] afforded unreacted 3rd generation disaccharide donor **246** (17.9 mg, 25.5 μmol, 35% recovered) and desired glycosidation product **275** (42.1 mg, 43.5 μmol, 59% yield, 90% yield based on recovered donor **246**).

^{1}H NMR (600 MHz, $CDCl_3$): δ 13.90 (s, 1H, C6OH/C11OH), 13.21 (C6OH/C11OH), 7.97 (d, *J* = 7.6 Hz, 1H, C1), 7.73 (app t, *J* = 8.0 Hz, 1H, H2), 7.36 (d, *J* = 8.4 Hz, 1H, H3), 6.27 (s, 1H, C3′NH), 5.99–5.79 (m, 2H, H3′c and H4″c), 5.50 (d, *J* = 4.8 Hz, 1H, H1′), 5.33–5.11 (m, 5H, H3′d, H4″d, and H7{5.19}), 4.93 (d, *J* = 10.0 Hz, 1H, C4″NH), 4.87 (br dd, *J* = 4.9, 4.1 Hz, 1H, H1″), 4.56 (d, *J* = 5.4 Hz, 2H, H3′b/H4″b), 4.47–4.43 (m, 2H, H3′b/H4″b), 4.36–4.23 (m, 2H, H5″{4.30}, H5′{4.27}), 4.06 (s, 3H, C4OMe), 3.67 (dd, *J* = 9.9, 4.1 Hz, 1H, H4″), 3.23–3.16 (m, 2H, H4′{3.20}, H10{3.18}), 2.86 (d, *J* = 18.6 Hz, 1H, H10), 2.44 (br d, *J* = 13.8 Hz, 1H, H2′), 2.39 (s, 3H, H14), 2.28 (br d, *J* = 14.7 Hz, 1H, H8), 2.14–2.04 (m, 2H, H8{2.11}, H2″{2.08}), 1.97–1.88 (m, 2H, H2′{1.92}, H2″{1.91}), 1.53 (s, 3H, C3′Me), 1.46 (s, 3H, C3″Me), 1.29 (d, *J* = 6.4 Hz, 3H, H6″), 1.26 (d, *J* = 6.5 Hz, 3H, H6′), 0.88 (s, 9H, H3″c), 0.14 (s, 6H, H3″a).

^{13}C NMR (151 MHz, $CDCl_3$): δ 212.1, 187.0, 186.8, 161.1, 156.6, 156.5, 155.8, 154.8, 135.7[f], 135.6[f], 134.4, 134.2, 133.3 (C3′c/C4 C3′c/C4″c), 133.0 (C3′c/C4″c), 121.1, 119.8 (C1), 118.6 (C3), 117.7 (C3′d/C4″d), 117.6 (C3′d/C4″d), 111.5, 111.4, 100.8 (C1′), 98.9 (C1″), 83.2 (C4′), 76.6 (C9), 73.2 (C3″), 69.6 (C7), 68.7 (C5″), 65.7 (C3′b/C4″b), 65.1 (C3′b/C4″b), 64.3 (C5′), 57.3 (C4″), 56.8 (C4OMe), 51.9 (C3′), 41.6 (C2″), 35.6 (C8/C2′), 35.5 (C8/C2′), 33.5 (C10), 28.6 (C3″Me), 25.9 (C3″c), 24.9 (C14), 23.9 (C3′Me), 18.3 (C3″b), 17.8 (C6′), 16.0 (C6″), –1.9 (C3″a), –2.0 (C3″a).[g]

R_f: 0.36 (50% EtOAc/Hex, red spot visible without UV or stain; spot is also visible under UV and is stained by CAM)

Preparation of Compound ***275*** *Continued*

Notes:

[a] Procedure adapted from patent CN102146108B.[24]

[b] Reaction run in a hood with the hood light turned off.

[c] The stock solution $BF_3{\bullet}OEt_2$ in dry CH_2Cl_2 was prepared as follows: a dry vial (1/2 dram) was charged with $BF_3{\bullet}OEt_2$ (41.6 mg) and dry CH_2Cl_2 (0.76 mL). As the density of $BF_3{\bullet}OEt_2$ is 1.15 g/mL, this gives a combined volume of 0.79622 mL (0.0362 mL $BF_3{\bullet}OEt_2$ + 0.76 mL CH_2Cl_2) and a concentration of 52 mg $BF_3{\bullet}OEt_2$ per 1 mL of stock solution.

[d] Bath was made with ca. 11:9 v/v ethylene glycol/EtOH plus some H_2O; temperature of bath ranged from −60 °C to −40 °C.

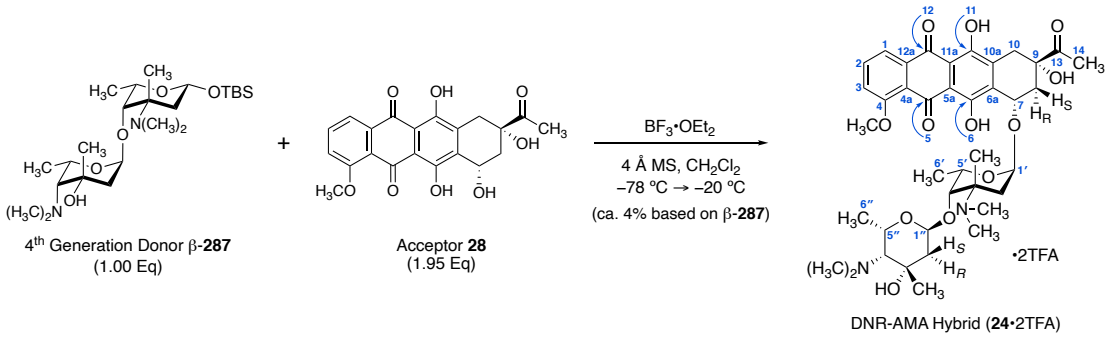
[e] Detailed gradient: 10% (100 mL) → 20% (100 mL) → 30% (100 mL) → 40% (100 mL) → 45% (100 mL) → 50% (100 mL) → 60% (100 mL) EtOAc/Hex → 20% (100 mL) CH_3OH/CH_2Cl_2; unreacted donor **246** eluted in 20% EtOAc/Hex and desired product **275** eluted in 45% → 50% EtOAc/Hex.

[f] One of these peaks results from C2; however, we were not able to make a confident assignment.

[g] Only peaks that lent themselves to confident assignment are assigned.

B.12 *Successful Syntheses of DNR-AMA Hybrid* ***24***

B.12.1 *Preparation of DNR-AMA Hybrid* ***24*** *from 4th Generation Disaccharide Donor* ***287***



4th Generation Donor β-**287** (1.00 Eq) + Acceptor **28** (1.95 Eq) → DNR-AMA Hybrid (**24**•2TFA)

Name: (8*S*,10*S*)-8-acetyl-10-(((2*R*,4*S*,5*S*,6*S*)-4-(dimethylamino)-5-(((2*S*,4*S*,5*R*,6*S*)-5-(dimethylamino)-4-hydroxy-4,6-dimethyltetrahydro-2H-pyran-2-yl)oxy)-4,6-dimethyltetrahydro-2H-pyran-2-yl)oxy)-6,8,11-trihydroxy-1-methoxy-7,8,9,10-tetrahydrotetracene-5,12-dione bis(2,2,2-trifluoroacetate)

Procedure[a]**:** A dried Anton Parr G10 microwave vial (referred to hereafter as the "reaction vial") charged with 4th generation donor **287** (19.9 mg, 41.9 μmol, 1.00 Eq) and aglycone acceptor **28** (32.6 mg, 81.8 μmol, 1.95 Eq) was dried over P_2O_5 in a vacuum desiccator on a high vacuum line for ≥ 24 hr. Then the reaction vial was equipped with a dried stir bar and charged with freshly activated powdered 4 Å molecular sieves (54 mg) followed by dry CH_2Cl_2 (1.1 mL, 38 mM). The reaction vial was flushed with argon, stoppered with an upside down 14/20 septum (secured with electrical tape then parafilm), and wrapped in foil.[b] The reaction was stirred at room temperature for 80 min. Then it was placed under a double balloon of argon and chilled in an acetone/dry ice bath (−78 °C). A freshly prepared stock solution of BF_3•OEt_2 in dry CH_2Cl_2 (0.11 mL, 17 mg, 0.12 mmol, 2.9 Eq | 150 mg/mL)[c] was added in one portion at −78 °C. The reaction was stirred for 15 min at -78 °C. Then it was removed from the acetone/dry ice bath, lowered into a H_2O/CH_3OH/dry ice bath (ca. −30 °C to −25 °C)[d], and stirred for an additional 7.1 hr before the reaction was quenched by the addition of a solution of pyridine (25 μL, 0.41 mmol, 7.4 Eq)[e] in dry CH_2Cl_2 (0.30 mL). After stirring for an additional 35 min, the reaction was directly loaded onto a column of SiO_2 using 5% CH_3OH/CH_2Cl_2. Elution via FCC (Column #1, SiO_2: 19:1:0:0/0 → 0:40:10:3/2 CH_2CH_2/CH_3OH/$CHCl_3$/H_2O/NEt_3 v/v/v/v/v)[f] afforded a mixture of unactivated donor **287** and α/β-linked DNR-AMA hybrid **24** free of excess aglycone **28**. Purification of this mixture by strong anion exchange chromatography (Column #2, SAX: 95:5:0 → 100:0:0 CH_3OH/NEt_3/AcOH)[g-h] afforded an impure mixture of α/β-linked DNR-AMA hybrid **24** free of **287**. Final purification by preparatory HPLC (Column #3, RP-C18: 25% → 60% B/A where A= 1000:1 H_2O:CF_3CO_2H v/v, B = 100% CH_3CN, 4.0 min run) afforded the α-linked **24**•2TFA (1.8 mg, 1.9 μmol, 4% yield based on donor β-**287**) as a red solid.

^{1}H NMR (600 MHz, CD_3OD): δ 7.99 (br d, *J* = 7.4 Hz, 1H, H1), 7.86 (app t, *J* = 8.1 Hz, 1H, H2), 7.59 (br d, *J* = 8.3 Hz, H3), 5.50 (m, 1H, H1′), 5.37 (dd, *J* = 8.0, 4.4 Hz, 1H, H1″), 5.01 (dd, *J* ~ 5, 2 Hz, 1H, H7), 4.67 (qd, *J* = 7.0, 3.6 Hz, 1H, H5″), 4.45 (q, *J* = 6.6 Hz, 1H, H5′), 4.04 (m, 4H, C4OMe and H4′), 3.42 (d, *J* = 3.6 Hz, 1H, H4″), 3.13 (br s, 6H, C3″NMe), 3.07 (ABq, *J* = 18.8 Hz, Δν = 22.9 Hz, 1H, H10), 3.03 (ABq, *J* = 18.8 Hz, Δν = 22.9 Hz, 1H, H10), 2.87 (s, 3H, C3′NMe), 2.77 (s, 3H, C3′NMe), 2.35 (s, 3H, H14), 2.27 (ABq, *J* = 14.5 Hz, Δν = 88.2 Hz, 1H, $H8_{pro\text{-}R}$), 2.22 (ABXdd, $^2J_{HH}$ = 14.7 Hz, $^3J_{HH}$ = 4.4 Hz, Δν = 36.8 Hz, 1H, $H2''_{pro\text{-}R}$), 2.16 (ABXdd, $^2J_{HH}$ = 14.7 Hz, $^3J_{HH}$ = 8.1 Hz, Δν = 36.8 Hz, 1H, $H2''_{pro\text{-}S}$), 2.13 (ABXdd, $^2J_{HH}$ = 14.5 Hz, $^3J_{HH}$ = 5.2 Hz, Δν = 88.2 Hz, 1H, $H8_{pro\text{-}S}$), 2.06-2.02 (m, 2H, H2′), 1.59 (d, *J* = 7.0 Hz, 3H, H6″), 1.57 (s, 3H, C3′Me), 1.56 (s, 3H, C3″Me), 1.38 (d, *J* = 6.7 Hz, 3H, H6′).

^{13}C NMR (151 MHz, CD_3OD): δ 213.9 (C13), 188.5 (C5)[i], 188.1 (C12), 162.6 (C4), 157.5 (C6), 156.4 (C11), 137.3 (C2), 136.6 (C12a), 136.1 (C6a), 135.7 (C10a), 121.8 (C4a), 120.6 (C1), 120.4 (C3), 112.5 (C5a/11a)[j], 112.2 (C5a/11a)[j], 100.7 ($^1J_{CH}$ = 171 Hz, C1′), 100.1 ($^1J_{CH}$ = 170 Hz, C1″), 78.4 (C4′), 71.6 ($^1J_{CH}$ = 145 Hz, C7), 70.5 (C4″), 70.1 (C3″), 67.4 (C5″), 66.7 (C3′) 65.9 (C5′), 57.1 (C4OMe), 44.5 (C3″NMe)[j], 44.1 (C3″NMe)[k], 42.2 (C2″), 38.2 (C3′NMe), 38.0 (C3′NMe), 37.1 (C8), 34.5 (C2′), 32.9 (C10), 31.0 (C3″Me), 24.4 (C14), 18.2 (C6′), 17.6 (C6″), 14.8 (C3′Me).

*Preparation of DNR-Hybrid **24** from 4th Generation Disaccharide Donor **287** Continued*

HRMS (ESI+, m/z): $[M+H]^+$ calculated for $[C_{39}H_{52}N_2O_{12}+H]^+$ 741.3593; found 733.3593 (error 0.0 ppm).

Notes:

[a] Procedure adapted from patent CN102146108B.[24]

[b] Reaction run in a hood with the hood light turned off.

[c] The stock solution $BF_3{\bullet}OEt_2$ in dry CH_2Cl_2 was prepared as follows: a dry vial (1 dram) was charged with $BF_3{\bullet}OEt_2$ (117.6 mg) and dry CH_2Cl_2 (0.68 mL). As the density of $BF_3{\bullet}OEt_2$ is 1.15 g/mL, this gives a combined volume of 0.782 mL (0.102 mL $BF_3{\bullet}OEt_2$ + 0.68 mL CH_2Cl_2) and a concentration of 150 mg $BF_3{\bullet}OEt_2$ per 1 mL of stock solution.

[d] Bath made by adding dry ice to 11:9 H_2O/CH_3OH (v/v). Dry ice was added over the course of the reaction with the goal of maintaining the bath temperature at -30 °C; a temperature range of -58 °C to -18 °C was noted through the reaction.

[e] Pyridine was measured out and dispensed using a Gilson P200 pipet.

[f] Detailed gradient for Column #1 (SiO_2): 19:1:0:0:0 (40 mL) → 0:10:40:1:0 (20.4 mL) → 0:25:25:3:0 (42.4 mL) → 0:40:10:3:0 (42.4 mL) → 0:40:10:3:2 $CH_2CH_2/CH_3OH/CHCl_3/H_2O/NEt_3$ v/v/v/v/v.

[g] SAX column ran with Amberlite IRA-400 (OH) ion-exchange resin (Aldrich catalog number 21,654-2).

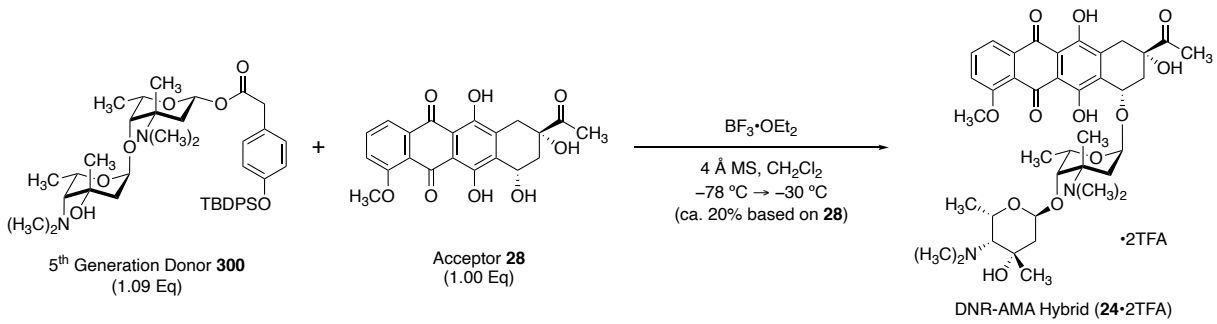
[h] Detailed gradient for Column #2 (SAX): 95:5:0 (5 mL) → 100:0:0 (80 mL) → 95:0:5 (160 mL) → 100:0:0 (300-400 mL) $CH_3OH/NEt_3/AcOH$ v/v/v; **287** eluted in the first portion of 100:0:0 $CH_3OH/NEt_3/AcOH$ while α/β-**24** eluted in 95:0:5 $CH_3OH/NEt_3/AcOH$ and the second portion of 100:0:0 $CH_3OH/NEt_3/AcOH$.

[i] No ^1H-^{13}C HMBC correlations to this quaternary ^{13}C nucleus; assignment made based on functional group analysis (carbonyl), process of elimination (not δ 188.1 – C12), and comparison with the assignment for daunorubicin•HCl (**5**•HCl, 151 MHz, DMSO-d_6) as detailed by Stancyzk, W. et al.[25]

[j] No ^1H-^{13}C HMBC correlations to these quaternary ^{13}C nuclei; assignments made based on process of elimination and comparison with the assignment for daunorubicin•HCl (**5** •HCl, 151 MHz, DMSO-d_6) as detailed by Stancyzk, K. et al.[25]

[k] Peak not visible in 1-D ^{13}C NMR; shift acquired from ^1H-^{13}C HSQC{^1H}{^{13}C}.

*B.12.2 Preparation of DNR-AMA Hybrid **24** from 5th Generation Donor **300***



5th Generation Donor **300** (1.09 Eq) + Acceptor **28** (1.00 Eq) → DNR-AMA Hybrid (**24**•2TFA)

Name: (8*S*,10*S*)-8-acetyl-10-(((2*R*,4*S*,5*S*,6*S*)-4-(dimethylamino)-5-(((2*S*,4*S*,5*R*,6*S*)-5-(dimethylamino)-4-hydroxy-4,6-dimethyltetrahydro-2H-pyran-2-yl)oxy)-4,6-dimethyltetrahydro-2H-pyran-2-yl)oxy)-6,8,11-trihydroxy-1-methoxy-7,8,9,10-tetrahydrotetracene-5,12-dione bis(2,2,2-trifluoroacetate)

Procedure: 5th generation donor **300** (53.5 mg, 73.0 μmol, 1.09 Eq) was transferred to a dried Anton Parr G10 microwave vial (referred to hereafter as the "reaction vial") using dry CH_2Cl_2. The solvent was removed *in vacuo*. Then the reaction vial containing donor **300**, a dried vial (1 dram) containing acceptor **28**, and a test tube equipped with a dried stir bar were dried over P_2O_5 in a vacuum desiccator on a high vacuum line for ca. 15 hr. Then the reaction vial was charged with dried acceptor **28** (26.6 mg, 66.8 μmol, 1.00 Eq), freshly activated powdered 4 Å MS (380.1 mg), the dried stir bar, and dry CH_2Cl_2 (1.5 mL, 45 mM), in that order. The reaction vial was flushed with argon, stoppered with an upside down 14/20 septum (secured with electrical tape then parafilm), and wrapped in foil.[a] The reaction was stirred at room temperature for 1 hr. Then it was placed under a double balloon of argon and chilled in an acetone/dry ice bath (-78 °C). A freshly prepared stock solution of BF_3•OEt_2 in dry CH_2Cl_2 (0.32 mL, 38 mg, 0.27 mmol, 4.0 Eq | 117 mg/mL)[b] was added in one portion at -78 °C. The reaction was stirred for an additional 15 min at -78 °C. Then it was removed from the acetone/dry ice bath and lowered into an ice/brine bath (ca. -5 °C). After stirring for 9.3 hr at ca. -5 °C, the reaction was removed from the ice/brine bath and lowered into a fresh acetone/dry ice bath (-78 °C). Then the reaction was quenched by the addition of a solution of pyridine (47 μL, 0.58 mmol, 8.7 Eq)[c] in dry CH_2Cl_2 (0.95 mL). 10 min later, the reaction was removed from the dry ice/acetone bath, briefly vortexed, and left to stir at room temperature for 45 min. Then the dark red reaction mixture was filtered through a pad of sand to remove the molecular sieves; the pad of sand was rinsed with CH_2Cl_2 and CH_3OH until the filtrate was colorless. The filtrate was concentrated *in vacuo*[d] and then purified in portions on a Teledyne Isco Combiflash system (RP-C18: 10% → 100% B/A where A= 1000:1 H_2O:CF_3CO_2H v/v, B = 100% CH_3CN). Fractions containing **24** with minimal impurities were combined, concentrated *in vacuo*, and co-evaporated with CH_3OH to remove H_2O. Impure fractions containing **24** were repurified in portions (Teledyne Isco Combiflash system, RP-C18: 10% → 100% B/A where A= 1000:1 H_2O:CF_3CO_2H v/v, B = 100% CH_3CN). Combination of all fractions containing **7** with minimal impurities and subsequent lyophilization from H_2O (+ 0.1% CF_3CO_2H) afforded DNR-AMA hybrid **24**•2TFA (14.6 mg, 15.1 μmol, ca. 20% yield based on acceptor **28**) as a blood red solid.

Notes:

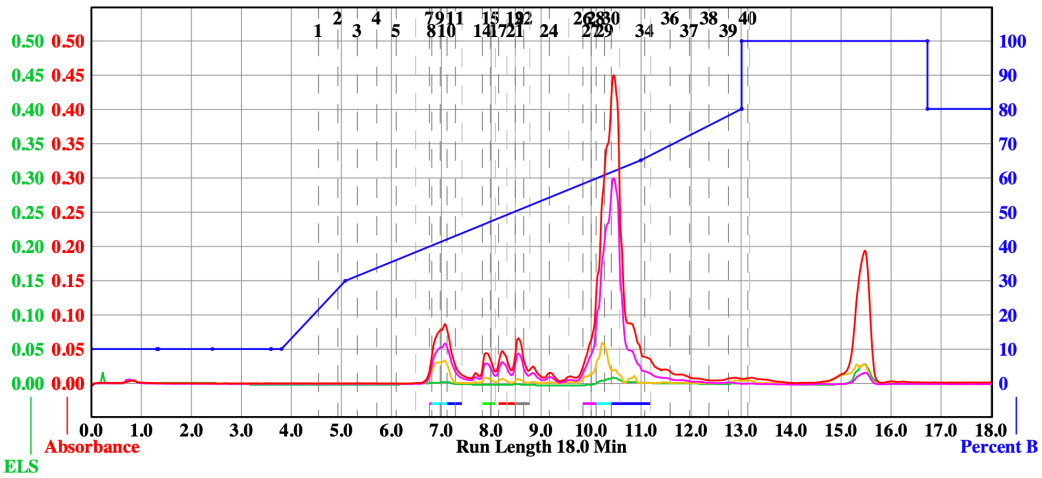
[a] Reaction run in a hood with the hood light turned off.

[b] The stock solution of $BF_3{\bullet}OEt_2$ in dry CH_2Cl_2 was prepared as follows: a dry vial (1 dram) was charged with $BF_3{\bullet}OEt_2$ (144.3 mg) and dry CH_2Cl_2 (1.1 mL). As the density of $BF_3{\bullet}OEt_2$ is 1.15 g/mL, this gives a combined volume (0.129 mL $BF_3{\bullet}OEt_2$ + 1.1 mL CH_2Cl_2) of 1.229 mL and a concentration of 117 mg $BF_3{\bullet}OEt_2$ per 1 mL of stock solution.

[c] Pyridine was measured out and dispensed using a Gilson P200 pipet.

[d] As previous experiments indicated that acceptor **28** and product **24** co-eluted under the reverse phase conditions noted above we made various attempts to remove unreacted **28** prior to reverse phase chromatography. The first involved adding CH_3OH (3 mL) to the initial crude product to give a heterogenous mixture (presumably **28** did not got into solution) that was filtered through a 0.2 μm syringe filter. After rinsing the flask and filter with additional CH_3OH (3 x 1 mL), the flask and filter were rinsed liberally with CH_2Cl_2 into a fresh flask. Finally, the filter was flushed with a combination of CH_2Cl_2 and CH_3OH into a third flask. Though LC/MS indicated the methanolic fraction contained little **28**, it also indicated that the CH_2Cl_2 fraction contained an appreciable amount of **24**. As such, all three fractions were ultimately purified by reverse phase. Further attempts to remove unreacted **28** leveraged the solubility of **24** and insolubility of **28** in acidic H_2O (H_2O + 0.1% CF_3CO_2H). We found that we could use liberal amounts of H_2O (+0.1% CF_3CO_2H) to load the crude mixture directly onto the reverse phase ISCO column without inducing band spreading. As such, by introducing a syringe filter into the loading process, we could remove insoluble **28**. However, due to encountered difficulties with syringe filter clogging when trying to use the filter for more than one injection, we found that prefiltration through a pad of cotton prior to loading the reverse phase ISCO column proved most effective.

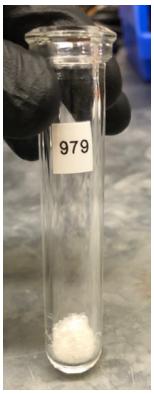
Representative RP-C18 Injection:



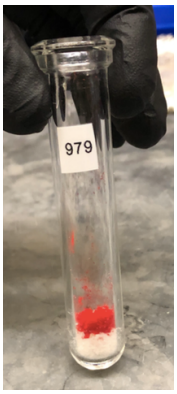
*Fractions 13-18 = pure **24**•2TFA; fractions 6-12 and 20-23 = impure **24**•2TFA.*

*Preparation of DNR-Hybrid **24** from 5th Generation Disaccharide Donor **300** Continued*

Visual Procedure:



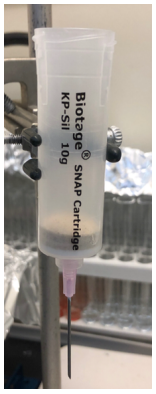
A. Donor **300** in reaction vial after drying overnight over P_2O_5.



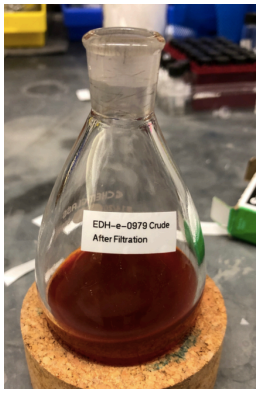
B. Donor **300** and acceptor **28** in reaction vial before 4Å MS added.

C. After dry CH_2Cl_2 was added, right before reaction vial wrapped in foil.

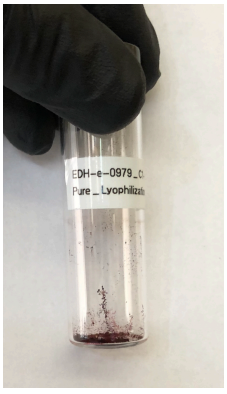
D. Quenched reaction mixture right before filtration through pad of sand to remove 4 Å MS.



E. Filtration set up for removing 4 Å MS.



F. Crude reaction mixture after filtration to remove 4 Å MS.



Gi. Pure DNR-AMA hybrid (**24**•2TFA) after lyophilization (side view).

≡

Gii. Pure DNR-AMA hybrid (**24**•2TFA) after lyophilization (bottom view).

B.13 *References for Chapter 6*

(1) Crich, D.; Smith, M.; Yao, Q.; Picione, J. 2,4,6-Tri-*Tert*-Butylpyrimidine (Ttbp): A Cost Effective, Readily Available Alternative to the Hindered Base 2,6-Di-*Tert*-Butylpyridine and Its 4-Substituted Derivatives in Glycosylation and Other Reactions. *Synthesis* **2001**, *2001*, 323-326.
(2) Soliman, S. E.; Bennett, C. S. Reagent-Controlled Synthesis of the Branched Trisaccharide Fragment of the Antibiotic Saccharomicin B. *Org. Lett.* **2018**, *20*, 3413-3417.
(3) Liang, X.; Lee, C. J.; Chen, X.; Chung, H. S.; Zeng, D.; Raetz, C. R.; Li, Y.; Zhou, P.; Toone, E. J. Syntheses, Structures and Antibiotic Activities of Lpxc Inhibitors Based on the Diacetylene Scaffold. *Bioorg. Med. Chem.* **2011**, *19*, 852-860.
(4) Mal, D.; Dinda, B.; Basak, S.; Ghosh, B. A Five-Step Cascade for the Modular and Regiodefined Synthesis of Naphth[2,1-*D*]Oxazoles. *Synthesis* **2016**, *48*, 1235-1245.
(5) Guanti, G.; Banfi, L.; Narisano, E.; Scolastico, C. Dibenzylaminoacetates as Useful Synthetic Equivalents of Glycine in the Synthesis of A-Amino-B-Hydroxyacids1. *Tetrahedron* **1988**, *44*, 3671-3684.
(6) Ren, F.; Hogan, P. C.; Anderson, A. J.; Myers, A. G. Synthesis of L-Kedarosamine in Protected Form and Its Efficient Incorporation into an Advanced Intermediate to Kedarcidin Chromophore. *Org. Lett.* **2007**, *9*, 1923-1925.
(7) Pragani, R.; Stallforth, P.; Seeberger, P. H. De Novo Synthesis of a 2-Acetamido-4-Amino-2,4,6-Trideoxy-D-Galactose (Aat) Building Block for the Preparation of a *Bacteroides Fragilis* A1 Polysaccharide Fragment. *Org. Lett.* **2010**, *12*, 1624-1627.
(8) Woodward, R. B.; Sondheimer, F.; Taub, D.; Heusler, K.; McLamore, W. M. The Total Synthesis of Steroids1. *J. Am. Chem. Soc.* **1952**, *74*, 4223-4251.
(9) Pihko, A. J.; Nicolaou, K. C.; Koskinen, A. M. P. An Expedient Synthesis of D-Callipeltose. *Tetrahedron Asymmetry* **2001**, *12*, 937-942.
(10) Corey, E. J.; Cho, H.; Rücker, C.; Hua, D. H. Studies with Trialkylsilyltriflates: New Syntheses and Applications. *Tetrahedron Lett.* **1981**, *22*, 3455-3458.
(11) Yoshida, A.; Akaiwa, M.; Asakawa, T.; Hamashima, Y.; Yokoshima, S.; Fukuyama, T.; Kan, T. Total Synthesis of (−)-Lemonomycin. *Chem. Eur. J.* **2012**, *18*, 11192-11195.
(12) Zhang, G.; Fang, L.; Zhu, L.; Zhong, Y.; Wang, P. G.; Sun, D. Syntheses and Biological Activities of 3'-Azido Disaccharide Analogues of Daunorubicin against Drug-Resistant Leukemia. *J. Med. Chem.* **2006**, *49*, 1792-1799.
(13) Brown, H. C.; Geoghegan, P. The Oxymercuration-Demercuration of Representative Olefins. A Convenient, Mild Procedure for the Markovnikov Hydration of the Carbon-Carbon Double Bond. *J. Am. Chem. Soc.* **1967**, *89*, 1522-1524.
(14) Szeja, W.; Fokt, I.; Grynkiewicz, G. Process for Synthesis of Complex 2-Deoxy-2-Iodo Pyranosides of High Purity, Particularly Suitable for Manufacturing Pharmaceutically Pure Annamycin. EP1634887A2 **2006**.
(15) Lear, M. J.; Yoshimura, F.; Hirama, M. A Direct and Efficient A-Selective Glycosylation Protocol for the Kedarcidin Sugar, L-Mycarose: $Agpf_6$ as a Remarkable Activator of 2-Deoxythioglycosides. *Angew. Chem. Int. Ed.* **2001**, *40*, 946-949.
(16) DeGoey, D. A.; Grampovnik, D. J.; Chen, H. J.; Flosi, W. J.; Klein, L. L.; Dekhtyar, T.; Stoll, V.; Mamo, M.; Molla, A.; Kempf, D. J. P1-Substituted Symmetry-Based Human Immunodeficiency Virus Protease Inhibitors with Potent Antiviral Activity against Drug-Resistant Viruses. *J. Med. Chem.* **2011**, *54*, 7094-7104.
(17) Sebesta, R.; Seebach, D. Preparation of (S,S)-Fmoc-B2hile-Oh, (S)-Fmoc-B2hmet-Oh, and (S)-Fmoc-B2htyr(Tbu)-Oh for Solid-Phase Syntheses Ofβ2- Andβ2/B3-Peptides. *Helv. Chim. Acta* **2003**, *86*, 4061-4072.
(18) Zeng, J.; Wang, R.; Zhang, S.; Fang, J.; Liu, S.; Sun, G.; Xu, B.; Xiao, Y.; Fu, D.; Zhang, W.; Hu, Y.; Wan, Q. Hydrogen-Bonding-Assisted Exogenous Nucleophilic Reagent Effect for B-Selective Glycosylation of Rare 3-Amino Sugars. *J. Am. Chem. Soc.* **2019**, *141*, 8509-8515.
(19) Wong, W. S. D.; Osuga, D. T.; Feeney, R. E. Pyridine Borane as a Reducing Agent for Proteins. *Anal. Biochem.* **1984**, *139*, 58-67.
(20) Rho, Y. S.; Kim, S. Y.; Kim, W.-J.; Yun, Y. K.; Sin, H. S.; Yoo, D. J. Convenient Syntheses of Daunomycinone-7-D-Glucuronides and Doxorubicinone-7-D-Glucuronides. *Synth. Commun.* **2004**, *34*, 3497-3511.
(21) Wright, L. H.; Chan, J. A.; Schroer, J. A.; Aszalos, A. A. A 13c Nuclear Magnetic Resonance Study of N-Acetyldaunorubicinol. *J. Org. Chem.* **1977**, *42*, 2344-5.
(22) Horton, D.; Priebe, W.; Varela, O. Synthesis and Antitumor Activity of 3'-Deamino-3'-Hydroxydoxorubicin. A Facile Procedure for the Preparation of Doxorubicin Analogs. *J Antibiot (Tokyo)* **1984**, *37*, 853-858.
(23) Li, T.-K.; Chan, N.-L.; Chein, T.-C. Human Topoisomerase Ii-Targeting Organoplatinum Compounds. WO 2020/037652 A1, **2020**.
(24) 张贵生; 刘青锋; 张志国 Synthesis of 3'-Triazoepirubicin with Anticancer Activity. CN102146108B, **2014**.
(25) Piorecka, K.; Stanczyk, W.; Florczak, M. Nmr Analysis of Antitumor Drugs: Doxorubicin, Daunorubicin and Their Functionalized Derivatives. *Tetrahedron Lett.* **2017**, *58*, 152-155.

APPENDIX C

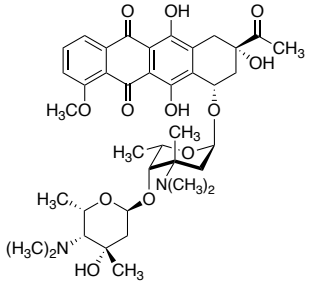
Experimental Procedures (Biology)

C.1 *Cytotoxicity Studies*

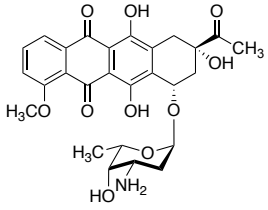
C.1.1 *Materials and Procedures for Cytotoxicity Studies*

Company: The cytotoxicity studies were performed by ImQuest BioSciences, Inc. (7340 Executive Way, Suite R Frederick, Maryland 21740 (USA)).

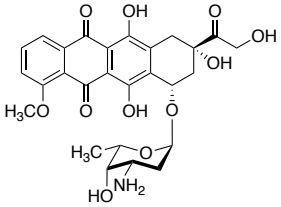
Compounds: DNR-AMA hybrid bis(trifluoroacetate) (**24**•2TFA) was submitted to ImQuest BioSciences as frozen 20 mM stock solutions in DMSO and stored at –20 °C. Daunorubicin hydrochloride (**5**•HCl) was submitted to ImQuest BioSciences as a solid, solubilized in DMSO at 20 mM, and stored at –20 °C. Doxorubicin (**6**) was purchased from Sigma-Aldrich by Imquest BioSciences and solubilized in DMSO at 20 mM. Staurosporine (**377**) was purchased from Sigma-Aldrich by Imquest BioSciences and solubilized in DMSO at 1 mM.



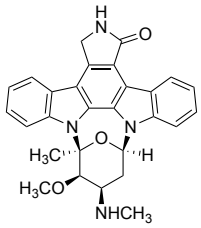
DNR-AMA Hybrid (**24**)



daunorubicin (**5**)



doxorubicin (**6**)



staurosporine (**377**)

Cell Lines: HCT116 cells (human colorectal carcinoma) from ATCC (CCL247) were cultured in McCoy's 5A media supplemented with 10% FBS and 2 mM L-glutamine. MDA-MB231 cells (human breast adenocarcinoma) from ATCC (HTB26) were cultured in DMEM media supplemented with 10% FBS and 2 mM L-glutamine. H69AR cells (human lung carcinoma) from ATCC (CRL11351) were cultured in RPMI 1640 media supplemented with 20% FBS and 2 mM L-glutamine.

***In Vitro* Anti-Cancer Evaluations:** Cells were counted by Trypan Blue Dye exclusion method and seeded in 96 well flat bottom microtiter plates at 1 x 10^4 cells per well in 100 µL/well. Cells were incubated overnight at 37 °C (5% CO_2) to allow the cells to adhere to the plates. Medium was removed and replaced with 100 µL/well of fresh medium. 100 µL of each compound at six concentrations was transferred to the 96 well plate containing the cells, in triplicate. Following 3 days incubation at 37 °C (5% CO_2), the test plates were stained with the tetrazolium dye XTT (2,3,-bis(2-methoxy-4-nitro-5-sulfophenyl)-[(phenylamino)carbonyl]-2*H*-tetrazolium hydroxide). XTT-tetrazolium was metabolized by the mitochondrial enzymes of metabolically active cells to a soluble formazan product, allowing rapid quantitative analysis of the inhibition of cell killing by test substances. XTT solution was prepared daily as a stock of 1 mg/mL in RPMI 1640. Phenazine methosulfate (PMS) solution was prepared at 0.15 mg/mL in PBS and stored in the dark at -20 °C. XTT/PMS stock was prepared immediately before use by adding 40 µL of PMS per mL of XTT solution. 50 µL of XTT/PMS was added to each well of the plate, and the plate was reincubated for 4 hours at 37 °C. Plates were sealed with adhesive plate sealers and shaken gently or inverted several times to mix the soluble formazan product, and the plate was read spectrophotometrically at 450/650 nm with a Molecular Devices Vmax plate reader. Microsoft Excel 2010 was used to analyze and graph the raw data.

C.2 *Cytotoxicity Data*

C.2.1 *Cytotoxicity Data for MDA-MB231 Cell Line*

DNR-AMA Hybrid (24) | TC_{50} = 0.09 μM

TOXICITY VALUES (XTT 450/650 nm)							
CONC (μM)	0	0.0032	0.01	0.032	0.1	0.32	1
SAMPLE 1	1.325	1.337	1.302	0.897	0.631	0.361	0.185
SAMPLE 2	1.374	1.313	1.280	0.897	0.650	0.342	0.192
SAMPLE 3	1.330	1.343	1.332	0.894	0.673	0.345	0.171
MEAN	1.343	1.331	1.305	0.896	0.651	0.349	0.183
% CC	100.0	99.1	97.1	66.7	48.49	26.01	13.60
STD DEV	2.00	1.15	1.94	0.12	1.60	0.75	0.80

Daunorubicin (5) | TC_{50} = 0.08 μM

TOXICITY VALUES (XTT 450/650 nm)							
CONC (μM)	0	0.0032	0.01	0.032	0.1	0.32	1
SAMPLE 1	1.325	1.289	1.132	0.922	0.591	0.316	0.144
SAMPLE 2	1.374	1.144	0.967	0.792	0.509	0.293	0.143
SAMPLE 3	1.330	1.265	1.126	1.004	0.617	0.305	0.158
MEAN	1.343	1.232	1.075	0.906	0.572	0.305	0.148
% CC	100.0	91.8	80.0	67.5	42.59	22.69	11.04
STD DEV	2.00	5.79	6.98	7.96	4.20	0.85	0.61

Doxorubicin (6) | TC_{50} = 0.47 μM

TOXICITY VALUES (XTT 450/650 nm)							
CONC (μM)	0	0.0032	0.01	0.032	0.1	0.32	1
SAMPLE 1	1.325	1.384	1.246	0.884	0.873	0.737	0.340
SAMPLE 2	1.374	1.286	1.271	0.867	0.938	0.771	0.341
SAMPLE 3	1.330	1.385	1.331	0.938	1.071	0.783	0.350
MEAN	1.343	1.352	1.283	0.896	0.961	0.764	0.343
% CC	100.0	100.7	95.5	66.8	71.55	56.87	25.58
STD DEV	2.00	4.25	3.28	2.79	7.52	1.77	0.39

Staurosporine (377, positive control) | TC_{50} = 0.005 μM

TOXICITY VALUES (XTT 450/650 nm)							
CONC (μM)	0	0.00032	0.001	0.0032	0.01	0.032	0.1
SAMPLE 1	1.325	1.097	0.972	0.760	0.505	0.174	0.186
SAMPLE 2	1.374	0.972	0.956	0.711	0.509	0.184	0.186
SAMPLE 3	1.330	1.025	0.951	0.683	0.482	0.179	0.177
MEAN	1.343	1.031	0.960	0.718	0.498	0.179	0.183
% CC	100.0	76.8	71.5	53.4	37.12	13.33	13.60
STD DEV	2.00	4.68	0.83	2.90	1.07	0.37	0.38

C.2.2 *Cytotoxicity Data for HCT 116 Cell Line*

DNR-AMA Hybrid (24) | TC_{50} = 0.09 µM

TOXICITY VALUES (XTT 450/650 nm)							
CONC (µM)	0	0.078	0.25	0.78	2.5	7.81	25
SAMPLE 1	1.025	0.457	0.443	0.469	0.417	0.259	0.300
SAMPLE 2	1.026	0.564	0.358	0.411	0.366	0.394	0.204
SAMPLE 3	1.050	0.545	0.390	0.296	0.270	0.351	0.222
MEAN	1.034	0.522	0.397	0.392	0.351	0.335	0.242
% CC	100.0	50.5	38.4	37.9	33.94	32.37	23.41
STD DEV	1.38	5.50	4.15	8.51	7.24	6.64	4.90

Daunorubicin (5) | TC_{50} = 0. µM

TOXICITY VALUES (XTT 450/650 nm)							
CONC (µM)	0	0.078	0.25	0.78	2.5	7.81	25
SAMPLE 1	1.025	0.524	0.558	0.283	0.344	0.379	0.250
SAMPLE 2	1.026	0.535	0.394	0.309	0.300	0.224	0.225
SAMPLE 3	1.050	0.655	0.556	0.214	0.205	0.258	0.259
MEAN	1.034	0.571	0.503	0.269	0.283	0.287	0.245
% CC	100.0	55.3	48.6	26.0	27.38	27.73	23.68
STD DEV	1.38	7.04	9.13	4.73	6.86	7.90	1.67

Doxorubicin (6) | TC_{50} = 0.09 µM

TOXICITY VALUES (XTT 450/650 nm)							
CONC (µM)	0	0.078	0.25	0.78	2.5	7.81	25
SAMPLE 1	1.025	0.514	0.470	0.535	0.340	0.452	0.255
SAMPLE 2	1.026	0.526	0.367	0.425	0.328	0.387	0.229
SAMPLE 3	1.050	0.535	0.440	0.433	0.410	0.447	0.250
MEAN	1.034	0.525	0.426	0.464	0.359	0.429	0.245
% CC	100.0	50.8	41.2	44.9	34.76	41.49	23.68
STD DEV	1.38	1.04	5.11	5.92	4.31	3.49	1.33

Staurosporine (377, positive control) | TC_{50} = 0.03 µM

TOXICITY VALUES (XTT 450/650 nm)							
CONC (µM)	0	0.00032	0.001	0.0032	0.01	0.032	0.1
SAMPLE 1	1.025	0.787	0.780	0.559	0.690	0.757	0.396
SAMPLE 2	1.026	0.698	0.742	0.680	0.729	0.355	0.401
SAMPLE 3	1.050	0.722	0.810	0.581	0.579	0.454	0.361
MEAN	1.034	0.736	0.777	0.607	0.666	0.522	0.386
% CC	100.0	71.2	75.2	58.7	64.44	50.49	37.37
STD DEV	1.38	4.44	3.33	6.22	7.50	20.24	2.10

C.2.3 *Cytotoxicity Data for H69AR Cell Line*

DNR-AMA Hybrid (24) | TC_{50} = 0.20 µM

TOXICITY VALUES (XTT 450/650 nm)							
CONC (µM)	0	0.156	0.5	1.56	5	15.6	50
SAMPLE 1	1.658	0.979	0.193	0.174	0.164	0.137	0.146
SAMPLE 2	1.646	0.876	0.187	0.171	0.164	0.129	0.145
SAMPLE 3	1.651	0.874	0.184	0.156	0.161	0.127	0.143
MEAN	1.652	0.910	0.188	0.167	0.163	0.131	0.145
% CC	100.0	55.1	11.4	10.1	9.86	7.93	8.76
STD DEV	0.39	3.65	0.28	0.58	0.11	0.32	0.12

Daunorubicin (5) | TC_{50} = 1.25 µM

TOXICITY VALUES (XTT 450/650 nm)							
CONC (µM)	0	0.156	0.5	1.56	5	15.6	50
SAMPLE 1	1.658	1.426	1.406	0.609	0.240	0.149	0.184
SAMPLE 2	1.646	1.470	1.395	0.522	0.219	0.146	0.187
SAMPLE 3	1.651	1.433	1.379	0.636	0.196	0.152	0.160
MEAN	1.652	1.443	1.393	0.589	0.219	0.149	0.177
% CC	100.0	87.4	84.4	35.7	13.24	9.03	10.72
STD DEV	0.39	1.44	0.80	3.60	1.34	0.18	0.91

Doxorubicin (6) | TC_{50} = 14.9 µM

TOXICITY VALUES (XTT 450/650 nm)							
CONC (µM)	0	0.156	0.5	1.56	5	15.6	50
SAMPLE 1	1.658	1.659	1.477	1.491	1.339	0.813	0.226
SAMPLE 2	1.646	1.686	1.506	1.451	1.371	0.730	0.232
SAMPLE 3	1.651	1.657	1.510	1.474	1.348	0.816	0.228
MEAN	1.652	1.667	1.498	1.472	1.353	0.786	0.229
% CC	100.0	100.9	90.7	89.1	81.89	47.59	13.84
STD DEV	0.39	0.97	1.07	1.20	0.98	2.95	0.19

Staurosporine (377, positive control) | TC_{50} = 0.15 µM

TOXICITY VALUES (XTT 450/650 nm)							
CONC (µM)	0	0.032	0.01	0.32	0.1	0.32	1
SAMPLE 1	1.658	1.500	1.307	0.163	0.152	0.137	0.115
SAMPLE 2	1.646	1.497	1.369	0.197	0.157	0.139	0.134
SAMPLE 3	1.651	1.514	1.452	0.206	0.162	0.150	0.145
MEAN	1.652	1.503	1.376	0.189	0.157	0.142	0.131
% CC	100.0	91.0	83.3	11.4	9.50	8.60	7.96
STD DEV	0.39	0.55	4.40	1.37	0.31	0.42	0.92

C.3 *Materials and Procedures for DNA Relaxation Studies*

Facilities and Personnel: DNA relaxation studies were performed by Jo Ann Byl (M.S., Senior Scientist) in the laboratory of Professor Neil Osheroff (Ph.D., Professor of Biochemistry, John Coniglio Chair in Biochemistry, Vanderbilt University).

Preparation of Enzymes: Recombinant human topoisomerase IIα (hTopIIα) was expressed in *Saccharomyces cerevisiae* and purified as described previously.[1-3] hTopIIα was stored at −80 °C as a 1.5 mg/mL stock in 50 mM Tris-HCl (pH 7.9), 0.1 mM EDTA, 750 mM KCl, and 40% (v/v) glycerol. *Archaeoglobus fulgidus* reverse gyrase was expressed in *E. coli* C41(DE3) and purified as described by Rodriguez.[4] *A. fulgidus* reverse gyrase was stored in liquid nitrogen; working stocks were stored at −80 °C.

Preparation of Negative Supercoiled (−SC) DNA: Negatively supercoiled pBR322 DNA was prepared according to the manufacturer's protocol from *Escherichia coli* using a Plasmid Mega Kit (Qiagen).

Preparation of Positively Supercoiled (+SC) DNA: Positively supercoiled pBR322 DNA was prepared by treating negatively supercoiled DNA with reverse gyrase[4] in an adaption of a previously described method.[5] Reaction mixtures contained 50 μg negatively supercoiled pBR322 DNA and 25 μg *A. fulgidus* reverse gyrase in a total of 500 μL 50 mM Tris•HCl (pH 9.0), 10 mM NaCl, 10 mM $MgCl_2$, and 0.3 mM ATP. Reactions were incubated at 95 °C for 5 min, halted by the addition of 13.6 μL 375 mM EDTA, and cooled on ice. Proteinase K was added (10 μL of 4 mg/mL), reactions were incubated at 45 °C for 30 min to digest the enzyme and then cooled on ice. Samples were extracted with phenol:chloroform:isoamyl alcohol (25:24:1) using a phase lock gel (vortex and microfuge at 13,000 rpm for 2 min). The supernatant was removed and divided into two 1.5 mL microfuge tubes. Precipitation involved addition of 0.1 volumes of NaOAc and 2-3 volumes of 100% ethanol. After chilling in a freezer for 20 min, the tubes were spun in a microcentrifuge at 13,000 rpm for 20 min at 4 °C. The majority of the supernatant was aspirated. 500 μL 70% ethanol was added, and the tubes were spun in a microcentrifuge at 13,000 rpm for 10 min at 4 °C. The supernatant was aspirated, and the pellet was dried. The positively supercoiled DNA was resuspended in 0.5x TE buffer and further purified using a Bio-Spin P-30 spin column in Tris buffer (Bio-Rad) according to the manufacturer's protocol using 0.5x TE buffer [buffer exchange to 0.5x TE buffer was accomplished via gravity flow prior to loading the crude, positively supercoiled DNA].

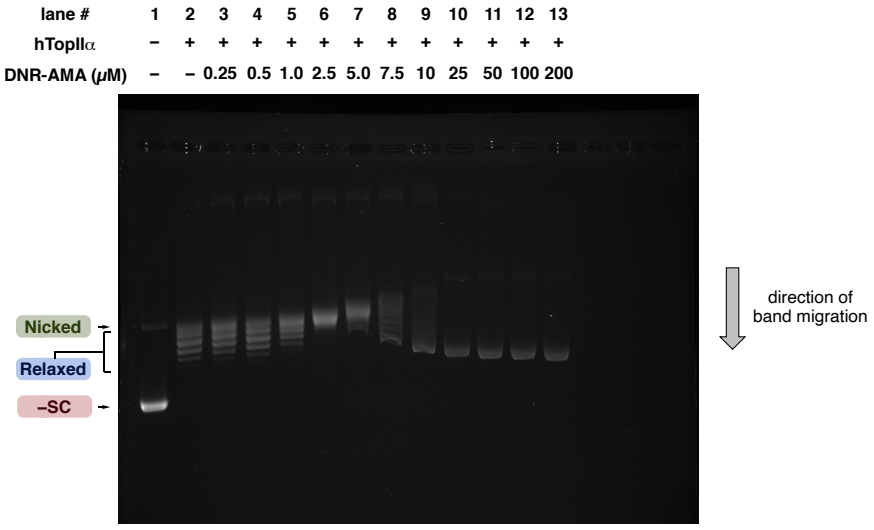
Assay 1 – DNA Relaxation Assays with Human Topoisomerase IIa (+hTopIIα): DNA relaxation reactions were carried out using the procedure of Fortune and Osheroff.[6] Reaction mixtures contained 5 nM negatively or positively supercoiled pBR322 DNA, 3 nM human topoisomerase IIα, 1 mM ATP, and DNR-AMA Hybrid (**24**•2TFA) or daunorubicin (**5**•HCl) in a total of 20 μL of relaxation buffer [10 mM Tris-HCl (pH 7.9) 5 mM $MgCl_2$, 175 mM KCl, 0.1 mM EDTA, and 2.5% (v/v) glycerol]. Mixtures were incubated at 37 °C for 4 min. Reactions were stopped by the addition of 3 μL of 0.77% SDS-77 mM EDTA, pH 8.0. Samples were mixed with 2 μL of agarose loading dye, heated for 2 min at 45 °C, and subjected to electrophoresis in a 1% agarose gel in 100 mM Tris-borate (pH 8.3) and 2 mM EDTA. Gels were stained for 30 min using 1.0 μg/mL ethidium bromide and rinsed in deionized water. DNA bands were visualized by UV light and quantified using an Alpha Innotech digital imaging system.

Assay 2 – DNA Relaxation Assay without Human Topoisomerase IIa (−hTopIIα): To determine how DNR-AMA Hybrid (**24**•2TFA) and daunorubicin (**5**•HCl) impacted the mobility of unrelaxed negatively or positively supercoiled pBR322 DNA, the DNA relaxation reactions were carried out as described in **Assay 1** except that topoisomerase diluent [50 mM Tris (pH 7.7), 0.5 mM DTT, 0.1 mM EDTA (pH 8.0), 750 mM KCl, and 40% (v/v) glycerol] was used instead of human topoisomerase IIα.

C.4 *Data from Assay 1*

C.4.1 *Inhibition of hTopIIα Mediated Relaxation of –SC DNA by DNR-AMA Hybrid (DNA-AMA, **24**•2TFA)*

Full Gel from Figure 3.6ai.



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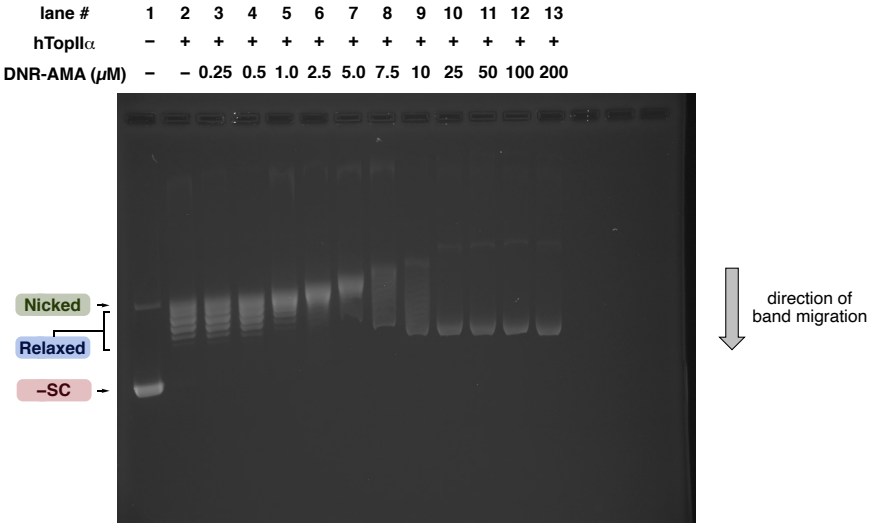
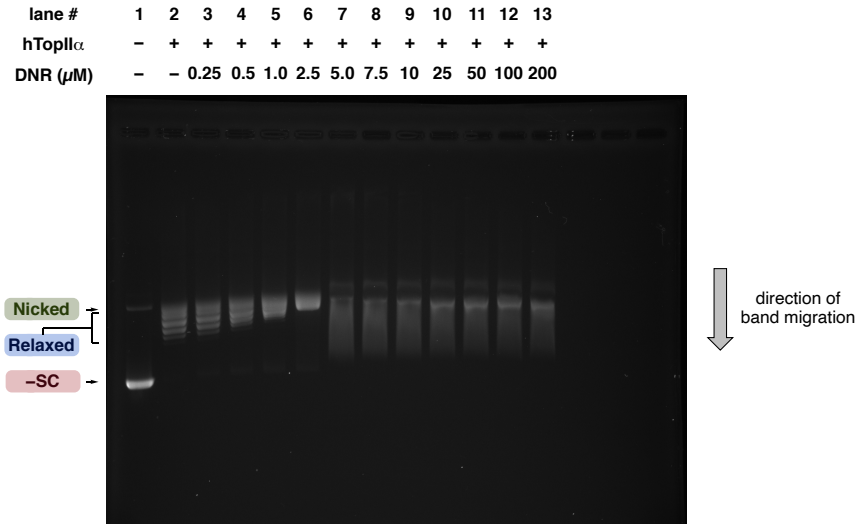


C.4.2 Inhibition of hTopIIα Mediated Relaxation of –SC DNA by Daunorubicin (DNR, **5**•*2TFA)*

Full Gel from Figure 3.6bi.



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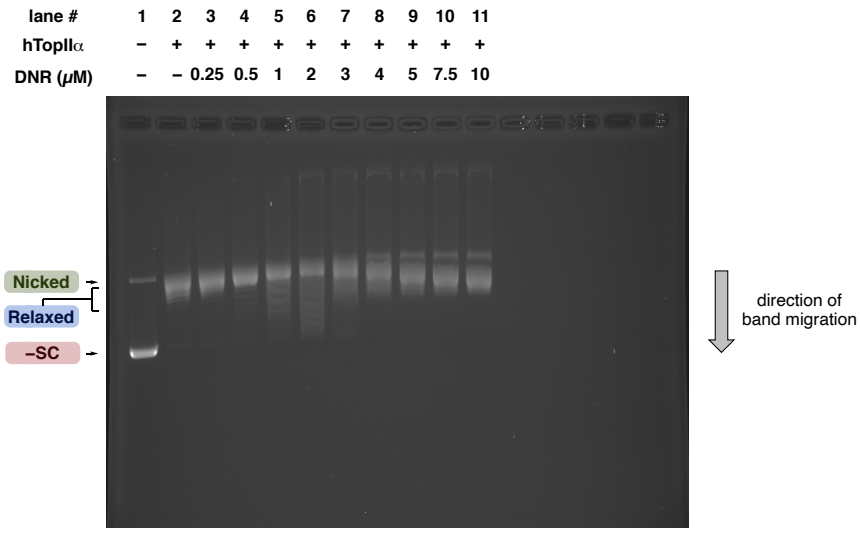
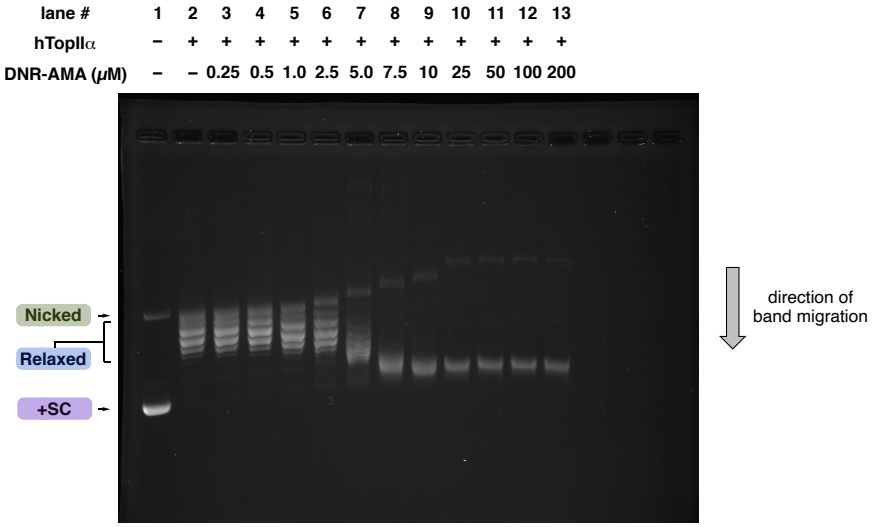


*C.4.3 Inhibition of hTopIIα Mediated Relaxation of +SC DNA by DNR-AMA Hybrid (DNA-AMA, **24**•2TFA)*

Full Gel from Figure 3.7ai.



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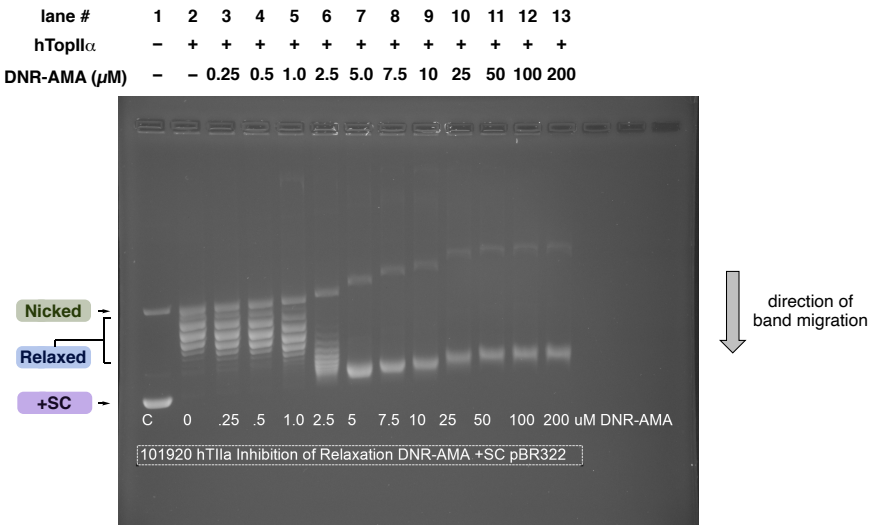
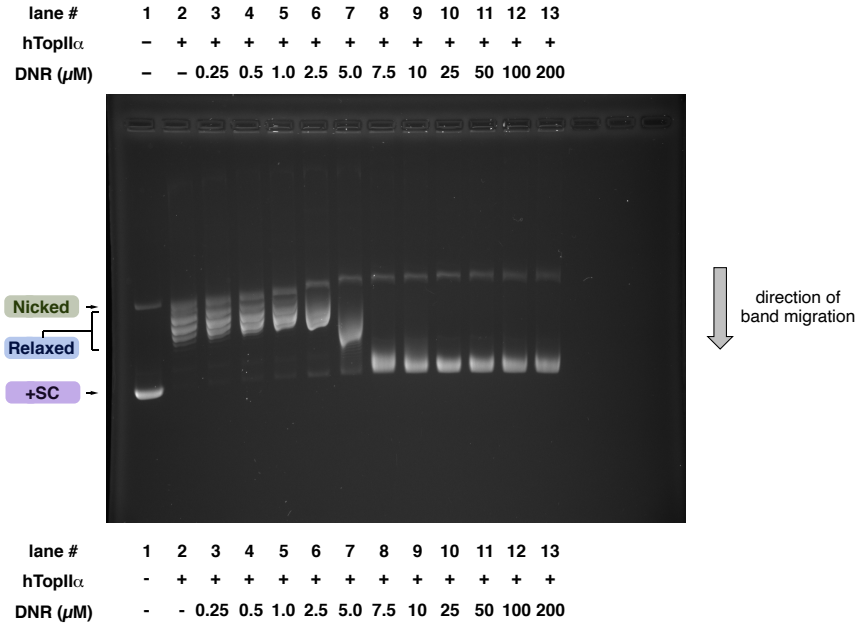


*C.4.4 Inhibition of hTopIIα Mediated Relaxation of +SC DNA by Daunorubicin (DNR, **5**•2TFA)*

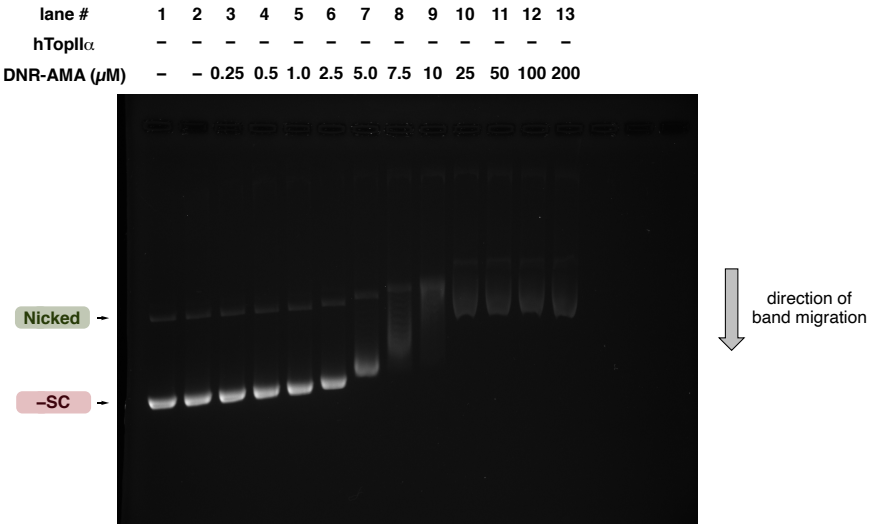
Full Gel from Figure 3.7bi.



C.5 Data from Assay 2

*C.5.1 Effect of DNR-AMA Hybrid (DNR-AMA, **24**•2TFA) on the Electrophoretic Mobility of –SC DNA.*

Full Gel from Figure 3.6aii.



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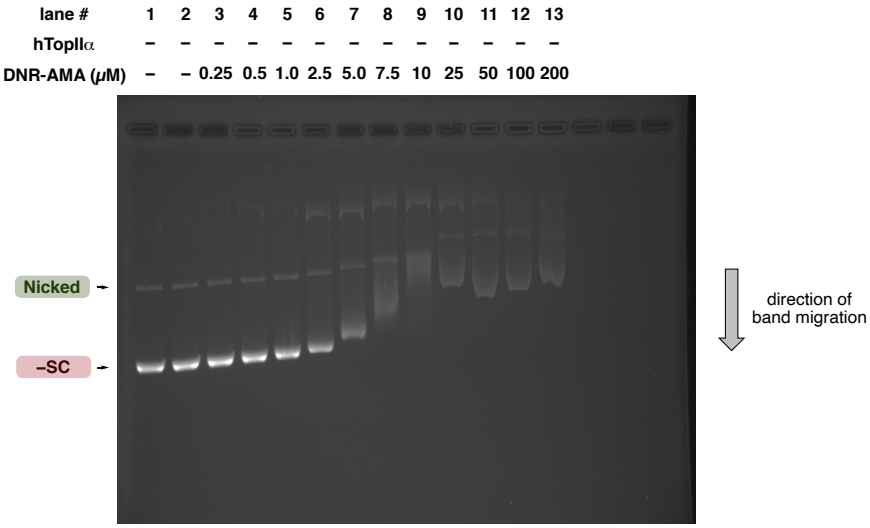
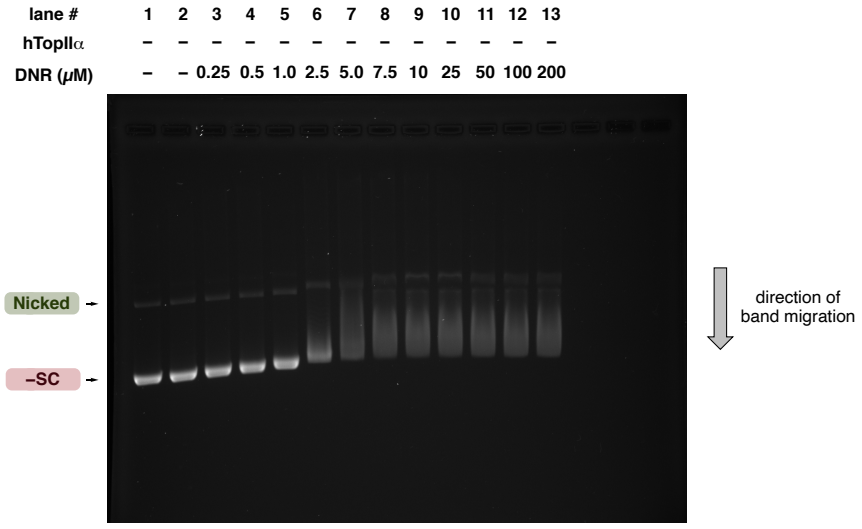


C.5.2 *Effect of Daunorubicin (DNR, **5**•HCl) on the Electrophoretic Mobility of –SC DNA.*

Full Gel from Figure 3.6bii.



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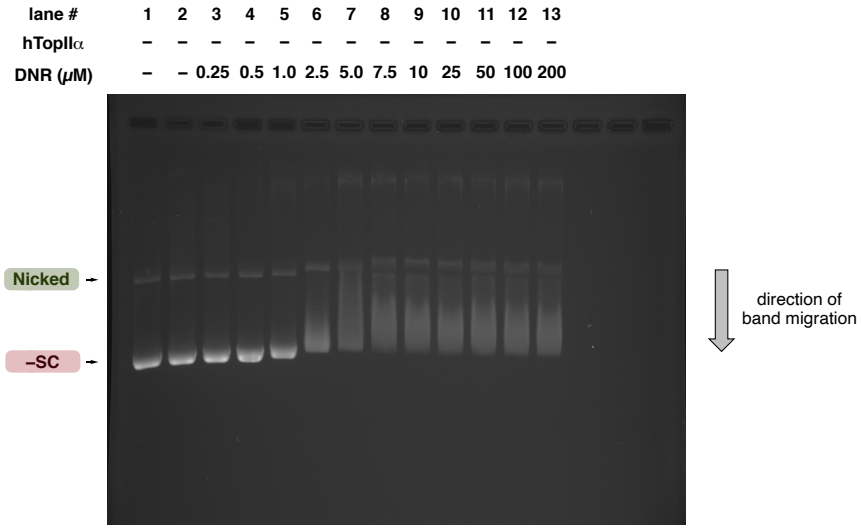
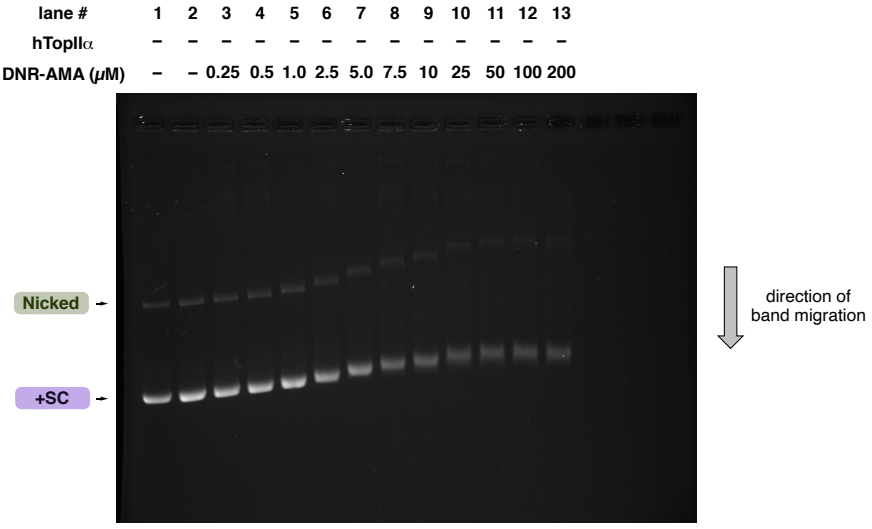
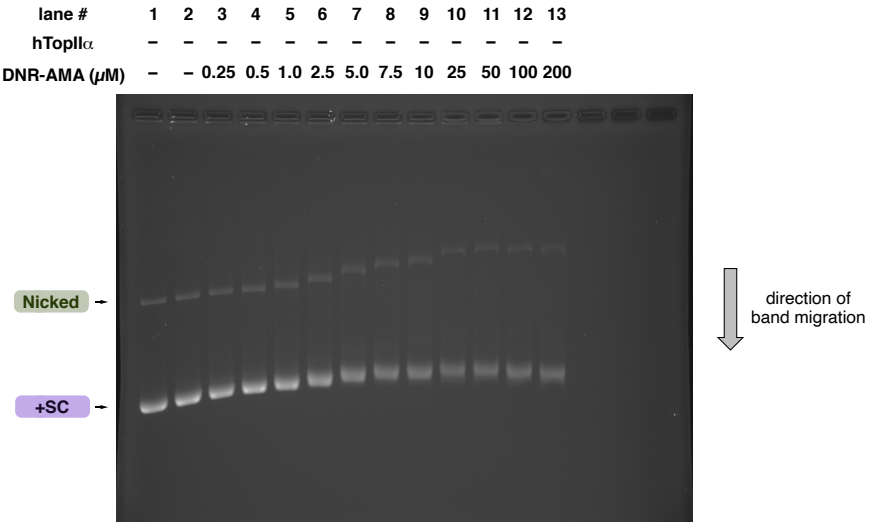


C.5.3 *Effect of DNR-AMA Hybrid (DNR-AMA, **24**•2TFA) on the Electrophoretic Mobility of +SC DNA.*

Full Gel from Figure 3.7aii.

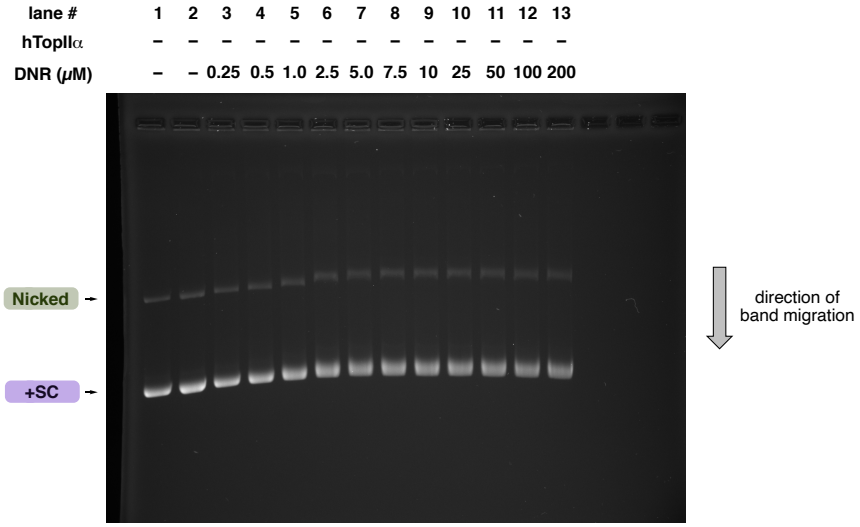


Replicate.

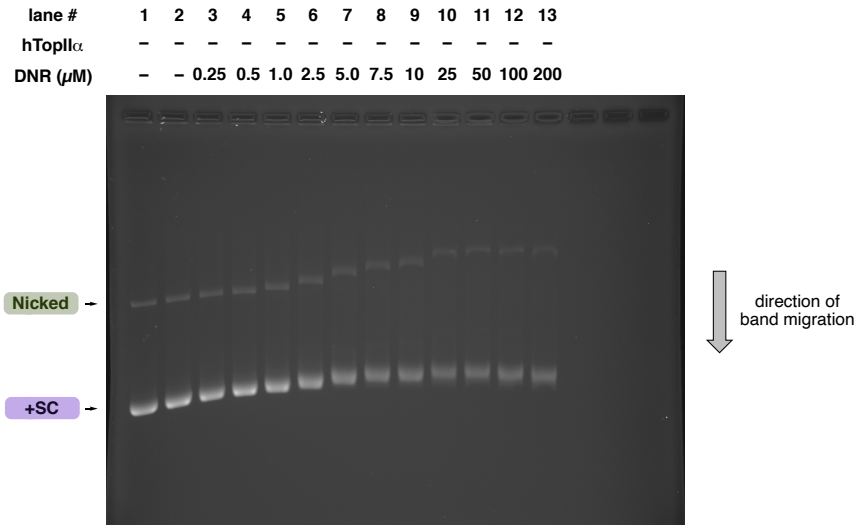


C.5.4 *Effect of Daunorubicin (DNR, **5**•HCl) on the Electrophoretic Mobility of +SC DNA.*

Full Gel from Figure 3.7bii.



Replicate.



C.6 *References for Appendix C*

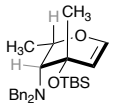
(1) Worland, S. T.; Wang, J. C. Inducible Overexpression,Purification, and Active Site Mapping of DNA Topoisomerasei1 from the Yeast *Saccharomyces Cereuisiae*. *J. Biol. Chem.* **1989**, *264*, 4412-4416.
(2) Wasserman, R. A.; Austin, C. A.; Fisher, M.; Wang, J. C. Use of Yeast in the Study of Anticancer Drugs Targeting DNA Topoisomerases:
Expression of a Functional Recombinant Human DNA Topoisomerase Ila in Yeas. *Cancer Res.* **1993**, *53*, 3591-3596.
(3) Kingma, P. S.; Greider, C. A.; Osheroff, N. Spontaneous DNA Lesions Poison Human Topoisomerase Iialpha and Stimulate Cleavage Proximal to Leukemic 11q23 Chromosomal Breakpoints. *Biochemistry* **1997**, *36*, 5934-9.
(4) Rodriguez, A. C. Studies of a Positive Supercoiling Machine. Nucleotide Hydrolysis and a Multifunctional "Latch" in the Mechanism of Reverse Gyrase. *J. Biol. Chem.* **2002**, *277*, 29865-73.
(5) McClendon, A. K.; Rodriguez, A. C.; Osheroff, N. Human Topoisomerase Iialpha Rapidly Relaxes Positively Supercoiled DNA: Implications for Enzyme Action Ahead of Replication Forks. *J. Biol. Chem.* **2005**, *280*, 39337-45.
(6) Fortune, J. M.; Osheroff, N. Merbarone Inhibits the Catalytic Activity of Human Topoisomerase Iialpha by Blocking DNA Cleavage. *J. Biol. Chem.* **1998**, *273*, 17643-50.

APPENDIX D

Crystallographic Data

D.1 *Crystallographic Data for Compound* ***225***

A suitable crystal was selected for analysis and mounted in a polyimide loop. All measurements were made on a Rigaku Oxford Diffraction Supernova EosS2 CCD with filtered Cu-Kα radiation at a temperature of 100.00(10) K. Using Olex2[1], the structure was solved with the ShelXT structure solution program using Direct Methods and refined with the ShelXL refinement package using Least Squares minimization.[2]



225

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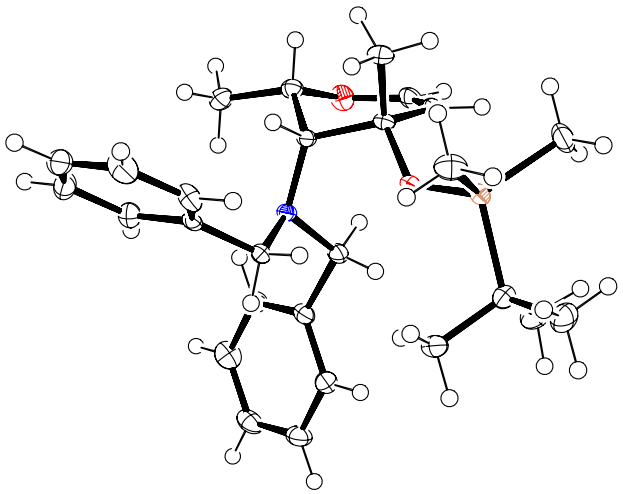
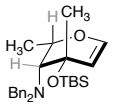


ORTEP diagram of **225** shown at 50% probability.

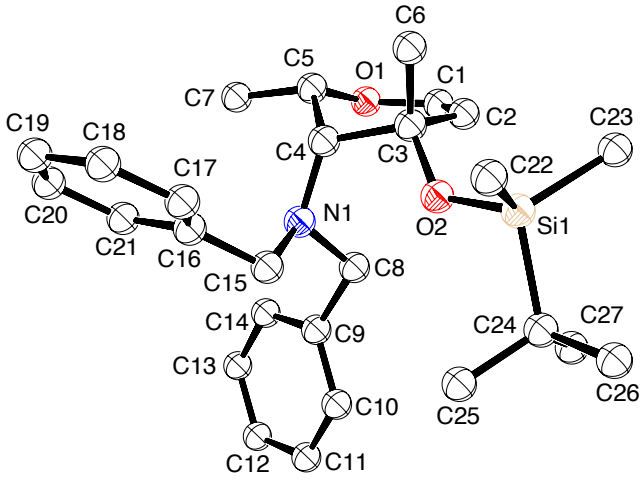
XRD Table D.1 Crystal data and structure refinement for compound **225**.



225

Parameter	Value
Empirical formula	$C_{27}H_{39}NO_2Si$
Formula weight	437.68
Temperature	100.00(10) K
Wavelength	1.54184 Å
Crystal system	Monoclinic
Space group	$P12_11$
Unit cell dimensions	a = 8.06293(9) Å b = 15.17197(14) Å c = 11.20793(14) Å α = 90° β = 110.8510(14)° γ = 90°
Volume	1281.28(3) $Å^3$
Z	2
Density (calculated)	1.134 Mg/m^3
Absorption coefficient	0.968 mm^{-1}
F(000)	476
Crystal size	0.179 x 0.132 x 0.09 mm^3
Theta range for data collection	4.221 to 73.437°.
Index ranges	-9<=h<=10, -18<=k<=18, -13<=l<=13
Reflections collected	14532
Independent reflections	5014 [R(int) = 0.0216]
Completeness to theta = 67.684°	100.0 %
Absorption correction	Gaussian
Max. and min. transmission	1.000 and 0.804
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	5014 / 1 / 287
Goodness-of-fit on F^2	1.051
Final R indices [I>2sigma(I)]	R1 = 0.0246, wR2 = 0.0643
R indices (all data)	R1 = 0.0249, wR2 = 0.0646
Absolute structure parameter	0.011(9)
Extinction coefficient	n/a
Largest diff. peak and hole	0.204 and -0.175 $e/Å^{-3}$

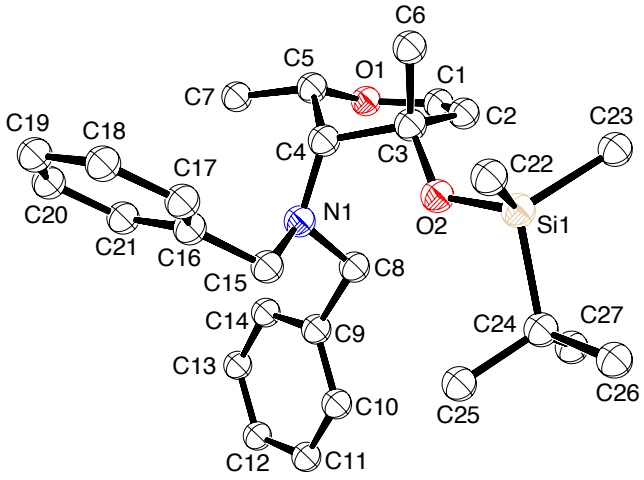
XRD Table D.2 Atomic Coordinates (x 10^5) and Equivalent Isotropic Displacement Parameters ($Å^2$x 10^4) for Compound **225**.U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor.



225

Atom	x	y	z	U(eq)
Si(1)	59883(5)	54992(3)	59602(4)	154(1)
O(1)	42606(16)	44653(8)	9076(12)	209(3)
O(2)	46783(14)	53195(7)	44679(11)	151(2)
N(1)	16576(17)	49254(9)	23168(13)	142(3)
C(1)	57340(20)	43388(12)	19781(17)	198(3)
C(2)	61010(20)	47803(11)	30592(17)	180(3)
C(3)	49330(19)	54892(11)	32803(14)	144(3)
C(4)	30757(18)	54721(11)	21726(14)	136(3)
C(5)	33600(20)	52912(10)	9076(16)	174(3)
C(6)	57490(20)	64044(11)	33205(17)	192(3)
C(7)	16640(20)	52703(12)	-2434(16)	212(4)
C(8)	21300(20)	40165(11)	27521(17)	183(3)
C(9)	5250(20)	34150(11)	23997(17)	174(3)
C(10)	980(20)	29781(12)	33413(18)	214(3)
C(11)	-13050(20)	23798(12)	30210(20)	251(4)
C(12)	-23040(20)	22159(12)	17530(20)	246(4)
C(13)	-18970(30)	26533(13)	8039(19)	257(4)
C(14)	-4920(20)	32436(12)	11271(18)	219(4)
C(15)	6920(20)	53667(11)	30439(15)	158(3)
C(16)	-2270(20)	62004(11)	24132(15)	147(3)
C(17)	1780(20)	70076(12)	30341(18)	223(4)
C(18)	-7000(30)	77735(12)	24600(20)	292(4)
C(19)	-19840(30)	77415(13)	12510(20)	278(4)
C(20)	-23840(20)	69400(13)	6146(17)	247(4)
C(21)	-15210(20)	61753(12)	11950(16)	193(3)
C(22)	57810(30)	66654(12)	64330(19)	253(4)
C(23)	83830(20)	52719(14)	62520(18)	274(4)
C(24)	51710(20)	47176(11)	69443(16)	179(3)
C(25)	31650(20)	48190(12)	66348(17)	228(4)
C(26)	61270(30)	49133(14)	83740(17)	278(4)
C(27)	55480(30)	37574(12)	66890(20)	286(4)

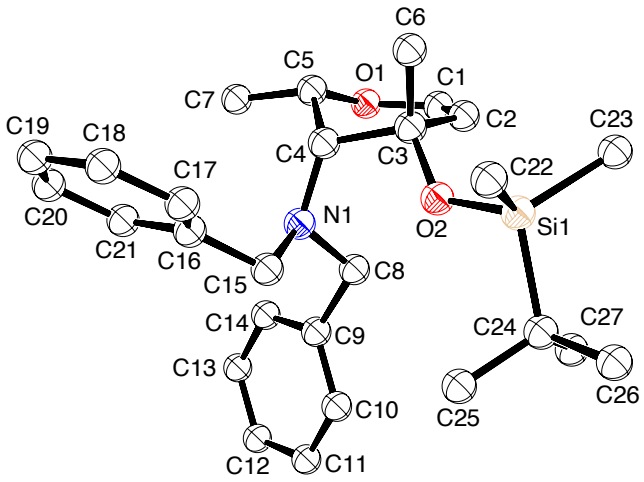
XRD Table D.3 Bond Lengths for Compound **225**.



225

Bond	Length (Å)	Bond	Length (Å)
Si(1)-O(2)	1.6522(12)	C(12)-C(13)	1.388(3)
Si(1)-C(22)	1.8715(19)	C(13)-H(13)	0.9300
Si(1)-C(23)	1.8719(18)	C(13)-C(14)	1.387(3)
Si(1)-C(24)	1.8908(17)	C(14)-H(14)	0.9300
O(1)-C(1)	1.369(2)	C(15)-H(15A)	0.9700
O(1)-C(5)	1.4483(19)	C(15)-H(15B)	0.9700
O(2)-C(3)	1.4404(18)	C(15)-C(16)	1.508(2)
N(1)-C(4)	1.4673(19)	C(16)-C(17)	1.389(2)
N(1)-C(8)	1.467(2)	C(16)-C(21)	1.393(2)
N(1)-C(15)	1.4735(19)	C(17)-H(17)	0.9300
C(1)-H(1)	0.9300	C(17)-C(18)	1.393(3)
C(1)-C(2)	1.323(3)	C(18)-H(18)	0.9300
C(2)-H(2)	0.9300	C(18)-C(19)	1.383(3)
C(2)-C(3)	1.507(2)	C(19)-H(19)	0.9300
C(3)-C(4)	1.569(2)	C(19)-C(20)	1.388(3)
C(3)-C(6)	1.530(2)	C(20)-H(20)	0.9300
C(4)-H(4)	0.9800	C(20)-C(21)	1.390(2)
C(4)-C(5)	1.539(2)	C(21)-H(21)	0.9300
C(5)-H(5)	0.9800	C(22)-H(22A)	0.9600
C(5)-C(7)	1.510(2)	C(22)-H(22B)	0.9600
C(6)-H(6A)	0.9600	C(22)-H(22C)	0.9600
C(6)-H(6B)	0.9600	C(23)-H(23A)	0.9600
C(6)-H(6C)	0.9600	C(23)-H(23B)	0.9600
C(7)-H(7A)	0.9600	C(23)-H(23C)	0.9600
C(7)-H(7B)	0.9600	C(24)-C(25)	1.536(2)
C(7)-H(7C)	0.9600	C(24)-C(26)	1.540(2)
C(8)-H(8A)	0.9700	C(24)-C(27)	1.536(2)
C(8)-H(8B)	0.9700	C(25)-H(25A)	0.9600
C(8)-C(9)	1.516(2)	C(25)-H(25B)	0.9600
C(9)-C(10)	1.390(2)	C(25)-H(25C)	0.9600
C(9)-C(14)	1.394(3)	C(26)-H(26A)	0.9600
C(10)-H(10)	0.9300	C(26)-H(26B)	0.9600
C(10)-C(11)	1.394(3)	C(26)-H(26C)	0.9600
C(11)-H(11)	0.9300	C(27)-H(27A)	0.9600
C(11)-C(12)	1.384(3)	C(27)-H(27B)	0.9600
C(12)-H(12)	0.9300	C(27)-H(27C)	0.9600

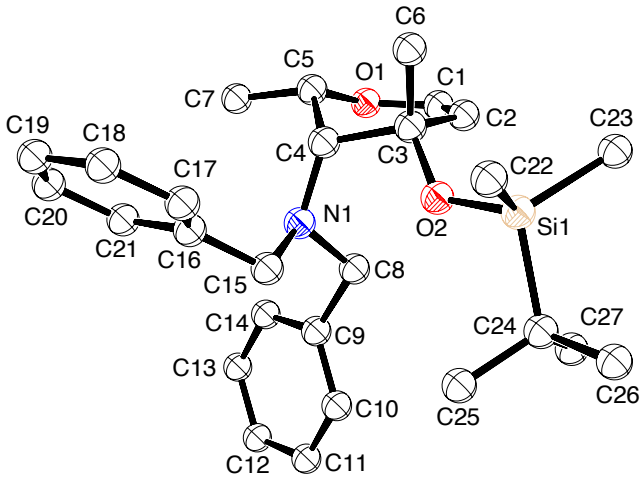
XRD Table D.4 Anisotropic Displacement Parameters ($Å^2 x\ 10^4$) for Compound **225**.The anisotropic displacement factor exponent takes the form: $-2\pi^2[\ h^2\ a^{*2}U^{11} + ...\ + 2\ h\ k\ a^*\ b^*\ U^{12}\ ]$



225

Atom	U^{11}	U^{22}	U^{33}	U^{23}	U^{13}	U^{12}
Si(1)	127(2)	162(2)	172(2)	-11(2)	48(2)	-12(2)
O(1)	217(6)	208(6)	216(6)	-43(5)	93(5)	29(5)
O(2)	123(5)	176(6)	164(5)	6(4)	62(4)	-12(4)
N(1)	131(6)	125(6)	188(7)	22(5)	79(5)	5(5)
C(1)	159(7)	205(8)	268(9)	3(7)	121(7)	30(6)
C(2)	131(7)	185(8)	238(8)	23(6)	86(6)	13(6)
C(3)	140(6)	152(7)	167(7)	-2(6)	85(5)	-13(6)
C(4)	131(6)	120(7)	172(7)	17(6)	74(5)	3(6)
C(5)	178(7)	178(8)	192(8)	10(6)	98(6)	5(6)
C(6)	201(8)	168(8)	231(8)	-5(6)	107(7)	-48(6)
C(7)	233(8)	242(9)	168(8)	27(6)	78(6)	-4(6)
C(8)	143(7)	139(8)	269(8)	40(6)	77(6)	4(6)
C(9)	145(7)	116(7)	275(9)	7(6)	92(6)	9(6)
C(10)	188(8)	201(8)	250(9)	42(7)	75(7)	5(6)
C(11)	226(8)	200(9)	371(11)	56(7)	159(8)	-11(7)
C(12)	193(8)	157(8)	407(11)	-33(7)	127(8)	-32(6)
C(13)	255(9)	221(9)	281(10)	-88(7)	76(7)	-35(7)
C(14)	245(9)	189(8)	253(9)	-19(6)	126(7)	-23(7)
C(15)	133(6)	181(8)	177(7)	25(6)	76(6)	23(6)
C(16)	116(7)	169(8)	181(8)	2(6)	84(6)	3(6)
C(17)	147(8)	224(9)	284(9)	-50(7)	59(7)	-6(6)
C(18)	254(9)	160(9)	473(12)	-74(8)	144(9)	-7(7)
C(19)	255(9)	200(9)	426(11)	99(8)	180(9)	80(7)
C(20)	237(9)	299(10)	201(8)	56(7)	75(7)	72(7)
C(21)	197(8)	204(8)	182(8)	-20(6)	72(6)	26(6)
C(22)	310(9)	192(9)	281(9)	-56(7)	136(8)	-73(7)
C(23)	139(7)	392(11)	266(9)	5(7)	41(6)	11(7)
C(24)	200(8)	166(8)	162(8)	3(6)	53(6)	-6(6)
C(25)	225(9)	257(9)	218(8)	33(7)	100(7)	-30(7)
C(26)	305(9)	310(10)	182(9)	14(7)	41(7)	-29(7)
C(27)	395(11)	172(9)	304(10)	21(7)	140(9)	29(7)

XRD Table D.5 Hydrogen Coordinates (x 10^4) and Isotropic Displacement Parameters ($Å^2$x 10^3) for Compound **225**.

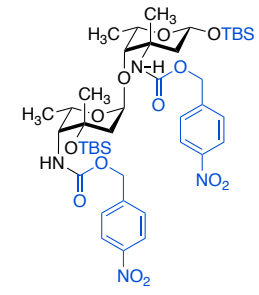


225

Atom	x	y	z	U(eq)
H(1)	6539	3910	1943	24
H(2)	7148	4644	3726	22
H(4)	2634	6079	2104	16
H(5)	4102	5766	778	21
H(6A)	5037	6834	3546	29
H(6B)	5791	6544	2496	29
H(6C)	6929	6410	3944	29
H(7A)	1941	5193	-1002	32
H(7B)	1035	5815	-298	32
H(7C)	936	4790	-162	32
H(8A)	2723	4015	3672	22
H(8B)	2957	3789	2376	22
H(10)	756	3086	4196	26
H(11)	-1571	2090	3662	30
H(12)	-3239	1816	1538	30
H(13)	-2568	2550	-50	31
H(14)	-225	3530	484	26
H(15A)	1520	5508	3890	19
H(15B)	-186	4964	3141	19
H(17)	1047	7037	3845	27
H(18)	-422	8308	2891	35
H(19)	-2575	8252	868	33
H(20)	-3233	6915	-204	30
H(21)	-1811	5641	765	23
H(22A)	6189	7057	5924	38
H(22B)	6486	6746	7319	38
H(22C)	4561	6791	6299	38
H(23A)	8523	4665	6064	41
H(23B)	9077	5390	7130	41
H(23C)	8776	5644	5712	41
H(25A)	2896	5425	6736	34
H(25B)	2808	4458	7205	34
H(25C)	2538	4637	5770	34
H(26A)	7386	4864	8583	42
H(26B)	5749	4498	8872	42
H(26C)	5842	5500	8559	42
H(27A)	5008	3634	5793	43
H(27B)	5064	3370	7159	43
H(27C)	6806	3668	6956	43

D.2 *Crystallographic Data for Compound* ***244***

A suitable crystal was selected for analysis and mounted in a polyimide loop. All measurements were made on a Rigaku Oxford Diffraction Supernova EosS2 CCD with filtered Cu-Kα radiation at a temperature of 100.00(10) K. Using Olex2[1], the structure was solved with the ShelXT structure solution program using Direct Methods and refined with the ShelXL refinement package using Least Squares minimization.[2]



244
(protecting groups denoted in blue)

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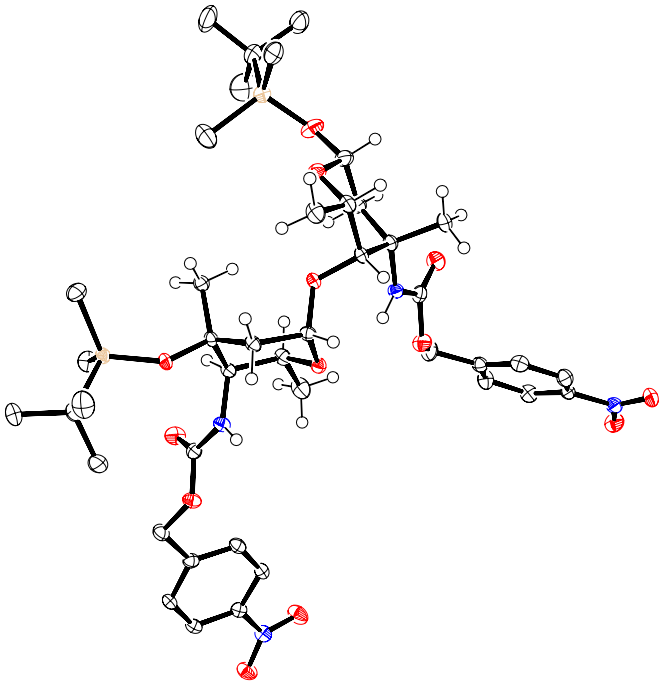
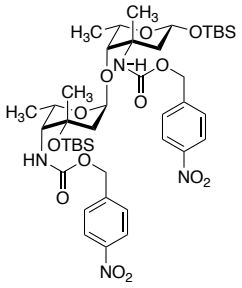


ORTEP diagram of **244** shown at 50% probability.
(protecting group hydrogens omitted for clarity)

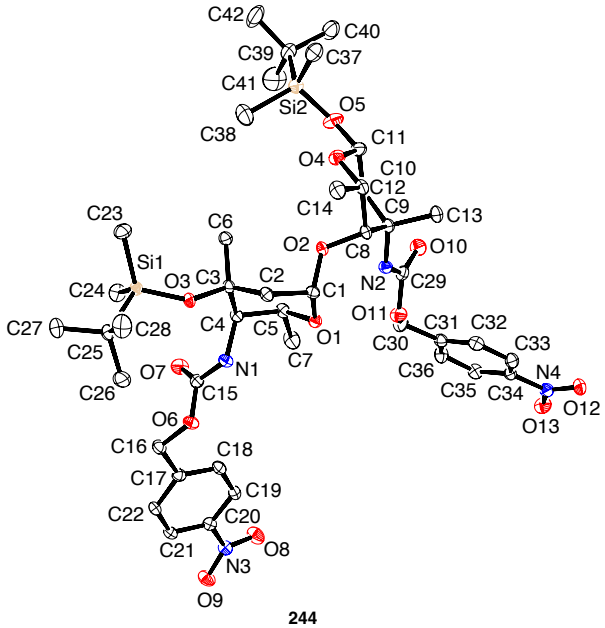
XRD Table D.6 Crystal data and structure refinement for compound **244**.



244

Empirical formula	$C_{42}H_{66}N_4O_{13}Si_2$
Formula weight	891.16
Temperature	100.00(10) K
Wavelength	1.54184 Å
Crystal system	Monoclinic
Space group	$P12_11$
Unit cell dimensions	a = 8.02112(11) Å
	b = 11.95004(14) Å
	c = 24.8127(4) Å
	α = 90°
	β = 94.4773(13)°
	γ = 90°
Volume	2371.10(5) $Å^3$
Z	2
Density (calculated)	1.248 Mg/m^3
Absorption coefficient	1.215 mm^{-1}
F(000)	956
Crystal size	0.386 x 0.215 x 0.026 mm^3
Theta range for data collection	3.574 to 73.445°.
Index ranges	-8<=h<=9, -14<=k<=14, -30<=l<=30
Reflections collected	24066
Independent reflections	9230 [R(int) = 0.0275]
Completeness to theta = 67.684°	100.0 %
Absorption correction	Gaussian
Max. and min. transmission	1.000 and 0.494
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	9230 / 1 / 564
Goodness-of-fit on F^2	1.026
Final R indices [I>2sigma(I)]	R1 = 0.0321, wR2 = 0.0812
R indices (all data)	R1 = 0.0338, wR2 = 0.0822
Absolute structure parameter	0.014(16)
Extinction coefficient	n/a
Largest diff. peak and hole	0.395 and -0.208 $e/Å^{-3}$

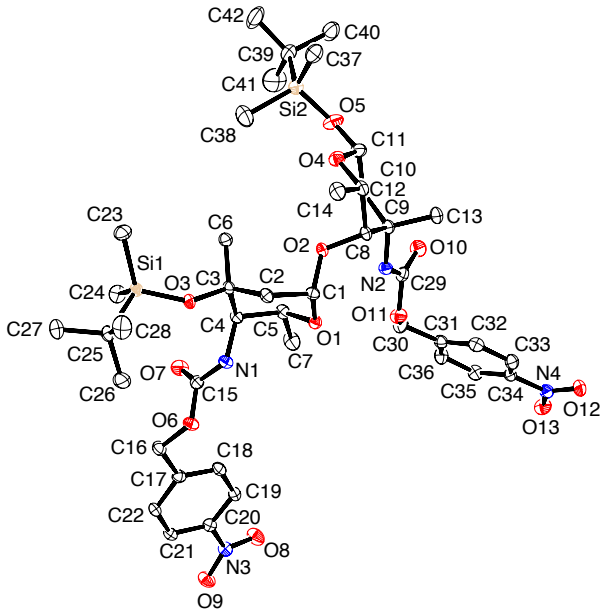
XRD Table D.7 Atomic Coordinates (x 10^5) and Equivalent Isotropic Displacement Parameters ($Å^2$x 10^4) for Compound **244**.U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor.



244

	x	y	z	U(eq)		x	y	z	U(eq)
Si(1)	107629(8)	44034(5)	84568(2)	169(1)	C(13)	46150(30)	106490(20)	69409(11)	228(5)
Si(2)	47602(9)	95108(6)	92491(3)	263(2)	C(14)	92650(30)	100160(20)	80089(11)	254(5)
O(1)	73340(20)	69281(14)	67852(7)	187(3)	C(15)	83100(30)	34760(20)	70160(9)	179(5)
O(2)	73700(20)	82548(13)	74756(6)	161(3)	C(16)	93120(30)	18070(20)	66293(10)	219(5)
O(3)	103280(20)	53006(14)	79607(7)	183(3)	C(17)	82230(30)	14090(20)	61423(9)	187(5)
O(4)	65060(20)	97730(15)	82660(7)	229(4)	C(18)	70090(30)	20890(20)	58817(10)	200(5)
O(5)	39680(20)	96068(19)	86137(7)	305(4)	C(19)	59990(30)	16820(20)	54488(10)	193(5)
O(6)	93540(20)	30024(14)	66684(7)	209(4)	C(20)	62120(30)	5840(20)	52908(10)	187(5)
O(7)	73020(20)	29559(16)	72505(8)	260(4)	C(21)	74160(30)	-1120(20)	55377(10)	197(5)
O(8)	38500(20)	6909(18)	46919(9)	311(5)	C(22)	84390(30)	3150(20)	59639(10)	192(5)
O(9)	54050(20)	-7719(16)	46527(8)	279(4)	C(23)	111230(40)	51350(20)	91197(11)	268(5)
O(10)	17760(20)	89865(16)	66096(8)	256(4)	C(24)	91140(30)	33220(20)	85116(11)	258(5)
O(11)	31170(20)	77780(17)	60833(7)	256(4)	C(25)	127660(30)	37470(20)	82641(10)	216(5)
O(12)	-14160(30)	97377(16)	37487(8)	291(4)	C(26)	125410(40)	32290(30)	77009(12)	363(7)
O(13)	-28280(20)	81835(18)	36963(8)	290(4)	C(27)	133550(40)	28460(30)	86729(12)	322(6)
N(1)	85880(20)	45937(17)	70379(8)	185(4)	C(28)	141300(40)	46550(30)	82644(13)	362(7)
N(2)	45960(30)	86501(18)	67445(8)	184(4)	C(29)	30600(30)	85300(20)	64993(10)	207(5)
N(3)	50810(30)	1386(18)	48466(8)	208(4)	C(30)	15600(30)	72600(20)	59046(11)	267(6)
N(4)	-17720(30)	88030(20)	39187(9)	234(5)	C(31)	7530(30)	77210(20)	53834(10)	199(5)
C(1)	83620(30)	76340(20)	71336(9)	167(5)	C(32)	12930(30)	86650(20)	51288(10)	214(5)
C(2)	96620(30)	69486(19)	74704(10)	176(5)	C(33)	4850(30)	90220(20)	46428(11)	221(5)
C(3)	89320(30)	59810(20)	77861(9)	161(4)	C(34)	-8690(30)	84220(20)	44233(10)	195(5)
C(4)	77130(30)	53030(20)	73957(9)	169(5)	C(35)	-14270(30)	74610(20)	46645(11)	231(5)
C(5)	64700(30)	60850(20)	70789(10)	193(5)	C(36)	-6110(30)	71280(20)	51485(11)	241(5)
C(6)	80430(30)	63990(20)	82700(9)	189(5)	C(37)	60160(40)	107600(30)	94645(12)	344(7)
C(7)	53260(40)	54660(20)	66662(12)	289(6)	C(38)	60780(50)	82330(30)	93441(15)	495(9)
C(8)	69240(30)	93770(20)	73279(9)	164(4)	C(39)	28210(40)	93890(30)	96321(11)	324(6)
C(9)	50170(30)	94760(20)	71732(9)	176(4)	C(40)	17100(40)	104300(30)	95332(13)	390(7)
C(10)	40980(30)	92190(20)	76786(9)	191(5)	C(41)	18200(50)	83540(40)	94533(17)	547(10)
C(11)	47490(30)	99340(20)	81564(10)	229(5)	C(42)	33380(50)	93140(40)	102436(12)	510(10)
C(12)	74220(30)	101100(20)	78211(10)	200(5)					

XRD Table D.8 Bond Lengths for Compound **244**.



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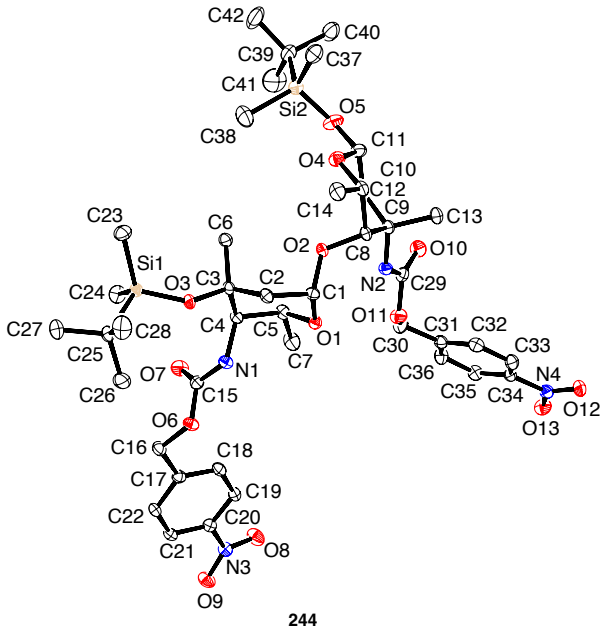
Bond	Length (Å)	Bond	Length (Å)	Bond	Length (Å)	Bond	Length (Å)
Si(1)-O(3)	1.6490(17)	N(4)-C(34)	1.469(3)	C(14)-H(14B)	0.9600	C(30)-H(30A)	0.9700
Si(1)-C(23)	1.865(3)	C(1)-H(1A)	0.9800	C(14)-H(14C)	0.9600	C(30)-H(30B)	0.9700
Si(1)-C(24)	1.862(3)	C(1)-C(2)	1.523(3)	C(16)-H(16A)	0.9700	C(30)-C(31)	1.505(3)
Si(1)-C(25)	1.883(3)	C(2)-H(2A)	0.9700	C(16)-H(16B)	0.9700	C(31)-C(32)	1.379(4)
Si(2)-O(5)	1.6574(19)	C(2)-H(2B)	0.9700	C(16)-C(17)	1.512(3)	C(31)-C(36)	1.393(3)
Si(2)-C(37)	1.856(3)	C(2)-C(3)	1.538(3)	C(17)-C(18)	1.389(3)	C(32)-H(32)	0.9300
Si(2)-C(38)	1.861(3)	C(3)-C(4)	1.550(3)	C(17)-C(22)	1.396(3)	C(32)-C(33)	1.391(4)
Si(2)-C(39)	1.891(3)	C(3)-C(6)	1.528(3)	C(18)-H(18)	0.9300	C(33)-H(33)	0.9300
O(1)-C(1)	1.424(3)	C(4)-H(4)	0.9800	C(18)-C(19)	1.382(3)	C(33)-C(34)	1.378(4)
O(1)-C(5)	1.451(3)	C(4)-C(5)	1.537(3)	C(19)-H(19)	0.9300	C(34)-C(35)	1.385(4)
O(2)-C(1)	1.417(3)	C(5)-H(5)	0.9800	C(19)-C(20)	1.383(3)	C(35)-H(35)	0.9300
O(2)-C(8)	1.429(3)	C(5)-C(7)	1.514(3)	C(20)-C(21)	1.382(3)	C(35)-C(36)	1.381(4)
O(3)-C(3)	1.423(3)	C(6)-H(6A)	0.9600	C(21)-H(21)	0.9300	C(36)-H(36)	0.9300
O(4)-C(11)	1.427(3)	C(6)-H(6B)	0.9600	C(21)-C(22)	1.384(3)	C(37)-H(37A)	0.9600
O(4)-C(12)	1.430(3)	C(6)-H(6C)	0.9600	C(22)-H(22)	0.9300	C(37)-H(37B)	0.9600
O(5)-C(11)	1.394(3)	C(7)-H(7A)	0.9600	C(23)-H(23A)	0.9600	C(37)-H(37C)	0.9600
O(6)-C(15)	1.371(3)	C(7)-H(7B)	0.9600	C(23)-H(23B)	0.9600	C(38)-H(38A)	0.9600
O(6)-C(16)	1.432(3)	C(7)-H(7C)	0.9600	C(23)-H(23C)	0.9600	C(38)-H(38B)	0.9600
O(7)-C(15)	1.205(3)	C(8)-H(8)	0.9800	C(24)-H(24A)	0.9600	C(38)-H(38C)	0.9600
O(8)-N(3)	1.224(3)	C(8)-C(9)	1.552(3)	C(24)-H(24B)	0.9600	C(39)-C(40)	1.538(4)
O(9)-N(3)	1.226(3)	C(8)-C(12)	1.532(3)	C(24)-H(24C)	0.9600	C(39)-C(41)	1.522(5)
O(10)-C(29)	1.216(3)	C(9)-C(10)	1.535(3)	C(25)-C(26)	1.526(4)	C(39)-C(42)	1.544(4)
O(11)-C(29)	1.372(3)	C(9)-C(13)	1.540(3)	C(25)-C(27)	1.528(3)	C(40)-H(40A)	0.9600
O(11)-C(30)	1.433(3)	C(10)-H(10A)	0.9700	C(25)-C(28)	1.542(4)	C(40)-H(40B)	0.9600
O(12)-N(4)	1.235(3)	C(10)-H(10B)	0.9700	C(26)-H(26A)	0.9600	C(40)-H(40C)	0.9600
O(13)-N(4)	1.224(3)	C(10)-C(11)	1.520(3)	C(26)-H(26B)	0.9600	C(41)-H(41A)	0.9600
N(1)-H(1)	0.8600	C(11)-H(11)	0.9800	C(26)-H(26C)	0.9600	C(41)-H(41B)	0.9600
N(1)-C(4)	1.448(3)	C(12)-H(12)	0.9800	C(27)-H(27A)	0.9600	C(41)-H(41C)	0.9600
N(1)-C(15)	1.354(3)	C(12)-C(14)	1.519(3)	C(27)-H(27B)	0.9600	C(42)-H(42A)	0.9600
N(2)-H(2)	0.8600	C(13)-H(13A)	0.9600	C(27)-H(27C)	0.9600	C(42)-H(42B)	0.9600
N(2)-C(9)	1.471(3)	C(13)-H(13B)	0.9600	C(28)-H(28A)	0.9600	C(42)-H(42C)	0.9600
N(2)-C(29)	1.339(3)	C(13)-H(13C)	0.9600	C(28)-H(28B)	0.9600		
N(3)-C(20)	1.472(3)	C(14)-H(14A)	0.9600	C(28)-H(28C)	0.9600		

XRD Table D.9 Anisotropic Displacement Parameters ($Å^2 x\ 10^4$) for Compound **244**.The anisotropic displacement factor exponent takes the form: $-2\pi^2[\ h^2\ a^{*2}U^{11} + ... + 2\ h\ k\ a^*\ b^*\ U^{12}\ ]$



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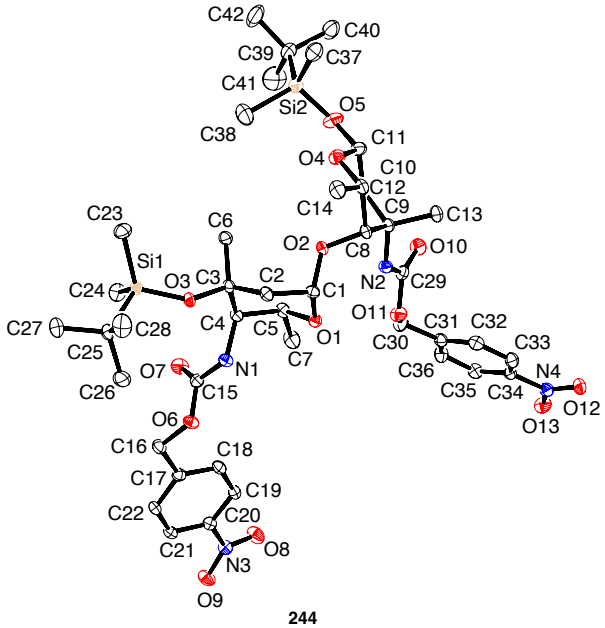
Atom	U^{11}	U^{22}	U^{33}	U^{23}	U^{13}	U^{12}	Atom	U^{11}	U^{22}	U^{33}	U^{23}	U^{13}	U^{12}
Si(1)	165(3)	166(3)	172(3)	-1(2)	-13(2)	8(2)	C(13)	208(12)	210(12)	259(13)	40(9)	-15(10)	63(9)
Si(2)	324(4)	262(3)	202(3)	-5(3)	16(3)	78(3)	C(14)	217(13)	235(13)	294(13)	-16(10)	-74(10)	-6(10)
O(1)	186(9)	187(8)	183(8)	-11(6)	-17(6)	22(6)	C(15)	161(11)	195(12)	175(11)	-19(9)	-29(9)	-10(9)
O(2)	151(8)	142(8)	188(8)	13(6)	6(6)	29(6)	C(16)	259(13)	161(11)	231(12)	-23(9)	-17(10)	19(9)
O(3)	166(8)	186(8)	194(8)	26(7)	-5(6)	59(7)	C(17)	191(12)	187(11)	187(11)	-9(9)	45(9)	-10(9)
O(4)	208(9)	274(9)	201(8)	-20(7)	-10(7)	-1(7)	C(18)	217(12)	138(11)	248(12)	-15(9)	30(10)	4(9)
O(5)	286(10)	437(12)	199(8)	-46(8)	57(7)	-24(9)	C(19)	177(12)	171(11)	232(12)	19(9)	22(9)	18(9)
O(6)	234(9)	159(8)	234(8)	-51(6)	25(7)	-14(7)	C(20)	173(12)	185(11)	204(11)	-8(9)	26(9)	-13(9)
O(7)	276(10)	215(9)	296(9)	-26(7)	71(8)	-61(7)	C(21)	202(12)	155(11)	237(12)	-24(9)	33(9)	12(9)
O(8)	198(9)	328(11)	392(11)	-69(9)	-70(8)	60(8)	C(22)	180(12)	173(11)	223(11)	4(9)	15(9)	54(9)
O(9)	270(10)	219(10)	340(10)	-99(8)	-34(8)	5(7)	C(23)	289(14)	300(14)	209(12)	-49(10)	-25(10)	4(11)
O(10)	154(9)	324(10)	288(9)	2(8)	-6(7)	51(7)	C(24)	228(13)	254(13)	291(13)	19(11)	13(10)	-56(10)
O(11)	200(9)	343(10)	213(8)	-49(8)	-57(7)	40(8)	C(25)	177(12)	246(12)	221(11)	26(10)	-12(9)	73(10)
O(12)	332(11)	248(10)	294(9)	65(8)	32(8)	102(8)	C(26)	359(16)	455(18)	269(14)	-40(13)	-10(12)	222(14)
O(13)	224(10)	386(11)	247(9)	-21(8)	-56(7)	14(8)	C(27)	325(15)	314(15)	319(14)	59(12)	-28(12)	124(12)
N(1)	189(10)	161(10)	208(9)	-20(8)	31(7)	2(8)	C(28)	203(13)	431(18)	455(17)	110(14)	53(12)	0(12)
N(2)	154(10)	218(10)	177(9)	-19(8)	-5(8)	48(8)	C(29)	208(13)	230(12)	180(11)	32(9)	-12(9)	15(10)
N(3)	174(10)	203(10)	247(10)	-12(8)	8(8)	-25(8)	C(30)	253(14)	281(13)	251(13)	12(10)	-88(11)	-23(11)
N(4)	204(11)	269(11)	227(10)	5(9)	12(9)	77(9)	C(31)	171(12)	216(12)	205(11)	-19(9)	-6(9)	37(9)
C(1)	134(11)	181(11)	187(10)	-13(9)	23(8)	10(9)	C(32)	176(12)	215(12)	247(12)	-44(10)	-8(9)	-30(9)
C(2)	139(11)	164(11)	222(11)	-8(9)	0(9)	8(9)	C(33)	216(13)	194(11)	254(12)	17(9)	20(10)	-15(9)
C(3)	133(11)	171(11)	175(10)	-2(9)	-15(9)	42(9)	C(34)	187(12)	209(12)	188(11)	3(9)	8(9)	55(9)
C(4)	164(11)	150(11)	192(11)	-12(9)	6(9)	14(9)	C(35)	190(12)	229(12)	267(12)	-18(10)	-24(10)	-26(9)
C(5)	144(11)	196(11)	233(12)	-16(9)	-21(9)	7(9)	C(36)	214(13)	216(12)	285(13)	39(10)	-20(10)	-40(10)
C(6)	167(12)	204(11)	194(11)	-6(9)	7(9)	34(9)	C(37)	372(17)	388(16)	254(13)	1(12)	-86(12)	35(13)
C(7)	236(13)	251(13)	359(15)	-40(11)	-115(11)	-4(10)	C(38)	600(20)	401(19)	490(20)	76(16)	95(17)	244(17)
C(8)	141(10)	147(10)	201(10)	18(9)	-2(8)	29(9)	C(39)	409(16)	346(15)	222(12)	56(12)	58(11)	65(13)
C(9)	152(11)	186(11)	188(10)	5(10)	-2(8)	31(9)	C(40)	374(17)	530(20)	276(14)	75(13)	73(12)	155(15)
C(10)	168(11)	210(12)	195(11)	-1(9)	13(9)	17(9)	C(41)	570(20)	520(20)	570(20)	48(18)	175(19)	-151(19)
C(11)	219(13)	276(13)	195(11)	-29(10)	25(9)	32(10)	C(42)	570(20)	720(30)	250(14)	177(17)	85(14)	230(20)
C(12)	195(12)	164(11)	237(12)	5(9)	-11(9)	21(9)							

XRD Table D.10 Hydrogen Coordinates (x 10^4) and Isotropic Displacement Parameters ($Å^2$x 10^3) for Compound **244**.



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Atom	x	y	z	U(eq)	Atom	x	y	z	U(eq)
H(1)	9296	4886	6836	22	H(24A)	8103	3674	8608	39
H(2)	5379	8222	6646	22	H(24B)	9480	2788	8785	39
H(1A)	8946	8160	6911	20	H(24C)	8909	2947	8171	39
H(2A)	10275	7443	7725	21	H(26A)	11730	2638	7699	54
H(2B)	10452	6644	7233	21	H(26B)	13590	2932	7605	54
H(4)	7064	4813	7617	20	H(26C)	12161	3792	7444	54
H(5)	5785	6461	7335	23	H(27A)	13453	3162	9030	48
H(6A)	7629	5771	8461	28	H(27B)	14423	2565	8587	48
H(6B)	7126	6873	8146	28	H(27C)	12559	2245	8659	48
H(6C)	8815	6815	8507	28	H(28A)	13770	5239	8016	54
H(7A)	4529	5979	6496	43	H(28B)	15146	4331	8156	54
H(7B)	4747	4885	6843	43	H(28C)	14323	4963	8621	54
H(7C)	5977	5140	6398	43	H(30A)	791	7348	6184	32
H(8)	7544	9614	7023	20	H(30B)	1748	6465	5859	32
H(10A)	2912	9357	7601	23	H(32)	2203	9066	5283	26
H(10B)	4243	8435	7771	23	H(33)	852	9655	4469	27
H(11)	4514	10725	8079	28	H(35)	-2322	7053	4505	28
H(12)	7157	10892	7732	24	H(36)	-980	6494	5321	29
H(13A)	5203	10762	6623	34	H(37A)	6887	10866	9225	52
H(13B)	4957	11205	7206	34	H(37B)	6504	10652	9826	52
H(13C)	3434	10711	6849	34	H(37C)	5307	11408	9453	52
H(14A)	9508	9267	8131	38	H(38A)	5466	7597	9199	74
H(14B)	9526	10531	8300	38	H(38B)	6371	8120	9723	74
H(14C)	9927	10192	7714	38	H(38C)	7077	8325	9159	74
H(16A)	8891	1501	6954	26	H(40A)	2322	11084	9656	59
H(16B)	10440	1529	6606	26	H(40B)	729	10356	9728	59
H(18)	6875	2820	5999	24	H(40C)	1388	10498	9154	59
H(19)	5196	2136	5268	23	H(41A)	1518	8395	9072	82
H(21)	7537	-845	5421	24	H(41B)	826	8317	9644	82
H(22)	9276	-131	6133	23	H(41C)	2486	7698	9531	82
H(23A)	11919	5728	9089	40	H(42A)	4079	8690	10312	76
H(23B)	11549	4612	9390	40	H(42B)	2360	9213	10438	76
H(23C)	10087	5442	9221	40	H(42C)	3896	9992	10362	76

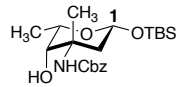
D.3 *References for Appendix D*

(1) Dolomanov, O. V.; Bourhis, L. J.; Gildea, R. J.; Howard, J. A. K.; Puschmann, H. Olex2: A Complete Structure Solution, Refinement and Analysis Program. *J. Appl. Crystallogr.* **2009**, *42*, 339-341.
(2) Sheldrick, G. M. A Short History of Shelx. *Acta Crystallogr A* **2008**, *64*, 112-22.

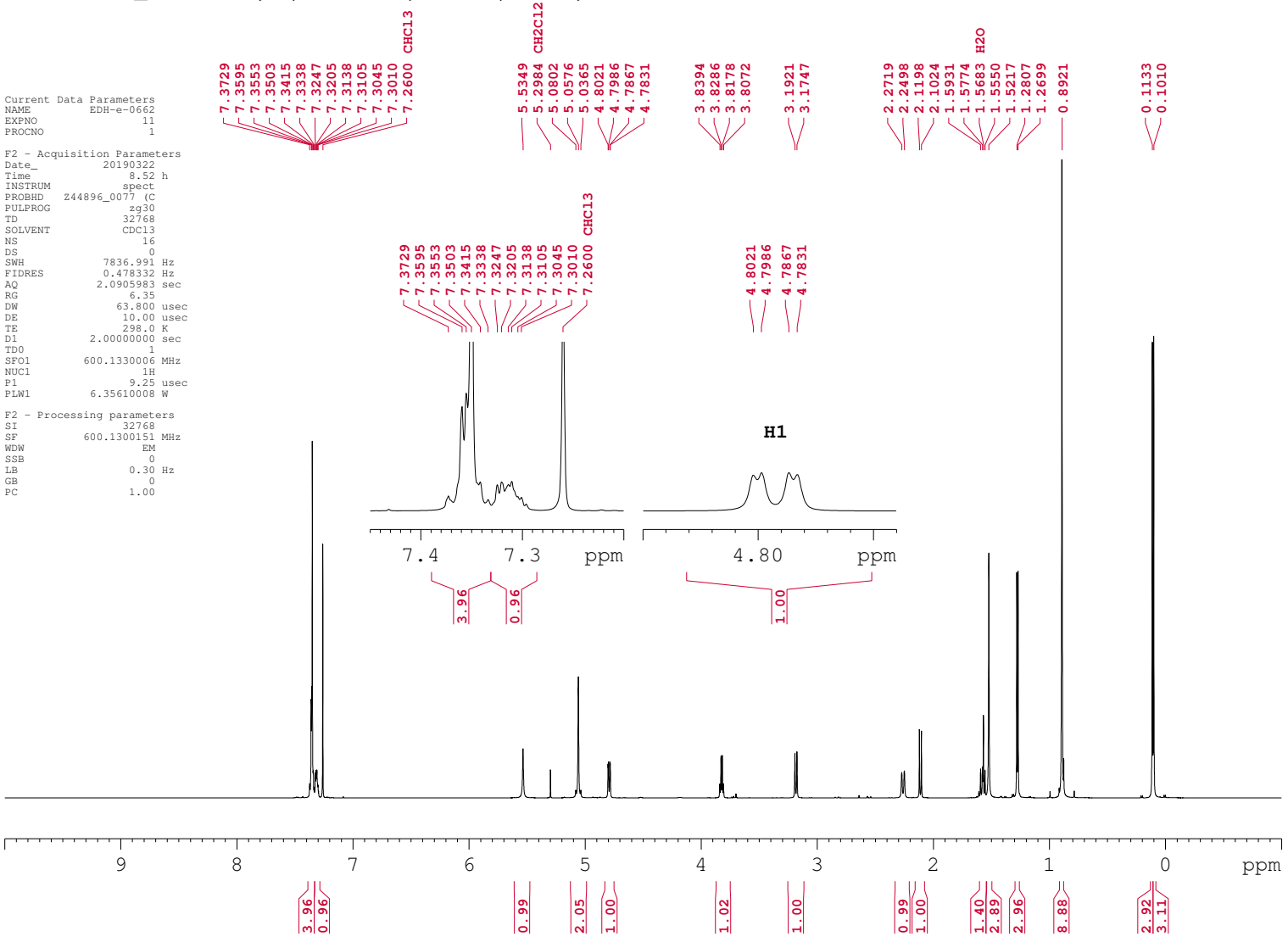
APPENDIX E

NMR Spectra

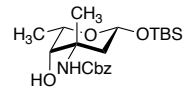
^{1}H NMR of Compound 218 (600 MHz, $CDCl_3$)



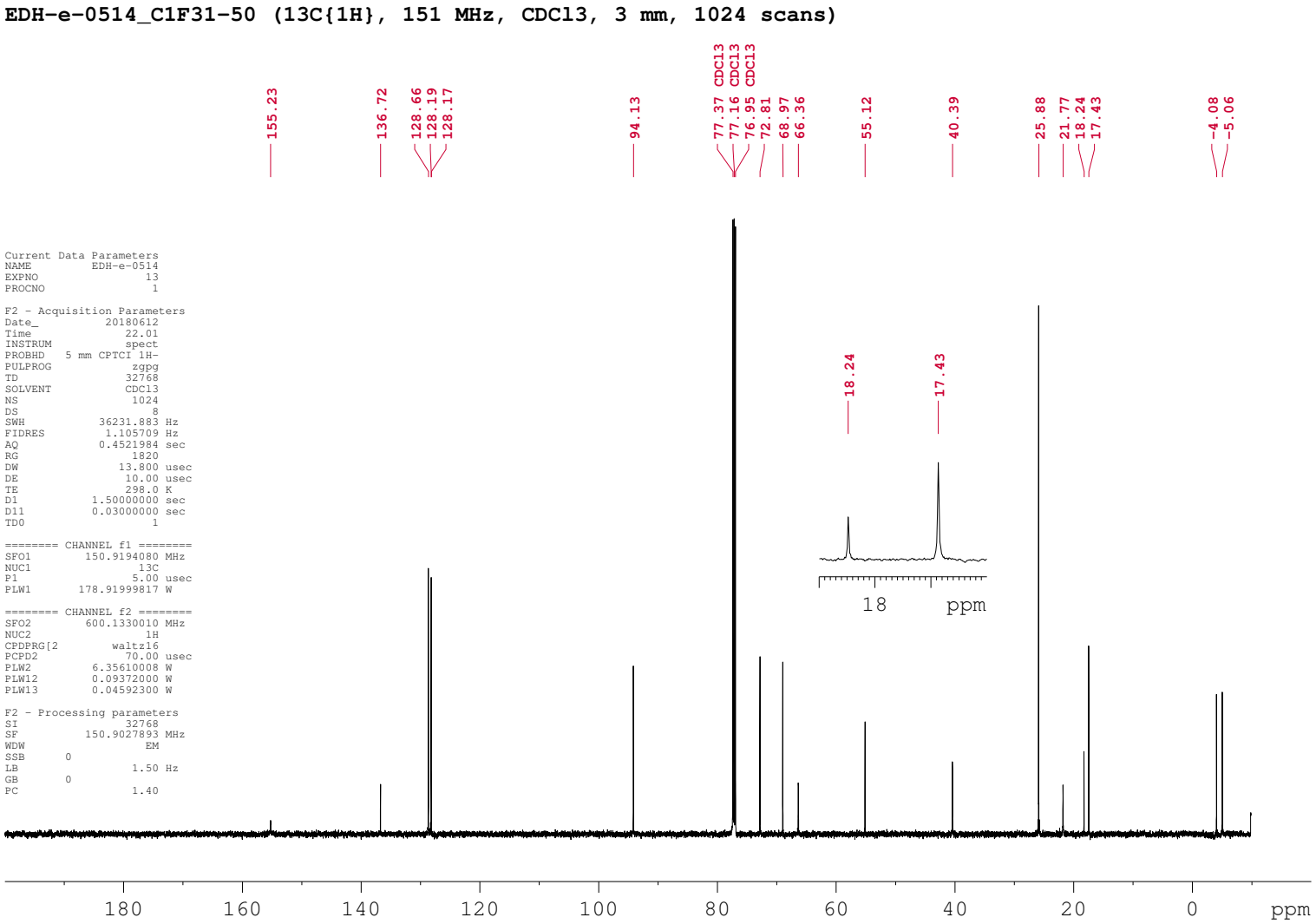
218



^{13}C NMR of Compound 218 (151 MHz, $CDCl_3$)

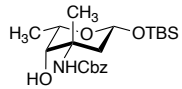


218



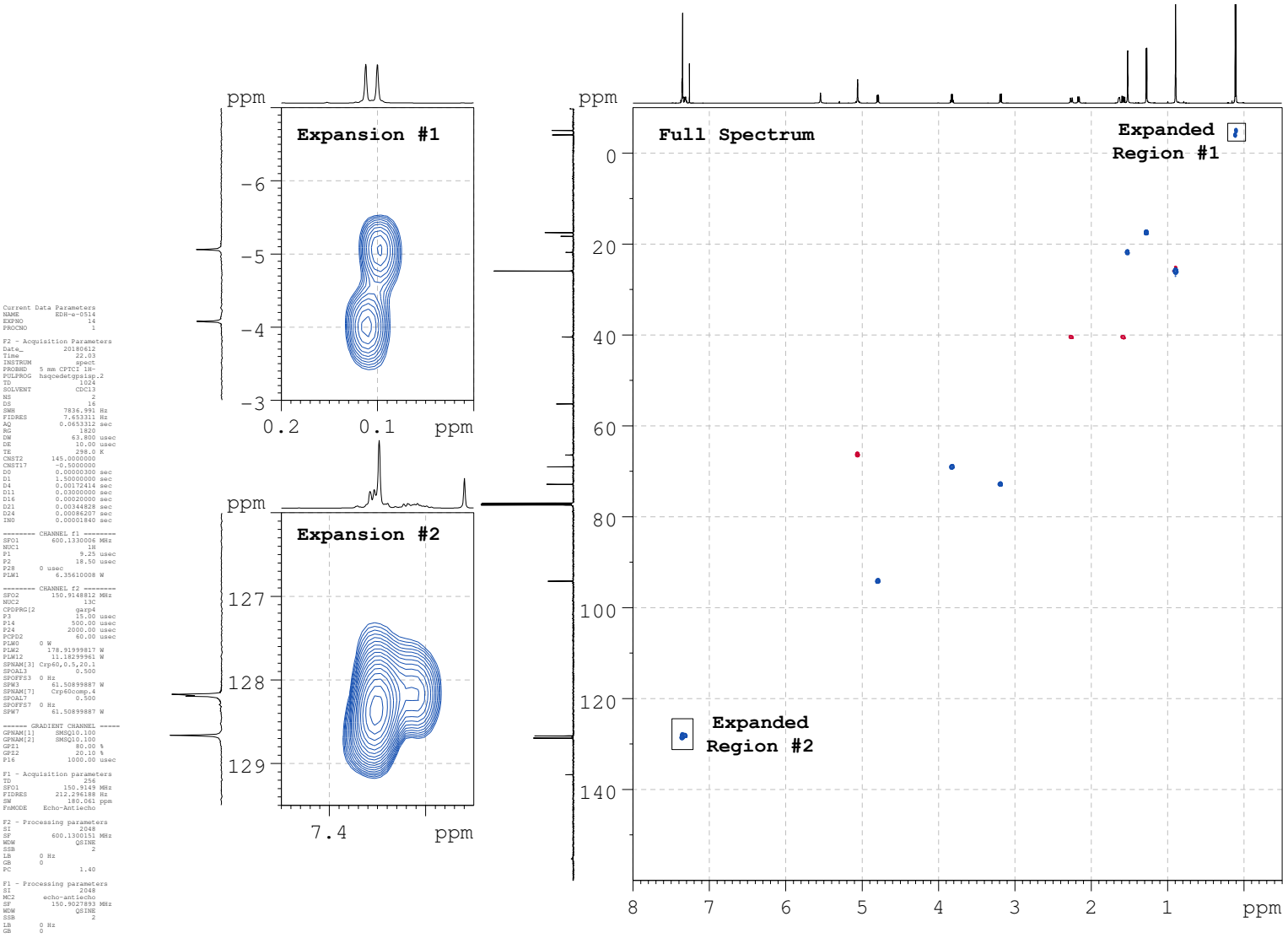
^{1}H-^{13}C HSQC{^{1}H}{^{13}C} NMR of Compound 218 (^{1}H-600 MHz, ^{13}C-151 MHz, $CDCl_3$)

Note: Blue = CH/CH_3 | Red = CH_2.



218

EDH-e-0514_C1F31-50 (HSQC{1H}{13C}, 600 MHz, CDCl3, 3 mm, O2P = 80 ppm)



Current Data Parameters
NAME EDH-e-0514
EXPNO 14
PROCNO 1

F2 - Acquisition Parameters
Date_ 20180612
Time 22.03
INSTRUM spect
PROBHD 5 mm CPTCI 1H-
PULPROG hsqcedetgpsisp.2
TD 1024
SOLVENT CDCl3
NS 2
DS 16
SWH 7836.991 Hz
FIDRES 7.653311 Hz
AQ 0.0653312 sec
RG 1820
DW 63.800 usec
DE 10.00 usec
TE 298.0 K
CNST2 145.0000000
CNST17 -0.5000000
D0 0.00000300 sec
D1 1.50000000 sec
D4 0.00172414 sec
D11 0.03000000 sec
D16 0.00020000 sec
D21 0.00344828 sec
D24 0.00086207 sec
IN0 0.00001840 sec

======== CHANNEL f1 ========
SFO1 600.1330006 MHz
NDC1 1H
P1 9.25 usec
P2 18.50 usec
P28 0 usec
PLW1 6.35610008 W

======== CHANNEL f2 ========
SFO2 150.9148812 MHz
NDC2 13C
CPDPRG[2 garp4
P3 15.00 usec
P14 500.00 usec
P24 2000.00 usec
PCPD2 60.00 usec
PLW0 0 W
PLW2 178.91999817 W
PLW12 11.18299961 W
SPNAM[3] Crp60,0.5,20.1
SPOAL3 0.500
SPOFFS3 0 Hz
SPW3 61.50899887 W
SPNAM[7] Crp60comp.4
SPOAL7 0.500
SPOFFS7 0 Hz
SPW7 61.50899887 W

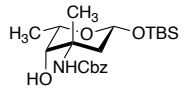
====== GRADIENT CHANNEL =====
GPNAM[1] SMSQ10.100
GPNAM[2] SMSQ10.100
GPZ1 80.00 %
GPZ2 20.10 %
P16 1000.00 usec

F1 - Acquisition parameters
TD 256
SFO1 150.9149 MHz
FIDRES 212.296188 Hz
SW 180.061 ppm
FnMODE Echo-Antiecho

F2 - Processing parameters
SI 2048
SF 600.1300151 MHz
WDW QSINE
SSB 2
LB 0 Hz
GB 0
PC 1.40

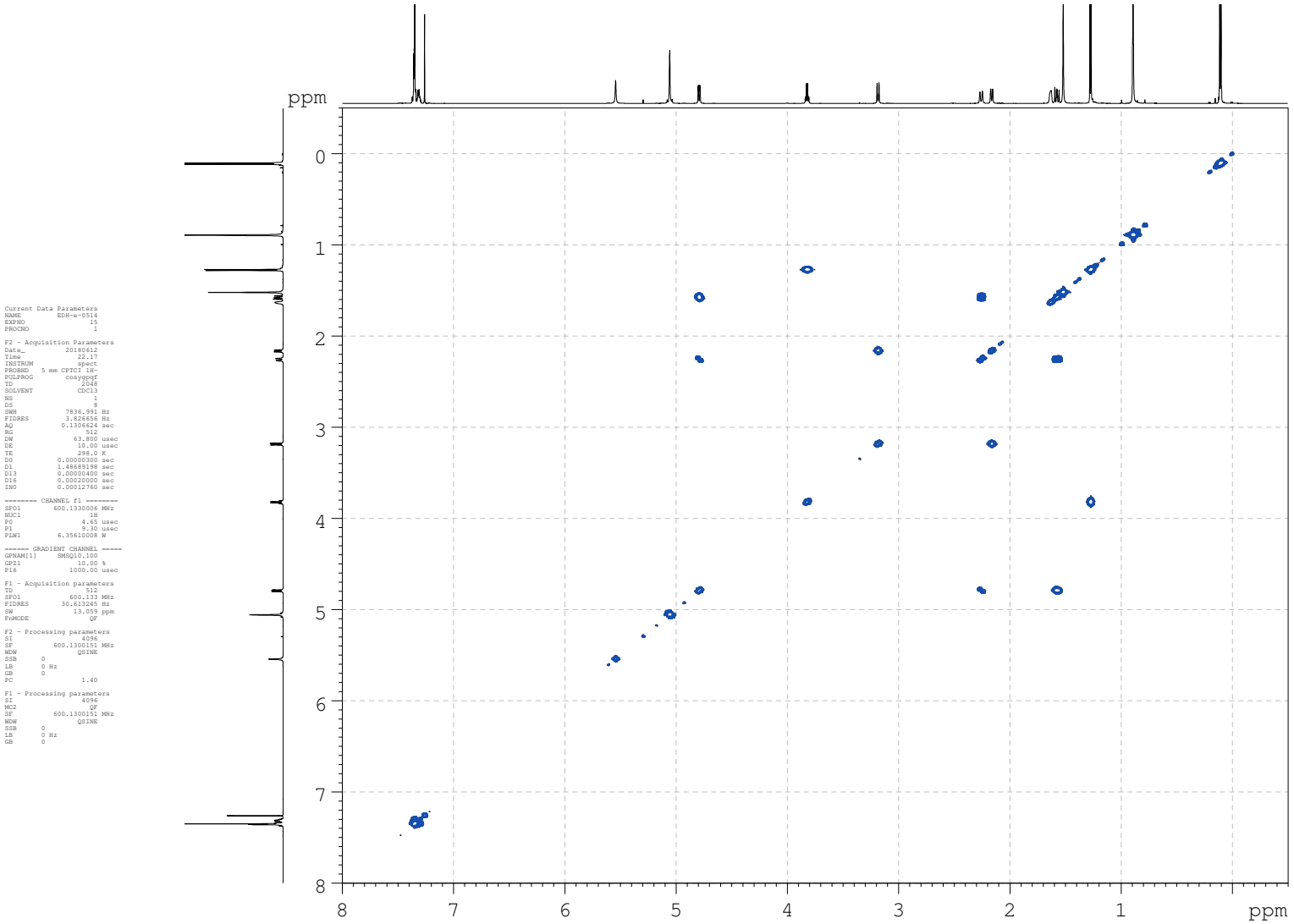
F1 - Processing parameters
SI 2048
MC2 echo-antiecho
SF 150.9027893 MHz
WDW QSINE
SSB 2
LB 0 Hz
GB 0

^{1}H-^{1}H COSY NMR Compound 218 (600 MHz, $CDCl_3$)



218

EDH-e-0514_C1F31-50 (COSY, 600 MHz, CDCl3, 3 mm)

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EXPNO                 15
PROCNO                 1

F2 - Acquisition Parameters
Date_          20180612
Time              22.17
INSTRUM             spect
PROBHD   5 mm CPTCI 1H-
PULPROG          cosygpqf
TD                 2048
SOLVENT            CDCl3
NS                    1
DS                    8
SWH            7836.991 Hz
FIDRES         3.826656 Hz
AQ             0.1306624 sec
RG                  512
DW               63.800 usec
DE               10.00 usec
TE                298.0 K
D0            0.00000300 sec
D1            1.48689198 sec
D13           0.00000400 sec
D16           0.00020000 sec
IN0           0.00012760 sec

======== CHANNEL f1 ========
SFO1         600.1330006 MHz
NUC1                   1H
P0                   4.65 usec
P1                   9.30 usec
PLW1         6.35610008 W

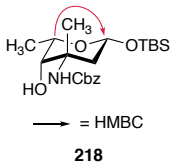
====== GRADIENT CHANNEL =====
GPNAM[1]      SMSQ10.100
GPZ1               10.00 %
P16              1000.00 usec

F1 - Acquisition parameters
TD                  512
SFO1         600.133 MHz
FIDRES       30.613245 Hz
SW              13.059 ppm
FnMODE               QF

F2 - Processing parameters
SI                 4096
SF           600.1300151 MHz
WDW                QSINE
SSB       0
LB        0 Hz
GB        0
PC                 1.40

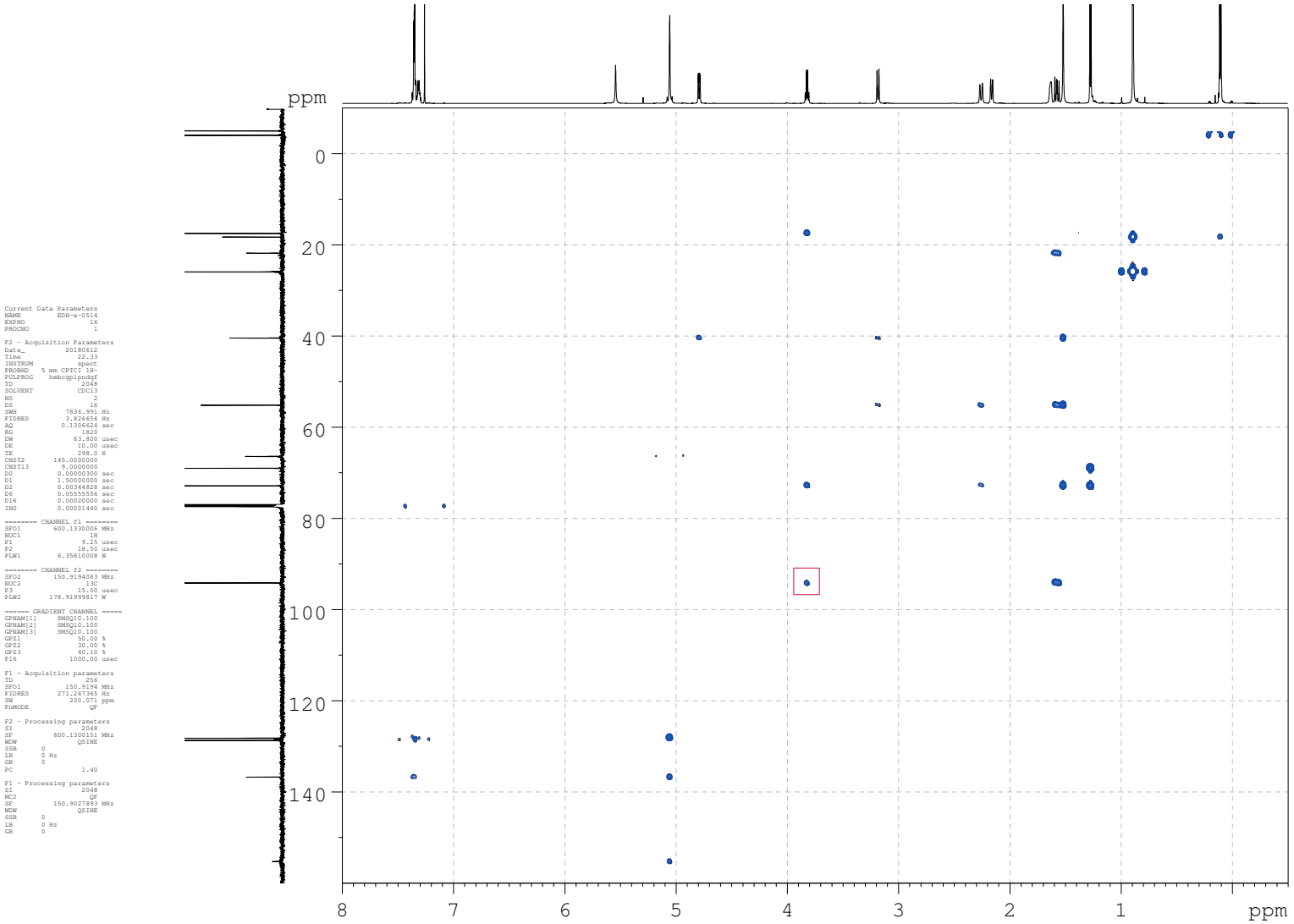
F1 - Processing parameters
SI                 4096
MC2                  QF
SF           600.1300151 MHz
WDW                QSINE
SSB       0
LB        0 Hz
GB        0
```

^{1}H-^{13}C HMBC NMR Compound 218 (^{1}H-600 MHz, ^{13}C-151 MHz, $CDCl_3$)



218

EDH-e-0514_C1F31-50 (HMBC, 600 MHz, CDCl3, 3 mm)



Current Data Parameters
NAME EDH-e-0514
EXPNO 16
PROCNO 1

F2 - Acquisition Parameters
Date_ 20180612
Time 22.33
INSTRUM spect
PROBHD 5 mm CPTCI 1H-
PULPROG hmbcgplpndqf
TD 2048
SOLVENT CDCl3
NS 2
DS 16
SWH 7836.991 Hz
FIDRES 3.826656 Hz
AQ 0.1306624 sec
RG 1820
DW 63.800 usec
DE 10.00 usec
TE 298.0 K
CNST2 145.0000000
CNST13 9.0000000
D0 0.00000300 sec
D1 1.50000000 sec
D2 0.00344828 sec
D6 0.05555556 sec
D16 0.00020000 sec
IN0 0.00001440 sec

======== CHANNEL f1 ========
SFO1 600.1330006 MHz
NUC1 1H
P1 9.25 usec
P2 18.50 usec
PLW1 6.35610008 W

======== CHANNEL f2 ========
SFO2 150.9194083 MHz
NUC2 13C
P3 15.00 usec
PLW2 178.91999817 W

====== GRADIENT CHANNEL =====
GPNAM[1] SMSQ10.100
GPNAM[2] SMSQ10.100
GPNAM[3] SMSQ10.100
GPZ1 50.00 %
GPZ2 30.00 %
GPZ3 40.10 %
P16 1000.00 usec

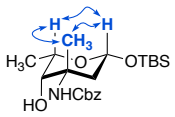
F1 - Acquisition parameters
TD 256
SFO1 150.9194 MHz
FIDRES 271.267365 Hz
SW 230.071 ppm
FnMODE QF

F2 - Processing parameters
SI 2048
SF 600.1300151 MHz
WDW QSINE
SSB 0
LB 0 Hz
GB 0
PC 1.40

F1 - Processing parameters
SI 2048
MC2 QF
SF 150.9027893 MHz
WDW QSINE
SSB 0
LB 0 Hz
GB 0

^{1}H-^{1}H NOESY NMR Compound 218 (600 MHz, $CDCl_3$)

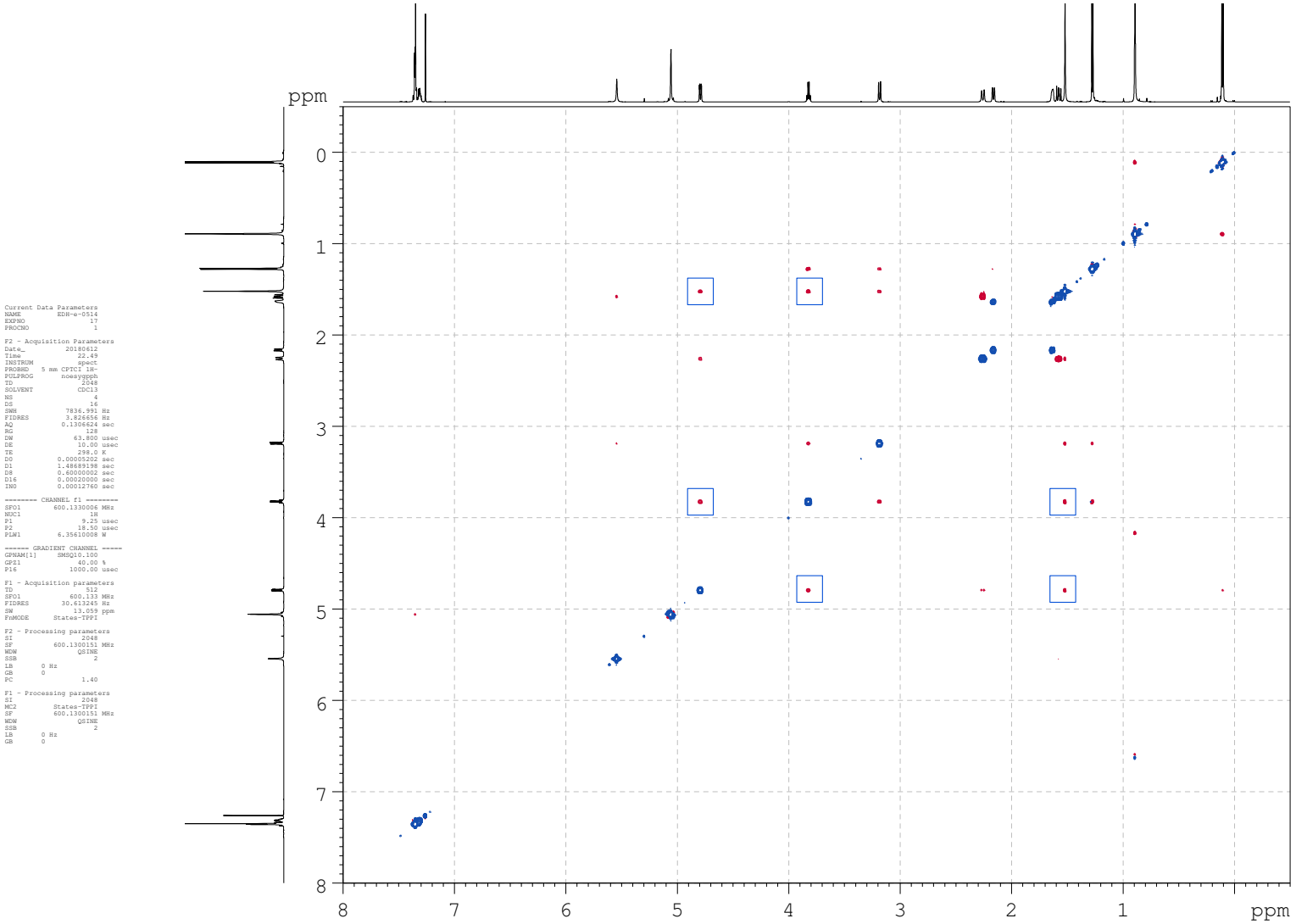
Note: Blue = diagonal/chemical exchange | Red = NOESY cross peaks.



= NOESY

218

EDH-e-0514_C1F31-50 (NOESY, 600 MHz, CDCl3, 3 mm)

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Current Data Parameters
NAME          EDH-e-0514
EXPNO                 17
PROCNO                 1

F2 - Acquisition Parameters
Date_          20180612
Time              22.49
INSTRUM             spect
PROBHD   5 mm CPTCI 1H-
PULPROG       noesygpph
TD                 2048
SOLVENT           CDCl3
NS                    4
DS                   16
SWH            7836.991 Hz
FIDRES         3.826656 Hz
AQ             0.1306624 sec
RG                  128
DW               63.800 usec
DE               10.00 usec
TE                298.0 K
D0            0.00005202 sec
D1            1.48689198 sec
D8            0.60000002 sec
D16           0.00020000 sec
IN0           0.00012760 sec

======== CHANNEL f1 ========
SFO1         600.1330006 MHz
NUC1                   1H
P1                   9.25 usec
P2                  18.50 usec
PLW1         6.35610008 W

====== GRADIENT CHANNEL =====
GPNAM[1]      SMSQ10.100
GPZ1               40.00 %
P16              1000.00 usec

F1 - Acquisition parameters
TD                  512
SFO1         600.133 MHz
FIDRES       30.613245 Hz
SW              13.059 ppm
FnMODE       States-TPPI

F2 - Processing parameters
SI                 2048
SF           600.1300151 MHz
WDW                QSINE
SSB                    2
LB         0 Hz
GB         0
PC                 1.40

F1 - Processing parameters
SI                 2048
MC2          States-TPPI
SF           600.1300151 MHz
WDW                QSINE
SSB                    2
LB         0 Hz
GB         0
```
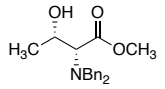
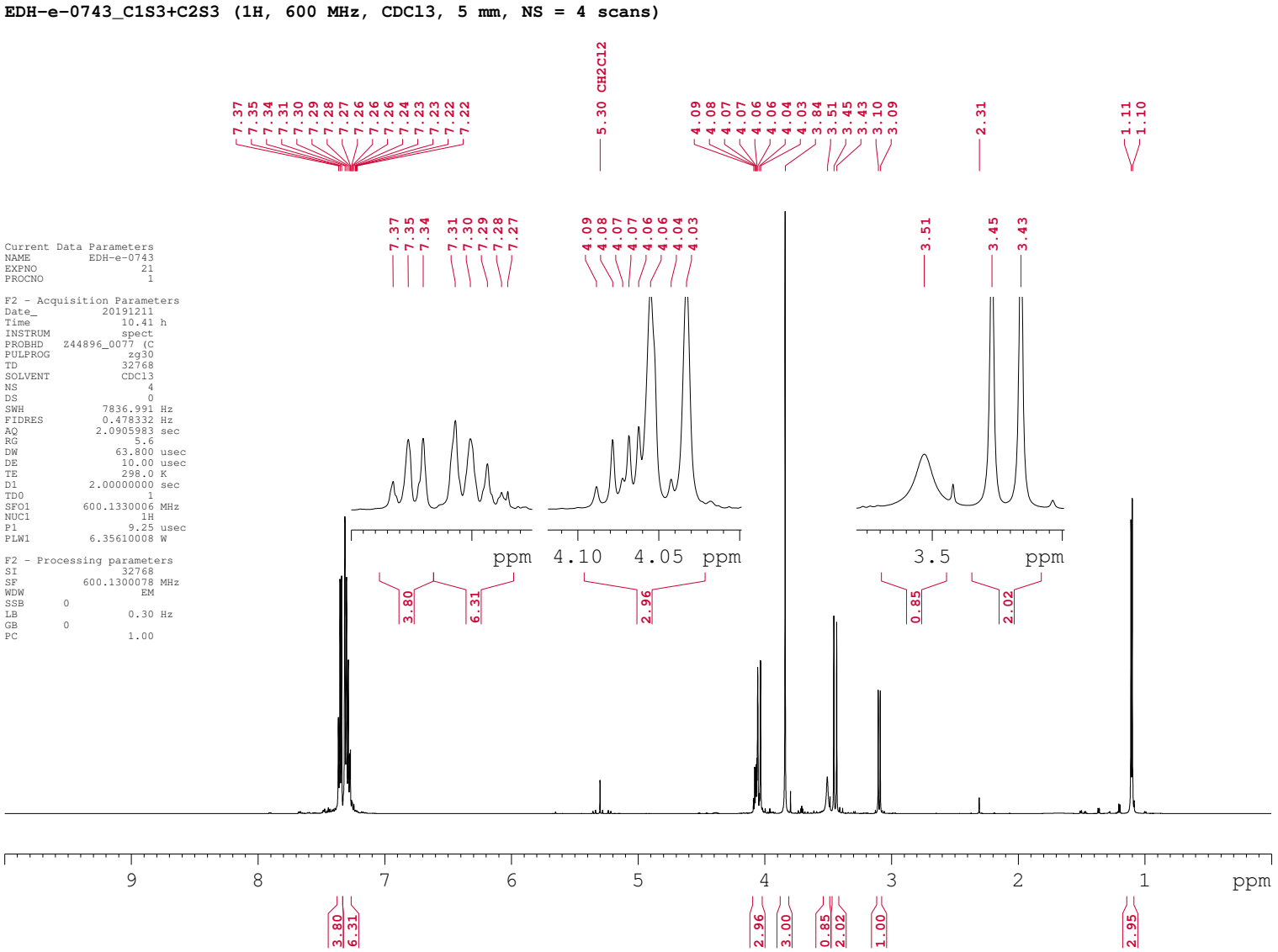

^{1}H NMR of Compound 219 (600 MHz, $CDCl_3$)

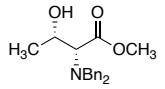
Note: Calibrated to residual CH_2Cl_2 (δ 5.30).



219

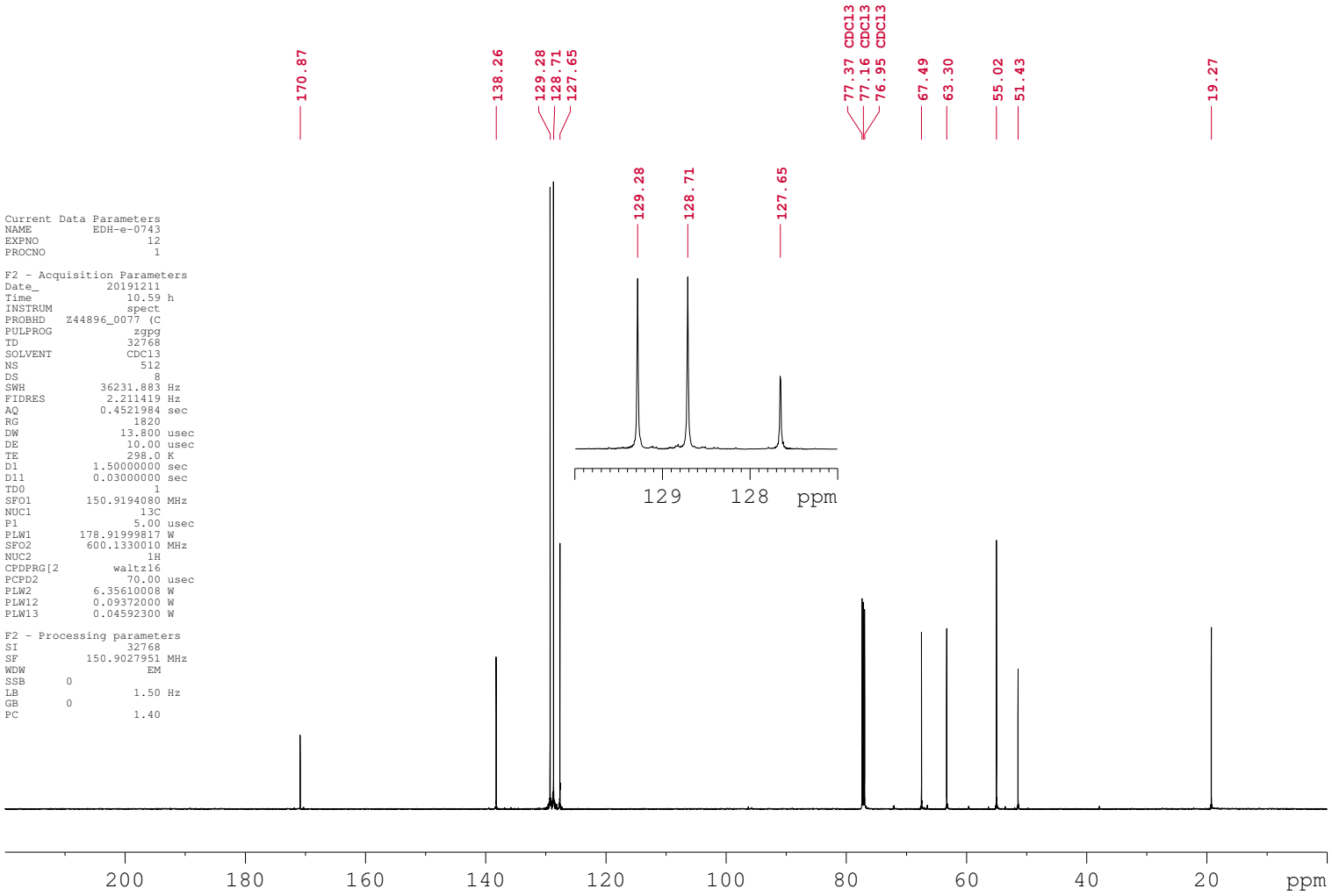


$^{13}C\{^{1}H\}$ NMR of Compound 219 (151 MHz, $CDCl_3$)



219

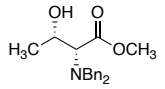
EDH-e-0743_C1S3+C2S3 (13C, 151 MHz, CDC13, 5 mm, NS = 512 scans)




```
Current Data Parameters
NAME          EDH-e-0743
EXPNO                 12
PROCNO                 1

F2 - Acquisition Parameters
Date_           20191211
Time               10.59 h
INSTRUM             spect
PROBHD    Z44896_0077 (C
PULPROG             zgpg
TD                 32768
SOLVENT            CDCl3
NS                   512
DS                     8
SWH            36231.883 Hz
FIDRES          2.211419 Hz
AQ             0.4521984 sec
RG                  1820
DW                13.800 usec
DE                10.00 usec
TE                298.0 K
D1           1.50000000 sec
D11          0.03000000 sec
TD0                    1
SFO1         150.9194080 MHz
NUC1                  13C
P1                  5.00 usec
PLW1        178.91999817 W
SFO2         600.1330010 MHz
NUC2                   1H
CPDPRG[2          waltz16
PCPD2              70.00 usec
PLW2           6.35610008 W
PLW12          0.09372000 W
PLW13          0.04592300 W

F2 - Processing parameters
SI                 32768
SF           150.9027951 MHz
WDW                   EM
SSB       0
LB                 1.50 Hz
GB        0
PC                 1.40
```

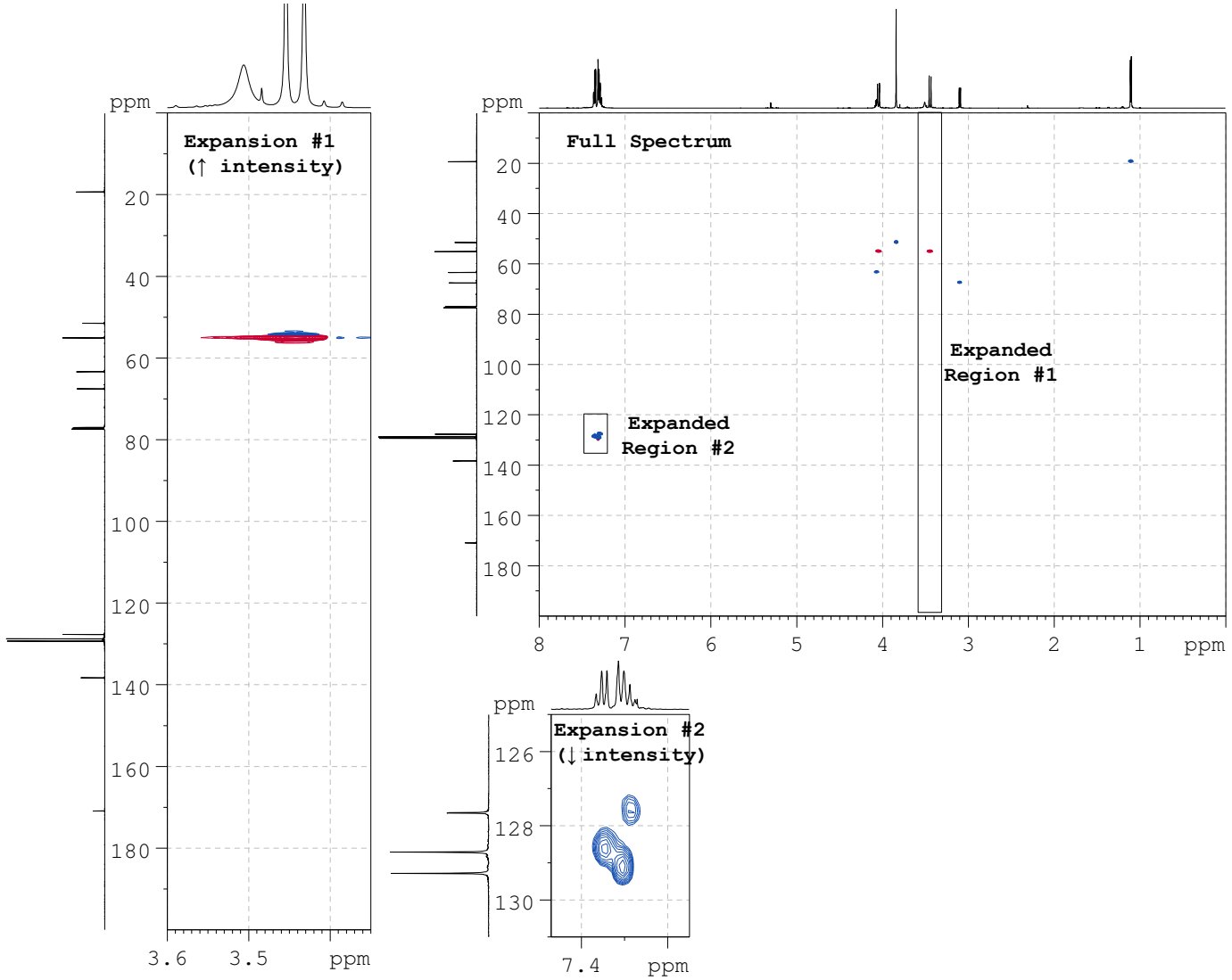
^{1}H-^{13}C HSQC{^{1}H}{^{13}C} of Compound 219 (600 MHz, $CDCl_3$)

Note: Blue = CH/CH_3 | Red = CH_2.



219

EDH-e-0743_C1S3+C2S3 (HSQC{1H}{13C}, 600 MHz, CDCl3, 5 mm, NS = 1 scan)



Current Data Parameters
NAME EDH-e-0743
EXPNO 13
PROCNO 1

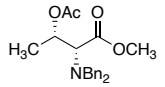
F2 - Acquisition Parameters
Date_ 20191211
Time 11.00 h
INSTRUM spect
PROBHD Z44896_0077 (C
PULPROG hsqcedetgpsisp.2
TD 1024
SOLVENT CDCl3
NS 1
DS 16
SWH 7836.991 Hz
FIDRES 15.306623 Hz
AQ 0.0653312 sec
RG 1820
DW 63.800 usec
DE 10.00 usec
TE 298.0 K
CNST2 145.0000000
CNST17 -0.5000000
D0 0.00000300 sec
D1 1.50000000 sec
D4 0.00172414 sec
D11 0.03000000 sec
D16 0.00020000 sec
D21 0.00344828 sec
D24 0.00086207 sec
IN0 0.00001840 sec
TDav 1
SFO1 600.1330006 MHz
NDC1 1H
P1 9.25 usec
P2 18.50 usec
P28 0 usec
PLW1 6.35610008 W
SFO2 150.9163903 MHz
NUC2 13C
CPDPRG[2 garp4
P3 15.00 usec
P14 500.00 usec
P24 2000.00 usec
PCPD2 60.00 usec
PLW0 0 W
PLW2 178.91999817 W
PLW12 11.18299961 W
SPNAM[3] Crp60,0.5,20.1
SPOAL3 0.500
SPOFFS3 0 Hz
SPW3 61.50899887 W
SPNAM[7] Crp60comp.4
SPOAL7 0.500
SPOFFS7 0 Hz
SPW7 61.50899887 W
GPNAM[1] SMSQ10.100
GPZ1 80.00 %
GPNAM[2] SMSQ10.100
GPZ2 20.10 %
P16 1000.00 usec

F1 - Acquisition parameters
TD 256
SFO1 150.9164 MHz
FIDRES 212.296188 Hz
SW 180.059 ppm
FnMODE Echo-Antiecho

F2 - Processing parameters
SI 1024
SF 600.1300078 MHz
WDW QSINE
SSB 2
LB 0 Hz
GB 0
PC 1.40

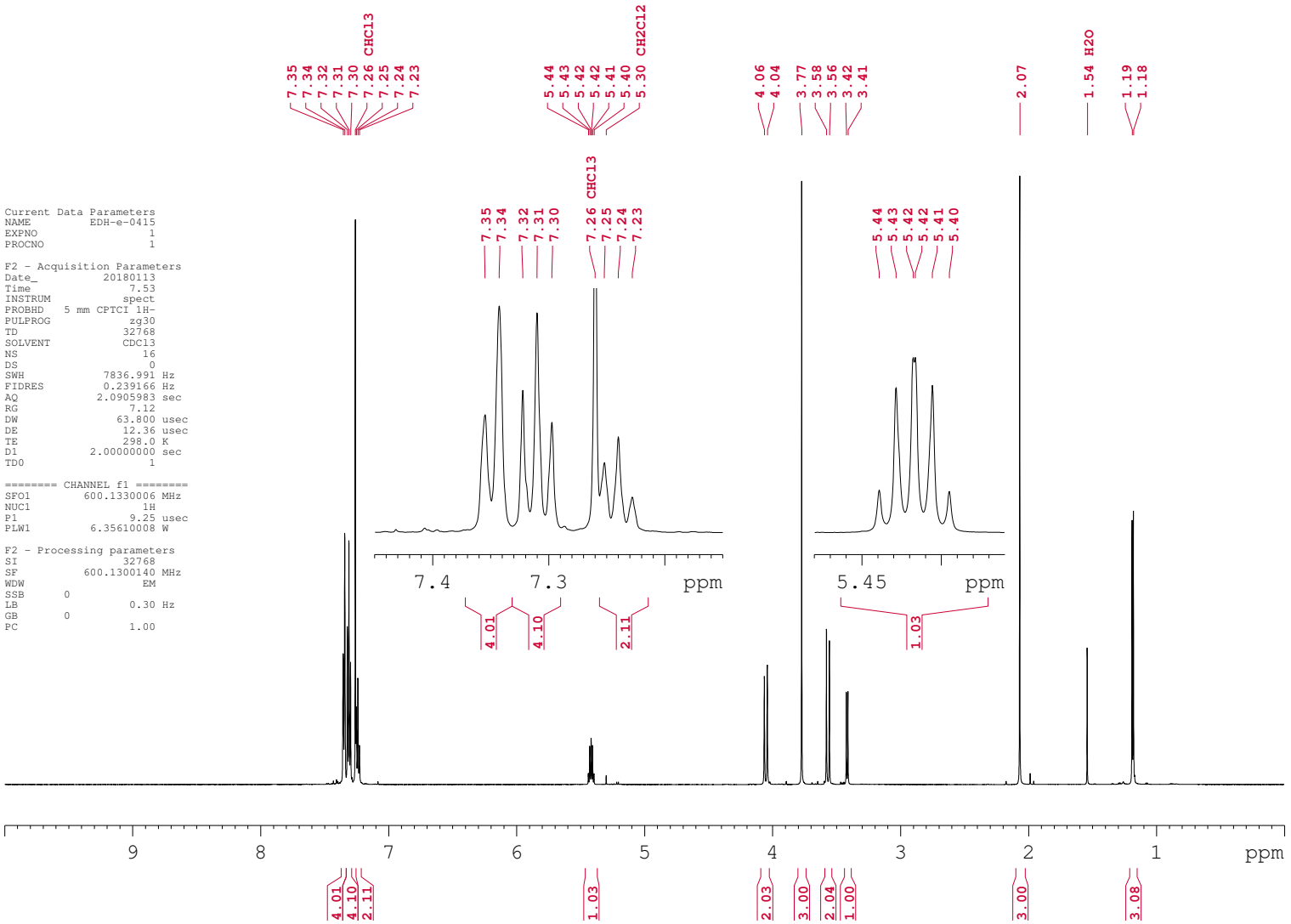
F1 - Processing parameters
SI 1024
MC2 echo-antiecho
SF 150.9027951 MHz
WDW QSINE
SSB 2
LB 0 Hz
GB 0

^{1}H NMR of Compound 220 (600 MHz, $CDCl_3$)

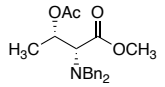


220

EDH-e-0415_C1F4-5 (1H, 600 MHz, CDCl3)

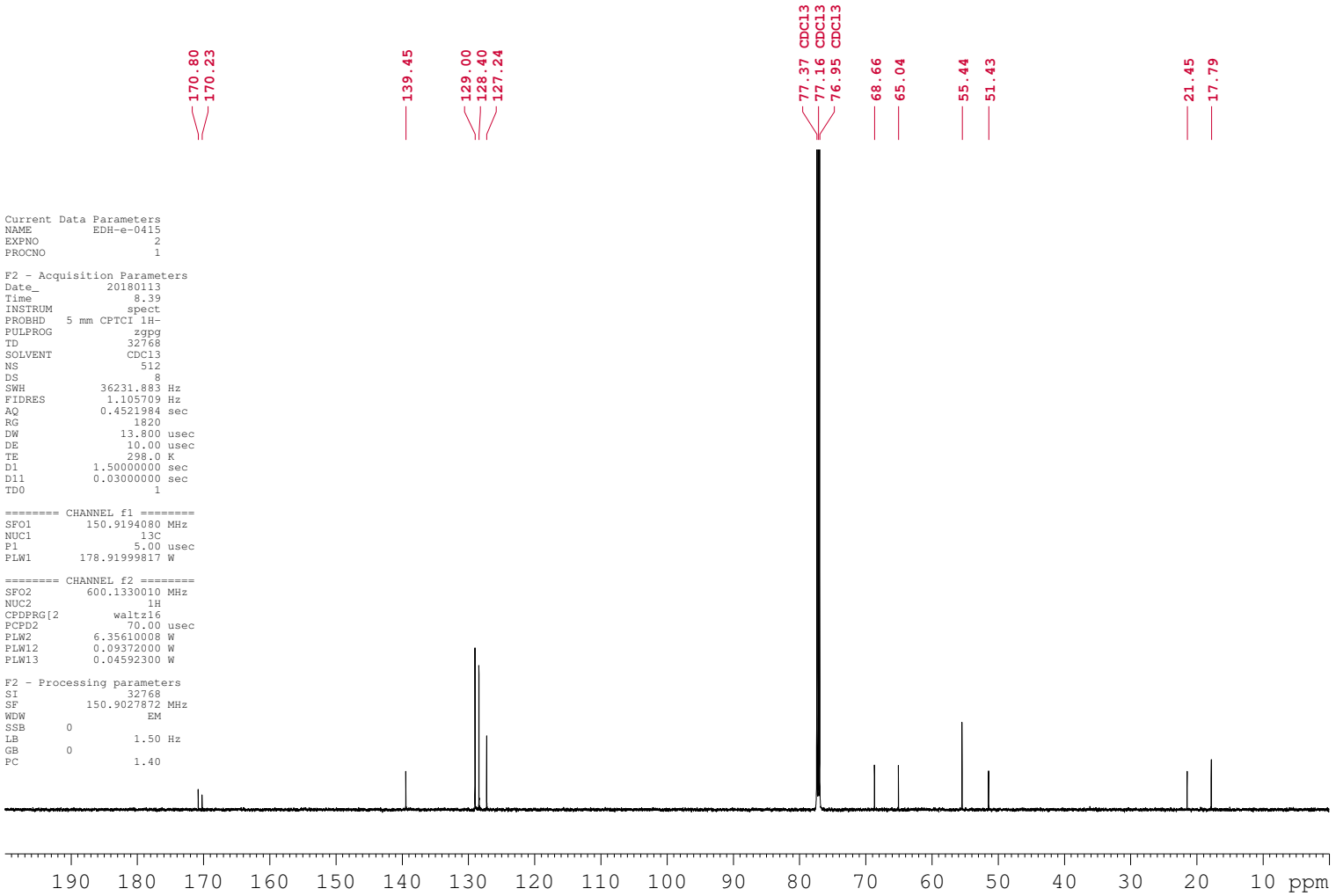


$^{13}C\{^{1}H\}$ NMR of Compound 220 (151 MHz, $CDCl_3$)



220

EDH-e-0415_C1F4-5 (13C, 151 MHz, CDCl3)

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Current Data Parameters
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EXPNO                  2
PROCNO                 1

F2 - Acquisition Parameters
Date_           20180113
Time                8.39
INSTRUM            spect
PROBHD   5 mm CPTCI 1H-
PULPROG            zgpg
TD                32768
SOLVENT           CDCl3
NS                  512
DS                    8
SWH           36231.883 Hz
FIDRES         1.105709 Hz
AQ            0.4521984 sec
RG                 1820
DW               13.800 usec
DE               10.00 usec
TE               298.0 K
D1           1.50000000 sec
D11          0.03000000 sec
TD0                    1

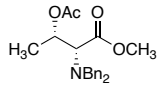
======== CHANNEL f1 ========
SFO1        150.9194080 MHz
NUC1               13C
P1                5.00 usec
PLW1       178.91999817 W

======== CHANNEL f2 ========
SFO2        600.1330010 MHz
NUC2               1H
CPDPRG[2         waltz16
PCPD2             70.00 usec
PLW2         6.35610008 W
PLW12        0.09372000 W
PLW13        0.04592300 W

F2 - Processing parameters
SI                32768
SF          150.9027872 MHz
WDW                EM
SSB      0
LB                1.50 Hz
GB       0
PC                1.40
```

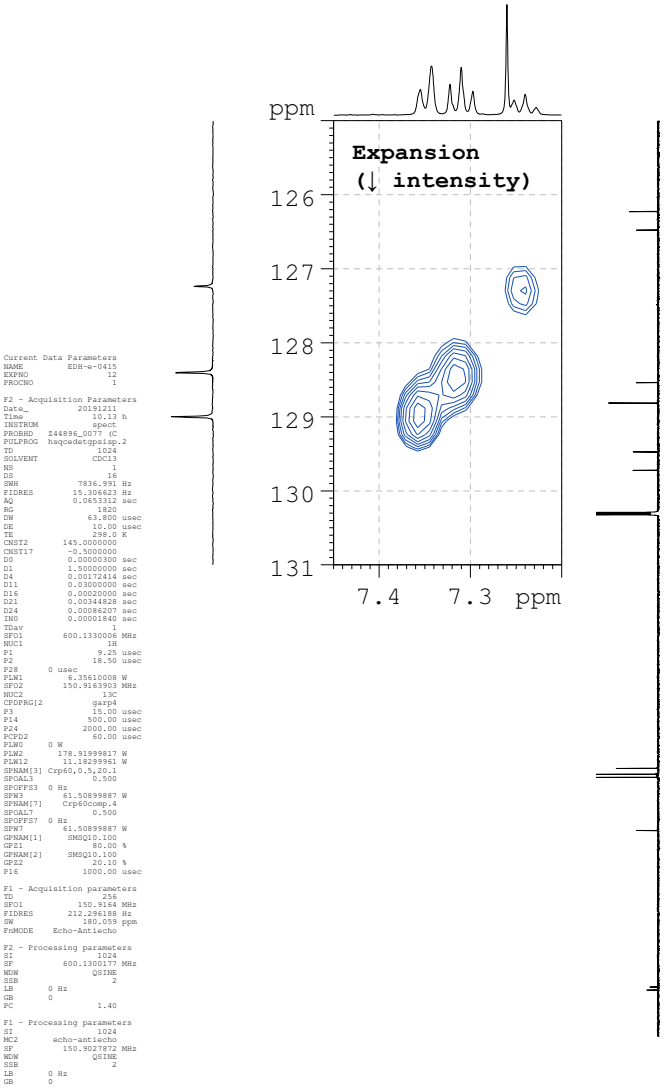
^{1}H-^{13}C HSQC{^{1}H}{^{13}C} NMR of Compound 220 (^{1}H-600 MHz, ^{13}C-151 MHz, $CDCl_3$)

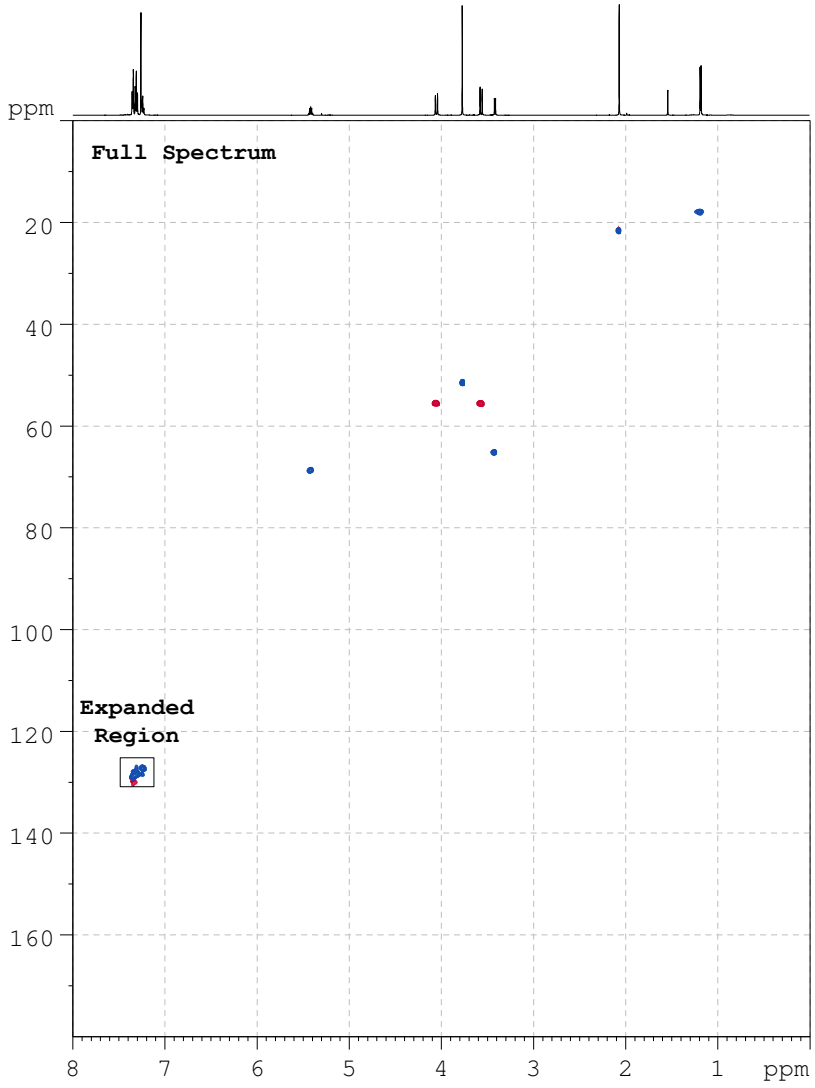
Note: Blue = CH/CH_3 | Red = CH_2.



220

EDH-e-0415_C1F4-5 (HSQC, 600 MHz, CDCl3, 5 mm, NS = 1 scan)






```
Current Data Parameters
NAME          EDH-e-0415
EXPNO                 12
PROCNO                 1

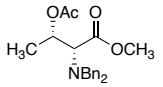
F2 - Acquisition Parameters
Date_          20191211
Time              10.13 h
INSTRUM             spect
PROBHD   I44896_0077 (C
PULPROG  hsqcedetgpsisp.2
TD                  1024
SOLVENT             CDCl3
NS                     1
DS                    16
SWH              7836.991 Hz
FIDRES          15.306623 Hz
AQ               0.0653312 sec
RG                  1820
DW                 63.800 usec
DE                 10.00 usec
TE                298.0 K
CNST2          145.0000000
CNST17          -0.5000000
D0               0.00000300 sec
D1               1.50000000 sec
D4               0.00172414 sec
D11              0.03000000 sec
D16              0.00020000 sec
D21              0.00344828 sec
D24              0.00086207 sec
IN0              0.00001840 sec
TDav                   1
SFO1           600.1330006 MHz
NDC1                  1H
P1                  9.25 usec
P2                 18.50 usec
P28        0 usec
PLW1            6.35610008 W
SFO2          150.9163903 MHz
NUC2                  13C
CPDPRG[2              garp4
P3                 15.00 usec
P14               500.00 usec
P24              2000.00 usec
PCPD2              60.00 usec
PLW0       0 W
PLW2         178.91999817 W
PLW12         11.18299961 W
SPNAM[3] Crp60,0.5,20.1
SPOAL3                0.500
SPOFFS3   0 Hz
SPW3           61.50899887 W
SPNAM[7]      Crp60comp.4
SPOAL7                0.500
SPOFFS7   0 Hz
SPW7           61.50899887 W
GPNAM[1]       SMSQ10.100
GPZ1                80.00 %
GPNAM[2]       SMSQ10.100
GPZ2                20.10 %
P16               1000.00 usec

F1 - Acquisition parameters
TD                    256
SFO1            150.9164 MHz
FIDRES         212.296188 Hz
SW              180.059 ppm
FnMODE     Echo-Antiecho

F2 - Processing parameters
SI                    1024
SF            600.1300177 MHz
WDW                 QSINE
SSB                     2
LB         0 Hz
GB         0
PC                  1.40

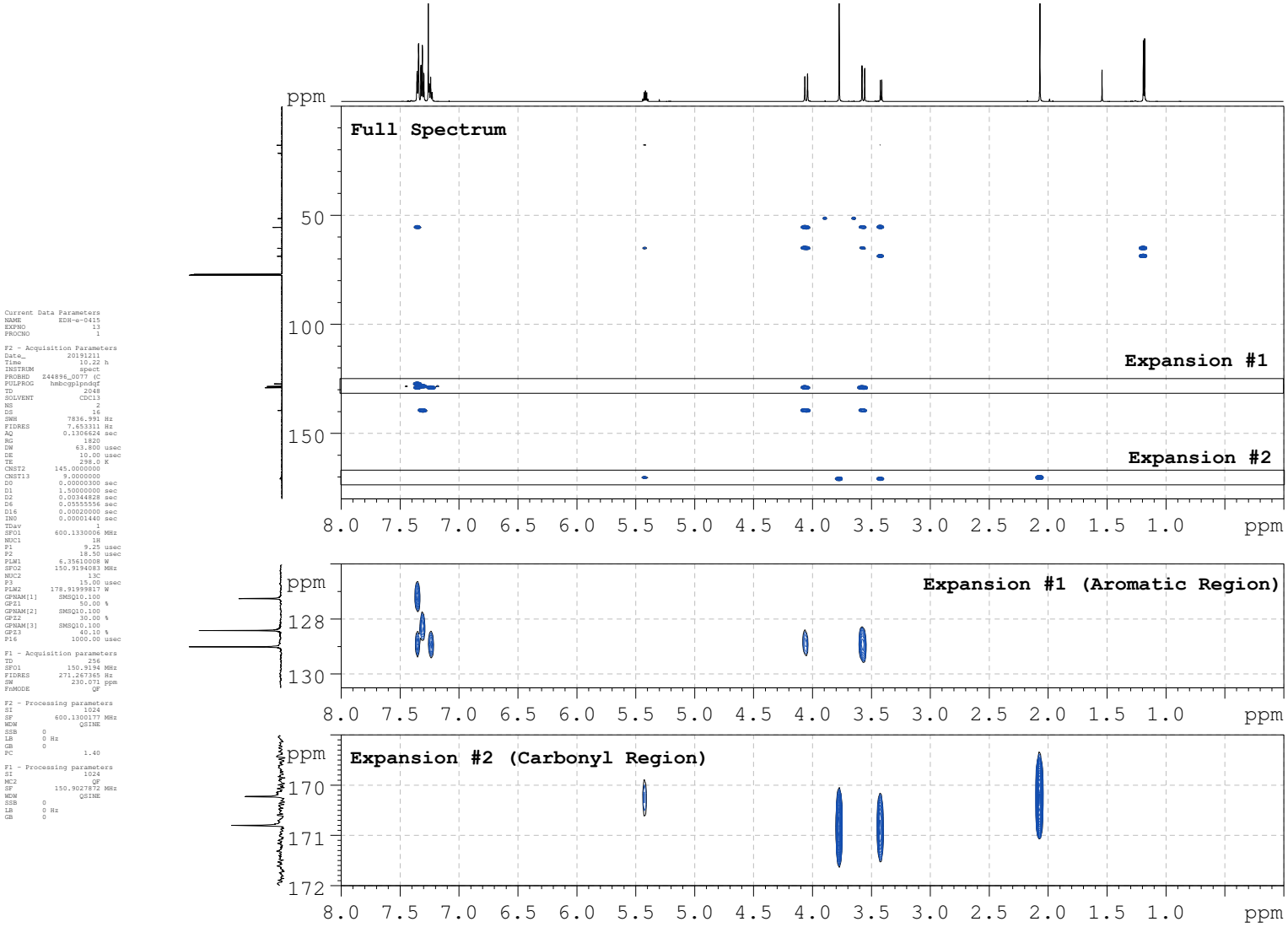
F1 - Processing parameters
SI                    1024
MC2         echo-antiecho
SF            150.9027872 MHz
WDW                 QSINE
SSB                     2
LB         0 Hz
GB         0
```

^{1}H-^{13}C HMBC NMR of Compound 220 (^{1}H-600 MHz, ^{13}C-151 MHz, $CDCl_3$)



220

EDH-e-0415_C1F4-5 (HMBC, 600 MHz, CDCl3, 5 mm, NS = 2 scans)



Current Data Parameters
NAME EDH-e-0415
EXPNO 13
PROCNO 1

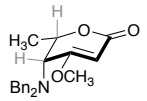
F2 - Acquisition Parameters
Date_ 20191211
Time 10.22 h
INSTRUM spect
PROBHD Z44896_0077 (C
PULPROG hmbcgplpndqf
TD 2048
SOLVENT CDCl3
NS 2
DS 16
SWH 7836.991 Hz
FIDRES 7.653311 Hz
AQ 0.1306624 sec
RG 1820
DW 63.800 usec
DE 10.00 usec
TE 298.0 K
CNST2 145.0000000
CNST13 9.0000000
D0 0.00000300 sec
D1 1.50000000 sec
D2 0.00344828 sec
D6 0.05555556 sec
D16 0.00020000 sec
IN0 0.00001440 sec
TDav 1
SFO1 600.1330006 MHz
NUC1 1H
P1 9.25 usec
P2 18.50 usec
PLW1 6.35610008 W
SFO2 150.9194083 MHz
NUC2 13C
P3 15.00 usec
PLW2 178.91999817 W
GPNAM[1] SMSQ10.100
GPZ1 50.00 %
GPNAM[2] SMSQ10.100
GPZ2 30.00 %
GPNAM[3] SMSQ10.100
GPZ3 40.10 %
P16 1000.00 usec

F1 - Acquisition parameters
TD 256
SFO1 150.9194 MHz
FIDRES 271.267365 Hz
SW 230.071 ppm
FnMODE QF

F2 - Processing parameters
SI 1024
SF 600.1300177 MHz
WDW QSINE
SSB 0
LB 0 Hz
GB 0
PC 1.40

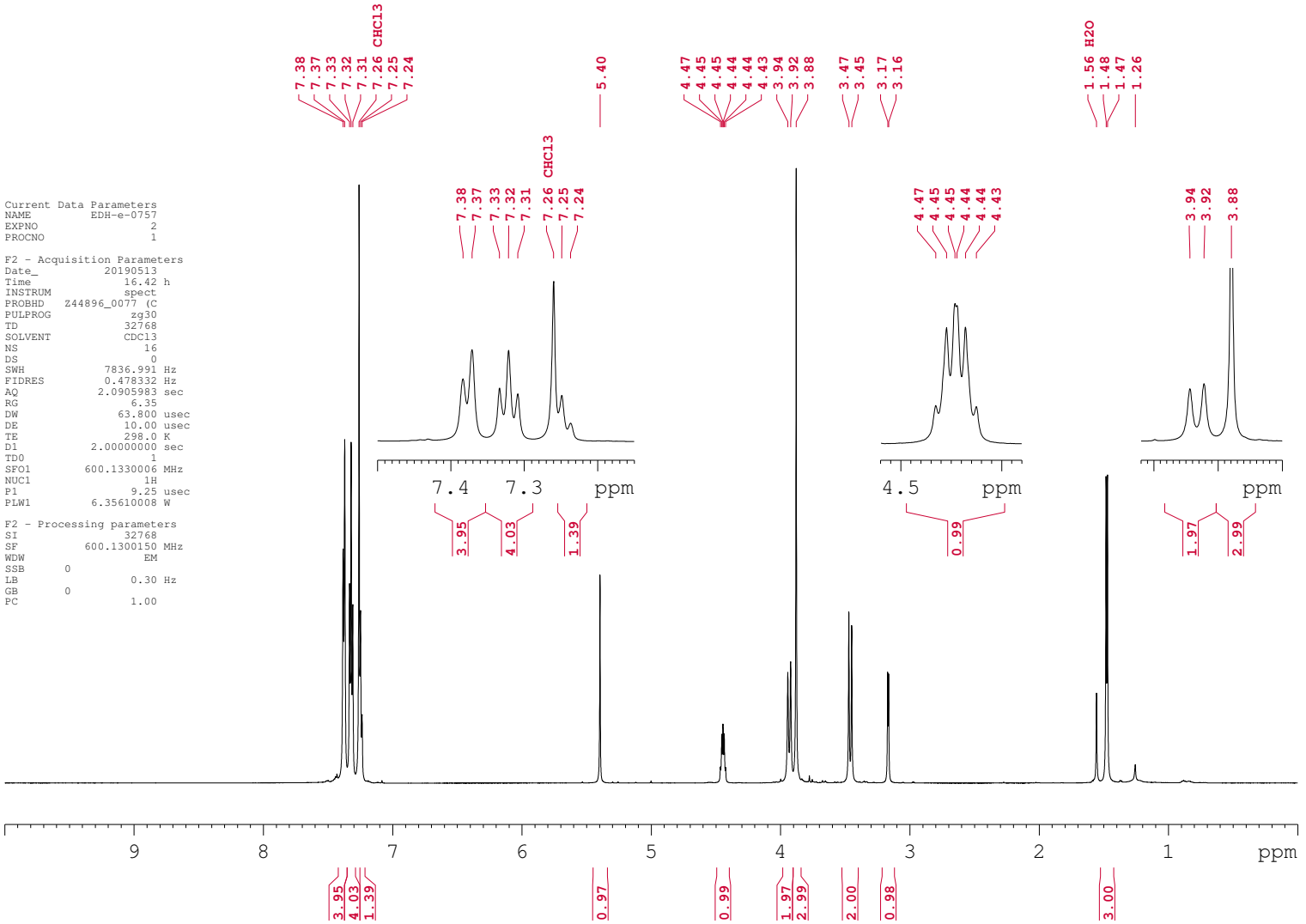
F1 - Processing parameters
SI 1024
MC2 QF
SF 150.9027872 MHz
WDW QSINE
SSB 0
LB 0 Hz
GB 0

^{1}H NMR of Compound 222 (600 MHz, $CDCl_3$)

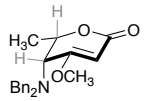


222

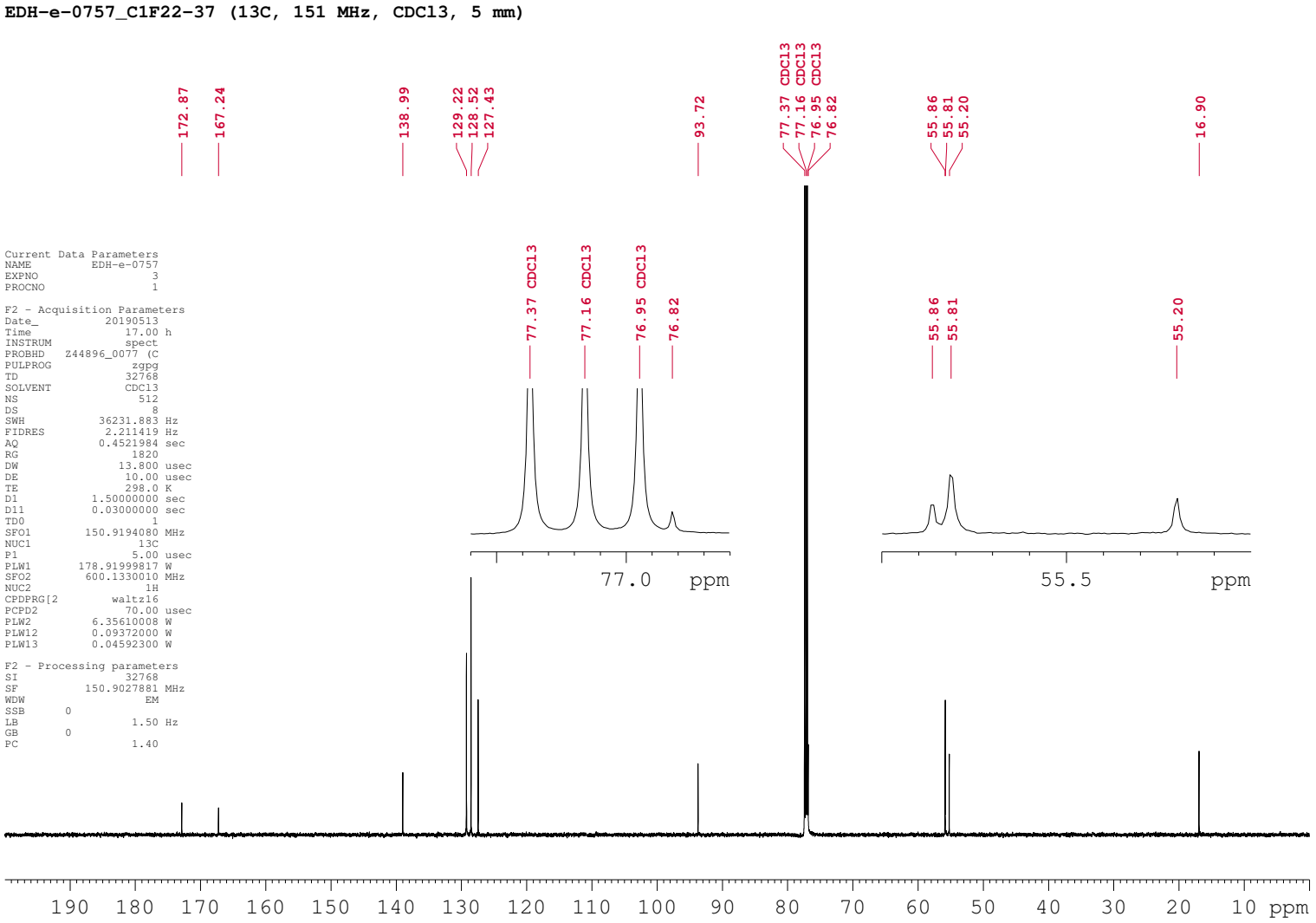
EDH-e-0757_C1F22-37 (1H, 600 MHz, CDCl3, 5 mm)



$^{13}C\{^{1}H\}$ NMR of Compound 222 (151 MHz, $CDCl_3$)

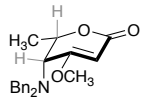


222



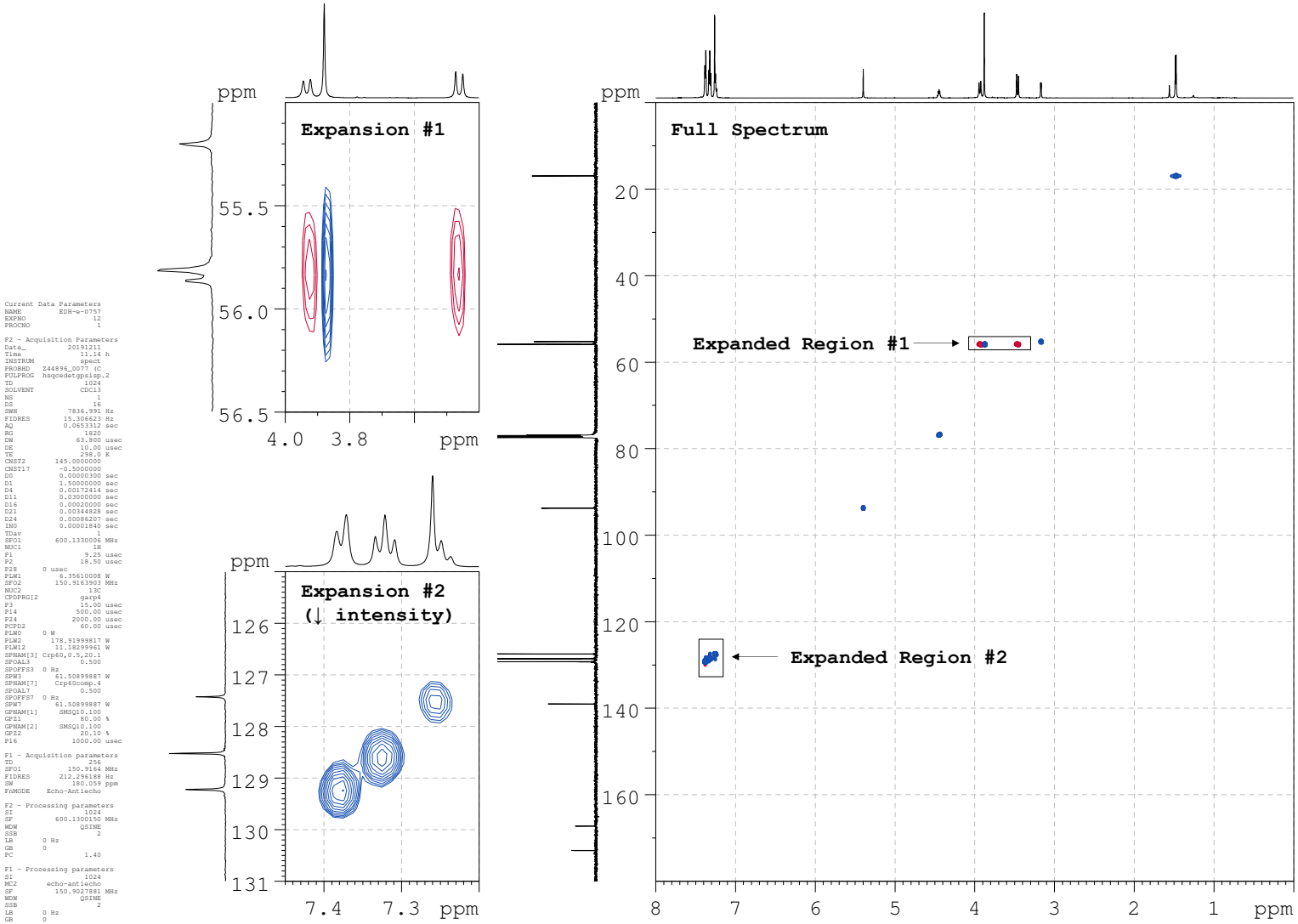
^{1}H-^{13}C HSQC{^{1}H}{^{13}C} NMR of Compound 222 (^{1}H-600 MHz, ^{13}C-151 MHz, $CDCl_3$)

Note: Blue = CH/CH_3 | Red = CH_2.



222

EDH-e-0757_C1F22-37 (HSQC{1H}{13C}, 600 MHz, CDCl3, 5 mm, NS = 1 scan)



Current Data Parameters
NAME EDH-e-0757
EXPNO 12
PROCNO 1

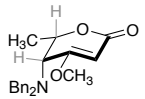
F2 - Acquisition Parameters
Date_ 20191211
Time 11.14 h
INSTRUM spect
PROBHD Z44896_0077 (C
PULPROG hsqcedetgpsisp.2
TD 1024
SOLVENT CDCl3
NS 1
DS 16
SWH 7836.991 Hz
FIDRES 15.306623 Hz
AQ 0.0653312 sec
RG 1820
DW 63.800 usec
DE 10.00 usec
TE 298.0 K
CNST2 145.0000000
CNST17 -0.5000000
D0 0.00000300 sec
D1 1.50000000 sec
D4 0.00172414 sec
D11 0.03000000 sec
D16 0.00020000 sec
D21 0.00344828 sec
D24 0.00086207 sec
IN0 0.00001840 sec
TDav 1
SFO1 600.1330006 MHz
NDC1 1H
P1 9.25 usec
P2 18.50 usec
P28 0 usec
PLW1 6.35610008 W
SFO2 150.9163903 MHz
NUC2 13C
CPDPRG[2 garp4
P3 15.00 usec
P14 500.00 usec
P24 2000.00 usec
PCPD2 60.00 usec
PLW0 0 W
PLW2 178.91999817 W
PLW12 11.18299961 W
SPNAM[3] Crp60,0.5,20.1
SPOAL3 0.500
SPOFFS3 0 Hz
SPW3 61.50899887 W
SPNAM[7] Crp60comp.4
SPOAL7 0.500
SPOFFS7 0 Hz
SPW7 61.50899887 W
GPNAM[1] SMSQ10.100
GPZ1 80.00 %
GPNAM[2] SMSQ10.100
GPZ2 20.10 %
P16 1000.00 usec

F1 - Acquisition parameters
TD 256
SFO1 150.9164 MHz
FIDRES 212.296188 Hz
SW 180.059 ppm
FnMODE Echo-Antiecho

F2 - Processing parameters
SI 1024
SF 600.1300150 MHz
WDW QSINE
SSB 2
LB 0 Hz
GB 0
PC 1.40

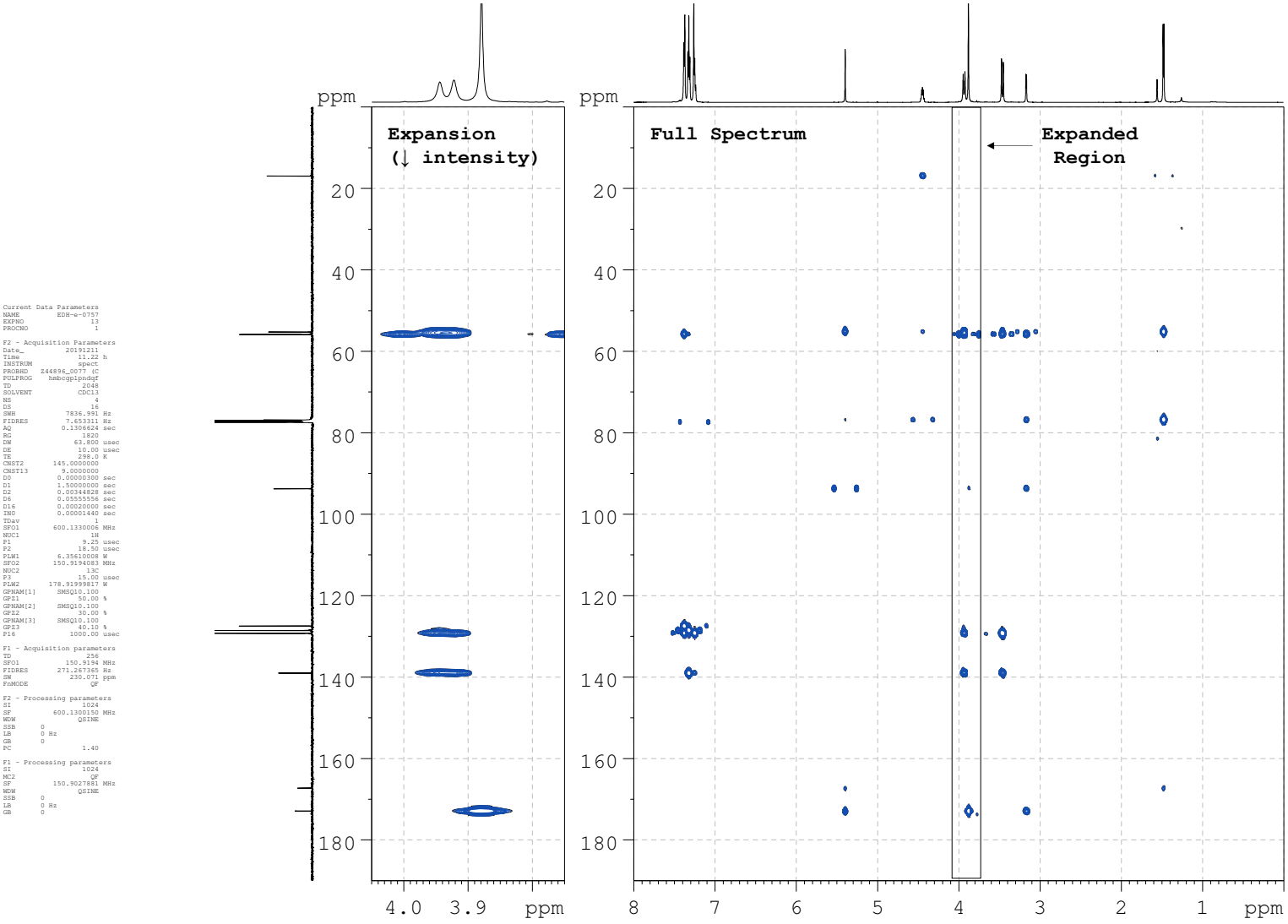
F1 - Processing parameters
SI 1024
MC2 echo-antiecho
SF 150.9027881 MHz
WDW QSINE
SSB 2
LB 0 Hz
GB 0

^{1}H-^{13}C HMBC NMR of Compound 222 (^{1}H-600 MHz, ^{13}C-151 MHz, $CDCl_3$)



222

EDH-e-0757_C1F22-37 (HMBC, 600 MHz, CDCl3, 5 mm, NS = 4 scans)



Current Data Parameters
NAME EDH-e-0757
EXPNO 13
PROCNO 1

F2 - Acquisition Parameters
Date_ 20191211
Time 11.22 h
INSTRUM spect
PROBHD Z44896_0077 (C
PULPROG hmbcgplpndqf
TD 2048
SOLVENT CDCl3
NS 4
DS 16
SWH 7836.991 Hz
FIDRES 7.653311 Hz
AQ 0.1306624 sec
RG 1820
DW 63.800 usec
DE 10.00 usec
TE 298.0 K
CNST2 145.0000000
CNST13 9.0000000
D0 0.00000300 sec
D1 1.50000000 sec
D2 0.00344828 sec
D6 0.05555556 sec
D16 0.00020000 sec
IN0 0.00001440 sec
TDav 1
SFO1 600.1330006 MHz
NUC1 1H
P1 9.25 usec
P2 18.50 usec
PLW1 6.35610008 W
SFO2 150.9194083 MHz
NUC2 13C
P3 15.00 usec
PLW2 178.91999817 W
GPNAM[1] SMSQ10.100
GPZ1 50.00 %
GPNAM[2] SMSQ10.100
GPZ2 30.00 %
GPNAM[3] SMSQ10.100
GPZ3 40.10 %
P16 1000.00 usec

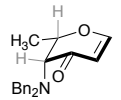
F1 - Acquisition parameters
TD 256
SFO1 150.9194 MHz
FIDRES 271.267365 Hz
SW 230.071 ppm
FnMODE QF

F2 - Processing parameters
SI 1024
SF 600.1300150 MHz
WDW QSINE
SSB 0
LB 0 Hz
GB 0
PC 1.40

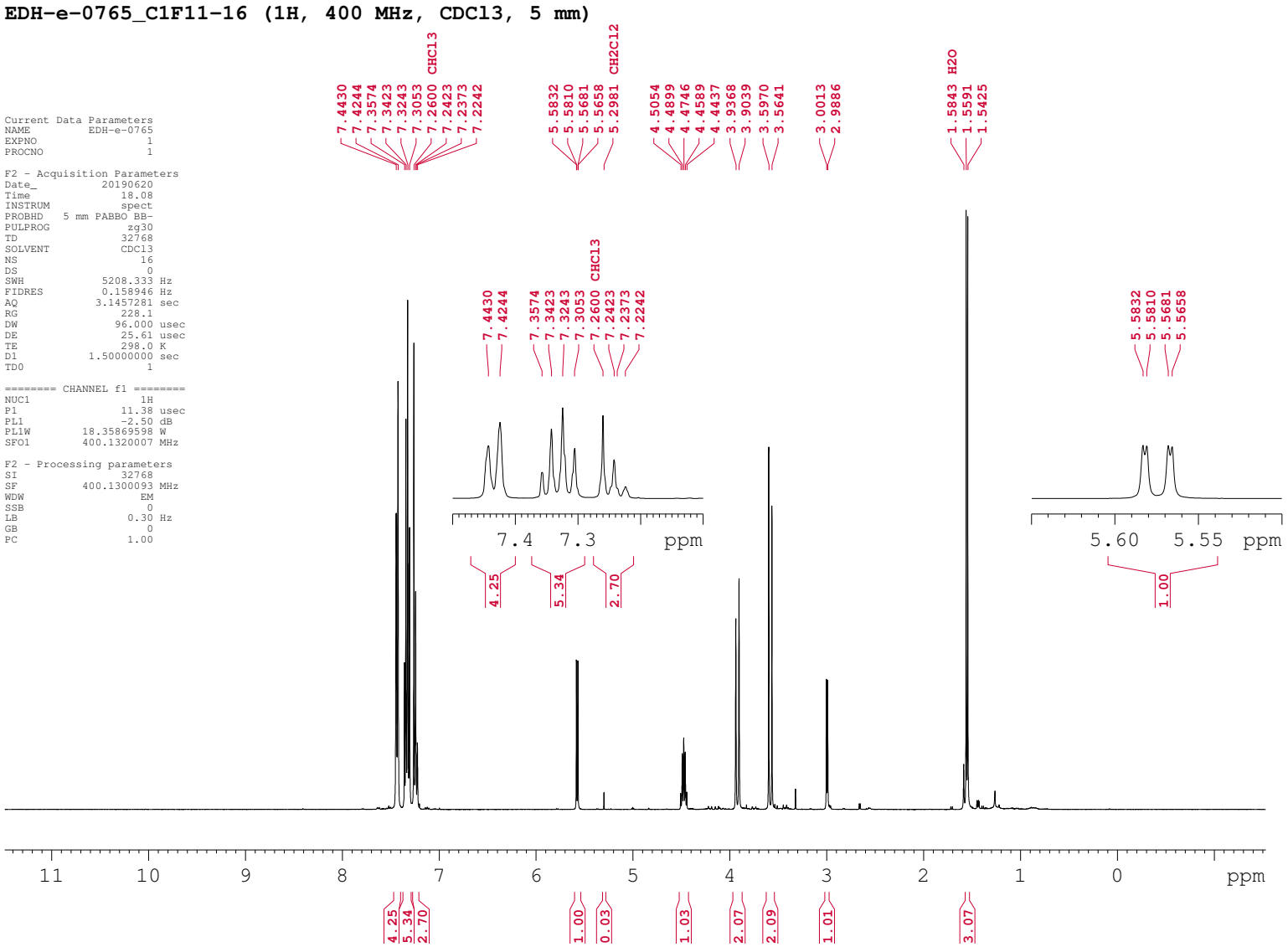
F1 - Processing parameters
SI 1024
MC2 QF
SF 150.9027881 MHz
WDW QSINE
SSB 0
LB 0 Hz
GB 0

^{1}H NMR of Compound 217 (400 MHz, $CDCl_3$)

Note: This is the ^{1}H NMR spectrum of **217** used to verify the purity of the 9.90 g of **217** obtained in the experimental procedure detailed above.

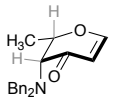


217

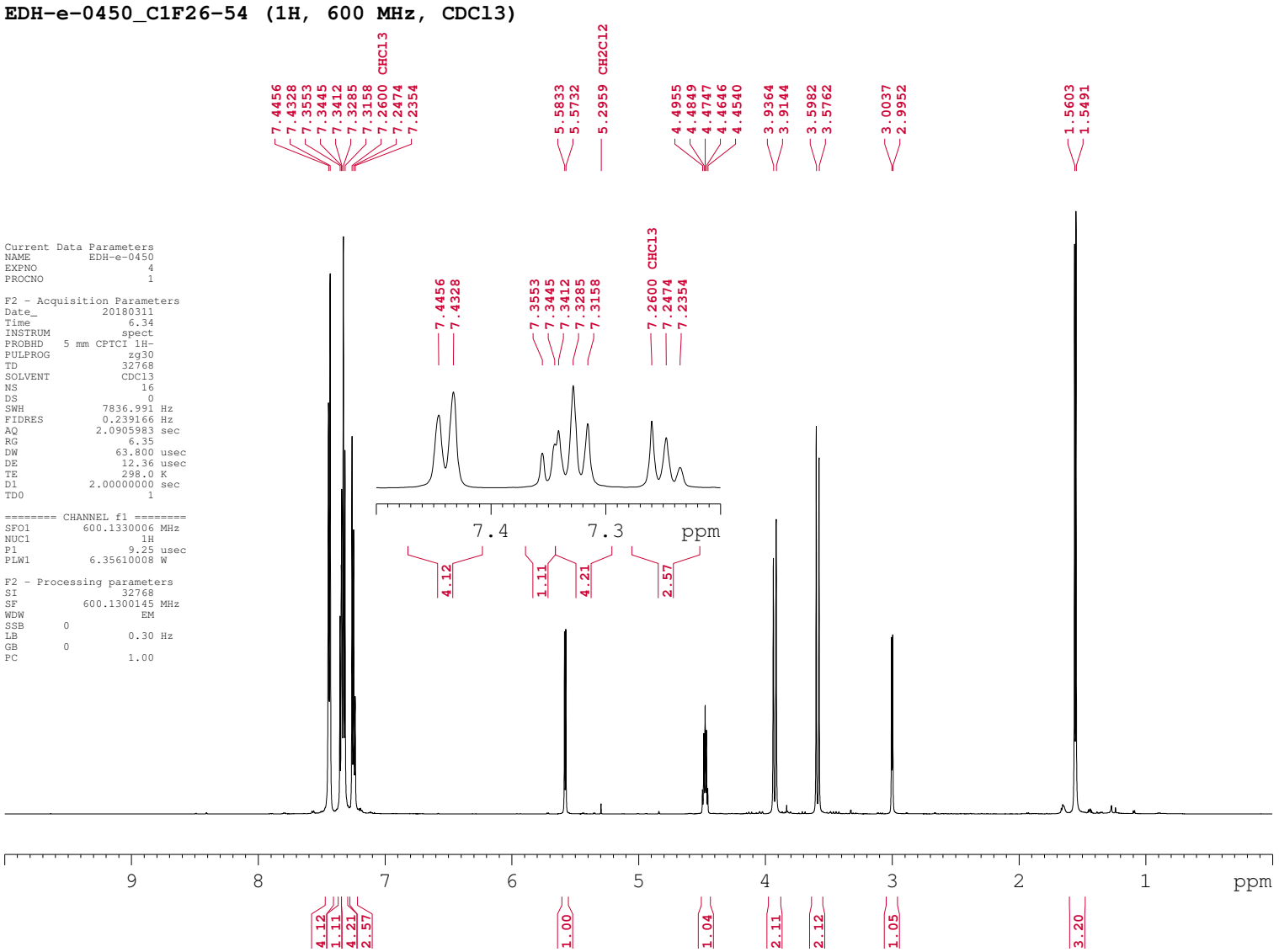


^{1}H NMR of Compound 217 (600 MHz, $CDCl_3$)

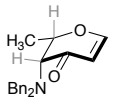
Note: This is the ^{1}H NMR of **217** used for the line listing and peak assignment.



217

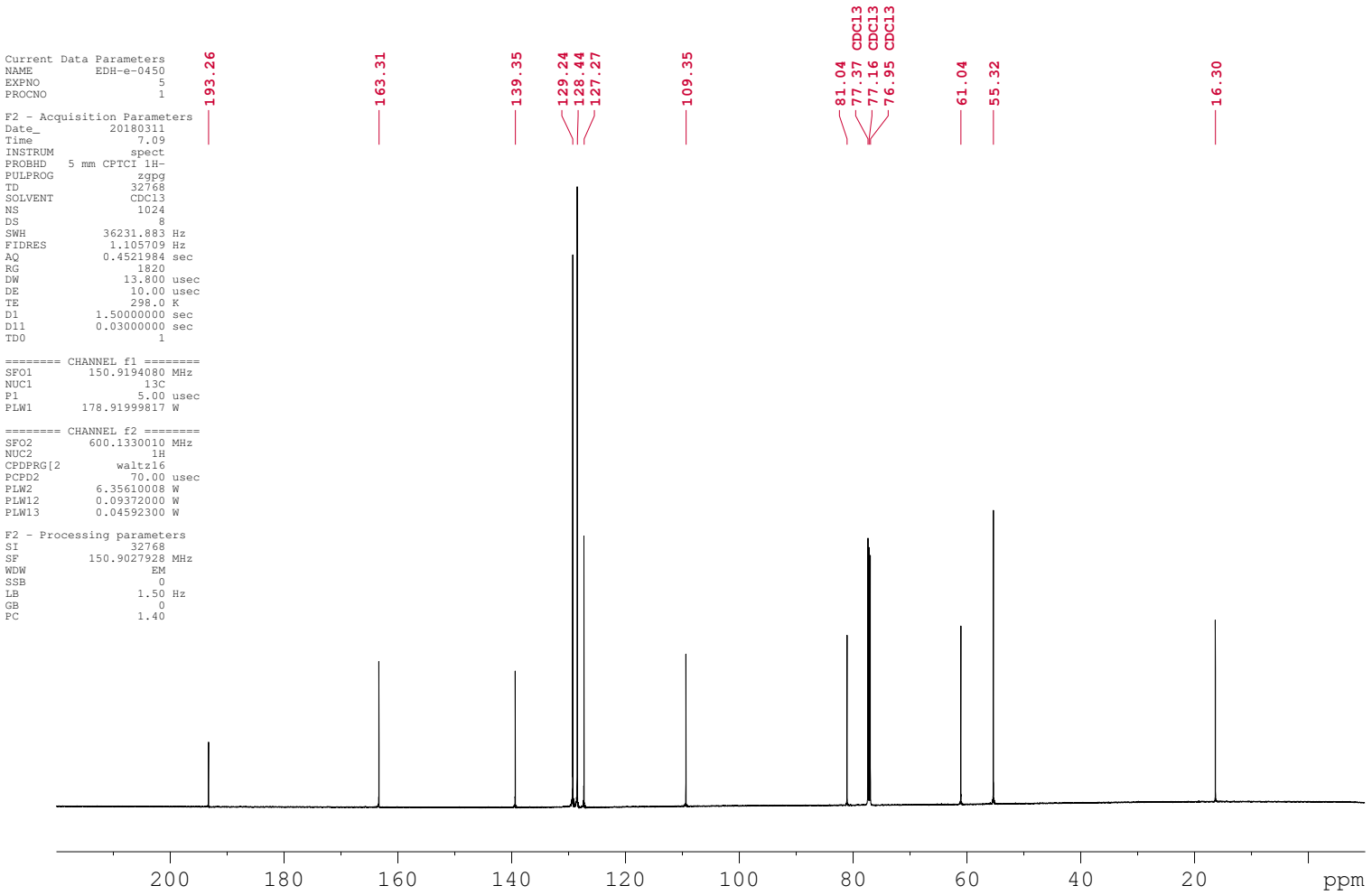


$^{13}C\{^{1}H\}$ NMR of Compound 217 (151 MHz, $CDCl_3$)



217

EDH-e-0450_C1F26-54 (13C, 151 MHz, CDCl3)




```
Current Data Parameters
NAME          EDH-e-0450
EXPNO                  5
PROCNO                 1

F2 - Acquisition Parameters
Date_           20180311
Time                7.09
INSTRUM            spect
PROBHD   5 mm CPTCI 1H-
PULPROG            zgpg
TD                32768
SOLVENT           CDCl3
NS                 1024
DS                    8
SWH           36231.883 Hz
FIDRES         1.105709 Hz
AQ            0.4521984 sec
RG                 1820
DW               13.800 usec
DE               10.00 usec
TE               298.0 K
D1           1.50000000 sec
D11          0.03000000 sec
TD0                    1

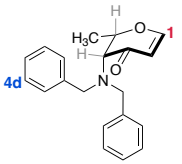
======== CHANNEL f1 ========
SFO1        150.9194080 MHz
NUC1               13C
P1                5.00 usec
PLW1       178.91999817 W

======== CHANNEL f2 ========
SFO2        600.1330010 MHz
NUC2               1H
CPDPRG[2         waltz16
PCPD2             70.00 usec
PLW2          6.35610008 W
PLW12         0.09372000 W
PLW13         0.04592300 W

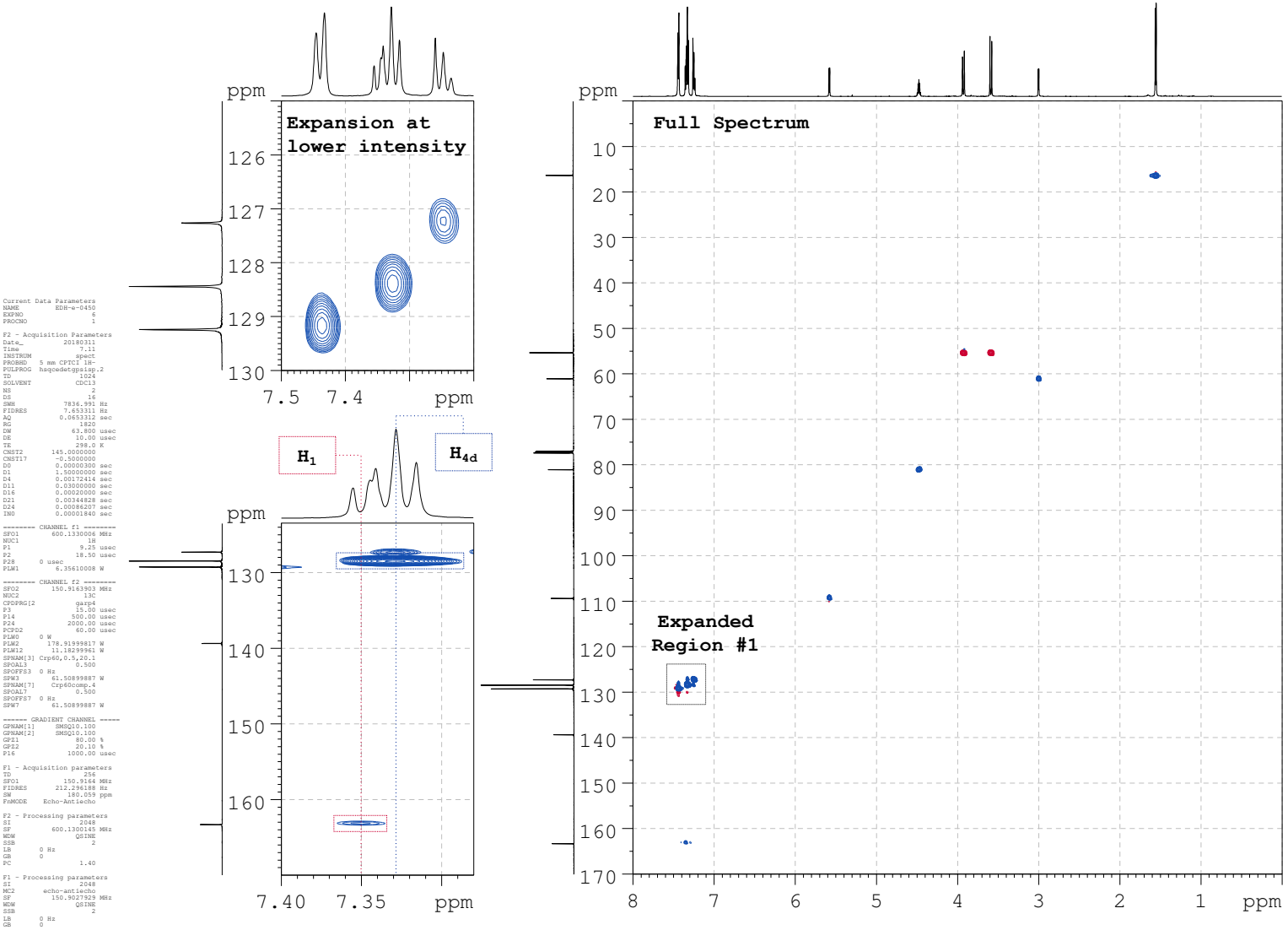
F2 - Processing parameters
SI                32768
SF          150.9027928 MHz
WDW                EM
SSB                   0
LB                1.50 Hz
GB                    0
PC                1.40
```

^{1}H-^{13}C HSQC{^{1}H}{^{13}C} NMR of Compound 217 (^{1}H-600 MHz, ^{13}C-151 MHz, $CDCl_3$)

Note: Blue = CH/CH_3 | Red = CH_2.



217



Current Data Parameters
NAME EDH-e-0450
EXPNO 6
PROCNO 1

F2 - Acquisition Parameters
Date_ 20180311
Time 7.11
INSTRUM spect
PROBHD 5 mm CPTCI 1H-
PULPROG hsqcedetgpsisp.2
TD 1024
SOLVENT CDCl3
NS 2
DS 16
SWH 7836.991 Hz
FIDRES 7.653311 Hz
AQ 0.0653312 sec
RG 1820
DW 63.800 usec
DE 10.00 usec
TE 298.0 K
CNST2 145.0000000
CNST17 -0.5000000
D0 0.00000300 sec
D1 1.50000000 sec
D4 0.00172414 sec
D11 0.03000000 sec
D16 0.00020000 sec
D21 0.00344828 sec
D24 0.00086207 sec
IN0 0.00001840 sec

======== CHANNEL f1 ========
SFO1 600.1330006 MHz
NUC1 1H
P1 9.25 usec
P2 18.50 usec
P28 0 usec
PLW1 6.35610008 W

======== CHANNEL f2 ========
SFO2 150.9163903 MHz
NUC2 13C
CPDPRG[2 garp4
P3 15.00 usec
P14 500.00 usec
P24 2000.00 usec
PCPD2 60.00 usec
PLW0 0 W
PLW2 178.91999817 W
PLW12 11.18299961 W
SPNAM[3] Crp60,0.5,20.1
SPOAL3 0.500
SPOFFS3 0 Hz
SPW3 61.50899887 W
SPNAM[7] Crp60comp.4
SPOAL7 0.500
SPOFFS7 0 Hz
SPW7 61.50899887 W

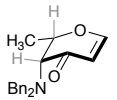
====== GRADIENT CHANNEL =====
GPNAM[1] SMSQ10.100
GPNAM[2] SMSQ10.100
GPZ1 80.00 %
GPZ2 20.10 %
P16 1000.00 usec

F1 - Acquisition parameters
TD 256
SFO1 150.9164 MHz
FIDRES 212.296188 Hz
SW 180.059 ppm
FnMODE Echo-Antiecho

F2 - Processing parameters
SI 2048
SF 600.1300145 MHz
WDW QSINE
SSB 2
LB 0 Hz
GB 0
PC 1.40

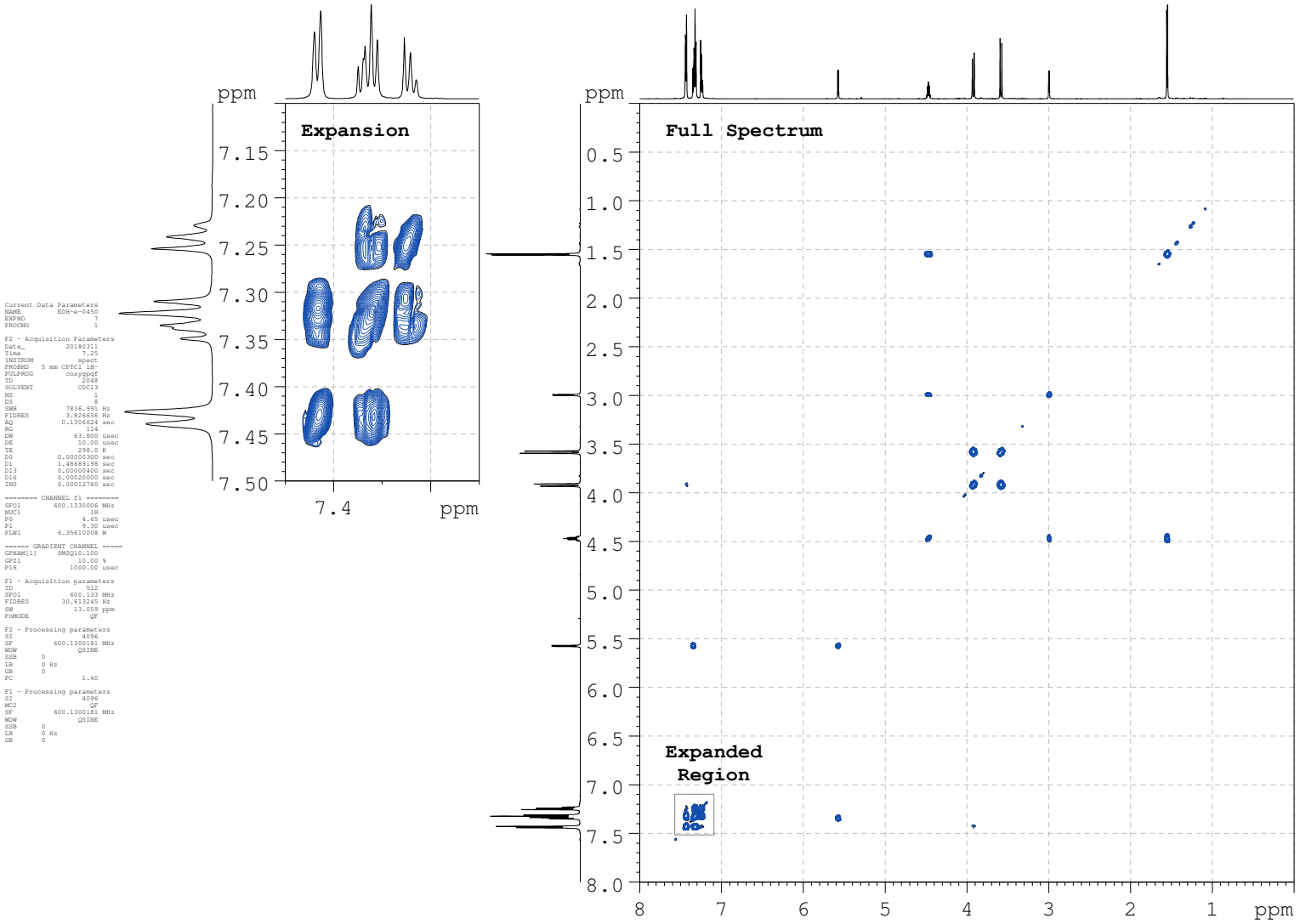
F1 - Processing parameters
SI 2048
MC2 echo-antiecho
SF 150.9027929 MHz
WDW QSINE
SSB 2
LB 0 Hz
GB 0

^{1}H-^{1}H COSY NMR of Compound 217 (600 MHz, $CDCl_3$)



217

EDH-e-0450_C1F26-54 (COSY, 600 MHz, CDCl3)



Current Data Parameters
NAME EDH-e-0450
EXPNO 7
PROCNO 1

F2 - Acquisition Parameters
Date_ 20180311
Time 7.25
INSTRUM spect
PROBHD 5 mm CPTCI 1H-
PULPROG cosygpqf
TD 2048
SOLVENT CDCl3
NS 1
DS 8
SWH 7836.991 Hz
FIDRES 3.826656 Hz
AQ 0.1306624 sec
RG 114
DW 63.800 usec
DE 10.00 usec
TE 298.0 K
D0 0.00000300 sec
D1 1.48689198 sec
D13 0.00000400 sec
D16 0.00020000 sec
IN0 0.00012760 sec

======== CHANNEL f1 ========
SFO1 600.1330006 MHz
NDC1 1H
P0 4.65 usec
P1 9.30 usec
PLW1 6.35610008 W

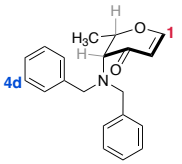
====== GRADIENT CHANNEL =====
GPNAM[1] SMSQ10.100
GPZ1 10.00 %
P16 1000.00 usec

F1 - Acquisition parameters
TD 512
SFO1 600.133 MHz
FIDRES 30.613245 Hz
SW 13.059 ppm
FnMODE QF

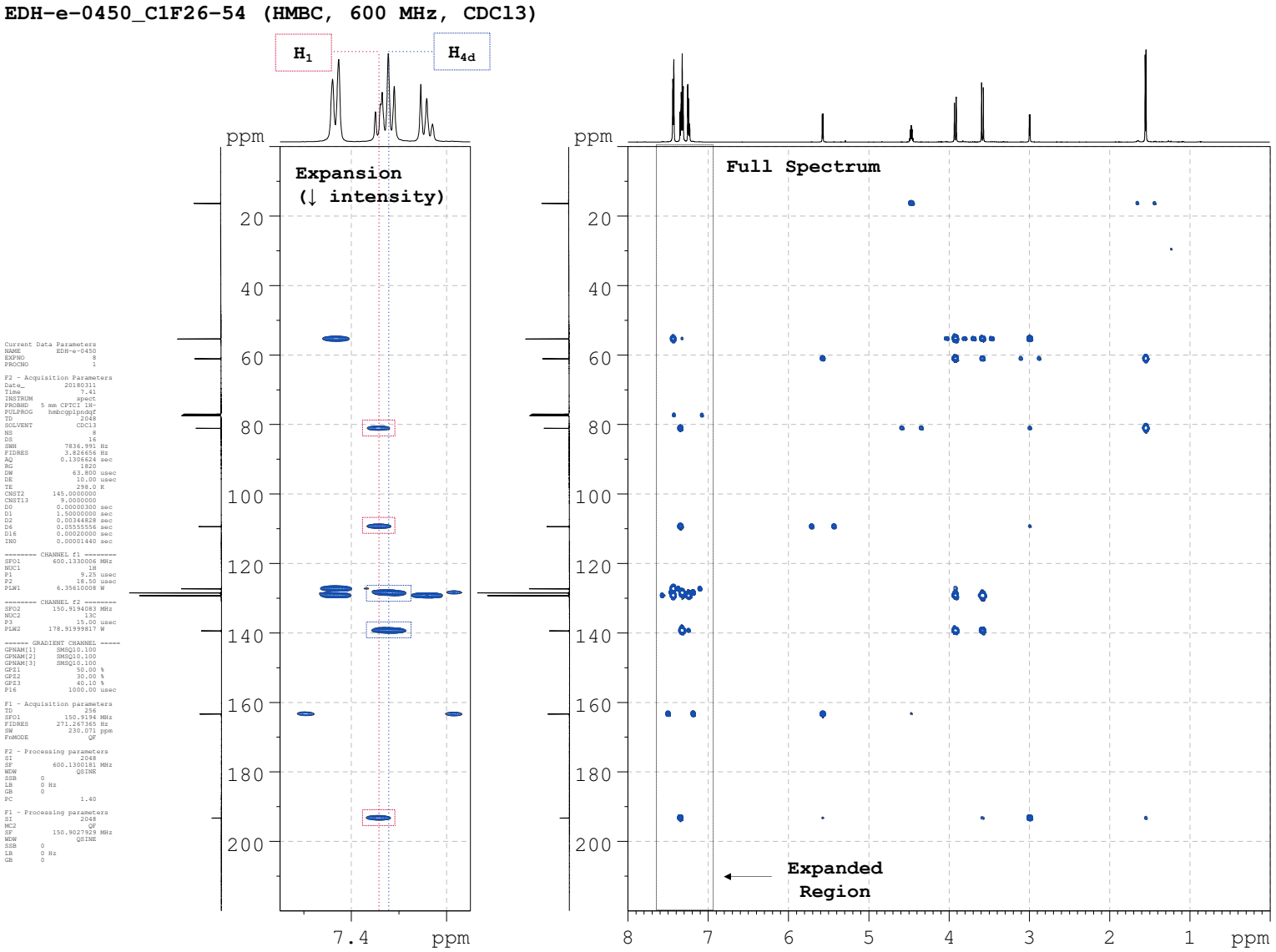
F2 - Processing parameters
SI 4096
SF 600.1300181 MHz
WDW QSINE
SSB 0
LB 0 Hz
GB 0
PC 1.40

F1 - Processing parameters
SI 4096
MC2 QF
SF 600.1300181 MHz
WDW QSINE
SSB 0
LB 0 Hz
GB 0

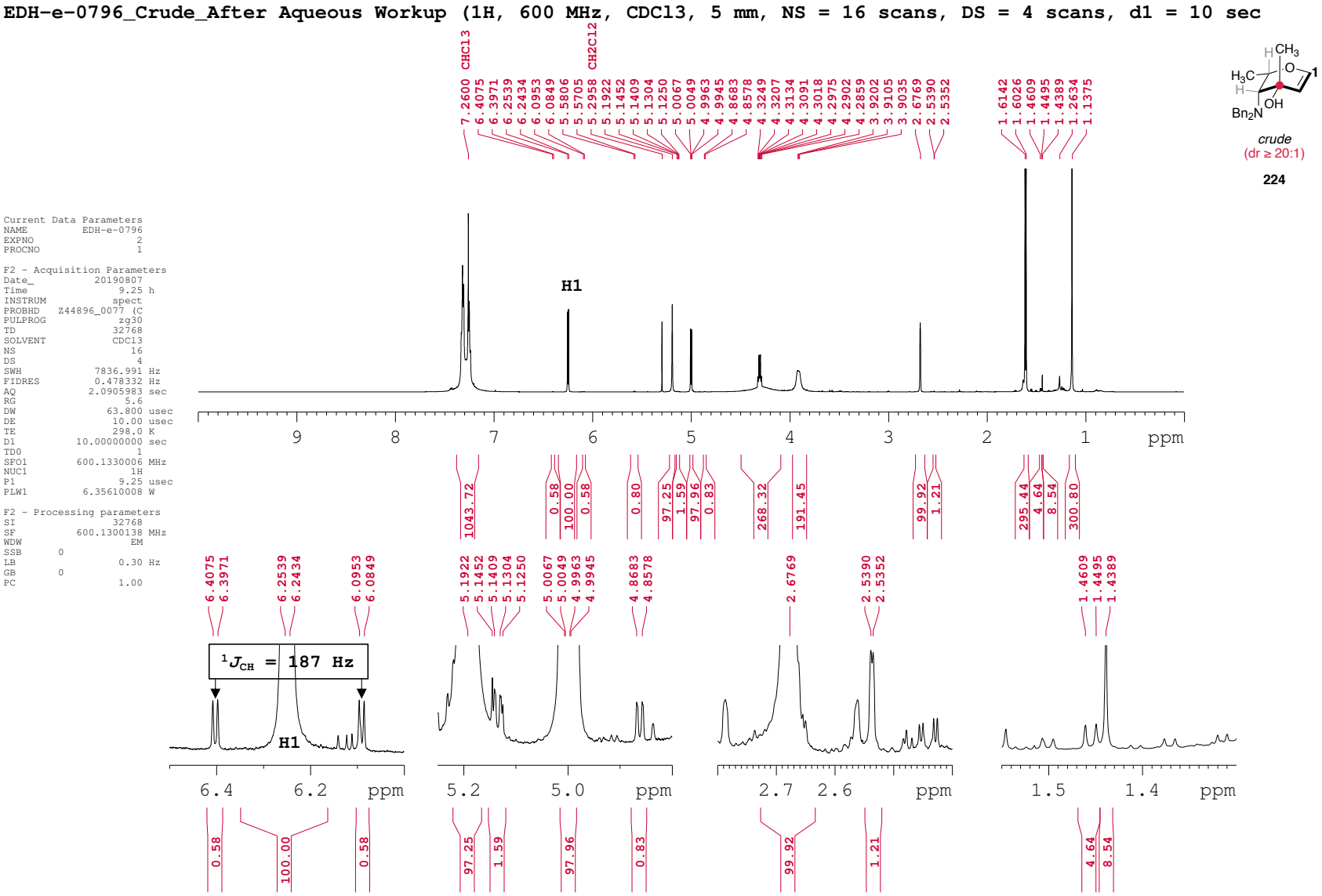
^{1}H-^{13}C HMBC NMR of Compound 217 (^{1}H-600 MHz, ^{13}C-151 MHz, $CDCl_3$)



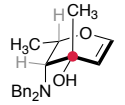
217



^{1}H NMR of Crude Compound 224 (600 MHz, $CDCl_3$)



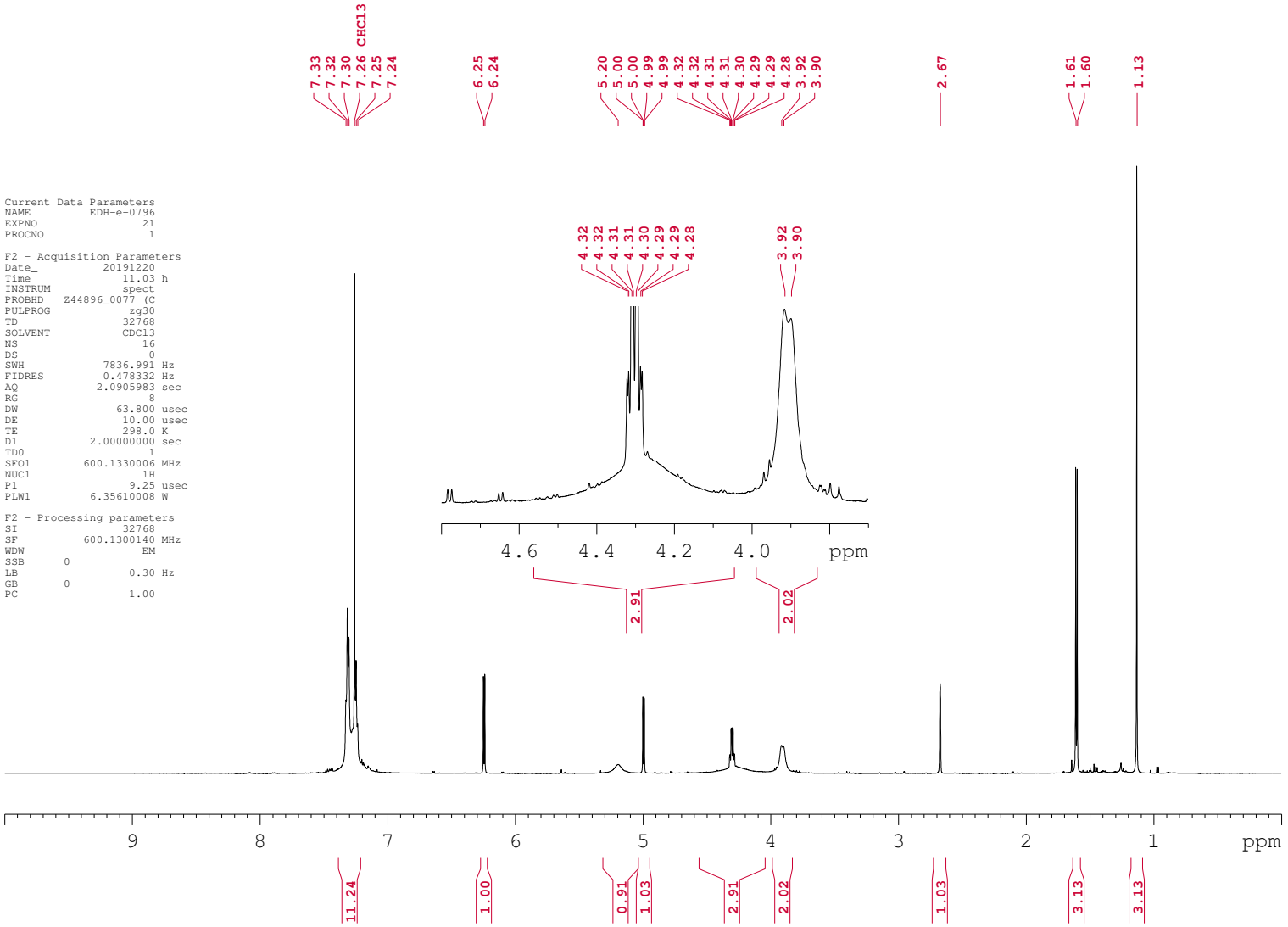
^{1}H NMR of Purified Compound 224 (600 MHz, $CDCl_3$)



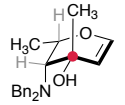
purified
(dr ≥ 20:1)

224

EDH-e-0796_C1F17-23 (1H. 600 MHz, CDCl3, 5 mm, NS = 16 scans)



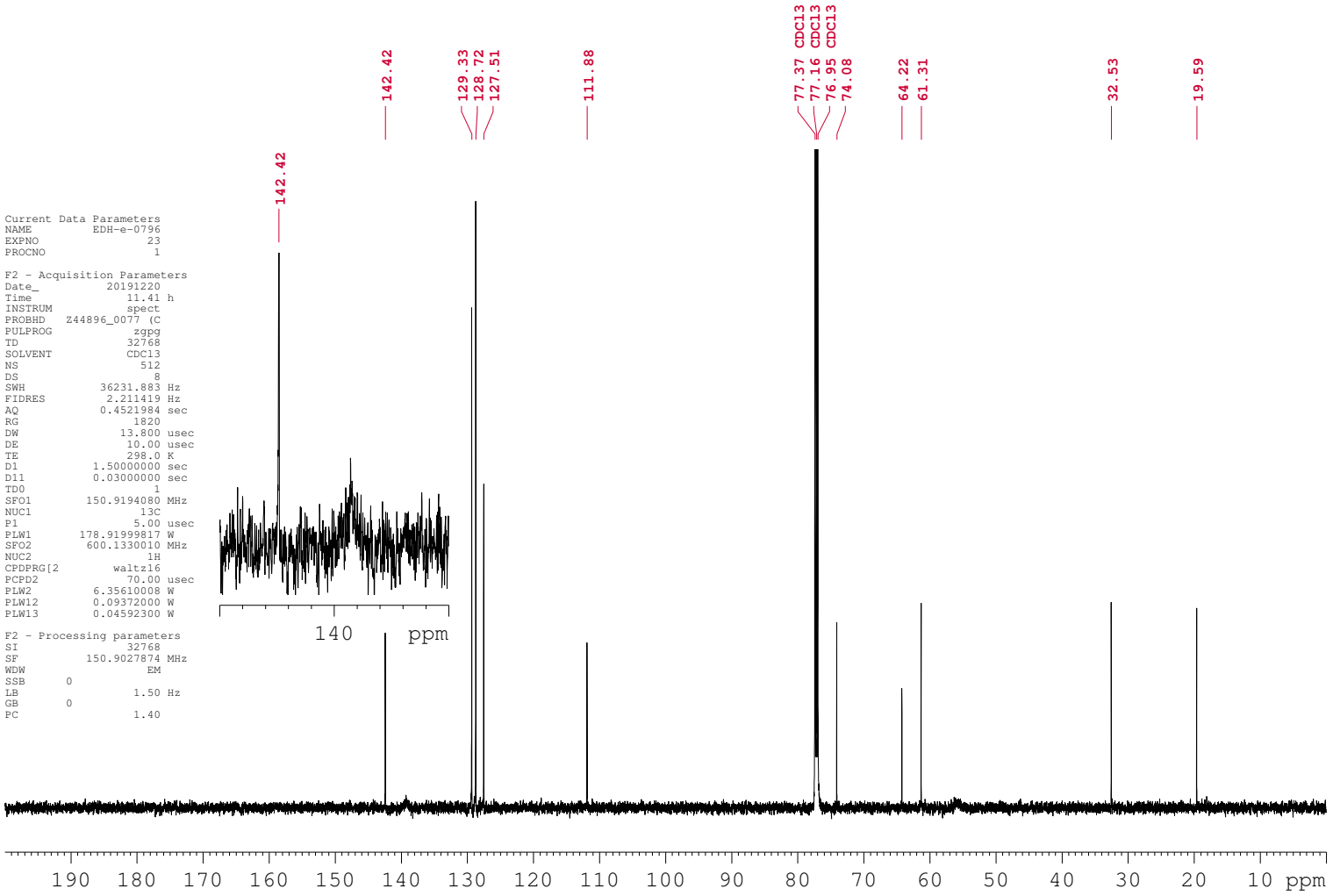
$^{13}C\{^{1}H\}$ NMR of Purified Compound 224 (151 MHz, $CDCl_3$)



purified
(dr ≥ 20:1)

224

EDH-e-0796_C1F17-23 (13C{1H}. 151 MHz, CDCl3, 5 mm, NS = 512 scans)



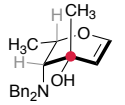

```
Current Data Parameters
NAME          EDH-e-0796
EXPNO                 23
PROCNO                 1

F2 - Acquisition Parameters
Date_           20191220
Time               11.41 h
INSTRUM             spect
PROBHD    Z44896_0077 (C
PULPROG             zgpg
TD                 32768
SOLVENT            CDCl3
NS                   512
DS                     8
SWH            36231.883 Hz
FIDRES          2.211419 Hz
AQ             0.4521984 sec
RG                  1820
DW                13.800 usec
DE                10.00 usec
TE                298.0 K
D1            1.50000000 sec
D11           0.03000000 sec
TD0                    1
SFO1         150.9194080 MHz
NUC1                13C
P1                 5.00 usec
PLW1        178.91999817 W
SFO2         600.1330010 MHz
NUC2                1H
CPDPRG[2         waltz16
PCPD2             70.00 usec
PLW2           6.35610008 W
PLW12          0.09372000 W
PLW13          0.04592300 W

F2 - Processing parameters
SI                 32768
SF           150.9027874 MHz
WDW                   EM
SSB       0
LB                 1.50 Hz
GB        0
PC                 1.40
```

^{1}H-^{13}C HSQC{^{1}H}{^{13}C} NMR of Purified Compound 224 (^{1}H-600 MHz, ^{13}C-151 MHz, $CDCl_3$)

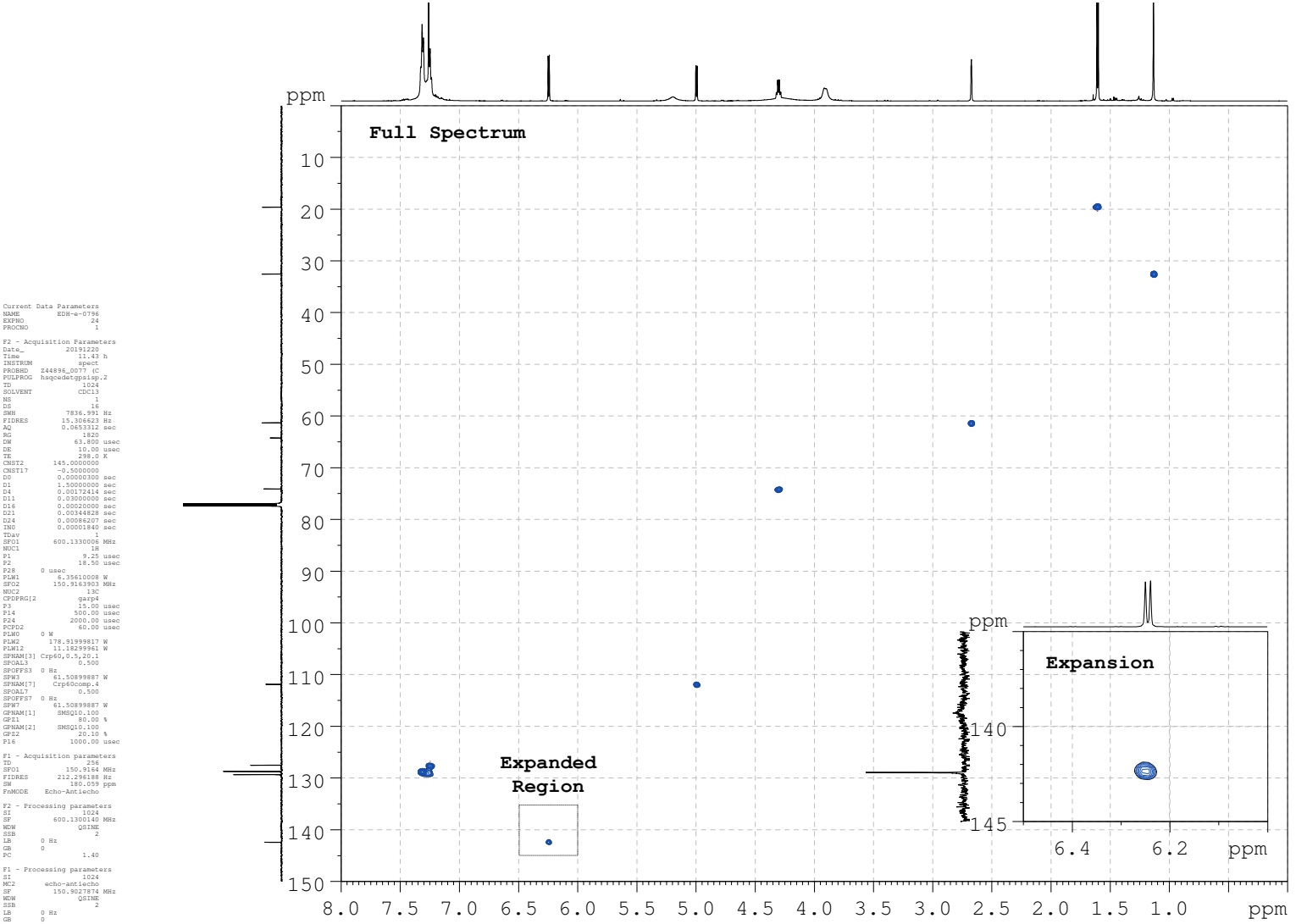
Note: Blue = CH/CH_3 | Red = CH_2.



purified
(dr ≥ 20:1)

224

EDH-e-0796_C1F17-23 (HSQC{1H}{13C}. 600 MHz, CDCl3, 5 mm, NS = 1 scan)




```
Current Data Parameters
NAME          EDH-e-0796
EXPNO                 24
PROCNO                 1

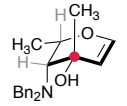
F2 - Acquisition Parameters
Date_           20191220
Time              11.43 h
INSTRUM             spect
PROBHD   Z44896_0077 (C
PULPROG  hsqcedetgpsisp.2
TD                  1024
SOLVENT            CDCl3
NS                     1
DS                    16
SWH              7836.991 Hz
FIDRES          15.306623 Hz
AQ               0.0653312 sec
RG                  1820
DW                 63.800 usec
DE                 10.00 usec
TE                298.0 K
CNST2          145.0000000
CNST17          -0.5000000
D0               0.00000300 sec
D1               1.50000000 sec
D4               0.00172414 sec
D11              0.03000000 sec
D16              0.00020000 sec
D21              0.00344828 sec
D24              0.00086207 sec
IN0              0.00001840 sec
TDav                   1
SFO1           600.1330006 MHz
NDC1                  1H
P1                  9.25 usec
P2                 18.50 usec
P28        0 usec
PLW1            6.35610008 W
SFO2          150.9163903 MHz
NUC2                  13C
CPDPRG[2              garp4
P3                 15.00 usec
P14               500.00 usec
P24              2000.00 usec
PCPD2             60.00 usec
PLW0       0 W
PLW2         178.91999817 W
PLW12         11.18299961 W
SPNAM[3] Crp60,0.5,20.1
SPOAL3                0.500
SPOFFS3    0 Hz
SPW3           61.50899887 W
SPNAM[7]     Crp60comp.4
SPOAL7                0.500
SPOFFS7    0 Hz
SPW7           61.50899887 W
GPNAM[1]       SMSQ10.100
GPZ1                80.00 %
GPNAM[2]       SMSQ10.100
GPZ2                20.10 %
P16               1000.00 usec

F1 - Acquisition parameters
TD                    256
SFO1            150.9164 MHz
FIDRES          212.296188 Hz
SW                180.059 ppm
FnMODE     Echo-Antiecho

F2 - Processing parameters
SI                   1024
SF            600.1300140 MHz
WDW                  QSINE
SSB                     2
LB         0 Hz
GB         0
PC                   1.40

F1 - Processing parameters
SI                   1024
MC2         echo-antiecho
SF            150.9027874 MHz
WDW                  QSINE
SSB                     2
LB         0 Hz
GB         0
```

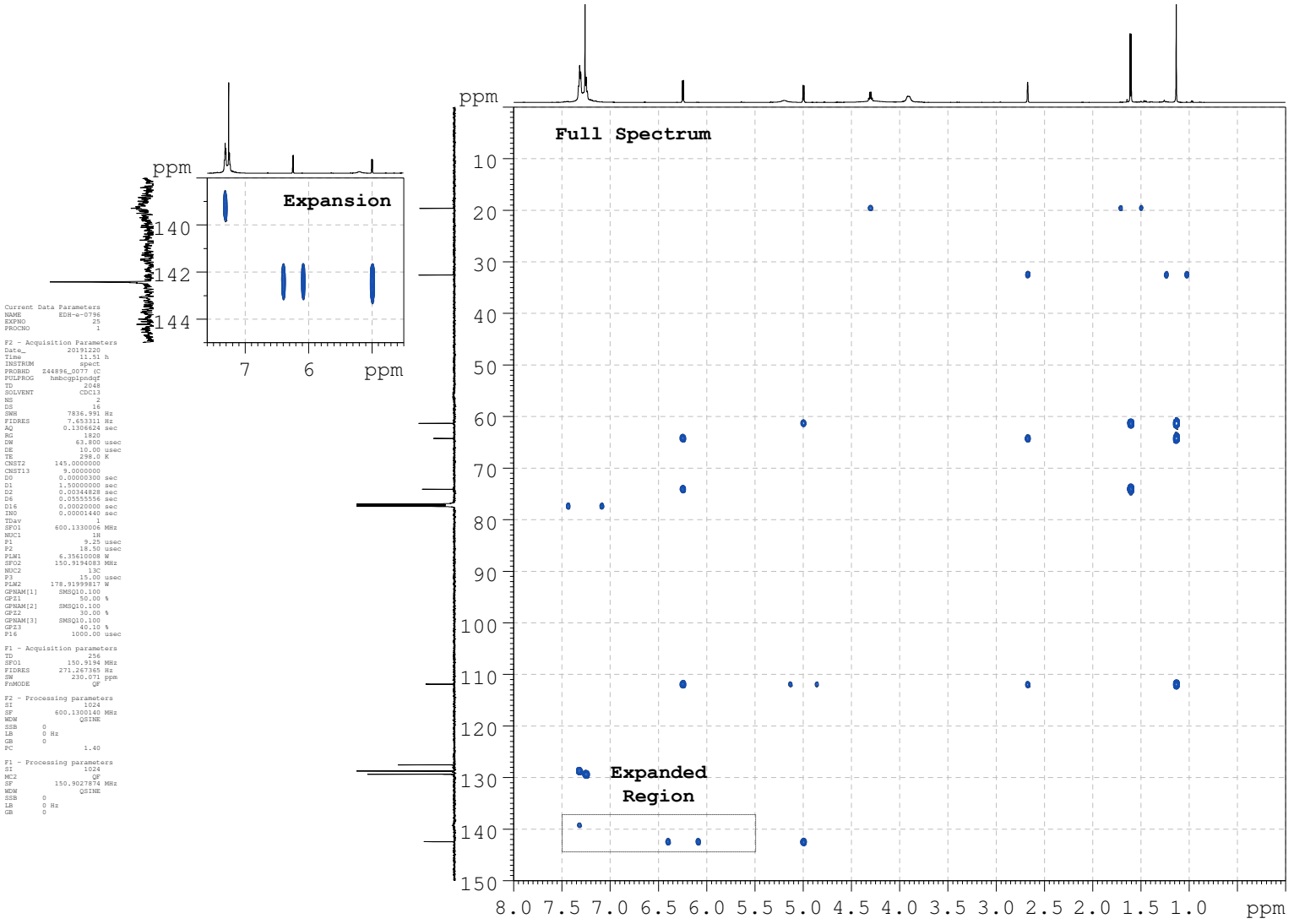
^{1}H-^{13}C HMBC NMR of Purified Compound 224(^{1}H-600 MHz, ^{13}C-151 MHz, $CDCl_3$)



purified
(dr ≥ 20:1)

224

EDH-e-0796_C1F17-23 (HMBC, 600 MHz, CDCl3, 5 mm, NS = 2 scans)



Current Data Parameters
NAME EDH-e-0796
EXPNO 25
PROCNO 1

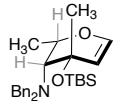
F2 - Acquisition Parameters
Date_ 20191220
Time 11.51 h
INSTRUM spect
PROBHD Z44896_0077 (C
PULPROG hmbcgplpndqf
TD 2048
SOLVENT CDCl3
NS 2
DS 16
SWH 7836.991 Hz
FIDRES 7.653311 Hz
AQ 0.1306624 sec
RG 1820
DW 63.800 usec
DE 10.00 usec
TE 298.0 K
CNST2 145.0000000
CNST13 9.0000000
D0 0.00000300 sec
D1 1.50000000 sec
D2 0.00344828 sec
D6 0.05555556 sec
D16 0.00020000 sec
IN0 0.00001440 sec
TDav 1
SFO1 600.1330006 MHz
NUC1 1H
P1 9.25 usec
P2 18.50 usec
PLW1 6.35610008 W
SFO2 150.9194083 MHz
NUC2 13C
P3 15.00 usec
PLW2 178.91999817 W
GPNAM[1] SMSQ10.100
GPZ1 50.00 %
GPNAM[2] SMSQ10.100
GPZ2 30.00 %
GPNAM[3] SMSQ10.100
GPZ3 40.10 %
P16 1000.00 usec

F1 - Acquisition parameters
TD 256
SFO1 150.9194 MHz
FIDRES 271.267365 Hz
SW 230.071 ppm
FnMODE QF

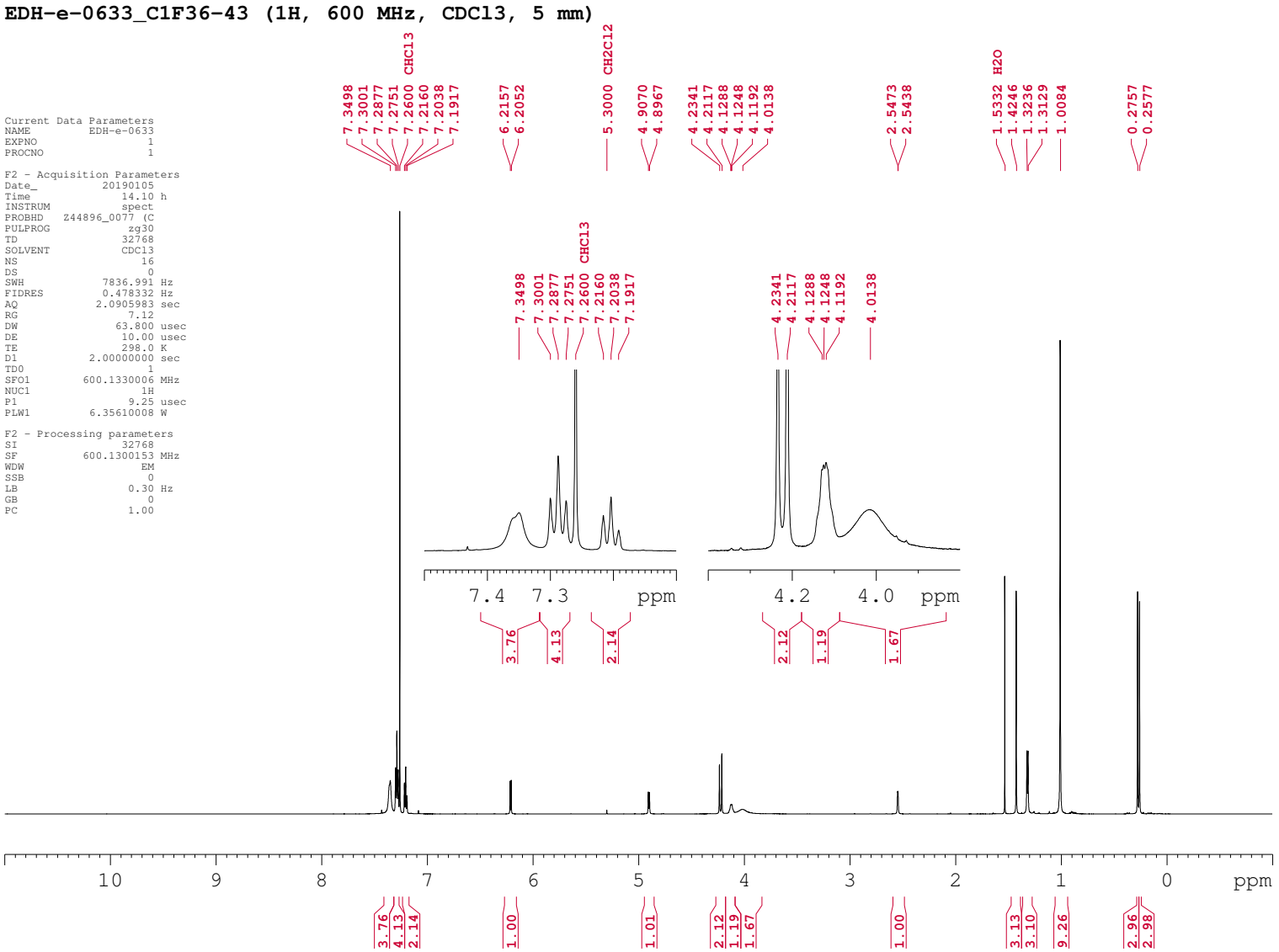
F2 - Processing parameters
SI 1024
SF 600.1300140 MHz
WDW QSINE
SSB 0
LB 0 Hz
GB 0
PC 1.40

F1 - Processing parameters
SI 1024
MC2 QF
SF 150.9027874 MHz
WDW QSINE
SSB 0
LB 0 Hz
GB 0

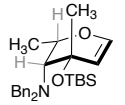
^{1}H NMR of Compound 225 (600 MHz, $CDCl_3$)



225

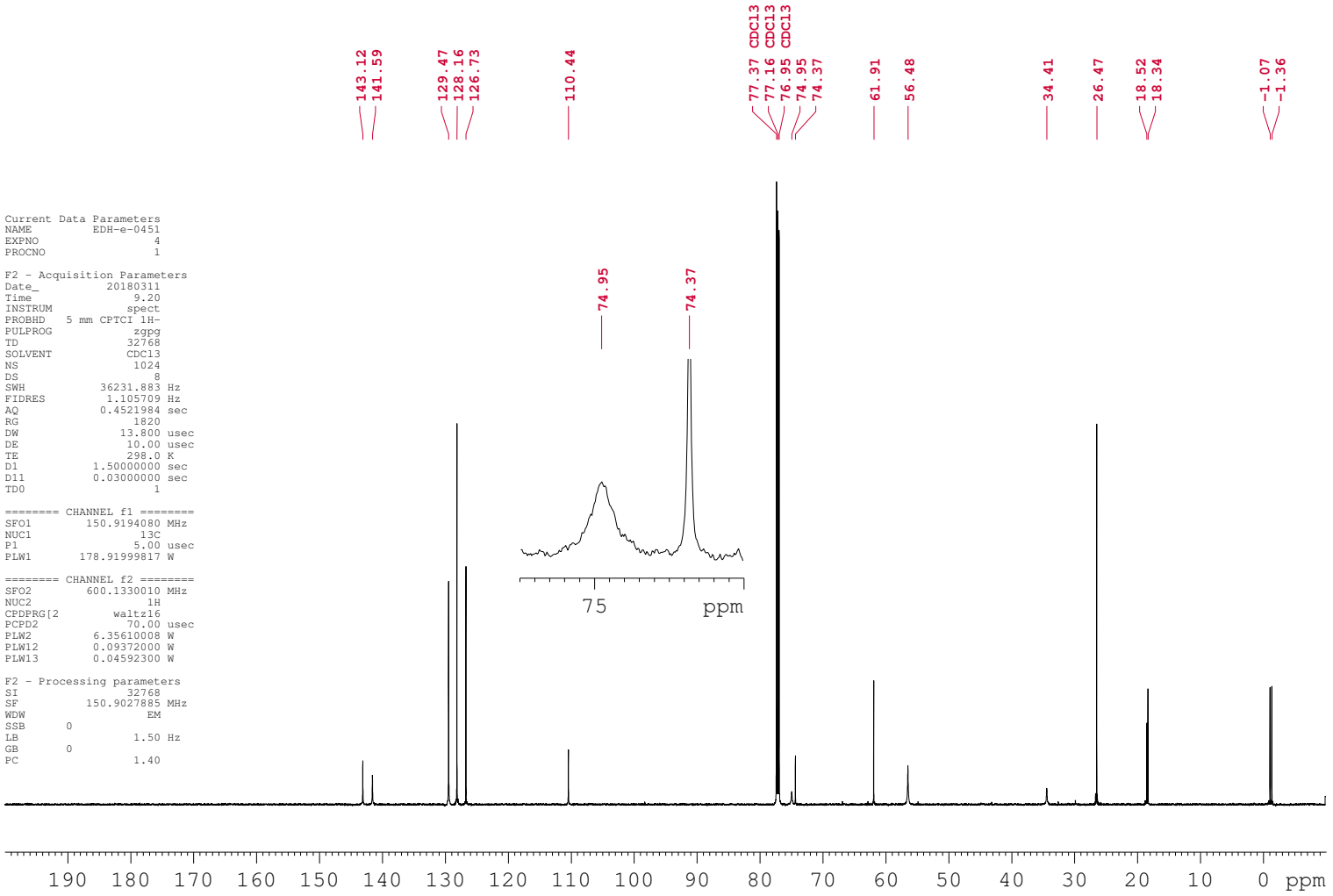


$^{13}C\{^{1}H\}$ NMR of Compound 225 (151 MHz, $CDCl_3$)



225

EDH-e-0451_C1F25-32_Bulk Material (13C, 151 MHz, CDCl3)




```
Current Data Parameters
NAME          EDH-e-0451
EXPNO                  4
PROCNO                 1

F2 - Acquisition Parameters
Date_           20180311
Time                9.20
INSTRUM            spect
PROBHD    5 mm CPTCI 1H-
PULPROG            zgpg
TD                32768
SOLVENT           CDCl3
NS                 1024
DS                    8
SWH           36231.883 Hz
FIDRES         1.105709 Hz
AQ            0.4521984 sec
RG                 1820
DW               13.800 usec
DE               10.00 usec
TE                298.0 K
D1           1.50000000 sec
D11          0.03000000 sec
TD0                    1

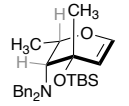
======== CHANNEL f1 ========
SFO1        150.9194080 MHz
NUC1                  13C
P1                 5.00 usec
PLW1       178.91999817 W

======== CHANNEL f2 ========
SFO2        600.1330010 MHz
NUC2                   1H
CPDPRG[2          waltz16
PCPD2             70.00 usec
PLW2          6.35610008 W
PLW12         0.09372000 W
PLW13         0.04592300 W

F2 - Processing parameters
SI                 32768
SF          150.9027885 MHz
WDW                   EM
SSB       0
LB                 1.50 Hz
GB        0
PC                 1.40
```

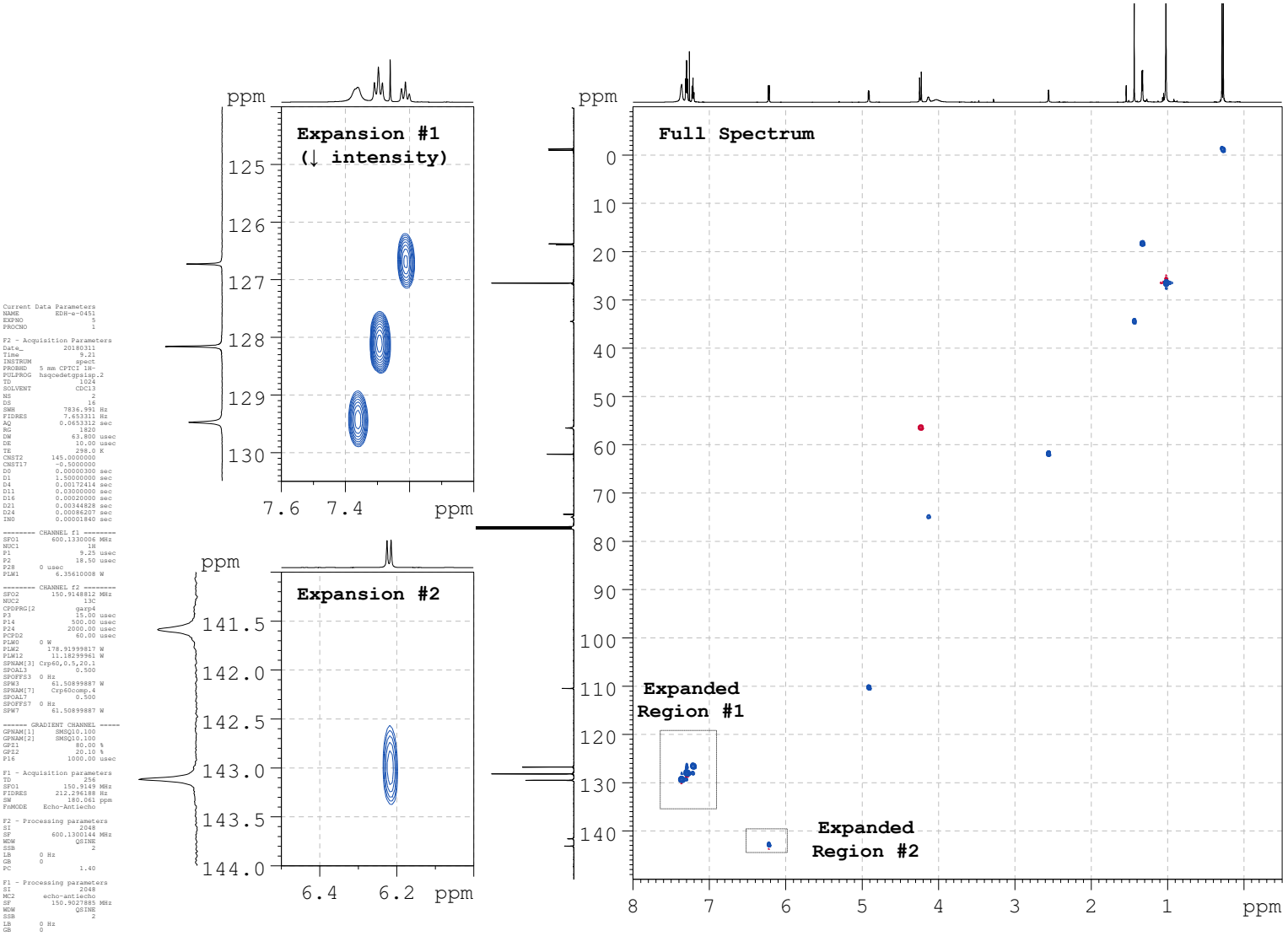
^{1}H-^{13}C HSQC{^{1}H}{^{13}C} NMR of Compound 225 (^{1}H-600 MHz, ^{13}C-151 MHz, $CDCl_3$)

Note: Blue = CH/CH_3 | Red = CH_2.

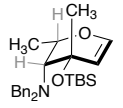


225

EDH-e-0451_C1F25-32_Bulk Material (HSQC{1H}{13C}, 600 MHz, CDCl3, O2P = 80 ppm)

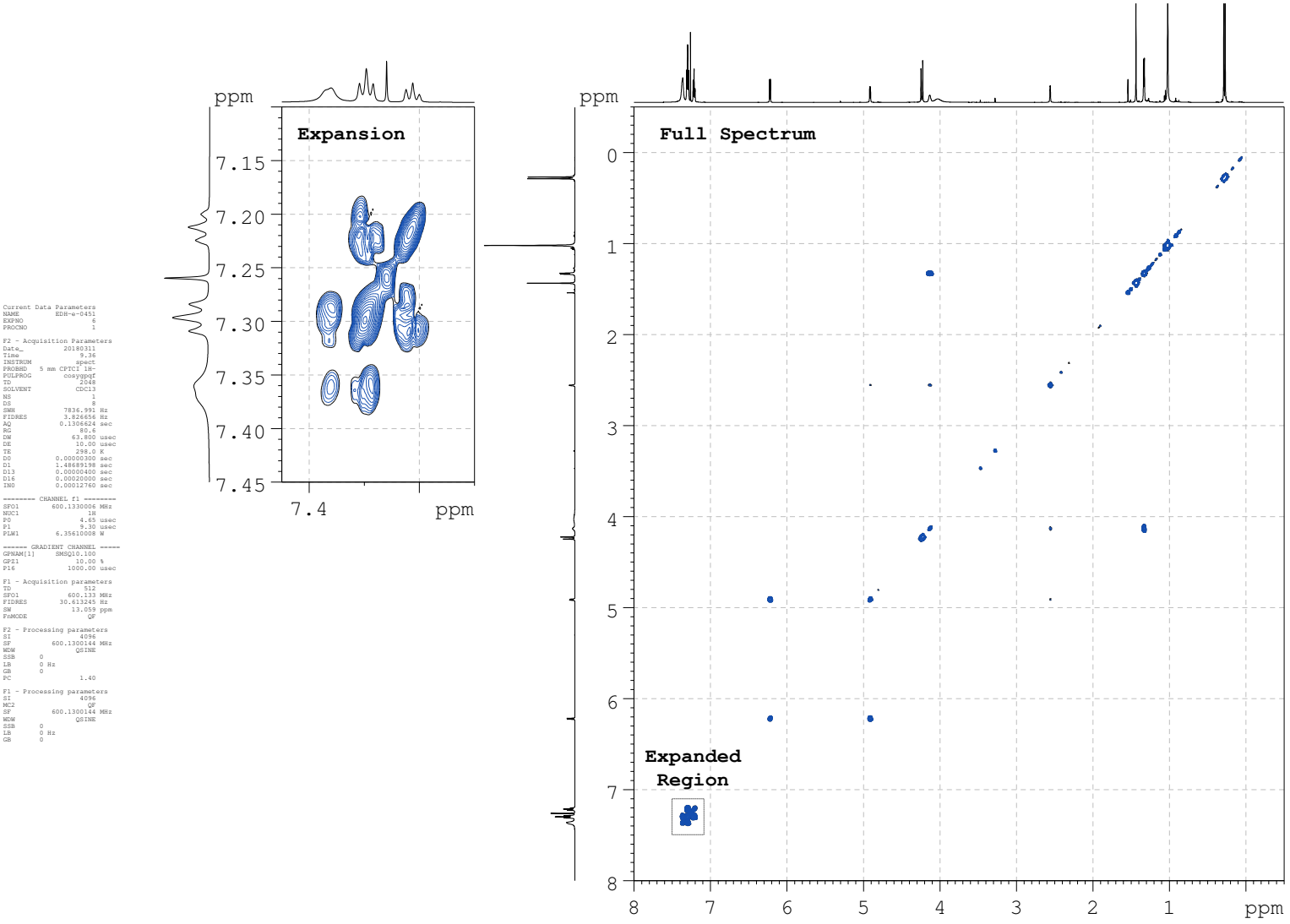


^{1}H-^{1}H COSY NMR of Compound 225 (600 MHz, $CDCl_3$)



225

EDH-e-0451_C1F25-32_Bulk Material (COSY, 600 MHz, CDCl3)



Current Data Parameters
NAME EDH-e-0451
EXPNO 6
PROCNO 1

F2 - Acquisition Parameters
Date_ 20180311
Time 9.36
INSTRUM spect
PROBHD 5 mm CPTCI 1H-
PULPROG cosygpqf
TD 2048
SOLVENT CDCl3
NS 1
DS 8
SWH 7836.991 Hz
FIDRES 3.826656 Hz
AQ 0.1306624 sec
RG 80.6
DW 63.800 usec
DE 10.00 usec
TE 298.0 K
D0 0.00000300 sec
D1 1.48689198 sec
D13 0.00000400 sec
D16 0.00020000 sec
IN0 0.00012760 sec

======== CHANNEL f1 ========
SFO1 600.1330006 MHz
NDC1 1H
P0 4.65 usec
P1 9.30 usec
PLW1 6.35610008 W

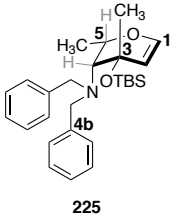
====== GRADIENT CHANNEL =====
GPNAM[1] SMSQ10.100
GPZ1 10.00 %
P16 1000.00 usec

F1 - Acquisition parameters
TD 512
SFO1 600.133 MHz
FIDRES 30.613245 Hz
SW 13.059 ppm
FnMODE QF

F2 - Processing parameters
SI 4096
SF 600.1300144 MHz
WDW QSINE
SSB 0
LB 0 Hz
GB 0
PC 1.40

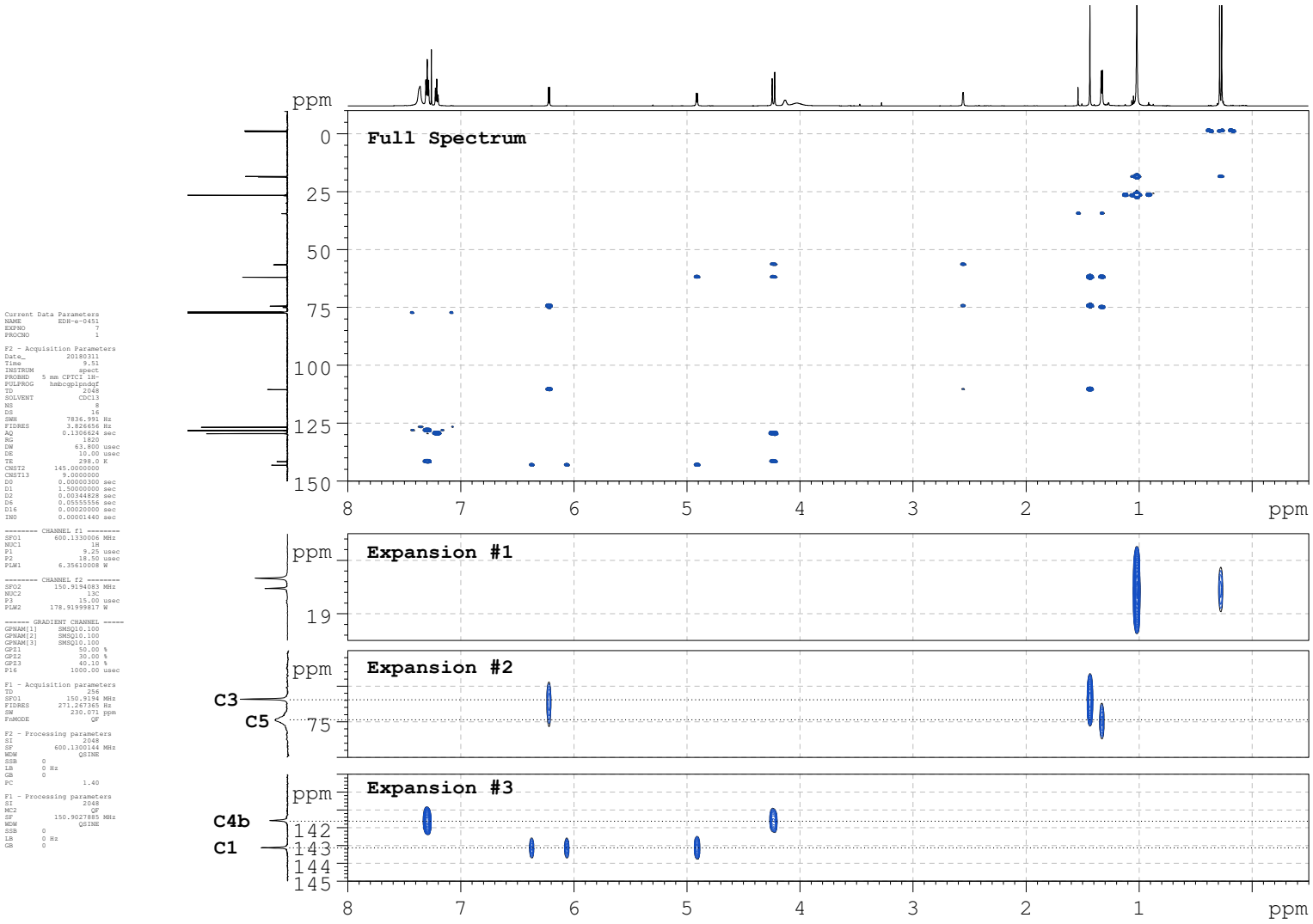
F1 - Processing parameters
SI 4096
MC2 QF
SF 600.1300144 MHz
WDW QSINE
SSB 0
LB 0 Hz
GB 0

^{1}H-^{13}C HMBC NMR of Compound 225 (^{1}H-600 MHz, ^{13}C-151 MHz, $CDCl_3$)



225

EDH-e-0451_C1F25-32_Bulk Material (HMBC, 600 MHz, CDCl3)



Current Data Parameters
NAME EDH-e-0451
EXPNO 7
PROCNO 1

F2 - Acquisition Parameters
Date_ 20180311
Time 9.51
INSTRUM spect
PROBHD 5 mm CPTCI 1H-
PULPROG hmbcgplpndqf
TD 2048
SOLVENT CDCl3
NS 8
DS 16
SWH 7836.991 Hz
FIDRES 3.826656 Hz
AQ 0.1306624 sec
RG 1820
DW 63.800 usec
DE 10.00 usec
TE 298.0 K
CNST2 145.0000000
CNST13 9.0000000
D0 0.00000300 sec
D1 1.50000000 sec
D2 0.00344828 sec
D6 0.05555556 sec
D16 0.00020000 sec
IN0 0.00001440 sec

======== CHANNEL f1 ========
SFO1 600.1330006 MHz
NUC1 1H
P1 9.25 usec
P2 18.50 usec
PLW1 6.35610008 W

======== CHANNEL f2 ========
SFO2 150.9194083 MHz
NUC2 13C
P3 15.00 usec
PLW2 178.91999817 W

====== GRADIENT CHANNEL =====
GPNAM[1] SMSQ10.100
GPNAM[2] SMSQ10.100
GPNAM[3] SMSQ10.100
GPZ1 50.00 %
GPZ2 30.00 %
GPZ3 40.10 %
P16 1000.00 usec

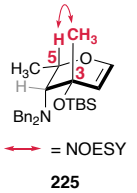
F1 - Acquisition parameters
TD 256
SFO1 150.9194 MHz
FIDRES 271.267365 Hz
SW 230.071 ppm
FnMODE QF

F2 - Processing parameters
SI 2048
SF 600.1300144 MHz
WDW QSINE
SSB 0
LB 0 Hz
GB 0
PC 1.40

F1 - Processing parameters
SI 2048
MC2 QF
SF 150.9027885 MHz
WDW QSINE
SSB 0
LB 0 Hz
GB 0

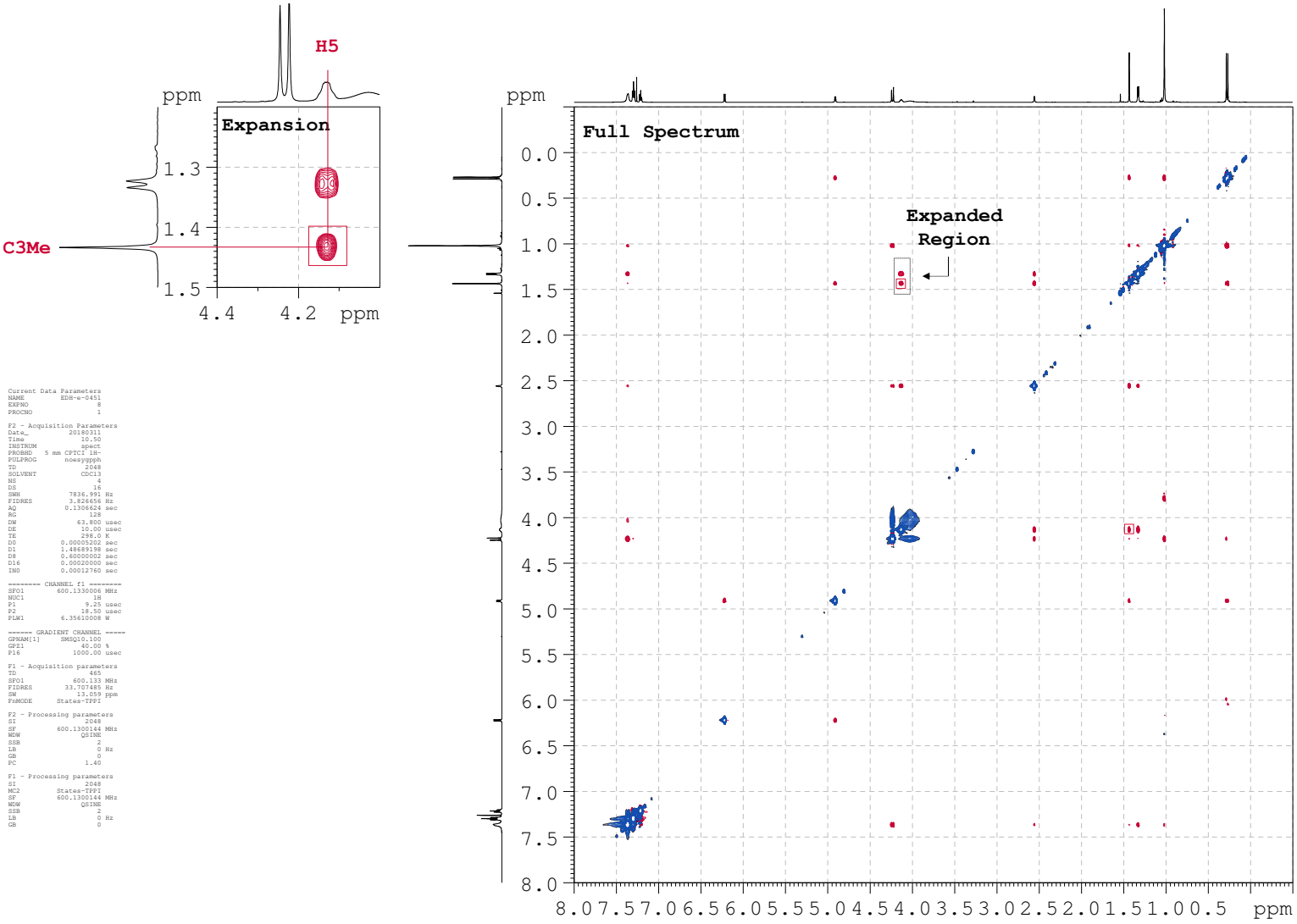
^{1}H-^{1}H NOESY NMR of Compound 225 (600 MHz, $CDCl_3$)

Note: Blue = diagonal/chemical exchange | Red = NOESY cross peaks.



225

EDH-e-0451_C1F25-32_Bulk Material (NOESY, 600 MHz, CDCl3)




```
Current Data Parameters
NAME          EDH-e-0451
EXPNO                  8
PROCNO                 1

F2 - Acquisition Parameters
Date_          20180311
Time              10.50
INSTRUM           spect
PROBHD   5 mm CPTCI 1H-
PULPROG       noesygpph
TD                 2048
SOLVENT           CDCl3
NS                    4
DS                   16
SWH            7836.991 Hz
FIDRES         3.826656 Hz
AQ             0.1306624 sec
RG                  128
DW                63.800 usec
DE                10.00 usec
TE                298.0 K
D0           0.00005202 sec
D1           1.48689198 sec
D8           0.60000002 sec
D16          0.00020000 sec
IN0          0.00012760 sec

======== CHANNEL f1 ========
SFO1        600.1330006 MHz
NUC1               1H
P1                9.25 usec
P2               18.50 usec
PLW1         6.35610008 W

====== GRADIENT CHANNEL =====
GPNAM[1]      SMSQ10.100
GPZ1               40.00 %
P16              1000.00 usec

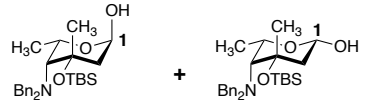
F1 - Acquisition parameters
TD                  465
SFO1          600.133 MHz
FIDRES       33.707485 Hz
SW           13.059 ppm
FnMODE       States-TPPI

F2 - Processing parameters
SI                 2048
SF          600.1300144 MHz
WDW                QSINE
SSB                   2
LB                 0 Hz
GB                    0
PC                 1.40

F1 - Processing parameters
SI                 2048
MC2          States-TPPI
SF          600.1300144 MHz
WDW                QSINE
SSB                   2
LB                 0 Hz
GB                    0
```

^{1}H NMR of Compound α/β-226 (ca. 2:3 α/β) (600 MHz, $CDCl_3$)

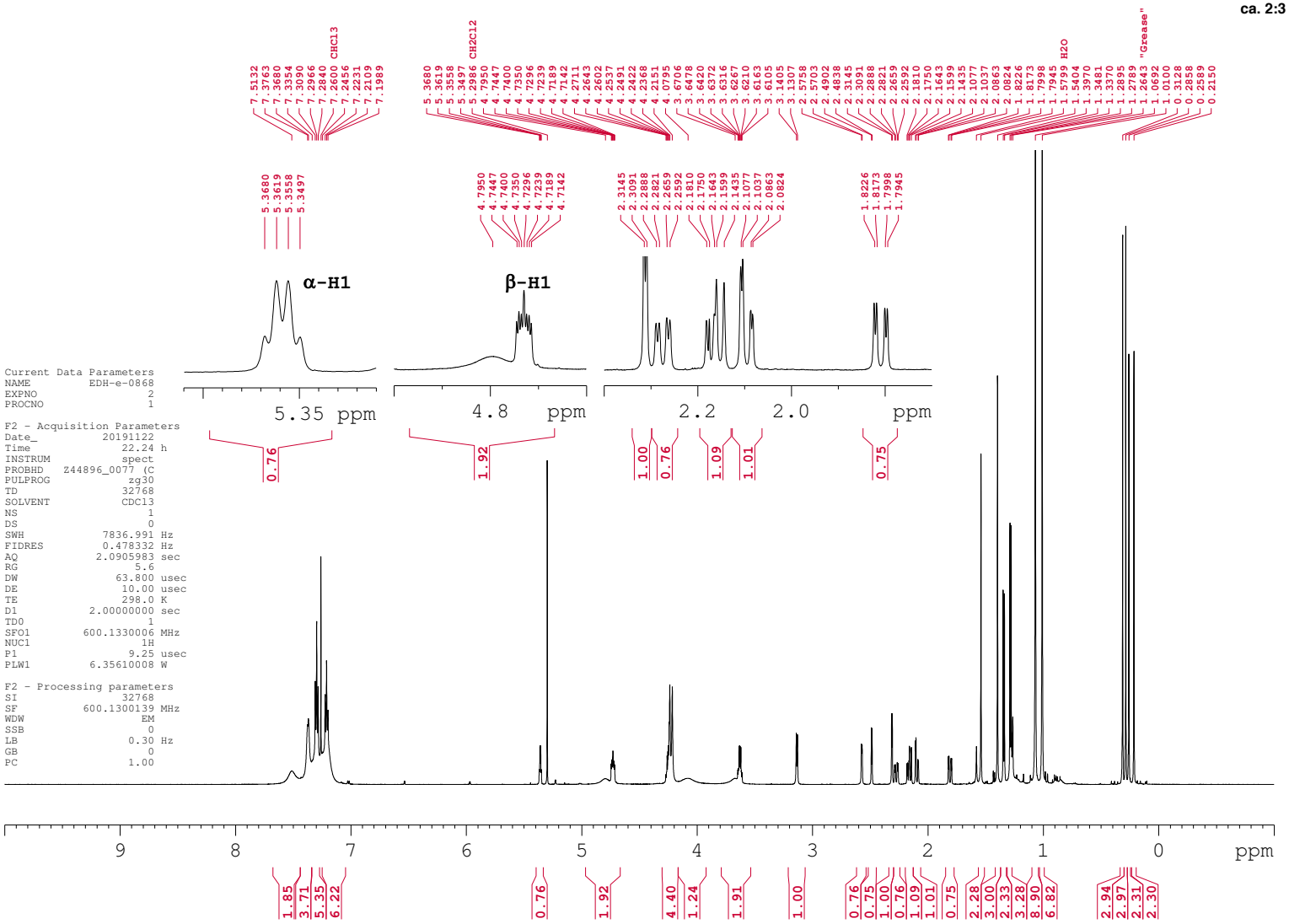
[1]Note: This is the ^{1}H NMR spectrum of α/β-**226** used for the line listing and peak assignment.



α-226 + β-226

ca. 2:3 α/β

EDH-e-0868_C1F16-19 (1H, 600 MHz, CDCl3, 5 mm, NS = 1 scan)

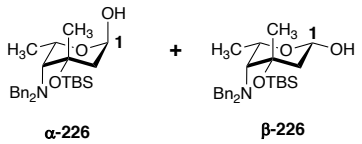



```
Current Data Parameters
NAME          EDH-e-0868
EXPNO                  2
PROCNO                 1

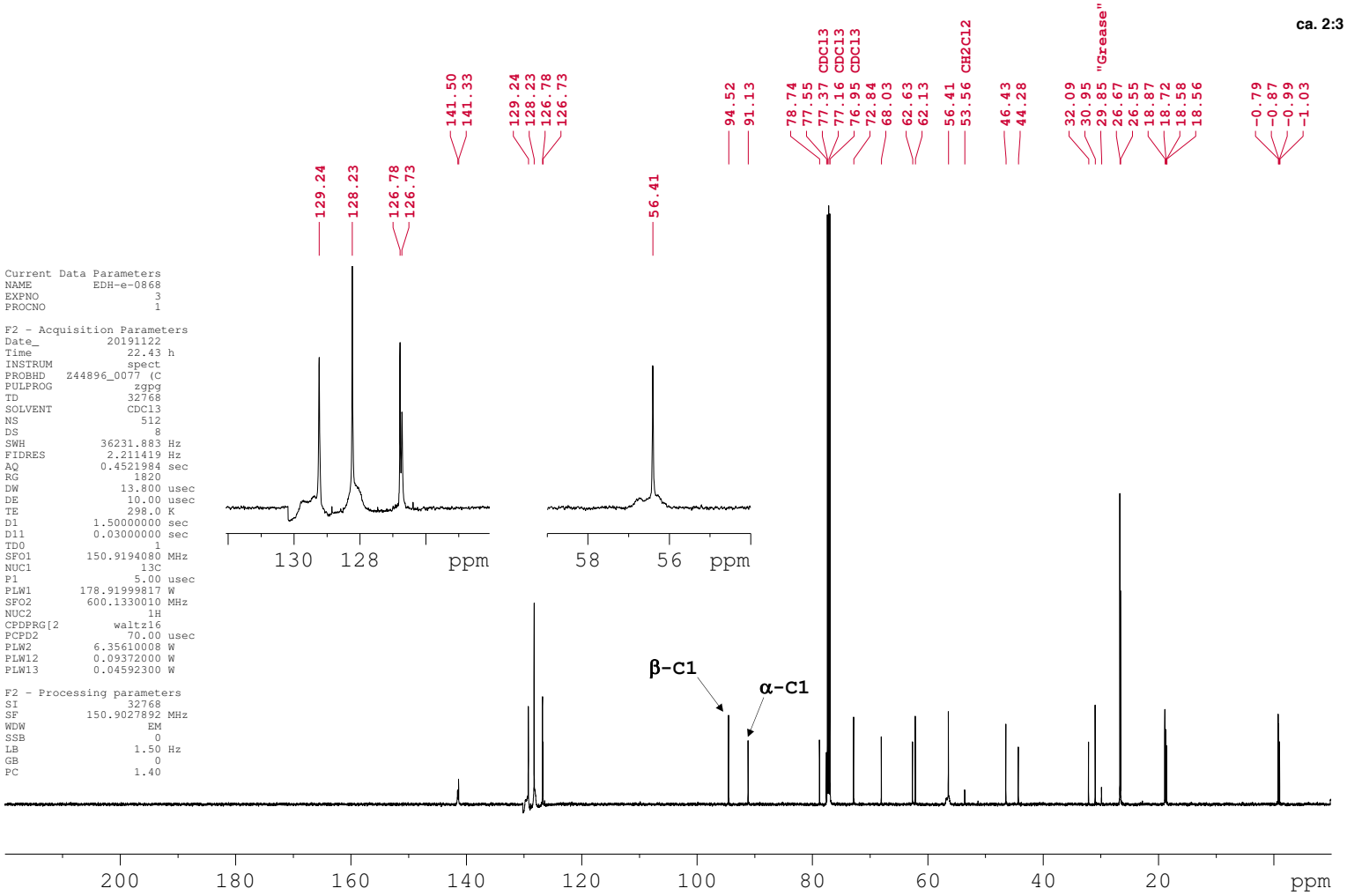
F2 - Acquisition Parameters
Date_           20191122
Time               22.24 h
INSTRUM             spect
PROBHD    Z44896_0077 (C
PULPROG             zg30
TD                 32768
SOLVENT            CDCl3
NS                     1
DS                     0
SWH             7836.991 Hz
FIDRES          0.478332 Hz
AQ              2.0905983 sec
RG                   5.6
DW                63.800 usec
DE                10.00 usec
TE                298.0 K
D1             2.00000000 sec
TD0                    1
SFO1         600.1330006 MHz
NUC1                  1H
P1                 9.25 usec
PLW1          6.35610008 W

F2 - Processing parameters
SI                 32768
SF           600.1300139 MHz
WDW                   EM
SSB                    0
LB                  0.30 Hz
GB                     0
PC                  1.00
```

$^{13}C\{^{1}H\}$ NMR of Compound α/β-226 (ca. 2:3 α/β) (151 MHz, $CDCl_3$)



EDH-e-0868_C1F16-19 (13C,{1H} 151 MHz, CDCl3, 5 mm, NS = 512 scans)



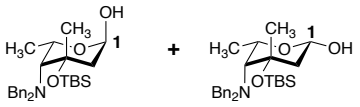

```
Current Data Parameters
NAME          EDH-e-0868
EXPNO                  3
PROCNO                 1

F2 - Acquisition Parameters
Date_           20191122
Time               22.43 h
INSTRUM             spect
PROBHD   Z44896_0077 (C
PULPROG             zgpg
TD                 32768
SOLVENT            CDCl3
NS                   512
DS                     8
SWH            36231.883 Hz
FIDRES          2.211419 Hz
AQ              0.4521984 sec
RG                  1820
DW                13.800 usec
DE                10.00 usec
TE                298.0 K
D1             1.50000000 sec
D11            0.03000000 sec
TD0                    1
SFO1         150.9194080 MHz
NUC1                 13C
P1                  5.00 usec
PLW1        178.91999817 W
SFO2         600.1330010 MHz
NUC2                 1H
CPDPRG[2          waltz16
PCPD2              70.00 usec
PLW2          6.35610008 W
PLW12         0.09372000 W
PLW13         0.04592300 W

F2 - Processing parameters
SI                 32768
SF           150.9027892 MHz
WDW                   EM
SSB                    0
LB                  1.50 Hz
GB                     0
PC                  1.40
```

^{1}H-^{13}C HSQC{^{1}H}{^{13}C} NMR of Compound α/β-226 (ca. 2:3 α/β) (^{1}H-600 MHz, ^{13}C-151 MHz, $CDCl_3$)

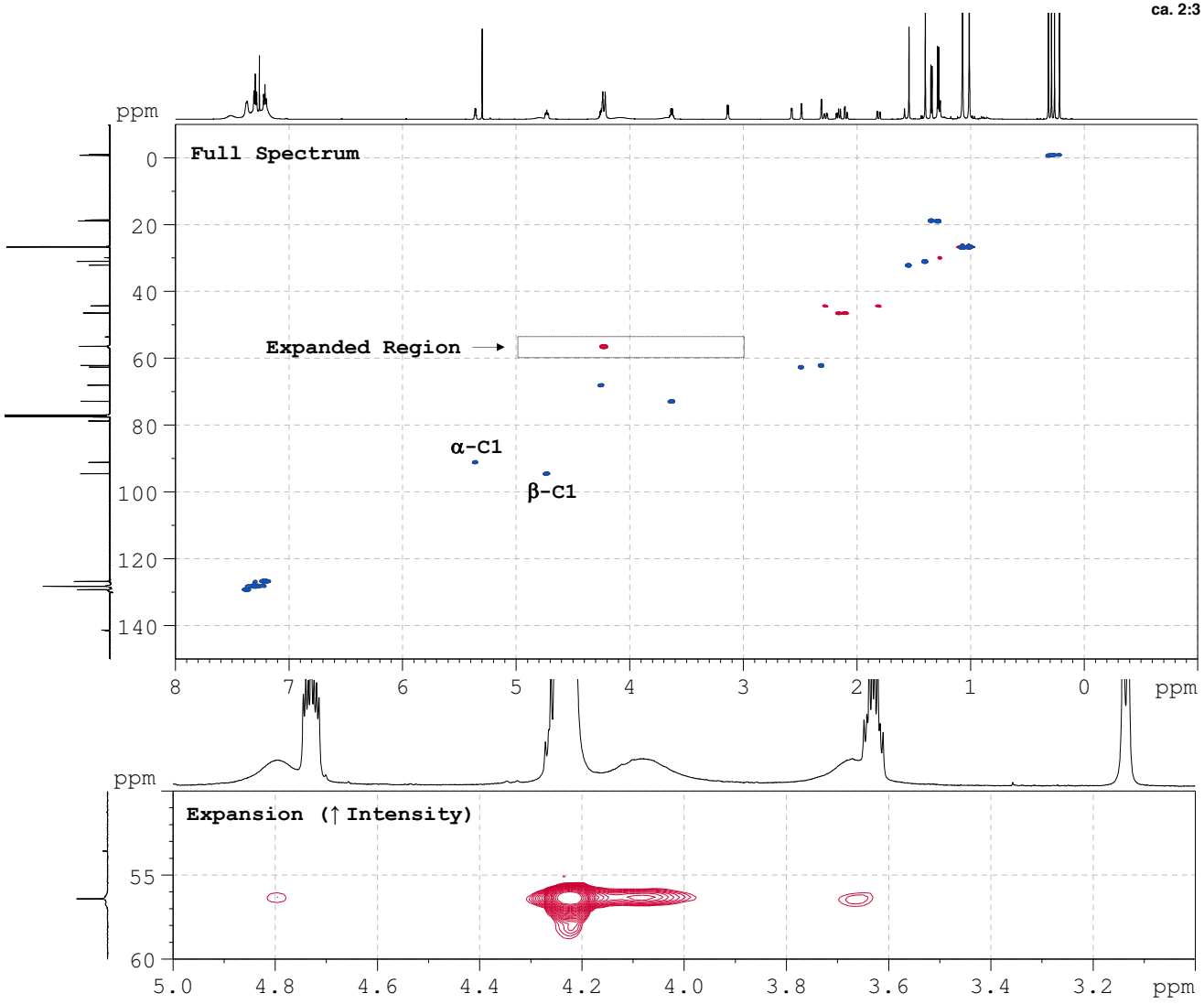
Note: Blue = CH/CH_3 | Red = CH_2.



α-226 β-226

ca. 2:3 α/β

EDH-e-0868_C1F16-19 (HSQC{1H}{13C}, 600 MHz, CDCl3, 5 mm, NS = 1 scan, O2P = 80 ppm)



Current Data Parameters
NAME EDH-e-0868
EXPNO 4
PROCNO 1

F2 - Acquisition Parameters
Date_ 20191122
Time 22.44 h
INSTRUM spect
PROBHD Z44896_0077 (C
PULPROG hsqcedetgpsisp.2
TD 1024
SOLVENT CDCl3
NS 1
DS 16
SWH 7836.991 Hz
FIDRES 15.306623 Hz
AQ 0.0653312 sec
RG 1820
DW 63.800 usec
DE 10.00 usec
TE 298.0 K
CNST2 145.0000000
CNST17 -0.5000000
D0 0.00000300 sec
D1 1.50000000 sec
D4 0.00172414 sec
D11 0.03000000 sec
D16 0.00020000 sec
D21 0.00344828 sec
D24 0.00086207 sec
IN0 0.00001840 sec
TDav 1
SFO1 600.1330006 MHz
NUC1 1H
P1 9.25 usec
P2 18.50 usec
P28 0 usec
PLW1 6.35610008 W
SFO2 150.9148812 MHz
NUC2 13C
CPDPRG[2 garp4
P3 15.00 usec
P14 500.00 usec
P24 2000.00 usec
PCPD2 60.00 usec
PLW0 0 W
PLW2 178.91999817 W
PLW12 11.18299961 W
SPNAM[3] Crp60,0.5,20.1
SPOAL3 0.500
SPOFFS3 0 Hz
SPW3 61.50899887 W
SPNAM[7] Crp60comp.4
SPOAL7 0.500
SPOFFS7 0 Hz
SPW7 61.50899887 W
GPNAM[1] SMSQ10.100
GPZ1 80.00 %
GPNAM[2] SMSQ10.100
GPZ2 20.10 %
P16 1000.00 usec

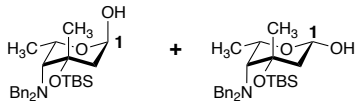
F1 - Acquisition parameters
TD 256
SFO1 150.9149 MHz
FIDRES 212.296188 Hz
SW 180.061 ppm
FnMODE Echo-Antiecho

F2 - Processing parameters
SI 1024
SF 600.1300139 MHz
WDW QSINE
SSB 2
LB 0 Hz
GB 0
PC 1.40

F1 - Processing parameters
SI 1024
MC2 echo-antiecho
SF 150.9027892 MHz
WDW QSINE
SSB 2
LB 0 Hz
GB 0

Coupled ^{1}H-^{13}C HSQC NMR of Compound α/β-226 (ca. 2:3 α/β) (^{1}H-600 MHz, ^{13}C-151 MHz, $CDCl_3$)

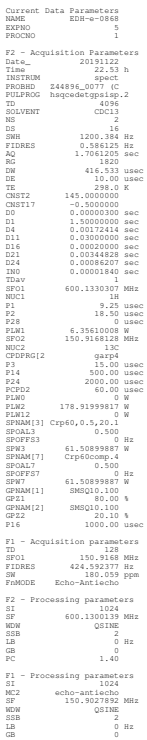
Note: Blue = CH/CH_3 | Red = CH_2.

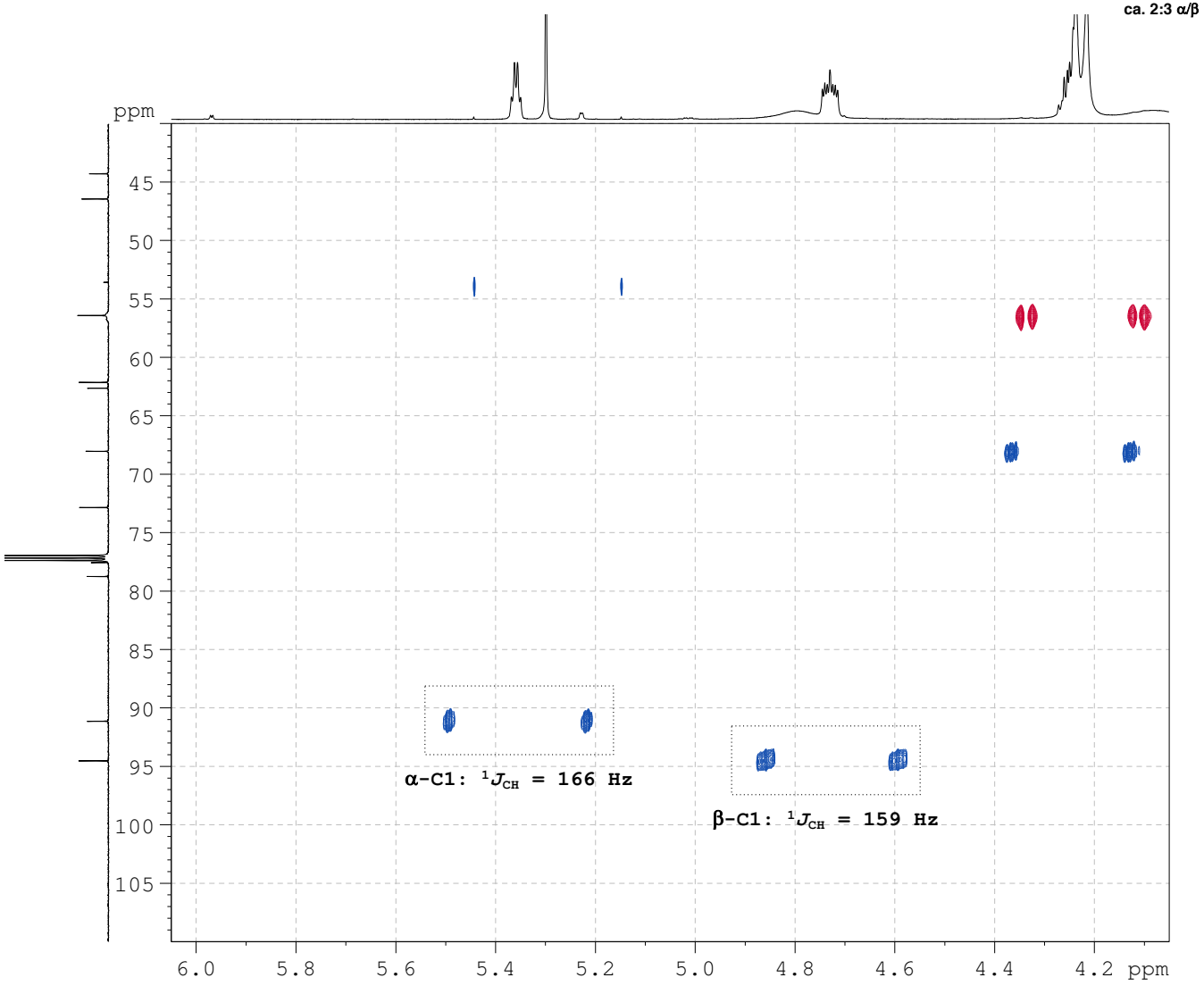


α-226 β-226

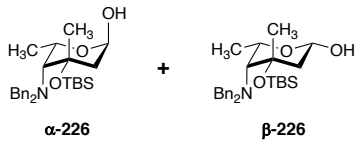
EDH-e-0868_C1F16-19 ("Coupled" - HSQC, 600 MHz, CDCl3, 5 mm)

ca. 2:3 α/β





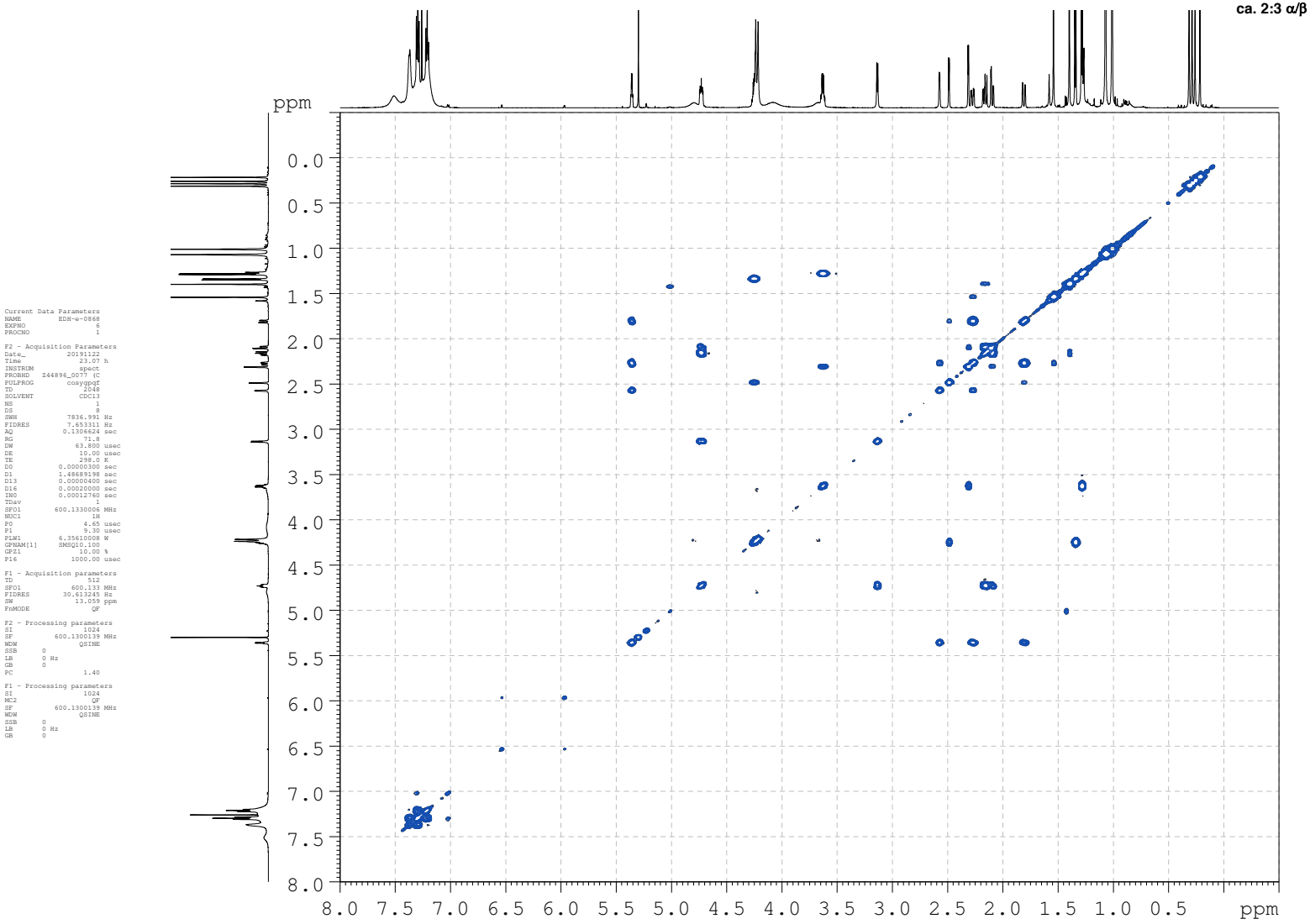
^{1}H-^{1}H COSY NMR of Compound α/β-226 (ca. 2:3 α/β) (600 MHz, $CDCl_3$)



α-226 β-226

EDH-e-0868_C1F16-19 (COSY, 600 MHz, CDCl3, 5 mm, NS = 1 scan)

ca. 2:3 α/β




```
Current Data Parameters
NAME            EDH-e-0868
EXPNO                    6
PROCNO                   1

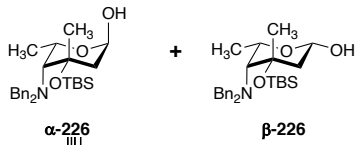
F2 - Acquisition Parameters
Date_          20191122
Time              23.07 h
INSTRUM             spect
PROBHD   Z44896_0077 (C
PULPROG          cosygpqf
TD                   2048
SOLVENT             CDCl3
NS                       1
DS                       8
SWH              7836.991 Hz
FIDRES           7.653311 Hz
AQ               0.1306624 sec
RG                    71.8
DW                 63.800 usec
DE                 10.00 usec
TE                 298.0 K
D0               0.00000300 sec
D1               1.48689198 sec
D13              0.00000400 sec
D16              0.00020000 sec
IN0              0.00012760 sec
TDav                     1
SFO1          600.1330006 MHz
NUC1                    1H
P0                   4.65 usec
P1                   9.30 usec
PLW1          6.35610008 W
GPNAM[1]      SMSQ10.100
GPZ1                10.00 %
P16               1000.00 usec

F1 - Acquisition parameters
TD                     512
SFO1            600.133 MHz
FIDRES         30.613245 Hz
SW                13.059 ppm
FnMODE                  QF

F2 - Processing parameters
SI                    1024
SF            600.1300139 MHz
WDW                 QSINE
SSB          0
LB         0 Hz
GB           0
PC                    1.40

F1 - Processing parameters
SI                    1024
MC2                     QF
SF            600.1300139 MHz
WDW                 QSINE
SSB          0
LB         0 Hz
GB           0
```

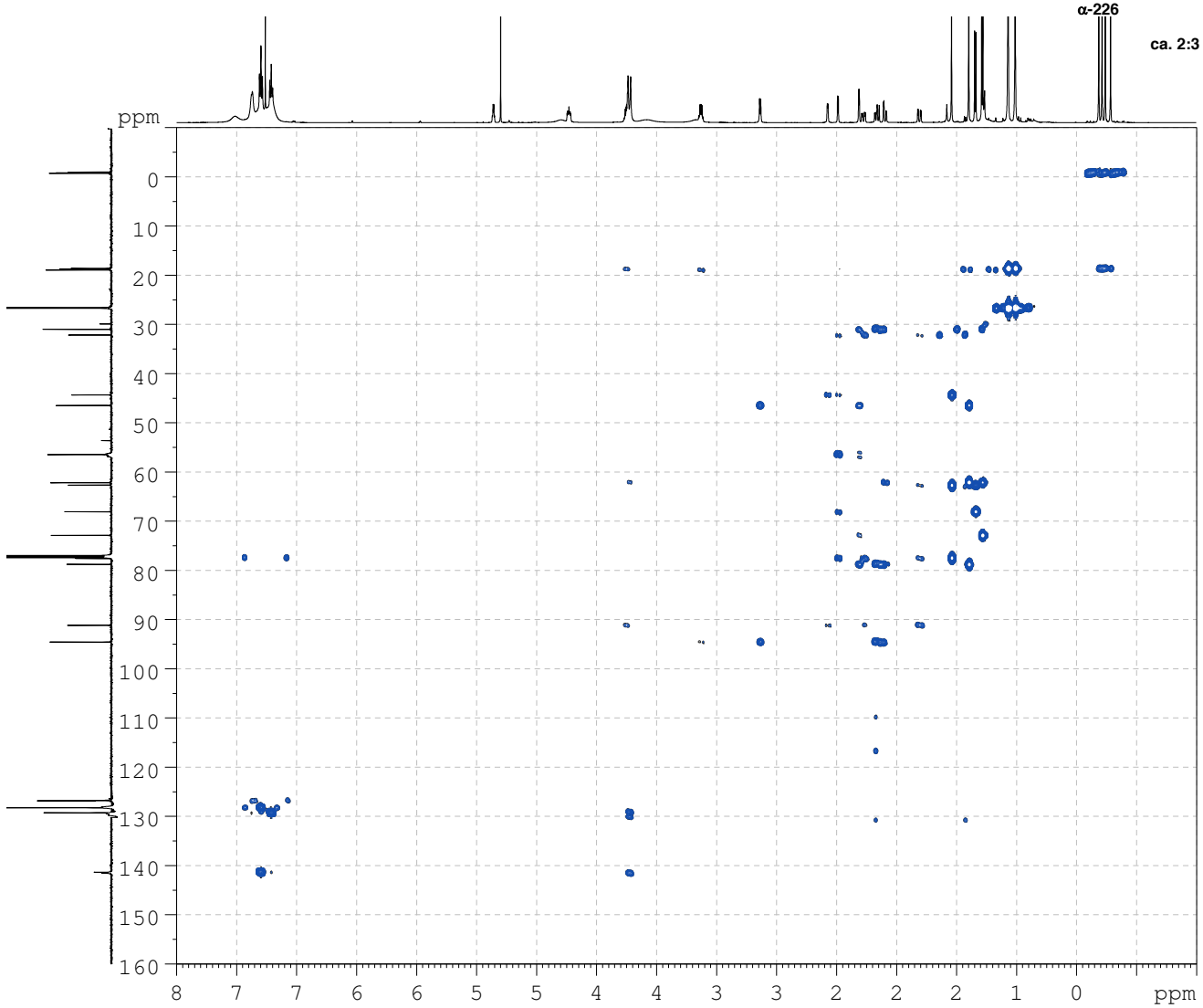
^{1}H-^{13}C HMBC NMR of Compound α/β-226 (ca. 2:3 α/β) (^{1}H-600 MHz, ^{13}C-151 MHz, $CDCl_3$)



α-226 β-226

ca. 2:3 α/β

EDH-e-0868_C1F16-19 (HMBC, 600 MHz, CDCl3, 5 mm, NS = 4 scans)




```
Current Data Parameters
NAME              EDH-e-0868
EXPNO                      7
PROCNO                     1

F2 - Acquisition Parameters
Date_           20191122
Time              23.23 h
INSTRUM             spect
PROBHD   I44896_0077 (C
PULPROG     hmbcgplpndqf
TD                 2048
SOLVENT           CDCl3
NS                    4
DS                   16
SWH            7836.991 Hz
FIDRES         7.653311 Hz
AQ             0.1306624 sec
RG                  1820
DW                63.800 usec
DE                10.00 usec
TE                298.0 K
CNST2         145.0000000
CNST13          9.0000000
D0             0.00000300 sec
D1             1.50000000 sec
D2             0.00344828 sec
D6             0.05555556 sec
D16            0.00020000 sec
IN0            0.00001440 sec
TDav                    1
SFO1          600.1330006 MHz
NUC1                   1H
P1                  9.25 usec
P2                 18.50 usec
PLW1           6.35610008 W
SFO2          150.9194083 MHz
NUC2                   13C
P3                 15.00 usec
PLW2         178.91999817 W
GPNAM[1]      SMSQ10.100
GPZ1               50.00 %
GPNAM[2]      SMSQ10.100
GPZ2               30.00 %
GPNAM[3]      SMSQ10.100
GPZ3               40.10 %
P16              1000.00 usec

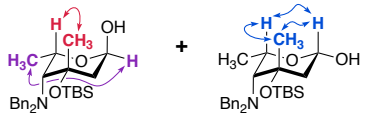
F1 - Acquisition parameters
TD                   256
SFO1          150.9194 MHz
FIDRES         271.267365 Hz
SW             230.071 ppm
FnMODE                  QF

F2 - Processing parameters
SI                  1024
SF            600.1300139 MHz
WDW                 QSINE
SSB                    0
LB                 0 Hz
GB                     0
PC                  1.40

F1 - Processing parameters
SI                  1024
MC2                   QF
SF            150.9027892 MHz
WDW                 QSINE
SSB                    0
LB                 0 Hz
GB                     0
```

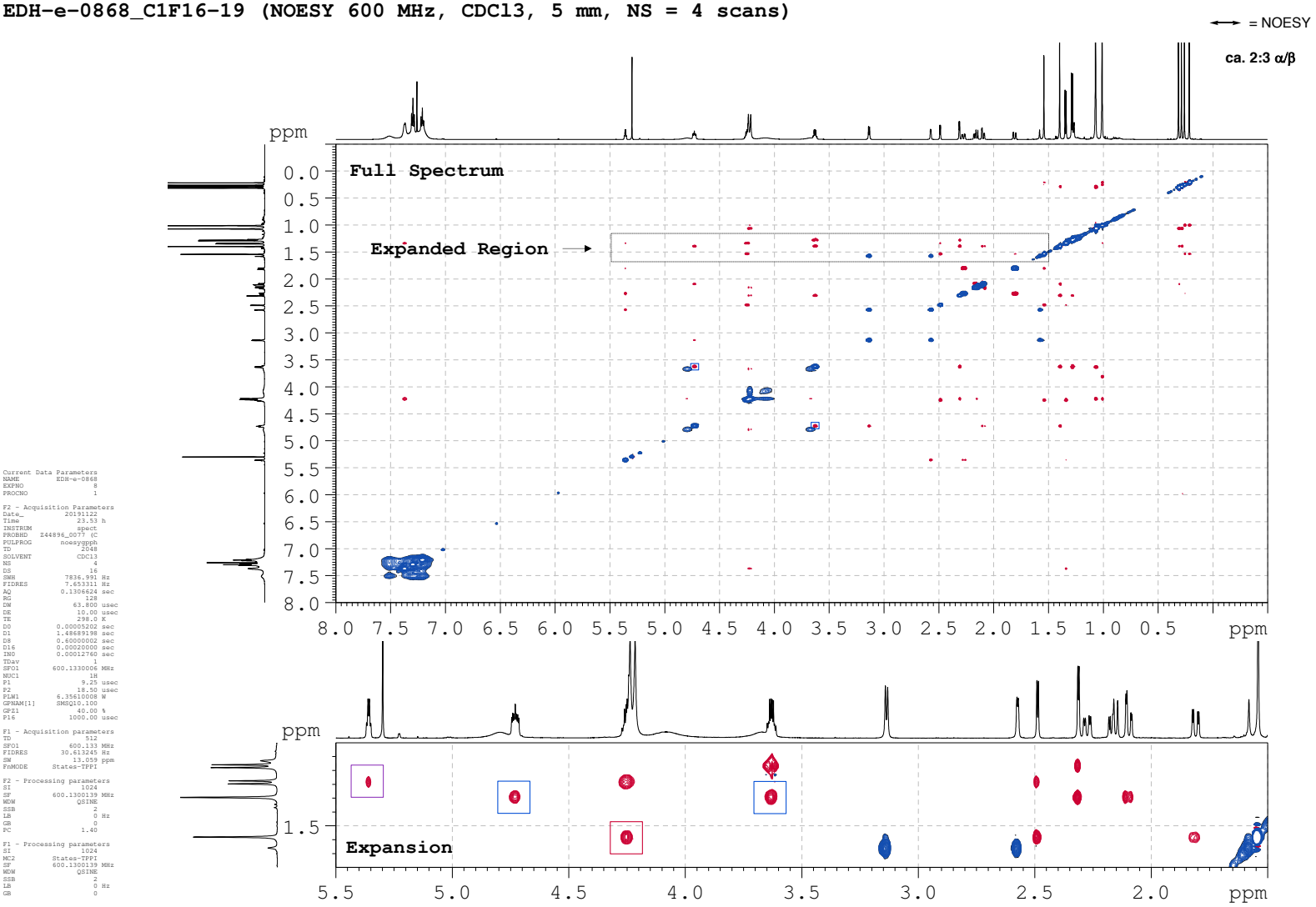
^{1}H-^{1}H NOESY NMR of Compound α/β-226 (ca. 2:3 α/β) (600 MHz, $CDCl_3$)

Note: Blue = diagonal/chemical exchange | Red = NOESY cross peaks.



α-226 + β-226

EDH-e-0868_C1F16-19 (NOESY 600 MHz, CDCl3, 5 mm, NS = 4 scans)




```
Current Data Parameters
NAME                 EDH-e-0868
EXPNO                         8
PROCNO                        1

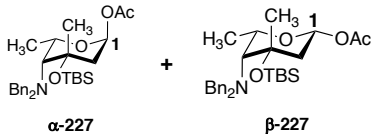
F2 - Acquisition Parameters
Date_               20191122
Time                    23.53 h
INSTRUM                 spect
PROBHD    Z44896_0077 (C
PULPROG              noesygpph
TD                        2048
SOLVENT                 CDCl3
NS                            4
DS                           16
SWH                  7836.991 Hz
FIDRES               7.653311 Hz
AQ                   0.1306624 sec
RG                          128
DW                     63.800 usec
DE                     10.00 usec
TE                     298.0 K
D0                   0.00005202 sec
D1                   1.48689198 sec
D8                   0.60000002 sec
D16                  0.00020000 sec
IN0                  0.00012760 sec
TDav                          1
SFO1                600.1330006 MHz
NUC1                        1H
P1                       9.25 usec
P2                      18.50 usec
PLW1                 6.35610008 W
GPNAM[1]             SMSQ10.100
GPZ1                    40.00 %
P16                   1000.00 usec

F1 - Acquisition parameters
TD                          512
SFO1                 600.133 MHz
FIDRES              30.613245 Hz
SW                   13.059 ppm
FnMODE             States-TPPI

F2 - Processing parameters
SI                         1024
SF                  600.1300139 MHz
WDW                      QSINE
SSB                          2
LB                           0 Hz
GB                           0
PC                        1.40

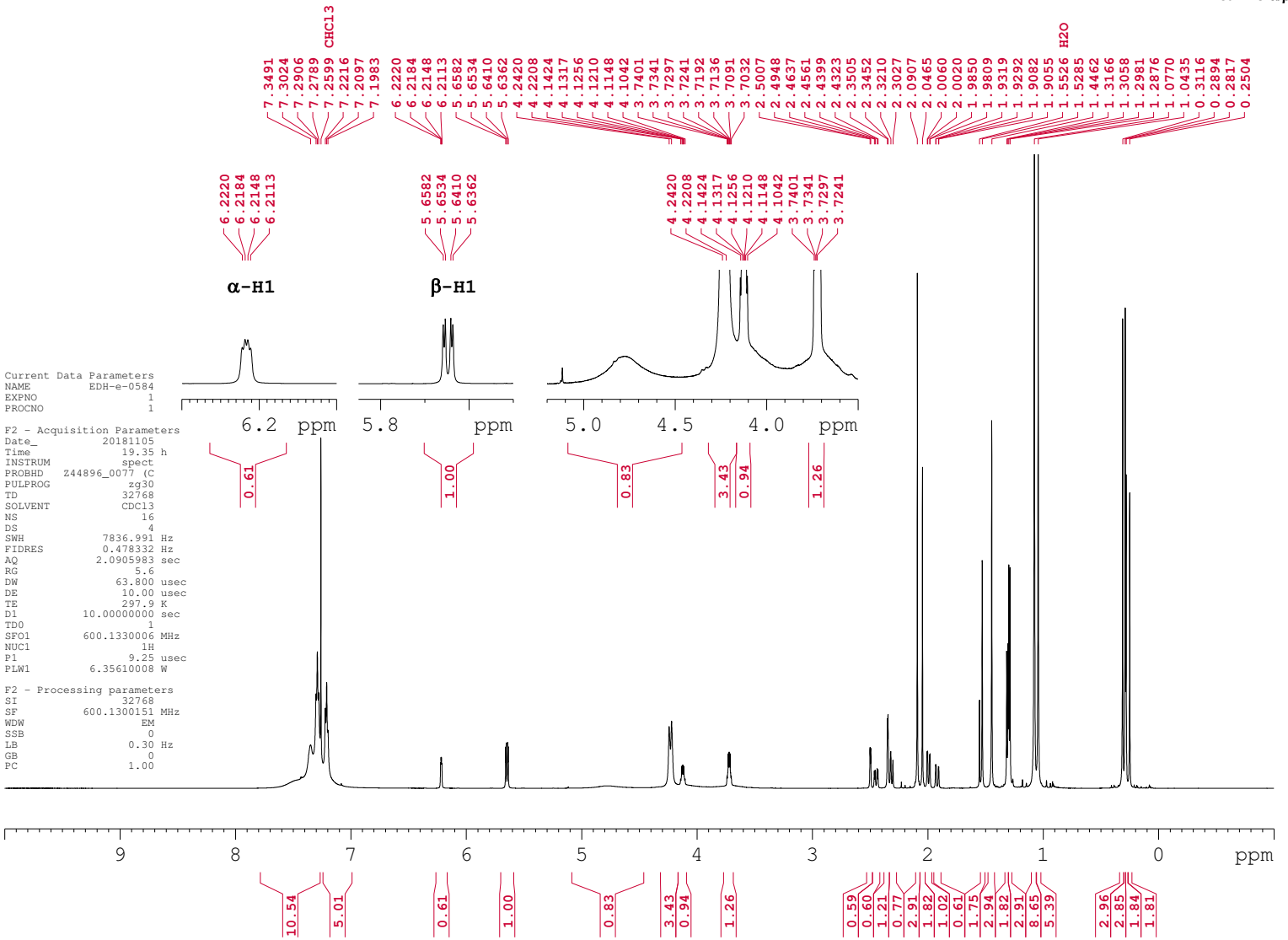
F1 - Processing parameters
SI                         1024
MC2                States-TPPI
SF                  600.1300139 MHz
WDW                      QSINE
SSB                          2
LB                           0 Hz
GB                           0
```

^{1}H NMR of Compound α/β-227 (ca. 2:3 α:β) (600 MHz, $CDCl_3$)

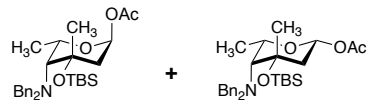


ca. 2:3 α/β

EDH-e-0584_C1F5-11 (1H, 600 MHz, CDCl3, 5 mm, NS = 16 scans, DS = 4 scans, d1 = 10 sec)



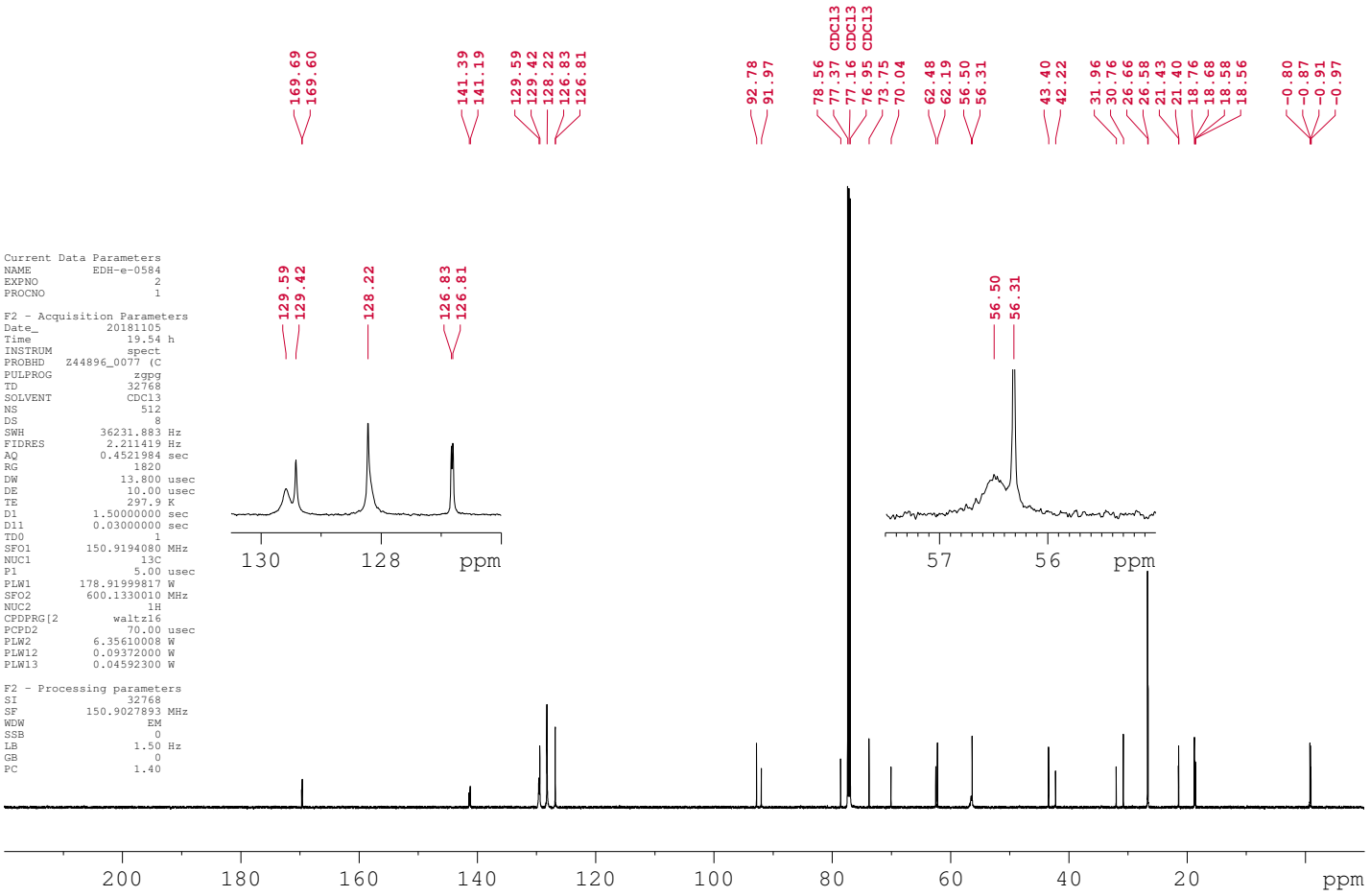
$^{13}C\{^{1}H\}$ NMR of Compound α/β-227 (ca. 2:3 α:β) (151 MHz, $CDCl_3$)



α-227 β-227

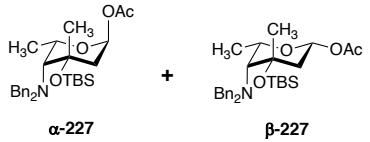
ca. 2:3 α/β

EDH-e-0584_C1F5-11 (13C,{1H} 151 MHz, CDCl3, 5 mm, NS = 512 scans)

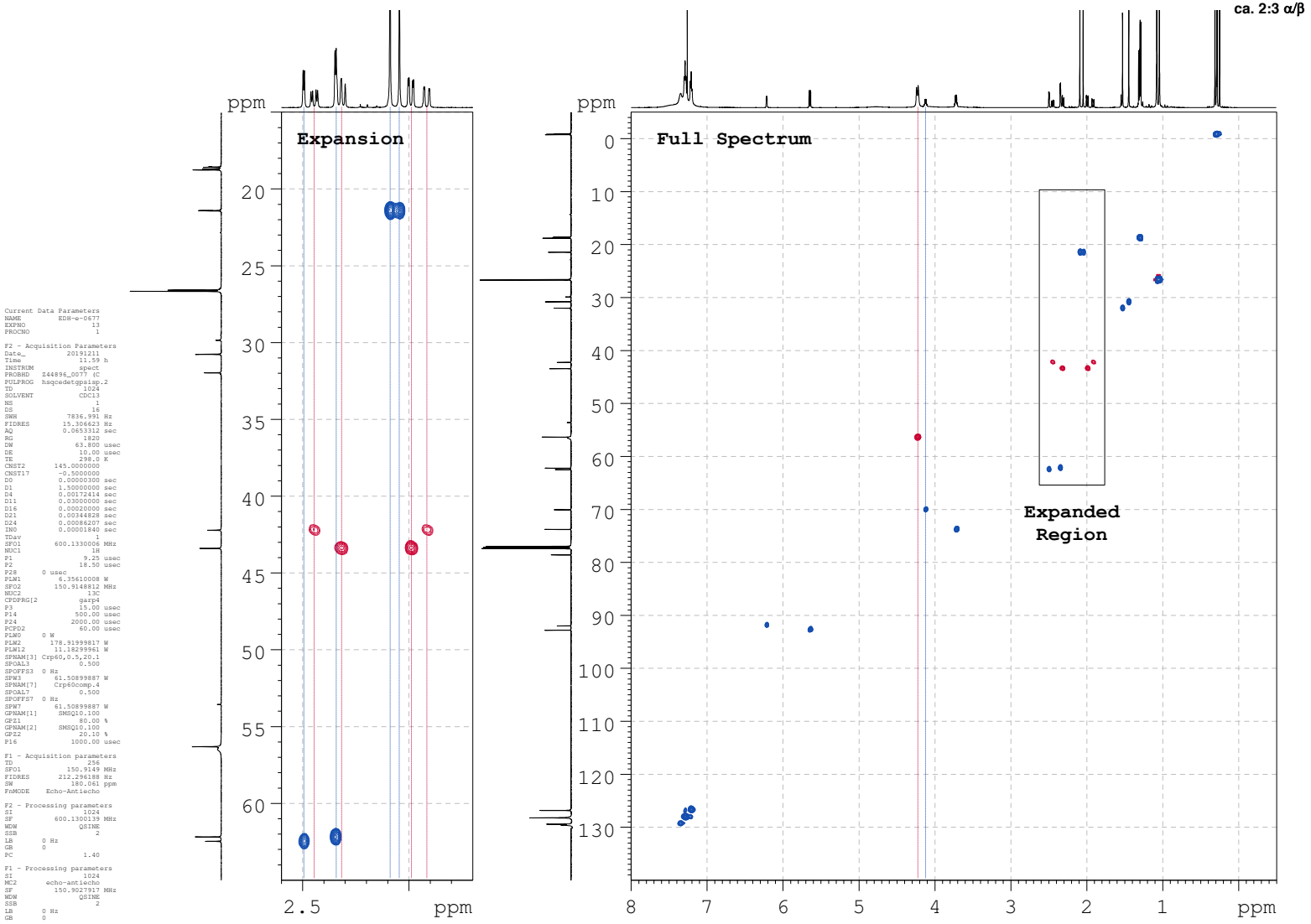


^{1}H-^{13}C HSQC{^{1}H}{^{13}C} NMR of Compound α/β-227 (ca. 2:3 α:β) (^{1}H-600 MHz, ^{13}C-151 MHz, $CDCl_3$)

Note: Blue = CH/CH_3 | Red = CH_2.



EDH-e-0677_C1F4-10 (HSQC{1H}{13C}, 600 MHz, CDCl3, 5 mm, NS = 1 scan, O2P = 80 ppm)



Current Data Parameters
NAME EDH-e-0677
EXPNO 13
PROCNO 1

F2 - Acquisition Parameters
Date_ 20191211
Time 11.59 h
INSTRUM spect
PROBHD Z44896_0077 (C
PULPROG hsqcedetgpsisp.2
TD 1024
SOLVENT CDCl3
NS 1
DS 16
SWH 7836.991 Hz
FIDRES 15.306623 Hz
AQ 0.0653312 sec
RG 1820
DW 63.800 usec
DE 10.00 usec
TE 298.0 K
CNST2 145.0000000
CNST17 -0.5000000
D0 0.00000300 sec
D1 1.50000000 sec
D4 0.00172414 sec
D11 0.03000000 sec
D16 0.00020000 sec
D21 0.00344828 sec
D24 0.00086207 sec
IN0 0.00001840 sec
TDav 1
SFO1 600.1330006 MHz
NUC1 1H
P1 9.25 usec
P2 18.50 usec
P28 0 usec
PLW1 6.35610008 W
SFO2 150.9148812 MHz
NUC2 13C
CPDPRG[2 garp4
P3 15.00 usec
P14 500.00 usec
P24 2000.00 usec
PCPD2 60.00 usec
PLW0 0 W
PLW2 178.91999817 W
PLW12 11.18299961 W
SPNAM[3] Crp60,0.5,20.1
SPOAL3 0.500
SPOFFS3 0 Hz
SPW3 61.50899887 W
SPNAM[7] Crp60comp.4
SPOAL7 0.500
SPOFF87 0 Hz
SPW7 61.50899887 W
GPNAM[1] SMSQ10.100
GPZ1 80.00 %
GPNAM[2] SMSQ10.100
GPZ2 20.10 %
P16 1000.00 usec

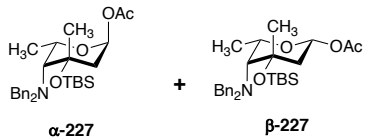
F1 - Acquisition parameters
TD 256
SFO1 150.9149 MHz
FIDRES 212.296188 Hz
SW 180.061 ppm
FnMODE Echo-Antiecho

F2 - Processing parameters
SI 1024
SF 600.1300139 MHz
WDW QSINE
SSB 2
LB 0 Hz
GB 0
PC 1.40

F1 - Processing parameters
SI 1024
MC2 echo-antiecho
SF 150.9027917 MHz
WDW QSINE
SSB 2
LB 0 Hz
GB 0

Coupled ^{1}H-^{13}C HSQC NMR of Compound α/β-227 (ca. 2:3 α:β) (^{1}H-600 MHz, ^{13}C-151 MHz, $CDCl_3$)

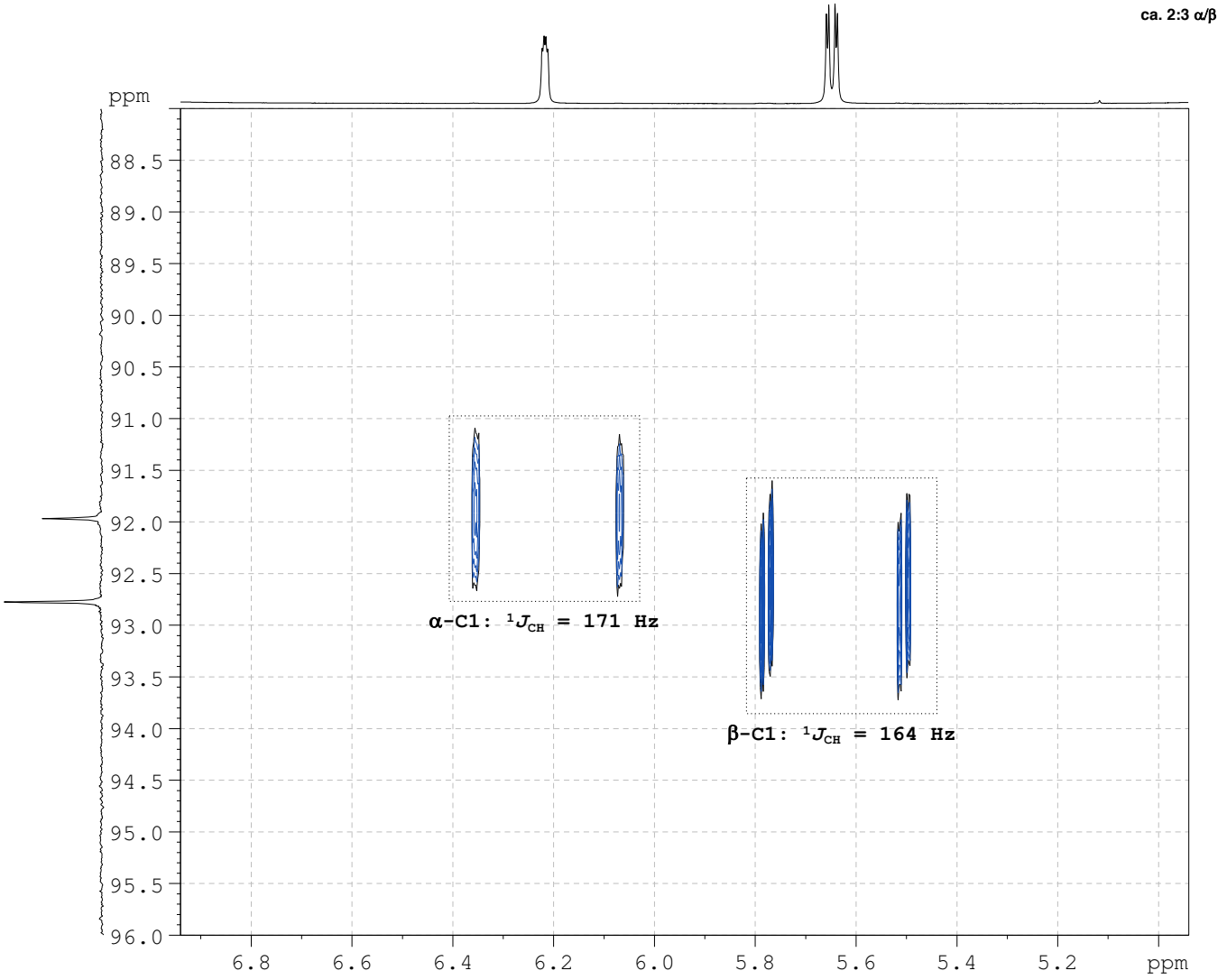
Note: Blue = CH/CH_3 | Red = CH_2.



α-227 + β-227

EDH-e-0677_C1F4-10 ("Coupled" HSQC, 600 MHz, CDCl3, 5 mm)

ca. 2:3 α/β




```
Current Data Parameters
NAME          EDH-e-0677
EXPNO             14
PROCNO             1

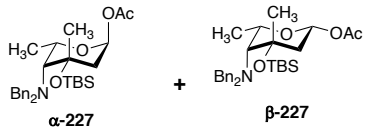
F2 - Acquisition Parameters
Date_          20191211
Time              12.07 h
INSTRUM           spect
PROBHD   Z44896_0077 (C
PULPROG  hsqcedetgpsisp.2
TD                4096
SOLVENT           CDCl3
NS                   2
DS                  16
SWH            1200.384 Hz
FIDRES         0.586125 Hz
AQ             1.7061205 sec
RG                1820
DW              416.533 usec
DE               10.00 usec
TE               298.0 K
CNST2        145.0000000
CNST17        -0.5000000
D0             0.00000300 sec
D1             1.50000000 sec
D4             0.00172414 sec
D11            0.03000000 sec
D16            0.00020000 sec
D21            0.00344828 sec
D24            0.00086207 sec
IN0            0.00001840 sec
TDav                  1
SFO1         600.1335648 MHz
NUC1                1H
P1                 9.25 usec
P2                18.50 usec
P28                0 usec
PLW1         6.35610008 W
SFO2         150.9167373 MHz
NUC2                13C
CPDPRG[2          garp4
P3                15.00 usec
P14              500.00 usec
P24             2000.00 usec
PCPD2             60.00 usec
PLW0                0 W
PLW2        178.91999817 W
PLW12               0 W
SPNAM[3] Crp60,0.5,20.1
SPOAL3            0.500
SPOFFS3             0 Hz
SPW3         61.50899887 W
SPNAM[7]    Crp60comp.4
SPOAL7            0.500
SPOFFS7             0 Hz
SPW7         61.50899887 W
GPNAM[1]     SMSQ10.100
GPZ1              80.00 %
GPNAM[2]     SMSQ10.100
GPZ2              20.10 %
P16             1000.00 usec

F1 - Acquisition parameters
TD                 128
SFO1          150.9167 MHz
FIDRES        424.592377 Hz
SW            180.059 ppm
FnMODE    Echo-Antiecho

F2 - Processing parameters
SI                1024
SF           600.1300139 MHz
WDW               QSINE
SSB                  2
LB                   0 Hz
GB                   0
PC                1.40

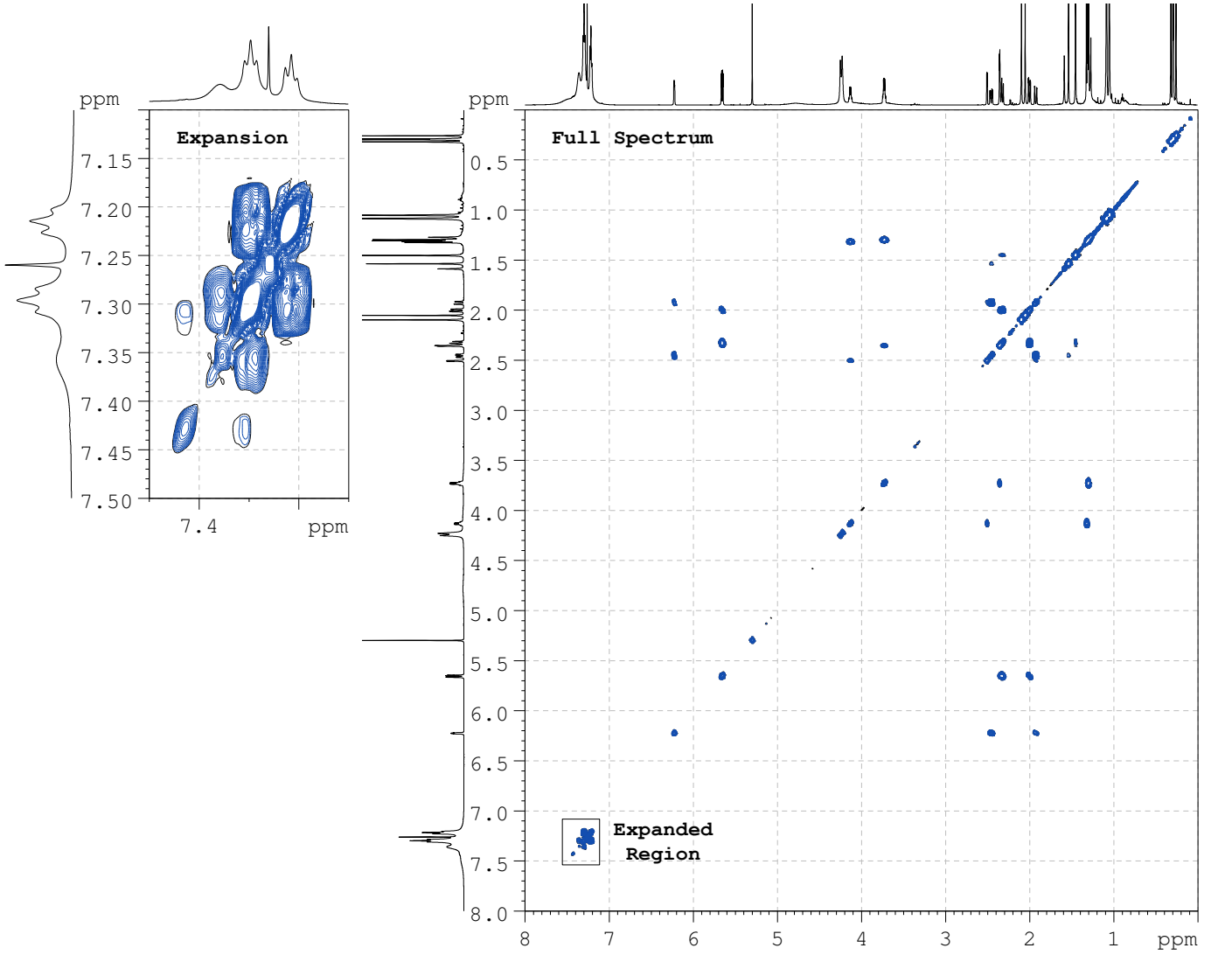
F1 - Processing parameters
SI                1024
MC2        echo-antiecho
SF           150.9027917 MHz
WDW               QSINE
SSB                  2
LB                   0 Hz
GB                   0
```

^{1}H-^{1}H COSY NMR Compound α/β-227 (ca. 2:3 α:β) (600 MHz, $CDCl_3$)



ca. 2:3 α/β

EDH-e-0500_C1F2-4 (COSY, 600 MHz, CDCl3, 5 mm)




```
Current Data Parameters
NAME              EDH-e-0500
EXPNO                      5
PROCNO                     1

F2 - Acquisition Parameters
Date_           20180517
Time              20.45
INSTRUM           spect
PROBHD   5 mm CPTCI 1H-
PULPROG         cosygpqf
TD                 2048
SOLVENT            CDCl3
NS                    1
DS                    8
SWH            7836.991 Hz
FIDRES         3.826656 Hz
AQ             0.1306624 sec
RG                 50.8
DW               63.800 usec
DE               10.00 usec
TE                298.0 K
D0            0.00000300 sec
D1             1.48689198 sec
D13           0.00000400 sec
D16           0.00020000 sec
IN0           0.00012760 sec

======== CHANNEL f1 ========
SFO1         600.1330006 MHz
NUC1                  1H
P0                  4.65 usec
P1                  9.30 usec
PLW1         6.35610008 W

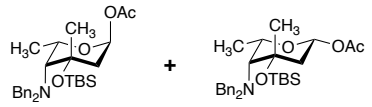
====== GRADIENT CHANNEL =====
GPNAM[1]      SMSQ10.100
GPZ1             10.00 %
P16            1000.00 usec

F1 - Acquisition parameters
TD                  512
SFO1         600.133 MHz
FIDRES       30.613245 Hz
SW              13.059 ppm
FnMODE             QF

F2 - Processing parameters
SI                 4096
SF           600.1300151 MHz
WDW                QSINE
SSB       0
LB        0 Hz
GB        0
PC                 1.40

F1 - Processing parameters
SI                 4096
MC2                QF
SF           600.1300151 MHz
WDW                QSINE
SSB       0
LB        0 Hz
GB        0
```

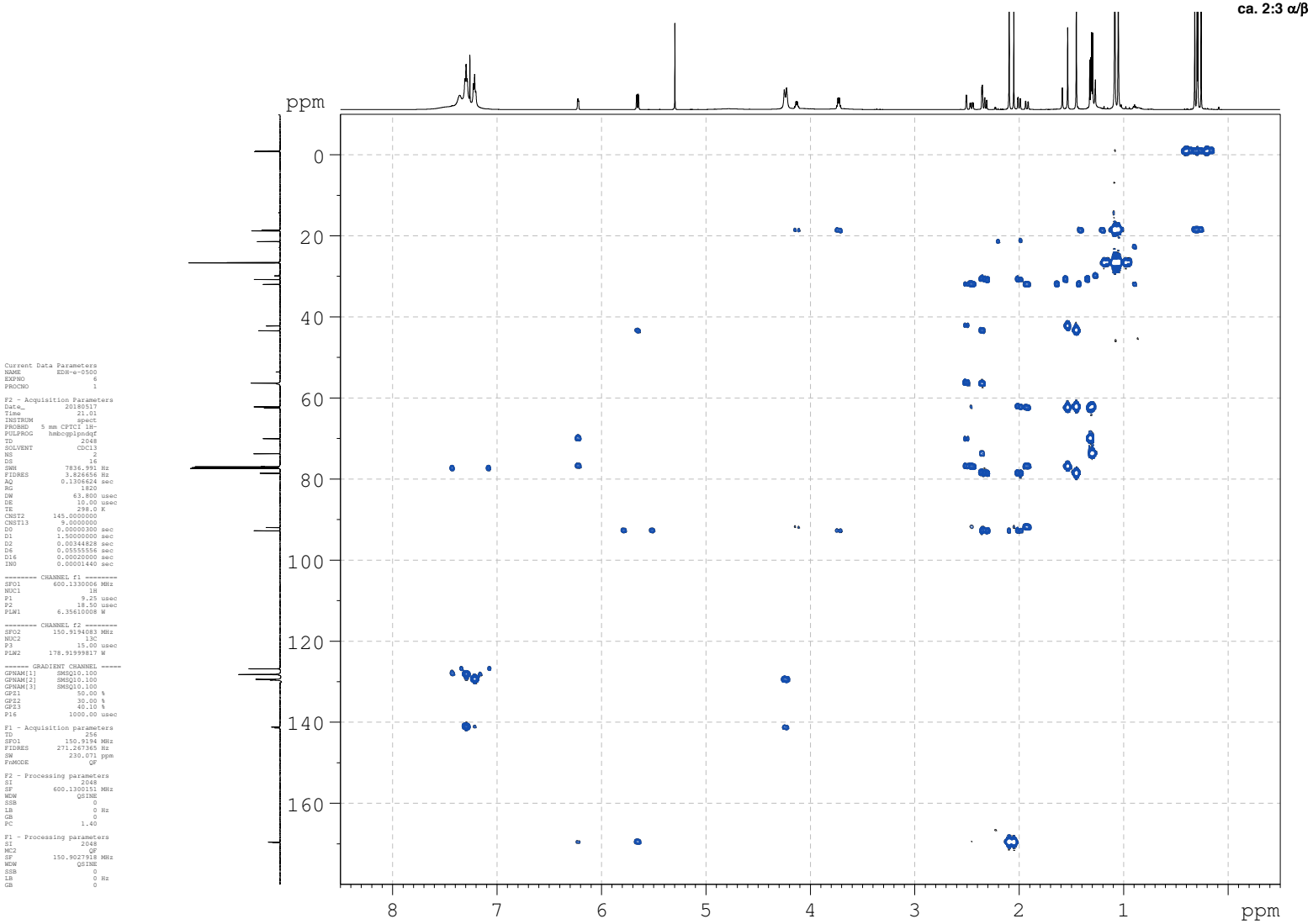
^{1}H-^{13}C HMBC NMR Compound α/β-227 (ca. 2:3 α:β) (^{1}H-600 MHz, ^{13}C-151 MHz, $CDCl_3$)



α-227 + β-227

ca. 2:3 α/β

EDH-e-0500_C1F2-4 (HMBC, 600 MHz, CDCl3, 5 mm, NS = 2 scans)




```
Current Data Parameters
NAME          EDH-e-0500
EXPNO                  6
PROCNO                 1

F2 - Acquisition Parameters
Date_           20180517
Time              21.01
INSTRUM            spect
PROBHD  5 mm CPTCI 1H-
PULPROG    hmbcgplpndqf
TD                 2048
SOLVENT           CDCl3
NS                    2
DS                   16
SWH           7836.991 Hz
FIDRES        3.826656 Hz
AQ            0.1306624 sec
RG                 1820
DW               63.800 usec
DE               10.00 usec
TE               298.0 K
CNST2       145.0000000
CNST13        9.0000000
D0            0.00000300 sec
D1            1.50000000 sec
D2            0.00344828 sec
D6            0.05555556 sec
D16           0.00020000 sec
IN0           0.00001440 sec

======== CHANNEL f1 ========
SFO1        600.1330006 MHz
NUC1                   1H
P1                   9.25 usec
P2                  18.50 usec
PLW1         6.35610008 W

======== CHANNEL f2 ========
SFO2        150.9194083 MHz
NUC2                  13C
P3                  15.00 usec
PLW2       178.91999817 W

====== GRADIENT CHANNEL =====
GPNAM[1]      SMSQ10.100
GPNAM[2]      SMSQ10.100
GPNAM[3]      SMSQ10.100
GPZ1               50.00 %
GPZ2               30.00 %
GPZ3               40.10 %
P16              1000.00 usec

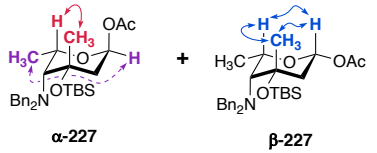
F1 - Acquisition parameters
TD                  256
SFO1          150.9194 MHz
FIDRES       271.267365 Hz
SW            230.071 ppm
FnMODE               QF

F2 - Processing parameters
SI                 2048
SF          600.1300151 MHz
WDW                QSINE
SSB                    0
LB                  0 Hz
GB                     0
PC                1.40

F1 - Processing parameters
SI                 2048
MC2                  QF
SF          150.9027918 MHz
WDW                QSINE
SSB                    0
LB                  0 Hz
GB                     0
```

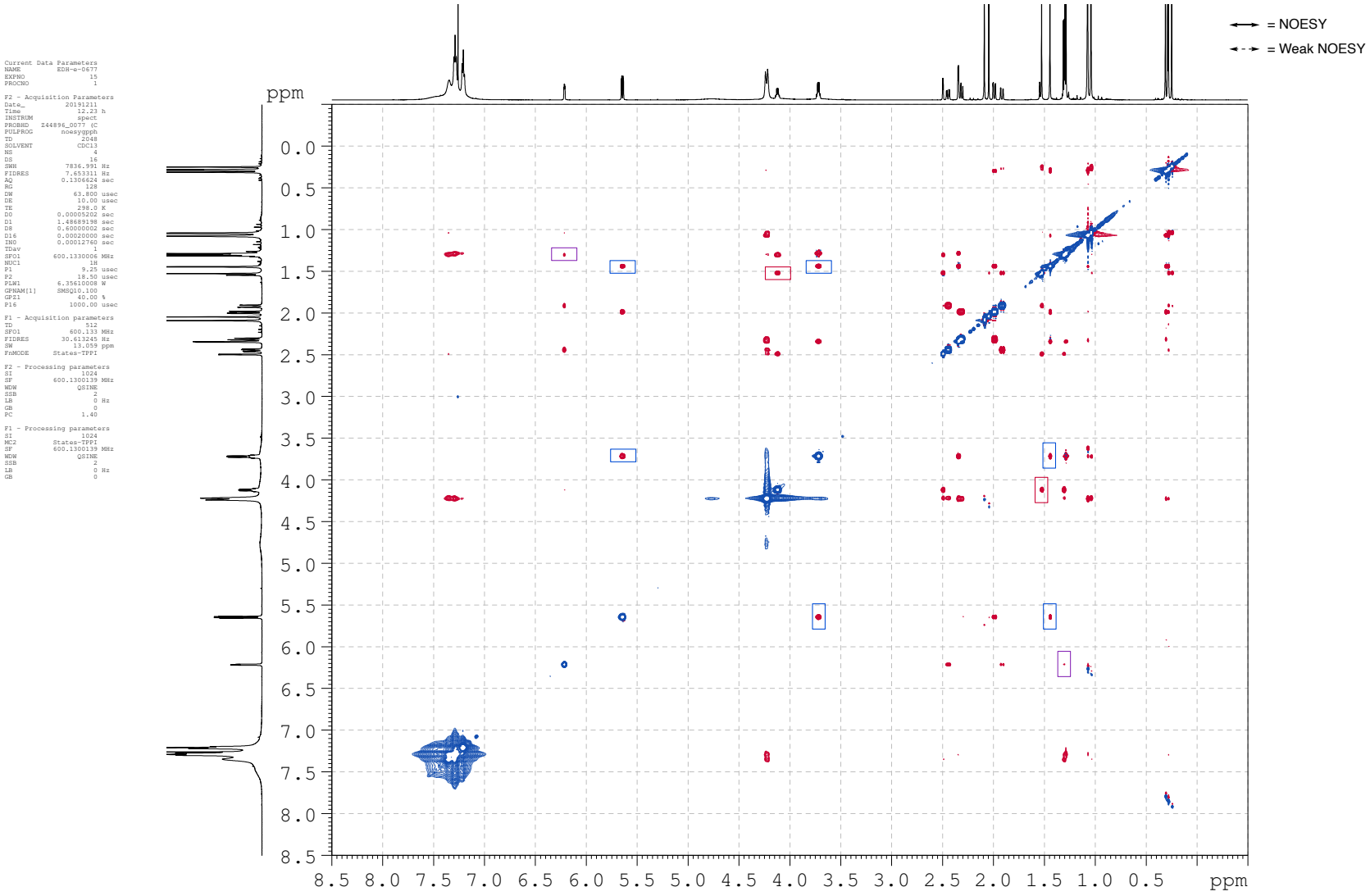
^{1}H-^{1}H NOESY NMR Compound α/β-227 (ca. 2:3 α:β) (600 MHz, $CDCl_3$)

Note: Blue = diagonal/chemical exchange | Red = NOESY cross peaks.

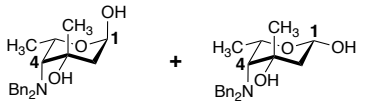


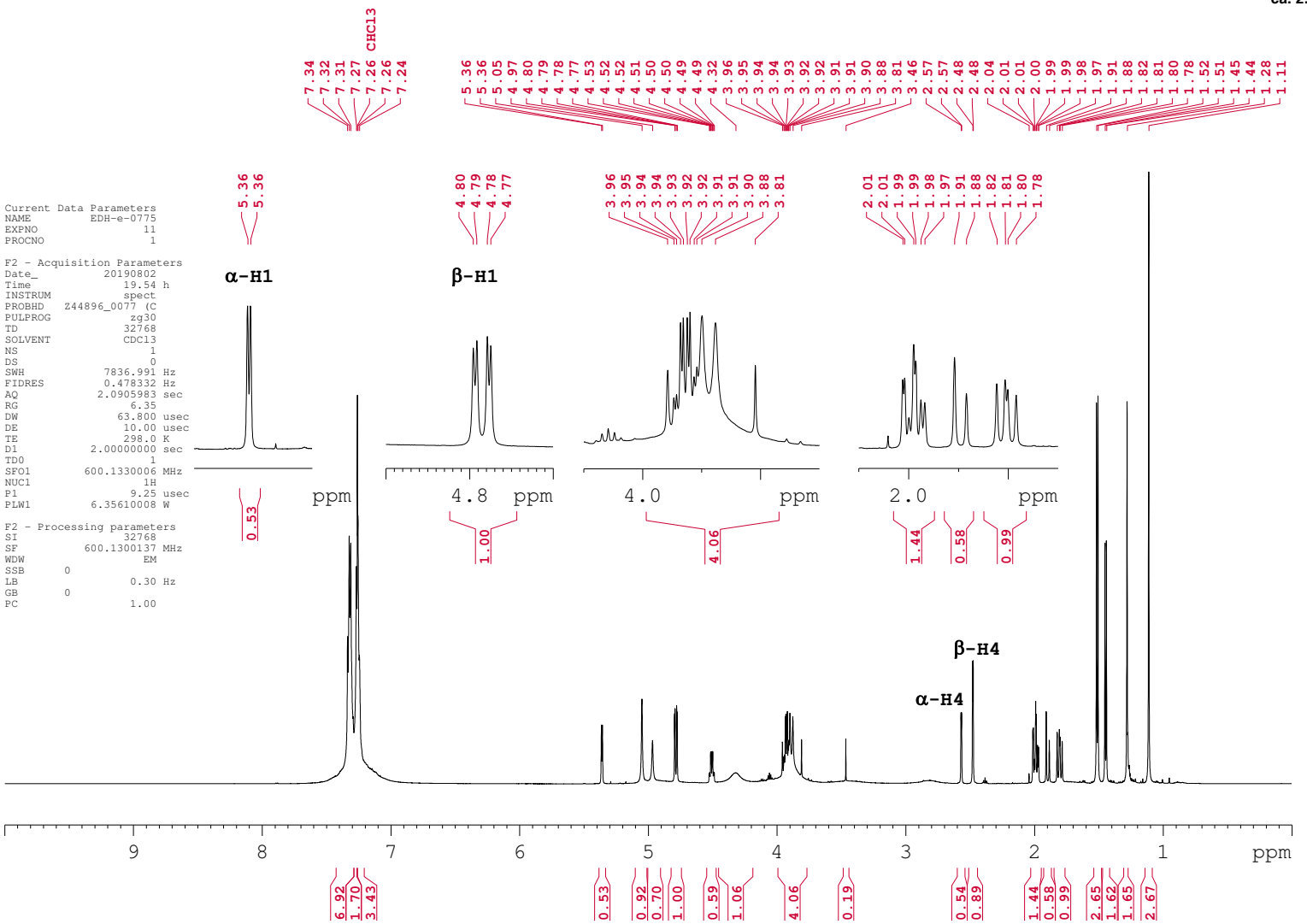
EDH-e-0677_C1F4-10 (NOESY, 600 MHz, CDCl3, 5 mm, NS = 4 scans)

ca. 2:3 α/β

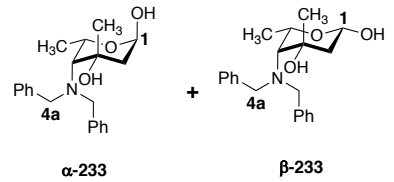


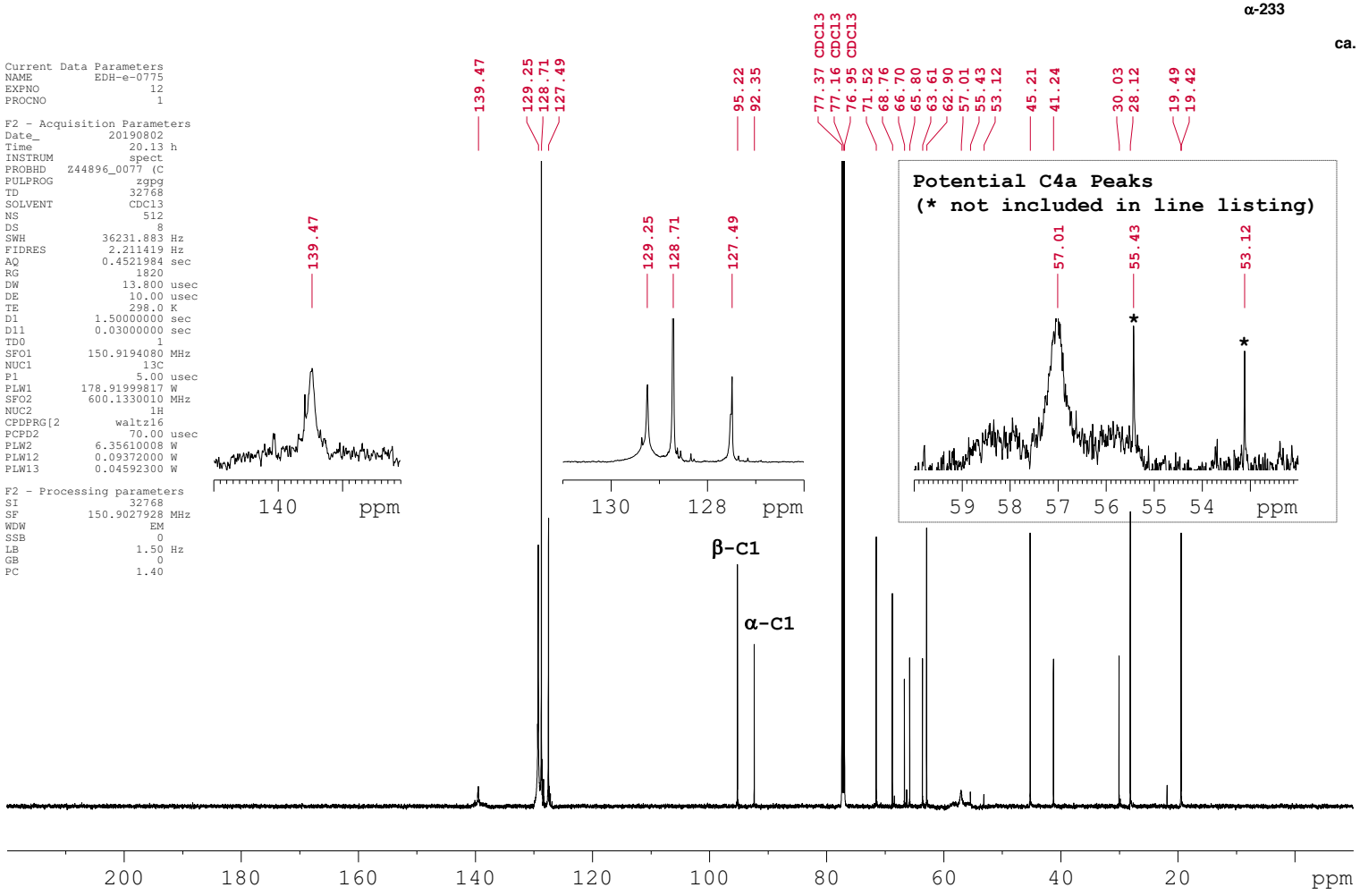
^{1}H NMR of Compound α/β-233 (ca. 2:3 α/β) (600 MHz, $CDCl_3$)





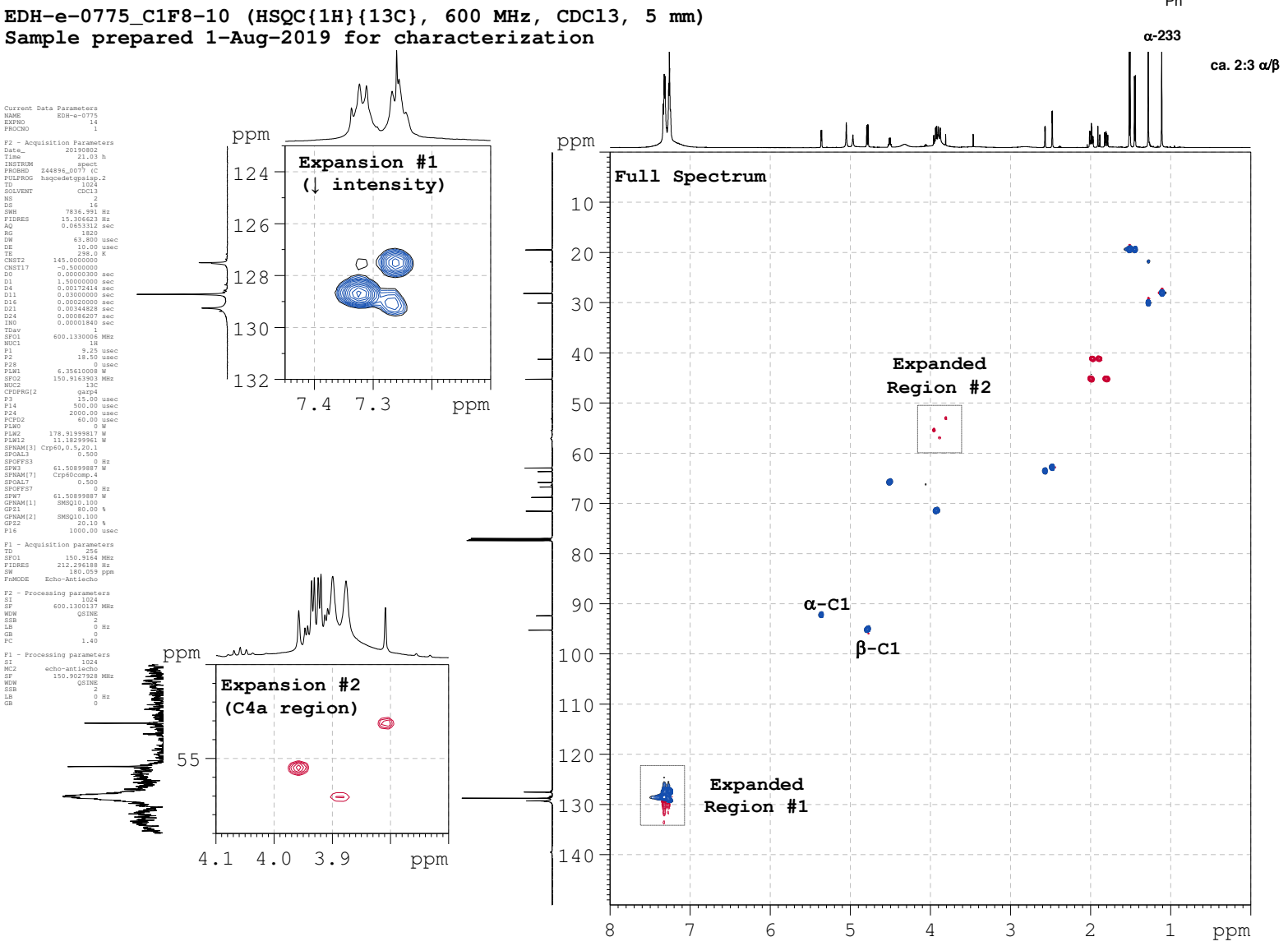
$^{13}C\{^{1}H\}$ NMR of Compound α/β-233 (ca. 2:3 α/β) (151 MHz, $CDCl_3$)





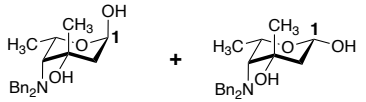
^{1}H-^{13}C HSQC{^{1}H}{^{13}C} NMR of Compound α/β-233 (ca. 2:3 α/β) (^{1}H-600 MHz, ^{13}C-151 MHz, $CDCl_3$)

Note: Blue = CH/CH_3 | Red = CH_2.



Coupled ^{1}H-^{13}C HSQC NMR of Compound α/β-233 (ca. 2:3 α/β) (^{1}H-600 MHz, ^{13}C-151 MHz, $CDCl_3$)

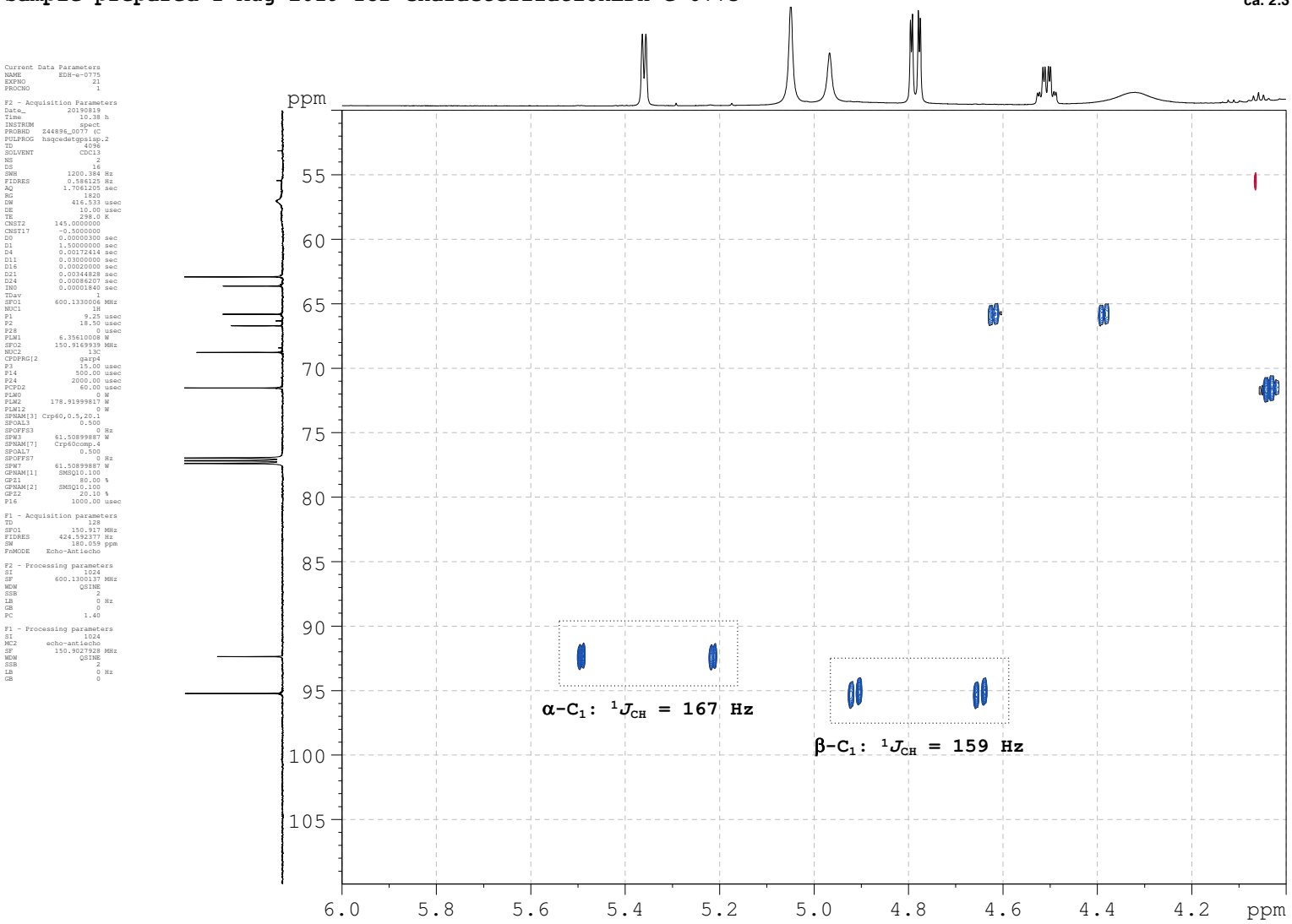
Note: Blue = CH/CH_3 | Red = CH_2.



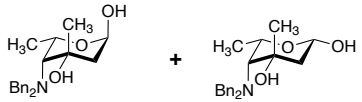
α-233 + β-233

ca. 2:3 α/β

EDH-e-0775_C1F8-10 (Coupled HSQC, 600 MHz, CDCl3, 5 mm)
Sample prepared 1-Aug-2019 for characterizationEDH-e-0775



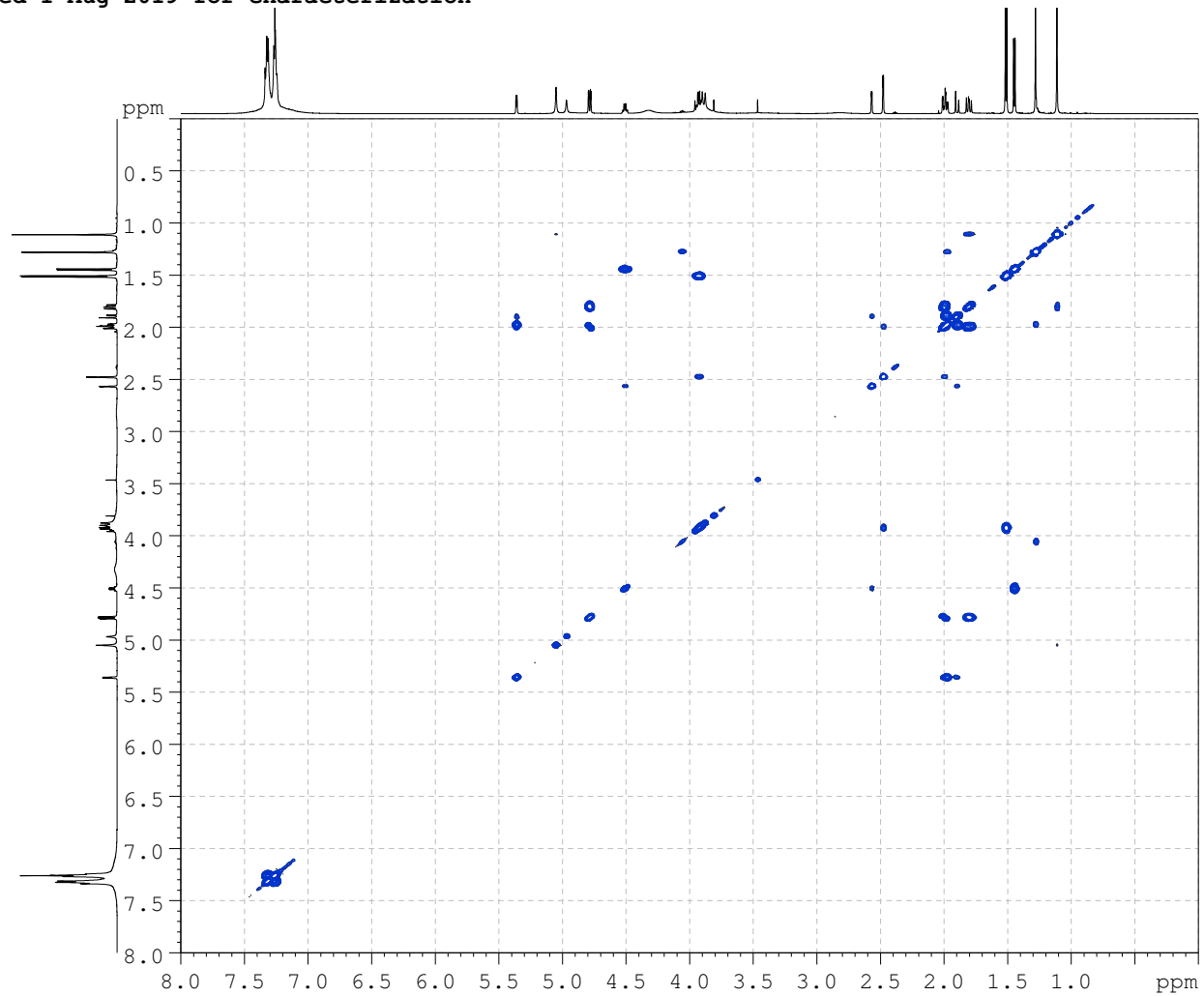
^{1}H-^{1}H COSY NMR of Compound α/β-233 (ca. 2:3 α/β) (600 MHz, $CDCl_3$)



α-233 β-233

ca. 2:3 α/β

EDH-e-0775_C1F8-10 (COSY, 600 MHz, CDCl3, 5 mm)
Sample prepared 1-Aug-2019 for characterization




```
Current Data Parameters
NAME              EDH-e-0775
EXPNO                     15
PROCNO                     1

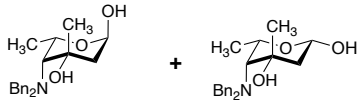
F2 - Acquisition Parameters
Date_           20190802
Time                 21.17 h
INSTRUM              spect
PROBHD   Z44896_0077 (C
PULPROG           cosygpqf
TD                    2048
SOLVENT               CDCl3
NS                        1
DS                        8
SWH               7836.991 Hz
FIDRES            7.653311 Hz
AQ                0.1306624 sec
RG                     71.8
DW                  63.800 usec
DE                  10.00 usec
TE                  298.0 K
D0                0.00000300 sec
D1                1.48689198 sec
D13               0.00002400 sec
D16               0.00020000 sec
ING               0.00012760 sec
TDav                      1
SFO1            600.1330006 MHz
NUC1                    1H
P0                     4.65 usec
P1                     9.30 usec
PLW1              6.35610008 W
GPNAM[1]     SMSQ10.100
GP21                 10.00 %
P16                1000.00 usec

F1 - Acquisition parameters
TD                      512
SFO1            600.133 MHz
FIDRES           30.613245 Hz
SW                  13.059 ppm
FnMODE                   QF

F2 - Processing parameters
SI                      1024
SF              600.1300137 MHz
WDW                   QSINE
SSB          0
LB           0 Hz
GB           0
PC                     1.40

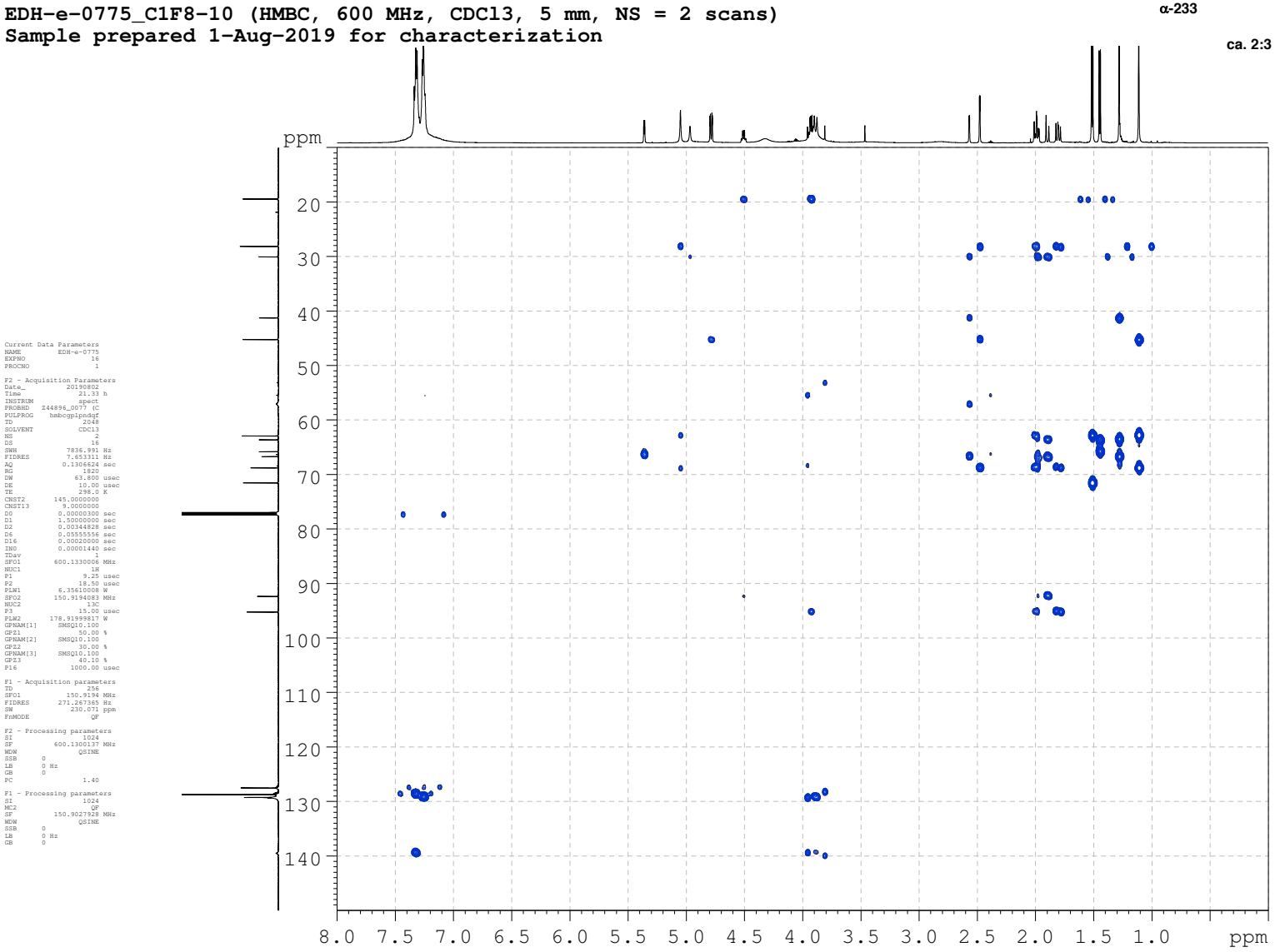
F1 - Processing parameters
SI                      1024
MC2                      QF
SF              600.1300137 MHz
WDW                   QSINE
SSB          0
LB           0 Hz
GB           0
```

^{1}H-^{13}C HMBC NMR of Compound α/β-233 (ca. 2:3 α/β) (^{1}H-600 MHz, ^{13}C-151 MHz, $CDCl_3$)



α-233 + β-233

ca. 2:3 α/β



Current Data Parameters
NAME EDH-e-0775
EXPNO 16
PROCNO 1

F2 - Acquisition Parameters
Date_ 20190802
Time 21.33 h
INSTRUM spect
PROBHD Z44896_0077 (C
PULPROG hmbcgplpndqf
TD 2048
SOLVENT CDCl3
NS 2
DS 16
SWH 7836.991 Hz
FIDRES 7.653311 Hz
AQ 0.1306624 sec
RG 1820
DW 63.800 usec
DE 10.00 usec
TE 298.0 K
CNST2 145.0000000
CNST13 9.0000000
D0 0.00000300 sec
D1 1.50000000 sec
D2 0.00344828 sec
D6 0.05555556 sec
D16 0.00020000 sec
IN0 0.00001440 sec
TDav 1
SFO1 600.1330006 MHz
NDC1 1H
P1 9.25 usec
P2 18.50 usec
PLW1 6.35610008 W
SFO2 150.9194083 MHz
NDC2 13C
P3 15.00 usec
PLW2 178.91999817 W
GPNAM[1] SMSQ10.100
GPZ1 50.00 %
GPNAM[2] SMSQ10.100
GPZ2 30.00 %
GPNAM[3] SMSQ10.100
GPZ3 40.10 %
P16 1000.00 usec

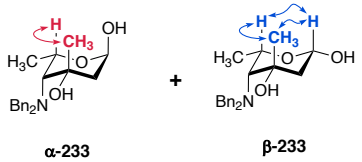
F1 - Acquisition parameters
TD 256
SFO1 150.9194 MHz
FIDRES 271.267365 Hz
SW 230.071 ppm
FnMODE QF

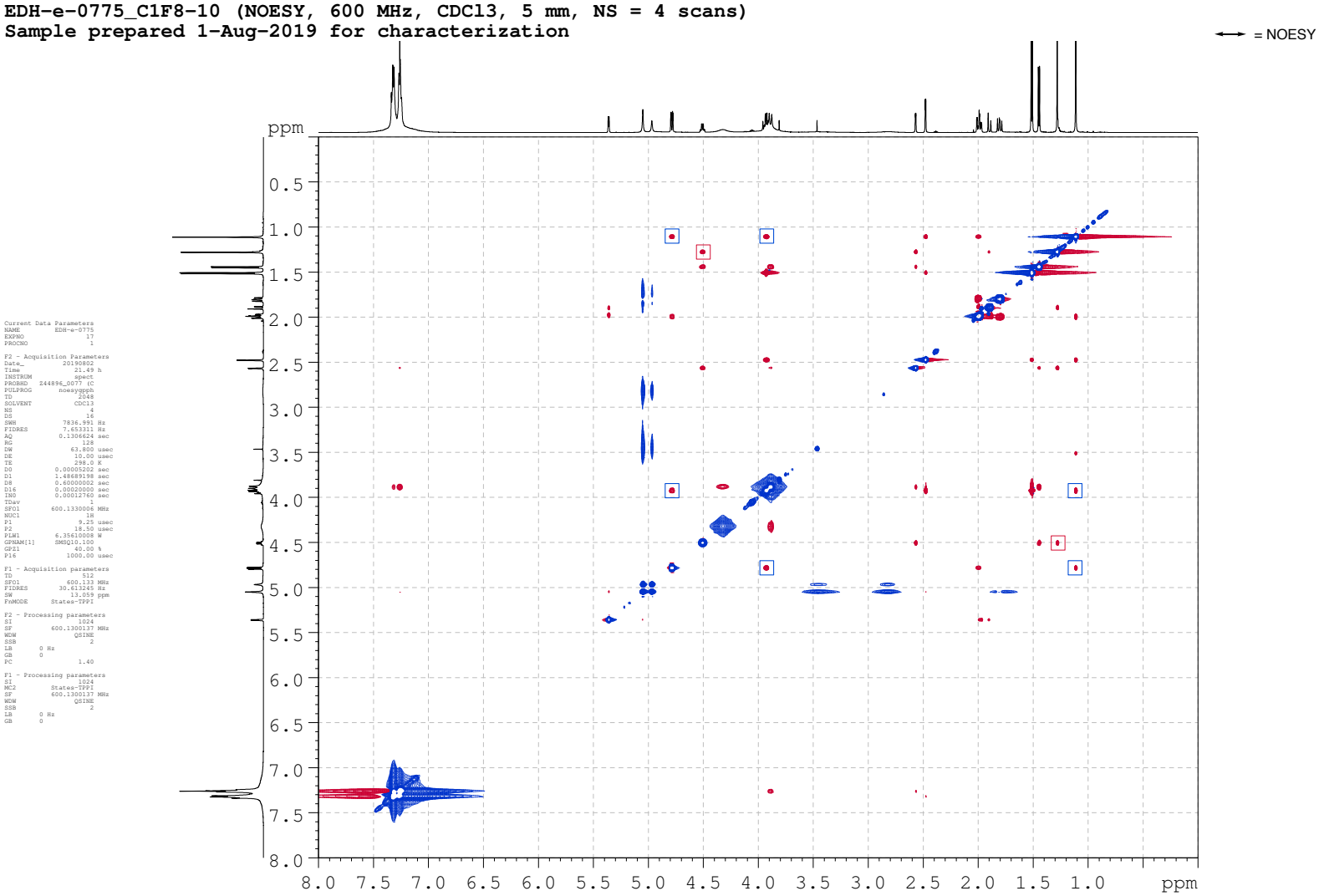
F2 - Processing parameters
SI 1024
SF 600.1300137 MHz
WDW QSINE
SSB 0
LB 0 Hz
GB 0
PC 1.40

F1 - Processing parameters
SI 1024
MC2 QF
SF 150.9027928 MHz
WDW QSINE
SSB 0
LB 0 Hz
GB 0

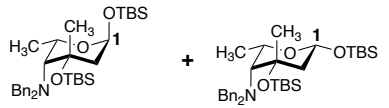
^{1}H-^{1}H NOESY NMR of Compound α/β-233 (ca. 2:3 α/β) (600 MHz, $CDCl_3$)

Note: Blue = diagonal/chemical exchange | Red = NOESY cross peaks.





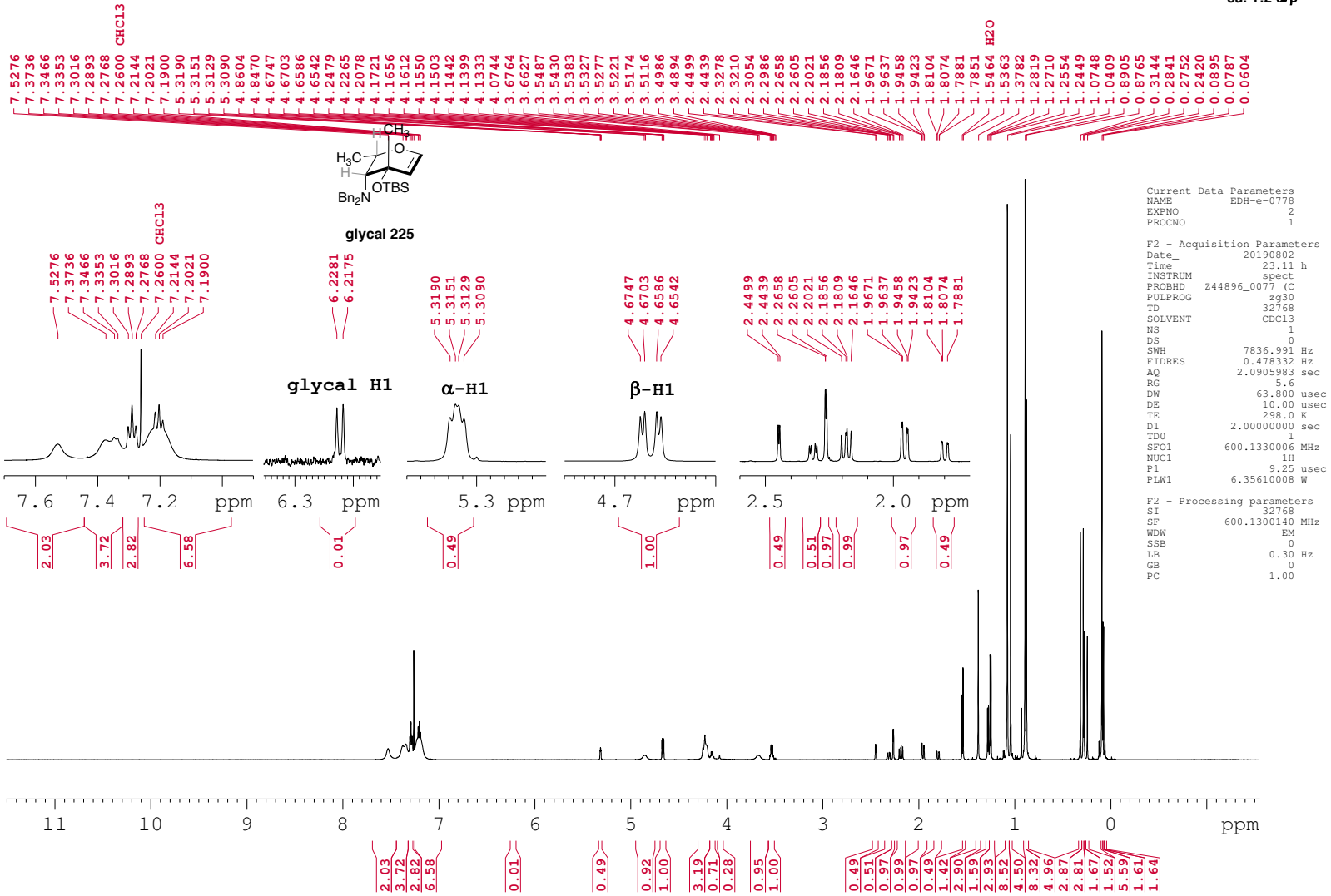
^{1}H NMR of Compound α/β-235 (ca. 1:2 α/β) (600 MHz, $CDCl_3$)



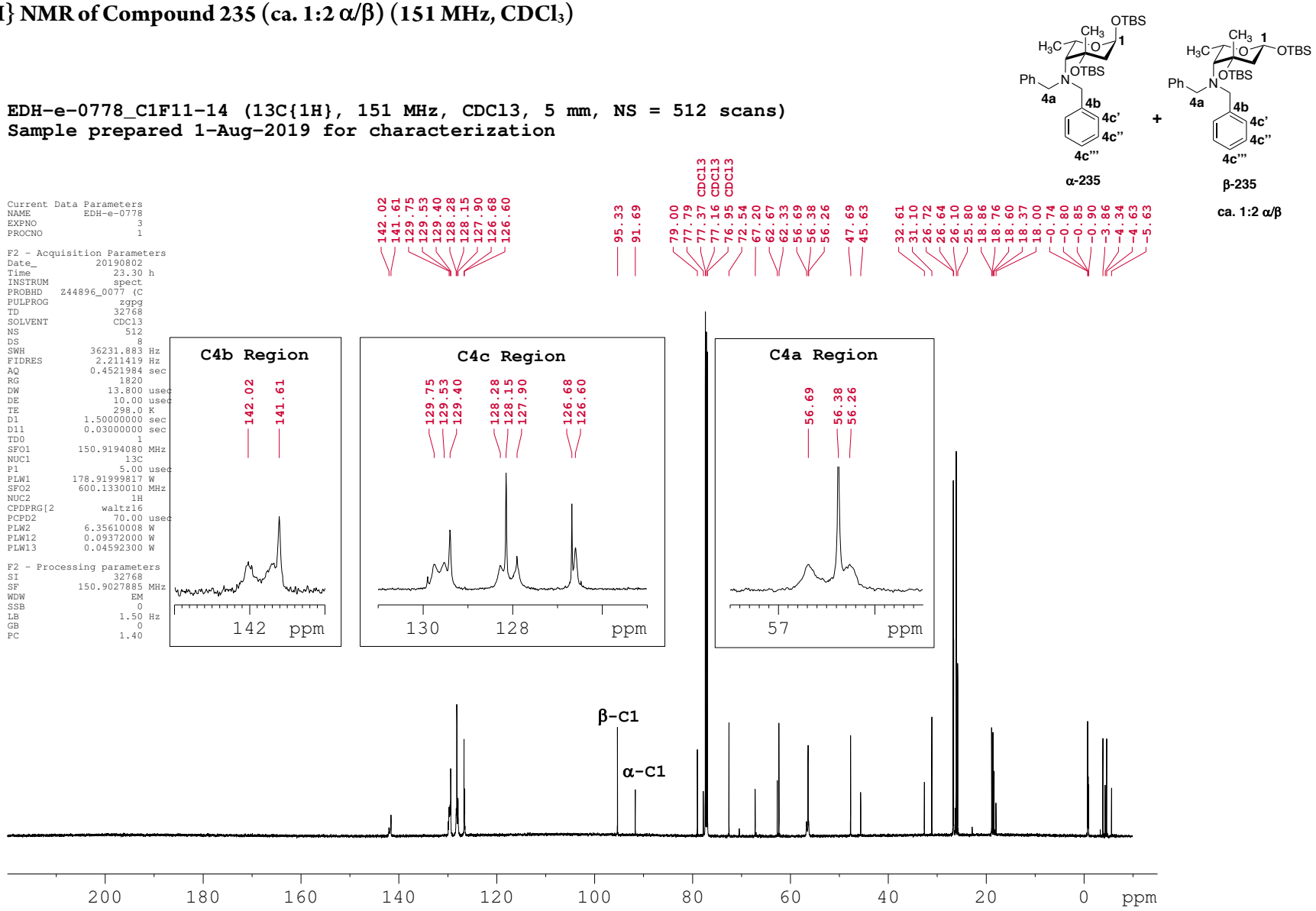
α-235 β-235

ca. 1:2 α/β

EDH-e-0778_C1F11-14 (1H, 600 MHz, CDCl3, 5 mm, NS = 1 scan)
Sample prepared 1-Aug-2019 for characterization

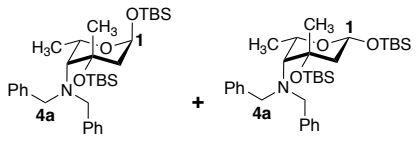


$^{13}C\{^{1}H\}$ NMR of Compound 235 (ca. 1:2 α/β) (151 MHz, $CDCl_3$)



^{1}H-^{13}C HSQC{^{1}H}{^{13}C} NMR of Compound 235 (ca. 1:2 α/β) (^{1}H-600 MHz, ^{13}C-151 MHz, $CDCl_3$)

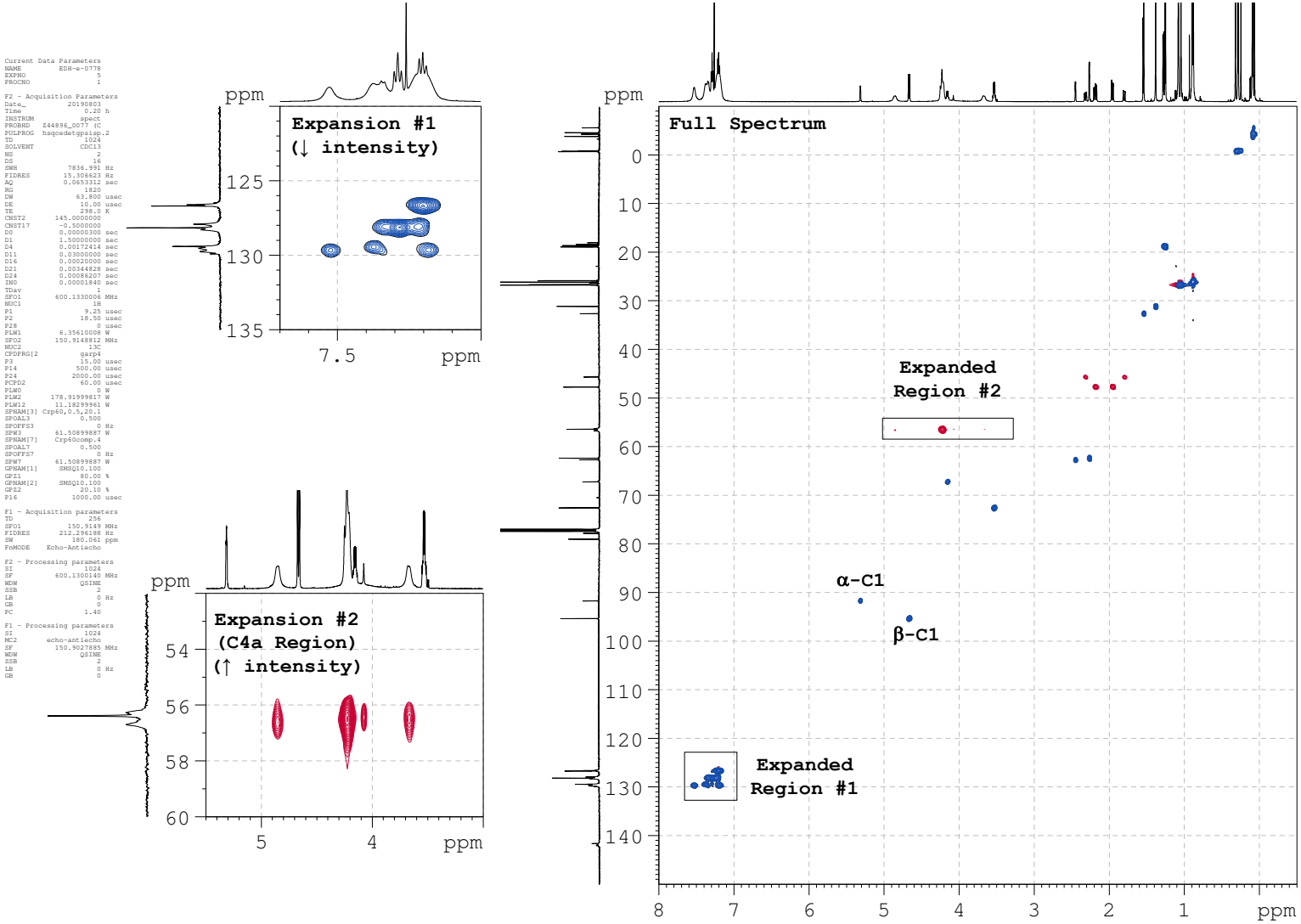
Note: Blue = CH/CH_3 | Red = CH_2.



α-235 + β-235

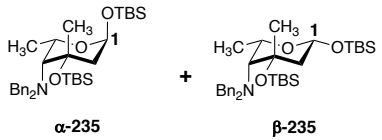
ca. 1:2 α/β

EDH-e-0778_C1F11-14 (HSQC{1H}{13C}, 600 MHz, CDCl3, 5 mm, O2P = 80 ppm)
Sample prepared 1-Aug-2019 for characterization



Coupled ^{1}H-^{13}C HSQC NMR of Compound 235 (ca. 1:2 α/β) (^{1}H-600 MHz, ^{13}C-151 MHz, $CDCl_3$)

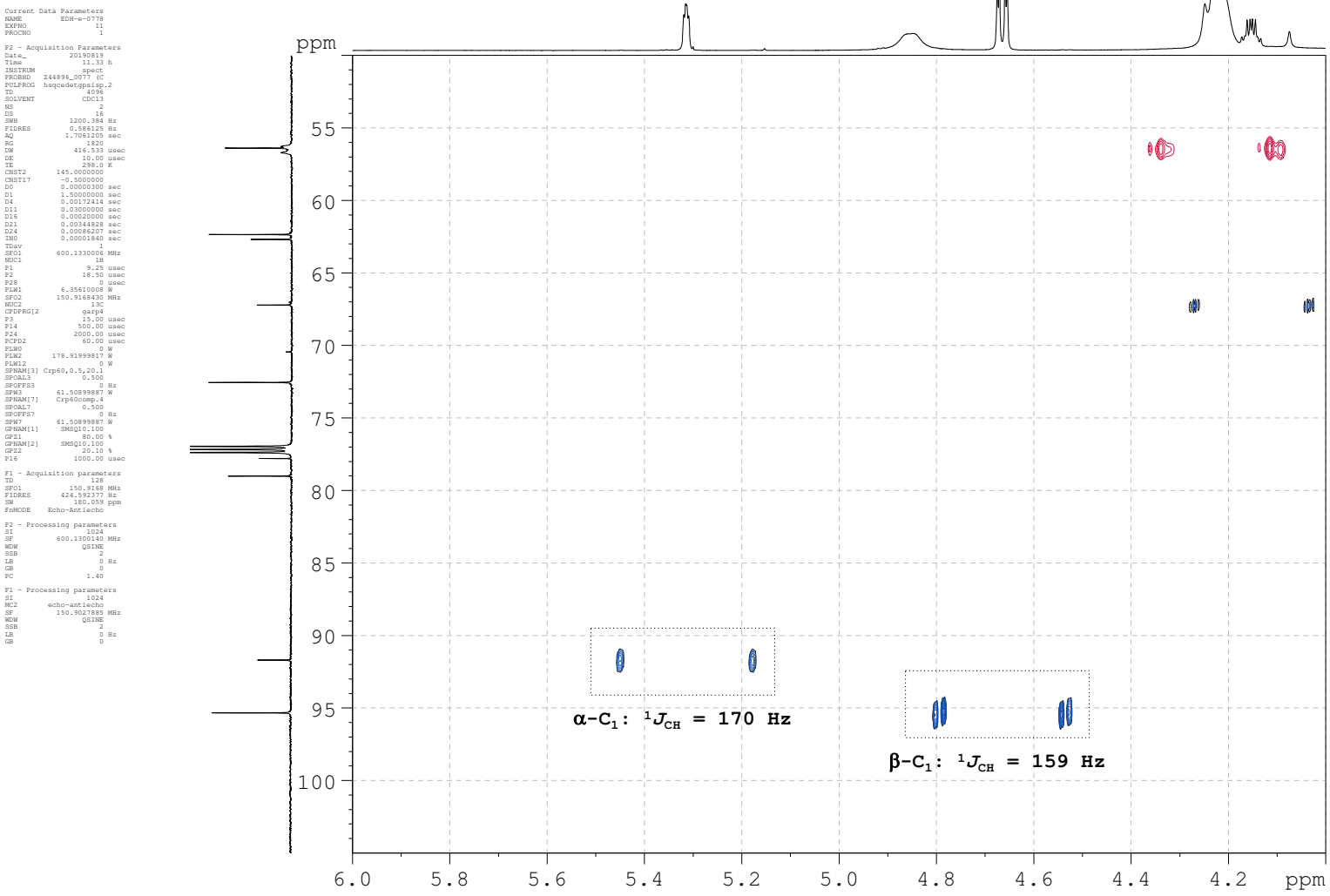
Note: Blue = CH/CH_3 | Red = CH_2.



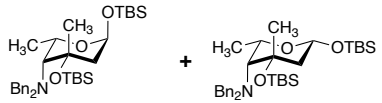
α-235 β-235

ca. 1:2 α/β

EDH-e-0778_C1F11-14 (Coupled HSQC, 600 MHz, CDCl3, 5 mm)
Sample prepared 1-Aug-2019 for characterization



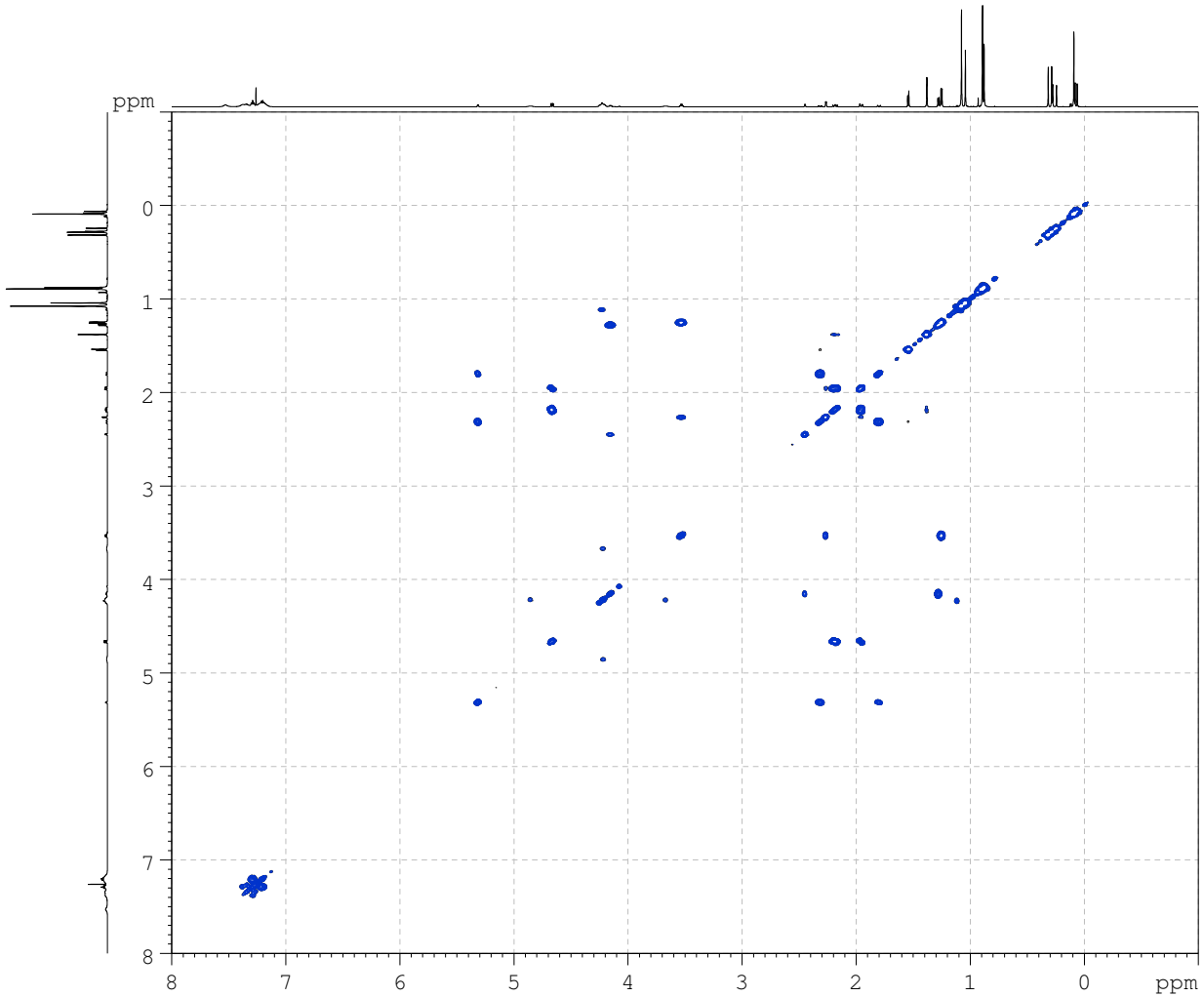
^{1}H-^{1}H COSY NMR of Compound 235 (ca. 1:2 α/β) (600 MHz, $CDCl_3$)



α-235 β-235

ca. 1:2 α/β

EDH-e-0778_C1F11-14 (COSY, 600 MHz, CDCl3, 5 mm)
Sample prepared 1-Aug-2019 for characterization



Current Data Parameters
NAME EDH-e-0778
EXPNO 6
PROCNO 1

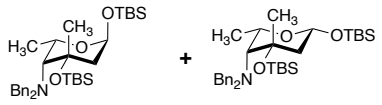
F2 - Acquisition Parameters
Date_ 20190803
Time 0.34 h
INSTRUM spect
PROBHD Z44896_0077 (C
PULPROG cosygpqf
TD 2048
SOLVENT CDCl3
NS 1
DS 8
SWH 7836.991 Hz
FIDRES 7.653311 Hz
AQ 0.1306624 sec
RG 57
DW 63.800 usec
DE 10.00 usec
TE 298.0 K
D0 0.00000300 sec
D1 1.48689198 sec
D13 0.00000400 sec
D16 0.00020000 sec
ING 0.00012760 sec
TDav 1
SFO1 600.1330006 MHz
NUC1 1H
P0 4.65 usec
P1 9.30 usec
PLW1 6.35610008 W
GPNAM[1] SMSQ10.100
GP21 10.00 %
P16 1000.00 usec

F1 - Acquisition parameters
TD 512
SFO1 600.133 MHz
FIDRES 30.613245 Hz
SW 13.059 ppm
FnMODE QF

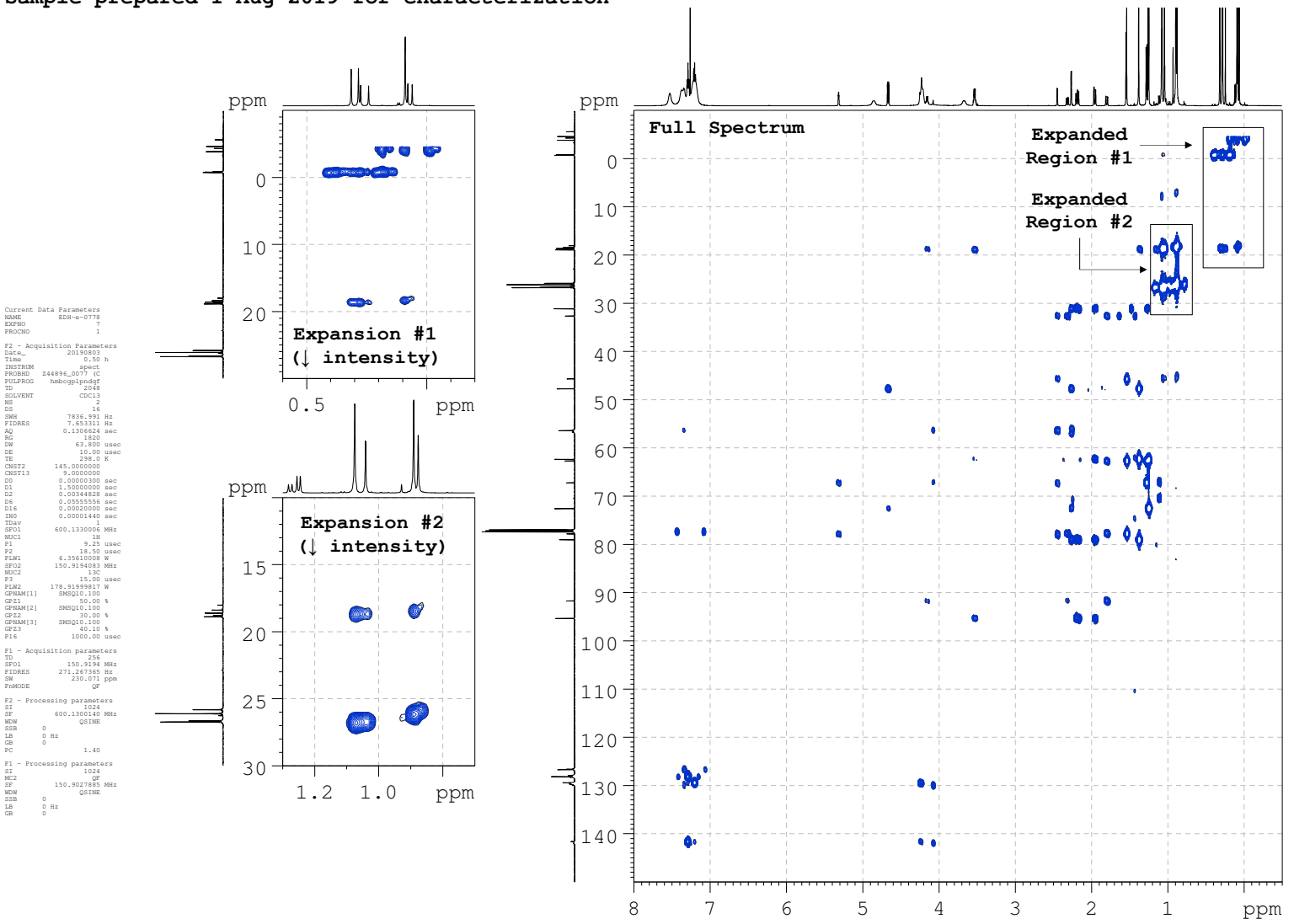
F2 - Processing parameters
SI 1024
SF 600.1300140 MHz
WDW QSINE
SSB 0
LB 0 Hz
GB 0
PC 1.40

F1 - Processing parameters
SI 1024
MC2 QF
SF 600.1300140 MHz
WDW QSINE
SSB 0
LB 0 Hz
GB 0

^{1}H-^{13}C HMBC NMR of Compound 235 (ca. 1:2 α/β) (^{1}H-600 MHz, ^{13}C-151 MHz, $CDCl_3$)



α-235 ca. 1:2 α/β β-235



Current Data Parameters
NAME EDH-e-0778
EXPNO 7
PROCNO 1

F2 - Acquisition Parameters
Date_ 20190803
Time 0.50 h
INSTRUM spect
PROBHD Z44896_0077 (C
PULPROG hmbcgplpndqf
TD 2048
SOLVENT CDCl3
NS 2
DS 16
SWH 7836.991 Hz
FIDRES 7.653311 Hz
AQ 0.1306624 sec
RG 1820
DW 63.800 usec
DE 10.00 usec
TE 298.0 K
CNST2 145.0000000
CNST13 9.0000000
D0 0.00000300 sec
D1 1.50000000 sec
D2 0.00344828 sec
D6 0.05555556 sec
D16 0.00020000 sec
IN0 0.00001440 sec
TDav 1
SFO1 600.1330006 MHz
NDC1 1H
P1 9.25 usec
P2 18.50 usec
PLW1 6.35610008 W
SFO2 150.9194083 MHz
NDC2 13C
P3 15.00 usec
PLW2 178.91999817 W
GPNAM[1] SMSQ10.100
GPZ1 50.00 %
GPNAM[2] SMSQ10.100
GPZ2 30.00 %
GPNAM[3] SMSQ10.100
GPZ3 40.10 %
P16 1000.00 usec

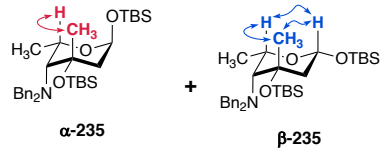
F1 - Acquisition parameters
TD 256
SFO1 150.9194 MHz
FIDRES 271.267365 Hz
SW 230.071 ppm
FnMODE QF

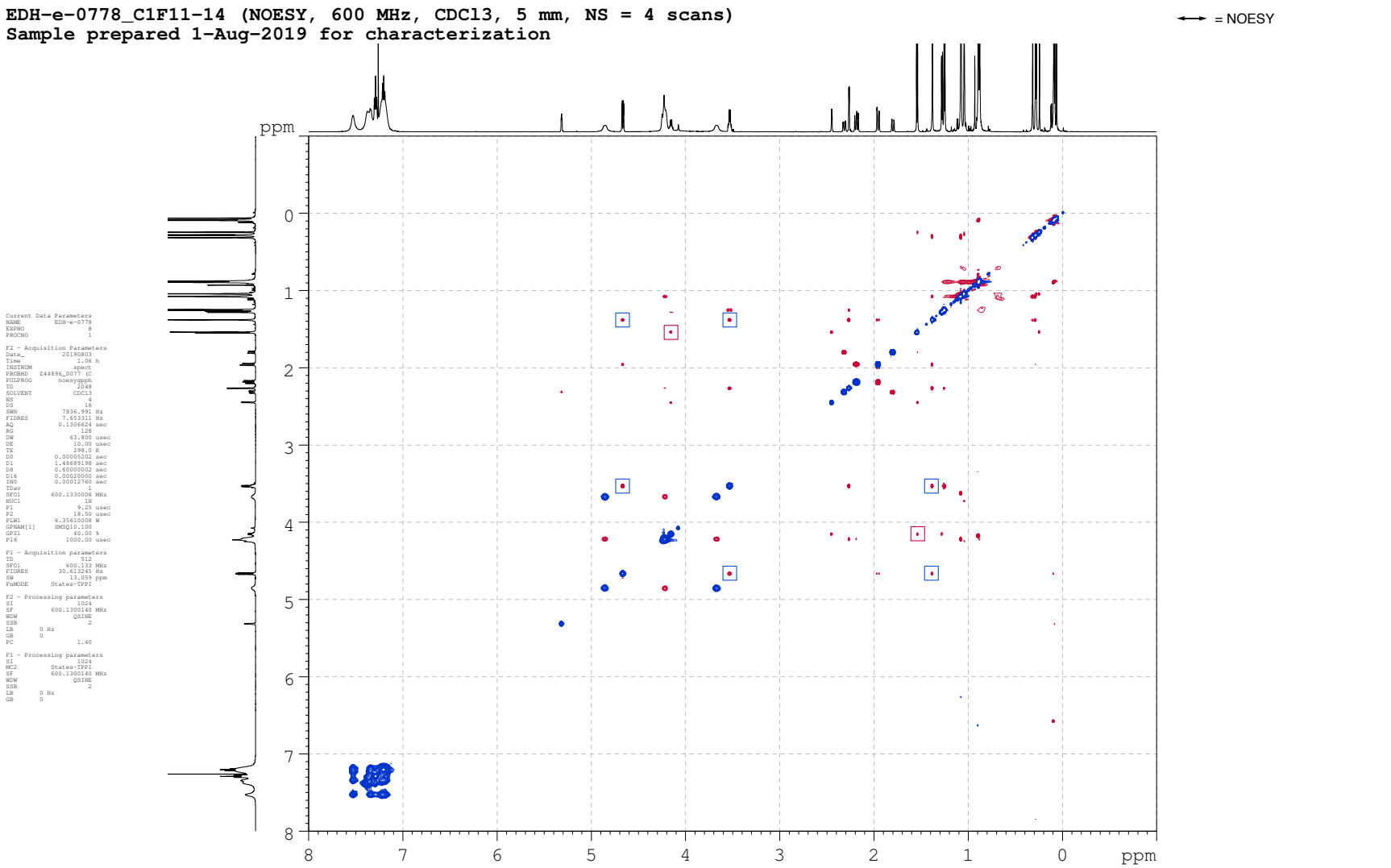
F2 - Processing parameters
SI 1024
SF 600.1300140 MHz
WDW QSINE
SSB 0
LB 0 Hz
GB 0
PC 1.40

F1 - Processing parameters
SI 1024
MC2 QF
SF 150.9027885 MHz
WDW QSINE
SSB 0
LB 0 Hz
GB 0

^{1}H-^{1}H NOESY NMR of Compound 235 (ca. 1:2 α/β) (600 MHz, $CDCl_3$)

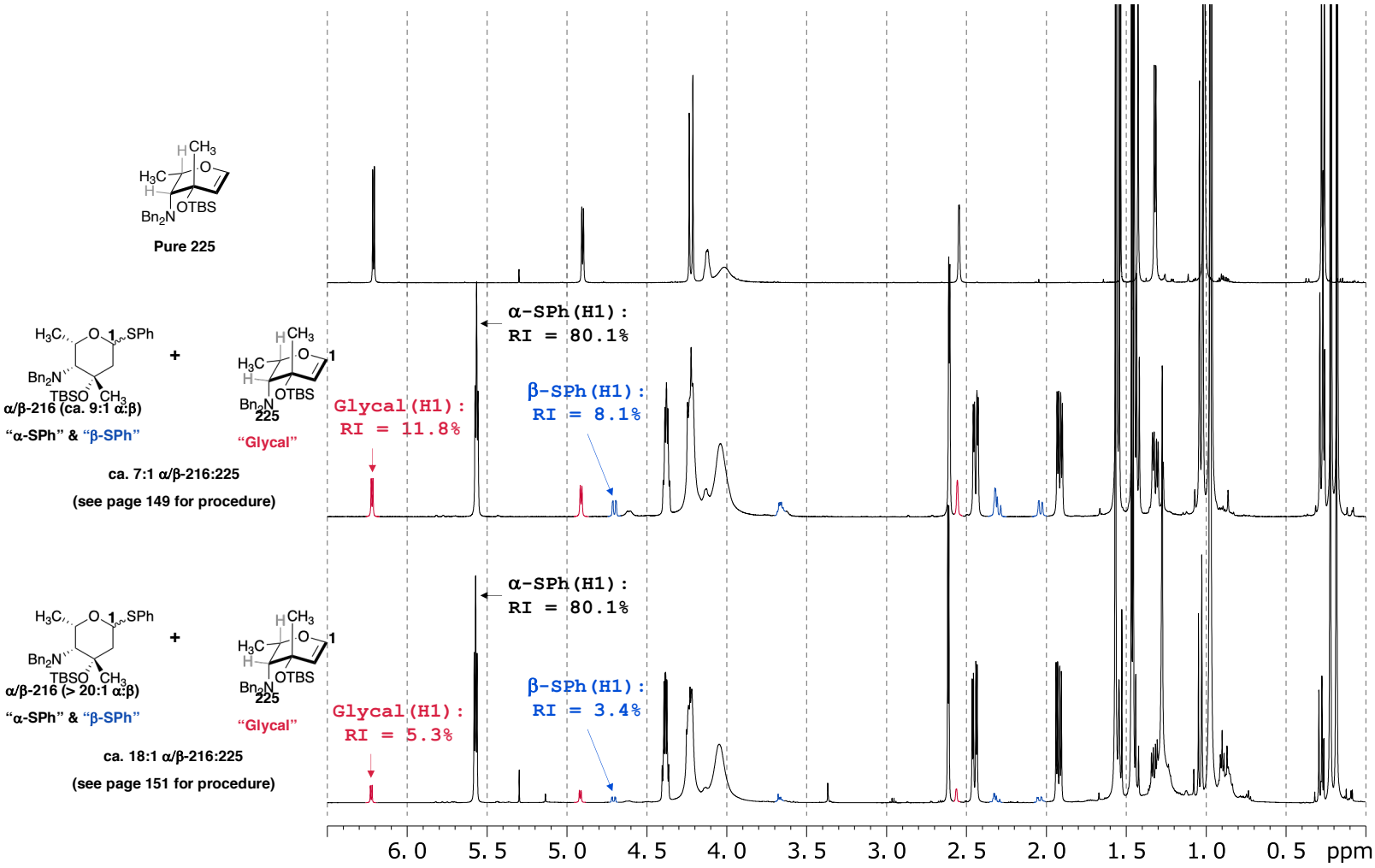
Note: Blue = diagonal/chemical exchange | Red = NOESY cross peaks.





^{1}H NMR Overlay Comparing Pure 225 to Mixtures of α/β-216 and 255 Prepared as Detailed on Pages 149 and 151 (600 MHz, $CDCl_3$)

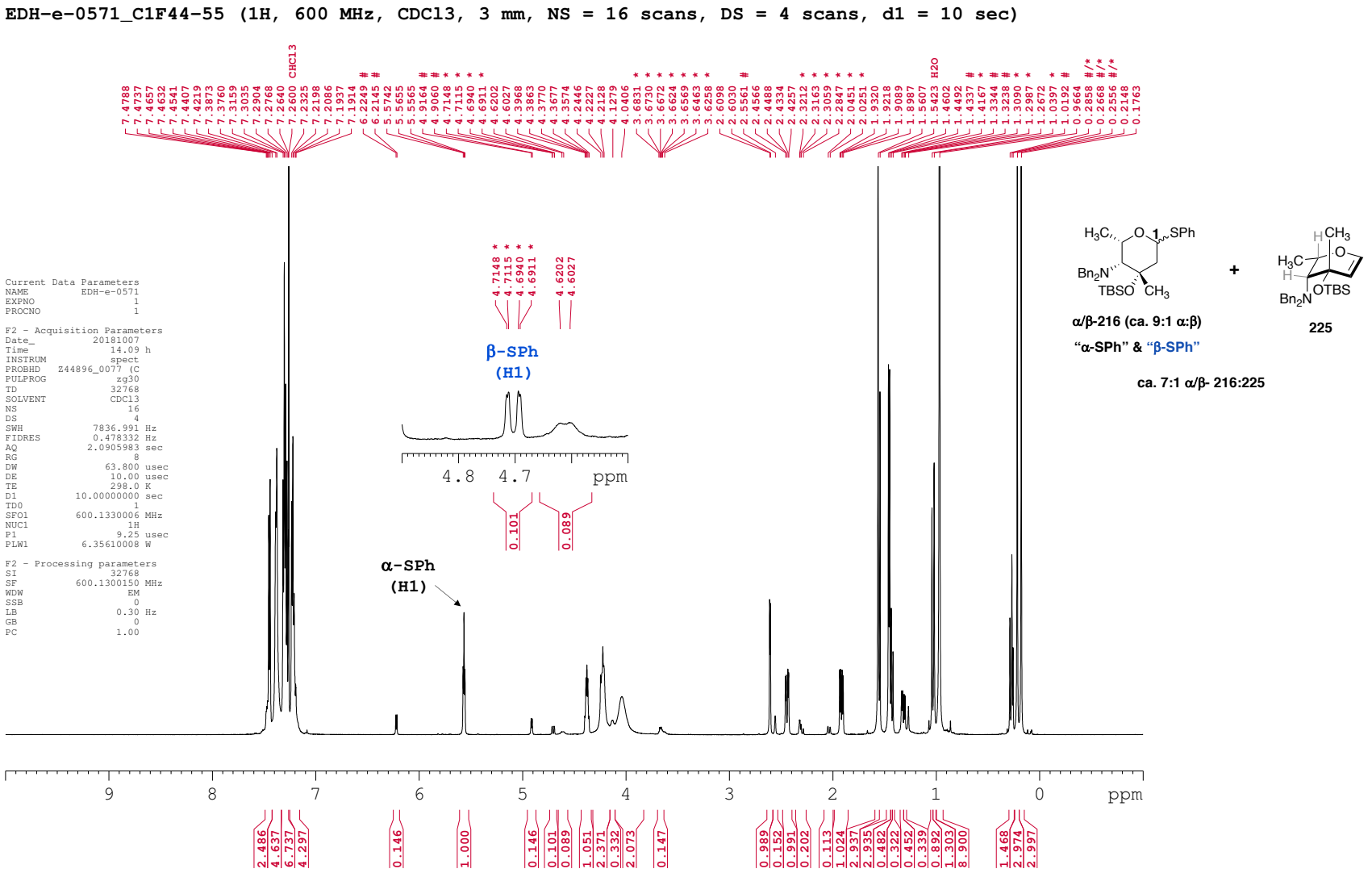
Note: RI = relative integration.



^{1}H NMR of Compound α/β-216 (ca. 9:1 α:β) (600 MHz, $CDCl_3$)

Note 1: ca. 7:1 mixture of compound thioglycoside α/β-**216** and glycal **225** prepared from acetate **227**; see page 149 for procedure.

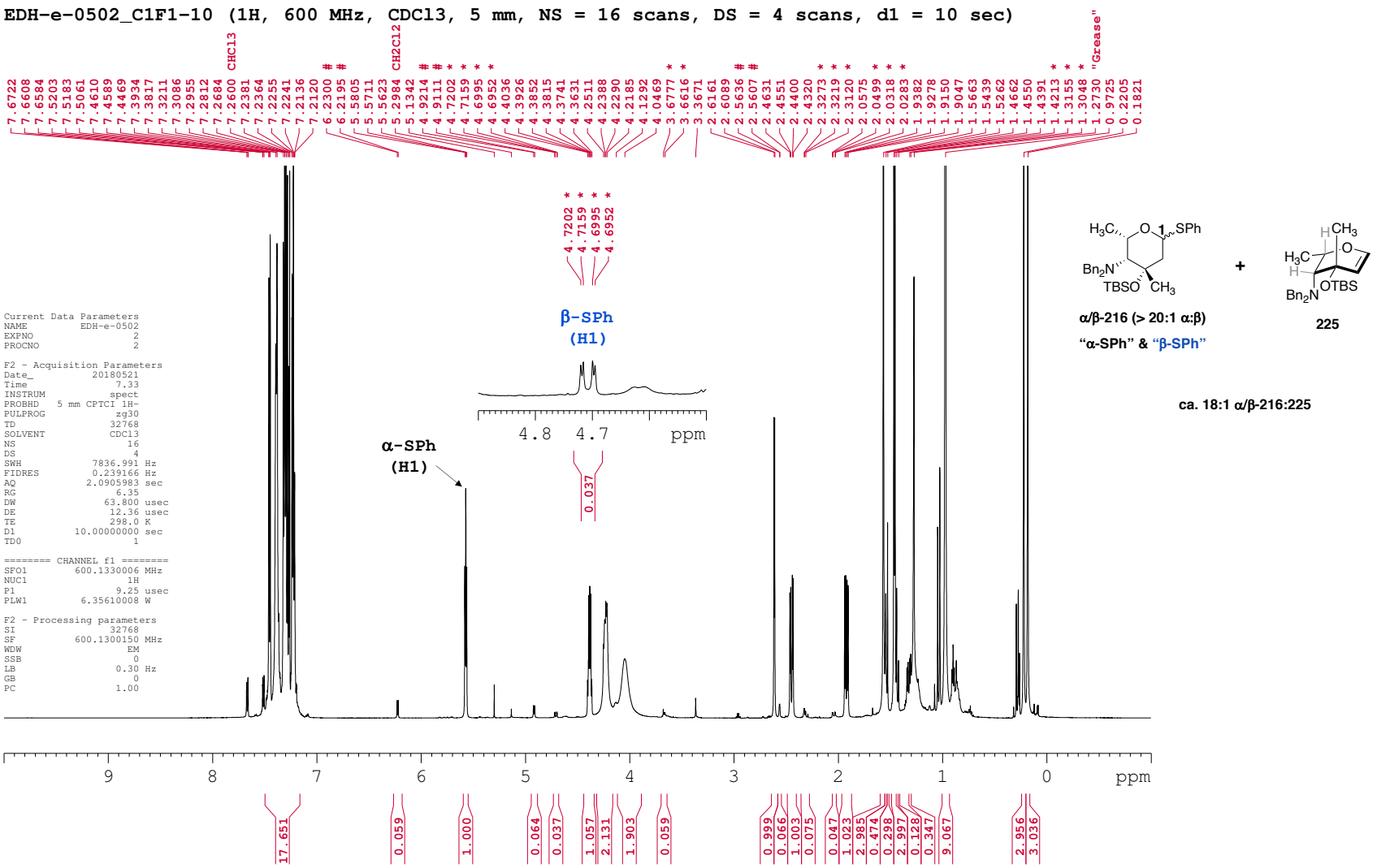
Note 2: Stars (*) denote peaks from β-**216**; hashes (#) denote peaks from **225**.



^{1}H NMR of Compound α/β-216 (> 20:1 α:β) (600 MHz, $CDCl_3$)

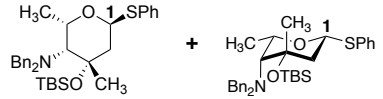
Note 1: ca. 18:1 mixture of compound thioglycoside α/β-**216** and glycal **225** prepared from acetate **227**; see page 151 for procedure.

Note 2: Stars (*) denote peaks from β-**216**; hashes (#) denote peaks from **225**.

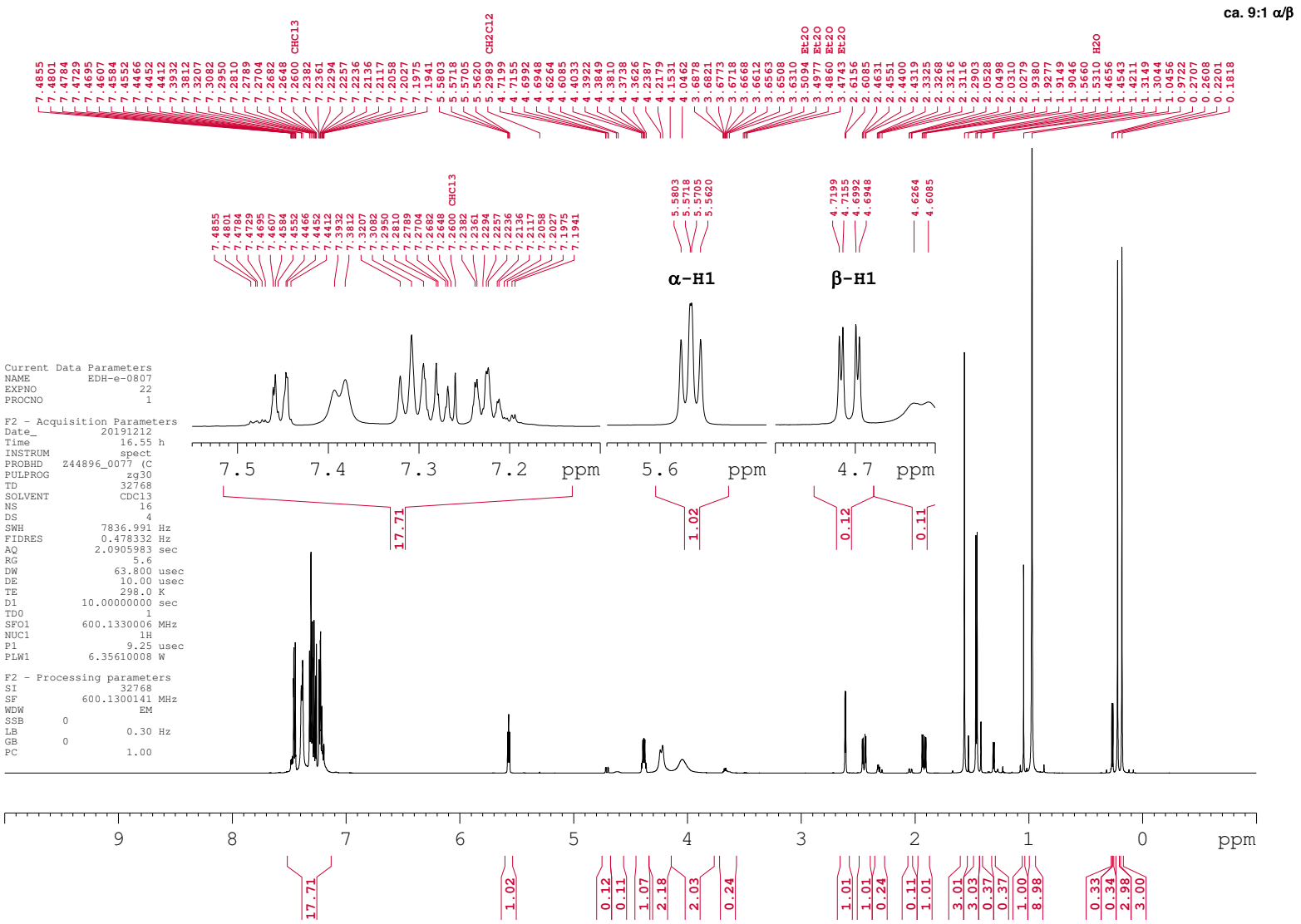


^{1}H NMR of Compound α/β-216 (ca. 9:1 α/β) (600 MHz, $CDCl_3$)

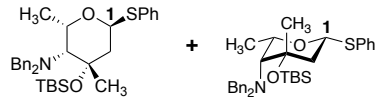
Note: Prepared from *O*-TBS Glycoside **235**; see page 157 for procedure.



ca. 9:1 α/β

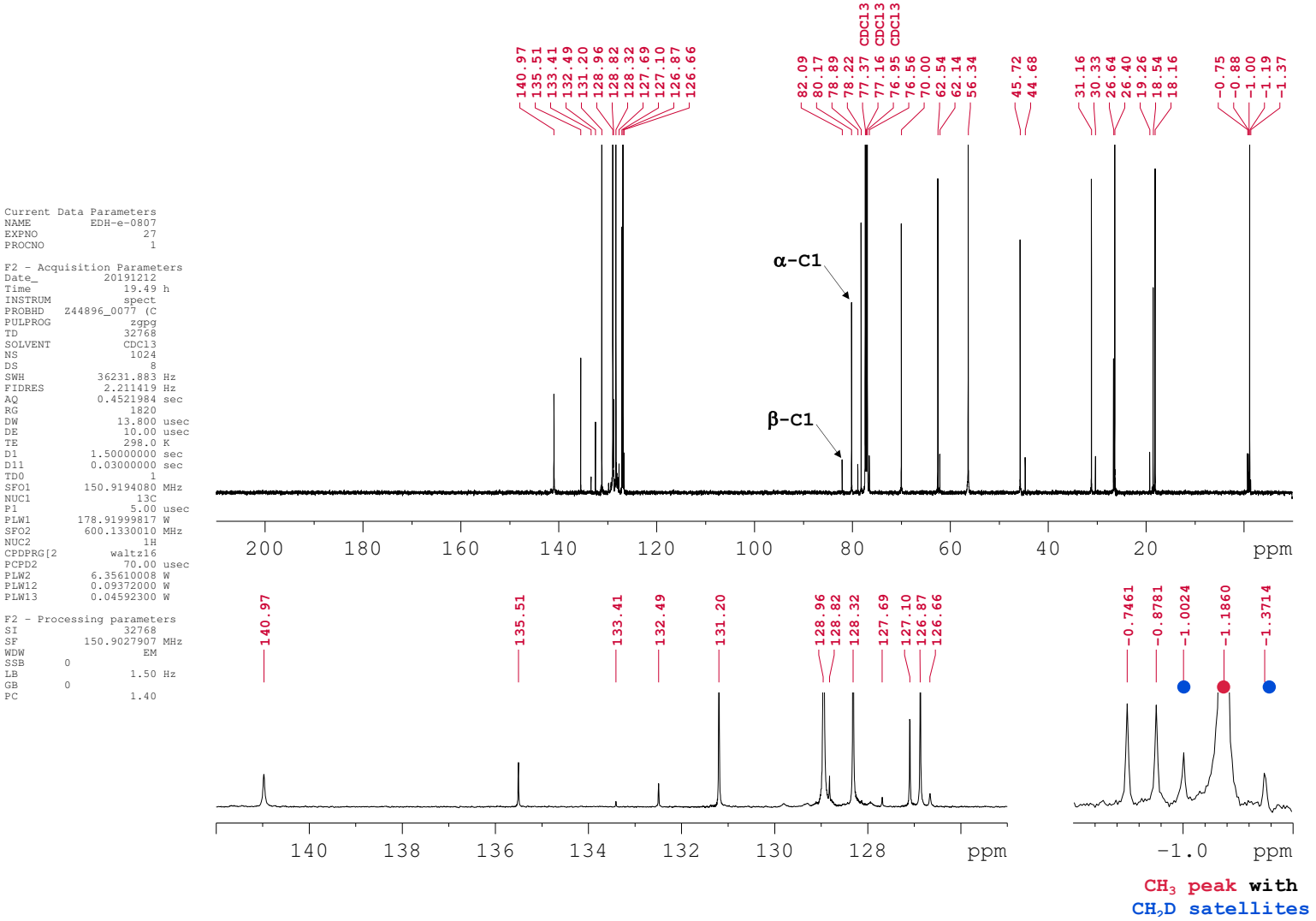


$^{13}C\{^{1}H\}$ NMR of Compound α/β-216 (ca. 9:1 α/β) (151 MHz, $CDCl_3$)



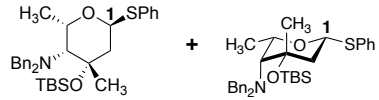
ca. 9:1 α/β

EDH-e-0807_C1F8-15 (13C{1H}, 151 MHz, CDCl3, NS = 1024 scans)



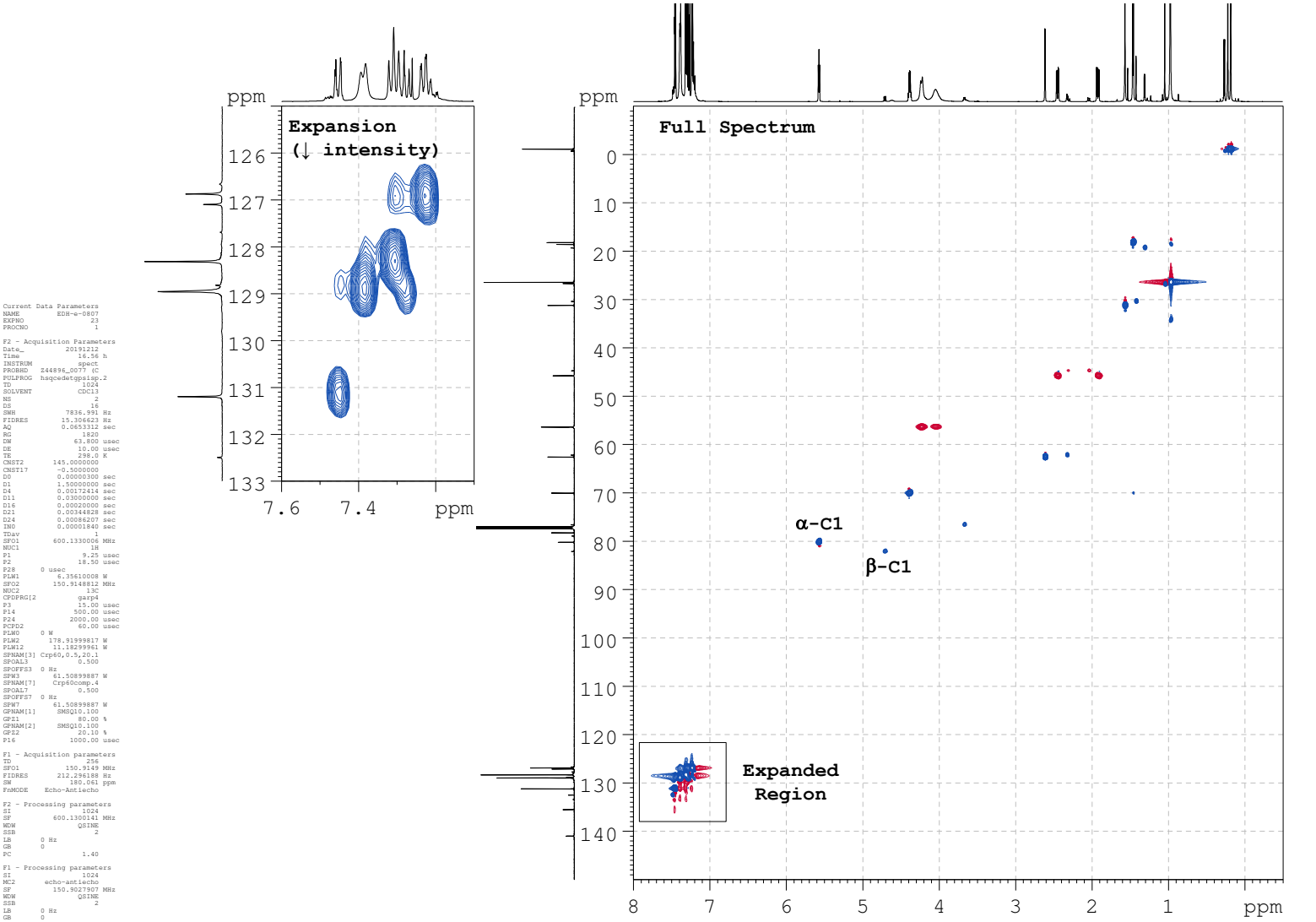
^{1}H-^{13}C HSQC{^{1}H}{^{13}C} NMR of Compound α/β-216 (ca. 9:1 α/β) (^{1}H-600 MHz, ^{13}C-151 MHz, $CDCl_3$)

Note: Blue = CH/CH_3 | Red = CH_2.



ca. 9:1 α/β

EDH-e-0807_C1F8-15 (HSQC{1H}{13C}, 600 MHz, CDCl3, NS = 2 scans, O2P = 80 ppm)



Current Data Parameters
NAME EDH-e-0807
EXPNO 23
PROCNO 1

F2 - Acquisition Parameters
Date_ 20191212
Time 16.56 h
INSTRUM spect
PROBHD Z44896_0077 (C
PULPROG hsqcedetgpsisp.2
TD 1024
SOLVENT CDCl3
NS 2
DS 16
SWH 7836.991 Hz
FIDRES 15.306623 Hz
AQ 0.0653312 sec
RG 1820
DW 63.800 usec
DE 10.00 usec
TE 298.0 K
CNST2 145.0000000
CNST17 -0.5000000
D0 0.00000300 sec
D1 1.50000000 sec
D4 0.00172414 sec
D11 0.03000000 sec
D16 0.00020000 sec
D21 0.00344828 sec
D24 0.00086207 sec
IN0 0.00001840 sec
TDav 1
SFO1 600.1330006 MHz
NUC1 1H
P1 9.25 usec
P2 18.50 usec
P28 0 usec
PLW1 6.35610008 W
SFO2 150.9148812 MHz
NUC2 13C
CPDPRG[2 garp4
P3 15.00 usec
P14 500.00 usec
P24 2000.00 usec
PCPD2 60.00 usec
PLW0 0 W
PLW2 178.91999817 W
PLW12 11.18299961 W
SPNAM[3] Crp60,0.5,20.1
SPOAL3 0.500
SPOFFS3 0 Hz
SPW3 61.50899887 W
SPNAM[7] Crp60comp.4
SPOAL7 0.500
SPOFF87 0 Hz
SPW7 61.50899887 W
GPNAM[1] SMSQ10.100
GPZ1 80.00 %
GPNAM[2] SMSQ10.100
GPZ2 20.10 %
P16 1000.00 usec

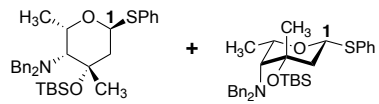
F1 - Acquisition parameters
TD 256
SFO1 150.9149 MHz
FIDRES 212.296188 Hz
SW 180.061 ppm
FnMODE Echo-Antiecho

F2 - Processing parameters
SI 1024
SF 600.1300141 MHz
WDW QSINE
SSB 2
LB 0 Hz
GB 0
PC 1.40

F1 - Processing parameters
SI 1024
MC2 echo-antiecho
SF 150.9027907 MHz
WDW QSINE
SSB 2
LB 0 Hz
GB 0

Coupled ^{1}H-^{13}C HSQC NMR of Compound α/β-216 (ca. 9:1 α/β) (^{1}H-600 MHz, ^{13}C-151 MHz, $CDCl_3$)

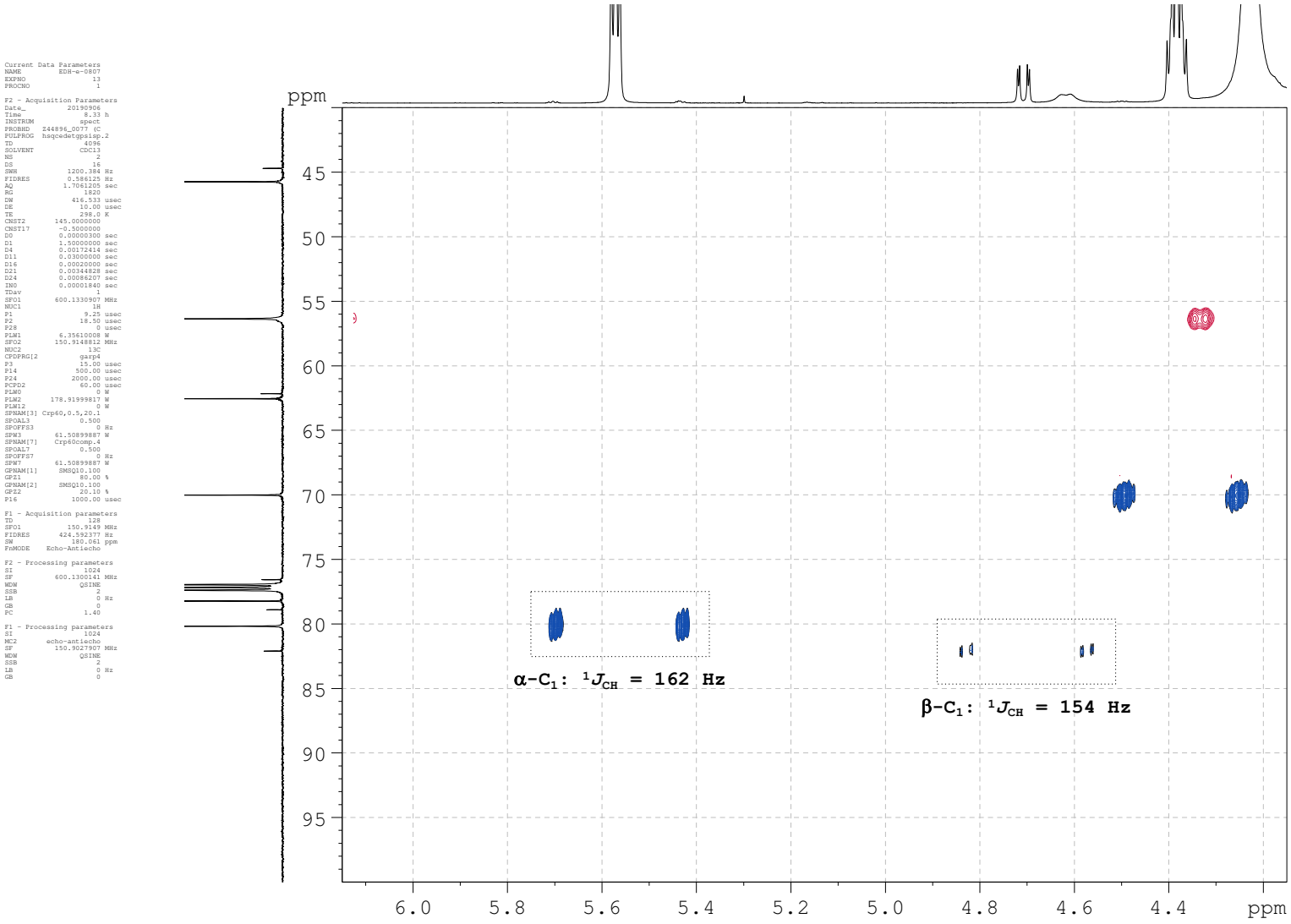
Note: Blue = CH/CH_3 | Red = CH_2.



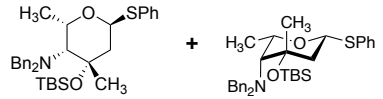
α-216 + β-216

ca. 9:1 α/β

EDH-e-0807_C1F8-15 ("Coupled" HSQC, 600 MHz, CDCl3, 5 mm)



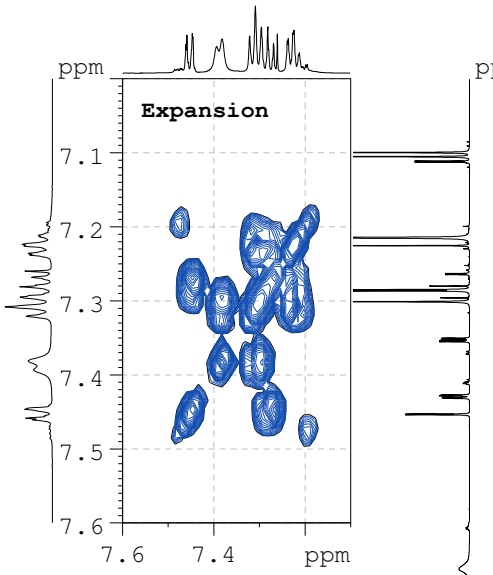
^{1}H-^{1}H COSY NMR of Compound α/β-216 (ca. 9:1 α/β) (600 MHz, $CDCl_3$)

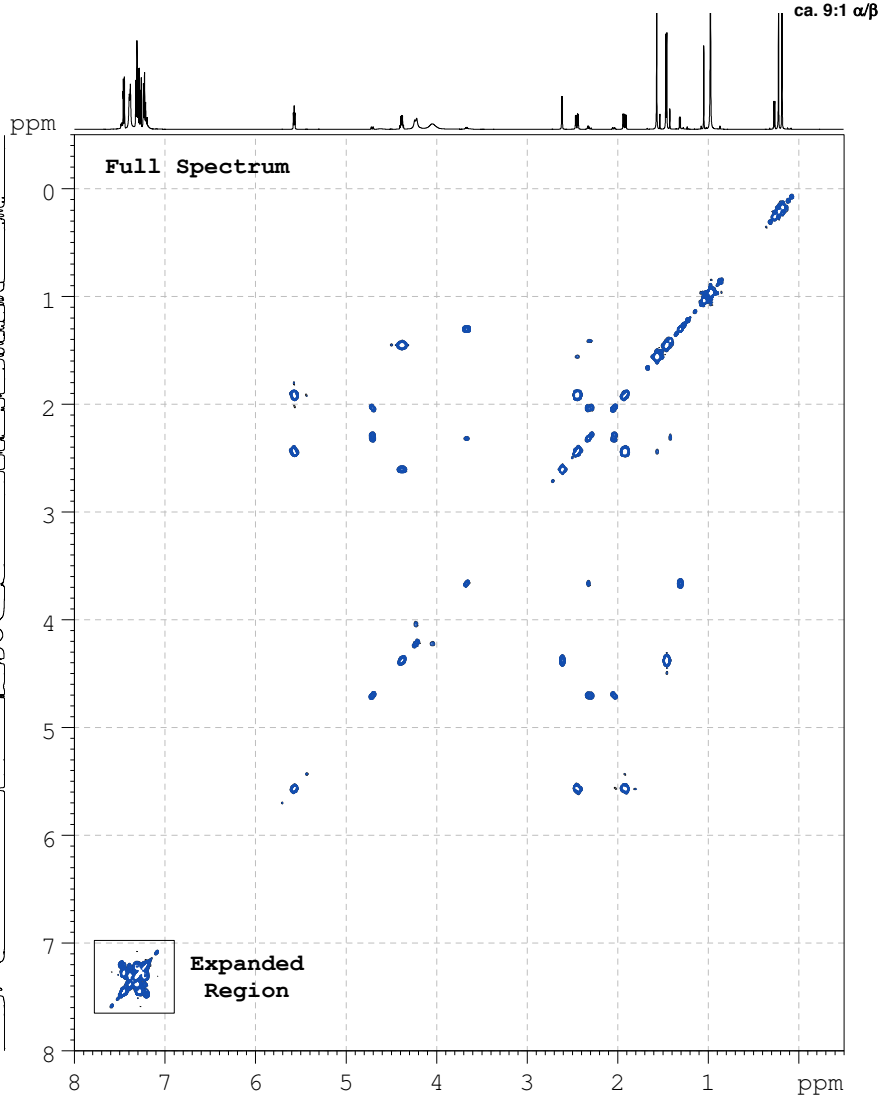


α-216 β-216

EDH-e-0807_C1F8-15 (COSY, 600 MHz, CDCl3, NS = 1 scan)

ca. 9:1 α/β






```
Current Data Parameters
NAME            EDH-e-0807
EXPNO                   24
PROCNO                   1

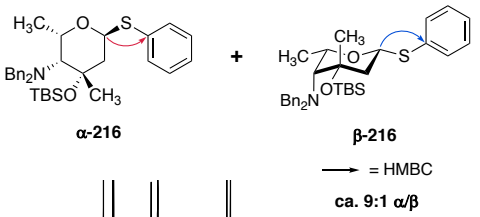
F2 - Acquisition Parameters
Date_         20191212
Time              17.11 h
INSTRUM             spect
PROBHD   Z44896_0077 (C
PULPROG          cosygpqf
TD                   2048
SOLVENT              CDCl3
NS                      1
DS                      8
SWH              7836.991 Hz
FIDRES           7.653311 Hz
AQ               0.1306624 sec
RG                     64
DW                 63.800 usec
DE                 10.00 usec
TE                298.0 K
D0              0.00000300 sec
D1              1.48689198 sec
D13             0.00000400 sec
D16             0.00020000 sec
IN0             0.00012760 sec
TDav                    1
SFO1         600.1330006 MHz
NUC1                   1H
P0                  4.65 usec
P1                  9.30 usec
PLW1         6.35610008 W
GPNAM[1]     SMSQ10.100
GPZ1               10.00 %
P16              1000.00 usec

F1 - Acquisition parameters
TD                    512
SFO1           600.133 MHz
FIDRES        30.613245 Hz
SW               13.059 ppm
FnMODE                QF

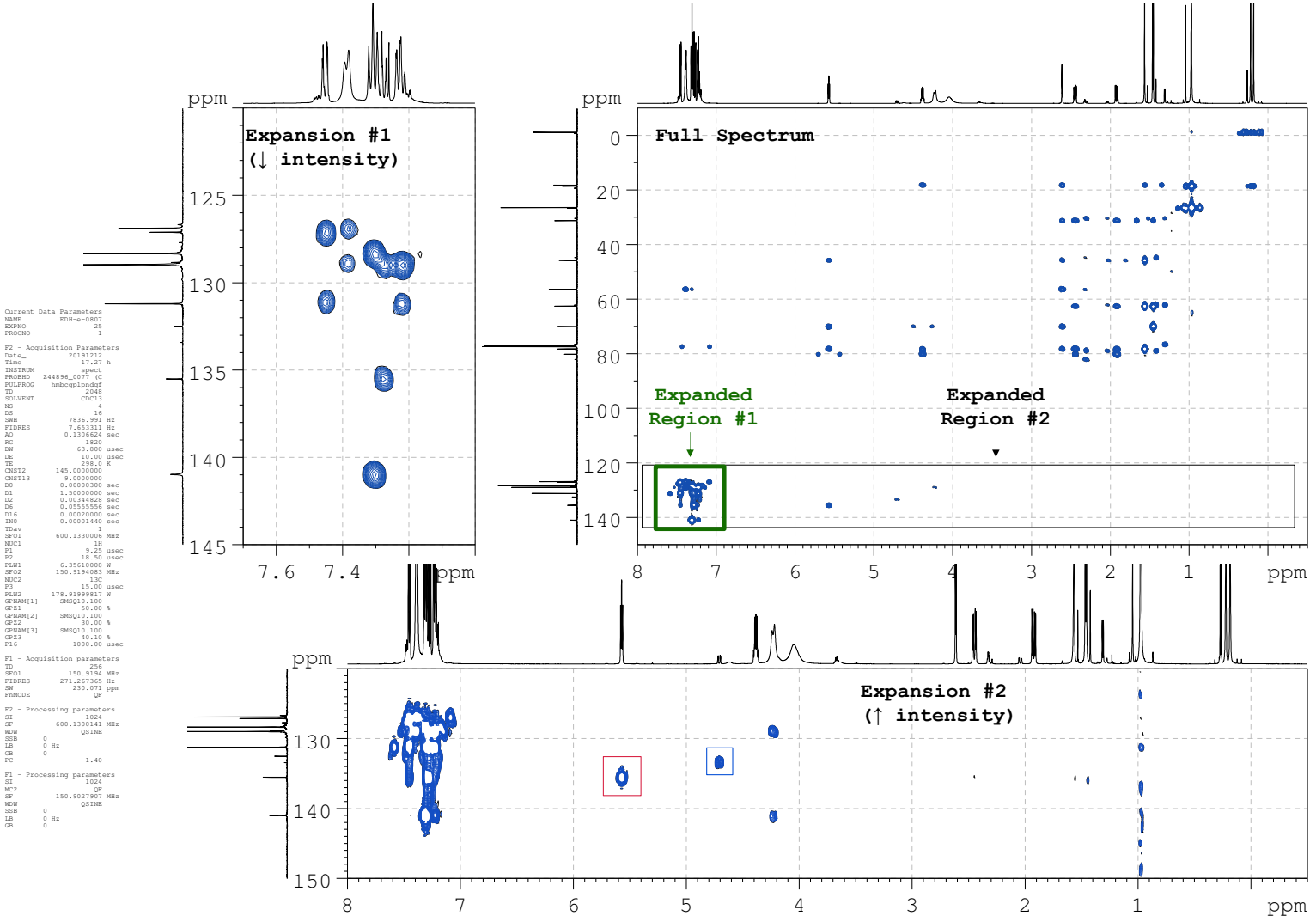
F2 - Processing parameters
SI                   1024
SF           600.1300141 MHz
WDW                  QSINE
SSB         0
LB        0 Hz
GB          0
PC                   1.40

F1 - Processing parameters
SI                   1024
MC2                    QF
SF           600.1300141 MHz
WDW                  QSINE
SSB         0
LB        0 Hz
GB          0
```

^{1}H-^{13}C HMBC NMR of Compound α/β-216 (ca. 9:1 α/β) (^{1}H-600 MHz, ^{13}C-151 MHz, $CDCl_3$)

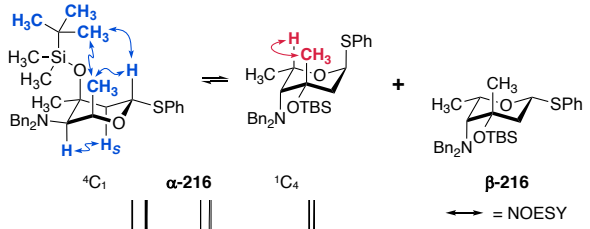


EDH-e-0807_C1F8-15 (HMBC, 600 MHz, CDCl3, NS = 4 scans)

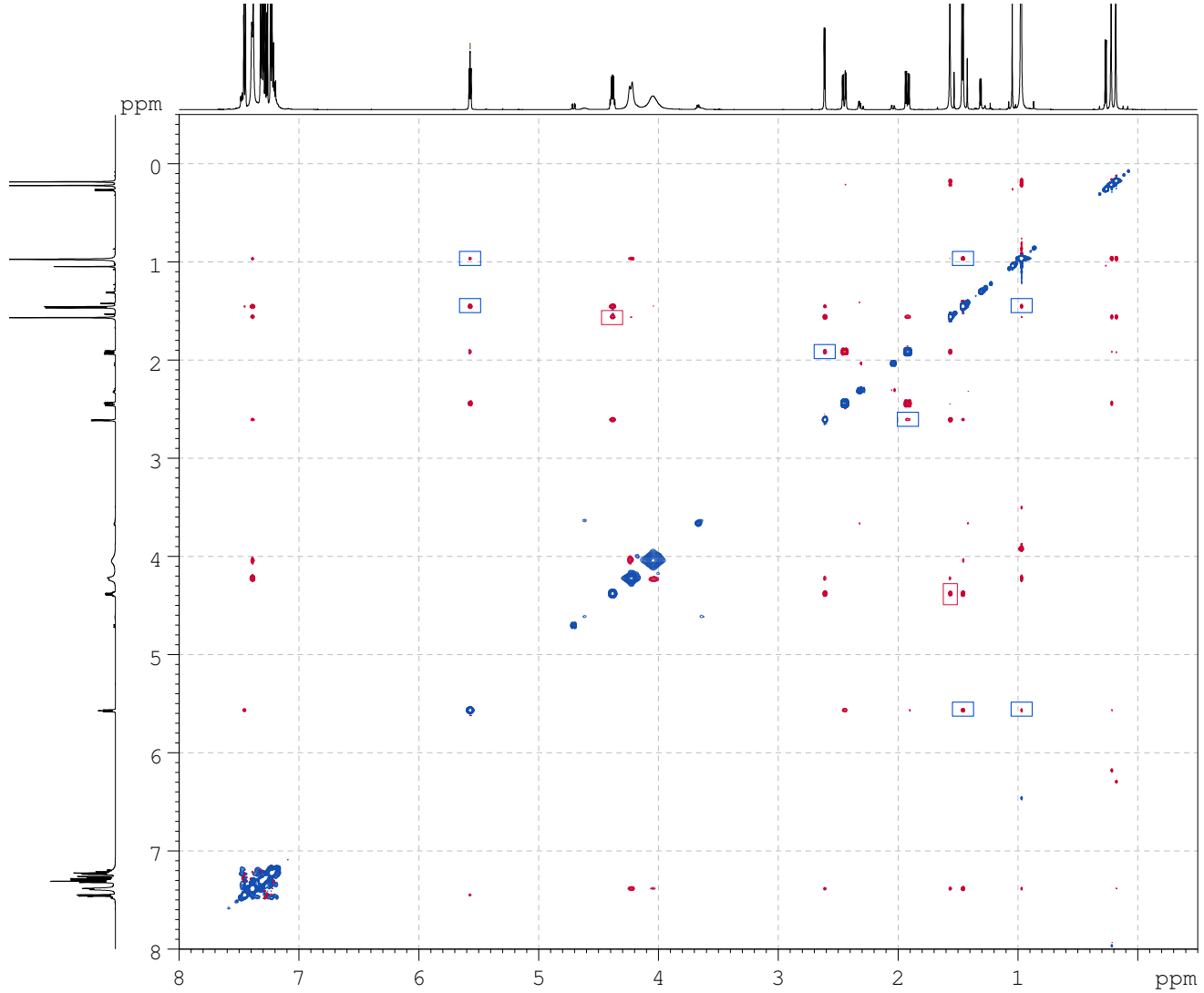


^{1}H-^{1}H NOESY NMR of Compound α/β-216 (ca. 9:1 α/β) (600 MHz, $CDCl_3$)

Note: Blue = diagonal/chemical exchange | Red = NOESY cross peaks.



EDH-e-0807_C1F8-15 (NOESY, 600 MHz, CDCl3, NS = 4 scans)




```
Current Data Parameters
NAME            EDH-e-0807
EXPNO                   26
PROCNO                   1

F2 - Acquisition Parameters
Date_            20191212
Time                17.57 h
INSTRUM              spect
PROBHD   Z44896_0077 (C
PULPROG          noesygpph
TD                    2048
SOLVENT             CDCl3
NS                       4
DS                      16
SWH               7836.991 Hz
FIDRES            7.653311 Hz
AQ                0.1306624 sec
RG                      128
DW                 63.800 usec
DE                 10.00 usec
TE                 298.0 K
D0                0.00005202 sec
D1                1.48689198 sec
D8                0.60000002 sec
D16               0.00020000 sec
IN0               0.00012760 sec
TDav                     1
SFO1           600.1330006 MHz
NUC1                    1H
P1                    9.25 usec
P2                   18.50 usec
PLW1           6.35610008 W
GPNAM[1]       SMSQ10.100
GPZ1                40.00 %
P16                1000.00 usec

F1 - Acquisition parameters
TD                       512
SFO1           600.133 MHz
FIDRES         30.613245 Hz
SW                 13.059 ppm
FnMODE         States-TPPI

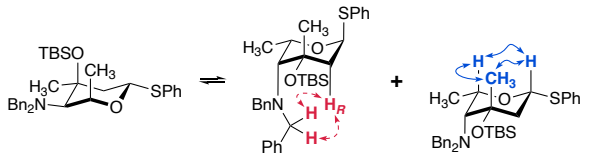
F2 - Processing parameters
SI                      1024
SF             600.1300141 MHz
WDW                    QSINE
SSB                        2
LB          0 Hz
GB          0
PC                     1.40

F1 - Processing parameters
SI                      1024
MC2            States-TPPI
SF             600.1300141 MHz
WDW                    QSINE
SSB                        2
LB          0 Hz
GB          0
```

^{1}H-^{1}H NOESY NMR of Compound α/β-216 (ca. 9:1 α/β) (600 MHz, $CDCl_3$)

Note 1: Blue = diagonal/chemical exchange | Red = NOESY cross peaks.

Note 2: Same spectrum with increased intensity as compared to NOESY on page S267.



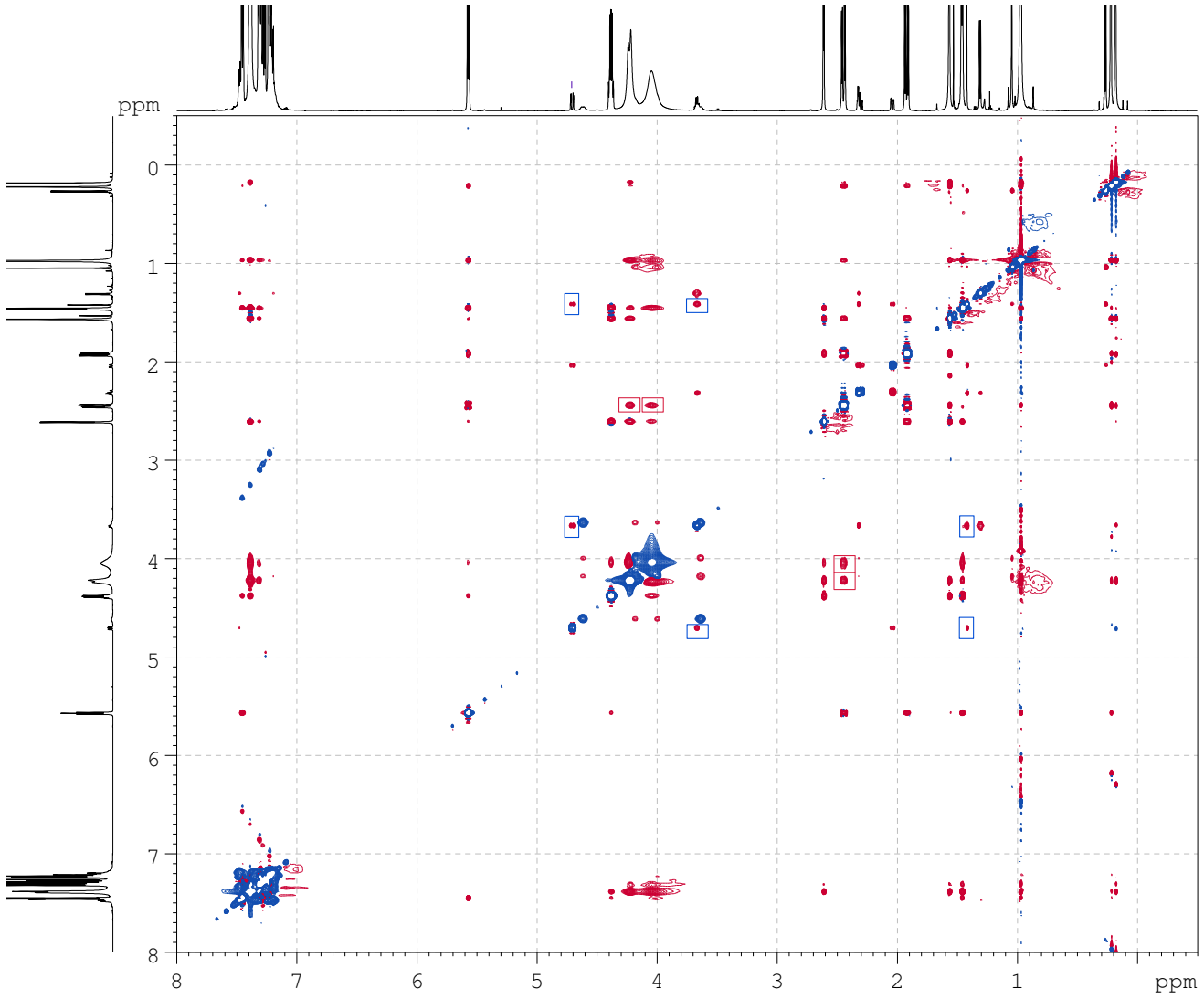
$^{4}C_1$ α-216 $^{1}C_4$ β-216

= NOESY

= Weak NOESY

ca. 9:1 α/β

EDH-e-0807_C1F8-15 (NOESY, 600 MHz, CDCl3, NS = 4 scans)




```
Current Data Parameters
NAME          EDH-e-0807
EXPNO                 26
PROCNO                 1

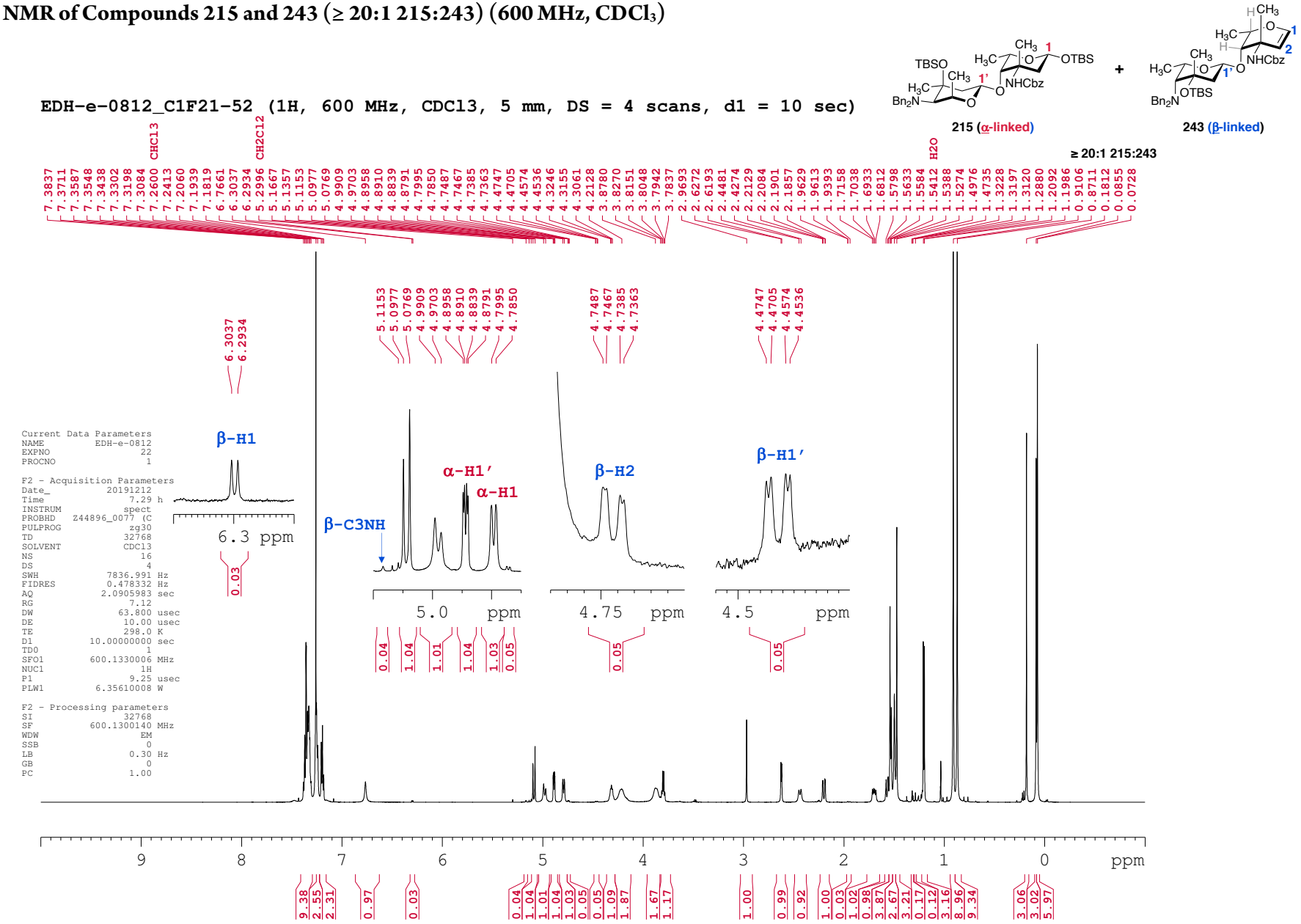
F2 - Acquisition Parameters
Date_           20191212
Time              17.57 h
INSTRUM             spect
PROBHD    Z44896_0077 (C
PULPROG         noesygpph
TD                  2048
SOLVENT             CDCl3
NS                     4
DS                    16
SWH             7836.991 Hz
FIDRES          7.653311 Hz
AQ              0.1306624 sec
RG                   128
DW                63.800 usec
DE                10.00 usec
TE                298.0 K
D0              0.00005202 sec
D1              1.48689198 sec
D8              0.60000002 sec
D16             0.00020000 sec
IN0             0.00012760 sec
TDav                   1
SFO1          600.1330006 MHz
NUC1                  1H
P1                 9.25 usec
P2                18.50 usec
PLW1            6.35610008 W
GPNAM[1]       SMSQ10.100
GPZ1                40.00 %
P16               1000.00 usec

F1 - Acquisition parameters
TD                   512
SFO1           600.133 MHz
FIDRES         30.613245 Hz
SW                13.059 ppm
FnMODE        States-TPPI

F2 - Processing parameters
SI                  1024
SF            600.1300141 MHz
WDW                 QSINE
SSB                     2
LB          0 Hz
GB                      0
PC                   1.40

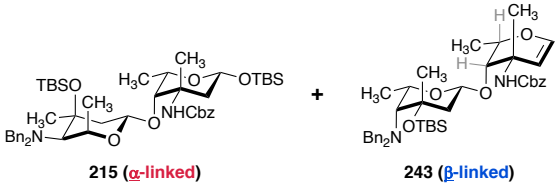
F1 - Processing parameters
SI                  1024
MC2           States-TPPI
SF            600.1300141 MHz
WDW                 QSINE
SSB                     2
LB          0 Hz
GB                      0
```

^{1}H NMR of Compounds 215 and 243 (≥ 20:1 215:243) (600 MHz, $CDCl_3$)



^{13}C NMR of Compounds 215 and 243 (≥ 20:1 215:243) (151 MHz, $CDCl_3$)

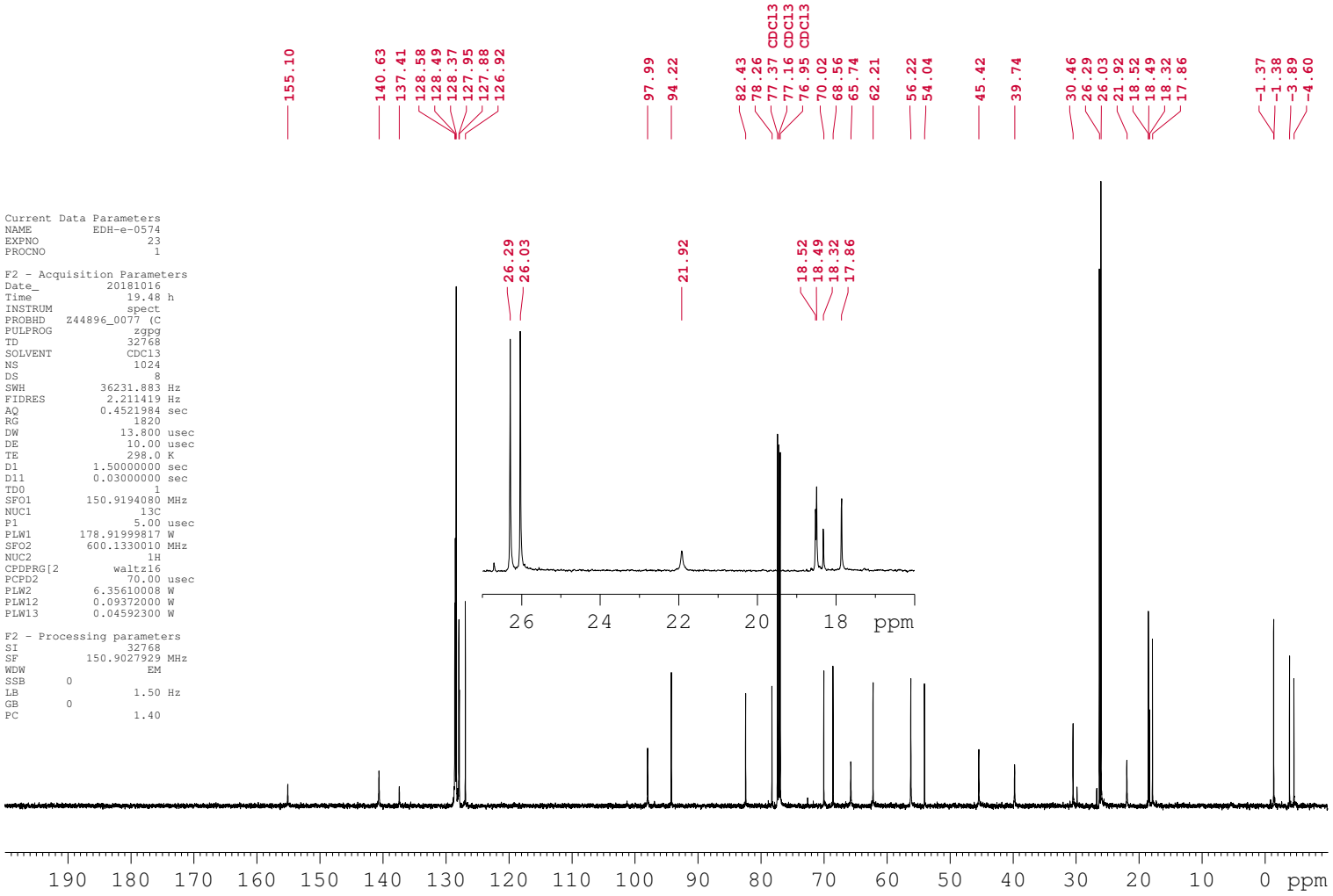
Note: Only peaks from major α-linked product **215** are visible.



215 (α-linked) **243 (β-linked)**

≥ 20:1 215:243

EDH-e-0574_C1F18-50 (13C, 151 MHz, CDCl3, 3 mm)




```
Current Data Parameters
NAME         EDH-e-0574
EXPNO                23
PROCNO                1

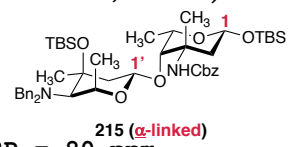
F2 - Acquisition Parameters
Date_          20181016
Time              19.48 h
INSTRUM             spect
PROBHD   Z44896_0077 (C
PULPROG             zgpg
TD                 32768
SOLVENT            CDCl3
NS                  1024
DS                     8
SWH            36231.883 Hz
FIDRES          2.211419 Hz
AQ              0.4521984 sec
RG                  1820
DW                 13.800 usec
DE                 10.00 usec
TE                 298.0 K
D1            1.50000000 sec
D11           0.03000000 sec
TD0                    1
SFO1         150.9194080 MHz
NUC1                13C
P1                  5.00 usec
PLW1        178.91999817 W
SFO2         600.1330010 MHz
NUC2                1H
CPDPRG[2          waltz16
PCPD2              70.00 usec
PLW2          6.35610008 W
PLW12         0.09372000 W
PLW13         0.04592300 W

F2 - Processing parameters
SI                 32768
SF           150.9027929 MHz
WDW                   EM
SSB       0
LB                 1.50 Hz
GB        0
PC                 1.40
```

^{1}H-^{13}C HSQC{^{1}H}{^{13}C} NMR of Compounds 215 and 243 (≥ 20:1 215:243) (^{1}H-600 MHz, ^{13}C-151 MHz, $CDCl_3$)

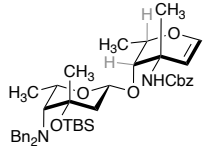
Note 1: Blue = CH/CH_3 | Red = CH_2.

Note 2: Only peaks from major α-linked product **20** are visible.



215 (α-linked)

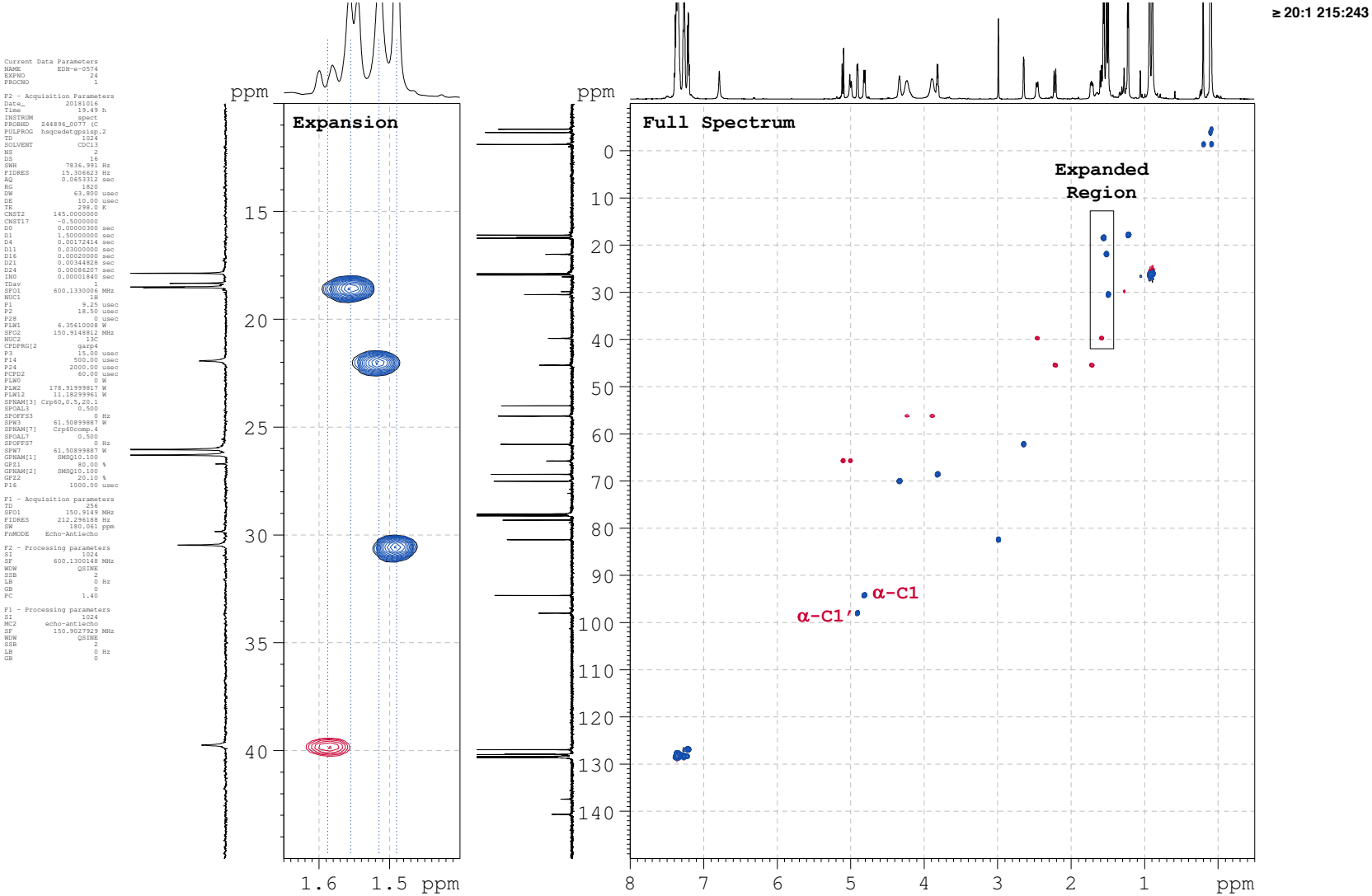
+



243 (β-linked)

≥ 20:1 215:243

EDH-e-0574_C1F18-50 (HSQC{1H}{13C}, 600 MHz, CDCl3, 3 mm, NS = 2 scans, O2P = 80 ppm

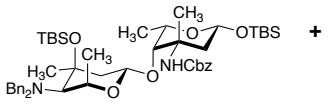


^{1}H-^{13}C HSQC{^{1}H}{^{13}C} NMR of Compounds 215 and 243 (≥ 20:1 215:243) (^{1}H-600 MHz, ^{13}C-151 MHz, $CDCl_3$)

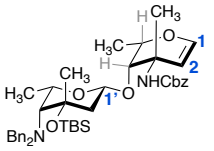
Note 1: Blue = CH/CH_3 | Red = CH_2.

Note 2: Same spectrum with increased intensity increased (and smaller window) as compared to spectrum on page S302.

Note 3: Correlations for β-linked **243** denoted with asterisk (*).



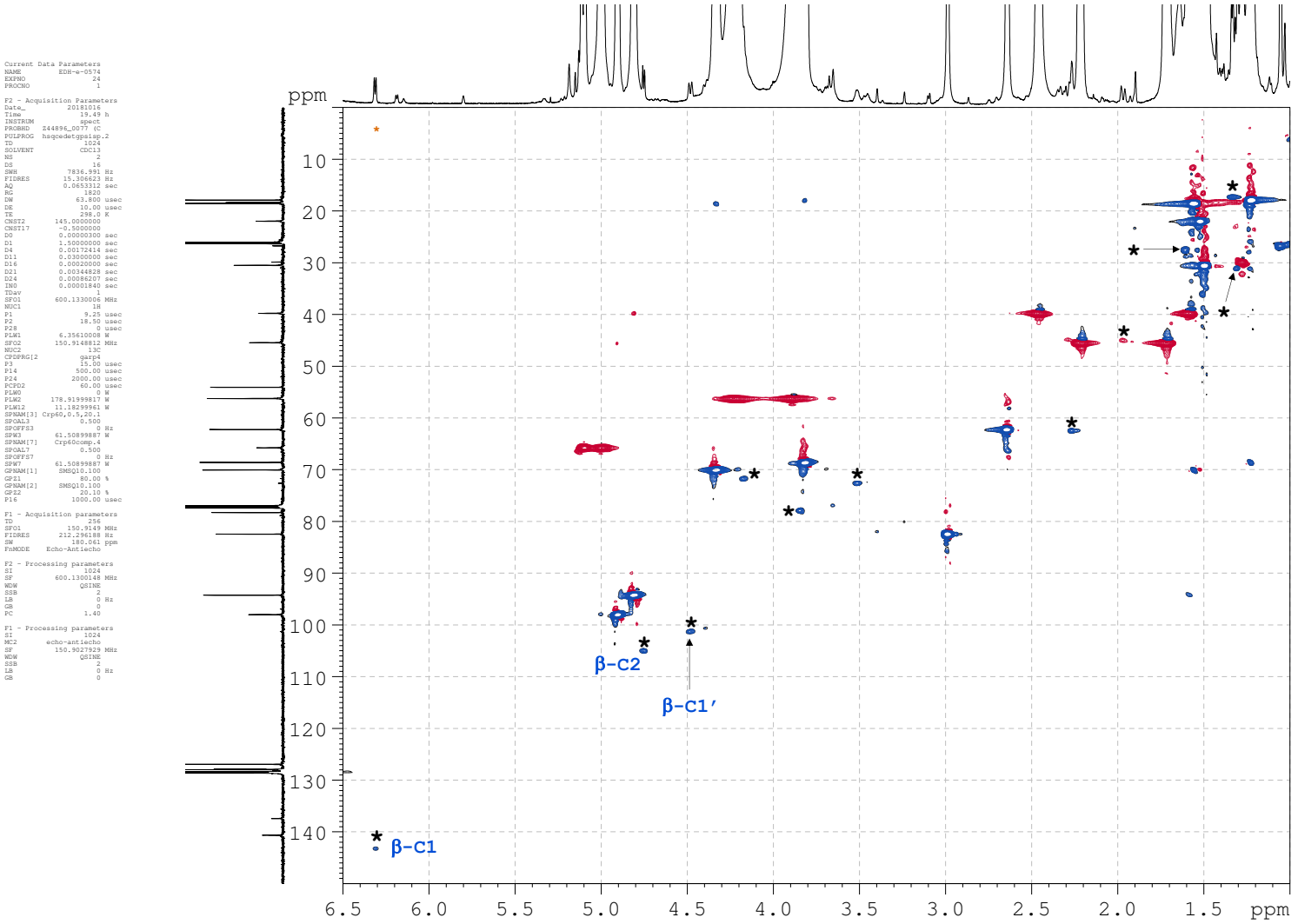
215 (α-linked) +



243 (β-linked)

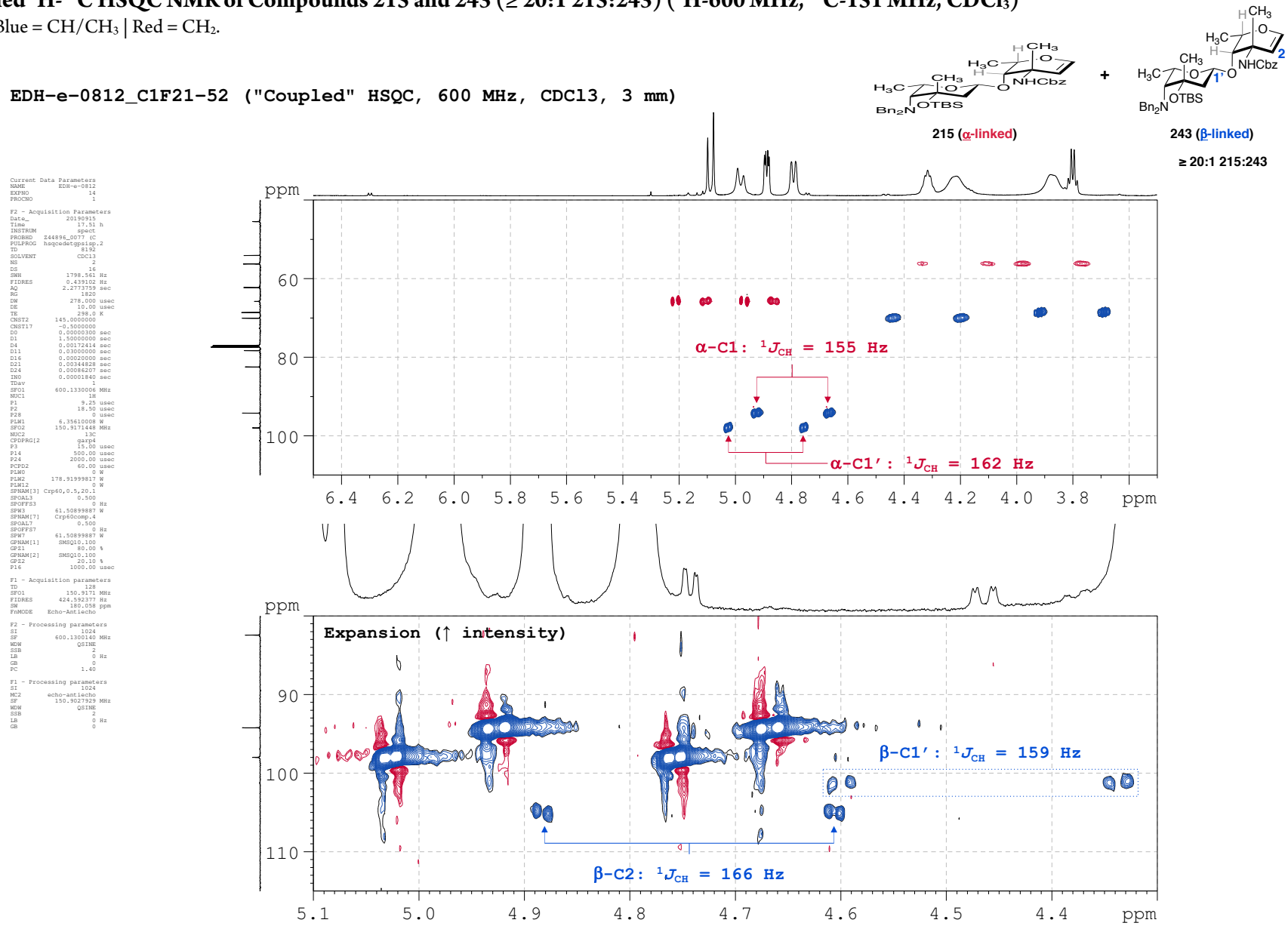
≥ 20:1 215:243

EDH-e-0574_C1F18-50 (HSQC{1H}{13C}, 600 MHz, CDCl3, 3 mm, NS = 2 scans, O2P = 80 ppm



Coupled ^{1}H-^{13}C HSQC NMR of Compounds 215 and 243 (≥ 20:1 215:243) (^{1}H-600 MHz, ^{13}C-151 MHz, $CDCl_3$)

Note: Blue = CH/CH_3 | Red = CH_2.




```
Current Data Parameters
NAME          EDH-e-0812
EXPNO                 14
PROCNO                 1

F2 - Acquisition Parameters
Date_           20190915
Time              17.51 h
INSTRUM             spect
PROBHD    Z44896_0077 (C
PULPROG  hsqcedetgpsisp.2
TD                  8192
SOLVENT             CDCl3
NS                     2
DS                    16
SWH              1798.561 Hz
FIDRES              0.439102 Hz
AQ                2.2773759 sec
RG                     1820
DW                 278.000 usec
DE                  10.00 usec
TE                  298.0 K
CNST2           145.0000000
CNST17           -0.5000000
D0               0.00000300 sec
D1               1.50000000 sec
D4               0.00172414 sec
D11              0.03000000 sec
D16              0.00020000 sec
D21              0.00344828 sec
D24              0.00086207 sec
IN0              0.00001840 sec
TDav                       1
SFO1            600.1330006 MHz
NUC1                     1H
P1                    9.25 usec
P2                   18.50 usec
P28                     0 usec
PLW1             6.35610008 W
SFO2            150.9171448 MHz
NUC2                     13C
CPDPRG[2                 garp4
P3                   15.00 usec
P14                 500.00 usec
P24                2000.00 usec
PCPD2                60.00 usec
PLW0                    0 W
PLW2           178.91999817 W
PLW12                   0 W
SPNAM[3] Crp60,0.5,20.1
SPOAL3                0.500
SPOFFS3                 0 Hz
SPW3           61.50899887 W
SPNAM[7]      Crp60comp.4
SPOAL7                0.500
SPOFFS7                 0 Hz
SPW7           61.50899887 W
GPNAM[1]       SMSQ10.100
GPZ1                 80.00 %
GPNAM[2]       SMSQ10.100
GPZ2                 20.10 %
P16                1000.00 usec

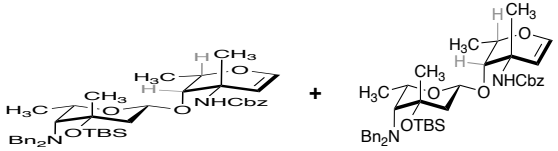
F1 - Acquisition parameters
TD                    128
SFO1            150.9171 MHz
FIDRES          424.592377 Hz
SW                180.058 ppm
FnMODE     Echo-Antiecho

F2 - Processing parameters
SI                    1024
SF              600.1300140 MHz
WDW                   QSINE
SSB                       2
LB                     0 Hz
GB                        0
PC                     1.40

F1 - Processing parameters
SI                    1024
MC2         echo-antiecho
SF              150.9027929 MHz
WDW                   QSINE
SSB                       2
LB                     0 Hz
GB                        0
```

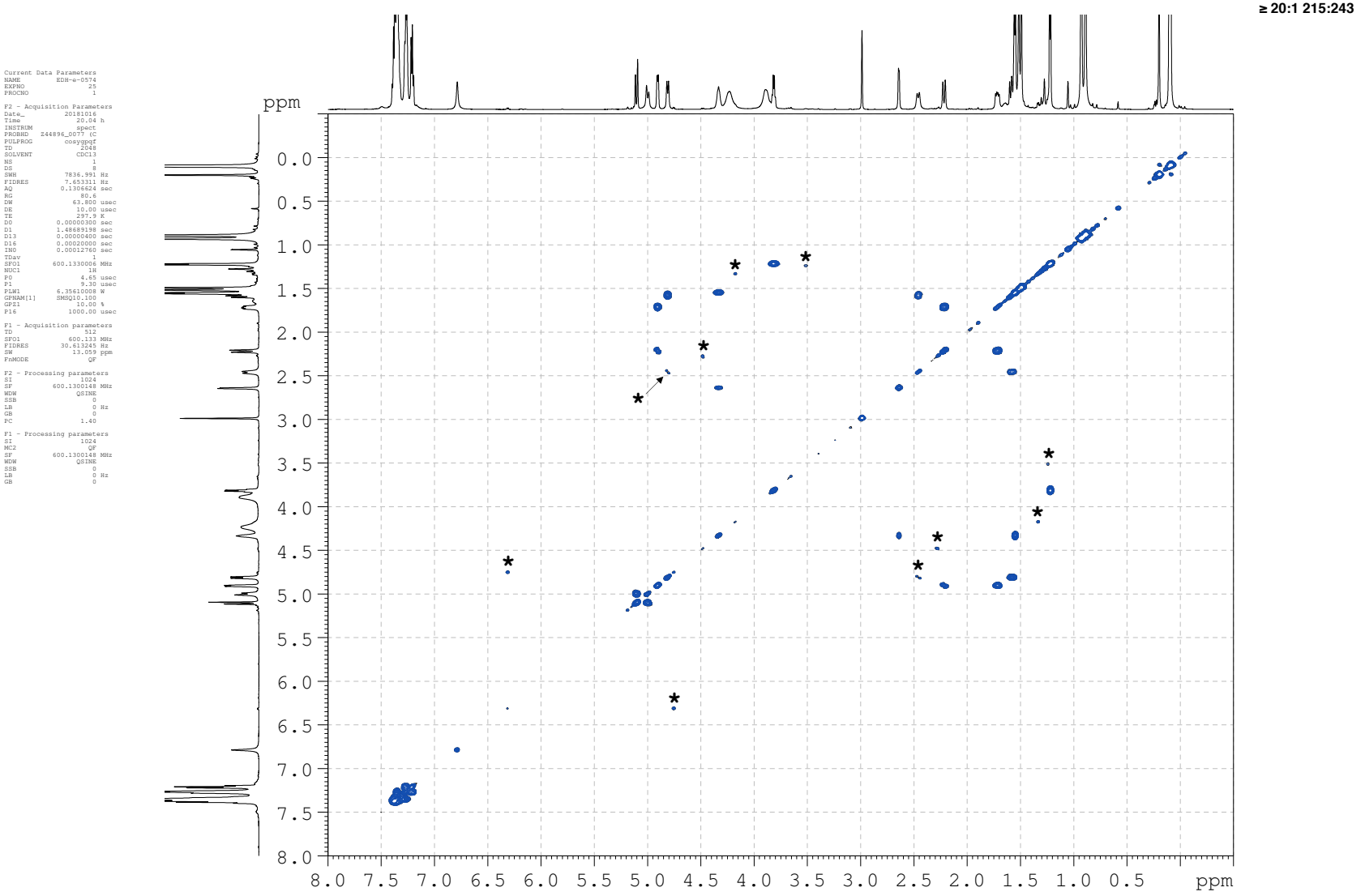
^{1}H-^{1}H COSY NMR of Compounds 215 and 243 (≥ 20:1 215:243) (600 MHz, $CDCl_3$)

Note: Cross peaks for β-linked **243** denoted with asterisk (*).

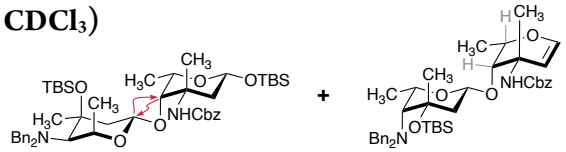


215 (α-linked) + 243 (β-linked)

≥ 20:1 215:243



^{1}H-^{13}C HMBC NMR of Compounds 215 and 243 (≥ 20:1 215:243) (^{1}H-600 MHz, ^{13}C-151 MHz, $CDCl_3$)

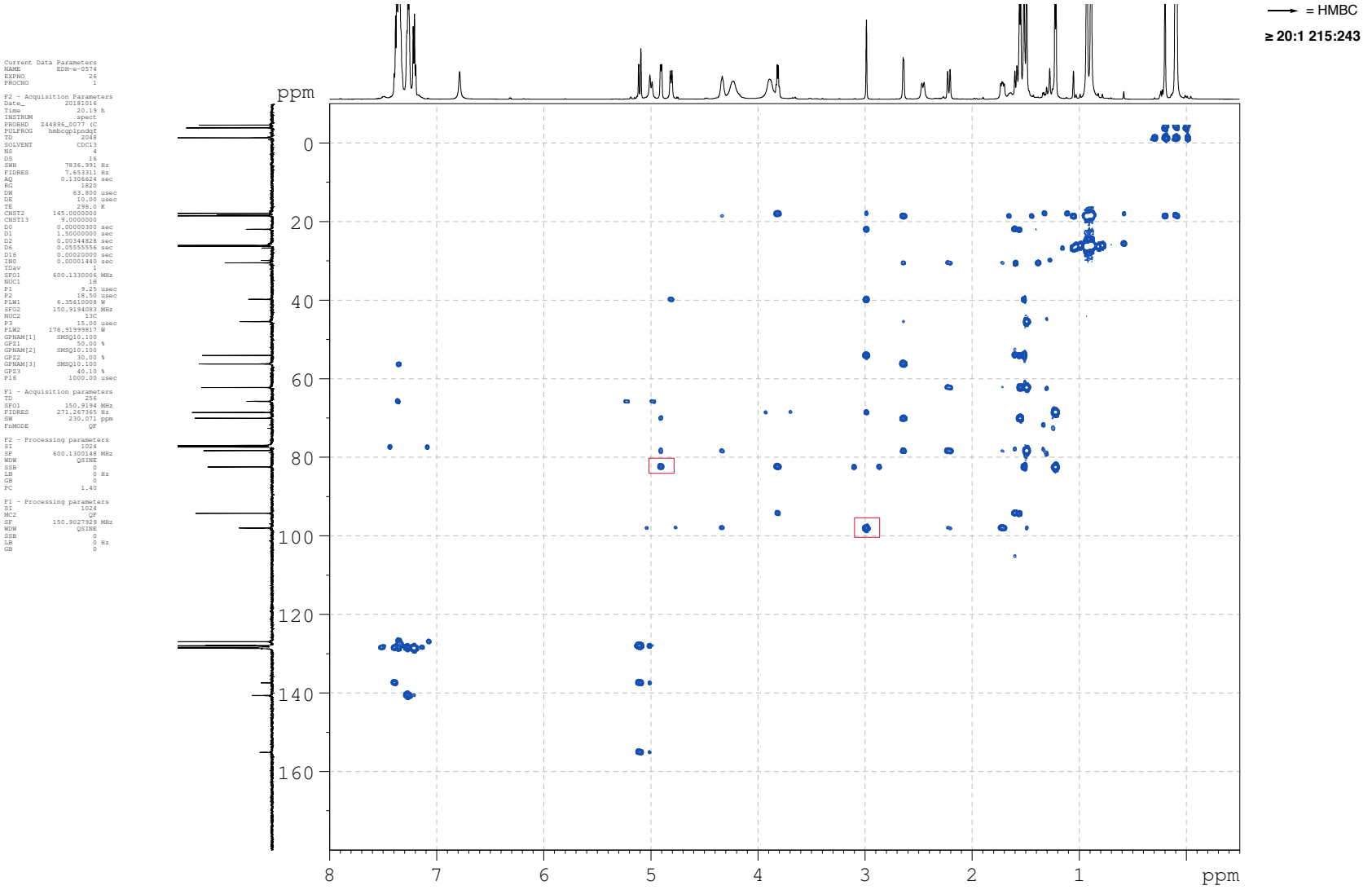


215 (α-linked) + 243 (β-linked)

EDH-e-0574_C1F18-50 (HMBC, 600 MHz, CDCl3, 3 mm, NS = 4 scans)

= HMBC

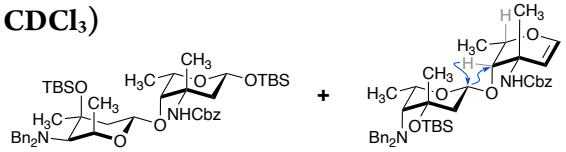
≥ 20:1 215:243



^{1}H-^{13}C HMBC NMR of Compounds 215 and 243 (≥ 20:1 215:243) (^{1}H-600 MHz, ^{13}C-151 MHz, $CDCl_3$)

Note 1: Same spectrum with increased intensity increased as compared to spectrum on page S306.

Note 2: Correlations for β-linked **243** denoted with asterisk (*).

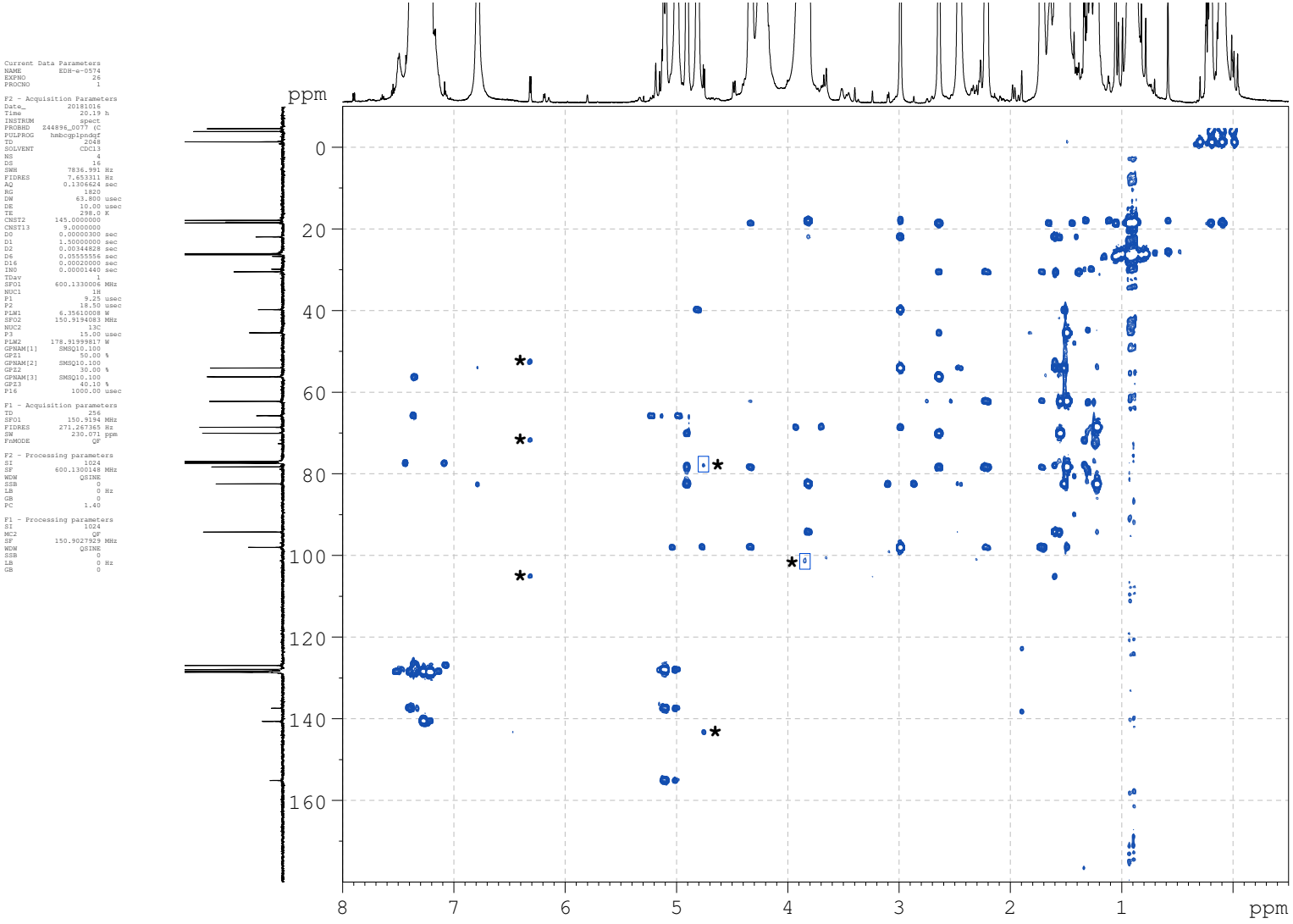


215 (α-linked) **243 (β-linked)**

= HMBC

≥ 20:1 215:243

EDH-e-0574_C1F18-50 (HMBC, 600 MHz, CDCl3, 3 mm, NS = 4 scans)



Current Data Parameters
NAME EDH-e-0574
EXPNO 26
PROCNO 1

F2 - Acquisition Parameters
Date_ 20181016
Time 20.19 h
INSTRUM spect
PROBHD Z44896_0077 (C
PULPROG hmbcgplpndqf
TD 2048
SOLVENT CDCl3
NS 4
DS 16
SWH 7836.991 Hz
FIDRES 7.653311 Hz
AQ 0.1306624 sec
RG 1820
DW 63.800 usec
DE 10.00 usec
TE 298.0 K
CNST2 145.0000000
CNST13 9.0000000
D0 0.00000300 sec
D1 1.50000000 sec
D2 0.00344828 sec
D6 0.05555556 sec
D16 0.00020000 sec
IN0 0.00001440 sec
TDav 1
SFO1 600.1330006 MHz
NDC1 1H
P1 9.25 usec
P2 18.50 usec
PLW1 6.35610008 W
SFO2 150.9194083 MHz
NDC2 13C
P3 15.00 usec
PLW2 178.91999817 W
GPNAM[1] SMSQ10.100
GPZ1 50.00 %
GPNAM[2] SMSQ10.100
GPZ2 30.00 %
GPNAM[3] SMSQ10.100
GPZ3 40.10 %
P16 1000.00 usec

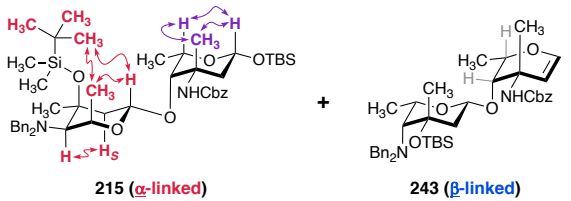
F1 - Acquisition parameters
TD 256
SFO1 150.9194 MHz
FIDRES 271.267365 Hz
SW 230.071 ppm
FnMODE QF

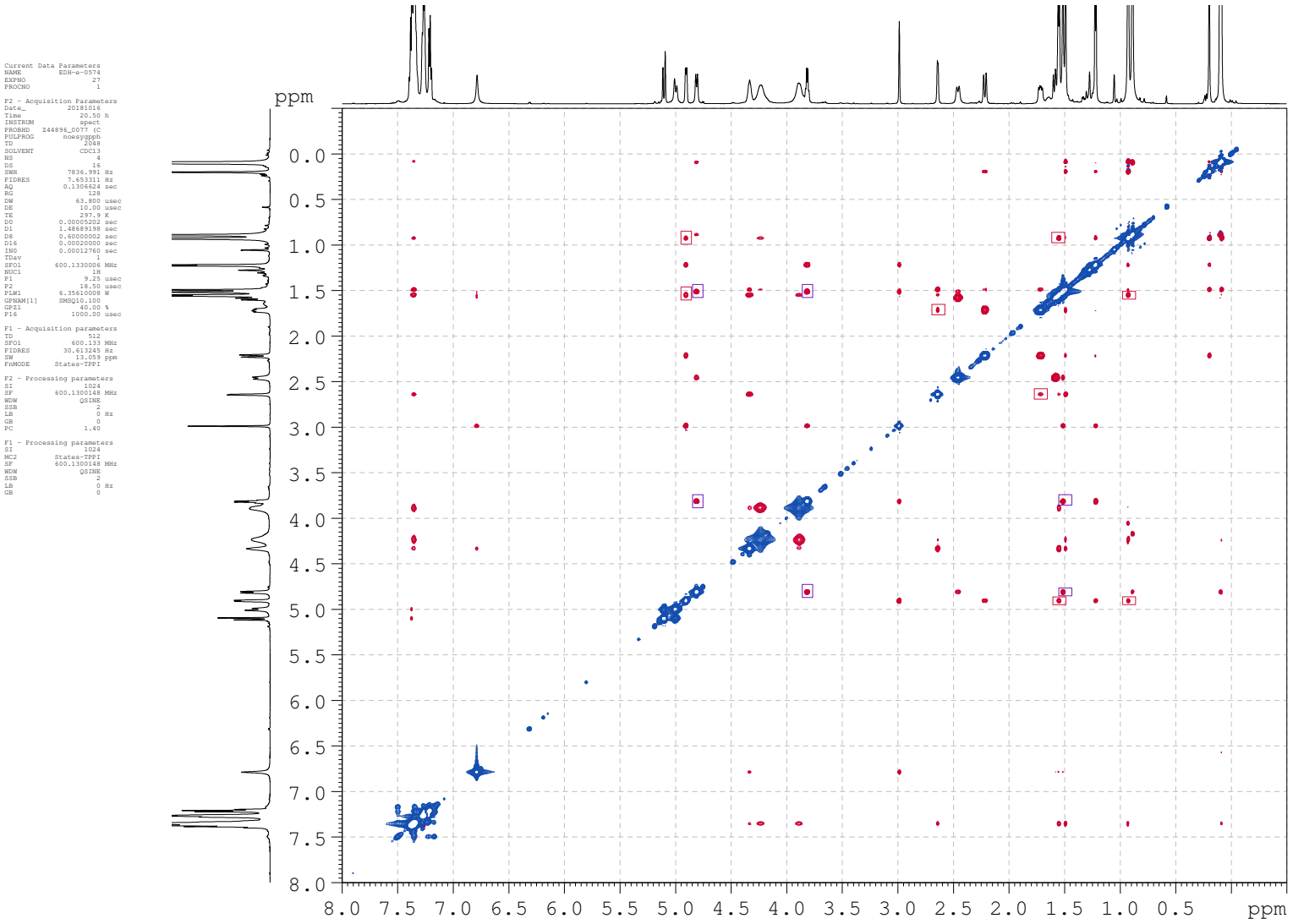
F2 - Processing parameters
SI 1024
SF 600.1300148 MHz
WDW QSINE
SSB 0
LB 0 Hz
GB 0
PC 1.40

F1 - Processing parameters
SI 1024
MC2 QF
SF 150.9027929 MHz
WDW QSINE
SSB 0
LB 0 Hz
GB 0

^{1}H-^{1}H NOESY NMR of Compounds 215 and 243 (≥ 20:1 215:243) (600 MHz, $CDCl_3$)

Note: Blue = diagonal/chemical exchange | Red = NOESY cross peaks.

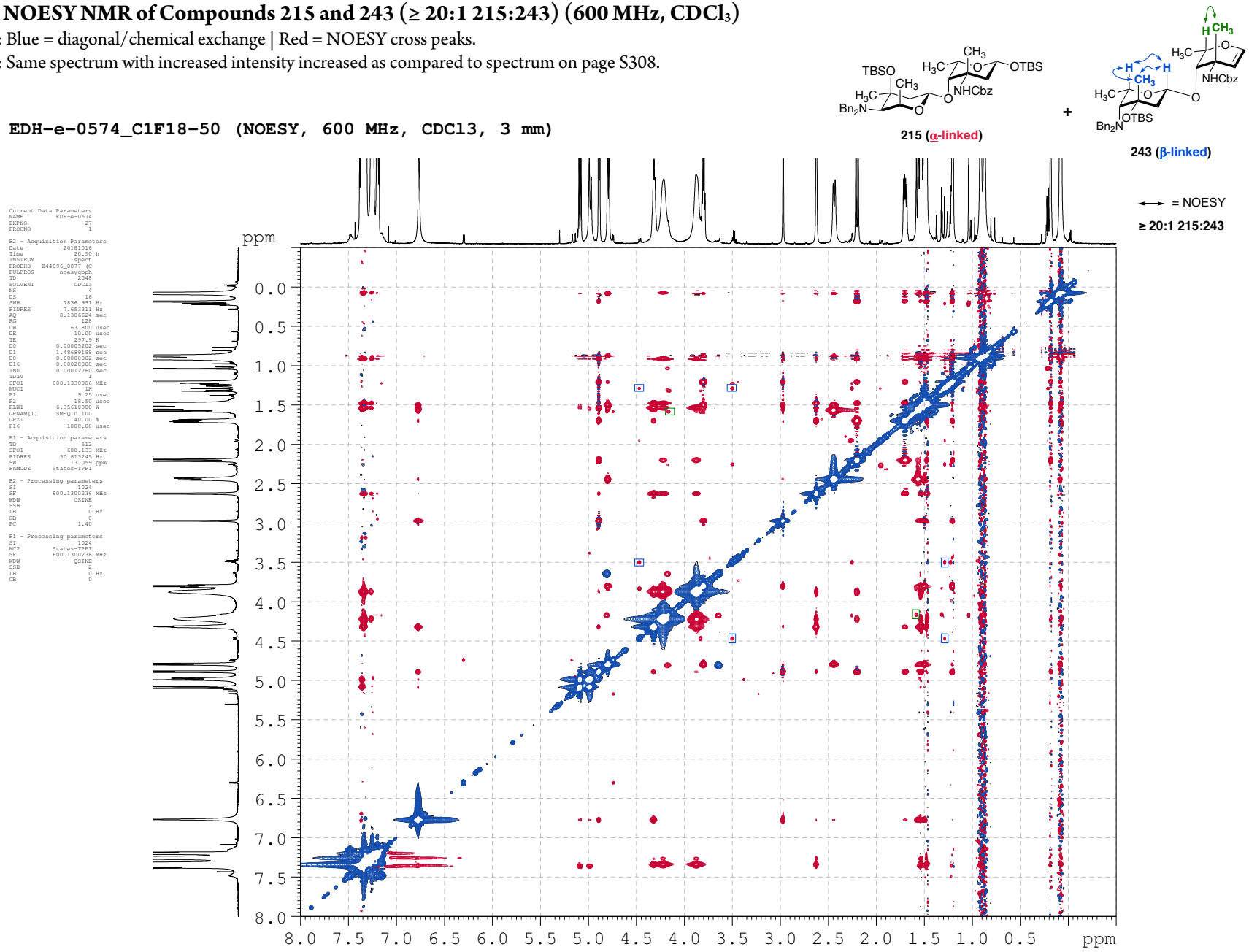




^{1}H-^{1}H NOESY NMR of Compounds 215 and 243 (≥ 20:1 215:243) (600 MHz, $CDCl_3$)

Note 1: Blue = diagonal/chemical exchange | Red = NOESY cross peaks.

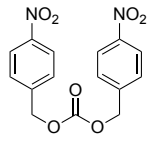
Note 2: Same spectrum with increased intensity increased as compared to spectrum on page S308.



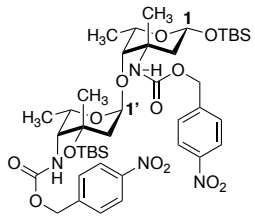
^{1}H NMR of Compound 244 (600 MHz, $CDCl_3$)

Note 1: ca. 1:1 mixture of compound **244** and tentatively assigned impurity **376**.

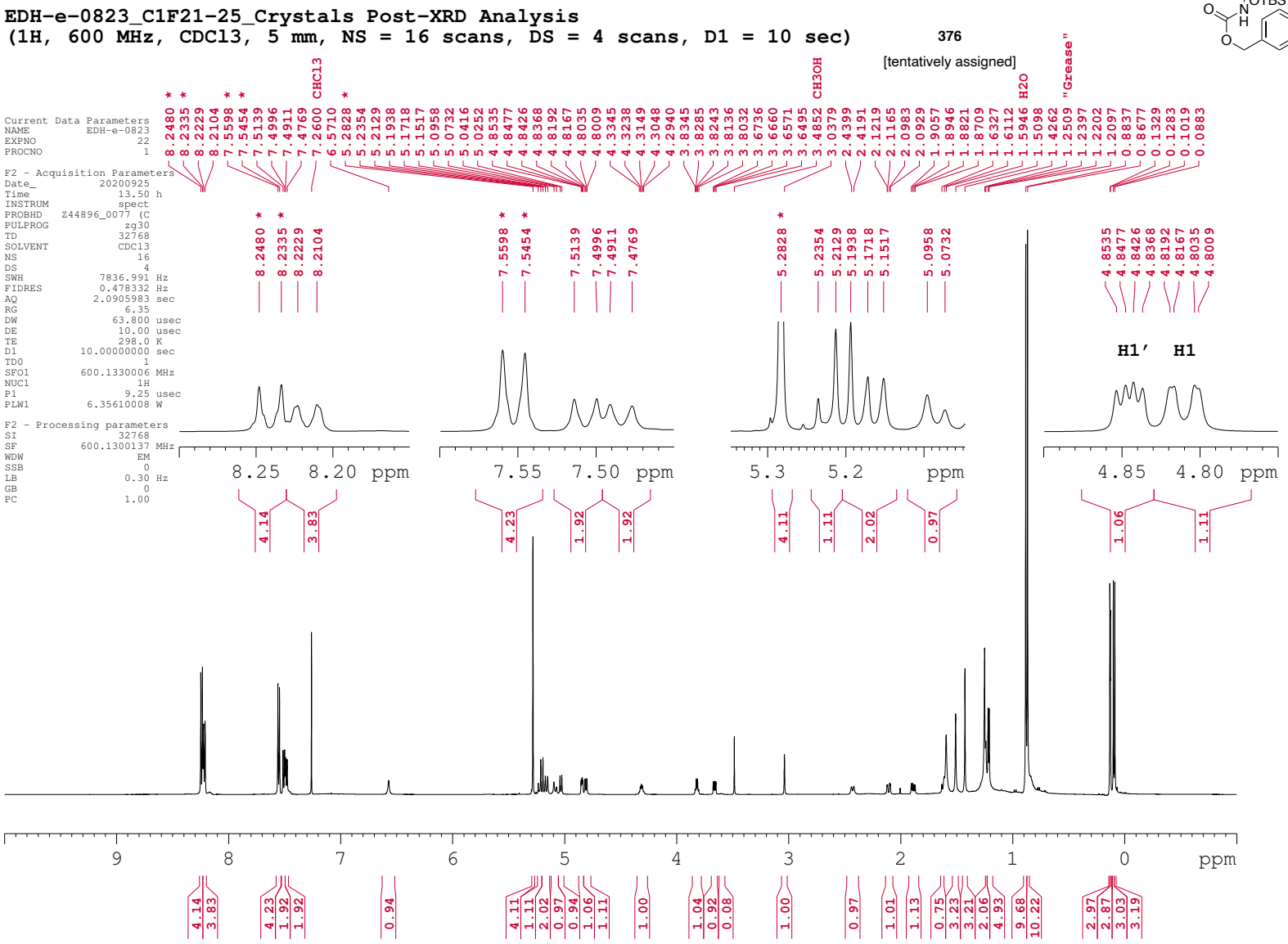
Note 2: Peaks corresponding to tentatively assigned impurity **376** denoted with asterisk (*).



376
[tentatively assigned]

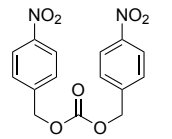


244

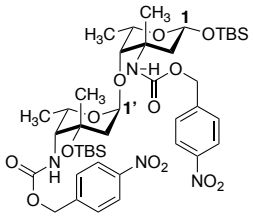


^{13}C NMR of Compound 244 (151 MHz, $CDCl_3$)

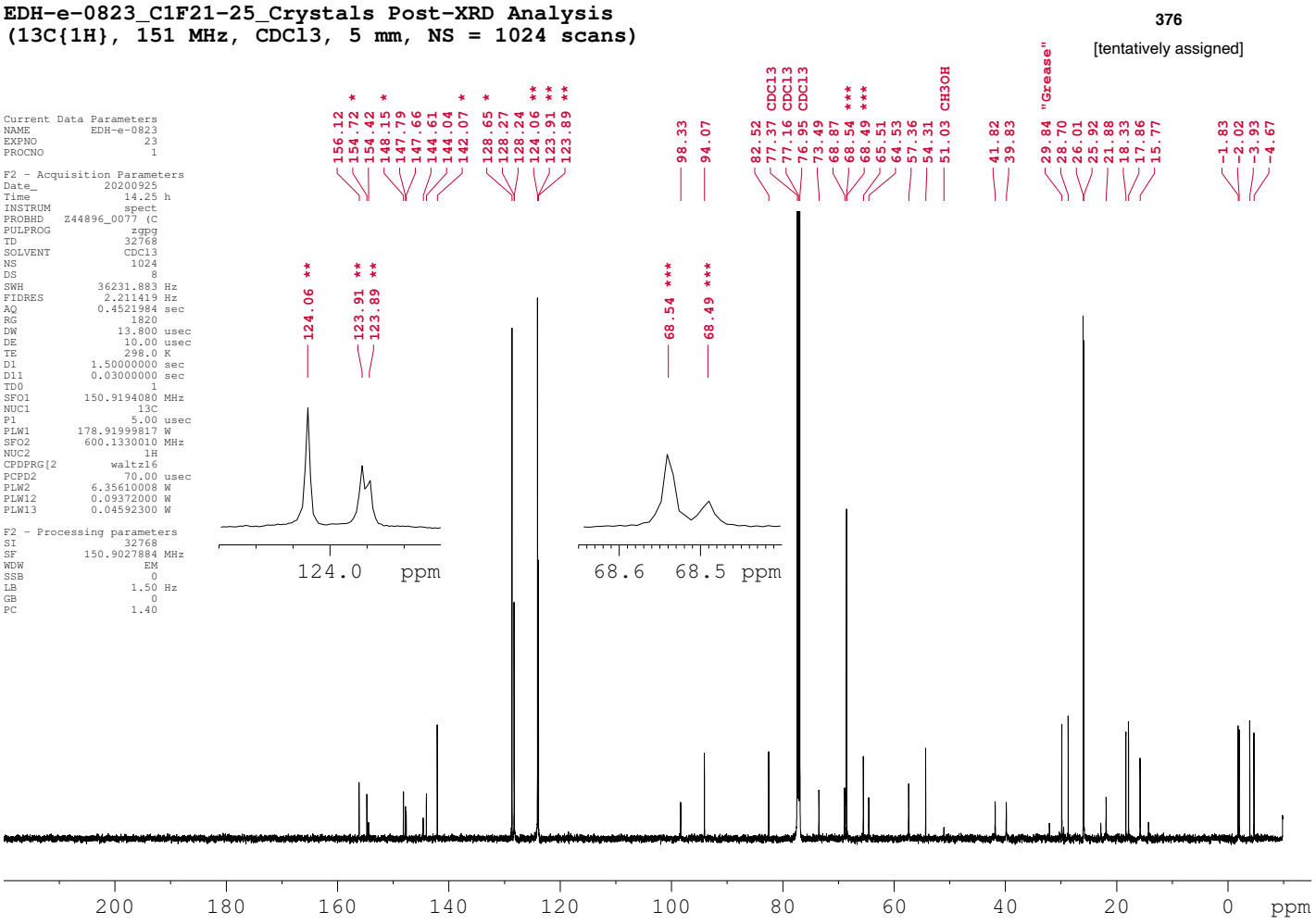
Note 1: ca. 1:1 mixture of compound **244** and tentatively assigned impurity **376**.
Note 2: Peaks corresponding to tentatively assigned impurity **376** denoted with asterisk (*).
Note 3: 2 of 3 peaks denoted with double asterisk (**) belong to **244**; the other belongs to **376**.
Note 4: 1 of 2 peaks denoted with triple asterisk (***) belong **244**; the other belongs to **376**.



376
[tentatively assigned]



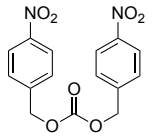
244



^{1}H-^{13}C HSQC{^{1}H}{^{13}C} NMR of Compound 244 (^{1}H-600 MHz, ^{13}C-151 MHz, $CDCl_3$)

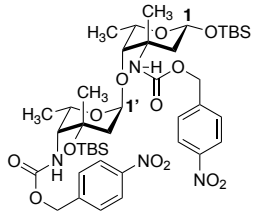
Note 1: Blue = CH/CH_3 | Red = CH_2.

Note 2: ca. 1:1 mixture of compound **244** and tentatively assigned impurity **376**.



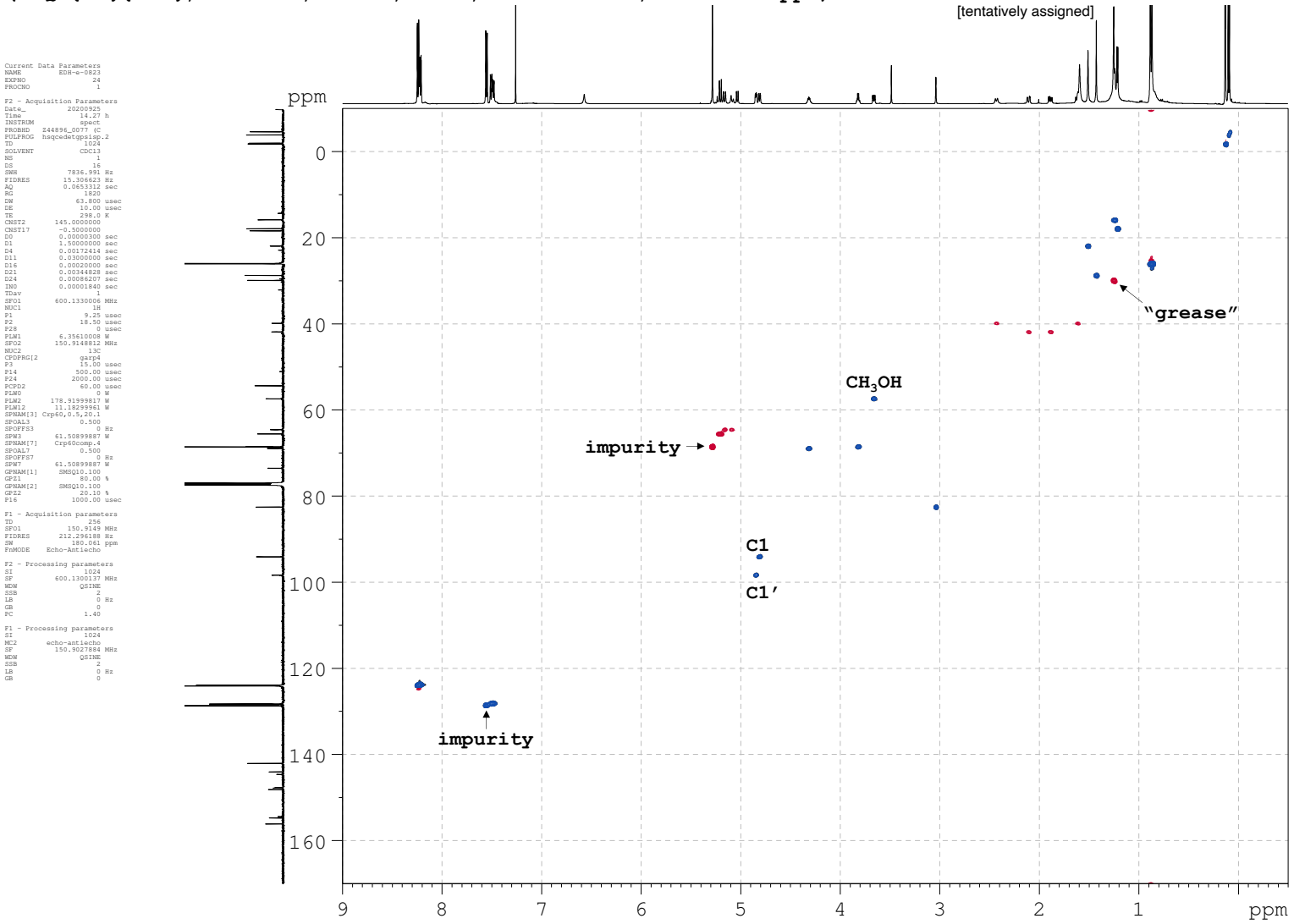
376

[tentatively assigned]



244

EDH-e-0823_C1F21-25_Crystals Post-XRD Analysis
(HSQC{1H}{13C}, 600 MHz, CDCl3, 5 mm, NS = 1 scan, O2P = 80 ppm)



Current Data Parameters
NAME EDH-e-0823
EXPNO 24
PROCNO 1

F2 - Acquisition Parameters
Date_ 20200925
Time 14.27 h
INSTRUM spect
PROBHD Z44896_0077 (C
PULPROG hsqcedetgpsisp.2
TD 1024
SOLVENT CDCl3
NS 1
DS 16
SWH 7836.991 Hz
FIDRES 15.306623 Hz
AQ 0.0653312 sec
RG 1820
DW 63.800 usec
DE 10.00 usec
TE 298.0 K
CNST2 145.0000000
CNST17 -0.5000000
D0 0.00000300 sec
D1 1.50000000 sec
D4 0.00172414 sec
D11 0.03000000 sec
D16 0.00020000 sec
D21 0.00344828 sec
D24 0.00086207 sec
IN0 0.00001840 sec
TDav 1
SFO1 600.1330006 MHz
NDC1 18
P1 9.25 usec
P2 18.50 usec
P28 0 usec
PLW1 6.35610008 W
SFO2 150.9148812 MHz
NUC2 13C
CPDPRG[2 garp4
P3 15.00 usec
P14 500.00 usec
P24 2000.00 usec
PCPD2 60.00 usec
PLW0 0 W
PLW2 178.91999817 W
PLW12 11.18299961 W
SPNAM[3] Crp60,0.5,20.1
SPOAL3 0.500
SPOFFS3 0 Hz
SPW3 61.50899887 W
SPNAM[7] Crp60comp.4
SPOAL7 0.500
SPOFFS7 0 Hz
SPW7 61.50899887 W
GPNAM[1] SMSQ10.100
GPZ1 80.00 %
GPNAM[2] SMSQ10.100
GPZ2 20.10 %
P16 1000.00 usec

F1 - Acquisition parameters
TD 256
SFO1 150.9149 MHz
FIDRES 212.296188 Hz
SW 180.061 ppm
FnMODE Echo-Antiecho

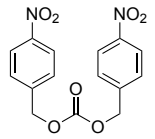
F2 - Processing parameters
SI 1024
SF 600.1300137 MHz
WDW QSINE
SSB 2
LB 0 Hz
GB 0
PC 1.40

F1 - Processing parameters
SI 1024
MC2 echo-antiecho
SF 150.9027884 MHz
WDW QSINE
SSB 2
LB 0 Hz
GB 0

Coupled ^{1}H-^{13}C HSQC NMR of Compound 244 (^{1}H-600 MHz, ^{13}C-151 MHz, $CDCl_3$)

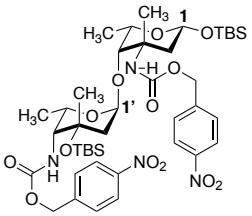
Note 1: Blue = CH/CH_3 | Red = CH_2.

Note 2: ca. 1:1 mixture of compound **244** and tentatively assigned impurity **376**.

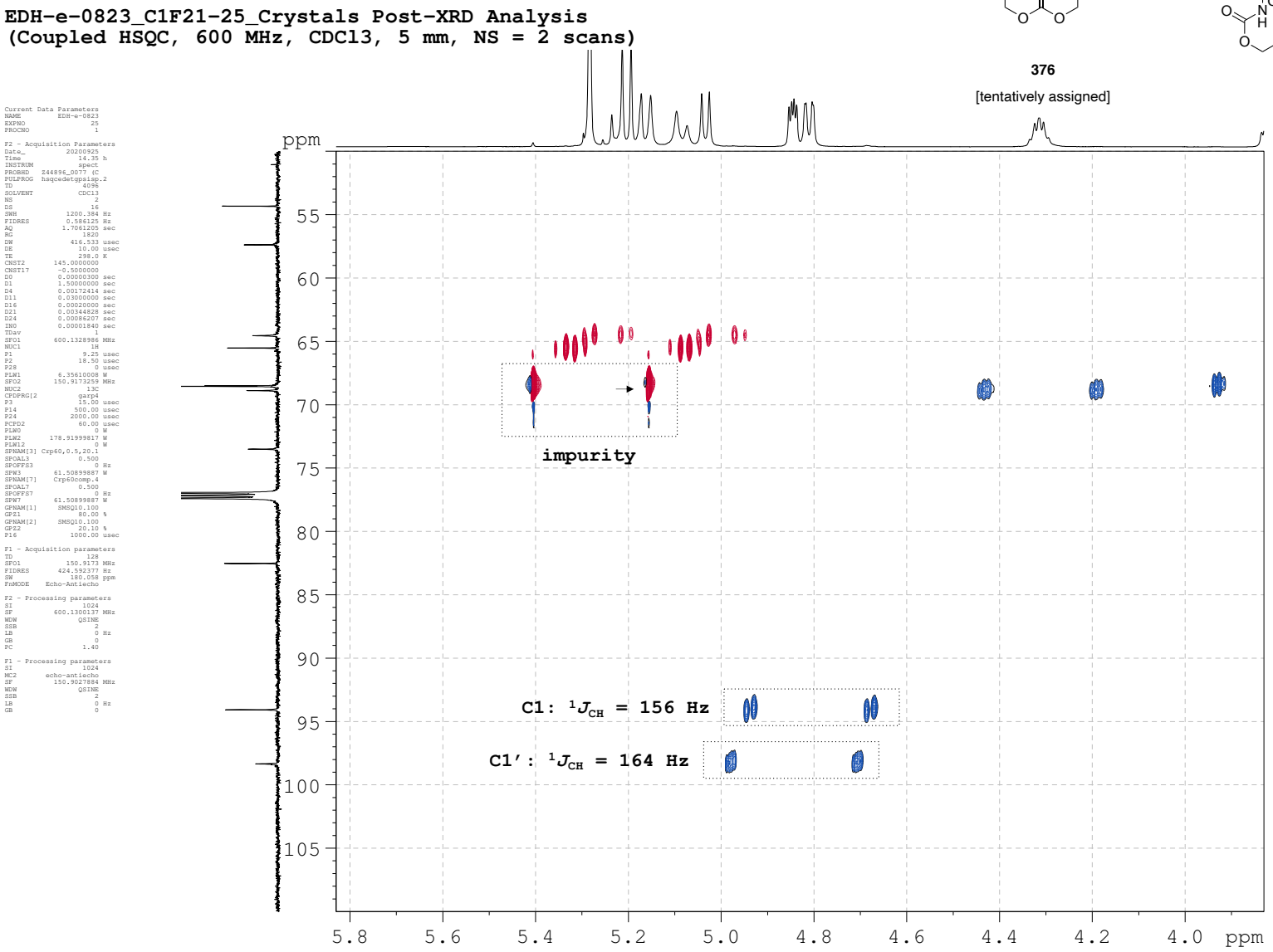


376

[tentatively assigned]

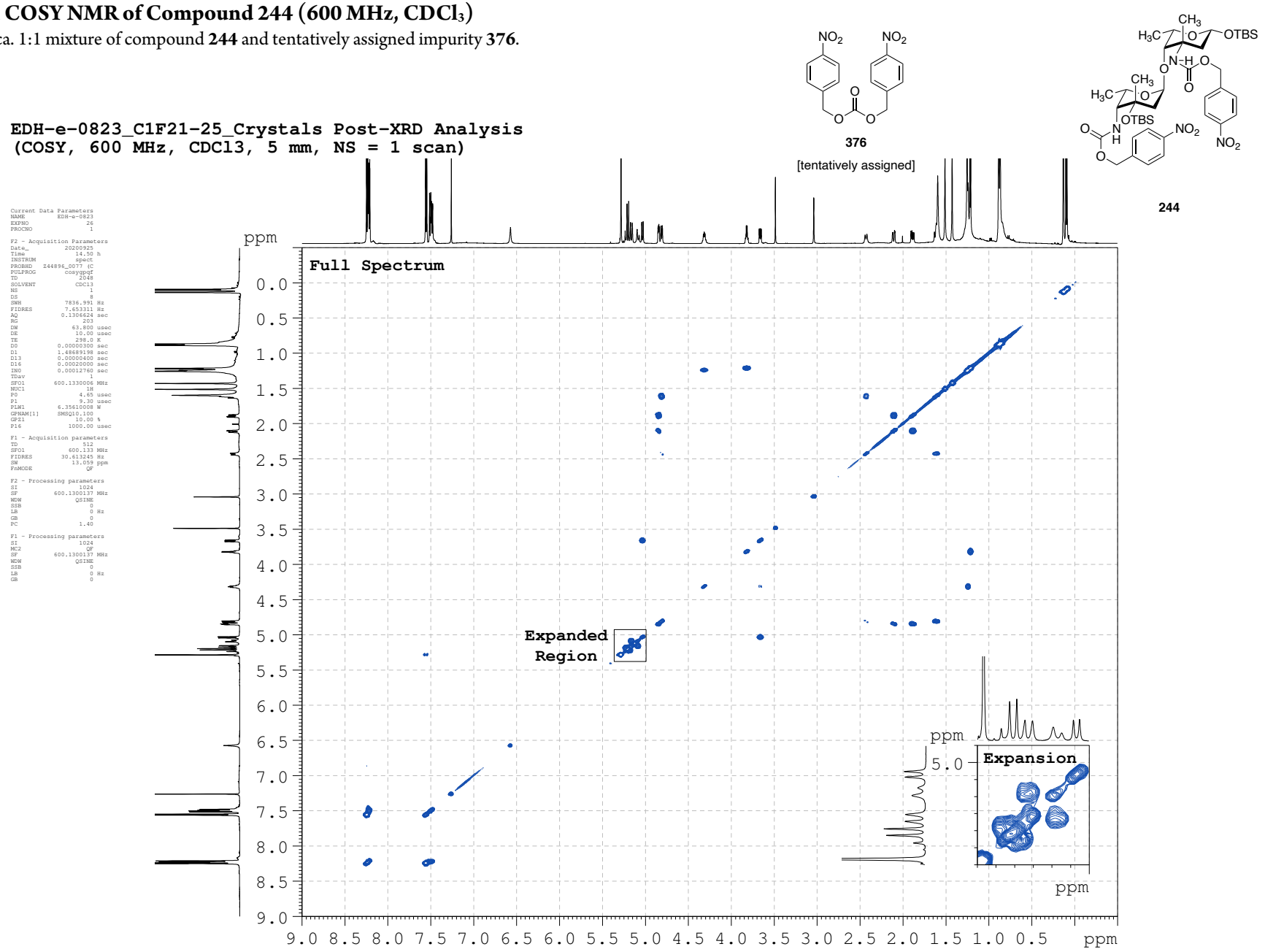


244



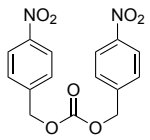
^{1}H-^{1}H COSY NMR of Compound 244 (600 MHz, $CDCl_3$)

Note: ca. 1:1 mixture of compound **244** and tentatively assigned impurity **376**.



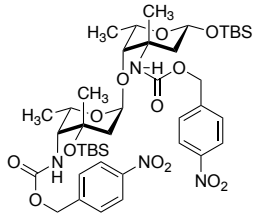
^{1}H-^{13}C HMBC NMR of Compound 244 (^{1}H-600 MHz, ^{13}C-151 MHz, $CDCl_3$)

Note: ca. 1:1 mixture of compound **244** and tentatively assigned impurity **376**.

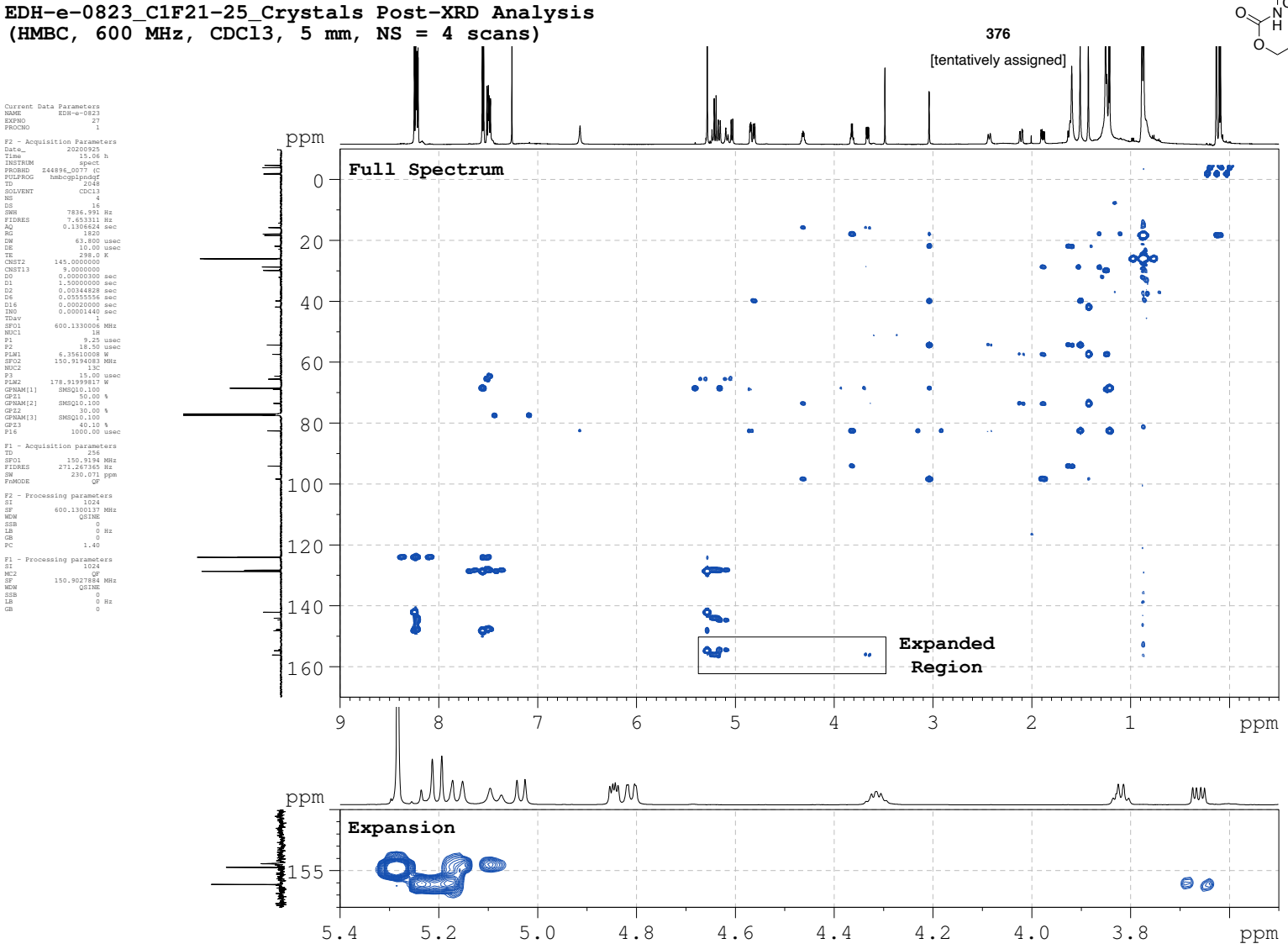


376

[tentatively assigned]



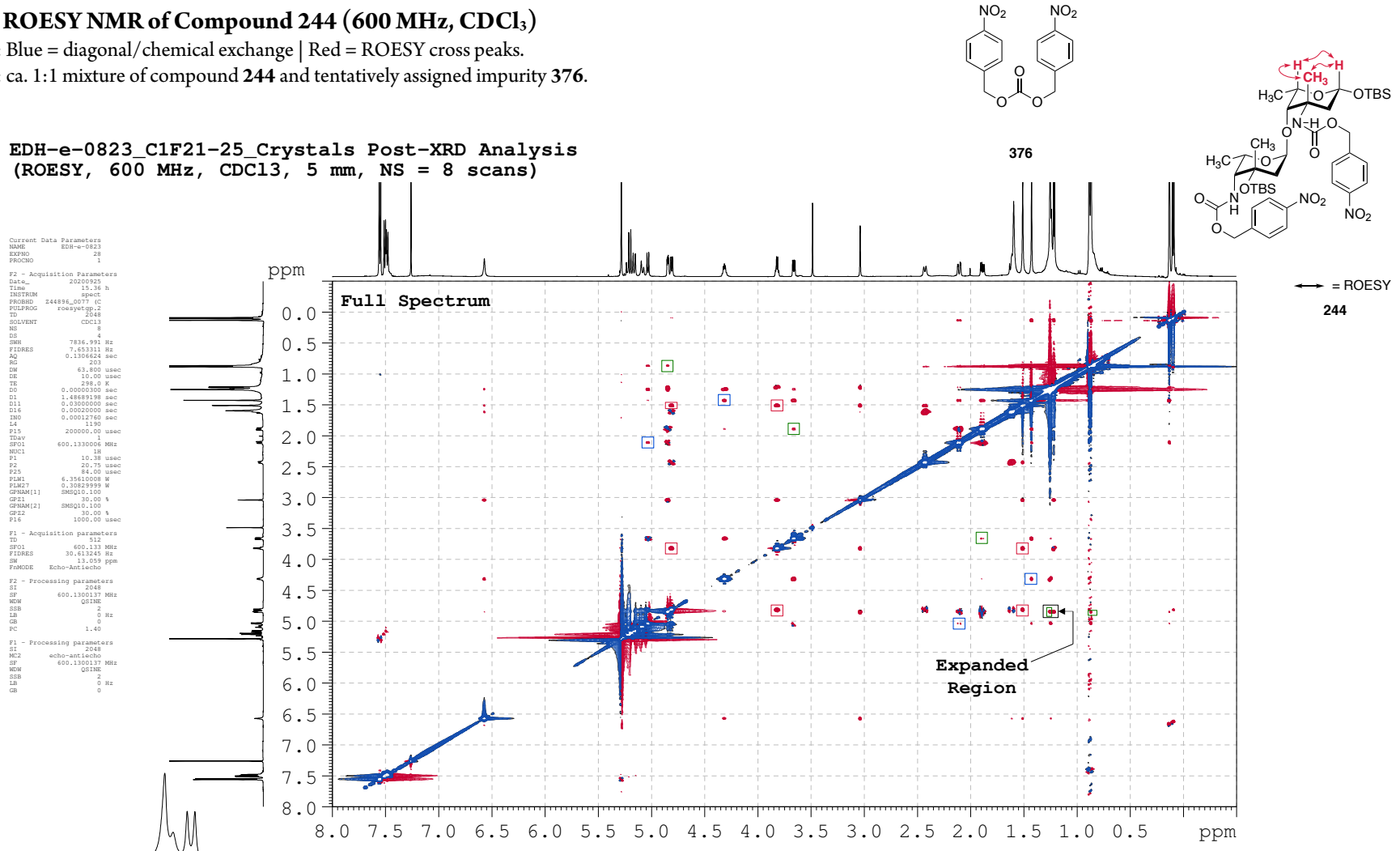
244



^{1}H-^{1}H ROESY NMR of Compound 244 (600 MHz, $CDCl_3$)

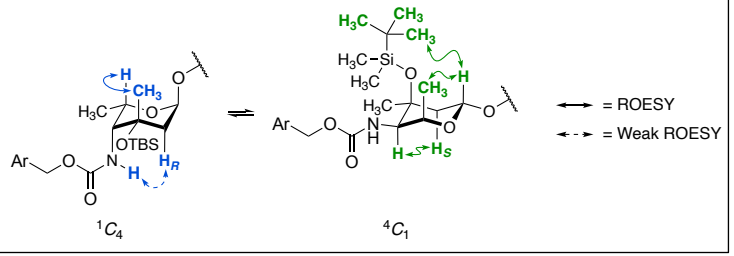
Note 1: Blue = diagonal/chemical exchange | Red = ROESY cross peaks.

Note 2: ca. 1:1 mixture of compound **244** and tentatively assigned impurity **376**.

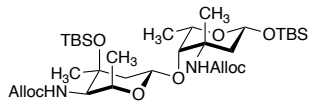


Compound 23: Conformational Analysis of the Non-Reducing Sugar

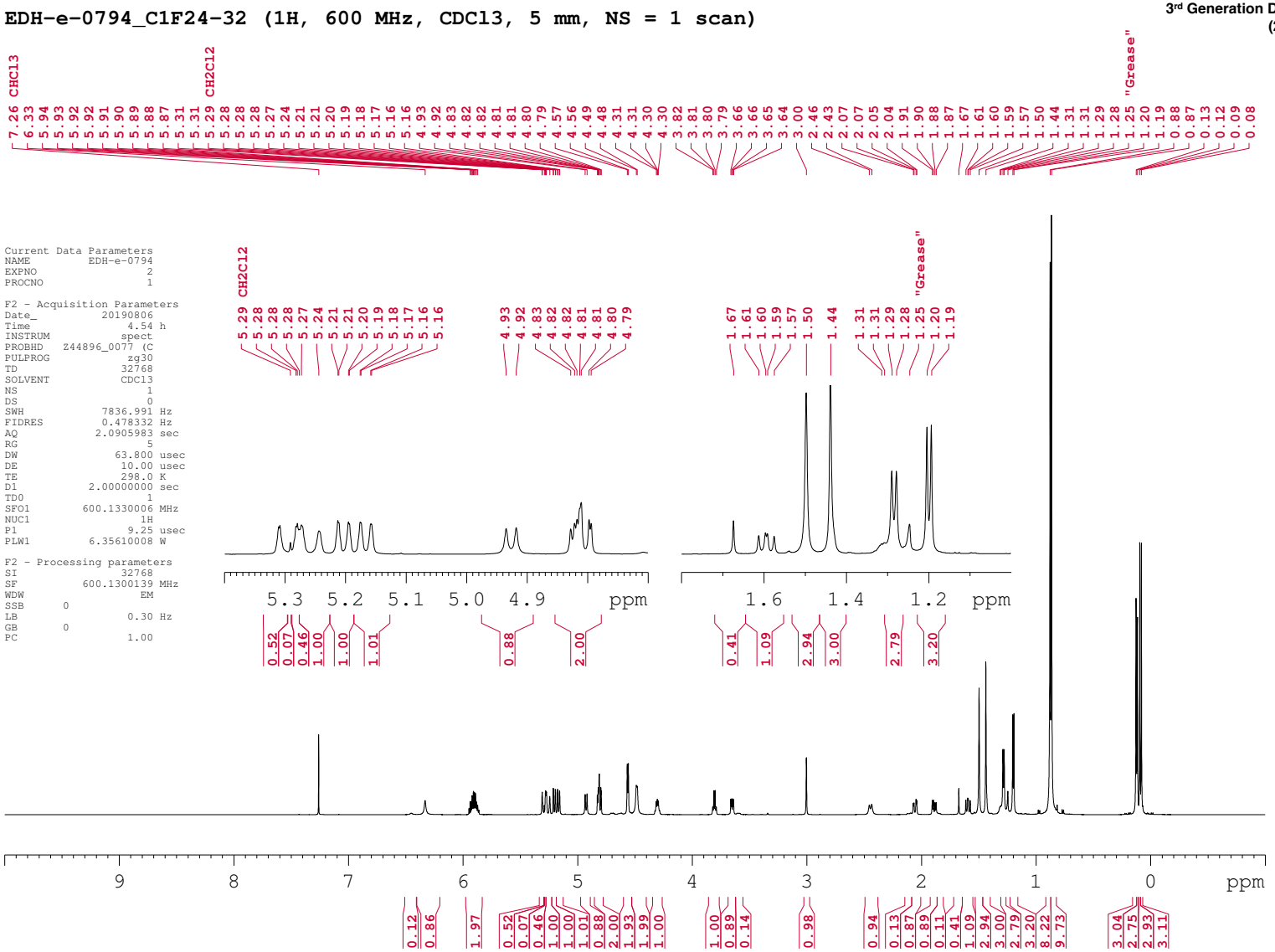
ROESY correlations (as well as the slightly anomalous $^3J_{H1'\text{-}H2'}$ coupling constants of 6.5 and 3.5 Hz) indicate that the reducing sugar does not solely occupy the canonical $^1C_4$ conformation; rather, as indicated by the ROESY correlations highlighted to the right (Ar = 4-NO_2Ph), the non-reducing sugar exists in conformational equilibrium and/or an intermediate conformation not pictured here.



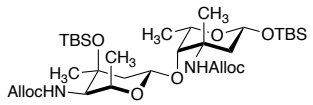
^{1}H NMR of 3rd Generation Disaccharide Donor (246) (600 MHz, $CDCl_3$)



3rd Generation Disaccharide Donor (246)

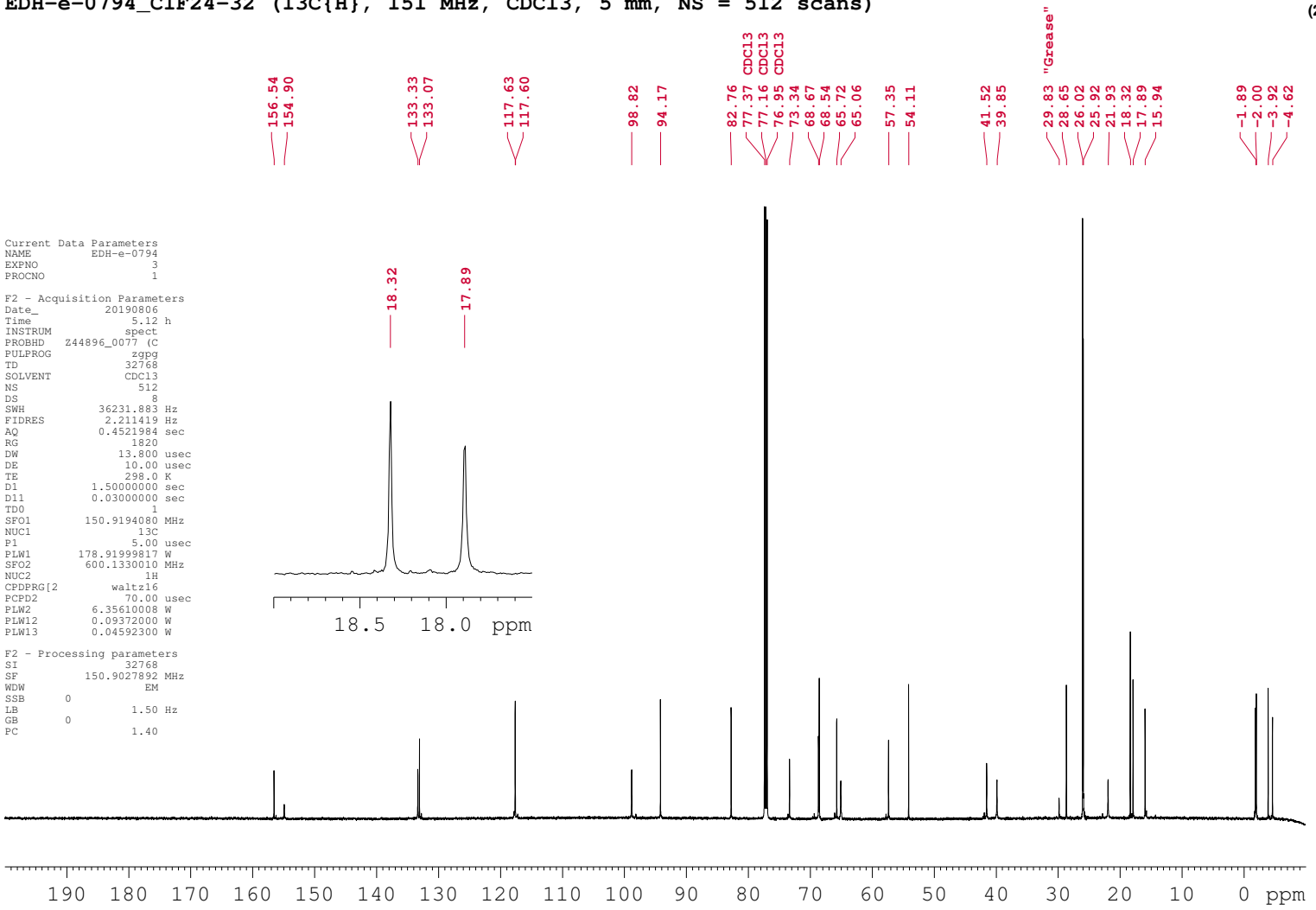


$^{13}C\{^{1}H\}$ NMR of 3rd Generation Disaccharide Donor (246) (151 MHz, $CDCl_3$)



3rd Generation Disaccharide Donor (246)

EDH-e-0794_C1F24-32 (13C{H}, 151 MHz, CDCl3, 5 mm, NS = 512 scans)



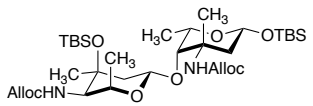

```
Current Data Parameters
NAME          EDH-e-0794
EXPNO                  3
PROCNO                 1

F2 - Acquisition Parameters
Date_           20190806
Time                5.12 h
INSTRUM            spect
PROBHD    Z44896_0077 (C
PULPROG             zgpg
TD                 32768
SOLVENT            CDCl3
NS                   512
DS                     8
SWH            36231.883 Hz
FIDRES          2.211419 Hz
AQ             0.4521984 sec
RG                  1820
DW                13.800 usec
DE                10.00 usec
TE                298.0 K
D1           1.50000000 sec
D11          0.03000000 sec
TD0                    1
SFO1         150.9194080 MHz
NUC1                 13C
P1                  5.00 usec
PLW1        178.91999817 W
SFO2         600.1330010 MHz
NUC2                 1H
CPDPRG[2          waltz16
PCPD2              70.00 usec
PLW2          6.35610008 W
PLW12         0.09372000 W
PLW13         0.04592300 W

F2 - Processing parameters
SI                 32768
SF           150.9027892 MHz
WDW                   EM
SSB       0
LB                  1.50 Hz
GB        0
PC                  1.40
```

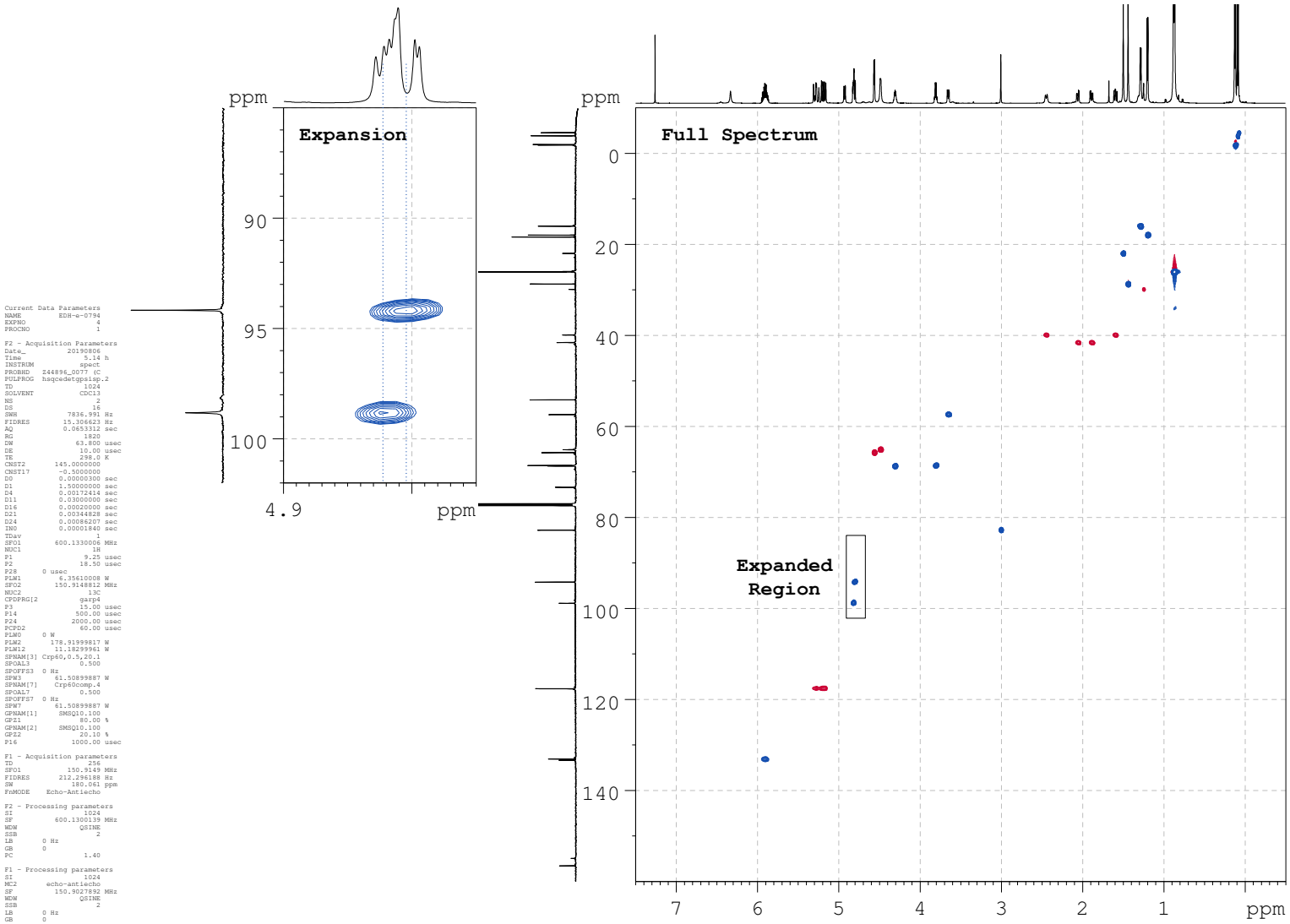
^{1}H-^{13}C HSQC{^{1}H}{^{13}C} NMR of 3rd Generation Disaccharide Donor (246) (^{1}H-600 MHz, ^{13}C-151 MHz, $CDCl_3$)

Note: Blue = CH/CH_3 | Red = CH_2.



3rd Generation Disaccharide Donor (246)

EDH-e-0794_C1F24-32 (HSQC{1H}{13C}, 600 MHz, CDCl3, 5 mm, O2P = 80 ppm)



Current Data Parameters
NAME EDH-e-0794
EXPNO 4
PROCNO 1

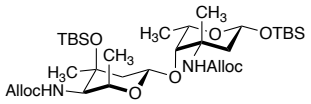
F2 - Acquisition Parameters
Date_ 20190806
Time 5.14 h
INSTRUM spect
PROBHD Z44896_0077 (C
PULPROG hsqcedetgpsisp.2
TD 1024
SOLVENT CDCl3
NS 2
DS 16
SWH 7836.991 Hz
FIDRES 15.306623 Hz
AQ 0.0653312 sec
RG 1820
DW 63.800 usec
DE 10.00 usec
TE 298.0 K
CNST2 145.0000000
CNST17 -0.5000000
D0 0.00000300 sec
D1 1.50000000 sec
D4 0.00172414 sec
D11 0.03000000 sec
D16 0.00020000 sec
D21 0.00344828 sec
D24 0.00086207 sec
IN0 0.00001840 sec
TDav 1
SFO1 600.1330006 MHz
NUC1 1H
P1 9.25 usec
P2 18.50 usec
PZ8 0 usec
PLW1 6.35610008 W
SFO2 150.9148812 MHz
NUC2 13C
CPDPRG[2 garp4
P3 15.00 usec
P14 500.00 usec
P24 2000.00 usec
PCPD2 60.00 usec
PLW0 0 W
PLW2 178.91999817 W
PLW12 11.18299961 W
SPNAM[3] Crp60,0.5,20.1
SPOAL3 0.500
SPOFFS3 0 Hz
SPW3 61.50899887 W
SPNAM[7] Crp60comp.4
SPOAL7 0.500
SPOFF87 0 Hz
SPW7 61.50899887 W
GPNAM[1] SMSQ10.100
GPZ1 80.00 %
GPNAM[2] SMSQ10.100
GPZ2 20.10 %
P16 1000.00 usec

F1 - Acquisition parameters
TD 256
SFO1 150.9149 MHz
FIDRES 212.296188 Hz
SW 180.061 ppm
FnMODE Echo-Antiecho

F2 - Processing parameters
SI 1024
SF 600.1300139 MHz
WDW QSINE
SSB 2
LB 0 Hz
GB 0
PC 1.40

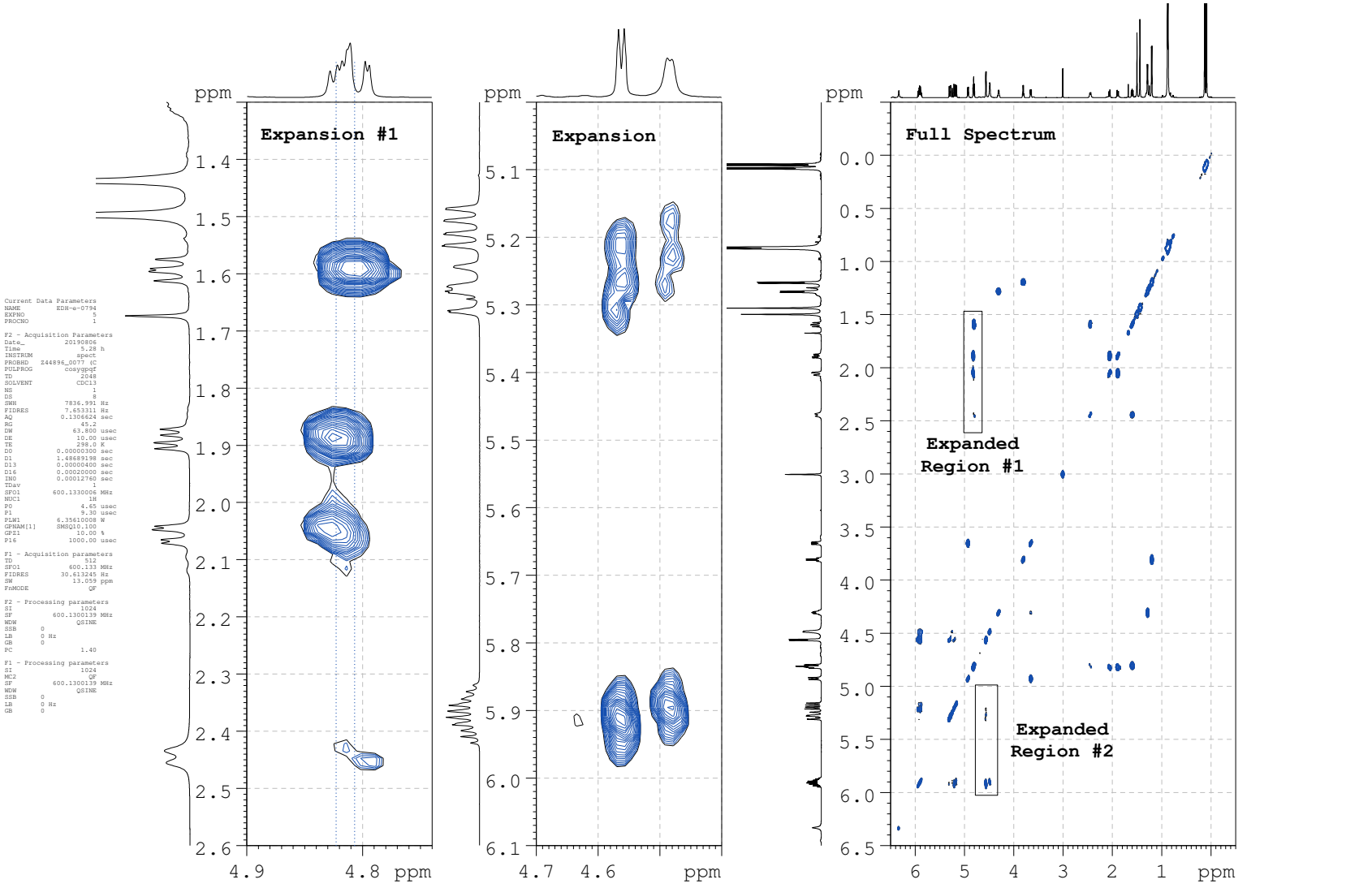
F1 - Processing parameters
SI 1024
MC2 echo-antiecho
SF 150.9027892 MHz
WDW QSINE
SSB 2
LB 0 Hz
GB 0

^{1}H-^{1}H COSY NMR of 3rd Generation Disaccharide Donor (246) (600 MHz, $CDCl_3$)



3rd Generation Disaccharide Donor (246)

EDH-e-0794_C1F24-32 (COSY, 600 MHz, CDCl3, 5 mm)



Current Data Parameters
NAME EDH-e-0794
EXPNO 5
PROCNO 1

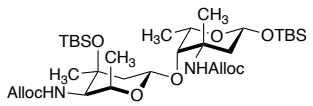
F2 - Acquisition Parameters
Date_ 20190806
Time 5.28 h
INSTRUM spect
PROBHD Z44896_0077 (C
PULPROG cosygpqf
TD 2048
SOLVENT CDCl3
NS 1
DS 8
SWH 7836.991 Hz
FIDRES 7.653311 Hz
AQ 0.1306624 sec
RG 45.2
DW 63.800 usec
DE 10.00 usec
TE 298.0 K
D0 0.00000300 sec
D1 1.48689198 sec
D13 0.00000400 sec
D16 0.00020000 sec
IN0 0.00012760 sec
TDav 1
SFO1 600.1330006 MHz
NDC1 1H
P0 4.65 usec
P1 9.30 usec
PLW1 6.35610008 W
GPNAM[1] SMSQ10.100
GPZ1 10.00 %
P16 1000.00 usec

F1 - Acquisition parameters
TD 512
SFO1 600.133 MHz
FIDRES 30.613245 Hz
SW 13.059 ppm
FnMODE QF

F2 - Processing parameters
SI 1024
SF 600.1300139 MHz
WDW QSINE
SSB 0
LB 0 Hz
GB 0
PC 1.40

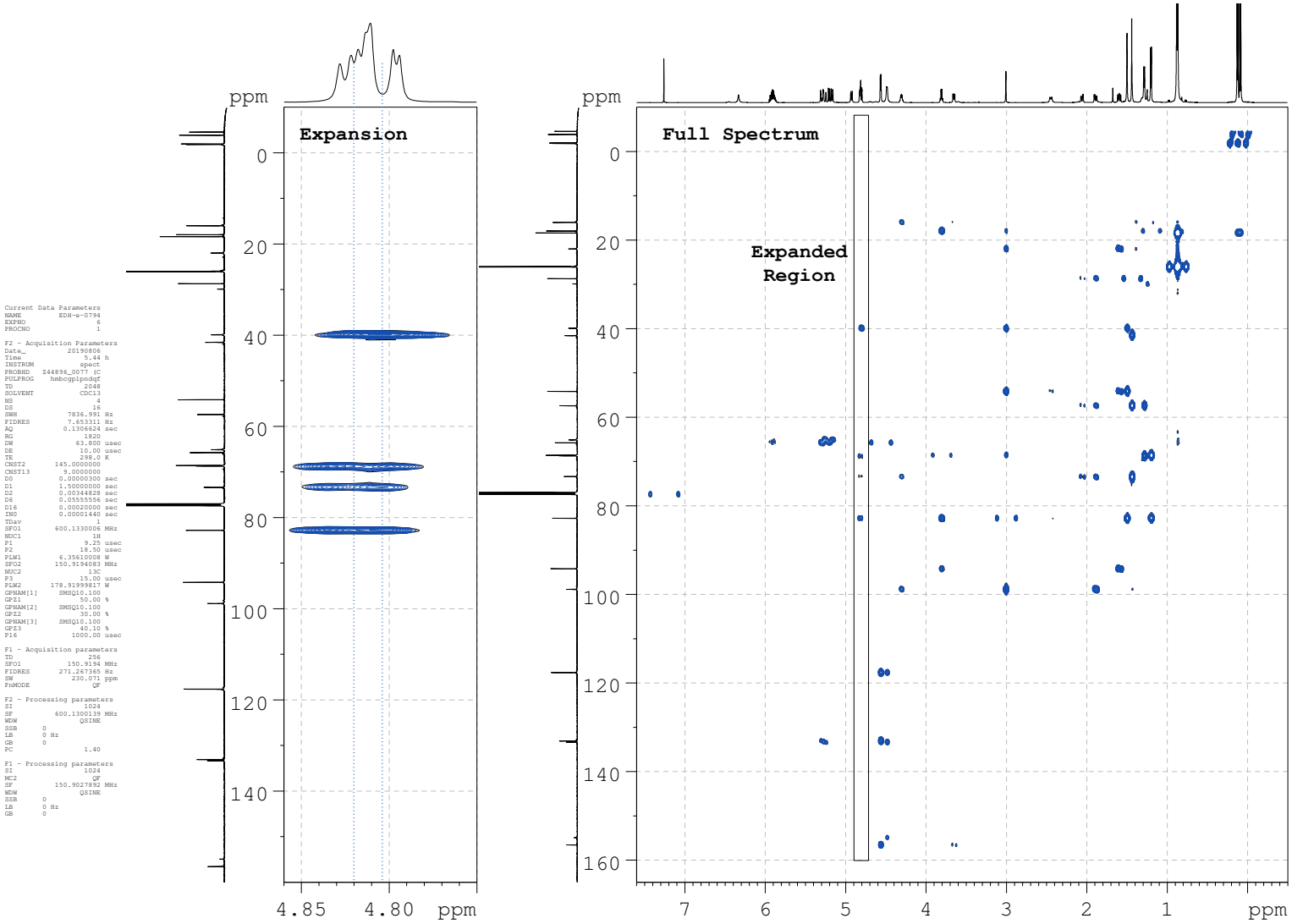
F1 - Processing parameters
SI 1024
MC2 QF
SF 600.1300139 MHz
WDW QSINE
SSB 0
LB 0 Hz
GB 0

^{1}H-^{13}C HMBC NMR of 3rd Generation Disaccharide Donor (246) (^{1}H-600 MHz, ^{13}C-151 MHz, $CDCl_3$)



3rd Generation Disaccharide Donor (246)

EDH-e-0794_C1F24-32 (HMBC, 600 MHz, CDCl3, 5 mm, NS = 4 scans)



Current Data Parameters
NAME EDH-e-0794
EXPNO 6
PROCNO 1

F2 - Acquisition Parameters
Date_ 20190806
Time 5.44 h
INSTRUM spect
PROBHD Z44896_0077 (C
PULPROG hmbcgplpndqf
TD 2048
SOLVENT CDCl3
NS 4
DS 16
SWH 7836.991 Hz
FIDRES 7.653311 Hz
AQ 0.1306624 sec
RG 1820
DW 63.800 usec
DE 10.00 usec
TE 298.0 K
CNST2 145.0000000
CNST13 9.0000000
D0 0.00000300 sec
D1 1.50000000 sec
D2 0.00344828 sec
D6 0.05555556 sec
D16 0.00020000 sec
IN0 0.00001440 sec
TDav 1
SFO1 600.1330006 MHz
NUC1 1H
P1 9.25 usec
P2 18.50 usec
PLW1 6.35610008 W
SFO2 150.9194083 MHz
NUC2 13C
P3 15.00 usec
PLW2 178.91999817 W
GPNAM[1] SMSQ10.100
GPZ1 50.00 %
GPNAM[2] SMSQ10.100
GPZ2 30.00 %
GPNAM[3] SMSQ10.100
GPZ3 40.10 %
P16 1000.00 usec

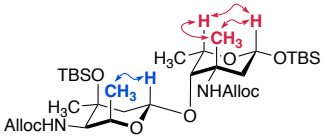
F1 - Acquisition parameters
TD 256
SFO1 150.9194 MHz
FIDRES 271.267365 Hz
SW 230.071 ppm
FnMODE QF

F2 - Processing parameters
SI 1024
SF 600.1300139 MHz
WDW QSINE
SSB 0
LB 0 Hz
GB 0
PC 1.40

F1 - Processing parameters
SI 1024
MC2 QF
SF 150.9027892 MHz
WDW QSINE
SSB 0
LB 0 Hz
GB 0

^{1}H-^{1}H NOESY NMR of 3rd Generation Disaccharide Donor (246) (^{1}H-600 MHz, $CDCl_3$)

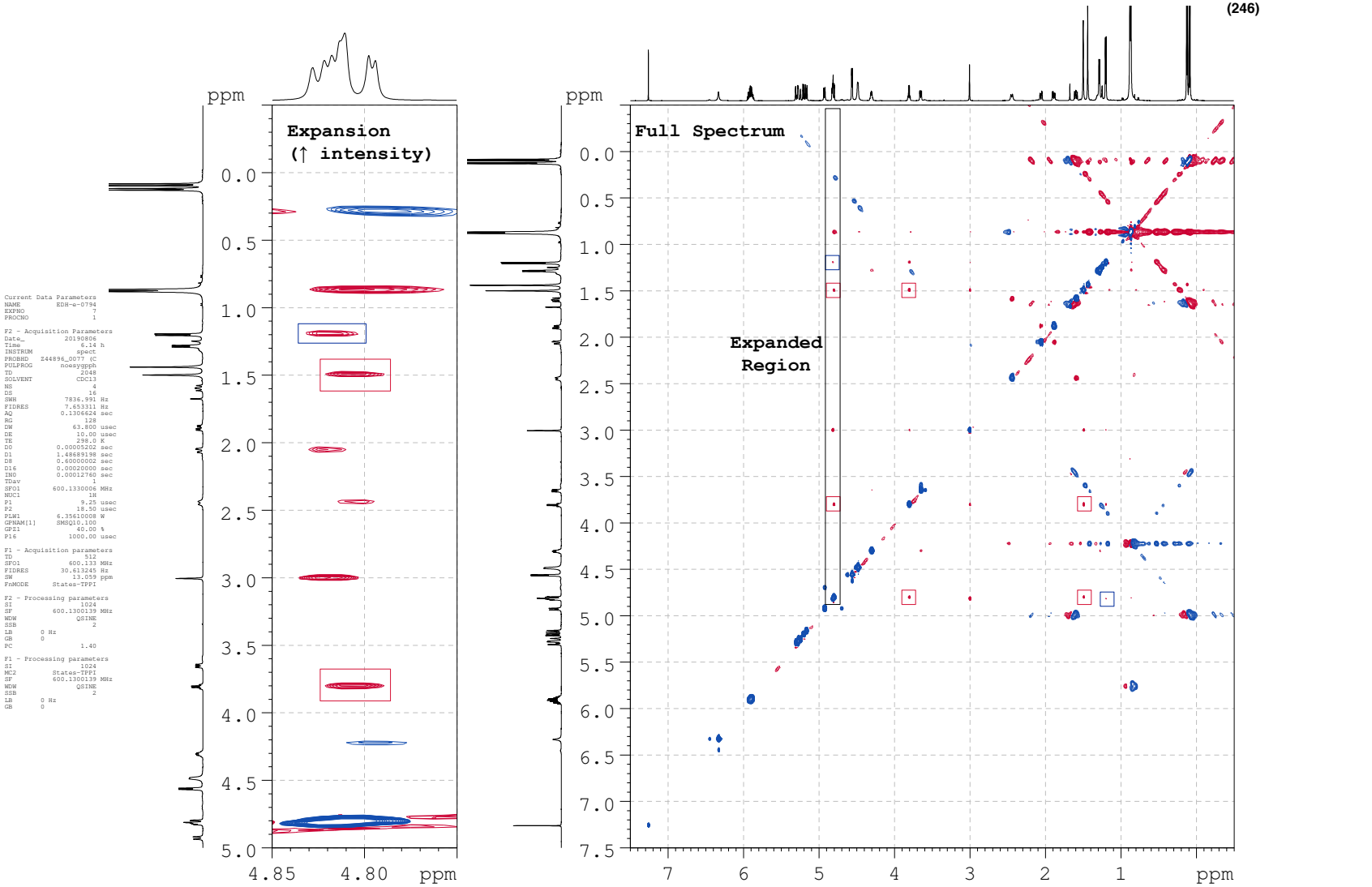
Note: Blue = diagonal/chemical exchange | Red = NOESY cross peaks.



= NOESY

3rd Generation Disaccharide Donor (246)

EDH-e-0794_C1F24-32 (NOESY, 600 MHz, CDCl3, 5 mm, NS = 4 scans)



Current Data Parameters
NAME EDH-e-0794
EXPNO 7
PROCNO 1

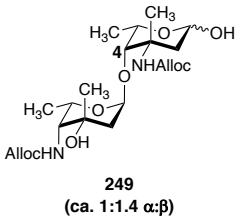
F2 - Acquisition Parameters
Date_ 20190806
Time 6.14 h
INSTRUM spect
PROBHD Z44896_0077 (C
PULPROG noesygpph
TD 2048
SOLVENT CDCl3
NS 4
DS 16
SWH 7836.991 Hz
FIDRES 7.653311 Hz
AQ 0.1306624 sec
RG 128
DW 63.800 usec
DE 10.00 usec
TE 298.0 K
D0 0.00005202 sec
D1 1.48689198 sec
D8 0.60000002 sec
D16 0.00020000 sec
IN0 0.00012760 sec
TDav 1
SFO1 600.1330006 MHz
NUC1 1H
P1 9.25 usec
P2 18.50 usec
PLW1 6.35610008 W
GPNAM[1] SMSQ10.100
GPZ1 40.00 %
P16 1000.00 usec

F1 - Acquisition parameters
TD 512
SFO1 600.133 MHz
FIDRES 30.613245 Hz
SW 13.059 ppm
FnMODE States-TPPI

F2 - Processing parameters
SI 1024
SF 600.1300139 MHz
WDW QSINE
SSB 2
LB 0 Hz
GB 0
PC 1.40

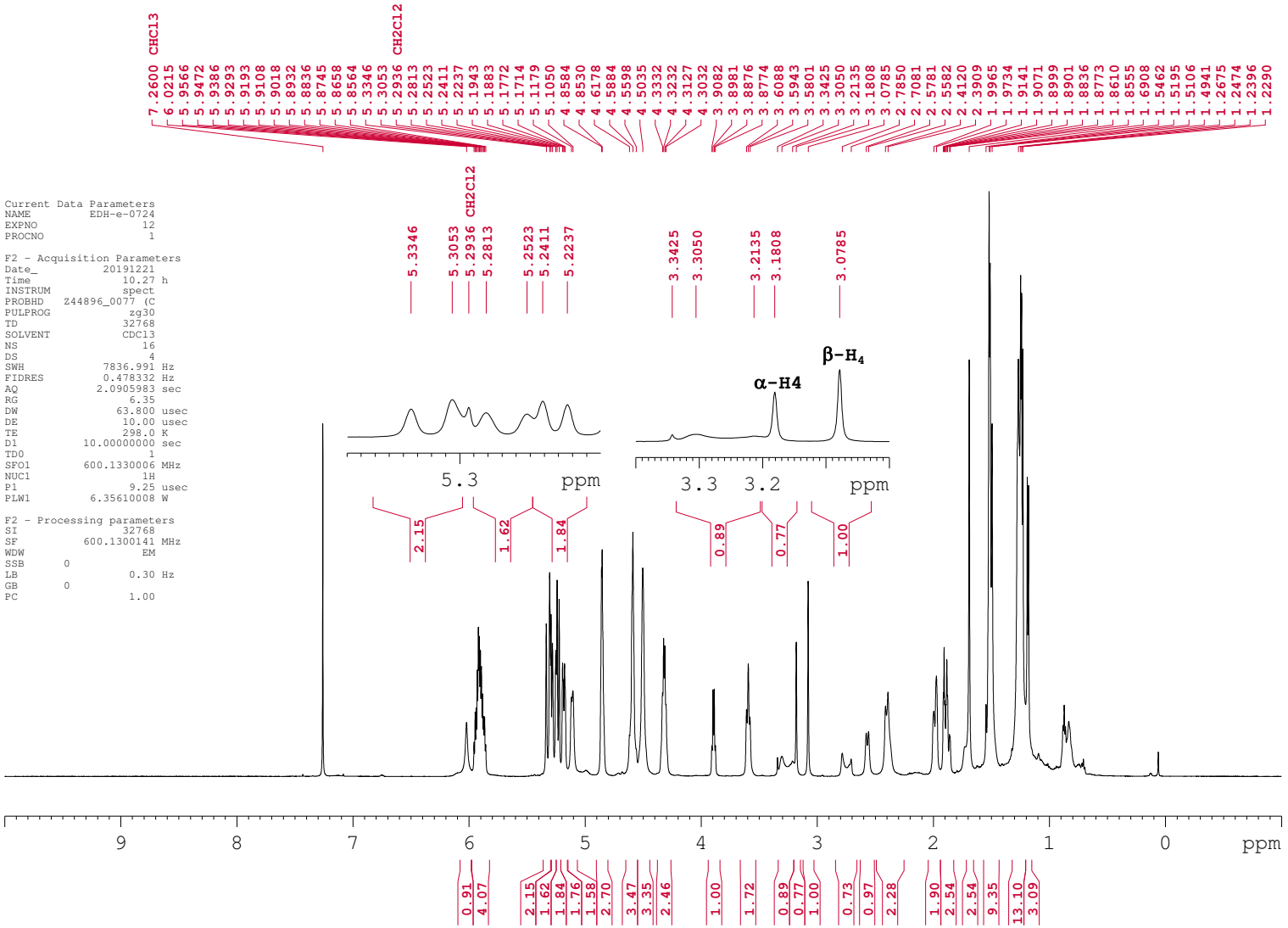
F1 - Processing parameters
SI 1024
MC2 States-TPPI
SF 600.1300139 MHz
WDW QSINE
SSB 2
LB 0 Hz
GB 0

^{1}H NMR of Compound 249 (ca. 1:1.4 α:β) (600 MHz, $CDCl_3$)

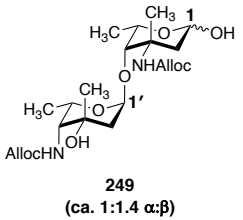


249
(ca. 1:1.4 α:β)

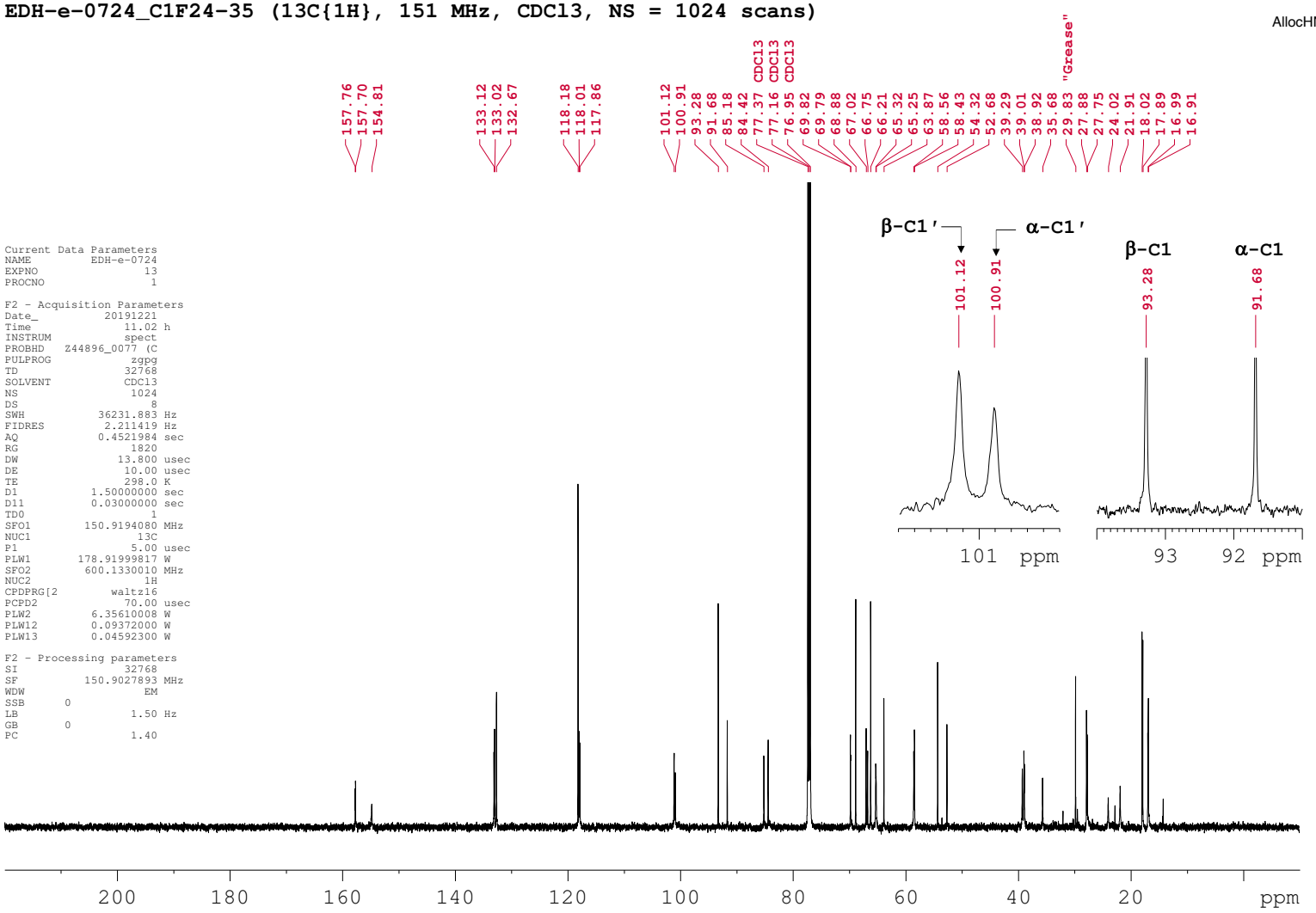
EDH-e-0724_C1F24-35 (1H, 600 MHz, CDCl3, NS = 16 scans, DS = 4 scans, d1 = 10 sec)



$^{13}C\{^{1}H\}$ NMR of Compound 249 (ca. 1:1.4 α:β) (151 MHz, $CDCl_3$)

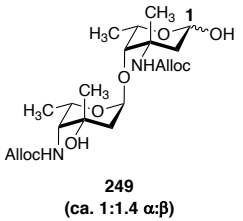


249
(ca. 1:1.4 α:β)



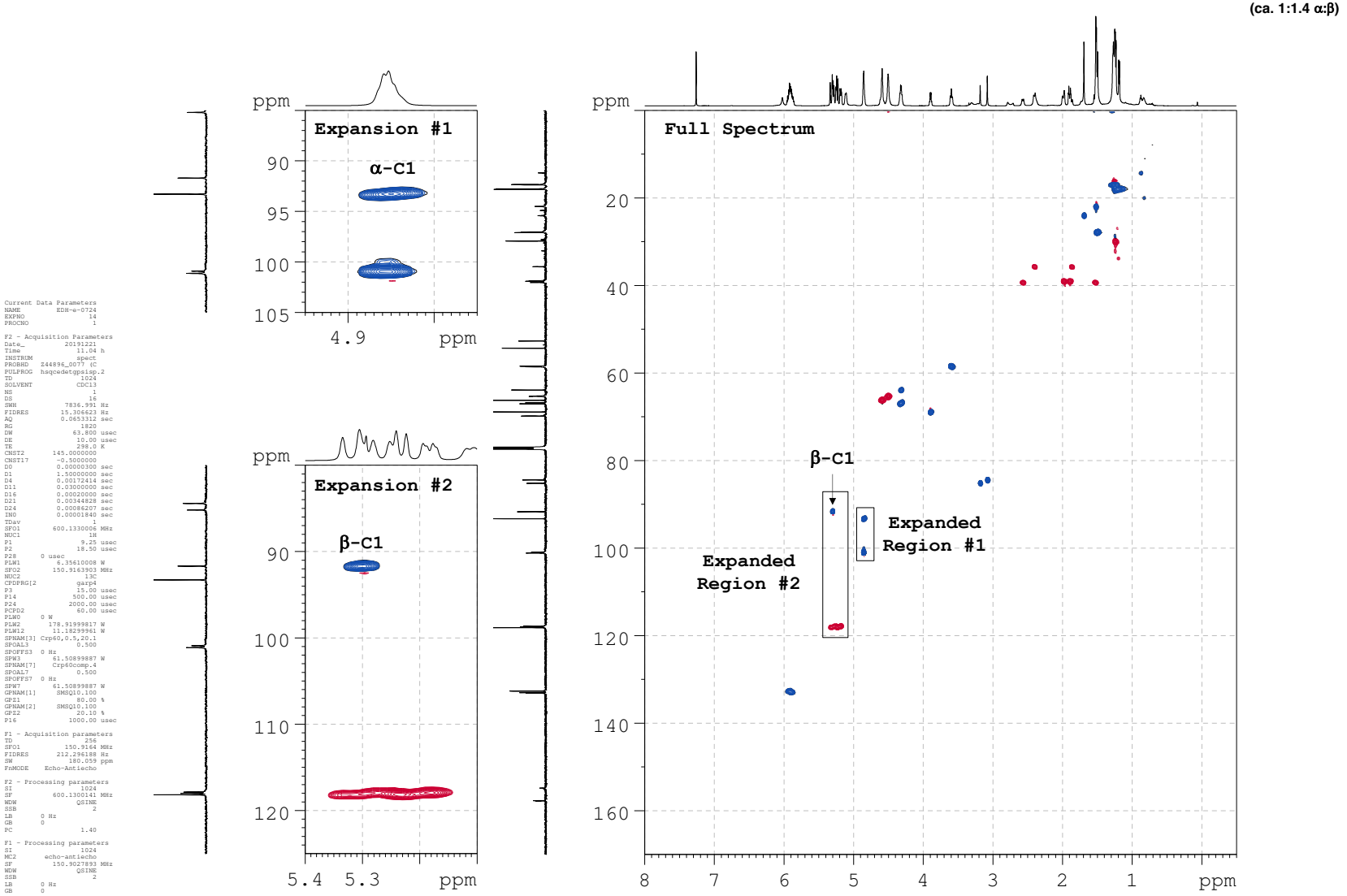
^{1}H-^{13}C HSQC{^{1}H}{^{13}C} NMR of Compound 249 (ca. 1:1.4 α:β) (^{1}H-600 MHz, ^{13}C-151 MHz, $CDCl_3$)

Note: Blue = CH/CH_3 | Red = CH_2.



249
(ca. 1:1.4 α:β)

EDH-e-0724_C1F24-35 (HSQC{1H}{13C}, 600 MHz, CDCl3, NS = 1 scan)



Current Data Parameters
NAME EDH-e-0724
EXPNO 14
PROCNO 1

F2 - Acquisition Parameters
Date_ 20191221
Time 11.04 h
INSTRUM spect
PROBHD Z44896_0077 (C
PULPROG hsqcedetgpsisp.2
TD 1024
SOLVENT CDCl3
NS 1
DS 16
SWH 7836.991 Hz
FIDRES 15.306623 Hz
AQ 0.0653312 sec
RG 1820
DW 63.800 usec
DE 10.00 usec
TE 298.0 K
CNST2 145.0000000
CNST17 -0.5000000
D0 0.00000300 sec
D1 1.50000000 sec
D4 0.00172414 sec
D11 0.03000000 sec
D16 0.00020000 sec
D21 0.00344828 sec
D24 0.00086207 sec
IN0 0.00001840 sec
TDav 1
SFO1 600.1330006 MHz
NUC1 1H
P1 9.25 usec
P2 18.50 usec
P28 0 usec
PLW1 6.35610008 W
SFO2 150.9163903 MHz
NUC2 13C
CPDPRG[2 garp4
P3 15.00 usec
P14 500.00 usec
P24 2000.00 usec
PCPD2 60.00 usec
PLW0 0 W
PLW2 178.91999817 W
PLW12 11.18299961 W
SPNAM[3] Crp60,0.5,20.1
SPOAL3 0.500
SPOFFS3 0 Hz
SPW3 61.50899887 W
SPNAM[7] Crp60comp.4
SPOAL7 0.500
SPOFFS7 0 Hz
SPW7 61.50899887 W
GPNAM[1] SMSQ10.100
GPZ1 80.00 %
GPNAM[2] SMSQ10.100
GPZ2 20.10 %
P16 1000.00 usec

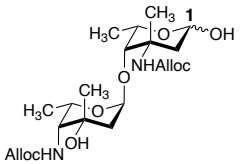
F1 - Acquisition parameters
TD 256
SFO1 150.9164 MHz
FIDRES 212.296188 Hz
SW 180.059 ppm
FnMODE Echo-Antiecho

F2 - Processing parameters
SI 1024
SF 600.1300141 MHz
WDW QSINE
SSB 2
LB 0 Hz
GB 0
PC 1.40

F1 - Processing parameters
SI 1024
MC2 echo-antiecho
SF 150.9027893 MHz
WDW QSINE
SSB 2
LB 0 Hz
GB 0

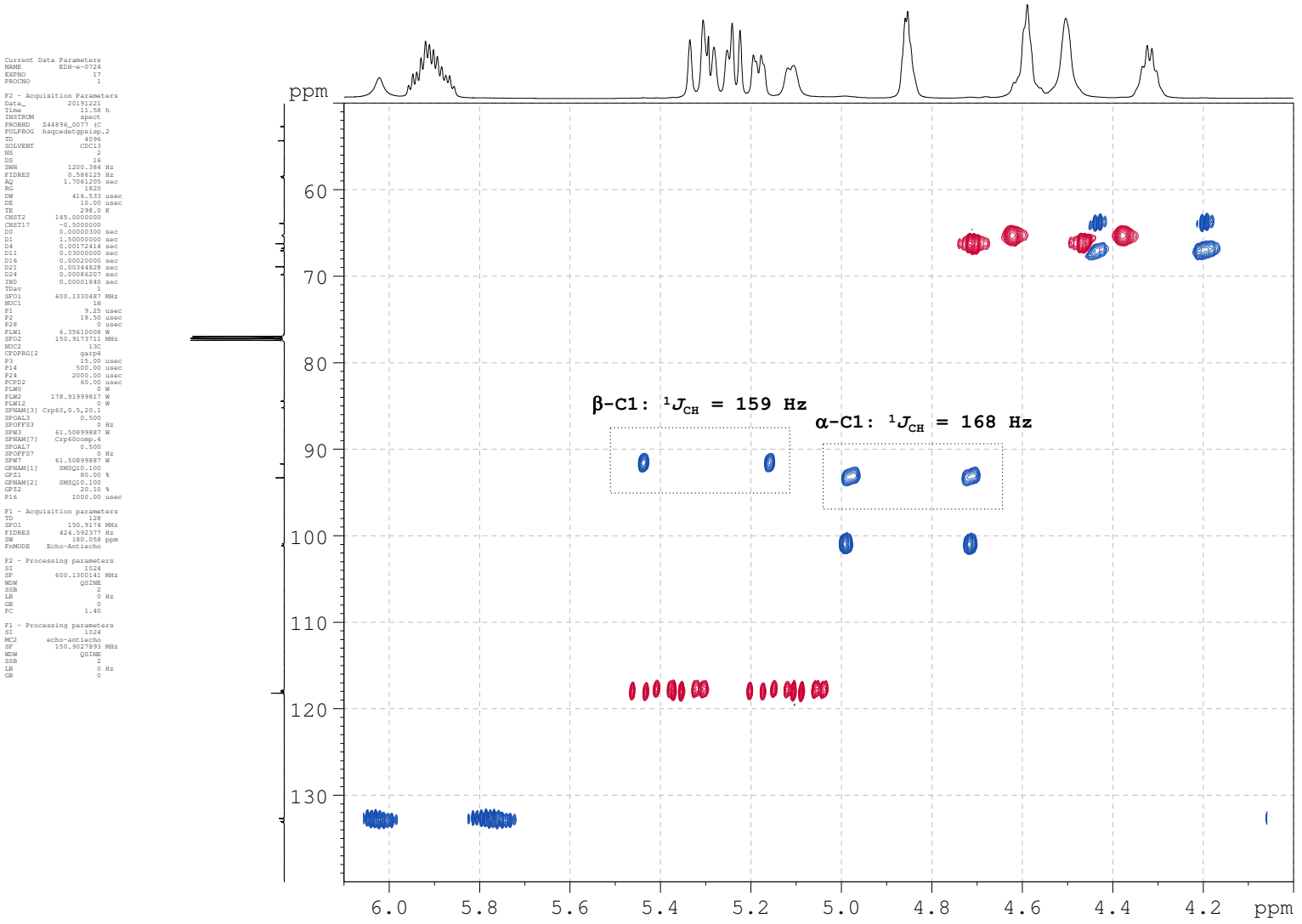
Coupled ^{1}H-^{13}C HSQC NMR of Compound 249 (ca. 1:1.4 α:β) (^{1}H-600 MHz, ^{13}C-151 MHz, $CDCl_3$)

Note: Blue = CH/CH_3 | Red = CH_2.



249
(ca. 1:1.4 α:β)

EDH-e-0724_C1F24-35 ("Coupled" HSQC, 600 MHz, CDCl3, NS = 2 scans)



Current Data Parameters
NAME EDH-e-0724
EXPNO 17
PROCNO 1

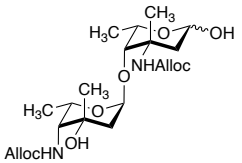
F2 - Acquisition Parameters
Date_ 20191221
Time 11.58 h
INSTRUM spect
PROBHD Z44896_0077 (C
PULPROG hsqcedetgpsisp.2
TD 4096
SOLVENT CDCl3
NS 2
DS 16
SWH 1200.384 Hz
FIDRES 0.586125 Hz
AQ 1.7061205 sec
RG 1820
DW 416.533 usec
DE 10.00 usec
TE 298.0 K
CNST2 145.0000000
CNST17 -0.5000000
D0 0.00000300 sec
D1 1.50000000 sec
D4 0.00172414 sec
D11 0.03000000 sec
D16 0.00020000 sec
D21 0.00344828 sec
D24 0.00086207 sec
IN0 0.00001840 sec
TDav 1
SFO1 600.1330487 MHz
NUC1 1H
P1 9.25 usec
P2 18.50 usec
P28 0 usec
PLW1 6.35610008 W
SFO2 150.9173711 MHz
NUC2 13C
CPDPRG[2 garp4
P3 15.00 usec
P14 500.00 usec
P24 2000.00 usec
PCPD2 60.00 usec
PLW0 0 W
PLW2 178.91999817 W
PLW12 0 W
SPNAM[3] Crp60,0.5,20.1
SPOAL3 0.500
SPOFFS3 0 Hz
SPW3 61.50899887 W
SPNAM[7] Crp60comp.4
SPOAL7 0.500
SPOFFS7 0 Hz
SPW7 61.50899887 W
GPNAM[1] SMSQ10.100
GPZ1 80.00 %
GPNAM[2] SMSQ10.100
GPZ2 20.10 %
P16 1000.00 usec

F1 - Acquisition parameters
TD 128
SFO1 150.9174 MHz
FIDRES 424.592377 Hz
SW 180.058 ppm
FnMODE Echo-Antiecho

F2 - Processing parameters
SI 1024
SF 600.1300141 MHz
WDW QSINE
SSB 2
LB 0 Hz
GB 0
PC 1.40

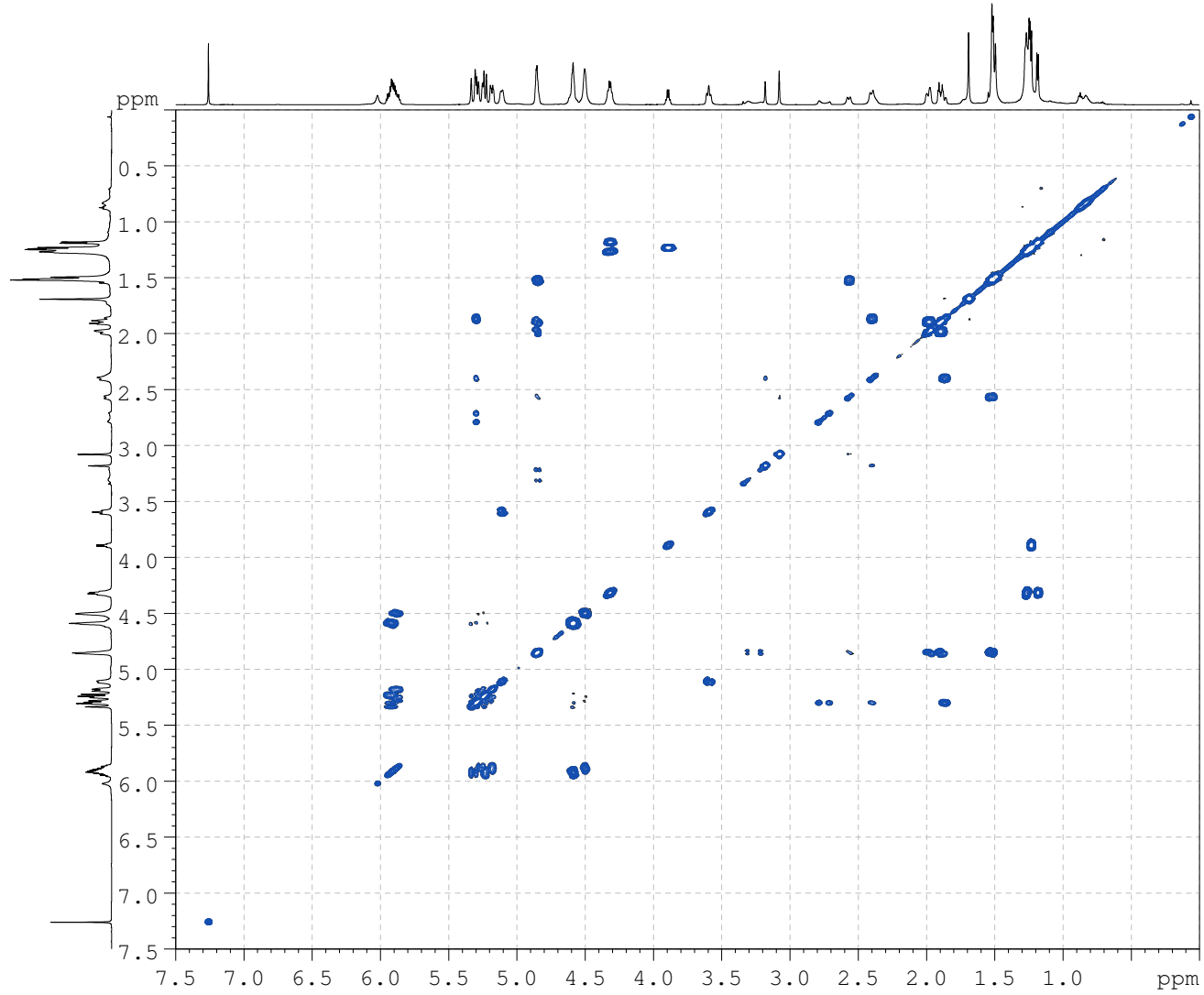
F1 - Processing parameters
SI 1024
MC2 echo-antiecho
SF 150.9027893 MHz
WDW QSINE
SSB 2
LB 0 Hz
GB 0

^{1}H-^{1}H COSY NMR of Compound 249 (ca. 1:1.4 α:β) (600 MHz, $CDCl_3$)



249
(ca. 1:1.4 α:β)

EDH-e-0724_C1F24-35 (COSY, 600 MHz, CDCl3, NS = 1 scan)




```
Current Data Parameters
NAME          EDH-e-0724
EXPNO                 15
PROCNO                 1

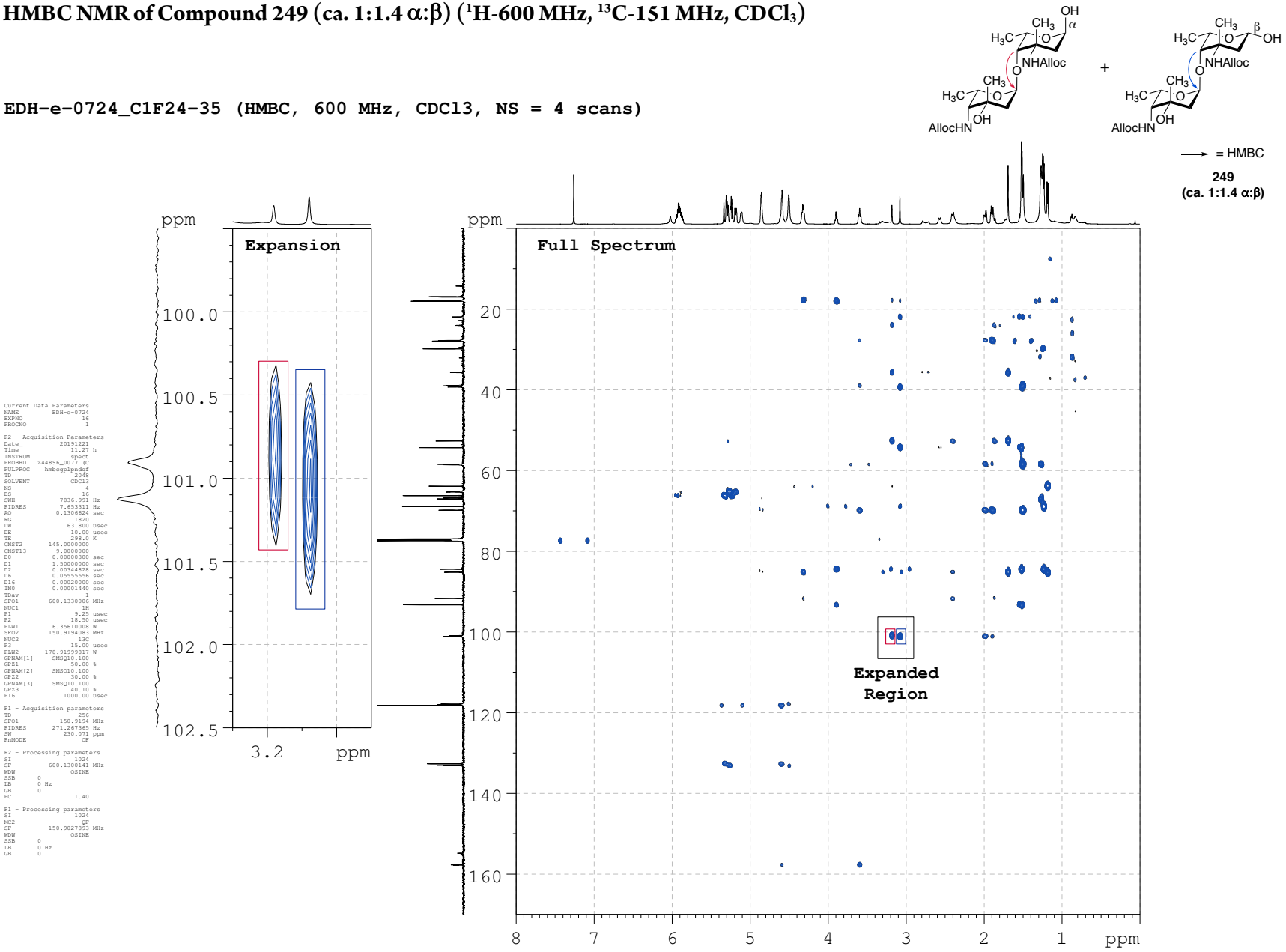
F2 - Acquisition Parameters
Date_           20191221
Time              11.12 h
INSTRUM             spect
PROBHD   Z44896_0077 (C
PULPROG          cosygpqf
TD                  2048
SOLVENT             CDCl3
NS                     1
DS                     8
SWH             7836.991 Hz
FIDRES          7.653311 Hz
AQ              0.1306624 sec
RG                  90.5
DW                 63.800 usec
DE                 10.00 usec
TE                298.0 K
D0              0.00000300 sec
D1              1.48689198 sec
D13             0.00000400 sec
D16             0.00020000 sec
IN0             0.00012760 sec
TDav                   1
SFO1          600.1330006 MHz
NUC1                  1H
P0                  4.65 usec
P1                  9.30 usec
PLW1           6.35610008 W
GPNAM[1]       SMSQ10.100
GPZ1               10.00 %
P16              1000.00 usec

F1 - Acquisition parameters
TD                   512
SFO1          600.133 MHz
FIDRES         30.613245 Hz
SW              13.059 ppm
FnMODE                QF

F2 - Processing parameters
SI                  1024
SF            600.1300141 MHz
WDW                 QSINE
SSB         0
LB        0 Hz
GB          0
PC                  1.40

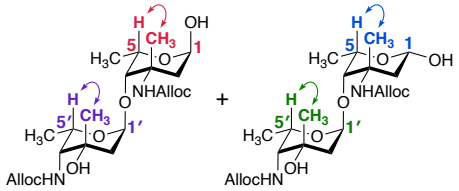
F1 - Processing parameters
SI                  1024
MC2                   QF
SF            600.1300141 MHz
WDW                 QSINE
SSB         0
LB        0 Hz
GB          0
```

^{1}H-^{13}C HMBC NMR of Compound 249 (ca. 1:1.4 α:β) (^{1}H-600 MHz, ^{13}C-151 MHz, $CDCl_3$)

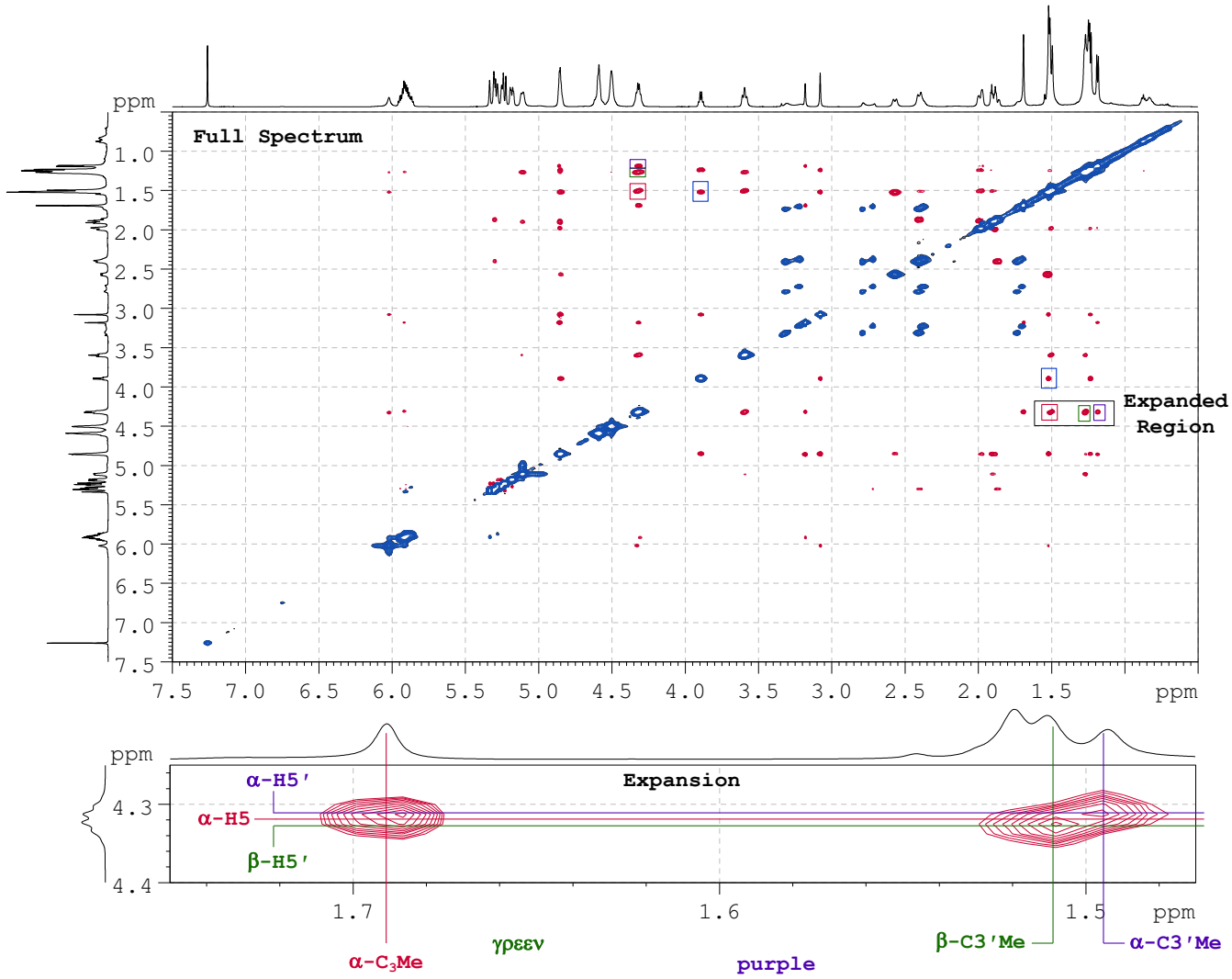


^{1}H-^{1}H NOESY NMR of Compound 249 (ca. 1:1.4 α:β) (^{1}H-600 MHz, $CDCl_3$)

Note: Blue = diagonal/chemical exchange | Red = NOESY cross peaks.



EDH-e-0724_C1F24-35 (NOESY, 600 MHz, CDCl3, NS = 4 scans)
Note: Sample was NOT degassed for this experiment.



Current Data Parameters
NAME EDH-e-0724
EXPNO 18
PROCNO 1

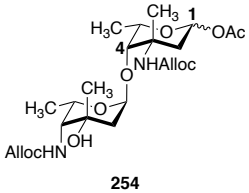
F2 - Acquisition Parameters
Date_ 20191221
Time 12.13 h
INSTRUM spect
PROBHD Z44896_0077 (C
PULPROG noesygpph
TD 2048
SOLVENT CDCl3
NS 4
DS 16
SWH 7836.991 Hz
FIDRES 7.653311 Hz
AQ 0.1306624 sec
RG 128
DW 63.800 usec
DE 10.00 usec
TE 298.0 K
D0 0.00005202 sec
D1 1.48689198 sec
D8 0.60000002 sec
D16 0.00020000 sec
IN0 0.00012760 sec
TDav 1
SFO1 600.1330006 MHz
NUC1 1H
P1 9.25 usec
P2 18.50 usec
PLW1 6.35610008 W
GPNAM[1] SMSQ10.100
GPZ1 40.00 %
P16 1000.00 usec

F1 - Acquisition parameters
TD 512
SFO1 600.133 MHz
FIDRES 30.613245 Hz
SW 13.059 ppm
FnMODE States-TPPI

F2 - Processing parameters
SI 1024
SF 600.1300141 MHz
WDW QSINE
SSB 2
LB 0 Hz
GB 0
PC 1.40

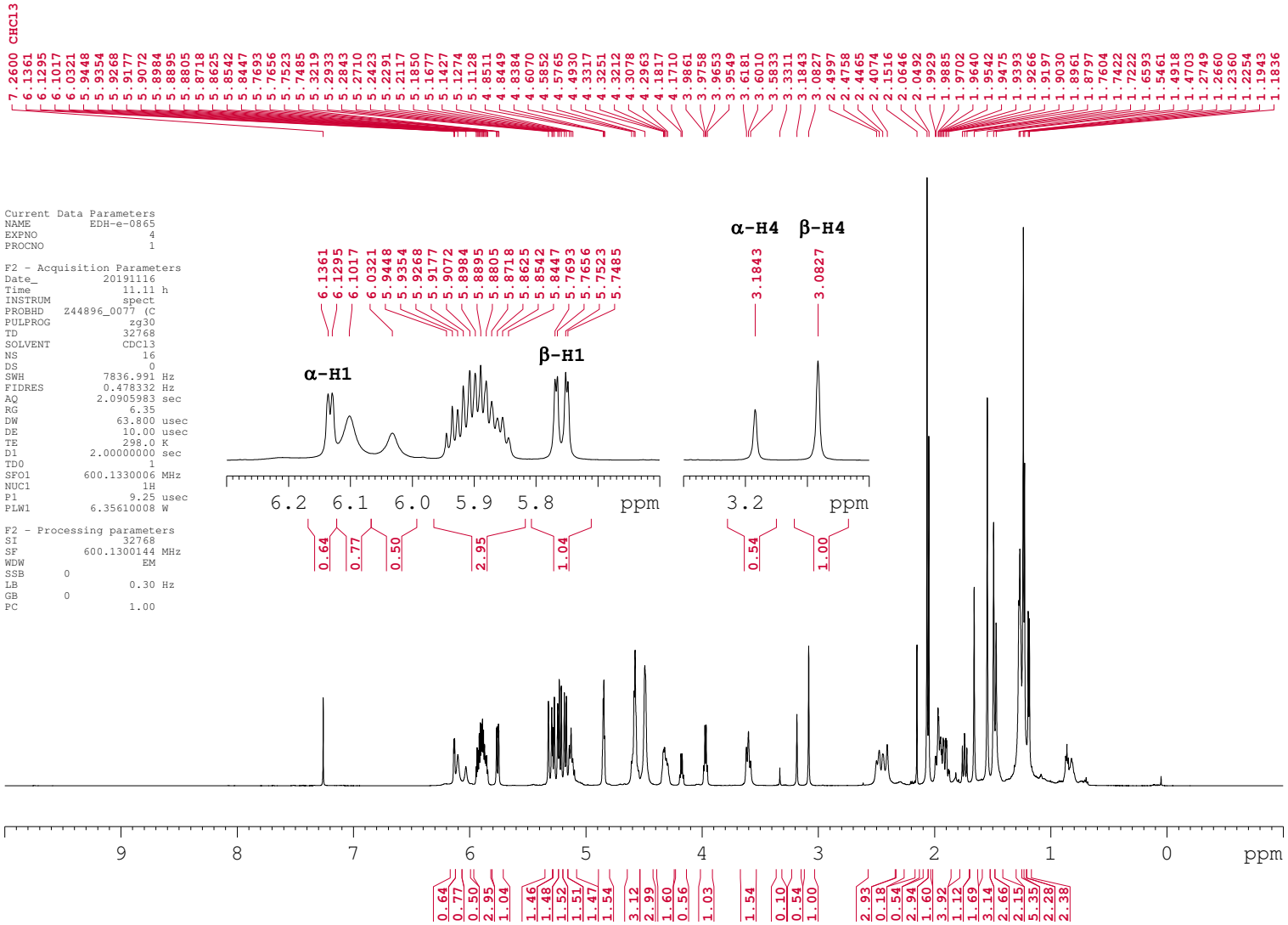
F1 - Processing parameters
SI 1024
MC2 States-TPPI
SF 600.1300141 MHz
WDW QSINE
SSB 2
LB 0 Hz
GB 0

^{1}H NMR of Compound 254 (ca. 1:1.7 α:β) (600 MHz, $CDCl_3$)

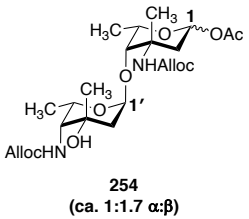


254
(ca. 1:1.7 α:β)

EDH-e-0865_C1F5-12 (1H, 600 MHz, CDCl3, 3 mm, NS = 16 scans)

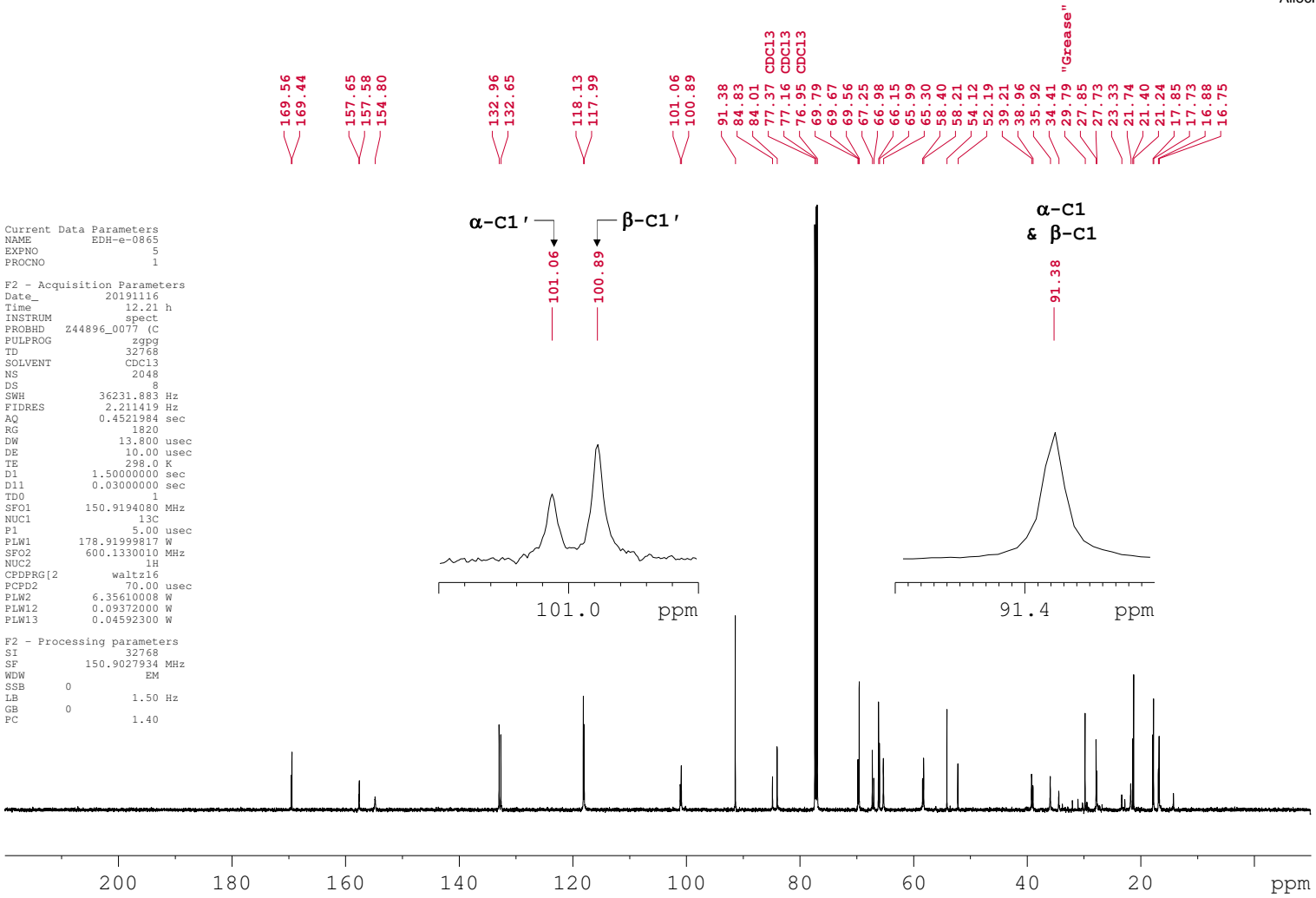


$^{13}C\{^{1}H\}$ NMR of Compound 254 (ca. 1:1.7 α:β) (151 MHz, $CDCl_3$)



254
(ca. 1:1.7 α:β)

EDH-e-0865_C1F5-12 (13C{1H}, 151 MHz, CDCl3, 3 mm, NS = 2048 scans)



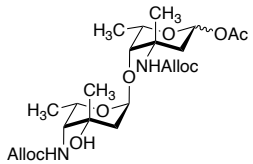
Current Data Parameters
NAME EDH-e-0865
EXPNO 5
PROCNO 1

F2 - Acquisition Parameters
Date_ 20191116
Time 12.21 h
INSTRUM spect
PROBHD Z44896_0077 (C
PULPROG zgpg
TD 32768
SOLVENT CDCl3
NS 2048
DS 8
SWH 36231.883 Hz
FIDRES 2.211419 Hz
AQ 0.4521984 sec
RG 1820
DW 13.800 usec
DE 10.00 usec
TE 298.0 K
D1 1.50000000 sec
D11 0.03000000 sec
TD0 1
SFO1 150.9194080 MHz
NUC1 13C
P1 5.00 usec
PLW1 178.91999817 W
SFO2 600.1330010 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 70.00 usec
PLW2 6.35610008 W
PLW12 0.09372000 W
PLW13 0.04592300 W

F2 - Processing parameters
SI 32768
SF 150.9027934 MHz
WDW EM
SSB 0
LB 1.50 Hz
GB 0
PC 1.40

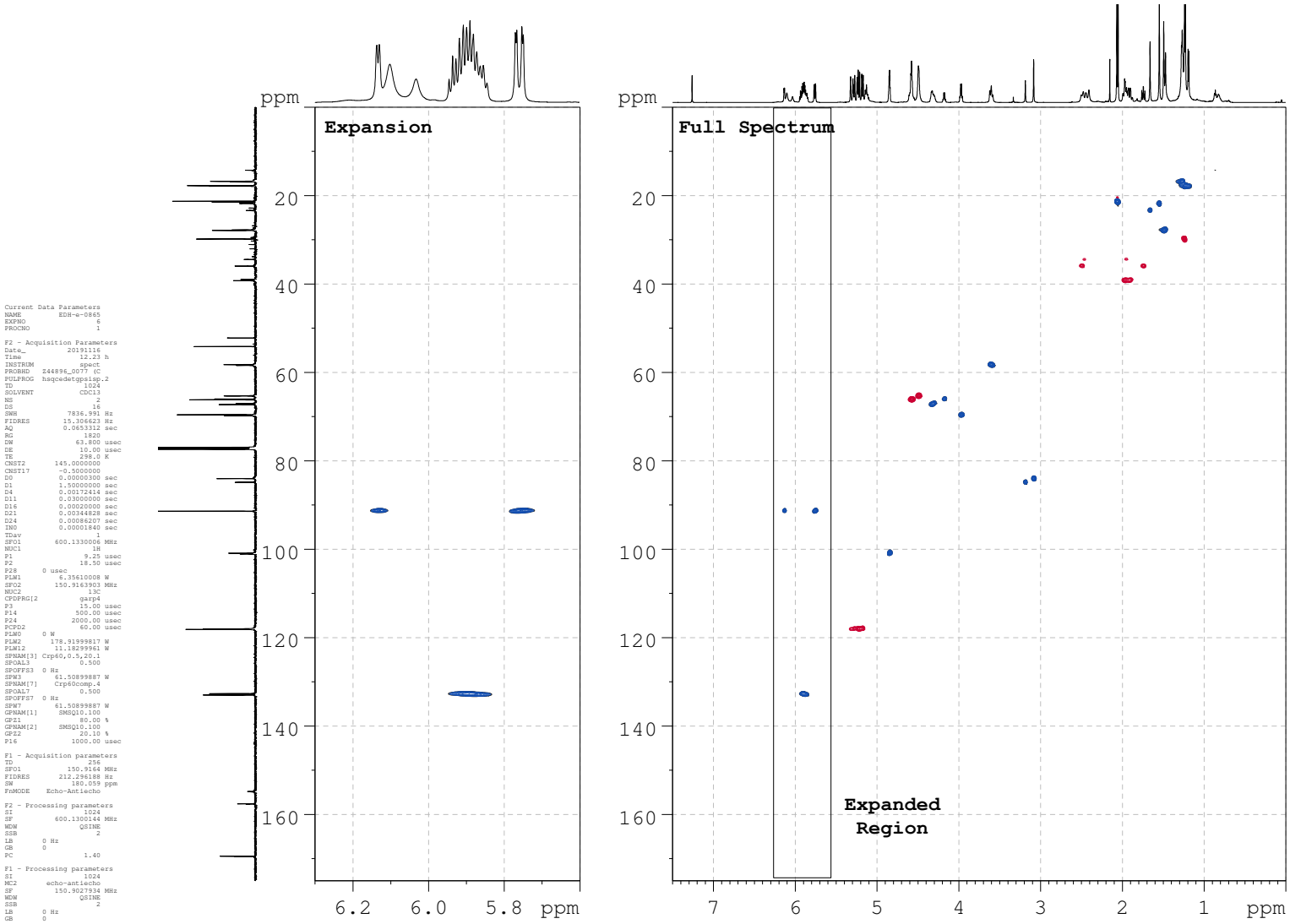
^{1}H-^{13}C HSQC{^{1}H}{^{13}C} NMR of Compound 254 (ca. 1:1.7 α:β) (^{1}H-600 MHz, ^{13}C-151 MHz, $CDCl_3$)

Note: Blue = CH/CH_3 | Red = CH_2.



254
(ca. 1:1.7 α:β)

EDH-e-0865_C1F5-12 (HSQC, 600 MHz, CDCl3, 3 mm)



Current Data Parameters
NAME EDH-e-0865
EXPNO 6
PROCNO 1

F2 - Acquisition Parameters
Date_ 20191116
Time 12.23 h
INSTRUM spect
PROBHD Z44896_0077 (C
PULPROG hsqcedetgpsisp.2
TD 1024
SOLVENT CDCl3
NS 2
DS 16
SWH 7836.991 Hz
FIDRES 15.306623 Hz
AQ 0.0653312 sec
RG 1820
DW 63.800 usec
DE 10.00 usec
TE 298.0 K
CNST2 145.0000000
CNST17 -0.5000000
D0 0.00000300 sec
D1 1.50000000 sec
D4 0.00172414 sec
D11 0.03000000 sec
D16 0.00020000 sec
D21 0.00344828 sec
D24 0.00086207 sec
IN0 0.00001840 sec
TDav 1
SFO1 600.1330006 MHz
NUC1 1H
P1 9.25 usec
P2 18.50 usec
P28 0 usec
PLW1 6.35610008 W
SFO2 150.9163903 MHz
NUC2 13C
CPDPRG[2 garp4
P3 15.00 usec
P14 500.00 usec
P24 2000.00 usec
PCPD2 60.00 usec
PLW0 0 W
PLW2 178.91999817 W
PLW12 11.18299961 W
SPNAM[3] Crp60,0.5,20.1
SPOAL3 0.500
SPOFFS3 0 Hz
SPW3 61.50899887 W
SPNAM[7] Crp60comp.4
SPOAL7 0.500
SPOFFS7 0 Hz
SPW7 61.50899887 W
GPNAM[1] SMSQ10.100
GPZ1 80.00 %
GPNAM[2] SMSQ10.100
GPZ2 20.10 %
P16 1000.00 usec

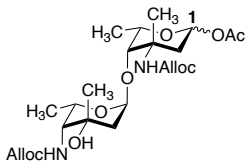
F1 - Acquisition parameters
TD 256
SFO1 150.9164 MHz
FIDRES 212.296188 Hz
SW 180.059 ppm
FnMODE Echo-Antiecho

F2 - Processing parameters
SI 1024
SF 600.1300144 MHz
WDW QSINE
SSB 2
LB 0 Hz
GB 0
PC 1.40

F1 - Processing parameters
SI 1024
MC2 echo-antiecho
SF 150.9027934 MHz
WDW QSINE
SSB 2
LB 0 Hz
GB 0

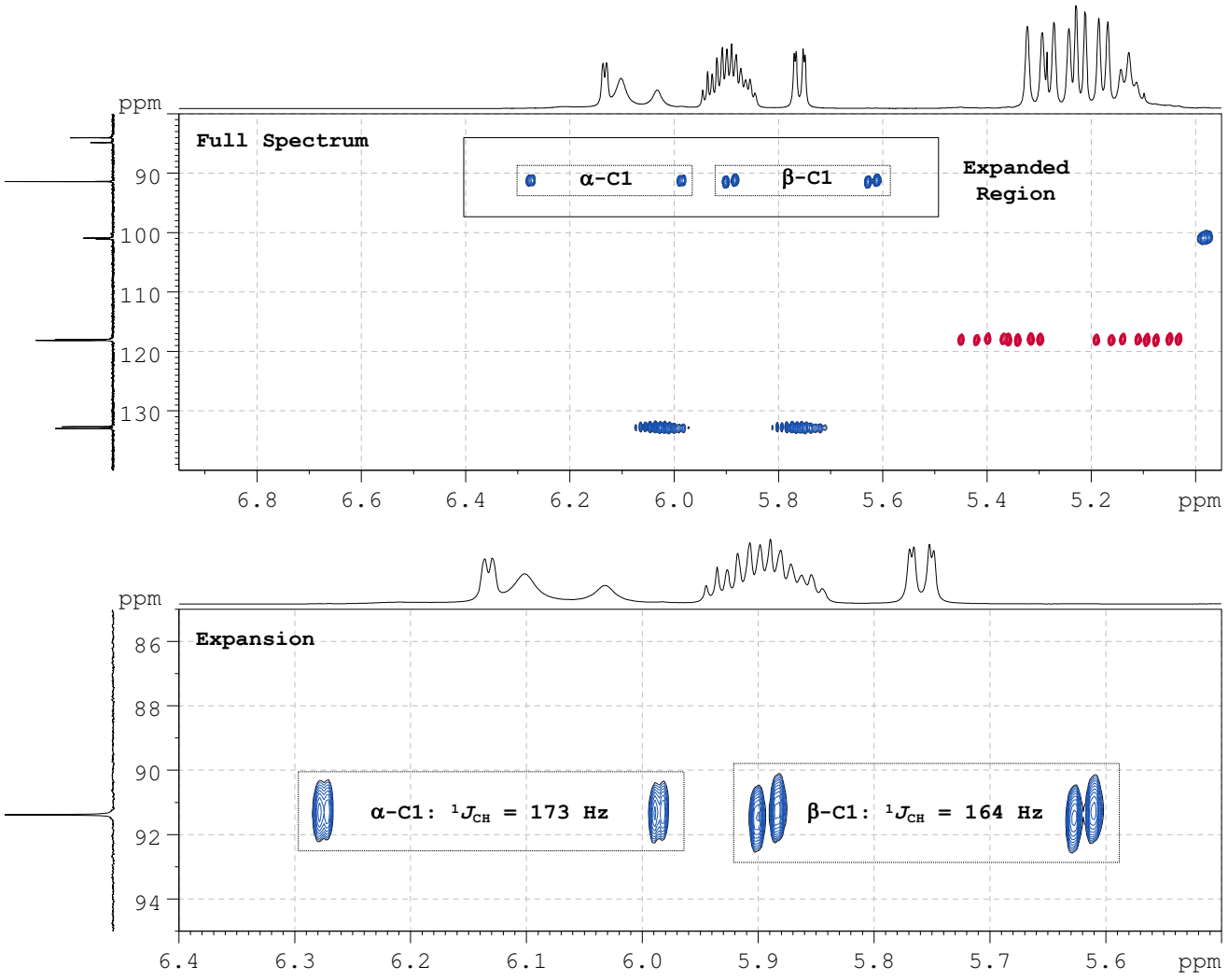
Coupled ^{1}H-^{13}C HSQC NMR of Compound 254 (ca. 1:1.7 α:β) (^{1}H-600 MHz, ^{13}C-151 MHz, $CDCl_3$)

Note: Blue = CH/CH_3 | Red = CH_2.



254
(ca. 1:1.7 α:β)

EDH-e-0865_C1F5-12 ("Coupled" HSQC, 600 MHz, CDCl3, 3 mm)




```
Current Data Parameters
NAME          EDH-e-0865
EXPNO                  7
PROCNO                 1

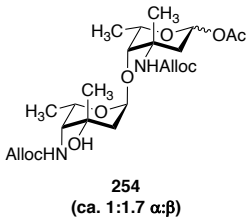
F2 - Acquisition Parameters
Date_          20191116
Time              12.38 h
INSTRUM             spect
PROBHD   Z44896_0077 (C
PULPROG  hsqcedetgpsisp.2
TD                  4096
SOLVENT             CDCl3
NS                     2
DS                    16
SWH             1200.384 Hz
FIDRES          0.586125 Hz
AQ              1.7061205 sec
RG                  1820
DW                416.533 usec
DE                 10.00 usec
TE                298.0 K
CNST2       145.0000000
CNST17       -0.5000000
D0            0.00000300 sec
D1            1.50000000 sec
D4            0.00172414 sec
D11           0.03000000 sec
D16           0.00020000 sec
D21           0.00344828 sec
D24           0.00086207 sec
IN0           0.00001840 sec
TDav                   1
SFO1         600.1335828 MHz
NUC1                1H
P1                 9.25 usec
P2                18.50 usec
PLW1          6.35610008 W
SFO2        150.9165412 MHz
NUC2                13C
CPDPRG[2            garp4
P3                15.00 usec
P14              500.00 usec
P24             2000.00 usec
PCPD2             60.00 usec
PLW0       0 W
PLW2       178.91999817 W
PLW12      0 W
SPNAM[3] Crp60,0.5,20.1
SPOAL3             0.500
SPOFFS3    0 Hz
SPW3       61.50899887 W
SPNAM[7]     Crp60comp.4
SPOAL7             0.500
SPOFFS7    0 Hz
SPW7       61.50899887 W
GPNAM[1]       SMSQ10.100
GPZ1              80.00 %
GPNAM[2]       SMSQ10.100
GPZ2              20.10 %
P16             1000.00 usec

F1 - Acquisition parameters
TD                   128
SFO1            150.9165 MHz
FIDRES        424.592377 Hz
SW             180.059 ppm
FnMODE     Echo-Antiecho

F2 - Processing parameters
SI                  1024
SF            600.1300144 MHz
WDW                 QSINE
SSB                    2
LB         0 Hz
GB         0
PC                  1.40

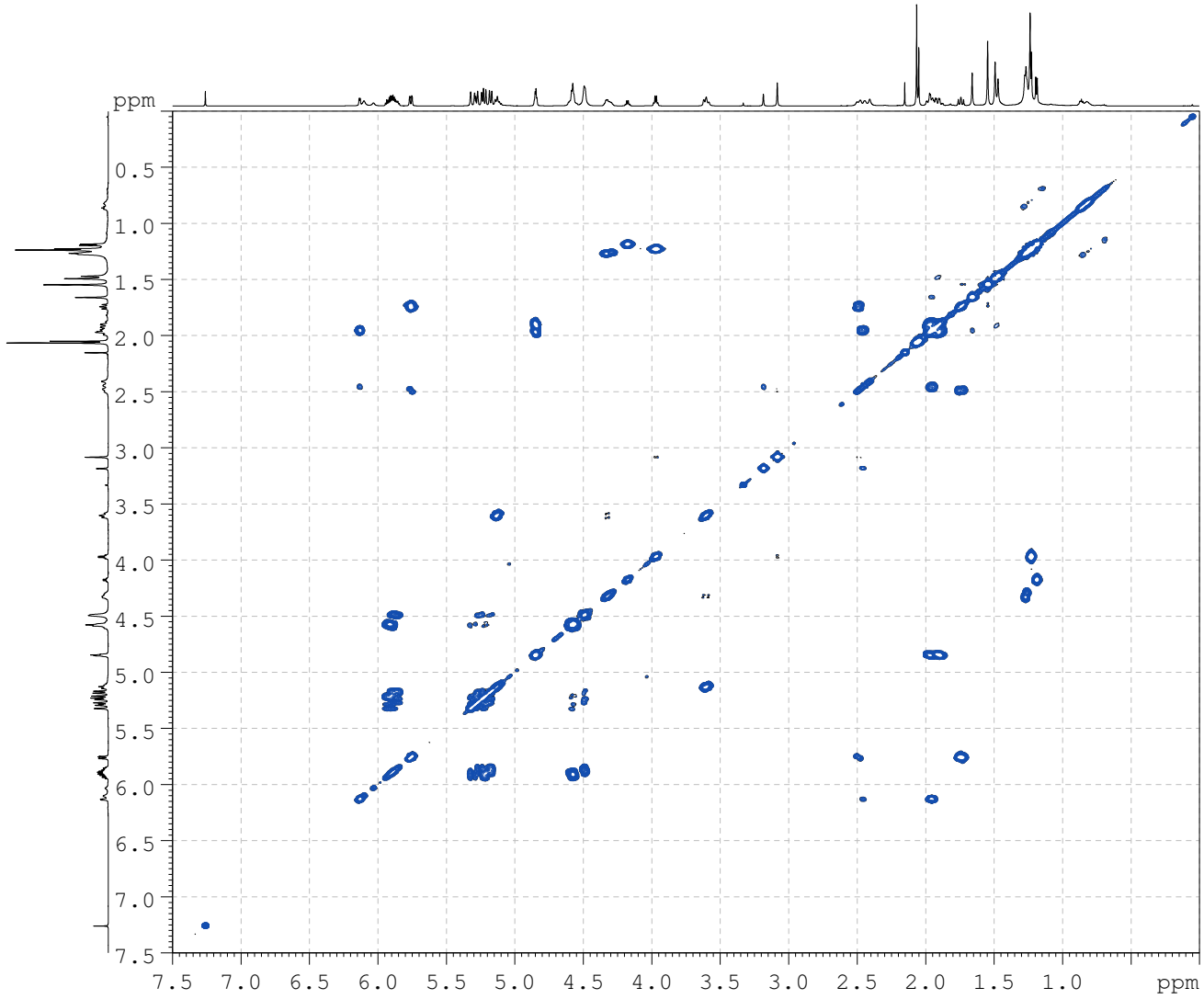
F1 - Processing parameters
SI                  1024
MC2        echo-antiecho
SF           150.9027934 MHz
WDW                 QSINE
SSB                    2
LB         0 Hz
GB         0
```

^{1}H-^{1}H COSY NMR of Compound 254 (ca. 1:1.7 α:β) (600 MHz, $CDCl_3$)



254
(ca. 1:1.7 α:β)

EDH-e-0865_C1F5-12 (COSY, 600 MHz, CDCl3, 3 mm)




```
Current Data Parameters
NAME          EDH-e-0865
EXPNO                  8
PROCNO                 1

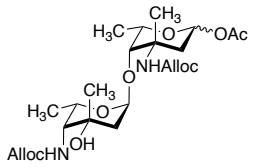
F2 - Acquisition Parameters
Date_          20191116
Time              12.53 h
INSTRUM             spect
PROBHD   Z44896_0077 (C
PULPROG         cosygpqf
TD                 2048
SOLVENT            CDCl3
NS                    1
DS                    8
SWH             7836.991 Hz
FIDRES          7.653311 Hz
AQ              0.1306624 sec
RG                  161
DW                63.800 usec
DE                10.00 usec
TE                298.0 K
D0              0.00000300 sec
D1              1.48689198 sec
D13             0.00000400 sec
D16             0.00020000 sec
IN0             0.00012760 sec
TDav                  1
SFO1          600.1330006 MHz
NUC1                  1H
P0                 4.65 usec
P1                 9.30 usec
PLW1           6.35610008 W
GPNAM[1]       SMSQ10.100
GPZ1              10.00 %
P16             1000.00 usec

F1 - Acquisition parameters
TD                  512
SFO1          600.133 MHz
FIDRES         30.613245 Hz
SW             13.059 ppm
FnMODE                QF

F2 - Processing parameters
SI                 1024
SF            600.1300144 MHz
WDW                QSINE
SSB                   0
LB                0 Hz
GB                    0
PC                 1.40

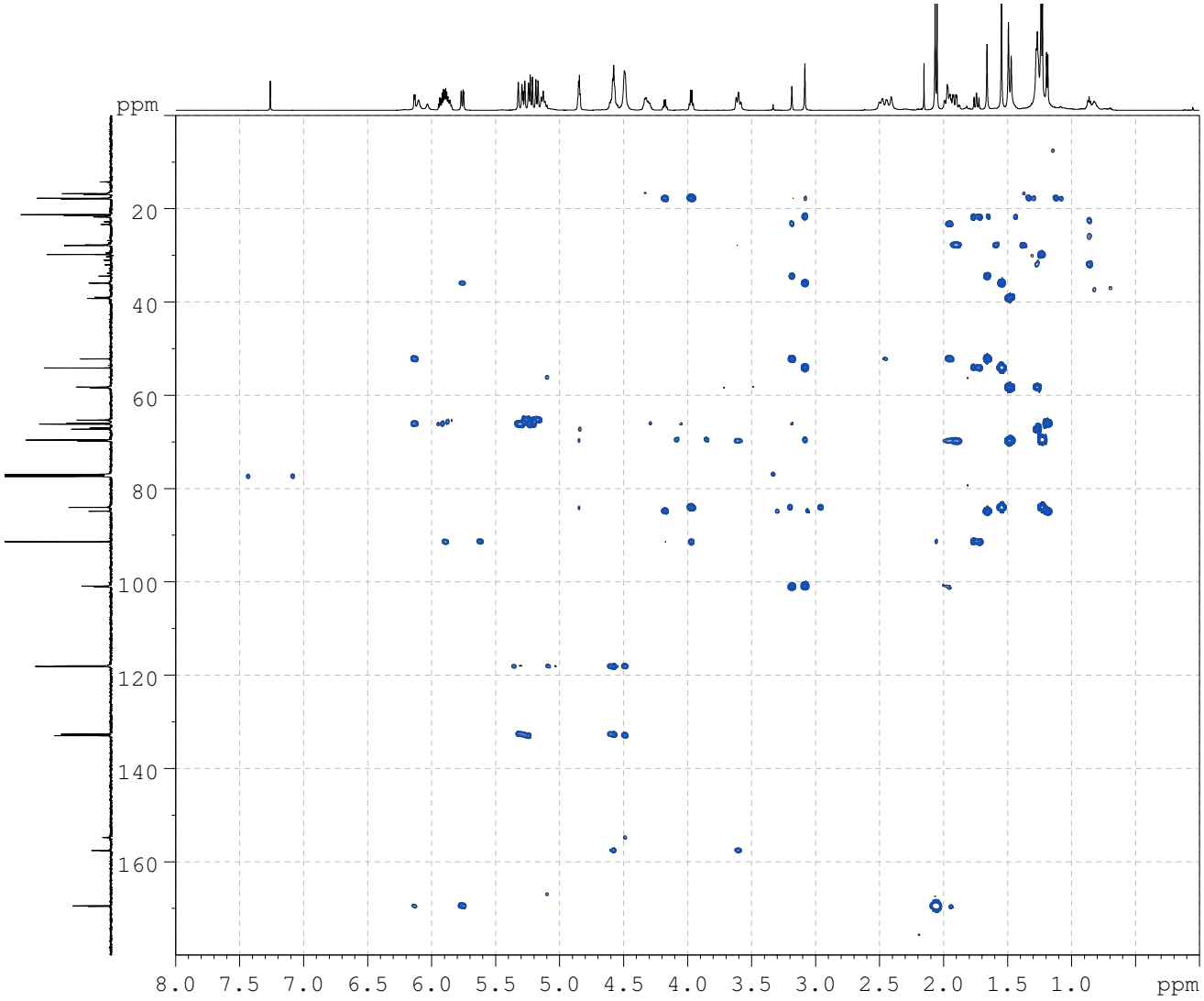
F1 - Processing parameters
SI                 1024
MC2                   QF
SF            600.1300144 MHz
WDW                QSINE
SSB                   0
LB                0 Hz
GB                    0
```

^{1}H-^{13}C HMBC NMR of Compound 254 (ca. 1:1.7 α:β) (^{1}H-600 MHz, ^{13}C-151 MHz, $CDCl_3$)



254
(ca. 1:1.7 α:β)

EDH-e-0865_C1F5-12 (HMBC, 600 MHz, CDCl3, 3 mm, NS = 4 scans)




```
Current Data Parameters
NAME          EDH-e-0865
EXPNO                  9
PROCNO                 1

F2 - Acquisition Parameters
Date_           20191116
Time              13.09 h
INSTRUM             spect
PROBHD   Z44896_0077 (C
PULPROG     hmbcgplpndqf
TD                  2048
SOLVENT             CDCl3
NS                     4
DS                    16
SWH              7836.991 Hz
FIDRES           7.653311 Hz
AQ               0.1306624 sec
RG                  1820
DW                 63.800 usec
DE                 10.00 usec
TE                298.0 K
CNST2         145.0000000
CNST13          9.0000000
D0             0.00000300 sec
D1             1.50000000 sec
D2             0.00344828 sec
D6             0.05555556 sec
D16            0.00020000 sec
IN0            0.00001440 sec
TDav                   1
SFO1          600.1330006 MHz
NUC1                  1H
P1                  9.25 usec
P2                 18.50 usec
PLW1           6.35610008 W
SFO2          150.9194083 MHz
NUC2                  13C
P3                 15.00 usec
PLW2         178.91999817 W
GPNAM[1]       SMSQ10.100
GPZ1               50.00 %
GPNAM[2]       SMSQ10.100
GPZ2               30.00 %
GPNAM[3]       SMSQ10.100
GPZ3               40.10 %
P16              1000.00 usec

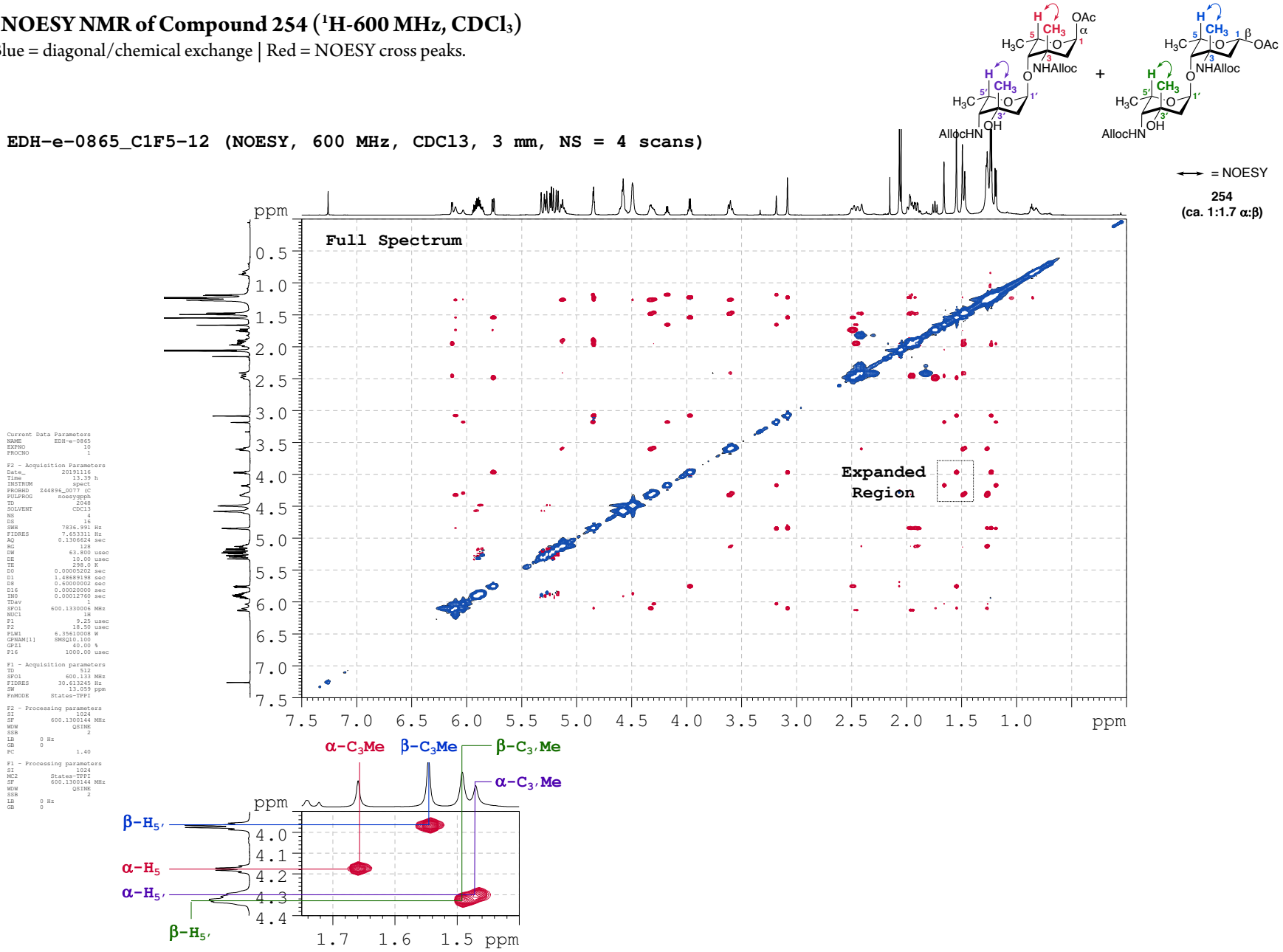
F1 - Acquisition parameters
TD                   256
SFO1          150.9194 MHz
FIDRES        271.267365 Hz
SW              230.071 ppm
FnMODE                QF

F2 - Processing parameters
SI                  1024
SF            600.1300144 MHz
WDW                 QSINE
SSB         0
LB          0 Hz
GB          0
PC                  1.40

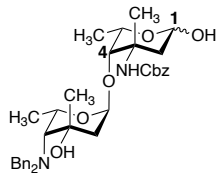
F1 - Processing parameters
SI                  1024
MC2                   QF
SF            150.9027934 MHz
WDW                 QSINE
SSB         0
LB          0 Hz
GB          0
```

^{1}H-^{1}H NOESY NMR of Compound 254 (^{1}H-600 MHz, $CDCl_3$)

Note: Blue = diagonal/chemical exchange | Red = NOESY cross peaks.

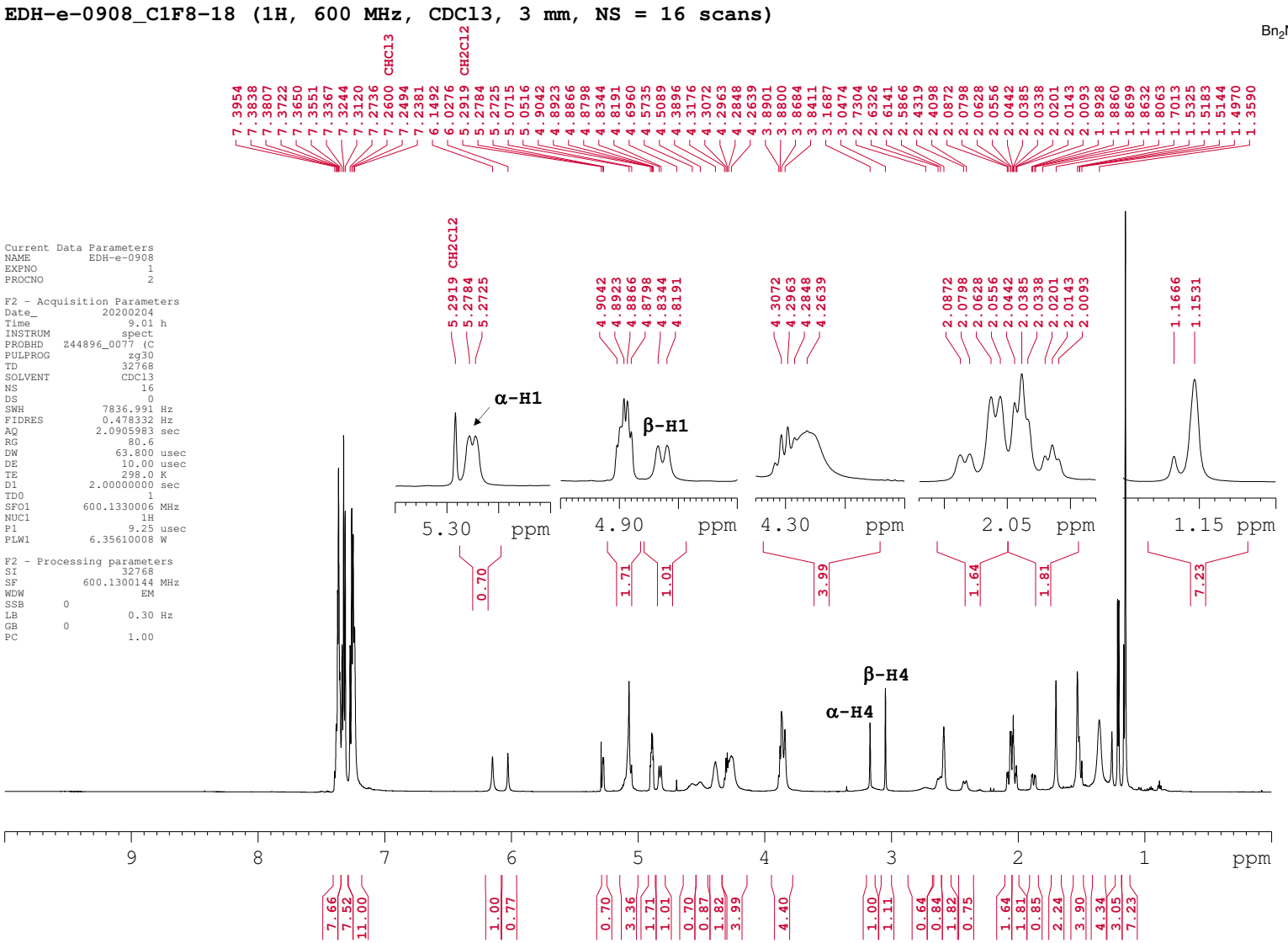


^{1}H NMR of Compound 288 (ca. 2:3 α/β) (600 MHz, $CDCl_3$)



α/β-288

ca. 2:3 α/β

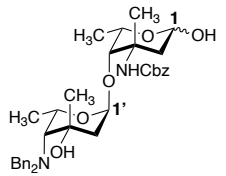


Current Data Parameters
NAME EDH-e-0908
EXPNO 1
PROCNO 2

F2 - Acquisition Parameters
Date_ 20200204
Time 9.01 h
INSTRUM spect
PROBHD Z44896_0077 (C
PULPROG zg30
TD 32768
SOLVENT CDCl3
NS 16
DS 0
SWH 7836.991 Hz
FIDRES 0.478332 Hz
AQ 2.0905983 sec
RG 80.6
DW 63.800 usec
DE 10.00 usec
TE 298.0 K
D1 2.00000000 sec
TD0 1
SFO1 600.1330006 MHz
NUC1 1H
P1 9.25 usec
PLW1 6.35610008 W

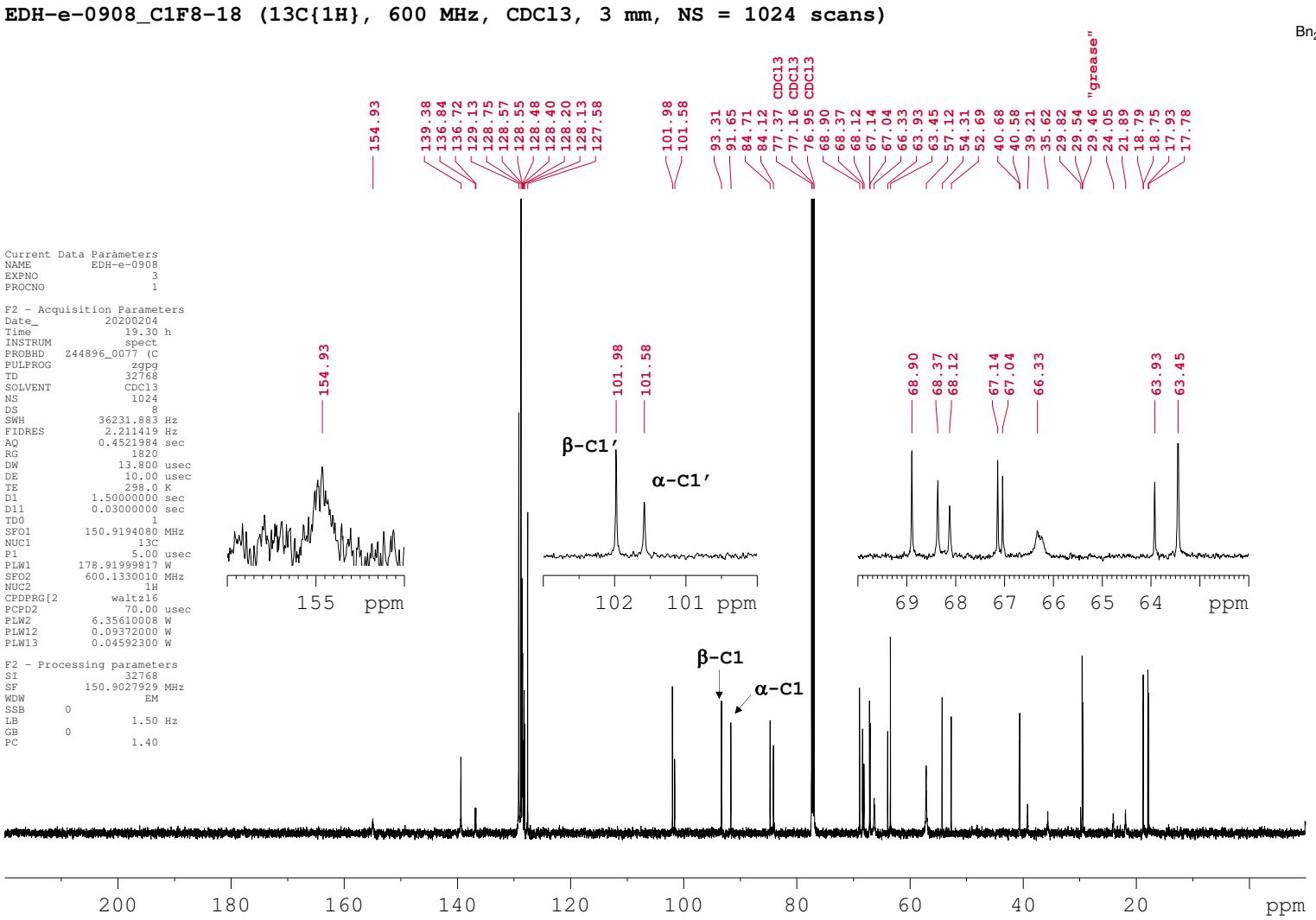
F2 - Processing parameters
SI 32768
SF 600.1300144 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

$^{13}C\{^{1}H\}$ NMR of Compound 288 (ca. 2:3 α/β) (151 MHz, $CDCl_3$)



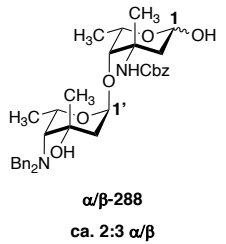
α/β-288

ca. 2:3 α/β



^{1}H-^{13}C HSQC{^{1}H}{^{13}C} NMR of Compound 288 (ca. 2:3 α/β) (^{1}H-600 MHz, ^{13}C-151 MHz, $CDCl_3$)

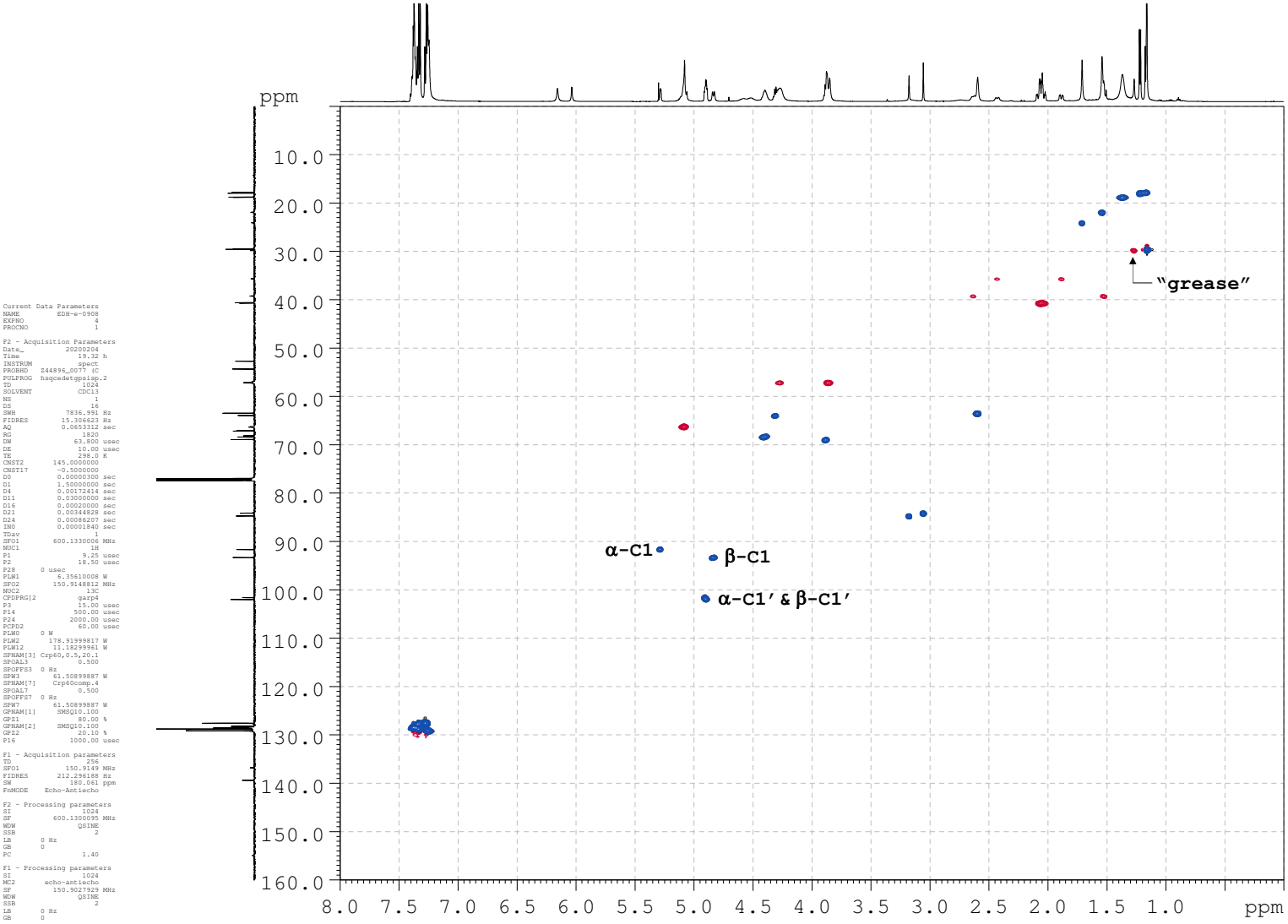
Note: Blue = CH/CH_3 | Red = CH_2.



α/β-288

ca. 2:3 α/β

EDH-e-0908_C1F8-18 (HSQC 600 MHz, CDCl3, 3 mm, NS = 1 scan, O2P = 80 ppm)



Current Data Parameters
NAME EDH-e-0908
EXPNO 4
PROCNO 1

F2 - Acquisition Parameters
Date_ 20200204
Time 19.32 h
INSTRUM spect
PROBHD Z44896_0077 (C
PULPROG hsqcedetgpsisp.2
TD 1024
SOLVENT CDCl3
NS 1
DS 16
SWH 7836.991 Hz
FIDRES 15.306623 Hz
AQ 0.0653312 sec
RG 1820
DW 63.800 usec
DE 10.00 usec
TE 298.0 K
CNST2 145.0000000
CNST17 -0.5000000
D0 0.00000300 sec
D1 1.50000000 sec
D4 0.00172414 sec
D11 0.03000000 sec
D16 0.00020000 sec
D21 0.00344828 sec
D24 0.00086207 sec
IN0 0.00001840 sec
TDav 1
SFO1 600.1330006 MHz
NUC1 1H
P1 9.25 usec
P2 18.50 usec
P28 0 usec
PLW1 6.35610008 W
SFO2 150.9148812 MHz
NUC2 13C
CPDPRG[2 garp4
P3 15.00 usec
P14 500.00 usec
P24 2000.00 usec
PCPD2 60.00 usec
PLW0 0 W
PLW2 178.91999817 W
PLW12 11.18299961 W
SPNAM[3] Crp60,0.5,20.1
SPOAL3 0.500
SPOFFS3 0 Hz
SPW3 61.50899887 W
SPNAM[7] Crp60comp.4
SPOAL7 0.500
SPOFFS7 0 Hz
SPW7 61.50899887 W
GPNAM[1] SMSQ10.100
GPZ1 80.00 %
GPNAM[2] SMSQ10.100
GPZ2 20.10 %
P16 1000.00 usec

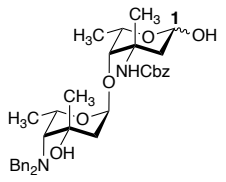
F1 - Acquisition parameters
TD 256
SFO1 150.9149 MHz
FIDRES 212.296188 Hz
SW 180.061 ppm
FnMODE Echo-Antiecho

F2 - Processing parameters
SI 1024
SF 600.1300095 MHz
WDW QSINE
SSB 2
LB 0 Hz
GB 0
PC 1.40

F1 - Processing parameters
SI 1024
MC2 echo-antiecho
SF 150.9027929 MHz
WDW QSINE
SSB 2
LB 0 Hz
GB 0

Coupled ^{1}H-^{13}C HSQC NMR of Compound 288 (ca. 2:3 α/β) (^{1}H-600 MHz, ^{13}C-151 MHz, $CDCl_3$)

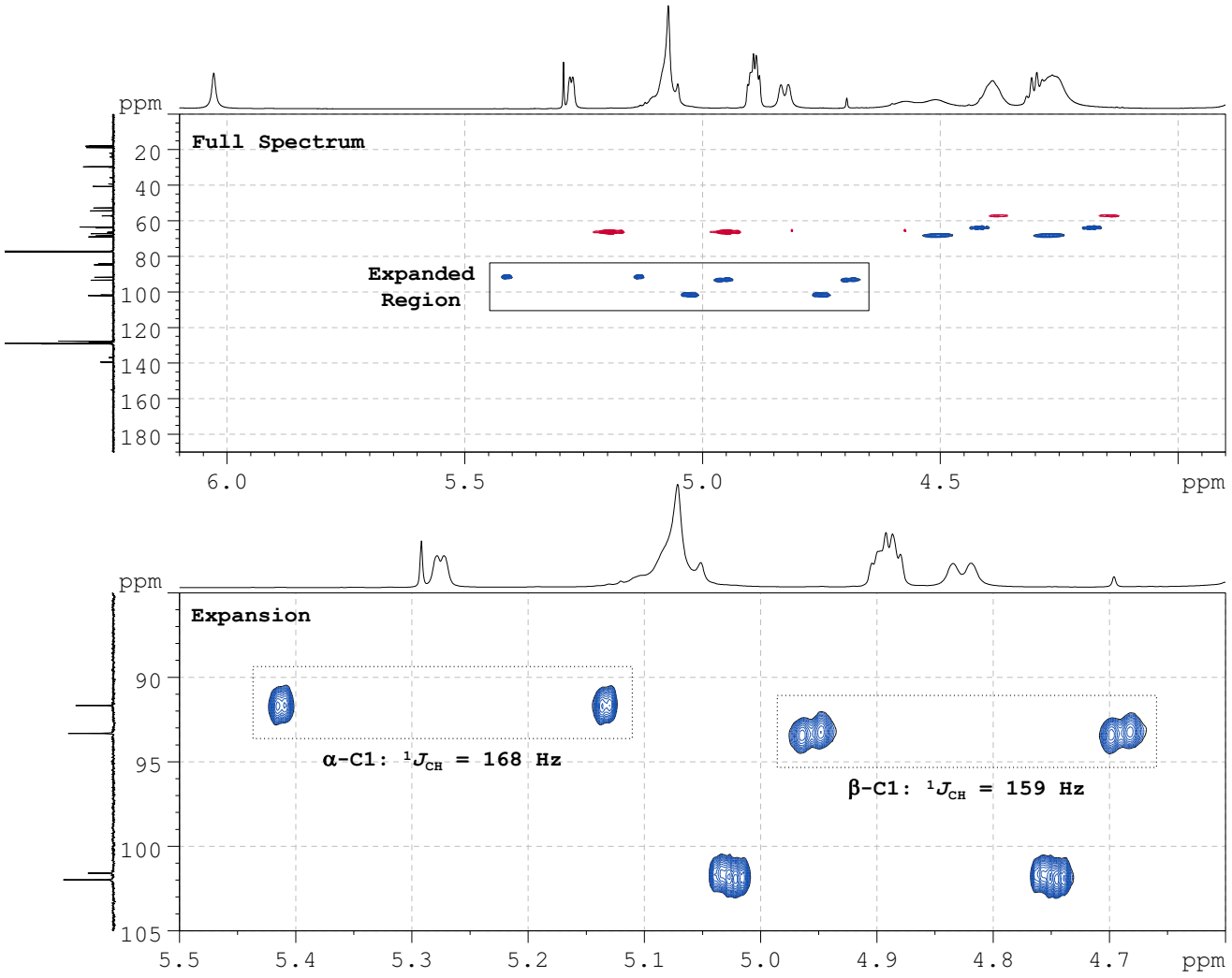
Note: Blue = CH/CH_3 | Red = CH_2.



α/β-288

ca. 2:3 α/β

EDH-e-0908_C1F8-18 ("Coupled" HSQC, 600 MHz, CDCl3, 3 mm)




```
Current Data Parameters
NAME          EDH-e-0908
EXPNO                  6
PROCNO                 1

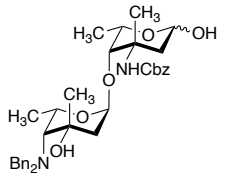
F2 - Acquisition Parameters
Date_           20200204
Time              19.56 h
INSTRUM             spect
PROBHD    Z44896_0077 (C
PULPROG  hsqcedetgpsisp.2
TD                  4096
SOLVENT             CDCl3
NS                     2
DS                    16
SWH           1200.384 Hz
FIDRES          0.586125 Hz
AQ              1.7061205 sec
RG                   1820
DW                416.533 usec
DE                 10.00 usec
TE                298.0 K
CNST2         145.0000000
CNST17         -0.5000000
D0              0.00000300 sec
D1              1.50000000 sec
D4              0.00172414 sec
D11             0.03000000 sec
D16             0.00020000 sec
D21             0.00344828 sec
D24             0.00086207 sec
IN0             0.00001840 sec
TDav                   1
SFO1          600.1330367 MHz
NUC1                  1H
P1                  9.25 usec
P2                 18.50 usec
P28         0 usec
PLW1         6.35610008 W
SFO2         150.9173862 MHz
NUC2                 13C
CPDPRG[2             garp4
P3                 15.00 usec
P14               500.00 usec
P24              2000.00 usec
PCPD2              60.00 usec
PLW0       0 W
PLW2       178.91999817 W
PLW12      0 W
SPNAM[3] Crp60,0.5,20.1
SPOAL3               0.500
SPOFFS3    0 Hz
SPW3         61.50899887 W
SPNAM[7]     Crp60comp.4
SPOAL7               0.500
SPOFFS7    0 Hz
SPW7         61.50899887 W
GPNAM[1]       SMSQ10.100
GPZ1                80.00 %
GPNAM[2]       SMSQ10.100
GPZ2                20.10 %
P16               1000.00 usec

F1 - Acquisition parameters
TD                   128
SFO1            150.9174 MHz
FIDRES         424.592377 Hz
SW              180.058 ppm
FnMODE     Echo-Antiecho

F2 - Processing parameters
SI                  1024
SF            600.1300144 MHz
WDW                 QSINE
SSB                    2
LB          0 Hz
GB          0
PC                  1.40

F1 - Processing parameters
SI                  1024
MC2         echo-antiecho
SF             150.9027929 MHz
WDW                 QSINE
SSB                    2
LB          0 Hz
GB          0
```

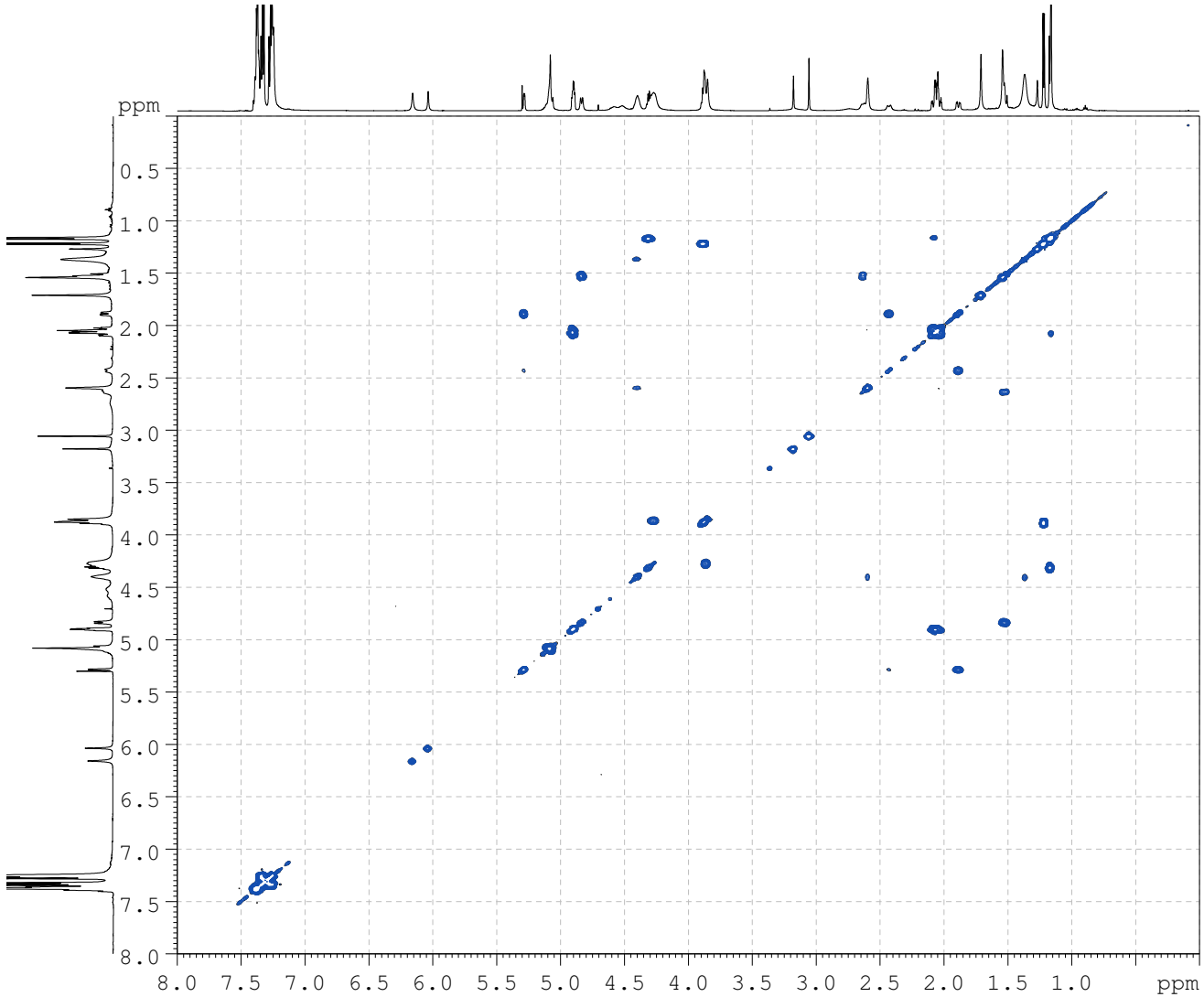
^{1}H-^{1}H COSY NMR of Compound 288 (ca. 2:3 α/β) (600 MHz, $CDCl_3$)



α/β-288

ca. 2:3 α/β

EDH-e-0908_C1F8-18 (COSY, 600 MHz, CDCl3, 3 mm, NS = 1 scan)




```
Current Data Parameters
NAME          EDH-e-0908
EXPNO                  5
PROCNO                 1

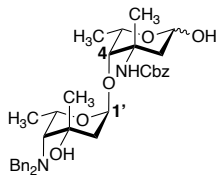
F2 - Acquisition Parameters
Date_           20200204
Time              19.40 h
INSTRUM             spect
PROBHD    Z44896_0077 (C
PULPROG          cosygpqf
TD                 2048
SOLVENT            CDCl3
NS                    1
DS                    8
SWH            7836.991 Hz
FIDRES         7.653311 Hz
AQ             0.1306624 sec
RG                  161
DW                63.800 usec
DE                10.00 usec
TE                298.0 K
D0           0.00000300 sec
D1           1.48689198 sec
D13          0.00000400 sec
D16          0.00020000 sec
IN0          0.00012760 sec
TDav                  1
SFO1       600.1330006 MHz
NUC1                1H
P0                 4.65 usec
P1                 9.30 usec
PLW1          6.35610008 W
GPNAM[1]      SMSQ10.100
GPZ1              10.00 %
P16             1000.00 usec

F1 - Acquisition parameters
TD                  512
SFO1         600.133 MHz
FIDRES       30.613245 Hz
SW            13.059 ppm
FnMODE               QF

F2 - Processing parameters
SI                 1024
SF         600.1300095 MHz
WDW              QSINE
SSB       0
LB        0 Hz
GB        0
PC                1.40

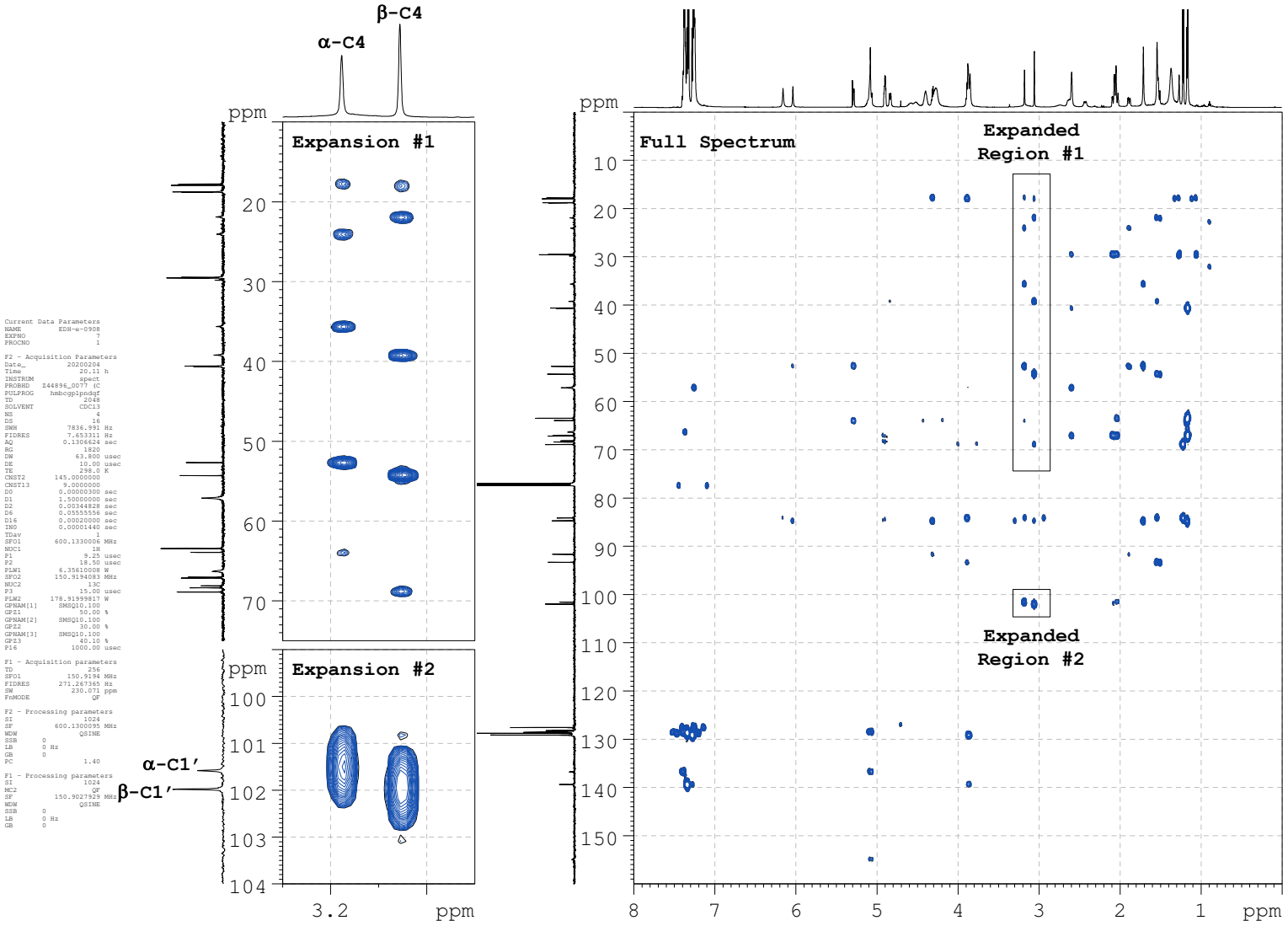
F1 - Processing parameters
SI                 1024
MC2                  QF
SF         600.1300095 MHz
WDW              QSINE
SSB       0
LB        0 Hz
GB        0
```

^{1}H-^{13}C HMBC NMR of Compound 288 (ca. 2:3 α/β) (^{1}H-600 MHz, ^{13}C-151 MHz, $CDCl_3$)



α/β-288

ca. 2:3 α/β



Current Data Parameters
NAME EDH-e-0908
EXPNO 7
PROCNO 1

F2 - Acquisition Parameters
Date_ 20200204
Time 20.11 h
INSTRUM spect
PROBHD Z44896_0077 (C
PULPROG hmbcgplpndqf
TD 2048
SOLVENT CDCl3
NS 4
DS 16
SWH 7836.991 Hz
FIDRES 7.653311 Hz
AQ 0.1306624 sec
RG 1820
DW 63.800 usec
DE 10.00 usec
TE 298.0 K
CNST2 145.0000000
CNST13 9.0000000
D0 0.00000300 sec
D1 1.50000000 sec
D2 0.00344828 sec
D6 0.05555556 sec
D16 0.00020000 sec
IN0 0.00001440 sec
TDav 1
SFO1 600.1330006 MHz
NUC1 1H
P1 9.25 usec
P2 18.50 usec
PLW1 6.35610008 W
SFO2 150.9194083 MHz
NUC2 13C
P3 15.00 usec
PLW2 178.91999817 W
GPNAM[1] SMSQ10.100
GPZ1 50.00 %
GPNAM[2] SMSQ10.100
GPZ2 30.00 %
GPNAM[3] SMSQ10.100
GPZ3 40.10 %
P16 1000.00 usec

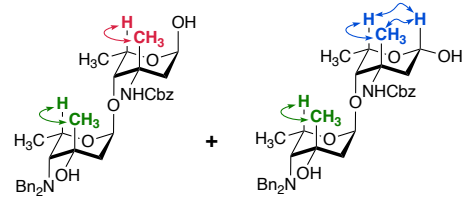
F1 - Acquisition parameters
TD 256
SFO1 150.9194 MHz
FIDRES 271.267365 Hz
SW 230.071 ppm
FnMODE QF

F2 - Processing parameters
SI 1024
SF 600.1300095 MHz
WDW QSINE
SSB 0
LB 0 Hz
GB 0
PC 1.40

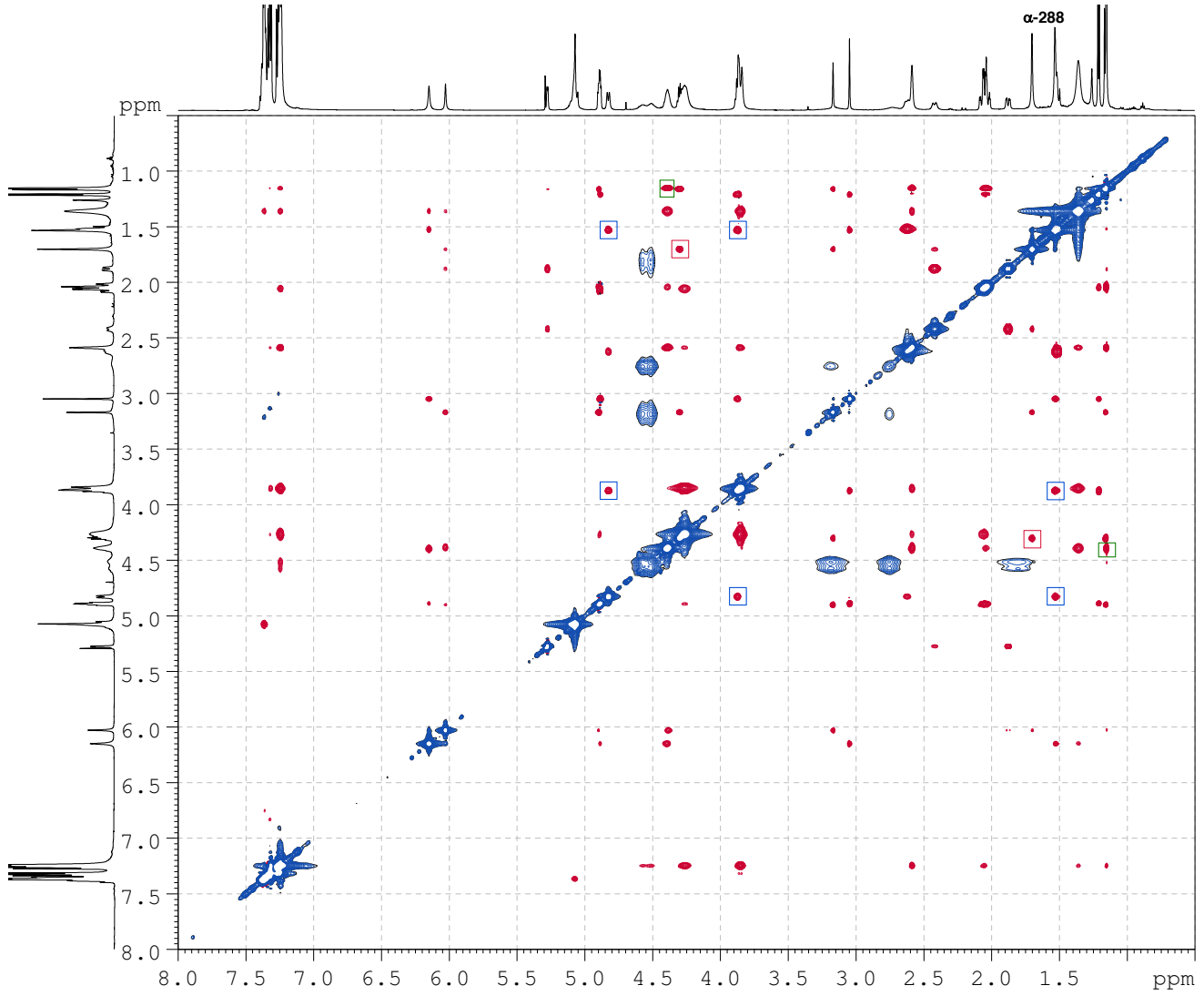
F1 - Processing parameters
SI 1024
MC2 QF
SF 150.9027929 MHz
WDW QSINE
SSB 0
LB 0 Hz
GB 0

^{1}H-^{1}H NOESY NMR of Compound 288 (ca. 2:3 α/β) (600 MHz, $CDCl_3$)

Note: Blue = diagonal/chemical exchange | Red = NOESY cross peaks.



EDH-e-0908_C1F8-18 (NOESY, 600 MHz, CDCl3, 3 mm, NS = 4 scans)




```
Current Data Parameters
NAME          EDH-e-0908
EXPNO                  8
PROCNO                 1

F2 - Acquisition Parameters
Date_           20200204
Time              20.41 h
INSTRUM             spect
PROBHD    Z44896_0077 (C
PULPROG         noesygpph
TD                  2048
SOLVENT            CDCl3
NS                     4
DS                    16
SWH             7836.991 Hz
FIDRES          7.653311 Hz
AQ              0.1306624 sec
RG                   128
DW                63.800 usec
DE                10.00 usec
TE                298.0 K
D0            0.00005202 sec
D1            1.48689198 sec
D8            0.60000002 sec
D16           0.00020000 sec
IN0           0.00012760 sec
TDav                   1
SFO1          600.1330006 MHz
NUC1                  1H
P1                 9.25 usec
P2                18.50 usec
PLW1           6.35610008 W
GPNAM[1]       SMSQ10.100
GPZ1                40.00 %
P16               1000.00 usec

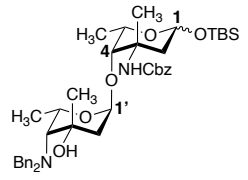
F1 - Acquisition parameters
TD                   512
SFO1           600.133 MHz
FIDRES        30.613245 Hz
SW                13.059 ppm
FnMODE       States-TPPI

F2 - Processing parameters
SI                   1024
SF            600.1300144 MHz
WDW                  QSINE
SSB                     2
LB         0 Hz
GB         0
PC                   1.40

F1 - Processing parameters
SI                   1024
MC2          States-TPPI
SF            600.1300144 MHz
WDW                  QSINE
SSB                     2
LB         0 Hz
GB         0
```

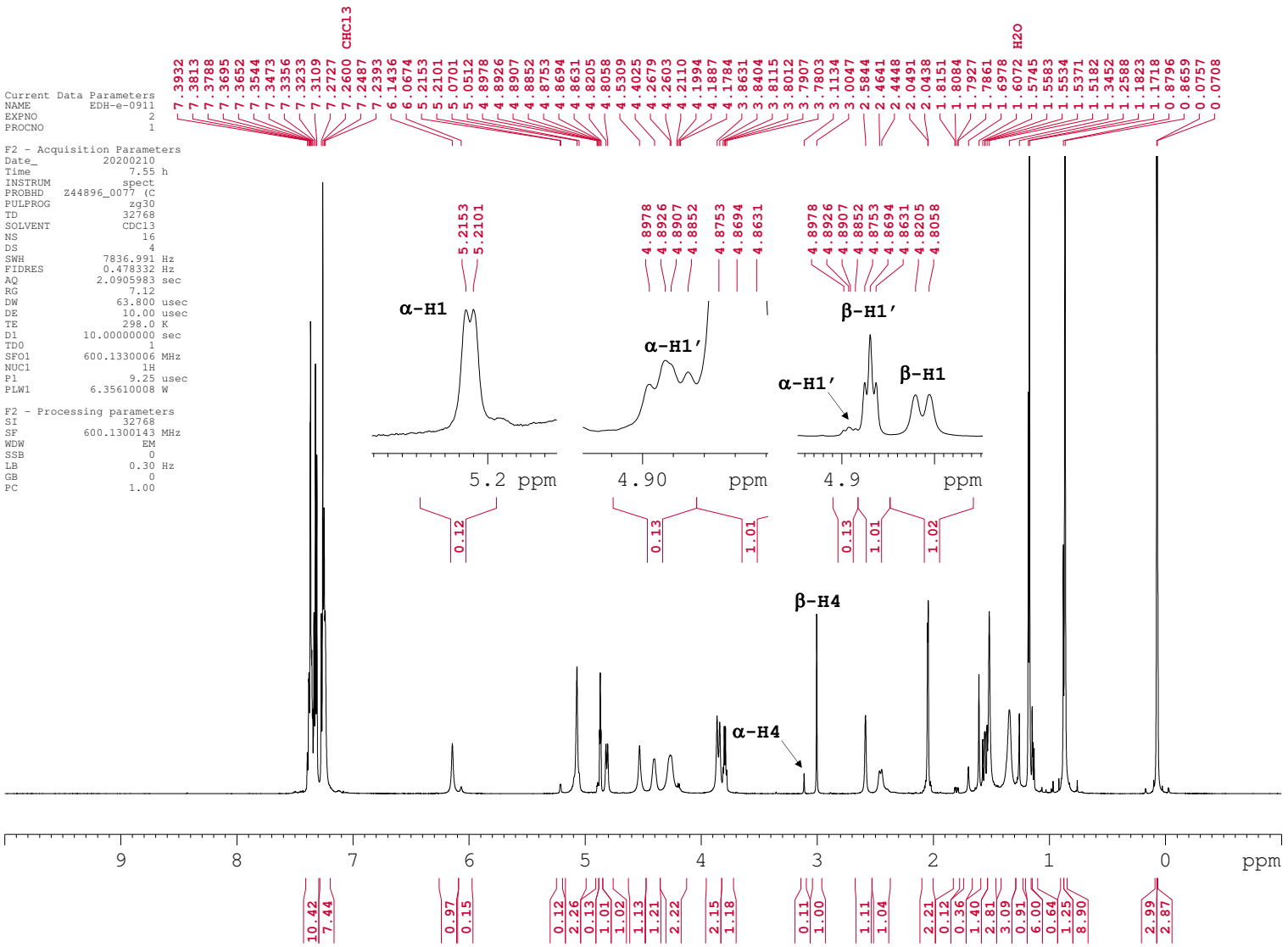
^{1}H NMR of Compound 289 (ca. 1:9 α/β) (600 MHz, $CDCl_3$)

Note: Spectrum of compound **289** from experimental procedure.



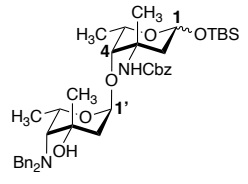
α/β-289

ca. 1:9 α/β



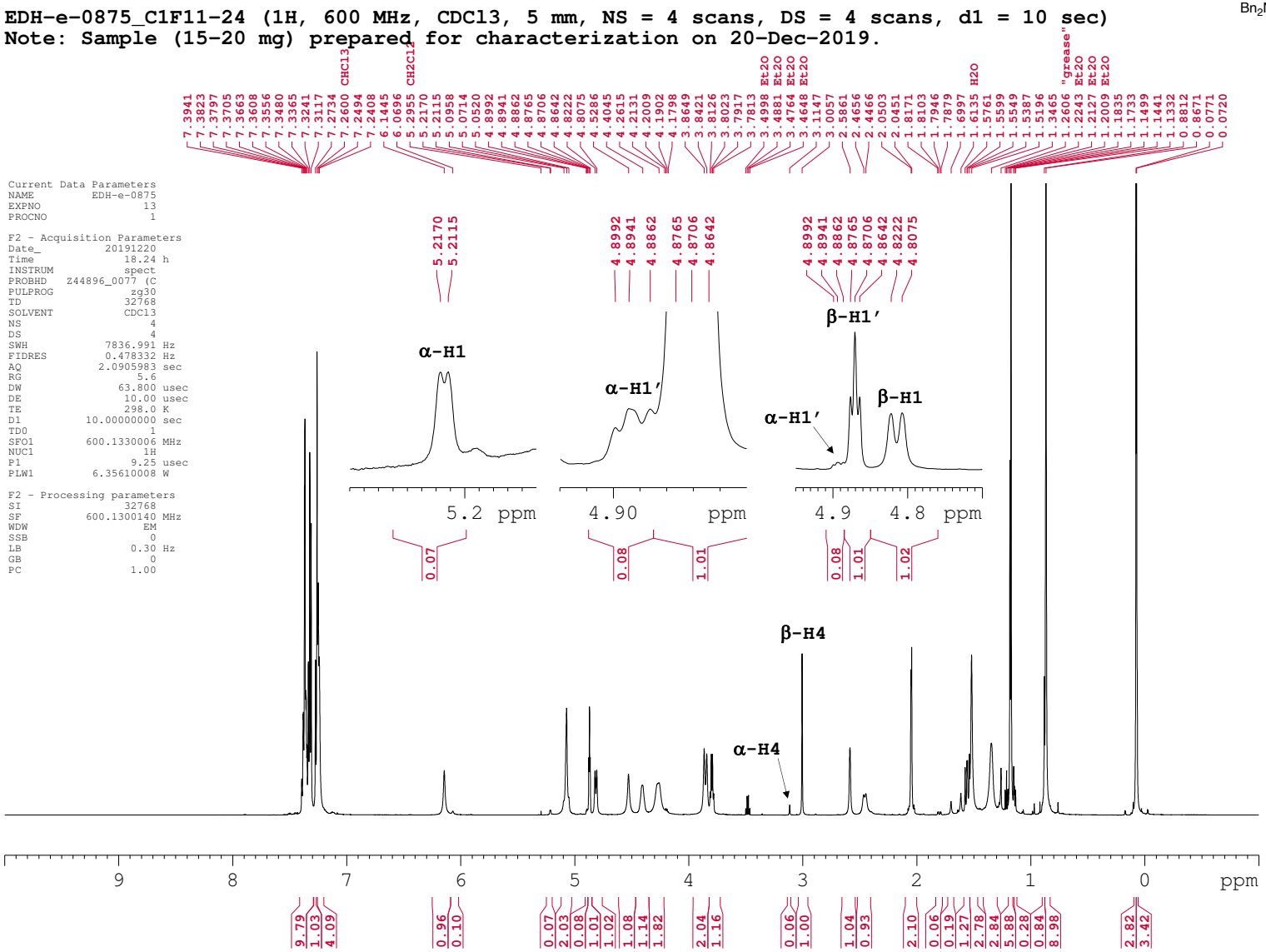
^{1}H NMR of Compound 289 (≥ 10:1 β/α) (600 MHz, $CDCl_3$)

Note: Spectrum of compound **289** used for ^{1}H NMR line listing and full NMR characterization.

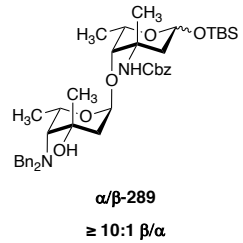


α/β-289

≥ 10:1 β/α



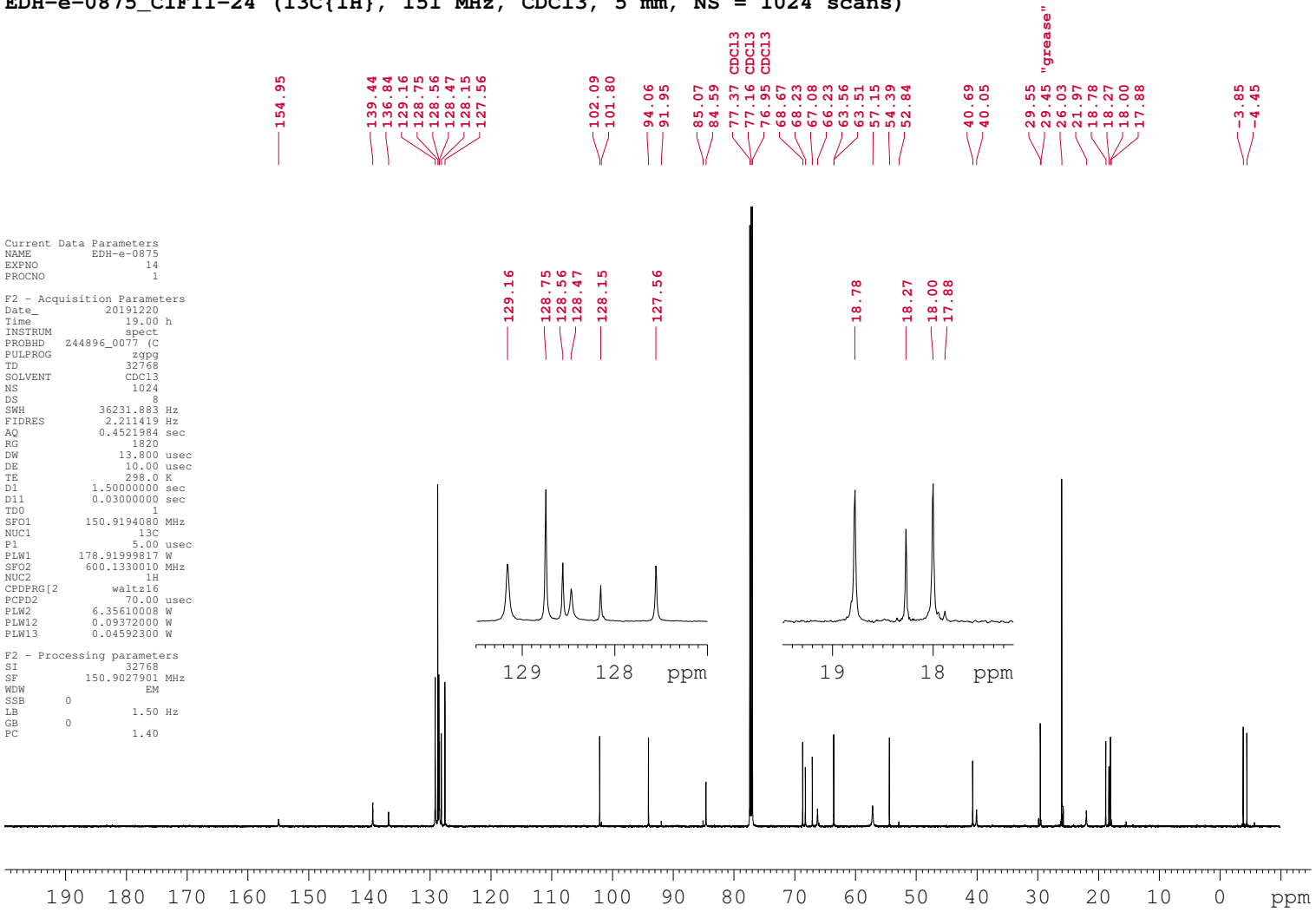
$^{13}C\{^{1}H\}$ NMR of Compound 289 (≥ 10:1 β/α) (151 MHz, $CDCl_3$)



α/β-289

≥ 10:1 β/α

EDH-e-0875_C1F11-24 (13C{1H}, 151 MHz, CDCl3, 5 mm, NS = 1024 scans)



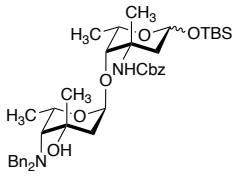

```
Current Data Parameters
NAME         EDH-e-0875
EXPNO                14
PROCNO                1

F2 - Acquisition Parameters
Date_          20191220
Time               19.00 h
INSTRUM            spect
PROBHD   Z44896_0077 (C
PULPROG            zgpg
TD                32768
SOLVENT           CDCl3
NS                 1024
DS                    8
SWH            36231.883 Hz
FIDRES          2.211419 Hz
AQ             0.4521984 sec
RG                 1820
DW                13.800 usec
DE                10.00 usec
TE                298.0 K
D1           1.50000000 sec
D11          0.03000000 sec
TD0                   1
SFO1        150.9194080 MHz
NUC1                13C
P1                 5.00 usec
PLW1       178.91999817 W
SFO2        600.1330010 MHz
NUC2                1H
CPDPRG[2         waltz16
PCPD2             70.00 usec
PLW2          6.35610008 W
PLW12         0.09372000 W
PLW13         0.04592300 W

F2 - Processing parameters
SI                32768
SF          150.9027901 MHz
WDW                EM
SSB       0
LB                1.50 Hz
GB        0
PC                1.40
```

^{1}H-^{13}C HSQC{^{1}H}{^{13}C} NMR of Compound 289 (≥ 10:1 β/α) (^{1}H-600 MHz, ^{13}C-151 MHz, $CDCl_3$)

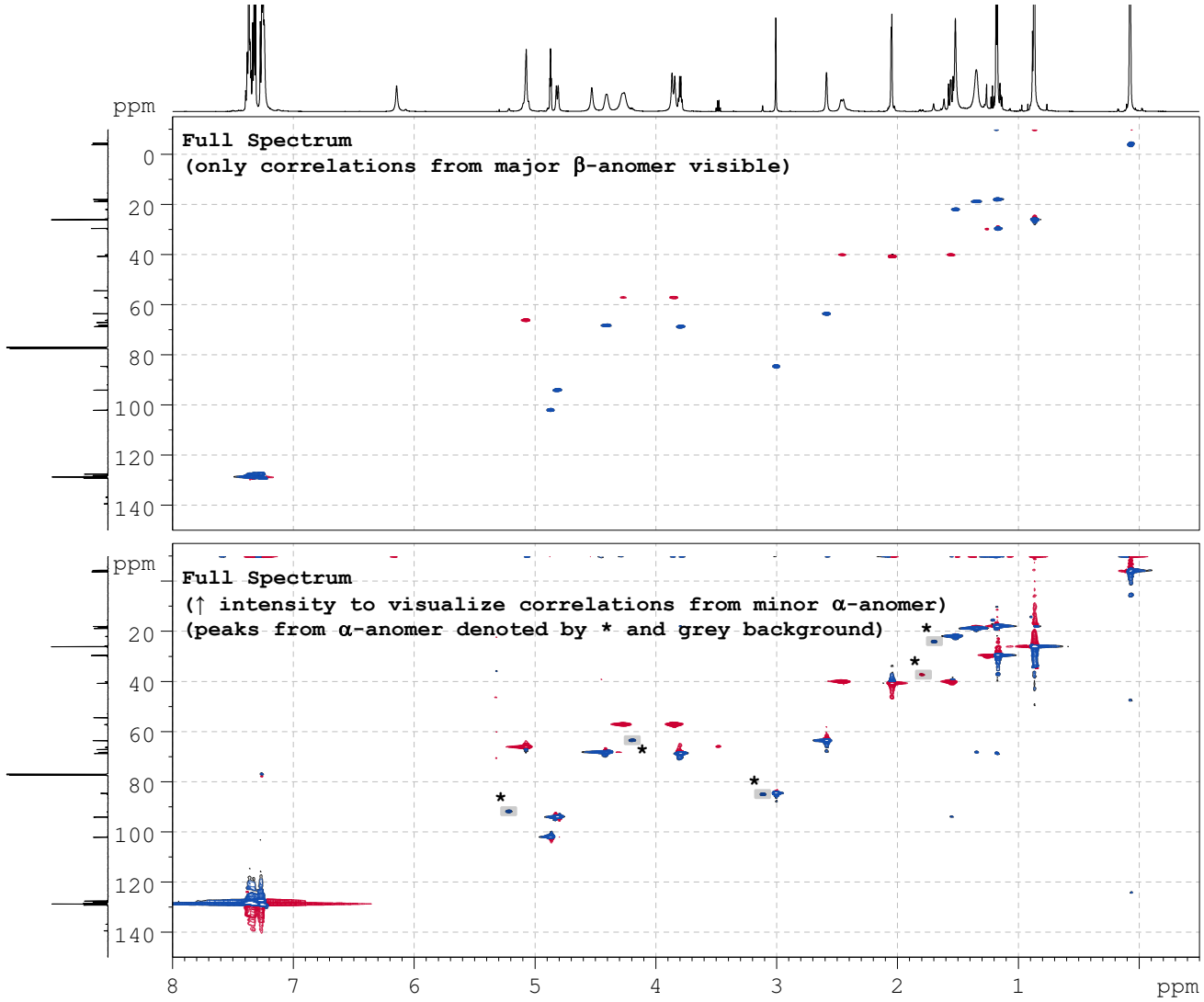
Note: Blue = CH/CH_3 | Red = CH_2.



α/β-289

≥ 10:1 β/α

EDH-e-0875_C1F11-24 (HSQC{1H}{13C}, 600 MHz, CDCl3, 5 mm, NS = 1 scan, O2P = 80 ppm)




```
Current Data Parameters
NAME          EDH-e-0875
EXPNO                 12
PROCNO                 1

F2 - Acquisition Parameters
Date_           20191220
Time              15.34 h
INSTRUM             spect
PROBHD    Z44896_0077 (C
PULPROG  hsqcedetgpsisp.2
TD                  1024
SOLVENT             CDCl3
NS                     1
DS                    16
SWH             7836.991 Hz
FIDRES         15.306623 Hz
AQ              0.0653312 sec
RG                   1820
DW                 63.800 usec
DE                 10.00 usec
TE                298.0 K
CNST2         145.0000000
CNST17         -0.5000000
D0              0.00000300 sec
D1              1.50000000 sec
D4              0.00172414 sec
D11             0.03000000 sec
D16             0.00020000 sec
D21             0.00344828 sec
D24             0.00086207 sec
IN0             0.00001840 sec
TDav                   1
SFO1          600.1330006 MHz
NUC1                  1H
P1                  9.25 usec
P2                 18.50 usec
P28         0 usec
PLW1          6.35610008 W
SFO2        150.9148812 MHz
NUC2                  13C
CPDPRG[2            garp4
P3                 15.00 usec
P14               500.00 usec
P24              2000.00 usec
PCPD2              60.00 usec
PLW0       0 W
PLW2         178.91999817 W
PLW12         11.18279961 W
SPNAM[3] Crp60,0.5,20.1
SPOAL3              0.500
SPOFFS3   0 Hz
SPW3          61.50899887 W
SPNAM[7]     Crp60comp.4
SPOAL7              0.500
SPOFFS7   0 Hz
SPW7          61.50899887 W
GPNAM[1]      SMSQ10.100
GPZ1               80.00 %
GPNAM[2]      SMSQ10.100
GPZ2               20.10 %
P16              1000.00 usec

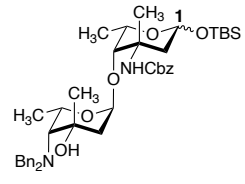
F1 - Acquisition parameters
TD                   256
SFO1        150.9149 MHz
FIDRES       212.296188 Hz
SW           180.061 ppm
FnMODE    Echo-Antiecho

F2 - Processing parameters
SI                  1024
SF          600.1300140 MHz
WDW                 QSINE
SSB                    2
LB          0 Hz
GB          0
PC                  1.40

F1 - Processing parameters
SI                  1024
MC2        echo-antiecho
SF          150.9027901 MHz
WDW                 QSINE
SSB                    2
LB          0 Hz
GB          0
```

Coupled ^{1}H-^{13}C HSQC NMR of Compound 289 (≥ 10:1 β/α) (^{1}H-600 MHz, ^{13}C-151 MHz, $CDCl_3$)

Note: Blue = CH/CH_3 | Red = CH_2.



α/β-289

≥ 10:1 β/α

EDH-e-0875_C1F11-24 ("Coupled" HSQC, 600 MHz, CDCl3, 5 mm)

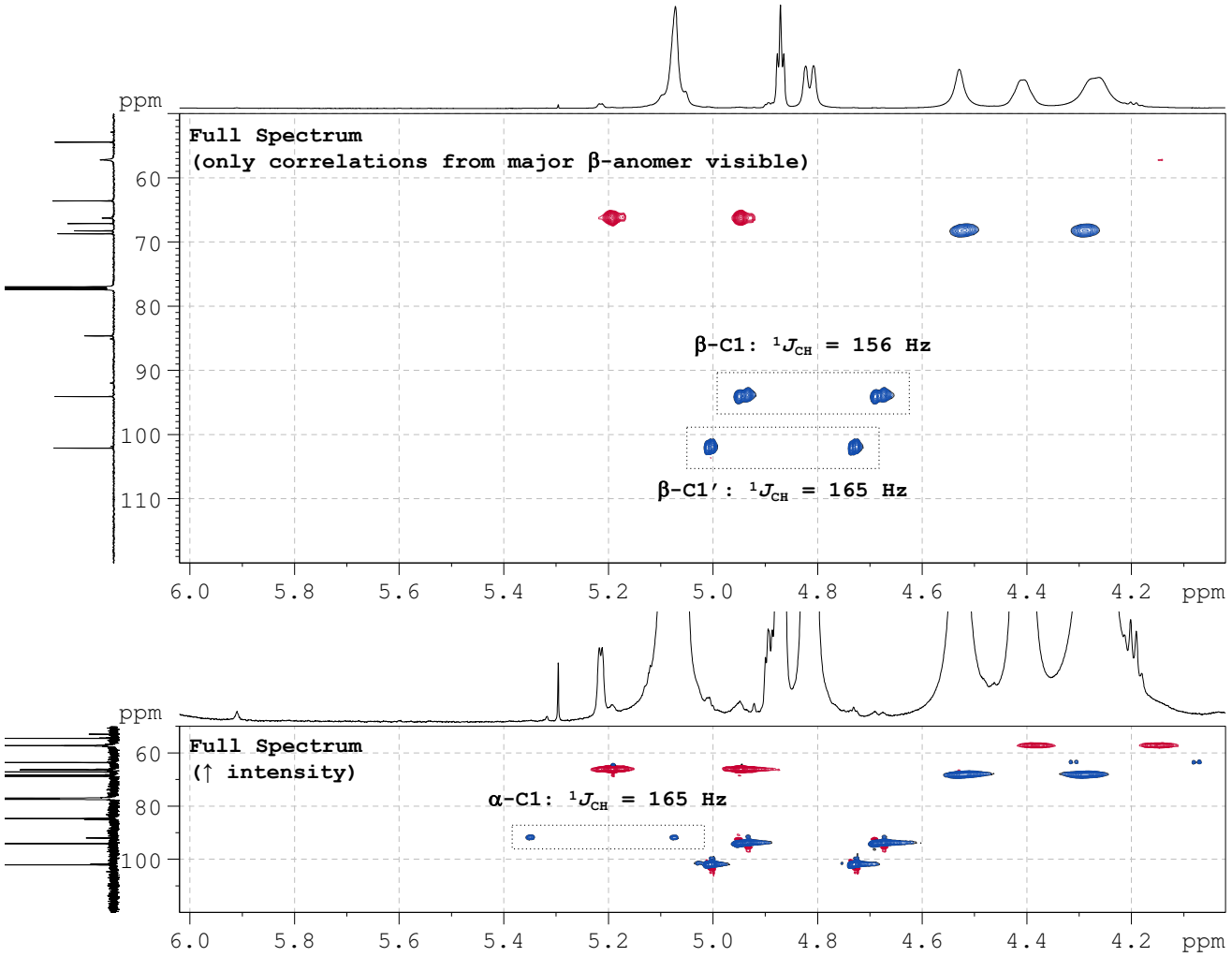
Current Data Parameters
NAME EDH-e-0875
EXPNO 17
PROCNO 1

F2 - Acquisition Parameters
Date_ 20191220
Time 19.48 h
INSTRUM spect
PROBHD Z44896_0077 (C
PULPROG hsqcedetgpsisp.2
TD 4096
SOLVENT CDCl3
NS 2
DS 16
SWH 1200.384 Hz
FIDRES 0.586125 Hz
AQ 1.7061205 sec
RG 1820
DW 416.533 usec
DE 10.00 usec
TE 298.0 K
CNST2 145.0000000
CNST17 -0.5000000
D0 0.00000300 sec
D1 1.50000000 sec
D4 0.00172414 sec
D11 0.03000000 sec
D16 0.00020000 sec
D21 0.00344828 sec
D24 0.00086207 sec
IN0 0.00001840 sec
TDav 1
SFO1 600.1330127 MHz
NUC1 1H
P1 9.25 usec
P2 18.50 usec
P28 0 usec
PLW1 6.35610008 W
SFO2 150.9174315 MHz
NUC2 13C
CPDPRG[2 garp4
P3 15.00 usec
P14 500.00 usec
P24 2000.00 usec
PCPD2 60.00 usec
PLW0 0 W
PLW2 178.91999817 W
PLW12 0 W
SPNAM[3] Crp60,0.5,20.1
SPOAL3 0.500
SPOFFS3 0 Hz
SPW3 61.50899887 W
SPNAM[7] Crp60comp.4
SPOAL7 0.500
SPOFF37 0 Hz
SPW7 61.50899887 W
GPNAM[1] SMSQ10.100
GPZ1 80.00 %
GPNAM[2] SMSQ10.100
GPZ2 20.10 %
P16 1000.00 usec

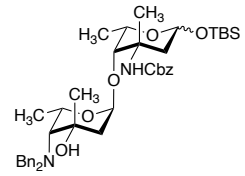
F1 - Acquisition parameters
TD 128
SFO1 150.9174 MHz
FIDRES 424.592377 Hz
SW 180.058 ppm
FnMODE Echo-Antiecho

F2 - Processing parameters
SI 1024
SF 600.1300140 MHz
WDW QSINE
SSB 2
LB 0 Hz
GB 0
PC 1.40

F1 - Processing parameters
SI 1024
MC2 echo-antiecho
SF 150.9027901 MHz
WDW QSINE
SSB 2
LB 0 Hz
GB 0



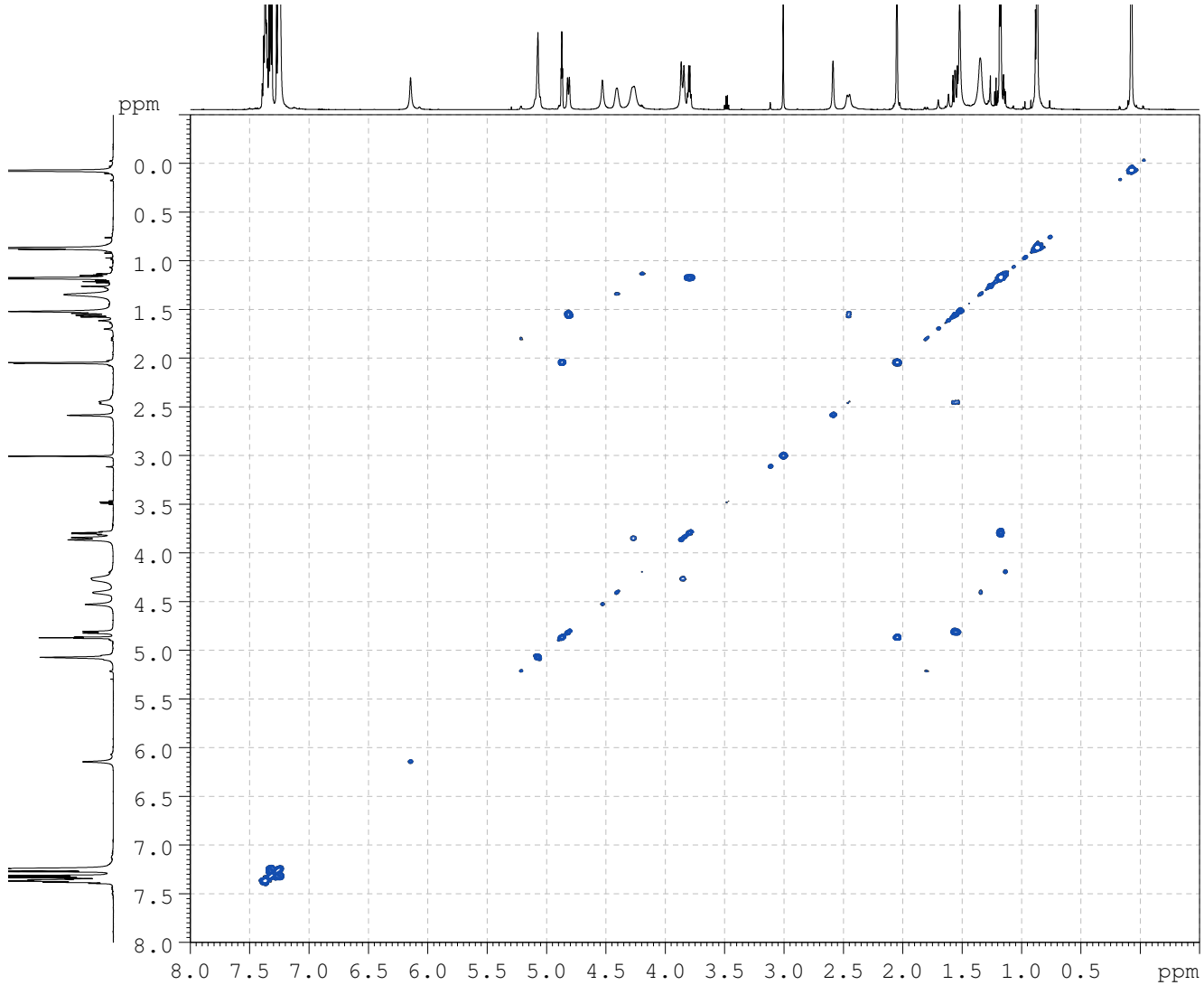
^{1}H-^{1}H COSY NMR of Compound 289 (≥ 10:1 β/α) (600 MHz, $CDCl_3$)



α/β-289

≥ 10:1 β/α

EDH-e-0875_C1F11-24 (COSY, 600 MHz, CDCl3, 5 mm, NS = 1 scan)




```
Current Data Parameters
NAME          EDH-e-0875
EXPNO                 15
PROCNO                 1

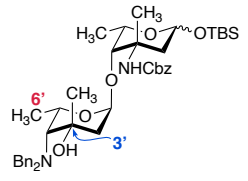
F2 - Acquisition Parameters
Date_          20191220
Time              19.01 h
INSTRUM             spect
PROBHD    Z44896_0077 (C
PULPROG          cosygpqf
TD                 2048
SOLVENT           CDCl3
NS                    1
DS                    8
SWH            7836.991 Hz
FIDRES         7.653311 Hz
AQ             0.1306624 sec
RG                 71.8
DW               63.800 usec
DE               10.00 usec
TE                298.0 K
D0           0.00000300 sec
D1           1.48689198 sec
D13          0.00000400 sec
D16          0.00020000 sec
IN0          0.00012760 sec
TDav                  1
SFO1       600.1330006 MHz
NUC1                 1H
P0                4.65 usec
P1                9.30 usec
PLW1         6.35610008 W
GPNAM[1]     SMSQ10.100
GPZ1             10.00 %
P16            1000.00 usec

F1 - Acquisition parameters
TD                  512
SFO1       600.133 MHz
FIDRES       30.61245 Hz
SW            13.059 ppm
FnMODE              QF

F2 - Processing parameters
SI                 1024
SF         600.1300140 MHz
WDW              QSINE
SSB       0
LB        0 Hz
GB        0
PC                 1.40

F1 - Processing parameters
SI                 1024
MC2                QF
SF         600.1300140 MHz
WDW              QSINE
SSB       0
LB        0 Hz
GB        0
```

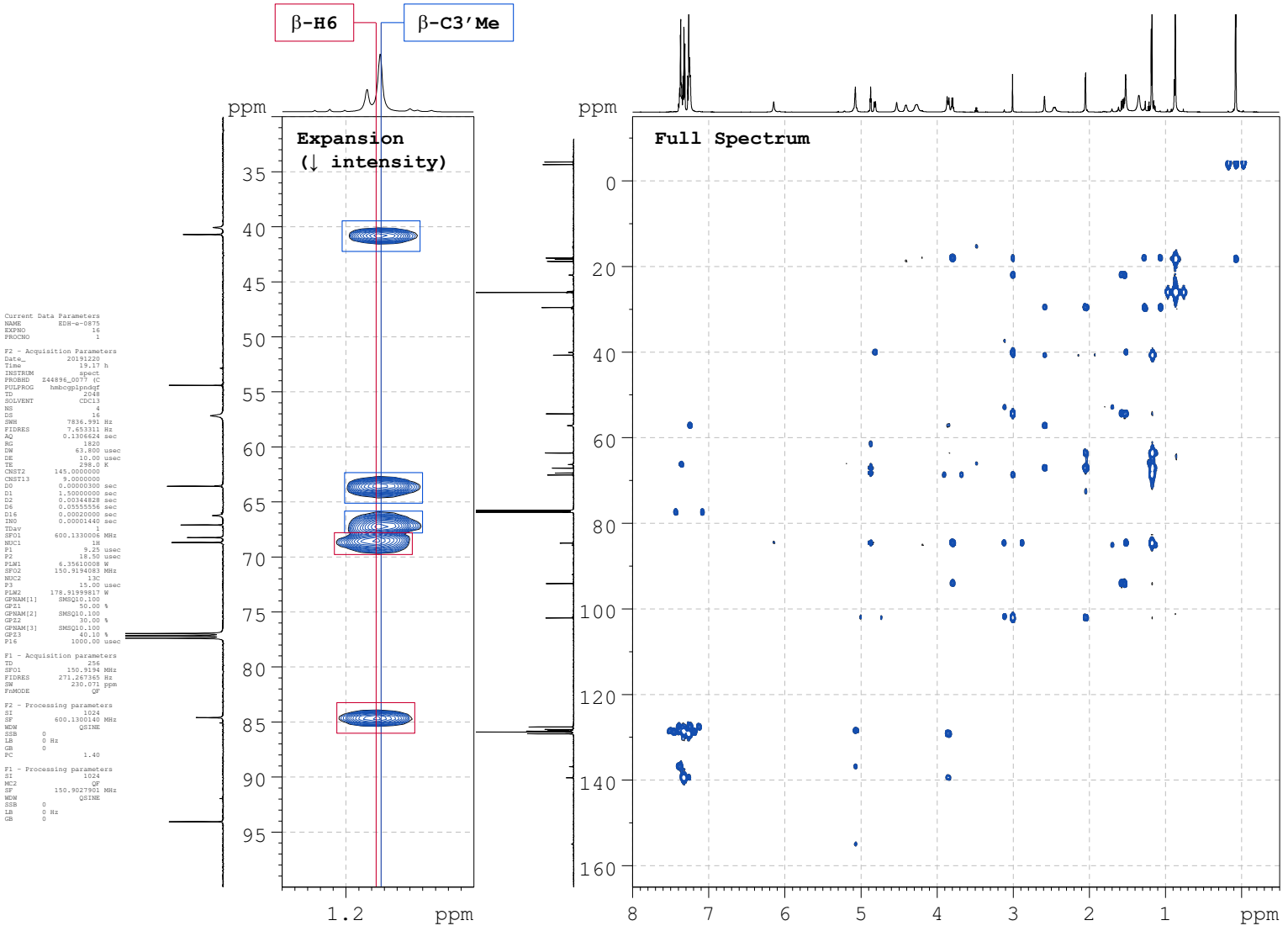
^{1}H-^{13}C HMBC NMR of Compound 289 (≥ 10:1 β/α) (^{1}H-600 MHz, ^{13}C-151 MHz, $CDCl_3$)



α/β-289

≥ 10:1 β/α

EDH-e-0875_C1F11-24 (HMBC, 600 MHz, CDCl3, 5 mm, NS = 4 scans)



Current Data Parameters
NAME EDH-e-0875
EXPNO 16
PROCNO 1

F2 - Acquisition Parameters
Date_ 20191220
Time 19.17 h
INSTRUM spect
PROBHD Z44896_0077 (C
PULPROG hmbcgplpndqf
TD 2048
SOLVENT CDCl3
NS 4
DS 16
SWH 7836.991 Hz
FIDRES 7.653311 Hz
AQ 0.1306624 sec
RG 1820
DW 63.800 usec
DE 10.00 usec
TE 298.0 K
CNST2 145.0000000
CNST13 9.0000000
D0 0.00000300 sec
D1 1.50000000 sec
D2 0.00344828 sec
D6 0.05555556 sec
D16 0.00020000 sec
IN0 0.00001440 sec
TDav 1
SFO1 600.1330006 MHz
NUC1 1H
P1 9.25 usec
P2 18.50 usec
PLW1 6.35610008 W
SFO2 150.9194083 MHz
NUC2 13C
P3 15.00 usec
PLW2 178.91999817 W
GPNAM[1] SMSQ10.100
GPZ1 50.00 %
GPNAM[2] SMSQ10.100
GPZ2 30.00 %
GPNAM[3] SMSQ10.100
GPZ3 40.10 %
P16 1000.00 usec

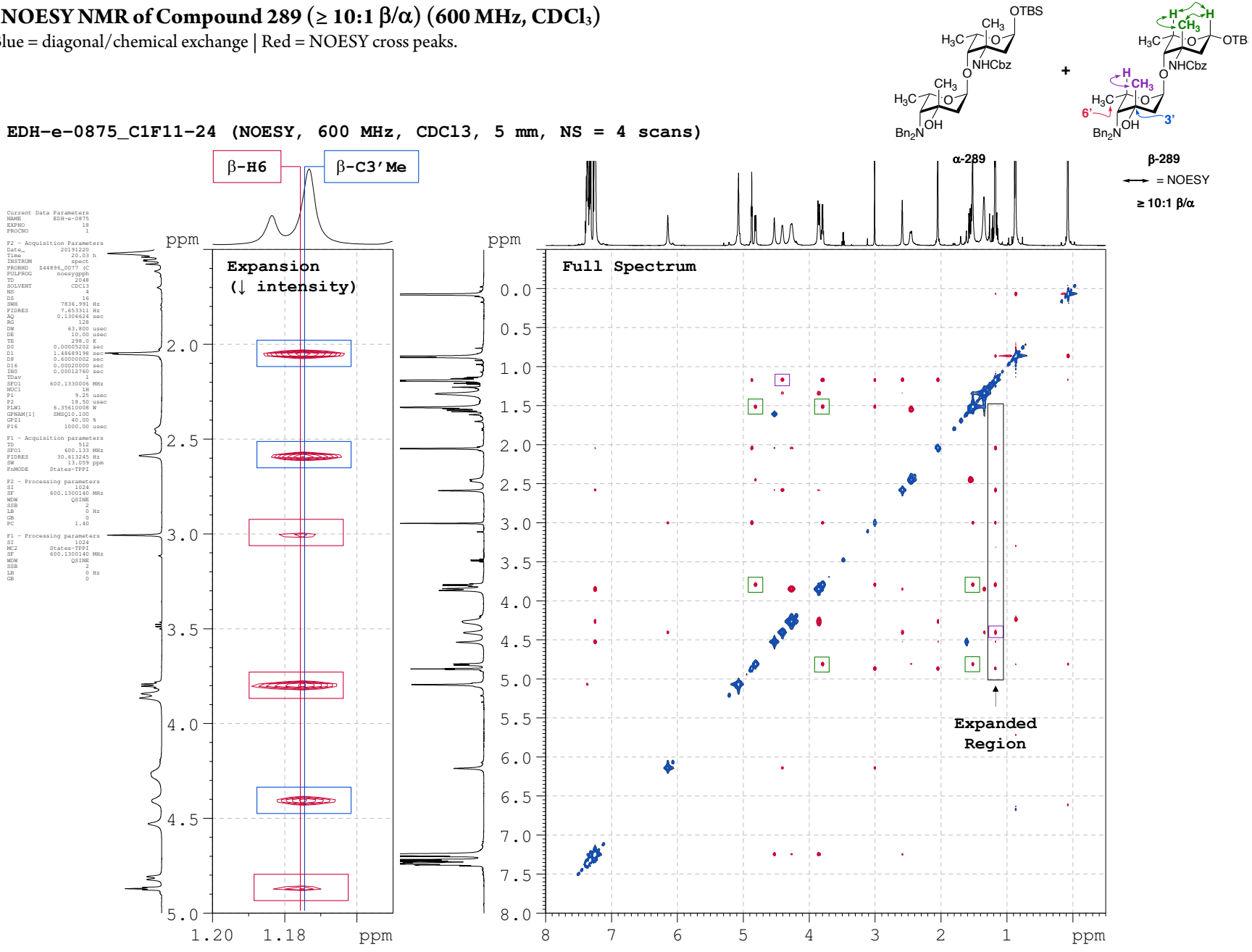
F1 - Acquisition parameters
TD 256
SFO1 150.9194 MHz
FIDRES 271.267365 Hz
SW 230.071 ppm
FnMODE QF

F2 - Processing parameters
SI 1024
SF 600.1300140 MHz
WDW QSINE
SSB 0
LB 0 Hz
GB 0
PC 1.40

F1 - Processing parameters
SI 1024
MC2 QF
SF 150.9027901 MHz
WDW QSINE
SSB 0
LB 0 Hz
GB 0

^{1}H-^{1}H NOESY NMR of Compound 289 (≥ 10:1 β/α) (600 MHz, $CDCl_3$)

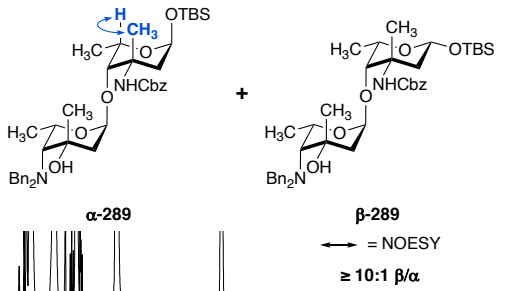
Note: Blue = diagonal/chemical exchange | Red = NOESY cross peaks.



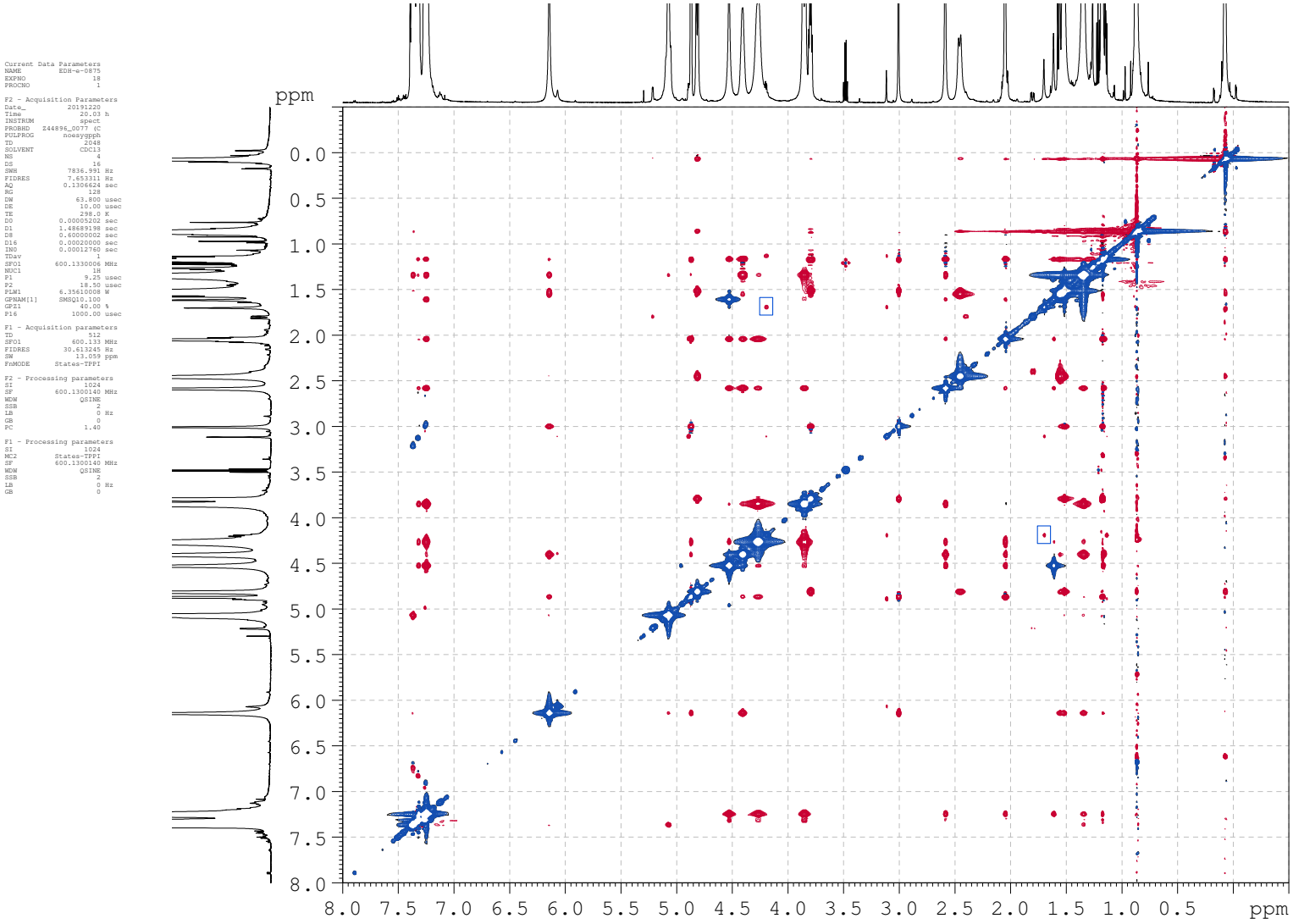
^{1}H-^{1}H NOESY NMR of Compound 289 (≥ 10:1 β/α) (600 MHz, $CDCl_3$)

Note 1: Blue = diagonal/chemical exchange | Red = NOESY cross peaks.

Note 2: Same spectrum with increased intensity as compared to NOESY on page S351.



EDH-e-0875_C1F11-24 (NOESY, 600 MHz, CDCl3, 5 mm, NS = 4 scans)



Current Data Parameters
NAME EDH-e-0875
EXPNO 18
PROCNO 1

F2 - Acquisition Parameters
Date_ 20191220
Time 20.03 h
INSTRUM spect
PROBHD Z44896_0077 (C
PULPROG noesygpph
TD 2048
SOLVENT CDCl3
NS 4
DS 16
SWH 7836.991 Hz
FIDRES 7.653311 Hz
AQ 0.1306624 sec
RG 128
DW 63.800 usec
DE 10.00 usec
TE 298.0 K
D0 0.00005202 sec
D1 1.48689198 sec
D8 0.60000002 sec
D16 0.00020000 sec
IN0 0.00012760 sec
TDav 1
SFO1 600.1330006 MHz
NUC1 1H
P1 9.25 usec
P2 18.50 usec
PLW1 6.35610008 W
GPNAM[1] SMSQ10.100
GPZ1 40.00 %
P16 1000.00 usec

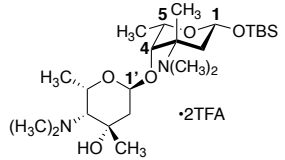
F1 - Acquisition parameters
TD 512
SFO1 600.133 MHz
FIDRES 30.613245 Hz
SW 13.059 ppm
FnMODE States-TPPI

F2 - Processing parameters
SI 1024
SF 600.1300140 MHz
WDW QSINE
SSB 2
LB 0 Hz
GB 0
PC 1.40

F1 - Processing parameters
SI 1024
MC2 States-TPPI
SF 600.1300140 MHz
WDW QSINE
SSB 2
LB 0 Hz
GB 0

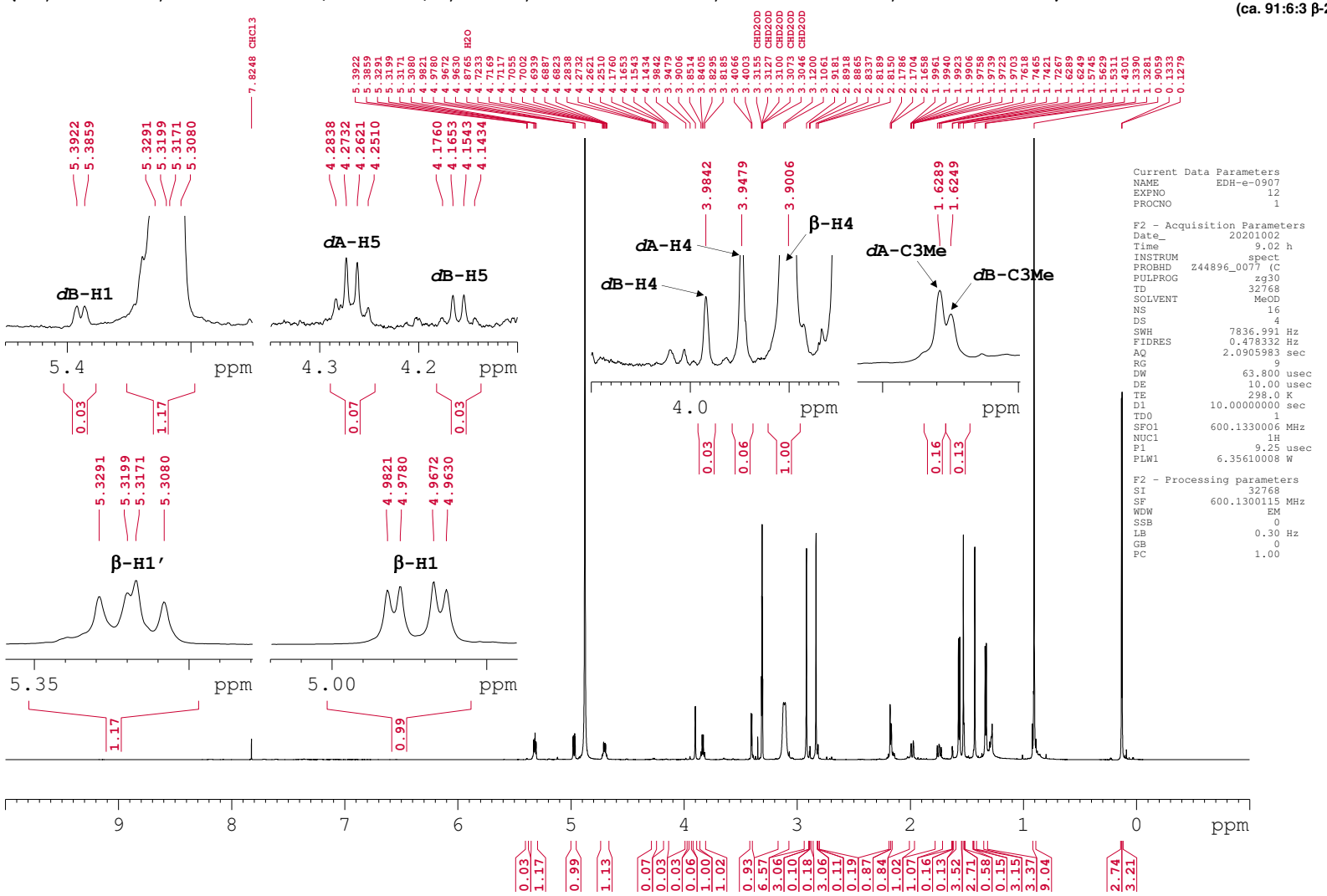
^{1}H NMR of Compound β-287•2TFA (≥ 10:1) (600 MHz, 4:1 CD3OD/$CDCl_3$ w/w + CF_3CO_2H)

Note: ***d*A** and ***d*B** are two unassigned disaccharides with structures similar to β-**287** present in the following ratio: ca. 91:6:3 β-**287**:***d*A**:***d*B**.



β-287•2TFA

(ca. 91:6:3 β-287:*d*A:*d*B)



Current Data Parameters
NAME EDH-e-0907
EXPNO 12
PROCNO 1

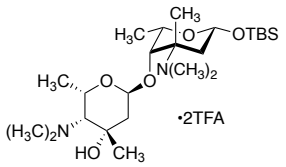
F2 - Acquisition Parameters
Date_ 20201002
Time 9.02 h
INSTRUM spect
PROBHD Z44896_0077 (C
PULPROG zg30
TD 32768
SOLVENT MeOD
NS 16
DS 4
SWH 7836.991 Hz
FIDRES 0.478332 Hz
AQ 2.0905983 sec
RG 9
DW 63.800 usec
DE 10.00 usec
TE 298.0 K
D1 10.00000000 sec
TD0 1
SFO1 600.1330006 MHz
NUC1 1H
P1 9.25 usec
PLW1 6.35610008 W

F2 - Processing parameters
SI 32768
SF 600.1300115 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

$^{13}C\{^{1}H\}$ NMR of Compound β-287•2TFA (≥ 10:1) (151 MHz, 4:1 CD3OD/$CDCl_3$ w/w + CF_3CO_2H)

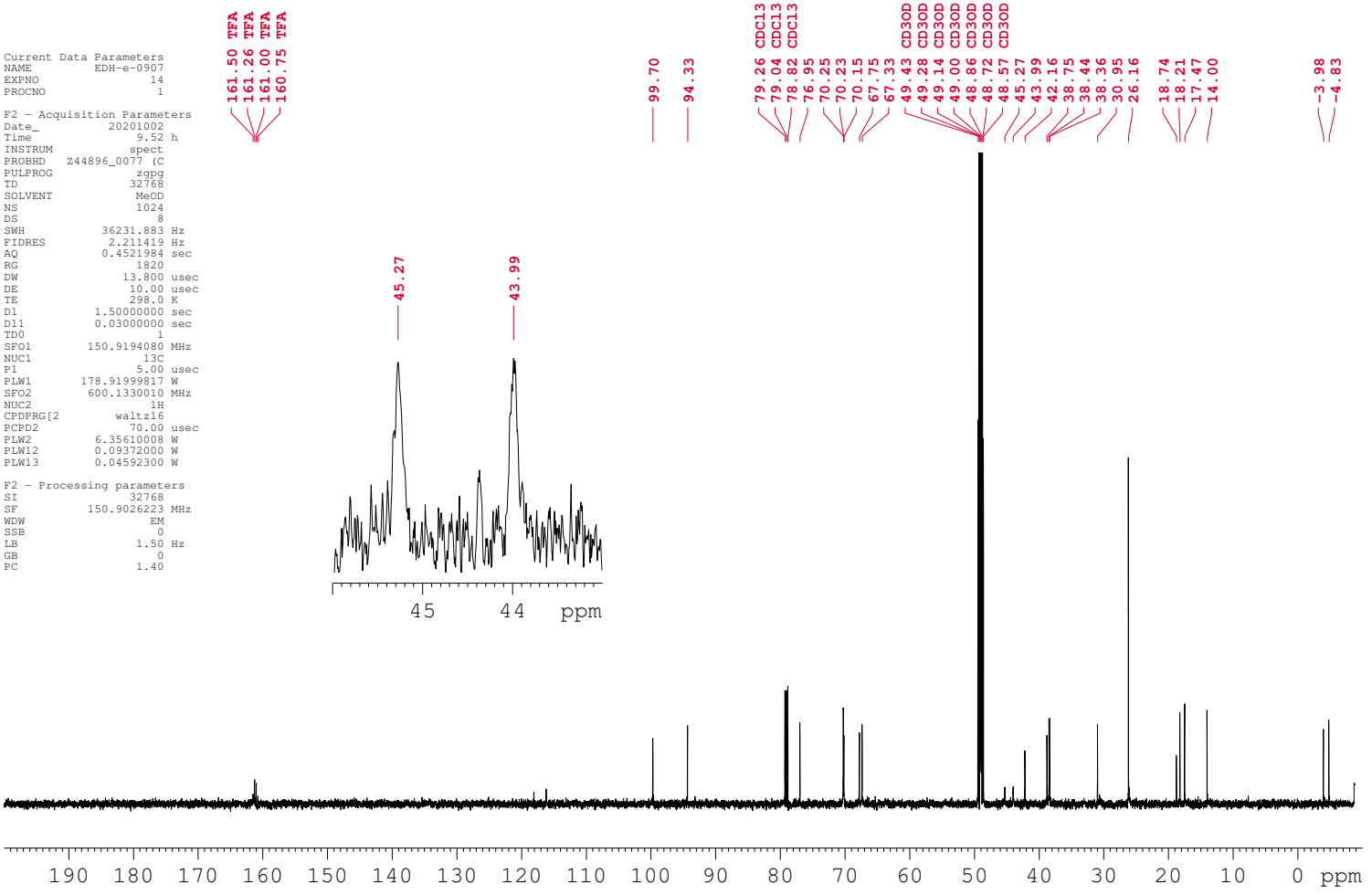
Note: ***d*A** and ***d*B** are two unassigned disaccharides with structures similar to β-**287** present in the following ratio: ca. 91:6:3 β-**287**:***d*A**:***d*B**.

Note 2: Only peaks from β-**287** are visible.



β-287•2TFA

(ca. 91:6:3 β-287:*d*A:*d*B)

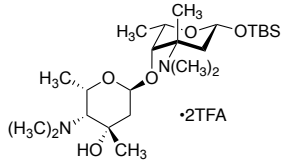


^{1}H-^{13}C HSQC{^{1}H}{^{13}C} NMR of Compound β-287•2TFA (≥ 10:1) (^{1}H-600 MHz, ^{13}C-151 MHz, 4:1 $CD_3OD/CDCl_3$ w/w + CF_3CO_2H)

Note 1: ***d*A** and ***d*B** are two unassigned disaccharides with structures similar to β-**287** present in the following ratio: ca. 91:6:3 β-**287**:***d*A**:***d*B**.

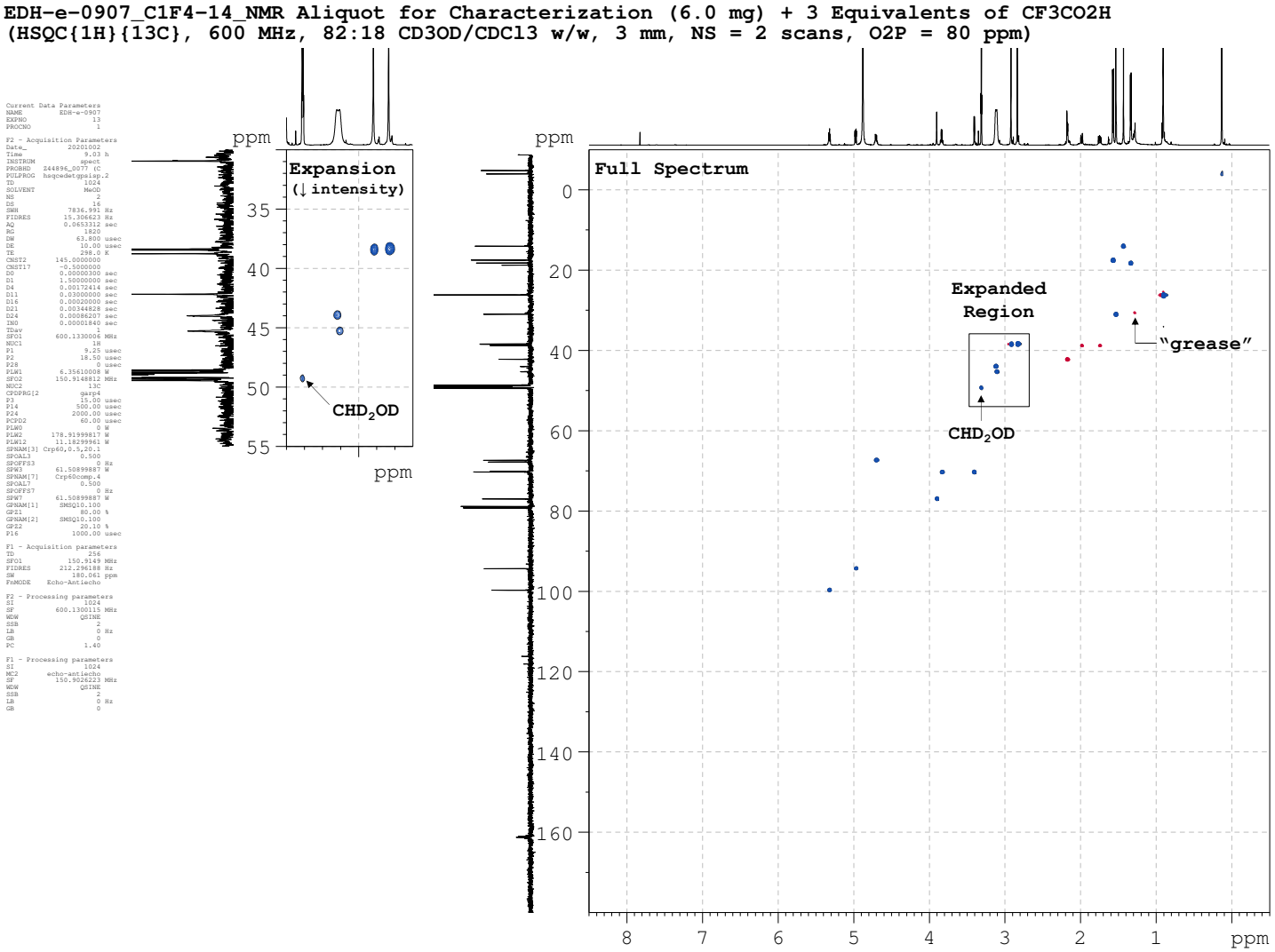
Note 2: Blue = CH/CH_3 | Red = CH_2.

Note 3: Only correlations from β-**287** are visible.



β-287•2TFA

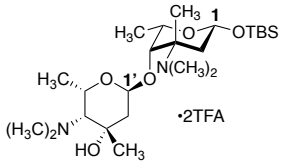
(ca. 91:6:3 β-287:*d*A:*d*B)



Coupled ^{1}H-^{13}C HSQC NMR of Compound β-287•2TFA (≥ 10:1) (^{1}H-600 MHz, ^{13}C-151 MHz, 4:1 $CD_3OD/CDCl_3$ w/w + CF_3CO_2H)

Note 1: ***d*A** and ***d*B** are two unassigned disaccharides with structures similar to β-**287** present in the following ratio: ca. 91:6:3 β-**287**:***d*A**:***d*B**.

Note 2: Blue = CH/CH_3 | Red = CH_2.



β-287•2TFA

(ca. 91:6:3 β-287:*d*A:*d*B)

EDH-e-0907_C1F4-14_NMR Aliquot for Characterization (6.0 mg) + 3 Equivalents of CF3CO2H ("Coupled" HSQC, 600 MHz, 82:18 CD3OD/CDCl3 w/w, 3 mm, NS = 4 scans)

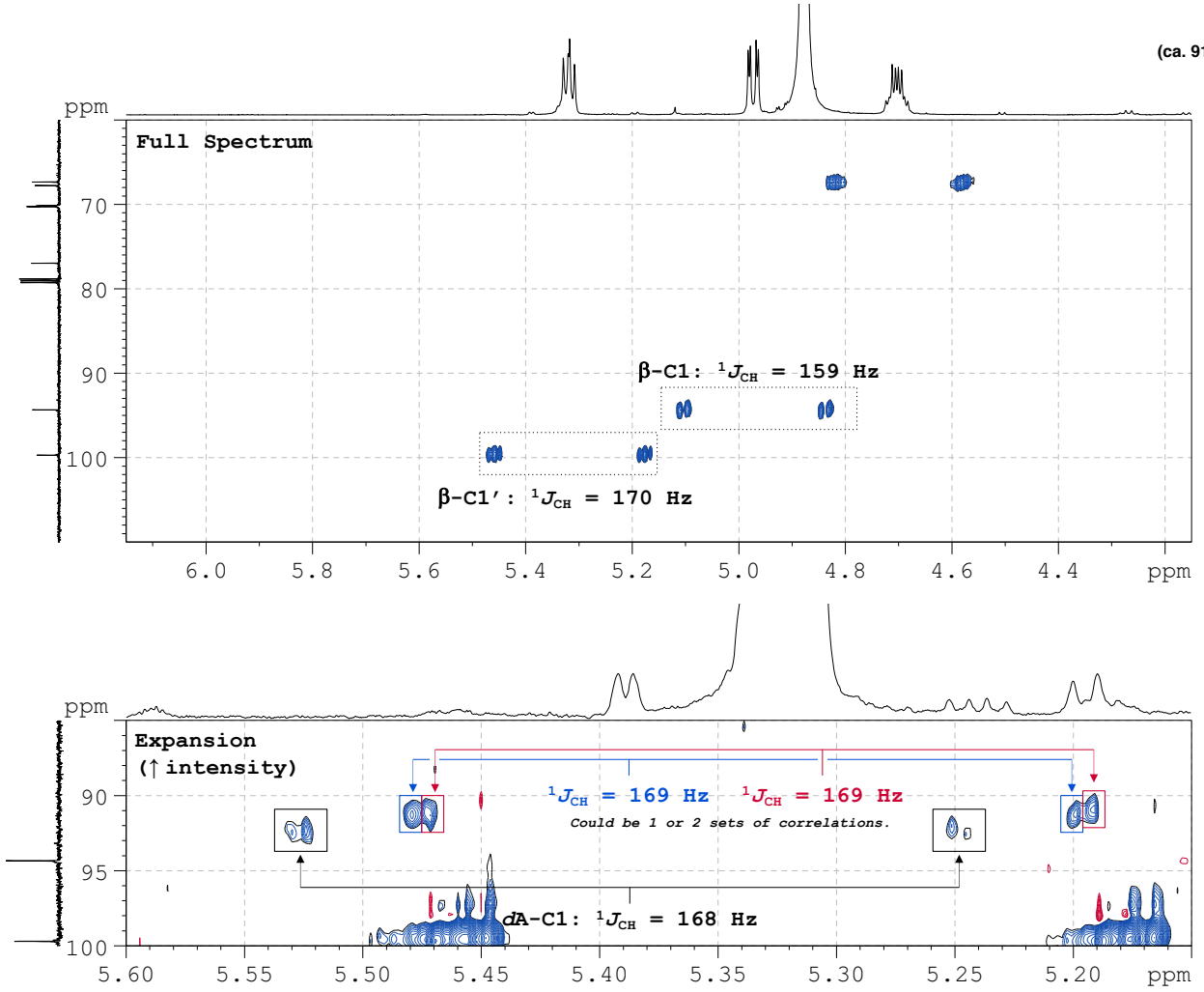

```
Current Data Parameters
NAME          EDH-e-0907
EXPNO                 18
PROCNO                 1

F2 - Acquisition Parameters
Date_          20201002
Time              11.58 h
INSTRUM             spect
PROBHD   24489E_0077 (C
PULPROG  hsqcedetgpsisp.2
TD                  4096
SOLVENT             MeOD
NS                     4
DS                    16
SWH             1200.384 Hz
FIDRES          0.586125 Hz
AQ              1.706125 sec
RG                  1820
DW               416.533 usec
DE                10.00 usec
TE                298.0 K
CNST2         145.0000000
CNST17         -0.5000000
D0              0.00000300 sec
D1              1.50000000 sec
D4              0.00172414 sec
D11             0.03000000 sec
D16             0.00020000 sec
D21             0.00344828 sec
D24             0.00086207 sec
IN0             0.00001840 sec
TDav                   1
SFO1          600.1330877 MHz
NUC1                  1H
P1                  9.25 usec
P2                 18.50 usec
P28                   0 usec
PLW1           6.35610008 W
SFO2          150.9174013 MHz
NUC2                 13C
CPDPRG[2           garp4
P3                 15.00 usec
P14               500.00 usec
P24              2000.00 usec
PCPD2              60.00 usec
PLW0                   0 W
PLW2         178.91999817 W
PLW12                  0 W
SPNAM[3] Crp60,0.5,20.1
SPOAL3             0.500
SPOFFS3                0 Hz
SPW3          61.50899887 W
SPNAM[7]    Crp60comp.4
SPOAL7             0.500
SPOFFS7                0 Hz
SPW7          61.50899887 W
GPNAM[1]      SMSQ10.100
GPZ1               80.00 %
GPNAM[2]      SMSQ10.100
GPZ2               20.10 %
P16              1000.00 usec

F1 - Acquisition parameters
TD                   128
SFO1          150.9174 MHz
FIDRES        424.592377 Hz
SW            180.058 ppm
FnMODE     Echo-Antiecho

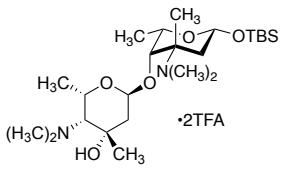
F2 - Processing parameters
SI                  1024
SF            600.1300115 MHz
WDW                QSINE
SSB                    2
LB                     0 Hz
GB                     0
PC                  1.40

F1 - Processing parameters
SI                  1024
MC2         echo-antiecho
SF            150.9026223 MHz
WDW                QSINE
SSB                    2
LB                     0 Hz
GB                     0
```

^{1}H-^{1}H COSY NMR of Compound β-287•2TFA (≥ 10:1) (600 MHz, 4:1 $CD_3OD/CDCl_3$ w/w + CF_3CO_2H)

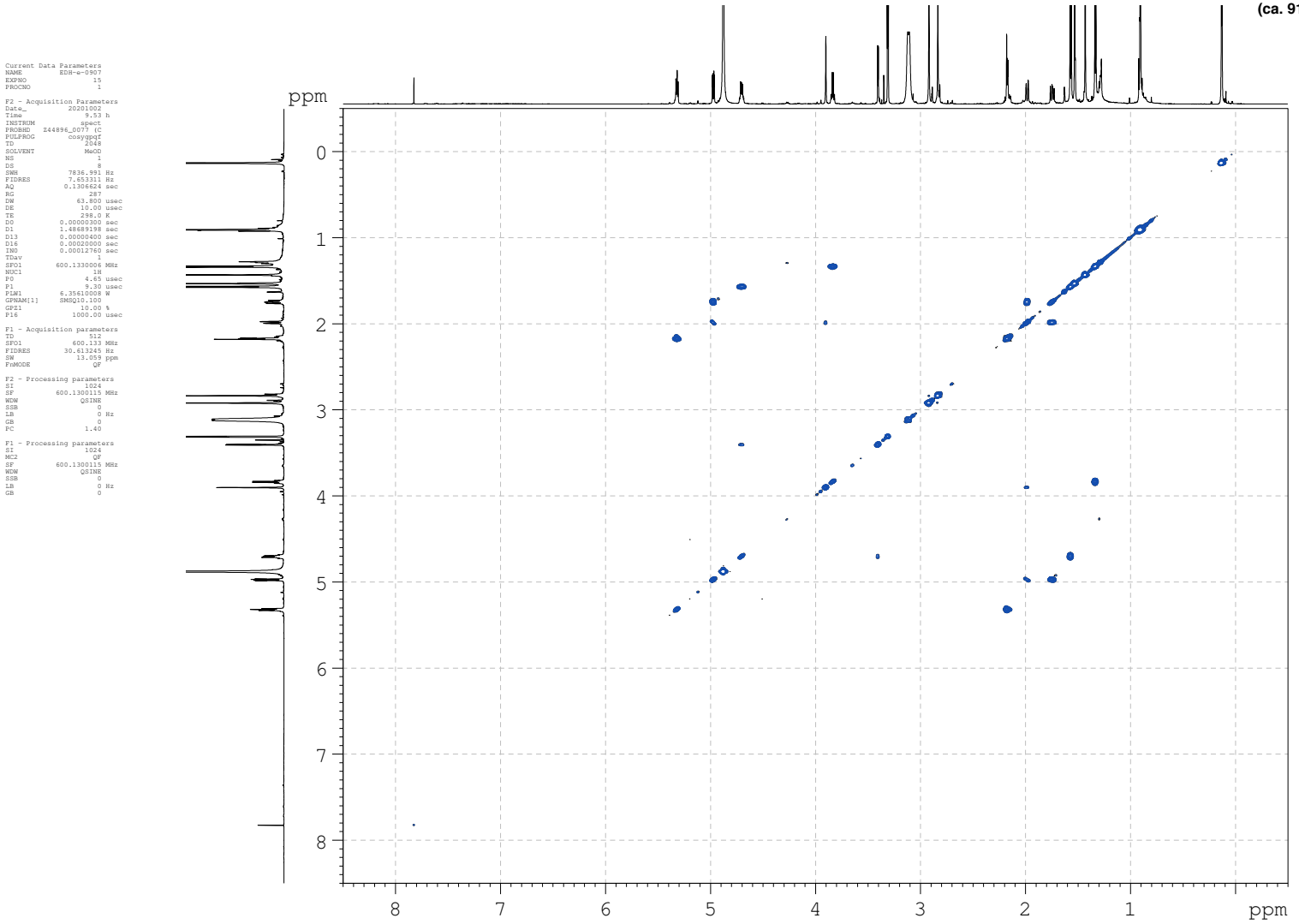
Note: ***d*A** and ***d*B** are two unassigned disaccharides with structures similar to β-**287** present in the following ratio: ca. 91:6:3 β-**287**:***d*A**:***d*B**.



β-287•2TFA

(ca. 91:6:3 β-287:*d*A:*d*B)

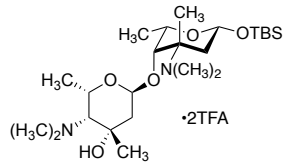
EDH-e-0907_C1F4-14_NMR Aliquot for Characterization (6.0 mg) + 3 Equivalents of CF3CO2H (COSY, 600 MHz, 82:18 CD3OD/CDCl3 w/w, 3 mm, NS = 1 scan)



^{1}H-^{1}H COSY NMR of Compound β-287•2TFA (≥ 10:1) (600 MHz, 4:1 $CD_3OD/CDCl_3$ w/w + CF_3CO_2H)

Note 1: ***d*A** and ***d*B** are two unassigned disaccharides with structures similar to β-**287** present in the following ratio: ca. 91:6:3 β-**287**:***d*A**:***d*B**.

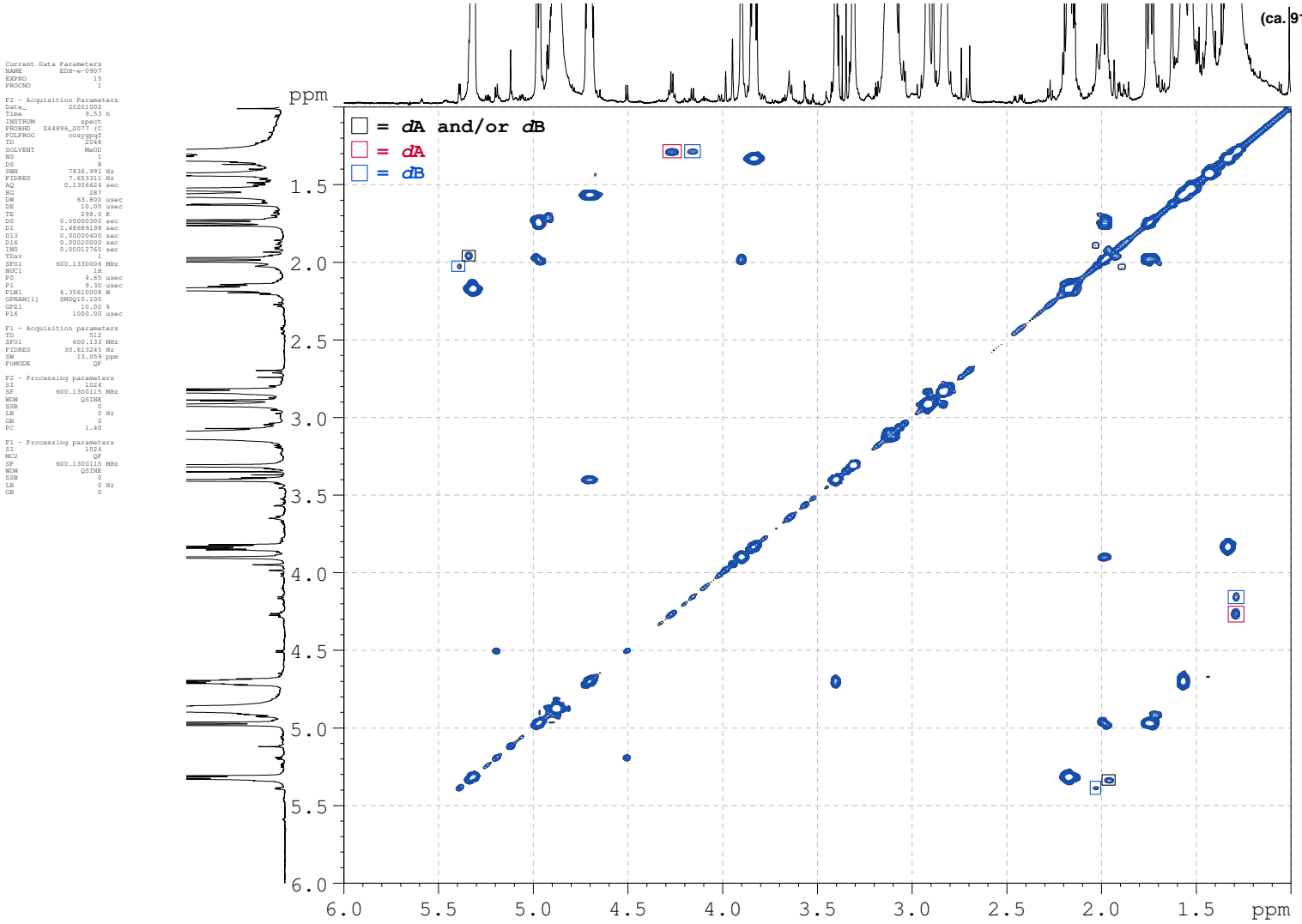
Note 2: Same spectrum with increased intensity and smaller spectral window as compared to COSY on page S357.



β-287•2TFA

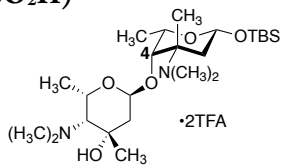
(ca. 91:6:3 β-287:*d*A:*d*B)

EDH-e-0907_C1F4-14_NMR Aliquot for Characterization (6.0 mg) + 3 Equivalents of CF3CO2H
(COSY, 600 MHz, 82:18 CD3OD/CDCl3 w/w, 3 mm, NS = 1 scan)



^{1}H-^{13}C HMBC NMR of Compound β-287•2TFA (≥ 10:1) (^{1}H-600 MHz, ^{13}C-151 MHz, 4:1 $CD_3OD/CDCl_3$ w/w + CF_3CO_2H)

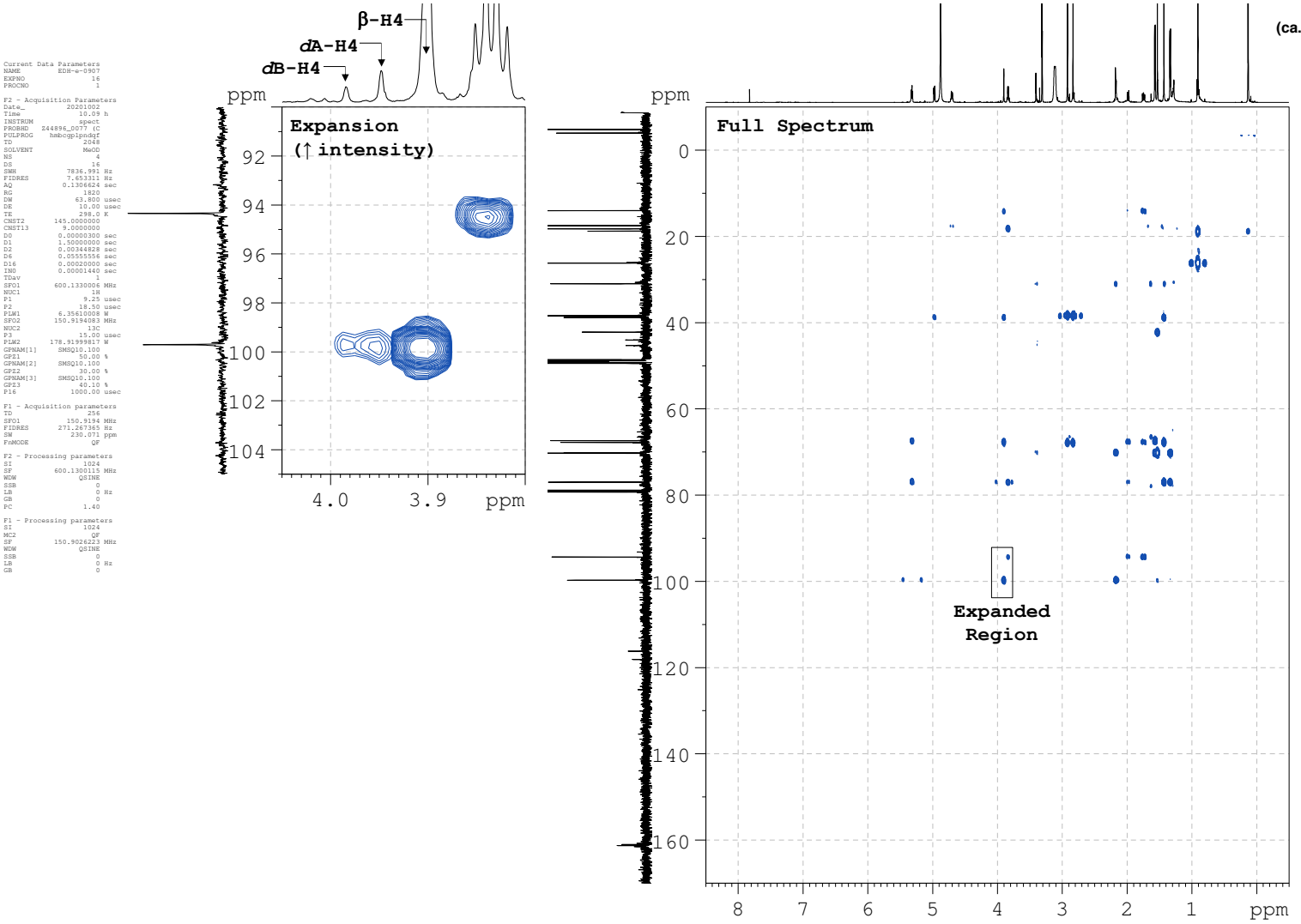
Note: ***d*A** and ***d*B** are two unassigned disaccharides with structures similar to β-**287** present in the following ratio: ca. 91:6:3 β-**287**:***d*A**:***d*B**.



β-287•2TFA

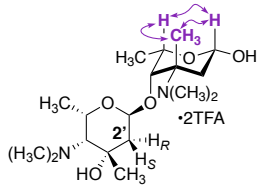
(ca. 91:6:3 β-287:*d*A:*d*B)

EDH-e-0907_C1F4-14_NMR Aliquot for Characterization (6.0 mg) + 3 Equivalents of CF3C (HMBC, 600 MHz, 82:18 CD3OD/CDCl3 w/w, 3 mm, NS = 4 scans)

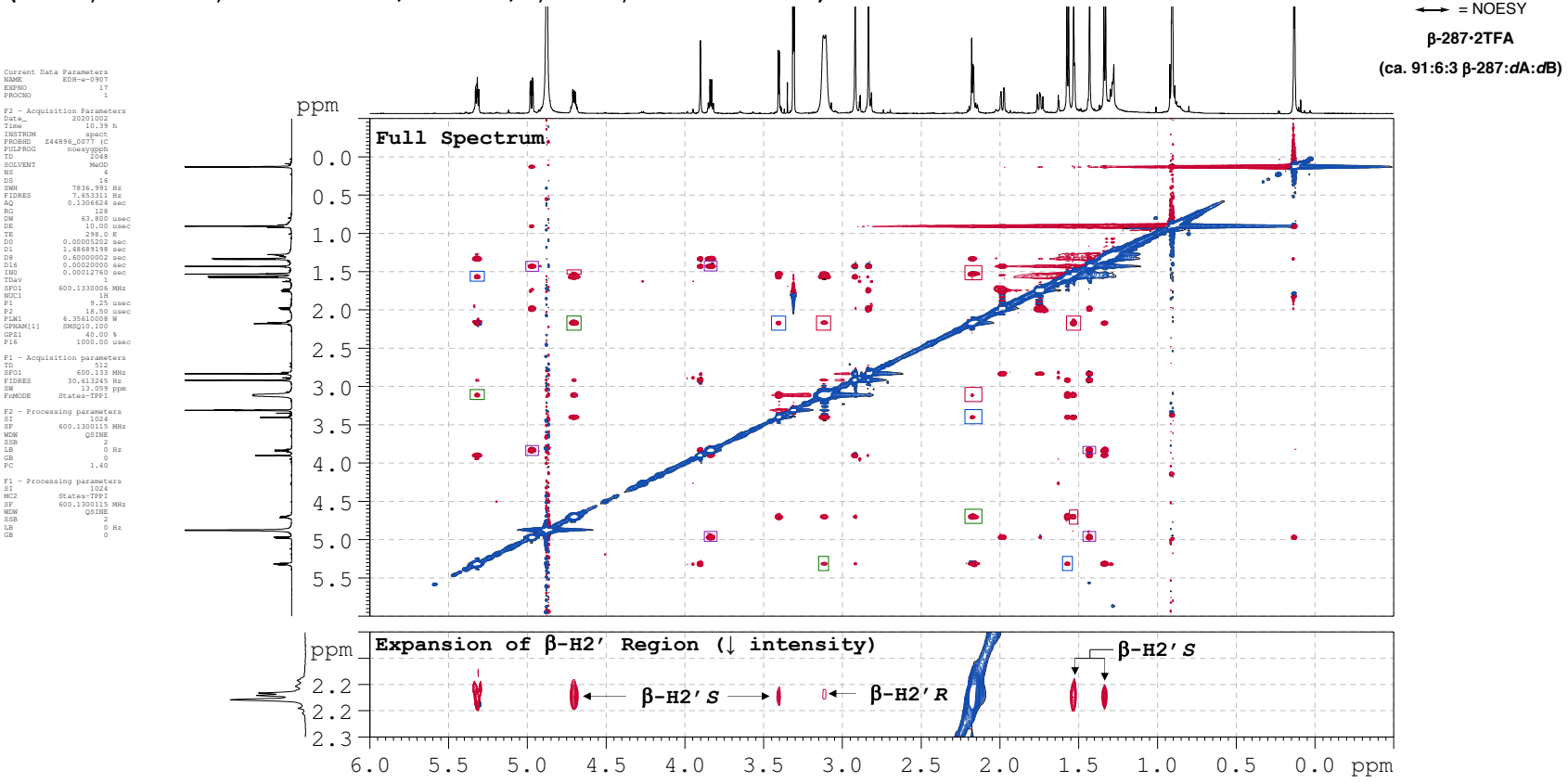


^{1}H-^{1}H NOESY NMR of Compound β-287•2TFA (≥ 10:1) (600 MHz, 4:1 $CD_3OD/CDCl_3$ w/w + CF_3CO_2H)

Note 1: ***d*A** and ***d*B** are two unassigned disaccharides with structures similar to β-**287** present in the following ratio: ca. 91:6:3 β-**287**:***d*A**:***d*B**.
Note 2: Blue = diagonal/chemical exchange | Red = NOESY cross peaks.



EDH-e-0907_C1F4-14_NMR Aliquot for Characterization (6.0 mg) + 3 Equivalents of CF3C (NOESY, 600 MHz, 82:18 CD3OD/CDC13 w/w, 3 mm, NS = 4 scans)



Current Data Parameters
NAME EDH-e-0907
EXPNO 17
PROCNO 1

F2 - Acquisition Parameters
Date_ 20201002
Time 10.39 h
INSTRUM spect
PROBHD Z44896_0077 (C
PULPROG noesygpph
TD 2048
SOLVENT MeOD
NS 4
DS 16
SWH 7836.991 Hz
FIDRES 7.653311 Hz
AQ 0.1306624 sec
RG 128
DW 63.800 usec
DE 10.00 usec
TE 298.0 K
D0 0.00005202 sec
D1 1.48689198 sec
D8 0.60000002 sec
D16 0.00020000 sec
IN0 0.00012760 sec
TDav 1
SFO1 600.1330006 MHz
NUC1 1H
P1 9.25 usec
P2 18.50 usec
PLW1 6.35610008 W
GPNAM[1] SMSQ10.100
GPZ1 40.00 %
P16 1000.00 usec

F1 - Acquisition parameters
TD 512
SFO1 600.133 MHz
FIDRES 30.613245 Hz
SW 13.059 ppm
FnMODE States-TPPI

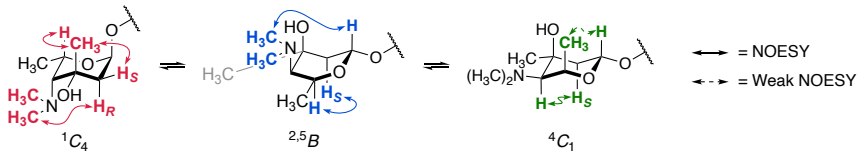
F2 - Processing parameters
SI 1024
SF 600.1300115 MHz
WDW QSINE
SSB 2
LB 0 Hz
GB 0
PC 1.40

F1 - Processing parameters
SI 1024
MC2 States-TPPI
SF 600.1300115 MHz
WDW QSINE
SSB 2
LB 0 Hz
GB 0

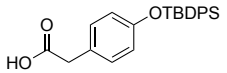
Compound β-287•2TFA: Conformational Analysis of the Non-Reducing Sugar[a]

NOESY correlations (as well as the anomalous $^{3}J_{H1'\text{-}H2'}$ coupling constants of 7.2 and 5.5 Hz) indicate that the reducing sugar does not solely occupy the canonical $^{1}C_4$ conformation; rather, as indicated by the NOESY correlations highlighted to the right, the non-reducing sugar exists in conformational equilibrium and/or an intermediate conformation not pictured here.

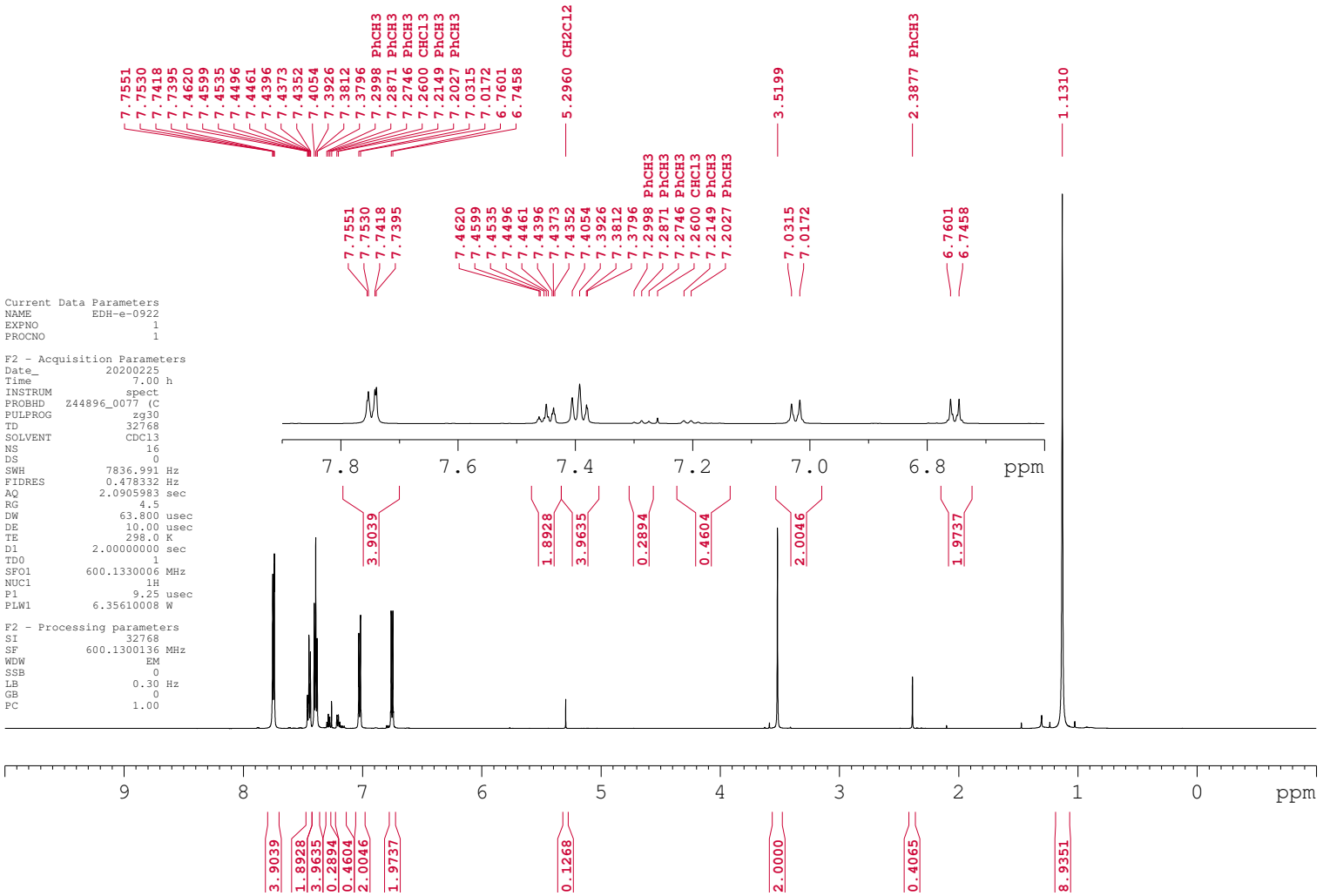
[a]For simplicity, the C4' nitrogen atom is not depicted in the cationic deuterated state that it likely assumed during the acquisition of this NOESY spectrum.



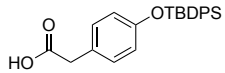
^{1}H NMR of Compound 303 (600 MHz, $CDCl_3$)



303

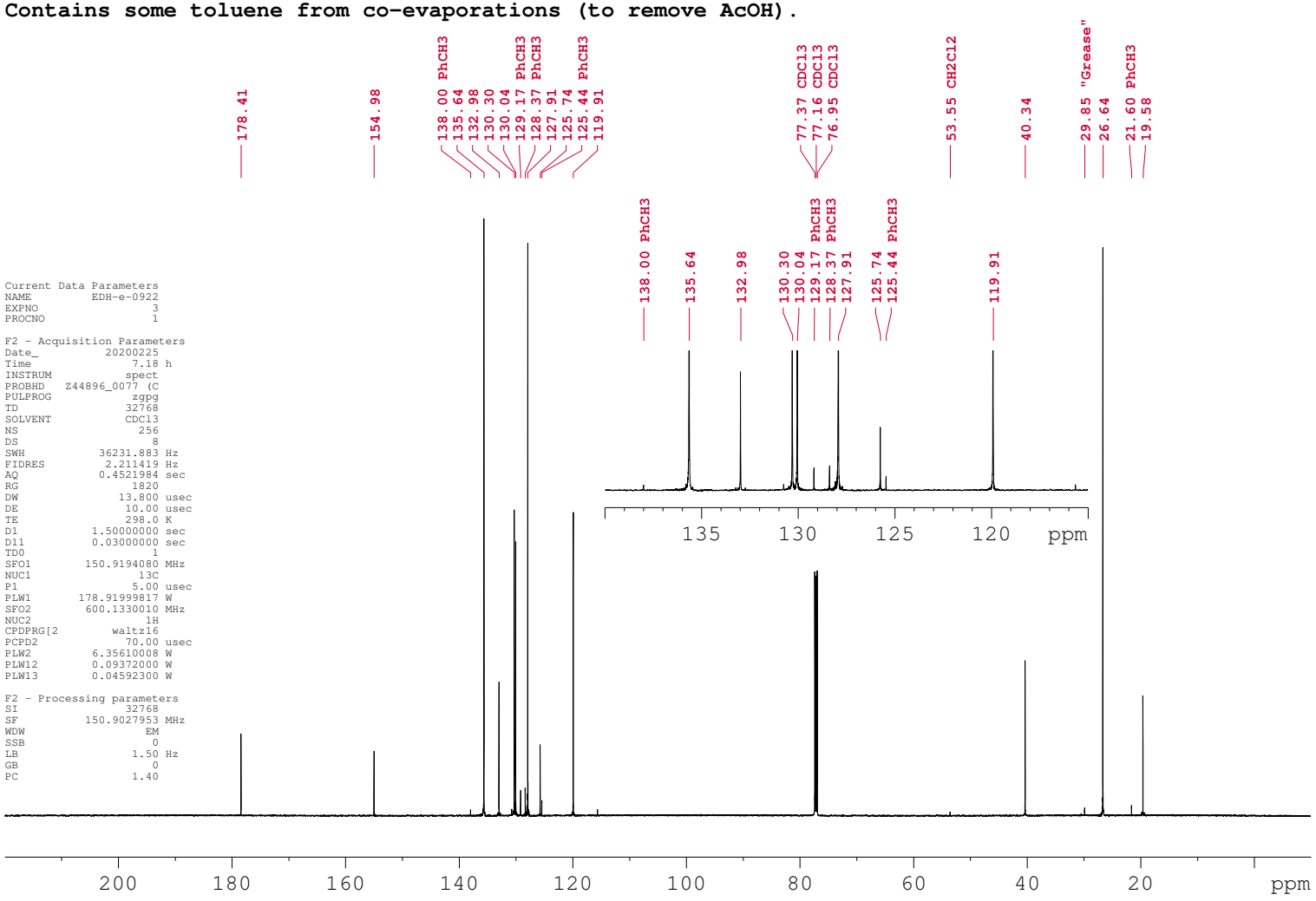


^{13}C NMR of Compound 303 (151 MHz, $CDCl_3$)



303

EDH-e-0922_C1F10-17 (13C{1H}, 151 MHz, CDCl3, 5 mm, NS = 256 scans)
Contains some toluene from co-evaporations (to remove AcOH).




```
Current Data Parameters
NAME         EDH-e-0922
EXPNO                 3
PROCNO                1

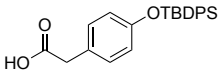
F2 - Acquisition Parameters
Date_          20200225
Time               7.18 h
INSTRUM            spect
PROBHD   Z44896_0077 (C
PULPROG            zgpg
TD                32768
SOLVENT           CDCl3
NS                  256
DS                    8
SWH           36231.883 Hz
FIDRES         2.211419 Hz
AQ             0.4521984 sec
RG                 1820
DW                13.800 usec
DE                10.00 usec
TE                298.0 K
D1           1.50000000 sec
D11          0.03000000 sec
TD0                   1
SFO1        150.9194080 MHz
NUC1                13C
P1                 5.00 usec
PLW1        178.91999817 W
SFO2        600.1330010 MHz
NUC2                1H
CPDPRG[2         waltz16
PCPD2             70.00 usec
PLW2         6.35610008 W
PLW12        0.09372000 W
PLW13        0.04592300 W

F2 - Processing parameters
SI                32768
SF          150.9027953 MHz
WDW                  EM
SSB                   0
LB                 1.50 Hz
GB                    0
PC                 1.40
```

^{1}H-^{13}C HSQC{^{1}H}{^{13}C} NMR of Compound 303 (^{1}H-600 MHz, ^{13}C-151 MHz, $CDCl_3$)

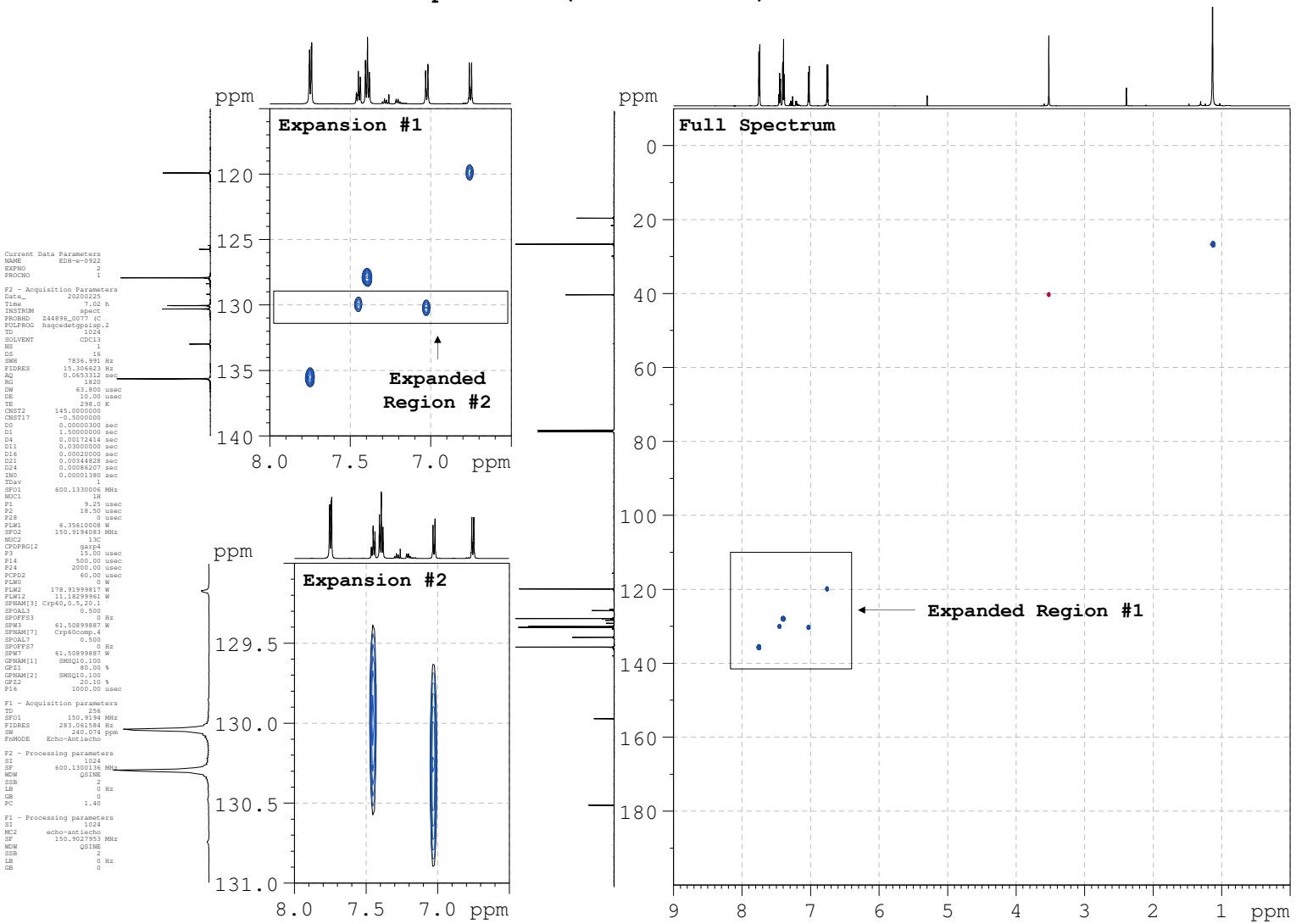
Note 1: Blue = CH/CH_3 | Red = CH_2.

Note 2: Only correlations from **303** are visible.



303

EDH-e-0922_C1F10-17 (HSQC, 600 MHz, CDCl3, 5 mm, NS = 1 scan, O2P = 110 ppm, SW2 = 240 ppm)
Contains some toluene from co-evaporations (to remove AcOH).



Current Data Parameters
NAME EDH-e-0922
EXPNO 2
PROCNO 1

F2 - Acquisition Parameters
Date_ 20200225
Time 7.02 h
INSTRUM spect
PROBHD Z44896_0077 (C
PULPROG hsqcedetgpsisp.2
TD 1024
SOLVENT CDCl3
NS 1
DS 16
SWH 7836.991 Hz
FIDRES 15.306623 Hz
AQ 0.0653312 sec
RG 1820
DW 63.800 usec
DE 10.00 usec
TE 298.0 K
CNST2 145.0000000
CNST17 -0.5000000
D0 0.00000300 sec
D1 1.50000000 sec
D4 0.00172414 sec
D11 0.03000000 sec
D16 0.00020000 sec
D21 0.00344828 sec
D24 0.00086207 sec
IN0 0.00001380 sec
TDav 1
SFO1 600.1330006 MHz
NUC1 1H
P1 9.25 usec
P2 18.50 usec
P28 0 usec
PLW1 6.35610008 W
SFO2 150.9194083 MHz
NUC2 13C
CPDPRG[2 garp4
P3 15.00 usec
P14 500.00 usec
P24 2000.00 usec
PCPD2 60.00 usec
PLW0 0 W
PLW2 178.91999817 W
PLW12 11.18299961 W
SPNAM[3] Crp60,0.5,20.1
SPOAL3 0.500
SPOFFS3 0 Hz
SPW3 61.50899887 W
SPNAM[7] Crp60comp.4
SPOAL7 0.500
SPOFFS7 0 Hz
SPW7 61.5089887 W
GPNAM[1] SMSQ10.100
GPZ1 80.00 %
GPNAM[2] SMSQ10.100
GPZ2 20.10 %
P16 1000.00 usec

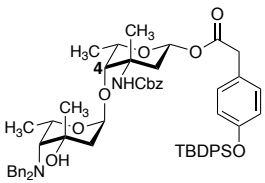
F1 - Acquisition parameters
TD 256
SFO1 150.9194 MHz
FIDRES 283.061584 Hz
SW 240.074 ppm
FnMODE Echo-Antiecho

F2 - Processing parameters
SI 1024
SF 600.1300136 MHz
WDW QSINE
SSB 2
LB 0 Hz
GB 0
PC 1.40

F1 - Processing parameters
SI 1024
MC2 echo-antiecho
SF 150.9027953 MHz
WDW QSINE
SSB 2
LB 0 Hz
GB 0

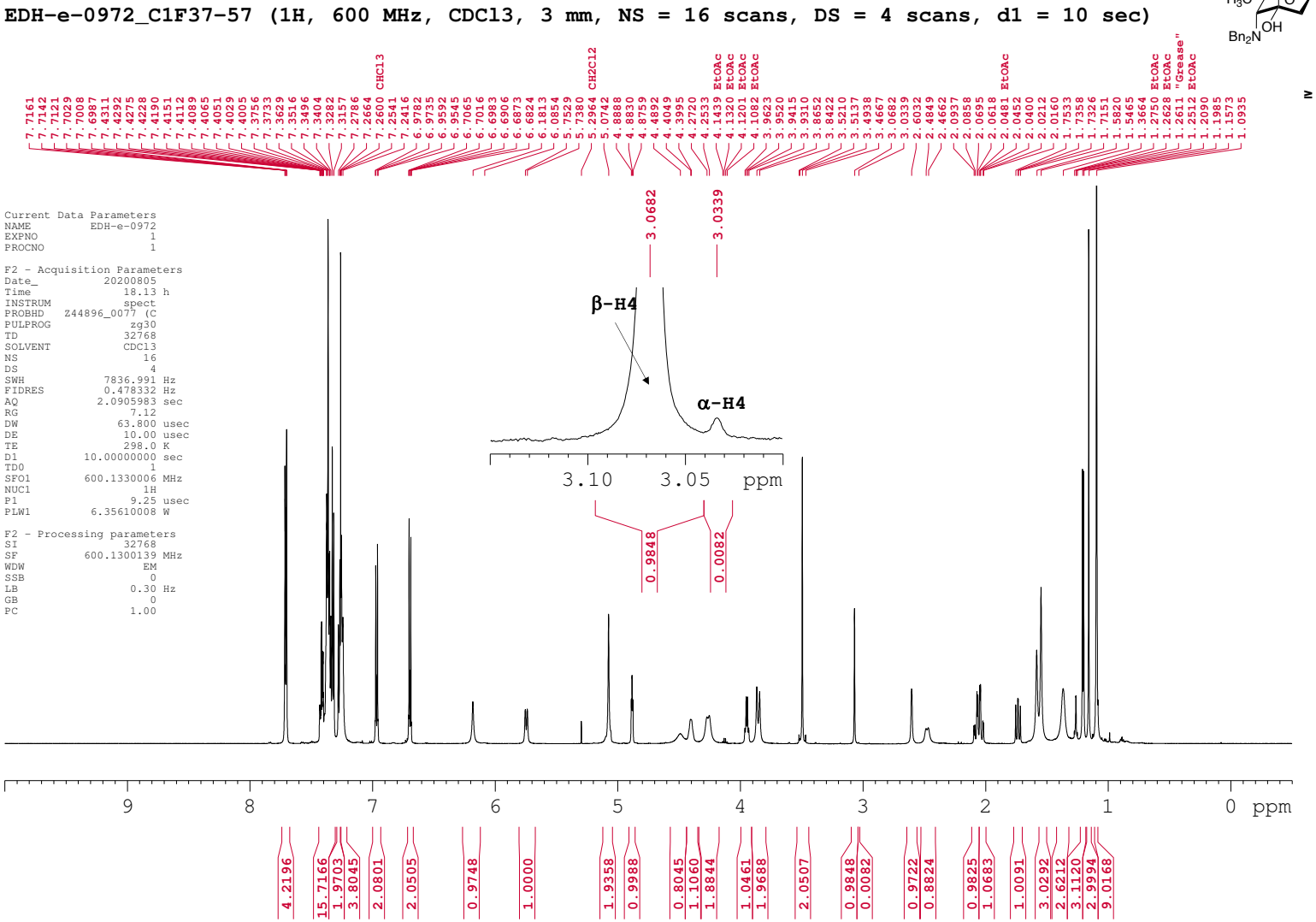
^{1}H NMR of Compound β-304 (≥ 20:1 β/α) (600 MHz, $CDCl_3$)

Note: This is the ^{1}H NMR spectrum of β-**304** used to verify the purity of the 245.4 mg of β-**304** obtained in the experimental procedure detailed above.



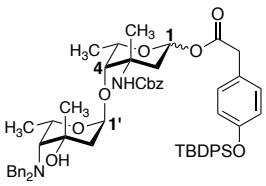
β-304

≥ 20:1 β/α



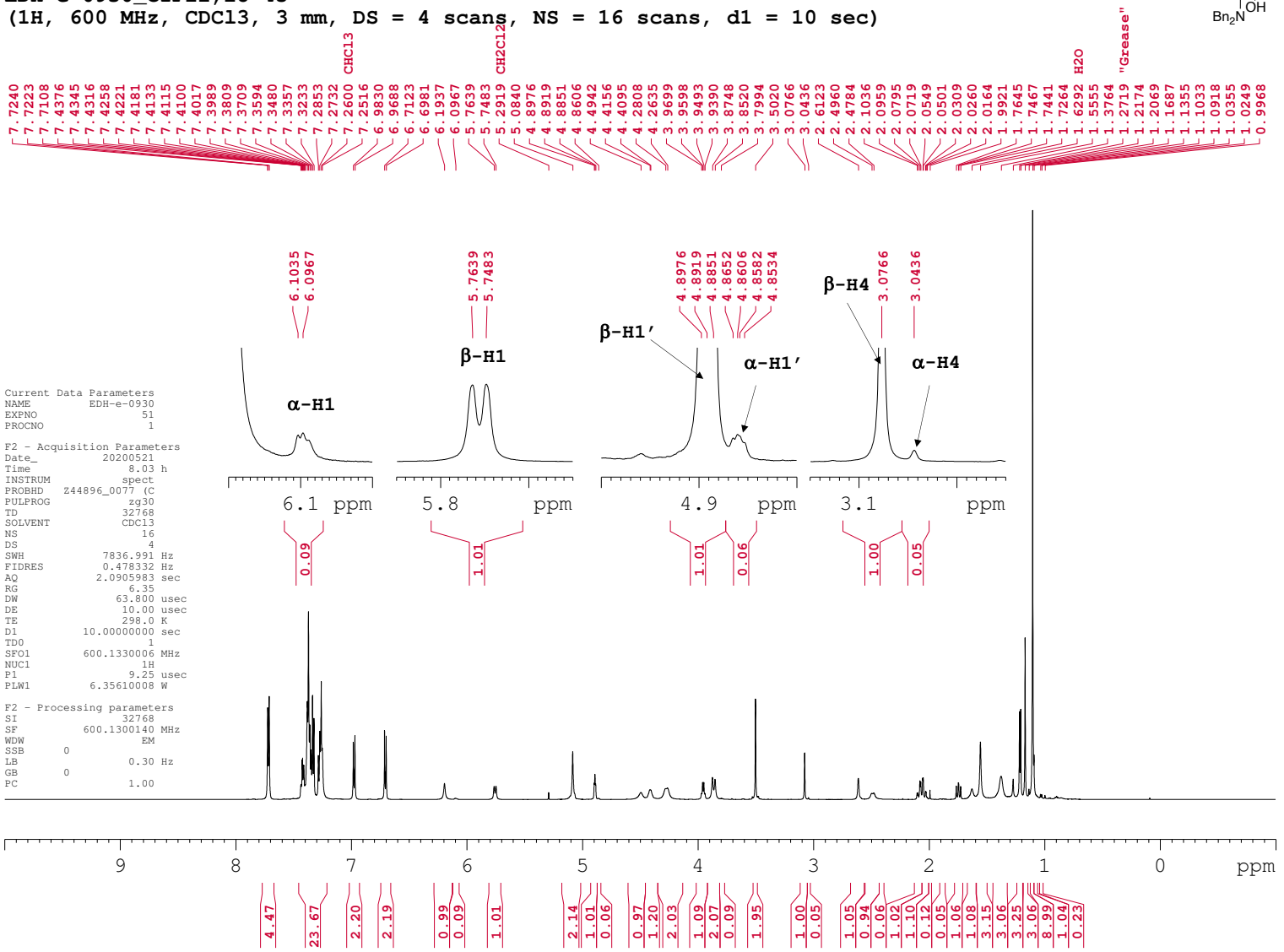
^{1}H NMR of Compound α/β-304 (≥ 10:1 β/α) (600 MHz, $CDCl_3$)

Note: The is the ^{1}H NMR of α/β-304 used for the line listing and peak assignment.



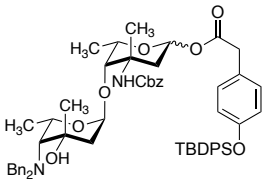
α/β-304

≥ 10:1 β/α



^{13}C NMR of Compound α/β-304 (≥ 10:1 β/α) (151 MHz, $CDCl_3$)

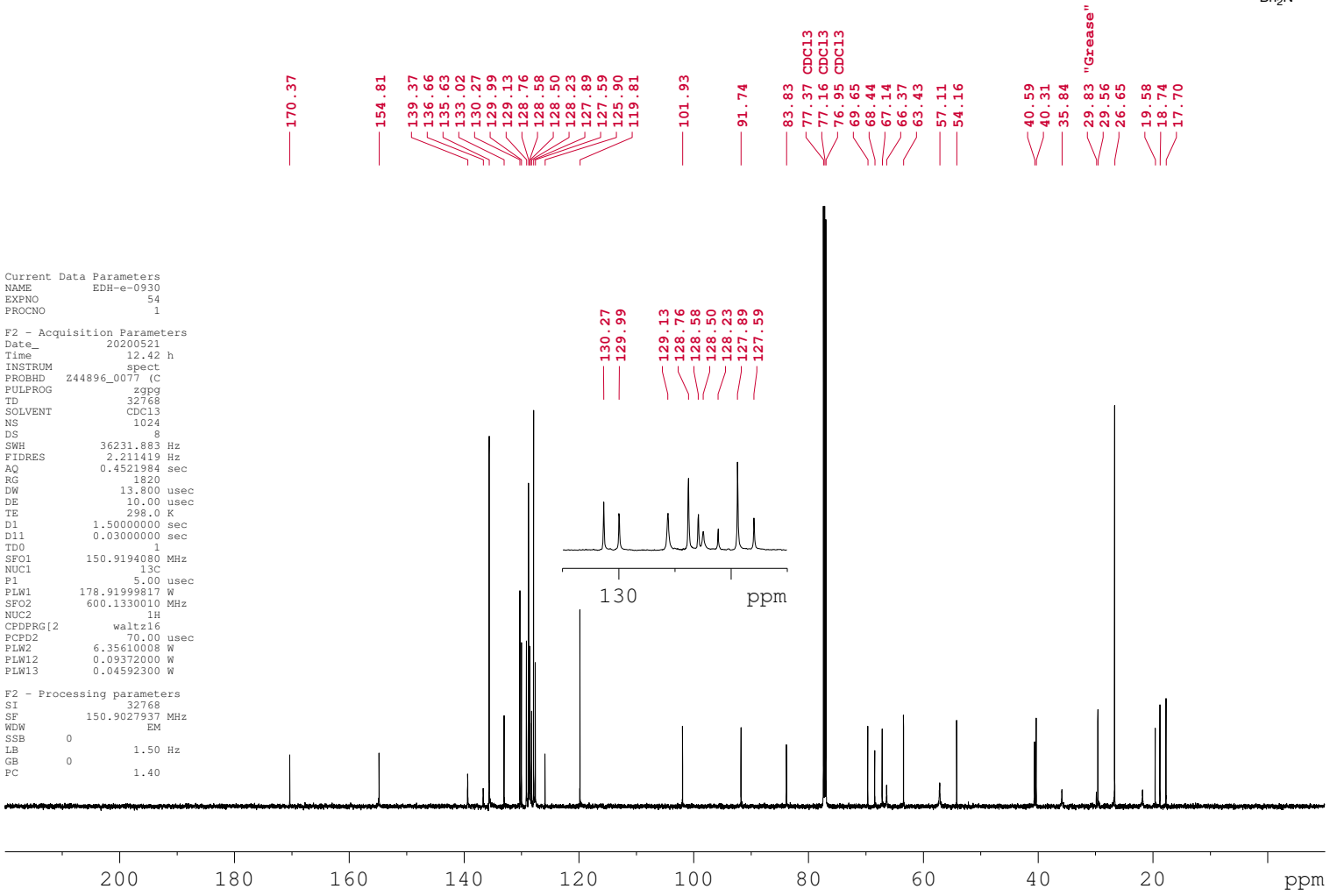
Note: Only peaks from major β-**304** are visible.



α/β-304

≥ 10:1 β/α

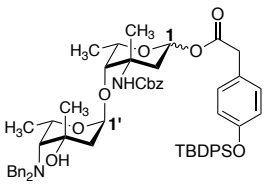
EDH-e-0930_C1F22,28-45 (13C{1H}, 151 MHz, CDCl3, 3 mm, NS = 1024 scans)



^{1}H-^{13}C HSQC{^{1}H}{^{13}C} NMR of Compound α/β-304 (≥ 10:1 β/α) (^{1}H-600 MHz, ^{13}C-151 MHz, $CDCl_3$)

Note 1: Blue = CH/CH_3 | Red = CH_2.

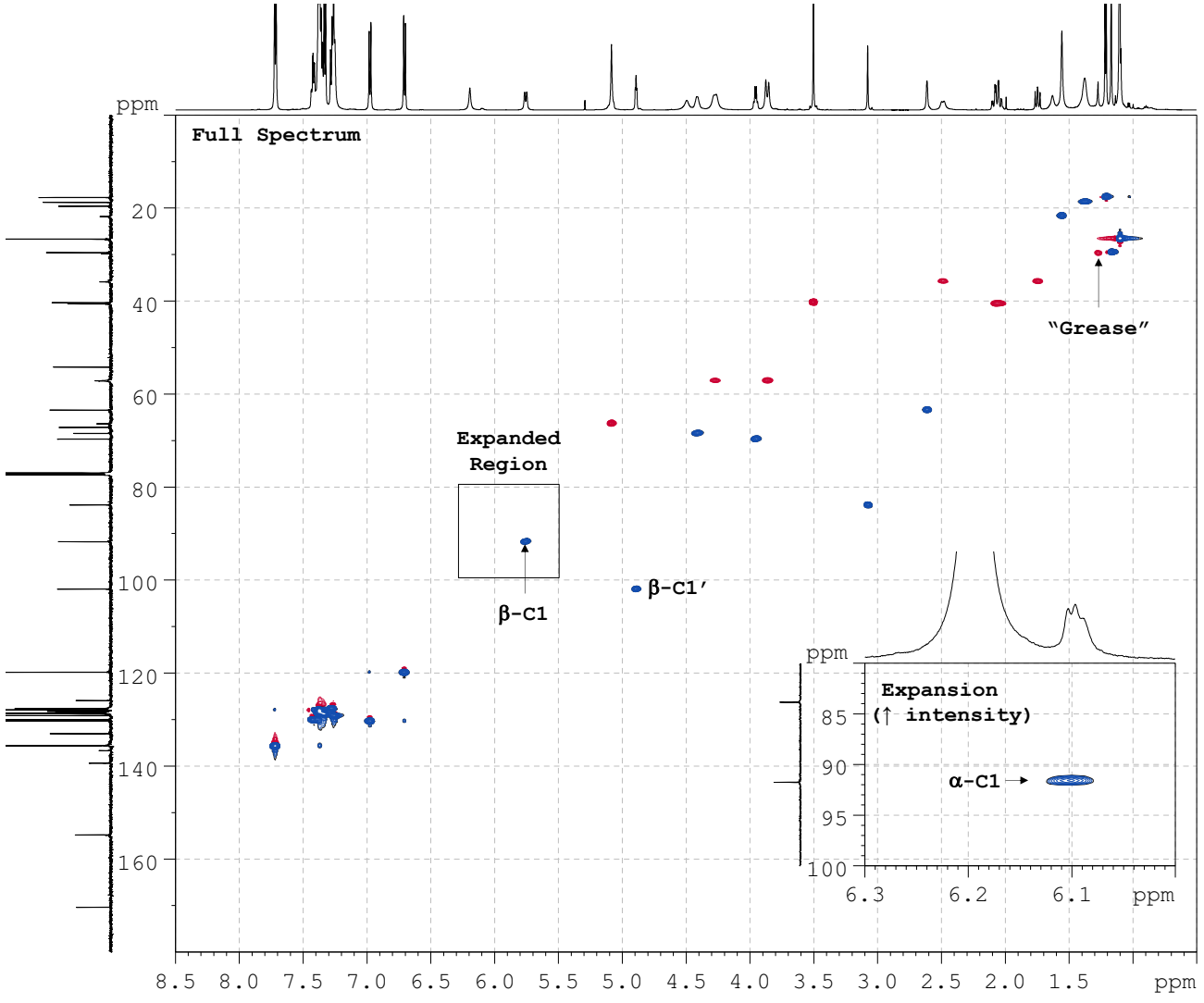
Note 2: Only correlations from major β-**304** are visible in full spectrum; α-**304** only visible in expansion with increased intensity.



α/β-304

≥ 10:1 β/α

EDH-e-0930_C1F22,28-45 ({1H}{13C}HSQC, 600 MHz, CDCl3, 3 mm, NS = 4 scans, O2p = 80



Current Data Parameters
NAME EDH-e-0930
EXPNO 52
PROCNO 1

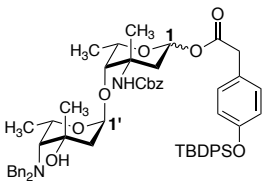
F2 - Acquisition Parameters
Date_ 20200521
Time 8.11 h
INSTRUM spect
PROBHD Z44896_0077 (C
PULPROG hsqcedetgpsisp.2
TD 1024
SOLVENT CDCl3
NS 4
DS 16
SWH 7836.991 Hz
FIDRES 15.306623 Hz
AQ 0.0653312 sec
RG 1820
DW 63.800 usec
DE 10.00 usec
TE 298.0 K
CNST2 145.0000000
CNST17 -0.5000000
D0 0.00000300 sec
D1 1.50000000 sec
D4 0.00172414 sec
D11 0.03000000 sec
D16 0.00020000 sec
D21 0.00344828 sec
D24 0.00086207 sec
IN0 0.00001840 sec
TDav 1
SFO1 600.1330006 MHz
NUC1 1H
P1 9.25 usec
P2 18.50 usec
P28 0 usec
PLW1 6.35610008 W
SFO2 150.9148812 MHz
NUC2 13C
CPDPRG[2 garp4
P3 15.00 usec
P14 500.00 usec
P24 2000.00 usec
PCPD2 60.00 usec
PLW0 0 W
PLW2 178.91999817 W
PLW12 11.18299961 W
SPNAM[3] Crp60,0.5,20.1
SPOAL3 0.500
SPOFFS3 0 Hz
SPW3 61.50899887 W
SPNAM[7] Crp60comp.4
SPOAL7 0.500
SPOFFS7 0 Hz
SPW7 61.50899887 W
GPNAM[1] SMSQ10.100
GPZ1 80.00 %
GPNAM[2] SMSQ10.100
GPZ2 20.10 %
P16 1000.00 usec

F1 - Acquisition parameters
TD 256
SFO1 150.9149 MHz
FIDRES 212.296188 Hz
SW 180.061 ppm
FnMODE Echo-Antiecho

F2 - Processing parameters
SI 1024

Coupled ^{1}H-^{13}C HSQC NMR of Compound α/β-304 (≥ 10:1 β/α) (^{1}H-600 MHz, ^{13}C-151 MHz, $CDCl_3$)

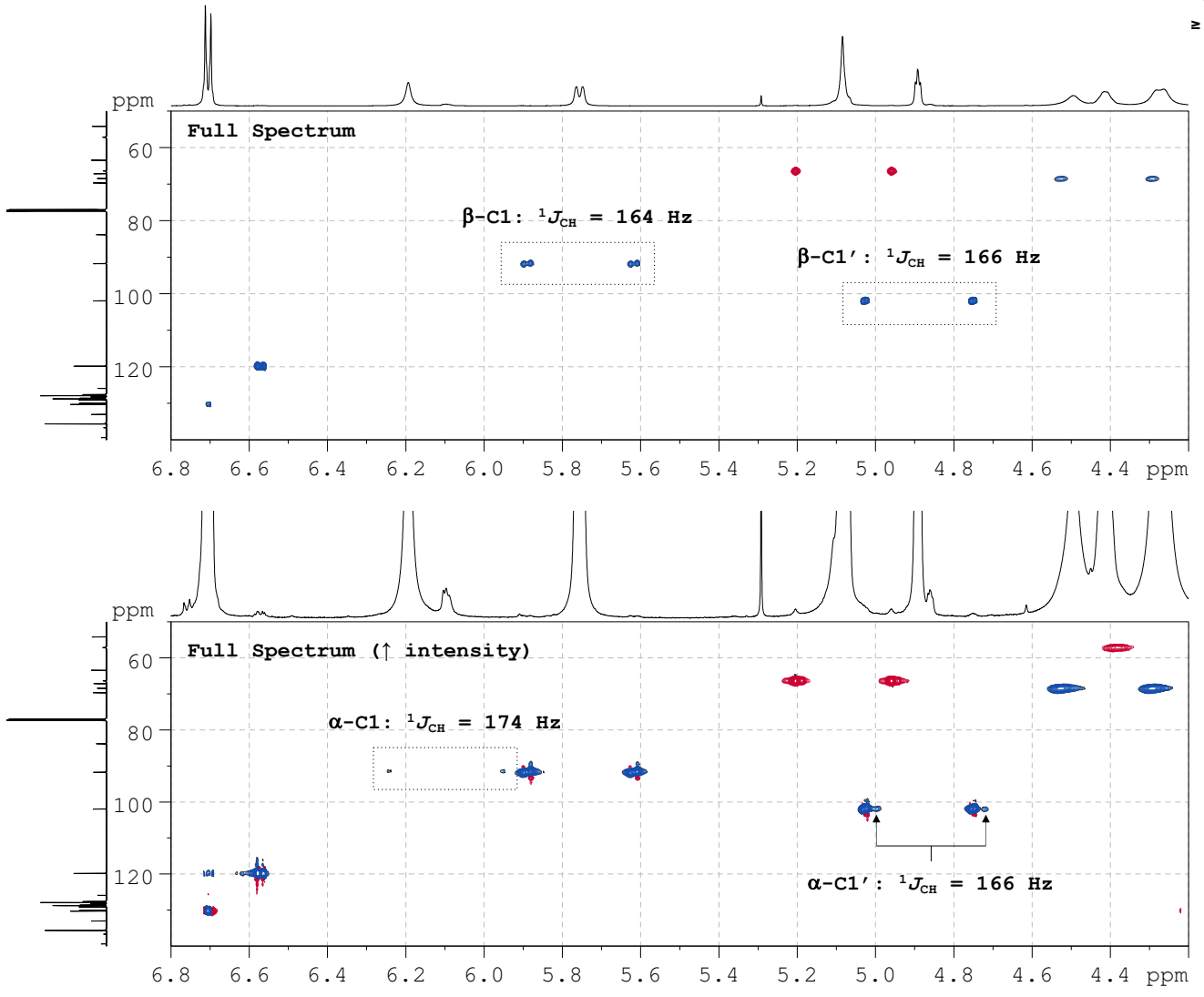
Note: Blue = CH/CH_3 | Red = CH_2.



α/β-304

≥ 10:1 β/α

EDH-e-0930_C1F22,28-45 ("Coupled" HSQC, 600 MHz, CDCl3, 3 mm, NS = 4 scans)



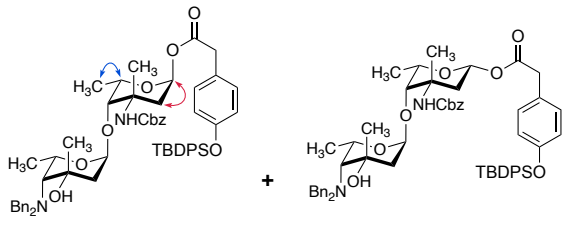
Current Data Parameters
NAME EDH-e-0930
EXPNO 56
PROCNO 1

F2 - Acquisition Parameters
Date_ 20200521
Time 13.13 h
INSTRUM spect
PROBHD Z44896_0077 (C
PULPROG hsqcedetgpsisp.2
TD 4096
SOLVENT CDCl3
NS 4
DS 16
SWH 1500.600 Hz
FIDRES 0.732715 Hz
AQ 1.3647872 sec
RG 1820
DW 333.200 usec
DE 10.00 usec
TE 298.0 K
CNST2 145.0000000
CNST17 -0.5000000
D0 0.00000300 sec
D1 1.50000000 sec
D4 0.00172414 sec
D11 0.03000000 sec
D16 0.00020000 sec
D21 0.00344828 sec
D24 0.00086207 sec
IN0 0.00001840 sec
TDav 1
SFO1 600.1332947 MHz
NUC1 1H
P1 9.25 usec
P2 18.50 usec
P28 0 usec
PLW1 6.35610008 W
SFO2 150.9173862 MHz
NUC2 13C
CPDPRG[2 garp4
P3 15.00 usec
P14 500.00 usec
P24 2000.00 usec
PCPD2 60.00 usec
PLW0 0 W
PLW2 178.91999817 W
PLW12 0 W
SPNAM[3] Crp60,0.5,20.1
SPOAL3 0.500
SPOFFS3 0 Hz
SPW3 61.50899887 W
SPNAM[7] Crp60comp.4
SPOAL7 0.500
SPOFFS7 0 Hz
SPW7 61.50899887 W
GPNAM[1] SMSQ10.100
GPZ1 80.00 %
GPNAM[2] SMSQ10.100
GPZ2 20.10 %
P16 1000.00 usec

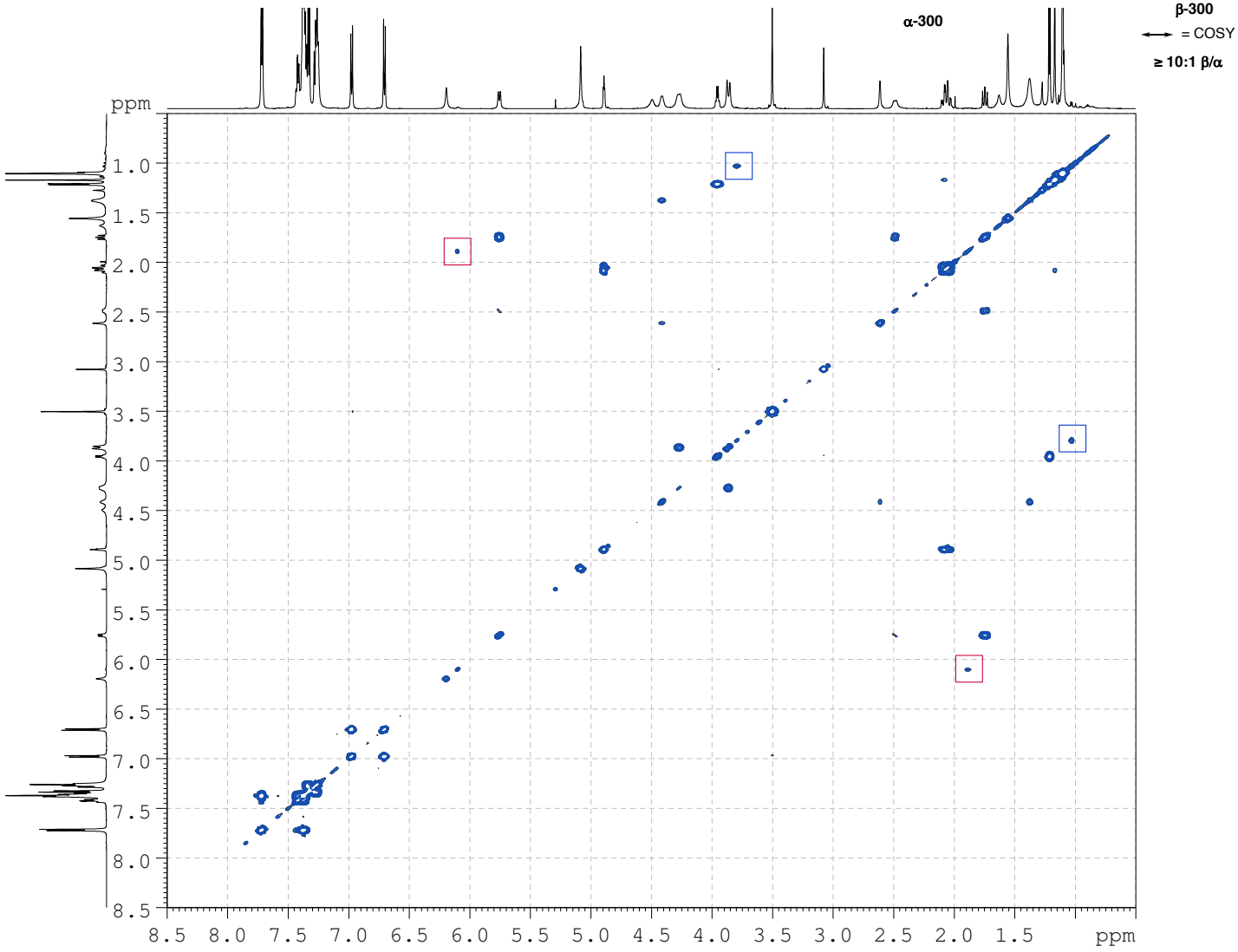
F1 - Acquisition parameters
TD 128
SFO1 150.9174 MHz
FIDRES 424.592377 Hz
SW 180.058 ppm
FnMODE Echo-Antiecho

F2 - Processing parameters
SI 1024

^{1}H-^{1}H COSY NMR of Compound α/β-304 (≥ 10:1 β/α) (600 MHz, $CDCl_3$)



EDH-e-0930_C1F22,28-45 (COSY, 600 MHz, CDCl3, 3 mm, NS = 2 scans)

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Current Data Parameters
NAME              EDH-e-0930
EXPNO                     55
PROCNO                     1

F2 - Acquisition Parameters
Date_           20200521
Time              12.43 h
INSTRUM             spect
PROBHD   Z44896_0077 (C
PULPROG          cosygpqf
TD                 2048
SOLVENT             CDCl3
NS                     2
DS                     8
SWH             7836.991 Hz
FIDRES          7.653311 Hz
AQ              0.1306624 sec
RG                   203
DW                63.800 usec
DE                10.00 usec
TE                298.0 K
D0             0.00000300 sec
D1             1.48689198 sec
D13            0.00000400 sec
D16            0.00020000 sec
IN0            0.00012760 sec
TDav                   1
SFO1           600.1330006 MHz
NUC1                  1H
P0                  4.65 usec
P1                  9.30 usec
PLW1           6.35610008 W
GPNAM[1]     SMSQ10.100
GPZ1                10.00 %
P16               1000.00 usec

F1 - Acquisition parameters
TD                   512
SFO1            600.133 MHz
FIDRES         30.613245 Hz
SW                13.059 ppm
FnMODE                QF

F2 - Processing parameters
SI                  1024
SF            600.1300140 MHz
WDW                 QSINE
SSB                    0
LB                  0 Hz
GB                     0
PC                  1.40

F1 - Processing parameters
SI                  1024
MC2                   QF
SF            600.1300140 MHz
WDW                 QSINE
SSB                    0
LB                  0 Hz
GB                     0
```
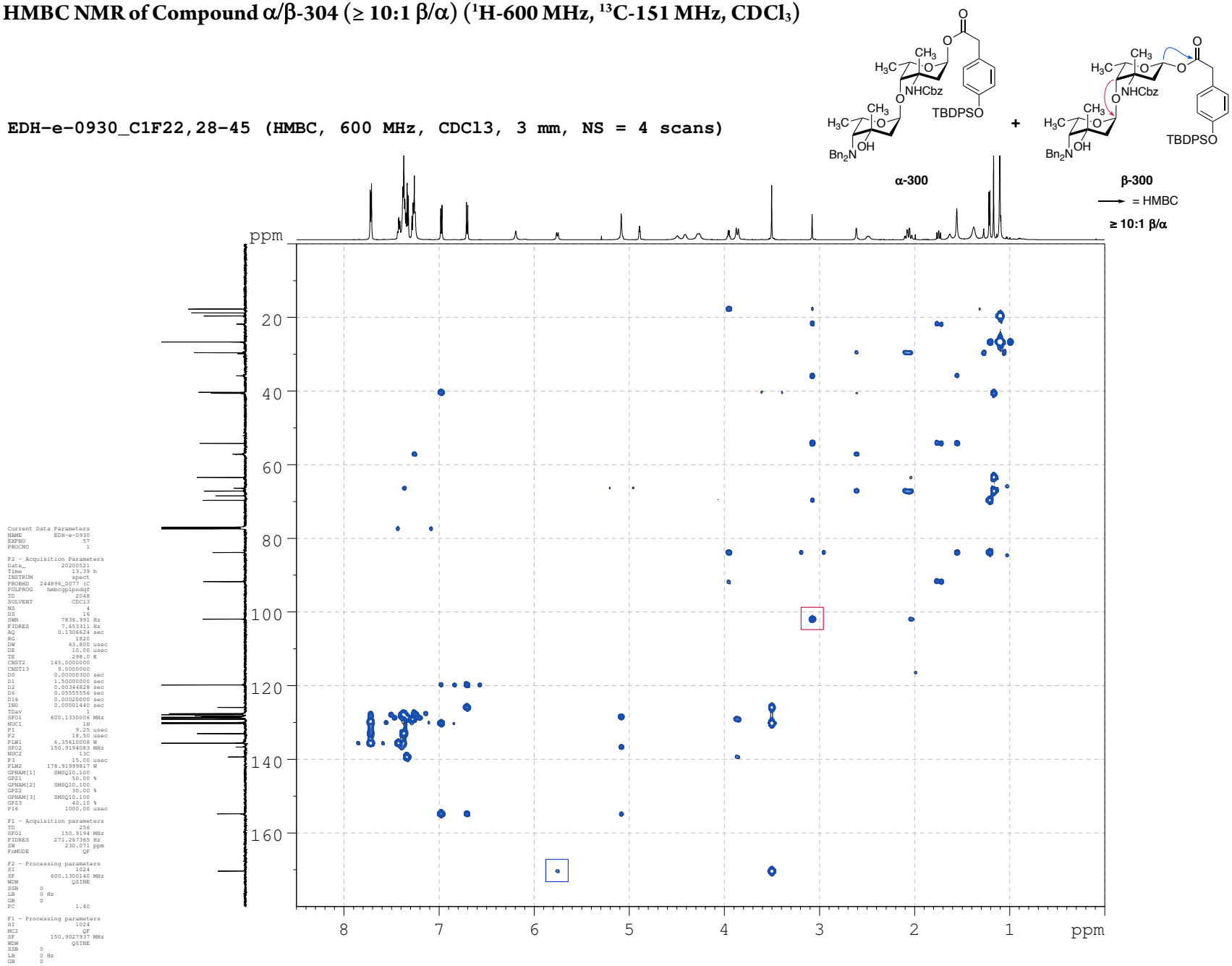

^{1}H-^{13}C HMBC NMR of Compound α/β-304 (≥ 10:1 β/α) (^{1}H-600 MHz, ^{13}C-151 MHz, $CDCl_3$)



Current Data Parameters
NAME EDH-e-0930
EXPNO 57
PROCNO 1

F2 - Acquisition Parameters
Date_ 20200521
Time 13.39 h
INSTRUM spect
PROBHD Z44896_0077 (C
PULPROG hmbcgplpndqf
TD 2048
SOLVENT CDCl3
NS 4
DS 16
SWH 7836.991 Hz
FIDRES 7.653311 Hz
AQ 0.1306624 sec
RG 1820
DW 63.800 usec
DE 10.00 usec
TE 298.0 K
CNST2 145.0000000
CNST13 9.0000000
D0 0.00000300 sec
D1 1.50000000 sec
D2 0.00344828 sec
D6 0.05555556 sec
D16 0.00020000 sec
IN0 0.00001440 sec
TDav 1
SFO1 600.1330006 MHz
NUC1 1H
P1 9.25 usec
P2 18.50 usec
PLW1 6.35610008 W
SFO2 150.9194083 MHz
NUC2 13C
P3 15.00 usec
PLW2 178.91999817 W
GPNAM[1] SMSQ10.100
GPZ1 50.00 %
GPNAM[2] SMSQ10.100
GPZ2 30.00 %
GPNAM[3] SMSQ10.100
GPZ3 40.10 %
P16 1000.00 usec

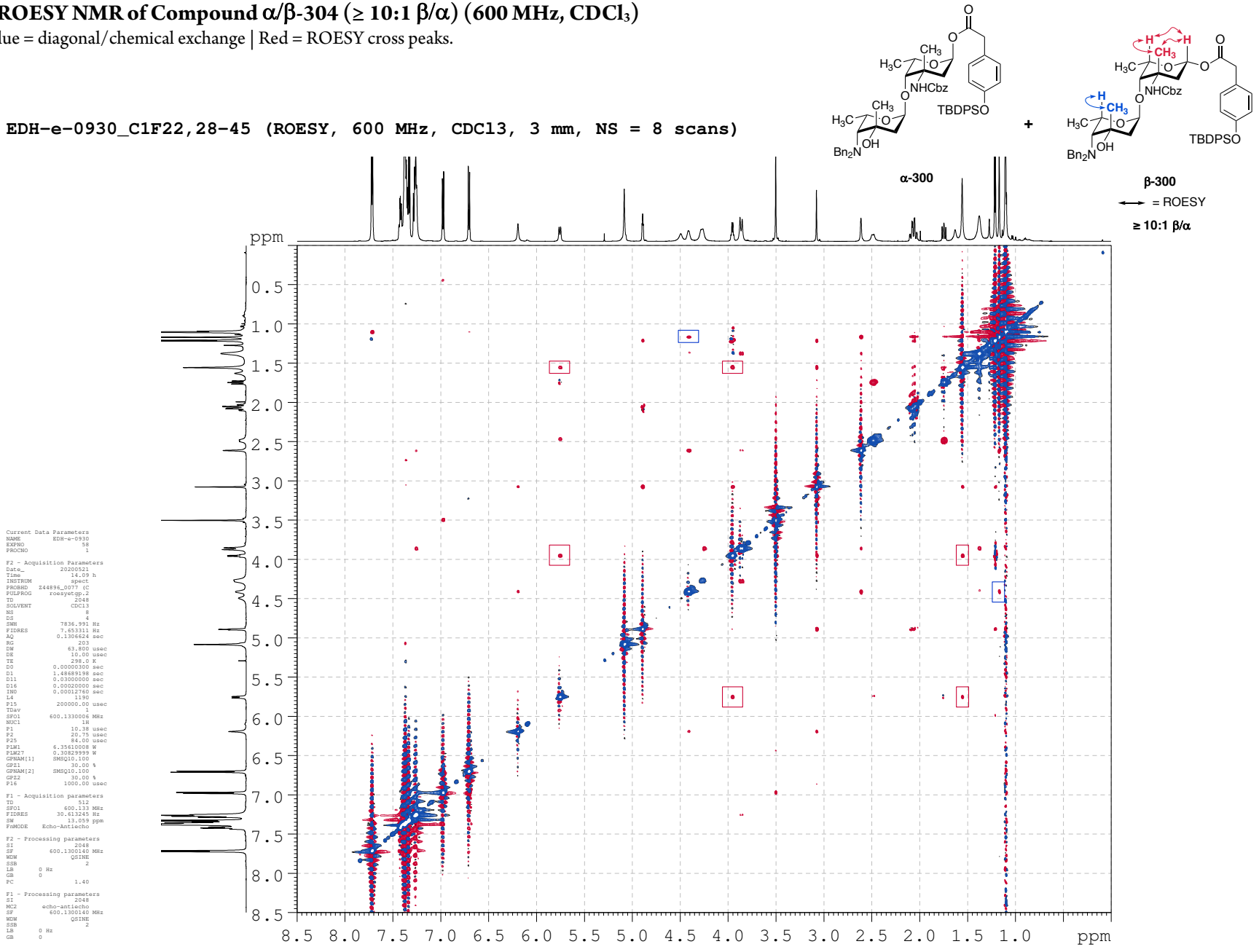
F1 - Acquisition parameters
TD 256
SFO1 150.9194 MHz
FIDRES 271.267365 Hz
SW 230.071 ppm
FnMODE QF

F2 - Processing parameters
SI 1024
SF 600.1300140 MHz
WDW QSINE
SSB 0
LB 0 Hz
GB 0
PC 1.40

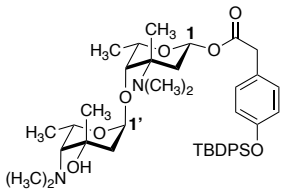
F1 - Processing parameters
SI 1024
MC2 QF
SF 150.9027937 MHz
WDW QSINE
SSB 0
LB 0 Hz
GB 0

^{1}H-^{1}H ROESY NMR of Compound α/β-304 (≥ 10:1 β/α) (600 MHz, $CDCl_3$)

Note: Blue = diagonal/chemical exchange | Red = ROESY cross peaks.

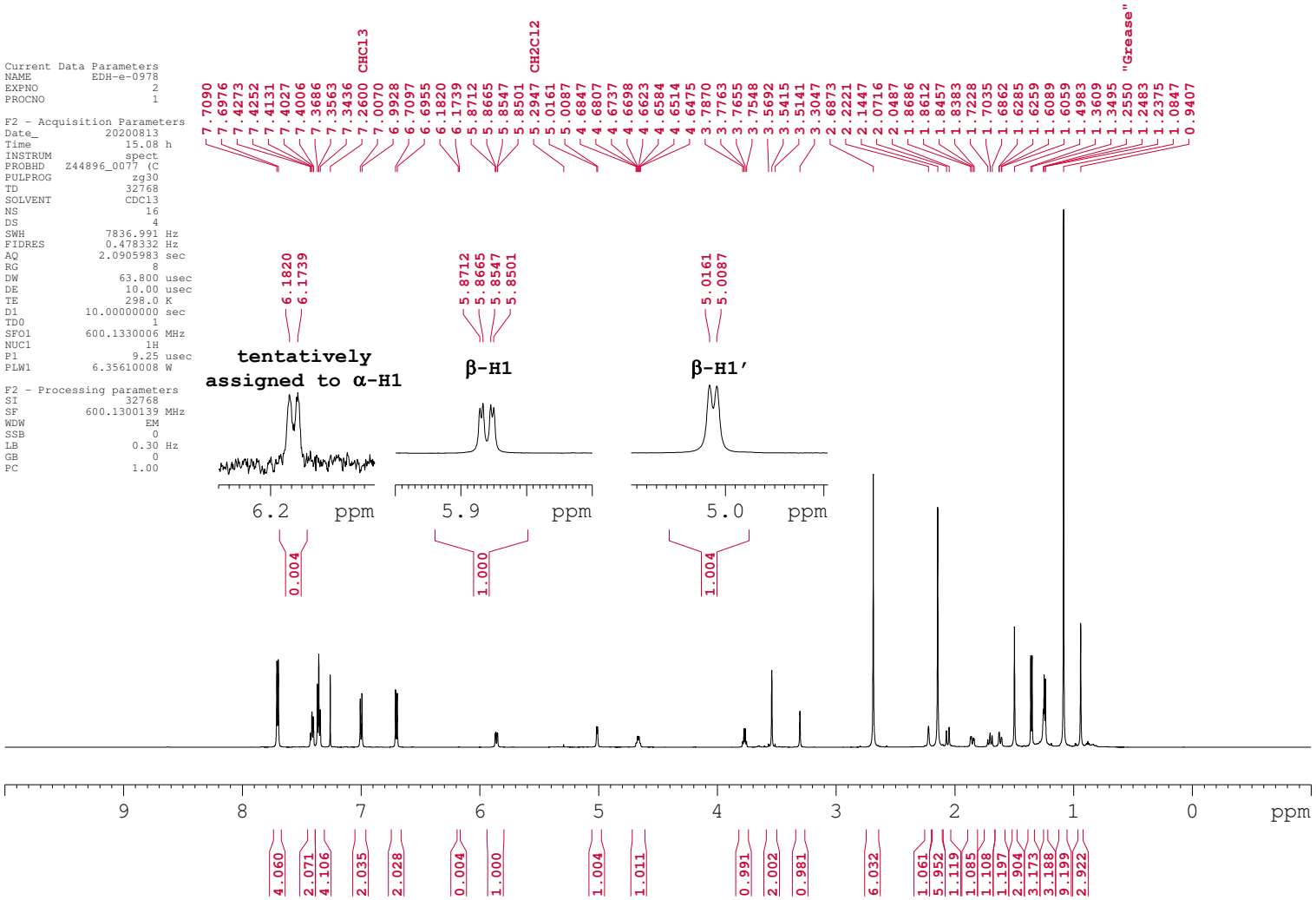


^{1}H NMR of Compound β-300 (≥ 20:1 β/α) (600 MHz, $CDCl_3$)

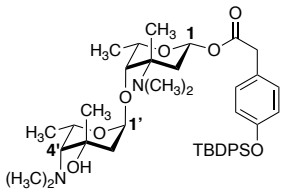


β-300

≥ 20:1 β/α

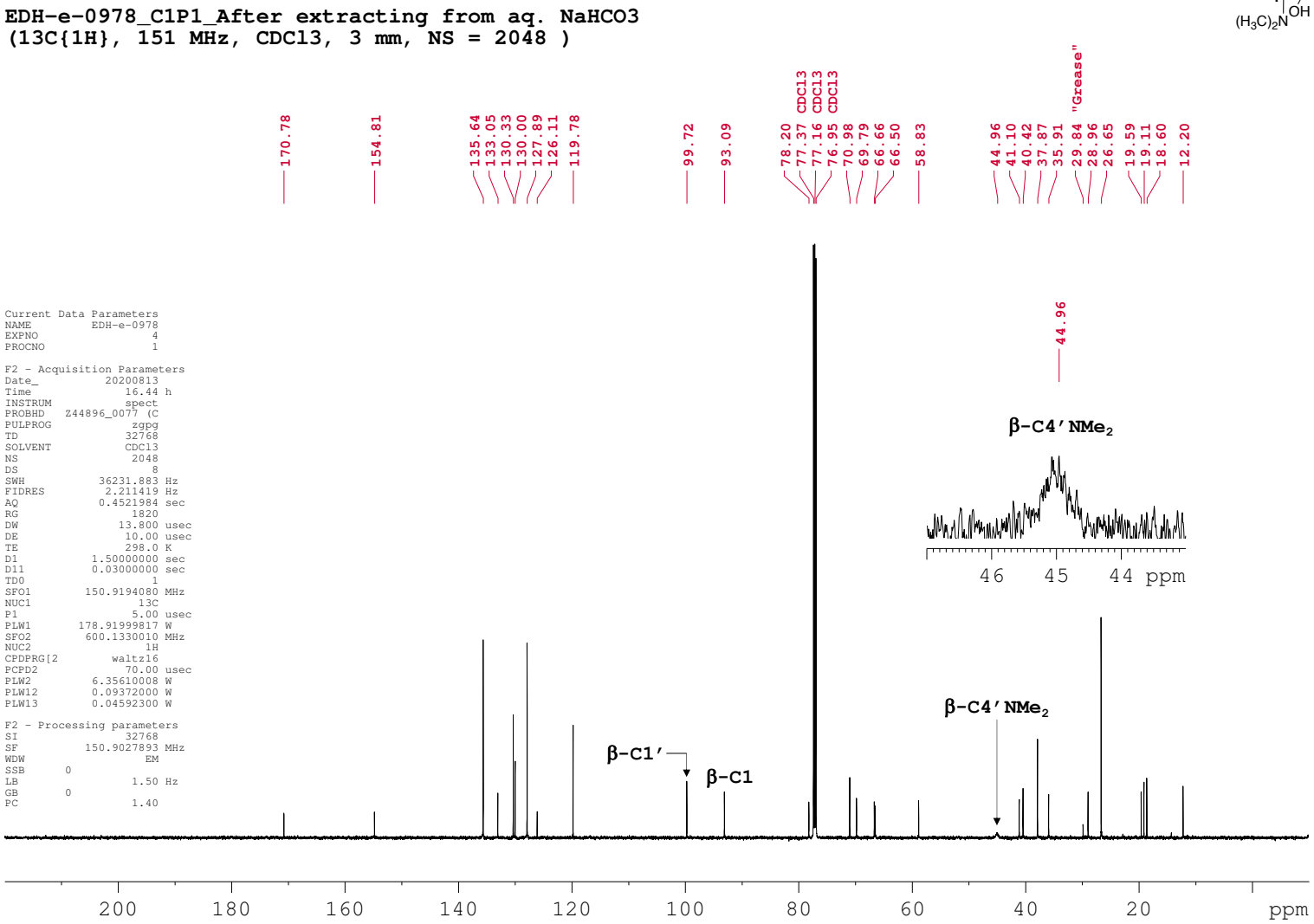


^{13}C NMR of Compound β-300 (≥ 20:1 β/α) (151 MHz, $CDCl_3$)



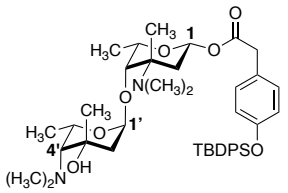
β-300

≥ 20:1 β/α



^{1}H-^{13}C HSQC{^{1}H}{^{13}C} NMR of Compound β-300 (≥ 20:1 β/α) (^{1}H-600 MHz, ^{13}C-151 MHz, $CDCl_3$)

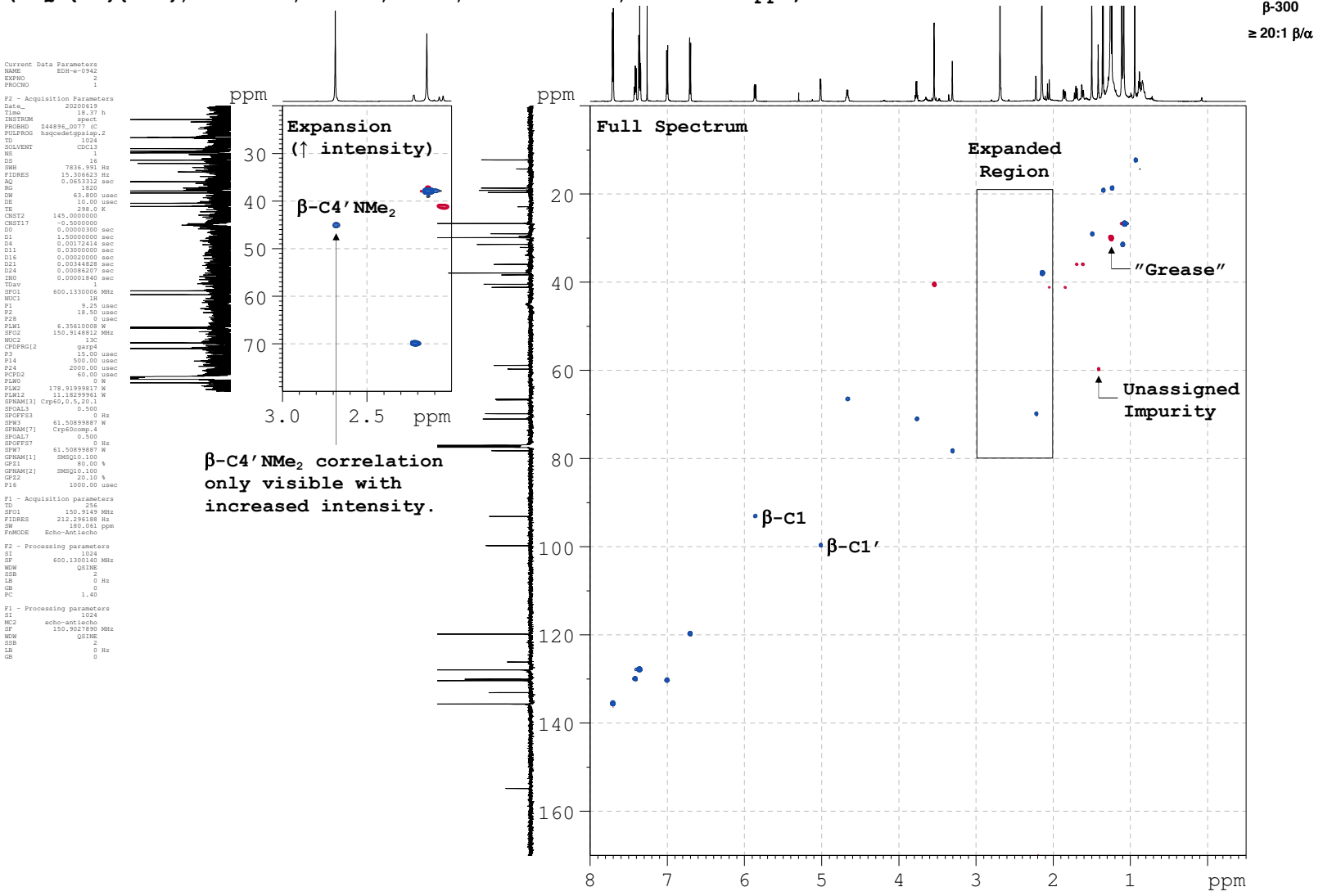
Note: Blue = CH/CH_3 | Red = CH_2.



β-300

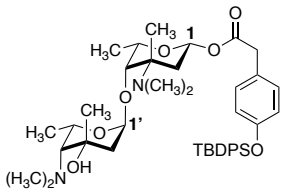
≥ 20:1 β/α

EDH-e-0942_C1F25-28_After extracting from aq. NaHCO3
(HSQC{1H}{13C}, 600 MHz, CDCl3, 3 mm, NS = 1 scan, O2P = 80 ppm)



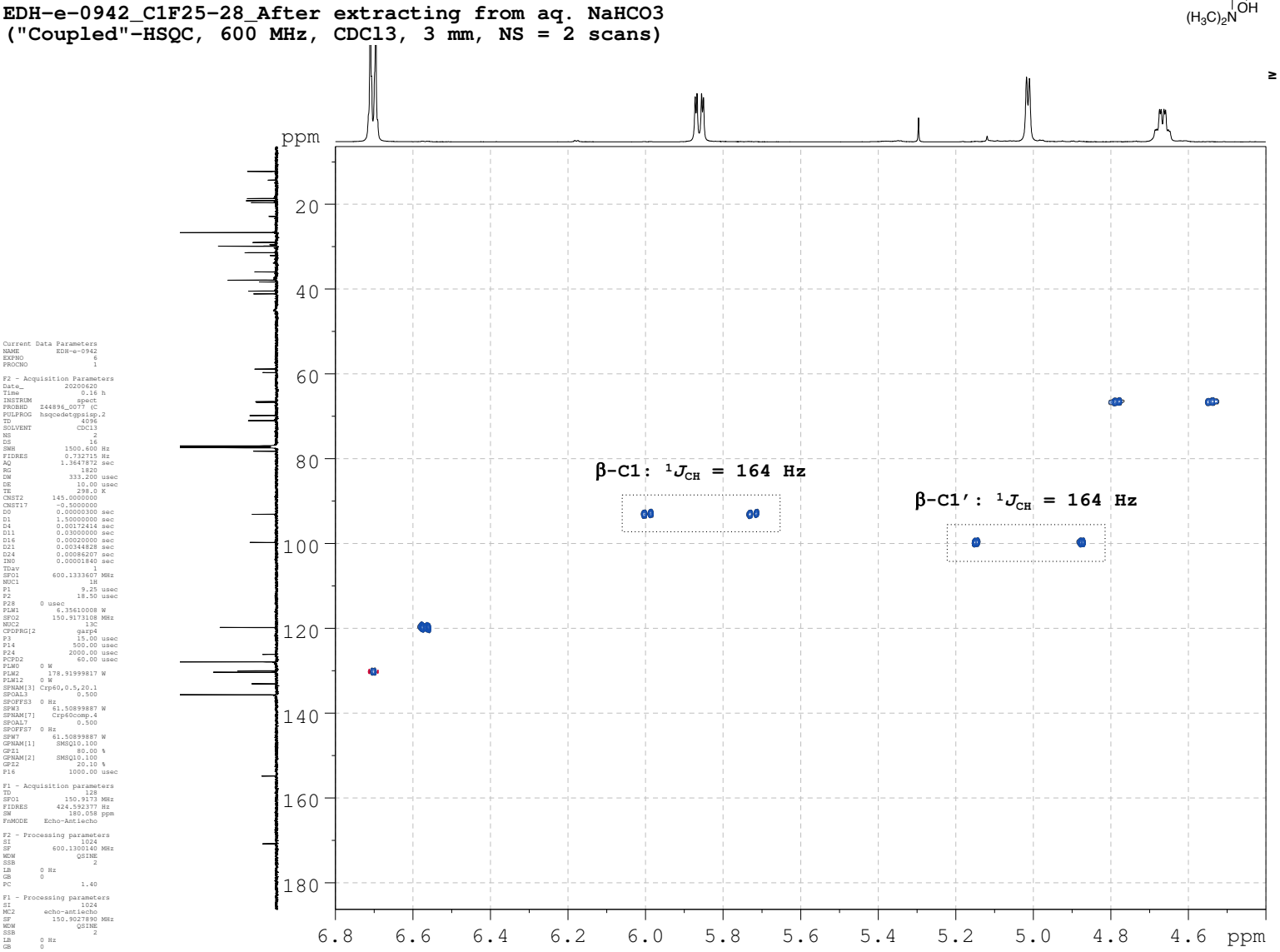
Coupled ^{1}H-^{13}C HSQC NMR of Compound β-300 (≥ 20:1 β/α) (^{1}H-600 MHz, ^{13}C-151 MHz, $CDCl_3$)

Note: Blue = CH/CH_3 | Red = CH_2.



β-300

≥ 20:1 β/α



Current Data Parameters
NAME EDH-e-0942
EXPNO 6
PROCNO 1

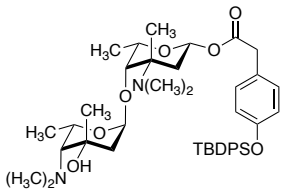
F2 - Acquisition Parameters
Date_ 20200620
Time 0.16 h
INSTRUM spect
PROBHD Z44896_0077 (C
PULPROG hsqcedetgpsisp.2
TD 4096
SOLVENT CDCl3
NS 2
DS 16
SWH 1500.600 Hz
FIDRES 0.732715 Hz
AQ 1.3647872 sec
RG 1820
DW 333.200 usec
DE 10.00 usec
TE 298.0 K
CNST2 145.0000000
CNST17 -0.5000000
D0 0.00000300 sec
D1 1.50000000 sec
D4 0.00172414 sec
D11 0.03000000 sec
D16 0.00020000 sec
D21 0.00344828 sec
D24 0.00086207 sec
IN0 0.00001840 sec
TDav 1
SFO1 600.1333607 MHz
NUC1 1H
P1 9.25 usec
P2 18.50 usec
P28 0 usec
PLW1 6.35610008 W
SFO2 150.9173108 MHz
NUC2 13C
CPDPRG[2 garp4
P3 15.00 usec
P14 500.00 usec
P24 2000.00 usec
PCPD2 60.00 usec
PLW0 0 W
PLW2 178.91999817 W
PLW12 0 W
SPNAM[3] Crp60,0.5,20.1
SPOAL3 0.500
SPOFFS3 0 Hz
SPW3 61.50899887 W
SPNAM[7] Crp60comp.4
SPOAL7 0.500
SPOFFS7 0 Hz
SPW7 61.50899887 W
GPNAM[1] SMSQ10.100
GPZ1 80.00 %
GPNAM[2] SMSQ10.100
GPZ2 20.10 %
P16 1000.00 usec

F1 - Acquisition parameters
TD 128
SFO1 150.9173 MHz
FIDRES 424.592377 Hz
SW 180.058 ppm
FnMODE Echo-Antiecho

F2 - Processing parameters
SI 1024
SF 600.1300140 MHz
WDW QSINE
SSB 2
LB 0 Hz
GB 0
PC 1.40

F1 - Processing parameters
SI 1024
MC2 echo-antiecho
SF 150.9027890 MHz
WDW QSINE
SSB 2
LB 0 Hz
GB 0

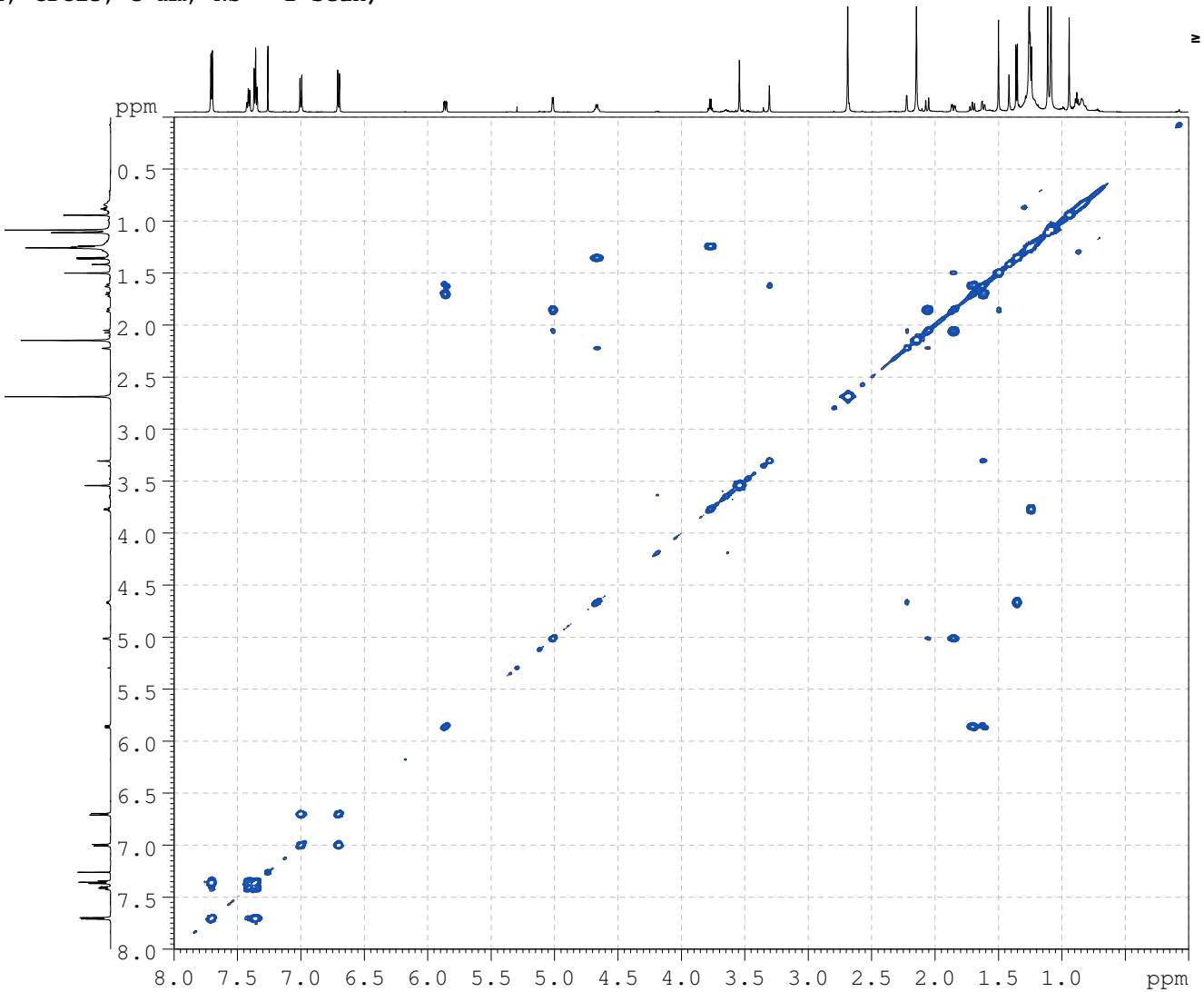
^{1}H-^{1}H COSY NMR of Compound β-300 (≥ 20:1 β/α) (600 MHz, $CDCl_3$)



β-300

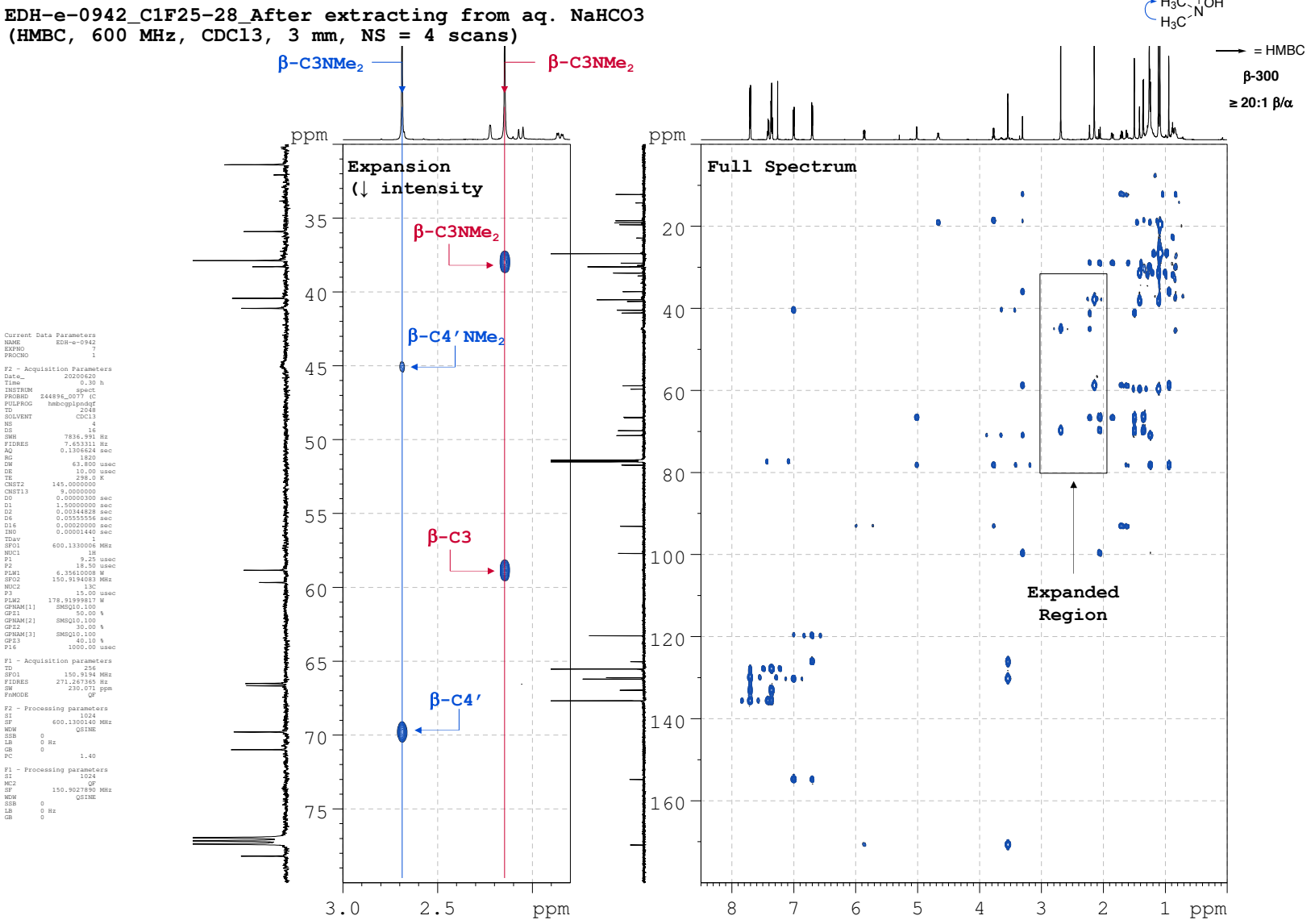
≥ 20:1 β/α

EDH-e-0942_C1F25-28_After extracting from aq. NaHCO3
(COSY, 600 MHz, CDCl3, 3 mm, NS = 1 scan)



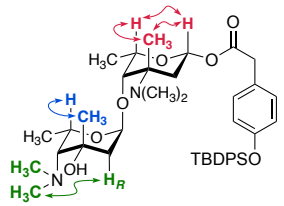

```
Current Data Parameters
NAME          EDH-e-0942
EXPNO                  5
PROCNO                 1

F2 - Acquisition Parameters
Date_          20200620
Time_      0 h
INSTRUM            spect
PROBHD    Z44896_0077 (C
PULPROG        cosygpqf
TD                 2048
SOLVENT           CDCl3
NS                    1
DS                    8
SWH           7836.991 Hz
FIDRES        7.653311 Hz
AQ            0.1306624 sec
RG                  228
DW               63.800 usec
DE               10.00 usec
TE                298.0 K
D0           0.00000300 sec
D1           1.48689198 sec
D13          0.00000400 sec
D16          0.00020000 sec
IN0          0.00012760 sec
TDav                  1
SFO1        600.1330006 MHz
NUC1                1H
P0                4.65 usec
P1                9.30 usec
PLW1         6.35610008 W
GPNAM[1]     SMSQ10.100
GPZ1             10.00 %
P16            1000.00 usec

F1 - Acquisition parameters
TD                  512
SFO1        600.133 MHz
FIDRES       30.613245 Hz
SW           13.059 ppm
FnMODE              QF

F2 - Processing parameters
SI                 1024
SF          600.1300140 MHz
WDW              QSINE
SSB       0
LB        0 Hz
GB        0
PC              1.40

F1 - Processing parameters
SI                 1024
MC2                QF
SF          600.1300140 MHz
WDW              QSINE
SSB       0
LB        0 Hz
GB        0
```

^{1}H-^{13}C HMBC NMR of Compound β-300 (≥ 20:1 β/α) (^{1}H-600 MHz, ^{13}C-151 MHz, $CDCl_3$)



^{1}H-^{1}H NOESY NMR of Compound β-300 (≥ 20:1 β/α) (600 MHz, $CDCl_3$)

Note: Blue = diagonal/chemical exchange | Red = NOESY cross peaks.

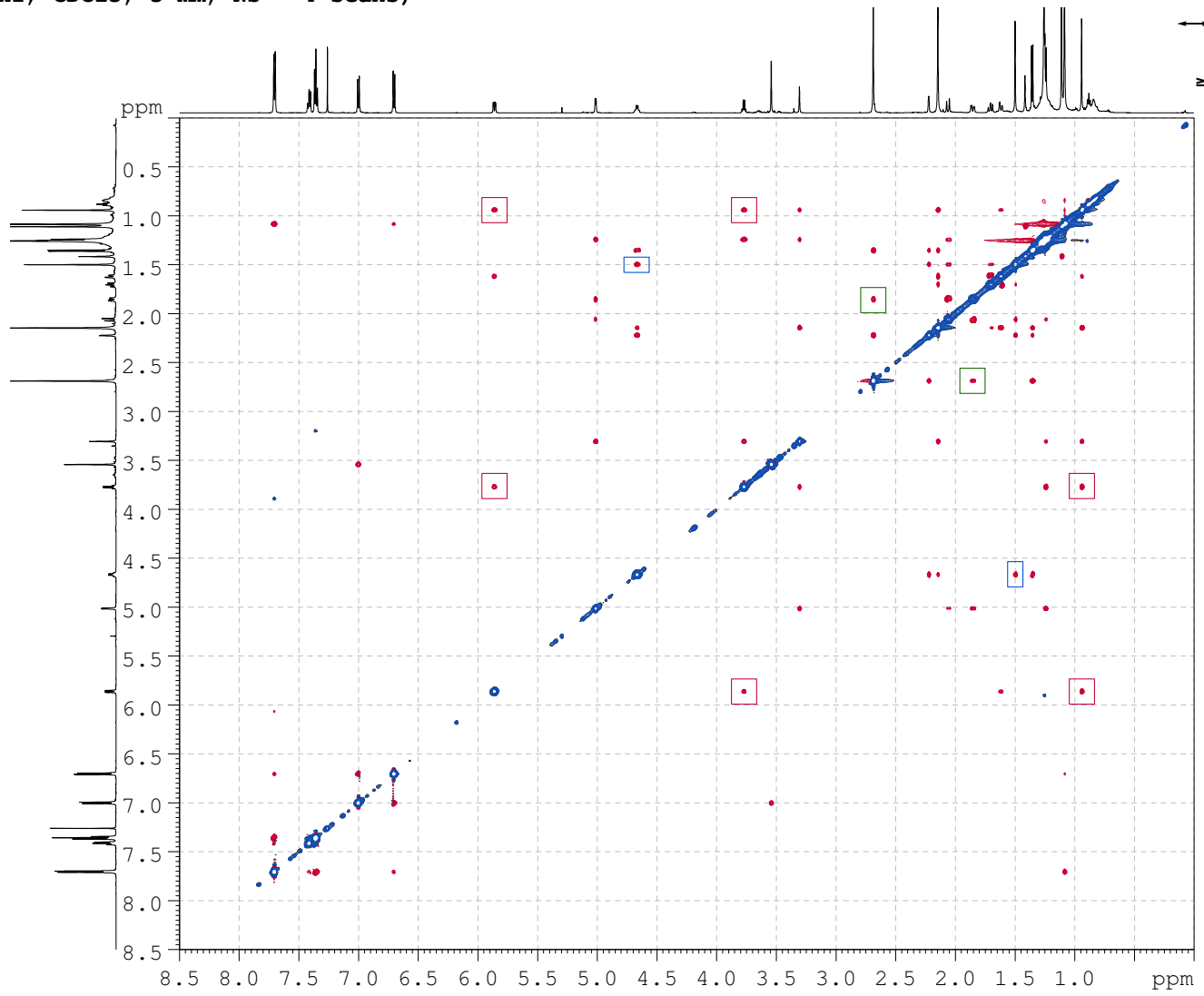


= NOESY

β-300

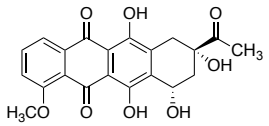
≥ 20:1 β/α

EDH-e-0942_C1F25-28_After extracting from aq. NaHCO3
(NOESY, 600 MHz, CDCl3, 3 mm, NS = 4 scans)

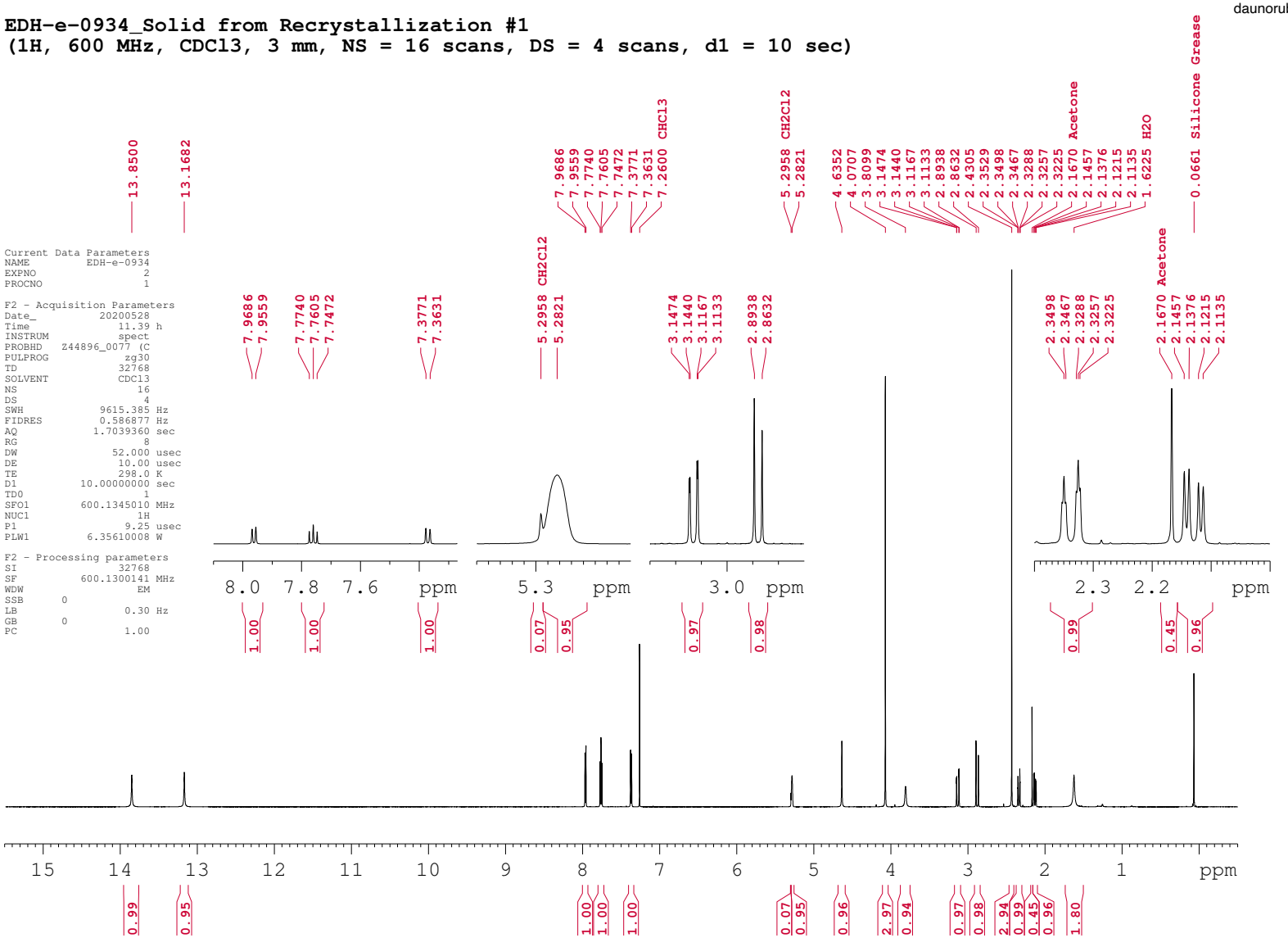



```
Current Data Parameters
NAME          EDH-e-0942
EXPNO                  8
PROCNO                 1

F2 - Acquisition Parameters
Date_           20200620
Time              1.00 h
INSTRUM             spect
PROBHD    Z44896_0077 (C
PULPROG         noesygpph
TD                  2048
SOLVENT            CDCl3
NS                     4
DS                    16
SWH            7836.991 Hz
FIDRES         7.653311 Hz
AQ             0.1306624 sec
RG                   128
DW                63.800 usec
DE                10.00 usec
TE                298.0 K
D0           0.00005202 sec
D1           1.48689198 sec
D8           0.60000002 sec
D16          0.00020000 sec
IN0          0.00012760 sec
TDav                   1
SFO1         600.1330006 MHz
NUC1                  1H
P1                 9.25 usec
P2                18.50 usec
PLW1          6.35610008 W
GPNAM[1]      SMSQ10.100
GPZ1               40.00 %
P16             1000.00 usec

F1 - Acquisition parameters
TD                   512
SFO1           600.133 MHz
FIDRES        30.613245 Hz
SW                13.059 ppm
FnMODE       States-TPPI

F2 - Processing parameters
SI                  1024
SF           600.1300140 MHz
WDW                 QSINE
SSB                     2
LB         0 Hz
GB         0
PC                  1.40

F1 - Processing parameters
SI                  1024
MC2          States-TPPI
SF           600.1300140 MHz
WDW                 QSINE
SSB                     2
LB         0 Hz
GB         0
```

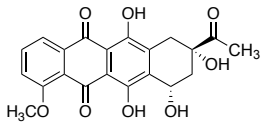
^{1}H NMR of Daunorubicinone (28) (600 MHz, $CDCl_3$)



daunorubicinone (**28**)

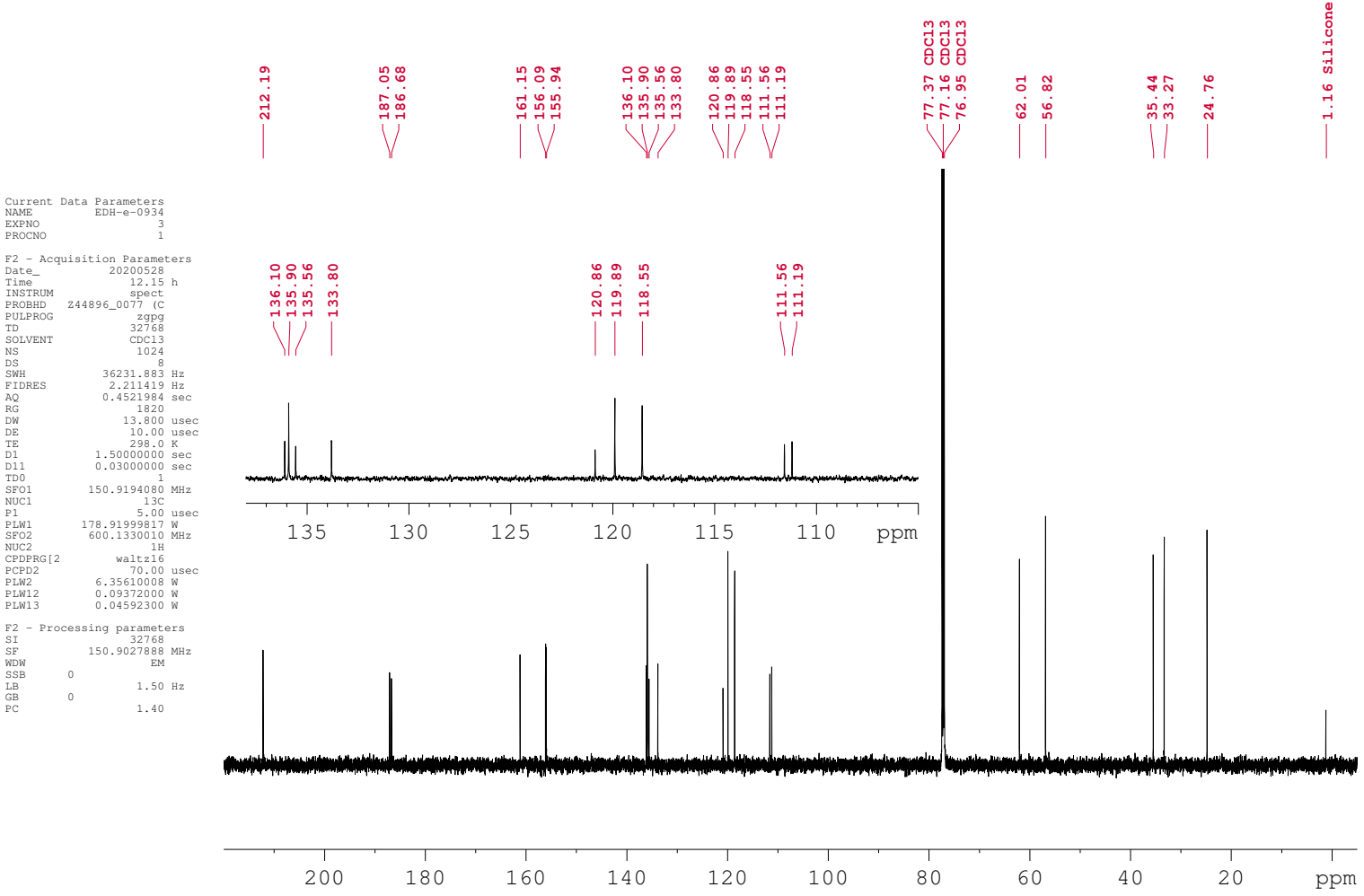


^{13}C NMR of Daunorubicinone (28) (151 MHz, $CDCl_3$)



daunorubicinone (**28**)

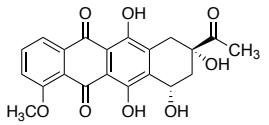
EDH-e-0934_Solid from Recrystallization #1 (13C{1H}, 151 MHz, CDCl3, 3 mm, NS = 1024 scans)



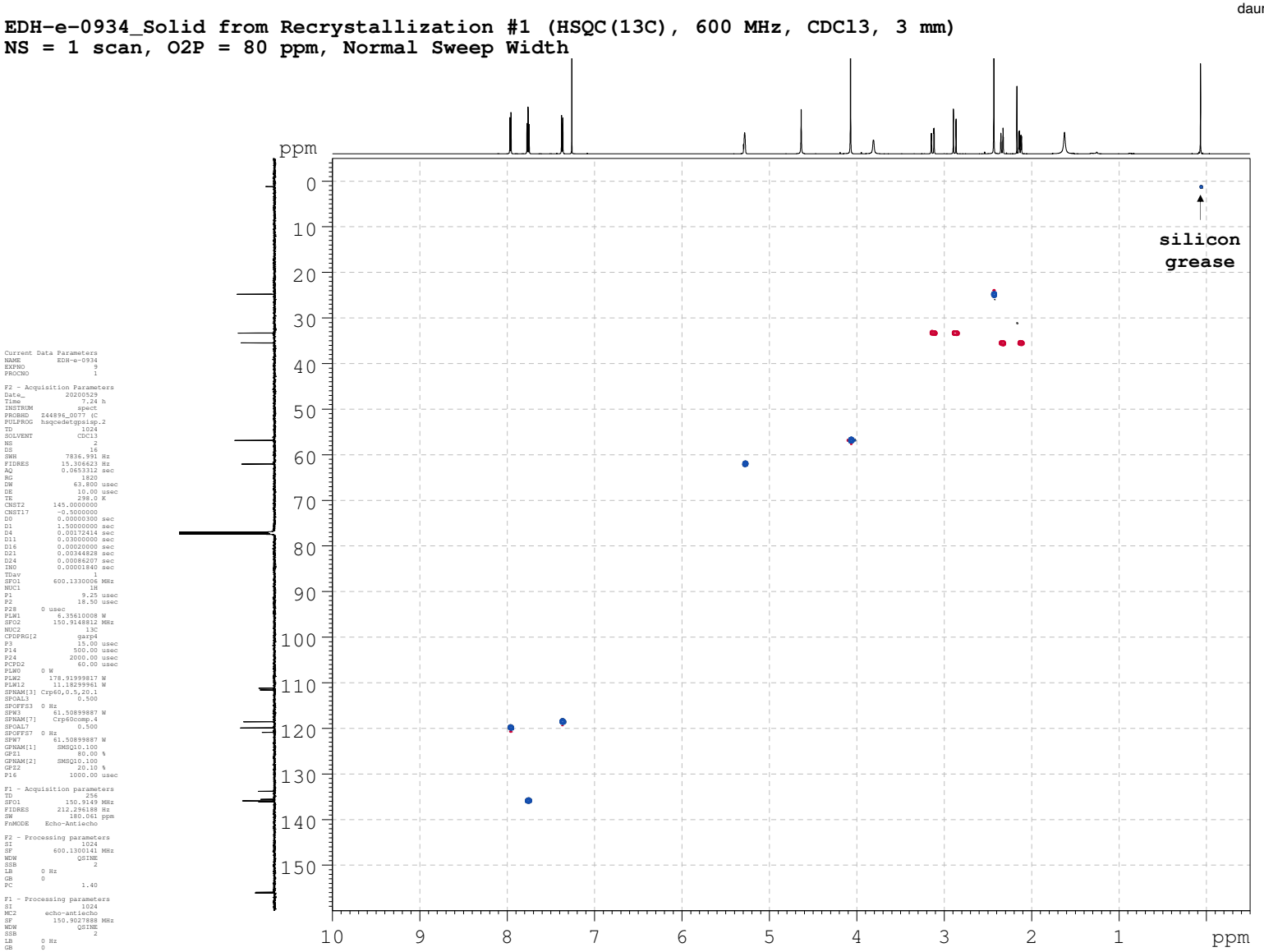
^{1}H-^{13}C HSQC{^{1}H}{^{13}C} NMR of Daunorubicinone (28) (^{1}H-600 MHz, ^{13}C-151 MHz, $CDCl_3$)

Note 1: Blue = CH/CH_3 | Red = CH_2.

Note 2: Unaltered ^{1}H spectral window (-1.5 to 11.5 ppm).



daunorubicinone (**28**)



Current Data Parameters
NAME EDH-e-0934
EXPNO 9
PROCNO 1

F2 - Acquisition Parameters
Date_ 20200529
Time 7.24 h
INSTRUM spect
PROBHD Z44896_0077 (C
PULPROG hsqcedetgpsisp.2
TD 1024
SOLVENT CDCl3
NS 2
DS 16
SWH 7836.991 Hz
FIDRES 15.306623 Hz
AQ 0.0653312 sec
RG 1820
DW 63.800 usec
DE 10.00 usec
TE 298.0 K
CNST2 145.0000000
CNST17 -0.5000000
D0 0.00000300 sec
D1 1.50000000 sec
D4 0.00172414 sec
D11 0.03000000 sec
D16 0.00020000 sec
D21 0.00344828 sec
D24 0.00086207 sec
IN0 0.00001840 sec
TDav 1
SFO1 600.1330006 MHz
NUC1 1H
P1 9.25 usec
P2 18.50 usec
P28 0 usec
PLW1 6.35610008 W
SFO2 150.9148812 MHz
NUC2 13C
CPDPRG[2 garp4
P3 15.00 usec
P14 500.00 usec
P24 2000.00 usec
PCPD2 60.00 usec
PLW0 0 W
PLW2 178.91999817 W
PLW12 11.18299961 W
SPNAM[3] Crp60,0.5,20.1
SPOAL3 0.500
SPOFFS3 0 Hz
SPW3 61.50899887 W
SPNAM[7] Crp60comp.4
SPOAL7 0.500
SPOFFS7 0 Hz
SPW7 61.50899887 W
GPNAM[1] SMSQ10.100
GPZ1 80.00 %
GPNAM[2] SMSQ10.100
GPZ2 20.10 %
P16 1000.00 usec

F1 - Acquisition parameters
TD 256
SFO1 150.9149 MHz
FIDRES 212.296188 Hz
SW 180.061 ppm
FnMODE Echo-Antiecho

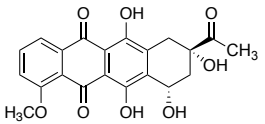
F2 - Processing parameters
SI 1024
SF 600.1300141 MHz
WDW QSINE
SSB 2
LB 0 Hz
GB 0
PC 1.40

F1 - Processing parameters
SI 1024
MC2 echo-antiecho
SF 150.9027888 MHz
WDW QSINE
SSB 2
LB 0 Hz
GB 0

^{1}H-^{13}C HSQC$\{^{1}H\}\{^{13}C\}$ NMR of Daunorubicinone (28) (^{1}H-600 MHz, ^{13}C-151 MHz, $CDCl_3$)

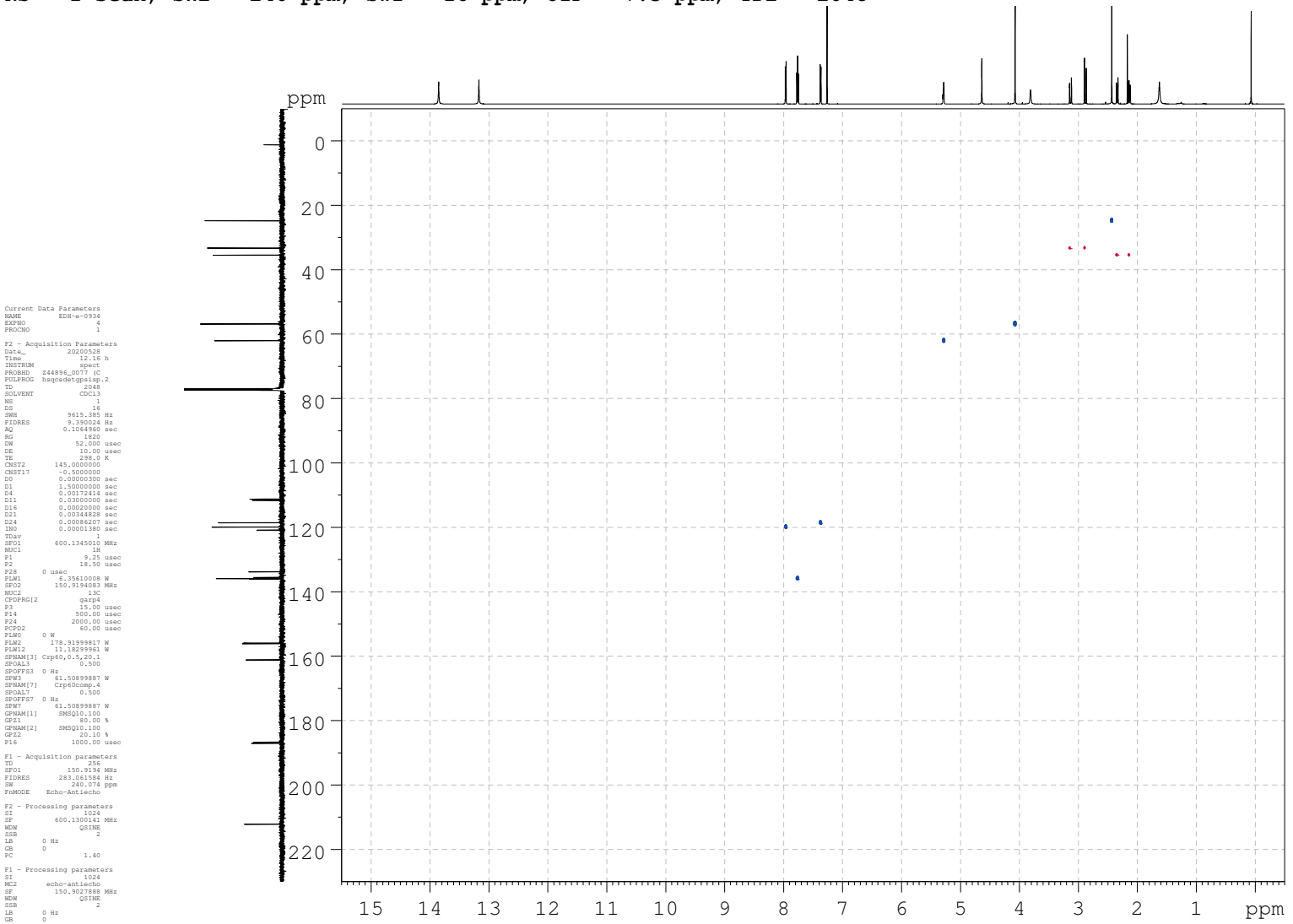
Note 1: Blue = CH/CH_3 | Red = CH_2.

Note 2: Extended ^{1}H spectral window (-0.5 to 15.5 ppm).



daunorubicinone (**28**)

EDH-e-0934_Solid from Recrystallization #1 (HSQC{1H}{13C}, 600 MHz, CDCl3, 3 mm)
NS = 1 scan, SW2 = 240 ppm, Sw1 = 16 ppm, O1P = 7.5 ppm, TD2 = 2048



Current Data Parameters
NAME EDH-e-0934
EXPNO 4
PROCNO 1

F2 - Acquisition Parameters
Date_ 20200528
Time 12.16 h
INSTRUM spect
PROBHD Z44896_0077 (C
PULPROG hsqcedetgpsisp.2
TD 2048
SOLVENT CDCl3
NS 1
DS 16
SWH 9615.385 Hz
FIDRES 9.390024 Hz
AQ 0.1064960 sec
RG 1820
DW 52.000 usec
DE 10.00 usec
TE 298.0 K
CNST2 145.0000000
CNST17 -0.5000000
D0 0.00000300 sec
D1 1.50000000 sec
D4 0.00172414 sec
D11 0.03000000 sec
D16 0.00020000 sec
D21 0.00344828 sec
D24 0.00086207 sec
IN0 0.00001380 sec
TDav 1
SFO1 600.1345010 MHz
NUC1 1H
P1 9.25 usec
P2 18.50 usec
P28 0 usec
PLW1 6.35610008 W
SFO2 150.9194083 MHz
NUC2 13C
CPDPRG[2 garp4
P3 15.00 usec
P14 500.00 usec
P24 2000.00 usec
PCPD2 60.00 usec
PLW0 0 W
PLW2 178.91999817 W
PLW12 11.18299961 W
SPNAM[3] Crp60,0.5,20.1
SPOAL3 0.500
SPOFFS3 0 Hz
SPW3 61.50899887 W
SPNAM[7] Crp60comp.4
SPOAL7 0.500
SPOFFS7 0 Hz
SPW7 61.50899887 W
GPNAM[1] SMSQ10.100
GPZ1 80.00 %
GPNAM[2] SMSQ10.100
GPZ2 20.10 %
P16 1000.00 usec

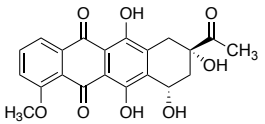
F1 - Acquisition parameters
TD 256
SFO1 150.9194 MHz
FIDRES 283.061584 Hz
SW 240.074 ppm
FnMODE Echo-Antiecho

F2 - Processing parameters
SI 1024
SF 600.1300141 MHz
WDW QSINE
SSB 2
LB 0 Hz
GB 0
PC 1.40

F1 - Processing parameters
SI 1024
MC2 echo-antiecho
SF 150.9027888 MHz
WDW QSINE
SSB 2
LB 0 Hz
GB 0

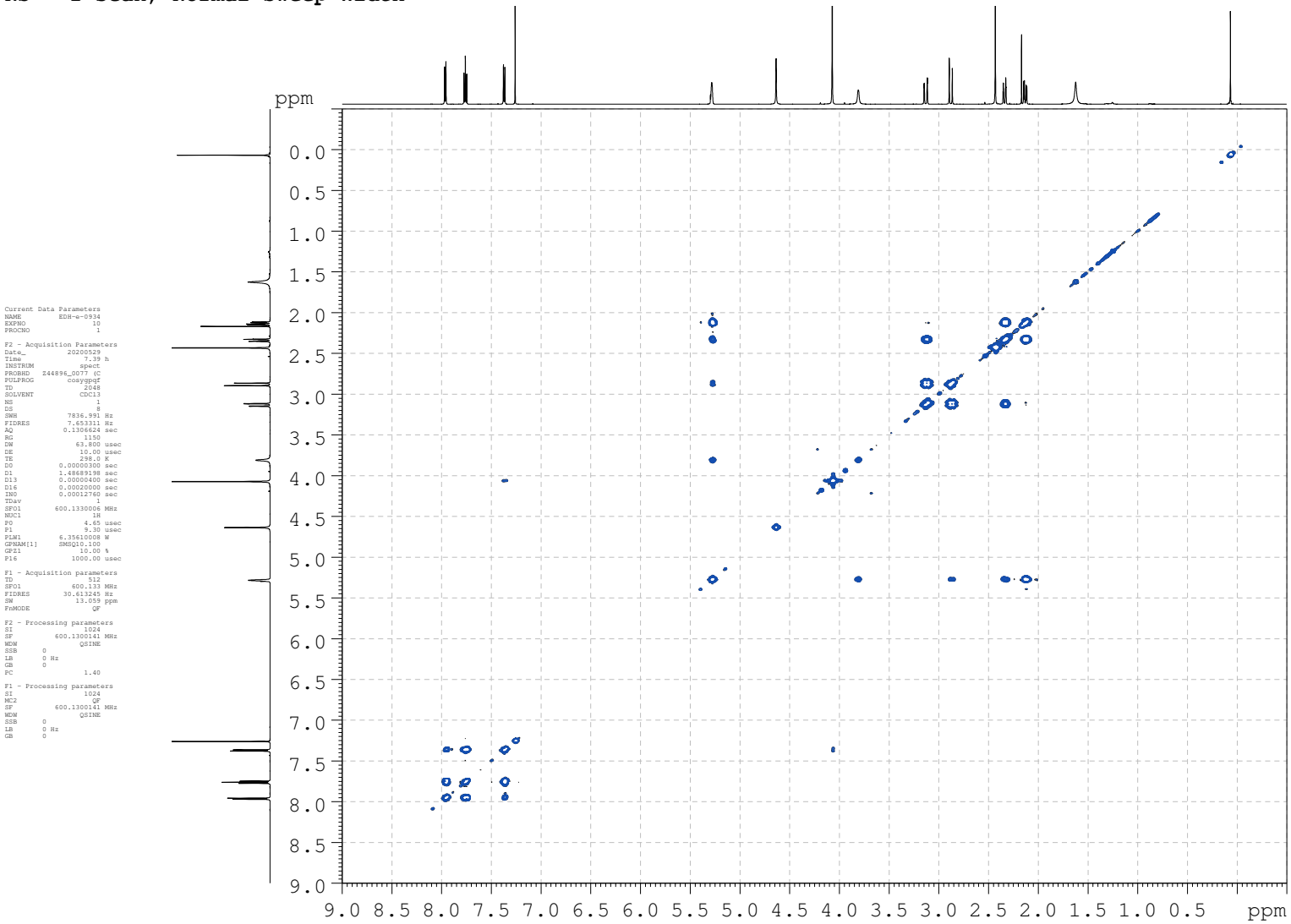
^{1}H-^{1}H COSY NMR of Daunorubicinone (28) (600 MHz, $CDCl_3$)

Note: Unaltered ^{1}H spectral window (-1.5 to 11.5 ppm).



daunorubicinone (**28**)

EDH-e-0934_Solid from Recrystallization #1 (COSY, 600 MHz, CDCl3, 3 mm)
NS = 1 scan, Normal Sweep Width

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Current Data Parameters
NAME          EDH-e-0934
EXPNO                 10
PROCNO                 1

F2 - Acquisition Parameters
Date_           20200529
Time              7.39 h
INSTRUM             spect
PROBHD   Z44896_0077 (C
PULPROG         cosygpqf
TD                  2048
SOLVENT             CDCl3
NS                     1
DS                     8
SWH             7836.991 Hz
FIDRES          7.653311 Hz
AQ              0.1306624 sec
RG                  1150
DW                 63.800 usec
DE                 10.00 usec
TE                298.0 K
D0              0.00000300 sec
D1              1.48689198 sec
D13             0.00000400 sec
D16             0.00020000 sec
IN0             0.00012760 sec
TDav                   1
SFO1          600.1330006 MHz
NUC1                  1H
P0                  4.65 usec
P1                  9.30 usec
PLW1          6.35610008 W
GPNAM[1]      SMSQ10.100
GPZ1               10.00 %
P16              1000.00 usec

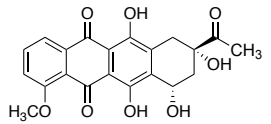
F1 - Acquisition parameters
TD                   512
SFO1          600.133 MHz
FIDRES         30.613245 Hz
SW                13.059 ppm
FnMODE                QF

F2 - Processing parameters
SI                  1024
SF            600.1300141 MHz
WDW                 QSINE
SSB         0
LB        0 Hz
GB          0
PC                  1.40

F1 - Processing parameters
SI                  1024
MC2                   QF
SF            600.1300141 MHz
WDW                 QSINE
SSB         0
LB        0 Hz
GB          0
```

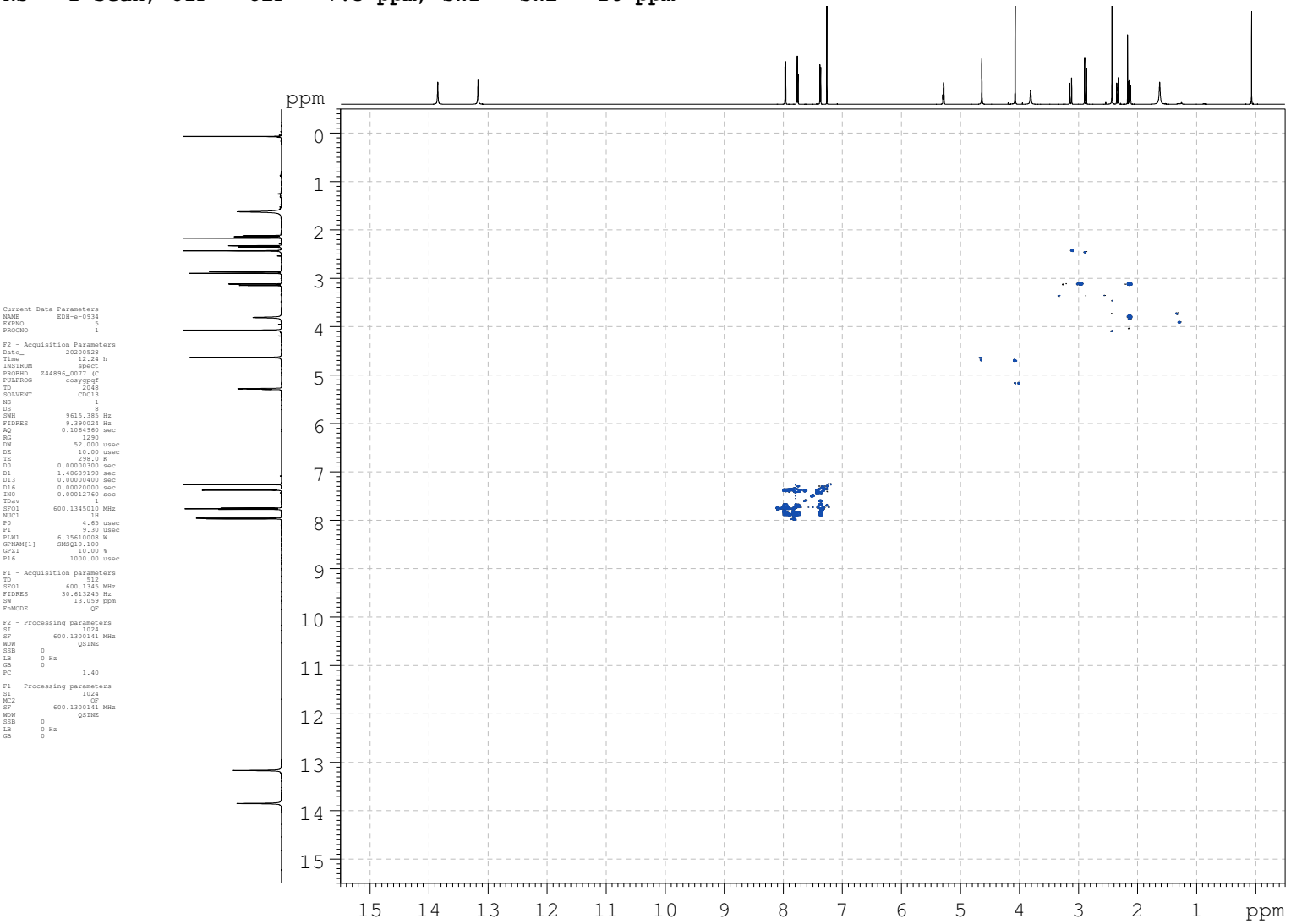
^{1}H-^{1}H COSY NMR of Daunorubicinone (28) (600 MHz, $CDCl_3$)

Note: Extended ^{1}H spectral window (-0.5 to 15.5 ppm).



daunorubicinone (**28**)

EDH-e-0934_Solid from Recrystallization #1 (COSY, 600 MHz, CDCl3, 3 mm)
NS = 1 scan, O1P = O2P = 7.5 ppm, SW1 = SW2 = 16 ppm

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Current Data Parameters
NAME          EDH-e-0934
EXPNO                  5
PROCNO                 1

F2 - Acquisition Parameters
Date_           20200528
Time              12.24 h
INSTRUM             spect
PROBHD   Z44896_0077 (C
PULPROG         cosygpqf
TD                 2048
SOLVENT            CDCl3
NS                    1
DS                    8
SWH            9615.385 Hz
FIDRES         9.390024 Hz
AQ             0.1064960 sec
RG                 1290
DW               52.000 usec
DE               10.00 usec
TE                298.0 K
D0            0.00000300 sec
D1            1.48689198 sec
D13           0.00000400 sec
D16           0.00020000 sec
IN0           0.00012760 sec
TDav                  1
SFO1         600.1345010 MHz
NUC1                 1H
P0                 4.65 usec
P1                 9.30 usec
PLW1          6.35610008 W
GPNAM[1]      SMSQ10.100
GPZ1             10.00 %
P16            1000.00 usec

F1 - Acquisition parameters
TD                   512
SFO1         600.1345 MHz
FIDRES        30.613245 Hz
SW               13.059 ppm
FnMODE                QF

F2 - Processing parameters
SI                  1024
SF           600.1300141 MHz
WDW                 QSINE
SSB       0
LB        0 Hz
GB        0
PC                  1.40

F1 - Processing parameters
SI                  1024
MC2                   QF
SF           600.1300141 MHz
WDW                 QSINE
SSB       0
LB        0 Hz
GB        0
```
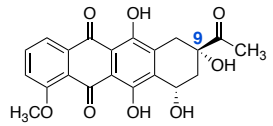
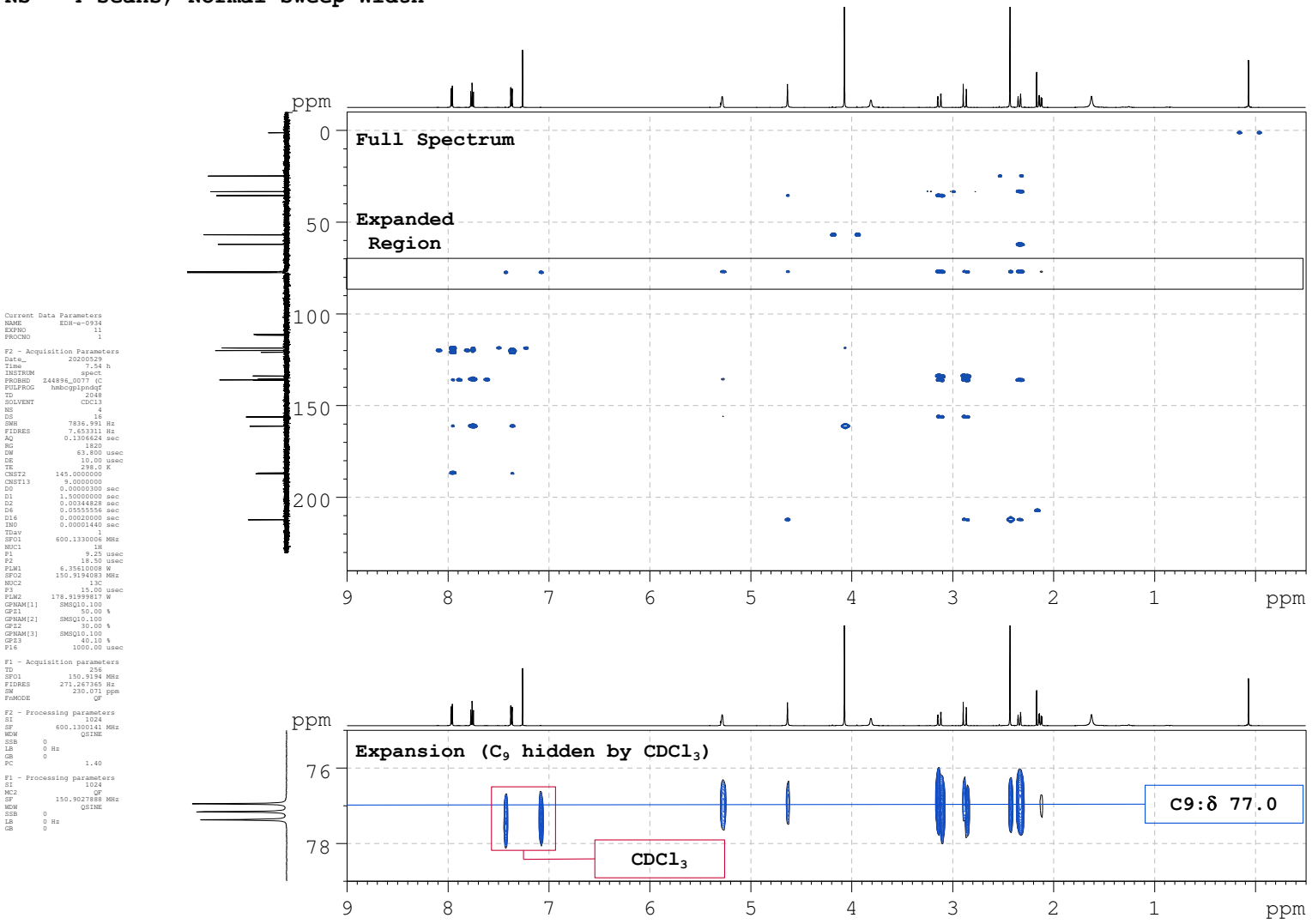

^{1}H-^{13}C HMBC NMR of Daunorubicinone (28) (^{1}H-600 MHz, ^{13}C-151 MHz, $CDCl_3$)

Note: Unaltered ^{1}H spectral window (-1.5 to 11.5 ppm).



daunorubicinone (**28**)

EDH-e-0934_Solid from Recrystallization #1 (HMBC, 600 MHz, CDCl3, 3 mm)
NS = 4 scans, Normal Sweep Width



Current Data Parameters
NAME EDH-e-0934
EXPNO 11
PROCNO 1

F2 - Acquisition Parameters
Date_ 20200529
Time 7.54 h
INSTRUM spect
PROBHD Z44896_0077 (C
PULPROG hmbcgplpndqf
TD 2048
SOLVENT CDCl3
NS 4
DS 16
SWH 7836.991 Hz
FIDRES 7.653311 Hz
AQ 0.1306624 sec
RG 1820
DW 63.800 usec
DE 10.00 usec
TE 298.0 K
CNST2 145.0000000
CNST13 9.0000000
D0 0.00000300 sec
D1 1.50000000 sec
D2 0.00344828 sec
D6 0.05555556 sec
D16 0.00020000 sec
IN0 0.00001440 sec
TDav 1
SFO1 600.1330006 MHz
NDC1 1H
P1 9.25 usec
P2 18.50 usec
PLW1 6.35610008 W
SFO2 150.9194083 MHz
NDC2 13C
P3 15.00 usec
PLW2 178.91999817 W
GPNAM[1] SMSQ10.100
GPZ1 50.00 %
GPNAM[2] SMSQ10.100
GPZ2 30.00 %
GPNAM[3] SMSQ10.100
GPZ3 40.10 %
P16 1000.00 usec

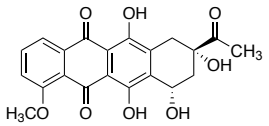
F1 - Acquisition parameters
TD 256
SFO1 150.9194 MHz
FIDRES 271.267365 Hz
SW 230.071 ppm
FnMODE QF

F2 - Processing parameters
SI 1024
SF 600.1300141 MHz
WDW QSINE
SSB 0
LB 0 Hz
GB 0
PC 1.40

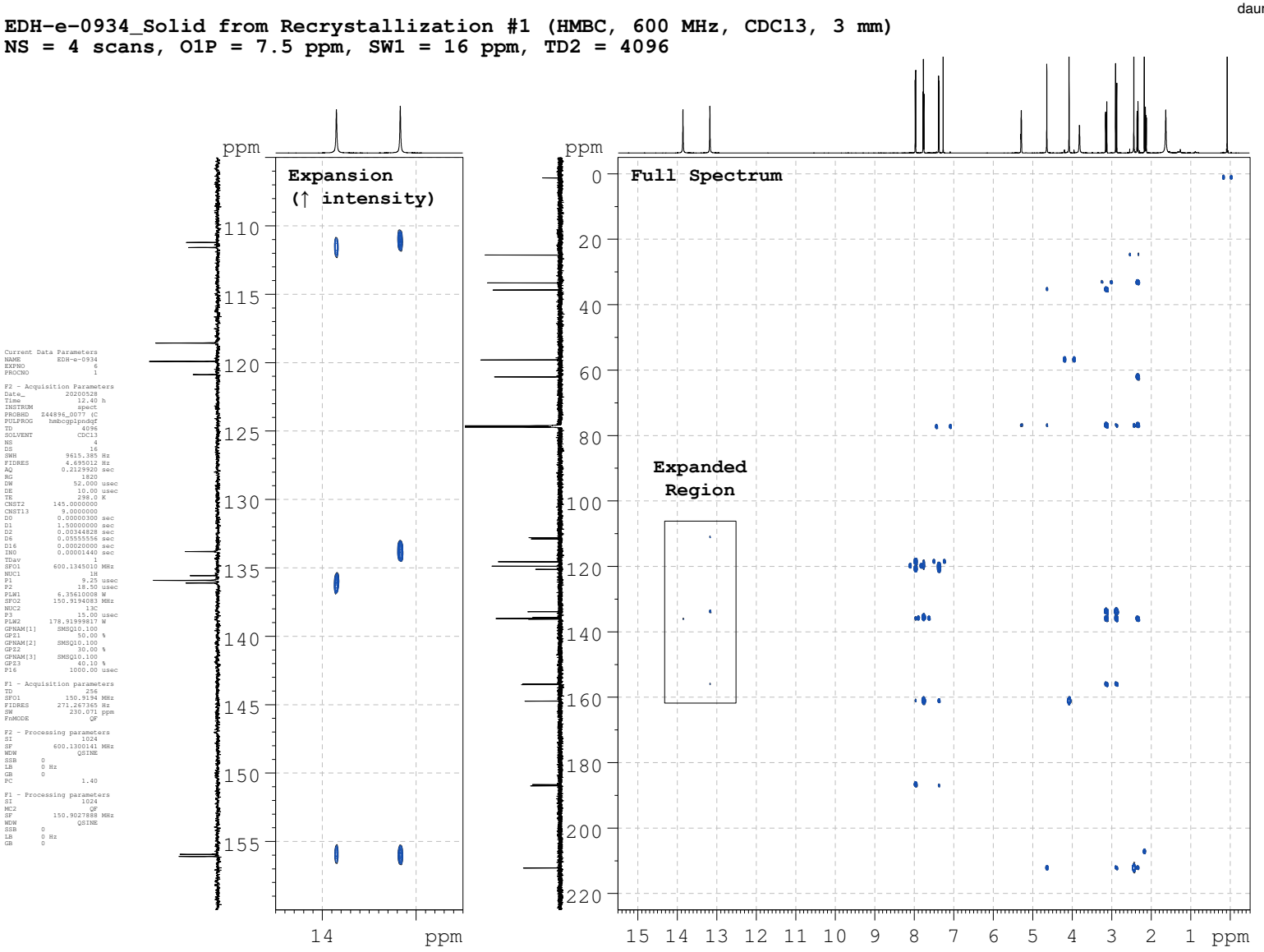
F1 - Processing parameters
SI 1024
MC2 QF
SF 150.9027888 MHz
WDW QSINE
SSB 0
LB 0 Hz
GB 0

^{1}H-^{13}C HMBC NMR of Daunorubicinone (28) (^{1}H-600 MHz, ^{13}C-151 MHz, $CDCl_3$)

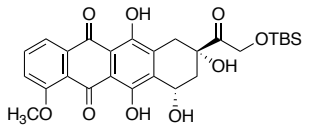
Note: Extended ^{1}H spectral window (-0.5 to 15.5 ppm).



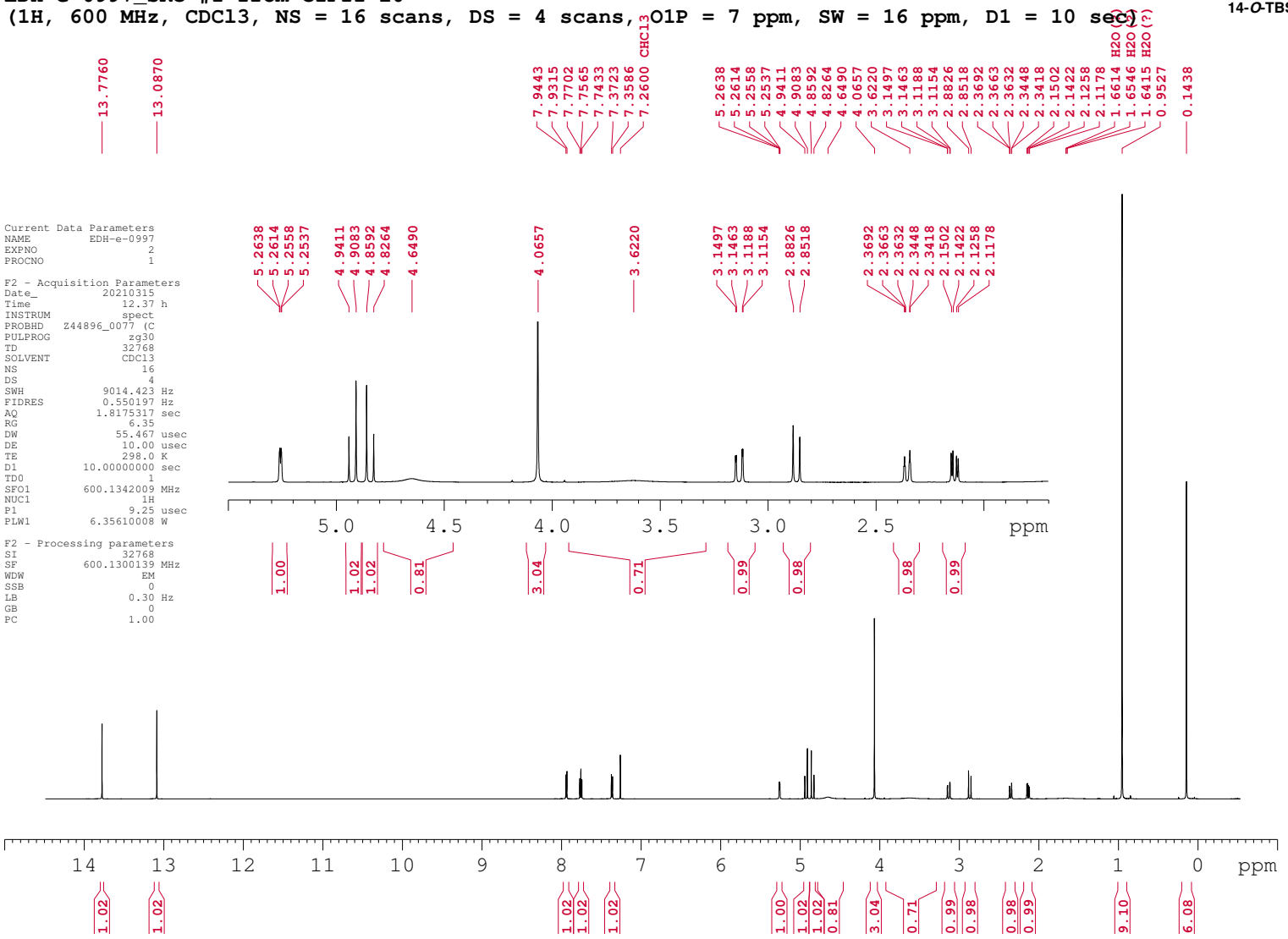
daunorubicinone (**28**)



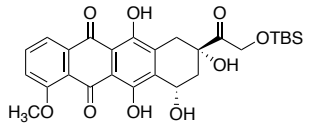
^{1}H NMR of Compound 14-*O*-TBS-Doxorubicinone (29) (600 MHz, $CDCl_3$)



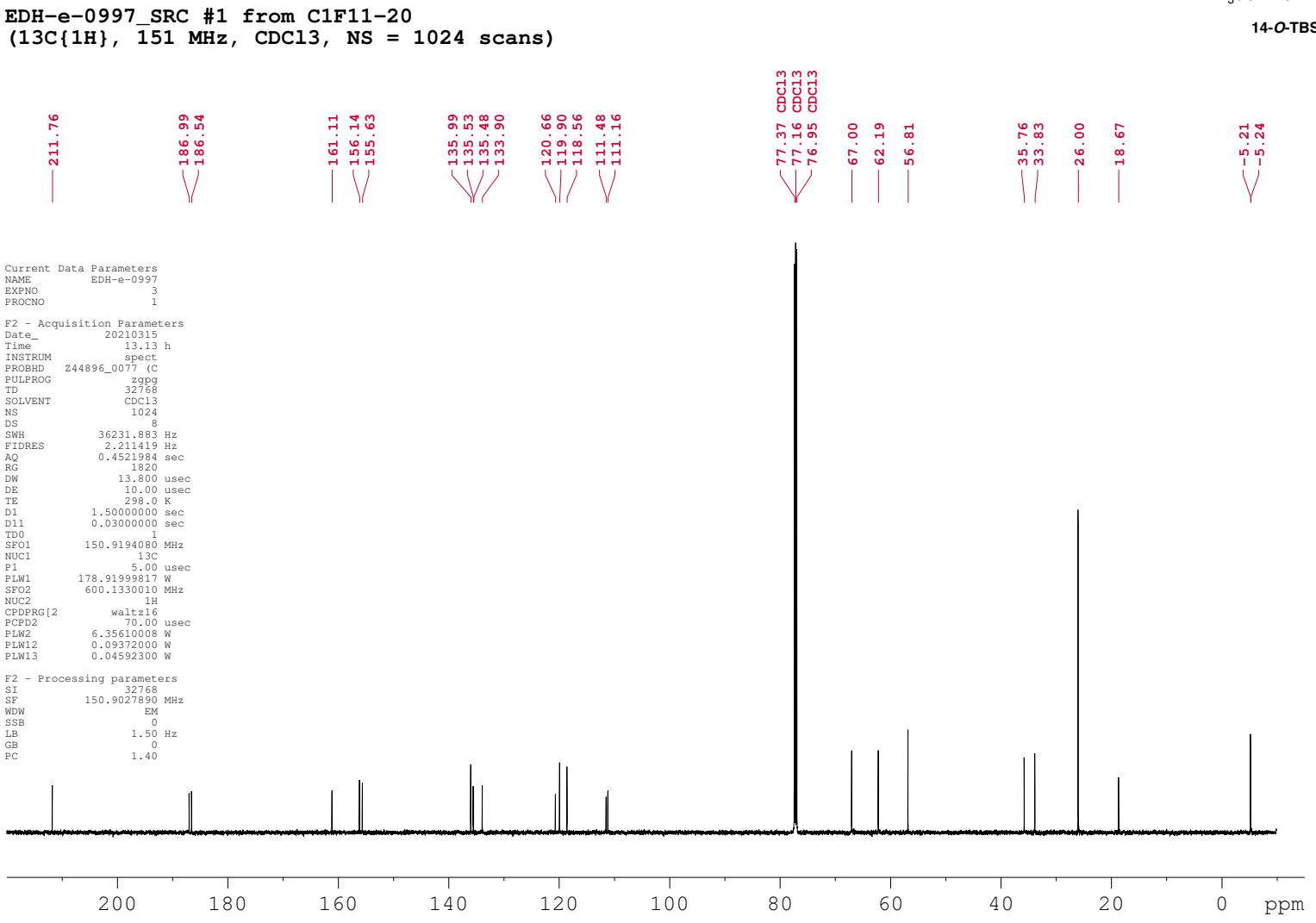
14-*O*-TBS-doxorubicinone (29)



$^{13}C\{^{1}H\}$ NMR of 14-*O*-TBS-Doxorubicinone (29) (151 MHz, $CDCl_3$)

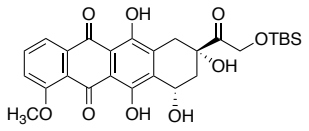


14-*O*-TBS-doxorubicinone (29)



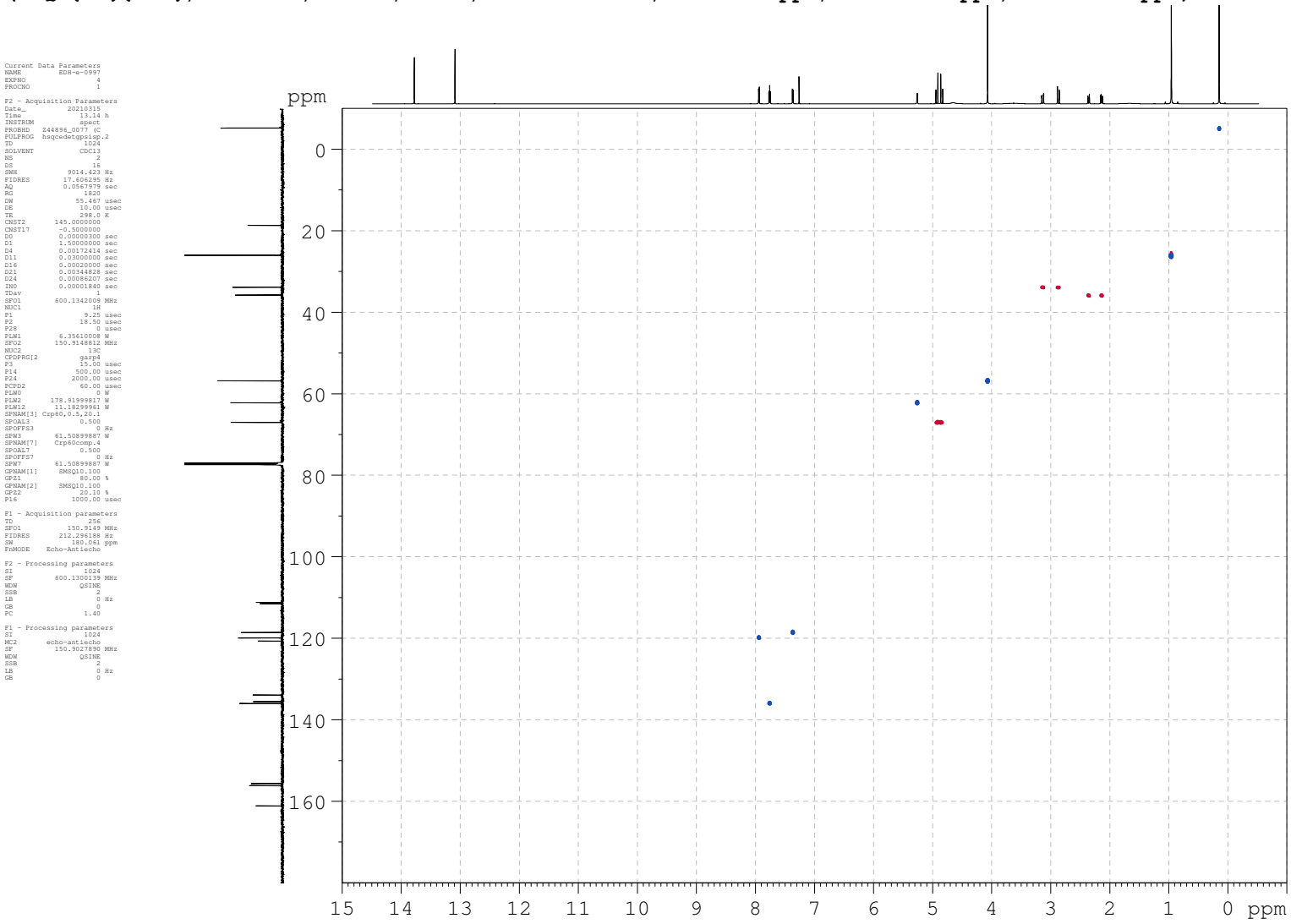
^{1}H-^{13}C HSQC{^{1}H}{^{13}C} NMR of 14-*O*-TBS-Doxorubicinone (29) (^{1}H-600 MHz, ^{13}C-151 MHz, $CDCl_3$)

Note: Blue = CH/CH_3 | Red = CH_2.



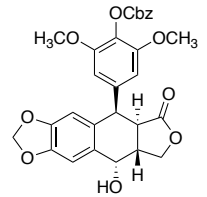
14-*O*-TBS-doxorubicinone (29)

EDH-e-0997_SRC #1 from C1F11-20
(HSQC{1H}{13C}, 600 MHz, CDC13, 3 mm, NS = 2 scans, O1P = 7 ppm, SW1 = 16 ppm, O2P = 80 ppm)

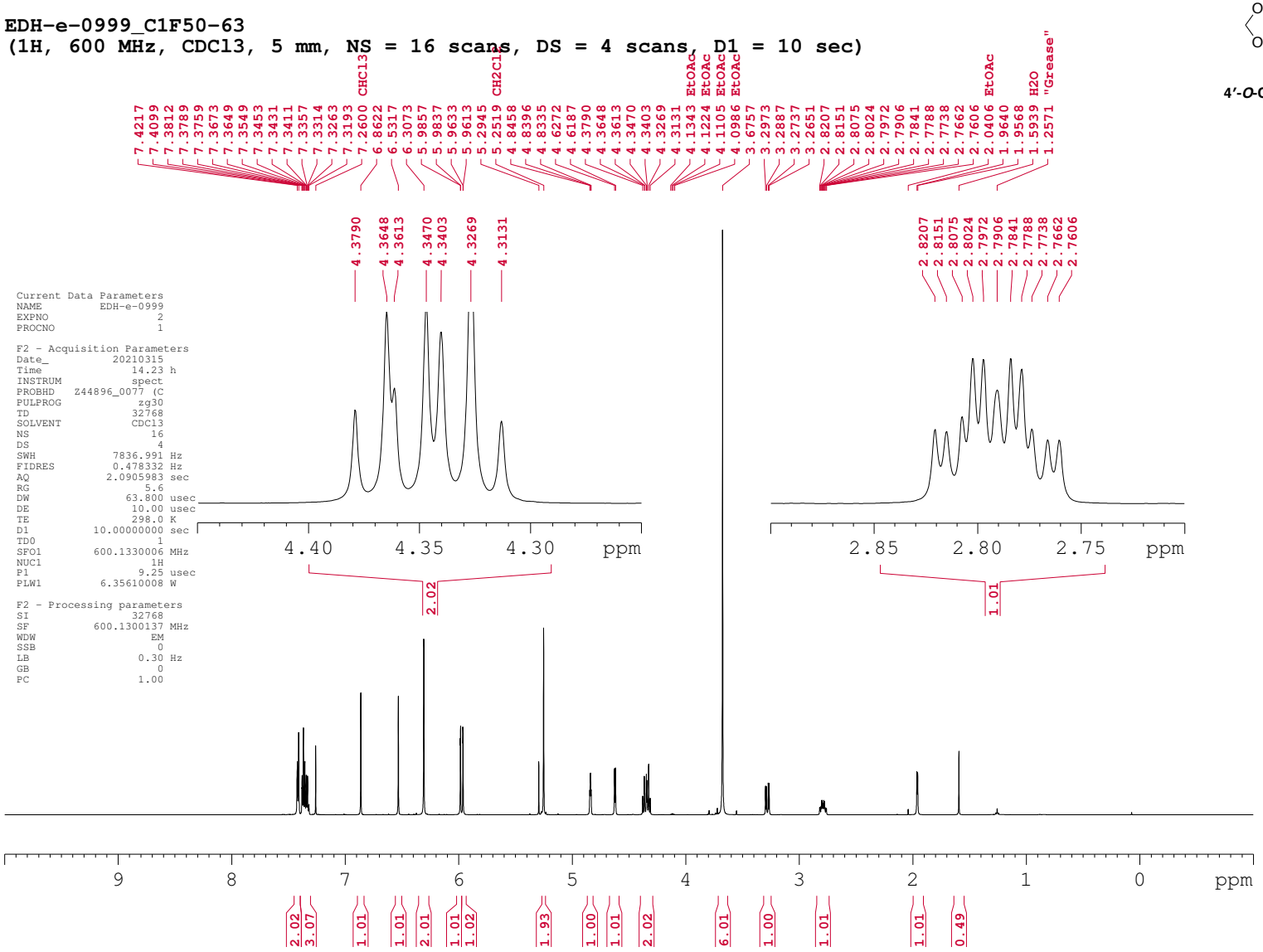



```
Current Data Parameters
NAME                  EDH-e-0997
EXPNO                          4
PROCNO                         1

F2 - Acquisition Parameters
Date_                   20210315
Time                      13.14 h
INSTRUM                     spect
PROBHD    Z44896_0077 (C
PULPROG   hsqcedetgpsisp.2
TD                          1024
SOLVENT                   CDCl3
NS                             2
DS                            16
SWH                     9014.423 Hz
FIDRES                 17.606295 Hz
AQ                     0.0567979 sec
RG                          1820
DW                        55.467 usec
DE                         10.00 usec
TE                        298.0 K
CNST2                145.0000000
CNST17                -0.5000000
D0                     0.00000300 sec
D1                     1.50000000 sec
D4                     0.00172414 sec
D11                    0.03000000 sec
D16                    0.00020000 sec
D21                    0.00344828 sec
D24                    0.00086207 sec
IN0                    0.00001840 sec
TDav                           1
SFO1                600.1342009 MHz
NUC1                         1H
P1                         9.25 usec
P2                        18.50 usec
P28                          0 usec
PLW1                 6.35610008 W
SFO2                150.9148812 MHz
NUC2                         13C
CPDPRG[2                 garp4
P3                        15.00 usec
P14                      500.00 usec
P24                     2000.00 usec
PCPD2                     60.00 usec
PLW0                         0 W
PLW2                178.91999817 W
PLW12                11.18299961 W
SPNAM[3]  Crp60,0.5,20.1
SPOAL3                   0.500
SPOFFS3                      0 Hz
SPW3                61.50899887 W
SPNAM[7]     Crp60comp.4
SPOAL7                   0.500
SPOFFS7                      0 Hz
SPW7                61.50899887 W
GPNAM[1]      SMSQ10.100
GPZ1                     80.00 %
GPNAM[2]      SMSQ10.100
GPZ2                     20.10 %
P16                    1000.00 usec

F1 - Acquisition parameters
TD                           256
SFO1                150.9149 MHz
FIDRES              212.296188 Hz
SW                      180.061 ppm
FnMODE     Echo-Antiecho

F2 - Processing parameters
SI                          1024
SF                  600.1300139 MHz
WDW                       QSINE
SSB                           2
LB                            0 Hz
GB                            0
PC                         1.40

F1 - Processing parameters
SI                          1024
MC2              echo-antiecho
SF                  150.9027890 MHz
WDW                       QSINE
SSB                           2
LB                            0 Hz
GB                            0
```

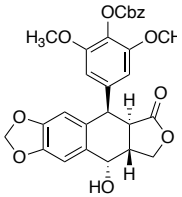
^{1}H NMR of Compound 4′-*O*-Cbz-Epipodophyllotoxin (30) (600 MHz, $CDCl_3$)



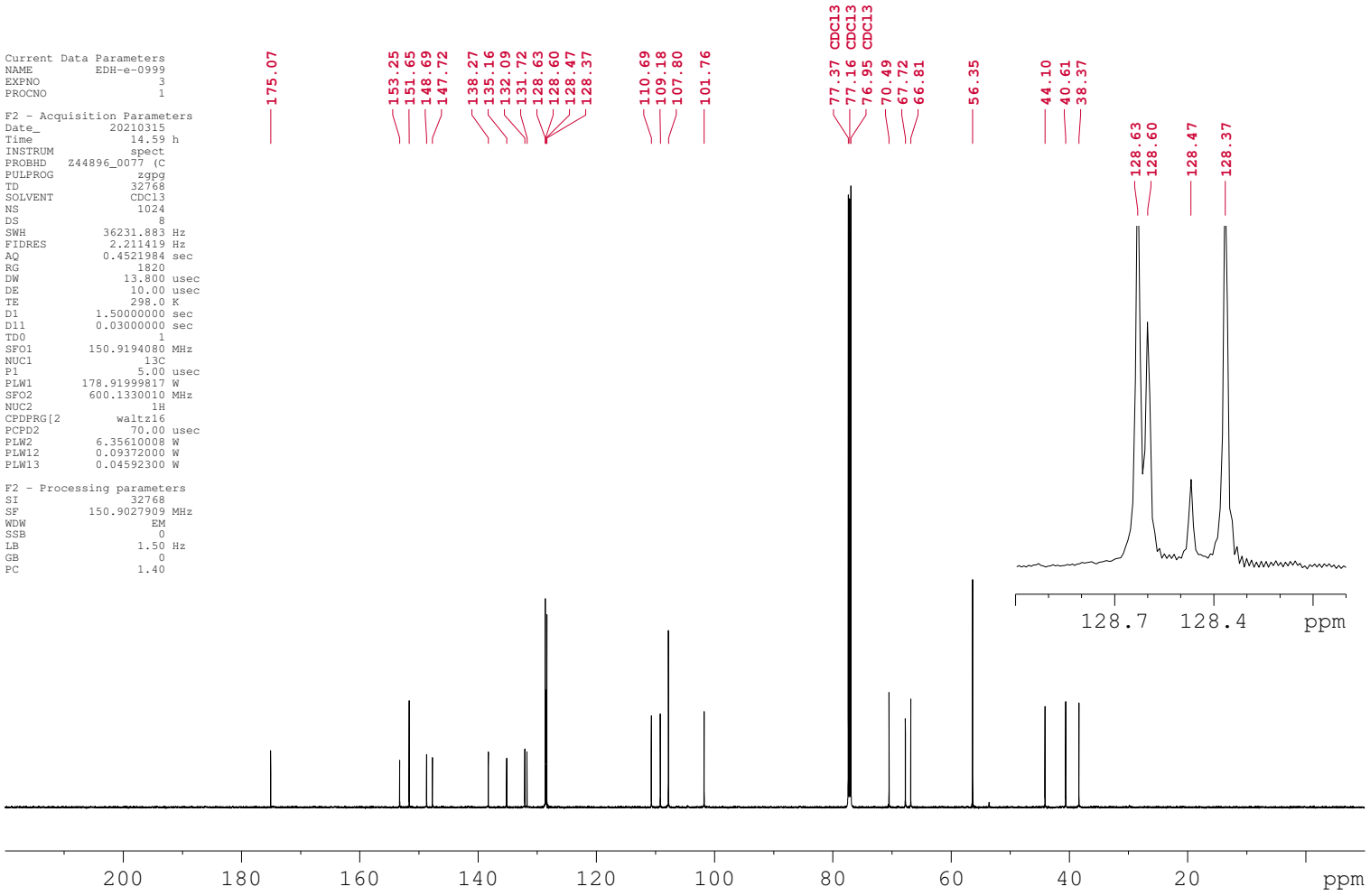
4′-*O*-Cbz-epipodophyllotoxin
(30)



$^{13}C\{^{1}H\}$ NMR of 4′-*O*-Cbz-Epipodophyllotoxin (30) (151 MHz, $CDCl_3$)

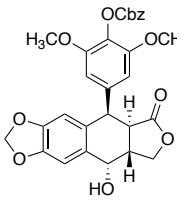


4′-*O*-Cbz-epipodophyllotoxin (30)

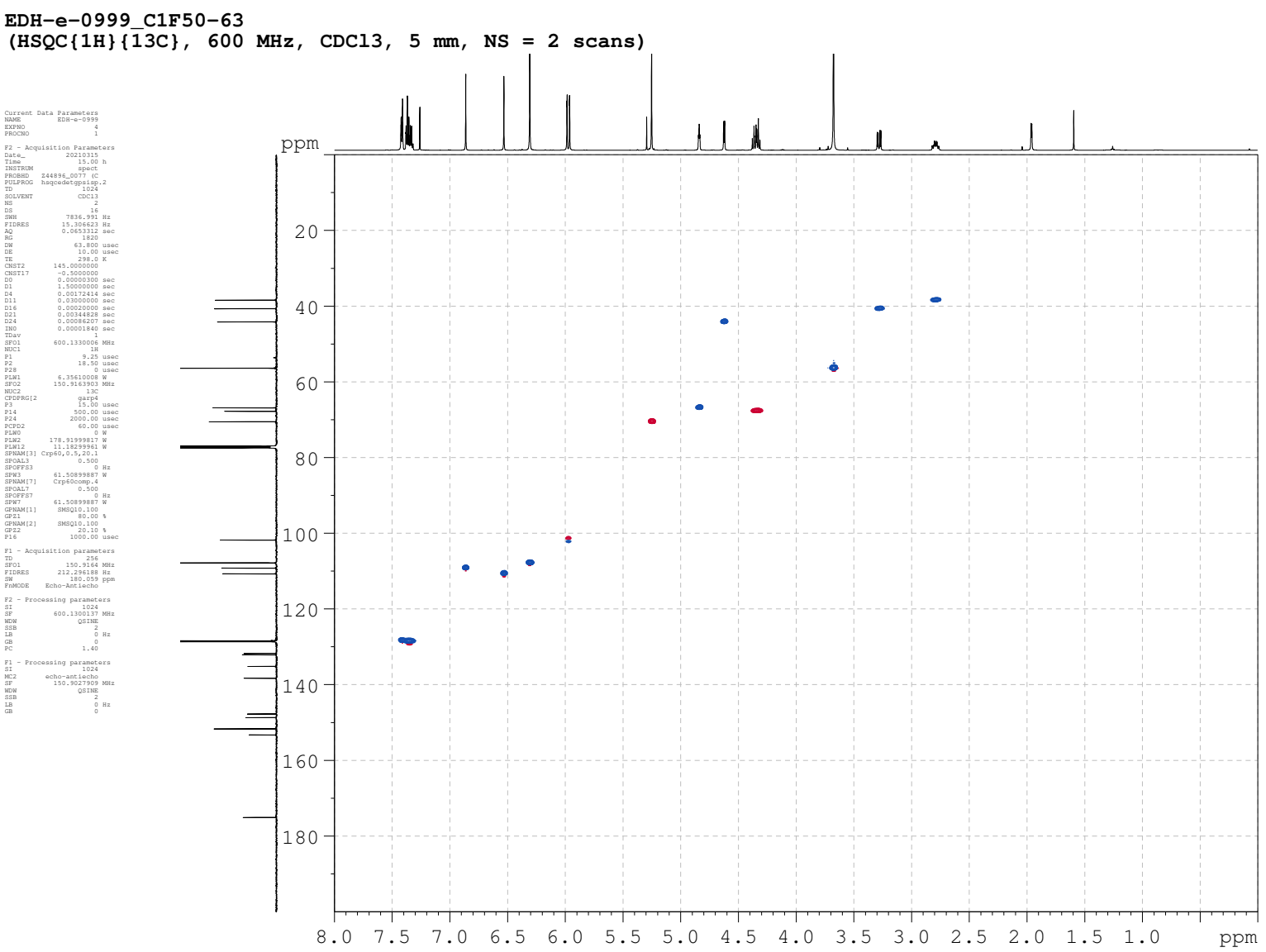


^{1}H-^{13}C HSQC{^{1}H}{^{13}C} NMR of 4′-*O*-Cbz-Epipodophyllotoxin (30) (^{1}H-600 MHz, ^{13}C-151 MHz, $CDCl_3$)

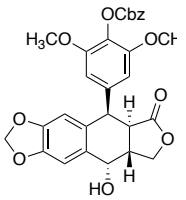
Note: Blue = CH/CH_3 | Red = CH_2.



4′-*O*-Cbz-epipodophyllotoxin (30)

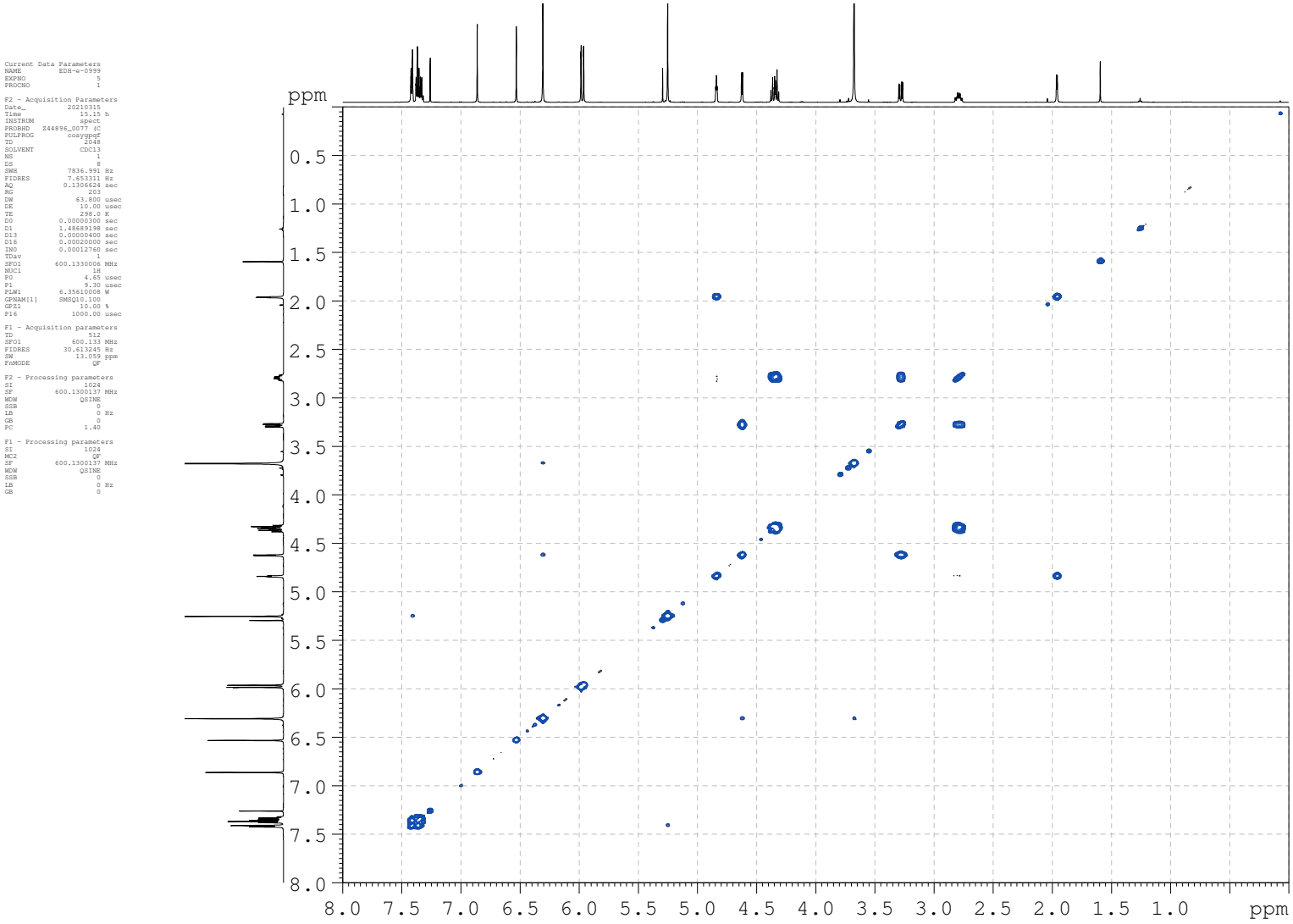


^{1}H-^{1}H COSY NMR of 4′-*O*-Cbz-Epipodophyllotoxin (30) (600 MHz, $CDCl_3$)



4′-*O*-Cbz-epipodophyllotoxin (30)

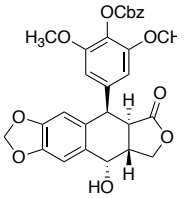
EDH-e-0999_C1F50-63
(COSY, 600 MHz, CDCl3, 5 mm, NS = 1 scan)




```
Current Data Parameters
NAME          EDH-e-0999
EXPNO                  5
PROCNO                 1

F2 - Acquisition Parameters
Date_           20210315
Time              15.15 h
INSTRUM             spect
PROBHD   Z44896_0077 (C
PULPROG         cosygpqf
TD                 2048
SOLVENT            CDCl3
NS                    1
DS                    8
SWH            7836.991 Hz
FIDRES         7.653311 Hz
AQ             0.1306624 sec
RG                  203
DW               63.800 usec
DE               10.00 usec
TE                298.0 K
D0           0.00000300 sec
D1           1.48689198 sec
D13          0.00000400 sec
D16          0.00020000 sec
IN0          0.00012760 sec
TDav                  1
SFO1         600.1330006 MHz
NUC1                  1H
P0                 4.65 usec
P1                 9.30 usec
PLW1          6.35610008 W
GPNAM[1]      SMSQ10.100
GPZ1              10.00 %
P16             1000.00 usec

F1 - Acquisition parameters
TD                  512
SFO1         600.133 MHz
FIDRES       30.61245 Hz
SW            13.059 ppm
FnMODE             QF

F2 - Processing parameters
SI                 1024
SF           600.1300137 MHz
WDW              QSINE
SSB                   0
LB                    0 Hz
GB                    0
PC                 1.40

F1 - Processing parameters
SI                 1024
MC2                QF
SF           600.1300137 MHz
WDW              QSINE
SSB                   0
LB                    0 Hz
GB                    0
```

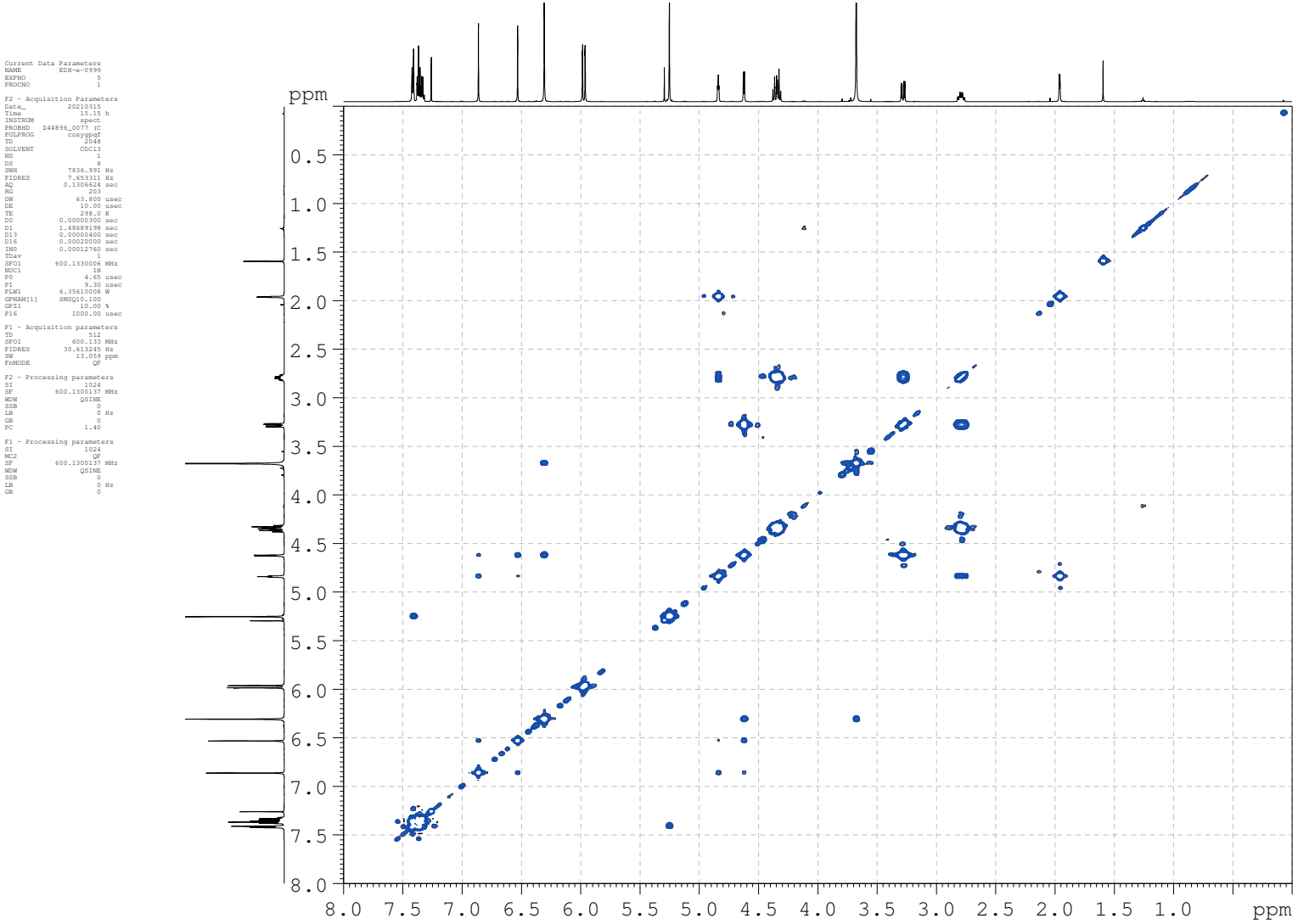
^{1}H-^{1}H COSY NMR of 4′-*O*-Cbz-Epipodophyllotoxin (30) (600 MHz, $CDCl_3$)

Note: Same spectrum with increased intensity as compared to COSY on page 259.

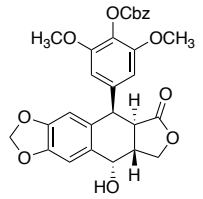


4′-*O*-Cbz-epipodophyllotoxin (30)

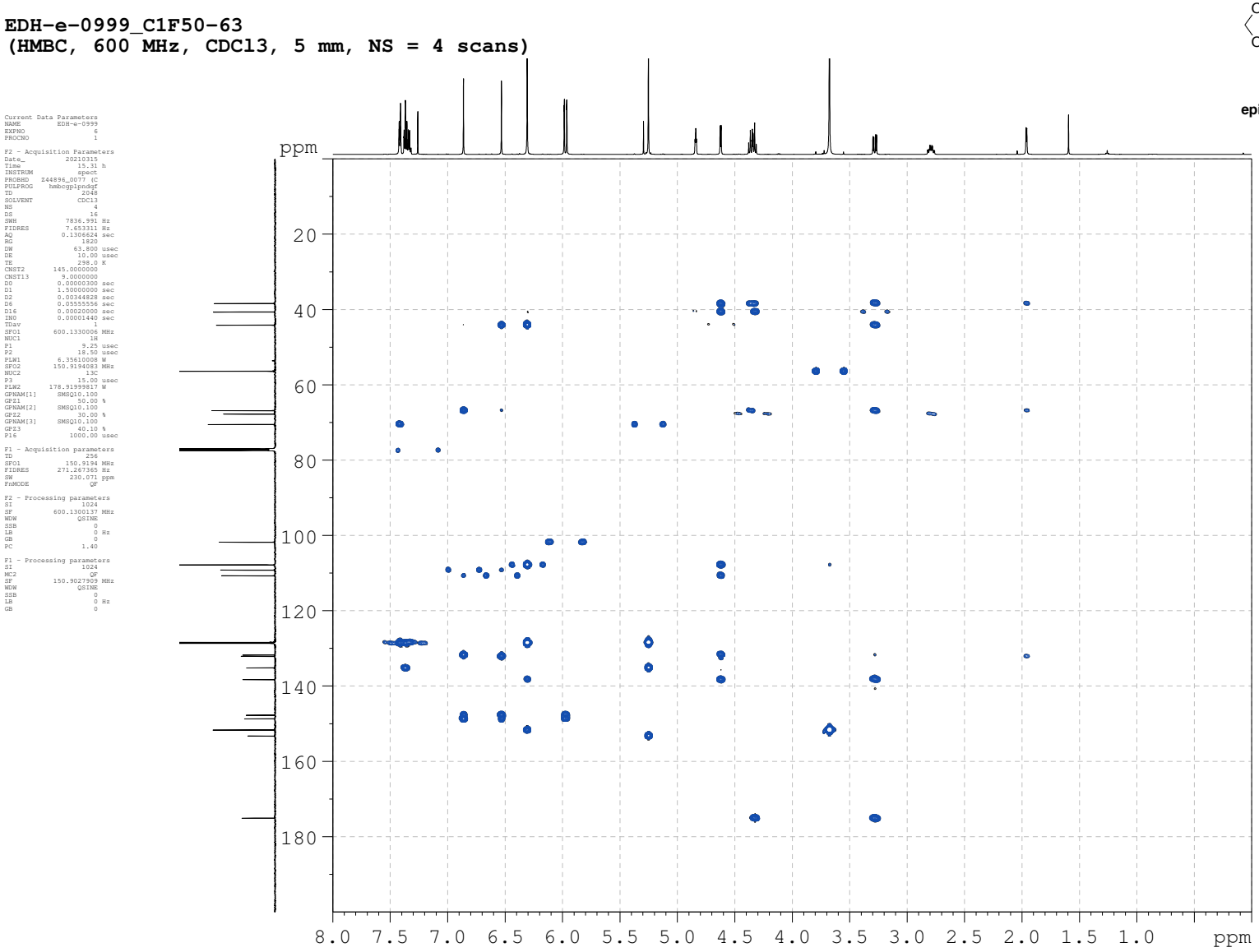
EDH-e-0999_C1F50-63
(COSY, 600 MHz, CDCl3, 5 mm, NS = 1 scan)



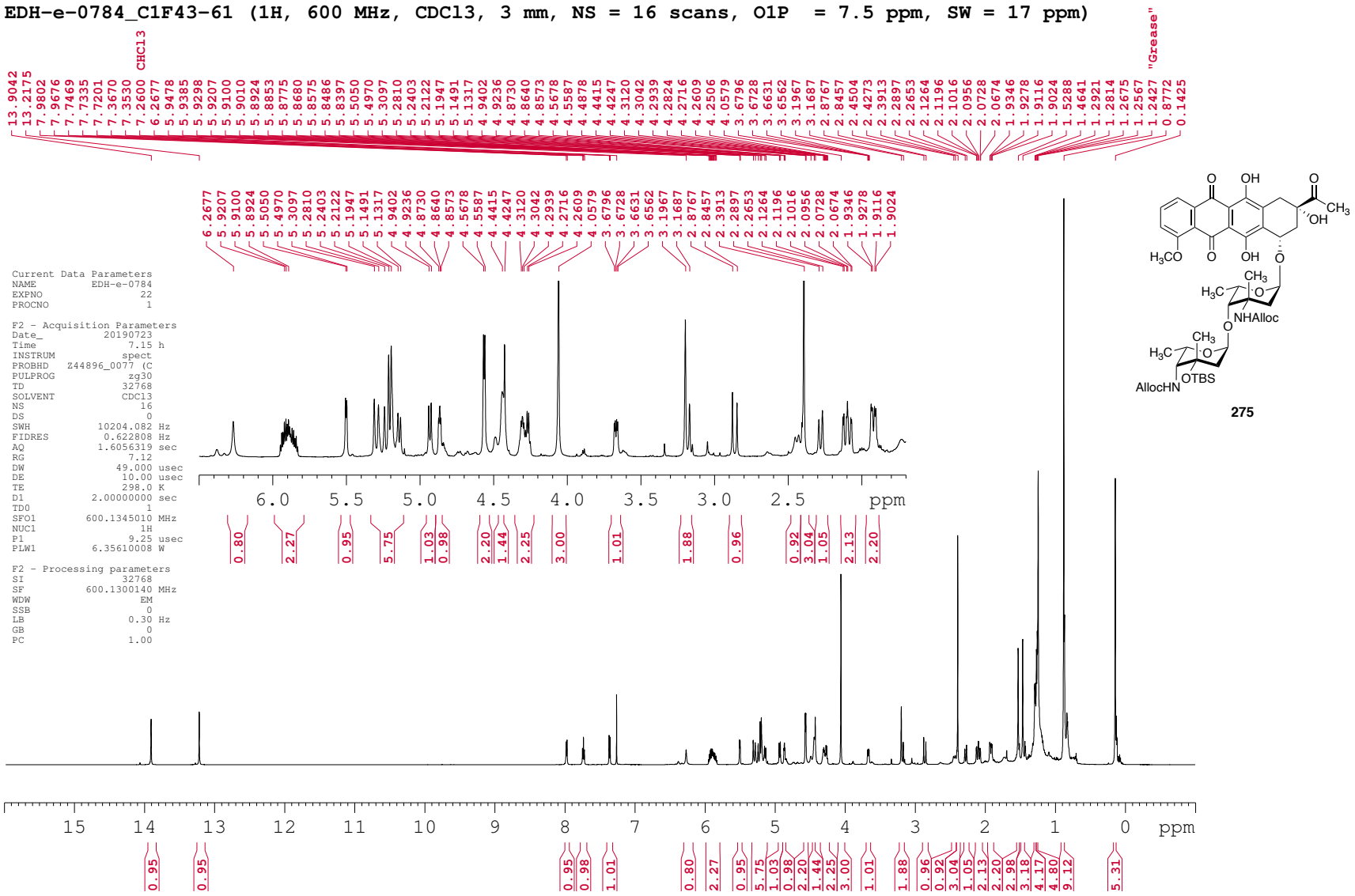
^{1}H-^{13}C HMBC NMR of 4′-*O*-Cbz-Epipodophyllotoxin (30) (^{1}H-600 MHz, ^{13}C-151 MHz, $CDCl_3$)



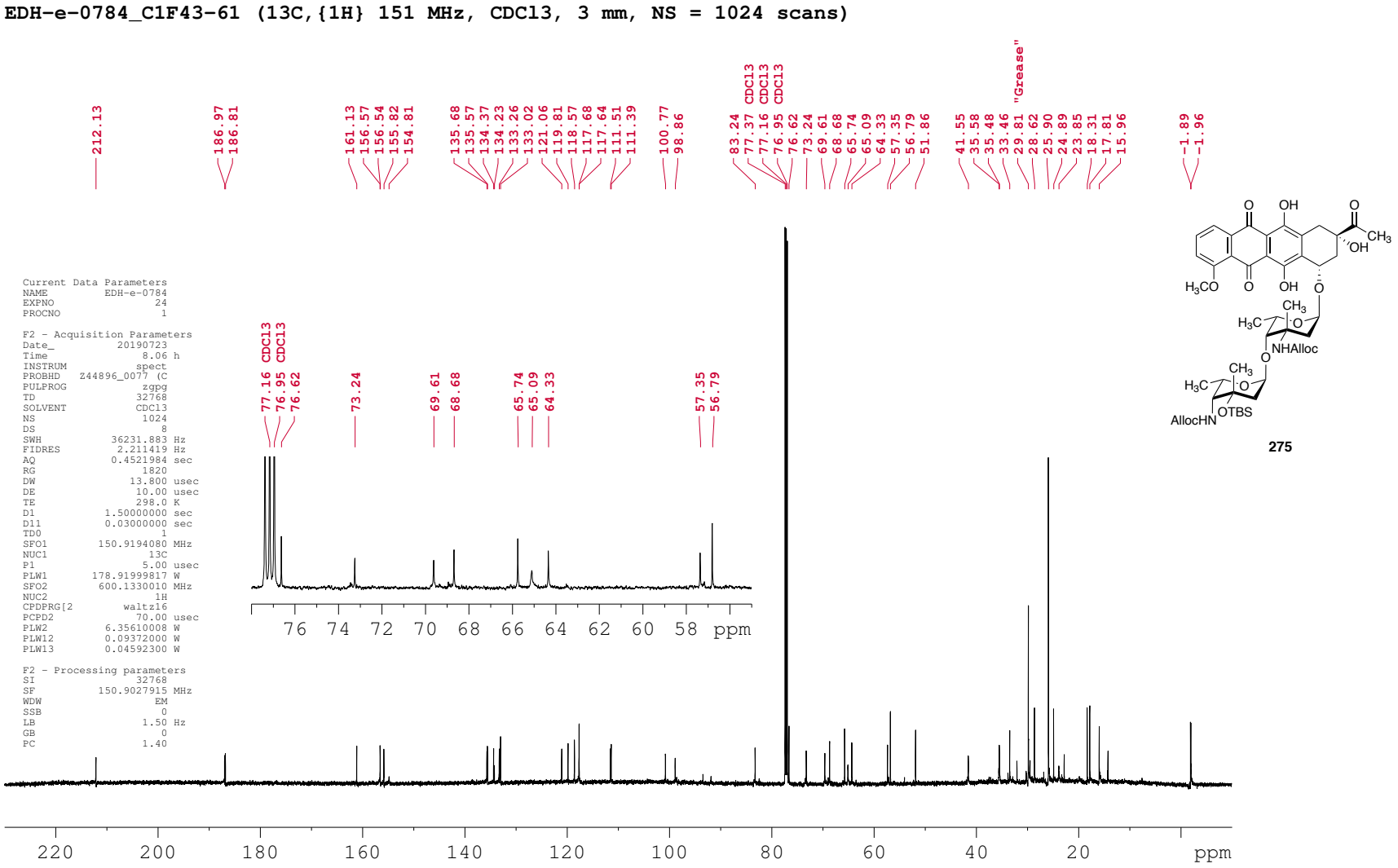
4′-*O*-Cbz-epipodophyllotoxin (30)



^{1}H NMR of Compound 275 (600 MHz, $CDCl_3$)



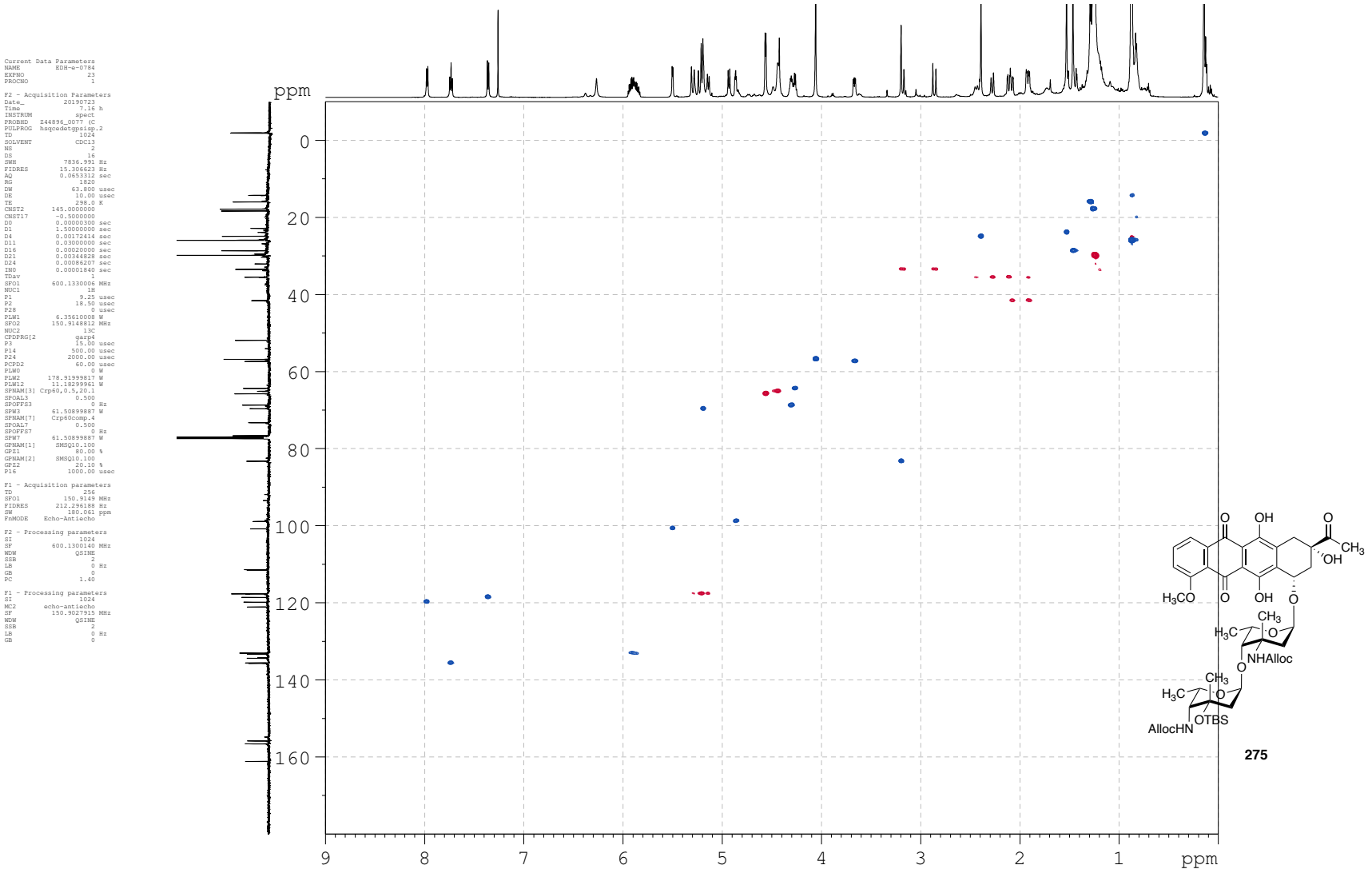
$^{13}C\{^{1}H\}$ NMR of Compound 275 ($CDCl_3$)



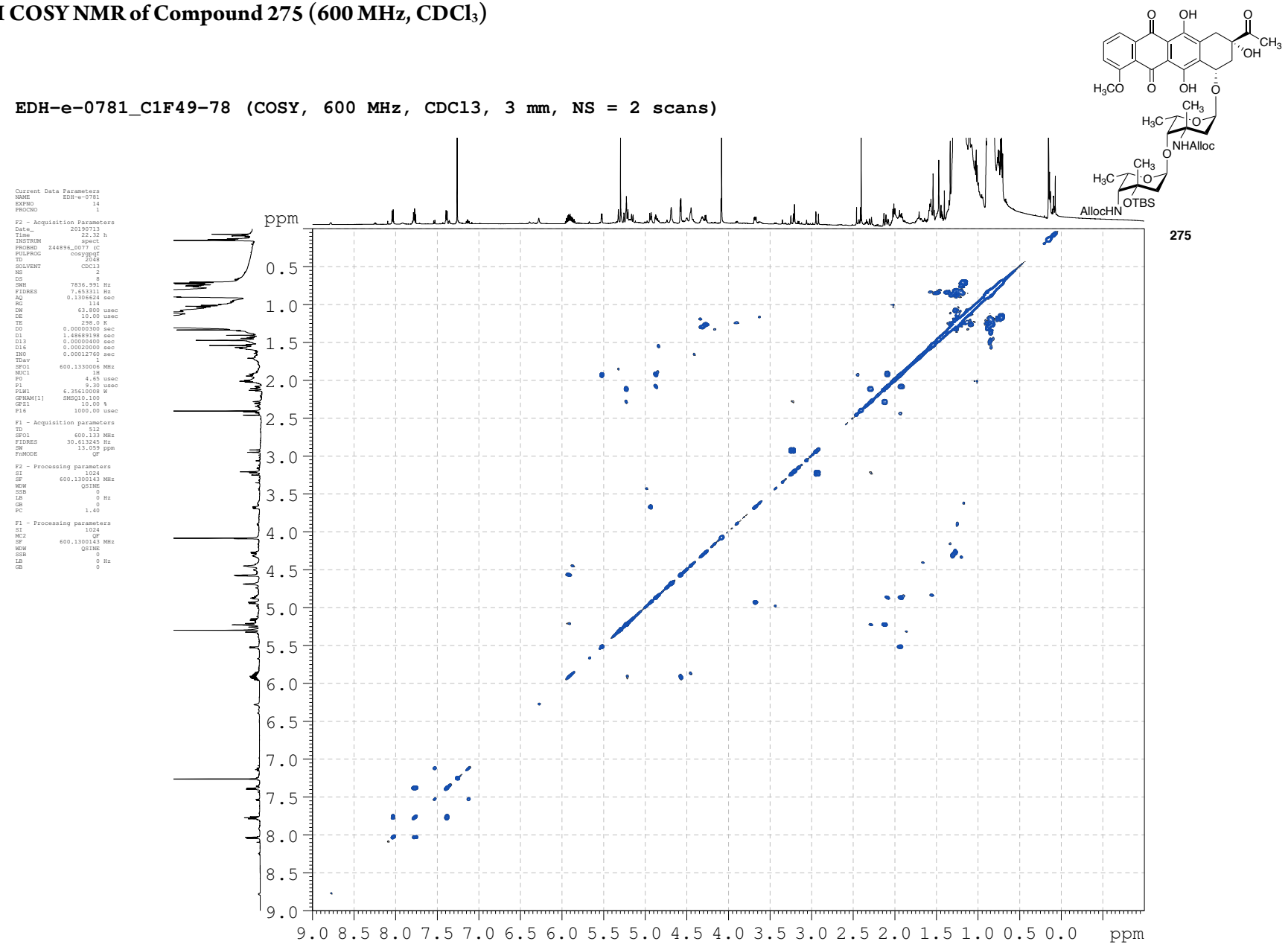
^{1}H-^{13}C HSQC{^{1}H}{^{13}C} NMR of Compound 275 (^{1}H-600 MHz, ^{13}C-151 MHz, $CDCl_3$)

Note: Blue = CH/CH_3 | Red = CH_2.

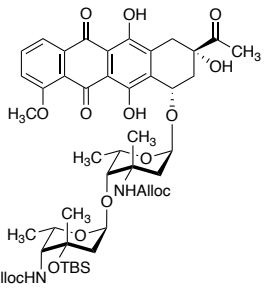
EDH-e-0784_C1F43-61 (HSQC{1H}{13C}, 600 MHz, CDCl3, 3 mm, O2P = 80 ppm)



^{1}H-^{1}H COSY NMR of Compound 275 ($600\ MHz$, $CDCl_3$)

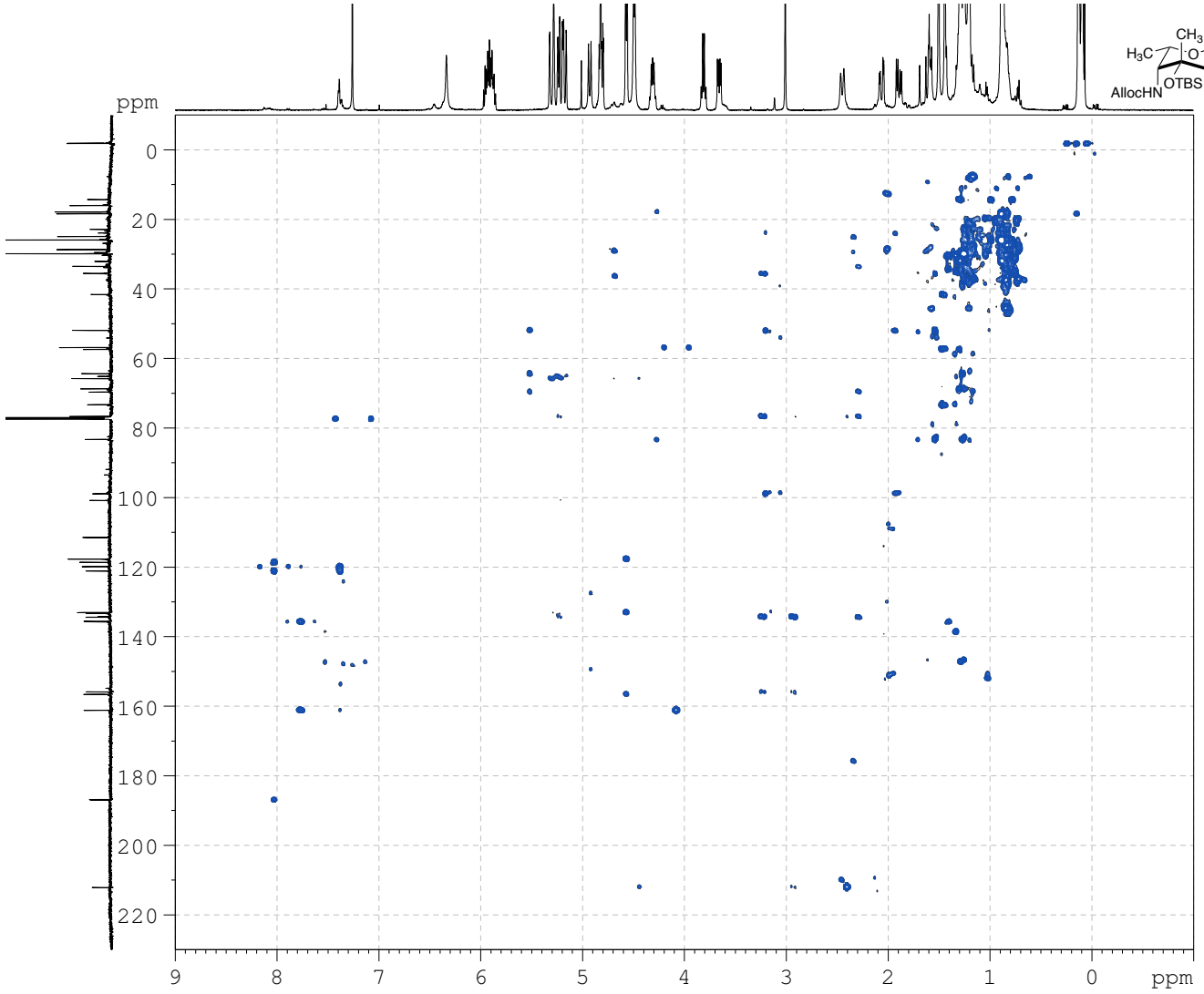


^{1}H-^{13}C HMBC NMR of Compound 275 (^{1}H-600 MHz, ^{13}C-151 MHz, $CDCl_3$)



275

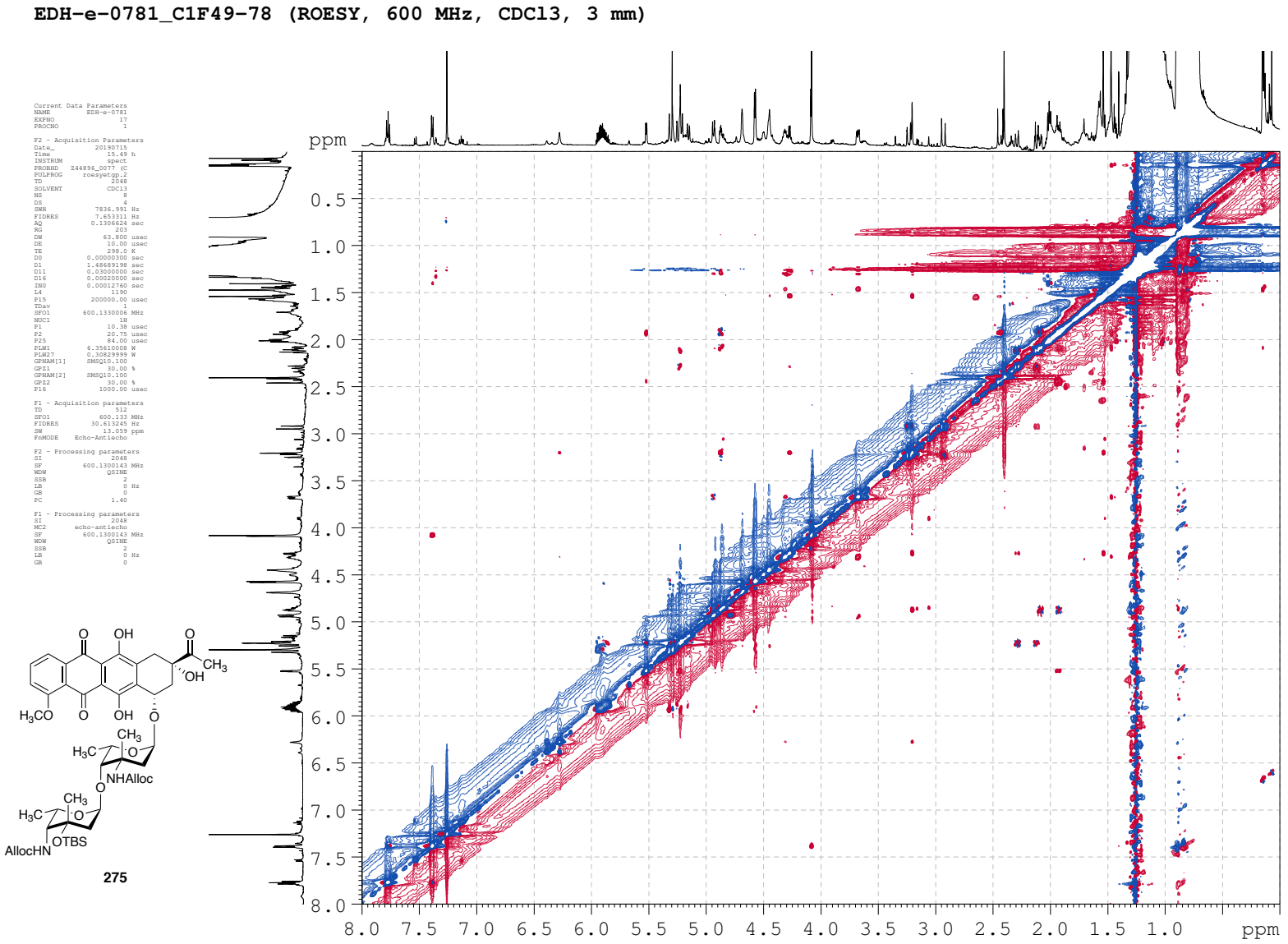
EDH-e-0781_C1F49-78 (HMBC, 600 MHz, CDCl3, 3 mm, NS = 16 scans)




```
Current Data Parameters
NAME          EDH-e-0781
EXPNO                 15
PROCNO                 1

F2 - Acquisition Parameters
Date_           20190713
Time              23.02 h
INSTRUM             spect
PROBHD    Z44896_0077 (C
PULPROG    hmbcgplpndqf
TD                  2048
SOLVENT             CDCl3
NS                    16
DS                    16
SWH              7836.991 Hz
FIDRES           7.653311 Hz
AQ               0.1306624 sec
RG                  1820
DW                 63.800 usec
DE                 10.00 usec
TE                298.0 K
CNST2         145.0000000
CNST13          9.0000000
D0             0.00000300 sec
D1             1.50000000 sec
D2             0.00344828 sec
D6             0.05555556 sec
D16            0.00020000 sec
IN0            0.00001440 sec
TDav                   1
SFO1          600.1330006 MHz
NUC1                  1H
P1                  9.25 usec
P2                 18.50 usec
PLW1           6.35610008 W
SFO2          150.9194083 MHz
NUC2                  13C
P3                 15.00 usec
PLW2         178.91999817 W
GPNAM[1]       SMSQ10.100
GPZ1                50.00 %
GPNAM[2]       SMSQ10.100
GPZ2                30.00 %
GPNAM[3]       SMSQ10.100
GPZ3                40.10 %
P16              1000.00 usec

F1 - Acquisition parameters
TD                    256
SFO1          150.9194 MHz
FIDRES        271.267365 Hz
SW               230.071 ppm
FnMODE                QF

F2 - Processing parameters
SI                   1024
SF            600.1300143 MHz
WDW                 QSINE
SSB                     0
LB                      0 Hz
GB                      0
PC                   1.40

F1 - Processing parameters
SI                   1024
MC2                    QF
SF            150.9027915 MHz
WDW                 QSINE
SSB                     0
LB                      0 Hz
GB                      0
```

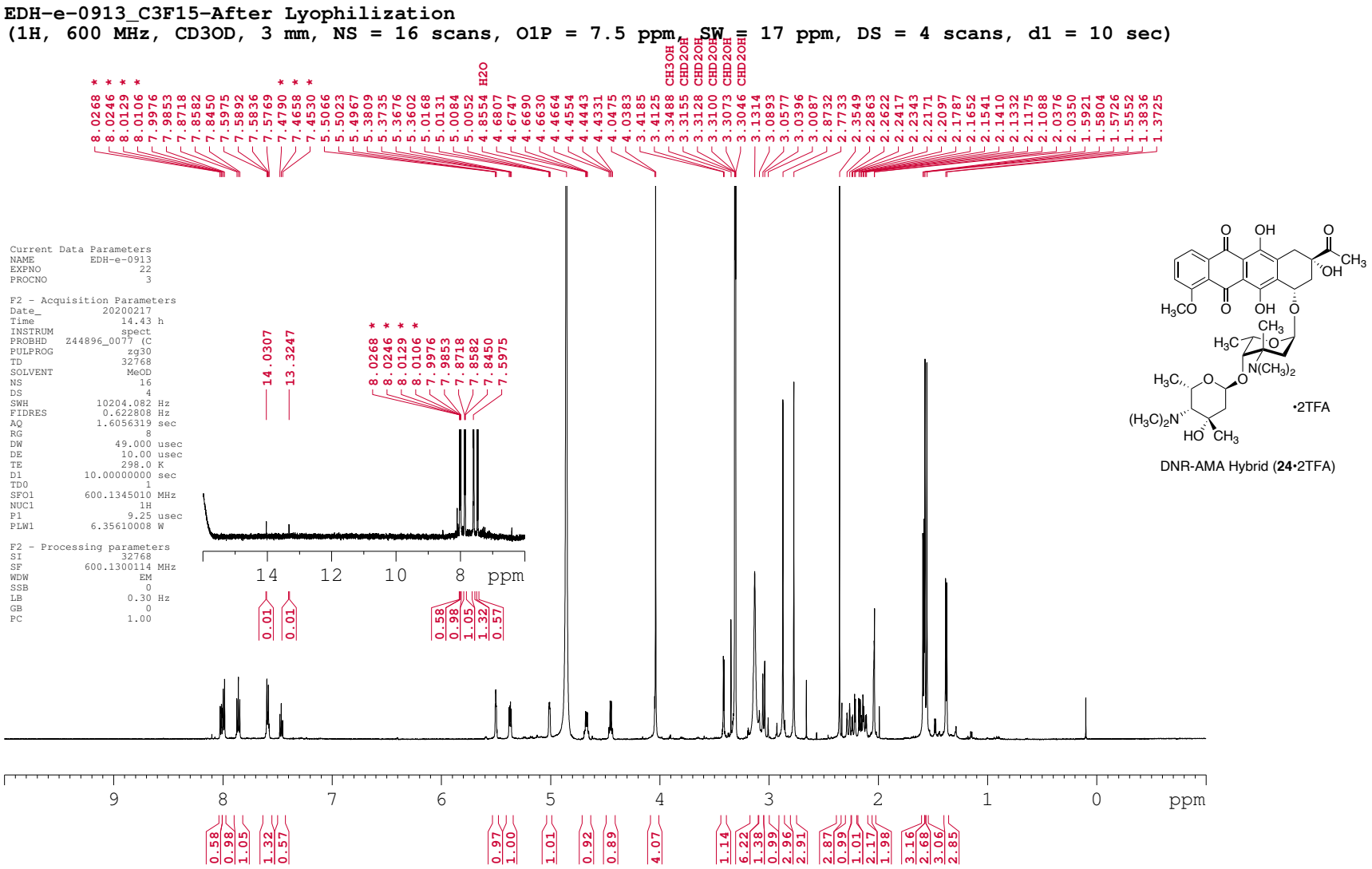
^{1}H-^{1}H ROESY NMR of Compound 275 (^{1}H-600 MHz, $CDCl_3$)

Note: Blue = diagonal/chemical exchange | Red = ROESY cross peaks.



^{1}H NMR of DNR-AMA Hybrid (24•2TFA) (600 MHz, CD_3OD)

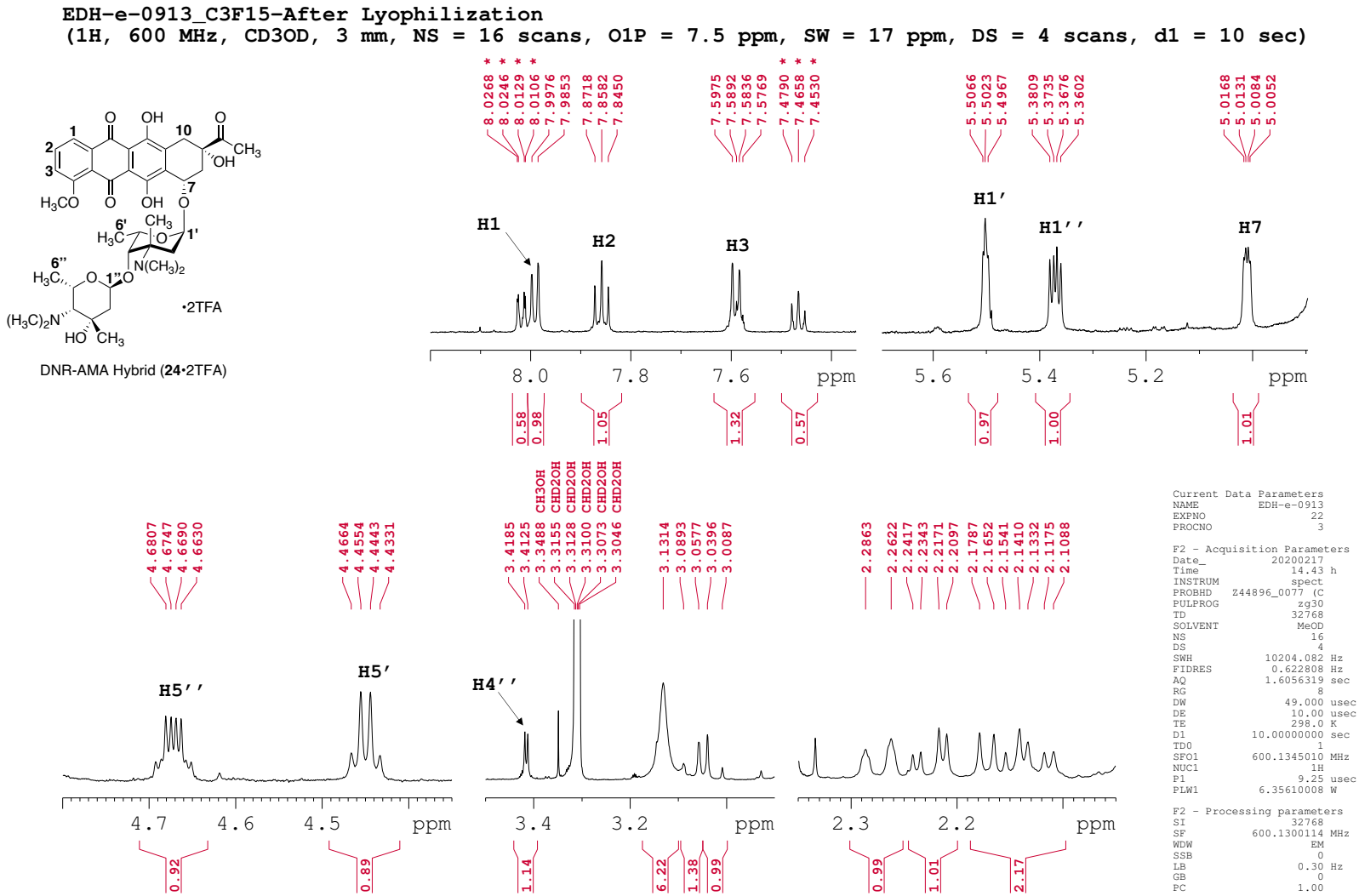
Note: Unassigned impurity(ies) indicated with asterisk (*).



^{1}H NMR of DNR-AMA Hybrid (24•2TFA) (600 MHz, CD_3OD)

Note 1: Selected expansions of spectrum on page S396.

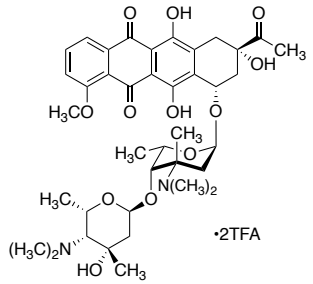
Note 2: Unassigned impurity(ies) indicated with asterisk (*).



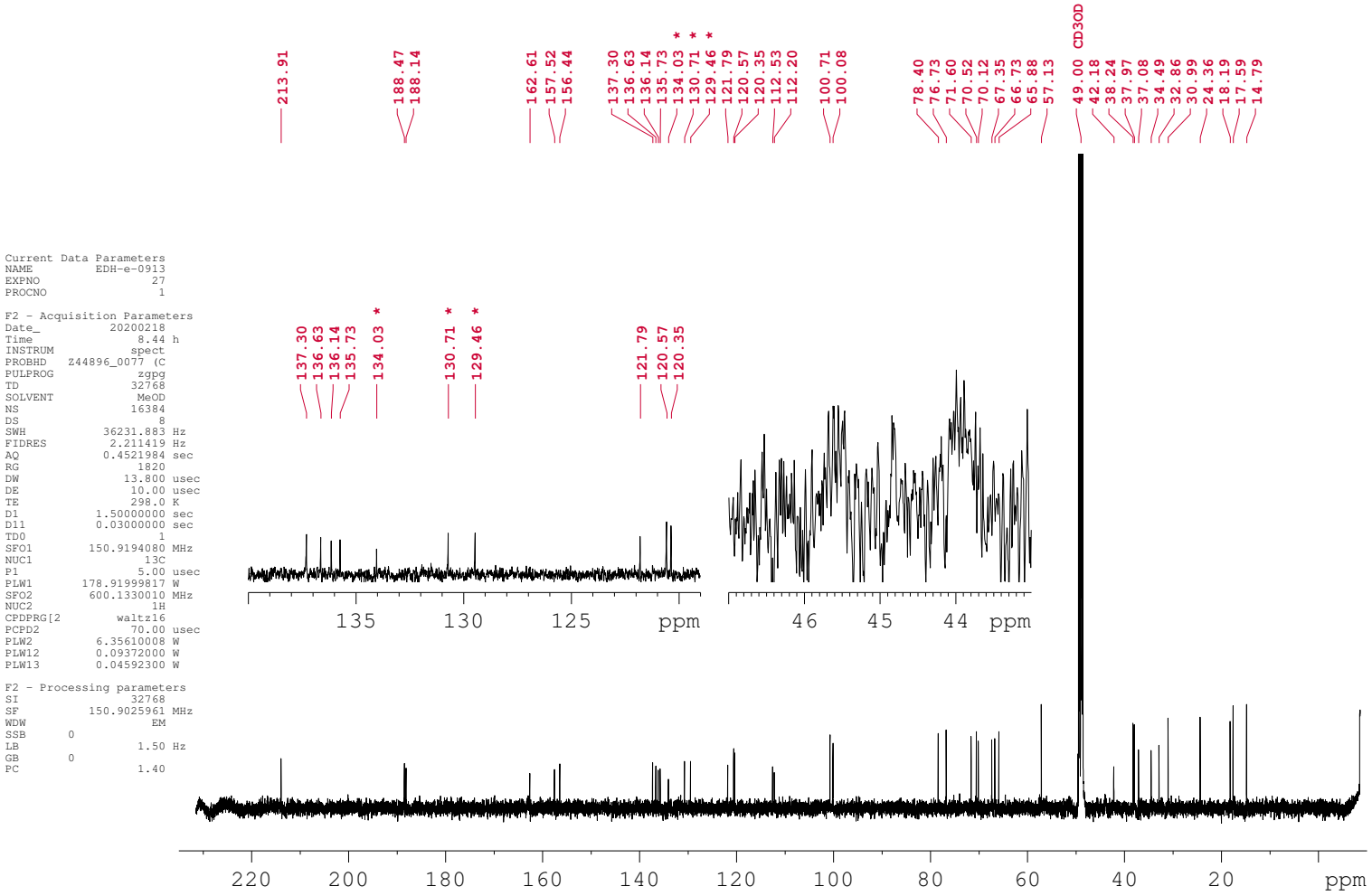

```
Current Data Parameters
NAME          EDH-e-0913
EXPNO                 22
PROCNO                 3

F2 - Acquisition Parameters
Date_           20200217
Time              14.43 h
INSTRUM             spect
PROBHD   Z44896_0077 (C
PULPROG             zg30
TD                 32768
SOLVENT             MeOD
NS                    16
DS                     4
SWH           10204.082 Hz
FIDRES         0.622808 Hz
AQ            1.6056319 sec
RG                     8
DW               49.000 usec
DE               10.00 usec
TE               298.0 K
D1           10.00000000 sec
TD0                    1
SFO1         600.1345010 MHz
NUC1                  1H
P1                 9.25 usec
PLW1          6.35610008 W

F2 - Processing parameters
SI                 32768
SF           600.1300114 MHz
WDW                   EM
SSB                    0
LB                  0.30 Hz
GB                     0
PC                  1.00
```

^{13}C NMR of DNR-AMA Hybrid (24•2TFA) (151 MHz, CD_3OD)

Note: Unassigned impurity(ies) indicated with asterisk (*).



EDH-e-0913_C3F15-After Lyophilization (13C, 151 MHz, CD3OD, 3 mm, NS = 16384 scans)

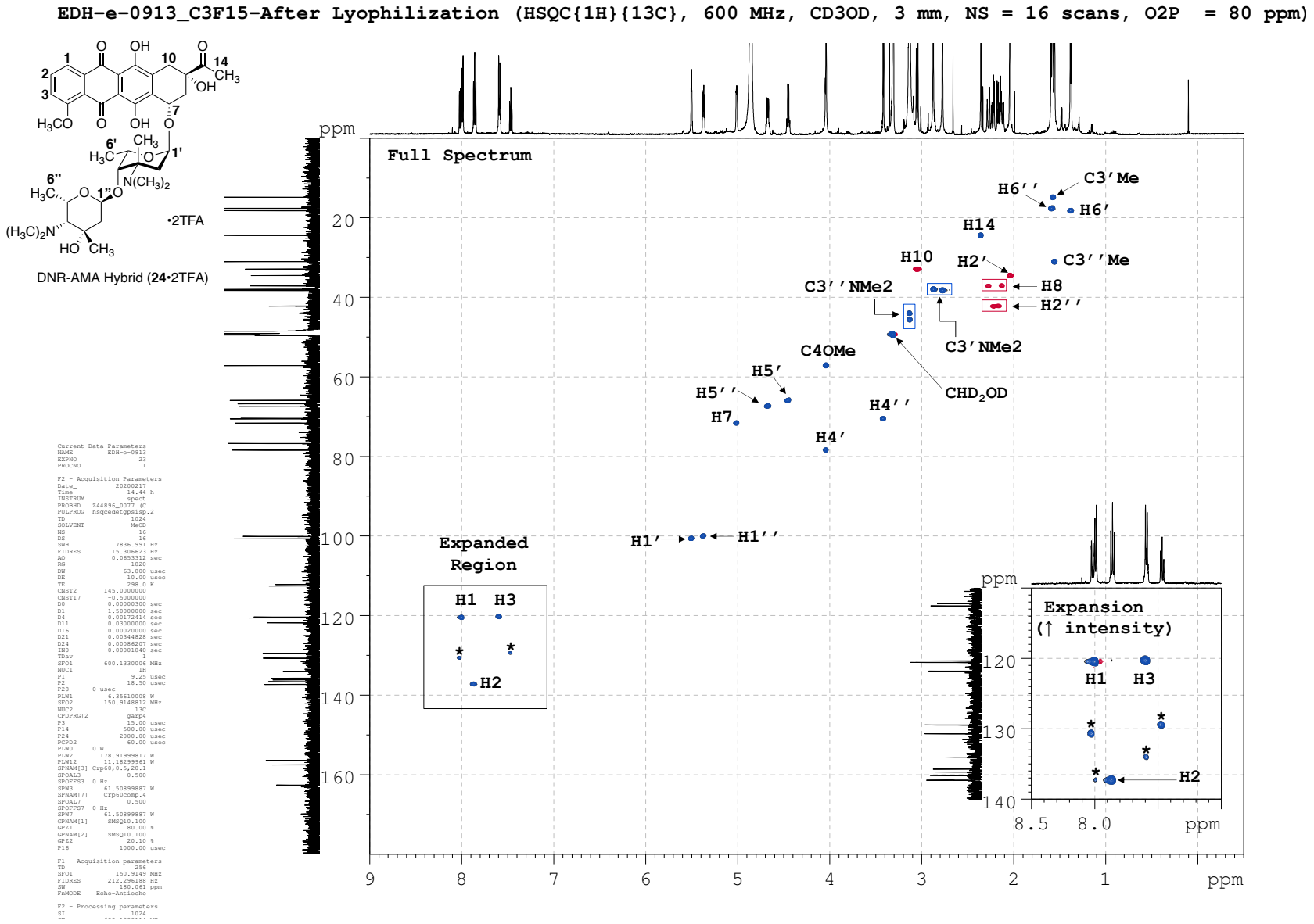



```
Current Data Parameters
NAME          EDH-e-0913
EXPNO                 27
PROCNO                 1

F2 - Acquisition Parameters
Date_           20200218
Time                8.44 h
INSTRUM             spect
PROBHD    Z44896_0077 (C
PULPROG             zgpg
TD                 32768
SOLVENT             MeOD
NS                 16384
DS                     8
SWH            36231.883 Hz
FIDRES         2.211419 Hz
AQ             0.4521984 sec
RG                  1820
DW                13.800 usec
DE                10.00 usec
TE                298.0 K
D1             1.50000000 sec
D11            0.03000000 sec
TD0                    1
SFO1         150.9194080 MHz
NUC1                13C
P1                  5.00 usec
PLW1        178.91999817 W
SFO2         600.1330010 MHz
NUC2                1H
CPDPRG[2          waltz16
PCPD2              70.00 usec
PLW2           6.35610008 W
PLW12          0.09372000 W
PLW13          0.04592300 W

F2 - Processing parameters
SI                 32768
SF           150.9025961 MHz
WDW                   EM
SSB       0
LB                 1.50 Hz
GB        0
PC                 1.40
```

^{1}H-^{13}C HSQC{^{1}H}{^{13}C} NMR of DNR-AMA Hybrid (24•2TFA) (^{1}H-600 MHz, ^{13}C-151 MHz, CD_3OD)

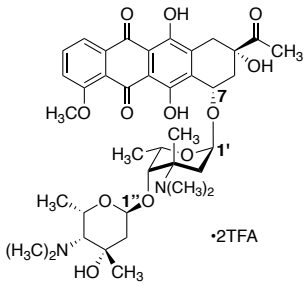
Note 1: Blue = CH/CH_3 | Red = CH_2.

Note 2: Unassigned impurity(ies) indicated with asterisk (*).



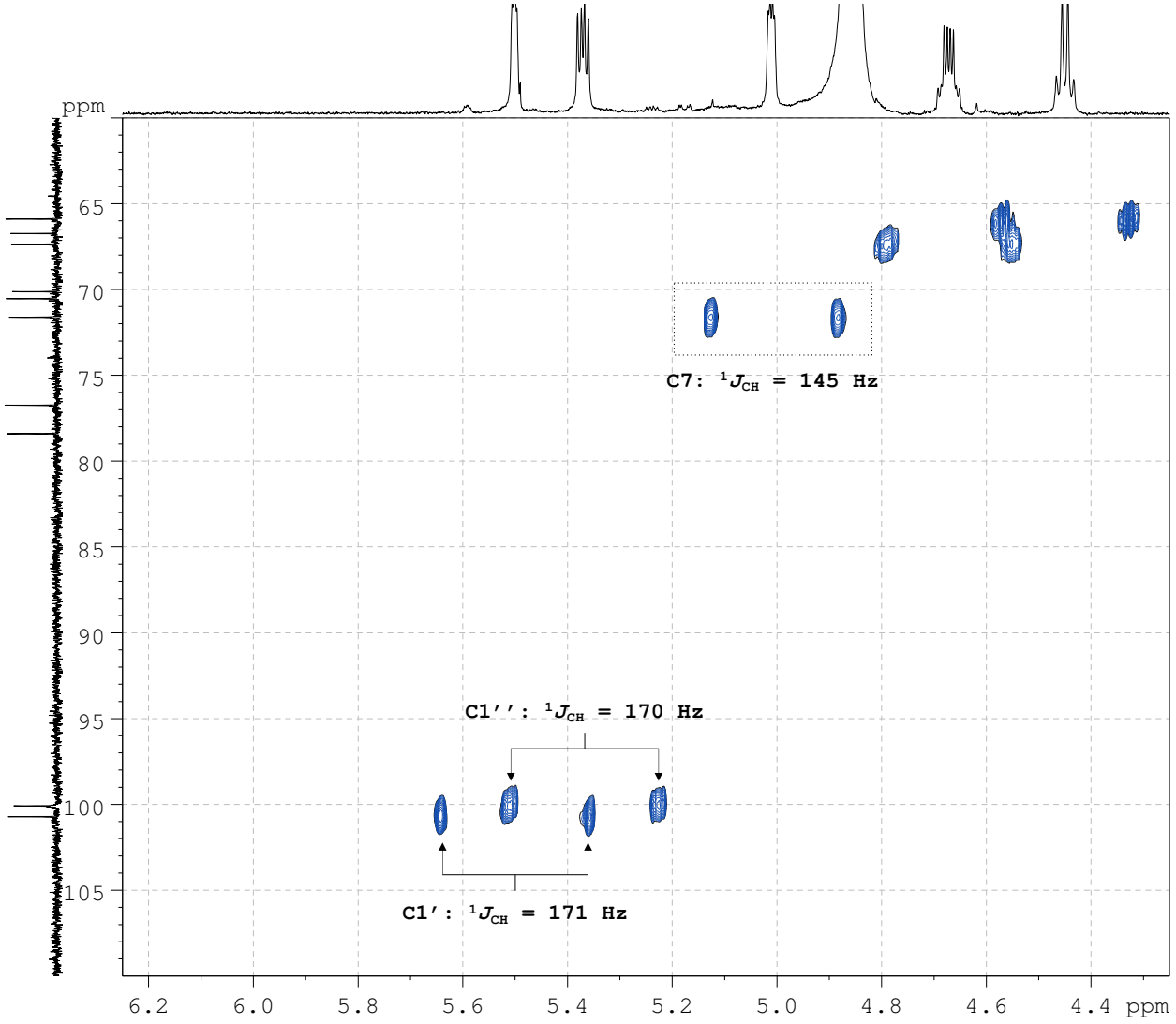
Coupled ^{1}H-^{13}C HSQC NMR of DNR-AMA Hybrid (24•2TFA) (^{1}H-600 MHz, ^{13}C-151 MHz, CD_3OD)

Note: Blue = CH/CH_3 | Red = CH_2.



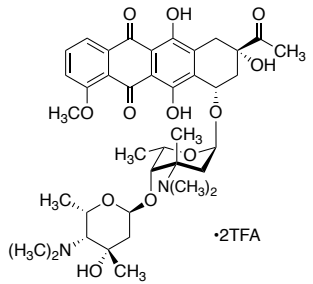
DNR-AMA Hybrid (**24**•2TFA)

EDH-e-0913_C1F15-After Lyophilization ("Coupled" HSQC, 600 MHz, CD3OD, 3 mm, NS = 32



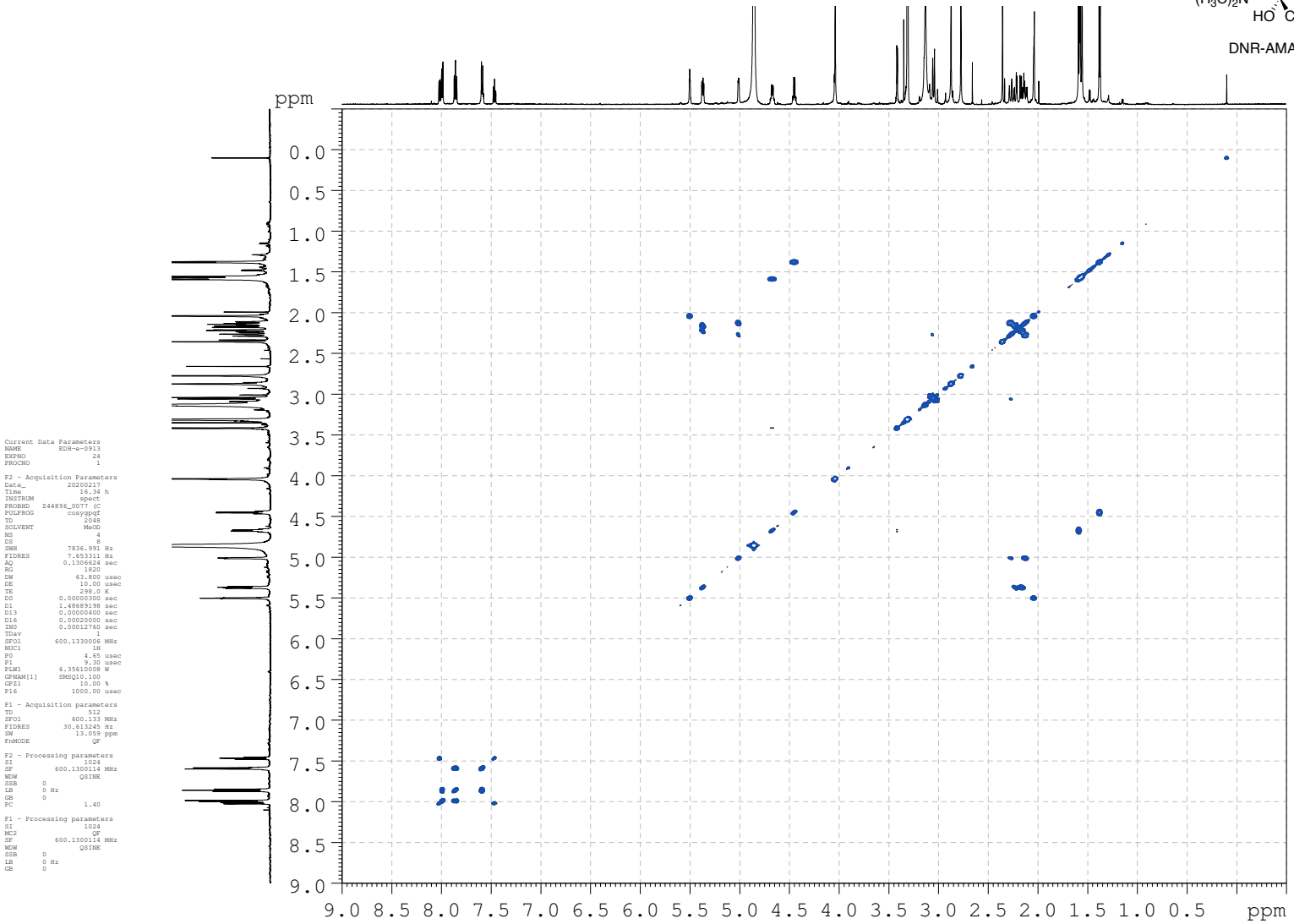

```
Current Data Parameters
NAME          EDH-e-0913
EXPNO                 29
PROCNO                 1

F2 - Acquisition Parameters
Date_           20200219
Time              9.11 h
INSTRUM             spect
PROBHD    Z44896_0077 (C
PULPROG   hsqcedetgpsisp.2
TD                  1024
SOLVENT             MeOD
NS                    32
DS                    16
SWH             1200.384 Hz
FIDRES          2.344500 Hz
AQ              0.4265301 sec
RG                  1820
DW                416.533 usec
DE                 10.00 usec
TE                298.0 K
CNST2       145.0000000
CNST17       -0.5000000
D0            0.00000300 sec
D1            1.50000000 sec
D4            0.00172414 sec
D11           0.03000000 sec
D16           0.00020000 sec
D21           0.00344828 sec
D24           0.00086207 sec
IN0           0.00001840 sec
TDav                  1
SFO1        600.1331507 MHz
NUC1                1H
P1                 9.25 usec
P2                18.50 usec
P28         0 usec
PLW1          6.35610008 W
SFO2        150.9158168 MHz
NUC2                13C
CPDPRG[2            garp4
P3                15.00 usec
P14              500.00 usec
P24             2000.00 usec
PCPD2             60.00 usec
PLW0       0 W
PLW2         178.91999817 W
PLW12      0 W
SPNAM[3] Crp60,0.5,20.1
SPOAL3              0.500
SPOFFS3    0 Hz
SPW3          61.50899887 W
SPNAM[7]    Crp60comp.4
SPOAL7              0.500
SPOFFS7    0 Hz
SPW7          61.50899887 W
GPNAM[1]      SMSQ10.100
GPZ1                80.00 %
GPNAM[2]      SMSQ10.100
GPZ2                20.10 %
P16               1000.00 usec

F1 - Acquisition parameters
TD                   128
SFO1            150.9158 MHz
FIDRES        424.592377 Hz
SW              180.060 ppm
FnMODE    Echo-Antiecho

F2 - Processing parameters
SI                   1024
```

^{1}H-^{1}H COSY NMR DNR-AMA Hybrid (24•2TFA) (600 MHz, CD_3OD)



DNR-AMA Hybrid (**24**•2TFA)

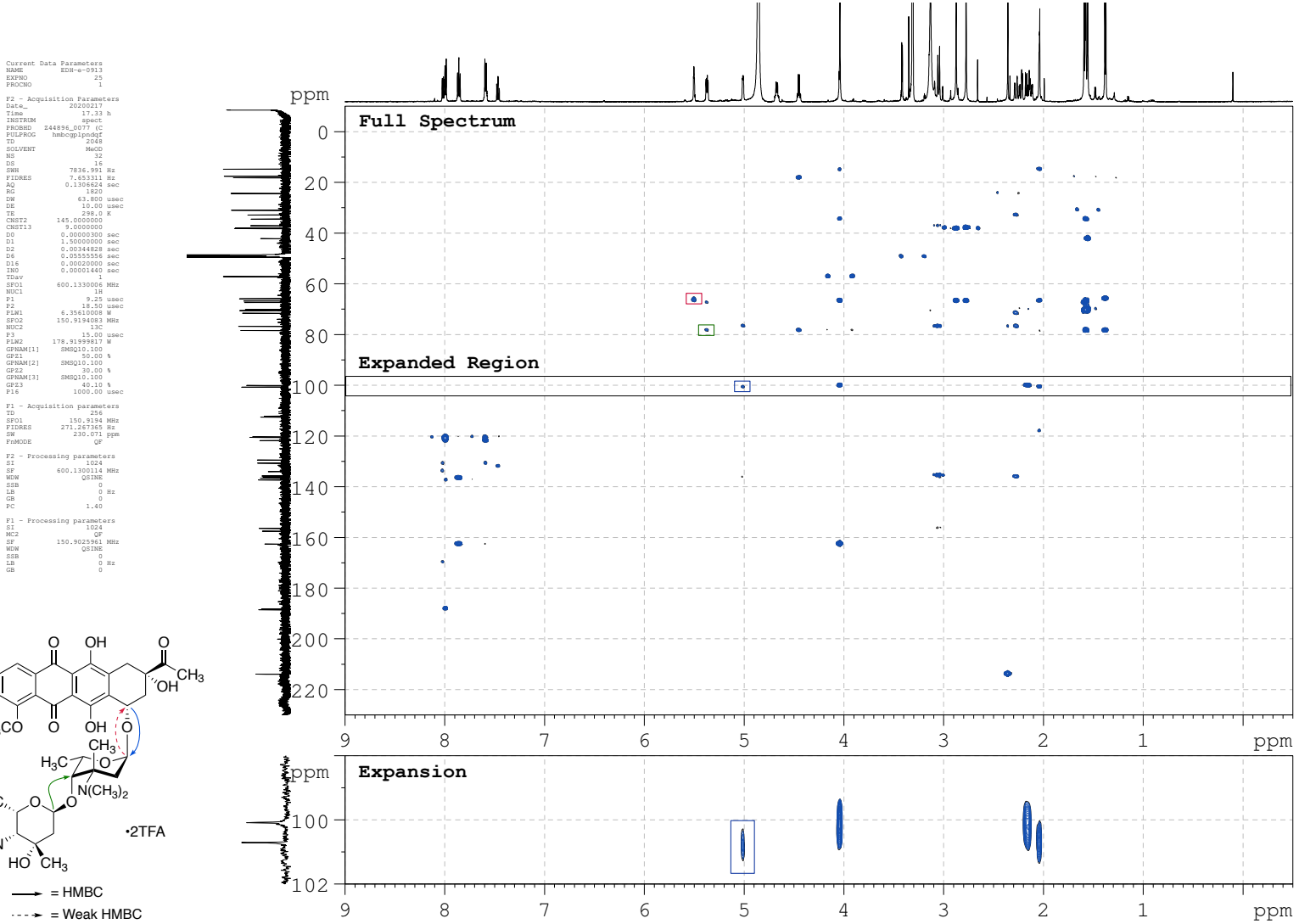
EDH-e-0913_C3F15-After Lyophilization (COSY, 600 MHz, CD3OD, 3 mm, NS = 4 scans)




```
Current Data Parameters
NAME          EDH-e-0913
EXPNO                 24
PROCNO                 1

F2 - Acquisition Parameters
Date_           20200217
Time              16.34 h
INSTRUM             spect
PROBHD    Z44896_0077 (C
PULPROG          coasygpqf
TD                  2048
SOLVENT             MeOD
NS                     4
DS                     8
SWH             7836.991 Hz
FIDRES             7.653311 Hz
AQ              0.1306624 sec
RG                   1820
DW                 63.800 usec
DE                 10.00 usec
TE                298.0 K
D0              0.00000300 sec
D1              1.48689198 sec
D13             0.00000400 sec
D16             0.00020000 sec
IN0             0.00012760 sec
TDav                   1
SFO1          600.1330006 MHz
NUC1                  1H
P0                  4.65 usec
P1                  9.30 usec
PLW1            6.35610008 W
GPNAM[1]      SMSQ10.100
GPZ1                10.00 %
P16              1000.00 usec

F1 - Acquisition parameters
TD                   512
SFO1            600.133 MHz
FIDRES          30.613245 Hz
SW                13.059 ppm
FnMODE                QF

F2 - Processing parameters
SI                   1024
SF            600.1300114 MHz
WDW                 QSINE
SSB                    0
LB                 0 Hz
GB                     0
PC                  1.40

F1 - Processing parameters
SI                   1024
MC2                   QF
SF            600.1300114 MHz
WDW                 QSINE
SSB                    0
LB                 0 Hz
GB                     0
```

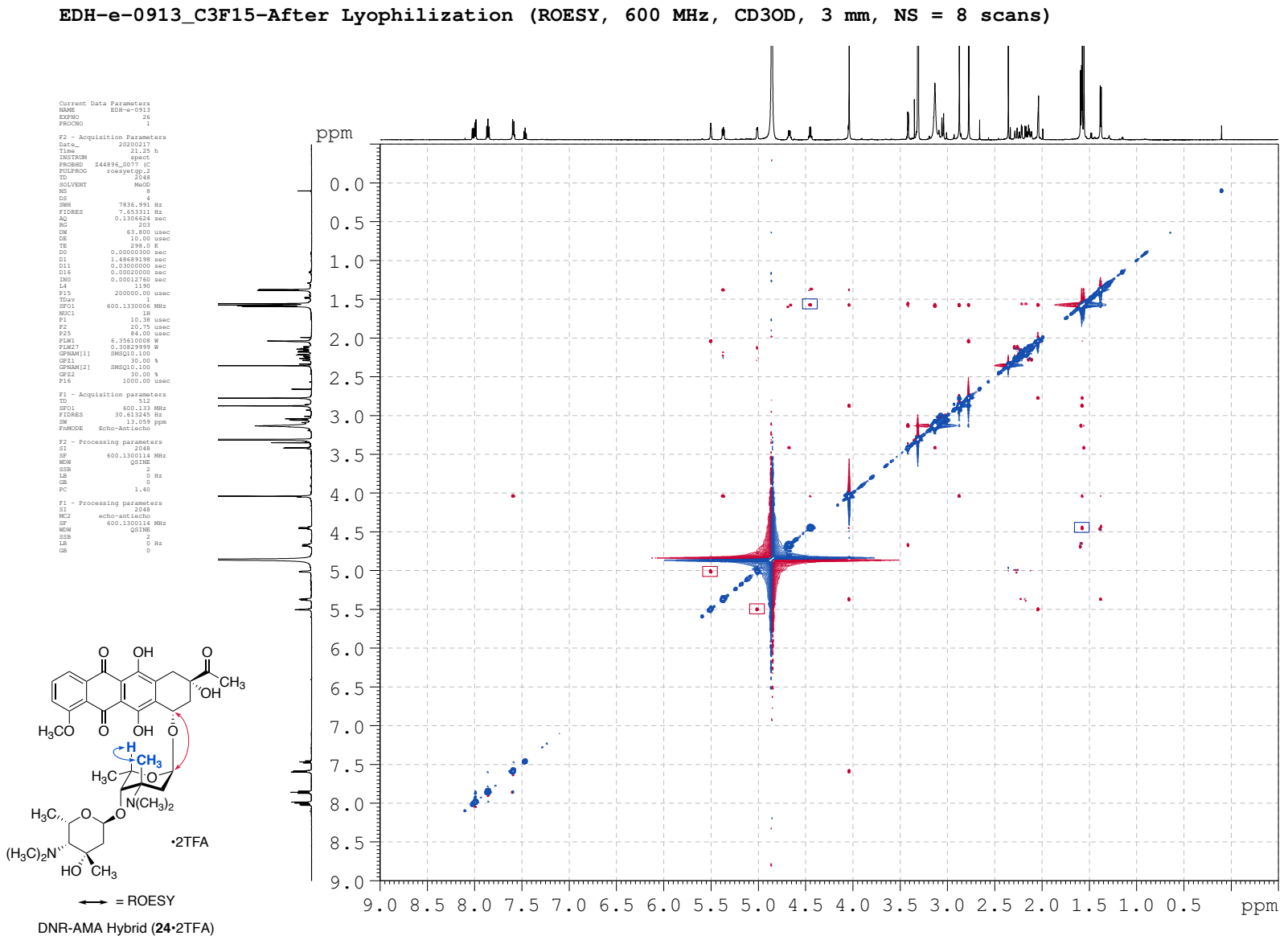
^{1}H-^{13}C HMBC NMR DNR-AMA Hybrid (24•2TFA) (^{1}H-600 MHz, ^{13}C-151 MHz, CD_3OD)

EDH-e-0913_C3F15-After Lyophilization (HMBC, 600 MHz, CD3OD, 3 mm, NS = 32 scans)



^{1}H-^{1}H ROESY NMR DNR-AMA Hybrid (24•2TFA) (600 MHz, CD_3OD)

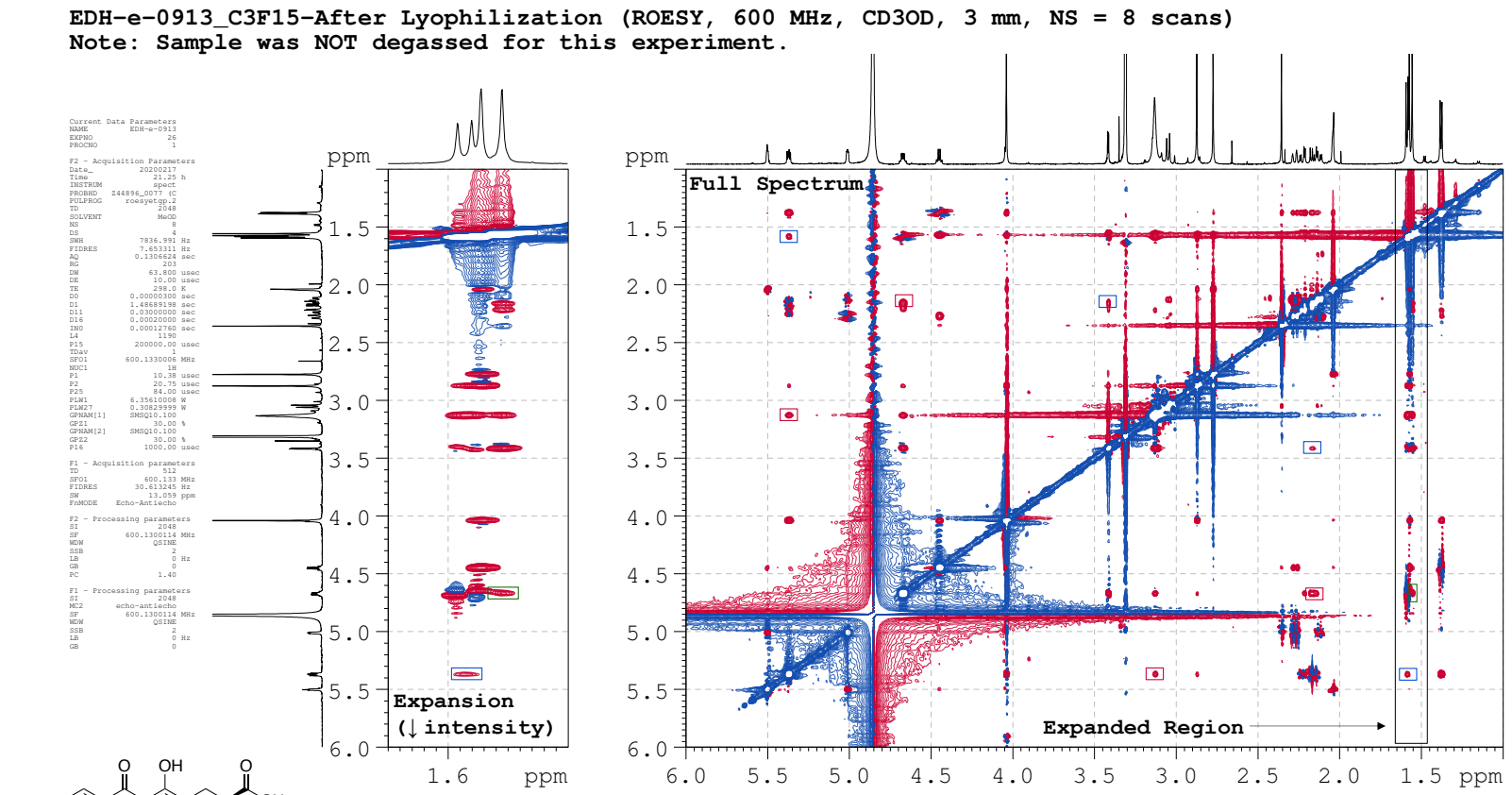
Note: Blue = diagonal/chemical exchange | Red = ROESY cross peaks.

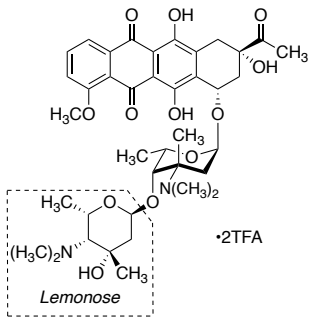


^{1}H-^{1}H ROESY NMR DNR-AMA Hybrid (24•2TFA) (600 MHz, CD_3OD)

Note 1: Blue = diagonal/chemical exchange | Red = ROESY cross peaks.

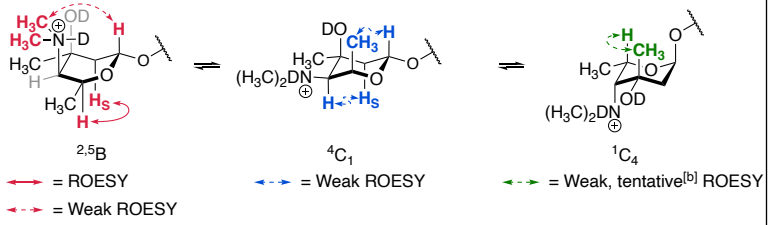
Note 2: Same spectrum with increased intensity and smaller spectral window as compared to ROESY on page S409.





DNR-AMA Hybrid (**24**•2TFA)

24•2TFA: Conformational Analysis of the Lemonose Sugar[a]

ROESY correlations (as well as the anomalous $^{3}J_{H1''\text{-}H2''}$ coupling constants of 8.0 and 4.0 Hz) indicate that the reducing sugar does not solely occupy the canonical $^{1}C_4$ conformation; rather, as indicated by the ROESY correlations highlighted to the right, the non-reducing sugar exists in conformational equilibrium and/or an intermediate conformation not pictured here.

[a] Full deuterium exchange (alcohol and protonated amine) was assumed.
[b] Tentative ROESY correlation defined as a correlation in which only 1 of the 2 possible cross peaks are easily discernible.