Total Synthesis of the *Morganella Morganii* Zwitterionic Polysaccharide Repeating Unit

By

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Chapter 1

A look into the field of zwitterionic polysaccharide (ZPS) synthesis

Part 1. ZPS Biological Significances

Zwitterionic polysaccharides (ZPSs) are a unique class of biopolymers comprised of carbohydrate based repeating units which bear positive and negatively charged moieties. For the most part, polysaccharides are generally devoid of charged functional groups, or are net negatively charged, and do not elicit an adaptive immune response.[1-3] An additional unique characteristic of ZPSs lies in their ability to elicit a T-cell stimulatory

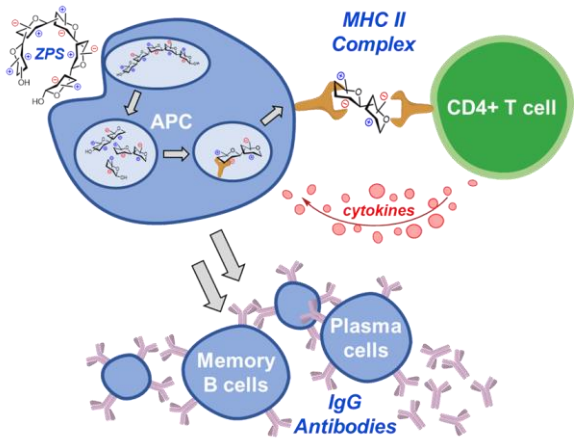


Figure 1. ZPS processing and presentation by the MHCII pathway

response; independent from peptides, protein, or lipids.[4] The ZPS-antigen can be processed by antigen presenting cells (APCs), bind with major histocompatibility complex

II (MHCII) and then presented on the surface to the α/ß-T-cell receptor of a naïve T-lymphocyte (depicted in Figure 1).[1] This process initiates when ZPSs, expressed as an MHCII-ZPS complex on the surface of B-cells, encounter ZPS primed T-cells and get necessary costimulation. The B-cells are then transformed into long-living plasma cells that can generate immunoglobulin from isotype switching and memory B-cells.[5-7]

ZPSs have been discovered and isolated from the capsule or the lipopolysaccharide (LPS) of various strains of bacteria: *Morganella morganii* (MM-ZPS),[8] *Bacteroides fragilis* (PS A1 & PS B),[9] *Staphylococcus aureus* (CP5 & CP8),[10] *Streptococcus pneumoniae* (SP1),[11] *Providencia alcalifaciens O22,*[12-13] *Plesiomonas shigelloides*,[14] *Photorhabdus temperate* subsp. *Cinereal* 3240,[15] *Shigella sonnei Plesiomonas shigelloides*.[16-17] In addition to the naturally occurring ZPSs, synthetic ZPSs have been produced by modifying neutral carbohydrate polymers with charged groups. This process has been shown to also elicit similar immunomodulatory response.[18-19] As expected, neutralization of the charged groups via chemical modification eliminates any T-cell independent response initially elicited by the ZPSs which reflects the importance of the zwitterionic character for the immune stimulation.[20-21]

Recently, many researchers have looked toward ZPS properties for inspiration in generating novel immunotherutics.[22-36] This has led to the development of new immunotherapeutics by conjugating antigens of interest, such as tumor associated carbohydrate antigens (TACAs), to ZPSs.[29] TACAs are truncated and overexpressed on the surface of malignant cells, lending them to be ideal candidates for immunotherapeutic development.[37-38] The aberrant glycosylation pattern found on tumor cells are a result of overexpression of glycosyltransferases in malignant cells.[39-43] Carcinogenesis is

accompanied by aberrant glycosylation of glycoproteins and glycolipids.[44] These events are correlated with multiple stages of tumor development, particularly the invasion and metastasis stages.[44] TACAs are expressed on myriad cancers while remaining essentially nonexistent on healthy cells; making them a promising target antigens immunotherapeutic development.[24, 45-46] However, because TACAs are endogenous carbohydrate antigens and are not zwitterionic, they elicit very low levels of immunogenicity.[22] TACAs can only stimulate the production of B-cells, and are not taken up by antigen presenting cells (APCs). Therefore, they are unable to induce a T-cell dependent immune response.[22, 24, 47-48] Without the presentation to T-cells, class switching from IgM to IgG and enhanced cell memory cannot be triggered.[34, 39, 42, 49]

One way to promote T-cell activation has been to conjugate TACA antigens to carrier proteins. Carrier proteins are strongly immunogenic molecules which can stimulate a long-lasting immune response. Many carriers have been reported, including proteins, peptides, nanoparticles, liposomes, and polysaccharides. The most well studied and used carriers include Bovine Serum Albumin (BSA),[50] Keyhole Limpet Hemocyanin (KLH),[51] CRM197, Diphtheria toxin (DT),[52] tetanus toxin (TT),[53] and most recently the PS A1 ZPS,[1, 32-35, 54-55] and the PS B ZPS.[56] Unfortunately, there are limitations to using large carrier proteins. Epitope suppression of the antigen can occur when a stronger immune response is elicited towards the carrier protein than the conjugated antigen. In addition, carrier proteins are large biomolecules which have an undefined chemical structure which leads to ambiguity in conjugation of antigen.

ZPSs are being developed as an alternative for large carrier proteins. ZPSs, such as PS A1, are an order of magnitude smaller than the average size of KLH (3 x 10^6 MW) and

can stimulate an equivalent immune response.[20, 57] In addition, ZPSs simultaneously interact with TLR-1 in addition to MHCII making them an attractive alternative to carrier proteins.[58-60] To date, two ZPSs have been used in the development of ZPS vaccine conjugates: PS A1 and PS B.[1, 32-35, 56] These ZPS-TACA conjugates are made using isolated and purified native ZPS. Currently this is the only synthetically feasible way to develop ZPS based vaccines due to the required size of ZPS needed to stimulate the immune system. It has been discovered that ZPSs smaller than 120 kDa (~20 monosaccharide residues) have little to no immunomodulatory properties.[3-4, 57, 61-67] While isolation is currently the most efficient option for construction of ZPS conjugate immunotherapeutics, total synthesis superior option for obtaining pure synthetic entities for definitive mechanistic and immunological studies. The development of chemical probes can include site specific modifications to access labeled compounds and microarray technology. Chemical probes and tool compounds can lead to be better understanding of chemical biology and the ZPS-immune system interactions which will in turn lead to better more effective therapeutics. In addition, total synthesis allows for structure validation of reported ZPSs. While this may seem as a trivial justification for the arduous task total synthesis, history shows that initial structural identification of compounds is not always accurate.[68-76] Total synthesis also allows for the ability to construct derivatives of the target molecule. Derivatization can lead to the development of more potent, safer, effective compounds and lead to discovering the minimum pharmacophore. For the broader field of ZPSs, the minimum pharmacophore is still yet to be determined.

Part 2. Synthetic studies towards a Sp1 ZPS repeating unit

Structural elucidation of the Sp1 ZPS was carried out by NMR analysis of degraded native polysaccharide in 1980 by Powell and co-workers at the University of Stockholm in Sweden.[77] Powell and co-workers determined the Sp1 polysaccharide is made up of trimer repeats composed of α-D-2-*N*-acetamido-4-amino-2,4,6-trideoxy-D-galatopyranose (D-AAT) and two α-D-galacturonic acid resides which differ at the site of glycosylation (C3 and C4).

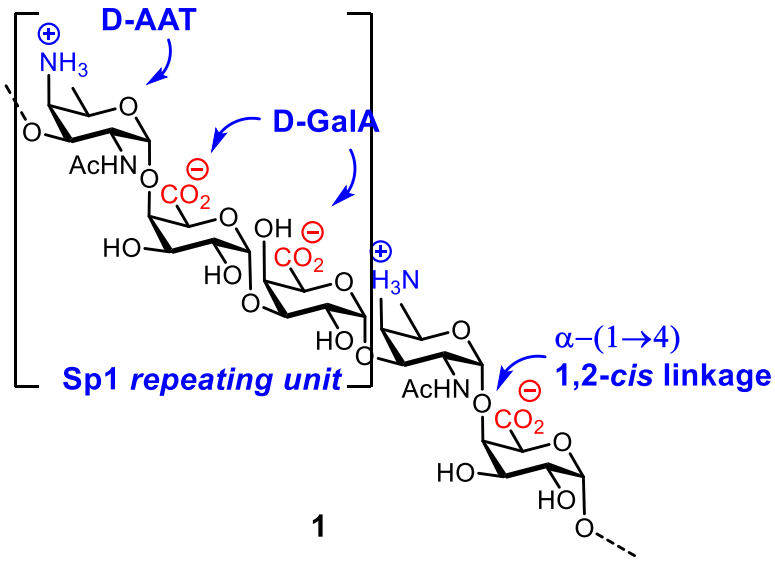


Figure 2. Sp1 structure

They were then able to report the structure of the Sp1 ZPS (**1**): →3)-α-Sug*p*-(1→4)-α-D-Gal*p*A-(1→3)-α-D-Gal*p*A-(1→ repeating structure (Figure 1). Total synthesis of the Sp1 ZPS poses three major challenges. Firstly, methods for incorporation of the rare monosaccharide 2-acetamido-4-amino-2,4,6-trideoxy-D-galactose (D-AAT) residue are

understudied.[78] These rare amino sugars contain a free amine at the C4 position and an acetamide at the C2 position. The D-AAT residues are found in such low quantities in nature, isolation is not a viable option for obtaining synthetic precursors. Because of this many procedures for preparation of D-AAT building blocks have been reported.[17, 79-86] Currently, the most common way to access the rare D-AAT building block is through manipulations of D-GlcNAc (Figure 2). Two major transformations must take place to convert a D-GlcNAc **2** residue to a D-AAT building block **3** (Figure 3, part a). One involves conversion of the equatorial C4 (*R*) alcohol into an axial C4 (*S*) amine. This is usually carried out by converting the C4 alcohol into a good leaving group (-OTs, -OMs, -OTf) followed by displacement by an amine nucleophile (NaN_3). Secondly the C6 alcohol needs to be removed. This is carried out by conversion into a suitable leaving group and then displaced by a hydride nucleophile to give the D-AAT residue, such as **3**. These methodologies were adopted by Sharon,[87] Lonngren,[88] Pozsgay,[82] Schmidt,[89] Grindley,[90] Van der Marel,[66] and their respective co-workers to synthesize building blocks for the synthesis of the Sp1 ZPS.

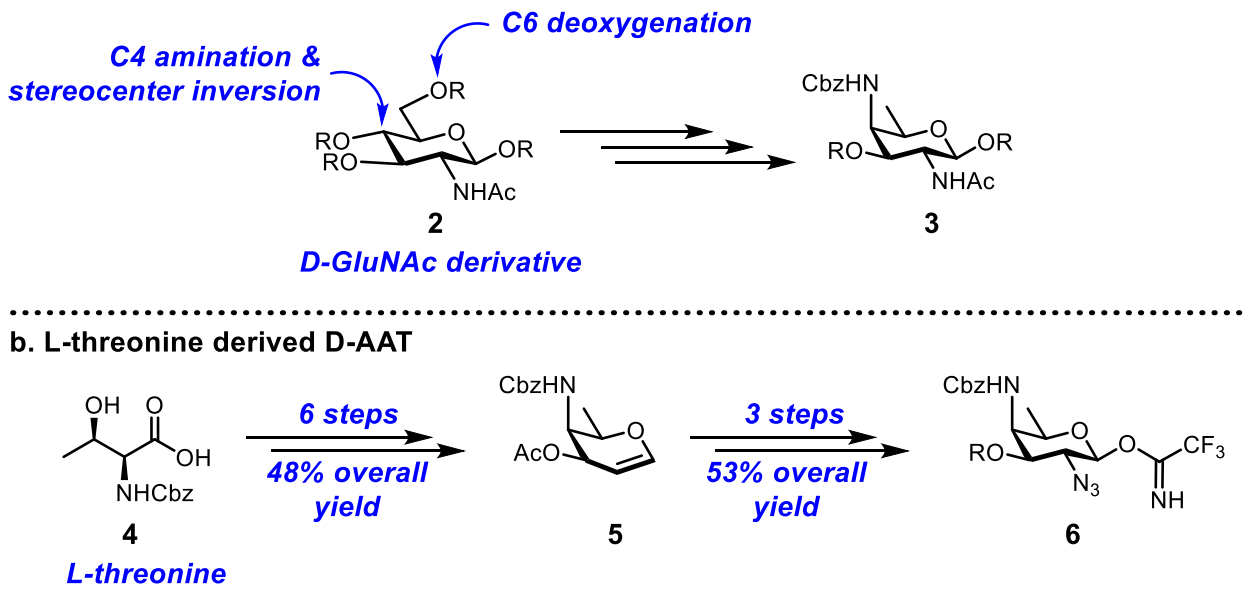


Figure 3. Synthesis of D-AAT glycosides

In addition, efforts to produce synthetic building blocks of the D-AAT residue have been developed by Seeberger and co-workers as a de novo strategy from Cbz-protected L-threonine **4** (Figure 3, part b).[83, 91] This procedure involved a Dieckmann cyclization of acetylated L-threonine **4** to form a β-ketoester product which under a series of steps is converted to the glycal **5**. Introduction of the C2 amine is carried out by azido-nitration or azido-selenation of the glycal **5**. Following two additional steps, the azido product can be converted into a suitable D-AAT donor building block **6**. As we will see in synthesis of Sp1 and PS A1 the C2-azide protected D-AAT donors are ideal building blocks for formation of the α-glyosidic linkages, due to the non-participatory nature of the azide functionality.

In contrast, when a D-AAT donor is required to form a β-glycosidic linkage it can be advantageous to use a -NHTCA or -NHAc protected amine at the C2 position to leverage the ability for neighboring group participation (NGP) by the acetamide functionality. D-glucal derivatives have also been used as a D-AAT precursor by Bundle[92] and D-mannose by van Boom[93] and Kulkarni.[94]

Another key synthetic consideration for the Sp1 ZPS is the D-galacturonic acids. D-galacturonic acids are generally regarded as both poor glycosyl donors and poor glycosyl acceptors due to the electron-withdrawing effects of the C6 carboxylic acid typically masked as esters.[95-96]

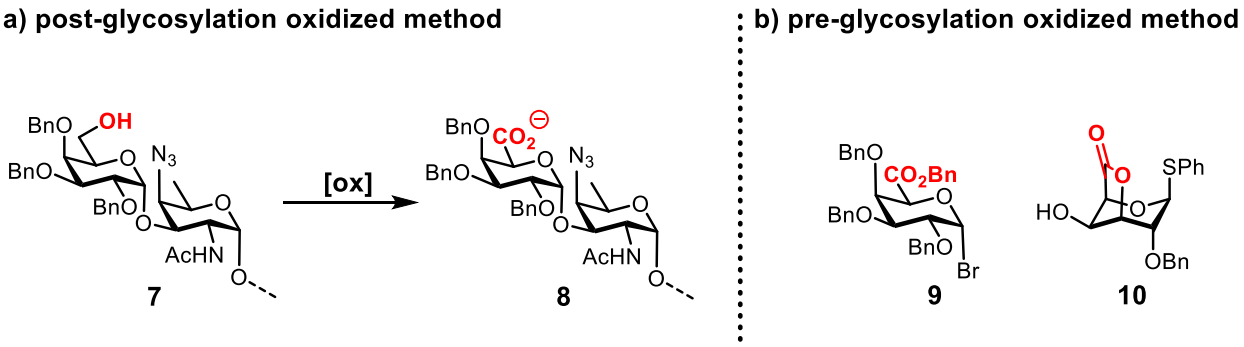


Figure 4. D-GalA donor derivatives and methods

Many synthetic methods have been developed to combat this lack of reactivity. The most common strategy involves a late stage oxidation of a D-Gal residue. For example, after construction of the core oligosaccharide using D-galactosyl residues, such as **7**, a late stage oxidation of the C6 alcohol to a carboxylic acid to provide the D-galacturonic acid

residue, such as **8** (Figure 4, part a). This method will be referred to as the *post-glycosylation oxidation method* involves the oxidation after the glycosidic bond has been formed. In contrast, the *pre-glycosylation oxidation method* uses C6-ester protected D-GalA donors, such as **9** to build the oligosaccharide core (Figure 4, part b). These can also be protected as the intramolecular lactone such as **10**.[97]

The third major synthetic challenge of the Sp1 ZPS repeating unit involves the 1,2-*cis* connectivity. The Sp1 ZPS is purely composed of 1,2-*cis*-glycosidic linkages which afford a reasonable degree of synthetic challenge.[98-99] The presence of a non-participating group is required for the synthesis of 1,2-*cis* glycosides, but non-participating group alone cannot ensure the stereoselectivity. Although the α-product is favored by the anomeric effect, this typically offset by steric factors of having adjacent *O*-linked substituents on the same face of the ring (1,2-*cis*). Therefore, stereoselectivity of glycosylation is typically poor and requires other modes of stereocontrol, such as participating solvent or reaction additives.[99]

Part 3. Partial synthesis of Sp1 repeating unit by Lonngren (1984)

Following this discovery of the Sp1 repeating unit structure by Powel, a synthesis was carried out by Lonngren and Lonn in 1984 at the University of Stockholm.[100] The target molecule for Lonngren and Lonn was a partial ZPS unit: methyl 2-acetamido-4-amino-2,4,6-trideoxy-3-*O*-(α-D-galactopyranosyl-uronic acid)-α-D-galactopyranoside **11** (Figure 5, part a).[100] The target disaccharide **12** derived from the greater polymer (**11**) contained an anomeric methyl group, which is not found in the isolated ZPS. This is referred to as a capping group. Capping groups are often utilized to prevent mutarotation of the reducing sugar.[101] To date, all synthetic ZPSs have employed capping groups to prevent the final

product from existing as a mixture of interconverting isomers. Retrosynthetic analysis of the target disaccharide **12** provided the monosaccharide building blocks **13** and **14** following a deprotection step and disconnection of the glycosidic bond (figure 5, part b, disconnection *i*). The D-GalA donor **13** was synthesized in a 31% yield over three steps from the known methyl (methyl α-D-galactopyranoside) urinate.[102] The D-AAT building block **14** was synthesized in a 9% yield over eight steps from D-GlcNAc.[103] This donor represented the *pre-glycosylation oxidation strategy* previously mentioned in part 2.

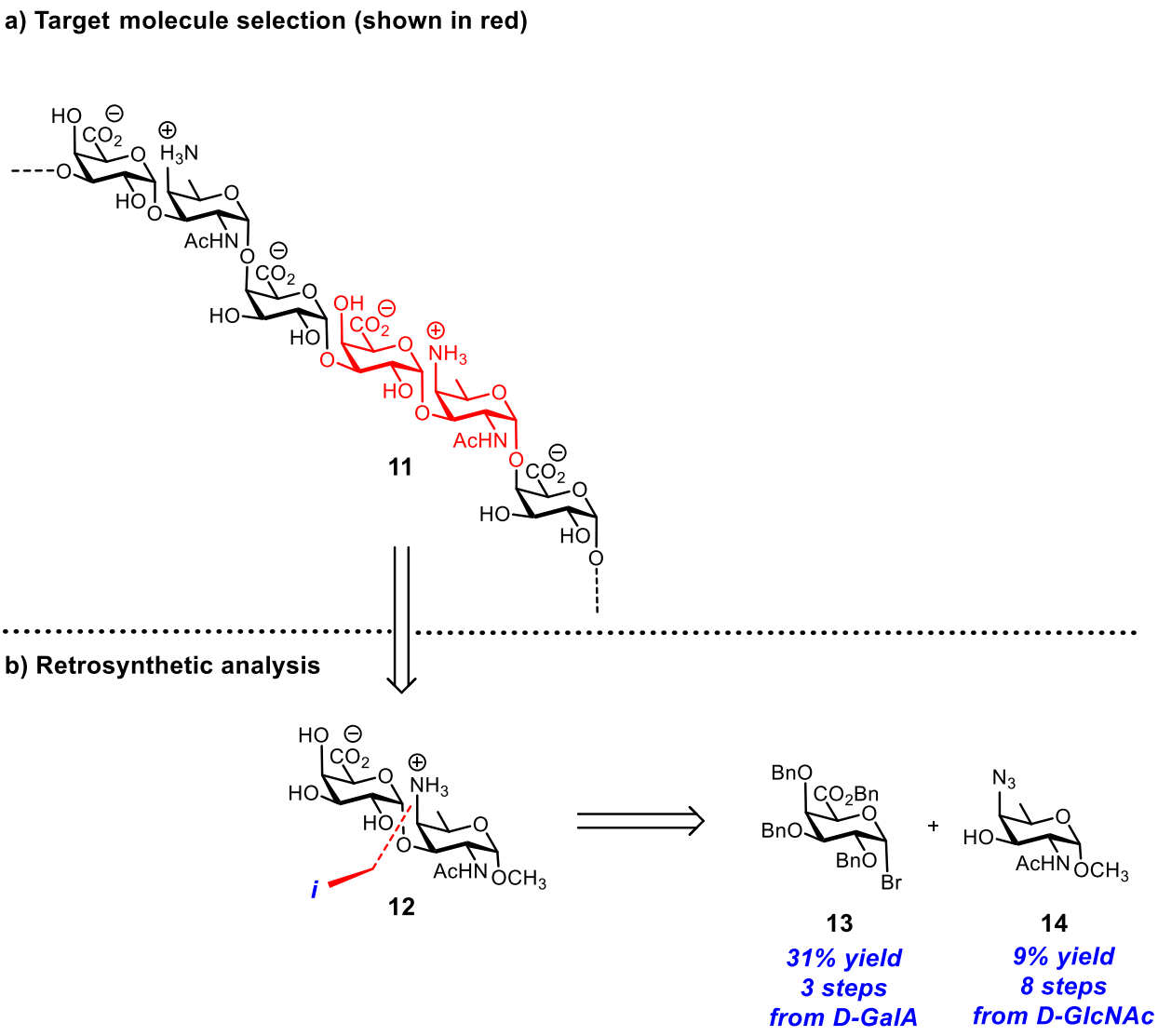


Figure 5. Retrosynthetic analysis (Lonngren, 1984)

The synthesis of **12** commenced with the construction of the bromide donor **13** and a D-AAT acceptor building blocks **14**. The glycosylation event between donor **13** and acceptor **14** was not trivial. Standard glycosylations conditions to favor an α-1,2-*cis* provided low yields and/or poor alpha/beta ratios of product (Scheme 1, entry 1 and 2). This could be

due to the use of the D-GalA residue which is known to poor glycosyl donor due to the disarming effects of the electron withdrawing group at the C6 position. Zinc bromide as a promotor system gave provided the desired disaccharide **15** with a good alpha beta ratio and good yield (entry 3). The fully protected disaccharide was hydrogenated over Pd/C which facilitated reduction of the azide residue to provide the C4 free amine and removal of the benzyl protecting groups. Following purification through a size exclusion column Lonngren and Lonn obtained their desired product **12**. While this route did not complete the Sp1 trisaccharide ZPS, it was incremental in laying the foundation for Sp1 synthesis to follow.

Scheme 1. Sp1 disaccharide synthetic route

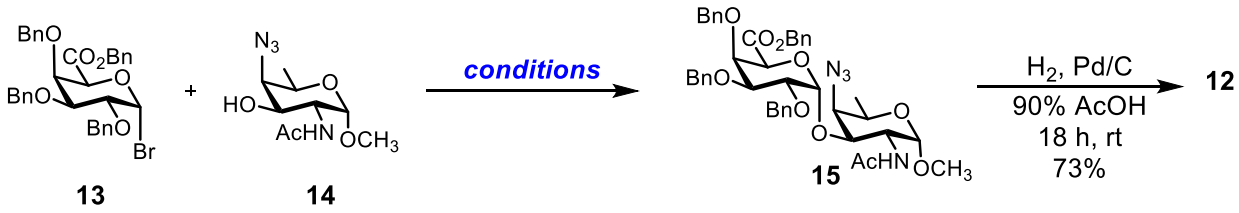


Table 1. reaction conditions for 15

entry	promotor	yield	αβ-ratio
1	$HgBr_2$ *-or-* AgOTf collidine	55%	3:1
2	TBAB	35%	8:1
3	ZnBr 4 Å MS	73%	7:1

Part 4. Partial synthesis of Sp1 repeating unit by Oscarson (2016)

Oscarson and co-workers, at the University College of Dublin in 2016, published a strategy for an iterative approach towards oligomers of Sp1.[79] Their approach was

focused on the synthesis of a trisaccharide repeating unit that contained the two sequential D-GalA residues with the D-AAT residues at the non-reducing end of the trisaccharide (figure 6, shown in red). Oscarson and co-workers planned two competing synthetic routes which could potentially achieve a Sp1 repeating units **17** and **18**. The differences in synthetic routes was the oxidation sate of the D-GalA precursor building blocks.

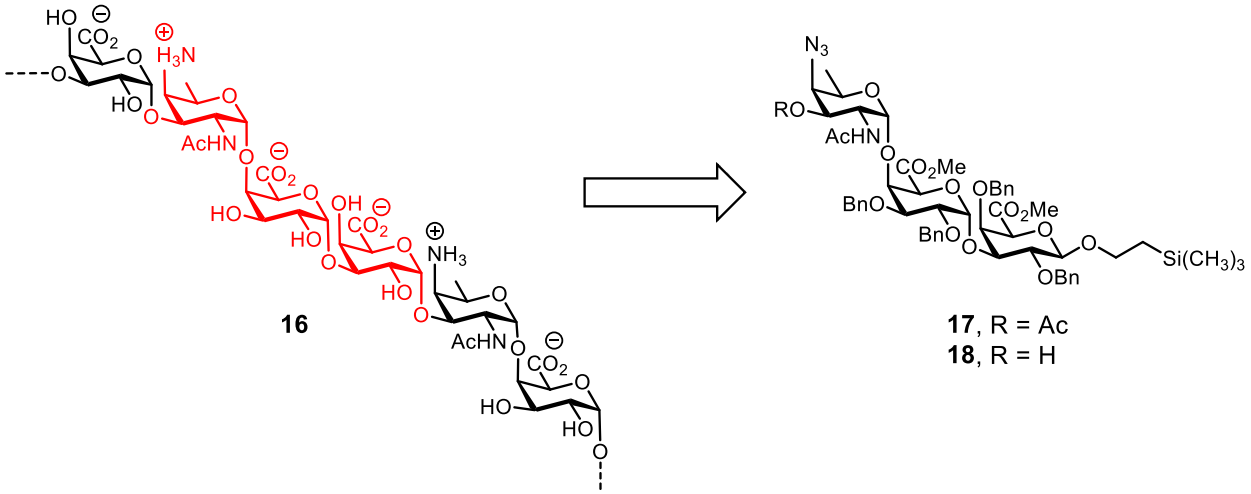


Figure 6. Oscarson and co-workers repeating unit selection

The first route, which incorporated a *pre-glycosylation oxidation strategy*, sought to obtain the protected trisaccharide **17** by utilizing D-GalA building blocks (**20** and **21**) to avoid a late stage oxidation of the C6 and C6' positions (figure 7, part a). The methyl ester D-GalA building blocks, **20** and **21**, which were derived from previously reported advanced intermediates.[104-105] This route incorporated the C2 *N*-aryl imine protected D-AAT

derivative **19**. The D-AAT thioglycoside **19** was obtained through seven steps from D-GlcN in a 48% yield.

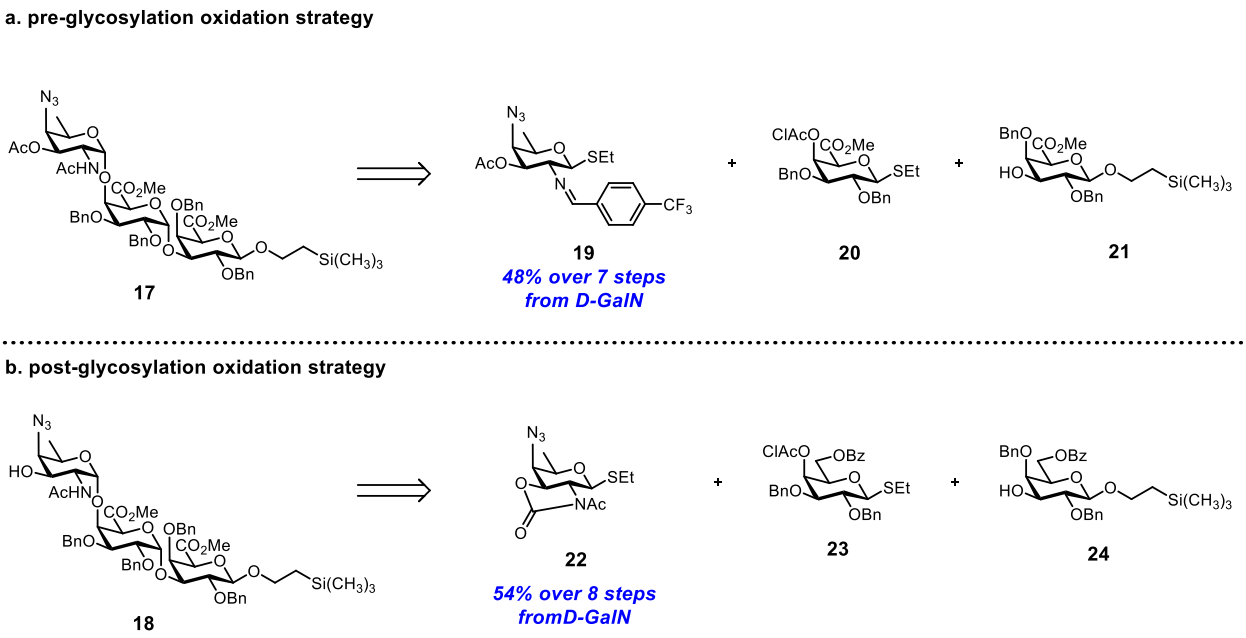


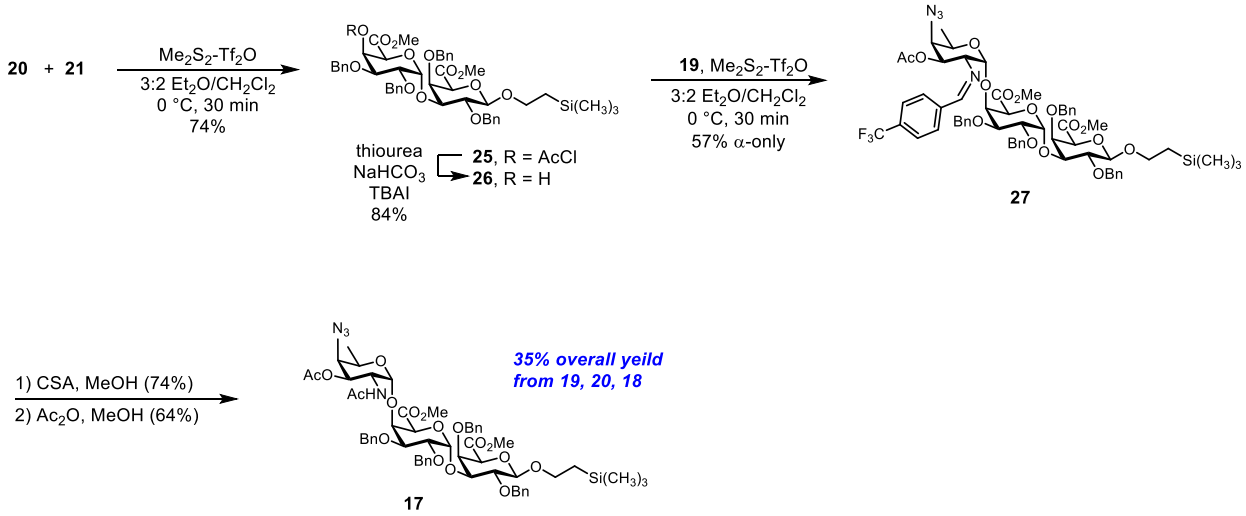
Figure 7. Retrosynthetic analysis by Oscarson and co-workers

The competing synthetic route used the *post-glycosylation oxidation strategy* to avoid the anticipated difficulties of D-GalA residues as glycosidic donors and acceptors to provide the desired trisaccharide **18** (Figure 7, part b). This route used D-Gal building blocks **23** and **24** and D-AAT thioglycoside donor **22** to obtain a fully protected trisaccharide which could subsequently undergo a late-stage oxidation to afford the protected trisaccharide **18** (figure 7, part b).

The *pre-glycosylation oxidation* approach began with glycosylation between thioglycoside donor **20** and D-GalA acceptor **21** which gave α-liked disaccharide **25** (Scheme 2). A

thiourea mediated saponification of **25** gave acceptor **26**. Next, a glycosylation with the thioglycoside donor **19** took place. Glycosylations with *N*-imine protected donor **19**, under nickel catalyzed stereoselective glycosylation conditions, developed by Nguyen to provide the 1,2-*cis*-2-amino glycosidic linkages.[106-107] Formation of the α-linked trisaccharide **27** proceeded in good yield, but with poor stereoselectivity. Acid mediated hydrolysis of the imine followed by acetylation to provide the C2'' acetamide afforded protected trisaccharide **17**. From the monosaccharide building blocks (**19**, **20**, and **21**), trisaccharide **17** was obtained with a 35% overall yield.

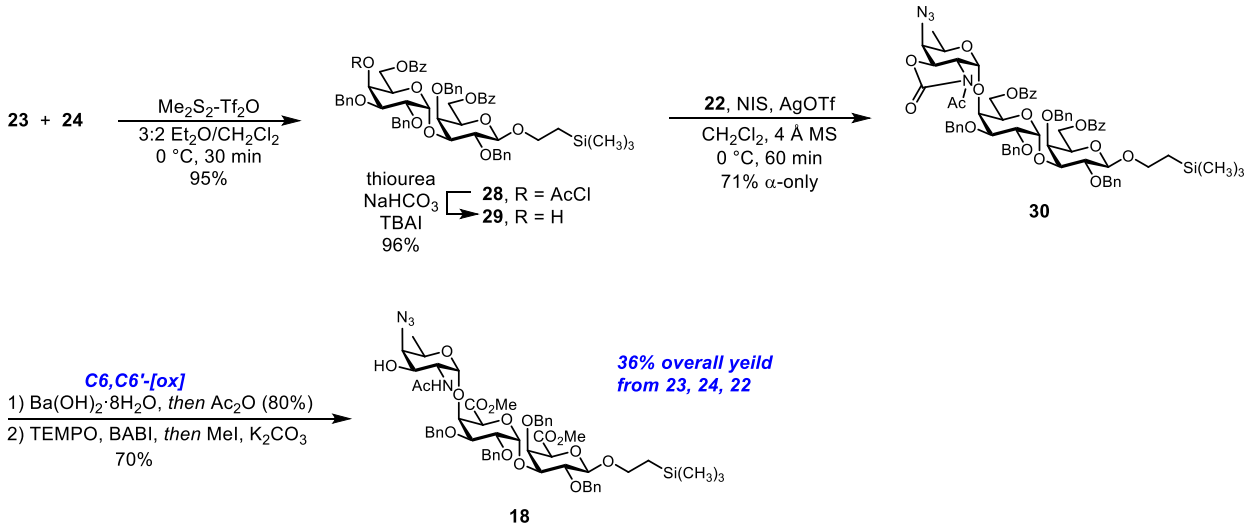
Scheme 2. pre-glycosylation oxidation route



Of the two *post-glycosylation oxidation* strategy discussed, the *optimized* route to synthesize **18** will be discussed here (scheme 3). The route commenced with a glycosylation between D-Gal thioglycoside donor **23** and D-Gal acceptor **24** to give α-linked disaccharide **28**. Following a saponification of the C4'-AcCl to give **29**, a glycosylation event with thioglycoside **22** gave trisaccharide **30**. In this case, the tunable reactivity of **22** was leveraged to provide the desired α-glycosidic linkage.[108] Following

the C2''-C3'' carbamate and C6 and C6' benzoyl groups were saponified followed by a C6 and C6' selective TEMPO oxidation and esterification to give protected trisaccharide **17**. From the monosaccharide building blocks (**22**, **23**, and **24**), trisaccharide **17** was obtained with a 35% overall yield.

Scheme 3. post-glycosylation oxidation route



It was found that both synthetic routes proceeded to the advanced trisaccharides (**17**, and **18**) in similar overall yield. However, building block synthesis was easier for the *post glycosylation oxidation* route to give trisaccharide **18** (scheme 3.). Next, the group wanted to transform the advanced trisaccharide intermediate **18** into a donor and an acceptor so they could obtain the hexasaccharide; Sp1 dimer (figure 8). Compound **18** was successfully transformed into trichloroacetimidate donor **31** in three steps using standard conditions (Figure 8, part a). In an additional two steps, the trisaccharide acceptor **32** was produced from **31**. Unfortunately, glycosylation events between donor **31** and acceptor **32** never afforded the desired product. Rather, they gave the undesired, β-linked,

hexasaccharide (Figure 8, part b). The group did not report a global deprotection of any trisaccharide or hexasaccharide material to give the Sp1 repeating unit.

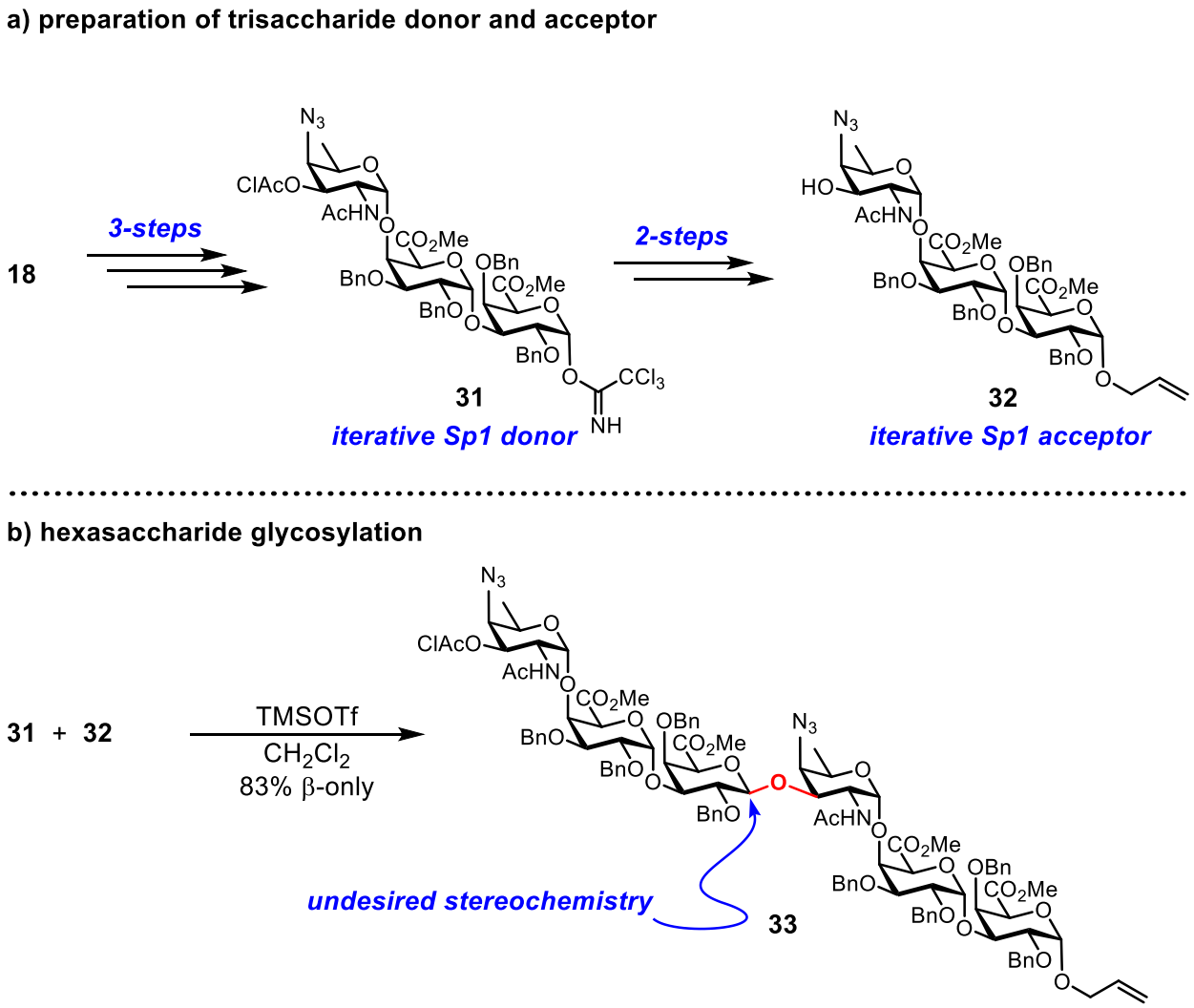


Figure 8. Attempted synthesis of an Sp1 ZPS hexasaccharide

The partial synthesis demonstrated by Oscarson and co-workers played on many of the strengths discovered by previous total (*vide infra*) and partial syntheses (*vide supra*). The

large number of total steps required and the difficulties of establishing the desired anomeric stereochemistry illustrates many of the inherent difficulties encountered when attempting such a difficult total synthesis.

Part 5. Bundle and co-worker's total synthesis of Sp1 (2010 report)

The first successful total synthesis of the Sp1 ZPS repeating unit was completed in 2010 by the Bundle group.[109] Bundle and co-workers selected the repeating unit frame which contains a central D-AAT reside and two flanking D-GalA residues (Figure 9. shown in red). From this greater oligosaccharide **34**, the repeating trisaccharide **35** was selected at the synthetic target. The repeating unit **35** contained a C1 methyl capping group to prevent mutarotation of the final product.

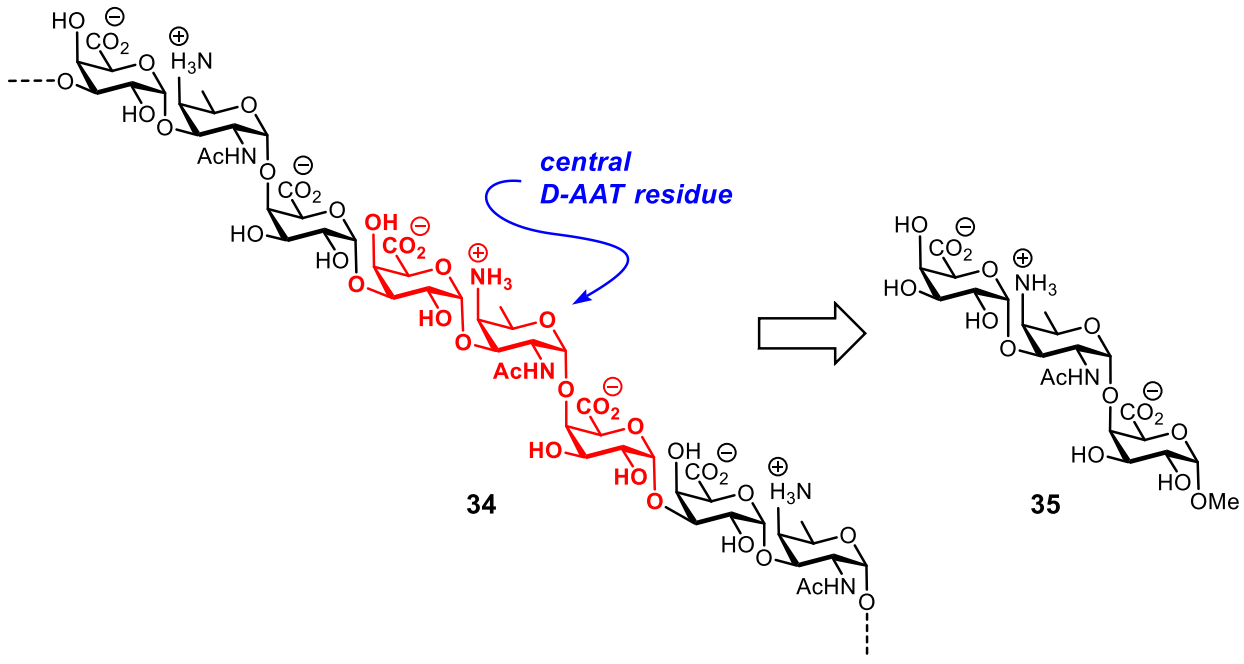


Figure 9. Bundle (2010) synthetic target selection of Sp1

The synthetic route was based on the *post-glycosidation oxidation strategy* and was planned to construct the target molecule. During the final synthetic stages, C6 and C6' alcohols would be deprotected and oxidized to the corresponding carboxylic acids. In contrast to other synthetic routes discussed thus far, the synthetic plan included production of the D-AAT residue by performing functional group interconversions of a protected D-Gal residue at an advanced stage of synthesis. Overall, the repeating unit **35** was planned to be synthesized from the reducing to non-reducing end.

Retrosynthetic analysis provided deprotection of **35** would give the protected trisaccharide **36** (Figure 10). Making the disconnection of the D-Gal-α-(1→3)-D-AAT glycosidic bond would provide the thioglycoside donor **37** and the disaccharide acceptor **38**. The thioglycoside donor **37** could be prepared in seven steps from D-Gal. The disaccharide acceptor **38** could come from disaccharide **39** following significant functional group interconversion to produce the D-AAT residue from a D-GlcN. Three major synthetic transformations needed to take place; 1) conversion of the C2' azide into an acetamide, 2) inversion of the C4' stereochemistry and installment of the an azide (masked amine), 3) deoxygenation of the C6' alcohol. Next, the D-AAT-α-(1→3)-D-GlcN glycosidic linkage could be disconnected to provide the building block donor **40** and acceptor **41**.

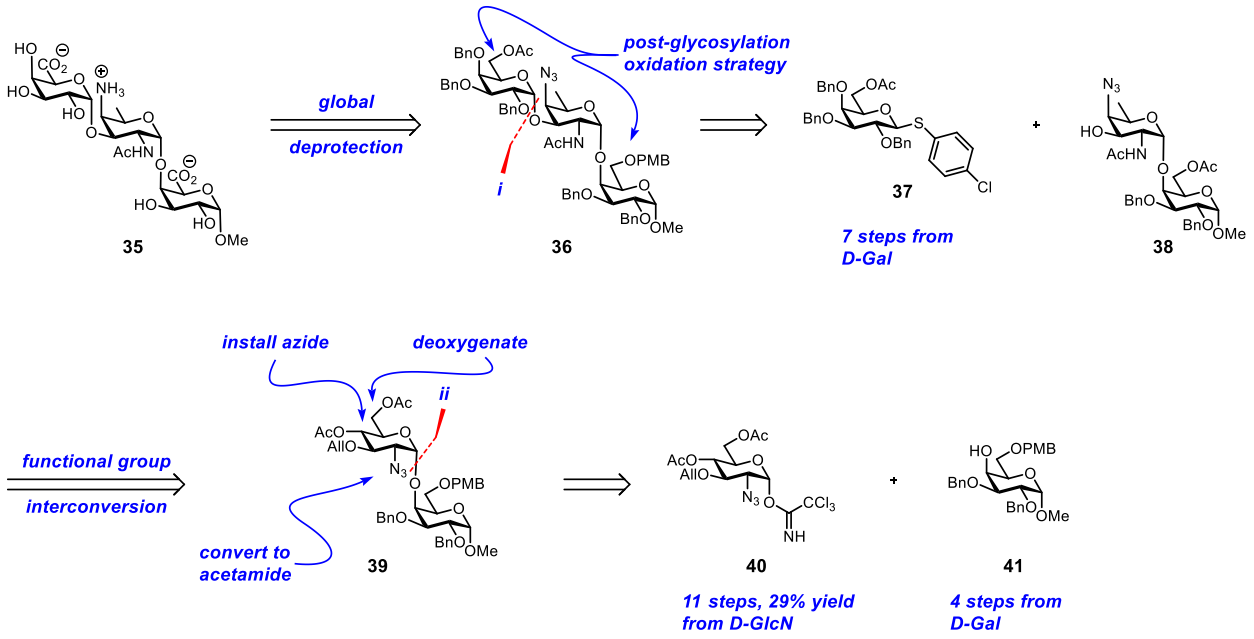
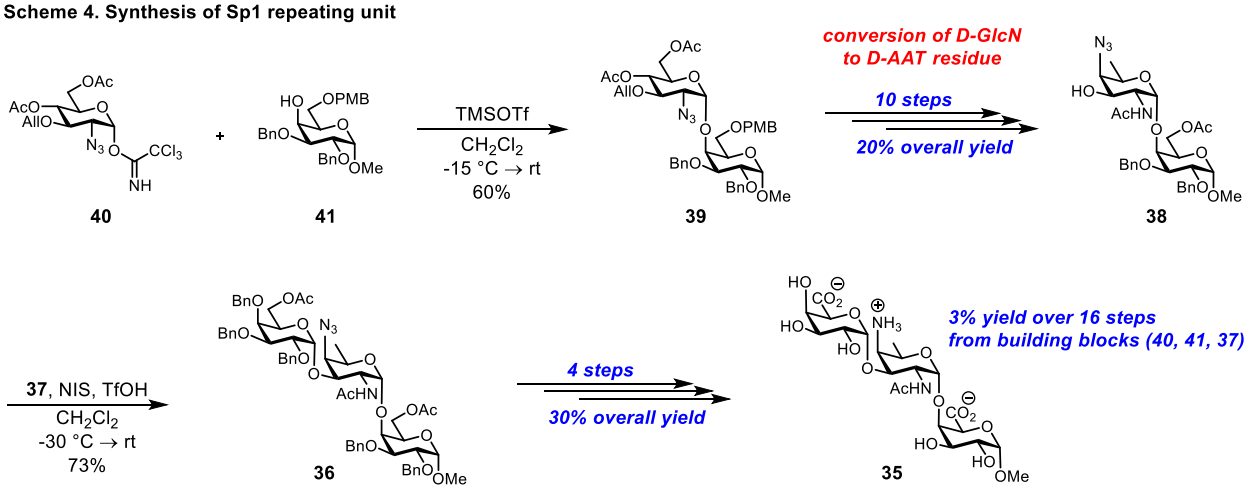


Figure 10. Bundle (2010) retrosynthetic analysis of Sp1 repeating unit

In the forward sense, a TMSOTf mediated glycosylation event between trichloroacetimidate donor **40** and acceptor **41** gave the α-linked disaccharide **39** (Scheme 4). The choice of the C2-azide in donor **4** is primarily for the formation of the1,2-*cis*-α-glycosidic bond. In general, the majority of amine protecting groups undergo participation, leading to the formation of the 1,2-*trans*-β-glycosidic linkage. The azide can act as a non-participatory group as it is small enough to not sterically hinder the formation of the 1,2-*cis* glycosidic linkage.

Scheme 4. Synthesis of Sp1 repeating unit



Following the formation of disaccharide **39**, a linear sequence of ten steps took place which converted the D-GlcN to a D-AAT residue and provided the C3' free alcohol of disaccharide acceptor **38**. First the C2'-N_3 was reduced then acetylated in the ten-step sequence. Then the acetates were removed and mesylation of the primary C6'-OH followed. Displacement of the by mesylate by a hydride then took place. Next, the C4'-OH was triflate and displaced by an azide nucleophile which inverted the C4' stereochemistry. Finally, the C6-PMB group was removed and replaced with an acetate protecting group. At this point in the synthetic route, the overall yield of acceptor **38** was 12% with a total of 11 steps from the monosaccharide building blocks **40** and **41**. Thioglycoside **37** was activated under NIS/TfOH conditions with acceptor **38** to give trisaccharide **36** in good yield. The core trisaccharide **36** then underwent a series of steps to oxidize the C6-OH and C6''-OH and globally deprotect give the Sp1 trisaccharide repeating unit, providing trisaccharide **35**. Synthesis of the trisaccharide **35** was produced in sixteen steps with a 3% overall yield from its constituent monosaccharide building blocks **40**, **41** and **47**.

The Bundle group sought to investigate immunomodulatory activity of larger Sp1 ZPSs. To facilitate this, they planned a synthesis to provide a hexasaccharide consisting of a dimer of repeating units (Figure 11). To access the Sp1 hexasaccharide **42**, modifications to the route of trisaccharide **35** were made to provide a trisaccharide donor **43** and a trisaccharide acceptor **44**. Retrosynthetic analysis of the trisaccharide donor **43** provided disconnection of the D-Gal-α-(1→3)-D-AAT linkage that could provide the previously used thioglycoside donor **37** and the disaccharide acceptor **45** (disconnection *i*). Following a series of functional group interconversions, as previously described to convert the D-GlcN to the D-AAT residue, a disconnection of the α-glycosidic bond in **45** could provide the building block acceptor **47** and reducing sugar donor **46** (disconnection *ii*). Conveniently, the building block donor **46** was synthesized by the route used to synthesize donor **40**.

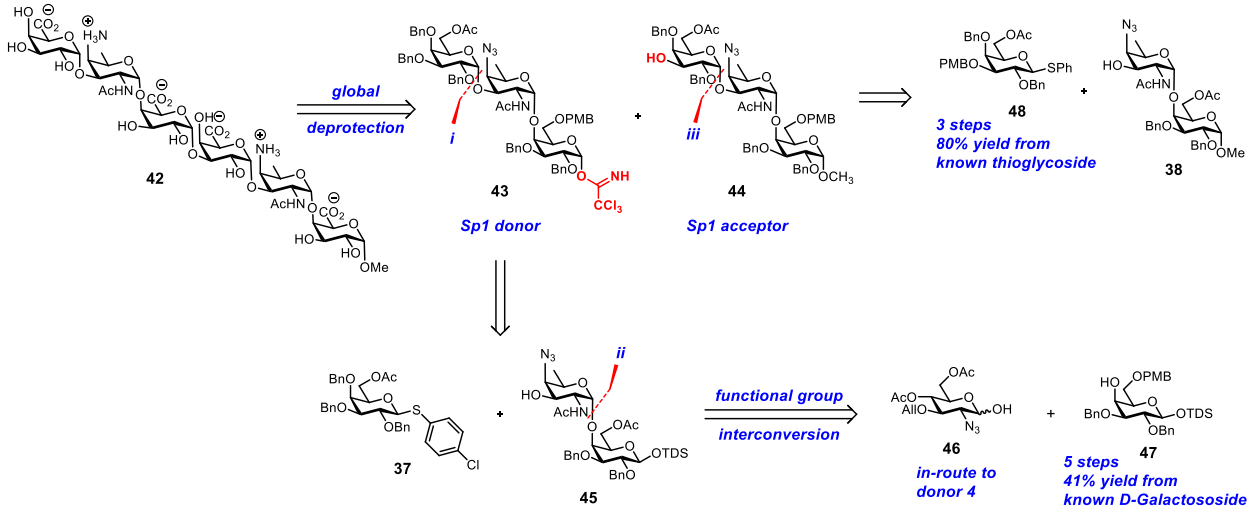
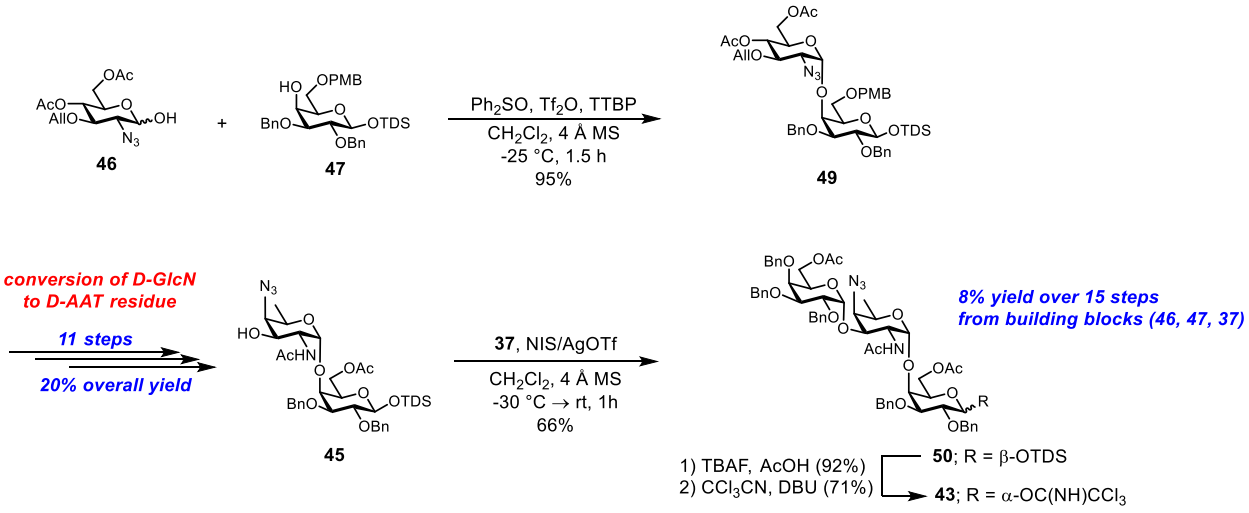


Figure 11. Sp1 hexasaccharide retrosynthetic analysis

Retrosynthetic analysis of acceptor **44** revealed disconnection of the D-Gal-α-(1→3)-D-AAT linkage could provide the D-galactosyl donor **48** and previously synthesized disaccharide acceptor **38** (disconnection *iii*).

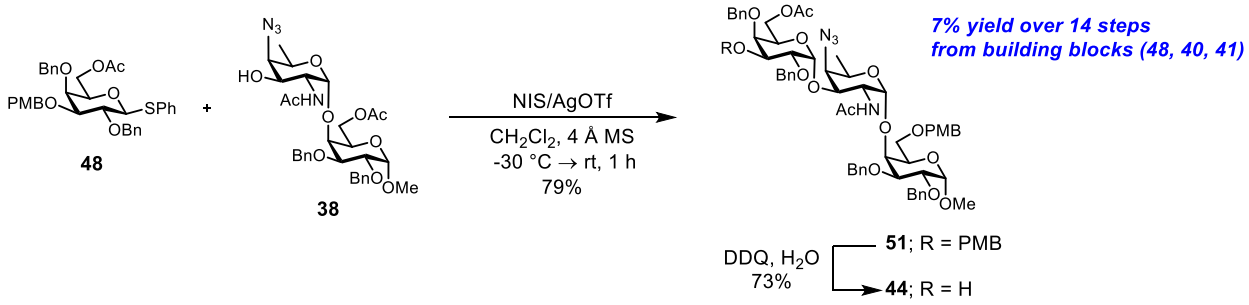
The synthesis of the trisaccharide donor **43** began attempted glycosylation events between the D-Gal acceptor **47** and the, previously used, trichloroacetimidate donor **40** (Scheme 5). However, this resulted only low yielding results. Instead, it was found the Gin's dehydrative glycosylation conditions using the free reducing sugar **46** as the glycosidic donor and acceptor **47** to give α-linked disaccharide **49** as the only detectable isomer (Scheme 5). Next a similar sequence of procedures, previously discussed to convert the D-GlcN residue to the D-AAT residue, was carried out. This procedure required eleven steps resulting in a 20% overall yield to provide the disaccharide acceptor **45**. The synthetic procedure, once again, caused a significant choke point during the synthetic route. Next, acceptor **45** underwent NIS/AgOTf mediated glycosylation conditions with thioglycoside **37** to complete the trisaccharide **50** in 66% yield with excellent stereoselectivity. The trisaccharide **50** was then converted into a trichloroacetimidate donor in a two-step sequence which involved removal of the silyl C1-silyl group (92% yield) followed by formation of the α-trichloroacetimidate **43** in 71% yield. Overall, the trisaccharide donor was produced in fifteen steps in an 8% overall yield from its constituent donor building blocks (**37**, **46** and **47**).

Scheme 5. Synthesis of the Sp1 trisaccharide donor



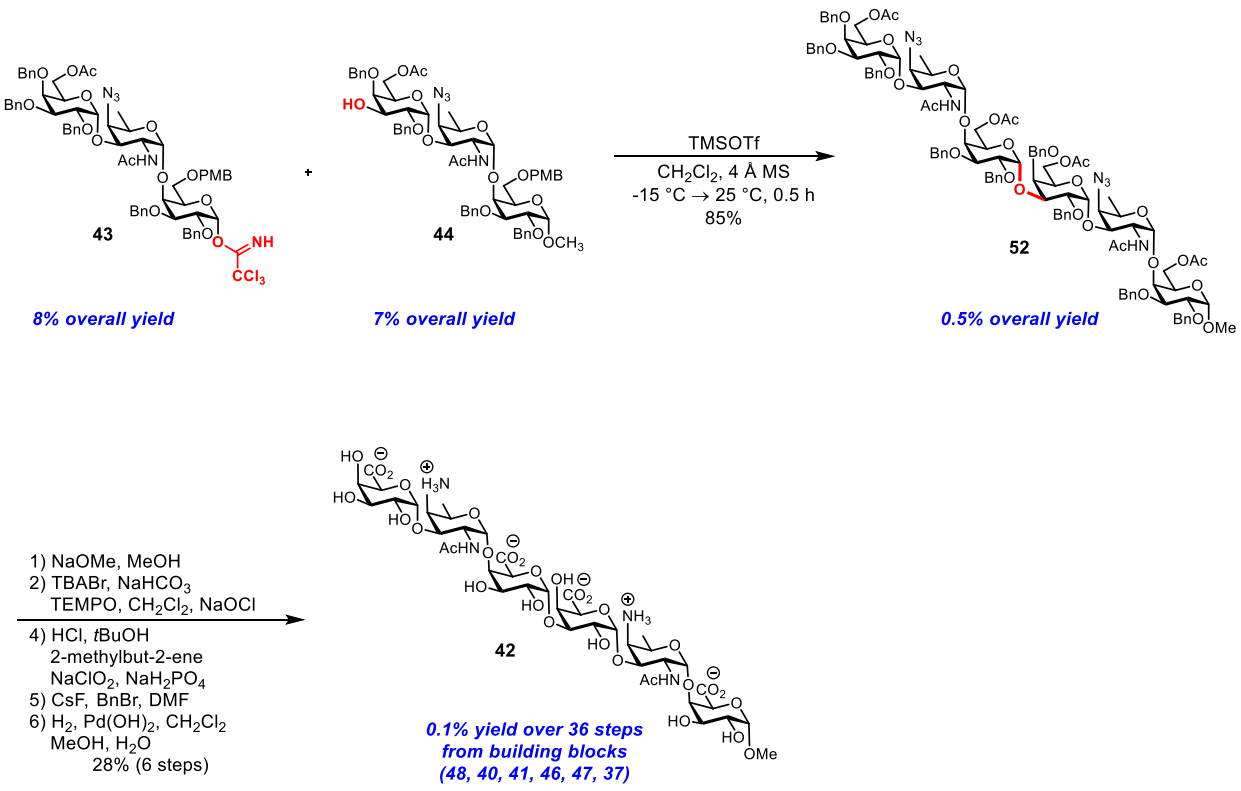
Synthesis of the trisaccharide acceptor **44** began with synthesis of the thioglycoside **48**, obtained in three steps in a 60% yield from the known *p*-toyl 4,6-*O*-benzylidene acetal-2-*O*-benzyl-1-thio-D-galactoside (Scheme 6). A NIS/AgOTf mediated glycosylation between disaccharide acceptor **38** and thioglycoside **48** provided the trisaccharide **51** in good yield and stereoselectivity. Following a DDQ mediated PMB removal of **51** gave trisaccharide acceptor **44**. The trisaccharide acceptor was produced in a total of fourteen steps from its constituent monosaccharide building blocks (**48**, **41** and **40**) in a 7% overall yield.

Scheme 6. Synthesis of the Sp1 trisaccharide acceptor



The [3+3] glycosylation between trisaccharide donor **43** and the trisaccharide acceptor **44** under TMSOTf mediated conditions provided hexasaccharide **52** in 85% yield with the newly formed α-linkage (Scheme 7). Following formation of the hexasaccharide, **39** was deacetylated then in a two-step sequence and then oxidized to the corresponding carboxylic acid and then benzyl protected to benzyl ester. The fully protected hexasaccharide which then underwent global deprotection by hydrogenation with Pearlman's catalyst to give the dimeric repeating unit, Sp1 ZPS **42**.

Scheme 7. Synthesis of the Sp1 hexasaccharide



Upon the completion of the first total synthesis of the Sp1 repeating unit, the Bundle group reported that the NMR spectra of the tri- and hexasaccharide did not fully match that of the native polysaccharide.[109] In addition, they found that both the tri- and hexasaccharide were unable to active T-cells.[109] This is thought to be due to the number of repeats

required to have immunomodulating properties (>120 kDa, ~5 trisaccharide repeats).[54, 110] Bundle concluded with the remarks that total synthesis will is not capable of producing a large enough ZPS capable of MHC-II.[109]

As a final analysis of the synthetic route used by the Bundle group, it is important to point out that many inefficiencies occurred during the linear post-glycosylation steps. For example, each synthesis of each advanced disaccharide (**38** and **45**) required around ten synthetic transformations to establish the D-AAT residue. Even a synthetic route with near quantitative yield will suffer in overall yield as the number of synthetic steps increase.[111] While these synthetic steps are necessary to produce the D-AAT residue, the steps could have been carried out on a monosaccharide building block rather than on advanced post-glycosylation disaccharide. This limits loss to only a single monosaccharide building block rather than both monosaccharide building blocks used to construct the disaccharide. Considering glycosylation reaction are convergent in nature, it can simply be stated: In regards to overall yield and material required for a synthetic campaign, in general, it is more efficient to limit the number of synthetic transformations carried out following glycosylation event(s) and best to carry out synthetic transformations on the monosaccharide building blocks instead.

Part 6. Codée and van der Marel's total synthesis of Sp1 (2011)

Synthetic efforts by the Codée, van der Marel and co-workers described the assembly of three repeating unit frames of the Sp1 ZPS **53**.[97] First, synthetic targets to represent each frame was identified (Figure 12). Synthetic target **54** ([abc]-frame) represents the same synthetic target of Bundle and co-workers in their 2010 report.[112] In addition, the synthetic target **55** ([bca]-frame) represents the same synthetic target of bundle's 2013 report,[113]

Oscarson 2016 report,[79] and Seeberger's 2014 and 2018 report.[114-115] The remaining, novel, synthetic target was trisaccharide **56** which represents the [cba]-frame repeating unit. A glycerol residue was chosen to facilitate capping of the representative repeating unit synthetic targets.

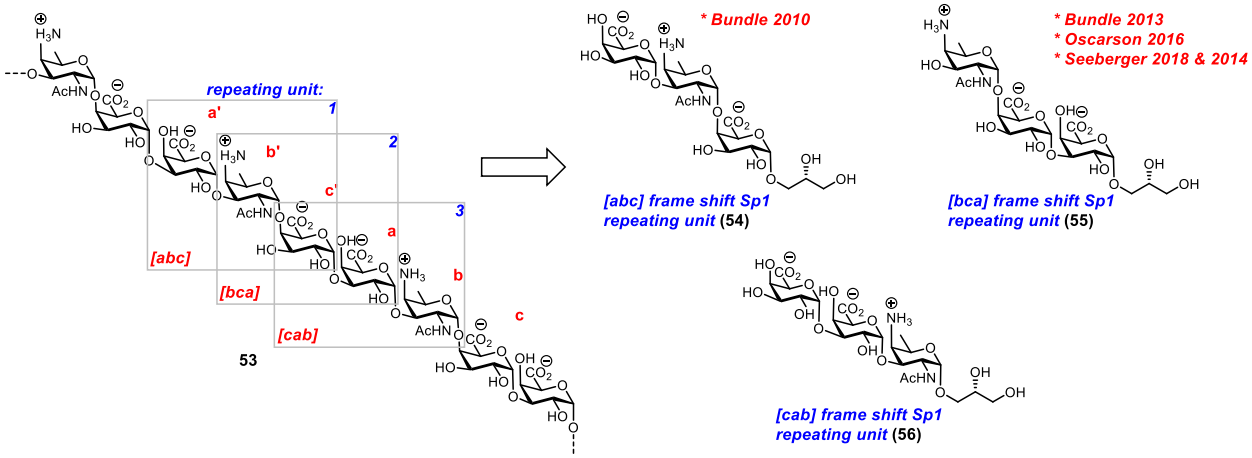


Figure 12. van der Marel and co-worker's synthetic target selection

In a highly convergent approach, each target molecule was planned to come from only two carbohydrate building blocks and a glycerol capping unit. The D-galacturonic acid-[3,6]-lactone building block was planned to provide both D-GalA residues in each of the target structures (Figure 13). This strategy is a form of the *pre-glycosylation oxidation method* which obviates the need for any late stage C6-OH oxidation. The 1,6-anhydro

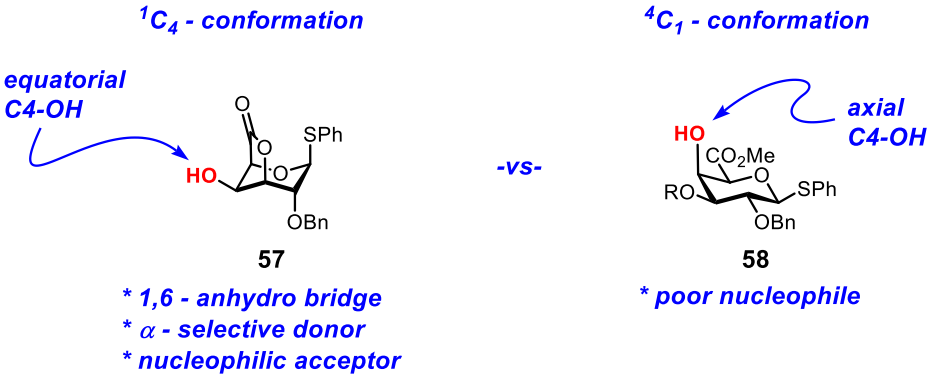


Figure 13. D-GalA [3,6]-lactone vs. D-GalA as a donor or acceptor

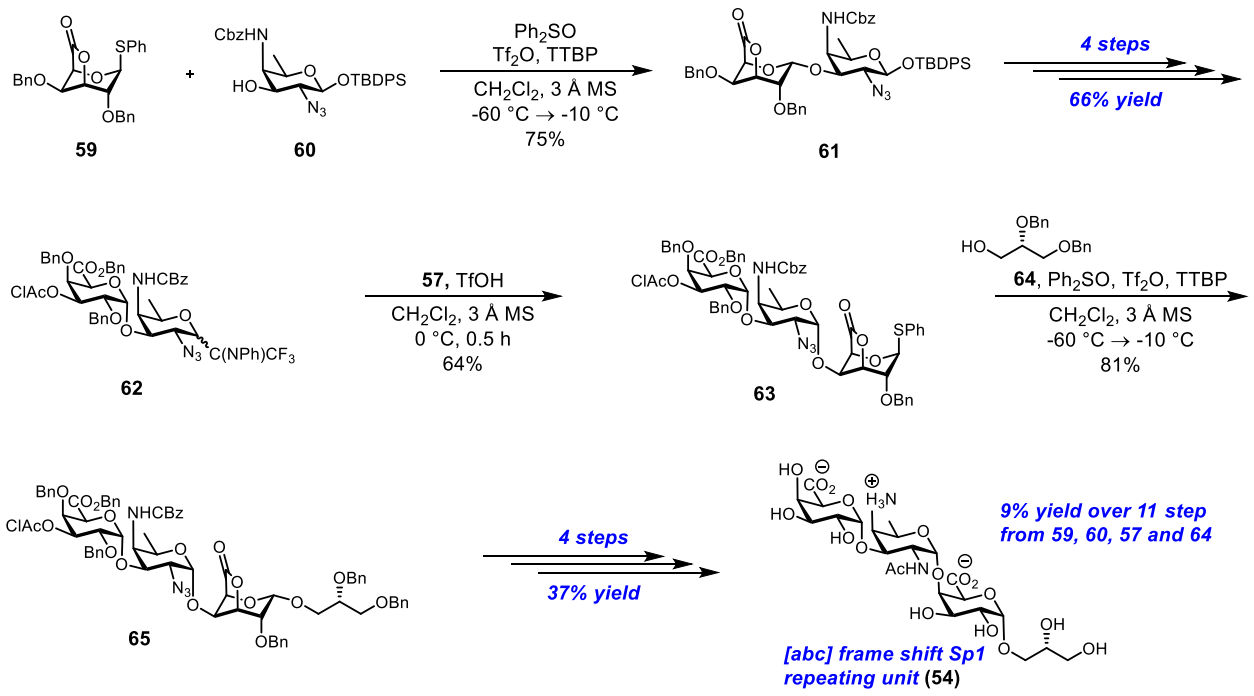
bridge forces the D-GalA residue to take a $^1C_4$-conformation (shown in **57**). This places the C4-OH in an equatorial position which greatly enhances its nucleophilicity as a glycosyl acceptor relative to its $^4C_1$-chair conformation (shown in **58**) where the C4-OH is axial and less nucleophilic, resulting in performing a poor acceptor. In addition, by protecting the C4 alcohol, the D-GalA lactone can provide an α-selective donor, which is advantageous for their synthetic route.

The D-AAT sugar and glycerol capping moiety would be synthesized according to known procedures. The D-AAT residue will be synthesized prior to any glycosylation event to limit late stage manipulations during the synthesis which could negatively affect over all yield.

Synthesis of the Sp1 [abc]-frame repeating unit **54** was to be carried out from the non-reducing end to the reducing end in a [1+1+1] glycosylation strategy. The route commenced with glycosylation between lactone **59** and the 2,4,6-trideoxy-4-amino-D-

$GalN_3$ monosaccharide **60** to give disaccharide **61** in good yield. Next followed a series of 4 steps: 1) opening of the lactone ring with benzyl alcohol (this provides the C6-benzyl ester and ring flips the D-GalA back to the $^4C_1$ conformation), 2) acylation of the C3'-OH, 3) desilylation to give the reducing sugar and 4) formation of the anomeric imidate to give donor **62** in 66% yield over 4 steps. Next, a triflic acid mediated glycosylation between donor **62** and lactone acceptor **57** afforded trisaccharide **63** in 64% yield. Capping of the repeating unit with protected glycerol moiety **64** proceeded in an 81% yield. Next, followed a deprotection sequence which ultimately resulted in the first of the trimer repeats, trisaccharide **54** in 37% over the four steps. The Sp1 [abc]-fame trisaccharide **54** was synthesized in a 9% yield over eleven steps from its constituent monosaccharide building blocks (**59**, **60**, **57** and **64**).

Scheme 8. Synthesis of Sp1 [abc] frame

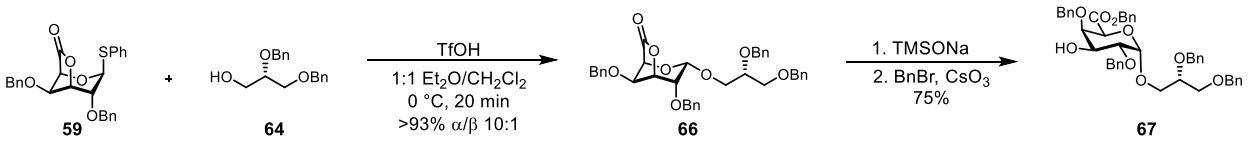


Next the [bca] trimer repeat **55** was selected as a synthetic target. Trisaccharide **55** contains the D-AAT sugar at the terminal non-reducing end of the trisaccharide. The synthetic strategy for **55** was a planned around a [2+1] glycosylation approach involving the D-GalA monosaccharide acceptor **67** and the disaccharide donor **33** (Scheme 9). The D-GalA monosaccharide acceptor **67** was produced in two steps from lactone **59**. First lactone **59** underwent a glycosidation alcohol **64** to give the lactone **66** (Scheme 9, part a). Second, the lactone ring was opened and protected as the benzyl ester in good yield to give acceptor **67**.

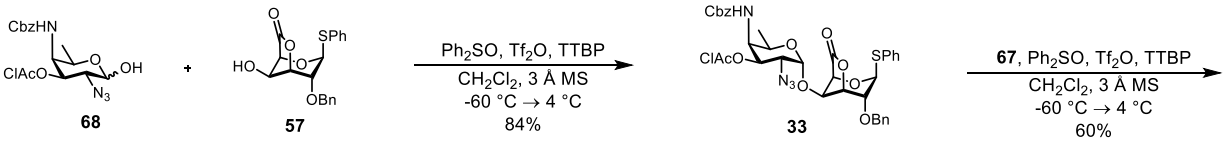
Synthesis of the disaccharide donor **33** was achieved via coupling of hemiacetal **68** and acceptor **57** under Gin's dehydrative glycosylation conditions in good yield as the α-anomer (Scheme 9, part b). The [2+1] glycosylation event between thioglycoside **33** and D-GalA acceptor **67** proceeded well to give the trisaccharide **69** in 60% yield as the α-linked product. To complete the synthesis of **55**, trisaccharide **69** underwent a lactone opening event followed by a global deprotection to give the [bca] frame shift trimeric repeating unit **55** in good overall yield. The Sp1 [bca]-fame trisaccharide **55** was synthesized in a 13% yield over eleven steps from its constituent monosaccharide building blocks (**59**, **60**, **57** and **64**).

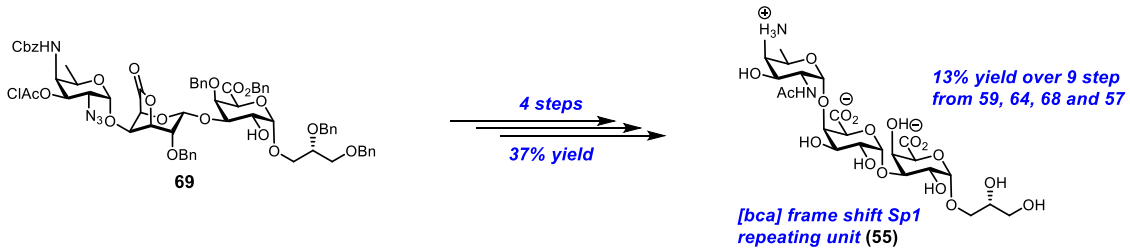
Scheme 9. Synthesis of Sp1 [bca] frame

a) synthesis of monosaccharide acceptor 67



b) synthesis of disaccharide acceptor 33 and completion of trisaccharide 55



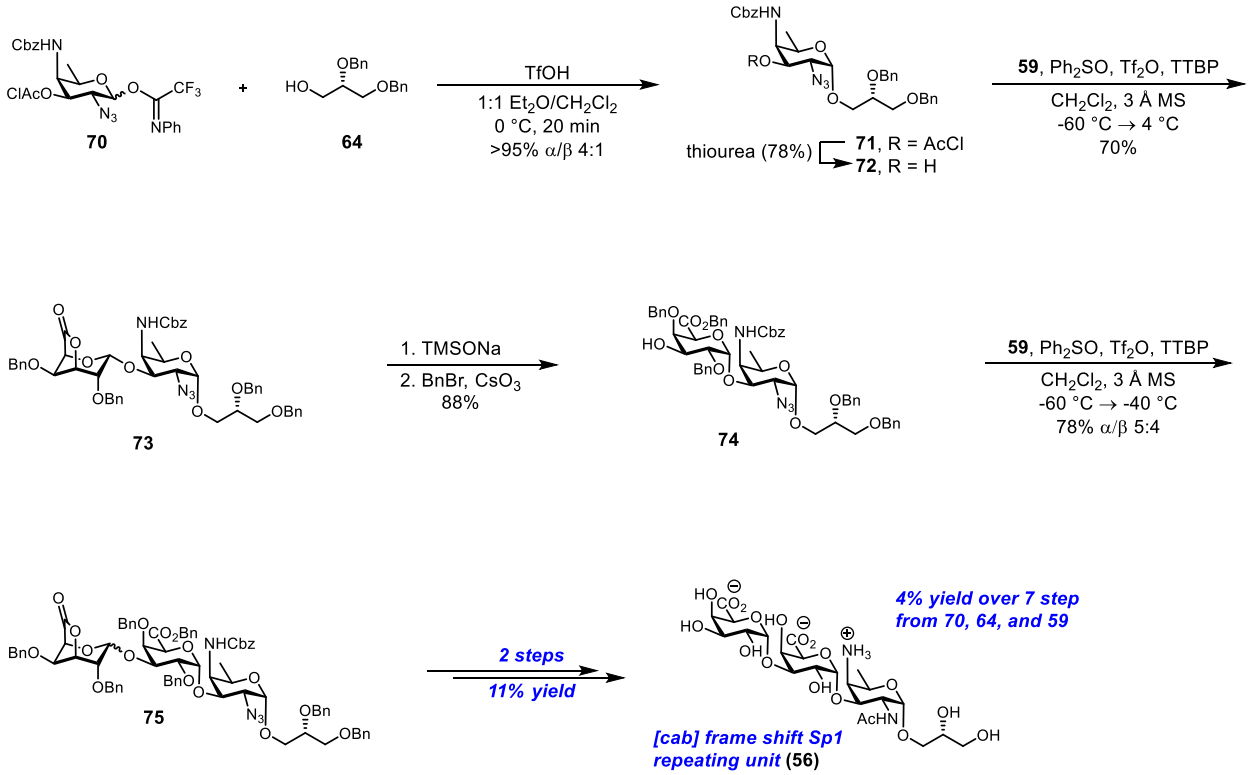


To complete the final trisaccharide in the series, the [*cba*] repeating unit **56** was selected as synthetic target (Scheme 10). The synthetic strategy for **56** was again a [1+1+1] strategy, however differing from **54** by building from reducing end of the trisaccharide to the non-reducing end. This method allows for the material that is carried though the longest linear sequence be an acceptor during each glycosylation event. In addition, the lactone donor **59** is planned to be used in back to back glycosylations to achieve **56**.

The route began with a glycosylation event between imidate **70** and glycerol capping unit **64** to give **71** in excellent yield with a 4:1 α/β selectivity. Following saponification of the C3-OAcCl in **71** to give acceptor **72**, a Tf_2O mediated glycosylation between donor **59** and acceptor **72** gave disaccharide **73** in good yield as the α-linked product. The lactone

in **73** was opened and protected to give acceptor **74** to be directly used in the following glycosylation with another D-GalA lactone donor **59** to give the complete trisaccharide core **75** in a good yield. Following a low yielding ring opening of the final lactone, global deprotection gave the [cba]-frame trisaccharide **56**, the final frame shift of repeating units of Sp1. The Sp1 [cba]-fame trisaccharide **56** was synthesized in a 4% yield over seven steps from its constituent monosaccharide building blocks (**70**, **64** and **59**).

Scheme 10. Synthesis of Sp1 [cab] frame



The work by Codée and van der Marel display a very powerful method to access each of the three fame shifts of the Sp1 trisaccharide. By modification of their route to accommodate for the ability to synthesize repeating unit trisaccharide donor and acceptor derivatives, one can see the potential ability to accessing dimer, trimer, and maybe even oligomeric Sp1 ZPS derivatives.

Part 7. Bundle Sp1 synthesis (2013)

In 2013, the Bundle and co-workers published their second synthetic efforts of a Sp1 ZPS monomeric repeating unit.[116] In this report the group decided to target a different frameshift then the one reported in the 2010 synthesis. The repeating unit shown in red in **76** was selected as the synthetic target (figure 14, part a). Rather than having the D-AAT residue the central residue (described herein as the [*abc*]-frame), it is now the non-reducing end terminal residue (described herein as the [*bca*]-frame).

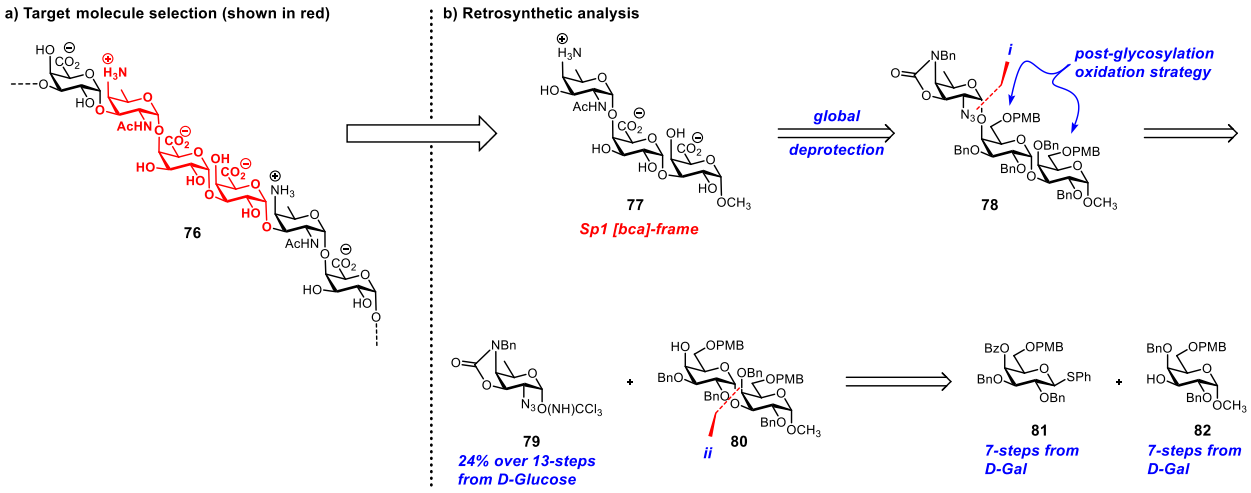


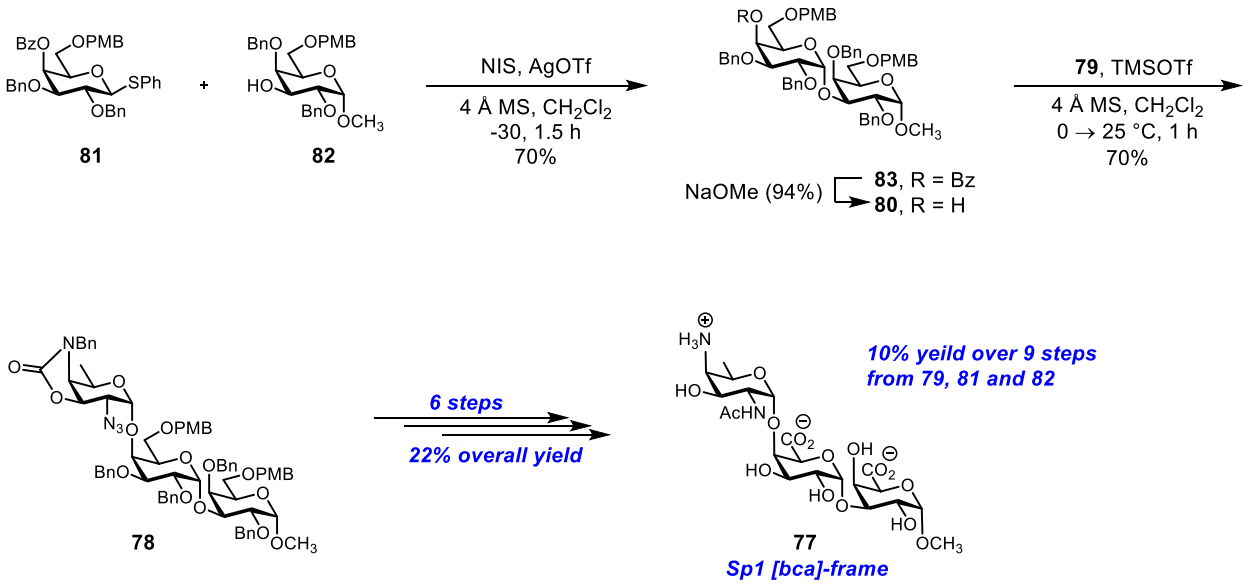
Figure 14. Synthesis of the [bca] Sp1 repeating unit by Bundle (2013)

The group determined the methyl-capped trisaccharide **77** could serve at the synthetic target (Figure 14, part b). A *post-glycosylation strategy* was planned to be used once again for their synthetic route. A [1+1+1] glycosylation strategy, proceeding from the reducing to the non-reducing end, was planned to construct the target molecule. Retrosynthetic analysis provided the protected trisaccharide **78** could come from the

trisaccharide **77** following a global deprotection, late stage C6 and C6' oxidation and a selective PMB removal. Disconnection of the D-AAT-α-(1→4)-D-GalA glycosidic linkage in **78** could provide the D-AAT trichloroacetimidate donor **79** and the disaccharide acceptor **80** (disconnection *i*). The D-AAT reside was planned to be accessed by the advanced trichloroacetimidate donor **79** which they previously reported to access in 24% yield over a thirteen step procedure from D-glucose.[117] Disconnection of the D-Gal-α-(1→3)-D-Gal glycosidic linkage in **80** provides the D-Gal donor **81** and the D-Gal acceptor **82** which both can be accessed in 7 steps from D-galactose (disconnection *ii*).

The synthesis commenced by glycosylation between thioglycoside **81** and acceptor **82** to give α-liked disaccharide **83** then saponified to reveal the C4'-OH to give acceptor **80** setting the stage for the final glycosylation (Scheme 11). The D-AAT trichloroacetimidate donor **79** was activated under TMSOTf mediated conditions in the presence of disaccharide acceptor **80** to give the core trisaccharide **78**.

Scheme 11. Synthesis of Sp1 [bca] repeating unit (Bundle, 2013)



A deprotection and protection sequence commenced, including: 1) base mediated cleavage of the oxazolidinone ring, 2) C3'' benzylation, 3) TfOH mediated C6 and C6' PMB removal, 4) reduction and acetylation of the C2'' azide, 5) C6 and C6' TEMPO/NaOCl oxidation to the D-GalA residues, 6) H_2/$Pd(OH)_2$ hydrogenation to give the [bca] Sp1 frame-shift **77**. The desired product was synthesized in nine steps with a 10% overall yield from its constituent building blocks (**81**, **82** and **79**).

Many improvements provided better synthetic yield and an expedited synthetic route over their original reports. A major improvement to the synthetic strategy included limiting the number of transformations between glycosylation events to provide a convergent synthetic sequence with increased overall yield. This was facilitated in part by installing the D-AAT directly instead of using the indirect glycosylation—epimerization method previously described.

Bundle *et al.* conclude that **77** as well as trisaccharide **35** and hexasaccharide **42** were tested for its ability to stimulate interleukin 10 (IL-10) and interferon-gamma (INF-γ) by Kasper and co-workers at Harvard medical School. Each of the responses is representative of the T-cell activating ability of zwitterionic antigens.[3] Unfortunately, neither the trisaccharides **35** or **77** or the hexasaccharide **42** were active. Published data describing the molecular weight required for activity[118] that appeared after synthetic work was initiated is consistent with this result. Based on their data, it appears that at least six repeating units (18 residues) are required for activity.

Part 8. Seeberger Sp1 synthesis (2014)

Seeberger and co-workers reported on the synthesis of a Sp1 trisaccharide in 2014 with a functionalized thiol handle for conjugation purposes.[114] Like the Bundle's 2013

synthesis, Seeberger and co-workers selected the [bca] frame-shift trisaccharide repeating unit **84** as the selected target molecule (Figure 15). However, their target molecule was planned to have a terminal thiol which could undergo dimerization forming a disulfide bond following deprotection. The ZPS can later be templated onto surfaces by reduction of the disulfides in the presence of gold nanoparticles or microarray plates.

Seeberger envisioned a synthetic route which used the *pre-glycosylation oxidation strategy* to provide the [*bca*]-frame. The route would follow a [1+1+1] glycosylation strategy which proceeded from the reducing end to the non-reducing end. In addition, the desired product would be capped with an aliphatic alkene linker attached to a protected thiol.

Retrosynthetic analysis provided the protected trisaccharide **85** could come from the desired product **84** following a global deprotection (figure 15). Disconnection of the D-AAT-α-(1$\rightarrow$4)-D-GalA glycosidic linkage in **85** could provide the D-AAT phosphate donor **86** and the disaccharide acceptor **87** (disconnection *i*). Previously, in 2011, Seeberger and co-workers reported a method to access the D-AAT donors though a *de novo* synthesis from Cbz-protected L-threonine in a nine-step sequence which was used to obtain donor **86** in an 18% overall yield.[86]

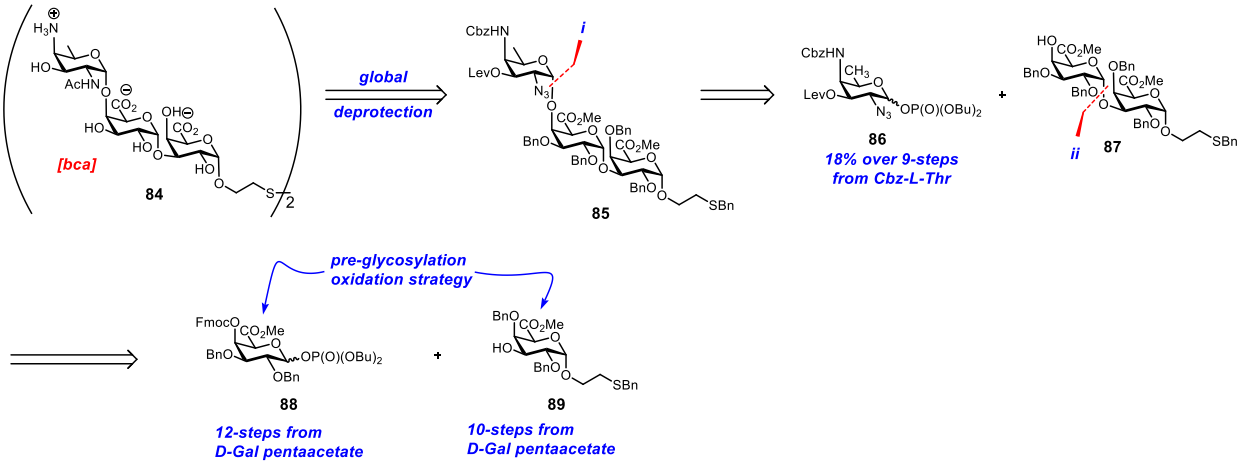
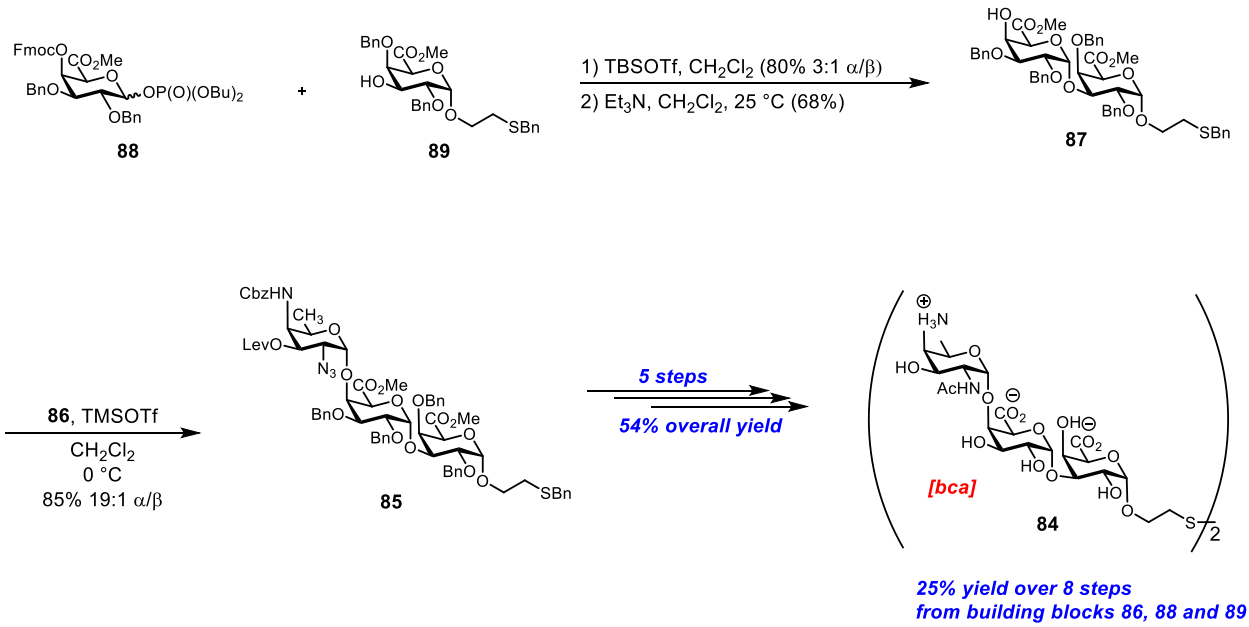


Figure 15. Retrosynthetic analysis (Seeberger, 2014)

Disconnection of the D-GalA-α-(1→3)-D-GalA glycosidic linkage provides the D-GalA donor **88** and acceptor **89** which both can be synthesized from D-galactose pentaacetate in twelve and ten steps respectively (disconnection *ii*).

A glycosylation event between donor **88** and acceptor **89** followed by a C4'-Fmoc deprotecting gave disaccharide acceptor **87** (Scheme 12). Acceptor **87** and D-AAT donor **86** were coupled under TMSOTf mediated conditions to give the completed core trisaccharide **85** in 85% yield with excellent stereoselectivity favoring the desired α-linked anomer.

Scheme 12. Sp1 [bca]-frame synthesis (Seeberger, 2014)



Following a series of steps including; 1) C3'' Lev deprotection **85** (>95%), 2) C3'' BOM protection (85%), 3) reduction of the C2'' azide and acetylation (72%), gave the fully protected trisaccharide [*bca*] repeating unit. Saponification followed by Birch reduction conditions gave **84** in excellent yield. The desired product **84** was synthesized in eight steps with a 25% overall yield from its constituent building blocks (**86**, **88** and **89**).

Following the total synthesis, **84** was reduced to fix to a microarray surface *via* a maleimide factionalized glass slide.[114] When the Sp1 repeating unit was fastened to the Sp1 microarray, it was used to interrogate rabbit sera raised against the Sp1 ZPS. This study found that binding was observed between the immobilized synthetic trisaccharide and the serum indicating that structural elements of the polysaccharide that are recognized by antibodies in the serum are also present in the trisaccharide.[114]

Later, the Sp1 trisaccharide **84** was used by Seeberger and co-workers in the development of glycoconjugate vaccine towards the highly invasive serotype 1 (ST1)

Streptococcus pneumoniae.[115] The Sp1 trisaccharide **84** was coupled with bromoacetate-activated CRM197, a nontoxic diphtheria toxin mutant carrier protein, to give the CRM197-**84** glycoconjugate. The CRM197-**84** glycoconjugate was found to induce a D-AAT-dependent immune response against native ST1 CPS that is more robust than that of the polysaccharide-based, multivalent block buster drug, Prevenar 13, in rabbits. Currently the CRM197 glycoconjugate is advancing in preclinical development for inclusion in semisynthetic vaccines covering multiple serotypes.[115]

Seeberger's 2014 synthesis of the Sp1 ZPS repeating unit outperformed all its predecessor's previous attempts. The improvement can be attributed to design of the most convergent synthetic route. This was accomplished by minimizing synthetic transformations between glycosylation events.

Part 9: PS A1 ZPS background and isolation

Bacteroides fragilis is a Gram negative anaerobic commensal bacterium which is the most frequently isolated species from clinical intra-abdominal abscesses.[119] Experimental induction of abscess in a rat model by intraperitoneal implantation of capsular polysaccharide complex (CPC) from *B*. *fragilis* revealed this CPC was a virulence factor.[120] As a result, this CPC can be utilized as an antigenic target. This hypothesis was further validated by immunizing a rat model with CPC after *B. fragilis* challenge. The CPC immunization protected rats from abscess formation.[121-122] Additionally, transfer of splenocytes from CPC immunized mice to non-immunized mice protected recipients from abscess formation.[123] This observation, is a direct indication of cellular immunity generated by CPC.[123] Isolation of the PS A1 ZPS from the CPC followed by inoculation of non-immunized mice also conferred protection.[123]

Among eight different polysaccharides isolated from *B*. *fragilis* (not all zwitterionic), ZPS PS A1 is the most abundant followed by ZPS PS B. Both of these capsular polysaccharides contain a zwitterionic character that is necessary for immunological T-cell mediated responses.[11, 124] PS A1 consists of a tetrasaccharide core repeating unit with a molecular weight of ~110 kDa (~120 repeating units). The repeating unit is comprised of [→3)-α-D-AAT*p*-(1→4)-[β-D-Gal*f*(1→3)-α-D-Gal*p*NAc(1→3)-β-D-Gal*p*-(1→] and has oppositely charged moieties on adjacent monosaccharides.[124]

Part 10: van der Marel's partial synthesis of PS A1 ZPS (2007)

The first published attempt to synthesize a PS A1 repeating unit was from the lab of van der Marel and co-workers in 2007 at Leinden University in the Netherlands.[66] The van der Marel group chose the representative tetrasaccharide repeating unit **90** (Figure 16. part a, repeating unit shown in red) containing the highly branched D-GalNAc residue as the central residue (figure 16). The target **91** tetrasaccharide contains two 1,2-*cis* glycosidic bonds and was planned to be capped with an *iso*propyl group.

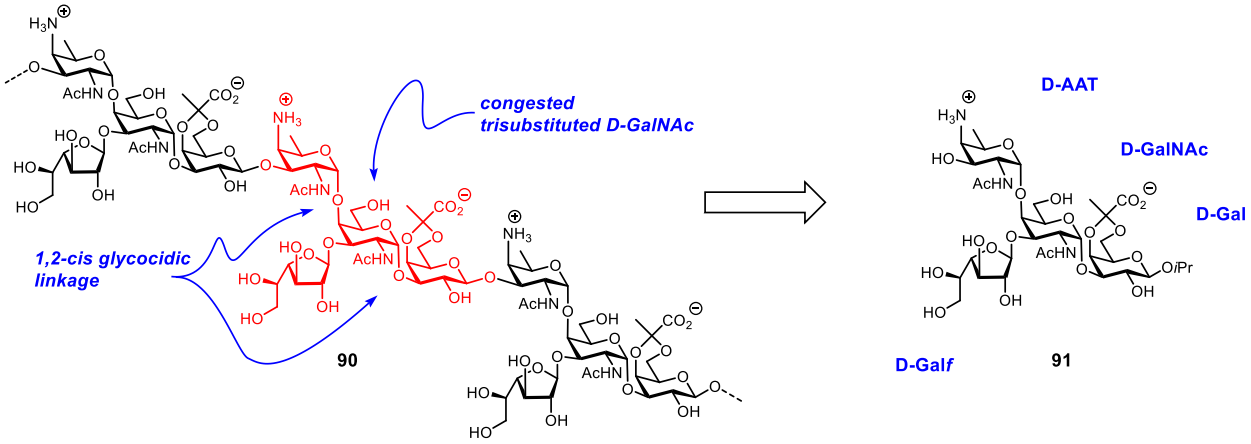
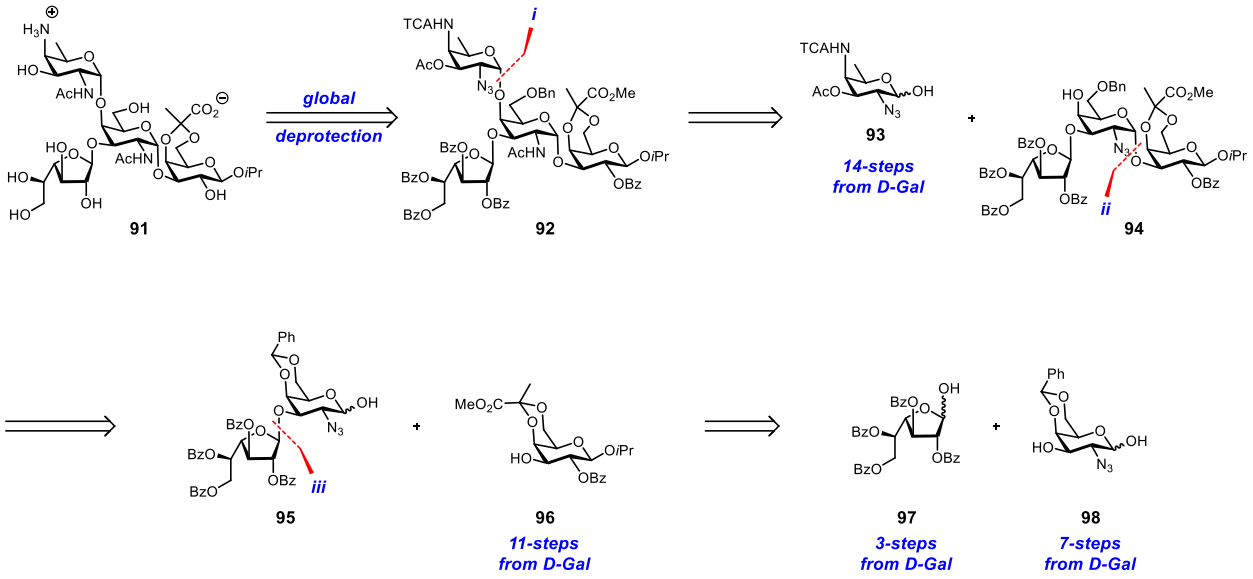


Figure 16. van der Marel PS A1 target molecule selection (2007)

Retrosynthetic analysis revealed that the desired product **91** could come from tetrasaccharide **92** following a global deprotection sequence (Figure 17). Disconnection of the D-AAT-α-(1→4)-D-GalNAc glycosidic linkage provided the trisaccharide acceptor **94** and the D-AAT donor **93** (disconnection *i*). The D-AAT sugar **93** was prepared through a series of fourteen steps from D-Gal.[125] Disconnection of the D-GalNAc-α-(1→3)-D-Gal glycosidic bond provided the disaccharide donor **95** and the D-Gal acceptor building block **96** (disconnection *ii*). The D-Gal acceptor **96** could be synthesized in eleven steps from D-Gal.[126] The final disconnection of the D-furanose residue could give the D-furanosyl building block **97** and the D-GalN acceptor **98** (disconnection *iii*). Building blocks **97** and **98** were both prepared in good yield from D-Gal in three and seven steps respectively.[127-128]

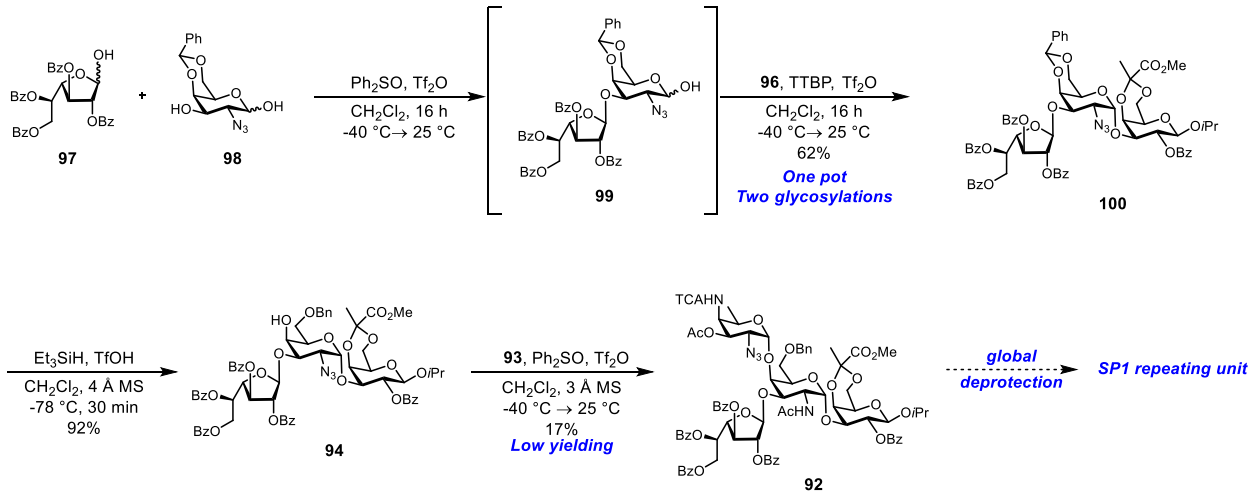
Figure 17. van der Marel PS A1 Retrosynthetic analysis



The van der Marel group described multiple methods utilizing alternative donor compounds in route to obtain their desired product. Herein, we chose to highlight the highest yielding and most notable methods used, which included a one-pot procedure

that facilitated two glycosylation events to obtain the trisaccharide **100** (Scheme 13). The one-pot glycosylation procedure started with a condensation between pre-activated donor **97** followed by the addition of acceptor **98** utilizing Gin's dehydrative glycosylation conditions.[129-130] This stepwise glycosylation procedure provided a 60% yield for the isolation of disaccharide **99**.

Scheme 13. van der Marel synthesis towards SP1 ZPS repeating unit



Continuing with the one-pot method, the intermediate disaccharide **99** was activated and added the D-Gal acceptor **96** to afford trisaccharide **100**. Following formation of the trisaccharide, **100** underwent reductive benzylidene acetal ring opening to give the final acceptor **94** in great yield and selectivity. Unfortunately, attempts to complete the core tetrasaccharide by a final dehydrative glycosylation between donor **93** and trisaccharide acceptor **94** provided only poor yields of tetrasaccharide **92**. The glycosylation presumably suffered due to the low nucleophilicity of the axial C4' hydroxyl. This dampened reactivity is likely due to a significant amount of 1,3-diaxial interaction and additional steric congestion. The group's effort was halted here at this final glycosylation providing the first partial synthesis of the PS A1 ZPS repeating unit.

Part 11. First total synthesis of the PS A1 ZPS repeating unit (Seeberger 2011)

In 2011, the Seeberger group from the Max Plank Institute in Berlin Germany, set out to complete the first total synthesis of the PS A1 ZPS repeating unit.[131] Seeberger's group selected the identical synthetic target as the one previously selected by the van der Marel lab. Retrosynthetic analysis of the successful route showed product **91** could come from the protected tetrasaccharide **101** following a global deprotection event (Figure 18).

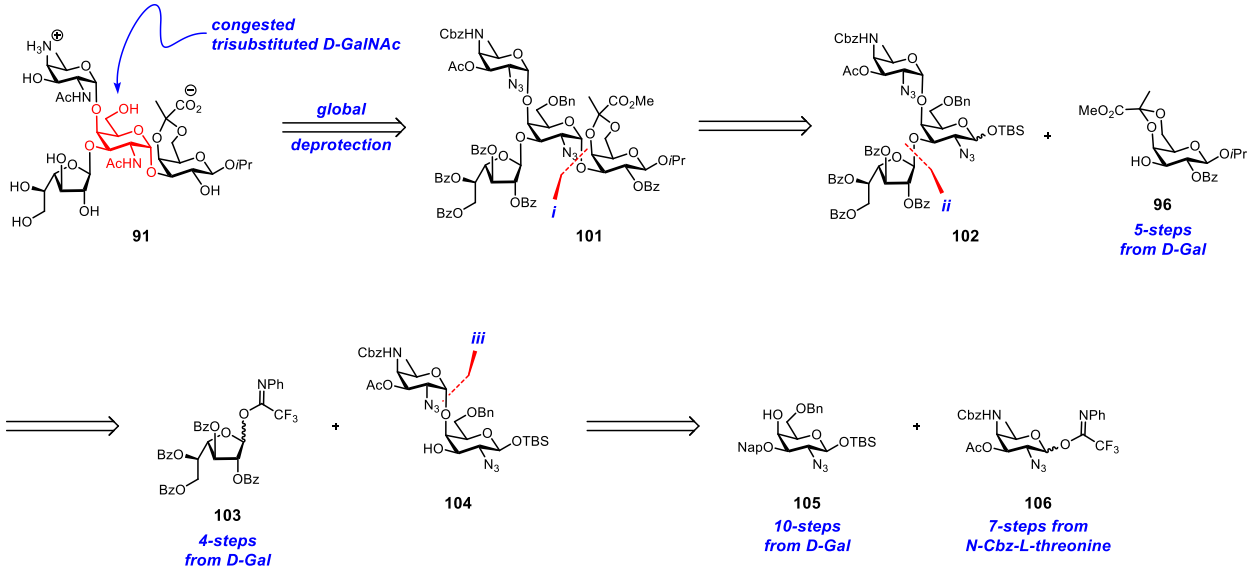


Figure 18. PS A1 ZPS retrosynthetic analysis (Seeberger, 2011)

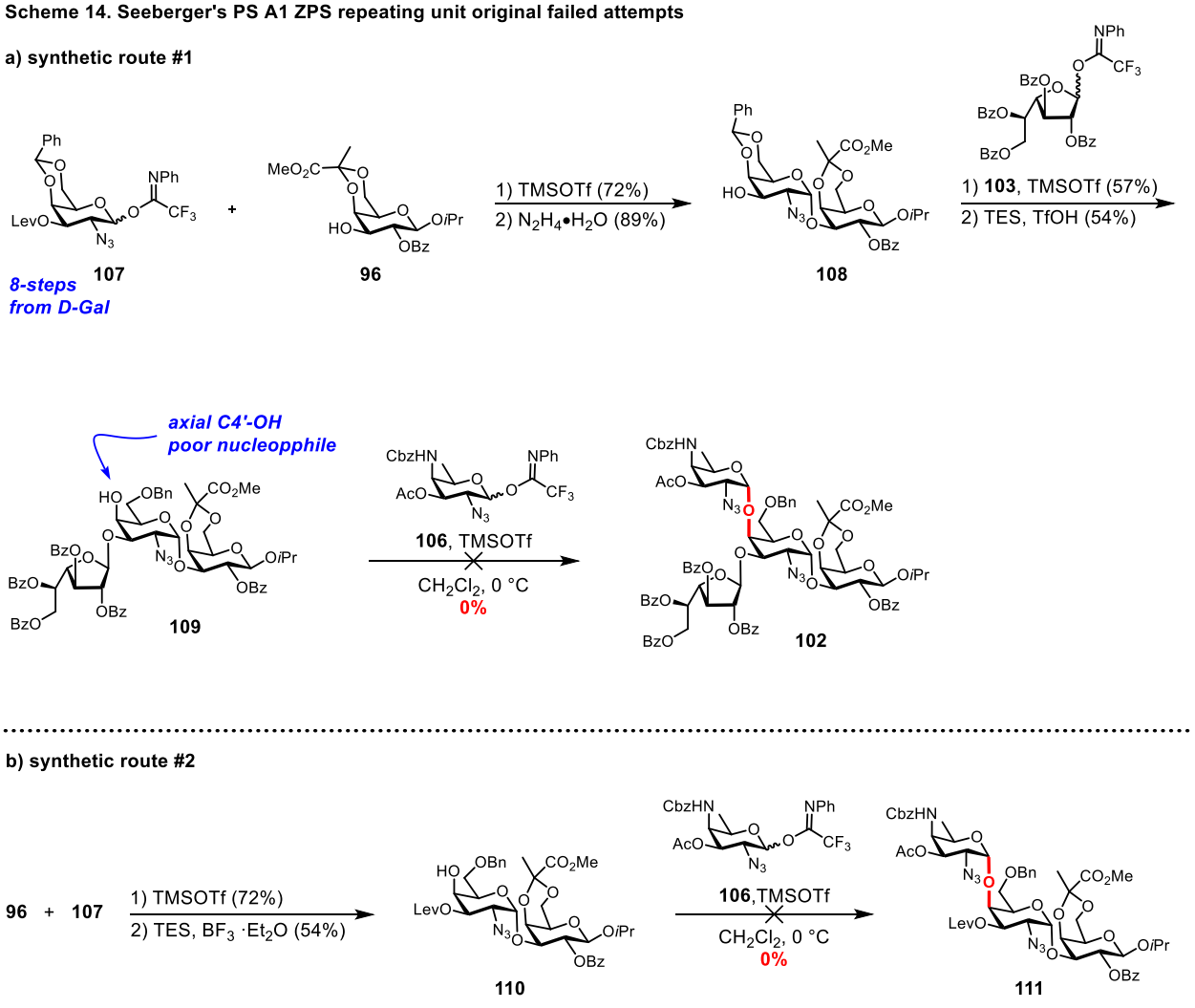
Disconnection of the D-GalNAc-α-(1→3)-D-Gal glycosidic linkage could provide the trisaccharide **102** and the D-Gal acceptor **96** (Figure 18, disconnection *i*). The D-Gal acceptor **96** could be derived in five steps from D-galactose. Next, the disconnection of the D-galactofuransoyl glycosidic bond from **102** could provide the monosaccharide donor **103** and the disaccharide acceptor **104** (disconnection *ii*). The D-galactofuranosyl imidate donor **103** could be derived from D-Gal in four steps using previously established

procedures.[132] The final disconnection, the D-AAT-α-(1→3)-D-Gal glycosidic bond could provide the monosaccharide building blocks **105** and **106** (disconnection *iii*). The D-Gal acceptor **105** could be derived from D-galactose in ten steps using previously established procedures.[133] The D-AAT building block was produced, in seven steps using a *de novo* method starting with Cbz-L-Threonine, utilizing a procedure developed within the Seeberger lab.[86]

The initial synthetic efforts by the Seeberger group were not successful (Scheme 14). The original planned synthetic route, as described in the paper as “path A”, was planned to synthesize the desired product from the reducing end to the non-reducing end of the tetrasaccharide. As previously mentioned, this is a powerful approach to oligosaccharide synthesis. This sentiment was reflected in the paper by the author, stating, “The modular nature of path A (Scheme 14, part a) was particularly attractive in light of a future automated synthesis.”[131] The original route began with formation of the D-GalNAc-α-(1→3)-D-Gal bond by a glycosylation event between donor **107** and the D-Gal acceptor **96** to provide a disaccharide which was then Lev deprotected to provide the disaccharide acceptor **108** (Scheme 14. part a). A glycosylation with the D-galactofuranosyl donor **106** followed by a benzylidene acetal ring opening provided the trisaccharide acceptor **109**.The following glycosylation between the axial C4-OH of the trisaccharide acceptor and D-AAT donor to produce the D-AAT-α-(1→4)-D-GalNAc bond was not successful. The Seeberger group previously discovered that imidate donor **106** had improved reactivity over the hemiacetal and thioglycoside D-AAT donors used by the van der Marel group, and therefore felt confident in overcoming the challenges previously encountered by van der Marel and co-workers. Unfortunately, the Seeberger group

experienced similar challenges as those experience by the van der Marel group when attempting glycosylation with a D-AAT donor and the axial C4' position, providing no desired product **102**.

Scheme 14. Seeberger's PS A1 ZPS repeating unit original failed attempts

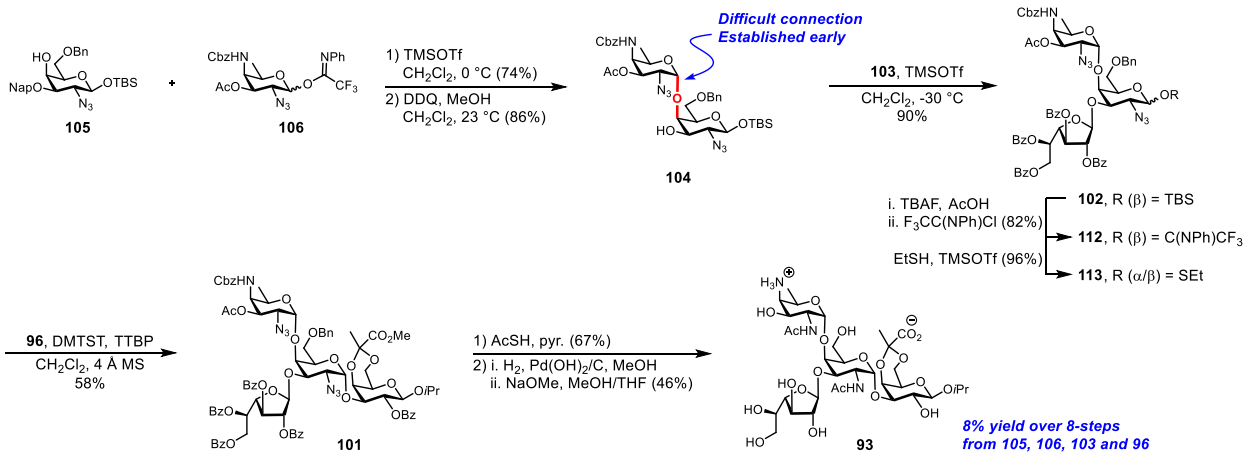


The Seeberger group decided that steric hindrance must be attributed to the difficult nature of the troublesome glycosylation. To overcome this synthetic challenge, they attempted to change the order of glycosylation events. To facilitate this, donor **107** and acceptor **96** were glycosylated together to provide a disaccharide product which subsequently underwent reductive benzylidene acetal ring opening disaccharide acceptor **110** (Scheme 14, part b). Acceptor **110** contained a small lev protecting group instead of

the large protected D-Gal*f* residue found in its trisaccharide counterpart **109**. Surprisingly, this modification ultimately had no positive outcome, because the glycosylation between acceptor **110** and donor **106** provided no desired trisaccharide product **111**. The Seeberger group speculated that the C4, C6 pyruvate ketal was interfering with the glycosylation.

With the difficulties encountered, the group then considered forming the troublesome 1,2-*cis*, D-AAT-α-(1→4)-D-Gal connection first (Scheme 15). This campaign began with a newly synthesized monosaccharide building block **105**. The smaller anomeric substituent (-OTBS) was speculated to not interfere with the glycosylation in the way the C4, C6 pyruvate ketal functionalized D-Gal residue did.

Scheme 15. Seeberger's synthetic route #3 to PS A1 ZPS repeating unit



Glycosylation between acceptor **105** and the D-AAT donor **106** fortunately provided a disaccharide with the difficult 1,2-*cis* connection in excellent yield and stereoselectivity. Moving forward, the disaccharide acceptor was produced by a DDQ mediated C3-Nap deprotection to give **104**. The D-galactofuranose imidate donor **103** underwent TMSOTf mediated glycosylation with acceptor **104** in fantastic yield to give trisaccharide **102** which then underwent a series of anomeric manipulations to provide the thioglycoside donor

113. The final glycosylation to install the terminal, reducing end, D-Gal residue proceeded by a DMTST mediated glycosylation between thioglycoside donor **96** and acceptor **113** in modest yield to establish the core, protected, tetrasaccharide **101**. Following azide reducing and acetylation of **101**, a global deprotection sequence provided the Seeberger group with **93**, completing the first total synthesis of the PS A1 ZPS repeating unit. The target molecule was produced in 8 steps with an 8% overall yield form its constituent building blocks **105**, **106**, **103** and **96**.

In 2014 the Seeberger group modified their initial route of the repeating unit **93** to incorporate an ethoxy thiol linker at C1 anomeric position rather than the alkane *iso*propyl capping unit.[114] The thiol functionalized linker allowed for the development of conjugation-ready ZPS (PS A1) probes, as seen previously.[114]

Part 12. Andreana's total synthesis of the PS A1 ZPS repeating unit (2018)

In 2018 Andreana and co-workers disclosed a synthesis of a PS A1 tetrasaccharide repeating unit.[55] Differing from the previously described PS A1 repeating units, the Andreana group selected an alternative repeating unit frame (Figure 19). From the selected ZPS frame (shown in red in **114**) the tetrasaccharide **115** was selected as the synthetic target for. The desired tetrasaccharide was capped with a methoxy phenyl group.

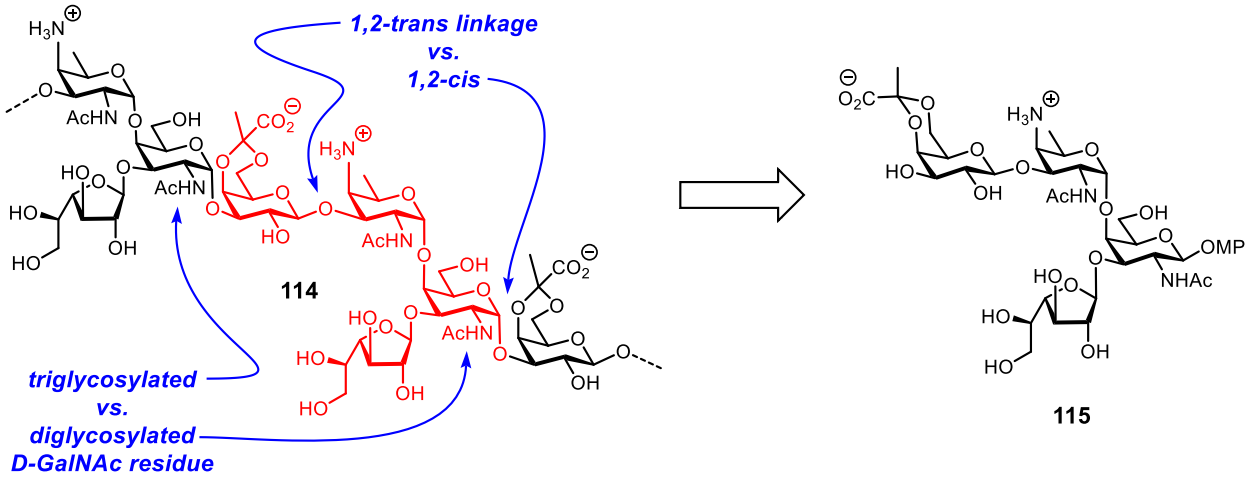


Figure 19. Andreana's PS A1 repeating unit frame and target molecule selection (2018)

The target molecule **115** contains several structural characteristics that could potentially ease synthesis. These include; 1) targeting a disubstituted D-GalNAc residue instead of the congested, branched, tri-substituted, D-galactosamine core residue; 2) NGP can be used in incorporating the β-glycosidic linkage of the D-Gal-β-(1→3)-D-AAT which is a 1,2-*trans* linkage. The previous synthetic target contained two 1,2-*cis* linkages, which are in general more difficult to synthesize. The Andreana group planned an expedient synthesis which built all the desired functionality into the protected monosaccharide building blocks prior to glycosylations. The target molecule was planned to be constructed from reducing end to non-reducing end. Retrosynthetic analysis showed that tetrasaccharide **115** could come from the fully protected tetrasaccharide **116** following a global deprotection event (Figure 20). Disconnecting the terminal D-Gal residue at the anomeric linkage could provide the monosaccharide building block **117** and the trisaccharide acceptor **118**

(disconnection *i*). The donor building block **117** could be derived from D-galactose in seven steps with a 65% overall yield.

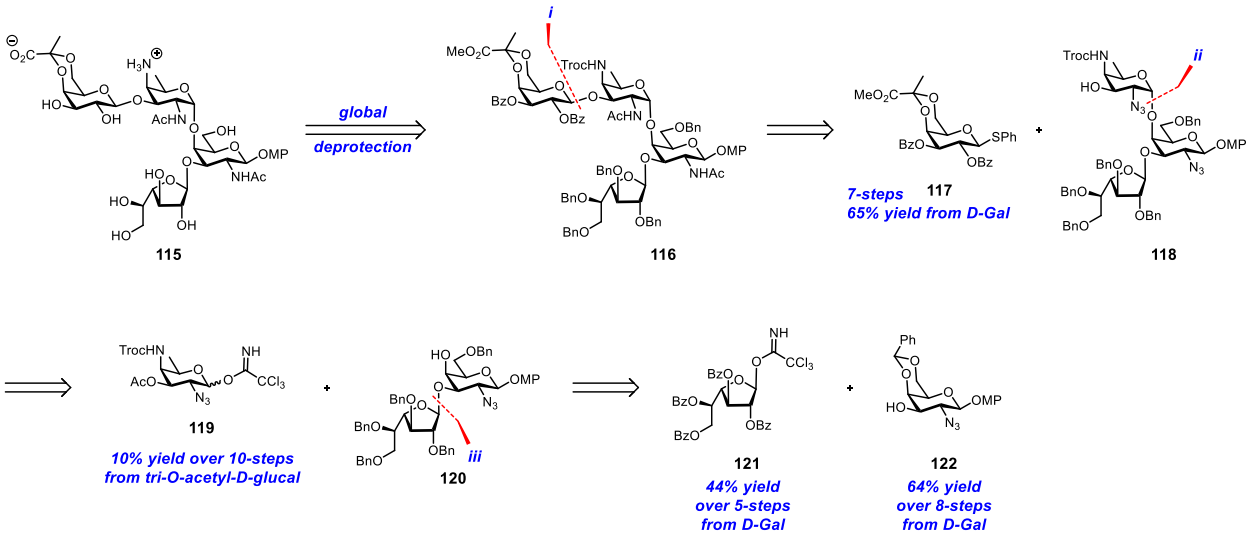


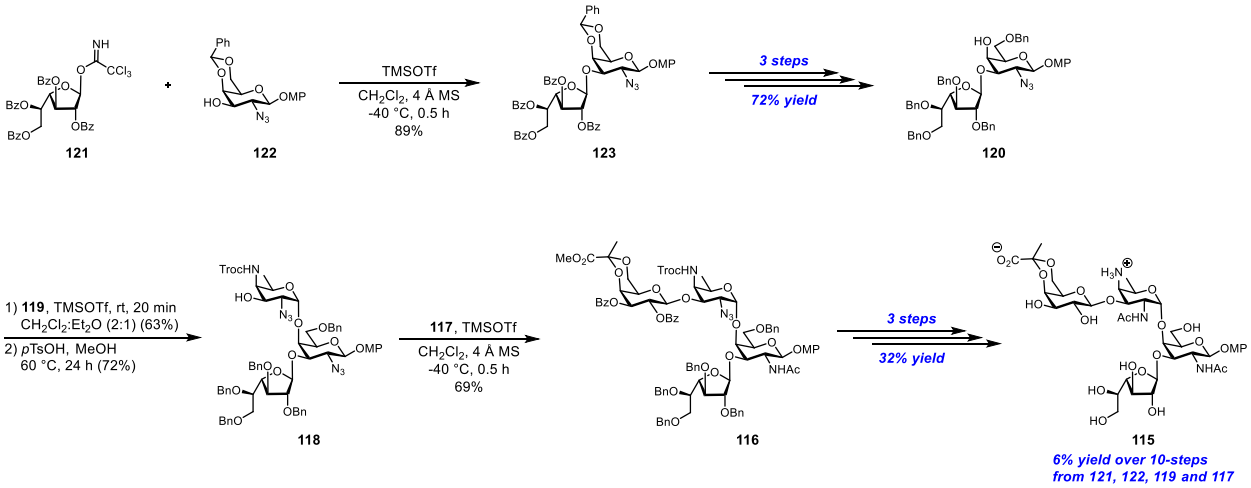
Figure 20. Andreana's PS A1 repeating unit retrosynthetic analysis (2018)

Disconnection of the D-ATT-α-(1→4)-D-GalNAc glycosidic bond could provide the trichloroacetimidate donor **119** and the disaccharide acceptor **120** (disconnection *ii*). The donor building block **119** could be produced in ten steps with a 10% overall yield from tri-*O*-acetyl-D-glucal. The final disconnection, D-galactofuranosyl glycosidic bond provided the trichloroacetimidate building block **121** and the D-GalN_3 building block **122**. The D-galactofuranosyl building block **121** could be synthesized in five steps with a 44% yield from D-Gal. The D-GalN_3 acceptor **122** could be synthesized in eight steps with a 64% yield from D-Gal.

Like the van der Marel synthesis, the Andreana group opted to make the D-Gal*f*-β-(1→3)-D-GalN_3 bond first. To do this D-galactofuanosyl imidate **121** and the D-

galactosamine accepter **122** were combined under acidic conditions to give the alpha glycosylated product **123** (Scheme 16).

Scheme 16. Andreana's 2018 synthetic route to PS A1 ZPS



Next, a sequence of three transformations took place. First, the benzoyl groups were removed under basic conditions and replaced by benzyl protecting groups. Next the C4, C6 benzylidene acetal selectively opened under reductive conditions to provide the C6-benzyl group and the free C4-hydroxyl in disaccharide acceptor **120**. Glycosylation of **120** with D-AAT trichloroacetimidate donor **119** was carried out to give trisaccharide **118**. This connection proved to be difficult for previous synthesis when the D-GalNAc residue was glycosylated at the anomeric position. However, as the Seeberger group concluded, this glycosylation was successful with a small anomeric substituent on the D-GalNAc residue. Following the glycosylation event, the C3' acetate protecting group was removed under acidic conditions, to avoid C3', C4' oxazolidinone formation, to give trisaccharide acceptor **118**. The final glycosylation between acceptor **118** and D-Gal thioglycoside donor **117** to form the D-Gal-β-(1→3)-D-AAT bond proceeded in 69% yield as a single beta anomer. Following the construction of the core tetrasaccharide the sequence provided the final

product **115** in 53% yield. The target molecule was produced in ten steps with an 6% overall yield form its constituent building blocks **121**, **122**, **119** and **117**.

The Andreana group also successfully synthesized an orthogonally fully protected repeating unit containing a C3'' Fmoc group. This derivative could selective deprotection to provide a PS A1 repeating unit acceptor which could be used to access higher order PS A1 oligosaccharides in the future.

Part 13. CP 5 ZPS from S. aureus (Adamo 2012)

The Gram-positive bacteria *Staphylococcus aureus* is an opportunistic bacterium which contains a polysaccharide capsule that includes the CP 5 and CP 8 ZPSs. Both have been identified as immunogenic by their ability to activate CD4+ T-cells *in vitro* and have been shown to be capable of inducing abscess formation in experimental animals.[134] The CP 8 ZPS has been shown to be capable of CD4+ T-cell activation and production of INF-γ at the site of infection in animal models.[135] Evaluation of antisera form attenuated *S. aureus* immunized rabbit revealed that type specific (CP 5 and CP 8) antibodies can facilitate killing of live bacteria via polymorphonuclear cell *in vitro*.[1] Immune responses from these polysaccharides in animals models is not well defined. However, the conjugate of CP 5 and CP 8 with *Pseudomonas aeruginosa* exotoxin A (Staphvax^{TM}) (Nabi Biopharmaceuticals, Rockville, MD, USA) has been observed to induce T-cell mediated immunity towards *S. aureus* infections.[136] Unfortunately Staphvax^{TM} failed in Phase III clinical studies due to poor efficacy.[137] Therefore, further studies are required to explore the efficacy of these polysaccharides in vaccine preparation.

The CP 5 ZPS (**124**) is composed of repeating trisaccharide structure shown in red in Figure 21. The CP 5 repeating unit consists of a: →4)-β-D-Man*p*NAcA-(1→4)-α-L-Fuc*p*NAc(3-OAc)-(1→3)-β-D-Fuc*p*NAc-(1→ (Figure 21).[138] Notably, the CP 5 contains a β-linked D-mannoside. β-mannosylations are considered one of the more difficult glycosidic bonds to form because they are disfavored kinetically and thermodynamically.[99, 139-143] In addition, the CP 5 contains acetamide moieties on every residue. Adamo and co-workers were the first group to complete the total synthesis of the CP 5 ZPS repeating unit in 2012 at the Novartis Vaccine and Diagnostics Research Center in Siena Italy.[144] From greater oligosaccharide **124**, the repeating frame **125** was selected as the synthetic target (Figure 21). The synthetic target **125** was capped with an amine functionalized linker to be used for conjugation and ELISA based studies.

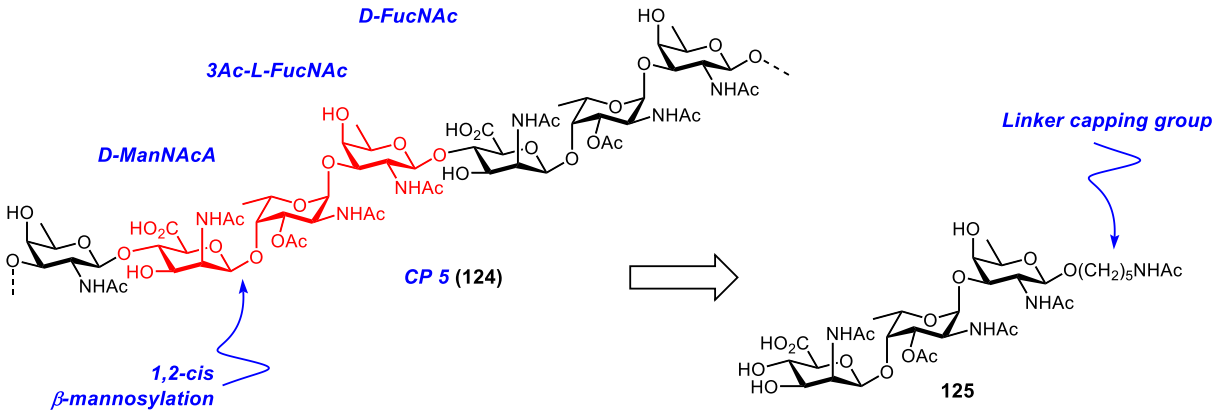


Figure 21. CP 5 ZPS repeating unit selection

Retrosynthetic analysis provided the desired product **125** could come from the fully protected product **126** (Figure 22). Disconnecting the C1'-β-glycosidic linkage in **126** could provide trichloroacetimidate donor **127** and acceptor **128** (Figure 22. disconnection

i). Following several functional group interconversions one could arrive at disaccharide **129**. Disconnecting the β-glycosidic linkage could provide donor **130** and L-Fuc acceptor **131**. Conversion of a D-GlcA residue to the D-ManNAcA reside via FGI to avoid performing a β-mannosylation was a key synthetic strategy planned by the group. This approach will be referred to as an indirect D-ManNac introduction via β-glycosylation—epimerization. It is important to note, the group planned to not incorporate a late-stage oxidation, but rather carry the C6-benzyl ester protected residues through the synthesis.

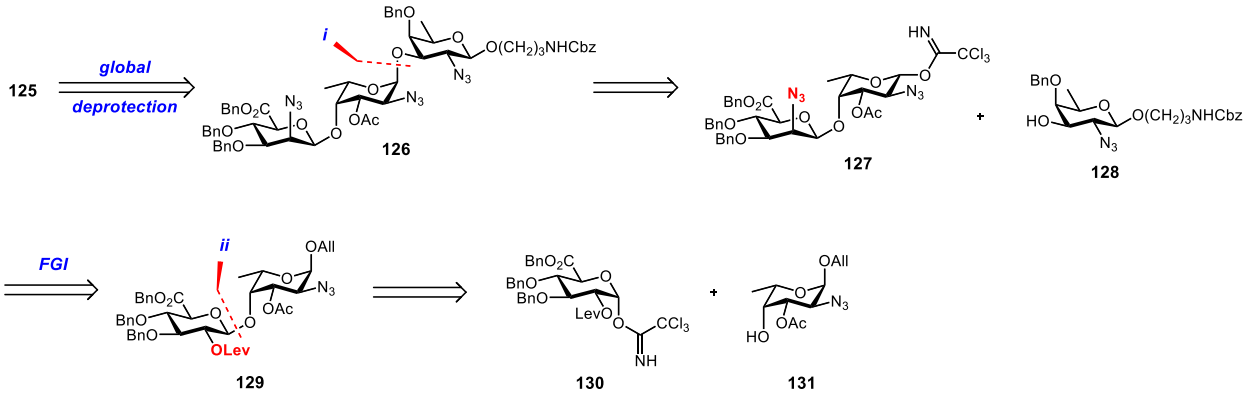
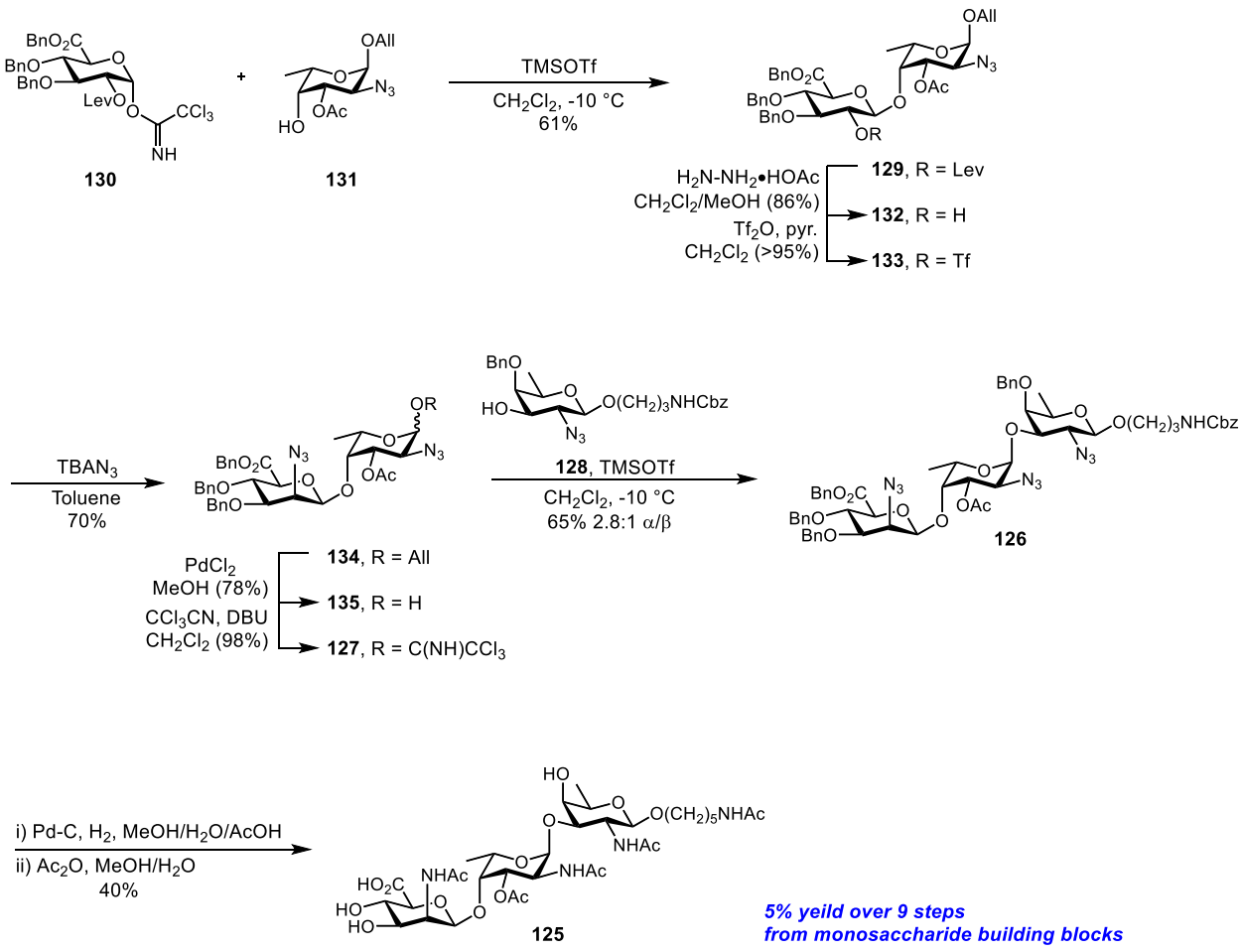


Figure 22. Retrosynthetic analysis of CP 5 by Adamo (2012)

The route began with a TMSOTf mediated glycosylation between donor **13**0 and acceptor **131** to provide the disaccharide **129** in good yield (Scheme 17). This step was complicated by C2 to C3 acyl migration of the acceptor **131**. With what will become the β-mannosyl linkage formed, the conversion of the D-GlcA to the D-ManNAcA residue followed. This was carried out by deprotection of the C2'-Lev group, to give **132,** and then triflation of the C2' free alcohol **133**. Next, displacement by an azide installed the C2'

amine functionality to provide **134**. Next the disaccharide needed to be converted into a glycosyl donor. To do this the anomeric allyl group was removed to provide the free reducing sugar **135**. Next the trichloroacetimidate **127** was produced. A glycosylation event with D-Fuc acceptor **128** provided the trisaccharide **126**. A global deprotection provided the desired product **125**, albeit in modest yield. The deprotection of the final compound was complicated due to 2'',6'' lactamization. The free amine produced, during the reduction of the azide, under Staudinger or hydrogen sulfide conditions underwent lactamization with the C6'' ester. Fortunately, hydrogenation conditions provided the best results in producing the desired product **125**.

Scheme 17. Synthesis of the CP 5 by Adamo and co-workers (2012)



Adamo and co-workers provided the first total synthesis of the CP 5 ZPS repeating unit **1** in a 5 % overall yield over a nine-step sequence from its constituent monosaccharide building blocks.

Part 14. CP 5 ZPS (G. J. Boons 2015)

To overcome the synthetic challenges associated with lactamization, G. J. Boons and co-workers planned developed a chemical synthesis of the CP 5 ZPS in 2015.[145] The target molecule for was selected to be the identical repeating unit frame consisting of the D-ManNAcA at the non-reducing end, a central L-FucNAc and a D-FucNAc residue at the reducing end (Figure 23).[145] Contrary to the Adamo, the Boons group opted for a late stage oxidation strategy to avoid complications of the C2'',C6''-lactamization of the D-Mannuronic residue. The synthetic route also proposed installment of the linker moiety into the trisaccharide rather than a monosaccharide. Retrosynthetic analysis provided the protected trisaccharide **137** could come from the target molecule **136** following a global deprotection event. Deprotection and oxidation of the C6'' alcohol could provide compound **138**. Prior to the oxidation step, the azides would be converted to acetamides to avoid lactamization complications. In addition, the linker would be installed at this stage of the synthesis. Protecting group manipulation of **138** removal of the linker would give trisaccharide **139**. Disconnection of the 1,2-*cis* β-mannosyl bond would provide donor **140** and disaccharide **141** (disconnection *i*). Donor **140** was produced in a 29% yield over a seven-step procedure from D-ManNAc. Next, disconnection of the α-glycosidic linkage of **141** would provide donor **142** and acceptor **143** (disconnection *ii*). Donor **142** was synthesized in a 27% yield with a thirteen-step procedure from L-Fuc. acceptor **143** was

produced in a 26% yield with a ten-step procedure from D-Fuc. The Boons group planned to synthesize the target molecule from the reducing to the non-reducing end in contrast to the Adamo group.

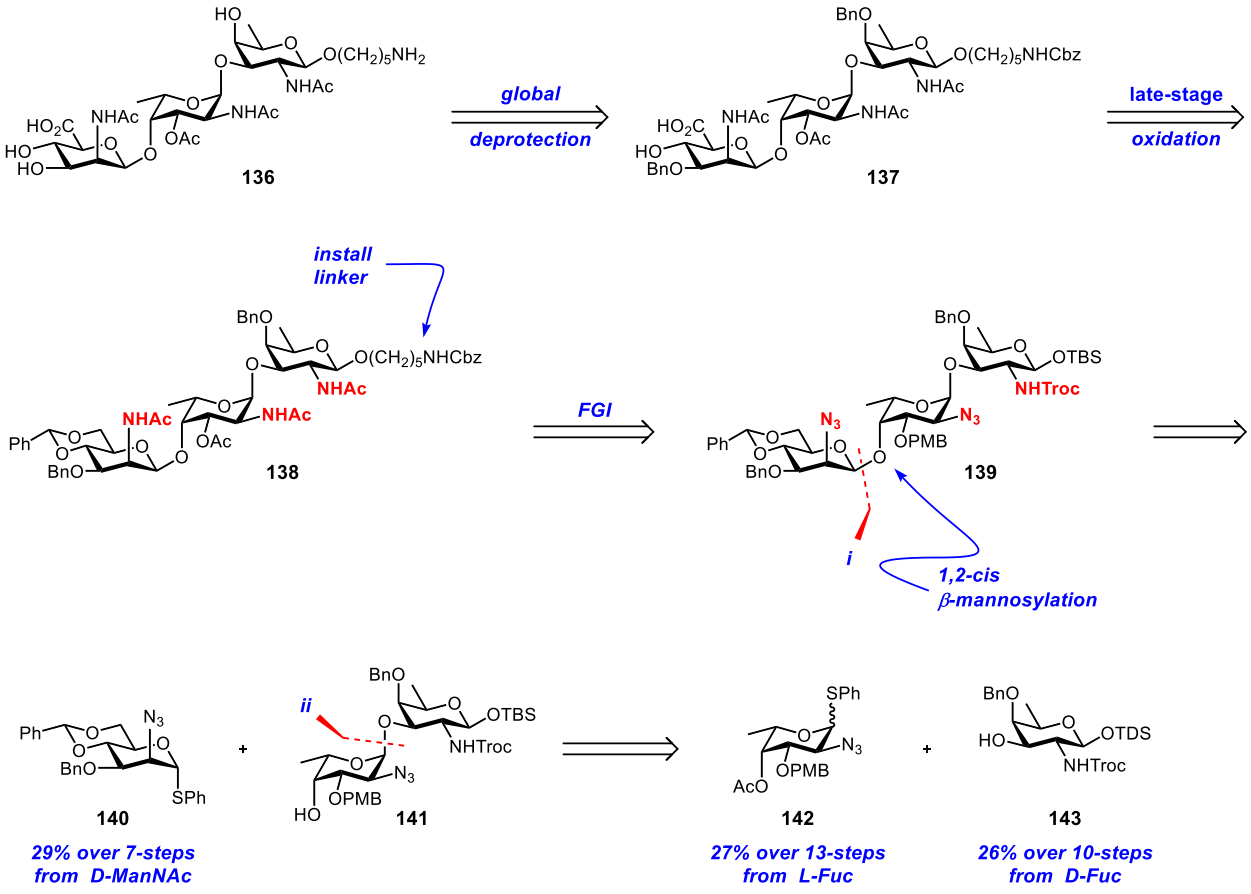
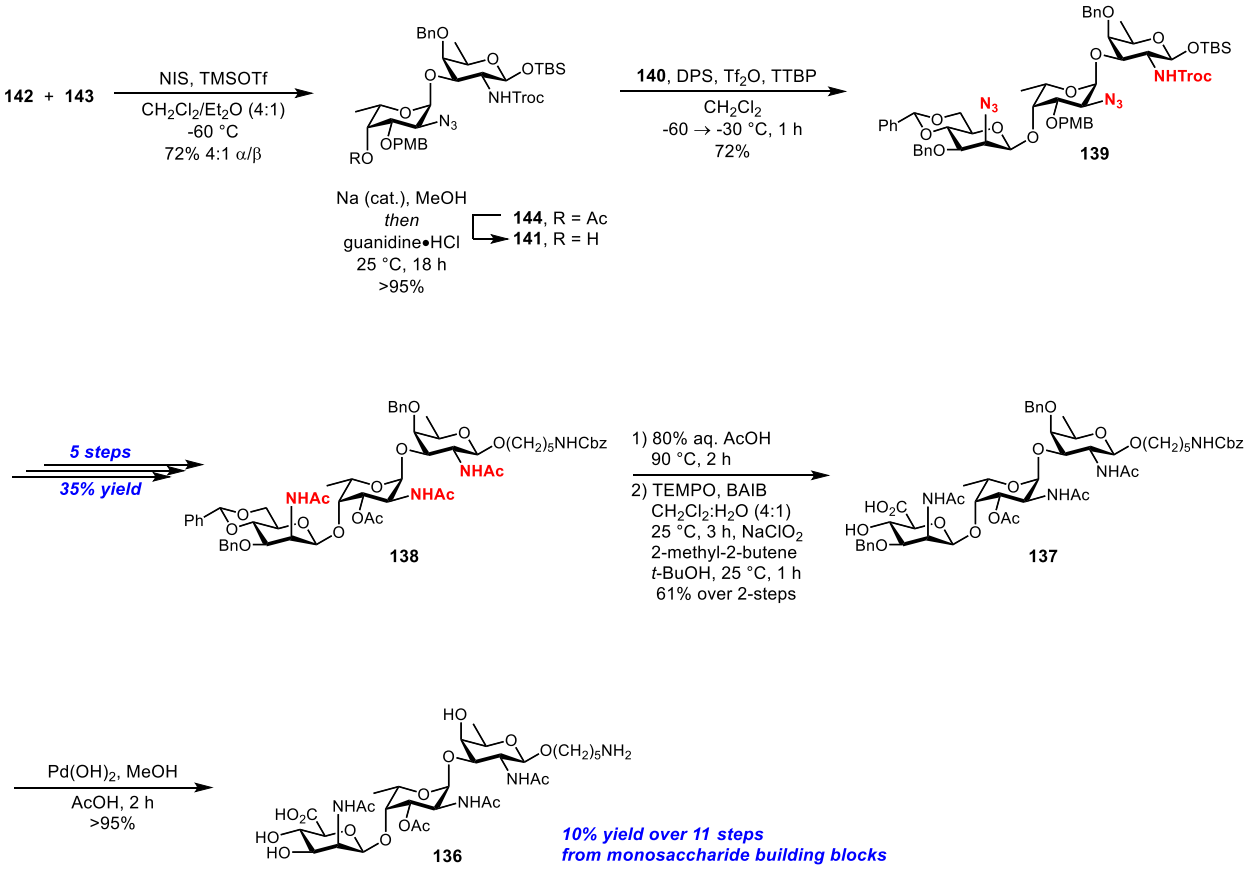


Figure 23. Retrosynthetic analysis of CP 5 by G. J. Boons (2015)

The forward synthesis began with glycosylation between **142** and **143** provided the disaccharide **144** (Scheme 18). As a note, the Boons group reported initial attempts with a C3 acetate protected derivative of **142** in place of the C3-OPMB protecting group. This derivative failed to undergo glycosylation events with **143** and the subsequent

glycosylation in any acceptable yields. The disarming effects of the acetate group were hypothesized to have caused the C3-acetylated derivative of **142** to be a poor glycosyl donor and disaccharide acceptor. Next, the C4' acetate was saponified to provide the disaccharide acceptor **141**. The following glycosylation between **141** and donor **140** was presumed to be a difficult step not only because it was a β-mannosylation, but because axial C4 hydroxyl of galactosides and fucosides are generally of low reactivity as glycosyl acceptor. [146] However, following optimization the Boons group was able to provide the trisaccharide **139** in good yield. Next, the trisaccharide **139** underwent a series of five steps to install the three acetamides, install the linker and exchange the C3'-PMB protecting group for an acetate to give trisaccharide **138**. With the acetamides installed prior to oxidation of the C6'' lactamization was avoided. Next C4'', C6'' benzylidene acetal was removed and the C6'' was selectively oxidized to give trisaccharide **137**. A final hydrogenation event provided the trisaccharide **136** in excellent yield. The synthesis of the CP 5 ZPS was completed in a 10% yield over an eleven-step procedure from its constituent monosaccharide building blocks.

Scheme 18. Synthesis of the CP 5 by G.J. Boons and co-workers (2015)



The work from the Boons lab provided the CP 5 ZPS in an improved yield compared to the Adamo group, in part due to the direction of synthesis (propagating from reducing end to the non-reducing end). In addition, their synthesis allows for incorporation of alternative linkers and multiple sites for derivatization. The Boons group provided experimental conditions to facilitate a direct β-mannosylation and avoided the undesired lactamization.

Part 15. CP 5 ZPS (Demchenko 2016)

Work by the Demchenko group in 2016 provided an alternative method to the CP 5 ZPS.[147] Demchenko selected the same CP 5 repeating frame as Boons and Adamo, but their target molecule contained an *O*-methyl capping group. The target molecule **145** also

contained an *O*-methyl group on the C4'' hydroxyl to delineate the point of propagation of the ZPS polymer (Figure 24). The synthetic strategy to **145** included a late stage oxidation to avoid lactamization, such as the method employed by the Boons group. Drawling similarities to the Adamo group, the Demchenko lab planned to employ the indirect D-ManNac introduction via β-glycosylation—epimerization. The synthesis would proceed elongation from reducing to non-reducing end. Retrosynthetic analysis demonstrated the target molecule **145** could come from trisaccharide **146** following a global deprotection and C6'' oxidation. Trisaccharide **146** could from **147** through a series of functional group interconversions. Disconnection of the L-fucosyl glycosidic linkage provided the disaccharide donor **148** and the monosaccharide building block **149** (disconnection *i*). Disconnection of the D-glucosidic bond provided the thioglycoside donor **151** and the L-fucosyl acceptor **152** (disconnection *ii*).

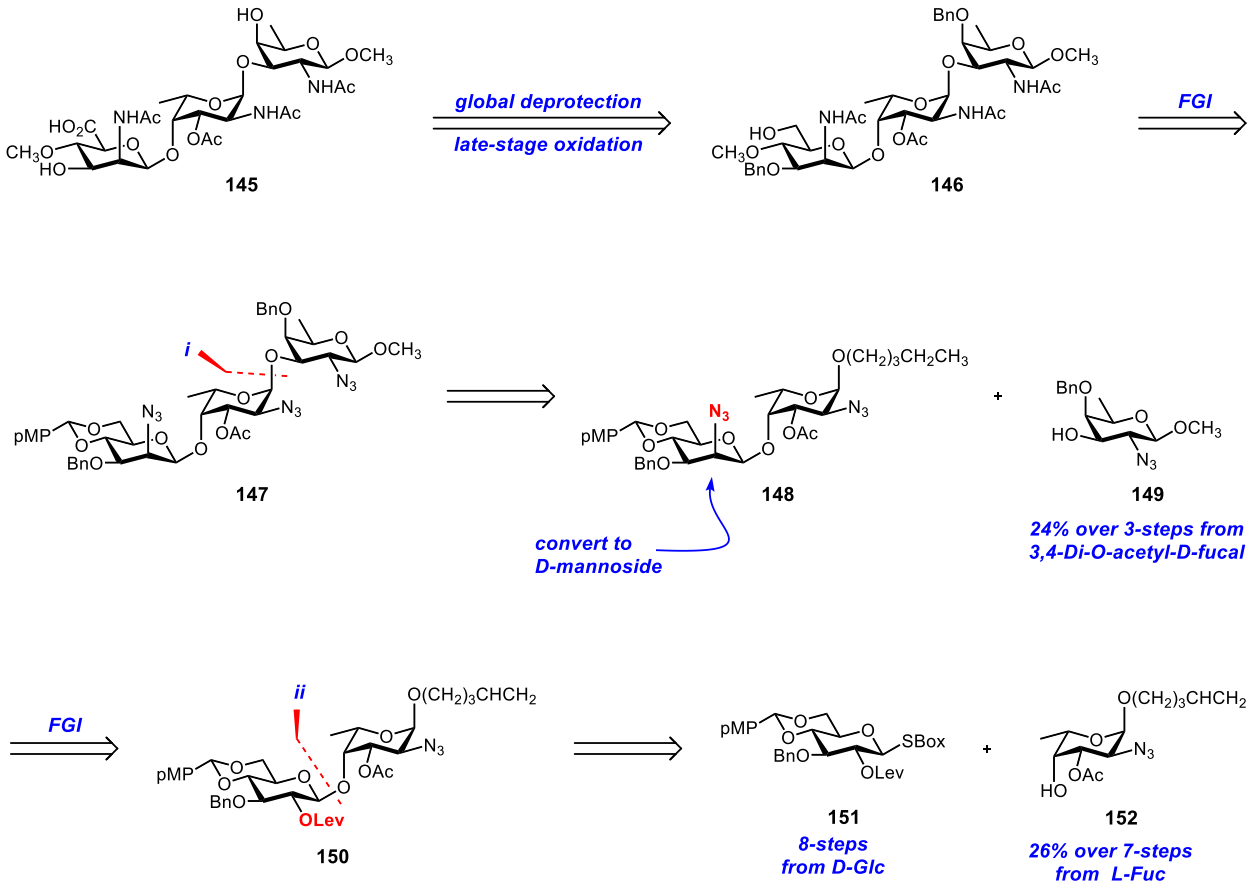


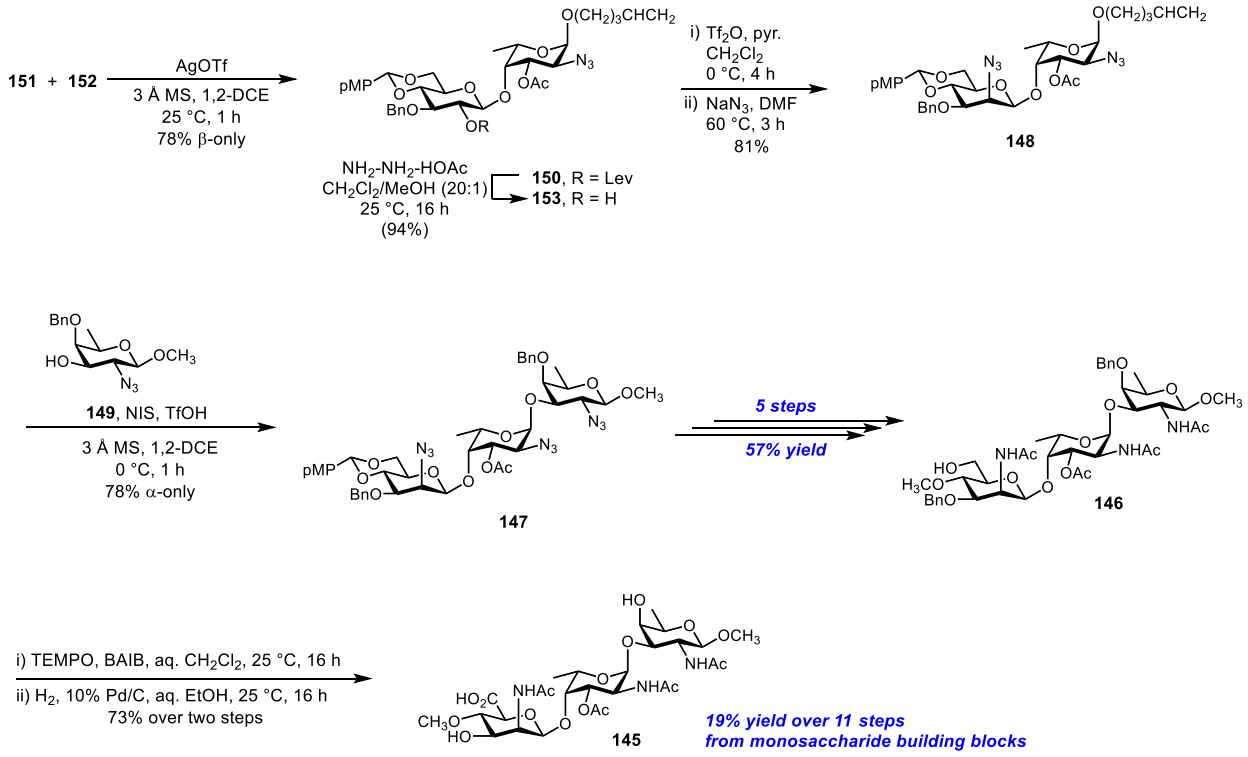
Figure 24. Retrosynthetic analysis of CP 5 by Demchenko (2016)

The synthesis began with the glycosylation between **151** and **152** to give **150** (Scheme 19). This provided the bond which would ultimately become the β-mannosyl glycosidic linkage. Conversion of the D-Glc residue to a D-Man residue began with deprotection of the Lev group in **150** to provide **153** The C2' hydroxyl was triflated then displaced by an azide to give disaccharide **148**. The *O*-pentenyl donor was activated with NIS/TfOH in the presence of the glycosyl acceptor **149** to provide trisaccharide **147**. Following a series of five steps the azides were converted into acetamides, the C4'' hydroxyl was methylated and C6'' hydroxyl was deprotected to provide trisaccharide **146**. With the acetamides set,

the trisaccharide was then oxidized to provide the D-ManNAcA residue, then deprotected by hydrogenation to provide the desired product **145**.

Demchenko *et al.* provided a route to access the CP 5 ZPS in a 19% overall yield over eleven steps from its monosaccharide building blocks. This route provides the highest yield of CP 5 ZPS to date.

Scheme 19. Synthesis of the CP 5 by Demchenko and co-workers (2016)



Part 16. CP 8 ZPS (Demchenko 2015)

In addition to CP 5, CP 8 ZPS if found as an isolate from *S. aureus*. The CP 5 ZPS is composed of a trisaccharide repeating unit structure of: →3)-β-D-ManNAcA(4OAc)-(1→3)-α-L-FucNAc-(1→3)-α-D-FucNAc-(1→.[138] It bears many similarities to the CP5 ZPS as shown below in Figure 25. The CP 8 and CP 5 contain the same glycosyl residues and order, however, differ in cite of acetylation and glycosidic connectivity.

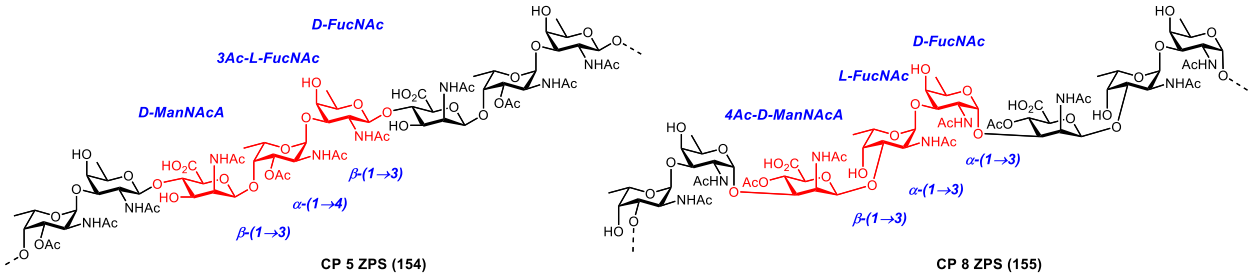


Figure 25. Comparison of CP 5 and CP 8 ZPS and CP8 repeating unit selection

Figure 25 depicts the targeted repeating unit, shown in red of **155**, selected by the Demchenko group for total synthesis. The *O*-methyl (C1 and C3'') capped target molecule **156** was planned from the repeating unit selection in ZPS **155** (Figure 26). Some aspects of the synthetic strategy include; an indirect β-mannosylation, late stage oxidation to avoid lactamization, and synthesis from reducing to non-reducing end.

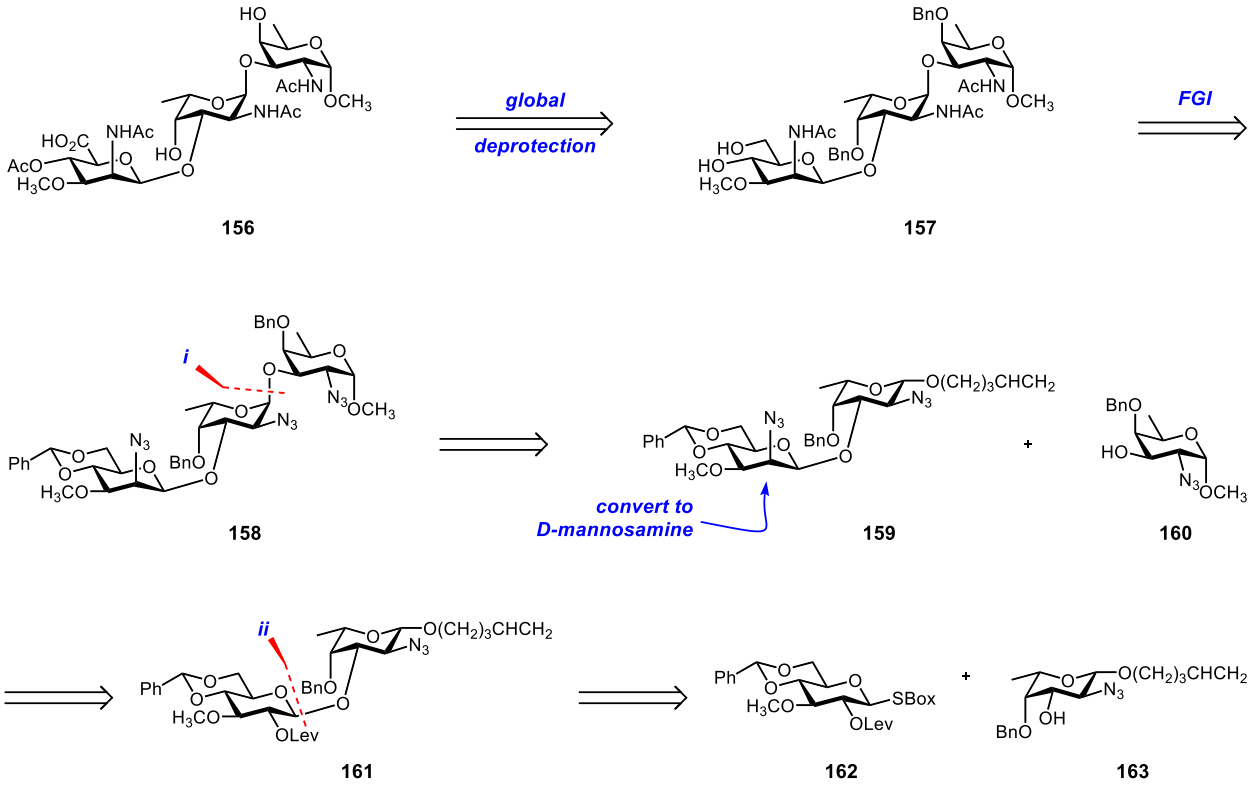


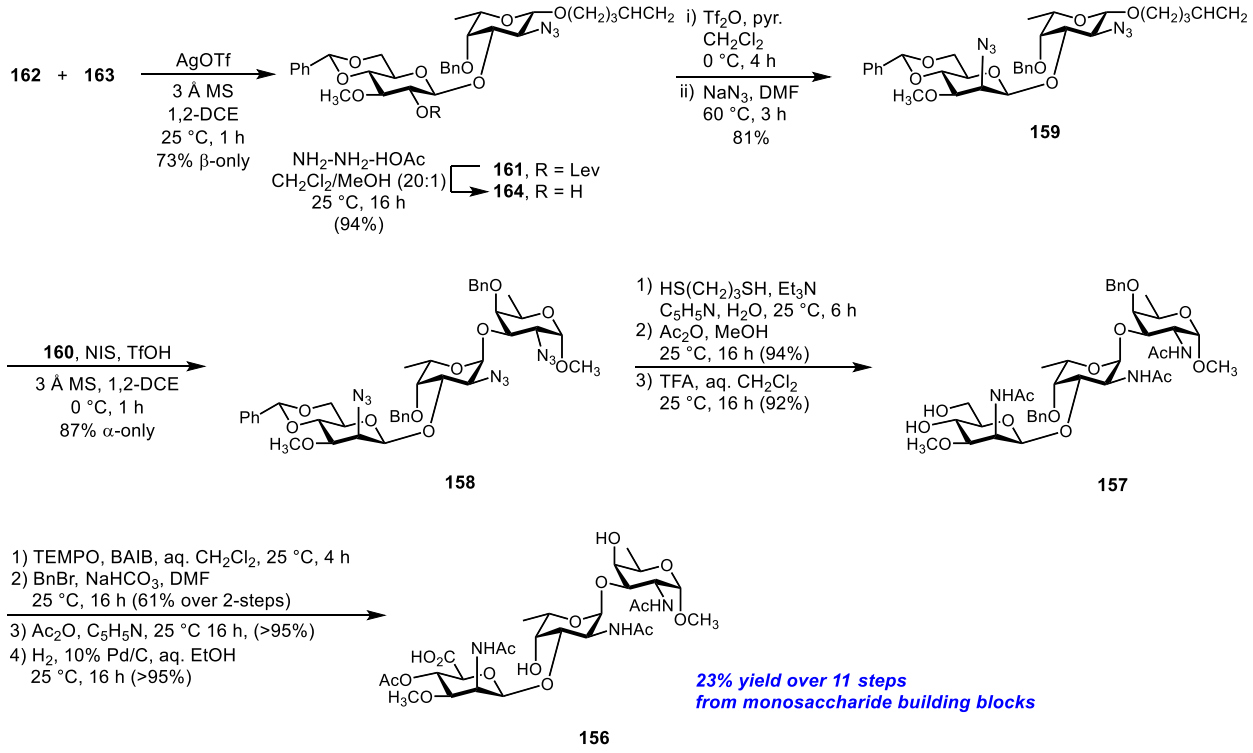
Figure 26. Retrosynthetic analysis of CP 8 by Demchenko (2015)

Retrosynthetic analysis showed that **156** could come from **157** following a global deprotection event. **158** could come from **157** following a series of function group interconversions. Disconnection of the C1' glycosidic bond would provide donor **159** and acceptor **160**. (disconnection *i*) Amination and epimerization of C2' would give disaccharide **161**. Disconnection of the D-Glc β-glycosidic bond would provide donor **162** and donor **163** (disconnection *ii)*.

Following synthesis of the rare glycosyl building blocks, a glycosylation between **162** and **163** provided disaccharide **161** (Scheme 20). Removal of the Lev protecting

group gave **164** which was converted into a triflate and was displaced by an azide to give **159**. Glycosylation between **159** and **160** gave trisaccharide **158**. Conversion of the azide functional groups to acetamides was carried out followed by removal of the benzylidene acetal to give **157**. Oxidation and benzyl ester protection of the C6'' hydroxyl followed by the C4'' acylation provided a fully protected trisaccharide. This trisaccharide could then be subjection to hydrogenation conditions to give the desired product **156**.

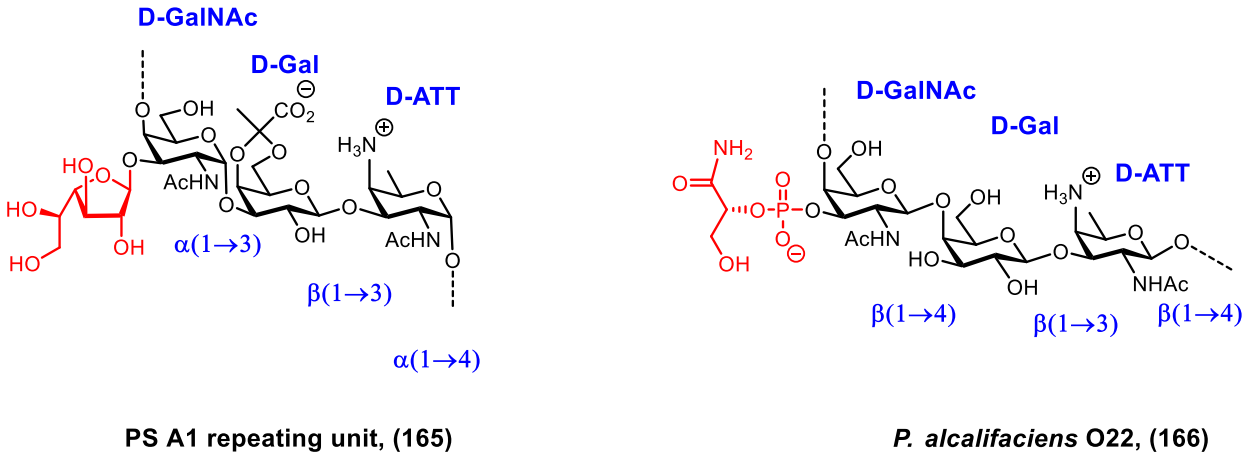
Scheme 20. Synthesis of the CP 8 by Demchenko and co-workers (2015)



Synthesis of the CP 8 ZPS was carried out in a 23% yield over a total of eleven steps from the monosaccharide building blocks. This method can access synthetically useful quantities of the CP 8 ZPS. With this ability, one can then develop derivatives of ZPS to probe immunological systems with synthetic ZPS tool compounds.

Part 17. Alcalifaciens O22 ZPS (Kulkarni 2017)

The *P. alcalifaniens* O22 ZPS was isolated and characterized by Ovchinnikova and co-workers.[13] They reported a structure of a phosphorylated *O*-polysaccharide from the S-form lipopolysaccharide of the *P. alcalifaniens* O22 which was composed of a trisaccharide repeating unit. The *P. alcalifaniens* O22 ZPS contained a D-glyceramide 2-phosphate (D-GroAN-2-P) residue residing at the terminal C3'' equatorial hydroxyl of the D-GalNAc residue (Figure 27). The repeating unit, like the previously discussed ZPSs, contained the rare 2-acetamido-4-amino-2,4,6-trideoxy-D-galactose (D-AAT) residue as well as a D-GalNAc and D-Gal residues. In addition, the *P. alcalifaniens* O22 ZPS only differs slightly in the core structure from the PS A1 ZPS repeating unit. The core trisaccharide residue of the two ZPSs are identical in composition and order: D-GalNAc, D-gal, D-AAT from non-reducing end to reducing end (Figure 27). The structure includes the D-Gal to D-AAT glycosidic linkages are both β-(1→3), and the D-AAT to D-GalNAc are both (1→4) linkages which are only different by the α/β configuration. A final parallel between the two core structures is the site of substitution. Both the PS A1 and the *P. alcalifaciens* O22 ZPS repeating units contain a branching site of substitution at the C3'' equatorial position on the D-GalNAc residues as the ZPS frame is shown below (Figure 27).



PS A1 repeating unit, (165)

***P. alcalifaciens* O22, (166)**

Figure 27. Comparison of PS A1 and P. alcalifaciens O22 repeating units

Kulkarni and co-workers planned an expedient synthesis from orthogonal building blocks **168**, **169**, **170** and **171**.[12] The D-galactosamine thioglycoside donor building block **169** was prepared though a number of steps including a C4' inversion of stereochemistry of D-glucosamine, because of the substantial price difference between the two monosaccharide starting materials. The D-AAT acceptor **171** was synthesized from a D-rhamnosyl compound, and the *H*-phosphonate **168** was produced in seven steps with a 33% yield from a dicyclohexylidene D-mannitol starting material (Figure 28).

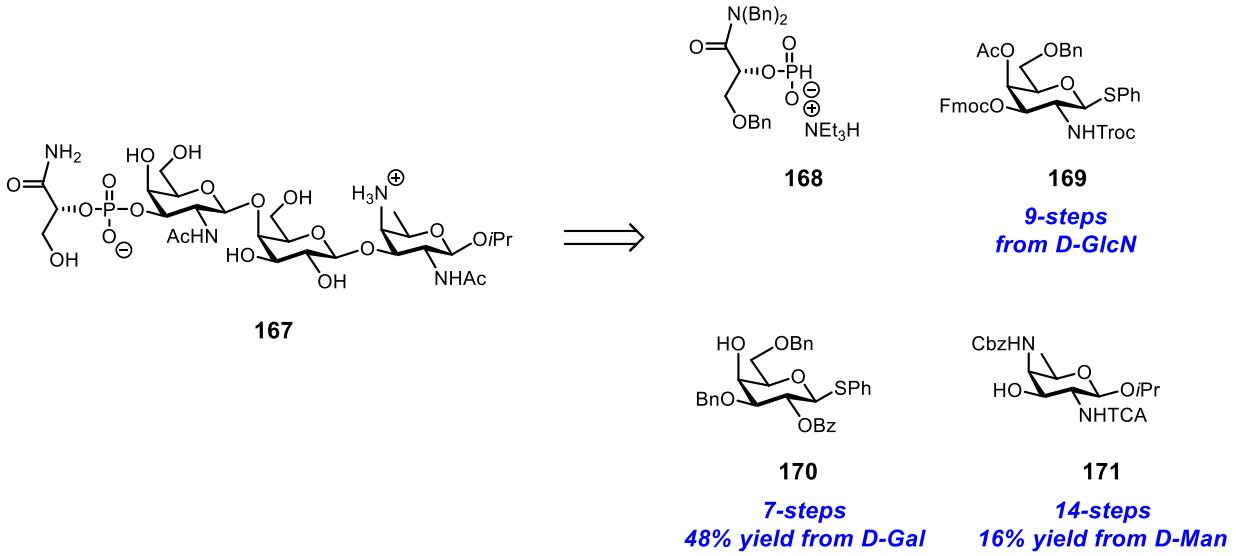
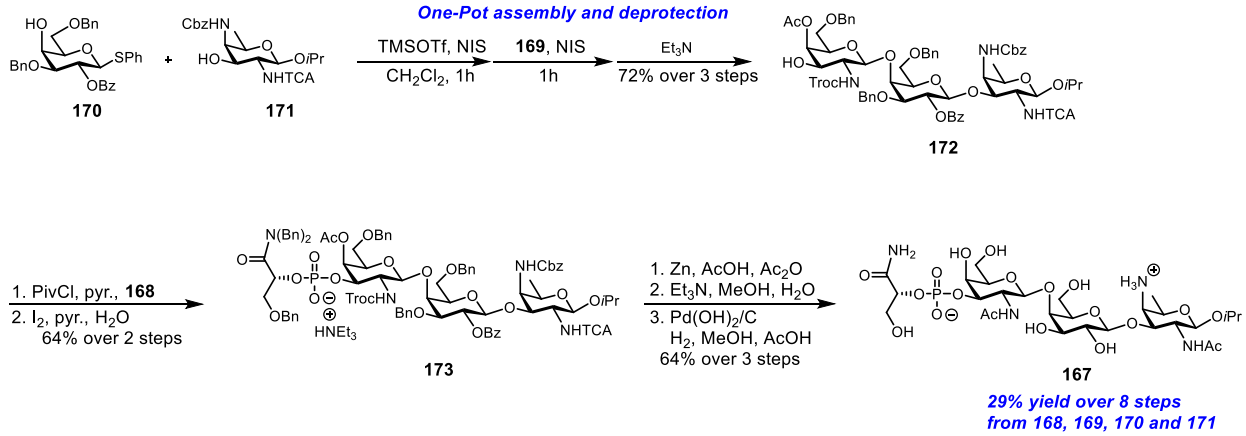


Figure 28. Kulkarni's retrosynthetic analysis of providencia alclafciens O22 repeating unit (2017)

The route was developed to allow for a one pot procedure which could produce the entire core trisaccharide followed by a deprotection in a one-pot method (Scheme 21). First the donor **170** and acceptor **171** were combined and activated under NIS/TMSOTf conditions to give the D-Gal-β-(1→3)-D-AAT disaccharide in high yield. It is thought that the enhanced nucleophilicity of the equatorial C3-OH of acceptor **171** over the axial C4-OH of donor **170** mitigates the formation of higher order oligosaccharides or self glycosylation between donor **170**. After 1 h, the group reports the addition of more equivalencies of NIS promotor along with glycosyl donor **169**. After an additional 1 h, the C3''-OH is deprotected by the addition of triethylamine to afford the trisaccharide core **3**. The trisaccharide core **172** was promptly coupled under pivaloyl chloride and pyridine followed by oxidation to furnish the phosphorylated trisaccharide **173** in 64% yield over

two steps. Following a global deprotection procedure, the ZPS repeating unit **167** was obtained in 63% yield over three steps. Biological evaluation of the *P. alcalifaciens* O22 ZPS repeating unit is currently underway to assess its immunological potential.

Scheme 21. Kulkarni's route to *providencia alclafciens* O22 repeating unit



Chapter 2

Total synthesis of the MM-ZPS repeating unit

Part 1. MM-ZPS introduction

Morganella morganii is an opportunistic, Gram-negative pathogen which causes biliary infections, bladder infections and bacteraemia.[148] *Morganella morganii* belongs to the tribe *Proteeae* of the family *Enterobacteriaceae*.[149] The first isolates were cultured by Morgan in 1906 from infants suffering from diarrhea.[149] In 1939, Sevin and Buttiaux showed that this species can also cause urinary tract infections following reports of isolation from feces, urine, and other pathological materials in hospitals.[150] In 1979, *O*-antigen grouping of *Morganella morganii* was investigated and reveled that several *O*-antigen serotypes were recognized by studies of organisms recovered from patients.[151] The *O*-antigen refers to repetitive glycan polymers making up the lipopolysaccharide which extends out of the outer core of the gram-negative bacteria cell wall.

The composition of these *O*-antigens can be determined through analytical methods and used to identify bacterial strains. In 2010, Young and co-workers began investigating the *O*-antigen of *Morganella morganii*. They discovered that hybridoma antibodies, produced from immunization with MM-ZPS, shared similarities to others hybridoma antibodies which recognize phosphocholine-containing antigens. This prompted Young and co-workers to undergo a structural and immunological analysis of the *O*-chain polysaccharide.[8] In 2011, the Young group were the first to discover and report the first

zwitterionic *O*-chain polysaccharide of the *Morganella morganii* (MM-ZPS). Their discovery reveled that the MM-ZPS does not only have a phosphocholine residue, but it also contains an additional phosphate and charged amino moiety on its structure giving rise to its charged, zwitterionic character. As with other ZPSs, the MM-ZPS was also discovered to elicit an immune response on its own, devoid of peptides or proteins which were previously thought to be required for immunomodulation. To determine the ability for the MM-ZPS to active the immune system, it was first hydrolyzed into fragments of varying lengths and purified. Binding affinity of the purified fragments was assessed by anti-*M. morganii* hybridoma enzyme immunoassay inhibition assay compared to glycerophosphocholine. To further validate that immunoactivation is being facilitated by the MM-ZPS, assays developed by Cobb and Kasper in 2008 for the evaluation of *B. fragilis* PS A were performed on the MM-ZPS.[110] The assay was conducted on the purified MM-ZPS fragments and recombinant human MHCII (HLA-DR1). It was found that 5 kDa MM-ZPS fragments are capable of MHCII binding with a similar binding affinity compared to 5 kDa fragments of *B. fragilis* PS A (K_D value of 3.66±0.63 μM for MM-ZPS vs. K_D value of 1.9±0.4 μM for PS A).[8] In addition, competitive binding studies between the MM-ZPS and the PS A ZPS confirmed comparable binding affinities between MM-ZPS and MHCII.[8] It was confirmed that binding of the monomeric repeating unit (trisaccharide) and dimeric repeating unit (hexasaccharide) failed to reproduce the 5 kDa MM-ZPS binding. This finding supported the long-tested concept of a length requirement for the MHCII interaction beyond the basic repeating unit. In addition, chemical quenching of the charged moieties (via acylation) completely removed the ability of the MM-ZPS to bind to

MHCII, indicating that the charged nature of the molecule impacts its immunomodulatory properties.

Bacteria capsule

Further investigation by Young and co-workers went on to describe the ability for the purified MM-ZPS to induce T-cell activation. Human peripheral blood mononuclear cells (PBMCs from a hepatitis B peptide antigen (HepB)) were used to isolate $CD4^+$ T-cells. This was achieved by using positive selection on magnetic beads as well as T-cell-depleted antigen-presenting cells (APCs) using CD3 negative selection. For activation, $CD4^+$ T-cells and APCs were co-cultured at a 1:1 ratio with MM-ZPS or PS A. Then the culture supernatants were collected and analyzed for interferon-γ (IFN-γ) by enzyme-linked immunosorbent assay (ELISA) as a measure of T-cell activation. Results showed the MM-ZPS activated $CD4^+$ T-cells to a similar extent as the PS A (3084 pg/mL vs. 5807 pg/mL after 1 week, respectively). Collectively, this data show for the first time that bacterial *O*-antigen can activate $CD4^+$ T-cells via the MHCII presentation. This is a unique qualifier for the MM-ZPS when compared to other studied ZPSs. At the time of its discovery, the MM-ZPS was the only known ZPS found as an *O*-antigen, a constituent of the LPS (lipopolysaccharide). LPS is found as the outer membrane of Gram-negative bacterial. The LPS is composed of four subunits: lipid A, inner core, outer core, and the O-antigen. In contrast, all previously discovered ZPSs (PS A1, Sp1, CP5, CP 8 etc.) had been discovered in the capsule of the bacteria.

The bacterial capsule is a polysaccharide layer that lies outside the cell envelope and is thus deemed part of the outer envelope of a bacterial cell. It is a well-organized layer, not easily washed off, and its can be the cause of various diseases. The capsule, which can

be found in both Gram-negative and Gram-positive bacteria, is different to the second lipid membrane (bacterial outer membrane) which contains lipopolysaccharides and lipoproteins and is found only in Gram-negative bacteria. The capsule is an amorphous secretion that diffuses into surrounding medium as a loose semi-ridged slime layer. This makes up the global glycocalyx. Capsules are a virulence factor, which serve multiple purposes during infection, including; protection from desiccation, shielding of surface antigens from complement deposition and prevention of interaction of surface-associated immunoglobulins with immune cells.

Therefore, in comparison, *O*-antigens are covalently attached to the bacteria while the capsule polysaccharides (CPSs) are not, are instead found in the slime layer. Accordingly, *O*-antigens are the superior choice of target vaccine development, promoting immune response towards the bacterial cell and not its capsule.

Structure of the Morganella morganii zwitterionic polysaccharide (MM-ZPS)

NMR analysis, performed by the Young lab, provided the chemical structure of the MM-ZPS.[8] Their findings determined that the MM-ZPS repeating unit was a trisaccharide composed of a D-GalN, D-Gal, and a D-GalNAc. In addition, the trisaccharide repeating unit contains two phosphate residues, one being a phosphocholine residue and the other being a phosphoglycerol residue which serves as a bridge between the repeating units. The MM-ZPS repeat structure continues by extending through the primary alcohol of the C4' phosphoglycerol arm affording the structure of: α-D-Gal*p*N-(1→3)-β-D-Gal*p*-(1→3)-β-Gal*p*NAc-(1→3)-glycerol (Figure 29). The structure of the reported MM-ZPS contains four charged groups (two positive and two negative) across the three monosaccharide

resides of the repeating unit. This represents, a much higher charge per sugar density than the heavily studied *B. fragilis* PS A1 ZPS which only contains two charged groups across the four monosaccharide residues of the repeating unit.

Part 2. MM-ZPS 1st-generation route retrosynthesis

We began by selecting **175** as the initial synthetic target, a representative repeating unit of the greater MM-ZPS polymer **174** (Figure 29). This was due to the level of synthetic challenge that would arise from the congested, tetra-substituted, central D-Gal residue. In fact, the central D-Gal residue is tetra-substituted, which is rare among oligosaccharides.[152] D-galactopyranosyl residues contain a minimum of three substituents syn to one another on the β-plane of the sugar

The repeating unit **175** was also an attractive synthetic target because it contained a continuous trisaccharide core rather than a repeating unit frame composed of a disaccharide and monosaccharide linked by a phosphoglycerol bridge.

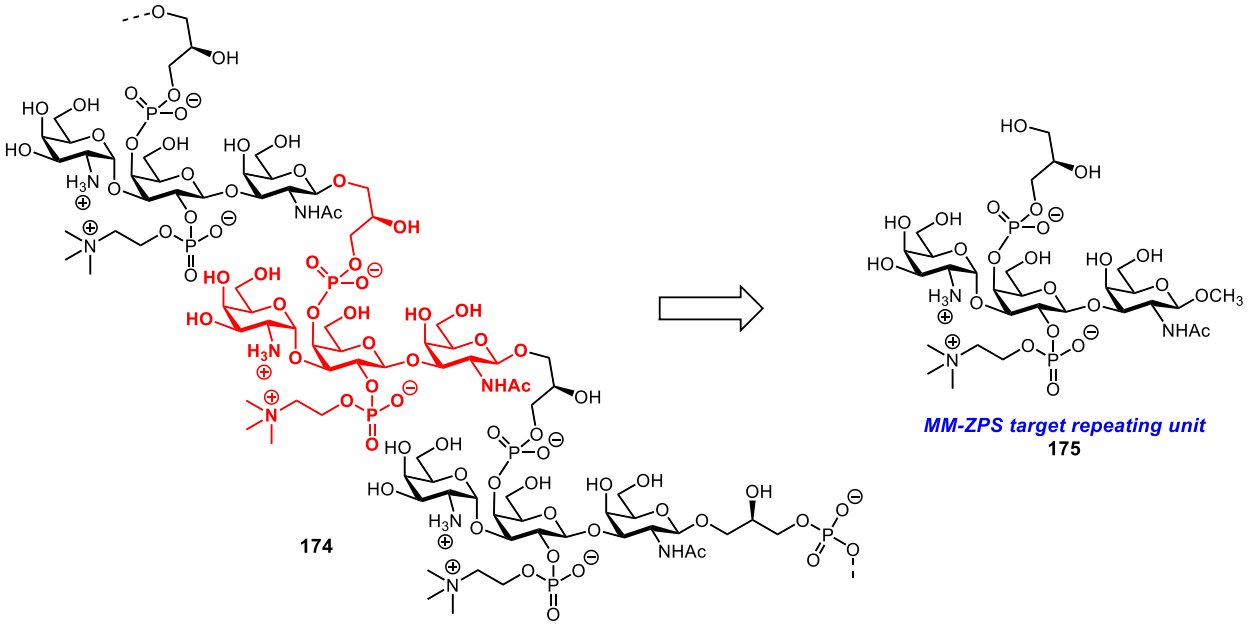


Figure 29. MM-ZPS synthetic target selection

Retrosynthetic analysis of the target MM-ZPS repeating unit trisaccharide **175**, revealed that the disconnection of the phosphocholine arm first, following global deprotection, could be made between trisaccharide **176** and phosphoramidite **177** (Figure 30 disconnection *i*). Phosphocholine moieties are charged structures which can cause many difficulties for chemical synthesis. Therefore, we elected to install the zwitterionic moiety late in the synthesis. Next, disconnection to complete the core trisaccharide by forming the D-Gal-β-(1→3)-D-GalNAc glycosidic linkage **176** was envisioned to arise from a ring opening event between the known D-GalNAc acceptor **179** and an intermediate epoxide formed between the C1 and C2 of the D-galactal disaccharide **178** (Figure 30, disconnection *ii*). In theory, the ring opening event would result in only a β-glycosidic linkage via an S_N2 pathway and then provide the unprotected C2' hydroxyl to be used

directly in the following step. This provides a clear advantage over alternative glycosylation strategy which may require a deprotection step after the initial glycosylation event. The epoxide ring opening would be facilitated by the D-GalNAc acceptor **179**, because it contained the acetamide required in the final product **175**. It is important to mention that the final product **175** contains a second amine functionality (C2''-NH_3^+) which must remain in a free amine state relative to the acetylated amine at the C2 position. Differentiating between the C2 and C2'' amines was a constant consideration during the planning, and execution, of the synthetic route. In addition, acceptor **179** was capped at the anomeric position with either an -OCH_3 or an -O*i*Pr alkyl group to prevent mutarotation of the reducing sugar. While the addition of the *O*-alkyl capping group may not be present in the native ZPS repeating unit, capping of the reducing end sugar has been incorporated in all previously published synthesis of ZPSs due to the final product existing as a mixture of isomers resulting from mutarotation of the free reducing sugar, which would exist in an equilibrium of products: linear, alpha furanosyl, beta furanosyl, alpha pyranosyl, beta pyranosyl.

Returning to the retrosynthetic analysis, disconnection of the phosphate ester in disaccharide **178** gave the known phosphoramidite **181** and the free axial C4-OH disaccharide **180** (Figure 30, disconnection *iii*).

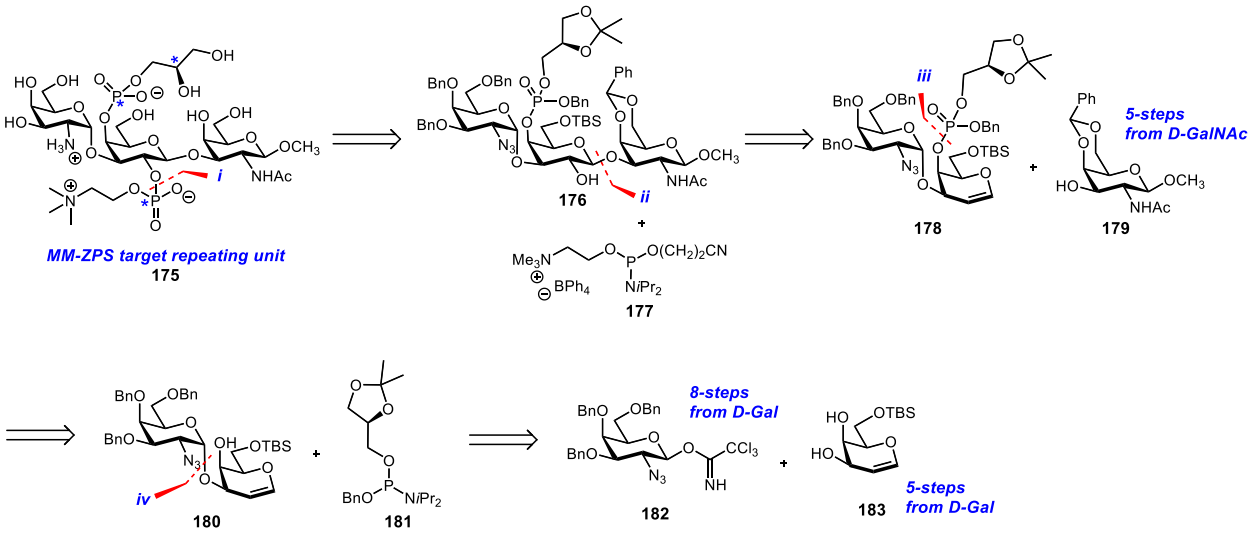


Figure 30. Figure 30. First-generation retrosynthetic analysis

It is important to note that the formation of the protected phosphate ester **181** will give rise to a pair of diastereomers which contain a chiral center at the newly formed phosphate. We reasoned that we could carry the diastereomeric pair of phosphates through the synthetic route where they would resolve into a single achiral phosphate following deprotection to afford the final product **175**.

The disconnection of the D-GalN_3-α-(1→3)-D-Gal glycosidic linkage in **180** would afford the known building blocks; trichloroacetimidate donor **182** and the D-galactal acceptor **183** which could both be derived from inexpensive and readily available D-galactose (Figure 30, disconnection *iv*). While it is generally not recommended to perform glycosylations with diols, in fear of getting a mixture of many possible products, the

glycosylation between donor **182** and acceptor **183** was planned to leverage the superior nucleophilicity of the allylic, pseudo equatorial, C3-OH over the pseudo-axial C4-OH.

Part 3. MM-ZPS 1st-generation synthetic route

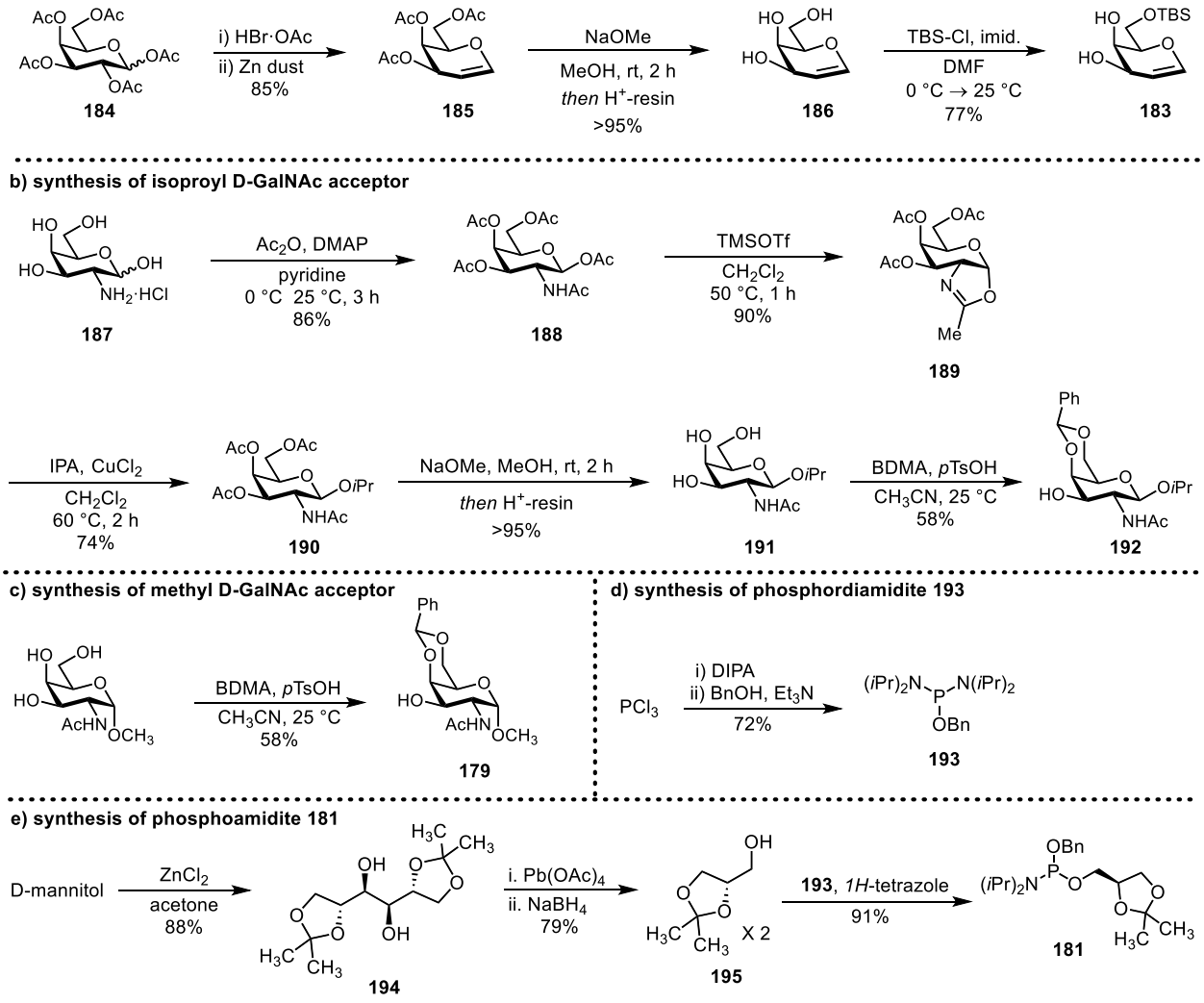
Synthetic work began with a sequence used to generate the first required building block, D-galactal **183** (Scheme 22. route a). Bromination and zinc mediated reductive elimination of the commercially available, and inexpensive, D-galactose pentaacetate **184** gave the D-galactal **185**. Saponification of triacetate **185** affords the triol **186** cleanly in quantitative yield. Selective silylation of the C6 primary alcohol of **186** gave the anhydro sugar **183**. Multi gram quantities of the first required building block, 6-*O*-*tert*-butyldimethylsilyl-D-Galactal **183**, was successfully synthesized utilizing this four-step procedure in a 62% overall yield.

The next building block to be synthesized was the reducing-end D-GalNAc acceptors **192** and **179** (Scheme 22, part b and c). To access the D-GalNAc acceptor **192**, acylation of D-galactosamine·HCl **187** gave the peracetylated D-GalNAc residue **188** (Scheme 22, route b). Acid promoted anchimeric assistance of the acetamide **188** gave oxazolidinone **189**. Opening of the oxazolidinone ring with isopropyl alcohol gave the isopropyl β-D-GalNAc **190** in a highly stereoselective fashion. Saponification of **190** gave the triol **191** which underwent acetal formation to afford the 4,6-*O*-benzylidene acetal **192** in decent yield on gram scales. In addition, the C1-methyl capped D-GalNAc acceptor **179** was synthesized in one step from commercially available material (Scheme 22, route c). With the acceptor D-GalNAc acceptor building blocks completed we moved onto synthesis of the glycerol-phosphate building block.

Synthesis of the phosphoramidite building block **193**, commenced by treatment of a solution of phosphorus oxychloride with diisopropyl amine to followed by the addition of benzyl alcohol to give phosphordiamidite **193** in good yield (Scheme 22, route d). The

protected glycerol is derived from D-mannitol which was initially protected at a diacetonide in the presence of zinc chloride to give diol **194**. The diacetonide **194** is then oxidatively cleaved using lead tetraacetate followed by a reduction using sodium borohydride of the resulting aldehydes to give two equivalence of alcohol **195** (Scheme 22, route e).

Scheme 22. Synthesis of MM-ZPS route 1 building blocks

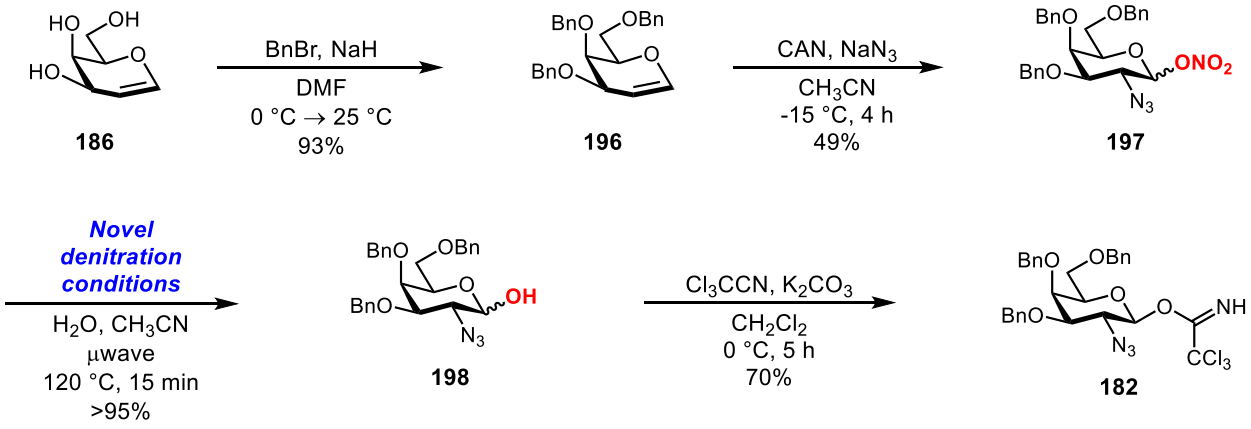


Utilizing tetrazole mediated conditions, the phosphordiamidite **193** combined with alcohol **195** gave the phosphoramidite building block **181** in excellent yield as an extremely non-polar oil which could be purified by base treated silica gel when using pure hexanes as

an eluent. As a note, phosphoramidite **181** is prone to oxidation and hydrolysis, complicating reaction monitoring and purification. Use of a *Combi Flash Rf* equipped with ELSD and a UV-vis detector was found to aid in identification of product positive fractions following expedited chromatography purification. Fractions which had appeared to contain material by ELSD and/or UV-vis where subjected to ^{31}P NMR analysis. The fractions containing a clean phosphorus signal at 149.37 ppm and 149.28 ppm were combined and concentrated down to afford the final phosphoramidite product **181**.

The final building block required for the synthesis towards **175** was the D-GalN_3 imidate building block **182** (Scheme 23). Benzylation of the previously described triol **186** afforded 3,4,6-tri-*O*-benzyl-D-galactal **196** in good yield. Azidonitration of D-galactal **196** gave nitrate ester **197** in modest yield. This reaction was complicated by CAN mediated de-benzylation of the starting material and product. Denitration of **197** was conducted by carrying out conditions developed in our lab utilizing aqueous organic solvent and microwave irradiation to give hemiacetal **198**.[153] Treatment of **198** under potassium carbonate mediated conditions in the presence of trichloro acetonitrile gave the β trichloroacetimidate **182**.

Scheme 23. synthesis of trichloroacetimidate donor 182



Conditions to facilitate the glycosylation between trichloroacetimidate donor **182** and C6-*O*-TBS protected D-galactal acceptor **183** were explored. First, triflic acid was examined as a glycosylation promotor to give disaccharide **180**, though gave only modest yields (Scheme 24, entry 1). It was found that the reaction would proceed to completion (consumption of starting material donor material **182**) while maintaining reduced temperatures and did not require warming to room temperature (entry 2). In addition, improved yields were obtained when the reaction was carried out below 25 °C. A similar trend was recognized when using TMSOTf as a promotor as well (entries 3 and 4), however TMSOTf provided a reversal in stereoselectivity, favoring the undesired β product. Presumably TMSOTf mediated conditions proceeds though an alpha triflate intermediate allowing for formation of the β product. A screen of alternative Lewis acid promotors found $BF_3{\cdot}Et_2O$ performed far superior to others. At elevated temperatures, increased amounts of decompositions products were observed (entry 6), however, at lower temperature yield of the desired product **180** improved (entry 7). The use of participating solvents where added to investigated for their ability to influence stereoselectivity in the reaction (entries 8-11).

Scheme 24. MM-ZPS synthetic route 1 continued

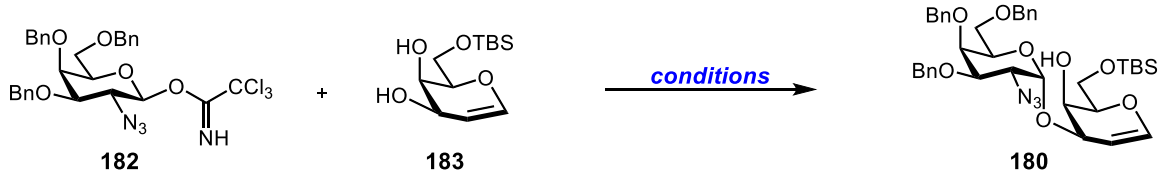


Table 2. Reaction conditions for 180

conditions

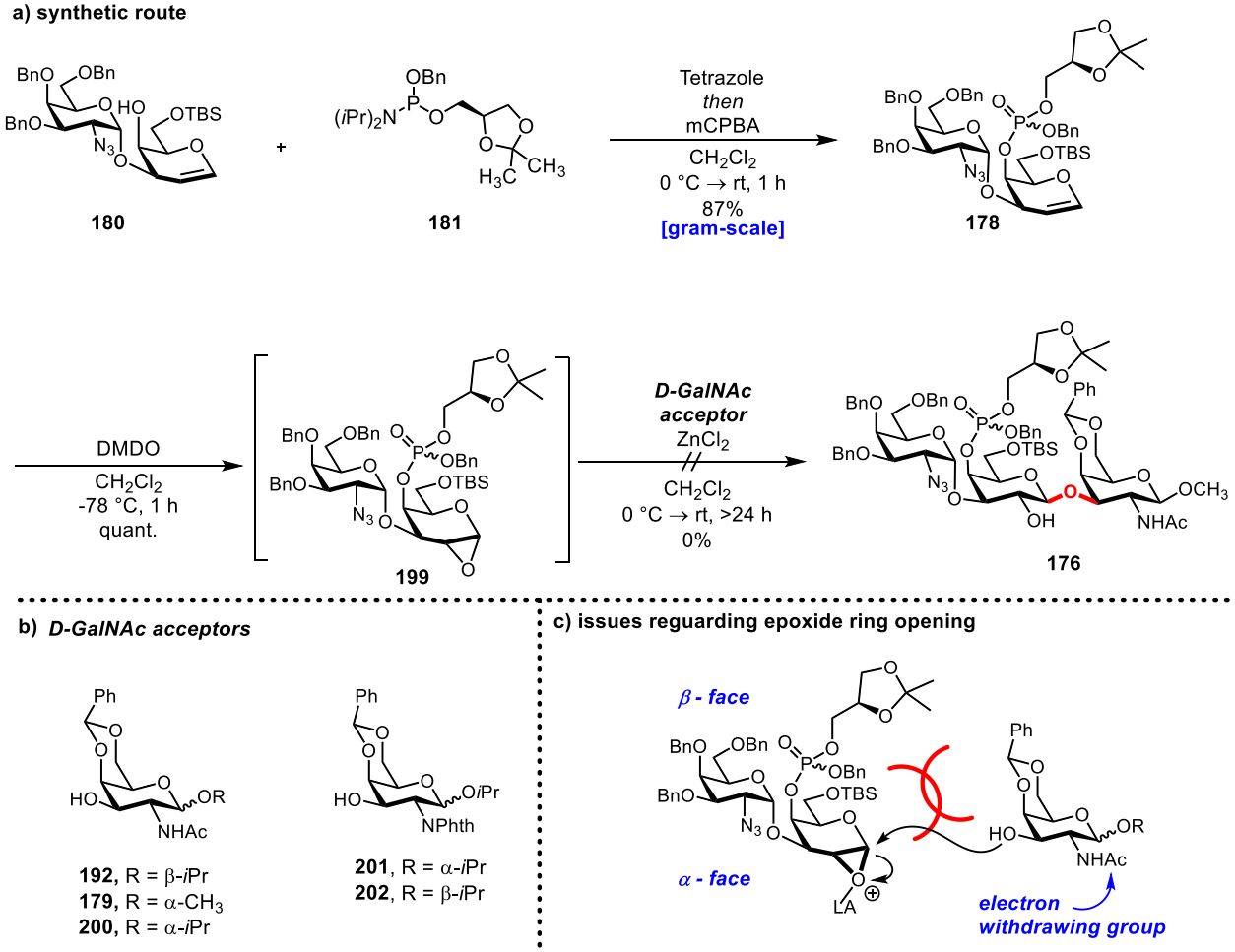
entry	*promotor*	*solvent*	*temperature (°C)*	time	*α/β ratio*	*yield*	
1	TfOH	CH_2Cl_2	-78 → 25	2 h	1.2:1	37	
2	TfOH	CH_2Cl_2	-78 → -40	4 h	1.5:1	43	
3	TMSOTf	CH_2Cl_2	-78 → 25	3 h	1:2	39	
4	TMSOTf	CH_2Cl_2	-78 → -20	1 h	1:2.4	47	
5	$SnCl_4$	CH_2Cl_2	-30 → -20	1 h	nd	0	
6	$BF_3•Et_2O$	CH_2Cl_2	-78 → 25	1 h	1.5:1	29	
7	$BF_3•Et_2O$	CH_2Cl_2	-78 → -55	3 h	1.4:1	56	
8	$BF_3•Et_2O$	CH_2Cl_2/Et_2O (1:1)	-78 → -55	3 h	1.8:1	66	
9	$BF_3•Et_2O$	CH_2Cl_2/iPr_2O (1:1)	-78 → -55	3 h	2.4:1	59	
10	$BF_3•Et_2O$	CH_2Cl_2/iPr_2O (1:1)	-40	1 h	3:1	65	
11	**$BF_3•Et_2O$**	**CH_2Cl_2/iPr_2O (2:1)**	**-40**	**1 h**	**3:1**	**77**	**[gram-scale]**

In general, ethereal solvents undergo solvent participation with the oxonium intermediate by coordination to the β face preferentially, due to the reverse anomeric effect, allowing for a nucleophilic attack by an acceptor at the α face.[111] Adding diethyl ether slightly improved the stereoselective outcome of the reaction (entry 7 vs. 8), however using the bulkier, diisopropyl ether performed far superior to diethyl ether, providing a 3:1 α/β ratio in a 65% yield (entry 10). These conditions were seen to be compatible with the reaction even on gram-scales, providing a 3:1 α/β ratio in a 77% yield (entry 11).

The synthetic route continued with installation of the phosphoglycerol motif using phosphoramidite **181** in a tetrazole mediated process (Scheme 25). The intermediate phosphorus (III) species was subsequently oxidized with *m*CPBA to provide an inseparable diastereomeric mixture of phosphate esters **178** in 87% over two steps (Scheme 25, part a). The diastereomeric mixture convoluted the NMR data through ^{31}P nuclei splitting of the protons and carbons. The diastereomeric mixture of phosphate **178** was carried forward by epoxidation of the glycal with DMDO to provide intermediate epoxide **199**. Initial attempts to simultaneously oxidize the phosphorus (III) center and the C1-C2 olefin led to significant decomposition, and thus it was elected to use a step wise oxidation procedure.

With **199** in hand, our plan was to engage the epoxide with the rigid D-GalNAc acceptor **179** to generate trisaccharide **176**. Despite our best efforts, synthetically useful quantities of **176** could not be obtained through this procedure. Rigorously anhydrous conditions and prolonged reaction times also failed to improve the yields of this glycosylation.

Scheme 25. MM-ZPS synthetic route 1 continued



As a remedy to this problem, we aimed to improve the nucleophilicity of the C3 alcohol by differing the nitrogen protecting groups. Acceptors **201**, **192**, **202** and **200** were synthesized and attempts were made with them in the ring opening reaction, however, provided no improvement (Scheme 25, part b). We hypothesized that these results were due to steric or electronic factors regarding the poor nucleophilicity of the C3 hydroxyl of the D-GalNAc acceptors. Precedent for C2-N-acyl or C2-NPhth D-GalNAc acceptors being poor glycosyl acceptors can easily be found in literature[154-167] and explicitly reported by Crich in a publication aptly titled "*Why are the hydroxy groups of partially protected N-acetylglucosamine derivatives such poor glycosyl acceptors, and what can be done about*

it? A comparative study of the reactivity of N-acetyl-, N-phthalimido-, and 2-azido-2-deoxy-glucosamine derivatives in glycosylation. 2-Picolinyl ethers as reactivity-enhancing replacements for benzyl ethers".[155] In addition to Crich's work, others report methods for establishing the D-Gal-β-(1→3)-D-GalNAc glycosidic bond in literature; the most successful methods include protected the C2-nitrogen of the D-galactosamine accepter as C2-N_3.[168-174] Unfortunately, this method would not be amenable to our current synthetic route due to incompatibility in subsequent steps of the route. Although, this strategy will be leveraged in following versions of the synthetic route (part 5 and 6). Other impeding factors hypothesized to impede the epoxide ring opening of glycal **199** included the large bulky substituents of the D-Gal residue blocking the β-face of the molecule. The phosphate ester, C6-*O*-TBS, and 3,4,6-tri-*O*-benzyl-2-deoxy-2-azido-D-galactoside all blocked the β-face of **199** which could impede the approach of the incoming C3-OH nucleophile of the acceptor which also needs to approach from the β-face of the molecule to establish the β glycosidic linkage (Scheme 25, part c).

With access to grams of galactal disaccharide **178** in hand, attempts were made to salvage the material and synthetic route by converting **178** into alternative glycosyl donors which could be used to obtain the D-Gal-β-(1→3)-D-GalNAc glycosidic bond. Though a three-step procedure, transformation into a C2-protected thioglycosides could be achieved. These steps included; 1) epoxidation of the anhydro sugar, 2) epoxide ring opening by a thiol, 3) protection of the newly formed C2-OH. This concession step was thought to be advantageous for the stereochemical outcome of the reaction. Acyl groups are known to provide good anchimeric assistance, resulting in a desired β-glycosidic bond. This phenomenon is also referred to as neighboring group participation (NGP). This

is contrary to the first glycosidic bond formed in the route which used a non-participating group (C2-azide) to form an α-glycosidic bond (*vide supra*).

Scheme 26. MM-ZPS route 1 alternative glycosylation attemept

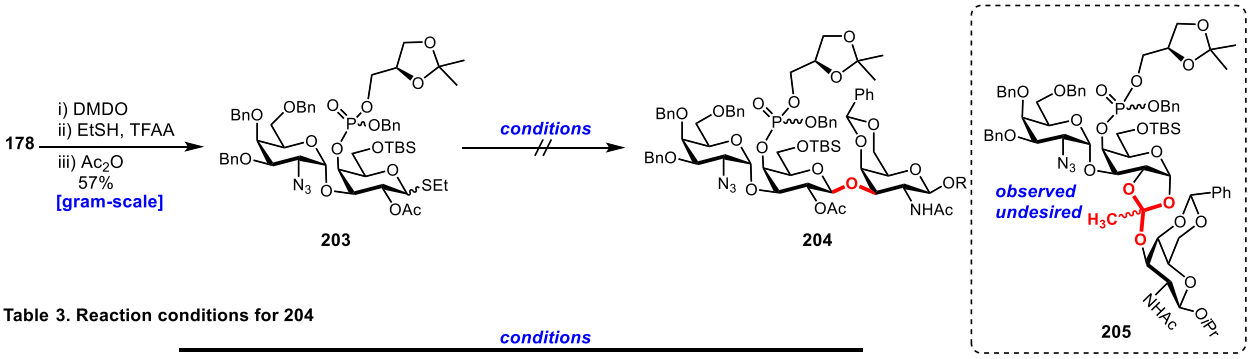


Table 3. Reaction conditions for 204

conditions

entry	acceptor	*promotor*	*solvent*	*temperature (°C)*	time
1	**192**	NIS, AgOTf	CH_2Cl_2	-40 → 25	12 h
2	**192**	NIS, TMSOTf	CH_2Cl_2	-40	6 h
3	**179**	NIS, TMSOTf	CH_2Cl_2	-40	3 h
4	**179**	NIS, TfOH	CH_2Cl_2	-78 → 25	1 h

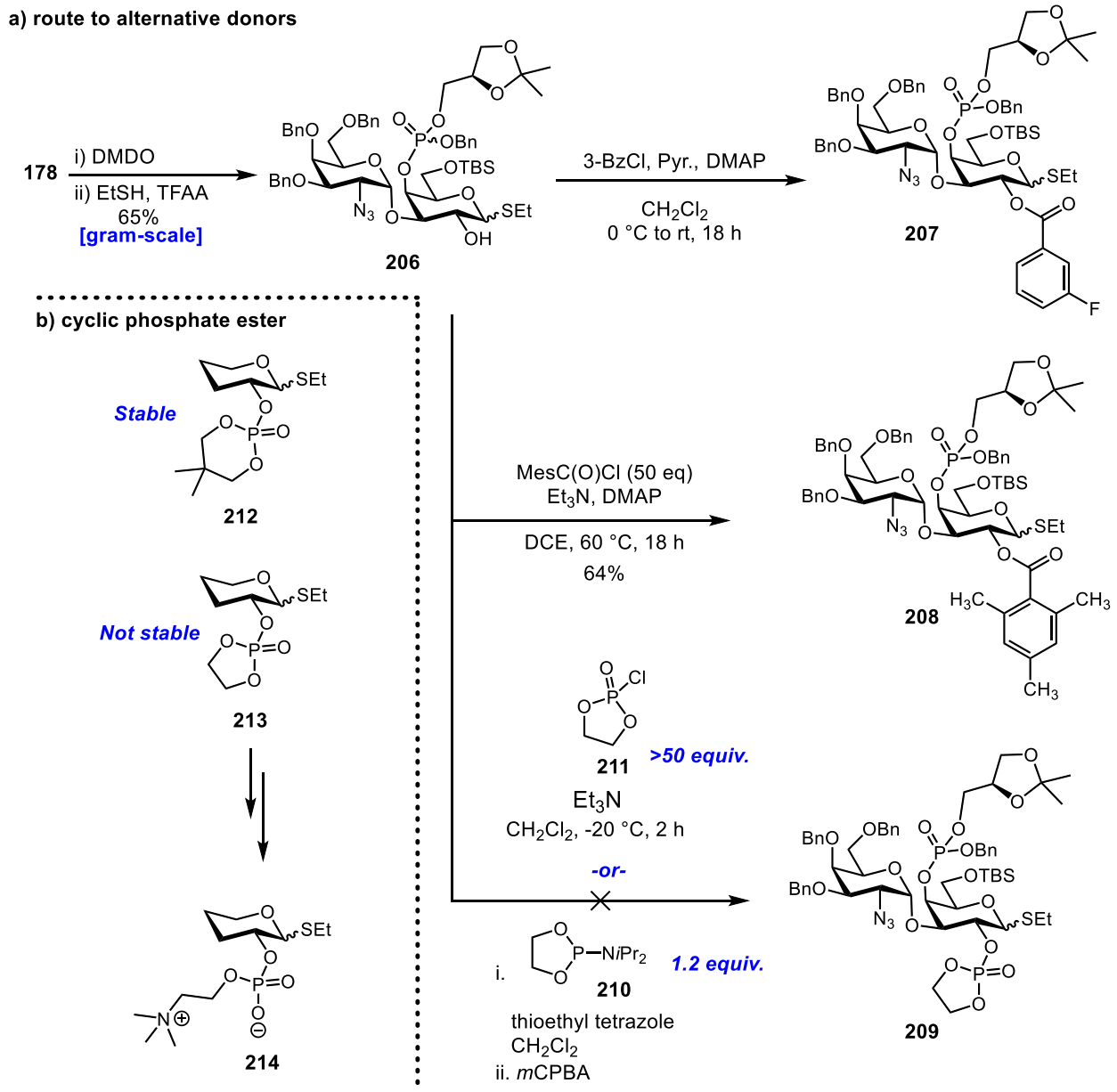
Disaccharide galactal **178** was transformed into its corresponding thioglycoside after oxidation with DMDO and subsequent treatment with ethanethiol in the presence of trifluoro acetic anhydride (Scheme 26). Next, the C2 alcohol was acetylated under standard conditions to give thioglycoside **203** in 57% yield over 3 steps.

Attempts of glycosylation with thioglycoside donor **203** was reacted with the D-galactosamine acceptor **192**, it failed to produce the desired trisaccharide **204**. Instead, the undesired orthoacetate **205** was isolated in 80% yield (Scheme 26, entry 1). Attempting this glycosylation again with a TMSOTf/NIS promotor system, which has been reported to limit ortho-ester formation,[175-177] also gave quantitative amounts of ortho-ester product. An alternative glycosyl acceptor (**179**) was then used. Unfortunately TMSOTf and TfOH as promotor systems only provided ortho-ester formation and decomposition under prolonged reaction times and elevated temperature (Scheme 26, entry 3 and 4).[176-

[177] As a note, purification of the orthoacetate product was complicated by hydrolysis which occurred on TLC plates or silica gel columns. Hydrolysis would inevitably result in a reducing sugar and recovered acceptor starting material. Material that was successfully purified provided a characteristic orthoacetate-associated signal at 124 ppm, appeared by ^{13}C NMR. In addition, chemical identification was carried out by treatment of the orthoacetate product with sodium methoxide over long reaction time (2 days), resulting in no loss of acetate methyl peaks or the indicative orthoacetate peak. In contrast, exposure for short reaction times (>1 h) of mildly acidic methanolic conditions resulted in hydrolysis and recovery of starting acceptor.

Based on literature precedence, we hypothesized the orthoacetate could be rearranged under Kochetkov conditions to trisaccharide.[178] However, exposure of orthoacetate **205** to acidic media resulted in decomposition over prolonged reaction times and hydrolysis if conditions were not meticulously anhydrous .

Scheme 27. MM-ZPS route 1 alternative disaccharide donors



We attempted to employ C2 participating groups reported to mitigate orthoacetate formation while still providing β-linkages through anchimeric assistance. The galactal **178** was converted to the thioglycoside **206**. The thioglycoside **206** was converted to the C2-3-fluorobenzoyl protected triglyceride **207** in 83% yield (Scheme 27 part a).[179] The C2-OH thioglycoside was also protected *via* acylation with a mesitylene acyl chloride to afford

thioglycoside **208**.[180] While this product was achieved, it required extreme excess of mesitylene acyl chloride and super stoichiometric quantities of DMAP to drive complete conversion. With this being the case, we decided to not move forward with this potential donor. A third option for a disaccharide donor included the cyclic phosphate protected **209**.[181] Cyclic phosphates, of the type **212**, are known to be, stable, adequate protecting groups which can undergo NGP to afford β-glycosidic linkages (Scheme 27, part b). On this basis, we speculated a convergent strategy that could incorporate the five-membered cyclic phosphate protecting group (shown in **213**) which could be treated with trimethylamine to afford the desired phosphocholine residue (shown in **214**) and found in the desired product. First, we attempted to install the cyclic phosphate to alcohol **206** by treatment with ethylene glycol chlorophosphate **211** under triethylamine mediated conditions, but it was found that extreme excess of **211** was required to fully consume the starting material disaccharide **206**. Using the ethylene glycol phosphoramidite **210** under thioethyl tetrazole mediated conditions followed by oxidation of the cyclic phosphate **209**, was much more successful. Unfortunately, when purification of each of these reactions by silica gel flash column chromatography was attempted the products quickly decomposed. Small amounts of material were obtained by filtration though a plug of Celite. When analyzed by ^{31}P NMR the characteristic shift of a five-membered cyclic phosphate appeared in the material obtained from filtration. However, in only a matter of hours, the same NMR sample showed a disappearance of the cyclic ^{31}P phosphorous peak in exchange for an upfield shift indicative a linear phosphate. These observations were supported by other literature findings regarding the decomposition/hydrolysis rates of five-, six-, seven-, and eight- membered cyclic phosphates were discovered.[182] It is

reported that five-membered cyclic phosphates (such as **213**) rapidly undergo decomposition and are unable to be isolated, agreeing with our anecdotal findings.

Moving forward, glycosylation of with the C2-O-*m*FBz protected thioglycoside **207** were attempted. Surprisingly, glycosylation event between **207** and the glycosyl acceptor **200** resulted primarily in the production of the unstable ortho-ester as well (Scheme 28 entry 1).

Scheme 28. MM-ZPS route 1 alternative glycosylation attemept continued

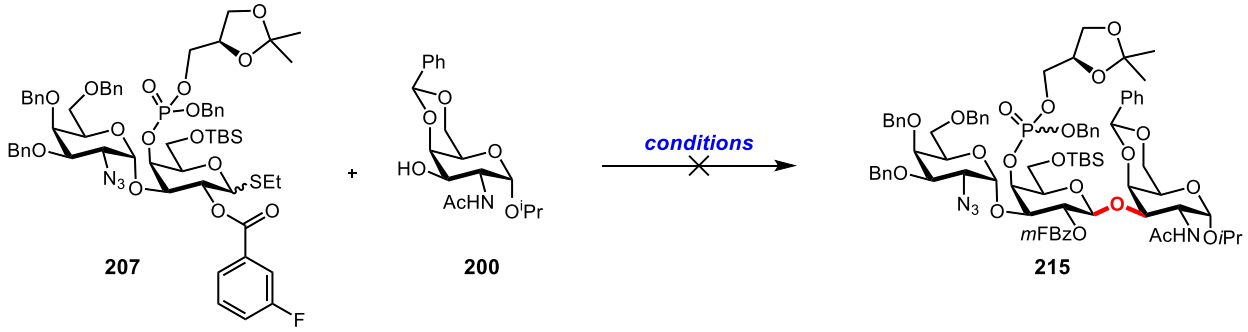


Table 4. Reaction conditions for 215

		conditions		
entry	***promotor***	***solvent***	***temperature (°C)***	**time**
1	NIS,TfOH	CH_2Cl_2	-78 → 25	5 h
2	Br_2 *then* AgOTf	CH_2Cl_2	0 *then* -50	18 h
3	Br_2 *then* $Hg(CN)_2$	CH_2Cl_2	-50	18 h
4	Br_2, $Hg(CN)_2$	CH_2Cl_2	-78 → 25	12 h
5	Br_2, AgOTf	CH_2Cl_2	-78 → 25	12 h

An alternative approach to installing the D-Gal-β-(1→3)-D-GalNAc glycosidic bond is to use Helferich glycosylation conditions.[183] These conditions require activation of glycosyl halides with mercury salts in toluene/nitromethane mixed solvent systems. Attempts were made to convert thioglycoside **207** to its glycosyl bromide. Unfortunately, both, *in situ* and

stepwise approaches to the anomeric halide resulted in loss of the C6-*O*-TBS protecting group and decomposition of the disaccharide (Scheme 28, entry 2-5).[184]

At this stage we concluded this motif was inherently prone to orthoacetate formation. We hypothesize the difficulties encountered during these glycosylation reactions are likely due to distortion of the galactoside donor. Conformational analysis suggests that rather than existing as its $^4C_1$ chair conformer **216**, the reducing end galactose residue likely undergoes a ring-flip and sits in a $^1C_4$ conformation as illustrated in conformer **217** (Figure 31).

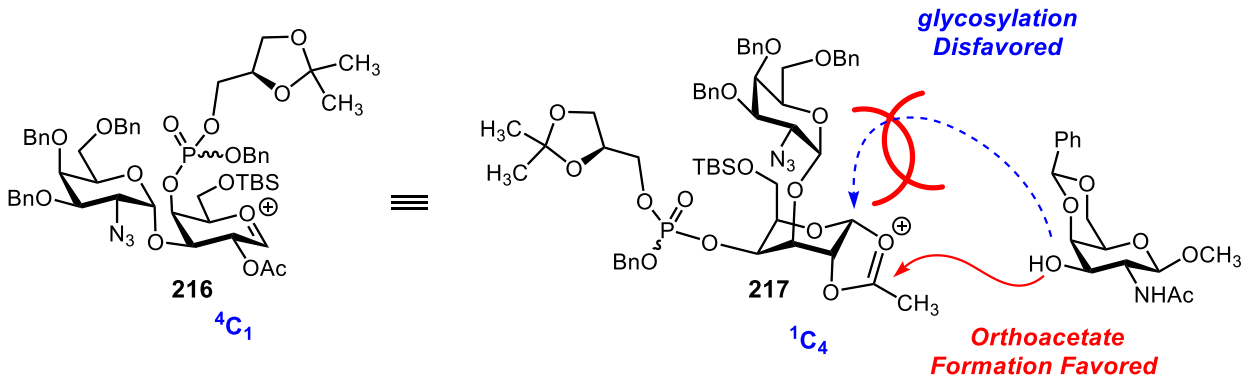


Figure 31. Conformational analysis of the oxonium/bicyclic oxocarbenium ion

This preference for the reactive intermediates to reside in a $^1C_4$ conformation leads to favorable attack at the dioxolenium carbonyl and disfavors approach at anomeric center by a transitory 1,3-diaxial interaction with the substituent at C3 position

At this juncture, we decided it was best to redesign the synthetic route from scratch. Through our search into chemical literature, we discovered the D-Gal*p*N-α-(1→3)-D-Gal*p*-β-(1→3)-D-Gal*p*NAc trisaccharide core structure, found in the MM-ZPS **175**, is relatively unique in nature.[185] A search of the *Glycan Structures Database* and the *Carbohydrate Structure Database* for this trisaccharide core structure leads a relatively limited number of results (>25). In addition, we found when pertaining to the mammalian carbohydrates space, the abundance of D-GalNAc residues are very low, making up only 4.8% of the carbohydrates in the body.[185] Meanwhile, the core structure (**218**) of interest to us contains two D-GalNAc residues, making it an increasingly rarer glycosidic pattern. However, this rare trisaccharide is found in one common place; carriers of the blood group determinant A type III and IV **219** (Figure 32).[174, 186-187]

Figure 32. Structural comparison of MM-ZPS and blood group antigen type A III and IV

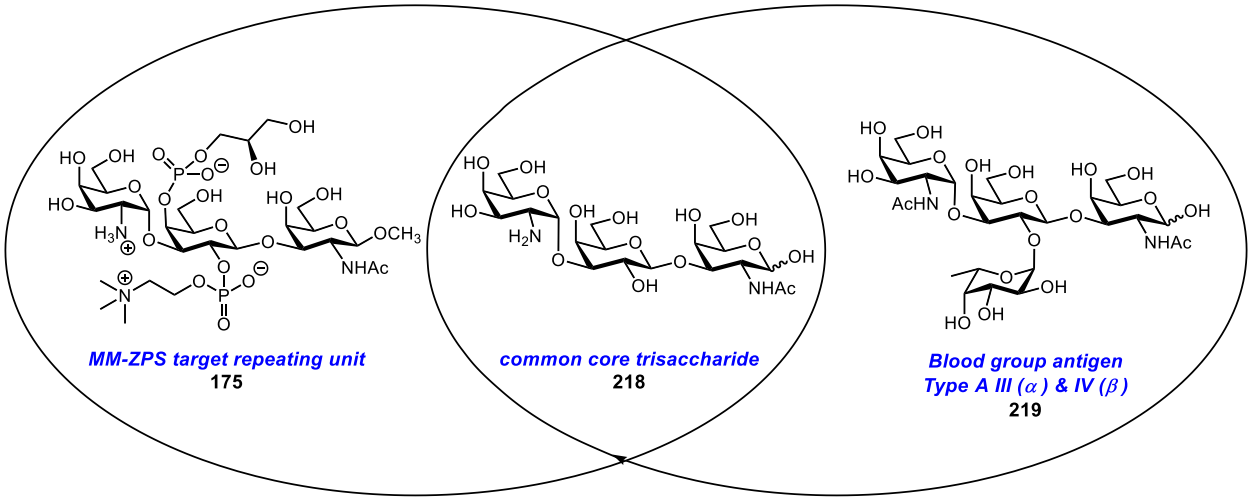


Figure 32. Structural comparison of MM-ZPS and blood group antigen type A III and IV

Since the discovery of ABO blood type determinants in 1900, synthetic efforts to construct the complete library of ABO antigens have been the focus for many groups.[168-174, 186-194] The blood group determinant A type III and IV was first completed by Bovin[172, 189, 192, 195] and co-workers then later by Ogawa[168] and co-workers and finally by Lowary[170-171] and co-workers. Each group successfully constructed the ABO antigen, which contains the D-Gal*p*NAc-α-(1→3)-D-Gal*p*-β-(1→3)-Gal*p*NAc trisaccharide core, with relatively simple building blocks. We were particularly inspired by the synthesis of Lowary and co-workers in 2011.[171] Retrosynthetic analysis performed by the Lowary group reveled that their target tetrasaccharide **219** would be achieved by first making the disconnection between the terminal non-reducing end D-GalN_3 residue **220** from the larger trisaccharide **221** (Figure 33, disconnection *i*).[171] Next the α-L-fucopyranoside disconnection was made to give the trichloroacetimidate **222** and D-Gal-β-(1→3)-D-GalN_3 disaccharide acceptor **223** (disconnection *ii*). The disaccharide **223** could be broken down into the imidate donor **224** and D-GalN_3 acceptor **225** (disconnection *iii*).

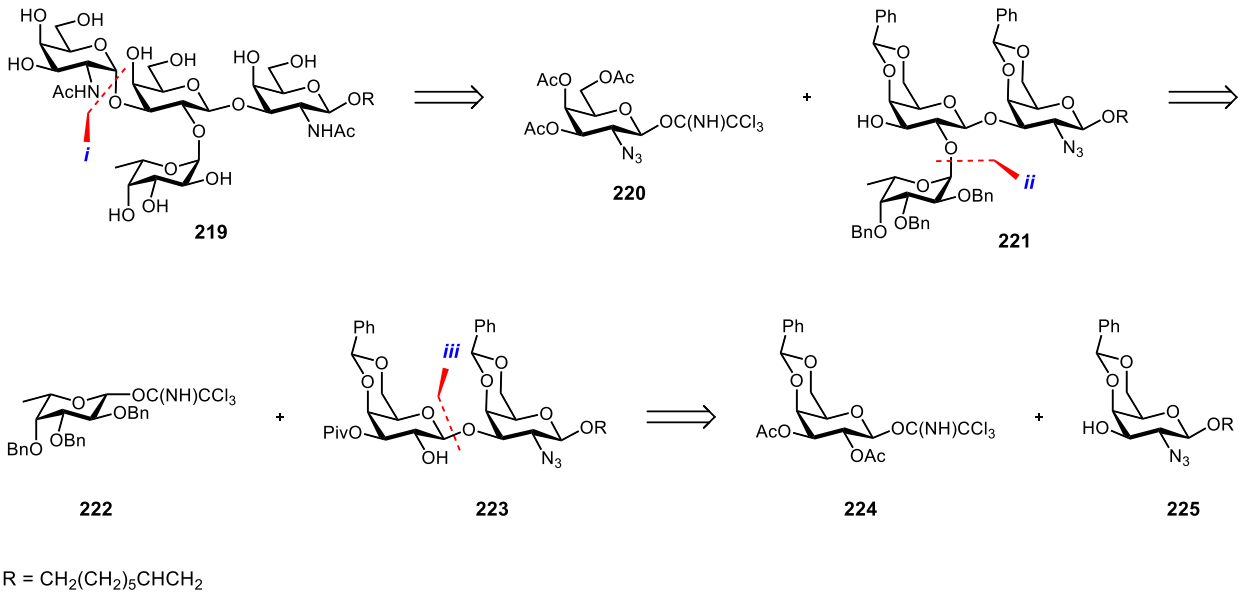


Figure 33. Retrosynthetic analysis of Lowary and co-workers synthesis of blood group antigen type A III and IV (2011)

When comparing what we had learned from our previous synthetic endeavors and studying the synthetic strategies employed from the synthesis of the blood group antigen A **219** by the Lowary's 2011synthesis, we identified three key lessons which we planned to incorporate in our *second-generation* route (part 5 and 6).

The first lesson being, we needed to establish the D-Gal*p*-β-(1→3)-Gal*p*NAc glycosidic linkage first. The most successful synthetic campaigns found in literature containing the D-Gal*p*N-α-(1→3)-D-Gal*p*-β-(1→3)-Gal*p*NAc trisaccharide core **218** established the D-Gal*p*-β-(1→3)-Gal*p*NAc glycosidic linkage first. We discovered that many groups

experienced difficulties when forming this bond, which resulted in primarily ortho-ester products or suffered low yields when using a terminal D-GalNAc residue.[157, 160-161, 163, 167]

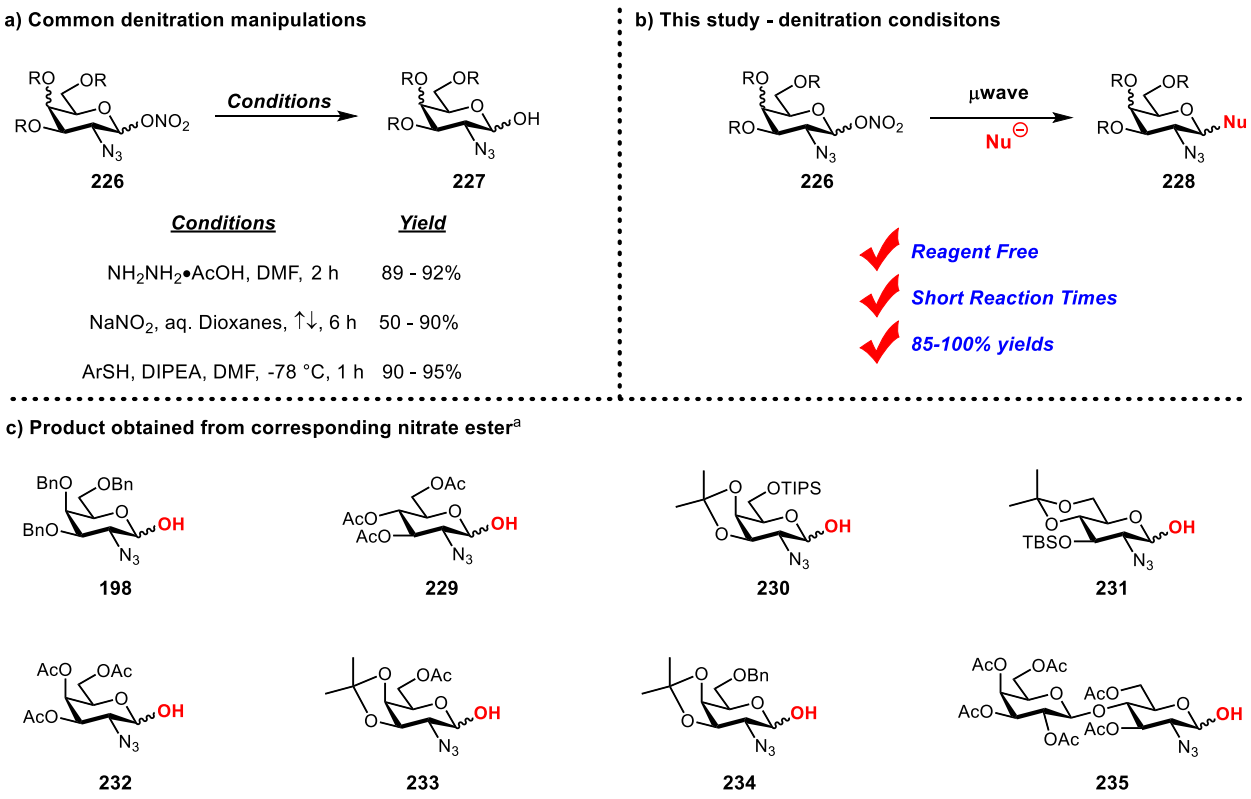
The second lesson regarded the use of a C2-azide for the D-GalNAc residue. The Lowary group successfully used an azide to mask the C2-NHAc of the terminal D-GalNAc residue which performed very well in this glycosylation (Figure 33, disconnection *iii*).[170-171, 194] Following the glycosylation events, a reduction and acylation and take place to afford the D-GalNAc residue. We plan to implement this strategy (use of a C2-N_3 D-galactosyl acceptor) into the design of our new synthetic route. For our applications, we needed to use a more complex protecting group strategy, than the one used by the Lowary group, for two reasons; 1) to facilitate the stepwise introduction of the phosphate moieties, 2) to retain differential acylation states between the two amino sugars (C2''-NH_3^+ vs. C2-NHAc).

The third general lesson we concluded from literature is to construct the molecule from the reducing end to the non-reducing end contrary to our first attempts. Carbohydrate synthesis where the molecule is elongated from the reducing end to the non-reducing end, is a powerful strategy that generally improves overall yield of the target molecule when multiple glycosylation events are required.

Part 4. Denitration methodology

During our synthetic work, on the *first-generation* route, we discovered several interesting findings which led to method development of novel denitration conditions for 2-deoxy-2-azido nitrate esters. The Lemieux azidonitration of protected *O*-glycals is a valuable tool for synthesizing 2-amino-2-deoxy sugars in which the newly installed amino group is masked as an azide.[196] However, the Lemieux procedure affords these 2-azido-2-deoxy

sugars in the form of an anomeric nitrate ester sugar. A variety of synthetic maneuvers are available to exchange the nitrate-ester for a useful reactive handle (Figure 34, a). Traditionally, the nitrate-ester is converted to a latent leaving group directly or via the intermediacy of an anomeric alcohol. We focused on manipulation of the nitrate-ester and commenced with the observation that converting the group to useful functionality is usually accomplished using strong nucleophiles under harsh reaction conditions. From a structural standpoint, we viewed the anomeric nitrate-ester as inherently unstable, which should render the group susceptible to reaction under milder conditions. Thus, we hypothesized direct nucleophilic substitution should be feasible if nitrate-esters are treated with nucleophiles at elevated temperatures (Figure 34, b).



a) 1:4 H_2O/acetone or 1:4 H_2O/MeCN, Et_3N or $NaHCO_3$, or pyridine (5.0 equiv.), 100 or 120 °C, 10 to 15 min, 86 to >95%

Figure 34. Direct, microwave-assisted substitution of anomeric nitrate-esters

This methodology began following as observation of the reagent free hydrolysis of a nitrate-ester upon sitting on silica gel of a TLC plate. We tested this observation by TLC analysis (and later full characterization) of spotting the nitrate ester 3,4,6-tri-*O*-benzyl-2-deoxy-2-azido-D-Gal **197** on a TLC plate which then sat for ~1 h. Afterwards, spotting fresh nitrate ester and the hemiacetal reducing sugar **198** followed by running the TLC plate revealed that the nitrate ester **197** originally spotted, which had time to sit on the TLC plate, had been completely consumed but now shared the polarity of the hemiacetal

product **198**. This discovery was quickly followed up by a range of studies involving the ability and hydrolysis rate of the nitrate esters by adsorption to silica gel. We found that armed sugars such as perbenzylated nitrate ester **197** would hydrolyze over hours when disarmed sugars such as the triacetate nitrate ester of **237** (nitrate ester 3,4,6-tri-*O*-acetyl -2-deoxy-2-azido-D-glucoside) would require days to fully hydrolyze (Figure 34, c). After determining that the silica gel was not inherently necessary for the process, we examined different conditions resulting in hydrolysis of the nitrate ester in wet organic solvents. Initially we became perplexed to why some substrates performed properly during the hydrolysis when others would result in significant decomposition. We quickly recognized the simple observation of reaction pH; the nitrate group was forming nitric acid causing the reactions to be extremely acidic leading to decomposition of acid labile substrates. This problem was quickly remedied by the addition of base to sequester the protons generated from the nitric acid. A variety of bases (Et_3N, DIPEA, $NaHCO_3$, NaOH, pyridine, DTBP) proved adequate, and regarding acid stable substrate, unnecessary. Ultimately, we developed a set of optimized general conditions; heating a nitrate ester to 100 to 120 °C by microwave irradiation in the presence of amine base in a 20% aqueous miscible organic solvent (acetone or acetonitrile) (Figure 34, c). Work-up is very simple, only requiring a wash with 1 N HCl to remove the added base in most cases. This is an advantage over previously described methods that require flash column chromatography to remove excess added reagents, such as aryl thiols.

For substrate scope we examined compatibility with the most common protecting groups used in carbohydrate synthesis (Figure 34, c). We started with examining the benzyl protected nitrate ester **197**, as previously mentioned, and then extended to the acetate.

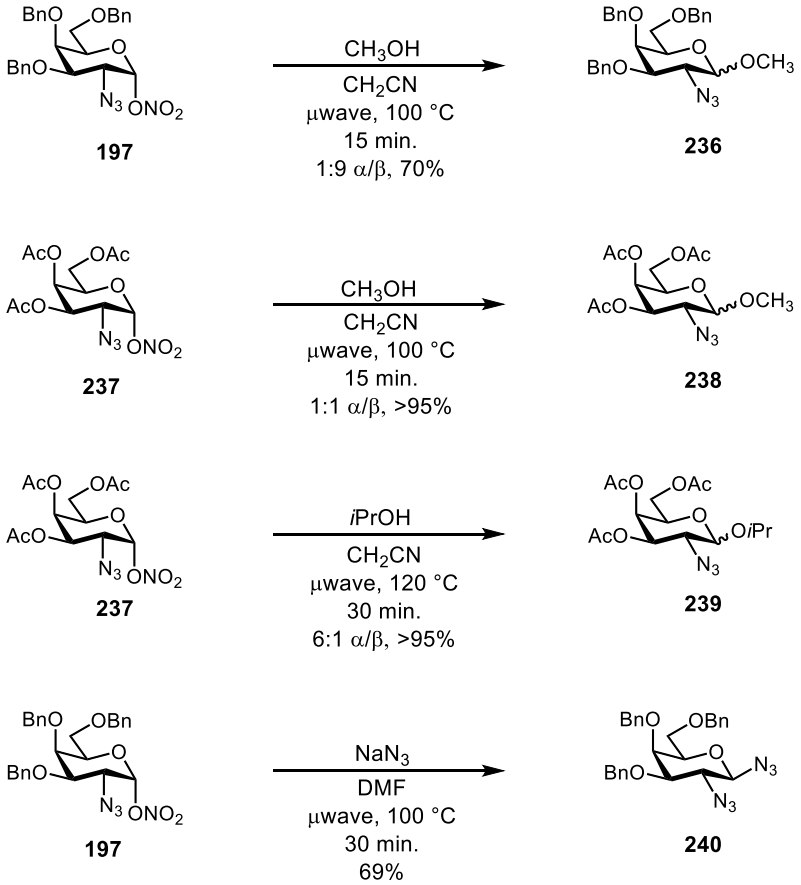
These derivatives (nitrate ester substrates of **229** and **232**) all performed well, given slightly longer reaction times, demonstrating no difference between D-Gal and D-Glu derivatives. To expand the utility of the reaction, we demonstrated that the conditions were amenable to acid-label protecting groups. We demonstrated this by using substrates which afforded the hemiacetal of **233**, **230**, **234** and **231**. In addition, the substrate which led to **231**, contained an acid-labile TBS group. In our hands it had no problem with our denitration conditions. To conclude the substrate scope, we prepared the nitrate ester 2-deoxy-2-azido peracetylated D-lactose which gave the product **235** under our denitration conditions, demonstrating that the acid labile glycosidic bond could withstand the reaction conditions.

The next segment of the program focused on using glycosyl acceptors as nucleophiles to study the direct glycosylation and glycosidation of the nitrate-ester. From the onset, it was our hope that a successful reaction between these substrates would produce a new glycosidic linkage, thereby enabling a nitrate-ester to serve as a latent leaving group in glycosylation chemistry. Unfortunately, thorough screening of this reaction never provided the glycosidic linkage in a reasonable yield. However, it was discovered that simple nucleophiles ran in high excess (>10 equiv.) could successfully undergo a glycosidation via attack at the anomeric carbon and displacement of the nitrate ester. Methanolysis of the perbenzylated nitrate ester **197** gave methyl 2-deoxy-2-azido D-galactoside **236** in excellent yield by proceeding primarily through a S_N2 type mechanism resulting in an inversion of stereochemistry in the product (Scheme 29). Methanolysis was then carried out on the peracylated substrate **237** to give methyl 2-deoxy-2-azido D-galactoside **238**

in good yield with a less stereoselective outcome reflecting a more S_N1 type mechanism. This is presumed to be due to its disarmed characteristic.

This trend was extended when examining the glycosidation between **237** and *iso*propyl alcohol to give **239** as almost exclusively an α product. Next, we examined nitrogenous nucleophiles. Sodium azide, as a hard nucleophile, and nitrate-ester **197** gave exclusively the S_N2 product of **240** with full conversion of stereochemistry. With these conditions, this method has been used to synthesize rare diamino sugars. Next, we felt it would be

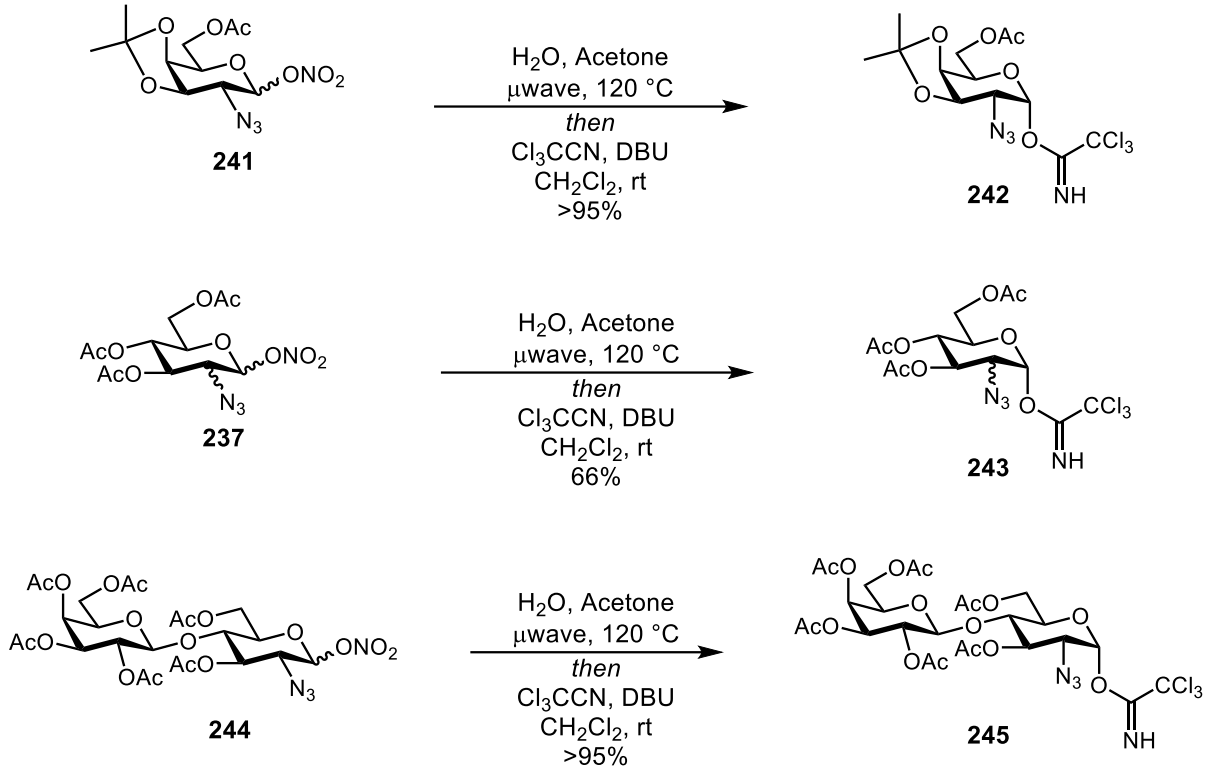
Scheme 29. one-pot microwave-assisted direct glycosidations of nitrate esters



advantageous to use the chemistry described above to improve on the conversion of a nitrate-ester to a trichloroacetimidate. Typically, this sequence is carried out in a two-step process requiring two individual chromatography purifications. Using microwave

mediated reaction conditions, this transformation can be achieved in quantitative yield using a one-pot procedure (Scheme 30).

Scheme 30. one-pot microwave-assisted conversion of anomeric nitrate-esters to trichloroacetimidate



While the second step of the reaction forms a biphasic solution, the addition of an amine base renders the product incapable of imidate hydrolysis. We demonstrated the direct nitrate hydrolysis followed by trichloroacetimidate formation in the conversion of substrates peracetylated substrates, acid labile substrates and disaccharides to their desired products (**242**, **243** and **245** respectively) from their nitrate-ester starting material all in acceptable yield and purity.

Part 5. MM-ZPS 2nd-generation route retrosynthesis

Highlighting some of the synthetic considerations we deemed necessary for the *second-generation* route, we planned a new retrosynthesis shown below (Figure 35). We envisioned the desired MM-ZPS repeating unit product **175** could come from the protected trisaccharide **246** after installation of the phosphocholine residue via a phosphocholine precursor type reagent such as **177** (Figure 35, disconnection *i*).

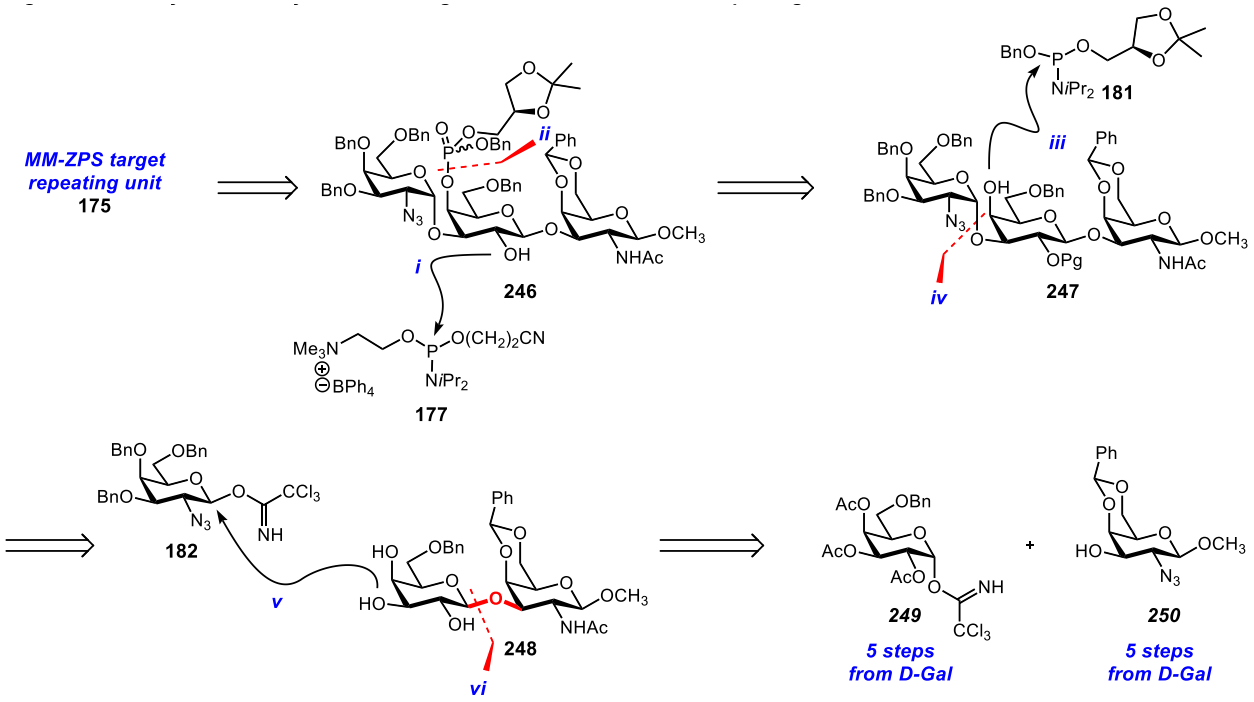


Figure 35. Retrosynthetic analysis of second-generation route to MM-ZPS repeating unit

With the C2'-OH protected, the phosphoglycerol residue disconnection would afford the core trisaccharide **247** and the phosphoramidite reagent **181** previously used in the *first-generation* route (Figure 35, disconnection *ii*). Installation of the phosphate ester residues at the late stage of synthesis, after the core trisaccharide was established would help deconvolute NMR data interpretations significantly as the protected molecule grows to a mass >1500 amu.

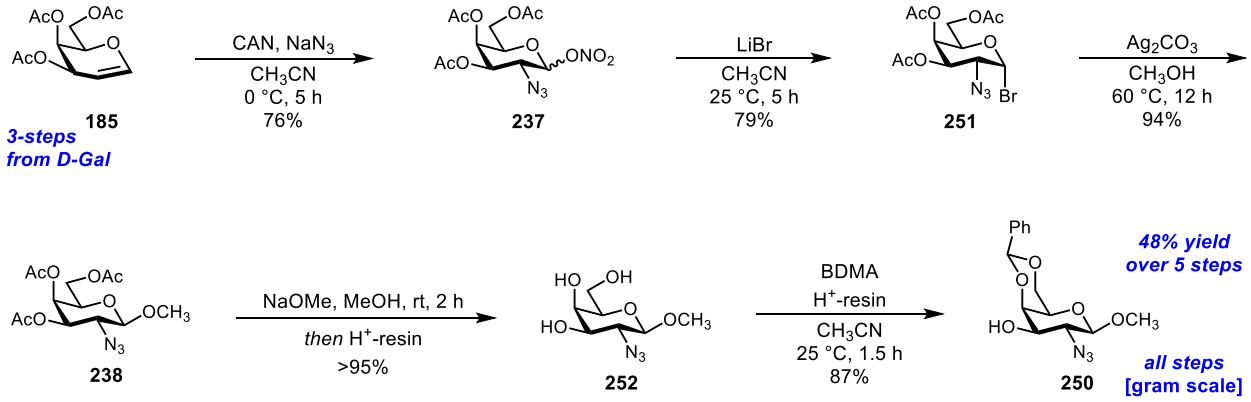
The core trisaccharide **247** could be completed by making the disconnection between the disaccharide glycosyl acceptor **248** and the trichloroacetimidate donor **182**, which was also used in the *first-generation* route (Figure 35, disconnection *iv*). This final disconnection represents the bond which ultimately caused us to forgo the *first-generation* route (high-lighted in red). It was key for us to establish this bond at an early stage in the synthetic route. From disaccharide **248**, the disconnection providing a D-Gal donor **249** and a D-GalN_3 acceptor **250** could be made (Figure 35. disconnection *vi*). The *second-generation route* was planned to incorporate the D-GalNAc residue by using the azido acceptor **250** which showed potential for forming the difficult glycosylation by Lowery *et al*.[171]

Part 6. MM-ZPS 2nd-generation synthetic route

Before major synthetic work could take place on the *second-generation* route, the starting materials needed to be synthesized. Synthesis of the D-GalN_3 imidate donor **182** and phosphoramidite **181** remained the same as in the *first-generation* route. The D-GalN_3 acceptor **250** and the D-Gal trichloroacetimidate donor **249** would need to be synthesized. The most successful route we found to produce the D-GalN_3 acceptor **250** from

inexpensive and readily available starting material was a five-step procedure which resulted in a 48% overall yield on gram scale (Scheme 31).

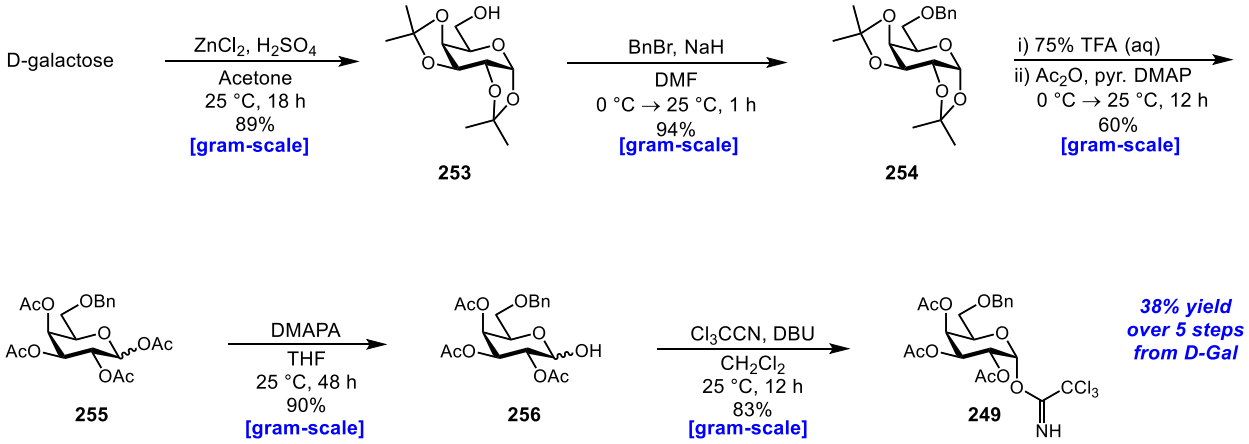
Scheme 31. Synthesis of D-GalN_3 acceptor 250



The route commenced with the azido nitration of 3,4,6-tri-*O*-acyl-D-Galactal **185,** which was obtained from D-galactose in three steps, in good yield to give the nitrate ester **237**. The nitrate ester **237** can be directly displaced by MeOH under microwave irradiation to afford **238** or treated with sodium methoxide to directly afford **252**. Both procedures resulting in a mixture of alpha and beta methyl galactosides. For reason we found it best to funnel the anomeric mixture of nitrate esters **237** to a single α-bromo galactoside **251**. Following, displacement of the bromide of **251** under halophilic mediated conditions in the presence of methanol provided the β-*O*-methyl-D-GalN_3 **238** in excellent yields as a single β-anomer. Saponification of **238** afforded the triol **252** which was subsequently protected as the 4,6-*O*-benzylidene acetal-D-GalN_3 acceptor **250** in an 87% yield on gram scales.

The remaining building block was the trichloroacetimidate D-Gal donor **249** which would become the central D-galactose residue of the core trisaccharide.

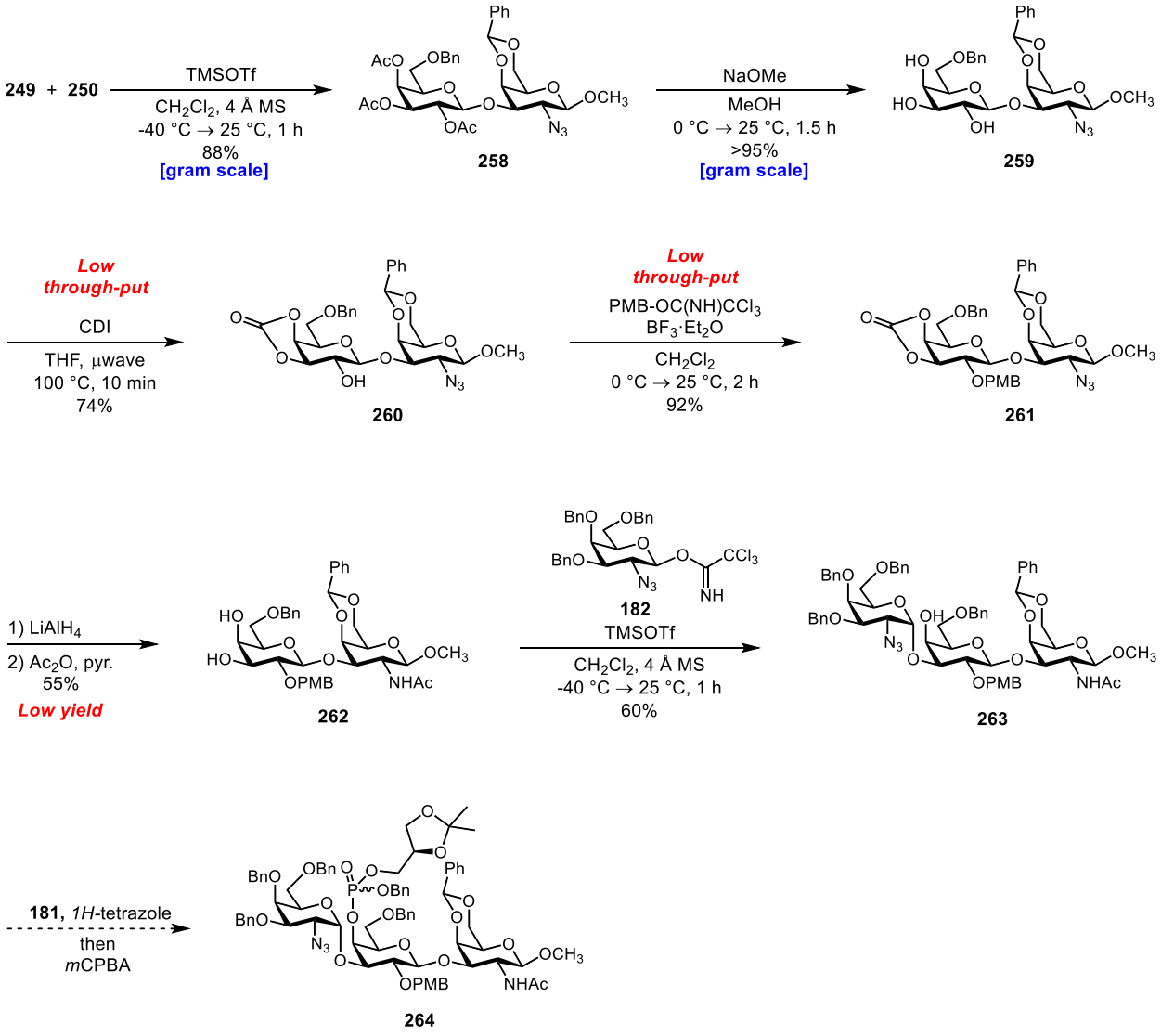
Scheme 32. Synthesis of D-Gal imidate donor 249



Starting from the inexpensive D-galactose, the diacetonide **253** was formed in good yield at multi-gram scales (Scheme 32). The acetonide **253** provided the free C6-OH which could then be permanently protected with a benzyl group rather than a TBS group which is prone to hydrolysis seen in the *first-generation* route. The diacetonide **253** was benzylated under Williamson etherification conditions to give the fully protected D-galactoside **254** in excellent yield at multi-gram scale. Aqueous TFA mediated hydrolysis of the diacetonide **254**, followed by acetylation gave the tetraacetate **255** in good yield. Surprisingly, refluxing acetonide **254** in aqueous acidic acid did not provide any desired acetonide de-protection. Selective anomeric deacetylation of **255** was achieved with DMAPA at room temperature after stirring for 2 days to give the free reducing sugar **256**. The reaction could be completed in shorter reaction times (~3 h) when >1.0 equivalences of DMAP was used, however this provided lower yields of alcohol **x** via formation of poly-deacylated products. The hemiacetal **256** was converted into the trichloroacetimidate donor **249** in good yield on gram scales. The trichloroacetimidate D-Gal donor **249** was synthesized in 5-steps with a 38% overall yield on gram scale.

With the trichloroacetimidate donor **249** and the D-GalN_3 acceptor **250** prepared, glycosylation attempts to forge the difficult D-Gal-β-(1→3)-D-GalN_3 bond began. TMSOTf mediated glycosylation between donor **249** and acceptor **250** gave the disaccharide product **258** in excellent yield as a single anomer, establishing the glycosidic linkage which previously gave us difficulties during the *first-generation* route (Scheme 33).

Scheme 33. Second-generation synthetic route towards MM-ZPS repeating unit



The next major synthetic task for the route was to protect the C2'-OH and prepare the disaccharide as a C3'-glycosyl acceptor for the final glycosylation. The C2'-protecting

group needed to be able to be removed following the formation of the trisaccharide core to allow for installation of the phosphoglycerol moiety. To this end, we selected a PMB protecting group which, in theory, could be removed selectively in the presence of the other protecting groups on the molecule. In addition, the orthogonality of the PMB protecting group allows for the incorporation of base labile protecting groups (acyl protecting groups -OAc, -OBz, etc.) as short-term, temporary, protecting groups in the event that we required protection of the free C4'-OH of the diol acceptor **x**.

To facilitate this synthetic goal, the acetates of **258** were removed by saponification to give the triol **259** as a white solid. The free C3', C4' alcohols of disaccharide **259** were carbonylated to give **260** in good yield. Best yields were obtained from atypical conditions involving microwave irradiation of the triol **x** with CDI in THF for short reaction times. The free C2'-OH of **260** was protected using the PMB-trichloroacetimidate to provide the fully protected disaccharide **261** in a 92% yield. Williamson etherification conditions proved too harsh for the base-labile carbonate functionality at the C3', C4' position. With the C2'-OH protected, the next synthetic tasks involved; conversion of the C2-azide to the acetamide and preparation of the glycosyl acceptor by deprotection of carbonate to provide the free C3' alcohol. As a note, the acetamide must be set before introduction of the final C2''-N_3 functionality to avoid acylation of both amines. Simultaneously, the carbonate was removed while the azide of **261** was reduced to a free amine by treatment with lithium aluminum hydride solution followed by an acylation of the C2 free amine to give the acetamide **262**. The disaccharide contained a diol, however the equatorial C3-OH is characteristically more nucleophilic then the axial C4-OH of D-Gal residues, therefore we postulated that we could use the diol **262** as an acceptor in a glycosylation

event. Glycosylation with acceptor **262** and the trichloroacetimidate donor **182** provided the complete trisaccharide core of **263** in a 60% yield.

Though we accessed the trisaccharide core, we did so in minimal quantities of material and in the next throughput we elected to change key elements of the synthesis (scheme 33, shown in red). The first modification involved use of an alternative C3', C4' protecting group. The carbonate protection produced a complex mixture of products which needed to be recycled to starting material under conventional heating methods regardless of equivalencies of reagent, reaction concentration, time, or temperature. Microwave conditions worked well but only at sub-gram scale. Furthermore, the PMB-trichloroacetimidate protection only provide consistently high yields on sub-gram scales while requiring excessive equivalence of PMB-trichloroacetimidate reagent. The final concern was the <60% yield which we were obtaining for the reduction/acylation step of the C2-azide. These combined issues led us to make modifications to this current *second-generation* route. The modifications would pertain to the disaccharide acceptor used in the final glycosylation.

Part 6.2 MM-ZPS 2nd-generation synthetic route version two

The 2nd-generation route version two disaccharide acceptor **265** became the target of total synthesis (Figure 36). The first apparent modification was the C2-NAcAc imide protection of **265**. This was planned because the C2-NAcAc can easily be transformed back to C2-NHAc during a late stage of synthesis while providing many advantages over the acetamide for chemical synthesis. In general, acetamides (-NHAc) elicit an extremely polar characteristic to the molecule while imides (-NAcAc) are relatively non-polar making. This change in polarity improves solubility which aids in chemical synthesis and

purification of the compound. In addition, diacylation also removes the nucleophilic characteristics of acetamide functionality, reducing the risk off-target reactions.

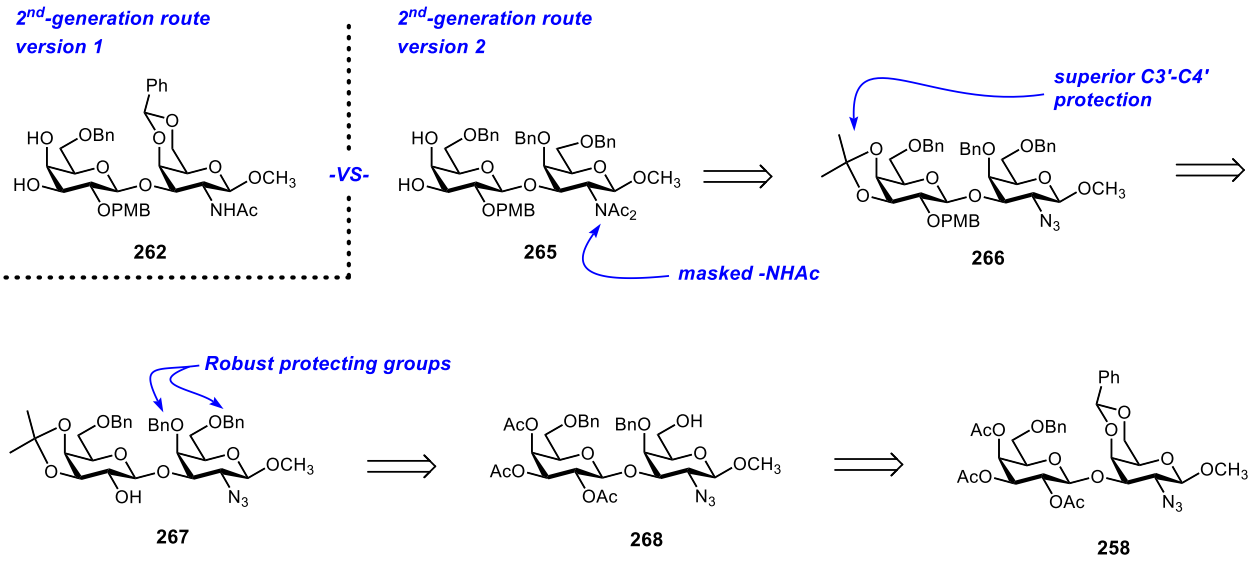


Figure 36. Comparison of 2nd-generation route disaccharide acceptors 262 and 265

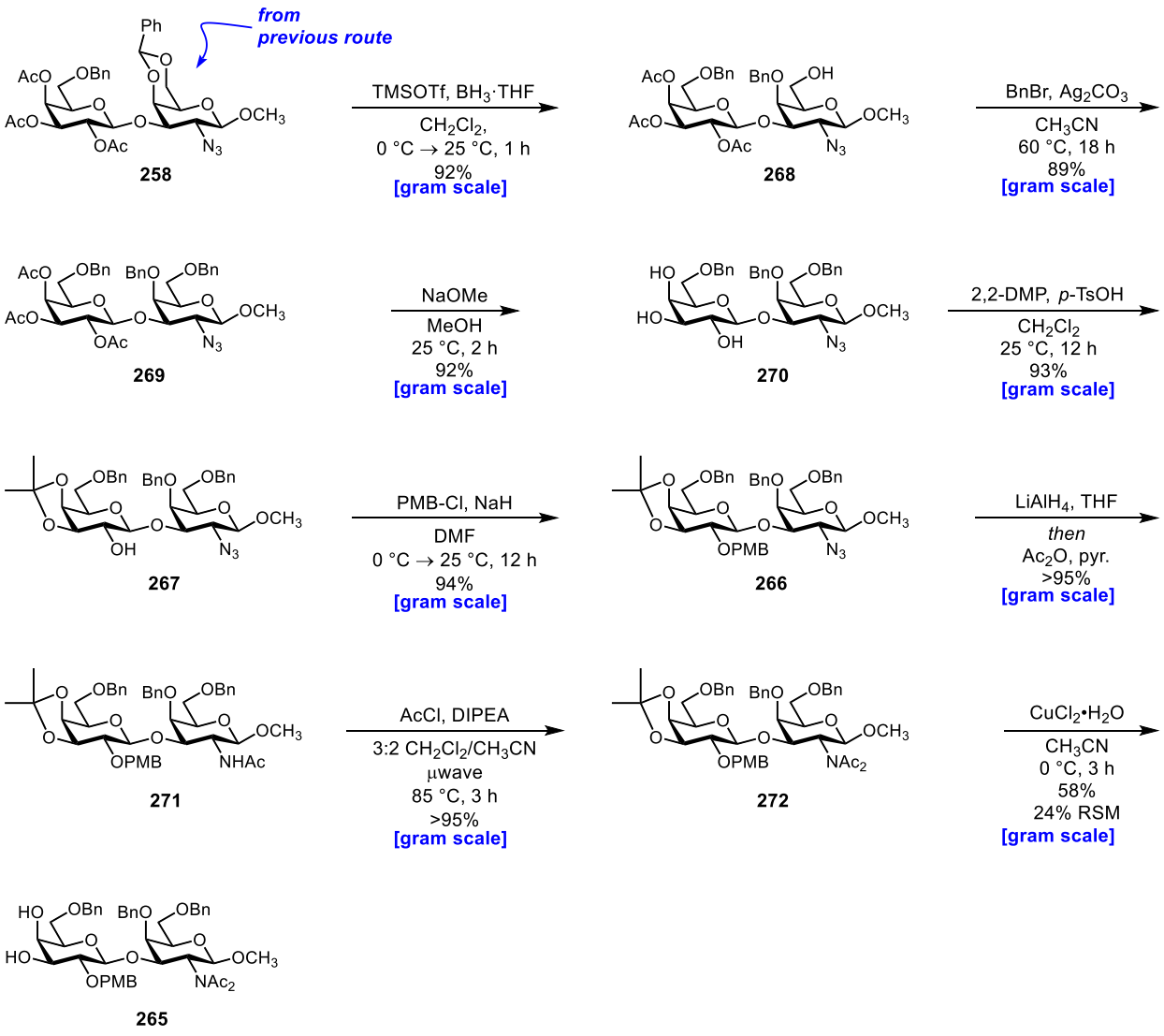
With these considerations in mind, the disaccharide acceptor **265** could come from the C2-azido disaccharide **266** following a reduction and diacylation. The C3', C4' acetonide was selected to replace the carbonate protection group. This alteration would provide three key improvements to the synthetic route; 1) PMB protection of the C2'-OH could now be carried out under, basic, Williamson etherification conditions removing the need to use the problematic PMB-trichloroacetimidate, 2) Williamson etherification

conditions using PMB-Cl and NaH in dry DMF are much more reliable at scale aiding in the high through put nature of the improved route, 3) acetonide protection of D-Gal residues generally are reliable and high yield relative to the carbonate counterpart. To facilitate the acetonide protection, the 4,6-*O*-benzylidene acetal must be removed and replaced with an acid stable protection group. Because the C4 and C6 alcohols did not need to be deprotected until the final step, we elected to protected them as benzyl protecting groups. The benzyl protecting groups (in **265**) would also increase the acid stability, relative to the 4,6-*O*-benzylidene acetal protected disaccharide (in **262**), which could help for subsequent glycosylations and intermediate deprotection steps.

To achieve these desired modifications, the previous synthetic route was intercepted at the and 4,6-*O*-benzylidene acetal **258**. The acetal was selectively opened using TMSOTf/borane conditions to afford the free C6 alcohol **268** (Scheme 34). The resulting alcohol was protected using benzyl bromide and silver carbonate while refluxing overnight to give the product **269** in good yield at gram scale. These protection conditions were favored over Williamson etherification conditions due to the base labile acetate protecting groups. Dudley's reagent[197-202] was originally employed as an alternative neutral method for benzyl protection and provided good yield (~70%) under conventional heating. Microwave irradiated conditions (120 °C, 30 min) provided the desired product in better yield (83%); however, we were limited to sub-gram scale due to the reaction volume of the microwave reaction vessels. Additionally, the benzyl-trichloroacetimidate reagent was screened for this reaction, but never provided synthetically useful yields (30-50%) provided that super-stoichiometric equivalencies of reagent were not used. Ultimately the halophilic conditions were optimal for this transformation. Following the C6 benzylation,

269 was saponified to provide the triol **270**. The triol **270** was protected as the C3'-C4' acetonide under acidic conditions to give acetonide **267** in 94% yield at gram scale. The free C2' alcohol **267** was selectively protected using PMB-Cl under Williamson etherification conditions to give the fully protected disaccharide **266** in excellent yield at gram scale.

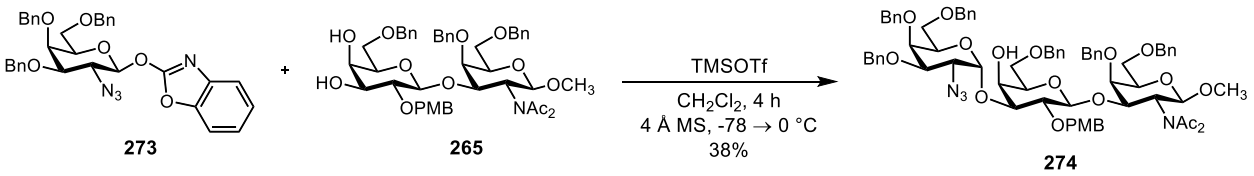
Scheme 34. Synthesis of 2nd-generation disaccharide acceptor 265



The azide of disaccharide **266** was reduced the intermediate C2 amine protected as an acetonide to give **271** in excellent yield (Scheme 34). The acetonide **271** acylated to provide the much less polar and more soluble imide **272** in excellent yield at gram scale. Hydrolysis of the acetonide of disaccharide **272** took place under copper chloride mediated conditions to provide diol **265** in decent yield along with considerable amount of starting material. The recovered starting material could be re-subjected to reaction conditions. Standard aqueous acetic acid conditions resulted in hydrolysis of the imine functionality, generating the C2-acetonide protected C3' C4'-diol and acetonide **271** . However, these by-products were not detected under copper mediated conditions, though long reaction times did generate unwanted byproducts possible due to the nucleophilic character of the diols produces in the desired product.

Nevertheless, we were determined to utilize the diol **265** as an acceptor to complete the core trisaccharide in a glycosylation event. As with the *first-generation* route, we reasoned the equatorial C3 alcohol of the D-galactose residue should express an enhanced nucleophilic character over the axial C4 alcohol. We hoped to utilize this characteristic to provide a selective C3' glycosylation between acceptor **265** and a suitable 2-deoxy-2-azido-D-galactoside donor. This would allow us to avoid a protection/deprotection step to provide the C4'-OH in subsequent steps. To investigate the final glycosylation, the diol acceptor **265** was first combined with the donor **273** and treated with TMSOTf at -78 °C and allowed to warm to 0 °C over the course of 4 h (Scheme 35).

Scheme 35. glycosylation of disaccharide acceptor 265



273 265 274

The desired D-GalN_3-α-(1→3)-D-Gal linked product **274** was isolated in a 38% yield. Confirmation of the desired product **274** was not trivial. HSQC ^{1}H-^{13}C HSCQ cross peaks were used to delineate the isolated material as tri- or tetrasaccharide in nature. Four trisaccharides, and two tetrasaccharides, were isolated from this reaction. Focusing on the trisaccharides of the material obtained, two of the isolated products clearly contained only β-anomeric linkages (J = > 6 Hz) indicating that they were not the desired C1''-α-linked product. The remaining two products contained an anomeric peak shifted up field, which is characteristic for α-linkages, and contained an α-characteristic ^{1}H coupling constant (J = < 4 Hz). However, the connectivity was unknown. Deductive reasoning provided that the compounds must have been the desired D-GalN_3-α-(1→3)-D-Gal linked product **274** and the undesired D-GalN_3-α-(1→4)-D-Gal isomer **275** (Figure 37). Of the two isolated products in question, we hypothesized that the major, of the two, was the desired product **274**, but we wanted further evidence to support this assumption. To support our hypothesis, full characterization of the D-Gal spin system was required. Unfortunately, 2-D NMR and selective 1-D experiments were not definitive in determining the position of the newly formatted glycosidic bond due to significant overlap of protons rendering the assignment of the C3' and C4' protons impossible. In addition, long-range HMBC correlations were too weak to be conclusive.

The major α-linked isolated product (unconfirmed to be **274** or **275** at the time) was acetylated to aid in characterization of the isolated material. Acylation of the free hydroxyl significantly shifted the adjacent proton downfield, away from the conjected region of the NMR spectra, and allowed for total proton assignment of the acylated derivative, providing the structure **276**. This confirmed our hypothesis that the major isolated product was **274** and contained the desired α-(1→3) glycosidic linkage.

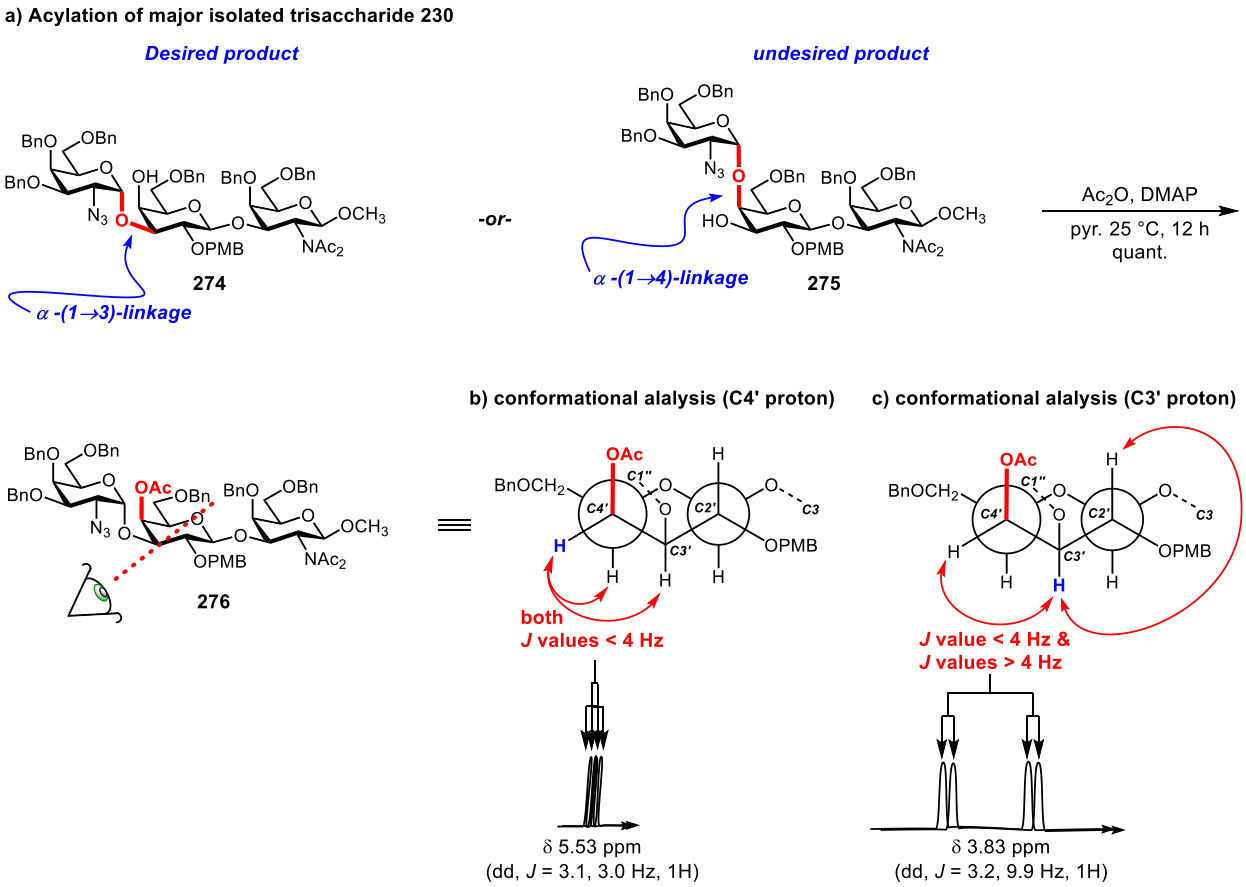


Figure 37. Characterization of major isolated trisaccharide 274

Two additional findings supported our chemical assignments. First, long-range HMBC cross peaks showed correlation between C1'' and C3' but not C4'. Secondly, the coupling constants of the afore mentioned proton adjacent to the -OAc (C4' in **276**) could properly be assigned. Coupling constant between C3 and C4 of D-Gal residues are unique to one another. The C4 proton has a cis/cis relationship to its neighboring protons while the C3 proton has a cis/trans which give rise to different ^{1}H NMR splitting patterns. This can be illustrated by looking at the Newman projection of **276** (Figure 37, part b). Simultaneously looking down the C4'-C5' and the C2'-C1' bonds show the downfield shifted C4' proton (adjacent to the acetate) should give rise to two small coupling constants with *J* values <4 Hz. In addition, the C3' proton (shifted upfield relative to C4' proton) should give rise to a coupling constant <4 Hz and one >4 Hz (Figure 37, part c). Our experimental findings agreed with this analysis. Confident with our characterization of the major isolated product, and subsequently the minor products, we moved forward to glycosylation optimization to improve on our 38% yield.

First, we investigated the effects of ethereal solvent additives. Our previous led us to try *iso*propyl ether as an additive to determine what effect it may have on the outcome of the ratio of desired vs. undesired products. Running the reaction in 80% ether to CH_2Cl_2 provided little improvement on overall reaction yield for the desired product. Unfortunately, the reaction was required to be initiated at an elevated temperature to keep the di*iso*propyl ether from freezing which added a temperature variable (Scheme 36, entry 2). performing the same reaction with a solvent solution of 1:1 di*iso*propyl ether/CH_2Cl_2 allowed for lower temperatures and resulted in the same overall yield for the desired product (Scheme 36, entry 5). Conducting the same reaction at elevated temperatures

slightly improved yield but showed minor effect of the ratio of products (Scheme 36, entry 6). The use of boron trifluoride was a promotor was examined but provided decreased overall yields and required longer reaction times (entry 3, 4 and 8). The trichloroacetimidate **182** was investigated under the improved reaction conditions and appeared to out-perform the *O*-benzoxazolyl imidate donor **273** (Table 5, entry 7). The addition of thiophene as an additive to provide stereochemical influence by participation was examined and did provide a reasonable degree of improved overall yield and ratio of desired product.

Scheme 36. glycosylation optimization of disaccharide acceptor 265

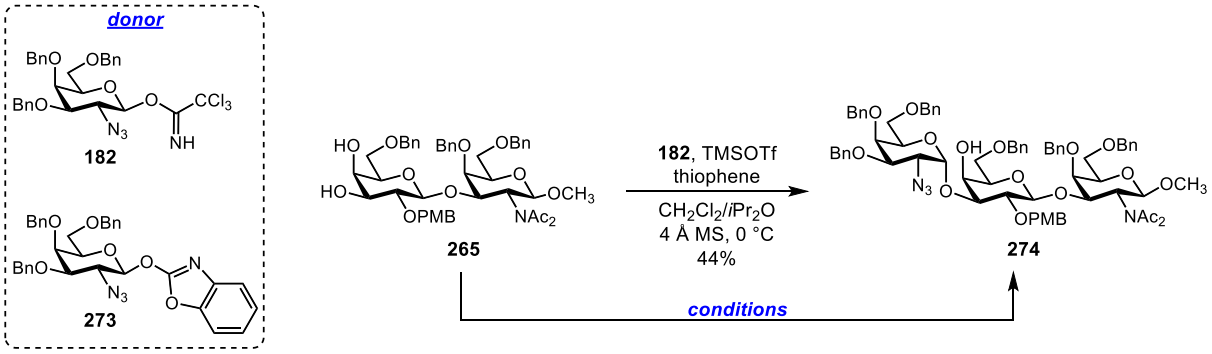


Table 5. Reaction conditions for 274

entry	*donor*	*donor equiv.*	*promotor*	*solvent/additive*	*temperature (°C)*	*time*	*yield desired product*	*yield (RSM)*	*yield other products*
1	**273**	1.2	TMSOTf	CH_2Cl_2	-78 → 0	4 h	38	ND	ND
2	**273**	1.5	TMSOTf	CH_2Cl_2/*i*Pr$_2$O (4:1)	-40	4 h	41	9	10
3	**273**	1.5	BF_3•Et_2O	CH_2Cl_2/*i*Pr$_2$O (1:1)	-78 → 0	4 h	22	19	50
4	**273**	2.5	BF_3•Et_2O	CH_2Cl_2/*i*Pr$_2$O (1:3)	-78 → -20	4 h	24	14	59
5	**273**	2.5	TMSOTf	CH_2Cl_2/*i*Pr$_2$O (1:1)	-78 → 25	4 h	38	33	26
6	**273**	1.8	TMSOTf	CH_2Cl_2/*i*Pr$_2$O (1:1)	-40 → 0	4 h	42	10	52
7	**182**	1.8	TMSOTf	CH_2Cl_2/*i*Pr$_2$O (1:1)	-40 → 0	4 h	55	25	40
8	**182**	1.8	BF_3•Et_2O	CH_2Cl_2/*i*Pr$_2$O (1:1)	-40 → 0	4 h	29	ND	66
9	**182**	1.0	TMSOTf	CH_2Cl_2/*i*Pr$_2$O (1:1)	-40 → 0	3 h	31	48	21
10	**182**	1.0	TMSOTf	CH_2Cl_2/*i*Pr$_2$O (1:1)	-78 → 25	6 h	31	33	10
11	**182**	1.0	TMSOTf	CH_2Cl_2/*i*Pr$_2$O (1:1)	-78 → 25	6 h	25	53	17
12	**182**	1.0	TMSOTf	CH_2Cl_2/*i*Pr$_2$O (1:1) thophene (25 equiv)	-15	2 h	44	33	25
13	**182**	1.0	TMSOTf	CH_2Cl_2/*i*Pr$_2$O (1:1) thophene (25 equiv)	-15	2 h	41	32	19
14	**182**	1.0	TMSOTf	CH_2Cl_2/*i*Pr$_2$O (1:1) thophene (25 equiv)	0	2 h	44	28	24

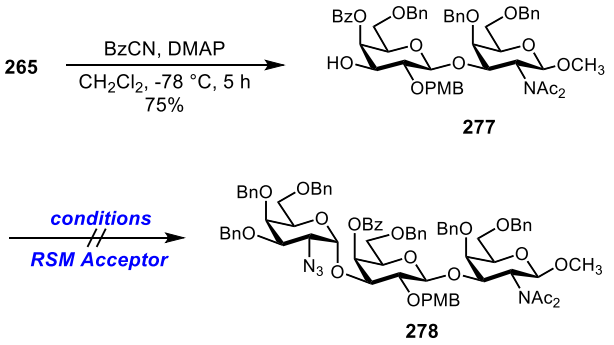
This glycosylation reaction was further complicated by the decomposition pathways of the trichloroacetimidate donors. Trichloroacetimidates are, such as **182**, are known to be able to undergo rearrangement to anomeric acetonide products.[203] In fact, armed donors, in particularly the per-benzylated glycosyl donors, are more prone to the rearrangement product. In the case of the glycosylation between acceptor **265** and donors **273** or **182**, rearrangement appeared to be the desired outcome relative to productive glycosylation

event. This prompted us to attempt the glycosylation with increased equivalences of glycosyl donor to offset this outcome and consume the starting material acceptor **273** (entries 4 and 5). Unfortunately, this modification did not only consume more of the acceptor starting material, but it also produced large amounts of diglycosylated product. With this finding we found it most efficient to stop the reaction prior to completion, and recover the useful acceptor starting material. To analyze the best conditions for this reaction we compared the yields by considering the ratios of desired product **274**, recovered starting material acceptor **265**, and the combined undesired products. The best conditions we found produced the desired product in a 44% yield consistently with the reaction going to nearly 75% (28% recovered starting material acceptor **265**) (entry 14).

Unsatisfied with the optimized results of the glycosylation with acceptor **265**, we decided to protect the C4' hydroxyl to eliminate the possibility of diglycosylation and C4' glycosylated products. To attempt this selective protection a procedure developed by Schmidt and co-workers was utilized.[204] The diol acceptor **265** was first treated with benzoyl cyanide and DMAP to give the C4'-OBz protected acceptor **277** (Scheme 37. part a). The acceptor **277** in a glycosylation event with donor **182** surprisingly produces no sign of the trisaccharide. Use of the alpha imidate donor **182** did not produce any observable quantity of trisaccharide **278** either. We suspected that the large benzoyl protecting group may have interfered with the glycosylation. In this light, we decided to protect the C4' hydroxyl with the smaller acetate protecting group. Diol **265** was treated with trimethyl orthoacetate then rearranged to the axial acetate by treatment with acetic acid to provide disaccharide acceptor **279** (part b).

Scheme 37. glycosylation attempts with disaccharide acceptors 277 and 279

a) glycosylation with C4'-OBz acceptor 277



b) glycosylation with C4'-OAc acceptor 279

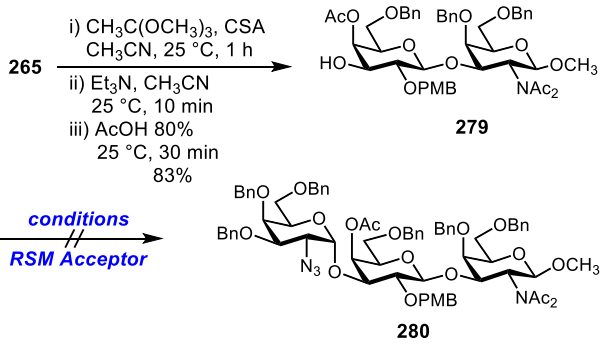


Table 6. Reaction conditions for 278

entry	*donor*	*equiv. of donor*	*conditions*
1	**182-β**	1.6	TMSOTf CH_2Cl_2, 4 Å MS -40 °C → rt, 12 h
2	**182-α**	1.5	BF_3•Et_2O CH_2Cl_2, 4 Å MS -40 °C → 25 °C, 12 h

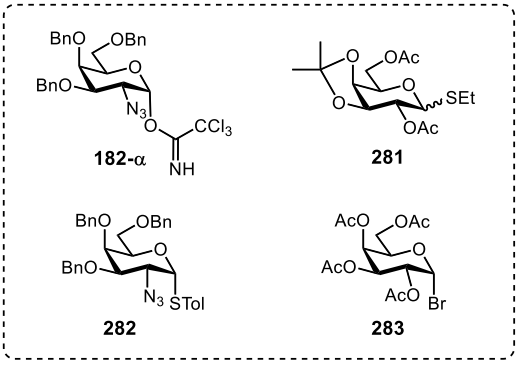


Table 7. Reaction conditions for 280

entry	*donor*	*equiv. of donor*	*conditions*
3	**182-β**	2.2	BF_3•Et_2O CH_2Cl_2, 4 Å MS -40 °C → 25 °C, 12 h
4	**282**	2.5	NIS, TMSOTf CH_2Cl_2, 4 Å MS -40 °C, 4 h
5	**282**	1.0	Ph_2SO, Tf_2O, DTBP CH_2Cl_2 , 4 Å MS -60 °C → 25 °C, 6 h
6	**182-α**	1.2	TMSOTf CH_2Cl_2, 4 Å MS -78 °C → 0 °C, 1 h
7	**281**	1.5	NIS, TMSOTf CH_2Cl_2, 4 Å MS -40 °C, 5 min
8	**283**	2.1	$Hg(CN)_2$, TBAI 3:1 CH_2Cl_2/CH_3NO_2 4 Å MS 0 °C → rt, 48 h

A variety of conditions and donors were employed with acceptor **279** to provide a trisaccharide. The trichloroacetimidate donor **182-β** with acceptor **279** produced no desired product (part b, entry 3). Thioglycoside **282** was synthesized and activated under NIS/TMSOTf conditions in the presence of acceptor **279**, but also provided no desired product (part b, entry 4 and 5). The *O*-benzyl oxazolyl imidate **273** also provided no desired product **280** as well with donor **282**. From here, model donors **281** and **283** were used to try and form any trisaccharide. Both resulted in no formation of trisaccharide, but

only recovered starting material. At this point we concluded that protection of the C4' hydroxyl resulted in severely impeding any nucleophilicity the disaccharide acceptor. These highly disappointing results caused us to move forward with the trisaccharide **274** produced from the glycosylation event between donor **182** and diol acceptor **265** in a 44% yield.

The trisaccharide **274** was coupled with the phosphoramidite **181** in good yield using previously described conditions to give **284** (Scheme 38). At this point in synthetic route, it was time to remove the PMB protection group to revel the C2' free hydroxyl to then install the final phosphocholine residue. PMB removal conditions began with most common DDQ oxidation conditions. The starting material was quickly consumed, and the reaction was worked up however only resulted in products which still contained a PMB protecting group. This indicated that other side reaction, including removal of alternative protecting groups, took place preferentially over loss of the PMB group. Prolonged reaction times (24 h) resulted in full baseline decomposition products only (entry 1).

Scheme 38. 2nd-generation route continued

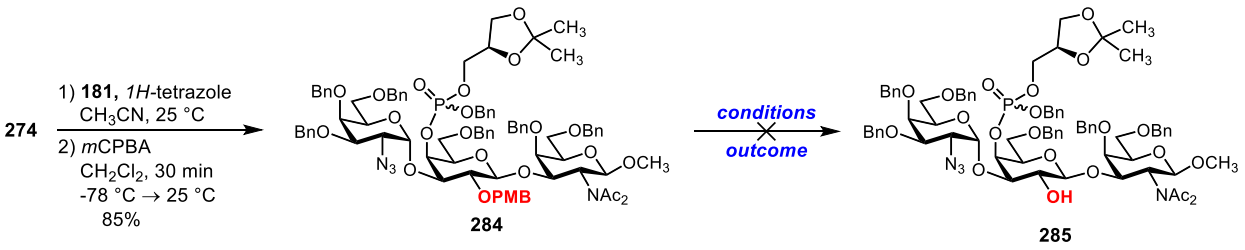
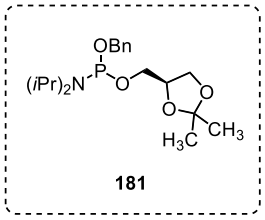


Table 8. Reaction conditions for 285



entry	*Conditions*	*Outcome*
1	DDQ, H_2O/ CH_2Cl_2/ 25 °C/ 24 h	decomposition*
2	DDQ, β-pinene, H_2O/ CH_2Cl/ -15 °C to 25 °C/ 24 h	decomposition*
3	CAN, H_2O/ acetone/ 0 °C/ 7 h	decomposition
4	HFIP, HCl/ CH_2Cl_2/ -78 °C/ 5 h	decomposition*
5	HFIP, HCl/ CH_2Cl_2/ -78 °C to 0 °C/ 6 h	decomposition
6	MgBr•Et_2O, SMe_2/ CH_2Cl_2/ 0 °C to 25 °C/ 80 h	NR
7	1,3-dimethoxybenzene, TfOH/ CH_2Cl_2/ 0 °C to 25 °C/ 22 min	decomposition
8	*t*BuBr/ CH_3CN, 4 Å MS/ 25 °C, 12 h	decomposition
9	oxalyl chloride/ CH_2Cl_2/ 25 °C/ 24 h	NR

* Starting material was consumed but isolated major product(s) contained PMB by NMR

The addition of beta pinene has been reported to help mitigate the acidic nature of DDQ reactions and provide improved yield.[205] Unfortunately, DDQ with beta pinene conditions had no change from the outcome of DDQ alone (entry 2). Alternative to DDQ, CAN mediated oxidation was used to attempt to remove the PMB group. CAN mediated oxidation of **284** produced none of the desired product but instead removed off target benzyl groups (entry 3). HFIP and HCl has been reported to remove PMB protecting groups in under 5 min at cold temperatures.[206] Surprisingly, using these conditions, the PMB protecting group was found intact on the major isolated products even over long reaction times (entries 4 and 5). Additional conditions found in literature to remove PMB protecting groups were used however none were successful in producing any desired product **285** (entries 6-9).[207-216]

Part 6.3 MM-ZPS 2nd-generation synthetic route version three

To overcome the hurdle of the C2' deprotection we decided to replace the PMB with an alternative protecting group. The PMB group was originally selected to allow for the installment of an orthogonal protecting group at the C4' hydroxyl (-OAc in **279** or -OBz in **277**) of the disaccharide acceptor (chapter 2 part 6.2). Because the C4' protected derivatives were poor glycosyl acceptors and we determined to move forward with the unprotected diol acceptor, the C2'-PMB protecting group was no longer necessary. Instead, we opted to synthesize a diol acceptor **286** which replaced the PMB with an acetate protecting group (Figure 38). Retrosynthetic analysis provided we could use the previous synthetic route to produce disaccharide acceptor **286** by protecting group manipulation of the triol **270**.

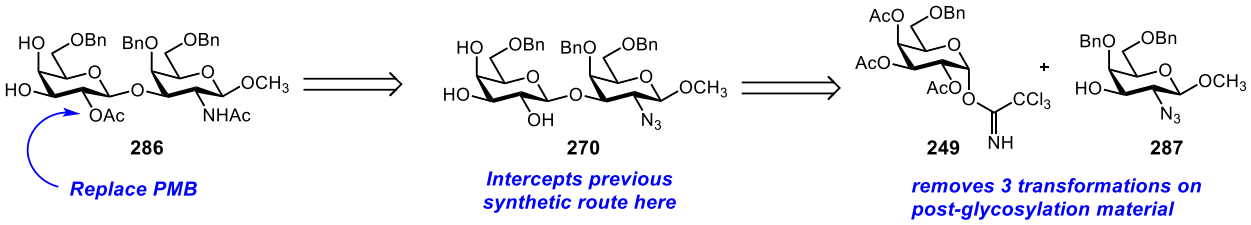


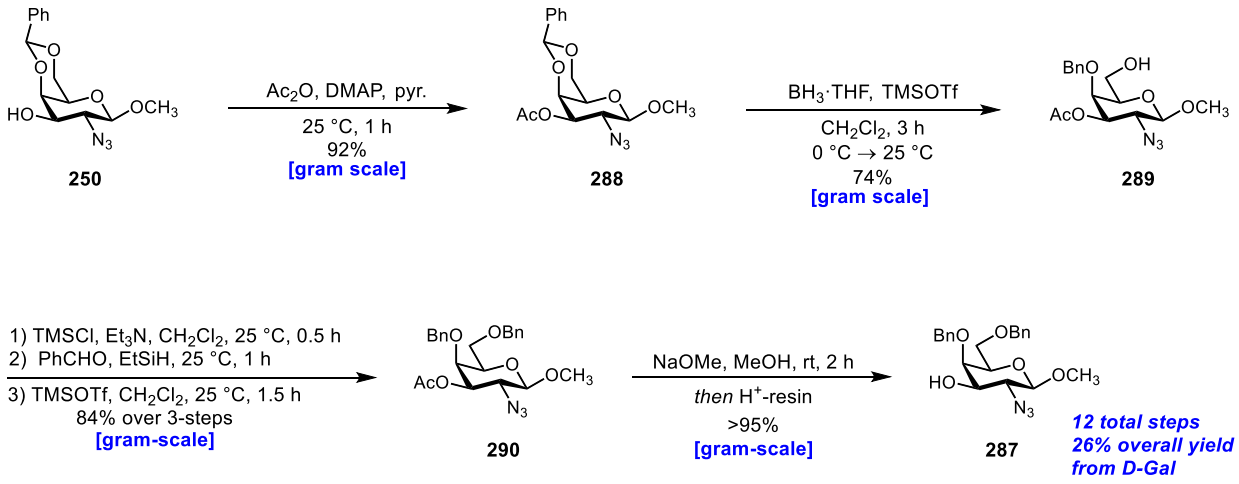
Figure 38. Retrosynthetic analysis of Improved, convergent, synthesis of disaccharide 270 in route to acceptor 287

We planned to cut down on the number of synthetic steps carried out on a post glycosylated molecule and provide a more convergent synthesis of **270** from the monosaccharide building blocks **249** and **287**. Conceptually, reducing the number of

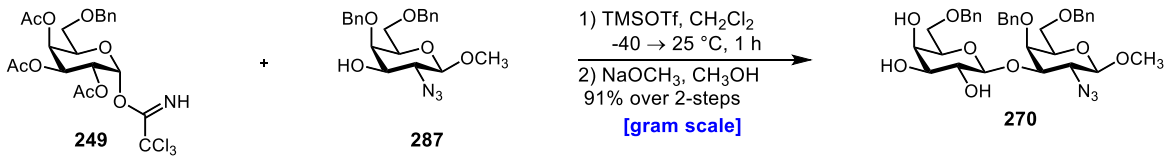
steps carried out on a molecule after a glycosylation event, can improve the overall yield if the number and performance of the synthetic steps are equivalent prior to a point of convergence. This is simply because loss in yield from each synthetic transformation after a convergence point affects both building blocks before the convergence point. To put this concept into practice, it is key to prepare each building block so that it can participate in a glycosylation event and then require minimal manipulations, preferentially only a single deprotection step, before it undergoes the following glycosylation. For us to facilitate this, we planned to synthesize the D-Gal acceptor **287** (Scheme 39).

Scheme 39. Synthesis of acceptor 287

a) Synthesis of acceptor 287



b) Improved route to triol 270



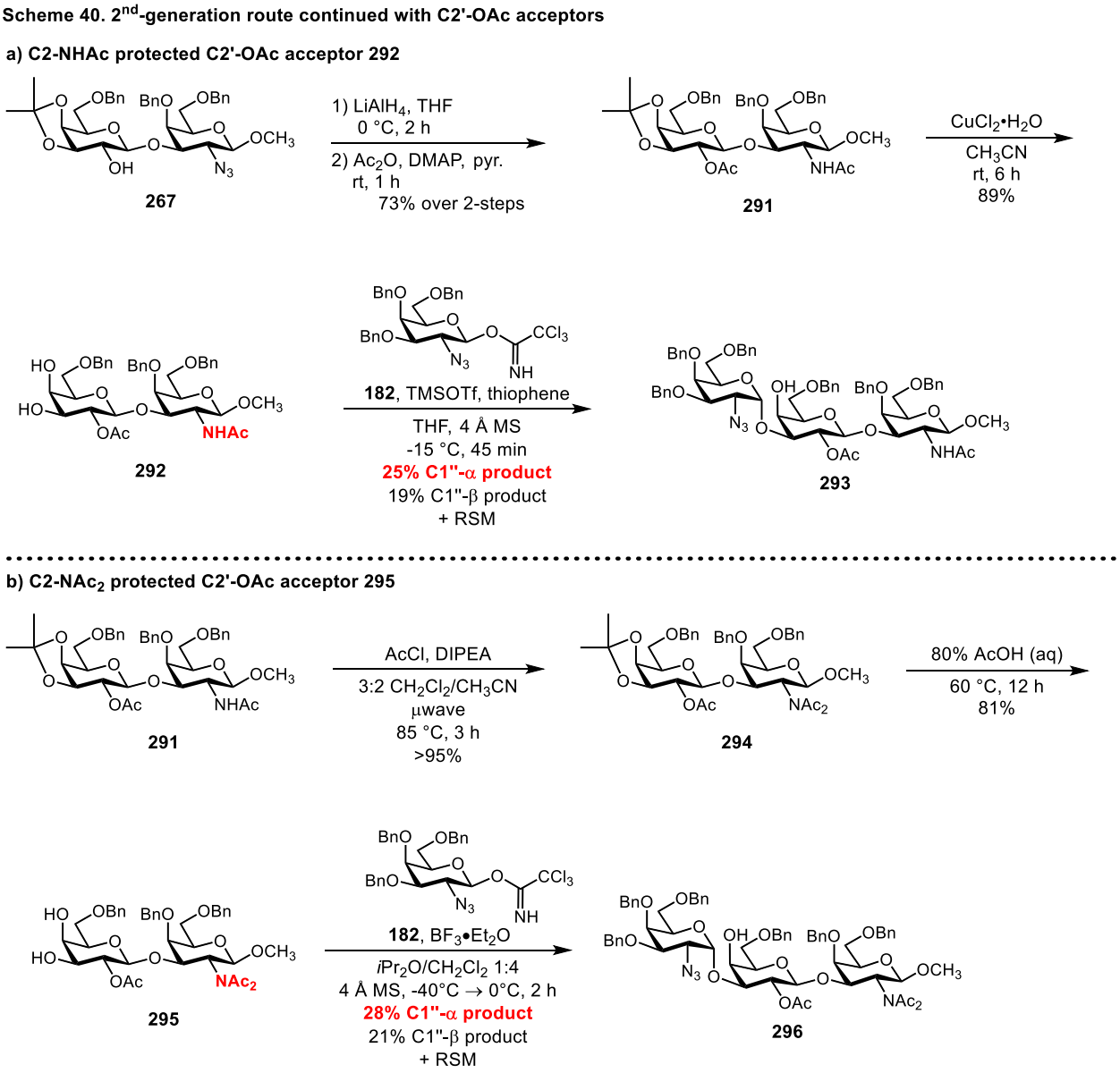
The D-GalN$_3$ **250** was acylated under standard conditions in excellent yield at gram scale to give fully protected D-Galactoside **288** (Scheme 39, part a). Treatment of the 4,6-*O*-

benzylidene acetal **288** with borane and TMSOTf selectively gave the free primary alcohol **289** in good yield at gram scale. To keep the acetate intact, neutral conditions to install the C6 benzyl group were employed. They performed well on gram scale to give the 4,6-di-*O*-benzyl product which was then saponified following the protection to give the acceptor **287** in excellent yield, over four steps, at gram scale. With the acceptor **287** in hand, glycosylation with the donor **249** utilizing TMSOTf mediated conditions gave the disaccharide which was directly saponified to give the triol **226** over two-steps in a 92% yield at gram scale (Scheme 39, part b). At this point, the triol **226** intercepted the previously described synthetic route. The new synthesis provided gram scales of the triol **226** in improved yields compared to the previous described route, allowing us to rapidly bring up material. Following the previous route from this point, the triol material **226** was protected using 2,2-DMP to give the C3', C4' *iso*propylidene **267** in excellent yield.

At this stage the C2 azide was reduced and then acylated to the acetamide while simultaneously protecting the C2' hydroxyl to provide the fully protected disaccharide **291** (Scheme 40, part a). The acetonide in **291** was removed using the copper chloride conditions to give a new diol acceptor **292**. The diol acceptor **292** was not soluble in the organic solvents previously used for similar glycosylation reactions but was soluble in THF. The use of THF is known to provide α-1,2-*cis* glycosidic linkages through solvent participation. Attempts at glycosylation between the diol acceptor **292** and the perbenzylated trichloroacetimidate **182** were carried out under TMSOTf mediated conditions in dry THF but provided only a mixture of products. The desired product **293** was obtained in a 25% yield along with a substantial amount of recovered starting material

acceptor. To improve the yields, we decided to synthesize and test the imide protected diol acceptor **296** (Scheme 40, part b).

Scheme 40. 2nd-generation route continued with C2'-OAc acceptors



The disaccharide **291** was treated with acetyl chloride in DIPEA under microwave irradiated conditions to provide **294**. The fully protected disaccharide **294** was deprotected using aqueous acetic acid to provide diol acceptor **295**. The imide protected acceptor **295** was much more soluble in typical polar aprotic solvents used for

glycosylations and less polar than the acetamide counterpart **292**. Glycosylation of acceptor **295** and the trichloroacetimidate **182** resulted in only a marginal improvement of yields, both giving nearly a 1:1 mixtures of alpha to beta products.

While the selectivity was low, it seemed a route incorporating a C2'-OAc protecting group provided advantages over the route utilizing the C2'-OPMB. However, to optimize the glycosylation forming the trisaccharide core, we once again found ourselves with limited supply of advanced material. We planned to synthesize gram quantities of trisaccharide material to allowed us to once again make changes based off of the reactivity explored during reaction optimization of the final phosphocholine coupling, optimization of global deprotection and to account of the significant loss in mass of the final product due to deprotection. To accomplish this goal, we needed to synthesize gram quantities of the disaccharide acceptor. Before beginning the material push, we decided to alter the synthetic route.

Over the course of working with the D-galactosamine derivatives we discovered the synthetic advantages that a trichloroacetate protecting group provides for amine protections over acetamides or azides. Trichloroacetamide (-NHTCA) protected sugars are very non-polar which aids in solubility, purification, and general handling. The electron-withdrawing character of the trichloro group reduces the nucleophilicity of the amide through inductive effects, which reduces the chance of many undesired side reactions. The three chlorines also give rise to unique chemical shifts which can be diagnostic for NMR and MS characterization of products. Due to all the clear advantages of the trichloroacetate protecting group, we elected to use a trichloroacetamide to protect the reducing-end D-galactosamine residue rather than our previous imide (-NAcAc or -

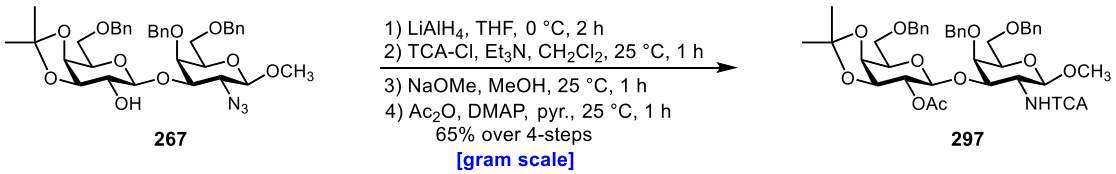
NAc_2) or acetamide (-NHAc) methods. For our purposes, we would still need to produce an acetamide (-NHAc) in the final product to provide the D-GalNAc residue. Fortunately, -NHTCA have been demonstrated by many groups to undergo de-chlorination under hydrogenation conditions to directly provide the -NHAc.

At the time, we assumed that exchange of the acetamide for the trichloro acetamide would be insignificant and not lead to any major changes in chemical reactivity throughout the route and instead only help improve yields because of the as mentioned positive characteristics of the TCA protecting group. First, disaccharide **267** from a previous route was converted to the C2-NHTCA derivative in a four-step procedure (Scheme 41, part a).

After initial synthesis of disaccharide **297**, a direct route to produce the C2-NHTCA derivative was developed. Treatment of our previously synthesized glycosyl acceptor **287** with lithium aluminum hydride followed by acylation with trichloro acetyl chloride and a sodium methoxide quench, to remove any *O*-acylated byproducts, provided the D-GalNTCA acceptor **298** (Scheme 41, part b). The acceptor **298** underwent a glycosylation event with the previously used D-Gal trichloroacetimidate donor **249**. As a note, protection of the C2-amine at the monosaccharide building block stage removed the need for the reduction/protection steps which were previously carried out on the disaccharide material. This lowers the number of synthetic transformations performed between the glycosylation events from eight, in the second version of the synthetic route, to only three in the current one.

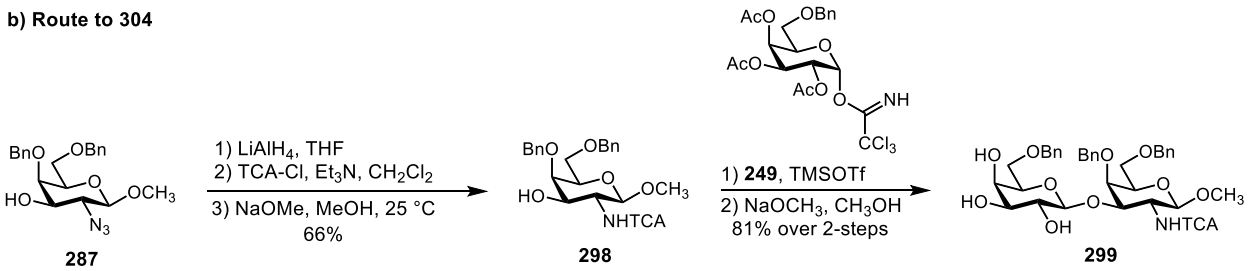
Scheme 41. Conclusion of 2nd-generation route

a) Route to 297

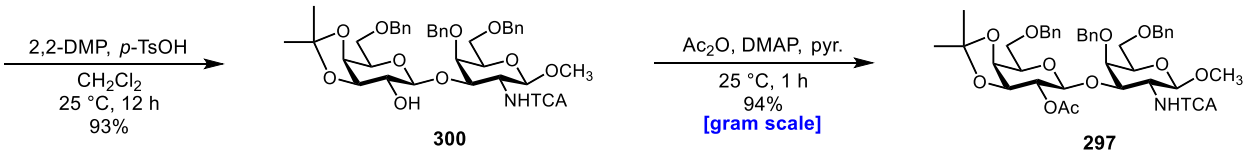


267 297

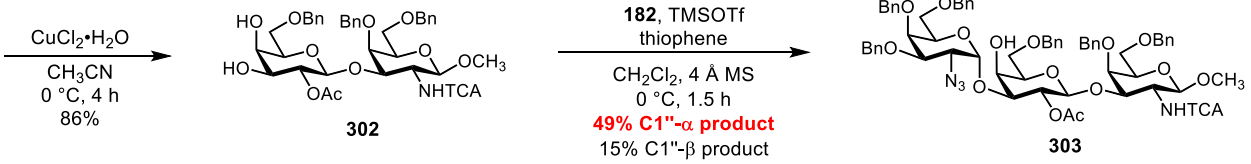
b) Route to 304



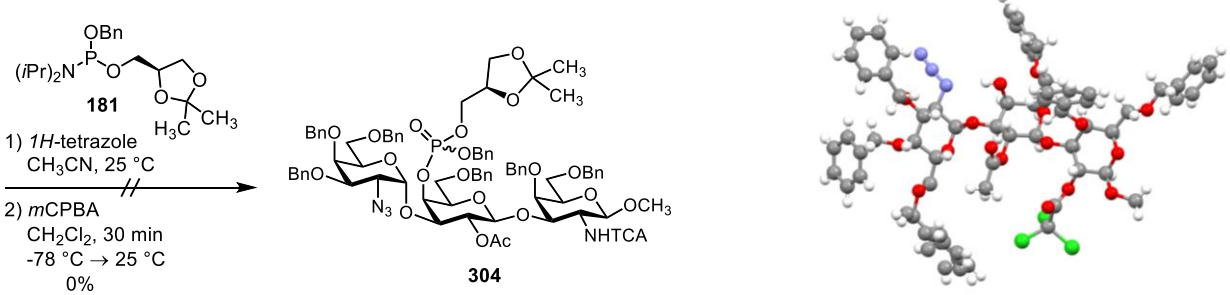
287 298 299



300 297



302 303



304

Continuing, the TMSOTf mediated glycosylation between donor **249** and TCA protected acceptor **298** was quenched and deprotected by sodium methoxide to provide the triol

disaccharide **299**. The disaccharide material was ready to be transformed into a proper glycosyl acceptor to be used in the final glycosylation event. The triol **299** was treated with 2,2-DMP to provide the C3'-C4' acetonide **300** in excellent yield at gram scale. The C2 free alcohol **300** was treated with acetic anhydride in pyridine to give the C2' acetate **297**. With a direct route to synthesize **297** in good yield achieved, the forward route to a trisaccharide product continued.

Next, hydrolysis of the acetonide group of **297** was achieved with copper chloride (or 80% AcOH) in to provide the disaccharide acceptor. By starting with the optimized conditions obtained from the previous synthetic routes, very little reaction optimization was required for the final glycosylation event. Treatment of disaccharide acceptor **302** and trichloroacetimidate donor **182** with TMSOTf in the presence of thiophene provided the desired trisaccharide **303** in a 49% yield consistently. In addition, the all β-linked trisaccharide was obtained from the reaction mixture in a 15% yield along with a mixture of the C4' glycosylated products and recovered starting material acceptor **302** as seen in the previous synthetic route. The trisaccharide **303** with the desired core stereochemistry was confirmed by 2-D long range NMR techniques, and a crystal structure was obtained by single crystal x-ray diffraction (Scheme 41). This crystal structure clearly shows the newly formed glycosidic linkage resides on the C3' position of the D-Gal residue and confirms the stereochemistry of the newly formed anomeric center.

Moving forward we needed to couple the first of two phosphorus containing moieties to the trisaccharide core. To do this, the trisaccharide **303** and phosphoramidite **181** were combined in dry acetonitrile and treated with tetrazole. The reaction progress was monitored by ^{31}P NMR and TLC. The phosphoramidite starting material has a

characteristic shift around 150 ppm and acyclic phosphites have characteristic chemical shifts around 100 ppm. This large difference in chemical shift allows for monitoring of the conversion from phosphoramidite to phosphite by substitution of the amine for the nucleophilic hydroxyl. Following this step, the reaction is oxidized to convert the phosphite to the corresponding phosphate. This step can also be monitored by ^{31}P NMR by looking for disappearance of the phosphite signal (100 ppm), and formation of a phosphate signal which typically are displayed around 0 ppm. However, when coupling trisaccharide **303** with phosphoramidite **181** no phosphite within the characteristic range was observed. In addition, TLC indicated that the trisaccharide starting material **303** was never consumed. The reaction was taken onto the final oxidation step by *m*CPBA. Unfortunately, no desired phosphate coupled product was observed, but instead only trisaccharide starting material **303** and phosphate byproducts of **181** were obtained. This reaction was repeated using alternative tetrazole sources, solvents, temperatures, equivalencies of reagent and reaction times, but none provided any alternative outcome resulting in the desired product **304**. After consumption of subsequent batches of phosphorus reagent **181**, we decided that significant steric and/or stereo-electronic factors must have attributed to the difference in reactivity between trisaccharide **303** and **181** towards the phosphate coupling reaction.

With this unfortunate conclusion we decided to close the door on the many versions of the *second-generation* synthetic route. We suspected to remedy the problem of phosphate coupling we would need to synthesize new trisaccharide derivatives with alternative protecting groups once again. Each version of the synthetic route required specific sequence of steps to accommodate the protecting groups planned to be used for

each route. With over forty-five steps from the D-Gal starting material, changes to the trisaccharide could result in weeks of synthetic work prior to the furthest forward step. All the versions of the *second-generation* route contained a long linear synthetic sequence between the two glycosylation events which diminished overall yield. Figure **39** generalizes how increased numbers of transformations negatively impacts overall yield. Yield from version 2 of the *second-generation* route are summarized below in pathway a. Following the first glycosylation, a series of eight transformations was carried out to produce the disaccharide acceptor in a 35% overall yield. In addition, the following glycosylation took place in <50% yield, making for an additional material choke point in synthesis. Conceptually, the loss in yield occurs after a point of convergence (glycosylation between **305** and **306** to give **307**) is more deleterious than before because it will affect the quantities of both downstream building blocks (**305** and **306**) needed to be synthesized to achieve the ultimate synthetic goal.

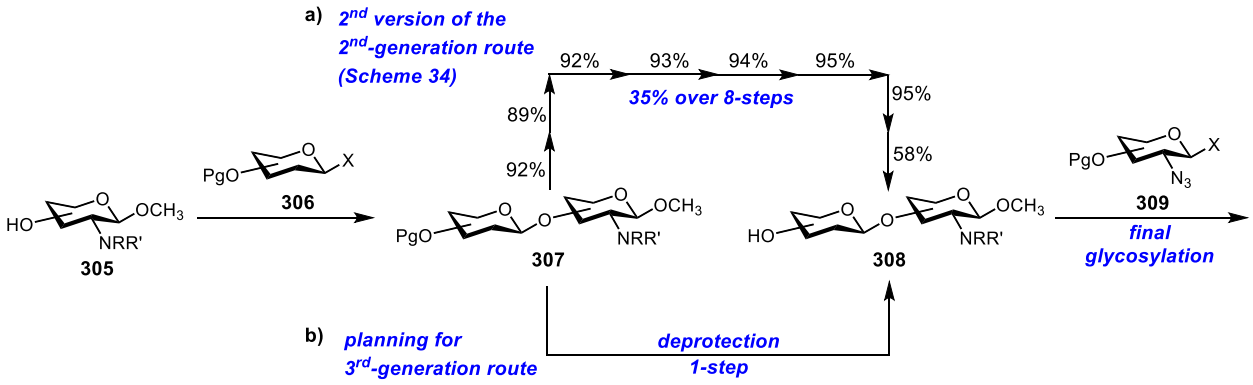


Figure 39. Planning for 3rd-generation route

Alternatively, many post-glycosylation steps can be carried out at earlier stages on the monosaccharide building blocks. At these early stages, low yielding transformations will only affect one of the building blocks rather than late stage (post glycosylation) material. In an ideal situation, donor and acceptor building blocks would be prepared for an iterative glycosylation strategy by building in the required protecting group strategy for each before a point of convergence (a glycosylation). For example, an acceptor **305** could undergo a glycosylation with donor **306** to provide a protected disaccharide **307** which could, in a single step, be deprotected resulting in an acceptor **308** ready for the final glycosylation with **309** (Figure 39, pathway b). This type of synthetic planning and preparation has recently become better recognized and utilized by the leaders in the oligosaccharide synthetic field; in particular by groups interested in iterative glycosylation utilizing automated carbohydrate synthesis.[217-222]

In addition to the improvement is overall yields, using this modular building block strategy allows for synthesis of differentially protected building blocks. These building blocks can be used to quickly generate multiple advanced intermediates that contain different protecting groups. The highest yielding routes can be selected moving forward to develop the most efficient synthetic campaign.

Even though the final version of the *second-generation* route was significantly more convergent than its predecessor (chapter 2, part 3 vs 1&2), the route was not convergent enough to provide modular building blocks which could be interchanged to quickly make a library of differentially protected trisaccharide derivatives. Moving forward, we decided to once again re-design the entire synthetic route to achieve a MM-ZPS repeating unit. Planning for the *third-generation* synthetic route, we decided that we wanted to develop

a highly convergent strategy. Ideally, we would like to protect each building block so they could be used in a glycosylation providing a product which could, in no more than one step, be deprotected and utilized in the following glycosylation event. By adhering to this strategy, loss in overall yield will be limited to early synthetic steps when material is inexpensive and plentiful, and less material will be required to be brought up for lengthy multi-step linear sequences.

Part 7. MM-ZPS 3rd-generation route planning and retrosynthetic analysis

The *third-generation* synthetic route was born out of lessons learned from our previous synthetic attempts towards a MM-ZPS repeating. These lessons included; synthesis from the reducing to the non-reducing end and limiting the number of synthetic transformations between glycosylation events. The most significant alteration of synthetic strategy dealt with selection of an alternative synthetic target. Owing to the polymeric nature of the ZPSs we were able to select an alternative repeating unit frame of the MM-ZPS which could serve as a synthetic target. The concept of a *frame-shift* is common in the field of ZPS syntheses and can be demonstrated in synthesis published by Seeberger,[114-115, 131] Andreanna,[55] Codée,[97] Bundle[112-113] and others.[66, 100, 223-224] To this end, we decided to shift from the first and *second-generation* synthetic target **310** to the alternative repeating unit **311** (Figure 40, synthetic target derived from the frame shown in red).

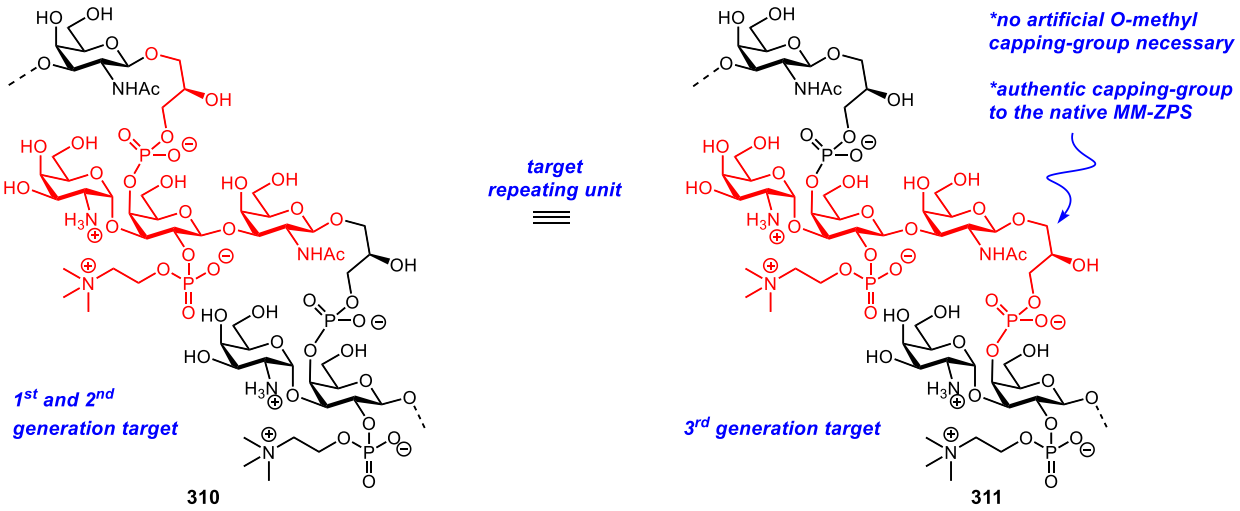


Figure 40. 3rd-generation MM-ZPS repeating unit route frameshift of target repeating unit

The new synthetic target frame **311** does not require an artificial alkyl capping-group. As previously mentioned, alkyl capping-groups are typically added to the C1-anomeric position of the reducing end of the sugar to prevent mutarotation, giving rise to a mixture of glycosidic products; alpha pyranose, beta pyranose, alpha furanose, beta furanose, open chain linear form sugars. In the case of the new synthetic target **311** the phosphoglycerol arm attached to the C1 position can prevent mutarotation preventing the need for an unnatural *O*-alkyl capping group. This renders our synthetic target **312** a more accurate synthetic representation of the native MM-ZPS repeating, over any C1-*O*-methyl derivatives (Figure 41). With the frame shift of **311** selected, **312** was determined to be the new synthetic target.

Retrosynthetic analysis revealed the trisaccharide **312** could come from the trisaccharide **313** following a global deprotection a disconnection of the phosphocholine residue providing the phosphoramidite **177** (Figure 41. disconnection *i*,bond formation *ii*).

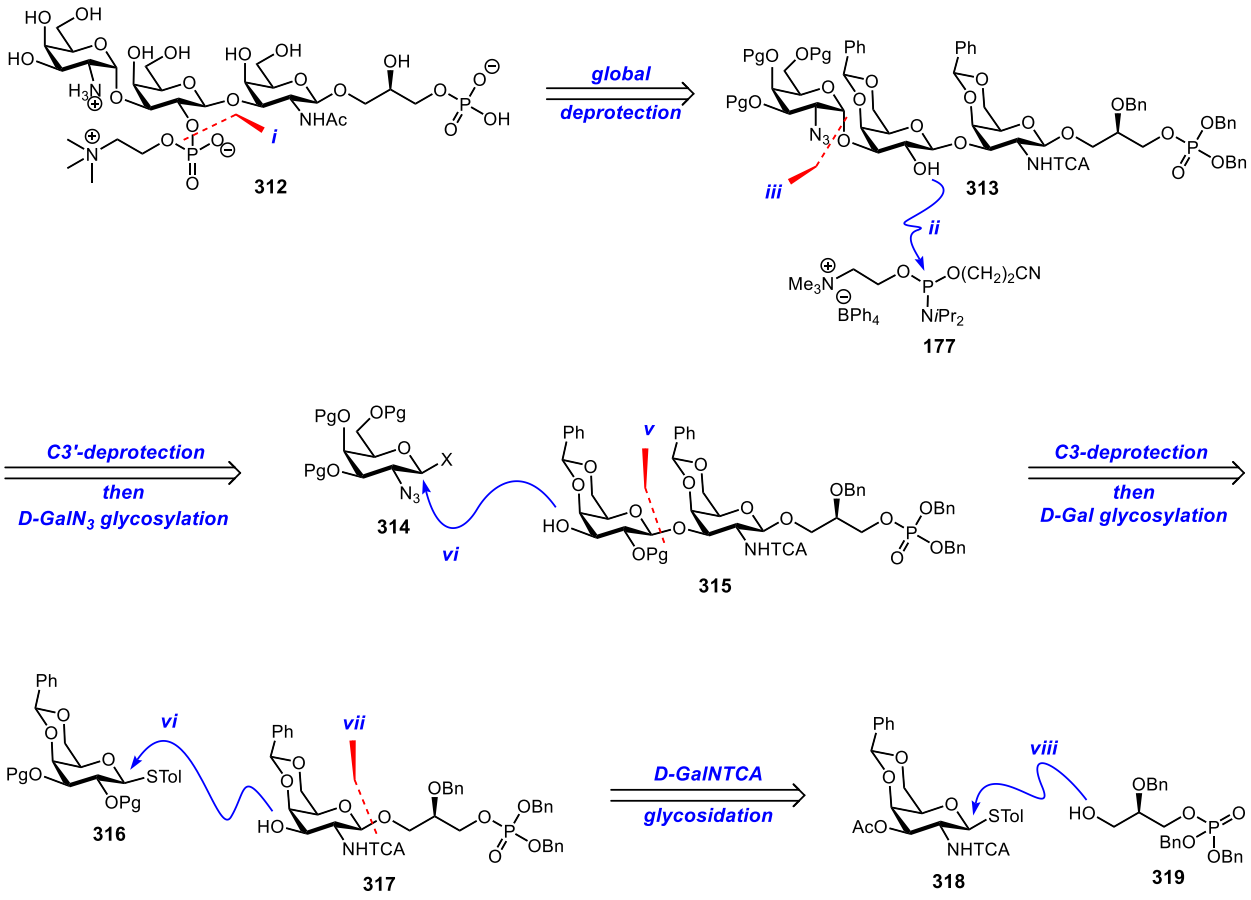


Figure 41. 3rd-generation retrosynthetic analysis of the MM-ZPS repeating unit

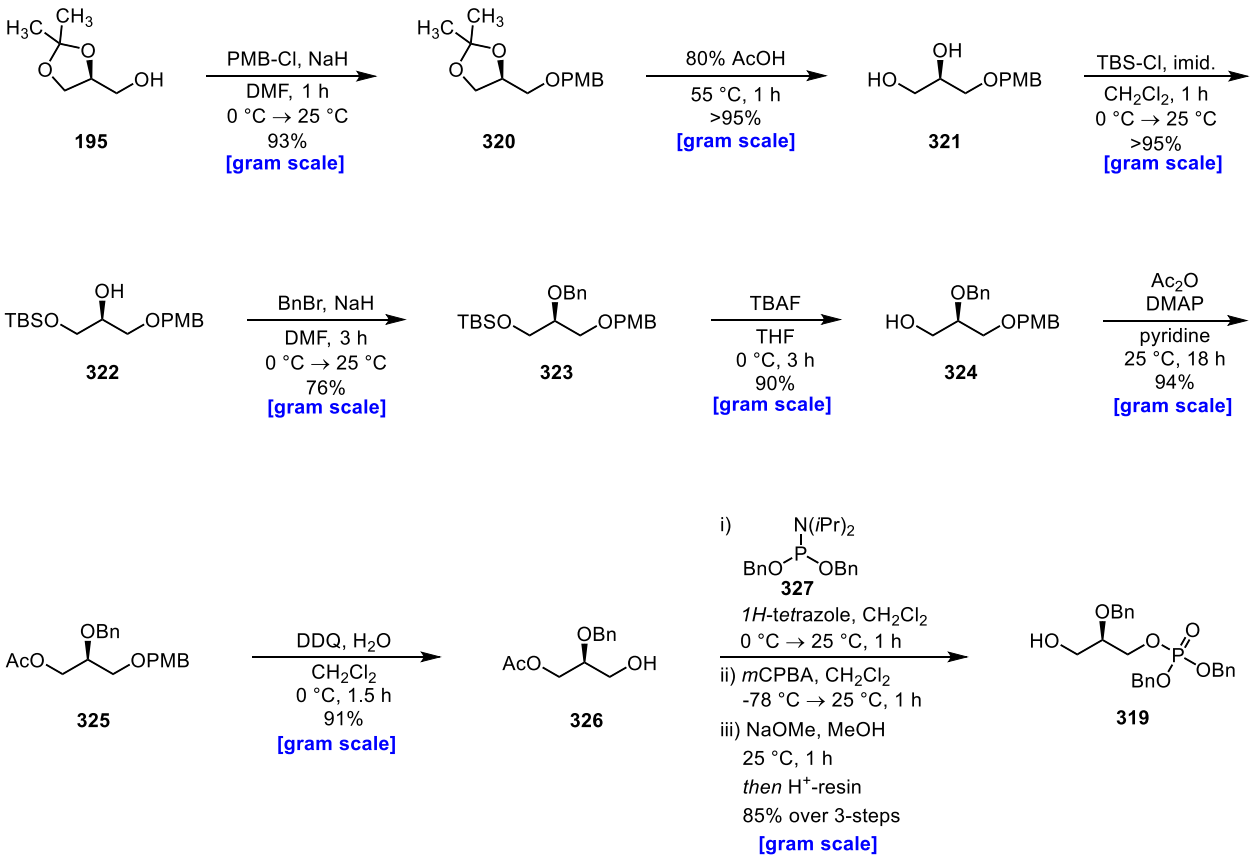
Disconnecting the GalN$_3$-α-(1→3)-D-Gal of the trisaccharide **313** provided a protected terminal D-GalN$_3$ donor **314** and the disaccharide acceptor **317** following a C3' deprotection (Figure 41. disconnection *iii*, bond formation *vi*). Disconnection of the D-Gal-

β-(1→3)-D-GalNTCA bond could provide a D-Gal donor **316** and the D-GalNTCA acceptor **317** following a C3 deprotection event (Figure 41. disconnection *v*, bond formation *vi*). Disconnection of the D-galactosamine anomeric linkage to the phosphoglycerol arm in **317** could provide the D-GalNTCA building block **318** and the alcohol **319** (Figure 41. disconnection *vii*, bond formation *viii*).

Part 8. MM-ZPS 3rd-generation synthetic route

Synthesis of the building blocks was first required to accommodate the newly designed synthetic strategy. We designed an eight-step sequence to provide the phosphoglycerol building block **319**. The previously described D-(+)-Solketal **195** was PMB protected in to give **320** (Scheme 42). The acetonide protecting group in **320** was removed by hydrolysis to give diol **321**. The diol **321** was selectively protected on the primary alcohol with TBS chloride to give alcohol **322**. This allowed for protection of the secondary alcohol of **322**. Benzyl protection of the secondary alcohol of **322** was successfully carried out to give **323**. It was discovered that during subsequent steps the TBS group was susceptible to hydrolysis under the PMB removal conditions employed leading to low yielding steps leading to **319** (route not shown). To work around this, the TBS protecting group in **323** was exchanged for an acetate protecting group by first removing the TBS group with TBAF to give the primary alcohol **324**. The alcohol **324** was then acetylated to give the fully protected glycerol moiety **325** The PMB group was removed with DDQ to give the free primary alcohol **326**. The alcohol **326** was then treated with the dibenzyl phosphoramidite **327** in the presence of *1H*-tetrazole to give the glycerol phosphite which was then oxidized to the phosphate **319**. This **319** would serve as the first building block of the synthesis.

Scheme 42. Synthesis of phosphoglycerol residue 319

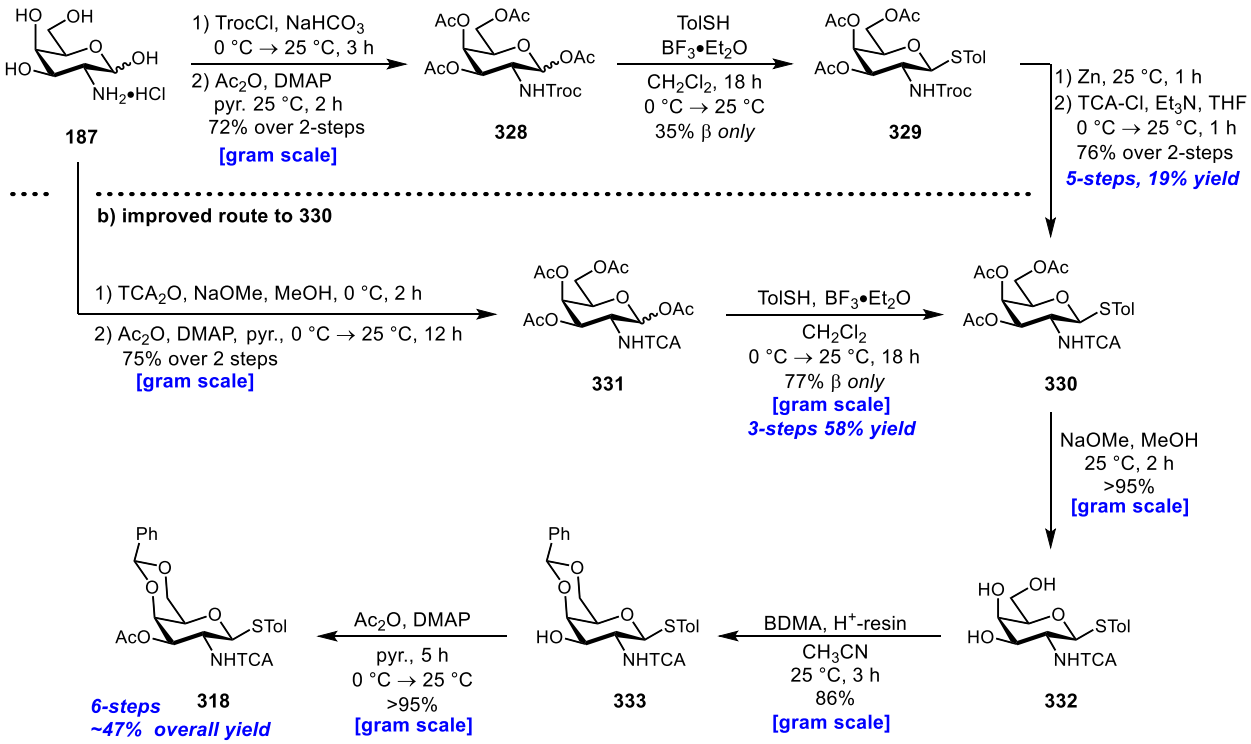


Next, work began on the D-GalNAc derivative which would become the reducing end residue of the core structure. The thioglycoside **318** was selected for synthesis (Scheme 43). The route to the thioglycoside building block **318** began with *N* protection of D-GalN$_2$·HCl **187** by treatment with Troc-chloride followed by the *O*-protection by treatment with acetic anhydride in pyridine to give **328** (Scheme 43, part a). The anomeric acetate in **328** was displaced to produce the thioglycoside **329** by treatment with thiocresol in a Lewis acid mediated process, however in a low yield. Due to the protecting group orthogonality required for late stages of the route, we exchanged the C2-NHTroc protecting group for a C2-NHTCA protecting group. This was accomplished by removing the Troc group of **329** by treatment with zinc dust followed by addition of thichloroacetyl

chloride and triethylamine to give thioglycoside **330** in good yield. As a note, activation of mortar and pestle ground zinc dust was key for Troc removal.

Schem 43. Synthesis of D-GalNHTCA donor 318

a) original route to 330



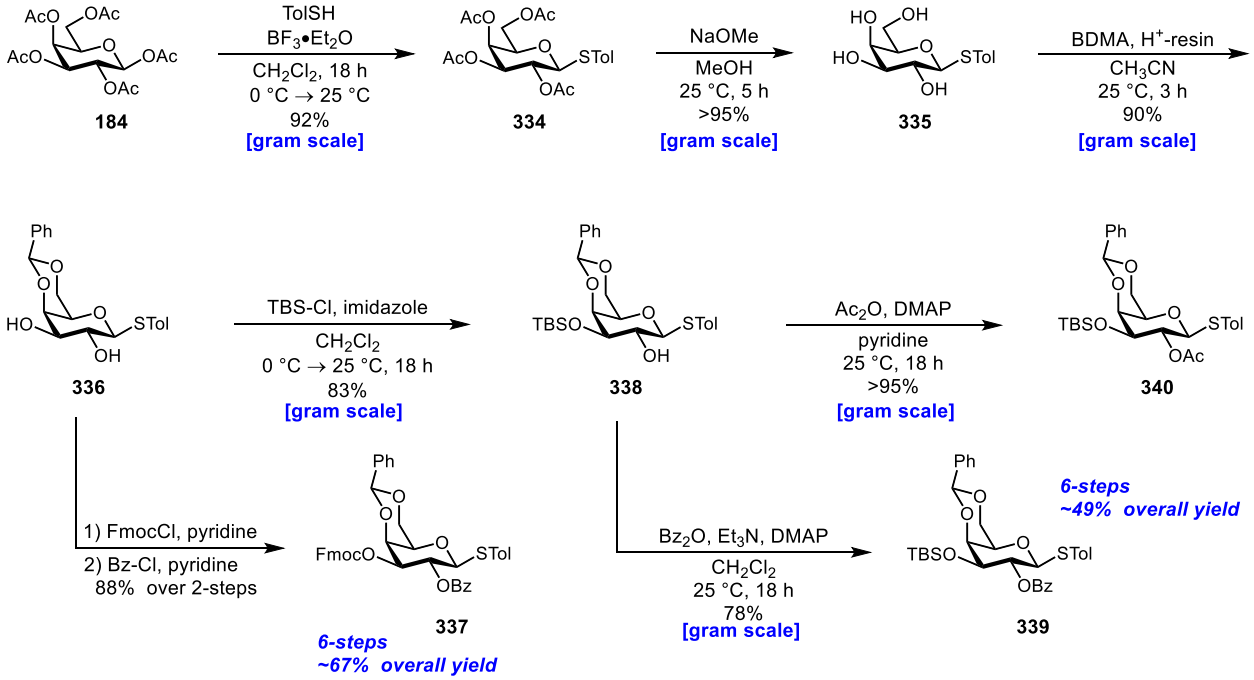
To afford thioglycoside **330**, the route had an overall yield of 19% over 5 steps. This yield was unacceptable and would impede production of the quantities of material required for the following steps to be carried out. Considering this hinderance, alternative methods of arriving at the thioglycoside **318** were utilized. Starting over once again, D-GalN·HCl **187** was directly C2-NHTCA protected by treatment with trichloroacetic anhydride in a sodium methoxide solution followed by a work-up and then *O*-acylation with acetic anhydride in pyridine to give acetate **331** (Scheme 43. part b). The acetate **331** was converted to a thioglycoside **330**. This route resulted in the thioglycoside **330** in

a three-step procedure with a 58% over all yield, which was much more capable for supplying the required quantities of material needed for subsequent steps.

Moving forward, thioglycoside **330** was saponified with sodium methoxide to give **332**. The resulting triol **332** was converted to the 4,6-*O*-benzylidene acetal **333** by treatment with BDMA and acidic resin. The C3 free alcohol of **333** was acetylated using general conditions to give the thioglycoside donor **318**. The thioglycoside donor was prepared in a total of 6 steps from D-GalN·HCl in a 47% overall yield.

For the central D-Gal residue, a series of three D-gal thioglycoside building blocks were prepared from inexpensive and radially available 1,2,3,4,6-penta-*O*-acyl-D-Gal **184**. D-galactose pentaacetate **184** was converted to the β-thioglycoside **334** (Scheme 44). The thioglycoside **334** was saponified to provide the tetraol **335**. The tetraol was protected as the 4,6-*O*-benzylidene acetal **336** which served as a common intermediate in route to each of the prepared D-Gal building block variants.

Scheme 44. Synthesis of thioglycoside D-Gal Donors

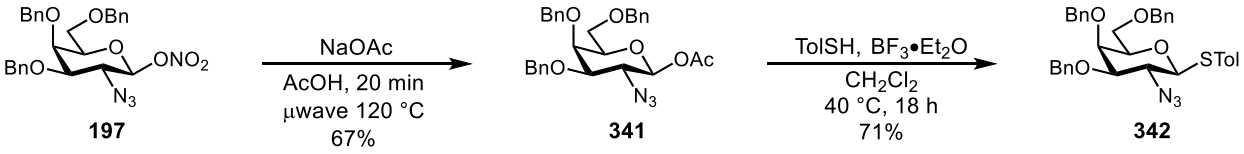


First, **336** was Fmoc protected at the C3 position followed by a C2-OBz protection to give the thioglycoside **337**. Then, **336** was C3-OTBS protected to give **338**. The alcohol **338** was C2-OBz protected to give **339** as well as C2 acylated to give **340**. Surprisingly, the benzoylation required superstochiometric equivalencies of benzoic anhydride and DMAP. Heating of the reaction gave rise to decomposition. In addition, as a note, both the C2-benzoyl protected **339** and C2-acetyl **340** were co-polar on TLC with their alcohol precursor **338** reaction progress was best monitored by crude NMR analysis. With the three potential building blocks of **337**, **339** and **340** in hand we moved onto the final monosaccharide building blocks needed for the terminal D-GalN_3 moiety.

Five D-GalN_3 donors were prepared and tested for the *third-generation* route. Quantities of the previous synthesized 3,4,6-tri-*O*-benzyl trichloroacetimidate donors, **182** and 3,4,6-

tri-*O*-benzyl *O*-benzoxazolyl imidate donor **273** were prepared. In addition, the tri-*O*-benzyl thioglycoside **342** was prepared (Scheme 45).

Scheme 45. Synthesis of 3,4,6-tri-*O*-benzyl-D-GalN_3 donors

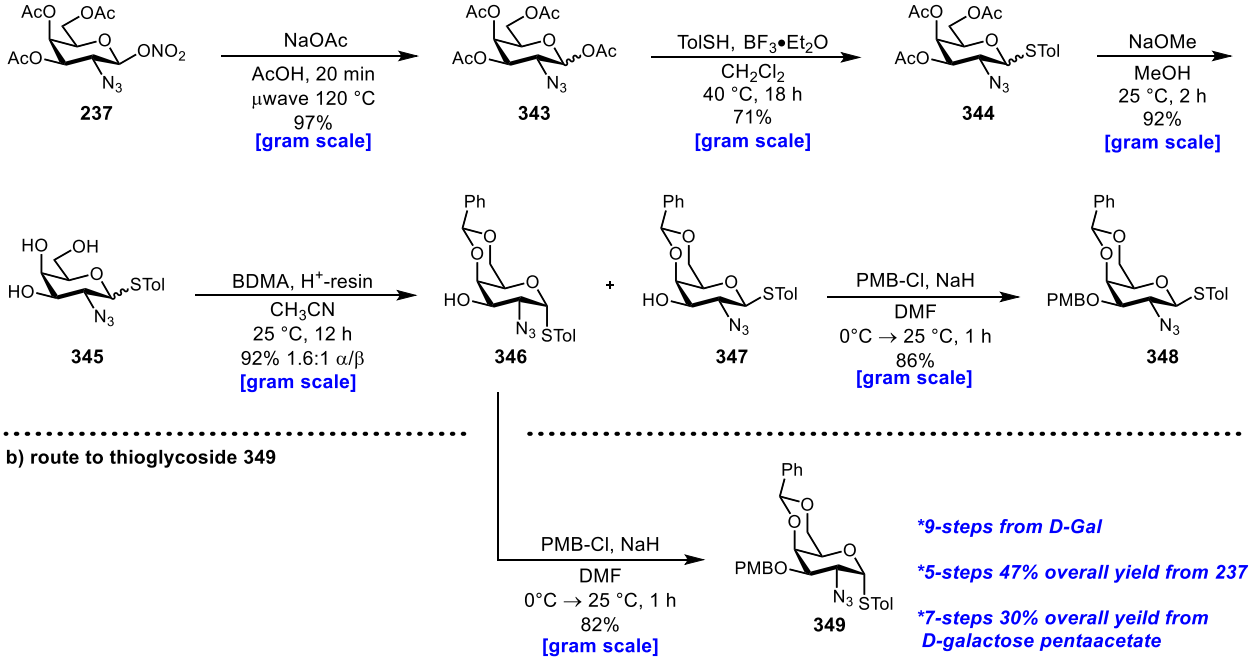


The azido nitrate ester **197** was treated with sodium acetate to give the anomeric acetate **341**. Displacement of the anomeric acetate in **341** with *p*-thiocresol gave thioglycoside donor **342**.

The final set of thioglycoside donors were prepared (Scheme 45, route a and b). Treatment of the 3,4,6-tri-*O*-acetyl azido nitrate ester **237** with sodium acetate, the tetra acetate **343** was obtained. Following, displacement of the acetate **343** was carried out with *p*-thiocresol under Lewis acid mediated conditions to give an inseparable mixture of α and β thioglycoside **344**. The mixture of thioglycosides **344** was saponified to give the mixture of triols **345** Protection of the triol mixture **345** as the 4,6-*O*-benzylidene acetals was carried out to give the **346** and **347** which could be separated using silica gel flash column chromatography. The pair of thioglycosides (**346** and **347**) were separately PMB protected to give resulting thioglycoside **348** and **349**. The synthesis of the alpha thioglycoside **349** was carried out in a total of nine steps from readily available and inexpensive D-galactose, and in five over all steps from known azido nitrate ester **237** in a 47% overall yield.

Scheme 46. Synthesis of D-GalN$_3$ donors

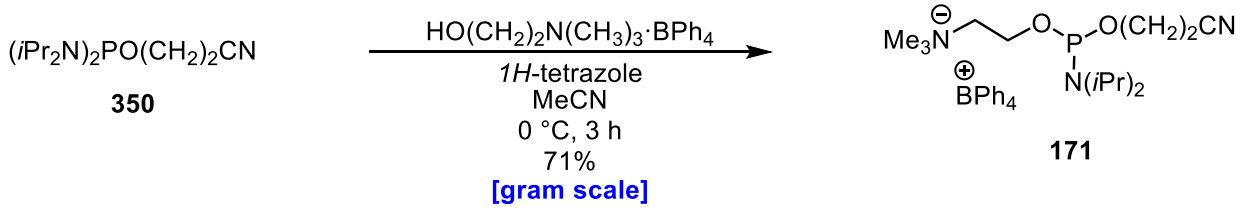
a) route to thioglycoside 348



The final building block was a phosphocholine precursor. For this we selected the phosphoramidite **171** as a synthetic target. A similar phosphoramidite (the tosylate salt) was utilized by the Schmidt group to install a phosphocholine moiety (Scheme 46).[225-226] Unfortunately, there was no published synthesis of the Schmidt phosphoramidite reagent. We hypothesized the phosphoramidite **171** cold come from the known compound **350**. Many procedures exist for the preparation of the phosphordiamidite **350**; in our hands it was best accomplished from an adapted procedure reported by Laneman and co-workers.[227] When access to phosphordiamidite **350** was achieved, we attempted synthesis of the tosylate salt of **171** used by the Schmidt group. The choline tosylate reagent proved to be incredible difficult to work with due to extremely low solubility. It required refluxing to go into an acetonitrile solution before adding the phosphorus reagent **350**. This procedure consistently provided mixtures of undesired products and low yields

of the desired tosylate. Rapid oxidation of the tosylate complicated attempts at purification of the reagent.

Scheme 47. Synthesis of phosphoramidite phosphocholination reagent 171

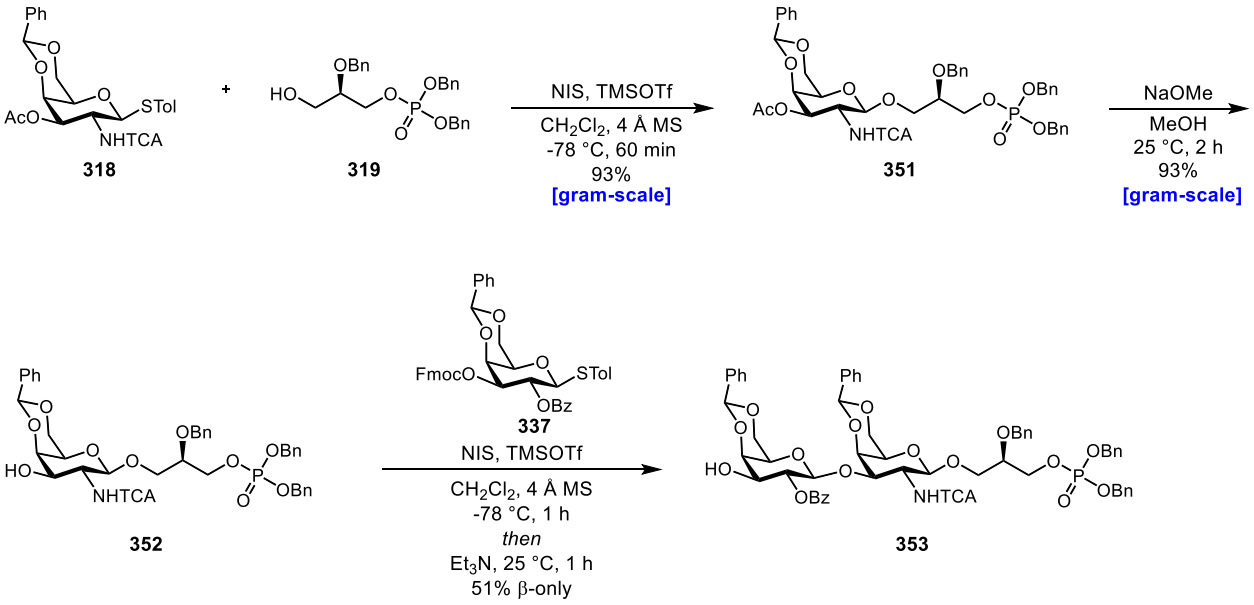


In a fortuitous turn of events, we discovered that the tetraphenyl borate salt **171** could be synthesized in high yield with high fidelity, primarily due to the enhanced solubility of the tetraphenyl borate-choline salt over the choline tosylate. Synthesis of the phosphoramidite **171** was achieved by treatment of phosphordiamidite **350** with choline tetra phenyl borate and tetrazole in acetonitrile. The phosphoramidite reagent **171** was surprisingly stable to oxidation. It could be handled in open air with minimal oxidation observed by NMR over several weeks. In addition, the reagent was stable to aqueous work up with helped to significantly purify the material. The choline tetraphenyl borate was prepared by a salt methathesis between the choline tosylate salt and sodium tetraphenyl borate in an aqueous solution at room temperature.

With the series of monosaccharide donor building blocks and phosphoramidite building blocks prepared, we were ready to begin the convergent, iterative glycosylation/deprotection, phase of synthesis towards the MM-ZPS repeating unit (Scheme 47). This began with the glycosidation between the alcohol **319** and the thioglycoside **318** to give beta linked phosphate product **351** in a 93% yield as a single anomer. Saponification of **351** lead to the acceptor **352** to be used in the following

glycosylation event. The glycosylation of **352** and the D-galactoside donor **337** resulted in a the disaccharide acceptor **353** which could be used directly in the following glycosylation due to Fmoc deprotection facilitated by triethylamine mediated quenching of the reaction.

Scheme 48. 3rd-generation route to MM-ZPS repeating unit



Unfortunately, the glycosylation/Fmoc deprotection procedure resulted in a low yield of product **353**. The reaction was complicated by an indiscernible co-polar by-product which possessed nearly identical NMR trace. Because the two products were unable to be separated, characterization of the two disaccharide species could not be confidently identified. The NMR yield of the major product of the mixture was 51% yield. Due to the similarities of the major and minor product, an no obvious sign of unwanted loss of any protecting group, we suspect the mixture may be attributed to conformationally locked rotamers. Previously, we have experienced this phenomenon when working with benzyl protected acetamido compounds (-NBnAc) which suffer from slow rotation around the C-

N bond as proven by Crich and co-workers.[155] Rather than committing additional time and resources to decipher what was giving rise to this anomaly we decided to move forward with an alternative glycosyl donor.

The acceptor **352** underwent a glycosylation event with thioglycoside donor **339** (Scheme 48). Fortunately, this donor/acceptor combination cleanly resulted in the protected disaccharide **354** in excellent yield as a single isolable diastereomer on milligram and gram scales. Treatment of the protected disaccharide **354** with HF-pyridine gave the C3'-acceptor **353**. Surprisingly, TBAF mediated conditions originally attempted resulted in very little conversion to the desired product. Protected phosphates can be deprotected under fluoride mediated conditions,[228-229] and this undesired outcome was experienced with both HF and TBAF mediated conditions over extended reaction times. Due to this unwanted reactivity, the best yields (77% desired product **353** with 10% RSM **354**) were obtained when prematurely working up the reaction and recovering the unreacted starting material which could then be re-subjected to reaction conditions.

Scheme 49. 3rd-generation route to MM-ZPS repeating unit continued

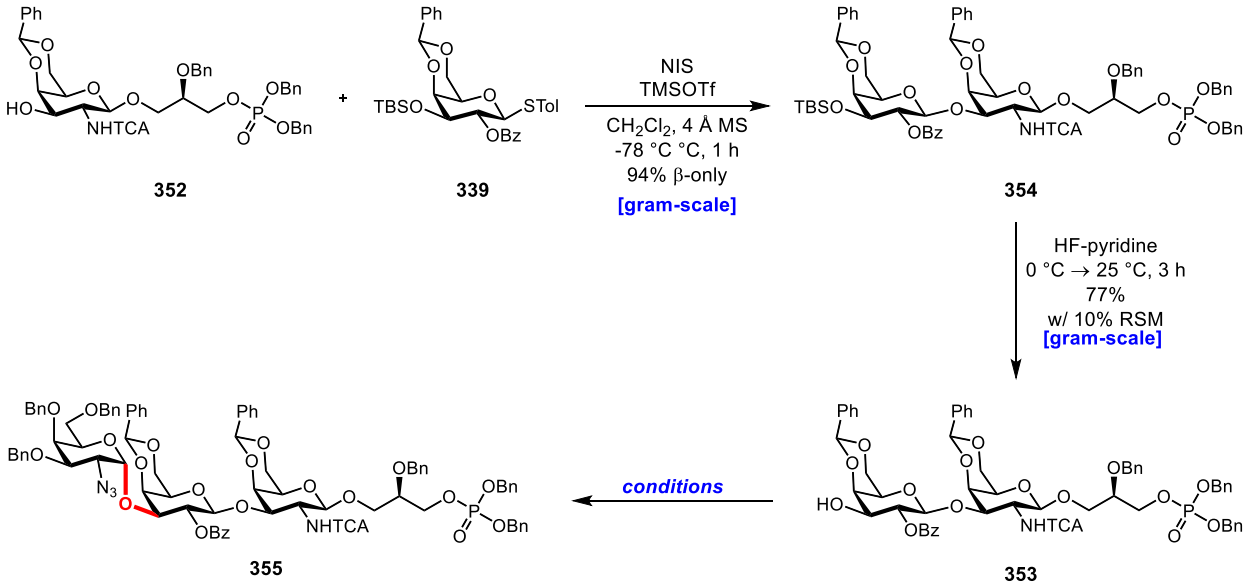


Table 9. Reaction conditions for 355

entry	*donor*	*equiv. of donor*	*conditions*	*yield*
1	182-β	2.2	TMSOTf, thiophene CH_2Cl_2, 4 Å MS -40 °C, 1 h	46
2	182-β	2.8	TMSOTf, thiophene CH_2Cl_2, 4 Å MS -78 °C, 2 h	39
3	182-α	1.5	TMSOTf, thiophene CH_2Cl_2, 4 Å MS -50 °C → -10 °C, 2 h	0[a]
4	182-α	4.0	TMSOTf, thiophene CH_2Cl_2, 4 Å MS -50 °C → -10 °C, 2 h	>15[a]
5	342	2.5	NIS, TMSOTf CH_2Cl_2, 4 Å MS -78 °C → -10 °C, 5 h	48
6	342	2.5	BF_3•Et_2O iPr_2O CH_2Cl_2, 4 Å MS -50 °C → 0 °C, 6 h	>10[a]

Glycosyl donors

182-β

182-α

342

undesired by-product

356

[a]quantative ammounts of RSM

Attempts at the final glycosylation began with the trichloroacetimidate **182-β** and acceptor **353** under TMSOTf mediated conditions in the presence of thiophene at -40 °C (scheme

48, table entry 1). This resulted in both alpha and beta products of **357**, as well as recovered acceptor **353**. To try to alter the reaction outcome, additional molar equivalence of donor **182-β** was used, but this had very little change in reaction outcome, only resulting in large amount of the known undesired rearrangement product **356** (entry 2). The α trichloroacetimidate donor **182-α** was next used, and as previously experienced with other routes, gave exclusively rearrangement product **356**. We then decided to use a different class of donor, once again, the thioglycoside. The tri-*O*-benzyl thioglycoside **342** was activated under NIS/TMSOTf conditions with acceptor **353** to give the desired alpha linked product **357** in 48% yield (scheme 48, table entry 5). Boron trifluoride mediated conditions were not successful at activating the thioglycoside donor **342** given the temperature and reaction time. While the TMSOTf/NIS mediated result was good, we decided to experimentally determine if the 4,6-*O*-benzylidene acetal protected thioglycosides donors (**348** and **349**) would perform in a glycosylation event with the disaccharide acceptor **353** superior to the 3,4,6-tri-*O*-benzyl-2-dexoy-2-azido galactoside donors (**182-α**, **182-β**, and **342**) (Scheme 49). It has been shown that the rigidity induced by the benzylidene acetal fused ring system can help stabilize the oxocarbenium reactive intermediate generated during a glycosylation event.[230] This is due to the greater strain imposed on the fused bicyclic structure; a phenomenon greatly exploited in the field of Crich's direct β-mannosylation via sulfoxide. [230-240] In contrast, tri-*O*-benzyl protected donors (**182-α**, **182-β**, and **342**) have no such additional conformation strain to help stabilize the reactive oxocarbenium intermediates.

Scheme 50. optimization of 3rd-generation route final glycosylation

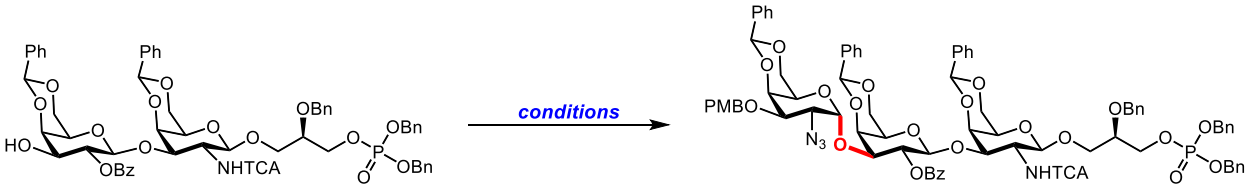
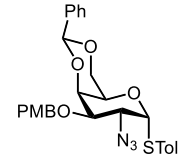
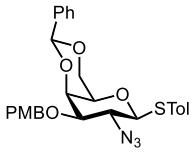


Table 10. Reaction conditions for 359

Glycosyl donors



349



348

entry	*donor*	*equiv. of donor*	*conditions*	*α/β ratio*	*yield*
1	**349**	**2.5**	NIS, TMSOTf *i*Pr$_2$O CH_2Cl_2, 4 Å MS -45 °C → -40 °C, 20 min	**2.2:1**	**96**
2	**349**	**2.1**	NIS, TMSOTf *i*Pr$_2$O CH_2Cl_2, 4 Å MS -78 °C, 2 h *then* -15 °C, 15 min	**2.8:1**	**61**
3	**349**	**2.5**	NIS, TMSOTf *i*Pr$_2$O CH_2Cl_2, 4 Å MS -15 °C, 20 min	**4.1:1**	**85**
4	**348**	**2.1**	NIS, TMSOTf *i*Pr$_2$O CH_2Cl_2, 4 Å MS -78 °C, 2 h	**1.2:1**	**85**
5	**348**	**2.5**	NIS, TMSOTf *i*Pr$_2$O CH_2Cl_2, 4 Å MS -15 °C, 20 min	**ND α-only**	**68**
6	**348**	**2.5**	NIS, TMSOTf *i*Pr$_2$O CH_2Cl_2, 4 Å MS -15 °C, 2 h	**3.7:1**	**82**

Initial attempts under NIS/TMSOTf mediated glycosylation conditions with the disaccharide acceptor **353** and the *p*-tolyl 3-*O*-p-methoxybenzoyl-4,6-*O*-benzylidene acetal-2-dexoy-2-azido-1-thio-α-D-galactoside donors **349** resulted in the 96% yield of a 2.2:1 alpha/beta ratio of product **359** (Scheme 49. entry 1). The alpha and beta linked products were separable by silica gel flash column chromatography resulting in around a 65% yield of the desired product **359**. We were ecstatic with this fantastic yield, however on a small scale. Determined to attempt this reaction on larger scales and even gram

scales, we first investigated factors which may positively influence the stereoselectivity of the product formation. The reaction was attempted under colder reaction conditions, but It was found that the donor did not adequately activate at -78 °C. Following, we allowed the reaction to warm to -15 °C. This resulted in a loss in yield however an increase in the desired stereoselectivity of the reaction. From these results we hypothesized that the reaction does not proceeded at -78 °C but does at, -15 °C and above. To test this hypothesis, we attempted the reaction again, with holding -15 °C constant; this resulted in highest selectivity for the alpha product **359** but did not maintain the yield of the first reaction attempt (entry 3). Next the beta thioglycoside **348** was tested in a glycosylation event with acceptor **353**. The beta thioglycoside was able to be activated at -78 °C in contrast to its alpha counterpart **349**, resulting a 1.2:1 alpha/beta ratio of products in good yield (entry 4). Like previous results, the beta thioglycoside performed well at elevated temperatures favoring the alpha linked desired product **359**. We concluded that optimized conditions could be achieved with either donor **348** or **349** at -15 °C in a NIS/TMSOTf mediated glycosylation in diisopropyl ether. These conditions held true when scaling up to over a gram scale (entry 6).

With these conditions determined, we synthesized gram quantities of the core trisaccharide **359**. The next stage of the planned synthetic route involved deprotecting of the C2'-OBz protecting group to provide the free C2' alcohol. The most standard conditions were first attempted (Scheme 50, entry 1). Methanolysis of the C2' benzoyl group with a sodium methoxide in methanol solution. Unfortunately, substochiometric equivalence, typically adequate for this type of deprotection, resulted in only untouched starting material. Additional NaOMe was added to the reaction (total of 1.0 equiv.). At 24

h, the reaction appeared to have consume all the starting material, however additionally resulted in obvious decomposition products. Other bases were tested, however most

Scheme 51. Continuation of 3rd-generation route and C2'-deprotection

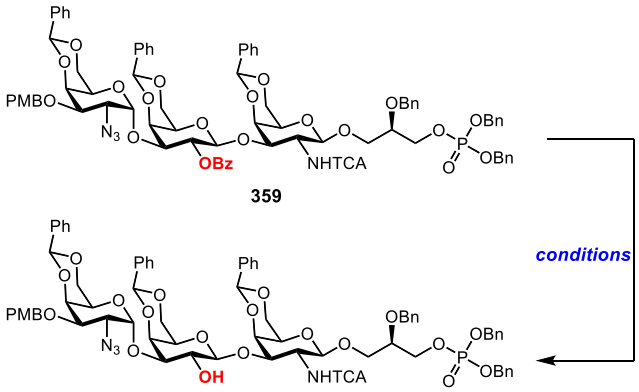


Table 11. Reaction conditions for 360

entry	*conditions*	*yield/outcome*
1	NaOMe (1.0 equiv.) MeOH/THF 1:1 25 °C, 24 h	**43%**
2	NaOH (5.0 equiv.) THF 25 °C, 1 h 40 °C, 20 min	**NR**
3	NaOH (5.0 equiv.) MeOH/THF 1:1 40 °C, 20 min	**64%**
4	hydrazine (10.0 equiv.) MeOH/THF 1:1 25 °C, 2 h	***decomposition***
5	NH_3 in MeOH (280 equiv.) THF 25 °C, 18 h	**NR**
6	NH_3 in MeOH (280 equiv.) THF 40 °C, 18 h	**NR**
7	NH_3 in MeOH (280 equiv.) THF μwave 120 °C, 20 min	***decompoisiton***
8	KCN (5.6 equiv.) MeOH/THF 1:1 40 °C, 18 h	**NR**
9	KOH (5.0 equiv.) THF 25 °C, 1 h 40 °C, 20 min	***decomposition***
10	KOH MeOH/THF 1:1 40 °C, 20 min	**54%**
11	K_2CO_3 (>1.0 equiv.) 1:1 MeOH/THF 40 °C, 3 h	**60%**
12	K_2CO_3 (10.0 equiv.) 1:1 MeOH/THF 40 °C, 12 h *then* rt, 16 h	**57%**
13	K_2CO_3 (25.0 equiv.) 2:1 MeOH/THF 60 °C, 45 min	**71%**

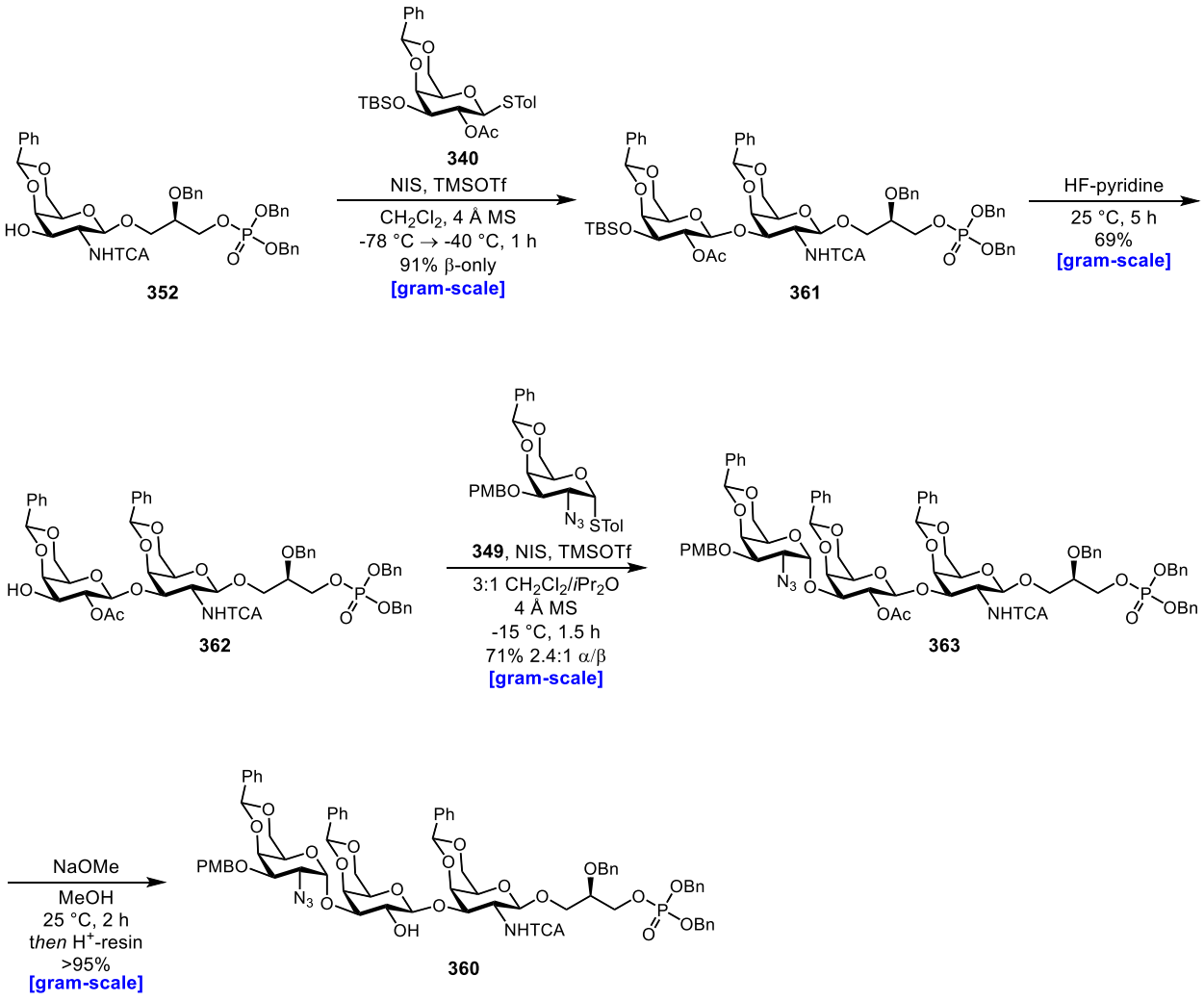
resulted in either no reaction or resulted in decomposition due to interference with the

phosphate residue (entries 2-11). The most productive reagent for the conversion of protected **359** to the alcohol **360** was potassium carbonate in a MeOH/THF solution heated to 60 °C for short reaction times (entry 13). Monitoring the reaction was key because extensive reaction times would ultimately result in complete decomposition and no recovery of the desired product **360**.

Screening of conditions to deprotect the C2'-OBz depleted the trisaccharide material, when bringing up material we decided to attempt to make the trisaccharide **360** which contains a C2'-OAc in place of the OBz protecting group. We initially planned to use the OBz over the OAc for the C2' protection to try and limit potential of orthoacetate formation. In general, the benzoyl group produced less ortho ester products while still having the ability to be a participating protecting group which leads to beta glycosidation products.[241] Since the C2-OAc thioglycoside donor **340** variant was already synthesized, we decided to see how it performed in a glycosylation with acceptor **352** (Scheme 51). Fortunately, no ortho-acetate was detected during the glycosylation with the C2-OAc thioglycoside donor **340** and monosaccharide acceptor **352**. The glycosylation worked well on small scale and on gram scale to provide the corresponding disaccharide product **361** in 91% yield as the β-linked glycoside as the only detectable product using conditions previously developed for an earlier route. Using developed conditions of TBS removal, the disaccharide acceptor **362** was produced in good yield from **361** by treatment with HF-pyridine. The final glycosylation proceeded using the previously developed conditions to give the core trisaccharide. Donor **349** was activated using NIS/TMSOTf conditions in the presence of acceptor **362** to provide the trisaccharide **363** in a 71% yield as a 2.4:1 mixture of alpha/beta-C1''-linked trisaccharides. The trisaccharides were able to be

separated using silica gel flash column chromatography, ultimately providing the desired product in a 50% yield. Given the late stage of synthesis we decided to move forward with this yield. Additionally, we were able to initially explore the final steps using the undesired C1''-β-linked trisaccharide.

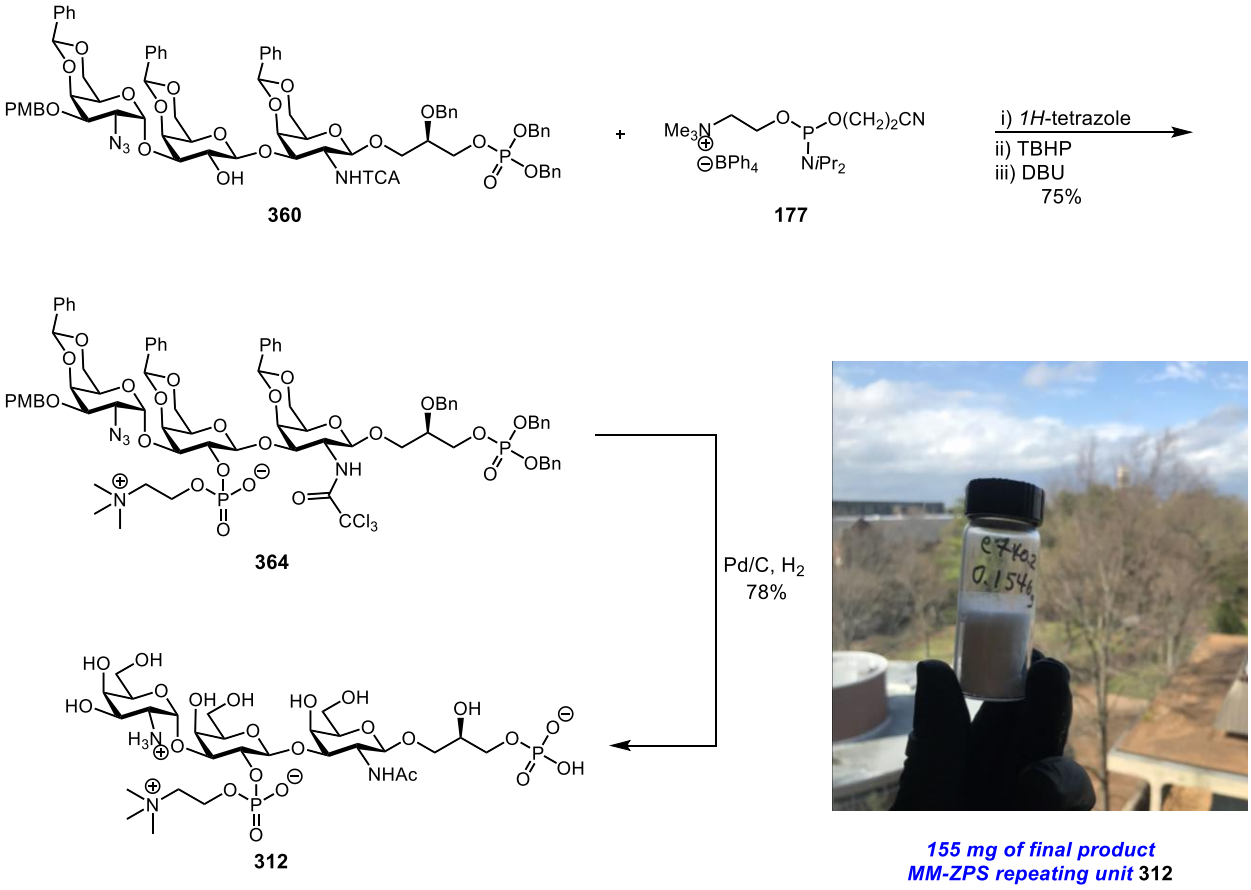
Scheme 52. Continuation of 3rd-generation route



With multigram quantities of the C2'-OAc protected trisaccharide **363** generated, we were ready to attempt the C2' deprotection which was previously difficult when attempting to remove the C2'-OBz protecting group. The acetate protecting group proved to be much

easier to remove. Treatment of acetate **363** with sodium methoxide in methanol removed the acetate protecting group to provide alcohol **360** (Scheme 52). The route was ready to attempt the final transformations including: phosphocholination and global deprotection. Given the numerous conditions which have been developed to install phosphocholine residues,[228, 242-254] we elected to use the tetraphenyl borate phosphoramidite **177** previously described. This is mainly to the simplistic coupling/oxidation procedure required for installation of the phosphocholine moiety. Many other phosphocholination procedures require multiple steps (coupling of the phosphate then trimethyl amine introductions) which typically lead to low yields. After developing our phosphocholine coupling reagent **177** and testing it on model systems, we were ready for the penultimate step, attempting the coupling to the trisaccharide **360**. The trisaccharide **360** was coupled with the phosphoramidite **177** in a tetrazole mediated process. This was then treated with *tert*-butyl hydroperoxide to oxidize the resulting phosphite to the phosphate. Finally, the resulting phosphate was treated with amine base to deprotect the cyano-ethyl group, resulting in a zwitterionic phosphocholine residue on the trisaccharide **364** in an excellent yield.

Scheme 53. Conclusion of 3rd-generation route



With the fully protected product **364** in hand we attempted the global deprotection of **364** by hydrogenation conditions. This reaction removed three benzylidene acetals, one PMB ether, and three benzyl ethers. The reductive environment also converted the C2-trichloroacetamide to the desired C2-acetamide and the C2'' azide to a desired C2-amine. Starting from an 0.33 mg (0.20 mmol) reaction, purification under size exclusion chromatography (P2-Biogel) gave 155 mg of MM-ZPS 36 in 89% isolated yield. Charge deconvoluted ESI FT ICR mass spectrometry revealed molecular mass spectral peaks in agreement with the mass calculated for **312** (calc'd [M+H] for $C_{28}H_{55}N_3O_{23}P_2$ = 864.2780, found 864.2772). ^{1}H NMR coupling constants revealed the presence of one α (C1'', 5.39

ppm, 3.82 Hz) and two β (C1', 4.69 ppm, 7.83 Hz; C1, 4.41 ppm, 7.56 Hz) glycosidic linkages. 2-D NMR experiments, including a ^{1}H-^{31}P HSQC experiment, allowed for full assignment of the ZPS's glycosidic bonds and phosphorus functionality. Additionally, the NMR data from the synthesized repeated unit **312** shows good agreement with that report-ed for the natural MM-ZPS polymer (See SI for NMR data comparison table).

In summary, we have completed the first total synthesis of the repeating unit of MM-ZPS **312**. Key steps include (1) early stage phosphoglycerol glycosylation occurring in high yield and excellent β-selectivity, (2) formation of the challenging D-Gal-β-(1→3)-D-GalNAc bond without generating any undesired orthoacetate byproduct, and (3) instillation of the phosphocholine group using tetraphenyl borate phosphocholine reagent **171**. As studies with the naturally occurring MM-ZPS demonstrated that single repeating units do not elicit an immune response, current efforts are focused on using the synthetic repeating units as an ink to 3-D print MM-ZPS materials of defined lengths. Studies regarding the synthesis and immunological properties of these materials are currently underway.

Chemical synthesis of complex tri- to oligosaccharides is still a challenging task for organic chemist of the day. Carbohydrates are unlike the other major classes of biopolymers often characterized by highly branched motifs. Each five- or six- membered ring monosaccharide unit has multiple sites of attachment to the next sugar moiety. Additionally, each glycosidic linkage connecting two sugar units can take on one of the two possible isomeric forms. There are more than 1,000 different trisaccharides possible when the nine mammalian monosaccharides are combined.[255] Given the structural complexity of carbohydrates, regio- and stereoselectivity of glycosylation reactions is the

key challenge for the assembly of oligosaccharides. Synthetic chemists have developed increasingly powerful and versatile methods that have resulted in the assembly of every-more complex oligosaccharides and glycosaminoglycans. Still, the preparation of such structures remained technically difficult, extremely time consuming and has been carried out by a relatively limited number of synthetic chemists.[256] The current state of oligosaccharide synthesis relies heavily on the use of protecting groups, which can substantially alter the outcome of key glycosylation reactions.[99, 257-263] Collectively, these synthetic hurdles render the vast field of oligosaccharide synthesis relatively unexplored despite that synthesis of oligosaccharides has been pursued for more than 100 years. There is currently no standard operating procedure for complex oligosaccharide synthesis; leading to a *de novo* approach of each synthetic target. To simplify the designing an oligosaccharide synthetic campaign we have complied a list of lessons learned from our endeavors. These integral lessons are apparent when comparing the progression of success between *first-, second-* and *third-generation* routes. The synthetic lessons learned primarily center around efficiency and chemical logistics of synthetic route. These lessons and synthetic strategies, which were key include: 1) utilization of previously published monosaccharide donor/acceptor building blocks; 2) increase convergency of the synthetic route by limiting synthetic steps between glycosylation events; 3) synthesis the target oligosaccharide from reducing end to non-reducing end; 4) use a slight excess of donor relative to the acceptor for glycosylation events and monitor the reaction progress by consumption of the acceptor; 5) ensure that the donor is less expensive and/or synthetically easier to access material in each glycosylation

event. Adherence to these key lessons ultimately allowed for our final synthetic attempt of the total synthesis of the MM-ZPS repeating unit to be successful.

Part 9. Proposed synthesis of the ZPS-PC3240

The zwitterionic *O*-polysaccharide from *Photorhabdus temperate* subsp. *Cinereal* 3240 (ZPS-PC3240) isolated any characterized by Knirel and co-workers in 2015.[15]

The repeating unit has a structure of: →3)-β-D-Gal*p*NAc-4-(PEtN)- β-(1→4)-D-Glc*p*A-(1→3)-β-D-FucNAc4N-(1→.[15] Shown below is the structure of the PC-3240 ZPS (Figure 42). Shown below, in red, is a representative repeating unit structure which could be selected for total synthesis. This selection benefits from the other options by providing the most simplistic combination of monosaccharide building blocks. This molecule benefits from containing only *1,2-trans* glycosidic linkages which could be formed from participating C2-protecting groups. Keeping these basic principles of oligosaccharide synthesis, laid out in the sections above in, mind, successful synthetic routes can quickly be developed.

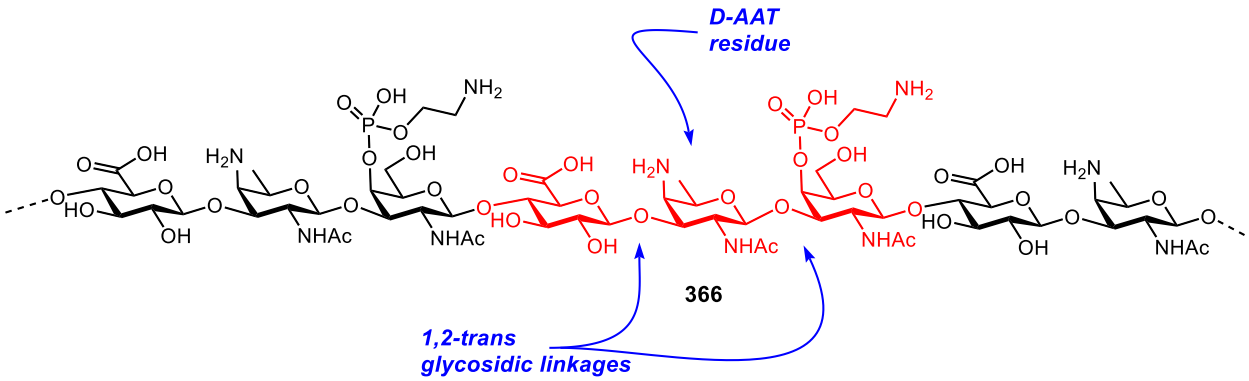


Figure 42. Repeating unit selection of proposed synthesis of the ZPS-PC3240 ZPS

Retrosynthetic analysis of the ZPS-PC40 **366** reveals that a repeating unit **367** could be selected from the polymeric structure of the ZPS **366** (Figure 42 shown in red).

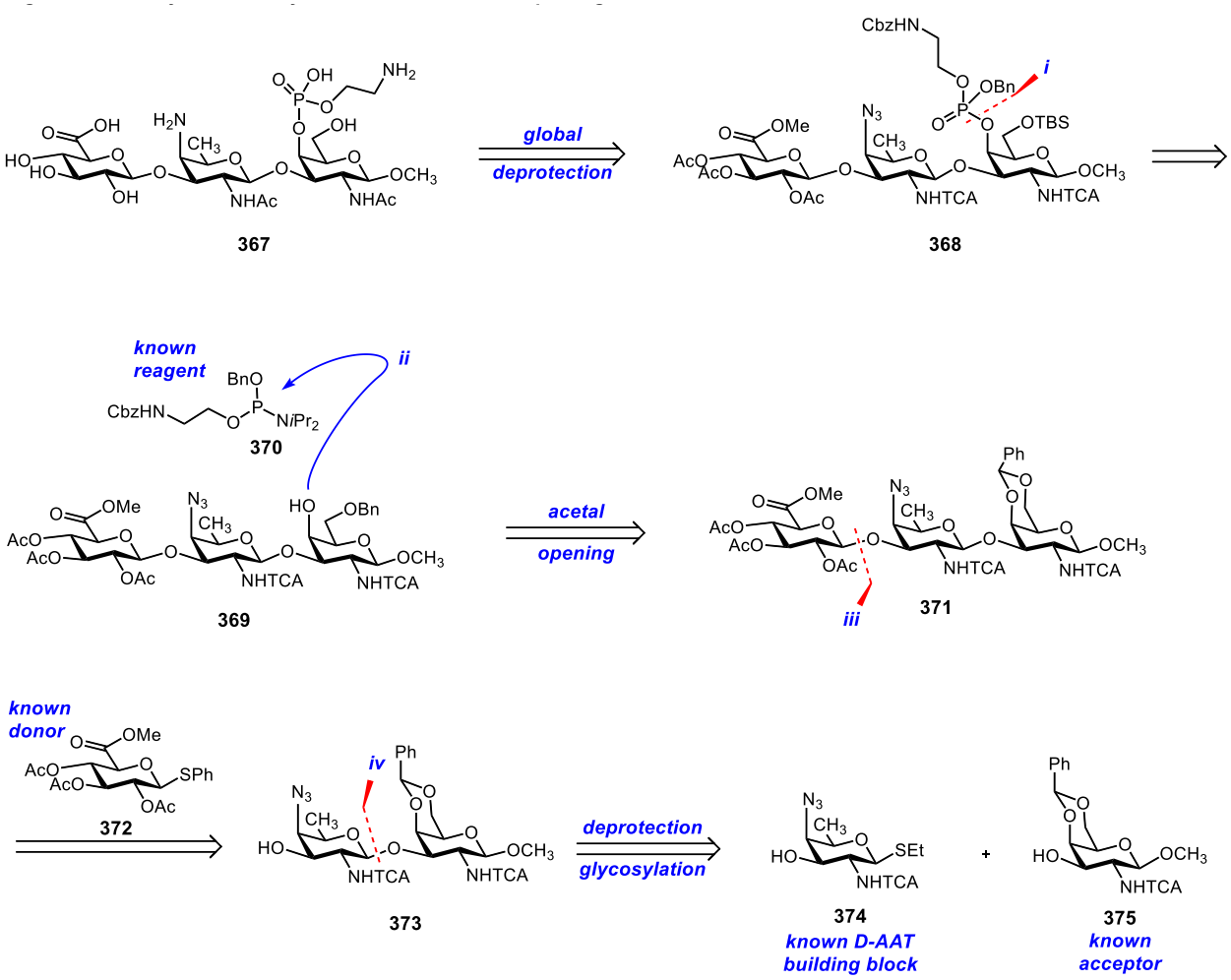


Figure 43. Retrosynthetic analysis of the ZPS-PC3240 repeating unit

Selecting **367** as the synthetic target, the trisaccharide **368** could come from a global deprotection (Figure 43). Disconnection of the phosphorus residue could provide

the known phosphoramidite reagent **370** and the core trisaccharide (disconnection *i*, bond forming step *ii*).[264-283] A selective benzylidene acetal ring opening of the C4, C6 protecting group could afford the C6 benzyl and the C4 free alcohol in **371**. Alternatively, the benzylidene acetal could be completely removed and the C6 position could be selectively protected by a TBS protected to provide the free C4-OH ready for phosphate incorporation at the C4 position. Disconnection of the D-GluAA-β-(1→3)-D-AAT bond could provide the disaccharide acceptor **373** and the known D-GluAA donor **372** (disconnection *iii*).[284-285] Alternatively, known D-glucuronide donors could be utilized.[286-294] Following a deprotection event, disconnection of the D-AAT-β-(1→3)-D-Gal bond of disaccharide **373** would give the D-ATT donor **374** known D-GalNTCA acceptor **375** (disconnection *iv*).

The D-GalNTCA acceptor **375** could come from a glycosidation of the thioglycoside **318** from the *third-generation* route or the acetamide protected derivative **179** from the *first-generation* route could be used as well. The known D-ATT thioglycoside **374** reported by Pozsgay and co-workers could be utilized for this synthetic route.[80, 82] In addition, relatively minor protecting group changes to the D-ATT thioglycoside intermediate reported by Oscarson and co-workers or Mulard could provide a suitable D-ATT donor.[17, 79] This route could successfully provide access to the repeating unit **367**, quickly and efficiently, by adhering to the basic principles learned over the synthesis **312** discussed above.

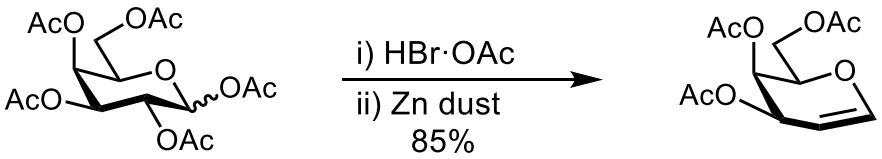
Chapter 3

Experimental section

Part 1. MM-ZPS 1st-generation synthetic route

Part 1 contains experimental procedures for compounds found within schemes 22-28

3,4,6-Tri-*O*-acetyl-D-galactal (185)

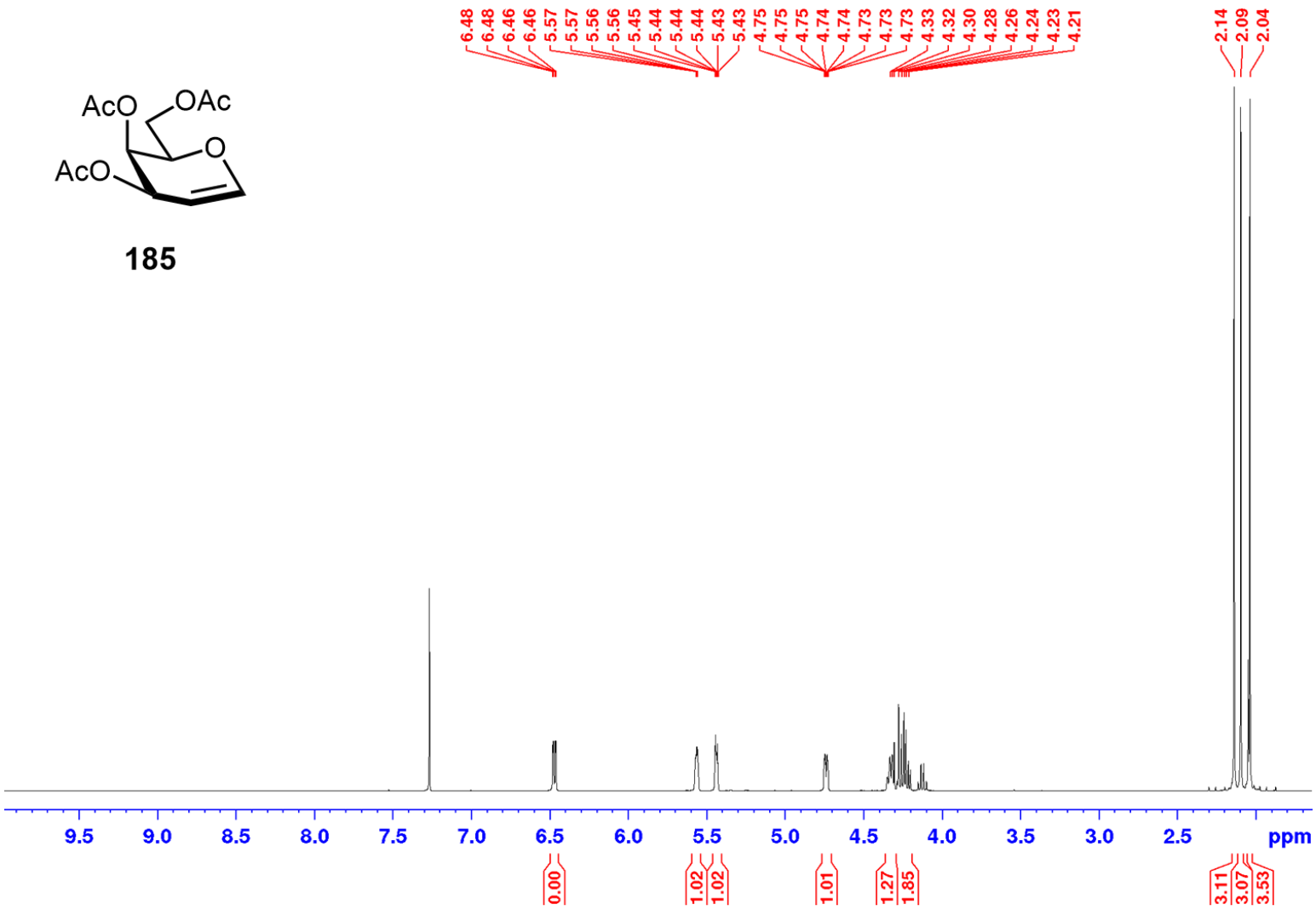


184 **185**

Commercially available 1,2,3,4,6-penta-*O*-acetyl-α/β-D-galactopyranoside **184** (1.0 equiv., 39.00 g, 100.00 mmol) was dissolved in anhydrous CH_2Cl_2 (300 mL) and added HBr (5.0 equiv., 82.0 mL, 500.0 mmol, 33% in AcOH) at 0 °C under an argon atmosphere. After 2 h, TLC indicated that the starting material had been complete consumed. The solution was diluted with CH_2Cl_2 (200 mL), washed with ice water (1 x 100 mL), satd. aq. $NaHCO_3$ (3 x 100 mL), brine (1 x 50 mL) and dried ($NaSO_4$). The organics were filtered and concentrated to give 2,3,4,6-tetra-*O*-acetyl-α-bromo-D-galactopyranoside (39.8 g, 96.8 mmol, >95% step 1) as a white foam. Spectroscopic data agreed with previously reported data. A solution of bromo 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranoside (1.0 equiv., 39.8 g, 96.8 mmol) in AcOH (500 mL) was added copper(II) sulfate (0.3 equiv., 4.78 mg, 30 mmol) and zinc dust (16.0 equiv., 104.6 g, 1.600 mmol) slowly over the course of 5 min at room temperature under and argon atmosphere. After 30 min, the reaction was diluted with EtOAc (350 mL) and transferred to a separatory funnel. The organics were washed with H_2O (1 x 100 mL), sat. aq., $NaHCO_3$ (3 x 100 mL), 40% sodium thiosulfate (2 x 100 mL), brine (1 x 50 mL), dried ($NaSO_4$), filtered through a glass fritted vacuum filter funnel to remove drying agent, and concentrated clear oil. Upon standing in a reduced atmosphere overnight, the oil produced a white crystallin solid 3,4,6-tri-*O*-acetyl-D-galactal **185** (24.424 g, 89.6 mmol, 90% over two steps) which was to be used with no further purification. Spectroscopic data agreed with previously reported

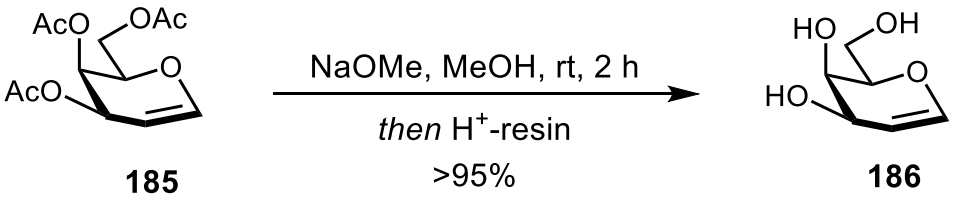
data.[295-300] ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.46 (dd, *J* = 1.7, 6.3 Hz, 1H), 5.57-5.56 (m, 1H), 5.44-5.43 (m, 1H), 4.74 (ddd, *J* = 1.3, 1.4, 3.6 Hz, 1H), 4.34-4.30 (m, 1H), 4.27-4.21 (m, 2H), 2.14 (s, 3H), 2.09 (s, 3H), 2.03 (s, 3H).

^{1}H NMR ($CDCl_3$, 400 MHz) of compound 185



185

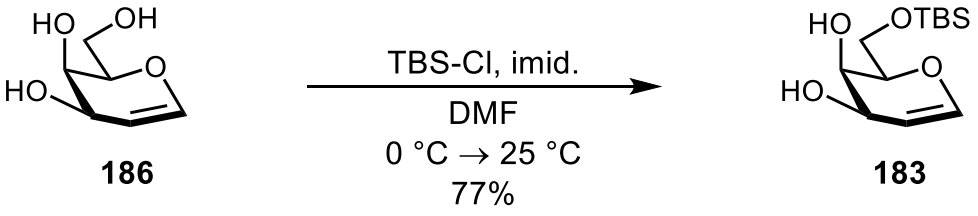
D-Galactal triol (186)



185 **186**

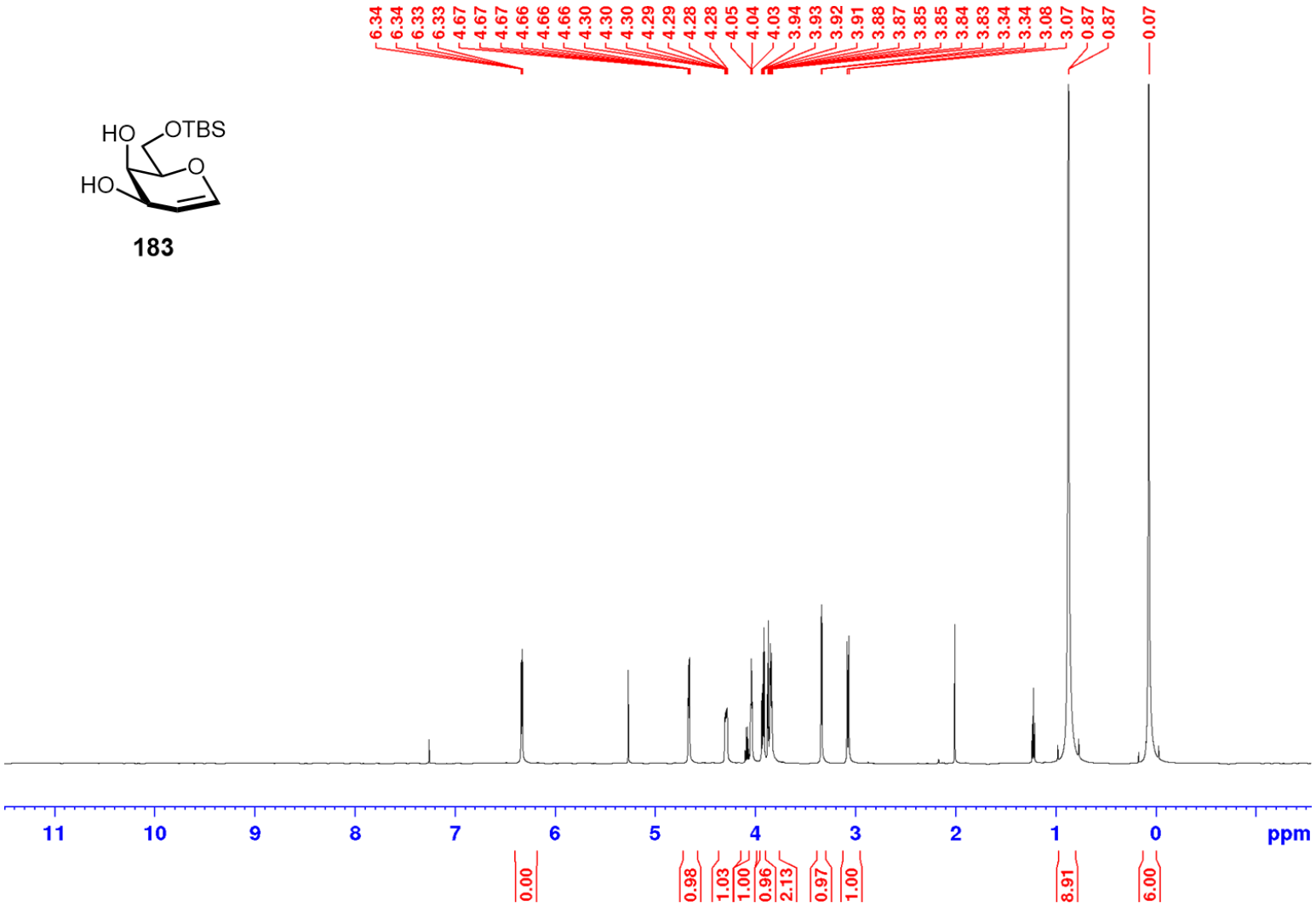
A solution of 3,4,6-tri-*O*-acetyl-D-galactal **185** (1.0 equiv., 24.4 g, 89.5 mmol) in anhydrous MeOH (250 mL) was treated with a freshly made solution of 2 N sodium methoxide in methanol (1.1 equiv., 50 mL, 100 mmol) at room temperature under an argon atmosphere. The pH of the solution was >11. At 2 h, the reaction was neutralized by the addition of Amberlite IR 120 (H^+) ion exchange resin. The solution was filtered through a glass fritted funnel with a pad of Celite to remove the resin. The filtrate was concentrated to dryness to give a crude triol. The crude material was passed through a plug of silica (70:30 to 85:15 EtOAc/hexanes) to give D-galactal triol **186** (12.7 g, 89.5 mmol, >95%) as a white solid without any need of further purification. Spectroscopic data matches previously reported data.[300-302]

6-*O*-tert-butyldimethylsilyl-D-galactal (183)

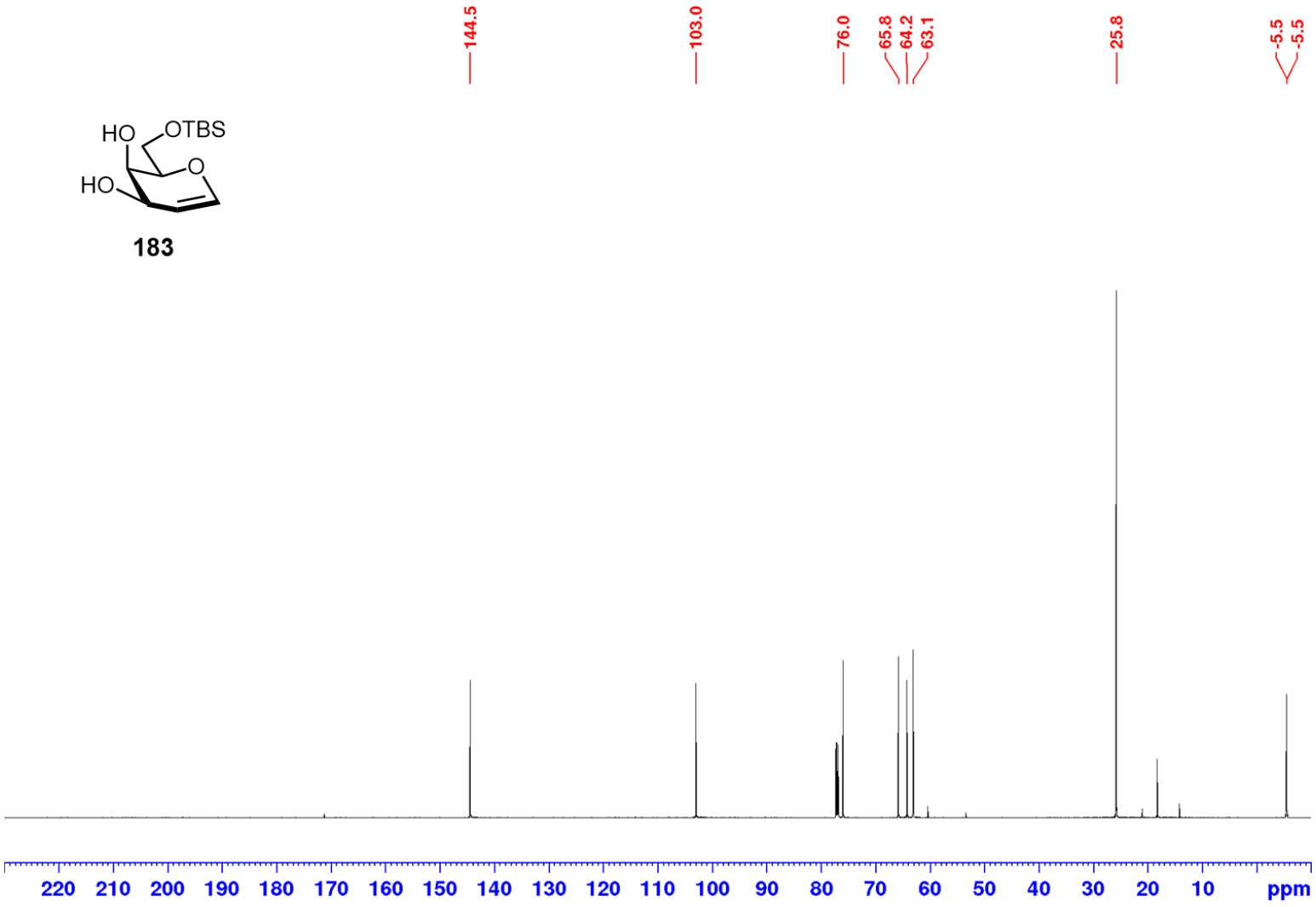


A solution of tert-butyldimethylsilyl chloride as a 50% wt. in toluene (1.0 equiv., 12.4 g, 41.1 mmol) was added dropwise to a solution of D-galactal triol **186** (1.0 equiv., 6.00 g, 41.1 mmol) and imidazole (2.2 equiv., 6.10 g, 89.7 mmol) in anhydrous DMF (30.0 mL) under an argon atmosphere at 0 °C. The solution warmed to room temperature while stirring. At 12 h, the mixture was partitioned between EtOAc (250 mL) and water (250 mL). The aqueous layer was extracted with EtOAc (2 x 250 mL) and the combined organic layers were dried ($MgSO_4$) and filtered through a glass fritted vacuum filter funnel to remove drying agent. The filtrate was concentrated *in vacuo* to a crude oil. The residue was purified by silica gel flash chromatography (80:20 hexanes/EtOAc) to give 6-*O-tert*-butyldimethylsilyl-D-galactal **183** (8.18 g, 31.4 mmol, 77%) as a white solid. The spectroscopic data agreed with the previously reported data.[303-305] R_f 0.54 (2:3 hexanes/EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.36 (dd, *J* = 1.28, 6.26 Hz, 1H), 4.70 (dt, *J* = 1.75, 6.16 Hz, 1H), 4.33-4.28 (m, 1H), 4.08 (t, *J* = 4.82 Hz, 1H), 3.98-3.85 (m, 3H), 3.23-3.21 (m, 1H), 2.84-2.79 (m, 1H), 0.90 (s, 9H), 0.09 (s, 6H); ^{13}C (100 MHz, $CDCl_3$) δ 144.9, 103.4, 76.1, 66.4, 64.5, 63.7, 26.2, 18.6, -5.14, -5.2; LRMS (ESI) calcd for $C_{12}H_{24}O_4SiNa$ $(M+Na)^+$ 282.989218 found 282.98 m/z.

^{1}H NMR ($CDCl_3$, 400 MHz) of compound 183

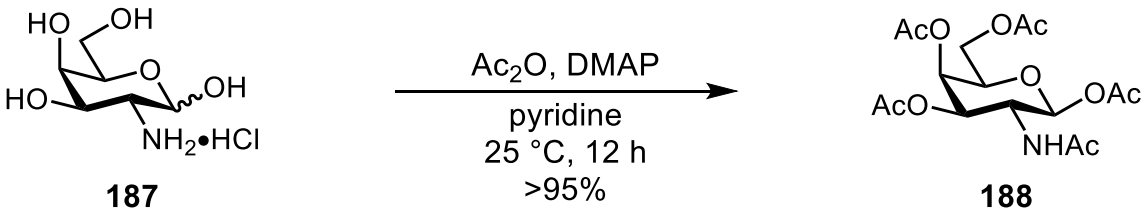


^{13}C NMR ($CDCl_3$, 100 MHz) of compound 183



183

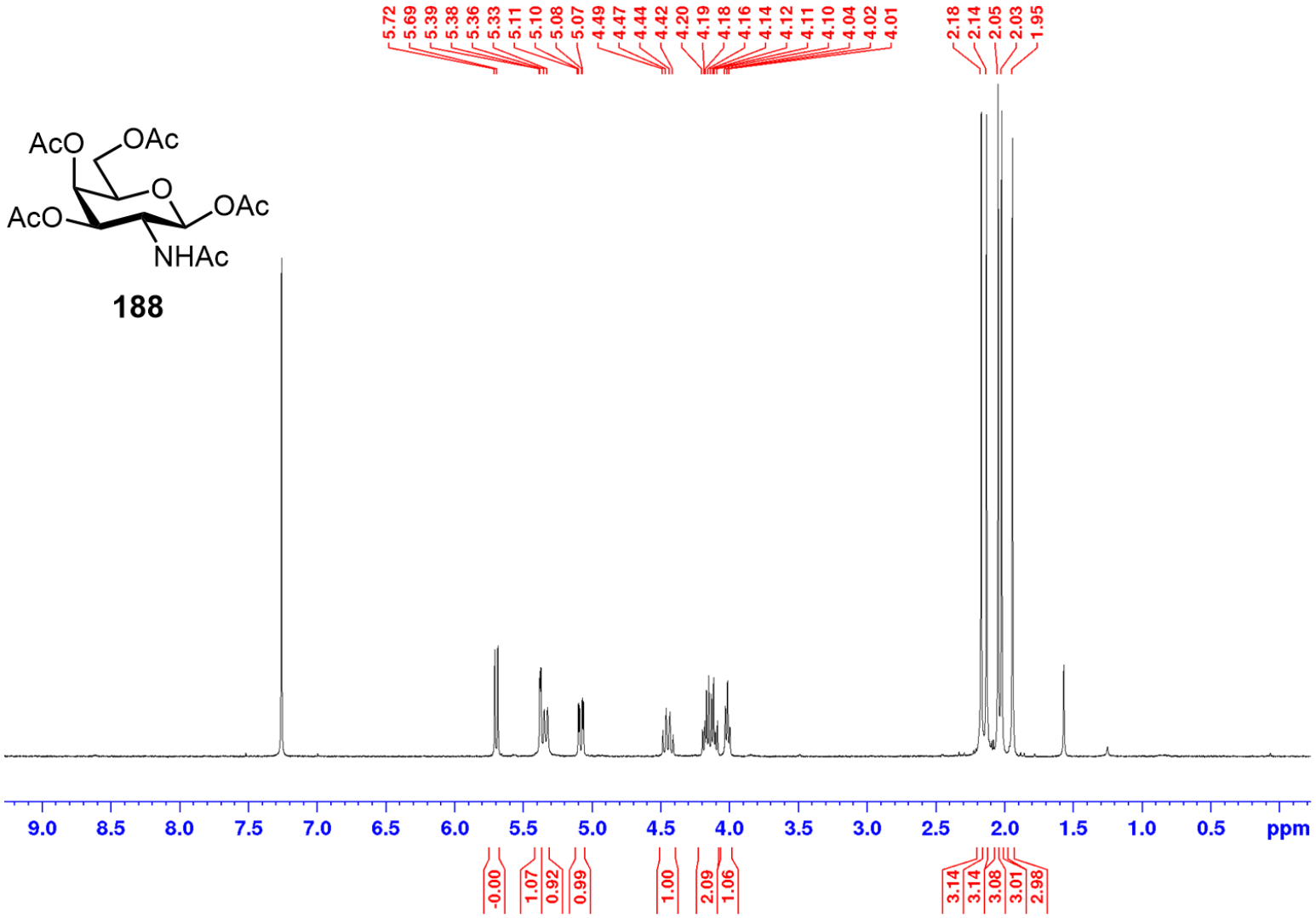
1,3,4,6-Tetra-*O*-acetyl-2-deoxy-2-acetamido-β-D-galactoside (188)



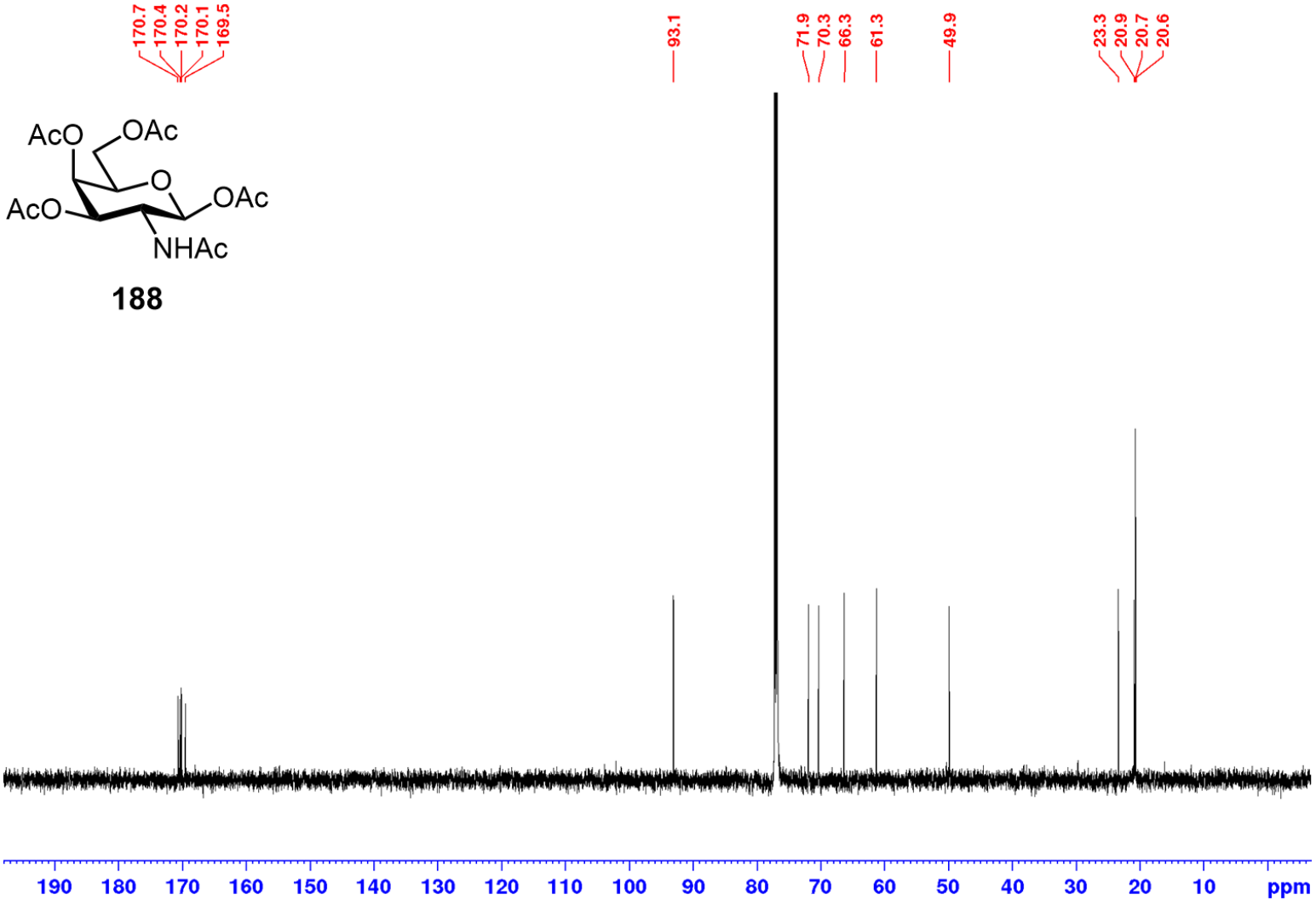
187 **188**

A solution of galactosamine HCl **187** (1.0 eq, 2.00 g, 9.28 mmol) in acetic anhydride (7.0 eq, 6.14 mL, 64.9 mmol) was added pyridine (29.0 eq, 30.0 mL, 269 mmol) at 0 °C and allowed to warm to room temperature. At 12 hours, the pyridine was removed *in vacuo* by co-evaporation with toluene (5 x 40 ml). The crude residue was recrystallized from methanol to give per-acetylated D-GalNAc **188** (3.47 g, 8.90 mmol, 96%) as a white solid. Our characterization data were consistent with previously reported data [306]. R_f 0.84 (9:1 CH_2Cl_2/MeOH); ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.69 (d, *J* = 8.73 Hz, 1H), 5.37 (d, *J* = 2.53 Hz, 1H), 5.32 (d, *J* = 9.19 Hz, 1H), 5.08 (dd, *J* = 3.44, 11.25 Hz, 1H), 4.48-4.41 (m, 1H), 4.19-4.08 (m, 2H), 4.03-3.99 (m, 1H), 2.17 (s, 3H), 2.13 (s, 3H), 2.04 (s, 3H), 2.02 (s, 3H), 1.94 (s, 3H); ^{13}C (100 MHz, $CDCl_3$) δ 171.1, 170.7, 170.6, 170.5, 169.9, 93.4, 72.3, 70.7, 66.7, 61.6, 50.2, 23.7, 21.3, 21.0, 21.0, 21.0.

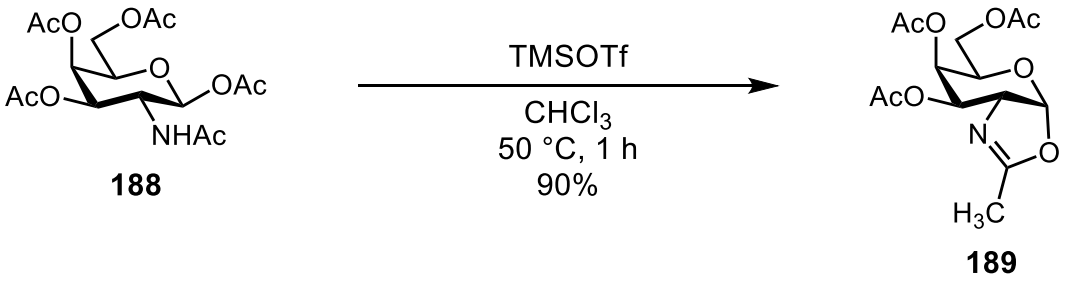
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 188



^{13}C NMR ($CDCl_3$, 100 MHz) of compound 188

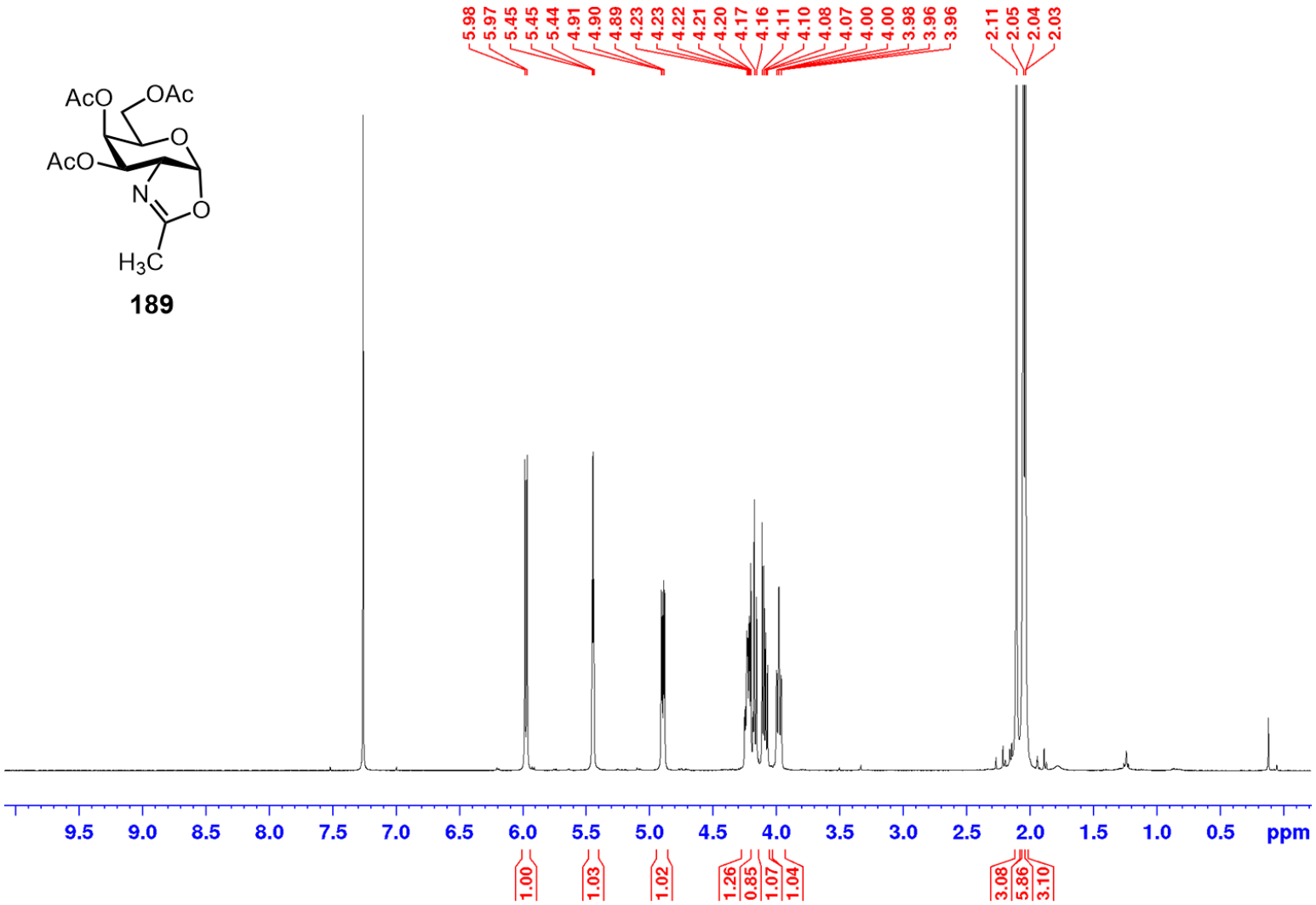


2,3-Dihydrooxazole-3,4,6-tri-*O*-acetyl-α-D-galactopyranoside (189)

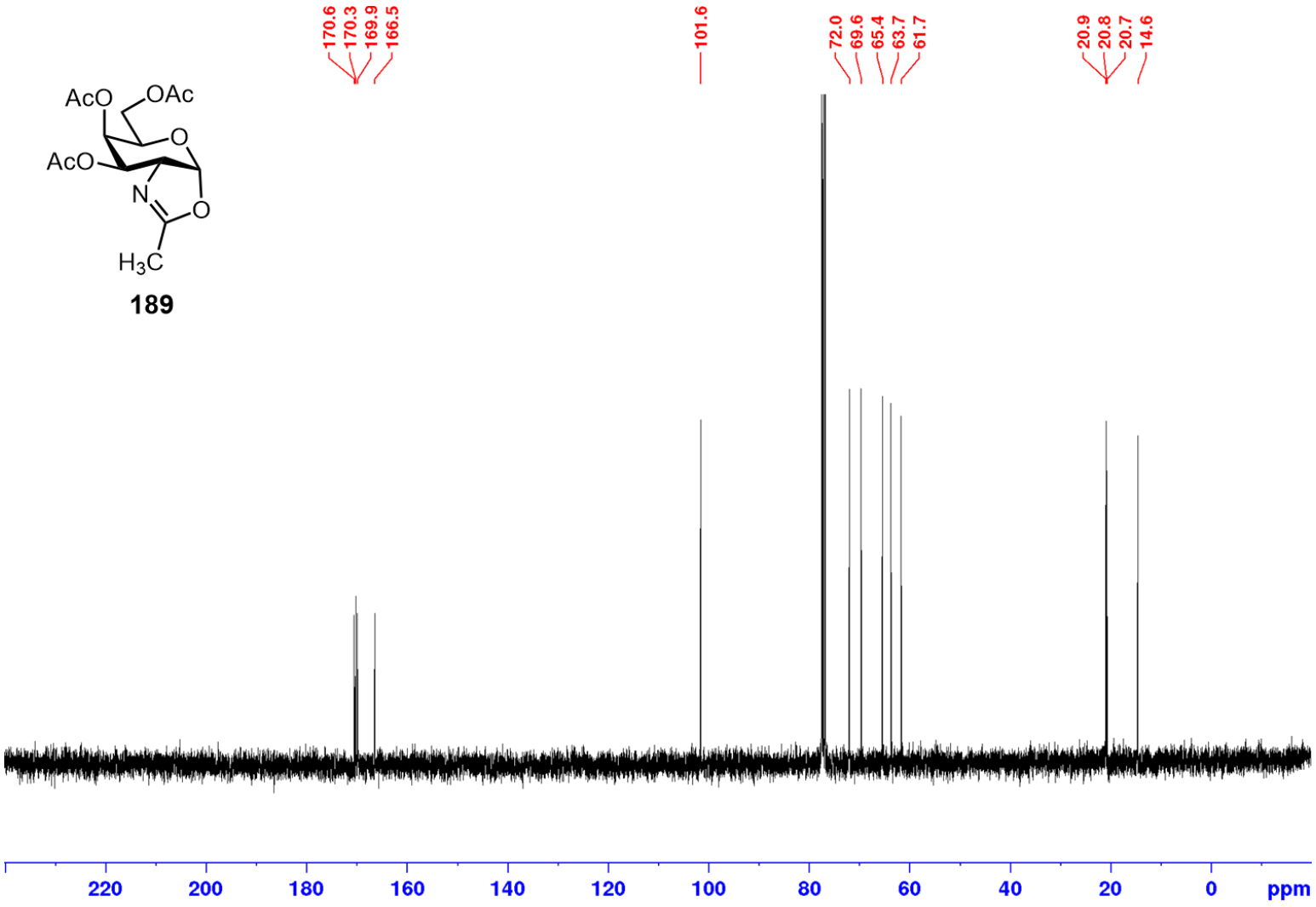


A solution of D-GalNAc **188** (1.0 equiv., 404 mg, 1.04 mmol) in $CHCl_3$ (28.6 mL) was treated with TMSOTf (1.0 equiv., 0.200 mL, 1.08 mmol) at room temperature, heated at 50 ºC. At 1 h the reaction was cooled and treated with NEt_3 (3.0 equiv., 0.440 mL, 3.08 mmol). The mixture was stirred at room temperature for 10 min and then filtered through a plug of silica. The filtrate was concentrated to an oil and purified by silica gel flash column chromatography (100% EtOAc, SiO_2 was base-washed with 1% NEt_3 prior to use) to yield D-galactoside **189** (0.308 g, 0.935 mmol, 90%) as an orange oil. Our spectroscopic data agreed with previous reports.[306] R_f 0.30 (EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.98 (d, *J* = 6.7 Hz, 1H), 5.44 (dd, *J* = 2.9, 3.0 Hz, 1H), 4.9 (dd, *J* = 3.4, 7.4 Hz, 1H), 4.25-4.20 (m, 1H), 4.17 (d, *J* = 6.7 Hz, 1H), 4.11-4.07 (m, 1H), 3.98 (dd, *J* = 1.0, 7.3 Hz, 1H), 2.1 (s, 3H), 2.05 (s, 6H), 2.03 (s, 3H); ^{13}C (100 MHz, $CDCl_3$) δ 170.6, 170.3, 169.9, 166.5, 101.6, 72, 69.6, 65.4, 63.7, 61.7, 20.9, 20.8, 20.7, 14.6.

^{1}H NMR ($CDCl_3$, 400 MHz) of compound 189

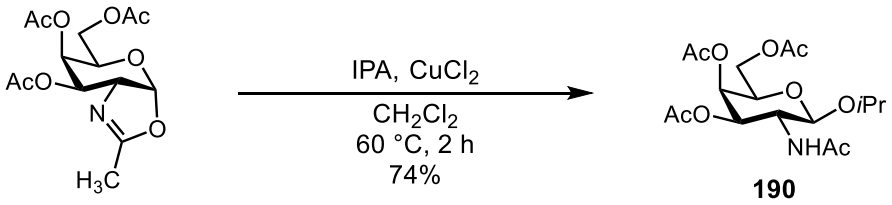


^{13}C NMR ($CDCl_3$, 100 MHz) of compound 189



189

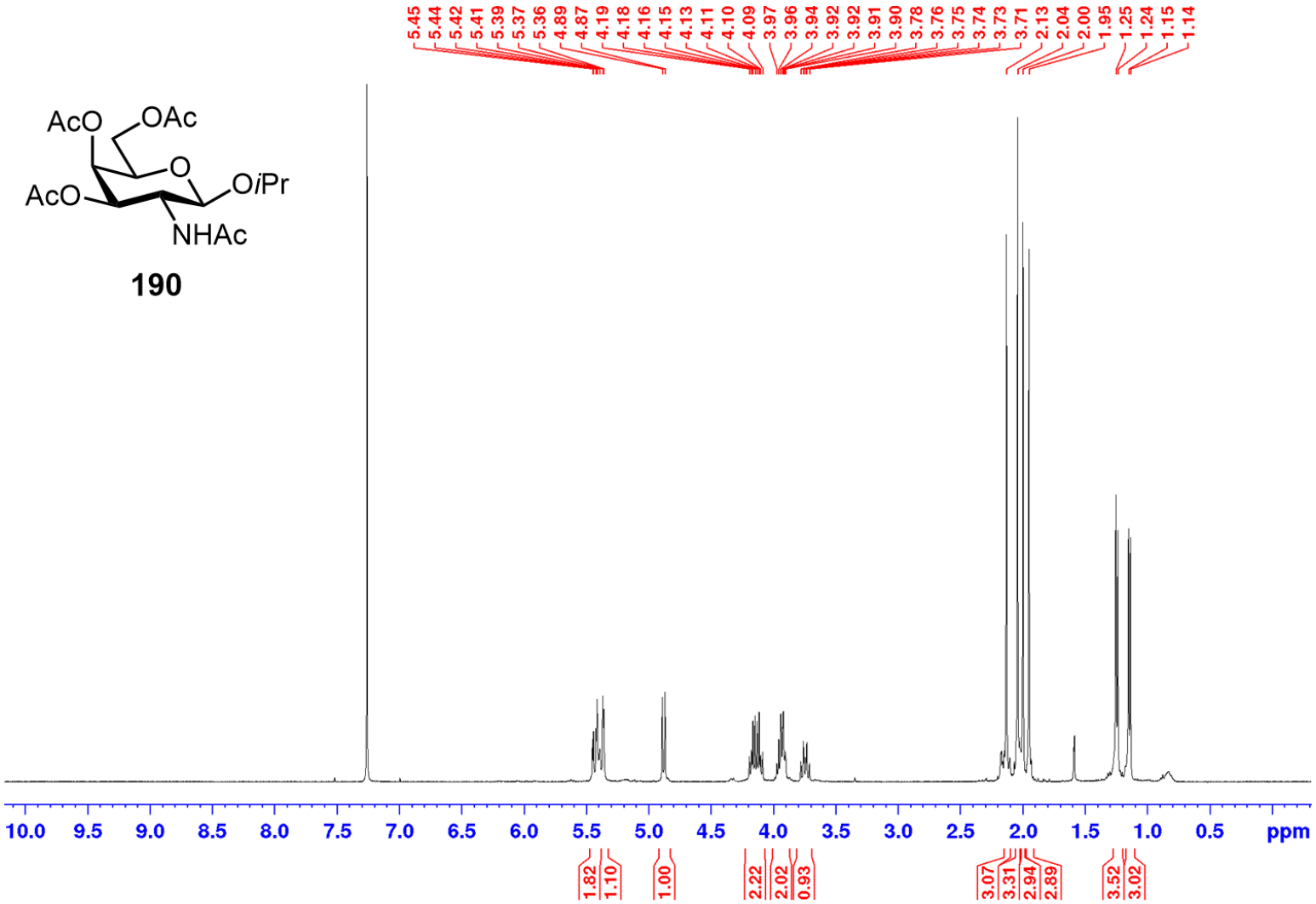
Isopropyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-acetamido-β-D-galactoside (190)



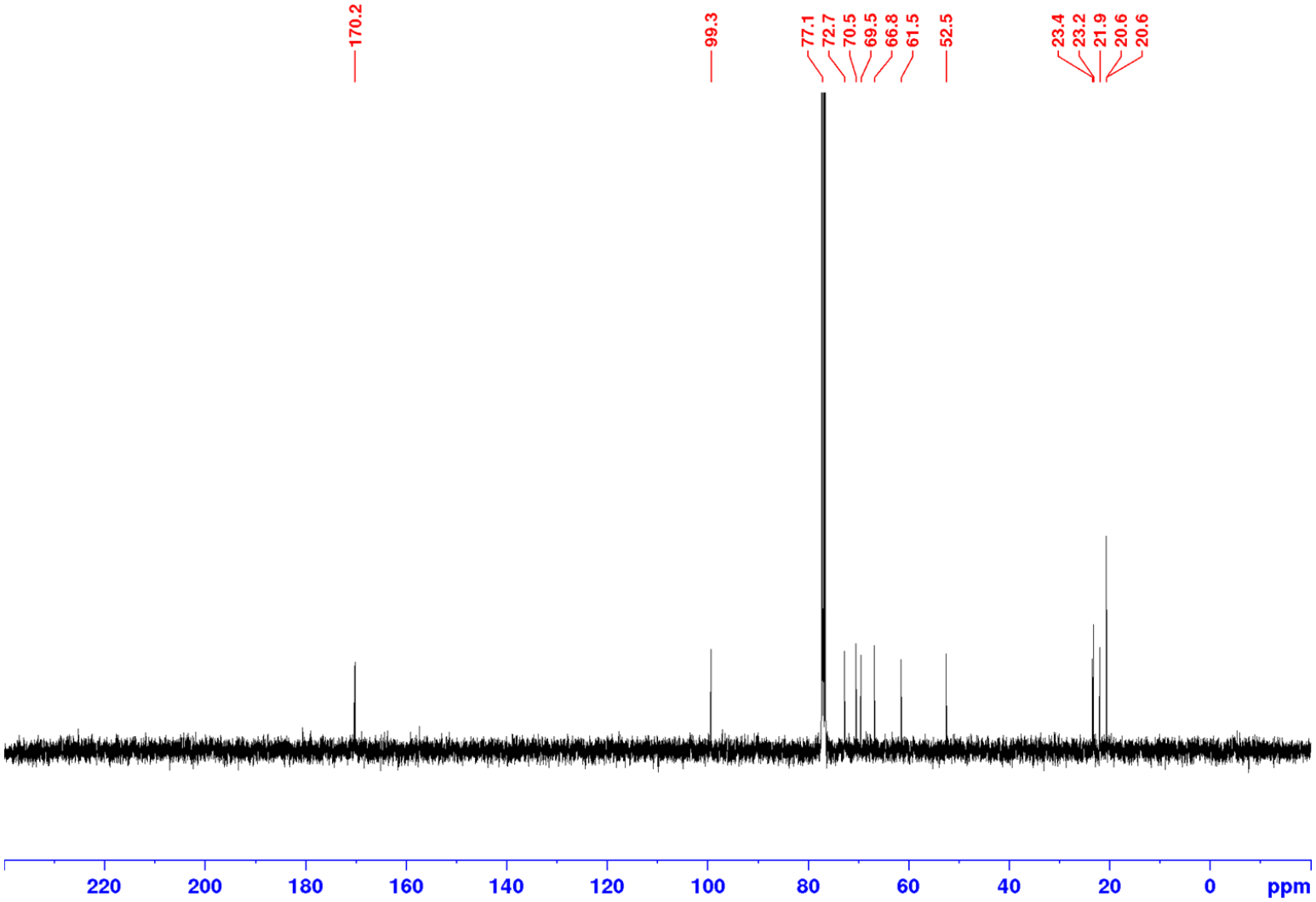
189 **190**

A solution of the D-galactoside **189** (1.0 equiv., 2.00 g, 5.14 mmol) and isopropyl alcohol (4.0 equiv., 1.6 mL, 20.5 mmol) in anhydrous CH_2Cl_2 (11.4 mL) was added anhydrous copper (II)chloride (1.0 equiv., 0.876 g, 5.14 mmol). The reaction mixture was heated to 60 °C. After 2 h, the reaction was cooled to 25 °C and added acetone (50 mL) and added aq. sat. sodium bicarbonate (50 mL). The reaction was then filtered through a pad of Celite on top of filter paper in a Büchner funnel with EtOAc. The filtrate was concentrated to dryness. The filtrate was transferred to a separatory funnel with EtOAc. The organics were washed with water (1x), 1 N HCL (1x), Rochelle's salt (1x), water again (3x) and with brine (1x). The organic layer was dried with $MgSO_4$ and filtered through a glass fritted vacuum filtration funnel to remove drying agent. The filtrate was concentrated to give isopropyl D-galactoside (1.474 g, 3.79 mmol, 74%). as a white solid. Our spectroscopic data agreed with previous reports.[306] R_f 0.30 (EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.45-5.38 (m, 2H), 5.35 (d, *J* = 3.1 Hz, 1H), 4.87 (d, *J* = 8.3 Hz, 1H), 4.15 (sept, *J* = 7.1, 11.7, 17.9 Hz, 2H), 3.97-3.90 (m, 2H), 3.78-3.71 (m, 1H), 2.13 (s, 3H), 2.04 (s, 3H), 2 (s, 3H), 1.94 (s, 3H), 1.24 (d, J = 6.2 Hz, 3H), 1.14 (d, *J* = 6.1 Hz, 3H); ^{13}C (100 MHz, $CDCl_3$) δ 170.2, 99.3, 77.1, 72.7, 70.5, 69.5, 66.8, 61.5, 52.5, 23.4, 23.2, 21.9, 20.6, 20.6 .

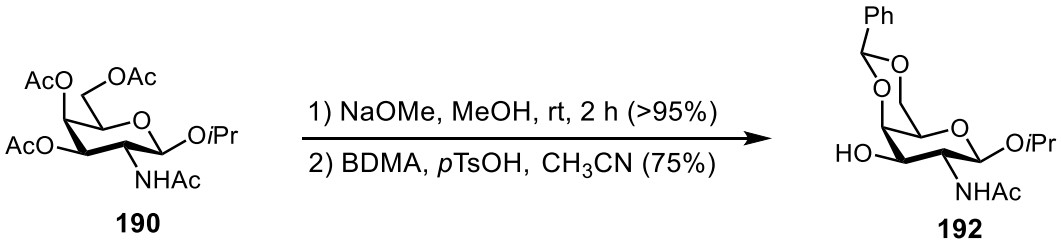
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 190



^{13}C NMR ($CDCl_3$, 100 MHz) of compound 190



Isopropyl 4,6-*O*-benzylidene acetal-2-deoxy-2-acetamido-β-D-galactopyranoside (192)

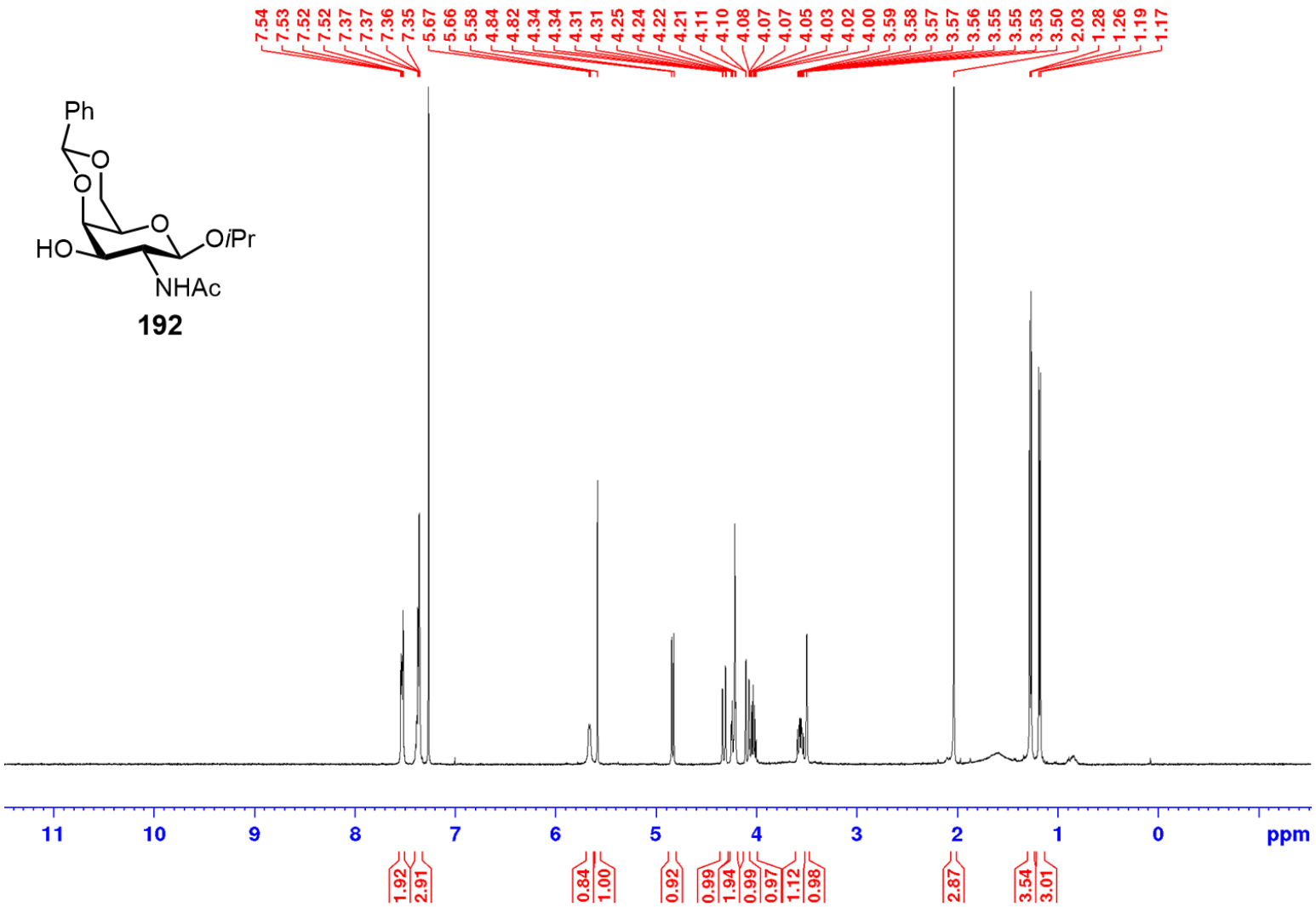


190 **192**

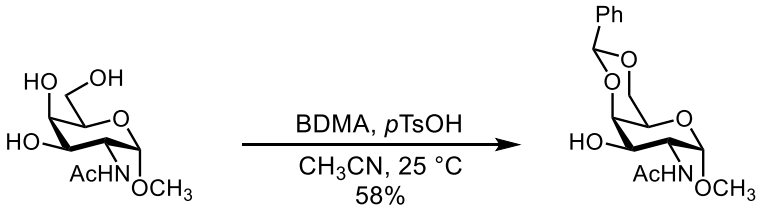
A solution of D-galactoside **190** (1.0 equiv., 1.23 g, 3.16 mmol) was treated with a freshly made solution of sodium methoxide in methanol until the pH of the solution was >11. At 1 hours, the reaction was neutralized by the addition of Amberlite IR 120 (H+) ion exchange resin. The solution was filtered through a glass fritted vacuum funnel equipped with a pad of Celite to remove the acidic resin. The filtrate collected was concentrated to dryness to give the crude triol **191** that was used in the next step without any further purification. A solution of triol **191** in dry acetonitrile (31 mL) was added Amberlite IR 120 (H+) ion exchange resin and benzaldehyde dimethyl acetal (1.5 eq, 0.74 mL, 4.74 mmol) dropwise at room temperature, under argon. At 18 hours, the reaction was then neutralized by the addition of triethylamine and concentrated down to a crude oil. The crude oil was purified by silica gel flash column chromatography (5:95 to 10:90 CH_3OH/CH_2Cl_2) to give Isopropyl 4,6-O-benzylidene-2-deoxy-2-acetamido-β-D-galactopyranoside **192** (0.912 g, 2.38 mmol, 75%) as a white solid. as a white solid. Our spectroscopic data agreed with previous reports.[306] R_f 0.33 (10:90 CH_3OH/CH_2Cl_2); ^1H NMR (400 MHz, $CDCl_3$): δ 7.54-7.52 (m, 2H), 7.37-7.35 (m, 3H), 5.66 (d, J = 4.7 Hz, 1H), 5.58 (s, 1H), 4.84 (d, *J* = 8.3 Hz, 1H), 4.32 (dd, *J* = 1.4, 12.4 Hz, 1H), 4.25-4.21 (m, 2H), 4.1 (dd, *J* = 1.8, 12.4 Hz, 1H), 4.03 (ddd, *J* = 6.2, 6.7, 12.4 Hz, 1H), 3.57 (sept, *J* = 5.8,

8.2, 9.6 Hz, 1H), 3.5 (br, 1H), 2.04 (s, 3H), 1.27 (d, *J* = 6.2 Hz, 6.1H), 1.17 (d, *J* = 6.1 Hz, 3H); ^{13}C (100 MHz, $CDCl_3$) δ 171.6, 137.6, 129, 128.1, 128.1, 126.4, 126.4, 101.2, 98.2, 77.1, 75.1, 71.3, 70.1, 69.2, 66.7, 56.3, 23.7, 23.3, 21.8.

^{1}H NMR ($CDCl_3$, 400 MHz) of compound 192



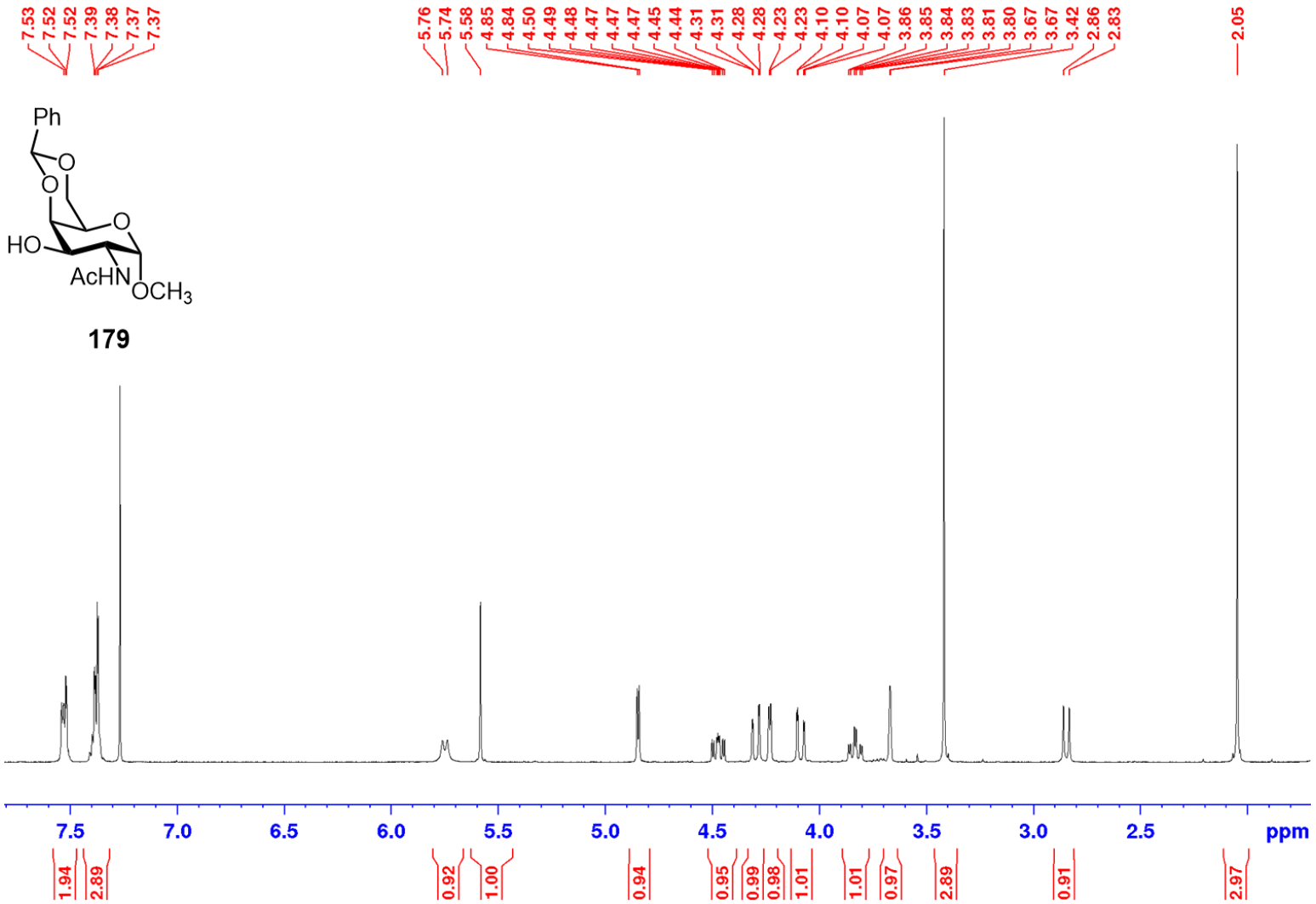
Methyl 4,6-*O*-benzylidene acetal-2-deoxy-2-acetamido-α-D-galactopyranoside (179)



179

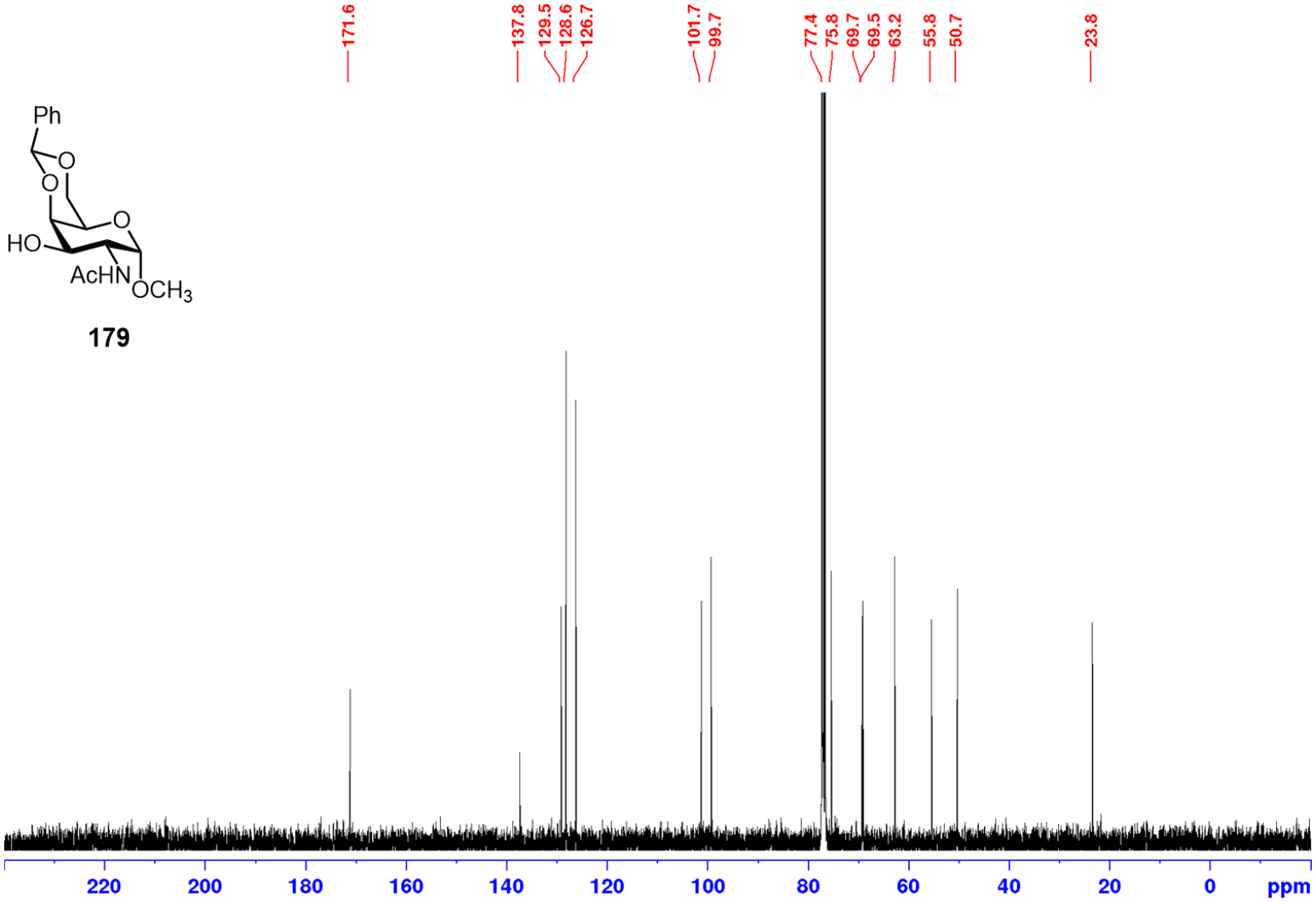
A solution of commercially available methyl 2-deoxy-2-acetamido-a-D-galactoside (1.0 equiv., 8.73 g, 37.1 mmol) and benzaldehyde dimethyl acetal (1.5 equiv., 6.8 mL, 44.5 mmol) in anhydrous acetonitrile (100 mL) was added *p*-TsOH (0.1 equiv., 0.70 g, 3.7 mmol). At 2.5 hours, the reaction mixture was quenched by the addition of triethyl amine and filtered through a pad of Celite. The filtrate was concentrated to a crude solid. The crude product was crystallized from hot ethanol and filtered to collect the D-galactoside **179** (6.96 g, 21.5 mmol, 58%) as a white solid. Our characterization data were consistent with previously reported data [307]. R_f 0.69 (9:1 CH_2Cl_2/CH_3OH); ^1H NMR (400 MHz, $CDCl_3$): δ 7.53-7.50 (m, 2H), 7.38-7.36 (m, 3 H), 5.70 (d, *J* = 9.17 Hz, 1H), 4.8 (d, *J* = 2.29 Hz, 1 H), 4.501-4.44 (m, 1H), 4.29 (dd, *J* = 1.59 Hz, 12.8, 1H), 4.23 (d, *J* = 3.67 Hz, 1H), 4.08 (dd, *J* = 1.84, 12.56 Hz, 1H), 3.83 (dd, *J* = 3.47, 10.81 Hz, 1H), 3.67 (s, 1H), 3.41 (s, 3H), 2.71 (d, *J* = 10.61 Hz, 1H), 2.04 (s, 3H); ^{13}C (100 MHz, $CDCl_3$) δ 171.6, 137.8, 129.5, 128.6, 126.7, 101.7, 99.7, 75.8, 63.2, 55.8, 50.7, 23.8.

^{1}H NMR ($CDCl_3$, 400 MHz) of compound 179



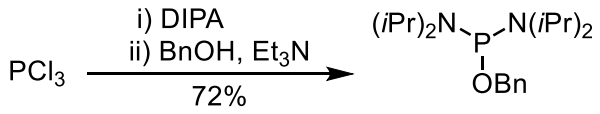
179

^{13}C NMR ($CDCl_3$, 100 MHz) of compound 179



179

1-(benzyloxy)-*N*,*N*,*N*',*N*'-tetraisopropyl phosphordiamidite (193)

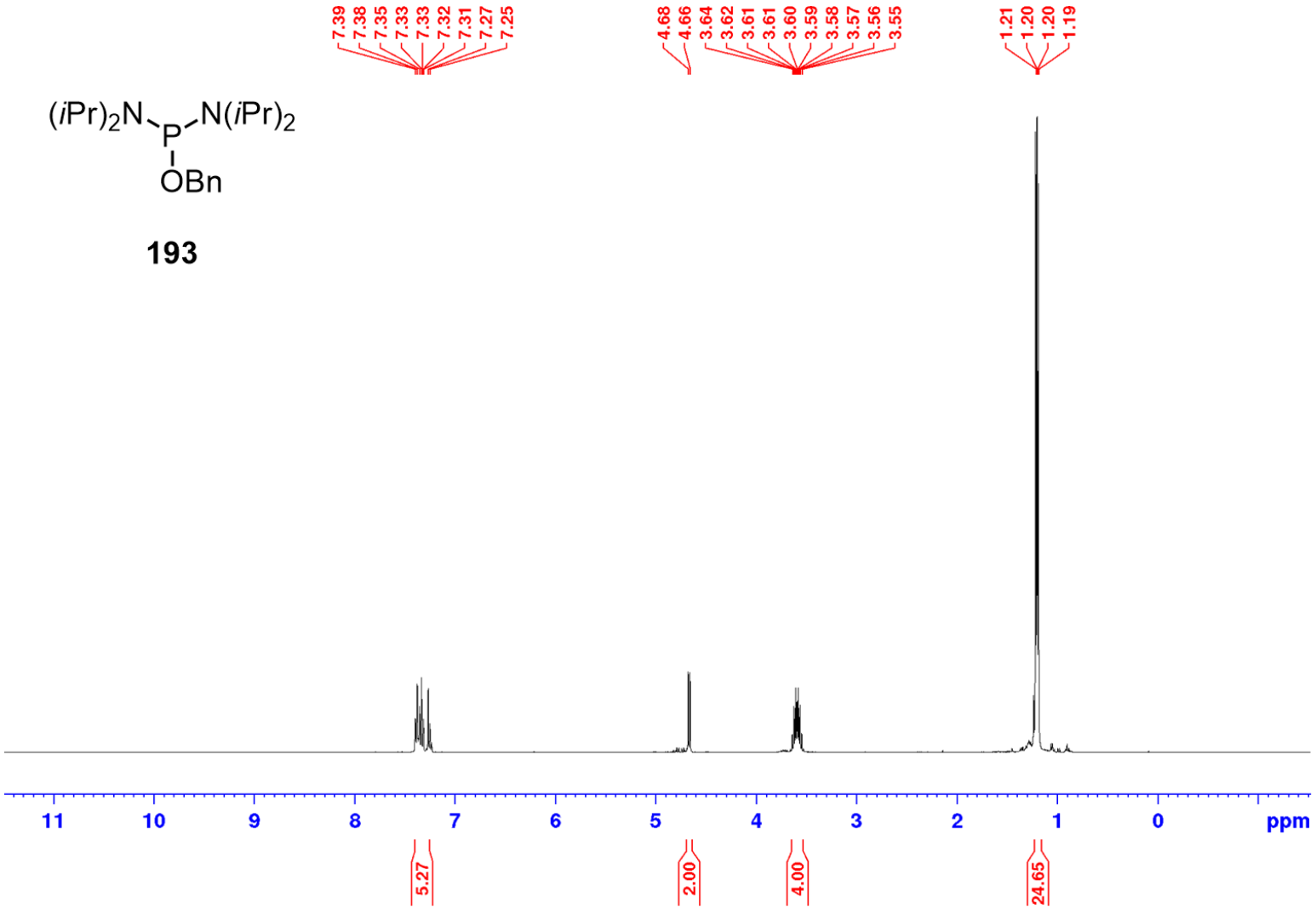


193

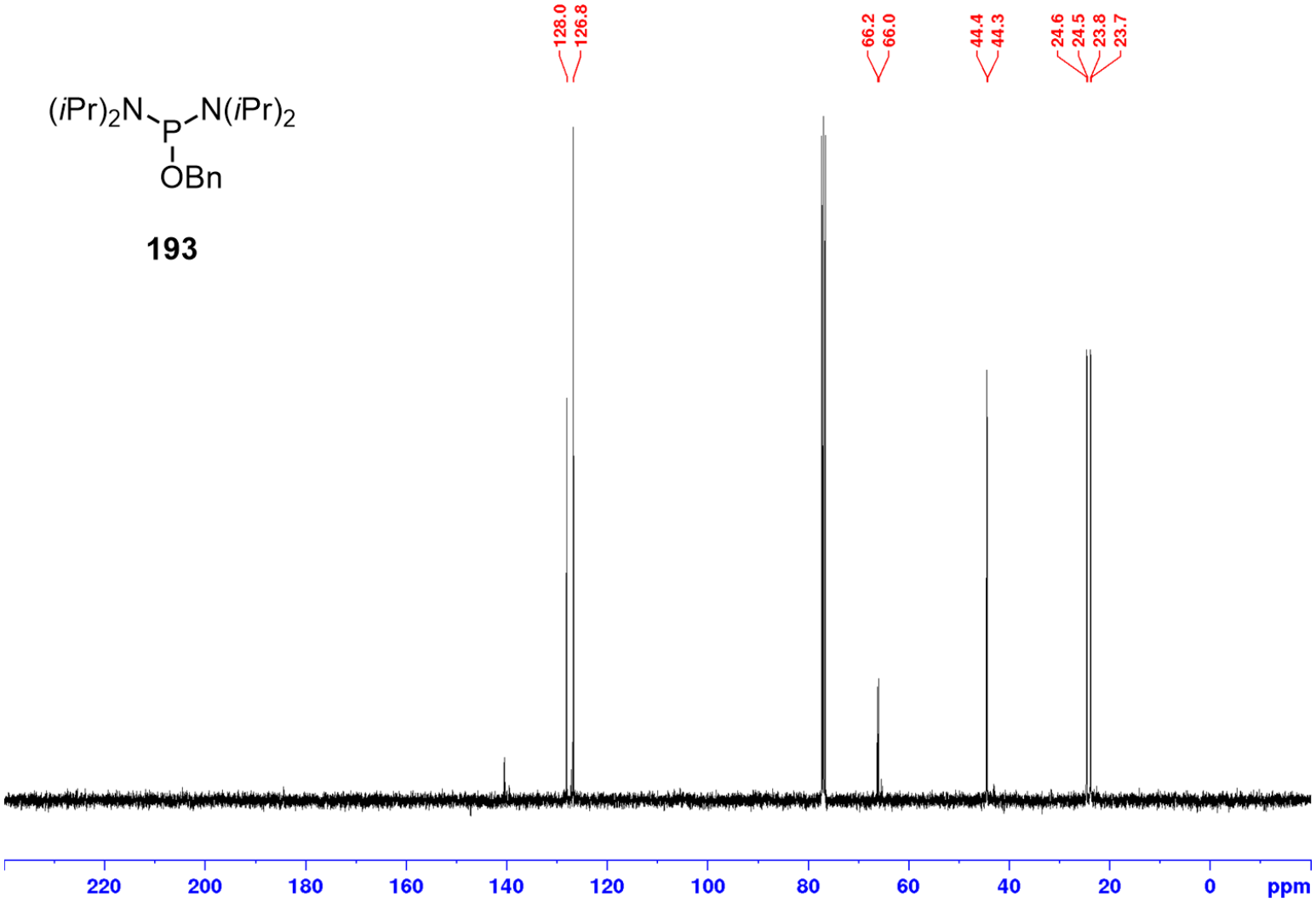
A solution of diisopropylamine (6.0 equiv., 49.0 mL, 346 mmol) in anhydrous hexanes (140 mL) were placed in a two-necked flask, equipped with an addition funnel and reflux condenser, under an argon atmosphere. The flask was cooled to 0 °C and treated dropwise, over 15 minutes, through the addition funnel with a solution of PCl_3 (1.0 equiv., 7.87 g, 57.0 mmol) in anhydrous hexanes (10 mL). After addition was complete, the addition funnel was rinsed with hexanes. The reaction mixture was stirred at room temperature. After 3 h, the milky suspension was refluxed for 18 h. The reaction was cooled to 0 °C and added Et_3N (3.0 equiv., 24.0 mL, 172 mmol) to neutralize the reaction mixture. Next, a solution of Et_3N (1.0 equiv., 8.00 mL, 57.4 mmol) and benzyl alcohol (1.0 equiv., 6.00 mL, 57.9 mmol) in anhydrous diethyl ether (20 mL) was added dropwise through the addition funnel at 0 °C. The reaction was stirred at room temperature. At 1 h, the mixture was filtered through a glass fritted filter funnel equipped with a pad of Celite. The filtrate was concentrated *in vacuo* to produce an orange oil. The crude oil was taken up in hexanes (100 mL), washed with anhydrous acetonitrile (3 x 50 mL), and concentrated to produce phosphordiamidite **193** (13.831 g, 57.01 mmol, 72%) as a yellow oil to be carried forward with no further purification. The spectroscopic data agreed with previously reported data.[308] R_f 0.2 (3:1 hexanes/EtOAc). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.38-7.22 (m, 5H), 4.65 (d, *J* = 7.54 Hz, 2H), 3.64-3.51 (m, 4H), 1.20-1.17 (m, 24H); ^{13}C

(100 MHz, $CDCl_3$) δ 140.9, 140.8, 128.4, 127.2, 66.6, 66.4, 44.9, 44.7, 25.0, 24.9, 24.4, 24.1; ^{31}P (162 MHz, $CDCl_3$) δ 124.91.

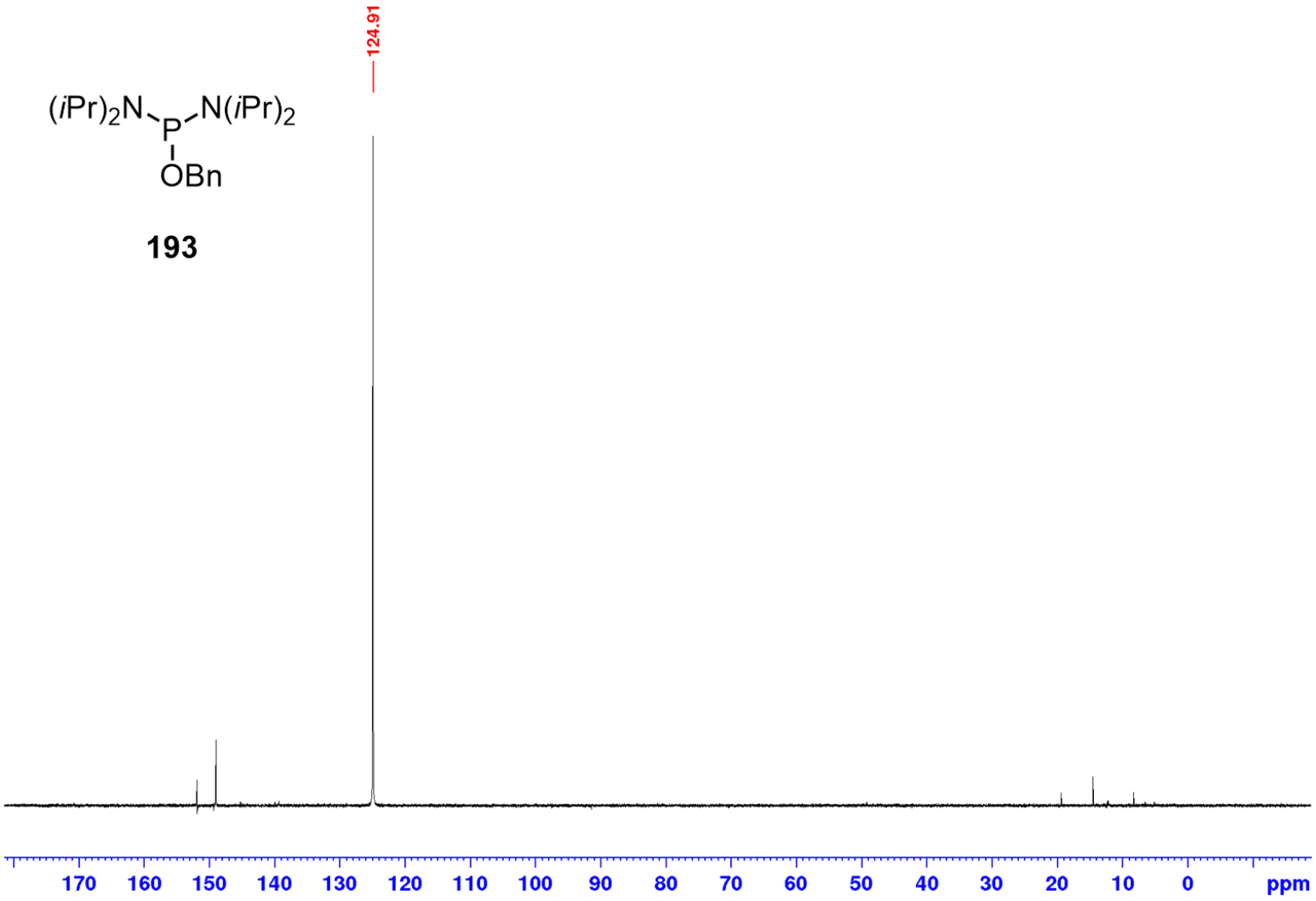
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 193



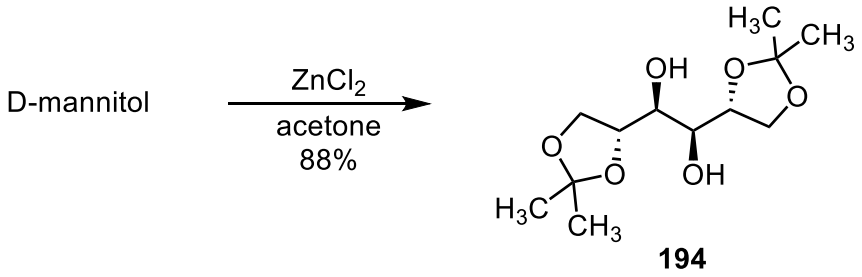
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 193



^{31}P NMR ($CDCl_3$, 162 MHz) of compound 193



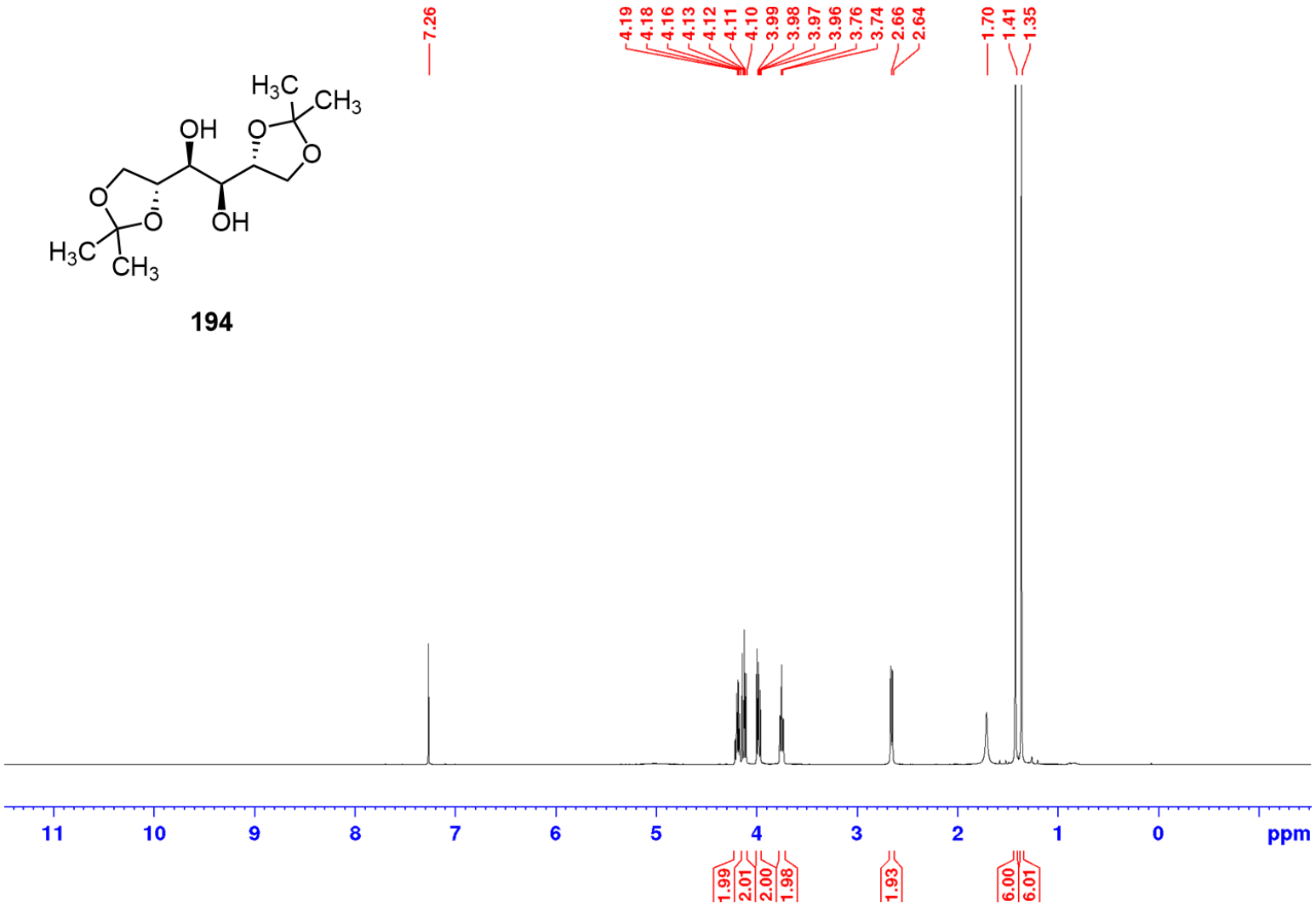
(1*S*,2*R*)-1-((*R*)-2,2-dimethyl-1,3-dioxolan-4-yl)-2-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)ethane-1,2-diol (194)



194

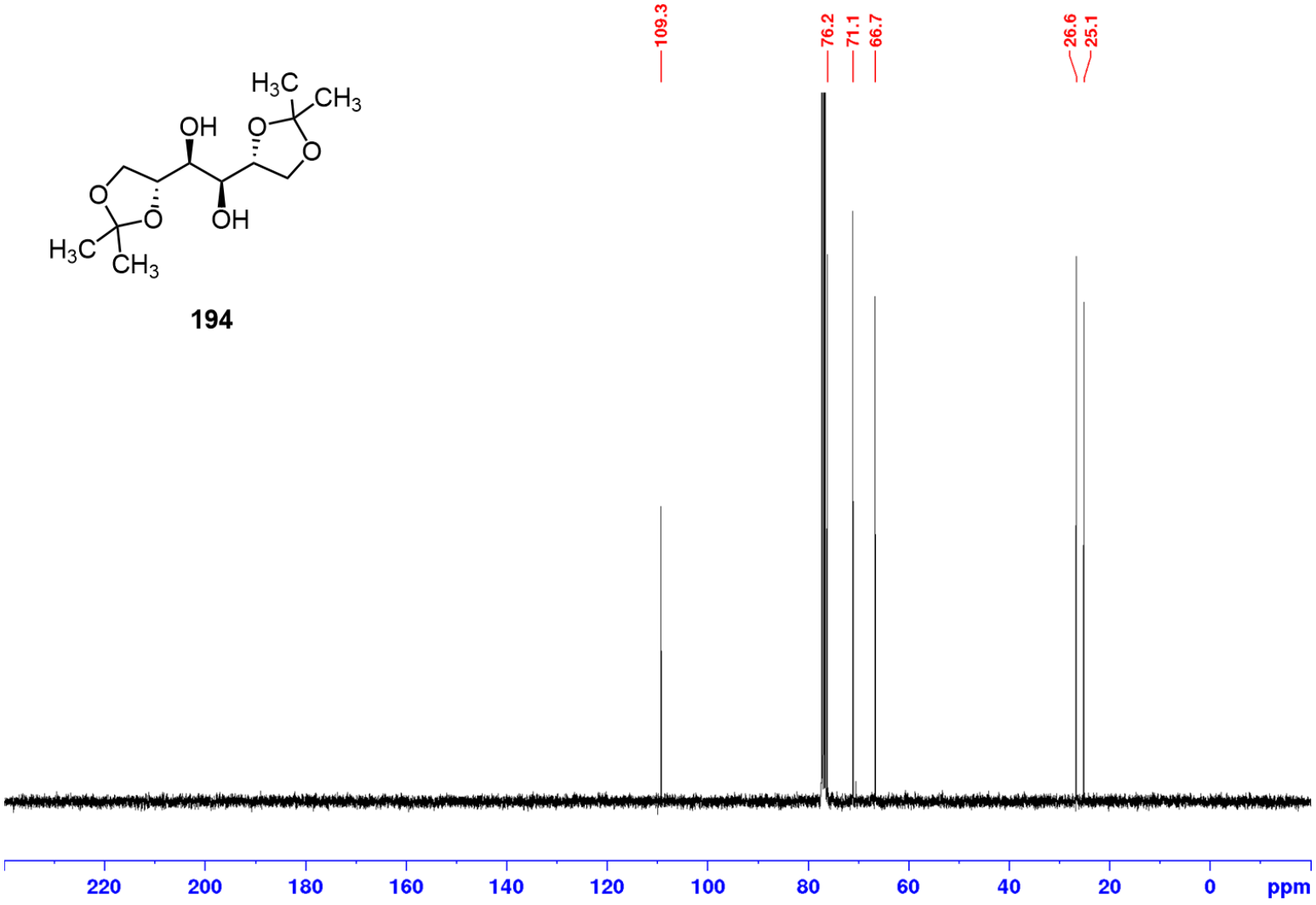
A mixture of zinc chloride (8.0 equiv., 50.6 g, 367 mol) in acetone (250 mL) was added D-mannitol (1.0 equiv., 8.35 g, 45.8 mmol) at room temperature under an argon atmosphere. At 15 h, saturated aqueous potassium carbonate (200 mL) was poured into the reaction mixture at room temperature. After 1 h, the reaction mixture was diluted with EtOAc (100 mL) and filtered through a glass fritted vacuum filter funnel equipped with a pad of Celite to remove zinc carbonate. The filter cake was then washed with EtOAc (50 mL). The filtrate was concentrated to dryness *in vacuo* to a crude anhydrous solid which was diluted with water (100 mL) and CH_2Cl_2 (100 mL) and transferred to a separatory funnel. The water layer was extracted with CH_2Cl_2 (3 x 100 mL). The combined organics were dried ($MgSO_4$) and filtered through a glass fritted filter funnel to remove drying agent. The filtrate was concentrated in vacuo to produce diacetonide **194** a white solid (10.326 g, 39.31 mmol, 88%) to be used without further purification. The spectroscopic data were in agreement with the reported data.[309-310] R_f 0.46 (3:1 hexanes/EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.22-4.17 (dd, *J* = 6.28, 11.77 Hz, 2H), 4.14-4.10 (dd, *J* = 6.28, 8.63 Hz, 2H), 3.97 (d, *J* = 6.28, 8.63 Hz, 2H), 3.75 (m, 2H), 2.54 (d, *J* = 6.28 Hz, 2H), 1.42 (s, 6H), 1.36 (s, 6H); ^{13}C (100 MHz, $CDCl_3$) δ 109.7, 76.6, 71.5, 6.1, 27.1, 25.5.

^{1}H NMR ($CDCl_3$, 400 MHz) of compound 194

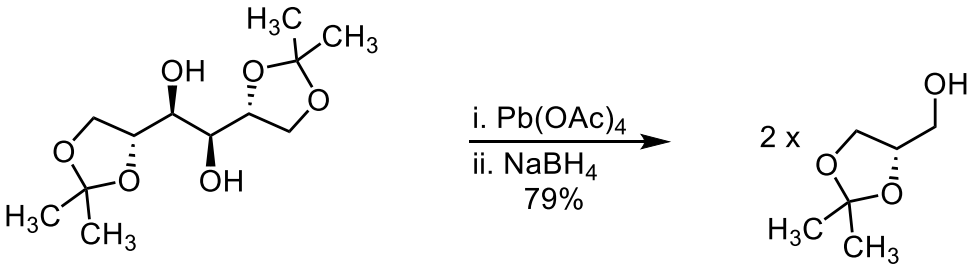


194

^{13}C NMR ($CDCl_3$, 100 MHz) of compound 194



(*S*)-(2,2-dimethyl-1,3-dioxolan-4-yl)methanol (195)

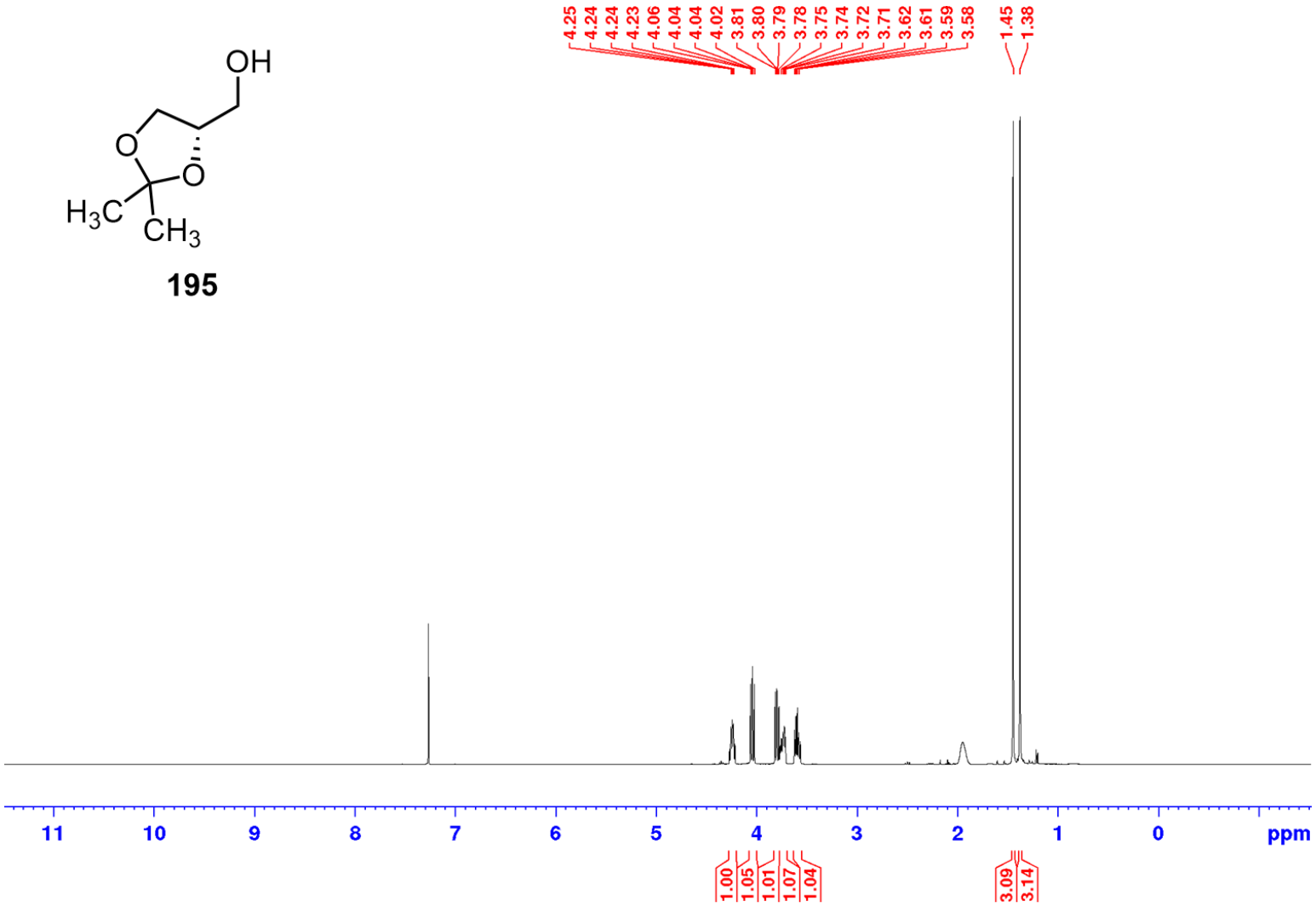


194 **195**

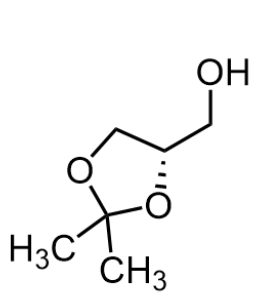
To a solution of diacetonide **194** (1.0 equiv., 8.0 g, 30 mmol) in anhydrous THF (40 mL) was added dried lead (IV) tetraacetate (1.0 equiv., 13.8 g, 30.0 mmol) under an argon atmosphere at 0 °C. The reaction was stirred at room temperature. At 2 h, the reaction mixture was filtered through a glass fritted vacuum filter funnel equipped with a pad of Celite. The filtrate was cooled to 0 °C under an argon atmosphere and treated with a solution of $NaBH_4$ (2.0 equiv., 2.29 g, 61.0 mmol) in 4% aqueous sodium hydroxide (20 mL) portion wise, slowly. After being stirred for 30 minutes at 0 °C and 2 h at ambient temperature, the reaction mixture was quenched by the addition of solid ammonium chloride (0.6 equiv., 1.0 g, 18.7 mmol) and concentrated to an oil. The mixture was diluted with EtOAc (200 mL) and washed with water (3 x 20 mL). The organics were separated, dried ($MgSO_4$), and filtered through a glass fritted filter funnel to remove drying agent. The filtrate was concentrated *in vacuo* to an oil. The resulting yellow oil was then purified by short path distillation under reduced atmosphere (20 mmHg, 175 °C) to furnish alcohol **195** as a colorless oil (6.234 g, 47.5 mmol, 79%). The spectroscopic data were in agreement with the reported data.[309-310] R_f 0.5 (7:3 hexanes/EtOAc); ^1H NMR (400 MHz, $CDCl_3$): δ 4.2-4.16 (m, 1H), 4.01-3.97 (dd, *J* = 6.58, 8.15 Hz, 1H), 3.75-3.52 (m, 3H), 2.4

(s, 2.45, 1H, OH), 1.39 (s, 3H), 1.32 (s, 3H); ^{13}C (100 MHz, $CDCl_3$) δ 109.7, 76.5, 66.0, 63.3, 27.0, 25.6.

^{1}H NMR ($CDCl_3$, 400 MHz) of compound 195

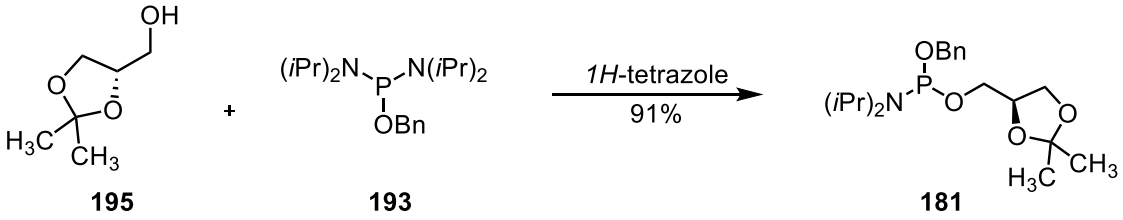


^{13}C NMR ($CDCl_3$, 100 MHz) of compound 195



195

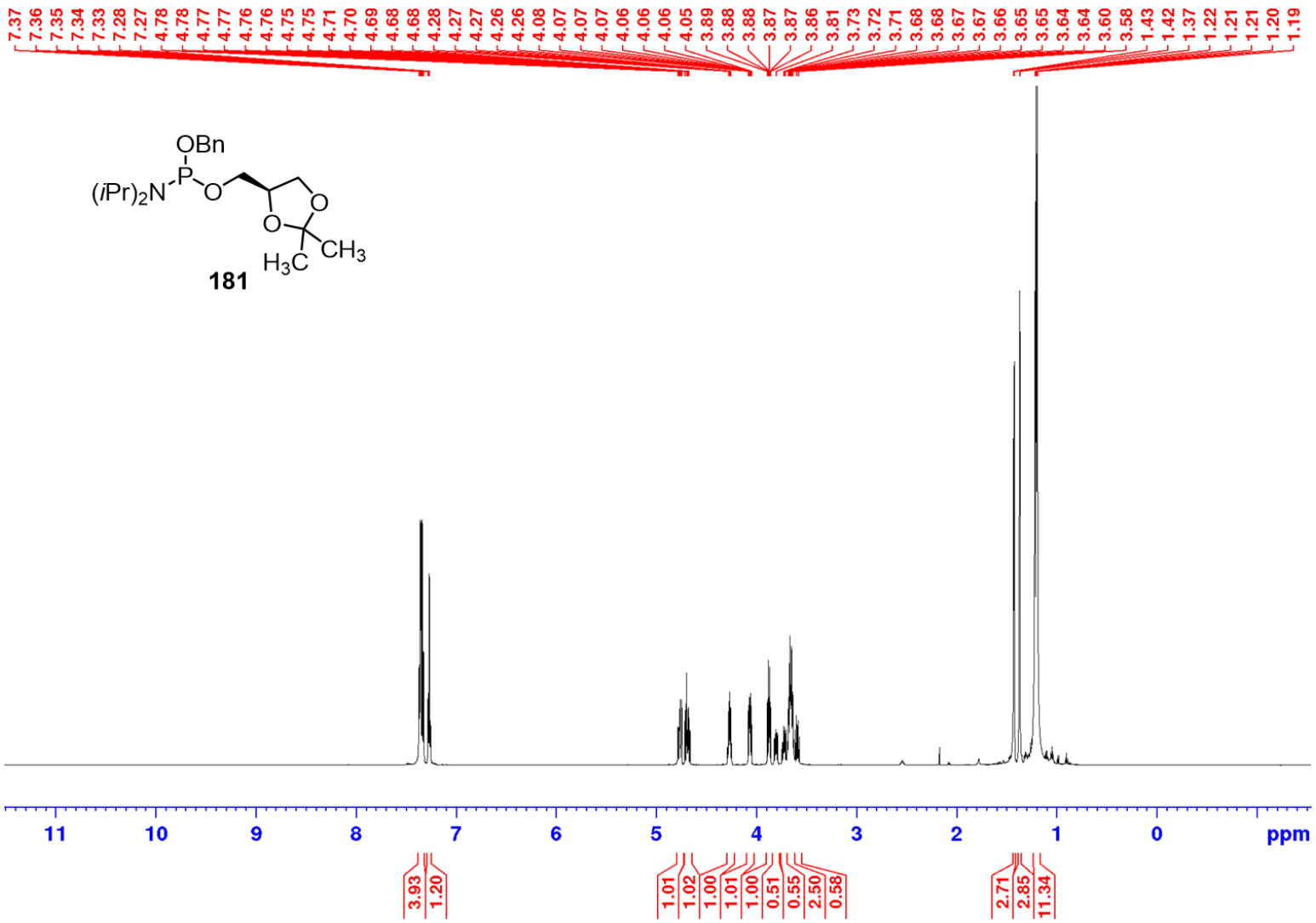
Benzyl (((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)methyl) diisopropylphosphoramidite (181)



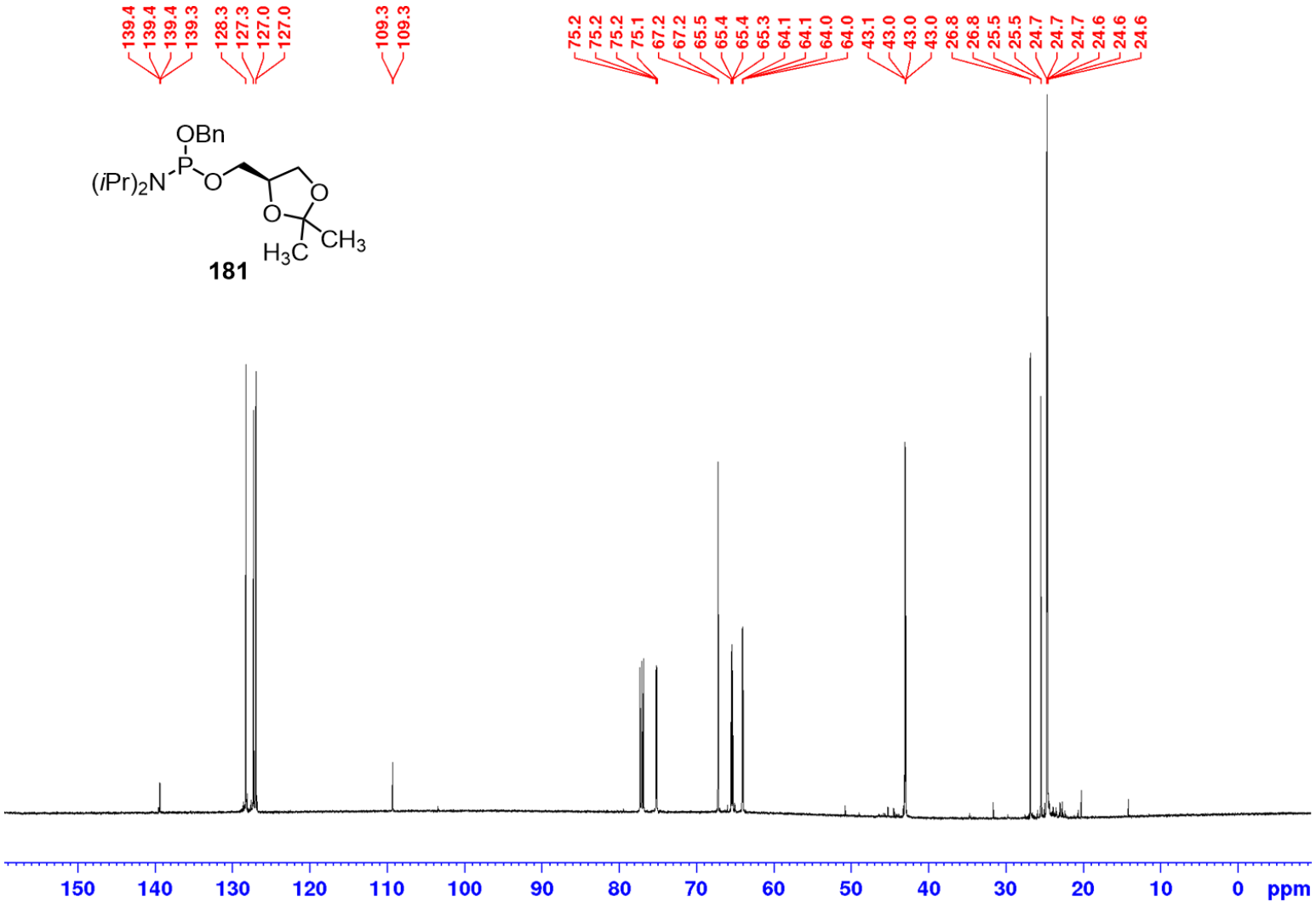
195 **193** **181**

To a solution of phosphordiamidite **194** (1.5 equiv., 1.92 g, 5.67 mmol) and *1H*-tetrazole (0.5 equiv., 0.133 g, 1.83 mmol) in anhydrous CH_2Cl_2 was added a solution of alcohol **195** (1.0 equiv., 0.500 g, 3.78 mmol) in anhydrous CH_2Cl_2 at room temperature under an argon atmosphere. At 3 h, the reaction mixture was and concentrated *in vacuo*. The crude oil was purified through a base treated silica gel column (10:1 hexane/Et_3N equilibration, 99:1 eluting solvent/Et_3N, eluding solvent 1:9 EtOAc/hexanes) to produce phosphoramidite **181** (1.354 g, 3.671 mmol, 91%). The spectroscopic data agreed with the previously reported data.[308] ^1H NMR (400 MHz, $CDCl_3$): δ 7.28-7.17 (m, 5H), 4.69-4.55 (m, 2H), 4.20-4.14 (m, 1H), 3.99-3.94 (m, 1H), 3.79-3.46 (m, 5H), 1.33 (s, 3H), 1.27 (s, 3H), 1.12-1.09 (m, 12H); ^{13}C (100 MHz, $CDCl_3$) δ 139.7, 139.7, 139.7, 139.6, 128.5, 127.6, 127.3, 127.3, 109.6, 109.6, 75.6, 75.5, 75.4, 67.5, 67.5, 65.8, 65.8, 65.7, 65.6, 64.5, 64.4, 64.3, 64.3, 43.4, 4.4, 43.3, 43.2, 27.1, 25.8, 25.8, 25.0, 24.9, 24.9; ^{31}P (162 MHz, $CDCl_3$) δ 149.4, 149.2.

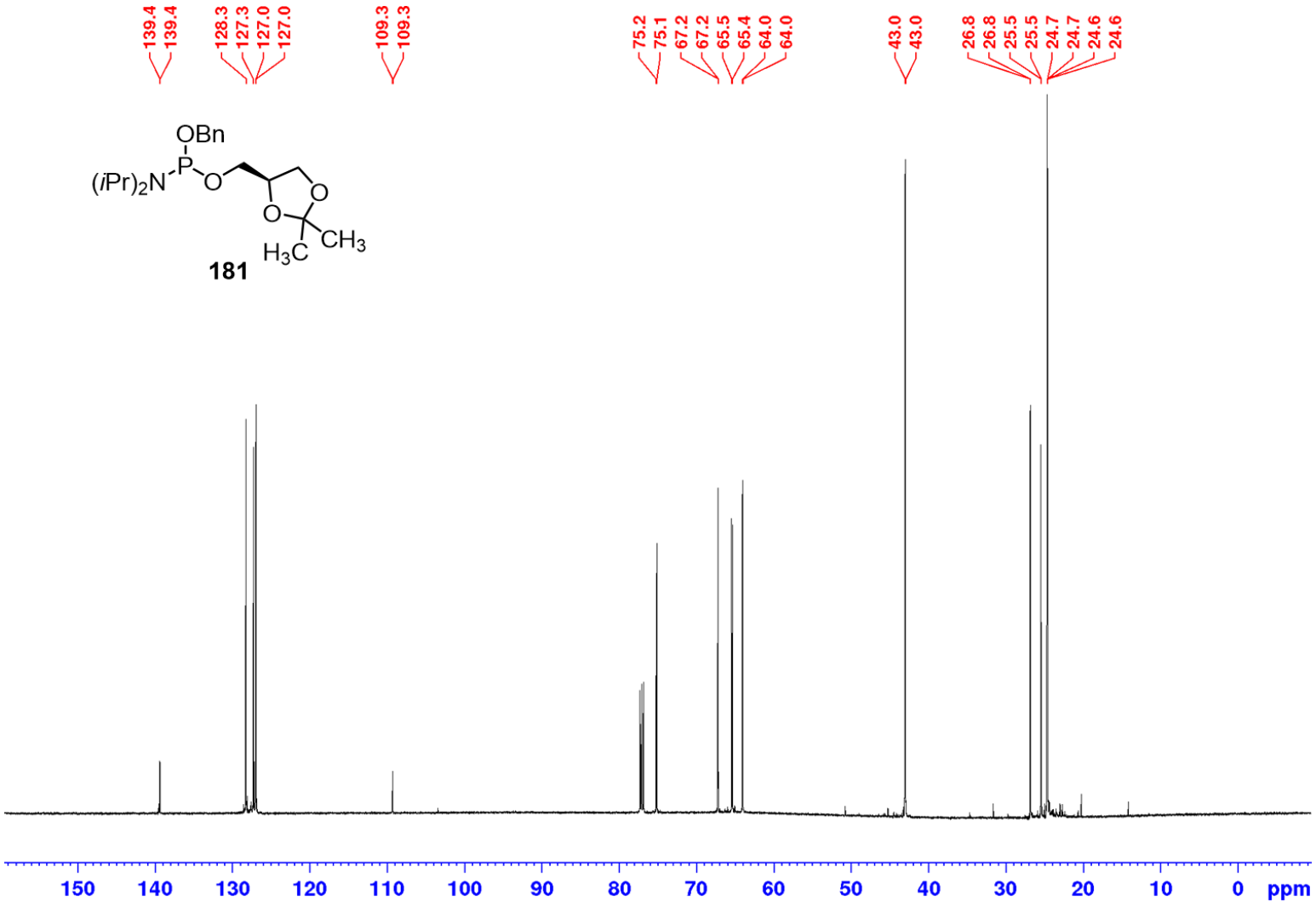
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 181



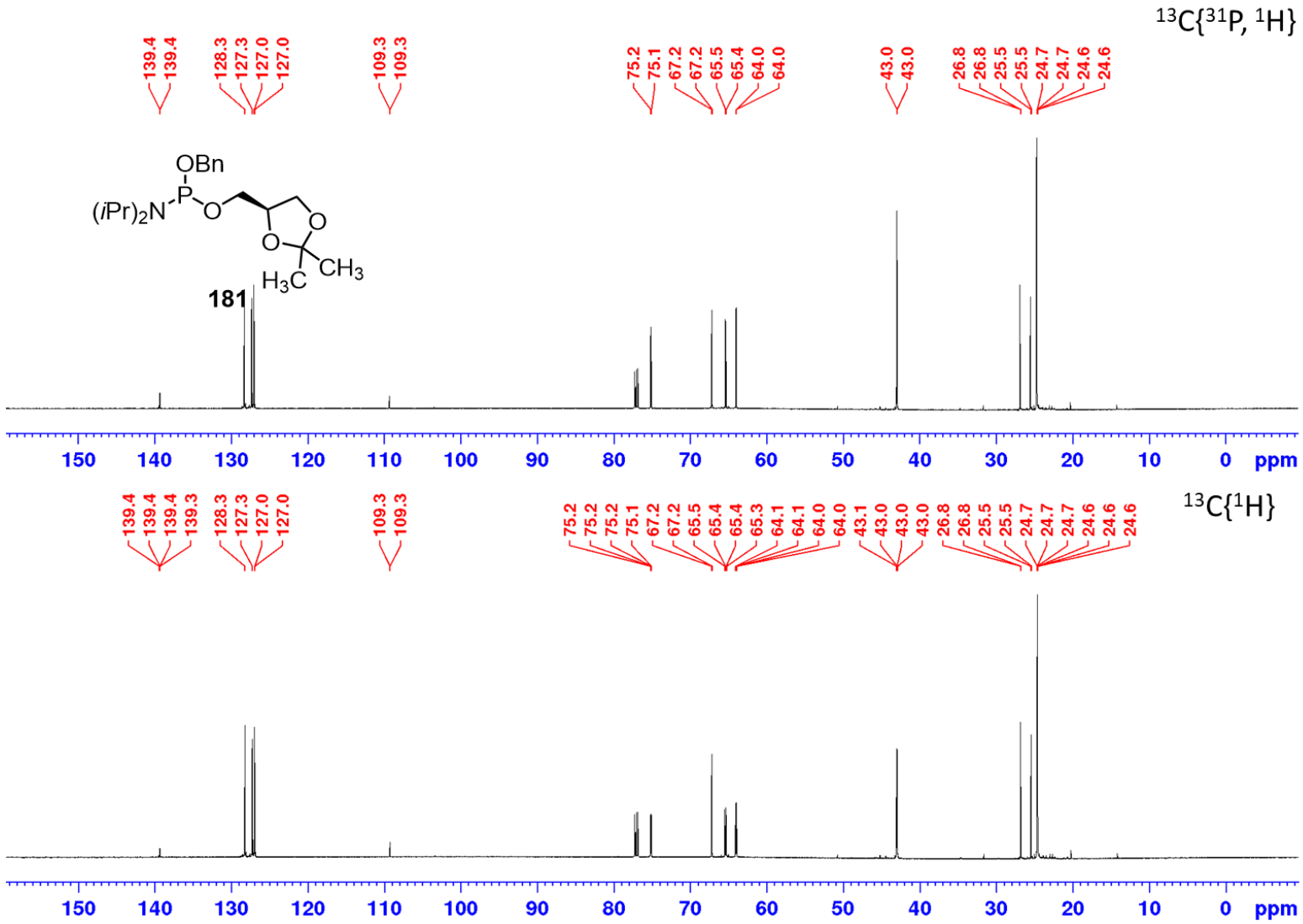
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 181



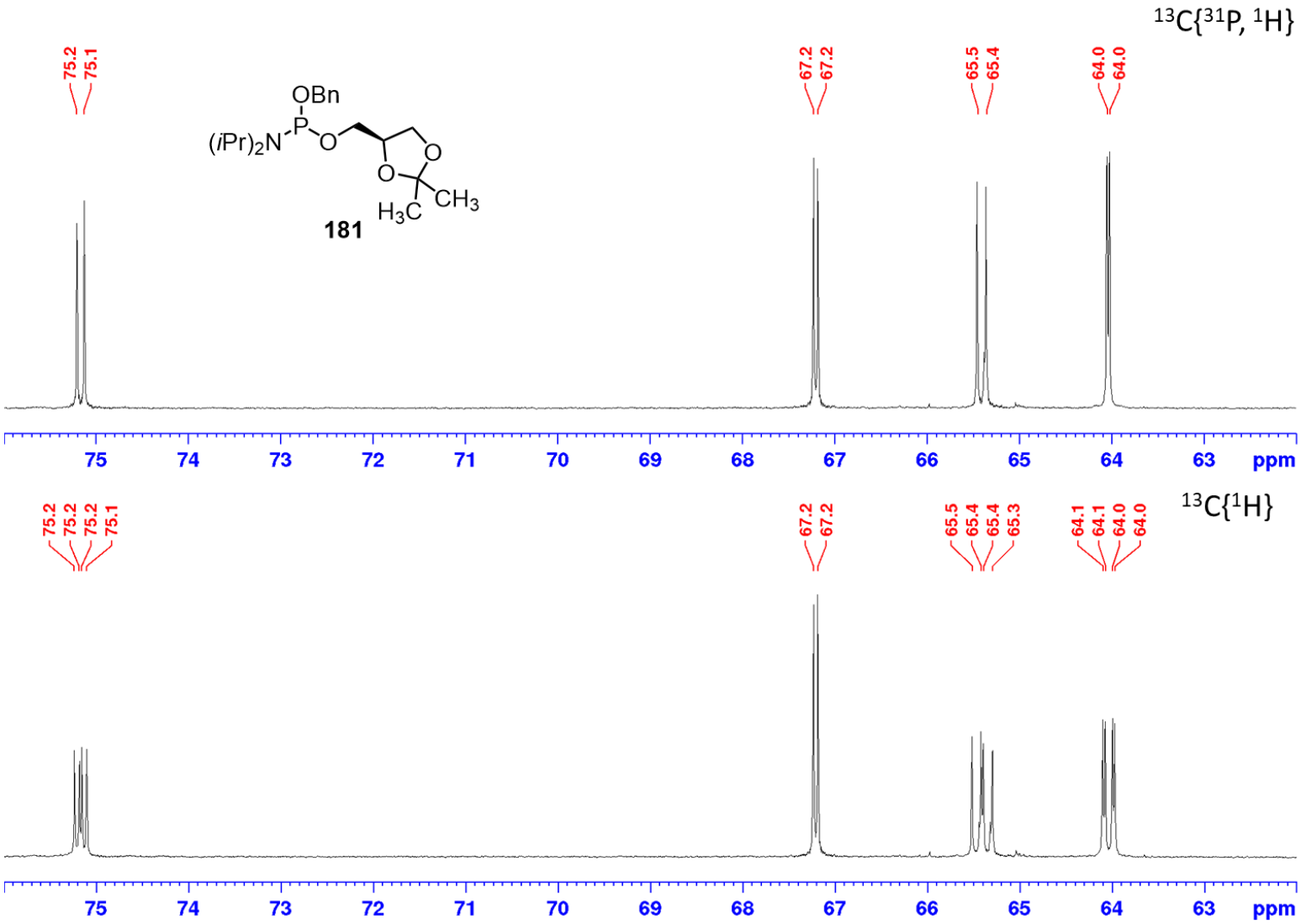
^{13}C {^{31}P, ^{1}H} NMR ($CDCl_3$, 100 MHz) of compound 181



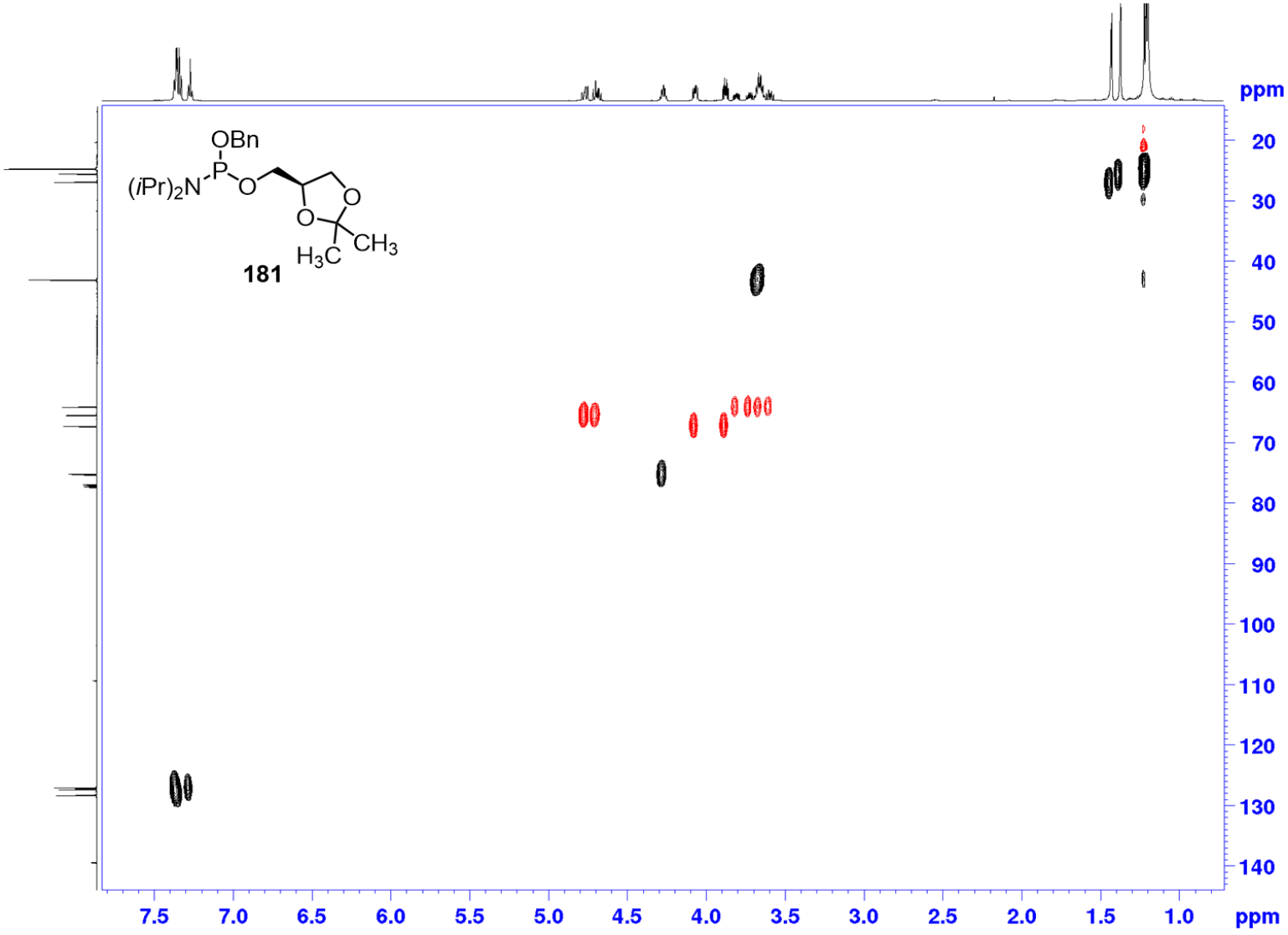
^{13}C {^{31}P, ^{1}H} (top spectra) vs.^{13}C {^{1}H} (bottom spectra) NMR ($CDCl_3$, 100 MHz) of compound 181



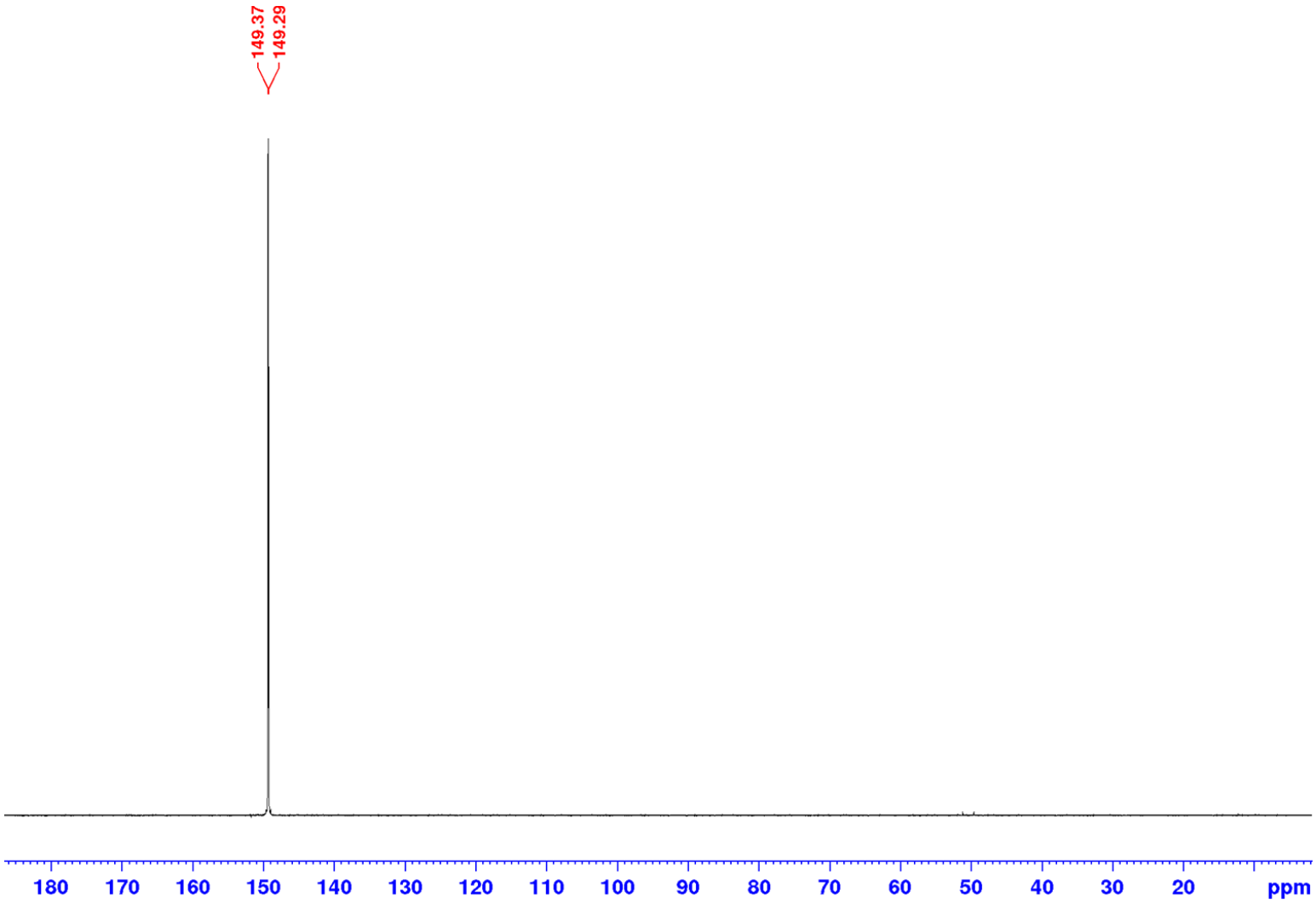
Zoom of ^{13}C {^{31}P, ^{1}H} (top spectra) vs.^{13}C {^{1}H} (bottom spectra) NMR ($CDCl_3$, 100 MHz) of compound 181



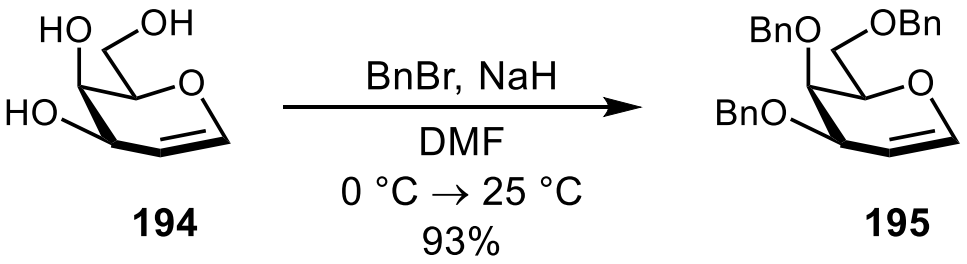
^{1}H-^{13}C HSCQ ($CDCl_3$) of compound 181



^{31}P NMR ($CDCl_3$, 162 MHz) of compound 181



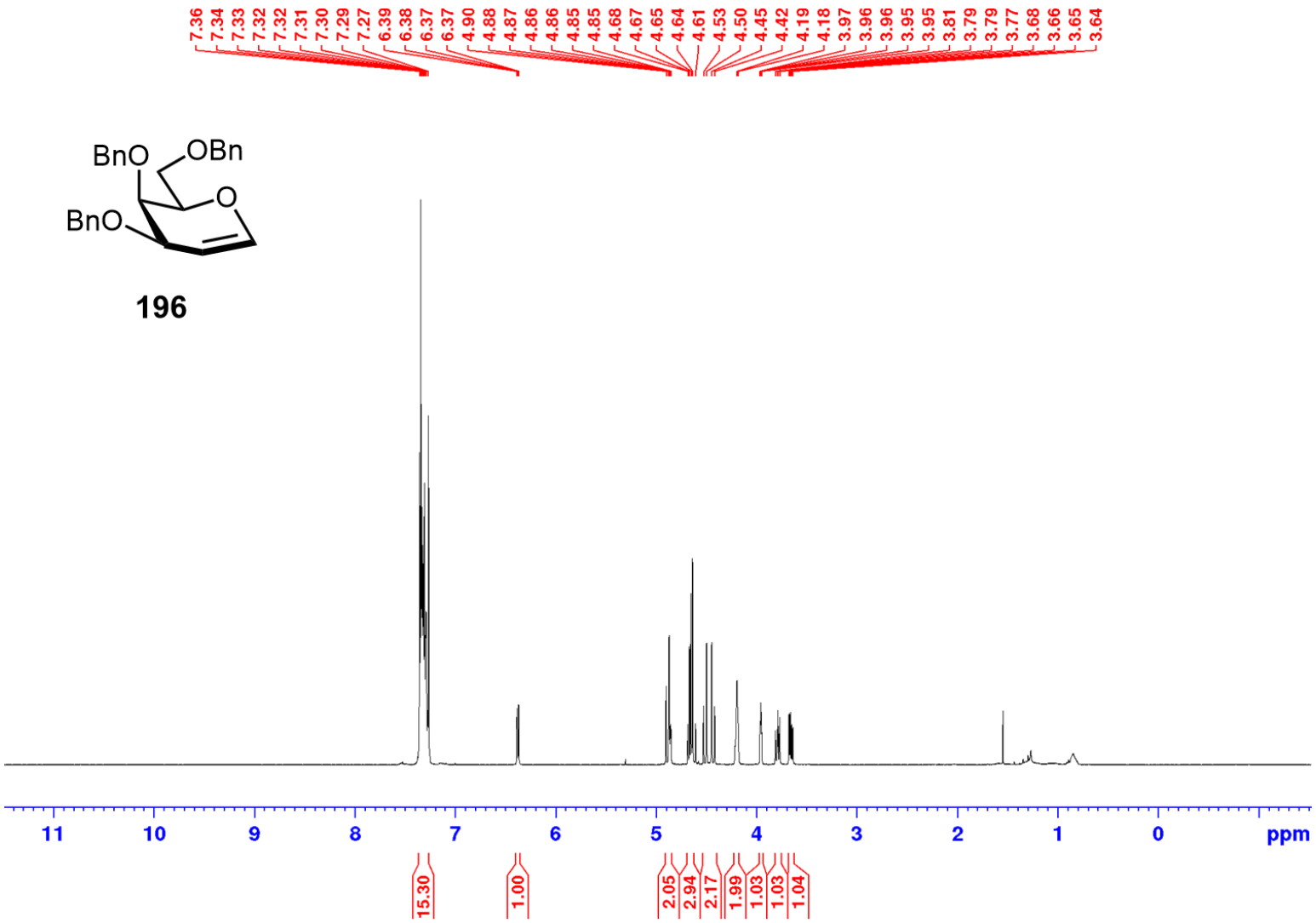
3,4,6-Tri-*O*-benzyl-D-galactal (196)



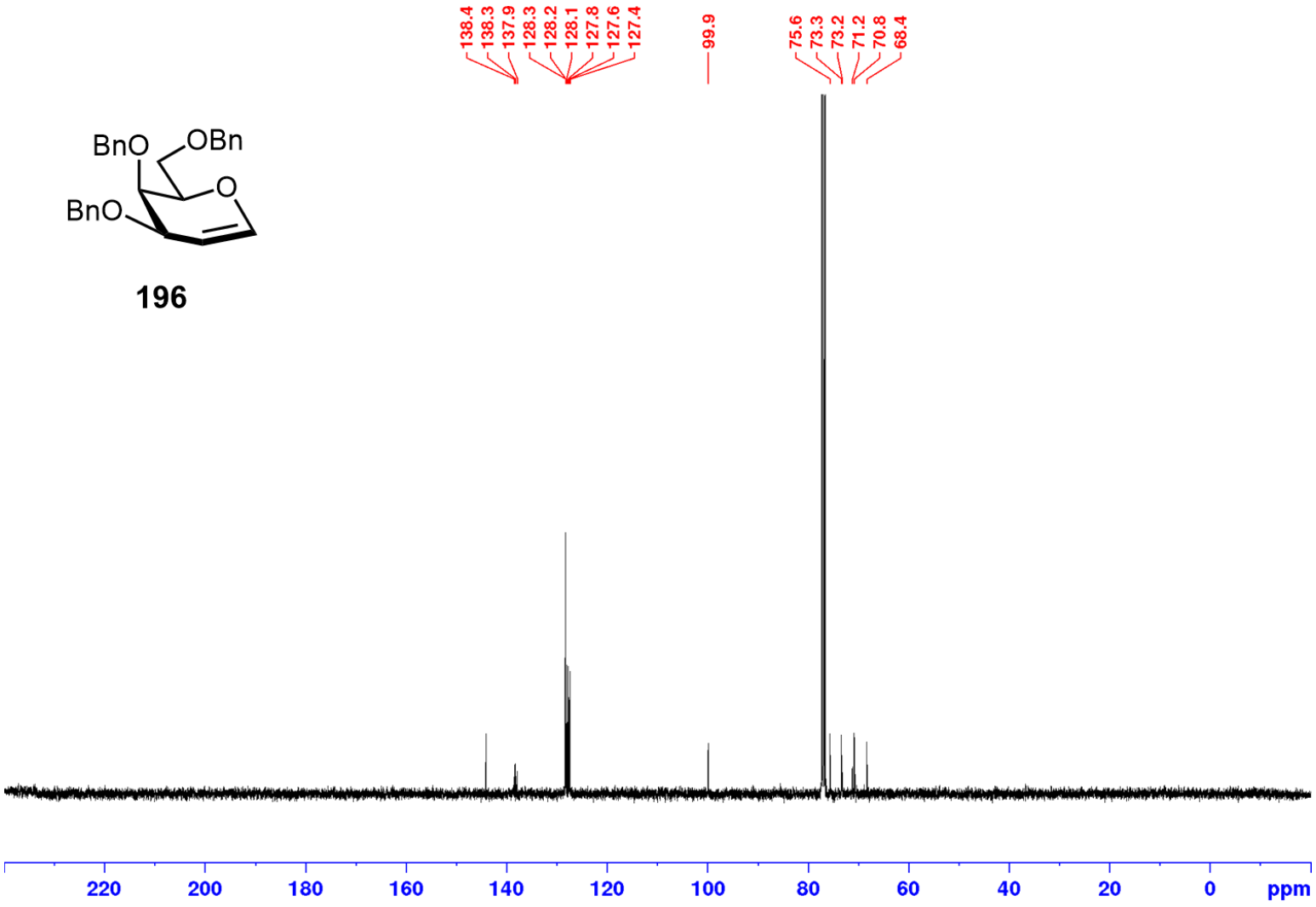
194 **195**

A solution of D-galactal **194** (1.0 equiv., 4.38 g, 30.0 mmol) in anhydrous DMF (300 mL) was added sodium hydride (4.0 equiv., 4.79 g, 120 mmol) at room temperature under an argon atmosphere. The reaction mixture was stirred at ambient temperature for 45 minutes under a constant stream of argon while the reaction was vented to allow for the emission of hydrogen gas. The reaction was cooled to 0 °C and benzyl bromide (4.0 equiv., 14.0 mL, 120 mmol) drop wise. The reaction mixture was stirred at room temperature. After 12 h, the reaction was cooled to 0 °C and added anhydrous CH_3OH (50 mL) to consume any residual sodium hydride. The reaction was diluted with EtOAc (450 mL), washed with water (3 x 150 mL), brine (150 mL x 1), dried ($MgSO_4$), filtered through a glass fritted funnel to remove drying agent. The filtrate was concentrated *in vacuo* to a crude oil. The crude residue was purified by flash column chromatography (90:10 hexanes/EtOAc) to provide tri-*O*-benzyl-D-galactal **195** (11.621 g, 27.9 mmol, 93%) as a white solid. The spectroscopic data agreed with the reported data.[300, 311-312] R_f 0.58 (3:1 hexanes/EtOAc). ^1H NMR (400 MHz, $CDCl_3$): δ 7.35-7.28 (m, 15H), 6.37 (dd, *J* = 1.27, 6.37 Hz, 1H), 4.89-4.84 (m, 2H), 4.67-4.59 (m, 3H), 4.45 (dd, *J* = 11.78 Hz, 2H), 4.18 (m, 2H), 3.94 (m, 1H), 3.78 (dd, *J* = 7.32, 10.19 Hz, 1H), 3.65 (dd, *J* = 5.15, 10.20 Hz, 1H); ^{13}C (100 MHz, $CDCl_3$) δ 144.5, 138.8, 138.6, 138.3, 128.7, 128.6, 128.4, 128.2, 128.0, 127.8, 127.7 (3 x Ph), 100.3, 76.0, 73.7, 73.6 (2 x CH_2Ph), 71.6, 71.2, 71.0, 68.7.

^{1}H NMR ($CDCl_3$, 400 MHz) of compound 196

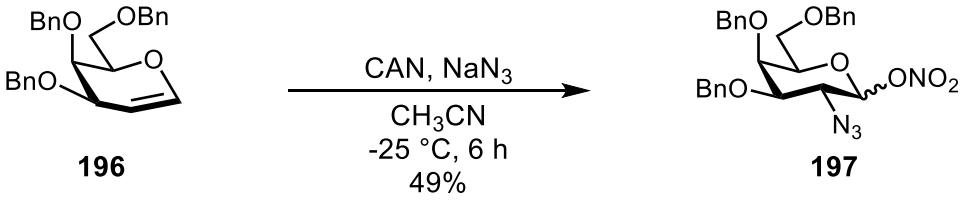


^{13}C NMR ($CDCl_3$, 100 MHz) of compound 196



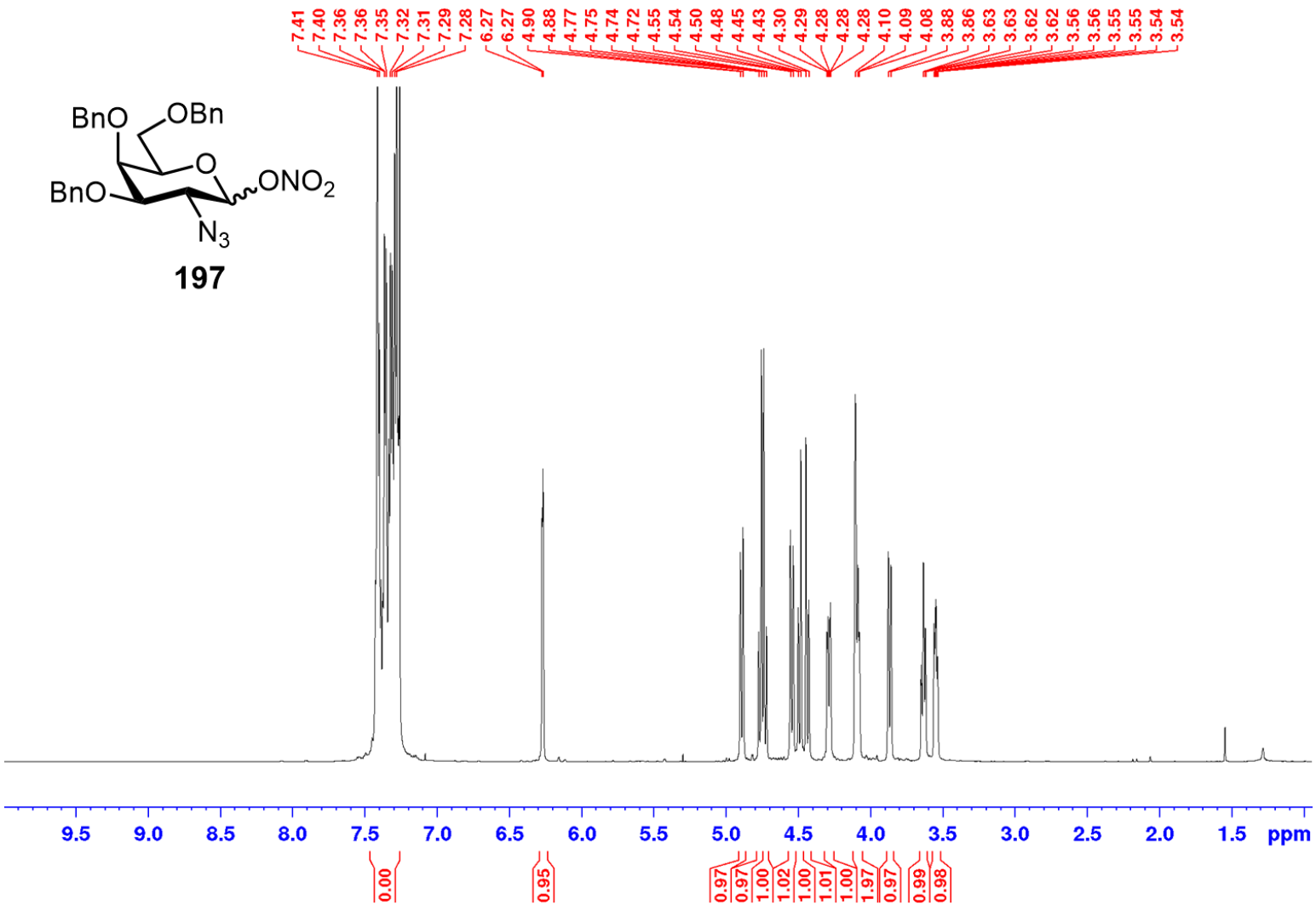
196

3,4,6-Tri-*O*-benzyl-2-deoxy-2-azido-α/β-D-galactopyranoside (197)

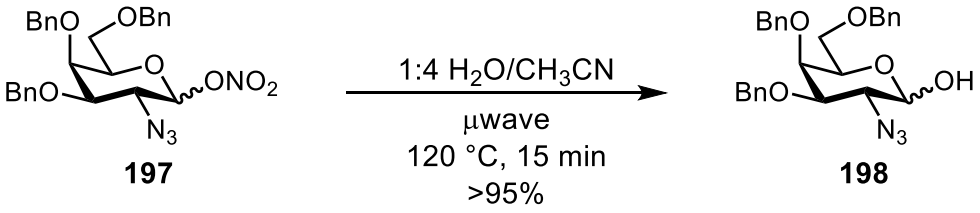


A solution of 3,4,6-tri-*O*-benyl-D-galactal **196** (1.0 equiv., 18.5 g, 44.4 mmol) in anhydrous CH_3CN (220 mL) at -25 °C was treated with cerium ammonium nitrate (3.0 equiv., 73.1 g, 133 mmol) followed by sodium azide (1.5 equiv., 4.33 g, 66.6 mmol). The mixture was stirred vigorously for 6 h at -25 °C and diluted with EtOAc (100 mL). The organic layer was washed with H_2O (3 x 100 mL), dried ($MgSO_4$), and filtered through a glass fritted filter funnel to remove drying agent. The filtrate was concentrated *in vacuo* to give a crude oil which was purified by silica gel flash column chromatography (10:90-25:75 EtOAc/hexanes) to give an inseparable mixture of nitrate esters 3,4,6-tri-*O*-benzyl-2-deoxy-2-azido-α/β-D-galactopyranoside **197** (11.3 g, 21.7 mmol, 49% yield) as a colorless oil. The spectroscopic data agreed with the reported data.[300, 313] ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.41-7.28 (m, 15H), 6.27 (d, *J* = 3.8 Hz, 1H), 4.89 (d, *J* = 11.7 Hz, 1H), 4.77 (d, *J* = 11.2 Hz, 1H), 4.73 (d, *J* = 11.2 Hz, 1H), 4.54 (d, *J* = 11.2 Hz, 1H), 4.49 (d, *J* = 11.2 Hz, 1H), 4.44 (d, *J* = 11.2 Hz, 1H), 4.29 (dd, *J* = 3.8, 10.8 Hz, 1H), 4.10-4.08 (m, 2H), 3.87 (d, *J* = 10.7 Hz, 1H), 3.63-3.62 (m, 1H), 3.56-3.54 (m, 1H).

^{1}H NMR ($CDCl_3$, 400 MHz) of compound 197

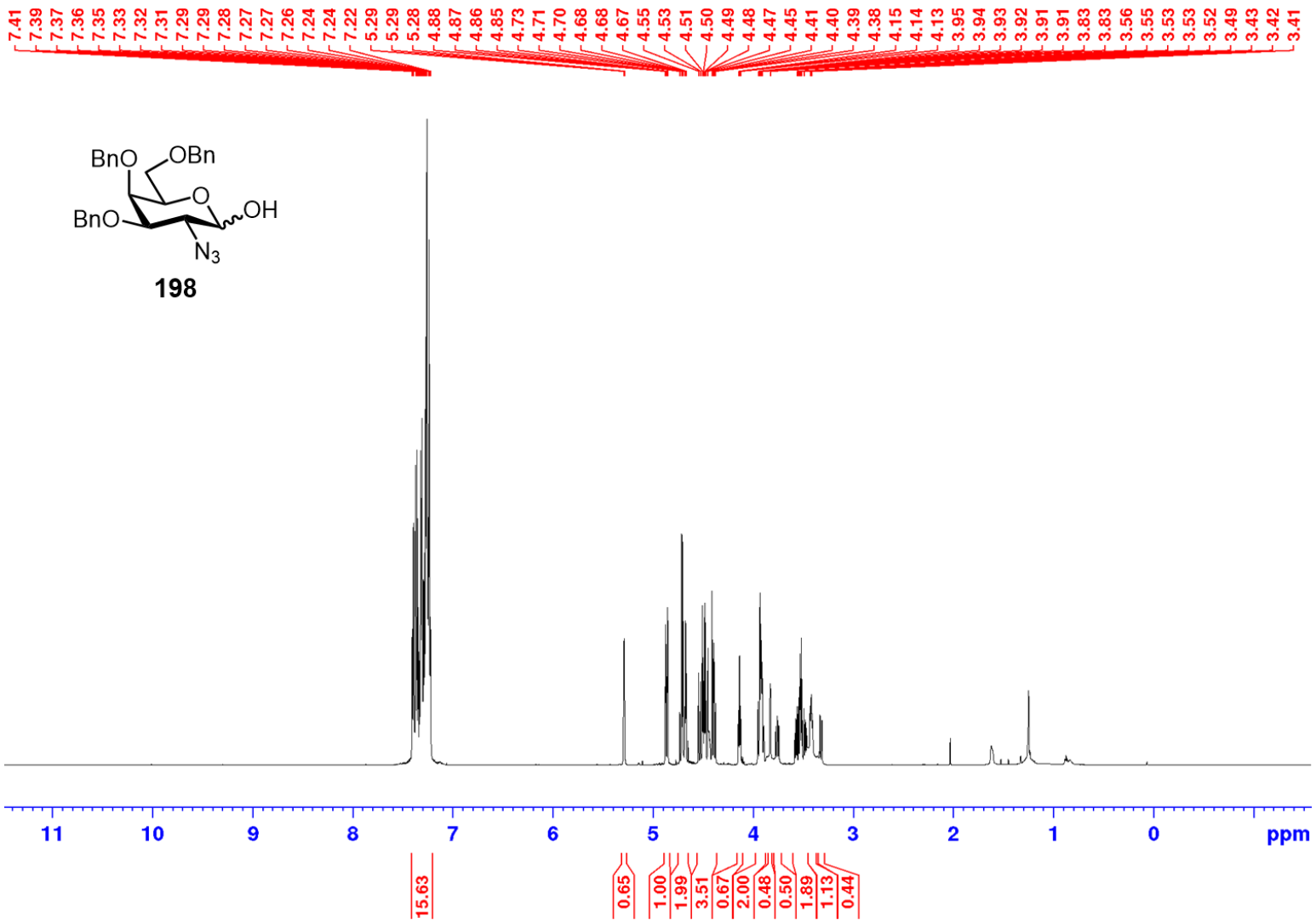


3,4,6-Tri-*O*-benzyl-2-deoxy-2-azido-D-galactosyl hemiacetal (198)

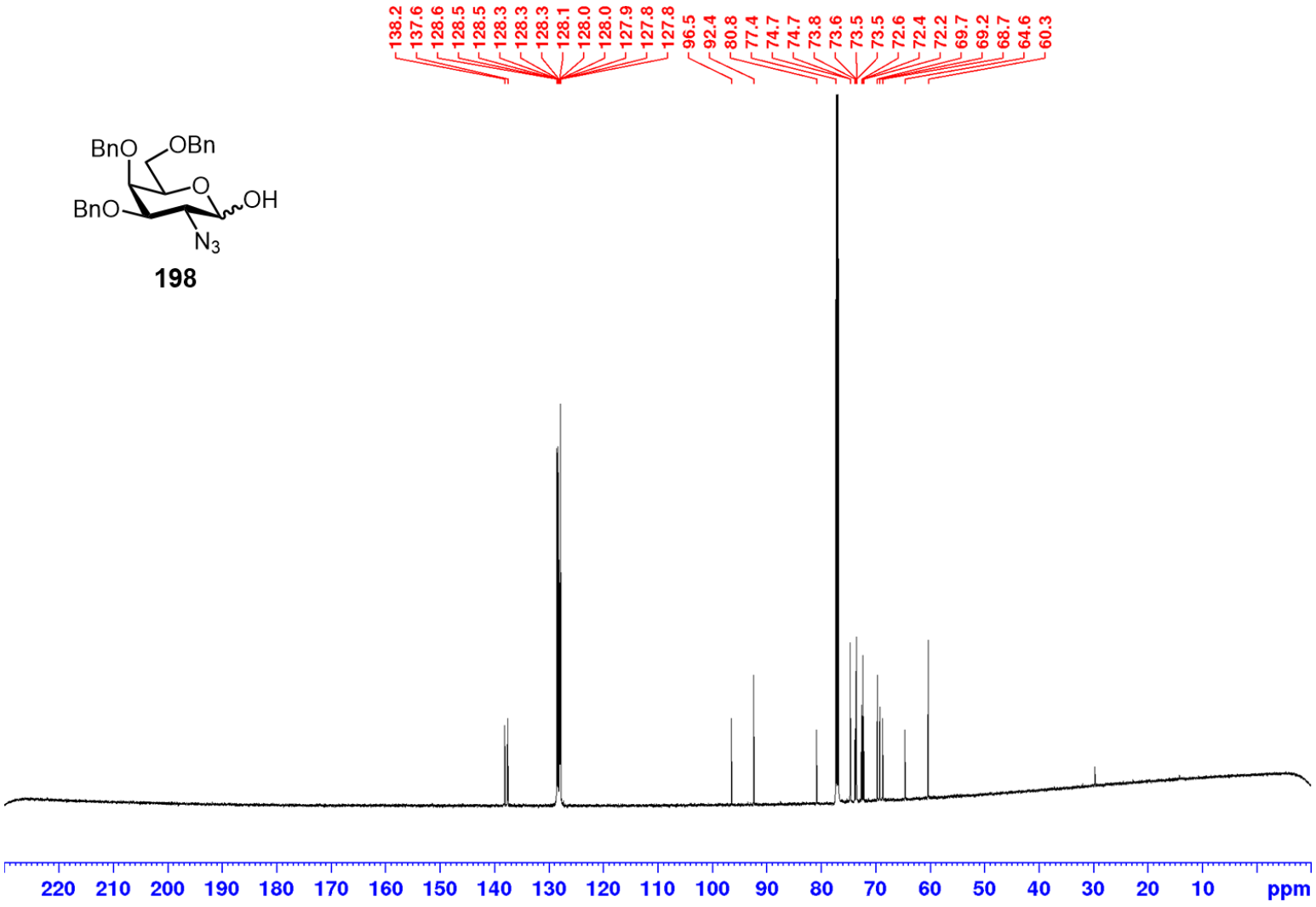


Prepared according to procedures developed in our lab.[153] A solution of nitrate esters 3,4,6-tri-*O*-benzyl-2-deoxy-2-azido-α/β-D-galactopyranoside **198** (1.0 equiv., 0.106 g, 0.204 mmol) in a 1:4 H_2O/acetone solution (2.0 mL) was heated by microwave irradiation to 120 °C for 15 minutes. The contents of each batches where combined and transferred to a separatory funnel. The organics where washed with water (2x), brine (1x), dried ($NaSO_4$) and filtered through a glass fritted vacuum filter funnel to remove drying agent. The filtrate was concentrated to a crude oil. The crude oil was purified by silica gel flash column chromatography (80:20 hexanes/EtOAc) to produce the reducing sugar 3,4,6-tri-*O*-benzyl-2-deoxy-2-azido-α/β-D-galactopyranoside **198** (0.97 g, 0.21 mmol, >95%) as a mixture of anomers. The spectroscopic data agreed with the reported data.[300, 313-314] HRMS (ESI) calcd for $C_{27}H_{29}O_5N_3Na$ $(M+Na)^+$ 498.2005, found 498.2018.

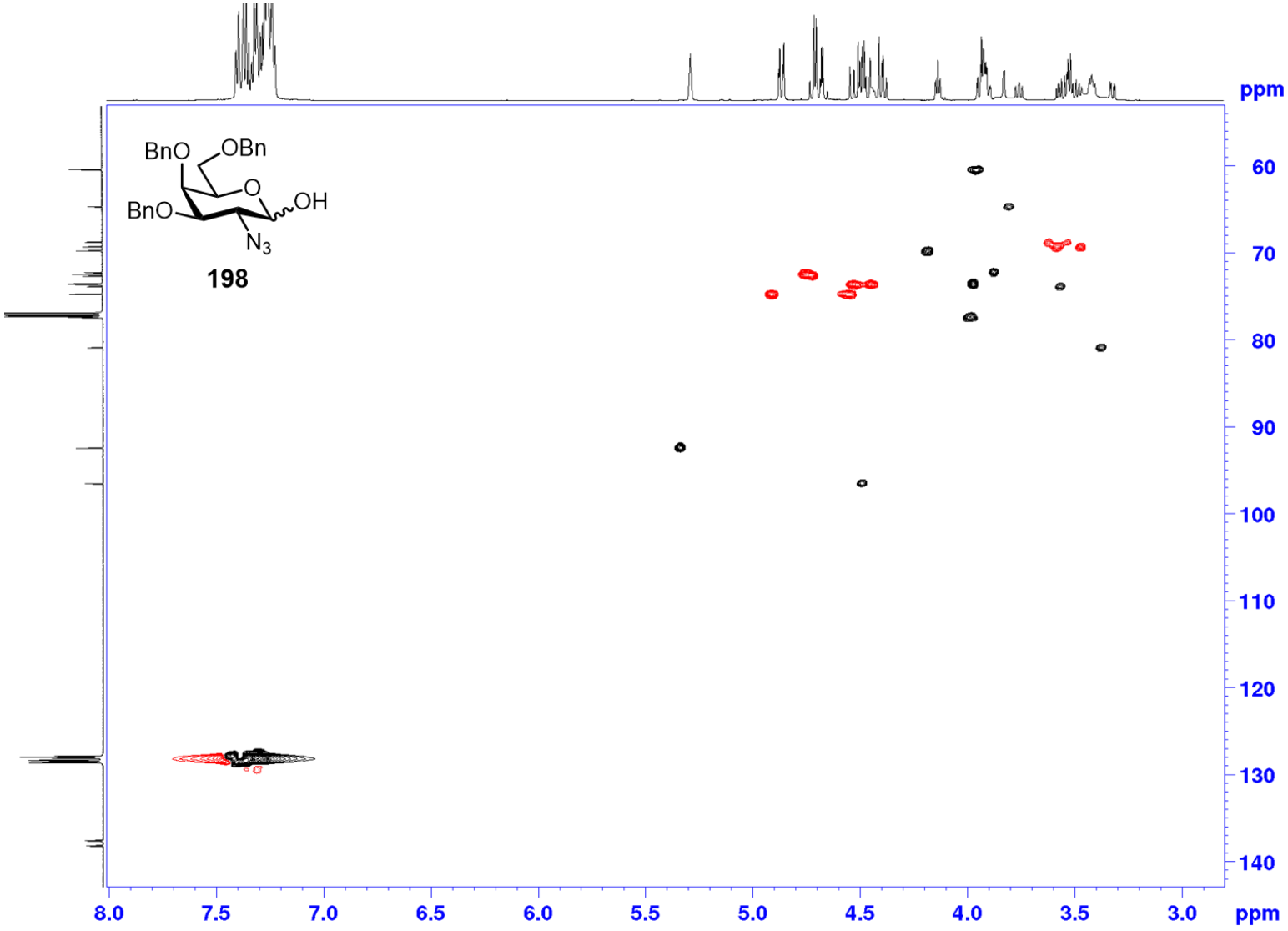
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 198



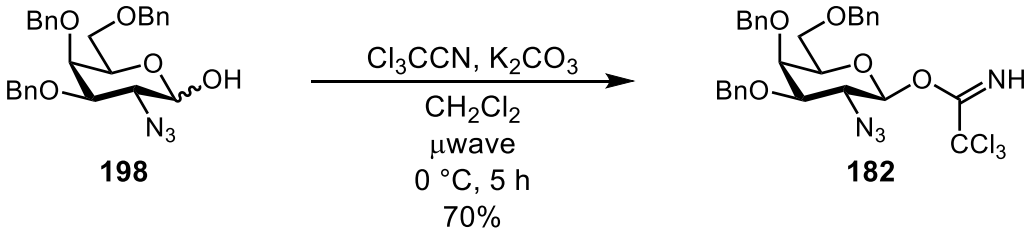
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 198



^{1}H-^{13}C HSCQ ($CDCl_3$) of compound S8

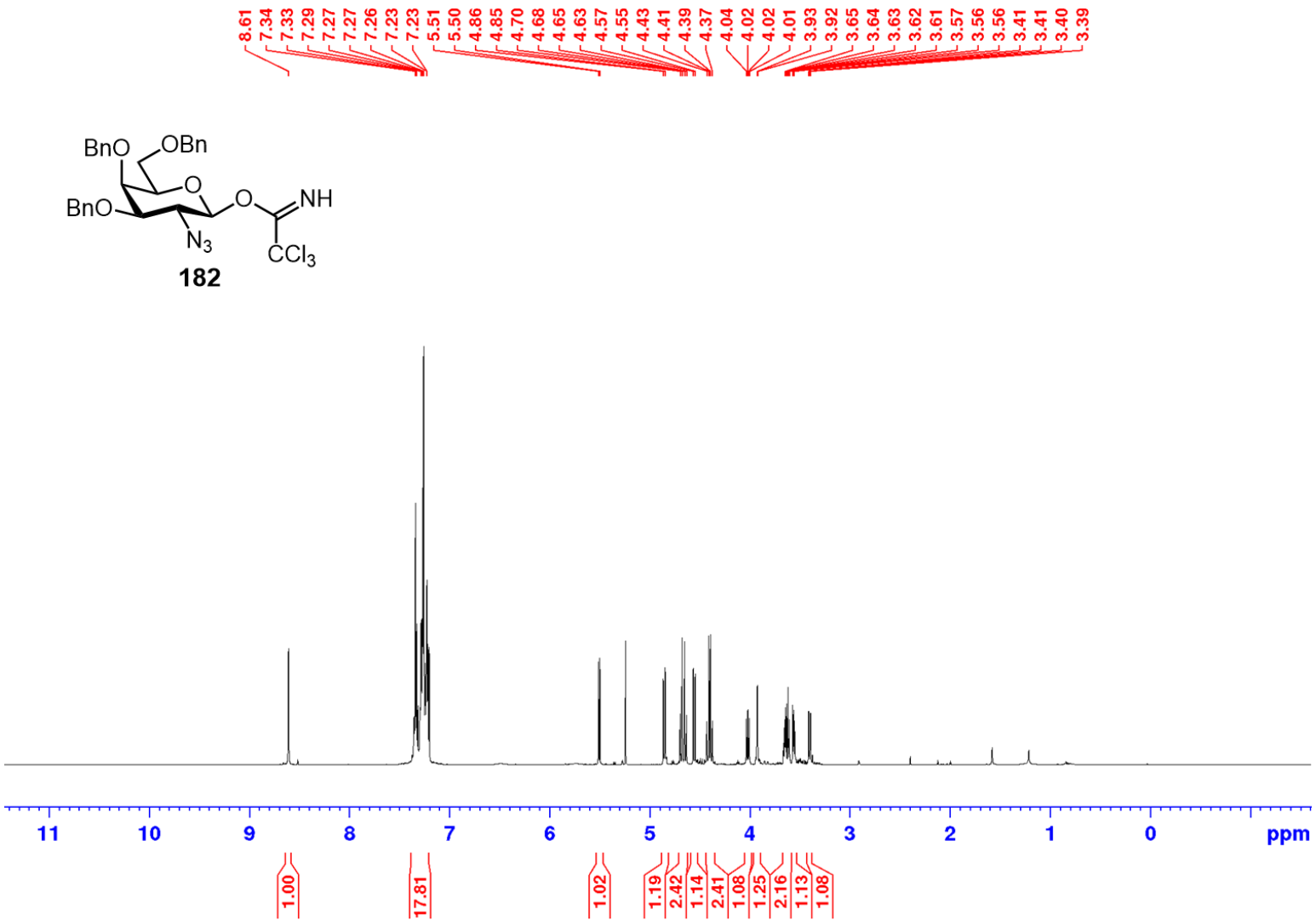


3,4,6-tri-*O*-benzyl-2-deoxy-2-azido-1-*O*-trichloroacetimidate-β-D-galactopyranoside (182)

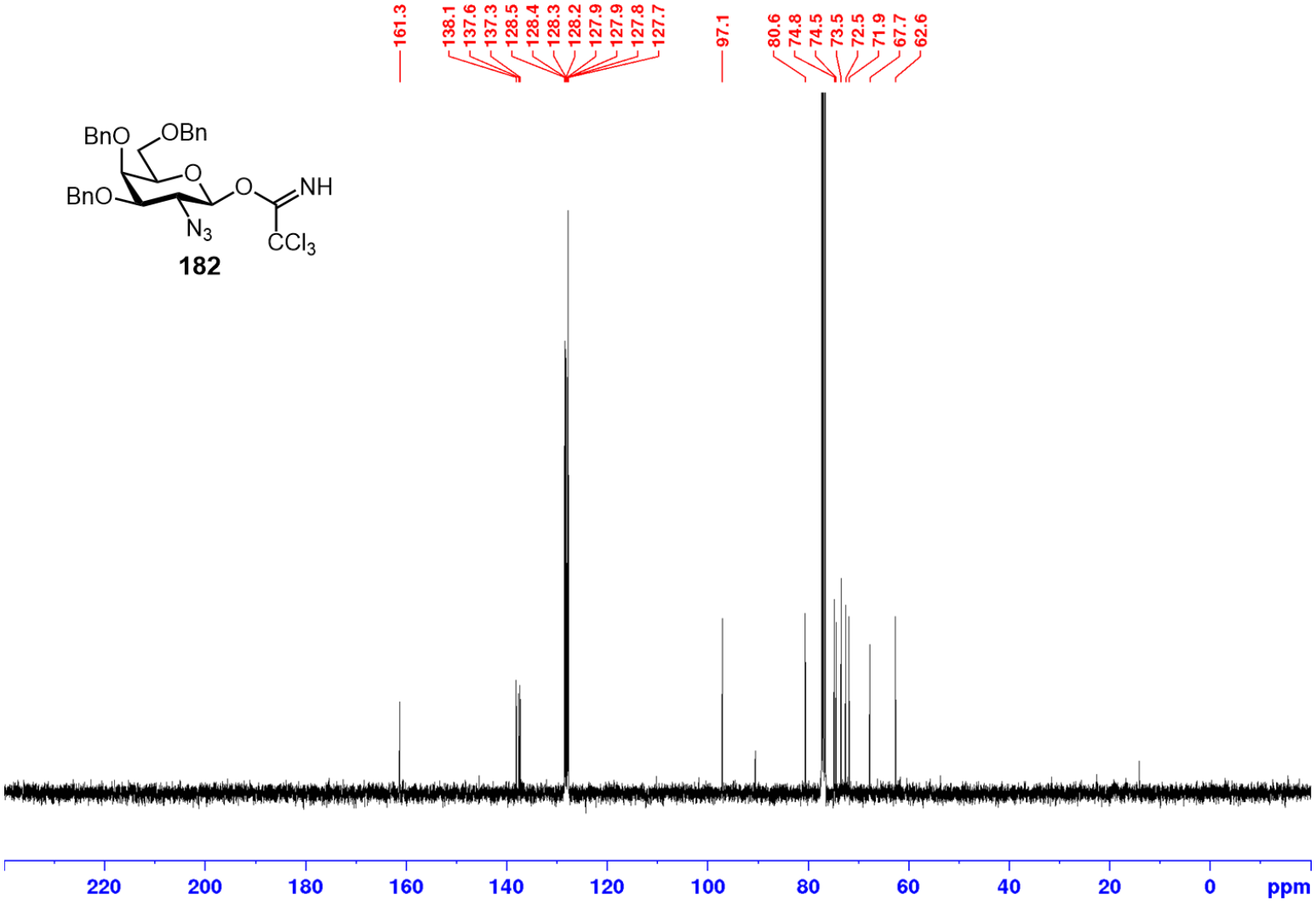


A solution of reducing sugar 3,4,6-tri-*O*-benzyl-2-deoxy-2-azido-α/β-D-galactopyranoside **198** (1.0 equiv., 10.0 g, 21.0 mmol) and potassium carbonate (5.0 equiv., 14.53 g, 105.0 mmol) in anhydrous CH_2Cl_2 (124 mL) was added trichloroacetonitrile (8.0 equiv., 16.9 mL, 168 mmol) at 0 °C under an argon atmosphere. After 5 h, the mixture was filtered through a glass fritted vacuum filter funnel equipped with a pad of over basic Celite with CH_2Cl_2 and concentrated *in vacuo*. The crude oil was purified by silica gel flash column chromatography (95:5 to 80:20 hexanes/EtOAc) to give β-trichloroacetimidate 3,4,6-tri-*O*-benzyl-2-deoxy-2-azido-β-D-galactopyranoside **182** (9.15 g, 14.7 mmol, 70%) as a white solid. Spectroscopic data were in agreement with the reported data.[313, 315] R_f 0.37 (4:1 hexanes/EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.60 (s, 1H), 7.34-7.20 (m, 15H), 5.50 (d, *J* = 8.30 Hz, 1H), 4.85 (d, *J* = 11.61 Hz, 1H), 4.66 (dd, *J* = 11.86 Hz, 2H), 4.55 (d, *J* = 11.5 Hz, 1H), 4.40 (dd, *J* = 11.50 Hz, 2H), 4.01 (dd, *J* = 8.5, 10.3 Hz), 3.92 (d, *J* = 2.7 Hz, 1H), 3.67-3.52 (m, 3H), 3.40 (dd, *J* = 2.97 Hz, 10.3, 1H); ^{13}C (100 MHz, $CDCl_3$) δ 161.7, 138.5, 138.0, 137.7, 128.9, 128.8, 128.7, 128.6, 128.3, 128.2, 128.1, 98.5, 90.9, 80.9, 77.5, 75.2, 74.8, 73.9, 72.9, 68.1, 63.0.

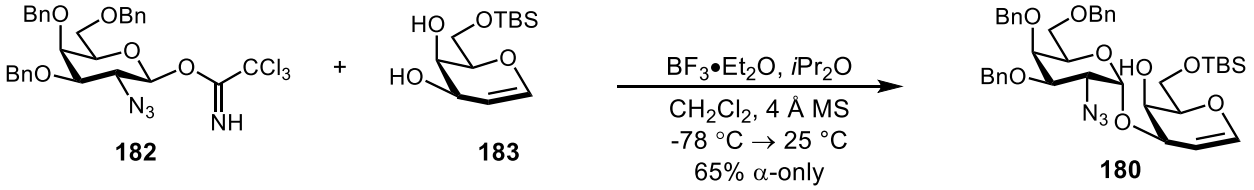
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 182



^{13}C NMR ($CDCl_3$, 100 MHz) of compound 182



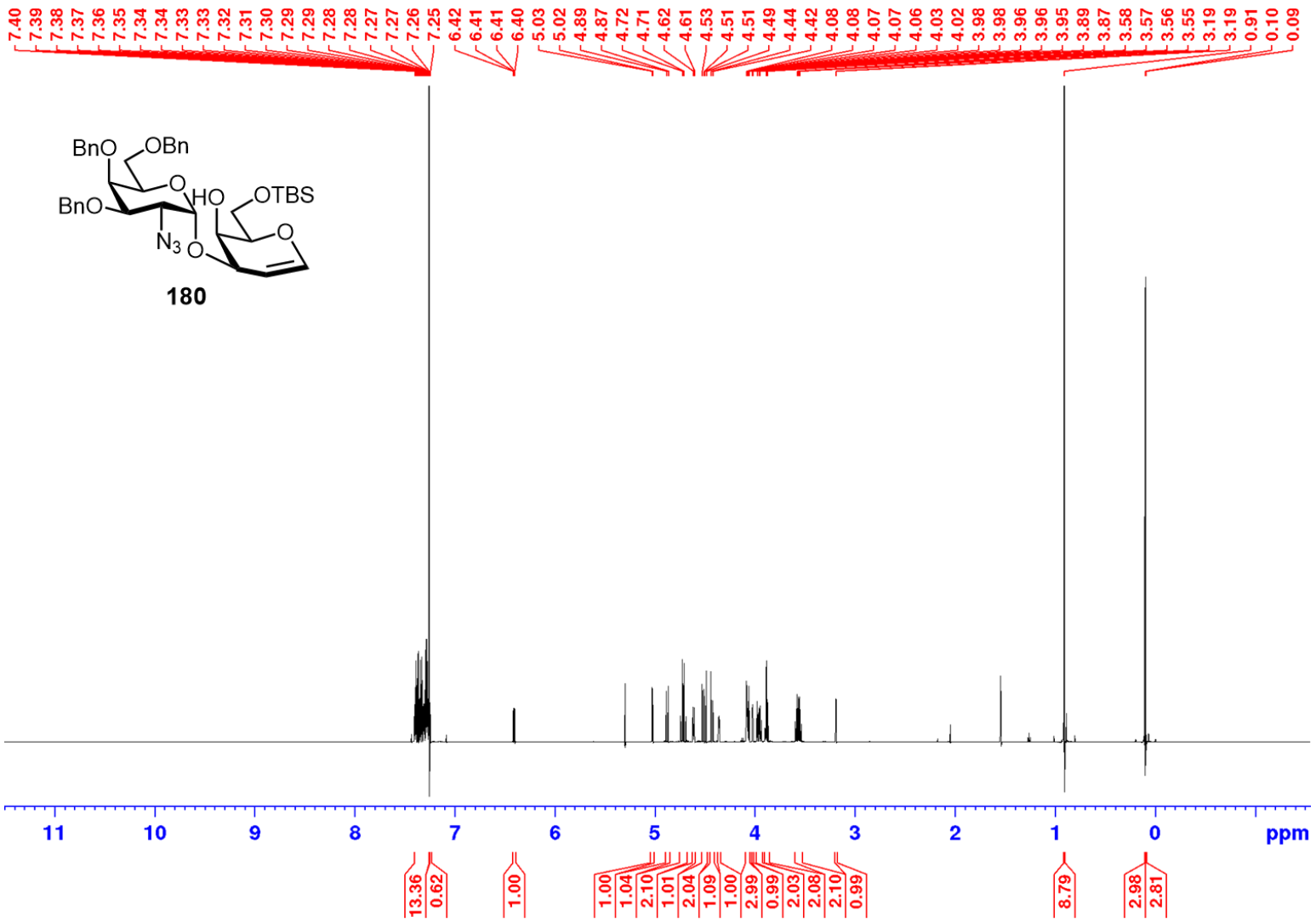
3,4,6-tri-*O*-benzyl-2-deoxy-2-azido-α-D-galactopyranosyl-(1→3)-6-*O*-*tert*-butyldimethylsilyl-D-galactal (180)



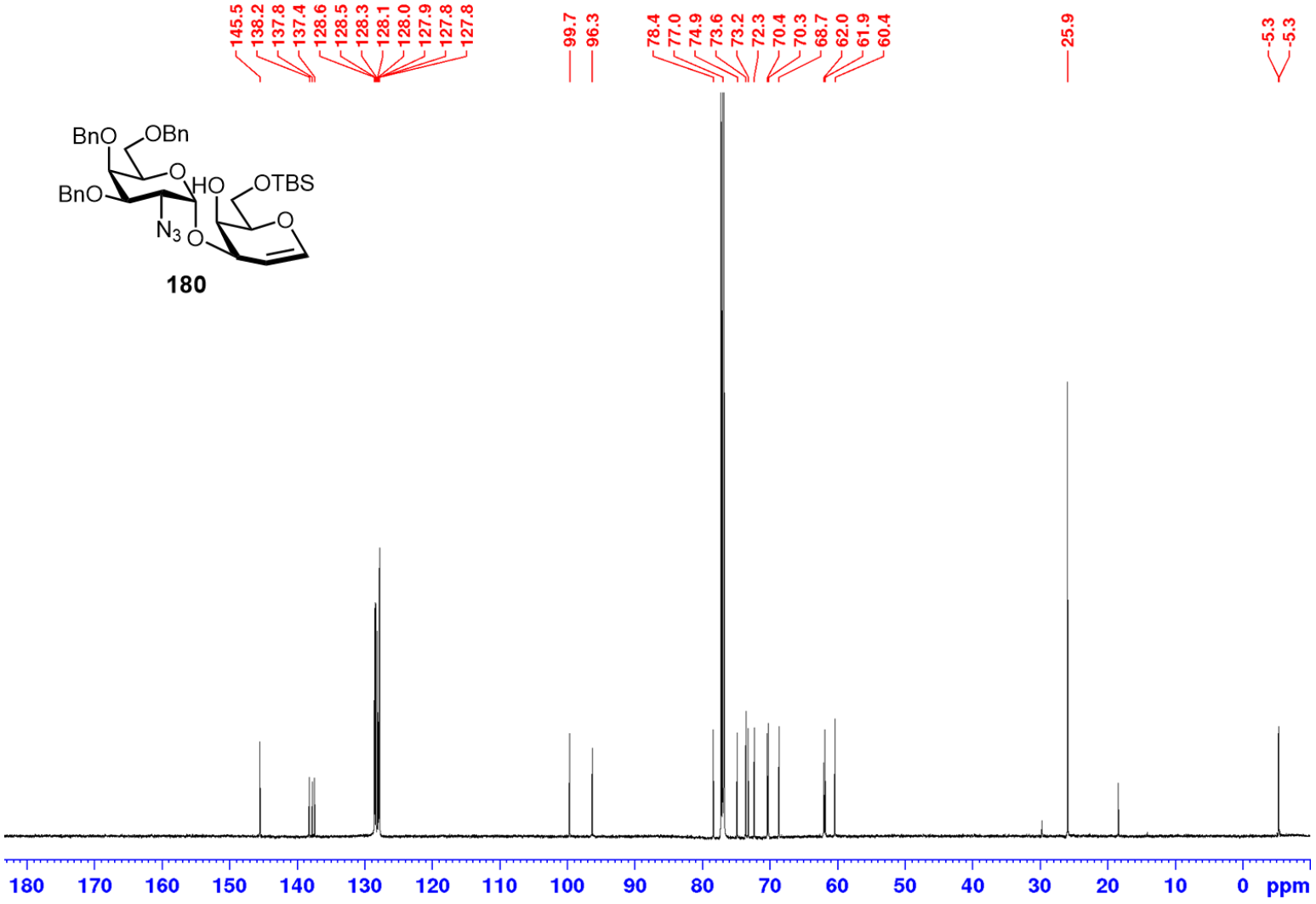
A solution of the β-trichloroacetimidate 3,4,6-tri-*O*-benzyl-2-deoxy-2-azido-β-D-galactopyranoside **182** (1.0 equiv., 1.44 g, 2.33 mmol) and 6-*O*-TBS-D-galactal acceptor **183** (1.0 equiv., 0.607 g, 2.33 mmol) were co-evaporated with benzene (3 x 20 mL) and placed under reduced pressure for 1 h. The dried mixture was diluted with anhydrous CH_2Cl_2 and cannulated into a, previously flame dried and cooled, round bottom flask under an argon atmosphere. The reaction flask was added pre-activated 4 Å molecular sieves (2.3 g) and a stir bar. The mixture was stirred at room temperature. After 1 h, the reaction mixture was cooled to -78 °C then added BF_3•EtO_2 (0.1 equiv.). The reaction mixture was stirred at room temperature. At 12 h, the reaction was neutralized by the addition of Et_3N (1 mL). The crude reaction mixture was filtered through a glass fritted filter funnel equipped with a pad of Celite. The filtrate was concentrated to a crude oil. The crude oil was purified by silica gel flash column chromatography (90:10 hexane/EtOAc) to give 3,4,6-tri-*O*-benzyl-2-deoxy-2-azido-α-D-galactopyranosyl-(1→3)-6-*O*-*tert*-butyldimethylsilyl-D-galactal **180** (1.088 g, 1.51 mmol, 66%) as a white solid. R_f 0.70 (3:2 hexanes/EtOAc); $[\alpha]D^{25}$ = -2.88° (c = 1.0, $CHCl_3$); IR ν_{max} (film) 3625.93, 1639.38, 1591.61, 1443.13, 1225.43, 1235.34, 1260.63, 982.90, 750.53, 695.41 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.4-7.25 (m, 15H), 6.40 (dd, *J* = 1.57, 6.30 Hz, 1H), 5.02 (d, *J* = 3.85 Hz, 1H), 4.87 (d, *J* = 11.2 Hz, 1H), 4.71 (dd, *J* = 11.37, 10.2 Hz, 2H), 4.61 (dd, *J* =

1.92, 6.30 Hz, 1H), 4.52-4.42 (m, 3H), 4.36 (m, 1H), 4.07 (m, 3H), 4.02 (m, 1H), 3.98-3.93 (m, 2H), 3.89-3.86 (m, 2H), 3.59-3.53 (m, 2H), 3.19 (d, *J* = 2.8 Hz, 1H), 0.90 (s, 9H), 0.099 (s, 3H), 0.094 (s, 3H); ^{13}C (100 MHz, $CDCl_3$) δ 145.9, 138.5, 138.1, 137.8, 128.9, 128.8, 128.7, 128.5, 128.2, 128.1, 100.0, 96.6, 78.7, 77.6, 75.2, 73.9, 73.6, 72.7, 70.7, 70.6, 69.0, 62.4, 62.2, 60.7, 26.3, 18.8, -4.9, -5.0; HRMS (ESI) calcd for $C_{39}H_{55}N_4O_8Si^+$ $(M+NH_4)^+$ 735.37832 found 735.37650 m/z

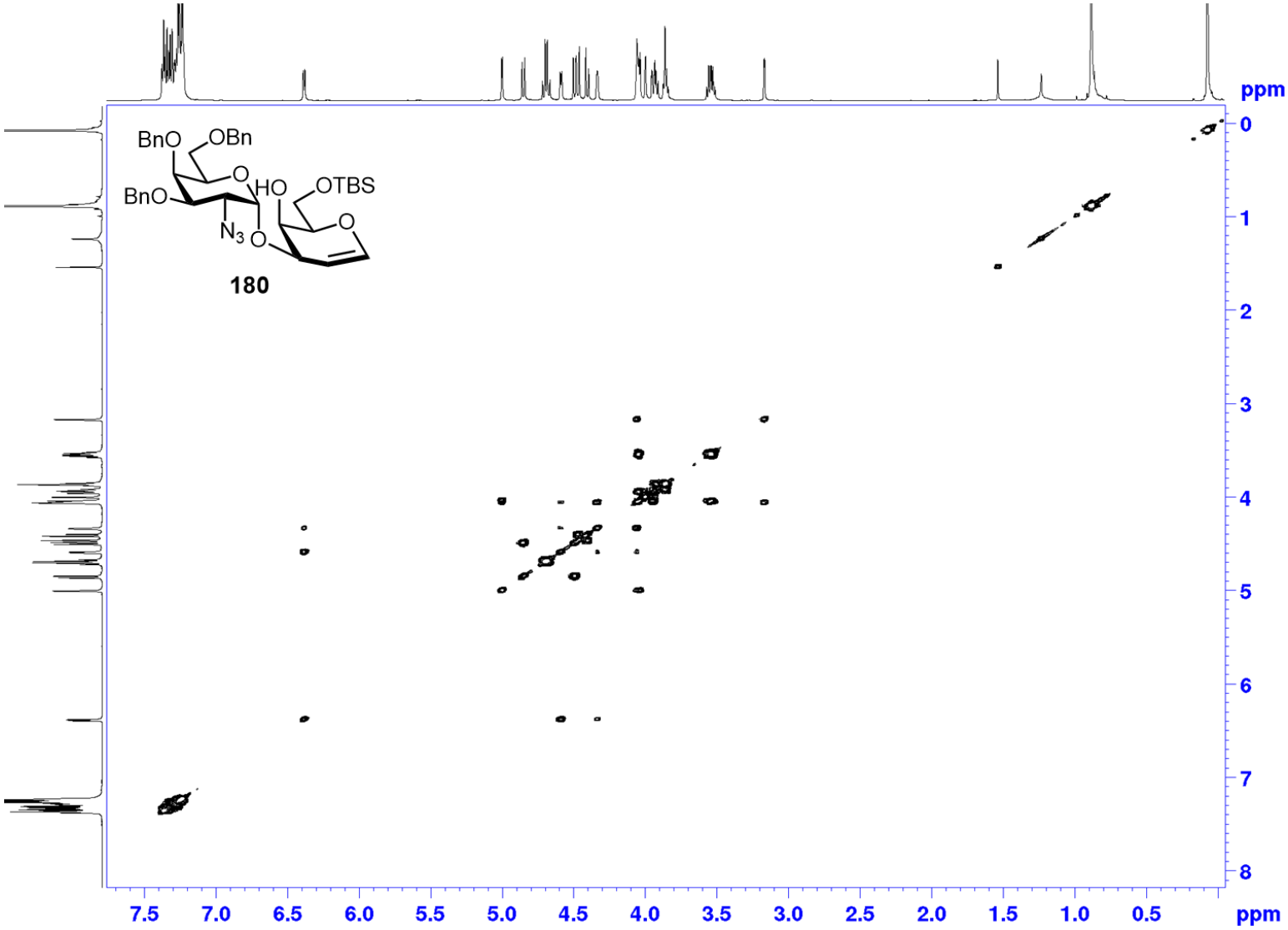
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 180



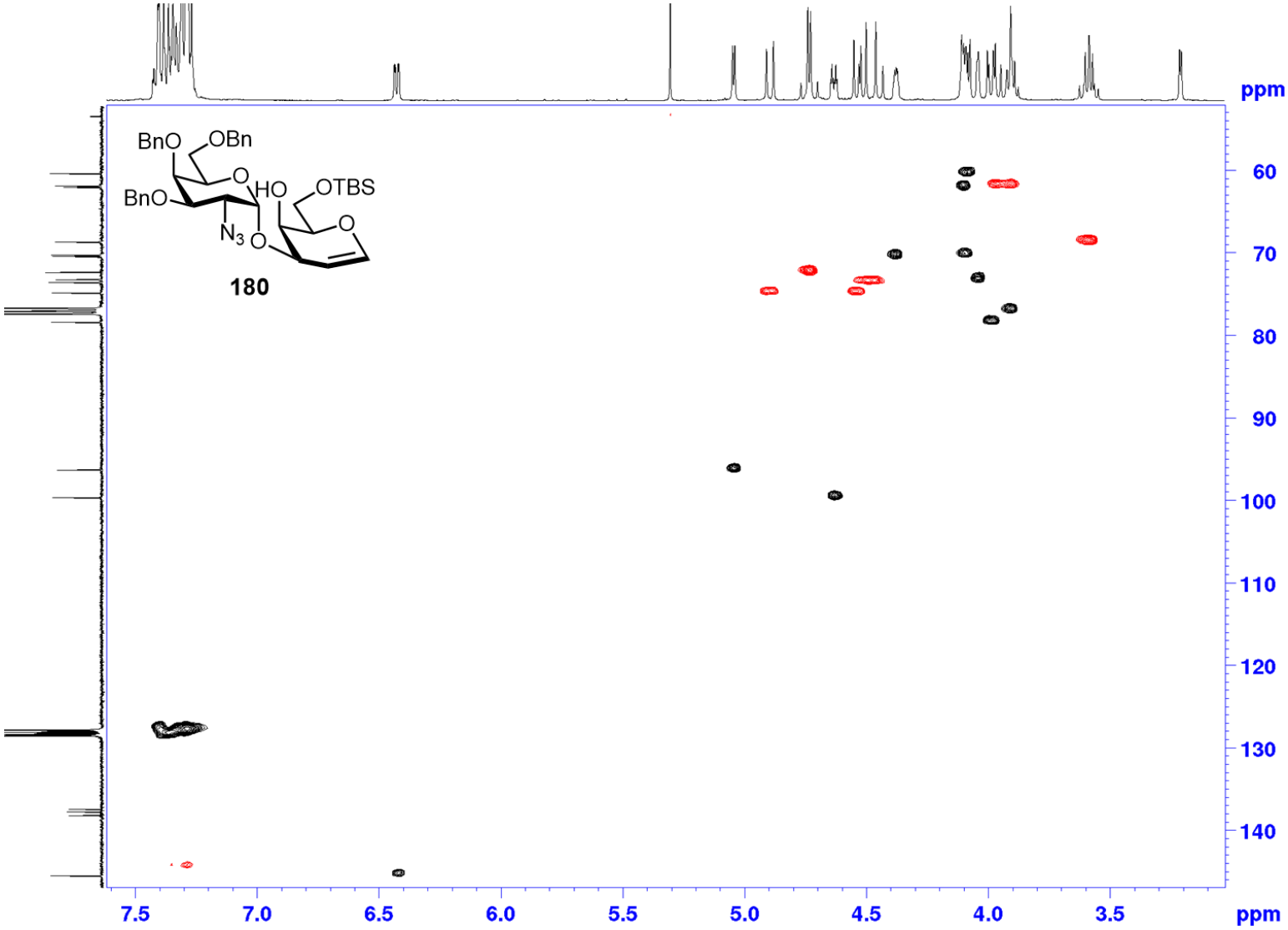
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 180

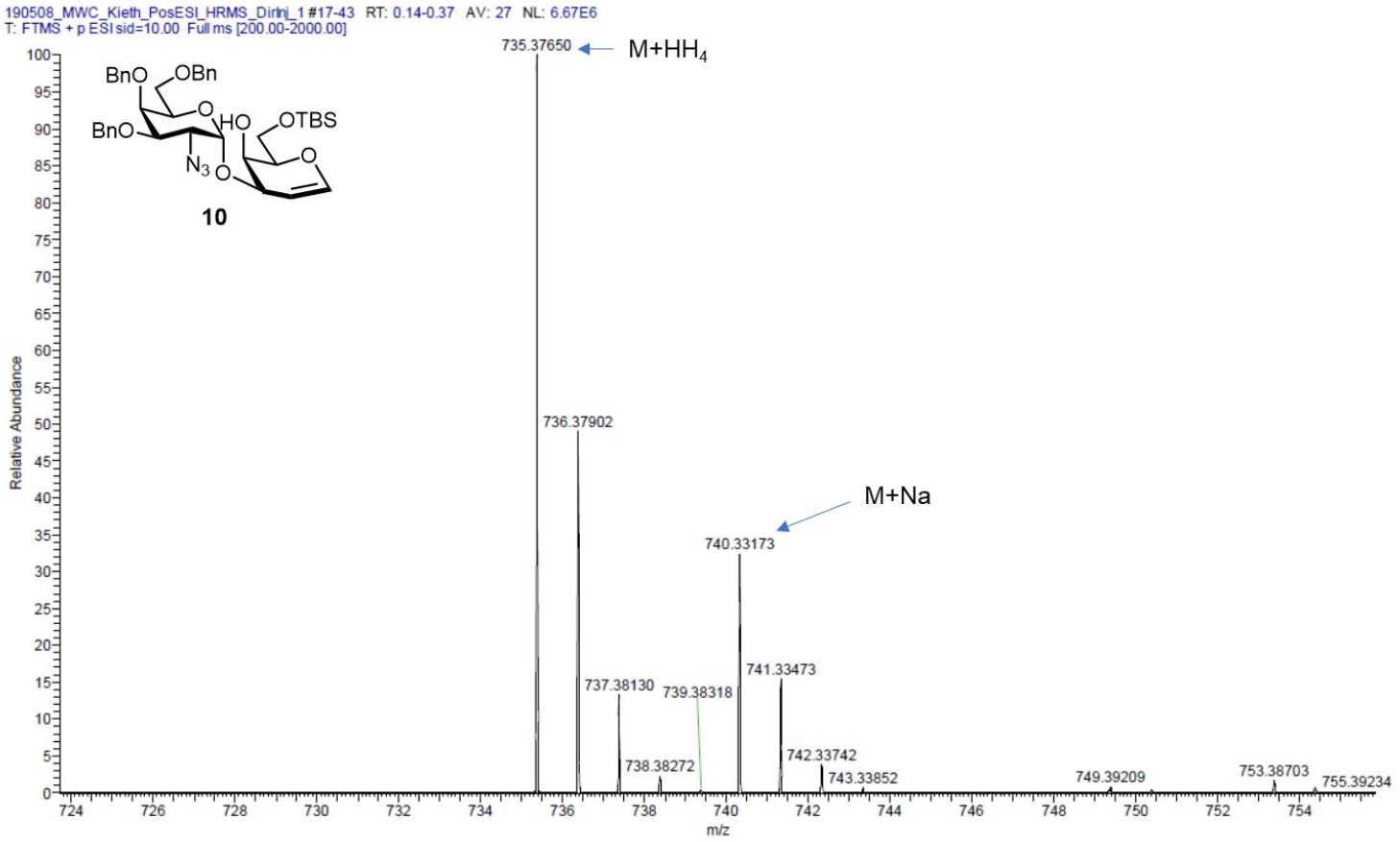


^{1}H-^{1}H COSY ($CDCl_3$) of compound 180



^{1}H-^{13}C HSCQ ($CDCl_3$) of compound 180




190508_MWC_Kieth_PosESI_HRMS_Dirlnj_1 #17-43 RT: 0.14-0.37 AV: 27 NL: 6.67E6
T: FTMS + p ESI sid=10.00 Full ms [200.00-2000.00]
BnO
OBn
BnO
HO
OTBS
N3
O
O
10
735.37650
M+HH4
736.37902
M+Na
740.33173
737.38130
739.38318
741.33473
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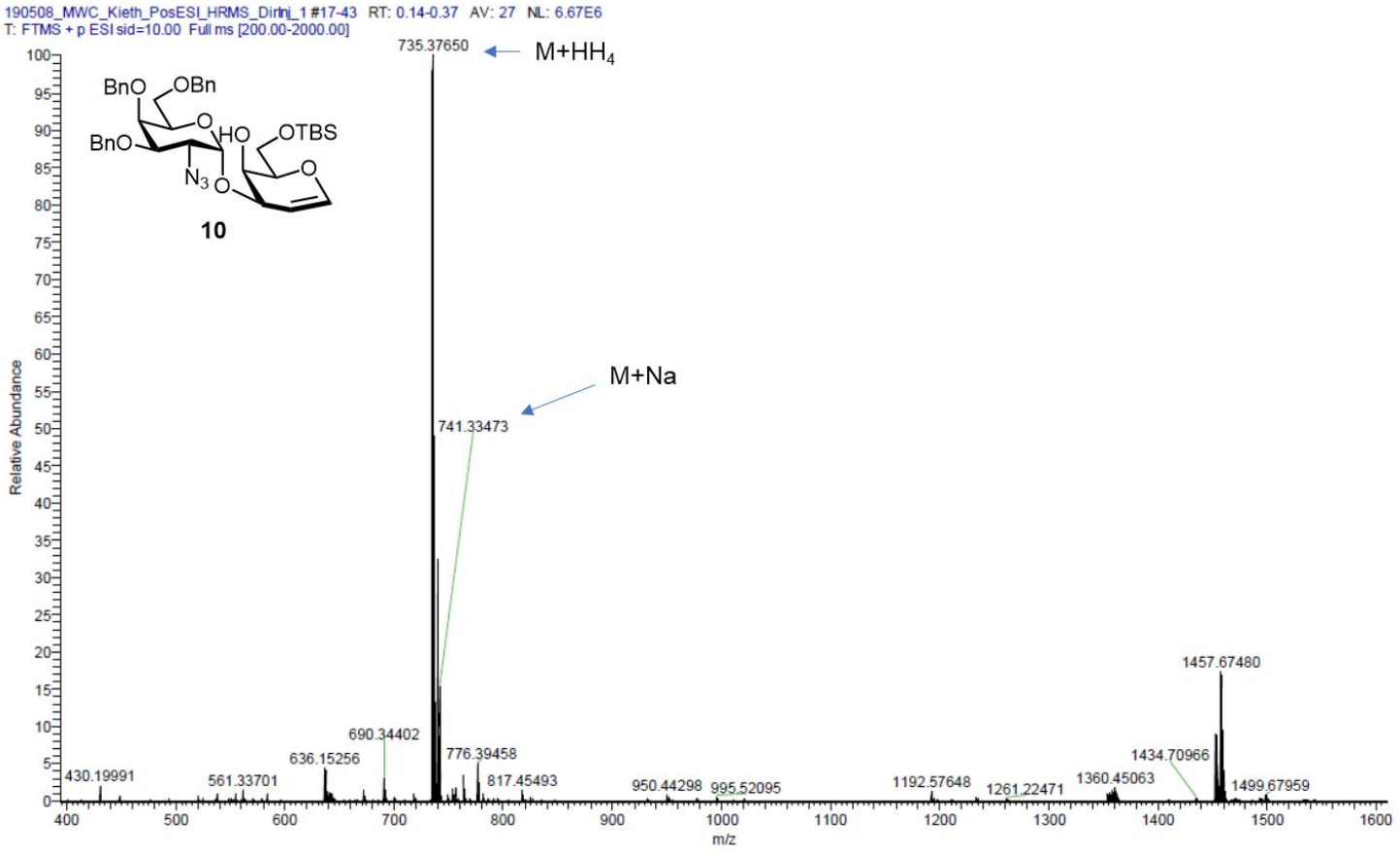
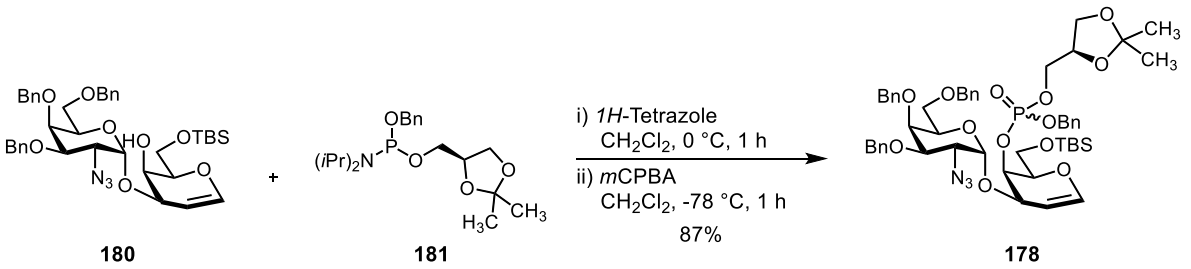
742.33742
743.33852
749.39209
753.38703
755.39234
Relative Abundance
m/z


190508_MWC_Kieth_PosESI_HRMS_DirInj_1 #17-43 RT: 0.14-0.37 AV: 27 NL: 6.67E6
T: FTMS + p ESI sid=10.00 Full ms [200.00-2000.00]
BnO
OBn
BnO
HO
OTBS
N3
10
735.37650
M+HH4
M+Na
741.33473
Relative Abundance
m/z
430.19991
561.33701
636.15256
690.34402
776.39458
817.45493
950.44298
995.52095
1192.57648
1261.22471
1360.45063
1434.70966
1457.67480
1499.67959

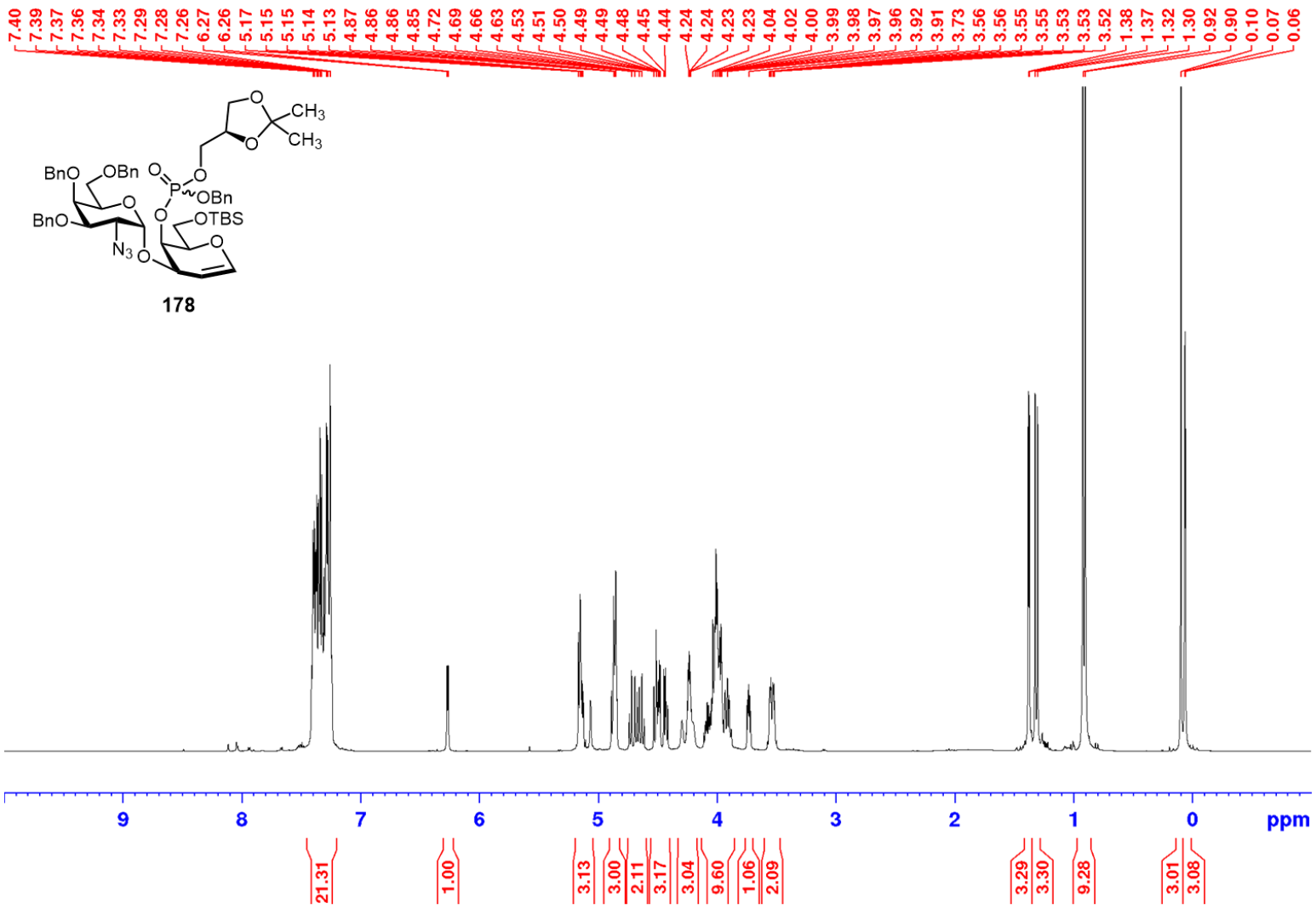
3,4,6-tri-*O*-benzyl-2-deoxy-2-azido-α-D-galactopyranosyl-(1→3)-6-*O*-*tert*-butyldimethylsilyl-4-*O*-(((*R*)-2,2-dimethyl-1,3-dioxolan-4-yl)methyl) phosphate-D-galactal (178)



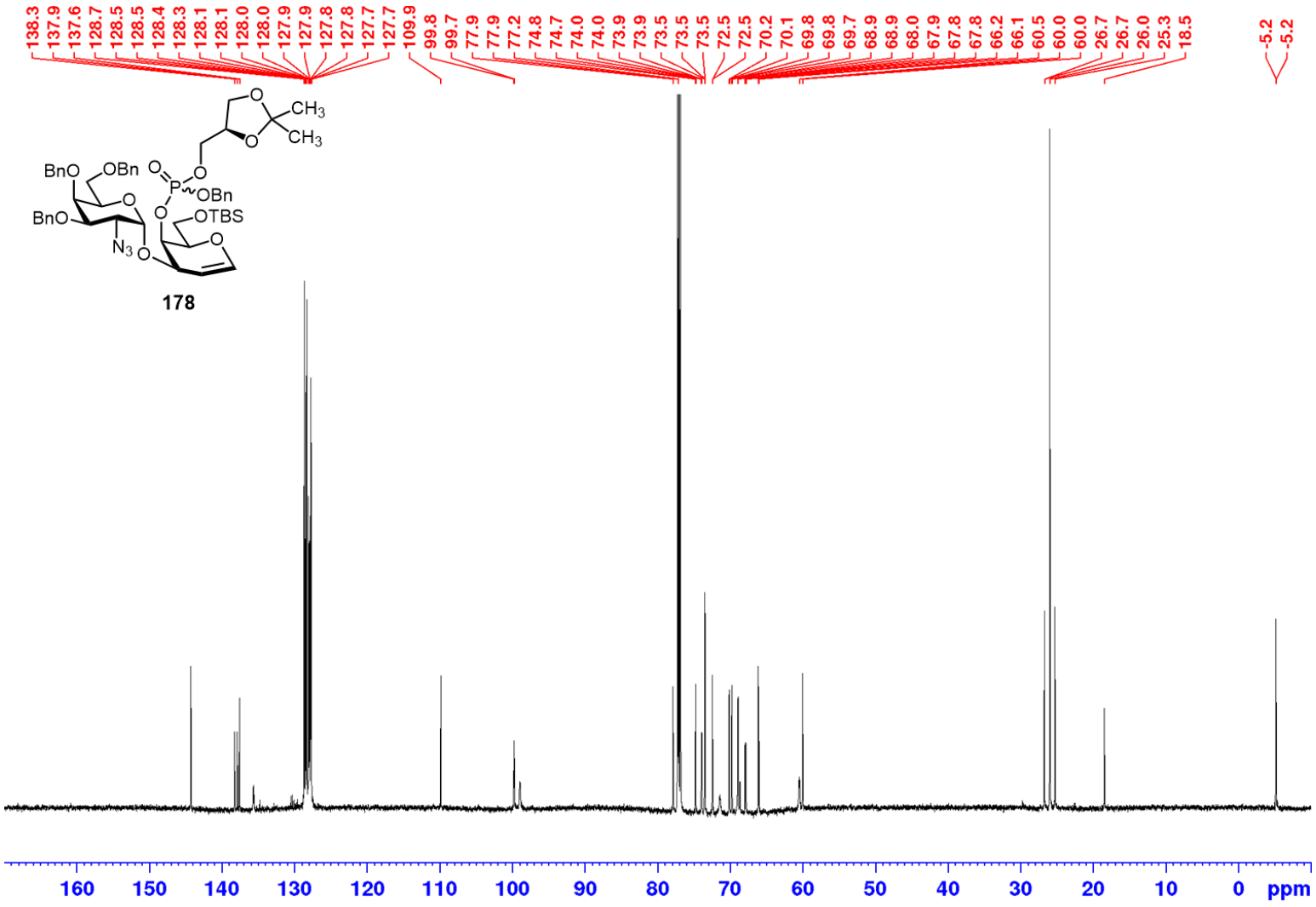
A solution of phosphoramidite **181** (2.0 equiv., 2.47 g, 6.69 mmol) in anhydrous CH_2Cl_2 (20 mL) was cooled to 0 °C under an argon atmosphere. *1H*-tetrazole (3.0 equiv., 0.7032 g, 10.118 mmol) was quickly added neat to the reaction. At 5 minutes, a solution of 3,4,6-tri-*O*-benzyl-2-deoxy-2-azido-α-D-galactopyranosyl-(1→3)-6-*O*-*tert*-butyldimethylsilyl-D-galactal **180** (1.0 equiv., 2.4 g, 3.34 mmol) in anhydrous CH_2Cl_2 (20 mL) was cannulated into the reaction mixture. The reaction warmed to room temperature. After 2 h, the reaction was cooled to -40 °C and a solution of *m*CPBA (2.0 equiv., 1.154 g, 6.690 mmol) in anhydrous CH_2Cl_2 (10 mL) dropwise. After 30 minutes, the reaction was diluted with anhydrous CH_2Cl_2 (10 mL) and sat. aq. $NaHCO_3$ (50 mL) was poured into the reaction mixture and allowed to stir. The reaction mixture was transferred to a separatory funnel with CH_2Cl_2 (25 mL). The aqueous layer was then extracted CH_2Cl_2 (3 x 20 mL). The organic layers were combined, dried ($MgSO_4$) and filtered through a glass fritted filter funnel. The filtrate was concentrated *in vacuo* to a crude oil. The crude oil was purified by silica gel flash column chromatography (80:20 hexanes/EtOAc) to give 3,4,6-tri-*O*-benzyl-2-deoxy-2-azido-α-D-galactopyranosyl-(1→3)-6-*O*-tert-butyldimethylsilyl-4-*O*-(((*R*)-2,2-dimethyl-1,3-dioxolan-4-yl)methyl) phosphate-D-galactal **178** (2.92 g, 2.91 mmol, 87%)

as an inseparable pair of diastereomers as a colorless oil: R_f 0.29 (7:3 hexanes/EtOAc); $[\alpha]D^{25}$ = -0.68° (c = 1.0, $CHCl_3$); IR ν_{max} (film) 3485.32, 3053.30, 2985.82, 2305.43, 2112.42, 1746.34, 1606.80, 1492.65, 1424.61, 1375.51, 1352.60, 1311.44, 1194.26, 1139.04, 1048.11, 804.82, 746.13, 704.12, 477.11 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.4-7.25 (m, 15H), 6.40 (dd, *J* = 1.57, 6.30 Hz, 1H), 5.02 (d, *J* = 3.85 Hz, 1H), 4.87 (d, *J* = 11.2 Hz, 1H), 4.71 (dd, *J* = 11.37, 10.2 Hz, 2H), 4.61 (dt, *J* = 1.92, 6.30 Hz, 1H), 4.52-4.42 (m, 3H), 4.36 (m, 1H), 4.07 (m, 3H), 4.02 (m, 1H), 3.98-3.93 (m, 2H), 3.89-3.86 (m, 2H), 3.59-3.53 (m, 2H), 3.19 (d, *J* = 2.8 Hz, 1H), 0.90 (s, 9H), 0.099 (s, 3H), 0.094 (s, 3H); ^{13}C (100 MHz, $CDCl_3$) δ 145.8, 138.5, 138.1, 137.7, 128.8, 128.8, 128.6, 128.4, 128.1, 128.1, 100.0, 96.6, 78.7, 77.5, 75.1, 73.8, 73.5, 72.6, 70.7, 70.5, 69.0, 62.3, 62.1, 60.7, 26.3, 18.8, -4.94, -5.0; ^{31}P NMR (162 MHz, $CDCl_3$): δ -1.6, -1.7; HRMS (ESI) calcd for $C_{52}H_{72}N_4O_{13}PSiNa^+$ $(M+Na)^+$ 1019.45972 found 1019.46105 m/z.

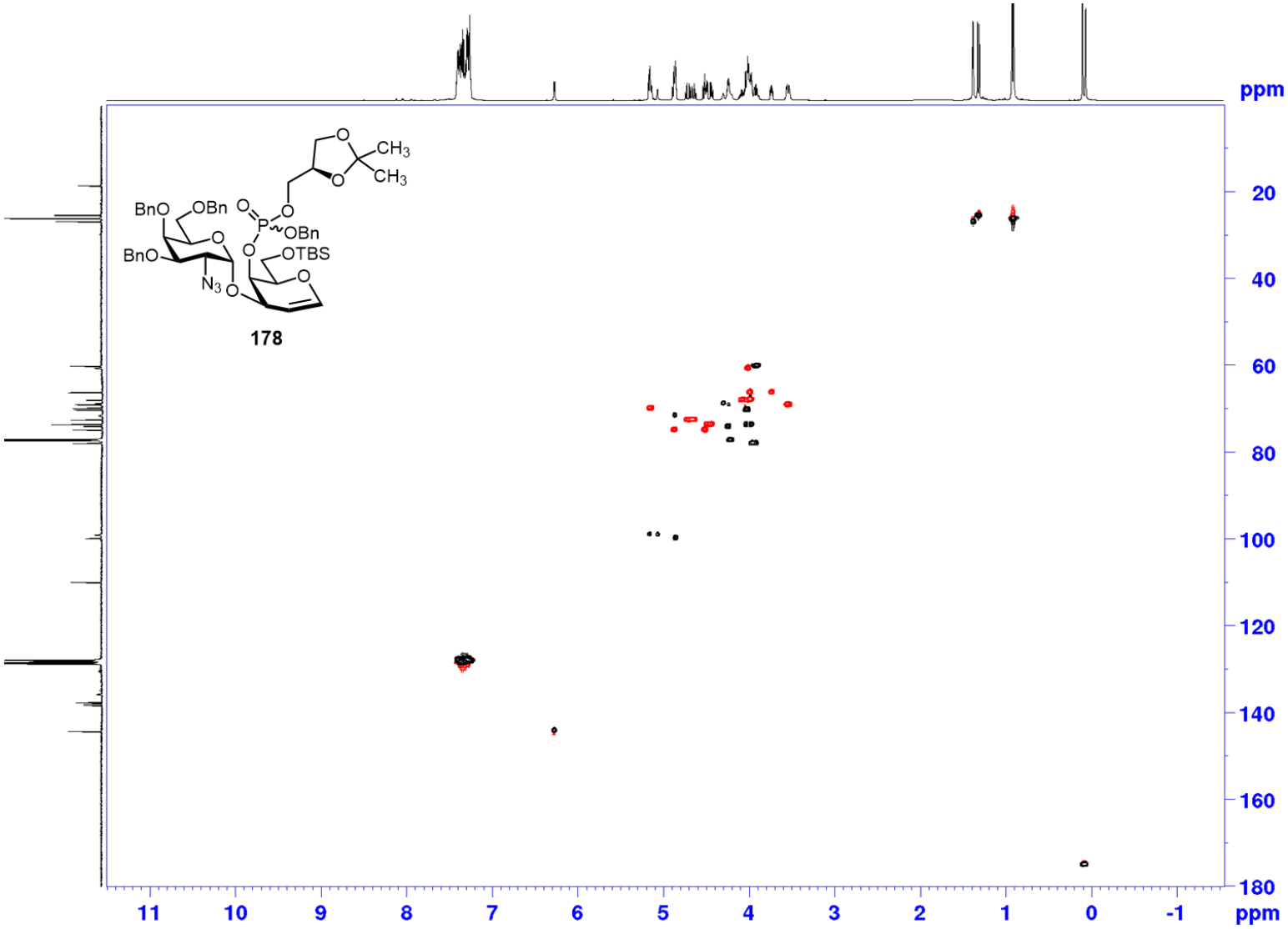
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 178



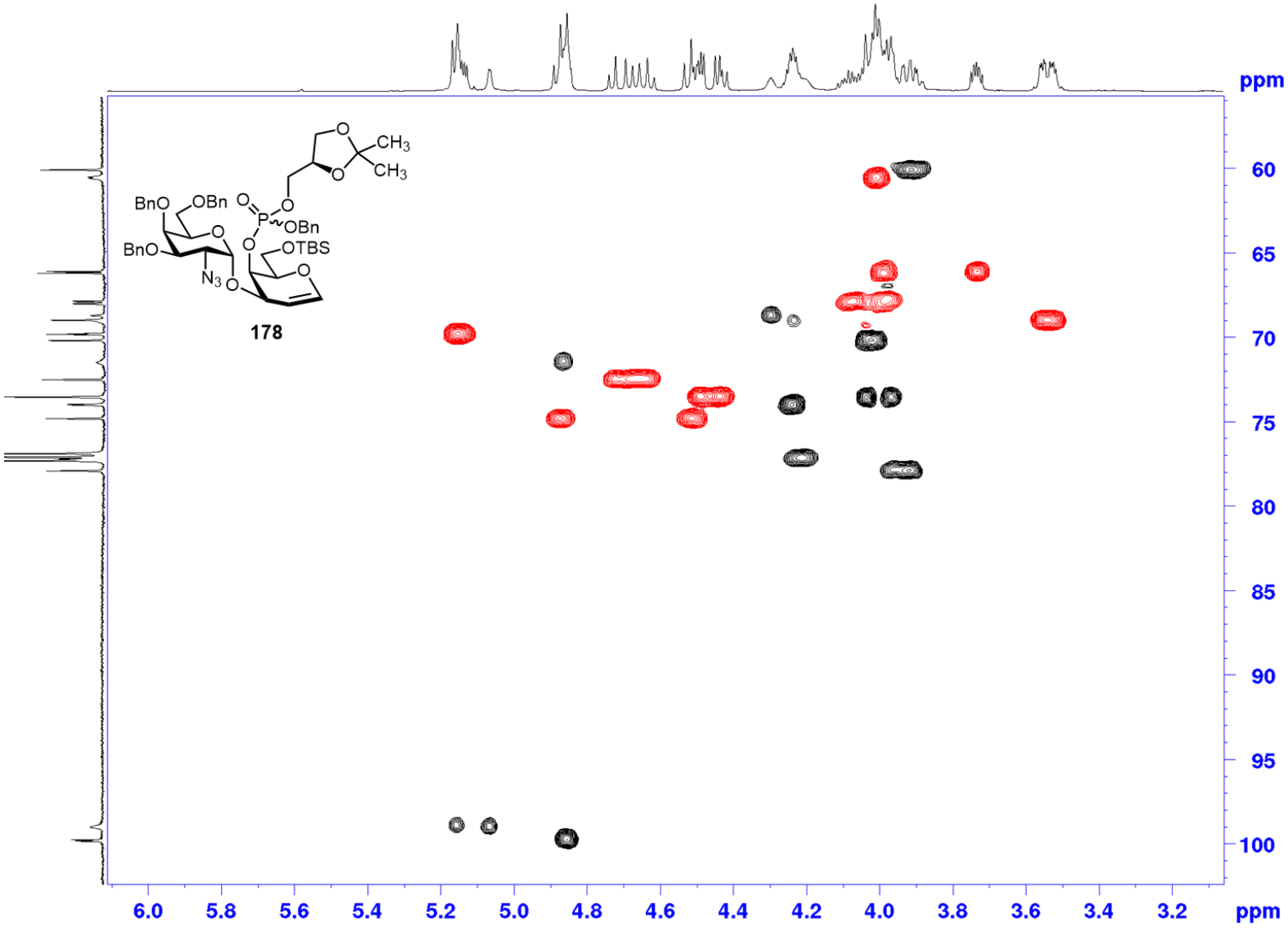
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 178



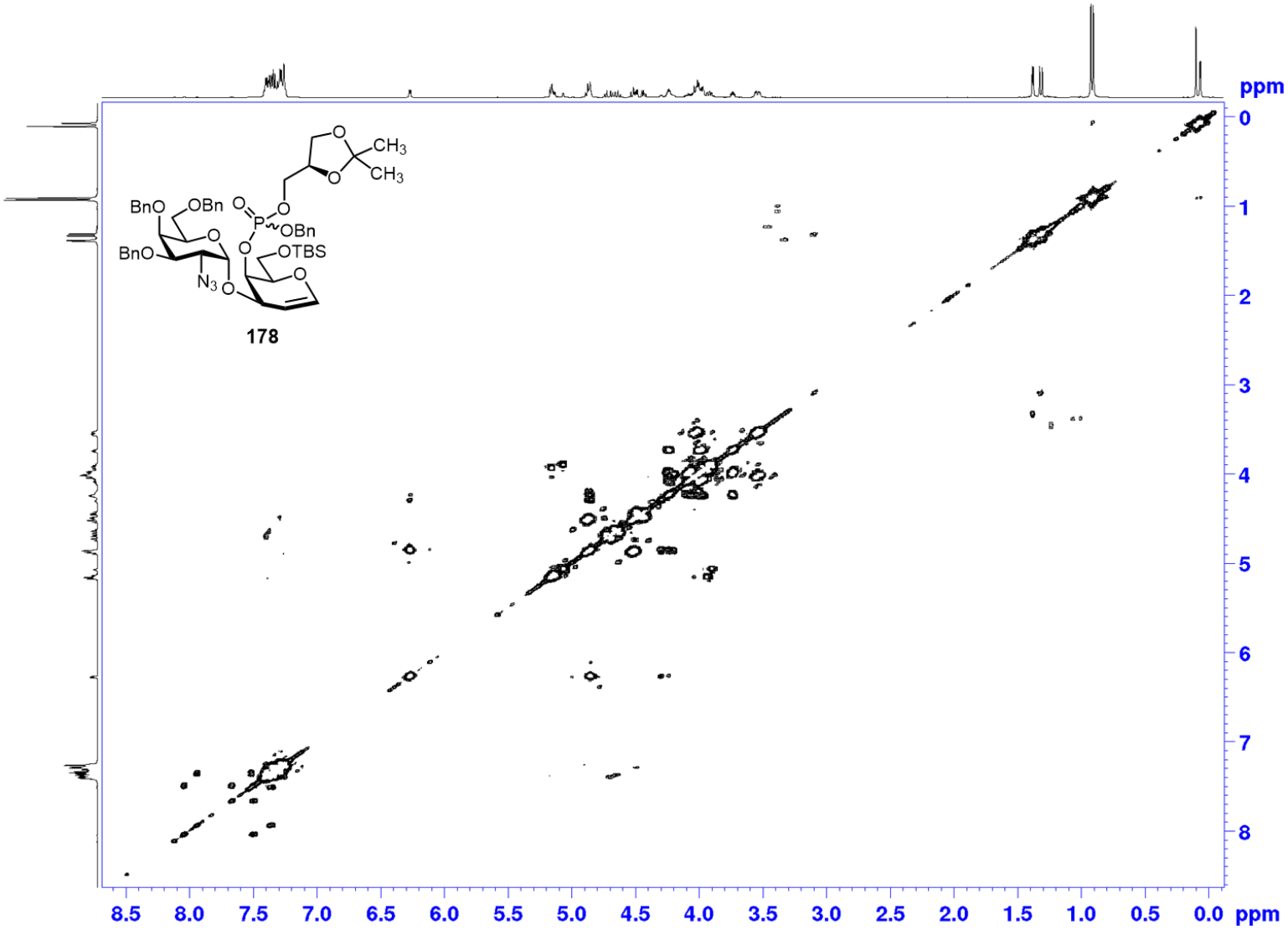
^{1}H-^{13}C HSCQ ($CDCl_3$) of compound 178



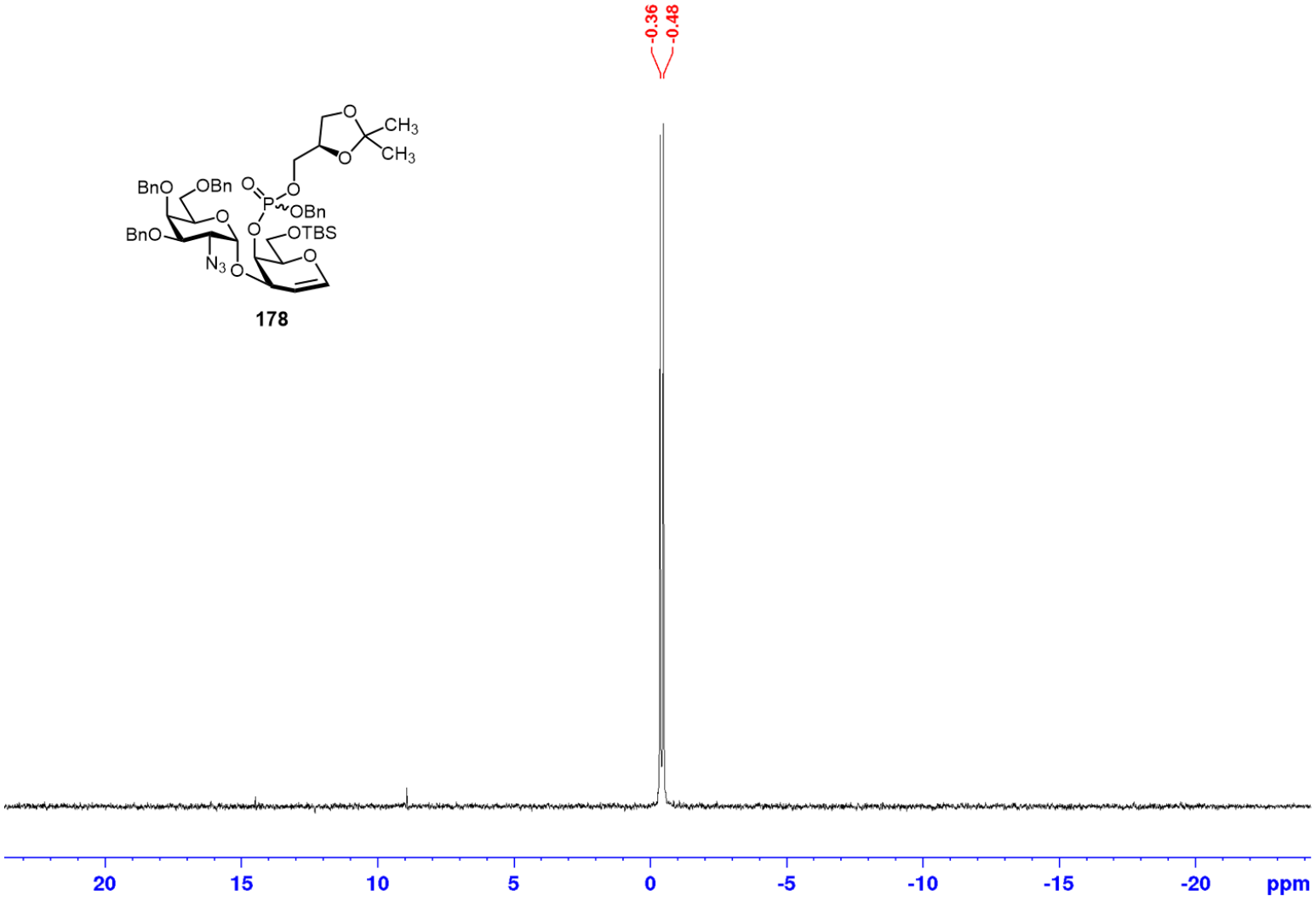
^{1}H-^{13}C HSCQ ($CDCl_3$) zoomed in region of compound 178



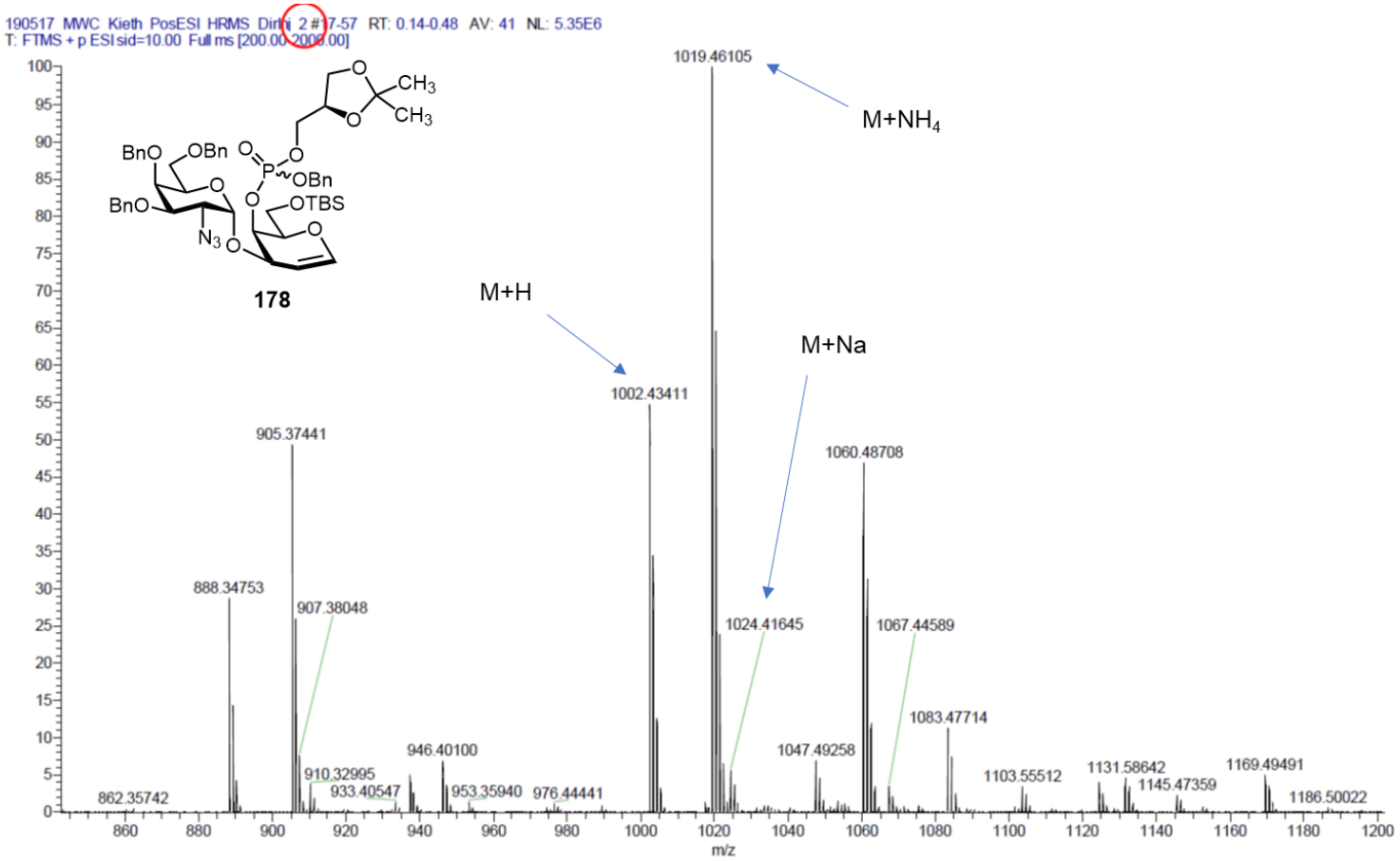
^{1}H-^{1}H COSY ($CDCl_3$) of compound 178



^{31}P NMR ($CDCl_3$, 162 MHz) of compound 178



178


190517 MWC Kieth PosESI HRMS Dirty 2 #17-57 RT: 0.14-0.48 AV: 41 NL: 5.35E6
T: FTMS + p ESI sid=10.00 Full ms [200.00-2000.00]
178
M+NH4
M+H
M+Na
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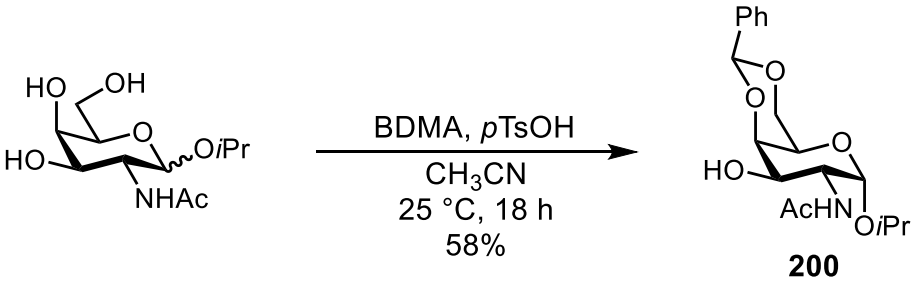
953.35940
976.44441
862.35742
1103.55512
1131.58642
1145.47359
1169.49491
1186.50022
m/z

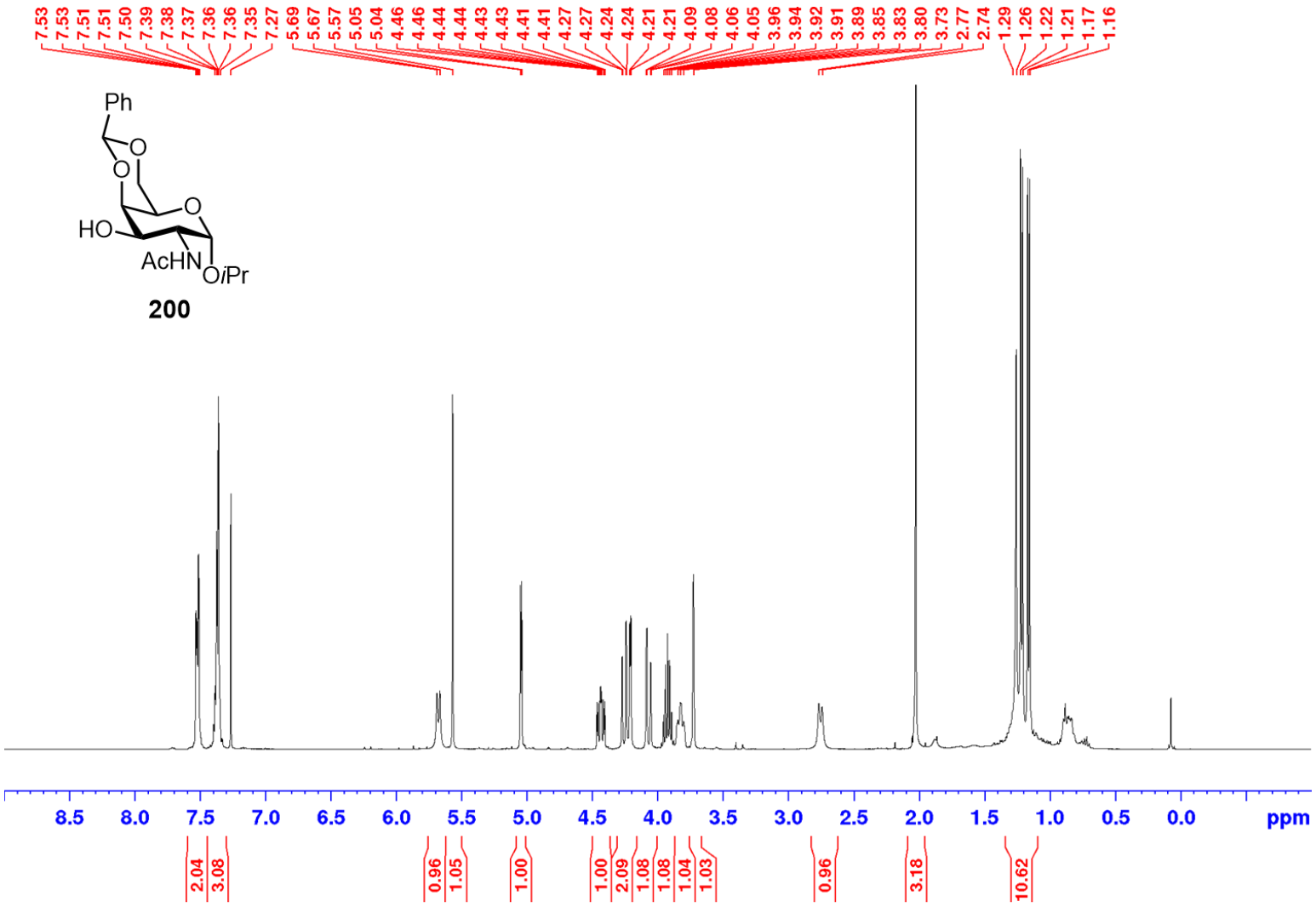
_Iso_propyl 4,6-_O_-benzylidene acetal-2-deoxy-2-acetamido-D-galactopyranoside (200)



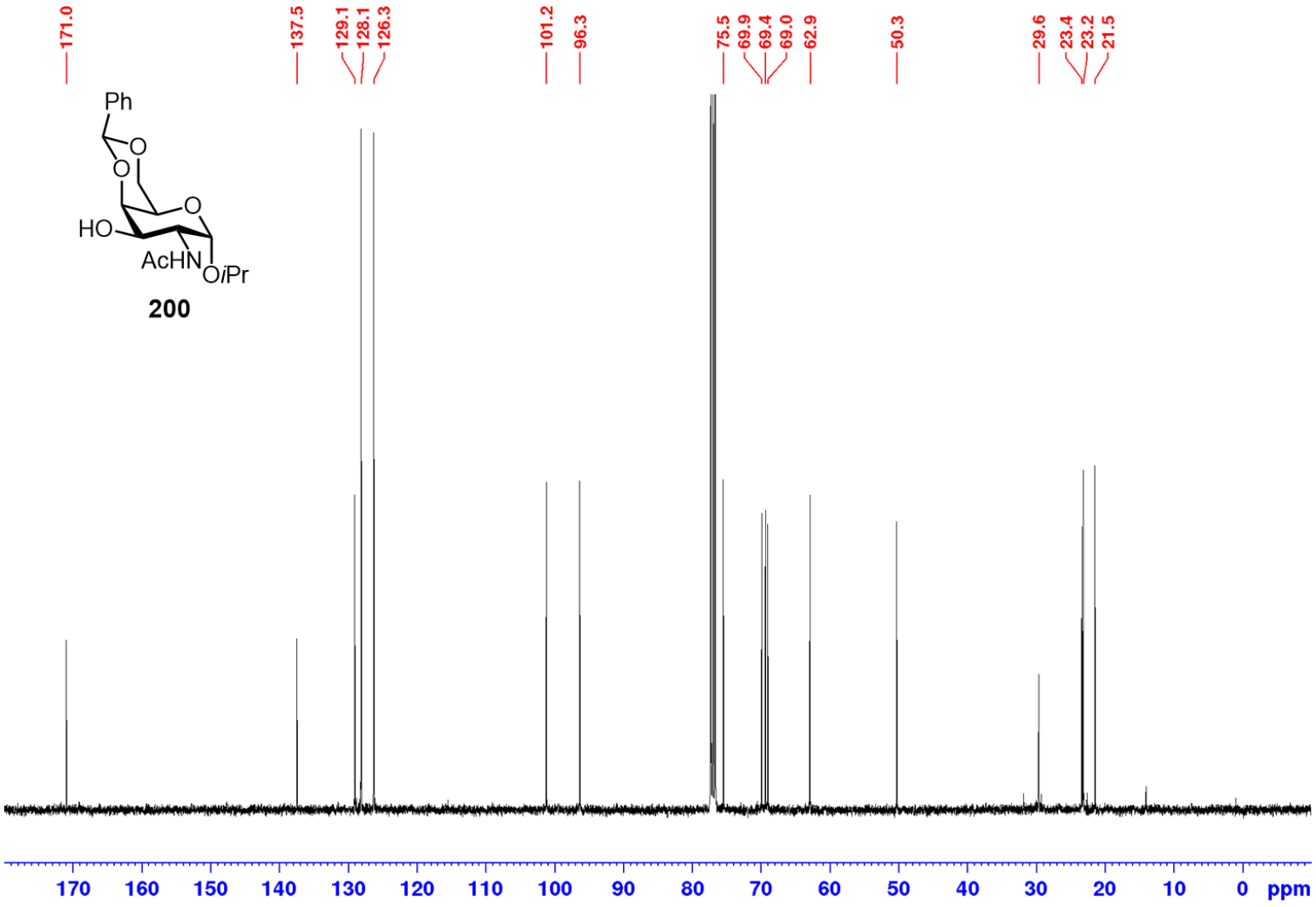
200

A solution of isopropyl 2-deoxy-2-acetamido-D-galactopyranoside[316-317] (1.0 equiv., 1.62 g, 6.15 mmol), benzaldehyde dimethyl acetal (1.2 equiv., 1.10 mL, 7.38 mmol) and acidic Amberlite IR 120 (H^+) ion exchange resin in anhydrous CH_3CN (60 mL) was stirred under an argon atmosphere at 25 °C. At 18 h, the reaction mixture was filtered through a glass fritted vacuum filter funnel equipped with a pad of Celite, to remove the acidic resin, then the filtrate was neutralized by the addition of Et_3N. The crude reaction mixture was concentrated then crystallized from hot ethanol and filtered to collect alpha acetamide **200** (1.24 g, 3.53 mmol, 58%) as a white solid. Our characterization data were consistent with previously reported data.[316-317] R_f = 0.4 (1:9 CH_3OH/CH_2Cl_2) visualized with ceric ammonium molybdate stain; ^1H NMR (400 MHz, $CDCl_3$): δ 7.53-7.50 (m, 2H), 7.39-7.34 (m, 3H), 5.68 (d, *J* =8.9 Hz, 1H), 5.56 (s, 1H), 5.05 (d, *J* = 3.7 Hz, 1H), 4.43 (m, 1H), 4.27 (dd, *J* = 1.3, 12.4 Hz, 1H), 4.21 (d, *J* = 3.4 Hz, 1H), 4.07 (dd, *J* = 1.4, 12.6 Hz, 1H), 3.93 (sept, *J* = 6.2, 12.7 Hz, 1H), 3.82 (m, 1H), 3.73 (s, 1H), 2.75 (d, *J* = 10.5 Hz, 1H), 2.03 (s, 3H), 1.21 (d, *J* = 6.3 Hz, 3H), 1.16 (d, *J* = 6.3Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.0, 137.5, 129.1, 128.2, 128.1, 126.3, 126.3, 101.2, 96.4, 75.5, 69.9, 69.4, 69.0, 62.87, 50.3, 23.4, 23.2, 21.5; LRMS (ESI) calcd for $C_{18}H_{25}NO_6Na$ $(M+Na)^+$ 374.1592, found 374.16 m/z.

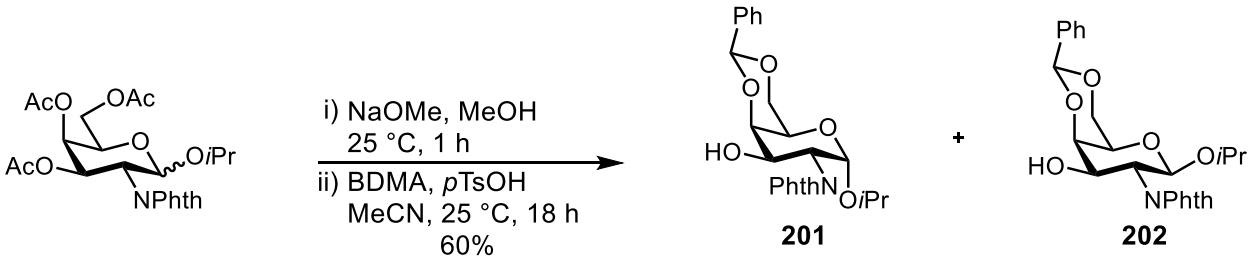
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 200



^{13}C NMR ($CDCl_3$, 100 MHz) of compound 200



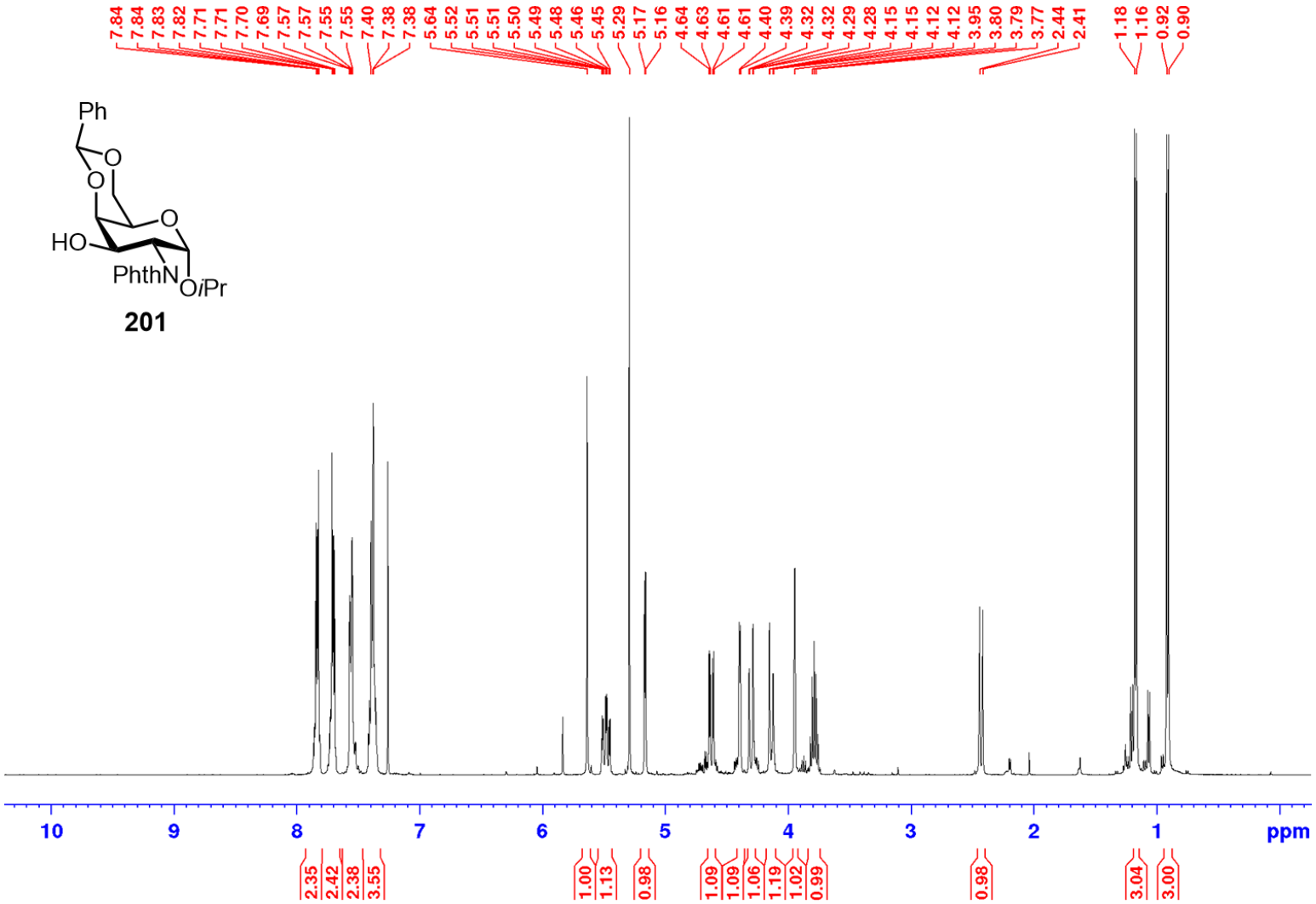
Isopropyl 4,6-*O*-benzylidene-2-deoxy-2-phthalimido-β-D-galactopyranoside (202) and Isopropyl 4,6-*O*-benzylidene-2-deoxy-2-phthalimido-α-D-galactopyranoside (201)



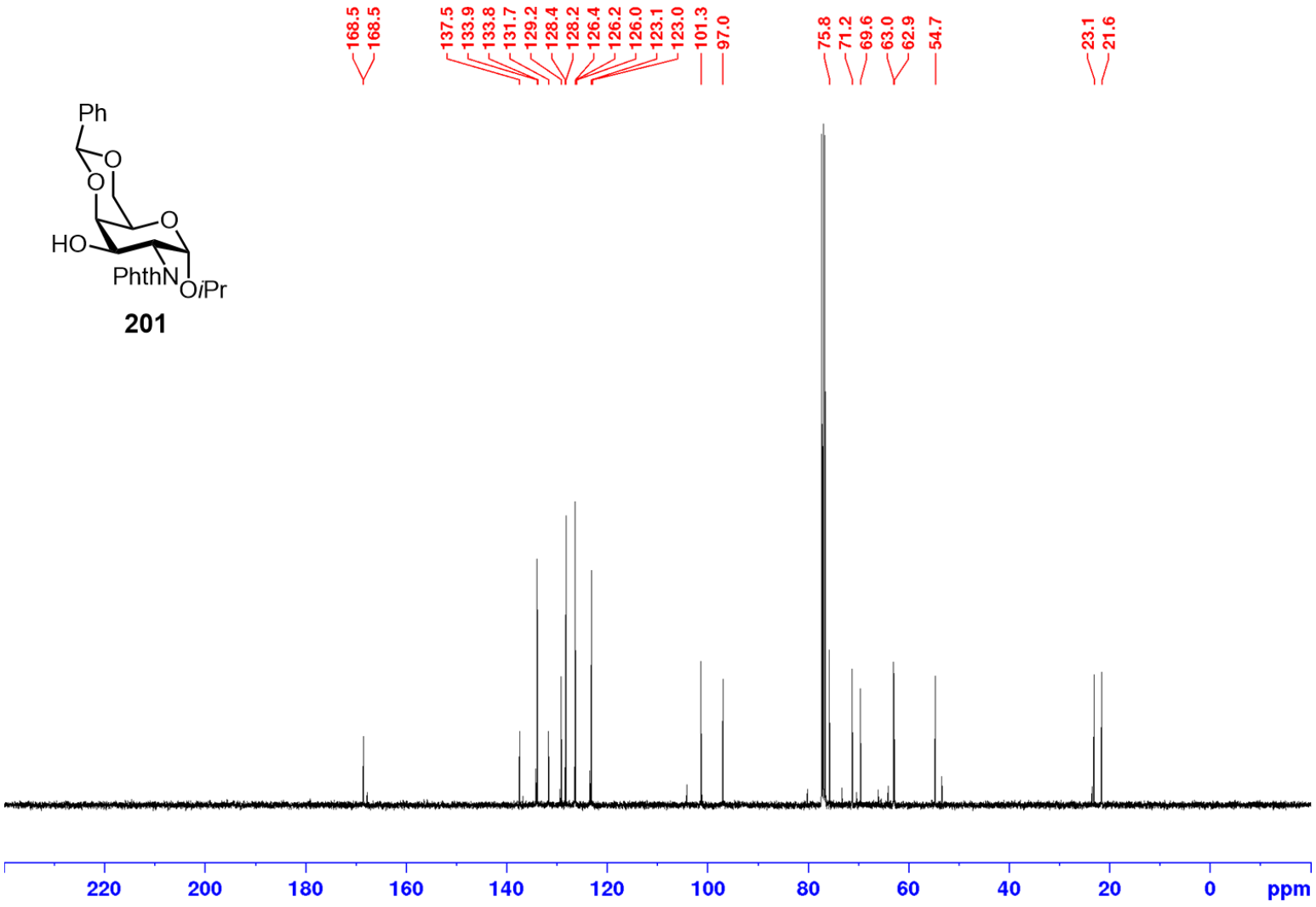
A solution of the an anomeric mixture of 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-phthalimido-α/β-D-galactopyranoside (1.0 equiv., 4.56 g, 12.92 mmol) was treated with a freshly made solution of sodium methoxide in methanol until the pH of the solution was >11. At 1 hours, the reaction was neutralized by the addition of Amberlite IR 120 (H+) ion exchange resin. The solution was filtered through a glass fritted vacuum funnel equipped with a pad of Celite to remove the acidic resin. The filtrate collected was concentrated to dryness to give a crude triol that was used in the next step without any further purification. A solution of triol (1.0 equiv., 1.51 g, 4.281 mmol) in dry acetonitrile (45 mL) was added *p*-TsOH (0.2 equiv., 0.163 g, 0.861 mmol) and benzaldehyde dimethyl acetal (1.5 eq, 1.0 mL, 6.5 mmol) dropwise at room temperature, under argon. At 12 hours, the reaction was then neutralized by the addition of triethylamine (1.0 equiv., 0.60 mL, 4.3 mmol) and concentrated down to a crude oil. The crude oil was purified by silica gel flash column chromatography (1:1 EtOAc/Hexanes) to give Isopropyl 4,6-O-benzylidene-2-deoxy-2-phthalimido-α-D-galactopyranoside **201** (0.951 g, 2.16 mmol, 51%) as a white solid and Isopropyl 4,6-O-benzylidene-2-deoxy-2-phthalimido-β-D-galactopyranoside **202** (0.810 g, 1.84 mmol, 43%) as a colorless foam. Rf = alpha 0.6 (1:4 EtOAc/hexanes), beta 0.5

(1:4 EtOAc/hexanes) visualized with ceric ammonium molybdate stain; ^{1}H NMR (400 MHz, $CDCl_3$): δ (alpha product, **201**) 7.85-7.83 (m, 2H), 7.73-7.70 (m, 2H), 7.58-7.55 (m, 2H), 7.43-7.36 (m, 3H), 5.64 (s, 1H), 5.53-5.45 (m, 1H), 5.18 (d, *J* = 3.5 Hz, 1H), 4.63 (dd, *J* = 3.4, 11.5 Hz, 1H), 4.41 (d, *J* = 3.4 Hz, 1H), 4.32 (dd, *J* = 1.4, 11.4 Hz, 1H), 4.15 (dd, *J* = 1.5, 11.4 Hz, 1H), 3.96 (s, 1H), 3.78 (sep, *J* = 6.2, 12.6 Hz, 1H), 2.42 (d, *J* = 10.4 Hz, 1H), 1.18 (d, *J* = 6.4 Hz, 3H), 0.92 (d, *J* = 6.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ (alpha product, **201**) 168.5, 137.4, 134.0, 133.9, 131.6, 129.1, 128.4, 128.3, 128.2, 126.4, 126.3, 123.4, 123.3, 123.1, 101.3, 96.9, 75.7, 71.2, 69.5, 62.9, 62.9, 54.7, 2.1, 21.6; ^{1}H NMR (400 MHz, $CDCl_3$): δ (beta product, **202**) 7.87-7.81 (m, 2H), 7.72-7.70 (m, 2H), 7.58-7.56 (m, 2H), 7.42-7.39 (m, 3H), 5.6 (s, 1H), 5.3 (d, *J* = 8.3 Hz, 1H), 4.56-4.49 (m, 1H), 4.44-4.36 (m, 2H), 4.27 (d, *J* = 3.9 Hz, 1H), 4.15 (dd, *J* = 1.7, 12.4 Hz, 1H), 3.94 (sept, *J* = 6.0, 12.3, 18.7 Hz, 1H), 3.63 (d, *J* = 1.1 Hz, 1H), 2.49 (d, *J* = 11.9 Hz, 1H), 1.14 (d, *J* = 6.4 Hz, 3H), 0.96 (d, *J* = 6.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ (beta product, **202**) 168.9, 168.4, 137.6, 134.1, 134.1, 132, 131.9, 129.4, 128.4, 126.7, 123.7, 123.1, 101.6, 97, 75.3, 71.8, 69.5, 68.1, 66.8, 55.2, 23.4, 21.9, LRMS (ESI) calcd for $C_{24}H_{25}NO_7Na$ $(M+Na)^+$ 462.149218, found 462.15 m/z.

^{1}H NMR ($CDCl_3$, 400 MHz) of compound 201

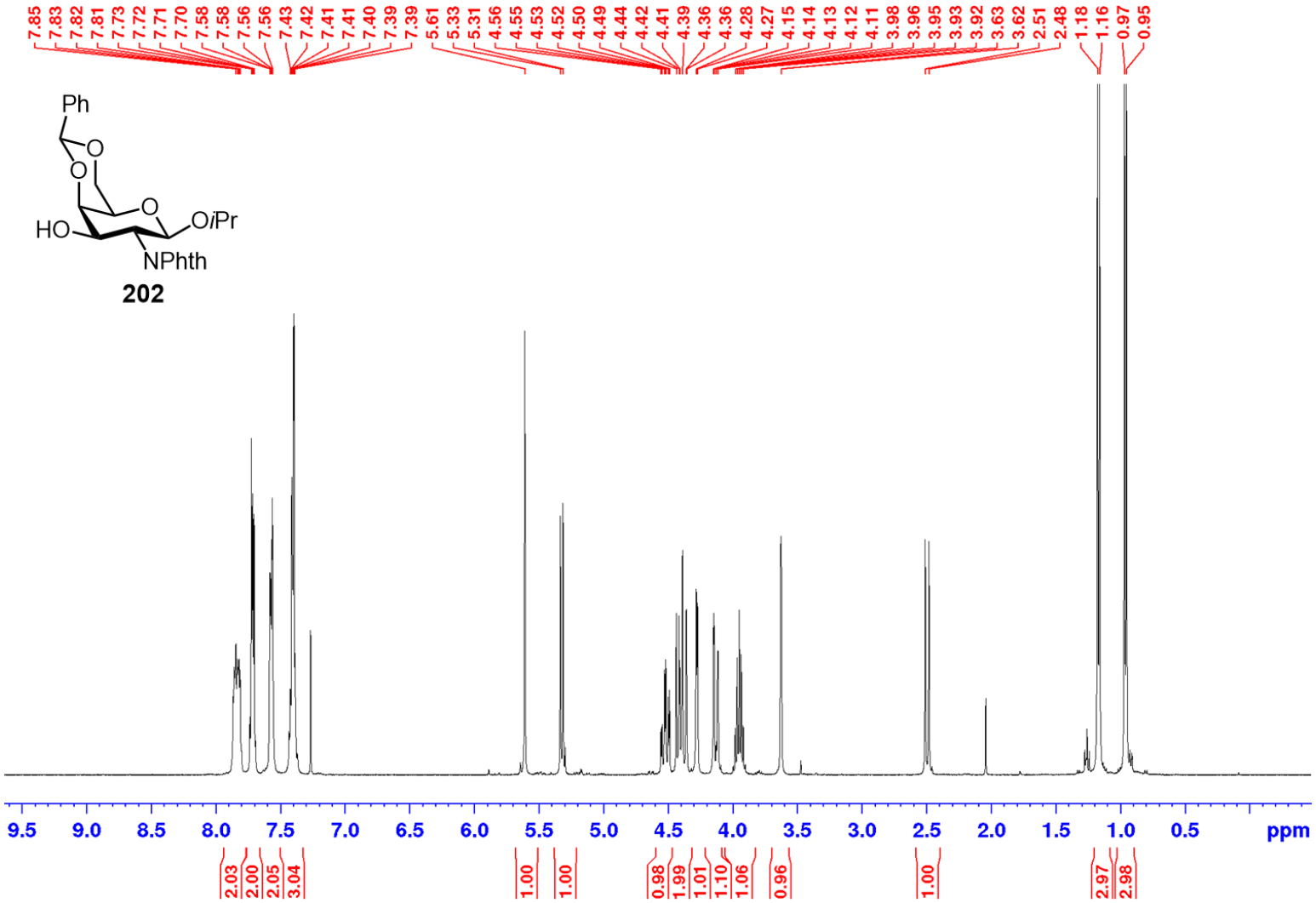


^{13}C NMR ($CDCl_3$, 100 MHz) of compound 201

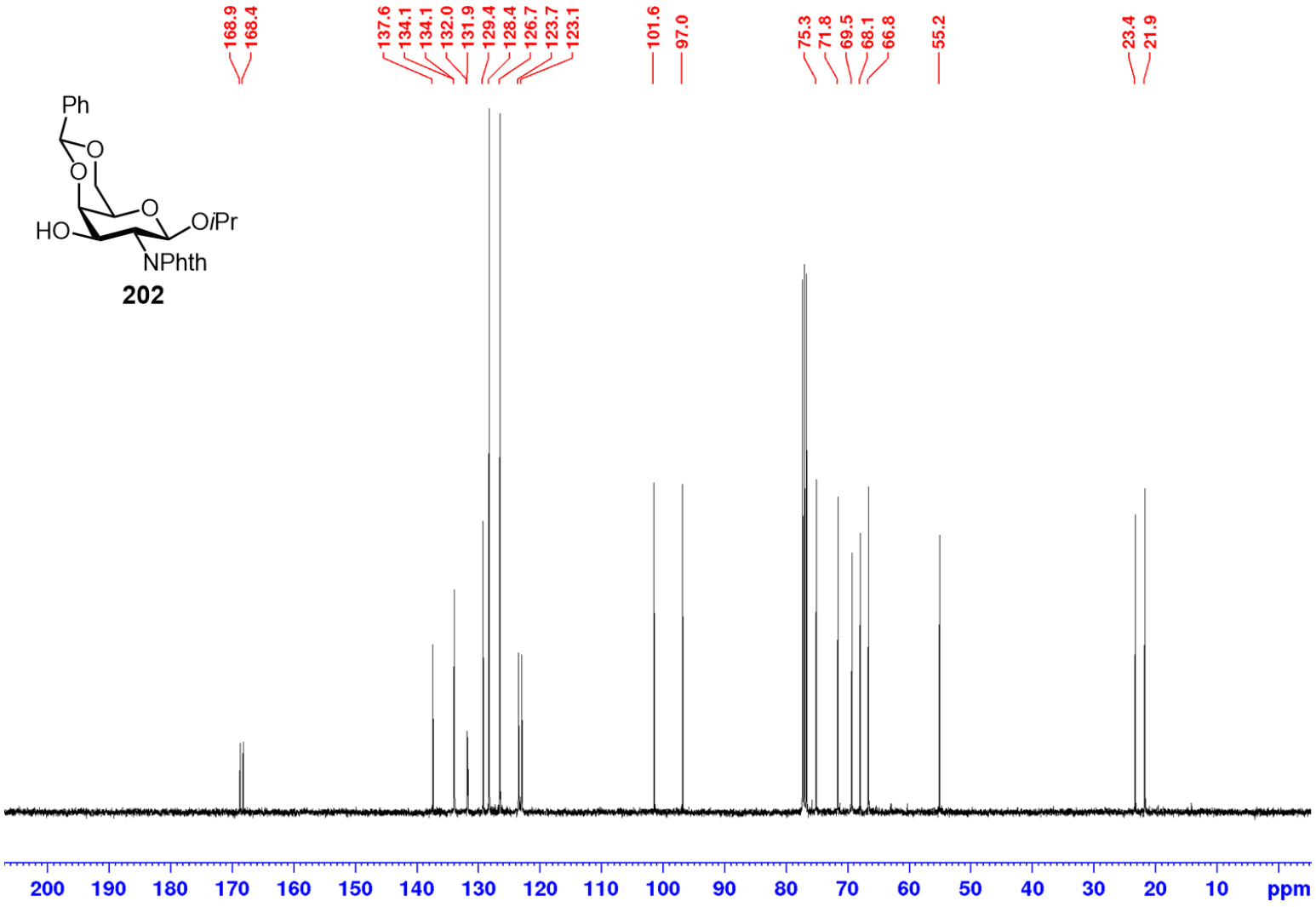


201

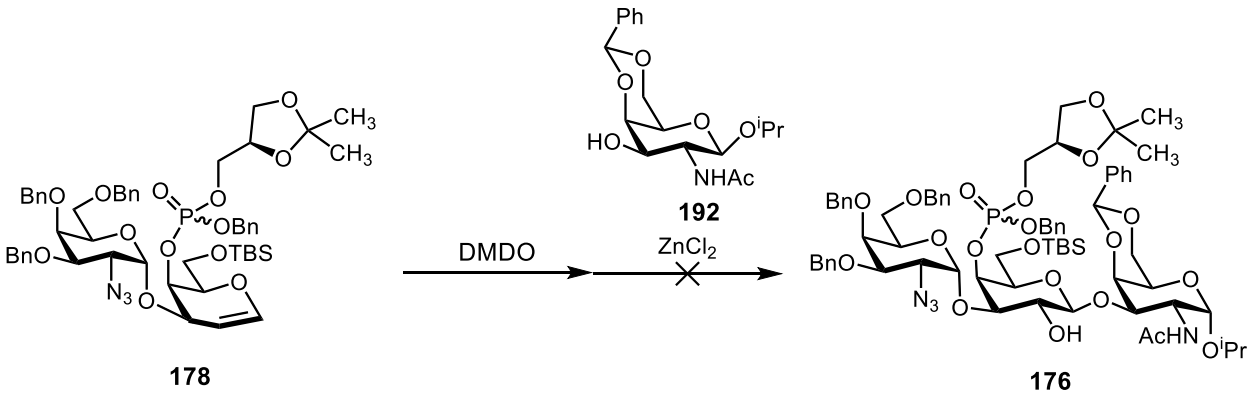
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 202



^{13}C NMR ($CDCl_3$, 100 MHz) of compound 202



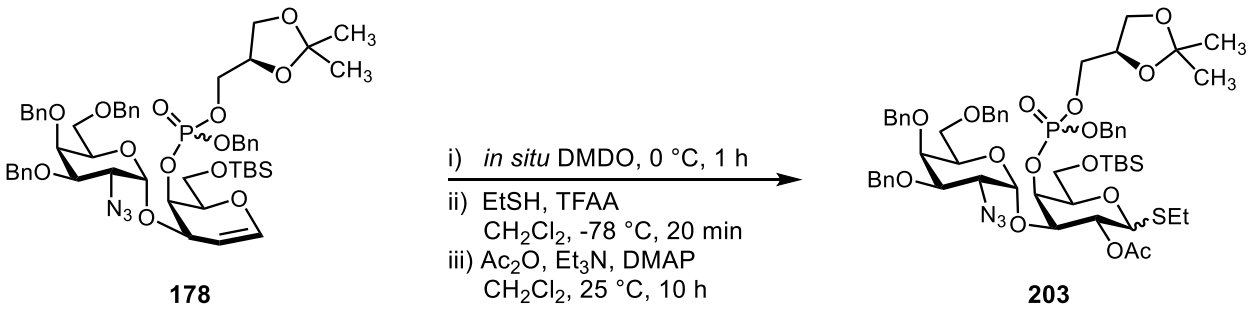
_Iso_propyl 3,4,6-tri-_O_-benzyl-2-deoxy-2-azido-α-D-galactopyranosyl-(1→3)-6-_O_-_tert_-butyldimethylsilyl-4-_O_-(((_R_)-2,2-dimethyl-1,3-dioxolan-4-yl)methyl) phosphate-β-D-galactopoyranosyl-(1→3)-2-deoxy-2-acetamido-4,6-O-benzylidene acetal-β-D-galactopyranoside (176)



To a solution of 3,4,6-tri-*O*-benzyl-2-deoxy-2-azido-α-D-galactopyranosyl-(1→3)-6-*O*-tert-butyldimethylsilyl-4-*O*-(((*R*)-2,2-dimethyl-1,3-dioxolan-4-yl)methyl) phosphate-D-galactal **178** (1.0 equiv., 0.200 g, 0.279 mmol) in anhydrous CH_2Cl_2 (3.0 mL), DMDO (5.0 equiv., 3.0 mL,1.39 mmol, [0.4 M] in acetone) was added at 0 °C. At 1 h, the reaction was concentrated down to a clear oil which contained the intermediate disaccharide epoxide. The crude reaction mixture was co-evaporated with toluene (3x) in a reaction flask, then the reaction flask was placed in a vacuum desiccator equipped with P_2O_5 and placed under reduced pressure for 1 h. The reaction flask was removed from the vacuum desiccator and filled with an argon atmosphere, diluted with anhydrous CH_2Cl_2 (3.0 mL) and added 4,6-*O*-benzylidene acetal-2-deoxy-2-acetamido-β-D-galactopyranoside **192** (1.5 equiv., 0.135 g, 0.418 mmol) or methyl 4,6-*O*-benzylidene acetal-2-deoxy-2-acetamido-D-α-galactopyranoside **S11** (for attempts at the C1-OMe product) and cooled to -78 °C. The reaction was then added a solution of $ZnCl_2$ (0.50 equiv., 0.1 mL, 0.139

mmol) dropwise. The reaction mixture was stirred at -78 °C for 20 minutes then at 0 °C. After 1 , consumption of the intermediate epoxide was apparent by TLC and NMR analysis. The reaction was concentrated down to a crude solid and purified by silica gel flash column chromatography. This reaction, and subsequent reactions incorporating prolonged reaction times, were both unsuccessful in producing any desired isopropyl 3,4,6-tri-*O*-benzyl-2-deoxy-2-azido-α-D-galactopyranosyl-(1→3)-6-*O*-*tert*-butyldimethylsilyl-4-*O*-(((*R*)-2,2-dimethyl-1,3-dioxolan-4-yl)methyl) phosphate-β-D-galactopoyranosyl-(1→3)-2-deoxy-2-acetamido-4,6-O-benzylidene acetal-β-D-galactopyranoside **176**.

Thioethyl 3,4,6-tri-*O*-benzyl-2-deoxy-2-azido-α-D-galactopyranosyl-(1→3)-6-*O*-*tert*-butyldimethylsilyl-4-*O*-(((*R*)-2,2-dimethyl-1,3-dioxolan-4-yl)methyl) phosphate-2-*O*-acetyl-1-thio-α/β-D-galactopyranoside (203)



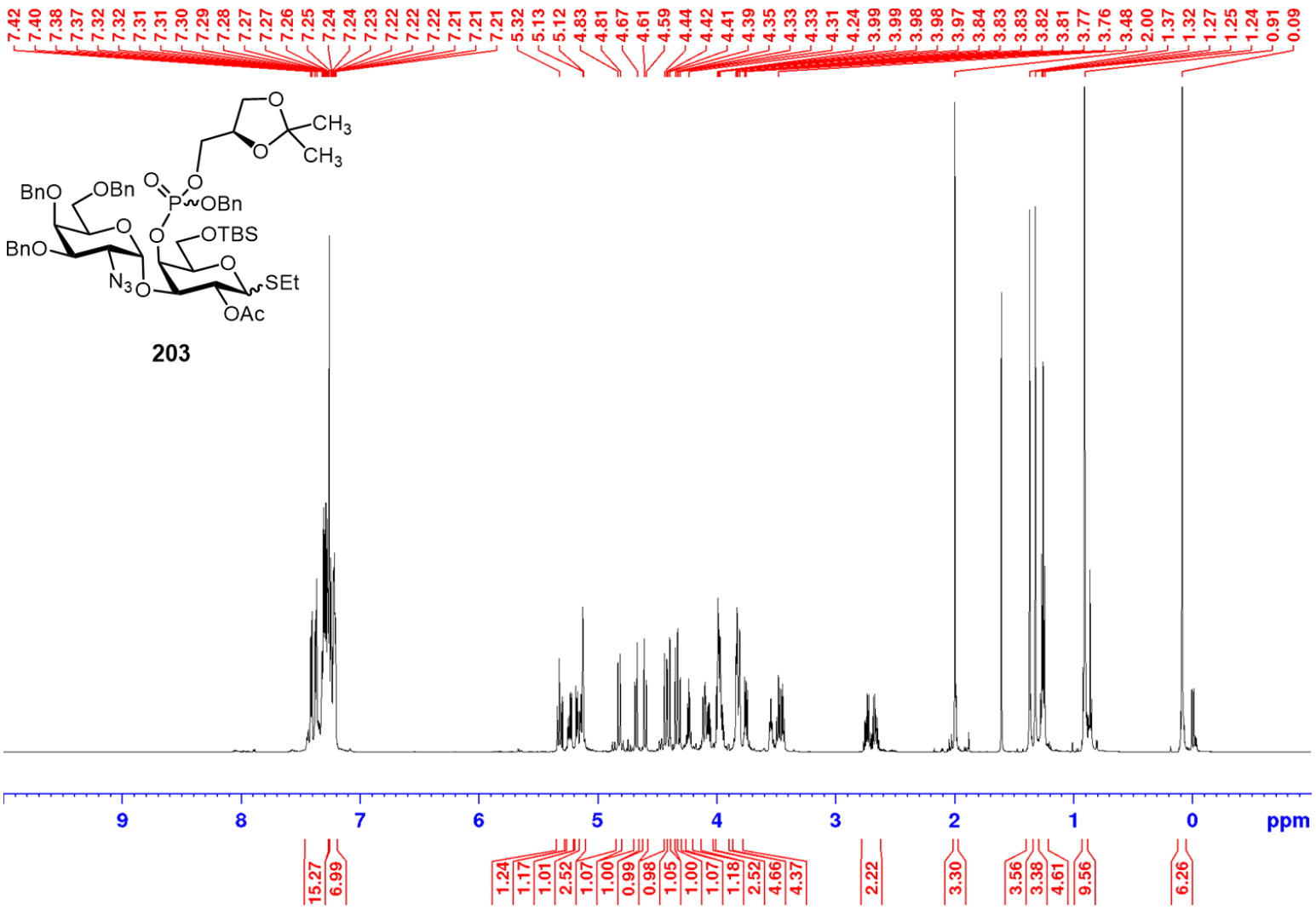
178 **203**

A biphasic solution of 3,4,6-tri-*O*-benzyl-2-deoxy-2-azido-α-D-galactopyranosyl-(1→3)-6-*O*-tert-butyldimethylsilyl-4-*O*-(((*R*)-2,2-dimethyl-1,3-dioxolan-4-yl)methyl) phosphate-D-galactal **178** (1.0 equiv., 1.05 g, 1.04 mmol) in 10:1 CH_2Cl_2/acetone (33.0 mL) and sat. aq. $NaHCO_3$ (50.0 mL) was added a solution of Oxone (13.7 equiv., 8.87 g, 14.42 mmol) in H_2O (35.0 mL) dropwise over 5 minutes at 0 °C. The mixture was vigorously stirred at 0 °C for 30 minutes and then allowed to warm to room temperature. At 2 h, the reaction mixture was transferred to a separatory funnel with CH_2Cl_2 (10 mL). The organic phase was separated, and the aqueous phase extracted with CH_2Cl_2 (3 × 20 mL). The combined organic phases were dried (Na_2SO_3), filtered through a glass fritted vacuum filter funnel to remove the drying agent, and concentrated to produce a clear oil. The crude oil, containing the intermediate epoxide, was dried in a vacuum desiccator under reduced pressure in the presence of P_2O_5 for 30 minutes. The reaction flask was then filled with an argon atmosphere, diluted with anhydrous CH_2Cl_2 (2.1 mL) and added ethane thiol (28.2 equiv., 2.20 mL, 29.5 mmol) and cooled to -78 °C. The reaction was then added trifluoroacetic acid anhydride (0.08 equiv., 0.013 mL, 0.090 mmol) dropwise. The reaction

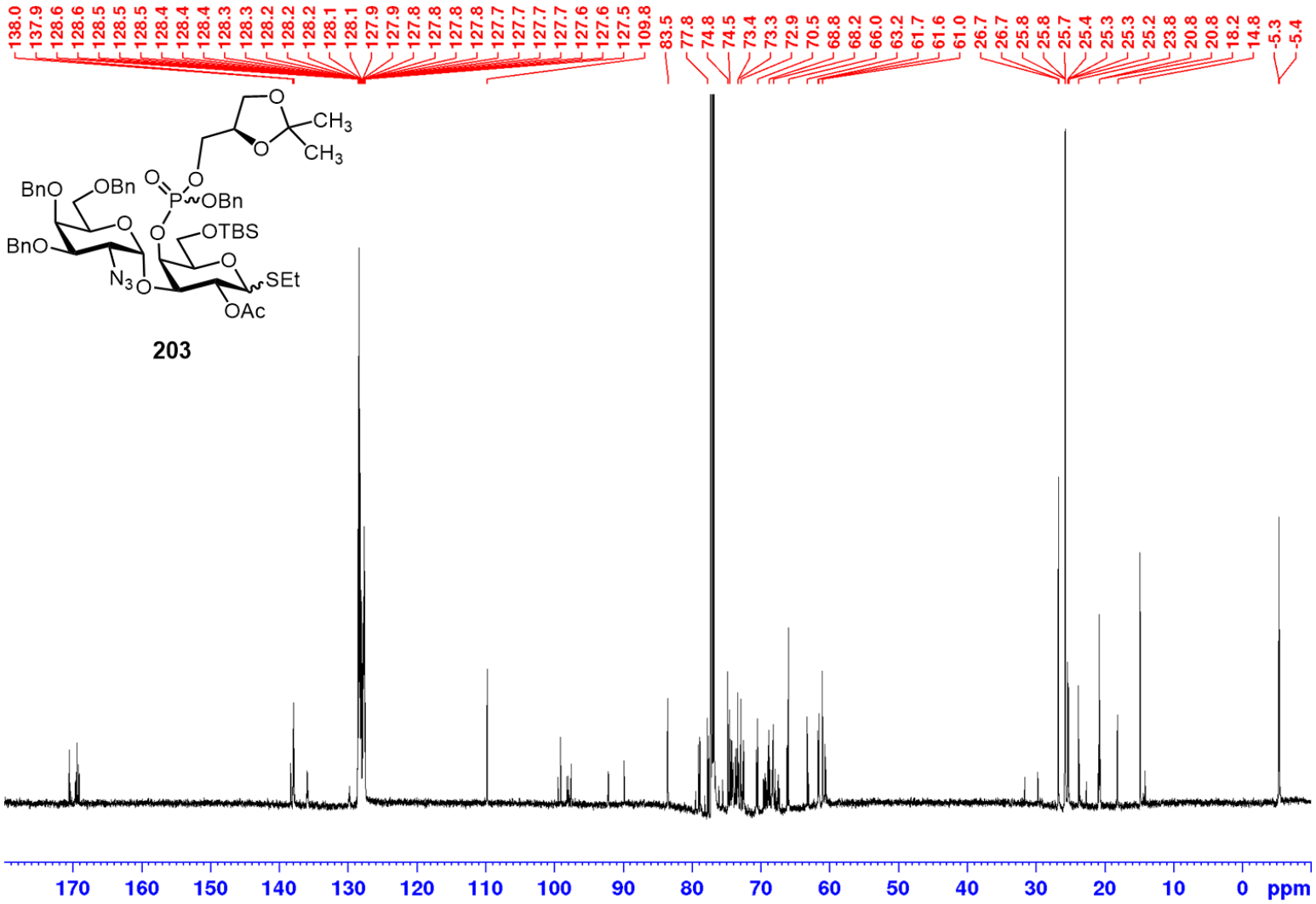
mixture was stirred at -78 °C for 20 minutes and then warmed to room temperature. After 1 h, the reaction mixture was neutralized by the addition of triethylamine and the solvent was evaporated by a stream of nitrogen gas in the hood until the malodorous thiol had dissipated. The reaction mixture was transferred to a larger flask without the stir bar and was co-evaporated with CH_2Cl_2 (3 x10 mL) to remove residual ethane thiol. The resulting crude oil was then diluted with CH_2Cl_2 (5.3 mL) and added Et_3N (10.0 equiv., 1.5 mL, 10.5 mmol) and added acetic anhydride (4.0 equiv., 0.40 mL, 4.2 mmol) and a DMAP (0.1 equiv.) under and argon atmosphere at 0 °C. The reaction mixture warmed to room temperature overnight. After 10 h, the reaction was cooled to 0 °C and added methanol (12.0 equiv., 0.50 mL, 12 mmol) to consume the excess acetic anhydride. The reaction was stirred at room temperature. After 5 min, the reaction was concentrated *in vacuo* to a crude oil. The crude oil was transferred to a separatory funnel and diluted with EtOAc (120 mL) and washed with water (3 x 10 mL) and brine (1 x 10 mL). The organic layer was separated, dried ($MgSO_4$), filtered through a glass fritted filter funnel to remove the drying agent. The filtrate was concentrated to a crude oil. The resulting crude oil was purified by silica gel chromatography (30:70 to 50:50 EtOAc/Hexanes) to give thioethyl 3,4,6-tri-*O*-benzyl-2-deoxy-2-azido-α-D-galactopyranosyl-(1→3)-6-*O*-*tert*-butyldimethylsilyl-4-*O*-(((*R*)-2,2-dimethyl-1,3-dioxolan-4-yl)methyl) phosphate-2-*O*-acetyl-1-thio-α/β-D-galactopyranoside **203** (0.668 g, 0.595 mmol, 57% yield) as inseparable pairs of anomers (four isomers; phosphate and C1 chiral centers) as a colorless oil; R_f = 0.6 (2:3 EtOAc/hexanes) visualized with ceric ammonium molybdate stain. $[\alpha]D^{25}$ = +5.88° (c = 1.0, $CHCl_3$); IR ν_{max} (film) 3854.04, 3840.54, 3751.40, 3433.13, 2927.22, 2362.26, 2113.32, 1750.63, 1654.67, 1497.56, 1456.50, 1371.85, 1259.13,

1227.22, 1094.22, 1054.30, 1008.47, 838.74, 779.65, 746.73, 698.28 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.44-7.26 (m, 15H), 7.24-7.19 (m, 5H), 5.34-5.11 (m, 5H), 4.82 (d, *J* = 11.5 Hz, 1H), 4.68 (d, *J* = 11.9 Hz, 1H), 4.60 (d, *J* = 11.5 Hz, 1H), 4.48-4.30 (m, 4H), 4.24-4.21 (m, 1H), 4.10 (dd, *J* = 2.6, 10.4 Hz, 1H), 4.08-4.05 (m, 1H), 4.00-3.94 (m, 4H), 3.84-3.80 (m, 4H), 3.76 (dd, *J* = 5.4, 8.4 Hz, 1H), 3.54 (dt, *J* = 1.8, 6.1 Hz, 1H), 3.49-3.42 (m, 2H),2.76-2.63 (m, 2H),1.99 (s, 3H),1.37 (s, 3H), 1.32 (s, 3H), 1.26 (t, *J* = 7.5 Hz, 3H), 0.91 (s, 9H), 0.09 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.6, 170.4, 169.7, 169.4, 169.2, 169.2, 169.1, 169.1, 138.3, 138, 138, 137.9, 136, 135.9, 128.6, 128.6, 128.5, 128.5, 128.5, 128.4, 128.4, 128.4, 128.3, 128.3, 128.2, 128.2, 128.2, 128.1, 128.1, 128, 127.9, 127.9, 127.8, 127.8, 127.8, 127.8, 127.7, 127.7, 127.7, 127.7, 127.6, 127.6, 127.5, 109.8, 109.8, 99.5, 99.1, 98.2, 98, 97.6, 97.5, 92.2, 92.2, 89.9, 83.6, 83.5, 79.1, 78.9, 78.8, 77.8, 77.6, 77.6, 76.6, 75.6, 75.5, 74.8, 74.5, 74.5, 74.4, 74.4, 74.3, 74.2, 74.1, 74.1, 74, 74, 73.7, 73.7, 73.7, 73.5, 73.4, 73.3, 73.2, 73.2, 72.9, 72.9, 72.6, 72.5, 72.5, 70.7, 70.6, 70.6, 70.5, 70.4, 69.6, 69.4, 69.3, 69.2, 69.2, 69, 68.9, 68.9, 68.8, 68.6, 68.4, 68.4, 68.3, 68.2, 68.2, 67.9, 67.5, 66.2, 66.2, 66.2, 66.1, 66, 63.2, 63.1, 61.7, 61.6, 61.5, 61, 61, 61, 60.7, 60.7, 60.6, 60.5, 31.6, 29.7, 26.7, 26.7, 25.8, 25.8, 25.7, 25.4, 25.3, 25.3, 25.2, 23.8, 23.7, 22.7, 21, 20.9, 20.8, 20.8, 20.7, 18.2, 18.1, 14.8, 14.1, -5.3, -5.3, -5.4, -5.4,; HRMS (ESI) calcd for $C_{56}H_{80}N_4O_{15}PSSi^{+}$ $(M+NH_4)^{+}$ 1139.48422, found 1139.48512 m/z.

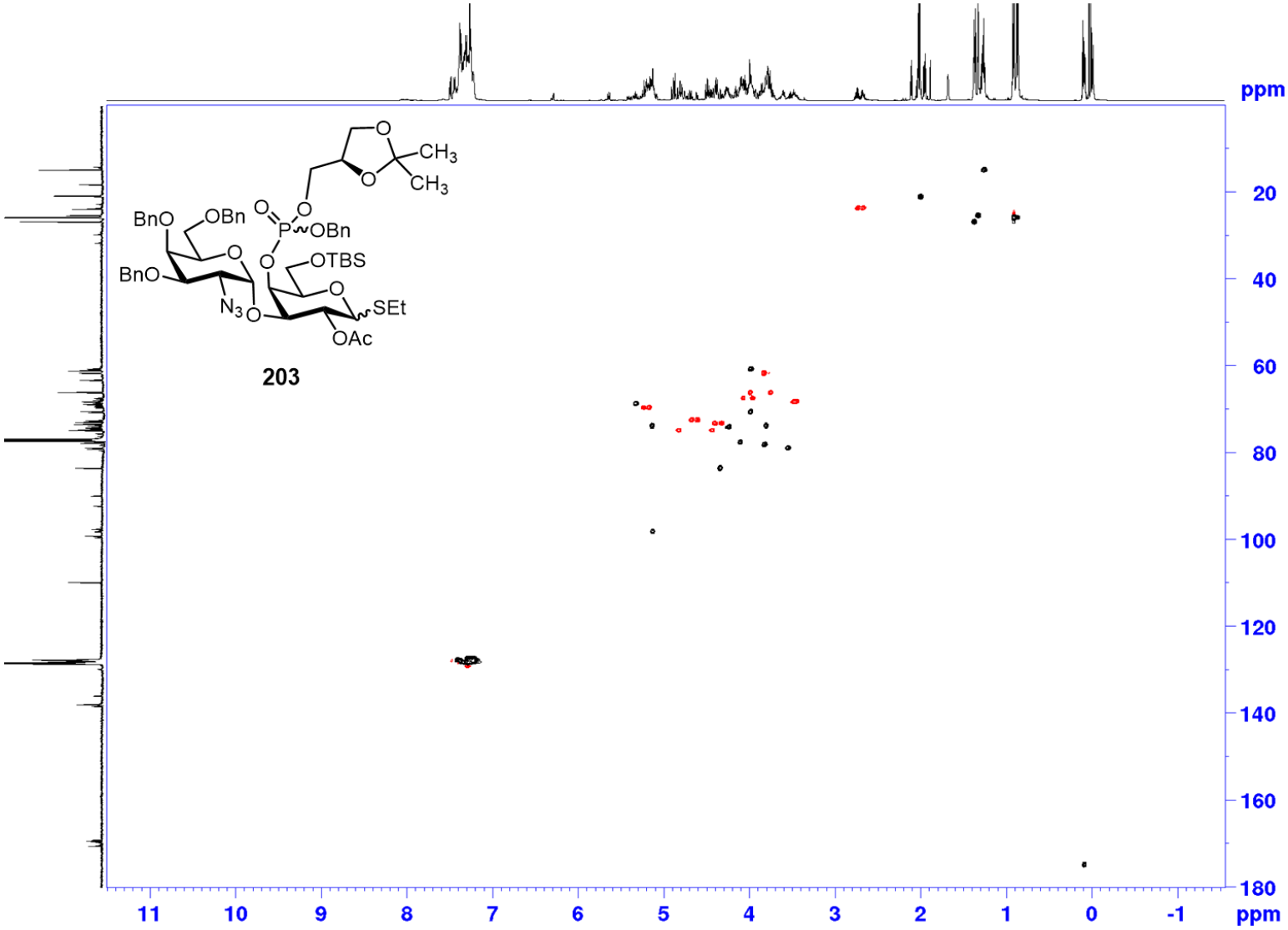
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 203



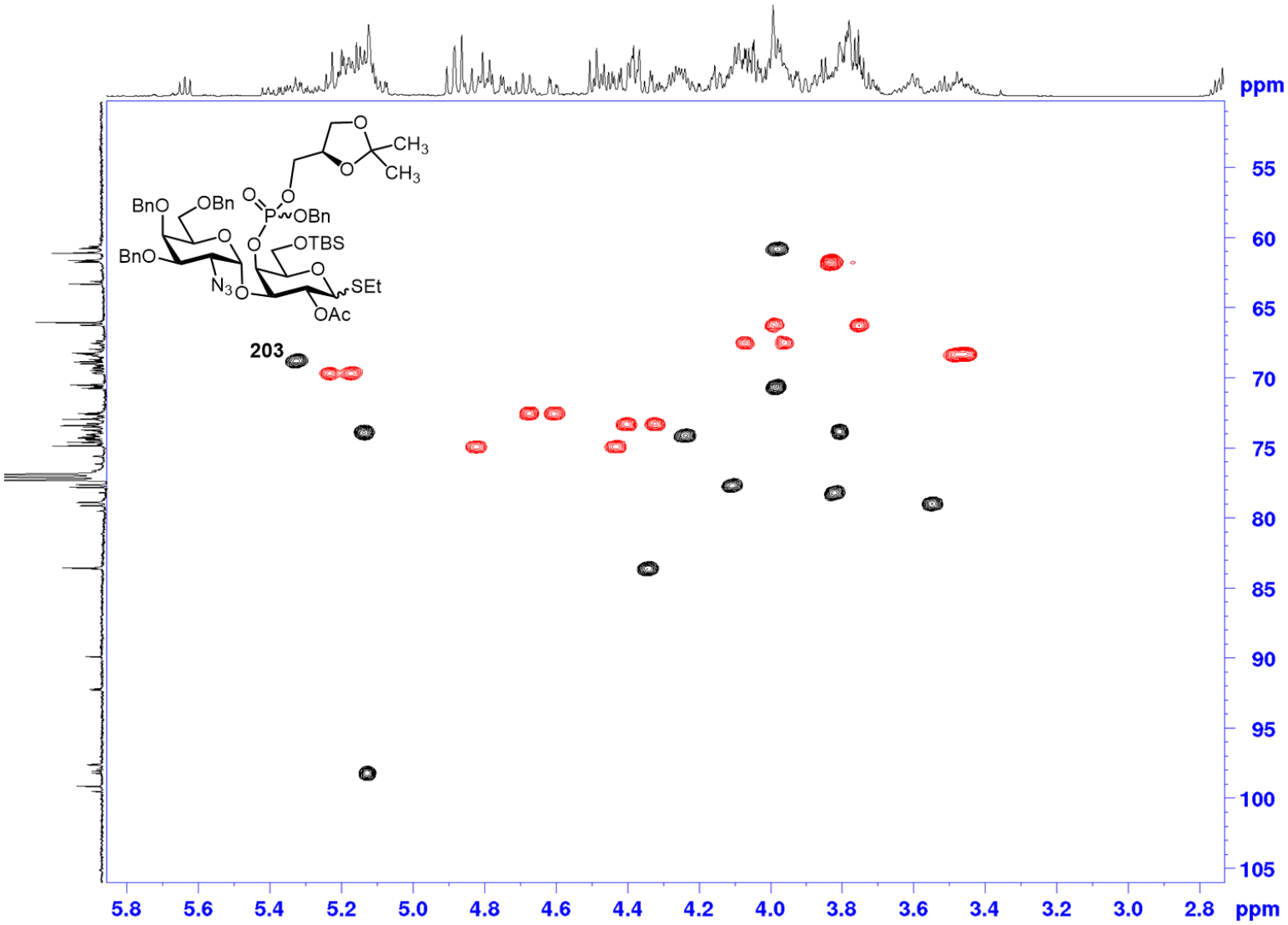
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 203



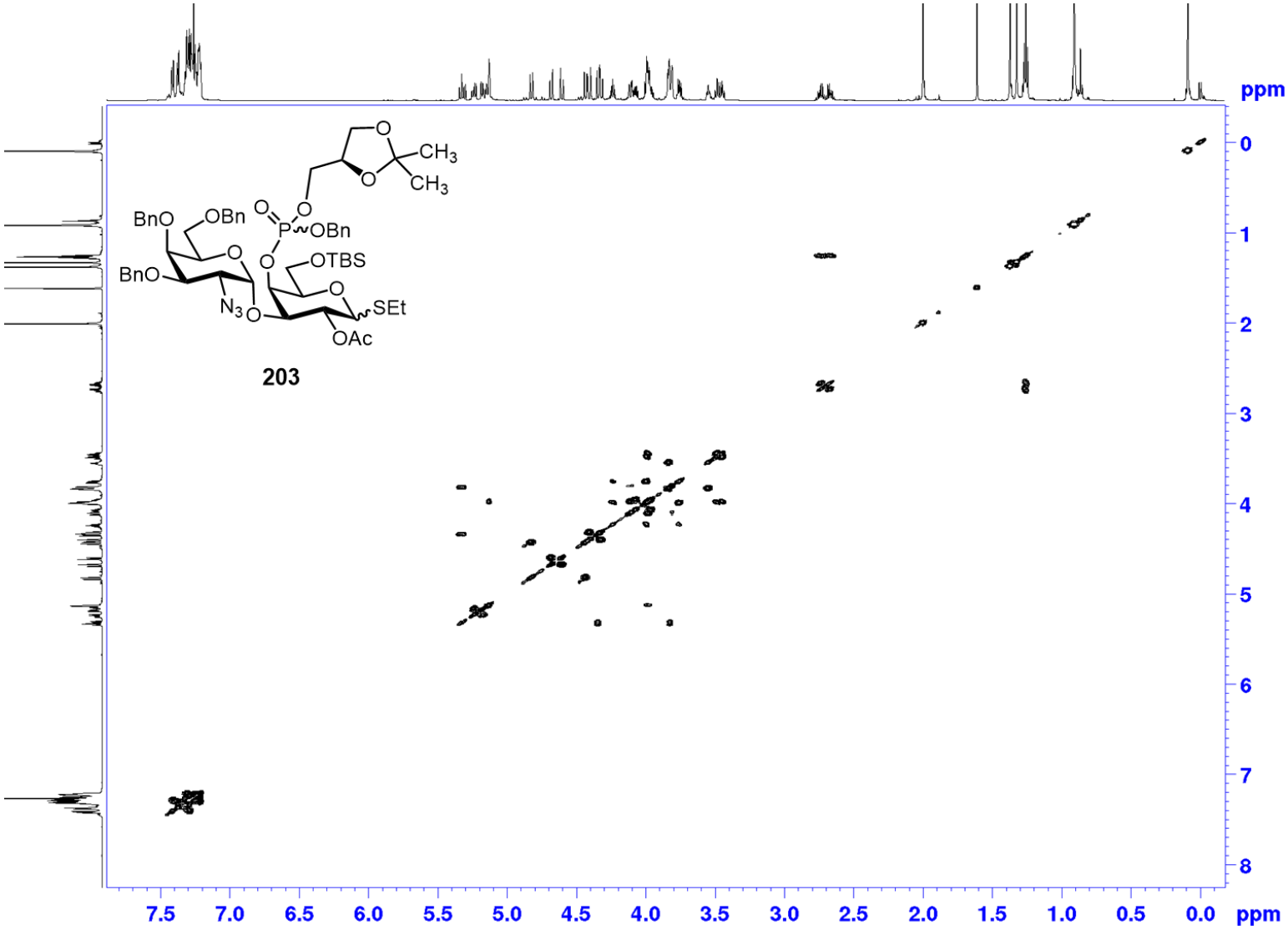
^{1}H-^{13}C HSCQ ($CDCl_3$) of compound 203



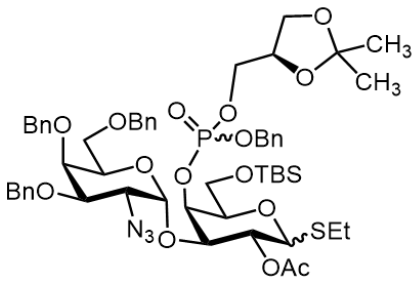
^{1}H-^{13}C HSCQ ($CDCl_3$) zoomed in region of compound 203



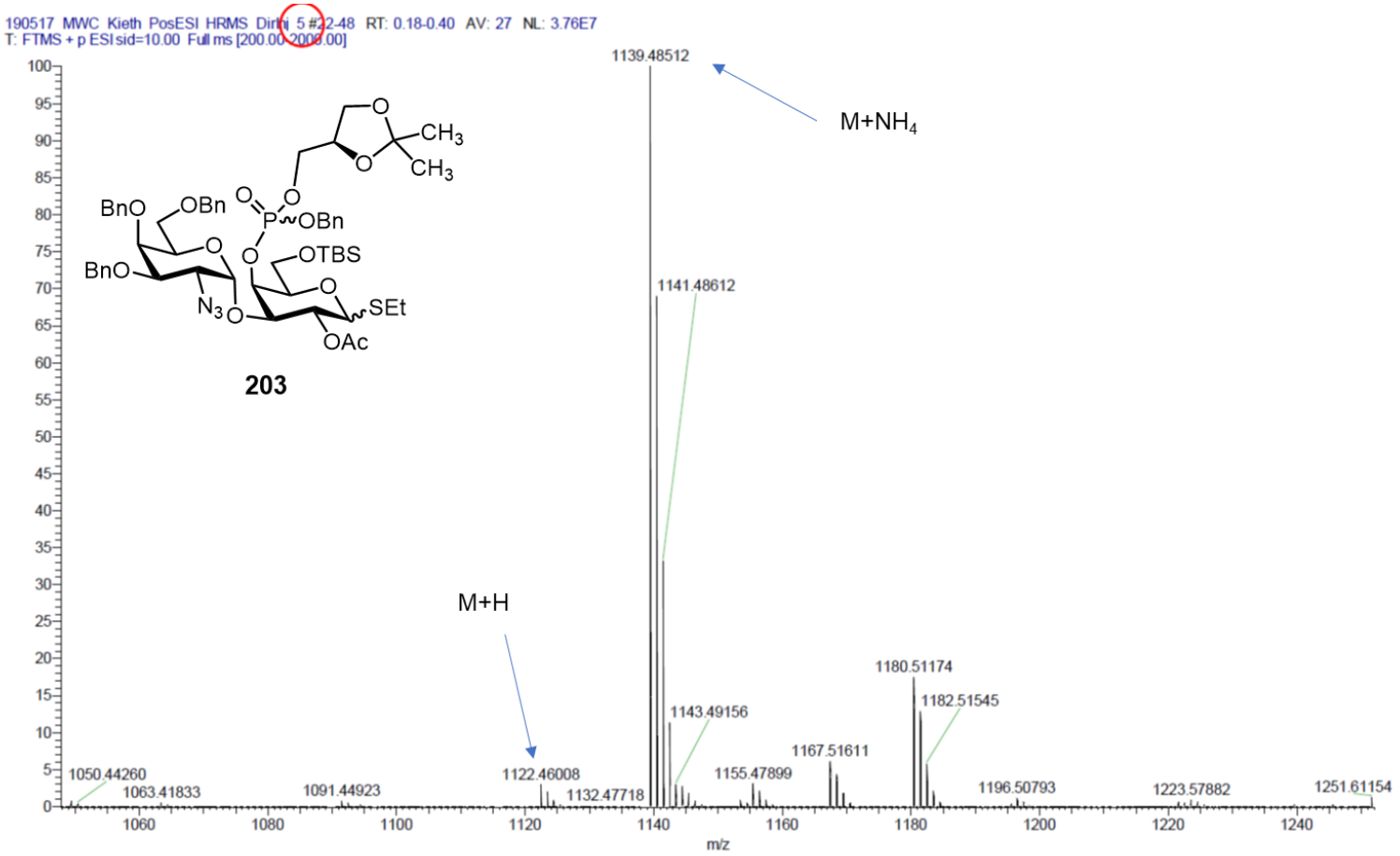
^{1}H-^{1}H COSY ($CDCl_3$) of compound 203



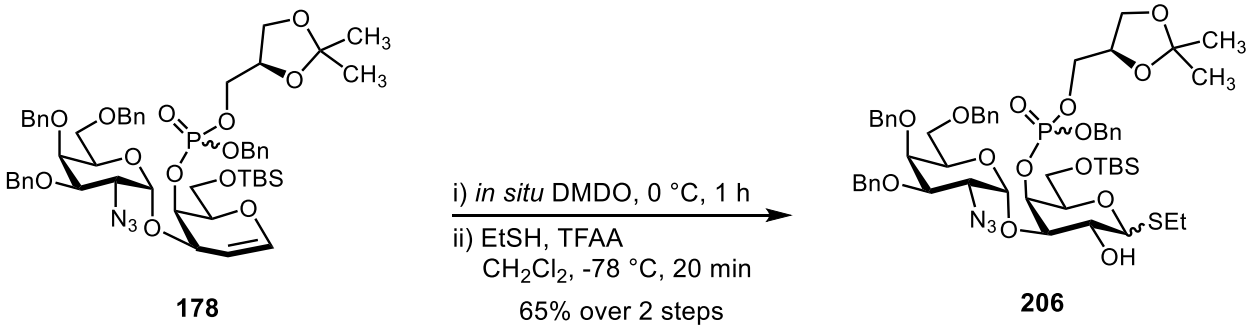
^{31}P NMR ($CDCl_3$, 162 MHz) of compound 203



203


190517 MWC Kieth PosESI HRMS Dirtn 5 #22-48 RT: 0.18-0.40 AV: 27 NL: 3.76E7
T: FTMS + p ESI sid=10.00 Full ms [200.00-2000.00]
203
M+NH4
M+H
1139.48512
1141.48612
1143.49156
1180.51174
1182.51545
1167.51611
1155.47899
1122.46008
1132.47718
1050.44260
1063.41833
1091.44923
1196.50793
1223.57882
1251.61154
m/z

Thioethyl 3,4,6-tri-*O*-benzyl-2-deoxy-2-azido-α-D-galactopyranosyl-(1→3)-6-*O*-*tert*-butyldimethylsilyl-4-*O*-(((*R*)-2,2-dimethyl-1,3-dioxolan-4-yl)methyl) phosphate-1-thio-α/β-D-galactopyranoside (206)



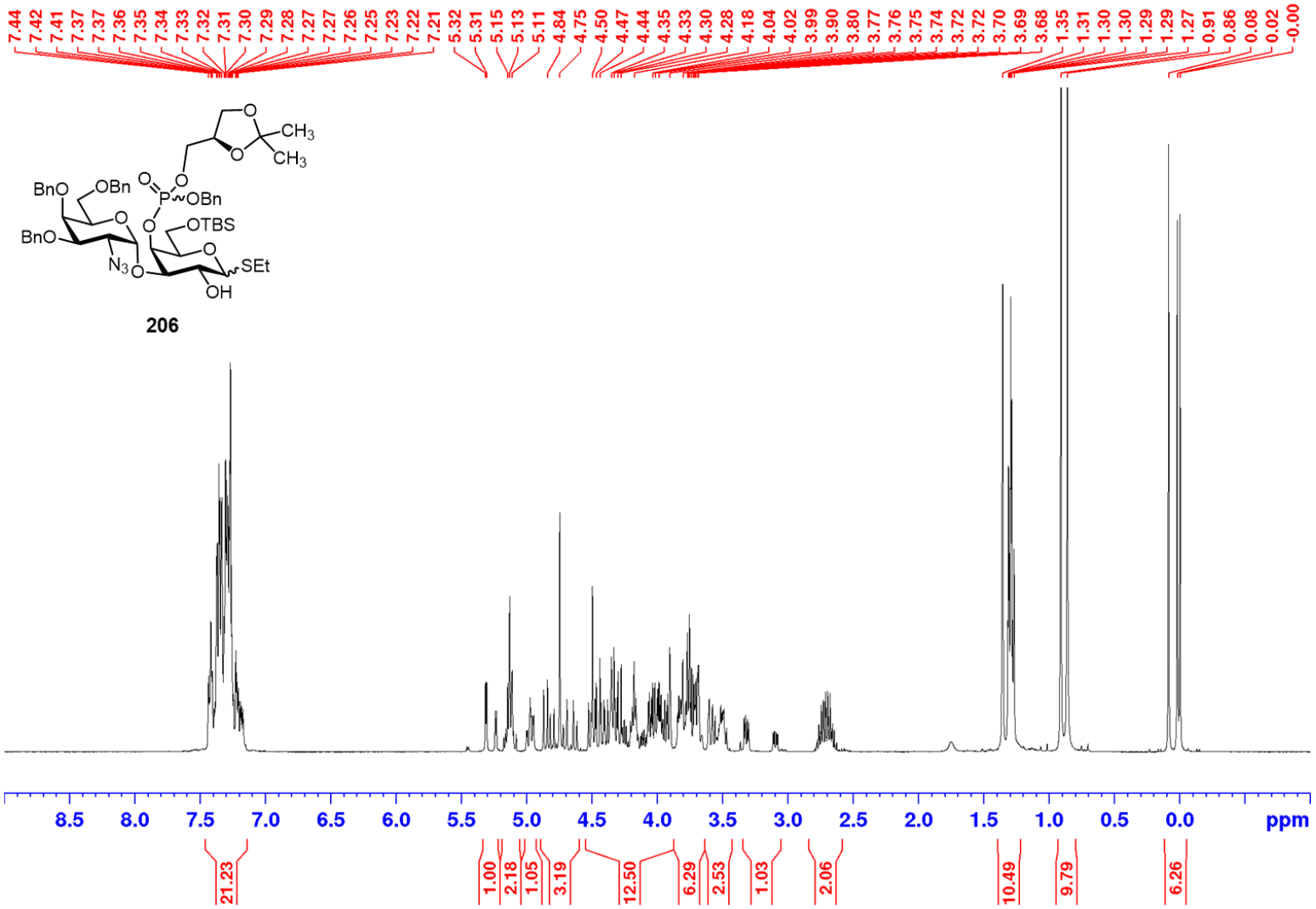
178 **206**

To a biphasic solution of 3,4,6-tri-*O*-benzyl-2-deoxy-2-azido-α-D-galactopyranosyl-(1→3)-6-*O*-tert-butyldimethylsilyl-4-*O*-(((*R*)-2,2-dimethyl-1,3-dioxolan-4-yl)methyl) phosphate-D-galactal **178** (1.0 equiv., 3.45 g, 3.44 mmol) in 10:1 CH_2Cl_2/acetone (33.0 mL) and sat. aq. $NaHCO_3$ (50 mL) was added a solution of Oxone (11.6 equiv., 8.87 g, 14.3 mmol) in H_2O (35 mL) dropwise, over 5 minutes, at 0 °C. The mixture was vigorously stirred at 0 °C for 30 minutes and then at room temperature. After 1.5 h, the starting material appeared to have been consumed by TLC. The crude reaction mixture was transferred to a separatory funnel with CH_2Cl_2 (10 mL). The organic phase was separated, and the aqueous phase extracted with CH_2Cl_2 (3 × 20 mL). The combined organics where dried ($NaSO_4$), filtered through a glass fritted vacuum filter funnel to remove drying agent. The filtrate was concentrated down to a clear oil which contained the epoxide intermediate. The crude oil was co-evaporated with toluene (3x) in a reaction flask. Then the reaction flask was placed inside of a vacuum desiccator equipped with P_2O_5 and left under reduced pressure for 1 h. The reaction flask removed from the vacuum desiccator and filled with an argon atmosphere, diluted with anhydrous CH_2Cl_2 (7.2 mL) and added

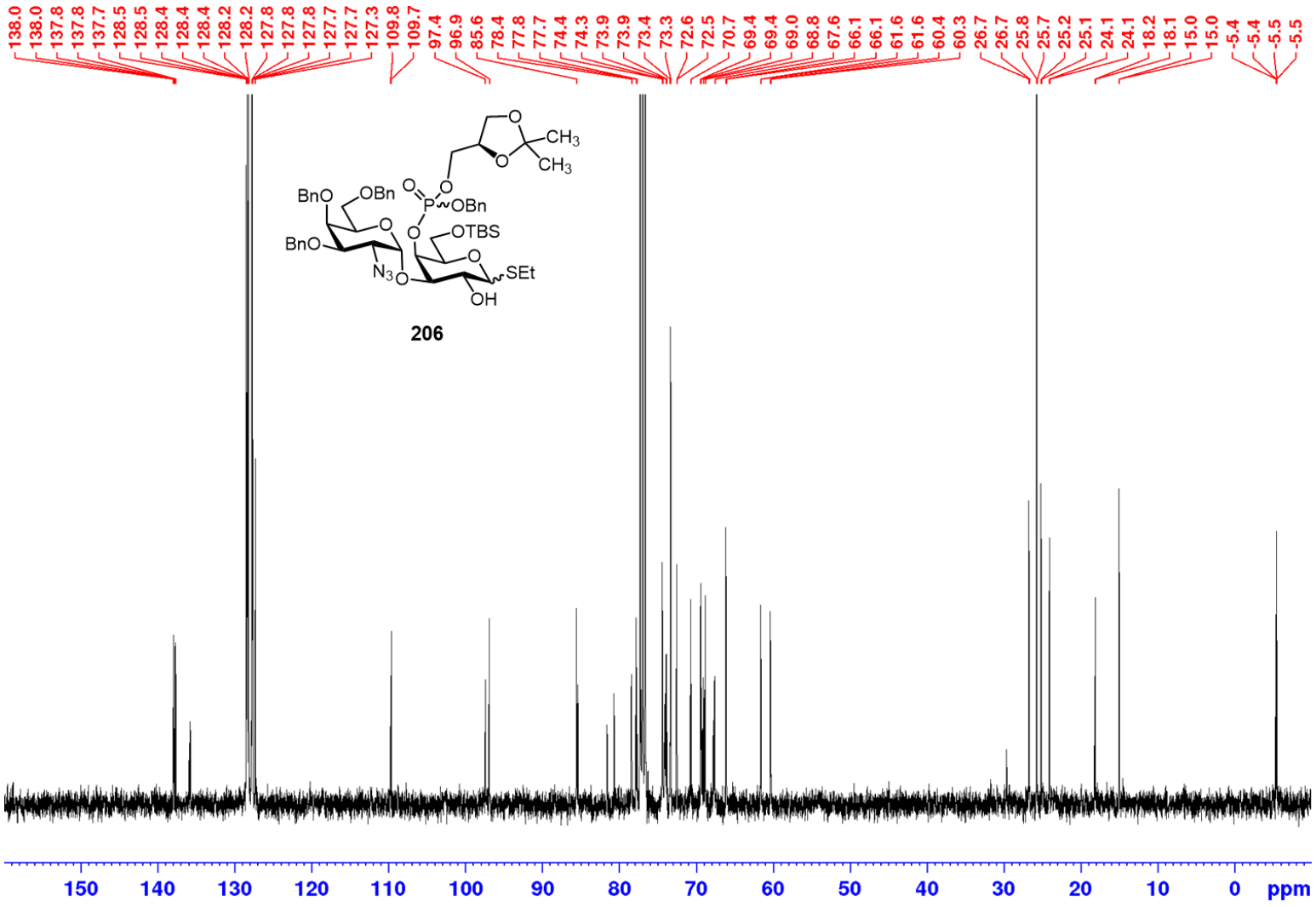
ethanethiol (28.2 equiv., 7.22 mL, 97.2 mmol) then cooled to -78 °C. The reaction was then charged with a trifluoroacetic anhydride (0.08 equiv., 0.04 mL, 0.28 mmol) dropwise. The reaction mixture was stirred at -78 °C for 20 minutes, then at 0 °C. After 1 h, the reaction mixture was neutralized by the addition of triethylamine and the solvent was evaporated by a stream of nitrogen gas in the hood until the malodorous thiol had dissipated. The reaction was furthered concentrated *in vacuo* and co-evaporated with toluene (2x) to produce a crude oil. The crude oil was purified by flash column chromatography (30:70 to 40:60 EtOAc/hexanes) to afford thioethyl 3,4,6-tri-*O*-benzyl-2-deoxy-2-azido-α-D-galactopyranosyl-(1→3)-6-*O*-*tert*-butyldimethylsilyl-4-*O*-(((*R*)-2,2-dimethyl-1,3-dioxolan-4-yl)methyl) phosphate-1-thio-α/β-D-galactopyranoside **206** (2.45 g, 2.22 mmol, 65%) as inseparable pairs of anomers as a colorless oil. R_f = 0.17 (1:3 EtOAc/hexanes) visualized with ceric ammonium molybdate stain; $[\alpha]D^{25}$ = +5.12° (c = 1.0, $CHCl_3$); IR ν_{max} (film) 3485.32, 2925.82, 2325.43, 2122.42, 1746.34, 1602.80, 1492.65, 1427.61, 1373.51, 1351.60, 1311.43, 1199.26, 1048.11, 807.82, 746.15, 701.12, cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.43-7.27 (m, 30H), 7.25-7.15 (m, 10H), 5.30 (d, *J* = 3.5 Hz, 1H), 5.24 (d, *J* = 3.5 Hz, 1H), 5.17-5.09 (m, 4H), 4.99-4.93 (m, 2H), 4.85 (d, *J* = 11.4 Hz, 1H), 4.79 (d, *J* = 11.5 Hz, 1H), 4.74 (s, 2H), 4.69 (d, *J* = 11.0 Hz, 1H), 4.62 (m, *J* = 11.2 Hz, 1H), 4.52-4.14 (m, 14H), 4.12-4.07 (m, 1H), 4.07-3.88 (m, 8H), 3.81-3.78 (m, 4H), 3.77-3.72 (m, 4H), 3.71-3.69 (m, 1H), 3.68-3.64 (m, 2H), 3.60-3.58 (m, 1H), 3.57-3.48 (m, 4H), 3.33-3.28 (m, 1H), 3.10-3.06 (m, 1H), 2.78-2.61 (m, 4H), 1.36-1.26 (m, 6H), 1.29 (s, 12H), 0.91 (s, 9H), 0.85 (s, 9H), 0.08 (s, 6H), 0.02 (s, 3H), -0.02 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 138.0, 180.0, 138.0, 137.8, 137.8, 137.8, 137.7, 137.7, 128.6, 128.5, 128.5, 128.5, 128.4, 128.4, 129.0, 128.4, 128.3, 128.2, 127.8, 127.8, 127.8, 127.8,

127.7, 127.7, 127.7, 127.6, 127.3, 127.3, 109.8, 109.6, 97.4, 96.9, 85.6, 85.3, 81.6, 80.7, 78.5, 78.4, 78.4, 77.9, 77.8, 77.7, 77.7, 77.2, 77.2, 74.5, 74.4, 74.4, 74.4, 74.1, 74.0, 74.0, 73.9, 74.0, 73.9, 73.3, 73.2, 73.2, 72.5, 72.5, 72.5, 72.4, 70.8, 70.7, 70.6, 70.6, 69.4, 69.3, 69.3, 69.2, 69.2, 69.1, 69.1, 69.0, 68.9, 68.8, 67.7, 67.7, 67.6, 67.6, 66.1, 66.1, 66.1, 66.1, 61.6, 61.6, 60.4, 60.3, 31.4, 31.3, 26.7, 26.7, 25.8, 25.8, 25.7, 25.7, 25.2, 25.1, 24.1, 24.1, 18.2, 18.1, 15.0, 15.0, -5.34, -5.4, 5.5, -5.5; ^{31}P NMR (162 MHz, $CDCl_3$): δ -1.4, -1.6; HRMS (ESI) calcd for $C_{54}H_{80}N_4O_{14}PSSi^+$ $(M+NH_4)^+$ 1097.47362, found 1097.47300 m/z.

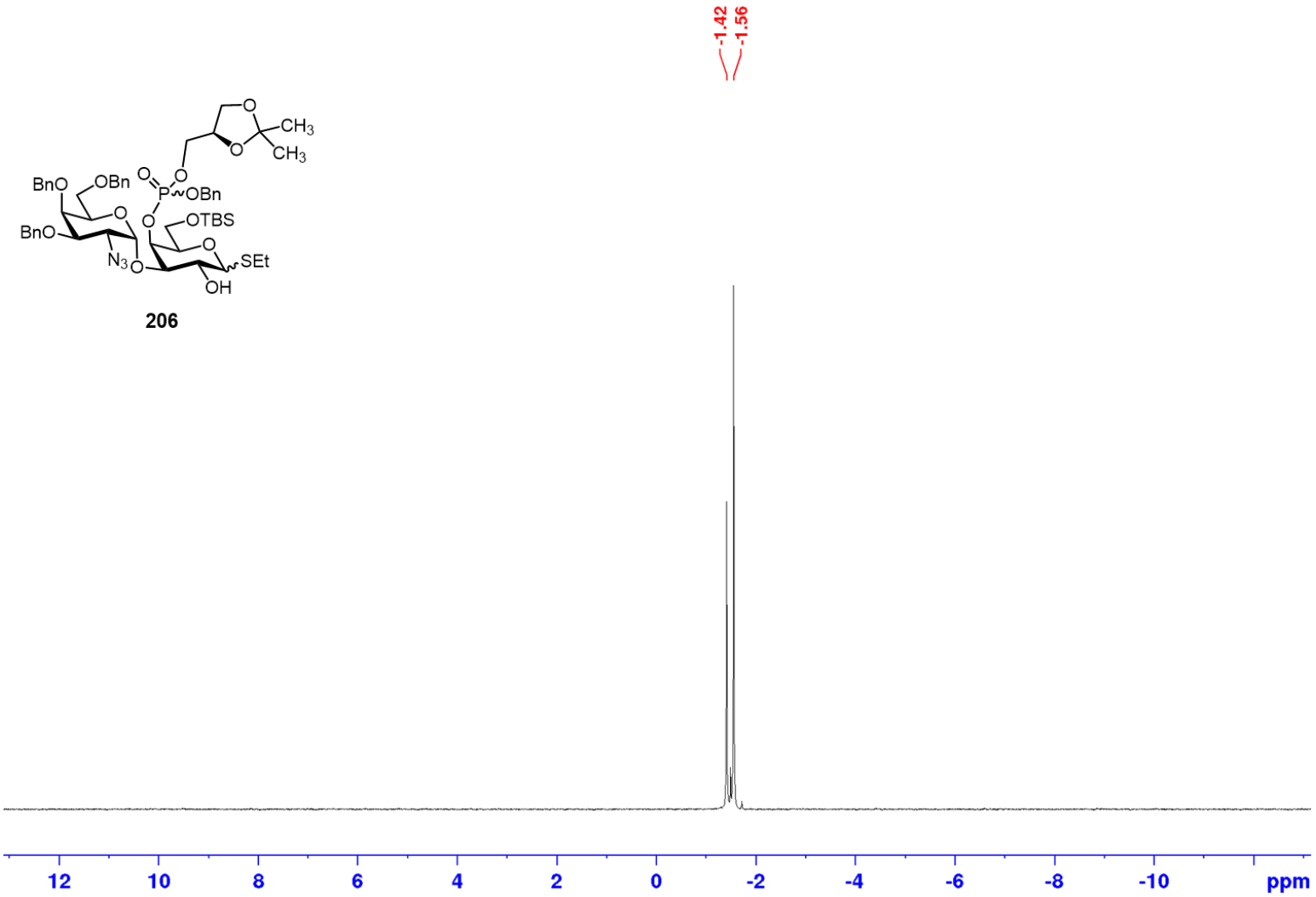
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 206



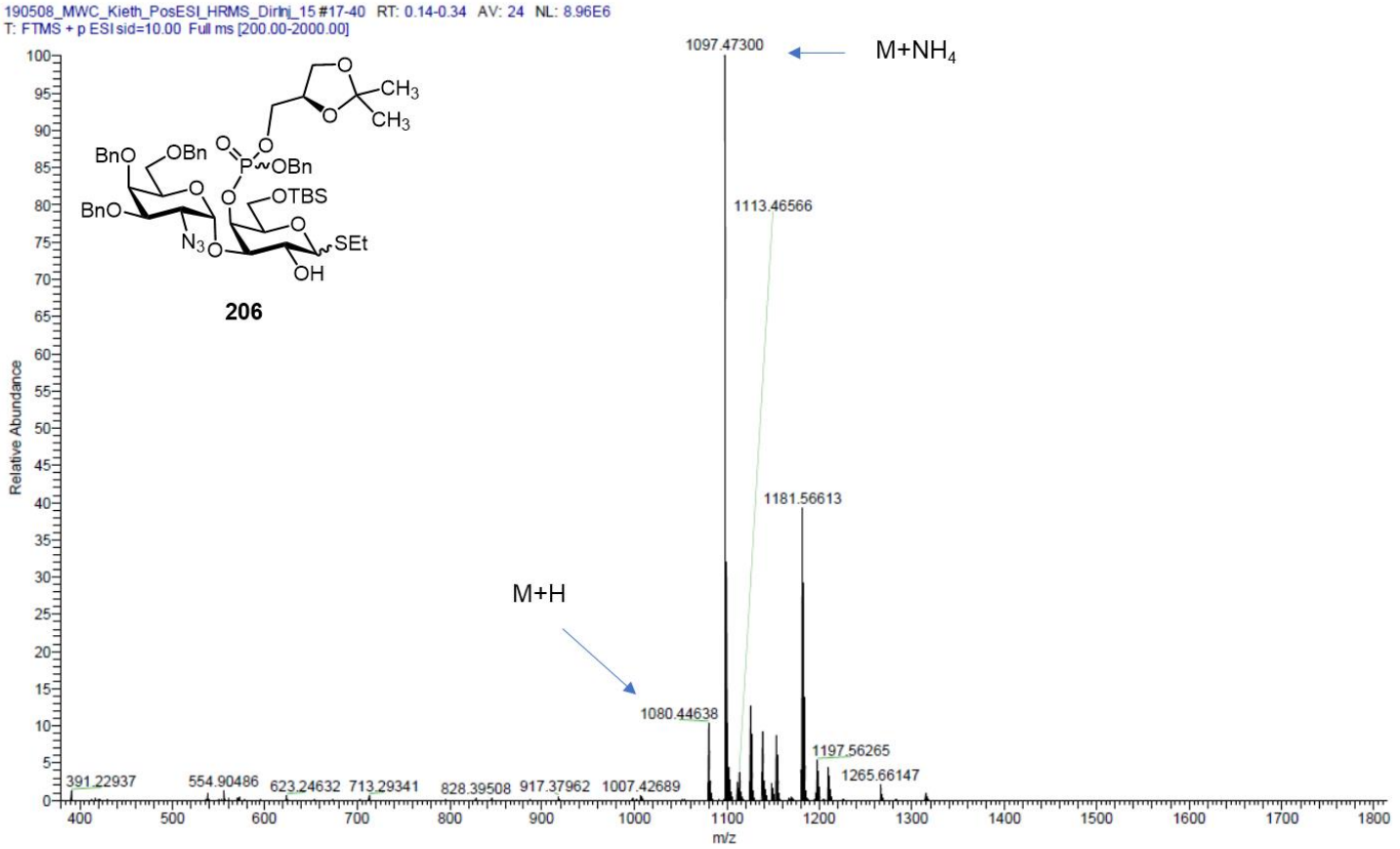
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 206

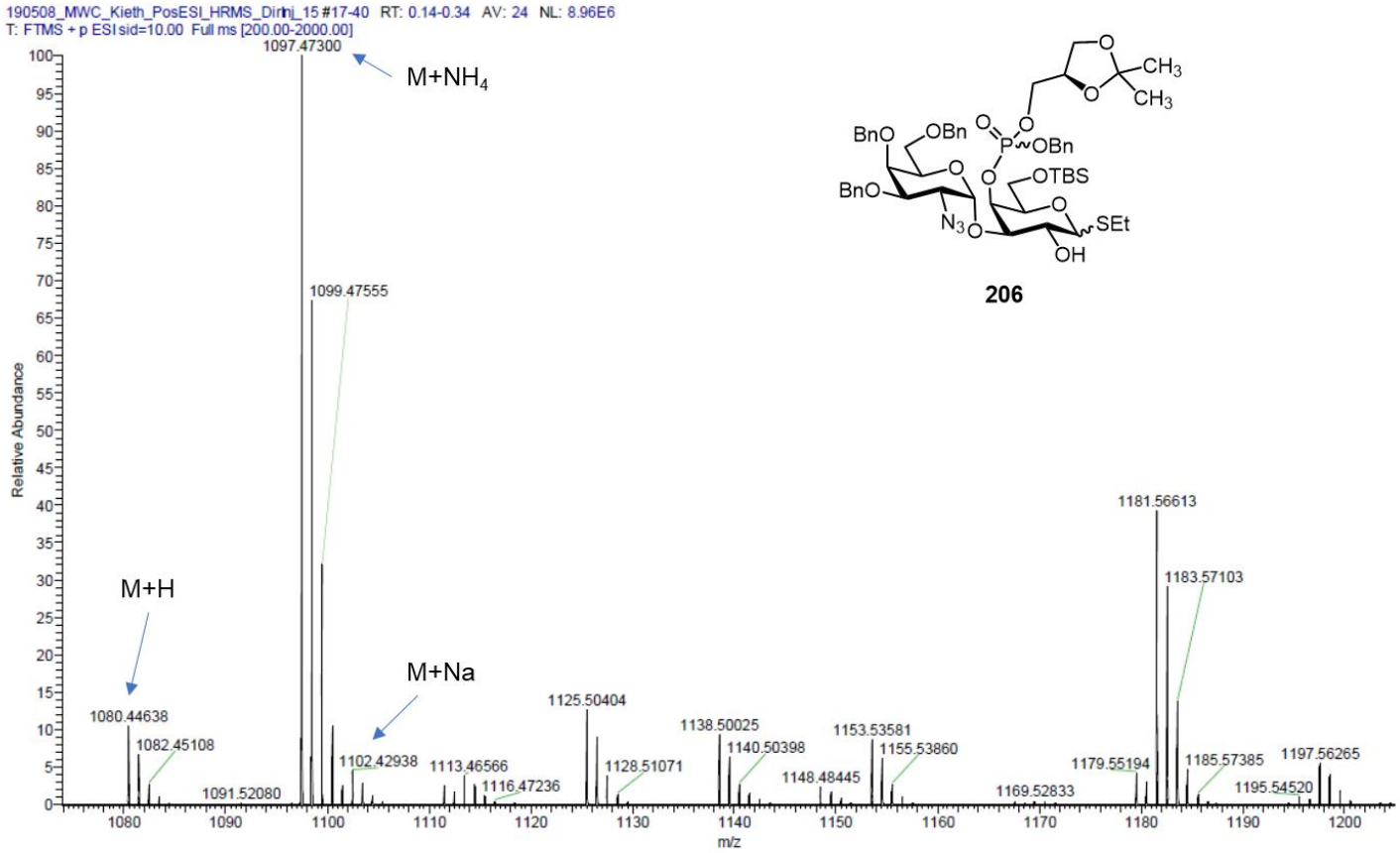


^{31}P NMR ($CDCl_3$, 162 MHz) of compound 206

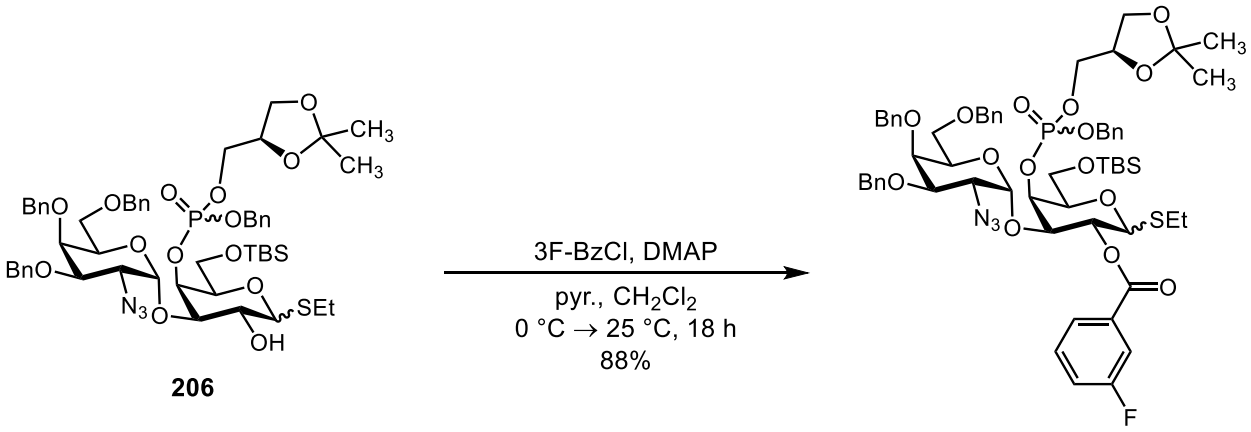


206


190508_MWC_Kieth_PosESI_HRMS_Dirlnj_15 #17-40 RT: 0.14-0.34 AV: 24 NL: 8.96E6
T: FTMS + p ESI sid=10.00 Full ms [200.00-2000.00]
206
M+NH4
M+H
1097.47300
1113.46566
1181.56613
1080.44638
1197.56265
1265.66147
391.22937
554.90486
623.24632
713.29341
828.39508
917.37962
1007.42689
Relative Abundance
m/z


190508_MWC_Kieth_PosESI_HRMS_Dinhj_15 #17-40 RT: 0.14-0.34 AV: 24 NL: 8.96E6
T: FTMS + p ESI sid=10.00 Full ms [200.00-2000.00]
1097.47300
M+NH4
1099.47555
M+H
M+Na
1080.44638
1082.45108
1091.52080
1102.42938
1113.46566
1116.47236
1125.50404
1128.51071
1138.50025
1140.50398
1148.48445
1153.53581
1155.53860
1169.52833
1179.55194
1181.56613
1183.57103
1185.57385
1195.54520
1197.56265
Relative Abundance
m/z
BnO
OBn
OBn
BnO
OTBS
SEt
OH
N3
CH3
CH3
206

Thioethyl 3,4,6-tri-*O*-benzyl-2-deoxy-2-azido-α-D-galactopyranosyl-(1→3)-6-*O*-*tert*-butyldimethylsilyl-4-*O*-(((*R*)-2,2-dimethyl-1,3-dioxolan-4-yl)methyl) phosphate-2-*O*-(3-fluorobenzoate)-1-thio-α/β-D-galactopyranoside (207)

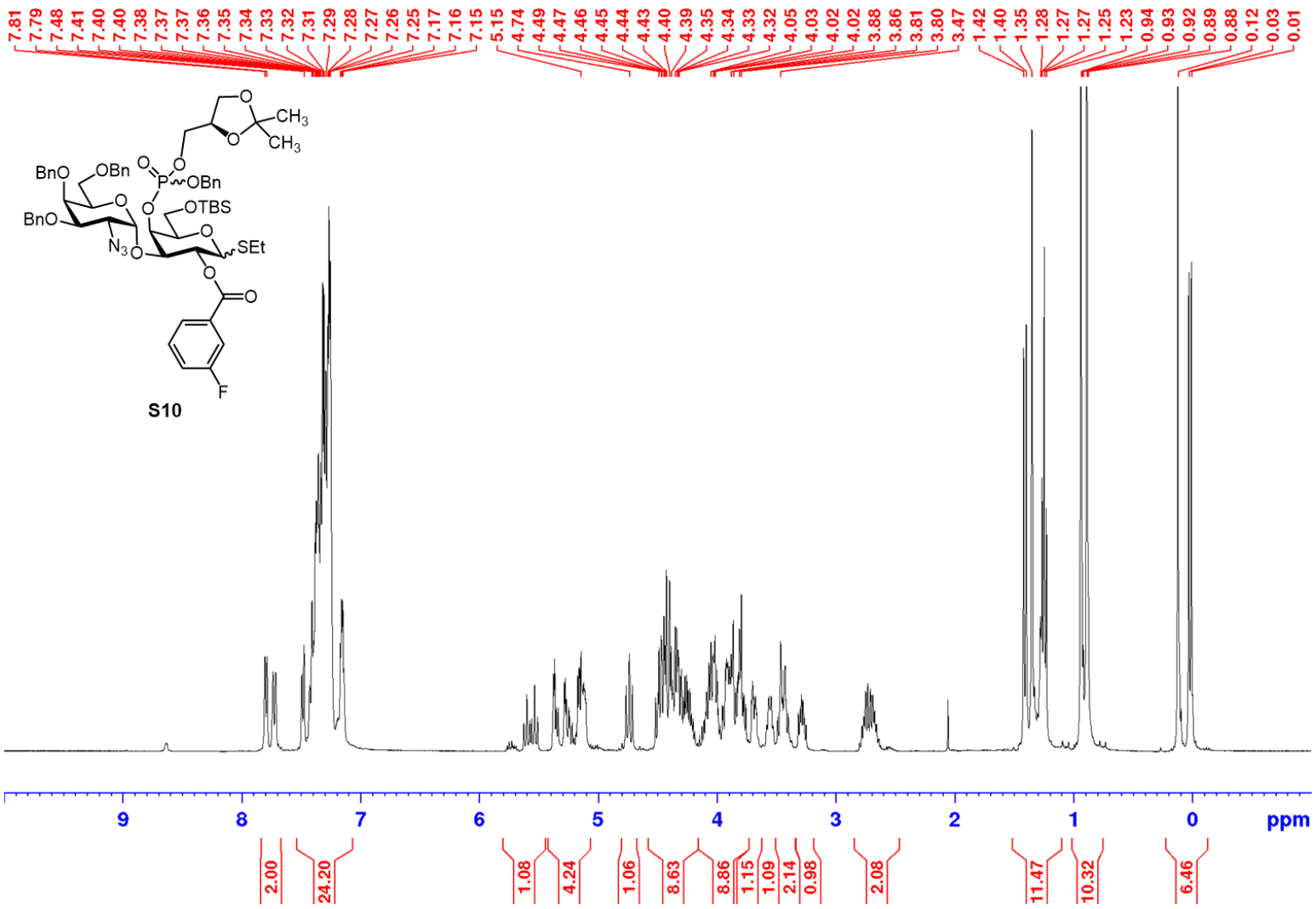


206 **207**

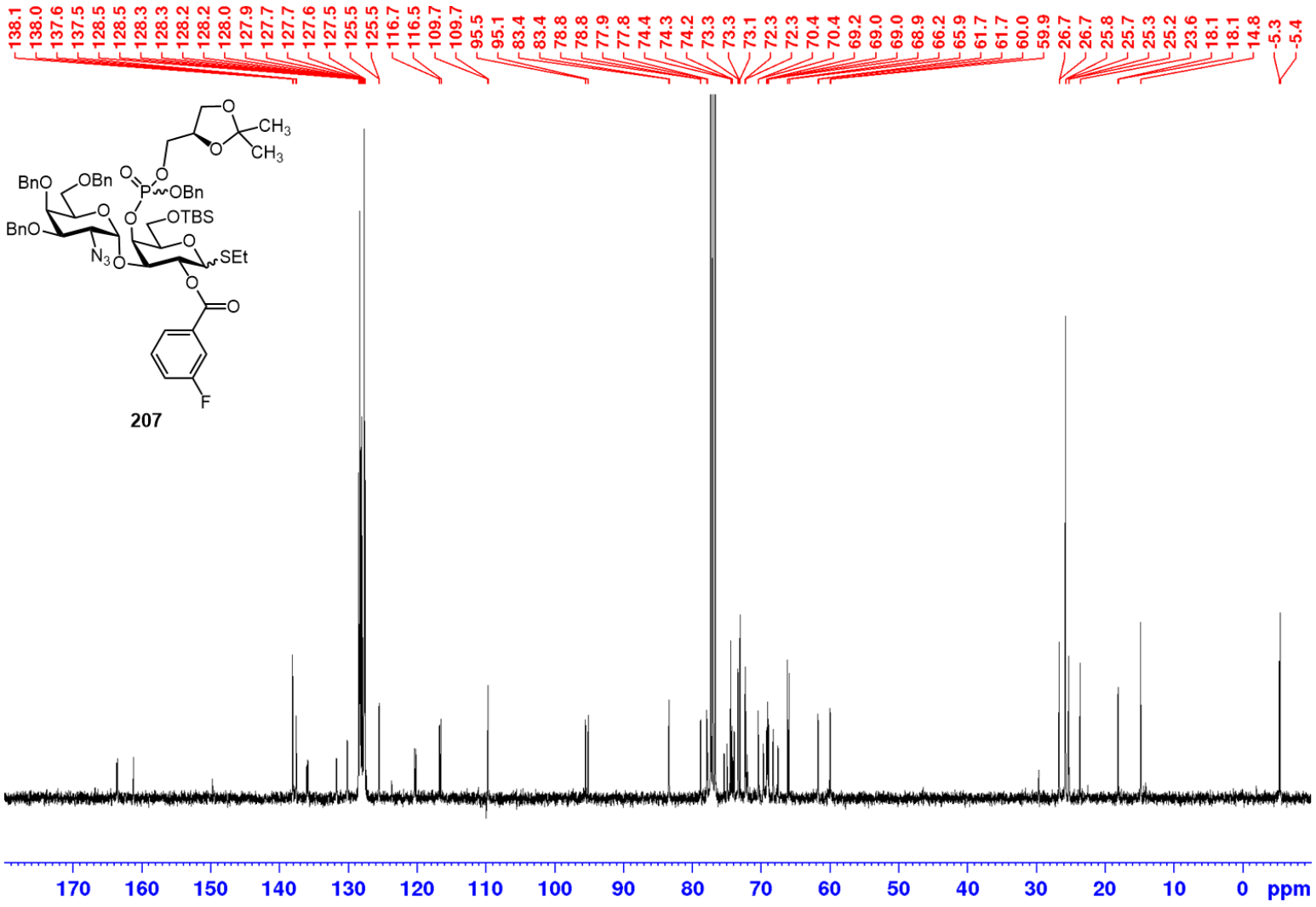
A solution of thioethyl 3,4,6-tri-*O*-benzyl-2-deoxy-2-azido-α-D-galactopyranosyl-(1→3)-6-*O*-*tert*-butyldimethylsilyl-4-*O*-(((*R*)-2,2-dimethyl-1,3-dioxolan-4-yl)methyl) phosphate-1-thio-α/β-D-galactopyranoside **206** (1.0 equiv., 0.94 g, 0.87 mmol) and DMAP (0.2 equiv., 0.021 g, 0.17 mmol) in pyridine (8.0 mL) was added 3-fluorobenzoyl chloride (5.0 equiv., 0.53 mL, 4.3 mmol) at 0 °C under an argon atmosphere. At 18 h, TLC analysis of the reaction mixture indicated complete consumption of the starting material. The reaction was added methanol (0.5 mL) and stirred for 5 minutes. The reaction was diluted with EtOAc (50 mL) and water (50 mL) and transferred to a separatory funnel. The layers where separated and the aqueous layer was extracted with EtOAc (3 x 20 mL). The organics combined, dried ($MgSO_4$), filtered through a glass frittered vacuum filter funnel to remove drying agent. The filtrate was concentrated to a crude oil. The crude oil was purified by silica gel flash column chromatography (25:75 to 40:60 EtOAc/hexanes) to give thioethyl 3,4,6-tri-*O*-benzyl-2-deoxy-2-azido-α-D-galactopyranosyl-(1→3)-6-*O*-*tert*-

butyldimethylsilyl-4-*O*-(((*R*)-2,2-dimethyl-1,3-dioxolan-4-yl)methyl) phosphate-2-*O*-(3-fluorobenzoate)-1-thio-α/β-D-galactopyranoside **207** (0.868 g, 0.722 mmol, 88% yield) as inseparable pairs of anomers as a colorless oil. R_f = 0.6 (2:3 EtOAc/hexanes) visualized with ceric ammonium molybdate stain. $[\alpha]D^{25}$ = -1.72° (c = 1.0, $CHCl_3$); IR ν_{max} (film) 3434.64, 2362.02, 211.91, 1637.55, 1266.31, 750.02cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.80 (d, *J* = 6.6 Hz, 2H), 7.71 (d, *J* = 8.6 Hz, 2H), 7.49 (d, *J* = 7.6 Hz, 2H), 7.49-7.27 (m, 31H), 7.26-7.22 (m, 7H), 7.19-7.10 (m, 4H), 5.61 (d, *J* = 10.0 Hz, 1H), 5.53 (d, *J* = 10.0 Hz, 1H), 5.39-5.33 (m, 2H), 5.29-5.20 (m, 2H), 5.18-5.06 (m, 4H), 4.76-4.70 (m, 2H), 4.51-4.19 (m, 16H), 4.52-4.19 (m, 6H), 3.94-3.86 (m, 6H), 3.84-3.76 (m, 4H), 3.71-3.66 (m, 2H), 3.58-3.52 (m, 2H), 3.49-3.37 (m, 4H), 3.18-3.25 (m, 2H), 2.79-2.63 (m, 4H), 1.43 (s, 3H), 1.39 (s, 3H), 1.36 (s, 6H), 1.24 (t, 6H), 0.94 (s, 9H), 0.88 (s, 9H), 0.11 (s, 6H), 0.03 (s, 3H), 0.01 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 163.7, 163.6, 163.6, 163.5, 161.2, 138.1, 138, 137.6, 137.5, 136.1, 136, 135.9, 135.8, 131.7, 131.7, 131.7, 130.2, 130.1, 128.5, 128.5, 128.3, 128.3, 128.2, 128.2, 128, 127.9, 127.9, 127.7, 127.7, 127.6, 127.5, 127.4, 125.5, 125.5, 120.4, 120.2, 116.7, 116.5, 109.7, 109.7, 95.5, 95.1, 83.4, 83.4, 78.8, 78.8, 77.9, 77.8, 77.2, 75.4, 74.9, 74.4, 74.3, 74.2, 74, 73.9, 73.3, 73.3, 73.1, 72.3, 72.3, 72.2, 72, 72, 70.4, 70.4, 69.7, 69.6, 69.2, 69.2, 69.1, 69, 69, 68.9, 68.3, 68.2, 67.6, 67.5, 66.2, 65.9, 61.7, 61.7, 60, 59.9, 29.6, 26.7, 26.7, 25.8, 25.7, 25.3, 25.2, 23.6, 18.1, 18.1, 14.8, -5.3, -5.4,; ^{31}P NMR (162 MHz, $CDCl_3$): δ -0.6, -0.8; HRMS (ESI) calcd for $C_{61}H_{81}FN_4O_{15}PSSi^+$ $(M+NH_4)^+$ 1219.49042, found 1219.49167 m/z.

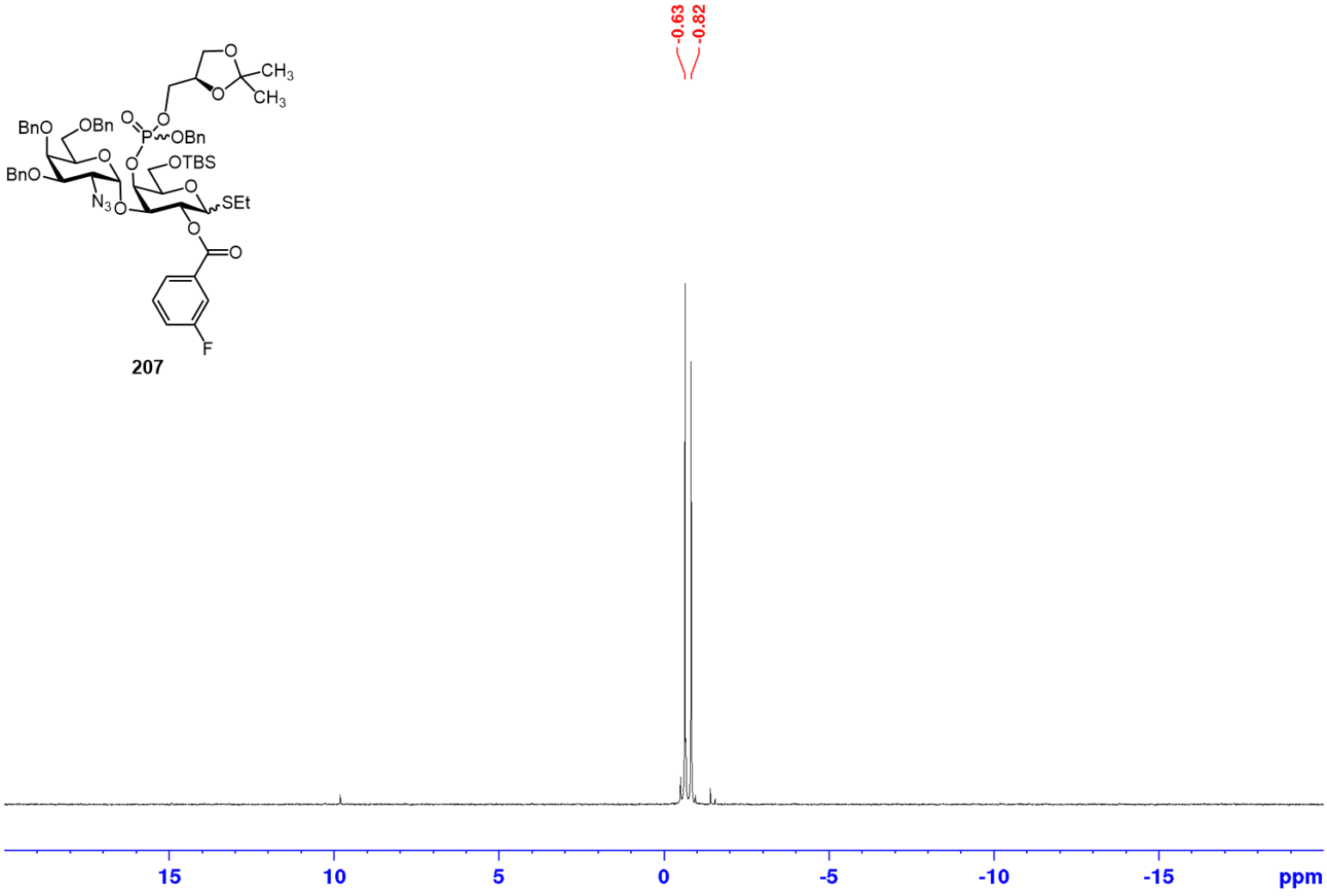
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 207



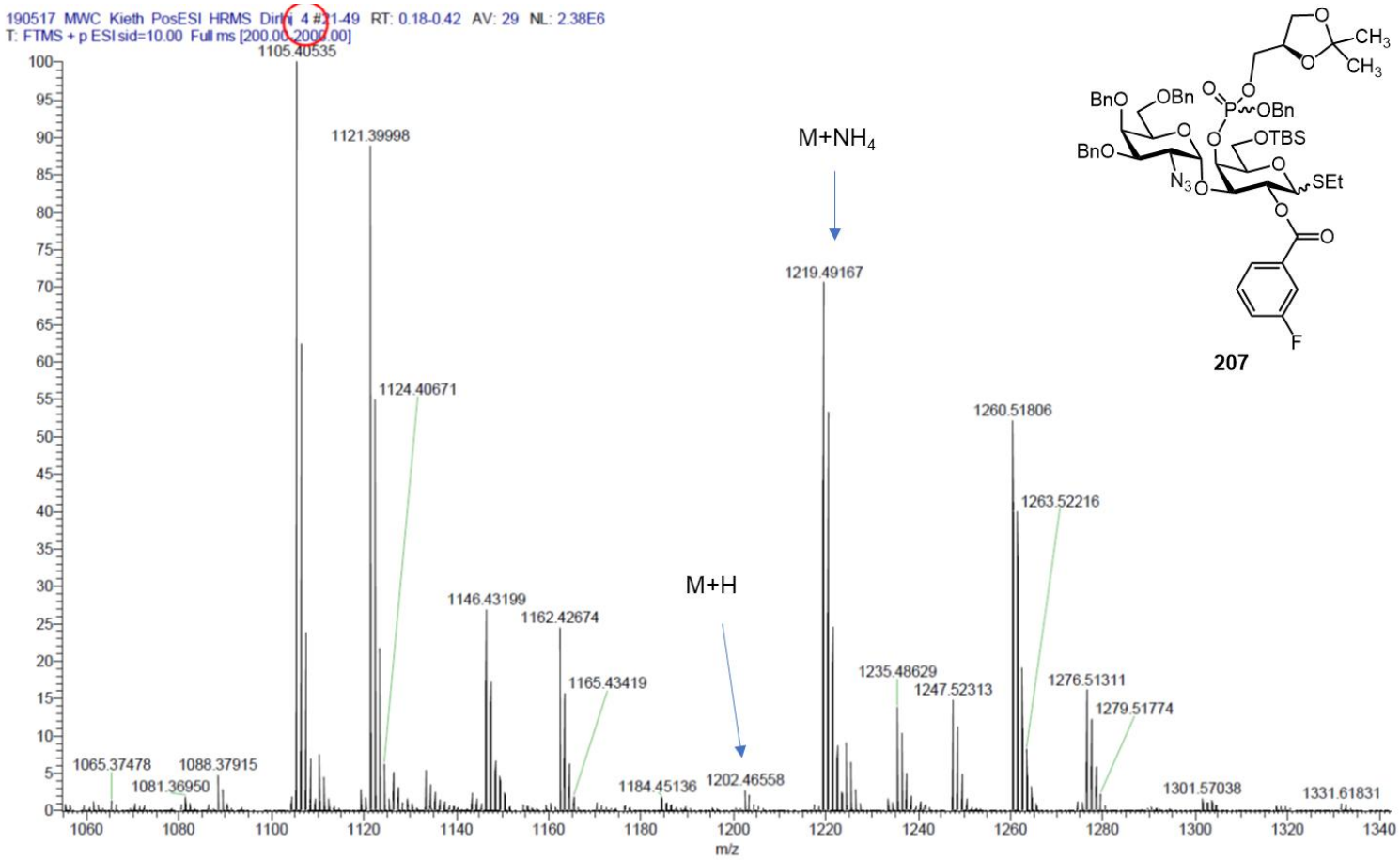
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 207



^{31}P NMR ($CDCl_3$, 162 MHz) of compound 207



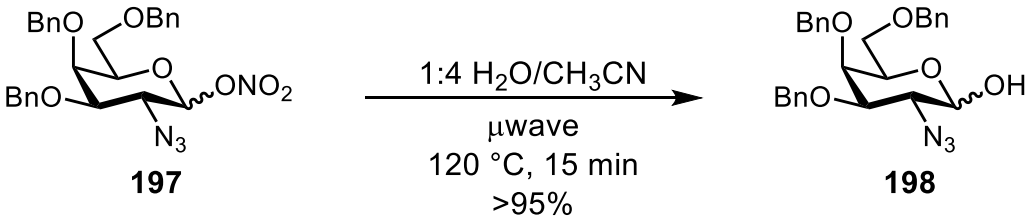
207


190517 MWC Kieth PosESI HRMS Dirty 4 #21-49 RT: 0.18-0.42 AV: 29 NL: 2.38E6
T: FTMS + p ESI sid=10.00 Full ms [200.00-2000.00]
1105.40535
1121.39998
1124.40671
1146.43199
1162.42674
1165.43419
1065.37478
1081.36950
1088.37915
1184.45136
1202.46558
M+H
M+NH4
1219.49167
1235.48629
1247.52313
1260.51806
1263.52216
1276.51311
1279.51774
1301.57038
1331.61831
m/z
207

Part 2. Denitration methodology

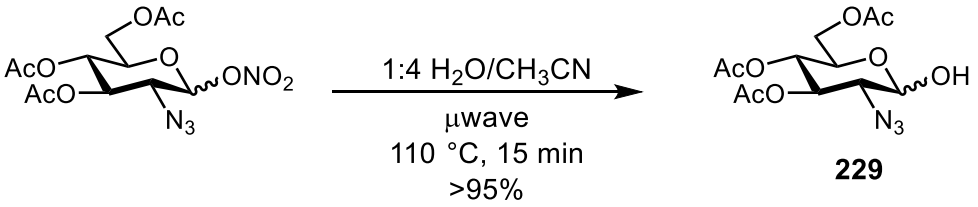
Part 2 contains experimental procedures for compounds found within figure 34 and schemes 29-30.

3,4,6-Tri-*O*-benzyl-2-deoxy-2-azido-D-galactose (198)



A solution of nitrate ester 3,4,6-tri-*O*-benzyl-2-deoxy-2-azido-1-D-galactoside **197** (1.0 equiv., 0.106 g, 0.204 mmol) in 20% aq. acetone (2.0 mL) and treated with pyridine (5.0 equiv., 0.08 mL, 1.0 mmol). The reaction was heated by microwave irradiation to 120 °C for 10 min. After complete consumption of the starting material, the reaction was transferred to a separatory funnel, diluted with EtOAc (20 mL) and washed with 1N HCl (1 x 5 mL), water (2 x 5 mL), and brine (1 x 5 mL). The organics were dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give 3,4,6-tri-*O*-benzyl-2-deoxy-2-azido-D-galactose **198** (0.97 g, 0.21 mmol, >95%) as a yellow oil. The spectral data was consistent with previously reported values.[314] HRMS (ESI) calcd for $C_{27}H_{29}O_5N_3$ $[M+Na]^+$, 498.2005, found 498.2018.

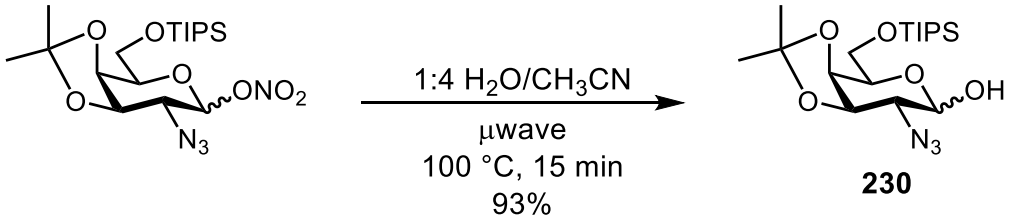
3,4,6-Tri-*O*-acetyl-2-deoxy-2-azido-D-glucose (229)



229

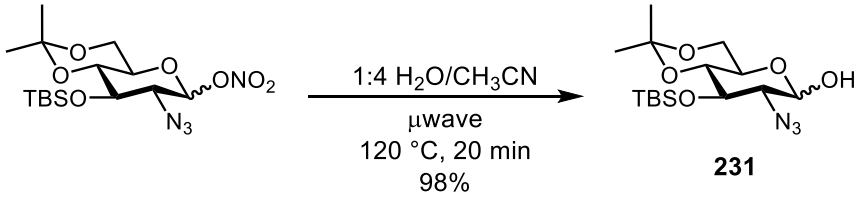
A solution of nitrate ester 3,4,6-tri-*O*-acetyl-2-deoxy-2-azido-1-D-glucoside (1.0 equiv., 0.075 g, 0.200 mmol) in 20% aq. acetone (2.0 mL) and treated with pyridine (5.0 equiv., 0.08 mL, 0.95 mmol). The reaction was heated at 110 °C for 15 min. After complete consumption of the starting material, the reaction was transferred to a separatory funnel, diluted with EtOAc (20 mL) and washed with 1 N HCl (1 x 5 mL), water (2 x 5 mL) and brine (1 x 5 mL). The organics were dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give 3,4,6-tri-*O*-acetyl-2-deoxy-2-azido-D-glucose **229** (0.066 g, 0.200 mmol, >95%) as a colorless oil. The spectral data was consistent with previously reported values.[133.] HRMS (ESI) calcd for $C_{12}H_{17}N_3O_8$ $[M+Na]^+$, 354.0913, found 354.0891.

3,4-*O*-isopropylidene-2-deoxy-2-azido-6-*O*-TIPS-D-galactose (230)



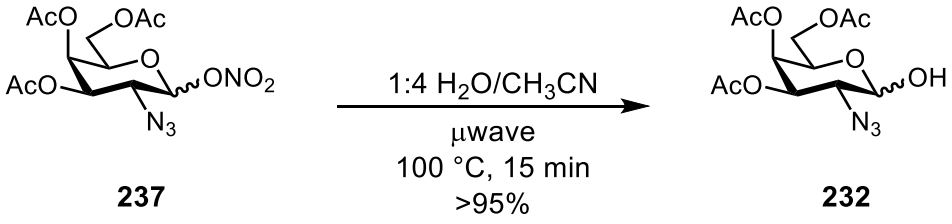
A solution of nitrate ester 3,4-O-isopropylidene-2-deoxy-2-azido-6-O-TIPS-1-D-galactoside (1.0 equiv., 0.100 g, 0.23 mmol) in 20% aq. acetonitrile (2.0 mL) and treated with pyridine (5.0 equiv., 0.08 mL, 1.0 mmol). The reaction was heated at 100 °C for 15 min. After complete consumption of the starting material, the reaction was transferred to a separatory funnel, diluted with EtOAc (20 mL) and washed with 1 N HCl (1 x 5 mL), water (2 x 5 mL) and brine (1 x 5 mL). The organics were dried over MgSO4, filtered, and concentrated in vacuo to give 3,4-O-isopropylidene-2-deoxy-2-azido-6-O-TIPS-D-galactose **230** (0.085 g, 0.210 mmol, 93%) as a yellow oil. The spectral data was consistent with previously reported values.[153] HRMS (ESI) calcd for $C_{18}H_{35}N_3O_5Si$ $[M+Na]^+$, 424.2244, found 424.2242.

4,6-*O*-isopropylidene-2-deoxy-2-azido-3-*O*-TBS-D-glucose (231)



A solution of nitrate ester 4,6-*O*-isopropylidene-2-deoxy-2-azido-3-*O*-TBS-1-D-glucoside (1.0 equiv., 0.111 g, 0.27 mmol) in 20% aq. acetone (2.0 mL) and treated with pyridine (5.0 eq, 0.11 mL, 1.35 mmol). The reaction was heated at heated at 120 °C for 20 min. After complete consumption of the starting material, the reaction was transferred to a separatory funnel, diluted with EtOAc (20 mL) and washed with 1N HCl (1 x 5 mL), water (2 x 5 mL) and brine (1 x 5 mL). The organics were dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give 4,6-O-isopropylidene-2-deoxy-2-azido-3-O-TBS-D-glucose **231** (96.2 mg, 0.27 mmol, 98%) as a yellow oil. The spectral data was consistent with previously reported values.[153]: HRMS (ESI) calcd for $C_{15}H_{29}N_3O_5Si$ $[M+Na]^+$, 382.1774, found 382.1739.

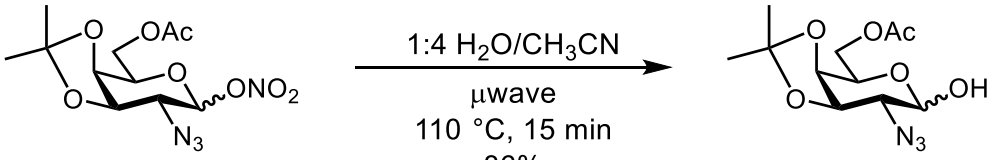
3,4,6-Tri-*O*-acetyl-2-deoxy-2-azido-D-galactose (232)



237 **232**

A solution of nitrate ester 3,4,6-tri-*O*-acetyl-2-deoxy-2-azido-1-D-galactoside **237** (1.0 equiv., 0.075 g, 0.19 mmol) in 20% aq. acetone (2.0 mL) and treated with pyridine (5.0 equiv., 0.08 mL, 0.95 mmol). The reaction was heated at 100 °C for 15 min. After complete consumption of the starting material, the reaction was transferred to a separatory funnel, diluted with EtOAc (20 mL) and washed with 1 N HCl (1 x 5 mL), water (2 x 5 mL) and brine (1 x 5 mL). The organics were dried over $MgSO_4$, filtered and concentrated *in vacuo* to give 3,4,6-tri-O-acetyl-2-deoxy-2-azido-D-galactose **232** (0.074 g, 0.19 mmol, >95%) as a colorless oil. The spectral data was consistent with previously reported values.[153] HRMS (ESI) calcd for $C_{12}H_{17}N_3O_8$ $[M+Na]^+$, 354.0913, found 354.0891.

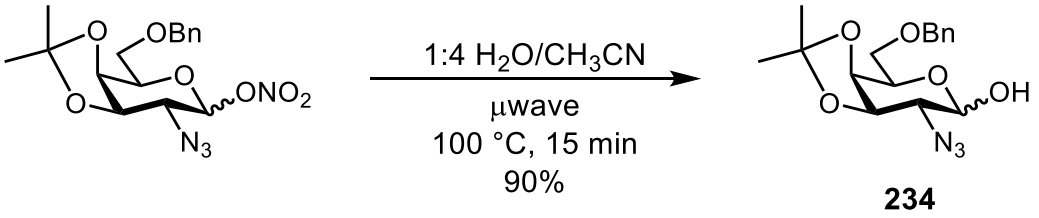
3,4-*O*-isopropylidene-2-deoxy-2-azido-6-*O*-acetate-D-galactose (233)



233

A solution of nitrate ester 3,4-*O*-isopropylidene-2-deoxy-2-azido-6-O-acetate-1-D-galactoside (1.0 equiv., 0.066 g, 0.20 mmol) in 20% aq. acetone (2.0 mL) and treated with pyridine (5.0 equiv., 0.08 mL, 0.95 mmol). The reaction was heated at 110 °C for 15 min. After complete consumption of the starting material, the reaction was transferred to a separatory funnel, diluted with EtOAc (20 mL) and washed with 1N HCl (1 x 5 mL), water (2 x 5 mL) and brine (1 x 5 mL). The organics were dried over $MgSO_4$, filtered, and concentrated *in vacuo*. Purification by flash chromatography (3:1 hexanes/EtOAc) gave 3,4-*O*-isopropylidene-2-deoxy-2-azido-6-*O*-acetate-D-galactose **233** (0.0495 g, 0.172 mmol, 86%) as a colorless oil. The spectral data was consistent with previously reported values[153]: HRMS (ESI) calcd for $C_{11}H_{17}N_3O_6$ $[M+Na]^+$,310.1015, found 310.0978.

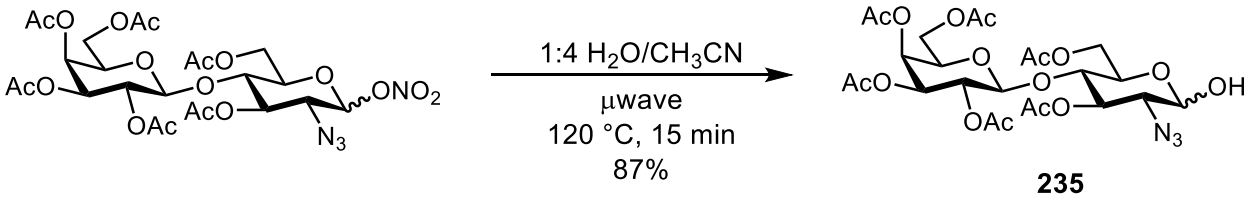
3,4-*O*-isopropylidene-2-deoxy-2-azido-6-*O*-benzyl-D-galactose (234)



234

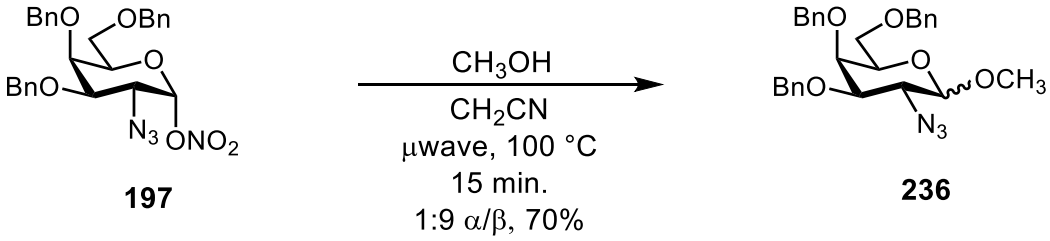
A solution of nitrate ester 3,4-*O*-isopropylidene-2-deoxy-2-azido-6-O-benzyl-1-D-galactoside (1.0 equiv., 0.076 g, 0.2 mmol) in 20% aq. acetone (2.0 mL) and treated with pyridine (5.0 equiv., 0.08 mL, 0.95 mmol). The reaction was heated at 100 °C for 15 min. After complete consumption of the starting material, the reaction was transferred to a separatory funnel, diluted with EtOAc (20 mL) and washed with 1 N HCl (1 x 5 mL), water (2 x 5 mL) and brine (1 x 5 mL). The organics were dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give 3,4-*O*-isopropylidene-2-deoxy-2-azido-6-*O*-benzyl-D-galactose **234** (60.1 mg, 0.17 mmol, 90%) as a colorless oil. The spectral data was consistent with previously reported values.[153] HRMS (ESI) calcd for $C_{16}H_{21}N_3O_5$ $[M+Na]^+$, 358.1379, found 358.1362.

3,6,2',3',4',6'-hexa-*O*-acetyl-D-lactosamine (235)



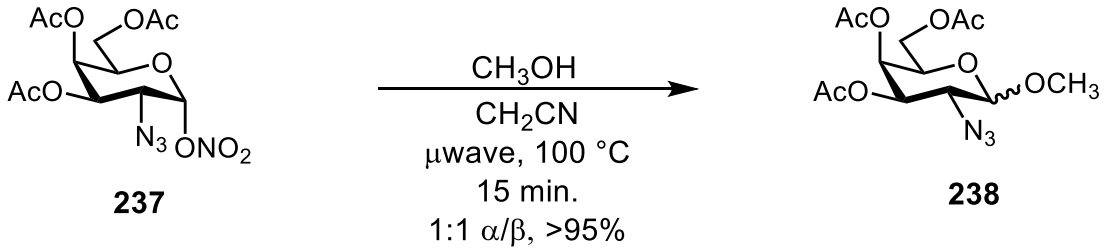
A solution of 1.0:2.4 (beta manno-/alpha gluco-) configured nitrate ester 3,6,2',3',4',6'-hexa-*O*-acetyl-1-D-lactosaminoside (1.0 equiv., 0.142 g, 0.21 mmol) was dissolved in 20% aq. acetone (2.0 mL) and treated with pyridine (5.0 equiv., 0.08 mL, 1.0 mmol). The reaction was heated at 120 °C for 15 min. After complete consumption of the starting material, the reaction was transferred to a separatory funnel, diluted with EtOAc (20 mL) and washed with 1 N HCl (1 x 5 mL), water (2 x 5 mL) and brine (1 x 5 mL). The organics were dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give 3,6,2',3',4',6'-hexa-*O*-acetyl-D-lactosamine **235** (0.128 g, 0.183 mmol) in 87% yield, while 95% of the gluco-configured starting material was successfully converted to the desired product. The manno- configured starting material is more resistant to hydrolysis conditions requiring longer reaction times to fully convert to desired product. The spectral data was consistent with previously reported values.[153] HRMS (ESI) calcd for $C_{24}H_{33}N_3O_{16}$ $[M+Na]^+$, 642.1758, found 642.1744.

Methyl 3,4,6-tri-*O*-benzyl-2-deoxy-2-azido-1-D-galactoside (236)



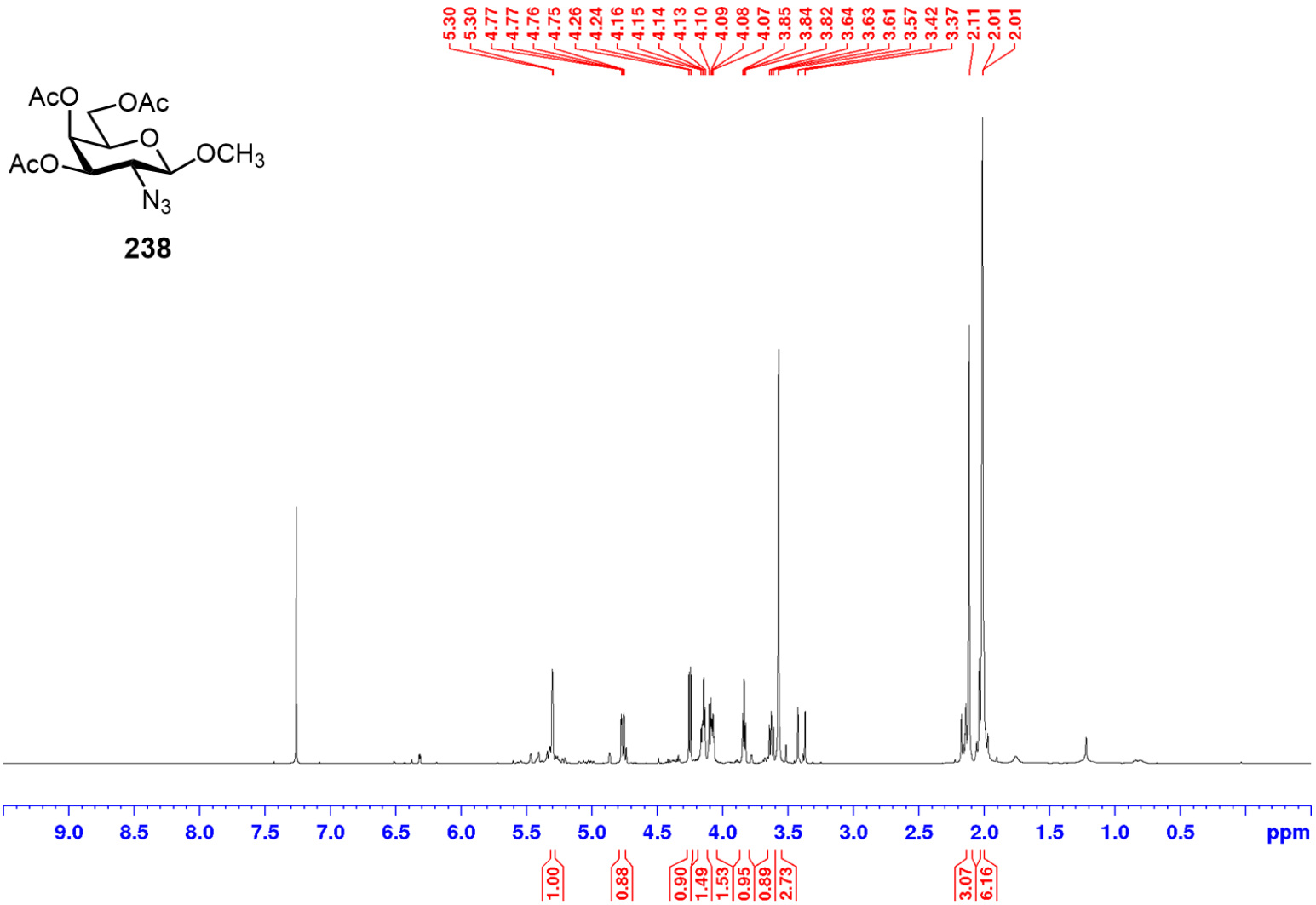
A solution of nitrate ester 3,4,6-tri-*O*-benzyl-2-deoxy-2-azido-1-α-D-galactoside **197** (1.0 equiv., 94.2 mg, 0.18 mmol) was dissolved in anhydrous CH_3OH (2.0 mL) and treated with pyridine (3.0 equiv., 0.07 mL, 0.90 mmol). The reaction mixture was heated at 100 °C for 15 min. After complete consumption of the starting material, the reaction was transferred to a separatory funnel, diluted with EtOAc (20 mL) and washed with 1 N HCl (1 x 5 mL), water (2 x 5 mL) and brine (1 x 5 mL). The organics were dried over $MgSO_4$, filtered, and concentrated *in vacuo*. Purification by flash chromatography (5:1 Hexanes/EtOAc) provided a 1:9 (alpha/beta) mixture of methyl D-galactoside **236** (62.0 mg, 0.127 mmol, 70%) as a colorless oil: R_f 0.60 (3:5 EtOAc/hexanes); $[\alpha]_D^{20}$ +2.02° (*c* 0.3, $CHCl_3$); IR (thin film, cm^{-1}) 3854.0, 2926.40, 2361.4, 2341.4, 2111.79, 1637.4, 1496.5, 1453.9, 1363.3, 1284.3, 1204.9, 1103.23, 1075.4, 735.8; ^1H NMR (600 MHz, $CDCl_3$): beta anomer δ 7.42-7.28 (m, 15H, CH Ar), 4.91-4.46 (m, 6H, 3 x CH_2OBn), 4.13 (d, 1H, *J* = 8.0, H-1), 3.92 (m, 1H, H-4), 3.83 (m, 1H, H-2), 3.63 (m, 2H, H-6a,6b), 3.55 (s, 3H), 3.53 (m, 1H), 3.36 (m, 1H, *J* = 2.6, 10.4, H-3); ^{13}C (100 MHz, $CDCl_3$): δ 138.3, 137.8, 137.6, 128.5-127.8, 103.1, 80.9, 74.6, 73.6, 73.5, 72.5, 72.1, 68.6, 63.3, 57.0; HR-Q-TOF/MS (*m/z*): cacld for $[M+Na]^+$, $C_{28}H_{31}N_3O_5$, 512.2161, found 512.2154.

Methyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-azido-1-D-galactoside (238)

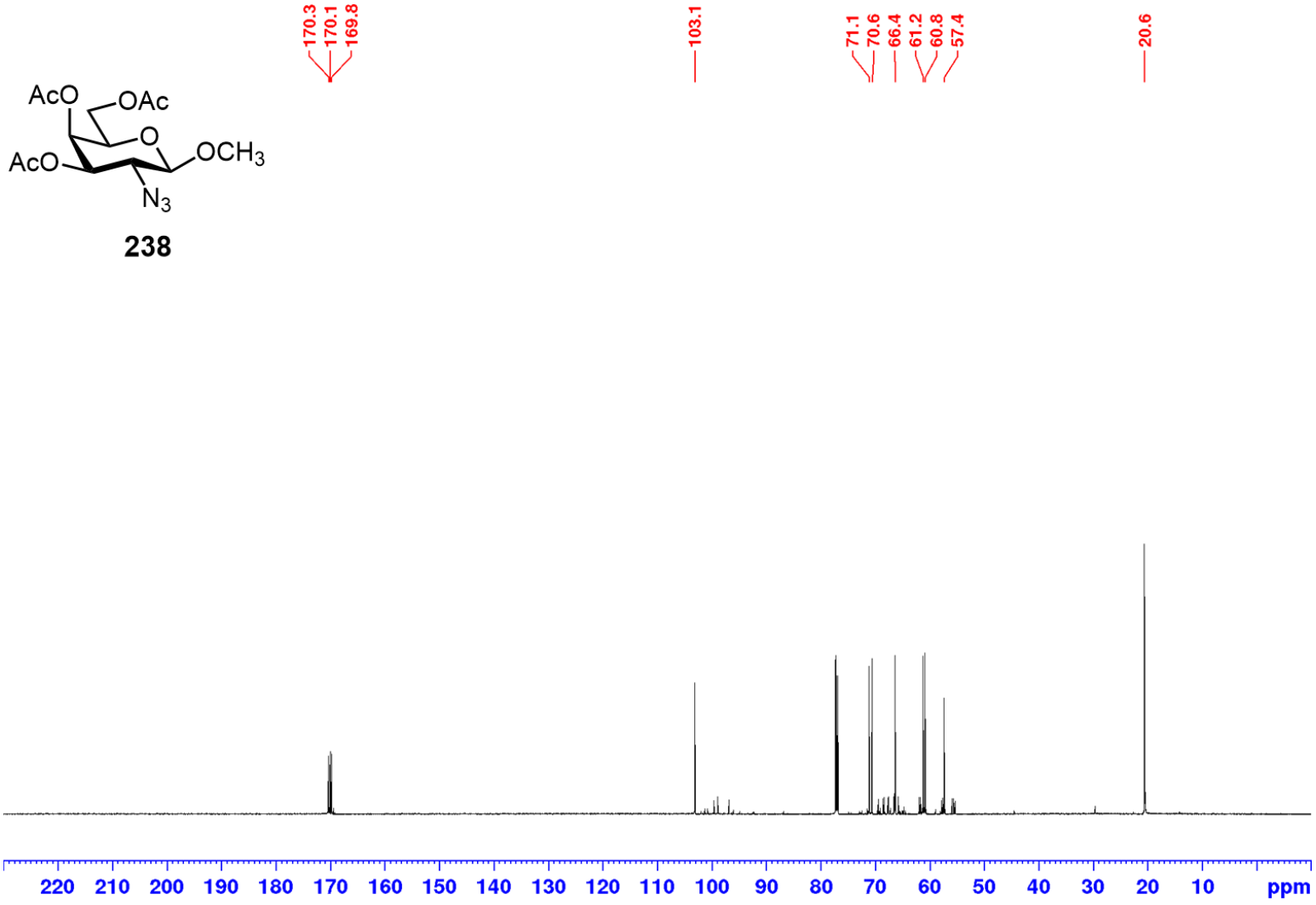


A solution of nitrate ester 3,4,6-tri-*O*-acetyl-2-deoxy-2-azido-1-α-D-galactoside **237** (1.0 equiv., 93.2 mg, 0.24 mmol) in anhydrous MeOH (2.0 mL) and treated with pyridine (5.0 equiv., 0.1 mL, 1.2 mmol). The reaction mixture was heated to 100 °C for 15 min. After complete consumption of the starting material, the reaction was transferred to a separatory funnel, diluted with EtOAc (20 mL) and washed with 1 N HCl (1 x 5 mL), water (2 x 5 mL) and brine (1 x 5 mL). The organics were dried over $MgSO_4$, filtered, and concentrated *in vacuo* to provide a 1.1:1.0 (alpha/beta) mixture of methyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-azido-1-D-galactoside **238** (858 mg, 0.24 mmol) in >95% yield as a colorless oil. The spectral data was consistent with previously reported values.[153] HR-Q-TOF/MS (*m/z*): cacld for $[M+Na]^+$, $C_{13}H_{19}N_3O_8$, 368.1070, found 368.1085.

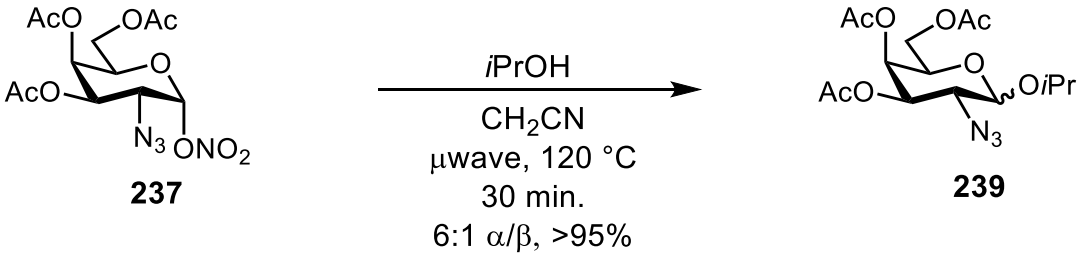
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 238



^{13}C NMR ($CDCl_3$, 100 MHz) of compound 238

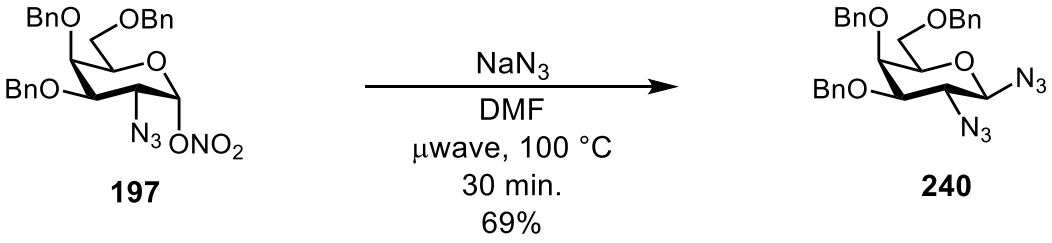


Isopropyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-azido-1-D-galactoside (239)



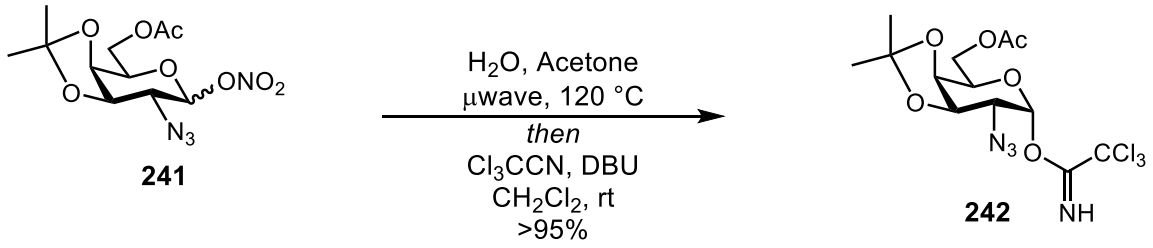
A solution of nitrate ester 3,4,6-tri-*O*-acetyl-2-deoxy-2-azido-1-α-D-galactoside **237** (1.0 equiv., 35.5 mg, 0.09 mmol) in anhydrous isopropanol (10 mL), diluted with CH_2Cl_2 (3 mL) and treated with pyridine (3.0 equiv., 0.02 mL, 0.27 mmol). The reaction mixture was heated to 120 °C for 30 min. After complete consumption of the starting material, the reaction was transferred to a separatory funnel, diluted with EtOAc (40 mL) and washed with 1 N HCl (1 x 5 mL), water (2 x 5 mL) and brine (1 x 5 mL). The crude product was purified by flash chromatography (5:1 Hexanes/ EtOAc) to give a 31:1 (alpha/beta) mixture of *iso*propyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-azido-1-D-galactoside **239** (0.7101 g, 1.91 mmol, >95%) as a colorless oil. The spectral data was consistent with previously reported values:[153] HR-Q-TOF/MS (*m/z*): cacld for $[M+Na]^+$, $C_{15}H_{23}N_3O_8$, 396.1383, found 396.1353.

Azido 3,4,6-tri-*O*-benzyl-2-deoxy-2-azido-1-D-galactoside (240)



A solution of nitrate ester 3,4,6-tri-*O*-benzyl-2-deoxy-2-azido-1-β-D-galactoside **197** (1.0 eq, 102.0 mg, 0.20 mmol) was dissolved in anhydrous DMF (2.0 mL) and treated with pyridine (2.5 equiv., 0.04 mL, 0.5 mmol). Sodium azide (11.8 equiv., 0.150 g, 2.3 mmol) was added and the reaction mixture heated to 100 °C for 30 min. After complete consumption of the starting material, the reaction was transferred to a separatory funnel, diluted with EtOAc (25 mL) and washed with 1 N HCl (1 x 5 mL), water (2 x 5 mL) and brine (1 x 5 mL). The crude product was purified by flash chromatography (9:1 Hexanes/EtOAc) to produce a azido 3,4,6-tri-*O*-benzyl-2-deoxy-2-azido-1-D-galactoside **240** as a single beta anomer (67.0 mg, 0.134 mmol, 69%) as a colorless oil: R_f 0.50 (1:3 EtOAc/hexanes); $[\alpha]_D^{20}$ -1.92° (*c* 0.3, $CHCl_3$); IR (thin film, cm^{-1}) 3350.9, 3030.7, 2920.5, 2870.23, 2111.90, 1727.8, 1604.7, 1496.2, 14504, 1306.9, 1260.55, 1152.9, 1103.4, 1027.6, 1004.5, 911.0, 763.3, 698.5; ^{1}H NMR (600 MHz, $CDCl_3$): beta anomer δ 7.40-7.28 (m, 15H, CH Ar), 4.90-4.43 (m, 6H, 3 x CH_2OBn), 4.43 (d, 1H, *J* = 8.9, H-1), 3.94 (d, 1H, *J* = 2.5 Hz, H-4), 3.74 (m, 1H, H-2), 3.62 (m, 3H, H-6a, H-6b,H-5), 3.39 (dd, *J* = 2.7, 10.2 Hz, 1H, H-3); ^{13}C (100 MHz, $CDCl_3$): δ 138.0, 137.5, 137.2, 128.5 – 127.8, 89.3, 81.0, 75.7, 74.6, 73.6, 72.4, 71.7, 68.2, 62.7; HR-Q-TOF/MS (*m/z*): cacld for $[M+Na]^+$, $C_{27}H_{28}N_6O_4$, 523.2069, found, 523.2029.

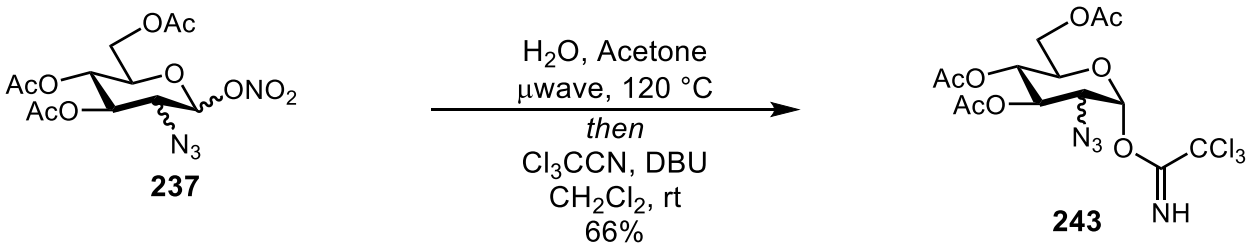
Trichloro acetamidine 3,4-*O*-isopropylidene-2-deoxy-2-azido-6-*O*-acetate-1-D-galactoside (242)



A solution of nitrate ester 3,4-*O*-isopropylidene-2-deoxy-2-azido-6-O-acetate-1-D-galactoside **241** (1.0 equiv., 0.150 g, 0.451 mmol) in a solution of 1:4 water/acetone (4.0 mL) and pyridine (5.0 equiv., 0.18 mL, 2.26 mmol) was heated in the microwave at 110 °C for 10 min. Then, the reaction was vial was concentrated under by a stream of air to reduce the solvent mixture to ~1/2 its initial volume. The crude reaction mixture was added CH_2Cl_2 (4.0 mL) and then added DBU (2.0 eq, 0.14 mL, 0.90 mmol), and 2,2,2-trichloroacetonitrile (10 eq, 0.45 mL, 4.51 mmol) at 0°C. At 30 min., the reaction was passed through a phase separator and concentrated to a crude dark brown oil. The Crude oil was passed through a plug of silica gel in a filter funnel and concentrated then purified by flash chromatography (2:5 EtOAc/hexanes) to give a 34:1 mixture (alpha/beta) trichloro acetamidine 3,4-*O*-isopropylidene-2-deoxy-2-azido-6-*O*-acetate-1-D-galactoside **242** (0.189 g, 0.438 mmol, >95%) as a yellow oil: R_f 0.26 (1:3 EtOAc/hexanes); $[\alpha]_D^{20}$ +6.63° (*c* 0.3, $CHCl_3$); IR (thin film, cm^{-1}) 3943.02, 3689.98, 3053.89, 2986.53, 2684.50, 2409.92, 2304.81, 2115.20, 1735.63, 1674.77, 1616.82, 1421.56, 1265.14, 741.47, 705.37; ^1H NMR (600 MHz, $CDCl_3$): alpha anomer δ 8.75 (s, 1H, NH), 6.34 (1H, d, *J* = 3.4 Hz, C1-H), 4.48 (dd, 1H, *J* = 5.5, 7.9), 4.44-4.24 (m, 4H),

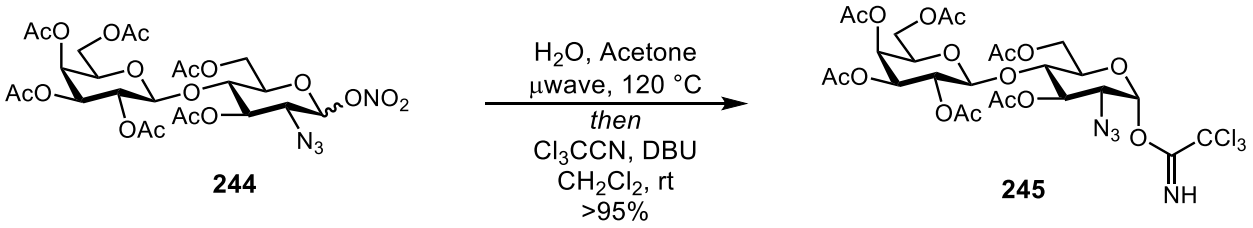
3.76 (dd, 1H, *J* = 3.4, 7.7, C2-H), 2.04 (s, 3H), 1.54 (s, 3H), 1.36 (s, 3H); ^{13}C (100 MHz, $CDCl_3$): δ 170.7, 160.5, 110.5, 94.3, 90.7, 73.4, 72.3, 68.1, 63.1, 60.1, 27.9, 26.0, 20.7; HR-Q-TOF/MS (*m/z*): cacld for $[M+Na]^+$, $C_{13}H_{17}Cl_3N_4O_6$, 453.0111, found 453.1277.

Trichloro acetamidine 3,4,6-tri-*O*-acetyl-2-deoxy-2-azido-1-D-glucoside (243)



A solution of a mixture of nitrate ester 3,4,6-tri-*O*-acetyl-2-deoxy-2-azido-1-D-glucoside 1:14.4:27.4 (beta gluco-/ alpha manno-/ alpha gluco-) **243** (1.0 equiv., 0.438 g, 1.164 mmol) in a solution of 1:4 water/acetone (10.0 mL) and pyridine (5.0 equiv., 0.47 mL, 5.82 mmol) was heated in the microwave at 120 °C for 20 min. Then, the reaction vial was concentrated under by a stream of air to reduce the initial amount of solvent mixture to ~1/2 of its initial volume. The crude reaction mixture was added CH_2Cl_2 (10.0 mL) and then added DBU (1.0 equiv., 0.18 mL, 0.1.164 mmol), and 2,2,2-trichloroacetonitrile (10 eq, 1.2 mL, 11.64 mmol) at 0 °C. At 1 h the reaction was a light orange and the pH of the was 7. Additional DBU (1.0 eq, 0.18 mL, 0.1.164 mmol) was added and the reaction quickly turned its characteristic dark brown color. At 3 h the reaction was passed through a phase separator and concentrated to a crude dark brown oil. The crude oil was purified by flash chromatography (20-2:5 EtOAc/hexanes) to give a 1:1.7 gluco-/manno-configured alpha imidate product mixture of imidate **243** (0.3758 g, 0.790 mmol, 68%) as a yellow oil. The spectral data was consistent with previously reported values. [153]

Trichloro acetamidine 3,6,2',3',4',6'-hexa-*O*-acetyl-D-lactosaminoside (245)

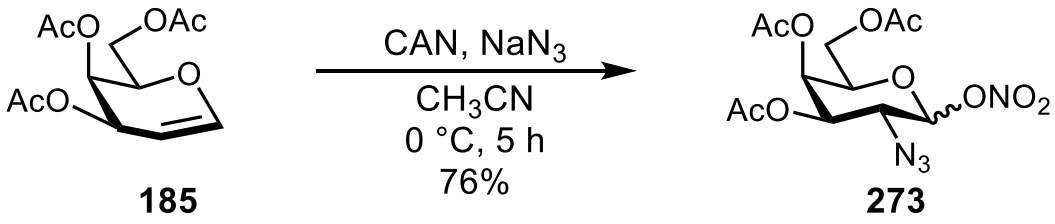


A solution of 1.0:2.4 (beta manno-/alpha gluco-) configured nitrate ester 3,6,2',3',4',6'-hexa-*O*-acetyl-1-D-lactosaminoside (1.0 equiv., 0.133 g, 0.20 mmol) in 20% aq. acetone (2.0 mL) and treated with pyridine (5.0 eq, 1.0 mmol, 0.08 mL). The reaction was heated by microwave irradiation to 120 °C for 10 min. Next, the reaction mixture was cooled to 0 °C and treated with CH_2Cl_2 (1.0 mL), DBU (10 eq, 0.3 mL, 1.9 mmol), and 2,2,2-trichloroacetonitrile (50 equiv., 1.0 mL, 10 mmol).[15] The reaction warmed to room temperature. After complete consumption of the starting material, the biphasic reaction mixture was filtered through a phase separator and concentrated *in vacuo*. The crude oil was purified by flash chromatography (10:3-1:1 hexanes/ EtOAc) to give a 5:1 (gluco-/manno-) mixture of alpha imidate products trichloro acetamidine 3,6,2',3',4',6'-hexa-O-acetyl-D-lactosaminoside **245** (0.133 g, 0.174 mmol, 87% overall). Under these reaction conditions the manno- configured starting material is more resistant to hydrolysis as previously mentioned. In this reaction, >95% of the gluco- configured starting material was successfully converted to alpha imidate **245** while 62% of the manno- configured starting material was converted to imidate product. The spectral data was consistent with previously reported values. [153]

Part 3. MM-ZPS 2nd-generation synthetic route

Part 3 contains experimental procedures for compounds found within schemes 31-33

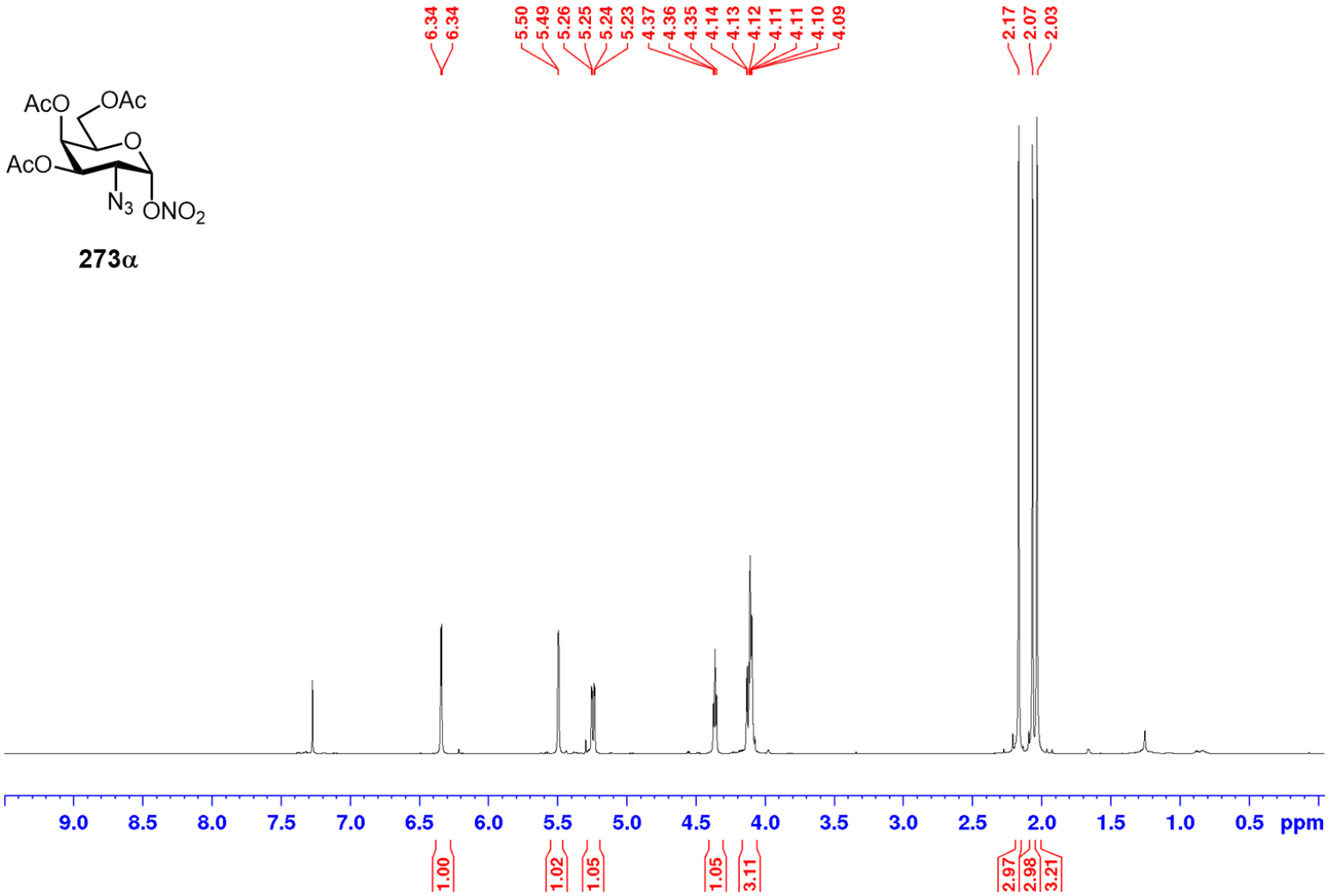
3,4,6-tri-*O*-acetyl-2-deoxy-2-azido-α/β-D-galactopyranosyl nitrate ester (273)



185 **273**

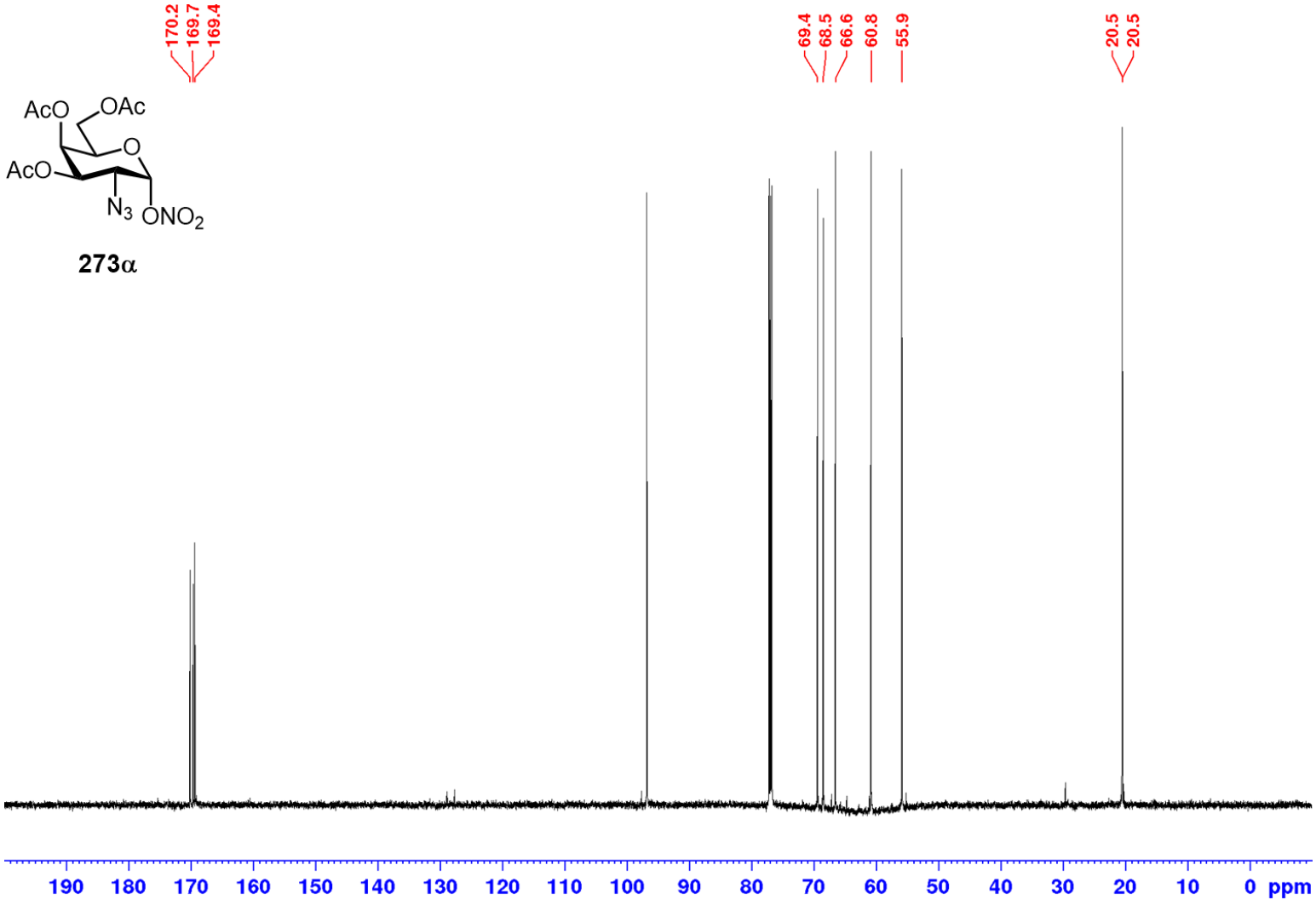
A solution of 3,4,6-tri-*O*-acetyl-D-galactal **185** (1.0 equiv., 5.00 g, 18.4 mmol) was diluted with anhydrous CH_3CN (92 mL) and cooled to 0 °C under an argon atmosphere. The mixture was added NaN_3 (1.6 equiv., 1.91 g, 29.4 mmol) followed by ceric ammonium nitrate (3.0 equiv., 30.2 g, 55.1 mmol). The reaction temperature was maintained at 0°C. At 8 h, the reaction was added water (50 mL) and EtOAc (50 mL) then transferred to a separatory funnel with additional EtOAc (250 mL). The organics were washed with water (4 x 50 mL), sat. aq. $NaHCO_3$ (2 x 50 mL), water (2 x 50 mL), brine (1 x 50 mL), dried ($NaSO_4$), filtered through a glass fritted funnel to remove drying agent. The filtrate was concentrated to a yellow oil. The crude oil was purified by silica gel flash column chromatography (30:70 to 40:60 EtOAc/hexanes) to give an inseparable mixture of anomers (1.0:0.85:0.23 alpha-gal/beta-gal/alpha-talo) of 3,4,6-tri-*O*-acetyl-2-deoxy-2-azido-α/β-D-galactopyranosyl nitrate ester **273** (5.326 g, 14.386 mmol, 76%) as a yellow solid. Our characterization data were consistent with previously reported data.[313]

^{1}H NMR ($CDCl_3$, 400 MHz) of compound 273

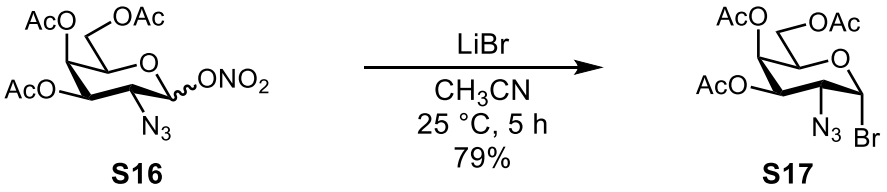


273α

^{13}C NMR ($CDCl_3$, 100 MHz) of compound 273



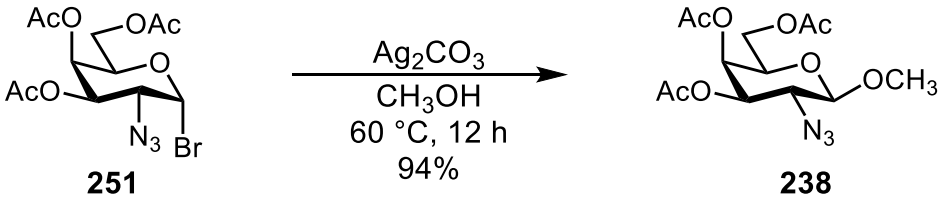
3,4,6-tri-*O*-acetyl-2-deoxy-2-azido-α-bromo-D-galactopyranoside (S17)



S16 **S17**

A solution of anomeric mixture of nitrate 3,4,6-tri-*O*-acetyl-2-deoxy-2-azido-α/β-D-galactoside mixture **S16** (1.0 equiv., 10.7 g, 28.4 mmol) in anhydrous CH_3CN (95 mL) at room temperature under an argon atmosphere was added LiBr (10.0 equiv., 24.7 g, 284 mmol). The reaction was stirred at room temperature. After 3 h, TLC indicated full conversion of starting material. The reaction mixture was added EtOAc (300 mL) then transferred to a separatory funnel. The organics were washed with water (4 x 50 mL), sat. aq. $NaHCO_3$ (2 x 50 mL), water (2 x 50 mL), brine (1 x 50 mL), dried ($NaSO_4$), filtered through a glass fritted vacuum filter funnel. The filtrate was concentrated to a white foam to give a crude mixture of material containing bromo 3,4,6-tri-*O*-acetyl-2-deoxy-2-azido-α-D-galactopyranoside **S17** (8.89 g, 22.6 mmol, 79%, ^1H NMR yield). Our spectroscopic data agrees with previously reported data.[313]

Methyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-azido-β-D-galactopyranoside (238)

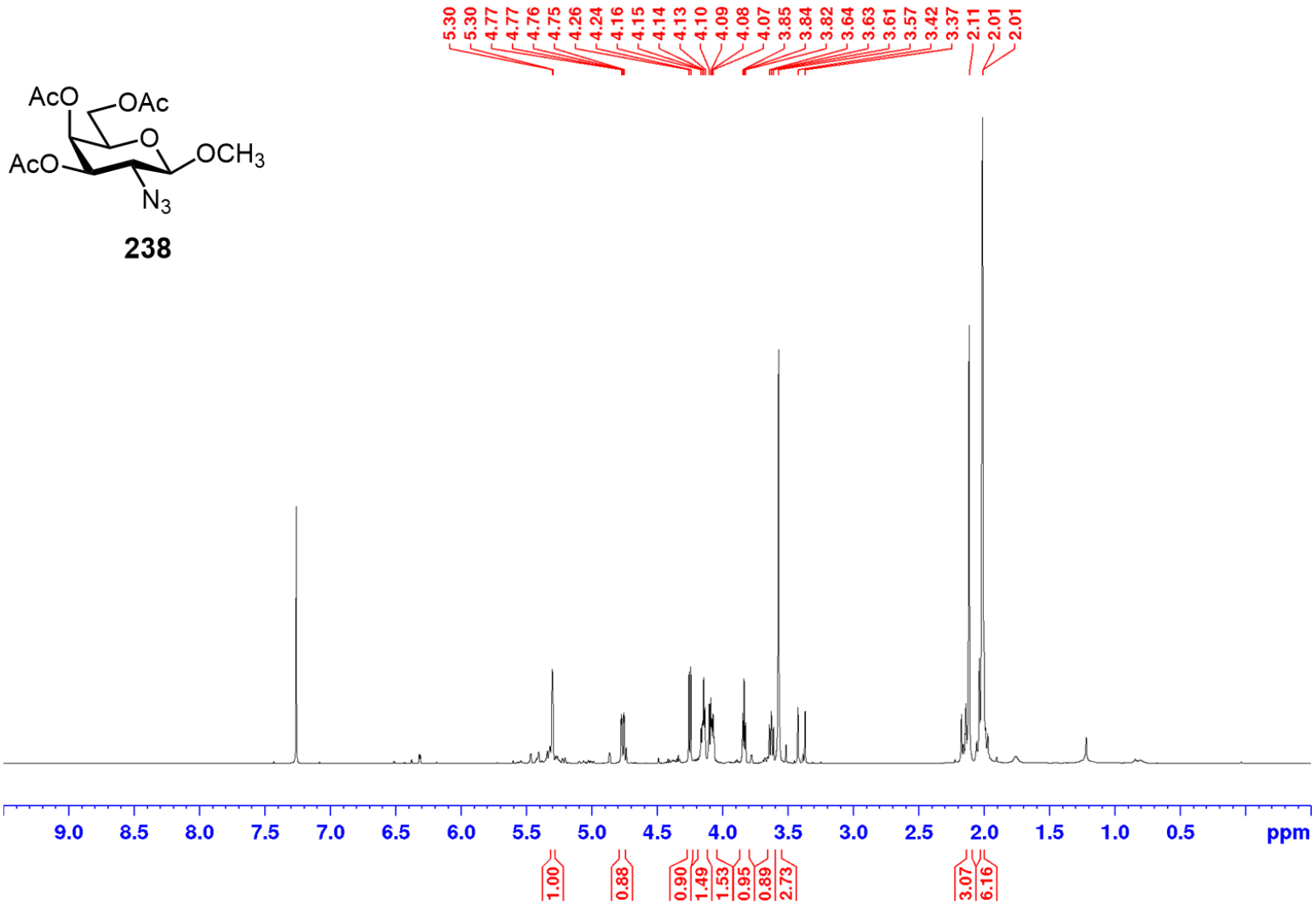


251 **238**

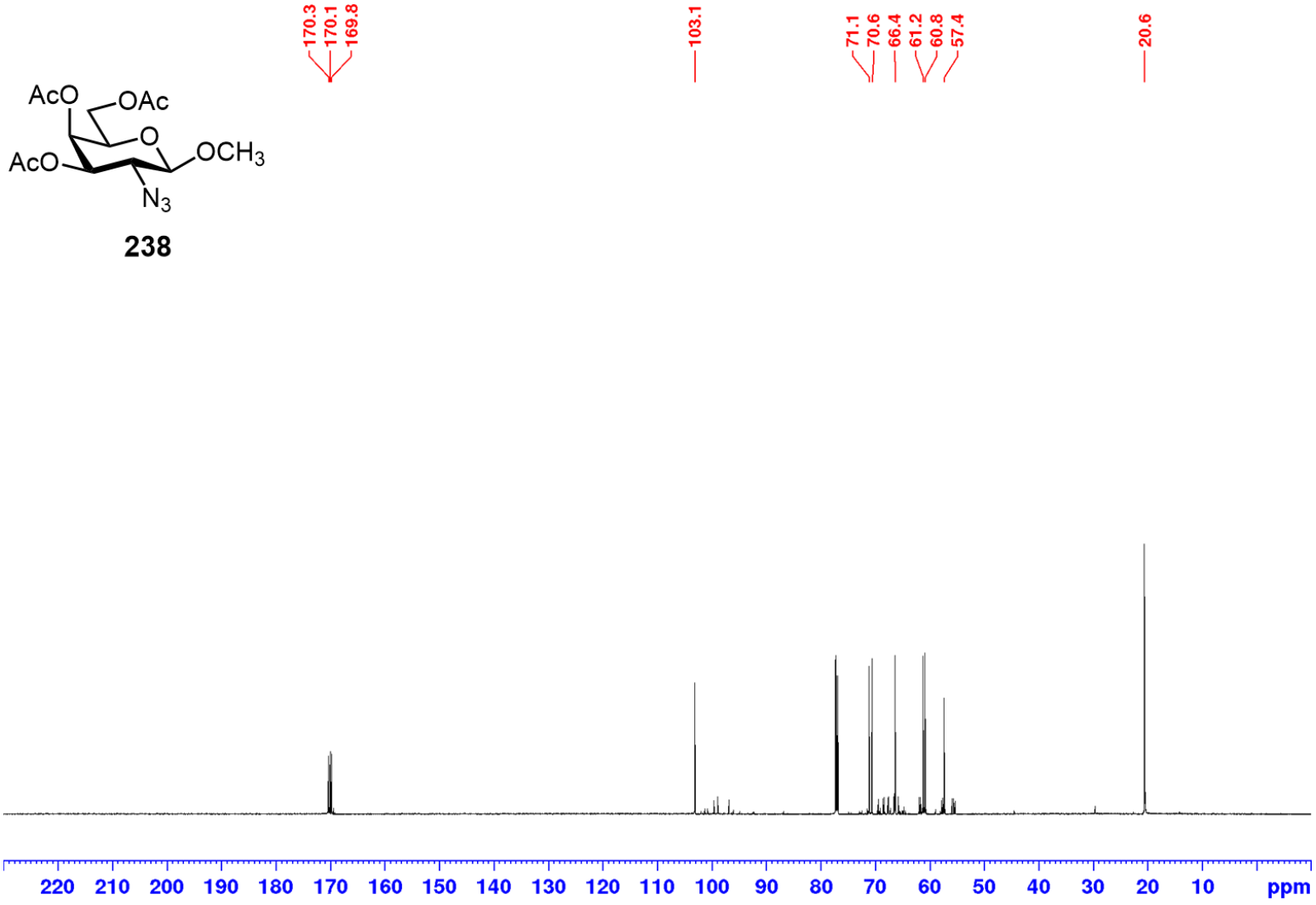
A solution of bromo 3,4,6-tri-*O*-acetyl-2-deoxy-2-azido-α-D-galactopyranoside **251** (1.0 equiv., 11.28 g, 28.4 mmol) in anhydrous CH_3OH (15 mL) was added Ag_2CO_3 (1.5 equiv., 11.7 g, 42.6 mmol) at room temperature, in the dark, under an argon atmosphere. The reaction mixture was then heated to 60 °C. At 12 h, TLC indicated full conversion of starting material. The reaction mixture was added EtOAc (100 mL) then transferred to a separatory funnel. The organics were washed with water (4 x 50 mL), sat. aq. $NaHCO_3$ (2 x 50 mL), 40% solution of sodium thiosulfate (2 x 50), water (2 x 50 mL), brine (1 x 50 mL), dried ($NaSO_4$) and filtered through a glass fritted vacuum filter funnel. The filtrate was concentrated to a white foam to give a crude solid, which was purified by silica gel flash column chromatography (20:80 to 30:70 EtOAc/hexanes) to give methyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-azido-β-D-galactopyranoside **238** (9.25 g, 26.8 mmol, 94%) as a white solid. Our spectroscopic data agrees with previously reported data.[300]

^{1}H NMR (400 MHz, $CDCl_3$): δ 5.3 (d, *J* = 0.1 Hz, 1H), 4.77 (dd, *J* = 3.1, 10.9 Hz, 1H), 4.25 (d, *J* = 8.1 Hz, 1H), 4.16-4.13 (m, 1H), 4.10-4.07 (m, 1H), 3.85-3.82 (m, 1H), 3.64-3.61 (m, 1H), 3.57 (s, 3H), 2.11 (s, 3H), 2.02 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.4, 170.1, 169.8, 103.1, 71.1, 70.6, 66.4, 61.2, 60.8, 57.4, 20.6.

^{1}H NMR ($CDCl_3$, 400 MHz) of compound 238

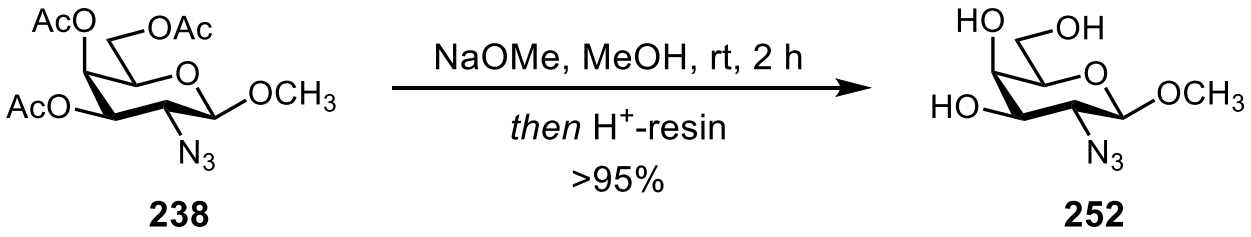


^{13}C NMR ($CDCl_3$, 100 MHz) of compound 238



238

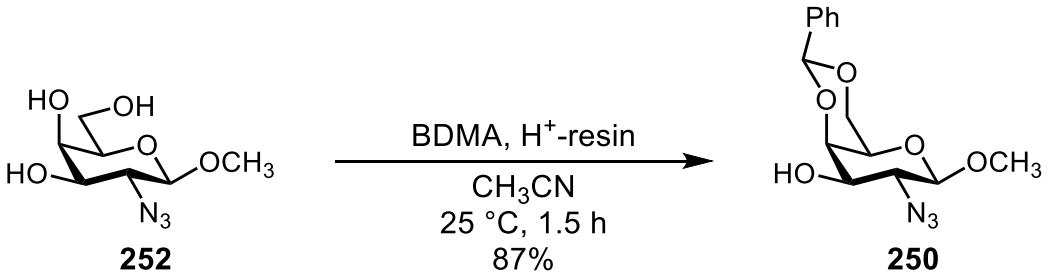
Methyl 2-deoxy-2-azido-β-D-galactopyranoside (252)



238 **252**

A solution of methyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-azido-β-D-galactopyranoside **238** (1.0 equiv., 5.36 g, 13.6 mmol) in anhydrous CH_3OH (75 mL) was treated with a freshly made solution sodium methoxide (1.0 equiv., 6.8 mL, 13.6 mmol, [2 N] in anhydrous CH_3OH) at room temperature under an argon atmosphere. The pH of the solution was >11. At 2 h, the reaction was neutralized by the addition of acidic Amberlite IR 120 (H^+) ion exchange resin. The solution was filtered through a glass fritted vacuum filter funnel equipped with a pad of Celite to remove the acidic resin. The filtrate was concentrated to dryness to give a crude triol. The crude material was passed through a plug of silica (40:60 to 50:50 EtOAc/hexanes) to give methyl 2-deoxy-2-azido-β-D-galactopyranoside **252** (4.52 g, 13.1 mmol, >95%) as a white amorphous solid without any need of further purification. Spectroscopic data matches previously reported data.[300, 318]

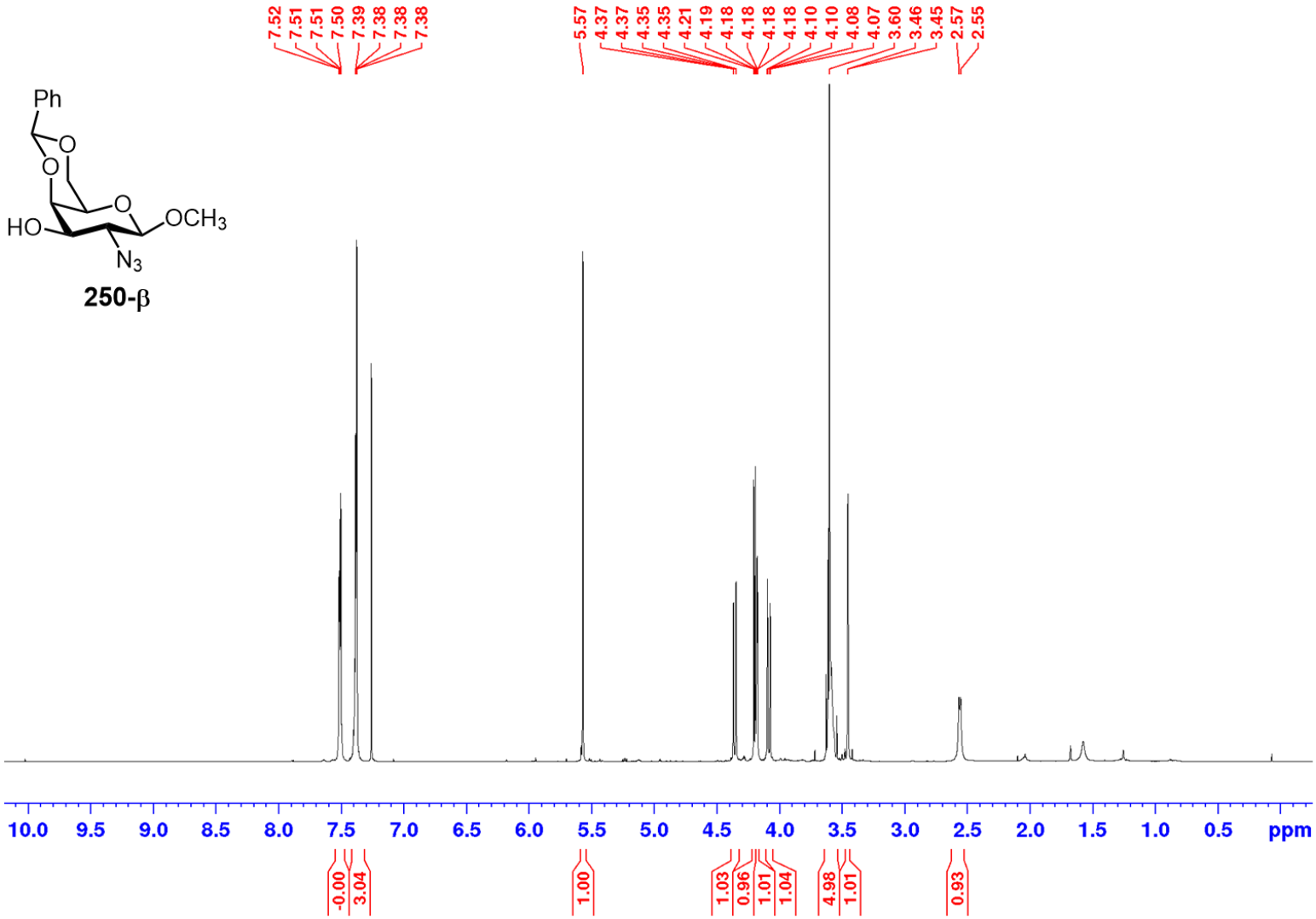
Methyl 4,6-*O*-benzylidene acetal-2-deoxy-2-azido-β-D-galactopyranoside (250)



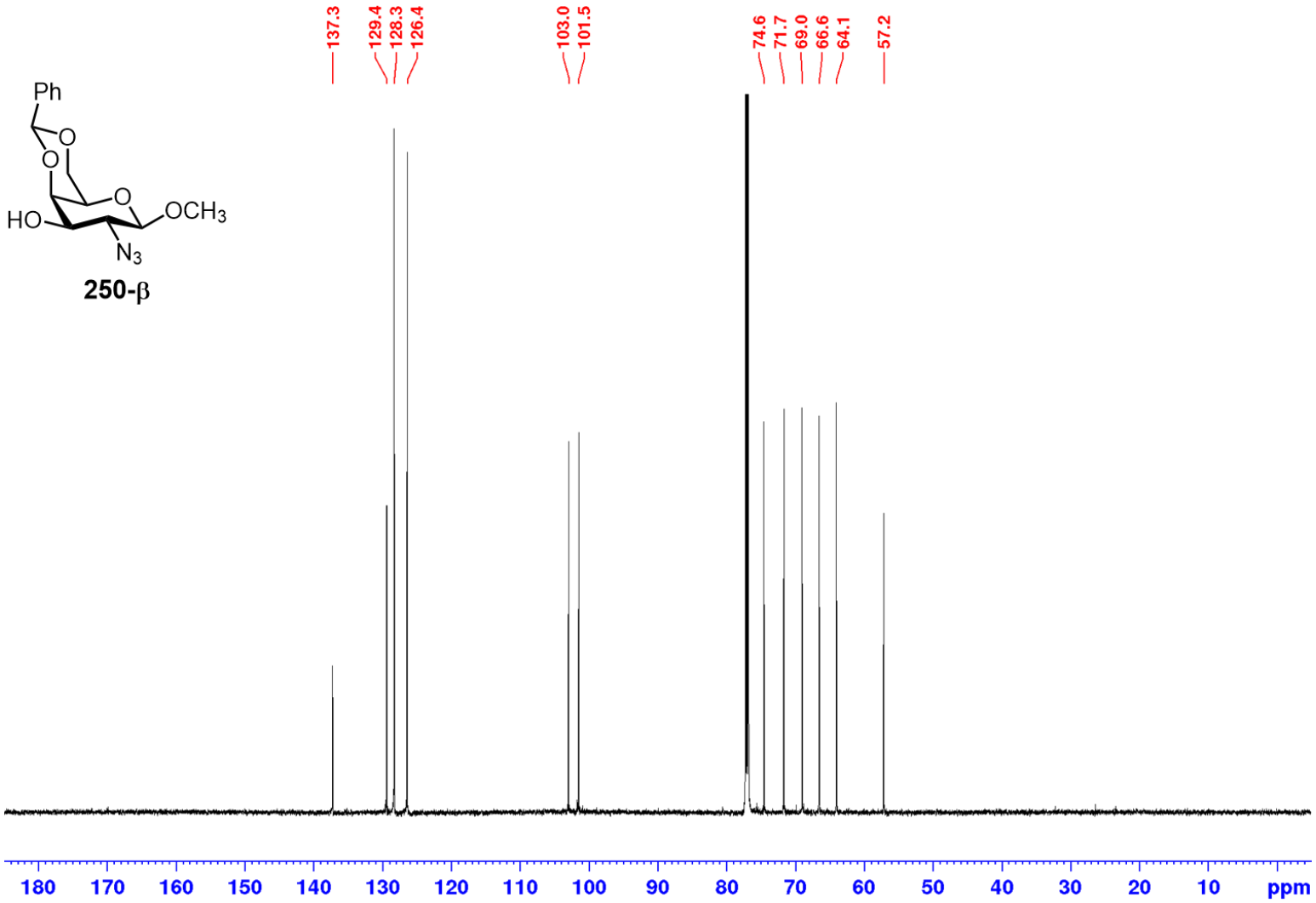
A solution of methyl 2-deoxy-2-azido-β-D-galactopyranoside **252** (1.0 equiv., 8.81 g, 40.2 mmol) and acidic Amberlite IR 120 (H^+) ion exchange resin in anhydrous CH_3CN (150 mL) was added benzaldehyde dimethyl acetal (1.2 equiv., 7.27 mL, 48.2 mmol) and stirred at room temperature under an argon atmosphere. At 18 h, the reaction was filtered through a glass fritted vacuum funnel equipped with a pad of Celite to remove the acidic resin. The filtrate was neutralized with Et_3N and concentrated to dryness. The reaction was purified by silica gel flash chromatography (50:50 to 70:30 EtOAc/hexanes) to give methyl 4,6-*O*-benzylidene acetal-2-deoxy-2-azido-β-D-galactopyranoside **250** (10.82 g, 35.12 mmol, 87%) was a white amorphous solid. Spectroscopic data matches previously reported data.[300, 319]

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.52-7.50 (m, 2H), 7.39-7.38 (m, 3H), 5.57 (s, 1H), 4.36 (dd, *J* = 1.3, 12.4 Hz, 1H), 4.21 (d, *J* = 7.5 Hz, 1H), 4.18 (dd, *J* = 0.8, 3.4 Hz, 1H), 4.08 (dd, *J* = 1.7, 12.6 Hz, 1H), 3.62-3.54 (m, 5H), 3.46 (d, *J* = 1.1 Hz, 1H), 2.56 (-OH, *J* = 7.8 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 137.3, 129.4, 128.3, 126.4, 103.0, 101.5, 74.6, 71.7, 69.0, 66.6, 64.1, 57.2.

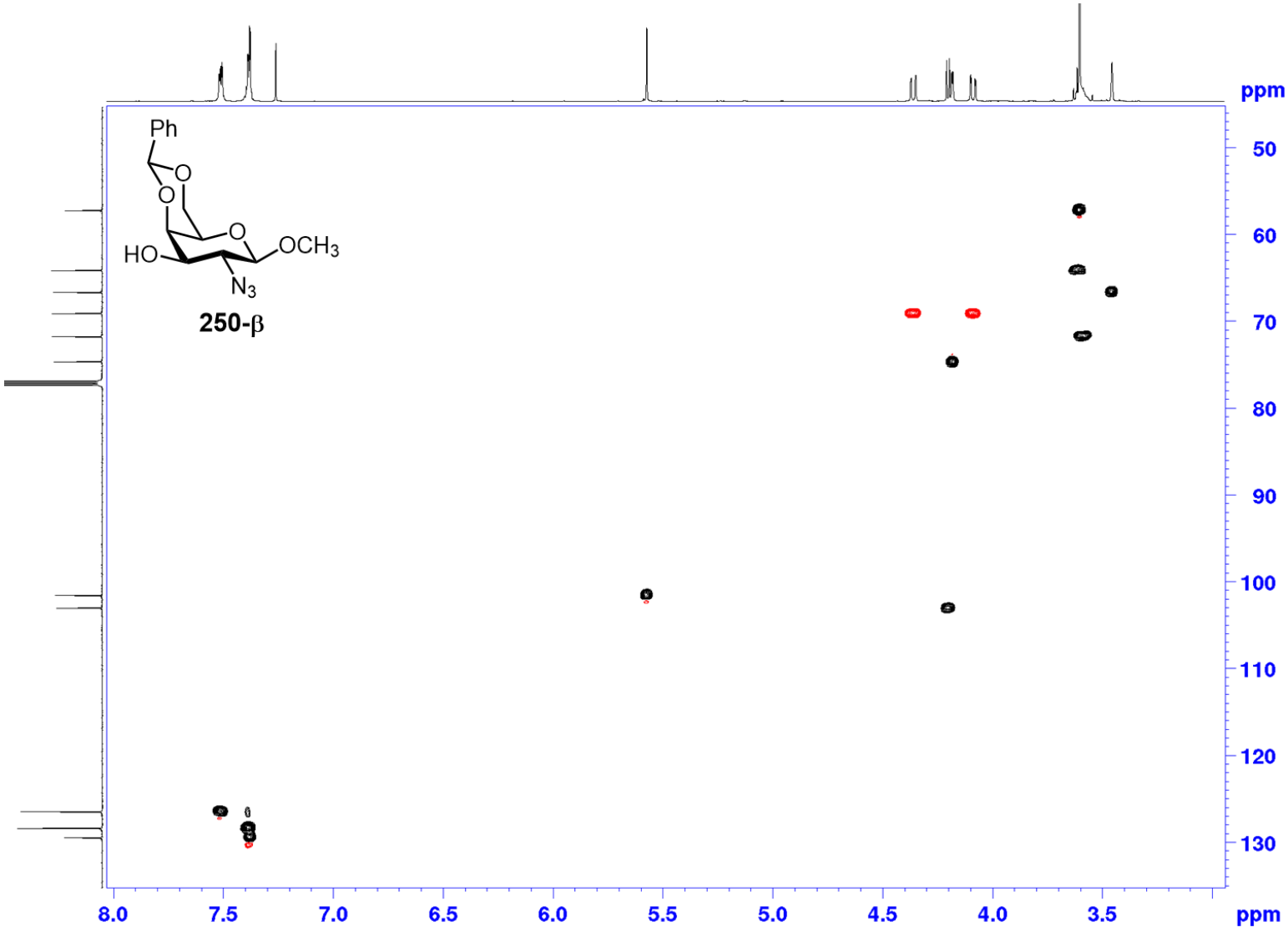
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 250-β



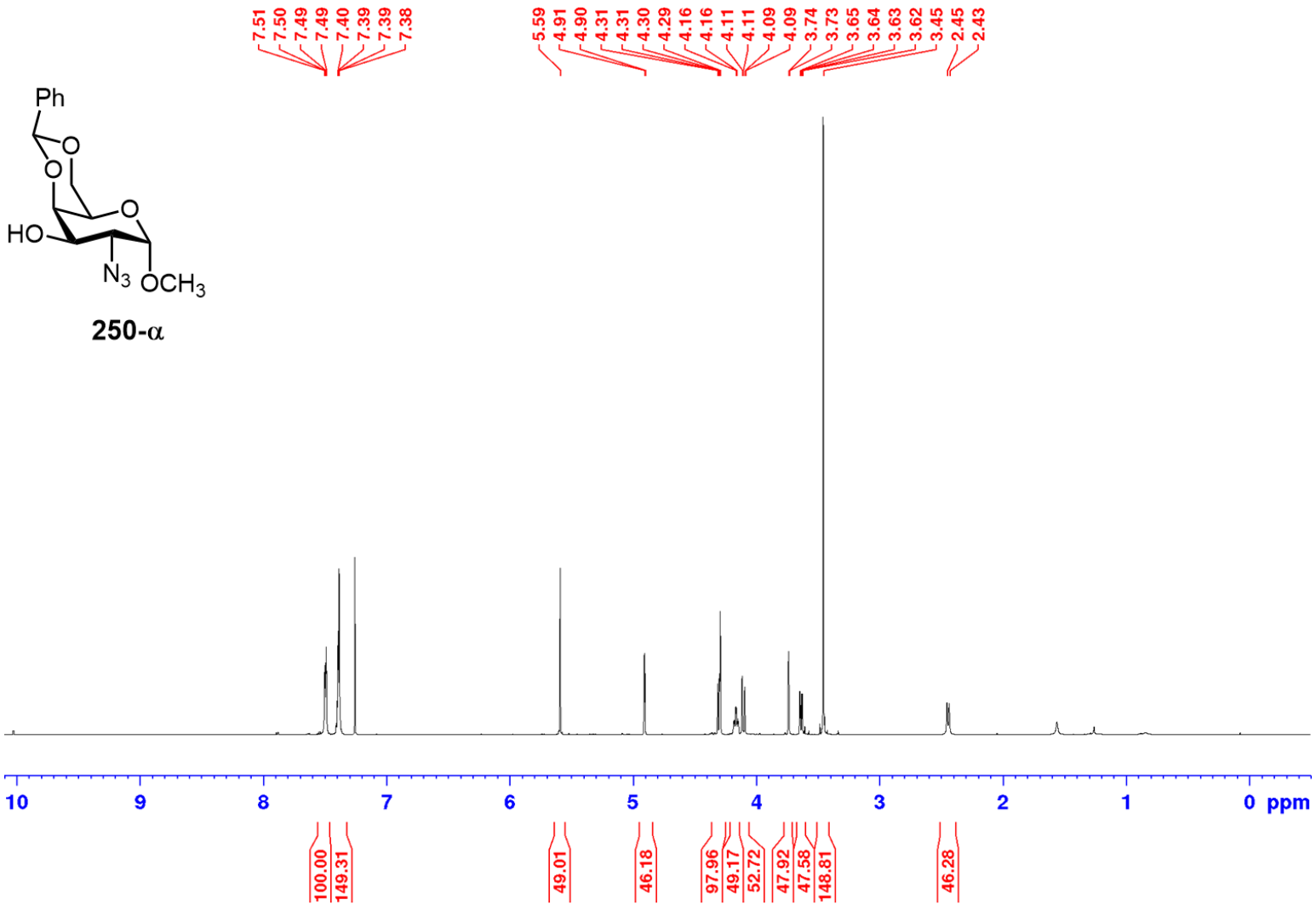
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 250-β



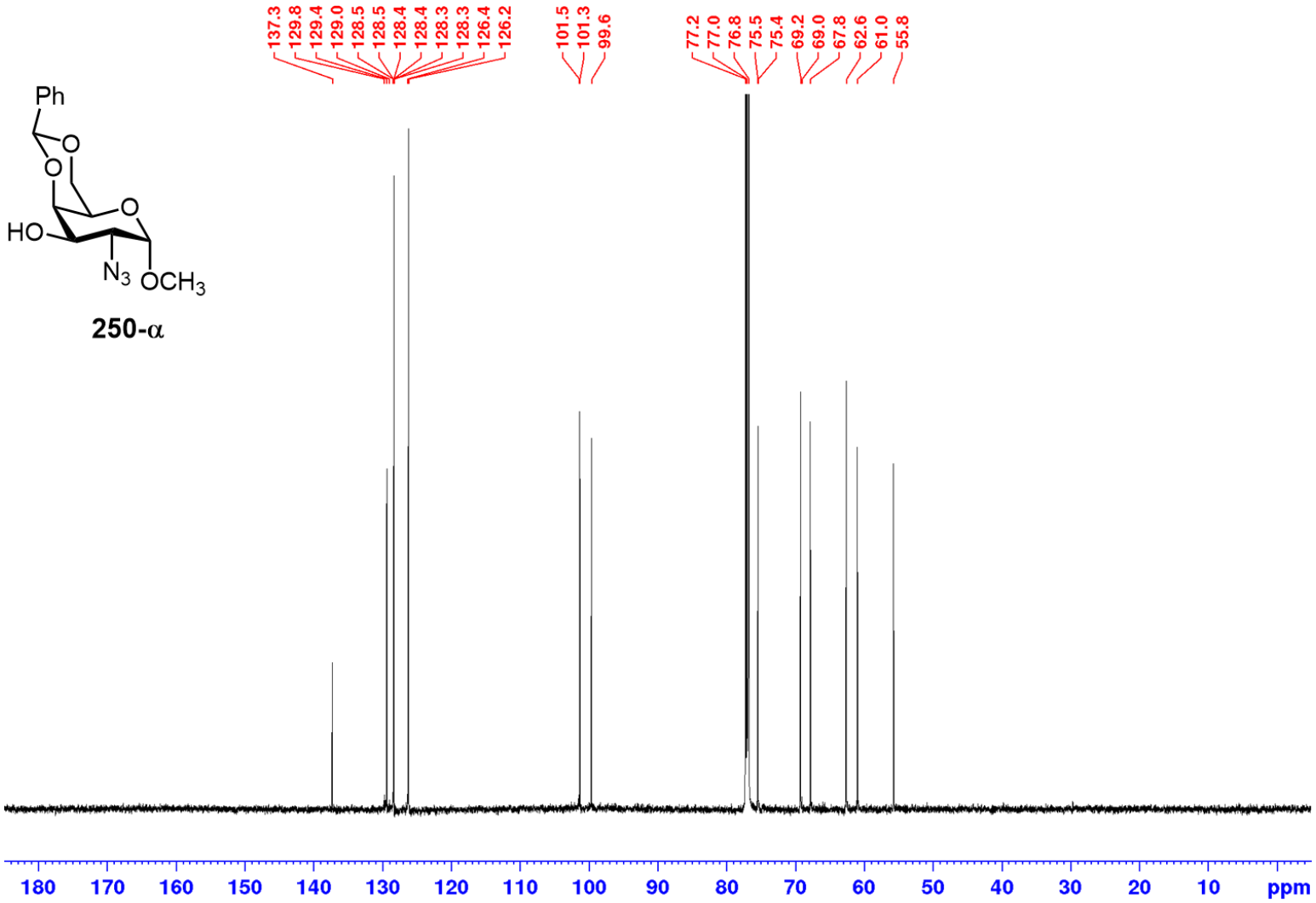
^{1}H-^{13}C HSCQ ($CDCl_3$) of compound 250-β



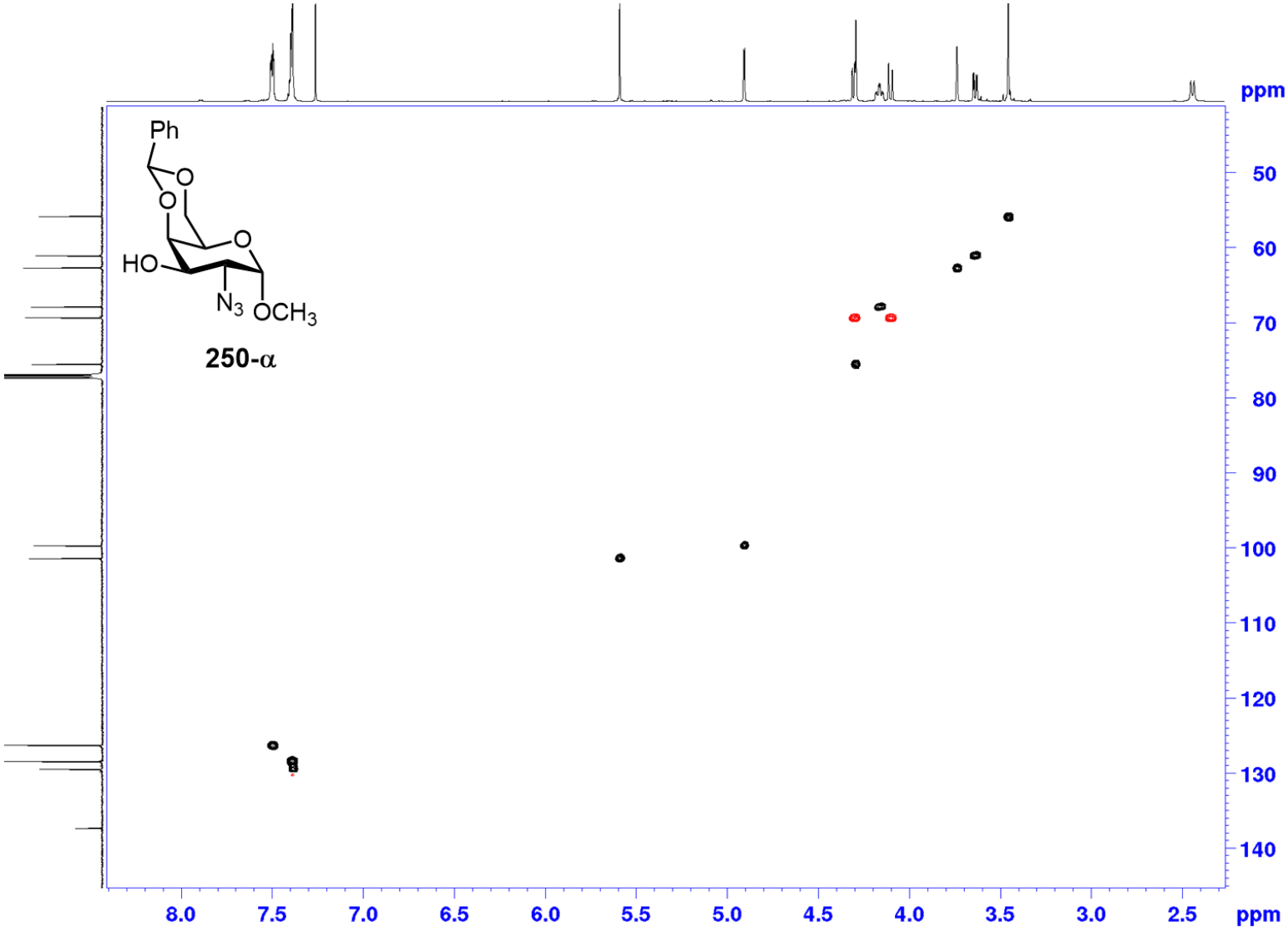
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 250-α



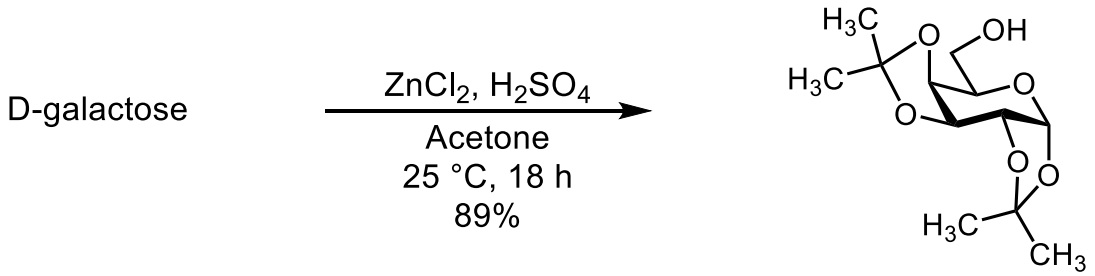
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 250-α



^{1}H-^{13}C HSCQ ($CDCl_3$) of compound 250-α



1,2:3,4-*O*-Di-isopropylidene-α-D-galactopyranoside (253)

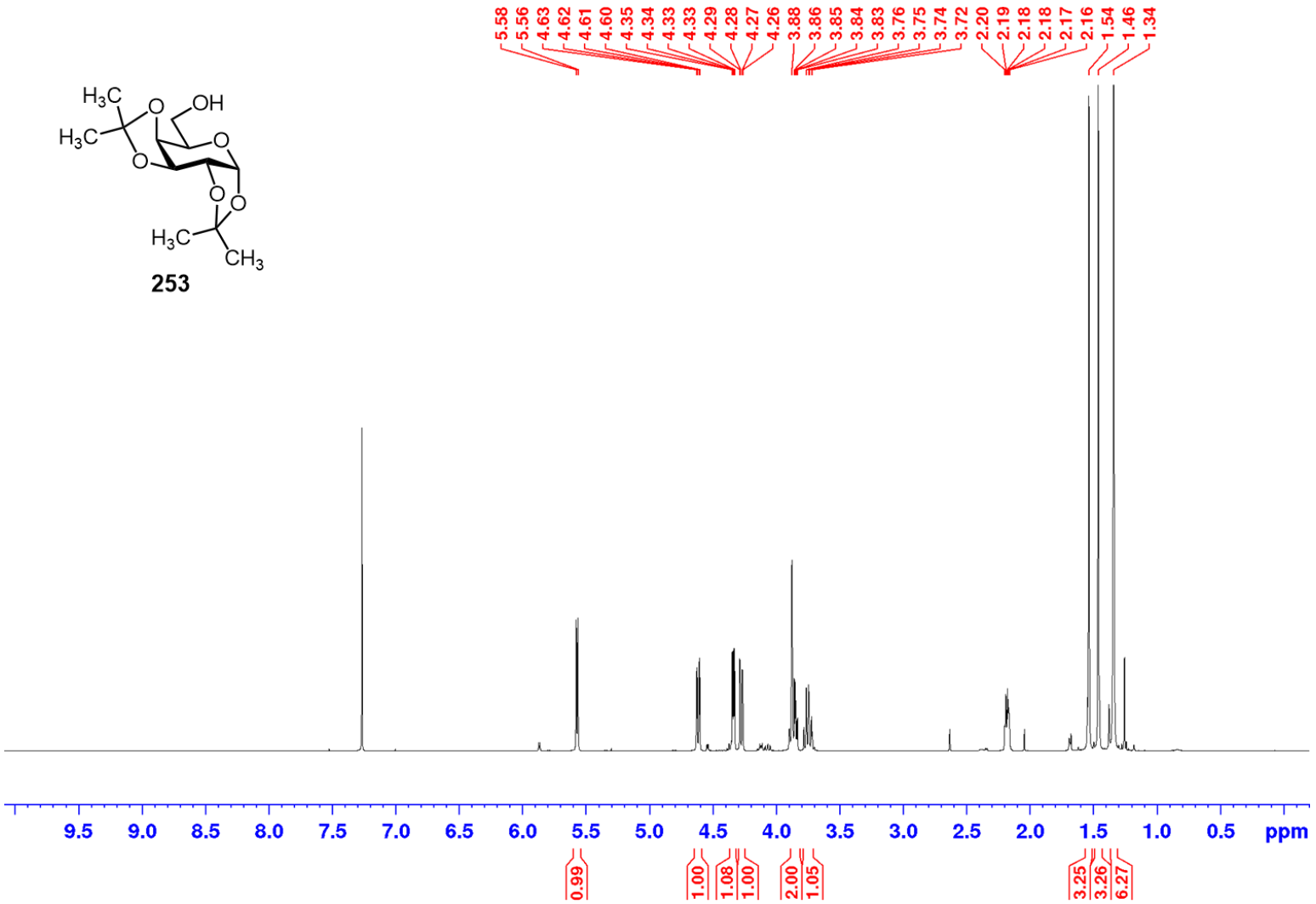


253

A suspension of D-galactose (1.0 equiv., 10.0 g, 56.0 mmol) in acetone (200 mL) with $ZnCl_2$ (1.0 equiv., 7.56 g, 56.0 mmol) was added concentrated H_2SO_4 (0.06 equiv., 0.20 mL, 3.74 mmol) at room temperature under an argon atmosphere. At 18 h, the reaction mixture was added solid potassium carbonate (1.0 equiv., 5.00 g, 36.2 mmol). After 30 minutes, the reaction was filtered through a glass fritted vacuum filter funnel equipped with a pad of Celite, and rinsed with acetone, to remove the solids from the reaction mixture. The filtrate was concentrated to an oil. The oil was transferred to a separatory funnel with EtOAc (400 mL) and washed with water (1 x 50 mL), sat. aq. $NaHCO_3$ (3 x 50 mL) and brine (1 x 50 mL). The organics where separated, dried ($MgSO_4$), filtered through a glass fritted vacuum filter funnel to remove drying agent. The filtrate was concentrated and co-evaporated with toluene (3x). The colorless oil was pure 1,2:3,4-*O*-di-isopropylidene-α-D-galactopyranoside **253** (13.0 g, 49.0 mmol, 89%) which was of sufficient purity to be used in the next step without any further purification required. Our characterization data were consistent with previously reported data.[320]

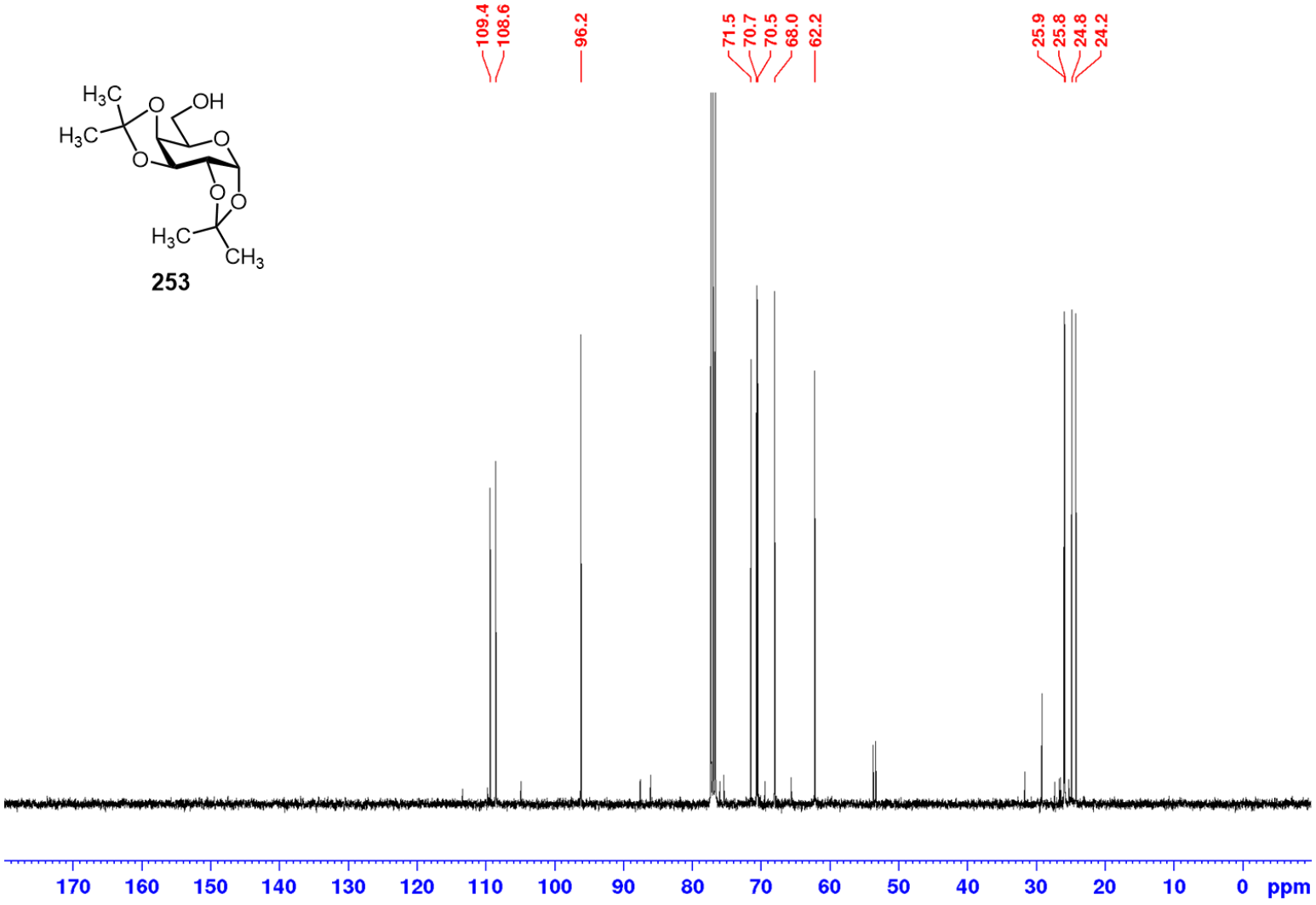
^{1}H NMR (400 MHz, $CDCl_3$): δ 5.57 (d, *J* = 5.1 Hz, 1H), 4.62 (dd, *J* = 2.4, 7.9 Hz, 1H), 4.34 (dd, *J* = 2.4, 4.99 Hz, 1H), 4.27 (dd, *J* = 1.5, 7.9 Hz, 1H), 3.89-3.82 (m, 2H), 3.78-3.72 (m, 1H), 1.54 (s, 3H), 1.46 (s, 3H), 1.34 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 109.4, 108.6, 96.2, 71.5, 70.7, 70.5, 68.0, 62.2, 25.9, 25.8, 24.8, 24.2.

^{1}H NMR ($CDCl_3$, 400 MHz) of compound 253

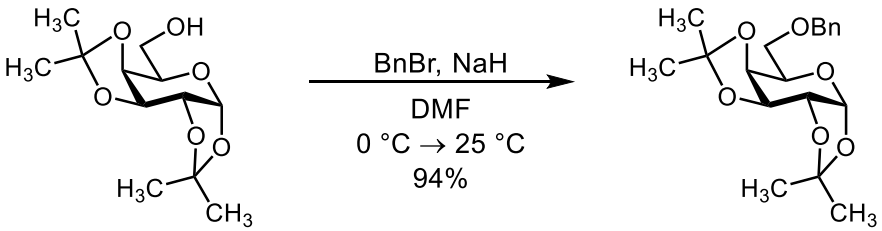


253

^{13}C NMR ($CDCl_3$, 100 MHz) of compound 253



1,2:3,4-Di-*O*-isopropylidene-6-*O*-benzyl-D-galactopyranoside (254)

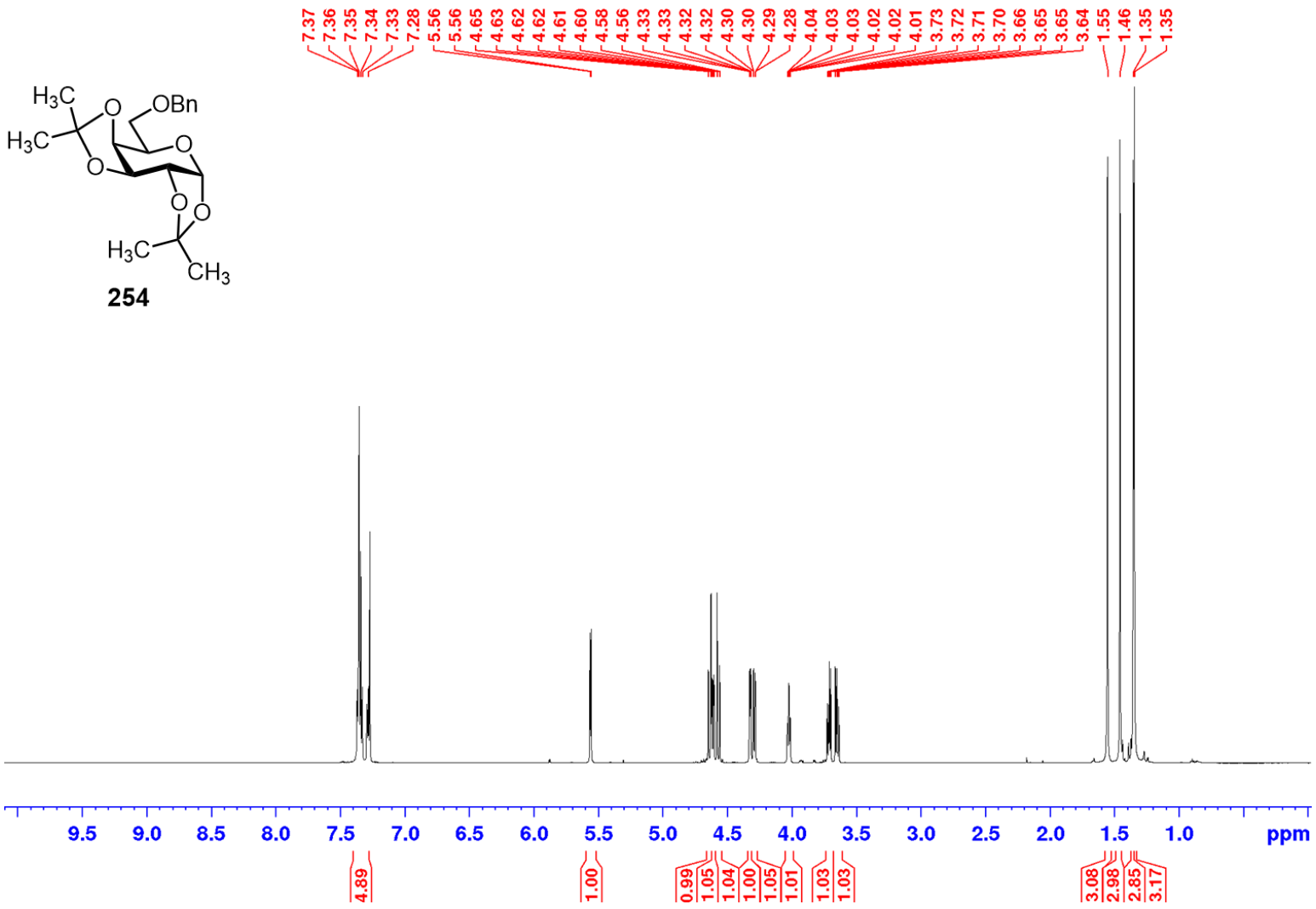


253 **254**

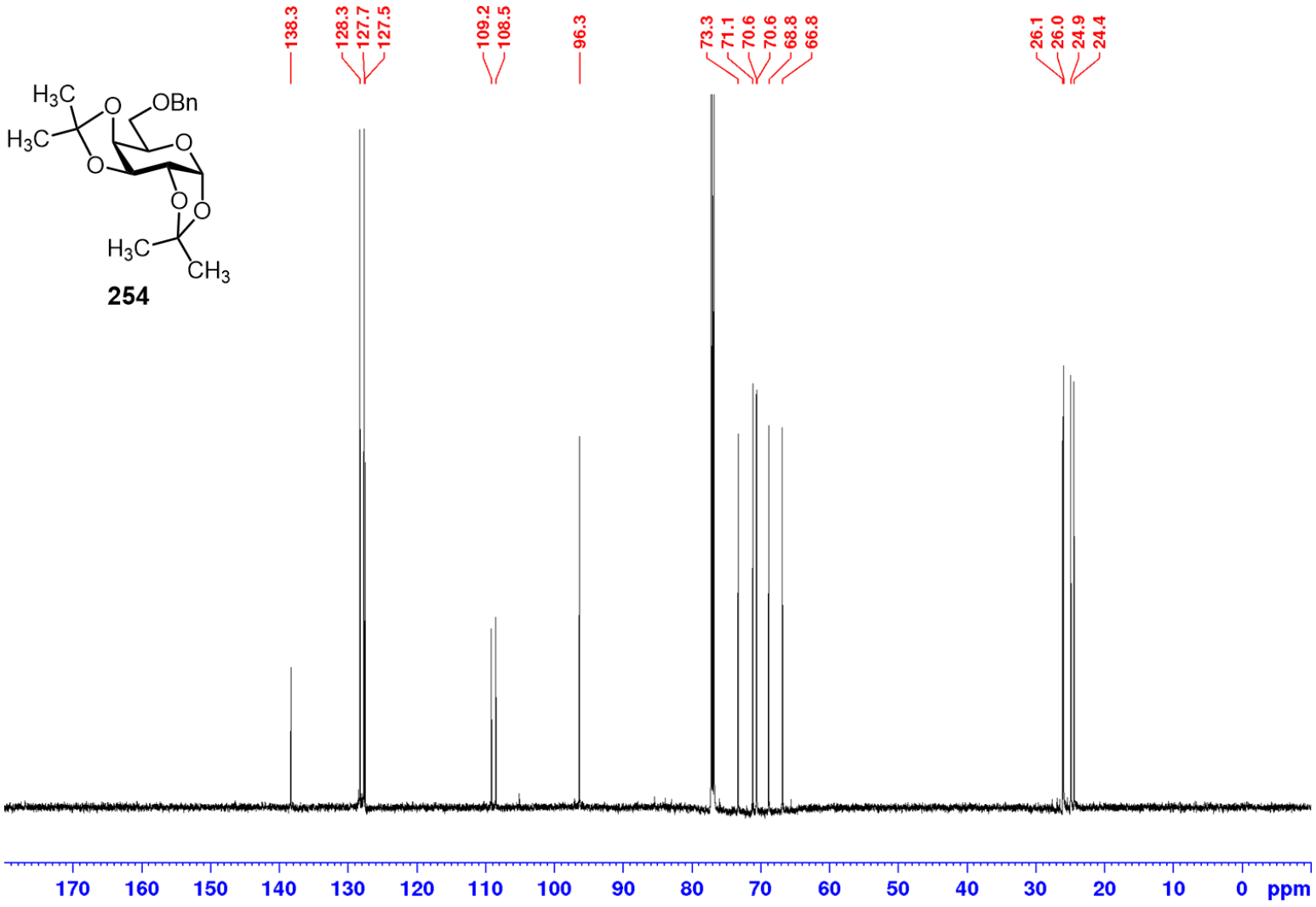
A solution of 1,2:3,4-*O*-di-isopropylidene-α-D-galactopyranoside **253** (1.0 equiv., 14.45 g, 55.50 mmol) in anhydrous DMF (185 mL) was treated with NaH (2.0 equiv., 4.40 g, 111 mmol, 60% in mineral oil) at room temperature under a constant stream of argon atmosphere which was allowed to flow through a vent needle to allow for the expulsion of H_2 gas generated from the reaction. After 30 minutes, the vent needle was removed, the reaction mixture was cooled to 0 °C and the reaction was added benzyl bromide (1.5 equiv., 9.90 mL, 83.0 mmol) dropwise. The reaction was stirred at room temperature. At 18 hours, the reaction was cooled to 0 °C, placed under a constant stream of argon and added a vent needle. The reaction was then quenched by the slow addition of water (s). When gas evolution was complete, the reaction was transferred to a separatory funnel with EtOAc (400 mL) and washed with 2 N HCl (3 x 100 mL), 40% solution of sodium thiosulfate (2 x 100 mL), water (2 x 50 mL), and brine (1 x 50 mL). The organics where separated, dried ($NaSO_4$), filtered through a glass fritted vacuum filter funnel to remove drying agent. The filtrate was concentrated to an orange oil which was purified by silica gel flash column chromatography (10:90 to 25:75 EtOAc/hexanes) to give 6-*O*-benzyl-1,2:3,4-di-isopropylidene-D-galactopyranoside **254** (18.70 g, 53.37 mmol, 96%) as a clear oil. Our characterization data were consistent with previously reported data.[321-326]

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.37-7.28 (m, 5H), 5.56 (d, *J* = 4.9 Hz, 1H), 4.64 (d, *J* = 12.2 Hz, 1H), 4.61 (dd, *J* = 2.3, 7.9 Hz, 1H), 4.57 (d, *J* = 12.1 Hz, 1H), 4.33 (dd, *J* = 2.4, 5.1 Hz, 1H), 4.29 (dd, *J* = 1.8, 7.9 Hz, 1H), 4.02 (ddd, *J* = 1.6, 6.6, 7.6 Hz, 1H), 3.71 (dd, *J* = 5.9, 10.5 Hz, 1H), 3.65 (dd, *J* = 6.7, 9.7 Hz, 1H), 1.55 (s, 3H), 1.47 (s, 3H), 1.36 (s, 3H), 1.34 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 138.3, 128.3, 127.7, 127.5, 109.2, 108.5, 96.3, 73.3, 71.2, 70.6, 70.6, 68.8, 66.8, 26.1, 26.0, 24.9, 24.4.

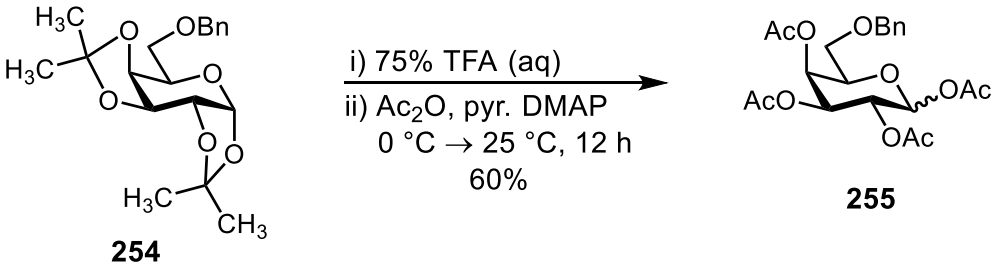
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 254



^{13}C NMR ($CDCl_3$, 100 MHz) of compound 254



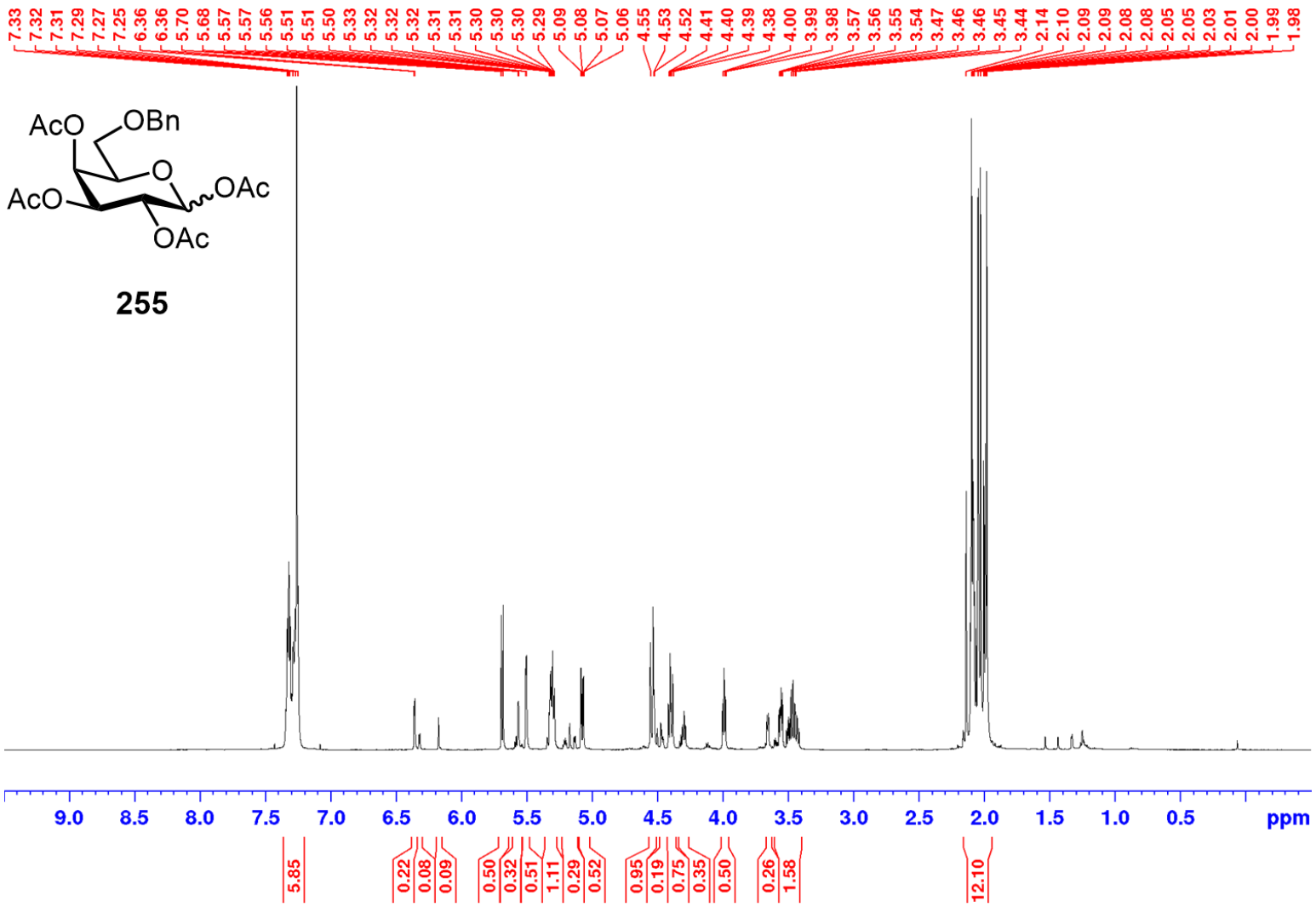
1,2,3,4-tetra-*O*-acetyl- 6-*O*-benzyl-D-galactopyranoside (255)



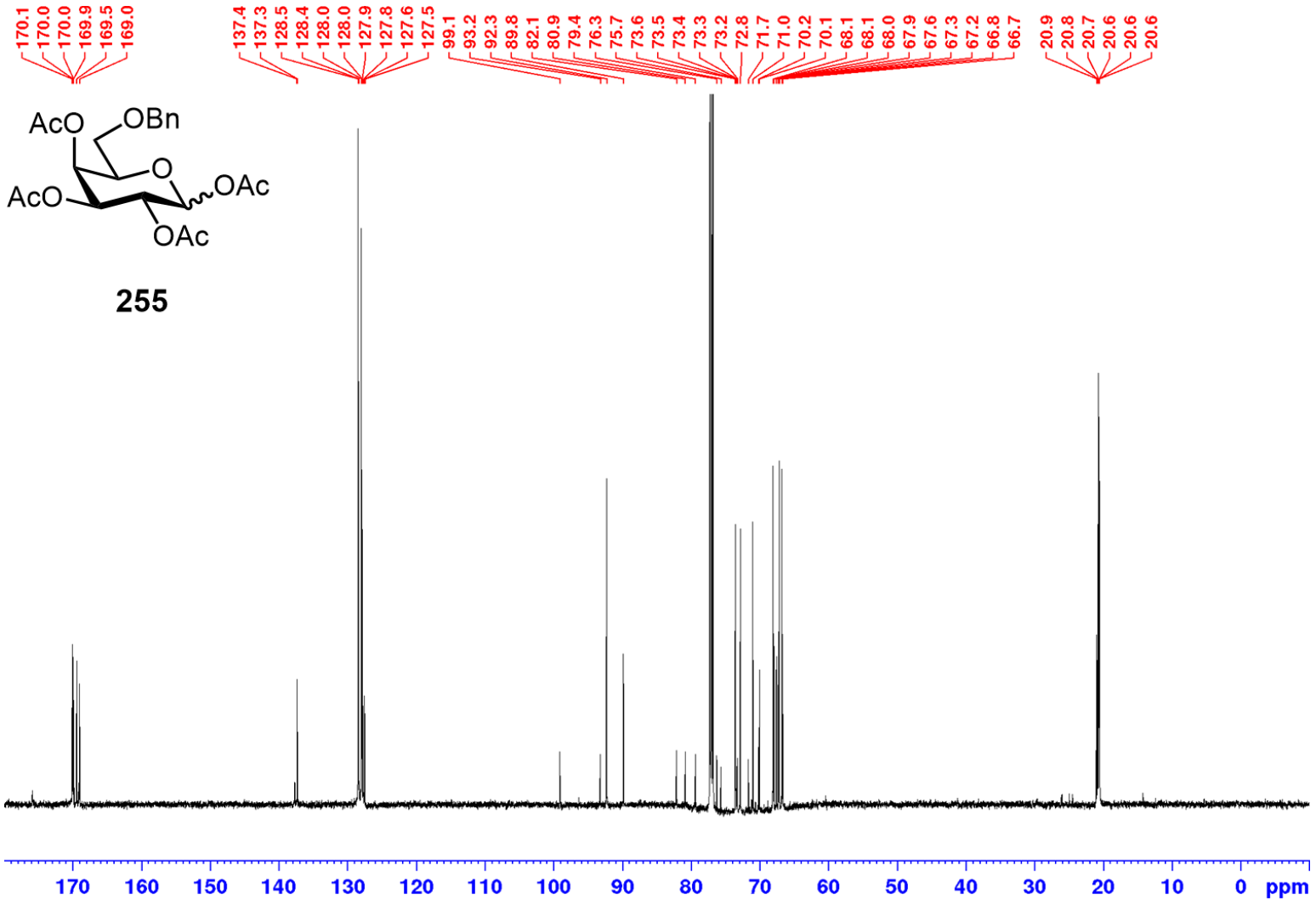
254 **255**

A solution of 6-*O*-benzyl-1,2:3,4-di-isopropylidene-D-galactopyranoside **254** (1.0 equiv., 18.7 g, 55.5 mmol) in anhydrous CH_2Cl_2 (10.0 mL) was added a cooled solution of aqueous 75% TFA solution (280 mL) at 0°C under an argon atmosphere. The reaction was stirred at room temperature. At 1.5 h, the reaction mixture was concentrated *in vacuo* then co-evaporated with toluene (3x). The resulting crude oil was added pyridine (21.0 equiv., 20.0 mL, 247 mmol), acetic anhydride (14.0 equiv., 15.0 mL, 159 mmol) and DMAP (0.1 equiv.) under an argon atmosphere at 0°C. The reaction was stirred at room temperature. After 12 h, the reaction was cooled to 0 °C and added methanol (10 mL) to consume the excess acetic anhydride. The reaction was stirred at room temperature. After 5 min, the reaction was concentrated to a crude oil. The crude oil was transferred to a separatory funnel with EtOAc (300 mL) and washed with 1 N HCl (3 x 25 mL), water (2 x 25 mL), brine (1 x 25 mL), dried ($NaSO_4$) and filtered through a glass fritted vacuum filter funnel to remove drying agent. The filtrate was concentrated to a crude oil. The crude oil was purified by a silica gel flash column chromatography (30:70 to 20:80 EtOAc/hexanes) to give an inseparable mixture of 0.45:1.0 α/β tetraacetate 1,2,3,4-tetra-*O*-acetyl-6-*O*-benzyl-D-galactopyranoside **255** (14.54 g, 33.23 mmol, 60%) as a yellow oil. Our characterization data were consistent with previously reported data.[325-328]

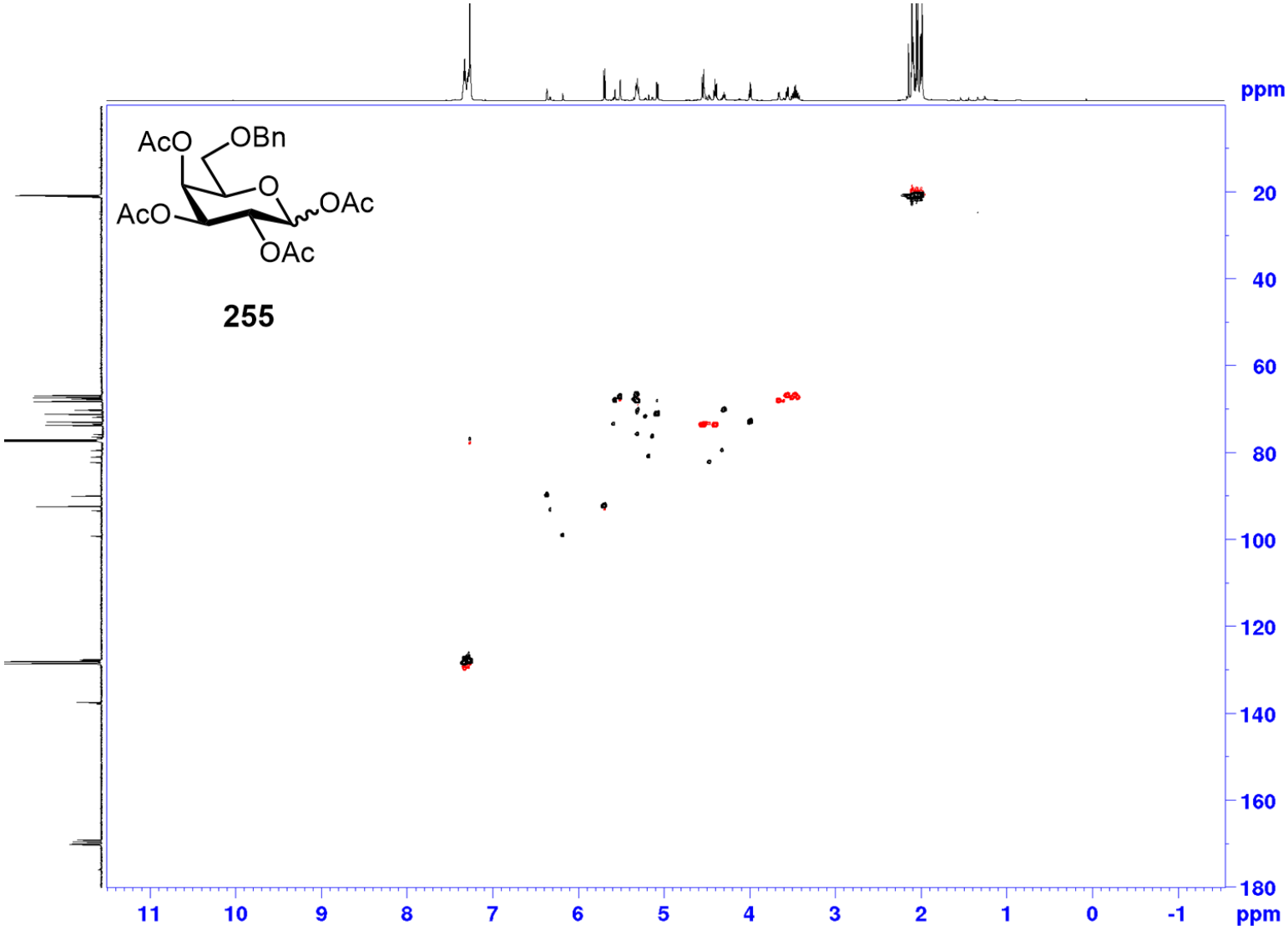
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 255



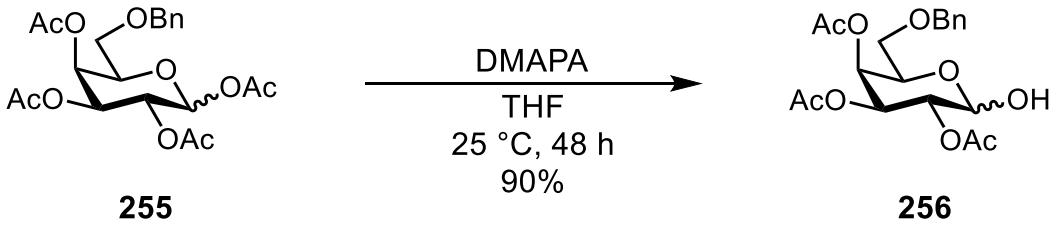
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 255



^{1}H-^{13}C HSCQ ($CDCl_3$) of compound 255



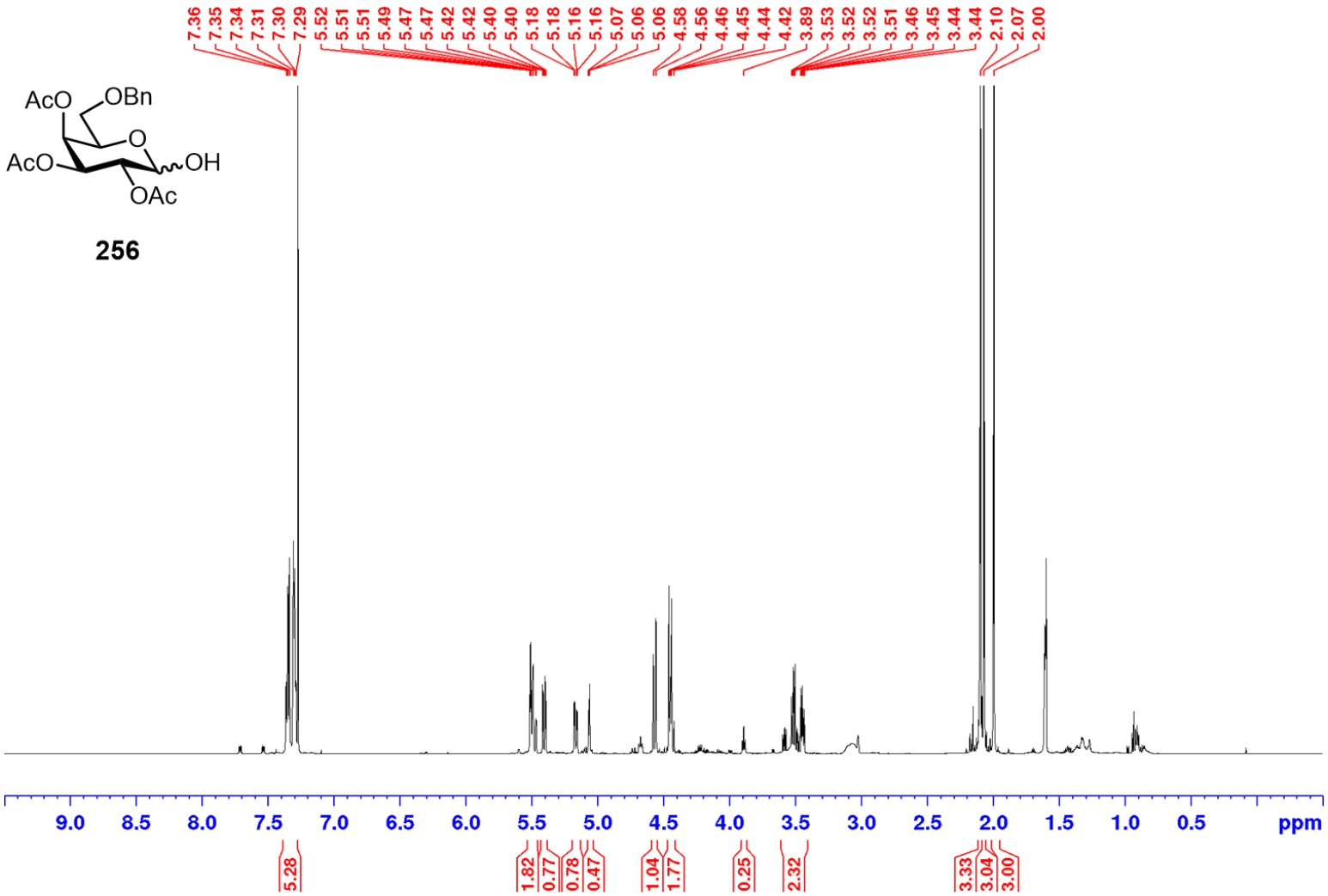
6-*O*-benzyl-2,3,4-tri-*O*-acetyl galactopyranose hemiacetal (256)



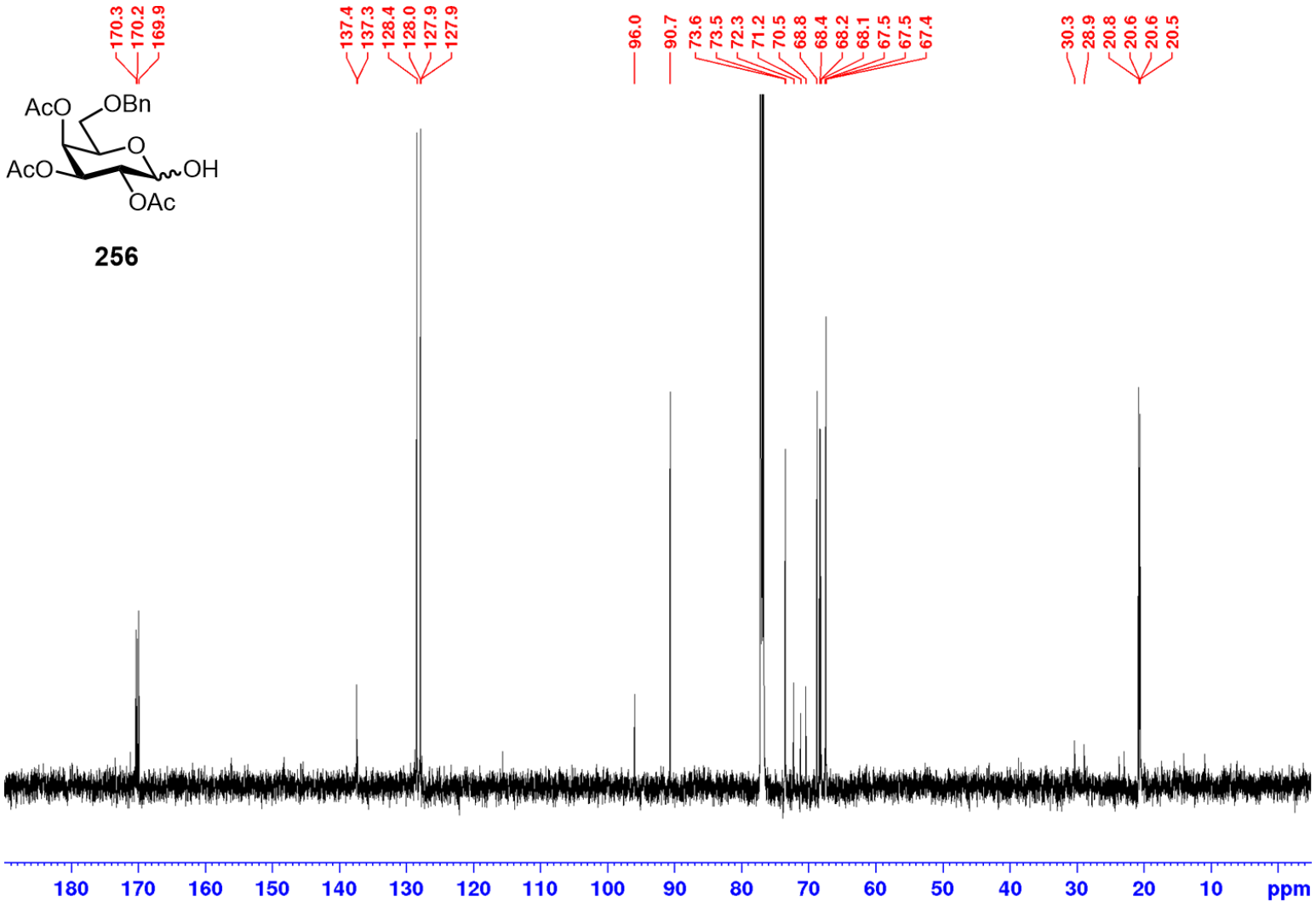
255 **256**

A solution of 1,2,3,4-tetra-*O*-acetyl-6-*O*-benzyl-D-galactopyranoside **255** (1.0 equiv., 14.5 g, 33.2 mmol) in anhydrous THF (166 mL) was added dimethylaminopropylamine (1.1 equiv., 4.63 mL, 36.5 mmol) at room temperature under an argon atmosphere. At 48 hours, the reaction was concentrated then diluted and transferred with EtOAc (400 mL) to a separatory funnel and washed with 1 N HCl (3 x 100 mL), water (2 x 100 mL), and brine (1 x 50 mL). The organics where separated, dried ($NaSO_4$) and filtered through a glass fritted vacuum filter funnel. The filtrate was concentrated to dryness. The crude solid was purified by silica gel flash column chromatography (25:70 to 30:70 EtOAc/hexanes) to give 6-*O*-benzyl-2,3,4-tri-*O*-acetyl galactopyranose hemiacetal **256** (11.8 g, 29.8 mmol 90%) as an amorphous solid. Our characterization data were consistent with previously reported data.[329-336]

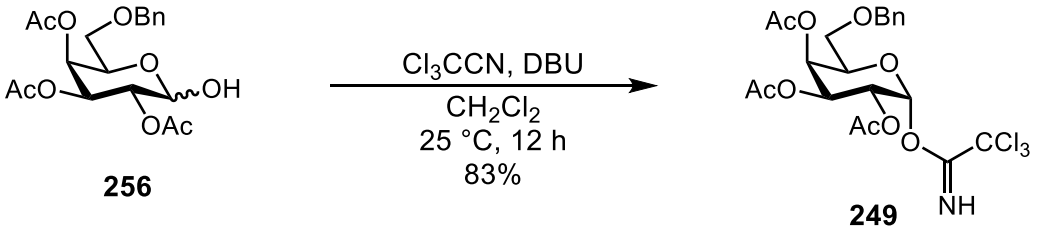
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 256



^{13}C NMR ($CDCl_3$, 100 MHz) of compound 256



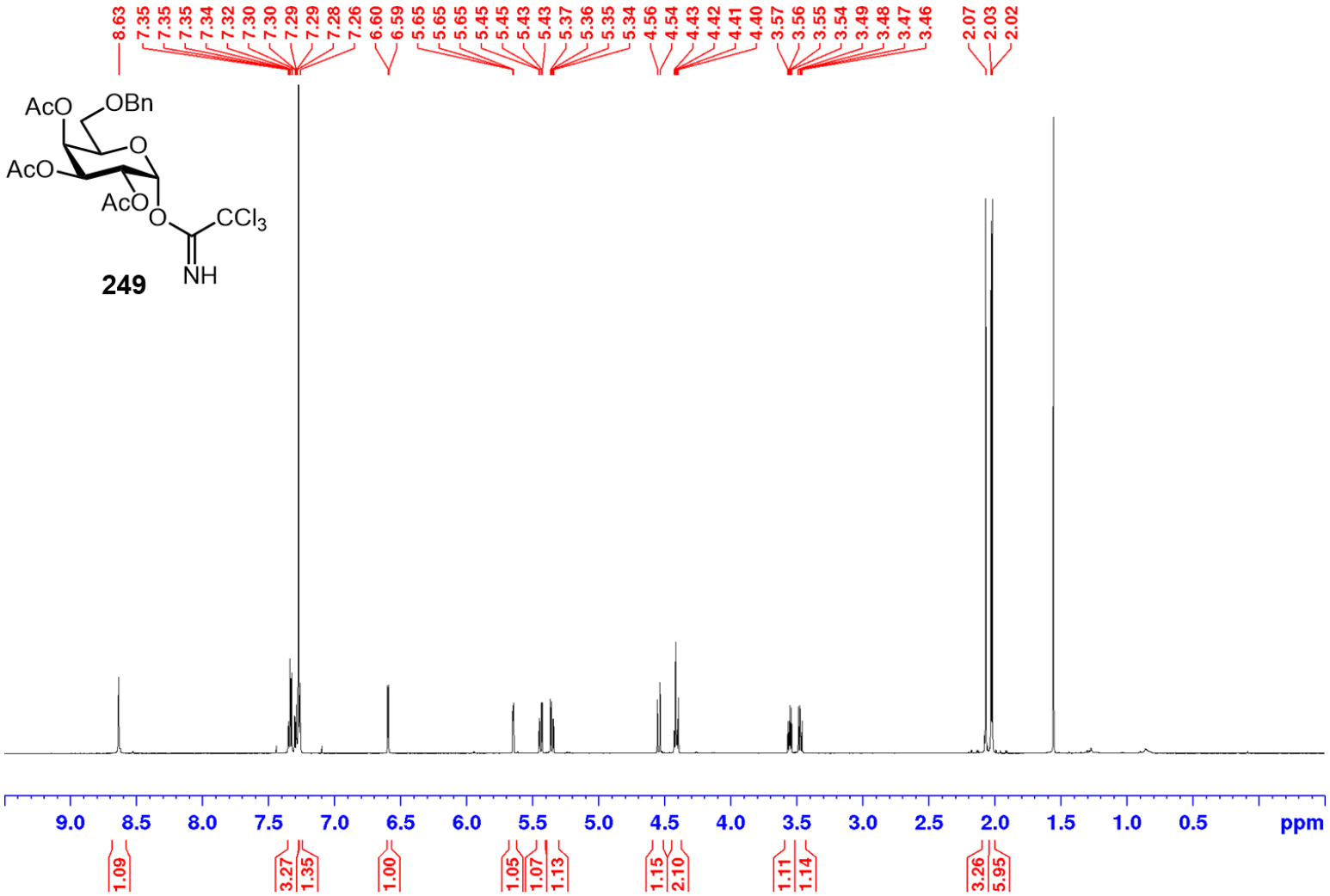
2,3,4-tri-*O*-acety-6-*O*-benzy-α-D-galactopyranosyl trichloroacetimidate (249)



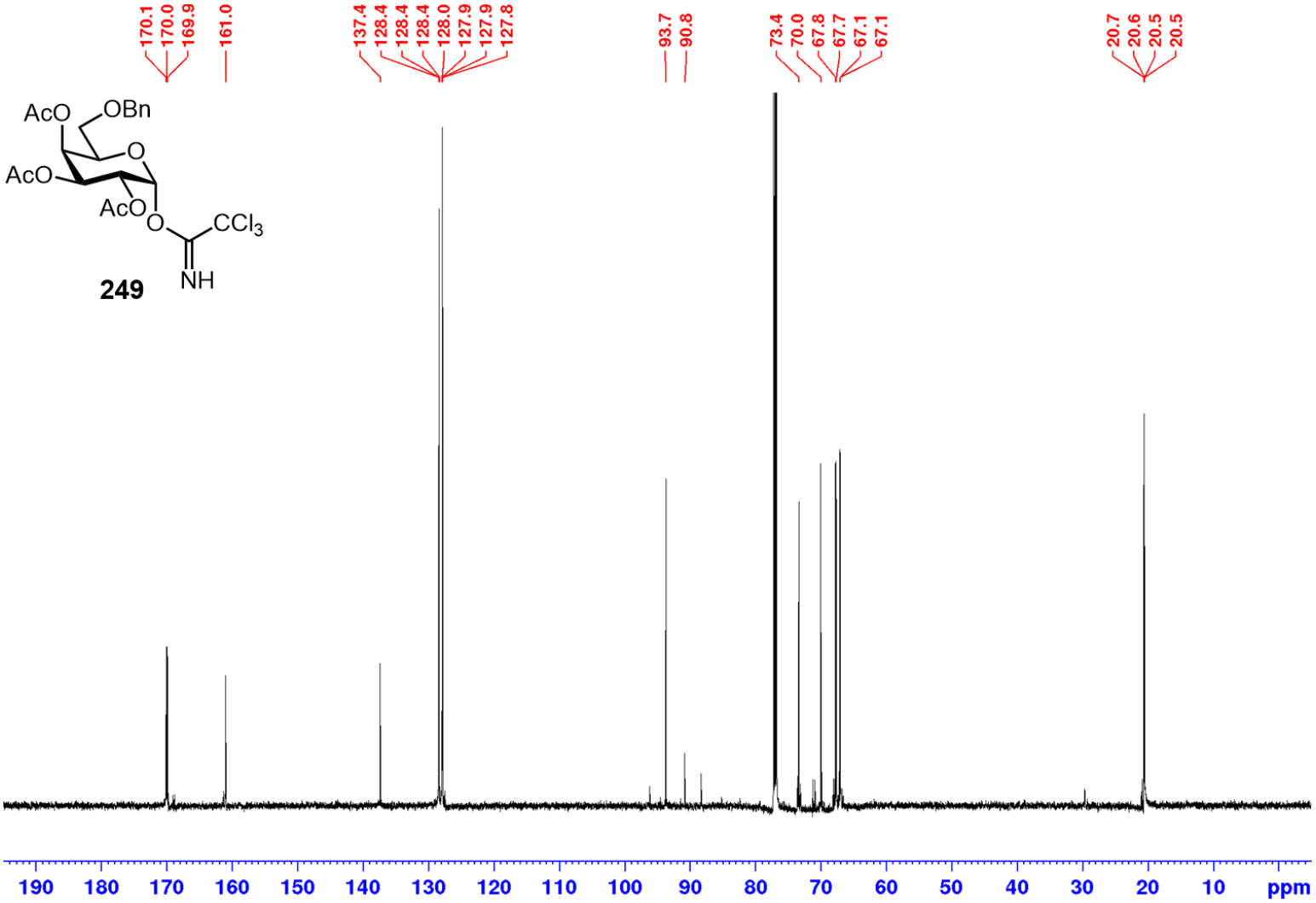
A solution of 6-*O*-benzyl-2,3,4-tri-*O*-acetyl D-galactopyranose **256** (1.0 equiv., 11.1 g, 25.09 mmol) in anhydrous CH_2Cl_2 (125 mL) under an argon atmosphere was cooled to 0 °C and added Cl_3CCN (10.0 equiv., 33.3 mL, 332 mmol) followed by DBU (0.26 equiv., 1.25 mL, 8.30 mmol). The reaction was stirred at room temperature. After 12 h, the dark brown crude reaction mixture was passed through a glass fritted vacuum filter funnel equipped with a plug of Celite. The filter cake and reaction flask were rinsed with CH_2Cl_2. The filtrate was concentrated to an oil. The crude oil was purified by silica gel flash chromatography (20:80 to 25:75 EtOAc/hexanes) to give the 2,3,4-tri-*O*-acety-6-*O*-benzy-α-D-galactopyranosyl trichloroacetimidate **249** (11.3 g, 20.9 mmol, 83%) as an amorphous solid. Our characterization data were consistent with previously reported data.[337]

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.63 (s, 1H), 7.35-7.28 (m, 3H), 7.26-7.25 (m, 2H), 6.59 (d, *J* = 3.6 Hz, 1H), 5.65 (dd, *J* = 1.1, 3.1 Hz, 1H), 5.44 (dd, *J* = 3.3, 10.8 Hz, 1H), 5.35 (dd, *J* = 3.7, 10.9 Hz, 1H), 4.55 (d, *J* = 12.1 Hz, 1H), 4.43-4.40 (m, 2H), 3.56 (dd, *J* = 5.8, 9.7 Hz, 1H), 3.48 (dd, *J* = 7.2, 9.6 Hz, 1H), 2.07 (s, 3H), 2.04 (s, 3H), 2.01 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.3, 170.2, 169.9, 137.4, 137.3, 128.4, 128.0, 127.9, 127.9, 96.0, 90.7, 73.6, 73.5, 72.3, 71.2, 70.5, 68.8, 68.4, 68.2, 68.1, 67.6, 67.5, 67.4, 30.3, 28.9, 20.8, 20.6, 20.6, 20.6.

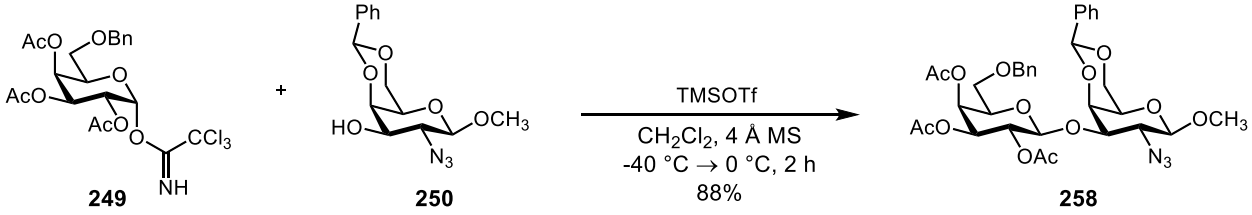
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 249



^{13}C NMR ($CDCl_3$, 100 MHz) of compound 249



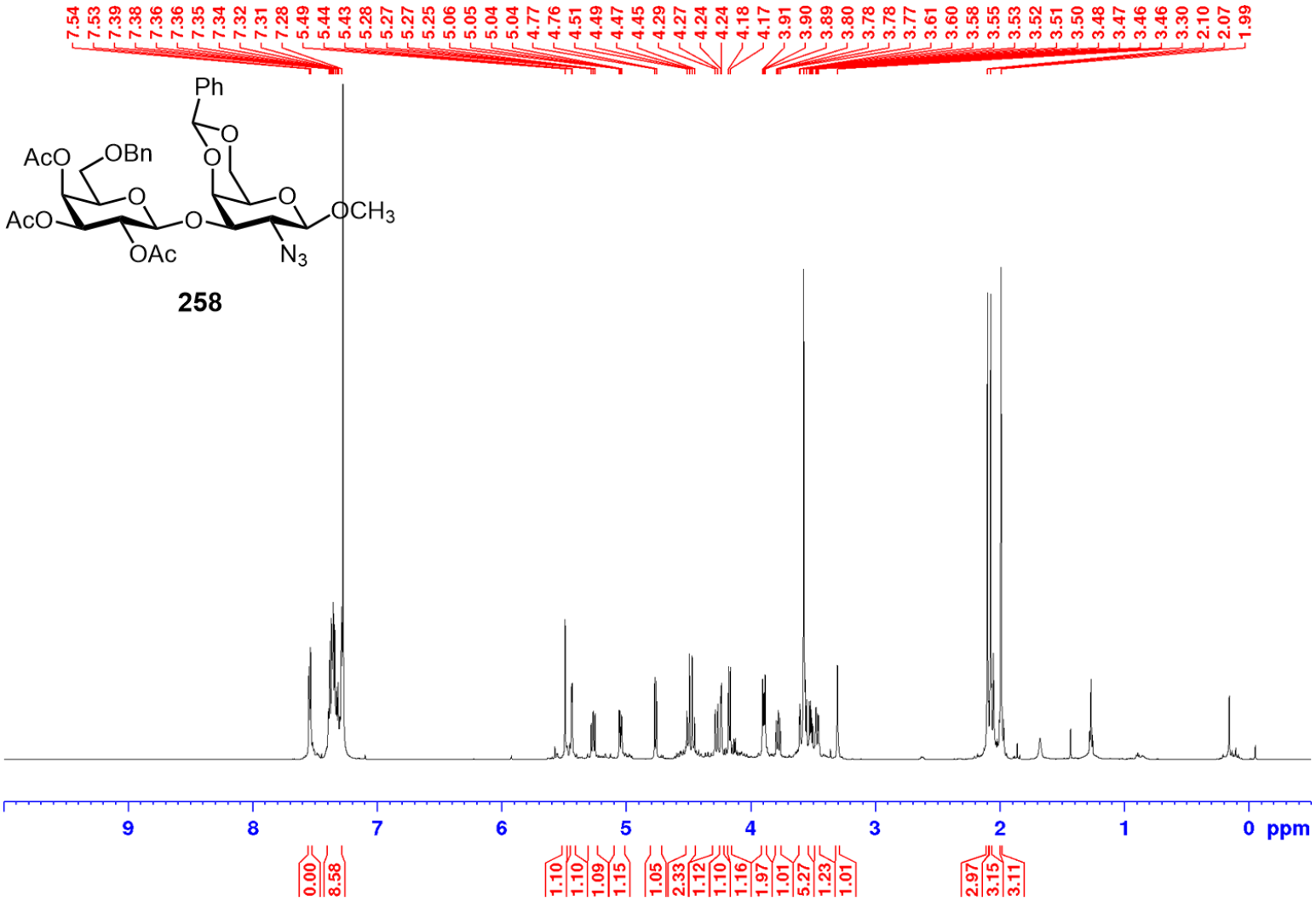
Methyl 6-*O*-benzyl-2,3,4-tri-*O*-acetyl-β-D-galactopyranosyl-(1→3)-4,6-*O*-benzylidene acetal-2-deoxy-2-azideo-β-D-galactopyranoside (258)



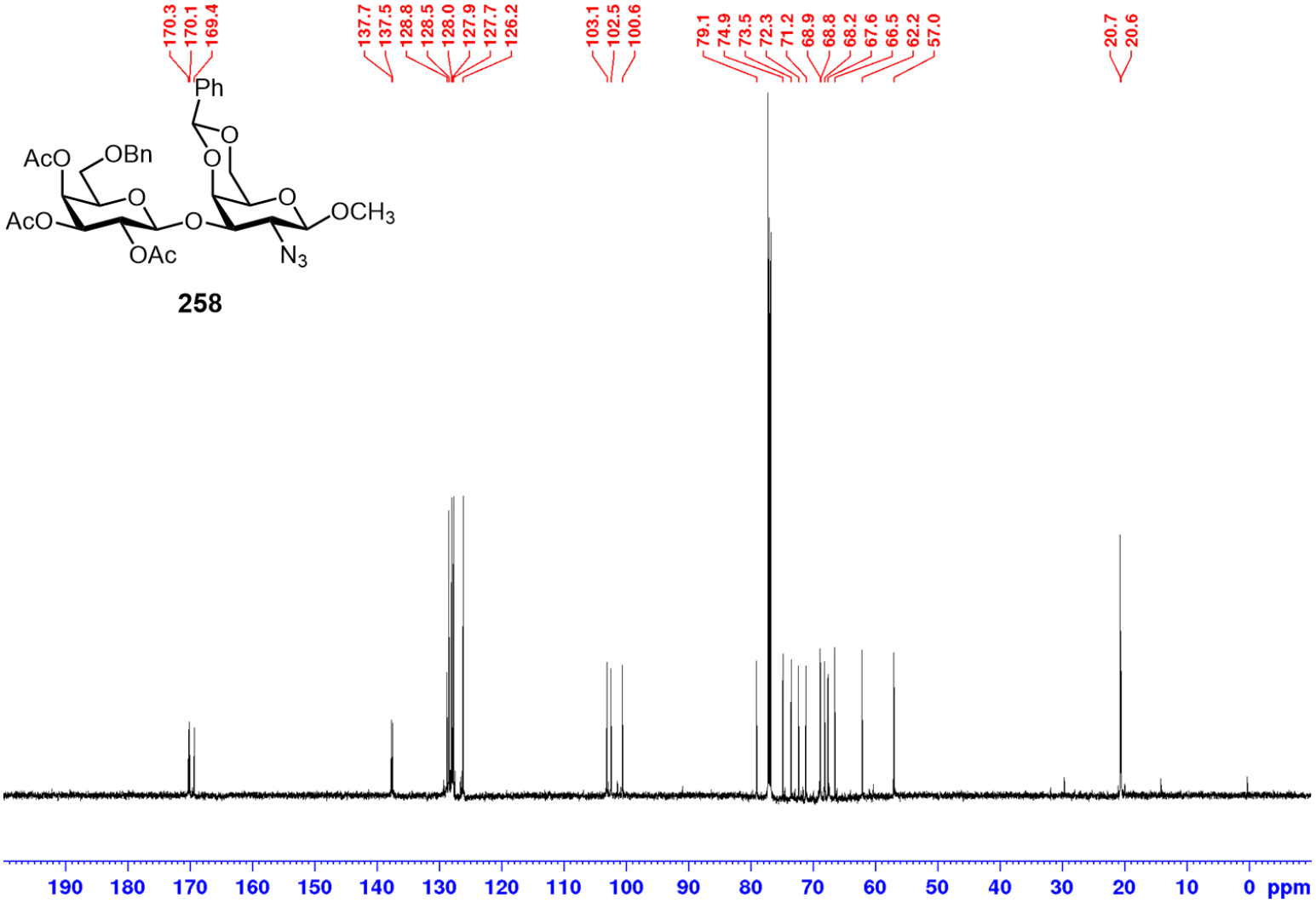
A solution of previously crystalized methyl 4,6-*O*-benzylidene acetyl-2-deoxy-2-azido-β-D-galactopyranoside **250** (1.0 equiv., 2.31 g, 7.53 mmol) in anhydrous CH_2Cl_2 (38.0 mL) was stirred over 4 Å molecular sieves (5.7 g, ~15% m/v) at room temperature for 12 hours. The solution was then cooled to -40°C, treated with TMSOTf (0.5 equiv., 0.68 mL, 3.8 mmol) followed by dropwise addition of the previously crystalized 2,3,4-tri-*O*-acety-6-*O*-benzy-α-D-galactopyranosyl trichloroacetimidate donor **249** (1.5 equiv., 6.11 g, 11.30 mmol) in a minimal amount of anhydrous CH_2Cl_2 (~10 mL). The reaction was stirred for 2 h while warming to, but not exceeding, 0 °C. The reaction was neutralized by the addition of with Et_3N (1.0 equiv., 1.0 mL, 7.2 mmol). The crude reaction mixture was passed through a glass fritted vacuum filter equipped with Celite to remove the molecular sieves. The filtrates was concentrated and subjected to silica gel flash column chromatography (3:10 to 2:5 EtOAc/hexanes) to give methyl 6-*O*-benzyl-2,3,4-tri-*O*-acetyl-β-D-galactopyranosyl-(1→3)-4,6-*O*-benzylidene acetal-2-deoxy-2-azideo-β-D-galactopyranoside **258** (4.68 g, 6.82 mmol, 88%) as a white amorphous solid. R_f = 0.4 (1:1 EtOAc/hexanes) visualized with ceric ammonium molybdate stain; $[\alpha]D^{25}$ = -1.52°(c = 1.0, $CHCl_3$); IR ν_{max} (film) 2114.94, 1748.27, 1367.98, 1221.17, 1178.50, 1136.23, 1058.49, 913.12, 821.70, 737.35, 699.61, 598.23 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.54-7.52 (m, 2H), 7.38-7.27 (m, 8H), 5.48 (s, 1H), 5.42 (dd, *J* = 0.8, 3.4 Hz, 1H), 5.25

(dd, *J* = 7.94, 10.2 Hz, 1H), 5.03 (dd, *J* = 3.3, 10.3 Hz, 1H), 4.75 (d, *J* = 8.1 Hz, 1H), 4.47 (dd, *J* = 11.8, 24.7 Hz, 2H), 4.27 (dd, *J* = 1.4, 12.2 Hz, 1H), 4.23 (d, *J* = 3.4 Hz, 1H), 4.16 (d, *J* = 8.0 Hz, 1H), 3.9-3.8 (m, 2H), 3.77 (dd, *J* = 8.4, 10.6 Hz, 1H), 3.56 (s, 3H), 3.55-3.49 (m, 2H), 3.45 (dd, *J* = 3.4, 10.2 Hz, 1H), 3.29 (d, *J* = 0.8 Hz, 1H), 2.09 (s, 3H), 20.6 (s, 3H), 1.98 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.3, 170.2, 169.4, 137.7, 137.5, 128.8, 128.5, 128.5, 128.1, 128.1, 128.0, 127.8, 127.8, 126.2, 126.2, 103.1, 102.5, 100.7, 79.1, 74.9, 73.6, 72.4, 71.2, 68.9, 68.9, 68.2, 67.7, 66.6, 62.2, 57.1, 20.7, 20.7, 20.6; HRMS (ESI) calcd for $C_{33}H_{43}N_4O_{13}^+$ $(M+NH_4)^+$ 703.28212, found 703.28198 m/z.

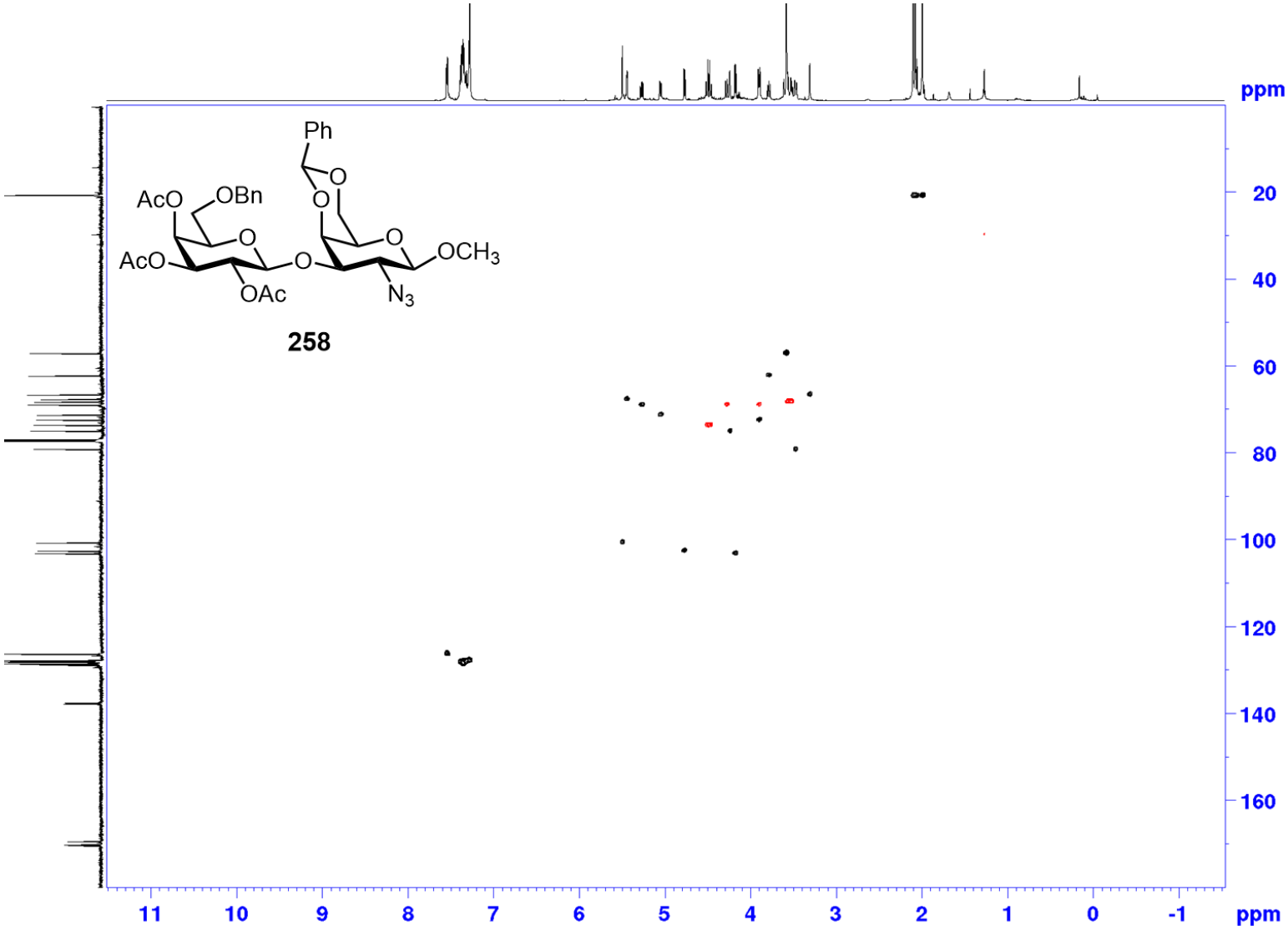
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 258



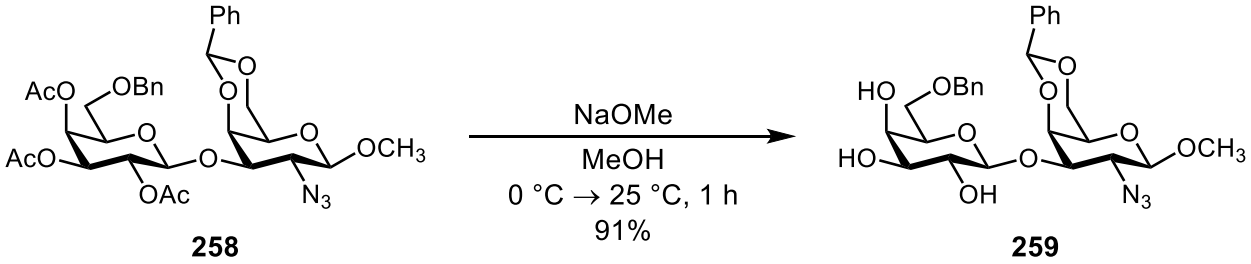
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 258



^{1}H-^{13}C HSCQ ($CDCl_3$) of compound 258

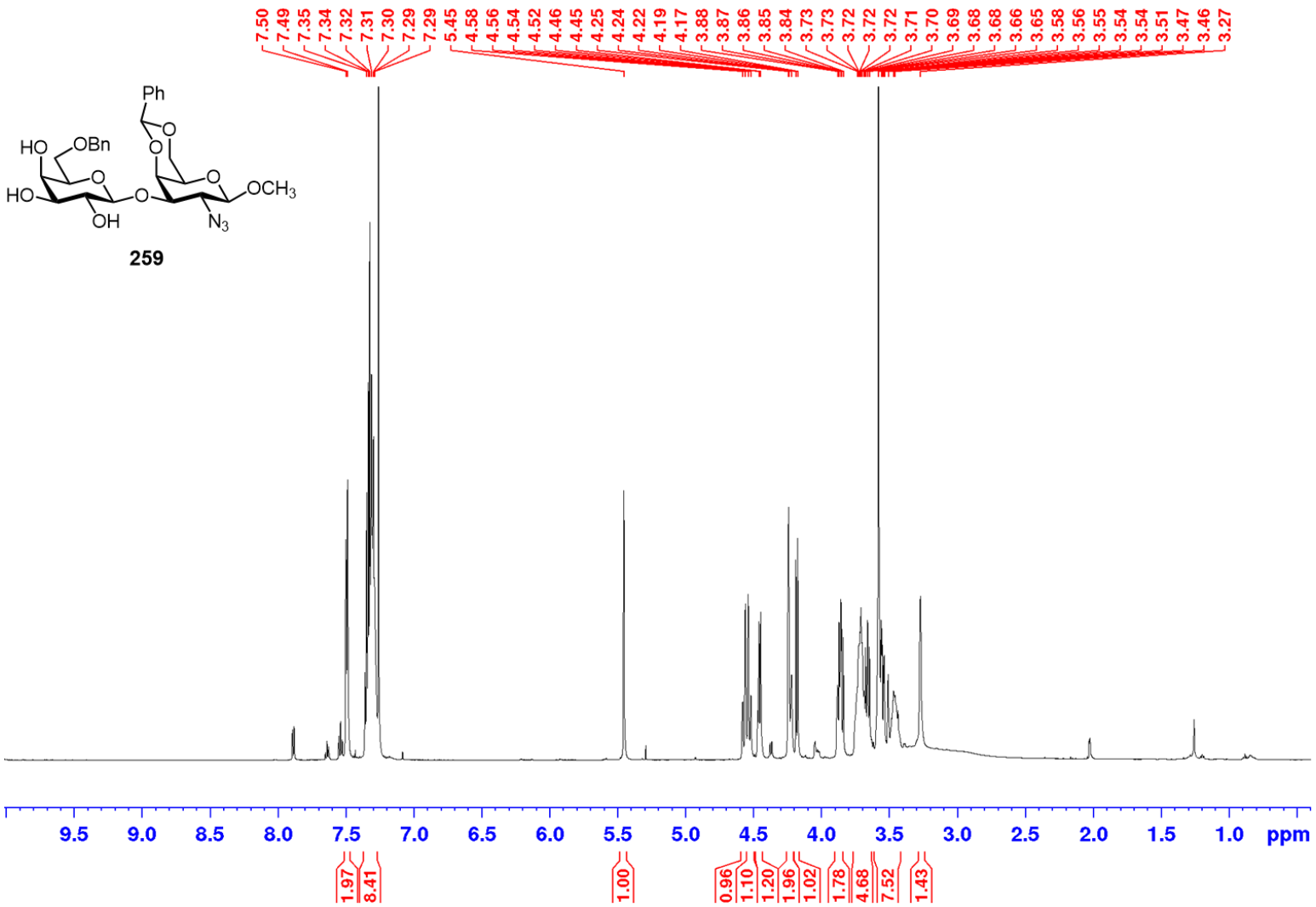


Methyl 6-*O*-benzyl-β-D-galactopyranosyl-(1→3)-4,6-di-*O*-benzylidene acetal-2-deoxy-2-azideo-β-D-galactopyranoside (259)

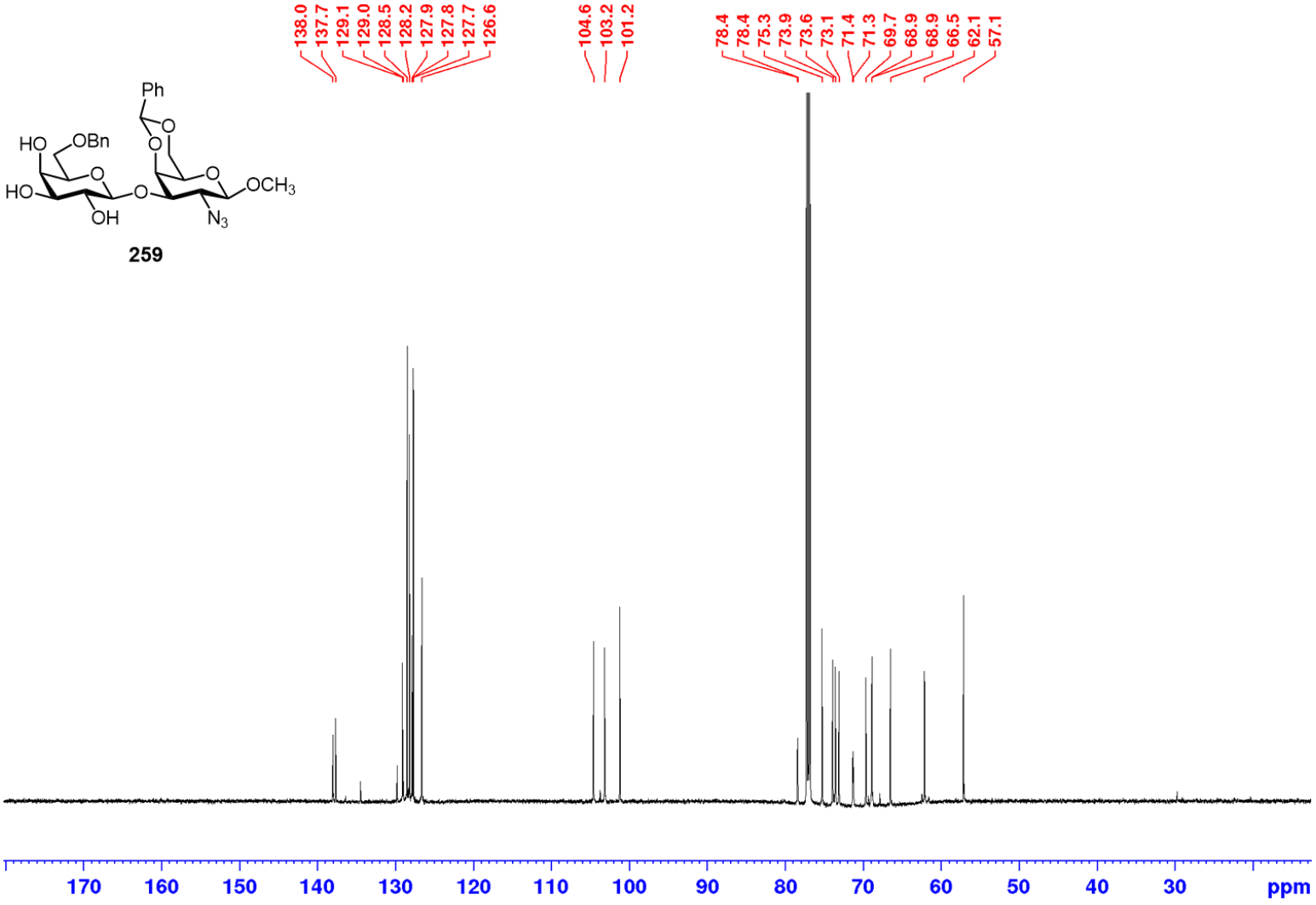


To a solution of methyl 2,3,4-tri-*O*-acetyl-6-*O*-benzyl-β-D-galactopyranosyl-(1→3)-4,6-di-*O*-benzylidene acetal-2-deoxy-2-azideo-β-D-galactopyranoside **258** (1.0 equiv., 2.696 g, 3.93 mmol) in dry methanol (20.0 mL) was added sodium methoxide solution (1.0 equiv., 2.0 mL, [2 N] in CH_3OH) at room temperature under an argon atmosphere. At 1 h, the reaction mixture was neutralized with the addition of acidic Amberlite IR 120 (H^+) ion exchange resin. The reaction was filtered through a glass frittered vacuum filter funnel equipped with pad Celite. The filter cake was rinsed with CH_2Cl_2. The filtrate was concentrated to an oil and purified by silica gel flash column chromatography (5:95 to 10:90 MeOH/CH_2Cl_2) to give methyl 6-*O*-benzyl-β-D-galactopyranosyl-(1→3)-4,6-di-*O*-benzyl-2-deoxy-2-azideo-β-D-galactopyranoside **259** (2.003 g, 3.58 mmol, 91%) as a white foam: Rf = 0.16 (5:95 CH_3OH/CH_2Cl_2) visualized with ceric ammonium molybdate stain; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.49 (d, *J* = 7.2 Hz, 2H), 7.35-7.29 (m, 8H), 5.46 (s, 1H), 4.57 (d, *J* = 11.6 Hz, 1H), 4.52 (d, *J* = 11.6 Hz, 1H), 4.46 (d, *J* = 7.9 Hz, 1H), 4.23 (d, *J* = 3.1 Hz, 2H), 4.18 (d, *J* = 8.7 Hz, 1H), 3.88-3.84 (m, 2H), 3.73-3.65 (m, 5H), 3.58-3.46 (m, 8H), 3.27 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 138.0, 137.6, 129.1, 129.0, 128.5, 128.2, 127.9, 127.8, 127.7, 126.6, 104.5, 103.1, 101.2, 78.4, 78.3, 75.2, 73.9, 73.5, 73.1, 71.36, 71.3, 69.7 68.9, 68.9, 66.5, 62.1, 57.1.

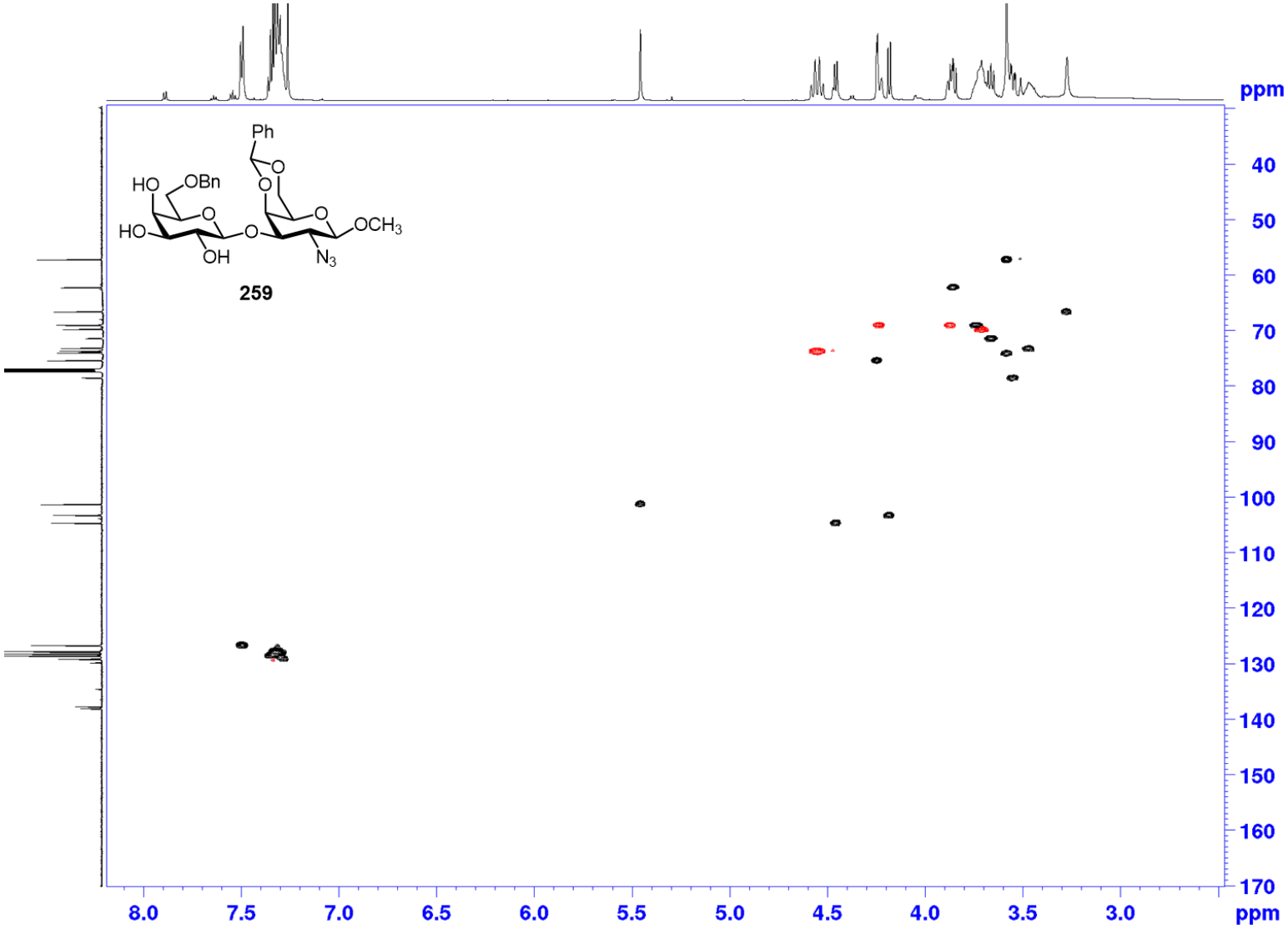
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 259



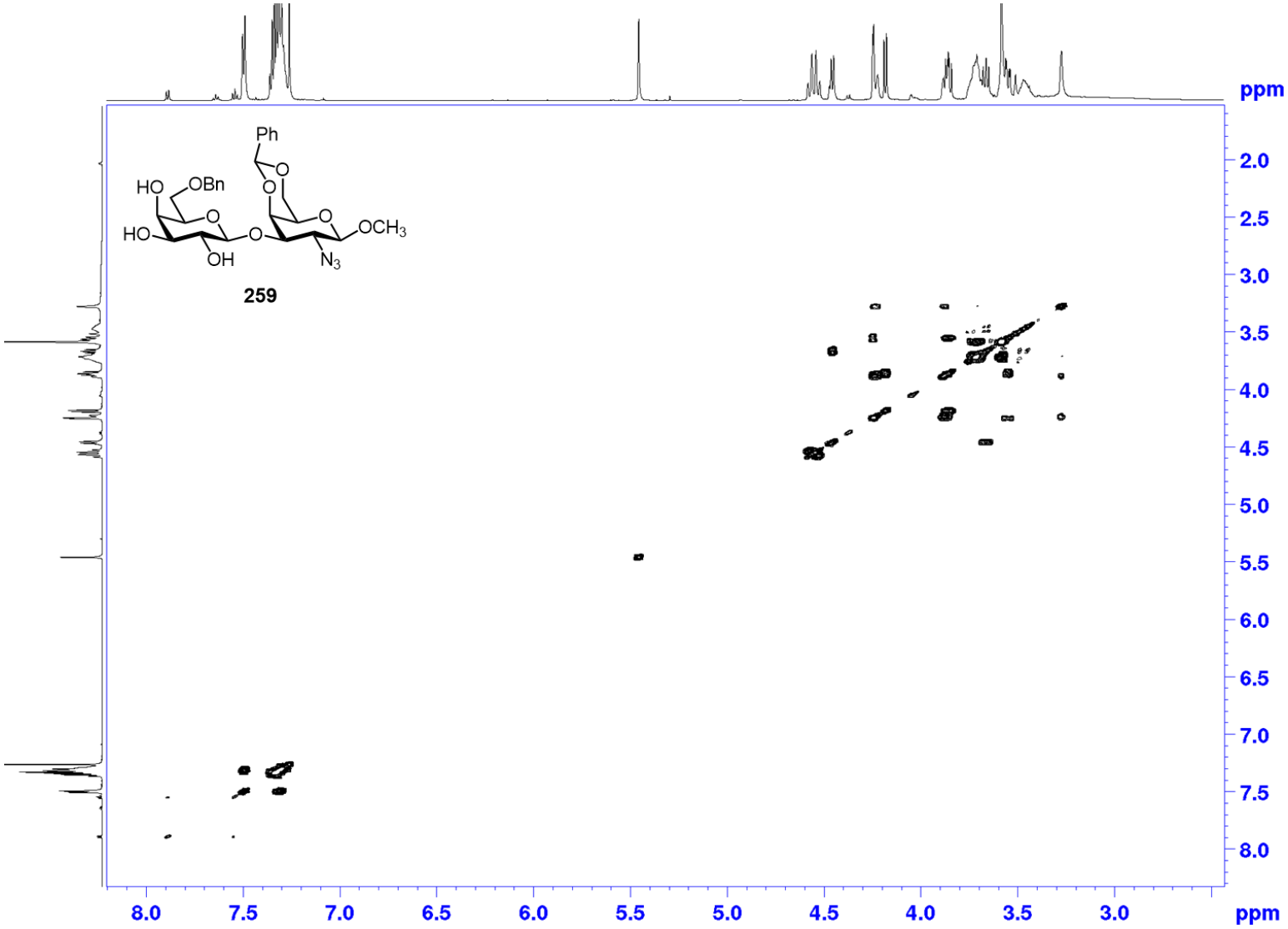
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 259



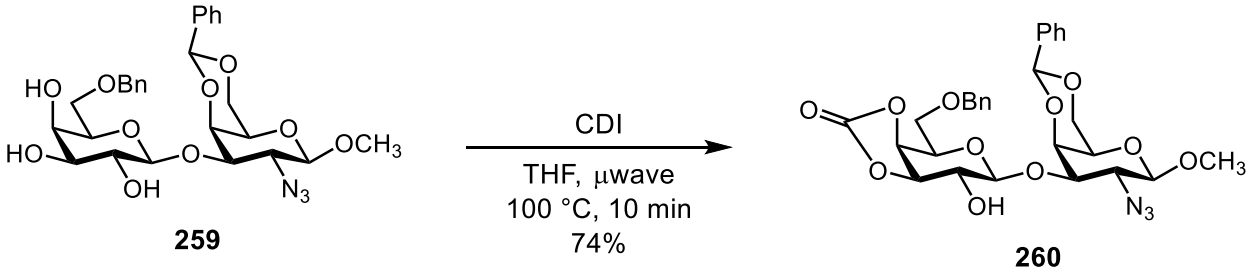
^{1}H-^{13}C HSCQ ($CDCl_3$) of compound 259



^{1}H-^{1}H COSY ($CDCl_3$) of compound 259



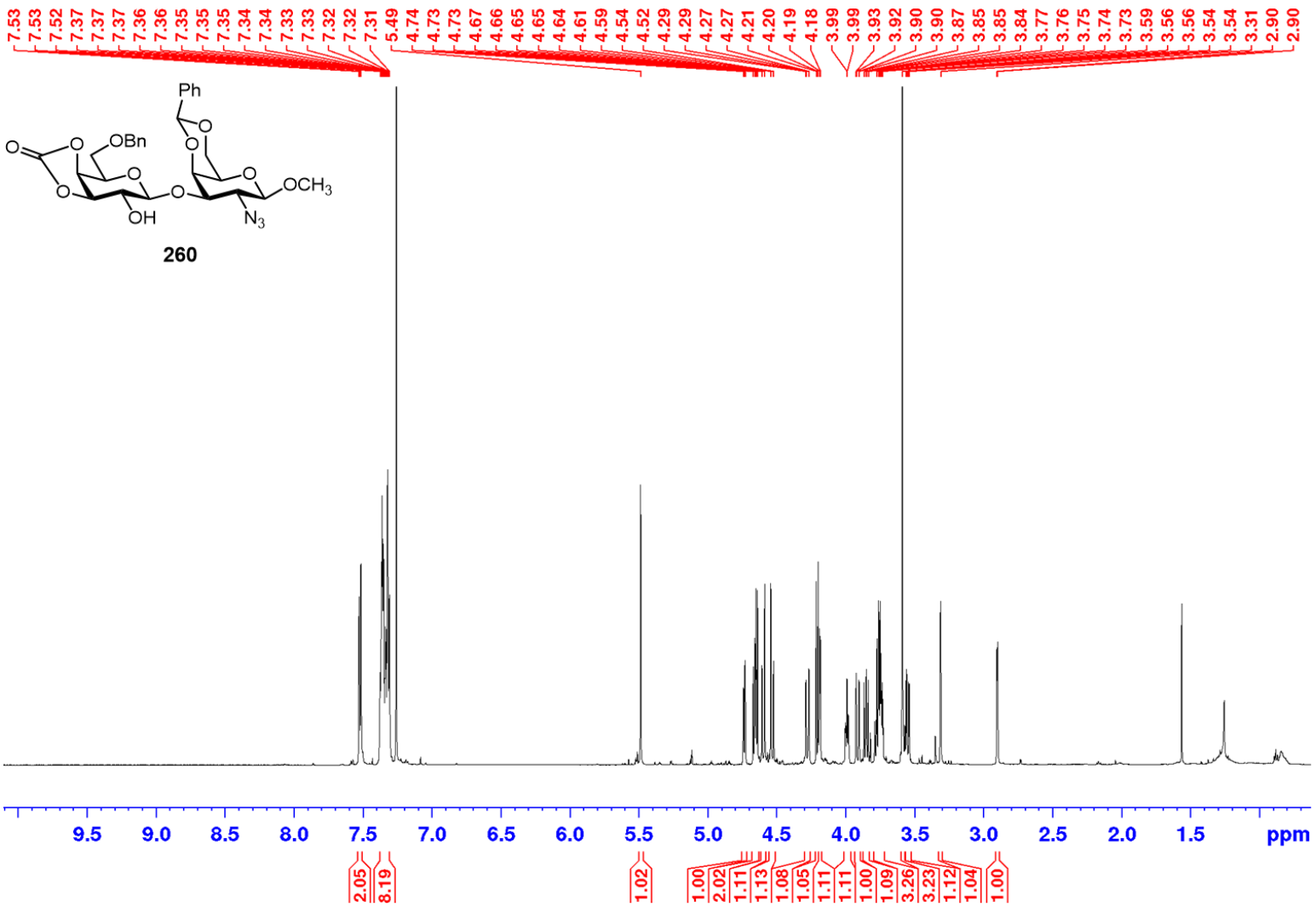
Methyl 6-*O*-benzyl-3,4-*O*-carbonyl-β-D-galactopyranosyl-(1→3)-4,6-di-*O*-benzylidene acetal-2-deoxy-2-azideo-β-D-galactopyranoside (260)



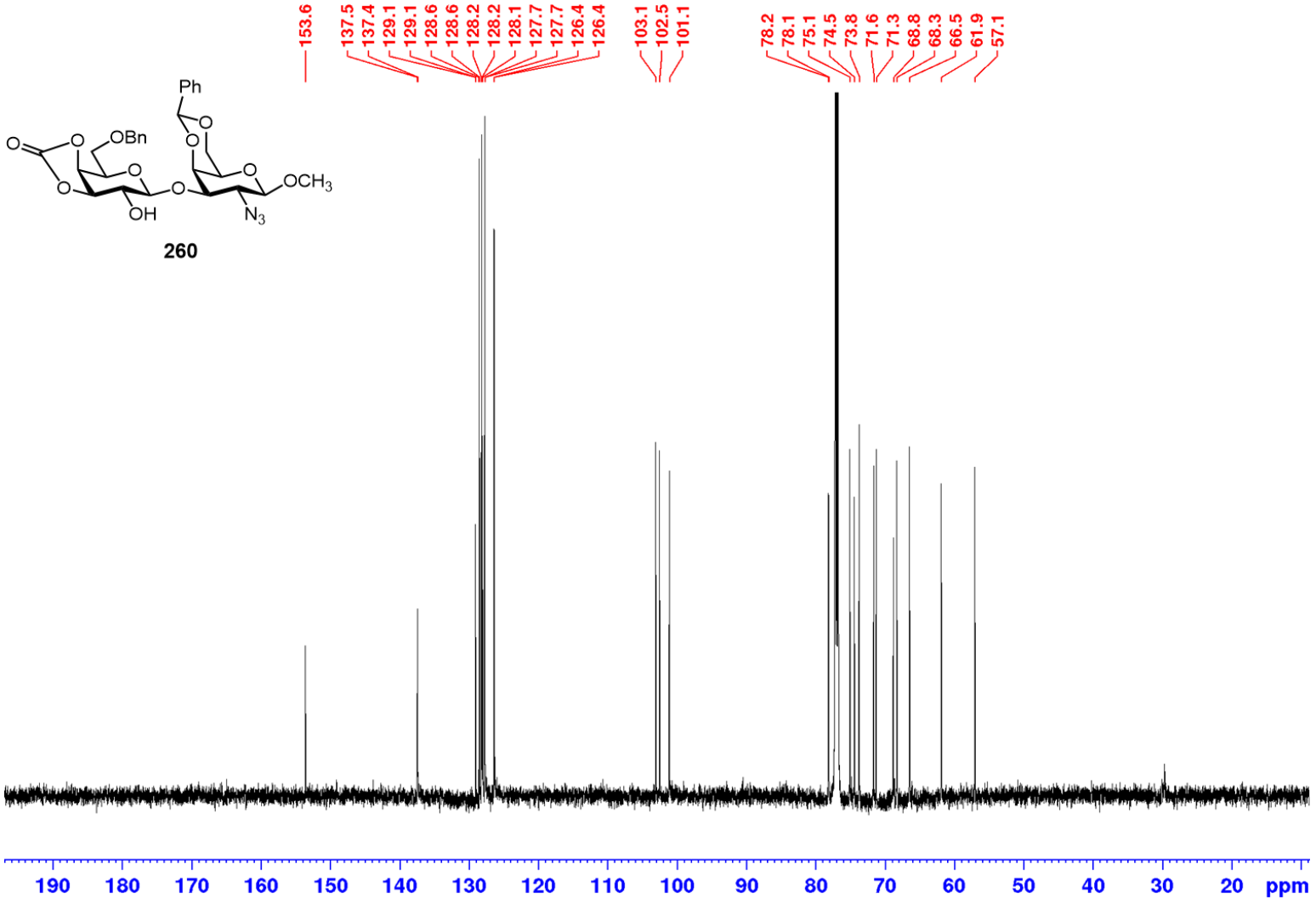
A solution of the methyl 6-*O*-benzyl-β-D-galactopyranosyl-(1→3)-4,6-di-*O*-benzyl-2-deoxy-2-azideo-β-D-galactopyranoside **259** (1.0 equiv., 2.477 g, 4.43 mmol) and CDI (1.5 equiv., 1.077 g, 6.64 mmol) in dry THF (20.0 mL, [0.23M]) was heated by microwave irradiation to 100°C for 15 min. The reaction was concentrated down then taken up in EtOAc and washed with sat. aq. NH_4Cl (2 x 20 mL), water (2 x 20 mL), and brine (1 x 20 mL). The organics where dried ($MgSO_4$), filtered through a glass fritted vacuum filter funnel to remove drying agent and concentrated to a white foam. The crude foam was purified by silica gel flash column chromatography (60:40 to 95:5 EtOAc/ hexanes) to give methyl 6-*O*-benzyl-3,4-*O*-carbonyl-β-D-galactopyranosyl-(1→3)-4,6-di-*O*-benzylidene acetal-2-deoxy-2-azideo-β-D-galactopyranoside **260** (1.9063 g, 3.26 mmol, 74%).; ^1H NMR (600 MHz, $CDCl_3$): δ 7.53-7.52 (m, 2H), 7.37-7.31 (m, 8H), 5.48 (s, 1H), 4.74 (dd, *J* = 1.9, 7.2 Hz, 1H), 4.67-4.64 (m, 2H), 4.6 (d, *J* = 11.6 Hz, 1H), 4.53 (d, *J* = 11.6 Hz, 1H), 4.28 (dd, *J* = 1.3, 12.4 Hz, 1H), 4.21 (d, *J* = 7.9 Hz, 1H), 4.19 (d, *J* = 3.3 Hz, 1H), 3.99 (dd, *J* = 1.9, 6.6 Hz, 1H), 3.92 (dd, *J* = 1.7, 12.8 Hz, 1H), 3.85 (dd, *J* = 7.8, 10.8 Hz, 1H), 3.77-3.73 (m, 3H), 3.59 (s, 3H), 3.55 (dd, *J* = 3.5, 10.5 Hz, 1H), 3.31 (s, 1H), 2.9 (d, *J* = 3.2 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 153.64, 137.47, 137.44, 129.1, 129.08,

128.58, 128.56, 128.21, 128.19, 128.08, 127.75, 127.73, 126.43, 126.41, 103.08, 102.53, 101.12, 78.2, 78.13, 75.08, 74.45, 73.75, 71.64, 71.28, 68.8, 68.31, 66.49, 61.88, 57.08.

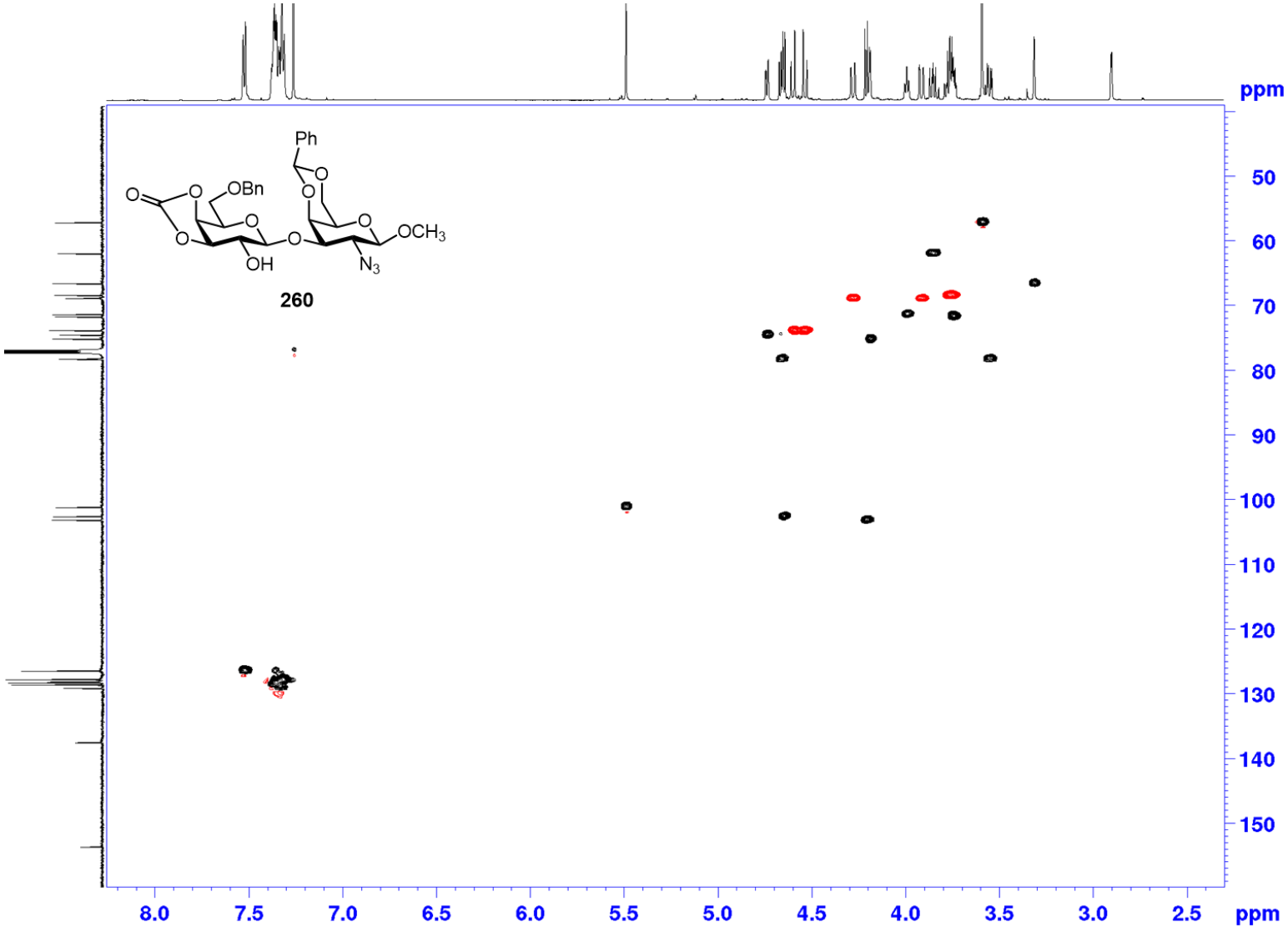
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 260



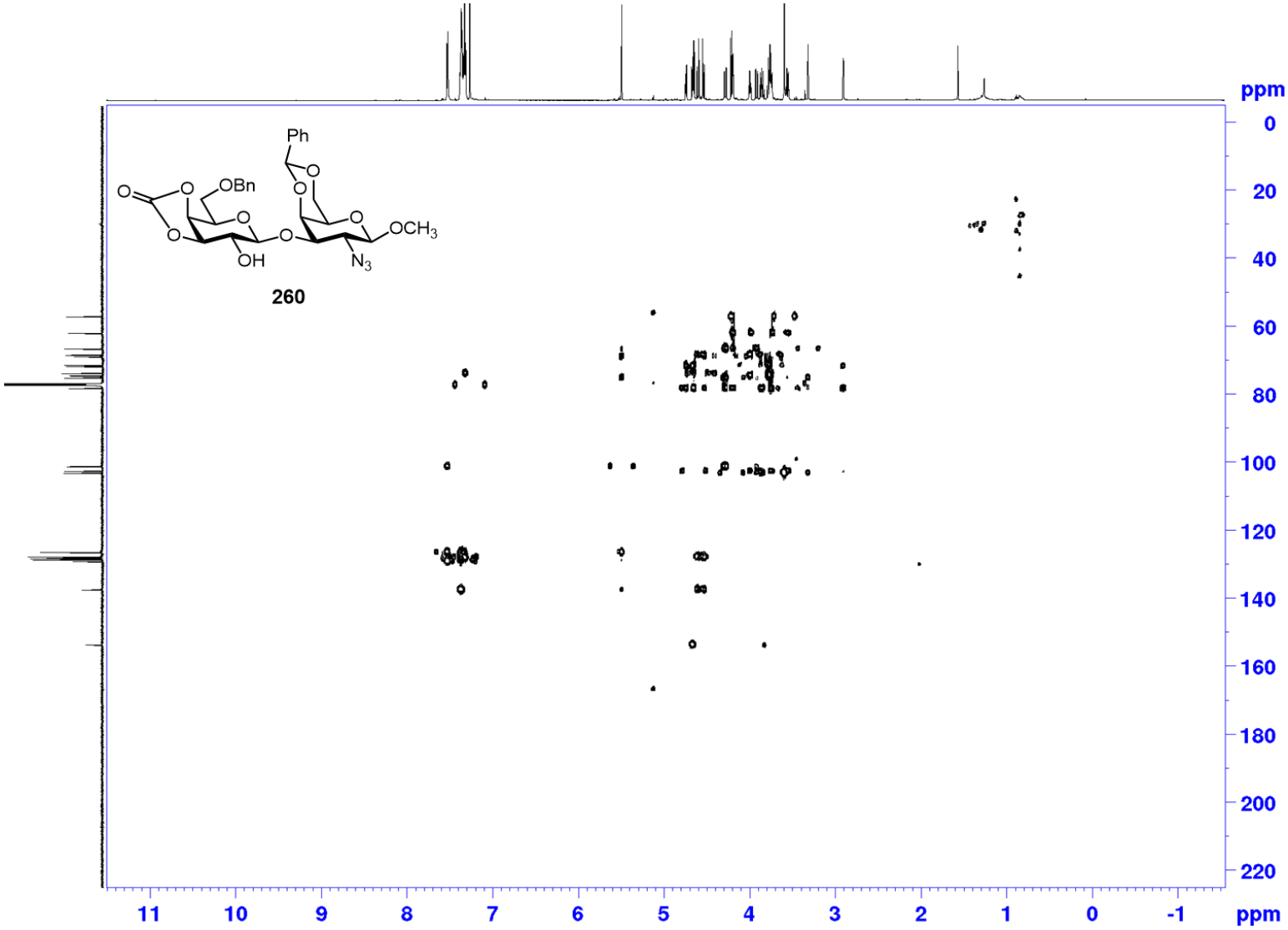
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 260



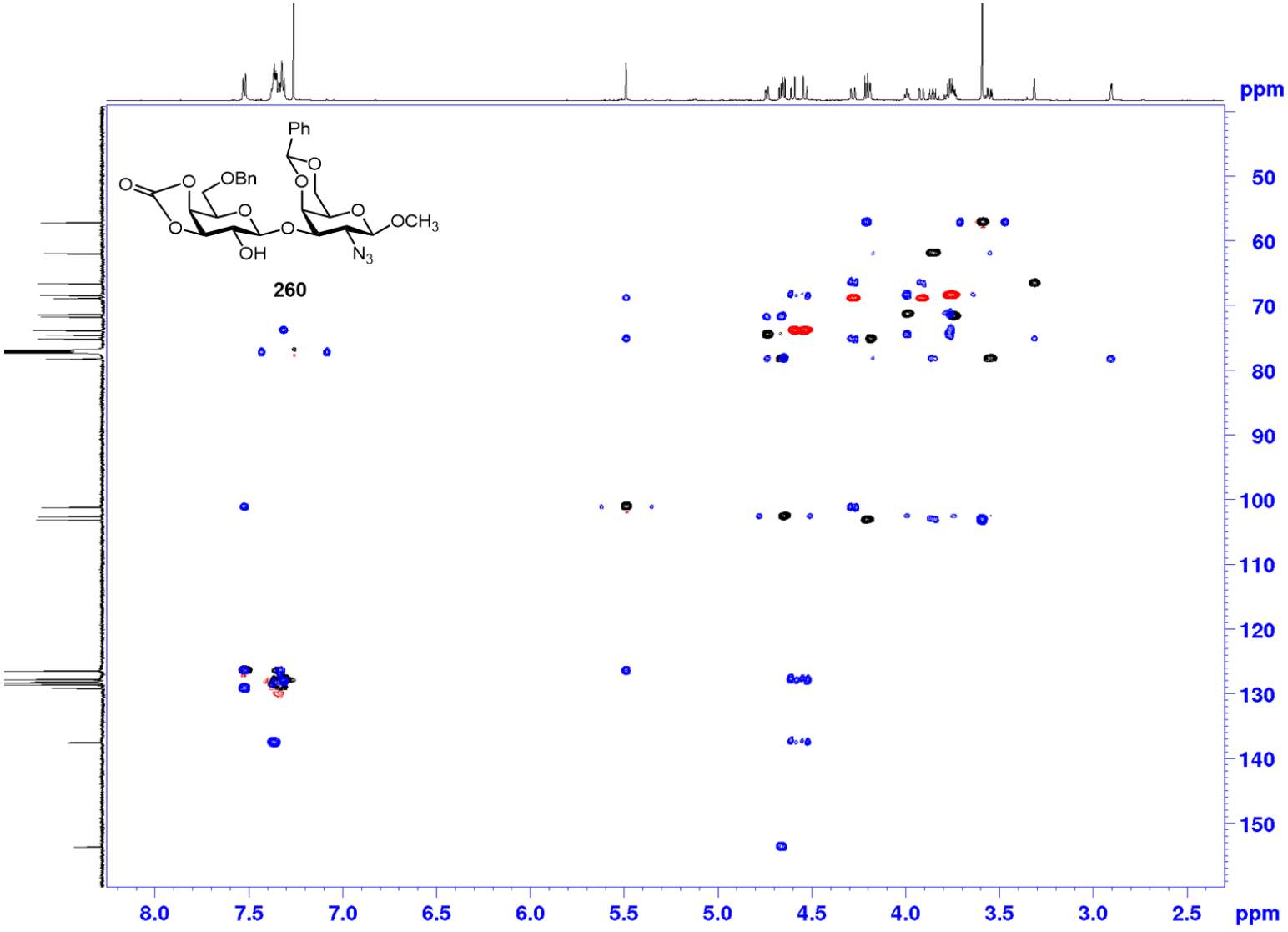
^{1}H-^{13}C HSCQ ($CDCl_3$) of compound 260



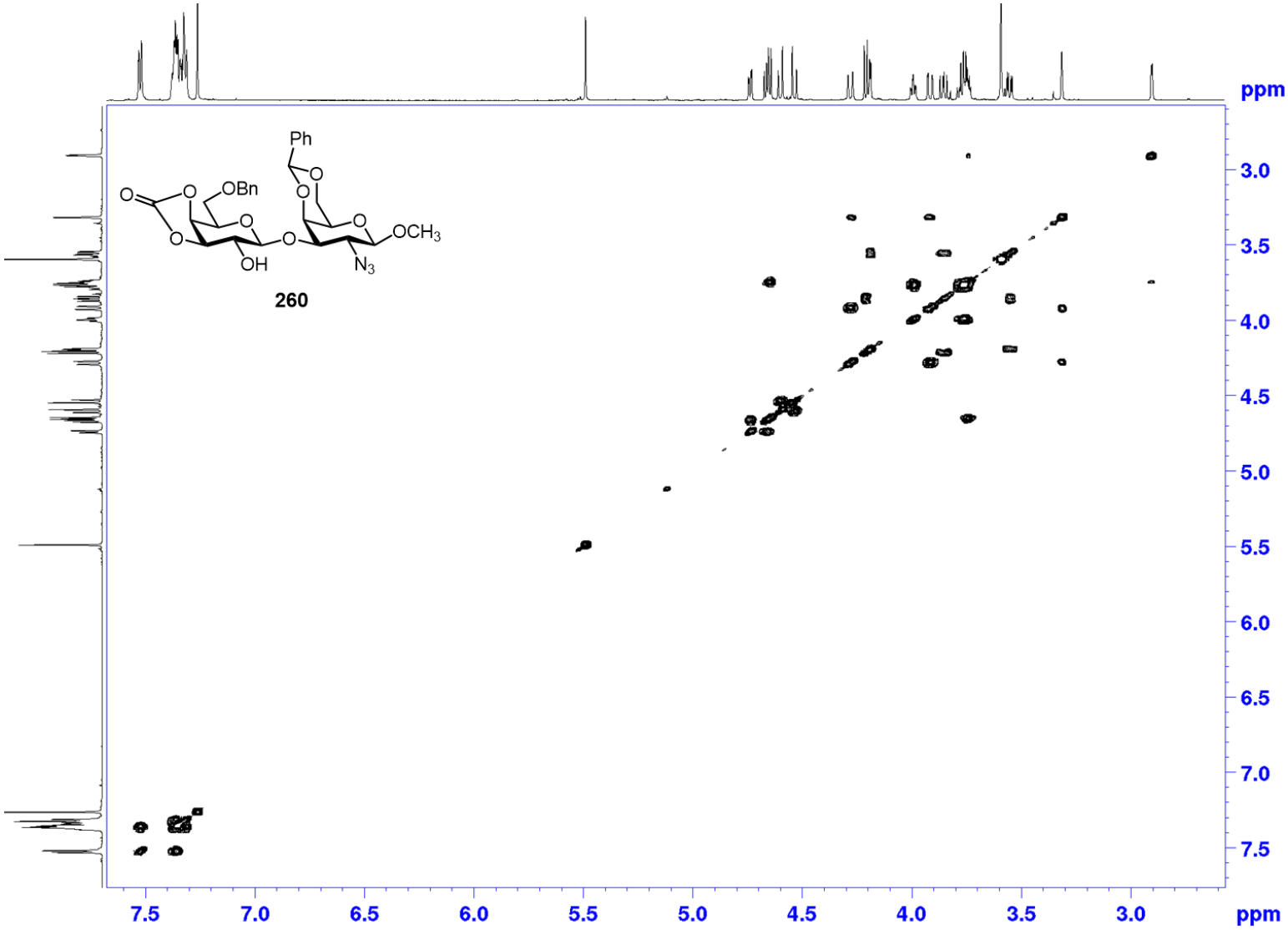
^{1}H-^{13}C HMBC ($CDCl_3$) of compound 260



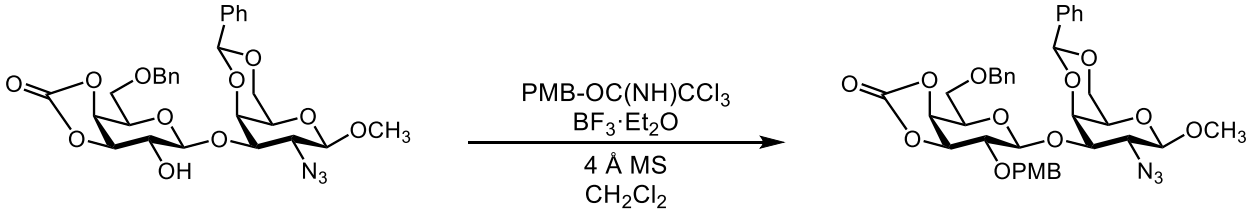
^{1}H-^{13}C HSCQ with ^{1}H-^{13}C HMBC overlay ($CDCl_3$) of compound 260



^{1}H-^{1}H COSY ($CDCl_3$) of compound 260



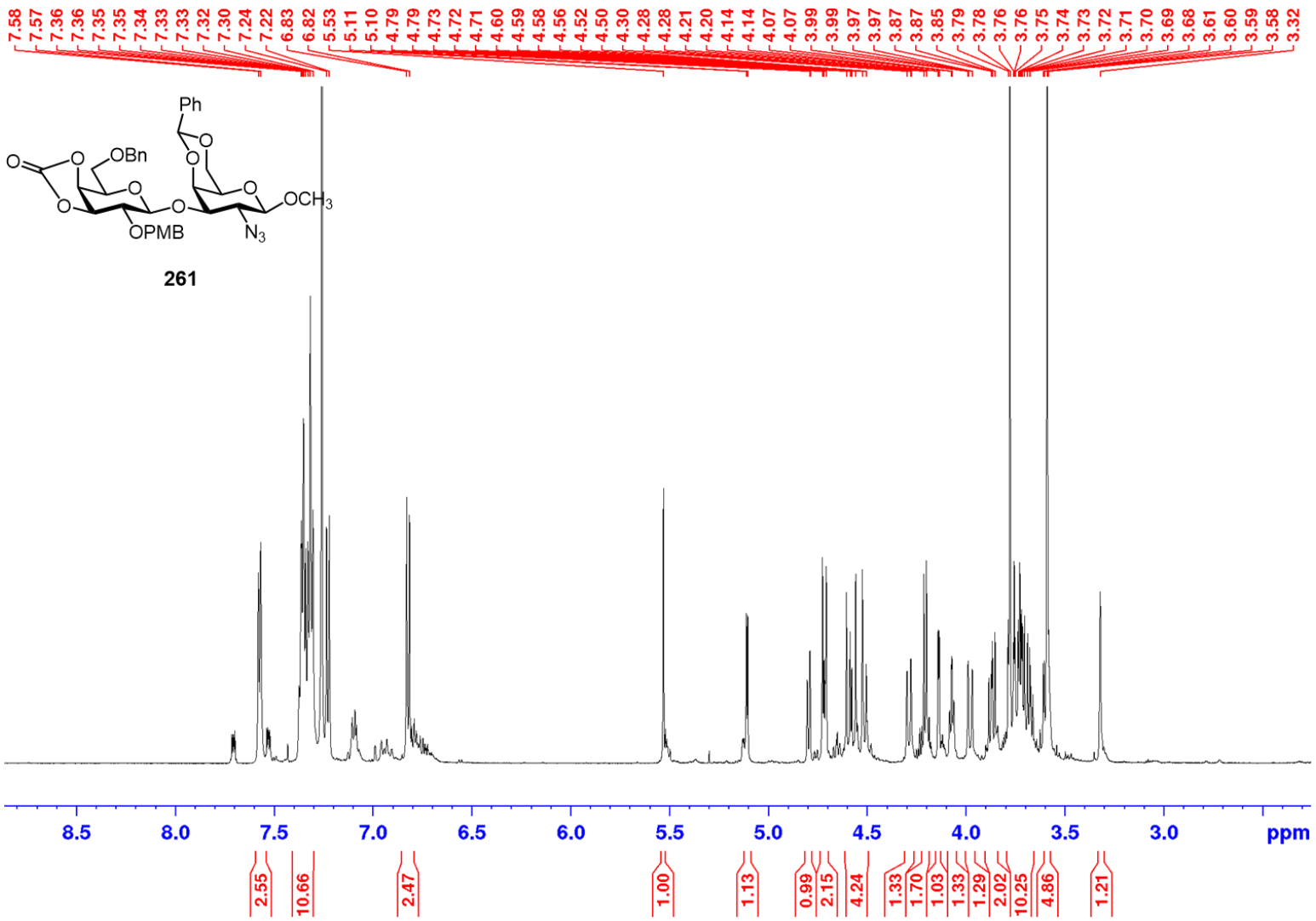
Methyl 6-O-benzyl-3,4-*O*-carbonyl-2-*O*-p-methoxybenzyl-β-D-galactopyranosyl-(1→3)-4,6-di-*O*-benzylidene acetal-2-deoxy-2-azideo-β-D-galactopyranoside (261)



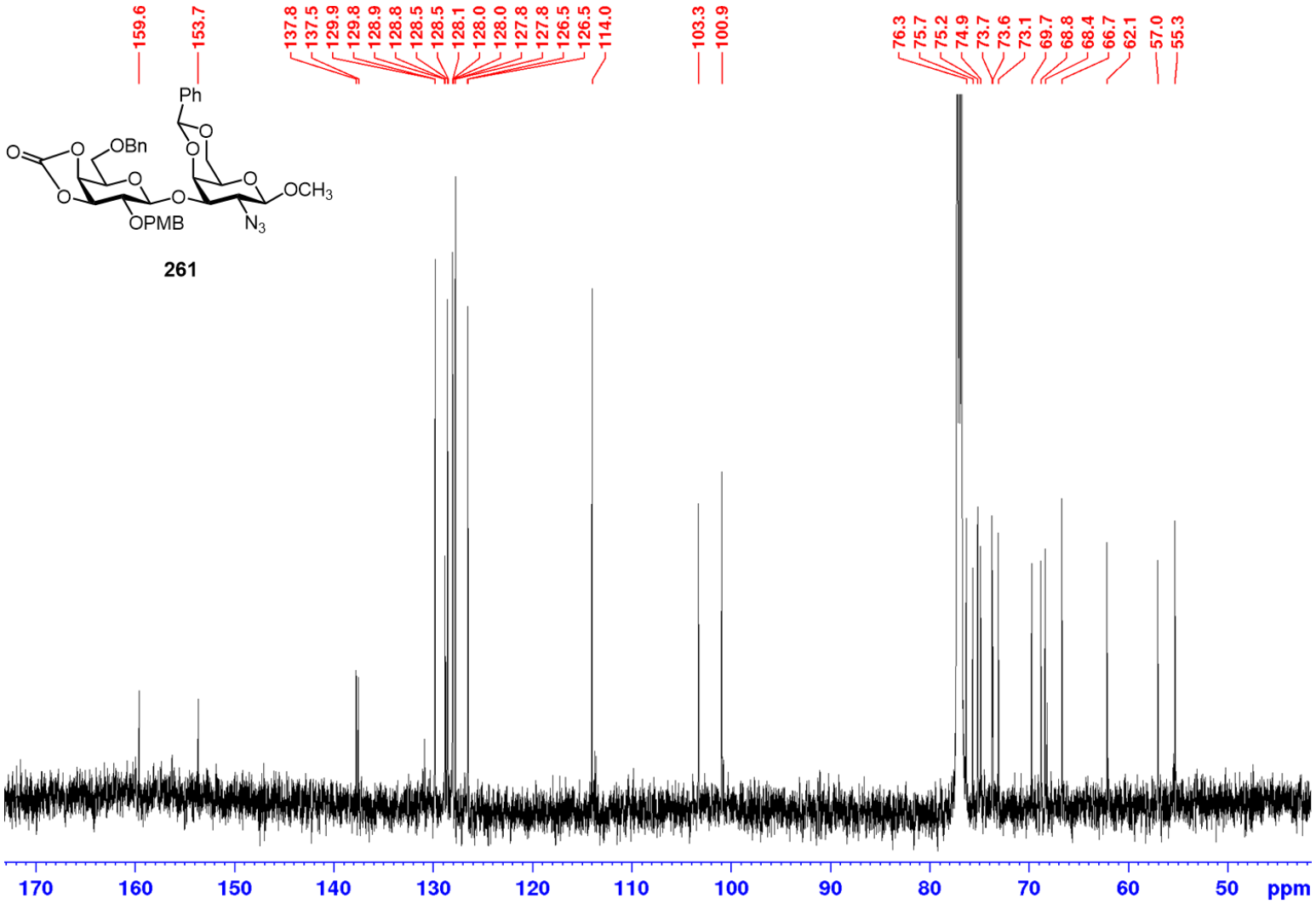
A solution of methyl 6-*O*-benzyl-3,4-*O*-carbonyl-β-D-galactopyranosyl-(1→3)-4,6-di-*O*-benzylidene acetal-2-deoxy-2-azideo-β-D-galactopyranoside **260** (1.0 equiv., 0.240 g, 0.410 mmol) in dry CH_2Cl_2 (4.0 mL), under argon, was stirred over powder 4 Å MS (600 mg) for 30 min. and cooled to -30 °C. The solution was added PMB-imidate (1.5 equiv., 0.200 g, 0.128 mL, 0.615 mmol) followed by $BF_3{\cdot}Et_2O$ (0.05 equiv., 2.6 µL, 0.020 mmol) dropwise. At 15 min., the starting material appeared to be mostly consumed by TLC. At 30 min, triethylamine was added to neutralize the crude reaciton mixture. The reaction was warmed to room temperature and filtered through glass fritted vacuum filter funnel equiped with a pad of celite. The filtrate was concentrated to a crude oil. The oil was purified by silica gel flash colimn chromatography (30:70 to 60:40 EtOAc/ hexanes) to give methyl 6-*O*-benzyl-3,4-*O*-carbonyl-2-O-*p*-methoxybenzyl-β-D-galactopyranosyl-(1→3)-4,6-di-*O*-benzylidene acetal-2-deoxy-2-azideo-β-D-galactopyranoside **261** (0.265 g, 0.376 mmol, 92%) as a white foam.; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.57 (d, *J* = 7.1 Hz, 3H), 7.36-7.30 (m, 10H), 6.82 (d, *J* = 8.66 Hz, 2H), 5.53 (s, 1H), 5.11 (d, *J* = 4.6 Hz, 1H), 4.8 (dd, *J* = 1.8, 8.6 Hz, 1H), 4.73-4.71 (m, 2H), 4.60-4.50 (m, 4H), 4.29 (d, *J* = 1.3, 12.1 Hz, 1H), 4.21 (d, *J* = 8 Hz, 2H), 4.14 (d, *J* = 3.5 Hz, 1H), 4.07, 4.06 (m, 1H), 3.98 (dd, *J* = 1.2, 12.8 Hz, 1H), 3.87 (m, 2H), 3.73 (dd, *J* = 7.8, 10.8 Hz, 10H), 3.59 (m, 5H), 3.32 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 159.6, 153.6, 137.7, 137.5, 129.8, 129.8, 128.8, 128.8, 128.5, 128.5, 128.0, 128.0,

127.9, 127.8, 127.7, 126.5, 126.5, 114.0, 103.2, 100.9, 76.3, 75.6, 75.1, 74.8, 73.7, 73.6, 73.1, 69.7, 68.8, 68.3, 66.6, 62.1, 57.0, 55.2.

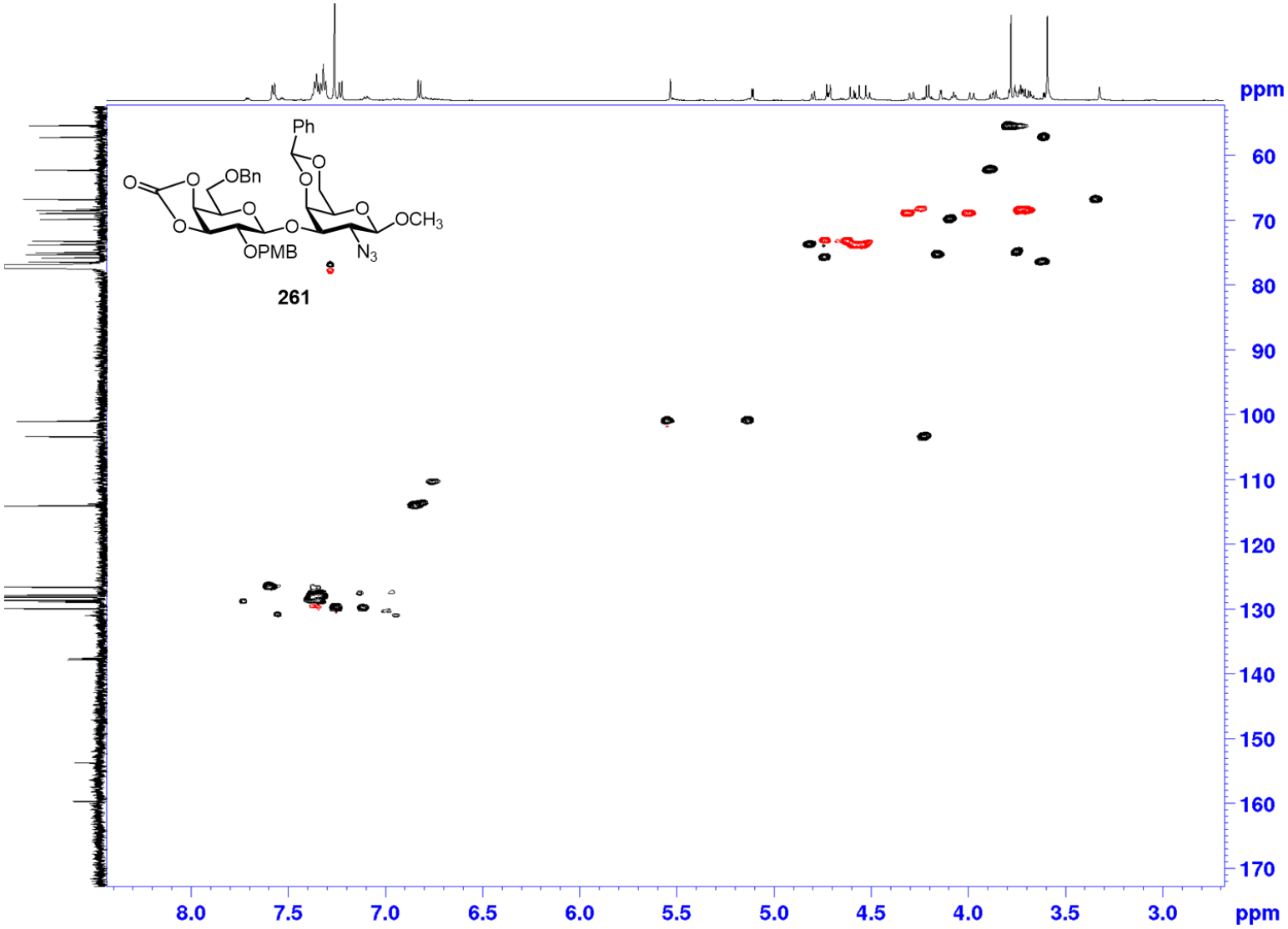
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 261



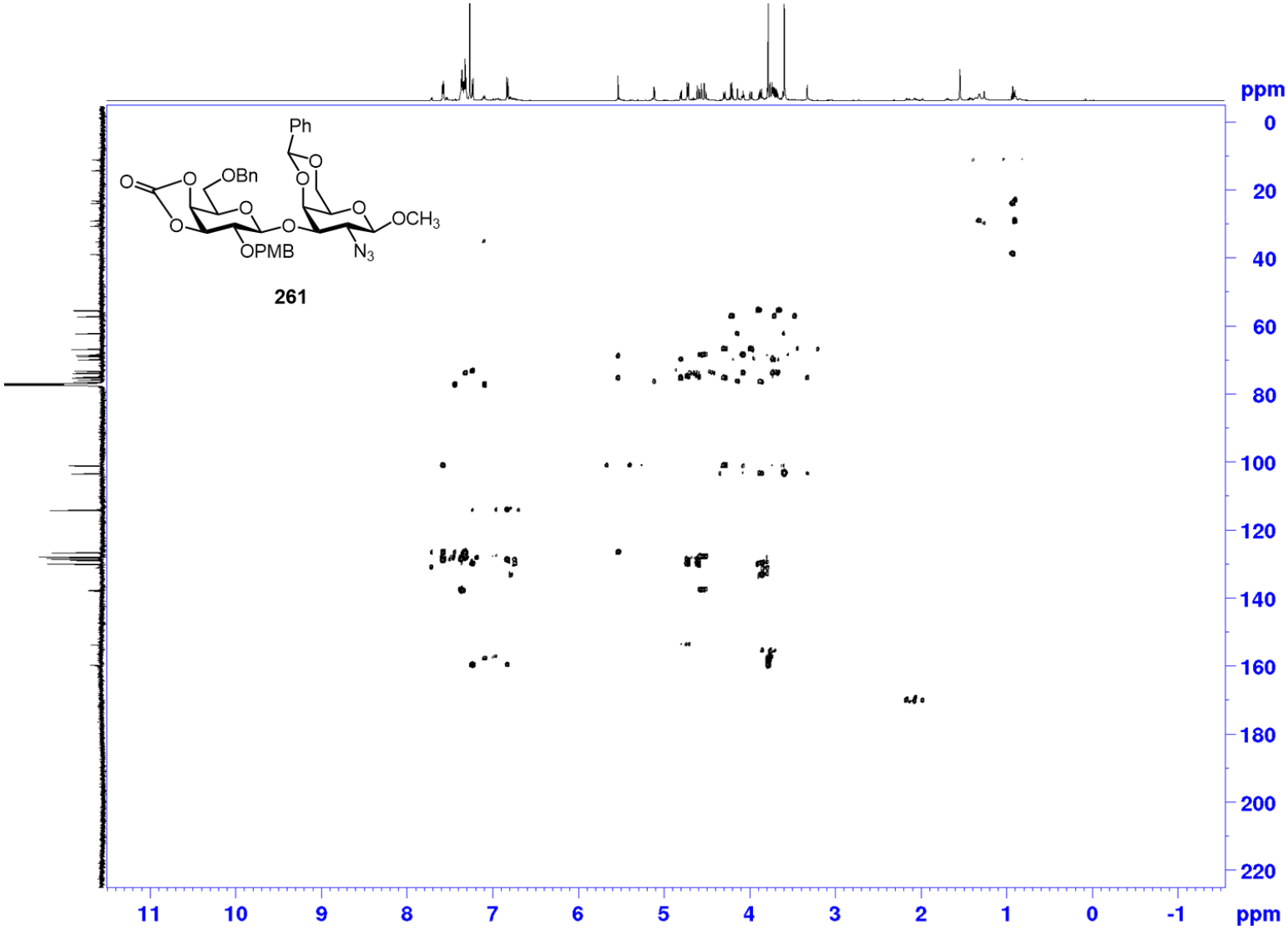
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 261



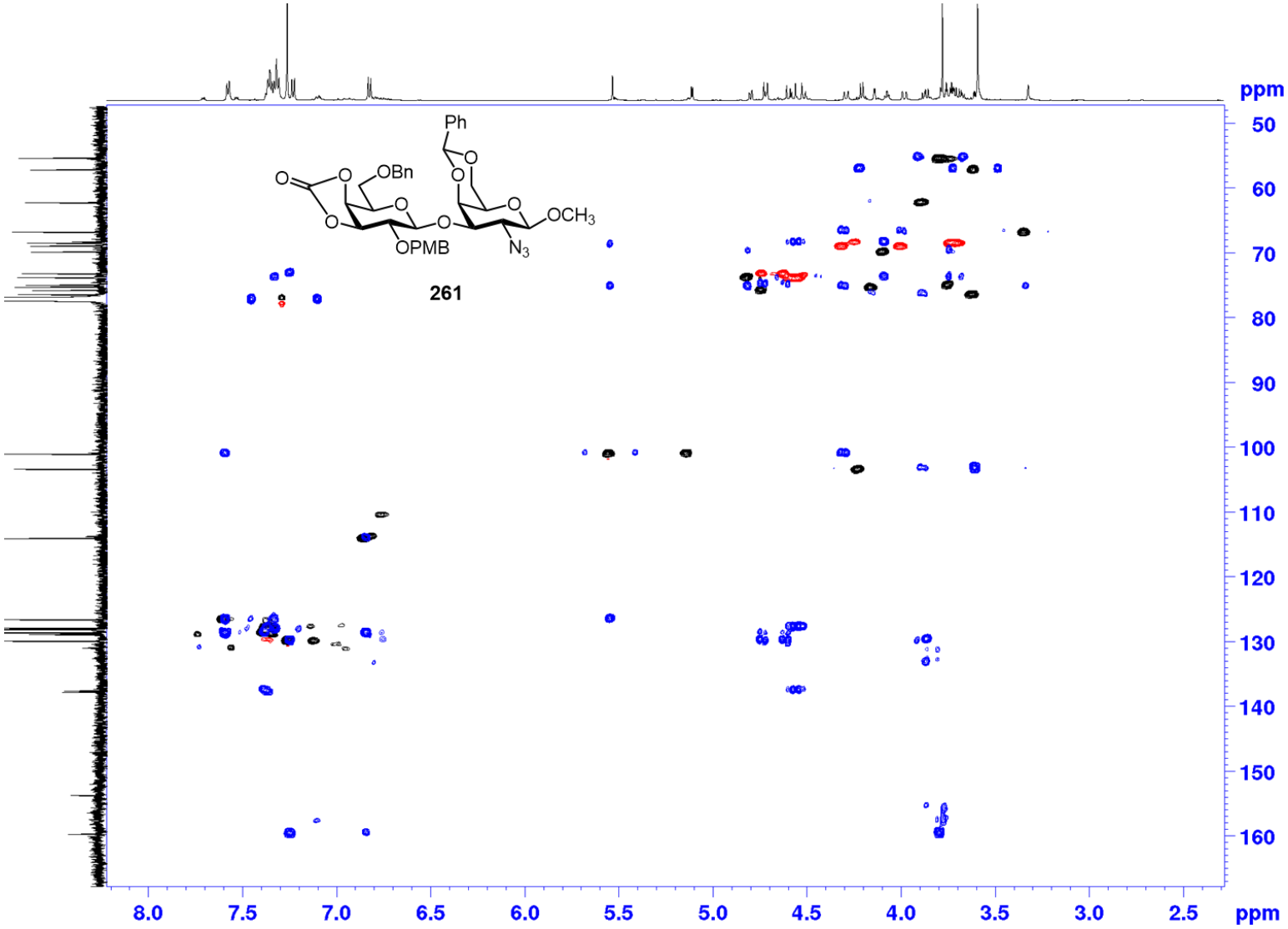
^{1}H-^{13}C HSCQ ($CDCl_3$) of compound 261



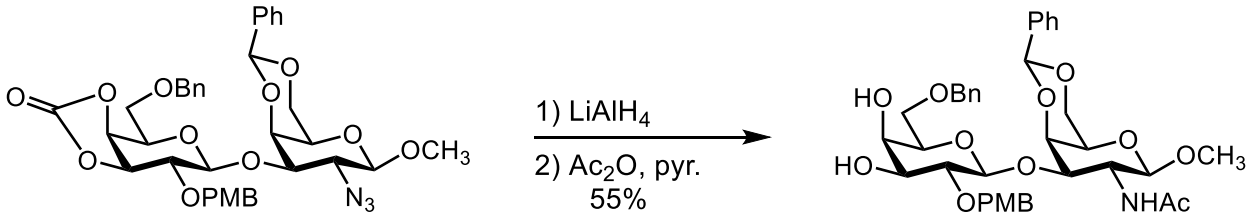
^{1}H-^{13}C HMBC ($CDCl_3$) of compound 261



^{1}H-^{13}C HSCQ with ^{1}H-^{13}C HMBC overlay ($CDCl_3$) of compound 261



Methyl 6-*O*-benzyl-2-O-*p*-methoxybenzyl-β-D-galactopyranosyl-(1→3)-4,6-di-*O*-benzylidene acetal-2-deoxy-2-acetamido-β-D-galactopyranoside (262)

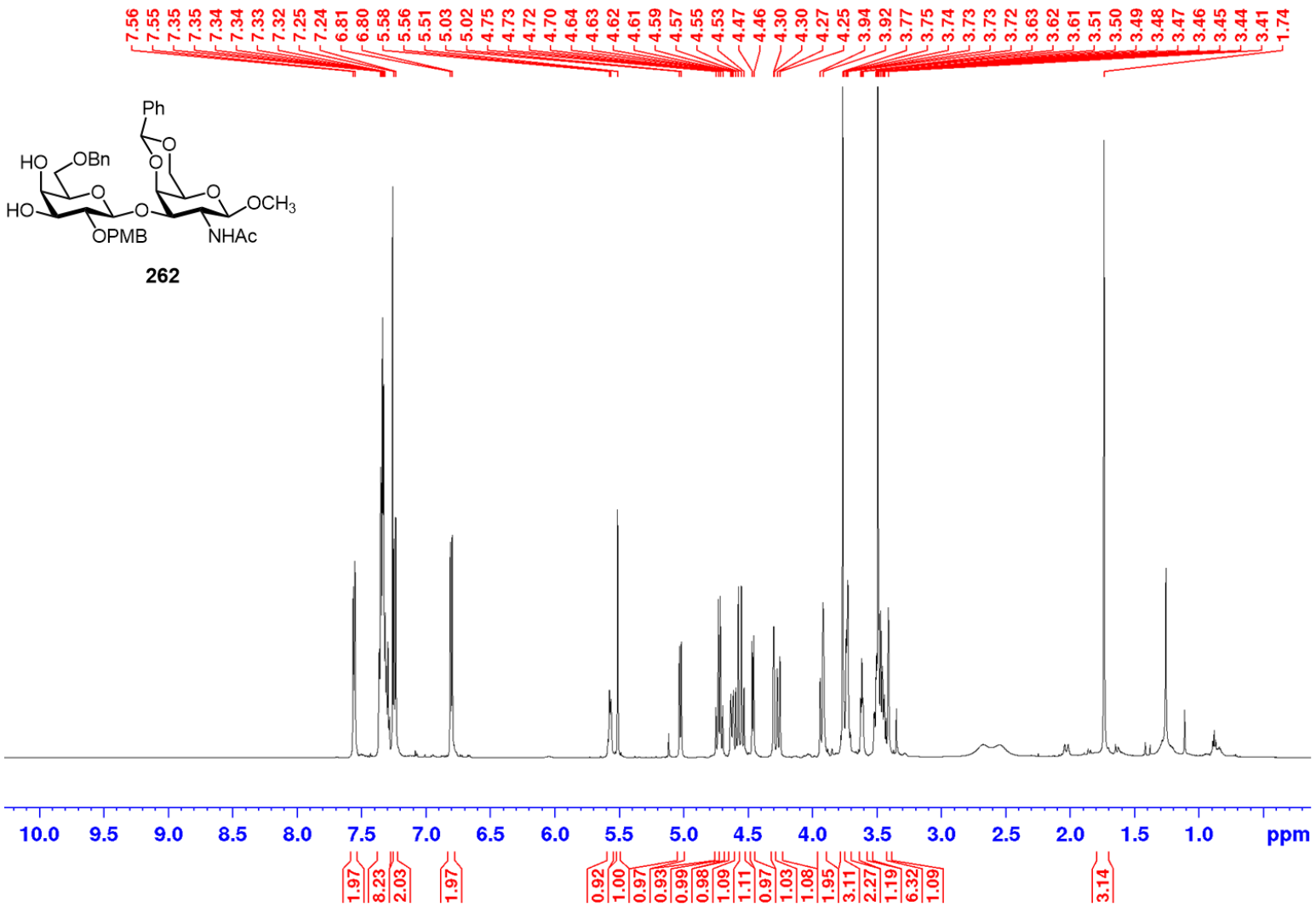


261 **262**

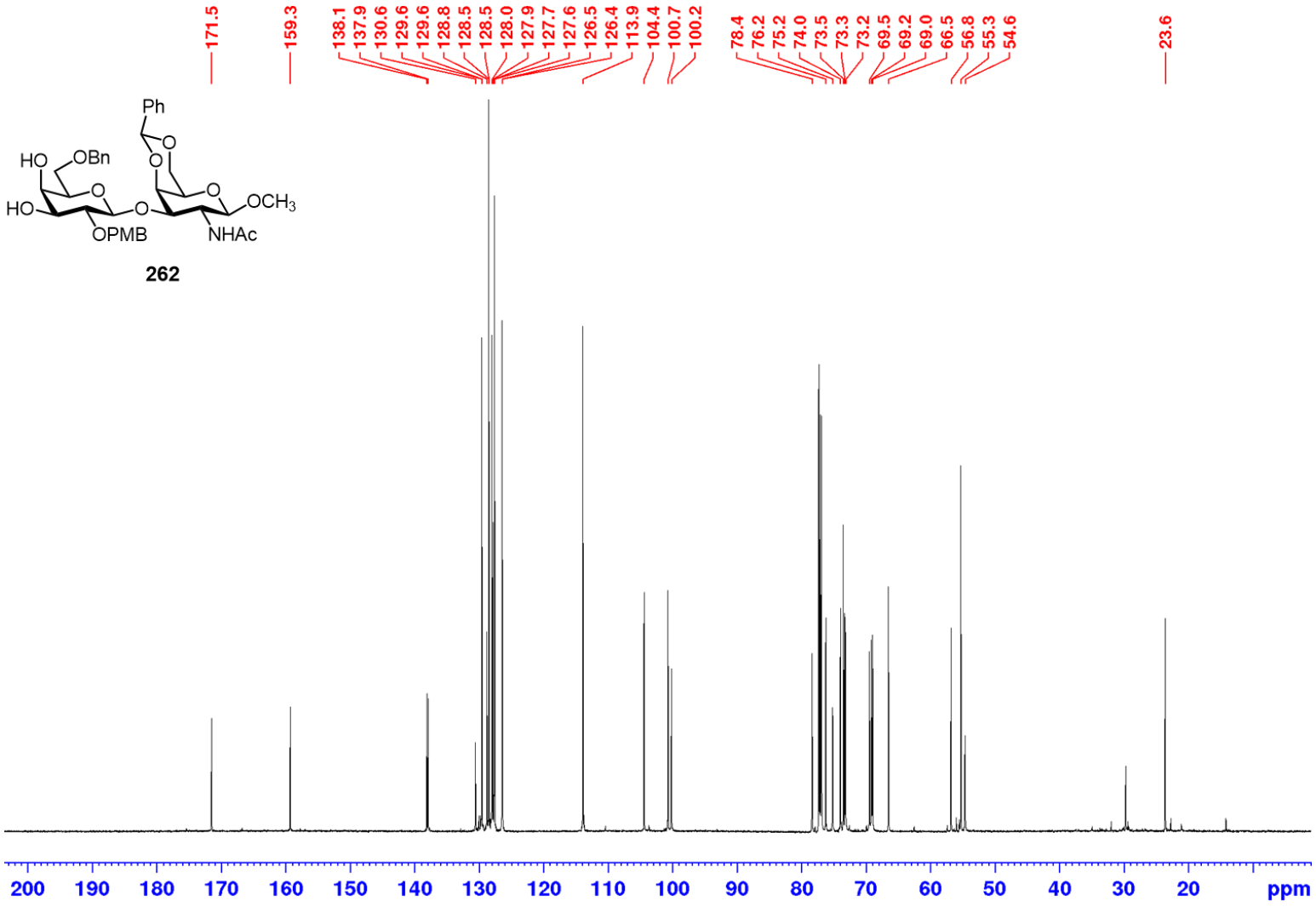
A solution of methyl 6-*O*-benzyl-3,4-*O*-carbonyl-2-O-*p*-methoxybenzyl-β-D-galactopyranosyl-(1→3)-4,6-di-*O*-benzylidene acetal-2-deoxy-2-azideo-β-D-galactopyranoside **261** (1.0 equiv., 0.2006 g, 0.295 mmol) was dissolved in dry THF (4.0 mL, [0.1 M]) and added a solution of lithium aluminium hydride (4.0 equiv., 0.49 mL, [2.4 M solution in THF]) dropwise at -15 °C and allowed to warm to room temperature. At 1.5 hours, the reaction was cooled to -15 °C and added water (0.05 mL), then 15% aq. NaOH (0.05 mL), followed by water again (0.15 mL). The reaction was warmed to room temperature and added $MgSO_4$. At 5 min, the crude reaction mixture was filtered through glass fritted vacuum filter funnel equiped with a pad of celite with MeOH to remove the solid and drying agent. The crude reaction filtrate was concentrated down to a white solid. The crude reaction was added Ac_2O (36 equiv., 1.0 mL, 10.6 mmol) and MeOH (2.0 mL) and stirred at room temperature for 1h. The reaction was concentrated down to a white solid and purified through a silica gel flash column chromatagraphy (3:97 to 5:95 MeOH/CH_2Cl_2) to give mthyl 6-*O*-benzyl-2-O-*p*-methoxybenzyl-β-D-galactopyranosyl-(1→3)-4,6-di-*O*-benzylidene acetal-2-deoxy-2-acetamido-β-D-galactopyranoside **262** (0.1121 g, 0.161 mmol, 55%) as a white solid. Rf = 0.35 (10:90 CH_3OH/CH_2Cl_2) visualized with ceric ammonium molybdate stain; ^1H NMR (400 MHz, $CDCl_3$): δ 7.56 (d, *J* = 6.8 Hz,

2H), 7.35-7.32 (m, 8H), 7.24 (d, *J* = 8.5 Hz, 2H), 6.8 (d, *J* = 8.5 Hz, 2H), 5.57 (d, *J* = 6.8 Hz, 1H), 5.51 (s, 1H), 5.02 (d, *J* = 7.9 Hz, 1H), 4.74 (d, *J* = 11 Hz, 1H), 4.71 (d, *J* = 11 Hz, 1H), 4.63 (dd, *J* = 3.1, 11 Hz, 1H), 4.58 (d, *J* = 10.5 Hz, 1H), 4.54 (d, *J* = 10.5 Hz, 2H), 4.47 (d, *J* = 7.5 Hz, 1H), 4.31 (d, *J* = 3.1 Hz, 1H), 4.26 (d, *J* = 11.8 Hz, 1H), 3.93-3.92 (m, 2H), 3.77 (s, 3H), 3.75-3.72 (m, 2H), 3.63-3.61 (m, 1H), 3.51-3.44 (m, 6H), 3.41-3.40 (m, 1H), 1.75 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.5, 159.3, 138.1, 137.9, 130.6, 129.6, 129.6, 128.8, 128.5, 128.5, 128.0, 127.8, 127.6, 127.6, 126.4, 126.4, 113.9, 104.4, 100.7, 100.1, 78.3, 76.2, 75.1, 73.9, 73.5, 73.2, 73.1, 69.4, 69.1, 68.9, 66.5, 56.8, 55.3, 54.6, 23.5.

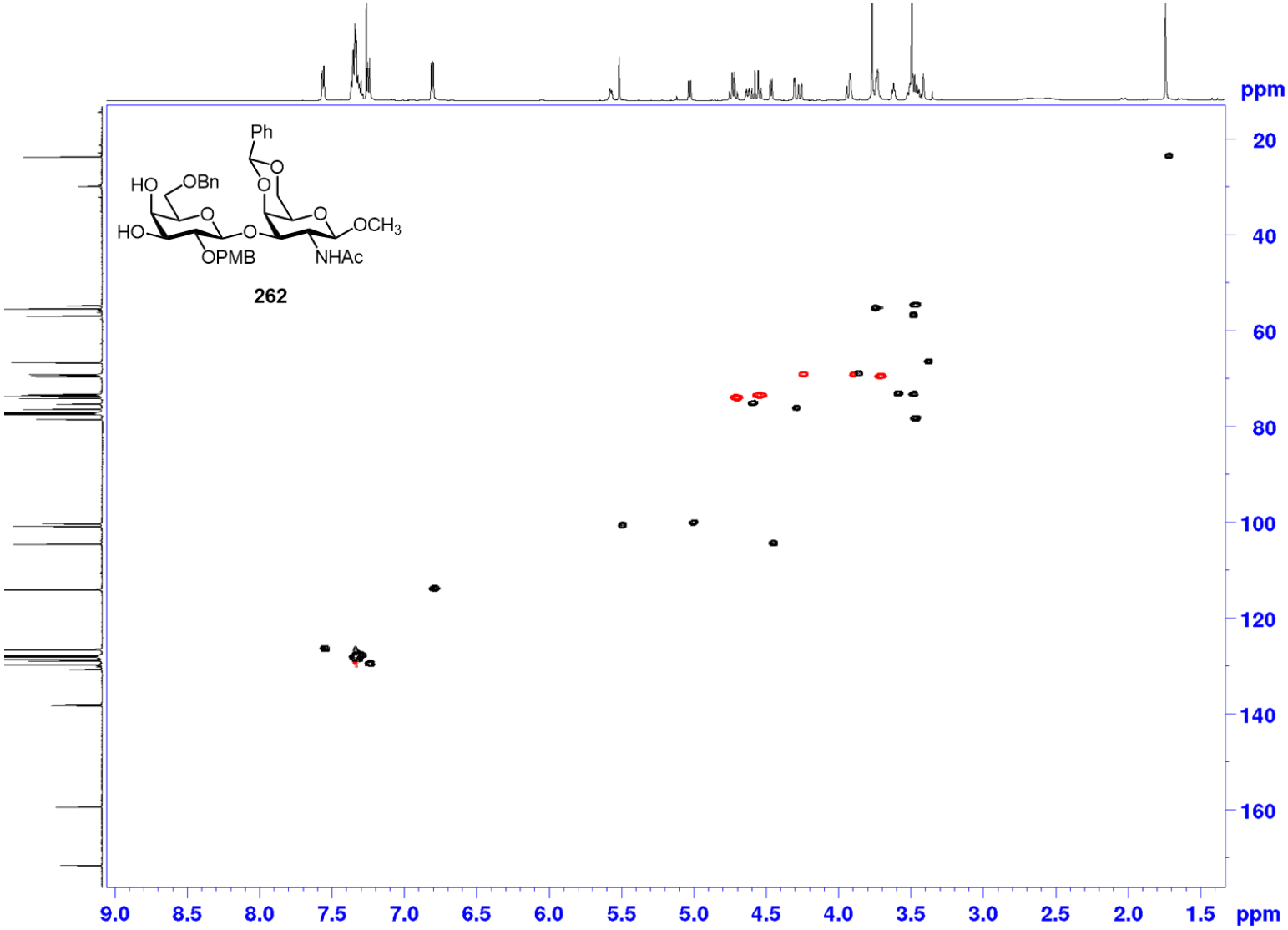
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 262



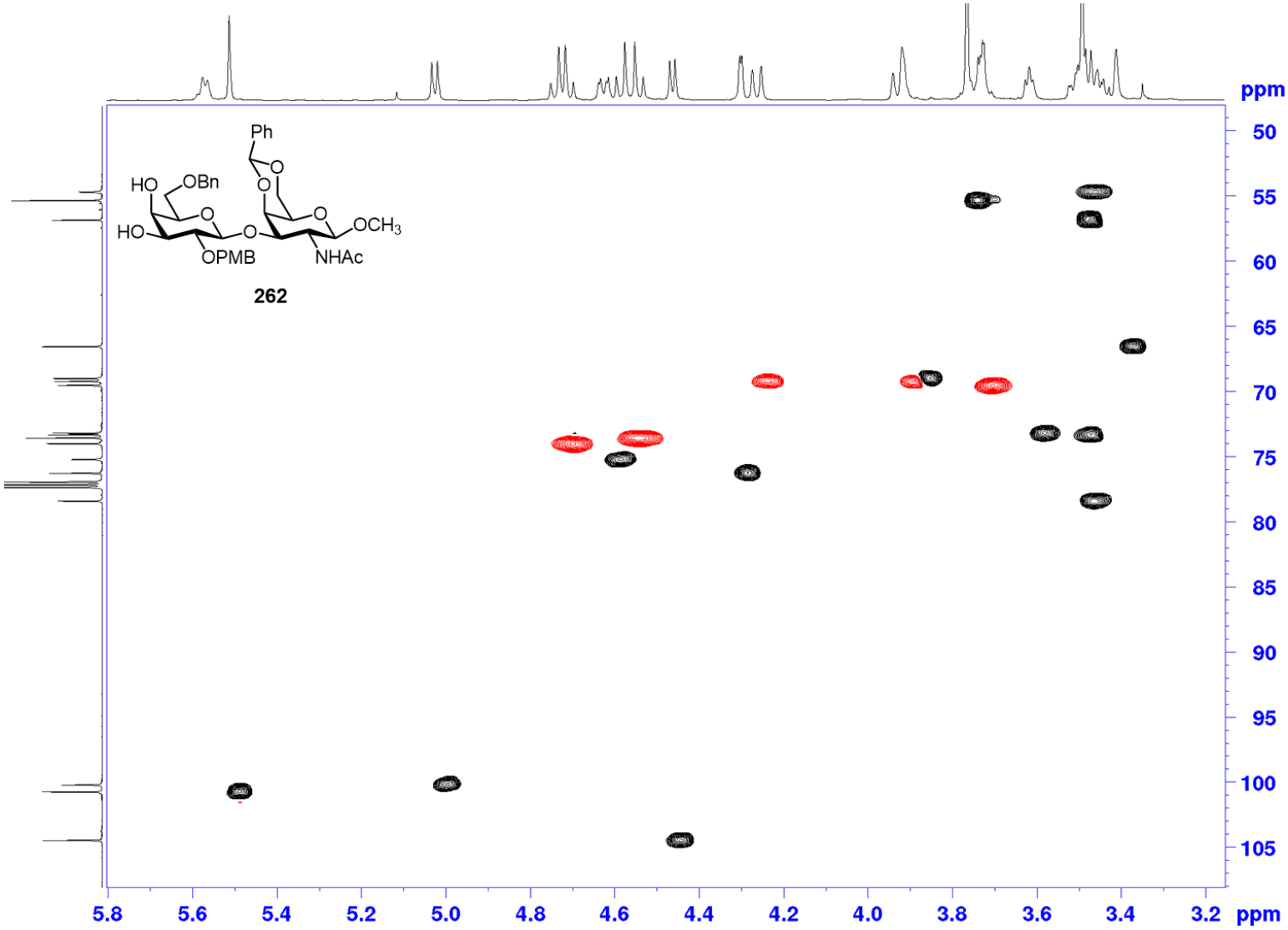
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 262



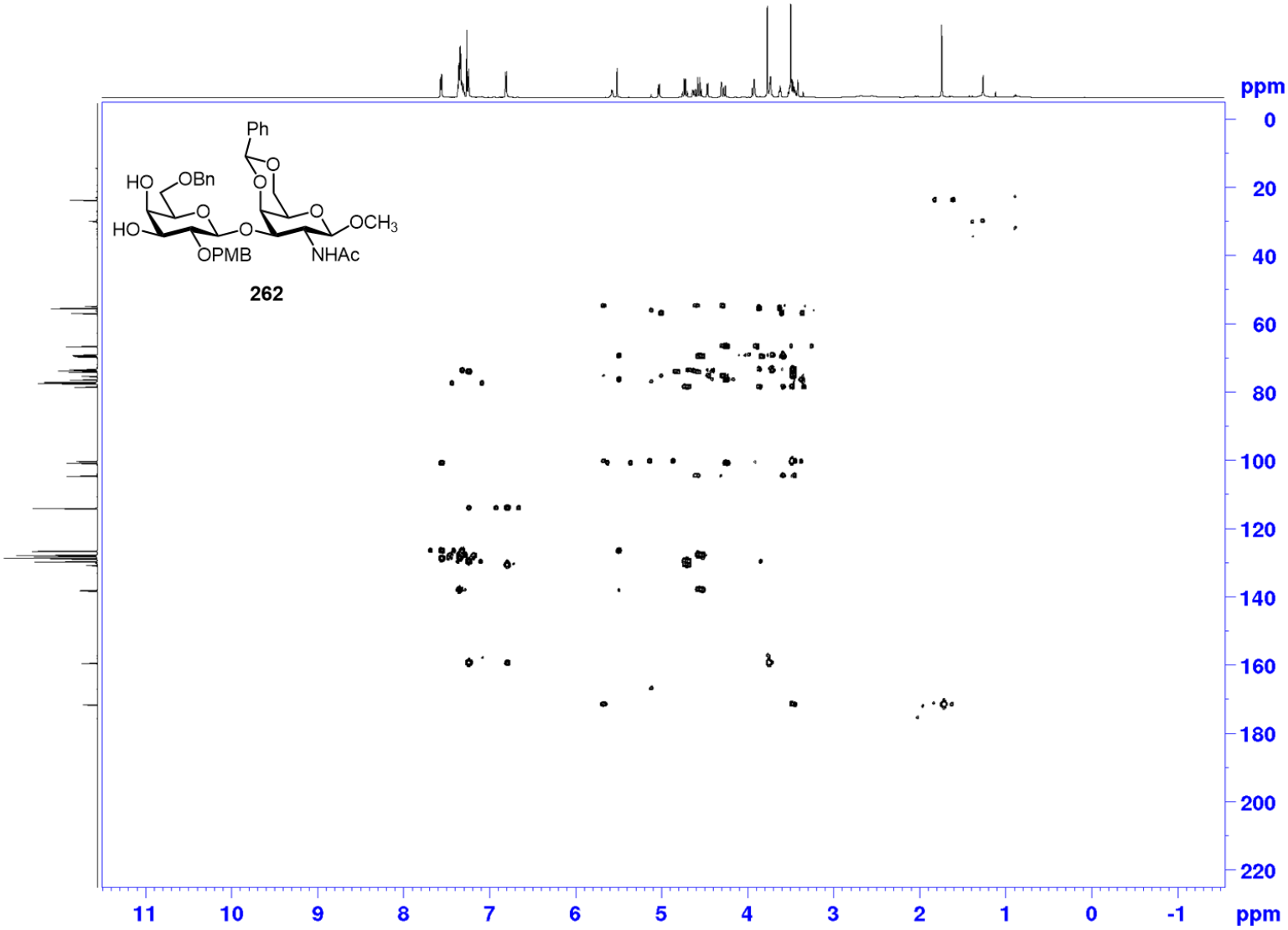
^{1}H-^{13}C HSCQ ($CDCl_3$) of compound 262



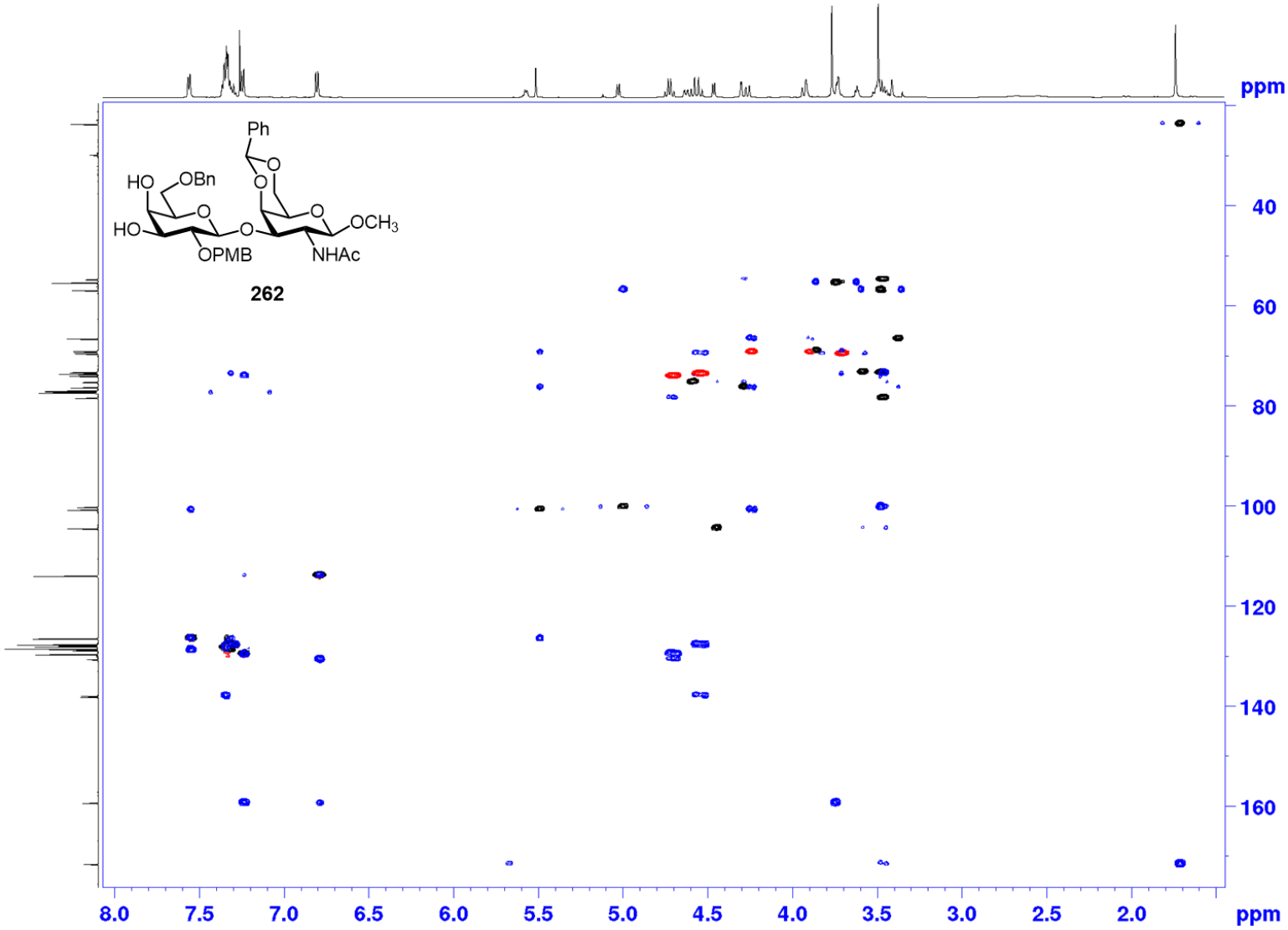
^{1}H-^{13}C HSCQ ($CDCl_3$) zoomed in region of compound 262



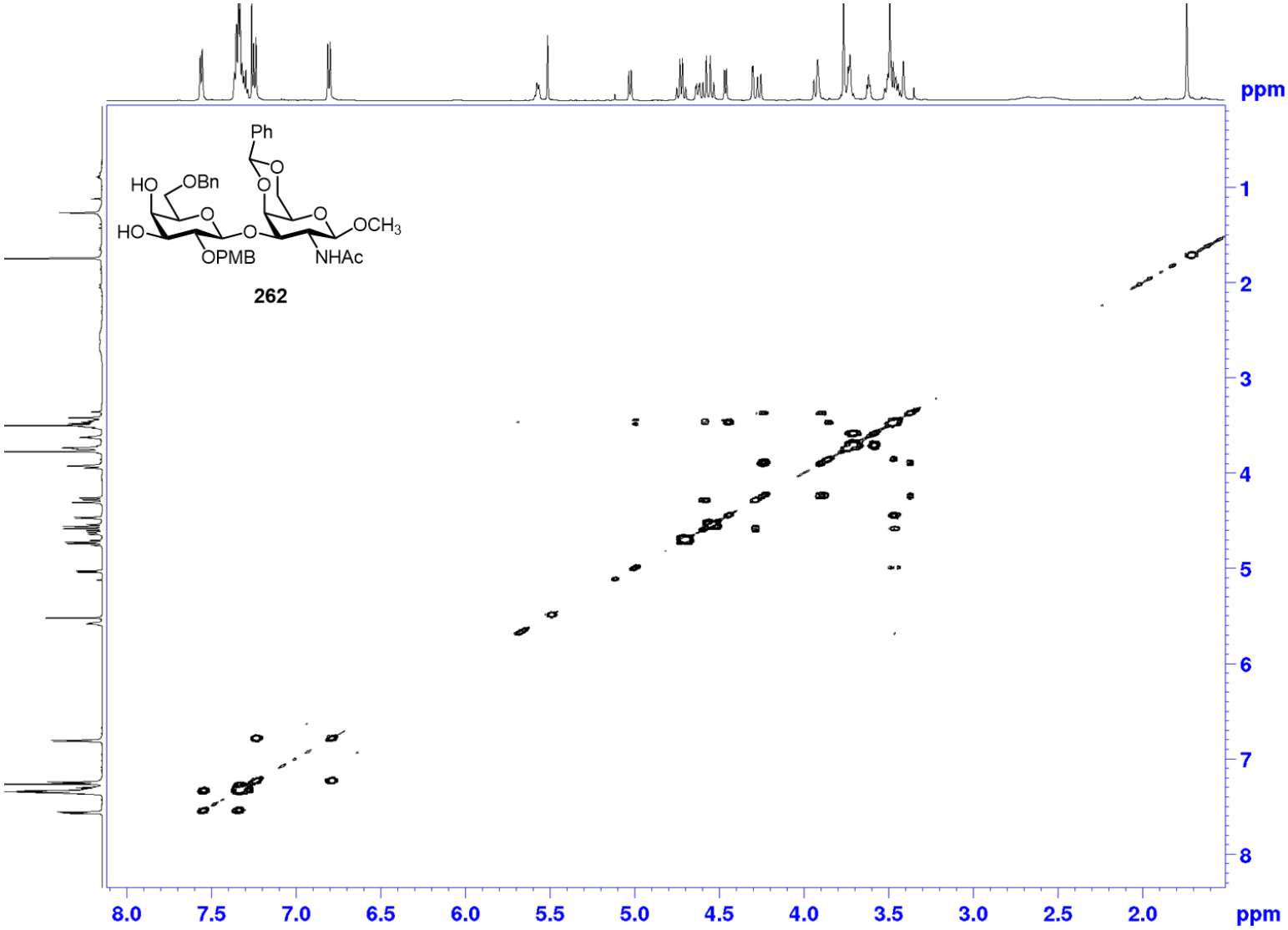
^{1}H-^{13}C HMBC ($CDCl_3$) of compound 262



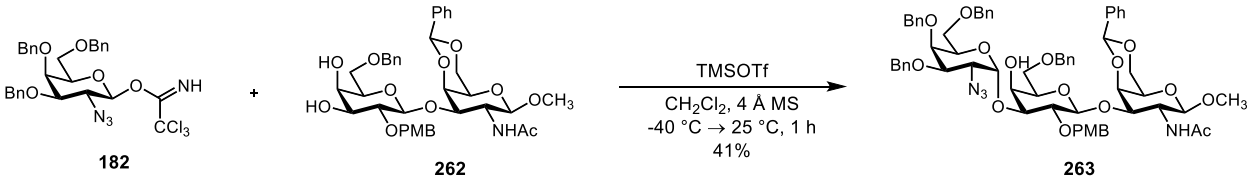
^{1}H-^{13}C HSCQ with ^{1}H-^{13}C HMBC overlay ($CDCl_3$) of compound 262



^{1}H-^{1}H COSY ($CDCl_3$) of compound 262



Methyl 3,4,6-tri-*O*-benzyl-2-deoxy-2-azido-α-D-galactopyranosyl-(1→3)-6-*O*-benzyl-2-*O*-(*p*-methoxy)-β-D-galactopyranosyl-(1→3)-4,6-*O*-benzylidene acetal-2-deoxy-2-acetamido-β-D-galactopyranoside (263)

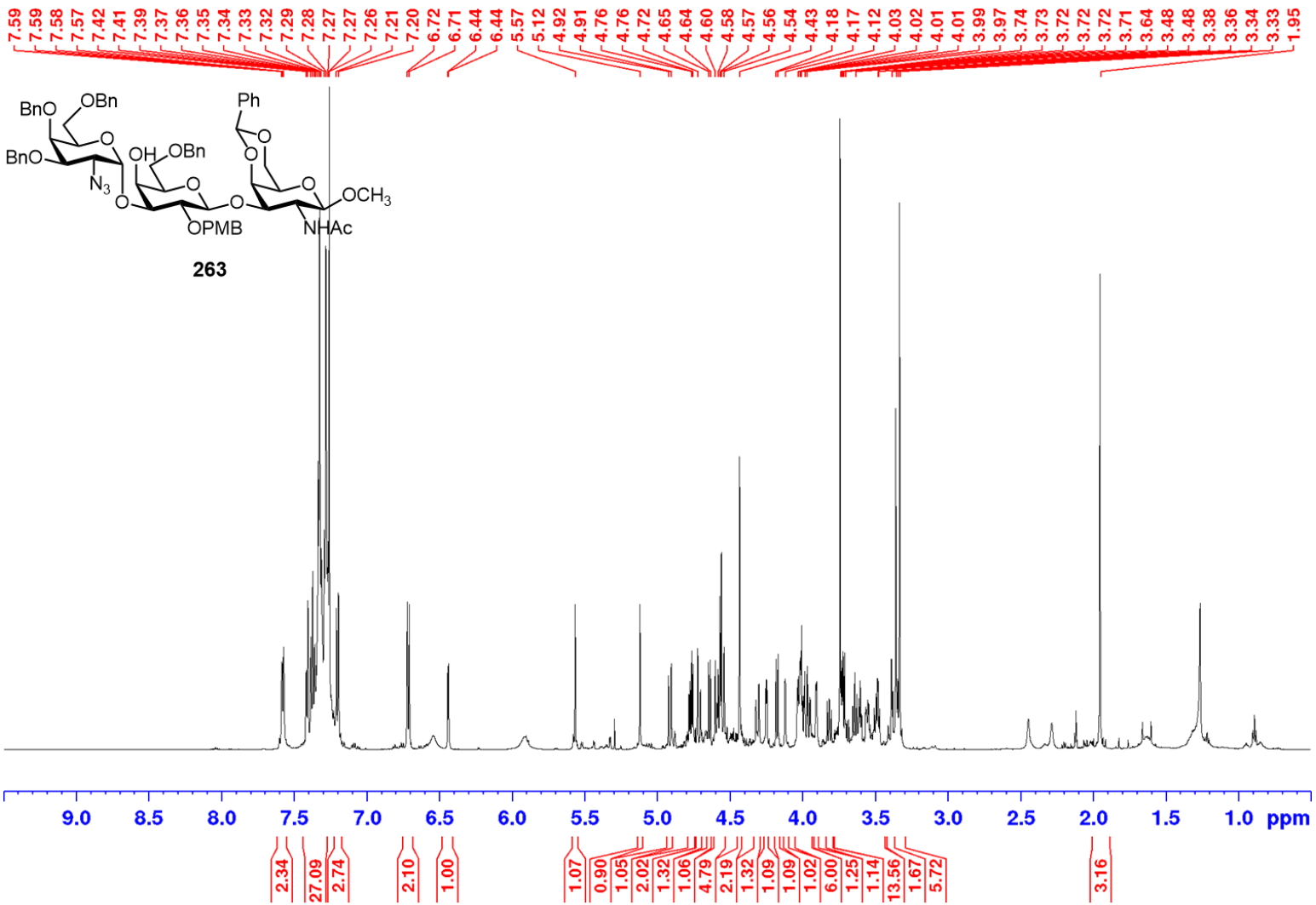


182 262 263

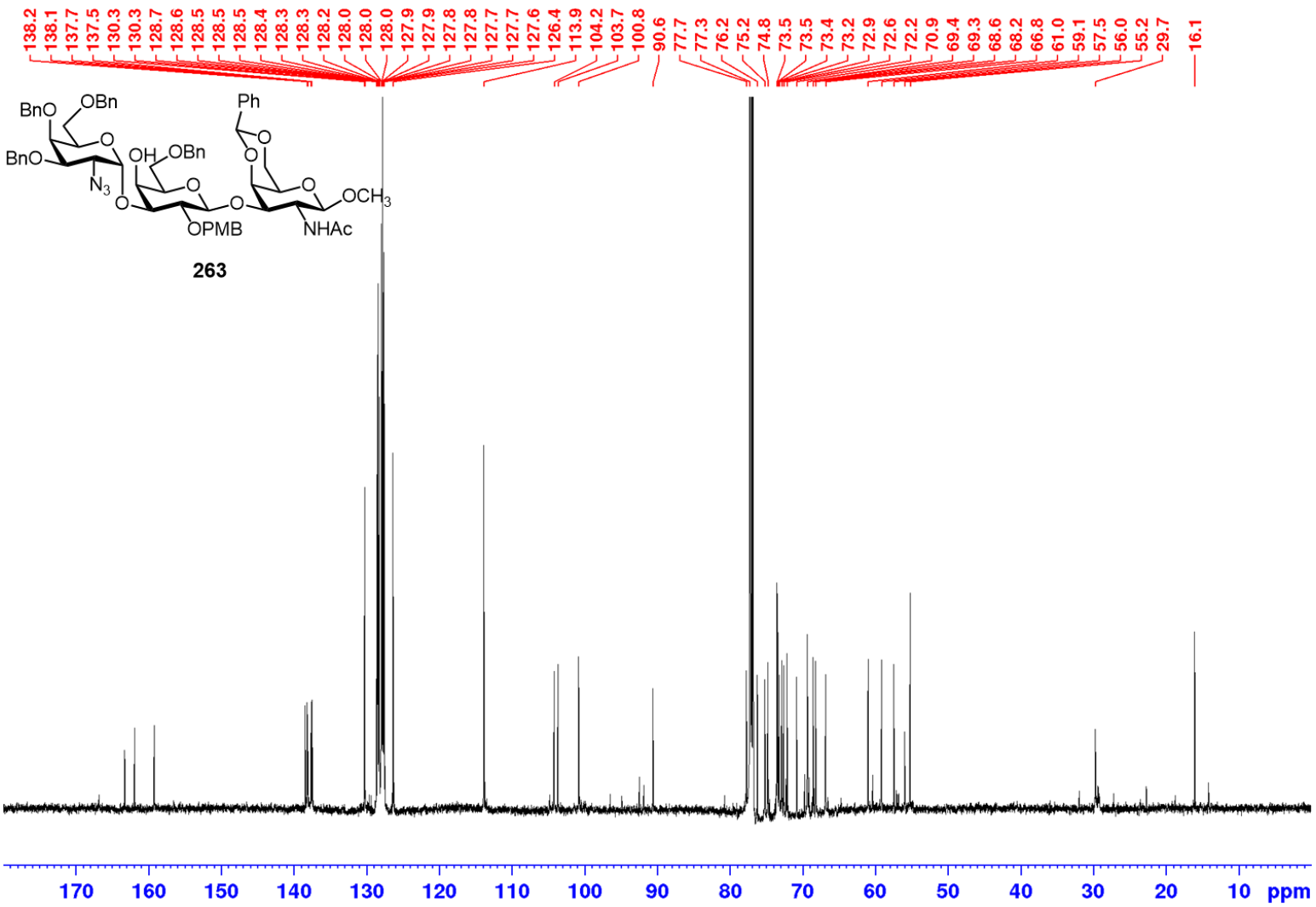
A solution of trichloroacetimidate 3,4,6-tri-*O*-benzyl-2-deoxy-2-azido-β-D-galactopyranoside 182 (1.5 equiv., 0.162 g, 0.261 mmol) and disaccharide acceptor methyl 6-*O*-benzyl-2-*O*-(*p*-methoxy)-β-D-galactopyranosyl-(1→3)-4,6-*O*-benzylidene acetal-2-deoxy-2-acetimido-β-D-galactopyranoside 262 (1.0 equiv., 0.123 g, 0.171 mmol) in a 1:4 solution of *i*Pr_2O/CH_2Cl_2 (3 mL). were added freshly activated and cooled 4 Å MS (450 mg, 15% m/v) under an argon atmosphere at room temperature. After 1 h, the reaction was cooled to -40 °C then added a solution of TMSOTf (0.05 equiv., 26 µL, 0.030 mmol, 15% in CH_2Cl_2). At 1 h, the reaction mixture was neutralized with Et_3N and added diluted with CH_2Cl_2. The reaction mixture was filtered through a pad of Celite within a glass fritted vacuum filter funnel to remove the 4 Å MS. The filtrate was concentrated to a crude oil and purified by silica gel flash column chromatography to give methyl 3,4,6-tri-O-benzyl-2-deoxy-2-azido-α-D-galactopyranosyl-(1→3)-6-*O*-benzyl-2-*O*-(*p*-methoxy)-β-D-galactopyranosyl-(1→3)-4,6-*O*-benzylidene acetal-2-deoxy-2-acetamido-β-D-galactopyranoside 263 (0.082 g, 0.071 mmol, 41%) as a white foam. Rf = 0.31 (60:40 EtOAc/hexanes) visualized with ceric ammonium molybdate stain; ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.58 (dd, *J* = 1.7, 8.0 Hz, 2H), 7.42-7.26 (m, 27H), 7.19 (m, 3H), 6.71 (d, *J* = 8.9 Hz, 2 H), 6.44 (d, *J* = 3.6 Hz, 1H), 5.56 (s, 1H), 5.12 (s, 1H), 4.91 (d, *J* = 11.3 Hz, 1H),

4.78-4.76 (m, 2H), 4.72-4.70 (m, 1H), 4.64 (d, *J* = 7.8 Hz, 1H), 4.60-5.54 (m, 5H), 4.44 (s, 2H), 4.32 (dd, *J* = 1.2, 11.9 Hz, 1H), 4.25 (d, *J* = 3.44 Hz, 1H), 4.18 (d, *J* = 7.52 Hz, 1H), 4.12 (d, *J* = 1.3 Hz, 1H) 4.04-3.95 (m ,6H), 3.91 (d, *J* = 3.0 Hz, 1H), 3.82 (dd, *J* = 7.6, 9.8 Hz, 1H), 3.78-3.43 (m, 14H), 3.41-3.37 (m, 2H), 3.36-3.29 (m, 6H), 1.95 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 163.2, 161.9, 159.2, 138.4, 138.2, 138.1, 137.6, 137.5, 130.2, 130.2, 128.6, 128.5, 128.5, 128.4, 128.4, 128.4, 128.3, 128.2, 128.2, 127.9, 127.9, 127.9, 127.9, 127.9, 127.8, 127.7, 127.7, 127.6, 127.6, 126.3, 113.8, 104.2, 103.6, 100.8, 90.6, 77.7, 77.3, 76.2, 75.2, 74.8, 73.5, 73.4, 73.3 ,73.2, 72.8, 72.6, 72.1, 70.8, 69.3, 69.3, 68.5, 68.2, 66.8, 61.0, 59.1, 57.4, 55.9, 55.2, 29.7, 16.05.

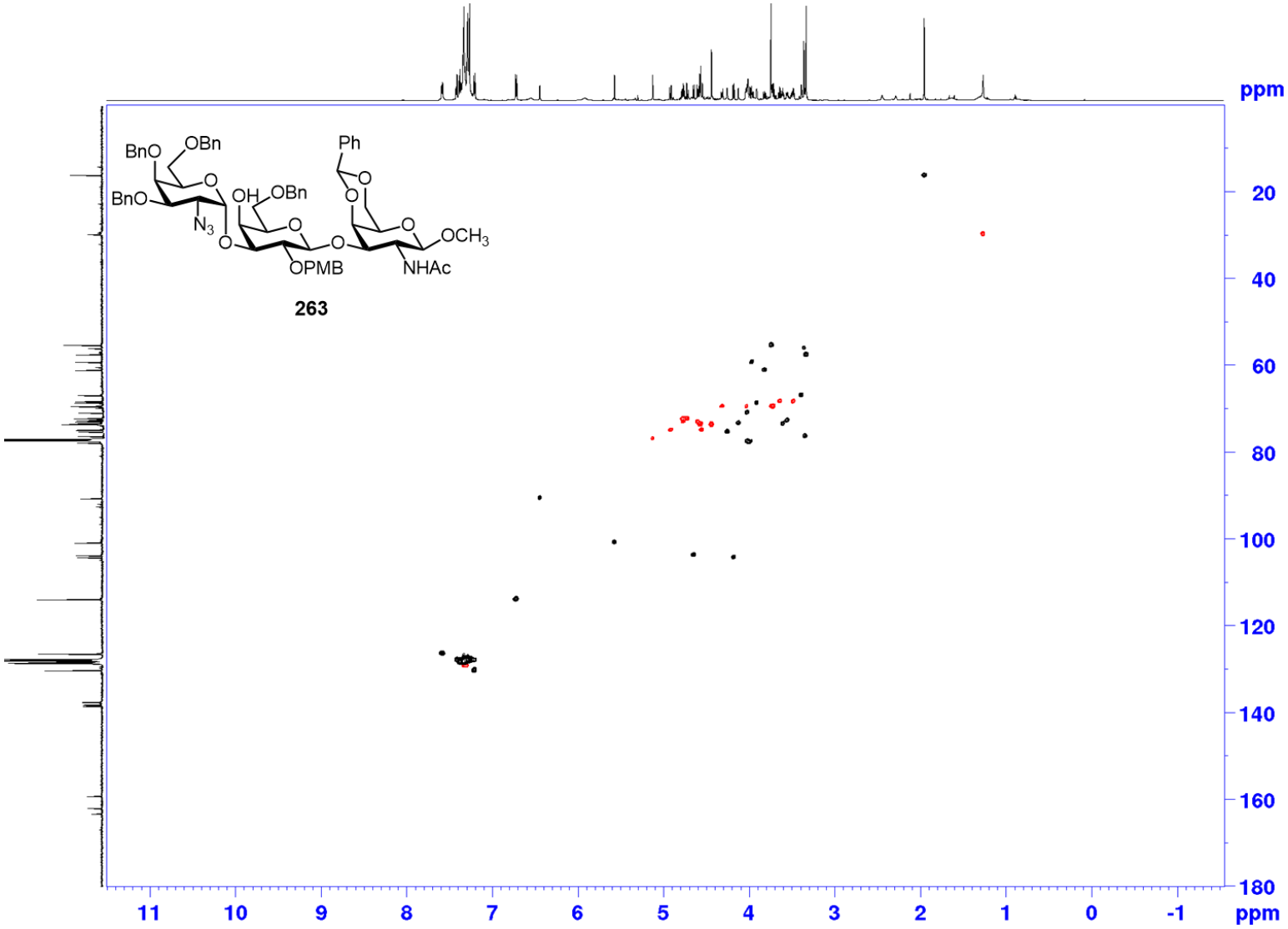
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 263



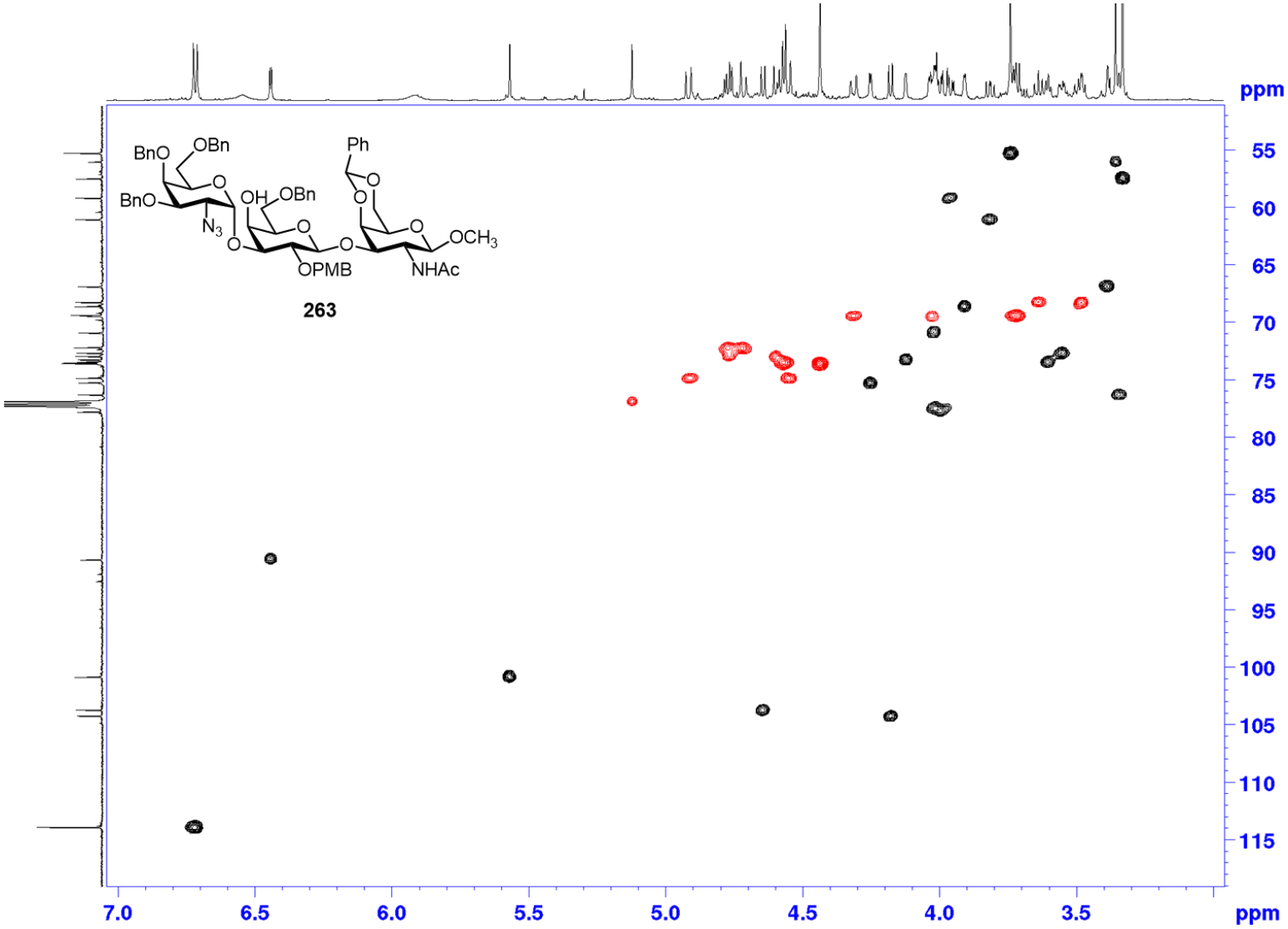
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 263



^{1}H-^{13}C HSCQ ($CDCl_3$) of compound 263



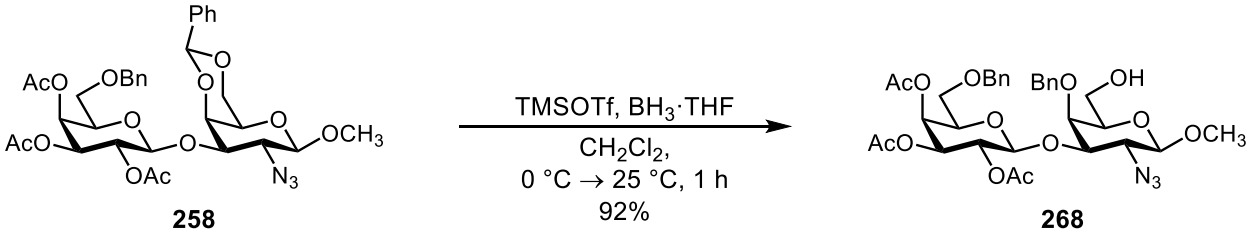
^{1}H-^{13}C HSCQ ($CDCl_3$) zoomed in region of compound 263



Part 4. MM-ZPS 2nd-generation version 2 synthetic route

Part 4 contains experimental procedures for compounds found within schemes 34-38

Methyl 6-*O*-benzyl-2,3,4-tri-*O*-acetyl-β-D-galactopyranosyl-(1→3)-4-*O*-benzyl-2-deoxy-2-azideo-β-D-galactopyranoside (268)

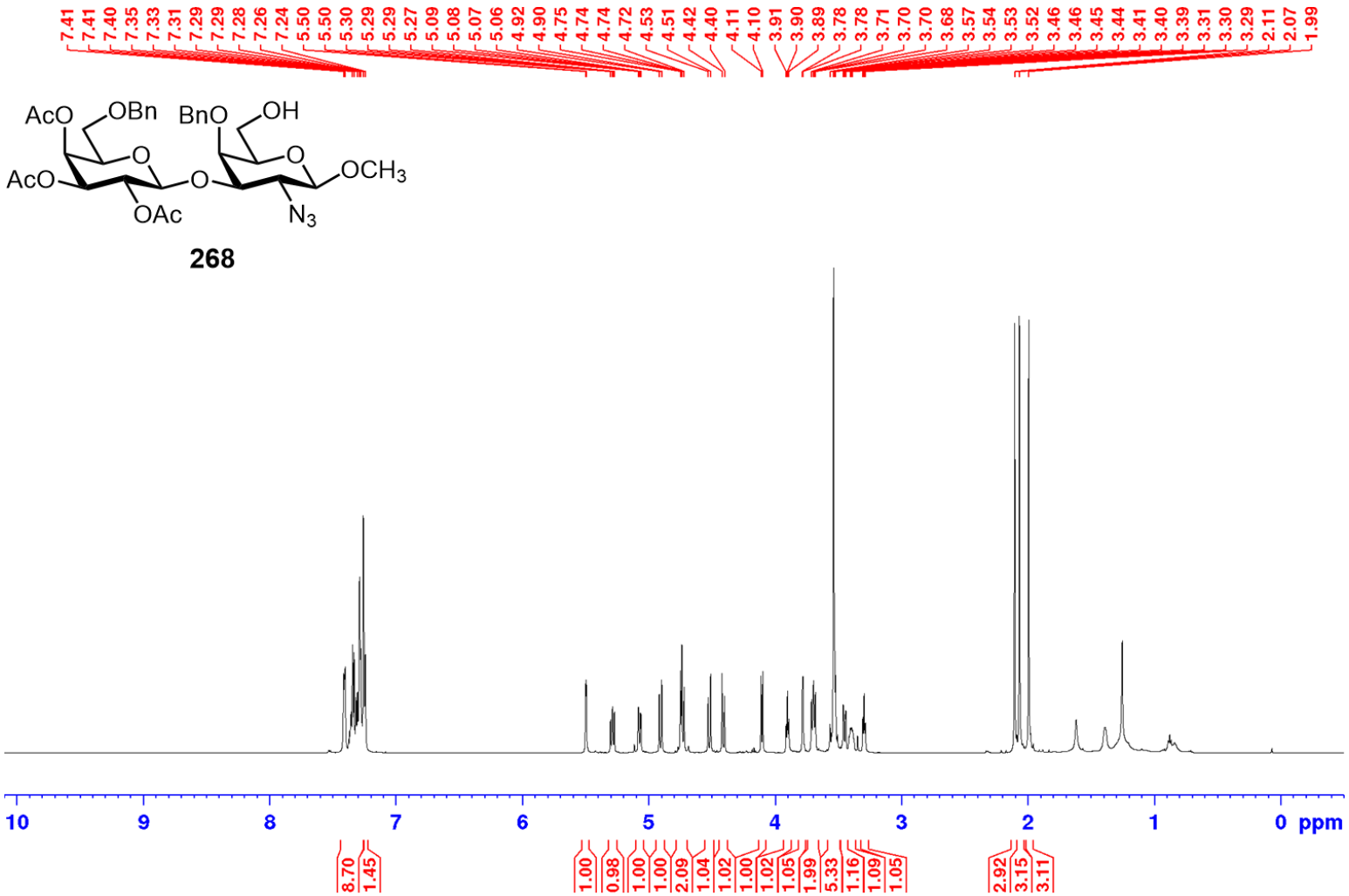


258 **268**

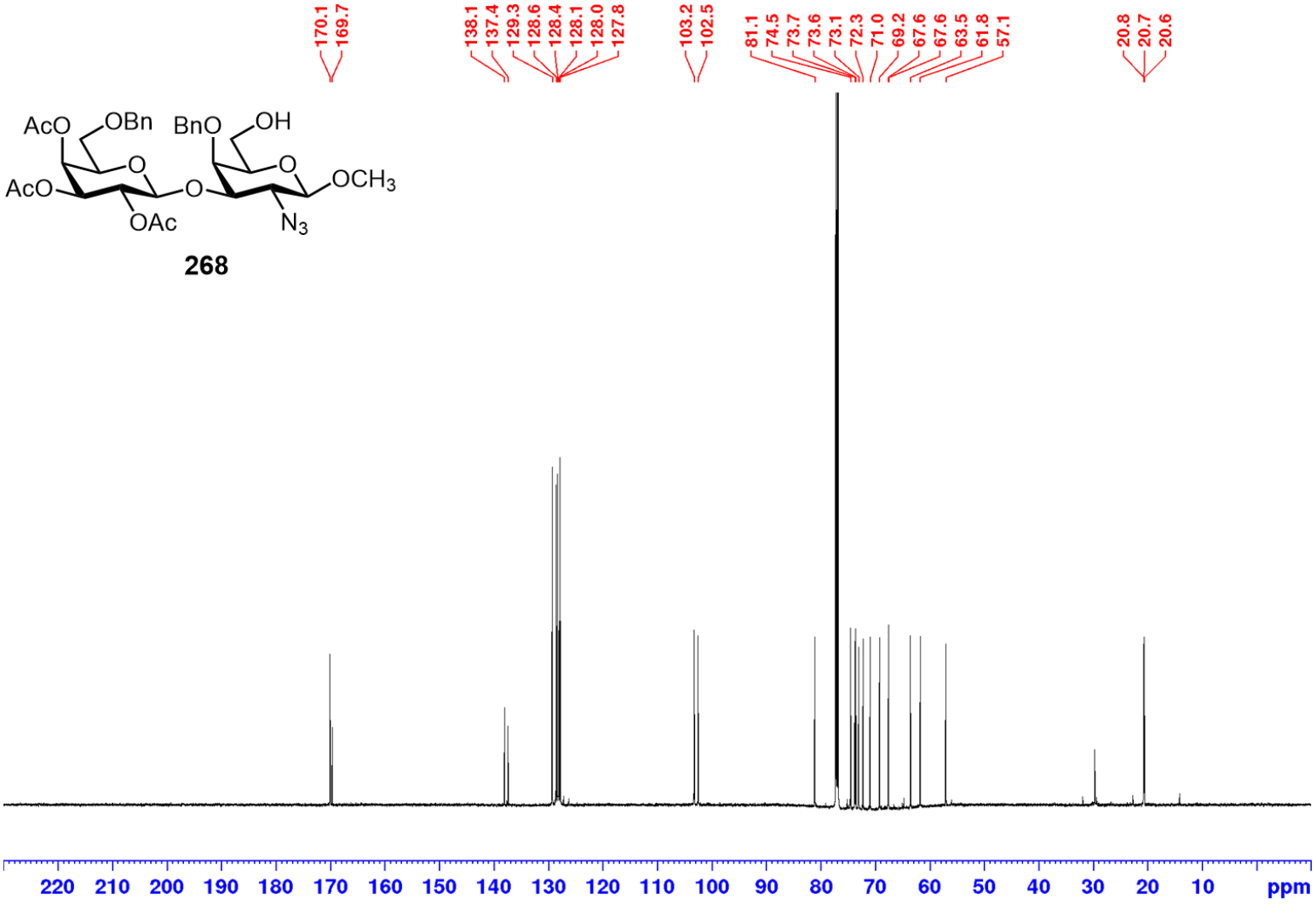
The reaction was carried out following adapted procedures developed by Hung and co-workers.[338] A solution of methyl 6-*O*-benzyl-2,3,4-tri-*O*-acetyl-β-D-galactopyranosyl-(1→3)-4,6-*O*-benzylidene acetal-2-deoxy-2-azideo-β-D-galactopyranoside **258** (1.0 equiv., 1.00 g, 4.35 mmol) in anhydrous CH_2Cl_2 (44.0 mL) was added BH_3·THF (5.0 equiv., 21.8 mL, 21.8 mmol) and a solution of TMSOTf (0.15 equiv., 0.65 mL, 0.652 mmol, 15 % in anhydrous CH_2Cl_2) at 0 °C under an argon atmosphere. The reaction mixture was stirred for 1.5 h while warming to room temperature. At 1.5 h, the reaction mixture was cooled to 0 °C and quenched by the addition of methanol and Et_3N. The reaction was concentrated to an oil then co-evaporated with methanol to remove residual borate esters. The crude reaction mixture was purified by silica gel flash column chromatography (30:70 to 60:40 EtOAc/hexanes) to give methyl 6-*O*-benzyl-2,3,4-tri-*O*-acetyl-β-D-galactopyranosyl-(1→3)-4-*O*-benzyl-2-deoxy-2-azideo-β-D-galactopyranoside **268** (0.900 g, 1.31 mmol, 90%) as a white amorphous solid.; R_f = 0.60 (3:1 EtOAc/hexanes) visualized with ceric ammonium molybdate stain; $[\alpha]D^{25}$ = -5.12° (c = 1.0, $CHCl_3$); IR ν_{max} (film) 3492.15, 2920.81, 2113.64, 1750.17, 1457.79, 1368.20, 1220.79, 1073.52 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.35 (m, 9H), 7.24 (m, 1H), 5.59 (d, *J* = 3.3 Hz, 1H), 5.29, (dd, *J* = 7.9, 10.3 Hz, 2H), 5.07 (dd, *J* = 3.4, 10.4 Hz, 1H), 4.92 (d, *J* = 11.9 Hz, 1H), 4.74 (dd,

J = 4.7, 7.7 Hz, 2H), 4.52 (d, *J* = 11.9 Hz, 1H), 4.40 (d, *J* = 11.9 Hz, 1H), 4.10 (d, *J* = 8.0 Hz, 1H), 3.90 (dd, *J* = 6.4, 0.8 Hz, 1H), 3.77 (d, *J* = 2.5 Hz, 1H), 3.70 (m, 2H), 3.54 (s, 3H), 3.53-3.52 (m, 2H), 3.45 (dd, *J* = 2.8, 10.5 Hz, 1H), 3.41 – 3.37 (m, 1H), 3.29 (dd, *J* = 6.2, 0.8 Hz, 1H), 2.11 (d, 3H), 2.07 (s, 3H), 2.00 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.1, 170.1, 169.7, 138.1, 137.4, 129.3, 129.3, 128.6, 128.6, 128.4, 128.4, 128.1, 128.0, 127.9, 127.8, 103.3, 102.5, 81.1, 74.5, 73.7, 73.6, 73.0, 72.3, 71.0, 69.2, 67.6, 67.6, 53.5, 61.8, 57.1, 20.8, 20.7, 20.6; HRMS (ESI) calcd for $C_{33}H_{45}N_4O_{13}^+$ $(M+NH_4)^+$ 705.29772, found 705.29792 *m/z*.

^{1}H NMR ($CDCl_3$, 400 MHz) of compound 268

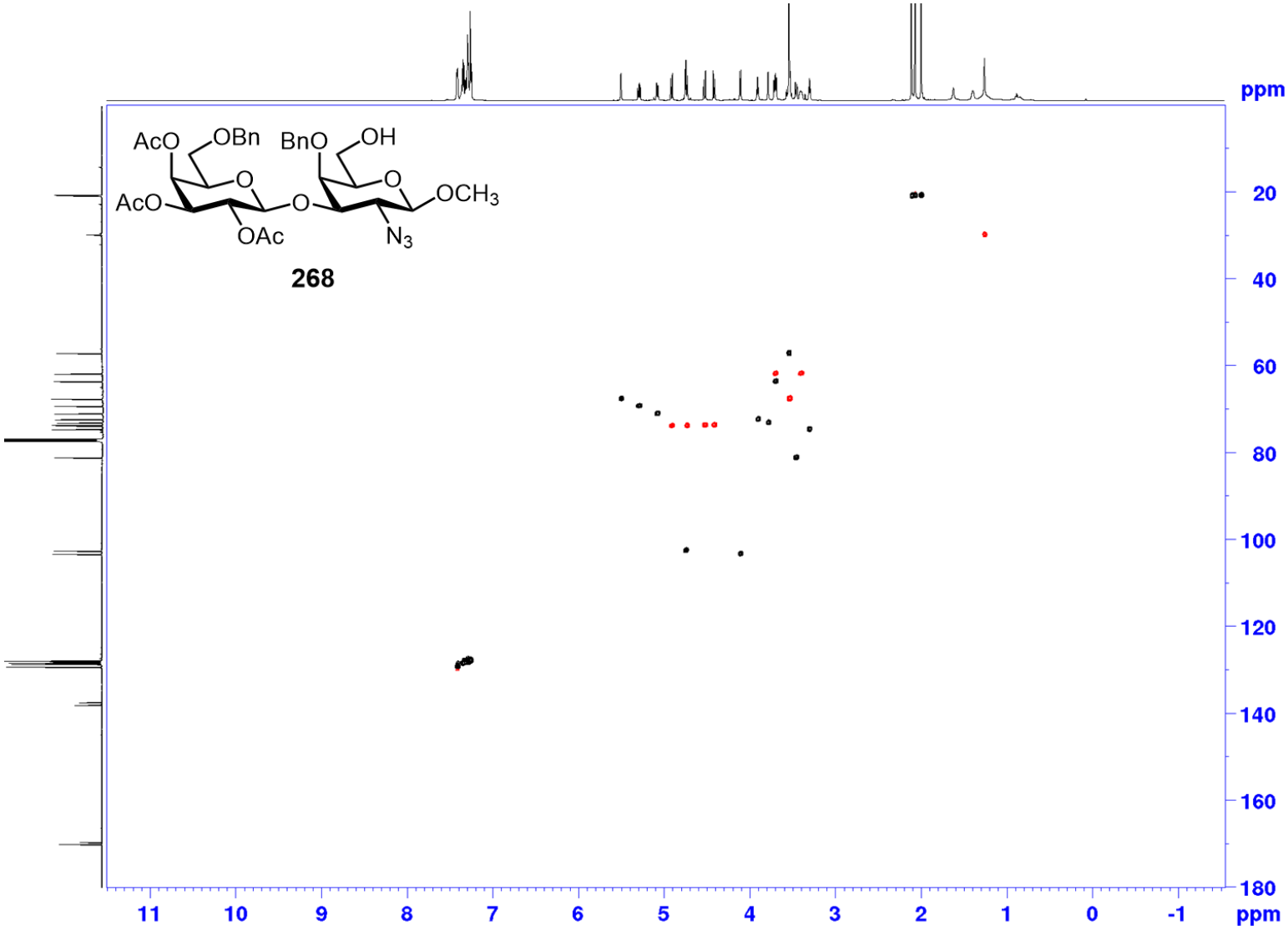


^{13}C NMR ($CDCl_3$, 100 MHz) of compound 268

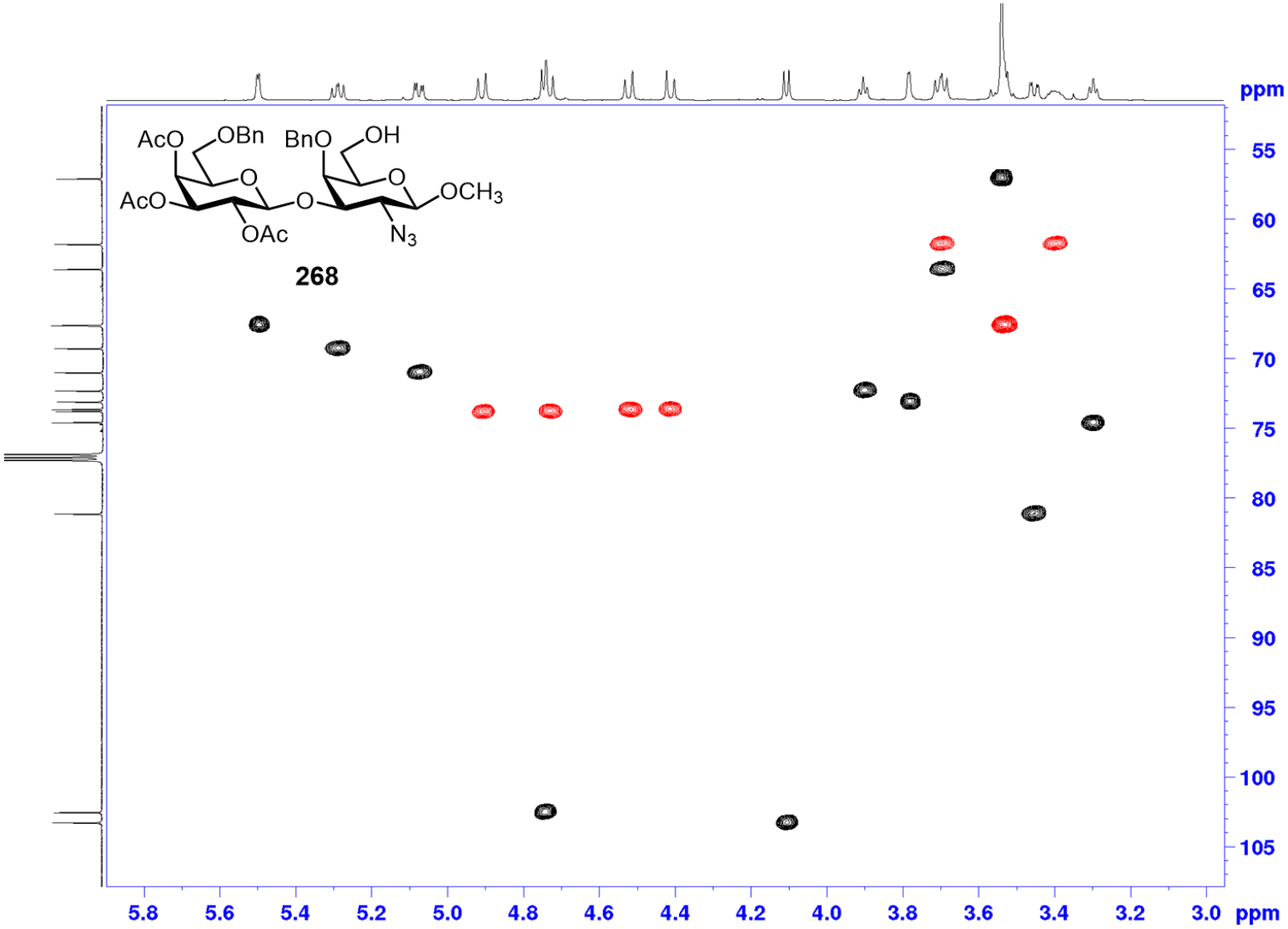


268

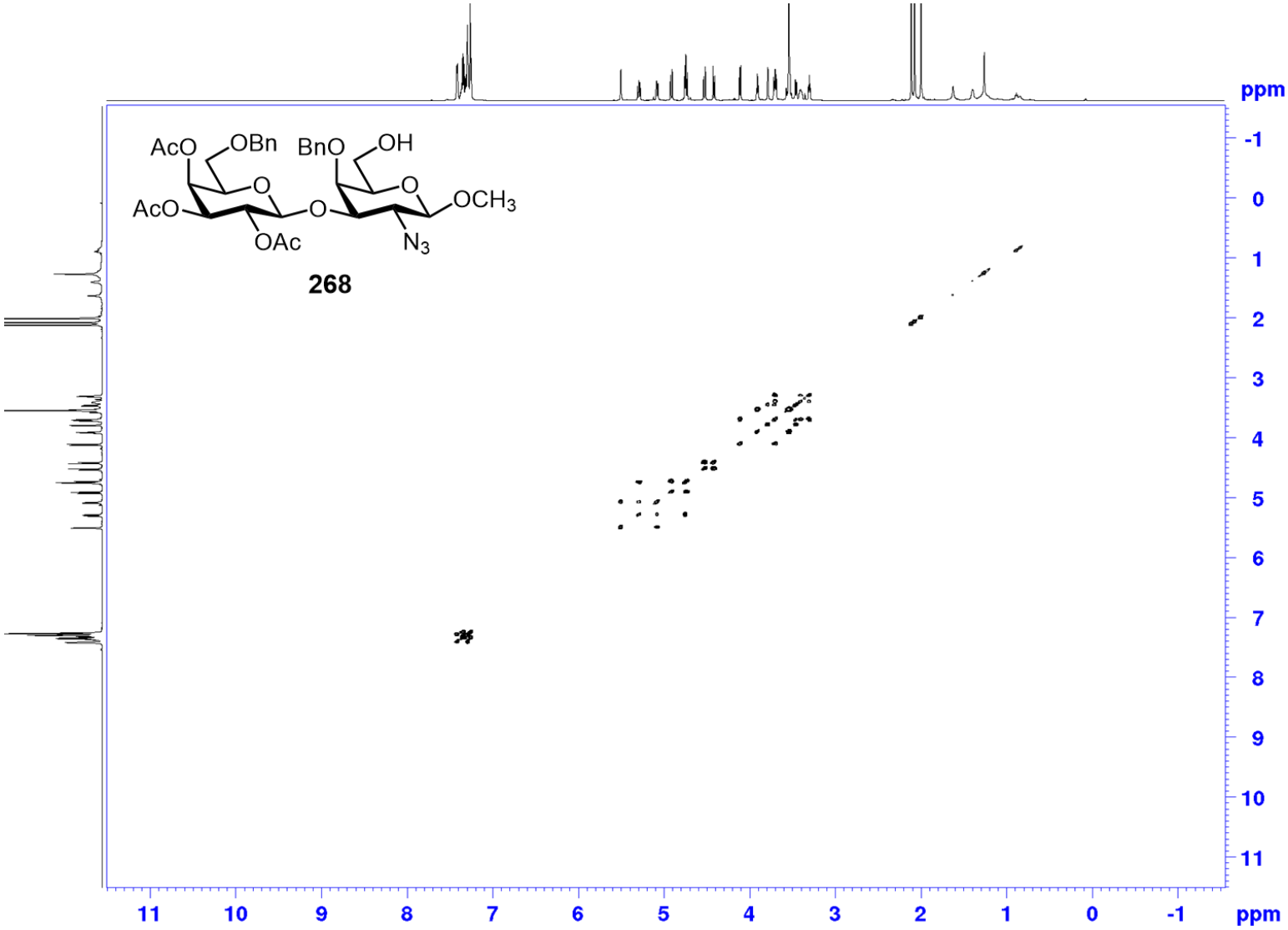
^{1}H-^{13}C HSCQ ($CDCl_3$) of compound 268



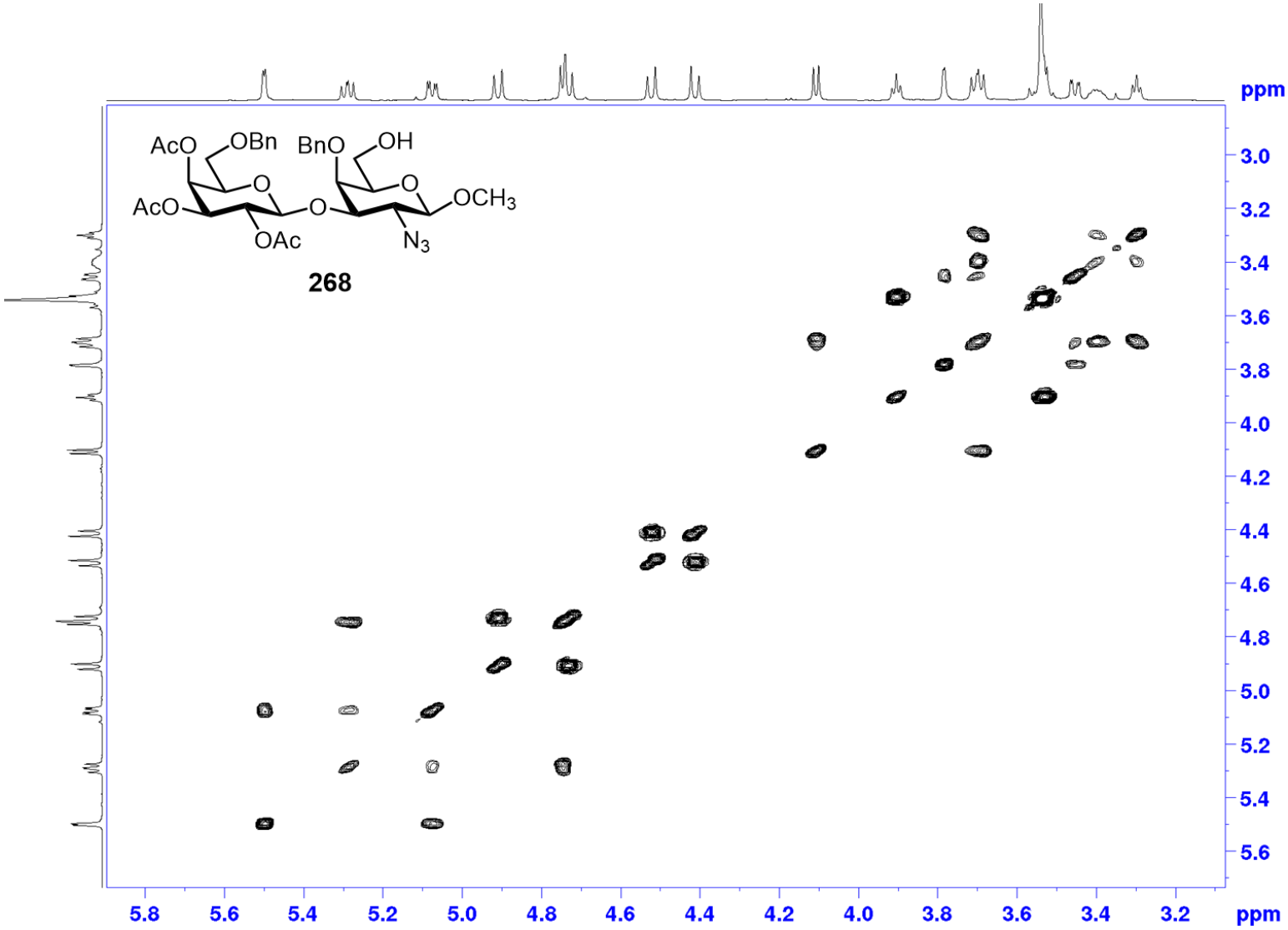
^{1}H-^{13}C HSCQ ($CDCl_3$) zoomed in region of compound 268

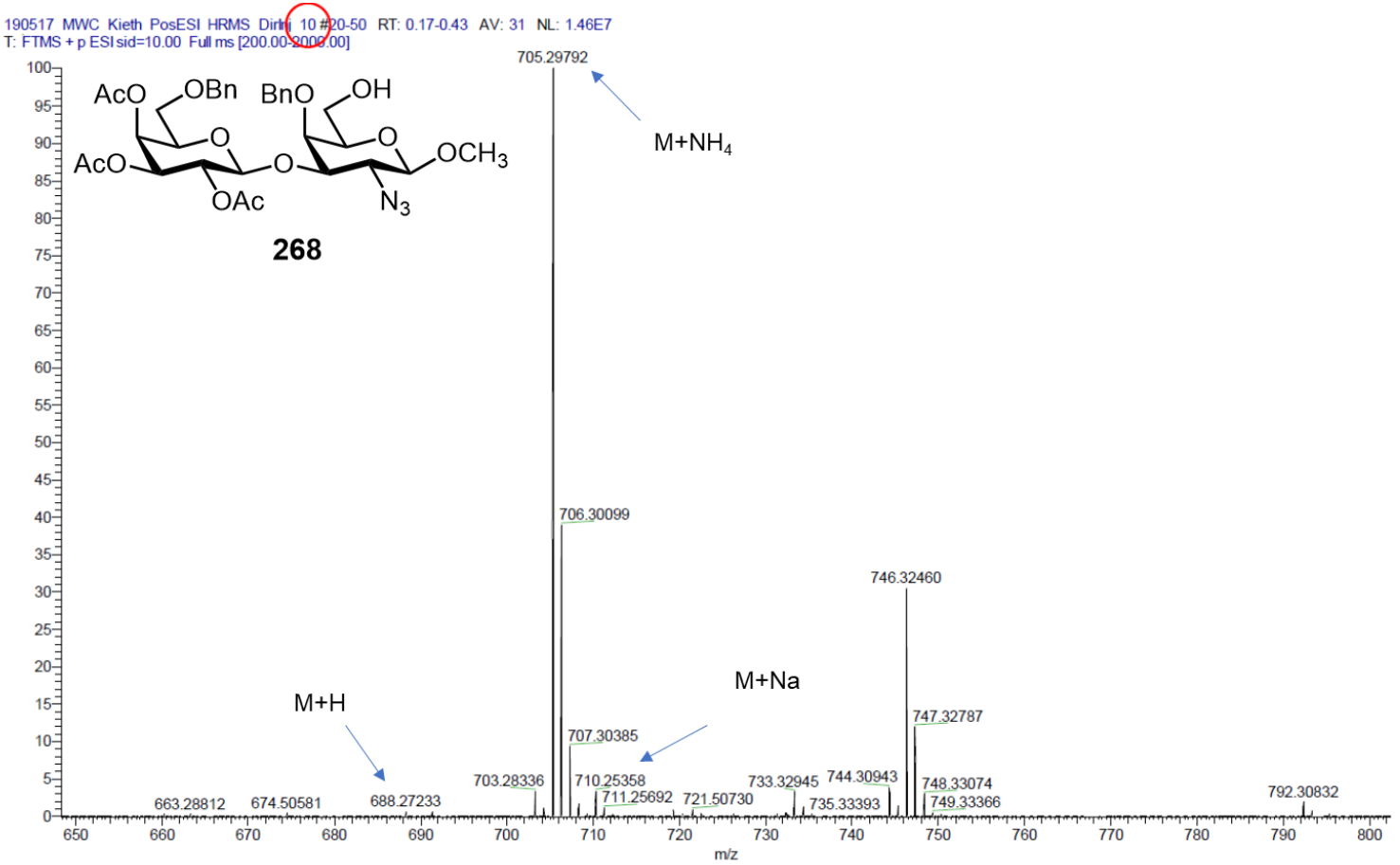


^{1}H-^{1}H COSY ($CDCl_3$) of compound 268

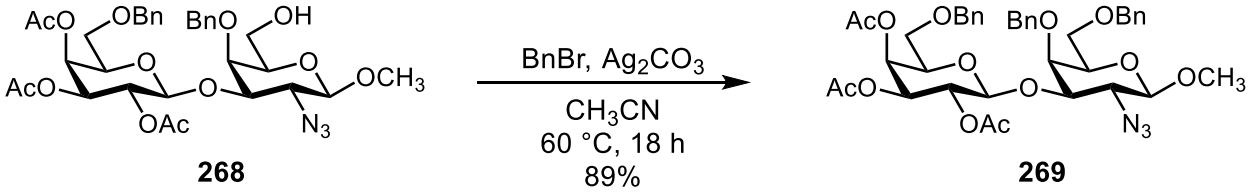


^{1}H-^{1}H COSY ($CDCl_3$) zoomed in region of compound 268




190517 MWC Kieth PosESI HRMS Dinh 10 #20-50 RT: 0.17-0.43 AV: 31 NL: 1.46E7
T: FTMS + p ESI sid=10.00 Full ms [200.00-2000.00]
AcO
OBn
BnO
OH
AcO
O
O
OCH3
OAc
N3
268
705.29792
M+NH4
706.30099
746.32460
M+Na
M+H
747.32787
707.30385
703.28336
710.25358
711.25692
721.50730
733.32945
744.30943
735.33393
748.33074
749.33366
663.28812
674.50581
688.27233
792.30832
m/z

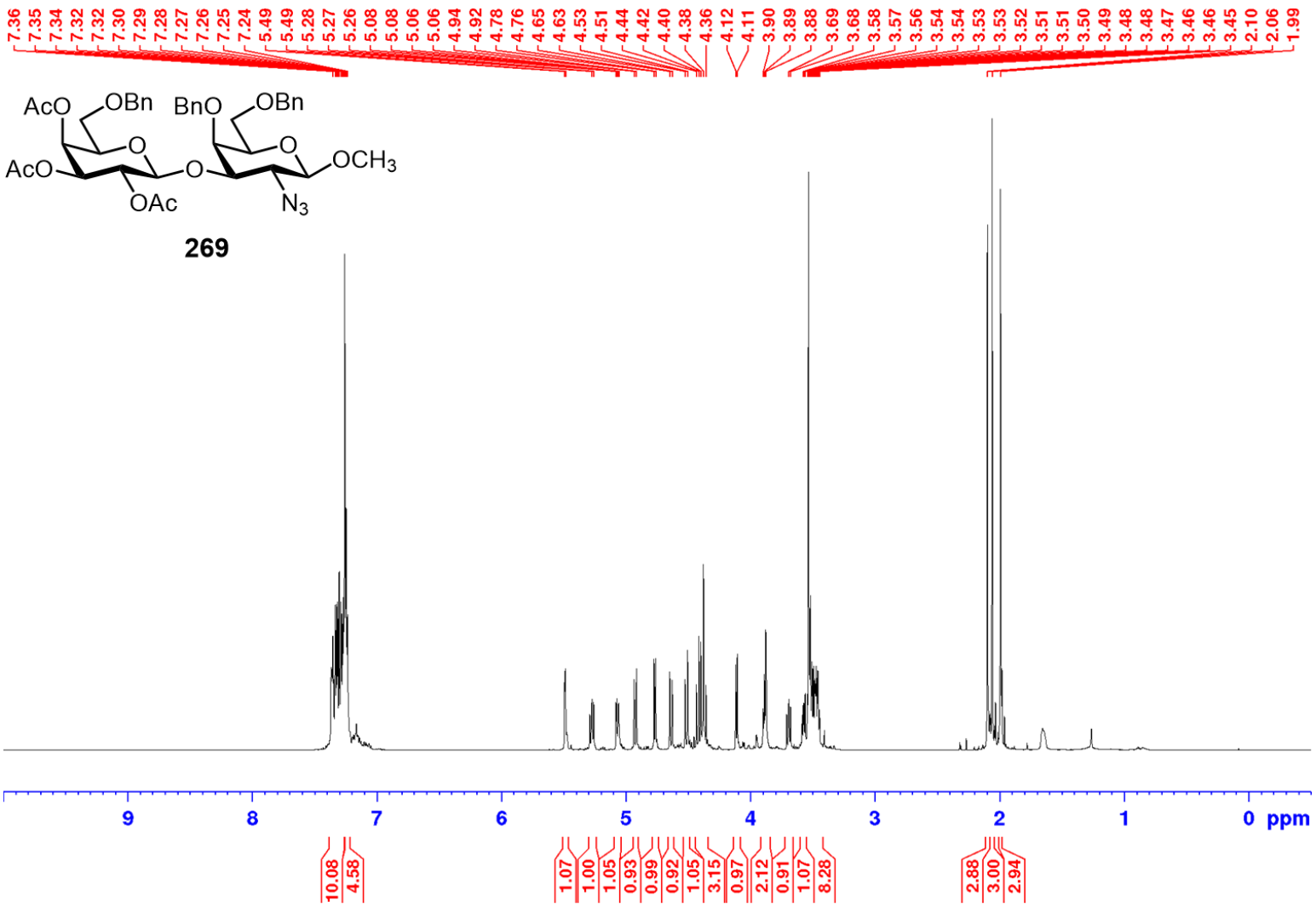
Methyl 6-*O*-benzyl-2,3,4-tri-*O*-acetyl-β-D-galactopyranosyl-(1→3)-4,6-di-*O*-benzyl-2-deoxy-2-azideo-β-D-galactopyranoside (269)



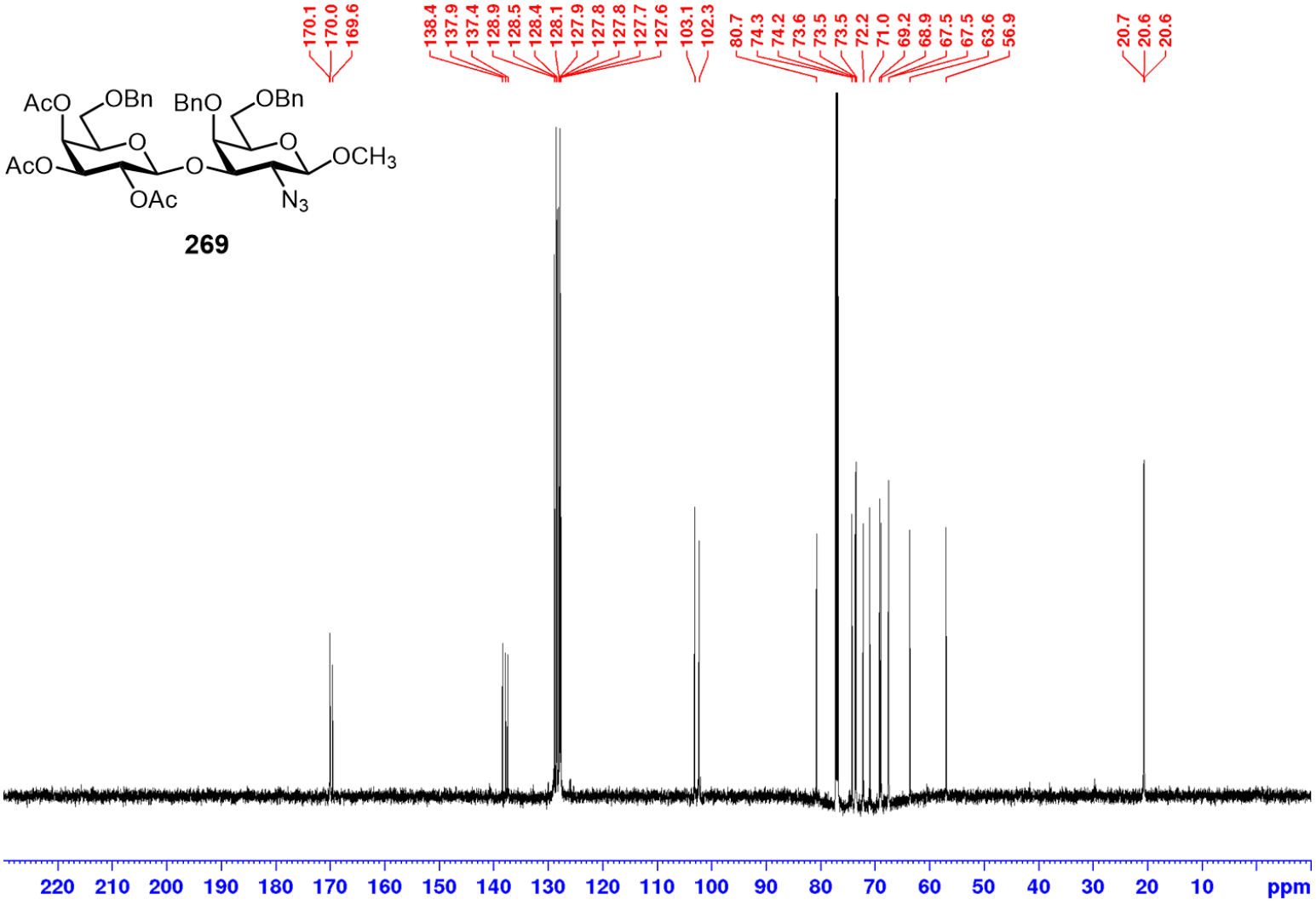
A solution of methyl 6-*O*-benzyl-2,3,4-tri-*O*-acetyl-β-D-galactopyranosyl-(1→3)-4-*O*-benzyl-2-deoxy-2-azideo-β-D-galactopyranoside **268** (1.0 equiv., 2.203, 3.203 mmol) and BnBr (5.0 equiv., 1.90 mL, 16.02 mmol) in anhydrous CH_3CN (15 mL) at room temperature, under argon, in the dark was treated with Ag_2CO_3 (5.0 equiv., 4.417 g, 16.02 mmol). The reaction mixture was heated to 60 °C. At 18 h, the crude reaction mixture was filtered through a pad of Celite in glass fritted filter funnel with EtOAc (100 mL). The filtrate was concentrated to a dark brown colored oil and washed with sat. aq., $NaHCO_3$ (2 x 25 mL), 40% sodium thiosulfate (2 x 25 mL), brine (1 x 25 mL), dried ($NaSO_4$), filtered through a glass fritted vacuum filter funnel to remove drying agent. The filtrate was concentrated to a brown oil was purified by silica gel flash column chromatography (20:80 to 50:50 EtOAc/hexanes) to give methyl 6-*O*-benzyl-2,3,4-tri-*O*-acetyl-β-D-galactopyranosyl-(1→3)-4,6-di-*O*-benzyl-2-deoxy-2-azideo-β-D-galactopyranoside **269** (2.209 g, 2.840 mmol, 89%) as a white amorphous solid. R_f = 0.60 (1:3 EtOAc/hexanes) visualized with ceric ammonium molybdate stain; $[\alpha]D^{25}$ = -16.12° (c = 1.0, $CHCl_3$); IR ν_{max} (film) 3568.07, 2113.74, 1750.16, 1454.65, 1368.11, 1220.26, 1071.99, 736.32, 699.42 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.37-7.23 (m, 15H), 5.49 (d, *J* = 3.1 Hz, 1H), 5.28 (dd, *J* = 7.9, 10.4 Hz, 1H), 5.07 (dd, *J* = 3.4, 10.4 Hz, 1H), 4.93 (d, *J* = 11.5 Hz, 1H), 4.77 (d, *J* = 7.8 Hz, 1H), 4.64 (d, *J* = 11.6 Hz, 1H), 4.52 (d, *J* = 11.9 Hz, 1H), 4.43-4.36 (m, 3H), 4.11 (d, *J* =

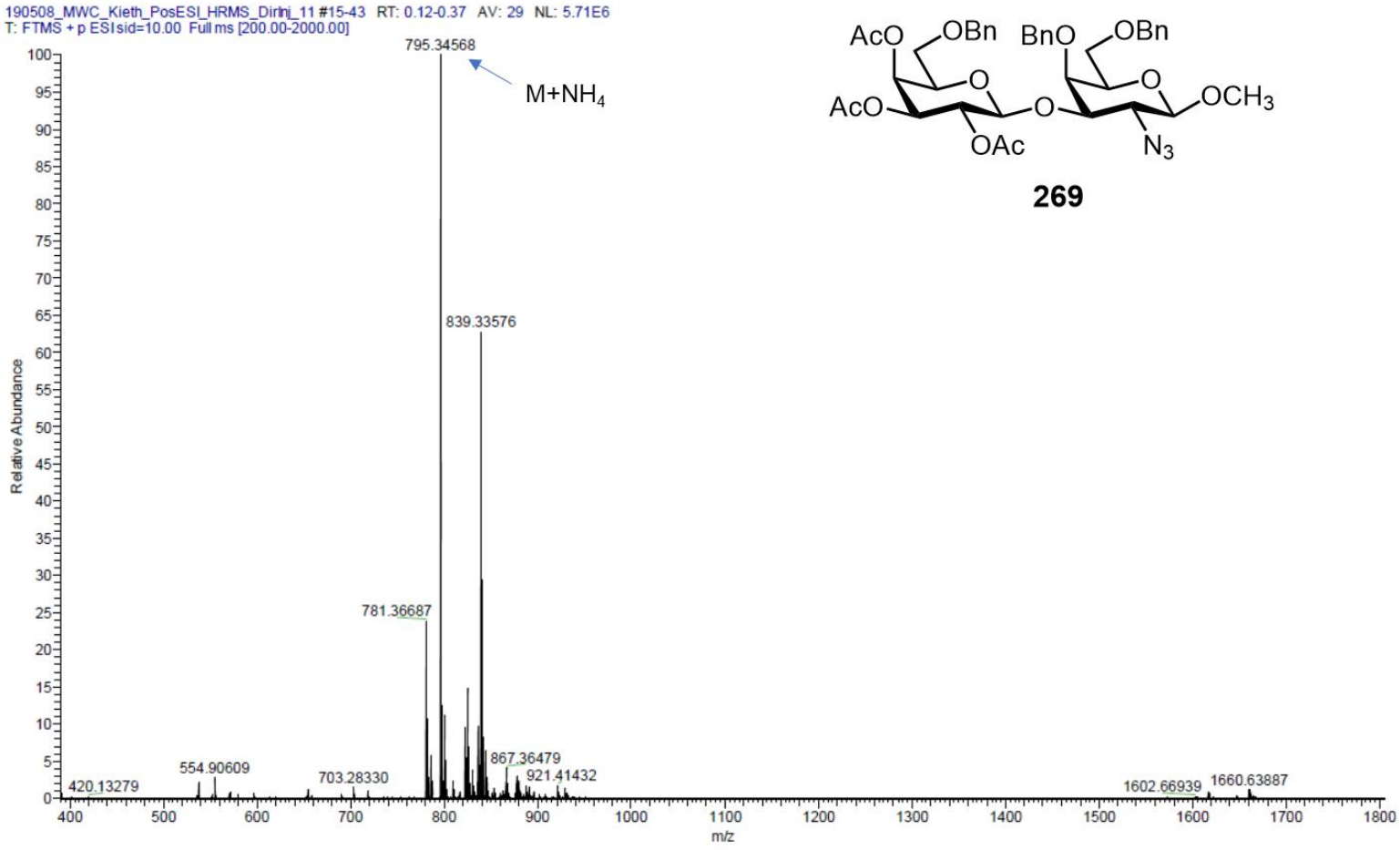
8.0 Hz, 1H), 3.90-3.8 (m, 2H), 3.70 (dd, *J* = 8.0, 10.4 Hz, 1H), 3.-3.49 (m, 9H), 2.11 (S, 3H), 2.07 (S, 3H), 2.00 (S, 3H);^{13}C NMR (100 MHz, $CDCl_3$): δ 170.1, 170.1, 169.6, 138.4, 137.9, 137.5, 128.9,128.9, 128.5, 128.5, 128.4, 128.4, 128.09, 128.1, 128.0, 127.9, 127.8, 127.8, 127.8, 127.8, 127.7, 103.1, 102.3, 80.8, 74.3, 74.3, 73.7, 73.6, 73.5, 72.2, 71.0, 69.2, 69.0, 67.6, 67.5, 63.7, 57.0, 20.7, 20.7, 20.6; HRMS (ESI) calcd for $C_{40}H_{51}N_4O_{13}^+$ $(M+NH_4)^+$ 795.34472, found 795.34568 m/z.

^{1}H NMR ($CDCl_3$, 400 MHz) of compound 269



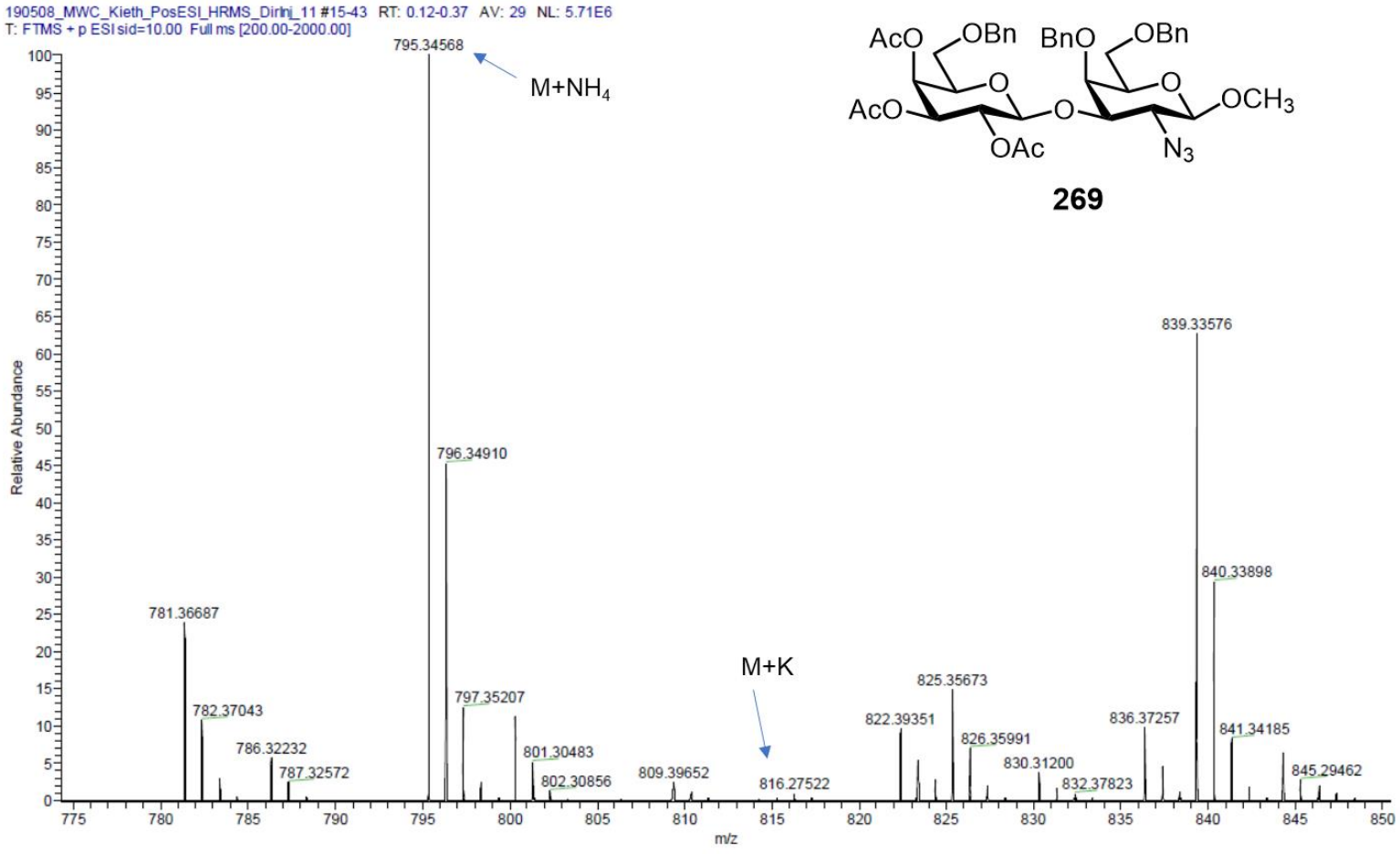
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 269




190508_MWC_Kieth_PosESI_HRMS_Dirthj_11 #15-43 RT: 0.12-0.37 AV: 29 NL: 5.71E6
T: FTMS + p ESI sid=10.00 Full ms [200.00-2000.00]
795.34568
M+NH4
839.33576
781.36687
867.36479
921.41432
554.90609
703.28330
420.13279
1602.66939
1660.63887
Relative Abundance
m/z

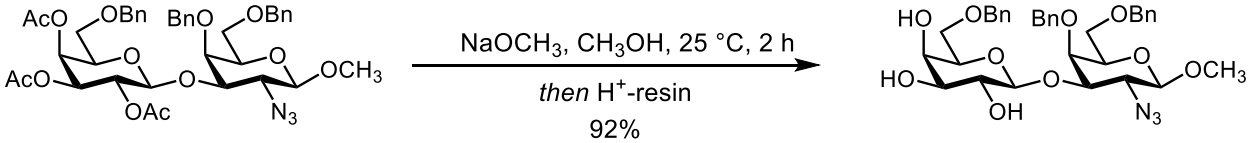
AcO
OBn
BnO
OBn
AcO
O
O
OCH3
OAc
N3

269


190508_MWC_Kieth_PosESI_HRMS_Dirlhj_11 #15-43 RT: 0.12-0.37 AV: 29 NL: 5.71E6
T: FTMS + p ESI sid=10.00 Full ms [200.00-2000.00]
795.34568
M+NH4
796.34910
781.36687
782.37043
786.32232
787.32572
797.35207
801.30483
802.30856
809.39652
816.27522
M+K
822.39351
825.35673
826.35991
830.31200
832.37823
836.37257
839.33576
840.33898
841.34185
845.29462
Relative Abundance
m/z
AcO
OBn
BnO
OBn
AcO
OAc
OCH3
N3

269

Methyl 6-*O*-benzyl-β-D-galactopyranosyl-(1→3)-4,6-di-*O*-benzyl-2-deoxy-2-azideo-β-D-galactopyranoside (270)

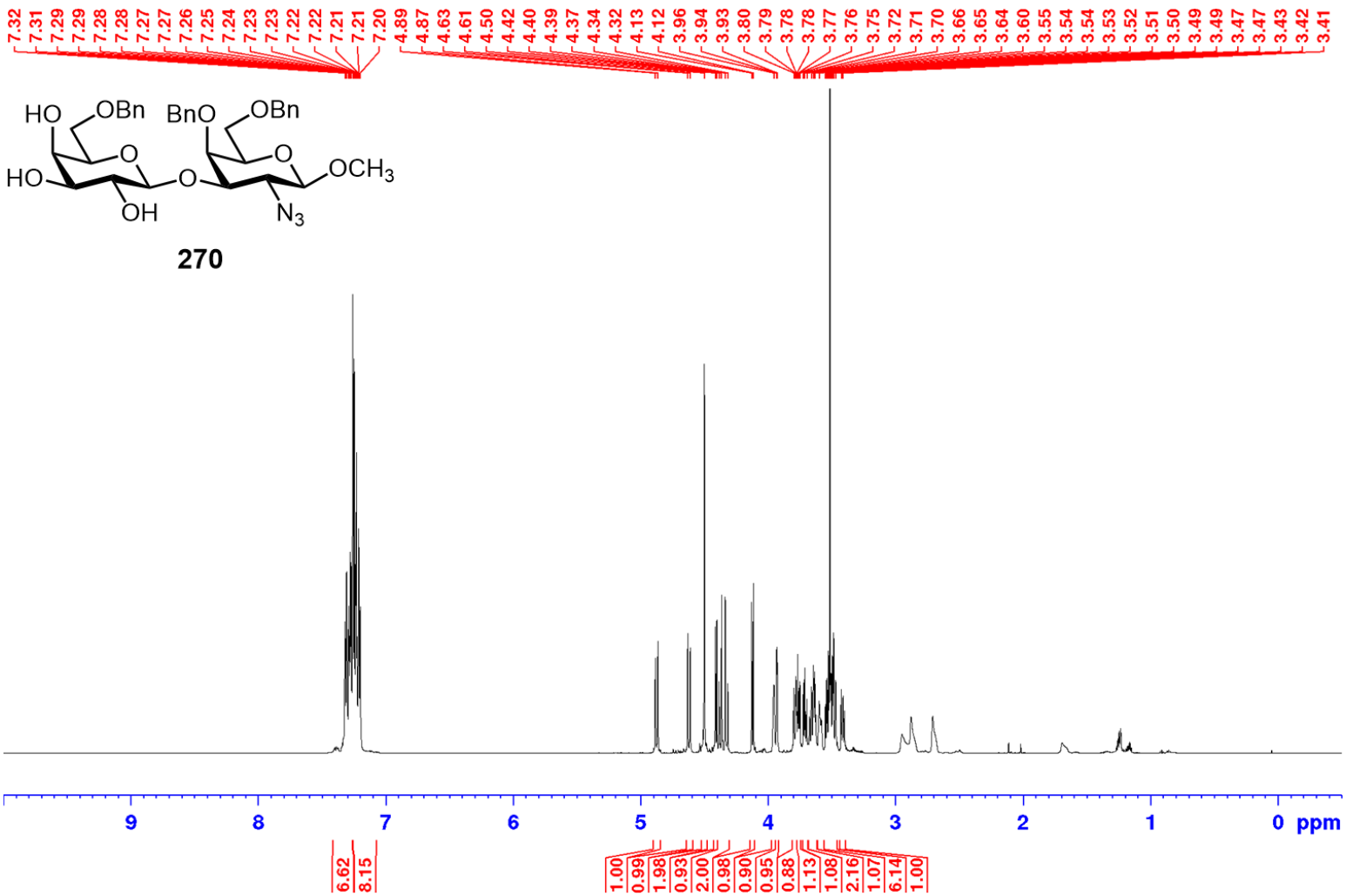


269 **270**

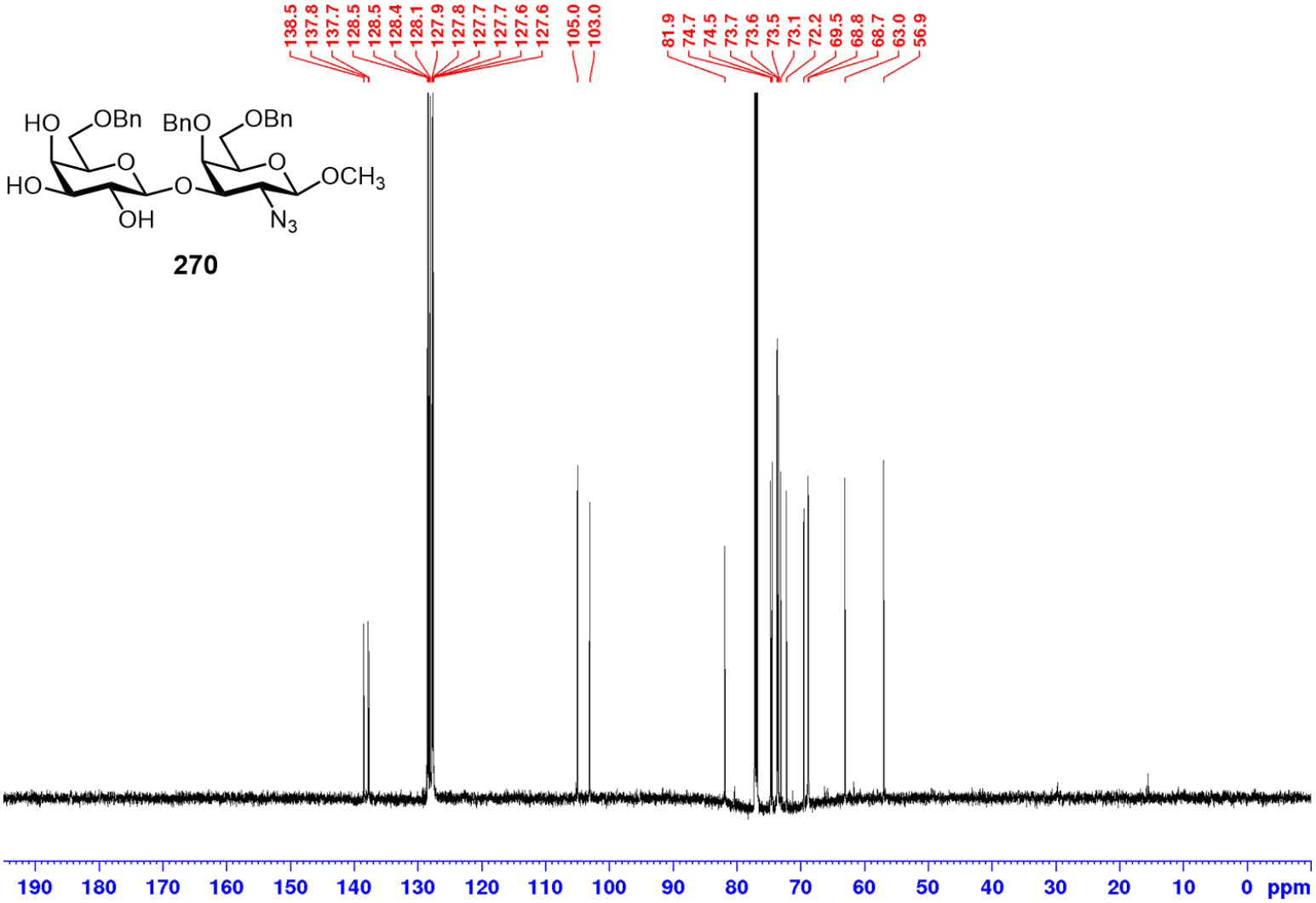
To a solution of methyl 4,6-di-*O*-benzyl-3-*O*-(6-*O*-benzyl-2,3,4-tri-*O*-acetyl-β-D-galactopyranosyl)-2-deoxy-2-azido-β-D-galactopyranoside **263** (1.0 equiv., 1.35 g, 1.74 mmol) in anhydrous methanol (3.2 mL) was added sodium methoxide solution (0.6 equiv., 0.3 mL, [2 N] in CH_3OH) at room temperature under an argon atmosphere. At 5 h, the reaction mixture was neutralized with the addition of acidic Amberlite IR 120 (H^+) ion exchange resin. The reaction was filtered through a glass frittered vacuum filter funnel equipped with pad Celite to remove the acidic resin. The filter cake was rinsed with CH_2Cl_2. The filtrate was concentrated to an oil and purified by silica gel flash column chromatography (55:45 to 70:30 EtOAc/hexanes) to give methyl 6-*O*-benzyl-β-D-galactopyranosyl-(1→3)-4,6-di-*O*-benzyl-2-deoxy-2-azideo-β-D-galactopyranoside **270** (0.500 g, 0.767 mmol, 95%) as a white foam. R_f = 0.16 (5:95 CH_3OH/CH_2Cl_2) visualized with ceric ammonium molybdate stain; $[\alpha]D^{25}$ = -4.52° (c = 1.0, $CHCl_3$); IR ν_{max} (film) 3433.56, 2115.95, 1637.12, 1073.15, 698.87 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.34-7.22 (m, 15H), 4.89 (d, *J* = 11.8 Hz, 1H), 4.65 (d, *J* = 11.8 Hz, 1H), 4.54 (m, 2H), 4.44 (d, *J* = 7.5 Hz, 1H), 4.38 (dd, *J* = 11.7, 28.7 Hz, 2H), 4.15 (d, *J* = 8.1 Hz, 1H), 4.02 (m, 1H), 3.96 (d, *J* = 2.6 Hz, 1H), 3.82-3.79 (m, 2H), 3.75-3.73 (m, 1H), 3.90-3.66 (m, 2H), 3.65-3.62 (m, 1H), 3.57-3.49 (m, 6H), 3.46-3.43 (dd, *J* = 6.1, 9.1 Hz, 1H), 2.75 (d, *J* = 1.79 Hz, 1H), 2.67 (d, *J* = 4.38 Hz, 1H), 2.60 (d, *J* = 2.6 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ

138.6, 137.9, 137.7, 128.6, 128.5, 128.5, 128.5, 128.4, 128.4, 128.16, 128.2, 127.9, 127.8, 127.8, 127.8, 127.7, 127.7, 127.6, 105.0, 103.1, 82.0, 74.8, 74.5, 73.8, 73.7, 73.7, 73.5, 73.2, 72.3, 69.5, 68.8, 68.7, 63.1, 57.0. HRMS (ESI) calcd for $C_{34}H_{45}N_4O_{10}^+$ $(M+NH_4)^+$ 669.31301 found 669.31319 m/z.

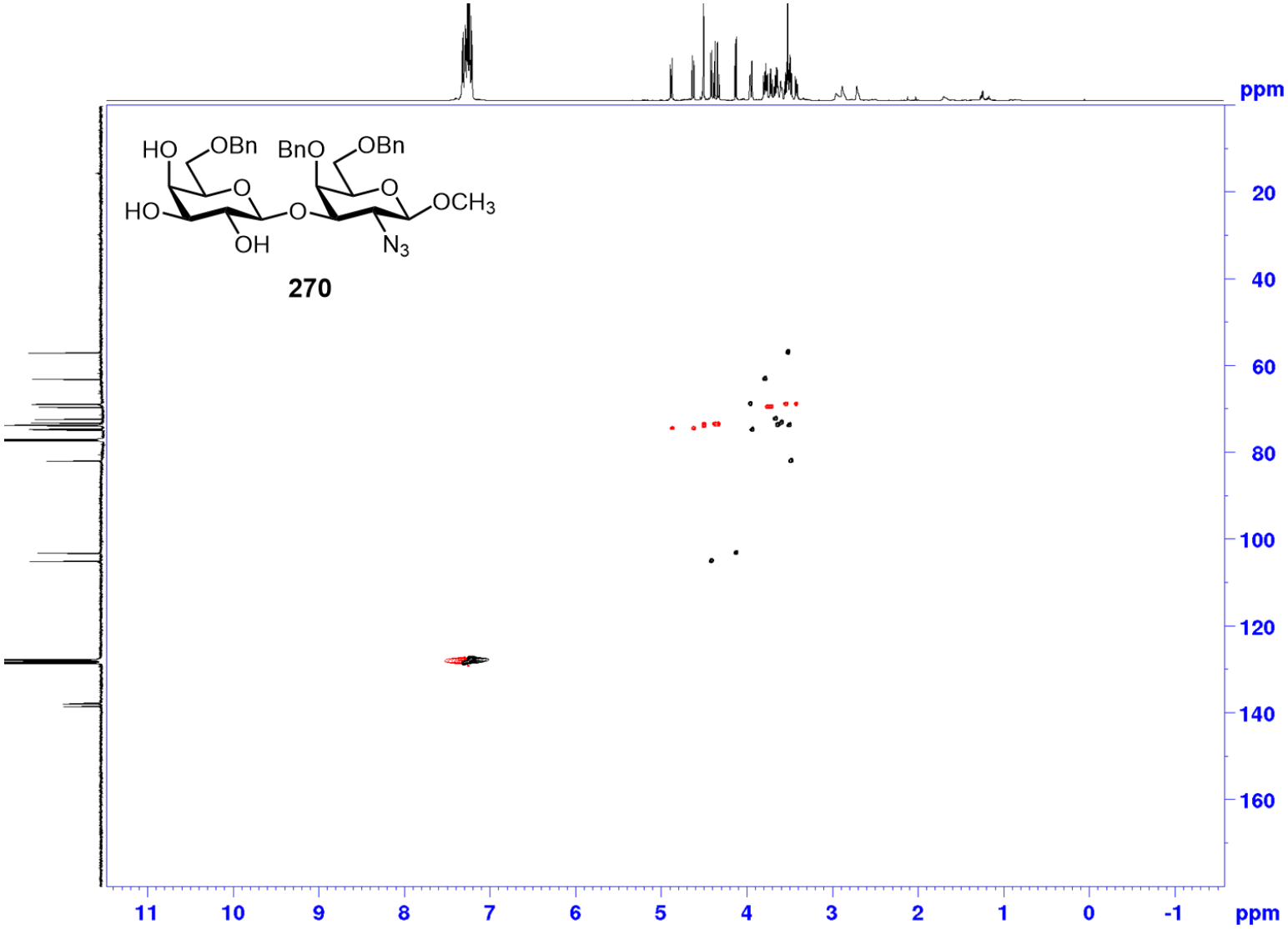
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 270



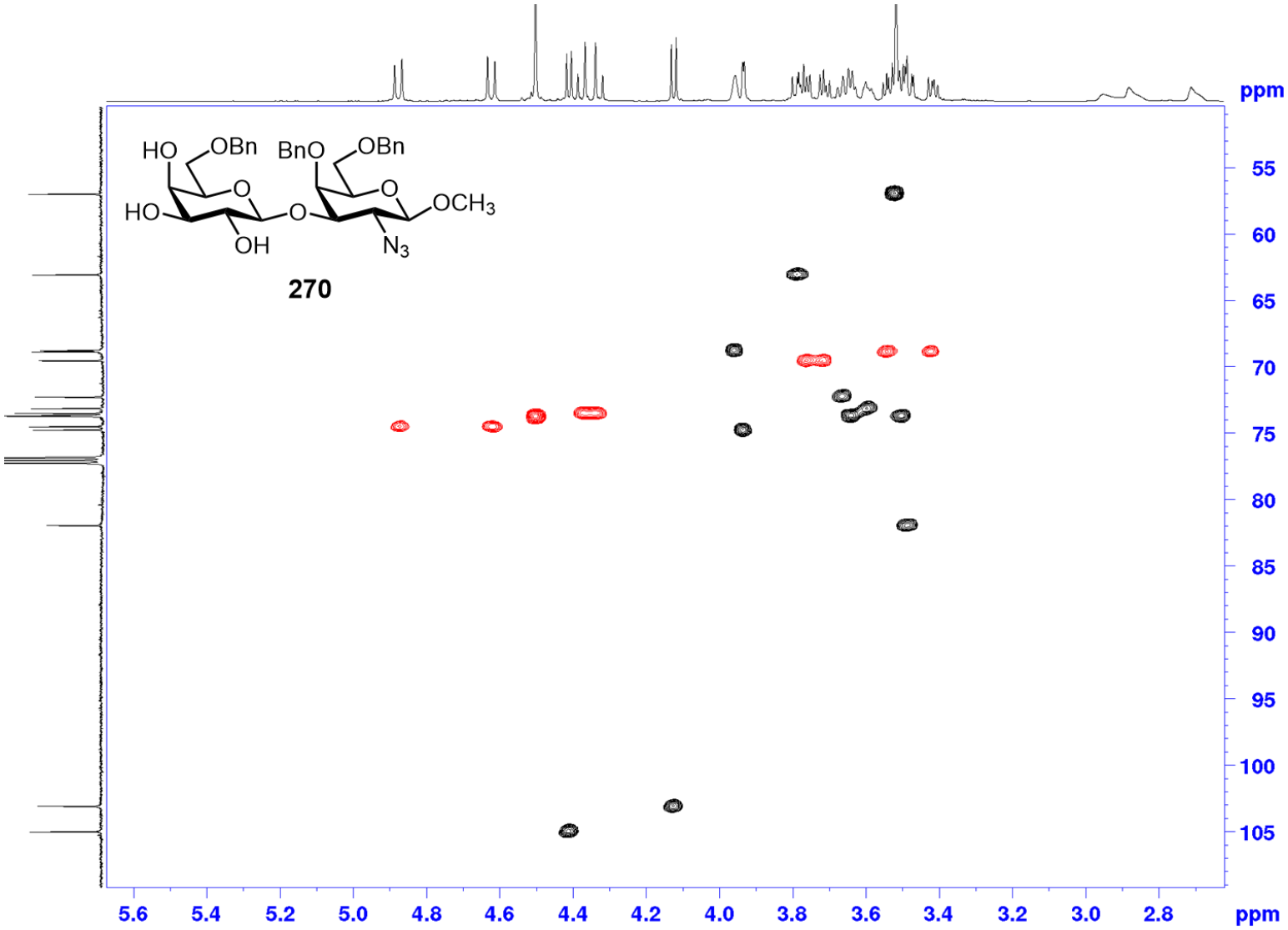
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 270



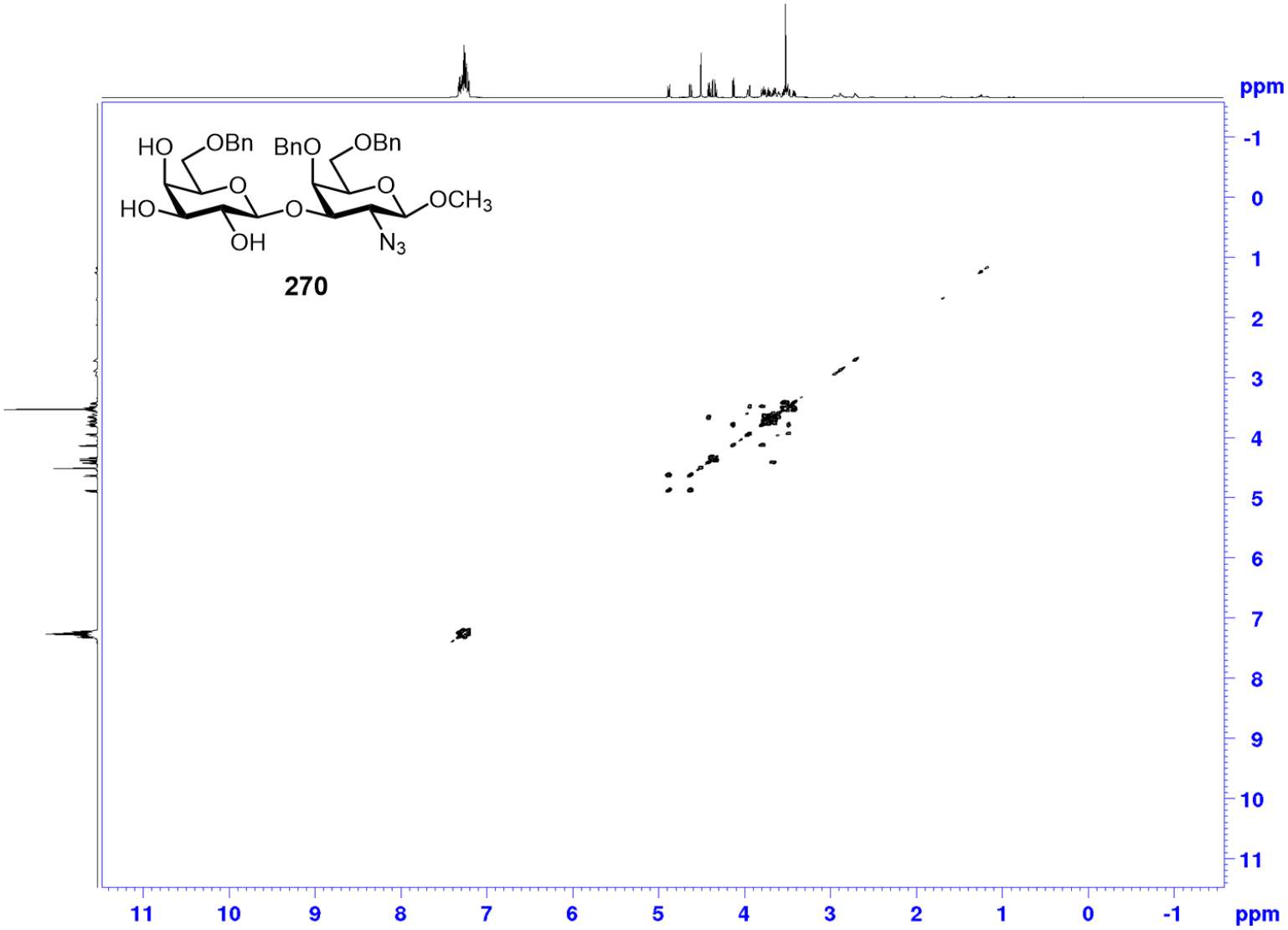
^{1}H-^{13}C HSCQ ($CDCl_3$) of compound 270



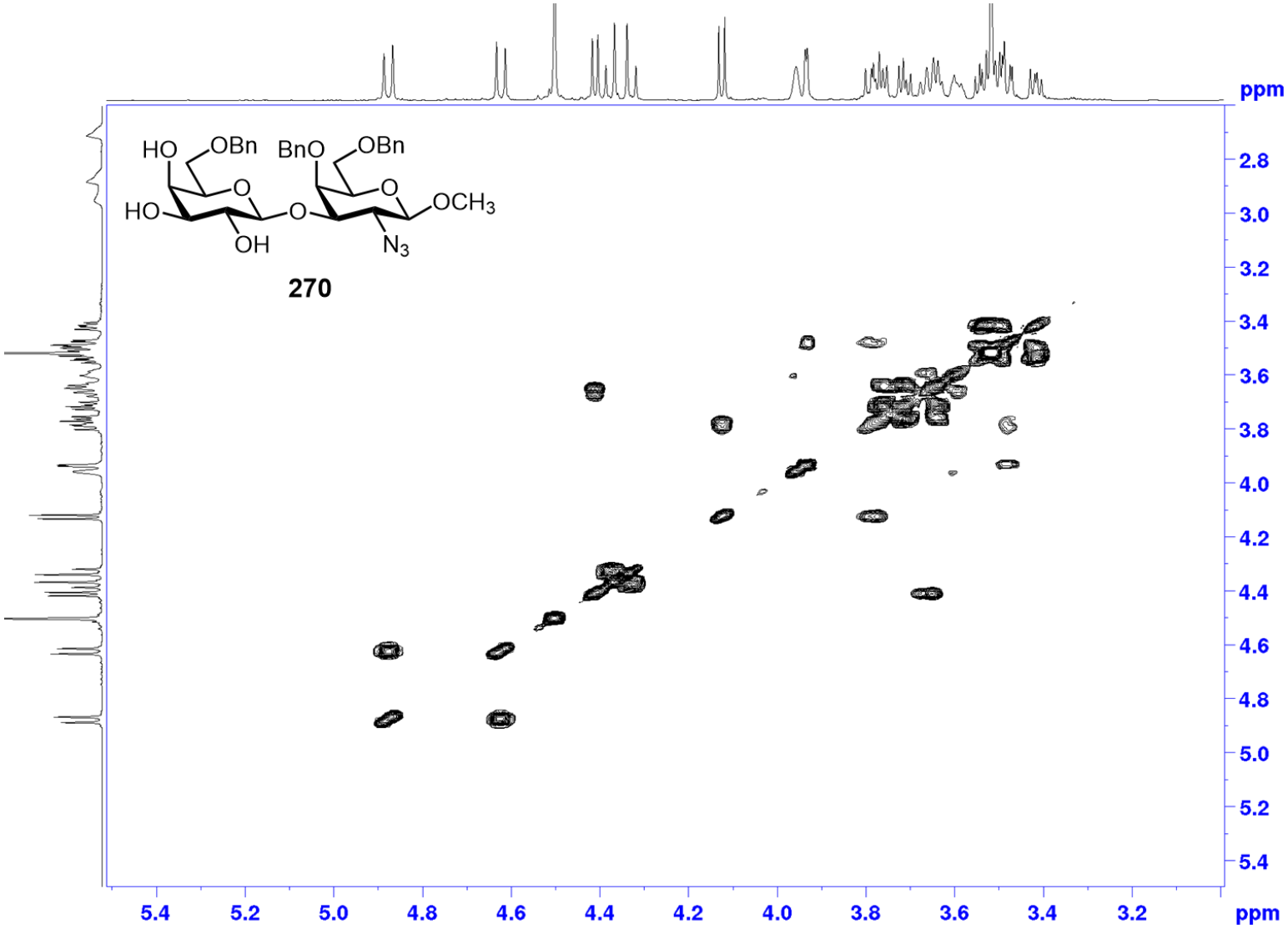
^{1}H-^{13}C HSCQ ($CDCl_3$) zoomed in region of compound 270

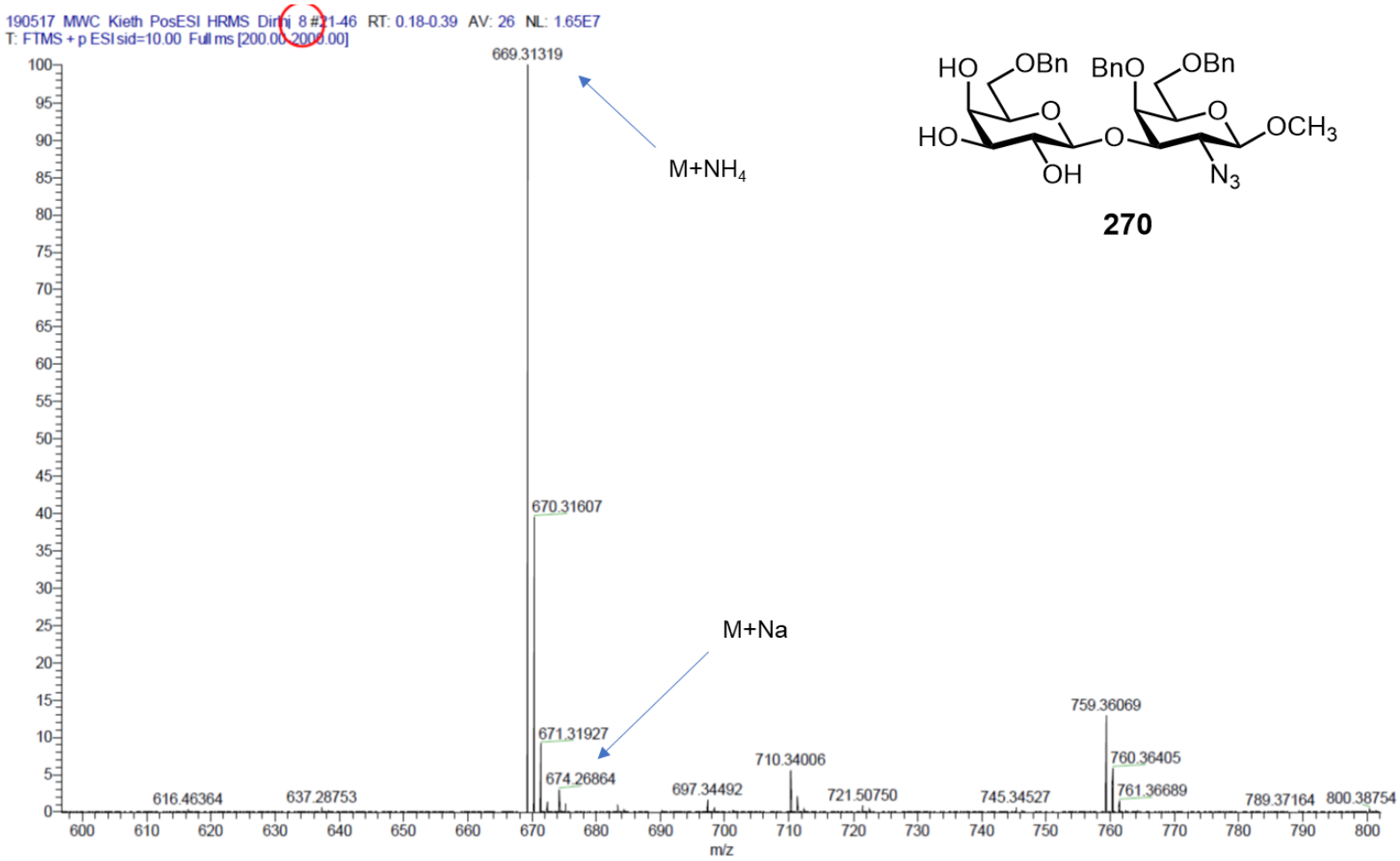


^{1}H-^{1}H COSY ($CDCl_3$) of compound 270



^{1}H-^{1}H COSY ($CDCl_3$) zoomed in region of compound 270

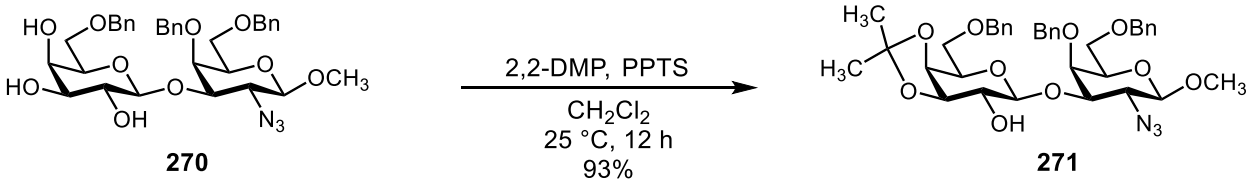



190517 MWC Kieth PosESI HRMS Dimj 8 #21-46 RT: 0.18-0.39 AV: 26 NL: 1.65E7
T: FTMS + p ESI sid=10.00 Full ms [200.00-2000.00]
669.31319
M+NH4
670.31607
M+Na
671.31927
674.26864
616.46364
637.28753
697.34492
710.34006
721.50750
745.34527
759.36069
760.36405
761.36689
789.37164
800.38754
m/z

HO
OBn
BnO
OBn
O
HO
O
O
OCH3
OH
N3

270

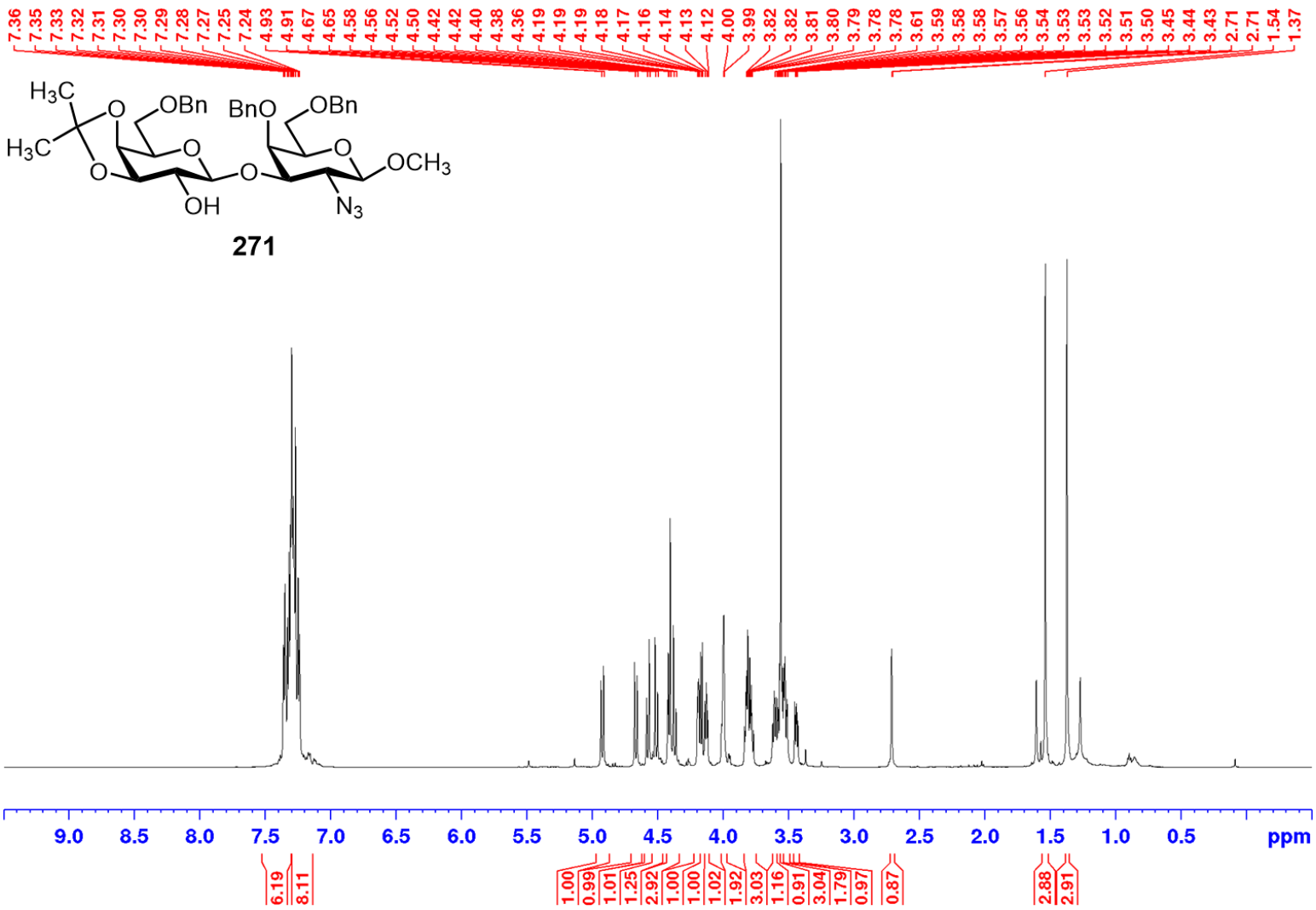
Methyl 6-*O*-benzyl-*O*-3,4-isopropylidene-β-D-galactopyranosyl-(1→3)-4,6-di-*O*-benzyl-2-deoxy-2-azideo-β-D-galactopyranoside (271)



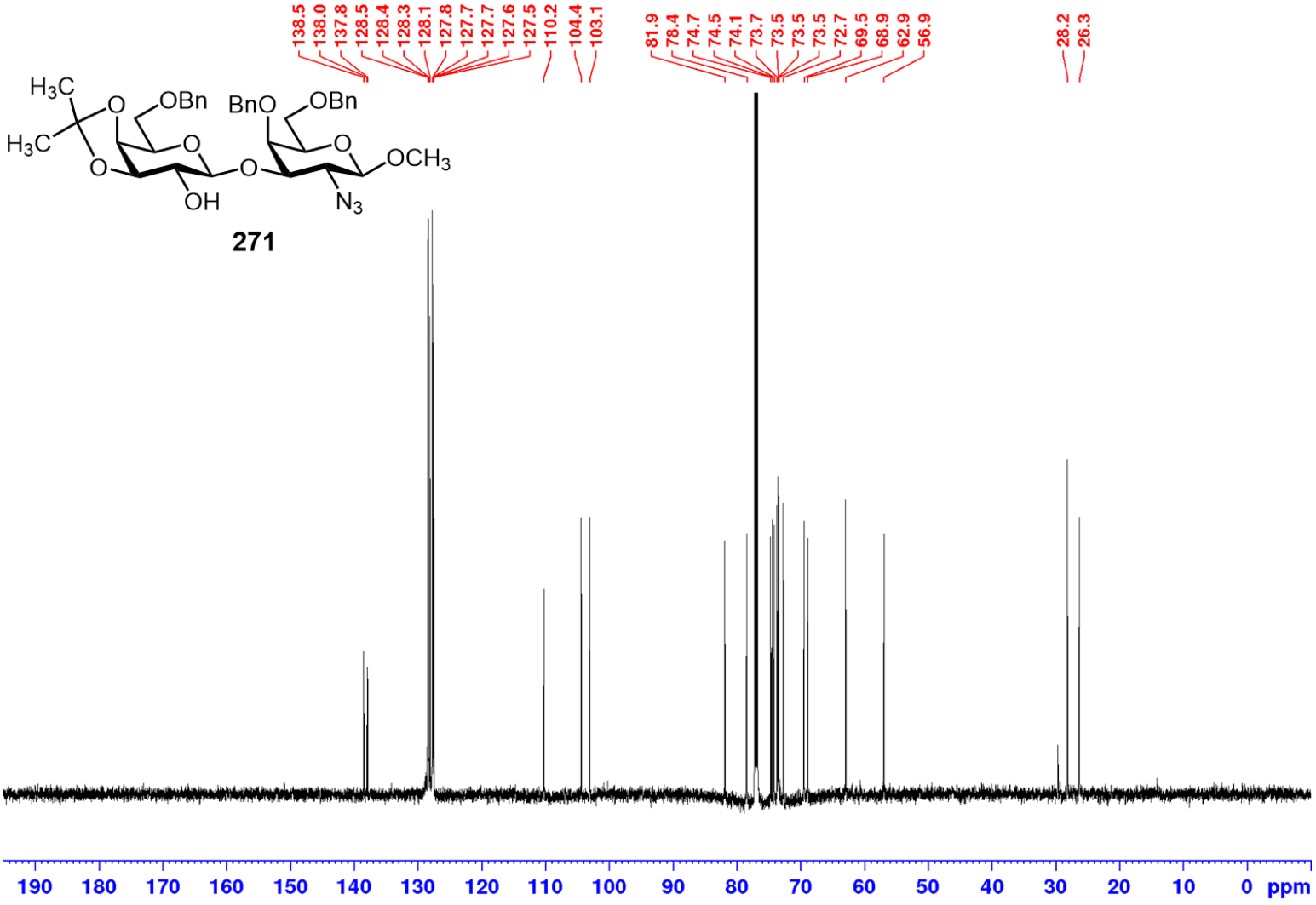
A solution of methyl 6-*O*-benzyl-β-D-galactopyranosyl-(1→3)-4,6-di-*O*-benzyl-2-deoxy-2-azideo-β-D-galactopyranoside **270** (1.0 equiv., 2.385 g, 3.660 mmol) in anhydrous CH_2Cl_2 (36 mL) was added 2,2-dimethoxypropane (6.0 equiv., 2.7 mL, 21.96 mmol) and PPTS (0.15 equiv., 0.137 g, 0.549 mmol) under an argon atmosphere at room temperature. At 4 h, the reaction mixture was neutralized by the addition of Et_3N (0.25 equiv., 0.13 mL, 0.915 mmol) added dropwise. The reaction mixture was concentrated to a syrup and purified by silica gel flash column chromatography (30:70 to 20:80 EtOAc/hexanes) to give methyl 6-*O*-benzyl-*O*-3,4-isopropylidene-β-D-galactopyranosyl-(1→3)-4,6-di-*O*-benzyl-2-deoxy-2-azideo-β-D-galactopyranoside **271** (2.355 g, 3.404 mmol, 93%) as a white amorphous solid. R_f = 0.50 (1:1 EtOAc/hexanes) visualized with ceric ammonium molybdate stain; $[\alpha]D^{25}$ = -0.12° (c = 1.0, $CHCl_3$); IR ν_{max} (film) 3409.18, 2922.49, 2114.01, 1646.57, 1455.37, 1376.27, 1261.73, 1162.76, 1073.12, 698.83 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.34-7.22 (m, 15H), 4.91 (d, *J* = 11.8 Hz, 1H), 4.65 (d, *J* = 11.8 Hz, 1H), 4.56 (d, *J* = 11.9 Hz, 1H), 4.50 (d, *J* = 12.0 Hz, 1H), 4.38 (m, 3H), 4.14 (m, 3H), 3.98 (m, 2H), 3.81-3.70 (m, 3H), 3.60-3.48 (m, 7H), 3.42 (dd, *J* = 5.9, 9.2 Hz, 1H), 2.67 (d, *J* = 1.9 Hz, 1H), 1.52 (s, 3H), 1.35 (s, 3H);^{13}C NMR (100 MHz, $CDCl_3$): δ 138.5, 138.0, 137.8, 128.5, 128.5, 128.4, 128.4, 128.3, 128.3, 128.1, 128.1, 127.7, 127.7, 127.7, 127.7, 127.6, 127.5, 127.5, 110.2, 104.4, 103.1, 81.9, 78.4, 74.7, 74.5, 74.1, 73.7, 73.5, 73.5, 73.5, 72.7, 69.5,

68.9, 62.9, 56.9, 28.2, 26.3; HRMS (ESI) calcd for $C_{37}H_{49}N_4O_{10}^+$ $(M+NH_4)^+$ 709.34432, found 709.34490 m/z.

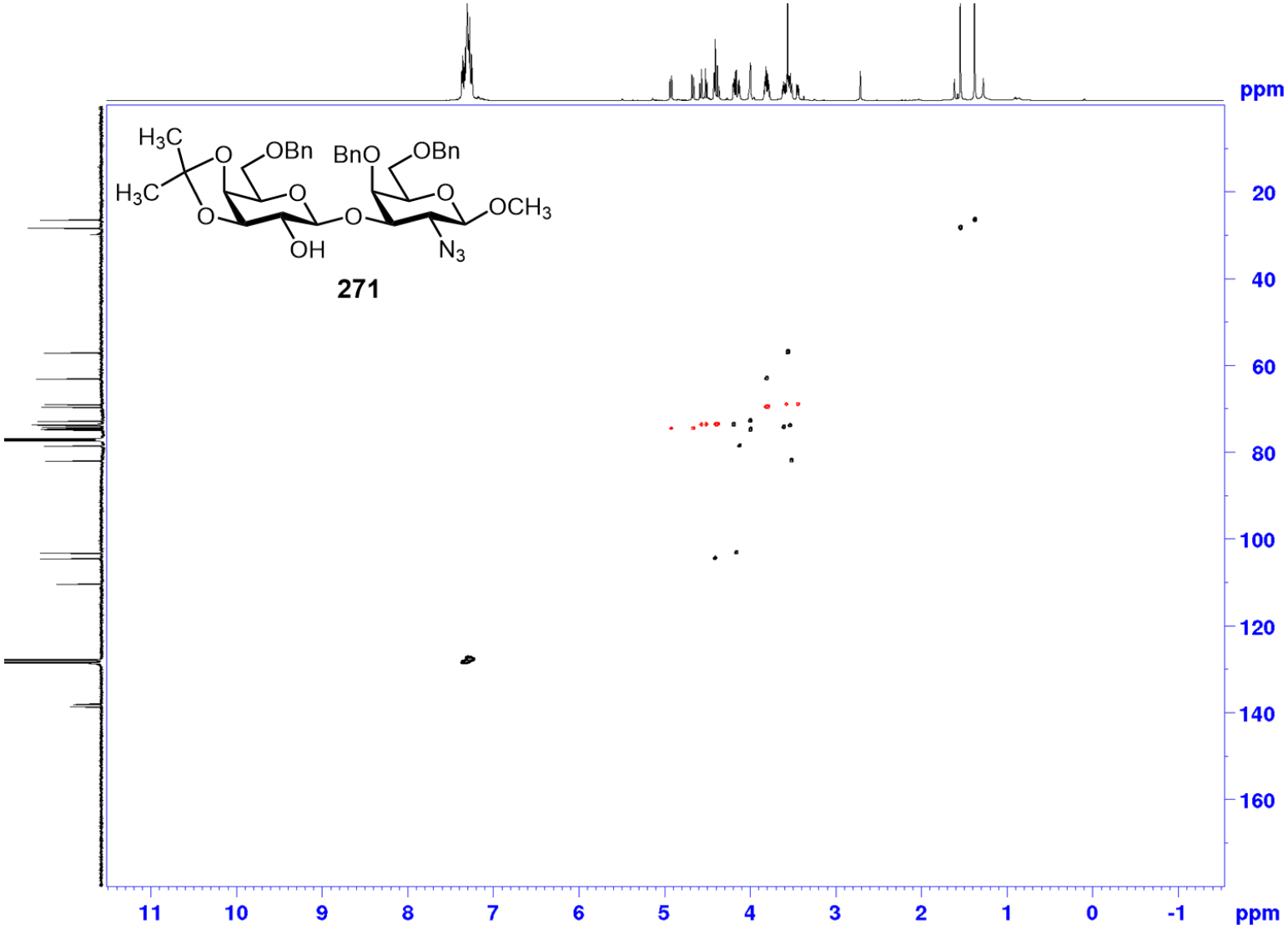
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 271



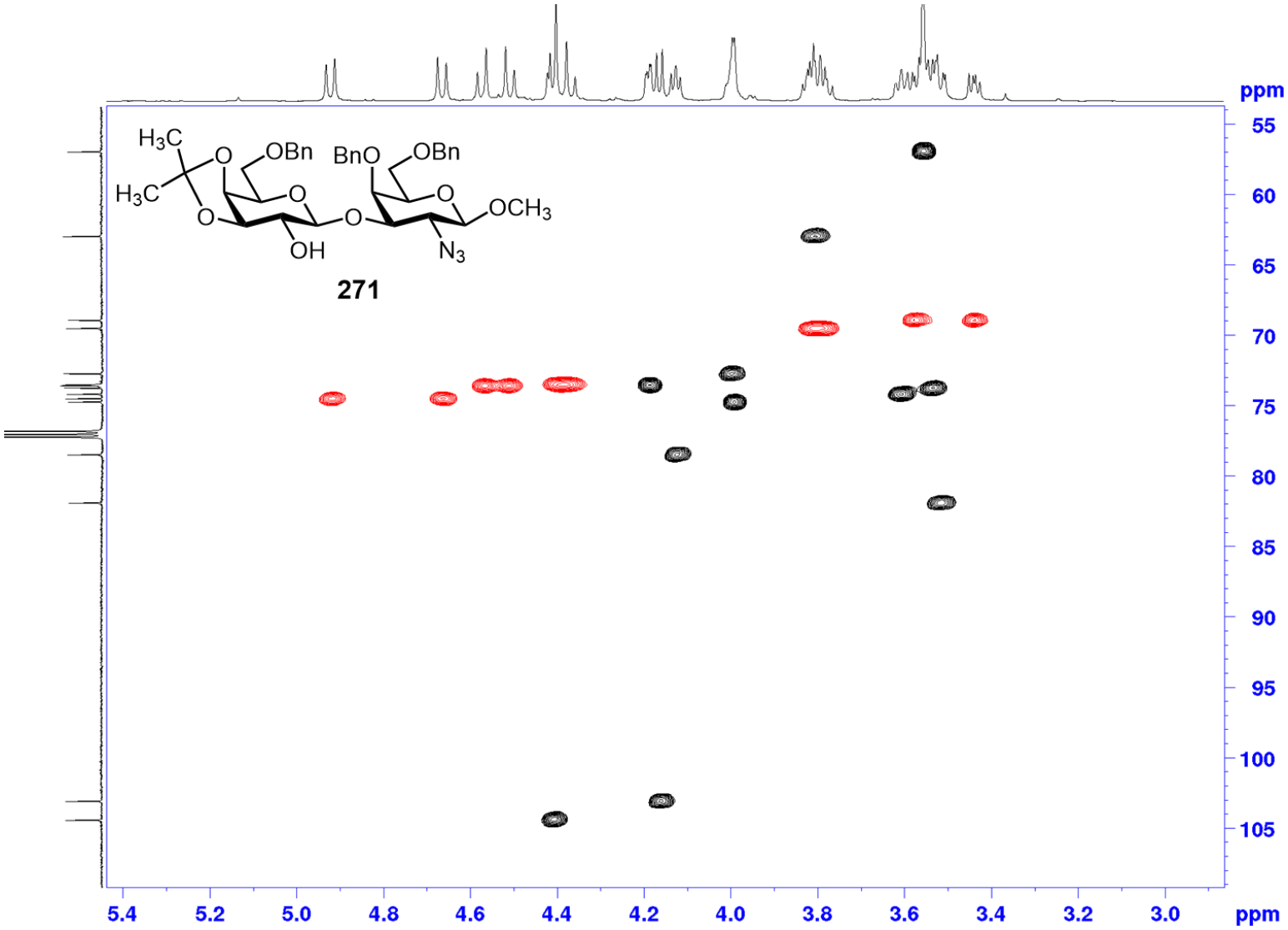
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 271



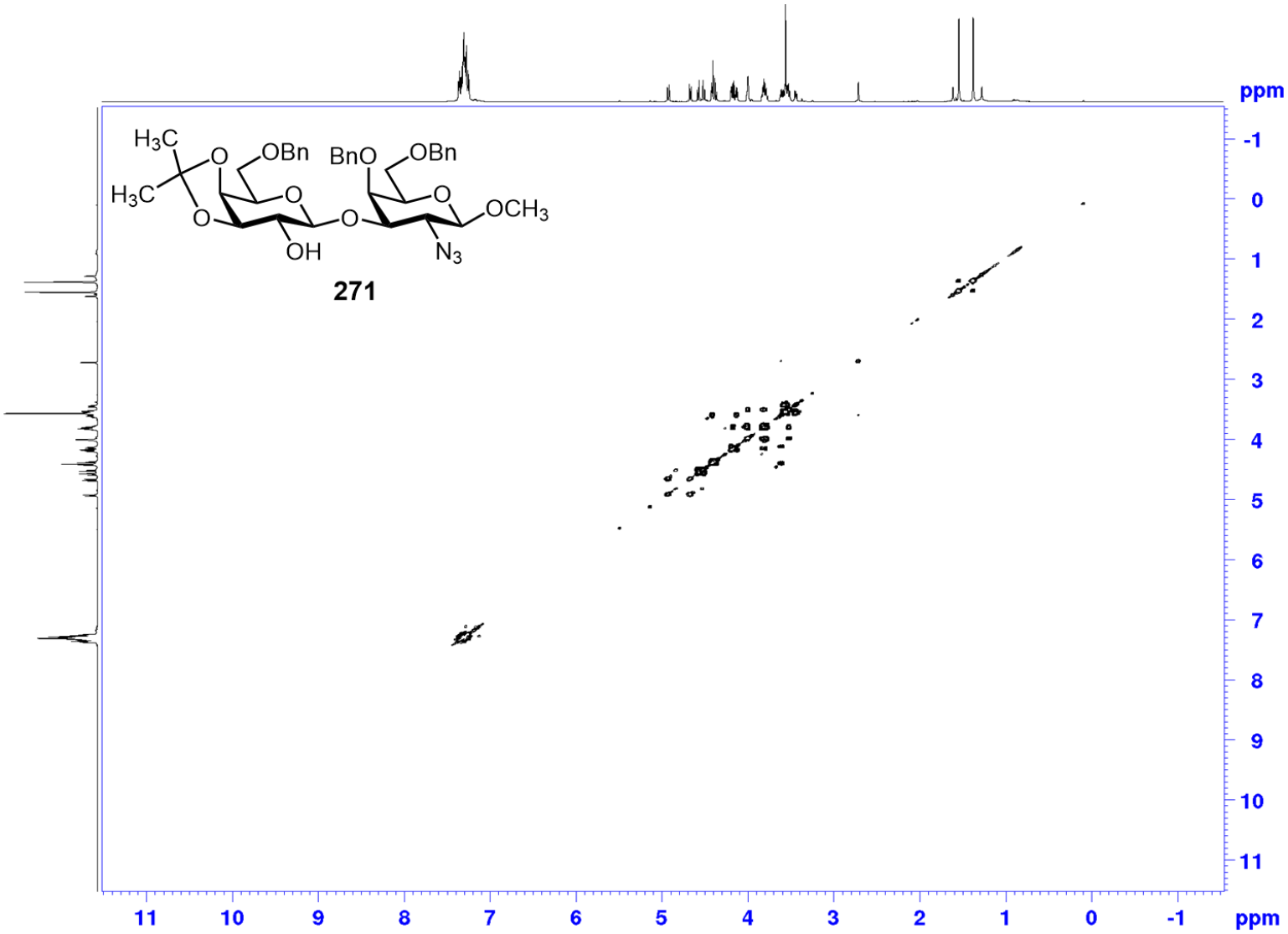
^{1}H-^{13}C HSCQ ($CDCl_3$) of compound 271



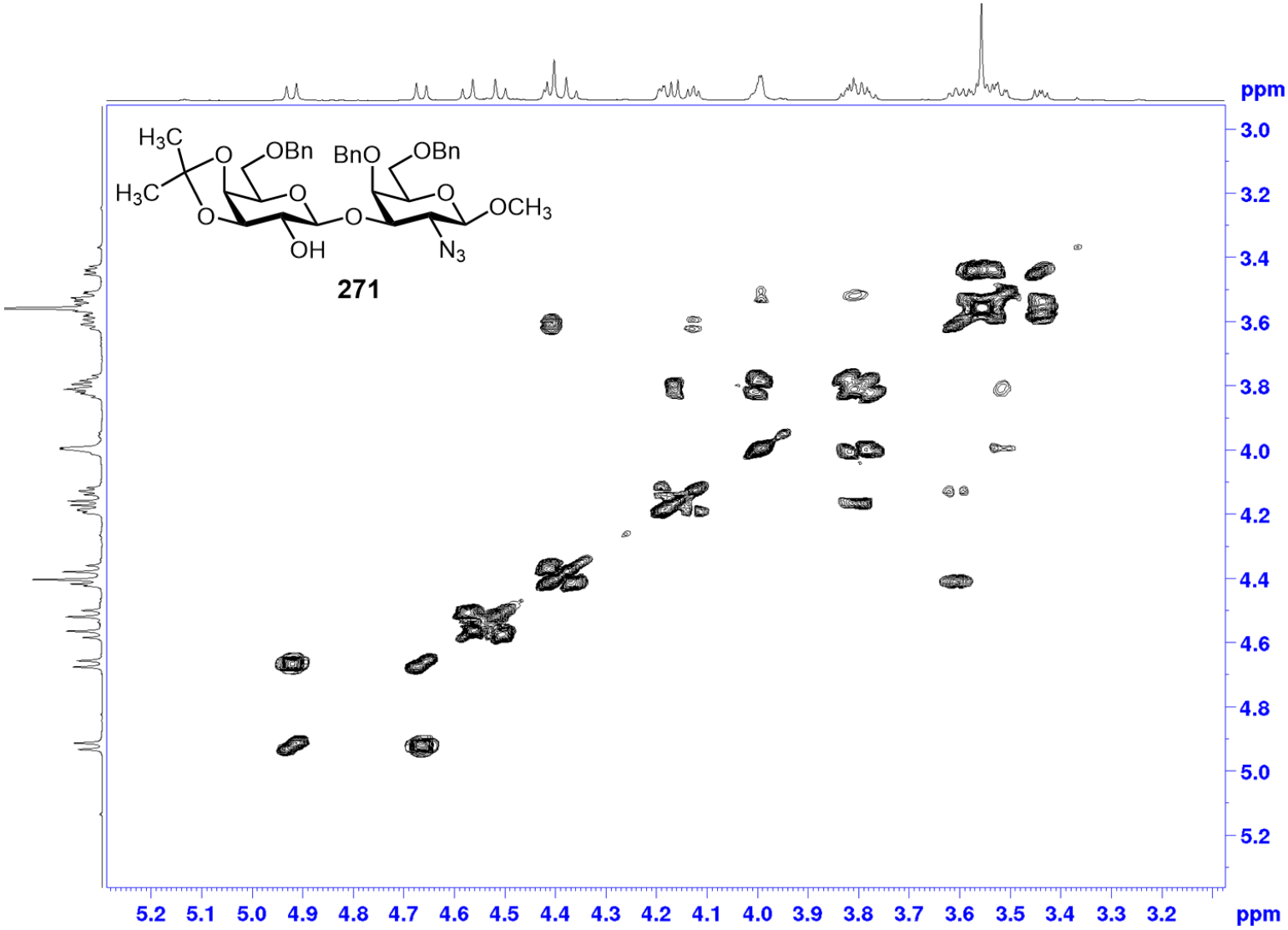
^{1}H-^{13}C HSCQ ($CDCl_3$) zoomed in region of compound 271

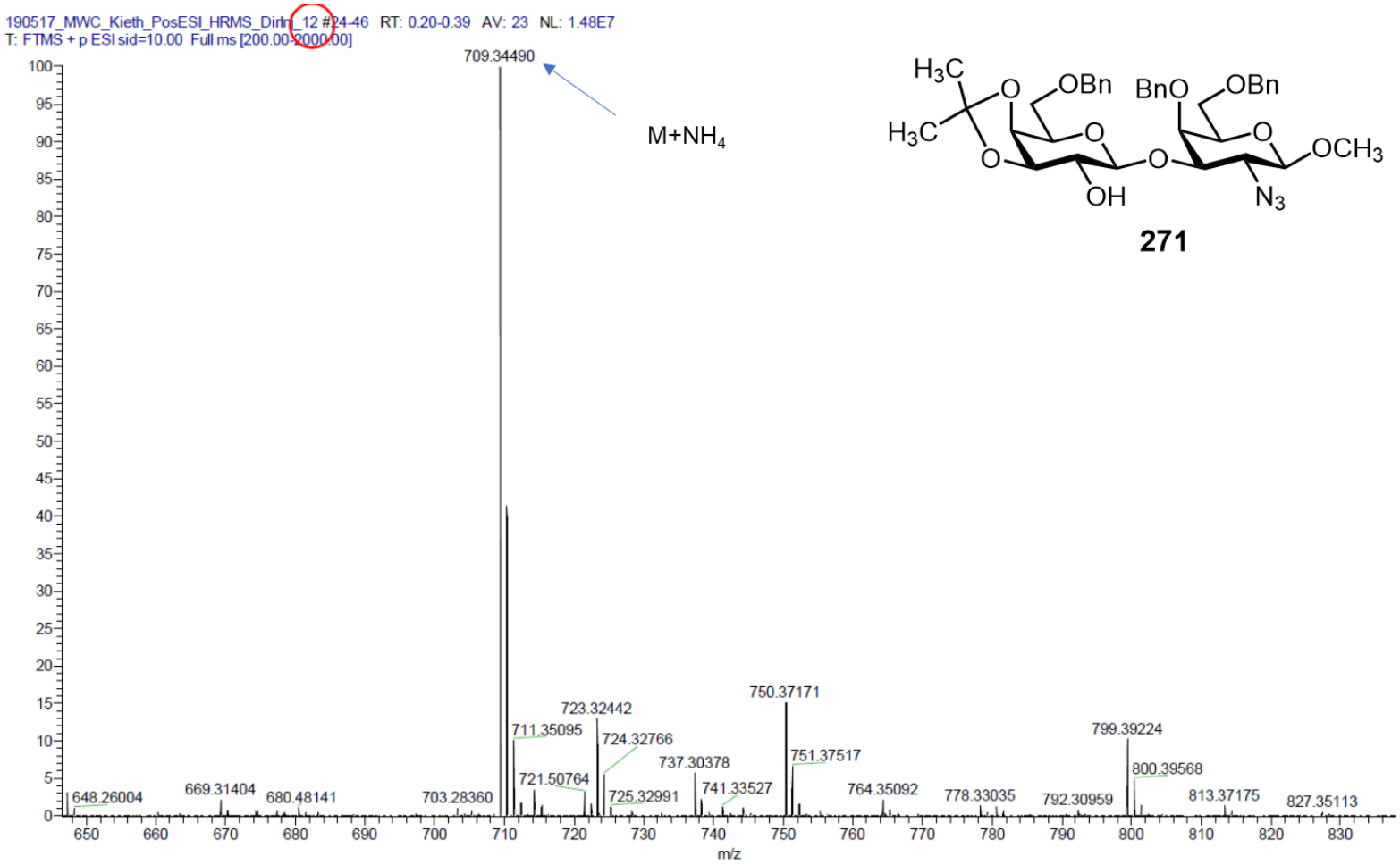


^{1}H-^{1}H COSY ($CDCl_3$) of compound 271

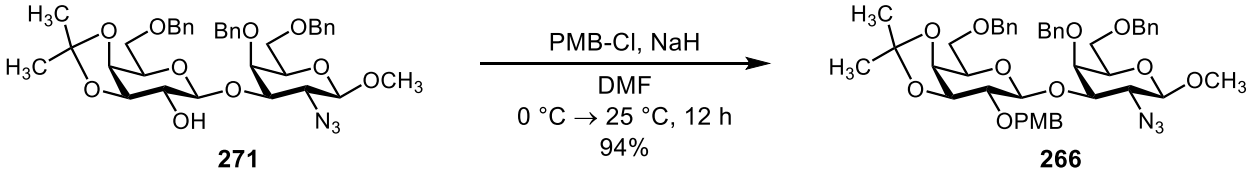


^{1}H-^{1}H COSY ($CDCl_3$) zoomed in region of compound 271




190517_MWC_Kieth_PosESI_HRMS_Dirln_12 #24-46 RT: 0.20-0.39 AV: 23 NL: 1.48E7
T: FTMS + p ESI sid=10.00 Full ms [200.00-2000.00]
709.34490
M+NH4
H3C
H3C
O
OBn
BnO
OBn
O
O
O
O
OCH3
OH
N3
271
750.37171
723.32442
711.35095
724.32766
799.39224
737.30378
751.37517
800.39568
721.50764
741.33527
764.35092
778.33035
792.30959
813.37175
827.35113
648.26004
669.31404
680.48141
703.28360
725.32991
m/z

Methyl 6-*O*-benzyl-*O*-3,4-isopropylidene-2-*O*-(*p*-methoxy)-β-D-galactopyranosyl-(1→3)-4,6-di-*O*-benzyl-2-deoxy-2-azideo-β-D-galactopyranoside (266)

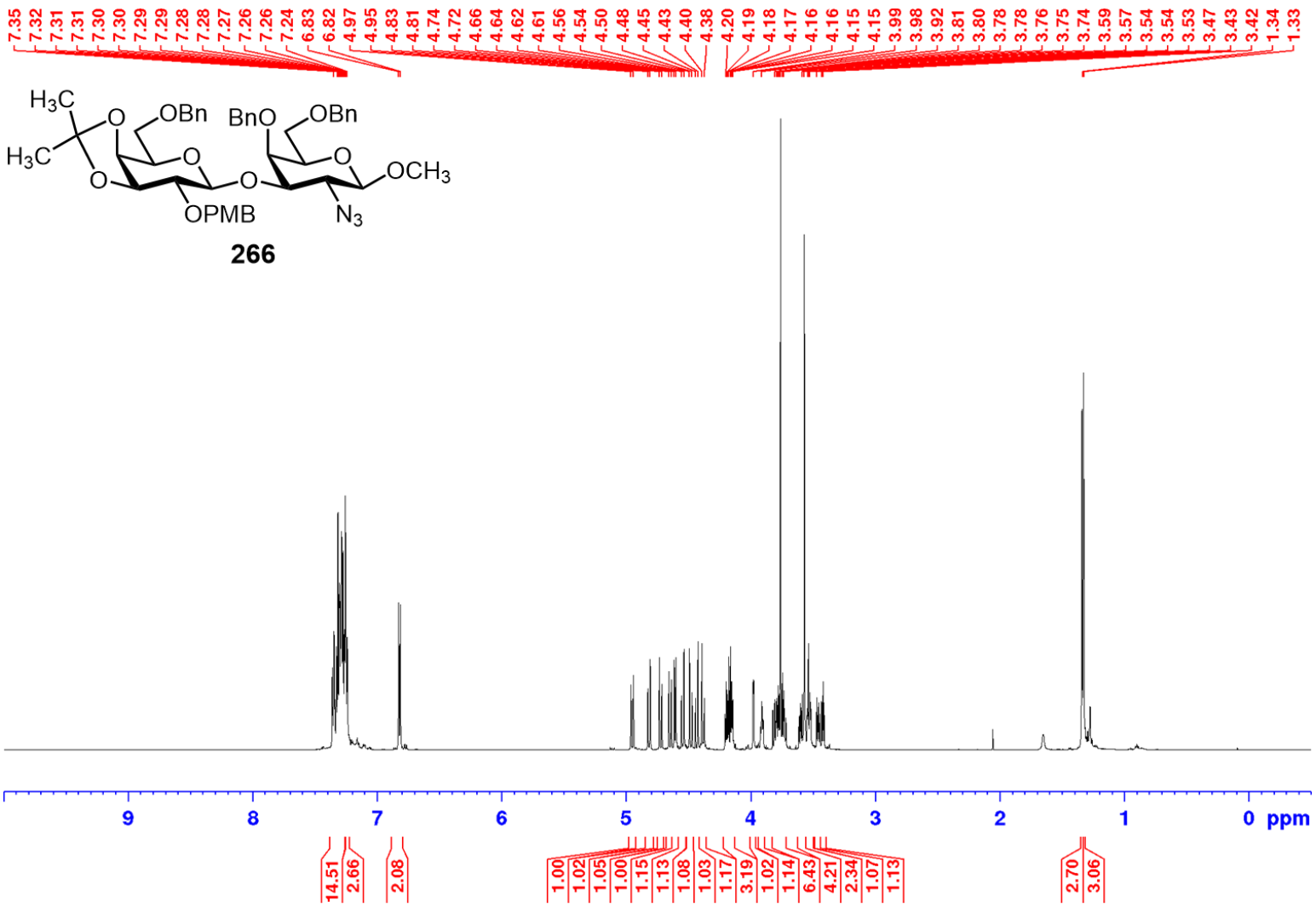


271 **266**

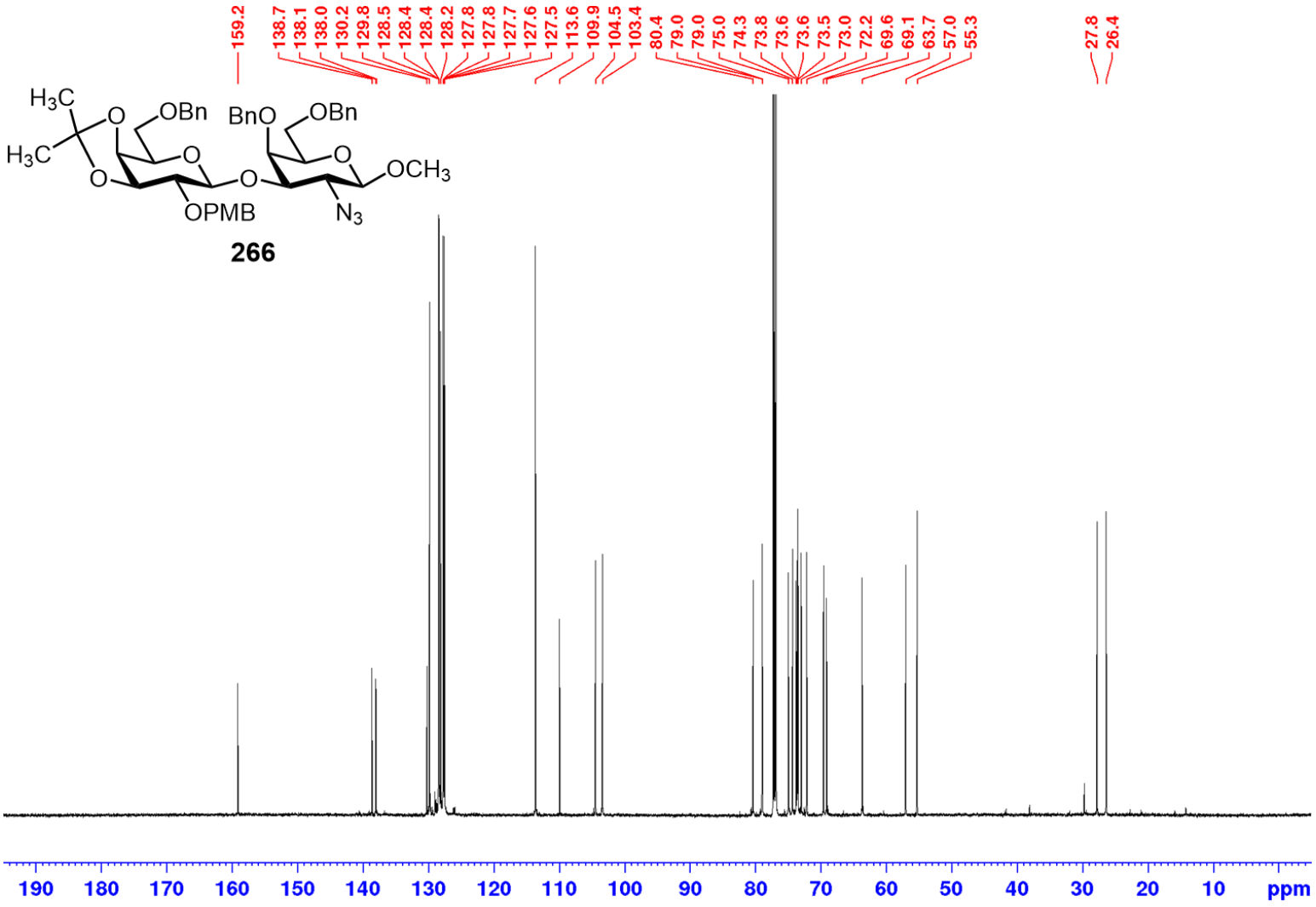
A solution of methyl 6-*O*-benzyl-*O*-3,4-isopropylidene-β-D-galactopyranosyl-(1→3)-4,6-di-*O*-benzyl-2-deoxy-2-azideo-β-D-galactopyranoside **271** (1.0 equiv., 2.579 g, 3.728 mmol) in anhydrous DMF (20 mL) was added solid NaH (3.0 equiv., 0.447 g, 11.18 mmol, 60% in mineral oil) at room temperature under a constant stream of argon that was allowed to exit the reaction flask through a vent needle. At 30 minutes, the reaction mixture was cooled to 0 °C and added 4-methoxybenzyl chloride (2.0 equiv., 1.02 mL, 7.45 mmol) dropwise. At 1.5 h, TLC indicated complete consumption of the starting material. The reaction was added CH_3OH dropwise until the evolution of H_2 gas was complete. The reaction was added EtOAc (100 mL) and transferred to a separatory funnel. The crude reaction mixture was washed with 1 N HCl (3 x 20 mL), sat. aq. $NaHCO_3$ (1 x 20 mL), water (1 x 20 mL) and brine (1 x 20 mL). The organics were dried ($NaSO_4$) and filtered through a glass fritted vacuum filter funnel to remove drying agent. The filtrate was concentrated to a syrup and purified by silica gel flash column chromatography (30:70 to 40:60 EtOAc/Hexanes) to methyl 6-*O*-benzyl-*O*-3,4-isopropylidene-2-*O*-(*p*-methoxy)-β-D-galactopyranosyl-(1→3)-4,6-di-*O*-benzyl-2-deoxy-2-azideo-β-D-galactopyranoside **266** (2.899 g, 3.570 mmol, >95% yield) as a white amorphous solid. R_f = 0.45 (30:70 EtOAc/hexanes) visualized with ceric ammonium molybdate stain; $[\alpha]D^{25}$ = -0.12° (c = 1.0, $CHCl_3$); IR ν_{max} (film) 3401.30, 2930.27, 2360.70, 2112.51, 1613.19,

1512.95, 1497.03, 1454.23, 1368.94, 1246.52, 1219.31, 1076.25, 871.19, 820.88, 736.87, 698.50 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.36-7.24 (m, 17H), 6.83 (d, *J* = 8.6 Hz, 2H), 4.96 (d, *J* = 11.9 Hz, 1H), 4.82 (d, *J* = 11.7 Hz, 1H), 4.73 (d, *J* = 11.6 Hz, 1H), 4.65 (d, *J* = 12.0 Hz, 1H), 4.61 (d, *J* = 7.8 Hz, 1H), 4.55 (d, *J* = 11.7 Hz, 1H), 4.49 (d, *J* = 12.0 Hz, 1H), 4.44 (d, *J* = 11.7 Hz, 1H), 4.39 (d, *J* = 11.9 Hz, 1H), 4.20-4.16 (m, 3H), 3.98 (d, *J* = 2.3 Hz, 1H), 3.92-3.90 (m, 1H), 3.82-3.72 (m, 6H), 3.61-3.52 (m, 6H), 3.47-3.41 (m, 2H), 1.34 (s, 3H), 1.32 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 159.2, 138.7, 138.1, 138.0, 130.3, 129.8, 129.8, 128.5, 128.5, 128.4, 128.4, 128.4, 128.4, 128.2, 128.2, 127.8, 127.8, 127.8, 127.7, 127.6, 127.6, 127.5, 113.7, 113.7, 110.0, 104.5, 103.4, 80.4, 79.0, 79.0, 75.0, 74.4, 73.8, 73.6, 73.6, 73.5, 73.0, 72.2, 69.6, 69.1, 63.7, 57.0, 55.23 27.8, 26.4; HRMS (ESI) calcd for $C_{45}H_{57}N_4O_{10}^{+}$ $(M+NH_4)^{+}$ 829.40182, found 829.40259 *m/z*.

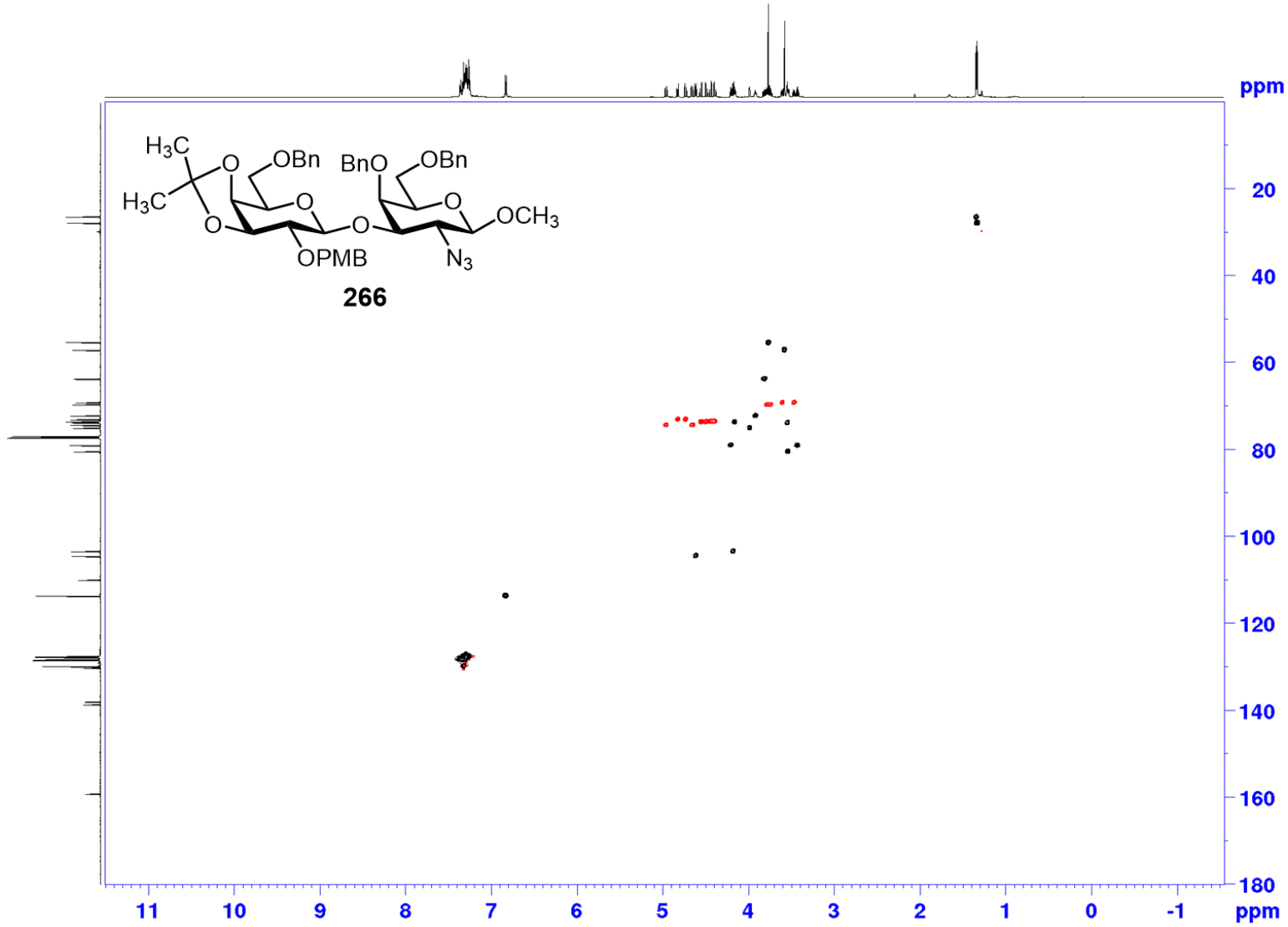
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 266



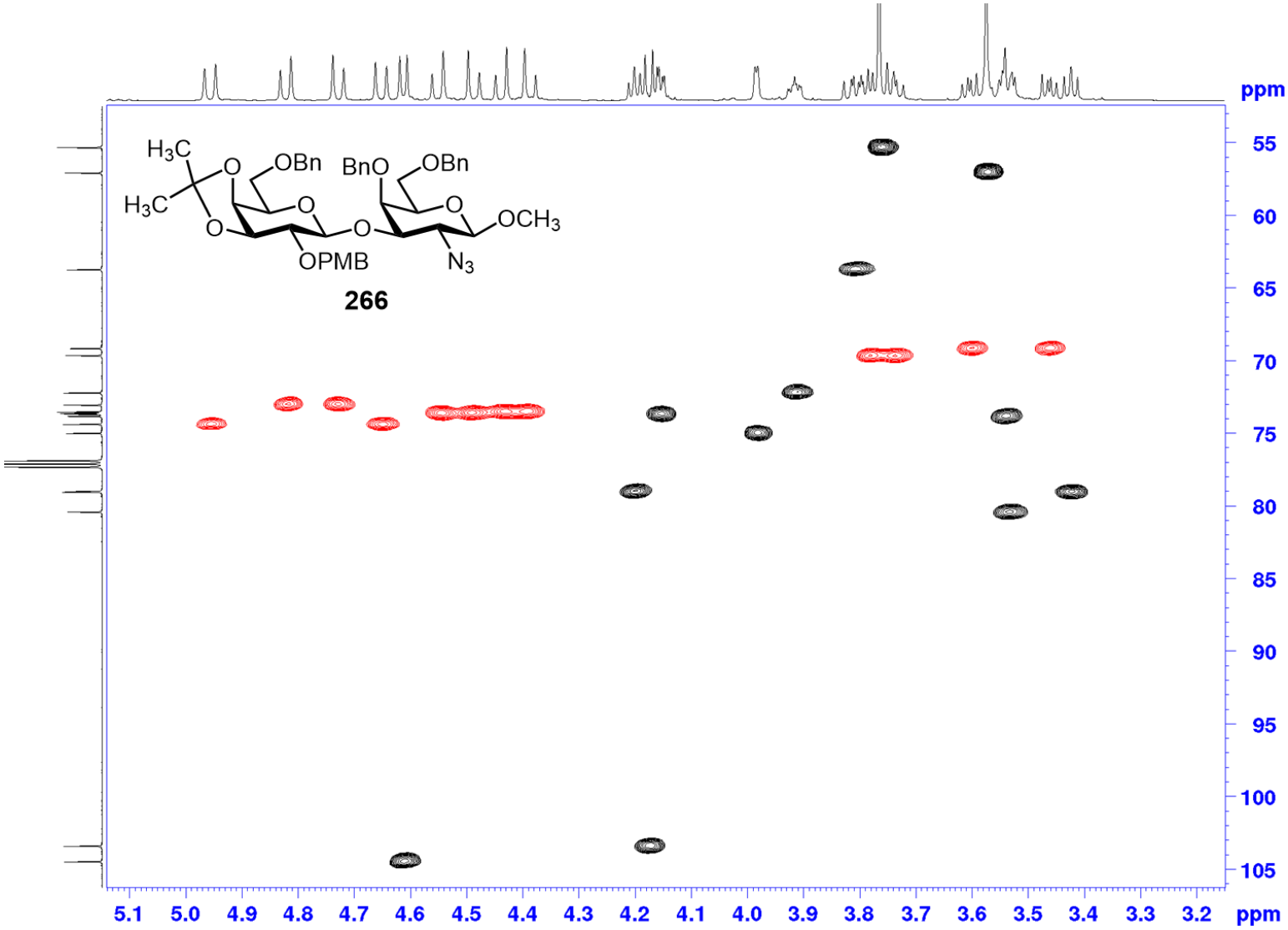
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 266

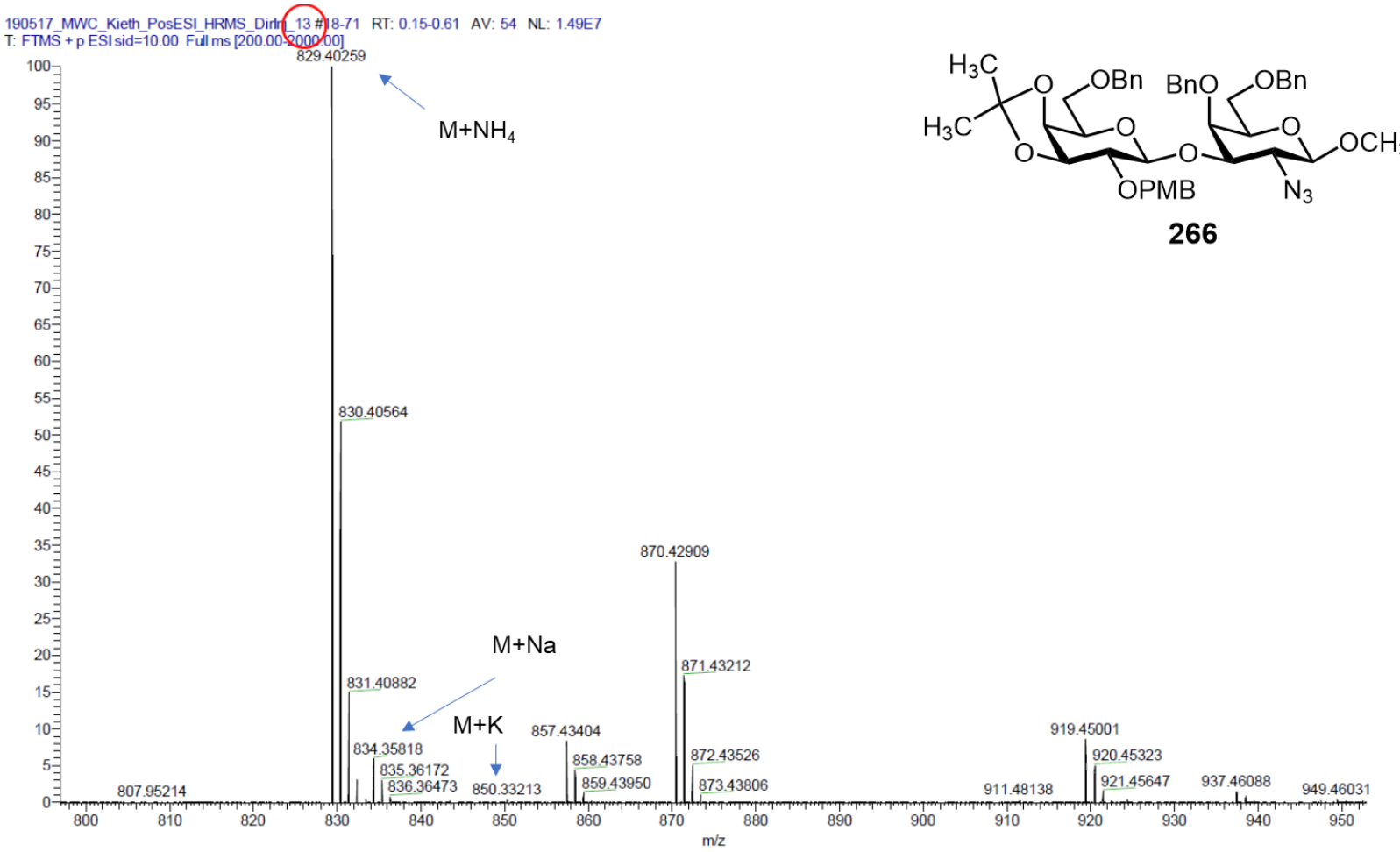


^{1}H-^{13}C HSCQ ($CDCl_3$) of compound 266



^{1}H-^{13}C HSCQ ($CDCl_3$) zoomed in region of compound 266

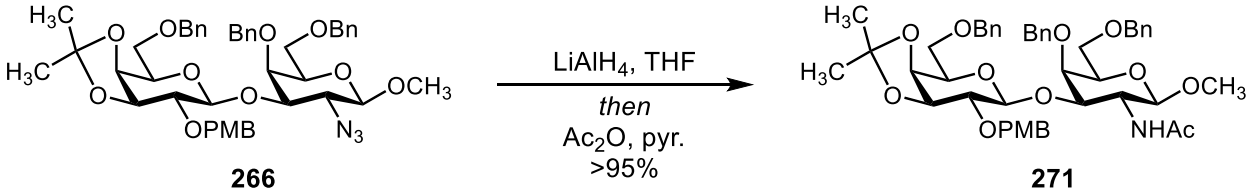



190517_MWC_Kieth_PosESI_HRMS_DirIn_13 #18-71 RT: 0.15-0.61 AV: 54 NL: 1.49E7
T: FTMS + p ESI sid=10.00 Full ms [200.00-2000.00]
829.40259
M+NH4
830.40564
870.42909
M+Na
871.43212
831.40882
M+K
857.43404
834.35818
858.43758
835.36172
872.43526
836.36473
850.33213
859.43950
873.43806
807.95214
911.48138
919.45001
920.45323
921.45647
937.46088
949.46031
m/z

H3C
H3C
O
OBn
BnO
OBn
O
O
O
O
OCH3
OPMB
N3

266

Methyl 6-*O*-benzyl-*O*-3,4-isopropylidene-2-*O*-(*p*-methoxy)-β-D-galactopyranosyl-(1→3)-4,6-di-*O*-benzyl-2-deoxy-2-acetamido-β-D-galactopyranoside (271)

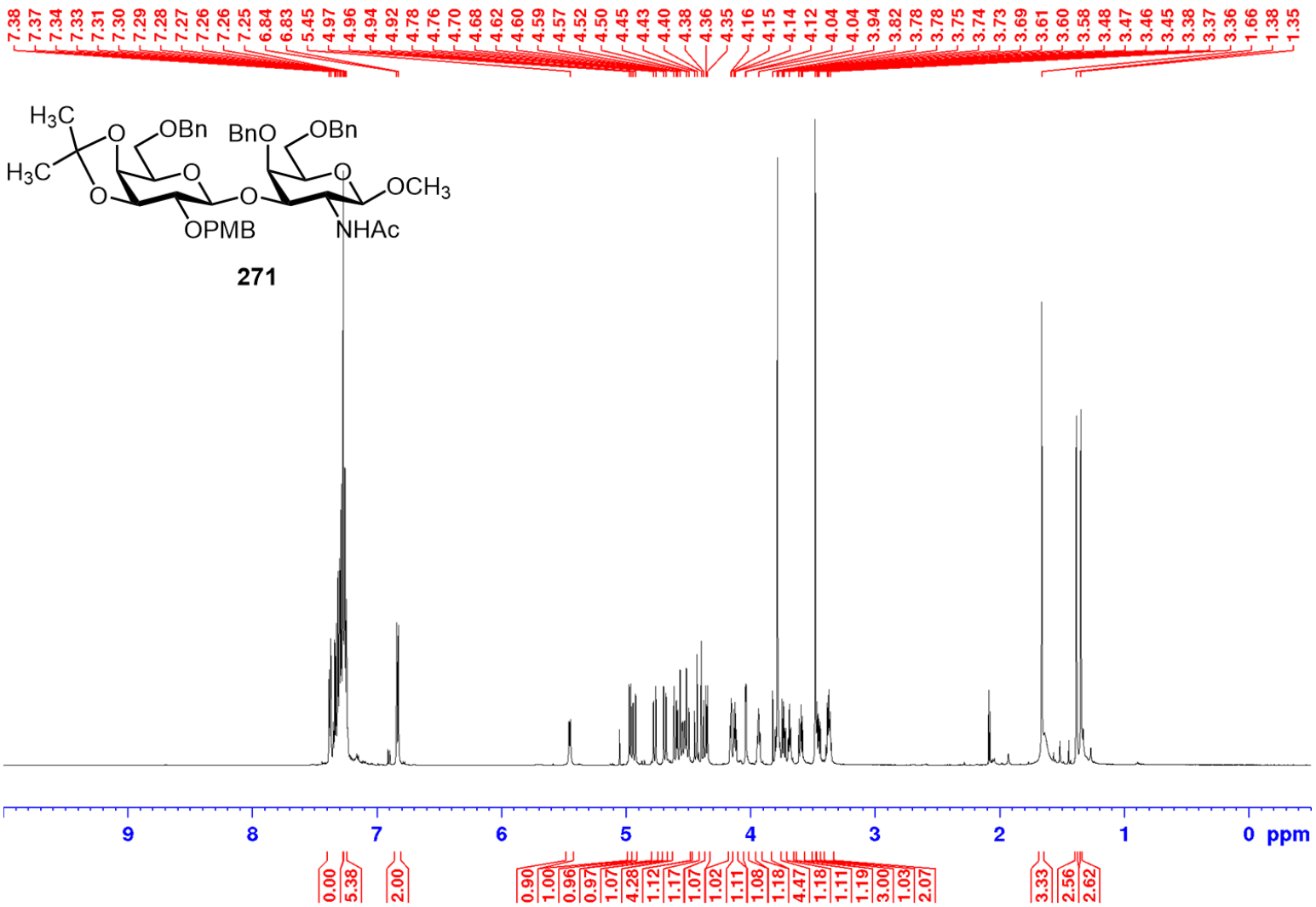


266 **271**

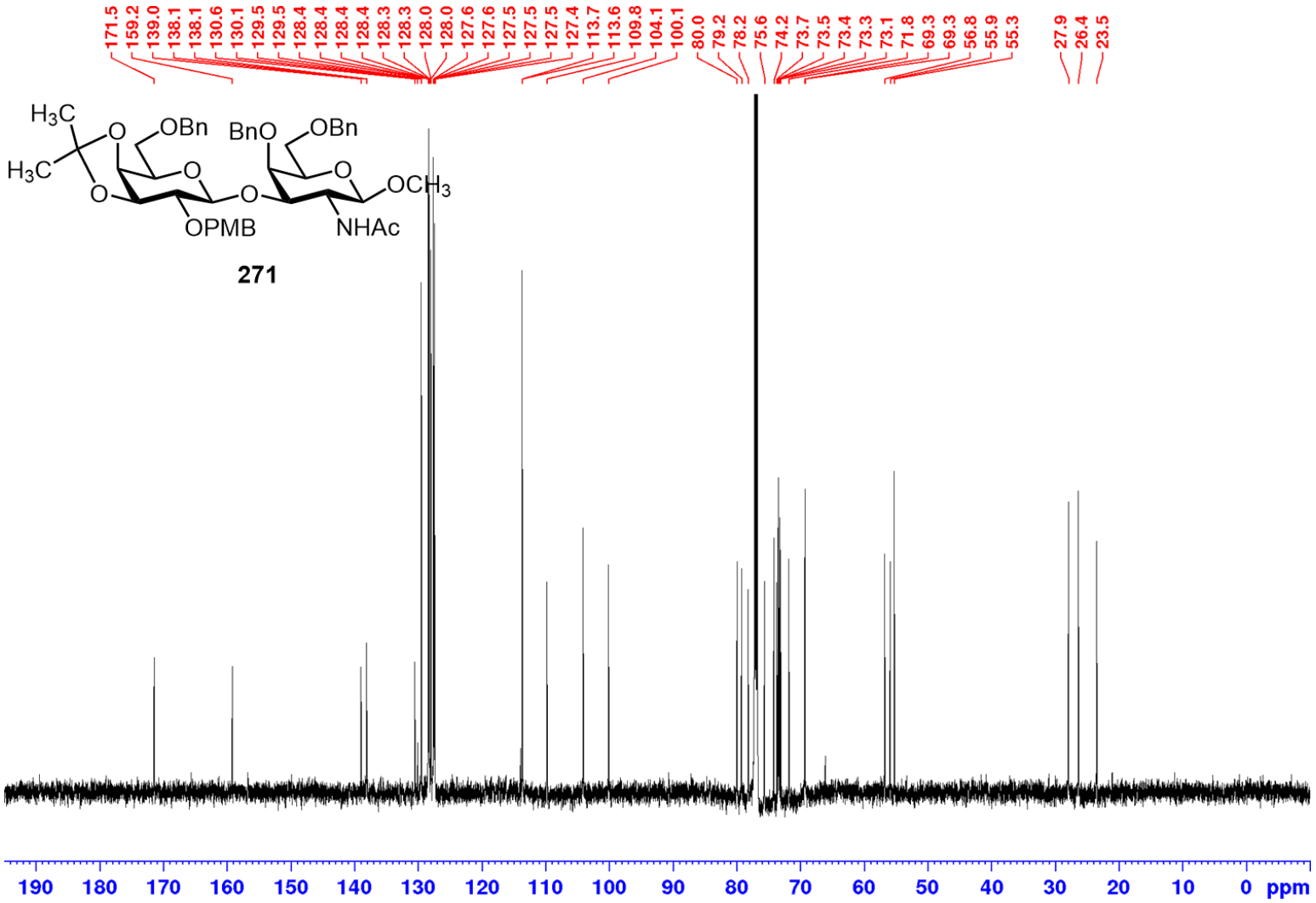
A solution of methyl 6-*O*-benzyl-*O*-3,4-isopropylidene-2-*O*-(*p*-methoxy)-β-D-galactopyranosyl-(1→3)-4,6-di-*O*-benzyl-2-deoxy-2-azideo-β-D-galactopyranoside **266** (1.0 equiv., 2.00 g, 2.46 mmol) in anhydrous THF (20.0 mL) under an argon atmosphere was cooled to -15 °C added a solution of $LiAlH_4$ (4.0 eq, 4.11 mL, 9.85 mmol, [1.0 M] in THF). The reaction was stirred for 2 h while warming to 0 °C. At 2 h, the reaction was cooled to -15 °C and added water (0.5 mL) dropwise followed by 15% NaOH aq. (0.5 mL) then water (1.5 mL) and allowed to warm to room temperature and stir for 15 minutes then added $NaSO_4$. The reaction mixture was filtered through a glass fritted vacuum filter funnel. The filtrate was concentrated to dryness then directly added Ac_2O (17.0 equiv., 4.00 mL, 42.4 mmol) and pyridine (20.0 equiv., 4.00 mL, 49.5 mmol) and added DMAP (0.1 equiv.) under an argon atmosphere at 0 °C. The reaction was stirred at room temperature. At 3 h, the reaction was cooled to 0 °C and added EtOAc and CH_3OH to consume excess acetic anhydride. The reaction warmed to room temperature while stirring for 10 min. Then reaction was concentrated to an oil and taken up in EtOAc (100 mL) and washed with 1 N HCl (2 x 20 mL), water (1 x 20 mL), brine (1 x 20 mL), dried ($NaSO_4$) and filtered through a glass fritted vacuum filter funnel to remove drying agent. The filtrate was concentrated to an oil. The reaction was then purified by silica gel flash column chromatography (75:25 to 10:00 EtOAc/hexanes) to give methyl 6-*O*-benzyl-*O*-

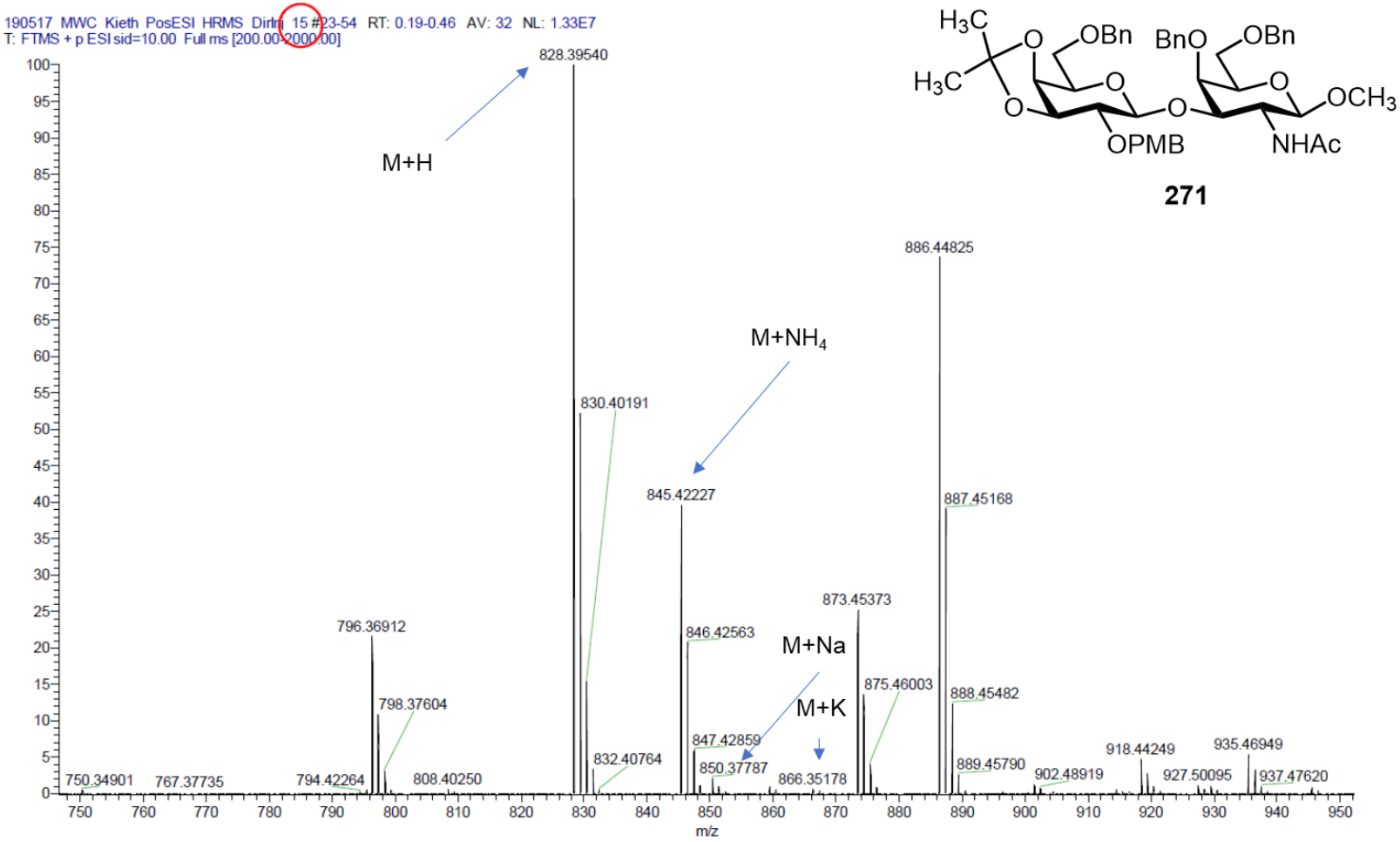
3,4-isopropylidene-2-*O*-(*p*-methoxy)-β-D-galactopyranosyl-(1→3)-4,6-di-*O*-benzyl-2-deoxy-2-acetamido-β-D-galactopyranoside **271** (1.96 g, 2.36 mmol, 96%) as a white foam. R_f = 0.4 (90:10 EtOAc/hexanes) visualized with ceric ammonium molybdate stain; $[\alpha]D^{25}$ = -6.88° (c = 1.0, $CHCl_3$); IR ν_{max} (film) 2929.36, 1653.91, 1611.12, 1512.18, 1453.52, 1320.25, 1305.81, 1242.64, 1074.66, 1032.15, 872.15, 824.03, 731.31, 693.17 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.38-7.27 (m, 12H), 7.26-7.23 (5H), 6.83 (d, *J* = 8.6 Hz, 2H), 5.45 (d, *J* = 6.7 Hz, 1H), 4.96 (d, *J* = 8.3 Hz, 1H), 4.93 (d, *J* = 11.8 Hz, 1H), 4.77 (d, *J* = 11.3 Hz, 1H), 4.68 (d, *J* = 11.9 Hz, 1H), 4.62-4.50 (m, 4H), 4.49 (d, *J* = 11.8 Hz, 1H), 4.38 (d, *J* = 11.8 Hz, 1H), 4.35 (d, *J* = 8.2 Hz, 1H), 4.16 (dd, *J* = 1.9, 5.5 Hz, 1H), 4.14-4.12 (m, 1H), 4.04 (d, *J* = 2.6 Hz, 1H), 3.95-3.92 (m, 1H), 3.82-3.75 (m, 4H), 3.75-3.71 (m, 1H), 3.70-3.67 (m, 1H), 3.59 (dd, *J* = 6.2, 9.7 Hz, 1H), 3.48 (s, 3H), 3.45 (dd, J = 6.1, 9.8 Hz, 1H), 3.40-3.35 (m, 2H), 1.66 (s, 3H), 1.38 (s, 3H), 1.35 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.5, 159.2, 139.0, 138.1, 138.1, 130.6, 130.1, 129.5, 129.5, 128.4, 128.4, 128.4, 128.4, 128.3, 128.3, 128.0, 128.0, 127.6, 127.6, 127.5, 127.5, 127.5, 127.4, 113.7, 113.7, 109.8, 104.2, 100.1, 80.0, 79.2, 78.2, 75.7, 74.2, 73.7, 73.5, 73.4, 73.3, 73.1, 71.8, 69.3, 69.3, 56.8, 55.9, 55.3, 27.9, 26.4, 23.5; HRMS (ESI) calcd for $C_{47}H_{58}NO_{12}^+$ $(M+H)^+$ 828.39538, found 828.39540 *m/z*.

^{1}H NMR ($CDCl_3$, 400 MHz) of compound 271



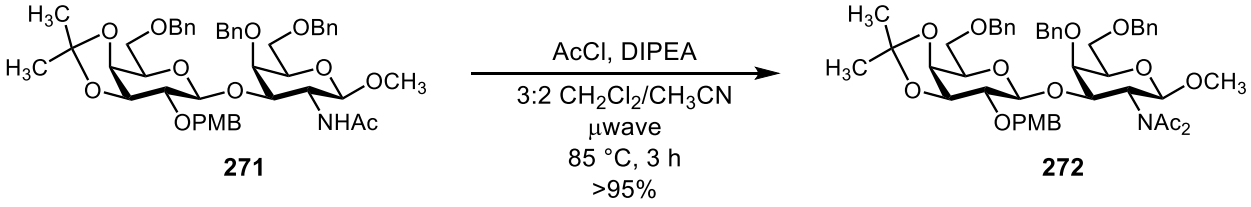
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 271




190517 MWC Kieth PosESI HRMS DirInj 15 #23-54 RT: 0.19-0.46 AV: 32 NL: 1.33E7
T: FTMS + p ESI sid=10.00 Full ms [200.00-2000.00]
828.39540
M+H
M+NH4
M+Na
M+K
886.44825
830.40191
845.42227
887.45168
873.45373
796.36912
846.42563
875.46003
888.45482
798.37604
847.42859
832.40764
850.37787
866.35178
889.45790
902.48919
918.44249
935.46949
927.50095
937.47620
750.34901
767.37735
794.42264
808.40250
m/z

271

Methyl 6-*O*-benzyl-*O*-3,4-isopropylidene-2-*O*-(*p*-methoxy)-β-D-galactopyranosyl-(1→3)-4,6-di-*O*-benzyl-2-deoxy-2-(*N*,*N*-diacetyl)-β-D-galactopyranoside (272)

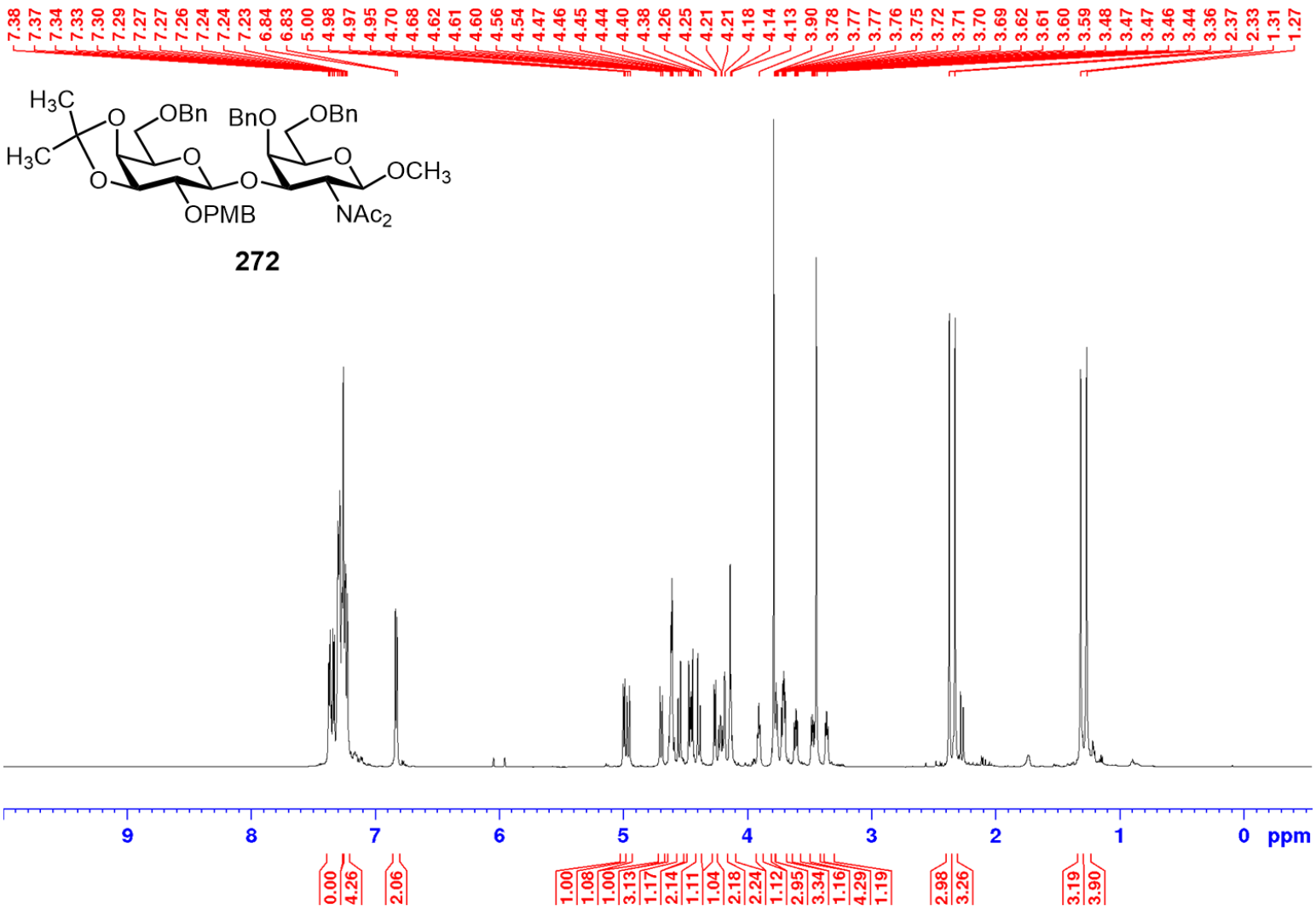


271 **272**

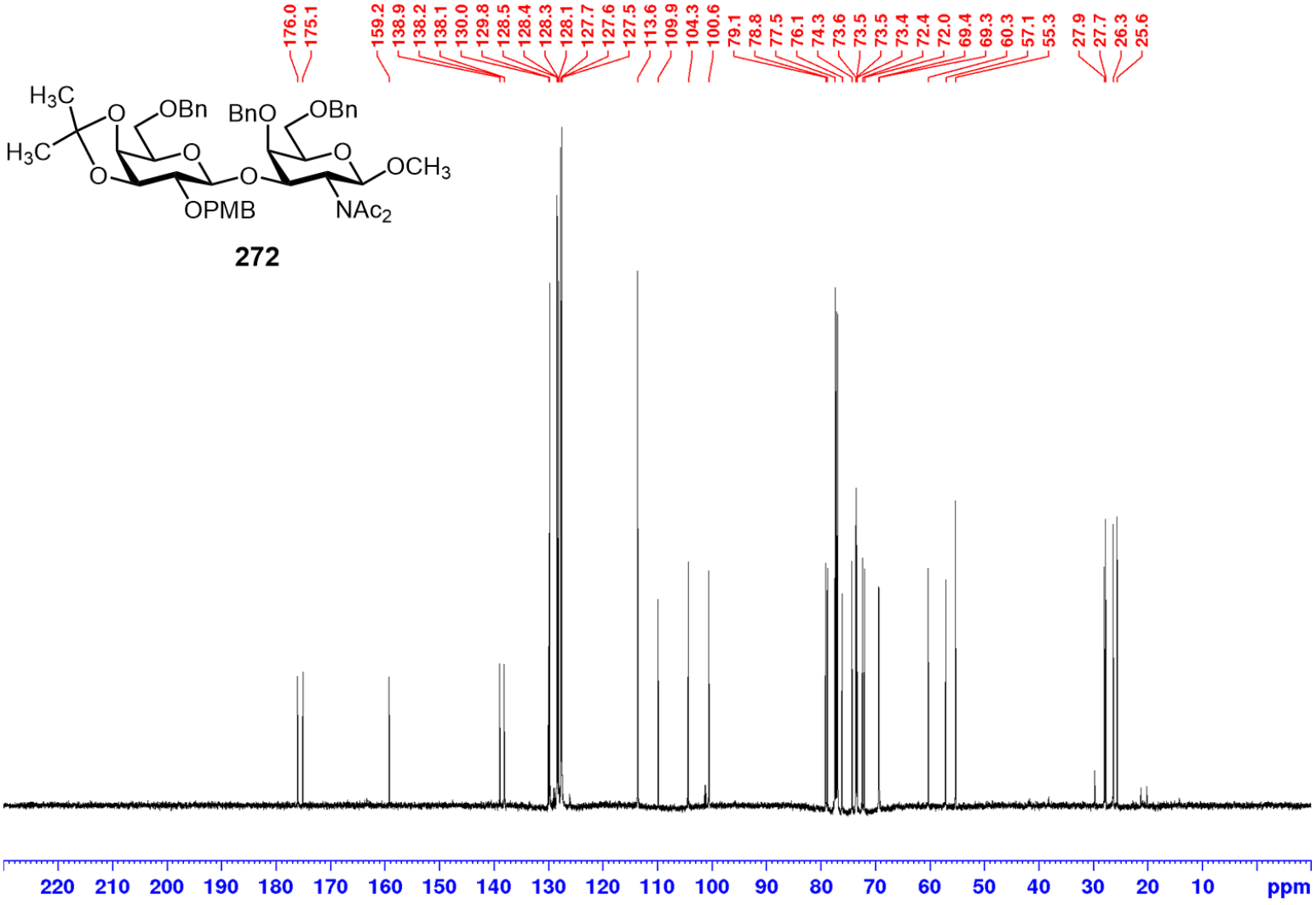
A solution of methyl 6-*O*-benzyl-*O*-3,4-isopropylidene-2-*O*-(*p*-methoxy)-β-D-galactopyranosyl-(1→3)-4,6-di-*O*-benzyl-2-deoxy-2-acetamido-β-D-galactopyranoside **271** (1.0 equiv., 1.47 g, 1.77 mmol) and *N*,*N*-diisopropylethylamine (5.0 equiv., 1.5 mL, 8.87 mmol) in a 3:2 mixture of CH_2Cl_2/CH_3CN (6 mL) was cooled to 0 °C, and added acetyl chloride (5.0 equiv., 0.63 mL, 8.87 mmol) under an argon atmosphere. The reaction mixture was warmed to room temperature then heated by μwave irradiation to 85 °C for 3 h. The reaction was concentrated to a crude oil and transferred to a separatory funnel with EtOAc (25 mL). The organics were washed with 1 N HCl (2 x 5 mL), 40% aq. solution of sodium thiosulfate (2 x 5 mL), aq. sat. $NaHCO_3$ (1 x 5 mL), brine (1 x 5 mL), dried ($NaSO_4$), filtered through a glass fritted filter funnel to remove the drying agents and concentrated to a foam. The crude reaction mixture was purified by silica gel flash chromatography (60:40 to 70:30 EtOAc/hexanes) to give methyl 6-*O*-benzyl-*O*-3,4-isopropylidene-2-*O*-(*p*-methoxy)-β-D-galactopyranosyl-(1→3)-4,6-di-*O*-benzyl-2-deoxy-2-(*N*,*N*-diacetyl)-β-D-galactopyranoside **272** (1.460 g, 1.682 mmol, >95% yield) as a yellow oil. R_f = 0.7 (1:1 EtOAc/hexanes) visualized with ceric ammonium molybdate stain; $[\alpha]D^{25}$ = +0.28° (c = 1.0, $CHCl_3$); IR ν_{max} (film) 2929.38, 1653.91, 1611.12, 1560.19, 1512.18, 1453.55, 1370.25, 1303.81, 1244.61, 1074.66, 1039.17, 872.17, 821.06, 737.30, 698.37 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.381-7.22 (m, 17H), 6.83 (d, *J* = 8.7

Hz, 2H), 5.00 (d, *J* = 7.8 Hz, 1H), 4.96 (d, *J* = 11.9 Hz, 1H), 4.70 (d, *J* = 11.8 Hz, 1H), 4.61 (m, 3H), 4.55 (d, *J* = 12.0 Hz, 1H), 4.45 (m, 2H), 4.39 (d, *J* = 12.1 Hz, 1H), 4.26 (d, *J* = 7.9 Hz, 1H), 4.20 (m, 2H), 4.13 (m, 2H), 3.90 (m, 1H), 3.78 (m, 4H), 3.71 (m, 2H), 3.6 (m, 1H), 3.45 (m, 4H), 3.36 (m, 1H), 2.38 (s, 3H), 2.33 (s, 3H), 1.32 (s, 3H), 1.27 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.5, 159.2, 139, 138.1, 138.1, 130.6, 130.1, 129.5, 129.5, 128.4, 128.4, 128.4, 128.4, 128.3, 128.3, 128, 128, 127.6, 127.6, 127.5, 127.5, 127.5, 127.4, 113.7, 113.6, 109.8, 104.1, 100.1, 80, 79.2, 78.2, 75.6, 74.2, 73.7, 73.5, 73.4, 73.3, 73.1, 71.8, 69.3, 69.3, 56.8, 55.9, 55.3, 27.9, 26.3, 23.6,; HRMS (ESI) calcd for $C_{49}H_{63}N_2O_{13}^+$ $(M+NH_4)^+$ 887.43242, found 887.43158 m/z.

^{1}H NMR ($CDCl_3$, 400 MHz) of compound 272

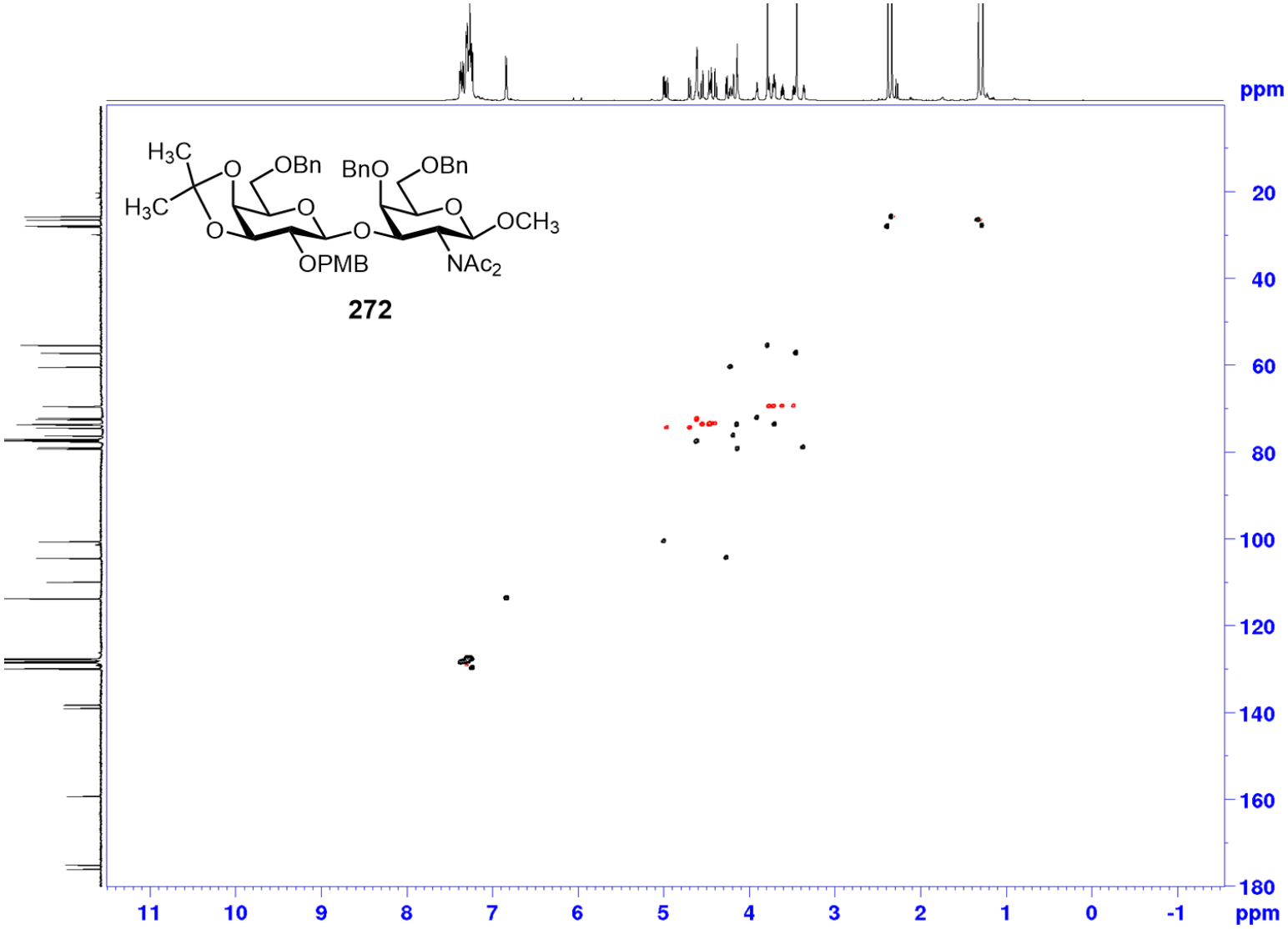


^{13}C NMR ($CDCl_3$, 100 MHz) of compound 272

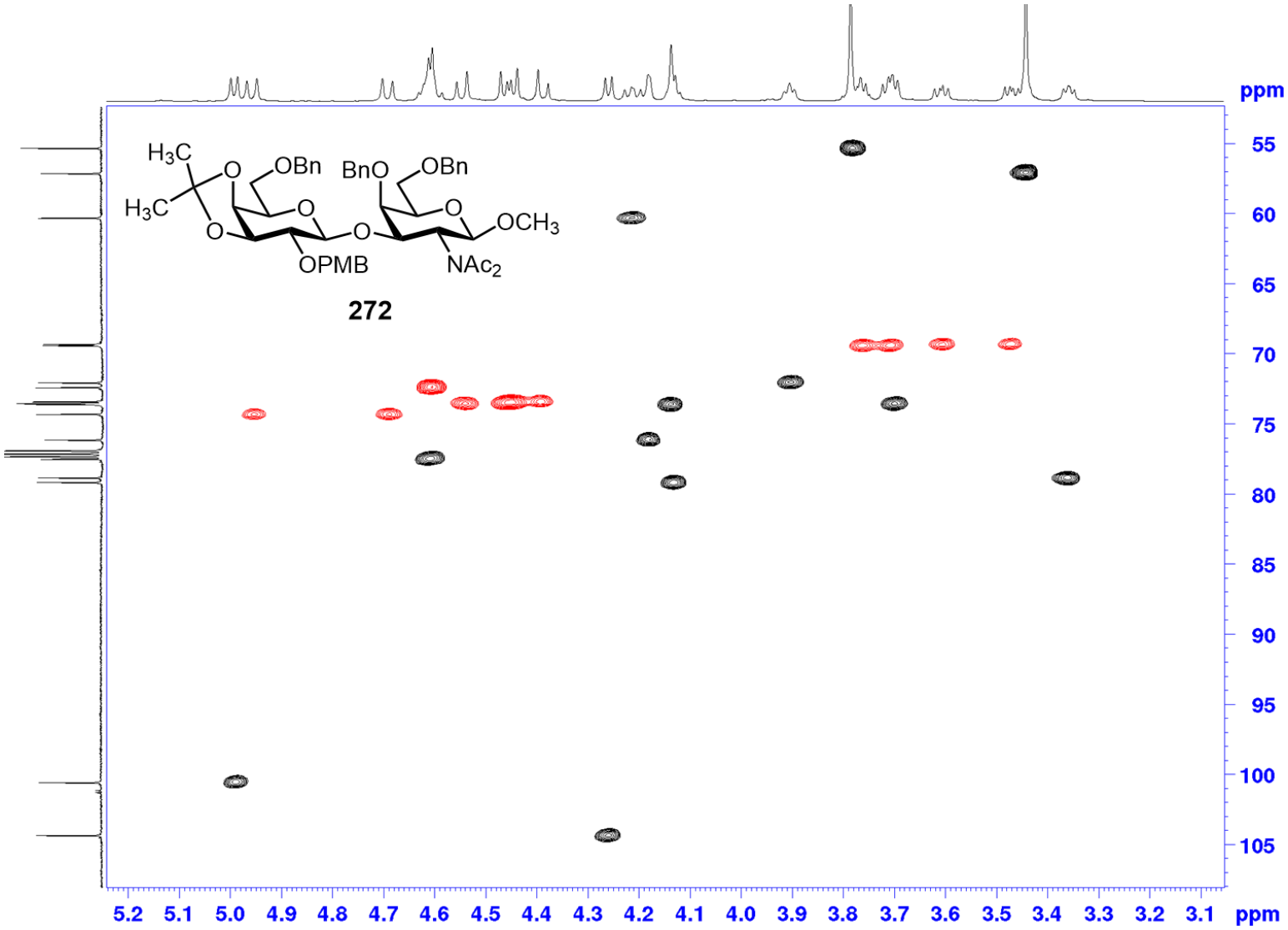


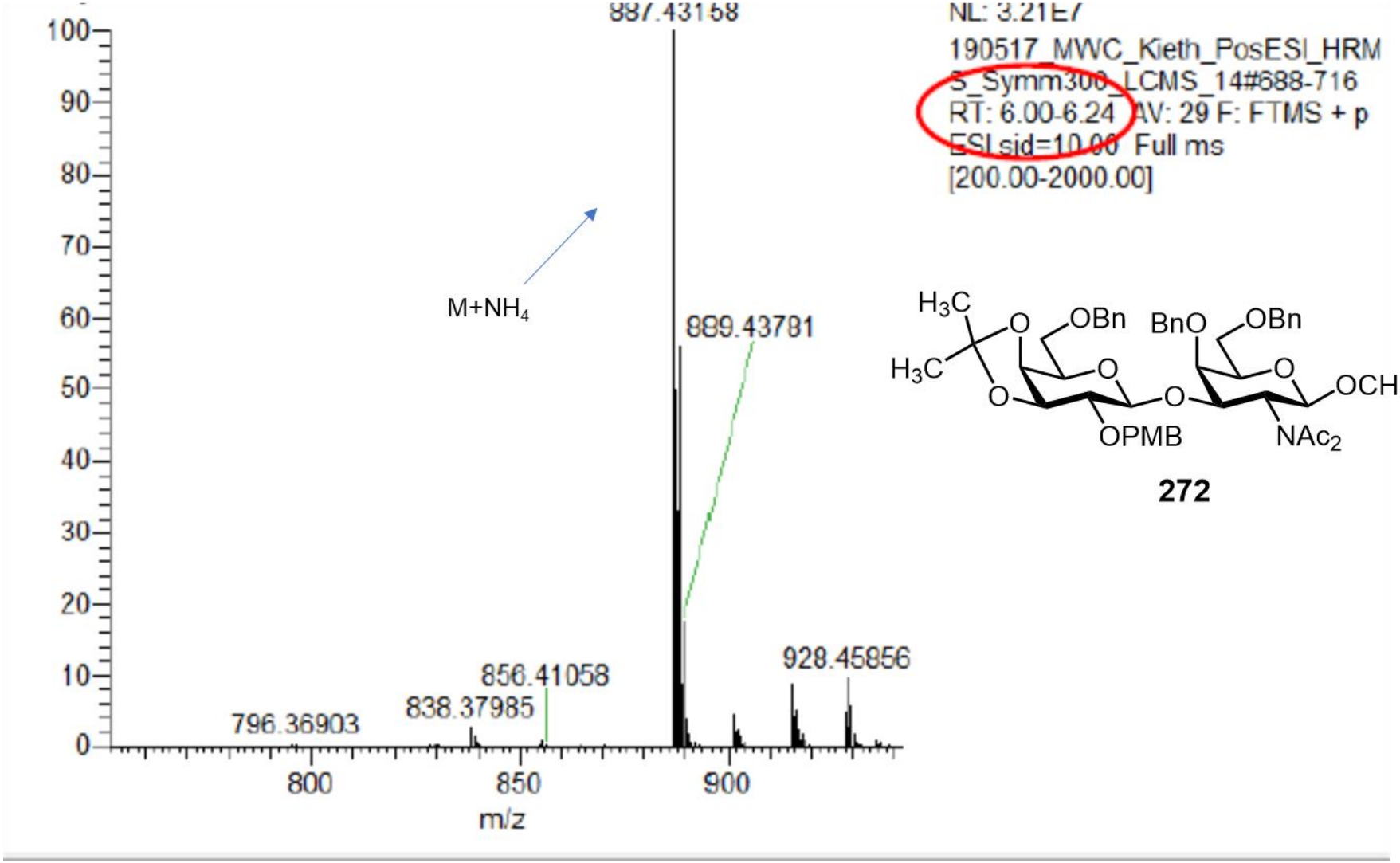
272

^{1}H-^{13}C HSCQ ($CDCl_3$) of compound 272



^{1}H-^{13}C HSCQ ($CDCl_3$) zoomed in region of compound 272




887.43158
NL: 3.21E7
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S_Symm300_LCMS_14#688-716
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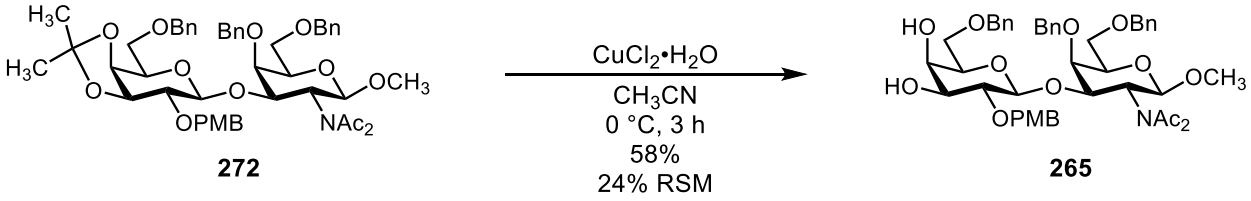
ESI sid=10.00 Full ms
[200.00-2000.00]
M+NH4
889.43781
928.45856
856.41058
838.37985
796.36903
100
90
80
70
60
50
40
30
20
10
0
800
850
900
m/z
H3C
H3C
O
OBn
BnO
OBn
O
O
O
O
O
OCH3
OPMB
NAc2

272

Methyl 6-*O*-benzyl-2-*O*-(*p*-methoxy)-β-D-galactopyranosyl-(1→3)-4,6-di-*O*-benzyl-2-deoxy-2-(*N*,*N*-diacetyl)-β-D-galactopyranoside (265)

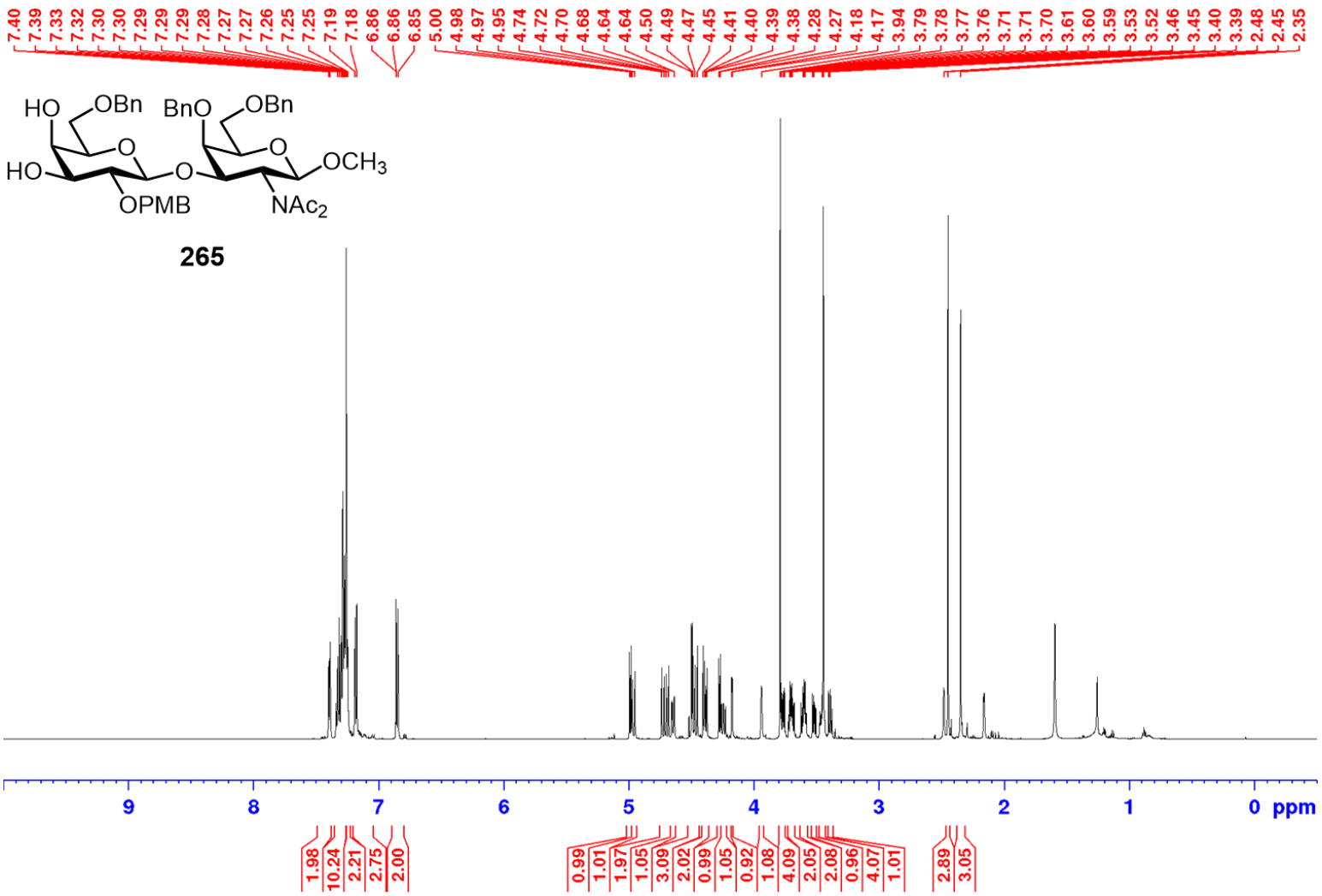


272 **265**

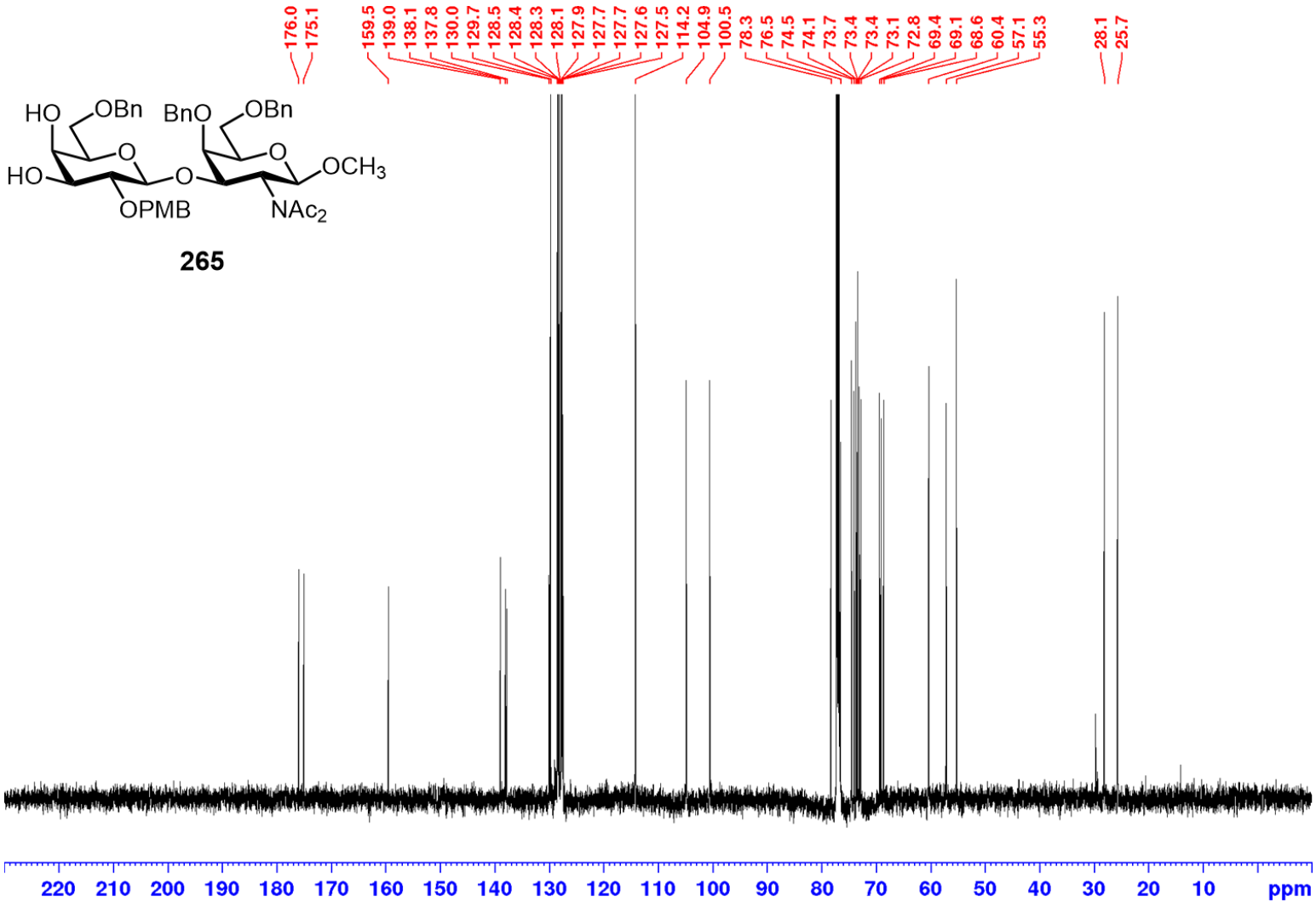
A solution of methyl 6-*O*-benzyl-*O*-3,4-isopropylidene-2-*O*-(*p*-methoxy)-β-D-galactopyranosyl-(1→3)-4,6-di-*O*-benzyl-2-deoxy-2-(*N*,*N*-diacetyl)-β-D-galactopyranoside **272** (1.0 equiv., 2.839 g, 3.263 mmol) and copper (II) chloride hydrate (5.0 equiv., 2.782 g, 16.32 mmol) in CH_3CN (32 mL) was stirred at 0 °C under an argon atmosphere. At 3 h, the crude reaction mixture was filtered through a glass fritted vacuum filter funnel equipped with a pad of Celite. The filtrate was transferred to a separatory funnel with EtOAc (100 mL). The organics were washed with 1 N HCl (3 x 20 mL), aq. sat. $NaHCO_3$ (1 x 20 mL), brine (1 x 20 mL), dried ($NaSO_4$), filtered through a glass fritted vacuum filter funnel to remove drying agent. The filtrate was concentrated to a crude syrup. The crude syrup was purified by silica gel flash column chromatography (20:80 to 66:33 EtOAc/hexanes) to give recovered starting material methyl 6-*O*-benzyl-*O*-3,4-isopropylidene-2-*O*-(*p*-methoxy)-β-D-galactopyranosyl-(1→3)-4,6-di-*O*-benzyl-2-deoxy-2-(*N*,*N*-diacetyl)-β-D-galactopyranoside **272** (0.6833 g, 0.785 mmol, 24%) as a yellow oil and the desired diol product methyl 6-*O*-benzyl-2-*O*-(*p*-methoxy)-β-D-galactopyranosyl-(1→3)-4,6-di-*O*-benzyl-2-deoxy-2-(*N*,*N*-diacetyl)-β-D-galactopyranoside **265** (1.5815 g, 1.9056 mmol, 58%) as an off-white amorphous solid. R_f = 0.30 (1:1 EtOAc/hexanes) visualized with ceric ammonium molybdate stain; $[\alpha]D^{25}$ = -7.32° (c = 1.0, $CHCl_3$); IR ν_{max} (film) 3854.54, 3841.03, 3751.26, 3649.46, 3422.38, 2365.02, 1654.65, 1510.72,

1457.96, 1366.63, 1264.87, 1070.03, 747.47, 702.39 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.40-7.38 (m, 2H), 7.34-7.27 (m, 10H), 7.25-7.24 (m, 3H), 7.19-7.17 (m, 2H), 6.85 (d, *J* = 8.61 Hz, 2H), 4.99 (d, *J* = 7.82 Hz, 1H), 4.9 (d, *J* = 11.85 Hz, 1H), 4.73 (d, *J* = 11.85 Hz, 1H), 4.69 (d, *J* = 11.73 Hz, 1H), 4.64 (dd, *J* = 2.9, 10.80 Hz,1H), 4.52-4.44 (m, 3H), 4.40-4.37 (m, 2H), 4.28 (d, *J* = 7.60 Hz, 1H), 4.24 (dd, *J* = 7.93, 10.84 Hz 1H), 4.17 (d, *J* = 2.46 Hz, 1H), 3.94 (m, 1H), 3.79-3.75 (m, 4H), 3.72-3.67 (m, 2H), 3.62-3.57 (m, 2H), 3.52 (dd, *J* = 6.48, 9.72 Hz, 1H), 3.47-3.45 (m, 4H), 3.39 (dd, *J* = 7.71, 9.28 Hz, 1H), 2.48 (-OH, 1H), 2.45 (s, 3H), 2.34 (s, 3H), 2.16 (-OH, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 176.0, 175.1, 159.5, 139.0, 138.1, 137.8, 130, 129.7, 128.5, 128.4, 128.3, 128.1, 127.9, 127.7, 127.7, 127.6, 127.5, 114.2, 104.9, 100.5, 78.3, 76.5, 74.5, 74.1, 73.7, 73.4, 73.4, 73.1, 72.8, 69.4, 69.1, 68.6, 60.4, 57.1, 55.3, 28.1, 25.7.; HRMS (ESI) calcd for $C_{46}H_{59}N_2O_{13}^{+}$ $(M+NH_4)^{+}$ 847.40112, found 847.40116 m/z

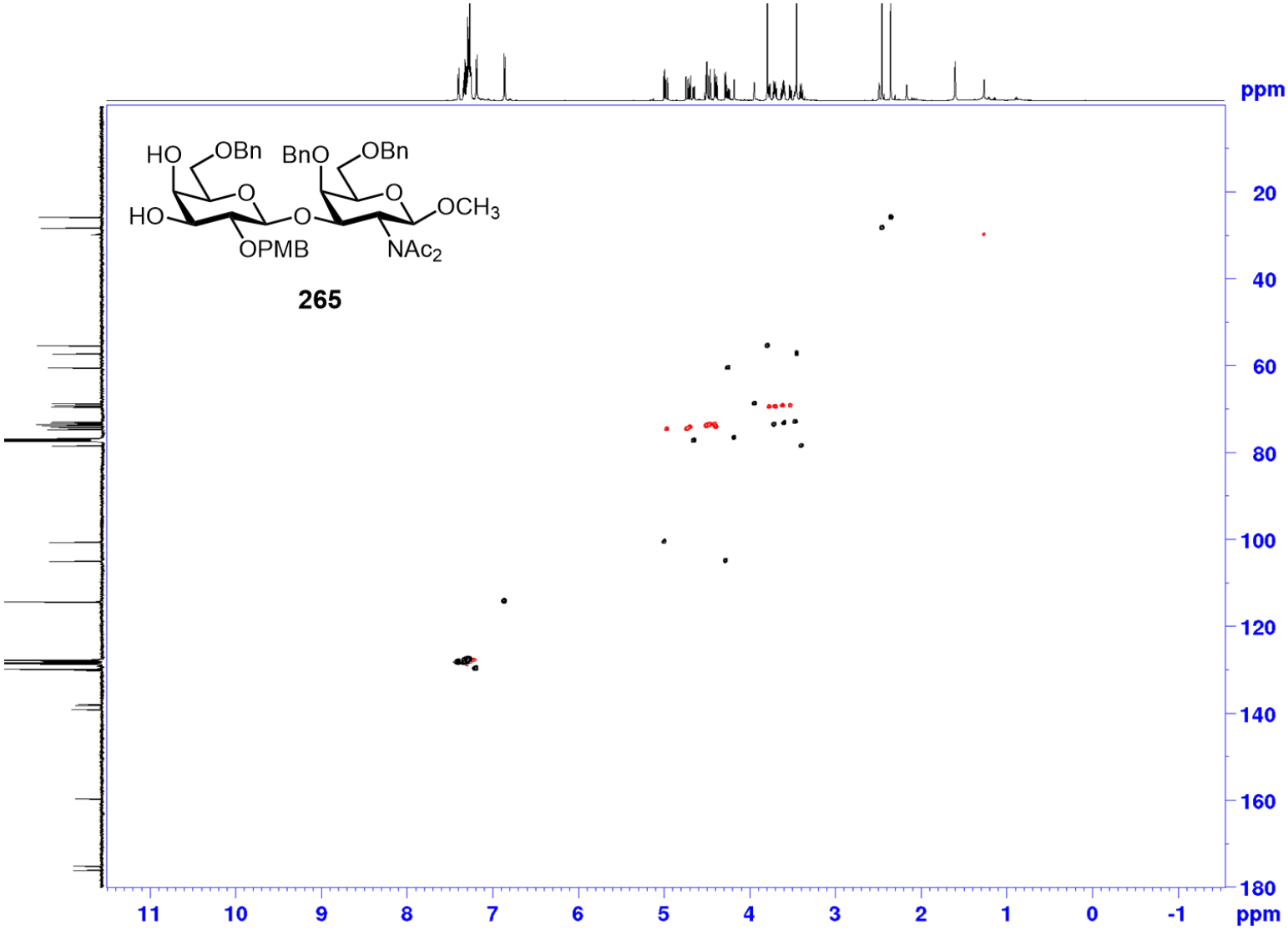
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 265



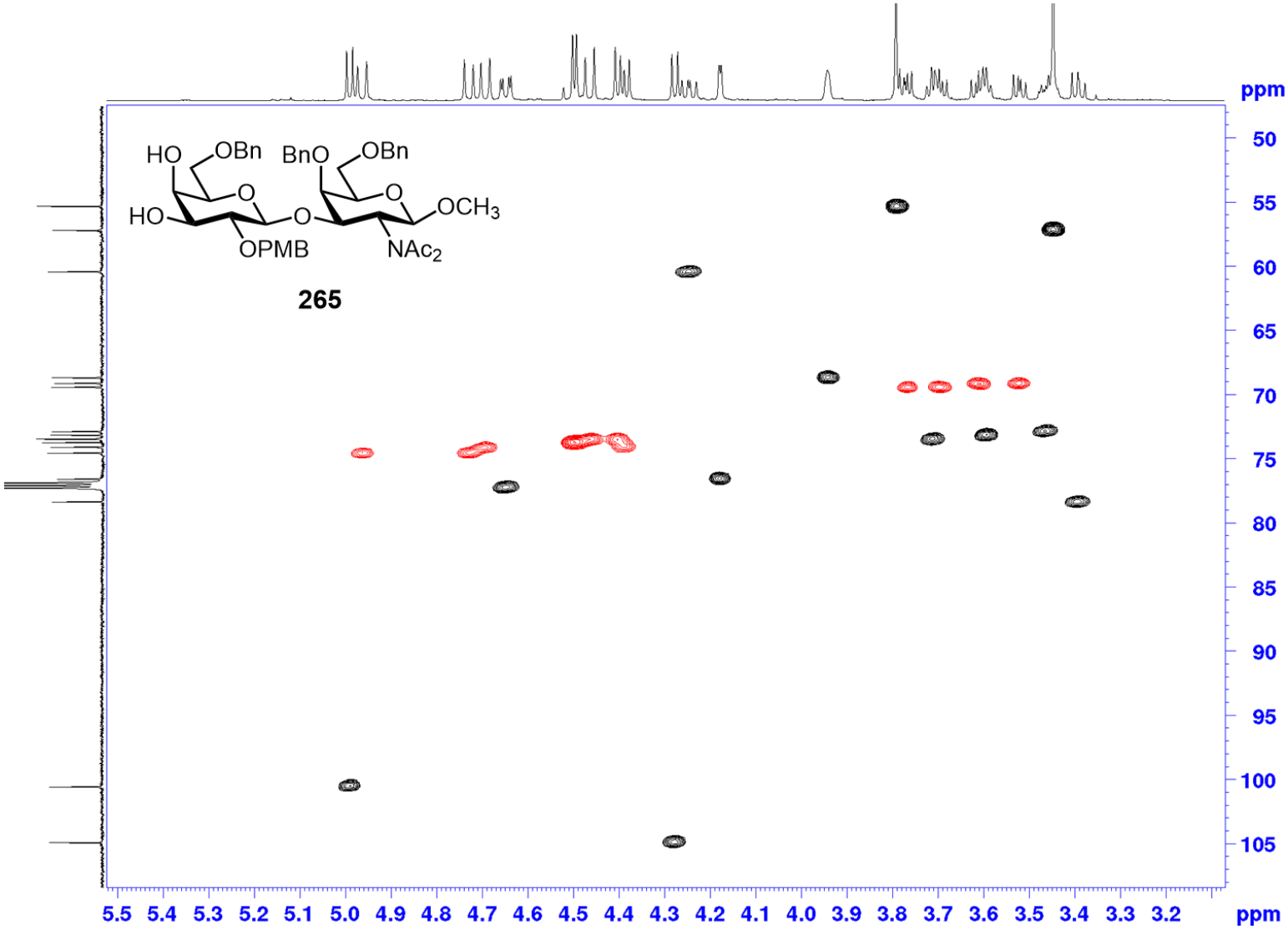
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 265

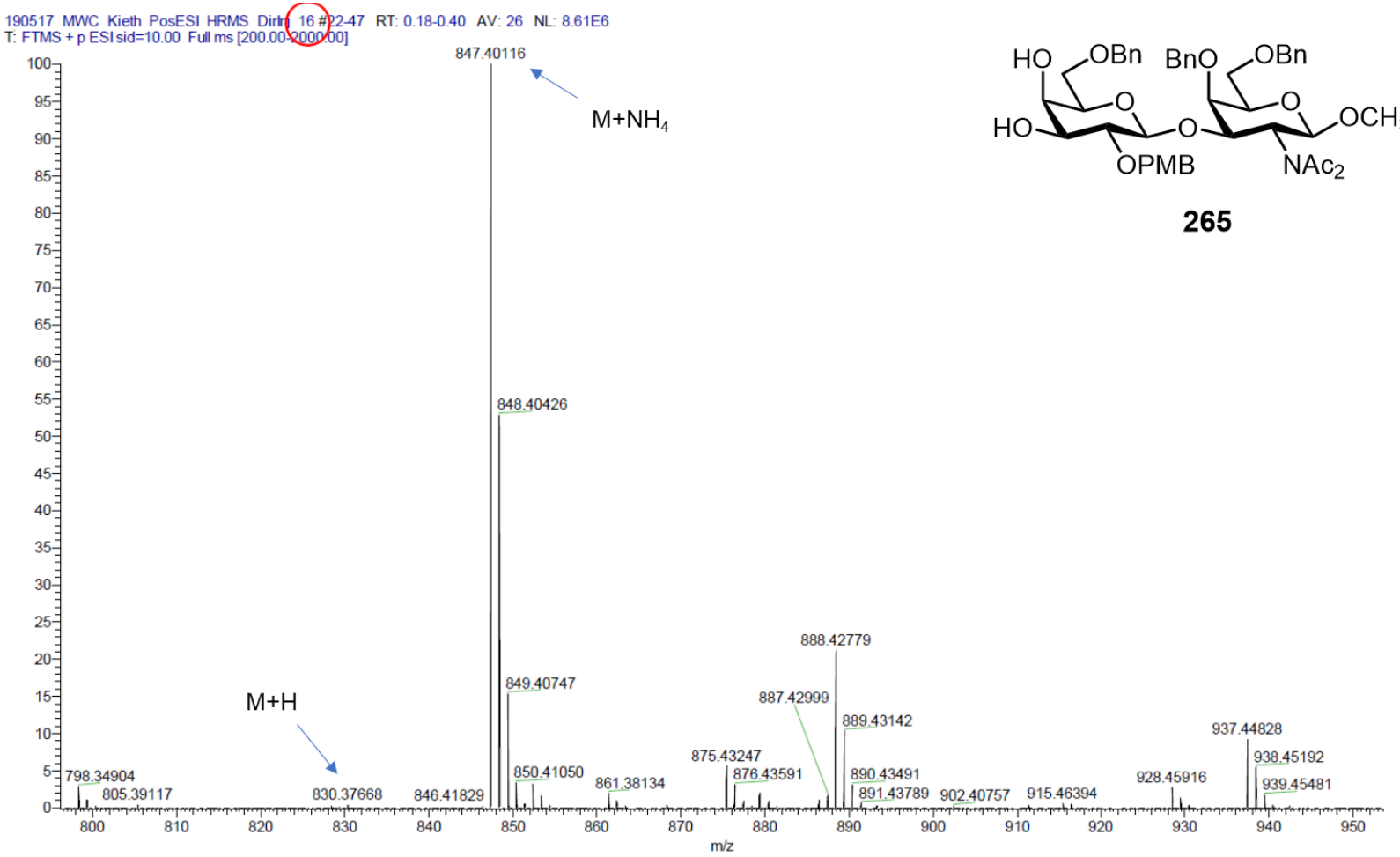


^{1}H-^{13}C HSCQ ($CDCl_3$) of compound 265



^{1}H-^{13}C HSCQ ($CDCl_3$) zoomed in region of compound 265

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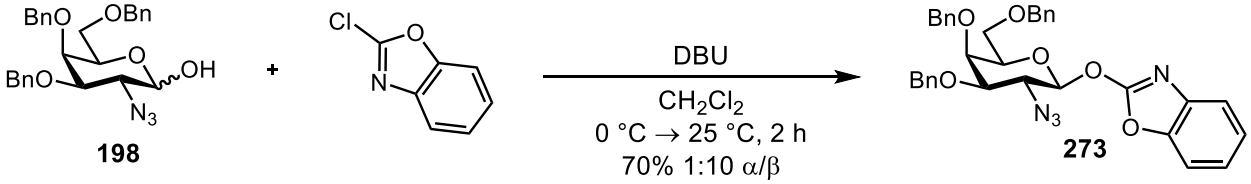
T: FTMS + p ESI sid=10.00 Full ms [200.00-2000.00]
847.40116
M+NH4
848.40426
849.40747
M+H
798.34904
805.39117
830.37668
846.41829
850.41050
861.38134
875.43247
876.43591
887.42999
888.42779
889.43142
890.43491
891.43789
902.40757
915.46394
928.45916
937.44828
938.45192
939.45481
m/z

HO
OBn
BnO
OBn
HO
O
O
O
OCH3
OPMB
NAc2

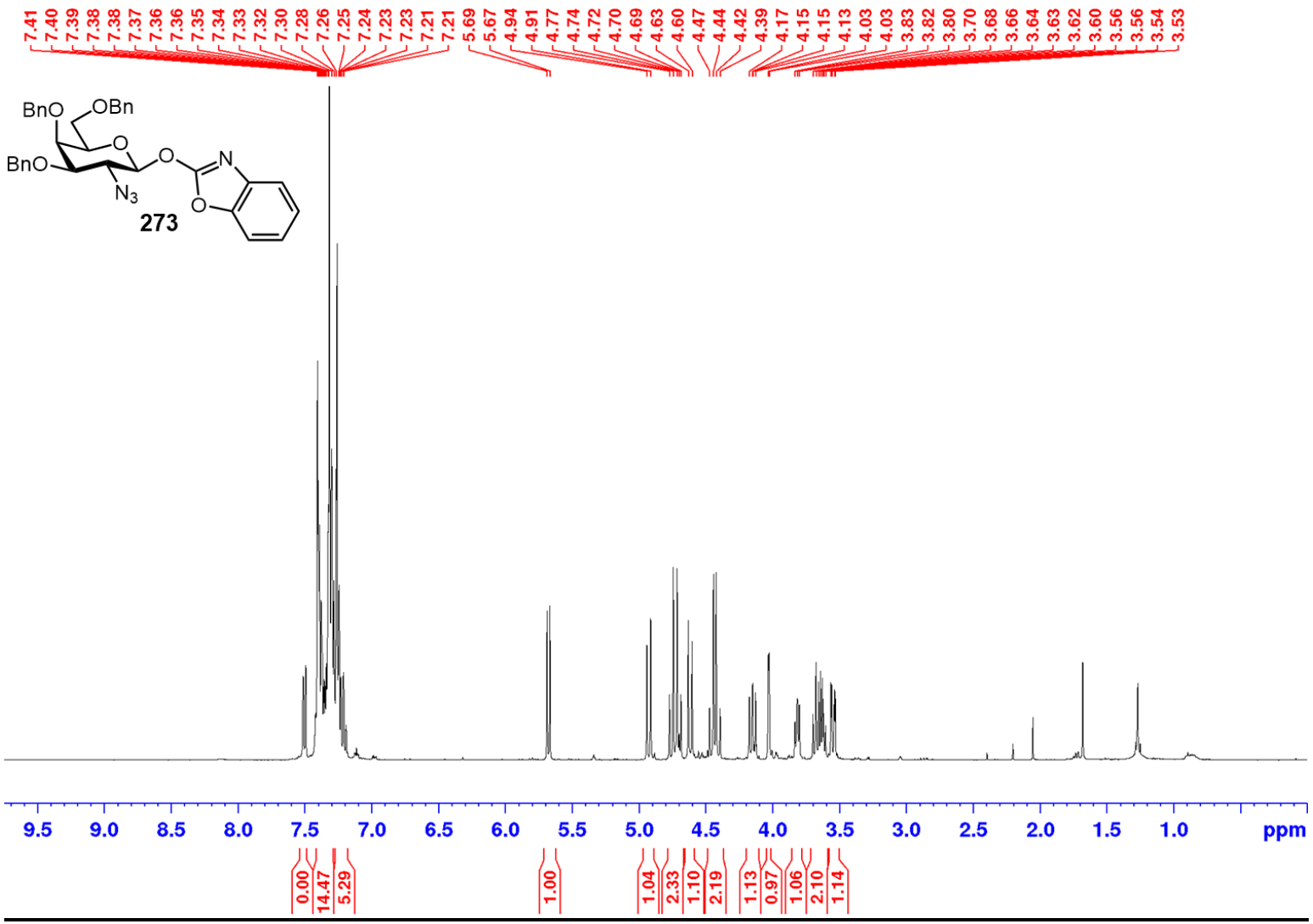
265

Benzoxazolyl 3,4,6-tri-*O*-benzyl-β-D-galactopyranoside (273)

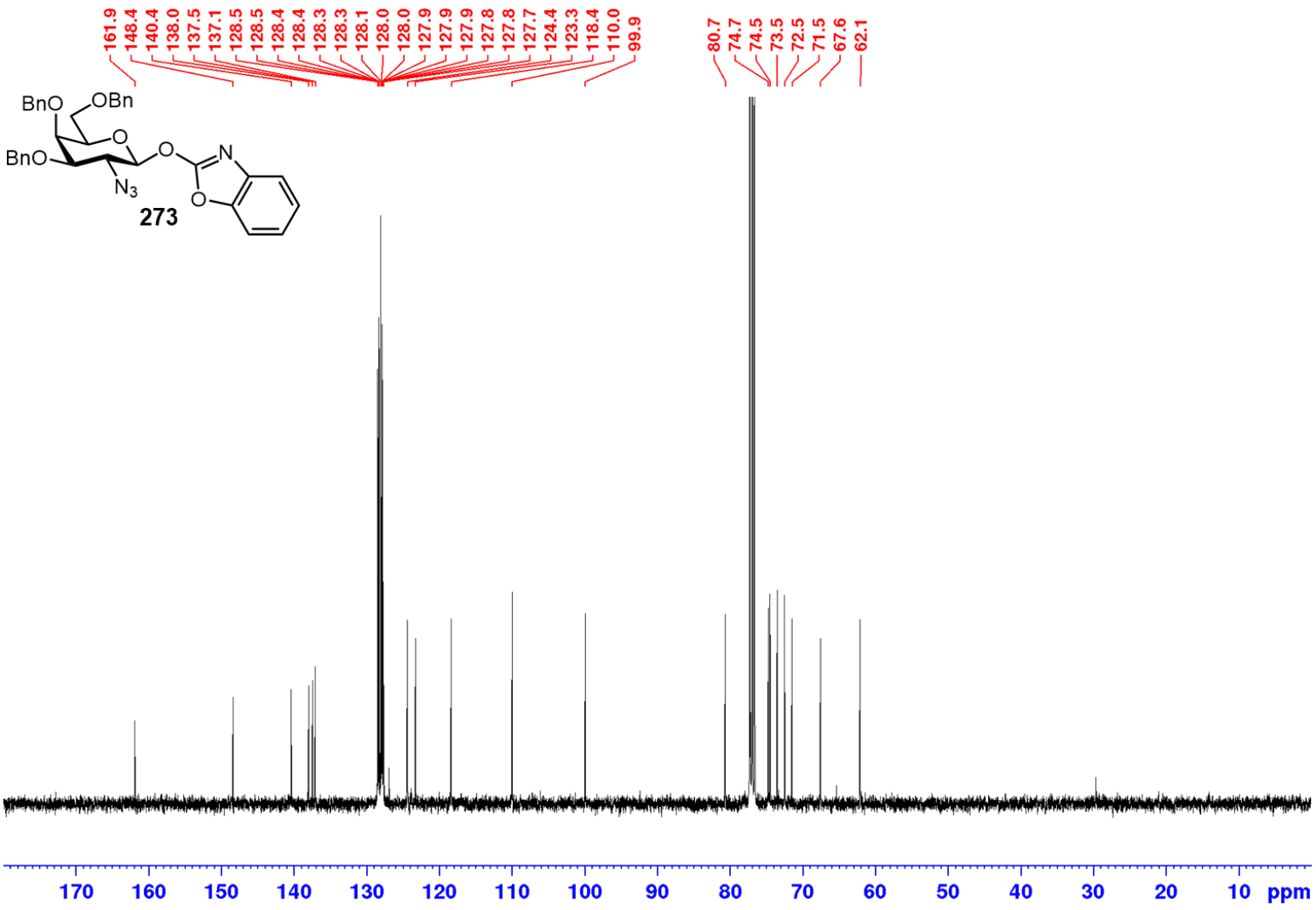


A solution of 3,4,6-tri-*O*-benzyl-β-D-galactopyranose **198** (1.0 equiv., 0.405 g, 0.852 mmol) and 2-chlorobenzoxazole (1.5 equiv., 0.146 mL, 1.27 mmol) in dry CH_2Cl_2 (5.0 mL) was added DBU (2.0 equiv., 0.23 mL, 1.70 mmol) at 0 °C under argon and stirred at room temperature. At 2 h, the reaction was passed through a glass fritted vacuum filter funnel equipped with a pad of Celite. The filtrate was concentrated down and purified by silica gel flash column chromatography (25:75 EtOAc/hexanes) to give 1:10 α/β mixture of benzoxazolyl 3,4,6-tri-*O*-benzyl-β-D-galactopyranoside **723** (0.3546 g, 0.600 mmol, 70%) which was easily separated by chromatography, as a clear oil;. R_f = 0.4 (30:70 EtOAc/hexanes) visualized with ceric ammonium molybdate stain; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.52 (d, *J* = 8.0 Hz, 1H), 7.42-7.27 (m, 16H), 7.25-7.19 (m, *J* = Hz, 2H), 5.68 (d, *J* = 8.4 Hz, 1H), 4.94 (d, *J* = 11.4 Hz, 1H), 4.74 (dd, *J* = 11.5, 22.4 Hz, 2H), 4.64 (d, *J* = 11.4Hz, 1H), 4.45 (dd, *J* = 11.8, 19.7 Hz, 2H), 4.16 (dd, *J* = 8.4, 10.3 Hz, 1H), 4.04 (d, *J* = 2.3 Hz, 1H), 3.84-3.80 (m, 1H), 3.70-3.61 (m, 2H), 3.55 (dd, *J* = 2.6, 10.3 Hz, 1H);^{13}C NMR (100 MHz, $CDCl_3$): δ 161.9, 148.4, 140.4, 138.0, 137.4, 137.0, 128.5, 128.5, 128.3, 128.3, 128.2, 128.2, 128.0, 128.0, 127.9, 127.9, 127.8, 127.8, 127.8, 127.8, 127.7, 124.4, 123.3, 118.3, 109.9, 99.9, 80.6, 74.7, 74.4, 73.5, 72.5, 71.4, 67.5, 62.1; LRMS (ESI) calcd for $C_{34}H_{32}N4O_6Na$ $(M+Na)^+$ 615.2322, found 615.24 *m/z*.

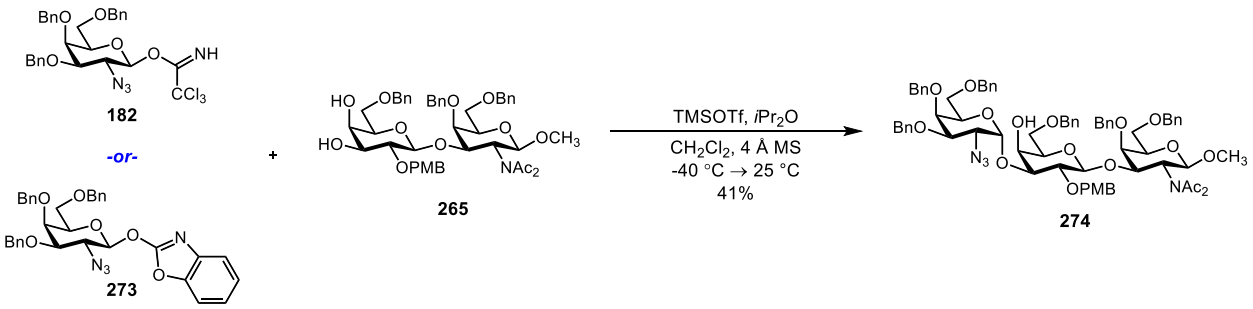
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 273



^{13}C NMR ($CDCl_3$, 100 MHz) of compound 273



Methyl 3,4,6-tri-*O*-benzyl-2-deoxy-2-azido-α-D-galactopyranosyl-(1→3)-6-*O*-benzyl-2-*O*-(*p*-methoxy)-β-D-galactopyranosyl-(1→3)-4,6-di-*O*-benzyl-2-deoxy-2-(*N*,*N*-diacetyl)-β-D-galactopyranoside (274)

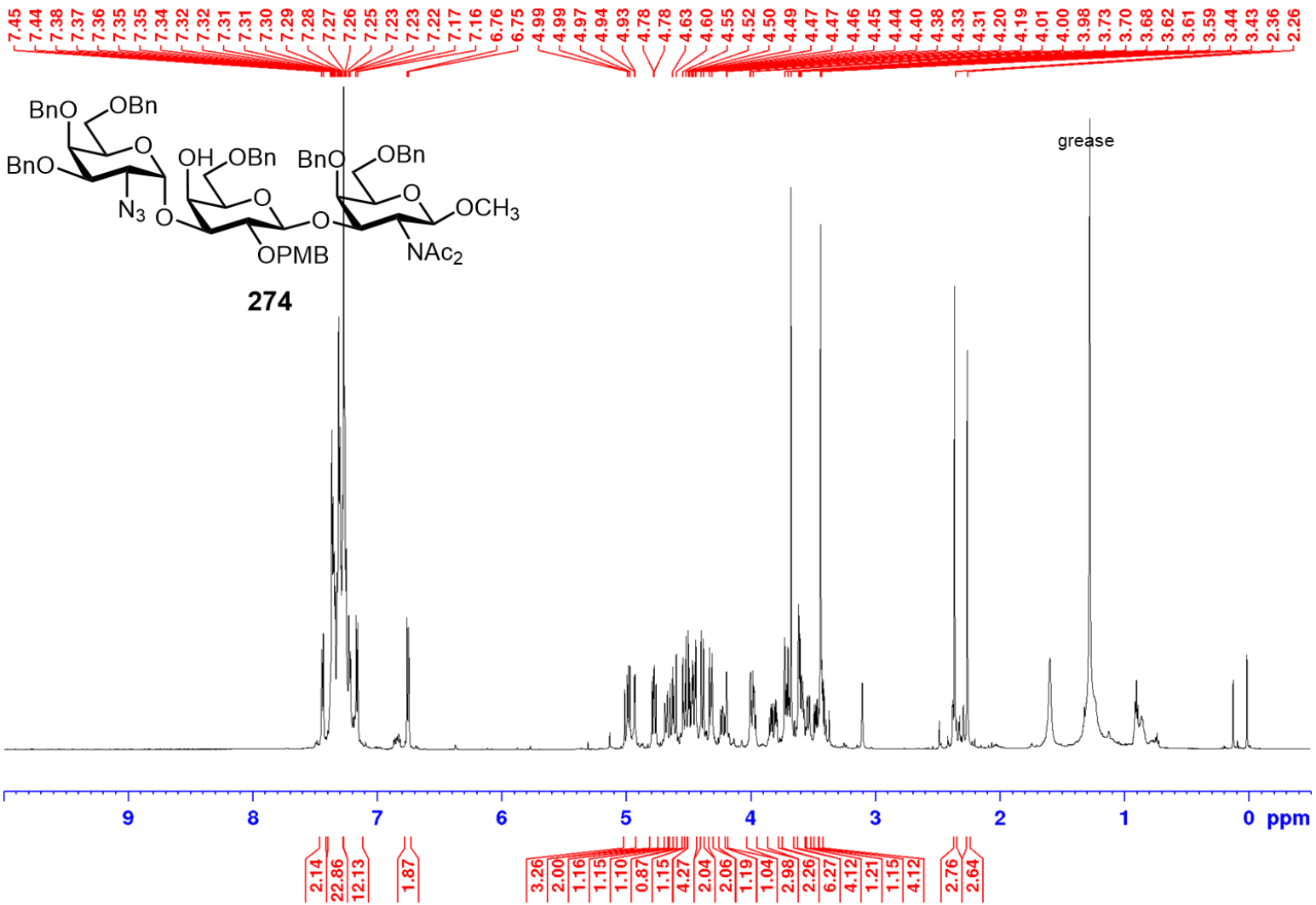


A solution of trichloroacetimidate 3,4,6-tri-*O*-benzyl-2-deoxy-2-azido-β-D-galactopyranoside **182** (1.5 equiv., 1.134 g, 0.183 mmol) or benzoxazolyl 3,4,6-tri-*O*-benzyl-β-D-galactopyranoside **273** (1.5 equiv.) and disaccharide acceptor methyl 6-*O*-benzyl-2-*O*-(*p*-methoxy)-β-D-galactopyranosyl-(1→3)-4,6-di-*O*-benzyl-2-deoxy-2-(*N*,*N*-diacetyl)-β-D-galactopyranoside **265** (1.0 equiv., 1.012 g, 0.121 mmol) in a 1:4 solution of iPr$_2$O/CH$_2$Cl$_2$ (3 mL) was added freshly activated, and cooled, 4 Å MS (450 mg, 15% m/v) under an argon atmosphere at room temperature. After 1 h, the reaction was cooled to -40 °C then added a solution of TMSOTf (0.05 equiv., 74 µL, 0.060 mmol, 15% in CH$_2$Cl$_2$). At 1 h, TLC indicated complete consumption of the starting material acceptor. The reaction mixture was neutralized with Et$_3$N and added diluted with CH$_2$Cl$_2$. The reaction mixture was filtered through a pad of Celite within a glass fritted vacuum filter funnel to remove the 4 Å MS. The filtrate was concentrated to a crude oil and purified by silica gel flash column chromatography to give methyl 3,4,6-tri-O-benzyl-2-deoxy-2-azido-α-D-galactopyranosyl-(1→3)-6-*O*-benzyl-2-*O*-(*p*-methoxy)-β-D-galactopyranosyl-(1→3)-4,6-di-*O*-benzyl-2-deoxy-2-(*N*,*N*-diacetyl)-β-D-galactopyranoside **274** (0.87 g, 0.50 mmol, 41%)[a] as a white foam. R_f = 0.75 (1:1 EtOAc/hexanes) visualized with ceric ammonium

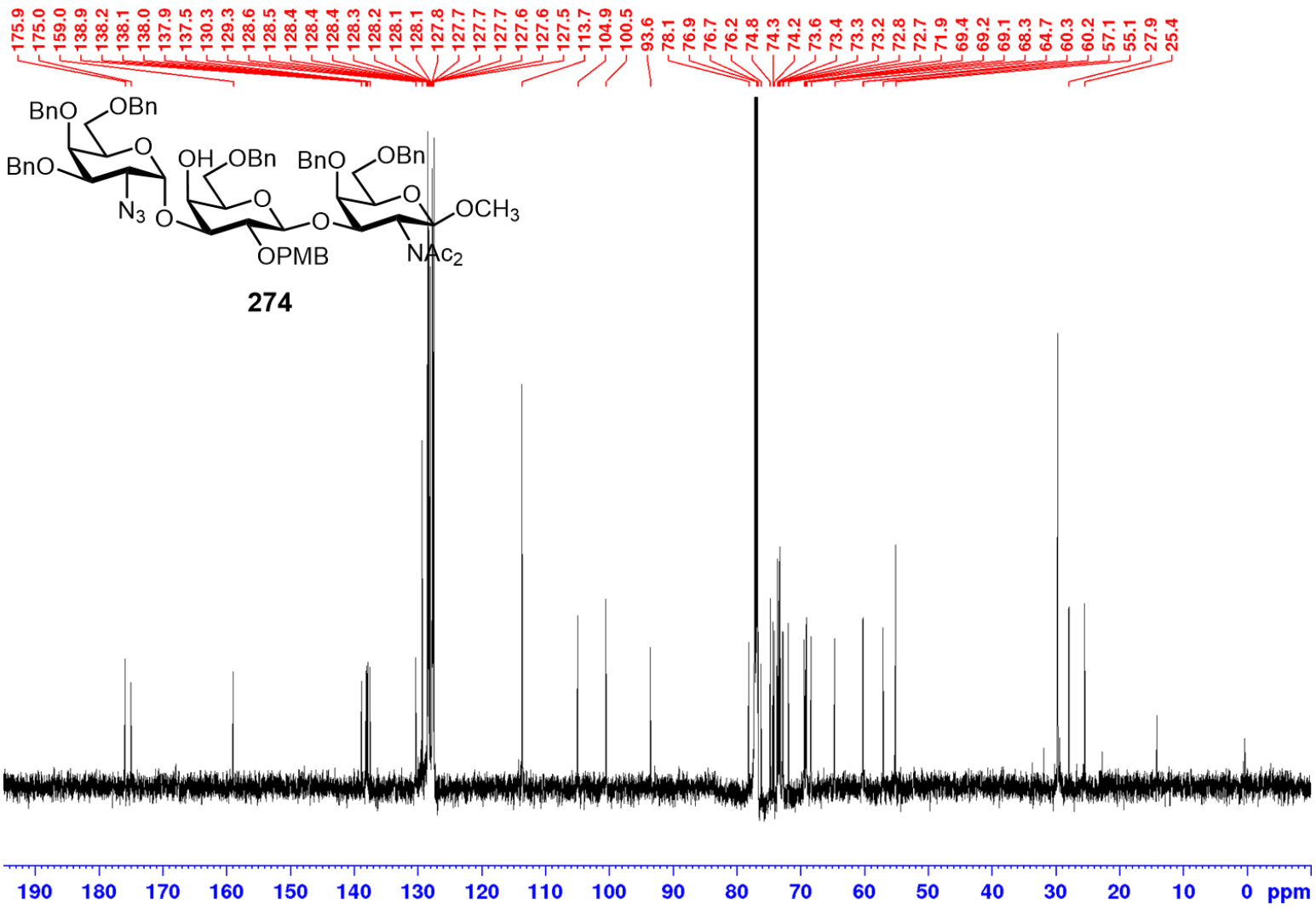
molybdate stain. $[\alpha]D^{25}$ = -10.92° (c = 1.0, $CHCl_3$); IR ν_{max} (film) 3750.98, 3421.02, 2923.44, 2362.99, 2112.84, 1698.18, 1513.82, 1496.98, 1455.06, 1365.54, 1246.96, 1102.04, 1067.68, 749.56, 698.45 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.44 (d, *J* = 7.26 Hz, 2H), 7.37-7.33 (m, 8H), 7.32-7.28 (m, 12H), 7.26-7.22 (m, 8H), 7.16 (d, *J* = 8.23 Hz, 2H), 6.76 (d, *J* = 8.72 Hz, 2H), 5.00 (d, *J* = 11.79 Hz, 1H), 4.98 (d, *J* = 7.88 Hz, 1H), 4.93 (d, *J* = 3.57 Hz, 1H), 4.78 (dd, *J* = 7.64, 11.20 Hz, 2H), 4.69-4.59 (m, 4H), 4.55-4.53 (m, 1H), 4.53-4.52 (m, 1H), 4.50-4.49 (m, 1H), 4.47-4.43 (m, 3H), 4.40-4.37 (m, 2H), 4.33 (m, 1H), 4.32 (d, *J* = 7.22 Hz, 1H, C1'), 4.23 (dd, *J* = 8.3, 11.0 Hz, 1H), 4.20 (d, *J* = 2.4 Hz, 1H), 4.01-3.95 (m, 3H), 3.85-3.82 (m, 1H), 3.81-3.78 (m, 1H), 3.73-3.68 (m, 3H), 3.68 (s, 3H), 3.62-3.60 (m, 2H), 3.59-3.57 (m, 1H), 3.54 (dd, *J* = 2.3, 10.4 Hz, 1H), 3.48 (dd, *J* = 6.1, 9.9 Hz, 1H), 3.45, 3.41 (m, 4H), 3.10 (-OH, 1H), 2.36 (s, 3H), 2.26 (s, 3H);^{13}C NMR (100 MHz, $CDCl_3$): δ 175.9, 175.0, 159.0, 138.9, 138.2, 138.1, 138.0, 137.9.0, 137.5, 130.3, 129.3, 128.6, 128.5, 128.4, 128.4, 128.4, 128.3, 128.2, 128.1, 128.1, 127.8, 127.7, 127.7, 127.7, 127.6, 127.6, 127.5, 113.7, 104.9, 100.5, 93.6, 78.1, 76.9, 76.7, 76.2, 74.8, 74.3, 74.2, 73.6, 73.4, 73.3, 73.2, 72.8, 72.7, 71.9, 69.4, 69.2, 69.1, 68.3, 64.7, 60.3, 60.2, 57.1, 55.1, 27.9, 25.4,; HRMS (ESI) calcd for $C_{73}H_{86}N_5O_{17}^+$ $(M+NH_4)^+$ 1304.60312, found 1304.59957 m/z.

[a] yield reported contains solvent impurities. see NMR spectra.

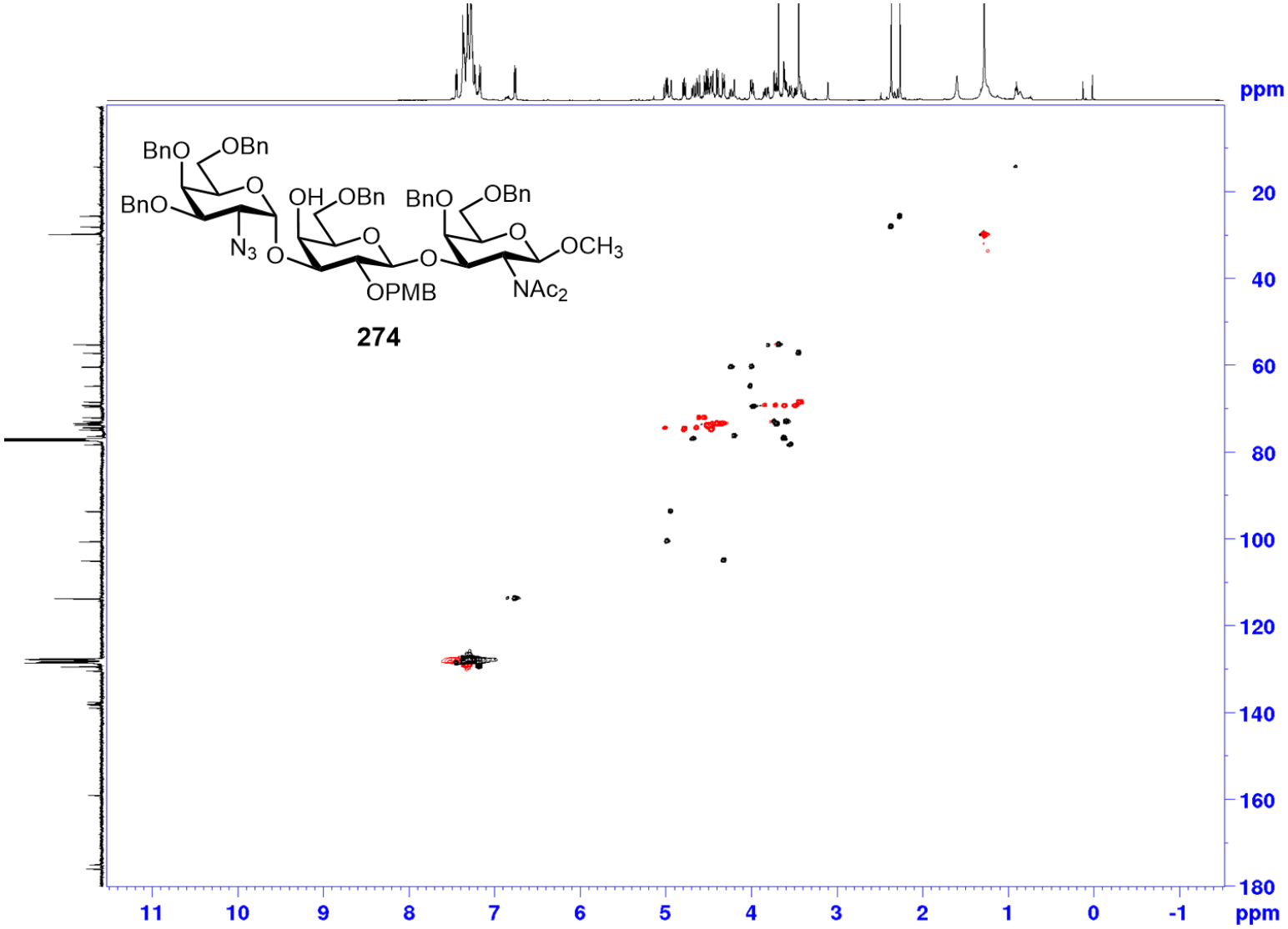
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 274



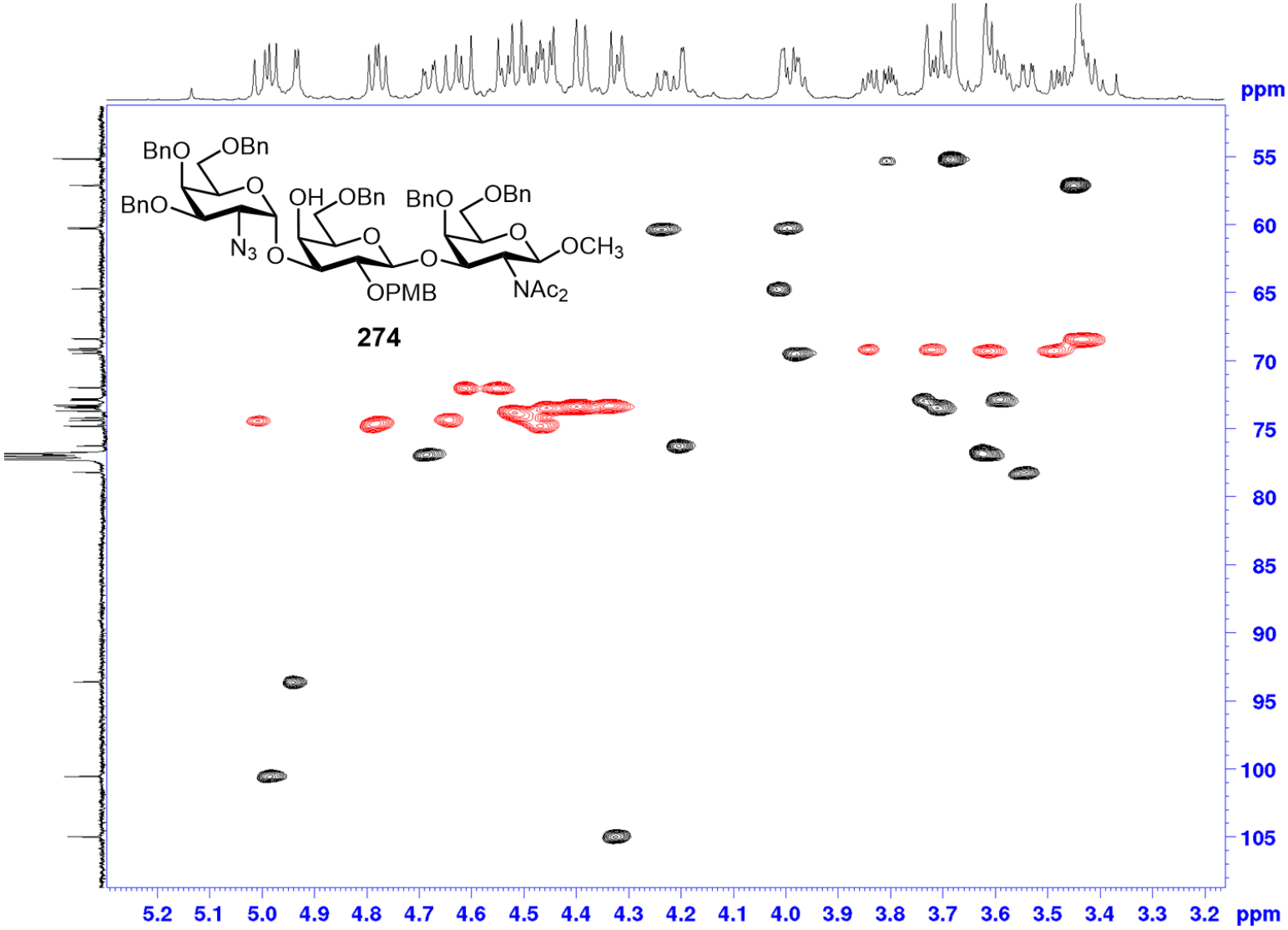
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 274



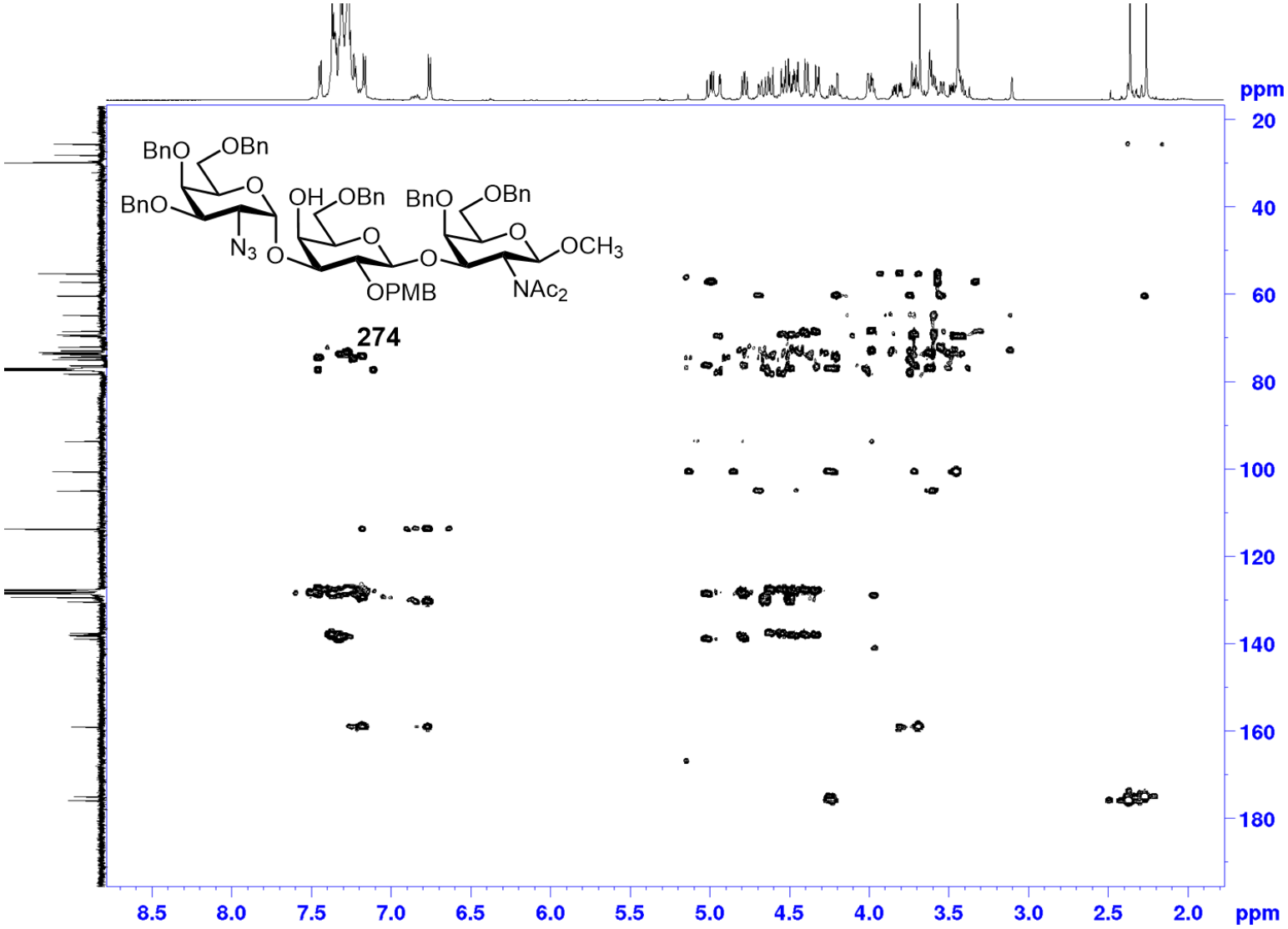
^{1}H-^{13}C HSCQ ($CDCl_3$) of compound 274



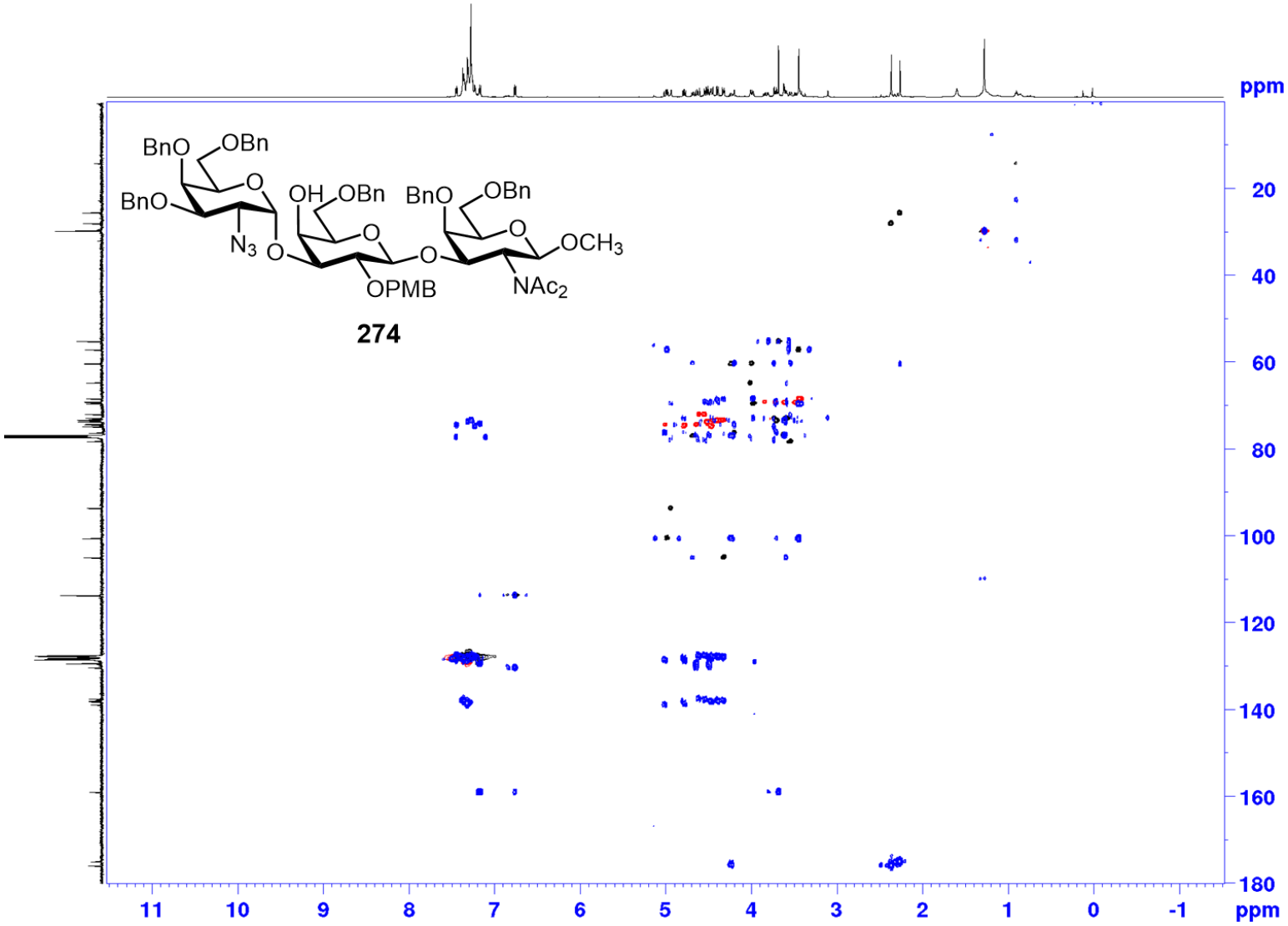
^{1}H-^{13}C HSCQ ($CDCl_3$) zoomed in region of compound 274



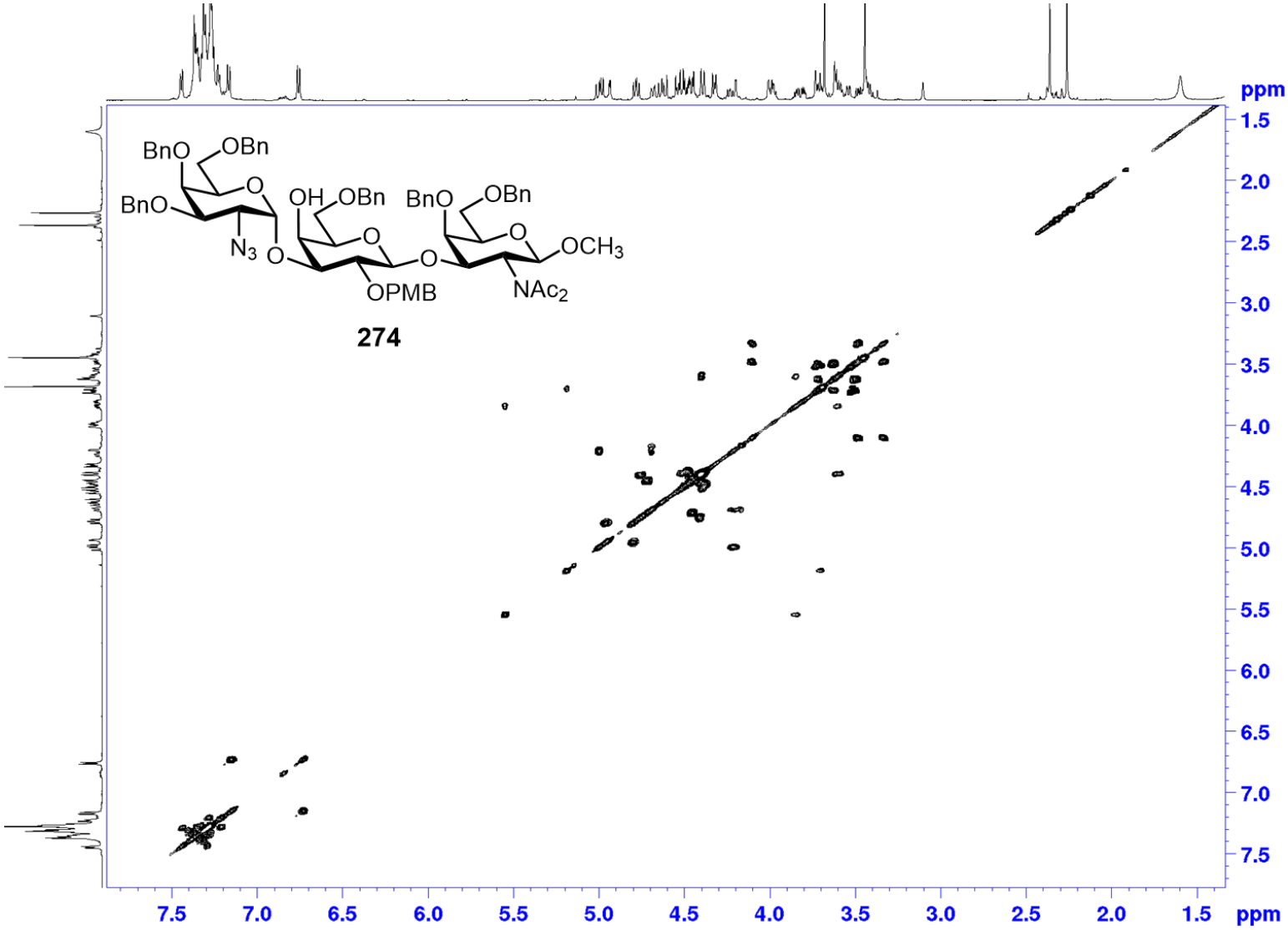
^{1}H-^{13}C HMBC ($CDCl_3$) of compound 274



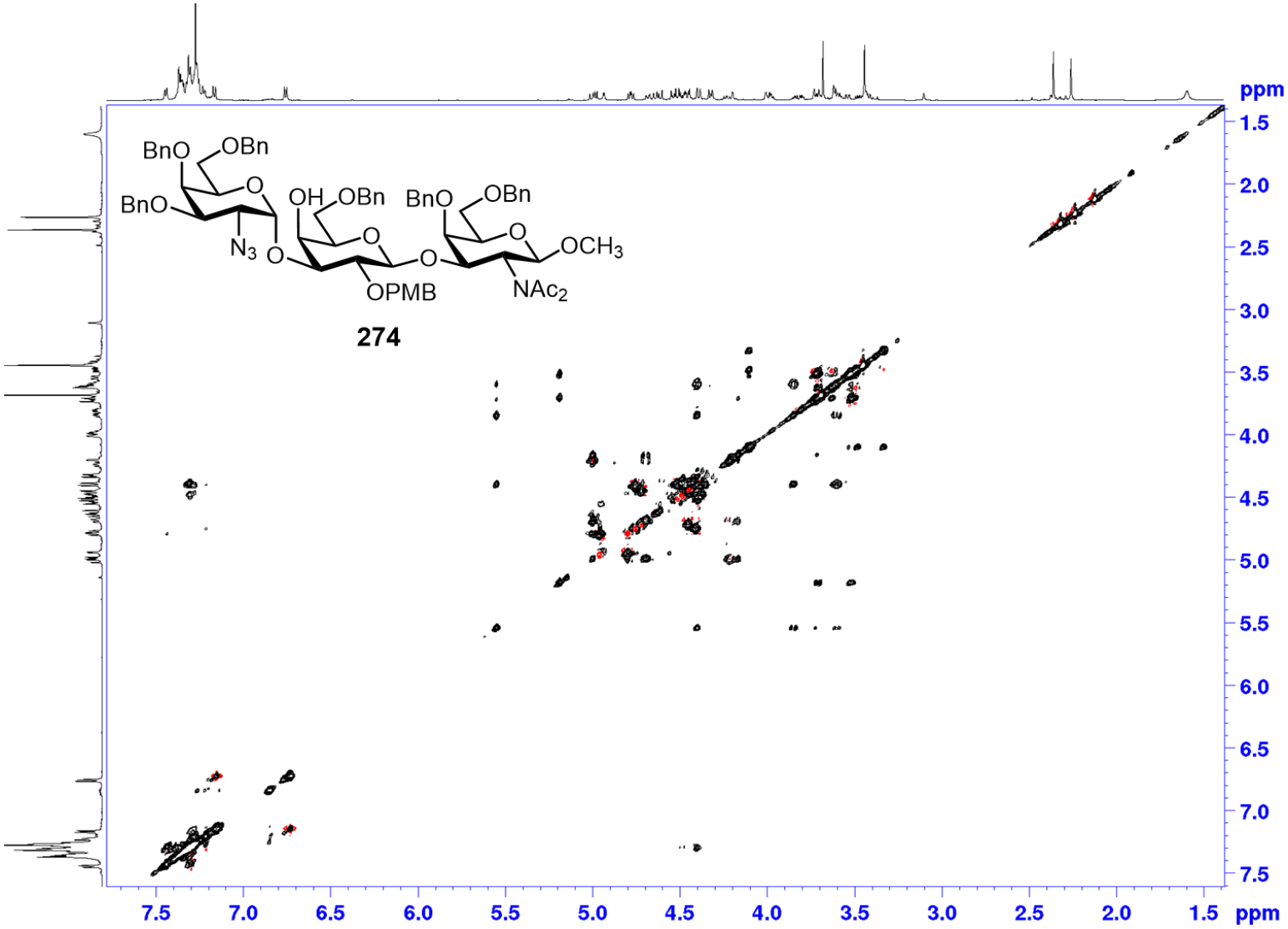
^{1}H-^{13}C HSCQ with ^{1}H-^{13}C HMBC overlay ($CDCl_3$) of compound 274



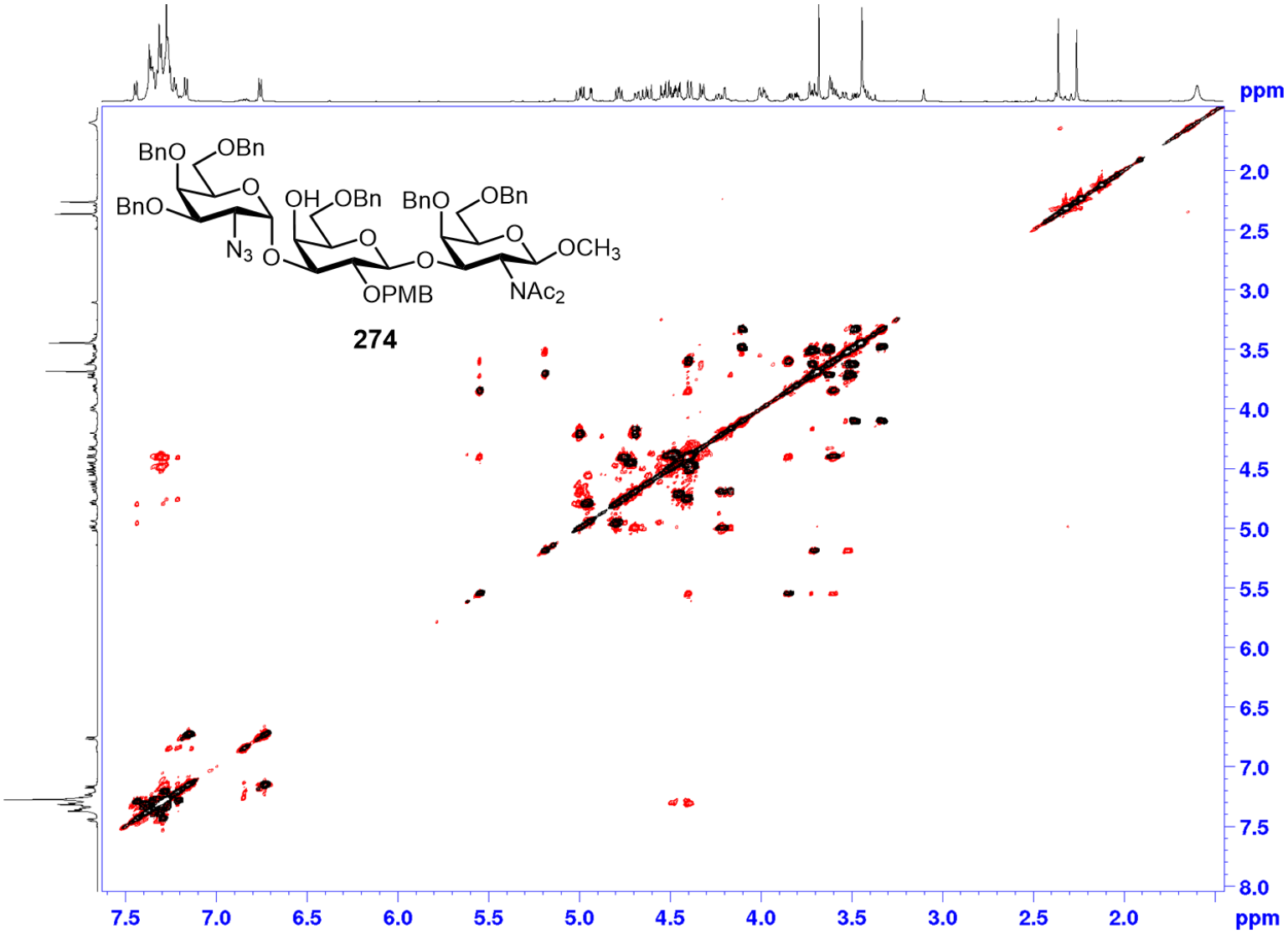
^{1}H-^{1}H COSY ($CDCl_3$) of compound 274

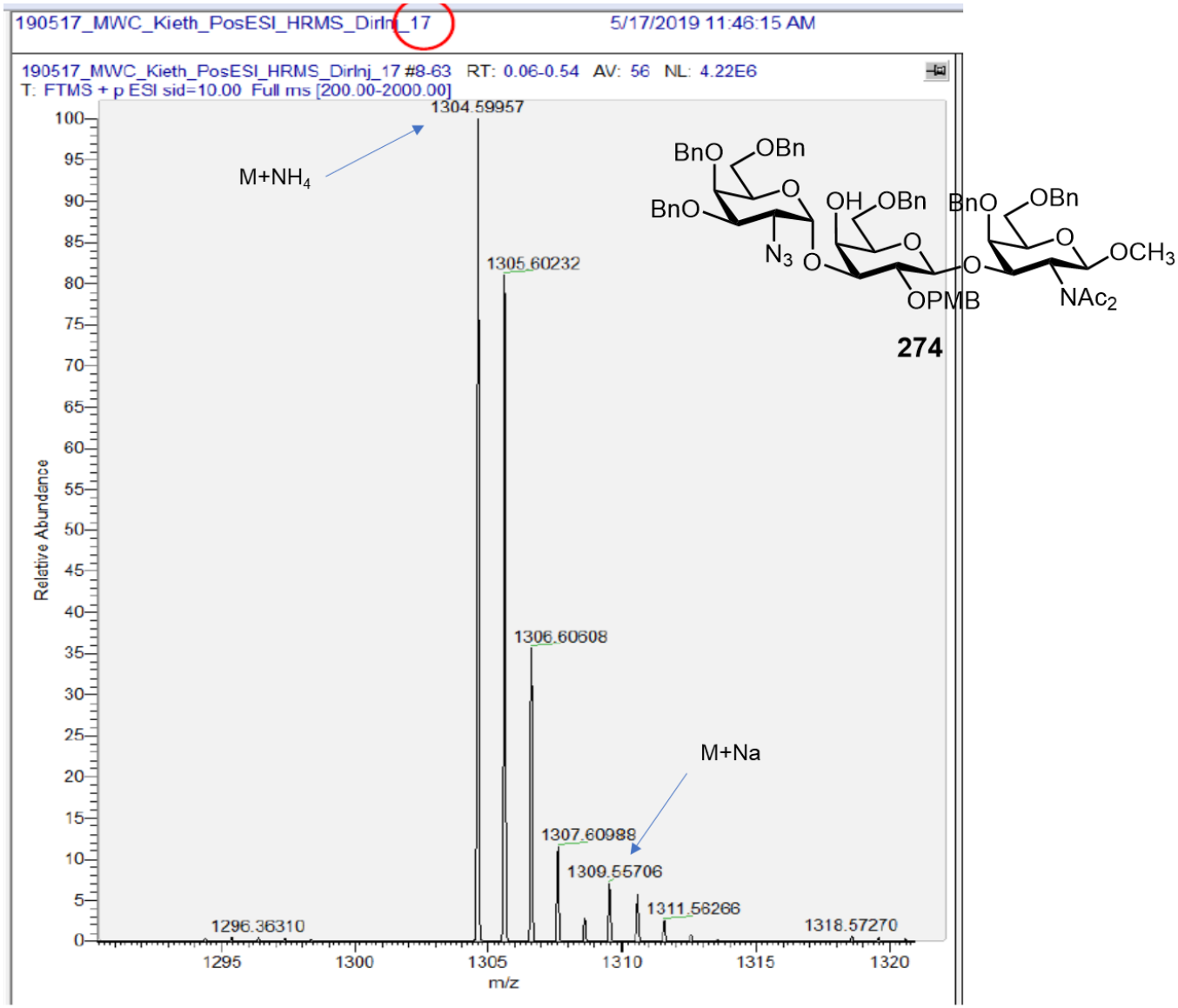


^{1}H-^{1}H TOSY ($CDCl_3$) of compound 274



^{1}H-^{1}H COSY with ^{1}H-^{1}H TOSY overlay ($CDCl_3$) of compound 274

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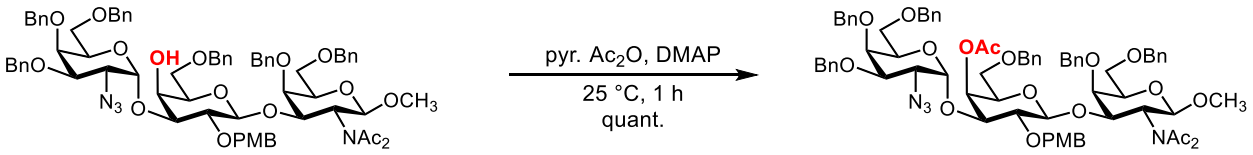
5/17/2019 11:46:15 AM
190517_MWC_Kieth_PosESI_HRMS_Dirlnj_17 #8-63 RT: 0.06-0.54 AV: 56 NL: 4.22E6
T: FTMS + p ESI sid=10.00 Full ms [200.00-2000.00]
1304.59957
M+NH4
1305.60232
1306.60608
M+Na
1307.60988
1309.55706
1311.56266
1296.36310
1318.57270
Relative Abundance
m/z
BnO
OBn
OH
N3
OPMB
OCH3
NAc2
274

Methyl 3,4,6-tri-*O*-benzyl-2-deoxy-2-azido-α-D-galactopyranosyl-(1→3)-4-*O*-acetyl-6-*O*-benzyl-2-*O*-(*p*-methoxy)-β-D-galactopyranosyl-(1→3)-4,6-di-*O*-benzyl-2-deoxy-2-(*N*,*N*-diacetyl)-β-D-galactopyranoside (276)

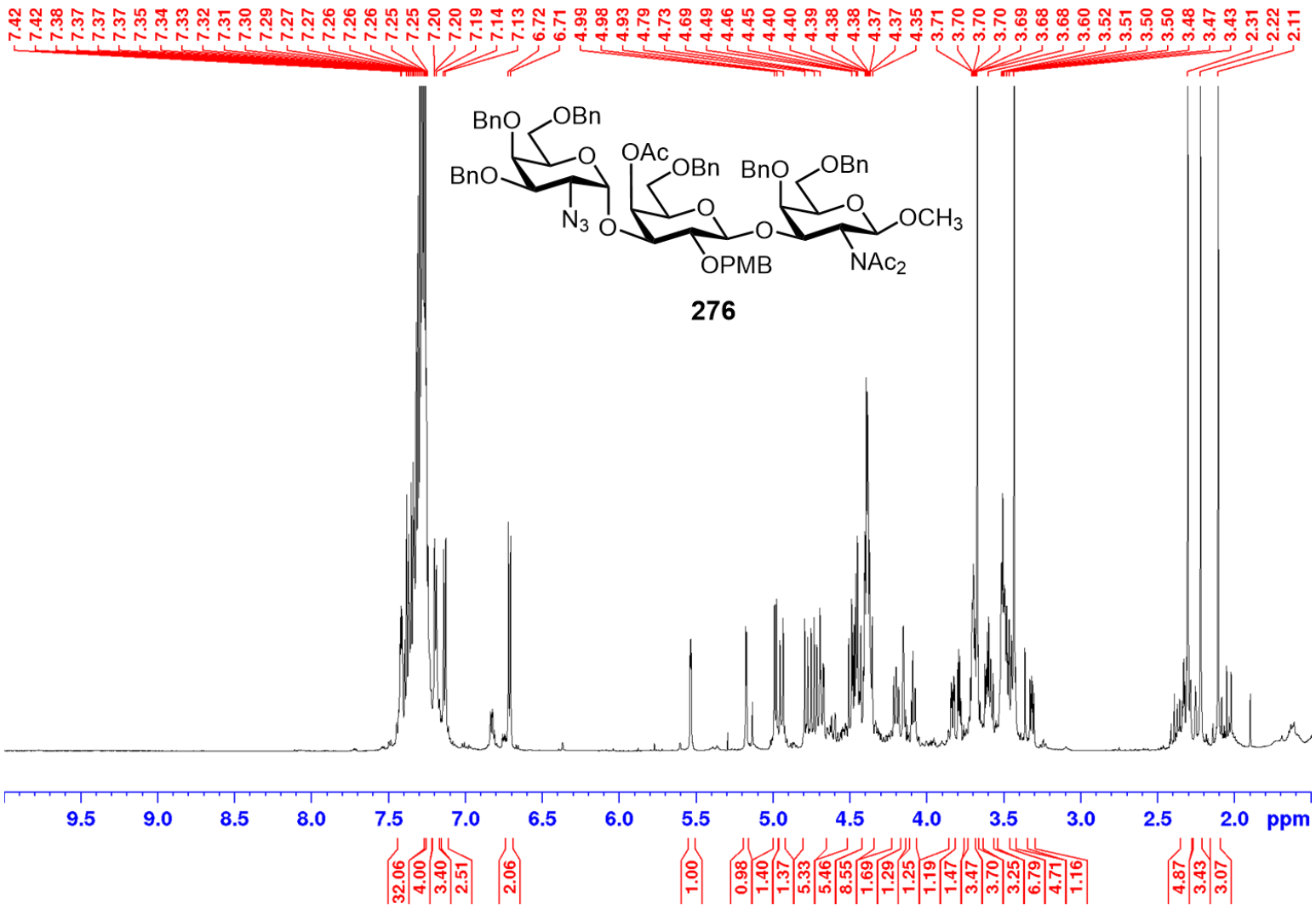


274 **276**

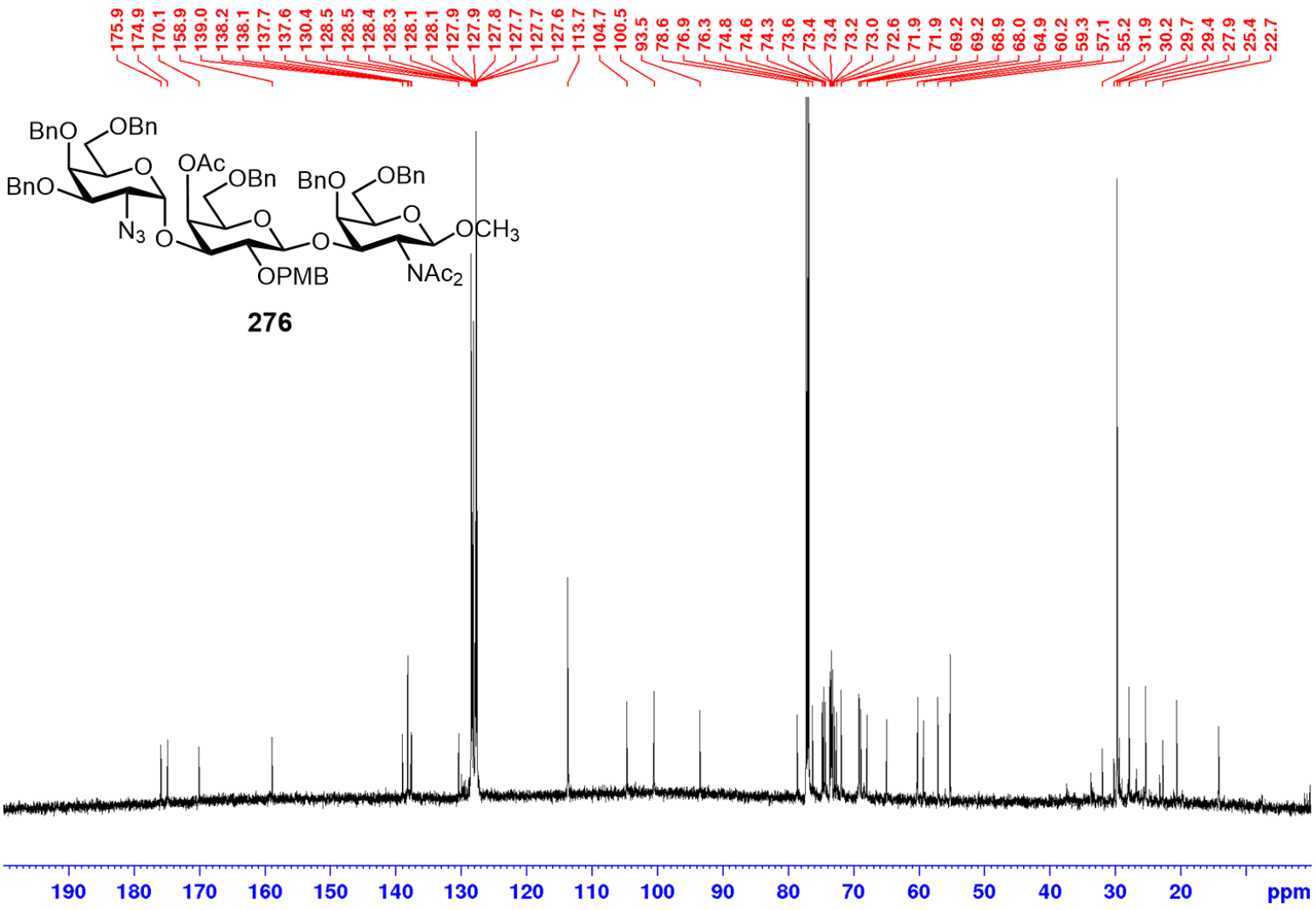
A solution of **274** (1.0 equiv.) in pyridine (1.0 mL) and acetic anhydride (0.2 mL) was treated with DMAP (cat.) at 0 °C under an argon atmosphere. The reaction was stirred at room temperature. At 1 h, the reaction was transferred to a separatory funnel and diluted with EtOAc (10 mL). The organics were washed with 1 N HCl (3 x 1.0 mL), sat. aq. $NaHCO_3$ (1 x 10 mL) and brine (1 x 10 mL). The organics were dried ($NaSO_4$) then filtered to remove drying agent. The filtrated was concentrated to a crude syrup which was analyzed by NMR without any further purification necessary. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.35-7.26 (m, 30H), 7.26-7.25 (m, 2H), 7.19 (d, *J* = 7.6 Hz, 2H), 7.13 (d, *J* = 8.1 Hz, 2H), 6.71 (d, *J* = 8.6 Hz, 2H), 5.53 (d, *J* = 3.1 Hz, 1H), 5.18 (d, *J* = 3.7 Hz, 1H), 4.98 (d, *J* = 7.8 Hz, 1H), 4.94 (d, *J* = 12 Hz, 1H), 4.79-4.67 (m, 5H), 4.51-4.43 (m, 5H), 4.41-4.35 (m, 9H), 4.2 (dd, *J* = 7.8, 10.7 Hz, 2H), 4.15 (d, *J* = 2.2 Hz, 1H), 4.09 (dd, *J* = 6.8, 6.9 Hz, 1H), 3.84 (dd, J = 3.2, 10.0 Hz, 1H), 3.80-3.78 (m, 1H), 3.72-3.66 (m, 3H), 3.63-3.57 (m, 4H), 3.52-3.46 (m, 3H), 3.45-3.42 (m, 6H), 3.41-3.34 (m, 5H), 3.32 (dd, *J* = 6.8, 10.4 Hz, 1H), 2.32 (s, 3H), 2.24 (s, 3H), 2.12 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 175.9, 174.9, 170.1, 158.9, 139, 138.2, 138.1, 137.7, 137.6, 130.4, 128.5, 128.5, 128.4, 128.3, 128.1, 128.1, 127.9, 127.9, 127.8, 127.7, 127.7, 127.6, 113.7, 104.7, 100.5, 93.5, 78.6, 76.9, 76.3, 74.8,

74.6, 74.3, 73.6, 73.4, 73.4, 73.2, 73, 72.6, 71.9, 71.9, 69.2, 69.2, 68.9, 68, 64.9, 60.2, 59.3, 57.1, 55.2, 31.9, 30.2, 29.7, 29.4, 27.9, 25.4, 22.7, 20.6, 14.1.

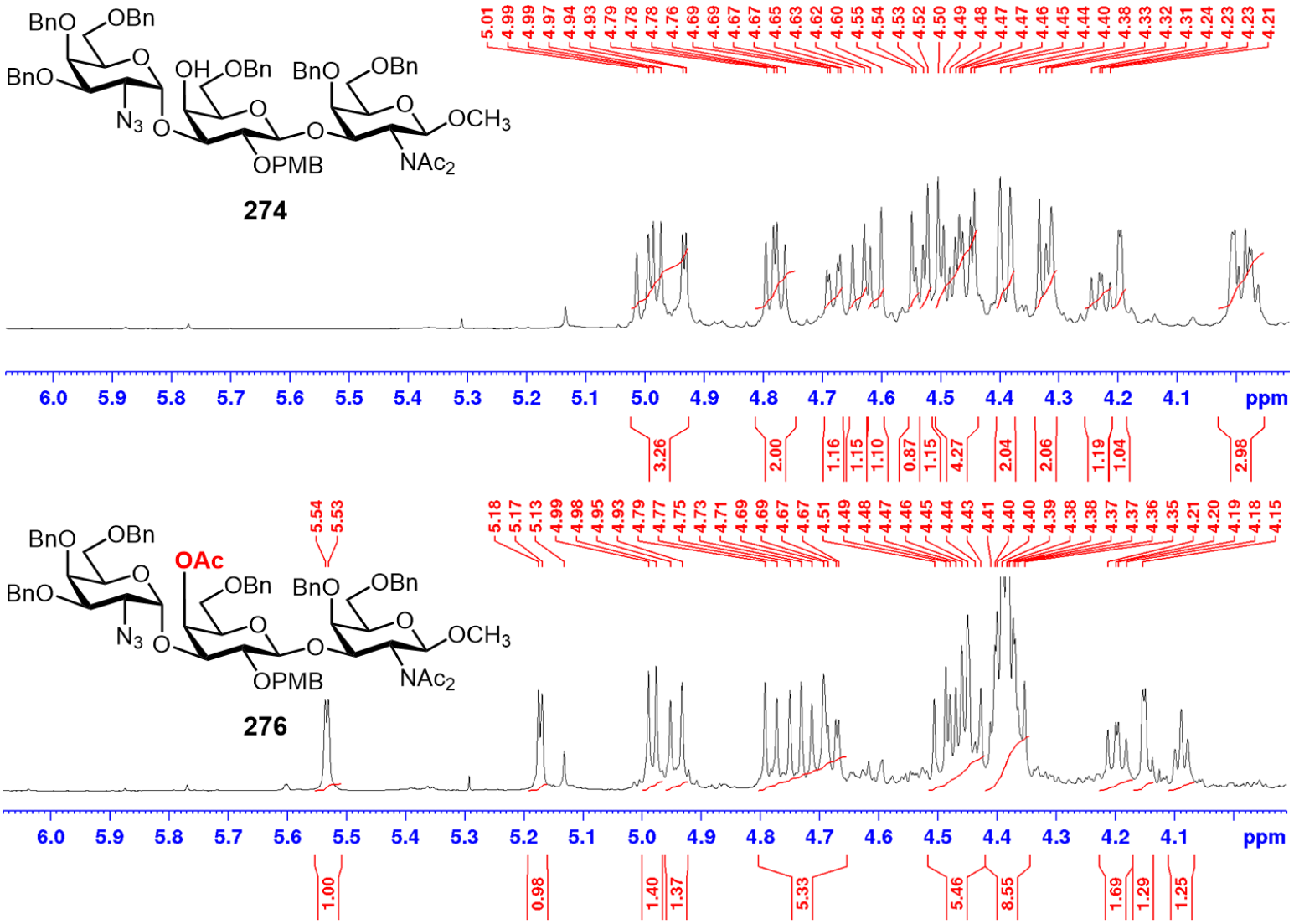
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 276



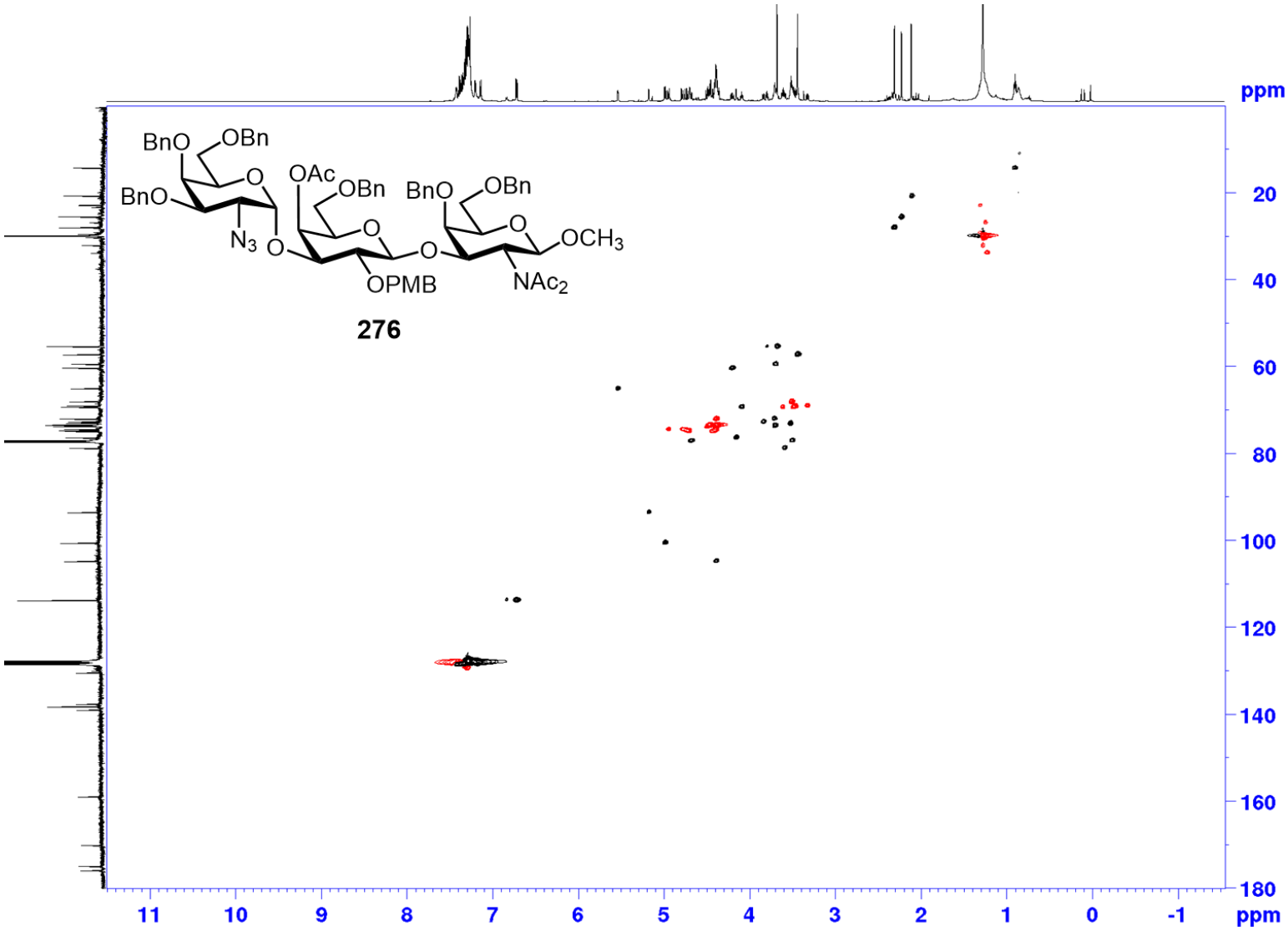
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 276



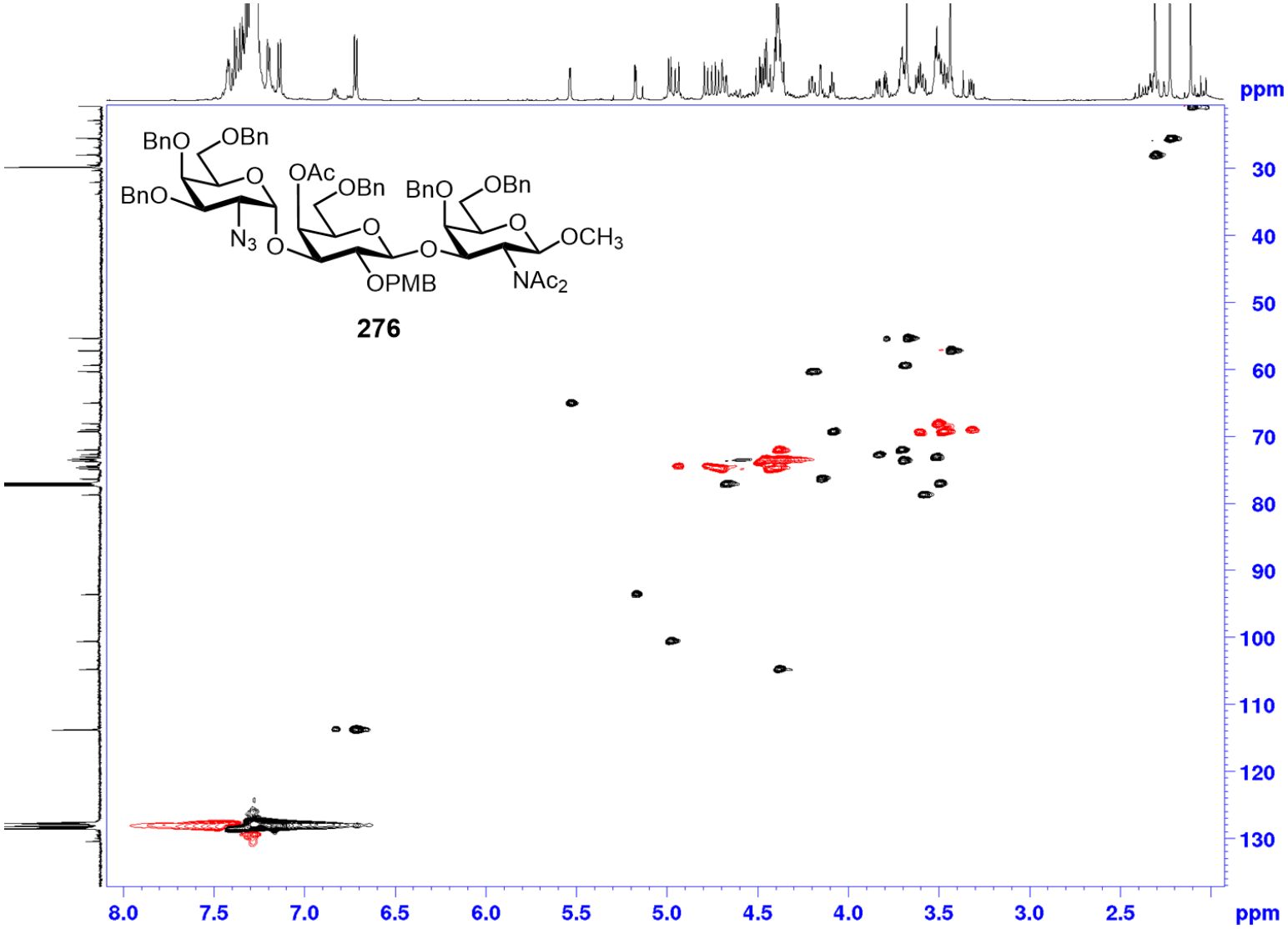
^{1}H NMR ($CDCl_3$, 400 MHz) zoomed in region of compound 274 (top) compared to 276 (bottom)



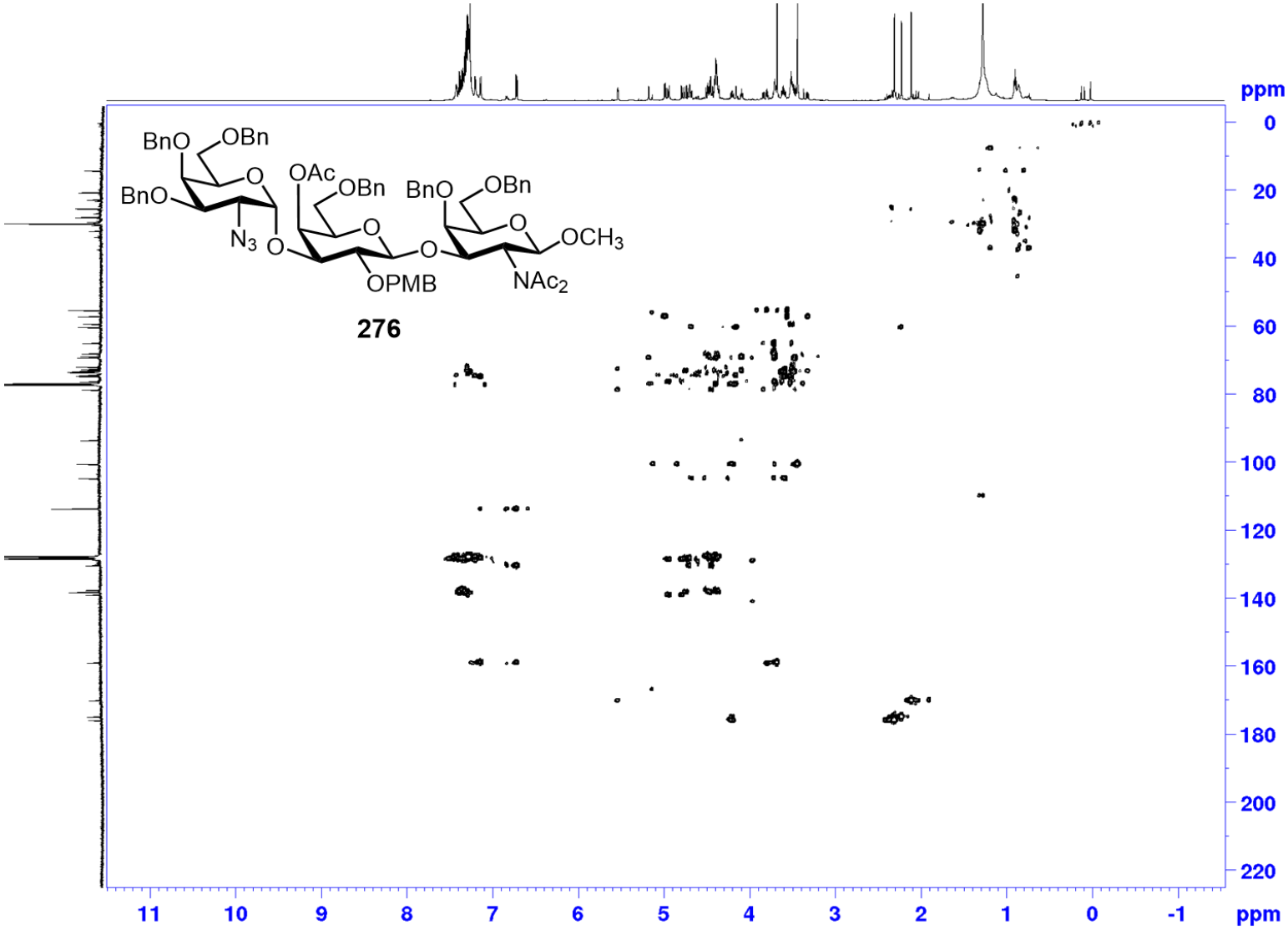
^{1}H-^{13}C HSCQ ($CDCl_3$) of compound 276



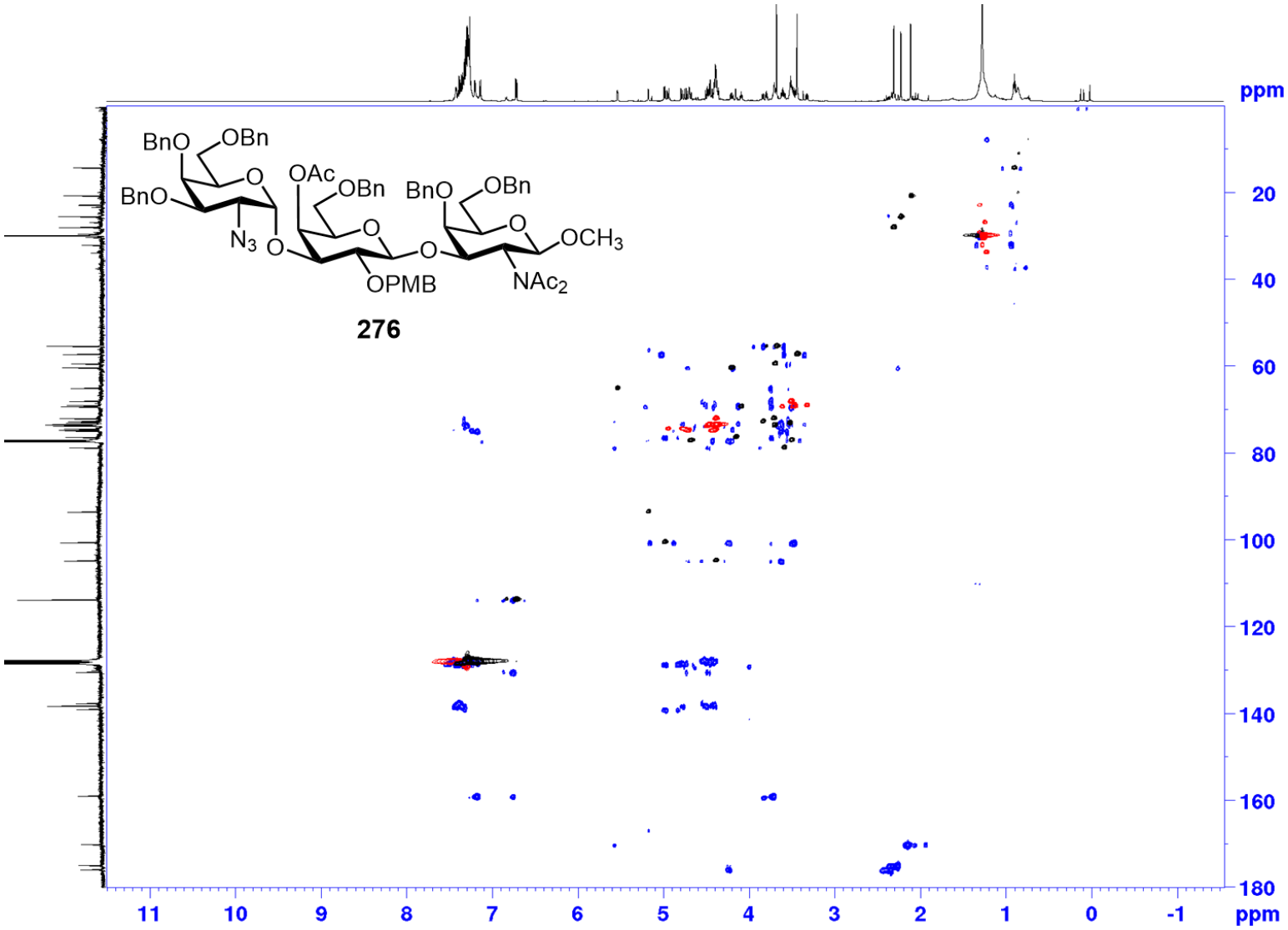
^{1}H-^{13}C HSCQ ($CDCl_3$) zoomed in region of compound 276



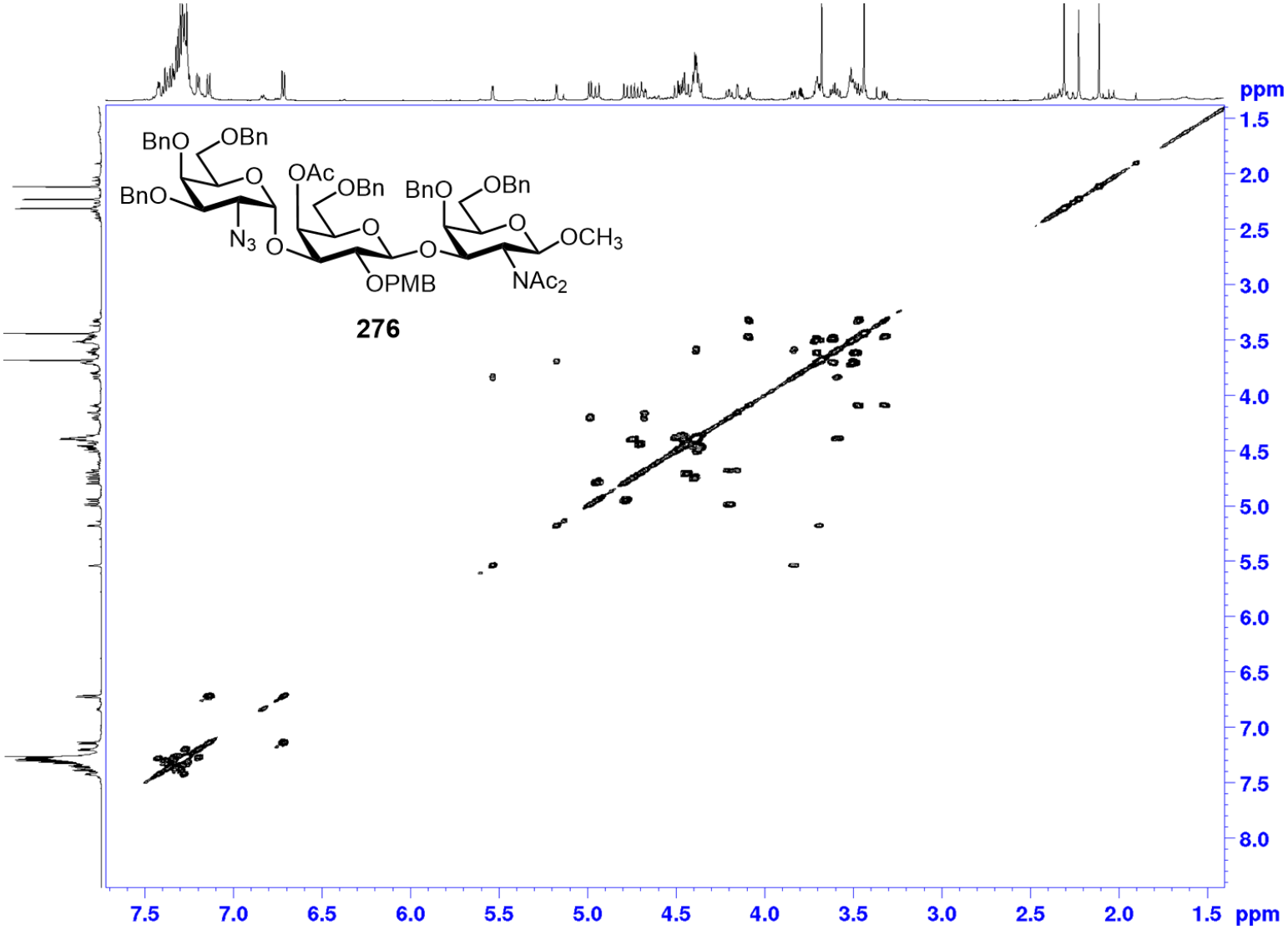
^{1}H-^{13}C HMBC ($CDCl_3$) of compound 276



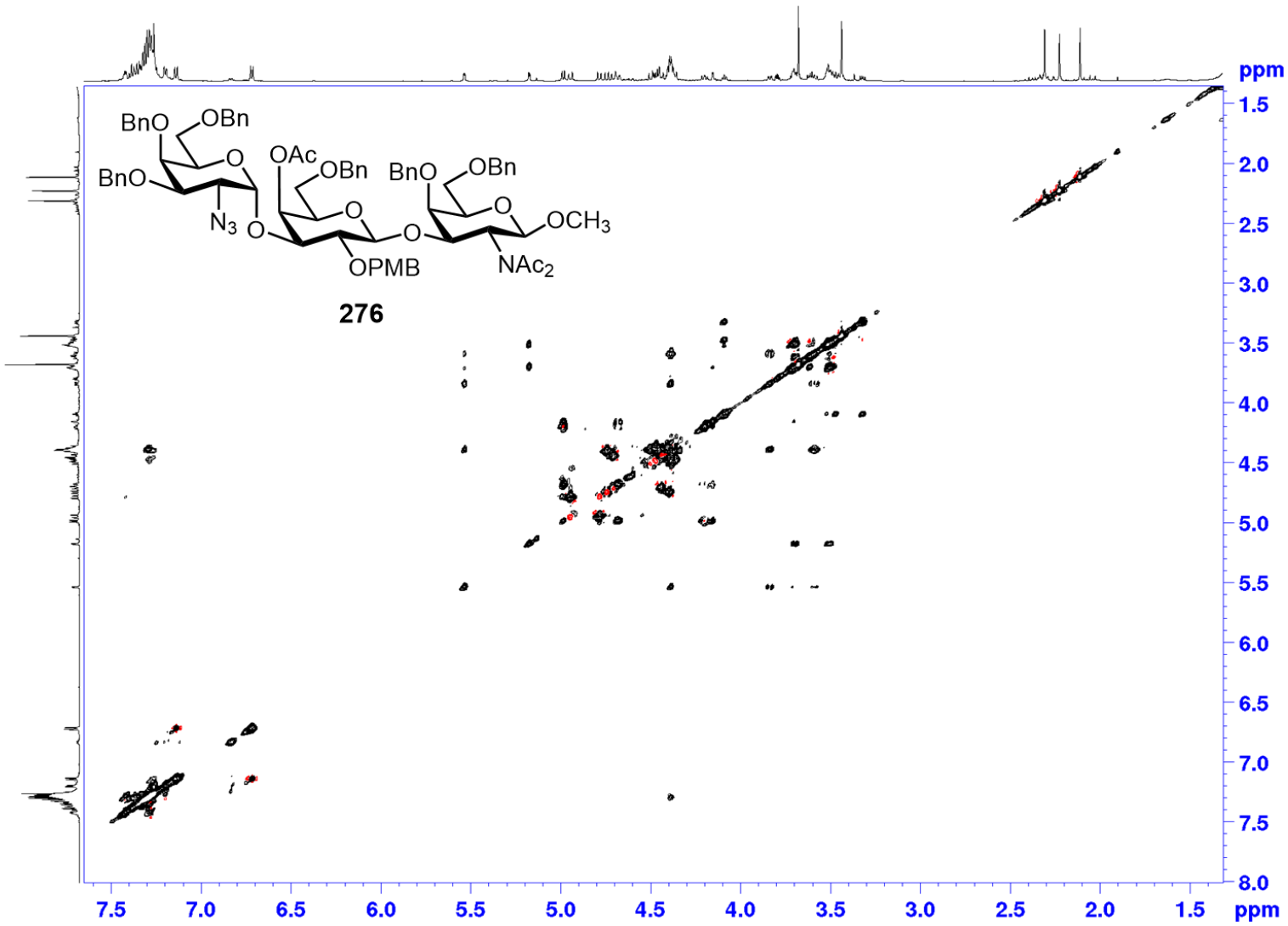
^{1}H-^{13}C HSCQ with ^{1}H-^{13}C HMBC overlay ($CDCl_3$) of compound 276



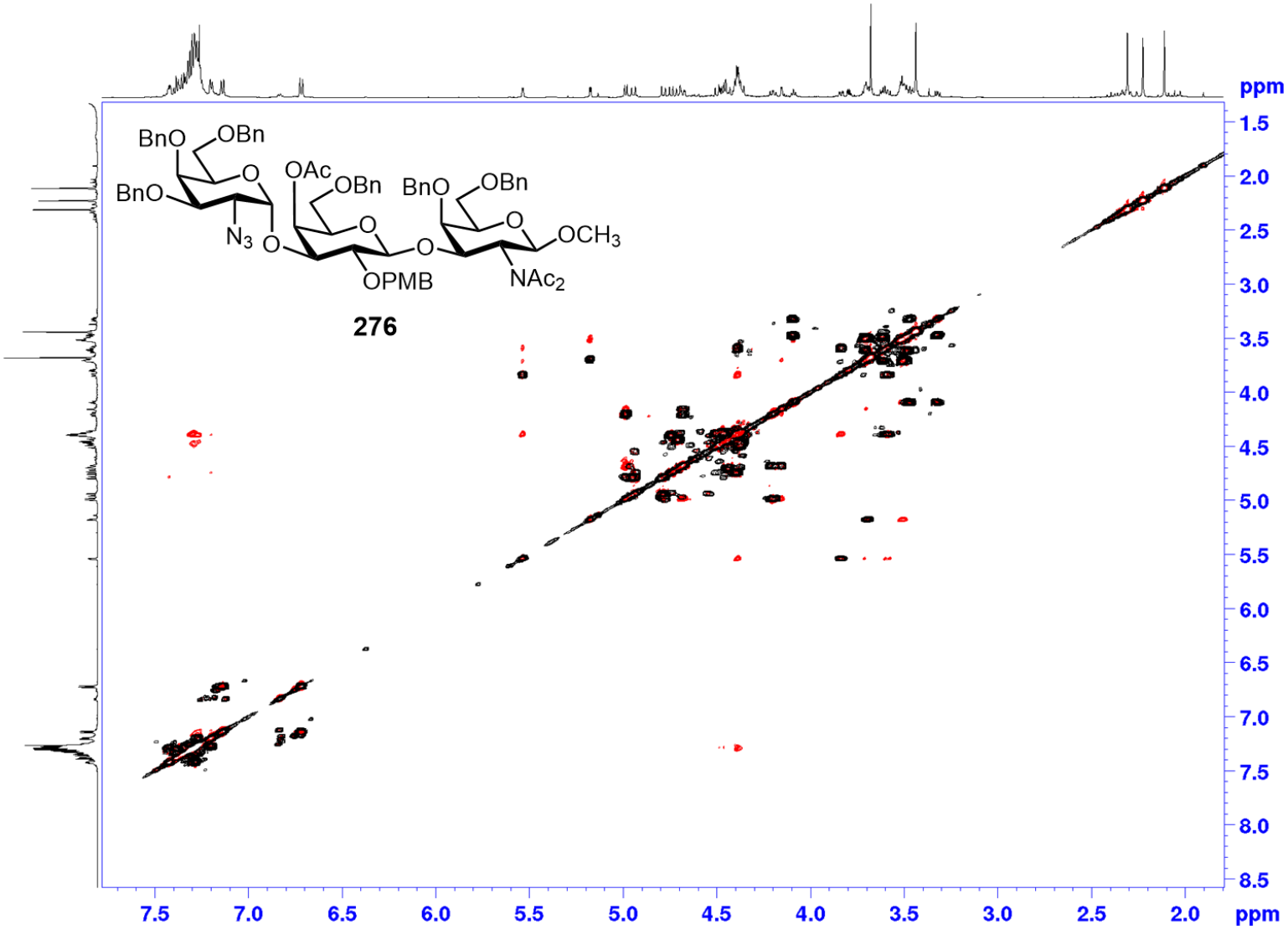
^{1}H-^{1}H COSY ($CDCl_3$) of compound 276



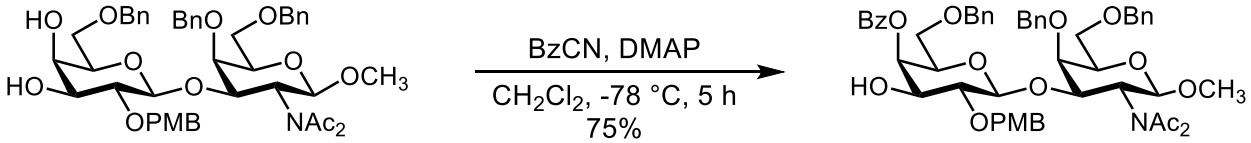
^{1}H-^{1}H TOSY ($CDCl_3$) of compound 276



^{1}H-^{1}H COSY with ^{1}H-^{1}H TOSY overlay ($CDCl_3$) of compound 276



6-*O*-benzyl-4-*O*-benzoyl-2-*O*-(*p*-methoxy)-β-D-galactopyranosyl-(1→3)-4,6-di-*O*-benzyl-2-deoxy-2-(*N*,*N*-diacetyl)-β-D-galactopyranoside (277)

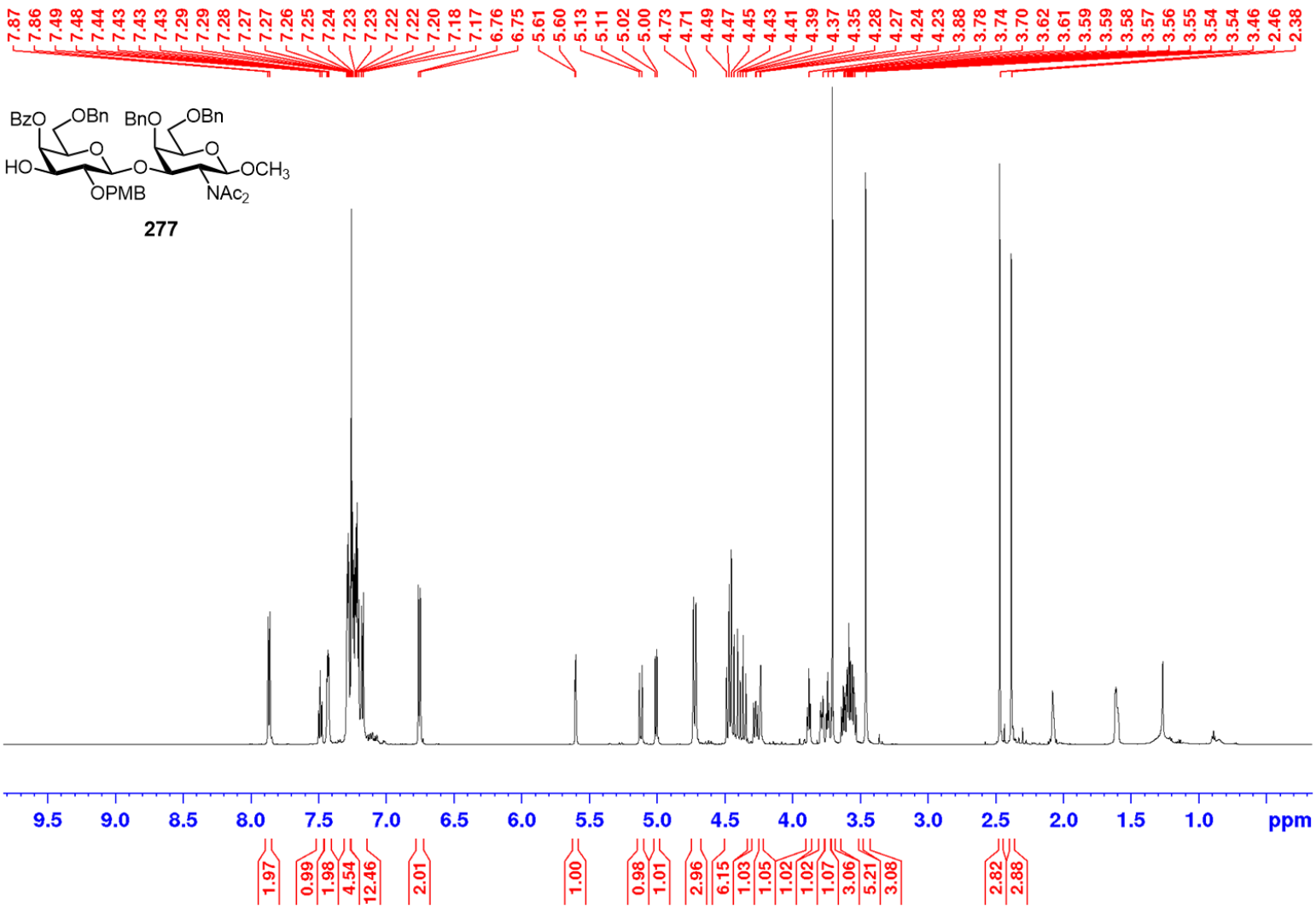


265 **277**

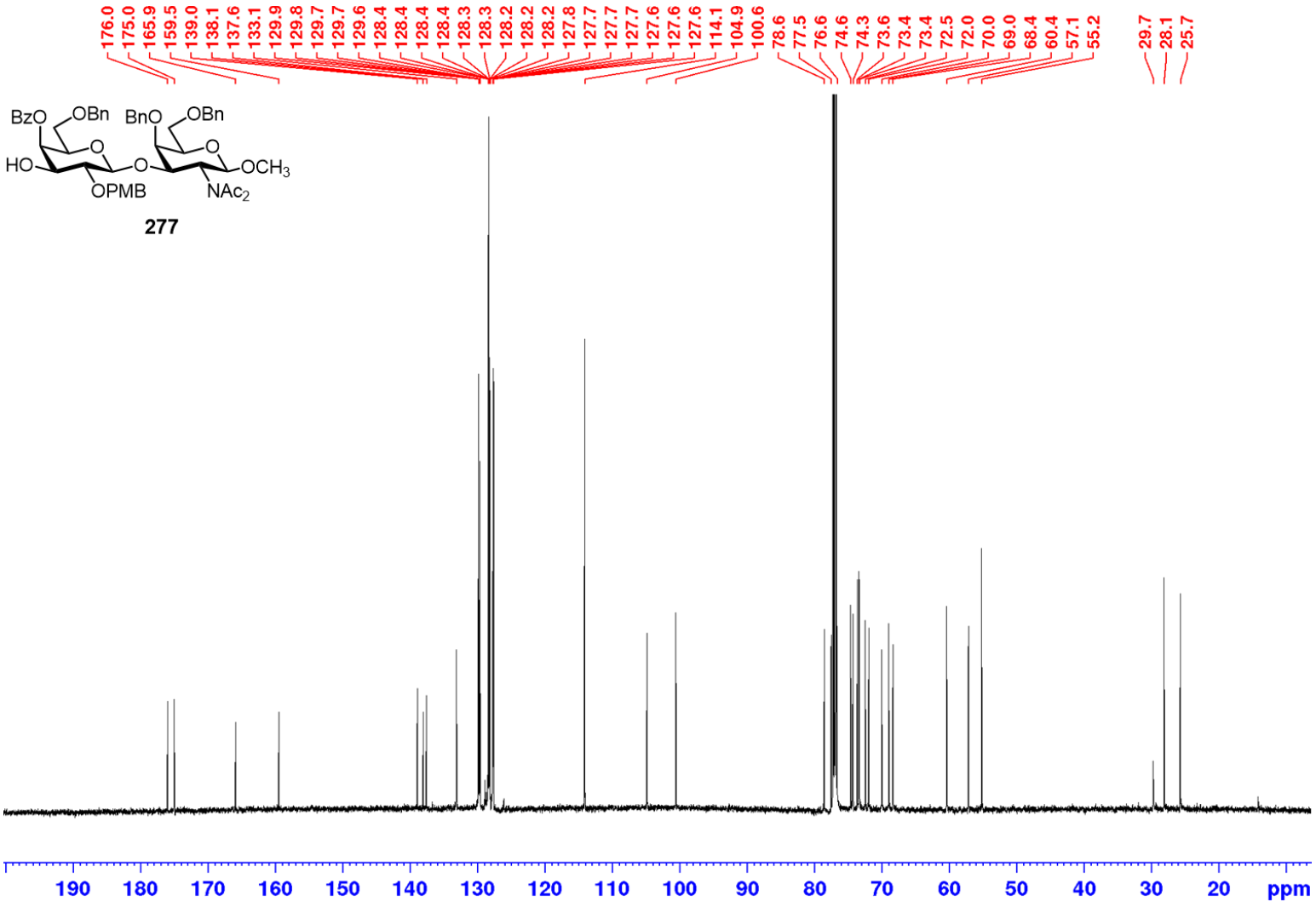
To a solution of diol 6-*O*-benzyl-2-*O*-(*p*-methoxy)-β-D-galactopyranosyl-(1→3)-4,6-di-*O*-benzyl-2-deoxy-2-(*N*,*N*-diacetyl)-β-D-galactopyranoside **265** (1.0 equiv., 0.138 g, 0.166 mmol) and 4 Å molecular sieves (800 mg) in dry dichloromethane (7.1 mL) was added benzoyl cyanide (1.1 equiv., 0.026 mL, 0.200 mmol, 90% pure) at room temperature under an argon atmosphere. At 1h, the reaction mixture was cooled to -78 °C, and 4-dimethylaminopyridine (DMAP) (0.1 equiv.) was added. The reaction was further stirred for 2 h at -78 °C temperature. After TLC analysis showed consumption of starting material, the reaction was quenched by addition of dat. aq. NH_4Cl and methanol (1.3 equiv.). Then the mixture was diluted with 100 mL of dichloromethane. The precipitate was filtered off through a pad of Celite in a glass fritted vacuum filter funnel. The organic filtrate was concentrated to an oil and taken up in EtOAc. The organic layer was washed with sat. aq. NH_4Cl (1x), aq. sodium thiosulfate (25%) (1x), brine (1x), dried ($MgSO_4$), filtered through a glass fritted vacuum filter funnel to remove drying agent, and concentrated. The residue was purified by silica gel flash column chromatography (20:80 to 40:60 EtOAc/hexanes) to give alcohol 6-*O*-benzyl-4-*O*-benzoyl-2-*O*-(*p*-methoxy)-β-D-galactopyranosyl-(1→3)-4,6-di-*O*-benzyl-2-deoxy-2-(*N*,*N*-diacetyl)-β-D-galactopyranoside **277** (0.1161 g, 0.124 mmol, 75%) as a white foam. R_f = 0.33 (30:70 EtOAc/hexanes) visualized with ceric ammonium molybdate stain; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.87 (d, *J* = 7.3 Hz, 2H), 7.49

(dd, *J* = 7.5, 7.6 Hz, 1H), 7.43 (dd, *J* = 3.5, 7.1 Hz, 2H), 7.29-7.27 (m, 5H), 7.26-7.17 (m, 12H), 6.75 (d, *J* = 8.6 Hz, 2H), 5.61 (d, *J* = 3.1 Hz, 1H), 5.12 (d, *J* = 11.5 Hz, 1H), 5 (d, *J* = 7.8 Hz, 1H), 4.71 (d, *J* = 11.2 Hz, 3H), 4.49-4.35 (m, 6H), 4.28 (dd, *J* = 8.1, 10.7 Hz, 1H), 4.23 (d, *J* = Hz, 1H), 3.88 (d, *J* = 2.3 Hz, 1H), 3.79 (dd, *J* = 5.6, 6.6 Hz, 1H), 3.74 (dd, *J* = 2.8, 5.6 Hz, 1H), 3.7 (s, 3H), 3.64-3.53 (m, 5H), 3.45 (s, 3H), 2.46 (s, 3H), 2.38 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 176.0, 175.0, 165.9, 159.5, 138.9, 138.1, 137.6, 133.1, 129.8, 129.8, 129.7, 129.6, 129.5, 128.4, 128.4, 128.4, 128.3, 128.3, 128.2, 128.2, 128.1, 128.1, 127.7, 127.7, 127.6, 127.6, 127.6, 127.6, 127.6, 114.1, 104.8, 100.5, 78.5, 77.5, 76.6, 74.6, 74.2, 73.6, 73.3, 73.3, 72.4, 71.9, 70.0, 68.9, 68.3, 60.3, 57.1, 55.1, 29.7, 28.1, 25.7.

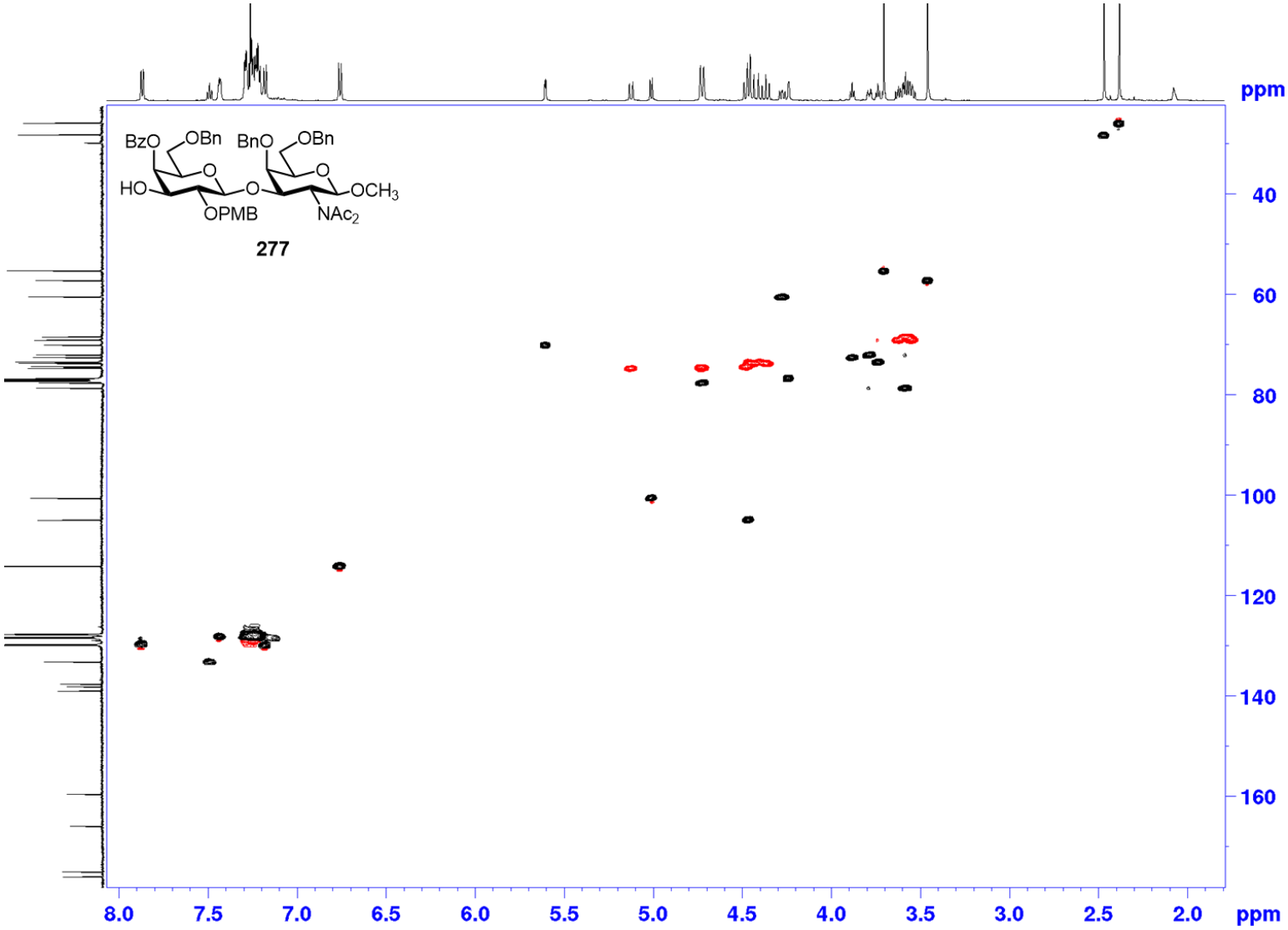
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 277



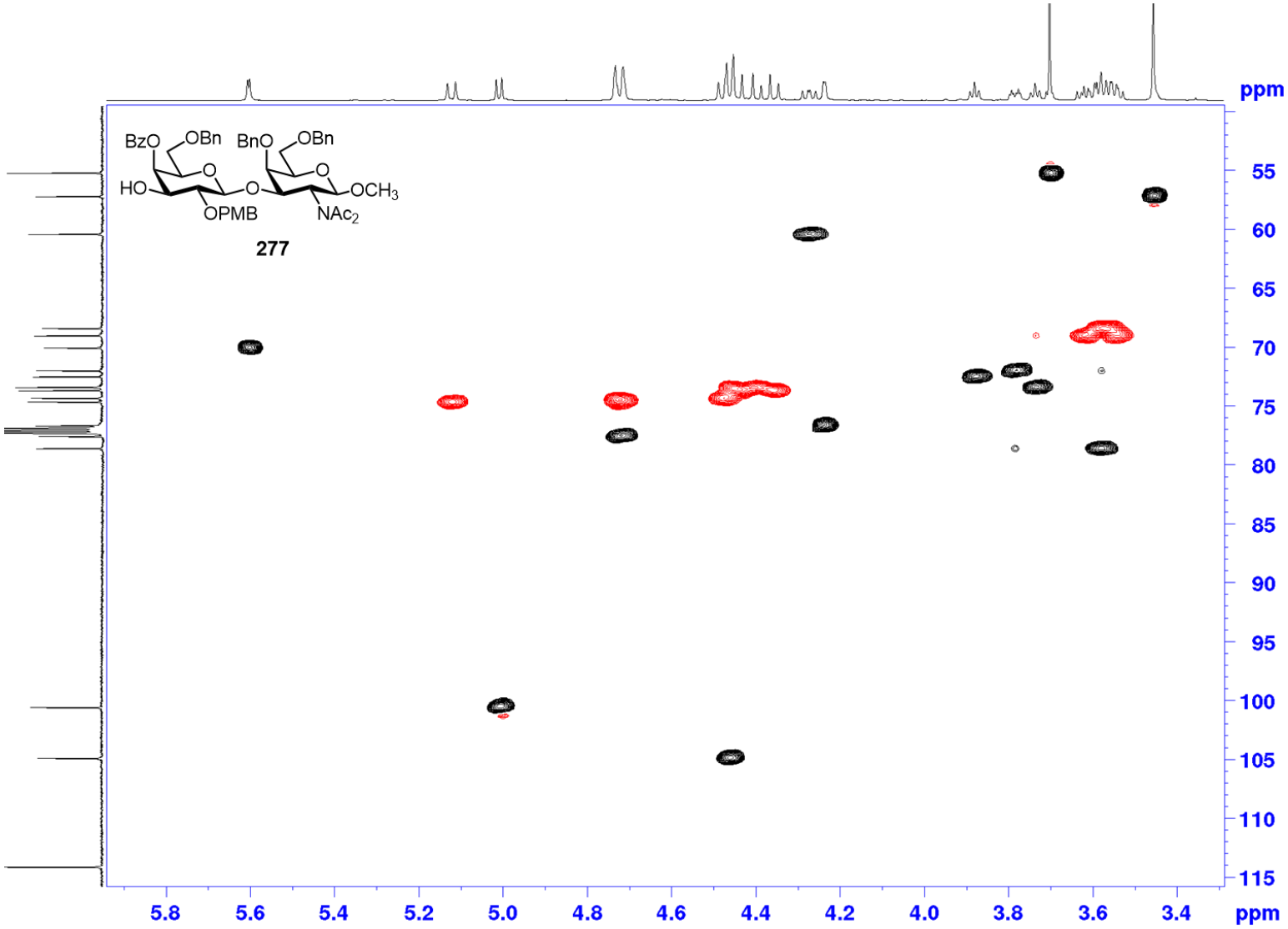
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 277



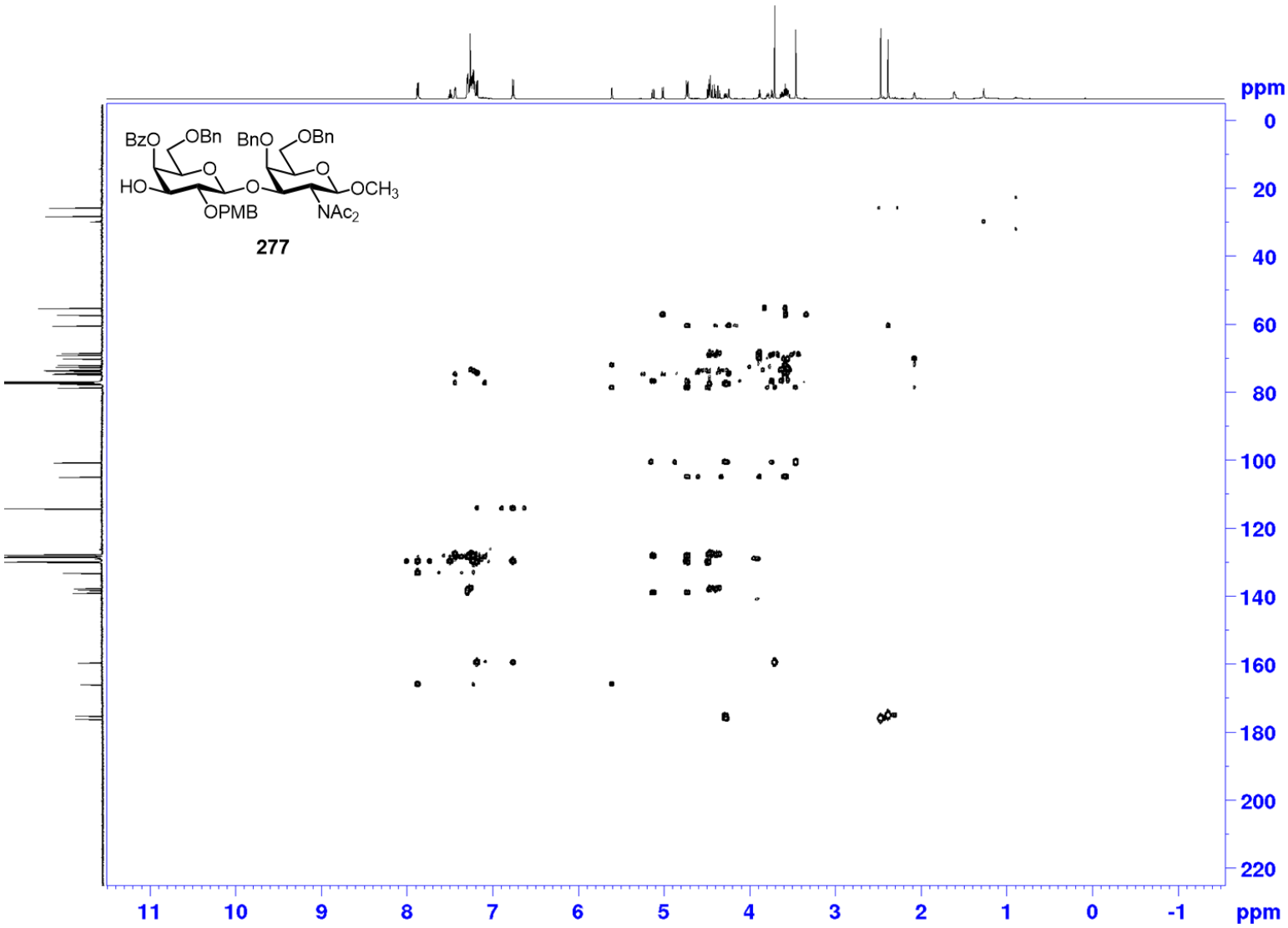
^{1}H-^{13}C HSCQ ($CDCl_3$) of compound 277



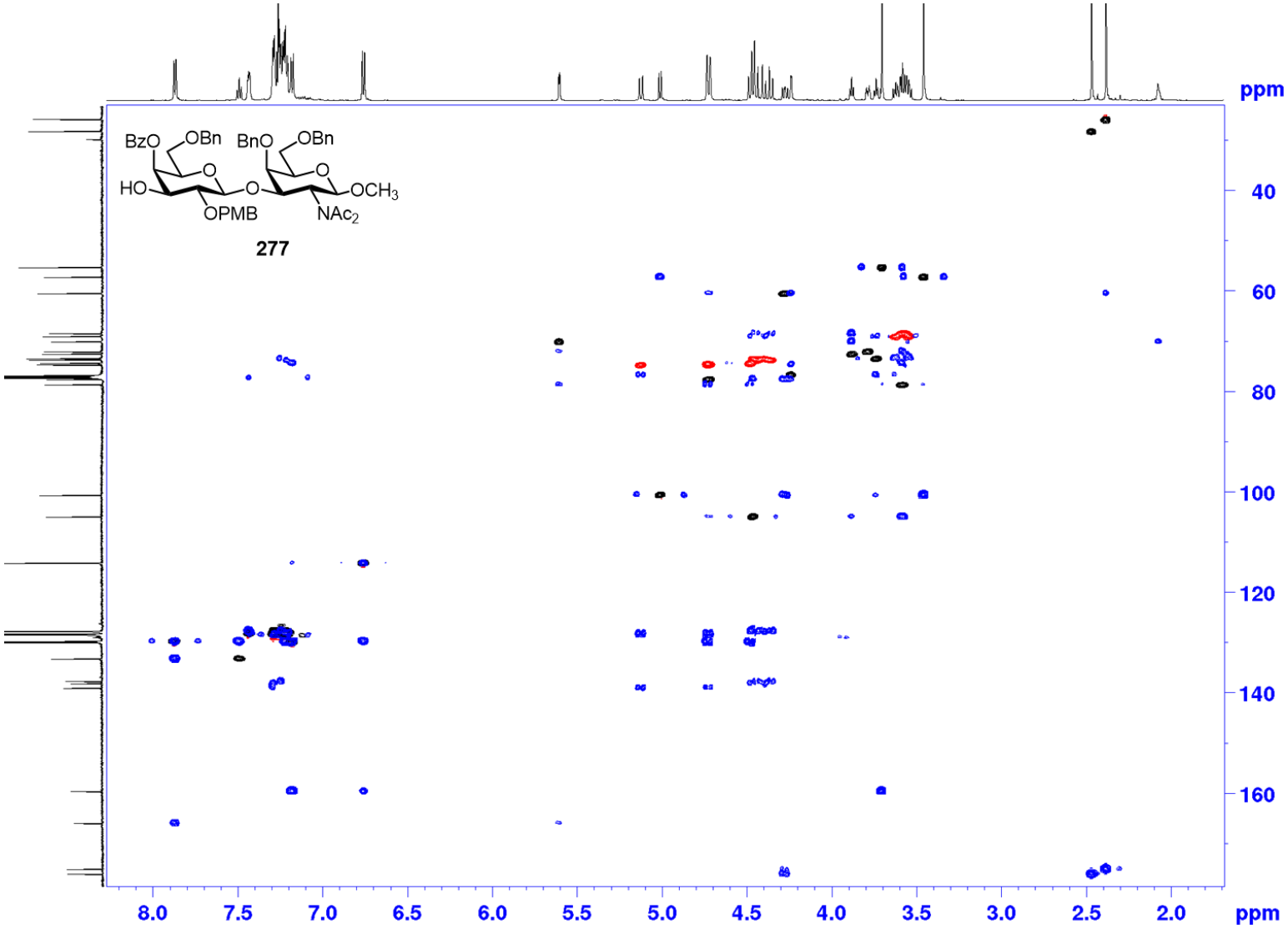
^{1}H-^{13}C HSCQ ($CDCl_3$) zoomed in region of compound 277



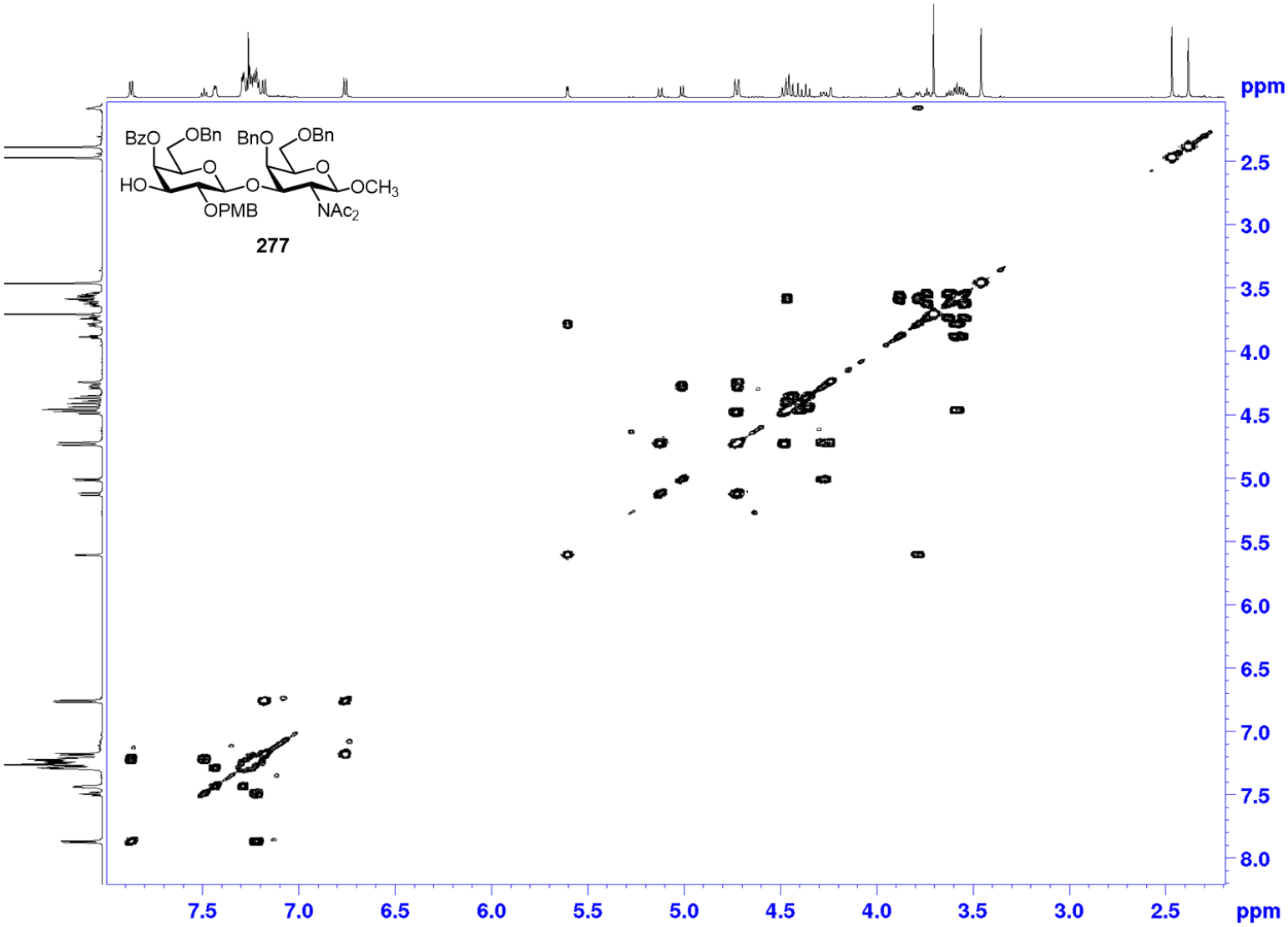
^{1}H-^{13}C HMBC ($CDCl_3$) of compound 277



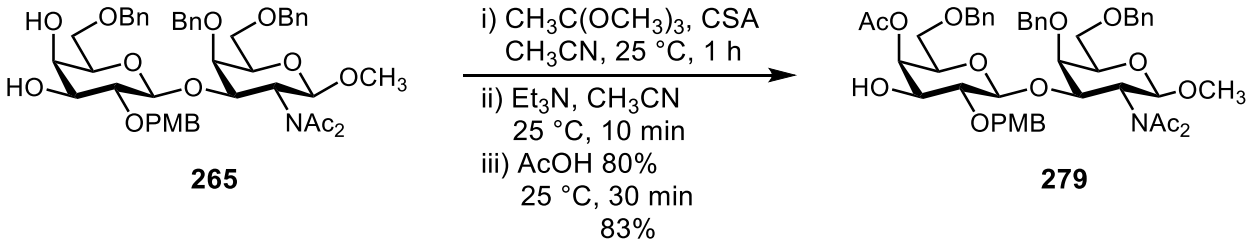
^{1}H-^{13}C HSCQ with ^{1}H-^{13}C HMBC overlay ($CDCl_3$) of compound 277



^{1}H-^{1}H COSY ($CDCl_3$) of compound 277



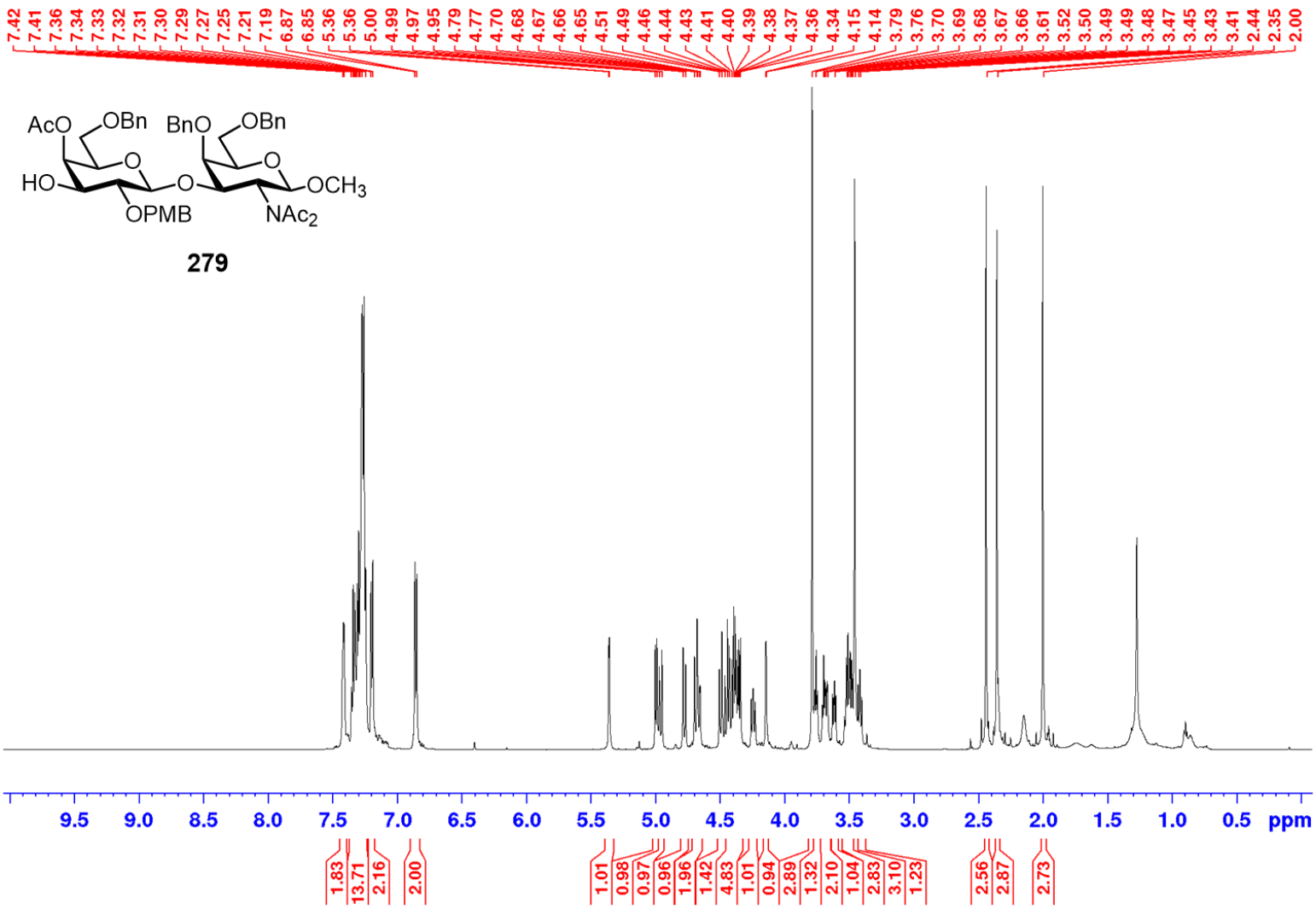
6-*O*-benzyl-4-*O*-acetyl-2-*O*-(*p*-methoxy)-β-D-galactopyranosyl-(1→3)-4,6-di-*O*-benzyl-2-deoxy-2-(*N*,*N*-diacetyl)-β-D-galactopyranoside (279)



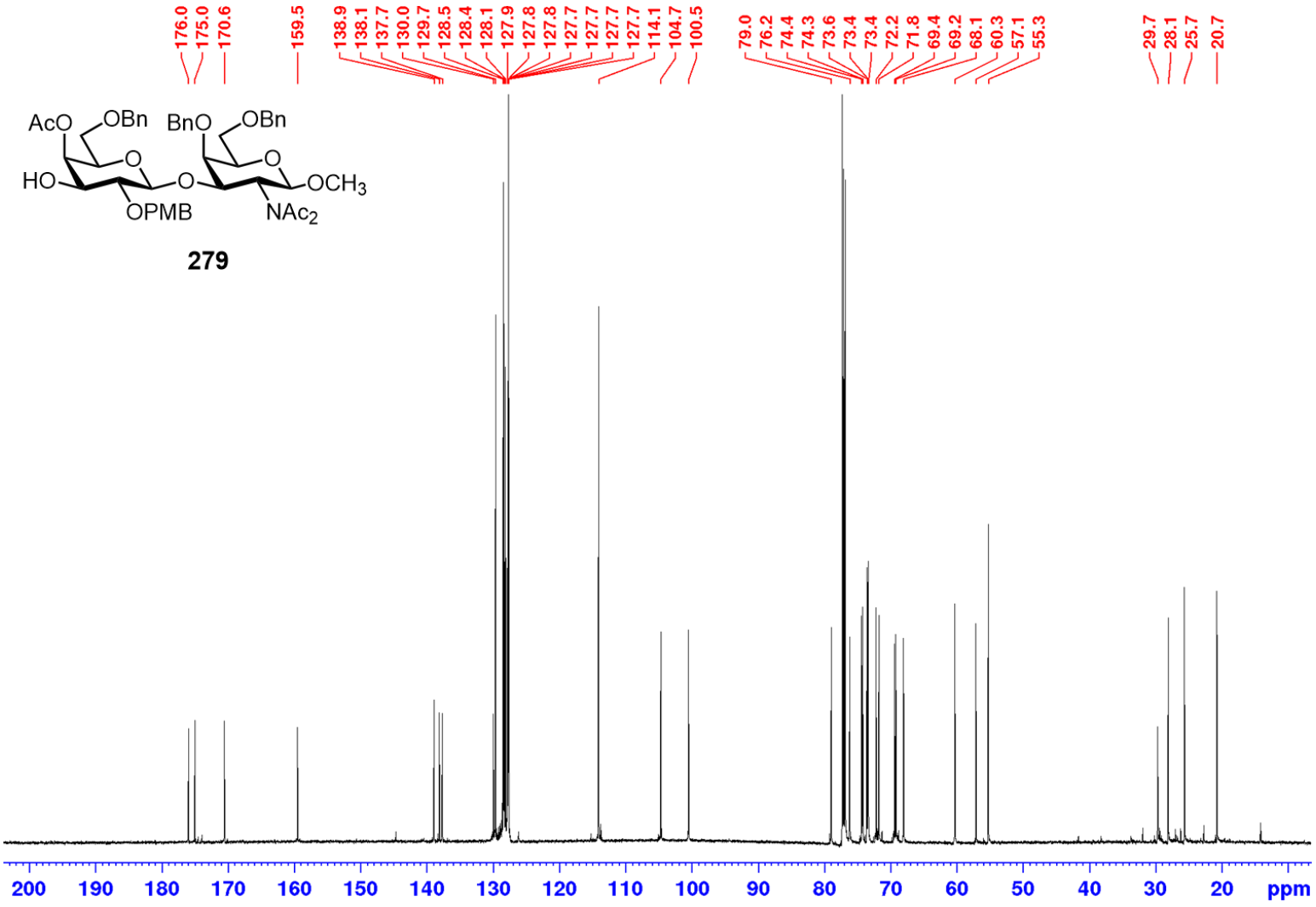
A solution of diol 6-*O*-benzyl-2-*O*-(*p*-methoxy)-β-D-galactopyranosyl-(1→3)-4,6-di-*O*-benzyl-2-deoxy-2-(*N*,*N*-diacetyl)-β-D-galactopyranoside **265** (1.0 equiv., 0.592 g, 0.713 mmol) in dry CH_3CN (10.0 mL) was added trimethyl orthoacetate (2.2 equiv., 0.20 mL, 1.563 mmol) and cooled to 0 °C. After 5 min, camphorsulfonic acid (0.05 equiv., 10.0 mg, 0.035 mmol) was added under an argon atmosphere. The reaction was stirred at room temperature. At 25 min, consumption of starting material was apparent by TLC. The reaction was added Et_3N (0.05 equiv., 0.05 mL, 0.36 mmol) and concentrated *in vacuo*. The reaction was then added 80% AcOH (aq) at room temperature. At 30 min, the reaction was concentrated *in vacuo* and co-evaporated with toluene (4x). The crude reaction mixture was purified by plug of silica gel (50:50 EtOAc/hexanes) to give alcohol 6-*O*-benzyl-4-*O*-acetyl-2-*O*-(*p*-methoxy)-β-D-galactopyranosyl-(1→3)-4,6-di-*O*-benzyl-2-deoxy-2-(*N*,*N*-diacetyl)-β-D-galactopyranoside **279** (0.516 mg, 0.592 mmol, 83% yield) as an off white foam. R_f = 0.40 (50:50 EtOAc/hexanes) visualized with ceric ammonium molybdate stain; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.42 (d, *J* = 3.6 Hz, 2H), 7.36-7.25 (m, 14H), 7.2 (d, *J* = 8.7 Hz, 2H), 6.86 (d, *J* = 8.7 Hz, 2H), 5.36 (d, J = 3.2 Hz, 1H), 4.99 (d, *J* = 7.8 Hz, 1H), 4.98 (d, *J* = 11.4 Hz, 1H), 4.78 (d, *J* = 12.5 Hz, 1H), 4.70-4.65 (m, 2H), 4.51-4.46 (m, 1H), 4.45-4.34 (m, 5H), 4.25 (dd, *J* = 8.2, 10.5 Hz, 1H), 4.15 (d, *J* = 2.3 Hz,

1H), 3.8 (s, 3H), 3.77-3.75 (m, 1H), 3.71-3.66 (m, 2H), 3.62-3.60 (m, 1H), 3.53-3.45 (m, 3H), 3.45 (s, 3H), 3.4 (dd, *J* = 7.3, 8.6 Hz, 1H), 2.44 (s, 3H), 2.35 (s, 3H), 2 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 175.9, 175.0, 170.5, 159.5, 138.9, 138.1, 137.6, 129.9, 129.6, 129.6, 128.5, 128.4, 128.4, 128.3, 128.3, 128.1, 128.1, 127.8, 127.8, 127.7, 127.7, 127.7, 127.7, 127.7, 127.6, 127.6, 114.0, 104.7, 100.5, 78.9, 76.1, 74.3, 74.2, 73.6, 73.4, 73.3, 72.2, 71.7, 69.4, 69.2, 68.0, 60.3, 57.1, 55.2, 29.7, 28.1, 25.6, 20.7.

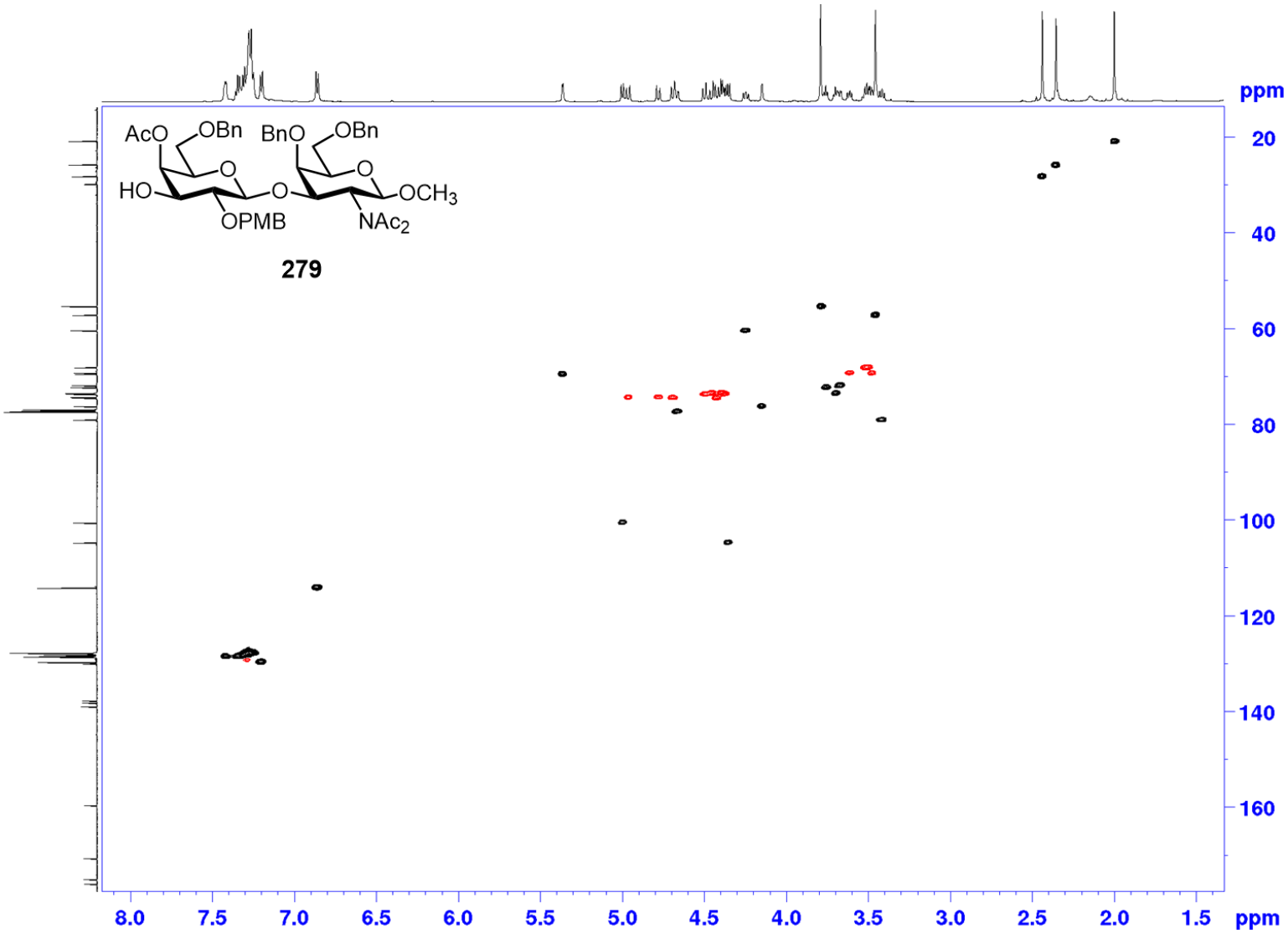
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 279



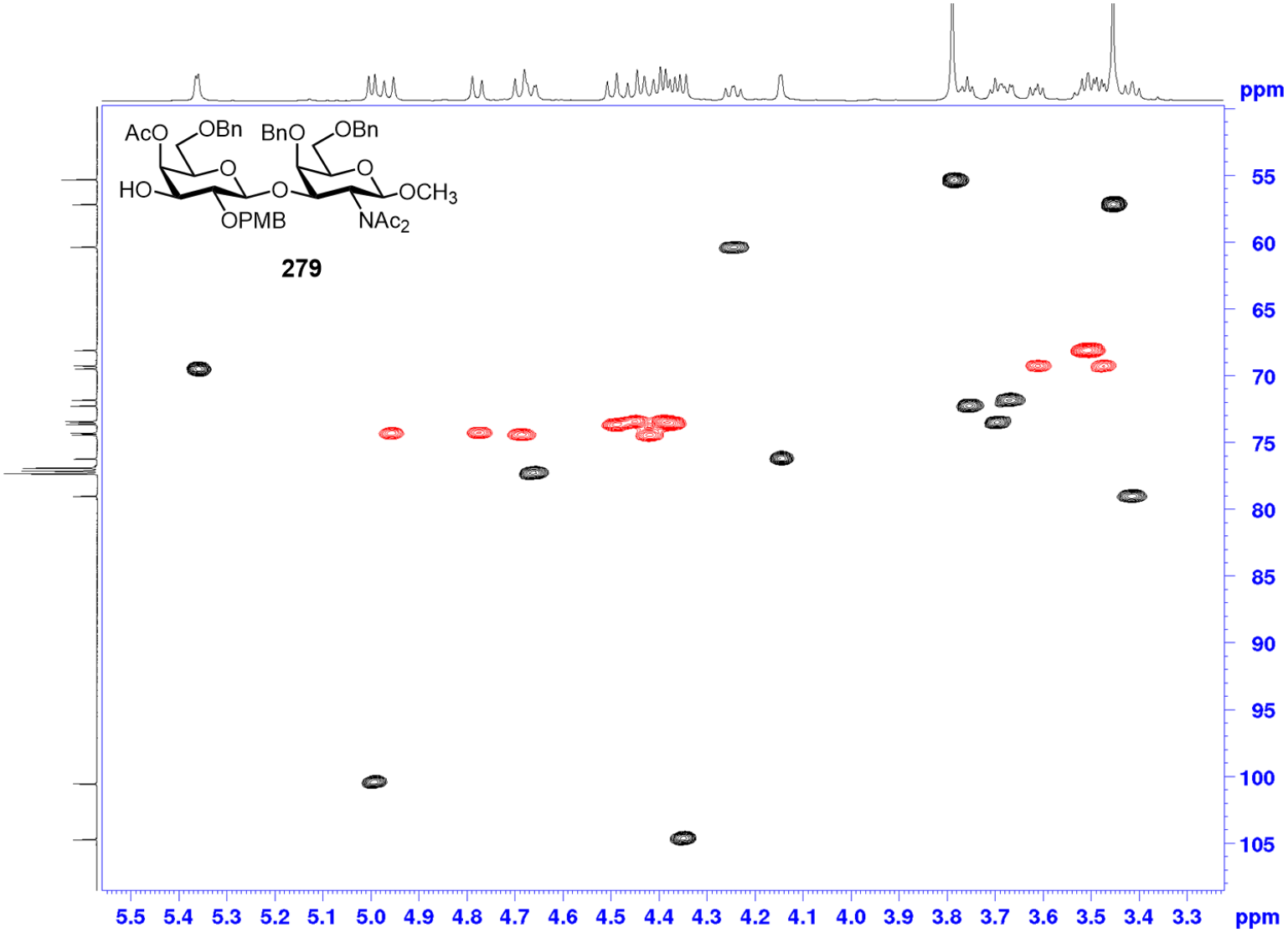
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 279



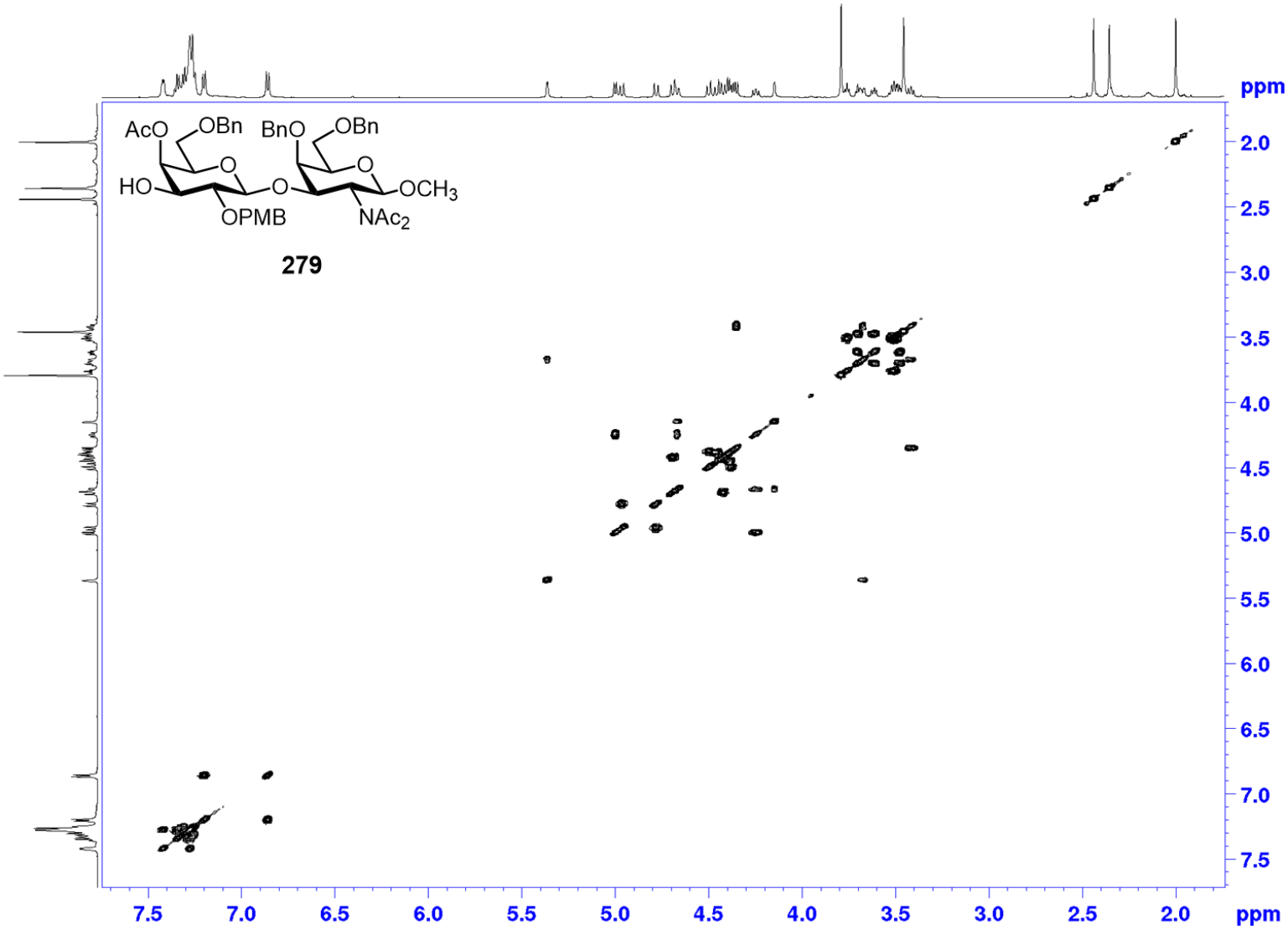
^{1}H-^{13}C HSCQ ($CDCl_3$) of compound 279



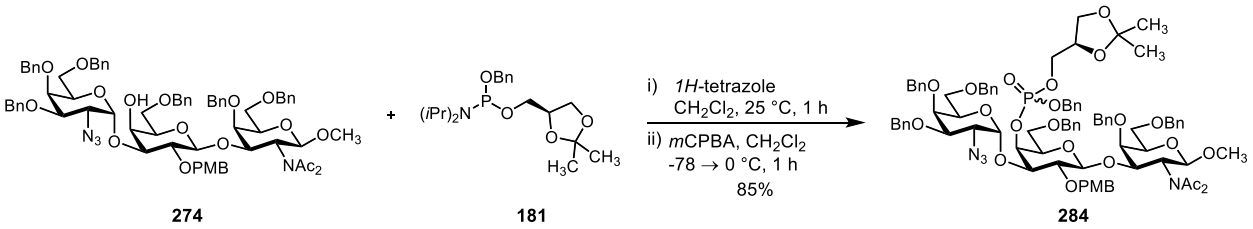
^{1}H-^{13}C HSCQ ($CDCl_3$) zoomed in region of compound 279



^{1}H-^{1}H COSY ($CDCl_3$) of compound 279



Methyl 3,4,6-tri-O-benzyl-2-deoxy-2-azido-α-D-galactopyranosyl-(1→3)-6-*O*-benzyl-4-*O*-(((*R*)-2,2-dimethyl-1,3-dioxolan-4-yl)methyl) phosphate-2-*O*-(*p*-methoxy)-β-D-galactopyranosyl-(1→3)-4,6-di-*O*-benzyl-2-deoxy-2-(*N*,*N*-diacetyl)-β-D-galactopyranoside (284)



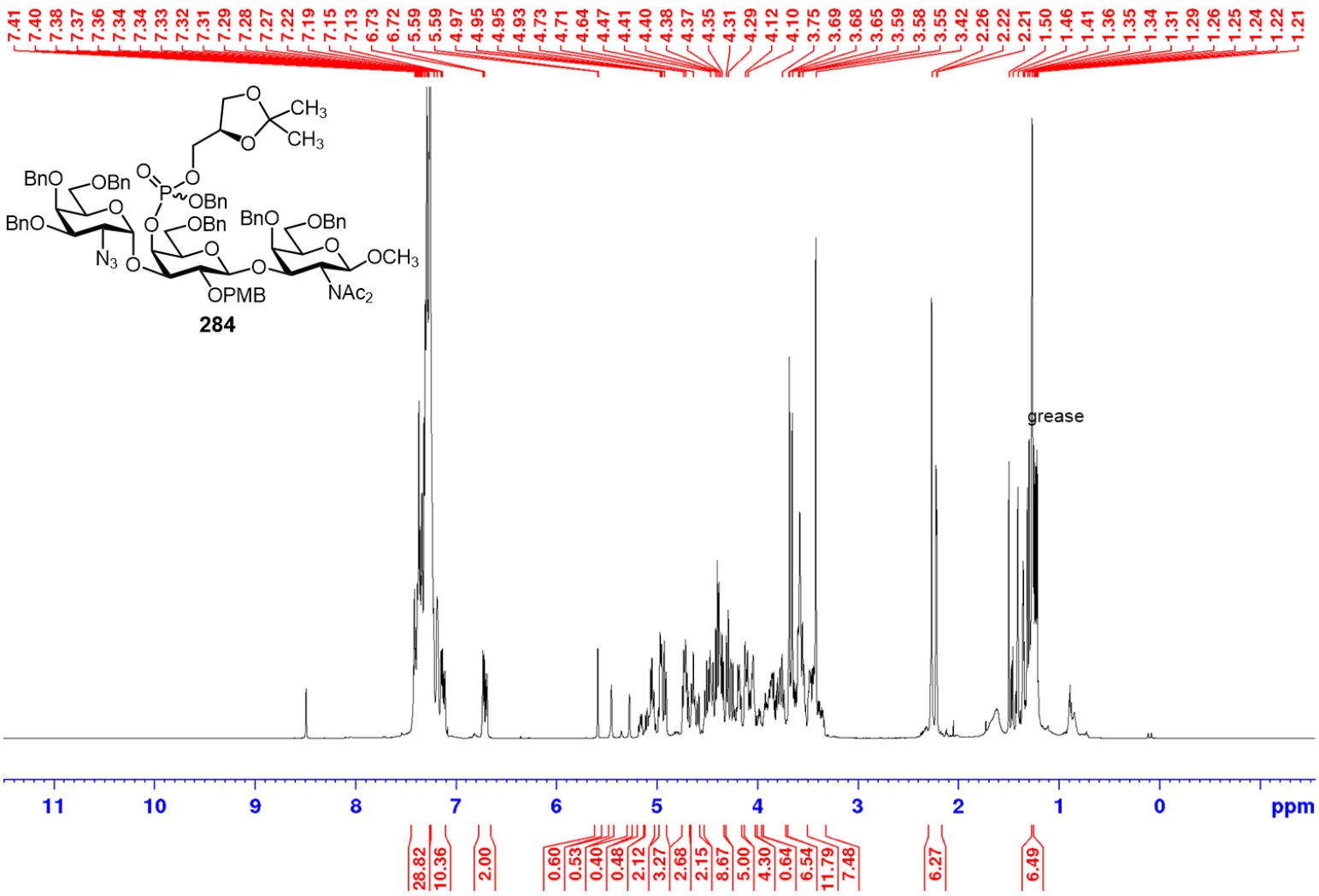
274 181 284

A solution of methyl 3,4,6-tri-O-benzyl-2-deoxy-2-azido-α-D-galactopyranosyl-(1→3)-6-*O*-benzyl-2-*O*-(*p*-methoxy)-β-D-galactopyranosyl-(1→3)-4,6-di-*O*-benzyl-2-deoxy-2-(*N*,*N*-diacetyl)-β-D-galactopyranoside **274** (1.0 equiv., 1.327 g, 1.031 mmol) and phosphoramidite **181** (1.2 equiv., 0.457 g, 1.237 mmol) in anhydrous CH_2Cl_2 (5.0 mL) was added tetrazole (1.2 equiv., 0.866 g, 1.237 mmol) under an argon atmosphere at room temperature. At 1 h, TLC analysis indicated that the starting material had been consumed. The reaction was filtered through a glass fritted vacuum filter funnel equipped with a pad of Celite to remove the amine salts. The filtrate cooled to -78 °C and treated a solution of *m*CPBA (1.2 equiv., 0.280 g, 1.237 mmol, in anhydrous CH_2Cl_2). At 1 h, the crude reaction mixture was concentrated and co-evaporated with toluene (3x) to give a white foam. The crude foam was purified by silica gel flash column chromatography (50:50 to 60:40 EtOAc/hexanes) to give an inseparable pair of phosphate diastereomeric methyl 3,4,6-tri-O-benzyl-2-deoxy-2-azido-α-D-galactopyranosyl-(1→3)-6-*O*-benzyl-4-*O*-(((*R*)-2,2-dimethyl-1,3-dioxolan-4-yl)methyl) phosphate-2-*O*-(*p*-methoxy)-β-D-galactopyranosyl-(1→3)-4,6-di-*O*-benzyl-2-deoxy-2-(*N*,*N*-diacetyl)-β-D-galactopyranoside **284** (1.377 g, 0.876 mmol, 85%)[a] as a white amorphous solid. R_f =

0.70 (1:1 EtOAc/hexanes) visualized with ceric ammonium molybdate stain; $[\alpha]D^{25}$ = -0.72° (c = 1.0, $CHCl_3$); IR ν_{max} (film) 3466.95, 2924.10, 2854.88, 2110.52, 1653.72, 1513.19, 1496.40, 1454.00, 1365.45, 1246.56, 1218.45, 1102.45, 1062.66, 736.32, 697.87 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.34-7.10 (m, 39H), 6.72 (d, *J* = 7.8 Hz, 1H), 6.69 (d, *J* = 7.9 Hz, 1H), 5.58 (d, *J* = 2.0, 1H), 5.45 (m, 1H), 5.27 (m, 1H), 5.18-5.14 (m, 1H), 5.11 (m, 2H), 4.98 (m, 3H), 4.74-4.67 (m, 2H), 4.65-4.57 (m, 2H), 4.52-4.33 (m, 8H), 4.31-4.23 (m, 3H), 4.18-4.15 (m, 2H), 4.12-4.00 (m, 4H), 4.00-3.96 (m,1H), 3.93-3.72 (m, 7H), 3.69-3.51 (m, 12H), 3.49-3.33 (m, 8H), 2.26 (s, 3H), 2.22 (s, 3H), 1.50 (s, 3H), 1.41 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 175.9, 174.9, 158.9, 138.8, 138.7, 138.2, 138.2, 138.1, 138.1, 138.0, 137.9, 137.7, 137.7, 135.9, 130.4, 130.3, 129.5, 129.5, 128.6, 128.5, 128.4, 128.3, 128.3, 128.2, 128.1, 128, 128, 127.8, 127.8, 127.6, 113.7, 113.7, 109.8, 109.7, 104.7, 100.5, 93.4, 93.3, 77.8, 77.7, 77.4, 76.1, 76.0, 74.5, 74.4, 74.4, 74.3, 74.1, 74.0, 74.0, 73.5, 73.4, 73.4, 73.3, 73.2, 72.8, 72.7, 72.3, 71.8, 69.6, 69.6, 69.5, 69.4, 69.2, 69.2, 69.0, 69.0, 68.0, 66.5, 66.4, 66.0, 66.0, 60.2, 59.7, 59.6, 57.1, 55.2, 55.2, 52.2, 46.1, 29.8, 29.8, 29.7, 27.8, 26.7, 26.7, 26.7, 25.4, 25.3, 25.2, 22.6, 22.5.; HRMS (ESI) calcd for $C_{86}H_{103}N_5O_4P^+$ $(M+NH_4)^+$ 1588.68272, found 1588.68282 m/z.

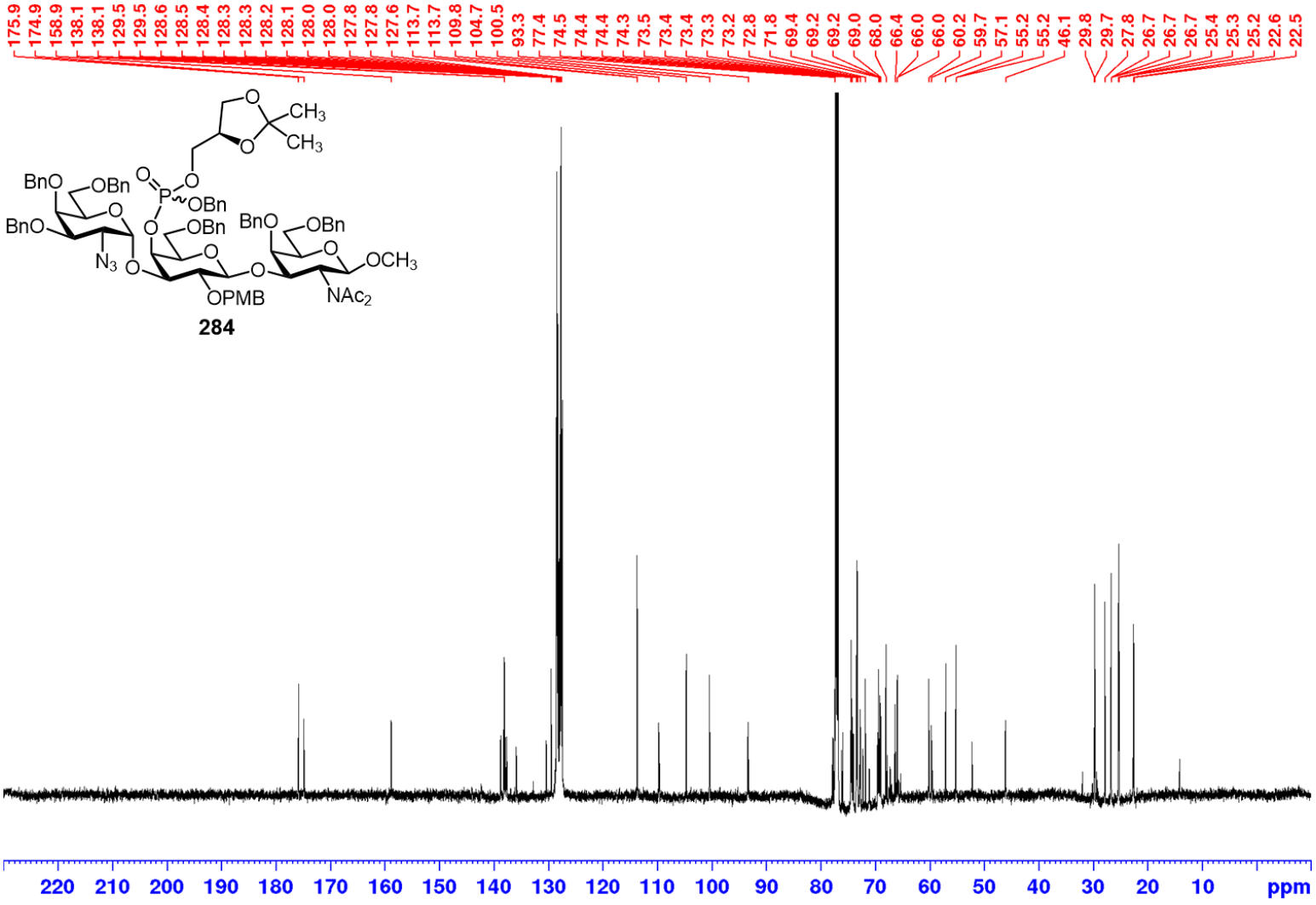
[a] yield reported contains solvent impurities. see NMR spectra.

^{1}H NMR ($CDCl_3$, 400 MHz) of compound 284

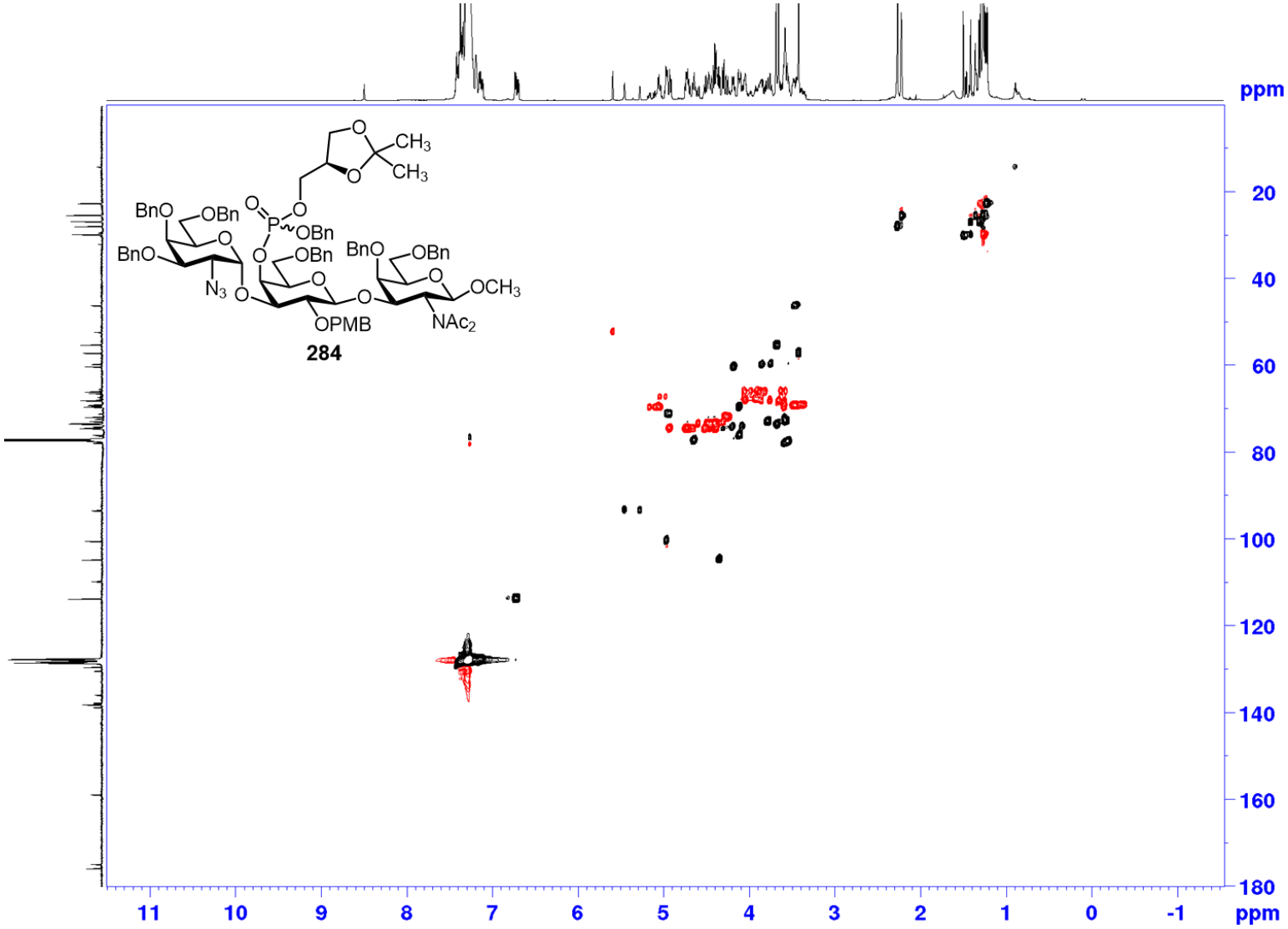


284

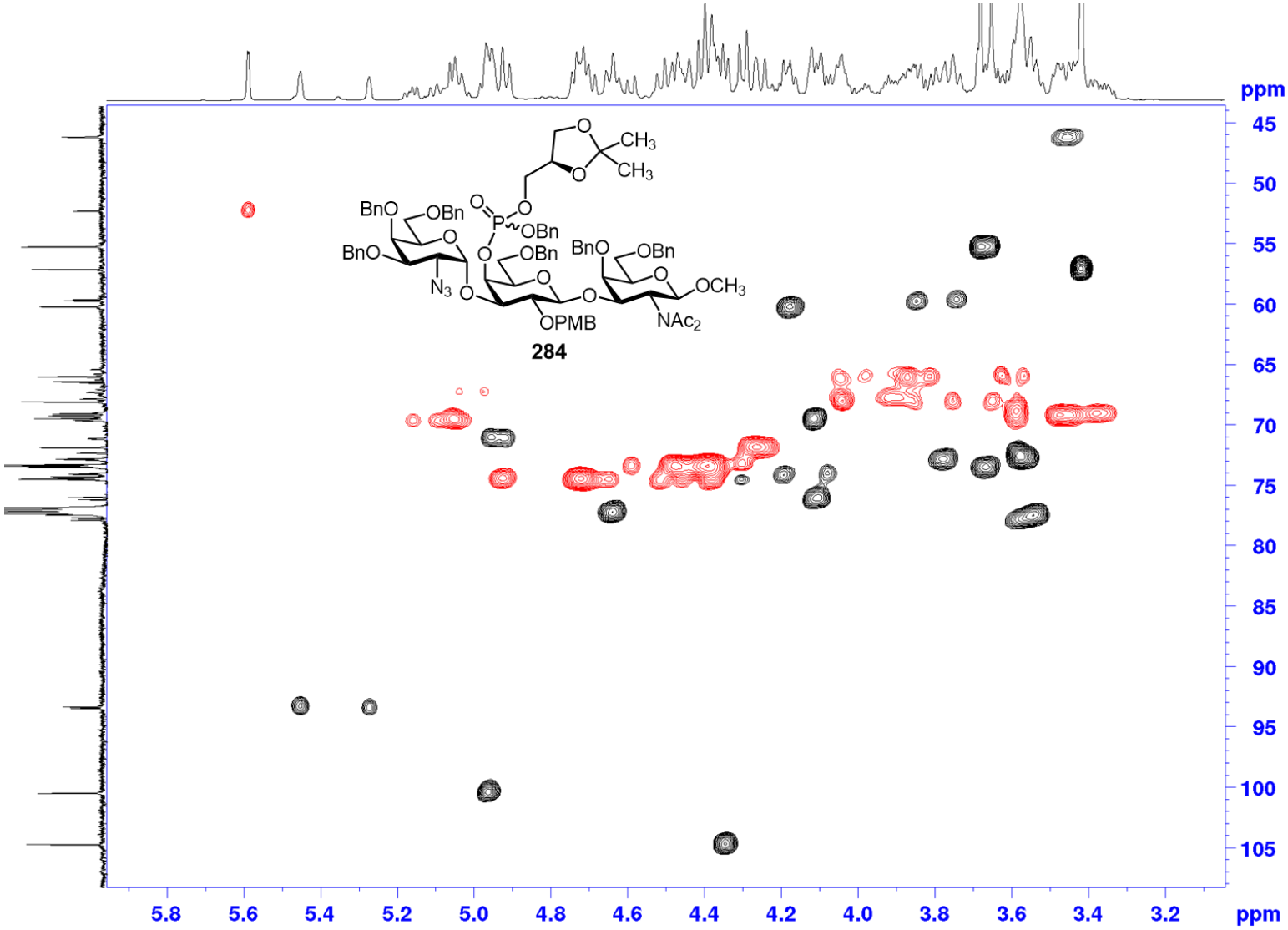
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 284



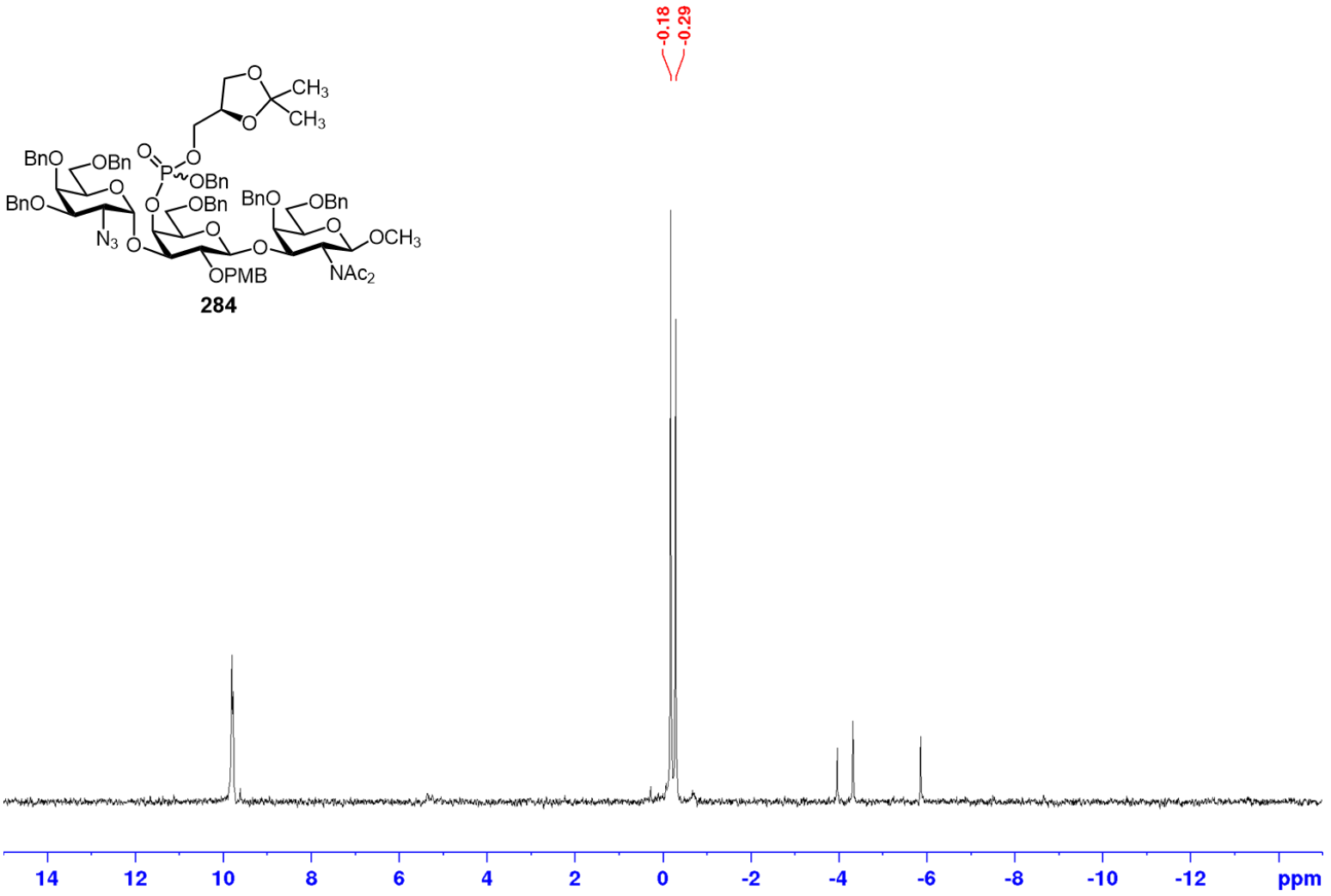
^{1}H-^{13}C HSCQ ($CDCl_3$) of compound 284



^{1}H-^{13}C HSCQ ($CDCl_3$) zoomed in region of compound 284



^{31}P NMR ($CDCl_3$, 162 MHz) of compound 284

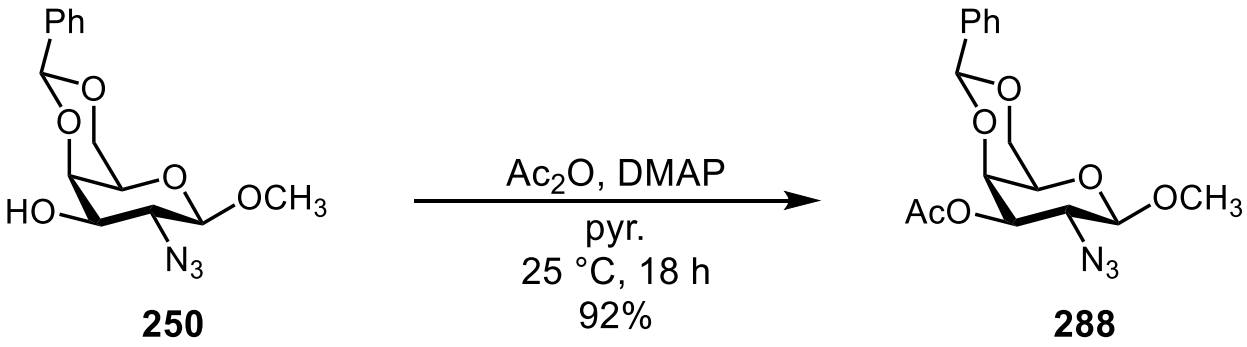


284

Part 5. MM-ZPS 2nd-generation version 3 synthetic route

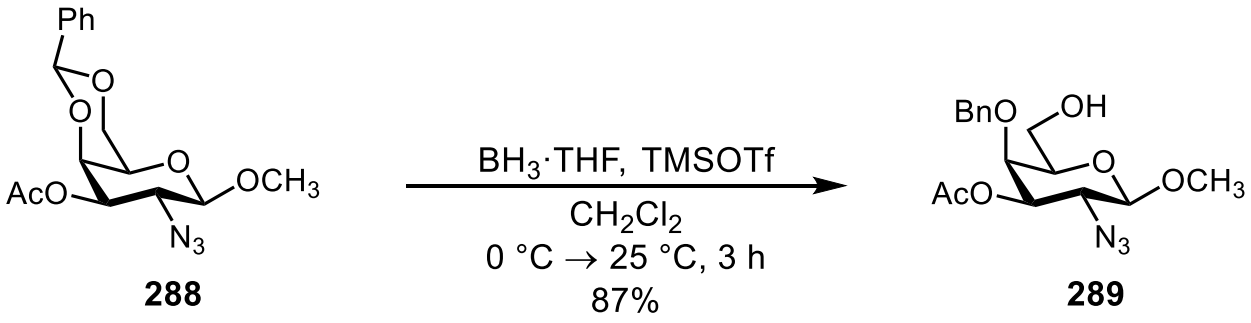
Part 5 contains experimental procedures for compounds found within schemes 39-41

Methyl 4,6-*O*-benzylidene acetal-3-*O*-acetyl-2-deoxy-2-azido-β-D-galactopyranoside (288)



To a solution of methyl 4,6-*O*-benzylidene acetyl-2-deoxy-2-azido-β-D-galactopyranoside **250** (1.0 equiv., 3.625 g, 11.80 mmol) in pyridine (10.0 equiv., 9.5 mL, 118.0 mmol) under argon was cooled to 0 °C and added acetic anhydride (1.5 equiv., 1.67 mL, 17.69 mmol) dropwise then added DMAP (cat.). The solution was stirred at room temperature. After 1 h, TLC indicated that starting material had been completely consumed. The reaction was cooled to 0 °C and the excess acetic anhydride was quenched by the addition of CH3OH (0.4 mL). After 5 min, the crude reaction mixture was warmed to room temperature then transferred to a separatory funnel with EtOAc (100 mL). The reaction was washed with 1 N HCl (3 x 25 mL), satd. aq. $NaHCO_3$ (1 x 25 mL), brine (1 x 125 mL), dried ($NaSO_4$), filtered through a glass fritted vacuum filter funnel to remove the drying agent. The filtrate was concentrated to a crude oil which was purified by flash column chromatography (20:80 to 40:60 EtOAc/hexanes) to give methyl 4,6-*O*-benzylidene acetal-3-*O*-acetyl-2-deoxy-2-azido-β-D-galactopyranoside **288** (3.75 g, 10.7 mmol, 92%) as a white amorphous solid. Our spectroscopic data agrees with previously reported data.[339] R_f: 0.7 in 50:50 EtOAc/hexanes, visualize with CAM stain.

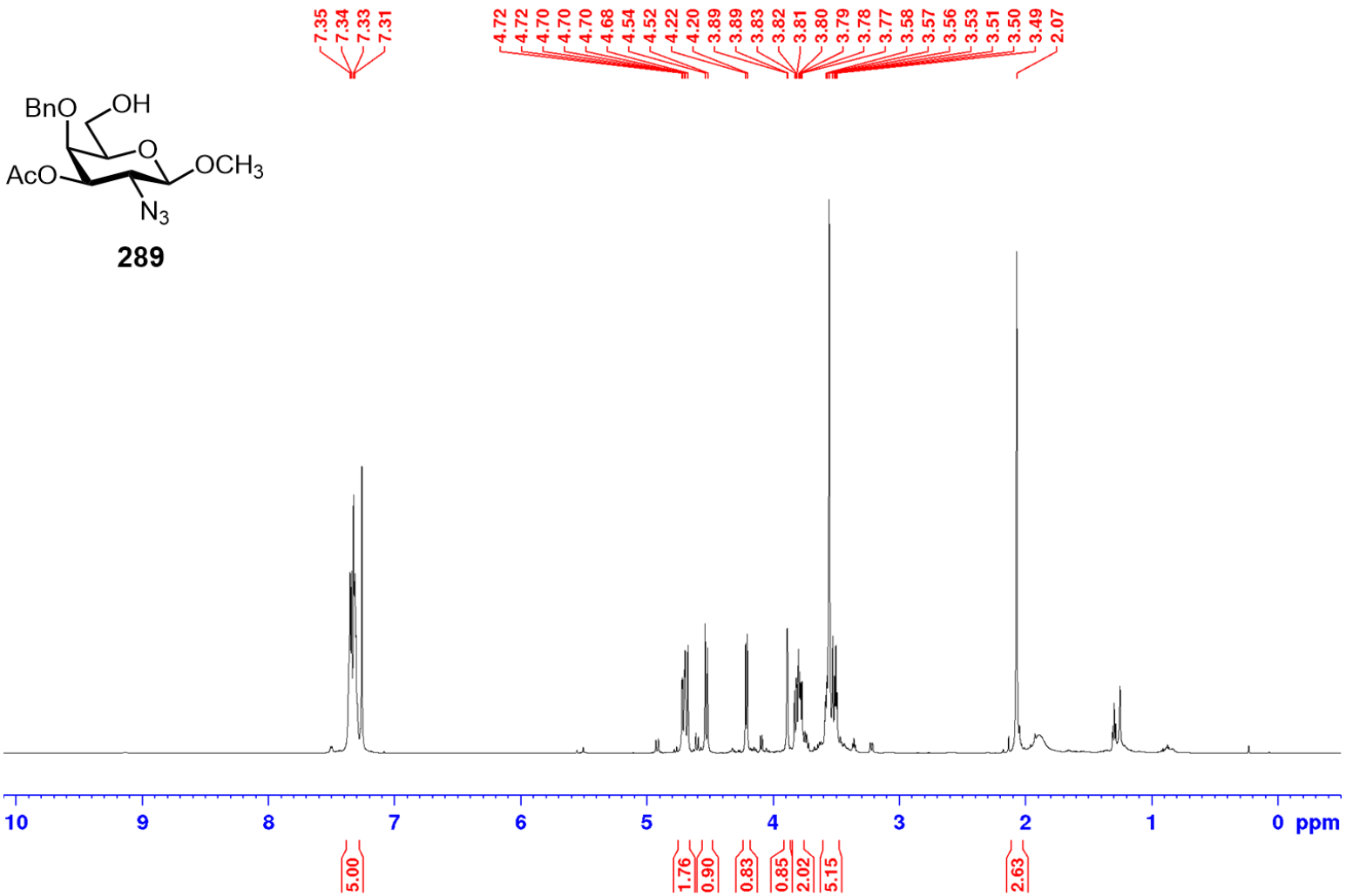
Methyl 4-*O*-benzyl-3-*O*-acetyl-2-deoxy-2-azido-β-D-galactopyranoside (289)



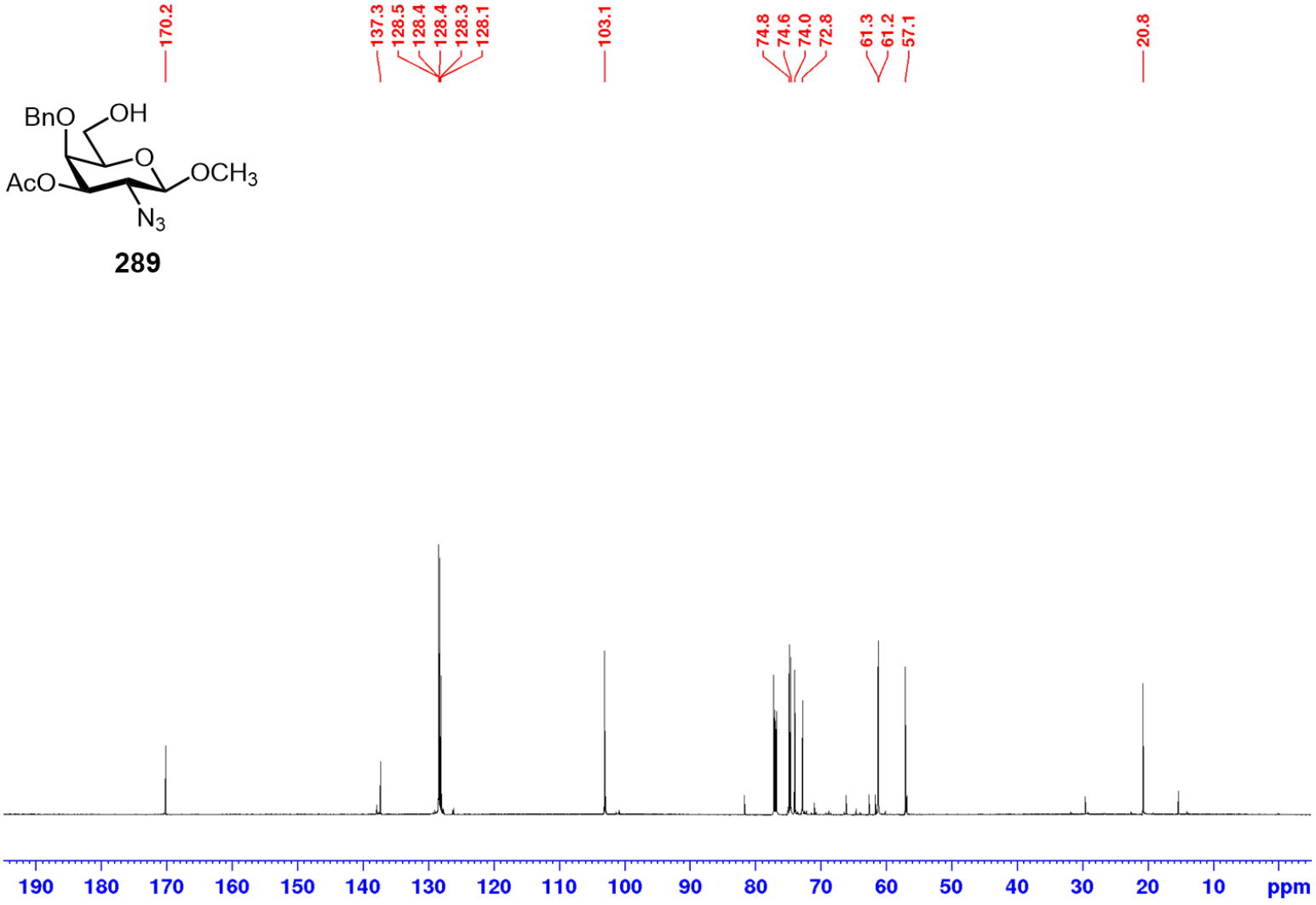
A solution methyl 4,6-*O*-benzylidene acetal-3-*O*-acetyl-2-deoxy-2-azido-β-D-galactopyranoside **288** (1.0 equiv., 3.756 g, 10.75 mmol) in anhydrous CH_2Cl_2 (54.0 mL) was added BH_3·THF (2.5 equiv., 26.9 mL, 26.87 mmol) and TMSOTf in anhydrous CH_2Cl_2 (0.25 equiv., 0.48 mL, 2.68 mmol) at 0 °C under an argon atmosphere. The reaction was stirred at room temperature for 3 h. The reaction mixture was quenched by the addition of anhydrous CH_3OH (10 equiv., 4.4 mL, 107.51 mmol) and Et_3N (0.50 equiv., 0.75 mL, 5.37 mmol) at 0 °C. The reaction was stirred at room temperature for 5 min. The crude reaction mixture was concentrated to an oil then co-evaporated with methanol to remove the residual borate esters. The crude reaction mixture was purified by silica gel flash column chromatography (15:85 to 30:70 EtOAc/hexanes) to give methyl 4-*O*-benzyl-3-*O*-acetyl-2-deoxy-2-azido-β-D-galactopyranoside **289** (3.274 g, 9.317 mmol, 87%) as a white amorphous solid. R_f = 0.35 (20:80 EtOAc/hexanes) visualized with ceric ammonium molybdate stain; $[\alpha]D^{25}$ = -4.12° (c = 1.0, $CHCl_3$); IR ν_{max} (film) 3435.27, 2926.26, 2109.34, 1745.11, 1646.37, 1496.36, 1453.21, 1372.12, 1316.53, 1227.19, 1133.42, 1109.16, 1048.50, 988.26., 906.23, 888.15, 740.73, 698.21, 475.21, 418.44 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.37-7.28 (m, 5H), 4.72-4.67 (m, 2H), 5.48 (s, 1H), 4.53 (d, *J* = 11.5 Hz, 1H), 4.21 (d, *J* = 7.8 Hz, 1H, C-1), 3.88 (d, *J* = 2.5 Hz, 1H), 3.83-3.76 (m, 2H), 3.58-3.49

(m, 5H), 2.07 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.2, 137.3, 128.4 (x2), 128.3, 128.1, 103.1, 74.8, 74.6, 74.0, 72.8, 61.3, 61.2 (x2), 57.1, 20.8.

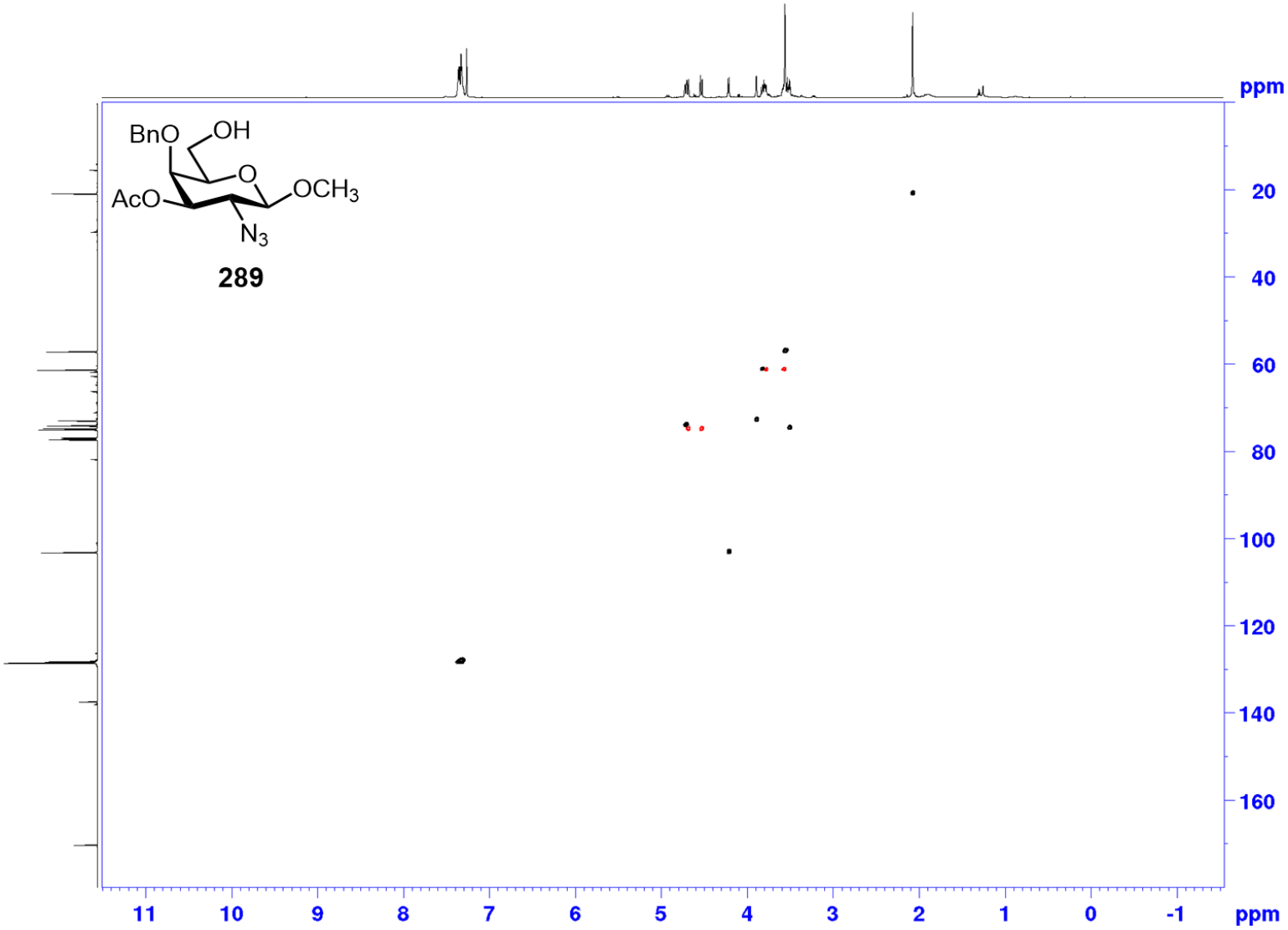
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 289



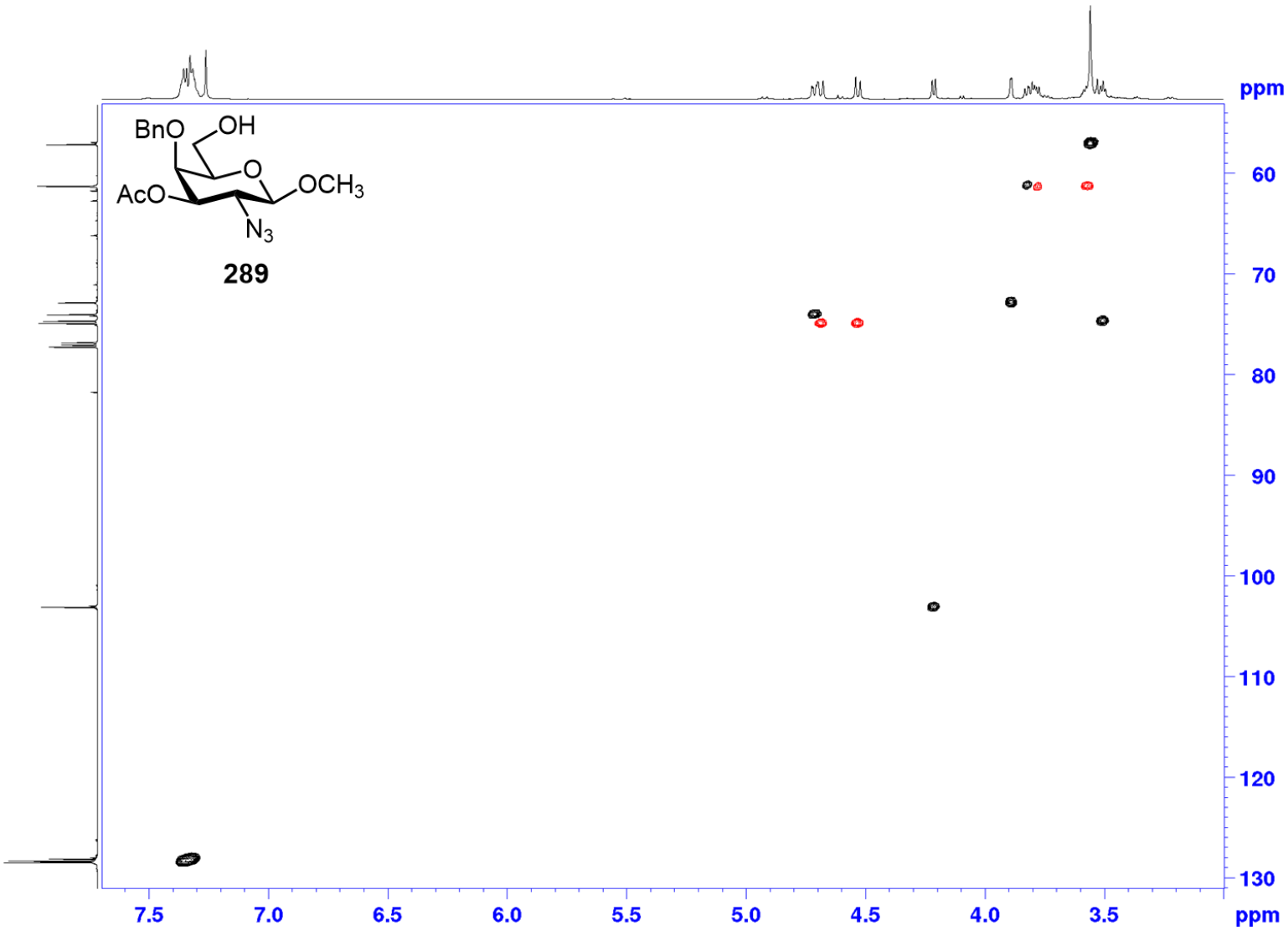
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 289



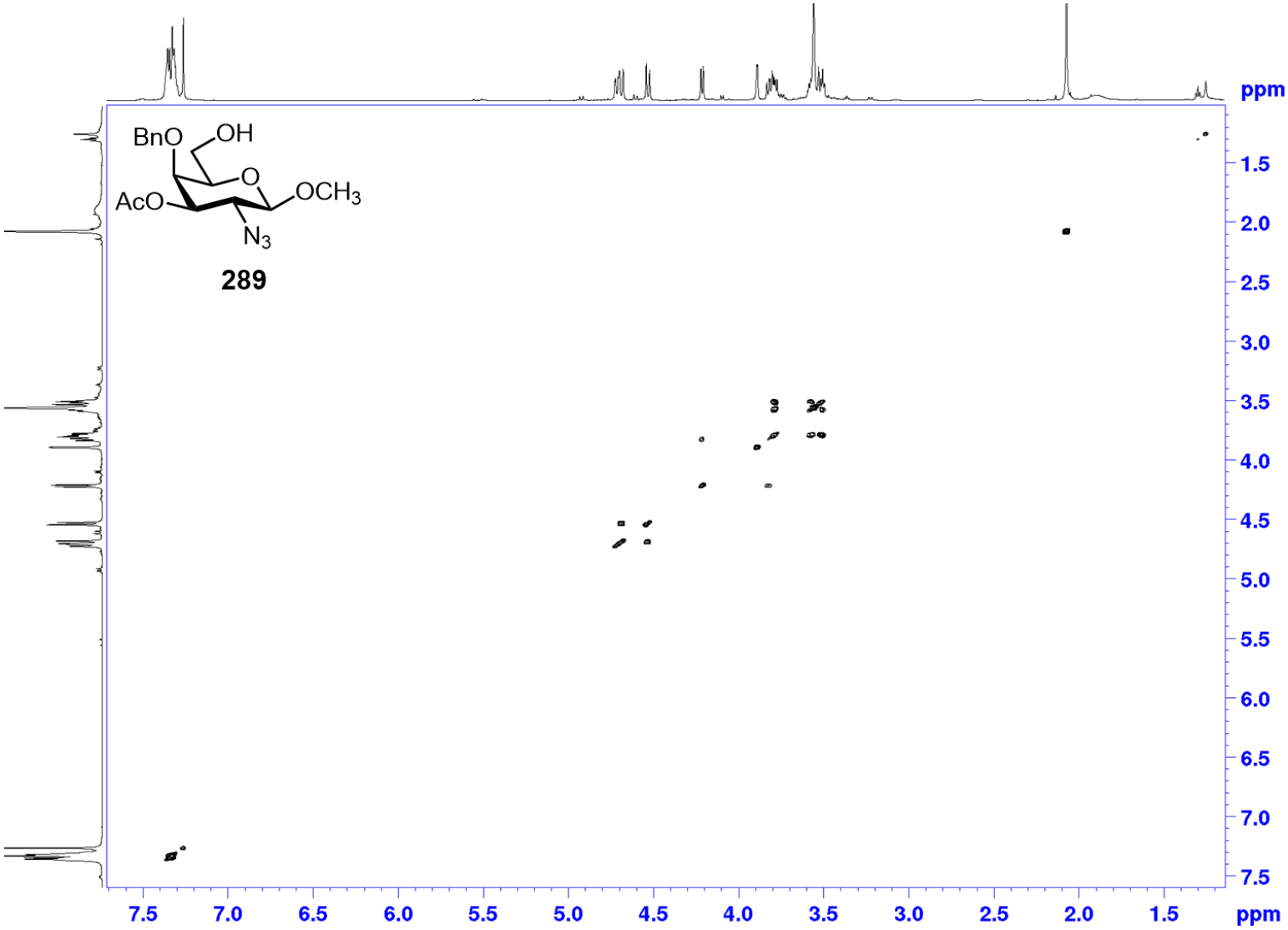
^{1}H-^{13}C HSCQ ($CDCl_3$) of compound 289

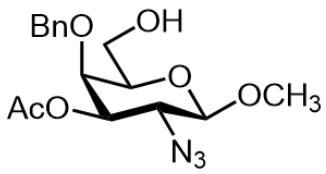


^{1}H-^{13}C HSCQ ($CDCl_3$) zoomed in region of compound 289

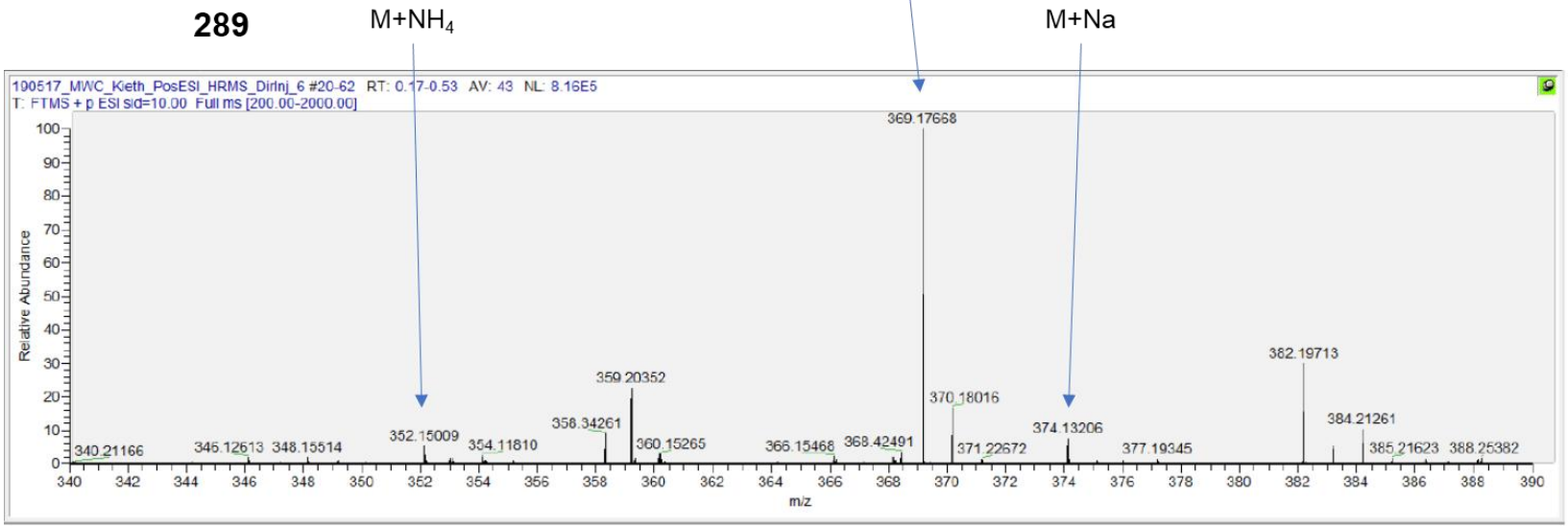


^{1}H-^{1}H COSY ($CDCl_3$) of compound 289

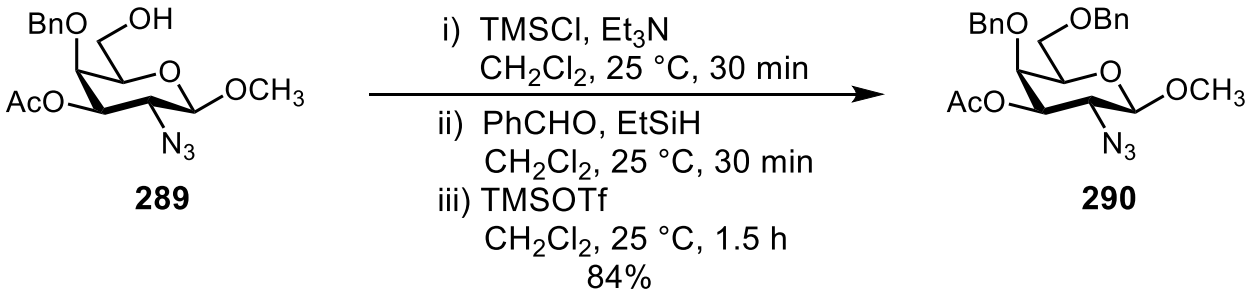



BnO
OH
O
AcO
OCH3
N3

289


M+NH4
M+NH4
M+Na
190517_MWC_Kieth_PosESI_HRMS_DirInj_6 #20-62 RT: 0.17-0.53 AV: 43 NL: 8.16E5
T: FTMS + p ESI sid=10.00 Full ms [200.00-2000.00]
Relative Abundance
m/z
369.17668
382.19713
359.20352
370.18016
384.21261
374.13206
358.34261
352.15009
354.11810
360.15265
366.15468
368.42491
371.22672
377.19345
385.21623
388.25382
340.21166
345.12613
348.15514

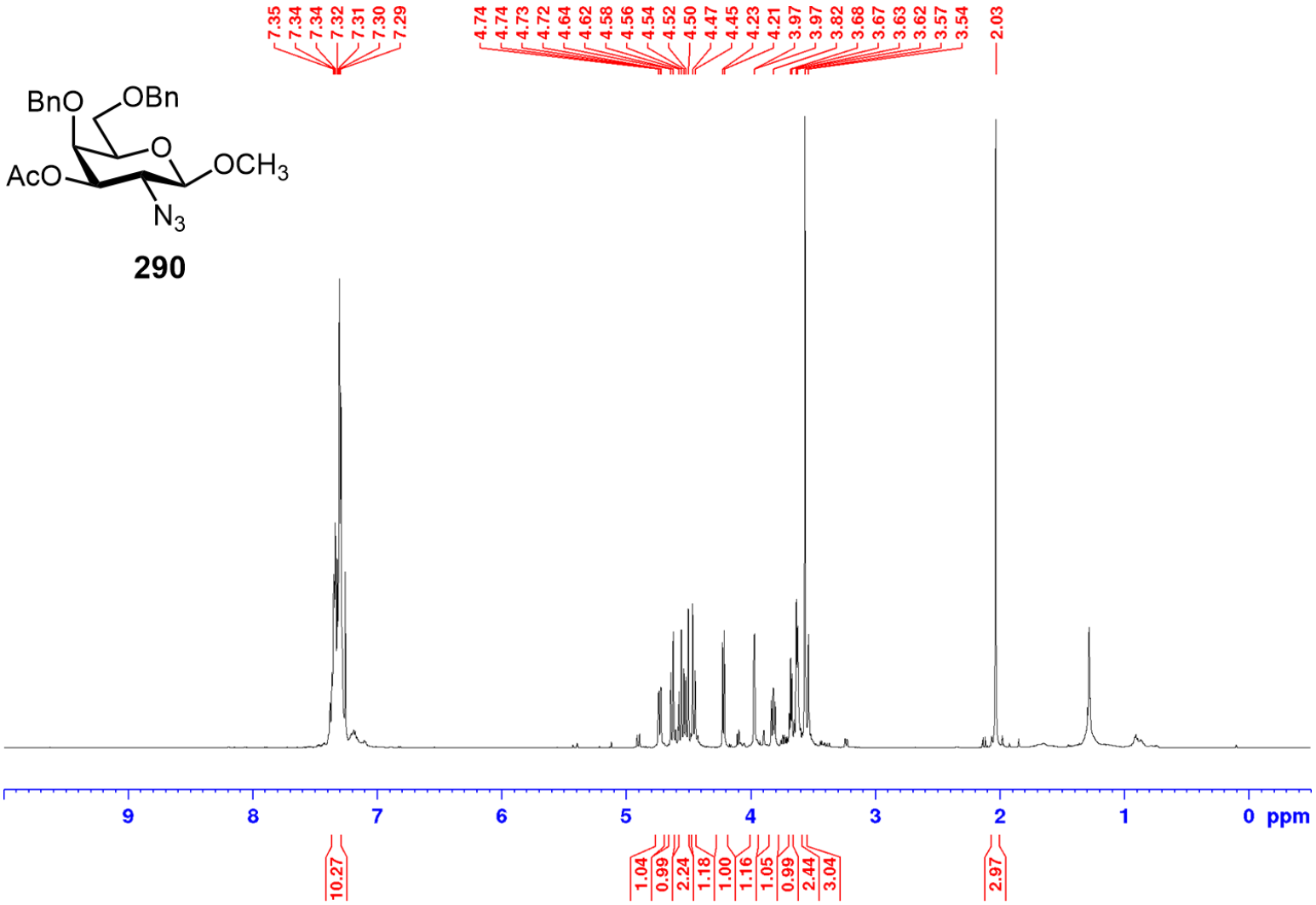
Methyl 4,6-di-*O*-benzyl-3-*O*-acetyl-2-deoxy-2-azido-β-D-galactopyranoside (290)



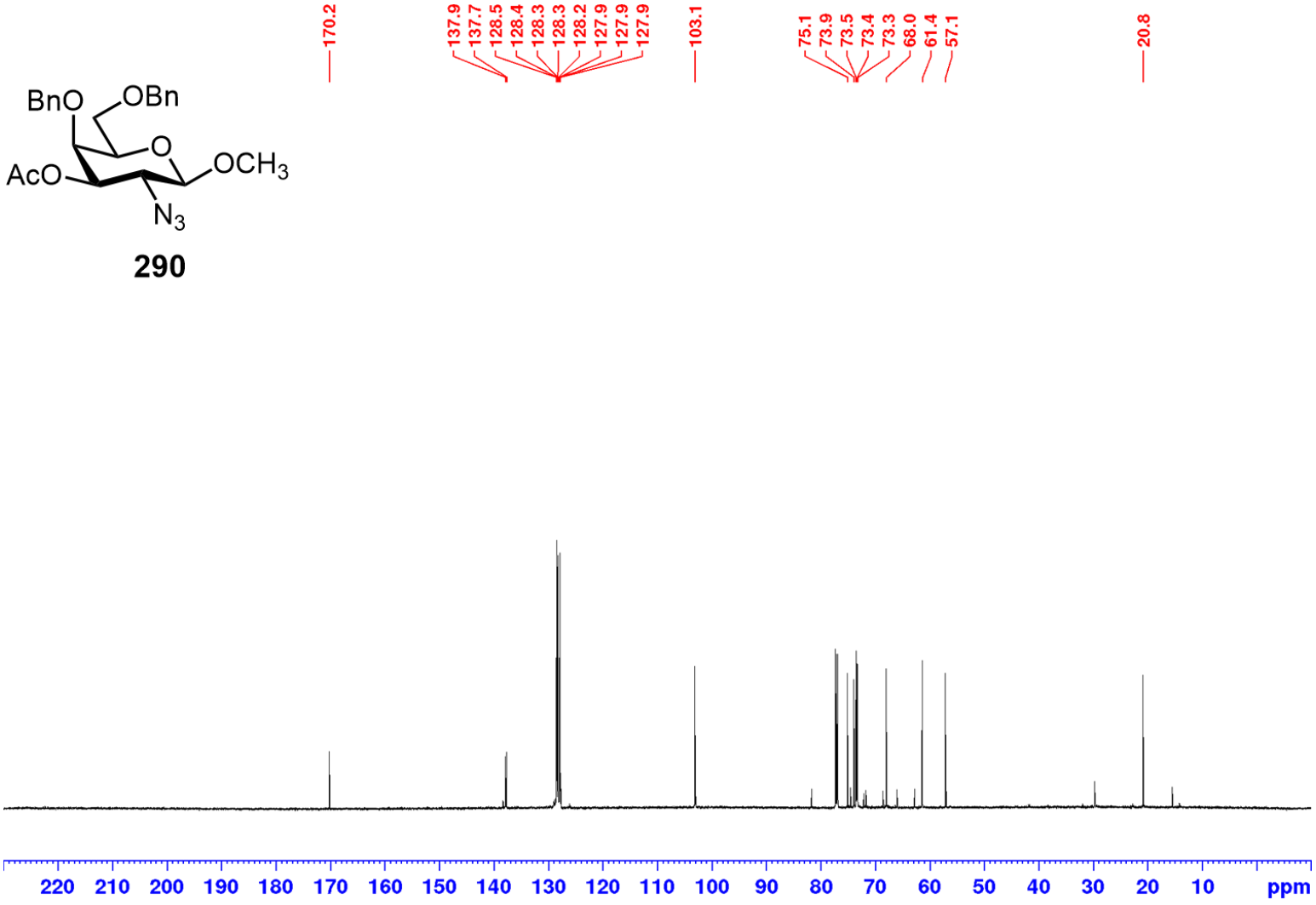
The reaction was carried out following adapted procedures developed by Hung and co-workers.[338] A solution of methyl 4-*O*-benzyl-3-*O*-acetyl-2-deoxy-2-azido-β-D-galactopyranoside **289** (1.0 equiv., 1.307 g, 3.720 mmol) and TMS-Cl (2.0 equiv., 0.95 mL, 7.440 mmol) in anhydrous CH_2Cl_2 (37 mL) was added Et_3N (4.0 equiv., 2.10 mL, 14.88 mmol) dropwise, at room temperature, under an argon atmosphere. At 30 min, the reaction mixture was added benzaldehyde (4.0 equiv., 1.52 mL, 14.88 mmol), Et_3Si-H (1.5 equiv., 0.89 mL, 5.580 mmol) and TMS-OTf (0.15 equiv., 0.101 mL, 0.558 mmol) dropwise. At 1.5 h, the crude reaction mixture was quenched by the addition of Et_3N (0. 25 equiv., 0.13 mL, 0.930 mmol) and concentrated to an oil. The crude oil was purified by silica gel flash column chromatography (20:80 to 30:70 EtOAc/hexanes) to give methyl 4,6-di-*O*-benzyl-3-*O*-acetyl-2-deoxy-2-azido-β-D-galactopyranoside **290** (1.379 g, 3.125 mmol, 84%) as a white amorphous solid. R_f = 0.5 (1:1 EtOAc/hexanes) visualized with ceric ammonium molybdate stain; $[\alpha]D^{25}$ = +100.28° (c = 1.0, $CHCl_3$); IR ν_{max} (film) 3435.37, 2926.16, 2109.54, 1745.31, 1646.77, 1496.16, 1453.41, 1372.42, 1316.73, 1227.89, 1133.22, 1109.86, 1048.20, 988.66., 906.23, 888.95, 740.73, 698.81, 475.61, 418.41 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.38-7.27 (m, 10H), 4.73 (dd, *J* = 2.6, 11.1 Hz, 1H), 4.63 (d, *J* = 11.6 Hz, 1H), 4.57 (m, 2H), 4.45 (d, *J* = 11.8 Hz, 1H), 4.22 (d, *J* = 7.9 Hz, 1H), 3.97 (d, *J* = 2.3 Hz, 2H), 3.82 (dd, *J* = 8.3, 10.3 Hz, 1H), 3.69-3.66 (m, 1H), 3.64-

3.61 (m, 2H), 3.56 (s, 3H), 2.03 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.2, 137.9, 137.7, 128.5 (x2), 128.4, 128.3, 128.2 (x2), 128.0, 127.9 (x2), 127.8, 103.1, 75.1, 73.9, 73.5, 73.4, 73.4, 61.3, 68.0, 57.2, 61.5, 57.2, 20.9 HRMS (ESI) calcd for $C_{23}H_{31}O_6N_4^+$ $(M+NH_4)^+$, 459.22382 found 459.22384.

^{1}H NMR ($CDCl_3$, 400 MHz) of compound 290

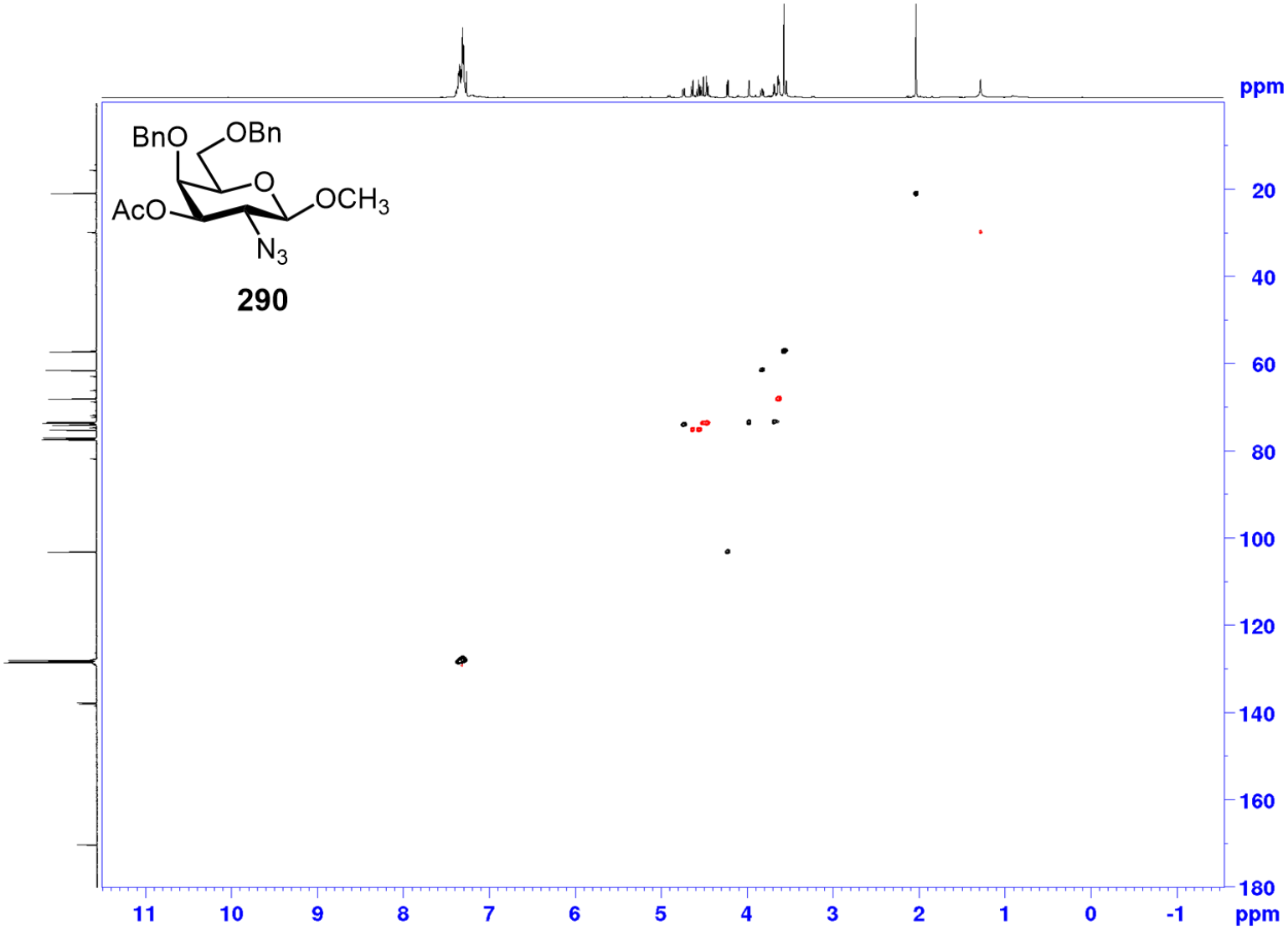


^{13}C NMR ($CDCl_3$, 100 MHz) of compound 290

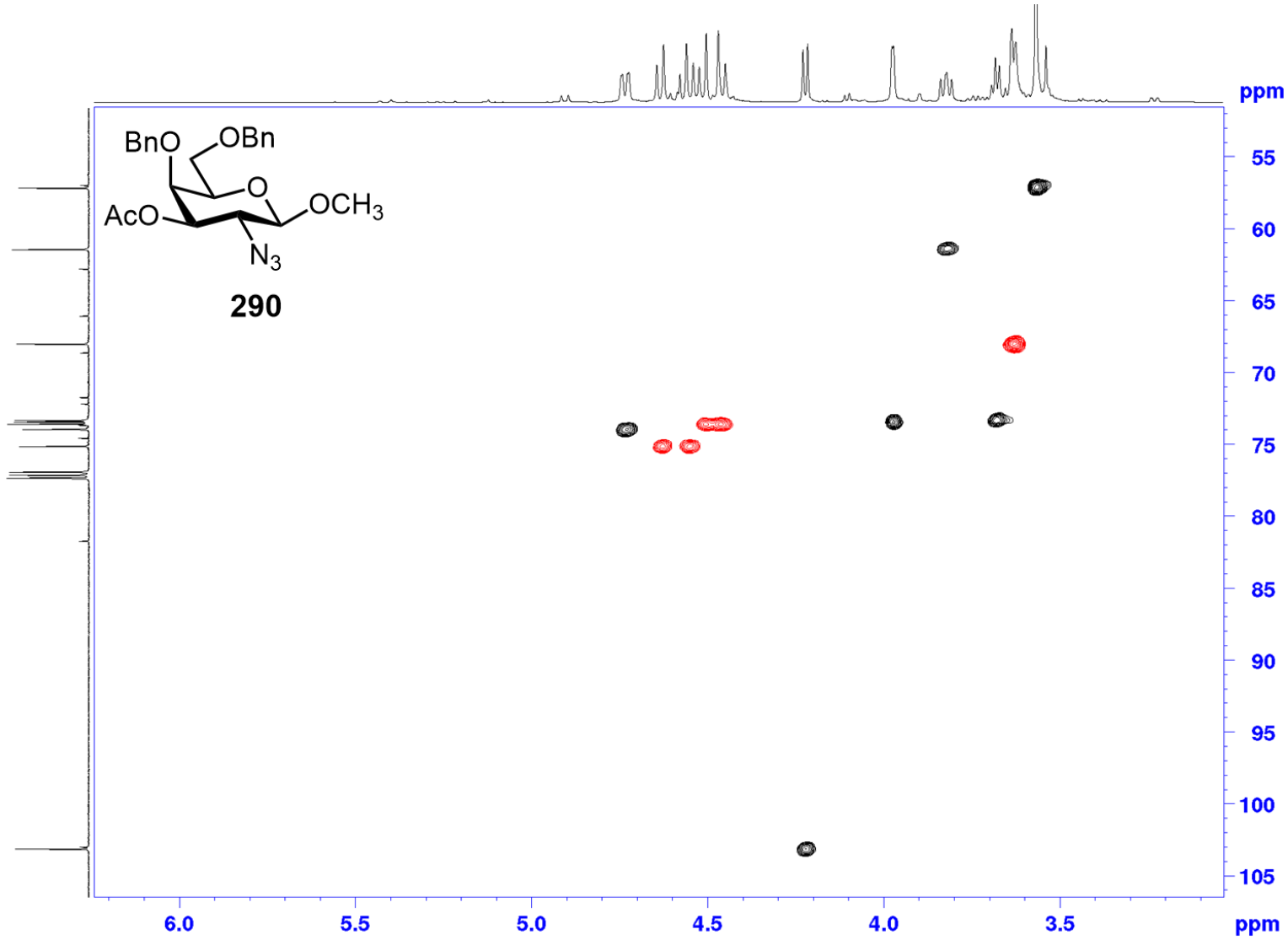


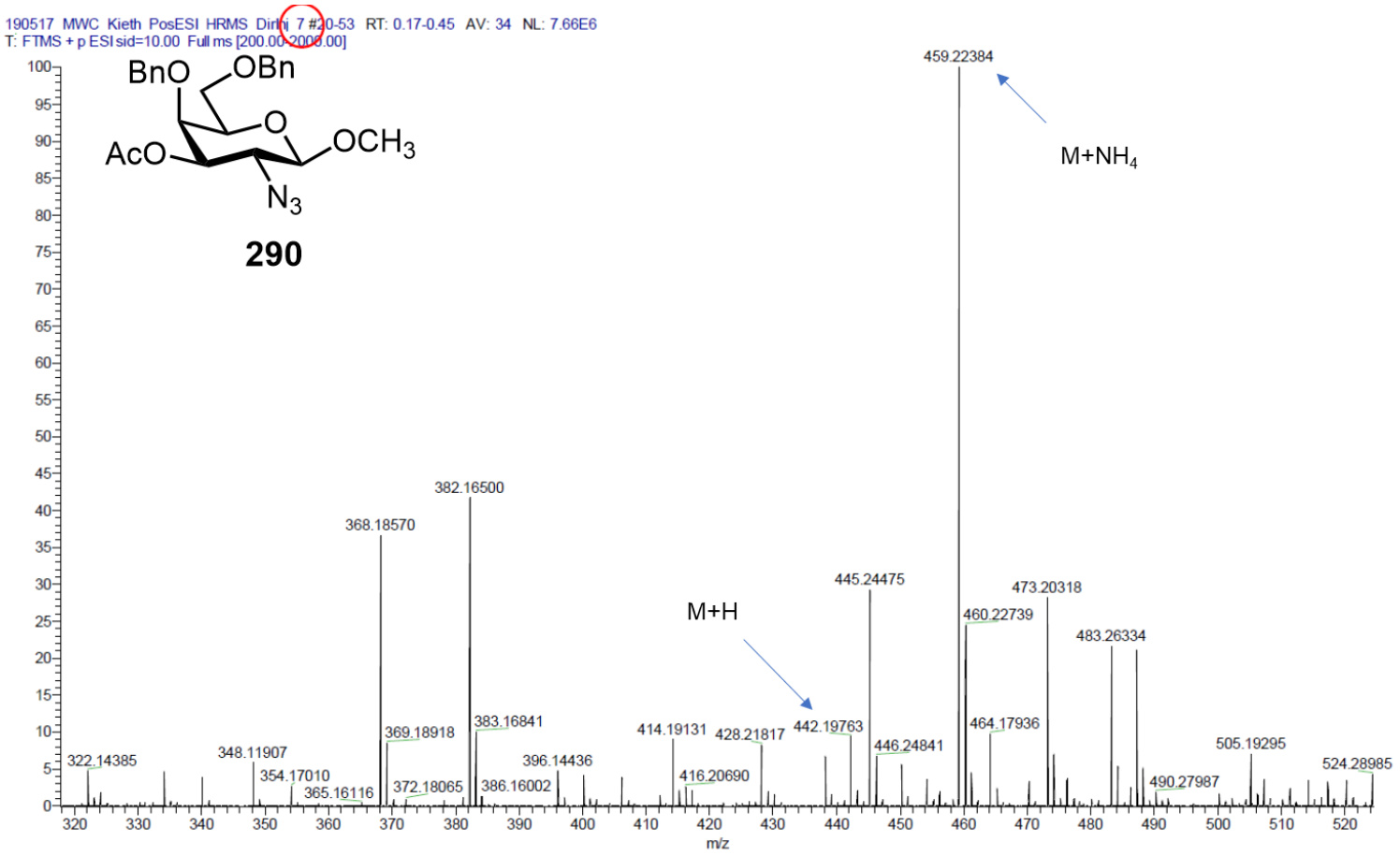
290

^{1}H-^{13}C HSCQ ($CDCl_3$) of compound 290

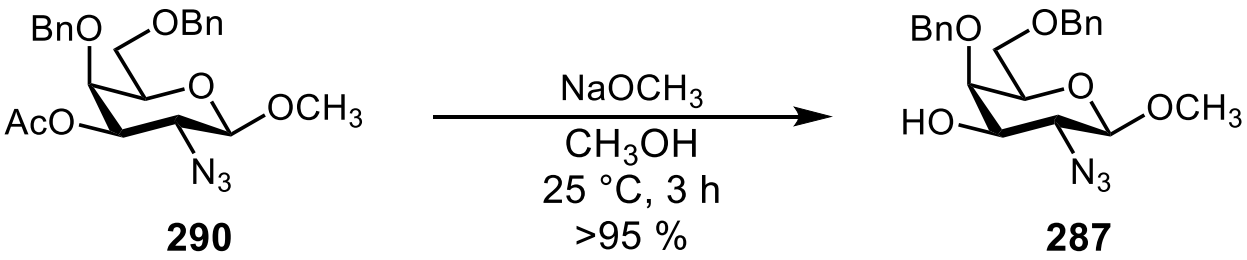


^{1}H-^{13}C HSCQ ($CDCl_3$) zoomed in region of compound 290




190517 MWC Kieth PosESI HRMS Dirtnj 7 #20-53 RT: 0.17-0.45 AV: 34 NL: 7.66E6
T: FTMS + p ESI sid=10.00 Full ms [200.00-2000.00]
BnO
OBn
O
AcO
OCH3
N3
290
459.22384
M+NH4
M+H
382.16500
368.18570
445.24475
473.20318
460.22739
483.26334
383.16841
369.18918
442.19763
414.19131
428.21817
464.17936
446.24841
505.19295
524.28985
322.14385
348.11907
354.17010
365.16116
372.18065
386.16002
396.14436
416.20690
490.27987
m/z

Methyl 4,6-di-*O*-benzyl-2-deoxy-2-azido-β-D-galactopyranoside (287)

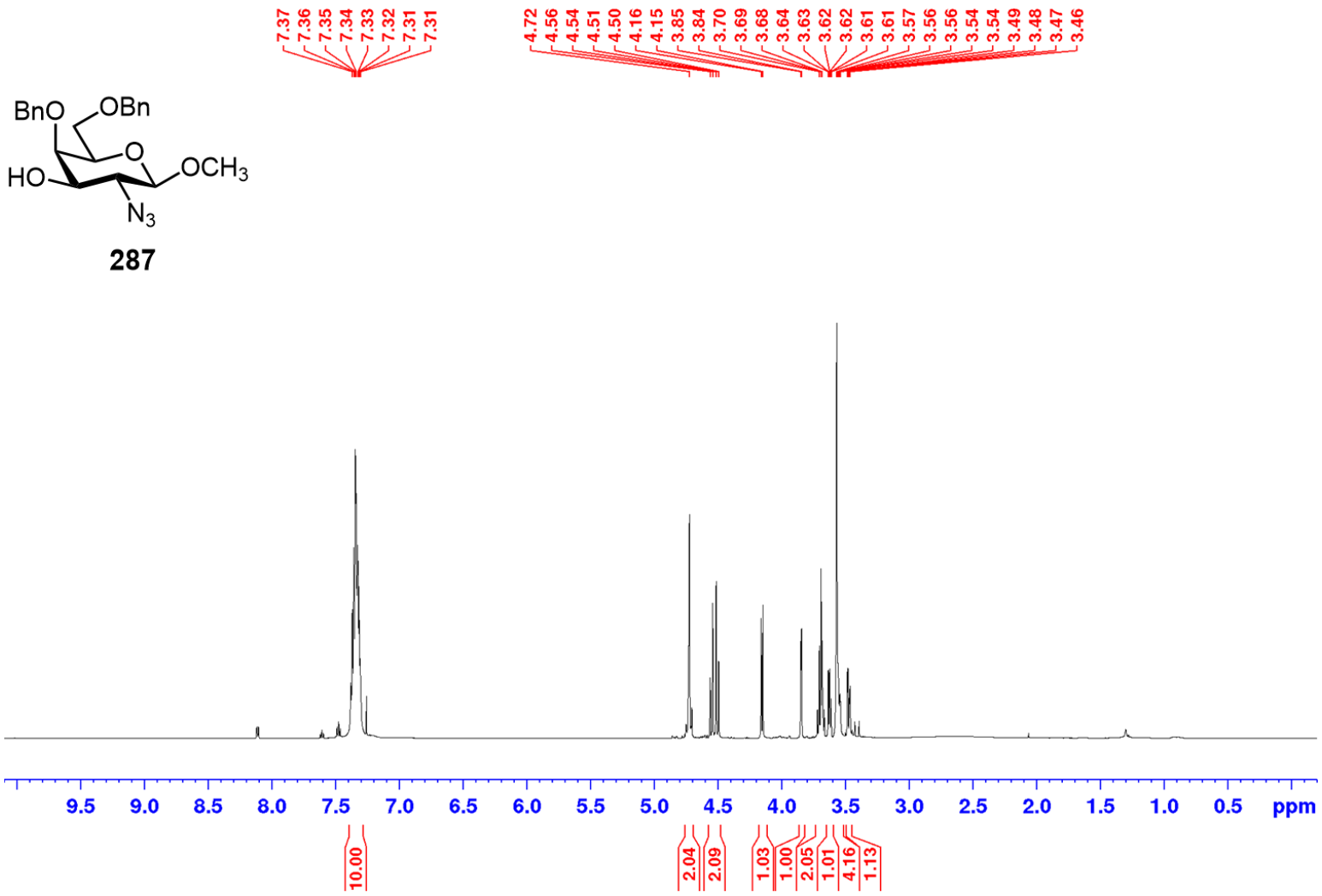


290 **287**

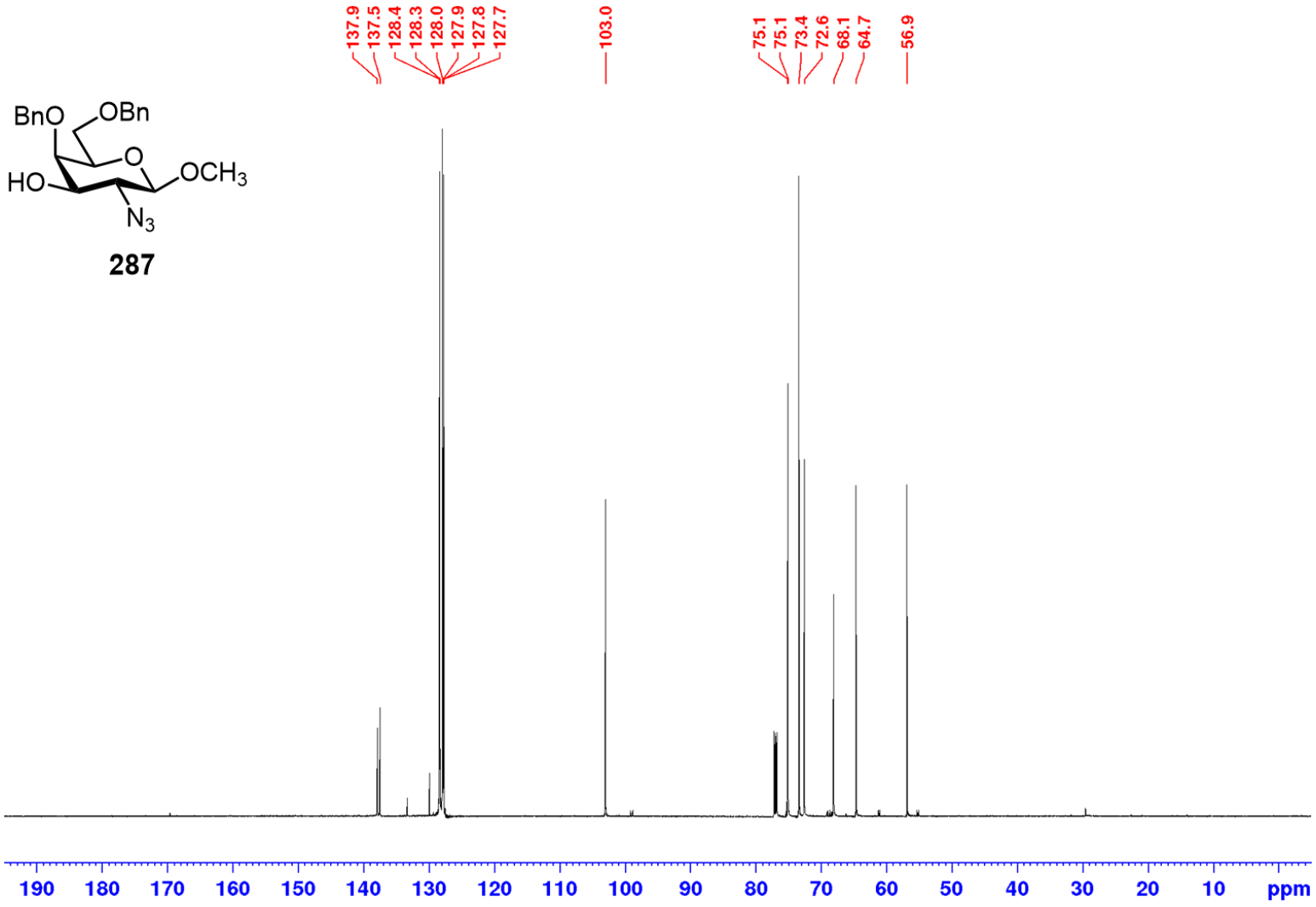
A solution of methyl 4,6-di-*O*-benzyl-3-*O*-acetyl-2-deoxy-2-azido-β-D-galactopyranoside **290** (1.0 equiv., 1.379 g, 3.125 mmol) in anhydrous CH_3OH (31 mL) was treated with a freshly made solution of sodium methoxide (0.5 equiv., 0.93 mL, 1.86 mmol, [2 N] in CH_3OH). The pH of the solution was >11. At 1 h, the reaction was neutralized by the addition of acidic Amberlite IR 120 (H^+) ion exchange resin. The solution was filtered through a glass fritted vacuum filter funnel equipped with a pad of Celite to remove the acidic resin. The filtrate was concentrated to dryness to give a crude triol. The crude material was purified by silica gel flash column chromatography (15:85 to 40:60 EtOAc/hexanes) to give methyl 4,6-di-*O*-benzyl-2-deoxy-2-azido-β-D-galactopyranoside **287** (1.358 g, 3.400 mmol, >95%) as a white solid. Our spectroscopic data agrees with previously reported data.[340-344]

^1H NMR (400 MHz, $CDCl_3$): δ 7.37-7.31 (m, 10H), 4.72-4.70 (m, 2H), 4.55 (d, *J* = 12.1 Hz, 1H), 4.5 (d, *J* = 11.6 Hz, 1H), 4.15 (d, *J* = 8.1 Hz, 1H), 3.84 (d, *J* = 3.4 Hz, 1H), 3.71-3.67 (m, 2H), 3.63-3.61 (m, 1H), 3.58-3.53 (m, 4H), 3.47 (dd, *J* = 3.1, 10.2 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 137.9, 137.5, 128.4, 128.3, 128.0, 127.9, 127.8, 127.7, 103.0, 75.1, 75.1, 73.4, 72.6, 68.1, 64.7, 56.9.

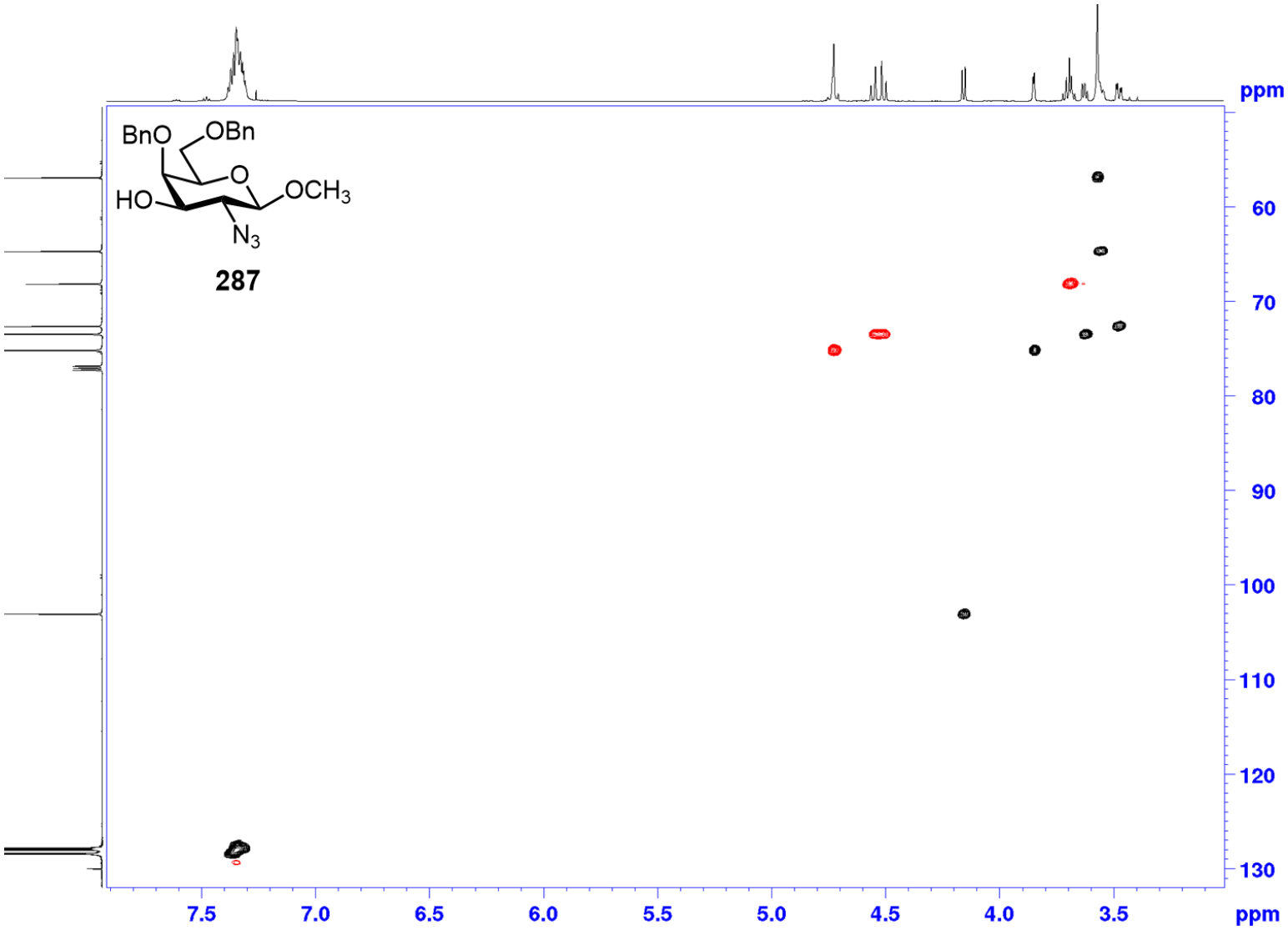
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 287



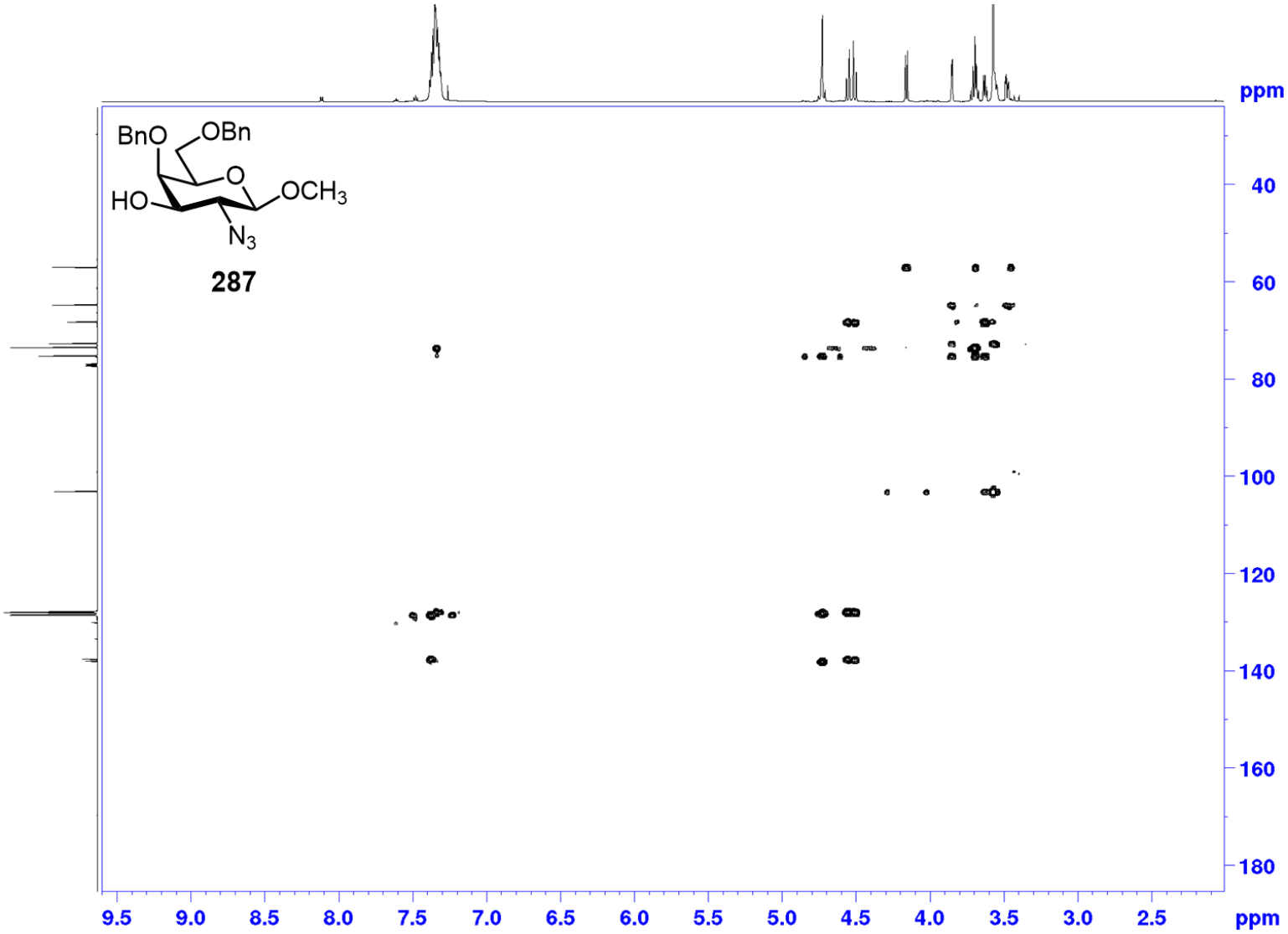
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 287



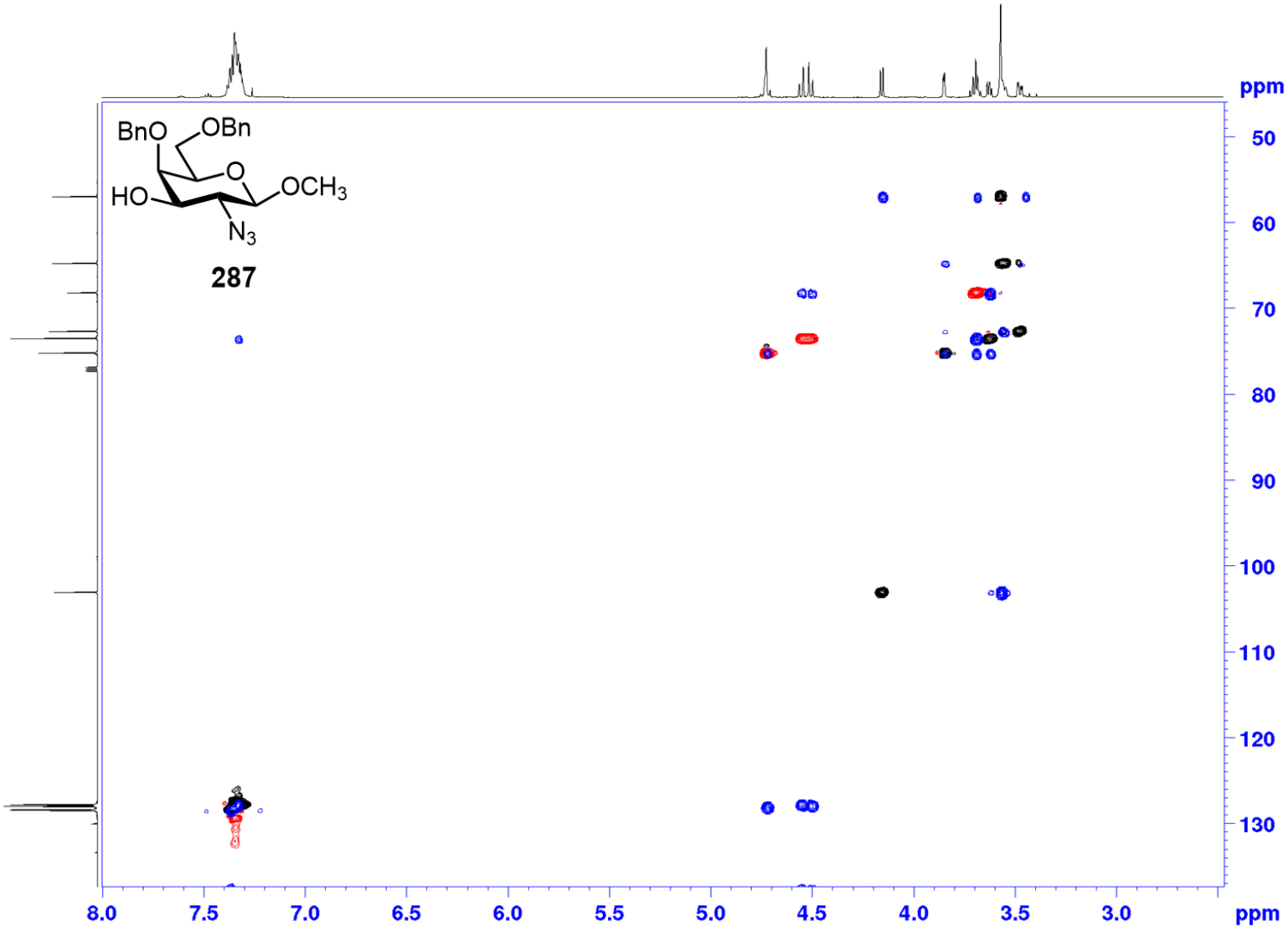
^{1}H-^{13}C HSCQ ($CDCl_3$) of compound 287



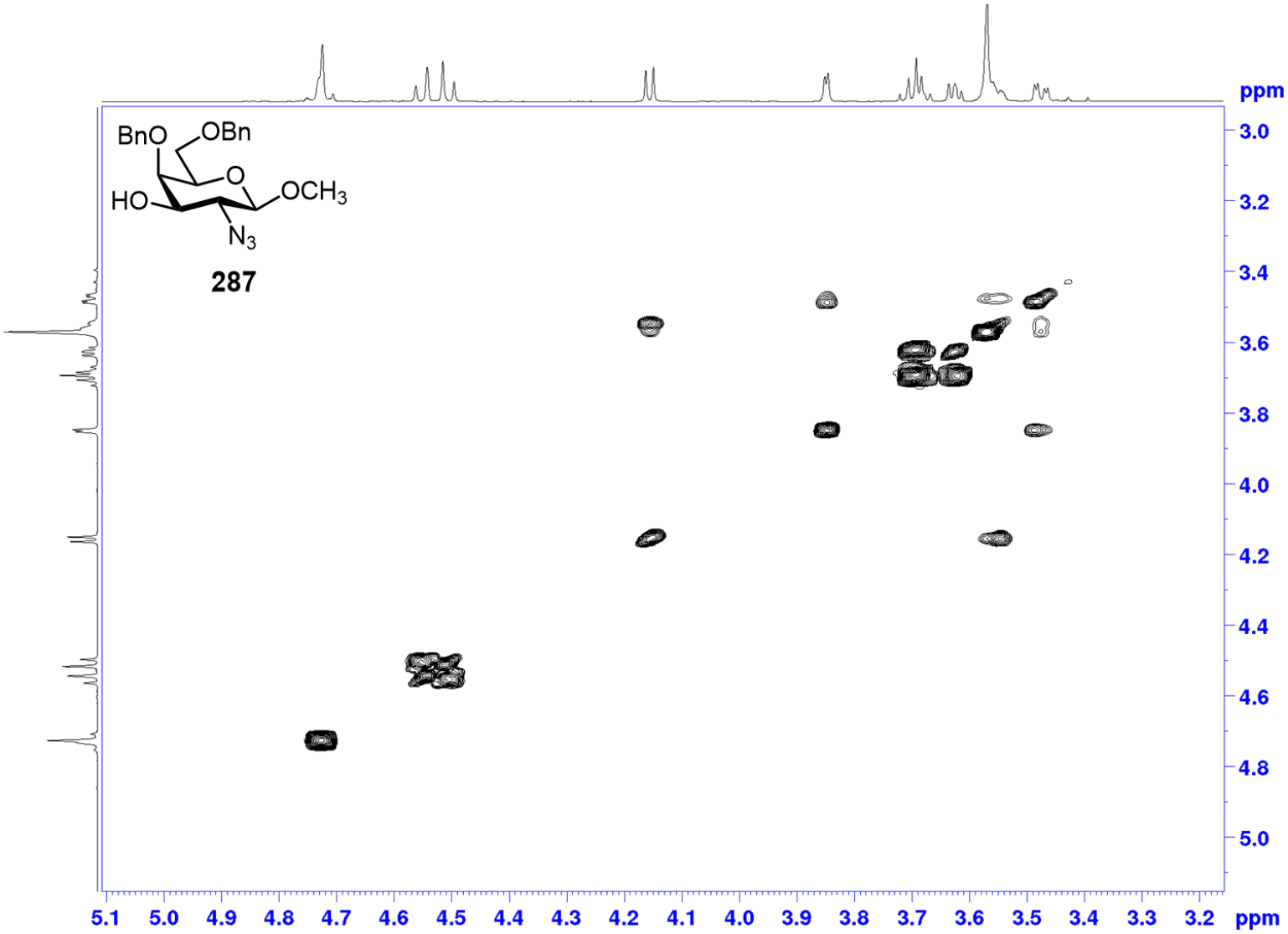
^{1}H-^{13}C HMBC ($CDCl_3$) of compound 287



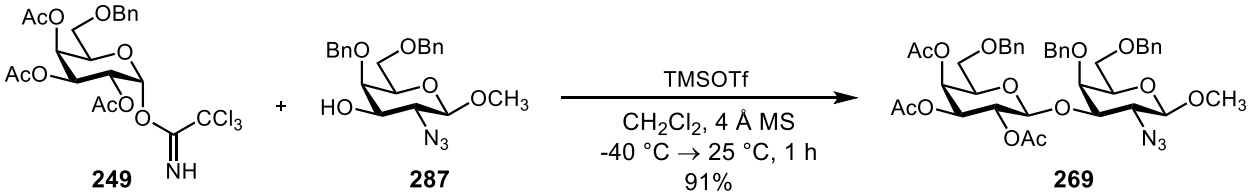
^{1}H-^{13}C HSCQ with ^{1}H-^{13}C HMBC overlay ($CDCl_3$) of compound 287



^{1}H-^{1}H COSY ($CDCl_3$) of compound 287



Methyl 6-*O*-benzyl-2,3,4-*O*-aceyl-β-D-galactopyranosyl-(1→3)-4,6-di-*O*-benzyl-2-deoxy-2-azideo-β-D-galactopyranoside (269)

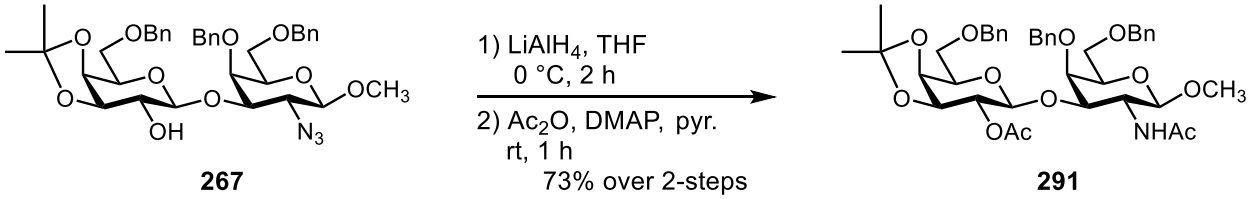


A solution of previously crystalized methyl 4,6-di-*O*-benzyl-2-deoxy-2-azido-β-D-galactopyranoside acceptor **287** (1.0 equiv., 2.24 g, 5.61 mmol) in anhydrous CH_2Cl_2 (50.0 mL) and previously crystalized 2,3,4-tri-*O*-acety-6-*O*-benzy-α-D-galactopyranosyl trichloroacetimidate donor **249** (1.5 equiv., 4.55 g, 8.41 mmol) was stirred over 4 Å molecular sieves (7.5 g, ~15% m/v) at room temperature for 12 hours. The solution was then cooled to -40 °C, treated with TMSOTf (0.5 equiv., 0.51 mL, 2.80 mmol). The reaction mixture was stirred for 1 h while warming to room temperature. The reaction was neutralized by the addition of Et_3N (0.5 equiv., 0.40 mL, 2.80 mmol), passed through a glass fritted vacuum filter funnel equipped with a pad of Celite to remove the molecule sieves. The filtrate was concentrated and subjected to silica gel flash column chromatography (20:80 to 50:50 EtOAc/hexanes) to give methyl 6-*O*-benzyl-2,3,4-*O*-aceyl-β-D-galactopyranosyl-(1→3)-4,6-di-*O*-benzyl-2-deoxy-2-azideo-β-D-galactopyranoside **269** (3.97 g, 5.61 mmol, 91%) as a white amorphous solid. R_f = 0.60 (1:3 EtOAc/hexanes) visualized with ceric ammonium molybdate stain; $[\alpha]D^{25}$ = -16.12° (c = 1.0, $CHCl_3$); IR ν_{max} (film) 3568.07, 2113.74, 1750.16, 1454.65, 1368.11, 1220.26, 1071.99, 736.32, 699.42cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.37-7.23 (m, 15H), 5.49 (d, *J* = 3.1 Hz, 1H), 5.28 (dd, *J* = 7.9, 10.4 Hz, 1H), 5.07 (dd, *J* = 3.4, 10.4 Hz, 1H), 4.93 (d, *J* = 11.5 Hz, 1H), 4.77 (d, *J* = 7.8 Hz, 1H), 4.64 (d, *J* = 11.6 Hz, 1H), 4.52 (d, *J* = 11.9 Hz,

1H), 4.43-4.36 (m, 3H), 4.11 (d, *J* = 8.0 Hz, 1H), 3.90-3.8 (m, 2H), 3.70 (dd, *J* = 8.0, 10.4 Hz, 1H), 3.-3.49 (m, 9H), 2.11 (S, 3H), 2.07 (S, 3H), 2.00 (S, 3H);^{13}C NMR (100 MHz, $CDCl_3$): δ 170.1, 170.1, 169.6, 138.4, 137.9, 137.5, 128.9,128.9, 128.5, 128.5, 128.4, 128.4, 128.1, 128.1, 128.0, 127.9, 127.8, 127.8, 127.8, 127.8, 127.7, 103.1, 102.3, 80.8, 74.3, 74.3, 73.7, 73.6, 73.5, 72.2, 71.0, 69.2, 69.0, 67.6, 67.5, 63.7, 57.0, 20.7, 20.7, 20.6; LRMS (ESI) calcd for $C_{40}H_{47}N_3O_{13}Na^+$ $(M+Na)^+$ 800.29918, found 800.23 m/z.

For NMR spectra *vide supra.*

Methyl 6-*O*-benzyl-*O*-3,4-isopropylidene-2-*O*-acetyl-β-D-galactopyranosyl-(1→3)-4,6-di-*O*-benzyl-2-deoxy-2-acetamido-β-D-galactopyranoside (291)

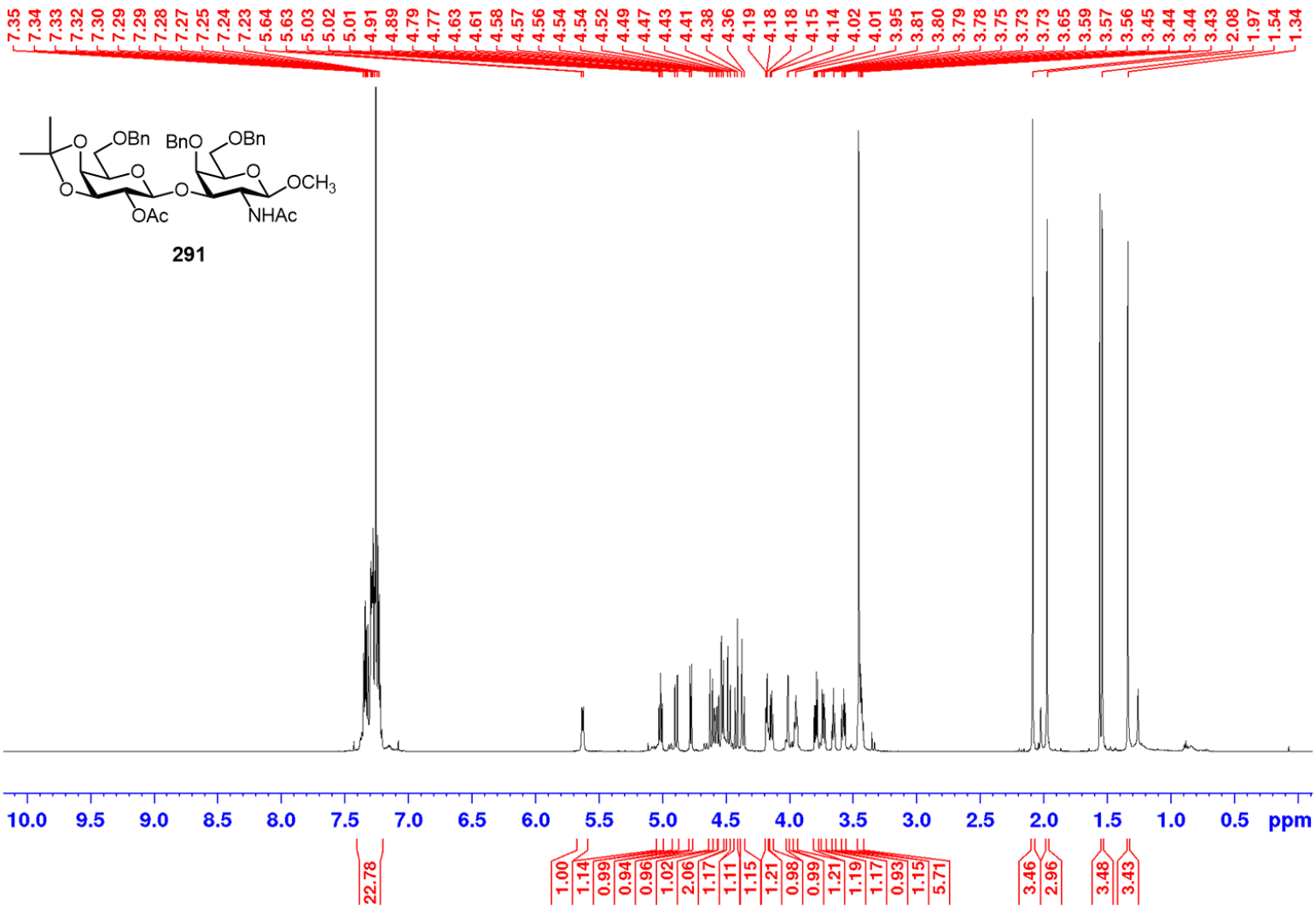


267 **291**

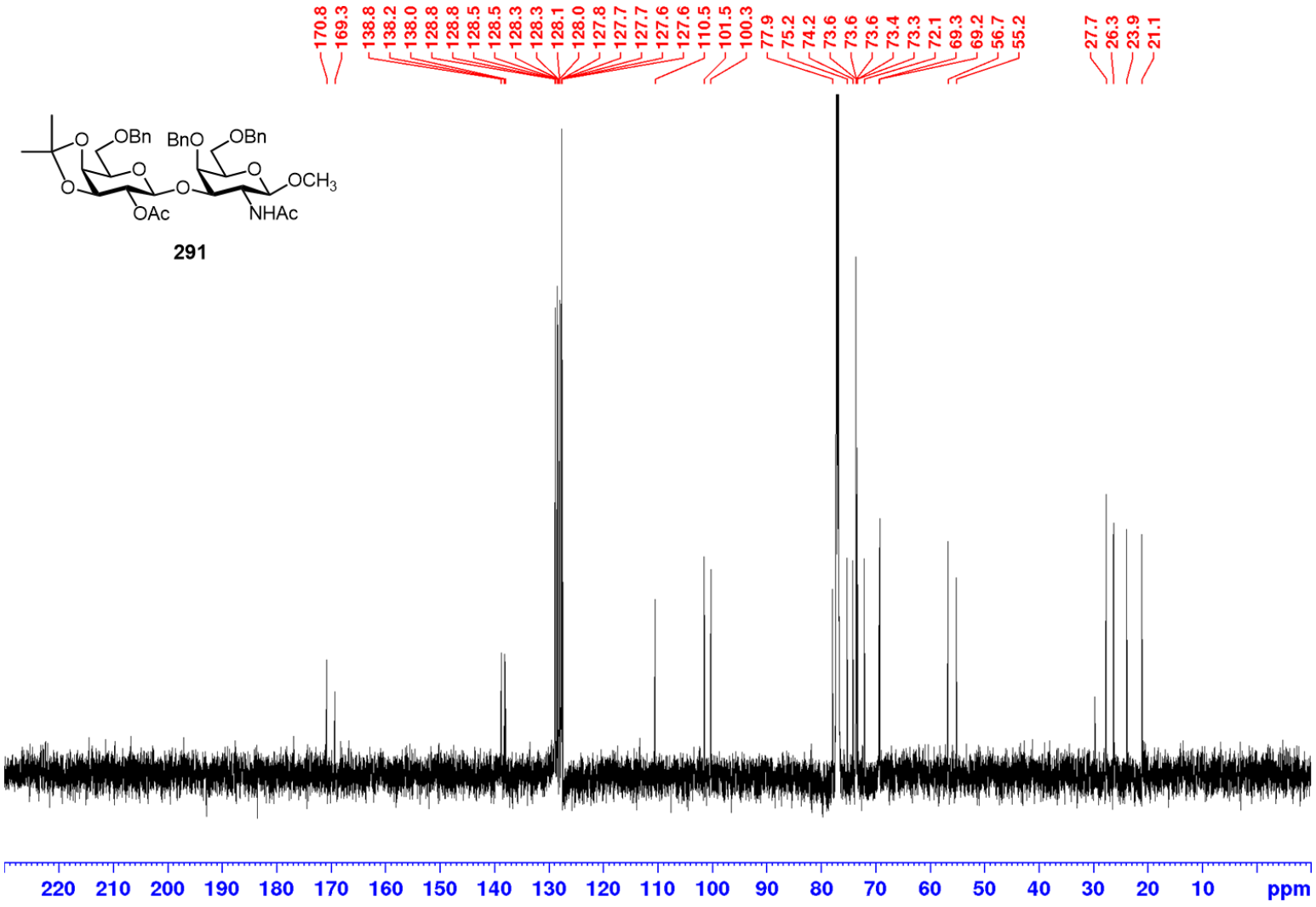
A soltution of methyl 6-*O*-benzyl-*O*-3,4-isopropylidene-β-D-galactopyranosyl-(1→3)-4,6-di-*O*-benzyl-2-deoxy-2-azideo-β-D-galactopyranoside **267** (1.0 equiv., 2.0124 g, 2.9090 mmol) was diluted with dry THF (30 mL) under an argon atmosphere at -15°C. The reaction was added a solution of lithium aluminum hydride in THF (4.0 equiv., 2.4 mL, 5.81 mmol, [2.4 M]) dropwise and stirred at 0°C. At 2 h, the reaction was cooled to -15°C and added water (0.13 mL) dropwise followed by 15% NaOH aq. (0.13 mL) then water again (0.4 mL) and warmed to room temperature and stirred. After 15 min of stirring at room temperature, the reaction was added $NaSO_4$ to absorb the water which was added to the reaction. The reaction was then filtered through a glass fritted vacuum filter funnel equipped with a pad of Celite. The filtrate was concentrated to dryness. The crude reaction mixture was added Ac_2O (2.5 equiv. 0.68 mL, 7.27 mmol) and pyridine (10 equiv., 3.0 mL, 40.0 mmol) at 0°C under an argon atmosphere. At 5 min, the crude reaction mixture was added DMAP (0.10 equiv.). At 1 h, the reaction was cooled to 0°C and added EtOAc and MeOH to consume the rest of the acetic anhydride. The reaction warmed and stirred at room temperature for 10 min. the reaction was concentrated to a thick oil and taken up in EtOAc and washed with 1N HCl (2x), water (1x), brine (1x), dried ($MgSO_4$), filtered through a glass fritted vacuum filter funnel to remove drying agent, and concentrated to an oil. The reaction was then purified by silica gel flash column

chromatography (75:25 to 100:0 EtOAc/hexanes) to give methyl 6-*O*-benzyl-*O*-3,4-isopropylidene-2-*O*-acetyl-β-D-galactopyranosyl-(1→3)-4,6-di-*O*-benzyl-2-deoxy-2-acetamido-β-D-galactopyranoside **291** (1.585 g, 2.114 mmol, 73%) as a white foam. R_f = 0.45 (10:90 CH_3OH/CH_2Cl_2) visualized with ceric ammonium molybdate stain; ^1H NMR (400 MHz, $CDCl_3$): δ 7.35-.23 (m, 23H), 5.63 (d, *J* = 6.8 Hz, 1H), 5.02 (d, *J* = 7.1 Hz, 1H), 4.9 (d, *J* = 11.9 Hz, 1H), 4.78 (d, *J* = 8.4 Hz, 1H), 4.62 (d, *J* = 11.9 Hz, 1H), 4.59 (dd, *J* = 2.8, 11.2 Hz, 1H), 4.56-4.52 (m, 2H), 4.48 (d, *J* = 12.1 Hz, 1H), 4.42 (d, *J* = 12.2 Hz, 1H), 4.37 (d, *J* = 11.9 Hz, 1H), 4.18 (dd, *J* = 2.1, 5.4 Hz, 1H), 4.14 (dd, *J* = 5.8, 6.1 Hz, 1H), 4.01 (d, *J* = 2.3 Hz, 1H), 3.95 (dd, *J* = 2.1, 6.1 Hz, 1H), 3.81-3.73 (m, 1H), 3.73 (dd, *J* = 7.1, 10.1 Hz, 1H), 3.65 (dd, *J* = 6.1, 6.2 Hz, 1H), 3.59-3.56 (m, 1H), 3.46-3.42 (m, 6H), 2.08 (s, 3H), 1.97 (s, 3H), 1.54 (s, 3H), 1.34 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.7, 169.3, 138.7, 138.1, 138.0, 128.8, 128.8, 128.4, 128.4, 128.3, 128.3, 128.0, 128.0, 127.7, 127.7, 127.7, 127.6, 127.5, 110.5, 101.4, 100.2, 77.9, 75.2, 74.1, 73.6, 73.6, 73.5, 73.3, 73.3, 72.0, 69.3, 69.2, 56.7, 55.2, 27.6, 26.2, 23.8, 21.0.

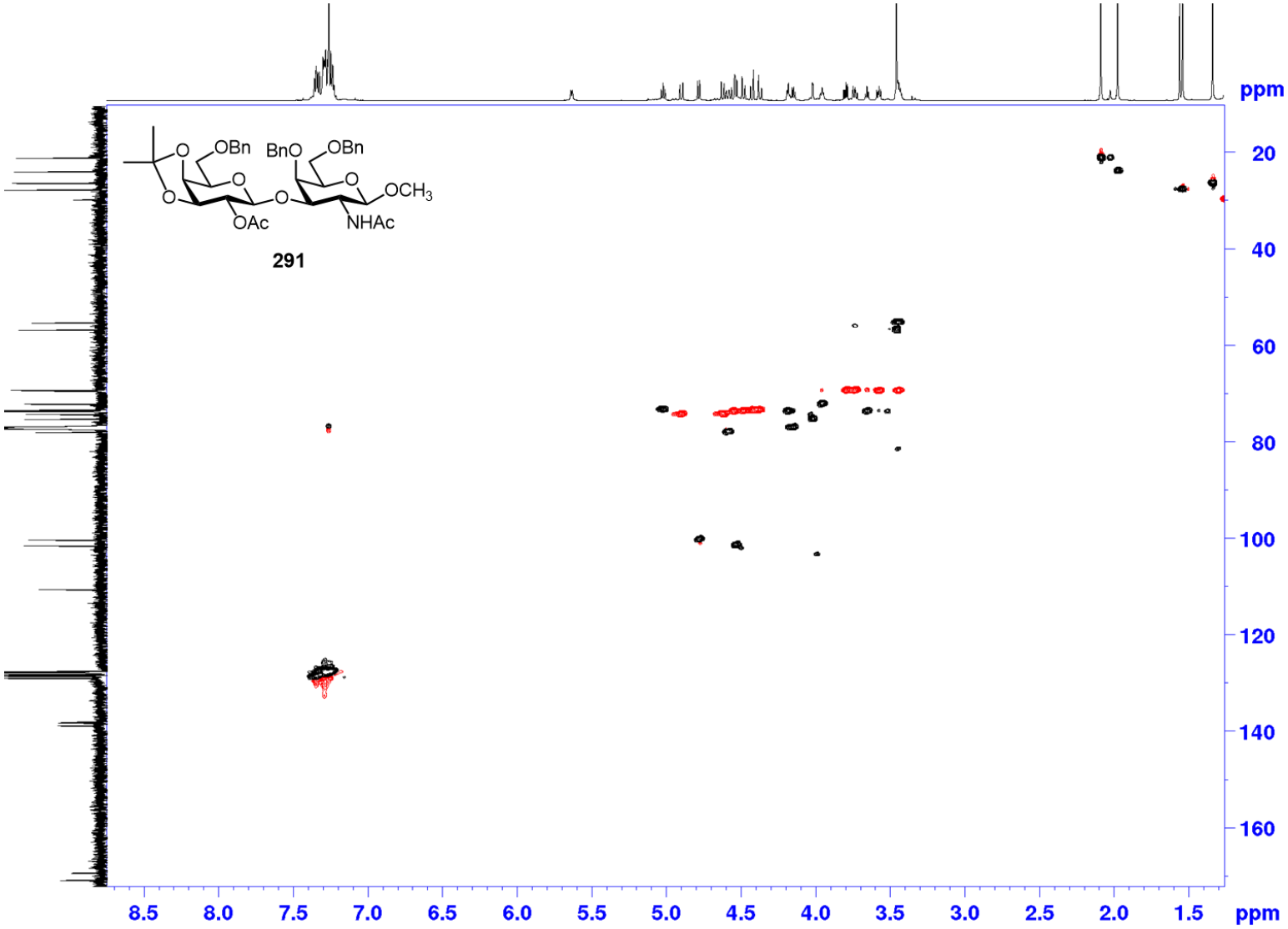
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 291



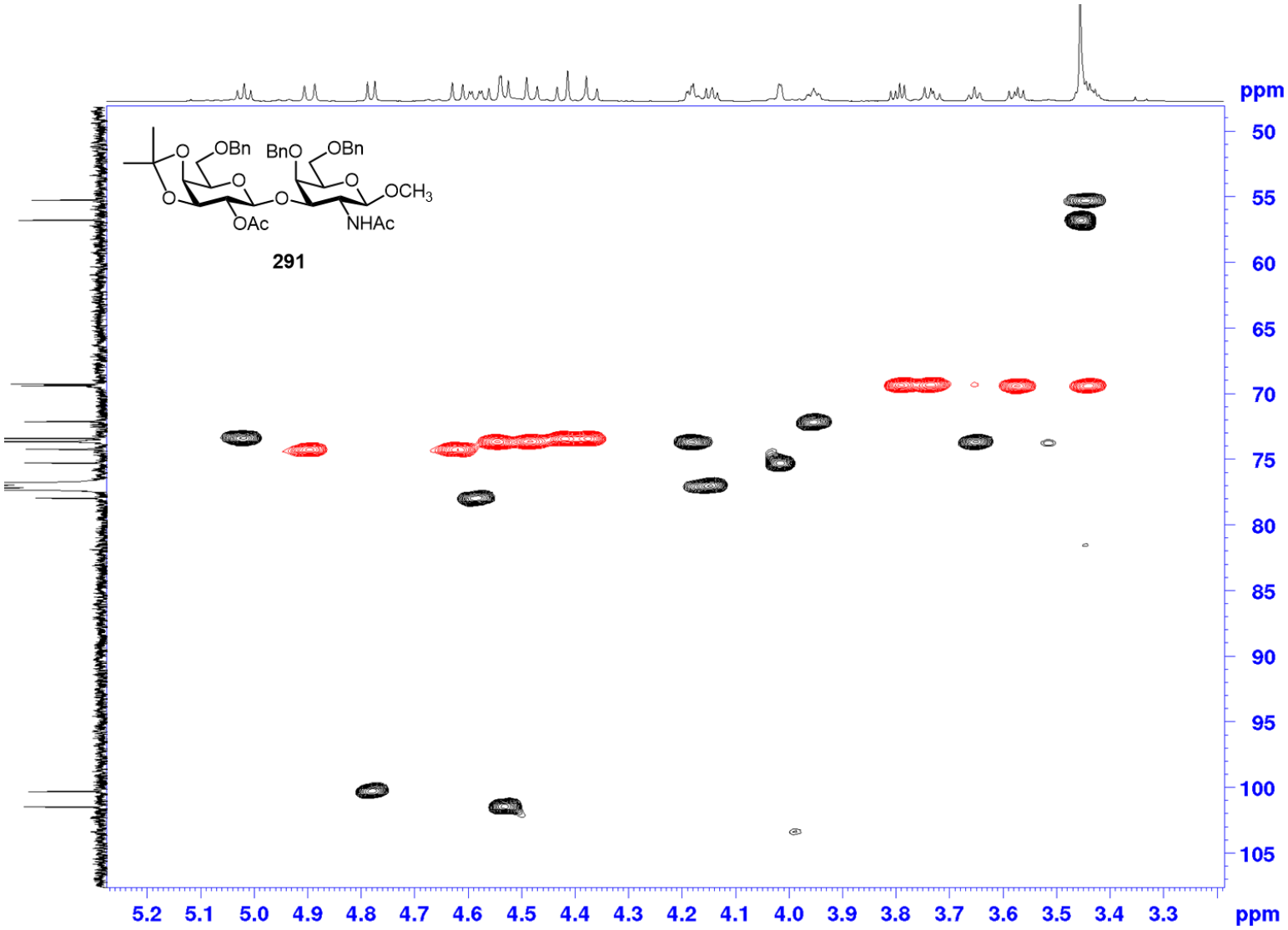
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 291



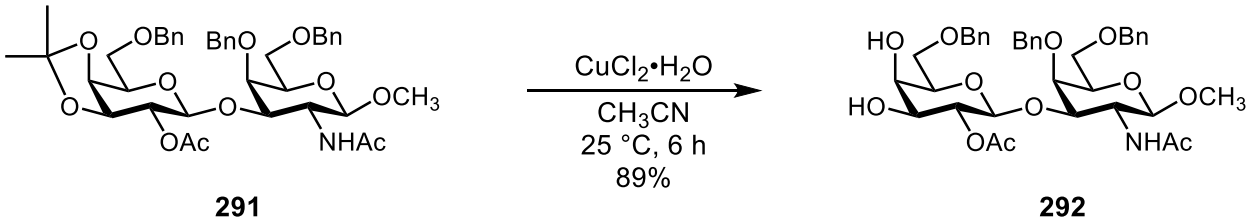
^{1}H-^{13}C HSCQ ($CDCl_3$) of compound 291



^{1}H-^{13}C HSCQ ($CDCl_3$) zoomed in region of compound 291



Methyl 6-*O*-benzyl-2-*O*-acetyl-β-D-galactopyranosyl-(1→3)-4,6-di-*O*-benzyl-2-deoxy-2-acetamido-β-D-galactopyranoside (292)

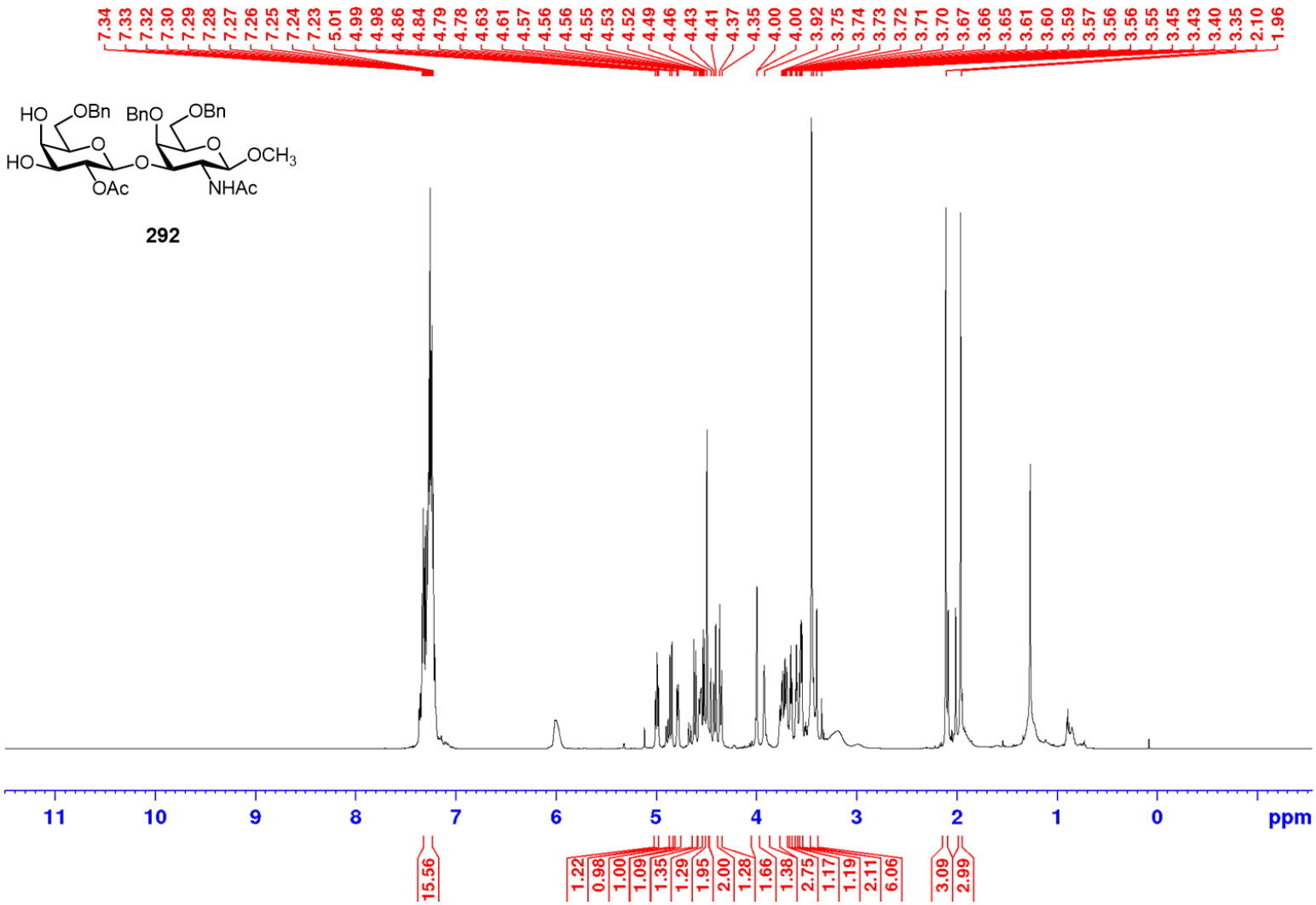


291 **292**

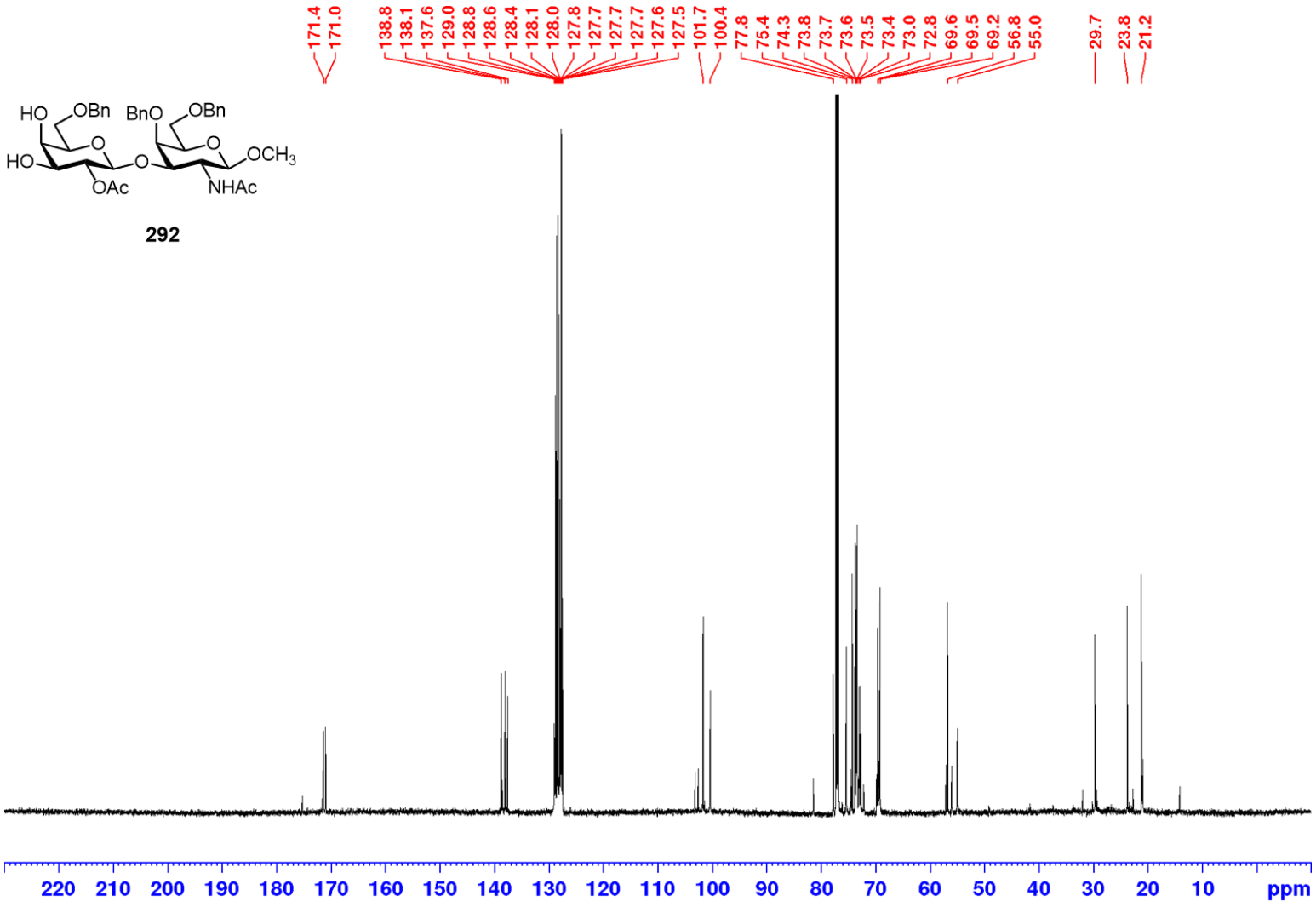
A solution of methyl 6-*O*-benzyl-*O*-3,4-isopropylidene-2-*O*-acetyl-β-D-galactopyranosyl-(1→3)-4,6-di-*O*-benzyl-2-deoxy-2-acetamido-β-D-galactopyranoside **191** (1.0 equiv., 0.269 g, 0.389 mmol) and copper (II) chloride hydrate (5.0 equiv., 0.331 g, 1.94 mmol) in CH_3CN (4 mL) was stirred at 0 °C under an argon atmosphere. At 3 h, the crude reaction mixture was filtered through a glass fritted vacuum filter funnel equipped with a pad of Celite. The filtrate was transferred to a separatory funnel with EtOAc (100 mL). The organics were washed with 1 N HCl (3 x 20 mL), aq. sat. $NaHCO_3$ (1 x 20 mL), brine (1 x 20 mL), dried ($NaSO_4$), filtered through a glass fritted vacuum filter funnel to remove drying agent, and concentrated to a crude syrup. The crude syrup was purified by silica gel flash column chromatography (20:80 to 66:33 EtOAc/hexanes) to give the diol product methyl 6-*O*-benzyl-2-*O*-acetyl-β-D-galactopyranosyl-(1→3)-4,6-di-*O*-benzyl-2-deoxy-2-acetamido-β-D-galactopyranoside **192** (0.248 g, 0.349 mmol, 89%) as an off-white amorphous solid. Rf = 0.40 (10:90 CH_3OH/EtOAc) visualized with ceric ammonium molybdate stain; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.34-7.23 (m, 15H), 5.01-4.98 (m, 1H), 4.85 (d, *J* = 11.7 Hz, 1H), 4.78 (d, *J* = 7.9 Hz, 1H), 4.62 (d, *J* = 12.1 Hz, 1H), 4.57-4.53 (m, 1H), 4.53 (d, *J* = 7.8 Hz, 1H), 4.50-4.48 (m, 2H), 4.43 (d, *J* = 12.3 Hz, 1H), 4.36 (d, *J* = 11.2 Hz, 2H), 4.01 (d, *J* = 1.3 Hz, 1H), 3.92 (d, *J* = 1.1 Hz, 1H), 3.75-3.65 (m, 4H), 3.61-3.55 (m, 3H), 3.52-3.35 (m, 5H), 2.12 (s, 3H), 1.97 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$):

δ 171.4, 171.0, 138.7, 138.0, 137.5, 129.0, 128.7, 128.5, 128.3, 128.0, 127.9, 127.7, 127.7, 127.7, 127.6, 127.6, 127.5, 101.6, 100.3, 77.8, 75.3, 74.2, 73.7, 73.7, 73.5, 73.4, 73.3, 73.0, 72.7, 69.5, 69.5, 69.1, 56.7, 54.9, 29.7, 23.7, 21.1.

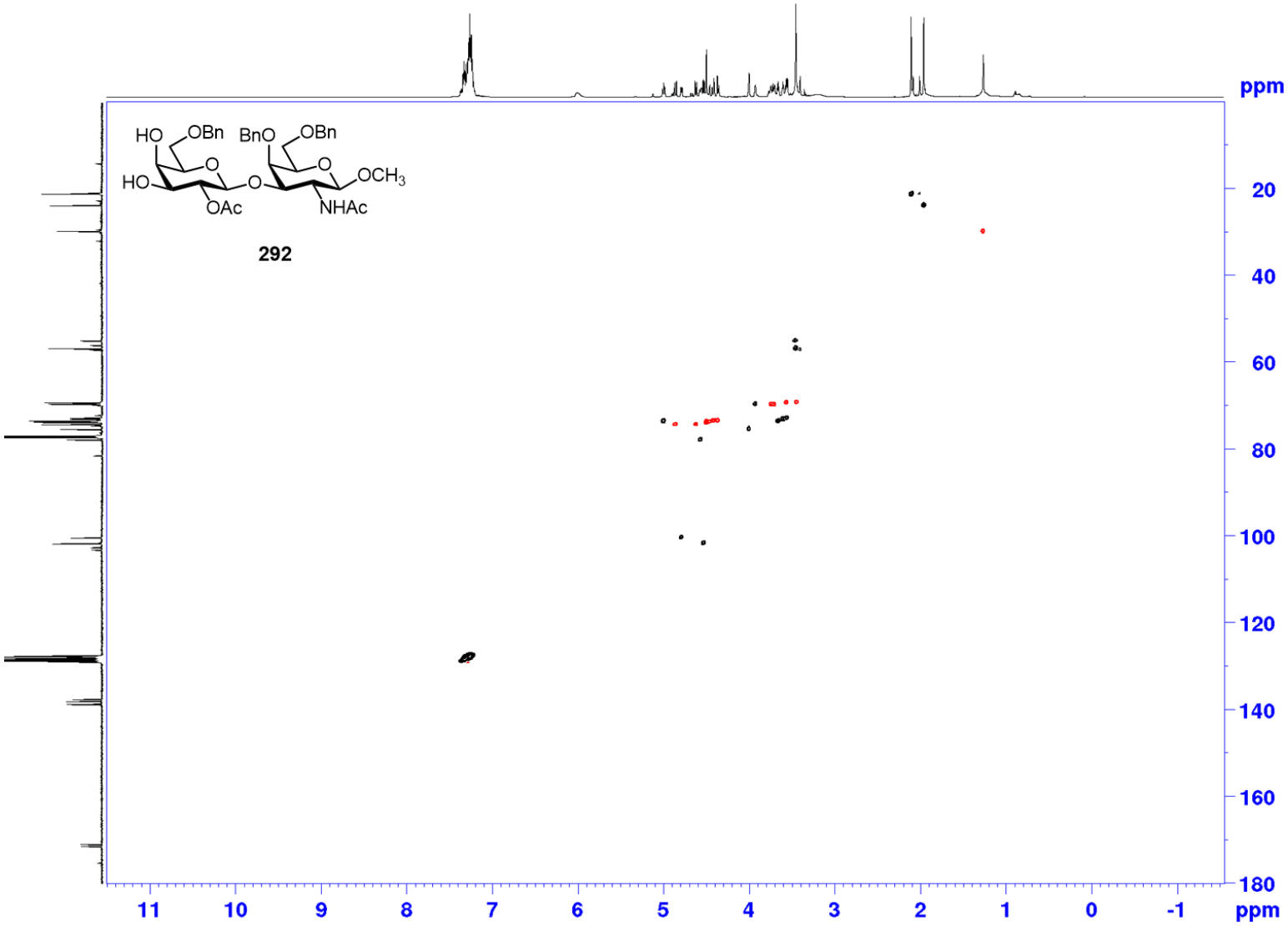
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 292



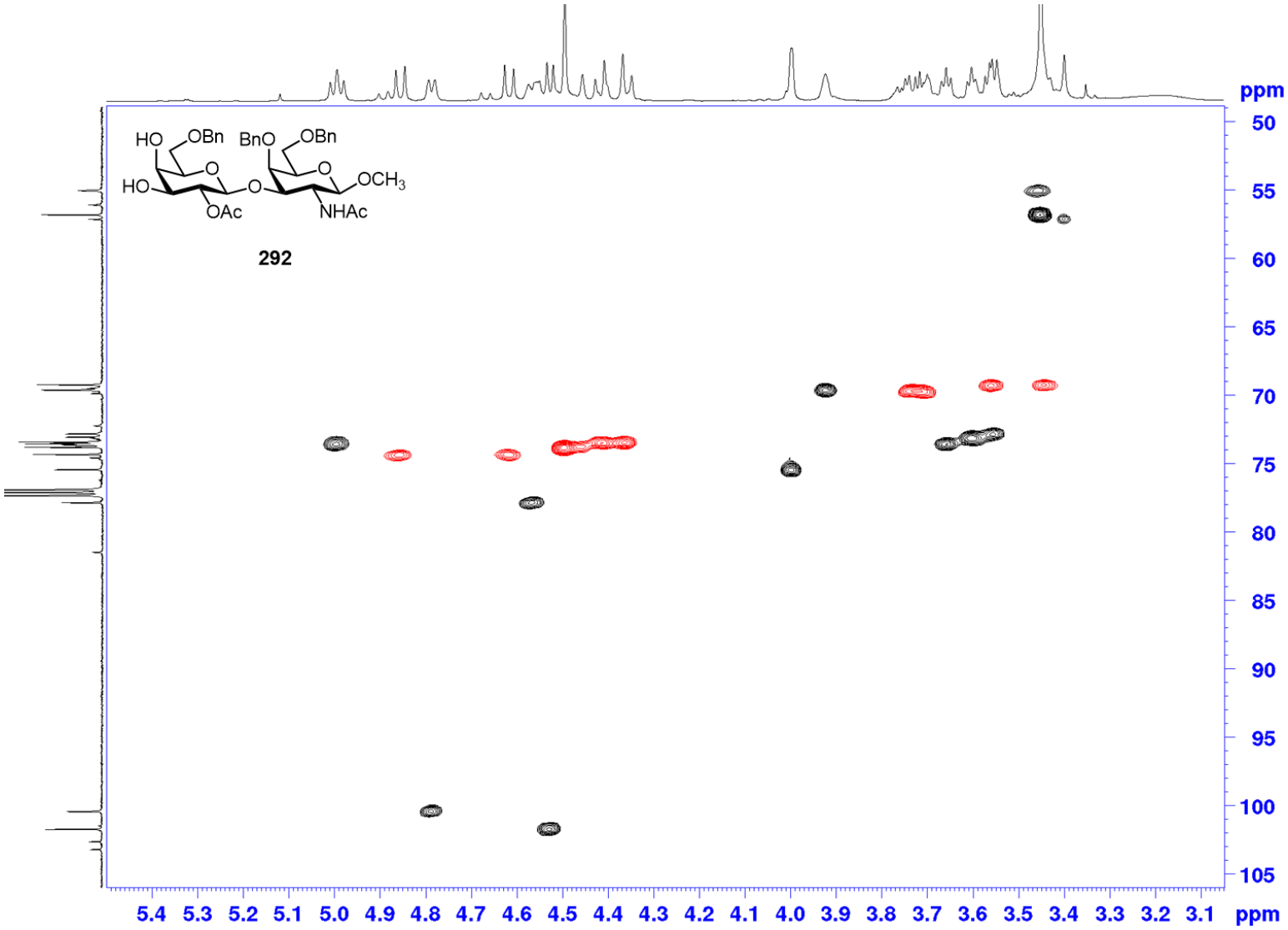
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 292



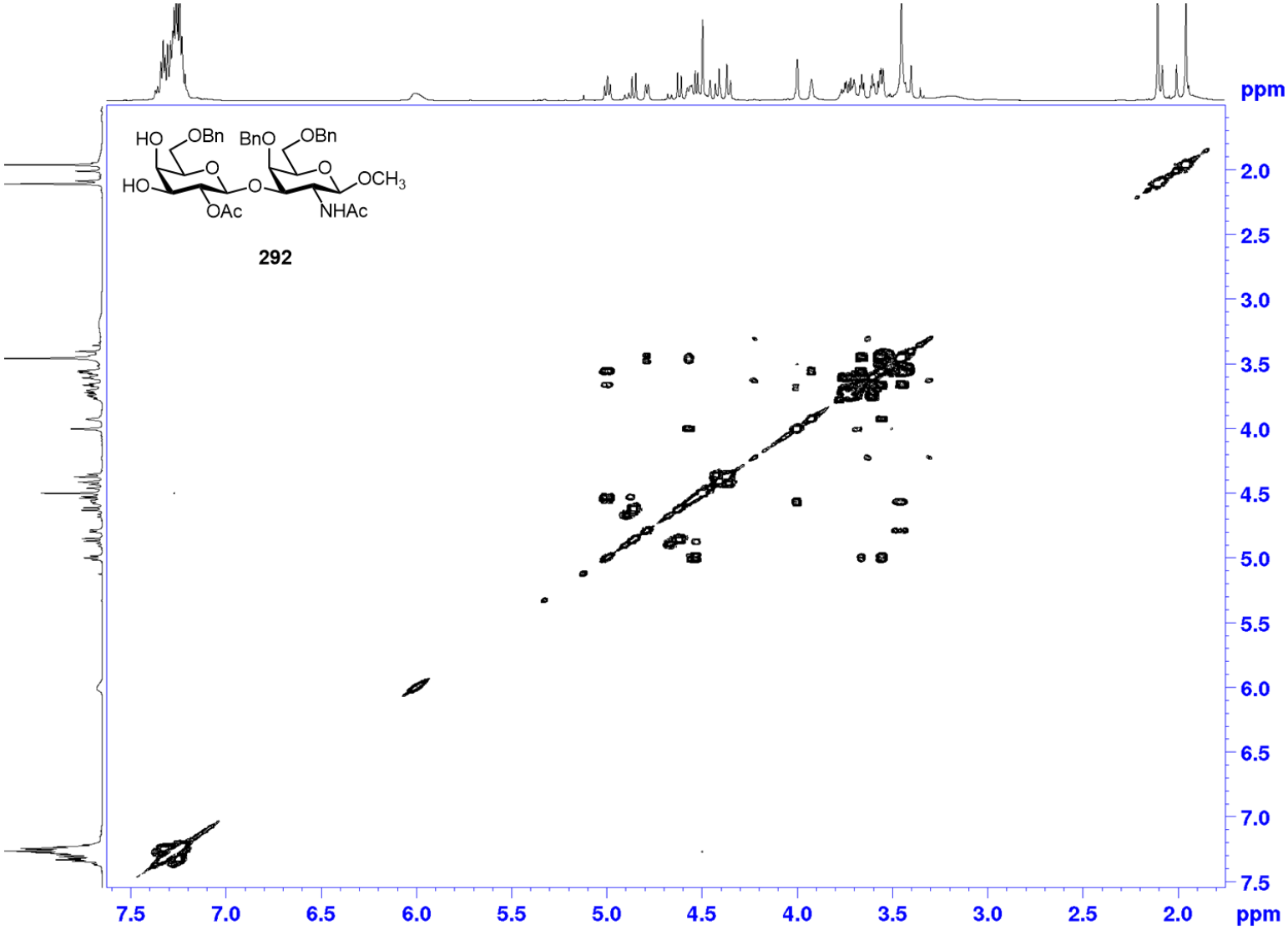
^{1}H-^{13}C HSCQ ($CDCl_3$) of compound 292



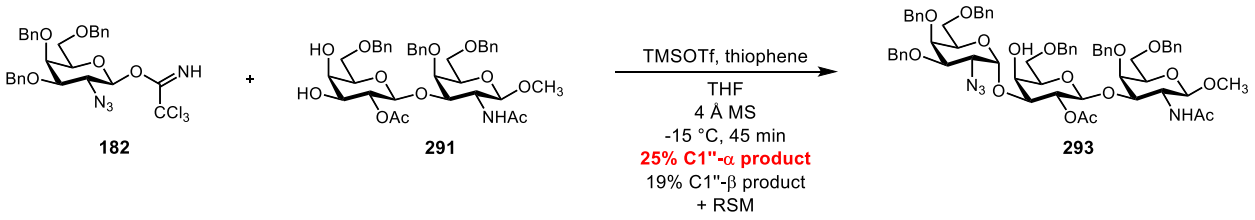
^{1}H-^{13}C HSCQ ($CDCl_3$) zoomed in region of compound 292



^{1}H-^{1}H COSY ($CDCl_3$) of compound 292



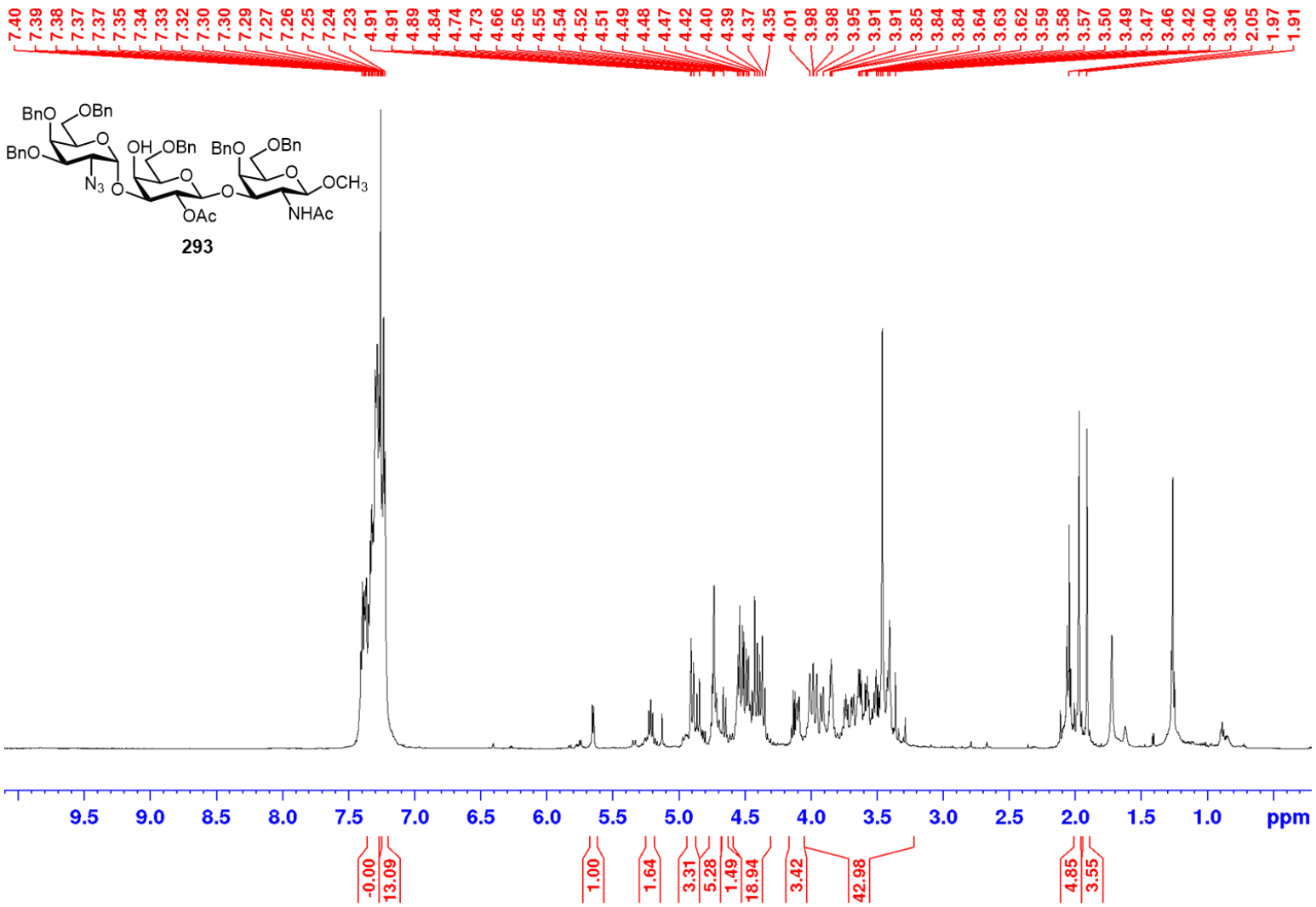
Methyl 3,4,6-tri-*O*-benzyl-2-deoxy-2-azido-α-D-galactopyranosyl-(1→3)-6-*O*-benzyl-2-*O*-(*p*-methoxy)-β-D-galactopyranosyl-(1→3)-4,6-di-*O*-benzyl-2-deoxy-2-acetamido-β-D-galactopyranoside (293)



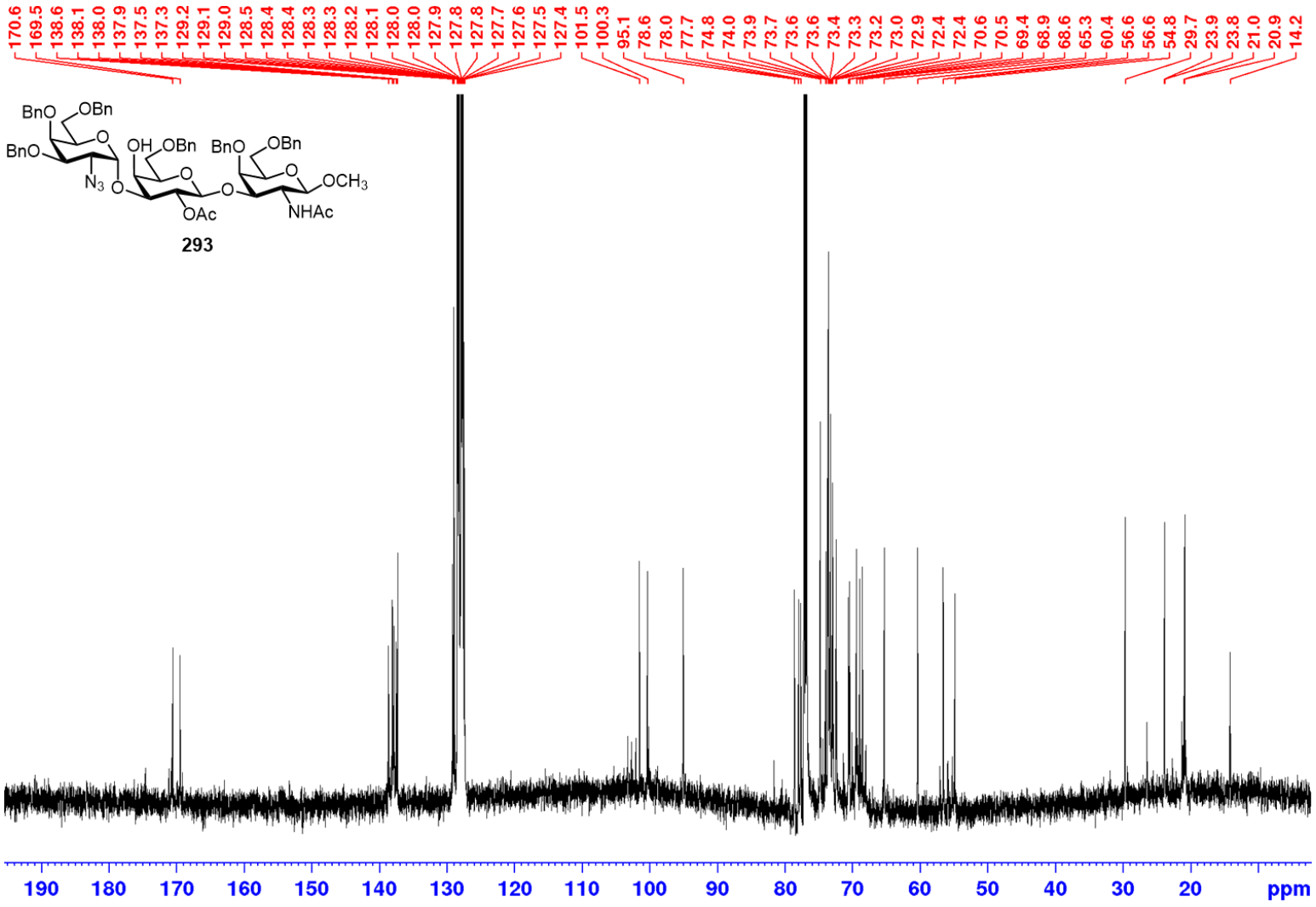
A solution of trichloroacetimidate 3,4,6-tri-*O*-benzyl-2-deoxy-2-azido-β-D-galactopyranoside **182** (1.5 equiv., 0.209 g, 0.338 mmol), disaccharide acceptor methyl 6-*O*-benzyl-2-*O*-(*p*-methoxy)-β-D-galactopyranosyl-(1→3)-4,6-*O*-benzylidene acetal-2-deoxy-2-acetimido-β-D-galactopyranoside **291** (1.0 equiv., 0.157 g, 0.221 mmol) in dry THF (2 mL) and thiophene (25 equiv., 0.465 g, 5.53 mmol) were added freshly activated and cooled 4 Å MS (300 mg, 15% m/v) under an argon atmosphere at room temperature. After 1 h, the reaction was cooled to -15 °C then added a solution of TMSOTf (0.10 equiv., 4.0 µL, 0.022 mmol). At 45 min, the reaction mixture was neutralized with Et_3N and added diluted with CH_2Cl_2. The reaction mixture was filtered through a pad of Celite within a glass fritted vacuum filter funnel to remove the 4 Å MS. The filtrate was concentrated to a crude oil and purified by silica gel flash column chromatography to give methyl 3,4,6-tri-O-benzyl-2-deoxy-2-azido-α-D-galactopyranosyl-(1→3)-6-*O*-benzyl-2-*O*-(*p*-methoxy)-β-D-galactopyranosyl-(1→3)-4,6-di-*O*-benzyl-2-deoxy-2-acetamido-β-D-galactopyranoside **293** (0.0631 g, 0.0541 mmol, 25%) as a white foam. Rf = 0.52 (70:30 EtOAc/hexanes) visualized with ceric ammonium molybdate stain. The trisaccharide methyl 3,4,6-tri-O-benzyl-2-deoxy-2-azido-β-D-galactopyranosyl-(1→3)-6-*O*-benzyl-2-*O*-(*p*-methoxy)-β-D-galactopyranosyl-(1→3)-4,6-di-*O*-benzyl-2-deoxy-2-acetamido-β-D-

galactopyranoside **293-C1''-β** (0.0486 g, 0.0416 mmol, 19%) was also isolated along with some unconsumed disaccharide starting material acceptor.; ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.41-7.22 (m, 16H), 7.23 (m, 14H), 5.65 (d, J = 6.6 Hz, 1H), 4.91-4.87 (m, 3H), 4.74-4.64 (m, 4H), 4.63-4.62 (m, 1H), 4.56-4.33 (m, 8H), 4.13-4.03 (m, 2H), 4.01-3.25 (m, 18H), 1.99 (s, 3H), 1.92 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.5, 169.5, 138.6, 138.1, 138.0, 137.8, 137.5, 137.3, 129.1, 129.0, 128.9, 128.4, 128.4, 128.3, 128.3, 128.2, 128.1, 128.1, 128.0, 127.9, 127.9, 127.8, 127.8, 127.7, 127.6, 127.5, 127.4, 101.5, 100.3, 95.0, 78.6, 77.9, 77.6, 74.7, 74.0, 73.9, 73.6, 73.6, 73.5, 73.4, 73.2, 73.2, 73.0, 72.9, 72.4, 72.3, 70.6, 70.4, 69.4, 68.9, 68.5, 65.3, 60.3, 56.6, 56.5, 54.8, 29.6, 26.4, 23.8, 23.8, 21.2, 21.0, 20.8, 14.1, 0.5, 0.27, 0.0.

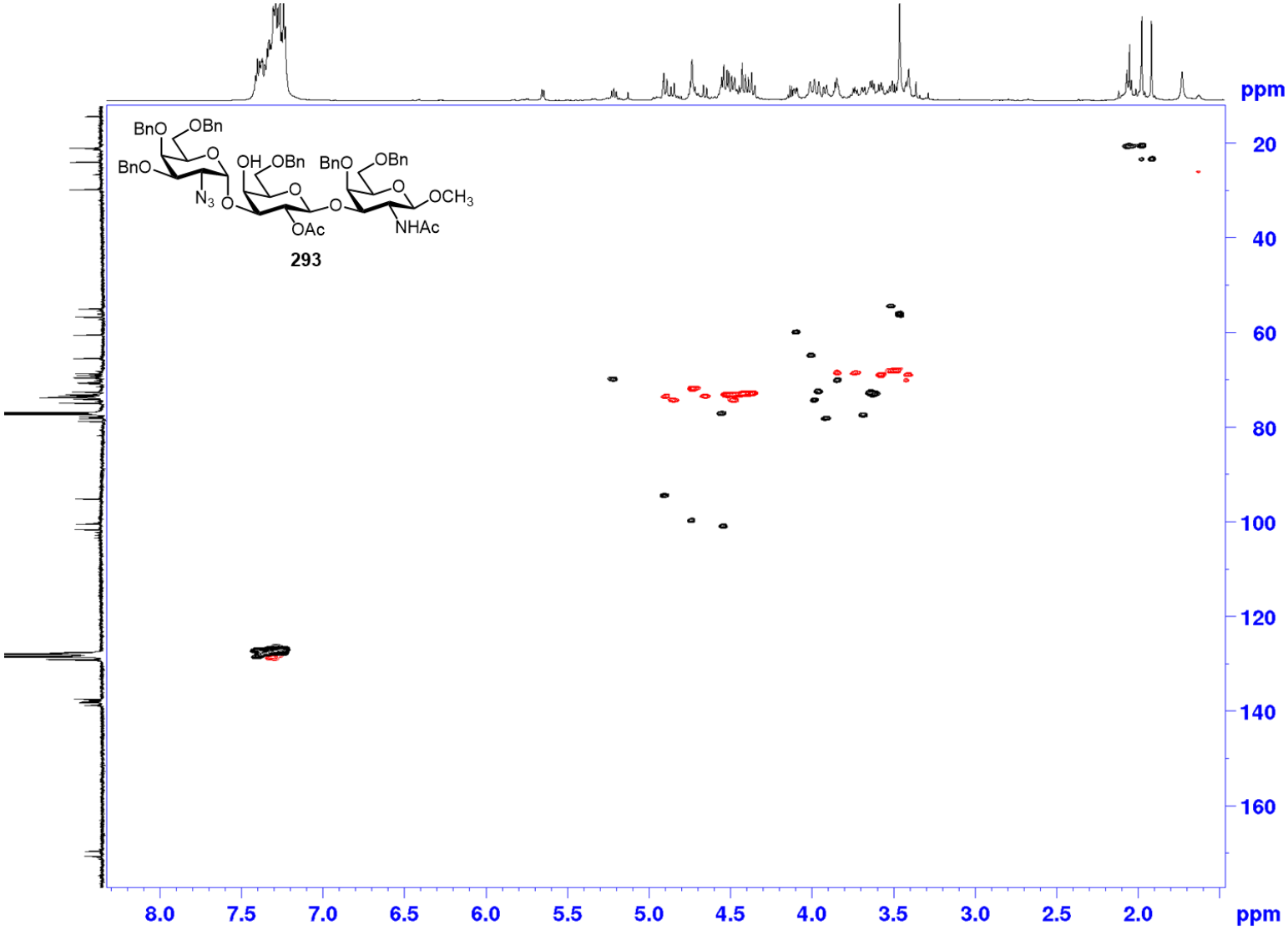
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 293



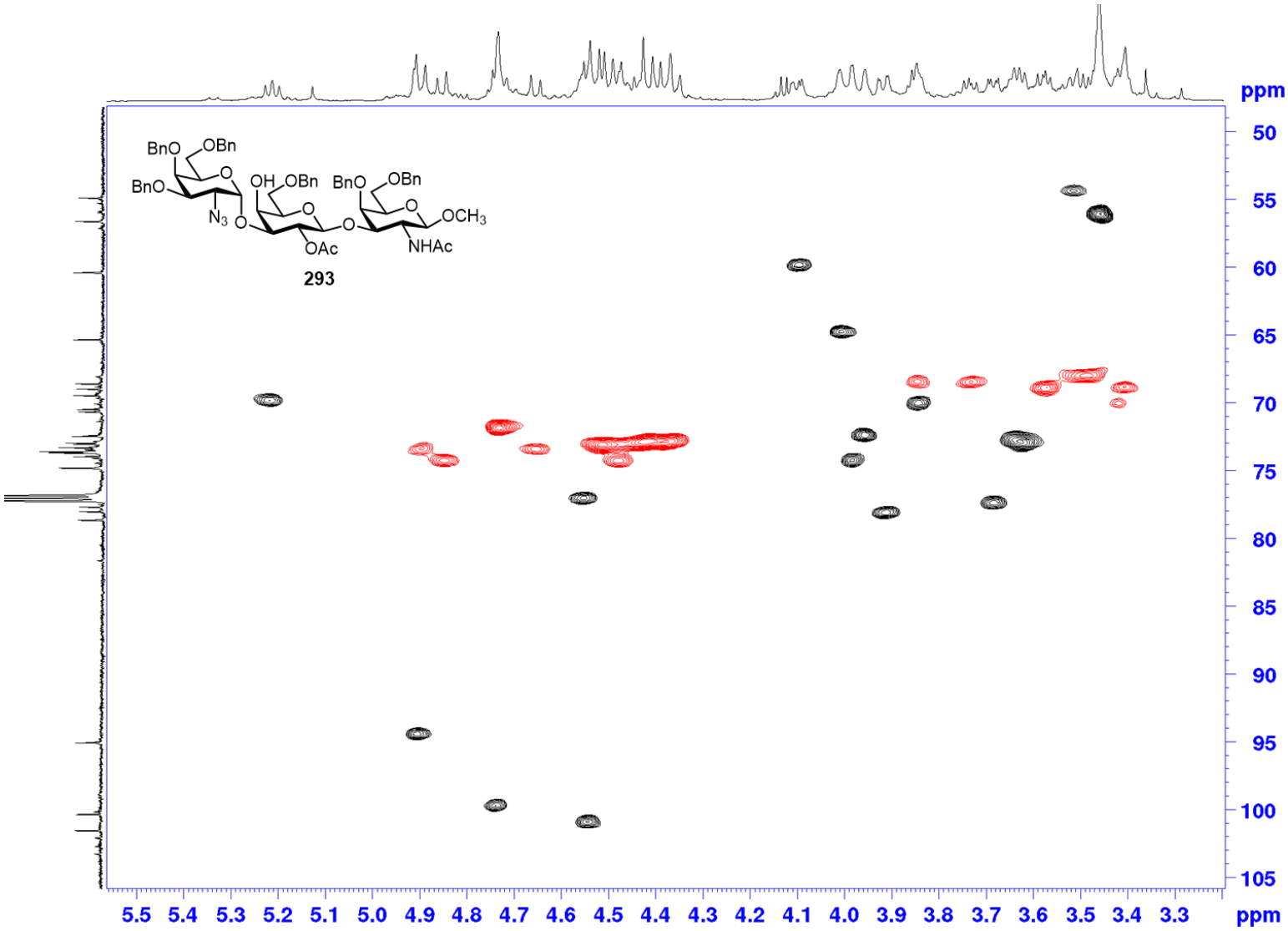
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 293



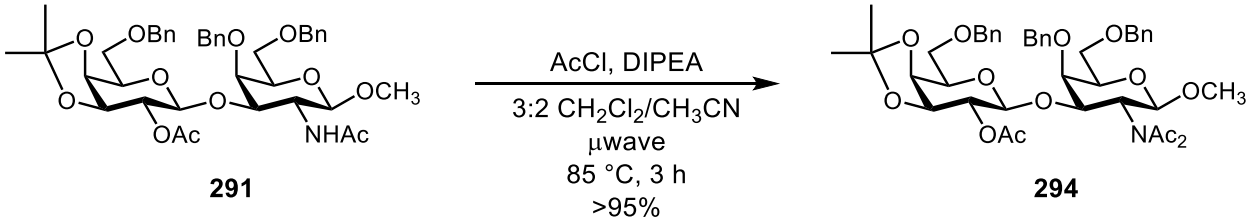
^{1}H-^{13}C HSCQ ($CDCl_3$) of compound 293



^{1}H-^{13}C HMBC ($CDCl_3$) zoomed in region of compound 293



Methyl 6-*O*-benzyl-*O*-3,4-isopropylidene-2-*O*-acetyl-β-D-galactopyranosyl-(1→3)-4,6-di-*O*-benzyl-2-deoxy-2-(*N*,*N*-diacetyl)-β-D-galactopyranoside (294)

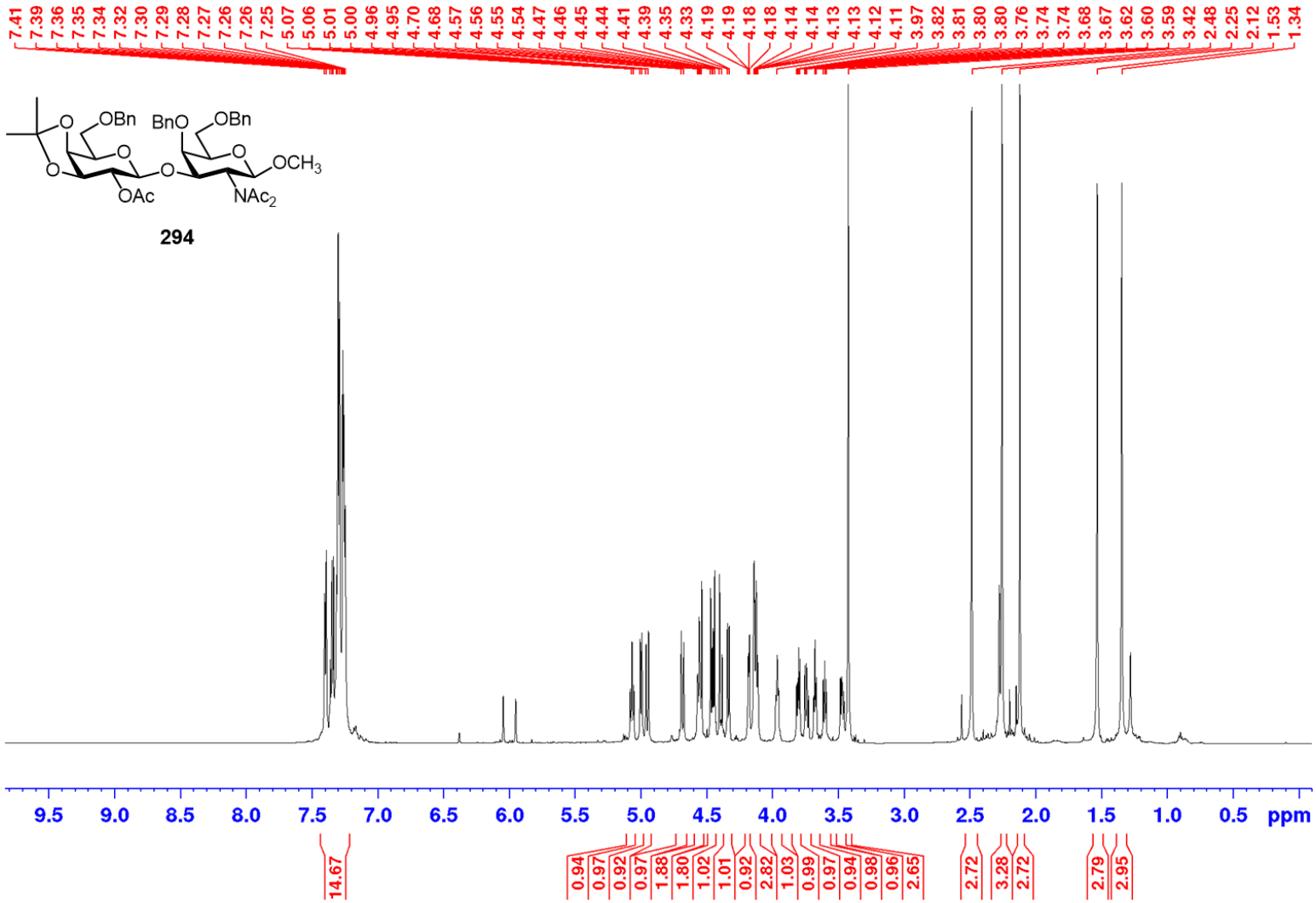


291 **294**

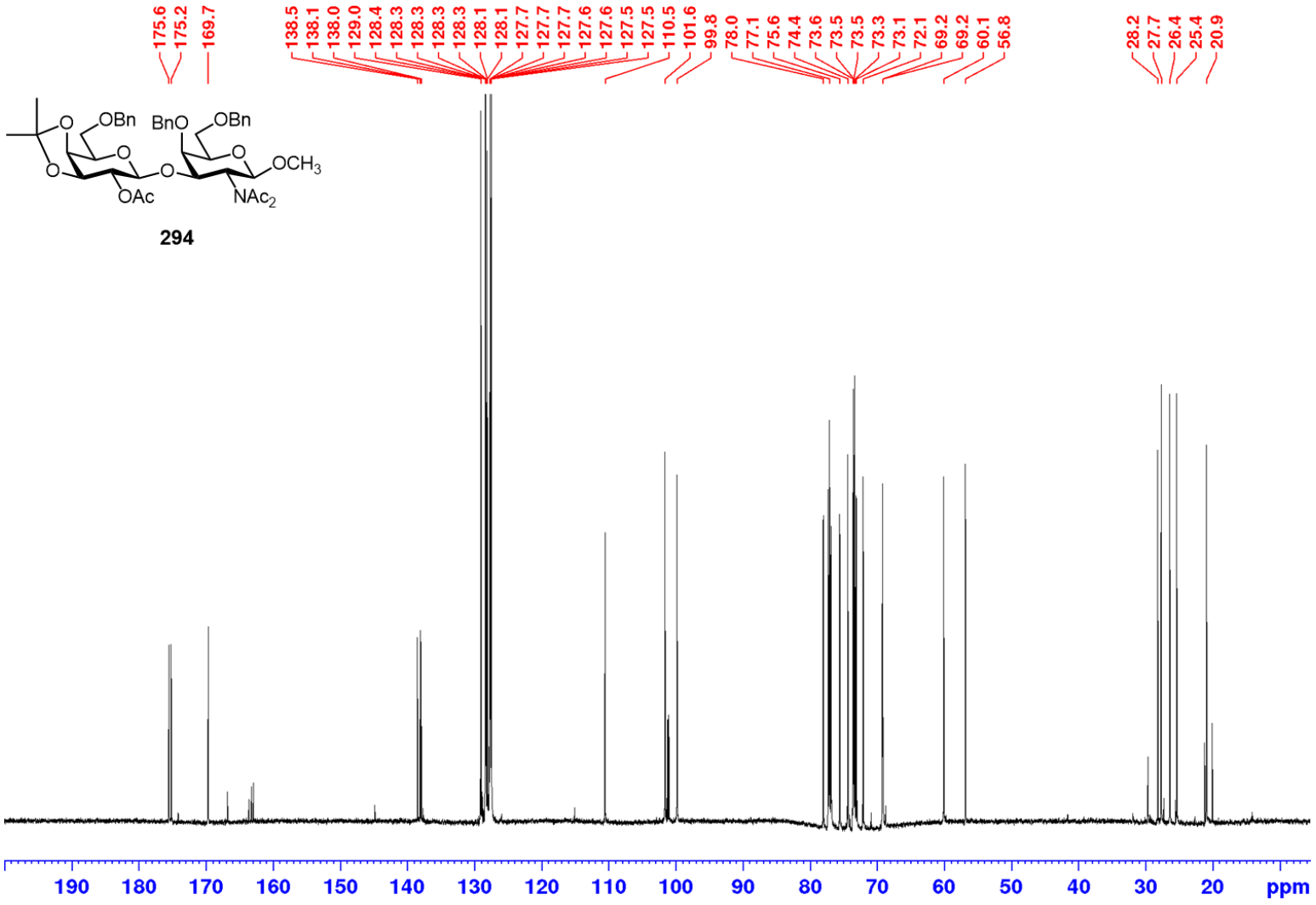
A solution of methyl 6-*O*-benzyl-*O*-3,4-isopropylidene-2-*O*-acetyl-β-D-galactopyranosyl-(1→3)-4,6-di-*O*-benzyl-2-deoxy-2-acetamido-β-D-galactopyranoside **291** (1.0 equiv., 0.132 g, 1.77 mmol) and *N*,*N*-diisopropylethylamine (2.0 equiv., 0.061 mL, 0.352 mmol) in a 3:2 mixture of CH_2Cl_2/CH_3CN (2 mL) was cooled to 0 °C, and added acetyl chloride (5.0 equiv., 0.062 mL, 0.880 mmol) under an argon atmosphere. The reaction mixture was warmed to room temperature then heated by μwave irradiation to 85 °C for 3 h. The reaction was concentrated to a crude oil and transferred to a separatory funnel with EtOAc (25 mL). The organics were washed with 1 N HCl (2 x 5 mL), 40% aq. solution of sodium thiosulfate (2 x 5 mL), aq. sat. $NaHCO_3$ (1 x 5 mL), brine (1 x 5 mL), dried ($NaSO_4$), filtered through a glass fritted filter funnel to remove the drying agents and concentrated to a foam. The crude reaction mixture was purified by silica gel flash chromatography (60:40 to 70:30 EtOAc/hexanes) to give methyl 6-*O*-benzyl-*O*-3,4-isopropylidene-2-*O*-acetyl-β-D-galactopyranosyl-(1→3)-4,6-di-*O*-benzyl-2-deoxy-2-(*N*,*N*-diacetyl)-β-D-galactopyranoside **294** (0.135 g, 0.170 mmol, 97% yield) as a yellow oil. Rf = 0.4 (1:1 EtOAc/hexanes) visualized with ceric ammonium molybdate stain; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.41-7.25 (m, 15H), 5.09-5.07 (m, 1H), 5.01 (d, *J* = 7.7 Hz, 1H), 4.95 (d, *J* = 11.3 Hz, 1H), 4.7 (d, *J* = 11.4 Hz, 1H), 4.57-4.54 (m, 2H), 4.47-4.44 (m, 2H), 4.4 (d, *J* =

11.8 Hz, 1H), 4.34 (d, *J* = 8.1 Hz, 1H), 4.19 (dd, *J* = 1.7, 5.1 Hz, 1H), 4.14-4.11 (m, 3H), 3.97 (dd, *J* = 1.2, 5.9 Hz, 1H), 3.82-3.80 (m, 1H), 3.75 (m, 1H), 3.67 (dd, *J* = 7.3, 9.8 Hz, 1H), 3.62-3.59 (m, 1H), 3.48 (dd, *J* = 5.9, 9.5 Hz, 1H), 3.42 (s, 3H), 2.49 (s, 3H), 2.24 (s, 3H), 2.11 (s, 3H), 1.53 (s, 3H), 1.35 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 175.6, 175.2, 169.7, 138.5, 138.1, 138, 129, 128.4, 128.3, 128.3, 128.3, 128.3, 128.1, 128.1, 127.7, 127.7, 127.7, 127.6, 127.6, 127.5, 127.5, 110.5, 101.6, 99.8, 78, 77.1, 75.6, 74.4, 73.6, 73.5, 73.5, 73.3, 73.1, 72.1, 69.2, 69.2, 60.1, 56.8, 28.2, 27.7, 26.4, 25.4, 20.9.

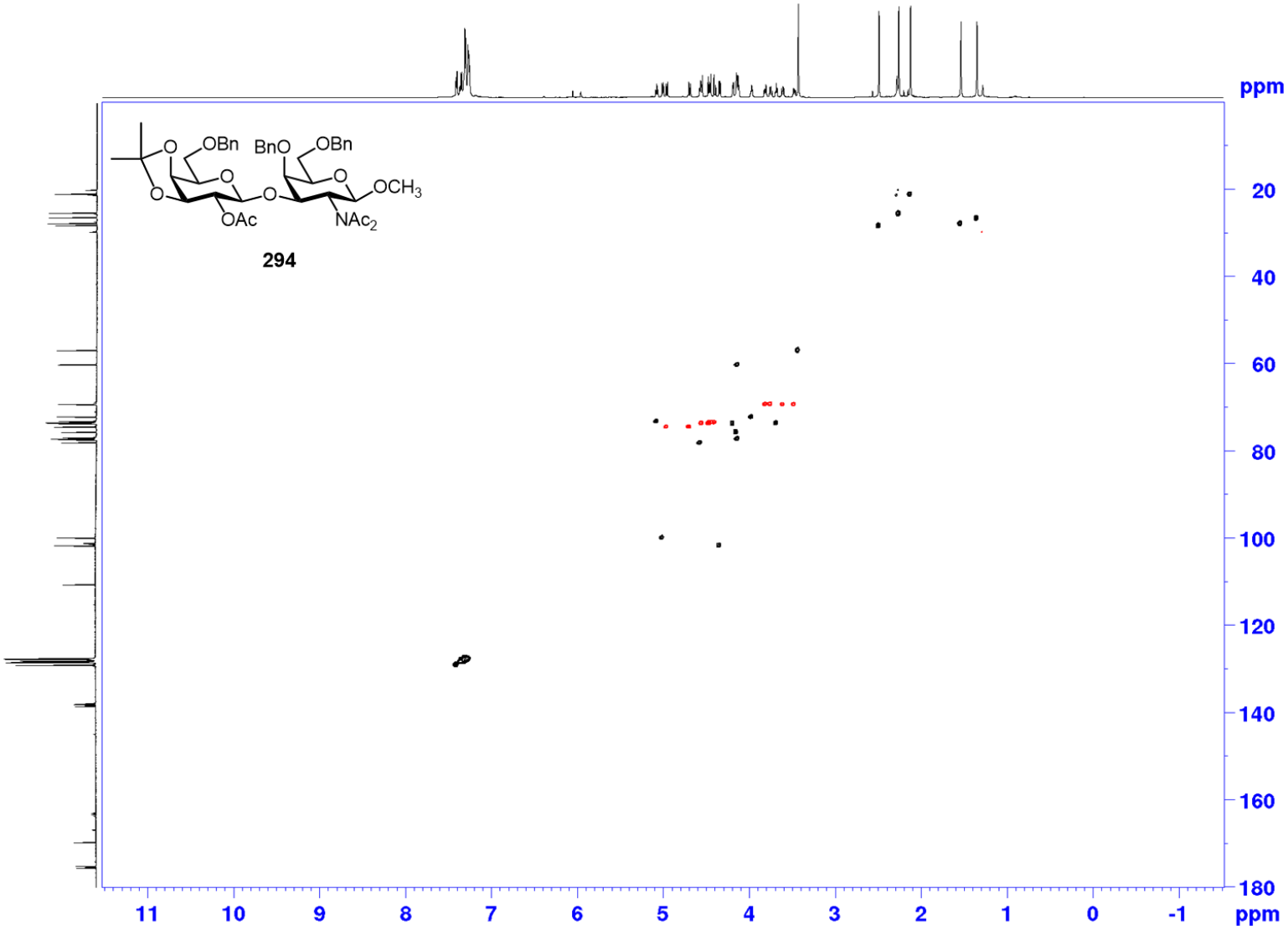
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 294



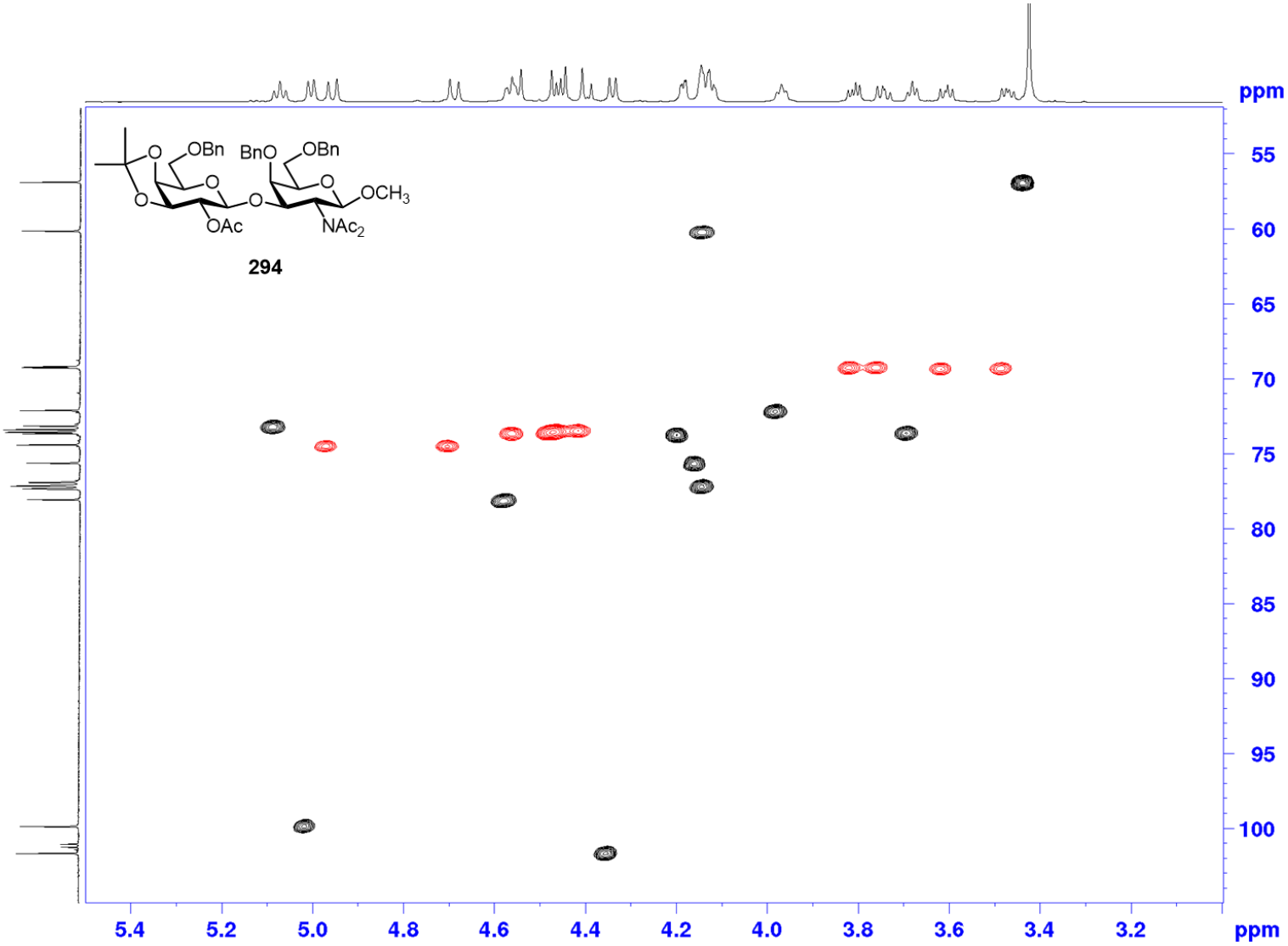
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 294



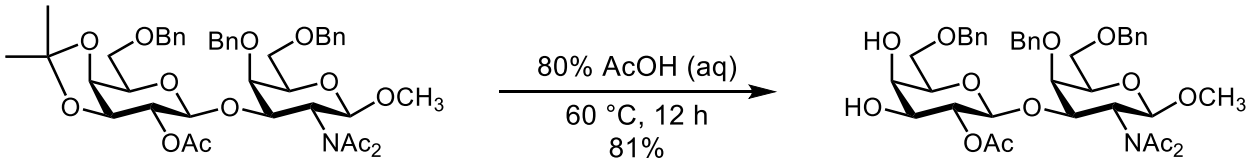
^{1}H-^{13}C HSCQ ($CDCl_3$) of compound 294



^{1}H-^{13}C HSCQ ($CDCl_3$) zoomed in region of compound 294



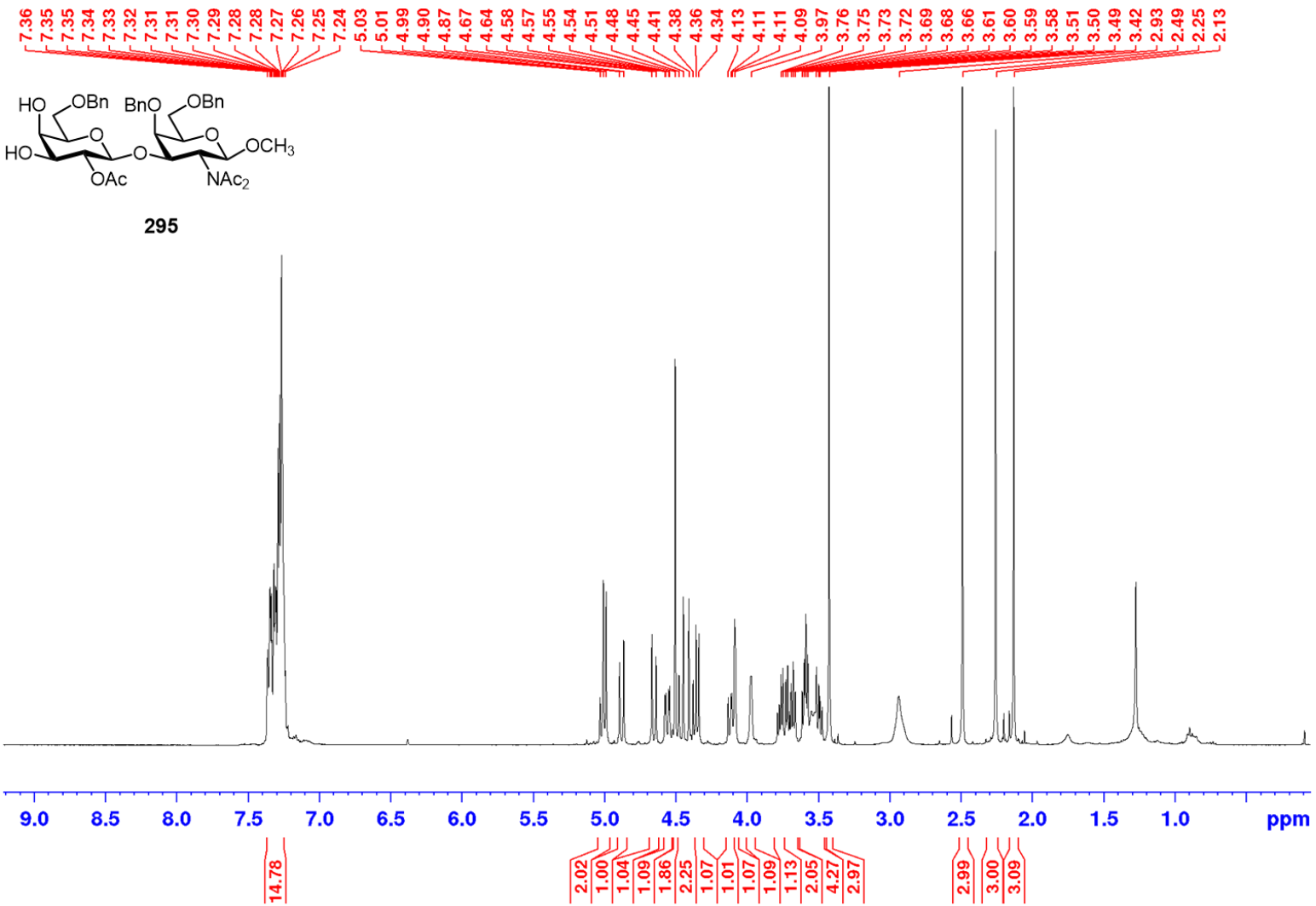
Methyl 6-*O*-benzyl-2-*O*-acetyl-β-D-galactopyranosyl-(1→3)-4,6-di-*O*-benzyl-2-deoxy-2-(*N*,*N*-diacetyl)-β-D-galactopyranoside (295)



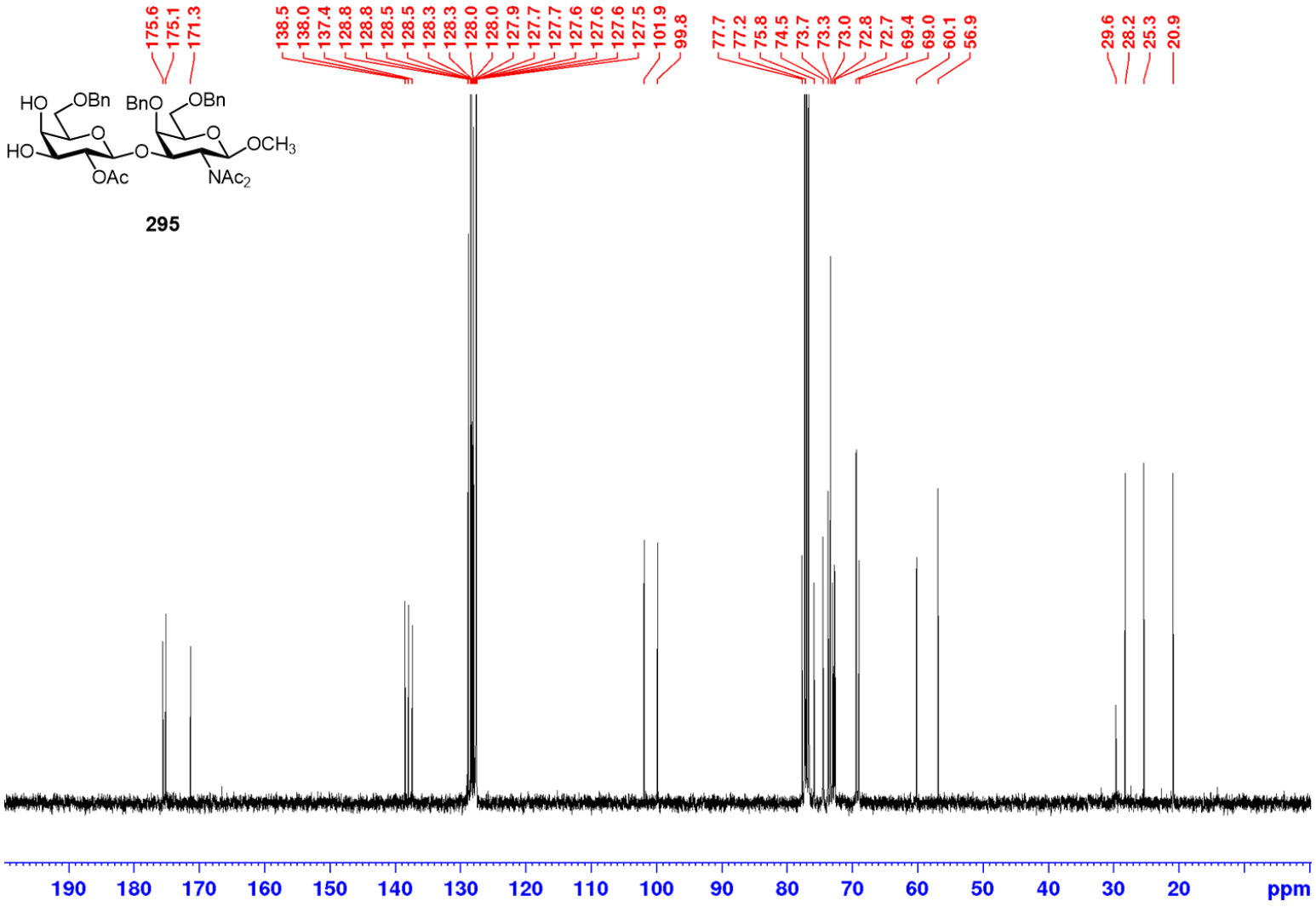
294 **295**

A solution of methyl 6-*O*-benzyl-*O*-3,4-isopropylidene-2-*O*-acetyl-β-D-galactopyranosyl-(1→3)-4,6-di-*O*-benzyl-2-deoxy-2-(*N*,*N*-diacetyl)-β-D-galactopyranoside **294** (1.0 equiv., 2.008 g, 7.96 mmol) in 80% (aq) AcOH was heated to 65 °C. At 12 h, TLC (100:0 EtOAc/hexanes, CAM stain) indicated complete consumption of starting material for a more polar spot. The reaction was concentrated to an oil which was purified by silica gel flash column chromatography (60:40 to 80:20 EtOAc/hexanes) to give diol methyl 6-*O*-benzyl-*O*-acetyl-β-D-galactopyranosyl-(1→3)-4,6-di-*O*-benzyl-2-deoxy-2-(*N*,*N*-diacetyl)-β-D-galactopyranoside **295** (1.664 g, 7.84 mmol, >95%) as a white foam. Rf = 0.30 (75:25 EtOAc/hexanes) visualized with ceric ammonium molybdate stain; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.37-7.24 (m, 15H), 5.03-4.99 (m, 2H), 4.88 (d, *J* = 11.5 Hz, 1H), 4.65 (d, *J* = 11.5 Hz, 1H), 4.56 (dd, *J* = 2.9, 10.7 Hz, 1H), 4.53-4.49 (m, 2H), 4.48-4.38 (m, 2H), 4.35 (d, *J* = 7.8 Hz, 1H), 4.12 (dd, *J* = 7.8, 10.9 Hz, 1H), 4.10-4.09 (m, 1H), 3.98 (d, *J* = 1.8 Hz, 1H), 3.78-3.74 (m, 1H), 3.73-3.66 (m, 2H), 3.61-3.49 (m, 4H), 3.42 (s, 3H), 2.49 (s, 3H), 2.28 (s, 3H), 2.13 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 175.6, 175.1, 171.3, 138.5, 138, 137.4, 128.8, 128.8, 128.5, 128.5, 128.3, 128.3, 128, 128, 127.9, 127.7, 127.7, 127.6, 127.6, 127.6, 127.5, 101.9, 99.8, 77.7, 77.2, 75.8, 74.5, 73.7, 73.3, 73, 72.8, 72.7, 69.4, 69, 60.1, 56.9, 29.6, 28.2, 25.3, 20.9.

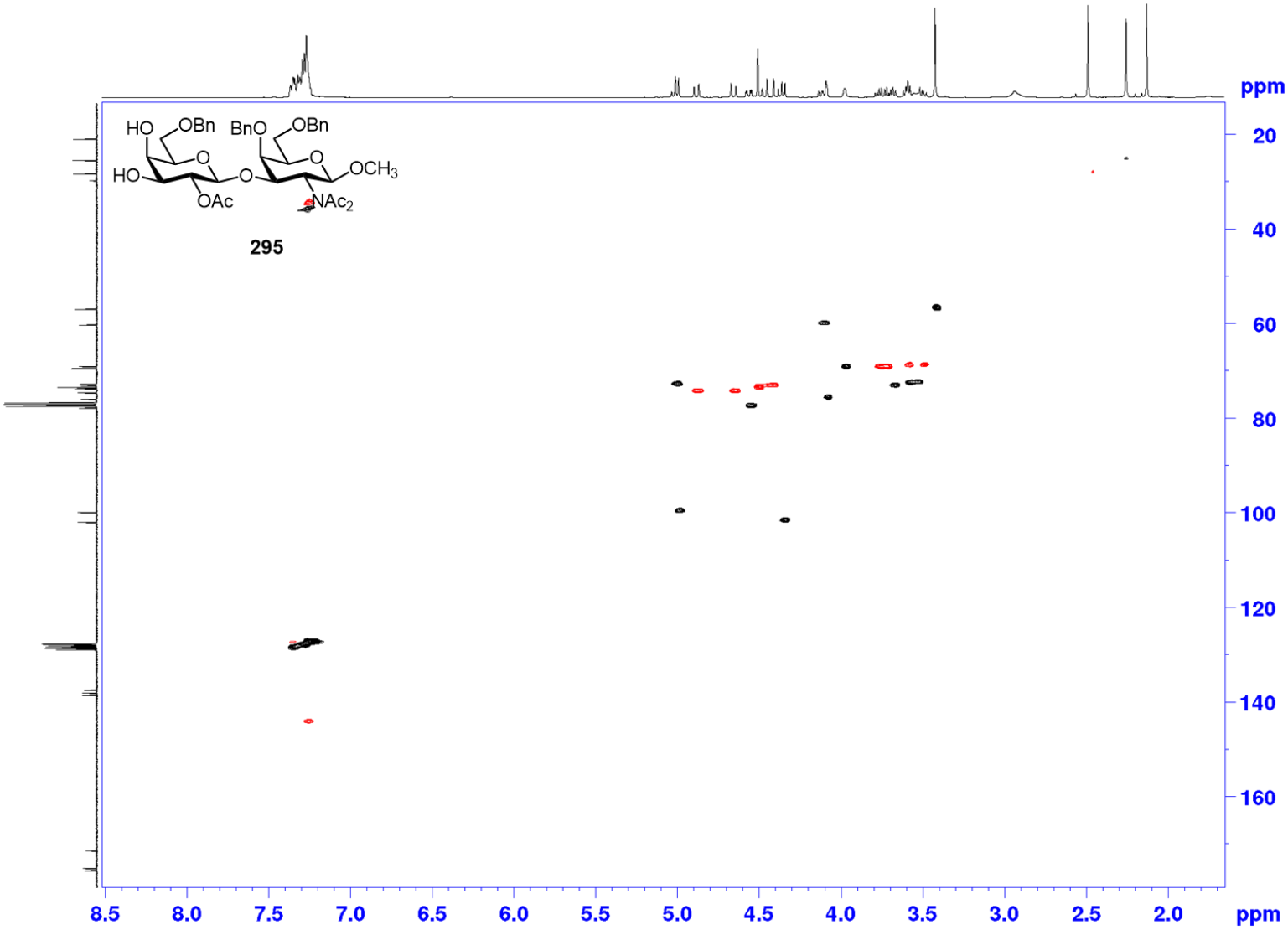
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 295



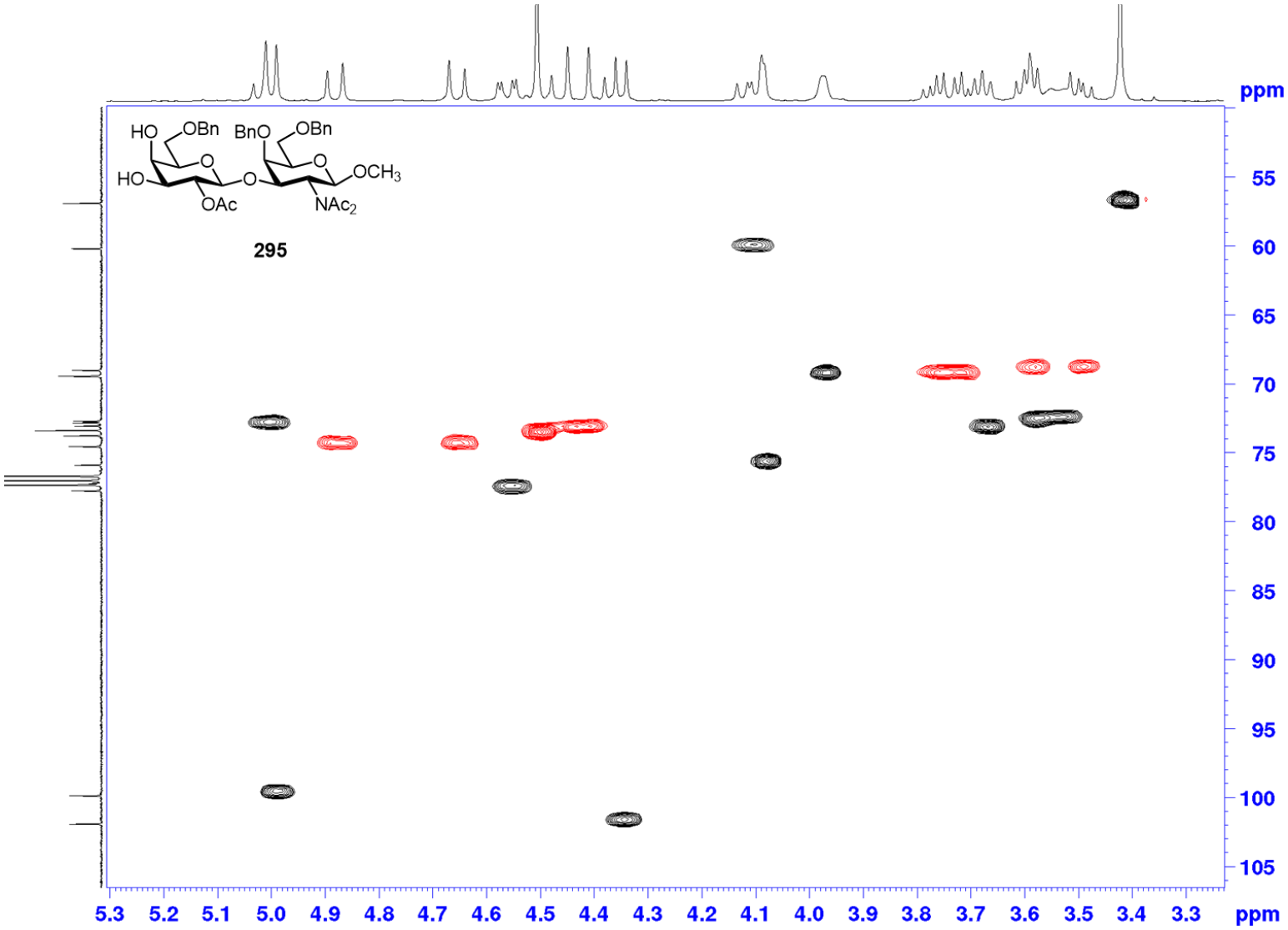
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 295



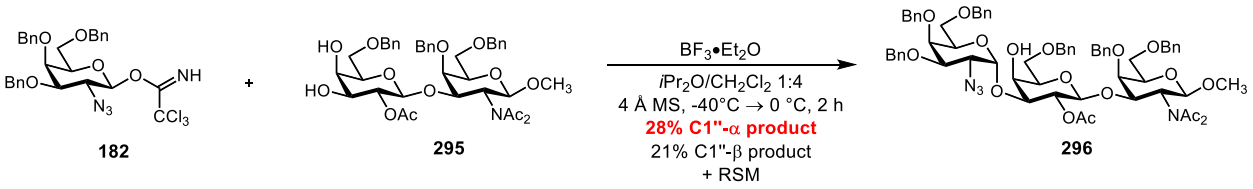
^{1}H-^{13}C HSCQ ($CDCl_3$) of compound 295



^{1}H-^{13}C HSCQ ($CDCl_3$) zoomed in region of compound 295



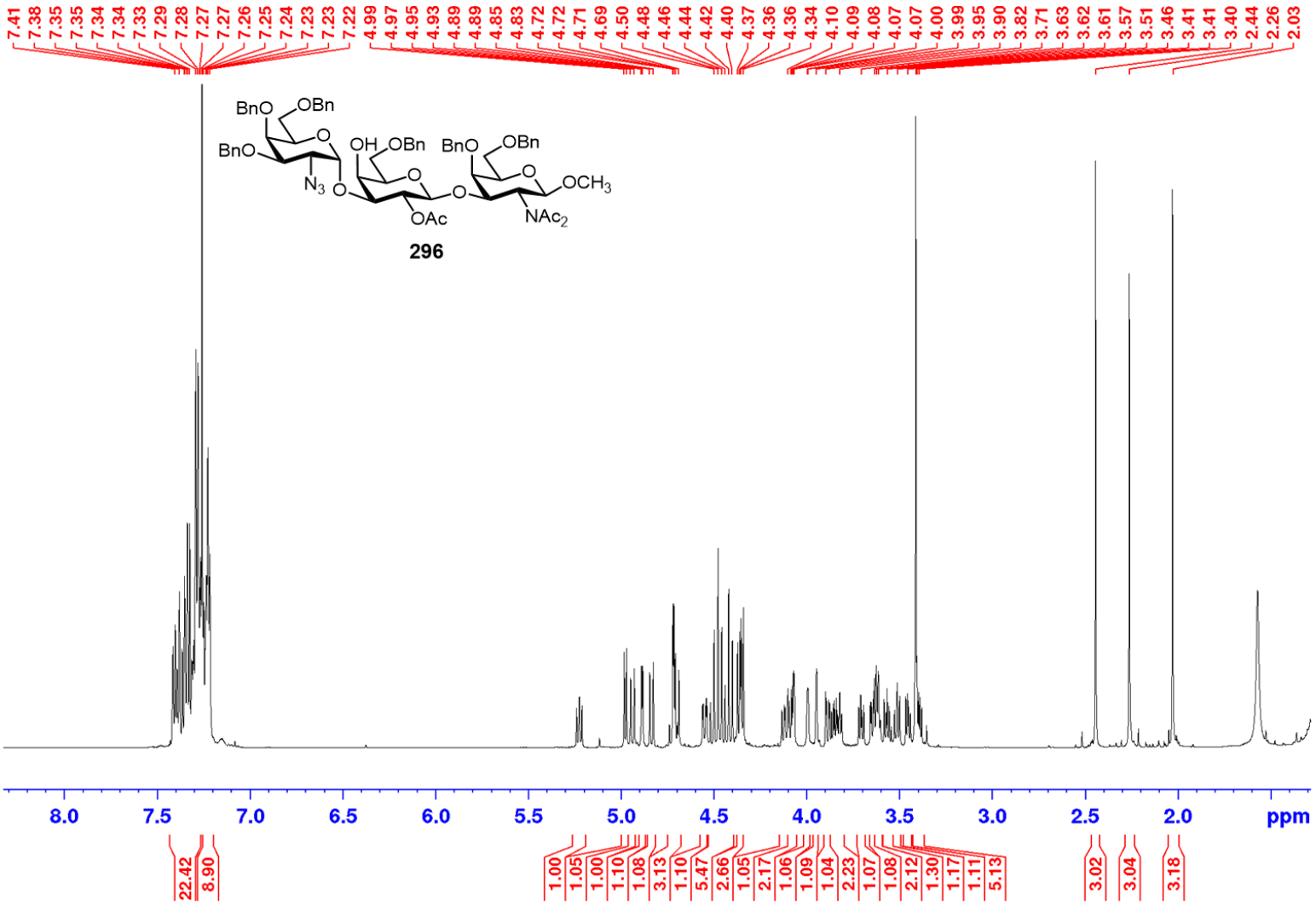
Methyl 3,4,6-tri-*O*-benzyl-2-deoxy-2-azido-α-D-galactopyranosyl-(1→3)-6-*O*-benzyl-2-*O*-(*p*-methoxy)-β-D-galactopyranosyl-(1→3)-4,6-di-*O*-benzyl-2-deoxy-2-(*N*,*N*-diacetyl)-β-D-galactopyranoside (296)



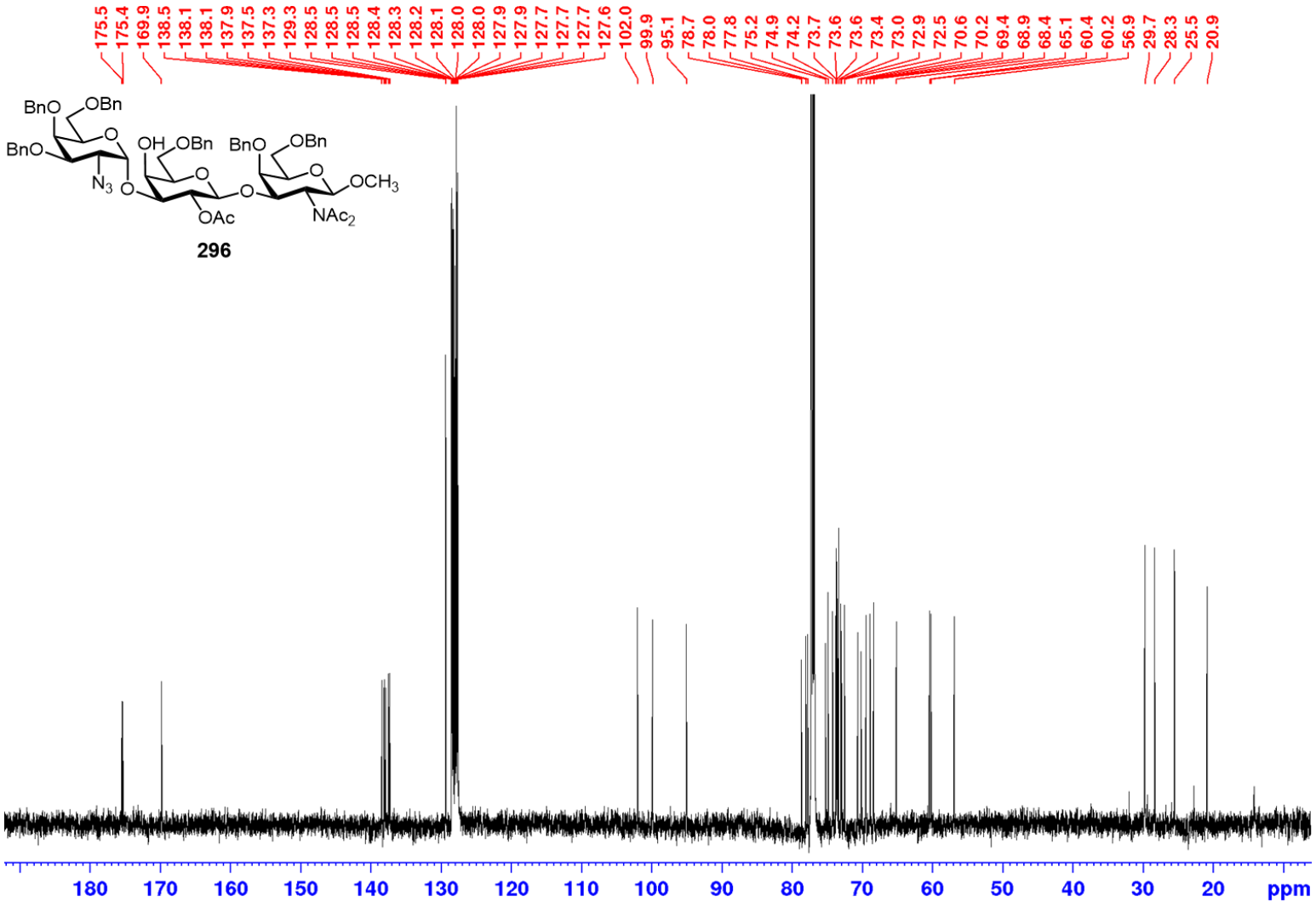
A solution of trichloroacetimidate 3,4,6-tri-*O*-benzyl-2-deoxy-2-azido-β-D-galactopyranoside **182** (1.5 equiv., 0.209 g, 0.338 mmol), disaccharide acceptor methyl 6-*O*-benzyl-2-*O*-(*p*-methoxy)-β-D-galactopyranosyl-(1→3)-4,6-*O*-benzylidene acetal-2-deoxy-2-(*N*,*N*-diacetyl)-β-D-galactopyranoside **295** (1.0 equiv., 0.157 g, 0.221 mmol) in a solution of dry 1:4 iPr$_2$O/CH$_2$Cl$_2$ (2 mL) and were added freshly activated and cooled 4 Å MS (175 mg, 15% m/v) under an argon atmosphere at room temperature. After 1 h, the reaction was cooled to -15 °C then added a solution of BF$_3$·Et$_2$O (0.10 equiv., 6.0 µL, 0.006 mmol). At 2 h, the reaction mixture was neutralized with Et$_3$N and added diluted with CH$_2$Cl$_2$. The reaction mixture was filtered through a pad of Celite within a glass fritted vacuum filter funnel to remove the 4 Å MS. The filtrate was concentrated to a crude oil and purified by silica gel flash column chromatography to give methyl 3,4,6-tri-O-benzyl-2-deoxy-2-azido-α-D-galactopyranosyl-(1→3)-6-*O*-benzyl-2-*O*-(*p*-methoxy)-β-D-galactopyranosyl-(1→3)-4,6-di-*O*-benzyl-2-deoxy-2-(*N*,*N*-diacetyl)-β-D-galactopyranoside **296** (0.0178 g, 0.0147 mmol, 27%) as a white foam. Rf = 0.65 (80:20 EtOAc/hexanes) visualized with ceric ammonium molybdate stain. The trisaccharide methyl 3,4,6-tri-*O*-benzyl-2-deoxy-2-azido-β-D-galactopyranosyl-(1→3)-6-*O*-benzyl-2-*O*-(*p*-methoxy)-β-D-galactopyranosyl-(1→3)-4,6-di-*O*-benzyl-2-deoxy-2-(*N*,*N*-diacetyl)-β-D-

galactopyranoside **296-C1''-β** (0.0137 g, 0.0113 mmol, 21%) was also isolated along with some unconsumed disaccharide starting material acceptor.; ^{1}H NMR (600 MHz, $CDCl_3$): 7.41-7.26 (m, 21H), 7.25-7.19 (m, 9H), 5.23 (dd, *J* = 8.1, 9.5 Hz, 1H), 4.98 (d, *J* = 7.8 Hz, 1H), 4.94 (d, *J* = 11.7 Hz, 1H), 4.89 (d, *J* = 3.8 Hz, 1H), 4.84 (d, *J* = 11.3 Hz, 1H), 4.74-4.69 (m, 3H), 4.56 (dd, *J* = 2.7, 10.8 Hz, 1H), 4.52-4.40 (m, 5H), 4.37-4.34 (m, 3H), 4.12 (dd, *J* = 7.8, 10.8 Hz, 1H), 4.10-4.07 (m, 2H), 4 (d, *J* = 1.5 Hz, 1H), 3.95 (d, *J* = 1.4 Hz, 1H), 3.89 (dd, *J* = 2.6, 10.2 Hz, 1H), 3.87-3.81 (m, 2H), 3.7 (dd, *J* = 6.4, 9.9 Hz, 1H), 3.64 (dd, *J* = 3.3, 9.7 Hz, 1H), 3.63-3.60 (m, 3H), 3.58-3.56 (m, 1H), 3.53-3.50 (m, 1H), 3.47-3.44 (m, 1H), 3.48-3.35 (m, 4H), 2.45 (s, 3H), 2.26 (s, 3H), 2.03 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 175.5, 175.4, 169.9, 138.5, 138.1, 138.1, 137.9, 137.5, 137.3, 129.3, 128.5, 128.5, 128.5, 128.4, 128.3, 128.2, 128.1, 128, 128, 127.9, 127.9, 127.7, 127.7, 127.7, 127.6, 102, 99.9, 95.1, 78.7, 78, 77.8, 75.2, 74.9, 74.2, 73.7, 73.6, 73.6, 73.4, 73, 72.9, 72.5, 70.6, 70.2, 69.4, 68.9, 68.4, 65.1, 60.4, 60.2, 56.9, 29.7, 28.3, 25.5, 20.9.

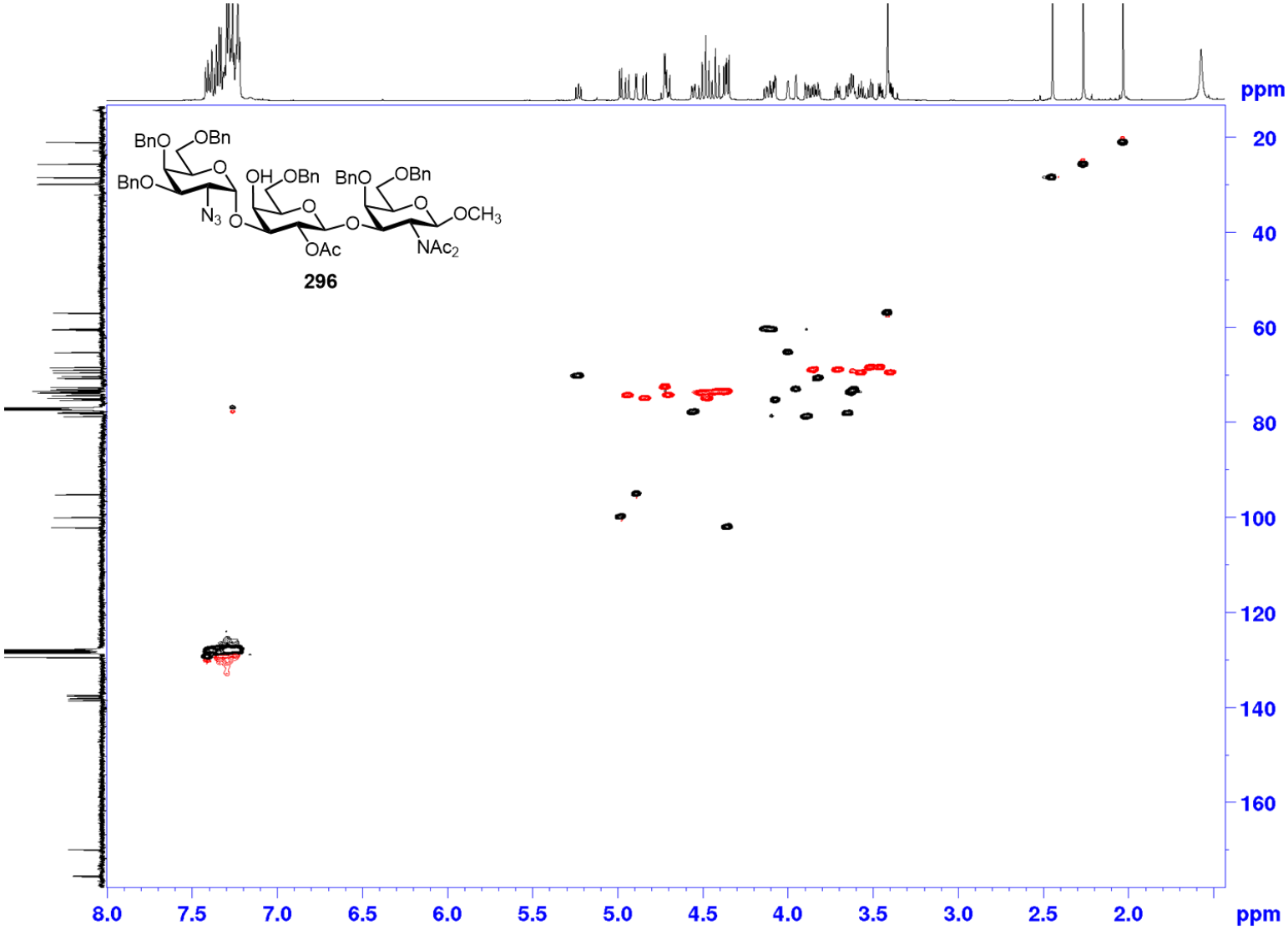
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 296



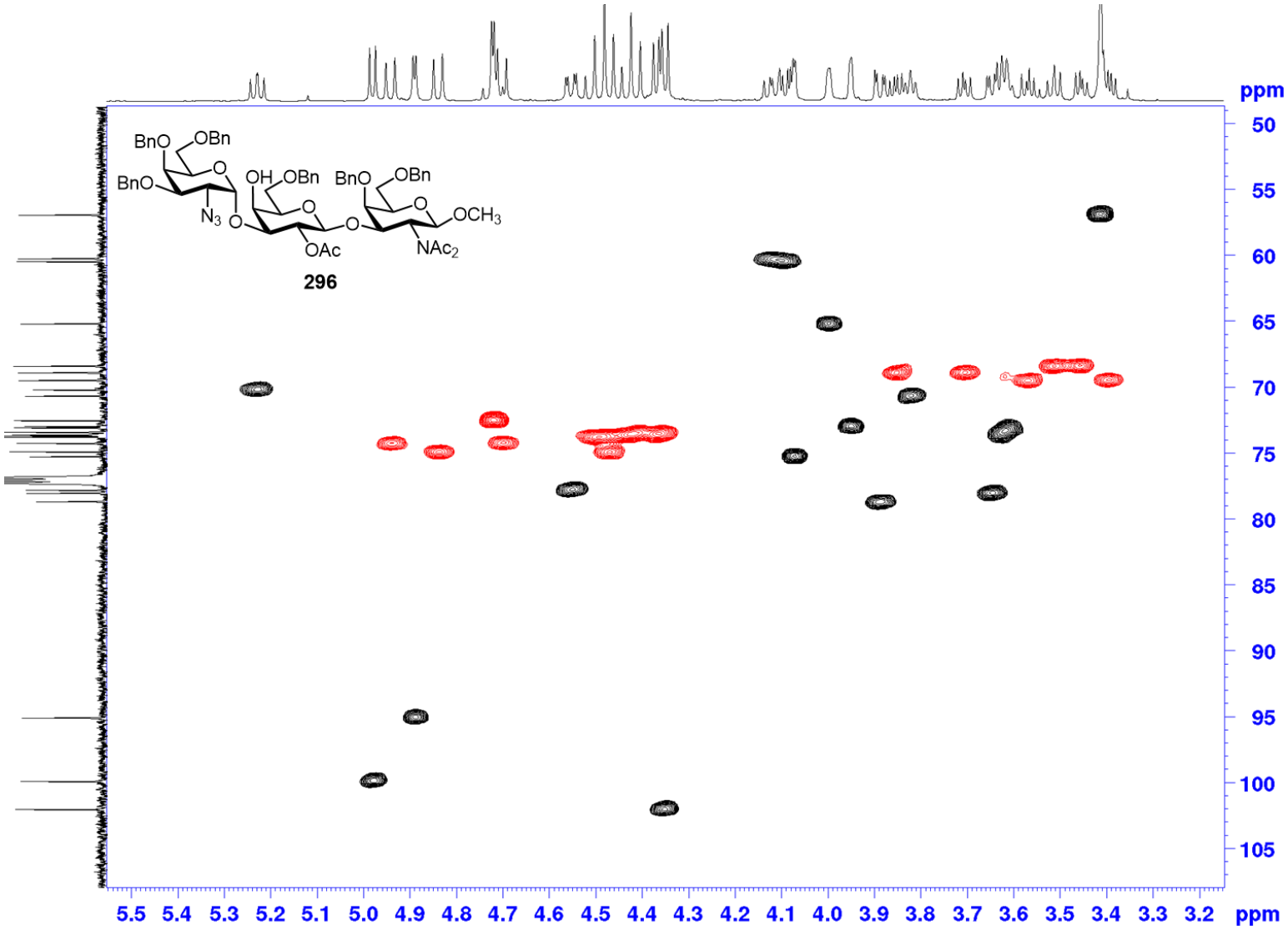
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 296



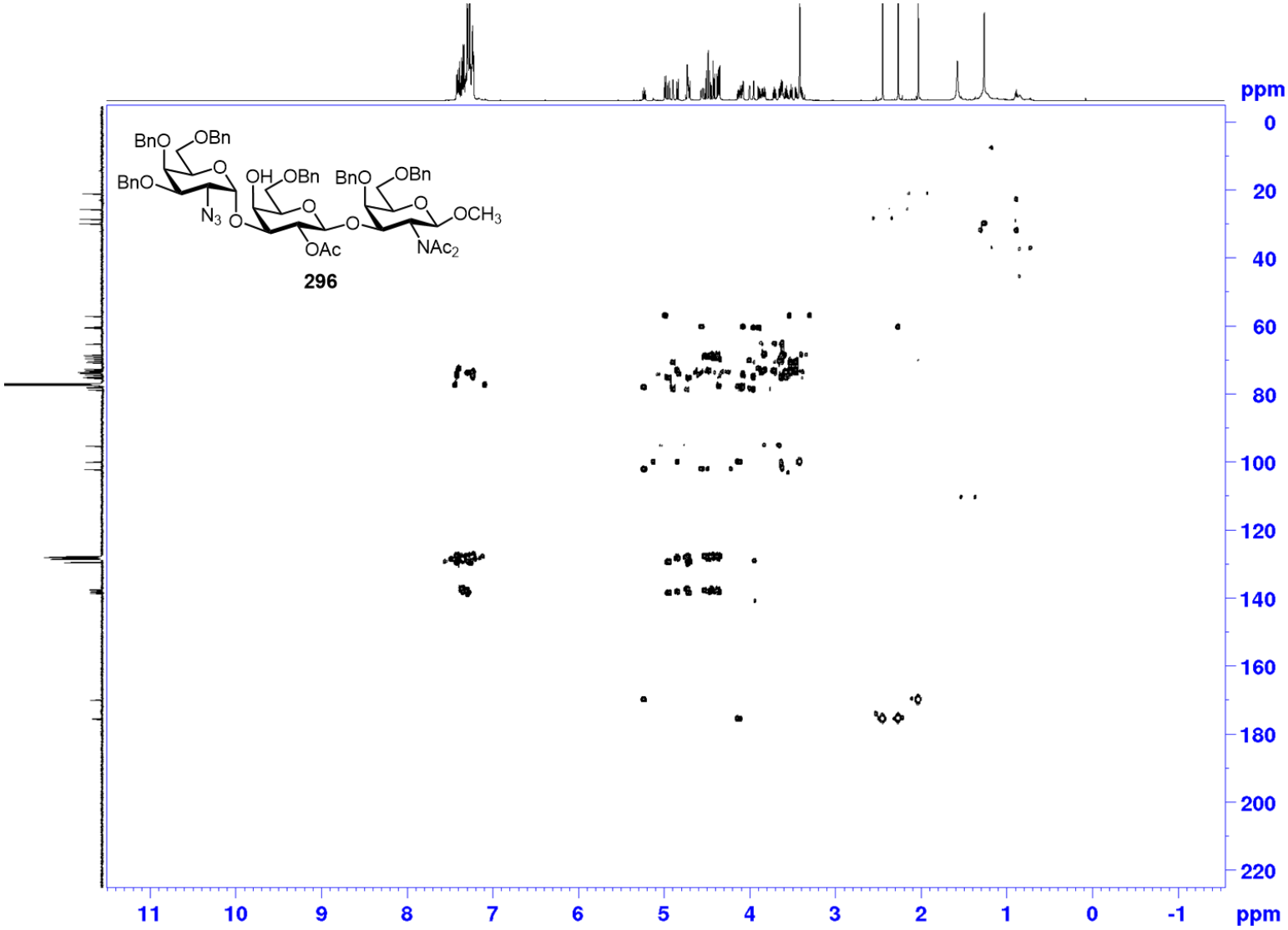
^{1}H-^{13}C HSCQ ($CDCl_3$) of compound 296



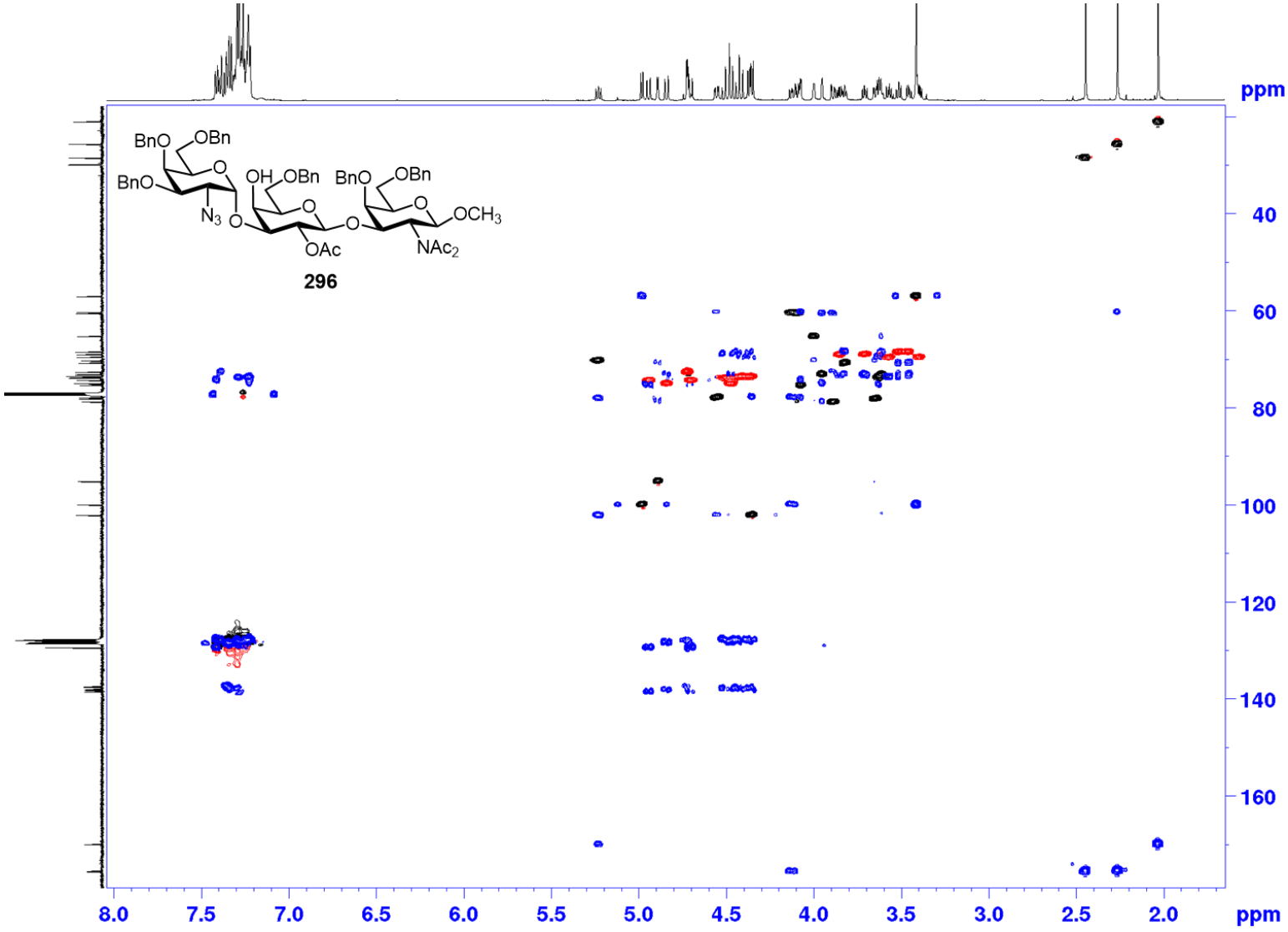
^{1}H-^{13}C HSCQ ($CDCl_3$) zoomed in region of compound 296



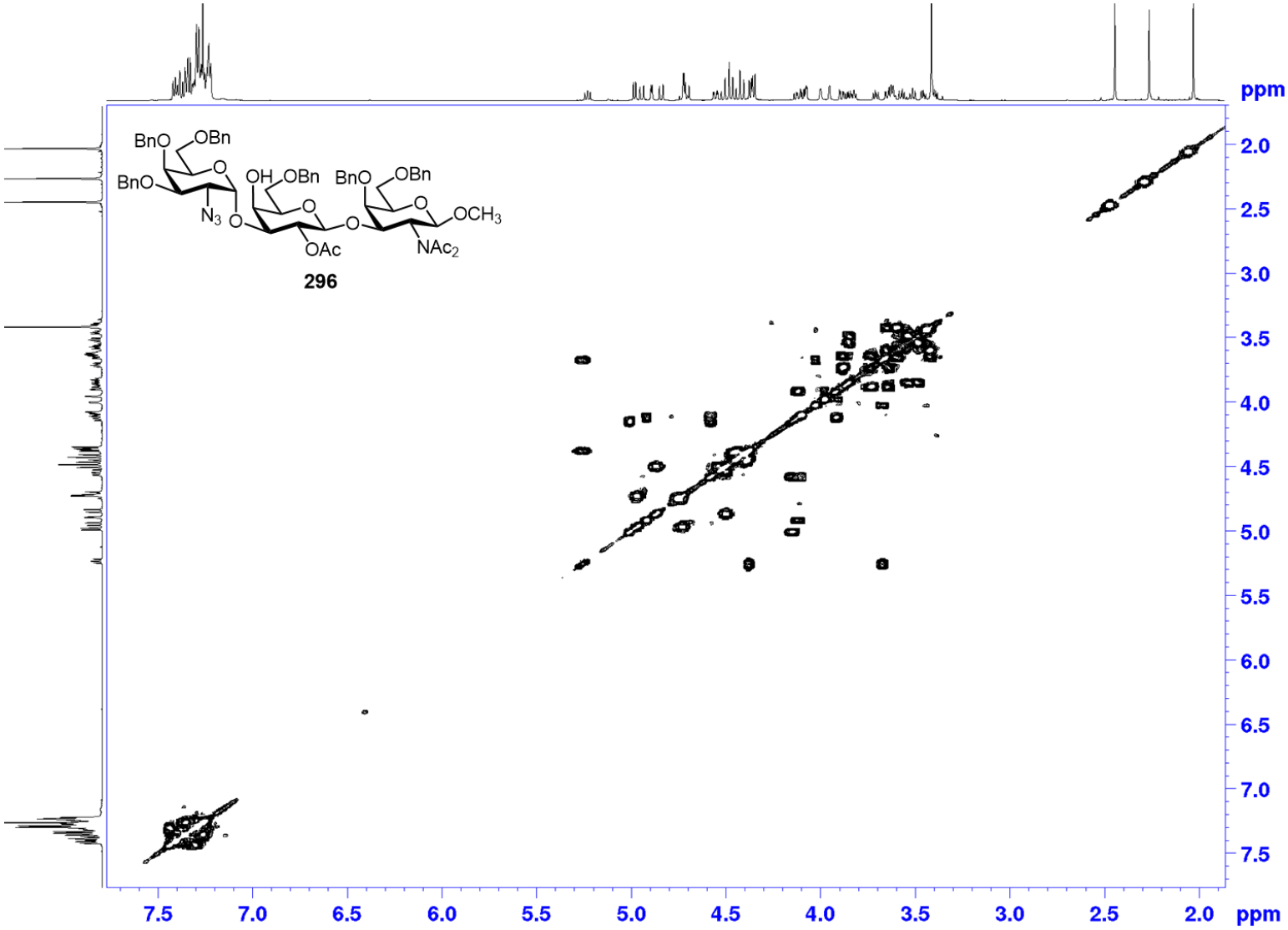
^{1}H-^{13}C HMBC ($CDCl_3$) of compound 296



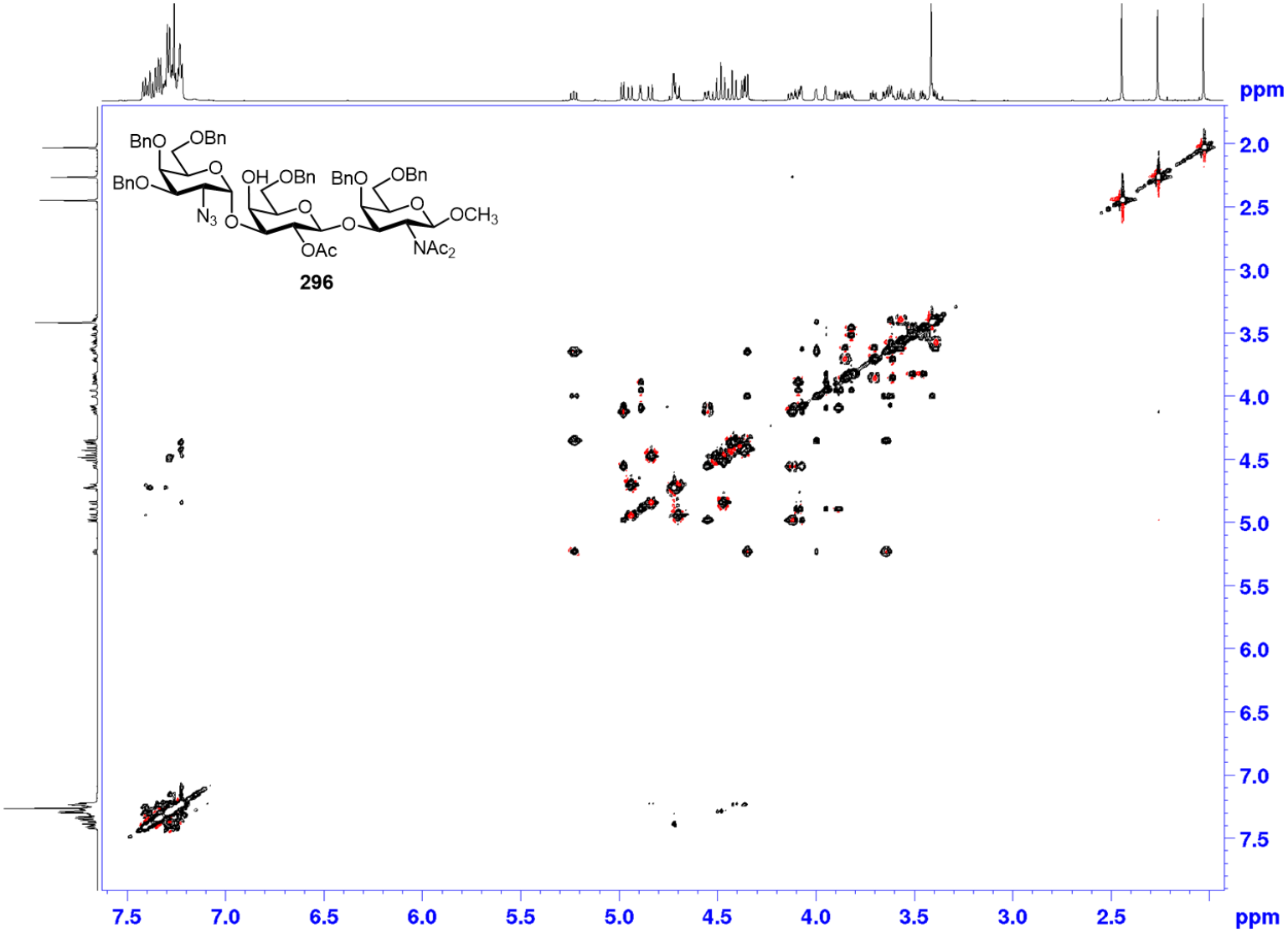
^{1}H-^{13}C HSCQ with ^{1}H-^{13}C HMBC overlay ($CDCl_3$) of compound 296



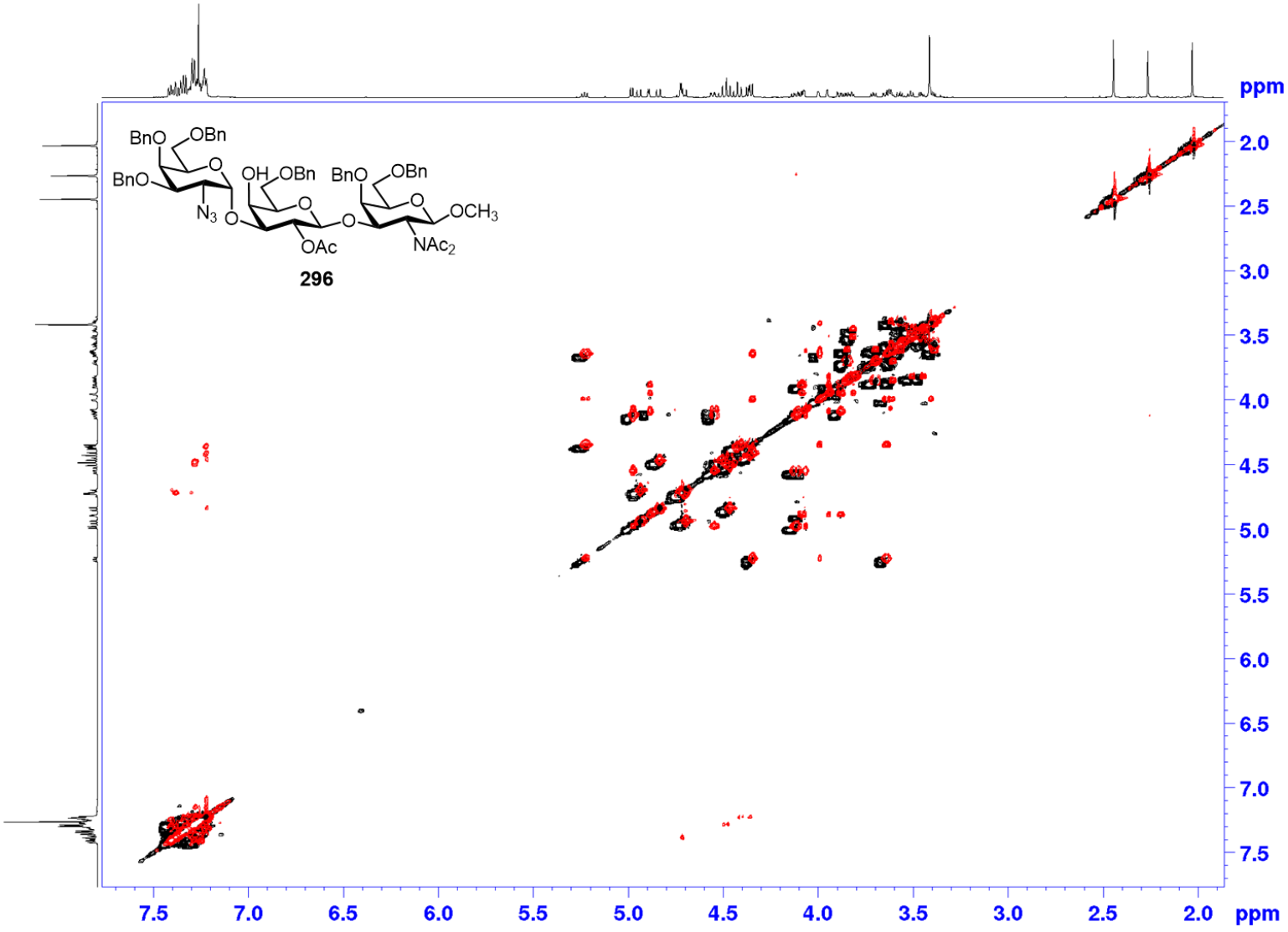
^{1}H-^{1}H COSY ($CDCl_3$) of compound 296



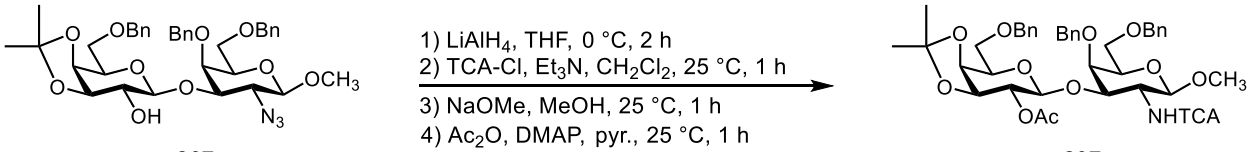
^{1}H-^{1}H TOSY ($CDCl_3$) of compound 296



^{1}H-^{1}H COSY with ^{1}H-^{1}H TOSY overlay ($CDCl_3$) of compound 296



Methyl 6-*O*-benzyl-3,4-*O*-isopropylidene-2-*O*-acetyl-β-D-galactopyranosyl-(1→3)-4,6-di-*O*-benzyl-2-deoxy-2-trichloro acetamido-β-D-galactopyranoside (297)



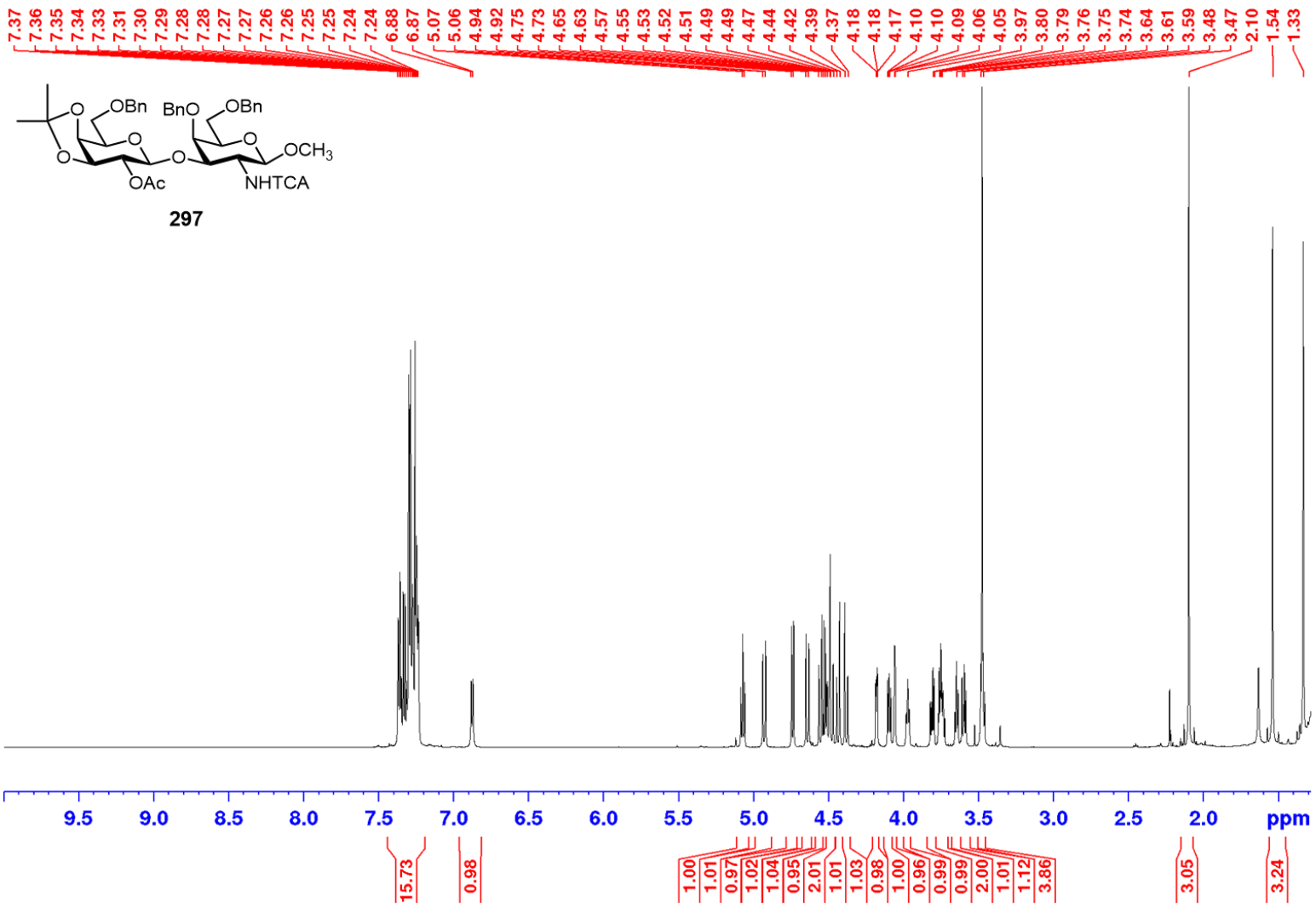
267 **297**

A soltution of methyl 6-*O*-benzyl-*O*-3,4-isopropylidene-β-D-galactopyranosyl-(1→3)-4,6-di-*O*-benzyl-2-deoxy-2-azideo-β-D-galactopyranoside **267** (1.0 equiv., 0.211 g, .274 mmol) was diluted with dry THF (30 mL) under an argon atmosphere at -15°C. The reaction was added a solution of lithium aluminum hydride in THF (1.0 equiv., 0.11 mL, 0.274 mmol, [2.4 M]) dropwise and stirred at 0 °C. At 2 h, the reaction was cooled to -15 °C and added water (0.13 mL) dropwise followed by 15% NaOH aq. (0.13 mL) then water again (0.4 mL) and warmed to room temperature and stirred. After 15 min of stirring at room temperature, the reaction was added $NaSO_4$ to absorb the water which was added to the reaction. The reaction was then filtered through a glass fritted vacuum filter funnel equipped with a pad of Celite. The filtrate was concentrated to dryness. The crude reaction mixture was diluted in dry CH_2Cl_2 (5.0 mL) and added Et_3N (5.0 equiv., 0.19 mL, 1.37 mmol) and TCA-Cl (2.0 equiv., 0.061 mL, 0.547 mmol) dropwise at 0 °C under an argon atmosphere. The reaction was stirred at room temperature. At 1 h, the reaction was added solution of NaOMe (1.0 mL) in dry methanol and stirred at room temperature. After 5 min, the reaction was concentrated to dryness. The reaction mixture was concentrated to dryness. The reaction was then diluted with Ac_2O (5.0 mL) and pyridine (5.0 mL) at 0 °C under an argon atmosphere. At 5 min, the crude reaction mixture was added DMAP (0.10 equiv.). At 1 h, the reaction was cooled to 0°C and added MeOH to consume the

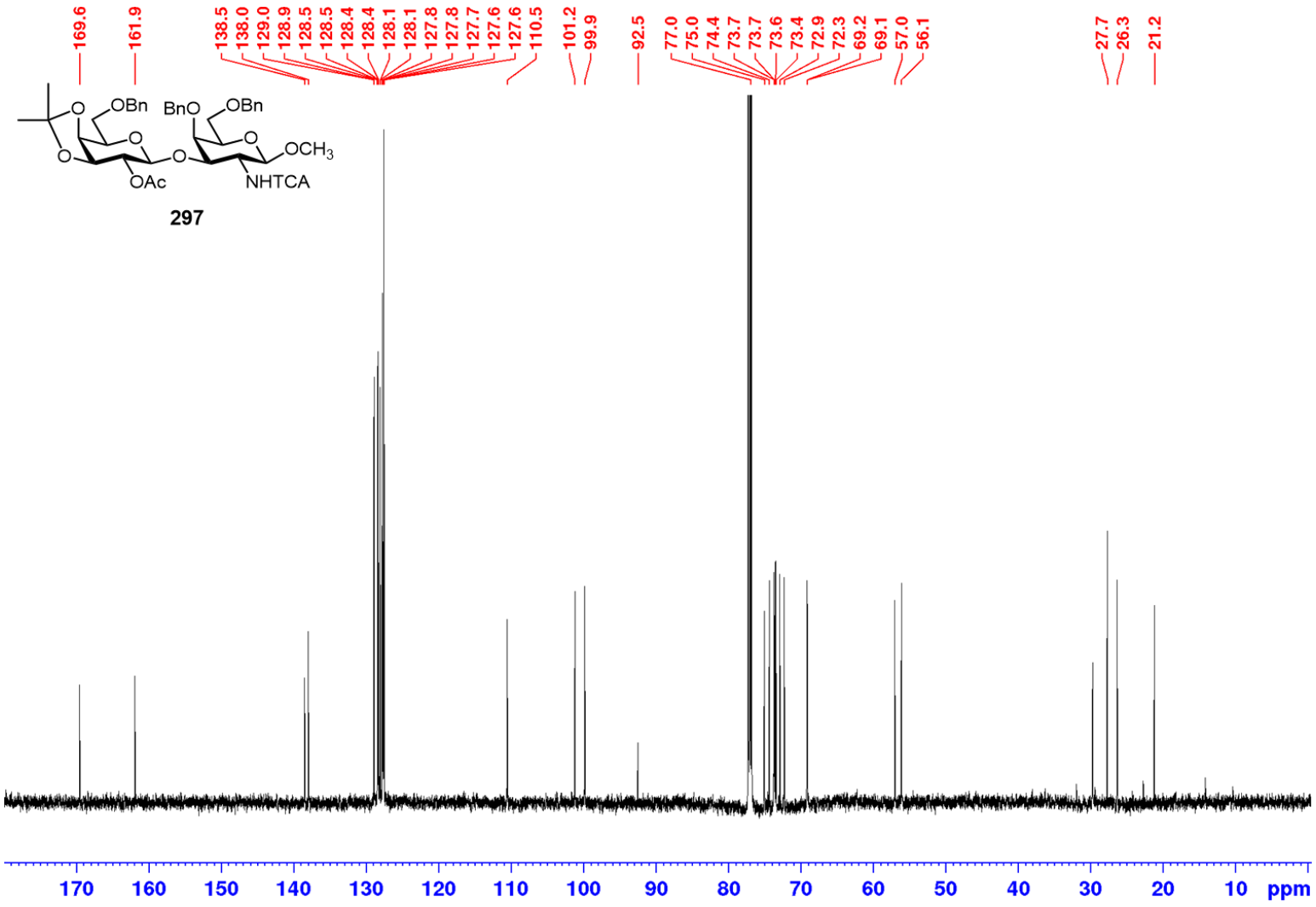
rest of the acetic anhydride. The reaction warmed and stirred at room temperature for 10 min. the reaction was concentrated to a thick oil and taken up in EtOAc and washed with 1 N HCl (2x), water (1x), brine (1x), dried ($NaSO_4$), filtered through a glass fritted vacuum filter funnel to remove drying agent, and concentrated to an oil. The reaction was then purified by silica gel flash column chromatography (75:25 to 100:0 EtOAc/hexanes) to give methyl 6-*O*-benzyl-3,4-*O*-isopropylidene-2-*O*-acetyl-β-D-galactopyranosyl-(1→3)-4,6-di-*O*-benzyl-2-deoxy-2-trichloro acetamido-β-D-galactopyranoside **297** (0.153 g, 0.274 mmol, 65%) as a white foam.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.37-7.23 (m, 15H), 6.89 (d, *J* = 7.1 Hz, 1H), 5.07 (dd, *J* = 8.3, 8.5 Hz, 1H), 4.93 (d, *J* = 11.6 Hz, 1H), 4.75 (d, *J* = 8.3 Hz, 1H), 4.64 (d, *J* = 11.6 Hz, 1H), 4.55 (d, *J* = 12.1 Hz, 1H), 4.52 (d, *J* = 7.9 Hz, 1H), 4.51-4.48 (m, 2H), 4.43 (d, *J* = 12.2 Hz, 1H), 4.37 (d, *J* = 11.9 Hz, 1H), 4.19 (dd, *J* = 2.0, 5.3 Hz, 1H), 4.10 (dd, *J* = 5.3, 7.3 Hz, 1H), 4.06 (d, *J* = 2.5 Hz, 1H), 3.98 (dd, *J* = 2.1, 5.6 Hz, 1H), 3.81 (dd, *J* = 5.3, 10.0 Hz, 1H), 3.76-3.73 (m, 2H), 3.64-3.63 (m, 1H), 3.61-3.58 (m, 1H), 3.48-3.46 (m, 4H), 2.11 (s, 3H), 1.51 (s, 3H), 1.34 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.6, 161.9, 138.5, 138.0, 128.9, 128.9, 128.4, 128.4, 128.3, 128.3, 128.1, 128.1, 127.7, 127.7, 127.6, 127.5, 127.5, 110.5, 101.2, 99.8, 92.5, 76.9, 75.0, 74.3, 73.7, 73.6, 73.5, 73.4, 72.9, 72.3, 69.1, 69.1, 57.0, 56.1, 27.6, 26.3, 21.1.

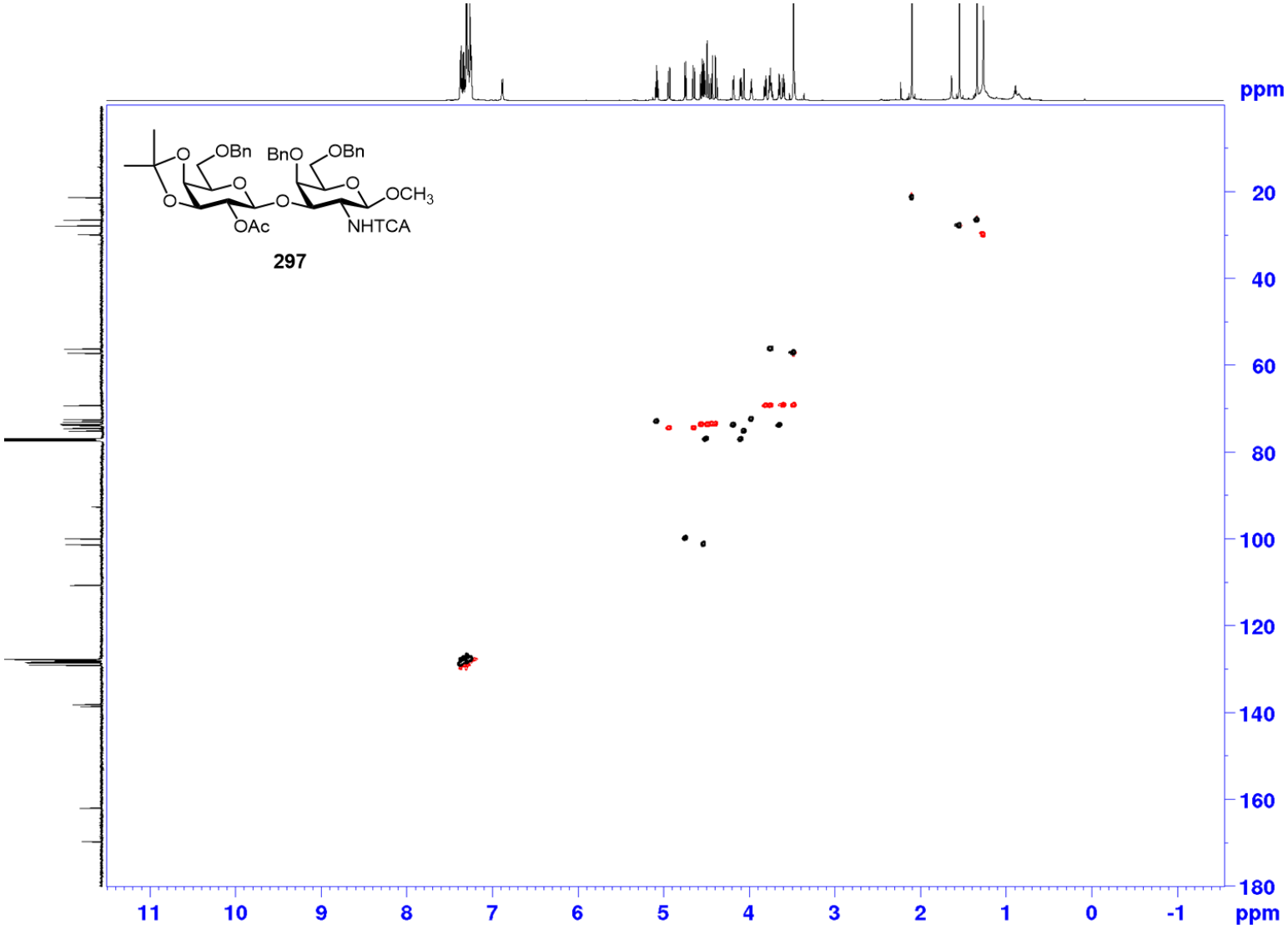
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 297



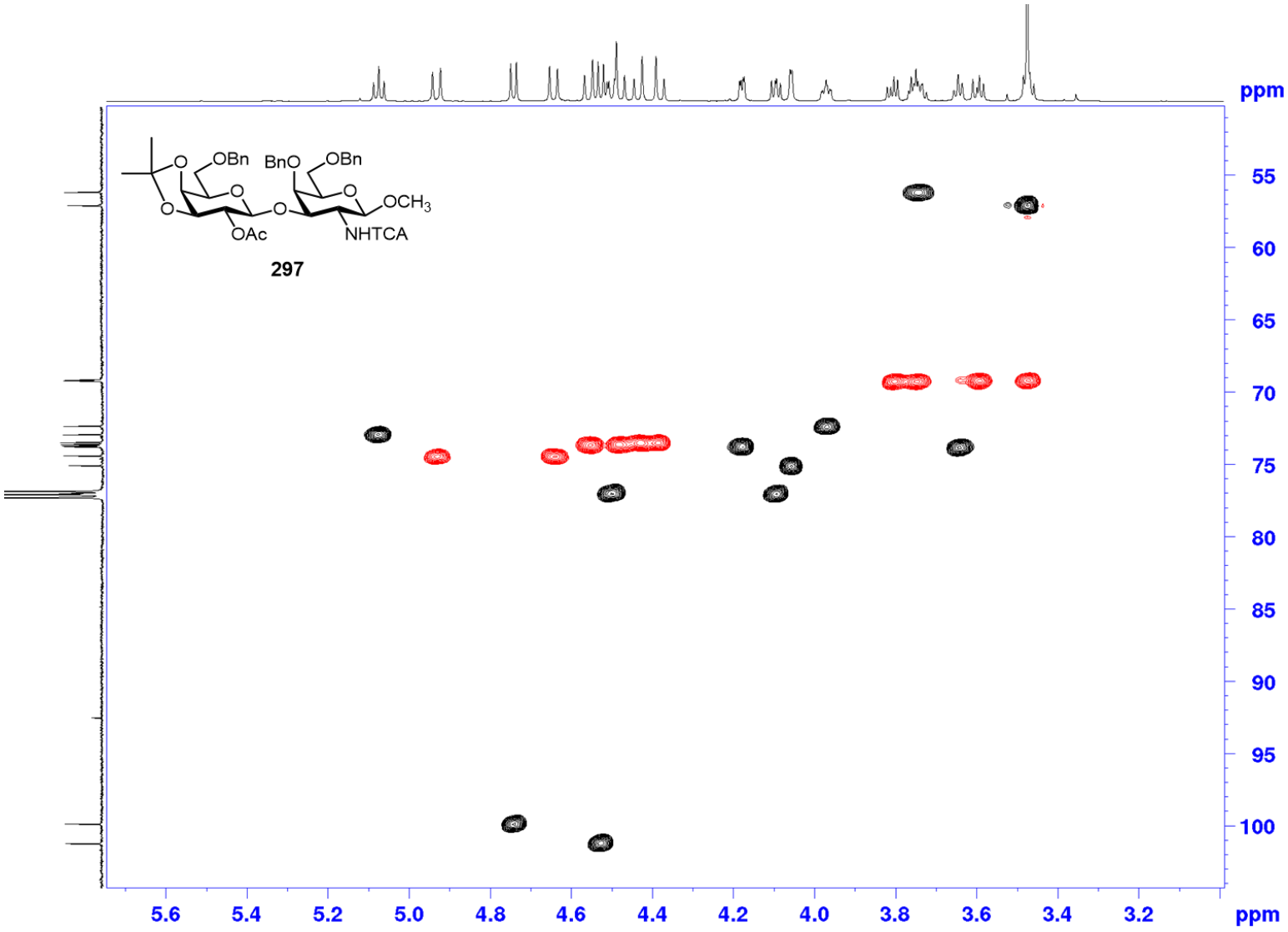
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 297



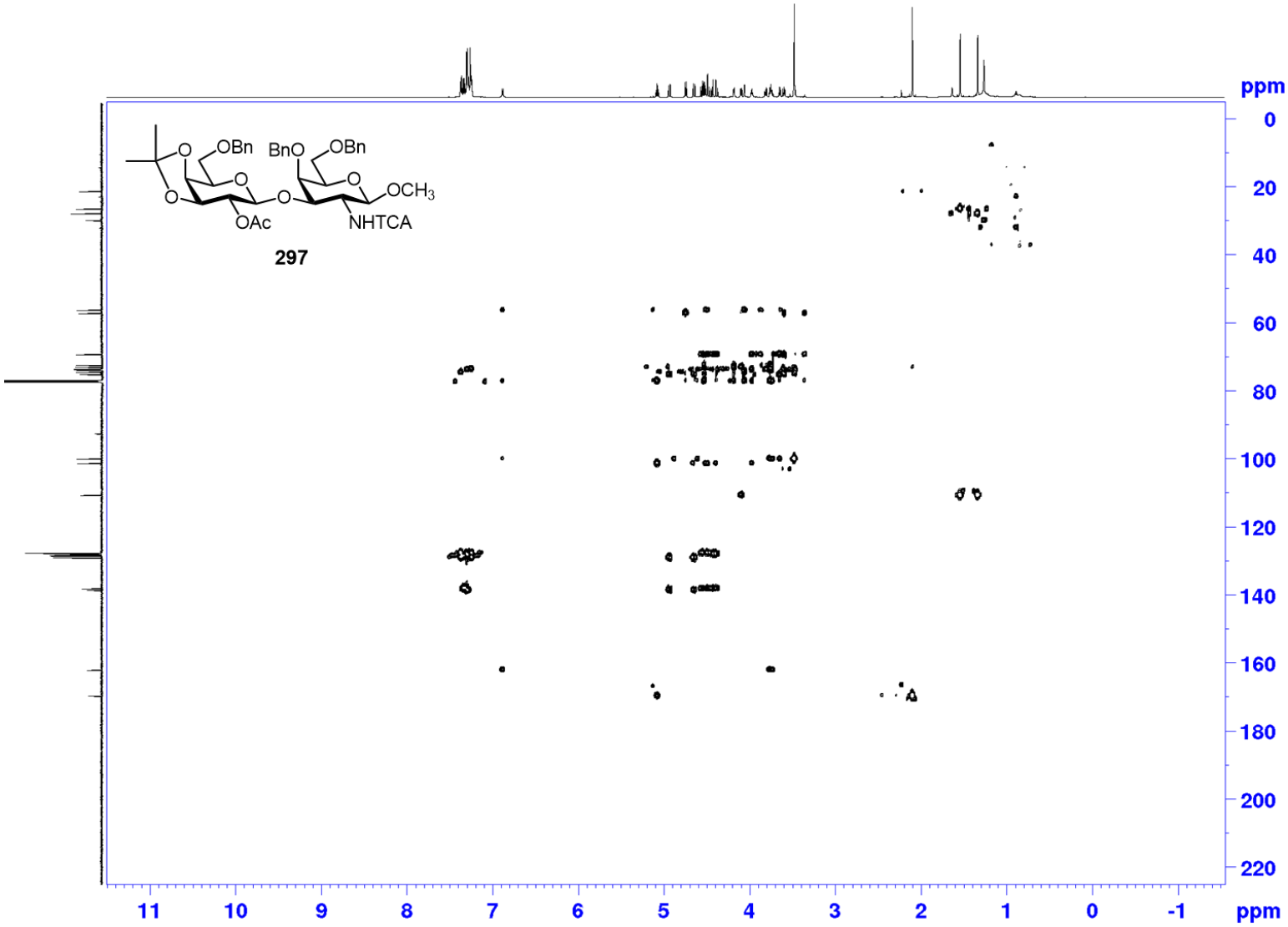
^{1}H-^{13}C HSCQ ($CDCl_3$) of compound 297



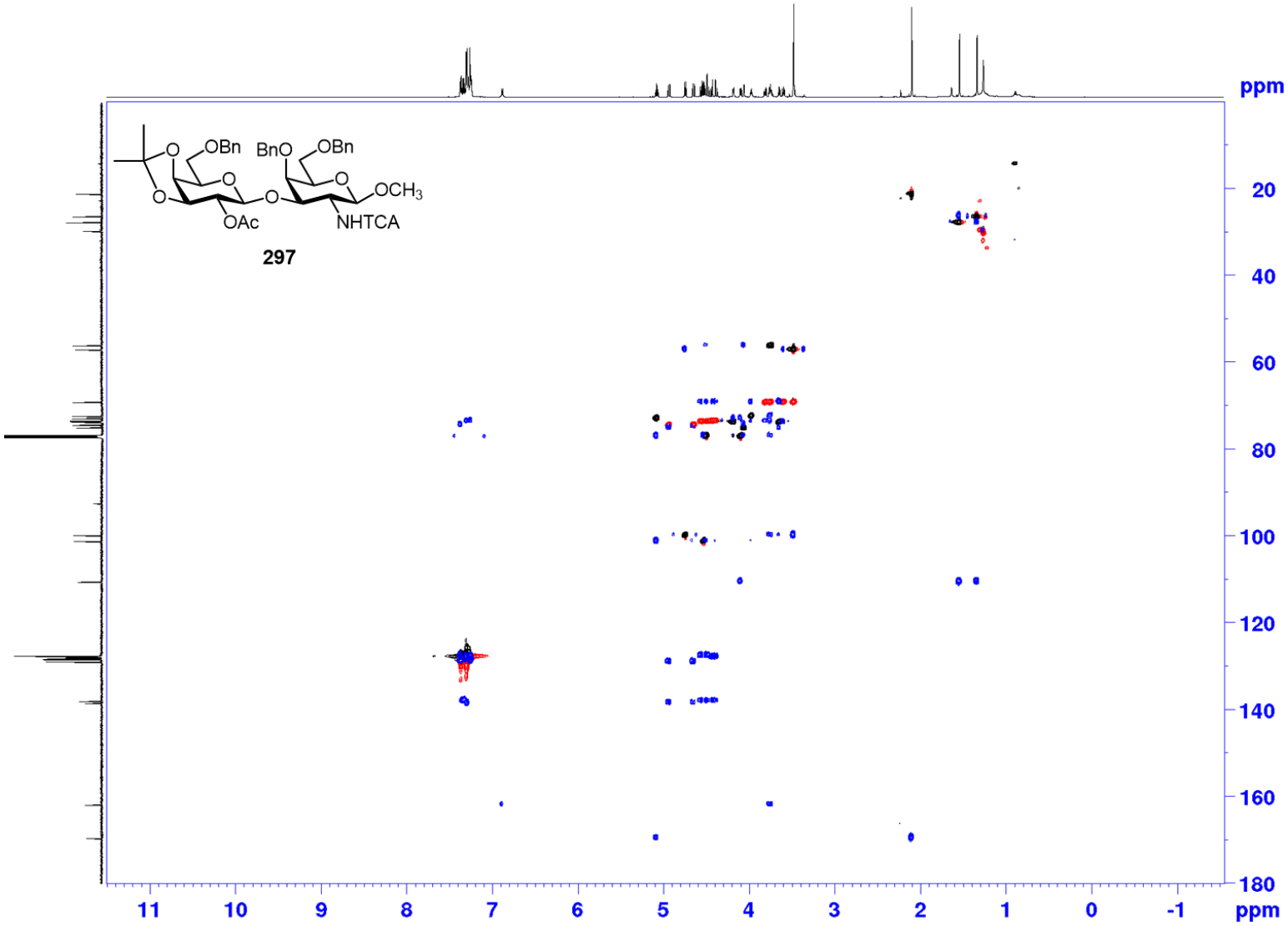
^{1}H-^{13}C HSCQ ($CDCl_3$) zoomed in region of compound 297



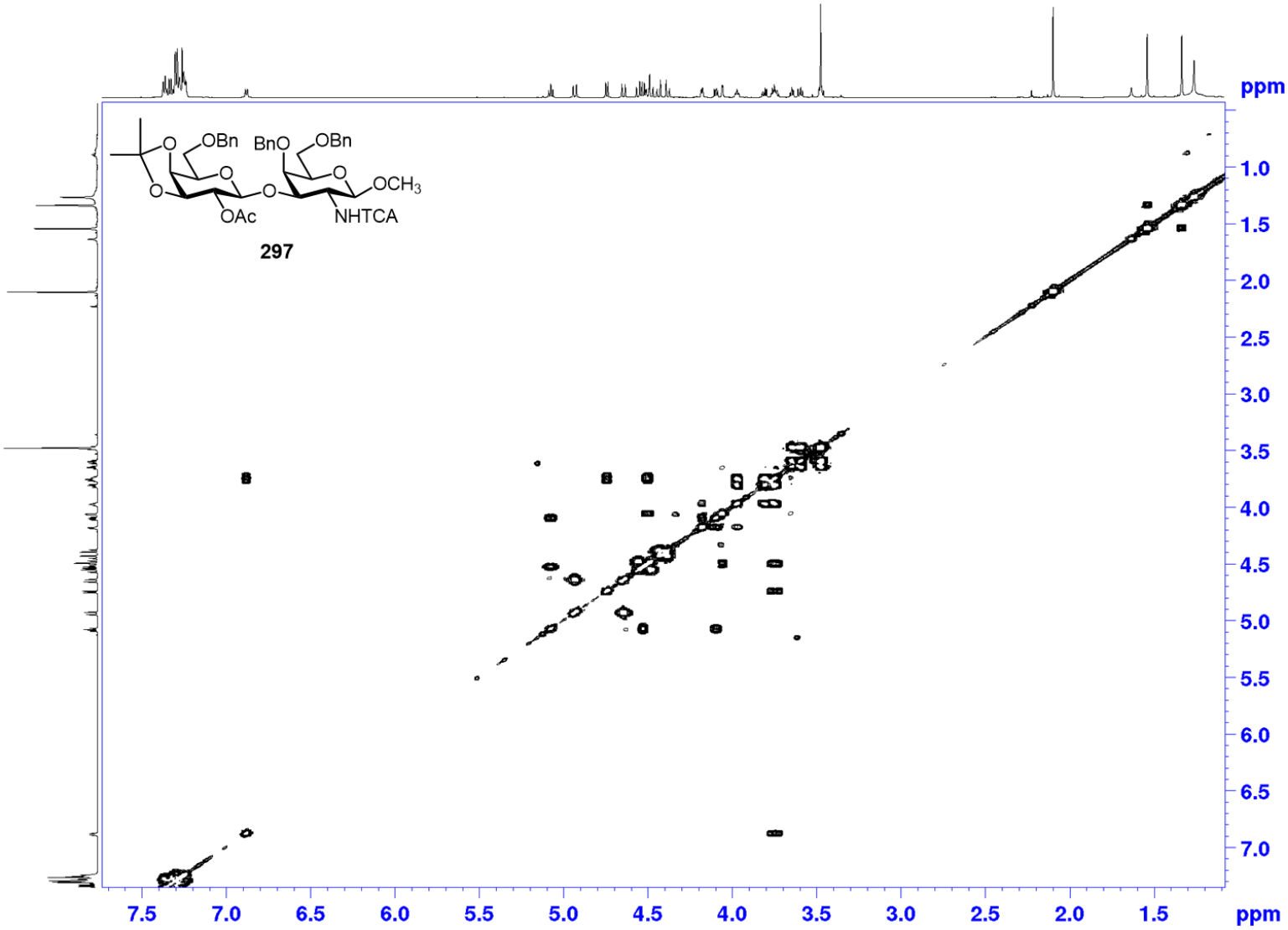
^{1}H-^{13}C HMBC ($CDCl_3$) of compound 297



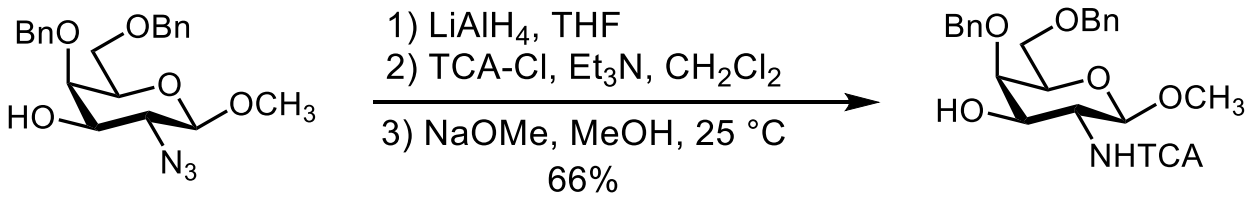
^{1}H-^{13}C HSCQ with ^{1}H-^{13}C HMBC overlay ($CDCl_3$) of compound 297



^{1}H-^{1}H COSY ($CDCl_3$) of compound 297



Methyl 4,6-di-*O*-benzyl-2-deoxy-2-trichloracetamido-β-D-galactopyranoside (DJK-e-584) (297)

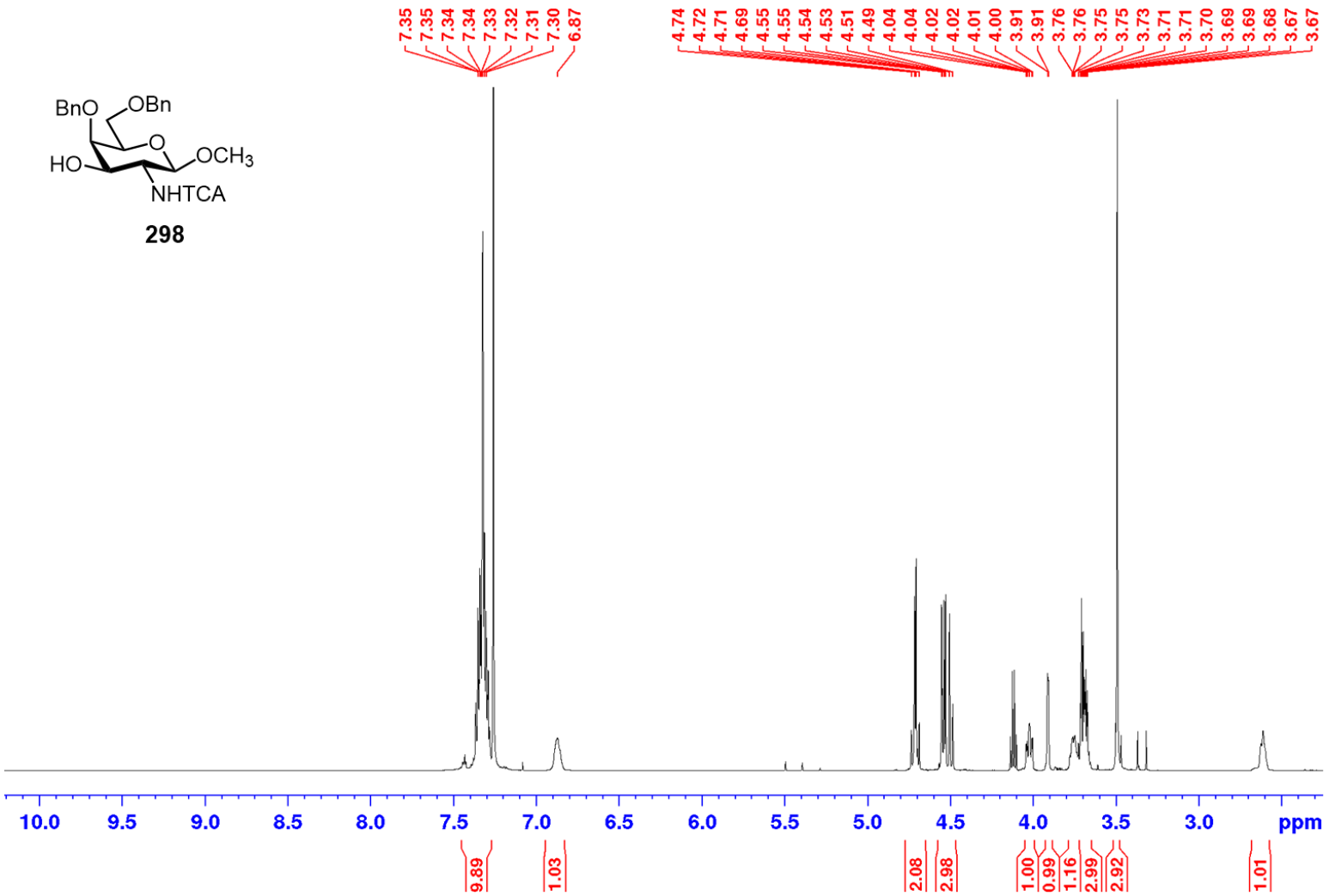


287 **298**

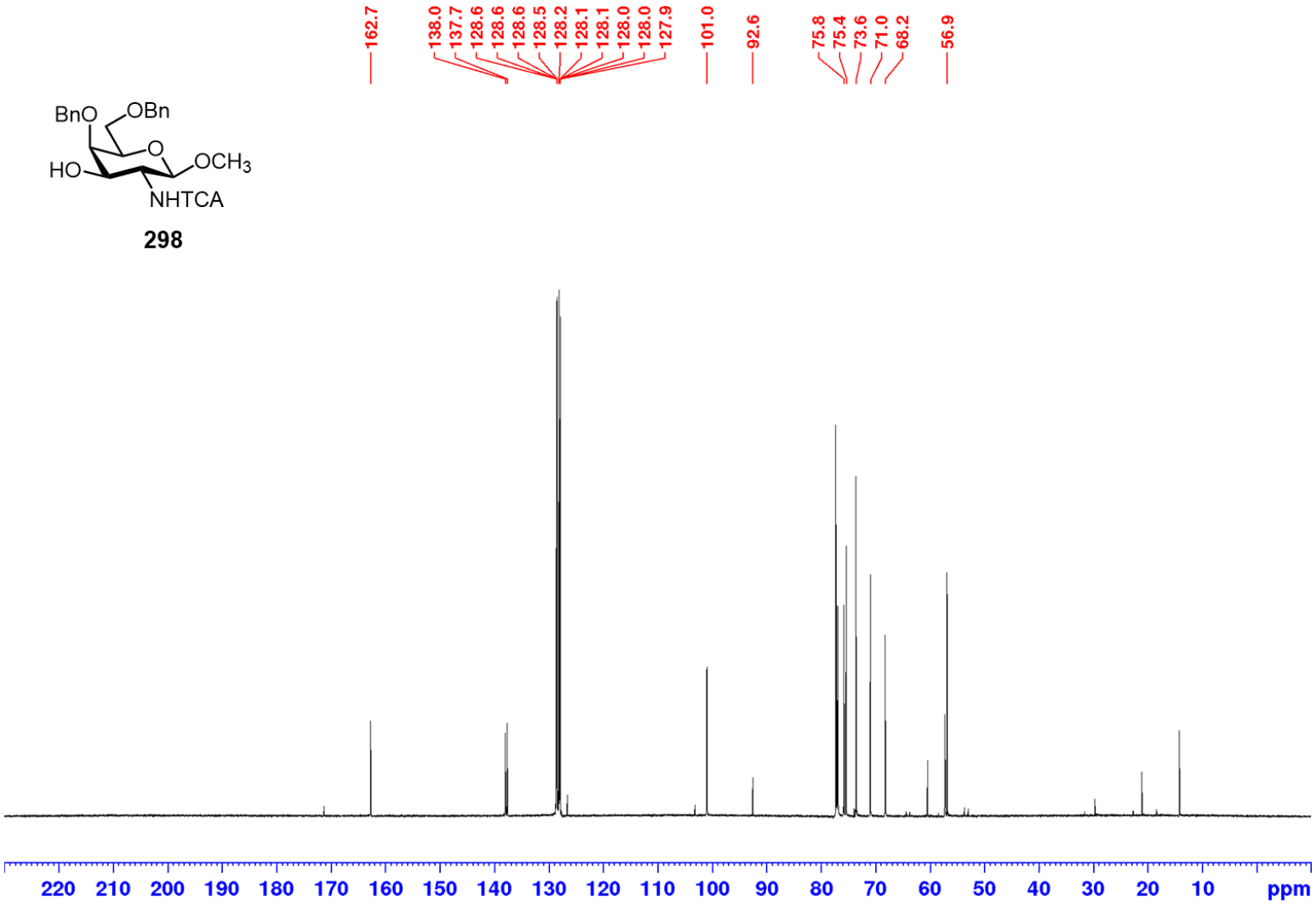
A solution of methyl 4,6-di-*O*-benzyl-3-*O*-acetyl-2-deoxy-2-azido-β-D-galactopyranoside **287** (1.0 equiv., 0.870 g, 2.18 mmol) in dry THF (20 mL) was treated with a solution of lithium aluminum hydride (1.0 equiv., 0.91 mL, 2.18 mmol) at -15 °C under an argon atmosphere. At 1 h, the reaction was quenched by the addition of water (0.15 equiv.), 15% NaOH (0.15 equiv.), then water (0.30 equiv) once again. Crude reaction was added $NaSO_4$ and stirred for 10 min. The crude reaction mixture was filtered though a glass fritted vacuum filter funnel to remove drying agent. The filtrate was concentrated to a white solid. The crude solid was dissolved in dry CH_2Cl_2 (10 mL) and added Et_3N (1.5 equiv., 0.45 mL, 3.27 mmol) and trichloro acetyl chloride (1.5 equiv., 0.37, 3.27 mmol) at 0 °C under an argon atmosphere. At 1 h, the reaction was added CH_3OH and concentrated to a crude syrup. The crude reaction mixture was diluted with dry CH_3OH (10 mL) and added a solution of sodium methoxide until the pH was >11 at room temperature under an argon atmosphere. At 1 hours, the reaction was neutralized by the addition of Amberlite IR 120 (H+) ion exchange resin. The solution was filtered through a glass fritted vacuum funnel equipped with a pad of Celite to remove the acidic resin. The filtrate was concentrated down to a crude oil. The crude oil was purified by silica gel flash column chromatography (20:80 to 40:60 EtOAc/hexanes) to give methyl 4,6-di-*O*-benzyl-2-deoxy-2-

trichloracetamido-β-D-galactopyranoside **298** (0.753 g, 1.45 mmol, 67%) as a white foam. Rf = 0.50 (50:50 EtOAc/hexanes) visualized with ceric ammonium molybdate stain; ^{1}H NMR (400 MHz, $CDCl_3$): δ 736-7.28 (m, 10 H), 6.89 (-OH, 1H), 4.74-4.69 (m, 2H), 4.55-4.49 (m, 3H), 4.02 (dd, *J* = 3.4, 11.0 Hz, 1H), 3.91 (d, *J* = 2.8 Hz, 1H), 3.76-3.73 (m, 1H), 3.71-3.66 (m, 3H), 3.50 (s, 3H), 2.63 (-OH, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 162.7, 138.0, 137.6, 128.6, 128.5 128.5, 128.5, 128.1, 128.1, 128.0, 128.0, 127.9, 127.9, 100.9, 92.5, 75.8, 75.3, 73.6, 70.9, 68.2, 56.8.

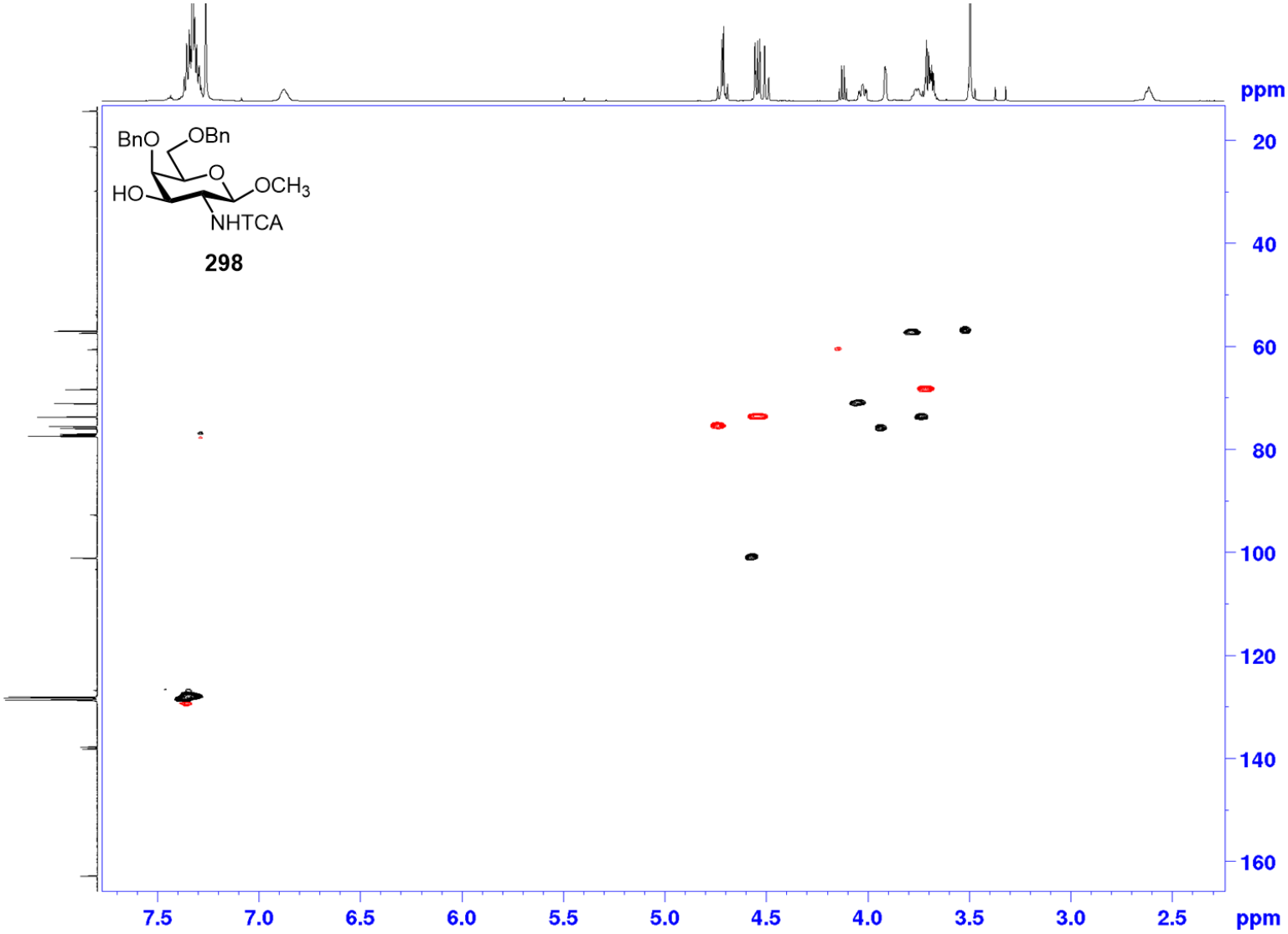
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 298



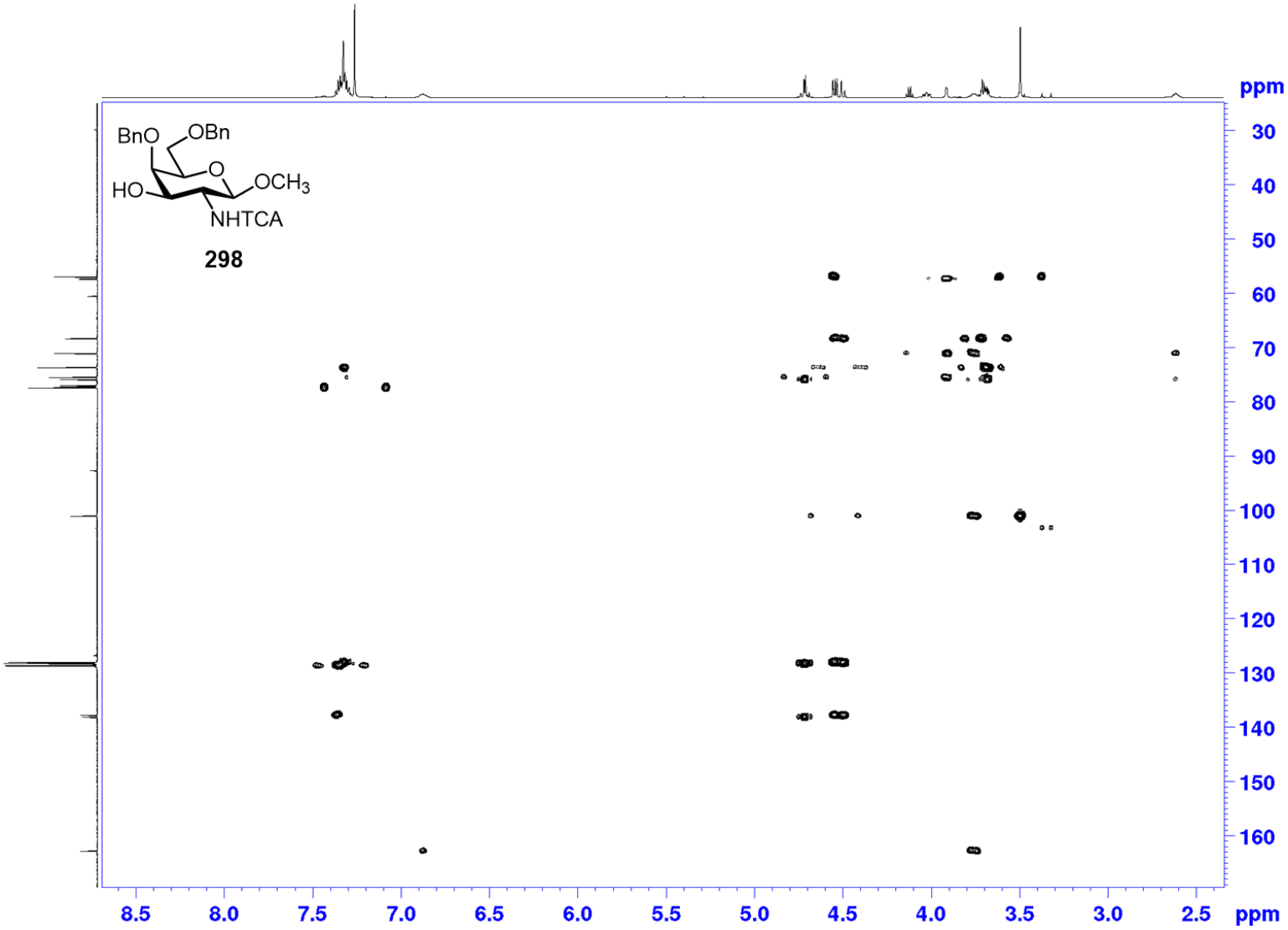
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 298



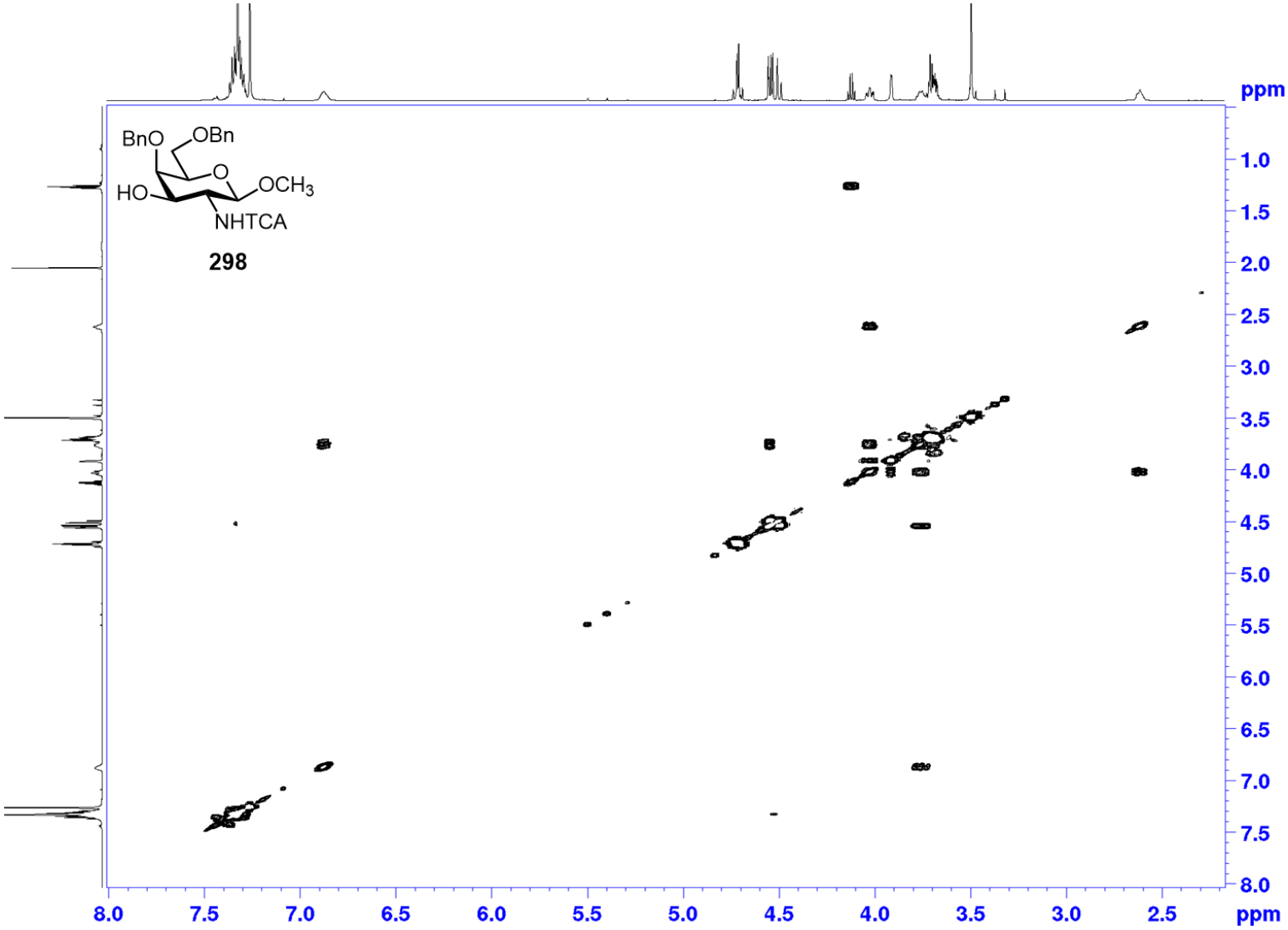
^{1}H-^{13}C HSCQ ($CDCl_3$) of compound 298



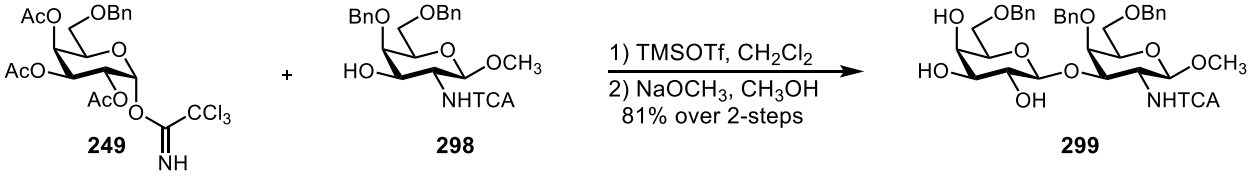
^{1}H-^{13}C HMBC ($CDCl_3$) of compound 298



^{1}H-^{1}H COSY ($CDCl_3$) of compound 298



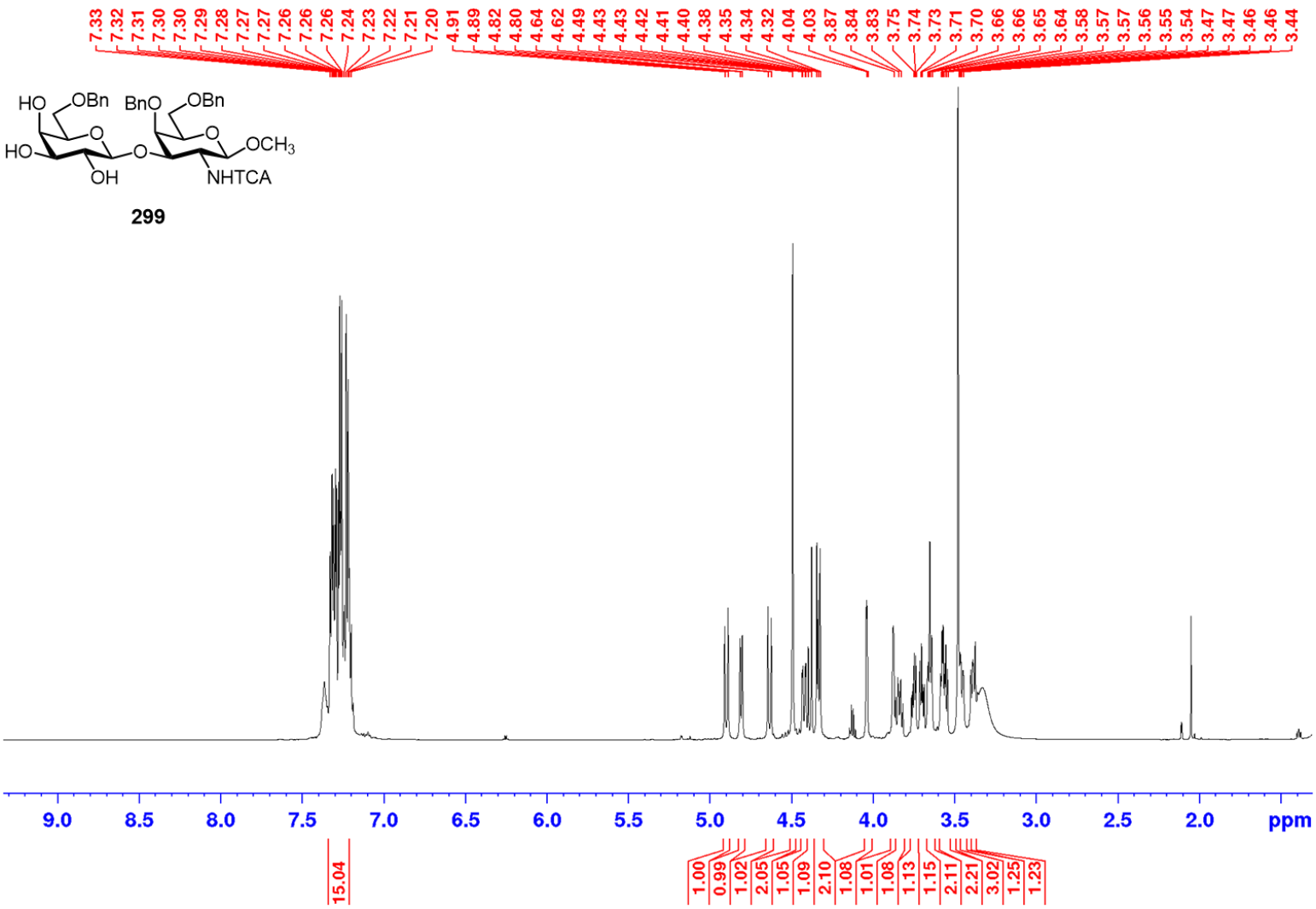
Methyl 6-*O*-benzyl-2-*O*-(*p*-methoxy)-β-D-galactopyranosyl-(1→3)-4,6-di-*O*-benzyl-2-deoxy-2-trichloracetamido-β-D-galactopyranoside (299)



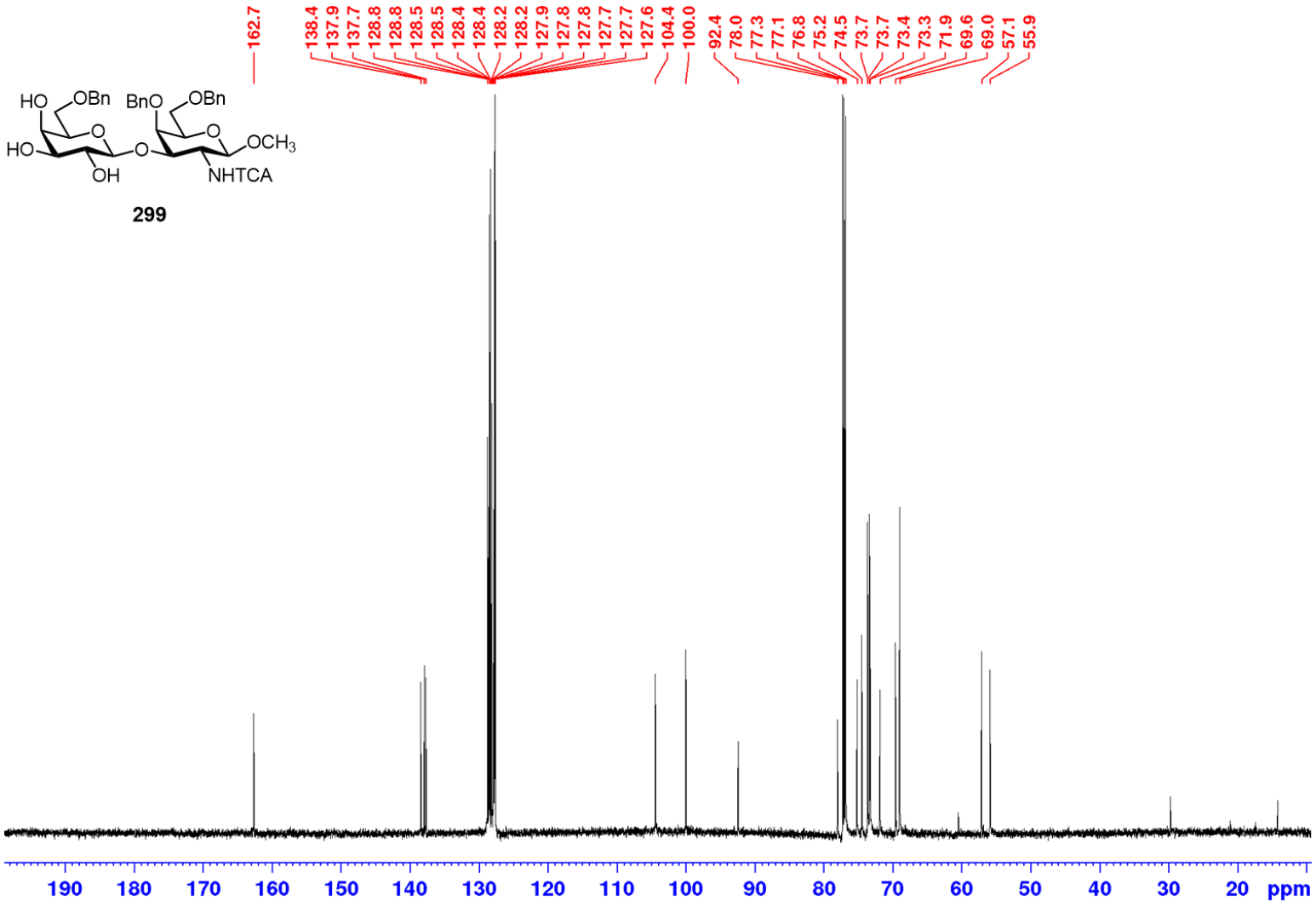
A solution of previously crystalized methyl 4,6-di-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranoside acceptor **298** (1.0 equiv., 0.311 g, 0.600 mmol) in dry CH_2Cl_2 (6.0 mL) and previously crystalized 2,3,4-tri-*O*-acety-6-*O*-benzy-α-D-galactopyranosyl trichloroacetimidate donor **249** (1.5 equiv., 0.487 g, 0.900 mmol) was stirred over 4 Å molecular sieves (450 g, ~15% m/v) at room temperature for 2 hours. The solution was then cooled to -40 °C, treated with TMSOTf (0.1 equiv., 0.011 mL, 0.060 mmol). The reaction mixture was stirred for 1 h at room temperature. The reaction was neutralized by the addition of Et_3N (0.5 equiv., 0.042 mL, 0.300 mmol), passed through a glass fritted vacuum filter funnel equipped with a pad of Celite, concentrated to a crude foam. The crude foam was diluted with dry CH_3OH (10 mL) and added a solution of sodium methoxide dropwise until the pH was > 11. After 3 h, the reaction mixture was neutralized by the addition of Amberlite IR 120 (H+) ion exchange resin. The solution was filtered through a glass fritted vacuum funnel equipped with a pad of Celite to remove the acidic resin. The filtrate was concentrated down to a crude oil. The crude oil was purified by silica gel flash column chromatagraphy (20:80 to 60:40 EtOAc/hexanes) and sub*J*ected to silica gel flash column chromatography (20:80 to 50:50 EtOAc/hexanes) to give methyl 6-*O*-benzyl-2,3,4-*O*-aceyl-β-D-galactopyranosyl-(1→3)-4,6-di-*O*-benzyl-2-deoxy-2-azideo-β-D-galactopyranoside **299** (0.261 g, 0.338 mmol, 81%) as a white amorphous

solid.: Rf = 0.45 (40:60 EtOAc/hexanes) visualized with ceric ammonium molybdate stain; ^{1}H NMR (400 MHz, $CDCl_3$): 7.33-7.20 (m, 15H), 4.9 (d, *J* = 11.7 Hz, 1H), 4.81 (d, *J* = 8.3 Hz, 1H), 4.64 (d, *J* = 11.7 Hz, 1H), 4.49 (d, *J* = 1.1 Hz, 2H), 4.42 (dd, *J* = 2.6, 10.9 Hz, 1H), 4.38 (d, *J* = 12.1 Hz, 1H), 4.35-4.32 (m, 2H), 4.03 (d, *J* = 2.6 Hz, 1H), 3.88-3.86 (m, 1H), 3.85-3.81 (m, 1H), 3.76-3.73 (m, 1H), 3.71-3.67 (m, 1H), 3.66-3.63 (m, 2H), 3.58-3.53 (m, 2H), 3.48 (s, 3H), 3.45 (dd, *J* = 2.8, 9.5 Hz, 1H), 3.39 (dd, *J* = 5.9, 9.6 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 162.7, 138.4, 137.9, 137.7, 128.8, 128.8, 128.5, 128.5, 128.4, 128.4, 128.2, 128.2, 127.9, 127.8, 127.8, 127.7, 127.7, 127.6, 104.4, 100.0, 92.4, 78.0, 77.3, 77.1, 76.8, 75.2, 74.5, 73.7, 73.7, 73.4, 73.3, 71.9, 69.6, 6, 57.1, 55.9.

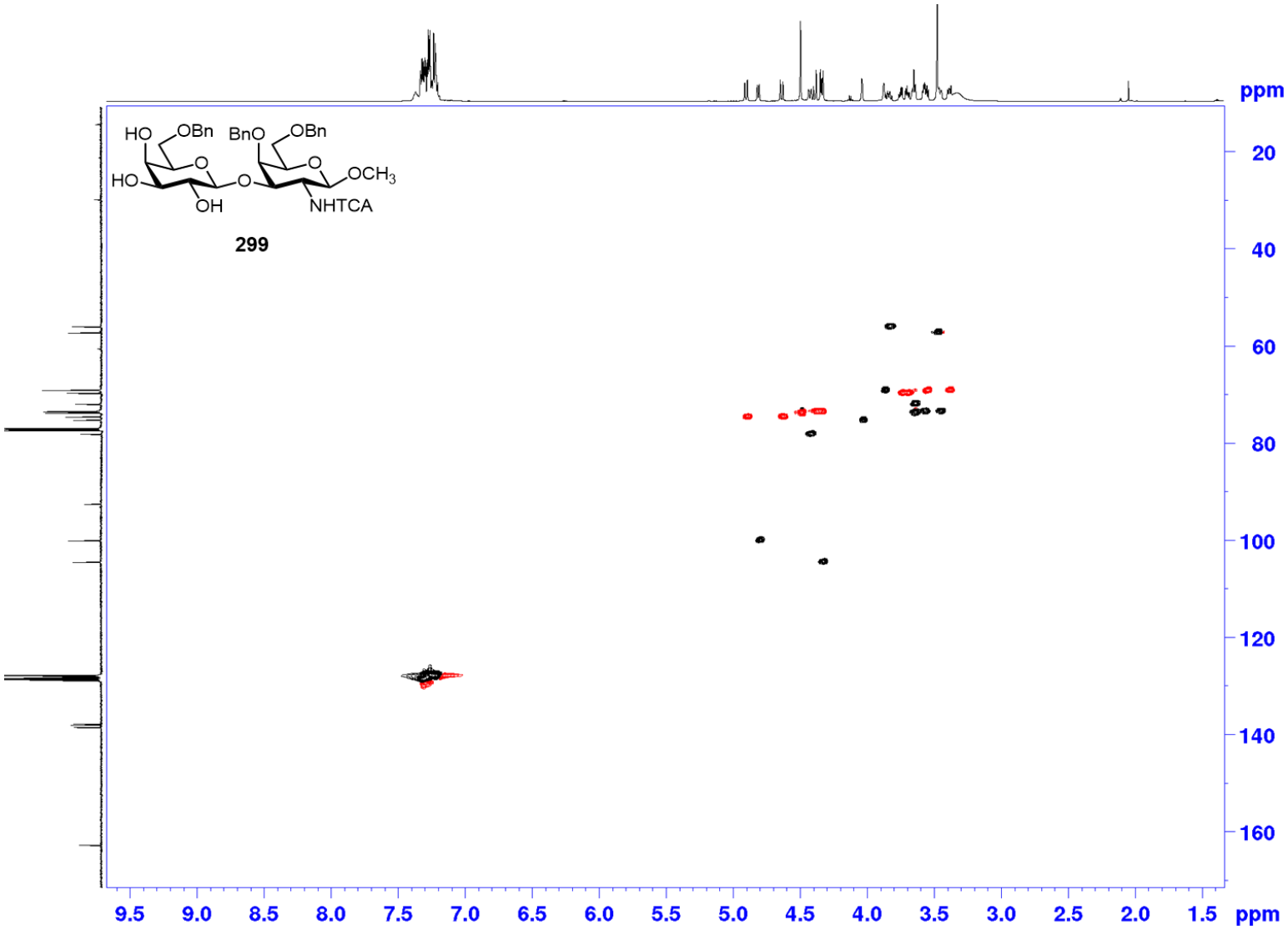
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 299



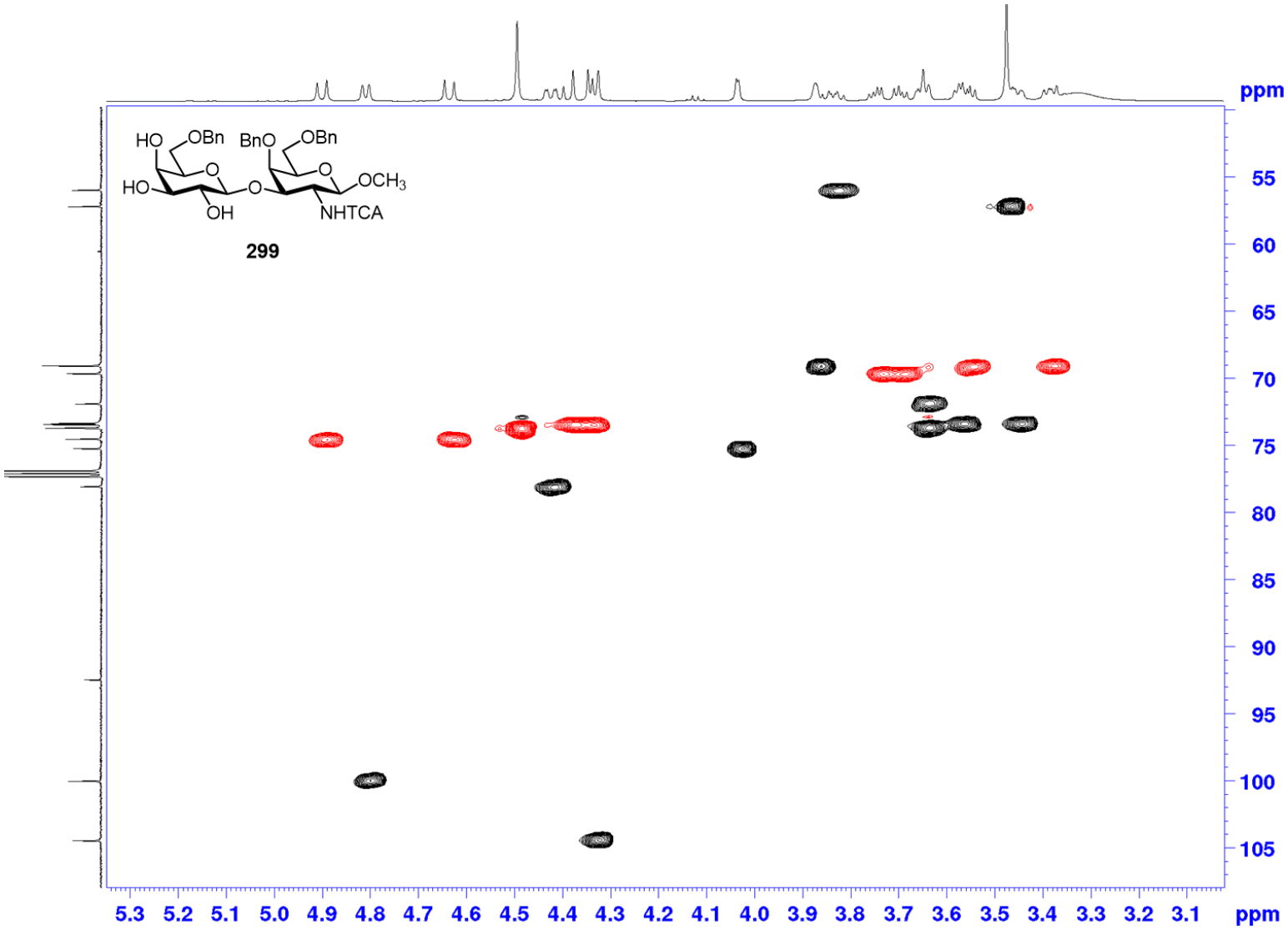
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 299



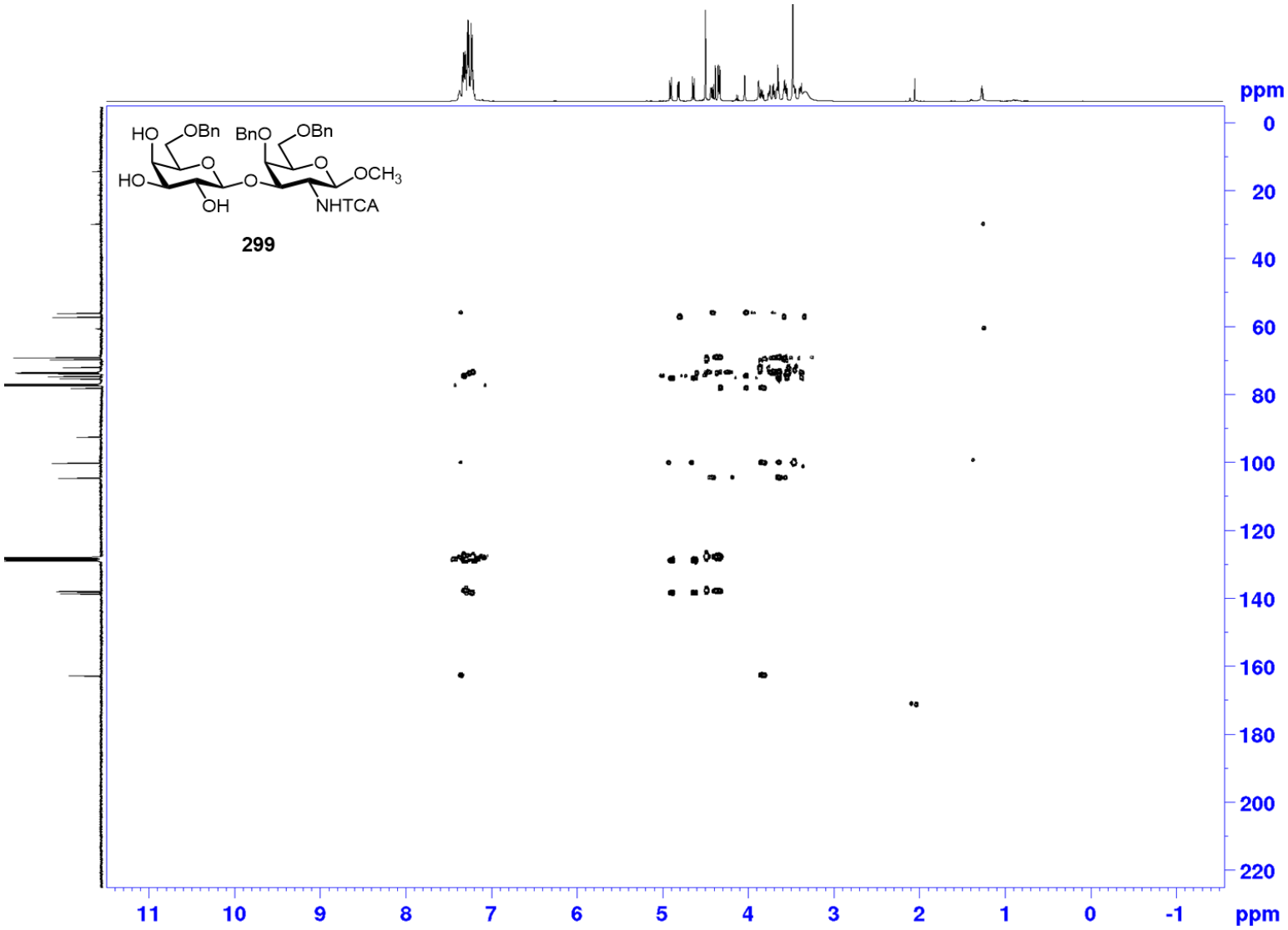
^{1}H-^{13}C HSCQ ($CDCl_3$) of compound 299



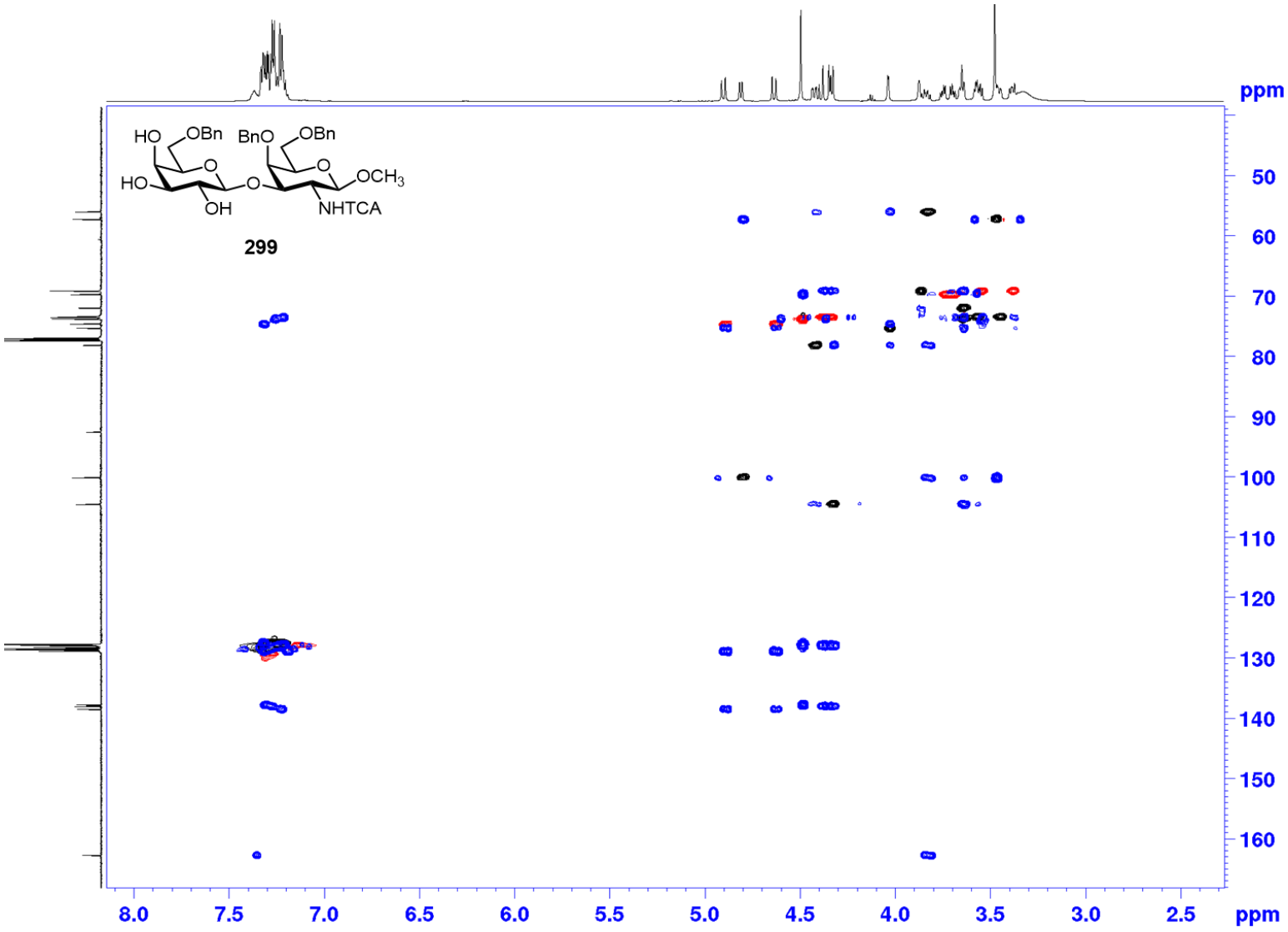
^{1}H-^{13}C HSCQ ($CDCl_3$) zoomed in region of compound 299



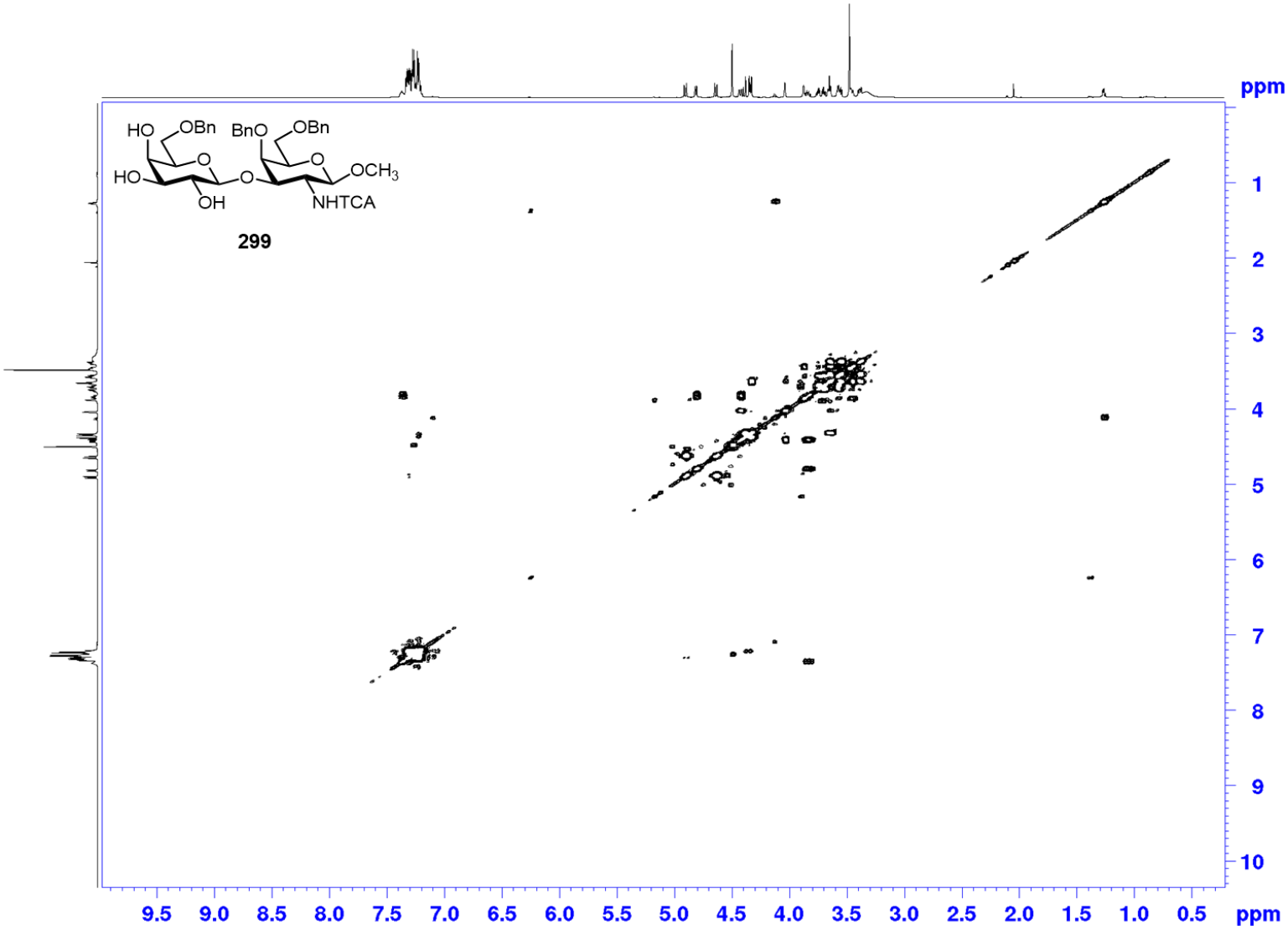
^{1}H-^{13}C HMBC ($CDCl_3$) of compound 299



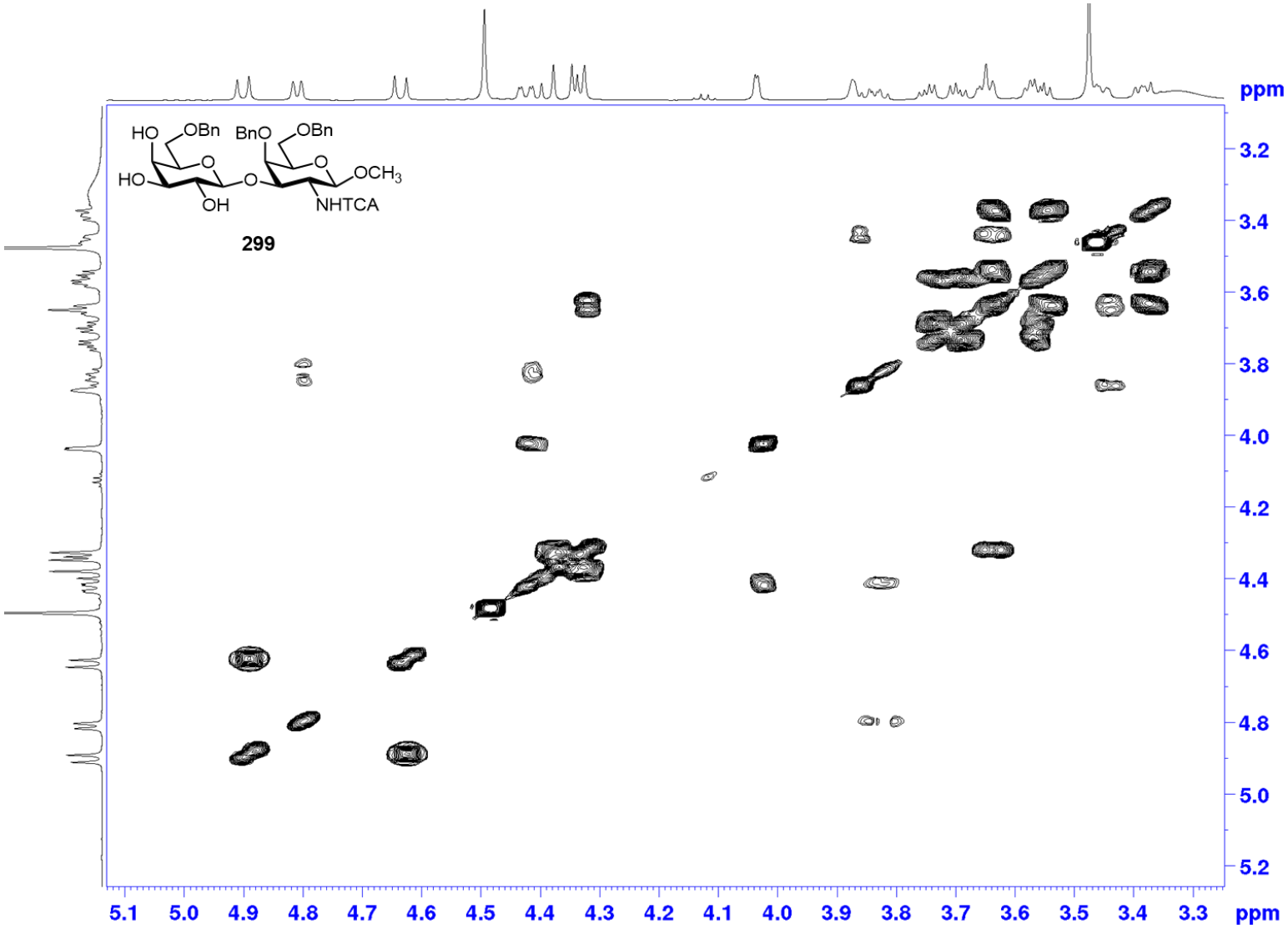
^{1}H-^{13}C HSCQ with ^{1}H-^{13}C HMBC overlay ($CDCl_3$) of compound 299



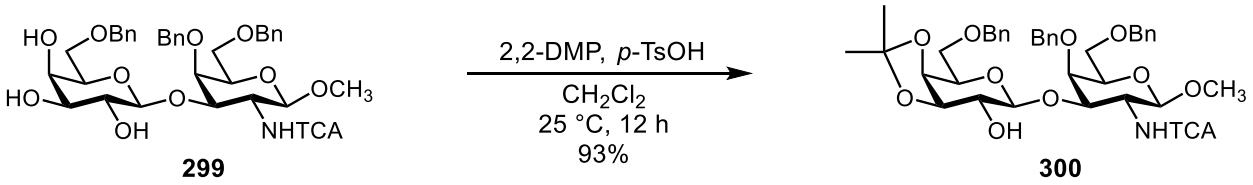
^{1}H-^{1}H COSY ($CDCl_3$) of compound 299



^{1}H-^{1}H COSY ($CDCl_3$) zoomed in region of compound 299



Methyl 6-*O*-benzyl-2-*O*-acetyl-β-D-galactopyranosyl-(1→3)-4,6-di-*O*-benzyl-2-deoxy-2-trichloro acetamido-β-D-galactopyranoside (300)

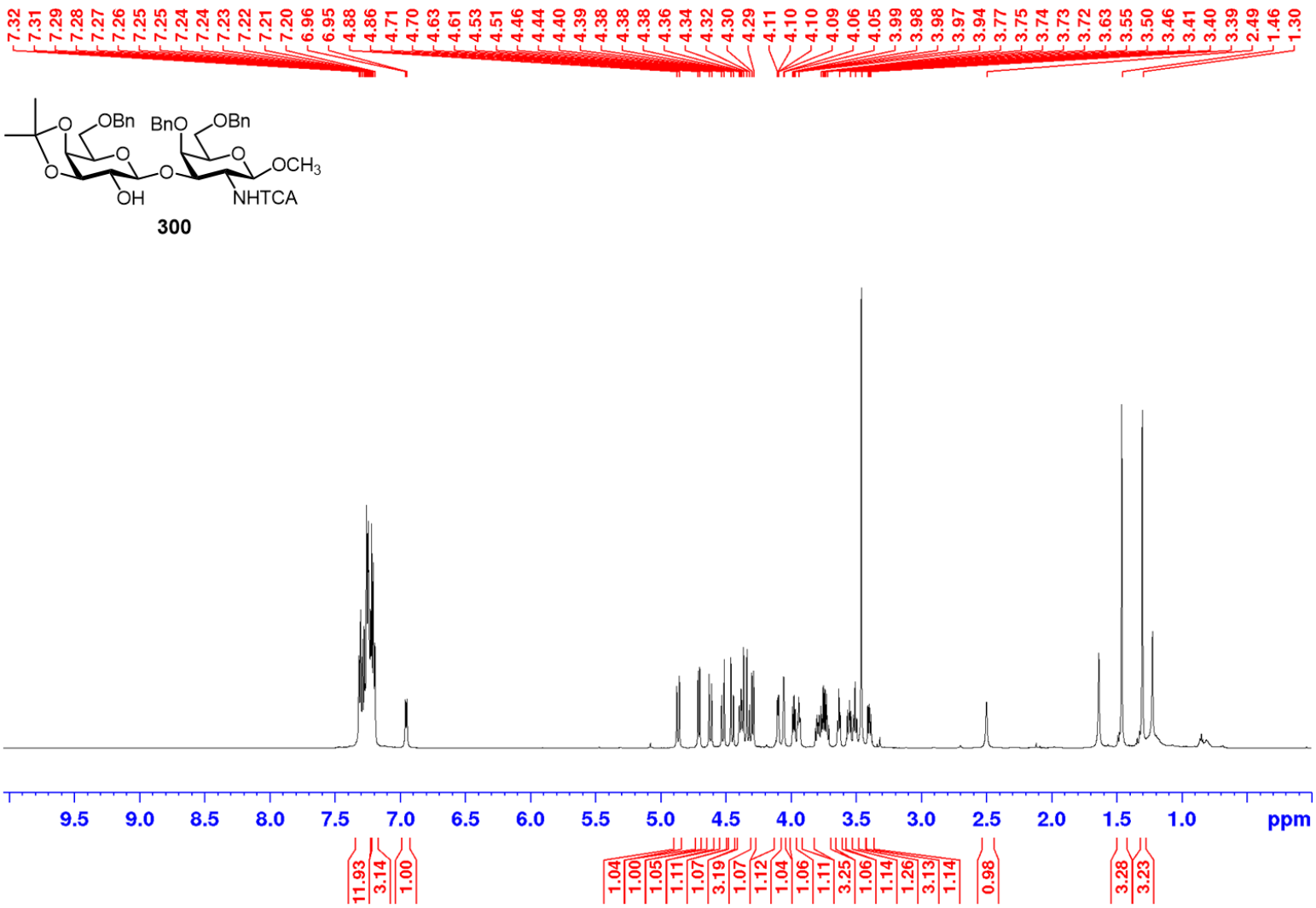


299 **300**

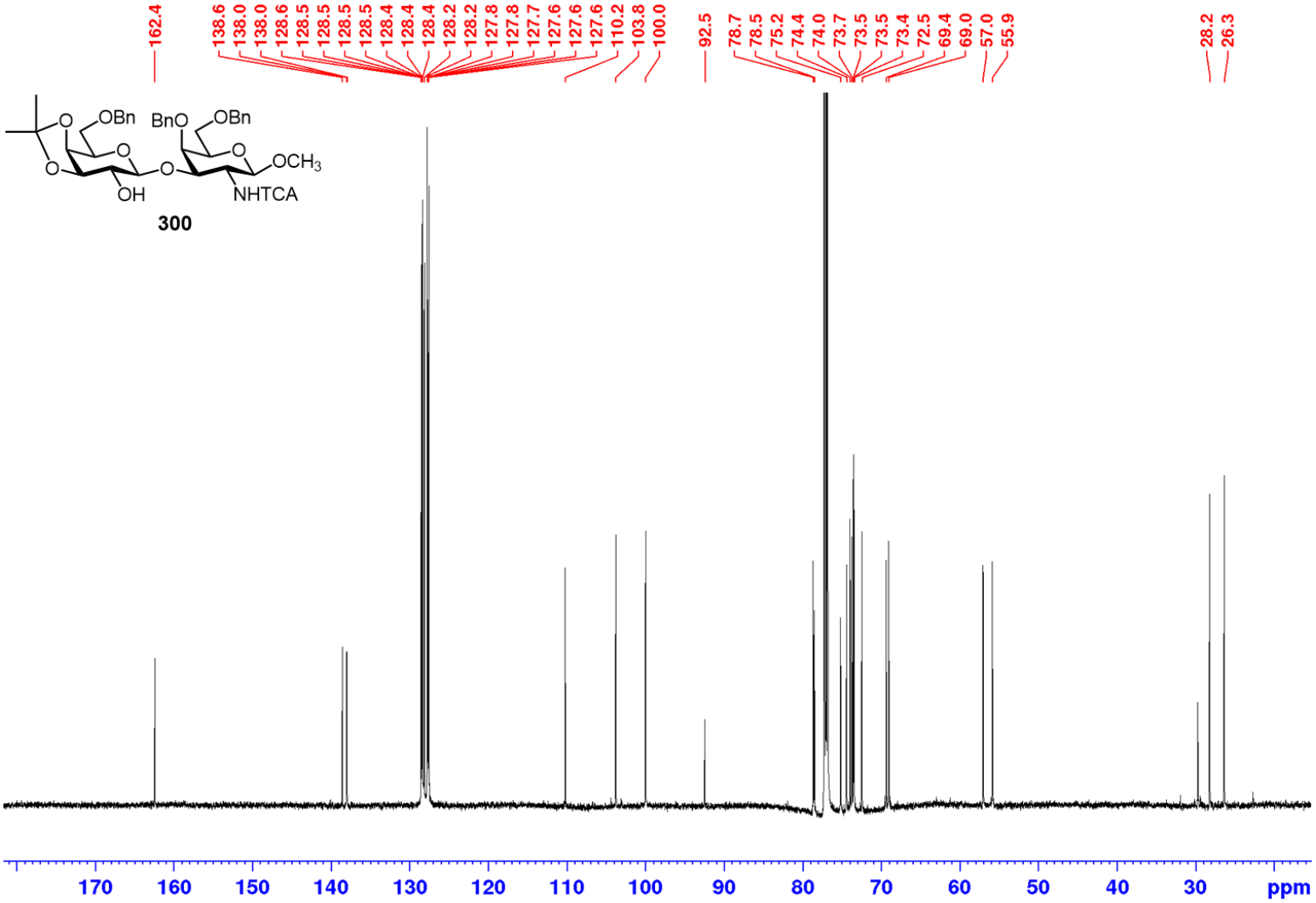
A solution of methyl 6-*O*-benzyl-β-D-galactopyranosyl-(1→3)-4,6-di-*O*-benzyl-2-deoxy-2-trichloro acetamido-β-D-galactopyranoside **299** (1.0 equiv., 0.211 g, 0.274 mmol) in CH_2Cl_2 (3 mL) was added 2,2-dimethoxypropane (6.0 equiv., 0.20 mL, 0.201 mmol) and *p*-TsOH (0.15 equiv) under argon at room temperature. At 12 h, the reaction mixture was neutralized by the addition of Et_3N (0.25 equiv.) added dropwise. The reaction mixture was concentrated to a syrup and purified by silica gel flash column chromatography (30:70 to 20:80 EtOAc/hexanes) to give methyl 6-*O*-benzyl-*O*-3,4-isopropylidene-β-D-galactopyranosyl-(1→3)-4,6-di-*O*-benzyl-2-deoxy-2-trichloro acetamido-β-D-galactopyranoside **300** (0.207 g, 0.255 mmol, 93%) as a white foam. Rf = 0.70 (1:1 EtOAc/hexanes) visualized with ceric ammonium molybdate stain; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.32-7.25 (m, 12H), 7.25-7.20 (m, 3H), 6.96 (d, *J* = 7.4 Hz, 1H), 4.87 (d, *J* = 11.8 Hz, 1H), 4.71 (d, *J* = 8.3 Hz, 1H), 4.62 (d, *J* = 11.8 Hz, 1H), 4.52 (d, *J* = 12.0 Hz, 1H), 4.40-4.29 (m, 3H), 4.29 (d, *J* = 8.3 Hz, 1H), 4.10 (dd, *J* = 2.1, 5.5 Hz, 1H), 4.06 (d, *J* = 2.4 Hz, 1H), 3.98 (dd, *J* = 5.9, 7.1 Hz, 1H), 3.94 (dd, *J* = 1.7, 6.6 Hz), 3.81-3.71 (m, 3H), 3.64-3.62 (m, 1H), 3.52-3.49 (m, 1H), 3.46 (s, 3H), 3.39 (dd, *J* = 6.1, 9.9 Hz, 1H), 2.49 (s, 3H), 1.46 (s, 3H), 1.30 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 162.4, 138.5, 138.0, 137.9, 128.5, 128.5, 128.5, 128.4, 128.4, 128.4, 128.3, 128.3, 128.1, 128.1, 127.7, 127.7, 127.7,

127.6, 127.5, 127.5, 110.2, 103.8, 100.0, 92.4, 78.6, 78.5, 75.1, 74.4, 73.9, 73.7, 73.5, 73.5, 73.4, 72.4, 69.3, 69.0, 57.0, 55.8, 28.2, 26.3.

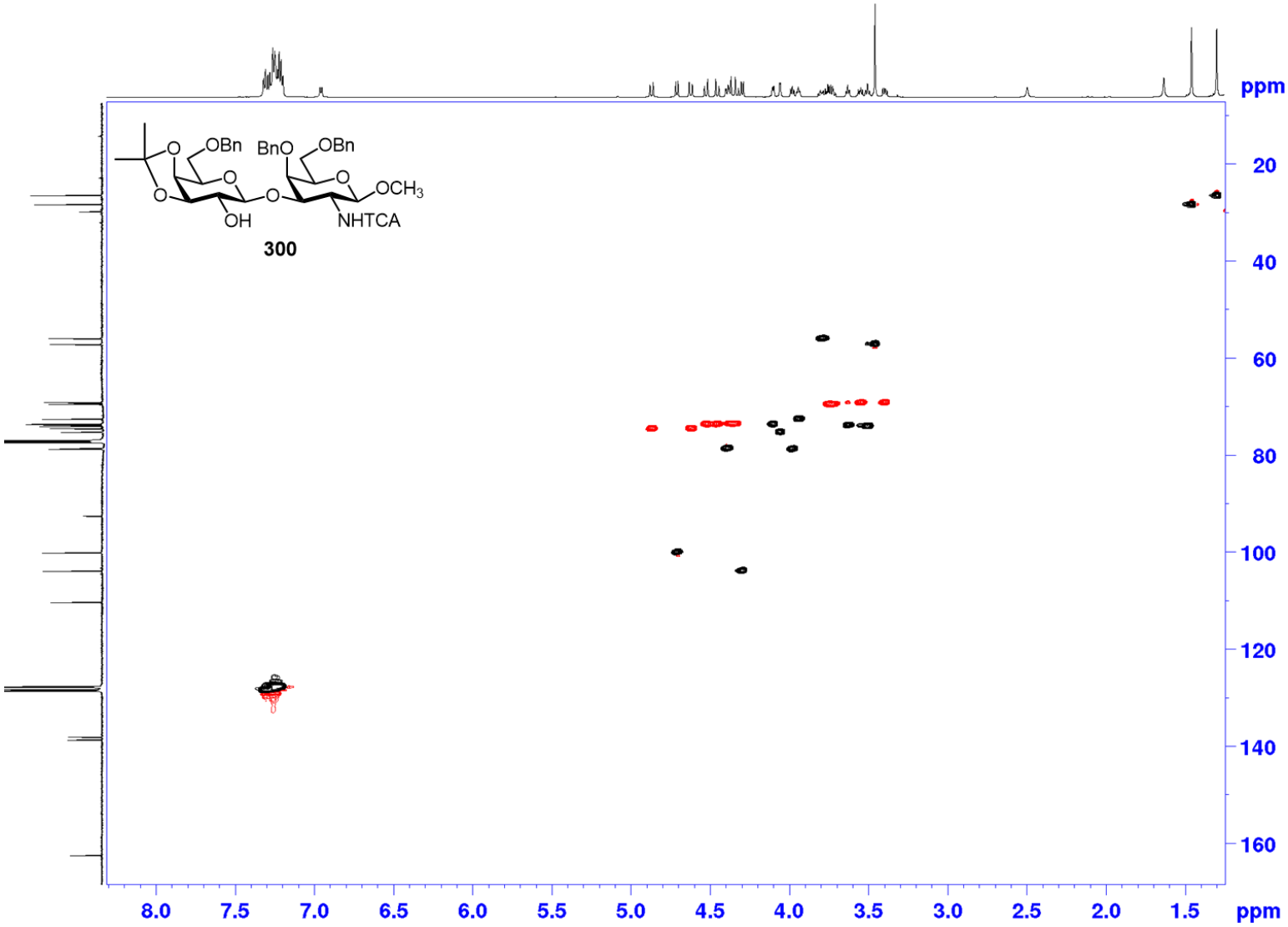
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 300



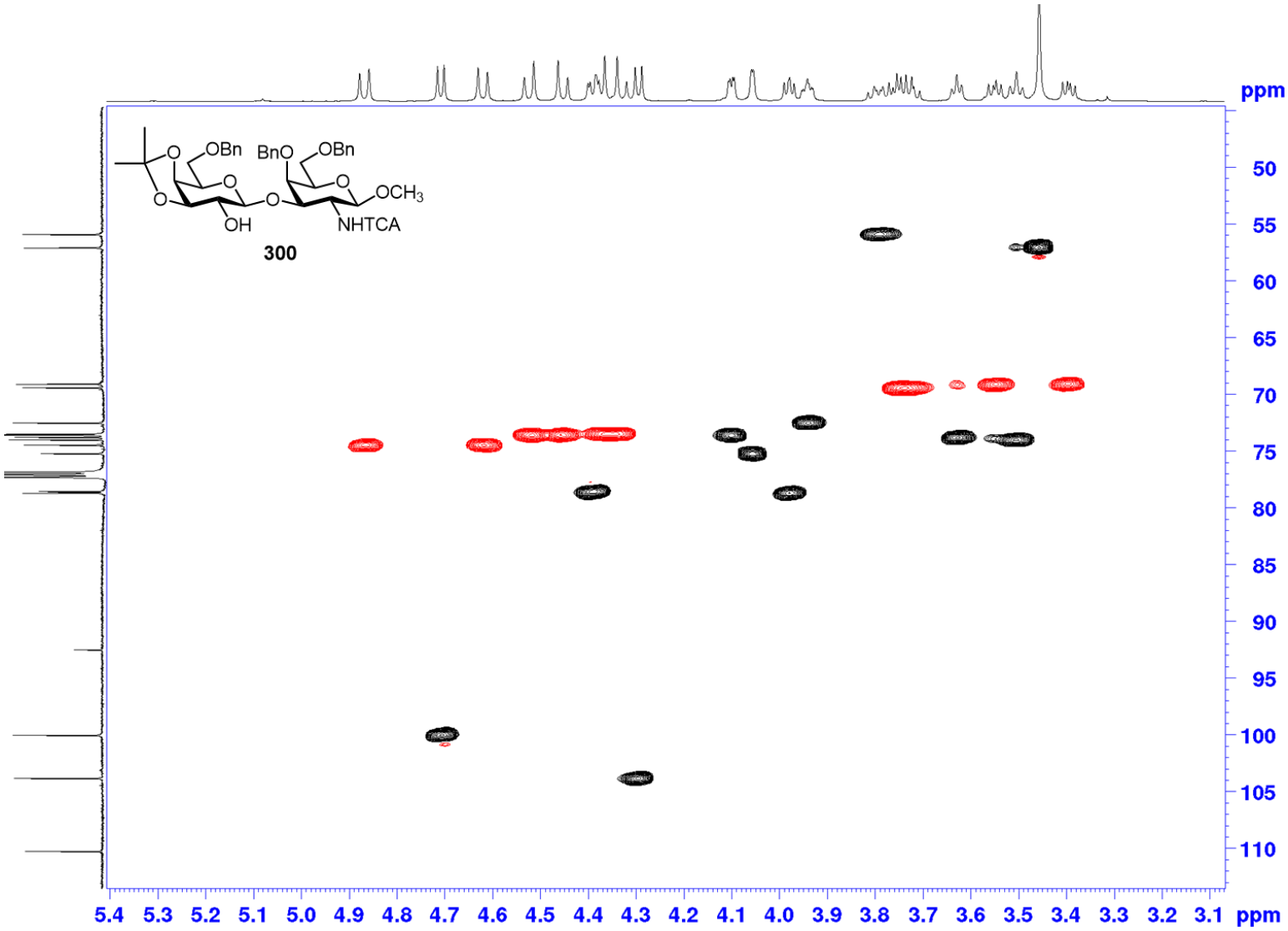
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 300



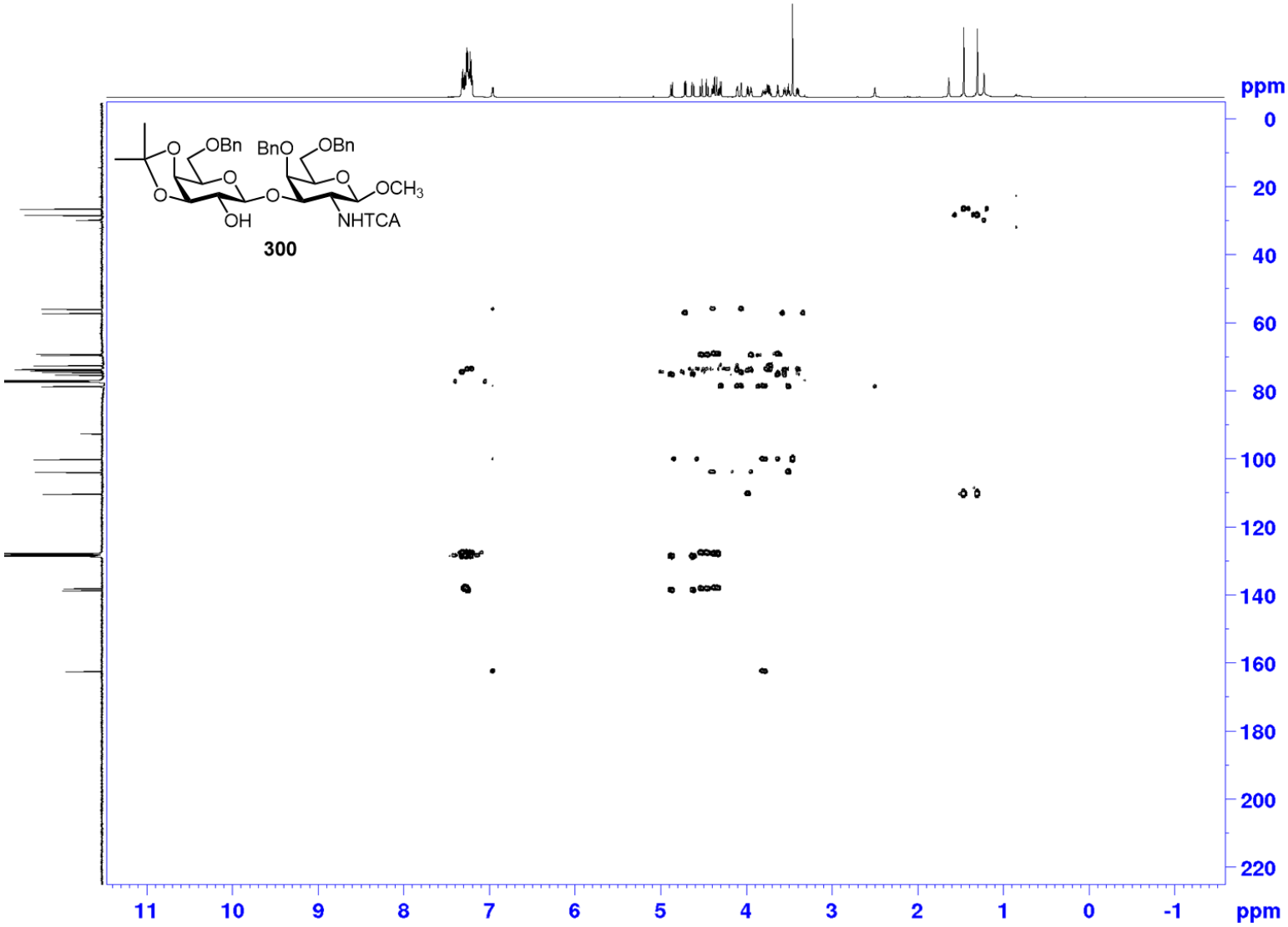
^{1}H-^{13}C HSCQ ($CDCl_3$) of compound 300



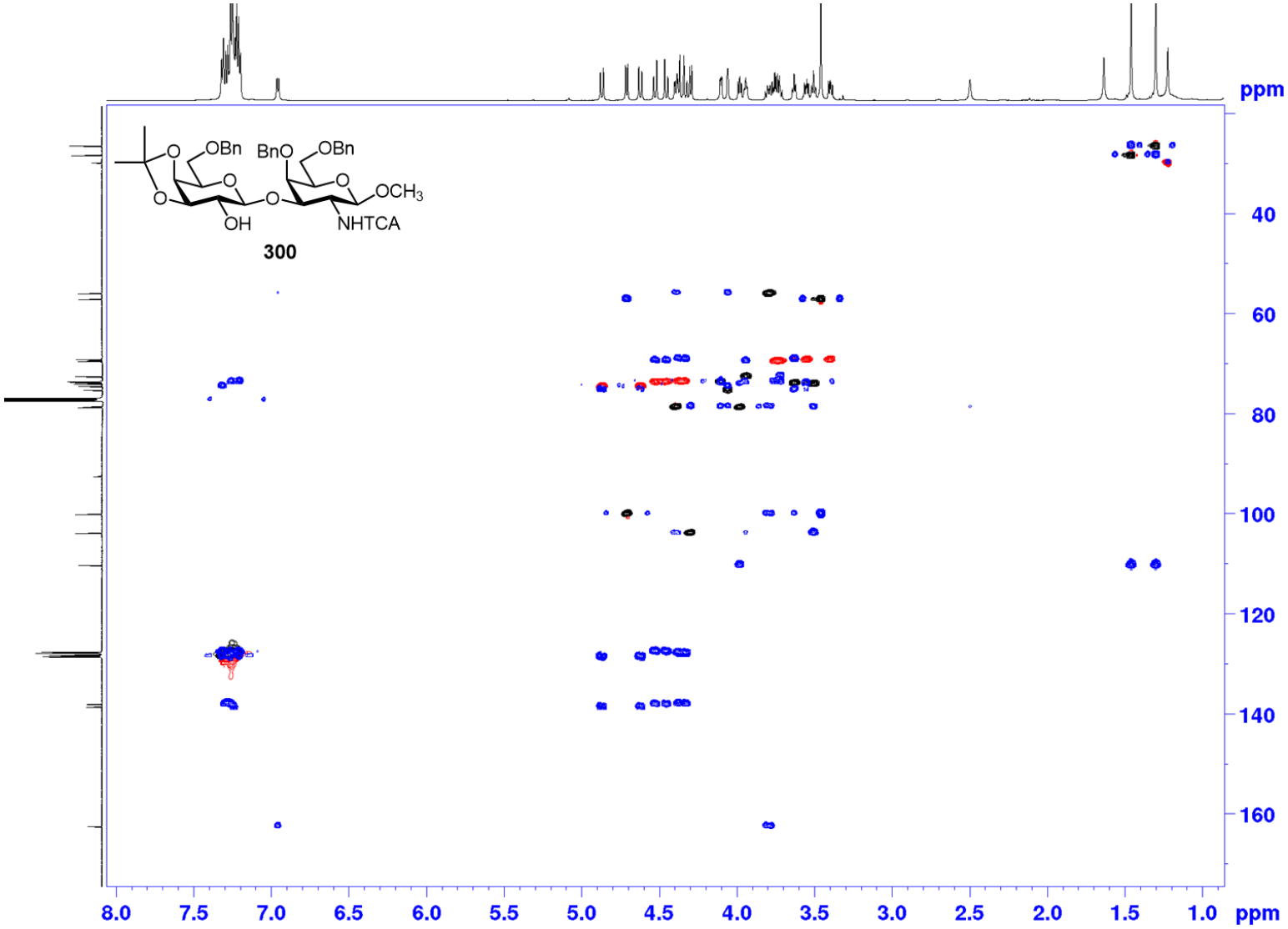
^{1}H-^{13}C HSCQ ($CDCl_3$) zoomed in region of compound 300



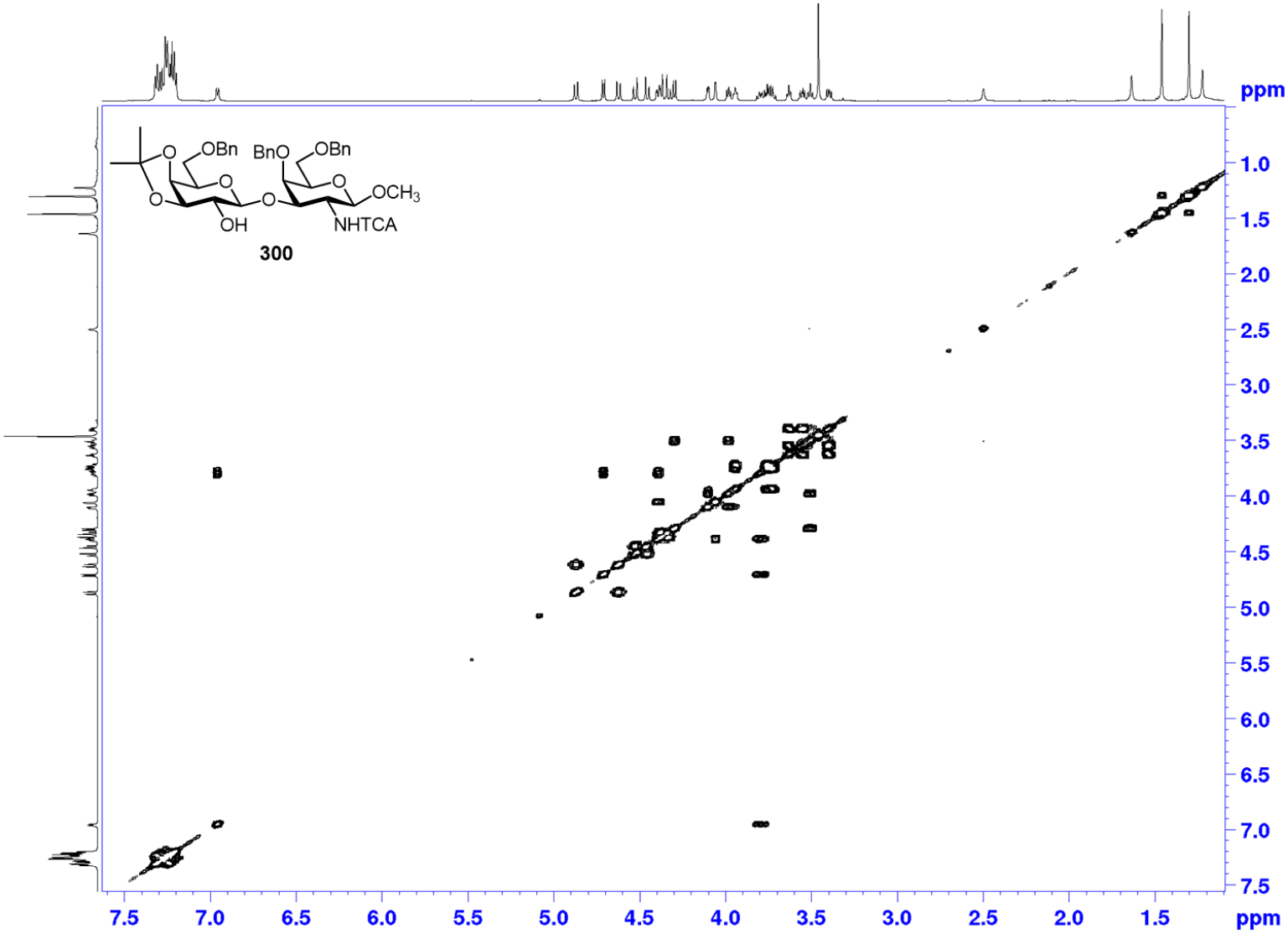
^{1}H-^{13}C HMBC ($CDCl_3$) of compound 300



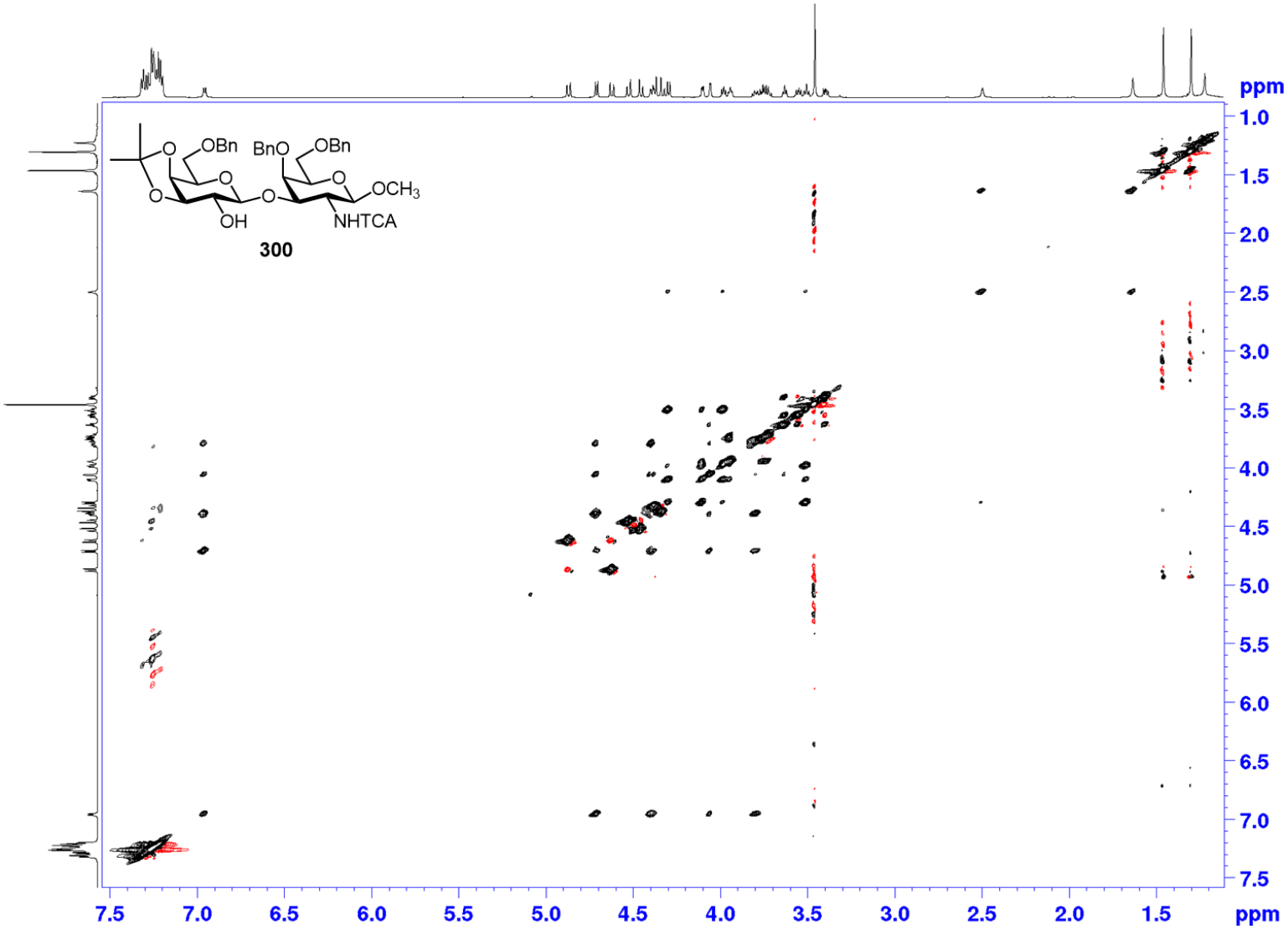
^{1}H-^{13}C HSCQ with ^{1}H-^{13}C HMBC overlay ($CDCl_3$) of compound 300



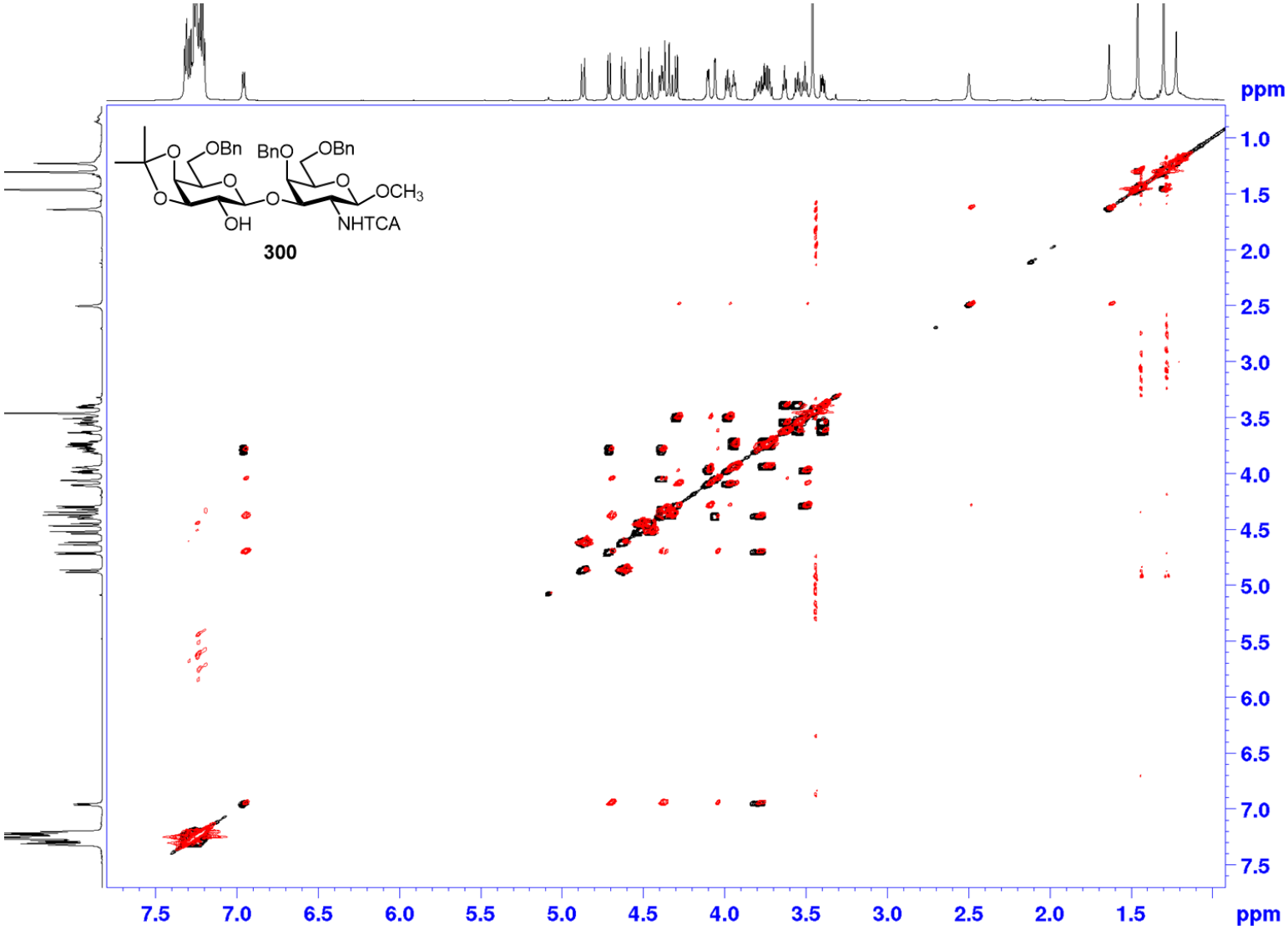
^{1}H-^{1}H COSY ($CDCl_3$) of compound 300



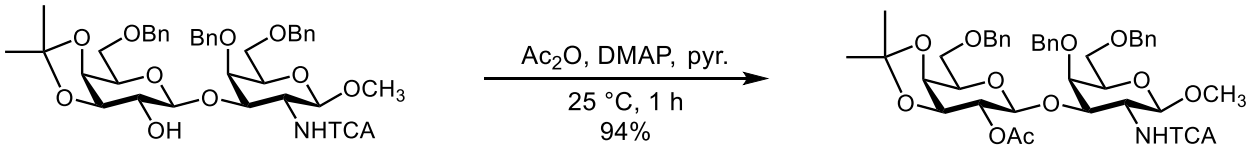
^{1}H-^{1}H TOSY ($CDCl_3$) of compound 300



^{1}H-^{1}H COSY with ^{1}H-^{1}H TOSY overlay ($CDCl_3$) of compound 300



Methyl 6-*O*-benzyl-3,4-*O*-isopropylidene-2-*O*-acetyl-β-D-galactopyranosyl-(1→3)-4,6-di-*O*-benzyl-2-deoxy-2-trichloro acetamido-β-D-galactopyranoside (297)



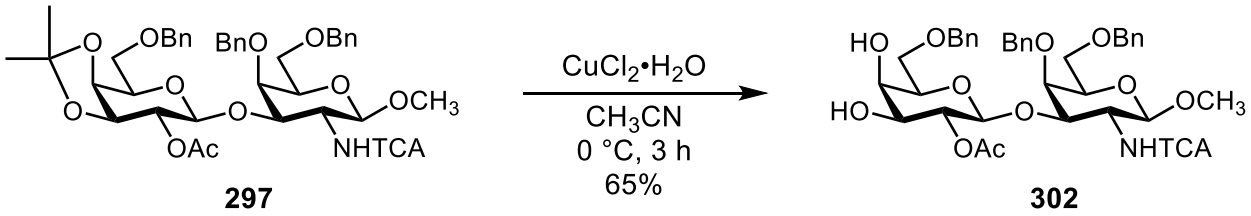
300 **297**

A soltution of methyl 6-*O*-benzyl-*O*-3,4-isopropylidene-β-D-galactopyranosyl-(1→3)-4,6-di-*O*-benzyl-2-deoxy-2-trichloro acetimido-β-D-galactopyranoside **300** (1.0 equiv., 0.211 g, 0.260 mmol) was diluted with dry THF (5 mL) under an argon atmosphere at -15°C. The reaction was added a solution of lithium aluminum hydride in THF (1.0 equiv., 0.11 mL, 0.274 mmol, [2.4 M]) dropwise and stirred at 0 °C. At 2 h, the reaction was cooled to -15 °C and added water (0.13 mL) dropwise followed by 15% NaOH aq. (0.10 mL) then water again (0.3 mL) and warmed to room temperature and stirred. After 15 min of stirring at room temperature, the reaction was added $NaSO_4$ to absorb the water which was added to the reaction. The reaction was then filtered through a glass fritted vacuum filter funnel equipped with a pad of Celite. The filtrate was concentrated to dryness. The crude reaction mixture was added Ac_2O (1.00 mL), pyridine (1.0 mL) and DMAP (cat.) at 0 °C under an argon atmosphere. At 1 h, the reaction was cooled to 0 °C and added EtOAc and MeOH to consume the rest of the acetic anhydride. The reaction warmed and stirred at room temperature for 10 min. the reaction was concentrated to a thick oil and taken up in EtOAc and washed with 1 N HCl (2x), water (1x), brine (1x), dried ($MgSO_4$), filtered through a glass fritted vacuum filter funnel to remove drying agent, and concentrated to an oil. The reaction was then purified by silica gel flash column chromatography (75:25 to 100:0 EtOAc/hexanes) to give methyl 6-*O*-benzyl-*O*-3,4-isopropylidene-2-*O*-acetyl-β-D-galactopyranosyl-(1→3)-4,6-di-*O*-benzyl-2-deoxy-2-acetamido-β-D-galactopyranoside **297** (0.213 g, 0.260 mmol, 94%) as a white solid.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.37-7.23 (m, 15H), 6.89 (d, *J* = 7.1 Hz, 1H), 5.07 (dd, *J* = 8.3, 8.5 Hz, 1H), 4.93 (d, *J* = 11.6 Hz, 1H), 4.75 (d, *J* = 8.3 Hz, 1H), 4.64 (d, *J* = 11.6 Hz, 1H), 4.55 (d, *J* = 12.1 Hz, 1H), 4.52 (d, *J* = 7.9 Hz, 1H), 4.51-4.48 (m, 2H), 4.43 (d, *J* = 12.2 Hz, 1H), 4.37 (d, *J* = 11.9 Hz, 1H), 4.19 (dd, *J* = 2.0, 5.3 Hz, 1H), 4.10 (dd, *J* = 5.3, 7.3 Hz, 1H), 4.06 (d, *J* = 2.5 Hz, 1H), 3.98 (dd, *J* = 2.1, 5.6 Hz, 1H), 3.81 (dd, *J* = 5.3, 10.0 Hz, 1H), 3.76-3.73 (m, 2H), 3.64-3.63 (m, 1H), 3.61-3.58 (m, 1H), 3.48-3.46 (m, 4H), 2.11 (s, 3H), 1.51 (s, 3H), 1.34 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.6, 161.9, 138.5, 138.0, 128.9, 128.9, 128.4, 128.4, 128.3, 128.3, 128.1, 128.1, 127.7, 127.7, 127.6, 127.5, 127.5, 110.5, 101.2, 99.8, 92.5, 76.9, 75.0, 74.3, 73.7, 73.6, 73.5, 73.4, 72.9, 72.3, 69.1, 69.1, 57.0, 56.1, 27.6, 26.3, 21.1.

For spectra *vide supra.*

Methyl 6-*O*-benzyl-2-*O*-acetyl-β-D-galactopyranosyl-(1→3)-4,6-di-*O*-benzyl-2-deoxy-2-trichloro acetamido-β-D-galactopyranoside (302)

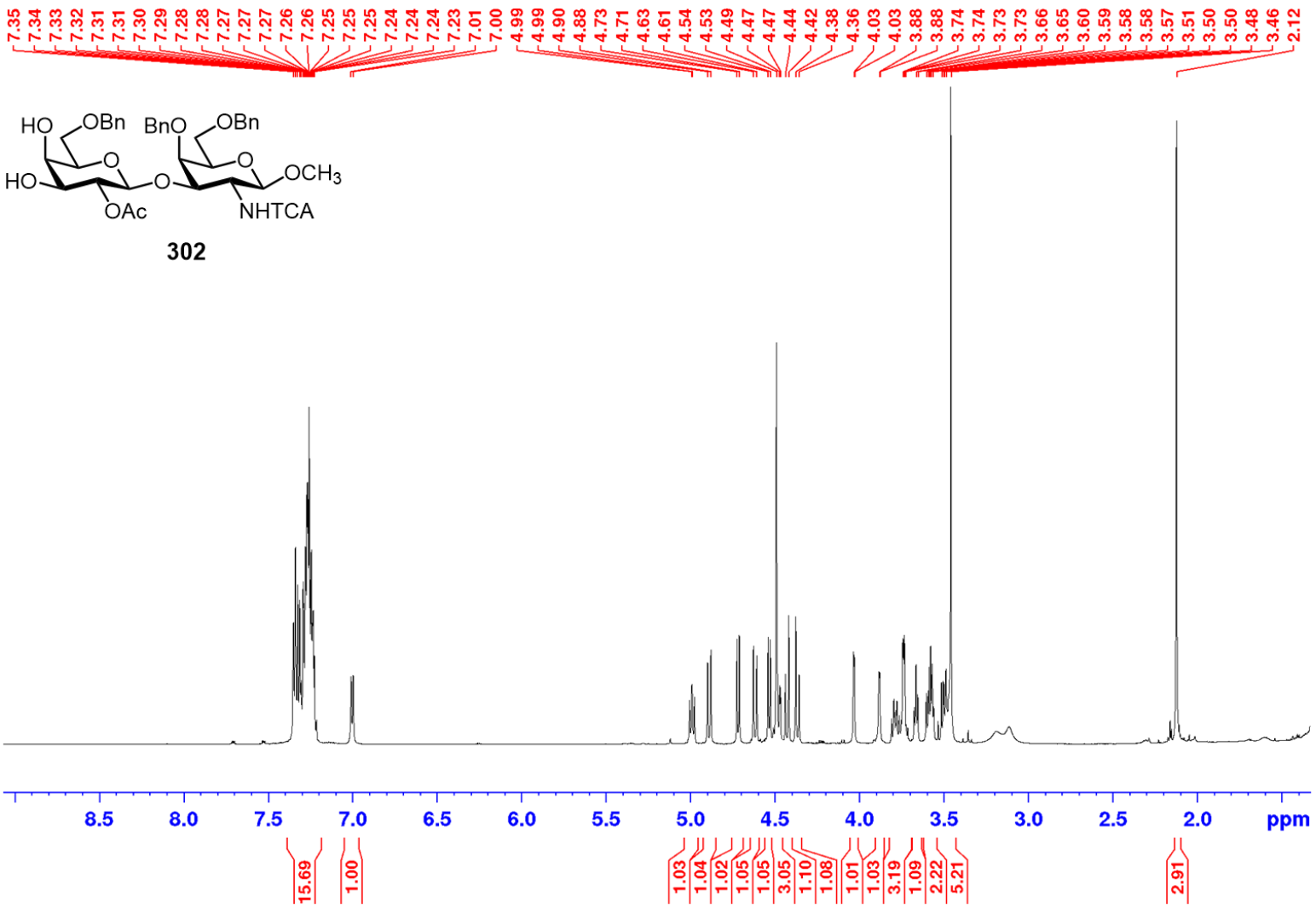


297 **302**

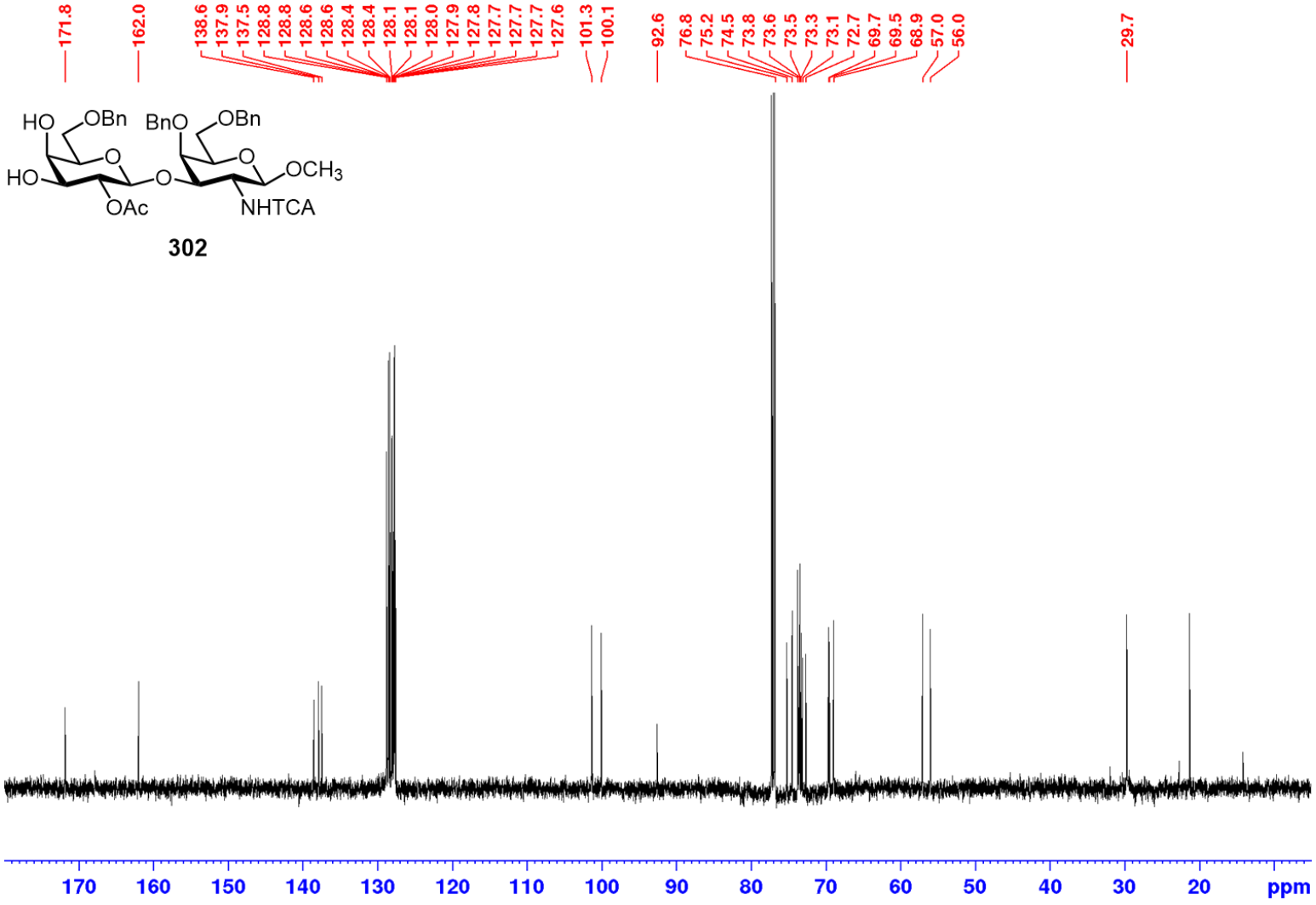
A solution of methyl 6-*O*-benzyl-*O*-3,4-isopropylidene-2-*O*-acetyl-β-D-galactopyranosyl-(1→3)-4,6-di-*O*-benzyl-2-deoxy-2-trichloro acetamido-β-D-galactopyranoside **297** (1.0 equiv., 1.77 g, 2.07 mmol) and copper (II) chloride hydrate (10.0 equiv., 2.78 g, 20.7 mmol) in CH_3CN (10 mL) was stirred at 0 °C under an argon atmosphere. At 3 h, the crude reaction mixture was filtered through a glass fritted vacuum filter funnel equipped with a pad of Celite. The filtrate was transferred to a separatory funnel with EtOAc (100 mL). The organics were washed with 1 N HCl (3 x 20 mL), aq. sat. $NaHCO_3$ (1 x 20 mL), brine (1 x 20 mL), dried ($NaSO_4$), filtered through a glass fritted vacuum filter funnel to remove drying agent, and concentrated to a crude syrup. The crude syrup was purified by silica gel flash column chromatography (20:80 to 70:30 EtOAc/hexanes) to give the diol product methyl 6-*O*-benzyl-2-*O*-acetyl-β-D-galactopyranosyl-(1→3)-4,6-di-*O*-benzyl-2-deoxy-2-trichloro acetamido-β-D-galactopyranoside **302** (1.084 g, 1.333 mmol, 65%) as an off-white amorphous solid. Rf = 0.40 (10:90 CH_3OH/EtOAc) visualized with ceric ammonium molybdate stain; ^1H NMR (400 MHz, $CDCl_3$): 7.35-7.22 (m, 16H), 7.01 (d, *J* = 7.3 Hz, 1H), 5.00 (dd, *J* = 7.6, 9.1 Hz, 1H), 4.89 (d, *J* = 11.8 Hz, 1H), 4.72 (d, *J* = 8.4 Hz, 1H), 4.62 (d, *J* = 11.8 Hz, 1H), 4.54 (d, *J* = 8.4 hz, 1H), 4.49 (s, 3H), 4.43 (d, *J* = 12.2 Hz, 1H), 4.37 (d, *J* = 11.8, 1H), 4.03 (d, *J* = 2.1 Hz, 1H), 3.88 (d, *J* = 2.4 Hz, 1H), 3.08-3.69 (m, 3H), 3.66 (dd, *J* = 5.6, 5.9 Hz, 1H), 3.60-3.55 (m, 2H), 3.53-3.44 (m, 5H), 2.12 (s, 3H);

^{13}C NMR (100 MHz, $CDCl_3$): δ 171.8, 162.0, 138.5, 137.9, 137.5, 128.8, 128.8, 128.5, 128.5, 128.4,128.3, 128.1, 128.1, 128.0, 127.8, 127.8, 127.7, 127.7, 127.7, 127.5, 101.3, 100.0, 92.5, 76.7, 75.2, 74.5, 73.7, 73.5, 73.4, 73.3, 73.1, 72.6, 69.6, 69.5, 68.9, 57.0, 56.0, 29.7.

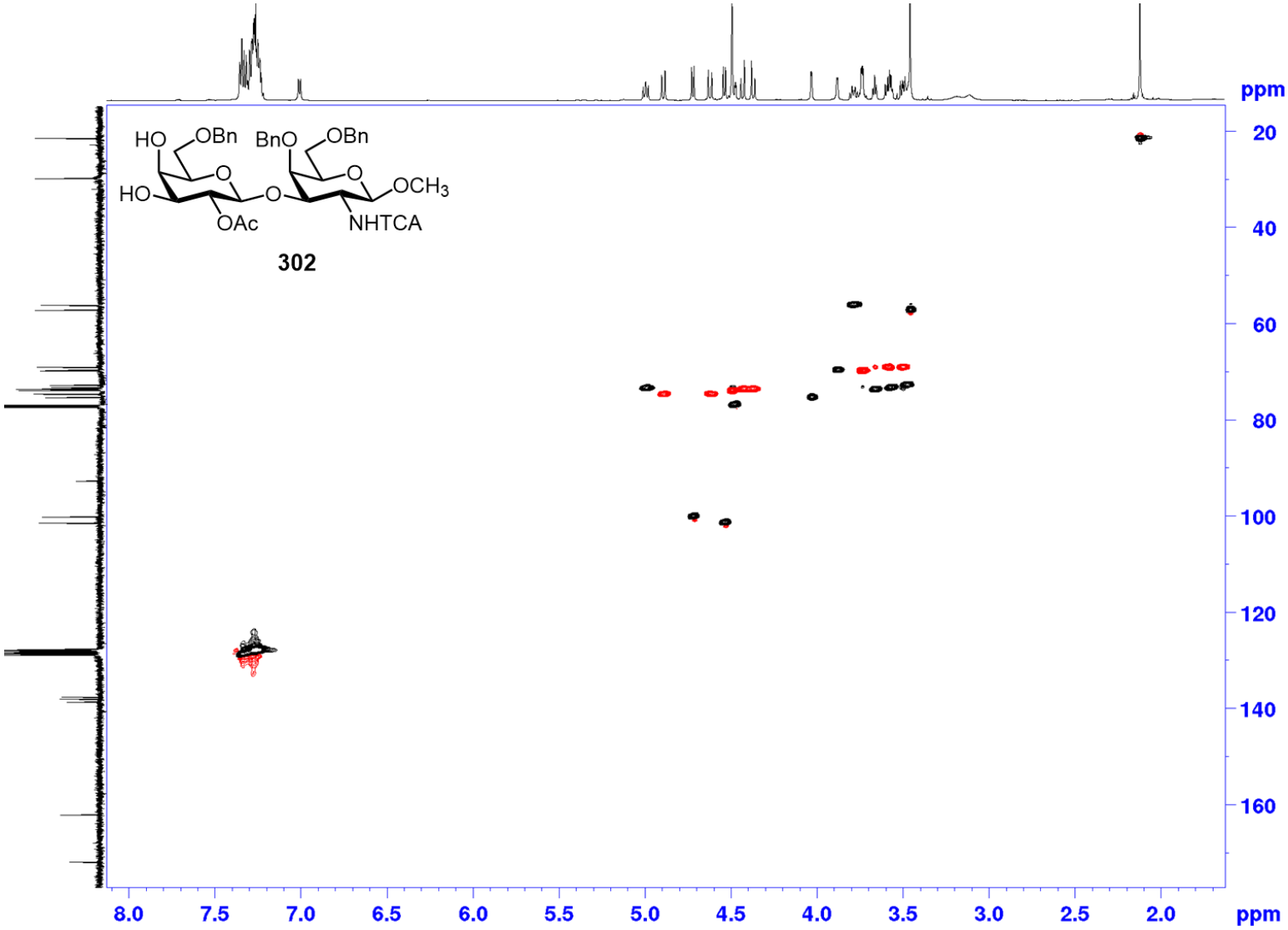
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 302



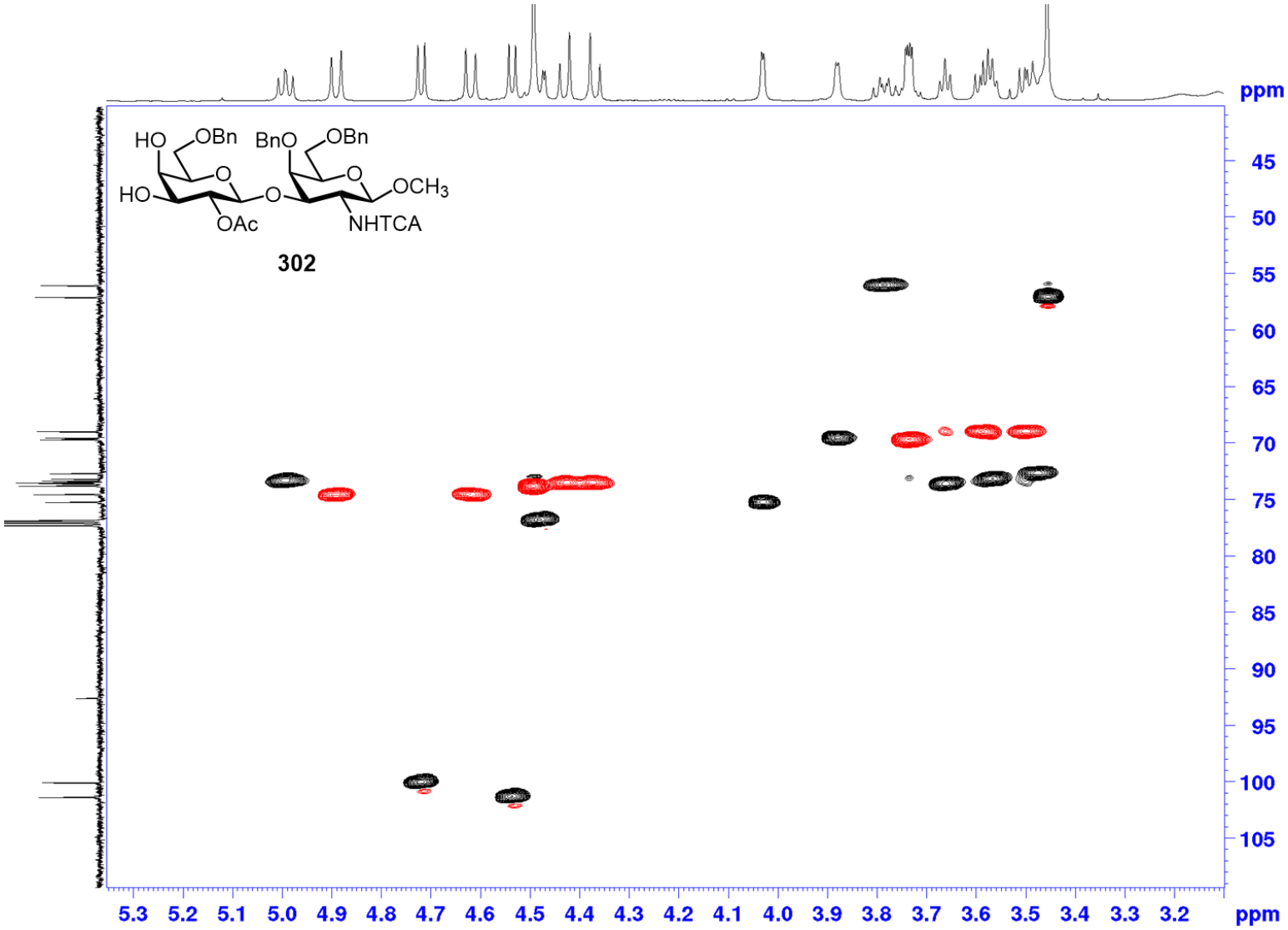
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 302



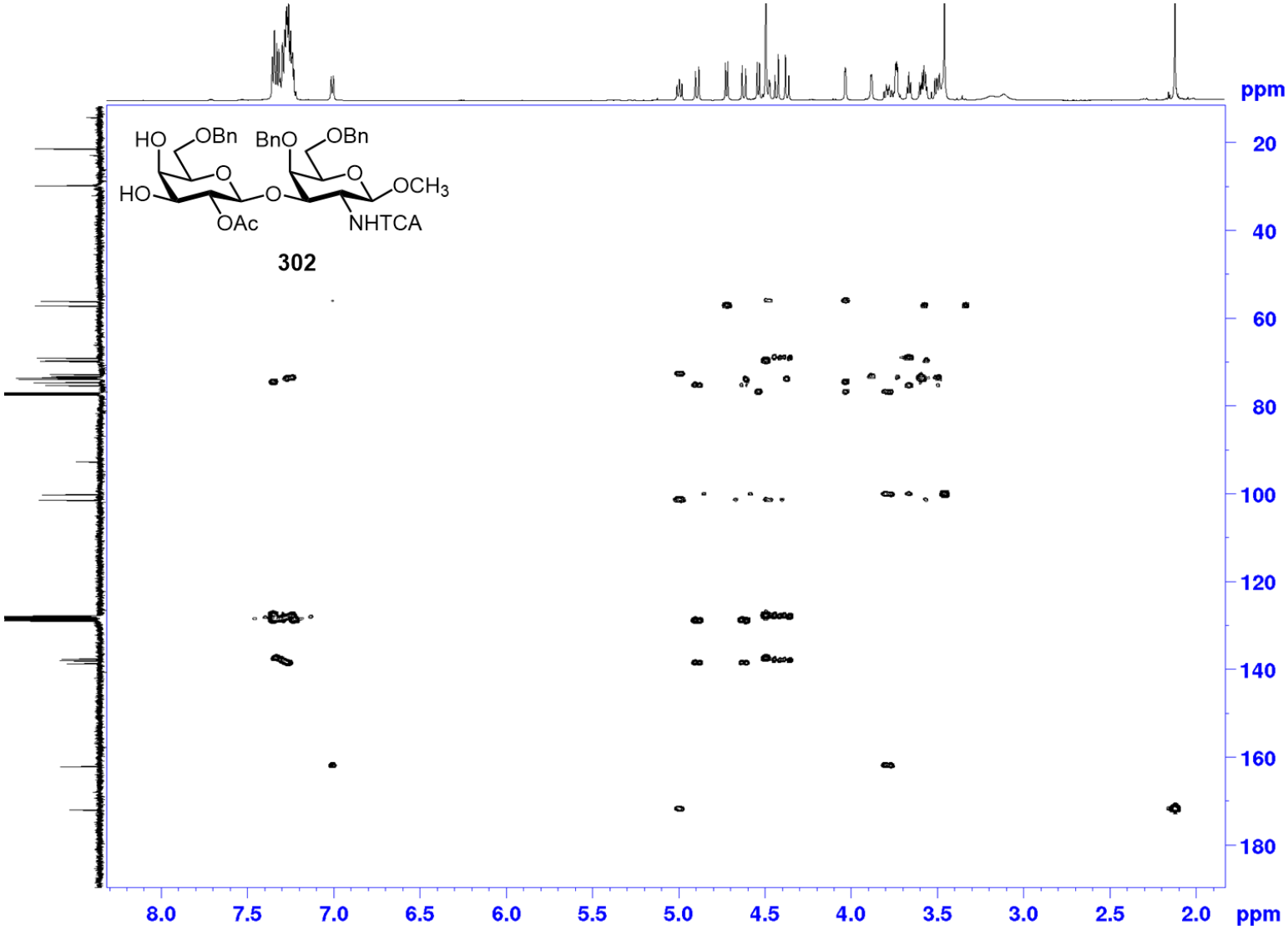
^{1}H-^{13}C HSCQ ($CDCl_3$) of compound 302



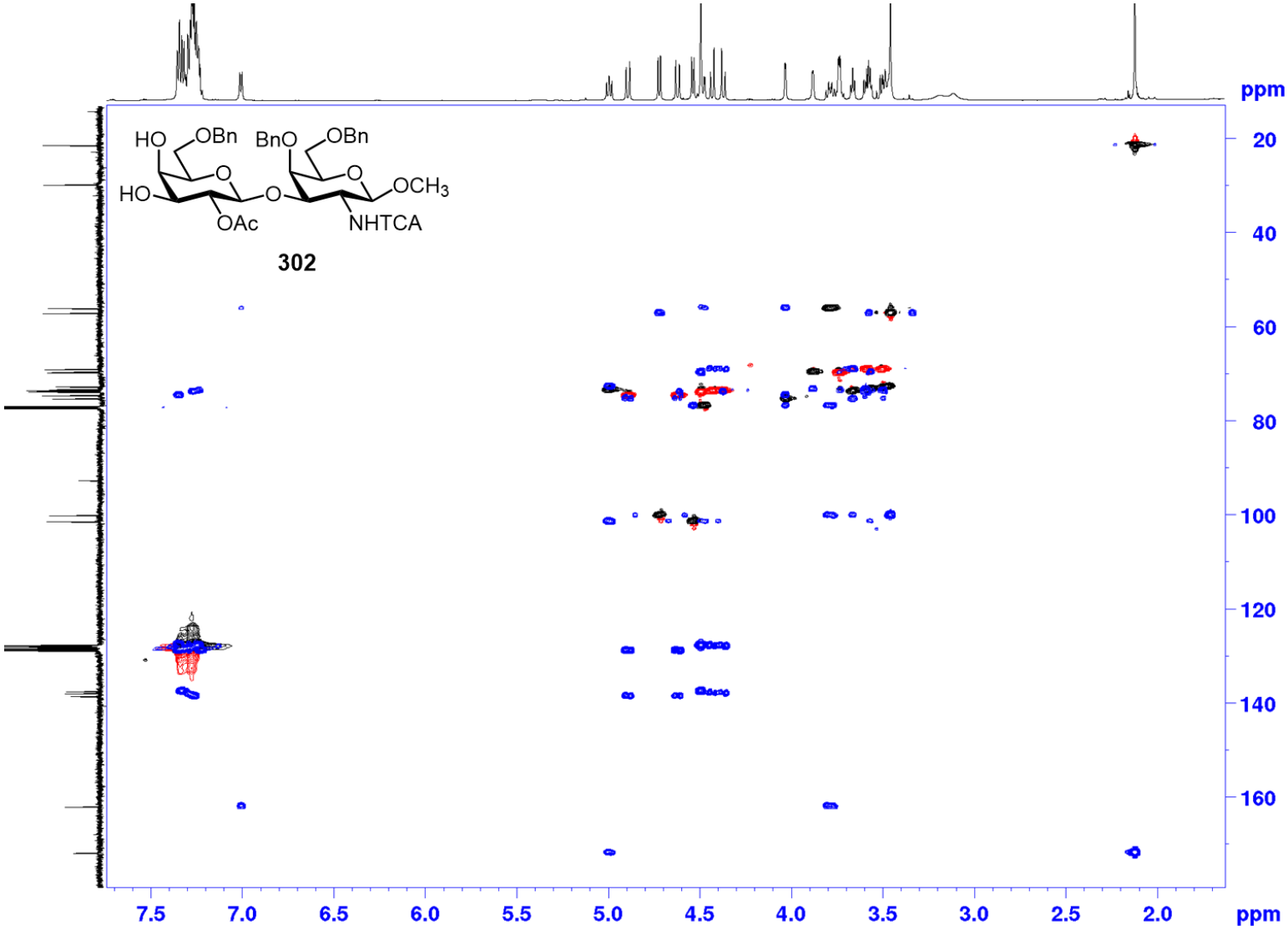
^{1}H-^{13}C HSCQ ($CDCl_3$) zoomed in region of compound 302



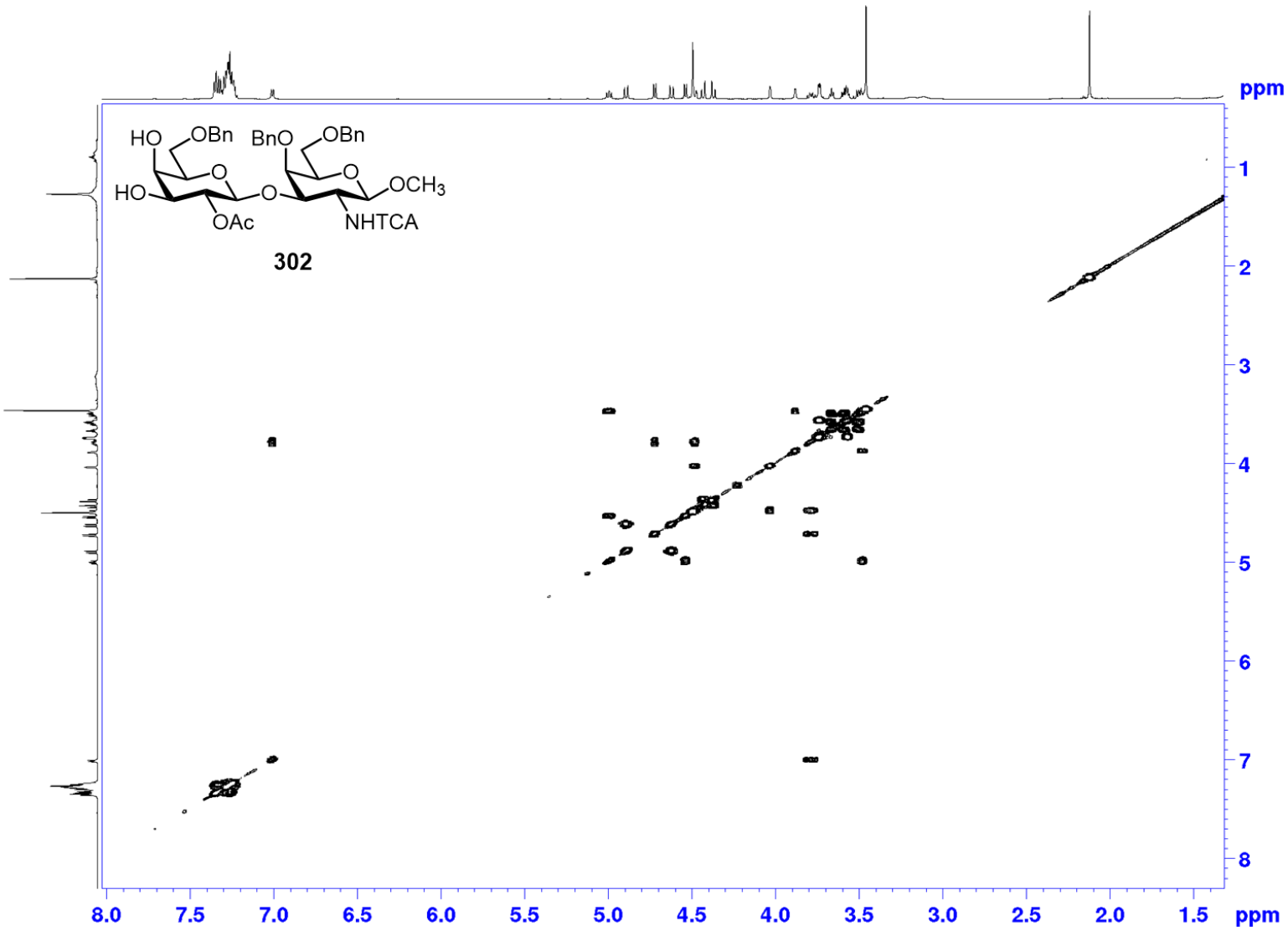
^{1}H-^{13}C HMBC ($CDCl_3$) of compound 302



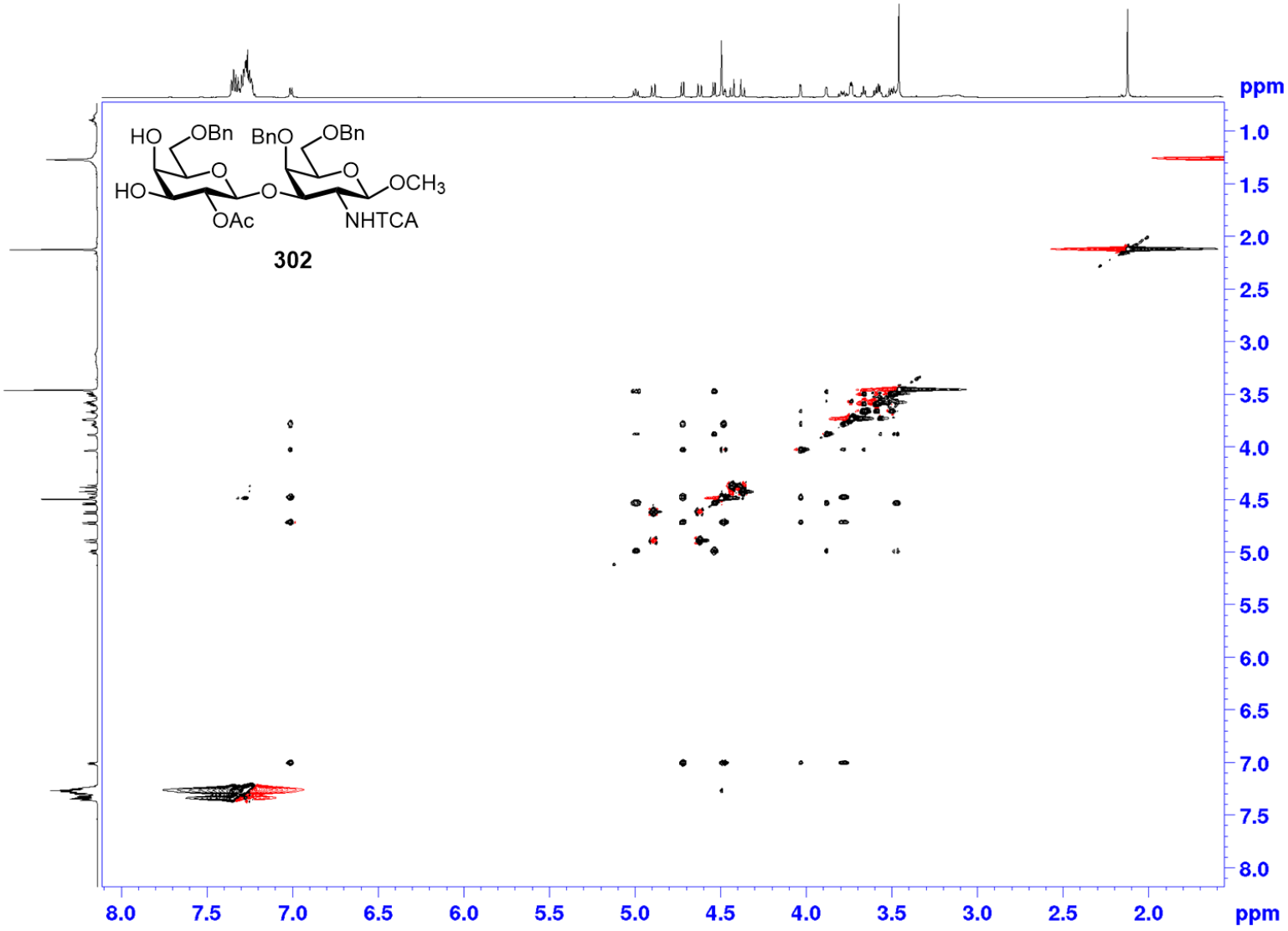
^{1}H-^{13}C HSCQ with ^{1}H-^{13}C HMBC overlay ($CDCl_3$) of compound 302



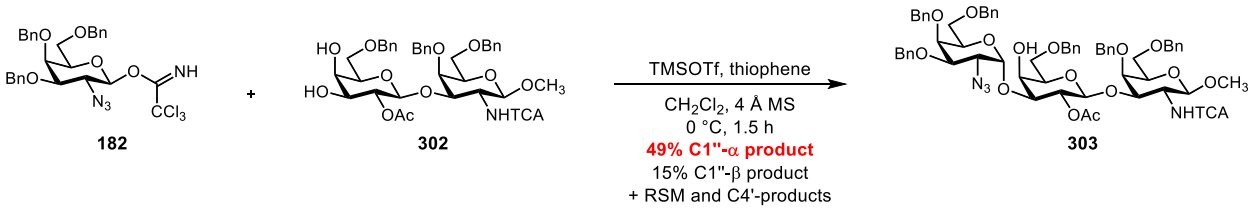
^{1}H-^{1}H COSY ($CDCl_3$) of compound 302



^{1}H-^{1}H TOSY ($CDCl_3$) of compound 302



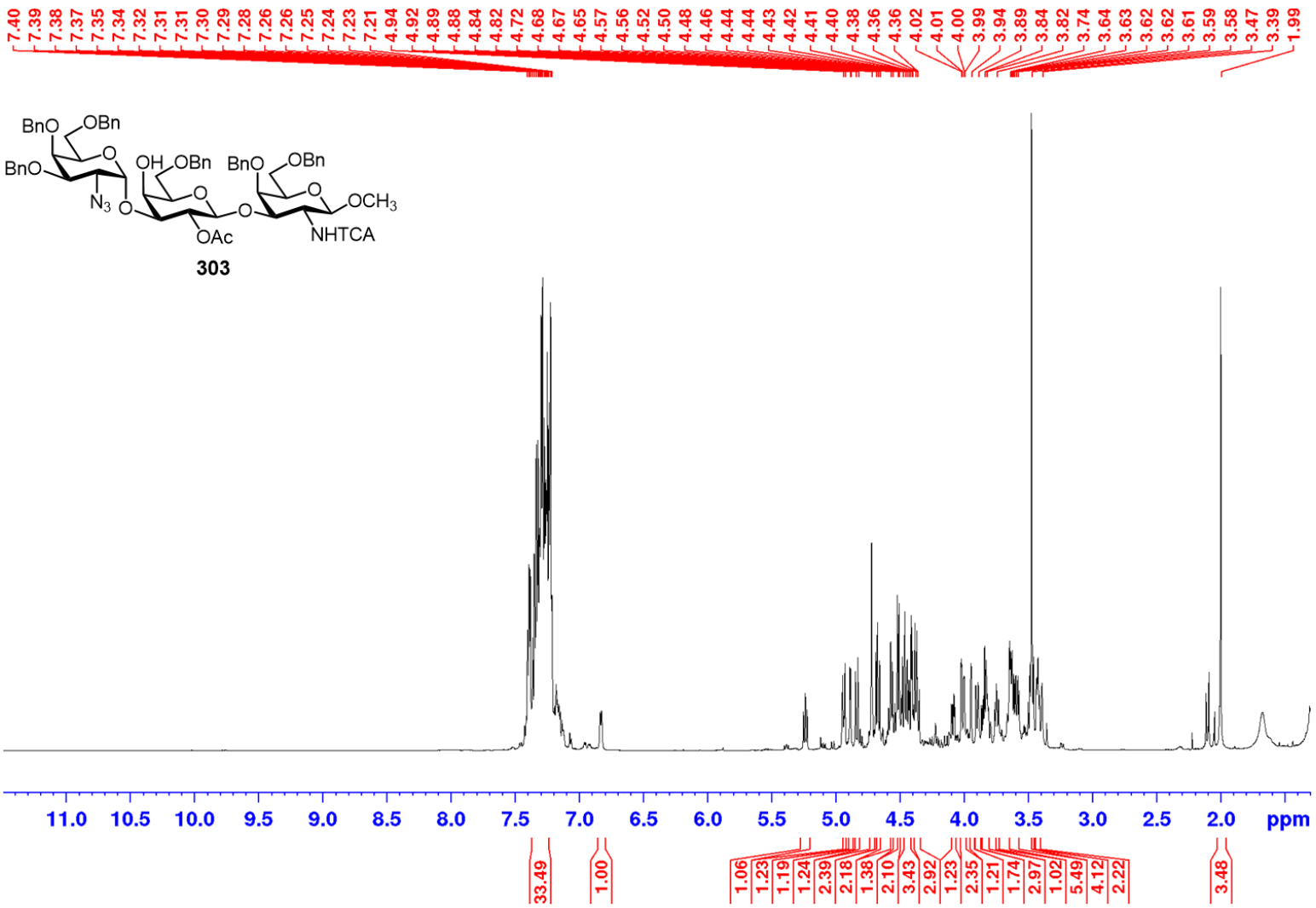
Methyl 3,4,6-tri-*O*-benzyl-2-deoxy-2-azido-α-D-galactopyranosyl-(1→3)-6-*O*-benzyl-2-*O*-(*p*-methoxy)-β-D-galactopyranosyl-(1→3)-4,6-di-*O*-benzyl-2-deoxy-2-trichloro acetamido-β-D-galactopyranoside (303)



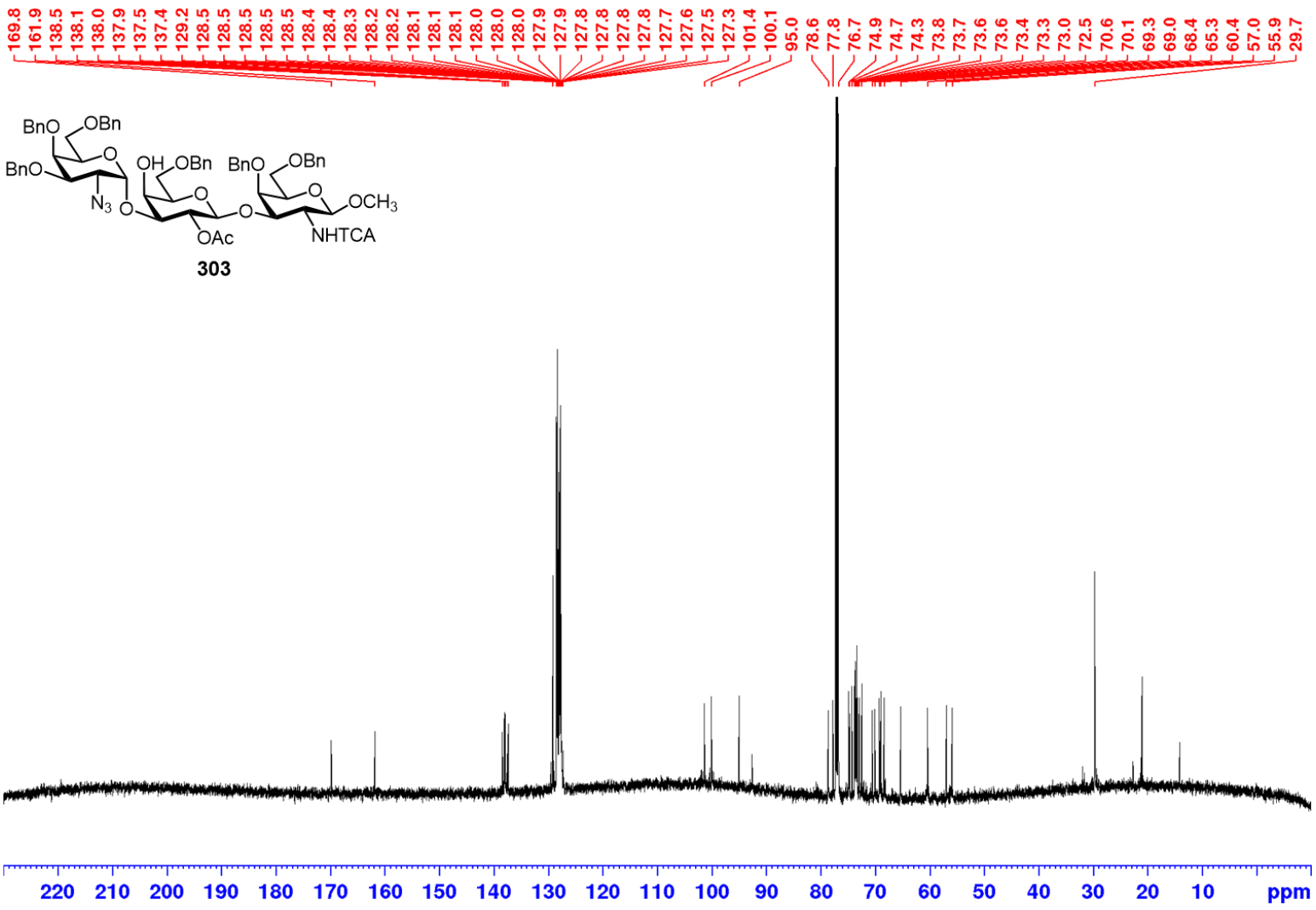
A solution of trichloroacetimidate 3,4,6-tri-*O*-benzyl-2-deoxy-2-azido-β-D-galactopyranoside 1**82** (1.5 equiv., 0.558 g, 0.900 mmol), disaccharide acceptor methyl 6-*O*-benzyl-2-*O*-(*p*-methoxy)-β-D-galactopyranosyl-(1→3)-4,6-*O*-benzylidene acetal-2-deoxy-2-trichloro acetamido-β-D-galactopyranoside **302** (1.0 equiv., 0.488 g, 0.600 mmol) in dry CH_2Cl_2 (6 mL) and thiophene (25 equiv., 1.26 g, 15.0 mmol) were added freshly activated and cooled 4 Å MS (450 mg, 15% m/v) under an argon atmosphere at room temperature. After 1 h, the reaction was cooled to 0 °C then added a solution of TMSOTf (0.10 equiv., 10 µL, 0.060 mmol). At 1.5 h, the reaction mixture was neutralized with Et_3N and added diluted with CH_2Cl_2. The reaction mixture was filtered through a pad of Celite within a glass fritted vacuum filter funnel to remove the 4 Å MS. The filtrate was concentrated to a crude oil and purified by silica gel flash column chromatography (25:75 to 30:70 EtOAc/hexanes) to give methyl 3,4,6-tri-O-benzyl-2-deoxy-2-azido-α-D-galactopyranosyl-(1→3)-6-*O*-benzyl-2-*O*-(*p*-methoxy)-β-D-galactopyranosyl-(1→3)-4,6-di-*O*-benzyl-2-deoxy-2-trichloro acetamido-β-D-galactopyranoside **303** (0.369 g, 0.291 mmol, 49%) as a white foam. Rf = 0.40 (40:60 EtOAc/hexanes) visualized with ceric ammonium molybdate stain. The trisaccharide methyl 3,4,6-tri-O-benzyl-2-deoxy-2-azido-β-D-galactopyranosyl-(1→3)-6-*O*-benzyl-2-*O*-(*p*-methoxy)-β-D-galactopyranosyl-

(1→3)-4,6-di-*O*-benzyl-2-deoxy-2-trichloro acetamido-β-D-galactopyranoside **303-C1''-β** (0.144 g, 0.114 mmol, 19%) was also isolated along with some unconsumed disaccharide starting material acceptor and the diglycosylated trteasaccharide products.; ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.40-7.22 (m, 29H), 6.83 (d, *J* = 7.1 Hz, 1H), 5.24 (d, *J* = 7.7, 9.1, 1H), 4.93 (d, *J* =11.3, 1H), 4.88 (d, *J* =3.6 Hz, 1H), 4.83 (d, *J* = 11.4, 1H), 4.72-4.71 (m, 2H), 4.68-4.65 (m, 2H), 4.58-4.55 (m, 2H), 4.58-4.55 (m, 3H), 4.52-4.50 (m, 2H), 4.46-4.41 (m, 3H), 4.38-4.34 (m, 3H), 4.08 (dd, *J* = 3.9, 10.4 Hz, 1H), 4.01 (dd, *J* = 2.4, 12.1 Hz, 2H), 3.94 (d, *J* = 1.8 Hz, 1H), 3.89 (dd, *J* = 2.2, 10.4 Hz, 1H), 3.86-3.80 (m, 3H), 3.75 (m, 3H), 3.66-3.57 (m, 5H), 3.47-3.45 (m, 4H), 3.43-3.41 (m, 2H), 2.00 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.8, 161.8, 138.4, 138.1, 138.0, 137.9, 137.5, 137.3, 129.5, 129.1, 129.0, 128.5, 128.5, 128.5, 128.4, 128.4, 128.3, 128.3, 128.2, 128.2, 128.1, 128.0, 128.0, 128.0, 128.0, 127.9, 127.9, 127.8, 127.8, 127.8, 127.7, 127.7, 127.7, 127.6, 127.6, 127.5, 127.3, 101.3, 100.0, 95.0, 92.5, 78.6, 77.7, 76.7, 74.8, 74.7, 74.2, 73.7, 73.7, 73.6, 73.5, 73.4, 73.3, 72.9, 72.4, 70.5, 70.1, 69.2, 68.9, 68.3, 65.3, 60.4, 56.9, 55.9, 29.7.

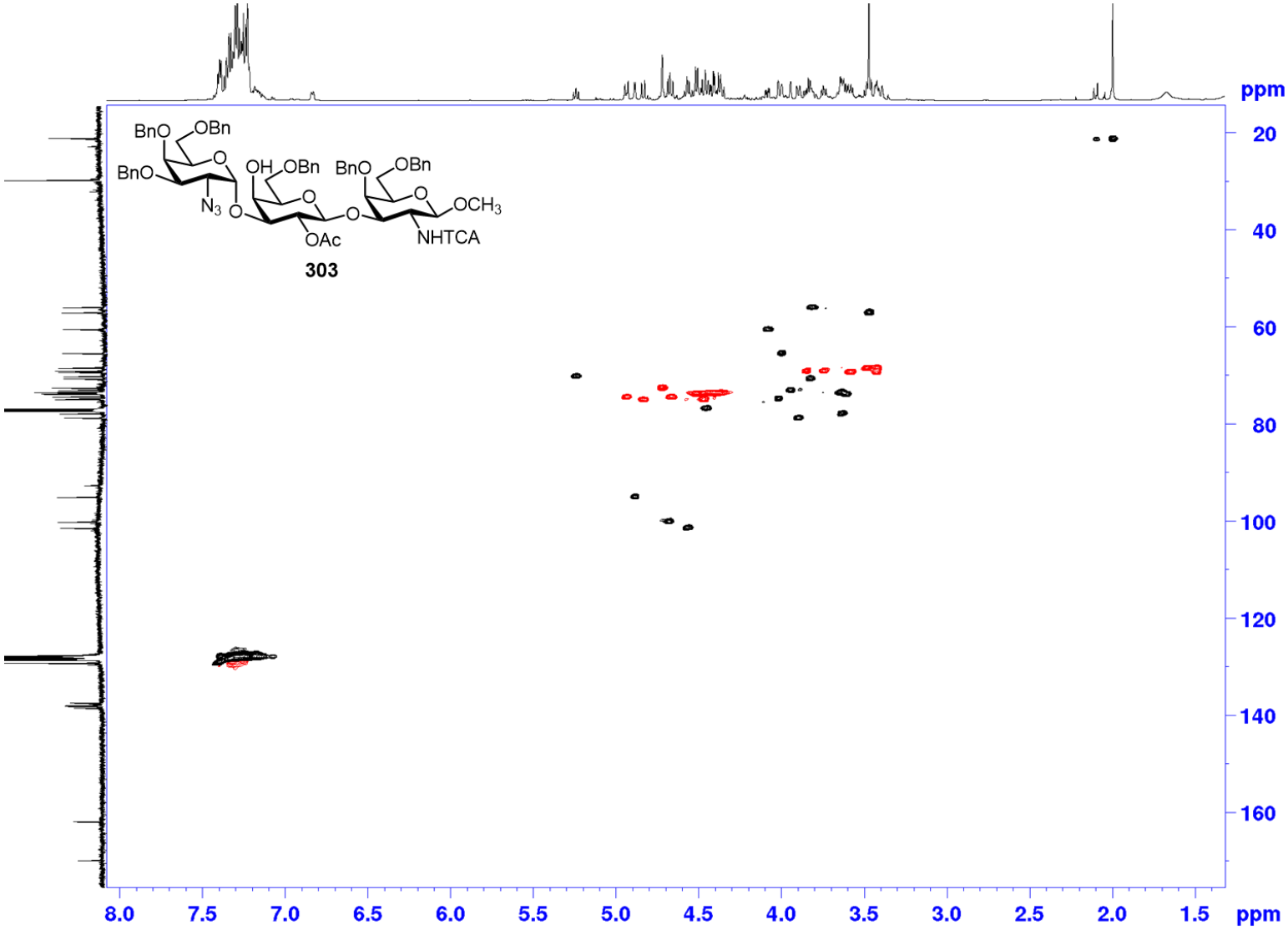
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 303



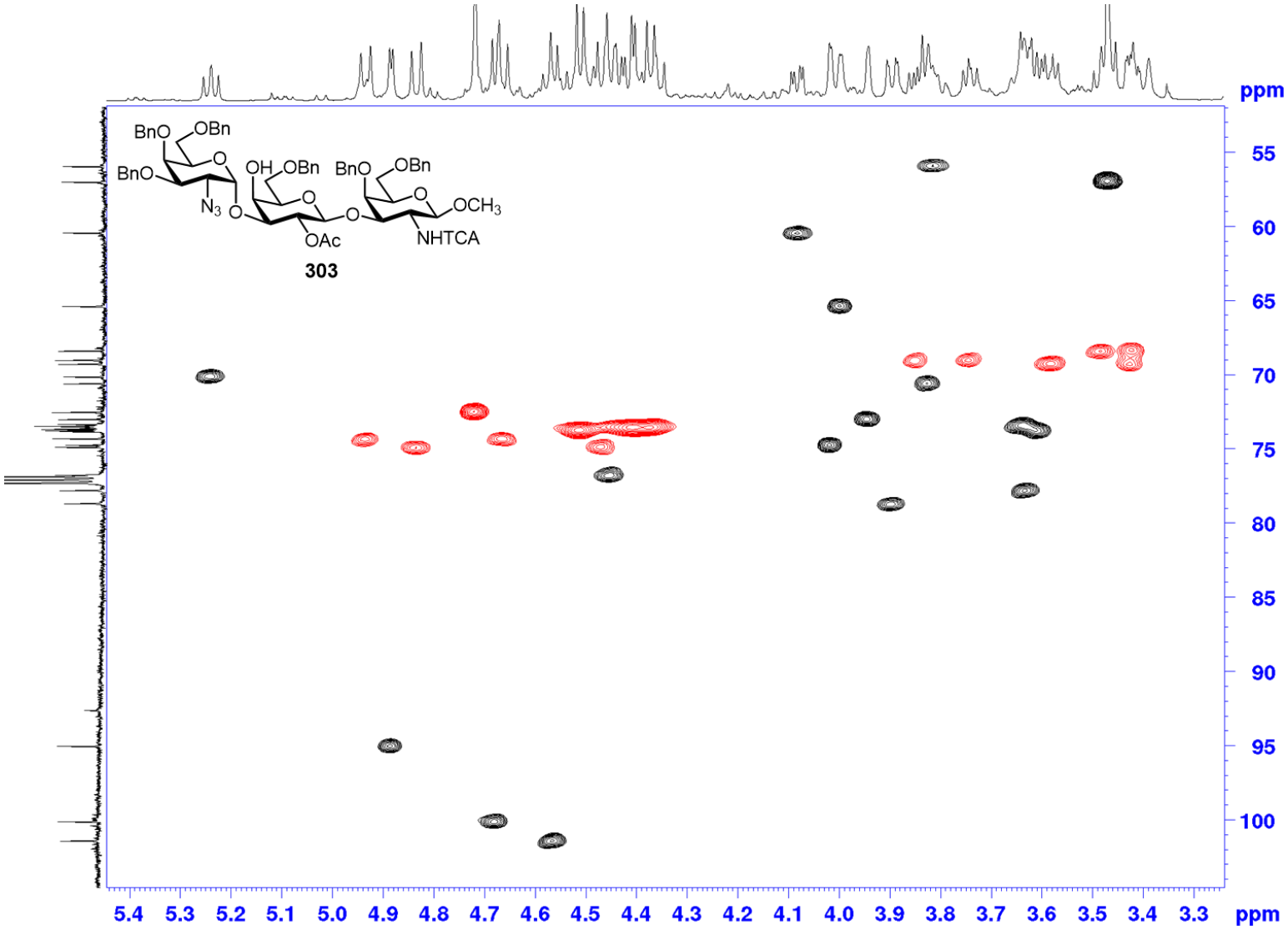
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 303



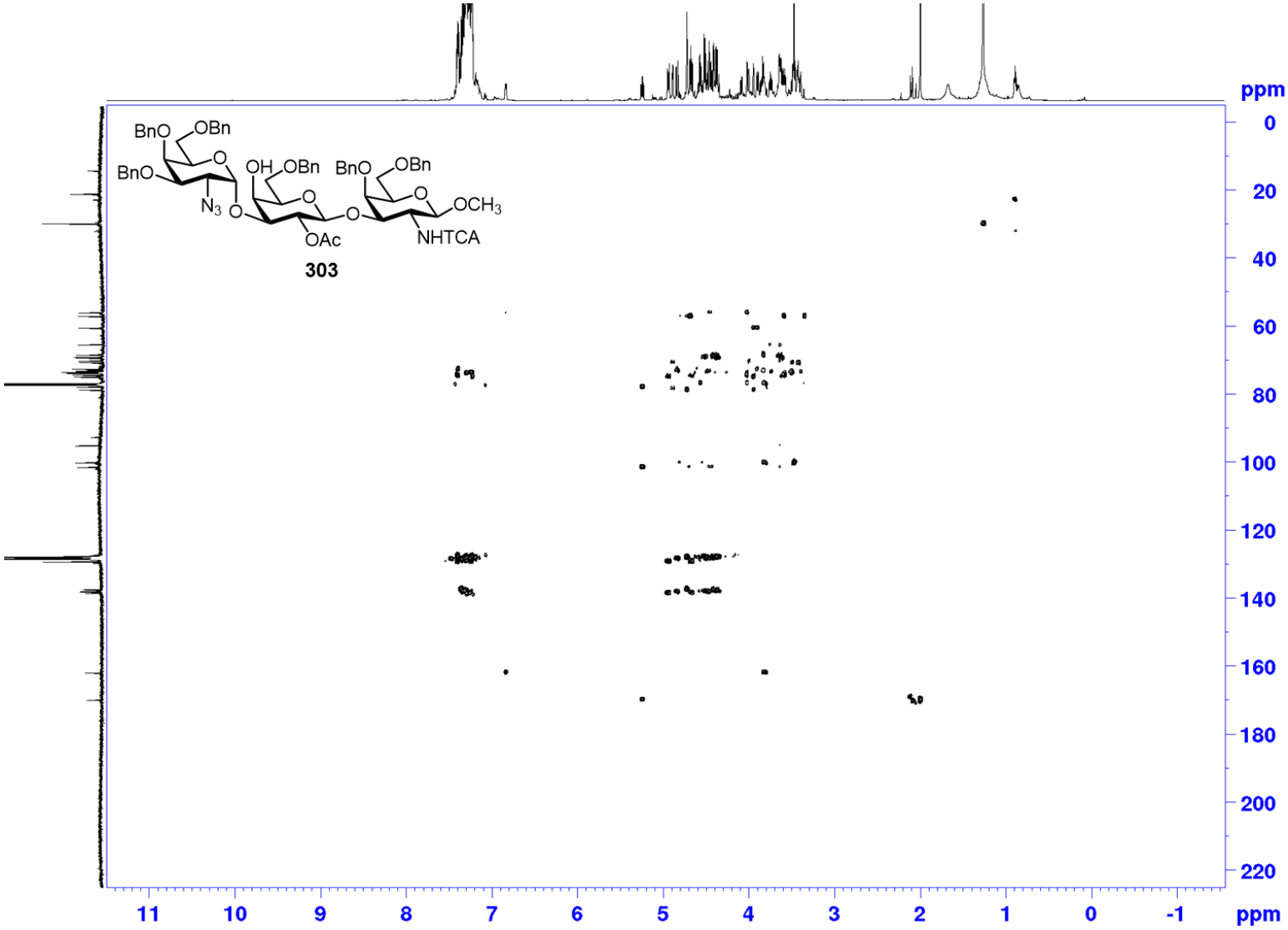
^{1}H-^{13}C HSCQ ($CDCl_3$) of compound 303



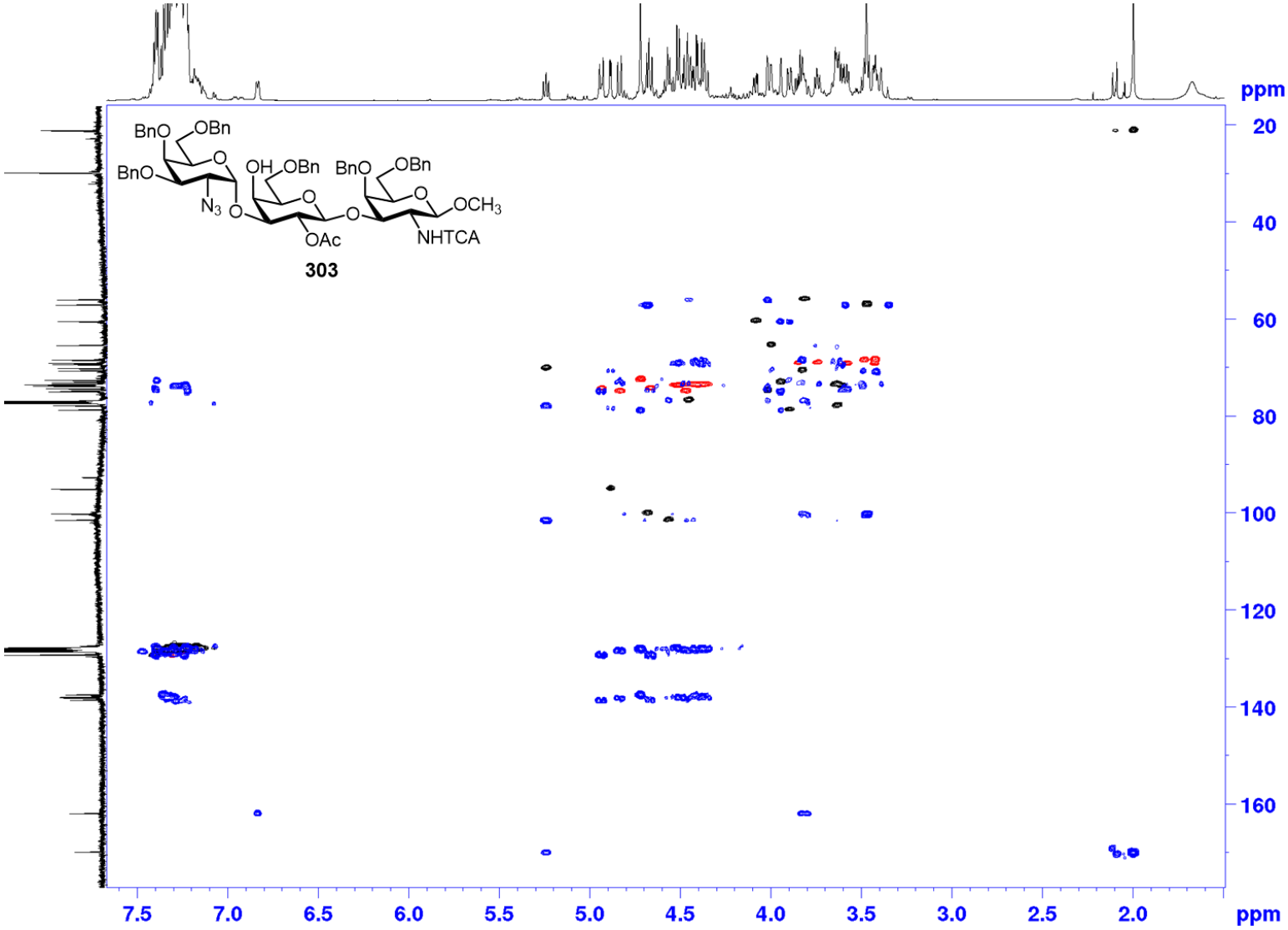
^{1}H-^{13}C HSCQ ($CDCl_3$) zoomed in region of compound 303



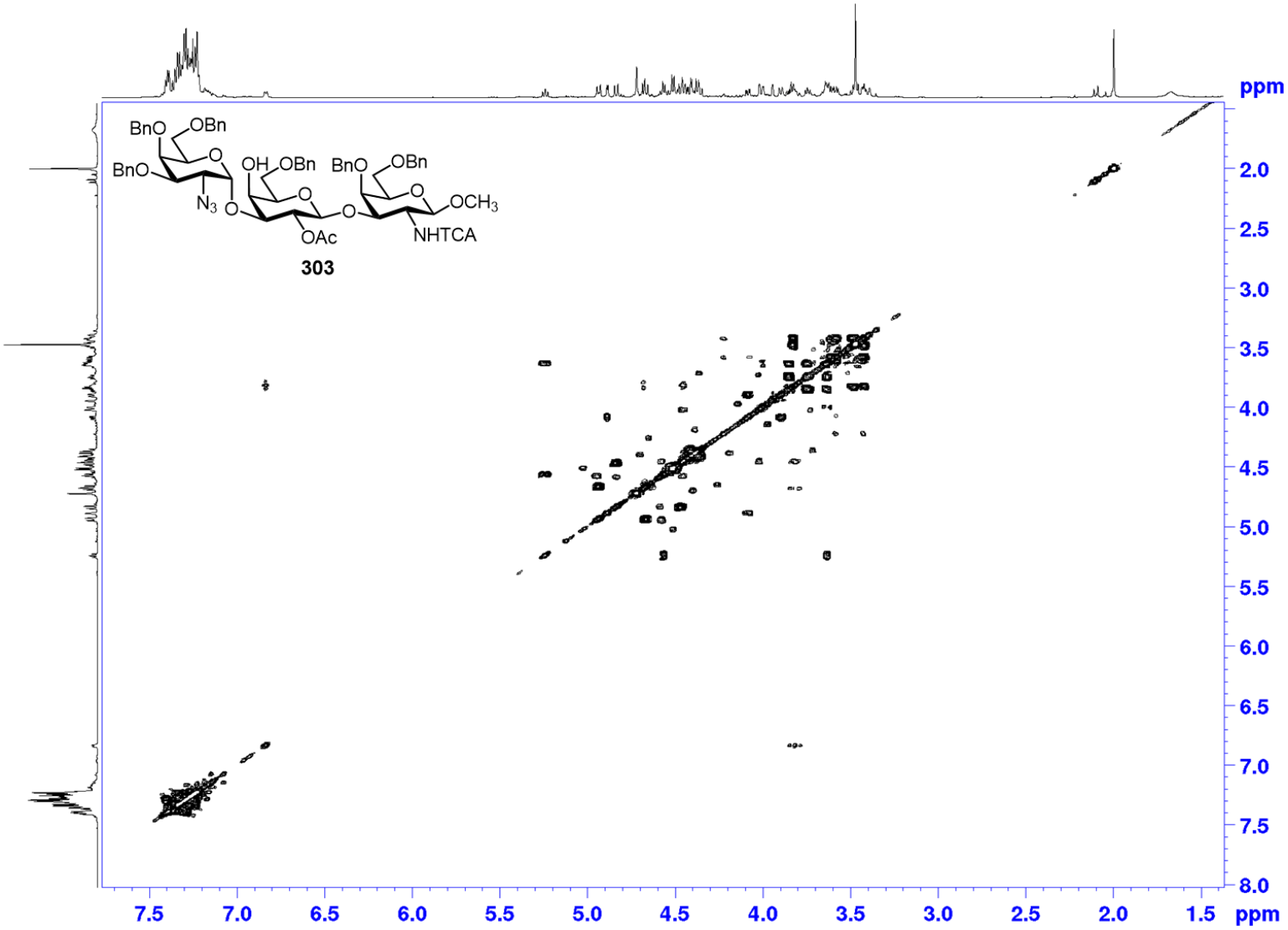
^{1}H-^{13}C HMBC ($CDCl_3$) of compound 303



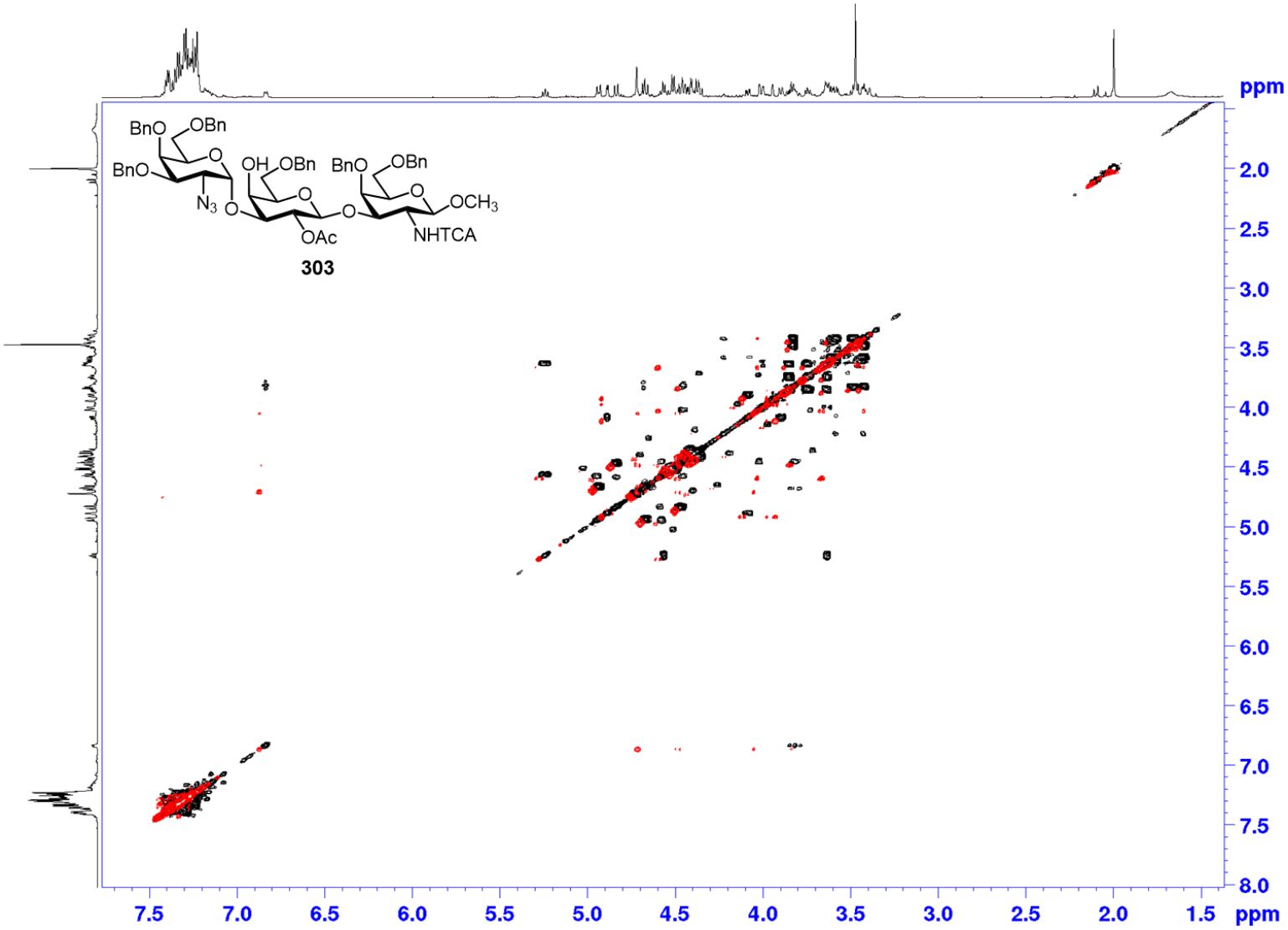
^{1}H-^{13}C HSCQ with ^{1}H-^{13}C HMBC overlay ($CDCl_3$) of compound 303

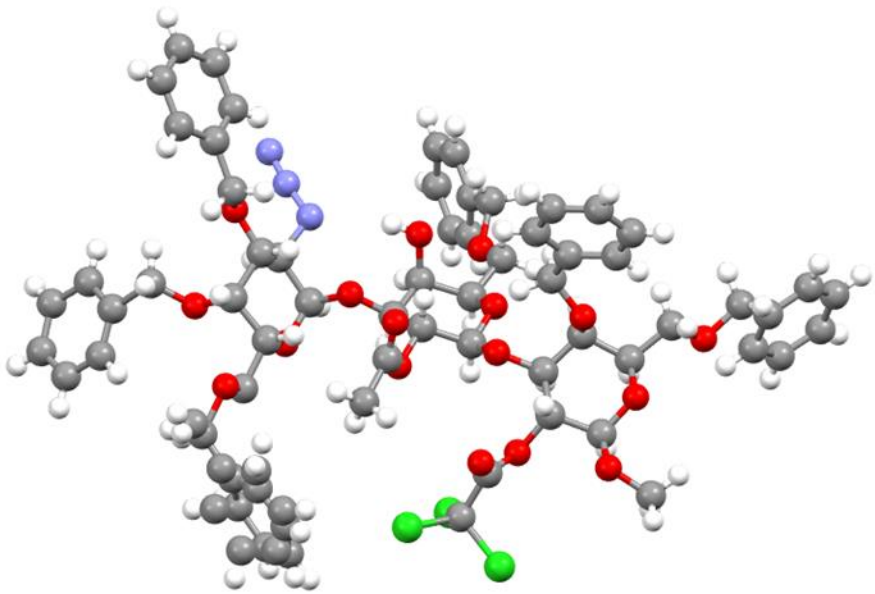


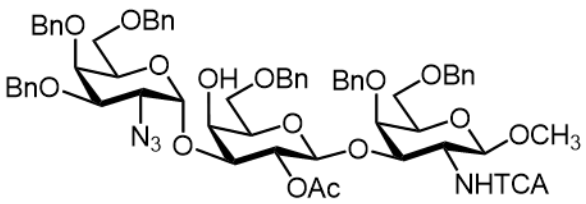
^{1}H-^{1}H COSY ($CDCl_3$) of compound 303



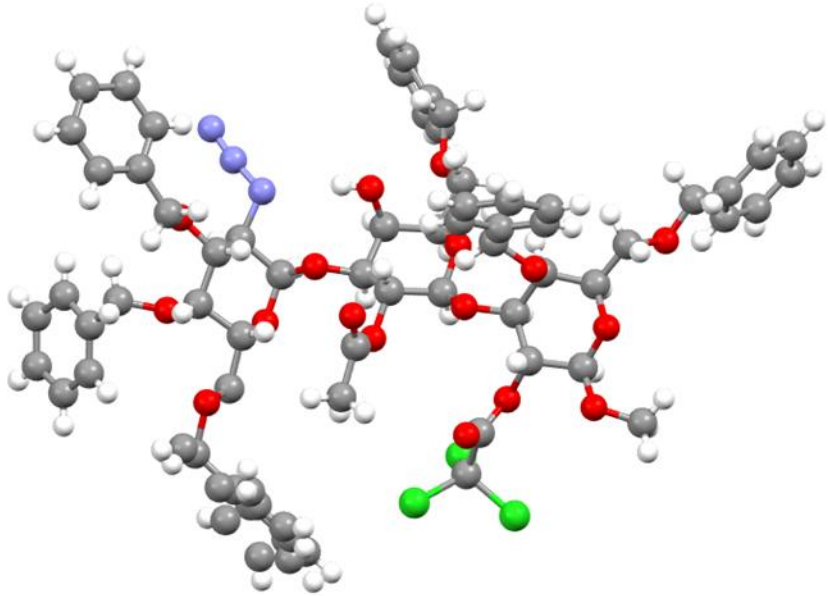
^{1}H-^{1}H COSY with ^{1}H-^{1}H TOSY overlay ($CDCl_3$) of compound 303

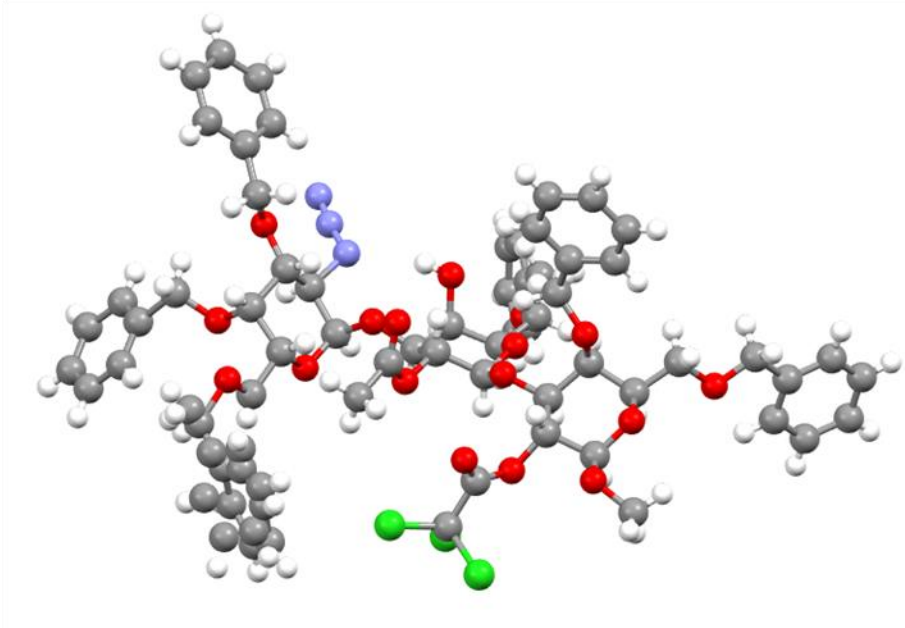






303

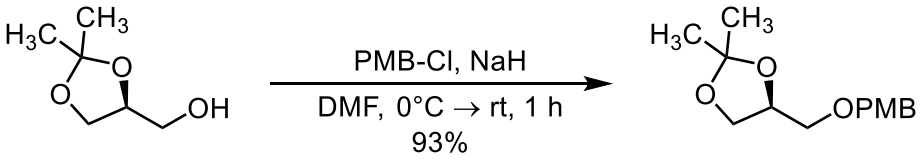




part 6. MM-ZPS 3rd-generation synthetic route

Part 6 contains experimental procedures for compounds found within schemes 42-53

(*S*)-4-(((4-methoxybenzyl)oxy)methyl)-2,2-dimethyl-1,3-dioxolane (320)

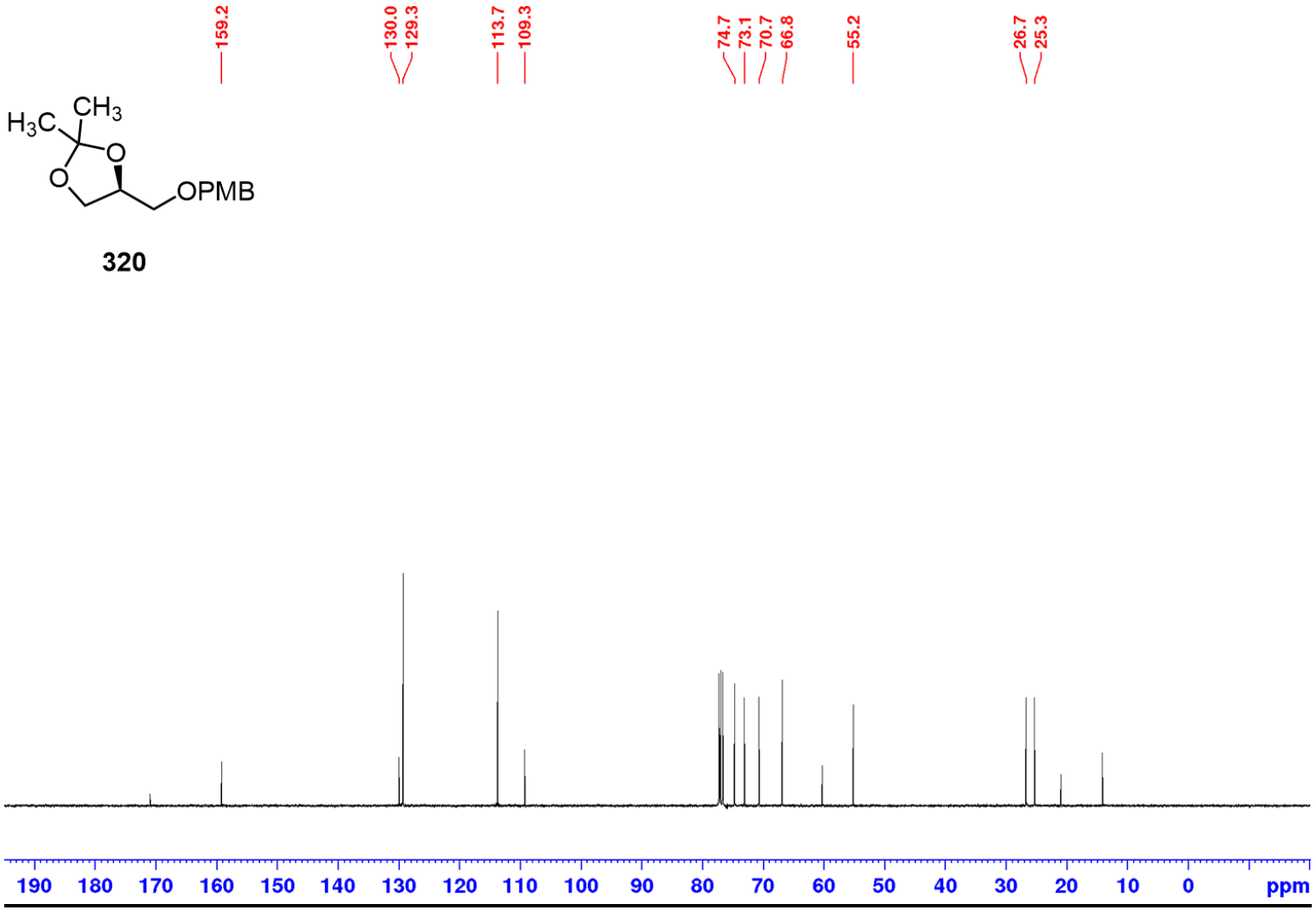


195 **320**

A solution of D-(+)-solketal **195** (1.0 equiv., 3.57 g, 27.0 mmol) in anhydrous DMF (90 mL) and was added NaH (3.0 equiv., 3.2 g, 81.0 mmol, 60% in mineral oil) at room temperature under an argon atmosphere. After 30 min, the reaction was cooled to 0 °C and PMB-Cl (1.25 equiv., 4.6 mL, 33.75 mmol) was added dropwise. The reaction mixture was stirred at room temperature for 12 h when TLC indicated that the starting material had been completely consumed. The reaction was then cooled to 0 °C and quenched by dropwise addition of anhydrous CH_3OH (1.0 mL). The reaction was diluted with EtOAc (200 mL) and transferred to a separatory funnel and washed with 1 N HCl (5 x 40 mL), satd. aq. $NaHCO_3$ (1 x 40 mL), brine (1 x 25), dried ($NaSO_4$) and filtered through a glass fritted vacuum filter funnel to remove drying agent. The filtrate was concentrated to a crude oil which was purified by flash column chromatography (10:90 to 20:80 EtOAc/hexanes) to give ketal **320** (6.33 g, 25.1 mmol, 93%)[a] as a clear oil. Our characterization data were consistent with previously reported data.[345-346]

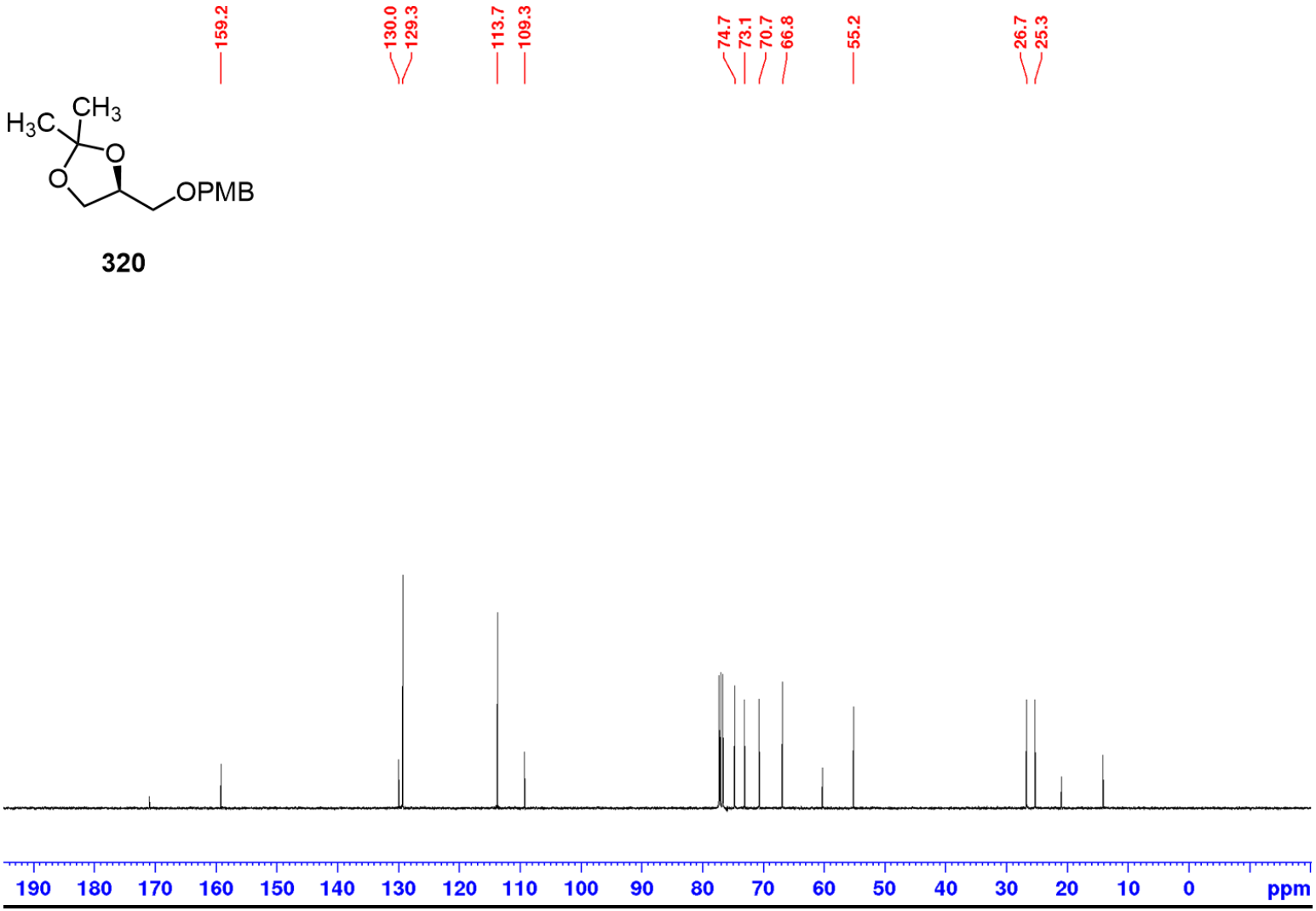
^{1}H NMR (400 MHz, $CDCl_3$): δ 7.23 (d, *J* = 10.1 Hz, 2H), 6.87 (d, *J* = 9.2 Hz, 2H), 4.52 (d, *J* = 11.5 Hz, 1H), 4.50 (d, *J* = 11.7 Hz, 1H), 4.28 (pentet, *J* = 5.9, 11.8 Hz, 1H), 4.04 (dd, *J* = 6.9, 8.7 Hz, 1H), 3.79 (s, 3H), 3.72 (dd, *J* = 6.3, 8.3 Hz, 1H), 3.52 (dd, *J* = 5.6, 10.1 Hz, 1H), 3.43 (dd, *J* = 5.6, 9.4 Hz, 1H), 1.42 (s, 3H), 1.37 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 159.2, 130.0, 129.3, 113.7, 109.3, 74.7, 73.1, 70.7, 66.9, 55.2, 26.7, 25.3. [a] yield reported contains solvent impurities. see NMR spectra.

^{1}H NMR ($CDCl_3$, 400 MHz) of compound 320



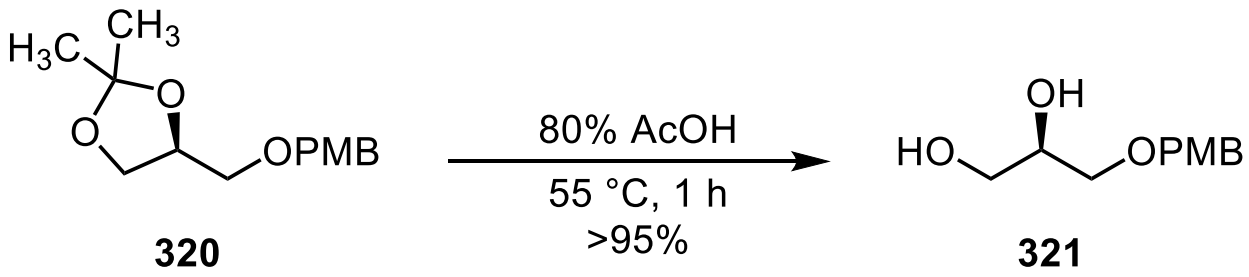
320

^{13}C NMR ($CDCl_3$, 100 MHz) of compound 320



320

(*R*)-3-((4-methoxybenzyl)oxy)propane-1,2-diol (321)

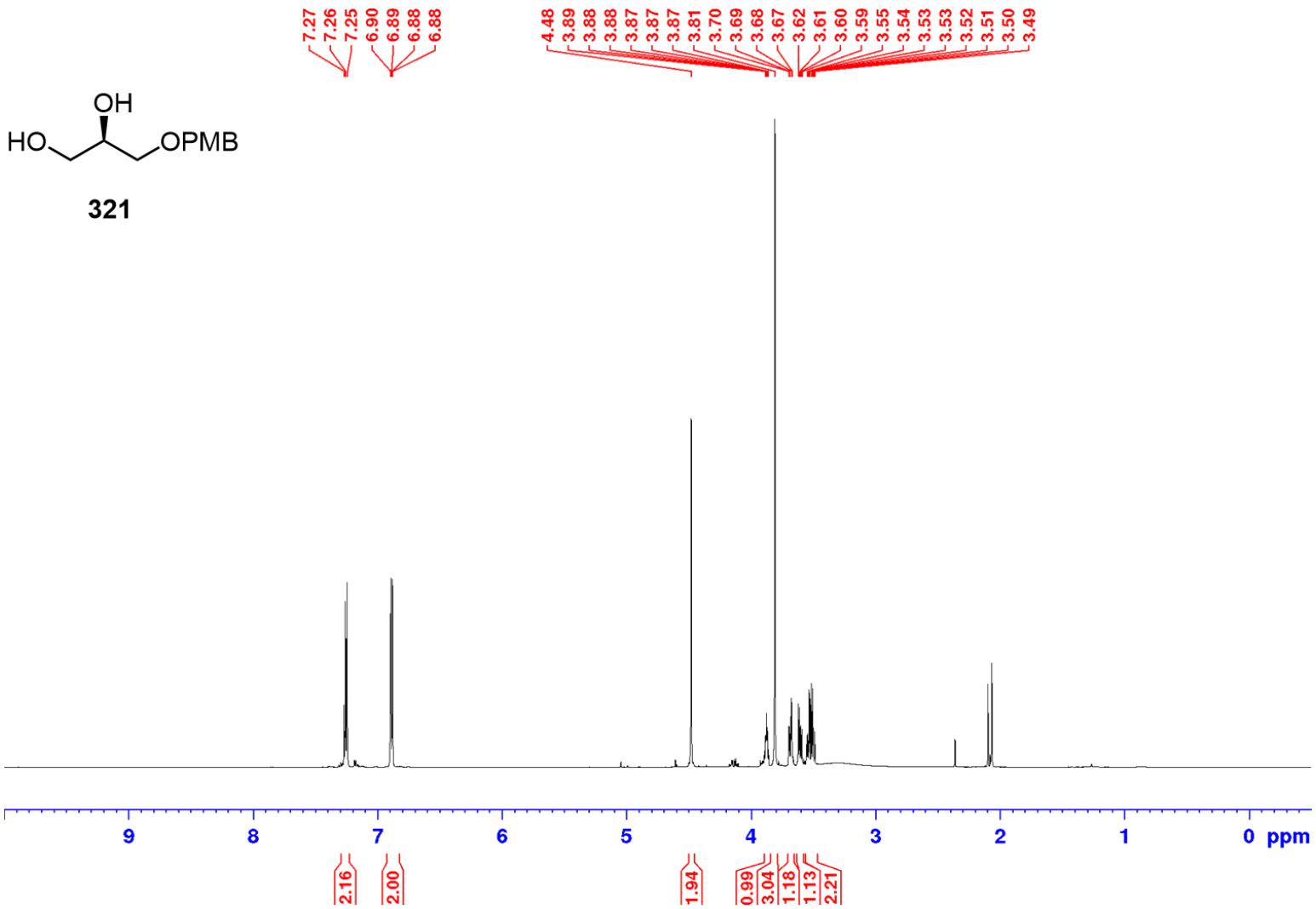


320 **321**

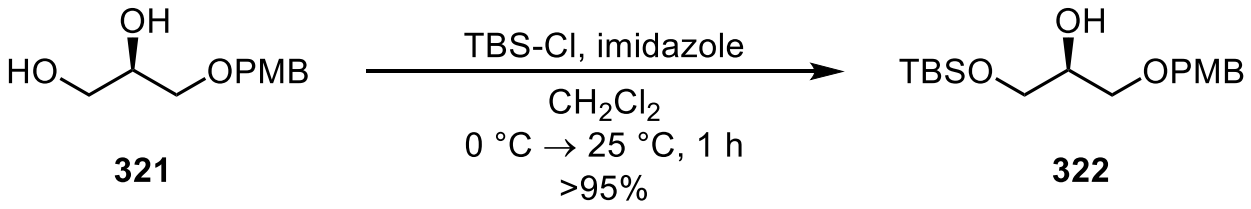
A solution of ketal **320** (1.0 equiv., 2.008 g, 7.96 mmol) in 80% (aq) AcOH was heated to 65 °C. At 1 h, TLC (85:15 EtOAc/hexanes, CAM stain) indicated complete consumption of starting material for a more polar spot. The reaction was concentrated to an oil to give diol **321** (1.664 g, 7.84 mmol, >95%) which was carried forward with no further purification. Spectroscopic data in in agreement with previously reported data.[345-346]

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.27 (d, *J* = 8.7 Hz, 2H), 6.88 (d, *J* = 8.7 Hz, 2H), 4.48 (s, 2H), 3.89-3.87 (m, 1H), 3.82 (s, 3H), 3.68 (dd, *J* = 3.2, 11.7 Hz, 1H), 3.61 (dd, *J* = 5.6, 11.2 Hz, 1H), 3.54 (dd, *J* = 3.5, 9.6 Hz, 1H), 3.5 (dd, *J* = 6.3, 9.6 Hz, 1H).

^{1}H NMR ($CDCl_3$, 400 MHz) of compound 321



(*S*)-1-((tert-butyldimethylsilyl)oxy)-3-((4-methoxybenzyl)oxy)propan-2-ol (322)

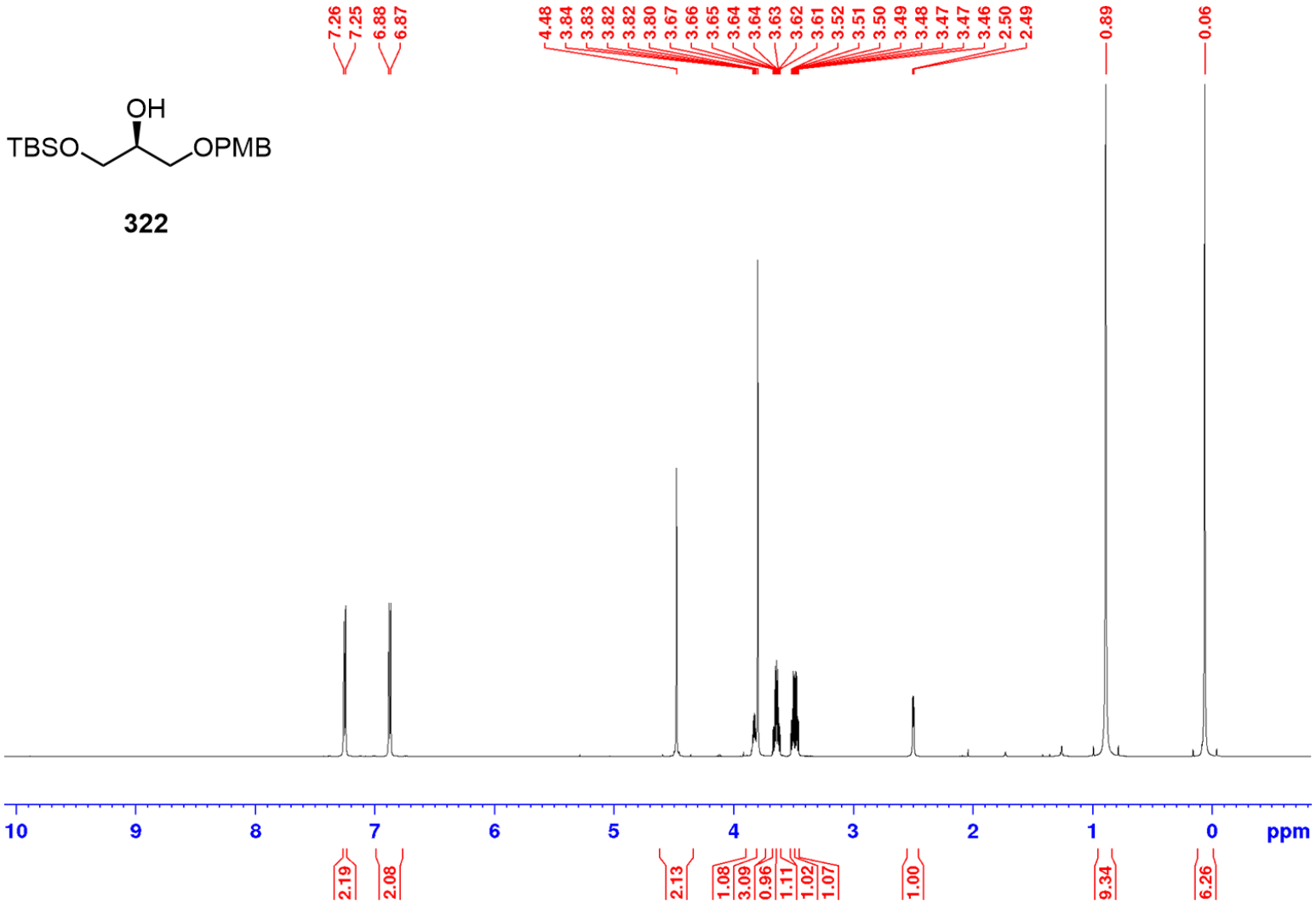


321 **322**

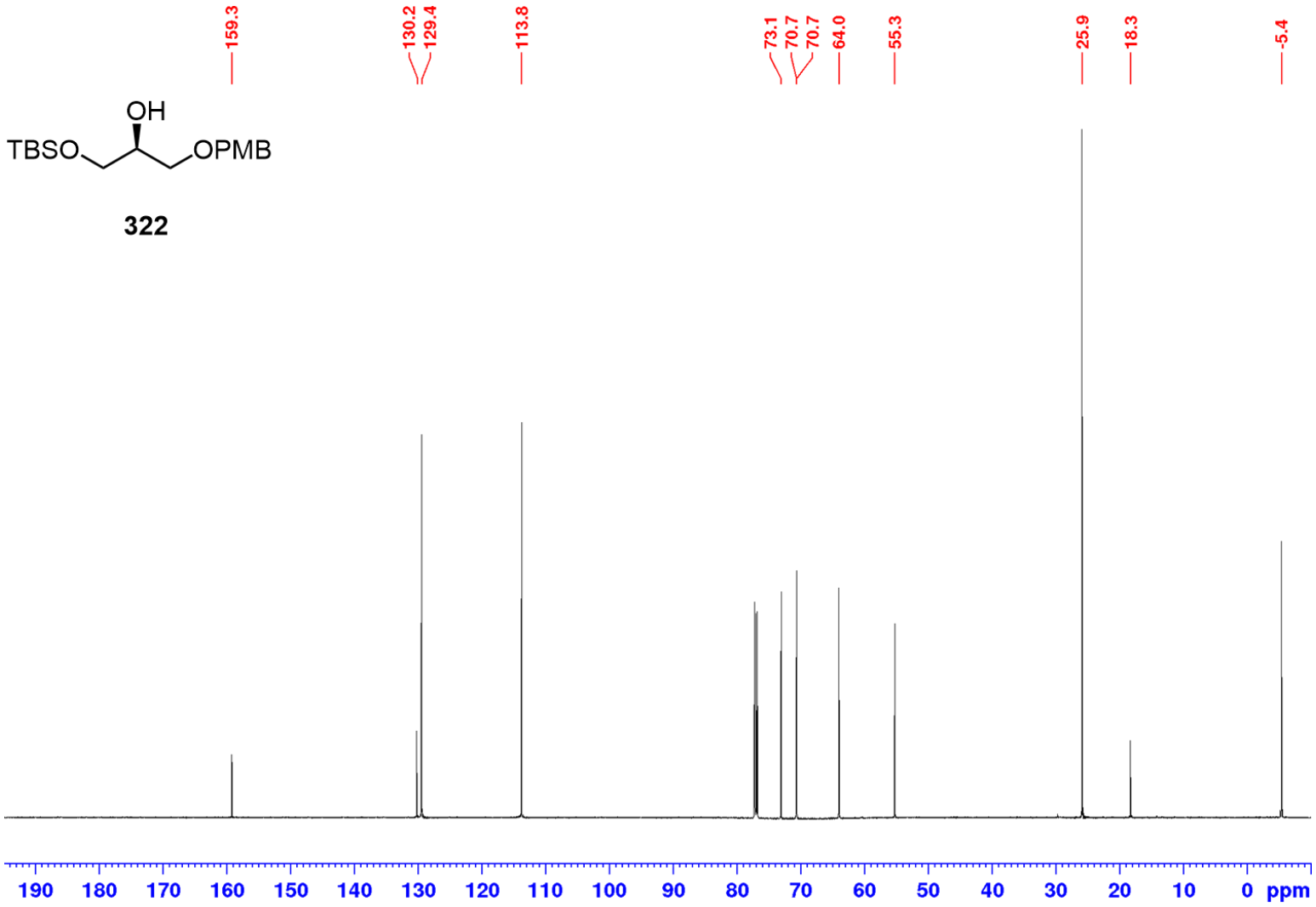
To a solution of diol **321** (1.0 equiv., 1.69 g, 7.96 mmol) and imidazole (2.5 equiv., 1.35 g, 19.9 mmol) in anhydrous CH_2Cl_2 (50 mL) was added solution of TBS-Cl (1.5 equiv., 1.80 g, 19.9 mmol) in anhydrous CH_2Cl_2 (10 mL) dropwise at 0 °C under an argon atmosphere. At 1 h, TLC (25:75 EtOAc/hexanes, CAM stain) indicated complete consumption of the starting material for a less polar spot. The crude reaction mixture was concentrated to dryness then transferred to a separatory funnel with EtOAc (100 mL). The reaction was washed with 1 N HCl (3 x 25 mL), satd. aq. $NaHCO_3$ (1 x 25 mL), brine (1 x 125 mL), dried ($NaSO_4$) and filtered through a glass fritted vacuum filter funnel to remove drying agent. The filtrate was concentrated to a crude oil which was purified by flash column chromatography (10:90 to 25:75 EtOAc/hexanes) to give alcohol **322** (2.59 g, 7.93 mmol, >95%) as a clear oil. Spectroscopic data in in agreement with previously reported data.[345-346]

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.25 (d, *J* = 8.4 Hz, 2H), 6.88 (d, *J* = 8.4 Hz, 2H), 4.48 (m, 2H), 3.85 (sextet, *J* = 5.2, 10.8, 15.2 Hz, 1H), 3.77 (s, 3H), 3.66 (dd, *J* = 10.1, 18.4 Hz, 1H), 3.63 (dd, *J* = 10.3, 18.3 Hz, 1H), 3.51 (dd, *J* = 9.8, 21.3 Hz, 1H), 3.47 (dd, *J* = 9.4, 21.9 Hz, 1H), 2.50 (s, 1H), 0.9 (s, 9H), 0.06 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 159.3, 130.2, 129.4, 113.8, 73.1, 70.7, 70.7, 64.1, 55.3, 25.9, 18.3, -5.4.

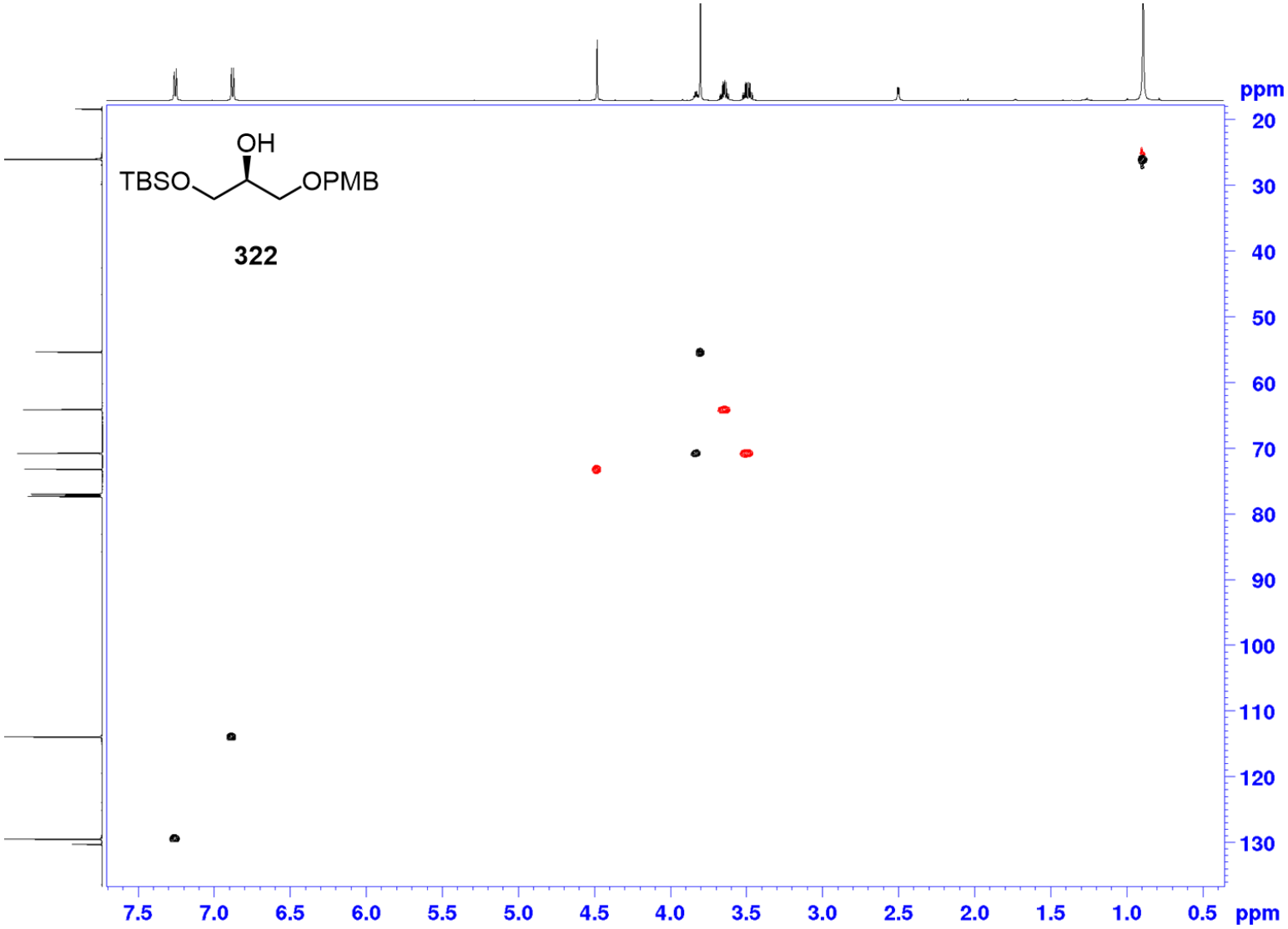
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 322



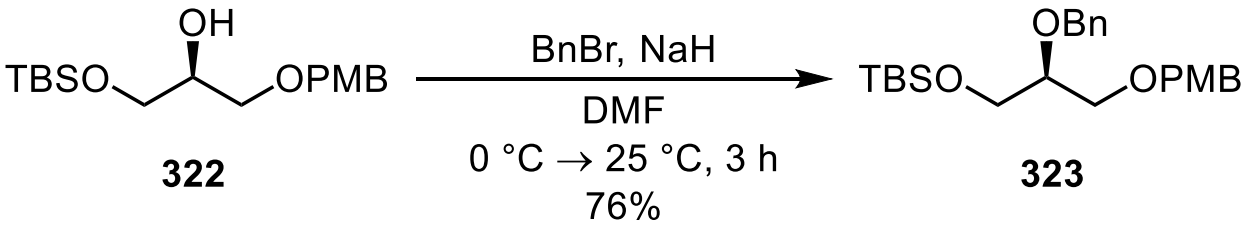
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 322



^{1}H-^{13}C HSCQ ($CDCl_3$) of compound 322



(*S*)-(2-(benzyloxy)-3-((4-methoxybenzyl)oxy)propoxy)(tert-butyl)dimethylsilane (323)



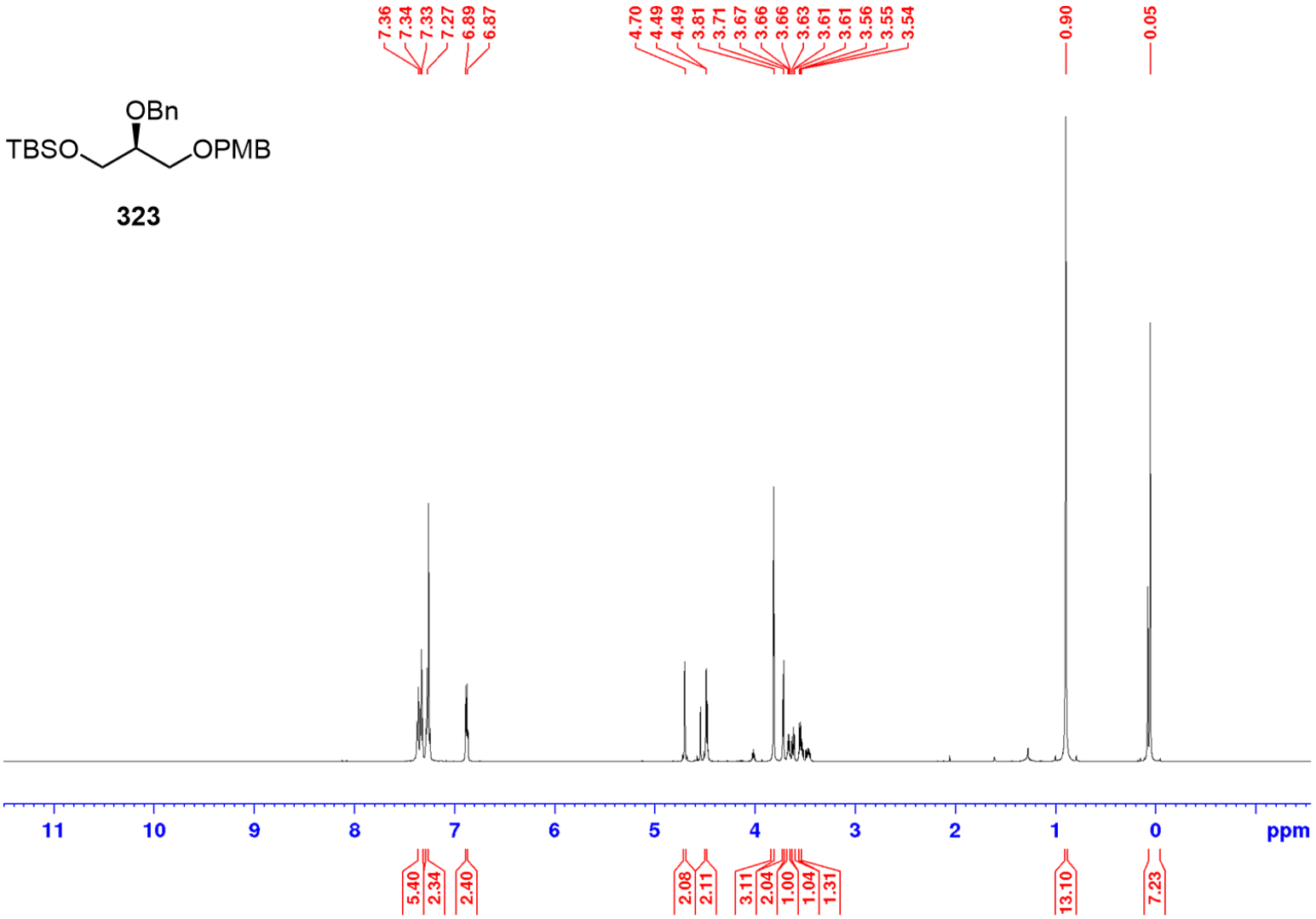
322 **323**

A solution of alcohol **322** (1.0 equiv., 1.795 g, 5.49 mmol) was dissolved win anhydrous DMF (25 mL) and added NaH (2.0 equiv., 0.44 g, 9.99 mmol, 60% in mineral oil) at room temperature under a stream argon which was allowed to evacuate the reaction flask through a vent needle. After 30 min, the reaction was cooled to 0 °C and benzyl bromide (1.2 equiv., 0.80 mL, 6.58 mmol) was added dropwise. The reaction was stirred at room temperature when TLC indicated that the starting material had been completely consumed. The reaction was then cooled to 0 °C and quenched by dropwise addition of CH_3OH (1.0 mL). The reaction was diluted with EtOAc (100 mL) and transferred to a separatory funnel and washed with 1 N HCl (5 x 25 mL), satd. aq. $NaHCO_3$ (1 x 10 mL), brine (1 x 25), dried ($NaSO_4$) and filtered through a glass fritted vacuum filter funnel to remove drying agent. The filtrate was concentrated to a crude oil which was purified by flash column chromatography (10:90 to 20:80 EtOAc/hexanes) to give protected glycerol **323** (1.7423 g, 1.302 mmol, 76%) as a clear oil. Our characterization data were consistent with previously reported data.[345-346]

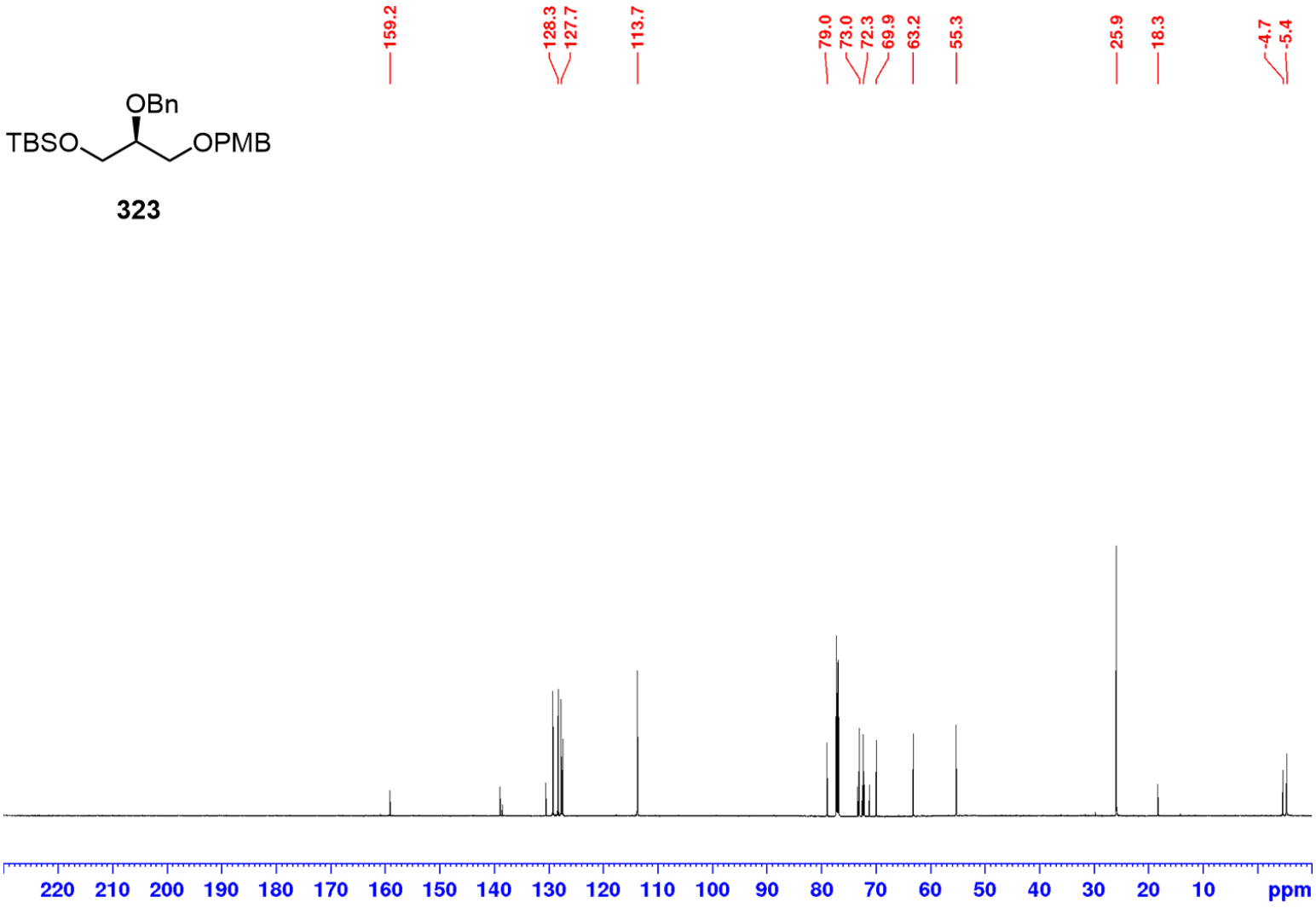
^1H NMR (400 MHz, $CDCl_3$): δ 7.36-7.34 (m, 5H), 7.32-7.30 (m, 2H), 6.88 (d, *J* = 8.7 Hz, 2H), 4.70 (s, 2H), 4.49 (d, *J* = 1.7 Hz, 2H), 3.83 (s, 3H), 3.72-3.70 (m, 2H), 3.68-3.66 (m, 1H), 3.61-3.59 (m, 1H), 3.56-3.54 (m, 1H), 0.90 (s, 9H), 0.01 (s, 6H); ^{13}C NMR (100 MHz,

$CDCl_3$): δ 159.2, 128.3, 127.7, 113.8, 78.0, 73.1, 72.3, 69.9, 63.2, 55.3, 25.9, 18.3, -4.7, -5.4, -5.4.

^{1}H NMR ($CDCl_3$, 400 MHz) of compound 323

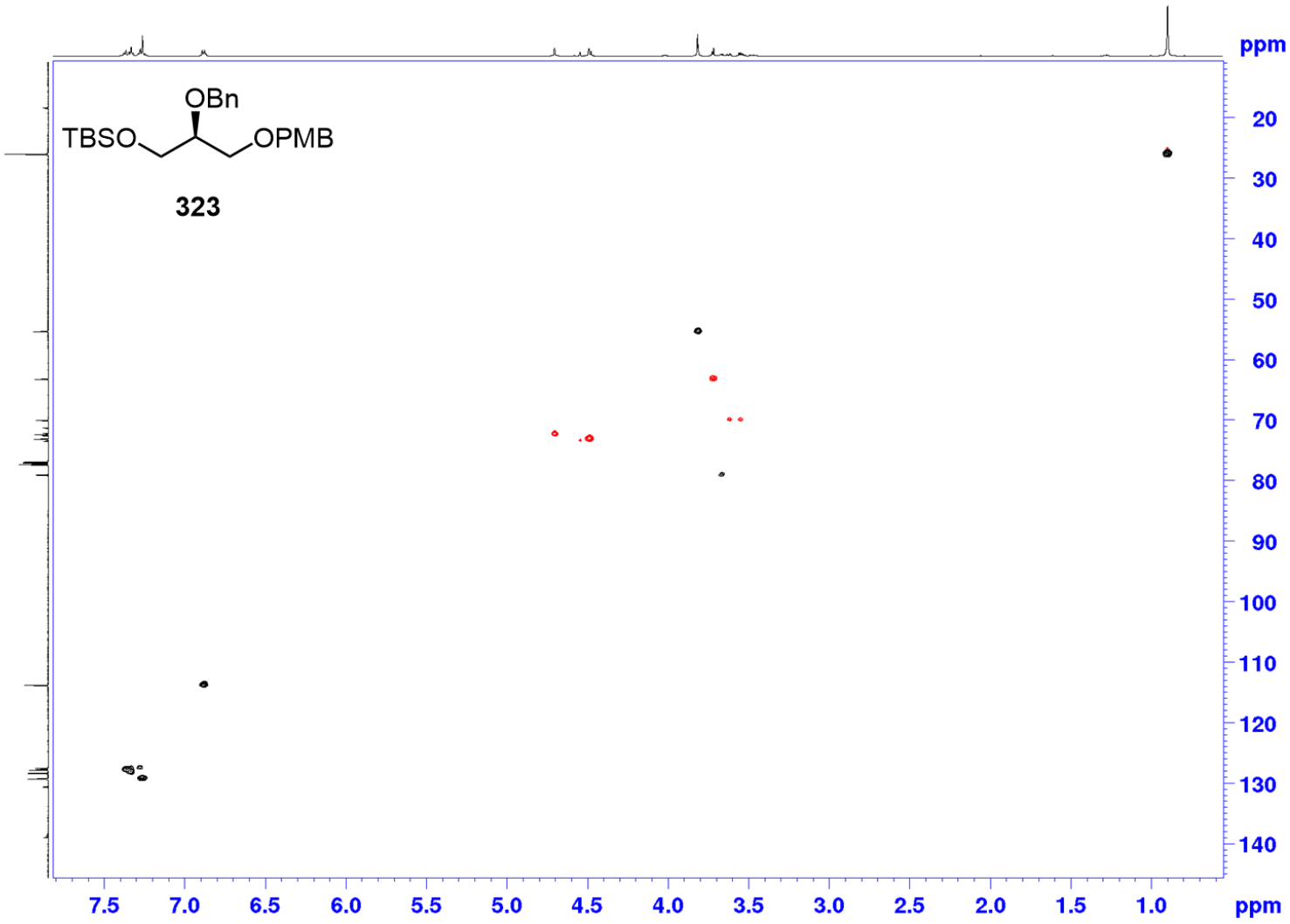


^{13}C NMR ($CDCl_3$, 100 MHz) of compound 323

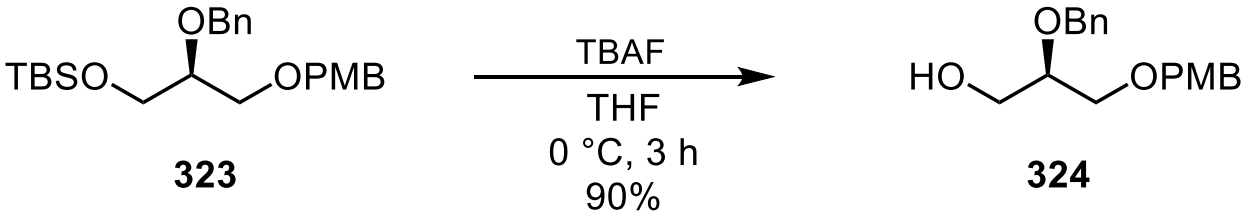


323

^{1}H-^{13}C HSCQ ($CDCl_3$) of compound 323



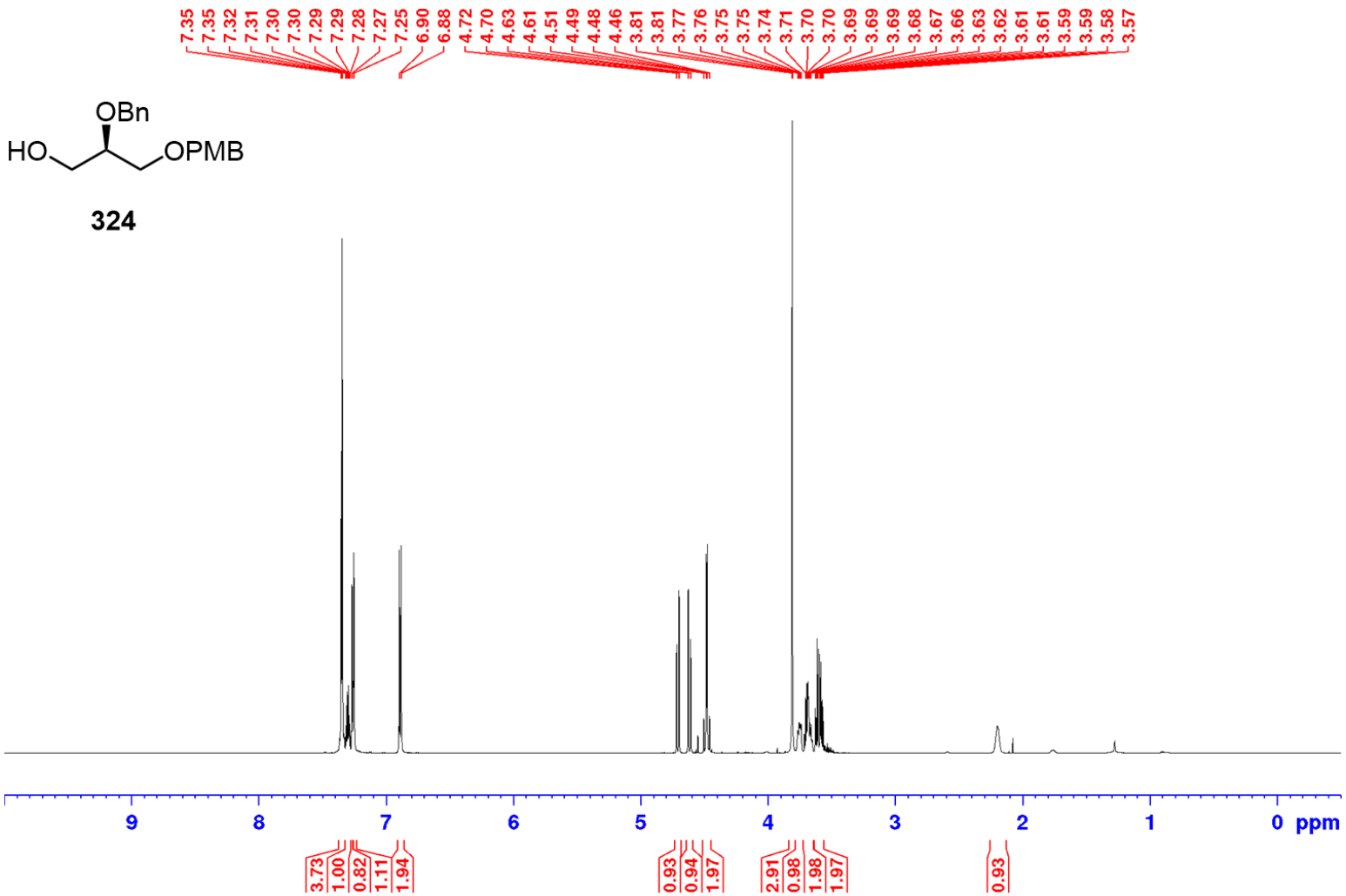
(*R*)-2-(benzyloxy)-3-((4-methoxybenzyl)oxy)propan-1-ol (324)



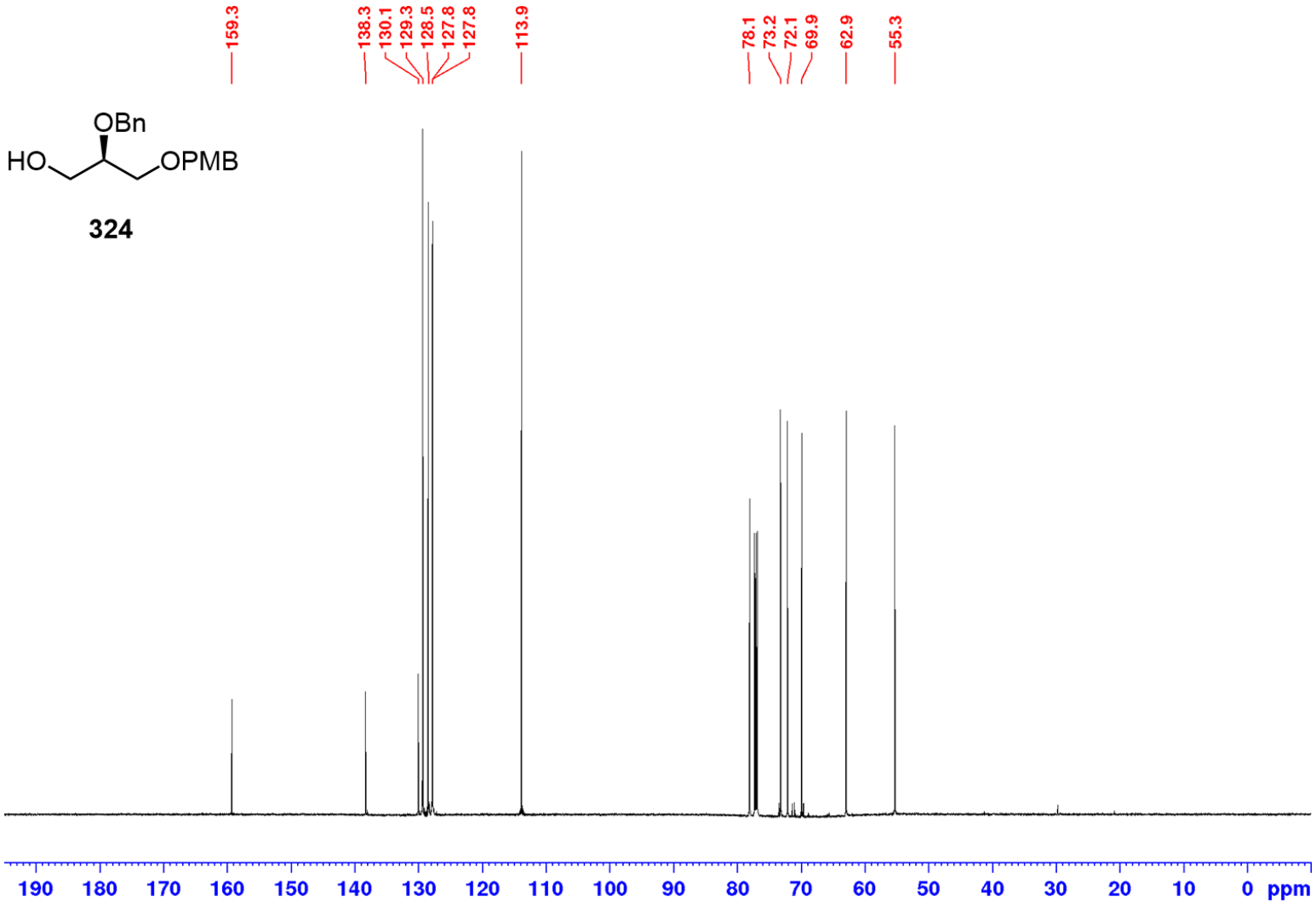
A solution of glycerol **323** (1.0 equiv., 1.7423 g, 1.302 mmol) in anhydrous THF (20 mL) was added a solution of TBAF (1.2 equiv., 4.7 mL, 1.56 mmol) dropwise at room temperature under an argon atmosphere. At 3 h, TLC indicated the starting material had been consumed and the reaction was transferred to a separatory funnel with EtOAc (50 mL) and washed with 1 N HCl (5 x 25 mL), satd. aq. $NaHCO_3$ (1 x 10 mL), brine (1 x 25), dried ($NaSO_4$) and filtered through a glass fritted vacuum filter funnel to remove drying agent. The filtrate was concentrated to a crude oil which was purified by flash column chromatography (25:75 to 40:60 EtOAc/hexanes) to give alcohol **324** (1.075 g, 3.555 mmol, 90%) as a clear oil. Our characterization data were consistent with previously reported data.[345-346]

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.35 (d, *J* = 4.6 Hz, 4H), 7.32-7.28 (m, 1H), 7.27 (d, *J* = 8.8 Hz, 1H), 7.25 (d, *J* = 8.8 Hz, 1H), 6.89 (d, *J* = 8.4 Hz, 2H), 4.71 (d, *J* = 12.4 Hz, 1H), 4.62 (d, *J* = 11.2 Hz, 1H), 4.50 (d, *J* = 11.5 Hz, 1H), 4.47 (d, *J* = 11.5 Hz, 1H), 3.81 (s, 3H), 3.77-3.74 (m, 1H), 3.71-3.66 (m, 2H), 3.62 (dd, *J* = 5.0, 10.1 Hz, 1H), 3.58 (dd, *J* = 4.8, 10.0 Hz, 1H), 2.19 (-OH, br, *J* = Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 159.3, 138.3, 130.1, 129.3, 128.5, 127.8, 127.8, 113.9, 78.1, 73.2, 72.1, 69.9, 62.9, 55.3.

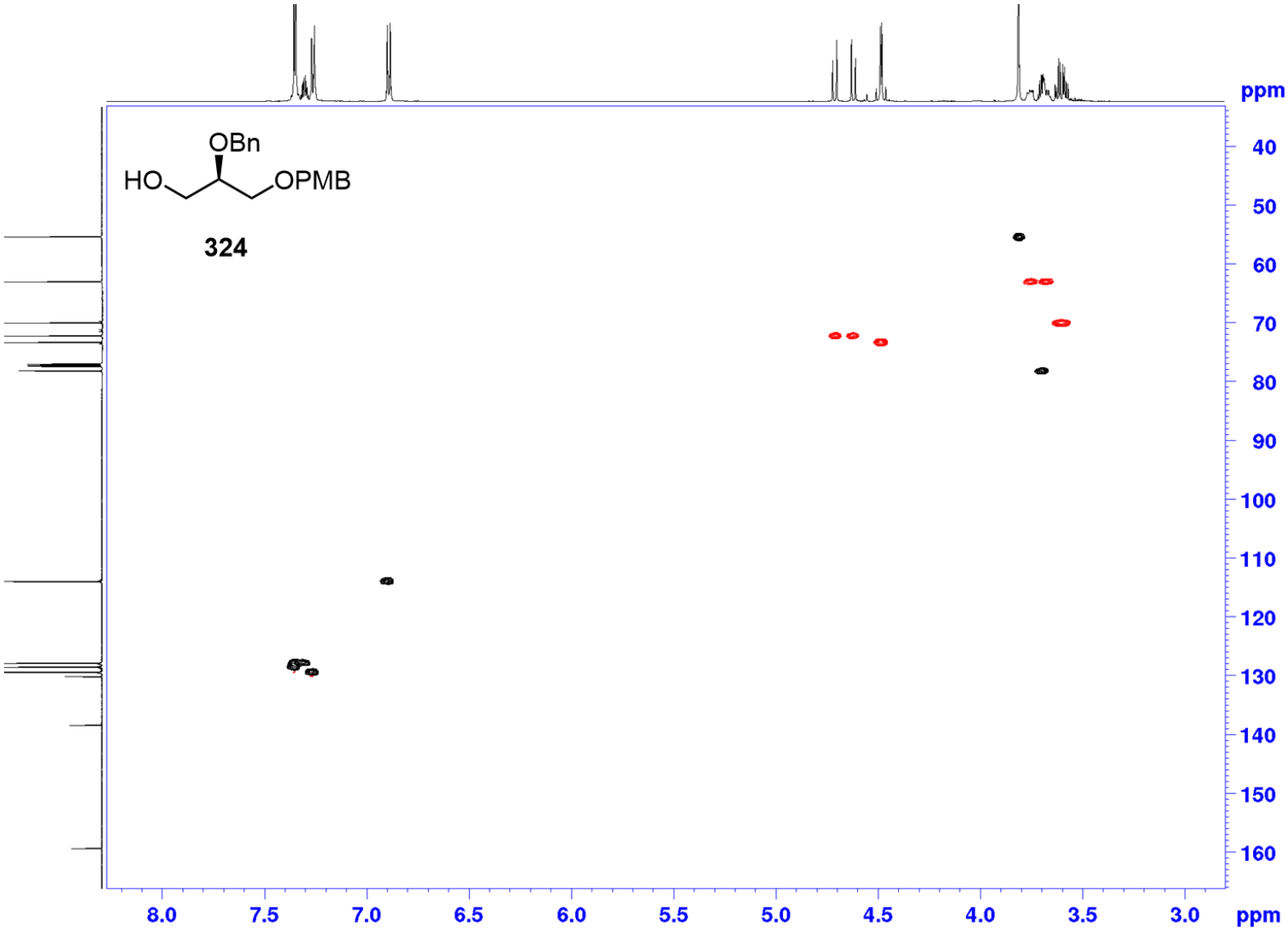
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 324



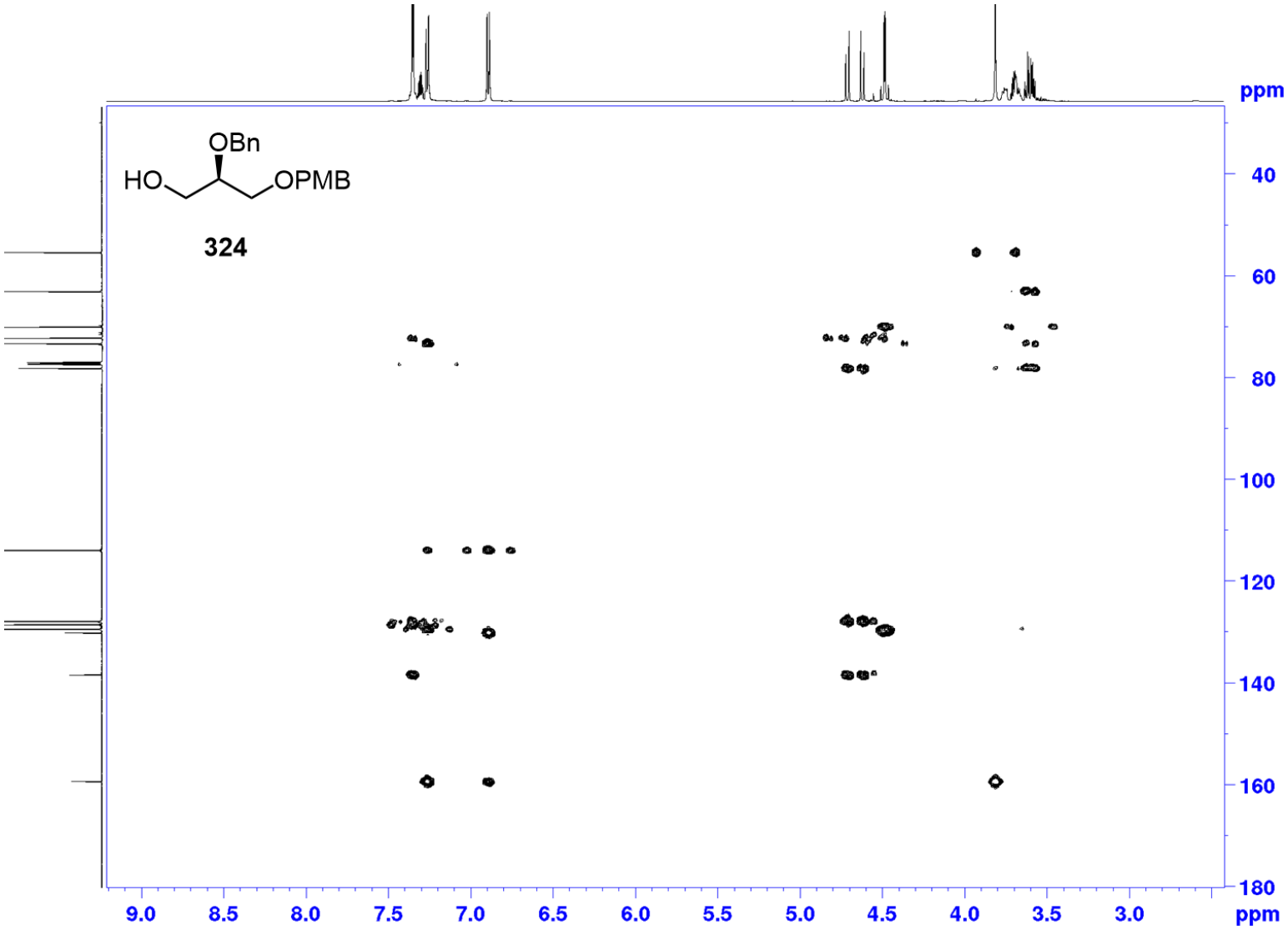
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 324



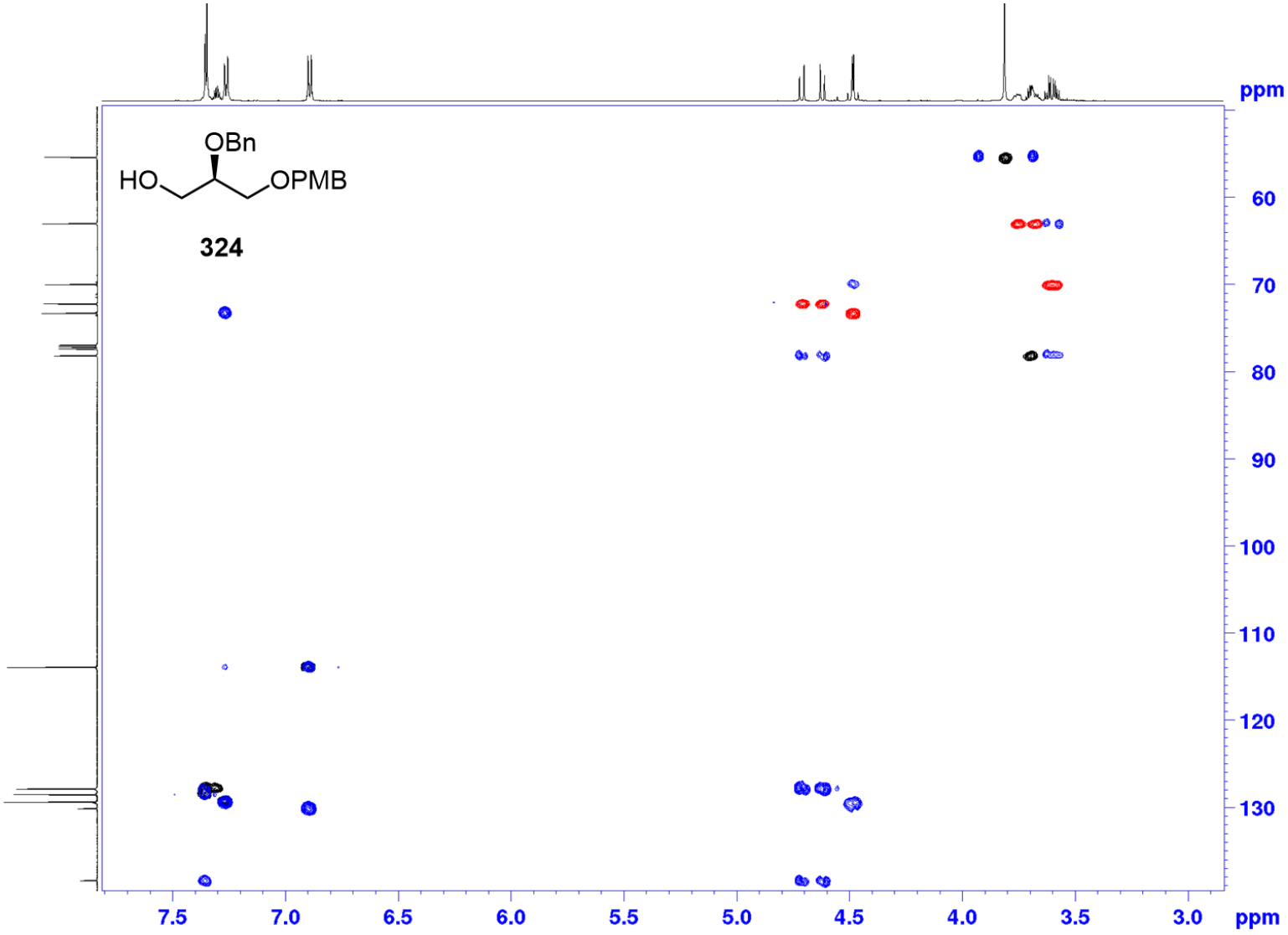
^{1}H-^{13}C HSCQ ($CDCl_3$) of compound 324



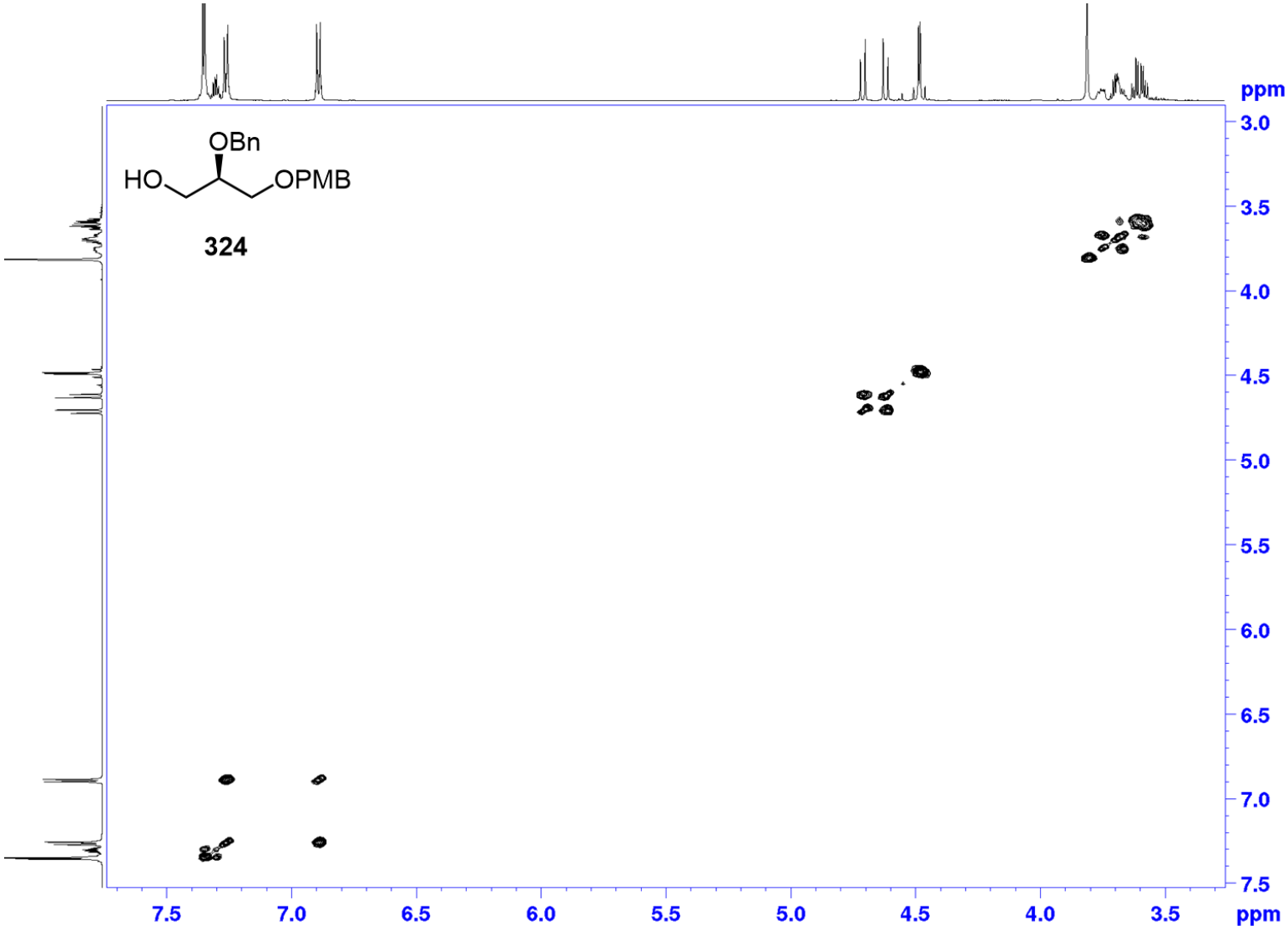
^{1}H-^{13}C HMBC ($CDCl_3$) of compound 324



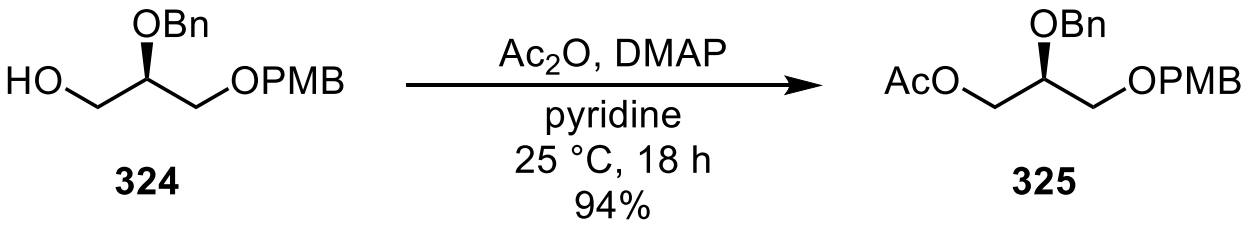
^{1}H-^{13}C HSCQ with ^{1}H-^{13}C HMBC overlay ($CDCl_3$) of compound 324



^{1}H-^{1}H COSY ($CDCl_3$) of compound 324



(*S*)-2-(benzyloxy)-3-((4-methoxybenzyl)oxy)propyl acetate (325)

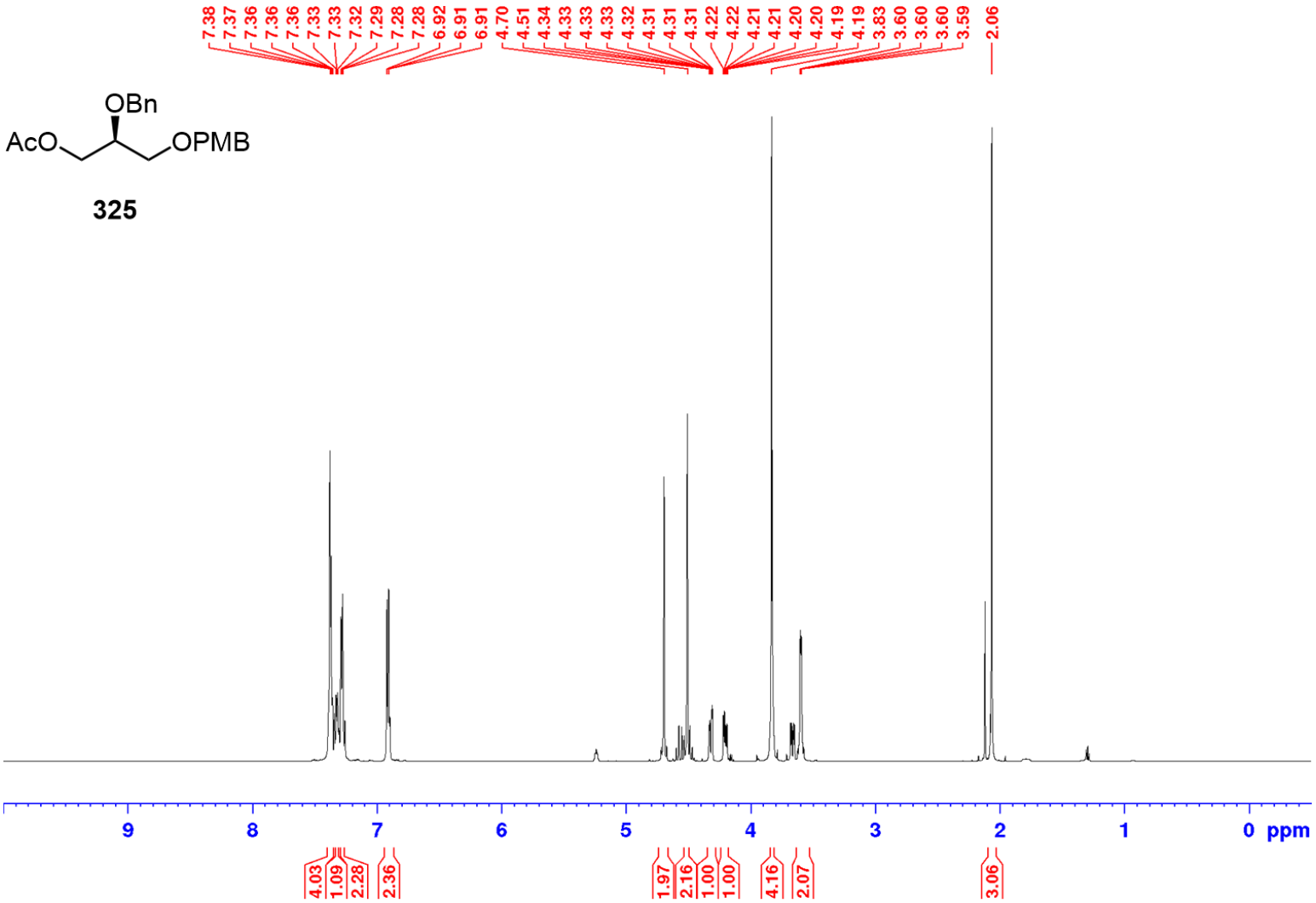


324 **325**

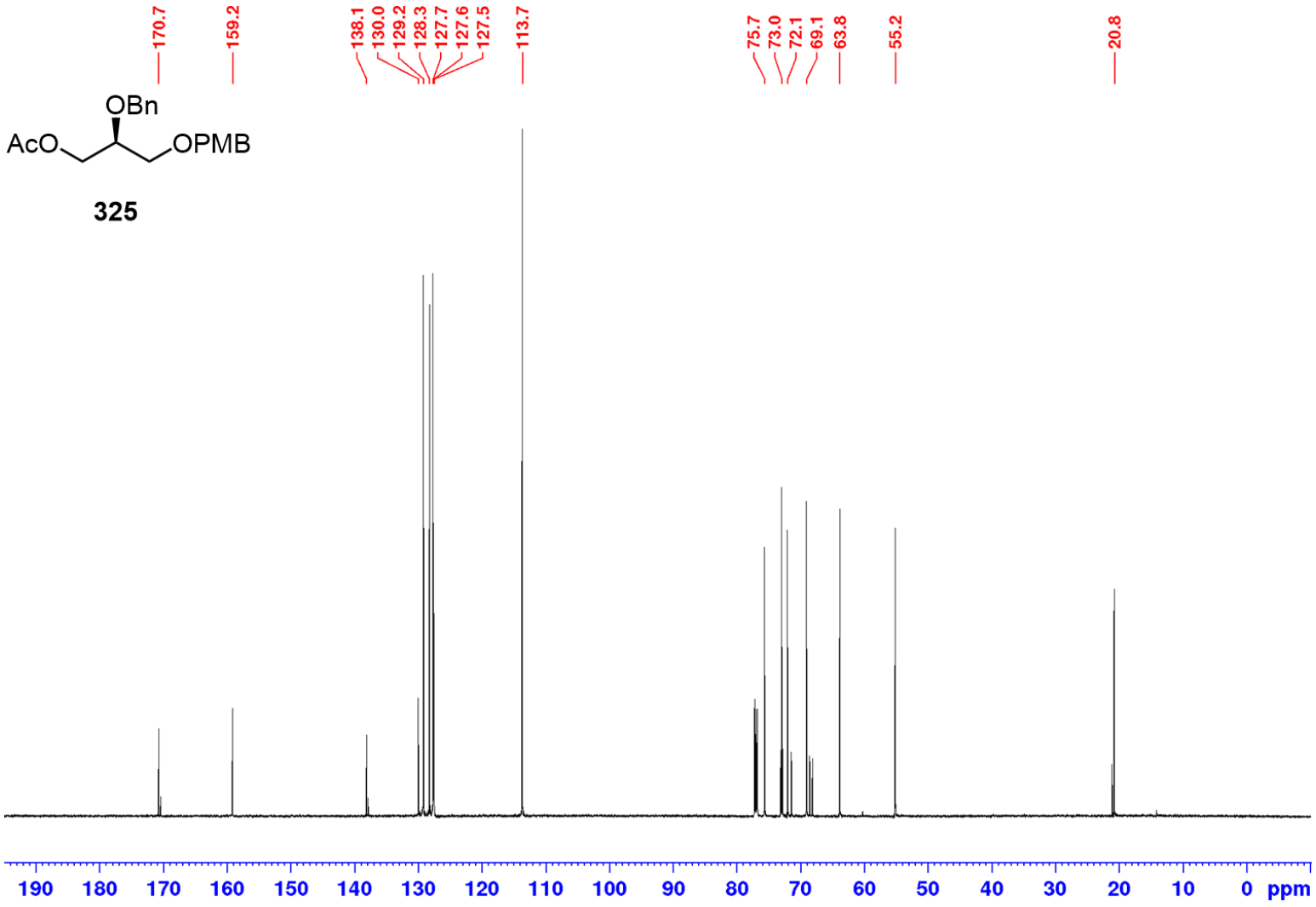
To a solution of alcohol **324** (1.0 equiv., 1.0045 g, 3.221 mmol) in pyridine (3.7 equiv., 1.0 mL, 11.91 mmol) under an argon atmosphere was cooled to 0 °C and added acetic anhydride (3.2 equiv., 1.0 mL, 10.32 mmol) dropwise then added DMAP (cat.). The solution was stirred and allowed to warm to room temperature. At 1 h, TLC indicated that the starting material was completely consumed. The reaction was cooled to 0 °C and the excess acetic anhydride was quenched by the addition of anhydrous CH_3OH (0.1 mL). After stirring at 0 °C for 5 min, the crude reaction mixture was stirred at room temperature for 5 min, then transferred to a separatory funnel with EtOAc (100 mL). The reaction was washed with 1 N HCl (3 x 25 mL), satd. aq. $NaHCO_3$ (1 x 25 mL), brine (1 x 125 mL), dried ($NaSO_4$) and filtered through a glass fritted vacuum filter funnel to remove drying agent. The filtrate was concentrated to a crude oil which was purified by flash column chromatography (10:90 to 25:75 EtOAc/hexanes) to give glycerol **325** (1.072 g, 3.113 mmol, 94%) as a clear oil. R_f = 0.3 (1:4 EtOAc/hexanes) visualized with ceric ammonium molybdate stain; $[\alpha]D^{25}$ = +15.68° (c = 1.0, $CHCl_3$); IR ν_{max} (film) 3030.46, 2863.75, 1739.26, 1612.01, 1585.54, 1512.99, 1454.66, 1367.89, 1301.77, 1246.41, 1174.17, 1097.90, 1035.65, 820.66, 738.42, 698.94, 604.83, 517.46 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.38-7.35 (m, 4H), 7.33-7.30 (m, 1H), 7.29-7.26 (m, 2H), 6.91 (d, *J* = 7.6 Hz, 2H), 4.70 (s, 2H), 4.51 (s, 2H), 4.33-4.30 (m, 2H), 4.22-4.19 (m, 1H), 3.83 (s, 3H), 3.82

(s, 1H), 3.60-3.59 (m, 2H), 2.06 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.7, 159.2, 138.1, 130.0, 129.2, 128.3, 127.67 127.6, 127.5, 113.7, 75.7, 73.0, 72.1, 69.1, 63.8, 55.2, 20.8. HR-ESI-MS (m/z): calcd for $C_{20}H_{27}N_1O_5^+$ $(M+NH_4)^+$ 362.19622, found 362.19528.

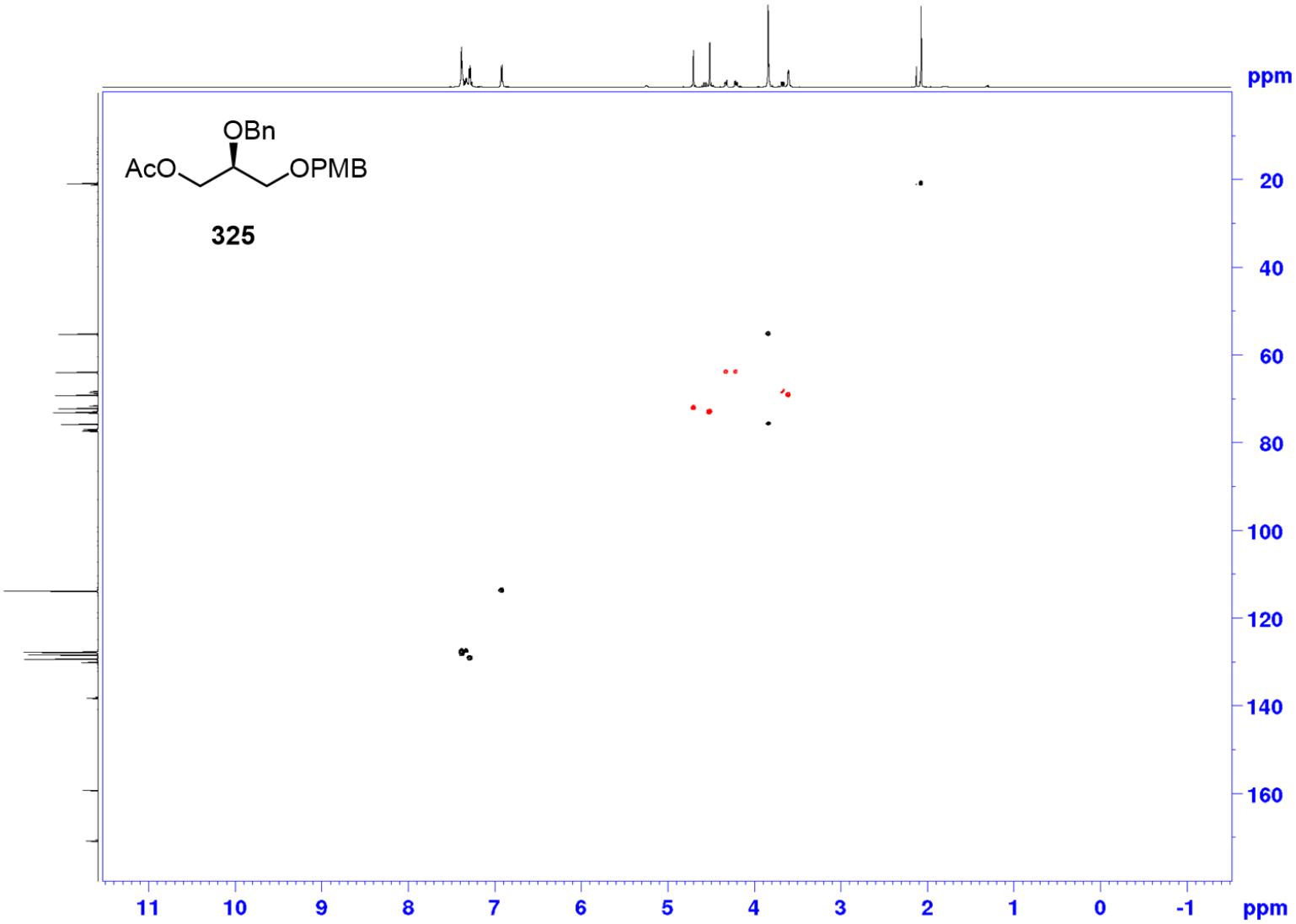
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 325

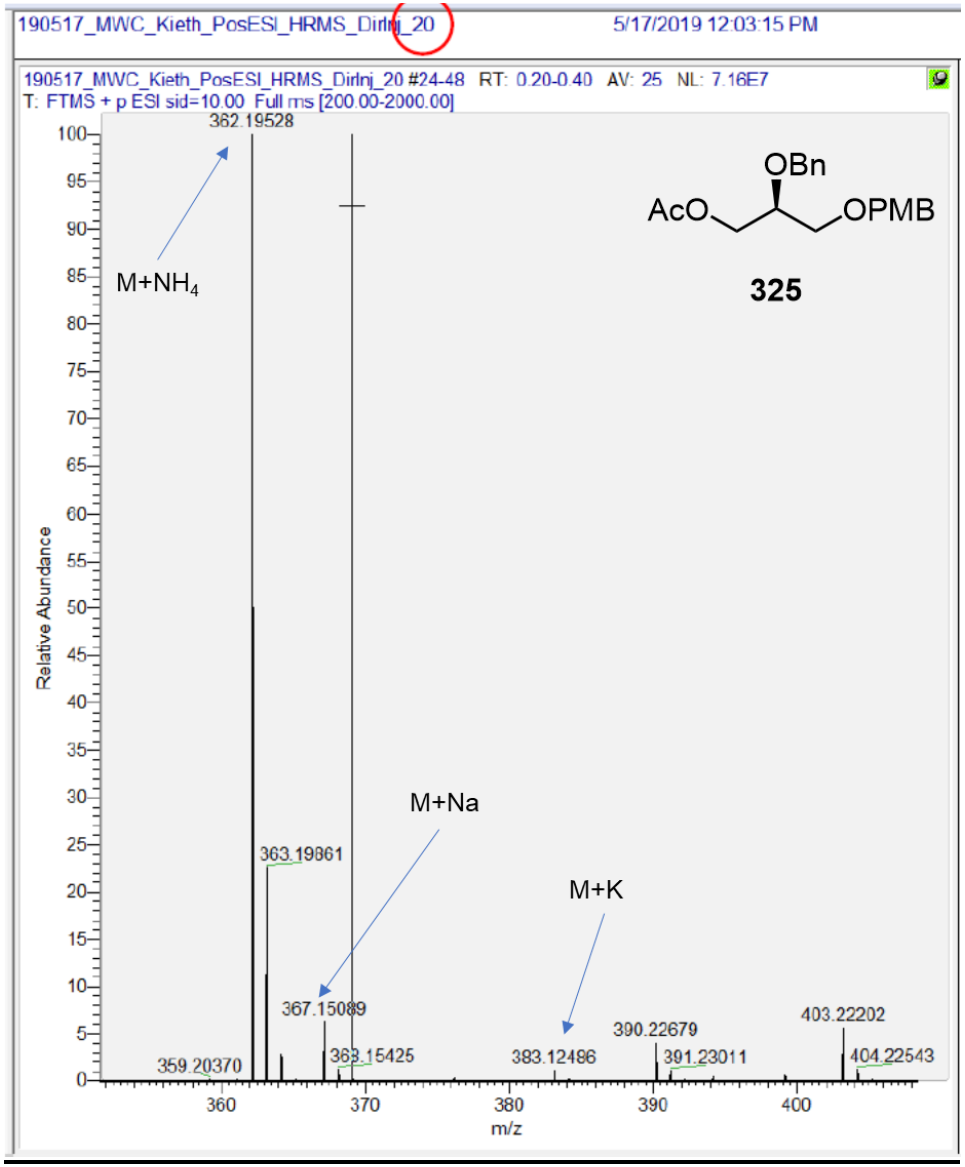


^{13}C NMR ($CDCl_3$, 100 MHz) of compound 325

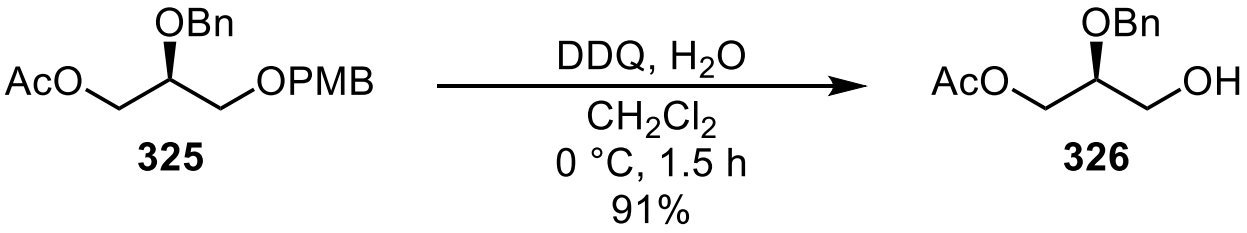


^{1}H-^{13}C HSCQ ($CDCl_3$) of compound 325



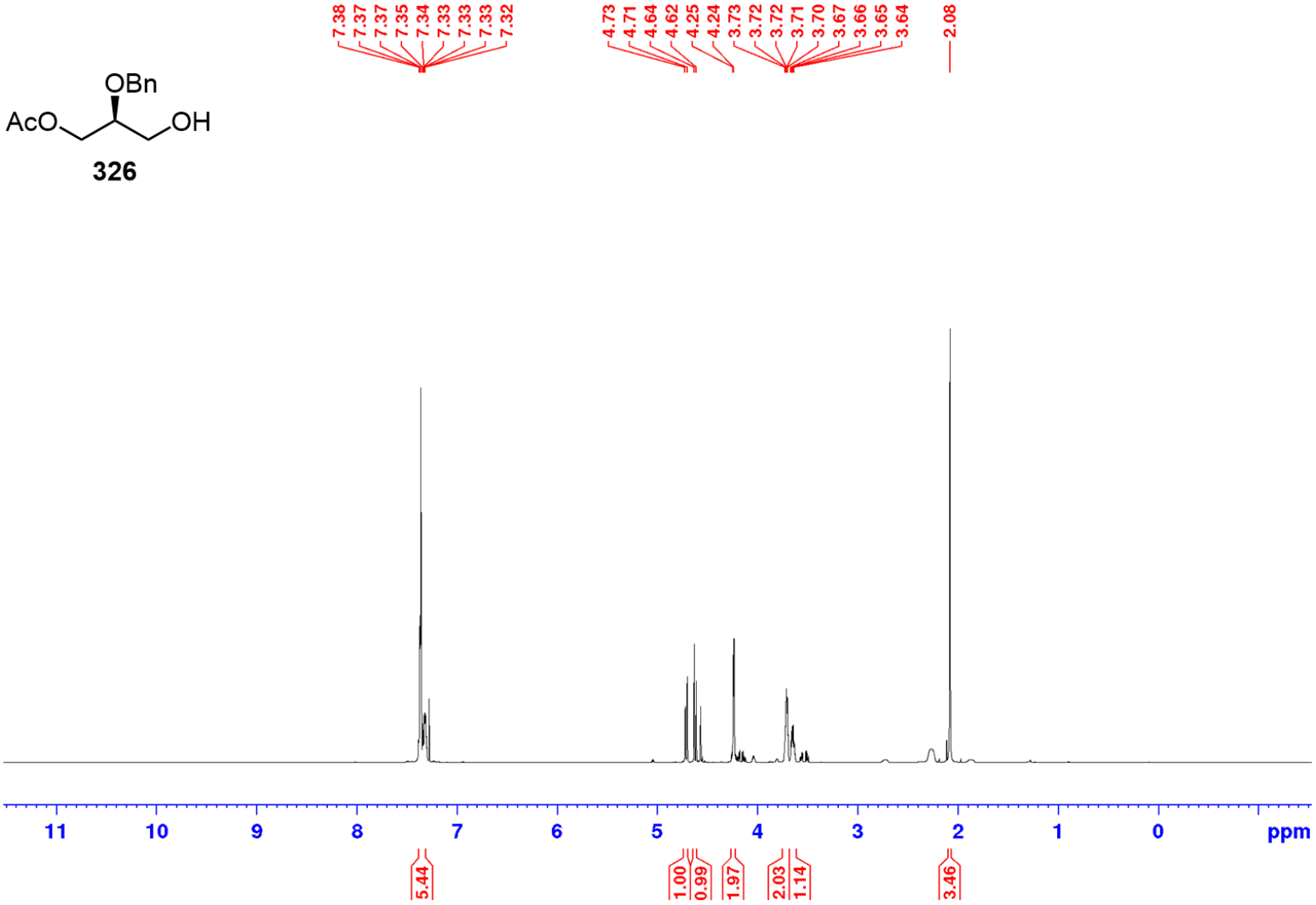

190517_MWC_Kieth_PosESI_HRMS_Dirlnj_20
5/17/2019 12:03:15 PM
190517_MWC_Kieth_PosESI_HRMS_Dirlnj_20 #24-48 RT: 0.20-0.40 AV: 25 NL: 7.16E7
T: FTMS + p ESI sid=10.00 Full ms [200.00-2000.00]
362.19528
M+NH4
OBn
AcO
OPMB
325
M+Na
M+K
363.19961
367.15089
359.20370
383.15425
383.12486
390.22679
391.23011
403.22202
404.22543
Relative Abundance
m/z

(*S*)-2-(benzyloxy)-3-hydroxypropyl acetate (326)

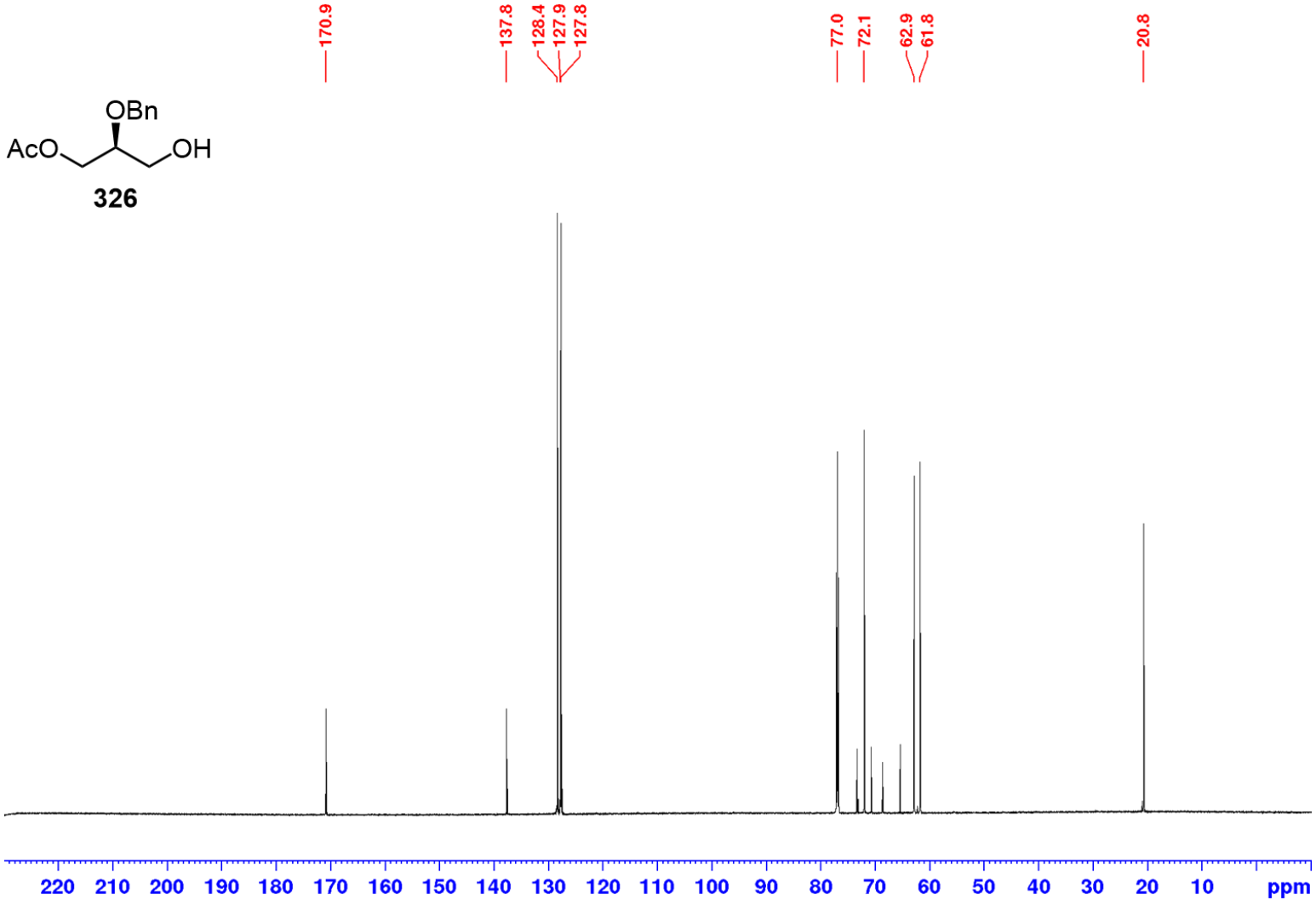


To a solution of glycerol **325** (1.0 equiv., 3.8404 g, 11.15 mmol) in anhydrous CH_2Cl_2 (106 mL) was added DDQ (1.5 equiv., 3.8 g, 16.72 mmol) at 0 °C under an argon atmosphere. The reaction was stirred at room temperature for 4 h when TLC indicated the complete consumption of starting material. The crude reaction mixture was filtered through a glass fritted vacuum filter funnel equipped with a pad of Celite to remove the solids. The filtrate was concentrated to a crude syrup. The crude reaction syrup was transferred to a separatory funnel with EtOAc (150 mL). The reaction was washed with $NaHCO_3$ (3 x 25 mL), brine (1 x 125 mL), dried ($NaSO_4$) and filtered through a glass fritted vacuum filter funnel to remove drying agent. The filtrate was concentrated to a crude oil which was purified by flash column chromatography (40:60 to 50:50 EtOAc/hexanes) to give alcohol **326** (2.278 g, 10.19 mmol, 91%) as a clear oil. Spectroscopic data matches previously reported data.[347-352] $[\alpha]D^{25}$ = -3.32° (c = 1.0, $CHCl_3$); IR ν_{max} (film) 3427.22, 2952.14, 1722.15, 1647.53, 1496.03, 1453.79, 1370.39, 1246.88, 1111.61, 1049.07, 910.58, 748.50, 700.04, 607.40 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.37-7.31 (m, 5H), 4.72 (d, *J* = 11.3 Hz, 1H), 4.63 (d, *J* = 11.4 Hz, 1H), 4.24 (d, *J* = 4.6 Hz, 2H), 3.73-3.71 (m, 2H), 3.67-3.63 (m, 1H), 2.09 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.1, 137.9, 128.6, 127.9, 77.3, 77.1, 77.1, 76.9, 72.2, 63.0, 61.9, 20.9.

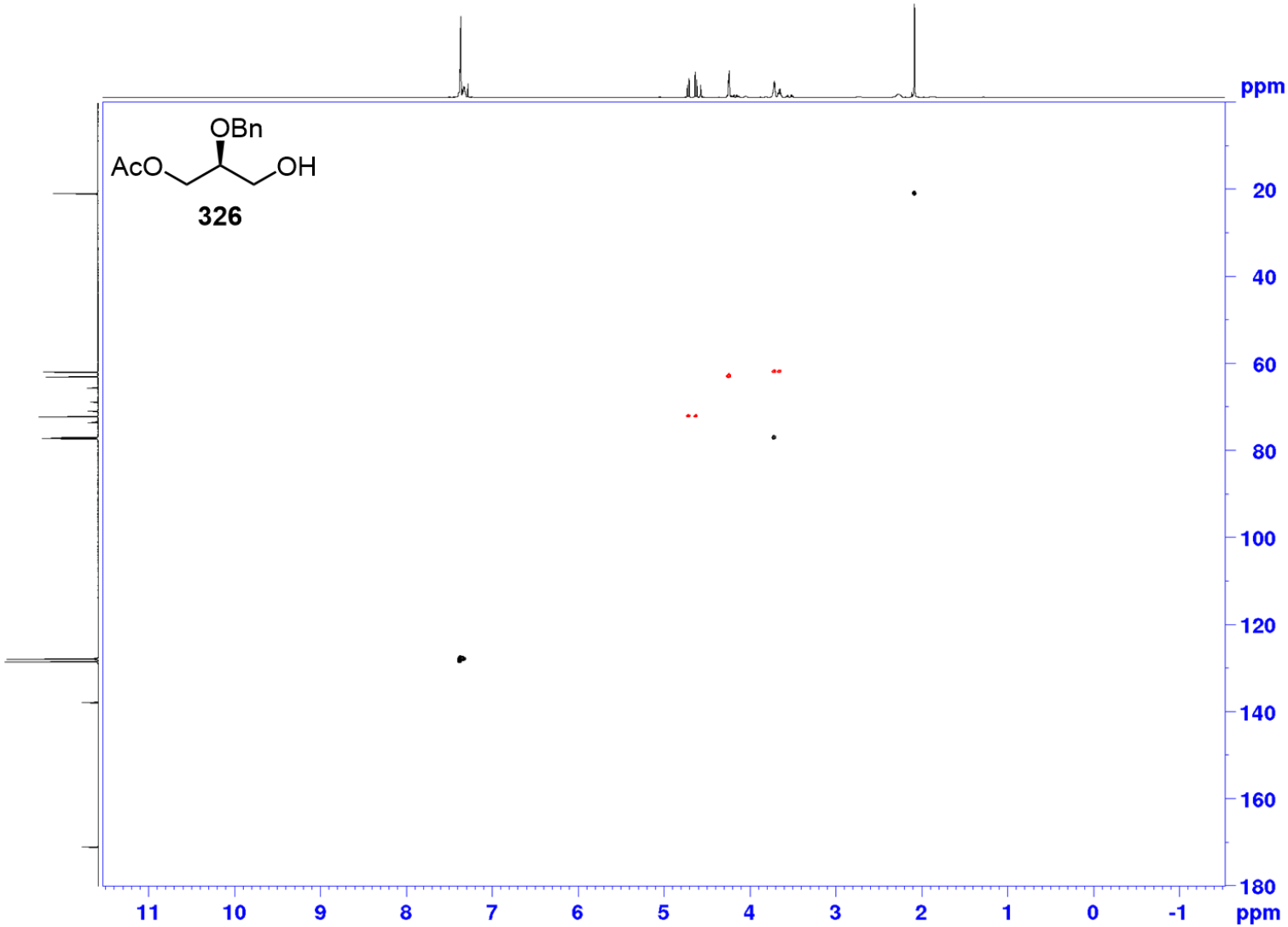
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 326



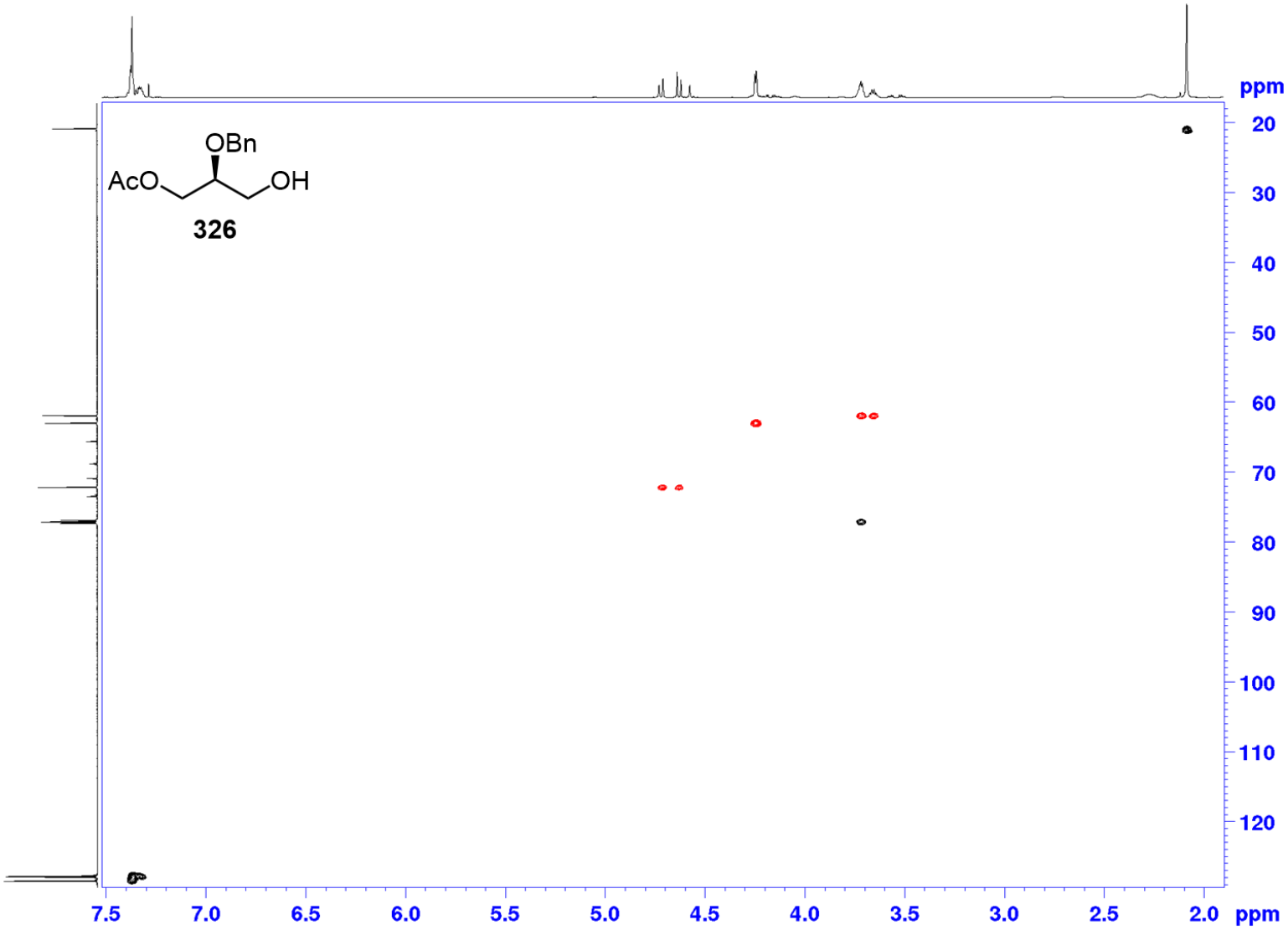
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 326



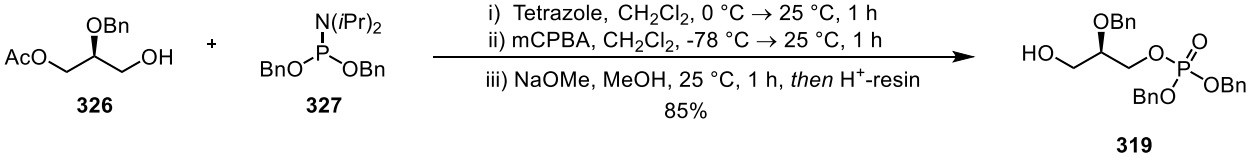
^{1}H-^{13}C HSCQ ($CDCl_3$) of compound 326



^{1}H-^{13}C HSCQ ($CDCl_3$) zoomed in region of compound 326



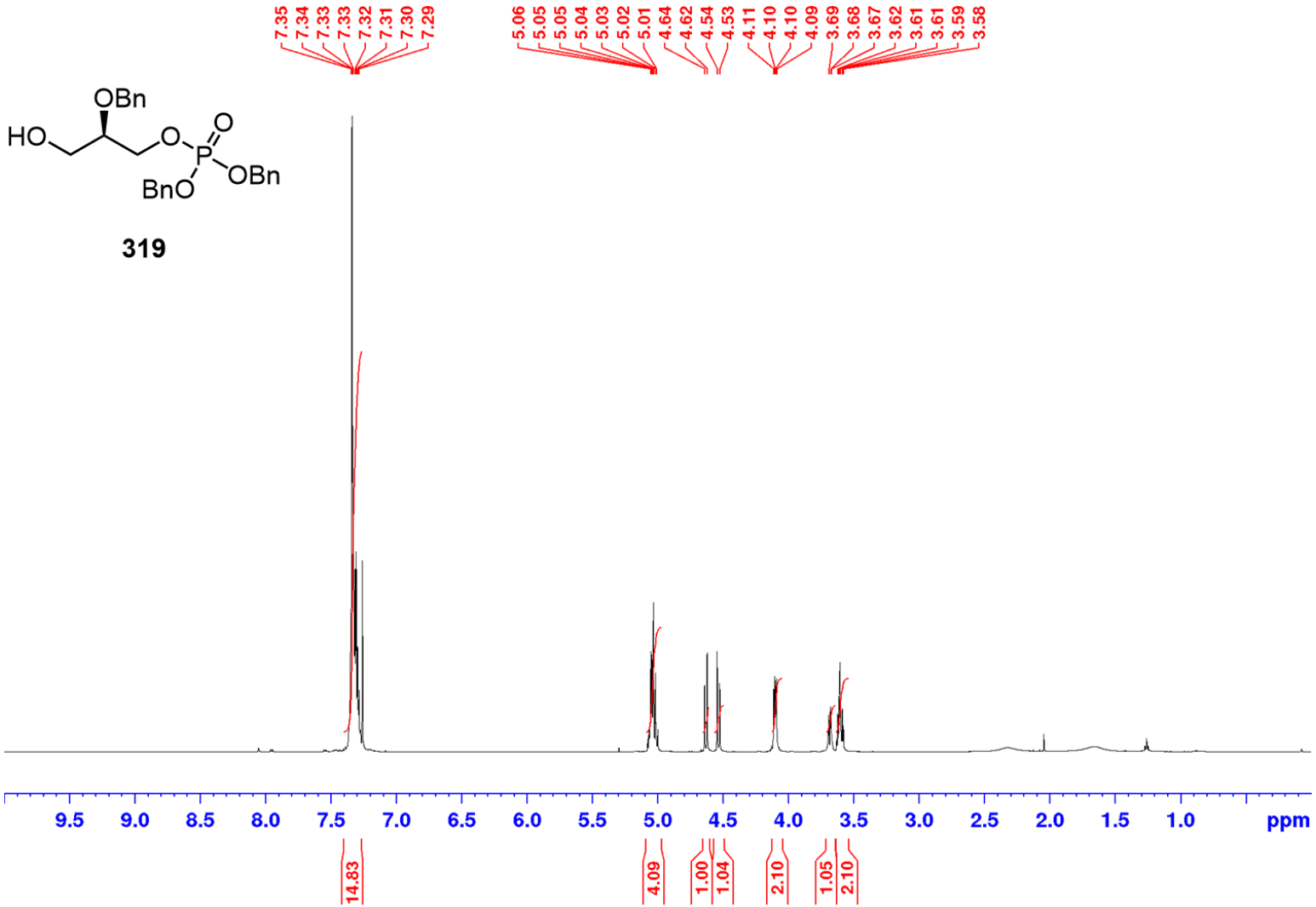
(*R*)-dibenzyl (2-(benzyloxy)-3-hydroxypropyl) phosphate (319)



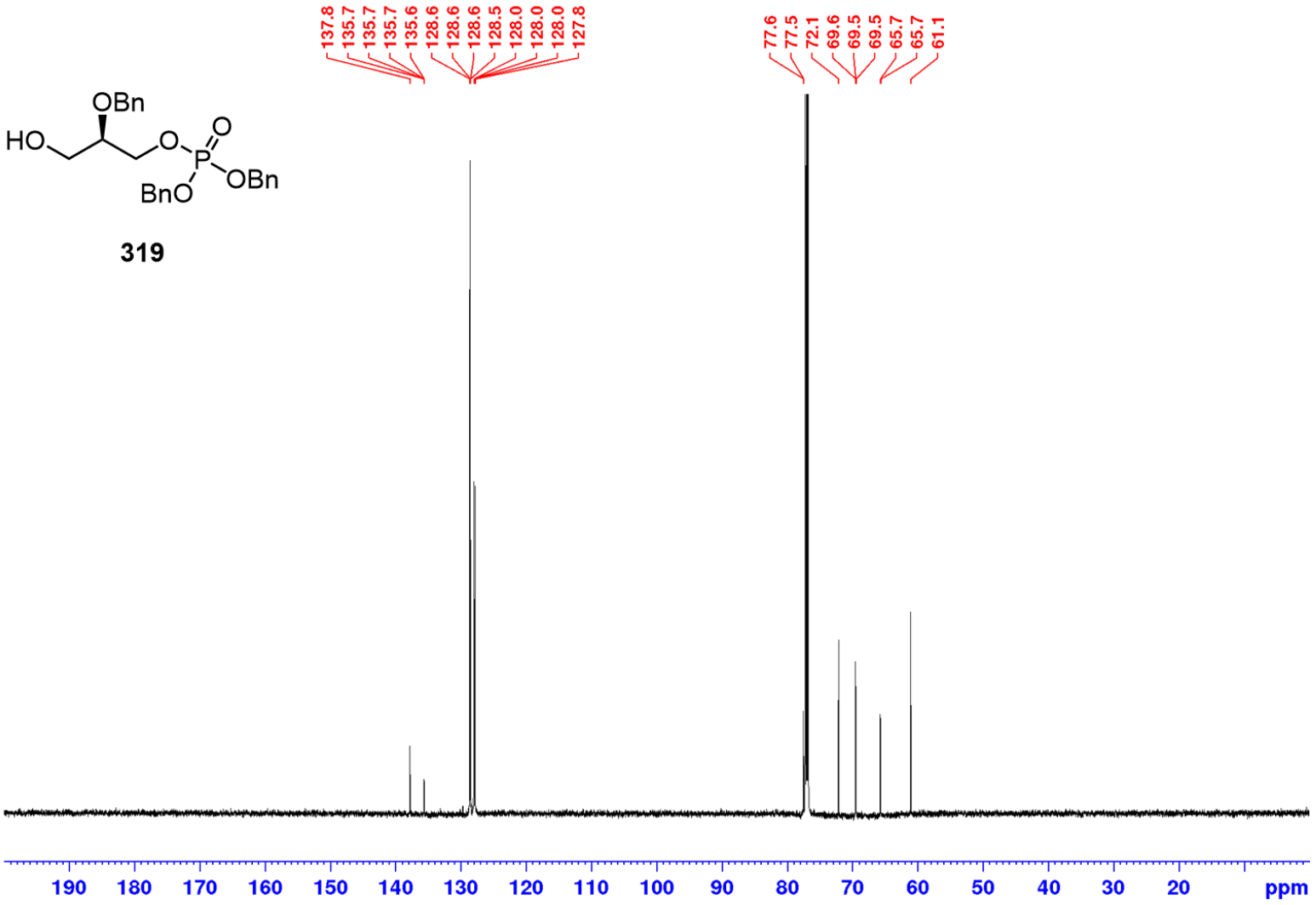
To a solution of alcohol **326** (1.0 equiv., 2.27 g, 10.19 mmol) in anhydrous CH_2Cl_2 (50 mL) under an argon atmosphere, was added commercially available dibenzyl *N*,*N*-diisopropylphosphoramidite **327** (2.0 equiv., 7.02 g, 20.38 mmol) dropwise. The mixture was cooled to 0 °C and added *1H*-tetrazole (2.0 equiv., 2.64 g, 20.38 mmol). The reaction mixture was stirred at room temperature for 4 h when TLC indicated that the starting material was completely consumed. The reaction was then cooled to -78 °C and added *m*CPBA (3.0 equiv., 7.00 g, 30.5 mmol) dropwise as a solution in CH_2Cl_2. The reaction mixture was stirred at room temperature for 1 h. The reaction was then transferred to a separatory funnel and washed with 1 N HCl (3 x 10 mL), satd. aq. $NaHCO_3$ (1 x 10 mL), brine (1 x 10 mL), dried ($NaSO_4$) and filtered through a glass fritted vacuum filter funnel to remove drying agent. The filtrate was concentrated to a crude oil. The oil was added anhydrous methanol (20 mL) and added 2 N sodium methoxide (1.0 equiv.) drop wise at room temperature. At 1 h, the reaction mixture was diluted with CH_2Cl_2 and neutralized by the addition of acidic Amberlite IR 120 (H^+) ion exchange resin. The resin was filtered by passing the crude reaction mixture though a glass fritted vacuum filter funnel equipped with a pad of Celite. The filtrate of the crude reaction mixture was concentrated to a crude oil which was purified by silica gel flash column chromatography (70:30 to 80:20 EtOAc/hexanes) to give alcohol **319** (3.845 g, 8.69 mmol, 85% over 2 steps) as a clear oil. R_f = 0.4 (3:4 EtOAc/hexanes) visualized with ceric ammonium molybdate stain; $[\alpha]D^{25}$

= -7.12° (c = 1.0, $CHCl_3$); IR ν_{max} (film) 3429.01, 1637.79, 1456.30, 1269.06, 1017.57, 782.63, 734.96, 697.30 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.28-7.18 (m, 15H), 5.00-4.92 (m, 4H), 4.55 (d, *J* = 12.1 Hz, 1H), 4.45 (d, *J* = 12.1 Hz, 1H), 4.03-4.01 (m, 2H), 3.62-3.59 (m, 1H), 3.55-3.50 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 137.8, 135.7, 135.7, 135.7, 135.6, 129.7, 128.6, 128.5, 128.4, 128.0, 128.0, 128.0 127.8, 72.1, 69.5, 69.5, 65.7, 65.7, 61.1; ^{31}P NMR (151 MHz, $CDCl_3$) δ 0.98; HR-ESI-MS (m/z): calcd for $C_{22}H_{28}O_6P^+$ $(M+H)^+$ 443.16178, found 443.16027.

^{1}H NMR ($CDCl_3$, 400 MHz) of compound 319

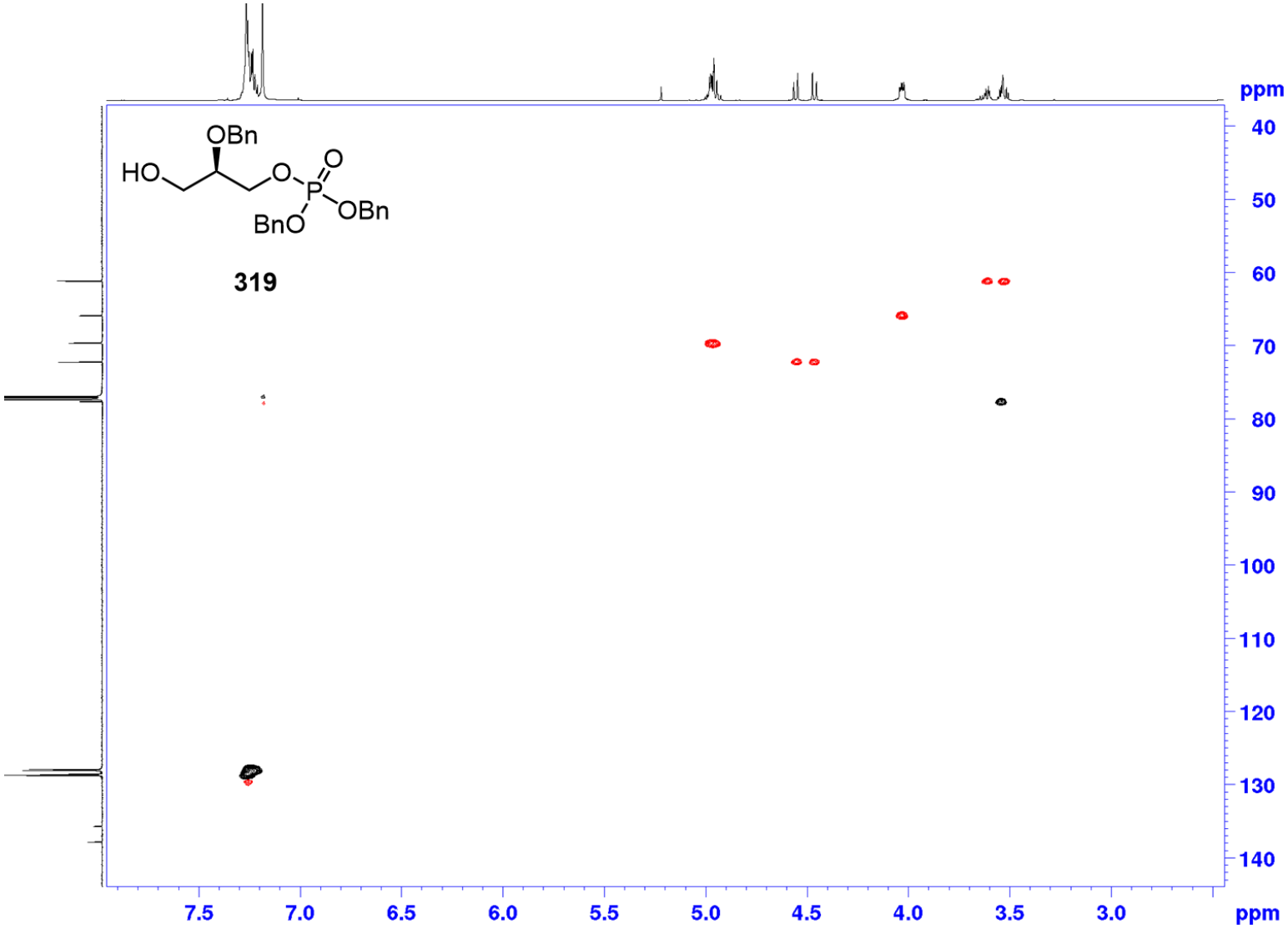


^{13}C NMR ($CDCl_3$, 100 MHz) of compound 319

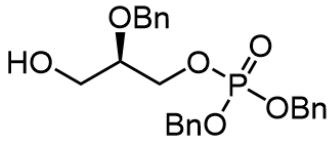


319

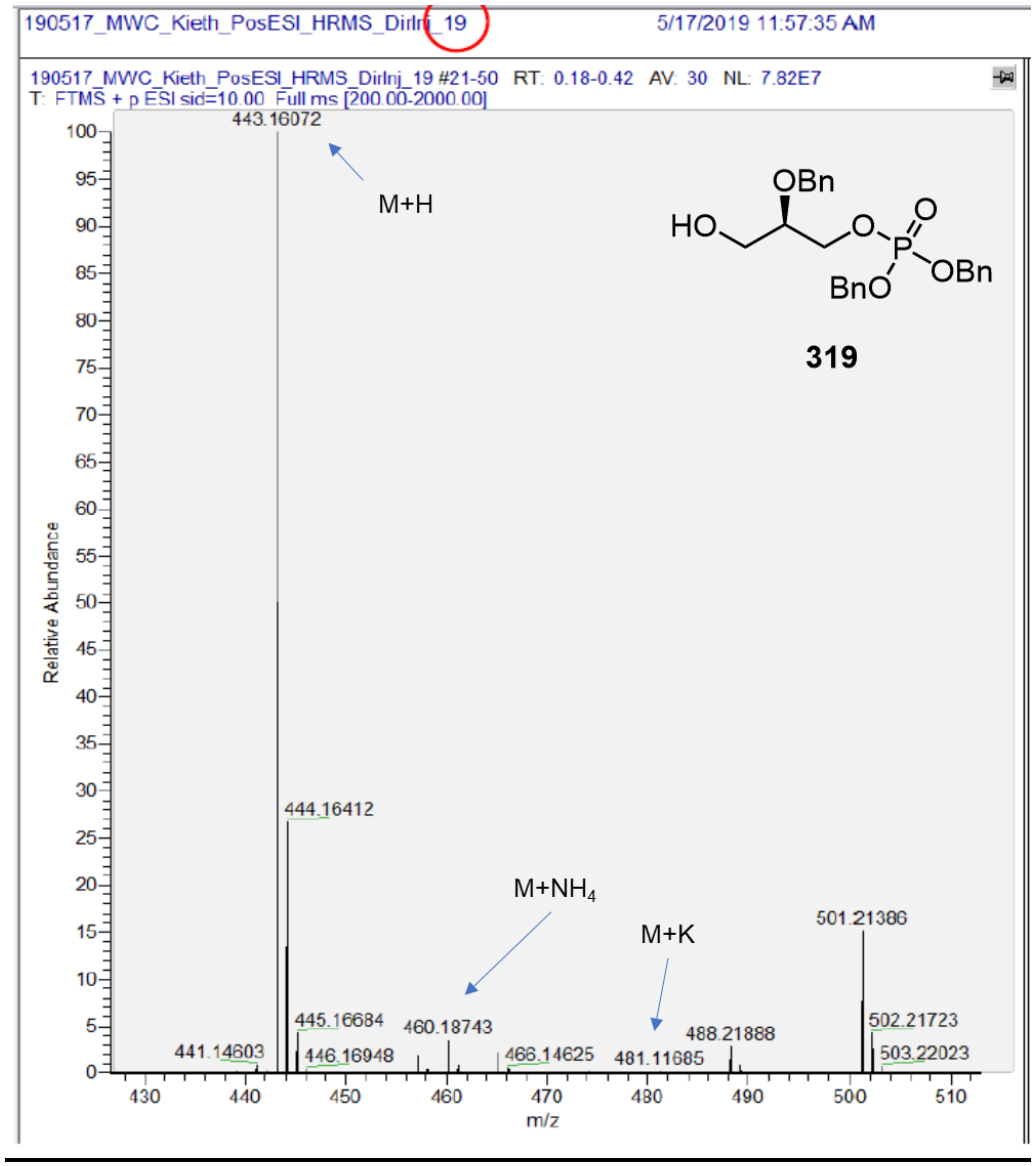
^{1}H-^{13}C HSCQ ($CDCl_3$) of compound 319



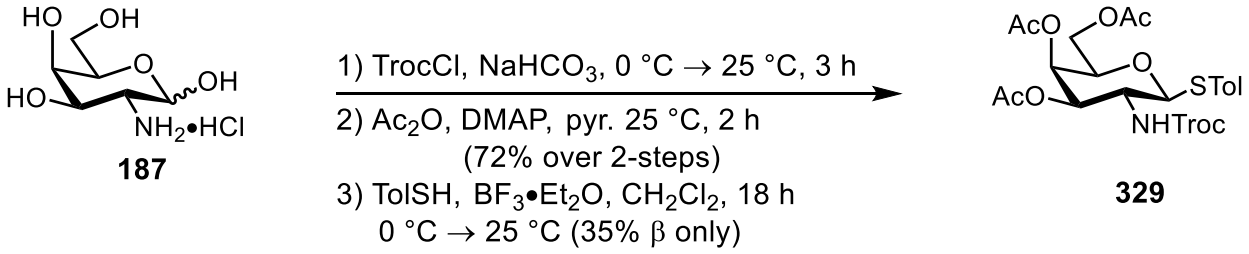
^{31}P NMR ($CDCl_3$, 162 MHz) of compound 319



319


190517_MWC_Kieth_PosESI_HRMS_DirInj_19
5/17/2019 11:57:35 AM
190517_MWC_Kieth_PosESI_HRMS_DirInj_19 #21-50 RT: 0.18-0.42 AV: 30 NL: 7.82E7
T: FTMS + p ESI sid=10.00 Full ms [200.00-2000.00]
443.16072
M+H
444.16412
M+NH4
M+K
501.21386
445.16684
460.18743
488.21888
502.21723
441.14603
446.16948
466.14625
481.11685
503.22023
Relative Abundance
m/z
OBn
HO
O
P
O
OBn
BnO
319

***p*-Tolyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-*N*-Troc-1-thio-β-D-galactopyranoside (329)**



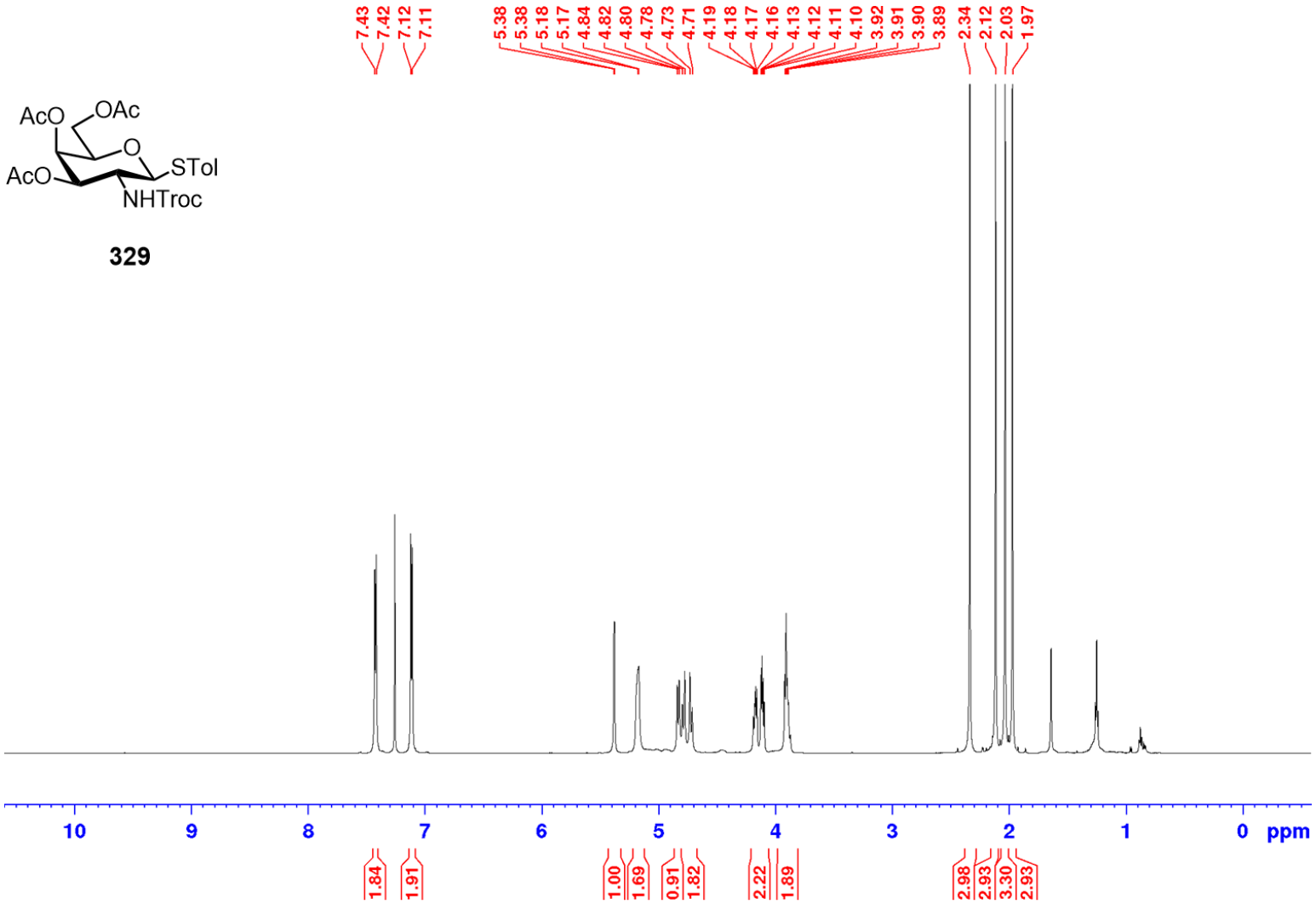
A solution of D-GalN·HCl **187** (1.0 equiv., 1.00 g, 4.637 mmol) in sat. aq. $NaHCO_3$ (40 mL) was added Troc-Cl (1.3 equiv., 0.766 mL, 5.565 mmol) at 0 °C under an argon atmosphere. The reaction was stirred at room temperature. At 3 h, the reaction was filtered to remove a white solid. The filter cake was washed with ice cold water. The white solid was removed and co-evaporated with toluene to remove residual water. The crude solid was diluted with pyridine (5.0 mL) and added acetic anhydride (5.0 mL) and DMAP (0.1 equiv.) at 0 °C under an argon atmosphere. The reaction was stirred at room temperature. At 2 h the reaction was added MeOH to consume the excess anhydride. After 5 min the reaction was transferred to a seperatory funnel with EtOAc. The orgains were washed with 1 N HCl (3 x 15 mL), aq. sat. $NaHCO_3$ (1 x 15 mL), brine (1 x 15 mL), dried ($NaSO_4$), filtered through a glass fritted filter funnel to remove the drying agents and concentrated to a foam. The crude reaction mixture was purified by silica gel flash chromatography (60:40 to 70:30 EtOAc/hexanes) to give the 1,3,4,6-tetra-*O*-acetyl-2-deoxy-(2',2',2'-trichloroethoxycarbonyl-amino-D-galactopyranoside **328** (1.184 g, 3.339 mmol 72% yield over 2 steps). The D-GalN residue was added *p*-thiocresol (2.0 equiv., 1.152 g, 9.275 mmol) in dry CH_2Cl_2 (25 mL) was added $BF_3{\cdot}Et_2O$ (2.5 equiv., 1.47 mL, 11.59 mmol) dropwise at room 0 °C under an argon atmosphere. The reaction was stirred at room temperature. At 18 h, the crude reaction mixture was quenched by the addition

of satd. aq. $NaHCO_3$ (100 mL) to the reaction flask. After stirring for 30 min the biphasic mixture was transferred to a separatory funnel and the organics were separated and the aqueous layer was extracted with CH_2Cl_2 (3 x 10 mL). The organics were combined and dried ($NaSO_4$), filtered through a glass fritted vacuum filter funnel to remove drying agent. The filtrate was concentrated to an oil. The crude oil was purified by silica gel flash column chromatography (10:90 to 30:70 EtOAc/hexanes) to give an mixture of anomers of *p*-tolyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-*N*-Troc-1-thio-β-D-galactopyranoside **329** (0.6763 g, 1.15 mmol, 35%) as an off white solid. Our characterization data were consistent with previously reported data.[353-356]

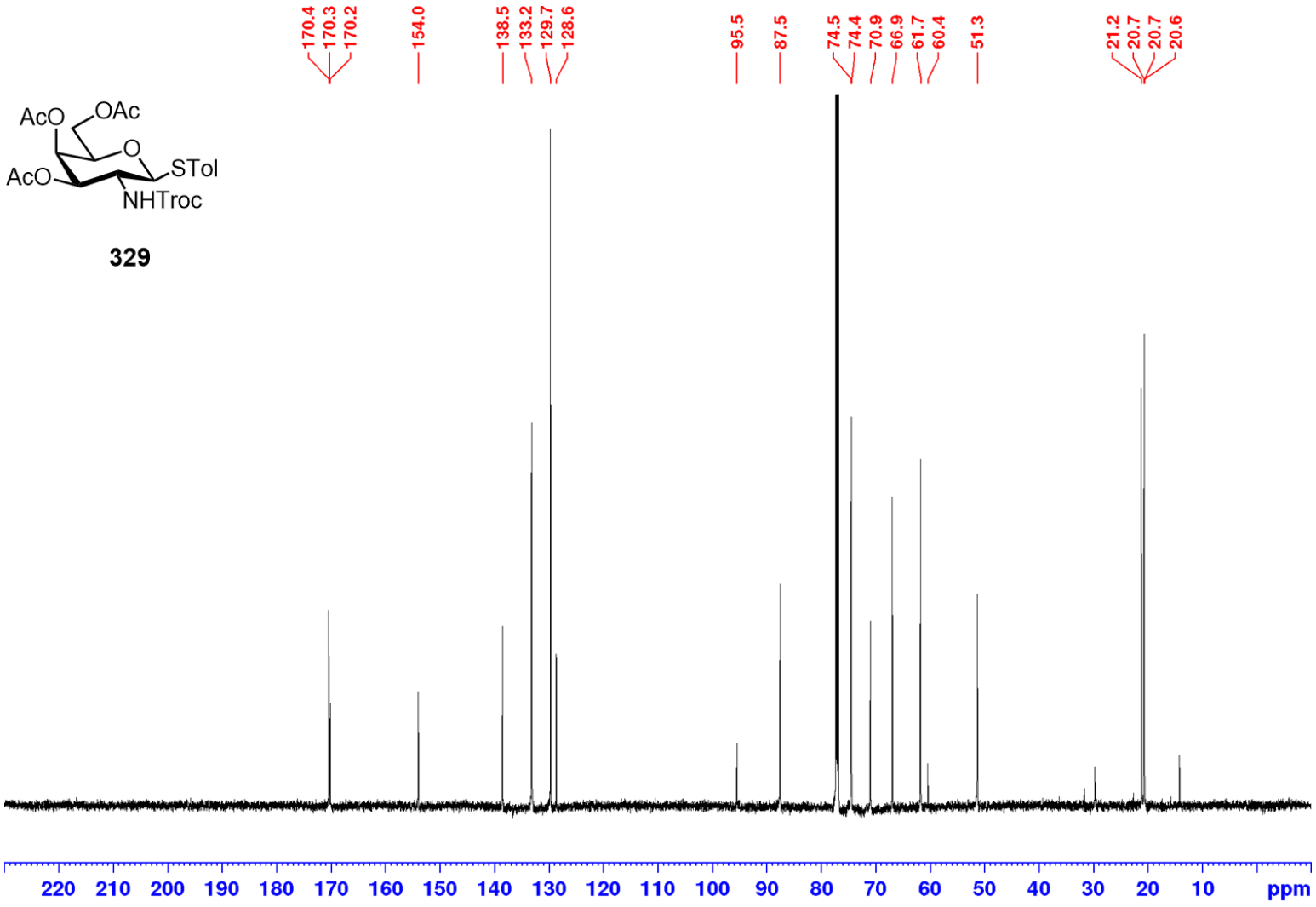
^{1}H NMR (600 MHz, $CDCl_3$): δ 7.43 (d, *J* = 7.8 Hz, 2H), 7.11 (d, *J* = 7.8 Hz, 2H), 5.38 (d, *J* = 2.6 Hz, 1H), 5.18-5.17 (m, 2H), 4.84 (d, *J* = 10.3 Hz, 1H), 4.80-4.71 (m, 2H), 4.19-4.10 (m, 2H), 3.92-3.89 (m, 2H), 2.33 (s, 3H), 2.13 (s, 3H), 2.04 (s, 3H), 1.98 (s, 3H);

^{13}C NMR (100 MHz, $CDCl_3$): δ 170.43, 170.34, 170.18, 170.17, 153.98, 138.49, 133.18, 129.7, 128.64, 95.47, 87.54, 74.49, 74.41, 70.94, 66.93, 61.72, 60.43, 51.31, 21.17, 20.68, 20.66, 20.63.

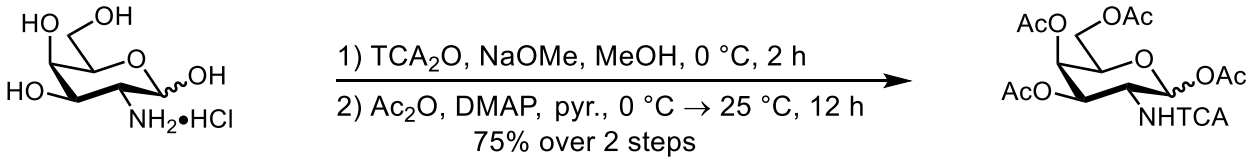
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 329



^{13}C NMR ($CDCl_3$, 100 MHz) of compound 329



1,3,4,6-Tetra-*O*-acetyl-2-deoxy-2-trichloracetamido-α/β-D-galactopyranoside (331)

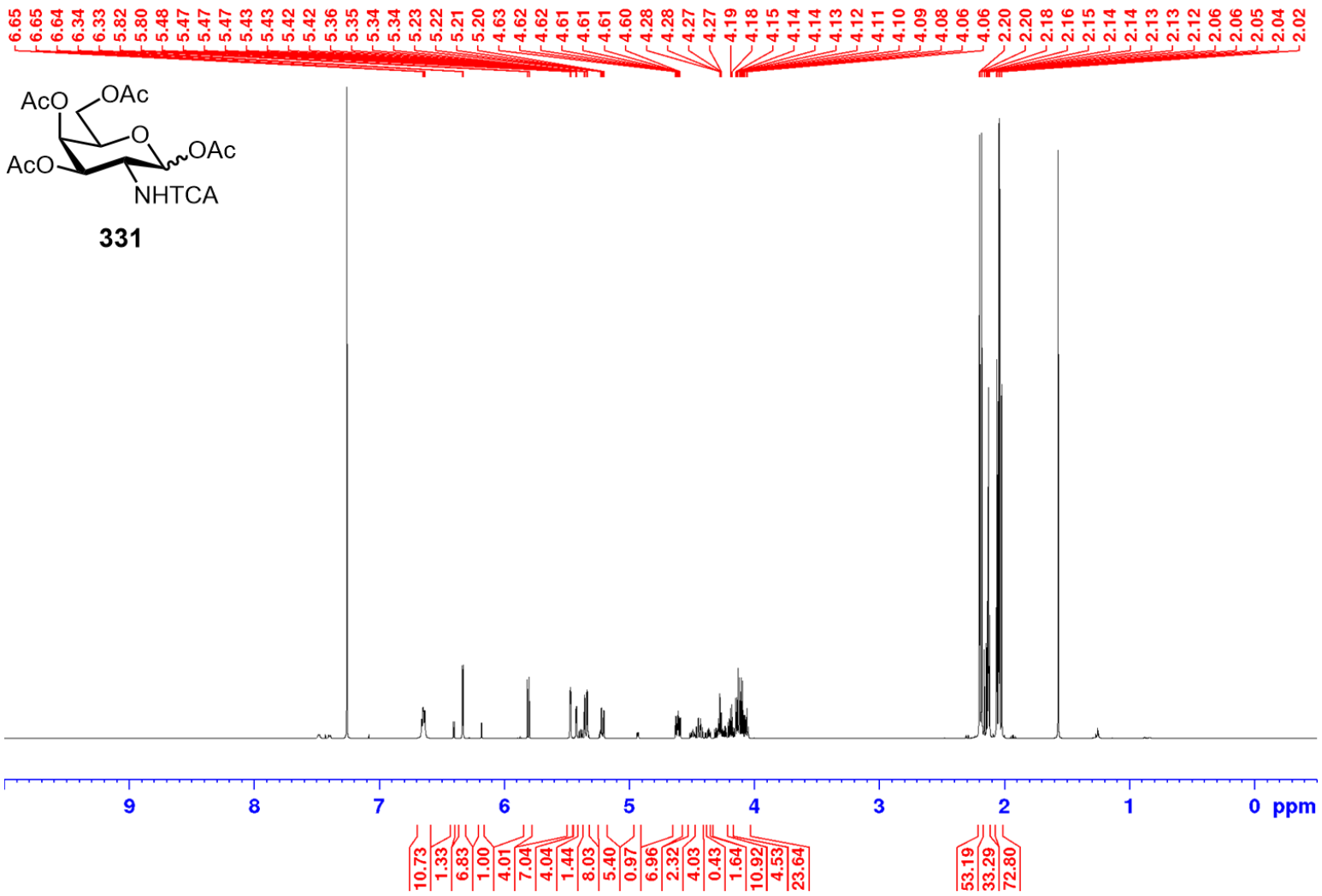


187 **331**

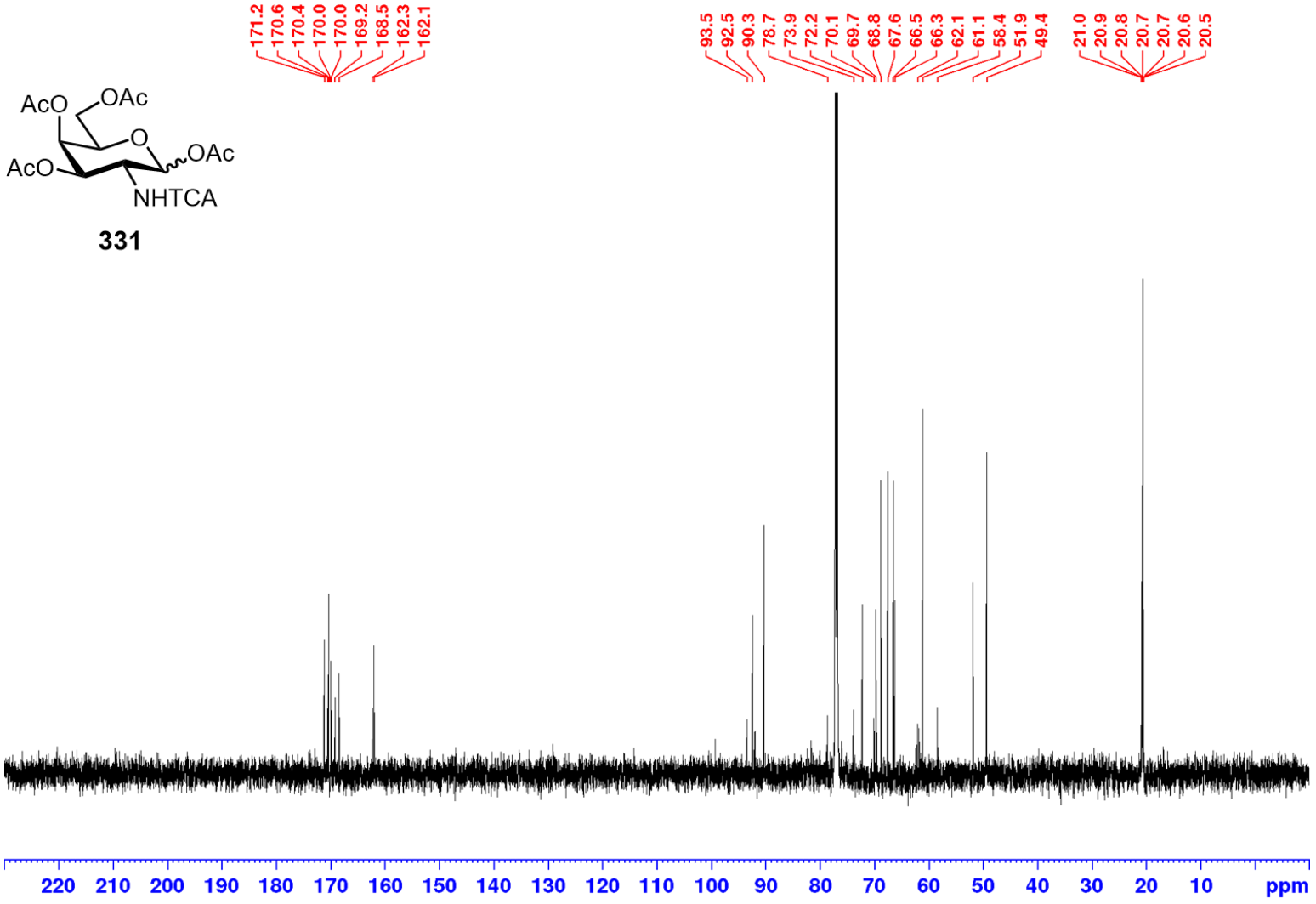
A suspension of D-galactosamine HCl **187** (1.0 equiv., 2.16 g, 10.0 mmol) in anhydrous CH_3OH (35 mL) was added a sodium methoxide solution (3.0 equiv., 15 mL, 30 mmol, [2 N] in CH_3OH) dropwise. After 30 min, all the starting material had been dissolved. The reaction mixture was then added trichloroacetic anhydride (1.5 equiv., 2.74 mL, 15 mmol) dropwise at 0 °C under an argon atmosphere. At 1 h, TLC (H_2O, CH_3OH, Et_3N 2:8:1, CAM stain) indicated that the starting material had been completely consumed for a less polar spot. The reaction was concentrated to dryness and taken to the next step with no further purification. The crude reaction mixture was suspended in pyridine (20 mL) and added acetic anhydride (10.0 equiv., 9.5 mL, 21.6 mmol) dropwise and DMAP (cat.) at 0 °C under an argon atmosphere. The solution was stirred while warming to room temperature. At 12 h, TLC (35:65 EtOAc/hexanes, CAM stain) indicated that the product of the first step was completely consumed for a less polar spot. The reaction was cooled to 0 °C and the excess acetic anhydride was quenched by the addition of CH_3OH (3.0 mL). After 5 min at 0 °C, the crude reaction mixture was stirred at room temperature for 5 min, then transferred to a separatory funnel with EtOAc (100 mL). The reaction was washed with 1 N HCl (3 x 25 mL), satd. aq. $NaHCO_3$ (1 x 25 mL), brine (1 x 125 mL), dried ($NaSO_4$) and filtered through a glass fritted vacuum filter funnel to remove drying agent. The filtrate was concentrated to a crude oil which was purified by flash column chromatography (25:75 to 35:65 EtOAc/hexanes) to give an inseparable mixture of 1,3,4,6-tetra-*O*-acetyl-2-deoxy-

2-trichloracetamido-α/β-D-galactopyranoside **331** (4.5229 g, 9.18 mmol, 92%) in a 30:52:6:12 β-pyranose/α-pyranose/β-furanose/α-furanose ratio of products. Spectroscopic data is in agreement with previously reported data.[357-360] HR-ESI-MS (m/z): calcd for $C_{80}H_{84}Cl_3N_5O_{22}P^+$ ($M+NH_4^+$) 1540.424923, found 1540.4260.

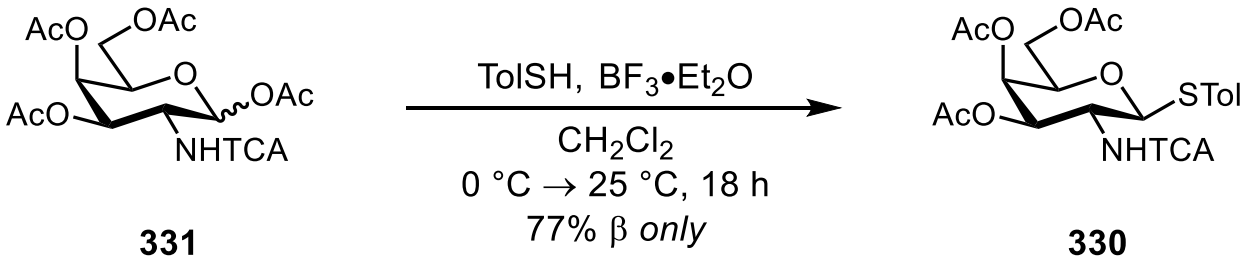
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 331



^{13}C NMR ($CDCl_3$, 100 MHz) of compound 331



***p*-Tolyl 3,4,6-tetra-*O*-acetyl-2-deoxy-2-trichloracetamido-1-thio-β-D-galactopyranoside (330)**

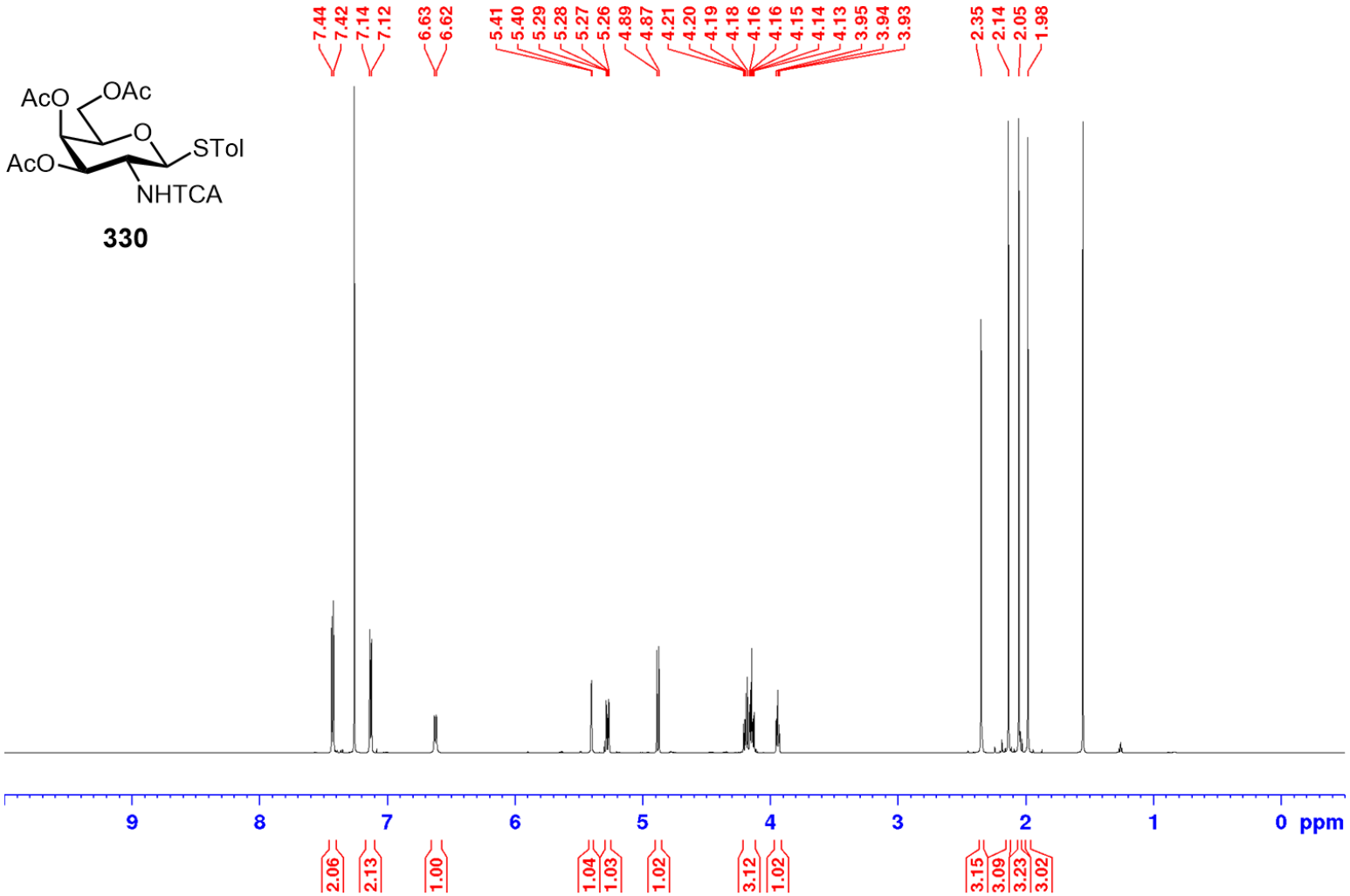


331 **330**

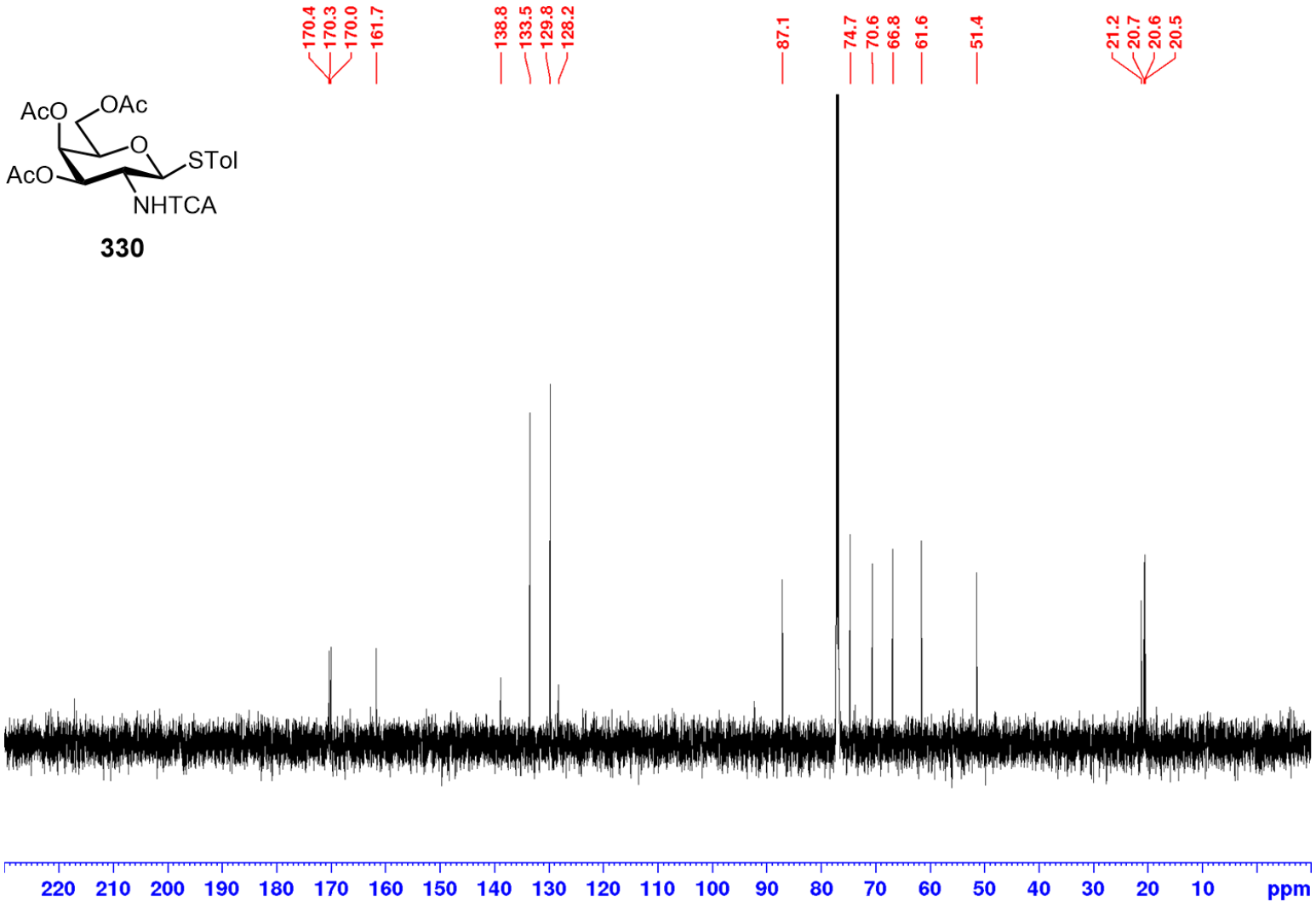
The mixture of 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-trichloracetamido-α/β-D-galactopyranoside **331** (1.0 equiv., 4.52 g, 9.17 mmol, 82% pyranoside) and *p*-thiocresol (1.5 equiv., 1.71 g, 13.75 mmol) in anhydrous CH_2Cl_2 (50 mL) were added $BF_3{\cdot}Et_2O$ (3.0 equiv., 3.4 mL, 27.51 mmol) dropwise at 0 °C under an argon atmosphere. The reaction was stirred while warming to room temperature. At 18 h, TLC indicated complete consumption of starting material. The crude reaction mixture was quenched by the addition of satd. aq. $NaHCO_3$ (30 mL) to the reaction flask. After stirring for 30 min the biphasic mixture was transferred to a separatory funnel and the organics were separated and the aqueous layer was extracted with CH_2Cl_2 (3 x 5 mL). The organics were combined and dried ($NaSO_4$) and filtered through a glass fritted vacuum filter funnel to remove drying agent. The filtrate was concentrated to an oil. The crude oil was purified by silica gel flash column chromatography (40:60 to 50:50 EtOAc/hexanes) then further purified by crystallization from hot EtOAc/hexanes the *p*-tolyl 3,4,6-tetra-*O*-acetyl-2-deoxy-2-trichloracetamido-1-thio-β-D-galactopyranoside **330** (3.2049 g, 5.754 mmol, 77%) as an off white solid. Our characterization data were consistent with previously reported data.[355, 361]

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.43 (d, *J* = 8.1 Hz, 2H), 7.13 (d, *J* = 8 Hz, 2H), 6.62 (d, *J* = 9 Hz, 1H), 5.40 (d, *J* = 2.9 Hz, 1H), 5.27 (dd, *J* = 3.3, 10.8 Hz, 1H), 4.88 (d, *J* = 10.3 Hz, 1H), 4.21-4.18 (m, 1H), 4.16-4.13 (m, 2H), 3.94 (dd, *J* = 0.2, 6.6 Hz, 1H), 2.35 (s, 3H), 2.14 (s, 3H), 2.05 (s, 3H), 1.98 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.4, 170.3, 170.05, 161.7, 138.8, 133.6, 133.4, 129.9, 129.8, 87.1, 74.7, 70.6, 66.8, 61.6, 51.4, 21.2, 20.7, 20.6, 20.5.

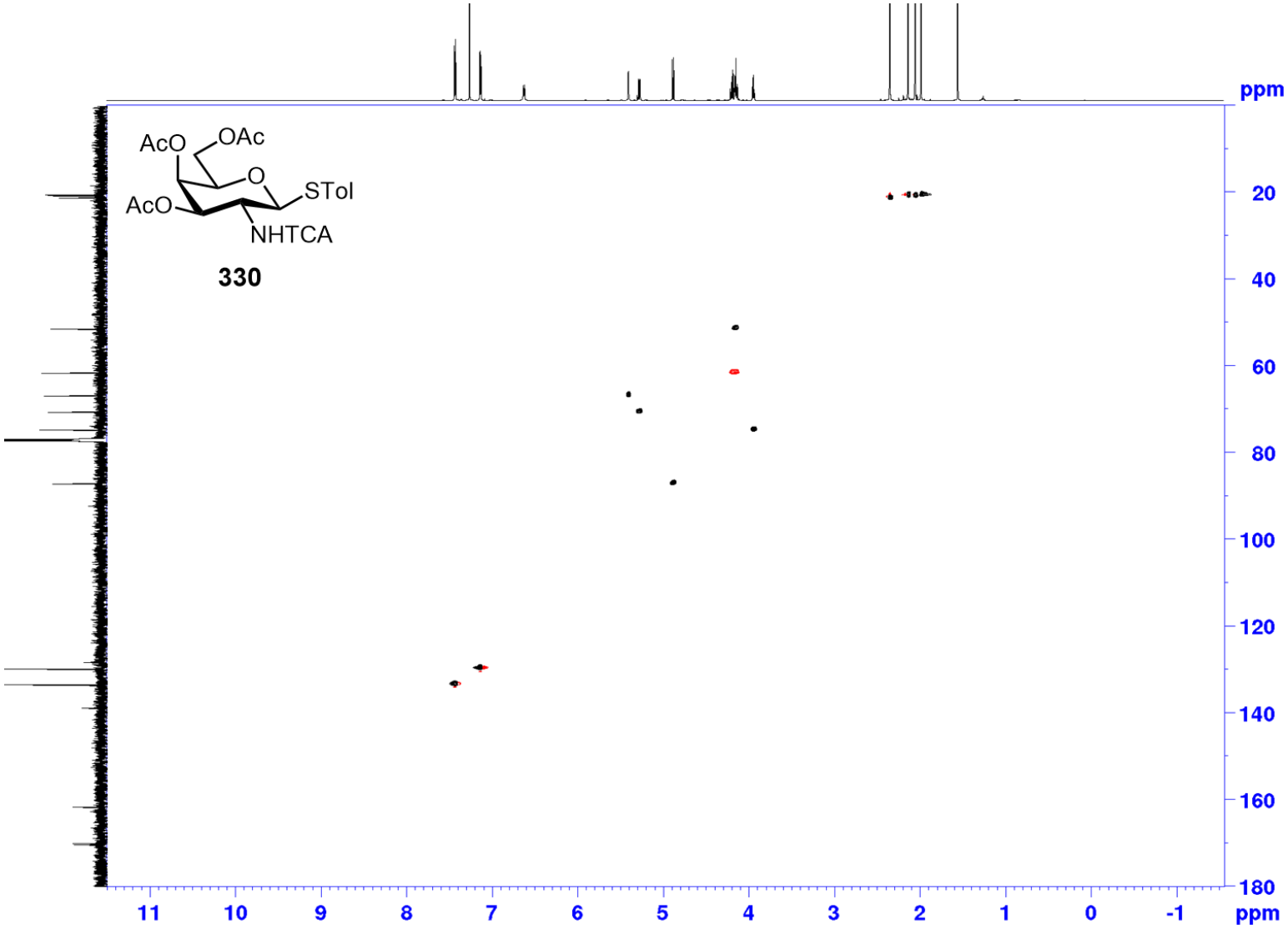
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 330



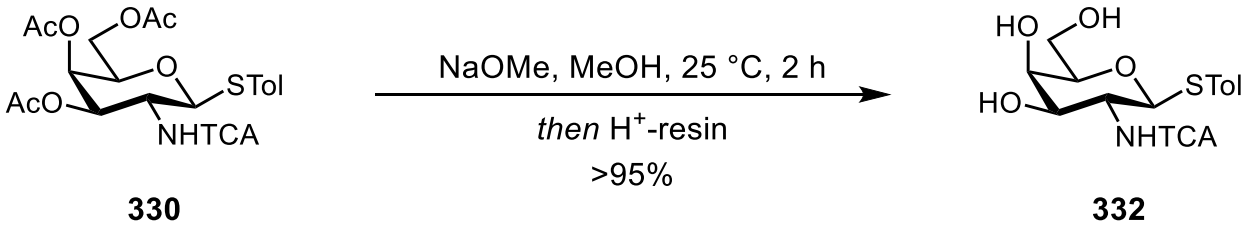
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 330



^{1}H-^{13}C HSCQ ($CDCl_3$) of compound 330



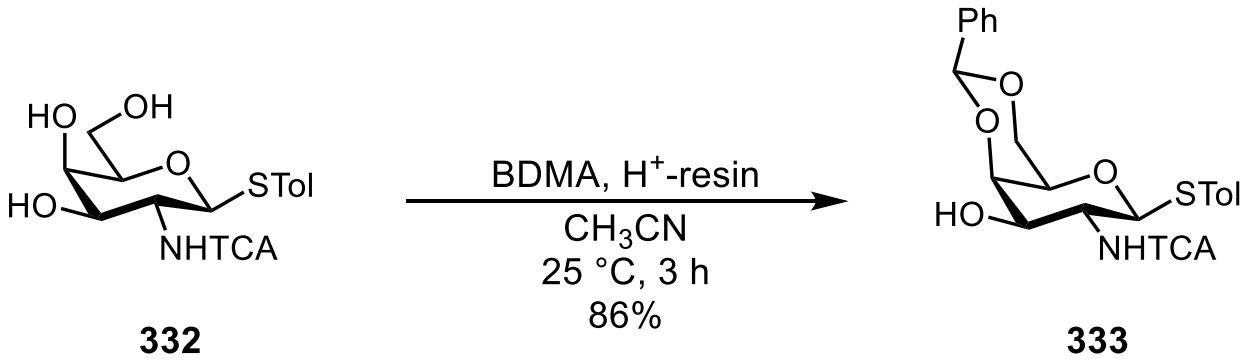
***p*-Tolyl 2-deoxy-2-trichloracetamido-1-thio-β-D-galactopyranoside (332)**



330 **332**

A solution of *p*-tolyl 3,4,6-tetra-*O*-acetyl-2-deoxy-2-trichloracetamido-1-thio-β-D-galactopyranoside **330** (1.0 equiv., 3.2049 g, 5.754 mmol) in anhydrous CH_3OH (50 mL) and added $NaOCH_3$ solution (1.0 equiv., 2 mL, 5.5 mmol, [2 N] in CH_3OH) dropwise at room temperature under an argon atmosphere. At 12 h, the pH of the reaction mixture was still >12 and TLC indicated that the product of the first step had been completely consumed for a more polar spot. The reaction was neutralized by the addition of acidic form Amberlite IR 120 (H+) ion exchange resin. The solution was filtered through a glass fritted vacuum filter funnel equipped with a pad of Celite to remove the acidic resin. The filtrate was concentrated to dryness to give a the triol *p*-tolyl 2-deoxy-2-trichloracetamido-1-thio-β-D-galactopyranoside **332** (2.475 g, 5.476 mmol, >95%) as an off-white solid with no further purification necessary. Our spectroscopic data is consistent with previously reported results.[355, 361]

***p*-Tolyl 4,6-*O*-benzylidene acetal-2-deoxy-2-trichloracetamido-1-thio-β-D-galactopyranoside (333)**



332 **333**

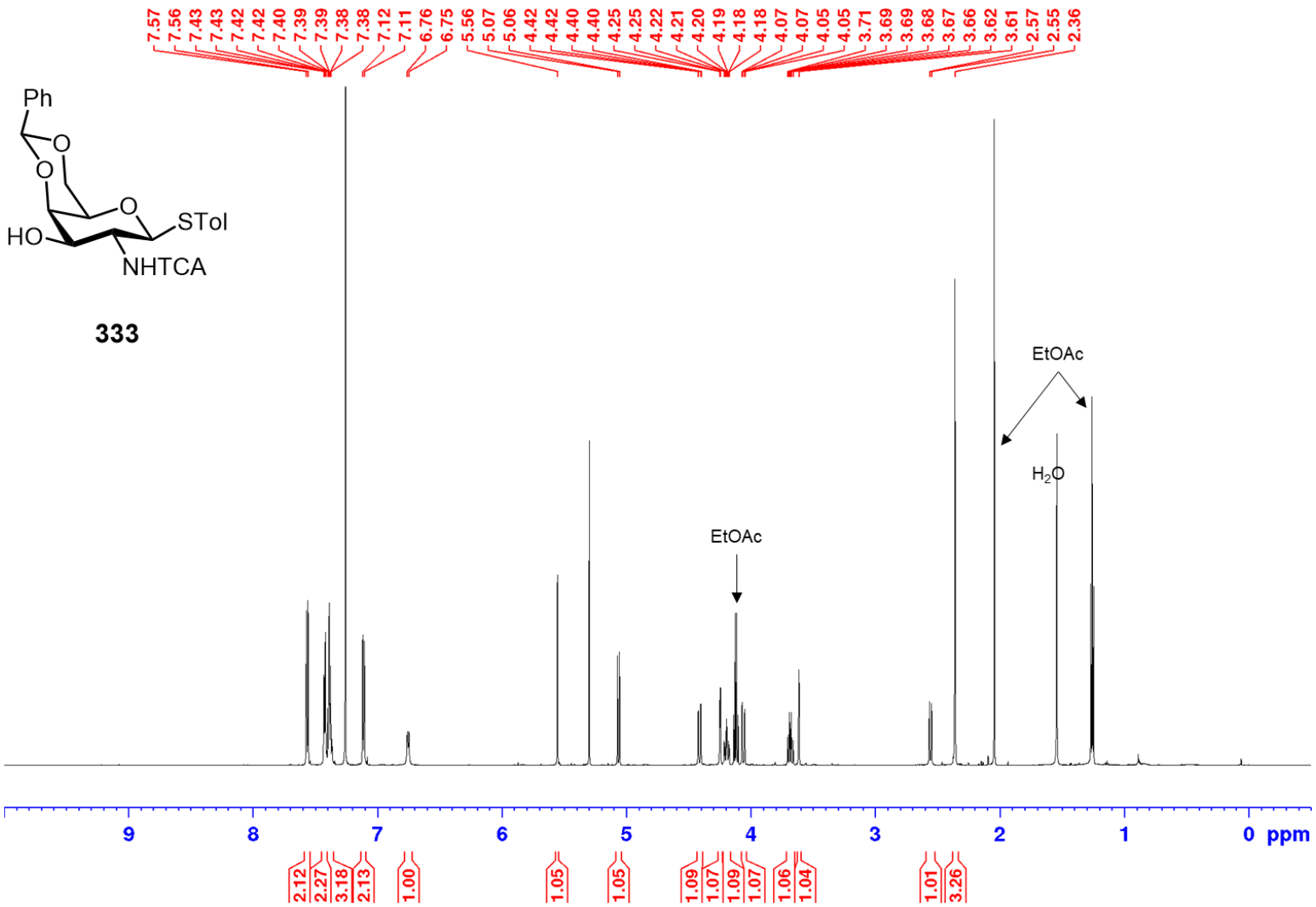
A solution of *p*-tolyl 2-deoxy-2-trichloracetamido-1-thio-β-D-galactopyranoside **332** (1.0 equiv. 2.475 g, 5.476 mmol) in anhydrous acetonitrile (50 mL) and added benzaldehyde dimethyl acetal (1.2 equiv., 1.11 mL, 7.0 mmol) dropwise at room temperature under argon. The pH of the reaction mixture was adjusted to < 4 by the addition of acidic Amberlite IR 120 (H^+) ion exchange resin. At 3 h, TLC indicated that the polar triol material had been completely consumed. The crude reaction mixture filtered through a glass fritted vacuum filter funnel equipped with a pad of Celite to remove the acid resin. The filtrate was further neutralized by the addition of triethylamine and crude mixture was concentrated down then purified by flash chromatography (30:70 to 40:60 EtOAc/Hexanes) to give *p*-tolyl 4,6-*O*-benzylidene acetal-2-deoxy-2-trichloracetamido-1-thio-β-D-galactopyranoside **333** (2.39 g, 4.60 mmol, 84%)[a] as a white solid. Our spectroscopic data is consistent with previously reported results.[355, 361]

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.57 (d, *J* = 8.3 Hz, 2H), 7.43-7.42 (m, 2H), 7.40-7.38 (m, 3H), 7.11 (d, *J* = 8.2 Hz, 2H), 6.75 (d, *J* = 7.5 Hz, 1H), 5.56 (s, 1H), 5.06 (d, *J* = 10.6 Hz, 1H), 4.41 (dd, *J* = 1.6, 12.5 Hz, 1H), 4.25 (d, *J* = 3.4 Hz, 1H), 4.19 (ddd, *J* = 3.7, 10.2,

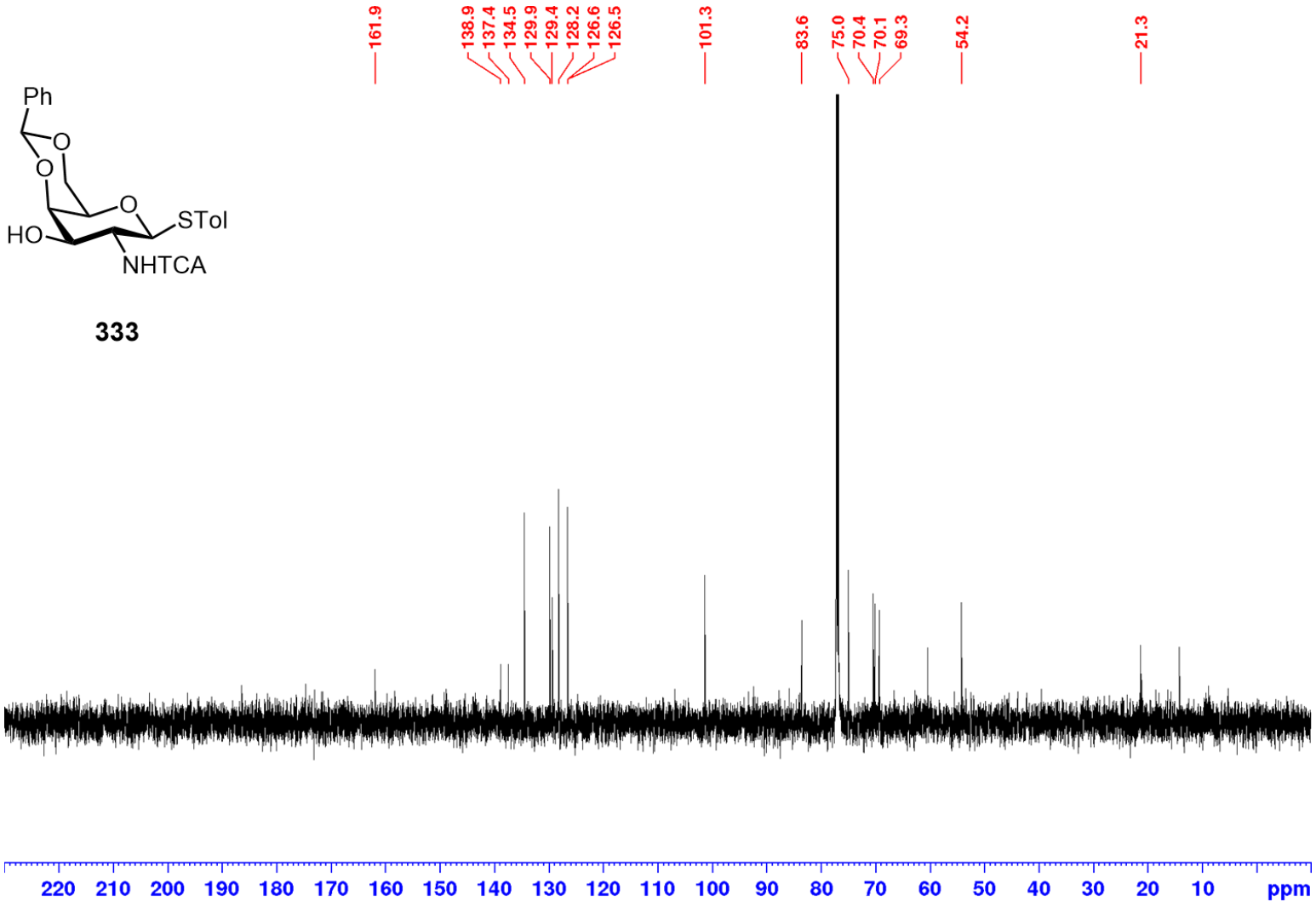
13.8 Hz, 1H), 4.06 (dd, *J* = 1.6, 12.4 Hz, 1H), 3.71-3.66 (m, 1H), 3.61 (d, *J* = 1 Hz, 1H), 2.56 (d, *J* = 10.1 Hz, 1H), 2.36 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 161.9, 138.9, 137.4, 134.5, 129.9, 129.4, 128.2, 126.6, 126.6, 101.3, 83.6, 75.0, 70.4, 70.1, 69.3, 54.2, 21.3.

[a] yield reported contains solvent impurities. see NMR spectra.

^{1}H NMR ($CDCl_3$, 400 MHz) of compound 333

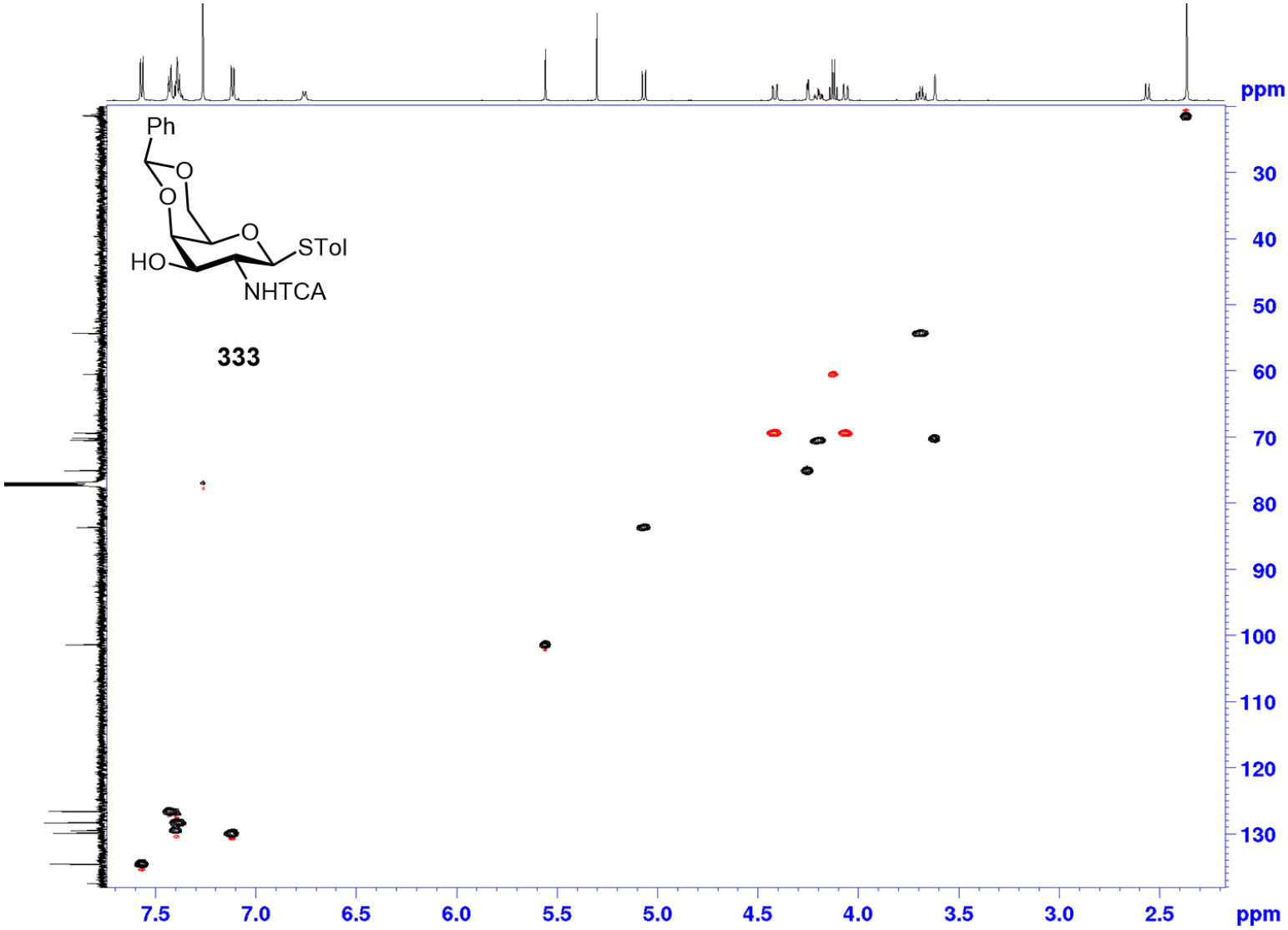


^{13}C NMR ($CDCl_3$, 100 MHz) of compound 333

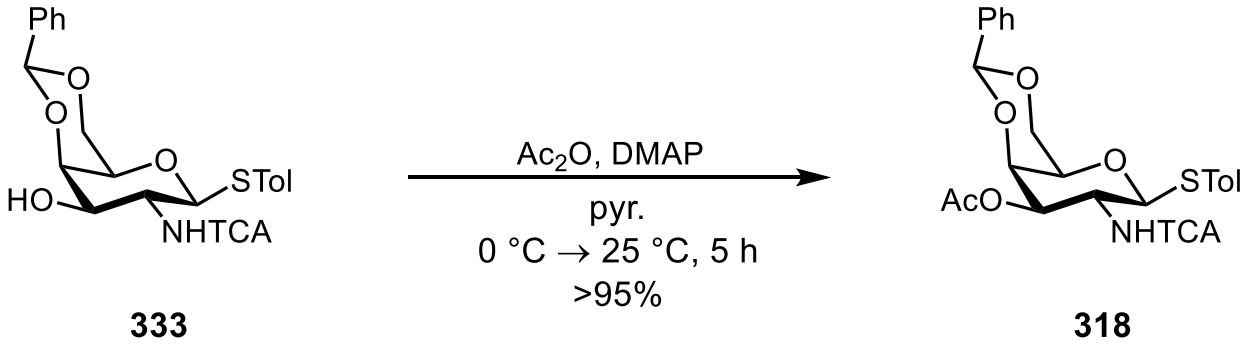


333

^{1}H-^{13}C HSCQ ($CDCl_3$) of compound 333



***p*-Tolyl 4,6-*O*-benzylidene acetal-3-*O*-acetyl-2-deoxy-2-trichloracetamido-1-thio-β-D-galactopyranoside (318)**

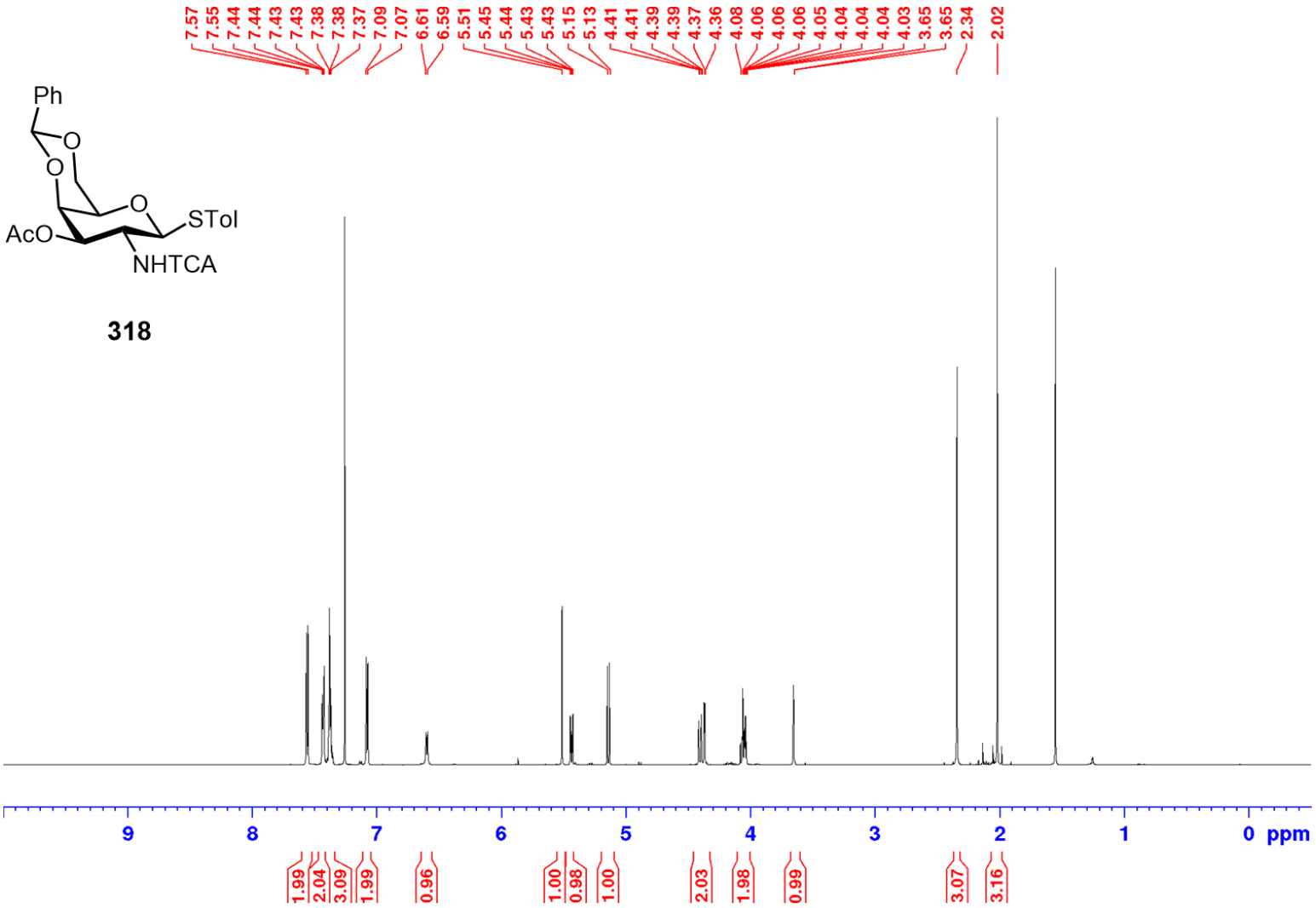


333 **318**

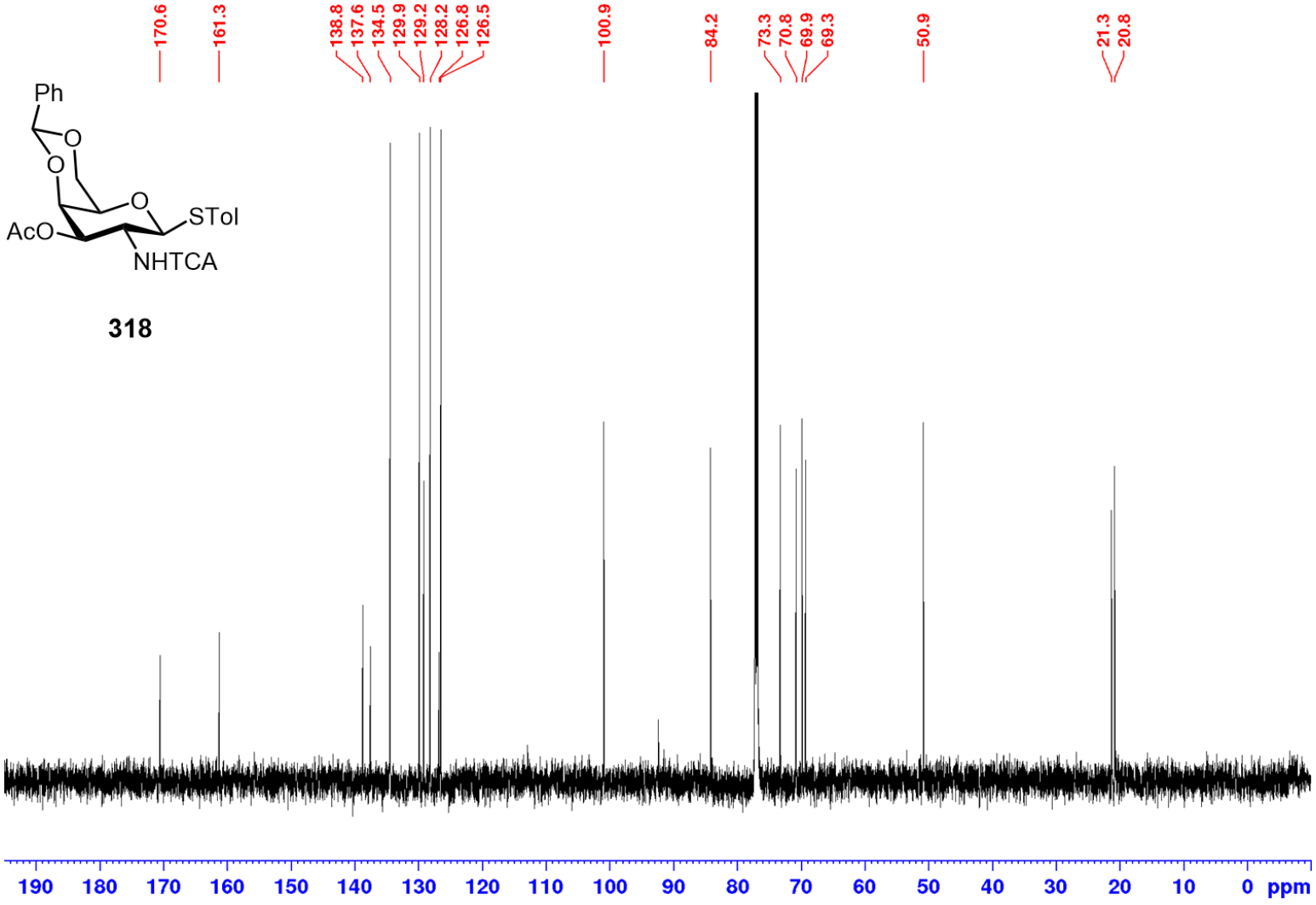
To a solution of *p*-tolyl 4,6-*O*-benzylidene acetal-2-deoxy-2-trichloracetamido-1-thio-β-D-galactopyranoside **333** (1.0 equiv., 3.2 g, 7.4 mmol) in pyridine (35 mL) was cooled to 0 °C under an argon atmosphere and added acetic anhydride (7.0 equiv., 4.6 mL, 51.8 mmol) and DMAP (0.1 equiv.). The reaction was stirred at room temperature. At 1 h, TLC indicated that the starting material was completely consumed. The reaction was cooled to 0 °C and the excess acetic anhydride was quenched by the addition of CH_3OH (5.0 mL). After 5 min at 0 °C, the crude reaction mixture was stirred at room temperature for 5 min and then transferred to a separatory funnel with EtOAc (100 mL). The reaction was washed with 1 N HCl (3 x 25 mL), satd. aq. $NaHCO_3$ (1 x 25 mL), brine (1 x 125 mL), dried ($NaSO_4$) and filtered through a glass fritted vacuum filter funnel to remove drying agent. The filtrate was concentrated to a crude oil which was purified by flash column chromatography (1:3 to 1:1 EtOAc/hexanes) then was crystalized from hot EtOAc and hexanes to give *p*-tolyl 4,6-*O*-benzylidene acetal-3-*O*-acetyl-2-deoxy-2-trichloracetamido-1-thio-β-D-galactopyranoside **318** (3.884 g, 6.92 mmol, 94%) as white crystals. Spectroscopic data is in agreement with previously reported data.[362] $[\alpha]D^{25}$ = -11.52° (c = 1.0, $CHCl_3$); IR ν_{max} (film) 3351.22, 2146.38, 1635.17, 1530.78, 1374.40,

1240.10, 1172.18, 1093.11, 1053.50, 816.61, 730.48, 730.48, 698.14 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.56 (d, *J* = 8.4 Hz, 2H), 7.44-7.43 (m, 2H), 7.38-7.37 (m, 3H), 7.08 (d, *J* = 7.4 Hz, 2H), 6.60 (d, *J* = 7.4 Hz, 1H), 5.52 (s, 1H), 5.45 (dd, *J* = 3.4, 10.9 Hz, 1H), 5.15 (d, *J* = 10 Hz, 1H), 4.42 (dd, *J* = 1.6, 12.4 Hz, 1H), 4.36 (d, *J* = 2.9 Hz, 1H), 4.08-4.03 (m, 2H), 3.64 (d, *J* = 0.8 Hz, 1H), 2.34 (s, 3H), 2.03 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.6, 161.3, 138.8, 137.6, 134.5, 129.9, 129.2, 128.2, 126.8, 126.5, 100.9, 84.2, 73.3, 70.8, 69.9, 69.3, 50.9, 21.3, 20.8; HR-ESI-MS (m/z): calcd for $C_{24}H_{28}Cl_3N_2O_6S^+$ ($M+NH_4^+$) 577.072823, found 577.0736.

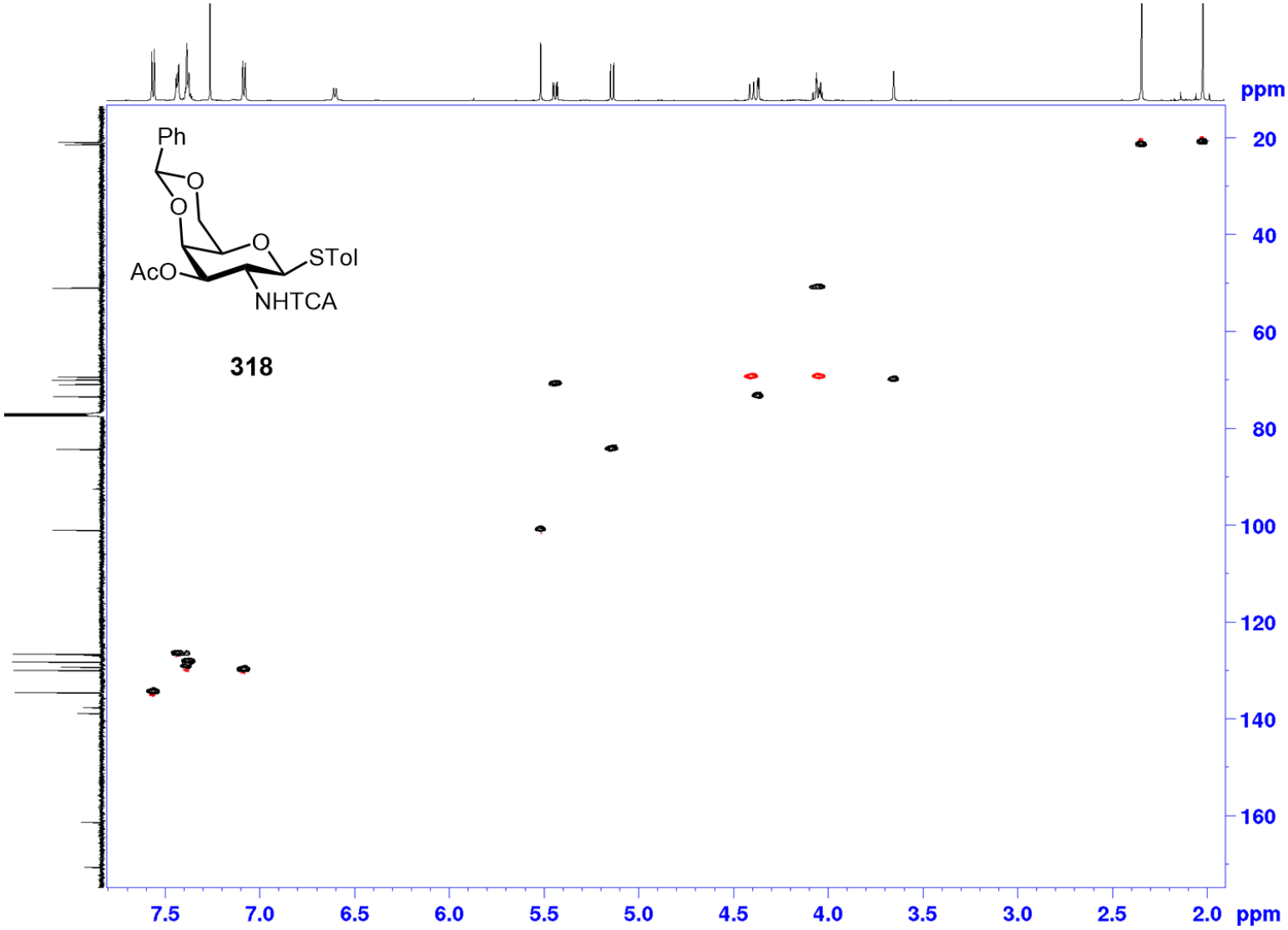
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 318



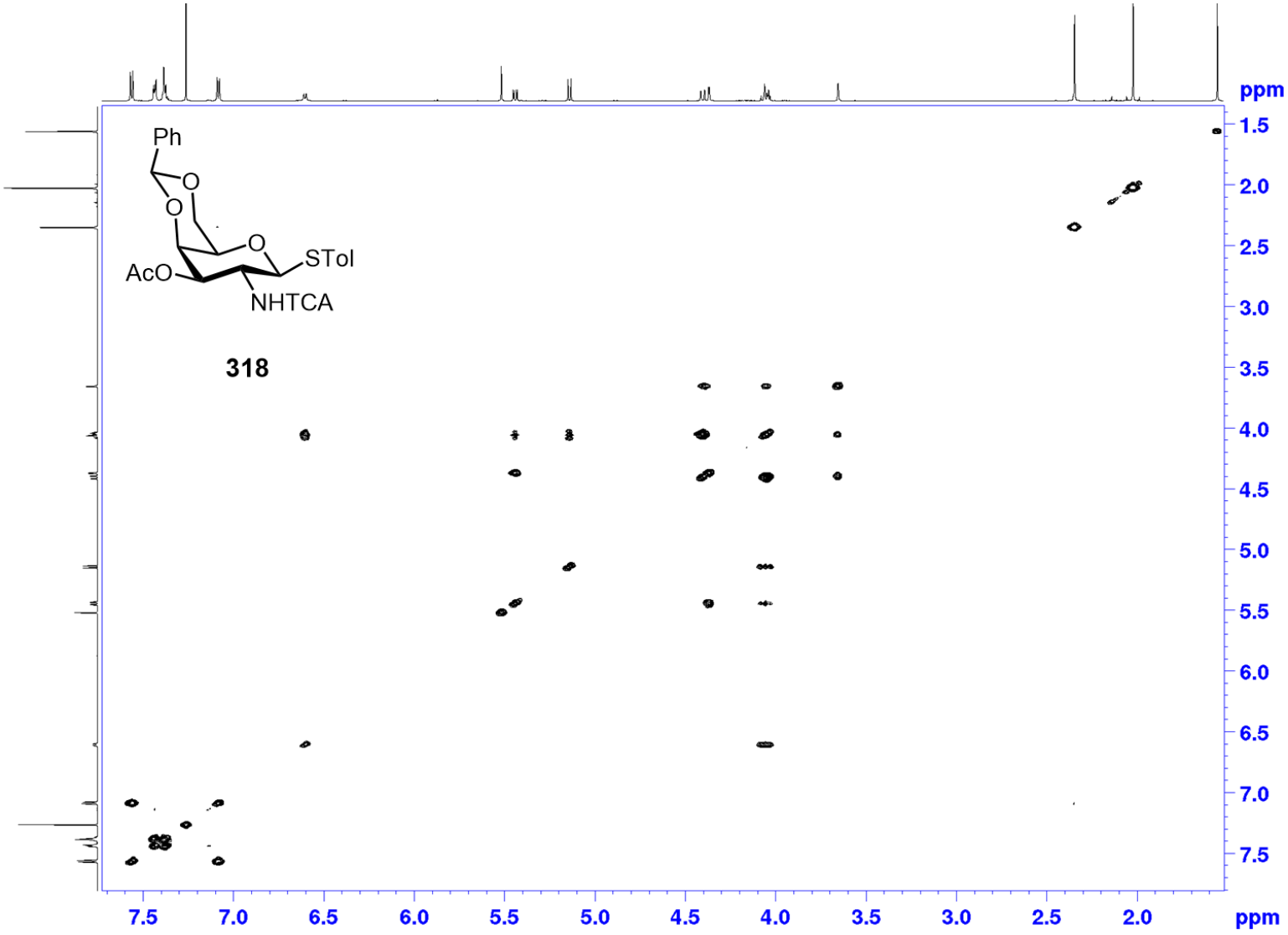
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 318



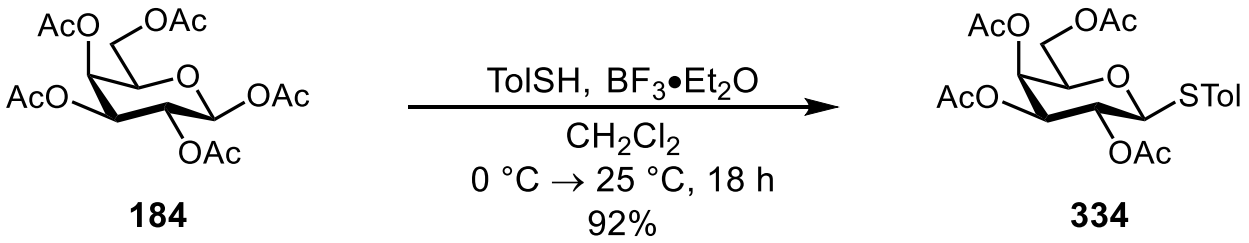
^{1}H-^{13}C HSCQ ($CDCl_3$) of compound 318



^{1}H-^{1}H COSY ($CDCl_3$) of compound 318

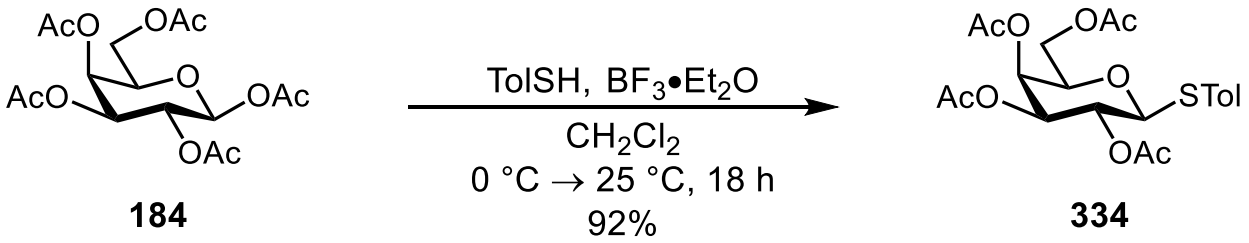


***p*-tolyl 2,3,4,6-tetra-*O*-aceyl-1-thio-β-D-galactopyranoside (334)**



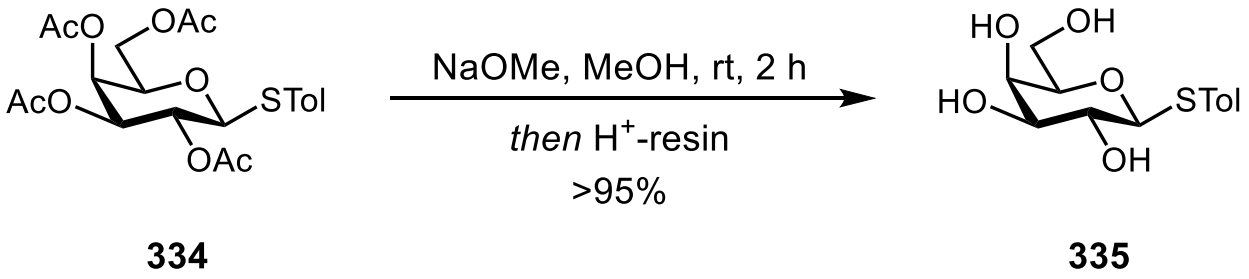
A solution of commercially available 1,2,3,4,6-penta-*O*-acyl-D-β-galactopyranoside **184** (1.0 equiv., 19.517 g, 50.00 mmol) and *p*-thiocresol (1.2 equiv., 9.13 g, 60.0 mmol) in anhydrous CH_2Cl_2 (250 mL) was added $BF_3{\cdot}Et_2O$ (3.0 equiv., 18.5 mL, 150.0 mmol) dropwise at 0 °C under an argon atmosphere. The reaction was stirred at room temperature for 18 h. TLC indicated complete consumption of starting material. The crude reaction mixture was quenched by the addition of satd. aq. $NaHCO_3$ (100 mL) to the reaction flask. After stirring for 30 min the biphasic mixture was transferred to a separatory funnel and the organics were separated and the aqueous layer was extracted with CH_2Cl_2 (3 x 20 mL). The organics were combined and dried ($NaSO_4$) and filtered through a glass fritted vacuum filter funnel to remove drying agent. The filtrate was concentrated to an oil. The crude oil was purified by silica gel flash column chromatography (30:70 to 50:50 EtOAc/hexanes) to give *p*-tolyl 2,3,4,6-tetra-*O*-acyl-β-D-galactopyranoside **334** (21.23 g, 46.71 mmol, 93%) as an off white solid. Our characterization data were consistent with previously reported data.[222, 363]

***p*-tolyl 2,3,4,6-tetra-*O*-aceyl-1-thio-β-D-galactopyranoside (334)**



A solution of commercially available 1,2,3,4,6-penta-*O*-acyl-D-β-galactopyranoside **184** (1.0 equiv., 19.517 g, 50.00 mmol) and *p*-thiocresol (1.2 equiv., 9.13 g, 60.0 mmol) in anhydrous CH_2Cl_2 (250 mL) was added $BF_3{\cdot}Et_2O$ (3.0 equiv., 18.5 mL, 150.0 mmol) dropwise at 0 °C under an argon atmosphere. The reaction was stirred at room temperature for 18 h. TLC indicated complete consumption of starting material. The crude reaction mixture was quenched by the addition of satd. aq. $NaHCO_3$ (100 mL) to the reaction flask. After stirring for 30 min the biphasic mixture was transferred to a separatory funnel and the organics were separated and the aqueous layer was extracted with CH_2Cl_2 (3 x 20 mL). The organics were combined and dried ($NaSO_4$) and filtered through a glass fritted vacuum filter funnel to remove drying agent. The filtrate was concentrated to an oil. The crude oil was purified by silica gel flash column chromatography (30:70 to 50:50 EtOAc/hexanes) to give *p*-tolyl 2,3,4,6-tetra-*O*-acyl-β-D-galactopyranoside **334** (21.23 g, 46.71 mmol, 93%) as an off white solid. Our characterization data were consistent with previously reported data.[222, 363]

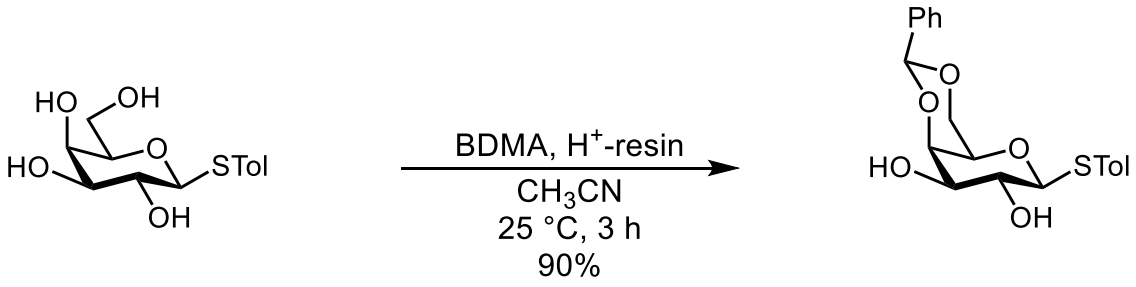
***p*-tolyl 1-thio-β-D-galactopyranoside (335)**



334 **335**

At a solution of *p*-tolyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside **334** (1.0 equiv., 21.23 g, 46.71 mmol mmol) in anhydrous CH_3OH (250 mL) was treated with a freshly made solution of sodium methoxide (1.0 equiv., 25.0 mL, 1.812 mmol, [2 N] in CH_3OH) at room temperature under an argon atmosphere. The pH of the solution was >11. At 2 h, the reaction was neutralized by the addition of acidic form acidic Amberlite IR 120 (H+) ion exchange resin. The solution was filtered through a glass fritted vacuum filter funnel with a pad of Celite to remove the acidic resin. The filtrate was concentrated to dryness to give a crude tetraol. The crude material was passed through a plug of silica (70:30 to 85:15 EtOAc/hexanes) to give *p*-tolyl 1-thio-β-D-galactopyranoside **335** (13.26 g, 46.54 mmol, >95%) as a white solid. Our characterization data were consistent with previously reported data.[222, 364]

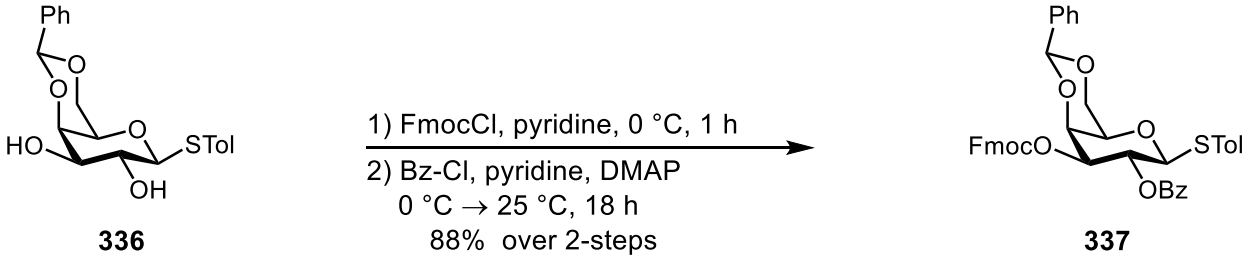
_p_-tolyl 4,6-_O_-benzylidene acetal-1-thio-β-D-galactopyranoside (336)



335 **336**

A solution of *p*-tolyl 1-thio-β-D-galactopyranoside **335** (1.0 equiv., 13.26 g, 46.54 mmol) and benzaldehyde dimethyl acetal (1.2 equiv., 8.38 mL, 55.85 mmol) in anhydrous CH_3CN (50 mL). The pH of the reaction mixture was adjusted to <4 by the addition of acidic Amberlite IR 120 (H^+) ion exchange resin at 25 °C under an argon atmosphere. At 12 h, TLC indicated that the tetraol material had been completely consumed in exchange for less polar spots. The crude reaction mixture filtered through a glass fritted vacuum filter funnel equipped with a pad of Celite to remove the acid resin. The filtrate was further neutralized by the addition of triethylamine and crude mixture was concentrated down then purified by flash chromatography (25:75 to 40:60 EtOAc/Hexanes) to give *p*-tolyl 4,6-*O*-benzylidene acetal-1-thio-β-D-galactopyranoside **336** (15.68 g, 41.89 mmol, 90%) as a white solid. The spectroscopic data were in agreement with the reported data.[222]

***p*-Tolyl 4,6-*O*-benzylidene acetal-3-*O*-Fmoc-2-*O*-benzoyl-1-thio-β-D-galactopyranoside (337)**



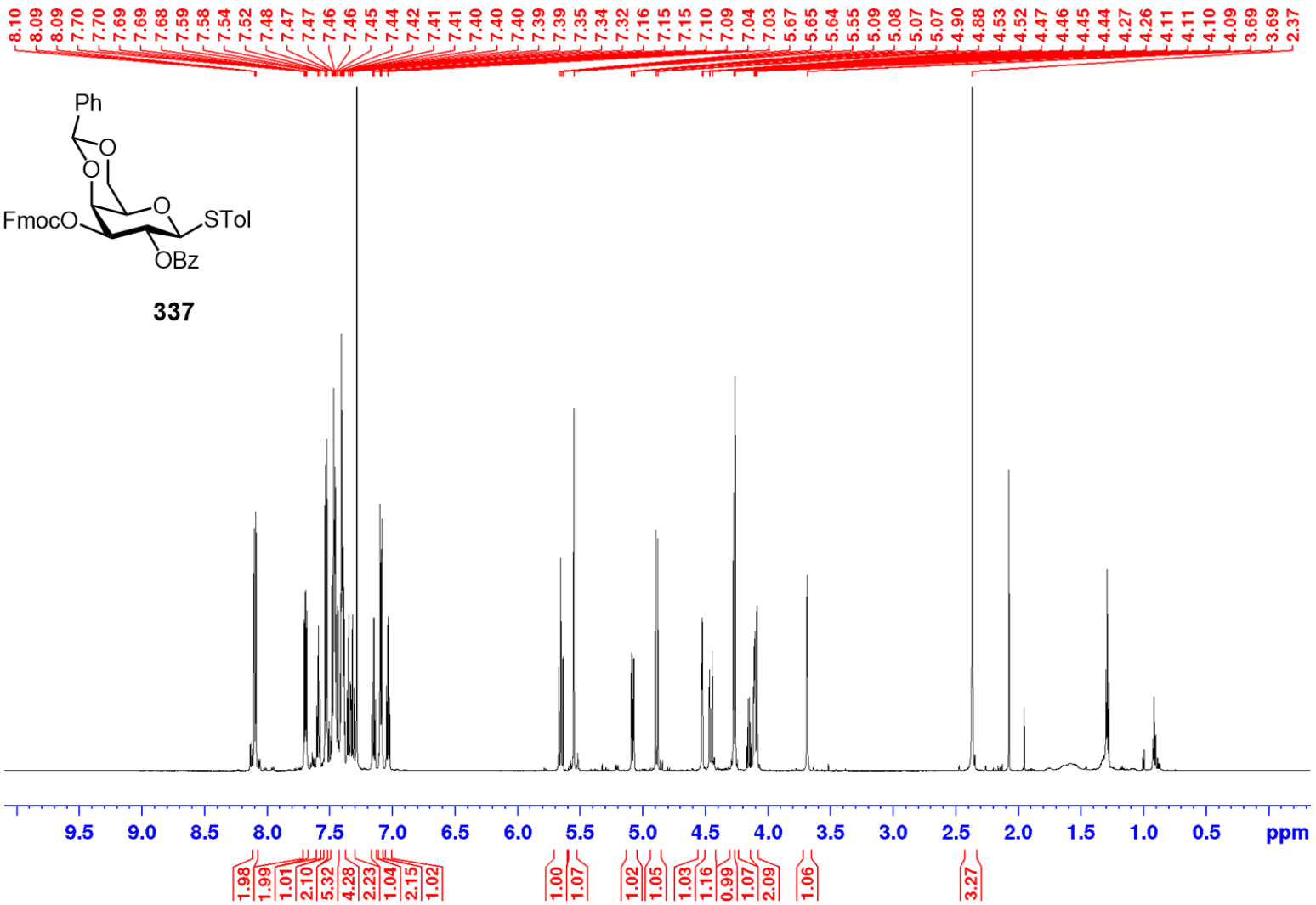
336 **337**

To a solution of *p*-tolyl 4,6-*O*-benzylidene acetal-1-thio-β-D-galactopyranoside **336** (1.0 equiv., 1.00 g, 2.67 mmol) in pyridine (13 mL) was added FmocCl (1.5 equiv., 1.060 g, 4.097 mmol) protionwise at 0 °C under an argon atmosphere. After 1 h, the reaction was added MeOH. The mixture was concentrated to a crude solid. The crude solid was co-evaporated with toluene (3x). The crude product was purified by a plug of silica gel (60:40 EtOAc/hexanes) to give the D-galactoside. The material was disolved in pyridine (13 mL) and added BzCl (3.0 equiv., 0.93 mL, 8.01 mmol) dropwise and DMAP (cat.) at 0 °C under an argon atmosphere. The reaction mixture was stirred at room temperature. After 18 h, the reaction was added MeOH (1.0 mL). The reaction mixture was concentrated to a crude oil. The crude oil was purified by silica gel flash column chromatography (50:50 to 80:20 EtOAc/hexanes) to give *p*-tolyl 4,6-*O*-benzylidene acetal-3-*O*-Fmoc-2-*O*-benzoyl-1-thio-β-D-galactopyranoside **337** (1.65 g, 2.35 mmol, 88%) as a white solid. Our spectroscopic data agreed with previously reported data.[91]

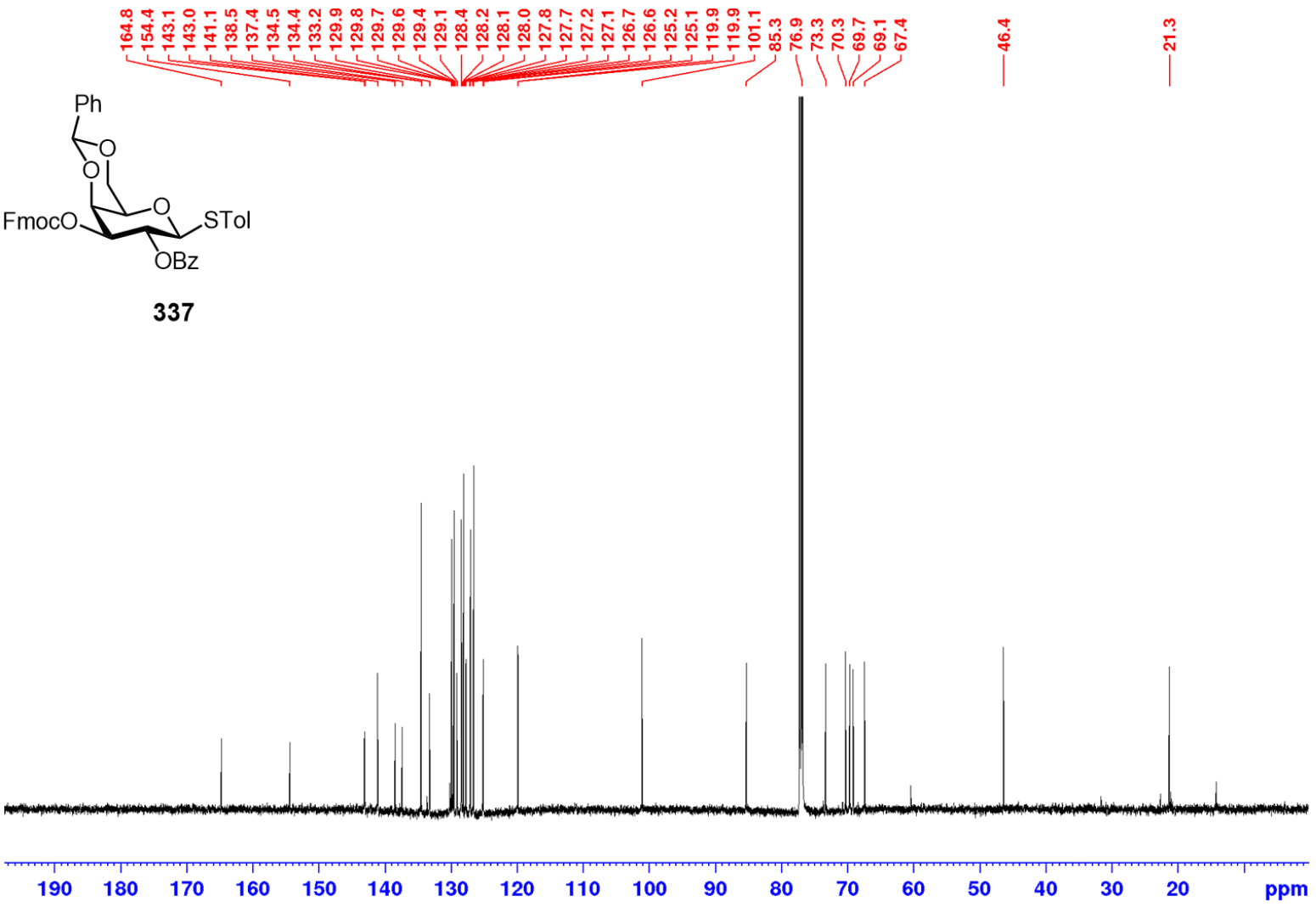
^{1}H NMR (600 MHz, $CDCl_3$): δ 8.10-8.09 (m, 2H), 7.70-7.69 (m, 2H), 7.60-7.58 (m, 1H), 7.53 (d, *J* = 8.1 Hz, 2H), 7.48-7.45 (m, 5H), 7.44-7.39 (m, 4H), 7.36-7.31 (m, 2H), 7.15 (dt, *J* = 0.8, 7.1 Hz, 1H), 7.09 (d, *J* = 7.8 Hz, 2H), 7.03 (dt, *J* = 0.8, 7.5 Hz, 1H), 5.66 (t, *J* = 9.2 Hz, 1H), 5.56 (s, 1H), 5.09 (dd, *J* = 3.5, 10.2 Hz, 1H), 4.9 (d, *J* = 9.2 Hz, 1H), 4.53

(dd, *J* = 0.7, 3.4 Hz, 1H), 4.46 (dd, *J* = 1.4, 12.4 Hz, 1H), 4.27 (s, 1H), 4.26 (s, 1H), 4.11-4.09 (m, 2H), 3.69 (s, 1H), 2.38 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 164.7, 154.4, 143.1, 143.0, 141.1, 138.4, 137.4, 134.5, 134.4, 133.2, 129.9, 129.8, 129.7, 129.5, 129.4, 129.1, 128.4, 128.2, 128.1, 127.9, 127.7, 127.7, 127.2, 127.0, 126.6, 126.6, 125.1, 125.1, 119.9, 119.8, 101.1, 85.3, 76.9, 73.3, 70.3, 69.6, 69.1, 67.4, 46.3, 21.2.

^{1}H NMR ($CDCl_3$, 400 MHz) of compound 337

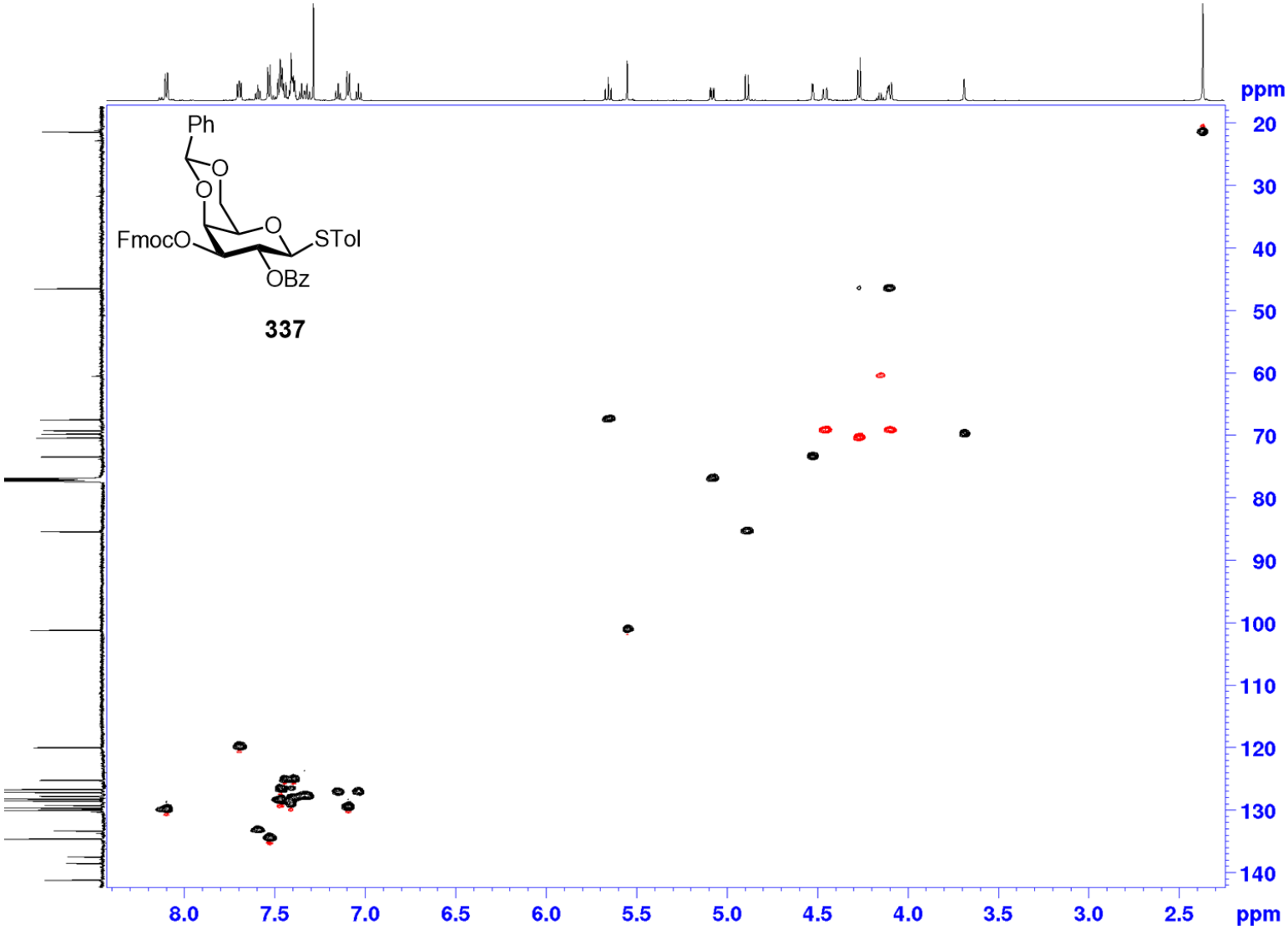


^{13}C NMR ($CDCl_3$, 100 MHz) of compound 337

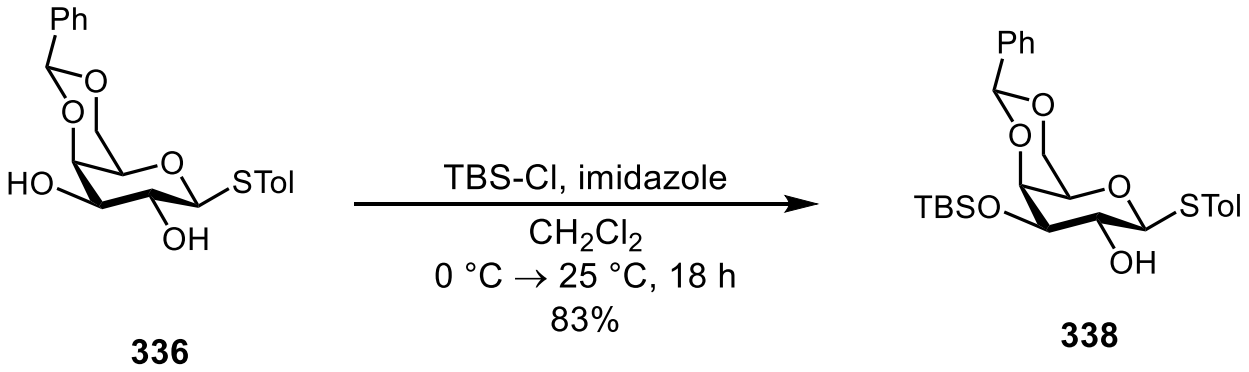


337

^{1}H-^{13}C HSCQ ($CDCl_3$) of compound 337



***p*-tolyl 4,6-*O*-benzylidene acetal-3-*O*-*tert*-butyldimethylsilyl-1-thio-β-D-galactopyranoside (338)**



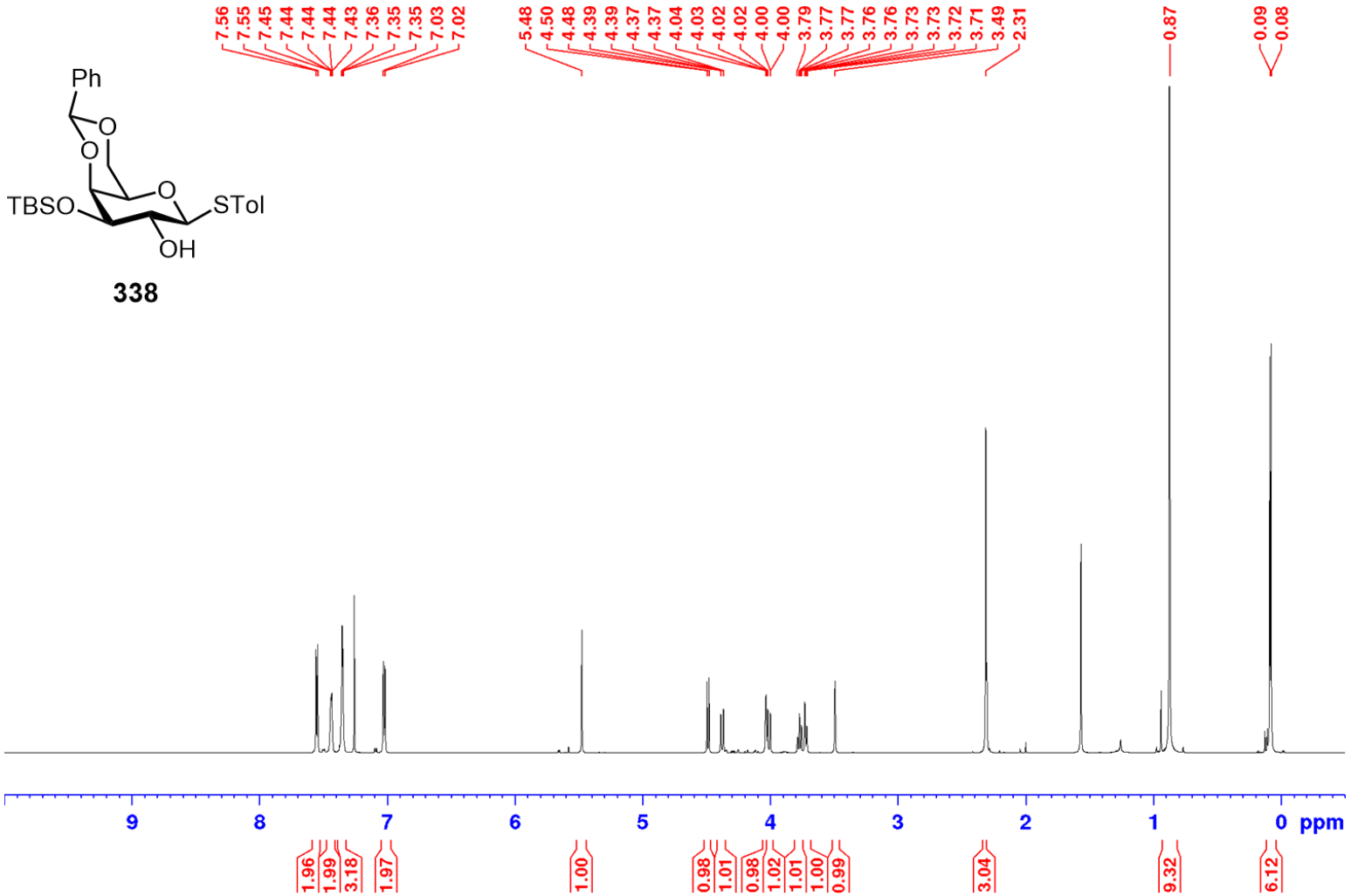
336 **338**

To a solution of *p*-tolyl 4,6-*O*-benzylidene acetal-1-thio-β-D-galactopyranoside **338** (1.0 equiv., 1.0 g, 2.67 mmol) and imidazole (2.5 equiv., 0.455 g, 6.67 mmol) in anhydrous CH_2Cl_2 (26 mL) was added solution of TBS-Cl (2.0 equiv., 0.80 g, 5.34 mmol) in CH_2Cl_2 (10 mL) dropwise at 0 °C under an argon atmosphere. The reaction was stirred at room temperature for 18 h. TLC indicated complete consumption of the starting material for a less polar spot. The crude reaction mixture was concentrated to dryness then transferred to a separatory funnel with EtOAc (100 mL). The reaction was washed with 1 N HCl (3 x 25 mL), satd. aq. $NaHCO_3$ (1 x 25 mL), brine (1 x 125 mL), dried ($NaSO_4$) and filtered through a glass fritted vacuum filter funnel to remove drying agent. The filtrate was concentrated to a crude oil which was purified by flash column chromatography (25:75 to 30:70 EtOAc/hexanes) to give *p*-tolyl 4,6-*O*-benzylidene acetal-3-*O*-*tert*-butyldimethylsilyl-1-thio-β-D-galactopyranoside **336** (1.088 g, 2.226 mmol, 83%) as white solid. The spectroscopic data were in agreement with the reported data.[222]

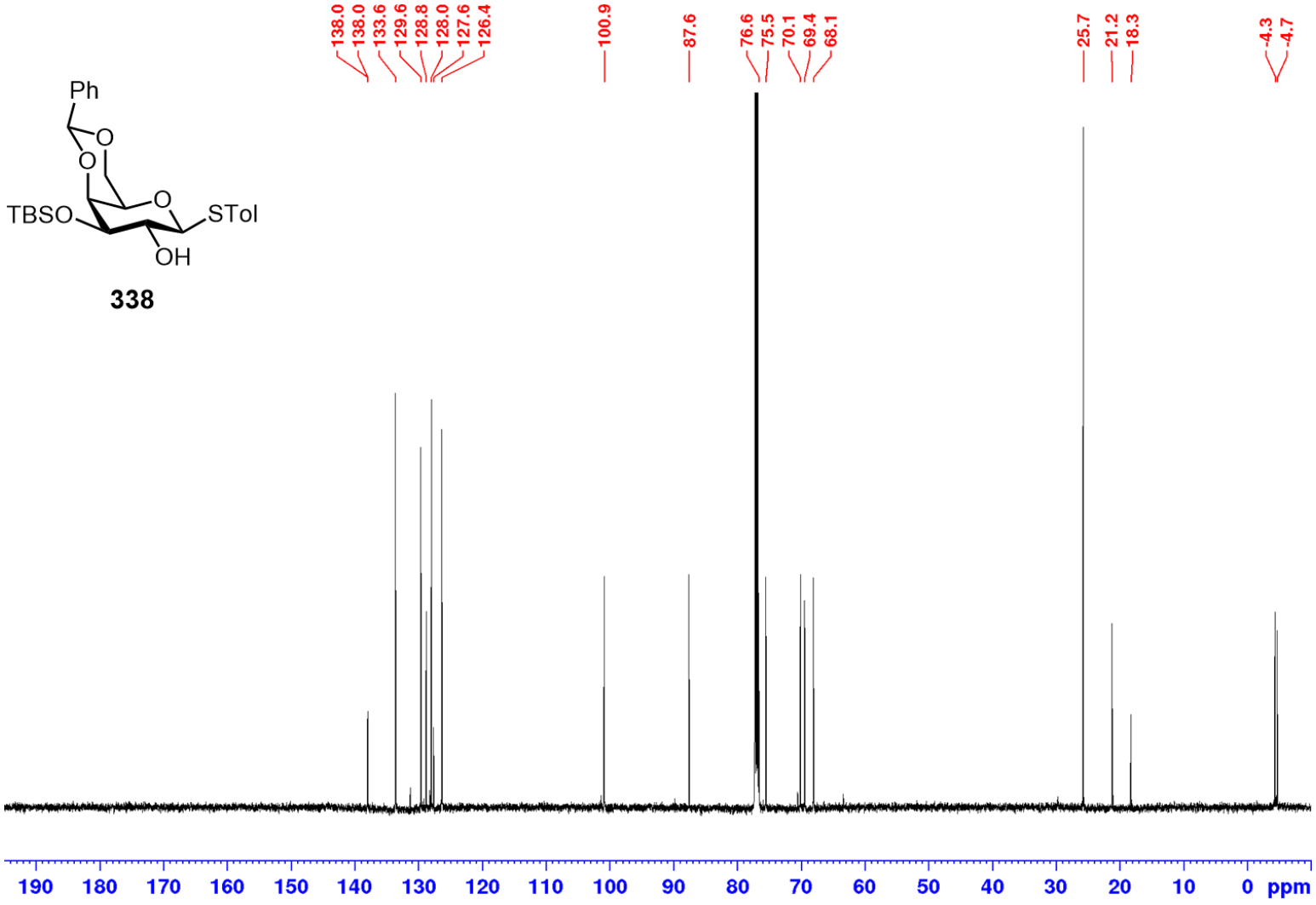
^{1}H NMR (400 MHz, $CDCl_3$): δ 7.56 (d, *J* = 8.1 Hz, 2H), 7.46-7.43 (m, 2H), 7.36-7.35 (m, 3H), 7.01 (d, *J* = 8.1 Hz, 2H), 5.48 (s, 1H), 4.50 (d, *J* = 9.1 Hz, 1H, C-1), 4.38 (dd, *J* = 1.2,

12.3 Hz, 1H, C-6'), 4.05 (d, *J* = 2.8 Hz, 1H, C-4), 4.01 (dd, *J* = 1.4, 12.6 Hz, 1H, C-6), 3.77 (ddd, *J* = 1.8, 9.0, 10.9 Hz, 1H, C-2), 3.72 (dd, *J* = 3.3, 9.1 Hz, 1H, C-3), 3.49 (s, 1H, C-5), 2.32 (s, 3H), 0.87 (s, 9H), 0.09 (s, 3H), 0.08 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 138.1, 138.0, 133.6, 129.6, 128.8, 128.0, 127.6, 126.4, 100.9, 87.6, 76.6, 75.5, 70.1, 69.4, 68.1, 25.8, 21.2, 18.3, -4.3, -4.7;

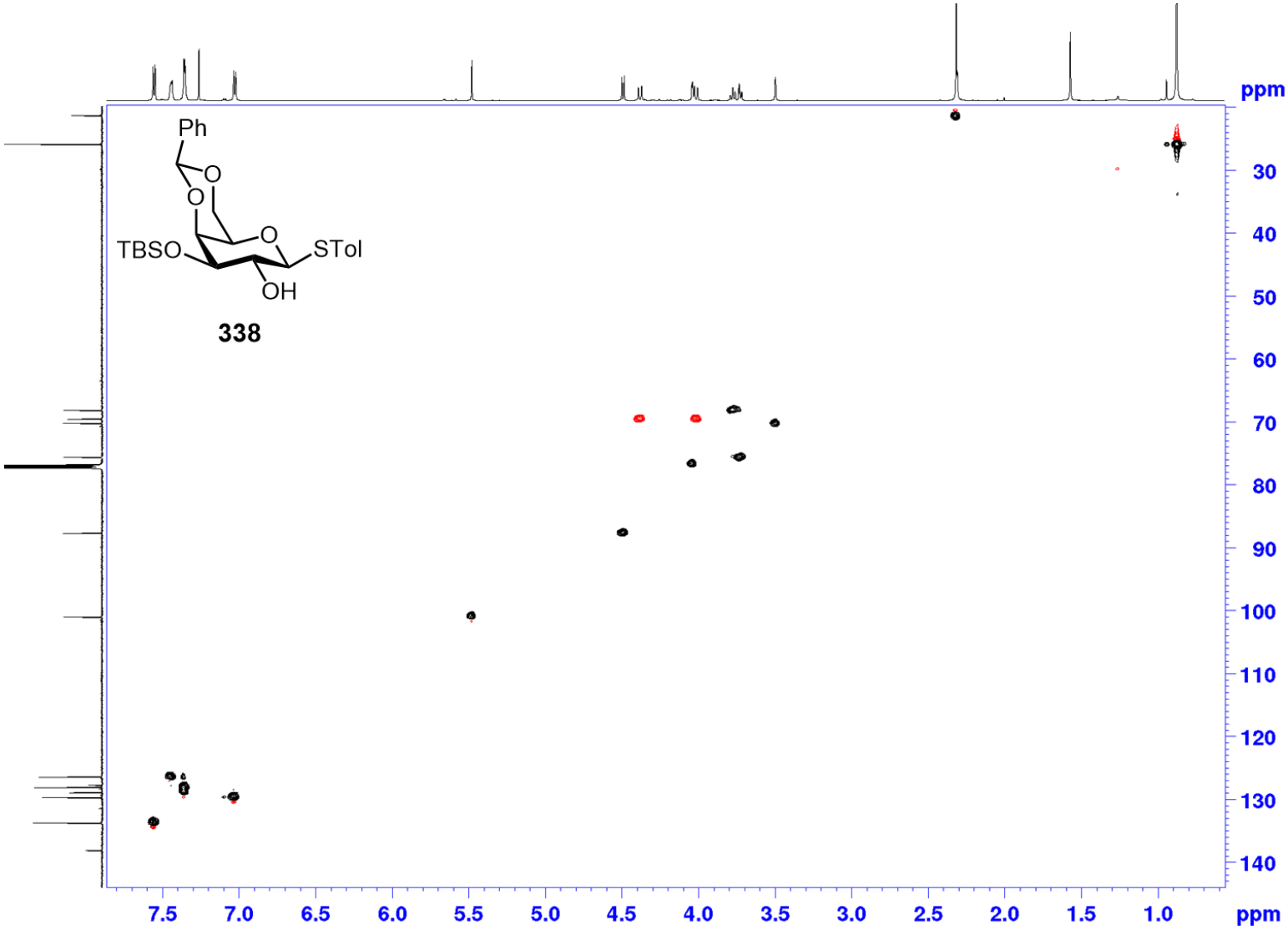
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 338



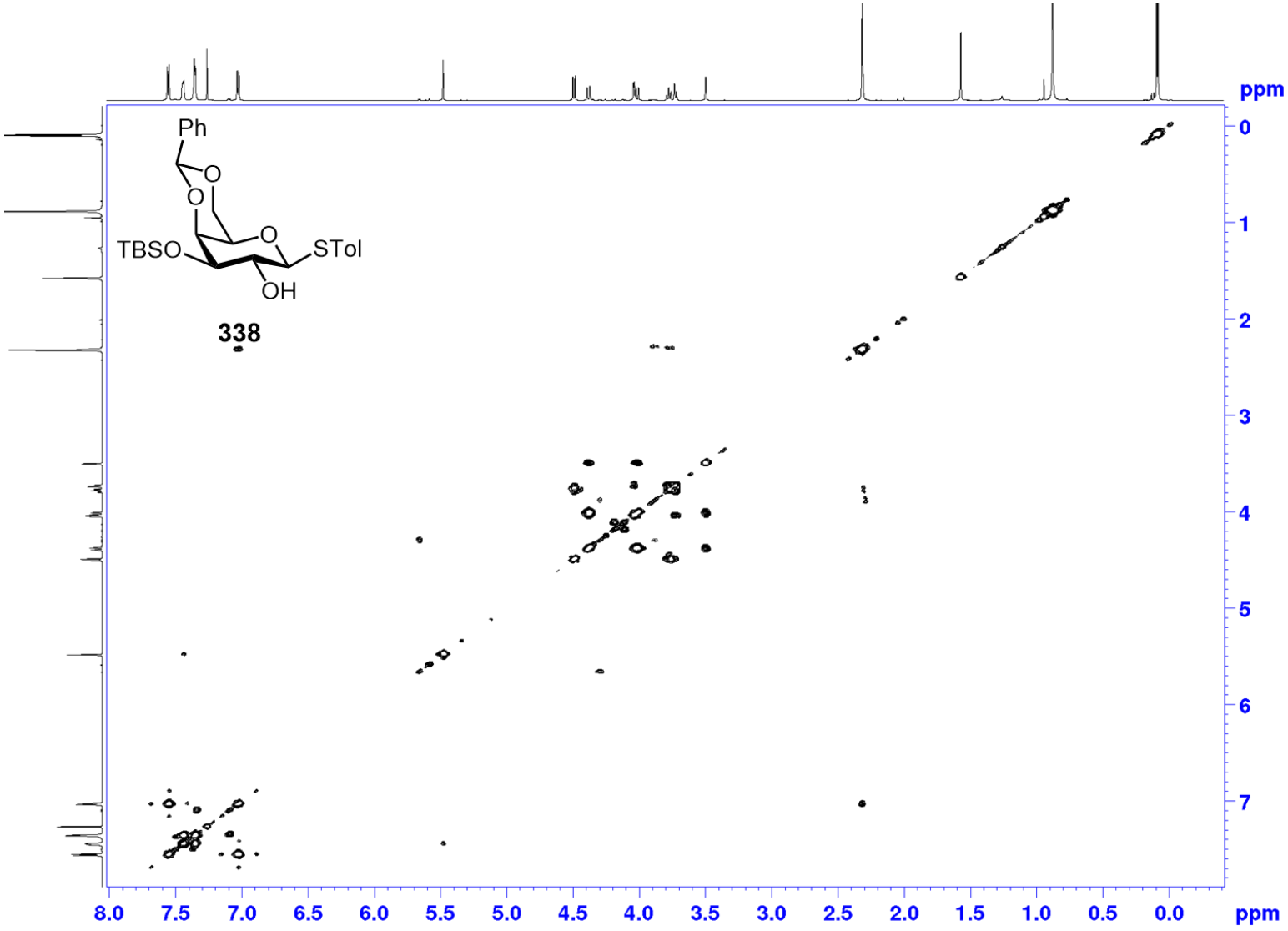
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 338



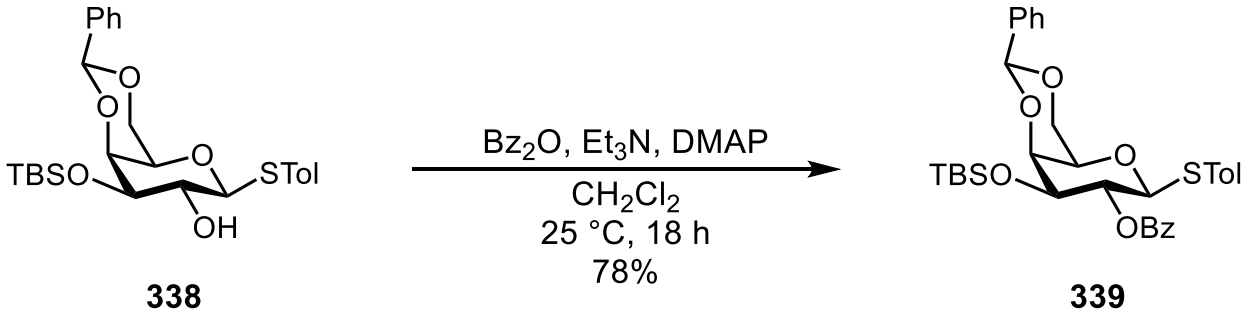
^{1}H-^{13}C HSCQ ($CDCl_3$) of compound 338



^{1}H-^{1}H COSY ($CDCl_3$) of compound 338



***p*-Tolyl 4,6-*O*-benzylidene acetal-3-*O*-*tert*-butyldimethylsilyl-2-*O*-benzoyl-1-thio-β-D-galactopyranoside (339)**

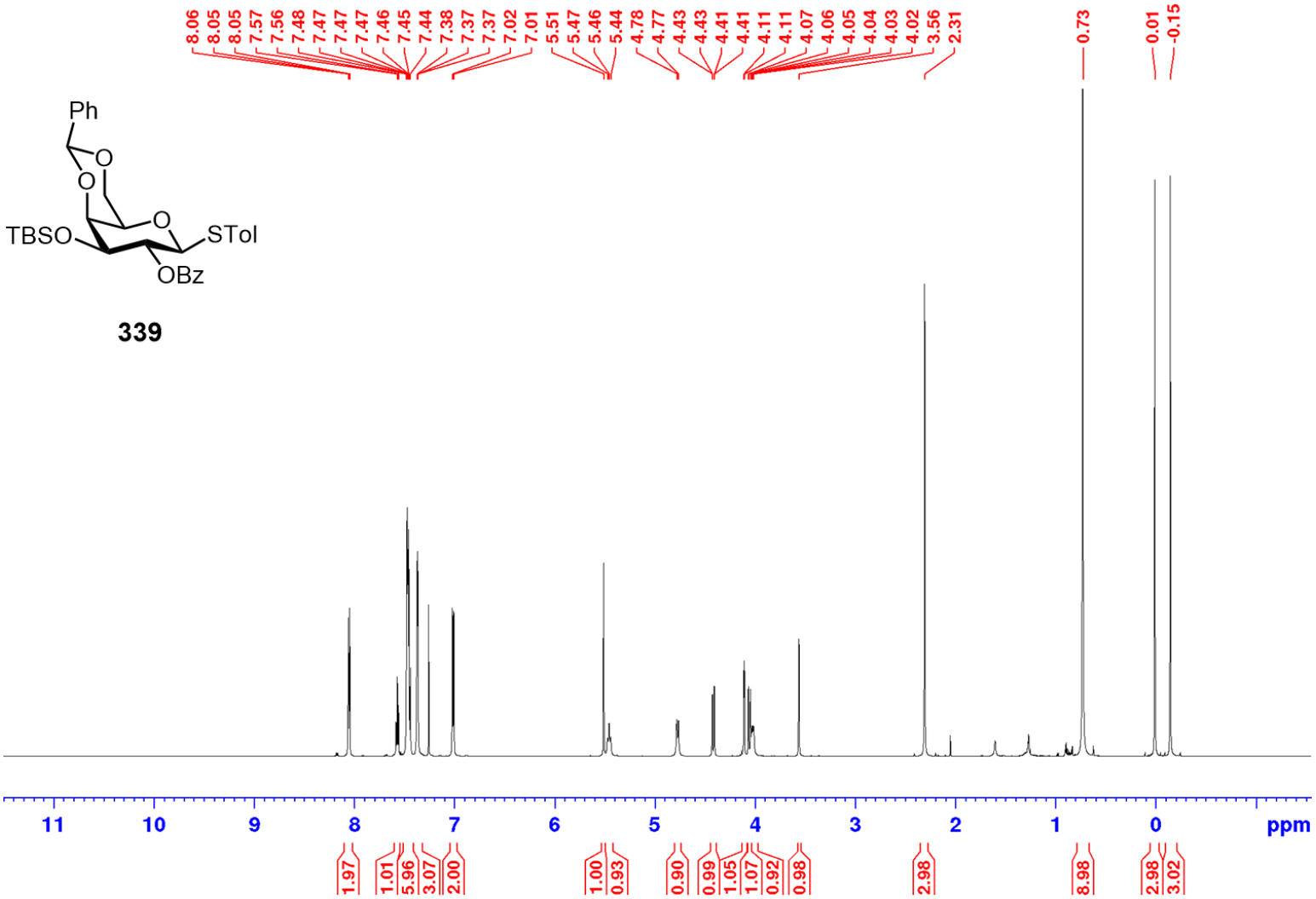


338 **339**

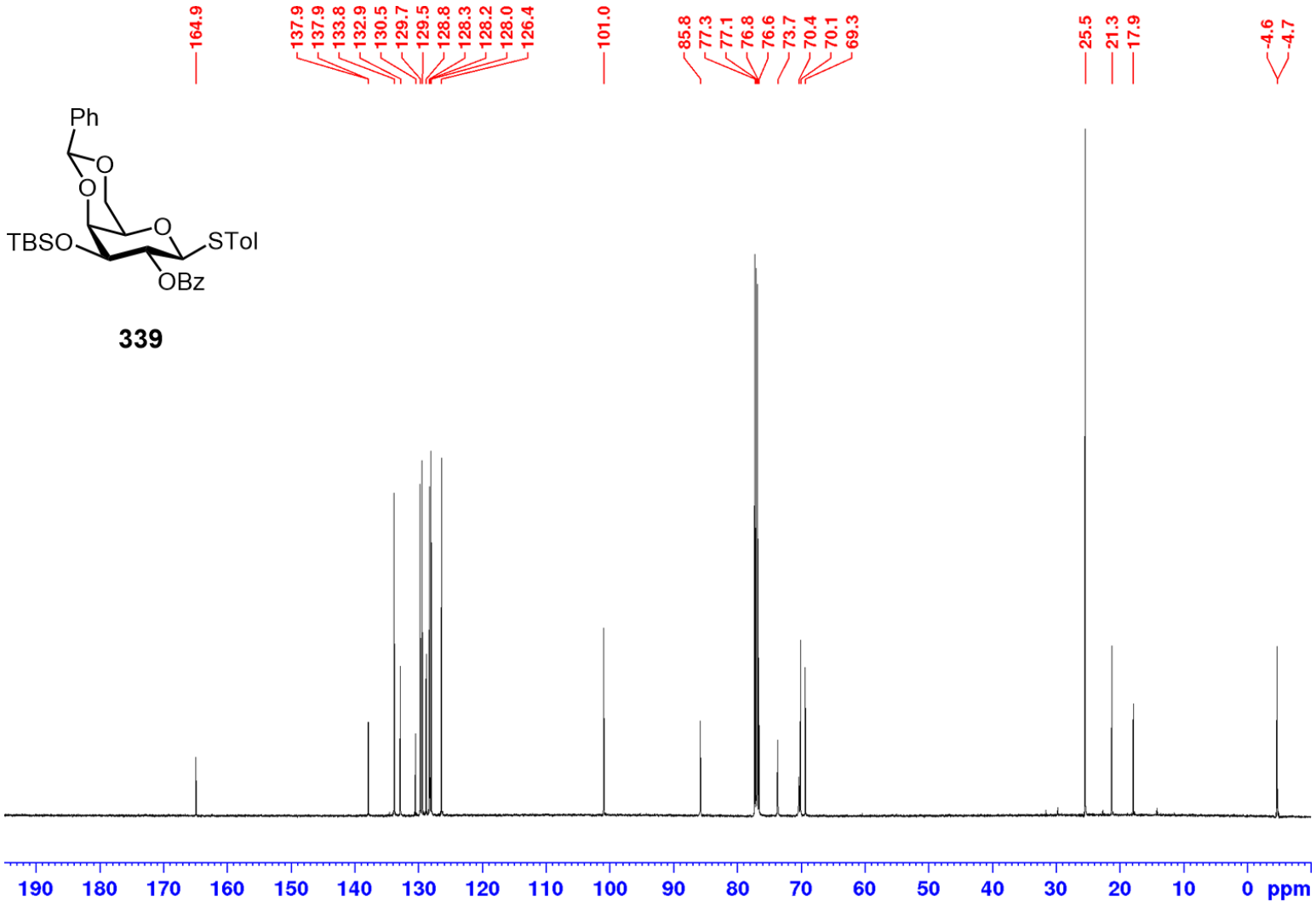
To a solution of thioglycoside *p*-tolyl 4,6-*O*-benzylidene acetal-3-*O*-*tert*-butyldimethylsilyl-1-thio-β-D-galactopyranoside **338** (1.0 equiv., 0.3576 g, 0.731 mmol), Et_3N (6.0 equiv., 0.6 mL, 4.38 mmol) in anhydrous CH_2Cl_2 (5.0 mL) and added benzoic anhydride (3.0 equiv., 0.486 g , 2.19 mmol) was added DMAP (0.5 equiv., 0.044 g, 0.038 mmol) under an argon atmosphere at 0 °C. The solution was stirred while warming to room temperature. At 18 h, crude NMR indicated that the starting material was completely consumed. The reaction was re-cooled to 0 °C and the excess benzoic anhydride was quenched by the addition of CH_3OH (5.0 mL). After 5 min at 0 °C, the crude reaction mixture was stirred at room for 5 min then transferred to a separatory funnel with EtOAc (100 mL). The reaction was washed with 1 N HCl (3 x 25 mL), satd. aq. $NaHCO_3$ (1 x 25 mL), brine (1 x 125 mL), dried ($NaSO_4$) and filtered through a glass fritted vacuum filter funnel to remove drying agent. The filtrate was concentrated to a crude oil which was purified by flash column chromatography (1:9 to 1:4 EtOAc/hexanes) then was crystalized from hot EtOAc and hexanes to give thioglycoside donor *p*-tolyl 4,6-*O*-benzylidene acetal-3-*O*-*tert*-butyldimethylsilyl-2-*O*-benzoyl-1-thio-β-D-galactopyranoside **339** (0.3326 g,

0.6510 mmol, 78%) as white crystals. The spectroscopic data agreed with previously reported data.[222, 365]

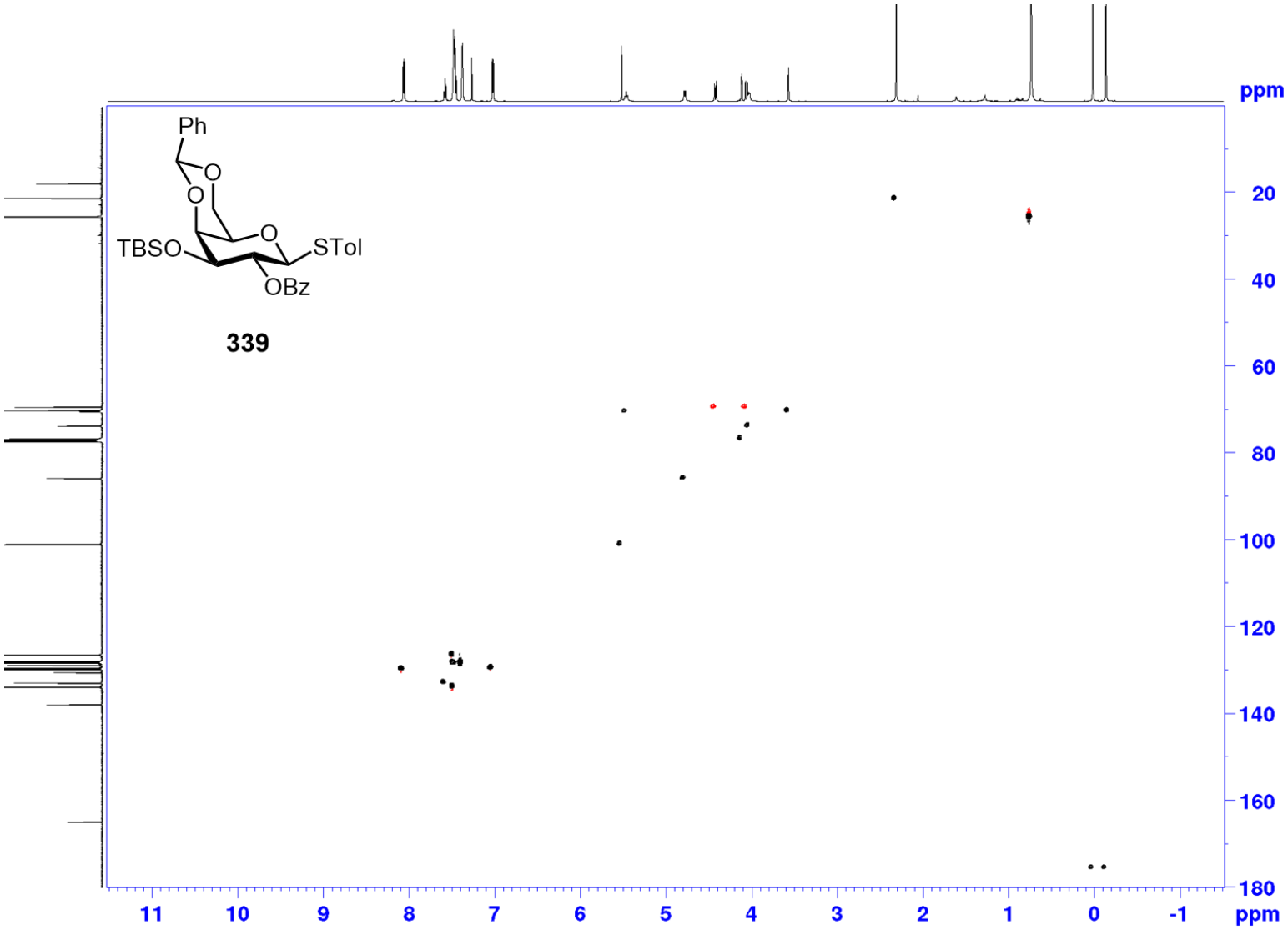
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 339



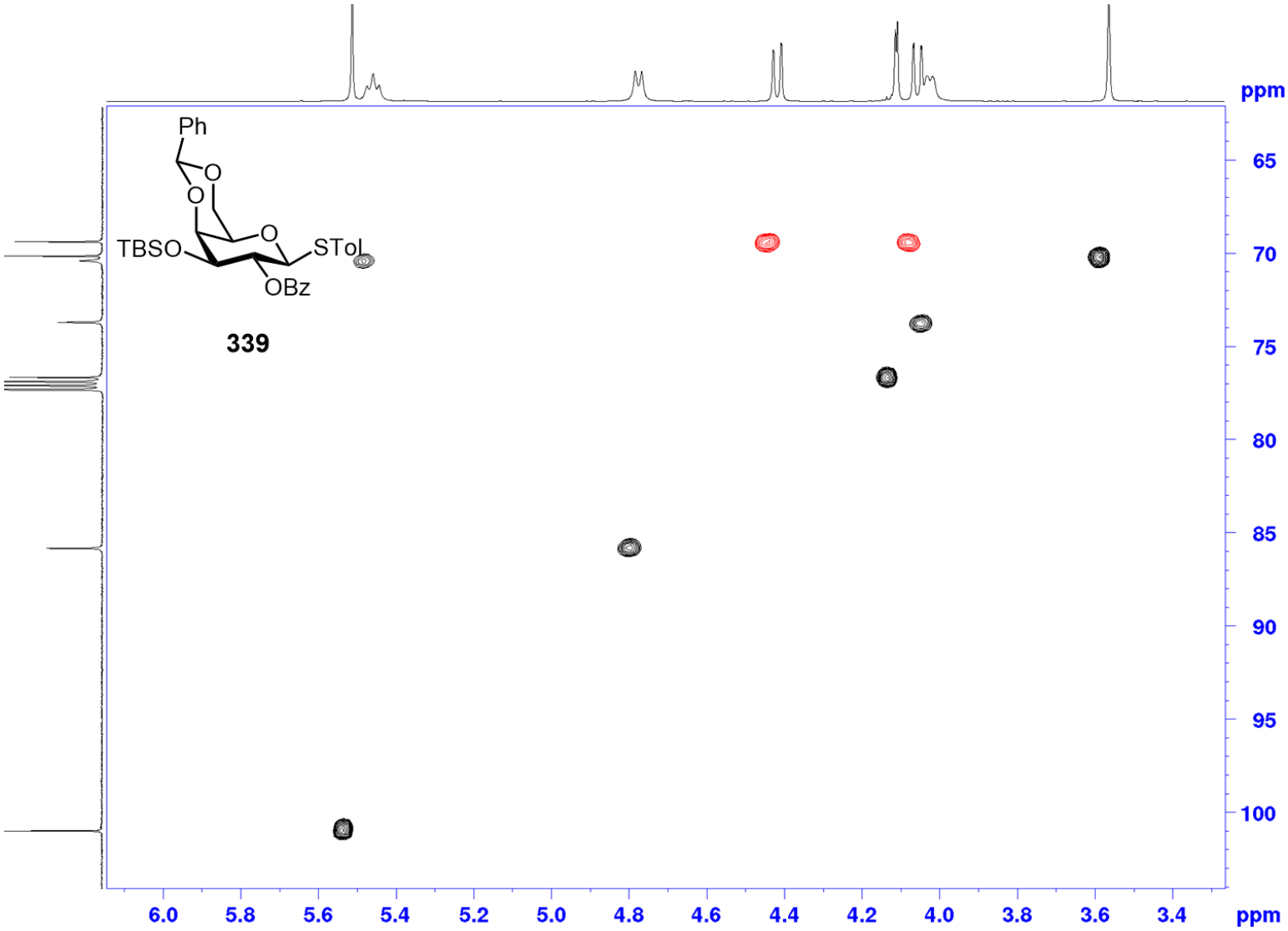
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 339



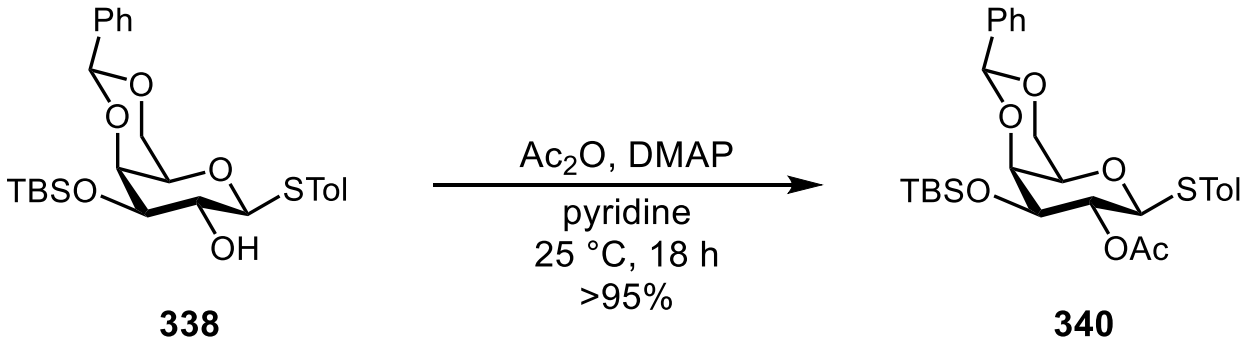
^{1}H-^{13}C HSCQ ($CDCl_3$) of compound 339



^{1}H-^{13}C HSCQ ($CDCl_3$) zoomed in region of compound 339



***p*-Tolyl 4,6-*O*-benzylidene acetal-3-*O*-*tert*-butyldimethylsilyl-2-*O*-acetyl-1-thio-β-D-galactopyranoside (340)**



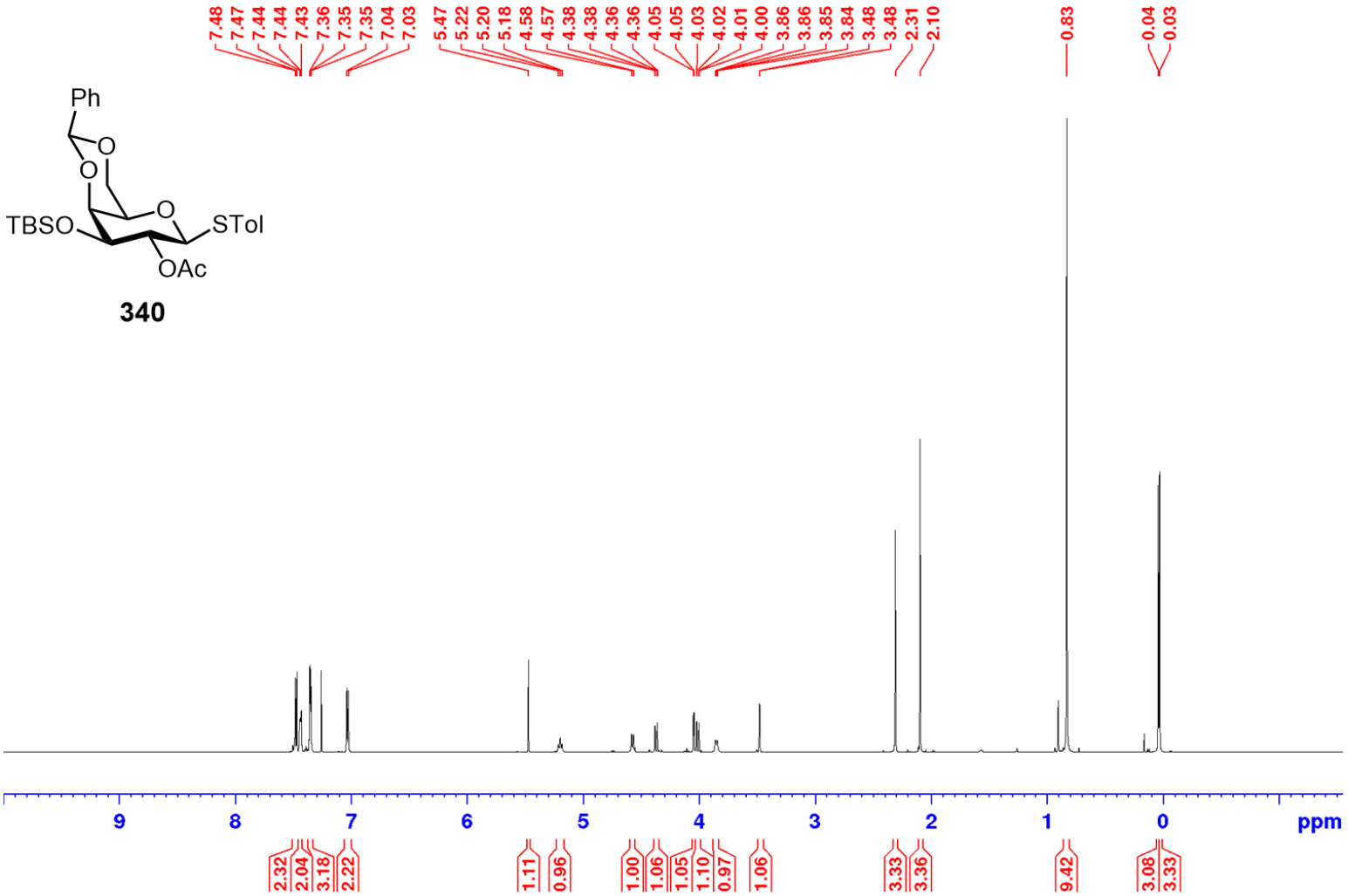
338 **340**

To a solution of *p*-tolyl 4,6-*O*-benzylidene acetal-3-*O*-*tert*-butyldimethylsilyl-1-thio-β-D-galactopyranoside **338** (1.0 equiv., 3.9 g, 8.01 mmol) in pyridine (15.0 equiv., 10 mL, 0.12 mmol) under argon was cooled to 0 °C and added acetic anhydride (13.0 equiv., 10 mL, 0.11 mmol) dropwise. After addition of DMAP (cat.), the solution was stirred at room temperature. Due to the co-polarity between the desired product and starting material, crude NMR was used to monitor the reaction progression. At 12 h, crude NMR indicated that the starting material was completely consumed. The reaction was cooled to 0 °C and the excess acetic anhydride was quenched by the addition of CH_3OH (5.0 mL). After 5 min at 0 °C, the crude reaction mixture was stirred at room temperature for 5 min, then transferred to a separatory funnel with EtOAc (100 mL). The reaction was washed with 1 N HCl (3 x 25 mL), satd. aq. $NaHCO_3$ (1 x 25 mL), brine (1 x 125 mL), dried ($NaSO_4$) and filtered through a glass fritted vacuum filter funnel to remove drying agent. The filtrate was concentrated to a crude oil which was purified by flash column chromatography (10:90 to 20:80 EtOAc/hexanes) then was crystalized from hot EtOAc and hexanes to give thioglycoside donor *p*-tolyl 4,6-*O*-benzylidene acetal-3-*O*-*tert*-butyldimethylsilyl-2-*O*-

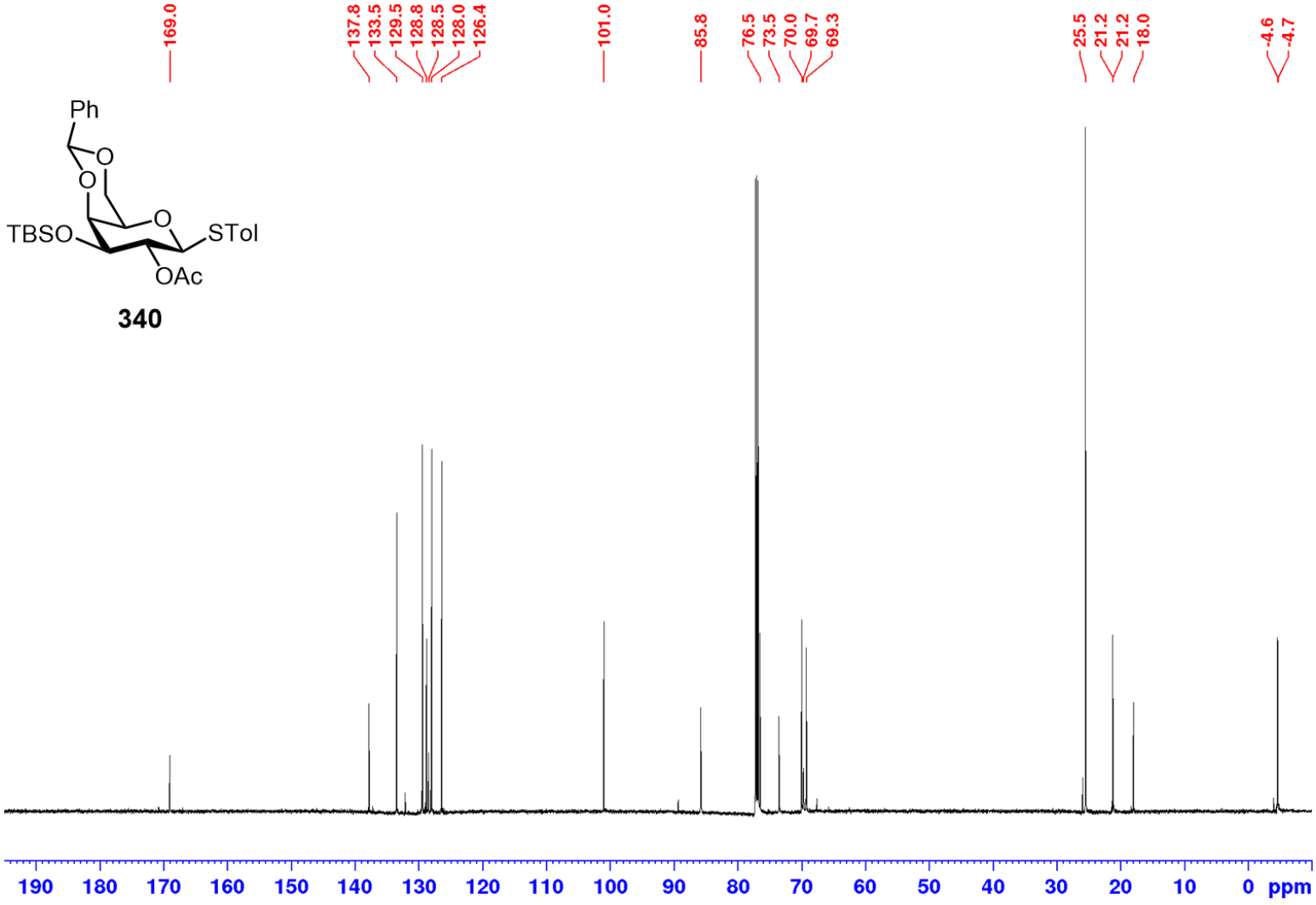
acetyl-1-thio-β-D-galactopyranoside **340** (3.4568 g, 6.51 mmol, 81%) as white crystals. The spectroscopic data were in agreement with the reported data.[362]

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.49 (d, *J* = 7.9 Hz, 2H), 7.44-7.43 (m, 2H), 7.36-7.35 (m, 3H), 7.03 (d, *J* = 7.9 Hz, 2H), 5.47 (s, 1H), 5.20 (dd, *J* = 0.2, 9.1 Hz, 1H), 4.58 (d, *J* = 9.6 Hz, 1H), 4.37 (dd, *J* = 1.5, 12.3 Hz, 1H), 4.05 (d, *J* = 3.5 Hz, 1H), 4.01 (dd, *J* = 1.8, 12.5 Hz, 1H), 3.86 (dd, *J* = 2.8, 9.5 Hz, 1H), 3.47 (s, 1H), 2.31 (s, 3H), 2.09 (s, 3H), 0.83 (s, 9H), 0.05 (s, 3H), 0.02 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.0, 137.8, 133.5, 129.5, 128.8, 128.5, 128.0, 126.4, 101.0, 85.8, 76.5, 73.5, 70.0, 69.7, 69.3, 25.5, 21.2, 21.2, 18.0, -4.6, -4.7.

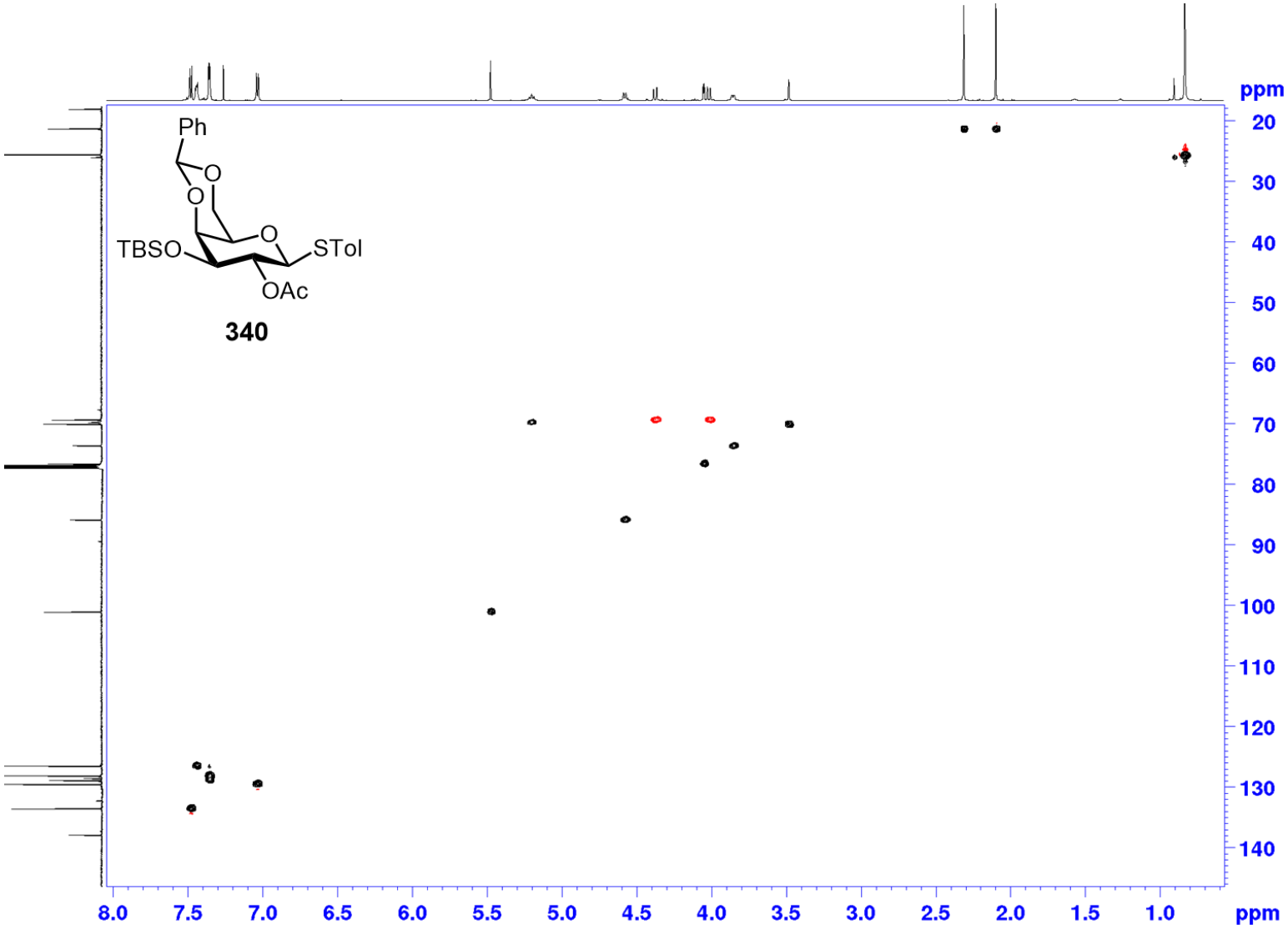
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 340



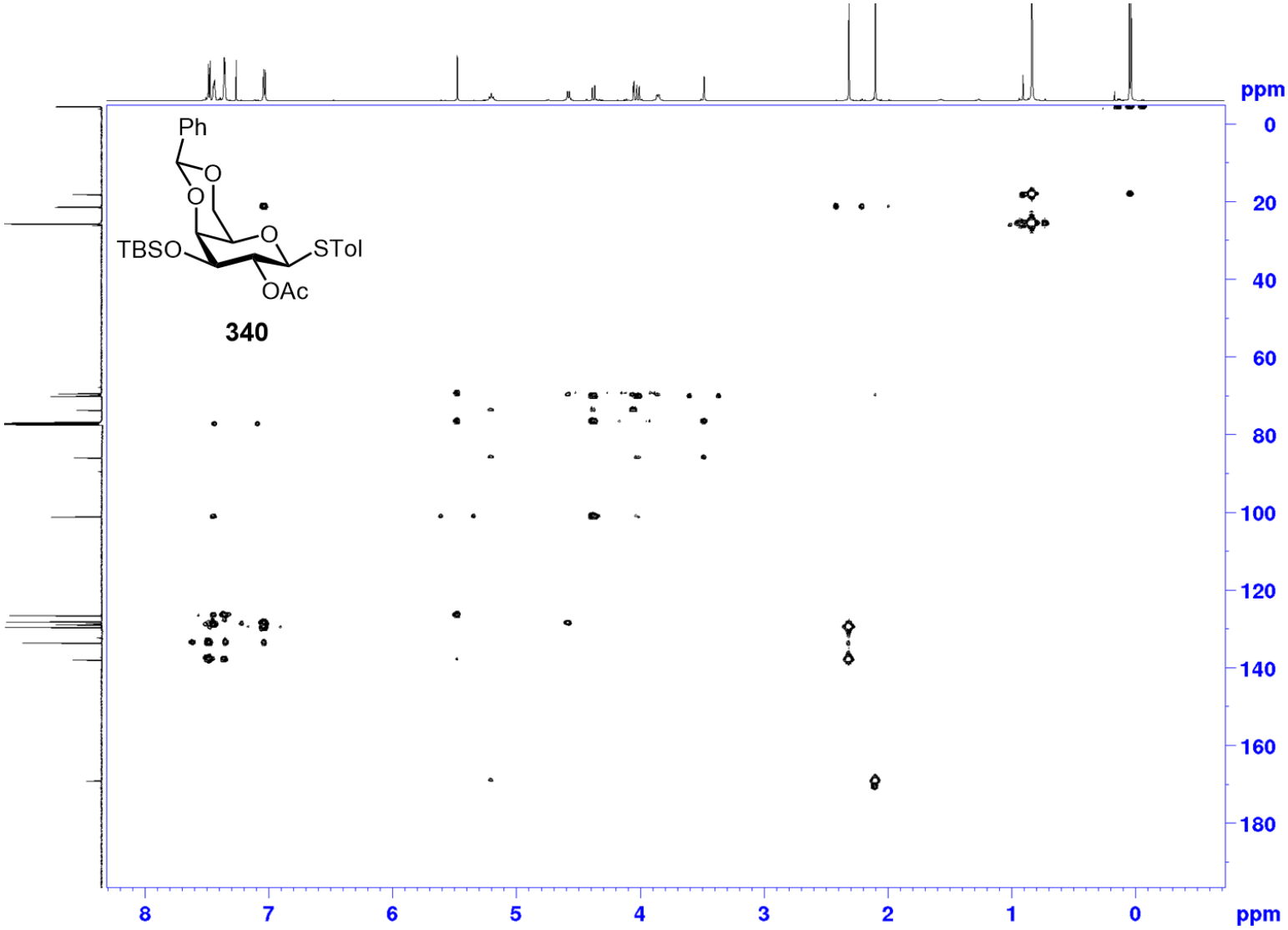
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 340



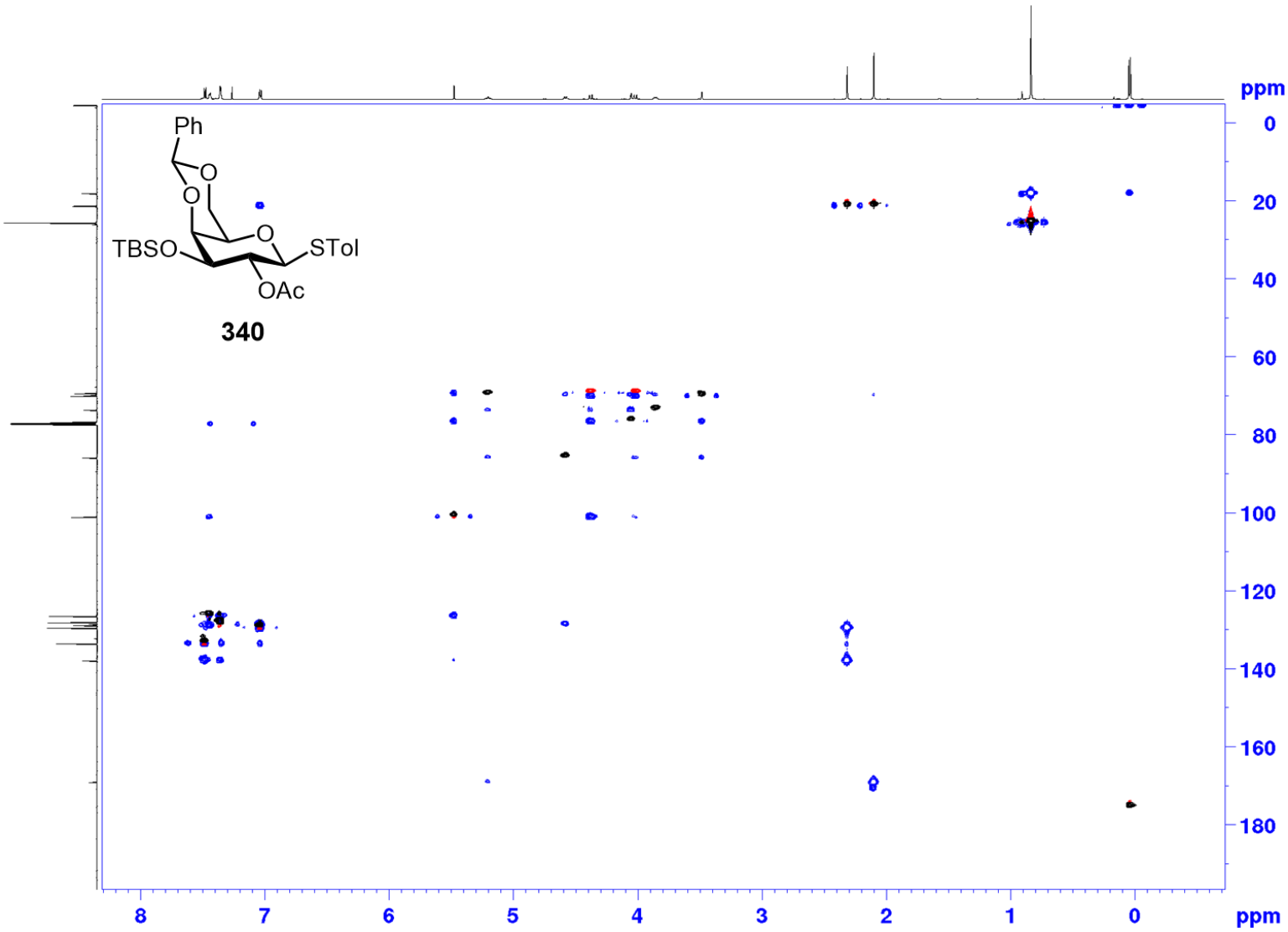
^{1}H-^{13}C HSCQ ($CDCl_3$) of compound 340



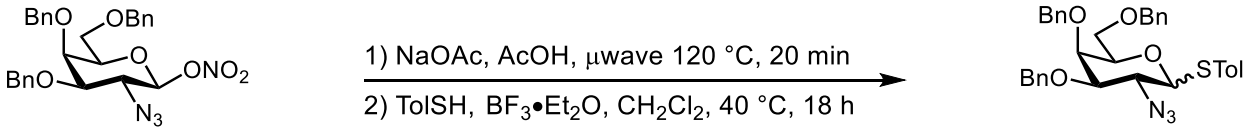
^{1}H-^{13}C HMBC ($CDCl_3$) of compound 340



^{1}H-^{13}C HSCQ with ^{1}H-^{13}C HMBC overlay ($CDCl_3$) of compound 340



_p_-tolyl 3,4,6-tri-_O_-benzyl-2-deoxy-2-azido-1-thio-α/β-D-galactopyranoside (342)



197 **342**

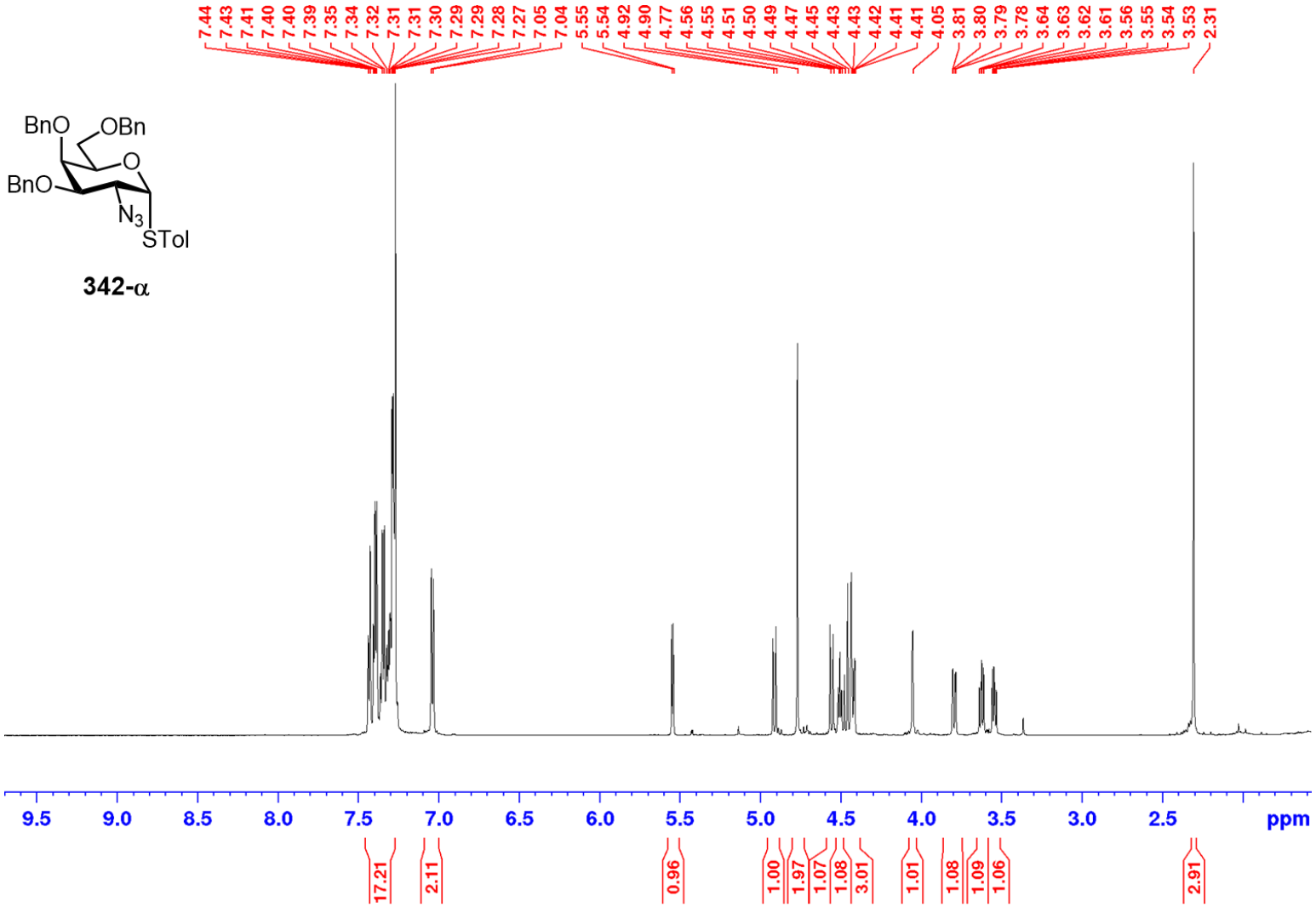
A solution of 3,4,6-tri-*O*-benzyl-2-deoxy-2-azido-α/β-D-galactopyranosyl nitrate ester **197** (1.0 equiv., 1.28 g, 2.46 mmol) and sodium acetate (3.0 equiv., 0.605 g, 7.38 mmol) in AcOH (20 mL) was heated by microwave irradiation to 120 °C for 25 min. The reaction mixture was transferred to a separatory funnel with additional EtOAc (50 mL). The organics were washed with sat. aq. $NaHCO_3$ (2 x 10 mL), brine (1 x 10 mL), dried ($NaSO_4$), filtered through a glass fritted vacuum filter funnel to remove drying agent. The filtrate was concentrated to a yellow oil. The crude oil was purified by silica gel flash column chromatography (10:90 to 30:70 EtOAc/hexanes) to give an inseparable mixture of anomers of the acetyl 3,4,6-tri-*O*-benzyl-2-deoxy-2-azido-D-α/β-galactopyranoside **341** to be carried on directly to the next step. A solution of acetyl 3,4,6-tri-*O*-benzyl-2-deoxy-2-azido-D-α/β-galactopyranoside **341** and *p*-thiocresol (2.0 equiv., 0.458 g, 3.69 mmol) in dry CH_2Cl_2 (20 mL) was added $BF_3{\cdot}Et_2O$ (2.5 equiv., 0.45 mL, 3.69 mmol) dropwise at room temperature under an argon atmosphere. The reaction was stirred and heated to 40 °C. At 18 h, TLC indicated complete consumption of starting material. The crude reaction mixture was quenched by the addition of satd. aq. $NaHCO_3$ (100 mL) to the reaction flask. After stirring for 30 min the biphasic mixture was transferred to a separatory funnel and the organics were separated and the aqueous layer was extracted with CH_2Cl_2 (3 x 10 mL). The organics were combined and dried ($NaSO_4$), filtered through a glass fritted vacuum filter funnel to remove drying agent. The filtrate was concentrated to an oil.

The crude oil was purified by silica gel flash column chromatography (10:90 to 30:70 EtOAc/hexanes) to give an mixture of anomers of *p*-tolyl 3,4,6-tri-*O*-benzyl-2-deoxy-2-azido-1-thio-α/β-D-galactopyranoside **342** (1.02 g, 1.75 mmol, 71%) as an off white solid. Our characterization data were consistent with previously reported data.[366-370]

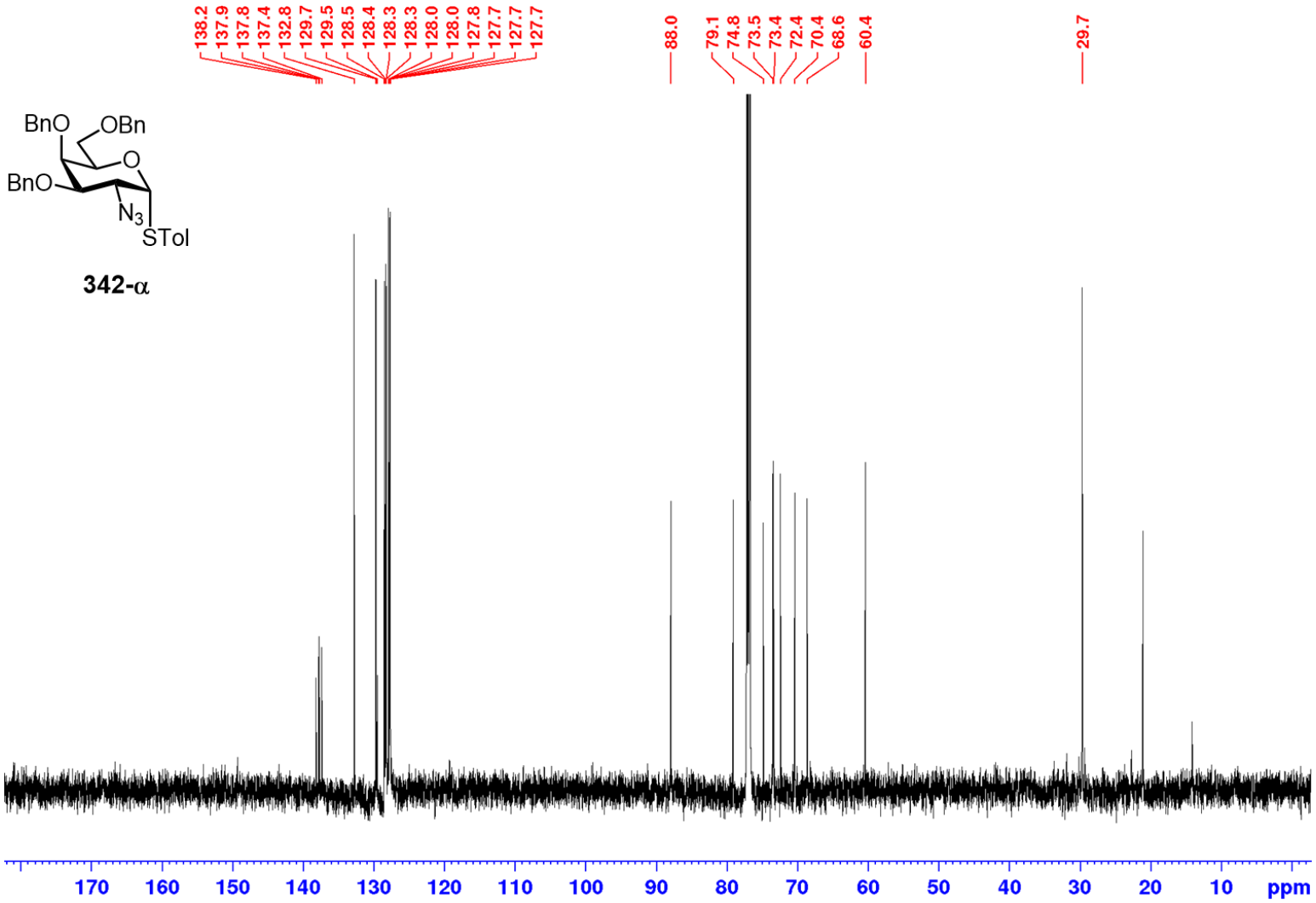
^{1}H NMR (600 MHz, $CDCl_3$): δ 7.42-7.28 (m, 17H), 7.05 (d, *J* = 7.9 Hz, 2H), 5.54 (d, *J* = 5.4 Hz, 1H), 4.92 (d, *J* = 11.4 Hz, 1H), 4.77 (s, 2H), 4.56 (d, *J* = 11.2 Hz, 1H), 4.51 (dd, *J* = 6.2, 7.3 Hz, 1H), 4.47-4.41 (m, 3H), 4.06 (d, *J* = 2.1 Hz, 1H), 3.81 (dd, *J* = 2.5, 10.4 Hz, 1H), 3.62 (dd, *J* = 7.1, 9.3 Hz, 1H), 3.55 (dd, *J* = 6.0, 9.1 Hz, 1H), 2.31 (s, 3H);

^{13}C NMR (100 MHz, $CDCl_3$): δ 138.2, 137.8, 137.7, 137.3, 132.8, 129.7, 129.5, 128.5, 128.3, 128.3, 128.2, 127.9, 127.9, 127.8, 127.7, 127.7, 127.6, 87.9, 79.1, 74.8, 73.4, 73.4, 72.4, 70.4, 68.6, 60.4, 29.6.

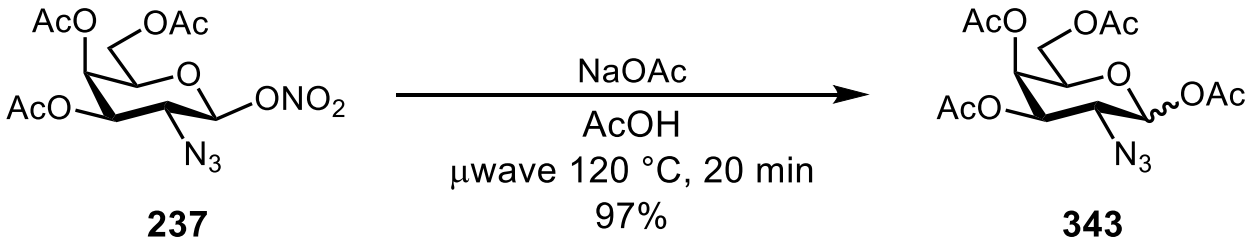
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 342-α



^{13}C NMR ($CDCl_3$, 100 MHz) of compound 342-α



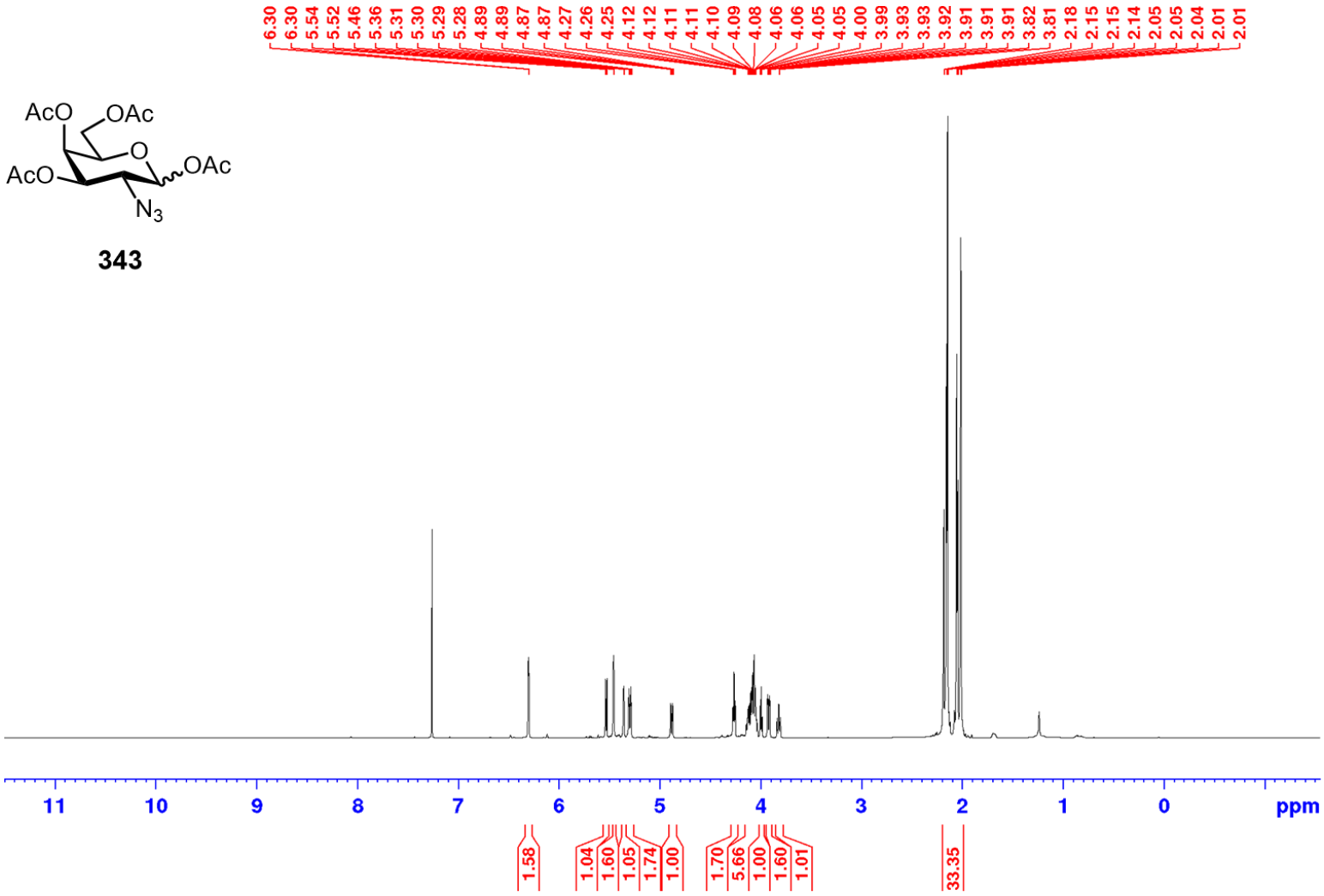
1,3,4,6-tetra-*O*-acyl-2-deoxy-2-azido-D-α/β-galactopyranoside (343)



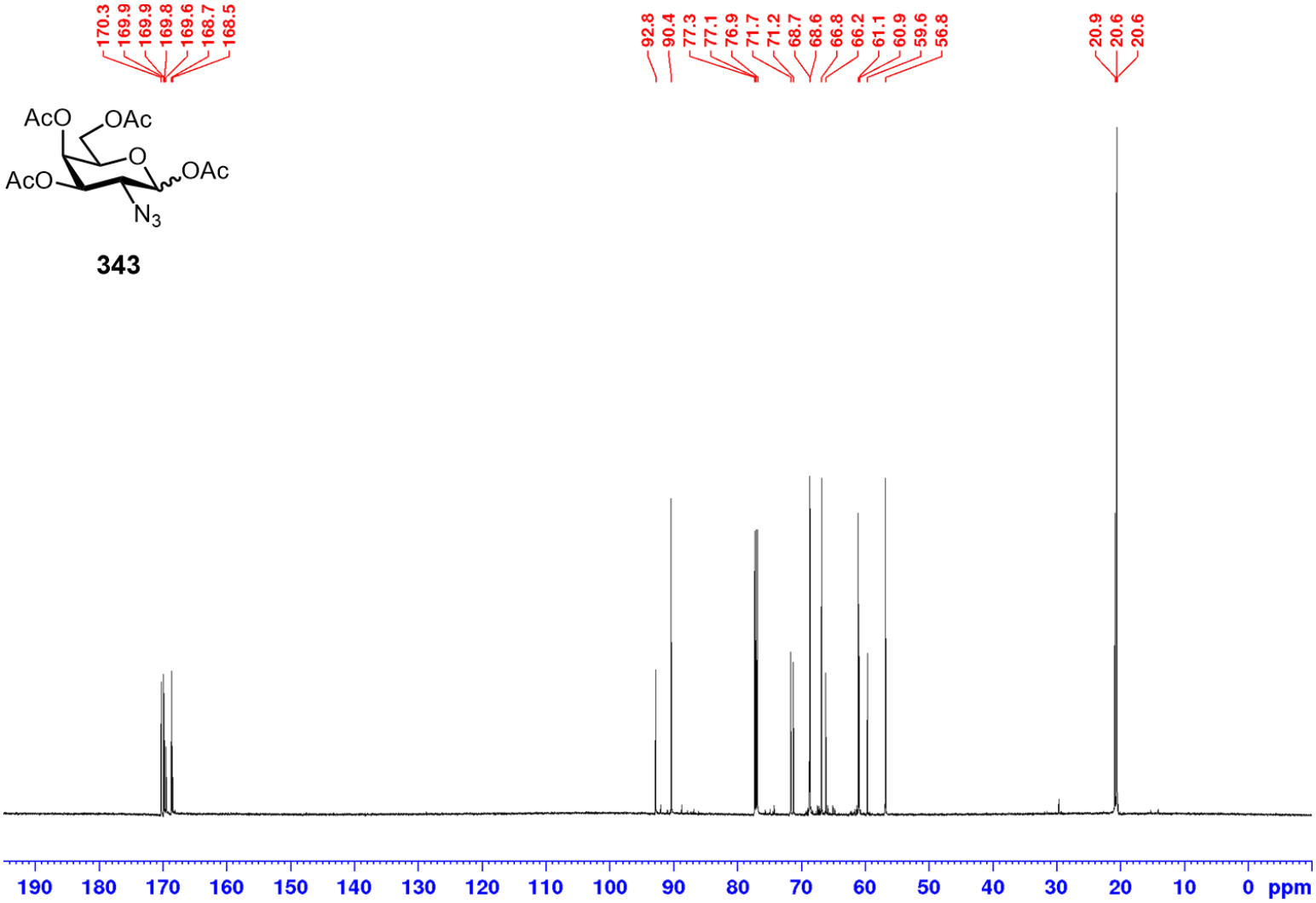
237 **343**

A solution of 3,4,6-tri-*O*-acetyl-2-deoxy-2-azido-α/β-D-galactopyranosyl nitrate ester **237** (1.0 equiv., 2.63 g, 7.00 mmol) and sodium acetate (3.0 equiv., 1.72 g, 21.0 mmol) in AcOH (18 mL) was heated by microwave irradiation to 120 °C for 25 min. The reaction mixture was transferred to a separatory funnel with additional EtOAc (50 mL). The organics were washed with sat. aq. $NaHCO_3$ (2 x 10 mL), brine (1 x 10 mL), dried ($NaSO_4$), filtered through a glass fritted vacuum filter funnel to remove drying agent. The filtrate was concentrated to a yellow oil. The crude oil was purified by silica gel flash column chromatography (25:75 to 40:60 EtOAc/hexanes) to give an inseparable mixture of anomers of the 1,3,4,6-tetra-*O*-acyl-2-deoxy-2-azido-D-α/β-galactopyranoside **343** (2.5451 g, 6.8175 mmol, >95%) as a yellow solid. Our characterization data were consistent with previously reported data.[313]

^{1}H NMR ($CDCl_3$, 400 MHz) of compound 343

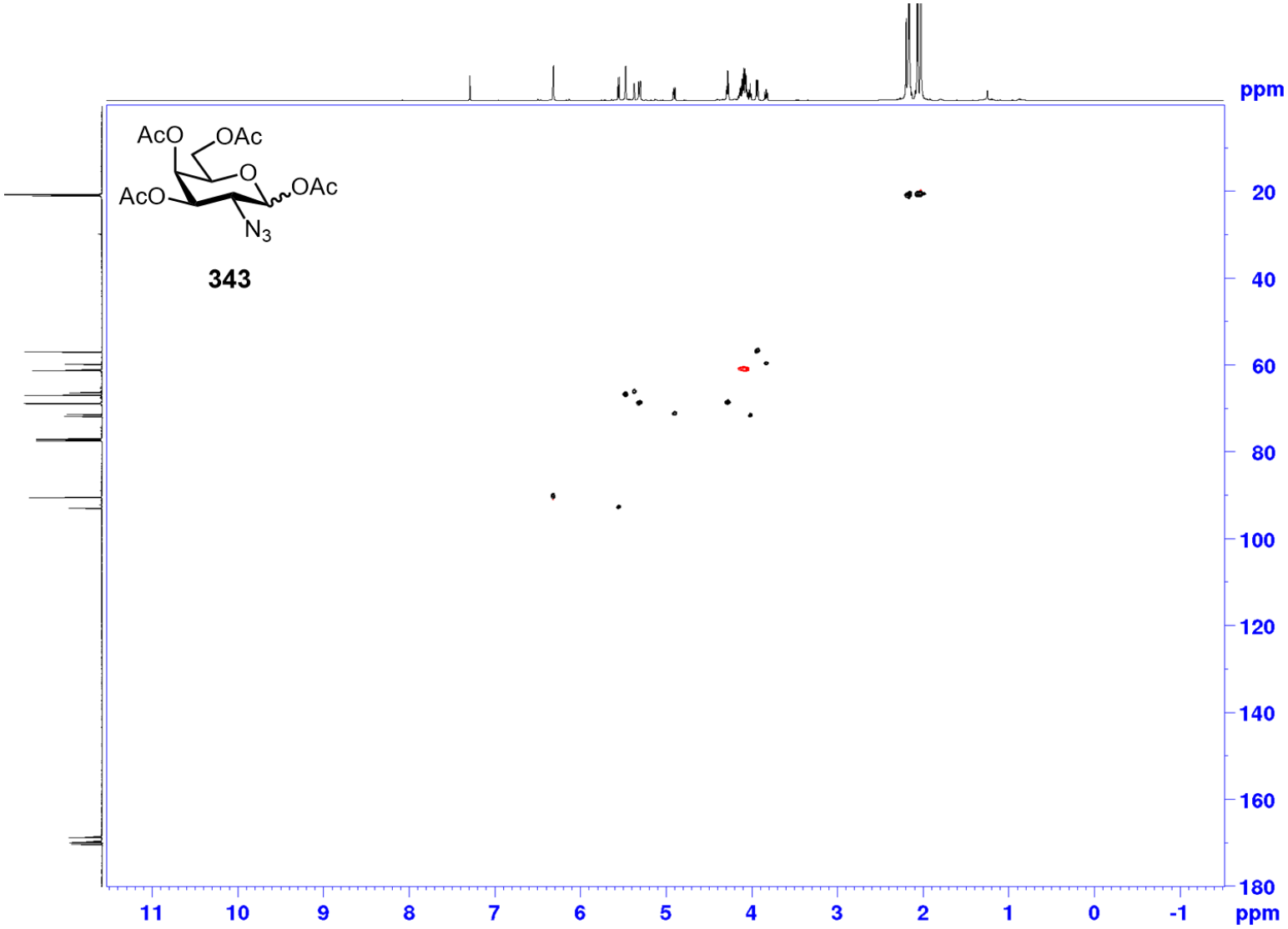


^{13}C NMR ($CDCl_3$, 100 MHz) of compound 343

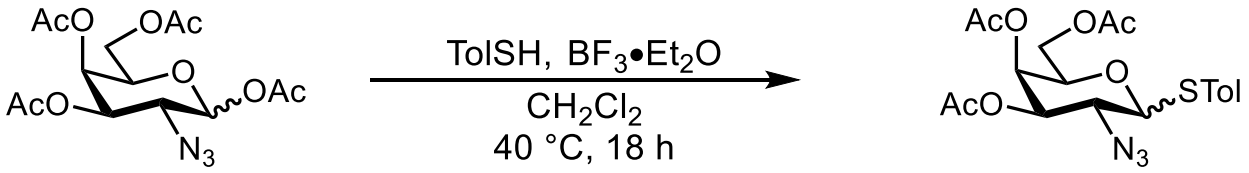


343

^{1}H-^{13}C HSCQ ($CDCl_3$) of compound 343



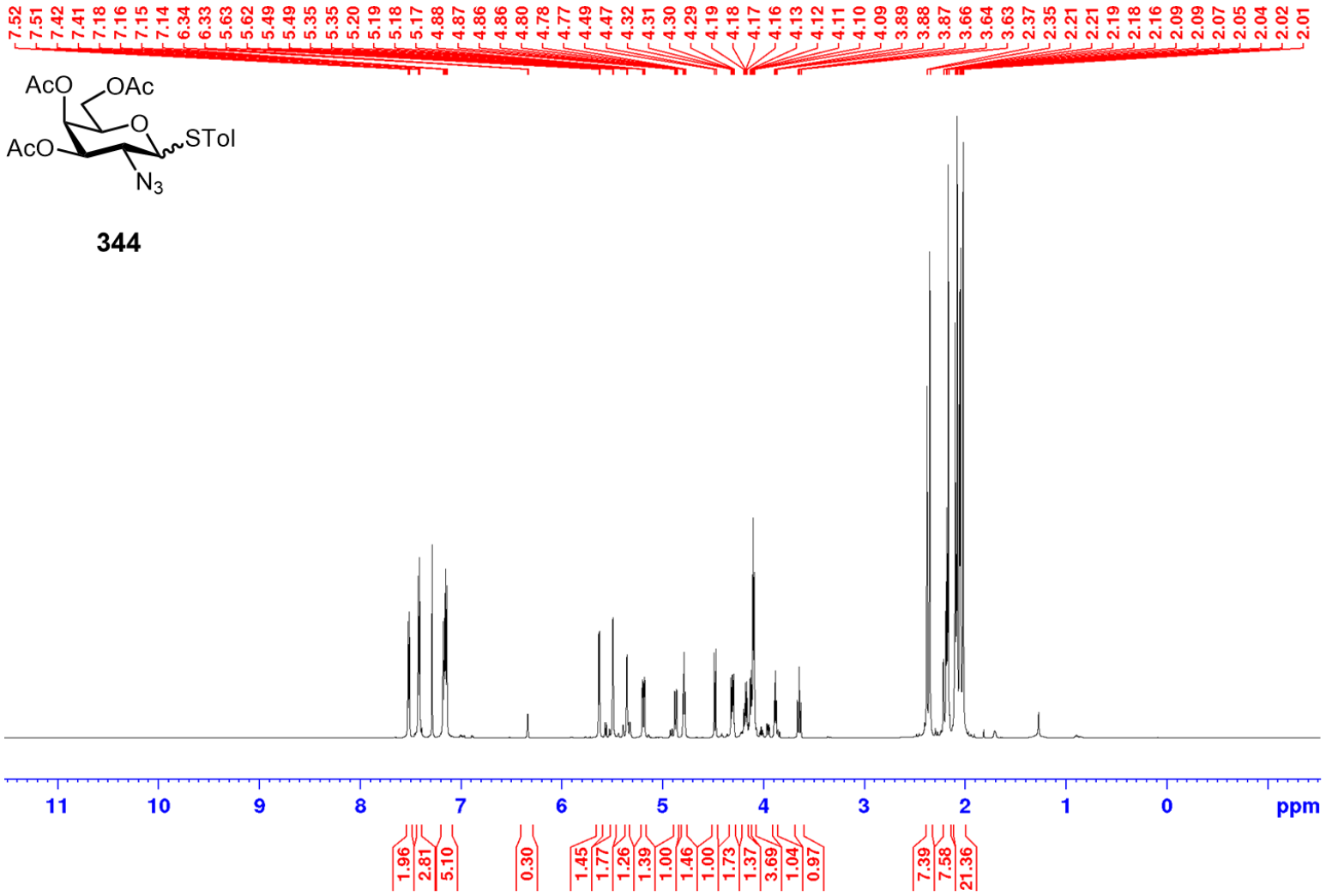
***p*-tolyl 3,4,6-tri-*O*-acyl-2-deoxy-2-azido-1-thio-α/β-D-galactopyranoside (344)**



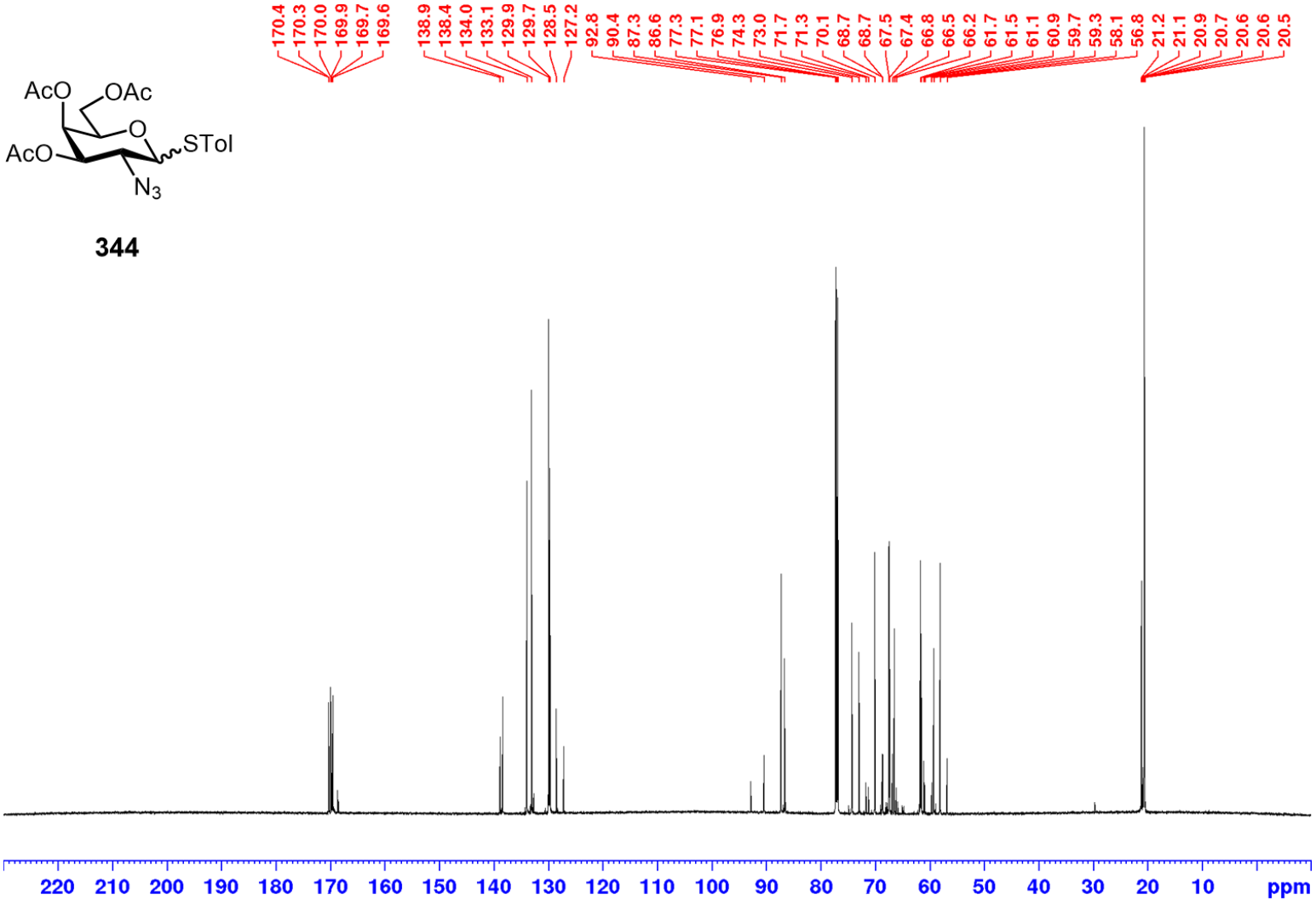
343 **344**

A solution of 1,3,4,6-tetra-*O*-acyl-2-deoxy-2-azido-D-α/β-galactopyranoside **343** (1.0 equiv., 2.448 g, 6.557 mmol) and *p*-thiocresol (2.0 equiv., 1.63 g, 13.1 mmol) in anhydrous CH_2Cl_2 (60 mL) was added $BF_3{\cdot}Et_2O$ (2.5 equiv., 2.0 mL, 16.4 mmol) dropwise at room temperature under an argon atmosphere. The reaction was stirred and heated to 40 °C. At 18 h, TLC indicated complete consumption of starting material. The crude reaction mixture was quenched by the addition of satd. aq. $NaHCO_3$ (100 mL) to the reaction flask. After stirring for 30 min the biphasic mixture was transferred to a separatory funnel and the organics were separated and the aqueous layer was extracted with CH_2Cl_2 (3 x 10 mL). The organics were combined and dried ($NaSO_4$), filtered through a glass fritted vacuum filter funnel to remove drying agent. The filtrate was concentrated to an oil. The crude oil was purified by silica gel flash column chromatography (20:80 to 30:70 EtOAc/hexanes) to give an inseparable mixture of anomers of *p*-tolyl 3,4,6-tri-*O*-acyl-2-deoxy-2-azido-1-thio-α/β-D-galactopyranoside **344** (2.0417 g, 4.66 mmol, 71%) as an off white solid. Our characterization data were consistent with previously reported data.[366, 371-375]

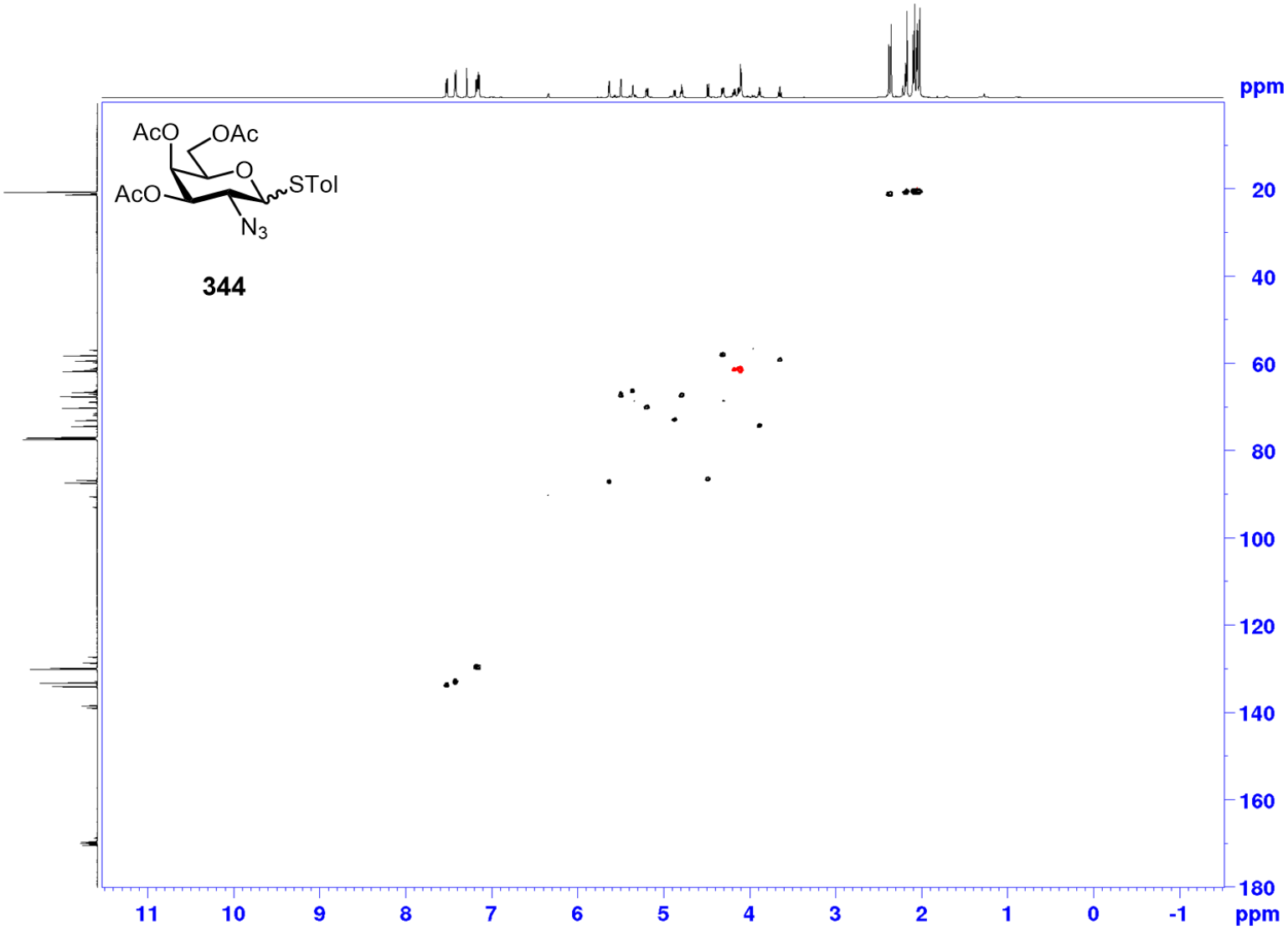
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 344



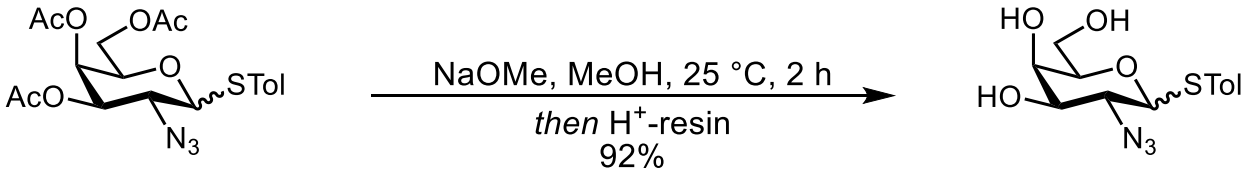
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 344



^{1}H-^{13}C HSCQ ($CDCl_3$) of compound 344



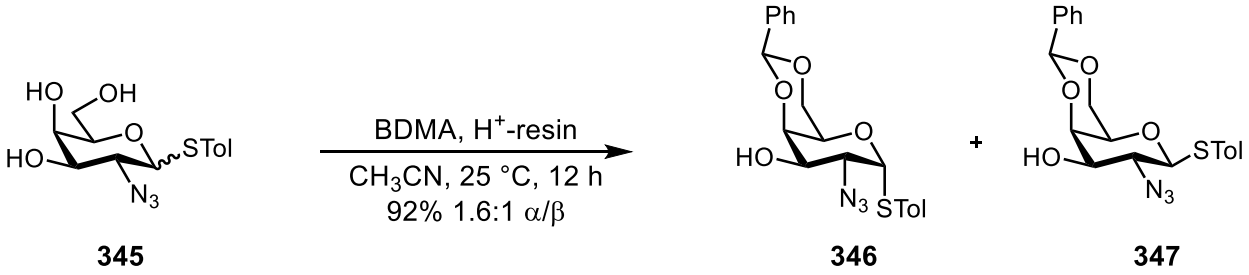
***p*-tolyl 2-deoxy-2-azido-1-thio-α/β-D-galactopyranoside (345)**



344 **345**

At a solution of *p*-tolyl 3,4,6-tri-*O*-acyl-2-deoxy-2-azido-1-thio-α/β-D-galactopyranoside **344** (1.0 equiv., 5.302 g, 12.12 mmol) in anhydrous CH_3OH (60 mL) was treated with a freshly made solution of sodium methoxide (1.0 equiv., 3.0 mL, 12.12 mmol, [2 N] in anhydrous CH_3OH) at room temperature under an argon atmosphere. The pH of the solution was >11. At 2 h, TLC indicated complete consumption of starting material for a more polar product. The reaction was neutralized by the addition of acidic form Amberlite IR 120 (H^+) ion exchange resin. The solution was filtered through a glass fritted vacuum filter funnel equipped with a pad of Celite to remove the acidic resin. The filtrate was concentrated to dryness to give a crude triol. The crude material was passed through a plug of silica (70:30 to 85:15 EtOAc/hexanes) to give *p*-tolyl 2-deoxy-2-azido-1-thio-α/β-D-galactopyranoside **345** (3.472 g, 11.15 mmol, 92%) as an off white solid. Our spectroscopic data agreed with previously reported data.[366, 371, 376-381]

***p*-tolyl 4,6-*O*-benzylidene acetal-2-deoxy-2-azido-1-thio-α/β-D-galactopyranoside (346 and 347)**



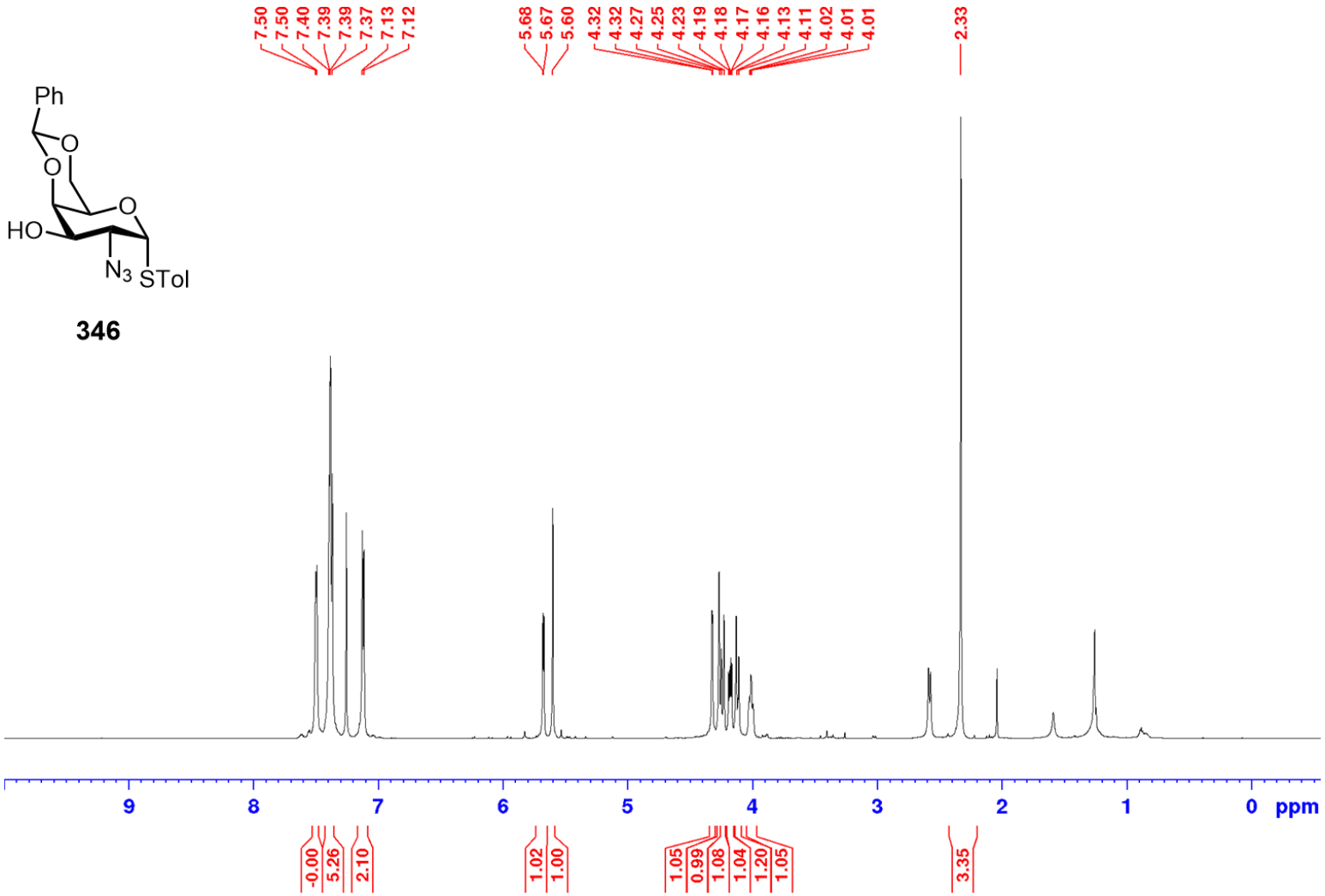
345 **346** **347**

A solution of *p*-tolyl 2-deoxy-2-azido-1-thio-α/β-D-galactopyranoside **S31** (1.0 equiv., 2.51 g, 8.05 mmol) and benzaldehyde dimethyl acetal (1.5 equiv., 1.82 mL, 12.1 mmol) in anhydrous CH_3CN (50 mL) was stirred at room temperature under an argon atmosphere. The pH of the reaction mixture was adjusted to < 4 by the addition of acidic Amberlite IR 120 (H^+) ion exchange resin at room temperature under an argon atmosphere. At 12 h, TLC indicated the triol starting material had been completely consumed in exchange for less polar spots. The crude reaction mixture filtered through a glass fritted vacuum filter funnel equipped with a pad of Celite to remove the acidic resin. The filtrate was further neutralized by the addition of triethylamine and crude mixture was concentrated down then purified by flash chromatography (25:75 to 40:60 EtOAc/Hexanes) to give anomericly pure *p*-tolyl 4,6-*O*-benzylidene acetal-2-deoxy-2-azido-1-thio-α-D-galactopyranoside **346** (1.82 g, 4.56 mmol, 57%) as a white solid and *p*-tolyl 4,6-*O*-benzylidene acetal-2-deoxy-2-azido-1-thio-β-D-galactopyranoside **347** (1.12 g, 2.80 mmol, 35%) as a white solid. Both, alpha and beta, could be further purified by crystallization from hot EtOAc/hexanes. The spectroscopic data collected were in agreement with the reported data.[366, 371, 374, 376, 378-379, 382-384]

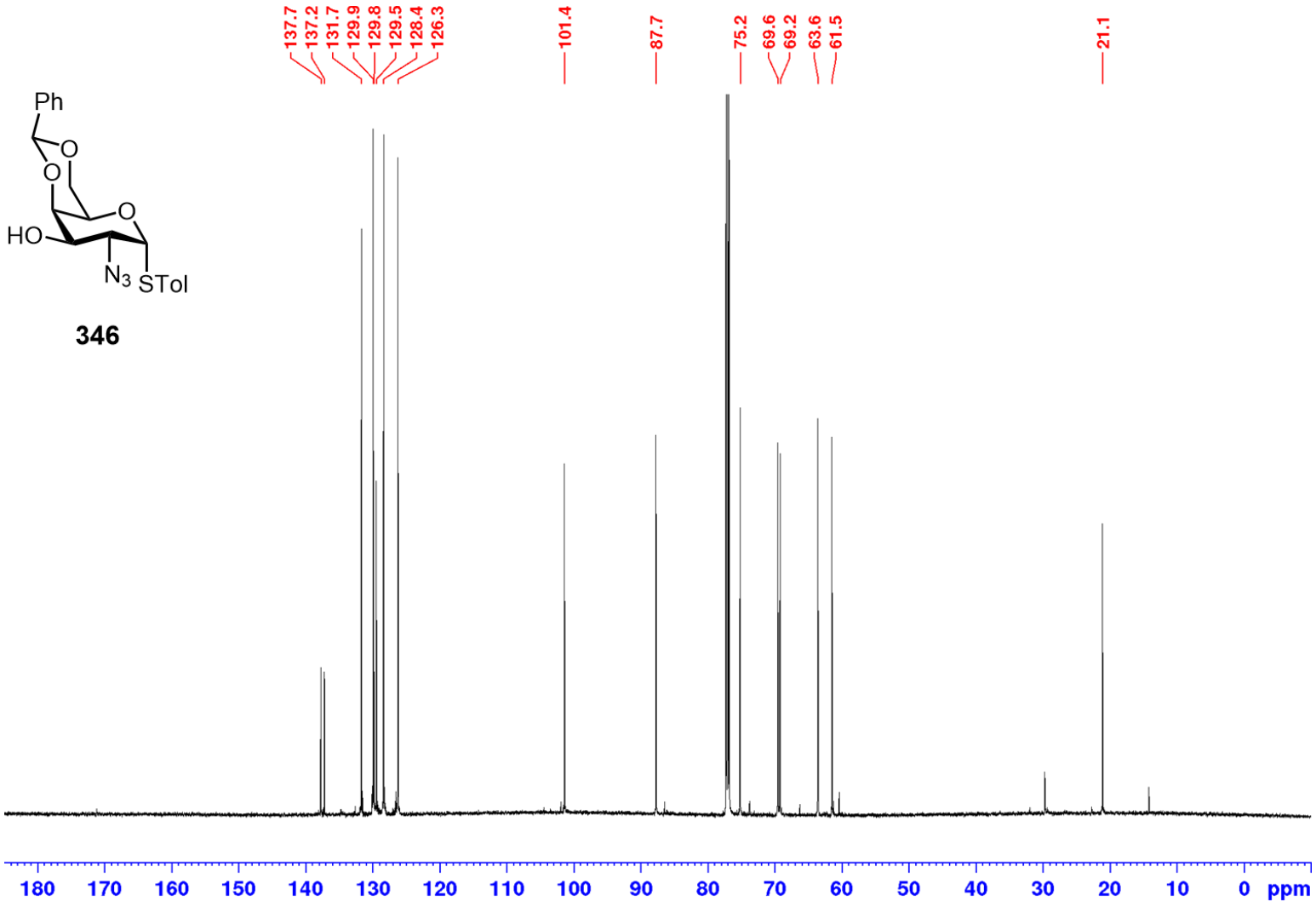
346: ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.64 (d, *J* = 8.1 Hz, 2H), 7.41-7.38 (m, 5H), 7.1 (d, *J* = 7.6 Hz, 2H), 5.53 (s, 1H), 4.40-4.36 (m, 2H), 4.18 (d, *J* = 2.9 Hz, 1H), 4.03 (dd, *J* = 1.2, 12.7 Hz, 1H), 3.63 (ddd, *J* = 3.4, 9.5, 12.8 Hz, 1H), 3.51-3.47 (m, 2H), 2.51 (d, -OH, *J* = 9.7 Hz, 1H), 2.37 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 138.7, 137.3, 134.8, 129.7, 129.5, 128.2, 126.5, 126.3, 101.4, 85.0, 74.4, 73.2, 69.8, 69.2, 62.0, 21.2.

347: ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.5049 (d, *J* = 4.3 Hz, 2H), 7.1269 (d, *J* = 7.7 Hz, 2H), 5.6927 (d, *J* = 5.1 Hz, 1H), 5.6141 (s, 1H), 4.3244 (d, *J* = 3.3 Hz, 1H), 4.2714 (s, 1H), 4.237 (d, *J* = 12.5 Hz, 1H), 4.1797 (dd, *J* = 5.3, 10.3 Hz, 1H), 4.118 (d, *J* = 11.8 Hz, 1H), 4.02-4.01 (m, 1H), 2.5852 (d, -OH, *J* = 9.6 Hz, 1H), 2.3146 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 137.7, 137.2, 131.7, 130.0, 129.8, 129.5, 128.4, 126.3, 101.4, 87.7, 75.2, 69.6, 69.2, 63.6, 61.5, 21.1.

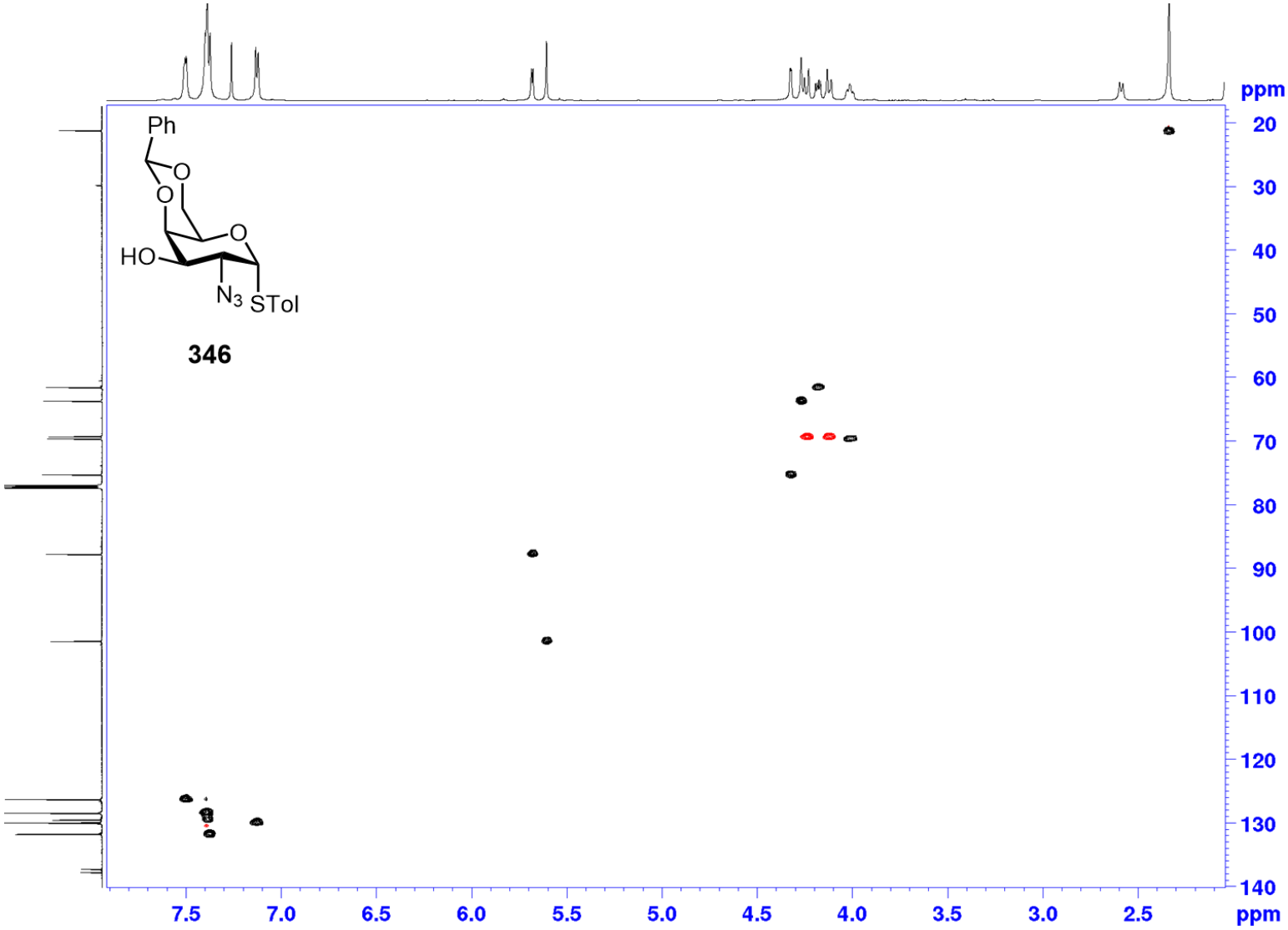
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 346



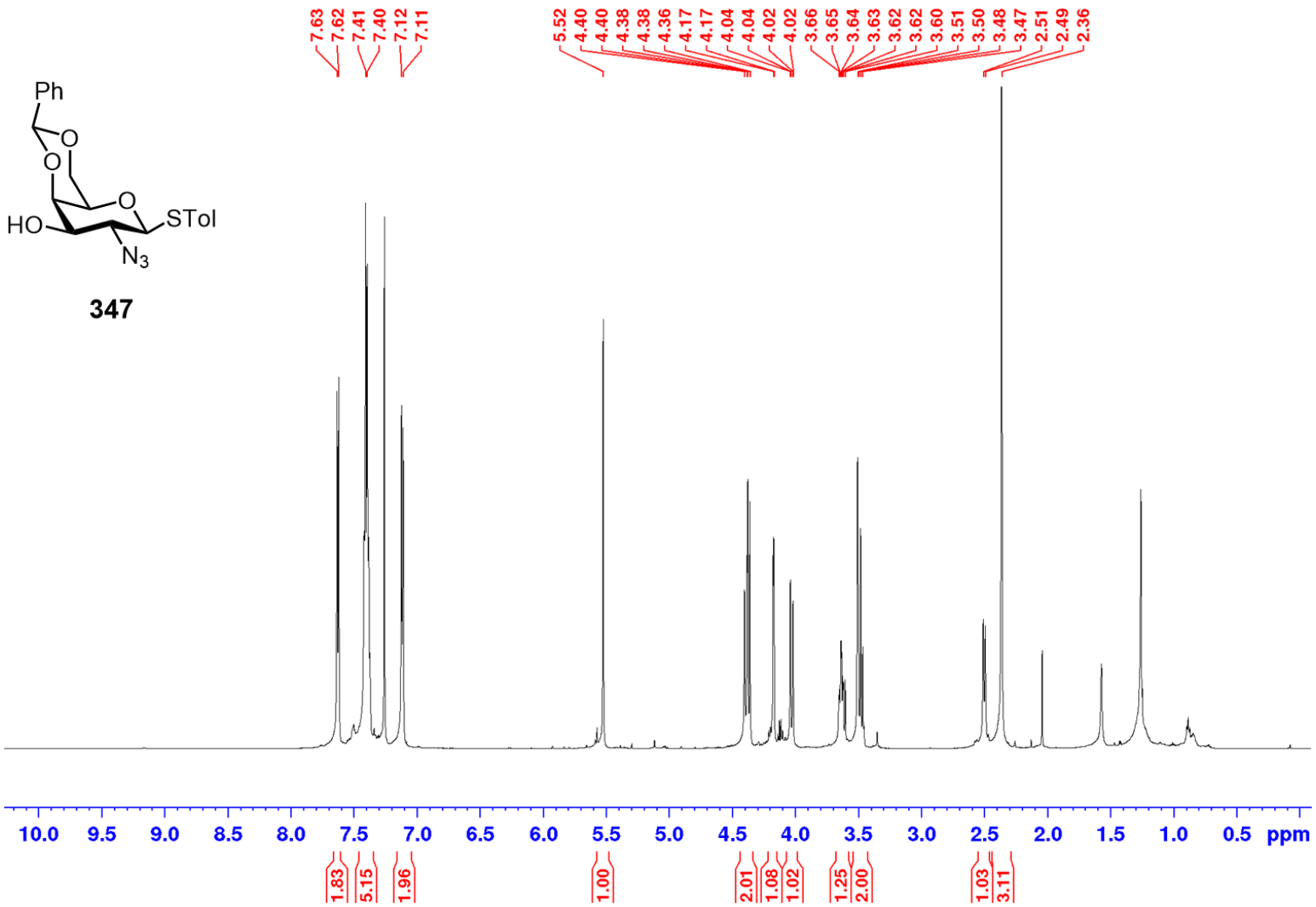
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 346



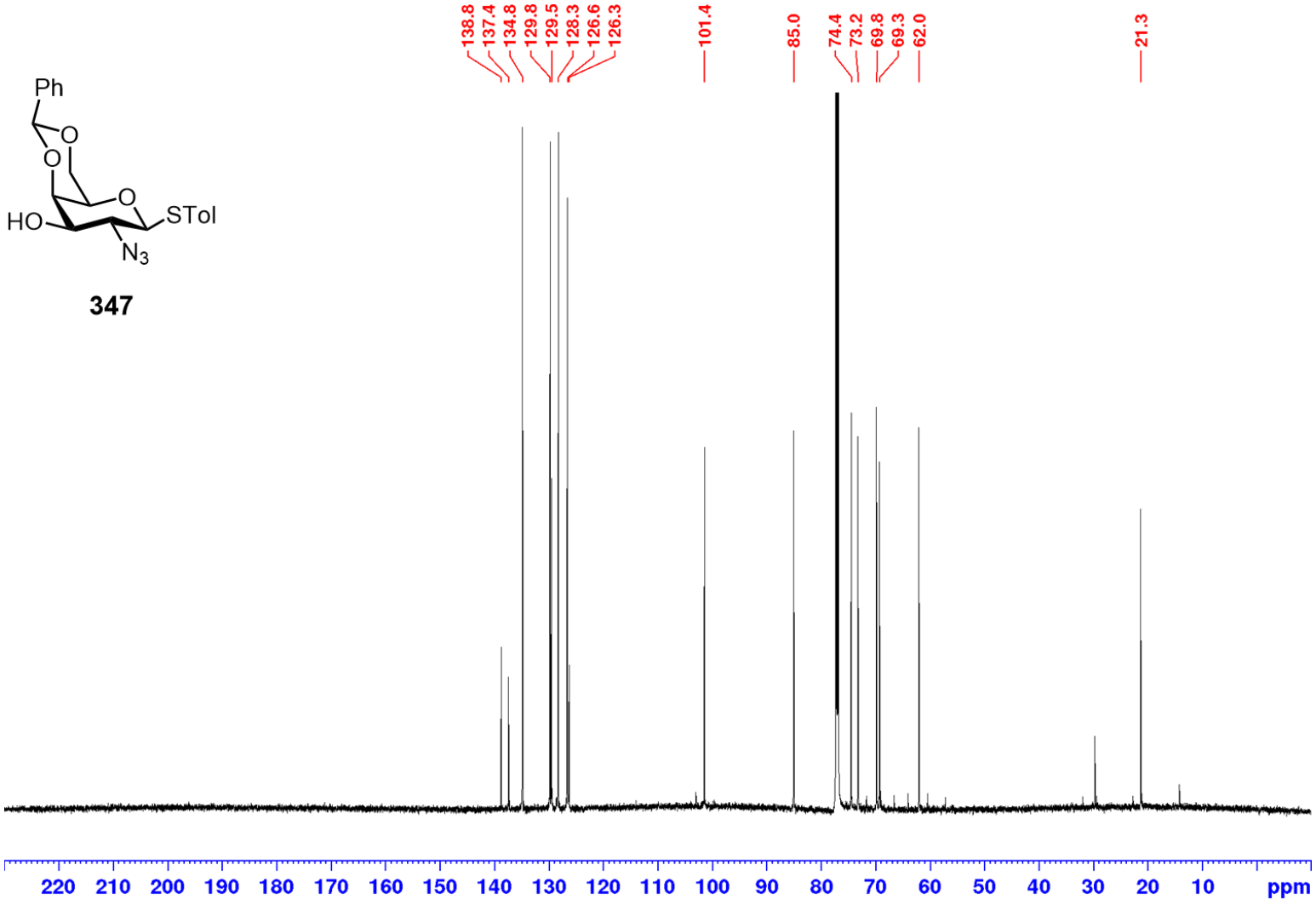
^{1}H-^{13}C HSCQ ($CDCl_3$) of compound 346



^{1}H NMR ($CDCl_3$, 400 MHz) of compound 347

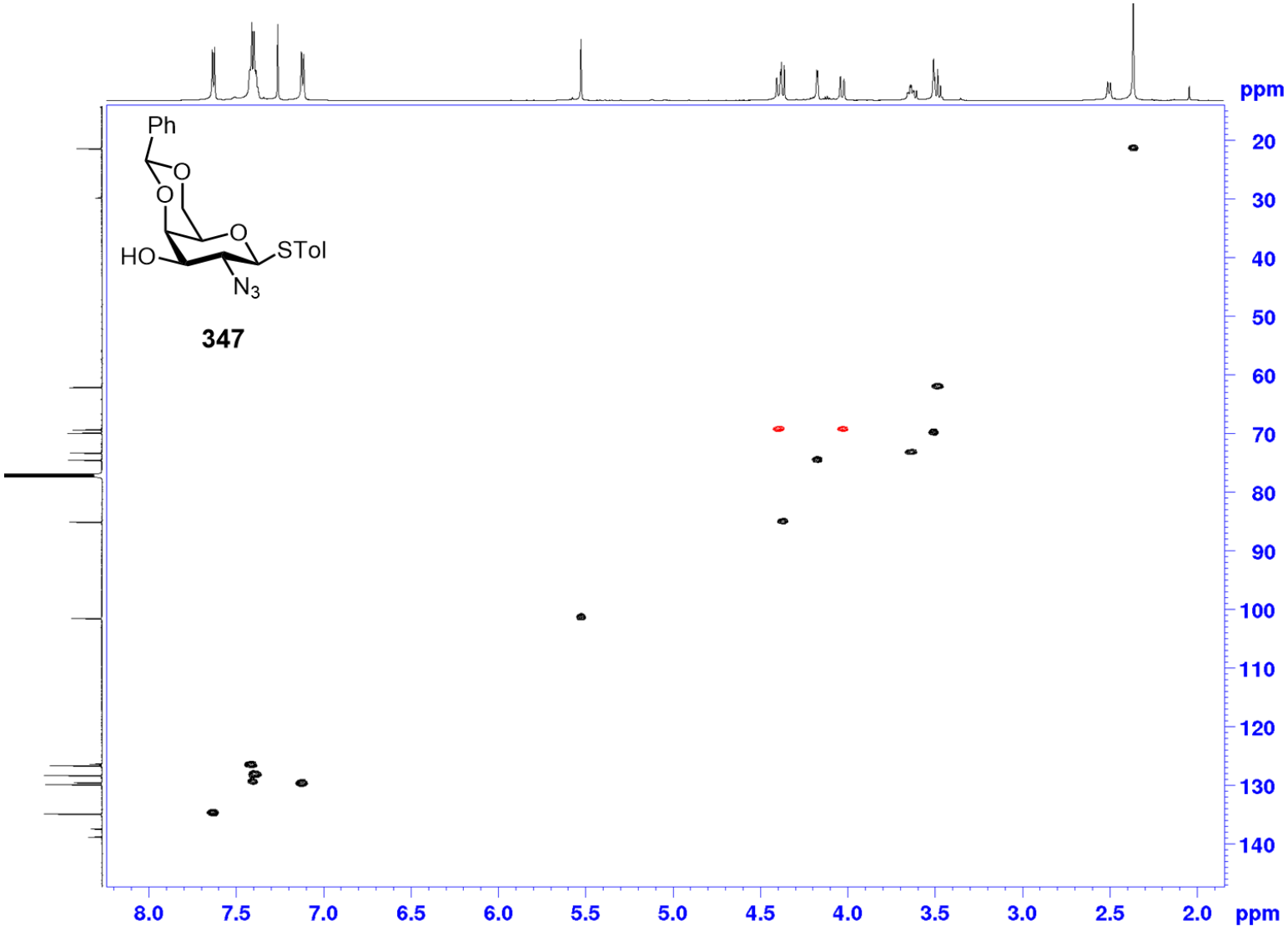


^{13}C NMR ($CDCl_3$, 100 MHz) of compound 347

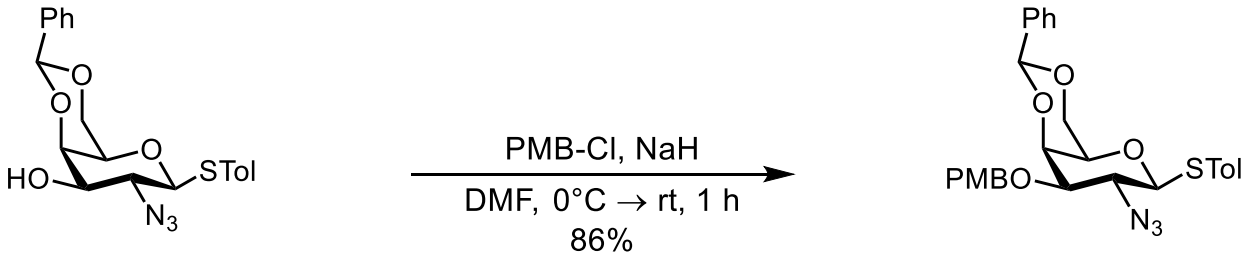


347

^{1}H-^{13}C HSCQ ($CDCl_3$) of compound 347



p-Tolyl 4,6-*O*-benzylidene acetal-3-*O*-(p-methoxy benzyl)-2-deoxy-2-azido-1-thio-β-D-galactopyranoside (348)

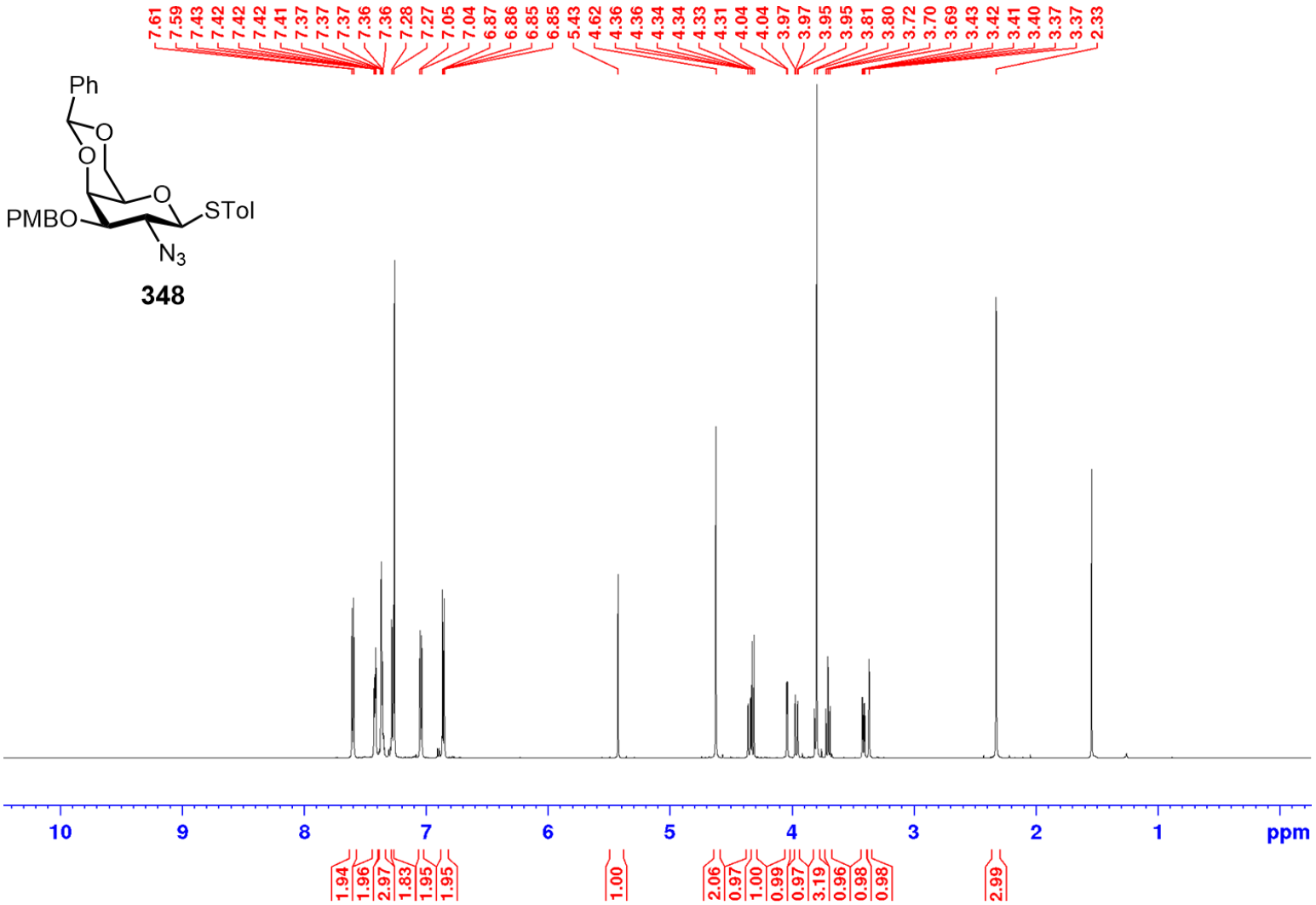


347 **348**

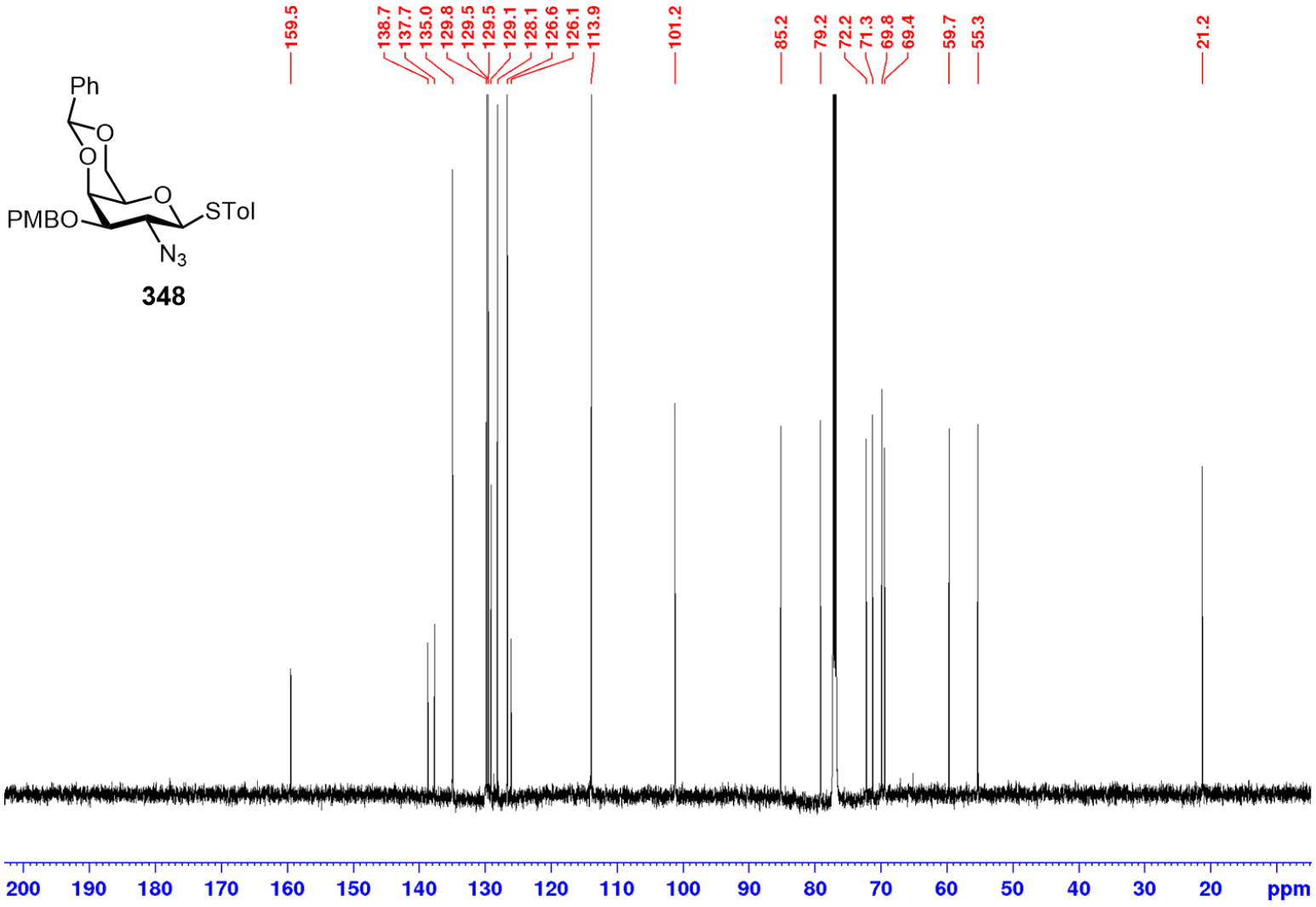
To a solution of *p*-tolyl 4,6-*O*-benzylidene acetal-2-deoxy-2-azido-1-thio-β-D-galactopyranoside **347** (1.0 equiv., 2.609 g, 6.531 mmol) in anhydrous DMF (30 mL) was added NaH (2.0 equiv., 0.52 g, 13.06 mmol, 60% in mineral oil) at room temperature under an argon atmosphere. After 30 min, the reaction was cooled to 0 °C and added PMB-Cl (1.2 equiv., 1.03 mL, 7.837 mmol) dropwise. The reaction mixture was stirred at room temperature for 1 h. TLC indicated that the starting material had been consumed in exchange for a less polar product. The reaction was then quenched by dropwise addition of CH_3OH (3.0 mL) at 0 °C. The reaction was stirred at room temperature for 10 min. The reaction was diluted with EtOAc (100 mL) and transferred to a separatory funnel and washed with 1 N HCl (5 x 30 mL), satd. aq. $NaHCO_3$ (1 x 20 mL), brine (1 x 20), dried ($NaSO_4$) and filtered to remove drying agent. The filtrate was concentrated to a crude oil which was purified by flash column chromatography (1:3 then 2:3 EtOAc/hexanes) which was then crystalized from hot EtOAc and hexanes give the *p*-tolyl 4,6-*O*-benzylidene acetal-3-*O*-(*p*-methoxy benzyl)-2-deoxy-2-azido-1-thio-β-D-galactopyranoside **348** (2.921 g, 5.623 mmol, 86%) as white solid. Our spectroscopic data was in agreement of previously reported data.[362]

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.6 (d, *J* = 8.1 Hz, 2H), 7.43-7.41 (m, 2H), 7.37-7.36 (m, 3H), 7.28 (d, *J* = 8.6 Hz, 2H), 7.04 (d, *J* = 7.8 Hz, 2H), 6.85 (d, *J* = 8.7 Hz, 2H), 5.44 (s, 1H), 4.62 (s, 2H), 4.36 (dd, *J* = 1.5, 12.2 Hz, 1H), 4.31 (d, *J* = 9.9 Hz, 1H), 4.04 (d, *J* = 2.8 Hz, 1H), 3.96 (dd, *J* = 1.4, 12.8 Hz, 1H), 3.8 (s, 3H), 3.71 (dd, *J* = 10.2, 10.3 Hz, 1H), 3.41 (dd, *J* = 3.3, 9.8 Hz, 1H), 3.37 (d, *J* = 0.9 Hz, 1H), 2.33 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 159.5, 138.7, 137.7, 135.0, 129.8, 129.5, 129.5, 129.1, 128.1, 126.6, 126.1, 113.9, 101.2, 85.2, 79.2, 72.2, 71.3, 69.9, 69.4, 59.7, 55.3, 21.3.

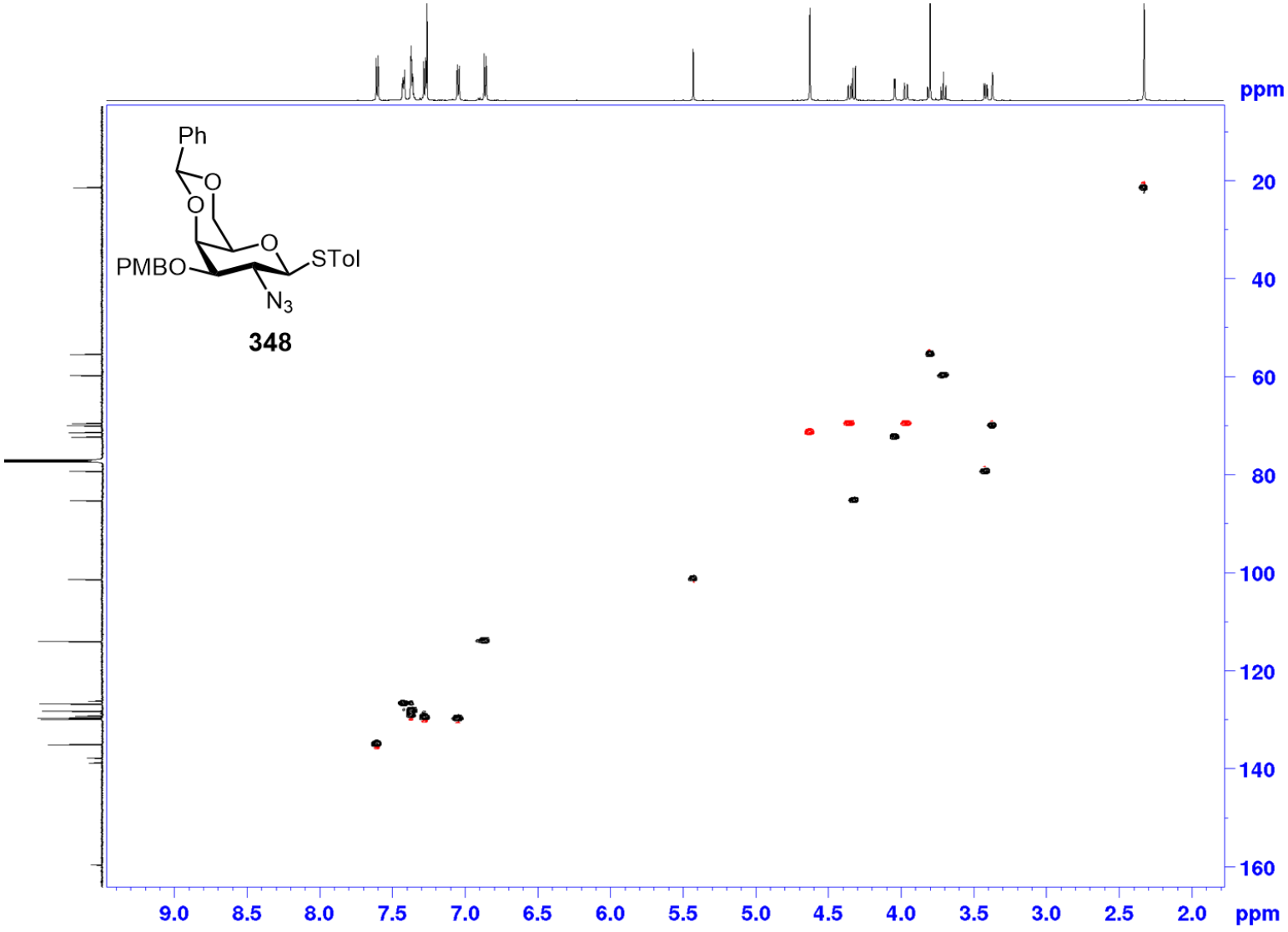
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 348



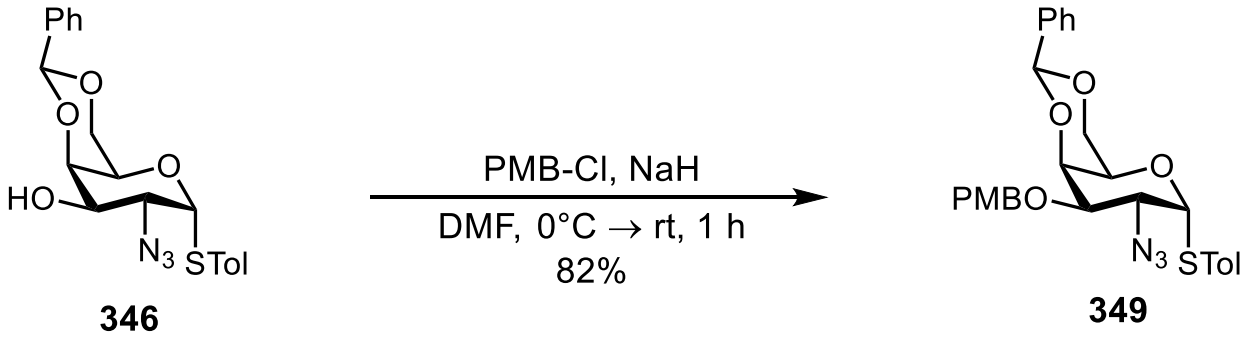
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 348



^{1}H-^{13}C HSCQ ($CDCl_3$) of compound 348



***p*-Tolyl 4,6-*O*-benzylidene acetal-3-*O*-(*p*-methoxy benzyl)-2-deoxy-2-azido-1-thio-α-D-galactopyranoside (349)**

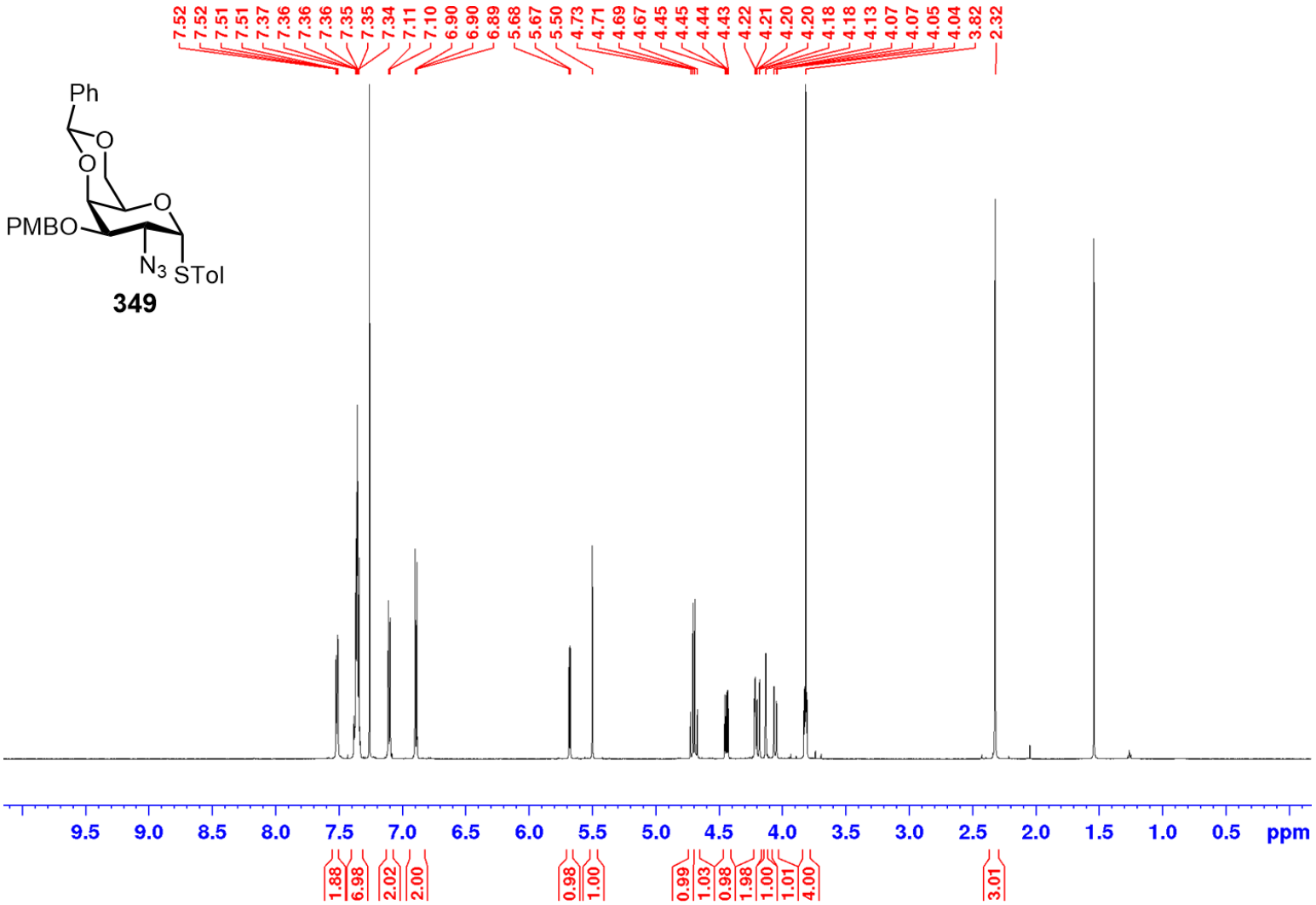


346 **349**

To a solution of *p*-tolyl 4,6-*O*-benzylidene acetal-2-deoxy-2-azido-1-thio-α-D-galactopyranoside **346** (1.0 equiv., 1.524 g, 3.816 mmol) in anhydrous DMF (25 mL) was added NaH (2.0 equiv., 0.31 g, 7.63 mmol, 60% in mineral oil) at room temperature under a stream of argon. After 30 min, the reaction was cooled to 0 °C and added PMB-Cl (1.2 equiv., 0.62 mL, 4.58 mmol) dropwise. The reaction mixture was stirred at room temperature for 1 h. TLC indicated that the starting material had been consumed in exchange for a less polar product. The reaction was then quenched by dropwise addition of CH_3OH (1.0 mL) at 0 °C. The reaction was stirred at room temperature for 10 min. The reaction was diluted with EtOAc (100 mL) and transferred to a separatory funnel and washed with 1 N HCl (5 x 20 mL), satd. aq. $NaHCO_3$ (1 x 20 mL), brine (1 x 20), dried ($NaSO_4$) and filtered to remove drying agent. The filtrate was concentrated to a crude oil which was purified by flash column chromatography (25:75 then 40:60 EtOAc/hexanes) which was then crystalized from hot EtOAc and hexanes give the *p*-tolyl 4,6-*O*-benzylidene acetal-3-*O*-(*p*-methoxy benzyl)-2-deoxy-2-azido-1-thio-α-D-galactopyranoside **349** (1.629 g, 3.13 mmol, 82%) as white solid. Our spectroscopic data was in agreement of previously reported data.[362]

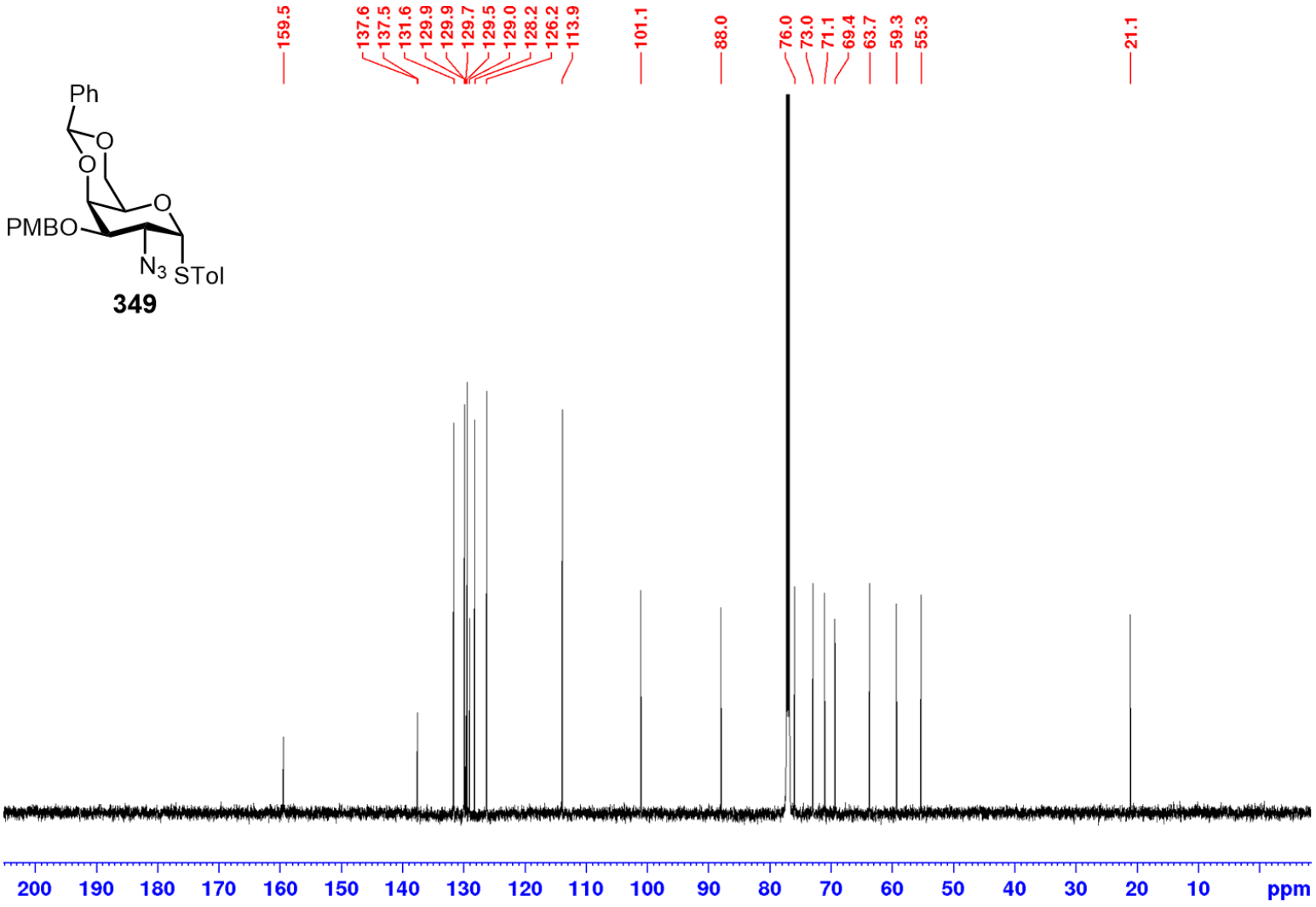
^{1}H NMR (400 MHz, $CDCl_3$): δ 7.51 (dd, *J* = 1.8, 7.8 Hz, 2H), 7.37-7.34 (m, 8H), 7.11 (d, *J* = 7.9 Hz, 2H), 6.9 (d, *J* = 8.9 Hz, 2H), 5.68 (d, *J* = 5.3 Hz, 1H), 5.51 (s, 1H), 4.71 (d, *J* = 11.8 Hz, 1H), 4.69 (d, *J* = 11.6 Hz, 1H), 4.44 (dd, *J* = 5.4, 10.7 Hz, 1H), 4.22 (d, *J* = 3.1 Hz, 1H), 4.19 (dd, *J* = 1.4, 12.8 Hz, 1H), 4.14 (s, 1H), 4.06 (dd, *J* = 1.8, 12.5 Hz, 1H), 3.82 (s, 5H), 2.33 (s, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 159.5, 137.6, 137.5, 131.6, 130.0, 129.9, 129.9, 129.7, 129.5, 129.0, 128.2, 128.1, 126.2, 113.9, 101.1, 88.0, 76.0, 73.0, 71.1, 7.4, 63.7, 59.3, 55.3, 21.1.

^{1}H NMR ($CDCl_3$, 400 MHz) of compound 349

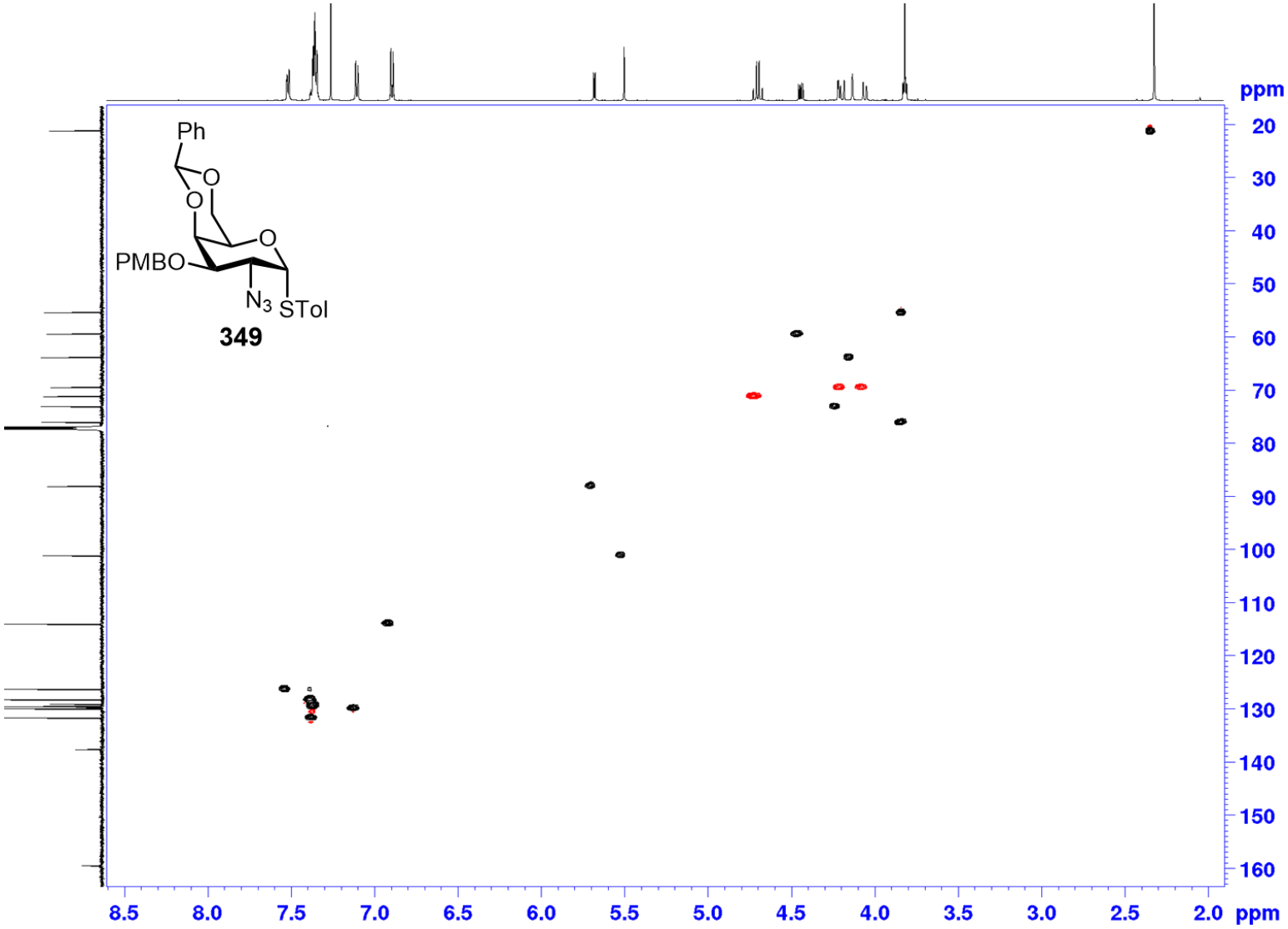


349

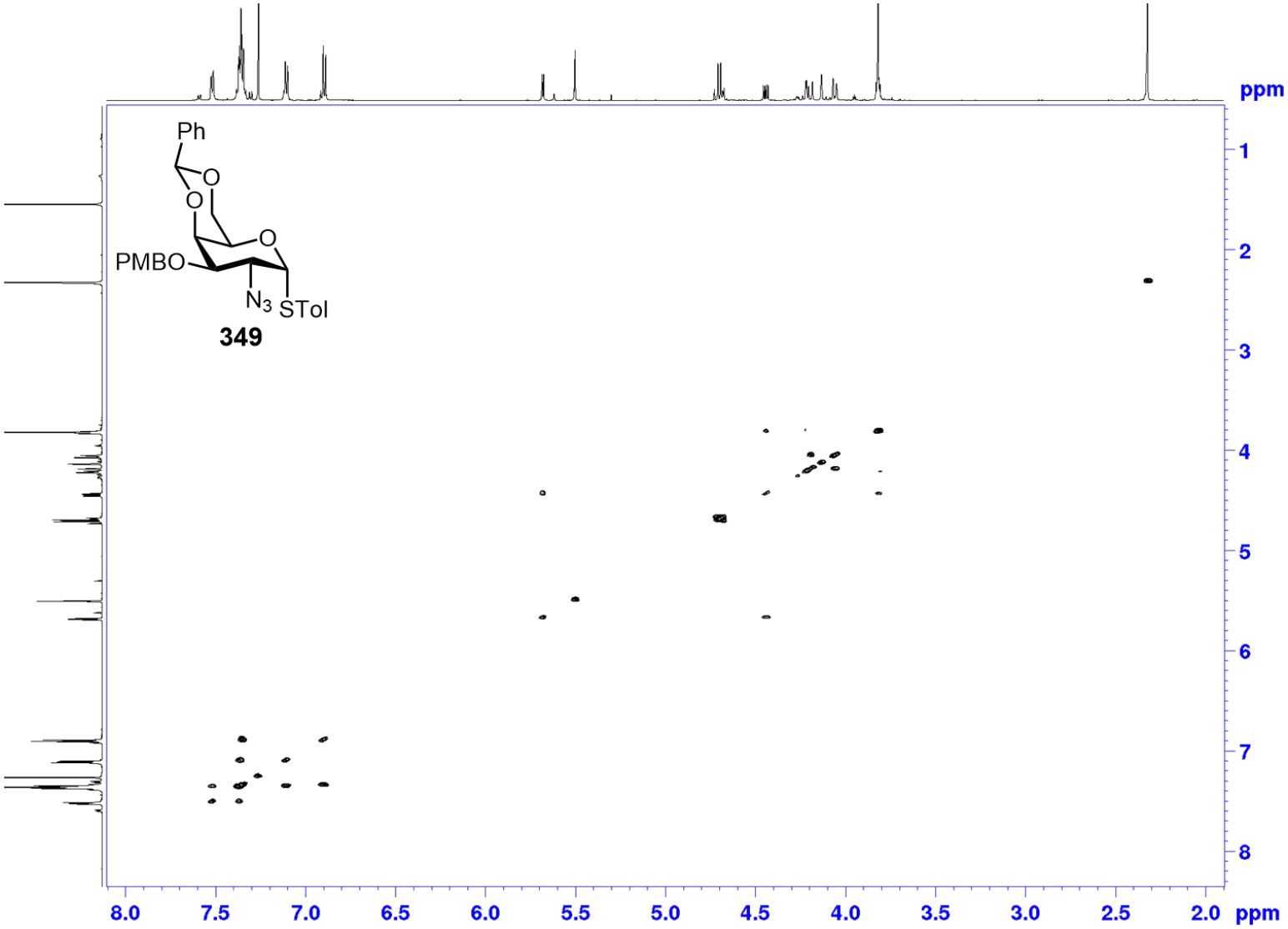
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 349



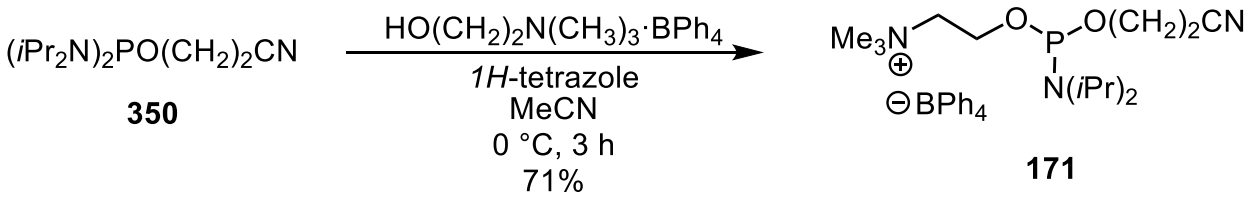
^{1}H-^{13}C HSCQ ($CDCl_3$) of compound 349



^{1}H-^{1}H COSY ($CDCl_3$) of compound 349



Choline-2-cyanoethyl *N*, *N*-diisopropylphosporamidite tetraphenylborate salt (171)

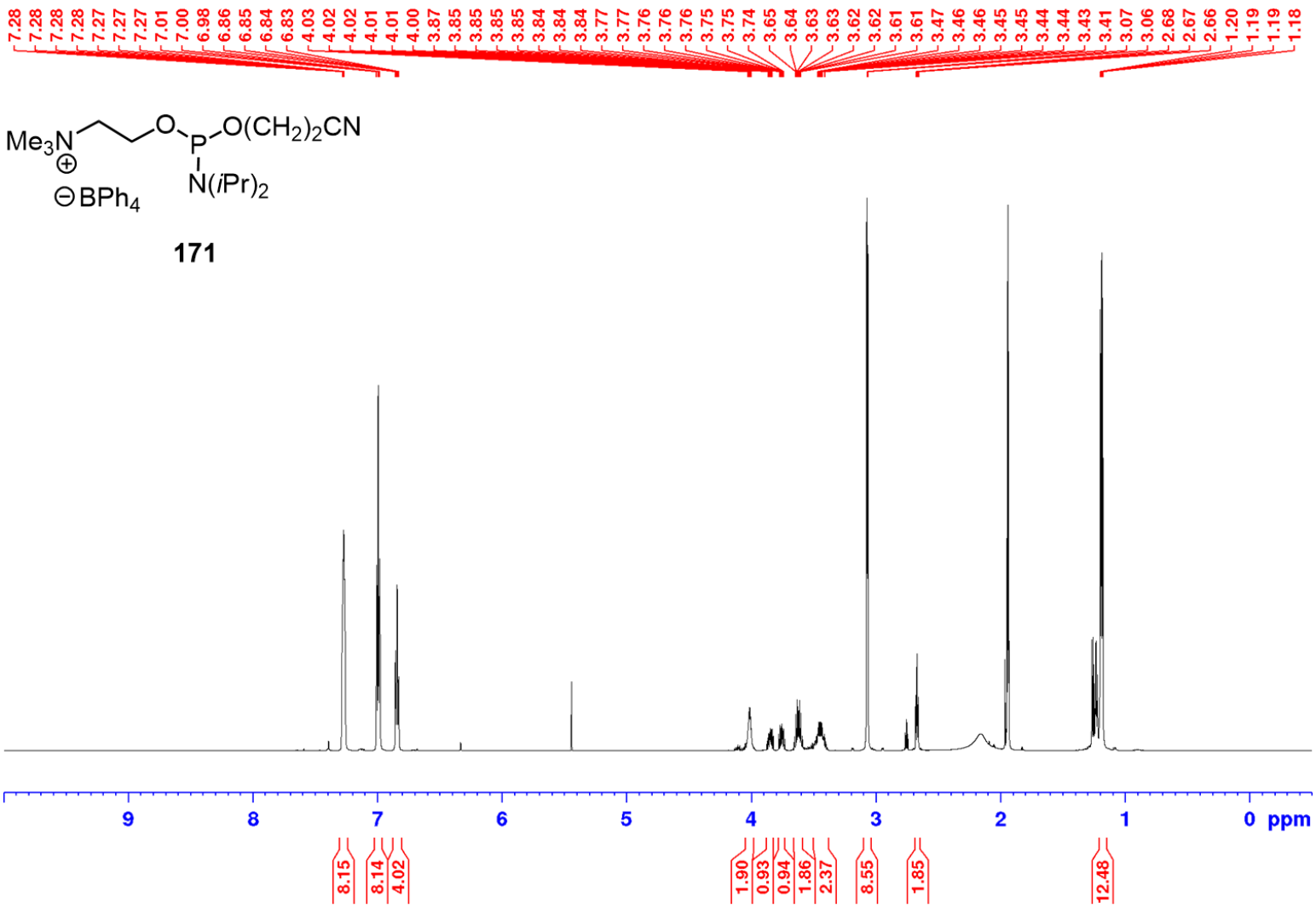


Synthesis of **350**: A solution of PCl_3 (1.0 equiv., 1.0 mL, 11.0 mmol) in anhydrous CH_3CN (110 mL) was added 2-cyanoethanol (1.0 equiv., 0.78 mL, 11.0 mmol) dropwise at 0 °C under an argon atmosphere. The reaction was vented and kept under a constant stream of nitrogen to expel the HCl (g) that was produced. The reaction warmed to room temperature while stirring. At 2 h, the reaction was cooled to 0 °C and added diisopropyl amine (4.0 equiv., 6.4 mL, 44.0 mmol) dropwise. The reaction was warmed to room temperature. At 12 h, the reaction was filtered to remove diisopropyl ammonium chloride salts with a glass fritted filter funnel using positive pressure of an argon balloon, to limit oxidation of the phosphoramidite, to give a crude yellow oil. The crude oil was analyzed by ^{31}P NMR (^{31}P: 124.4 ppm for **350**, 150.1 ppm for $(iPr_2N)P((CH_2)_2CN)_2$ impurity) and integration of ^{31}P indicated that the reaction was 72% pure phosphordiamidite **350** (which is commercially available), reagents equivalencies for the last step are normalized for this purity.

Synthesis of **171**: The crude oil containing phosphordiamidite **350** (1.0 equiv.,2.39 g 7.92 mmol) was diluted in anhydrous CH_3CN (40 mL) and added choline tetraphenylborate salt (1.0 equiv., 4.5 g, 7.86 mmol) and a 4% tetrazole solution (0.5 equiv., 6.74 g, 8.5 mL, 3.85 mmol) dropwise to the reaction at 0 °C under an argon atmosphere. The reaction warmed to room temperature while stirring. At 12 h, the reaction was diluted with EtOAc (100 mL) and transferred to a separatory funnel and washed with sat. aq. $NaHCO_3$ (3 x

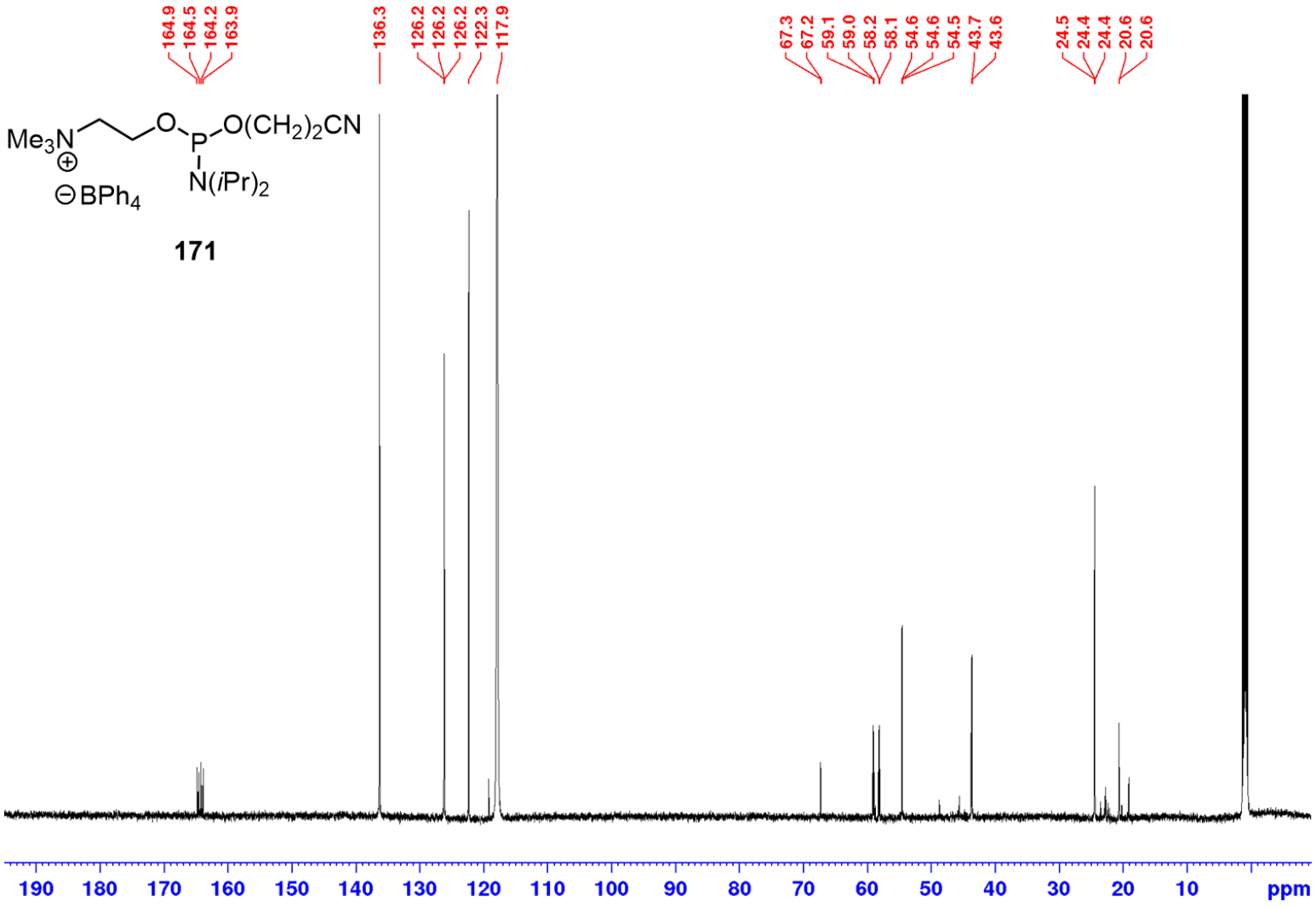
20 mL), brine (1 x 20 mL), dried ($NaSO_4$) and filtered through a glass fritted vacuum filter funnel to remove drying agent. The filtrate was concentrated to give phosphoramidite **171** (4.89 g, 7.84 mmol, 71%) as an off-white foam. $[\alpha]D^{25}$ = -8.32° (c = 1.0, $CHCl_3$); IR ν_{max} (film) 3434.95, 2363.71, 1654.29, 1510.67, 1457.45, 1371.05, 1245.52, 1074.92, 748.70, 698.47 cm^{-1}; ^1H NMR (400 MHz, CD_3CN): δ 7.28-7.27 (m, 8H), 7.00 (t, *J* = 7.0 Hz, 8H), 6.85 (dt, *J* = 1.2, 8.3 Hz, 4H), 4.03-3.99 (m, 2H), 3.87-3.82 (m, 1H), 3.78-3.73 (m, 1H), 3.65-3.59 (m, 2H), 3.49-3.38 (m, 2H), 3.06 (d, *J* = 5.6 Hz, 8H), 2.66 (t, *J* = 6.0 Hz, 2H), 1.18 (dd, *J* = 4.7, 7.0 Hz, 12 H);^{13}C NMR (100 MHz, CD_3CN): δ 164.9, 164.5, 164.2, 163.9, 136.3, 126.2, 126.2, 126.2, 122.3, 117.9, 67.3, 67.2, 59.1, 59.0, 58.2, 58.1, 54.6, 54.6, 54.5, 43.7, 43.6, 24.5, 24.4, 24.4, 20.6, 20.6; δ; ^{31}P NMR (151 MHz, CD_3CN) δ 150.3 ; HR-ESI-MS (m/z): calcd for $C_{14}H_{31}N_3O_2P^+$ $(M)^+$ 304.21480, found 304.21385.

^{1}H NMR ($CDCl_3$, 400 MHz) of compound 171

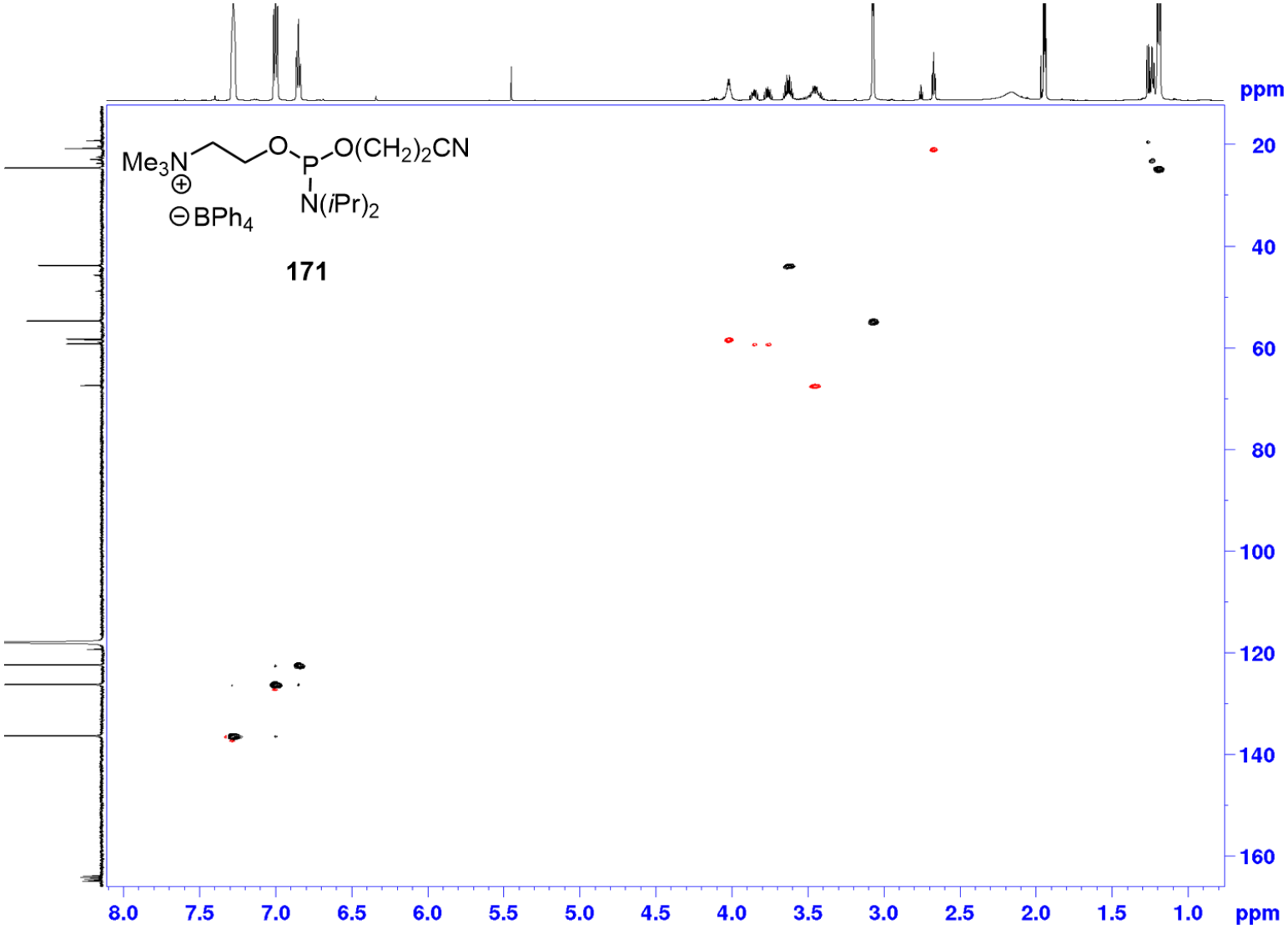


171

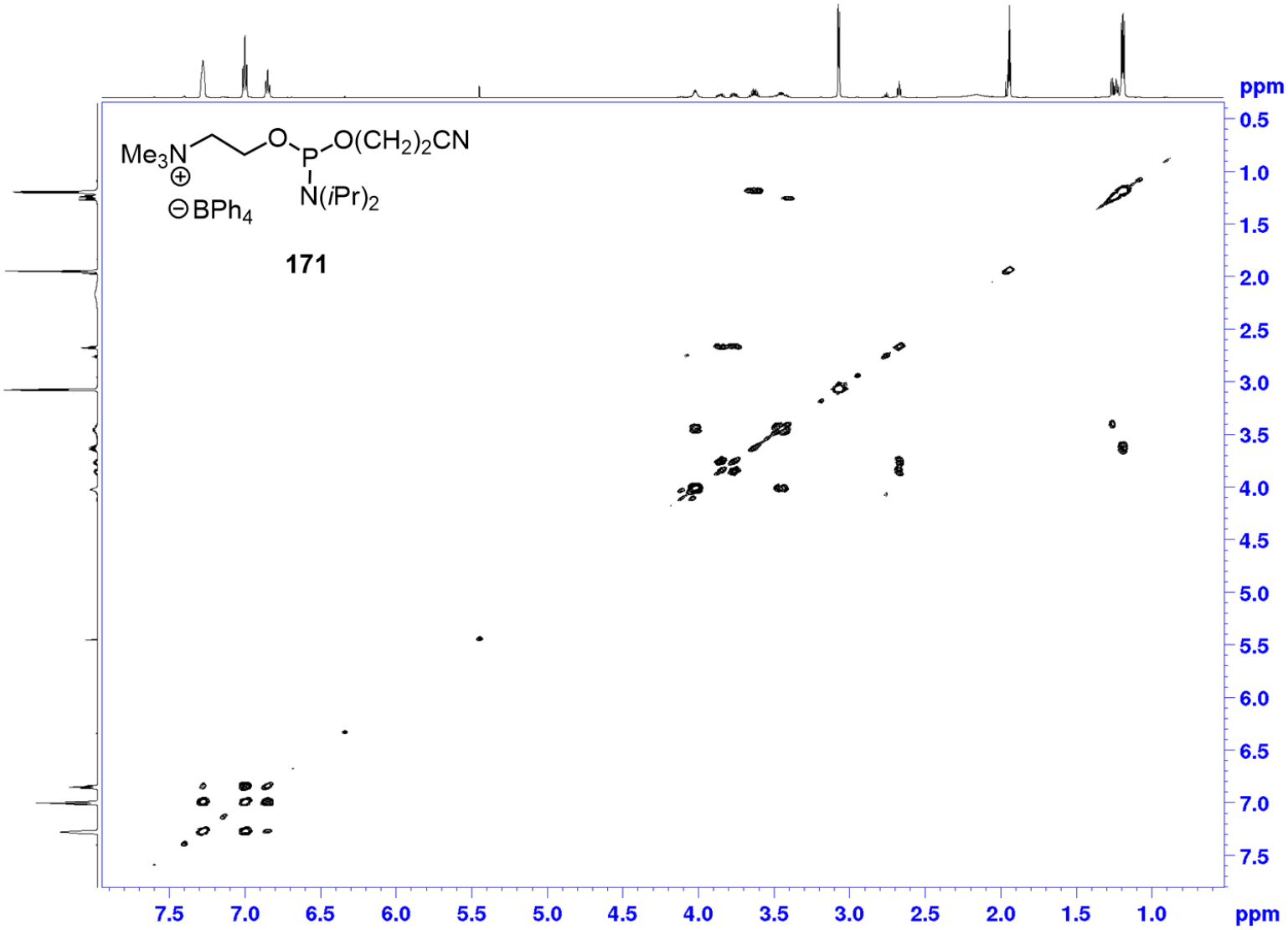
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 171



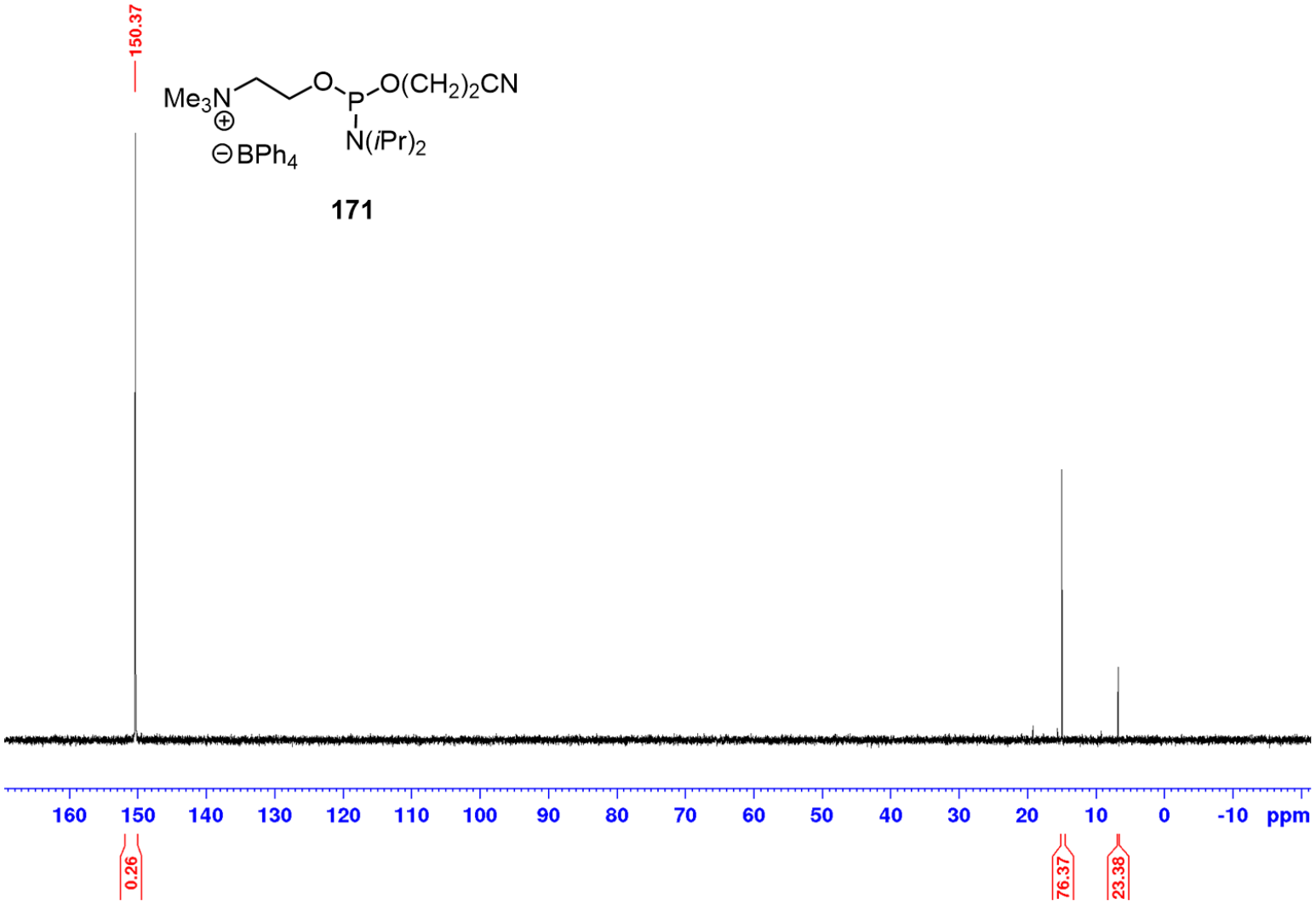
^{1}H-^{13}C HSCQ ($CDCl_3$) of compound 171

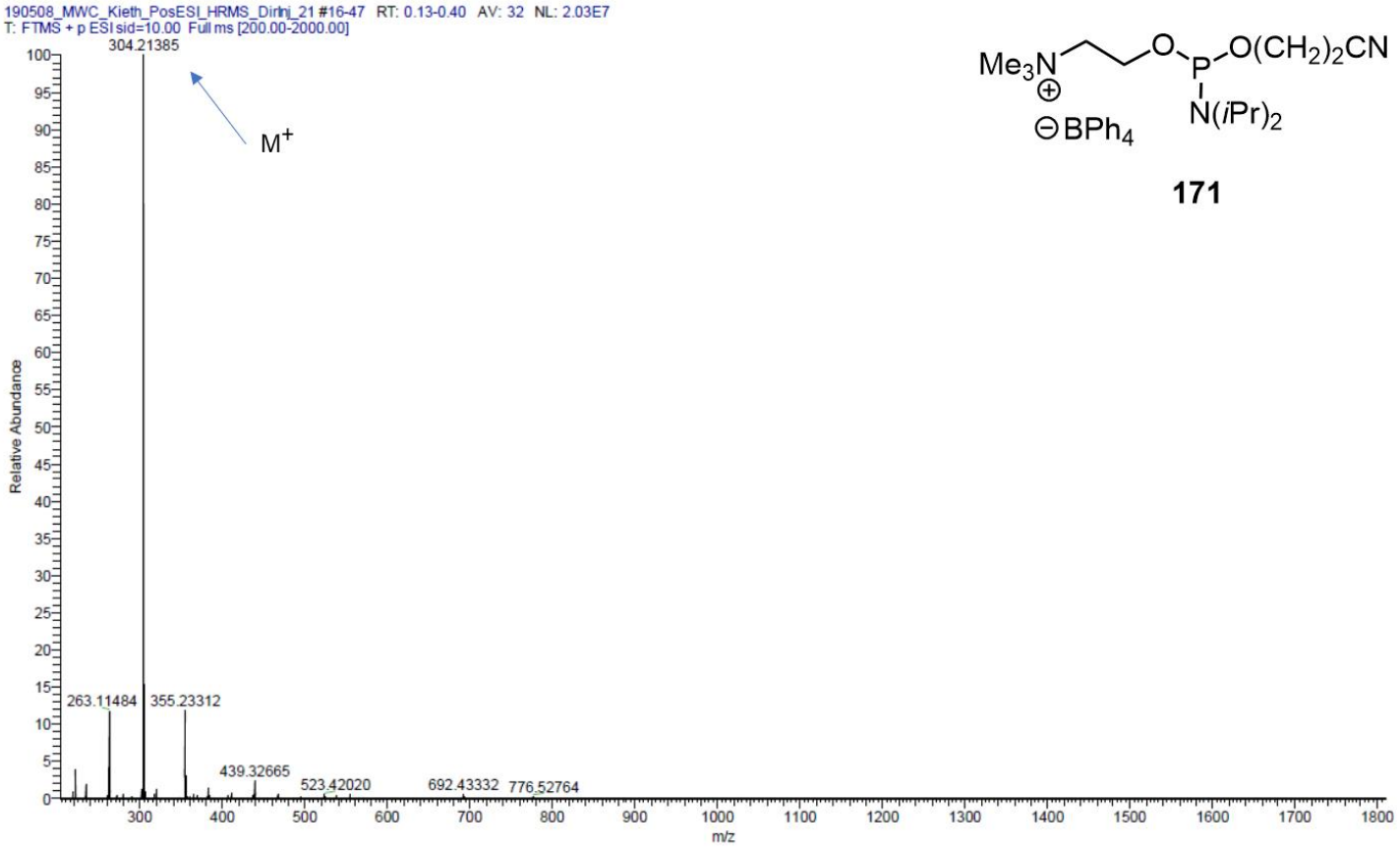


^{1}H-^{1}H COSY ($CDCl_3$) of compound 171



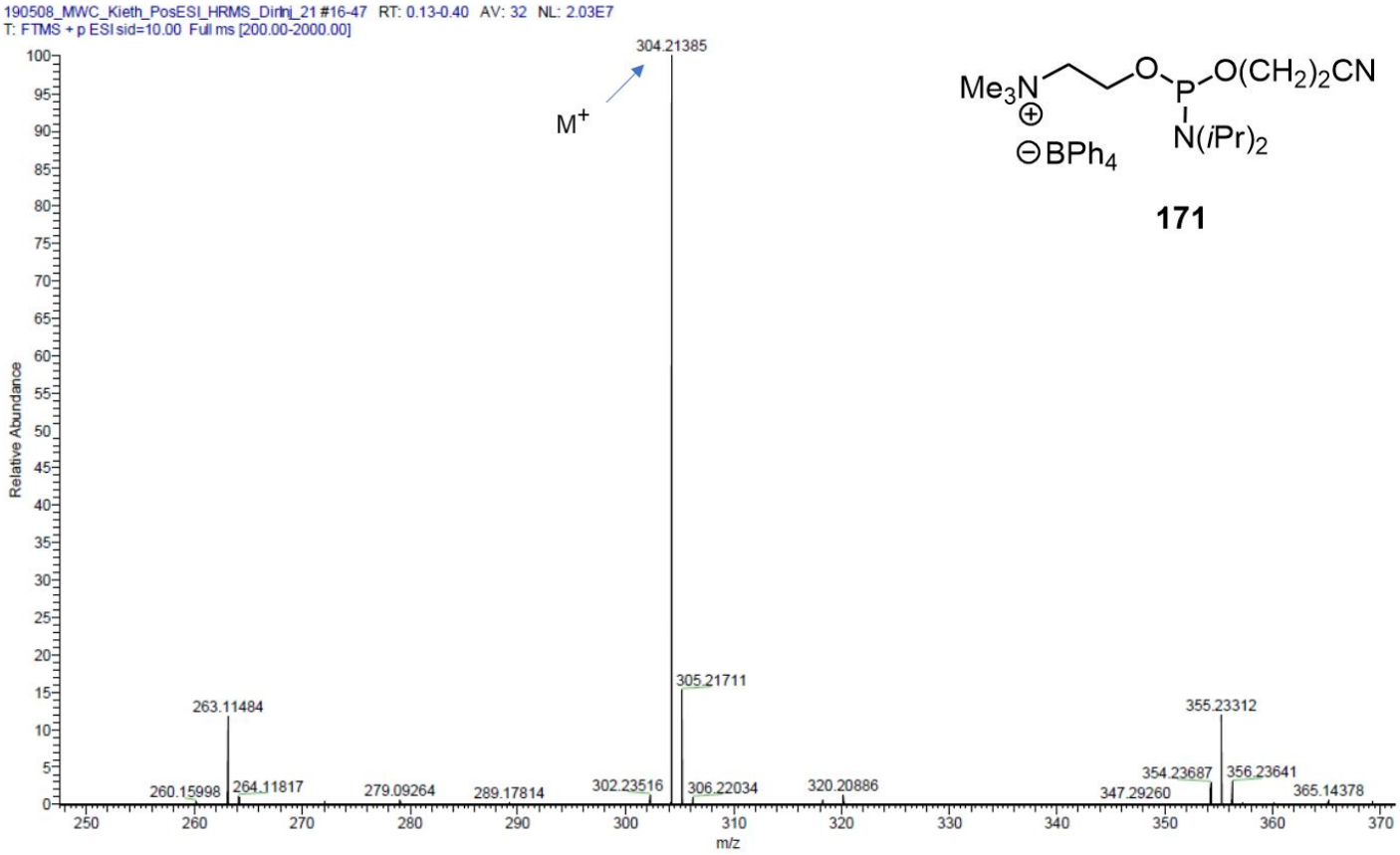
^{31}P NMR ($CDCl_3$, 162 MHz) of compound 171




190508_MWC_Kieth_PosESI_HRMS_DirInj_21 #16-47 RT: 0.13-0.40 AV: 32 NL: 2.03E7
T: FTMS + p ESI sid=10.00 Full ms [200.00-2000.00]
304.21385
M+
263.11484
355.23312
439.32665
523.42020
692.43332
776.52764
Relative Abundance
m/z

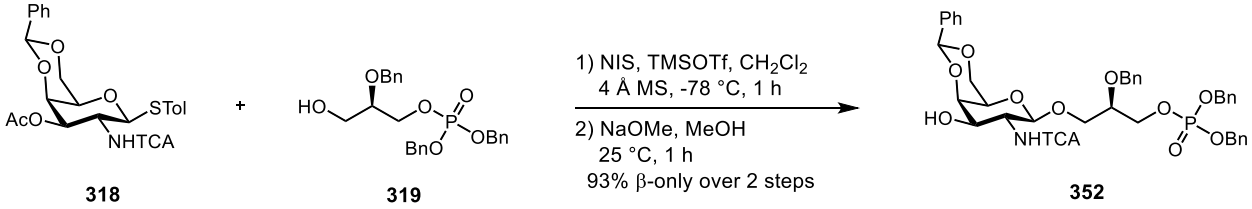
Me3N
O
P
O(CH2)2CN
N(iPr)2
BPh4

171


190508_MWC_Kieth_PosESI_HRMS_DirInj_21 #16-47 RT: 0.13-0.40 AV: 32 NL: 2.03E7
T: FTMS + p ESI sid=10.00 Full ms [200.00-2000.00]
304.21385
M+
263.11484
305.21711
355.23312
260.15998
264.11817
279.09264
289.17814
302.23516
306.22034
320.20886
354.23687
356.23641
347.29260
365.14378
Relative Abundance
m/z
Me3N
O
P
O(CH2)2CN
N(iPr)2
BPh4

171

(*R*)-dibenzyl (2-(benzyloxy)-3-hydroxypropyl) phosphate 4,6-*O*-benzylidene acetal-2-deoxy-2-trichloroacetamido-β-D-galactopyranoside (352)

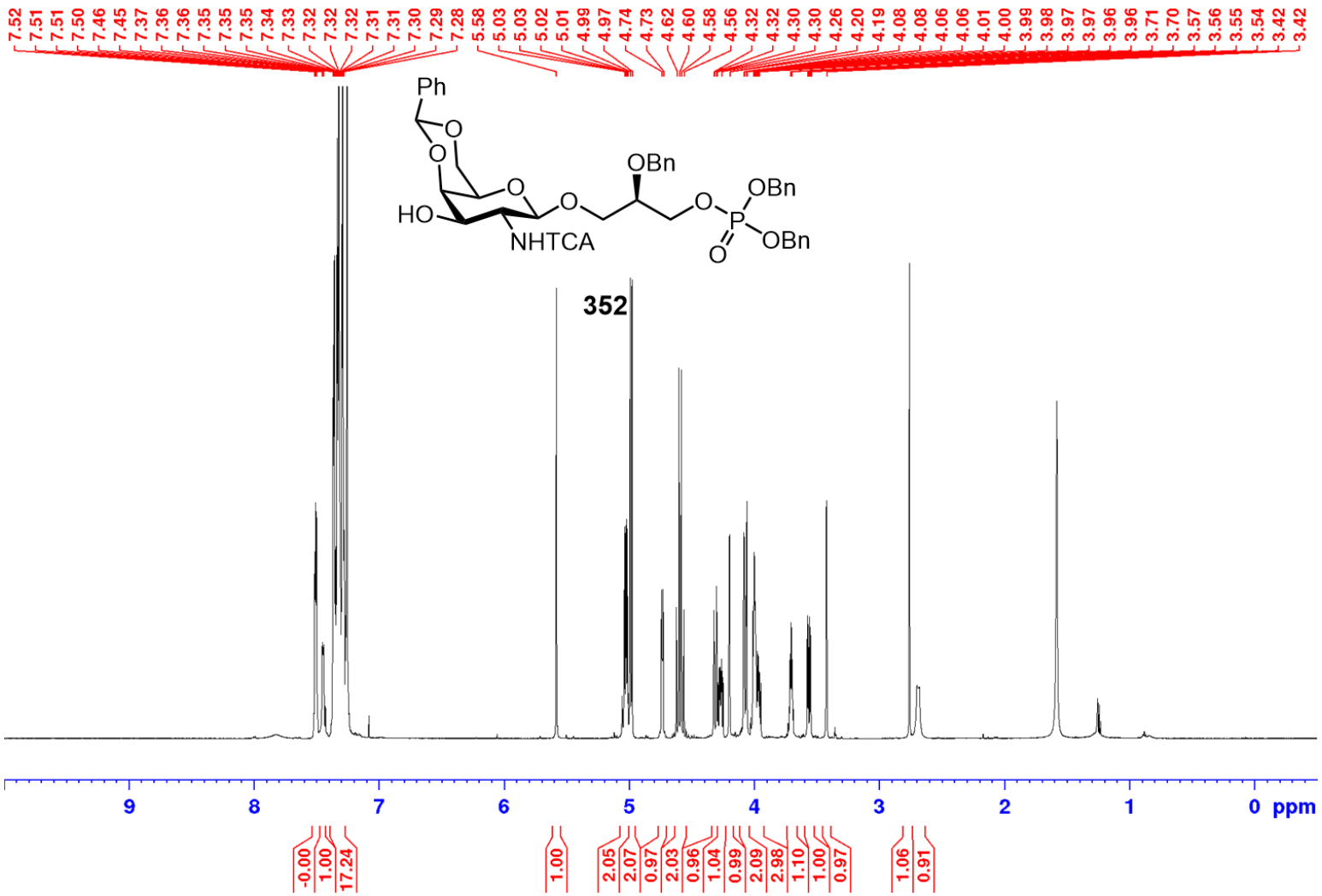


A solution of (*R*)-dibenzyl (2-(benzyloxy)-3-hydroxypropyl) phosphate **319** (1.0 equiv., 1.60 g, 3.63 mmol) and *p*-tolyl 4,6-*O*-benzylidene acetal-3-*O*-acetyl-2-deoxy-2-trichloracetamido-1-thio-β-D-galactopyranoside **318** (1.2 equiv., 2.46 g, 4.36 mmol) in anhydrous CH_2Cl_2 (36 mL) under an argon atmosphere was added 4 Å MS (5.0 g) at room temperature and allowed to stir for 1 h. The reaction mixture was cooled to -78 °C and added NIS (2.0 equiv., 1.63 g, 7.26 mmol). After 2 min, the crude reaction mixture was added a solution of TMSOTf (0.15 equiv., 2.0 mL, 5% in anhydrous CH_2Cl_2) dropwise. The reaction was stirred at -50 °C for 5 h when TLC indicated complete consumption of the alcohol **319** starting material. The reaction was quench by the addition of dilute Et_3N in CH_2Cl_2 while warming to room temperature. The reaction was filtered through a glass fritted filter funnel equipped with a pad of Celite to remove the 4 Å MS and then concentrated to a crude oil. The crude oil was then diluted with EtOAc and transferred to a separatory funnel and washed with satd. aq. Na_2SO_4 (5 x 20 mL), brine (1 x 20), dried ($NaSO_4$) and filtered through a glass fritted vacuum filter funnel to remove drying agent. The filtrate was concentrated to a crude oil. The crude oil was purified by plug of silica (40:60 to 60:40 EtOAc/hexanes) to give crude reaction mixture containing (*R*)-dibenzyl (2-(benzyloxy)-3-hydroxypropyl) phosphate 3-*O*-acetyl-4,6-*O*-benzylidene acetal-2-deoxy-2-trichloroacetamido-β-D-galactopyranoside (**351)** which was taken

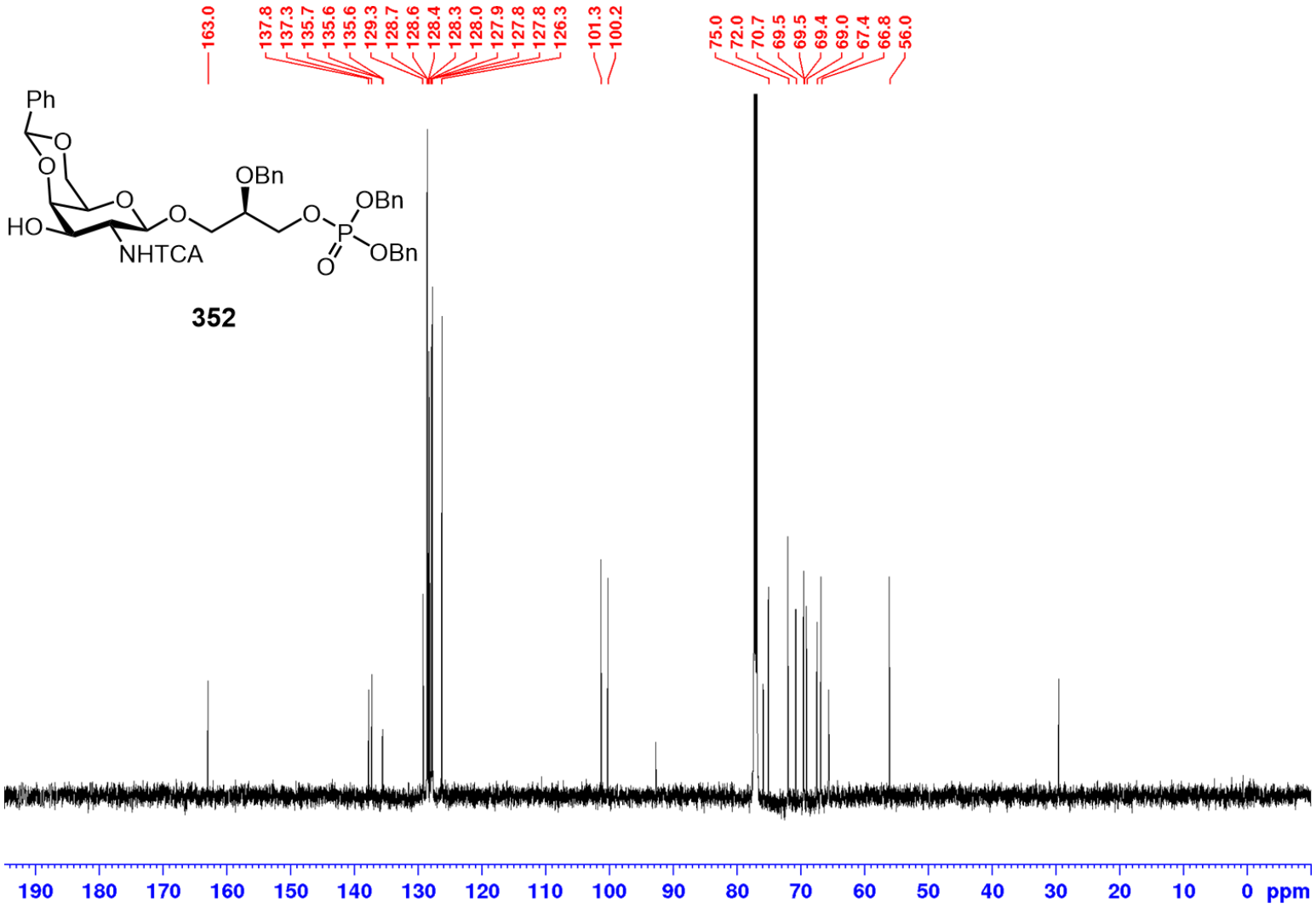
directly to the next step. The crude reaction mixture was directly subjected to saponification conditions. The crude reaction mixture in anhydrous CH_3OH (30 mL) was added a solution of $NaOCH_3$ (1.0 equiv., 1.5 mL, 3.6 mmol, [2 N] in CH_3OH) dropwise at room temperature. At 2 h, the pH of the reaction mixture was still >12 and TLC indicated that the product of the first step had been completely consumed for a more polar spot. The reaction was neutralized by the addition of the acidic form Amberlite IR 120 (H+) ion exchange resin. The solution was filtered through a glass fritted vacuum filter funnel equipped with a pad of Celite to remove the resin. The filtrate was concentrated to dryness the purified by silica gel flash column chromatography (60:40 to 95:5 EtOAc/hexanes) to give (*R*)-dibenzyl (2-(benzyloxy)-3-hydroxypropyl) phosphate 4,6-*O*-benzylidene acetal-2-deoxy-2-trichloroacetamido-β-D-galactopyranoside **352** (2.35 g, 2.81 mmol, 93%) as a white foam in quantitative yield. R_f = 0.25 (80:20 EtOAc/hexanes) visualized with ceric ammonium molybdate stain; $[\alpha]D^{25}$ = -4.72° (c = 1.0, $CHCl_3$); IR ν_{max} (film) 3434.53, 2920.89, 1706.16, 1637.75, 1531.40, 1479.36, 1455.46, 1368.28, 1260.46, 1216.59, 1169.79, 1019.40, 821.44, 734.27, 697.77, 670.16, 503.01 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.52-7.50 (m, 2H), 7.46-7.45 (m, 1H), 7.37-7.28 (m, 17H), 5.58 (s, 1H), 5.02 (d, *J* = 8.7 Hz, 1H), 5.01 (d, *J* = 8.1 Hz, 1H), 4.98 (d, *J* = 8.2 Hz, 2H), 4.73 (d, *J* = 7.9 Hz, 1H), 4.60 (*J* = 12.1 Hz, 1H), 4.57 (d, *J* = 11.9 Hz, 1H), 4.30 (dd, *J* = 1.0, 12.5 Hz, 1H), 4.28-4.24 (m, 1H), 4.29 (d, *J* = 1.3 Hz, 1H), 4.08-4.05 (m, 2H), 4.00-3.94 (m, 3H), 3.71-3.68 (m, 1H), 3.55 (dd, *J* = 4.7, 11.2 Hz, 1H), 3.41 (d, *J* = 0.8 Hz, 1H), 2.75 (s, 1H, -NH), 2.68 (d, *J* = 7.9 Hz, -OH); ^{13}C NMR (100 MHz, $CDCl_3$): δ 163.0, 137.8, 137.3, 135.7, 135.6, 135.6, 129.3, 128.7, 128.6, 128.6, 128.6, 128.6, 128.4, 128.4, 128.4, 128.3, 128.3, 128.3, 128.0, 128.0, 128.0, 127.9, 127.9, 127.9, 127.8, 127.8, 127.8, 127.8, 126.3, 126.3, 101.3,

100.2, 75.0, 72.0, 70.7, 69.5, 69.5, 69.4, 69.0, 67.4, 66.8, 56.0; ^{31}P NMR (151 MHz, $CDCl_3$) δ 0.14; HR-ESI-MS (m/z): calcd for $C_{39}H_{42}Cl_3NO_{11}P^+$ ($M+H^+$) 836.155576, found 836.1564.

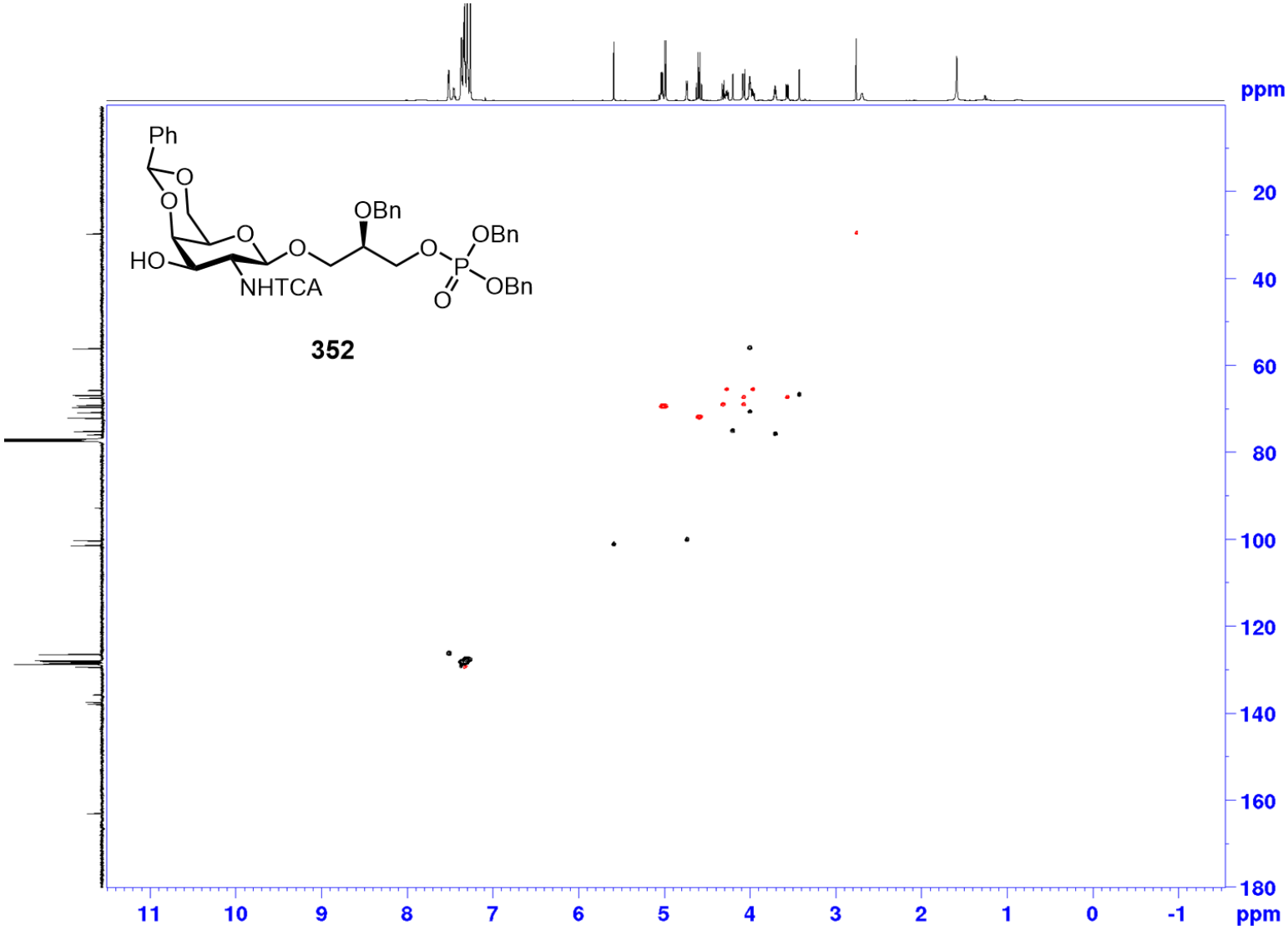
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 352



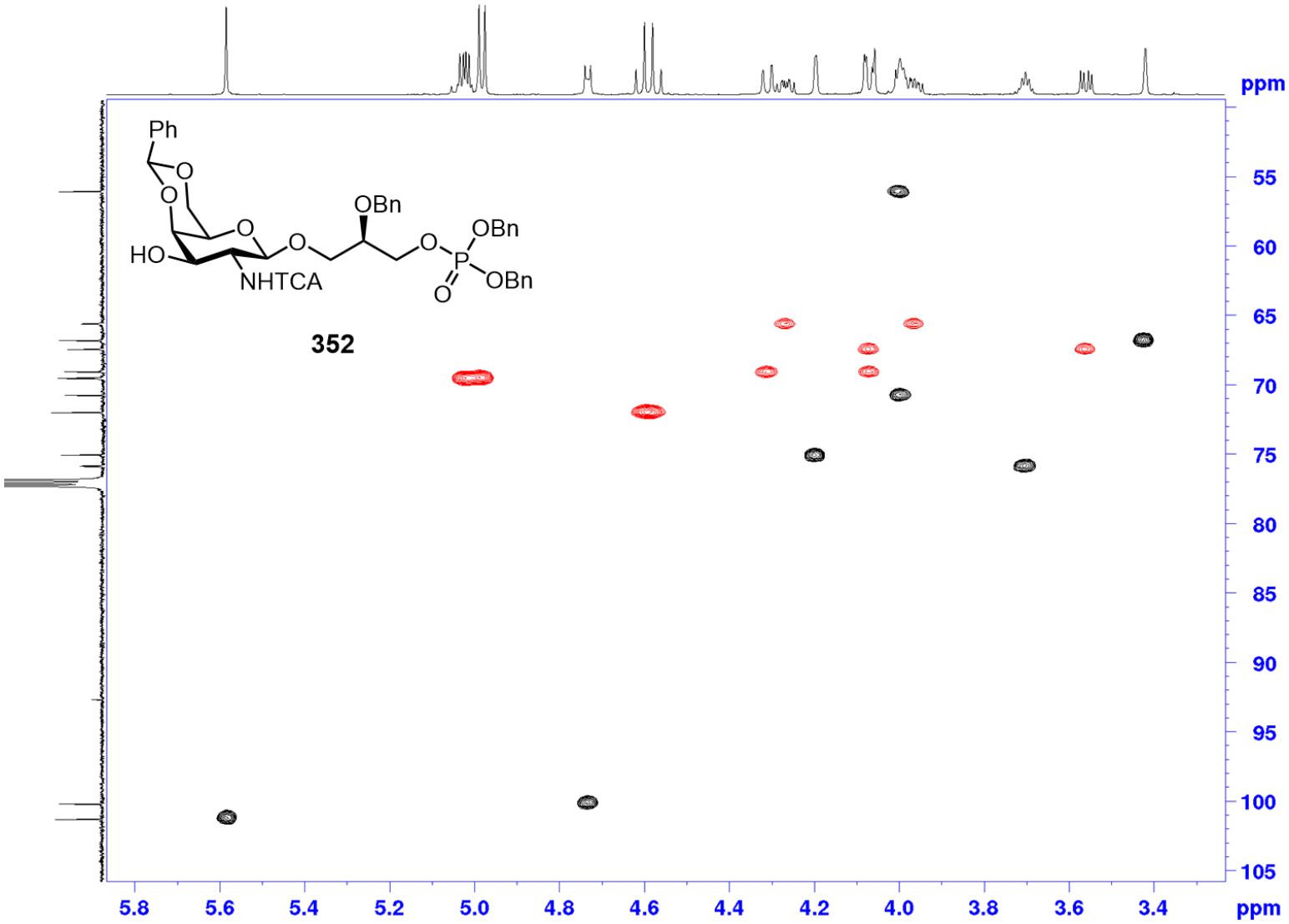
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 352



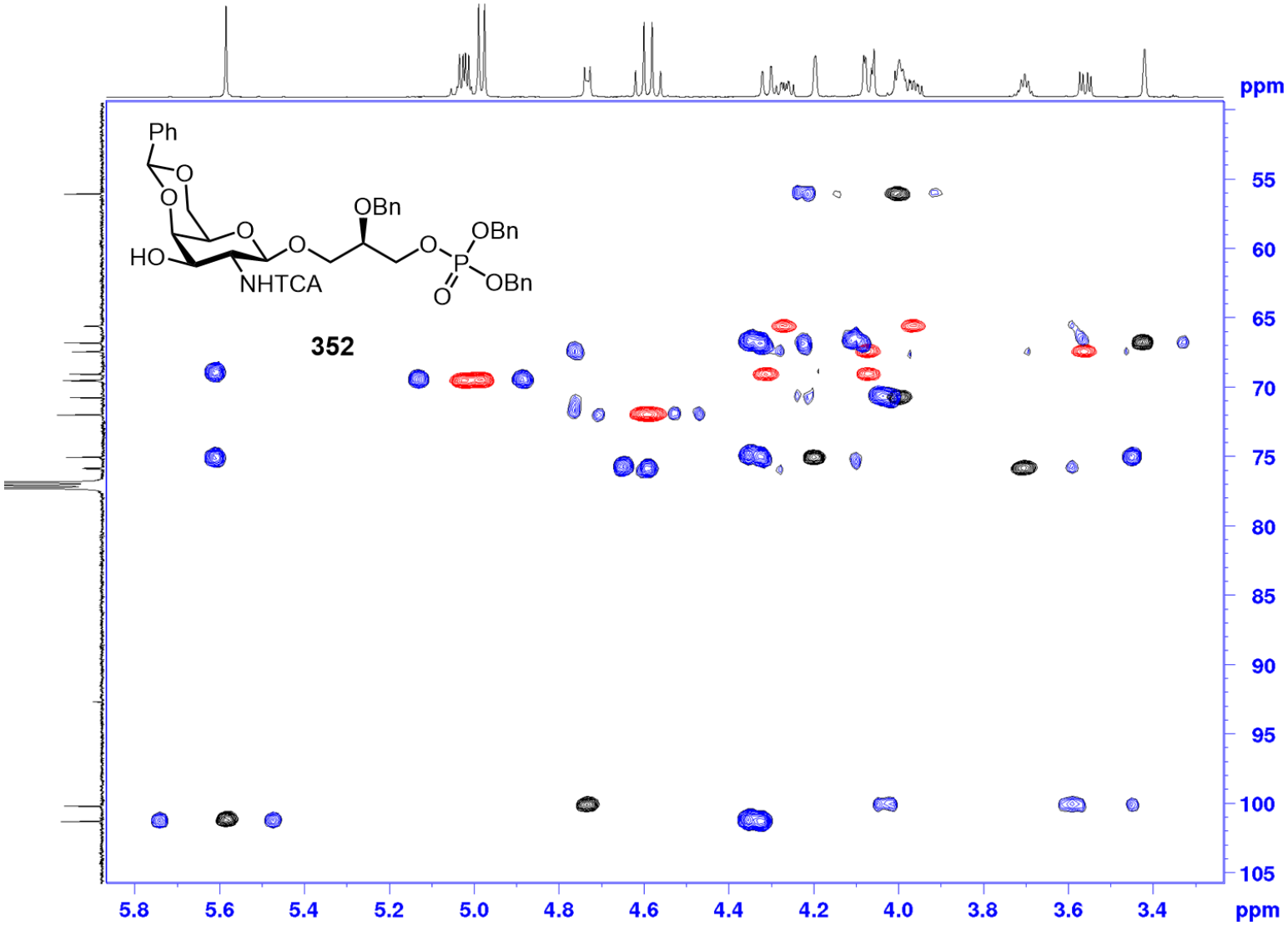
^{1}H-^{13}C HSCQ ($CDCl_3$) of compound 352



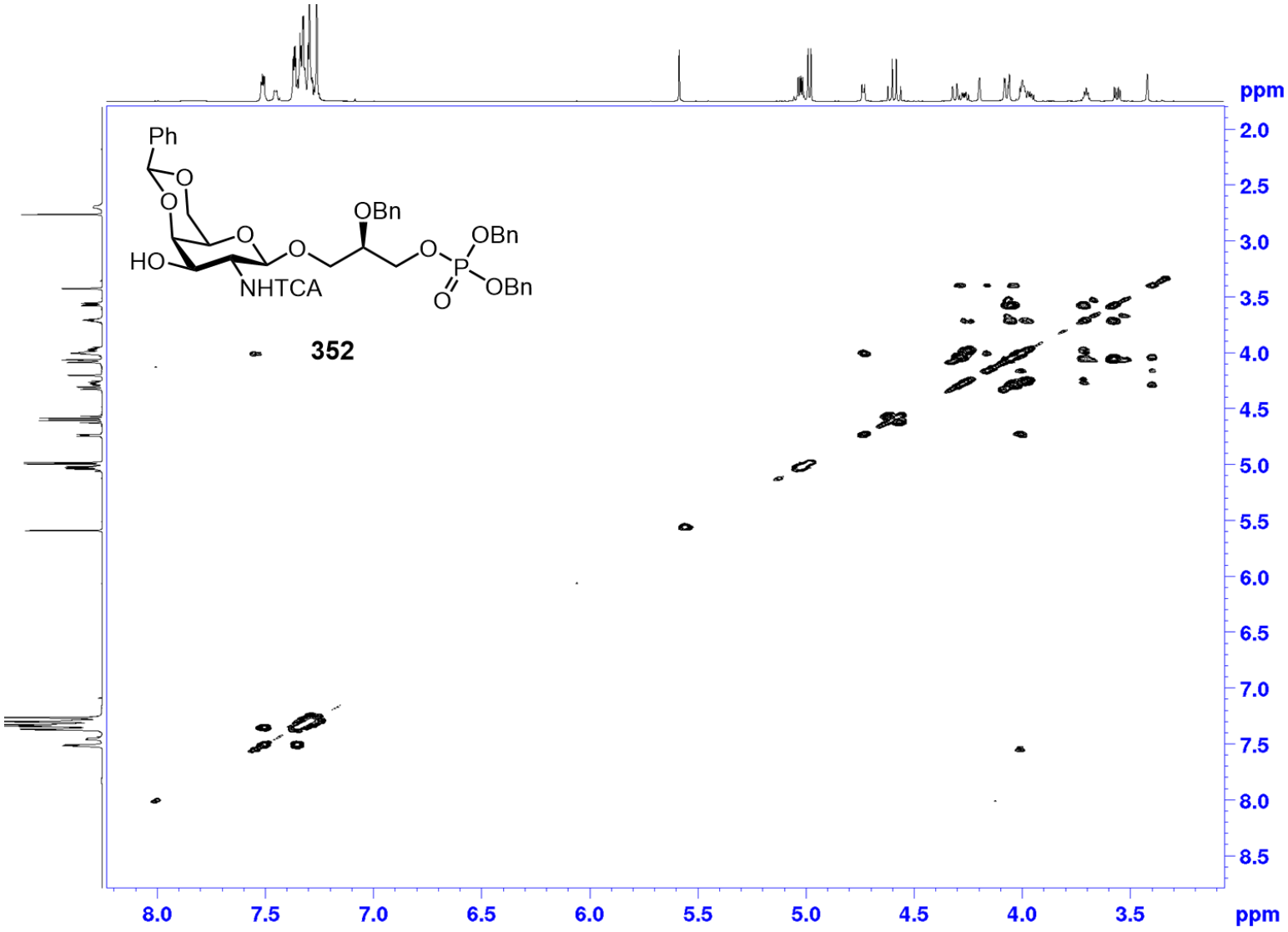
^{1}H-^{13}C HSCQ ($CDCl_3$) zoomed in region of compound 352



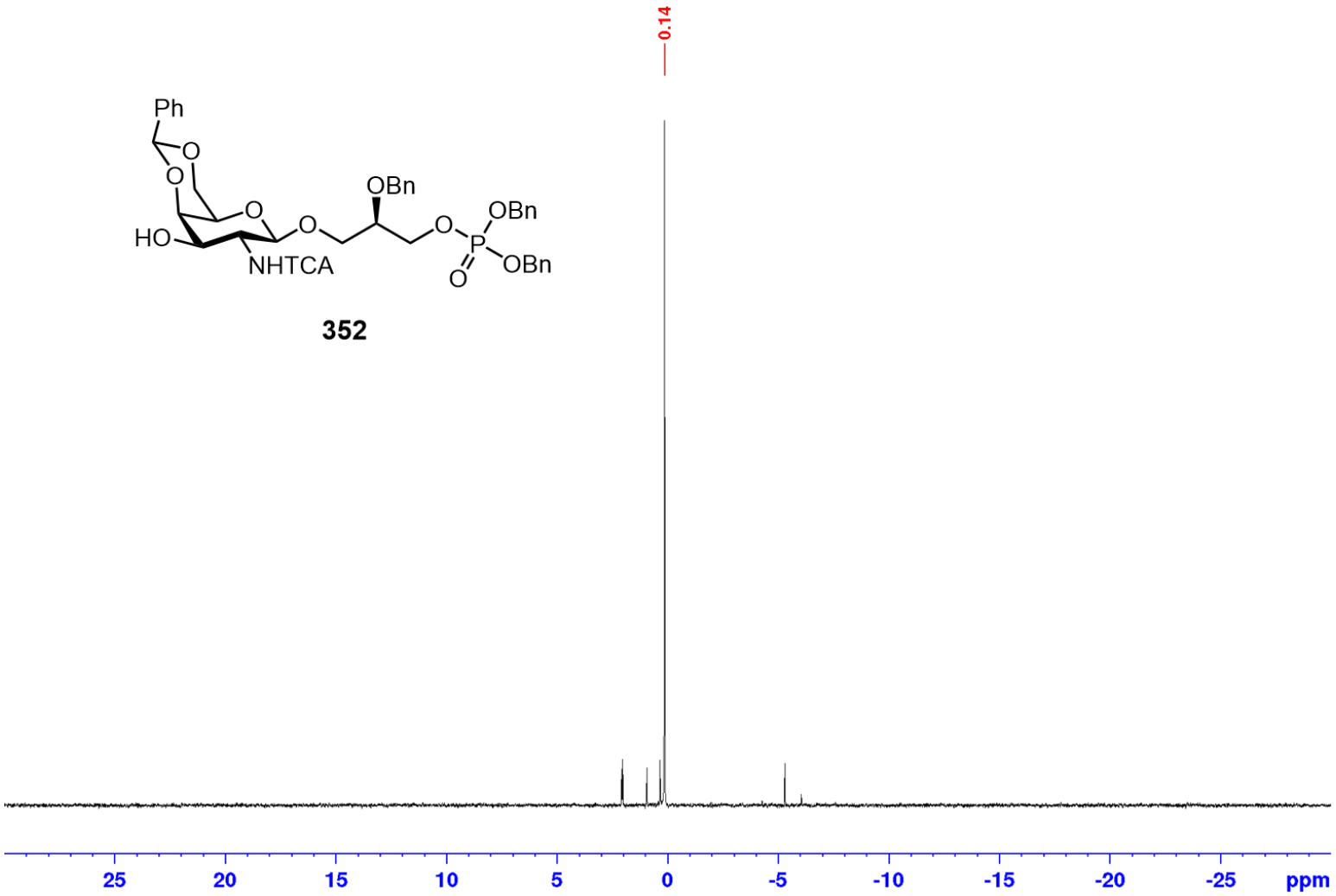
^{1}H-^{13}C HSCQ with ^{1}H-^{13}C HMBC overlay ($CDCl_3$) of compound 352



^{1}H-^{1}H COSY ($CDCl_3$) of compound 352



^{31}P NMR ($CDCl_3$, 162 MHz) of compound 352



352

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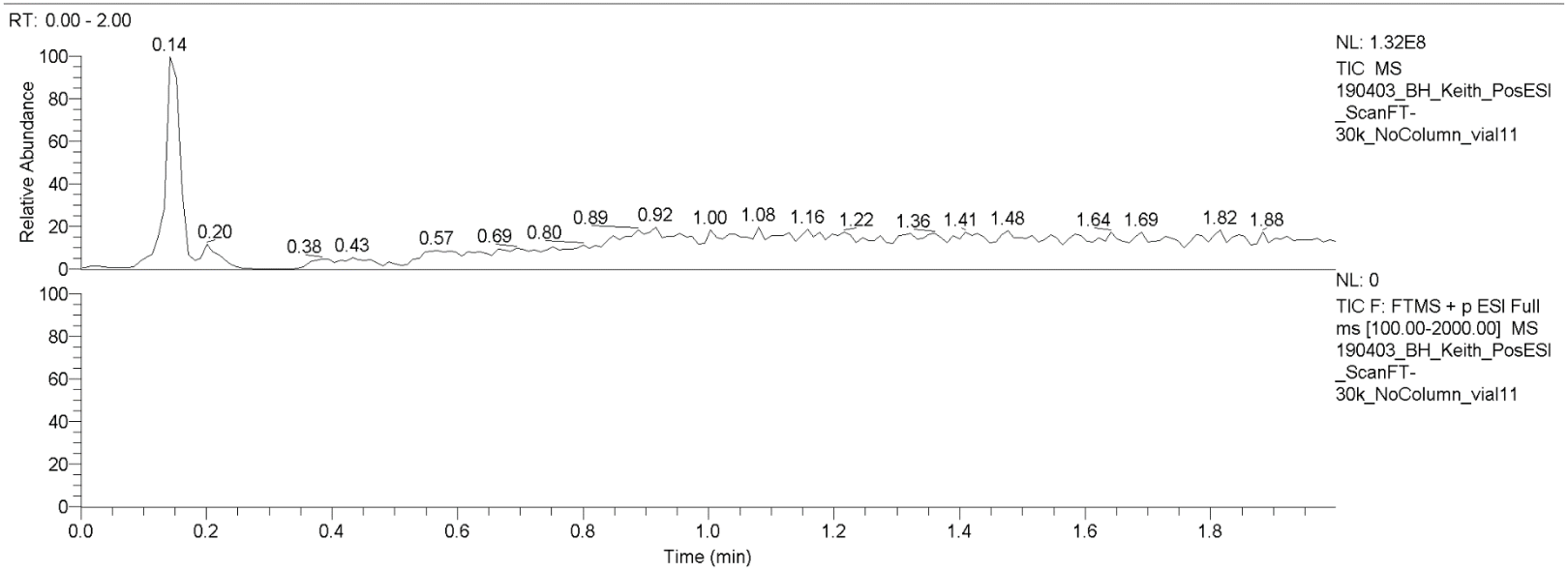
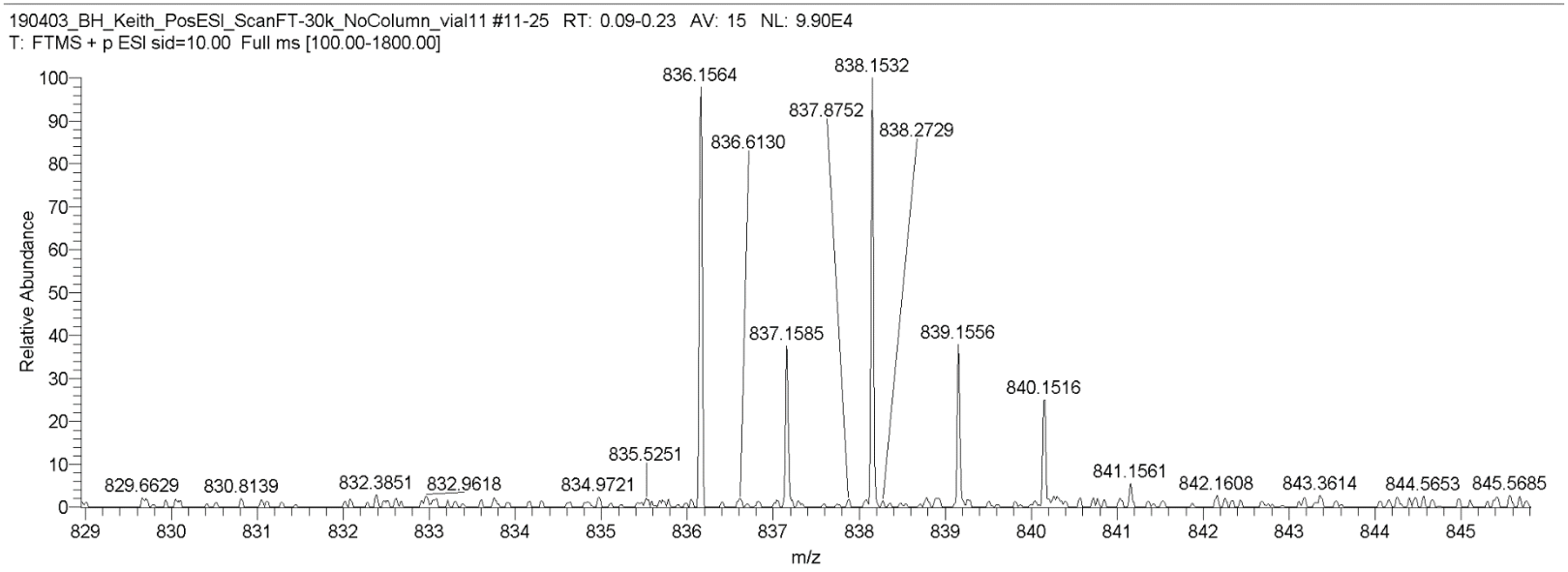
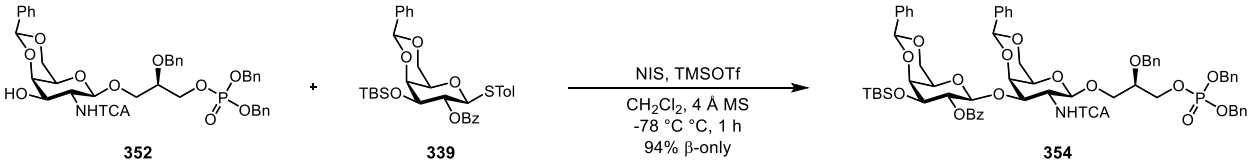




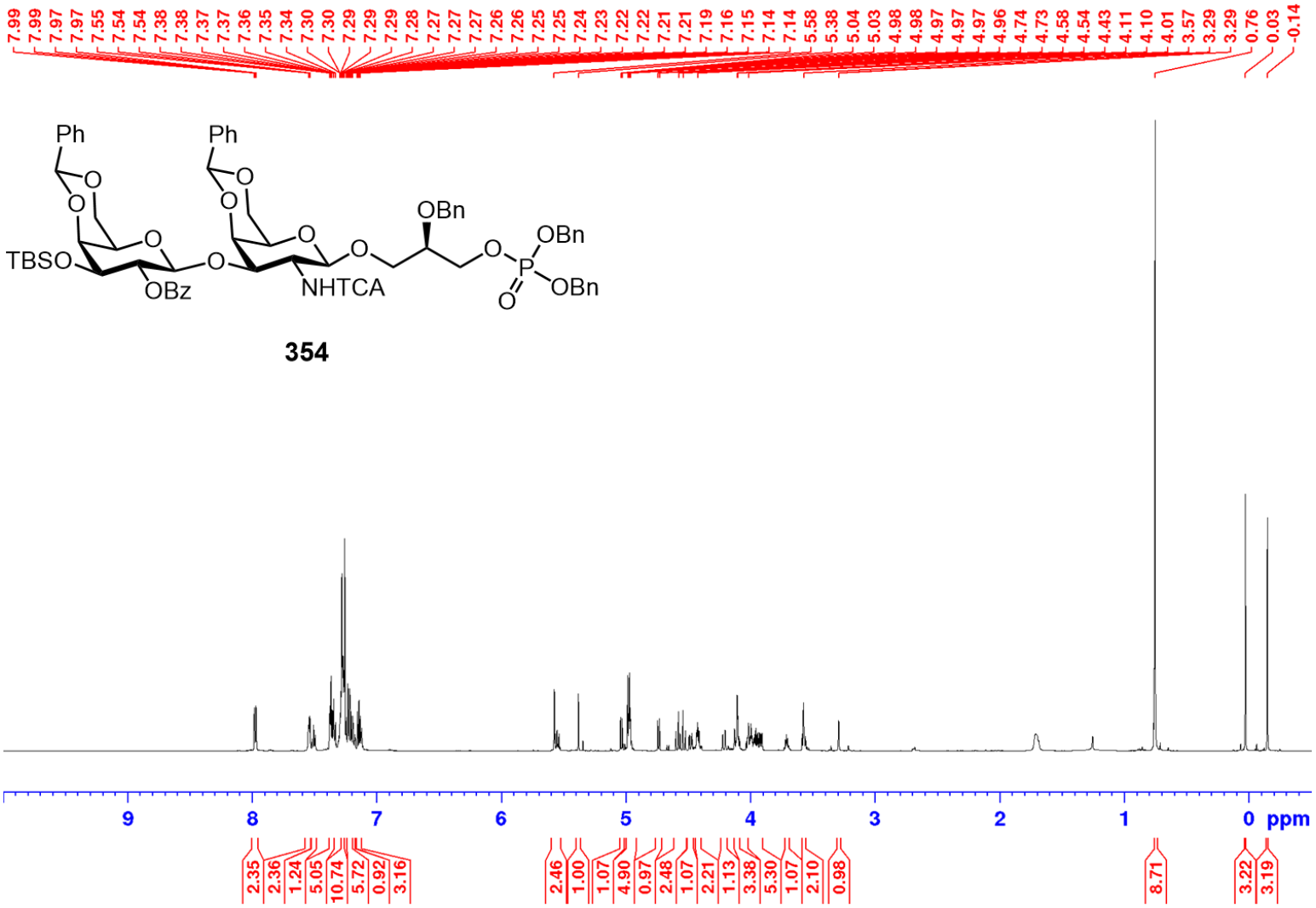
(*R*)-dibenzyl (2-(benzyloxy)-3-hydroxypropyl) phosphate 4,6-*O*-benzylidene acetal-2-*O*-benzoyl-3-*O*-*tert*-butyldimethylsilyl-β-(1→3)-D-galactopyranosyl-4,6-*O*-benzylidene acetal-2-deoxy-2-trichloroacetamido-β-D-galactopyranoside (354)



A solution of acceptor (*R*)-dibenzyl (2-(benzyloxy)-3-hydroxypropyl) phosphate 4,6-*O*-benzylidene acetal-2-deoxy-2-trichloroacetamido-β-D-galactopyranoside **352** (1.0 equiv., 1.866 g, 2.229 mmol) and donor *p*-tolyl 4,6-*O*-benzylidene acetal-3-*O*-*tert*-butyldimethylsilyl-2-*O*-benzoyl-1-thio-β-D-galactopyranoside **339** (1.5 equiv., 1.98 g, 3.34 mmol) in anhydrous CH_2Cl_2 (30 mL) under an argon atmosphere was added 4 Å MS (4.0 g) at room temperature and allowed to stir for 1h. The reaction mixture was cooled to -78 °C and added NIS (2.0 equiv., 1.003 g, 4.458 mmol). After 2 min, the crude reaction mixture was added a solution of TMSOTf (0.15 equiv., 1.2 mL, 5% in anhydrous CH_2Cl_2) dropwise. The reaction was held at -78 °C. At 1 h, TLC indicated complete consumption of the acceptor **352** starting material. The reaction was quench by the addition of dilute Et_3N in CH_2Cl_2 while warming to room temperature. The reaction was filtered through a glass fritted vacuum filter funnel equipped with a pad of Celite to remove the 4 Å MS, concentrated to a crude oil. The crude oil was then diluted with EtOAc (100 mL) and transferred to a separatory funnel and washed with satd. aq. Na_2SO_4 (5 x 20 mL), brine (1 x 20 mL), dried ($NaSO_4$) and filtered through a glass fritted vacuum filter funnel to remove drying agent. The filtrate was concentrated to a crude oil which was purified by flash column chromatography (5:1 to 9:1 EtOAc/hexanes) give the disaccharide (*R*)-

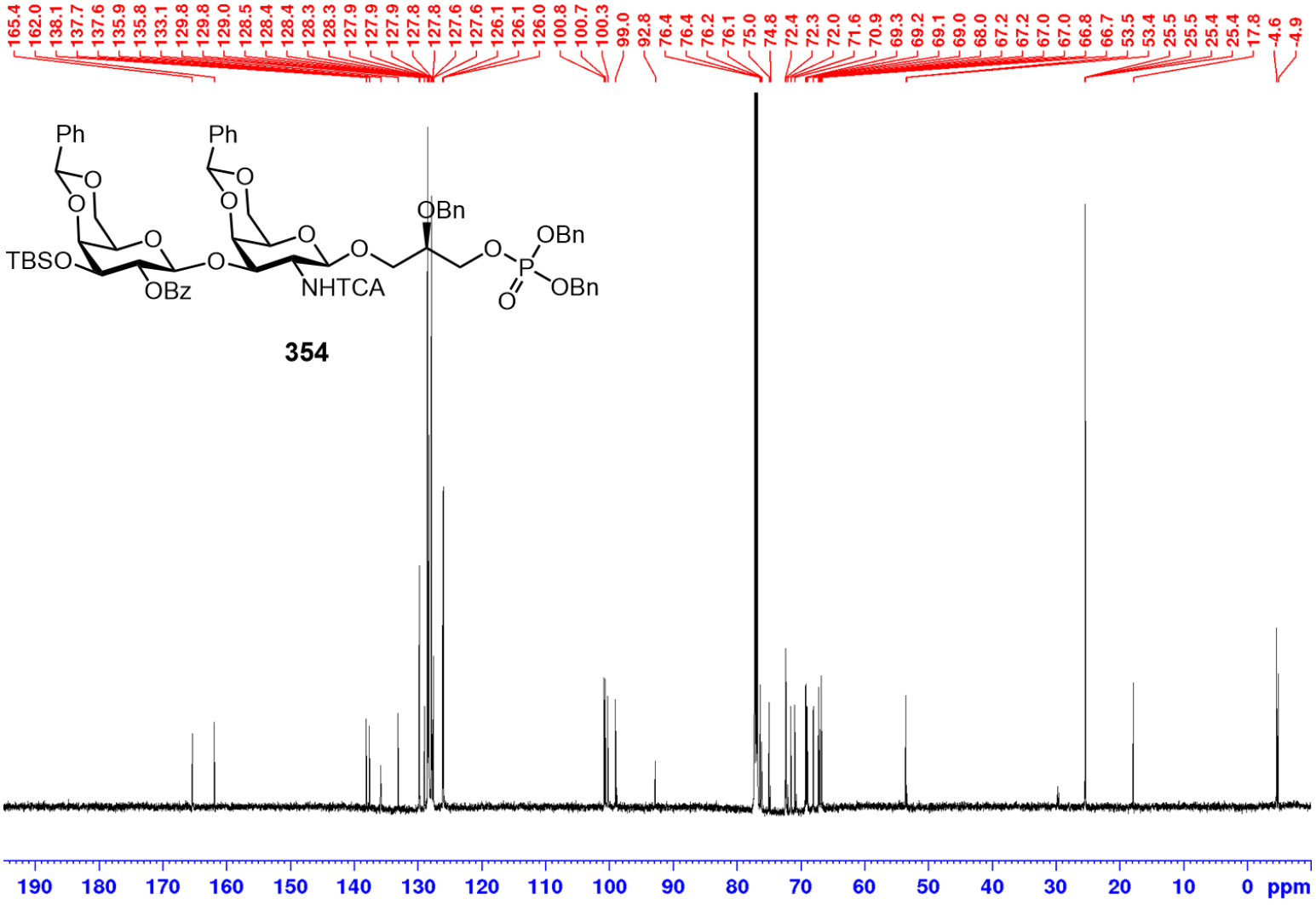
dibenzyl (2-(benzyloxy)-3-hydroxypropyl) phosphate 4,6-*O*-benzylidene acetal-2-*O*-benzoyl-3-*O*-*tert*-butyldimethylsilyl-β-(1→3)-D-galactopyranosyl-4,6-*O*-benzylidene acetal-2-deoxy-2-trichloroacetamido-β-D-galactopyranoside **354** (2.631 g, 2.015 mmol, 94%, β-only) as white solid. R_f = 0.40 (80:20 EtOAc/hexanes) visualized with ceric ammonium molybdate stain; $[\alpha]D^{25}$ = +6.88° (c = 1.0, $CHCl_3$); IR ν_{max} (film) 3409.24, 1708.02, 1639.38, 1453.83, 1368.21, 1267.92, 1187.82, 1087.65, 1011.56, 837.17, 821.84, 731.64, 697.13 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.98 (dd, *J* = 1,3 8.1 Hz, 2H), 7.55-7.54 (m, 23H), 7.52-7.50 (m, 1H), 7.38-7.34 (m, 5H), 7.28-7.26 (m, 10H), 7.24-7.19 (m, 5H), 7.19-7.17 (m, 1H), 7.16-7.12 (m, 3H), 5.58-5.52 (m, 2H), 5.38 (s, 1H), 5.03 (d, *J* = 8.3 Hz, 1H), 4.98-4.95 (m, 5H), 4.73 (d, *J* = 8.3 Hz, 1H), 4.58 (d, *J* = 11.8, 1H), 4.53 (d, 11.8 Hz, 1H), 4.48 (dd, *J* = 3.40, 11.0 Hz, 1H), 4.43-4.40 (m, 2H), 4.21 (dd, *J* = 1.2, 12.5 Hz, 1H), 4.13-4.09 (m, 3H), 4.03-3.91 (m, 5H), 3.72-3.68 (m, 1H), 3.58-3.55 (m, 2H), 3.29 (s, 1H), 0.76 (s, 9H), 0.03 (s, 3H), -.14 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 165.4, 162.0, 138.1, 137.7, 137.6, 135.9, 135.8, 133.1, 129.8, 129.8, 129.0, 128.5, 128.5, 128.4, 128.4, 128.4, 128.4, 128.3, 128.3, 128.3, 128.0, 127.9, 127.9, 127.9, 127.9, 127.8, 127.8, 127.8, 127.6, 127.6, 126.1, 126.1, 126.0, 100.8, 100.7, 100.3, 99.0, 92.8, 76.4, 76.4, 76.2, 76.1, 75.0, 74.8, 72.4, 72.3, 72.0, 71.6, 70.9, 69.3, 69.2, 69.1, 69.0, 68.0, 67.2, 67.2, 67.0, 67.0, 66.8, 66.7, 53.5, 53.4, 29.7, 25.5, 25.5, 25.4, 25.4, 17.8, -4.6, -4.9; HR-ESI-MS (m/z): calcd for $C_{65}H_{74}Cl_3NO_{17}PSi^+$ $(M+H)^+$ 1304.352376, found 1304.3537.

^{1}H NMR ($CDCl_3$, 400 MHz) of compound 354

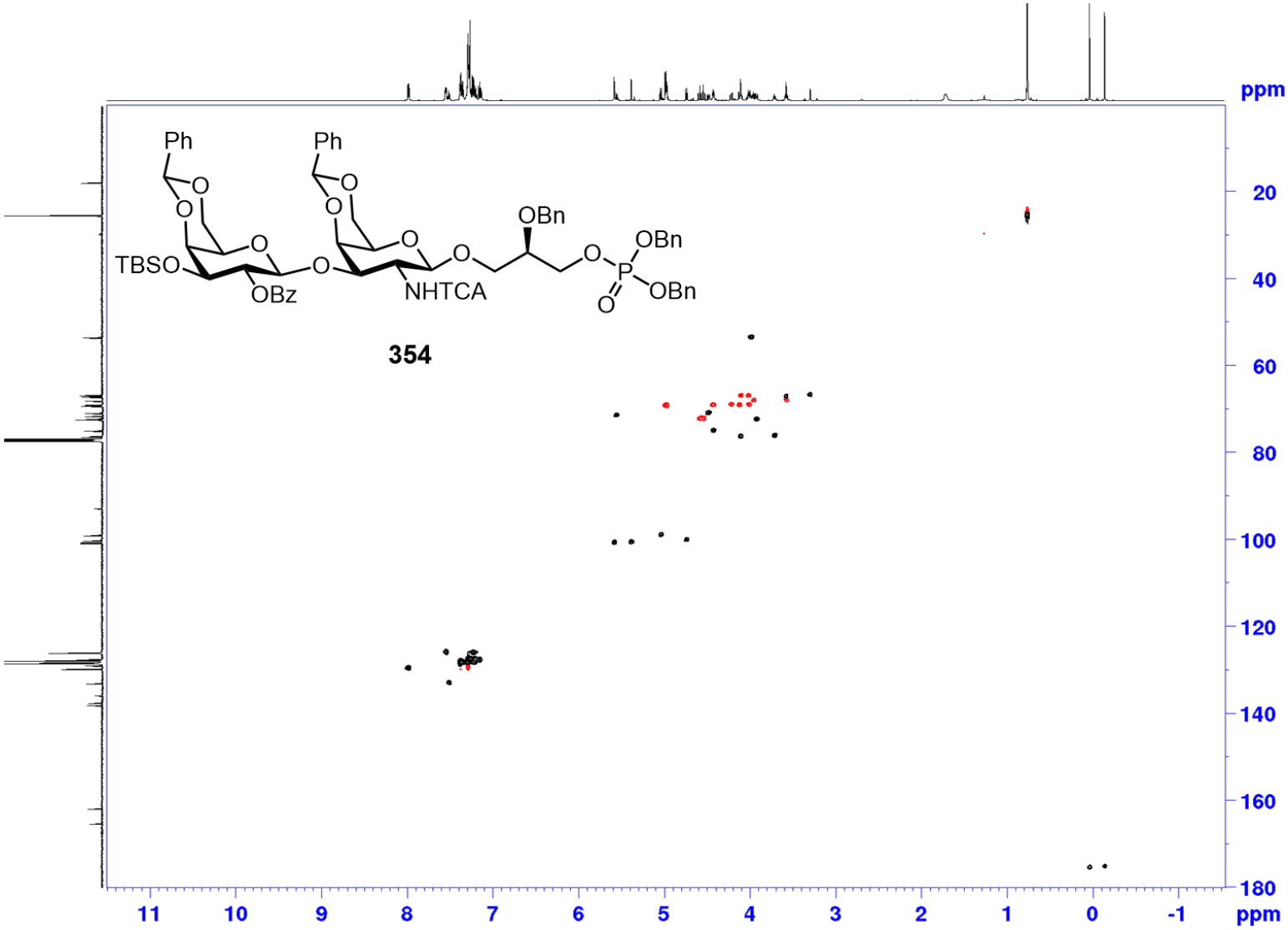


354

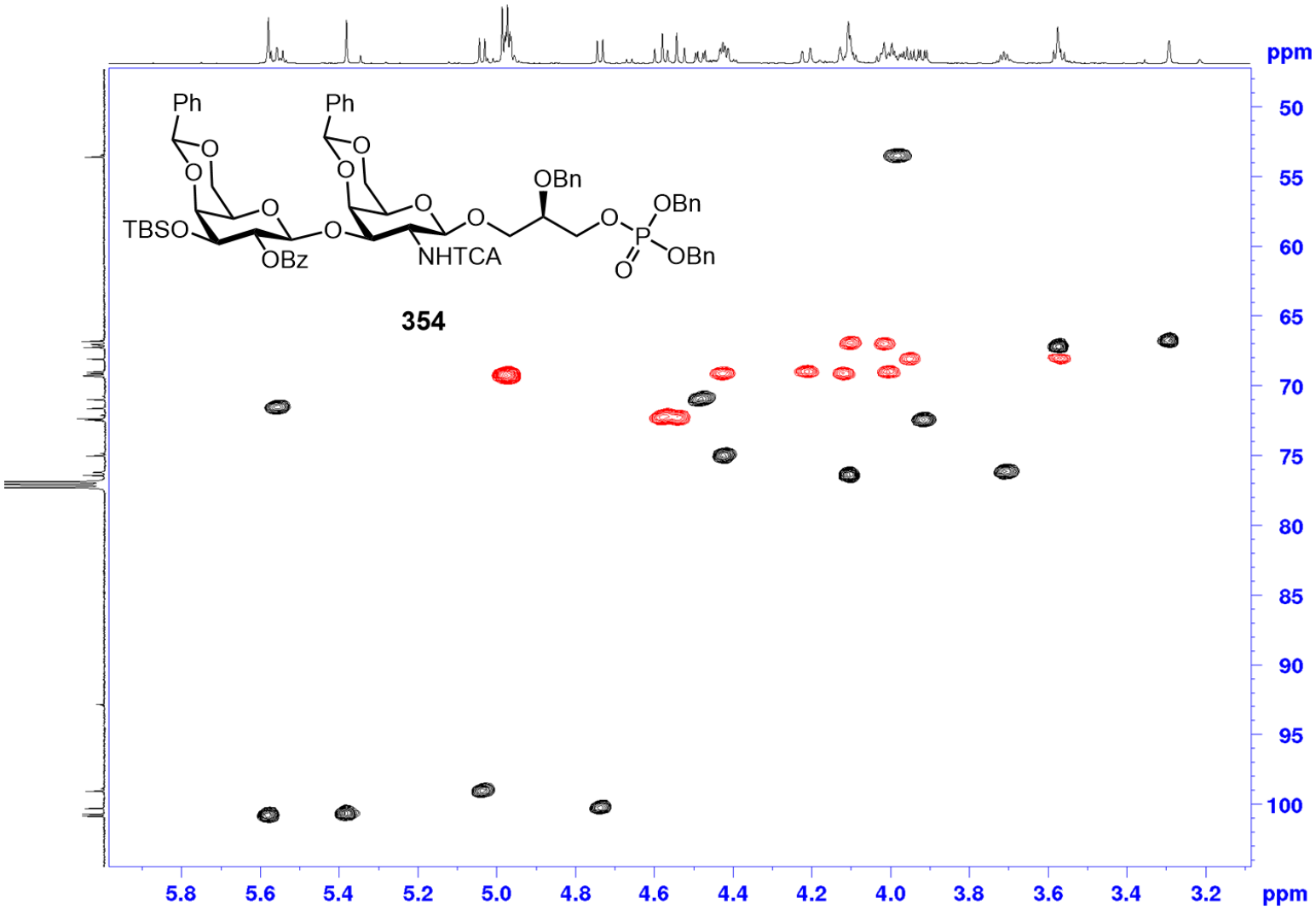
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 354



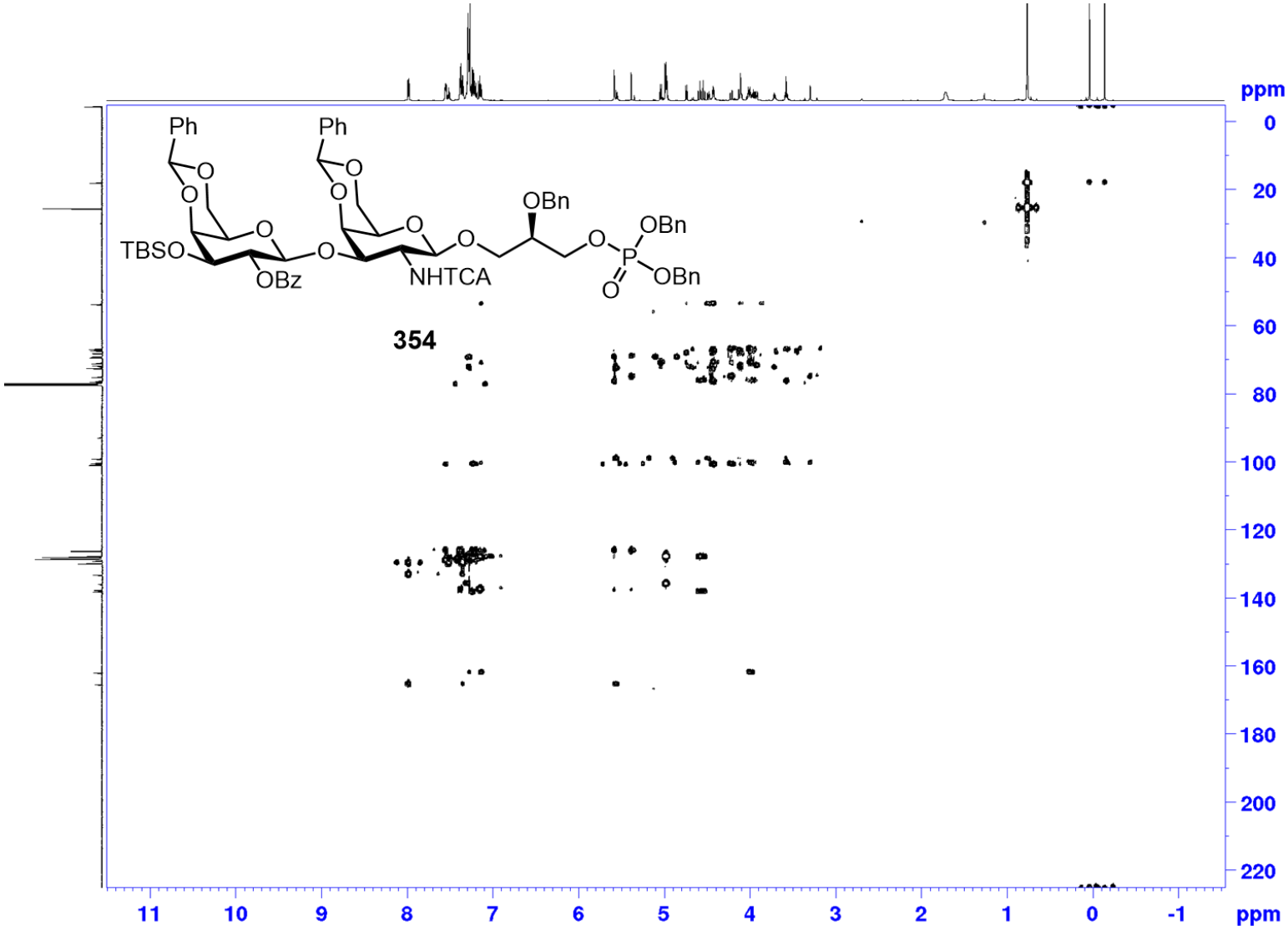
^{1}H-^{13}C HSCQ ($CDCl_3$) of compound 354



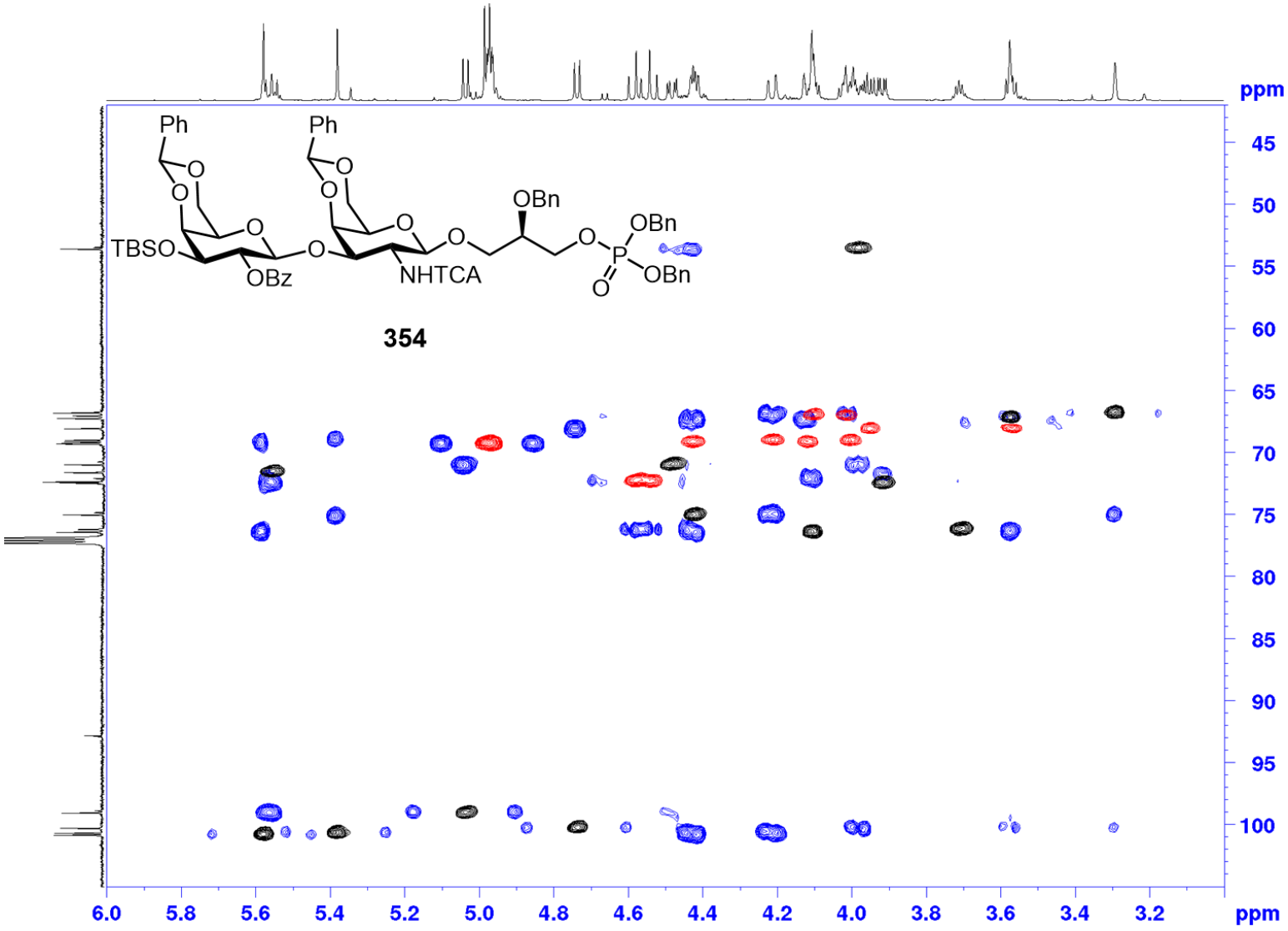
^{1}H-^{13}C HSCQ ($CDCl_3$) zoomed in region of compound 354



^{1}H-^{13}C HMBC ($CDCl_3$) of compound 354



^{1}H-^{13}C HSCQ with ^{1}H-^{13}C HMBC overlay ($CDCl_3$) of compound 354



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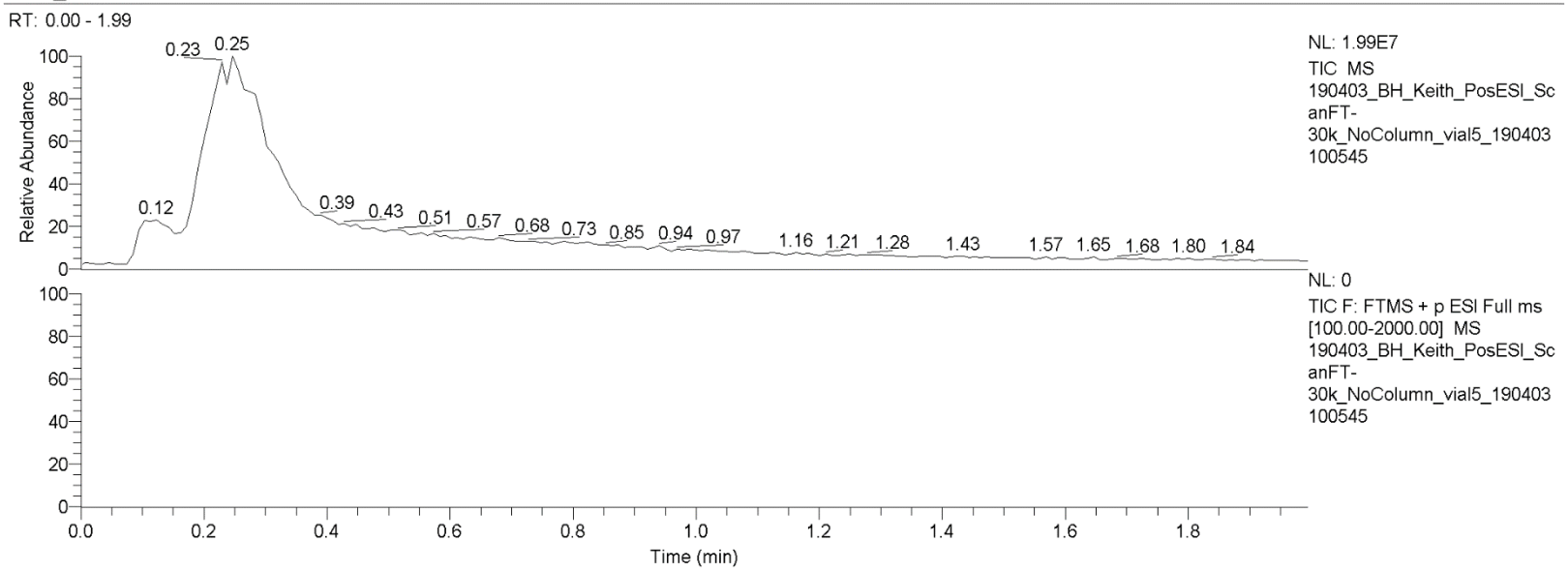
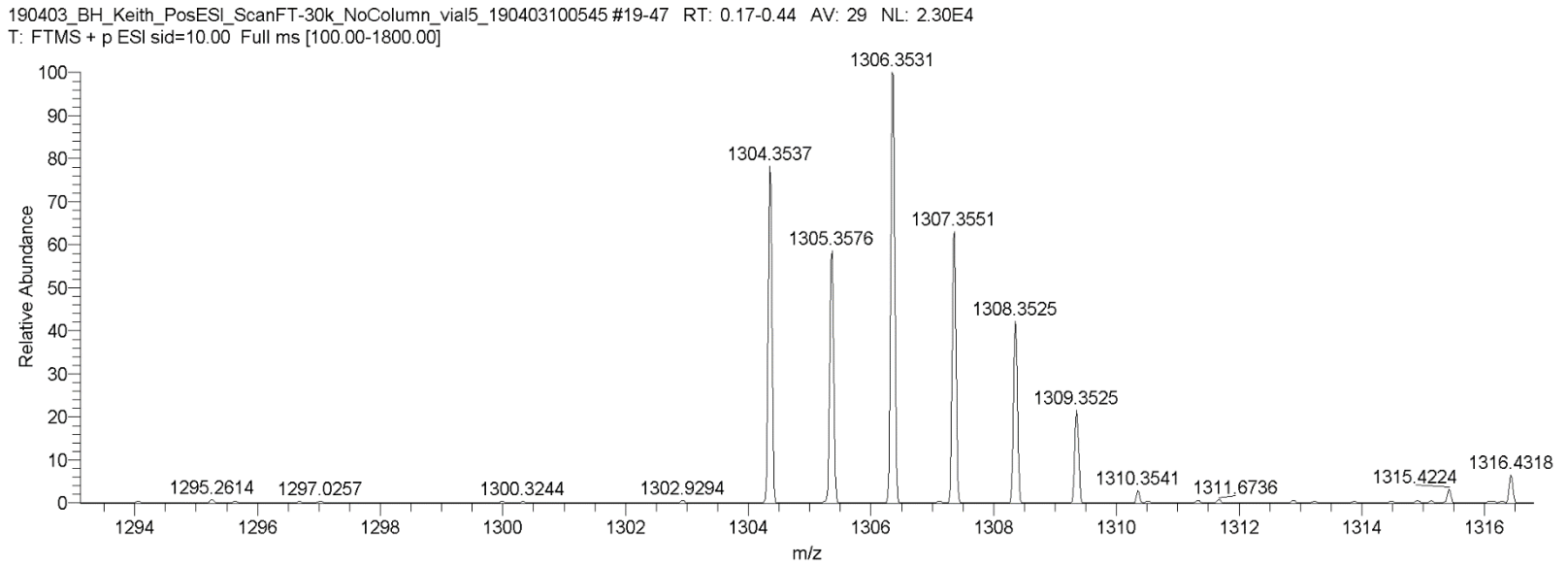
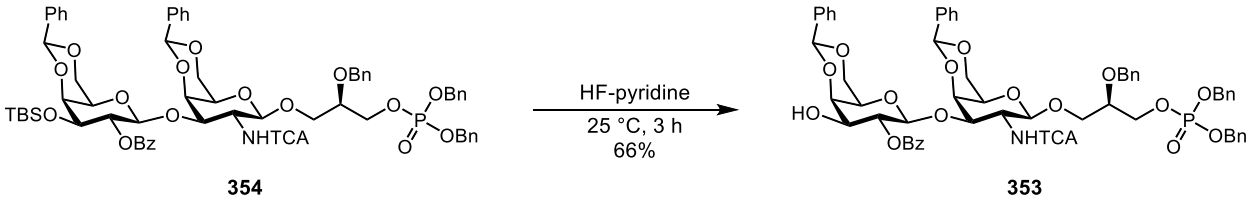




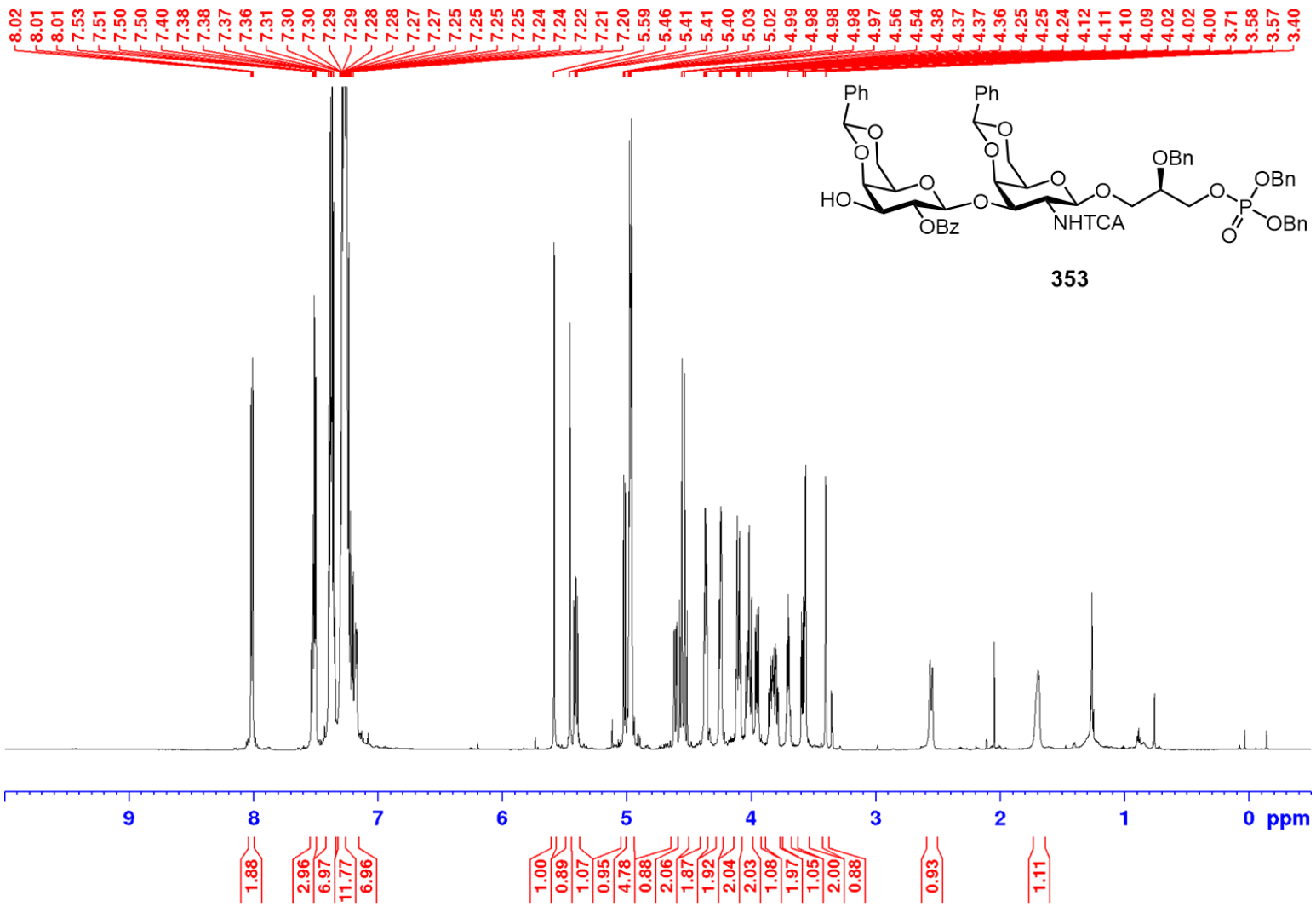
(*R*)-dibenzyl (2-(benzyloxy)-3-hydroxypropyl) phosphate 4,6-*O*-benzylidene acetal-2-*O*-benzoyl-β-(1→3)-D-galactopyranosyl-4,6-*O*-benzylidene acetal-2-deoxy-2-trichloroacetamido-β-D-galactopyranoside (353)



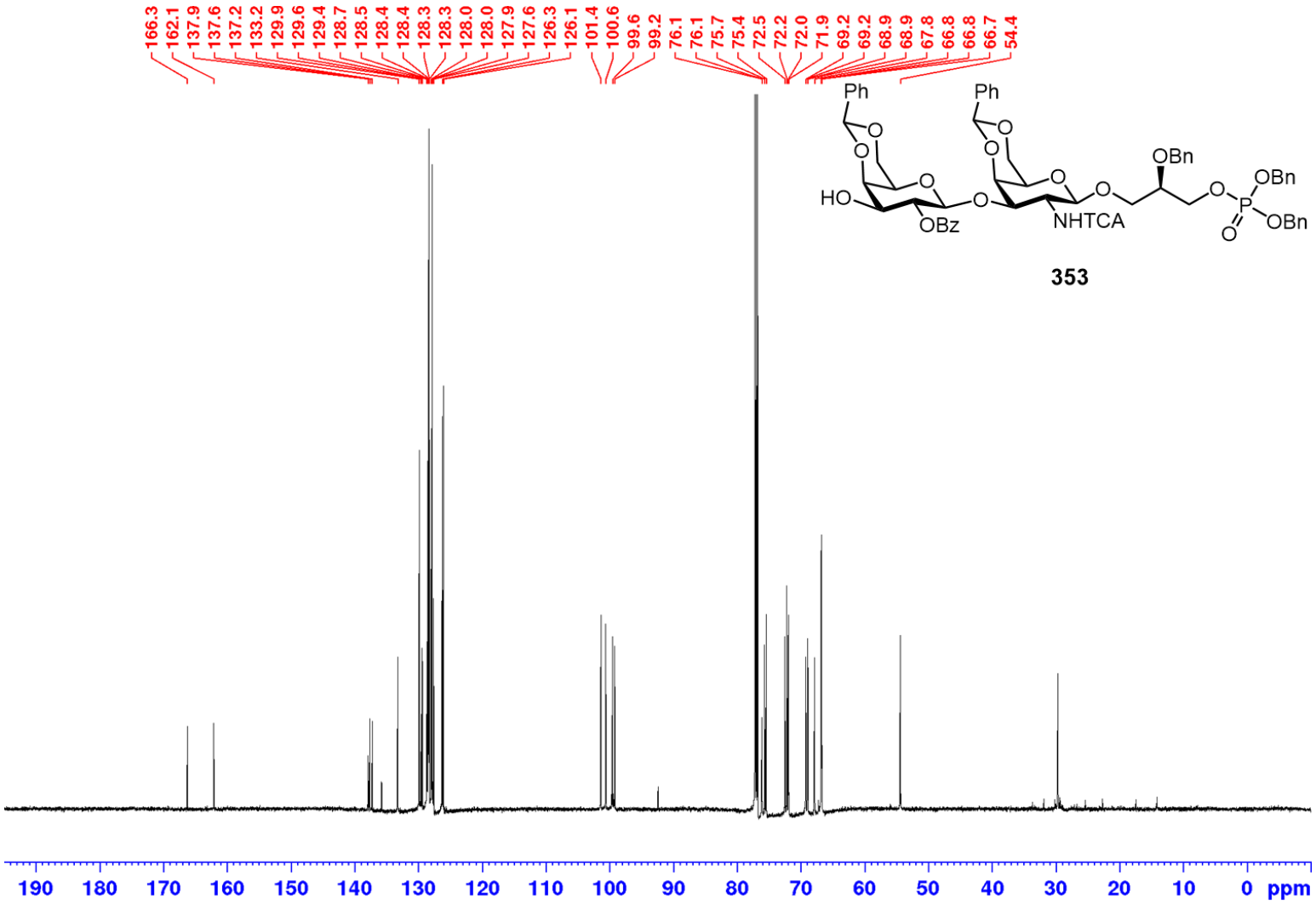
A solution of disaccharide (*R*)-dibenzyl (2-(benzyloxy)-3-hydroxypropyl) phosphate 4,6-*O*-benzylidene acetal-2-*O*-benzoyl-3-*O*-*tert*-butyldimethylsilyl-β-(1→3)-D-galactopyranosyl-4,6-*O*-benzylidene acetal-2-deoxy-2-trichloroacetamido-β-D-galactopyranoside **354** (1.0 equiv., 2.611 g, 2.0151 mmol) in pyridine (20.0 mL) was added a 70% HF-pyridine solution (10 equiv., 2.6 mL) at 0 °C under an argon atmosphere. The reaction was stirred at room temperature for 3 h when TLC indicated complete consumption of starting material. The reaction was diluted with EtOAc (100 mL) and transferred to a separatory funnel and washed with 1 N HCl (5 x 20 mL), satd. aq. $NaHCO_3$ (1 x 20 mL), brine (1 x 20 mL), dried ($NaSO_4$) and filtered through a glass fritted vacuum filter funnel to remove drying agent. The filtrate was concentrated to a crude oil which was purified by flash column chromatography (50:50 then 55:45 to 90:10 EtOAc/hexanes) give the disaccharide acceptor (*R*)-dibenzyl (2-(benzyloxy)-3-hydroxypropyl) phosphate 4,6-*O*-benzylidene acetal-2-*O*-benzoyl-β-(1→3)-D-galactopyranosyl-4,6-*O*-benzylidene acetal-2-deoxy-2-trichloroacetamido-β-D-galactopyranoside **353** (1.5843 g, 1.330 mmol, 66%) as white foam. R_f = 0.30 (10:90 CH_3OH/CH_2Cl_2) visualized with ceric ammonium molybdate stain; $[\alpha]D^{25}$ = -5.32° (c = 1.0, $CHCl_3$); IR ν_{max} (film) 3445.77, 1717.67, 1637.37, 1541.63, 1454.39, 1367.27, 1267.72, 1173.90, 1089.43, 1023.37, 821.32, 734.42, 698.13

cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.02-8.01 (m, 2H), 7.54-7.50 (m, 3H), 7.40-7.35 (m, 7H), 7.32-7.27 (m, 11H), 7.25-7.17 (m, 7H), 5.59 (s, 1H), 5.46 (s, 1H), 5.41 (dd, *J* = 8.1, 9.7 Hz, 1H), 5.02 (d, *J* = 8.5 Hz, 1H), 4.99-4.97 (m, 5H), 4.61 (dd, *J* = 3.5, 11.3 Hz, 1H), 4.56 (dd, *J* = 11.8 Hz, 1H), 4.52 (dd, *J* = 11.8 Hz, 1H), 4.38-4.36 (m, 2H), 4.26-4.24 (m, 2H), 4.12-4.09 (m, 2H), 4.05-4.00 (m, 1H), 3.97-3.94 (m, 2H), 3.86-3.79 (m, 1H), 3.72-3.69 (m, 2H), 3.60-3.56 (s, 2H), 3.40 (s, 1H), 1.68 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.3, 162.1, 137.9, 137.6, 137.2, 133.2, 129.9, 129.9, 129.6, 129.4, 128.7, 128.5, 128.5, 128.4, 128.4, 128.3, 128.3, 128.2, 128.2, 128.0, 128.0, 128.0, 127.9, 127.9, 127.8, 127.6, 126.3, 126.1, 101.4, 100.6, 99.6, 99.2, 76.1, 76.1, 75.7, 75.4, 72.5, 72.2, 72.0, 71.9, 69.2, 69.2, 68.9, 68.9, 67.8, 66.8, 66.8, 66.7, 54.4; ^{31}P NMR (151 MHz, $CDCl_3$) δ 0.20; HR-ESI-MS (m/z): calcd for $C_{59}H_{60}Cl_3NO_{17}P^+$ $(M+H)^+$ 1190.265876, found 1190.2669.

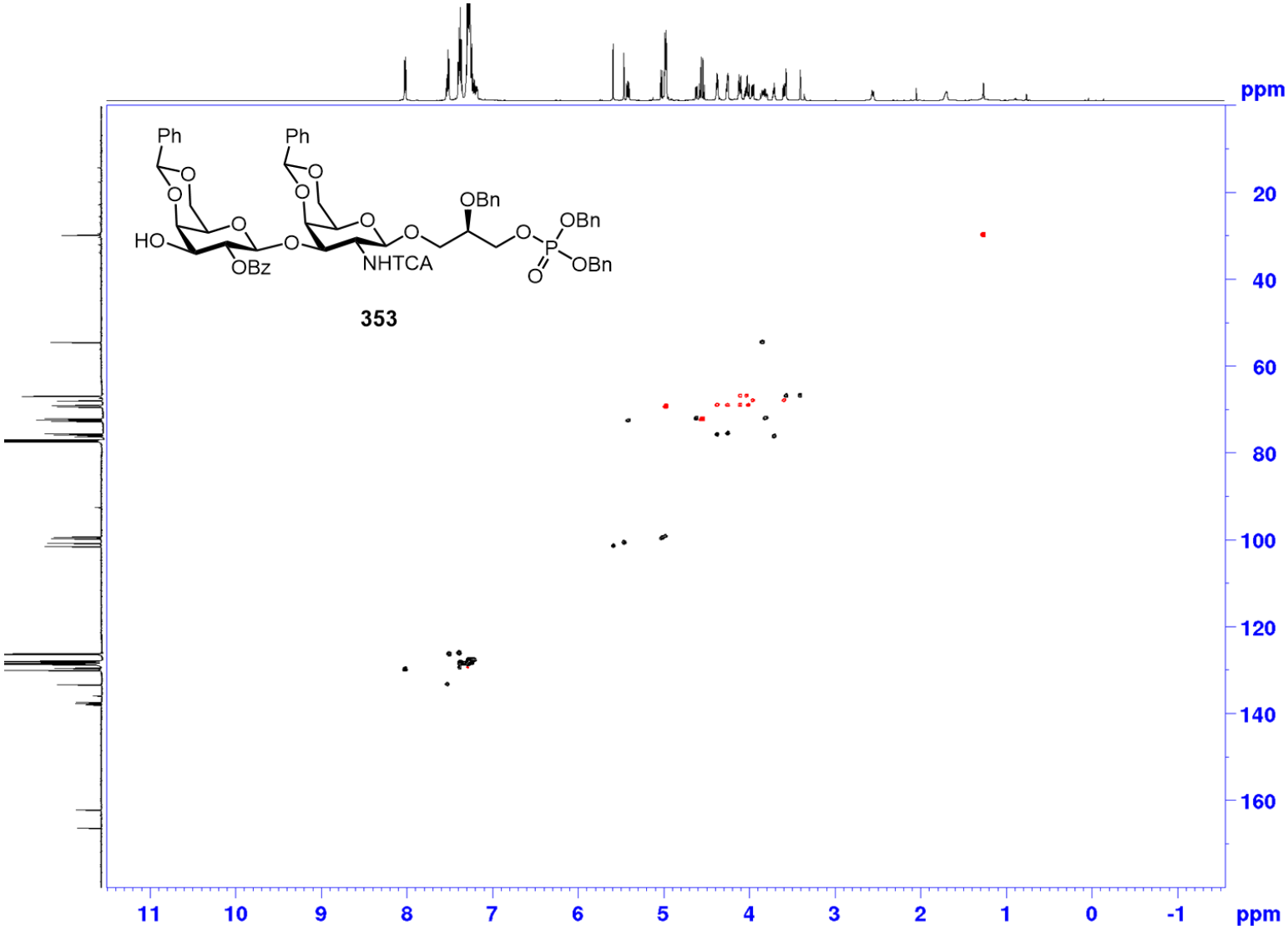
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 353



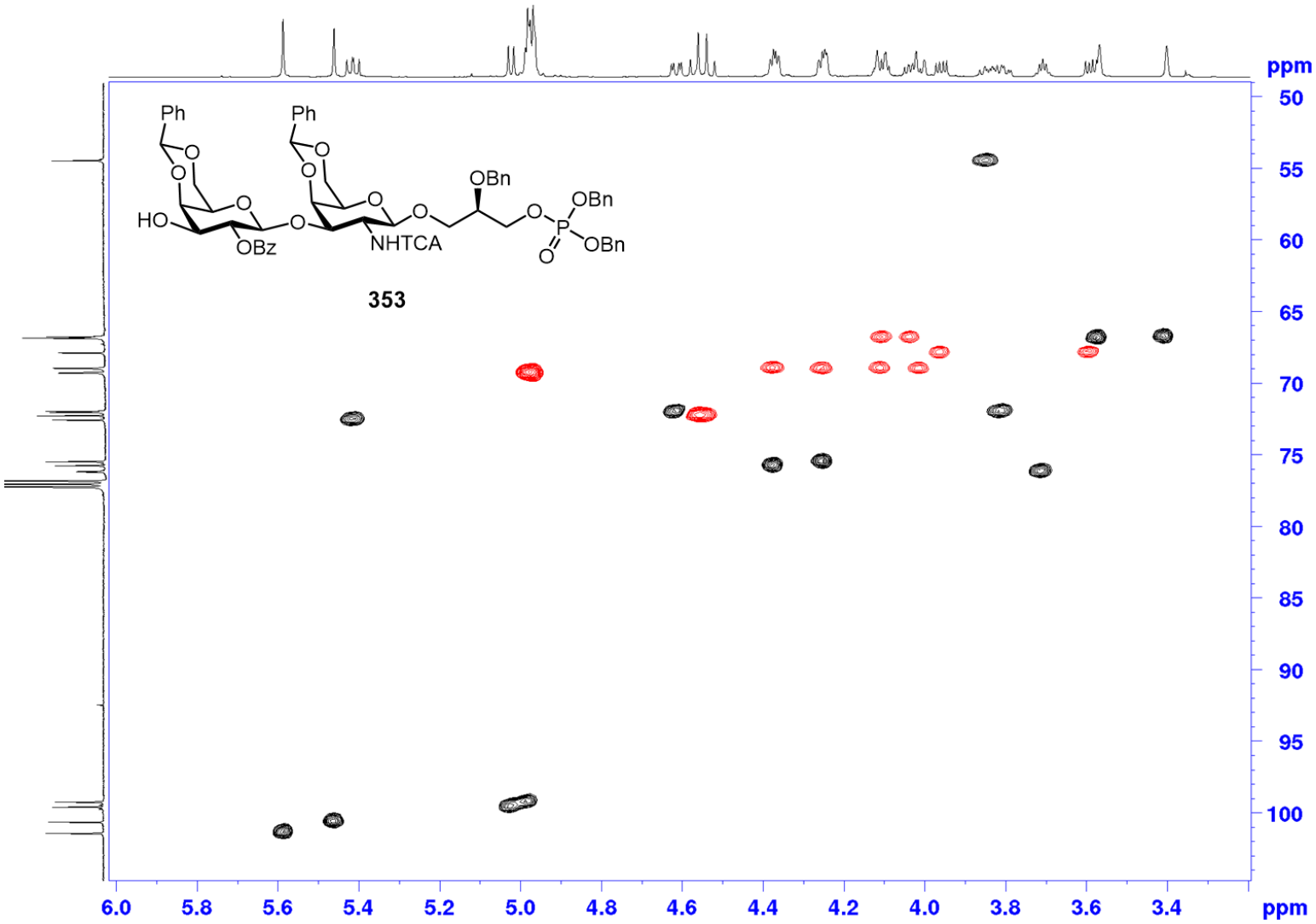
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 353



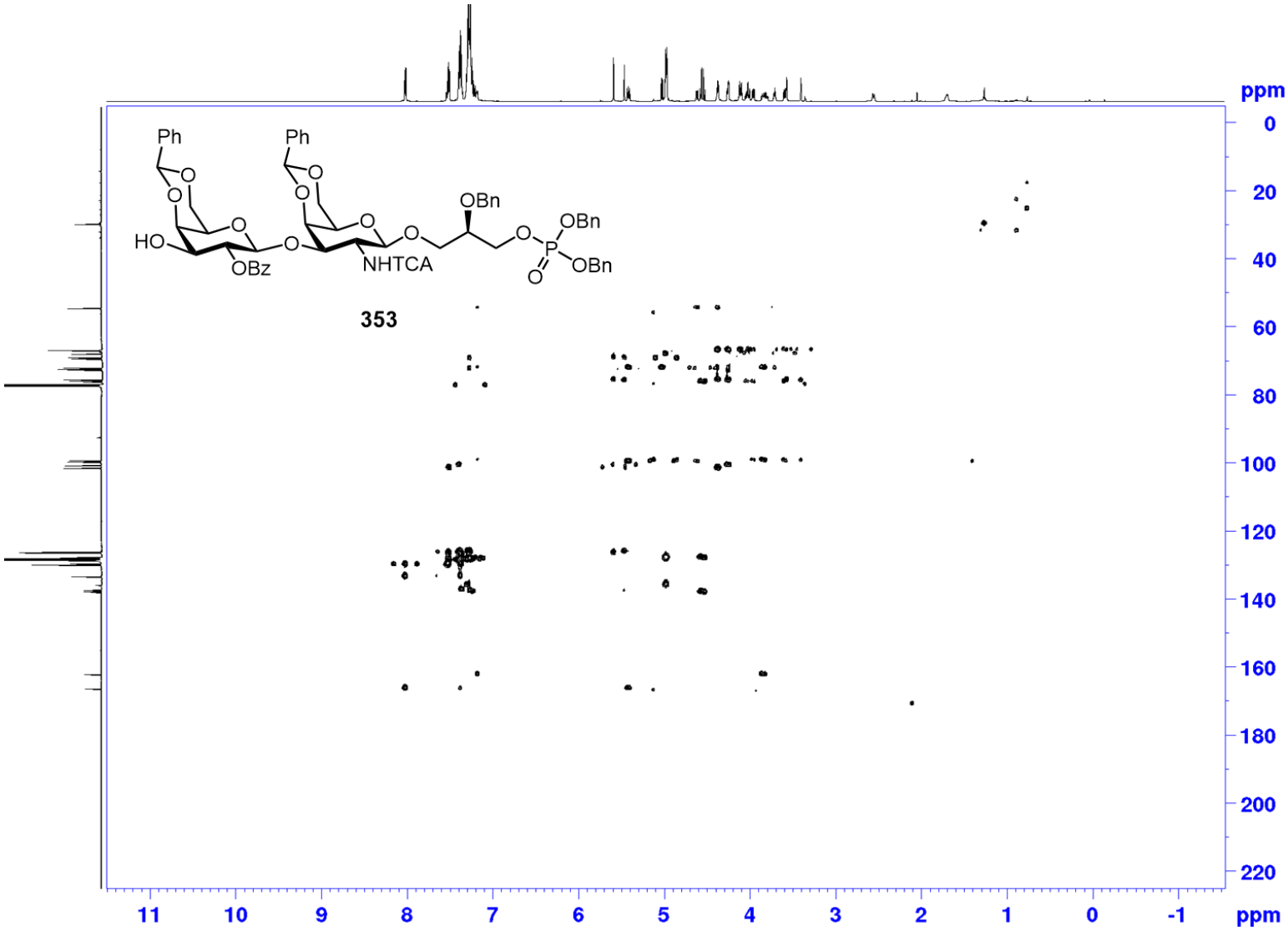
^{1}H-^{13}C HSCQ ($CDCl_3$) of compound 353



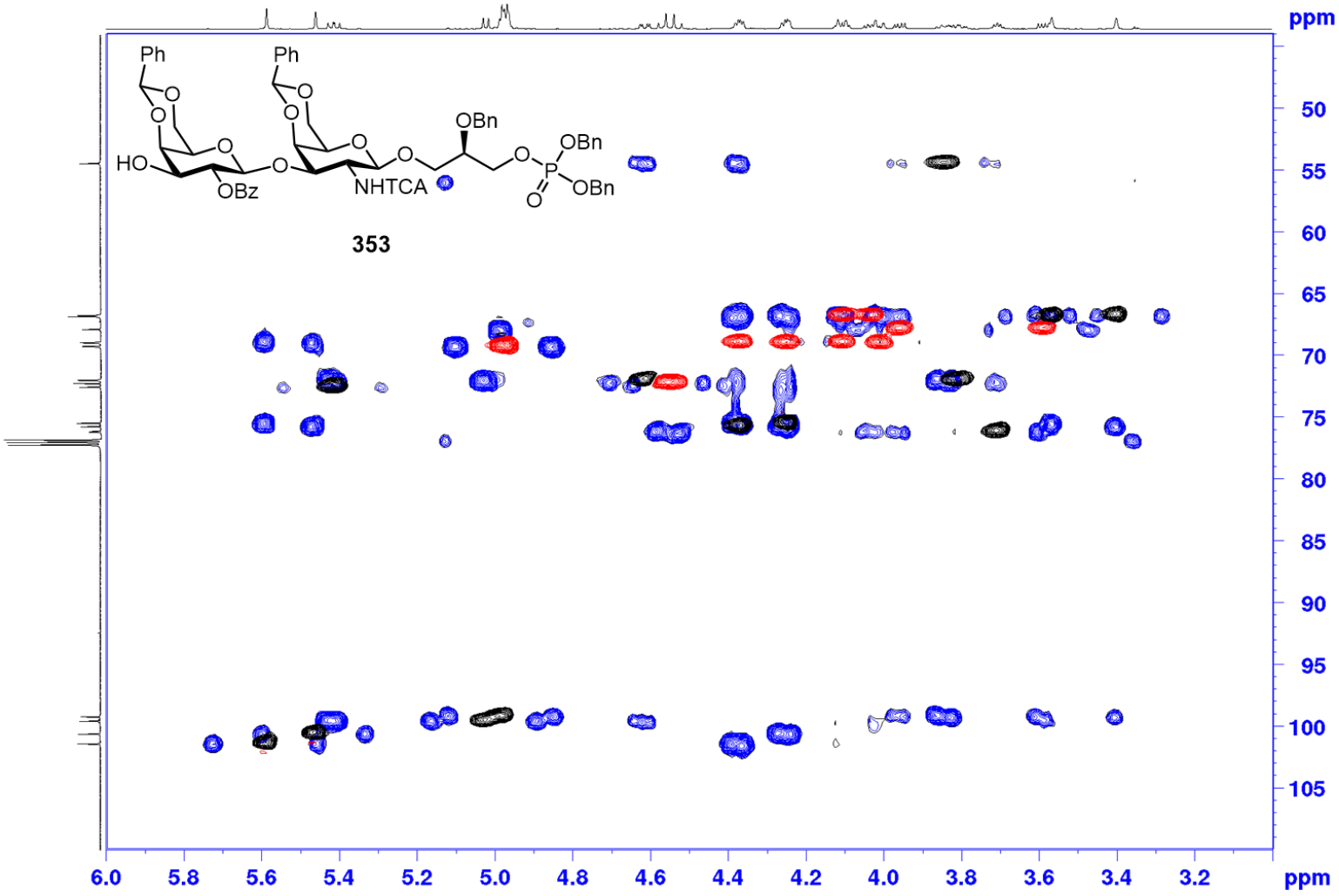
^{1}H-^{13}C HSCQ ($CDCl_3$) zoomed in region of compound 353



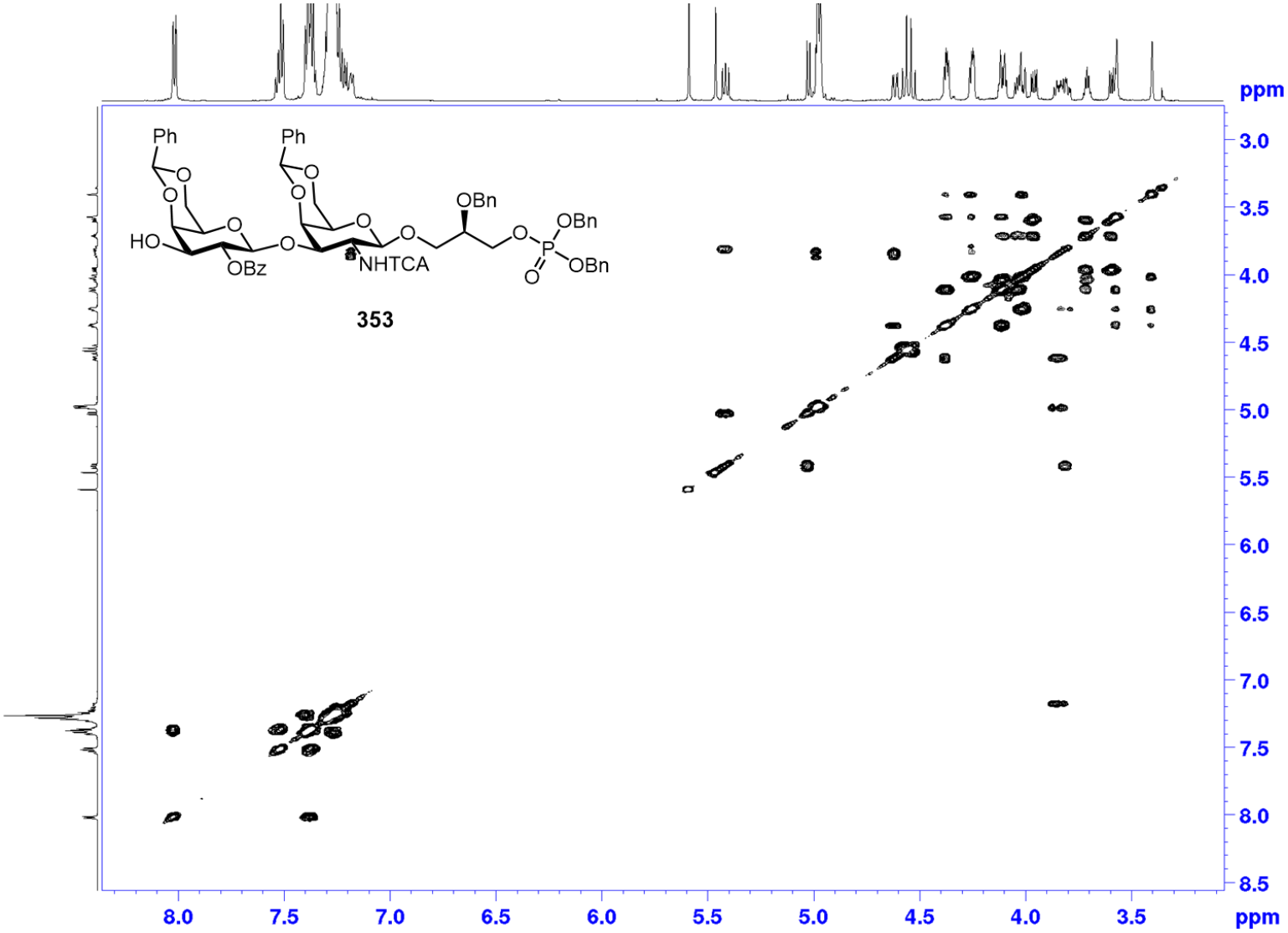
^{1}H-^{13}C HMBC ($CDCl_3$) of compound 353



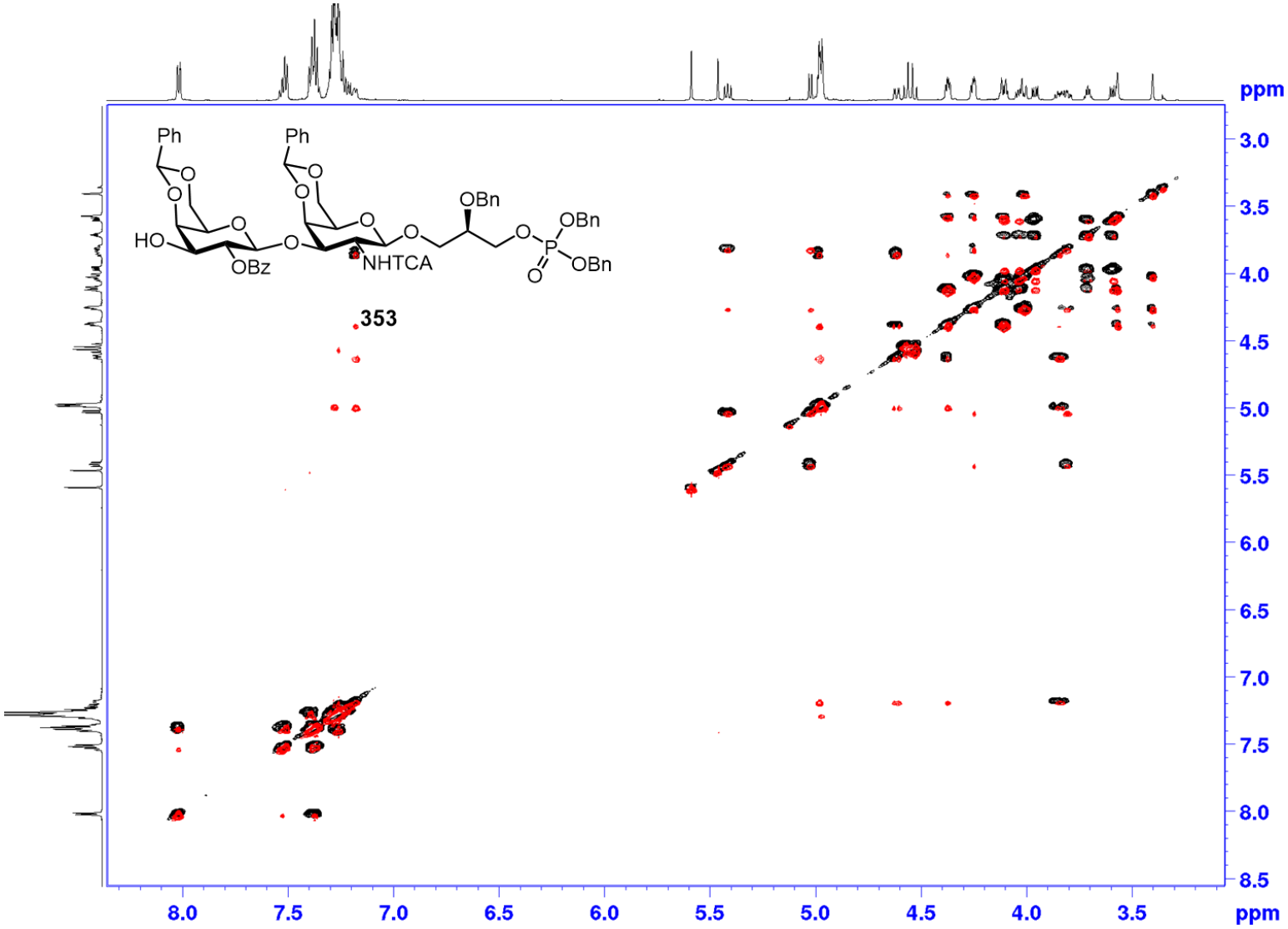
^{1}H-^{13}C HSCQ with ^{1}H-^{13}C HMBC overlay ($CDCl_3$) of compound 353



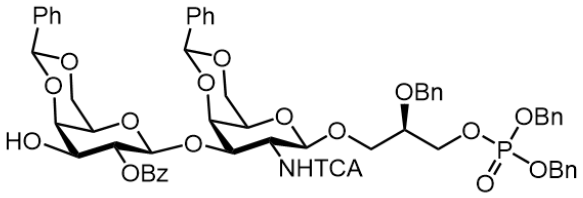
^{1}H-^{1}H COSY ($CDCl_3$) of compound 353



^{1}H-^{1}H COSY with ^{1}H-^{1}H TOSY overlay ($CDCl_3$) of compound 353



^{31}P NMR ($CDCl_3$, 162 MHz) of compound 353



353

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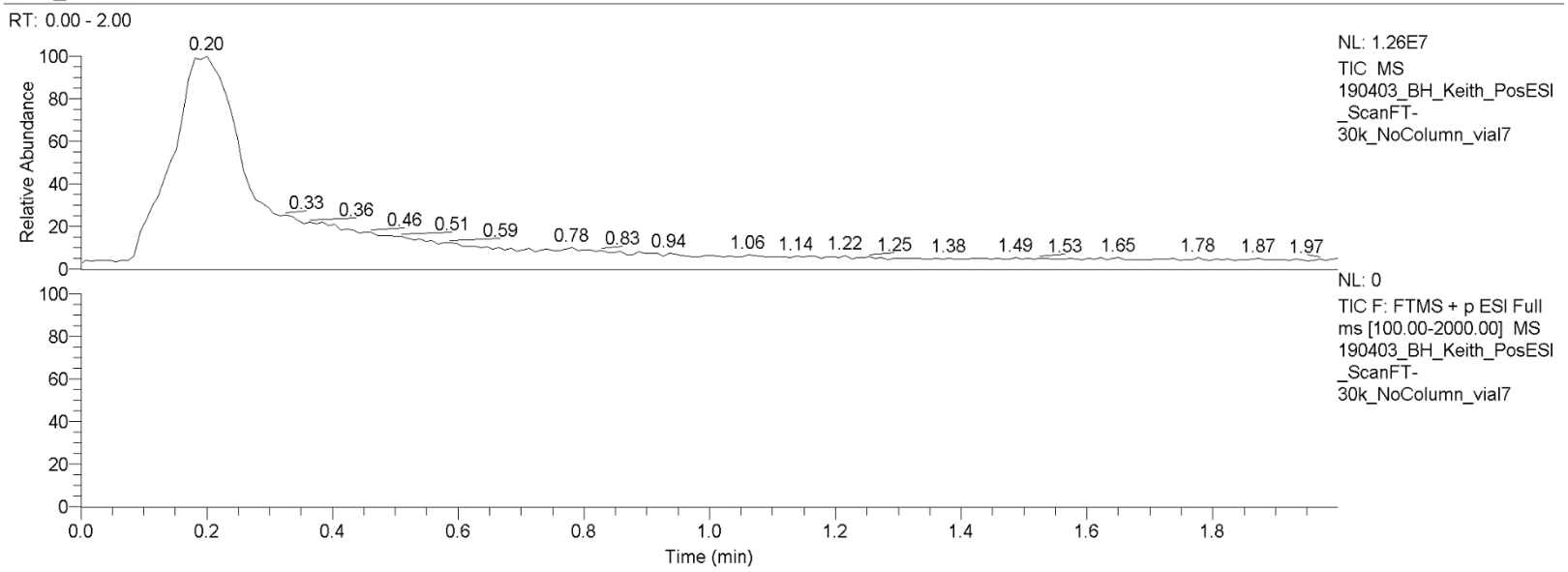
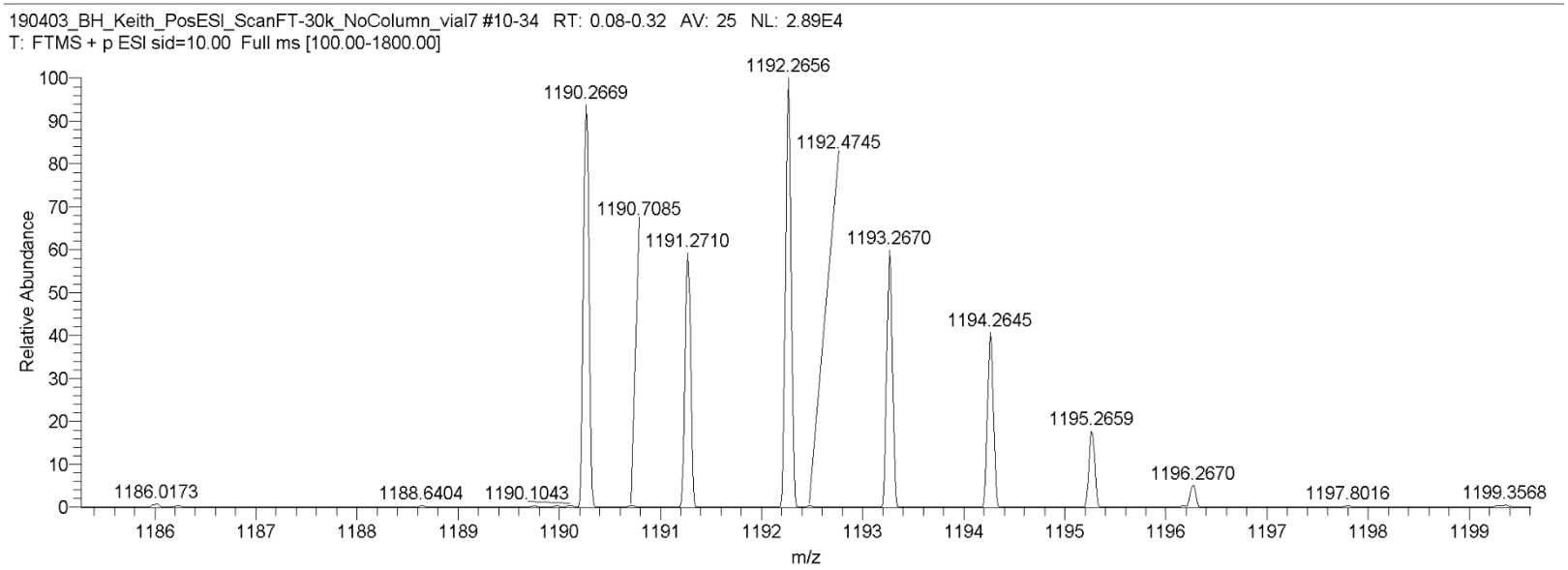
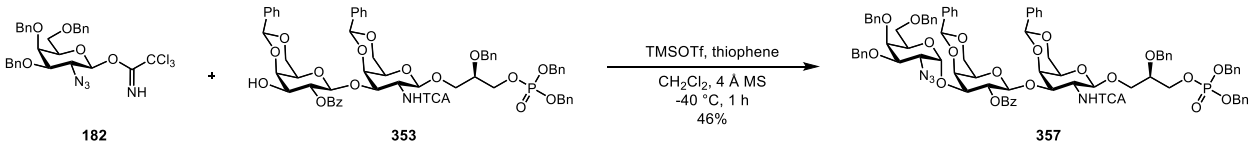




(*R*)-dibenzyl (2-(benzyloxy)-3-hydroxypropyl) phosphate 3,4,6-tri-*O*-benzy-2-deoxy-2-azido-3-*O*-*para*-methoxy benzyl-α-(1→3)-D-galactopyranosyl-4,6-*O*-benzylidene acetal-2-*O*-acetyl-β-(1→3)-D-galactopyranosyl-4,6-*O*-benzylidene acetal-2-deoxy-2-trichloroacetamido-β-D-galactopyranoside (357)



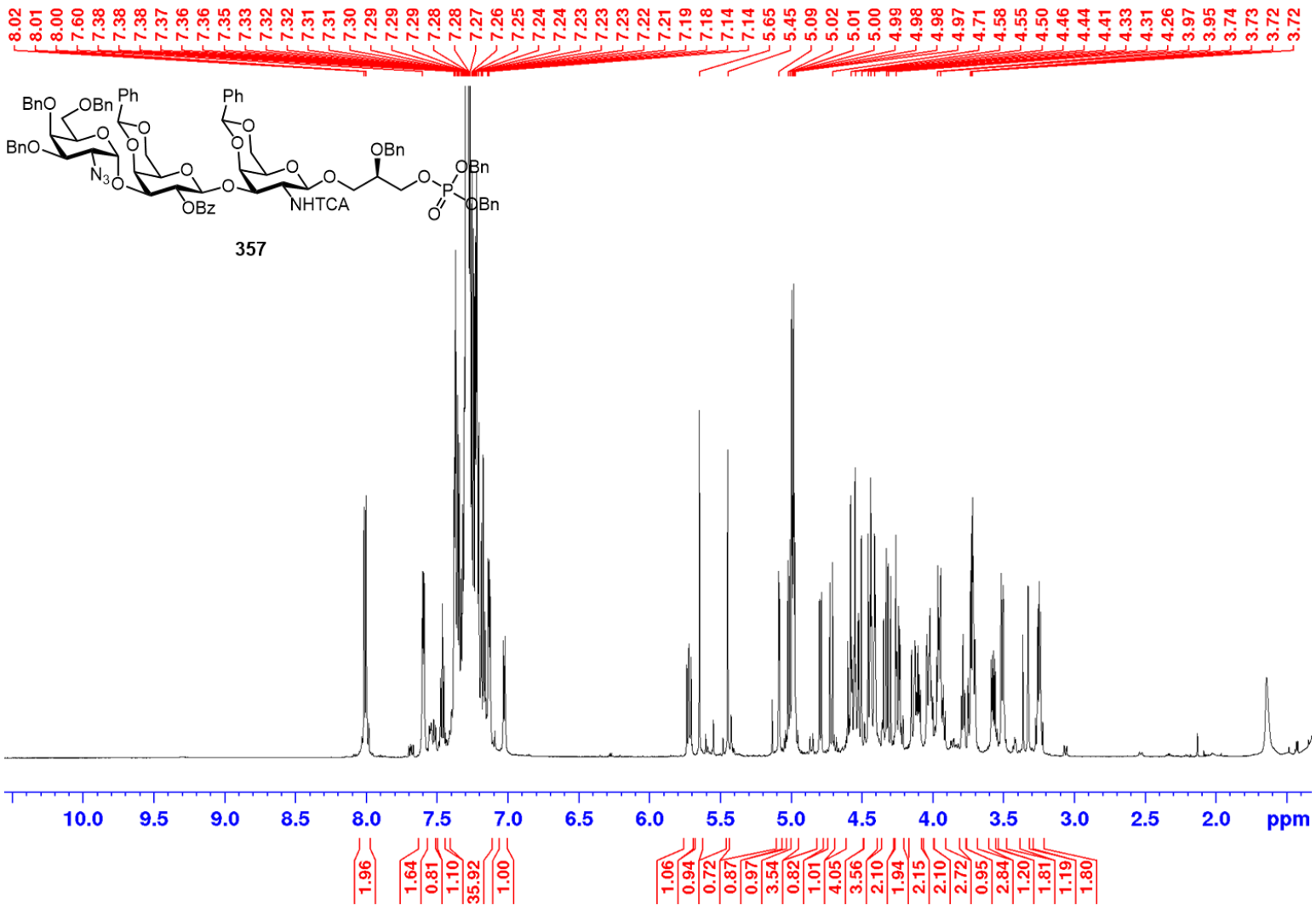
182 353 357

A solution of disaccharide acceptor (*R*)-dibenzyl (2-(benzyloxy)-3-hydroxypropyl) phosphate 4,6-*O*-benzylidene acetal-2-*O*-benzoyl-β-(1→3)-D-galactopyranosyl-4,6-*O*-benzylidene acetal-2-deoxy-2-trichloroacetamido-β-D-galactopyranoside 353 (1.0 equiv., 0.088 g, 0.0739 mmol) and a trichloroacetimidate donor 182 (2.6 equiv., 0.120 g, 0.190 mmol) in dry CH_2Cl_2 (1 mL) under an argon atmosphere was added preactivated, and cooled, 4 Å MS (0.150 g) at room temperature and allowed to stir for 1 h. The reaction mixture was cooled to -35 °C and added a solution of TMSOTf (0.20 equiv., 0.077 mL, 0.021 mmol) dropwise. The reaction was held a constant temperature at -35 °C. At 40 min, TLC indicated complete consumption of the disaccharide acceptor 353 starting material. The reaction was quench by the addition of dilute Et_3N in CH_2Cl_2. The reaction was filtered through a glass fritted vacuum filter funnel equipped with a pad of Celite to remove the 4 Å MS. The filtrate was concentrated to a crude oil which was purified by flash column chromatography (5:95 to 20:80 MeOH/CH_2Cl_2) give the C1''-α-linked trisaccharide (*R*)-dibenzyl (2-(benzyloxy)-3-hydroxypropyl) phosphate 3,4,6-tri-*O*-benzyl-2-deoxy-2-azido-3-*O*-para-methoxy benzyl-α-(1→3)-D-galactopyranosyl-4,6-*O*-benzylidene acetal-2-*O*-benzoyl-β-(1→3)-D-galactopyranosyl-4,6-*O*-benzylidene acetal-2-deoxy-2-trichloroacetamido-β-D-galactopyranoside 357 (0.0558 g, 0.034 mmol, 46%)

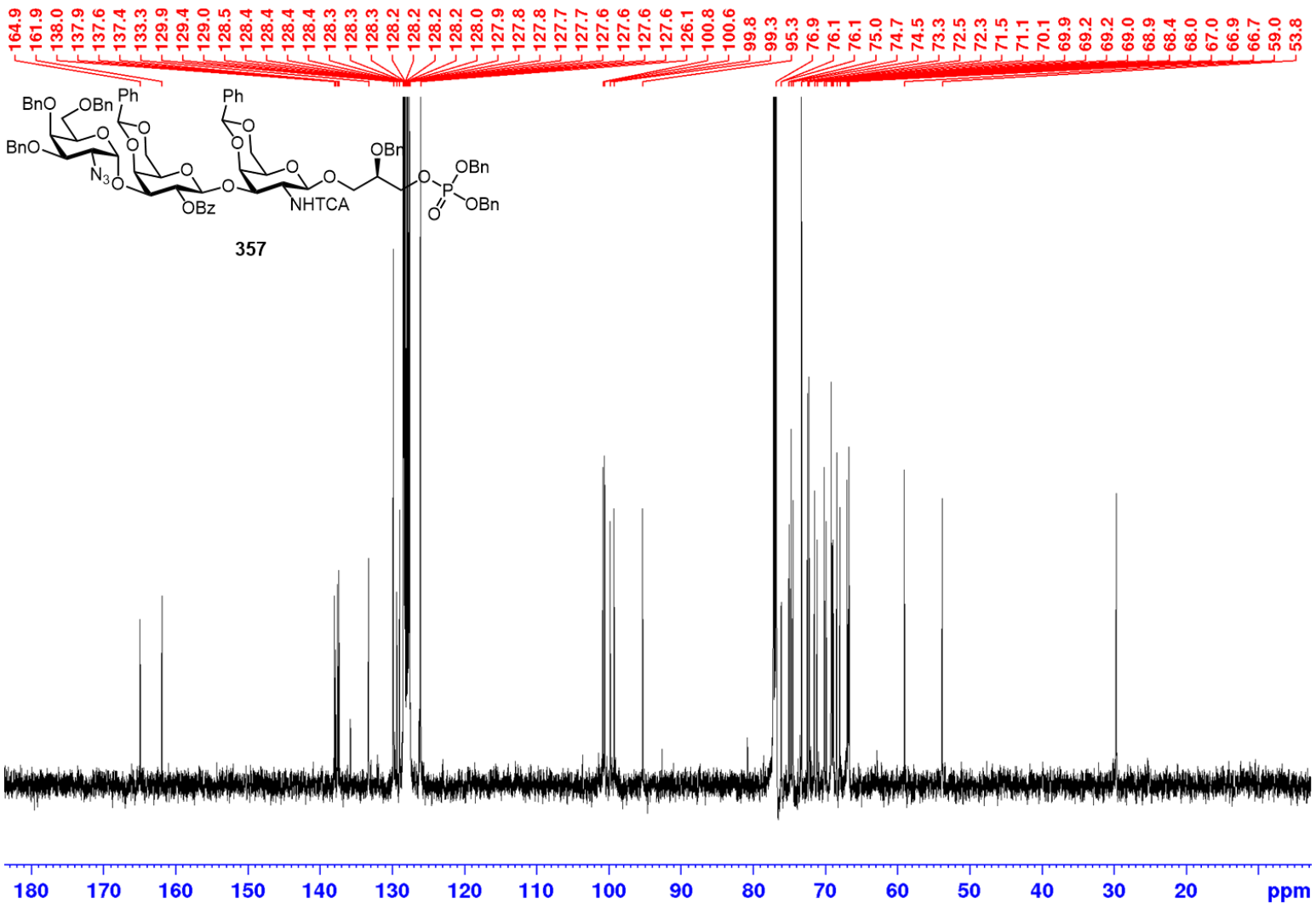
as a white solid. IR ν_{max} (film) 2919.70, 2110.67, 1719.36, 1542.19, 1454.19, 1367.55, 1266.72, 1090.82, 1056.21, 1024.97, 821.85, 737.96, 697.50 cm^{-1};

^{1}H NMR (600 MHz, $CDCl_3$): δ 8.01 (d, *J* = 8.3 Hz, 2H), 7.61-7.59 (m, 2H), 7.56-7.51 (m, 1H), 7.48-7.45 (m, 1H), 7.38-7.13 (m, 36H), 7.03 (d, *J* = 7.5 Hz, 1H), 5.72 (dd, *J* = 8.2, 9.7 Hz, 1H), 5.65 (s, 1H), 5.45 (s, 1H), 5.09 (d, *J* = 2.9 Hz, 1H), 5.02 (d, *J* = 8.1 Hz, 1H), 5.00-4.97 (m, 4H), 4.8 (d, *J* = 8.4 Hz, 1H), 4.72 (d, *J* = 11.1 Hz, 1H), 4.60-4.50 (m, 4H), 4.46-4.40 (m, 4H), 4.35-4.30 (m, 2H), 4.26-4.23 (m, 2H), 4.15-4.09 (m, 2H), 4.04-4.00 (m, 2H), 3.97-3.93 (m, 3H), 3.79 (dd, *J* = 6.3, 7.4 Hz, 1H), 3.75-3.70 (m, 3H), 3.58 (dd, *J* = 5.6, 10.9 Hz, 1H), 3.52-3.51 (m, 2H), 3.35 (d, *J* = 0.8 Hz, 1H), 3.26-3.24 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 164.9, 161.9, 138.0, 137.8, 137.5, 137.4, 133.2, 129.8, 129.3, 129.0, 128.5, 128.4, 128.4, 128.4, 128.3, 128.3, 128.3, 128.3, 128.2, 128.2, 128.2, 128.1, 128.1, 128.03, 127.8, 127.8, 127.8, 127.7, 127.6, 127.6, 127.6, 127.5, 127.5, 126.0, 100.8, 100.5, 99.8, 99.2, 95.3, 76.8, 76.1, 76.0, 75.0, 74.7, 74.4, 73.2, 72.4, 72.2, 71.5, 71.1, 70.1; HR-ESI-MS (m/z): calcd for $C_{86}H_{86}Cl_3N_4O_{21}P^-$ $(M-H)^-$ 1645.4513, found 1645.452.

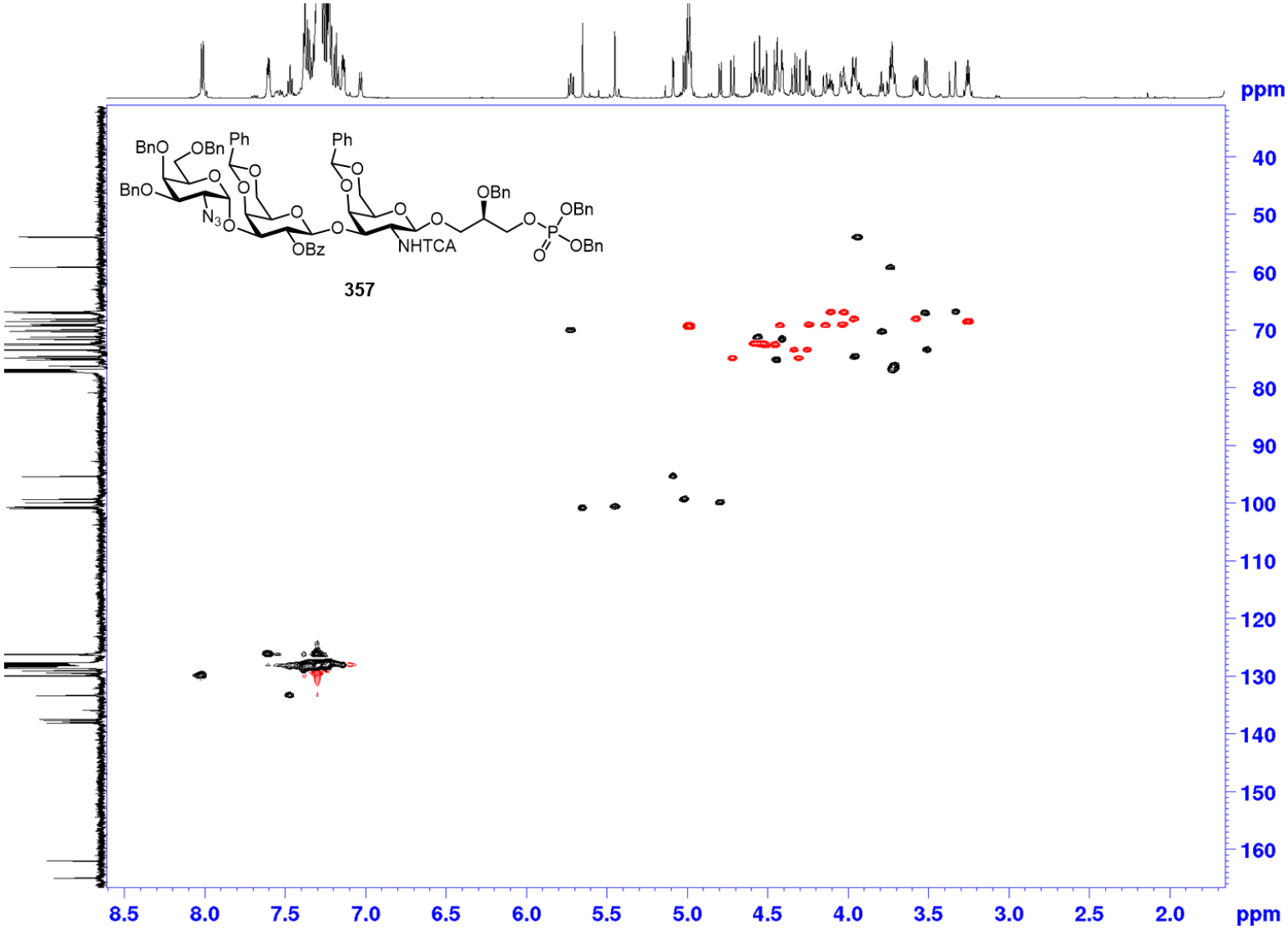
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 357



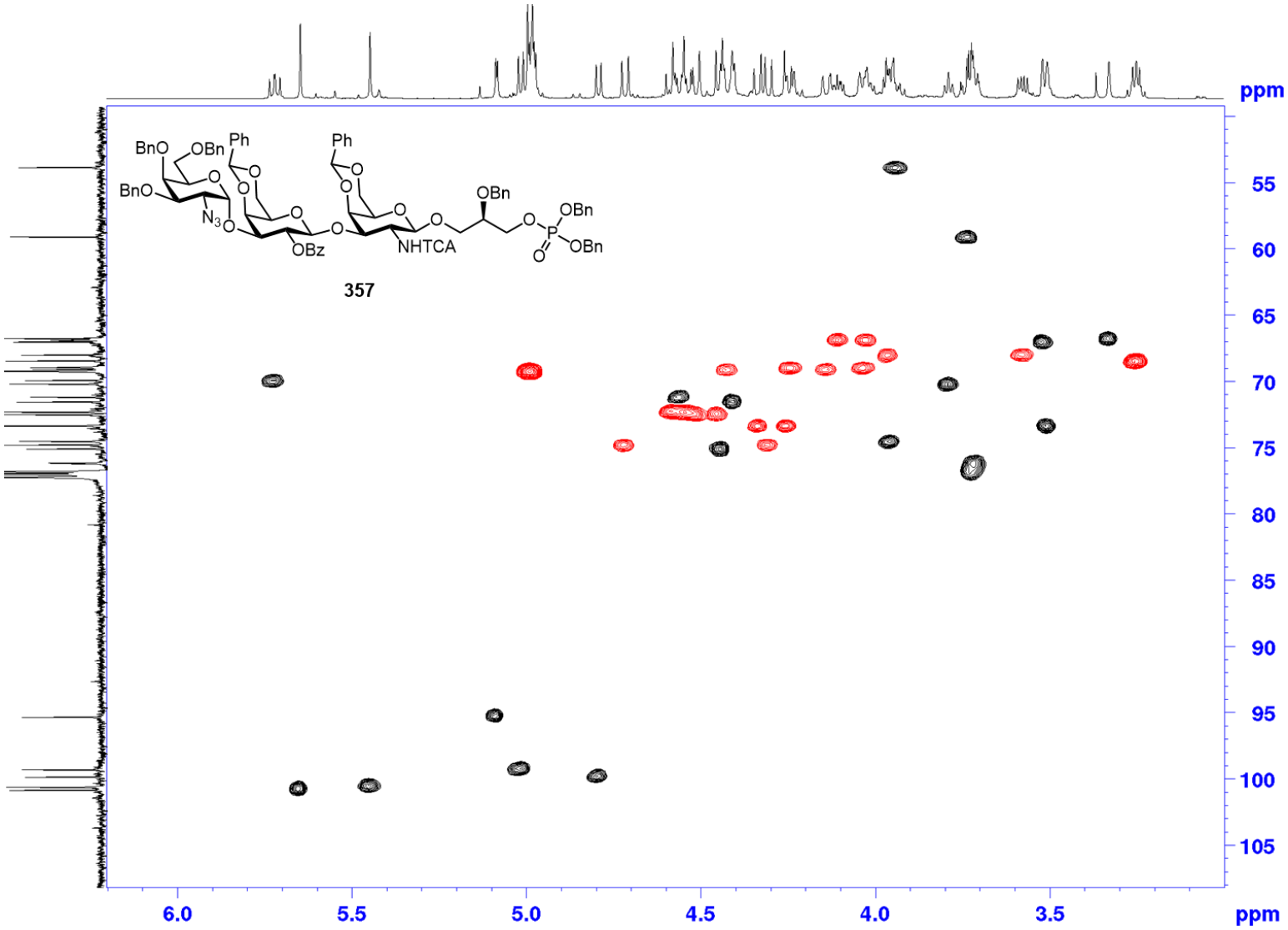
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 357



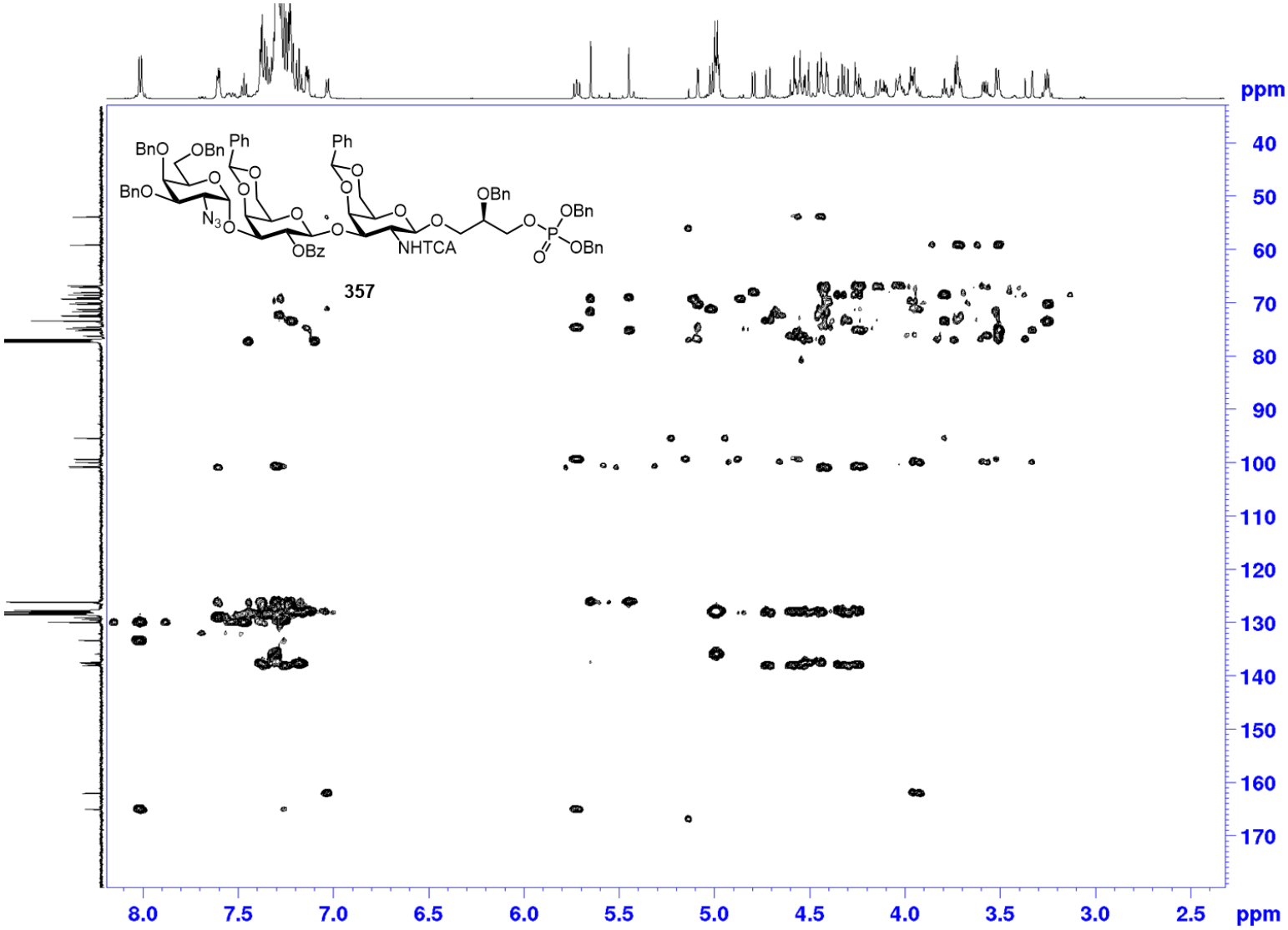
^{1}H-^{13}C HSCQ ($CDCl_3$) of compound 357



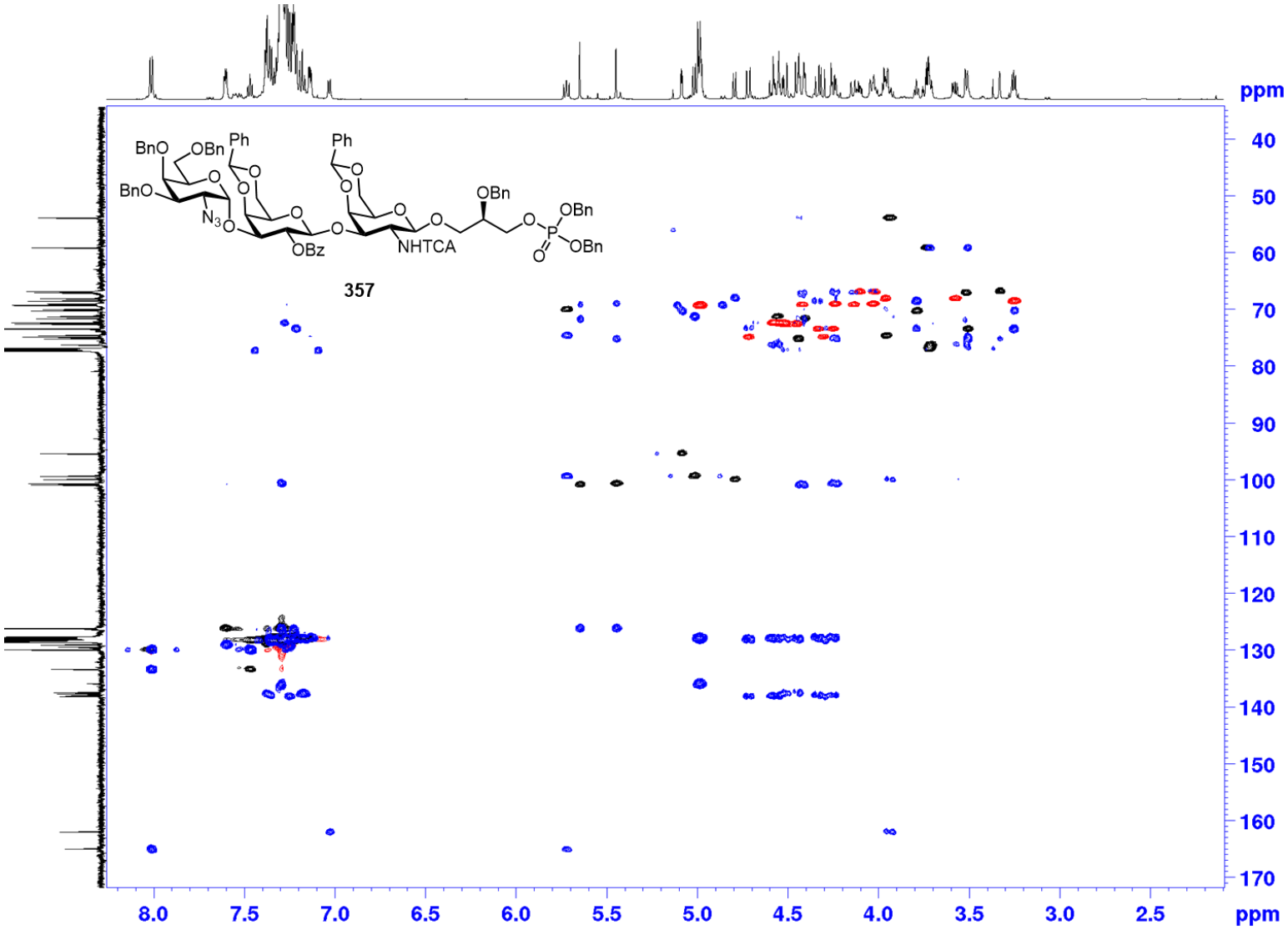
^{1}H-^{13}C HSCQ ($CDCl_3$) zoomed in region of compound 357



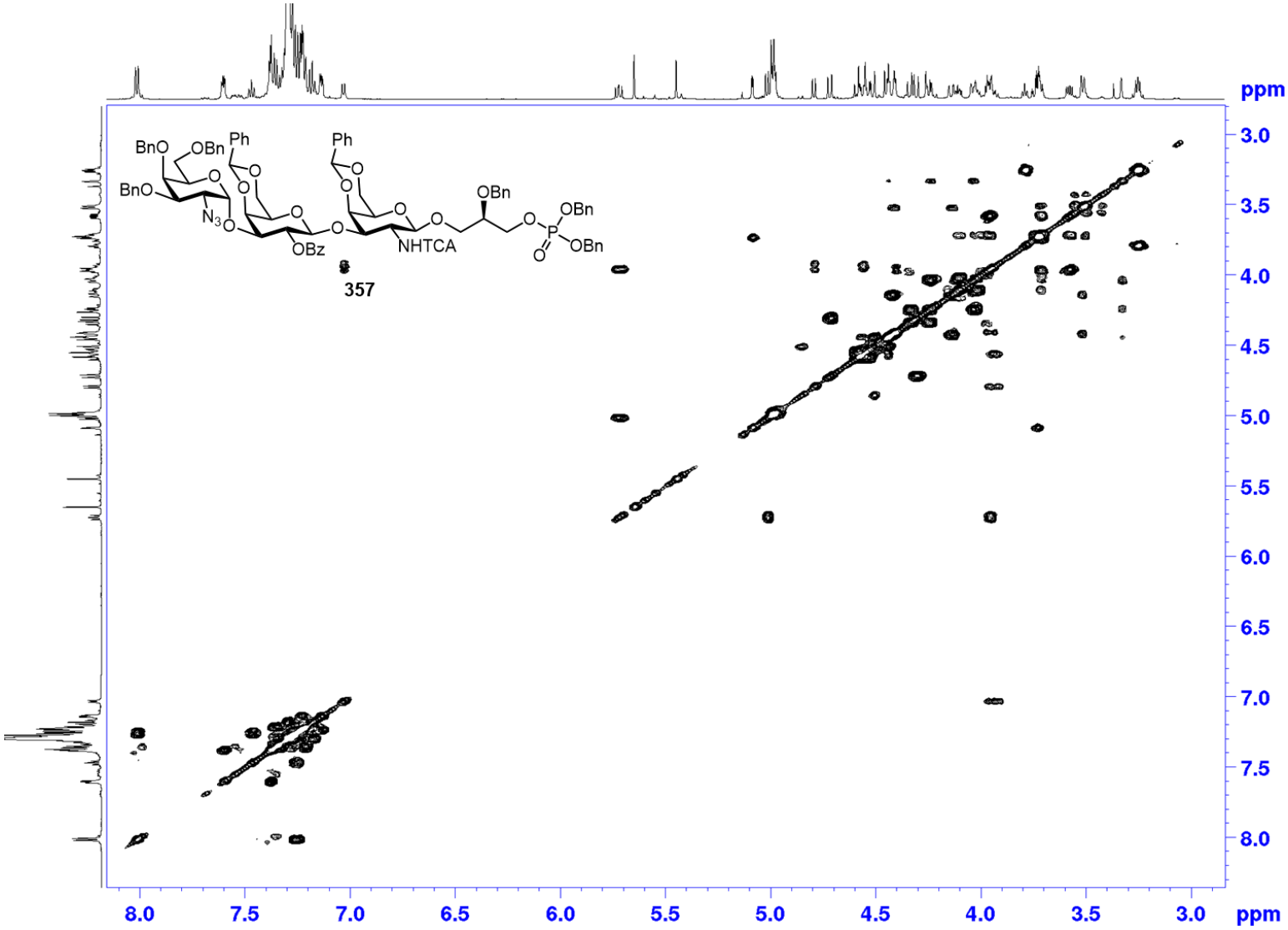
^{1}H-^{13}C HMBC ($CDCl_3$) of compound 357



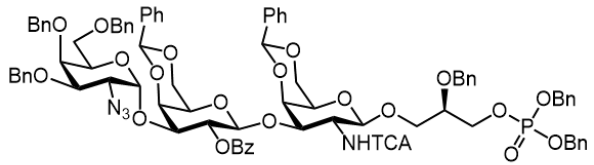
^{1}H-^{13}C HSCQ with ^{1}H-^{13}C HMBC overlay ($CDCl_3$) of compound 357



^{1}H-^{1}H COSY ($CDCl_3$) of compound 357

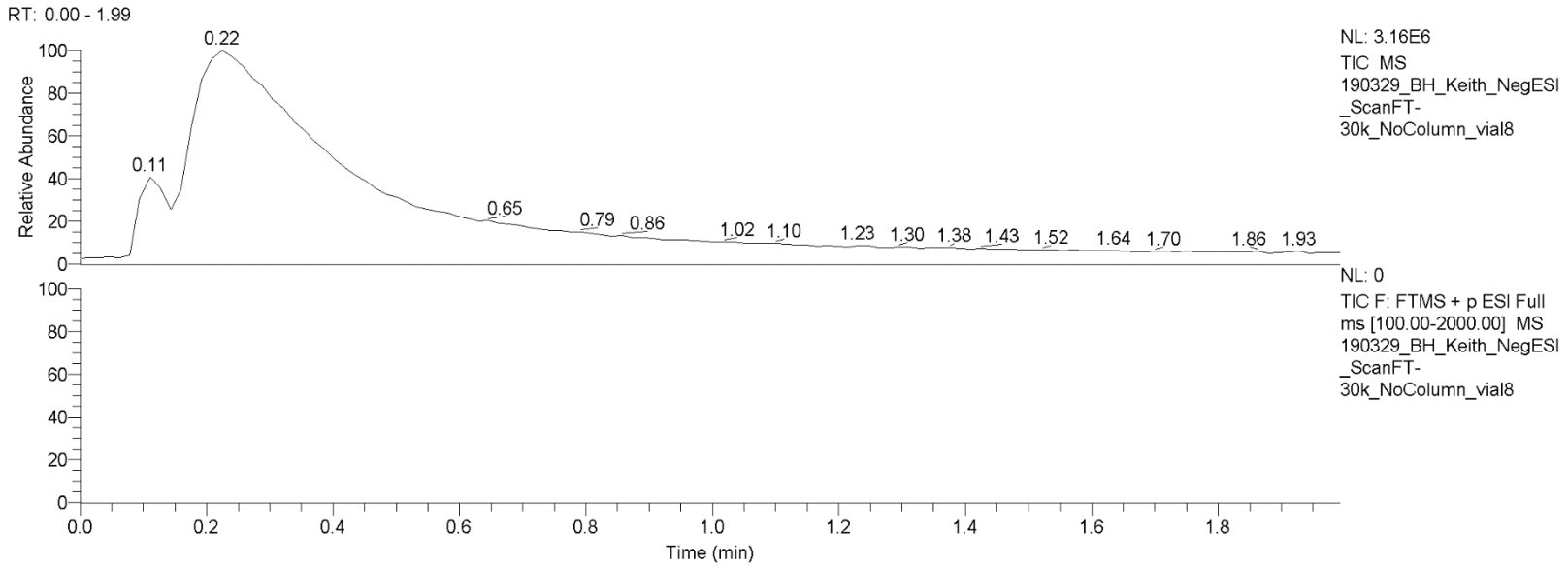


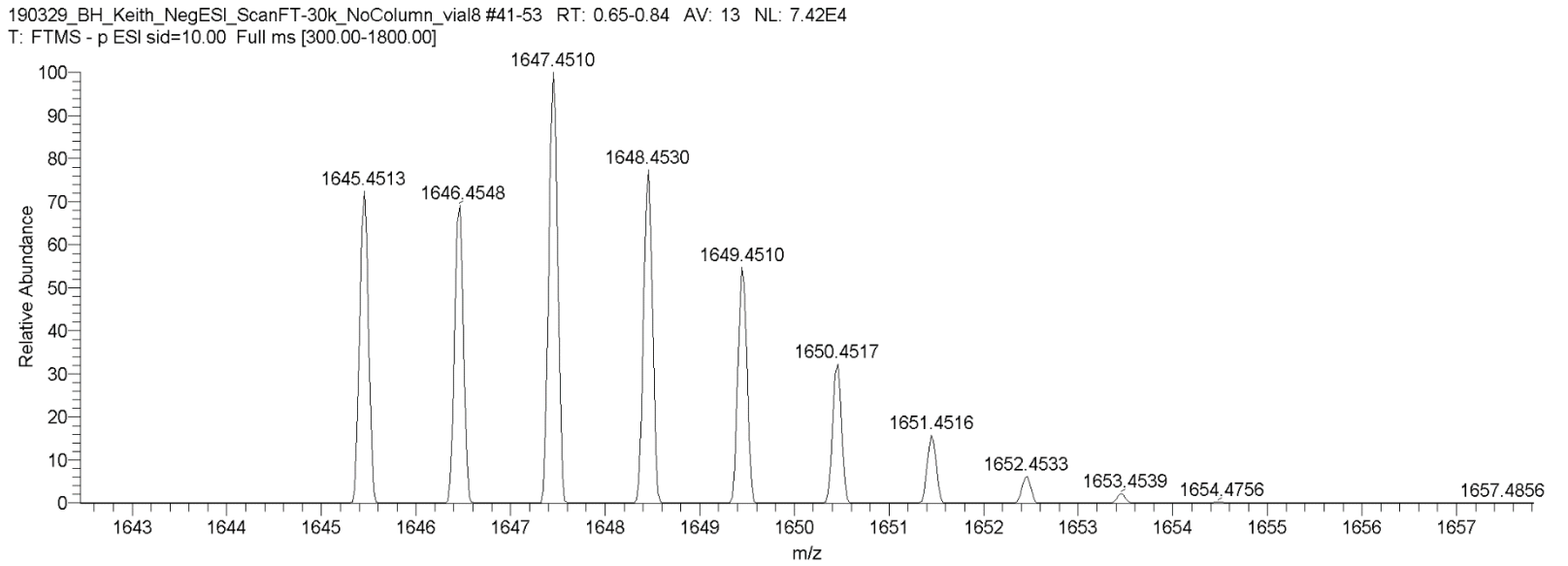
^{31}P NMR ($CDCl_3$, 162 MHz) of compound 357



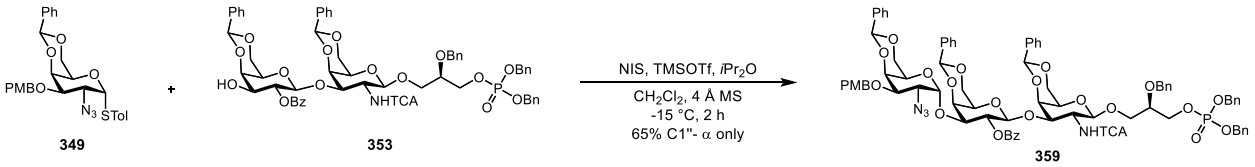
357

190329_BH_Keith_NegESI_ScanFT-30k_NoC... 3/29/2019 3:59:23 PM
e726_C2-OBz_Neg





(*R*)-dibenzyl (2-(benzyloxy)-3-hydroxypropyl) phosphate 4,6-*O*-benzylidene acetal-2-deoxy-2-azido-3-*O*-*para*-methoxy benzyl-α-(1→3)-D-galactopyranosyl-4,6-*O*-benzylidene acetal-2-*O*-benzoyl-β-(1→3)-D-galactopyranosyl-4,6-*O*-benzylidene acetal-2-deoxy-2-trichloroacetamido-β-D-galactopyranoside (359)

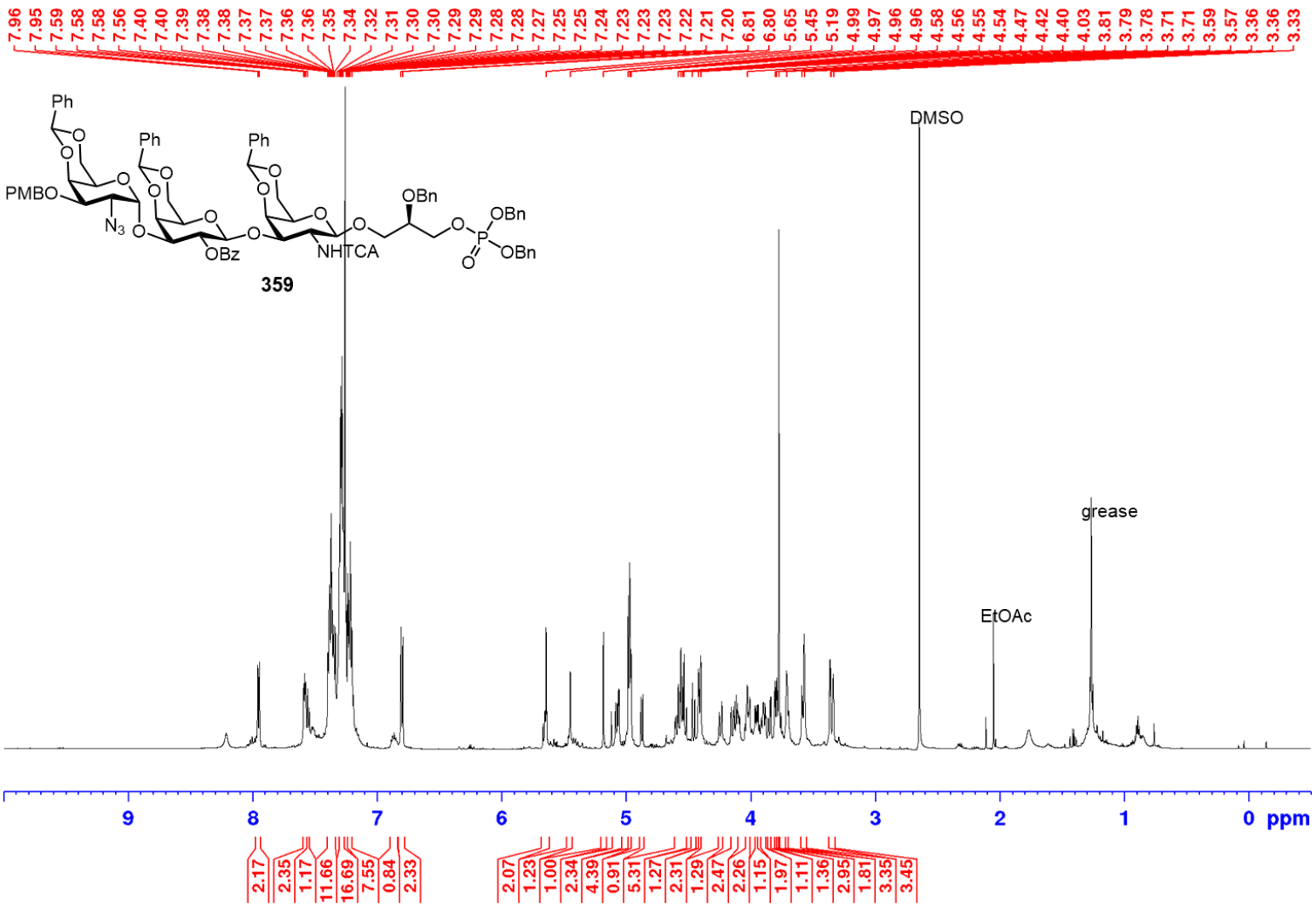


A solution of disaccharide acceptor (*R*)-dibenzyl (2-(benzyloxy)-3-hydroxypropyl) phosphate 4,6-*O*-benzylidene acetal-2-*O*-benzoyl-β-(1→3)-D-galactopyranosyl-4,6-*O*-benzylidene acetal-2-deoxy-2-trichloroacetamido-β-D-galactopyranoside **353** (1.0 equiv., 0.253 g, 0.213 mmol) and a thioglycoside donor *p*-tolyl 4,6-*O*-benzylidene acetal-3-*O*-(*p*-methoxy benzyl)-2-deoxy-2-azido-1-thio-α/β-D-galactopyranoside **349** (2.5 equiv., 0.276 g, 0.532 mmol) in a mixture of anhydrous 3:1 CH_2Cl_2/*i*Pr_2O (4 mL) under an argon atmosphere was added preactivated, and cooled, 4 Å MS (0.450 g) at room temperature and allowed to stir for 1 h. The reaction mixture was cooled to -15 °C and added NIS (3.0 equiv., 0.144 g, 0.65 mmol). After 2 min, the crude reaction mixture was added a solution of TMSOTf (0.10 equiv., 0.077 mL, 0.021 mmol, 5% in anhydrous CH_2Cl_2) dropwise. The reaction was held a constant temperature at -10 °C. At 2 h, TLC indicated complete consumption of the disaccharide acceptor **353** starting material. The reaction was quench by the addition of dilute Et_3N in CH_2Cl_2. The reaction was filtered through a glass fritted vacuum filter funnel equipped with a pad of Celite to remove the 4 Å MS. The filtrate was concentrated to a crude oil. The crude oil was then diluted and transferred with EtOAc (25 mL) to a separatory funnel and washed with satd. aq. Na_2SO_4 (5 x 5 mL), brine (1 x 5

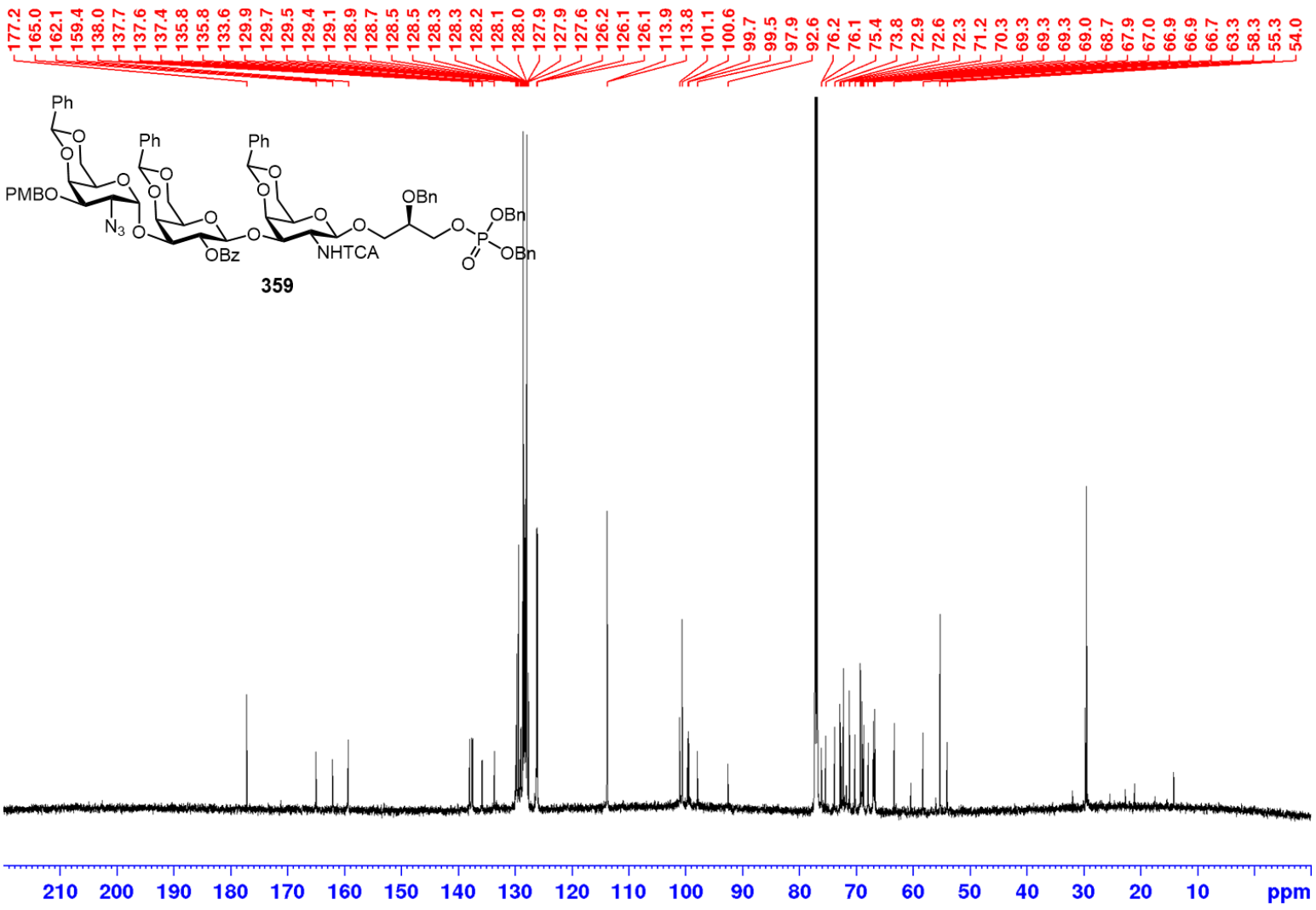
mL), dried ($NaSO_4$) and filtered through a glass fritted vacuum filter funnel to remove drying agent. The filtrate was concentrated to a crude oil and purified by flash column chromatography (5:95 to 20:80 CH_3OH/CH_2Cl_2) give the C1''-α-linked trisaccharide (*R*)-dibenzyl (2-(benzyloxy)-3-hydroxypropyl) phosphate 4,6-*O*-benzylidene acetal-2-deoxy-2-azido-3-*O*-para-methoxy benzyl-α-(1→3)-D-galactopyranosyl-4,6-*O*-benzylidene acetal-2-*O*-benzoyl-β-(1→3)-D-galactopyranosyl-4,6-*O*-benzylidene acetal-2-deoxy-2-trichloroacetamido-β-D-galactopyranoside **359** (0.2187 g, 0.138 mmol, 65%)[a] as yellow oil. R_f = 0.40 (25:80 CH_3OH/CH_2Cl_2) visualized with ceric ammonium molybdate stain; $[\alpha]D^{25}$ = -2.72° (c = 1.0, $CHCl_3$); IR ν_{max} (film) 3840.85, 3629.96, 1649.68, 1591.61, 1448.23, 1275.44, 1275.44, 1260.63, 982.90, 750.53, 695.41 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.95 (d, *J* = 7.3 Hz, 2H), 7.95-7.55 (m, 3H), 7.40-7.34 (m, 12H), 7.32-7.24 (m, 17H), 7.25-7.20 (m, 8H), 6.80 (d, *J* = 8.8 Hz, 2H), 5.64 (s, 2H), 5.45 (s, 1H), 5.18 (s, 1H), 5.09-5.06 (s, 2H), 4.99-4.96 (m, 4H), 4.97 (d, *J* = 7.9 Hz, 1H), 4.60-4.52 (m, 5H), 4.46 (d, *J* = 11.4 Hz, 1H), 4.42-4.40 (m, 2H), 4.24 (d, *J* = 11.9 Hz, 1H), 4.16-4.09 (m, 2H), 4.03-4.01 (m, 2H), 3.97-3.94 (m, 1H), 3.90-3.88 (m, 2H), 3.85 (dd, *J* = 2.8, 10.6 Hz, 1H), 3.81-3.79 (m, 1H), 3.77 (s, 3H), 3.71 (s, 1H),3.59 (s, 3H), 3.36-3.33 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 177.2, 165.0, 162.1, 159.4, 138.0, 137.7, 137.6, 137.4, 135.8, 135.8, 133.6, 129.9, 129.7, 129.5, 129.4, 129.3, 129.2, 129.1, 129.0, 128.9, 128.8, 128.7, 128.6, 128.5, 128.4, 128.3, 128.3, 128.2, 128.1, 128.0, 127.9, 127.9, 127.6, 126.3, 126.2, 126.1, 126.1, 113.9, 113.8, 101.1, 100.6, 99.7, 99.5, 97.9, 92.6, 76.2, 76.1, 75.4, 73.8, 72.9, 72.6, 72.3, 71.2, 70.3, 69.3, 69.3, 69.3, 69.0, 68.7, 67.9, 67.0, 66.9, 66.9, 66.7, 63.3, 58.3, 55.3, 54.0; ^{31}P NMR (151 MHz, $CDCl_3$) δ 0.18; HR-ESI-MS (m/z): calcd for

$C_{75}H_{82}Cl_3N_5O_{22}P^+$ $(M+NH_4)^+$ 1602.440523, found 1602.4420. [a] yield reported contains solvent impurities. see NMR spectra.

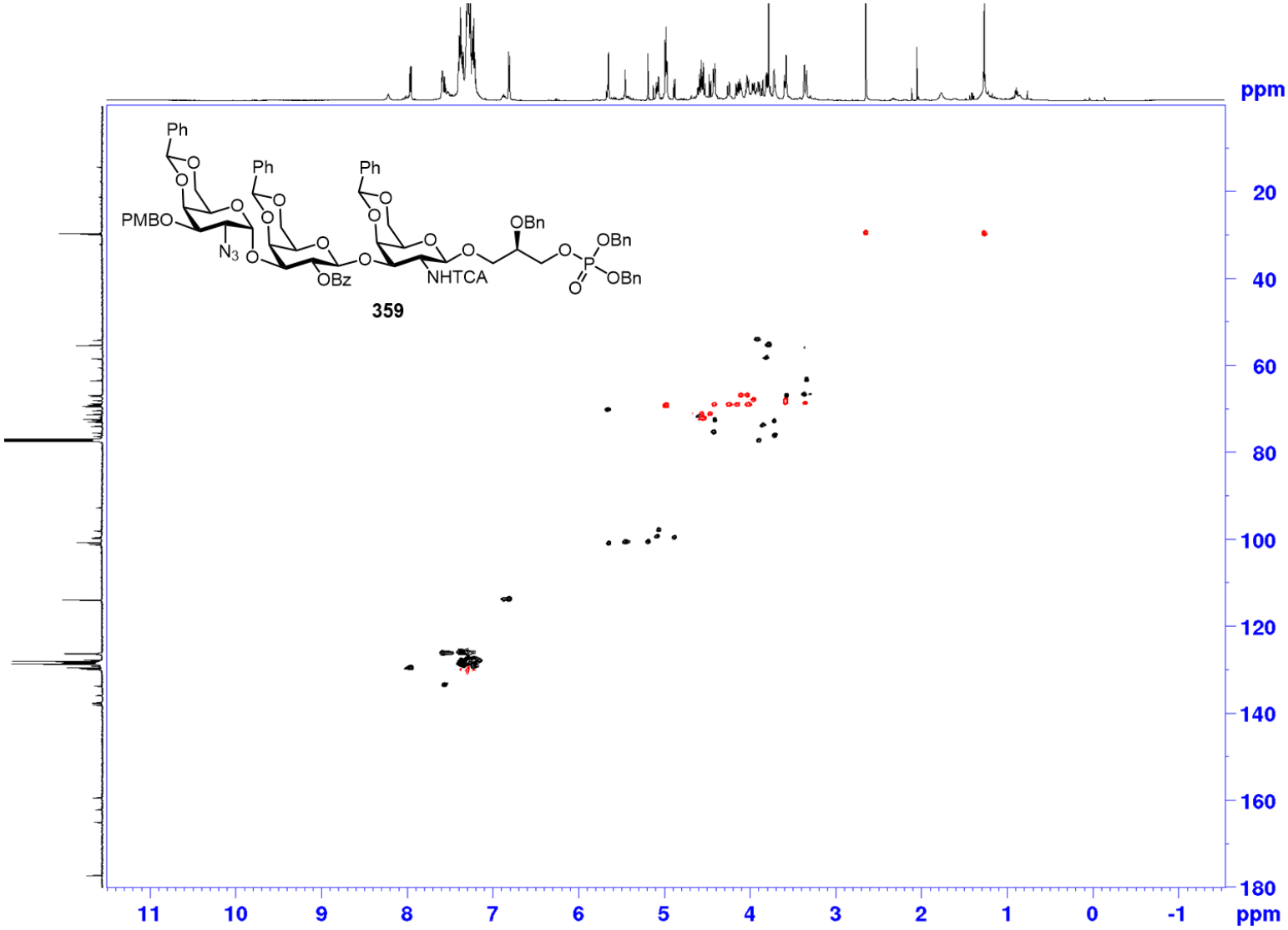
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 359



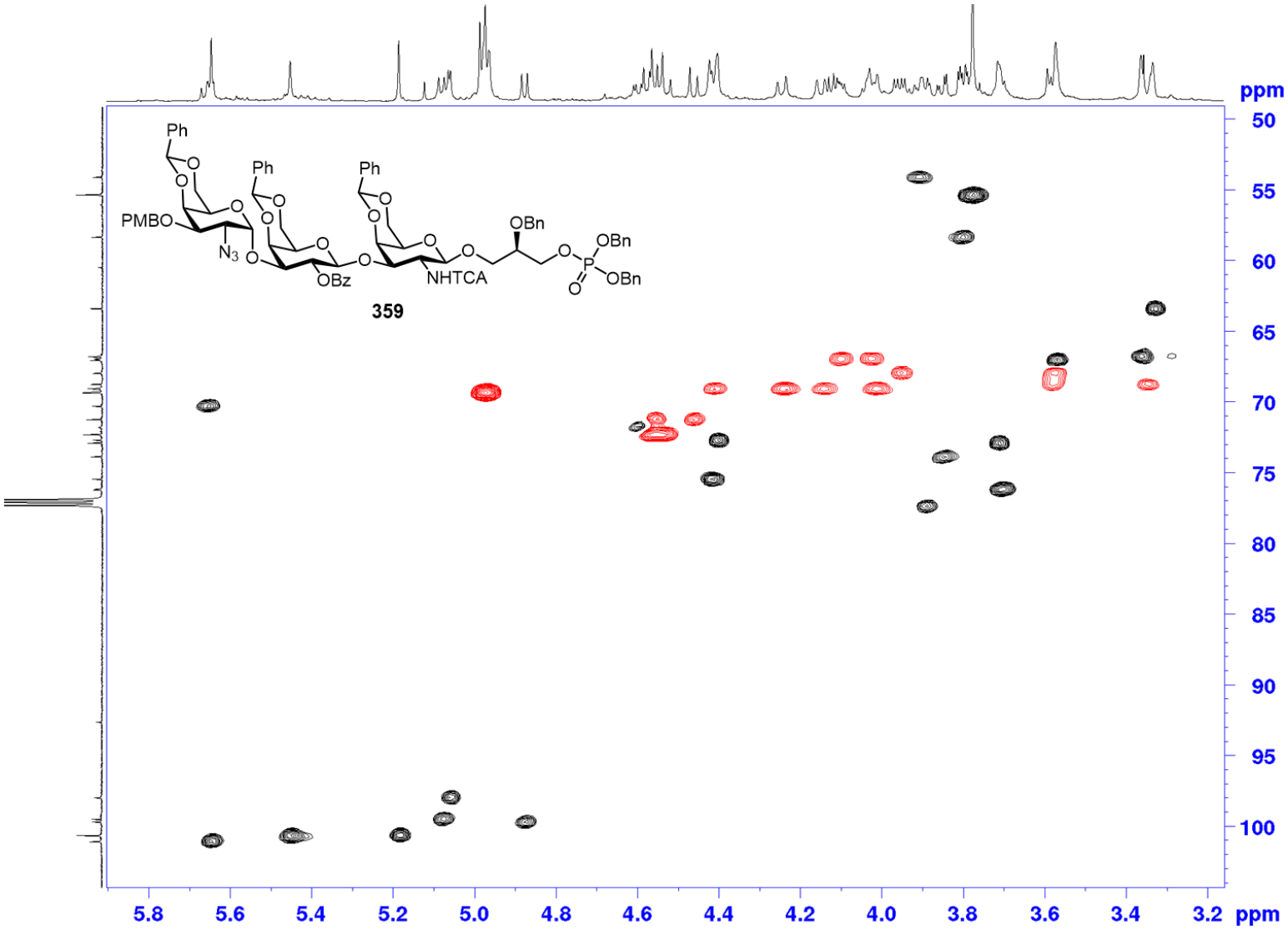
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 359



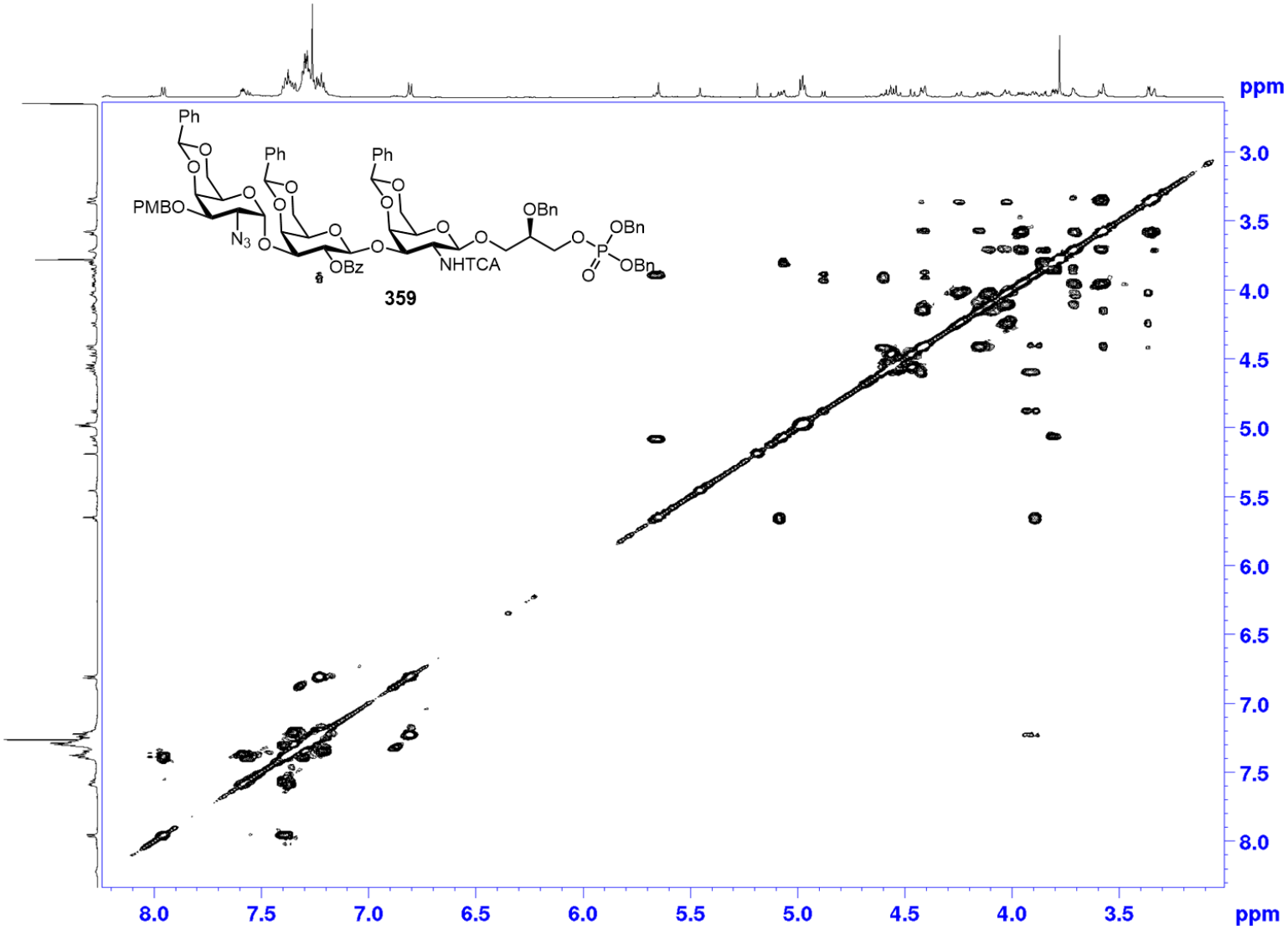
^{1}H-^{13}C HSCQ ($CDCl_3$) of compound 359



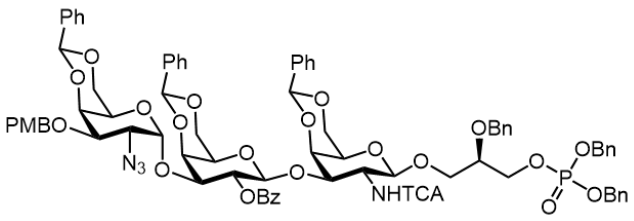
^{1}H-^{13}C HSCQ ($CDCl_3$) zoomed in region of compound 359



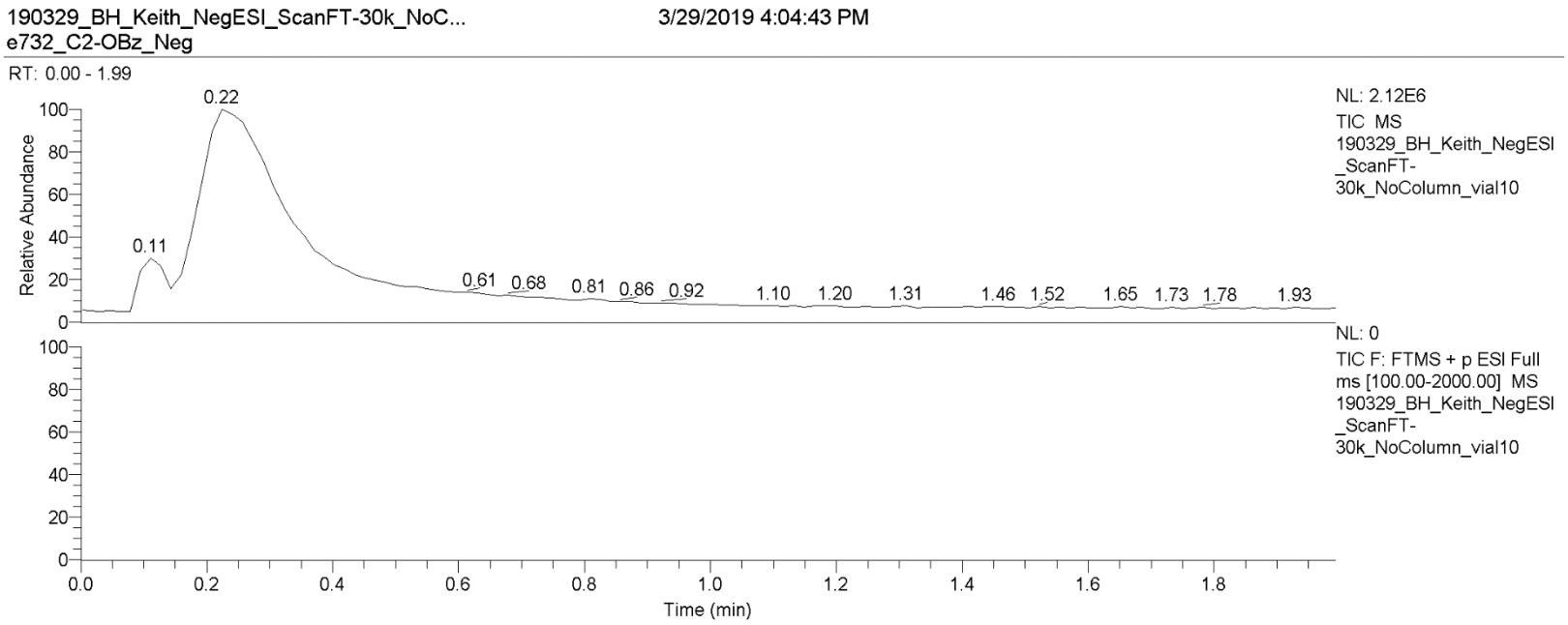
^{1}H-^{1}H COSY ($CDCl_3$) of compound 359

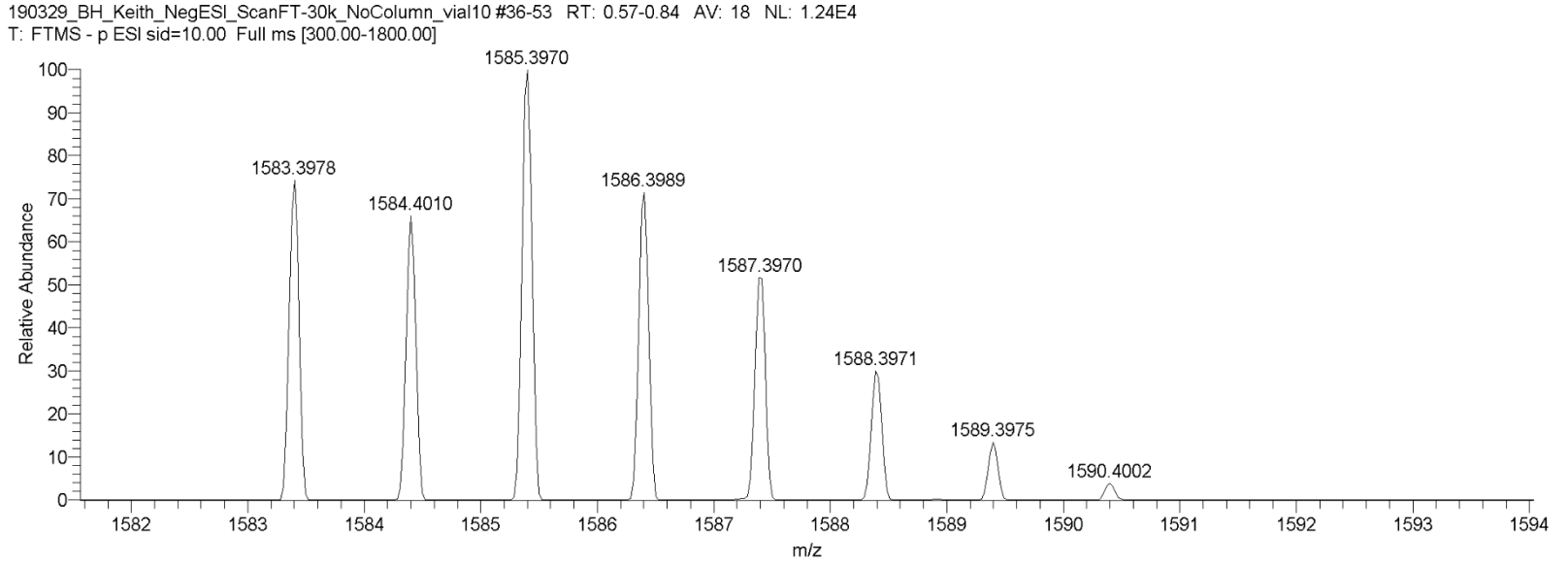


^{31}P NMR ($CDCl_3$, 162 MHz) of compound 359

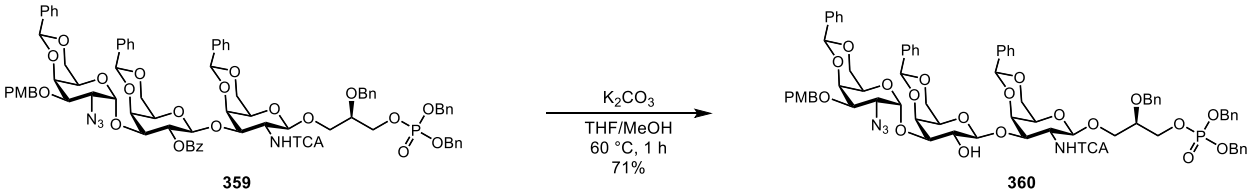


359


190329_BH_Keith_NegESI_ScanFT-30k_NoC...
3/29/2019 4:04:43 PM
e732_C2-OBz_Neg
RT: 0.00 - 1.99
0.22
0.11
0.61
0.68
0.81
0.86
0.92
1.10
1.20
1.31
1.46
1.52
1.65
1.73
1.78
1.93
Relative Abundance
NL: 2.12E6
TIC MS
190329_BH_Keith_NegESI
_ScanFT-
30k_NoColumn_vial10
NL: 0
TIC F: FTMS + p ESI Full
ms [100.00-2000.00] MS
190329_BH_Keith_NegESI
_ScanFT-
30k_NoColumn_vial10
Time (min)


190329_BH_Keith_NegESI_ScanFT-30k_NoColumn_vial10 #36-53 RT: 0.57-0.84 AV: 18 NL: 1.24E4
T: FTMS - p ESI sid=10.00 Full ms [300.00-1800.00]
1585.3970
1583.3978
1584.4010
1586.3989
1587.3970
1588.3971
1589.3975
1590.4002
Relative Abundance
m/z

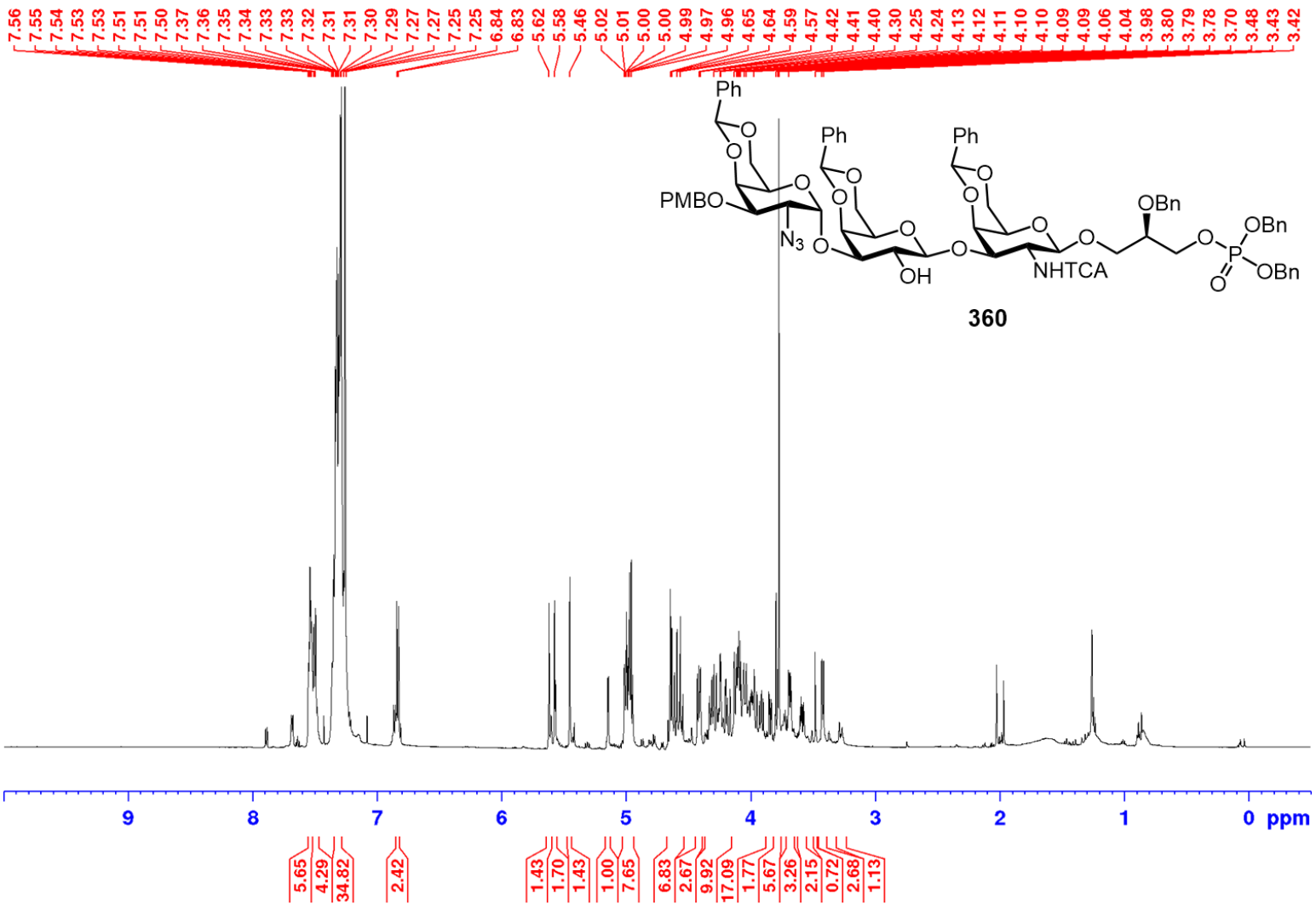
(*R*)-dibenzyl (2-(benzyloxy)-3-hydroxypropyl) phosphate 4,6-*O*-benzylidene acetal-2-deoxy-2-azido-3-*O*-*para*-methoxy benzyl-α-(1→3)-D-galactopyranosyl-4,6-*O*-benzylidene acetal-β-(1→3)-D-galactopyranosyl-4,6-*O*-benzylidene acetal-2-deoxy-2-trichloroacetamido-β-D-galactopyranoside (360)



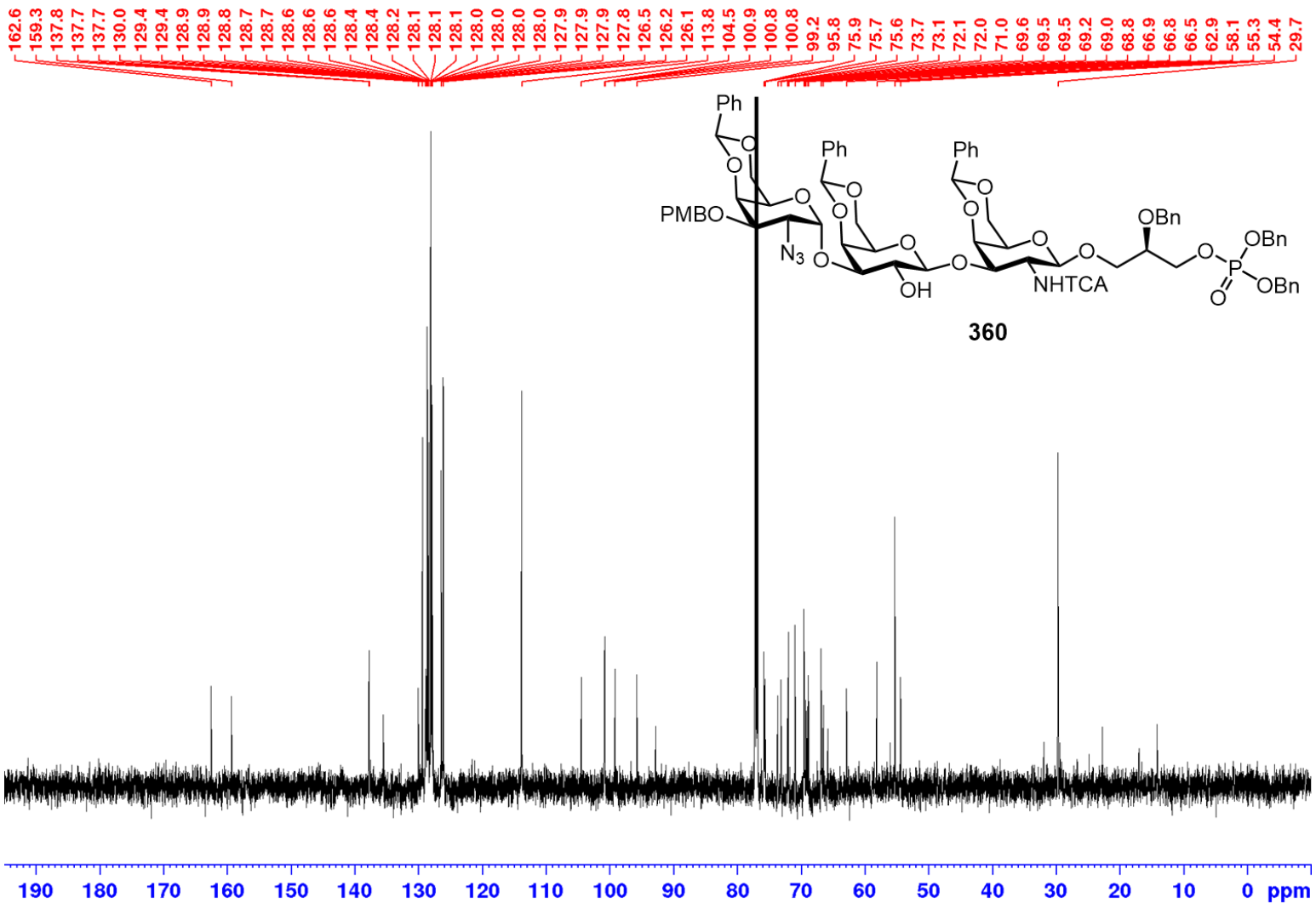
A solution of trisaccharide (*R*)-dibenzyl (2-(benzyloxy)-3-hydroxypropyl) phosphate 4,6-*O*-benzylidene acetal-2-deoxy-2-azido-3-*O*-para-methoxy benzyl-α-(1→3)-D-galactopyranosyl-4,6-*O*-benzylidene acetal-2-*O*-benzoyl-β-(1→3)-D-galactopyranosyl-4,6-*O*-benzylidene acetal-2-deoxy-2-trichloroacetamido-β-D-galactopyranoside **359** (1.0 equiv., 0.4341 g, 0.2736 mmol) in a mixture of anhydrous 2:1 CH_3OH/THF (6 mL) was added previously dried K_2CO_3 (25.0 equiv., 0.945 g, 6.84 mmol) and heated to 60 °C under an argon atmosphere. At 1 h, TLC indicated that the starting material had been consumed. The reaction was transferred with EtOAc (20 mL) to a separatory funnel and washed with brine (1 x 5 mL), dried ($NaSO_4$) and filtered through a glass fritted vacuum filter funnel to remove drying agent. The filtrate was concentrated to white solid. The material was purified by flash column chromatography (5:95 to 1:9 CH_3OH/CH_2Cl_2) give the trisaccharide **(***R*)-dibenzyl (2-(benzyloxy)-3-hydroxypropyl) phosphate 4,6-*O*-benzylidene acetal-2-deoxy-2-azido-3-*O*-*para*-methoxy benzyl-α-(1→3)-D-galactopyranosyl-4,6-*O*-benzylidene acetal-β-(1→3)-D-galactopyranosyl-4,6-*O*-benzylidene acetal-2-deoxy-2-trichloroacetamido-β-D-galactopyranoside **360** (0.2884 g, 0.195 mmol, 71%) as white amorphous solid. R_f = 0.25 (80:20 EtOAc/hexanes) visualized

with ceric ammonium molybdate stain; $[\alpha]D^{25}$ = +26.68° (c = 1.0, $CHCl_3$); IR ν_{max} (film) 3391.01, 2111.51, 1636.01, 1514.17, 1455.61, 1367.11, 1249.13, 1173.55, 1095.85, 1051.43, 820.57, 736.33, 698.32 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.56-7.50 (m, 9H), 7.37-7.26 (m, 34H), 6.83 (d, *J* = 8.7 Hz, 2H), 5.62 (s, 1H), 5.58 (s, 1H), 5.46 (s, 1H), 5.14 (d, *J* = 3.4 Hz, 1H), 5.02-4.9 (m, 7H), 4.67-4.55 (m, 6H), 4.43-4.40 (m, 3H), 4.37-4.17 (m, 9H), 4.13-3.89 (m, 17H), 3.84 (dd, *J* = 3.1, 10.4 Hz, 2H), 3.81-3.76 (m, 6H), 3.71-3.65 (m, 3H), 3.62-3.57 (m, 2H), 3.48 (s, 1H), 3.42 (d, *J* = 9.1 Hz, 3H), 3.29-3.26 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 162.6, 159.3, 137.8, 137.7, 137.7, 135.6, 135.5, 130.0, 129.4, 129.4, 129.4, 128.9, 128.9, 128.8, 128.7, 128.7, 128.6, 128.6, 128.6, 128.6, 128.6, 128.4, 128.4, 128.4, 128.2, 128.1, 128.1, 128.1, 128.1, 128.0, 128.0, 128.0, 128.0, 128.0, 127.9, 127.9, 127.9, 127.8, 127.8, 126.5, 126.2, 126.1, 113.8, 104.5, 100.9, 100.8, 100.8, 99.2, 95.8, 92.8, 75.9, 75.8, 75.7, 75.6, 73.7, 73.1, 72.1, 72.0, 71.0, 69.6, 69.5, 69.5, 69.2, 69.0, 68.8, 66.9, 66.8, 66.5, 65.8, 62.9, 58.1, 55.3, 54.4, 31.9, 29.7, 29.4; ^{31}P NMR (151 MHz, $CDCl_3$) δ 0.16; HR-ESI-MS (m/z): calcd for $C_{73}H_{75}Cl_3N_4O_{21}P^-$ $(M-H)^-$ 1479.3732, found 1479.3735.

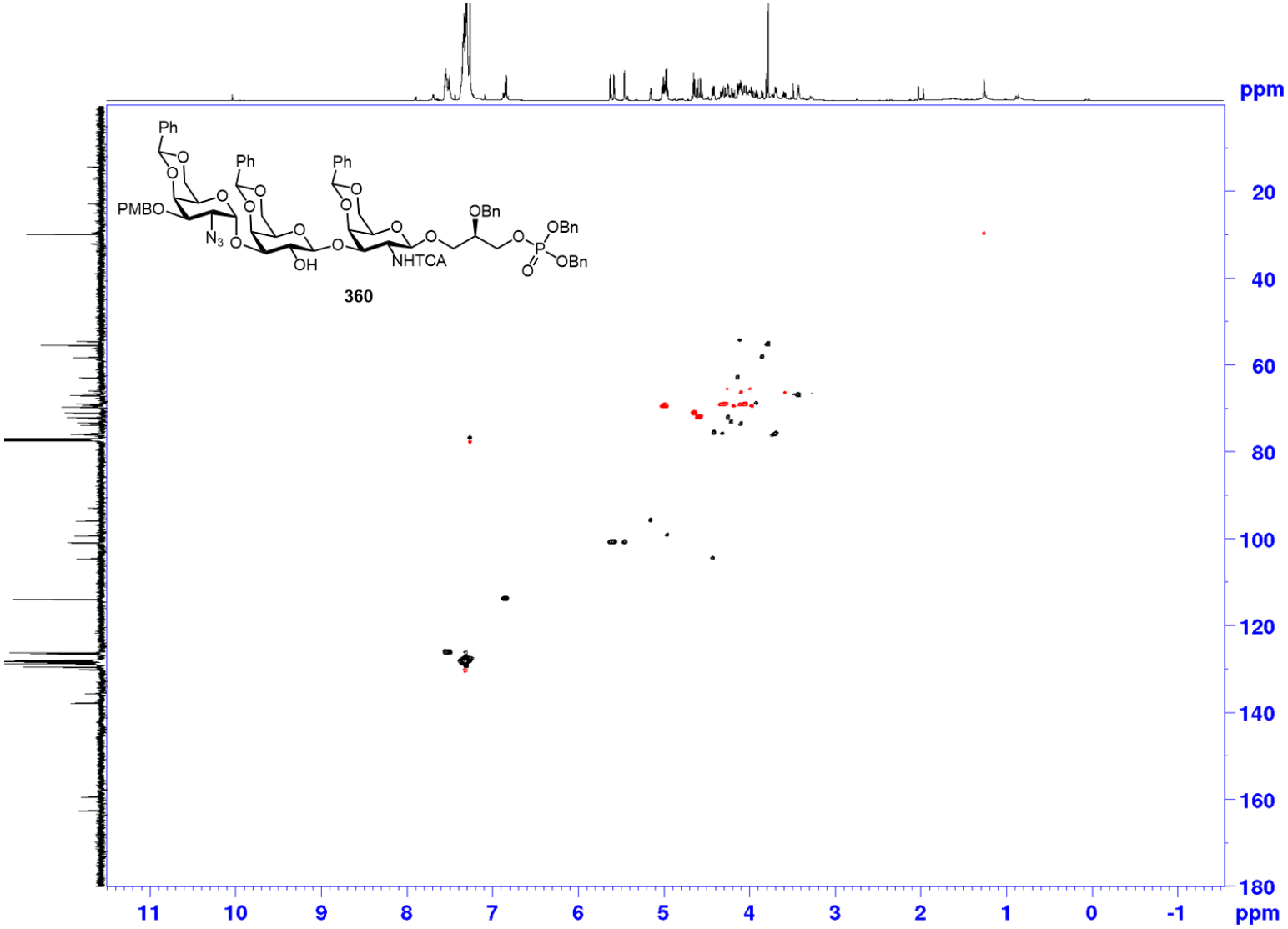
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 360



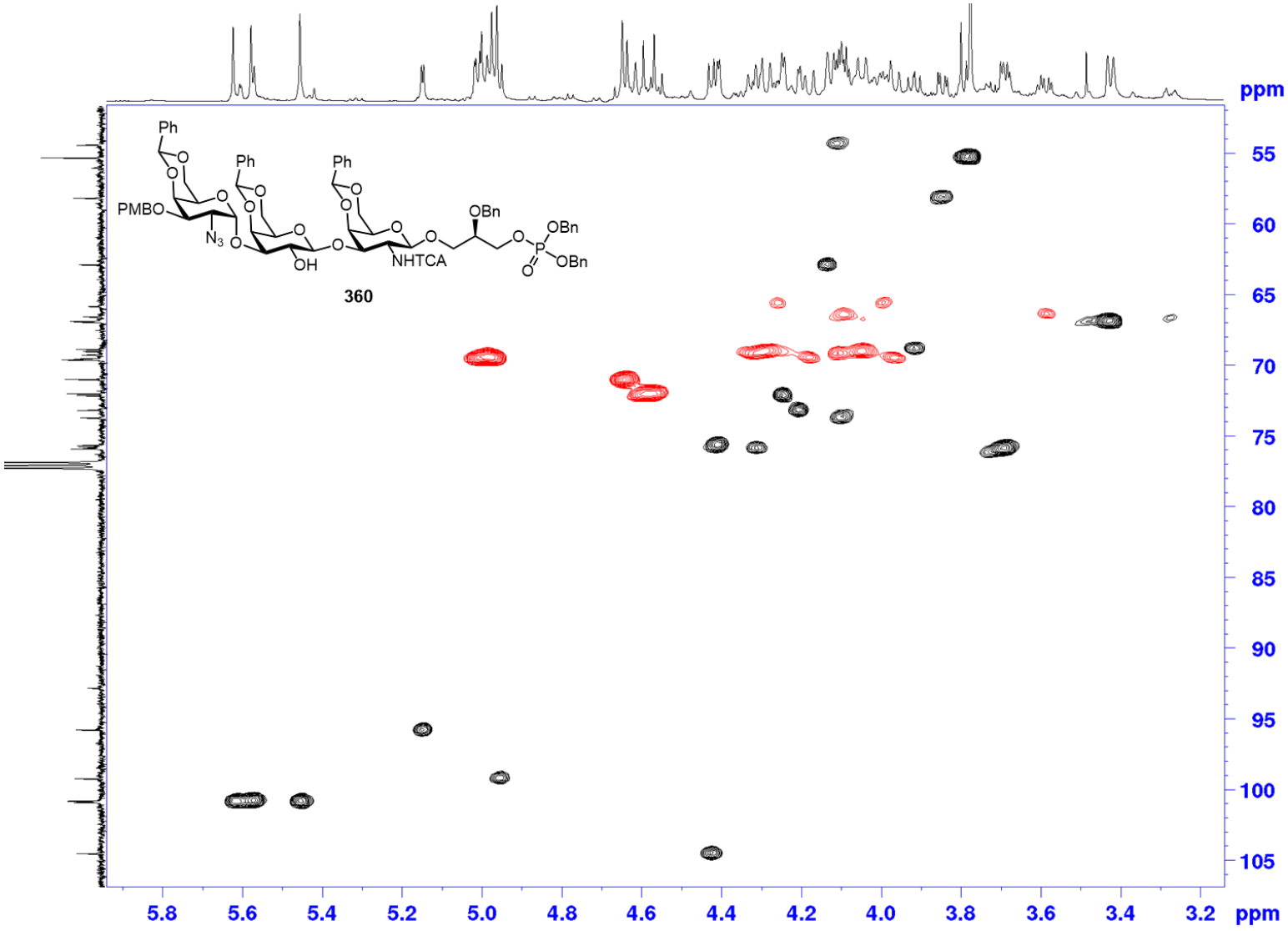
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 360



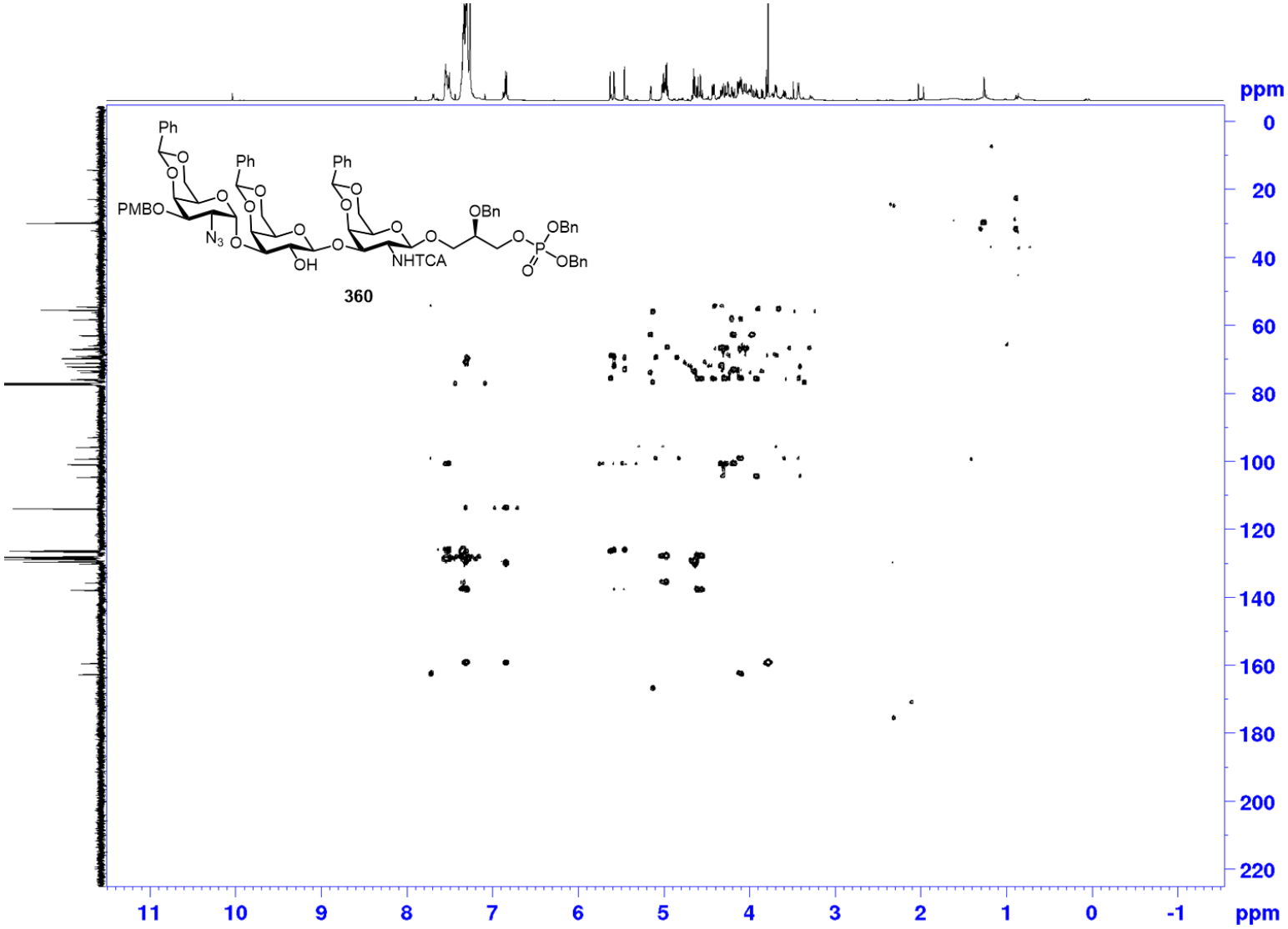
^{1}H-^{13}C HSCQ ($CDCl_3$) of compound 360



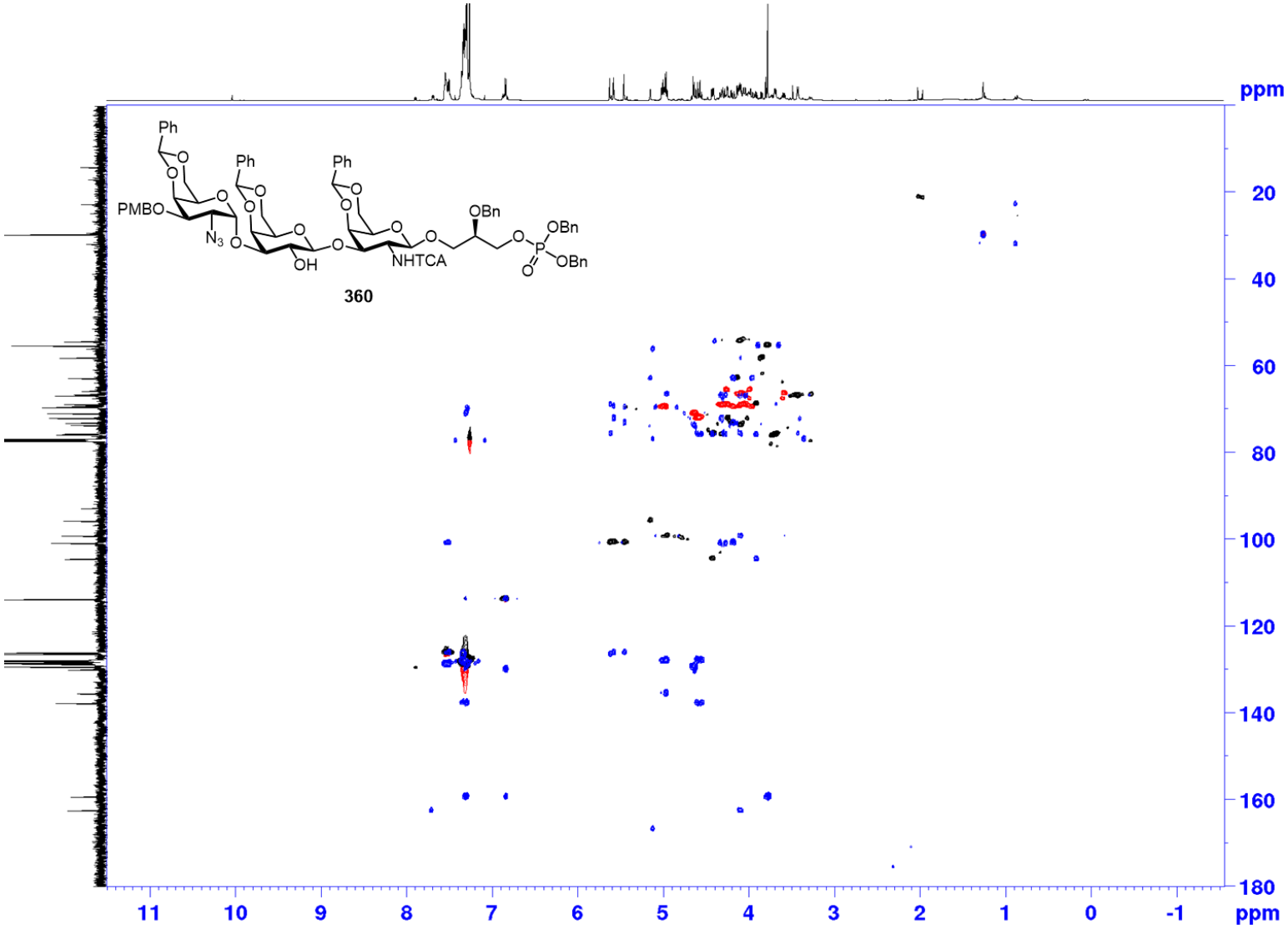
^{1}H-^{13}C HSCQ ($CDCl_3$) zoomed in region of compound 360



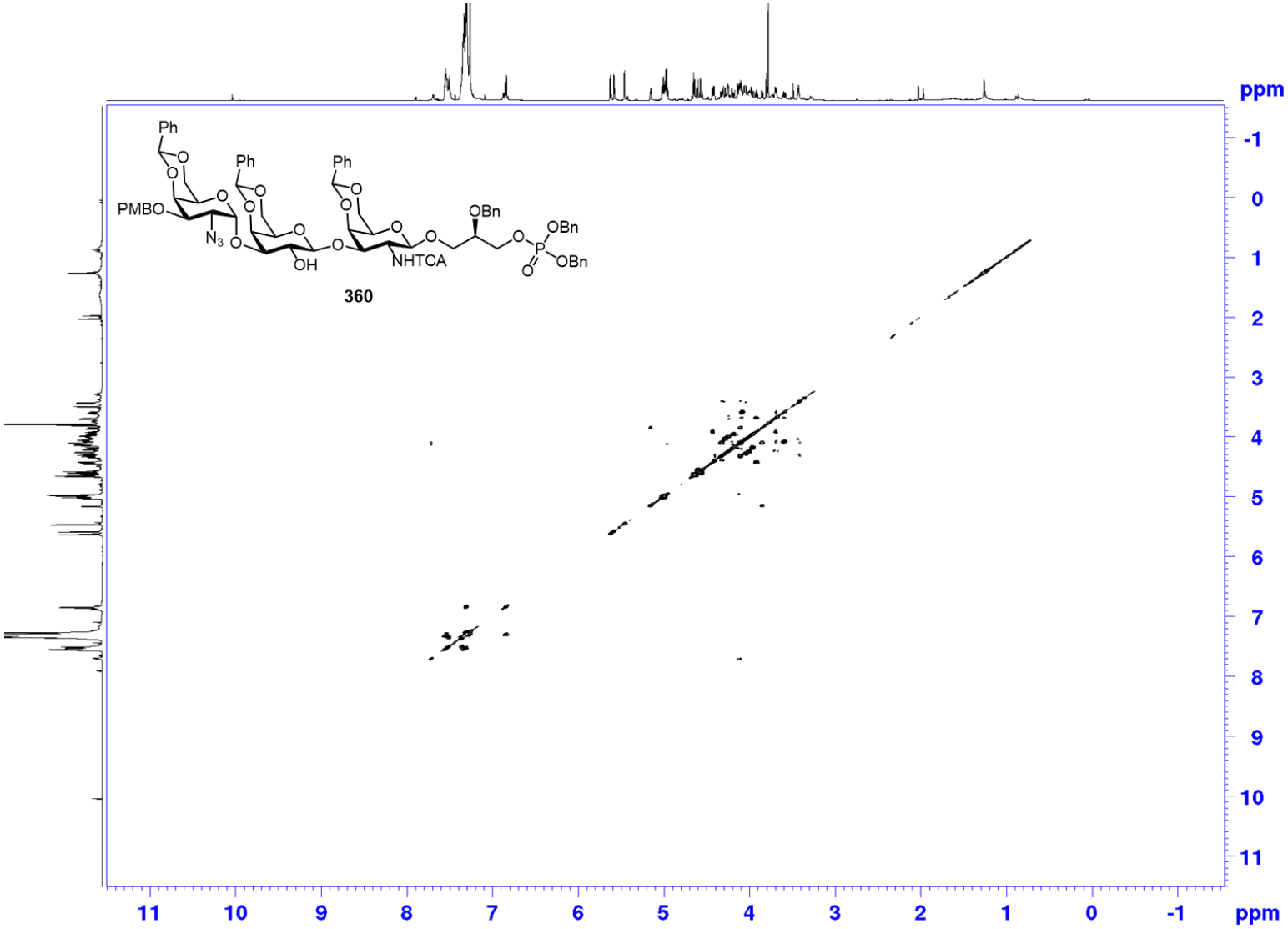
^{1}H-^{13}C HMBC ($CDCl_3$) of compound 360



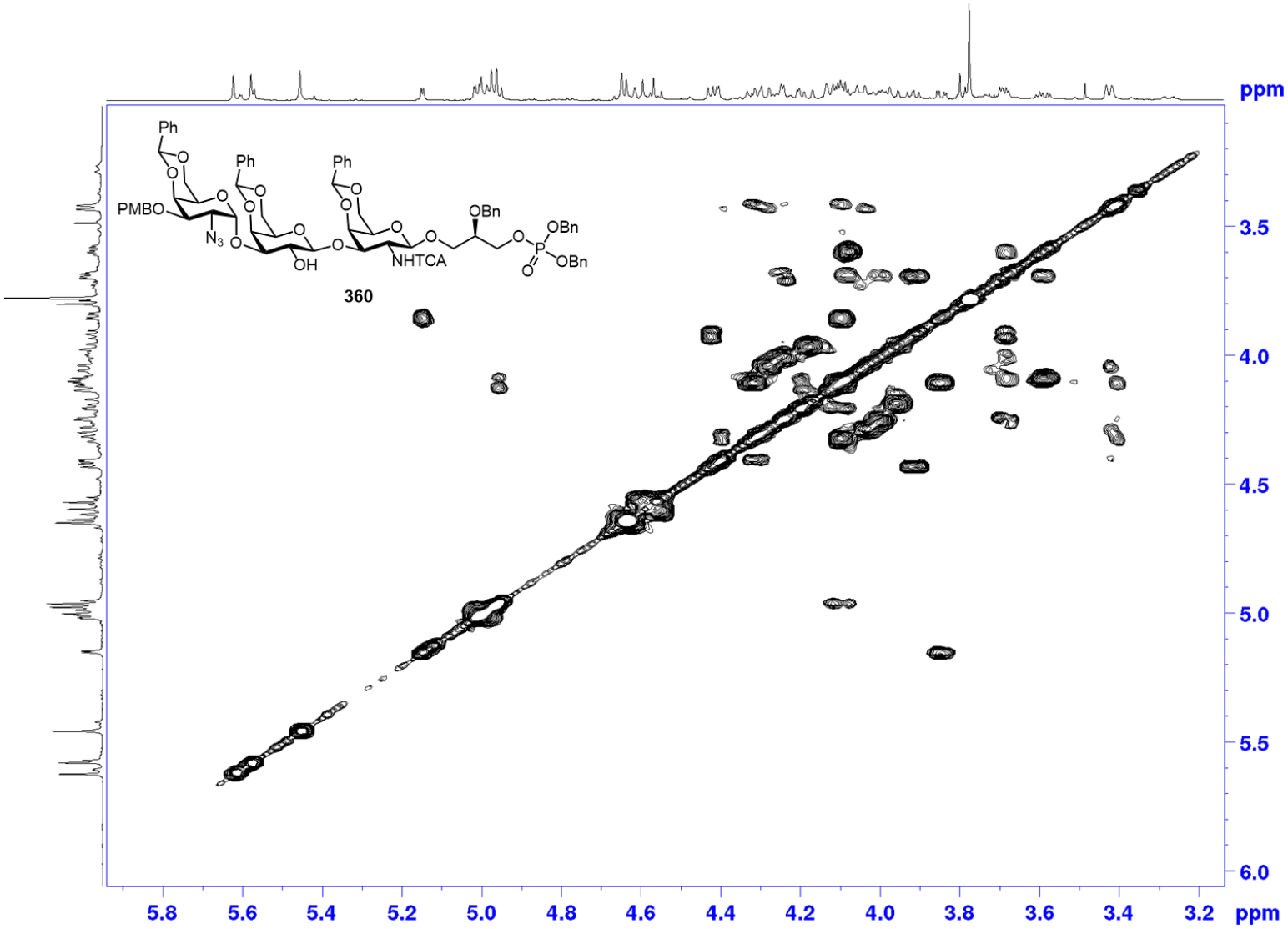
^{1}H-^{13}C HSCQ with ^{1}H-^{13}C HMBC overlay ($CDCl_3$) of compound 360



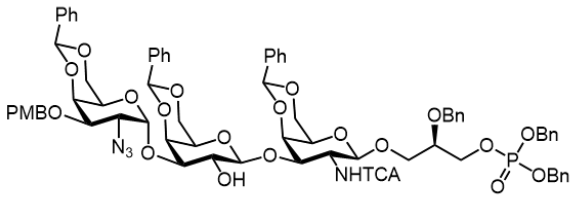
^{1}H-^{1}H COSY ($CDCl_3$) of compound 360



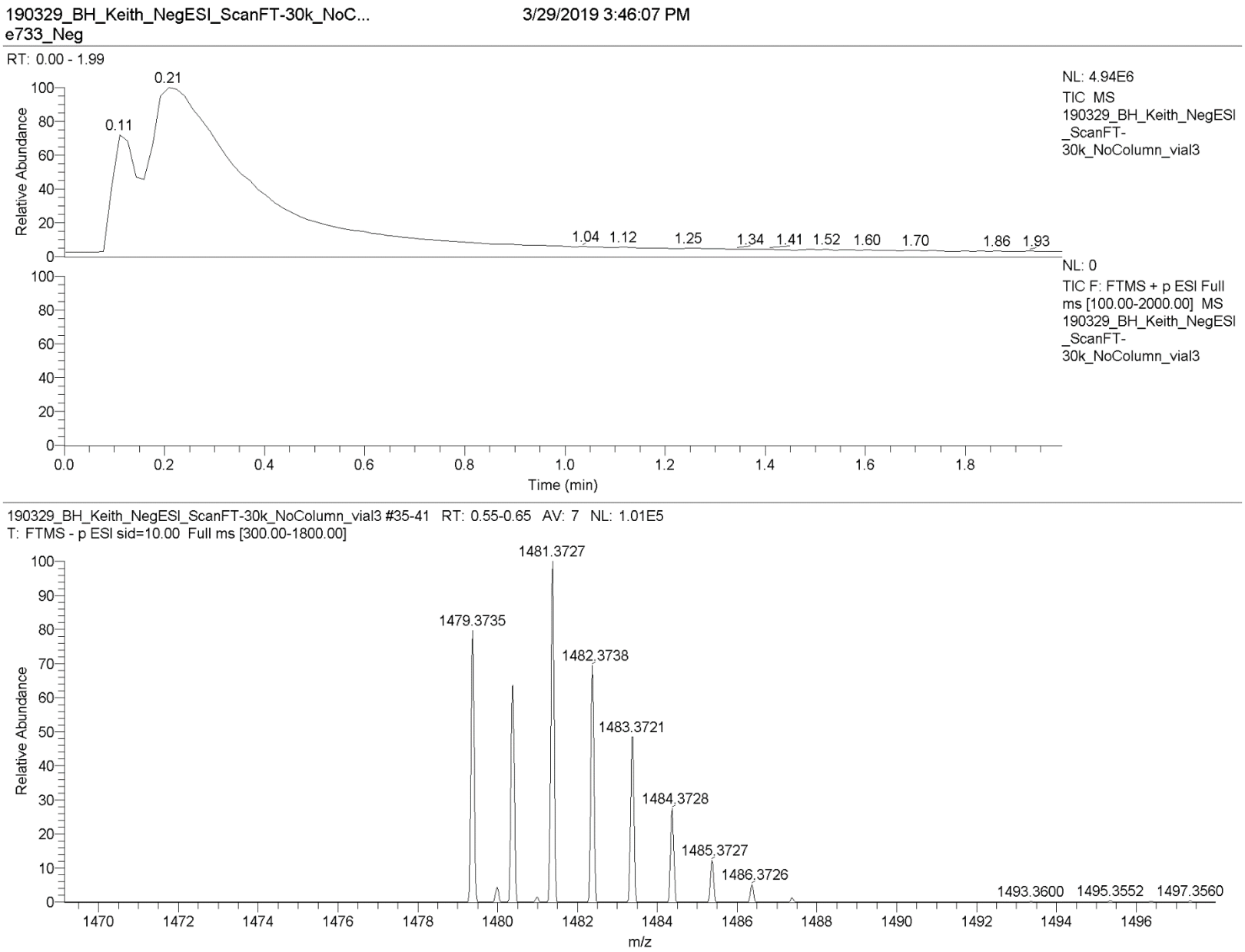
^{1}H-^{1}H COSY ($CDCl_3$) zoomed in region of compound 360



^{31}P NMR ($CDCl_3$, 162 MHz) of compound 360



360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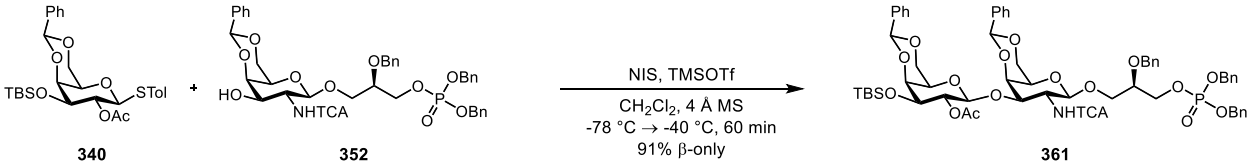
3/29/2019 3:46:07 PM
e733_Neg
RT: 0.00 - 1.99
0.21
0.11
1.04 1.12 1.25 1.34 1.41 1.52 1.60 1.70 1.86 1.93
Relative Abundance
NL: 4.94E6
TIC MS
190329_BH_Keith_NegESI
_ScanFT-
30k_NoColumn_vial3
NL: 0
TIC F: FTMS + p ESI Full
ms [100.00-2000.00] MS
190329_BH_Keith_NegESI
_ScanFT-
30k_NoColumn_vial3
Time (min)
190329_BH_Keith_NegESI_ScanFT-30k_NoColumn_vial3 #35-41 RT: 0.55-0.65 AV: 7 NL: 1.01E5
T: FTMS - p ESI sid=10.00 Full ms [300.00-1800.00]
1481.3727
1479.3735
1482.3738
1483.3721
1484.3728
1485.3727
1486.3726
1493.3600 1495.3552 1497.3560
Relative Abundance
m/z

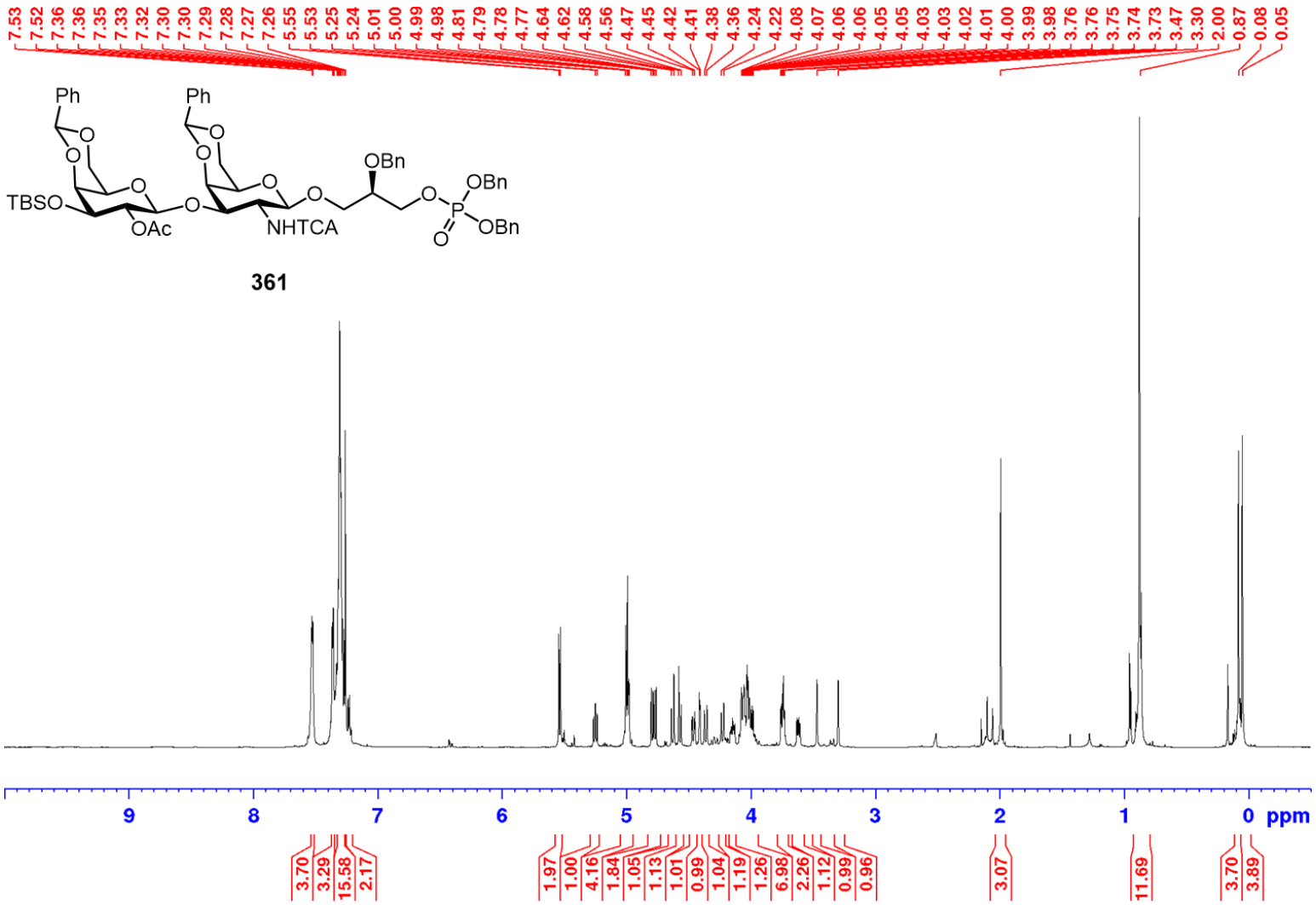
(*R*)-dibenzyl (2-(benzyloxy)-3-hydroxypropyl) phosphate 4,6-*O*-benzylidene acetal-2-*O*-acetyl-3-*O*-*tert*-butyldimethylsilyl-β-(1→3)-D-galactopyranosyl-4,6-*O*-benzylidene acetal-2-deoxy-2-trichloroacetamido-β-D-galactopyranoside (361)



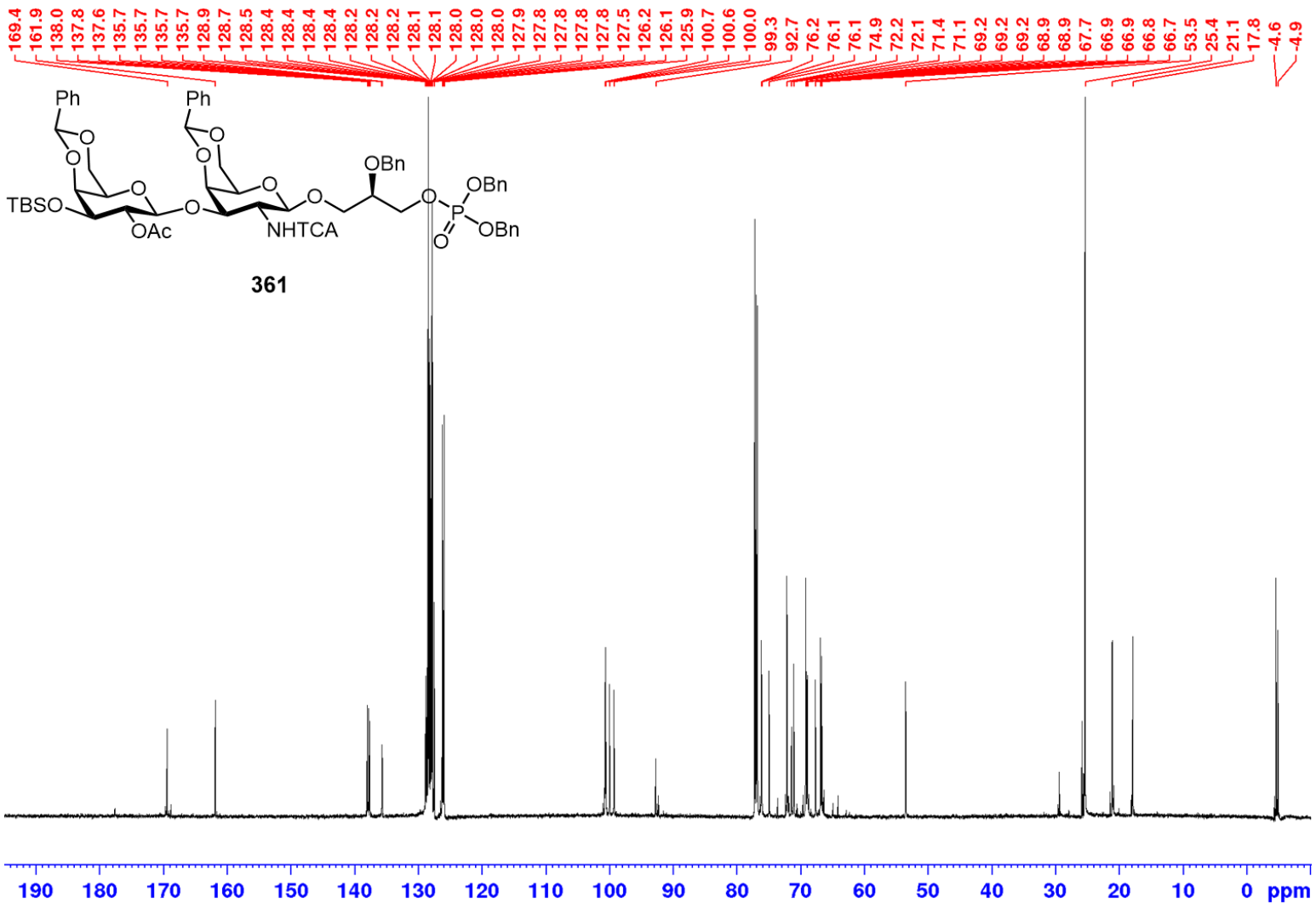
A solution of acceptor (*R*)-dibenzyl (2-(benzyloxy)-3-hydroxypropyl) phosphate 4,6-*O*-benzylidene acetal-2-deoxy-2-trichloroacetamido-β-D-galactopyranoside **352** (1.0 equiv., 1.866 g, 2.229 mmol) and donor *p*-tolyl 4,6-*O*-benzylidene acetal-3-*O*-*tert*-butyldimethylsilyl-2-*O*-acetyl-1-thio-β-D-galactopyranoside **340** (1.5 equiv., 1.98 g, 3.34 mmol) in anhydrous CH_2Cl_2 (30 mL) under an argon atmosphere was added preactivated, and cooled, 4 Å MS (4.0 g) at room temperature and allowed to stir for 1 h. The reaction mixture was cooled to -78 °C and added NIS (2.0 equiv., 1.003 g, 4.458 mmol). After 2 min, the crude reaction mixture was added a solution of TMSOTf (0.15 equiv., 1.2 mL, 5% in anhydrous CH_2Cl_2) dropwise. The reaction was held at -78 °C. At 1 h, TLC indicated complete consumption of the acceptor **352** starting material. The reaction was quench by the addition of dilute Et_3N in CH_2Cl_2 while warming to room temperature. The reaction was filtered through a pad of Celite to remove the 4 Å MS, concentrated to a crude oil. The crude oil was then diluted with EtOAc (100 mL) and transferred to a separatory funnel and washed with satd. aq. Na_2SO_4 (5 x 20 mL), brine (1 x 20 mL), dried ($NaSO_4$) and filtered through a glass fritted vacuum filter funnel to remove drying agent. The filtrate was concentrated to a crude oil which was purified by silica gel flash column chromatography (80:20 to 90:10 EtOAc/hexanes) give the disaccharide (*R*)-dibenzyl (2-(benzyloxy)-3-

hydroxypropyl) phosphate 4,6-*O*-benzylidene acetal-2-*O*-acetyl-3-*O*-*tert*-butyldimethylsilyl-β-(1→3)-D-galactopyranosyl-4,6-*O*-benzylidene acetal-2-deoxy-2-trichloroacetamido-β-D-galactopyranoside **361** (2.631 g, 2.015 mmol, 91%, β-only) anomericly pure as a white foam. R_f = 0.30 (80:20 EtOAc/hexanes) visualized with ceric ammonium molybdate stain.; $[\alpha]D^{25}$ = +7.08° (c = 1.0, $CHCl_3$); IR ν_{max} (film) 3053.52, 2986.50, 1718.81, 1421.41, 1264.44, 1054.44, 895.92, 839.76, 748.01, 702.66 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.52 (d, *J* = 3.9 Hz, 4H), 7.36-7.35 (m, 3H), 7.33-7.27 (m, 16H), 7.25-7.21 (m, 2H), 5.54 (d, *J* = 8.4 Hz, 2H), 5.25 (dd, *J* = 8.3, 9.2 Hz, 1H), 5.01-4.97 (m, 4H), 4.79 (d, *J* = 15.5 Hz, 1H), 4.78 (d, *J* = 15.8 Hz, 1H), 4.63 (d, *J* = 11.9 Hz, 1H), 4.57 (d, *J* = 12.3, 1H), 4.46 (dd, *J* = 3.5, 11.1 Hz, 1H), 4.41 (d, *J* = 3.3 Hz, 1H), 4.36 (d, *J* = 11.6 Hz, 1H), 4.23 (d, *J* = 11.8 Hz, 11H), 4.17-4.12 (m, 1H), 4.08-3.98 (m, 7H), 3.76-3.73 (m, 2H), 3.63-3.60 (m, 1H), 3.47 (s, 1H), 3.30 (s, 1H), 2.00 (s, 2H), 0.87 (s, 9H), 0.08 (s, 3H), 0.05 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.4, 161.9, 138.0, 137.8, 137.6, 135.7, 135.7, 135.7, 135.7, 128.9, 128.7, 128.5, 128.4, 128.4, 128.4, 128.4, 128.3, 128.3, 128.2, 128.2, 128.2, 128.1, 128.1, 128.0, 128.0, 128.0, 127.9, 127.8, 127.8, 127.8, 127.8, 127.8, 127.7, 127.5, 126.2, 126.1, 125.9, 100.7, 100.6, 100.0, 99.3, 92.7, 76.2, 76.1, 76.1, 74.9, 72.2, 72.1, 71.4, 71.1, 69.2, 69.2, 69.2, 68.9, 68.9, 67.7, 66.9, 66.9, 66.8, 66.7, 53.5, 25.4, 21.1, 17.8, -4.6, -4.9; ^{31}P NMR (151 MHz, $CDCl_3$) δ 0.15; HR-ESI-MS (m/z): calcd for $C_{60}H_{71}Cl_3NO_{17}PSiNa^+$ $(M+Na)^+$ 1264.318618, found 1264.3195.

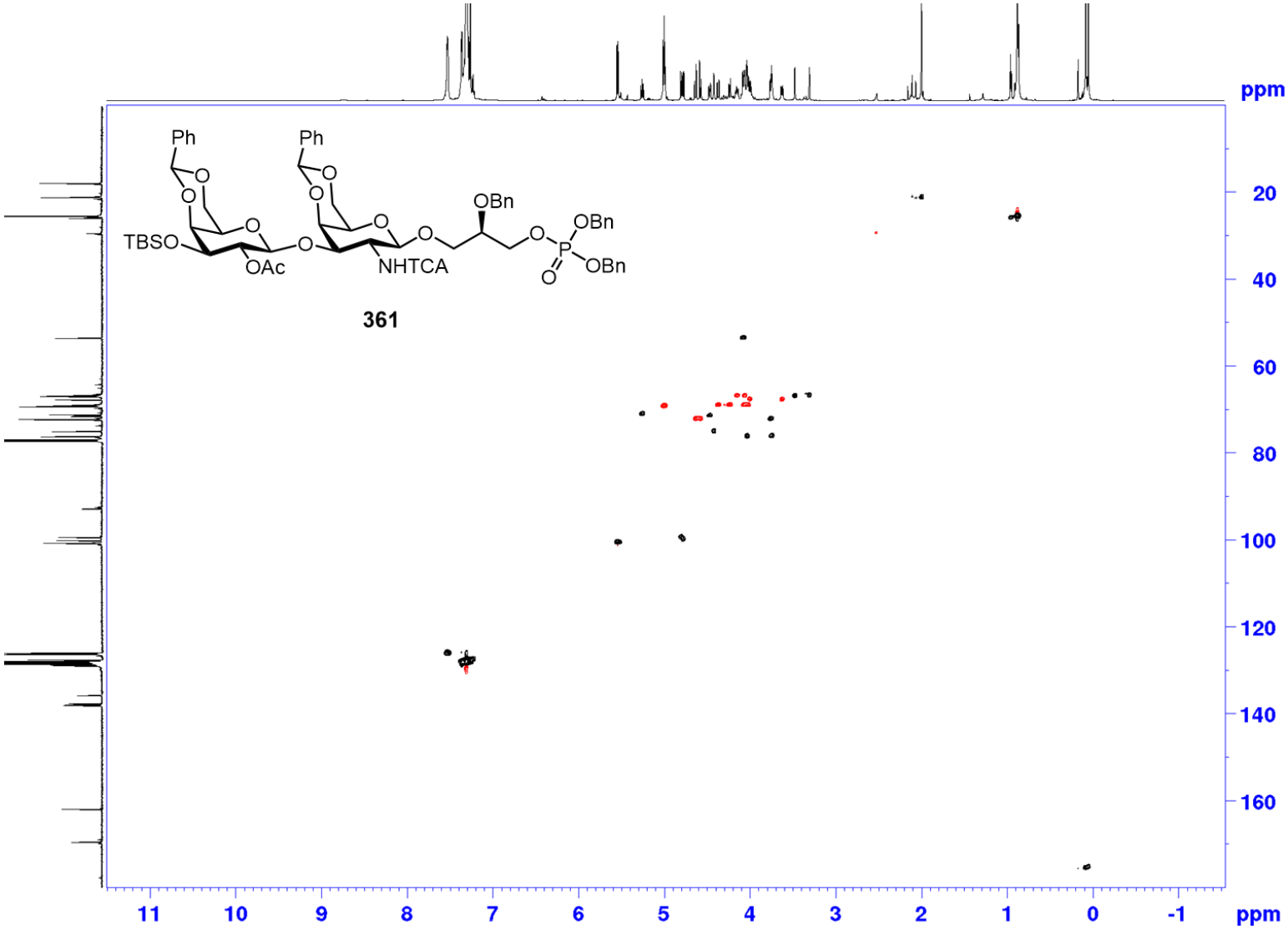
^{1} NMR ($CDCl_3$, 400 MHz) of compound 361



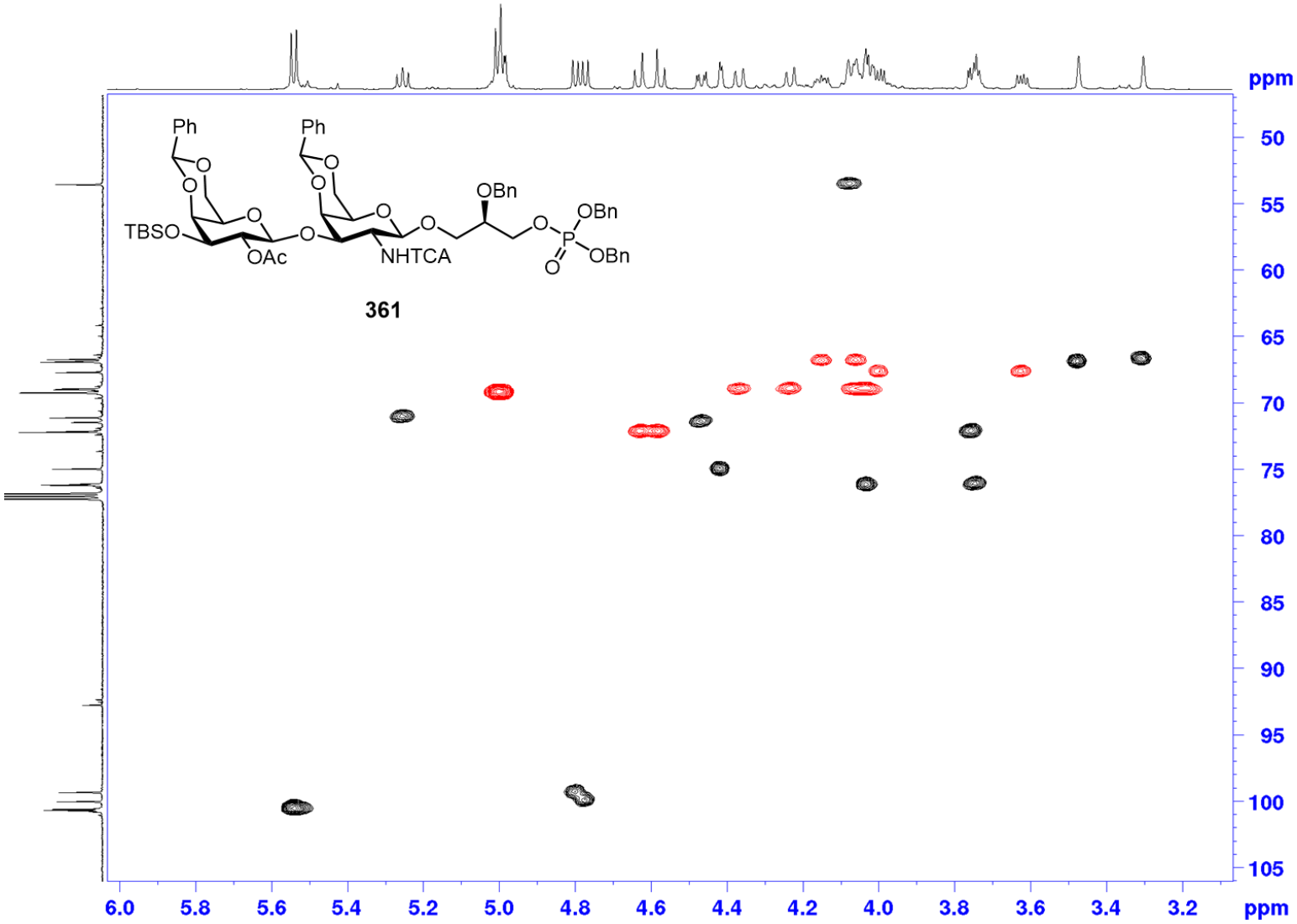
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 361



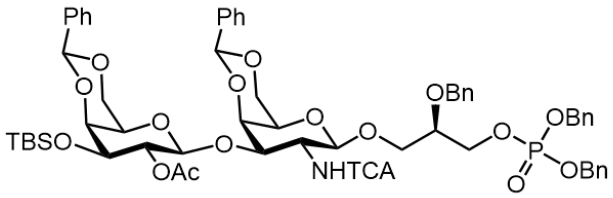
^{1}H-^{13}C HSCQ ($CDCl_3$) of compound 361



^{1}H-^{13}C HSCQ ($CDCl_3$) zoomed in region of compound 361



^{31}P NMR ($CDCl_3$, 162 MHz) of compound 361



361

190403_BH_Keith_PosESI_ScanFT-30k_NoC... 4/3/2019 9:37:10 AM
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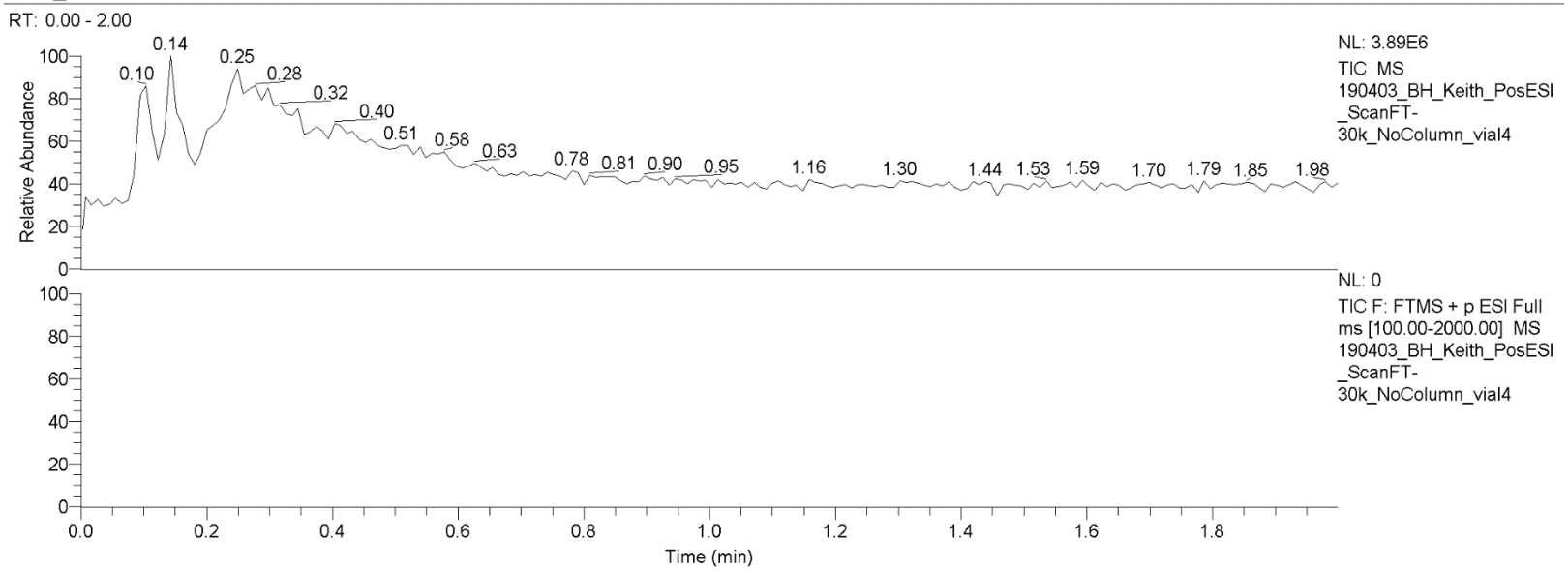

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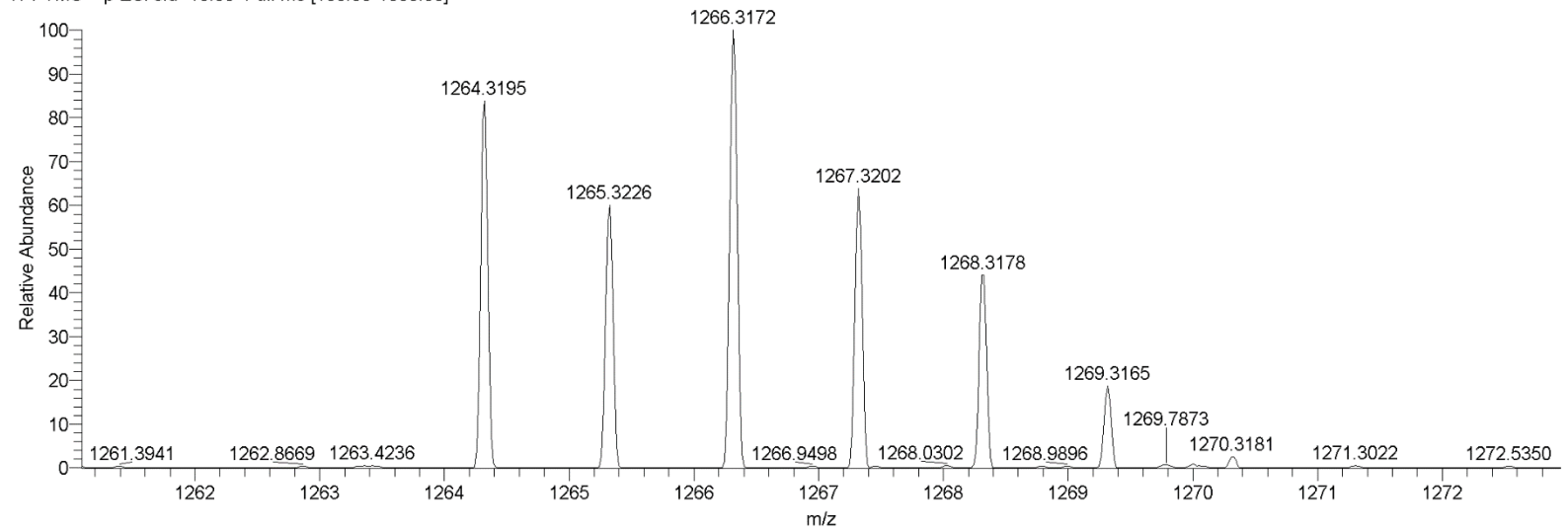
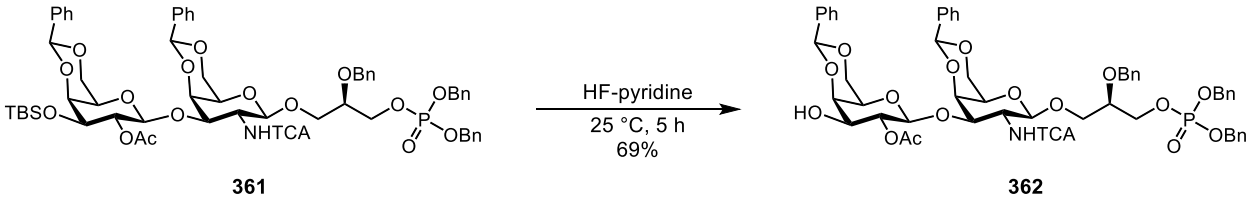


(*R*)-dibenzyl (2-(benzyloxy)-3-hydroxypropyl) phosphate 4,6-*O*-benzylidene acetal-2-*O*-acetyl-β-(1→3)-D-galactopyranosyl-4,6-*O*-benzylidene acetal-2-deoxy-2-trichloroacetamido-β-D-galactopyranoside (362)

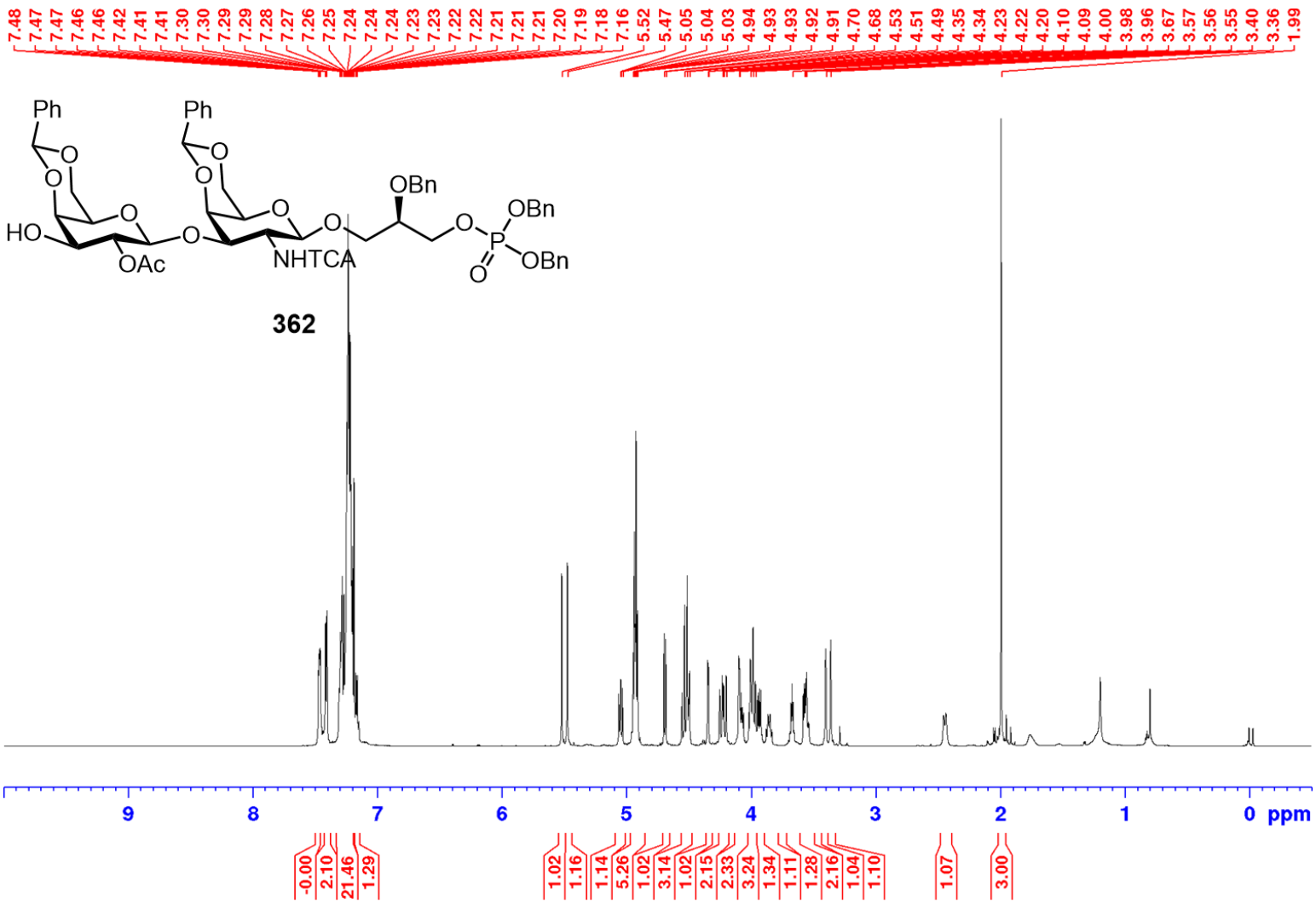


361 **362**

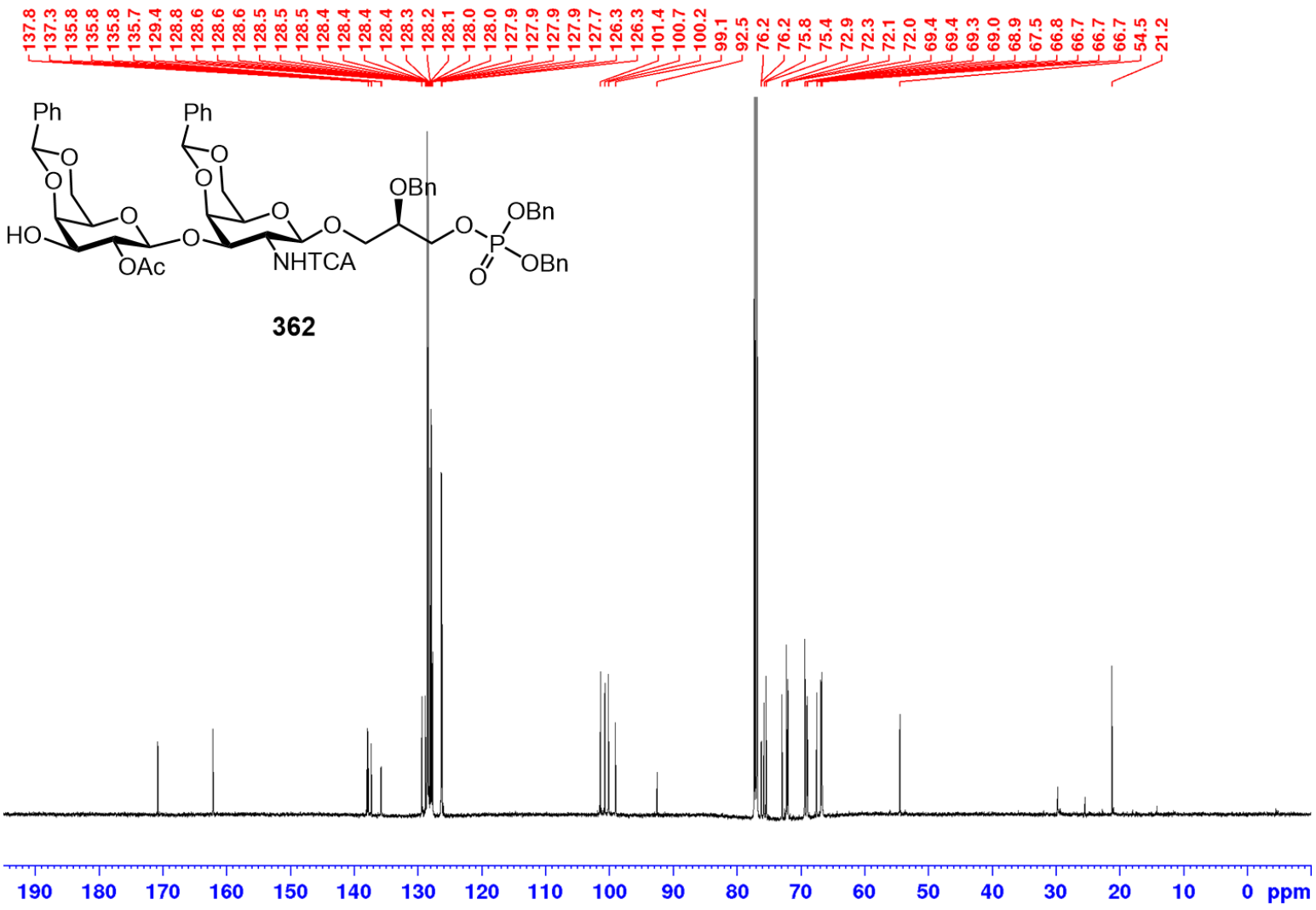
A solution of disaccharide (*R*)-dibenzyl (2-(benzyloxy)-3-hydroxypropyl) phosphate 4,6-*O*-benzylidene acetal-2-*O*-acetyl-3-O-*tert*-butyldimethylsilyl-β-(1→3)-D-galactopyranosyl-4,6-*O*-benzylidene acetal-2-deoxy-2-trichloroacetamido-β-D-galactopyranoside **361** (1.0 equiv., 2.631 g, 2.0151 mmol) in pyridine (20.0 mL) was added a 70% HF-pyridine solution (10 equiv., 2.6 mL) at 0 °C under an argon atmosphere. The reaction was stirred at room temperature. At 9 h, TLC indicated complete consumption of starting material. The reaction was diluted with EtOAc (100 mL) and transferred to a separatory funnel and washed with 1 N HCl (5 x 20 mL), satd. aq. $NaHCO_3$ (1 x 20 mL), brine (1 x 20 mL), dried (NaSO4) and filtered through a glass fritted vacuum filter funnel to remove drying agent. The filtrate was concentrated to a crude oil which was purified by flash column chromatography (1:1 then 4:3 to 9:1 EtOAc/hexanes) give the disaccharide acceptor (*R*)-dibenzyl (2-(benzyloxy)-3-hydroxypropyl) phosphate 4,6-*O*-benzylidene acetal-2-*O*-acetyl-β-(1→3)-D-galactopyranosyl-4,6-*O*-benzylidene acetal-2-deoxy-2-trichloroacetamido-β-D-galactopyranoside **362** (1.669 g, 1.401 mmol, 69%) as white foam. R_f = 0.20 (80:20 EtOAc/hexanes) visualized with ceric ammonium molybdate stain; $[\alpha]D^{25}$ = +43.68° (c = 1.0, $CHCl_3$); IR ν_{max} (film) 3489.32, 3054.30, 2985.82, 2305.41, 2112.43, 1746.30, 1608.80, 1496.65, 1421.61, 1373.51, 1351.60, 1318.44,

1264.78, 1194.24, 1139.04, 1107.32, 1048.12, 895.82, 804.88, 746.16, 704.11, 477.16 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.48-7.46 (m, 2H), 7.42-7.41 (m, 2H), 7.31-7.16 (m, 21H), 5.52 (s, 1H), 5.47 (s, 1H), 5.04 (dd, *J* = 8.1, 9.8 Hz, 1H), 4.49-4.91 (m, 5H), 4.68 (d, *J* = 8.0 Hz, 1H), 4.55-4.49 (m, 3H), 4.34 (d, *J* = 3.3 Hz, 1H), 4.22 (dd, *J* = 12.36, 19.2 Hz, 2H), 4.10-4.06 (m, 2H), 4.02-3.96 (m, 3H), 3.95-3.92 (dd, *J* = 5.1, 10.8 Hz, 1H), 3.87-3.83 (m, 1H), 3.68-3.66 (m, 1H), 3.58-3.53 (m, 2H), 3.40 (s, 1H), 3.36 (s, 1H), 2.44 (d, *J* = 10.5, 1H), 1.99 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.8, 162.1, 137.9, 137.8, 137.3, 135.8, 135.8, 135.8, 135.7, 129.4, 128.8, 128.6, 128.6, 128.6, 128.5, 128.5, 128.5, 128.4, 128.4, 128.4, 128.4, 128.3, 128.2, 128.1, 128.0, 128.0, 127.9, 127.9, 127.9, 127.9, 127.7, 126.3, 126.3, 101.4, 100.7, 100.2, 99.1, 92.5, 76.2, 76.2, 75.8, 75.4, 72.9, 72.3, 72.1, 72.0, 69.4, 69.4, 69.3, 69.0, 68.9, 67.5, 66.8, 66.7, 66.7, 66.7, 54.5, 21.2; HR-ESI-MS (m/z): calcd for $C_{54}H_{58}Cl_3NO_{17}P^+$ ($M+H^+$) 1128.250276, found 1128.2515.

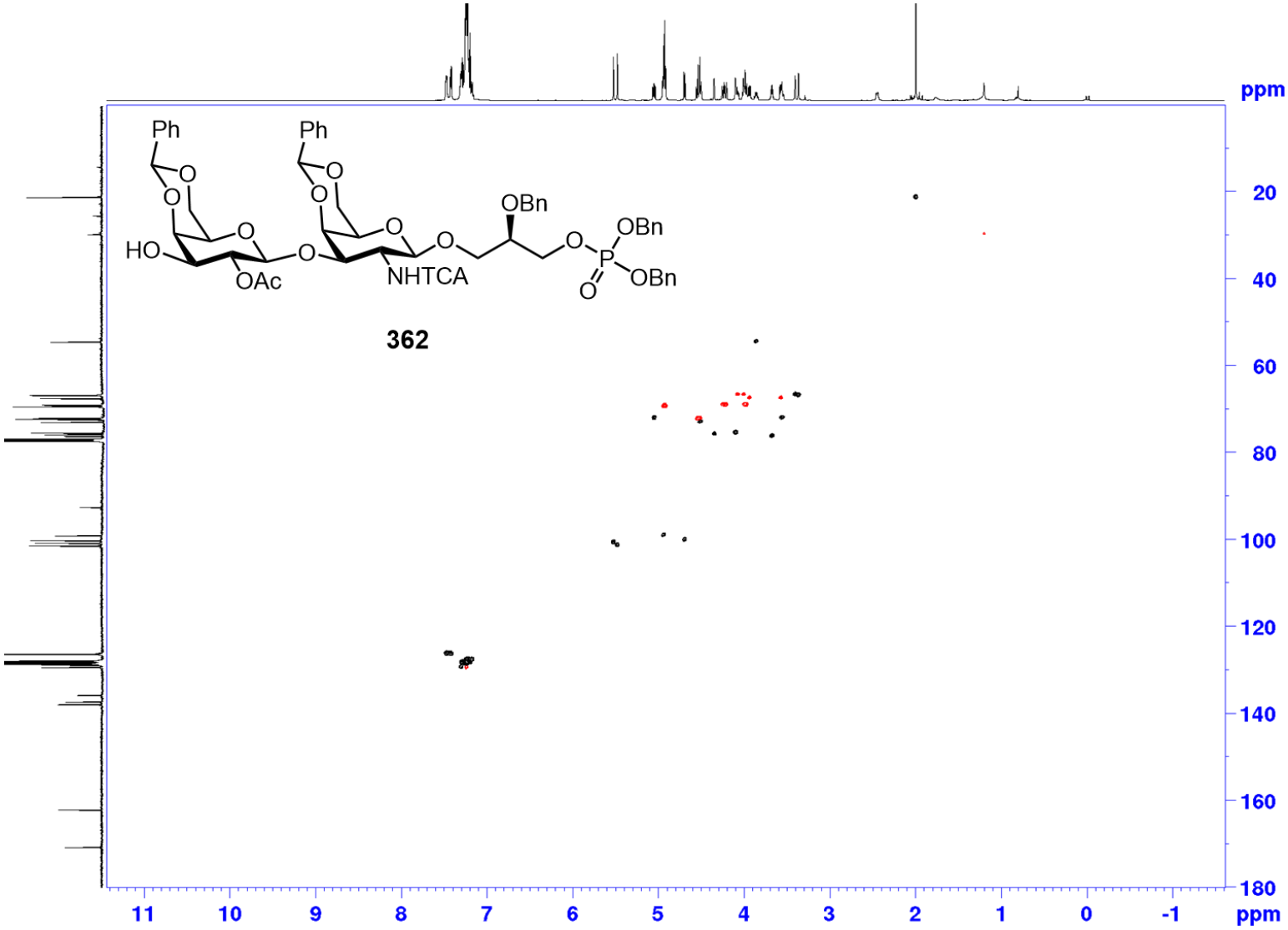
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 362



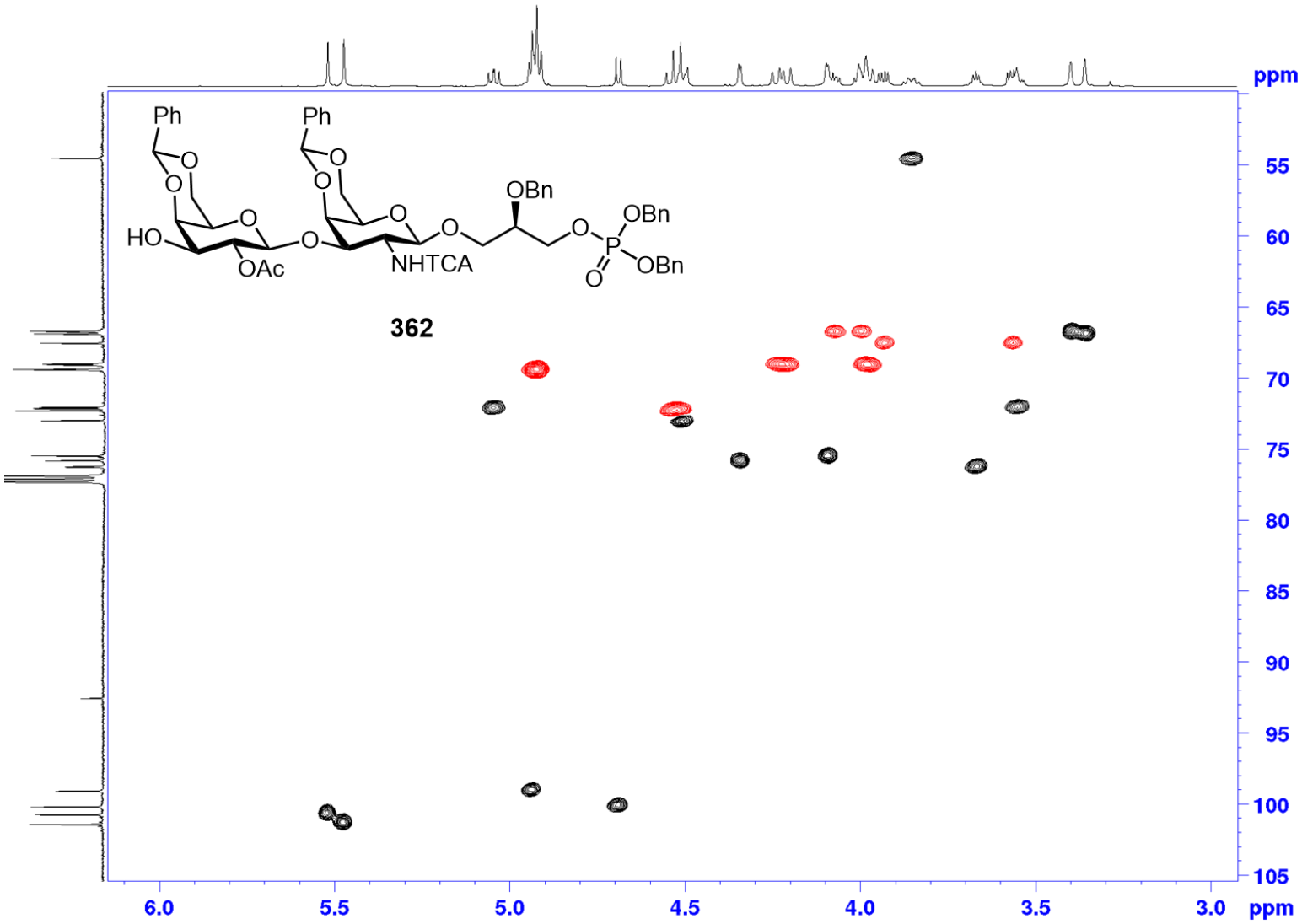
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 362



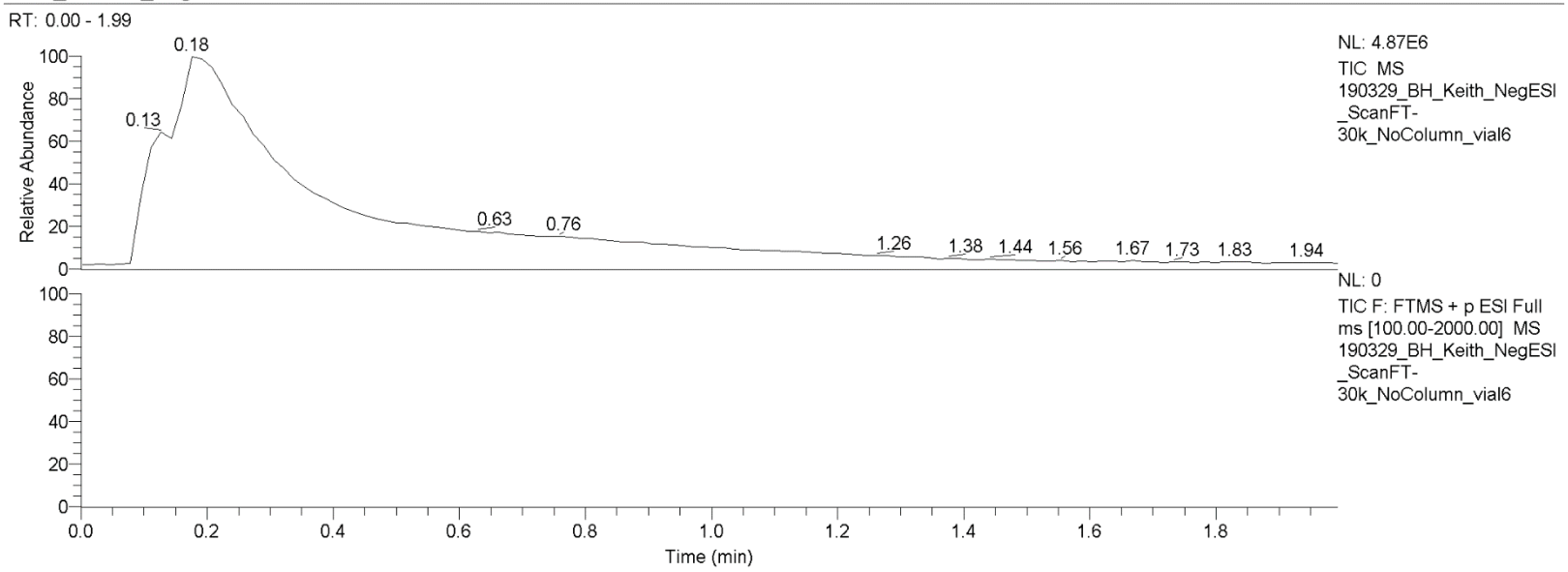
^{1}H-^{13}C HSCQ ($CDCl_3$) of compound 362

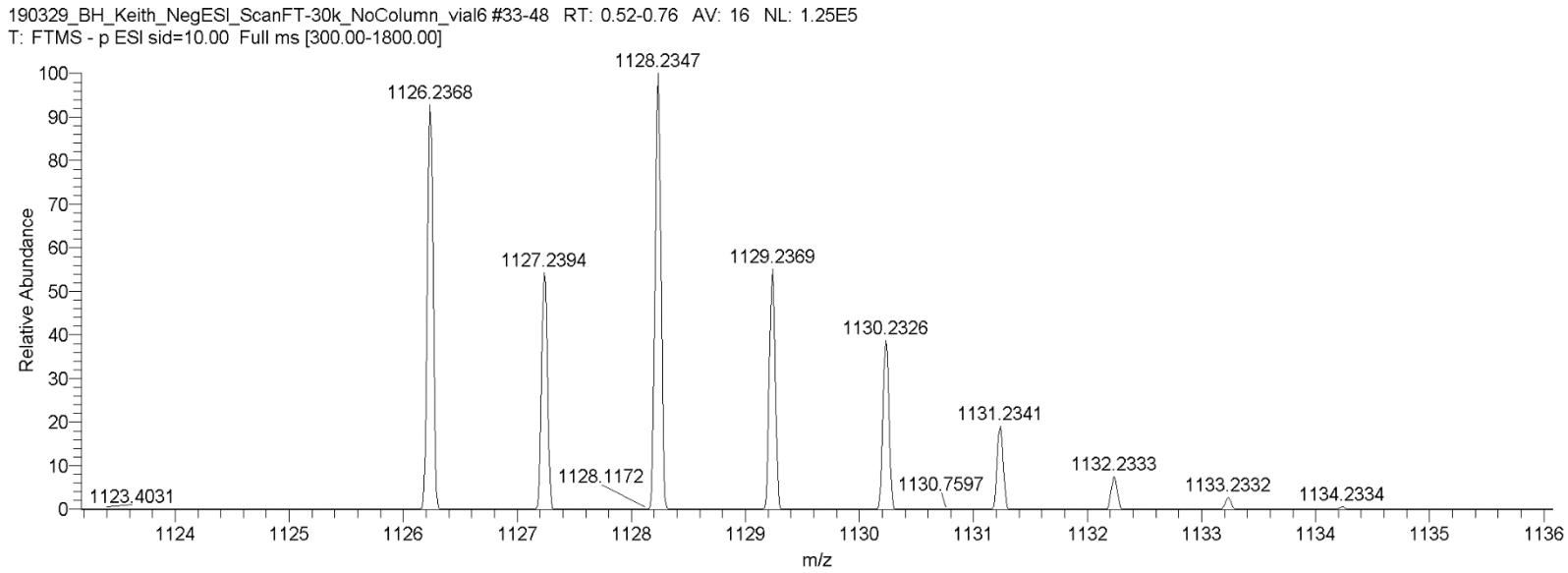


^{1}H-^{13}C HSCQ ($CDCl_3$) zoomed in region of compound 362

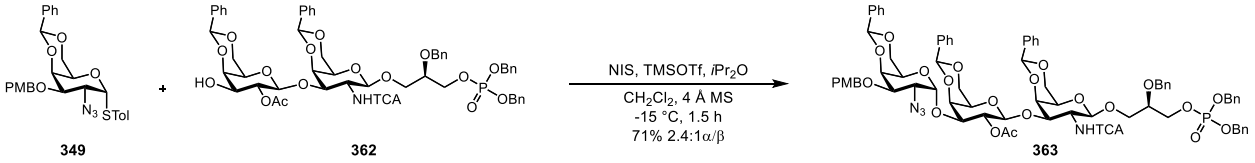


190329_BH_Keith_NegESI_ScanFT-30k_NoC... 3/29/2019 3:54:06 PM
e724_C2-OAc_Neg





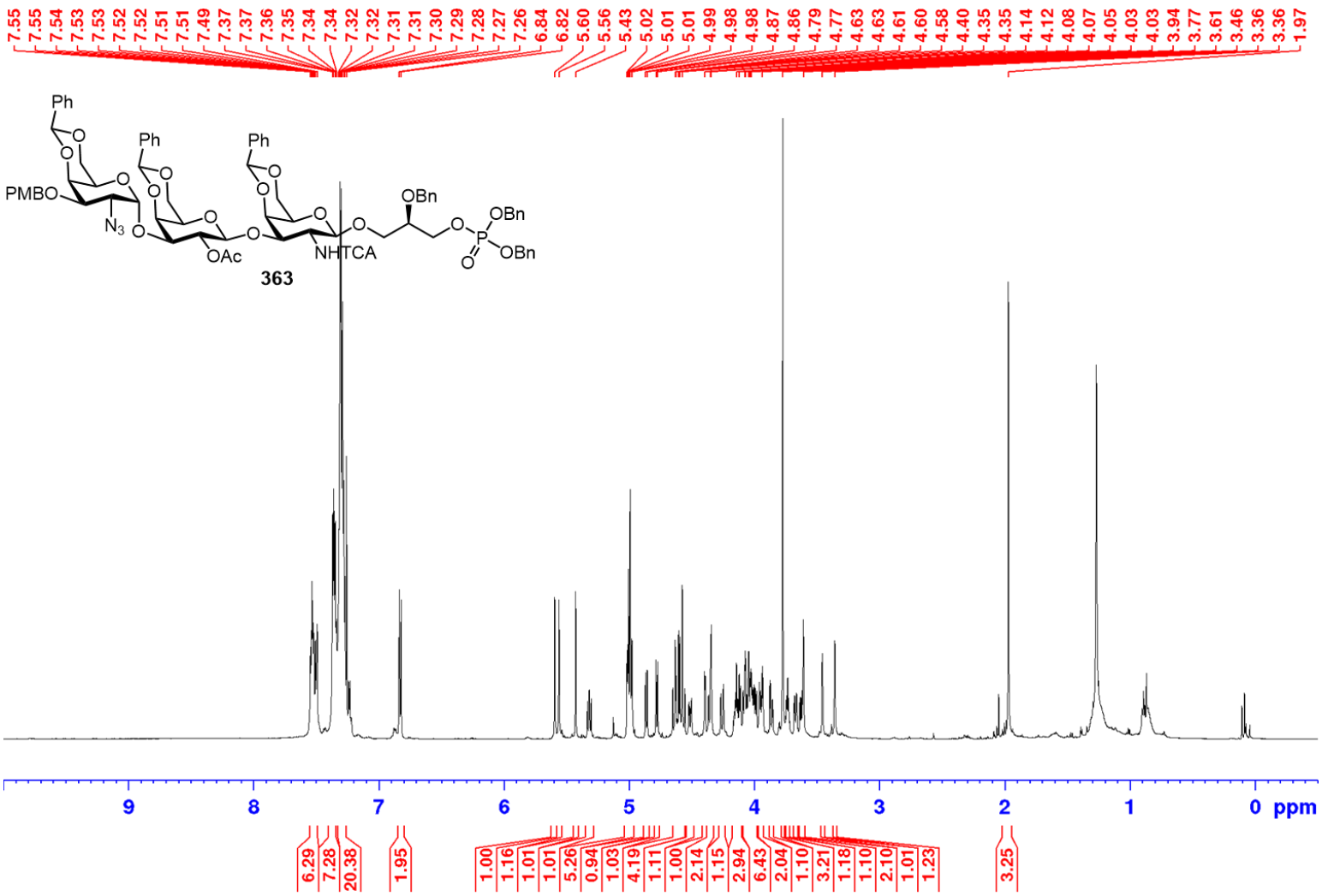
acetal-2-deoxy-2-trichloroacetamido-β-D-galactopyranoside (363)



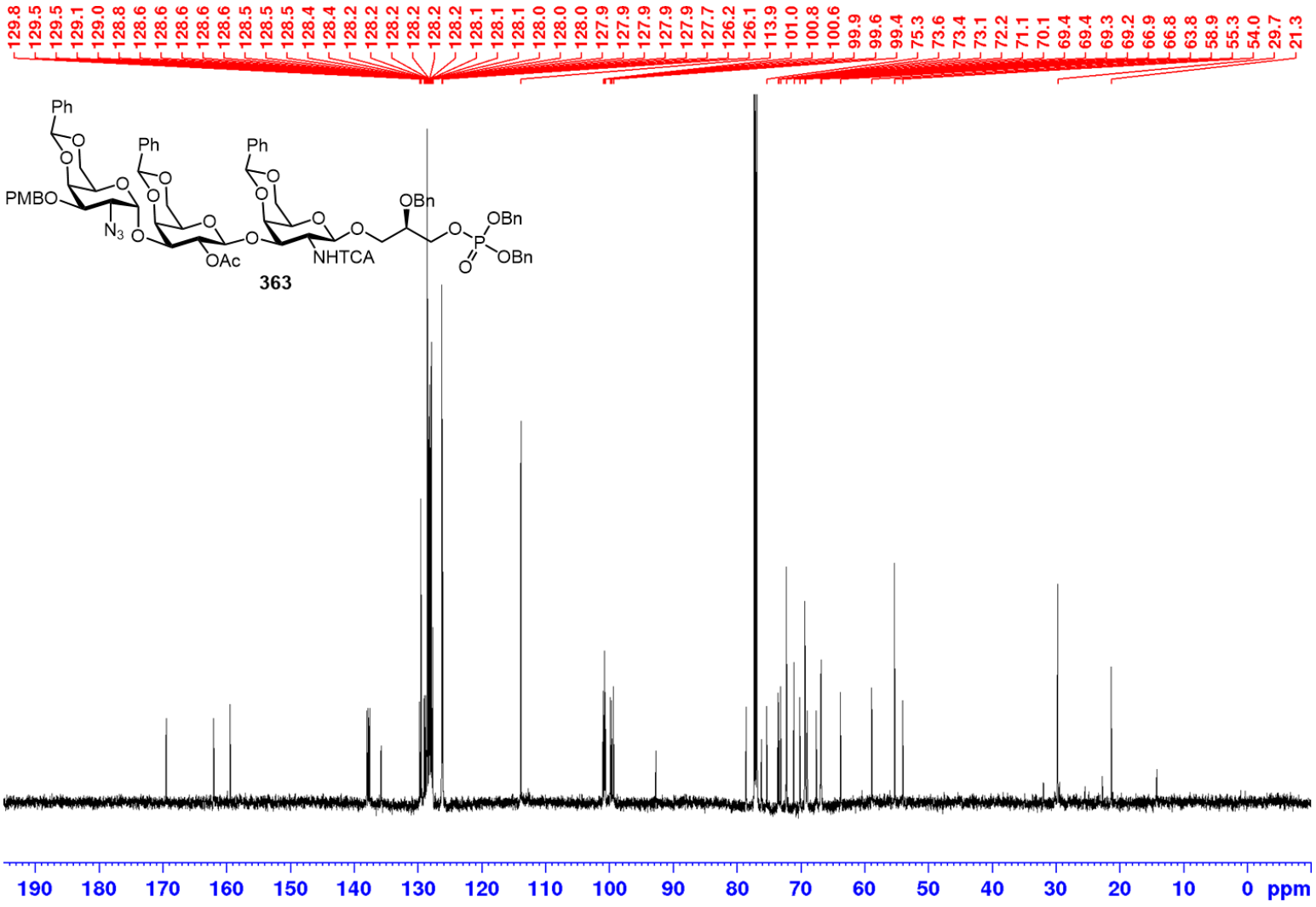
A solution of disaccharide acceptor (*R*)-dibenzyl (2-(benzyloxy)-3-hydroxypropyl) phosphate 4,6-*O*-benzylidene acetal-2-*O*-acetyl-β-(1→3)-D-galactopyranosyl-4,6-*O*-benzylidene acetal-2-deoxy-2-trichloroacetamido-β-D-galactopyranoside **362** (1.0 equiv., 1.5737 g, 1.393 mmol) and donor *p*-tolyl 4,6-*O*-benzylidene acetal-3-*O*-(*p*-methoxy benzyl)-2-deoxy-2-azido-1-thio-α/β-D-galactopyranoside **349** (1.4 equiv., 1.0137 g, 1.951 mmol) in a mixture of anhydrous *i*Pr$_2$O, CH$_2$Cl$_2$ (0.80 mL, 1:4) was added preactivated, and cooled, 4 Å MS (0.150 g) at room temperature under an argon atmosphere. The reaction mixture was stirred for 1 h. The reaction mixture was cooled to -15 °C and then added a solution of TMSOTf (0.20 equiv., 0.054 mL, 0.014 mmol, 5% in anhydrous CH$_2$Cl$_2$) dropwise. The reaction temperature was held constant. At 1.5 h, TLC indicated that all the acceptor **362** starting material had been consumed. The reaction was quench by the addition of dilute Et$_3$N in CH$_2$Cl$_2$. The reaction was filtered through a pad of Celite to remove the 4 Å MS. The filtrate was concentrated to a crude oil and purified by silica gel flash column chromatography (5:95 to 20:80 CH$_3$OH/CH$_2$Cl$_2$) give the C1''-α-linked trisaccharide (*R*)-dibenzyl (2-(benzyloxy)-3-hydroxypropyl) phosphate 4,6-*O*-benzylidene acetal-2-deoxy-2-azido-3-*O*-*para*-methoxy benzyl-α-(1→3)-D-galactopyranosyl-4,6-*O*-benzylidene acetal-2-*O*-acetyl-β-(1→3)-D-galactopyranosyl-4,6-*O*-benzylidene acetal-2-deoxy-2-trichloroacetamido-β-D-galactopyranoside **363** (1.516 g, 0.994 mmol, 71% as a 2.4:1 C1''-α/β mixture total, 1.068 g, 0.700 mmol, 50% C1''-α-only) as white solid. R_f =

0.20 (80:20 EtOAc/hexanes) visualized with ceric ammonium molybdate stain; $[\alpha]D^{25}$ = +16.28° (c = 1.0, $CHCl_3$); IR ν_{max} (film) 3409.96, 2110.66, 1641.47, 1513.47, 1454.95, 1368.00, 1247.70, 1174.27, 1023.22, 821.11, 734.38, 698.04 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.55-7.49 (m, 6H), 7.37-7.32 (m, 7H), 7.31-7.27 (m, 19H), 6.83 (d, *J* = 8.6 Hz, 2H), 5.60 (s, 1H), 5.56 (s, 1H), 5.45 (s, 1H), 5.32 (dd, *J* = 8.3, 9.8 Hz, 1H), 5.02-4.98 (m, 5H), 4.86 (d, *J* = 8.5 Hz, 1H), 4.65-4.56 (m, 4H), 4.51 (dd, *J* = 3.3, 11.1 Hz, 1H), 4.39 (d, 3.30 Hz, 1H), 4.36-4.33 (m, 2H), 4.27-4.25 (m, 1H), 4.17-4.11 (m, 3H), 4.09-3.98 (m, 6H), 3.96-3.93 (m, 2H), 3.86 (dd, *J* = 3.1, 10.6 Hz, 1 H), 3.77 (s, 3H), 3.75-3.73 (m, 1H), 3.67 (dd, *J* = 3.5, 9.8 1H), 3.64-3.61 (m, 2H), 3.46 (s, 1H), 3.36 (m, 1H), 1.97 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 177.2, 165.0, 162.1, 159.4, 138.0, 137.7, 137.6, 137.4, 135.8, 135.8, 133.6, 129.9, 129.7, 129.5, 129.4, 129.1, 128.9, 128.7, 128.5, 128.5, 128.5, 128.4, 128.4, 128.3, 128.3, 128.2, 128.2, 128.1, 128.0, 127.9, 127.9, 127.9, 127.9, 127.8, 127.6, 126.3, 126.2, 126.1, 126.1, 113.9, 113.8, 101.1, 100.6, 99.7, 99.5, 97.9, 92.6, 76.2, 76.1, 75.4, 73.8, 72.9, 72.6, 72.3, 71.2, 70.3, 69.3, 69.3, 69.3, 69.0, 68.7, 67.9, 67.0, 66.9, 66.9, 66.7, 63.3, 58.3, 55.3, 54.0; ^{31}P NMR (151 MHz, $CDCl_3$) δ 0.17; HR-ESI-MS (m/z): calcd for $C_{80}H_{81}Cl_3N_4O_{22}P^+$ ($M+H^+$) 1540.424923, found 1540.4260.

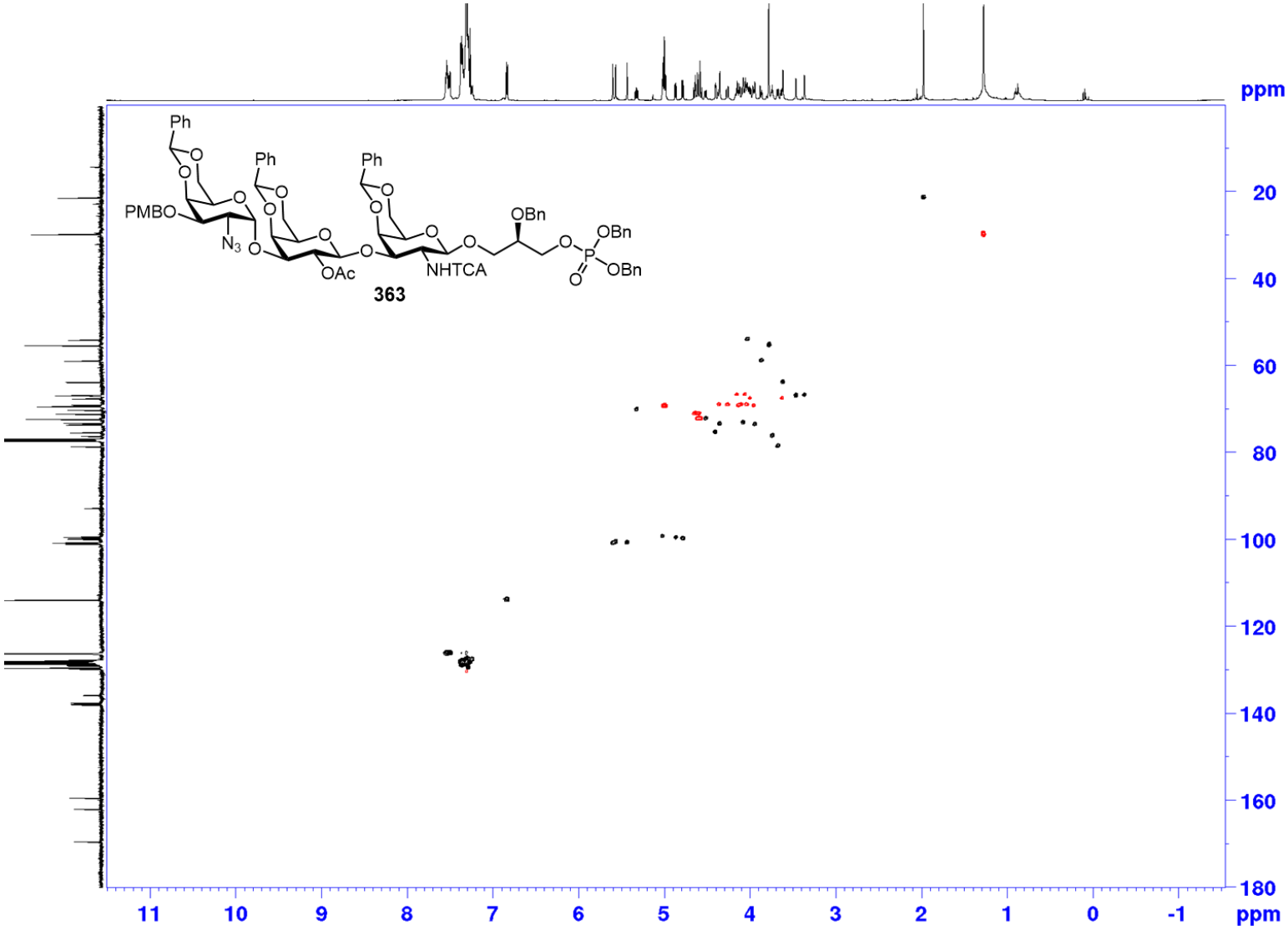
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 363



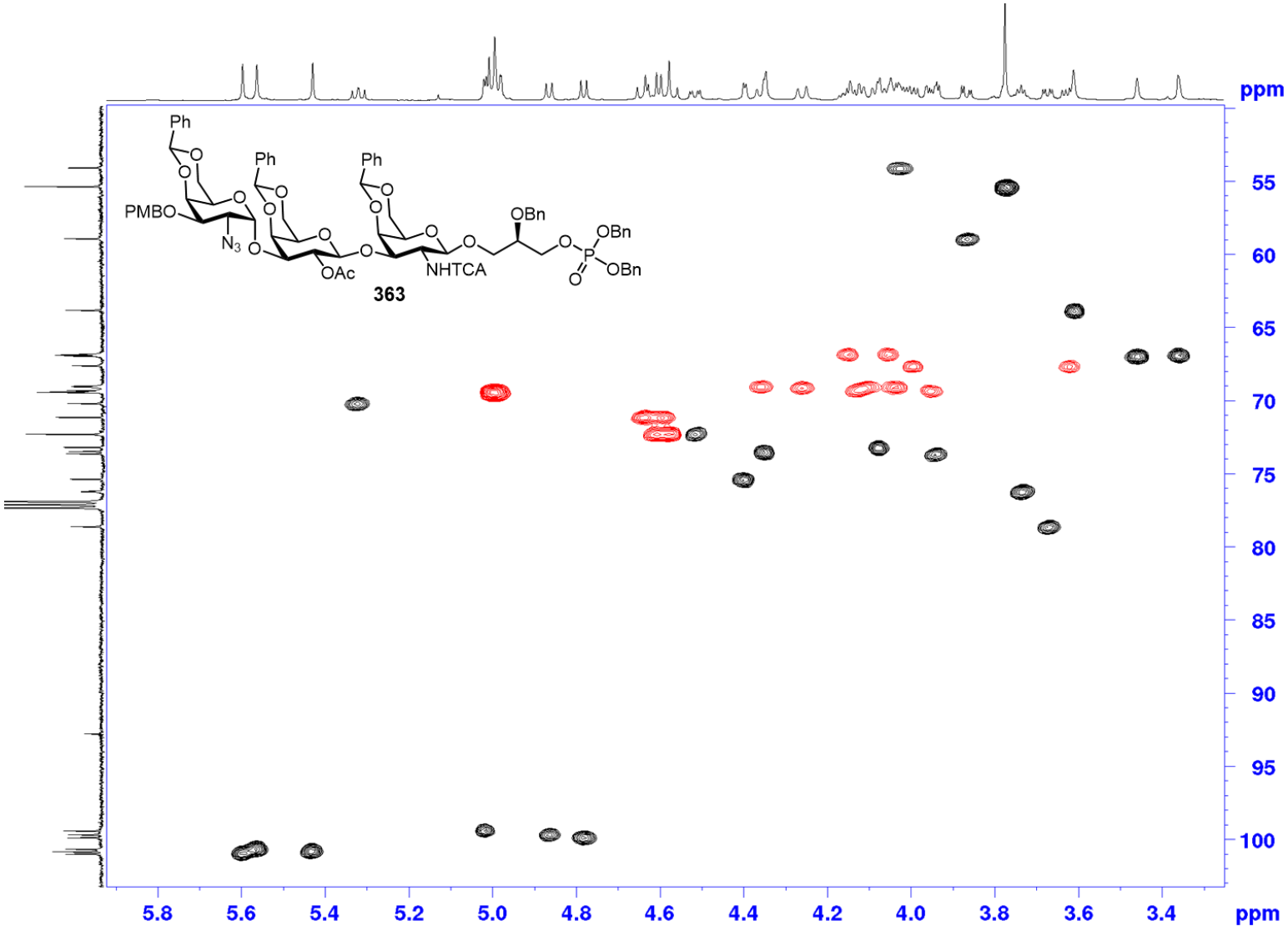
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 363



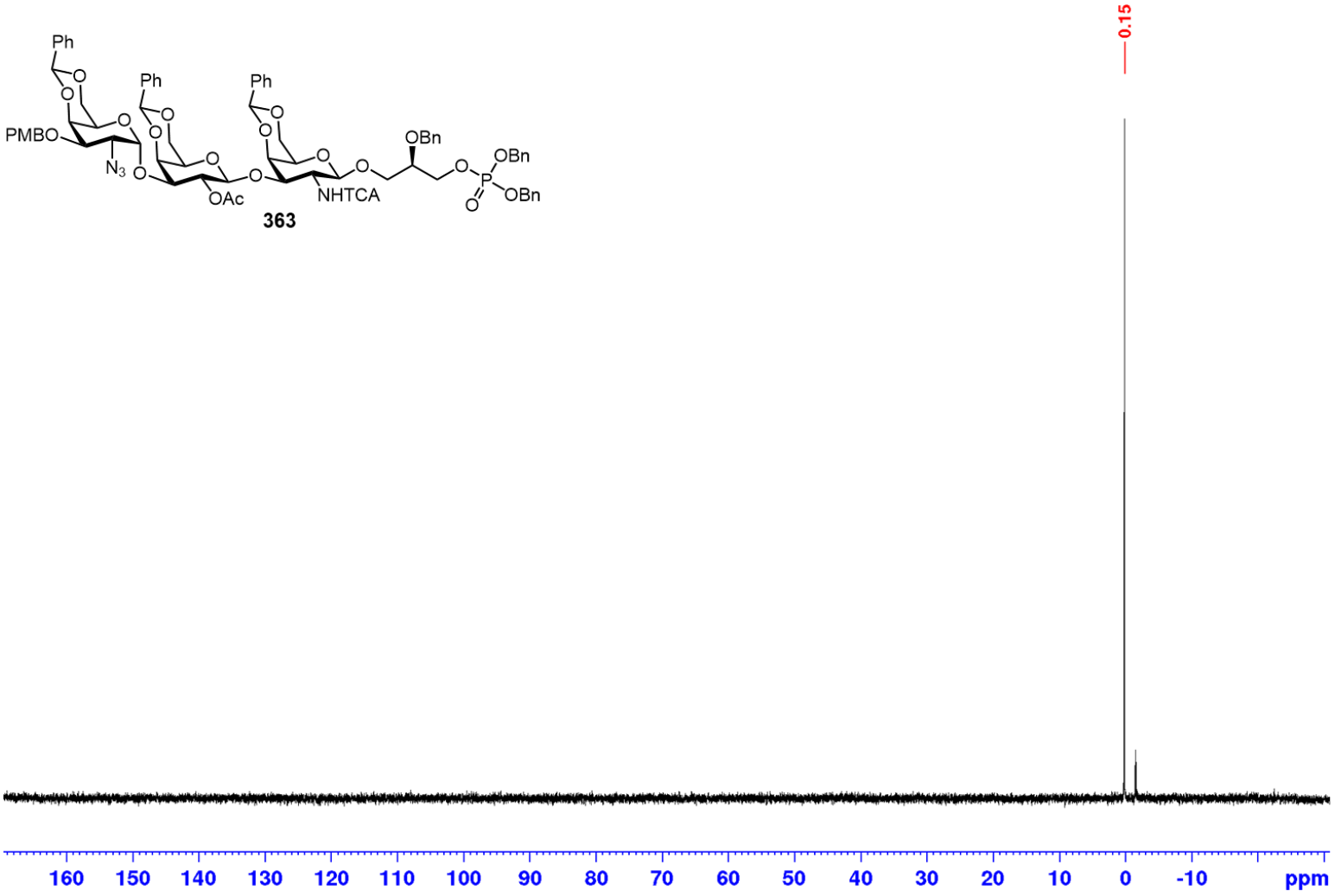
^{1}H-^{13}C HSCQ ($CDCl_3$) of compound 363



^{1}H-^{13}C HSCQ ($CDCl_3$) zoomed in region of compound 363



^{31}P NMR ($CDCl_3$, 162 MHz) of compound 363



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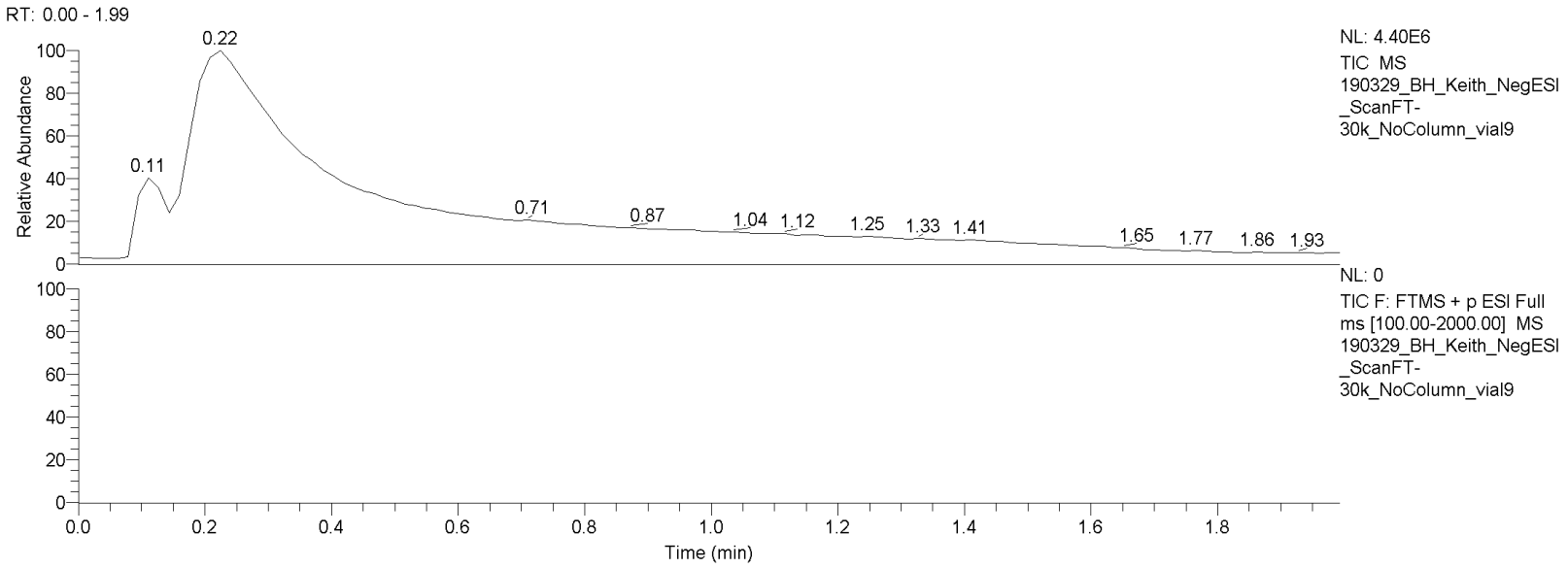
e732_C2-OAc_Neg



190329_BH_Keith_NegESI_ScanFT-30k_NoColumn_vial9 #29-45 RT: 0.45-0.71 AV: 17 NL: 1.76E5
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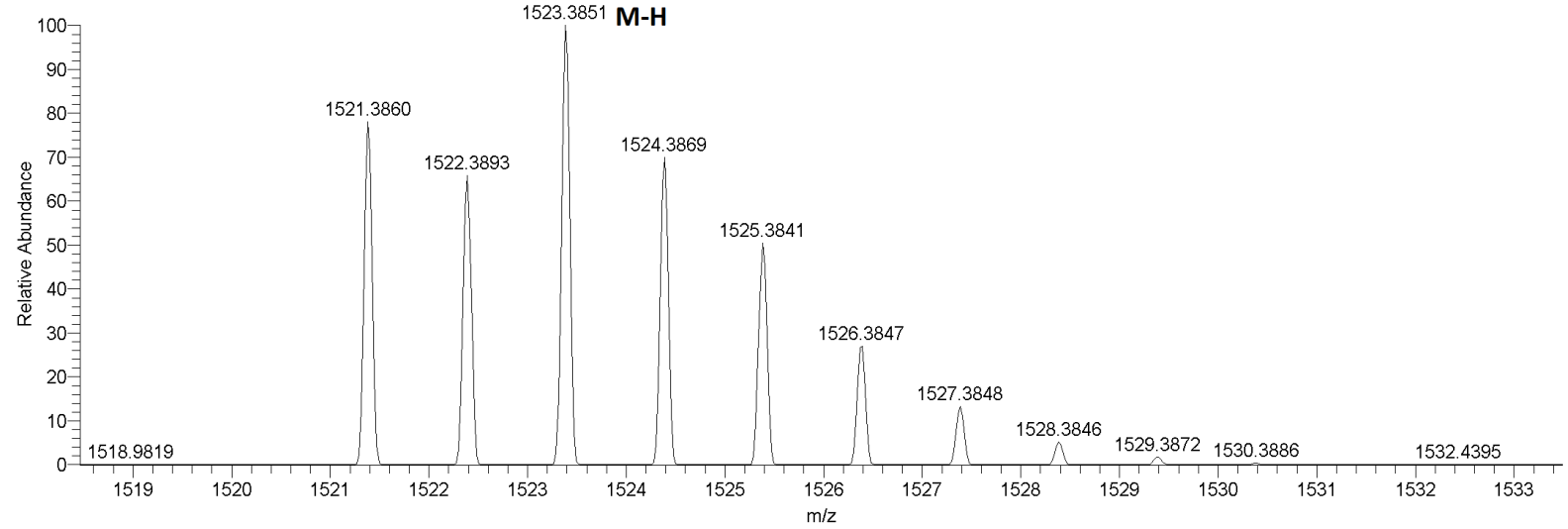
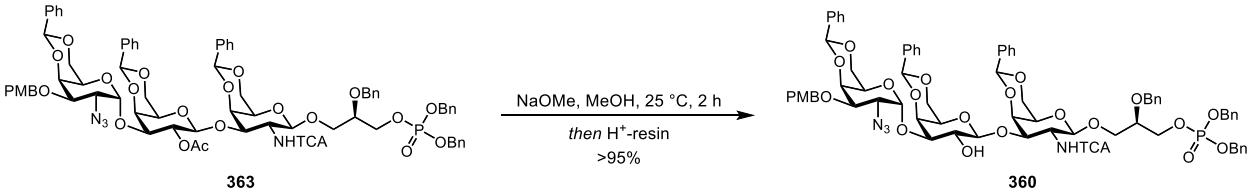


(*R*)-dibenzyl (2-(benzyloxy)-3-hydroxypropyl) phosphate 4,6-*O*-benzylidene acetal-2-deoxy-2-azido-3-*O*-*para*-methoxy benzyl-α-(1→3)-D-galactopyranosyl-4,6-*O*-benzylidene acetal-β-(1→3)-D-galactopyranosyl-4,6-*O*-benzylidene acetal-2-deoxy-2-trichloroacetamido-β-D-galactopyranoside (364)



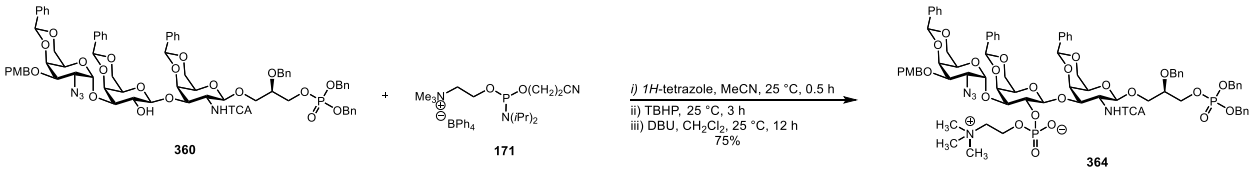
363 **360**

A solution of (*R*)-dibenzyl (2-(benzyloxy)-3-hydroxypropyl) phosphate 4,6-*O*-benzylidene acetal-2-deoxy-2-azido-3-*O*-*para*-methoxy benzyl-α-(1→3)-D-galactopyranosyl-4,6-*O*-benzylidene acetal-2-*O*-acetyl-β-(1→3)-D-galactopyranosyl-4,6-*O*-benzylidene acetal-2-deoxy-2-trichloroacetamido-β-D-galactopyranoside **363** (1.0 equiv., 1.516 g, 0.994 mmol) in anhydrous CH_3OH (20 mL) and added $NaOCH_3$ solution (1.0 equiv., 1.8 mL, 3.6 mmol, [2 N] in CH_3OH) dropwise at room temperature. At 2 h, the pH of the reaction mixture was still >12 and TLC indicated that the product of the first step had been completely consumed for a more polar spot. The reaction was neutralized by the addition of acidic form Amberlite IR 120 (H+) ion exchange resin. The solution was filtered through a glass fritted funnel with a pad of Celite to remove the resin. The filtrate was concentrated to dryness to give (*R*)-dibenzyl (2-(benzyloxy)-3-hydroxypropyl) phosphate 4,6-*O*-benzylidene acetal-2-deoxy-2-azido-3-*O*-*para*-methoxy benzyl-α-(1→3)-D-galactopyranosyl-4,6-*O*-benzylidene acetal-β-(1→3)-D-galactopyranosyl-4,6-*O*-benzylidene acetal-2-deoxy-2-trichloroacetamido-β-D-galactopyranoside **364** (1.3904 g, 0.9410 mmol, >95%) as a white foam in quantitative yield. R_f = 0.25 (80:20 EtOAc/hexanes) visualized with ceric ammonium molybdate stain; $[\alpha]D^{25}$ = +26.68° (c =

1.0, $CHCl_3$); IR ν_{max} (film) 3391.01, 2111.51, 1636.01, 1514.17, 1455.61, 1367.11, 1249.13, 1173.55, 1095.85, 1051.43, 820.57, 736.33, 698.32 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.56-7.50 (m, 9H), 7.37-7.26 (m, 34H), 6.83 (d, *J* = 8.7 Hz, 2H), 5.62 (s, 1H), 5.58 (s, 1H), 5.46 (s, 1H), 5.14 (d, *J* = 3.4 Hz, 1H), 5.02-4.9 (m, 7H), 4.67-4.55 (m, 6H), 4.43-4.40 (m, 3H), 4.37-4.17 (m, 9H), 4.13-3.89 (m, 17H), 3.84 (dd, *J* = 3.1, 10.4 Hz, 2H), 3.81-3.76 (m, 6H), 3.71-3.65 (m, 3H), 3.62-3.57 (m, 2H), 3.48 (s, 1H), 3.42 (d, *J* = 9.1 Hz, 3H), 3.29-3.26 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 162.6, 159.3, 137.8, 137.7, 137.7, 135.6, 135.5, 130.0, 129.4, 129.4, 129.4, 128.9, 128.9, 128.8, 128.7, 128.7, 128.6, 128.6, 128.6, 128.6, 128.6, 128.4, 128.4, 128.4, 128.2, 128.1, 128.1, 128.1, 128.1, 128.0, 128.0, 128.0, 128.0, 128.0, 127.9, 127.9, 127.9, 127.8, 127.8, 126.5, 126.2, 126.1, 113.8, 104.5, 100.9, 100.8, 100.8, 99.2, 95.8, 92.8, 75.9, 75.8, 75.7, 75.6, 73.7, 73.1, 72.1, 72.0, 71.0, 69.6, 69.5, 69.5, 69.2, 69.0, 68.8, 66.9, 66.8, 66.5, 65.8, 62.9, 58.1, 55.3, 54.4, 31.9, 29.7, 29.4; ^{31}P NMR (151 MHz, $CDCl_3$) δ 0.16; HR-ESI-MS (m/z): calcd for $C_{73}H_{75}Cl_3N_4O_{21}P^-$ $(M-H)^-$ 1479.3732, found 1479.3735.

For NMR spectra, *vide supra*.

(*R*)-dibenzyl (2-(benzyloxy)-3-hydroxypropyl) phosphate 4,6-*O*-benzylidene acetal-2-deoxy-2-azido-3-*O*-*para*-methoxy benzyl-α-(1→3)-D-galactopyranosyl-4,6-*O*-benzylidene acetal-2-*O*-phosphocholine-β-(1→3)-D-galactopyranosyl-4,6-*O*-benzylidene acetal-2-deoxy-2-trichloroacetamido-β-D-galactopyranoside (364)

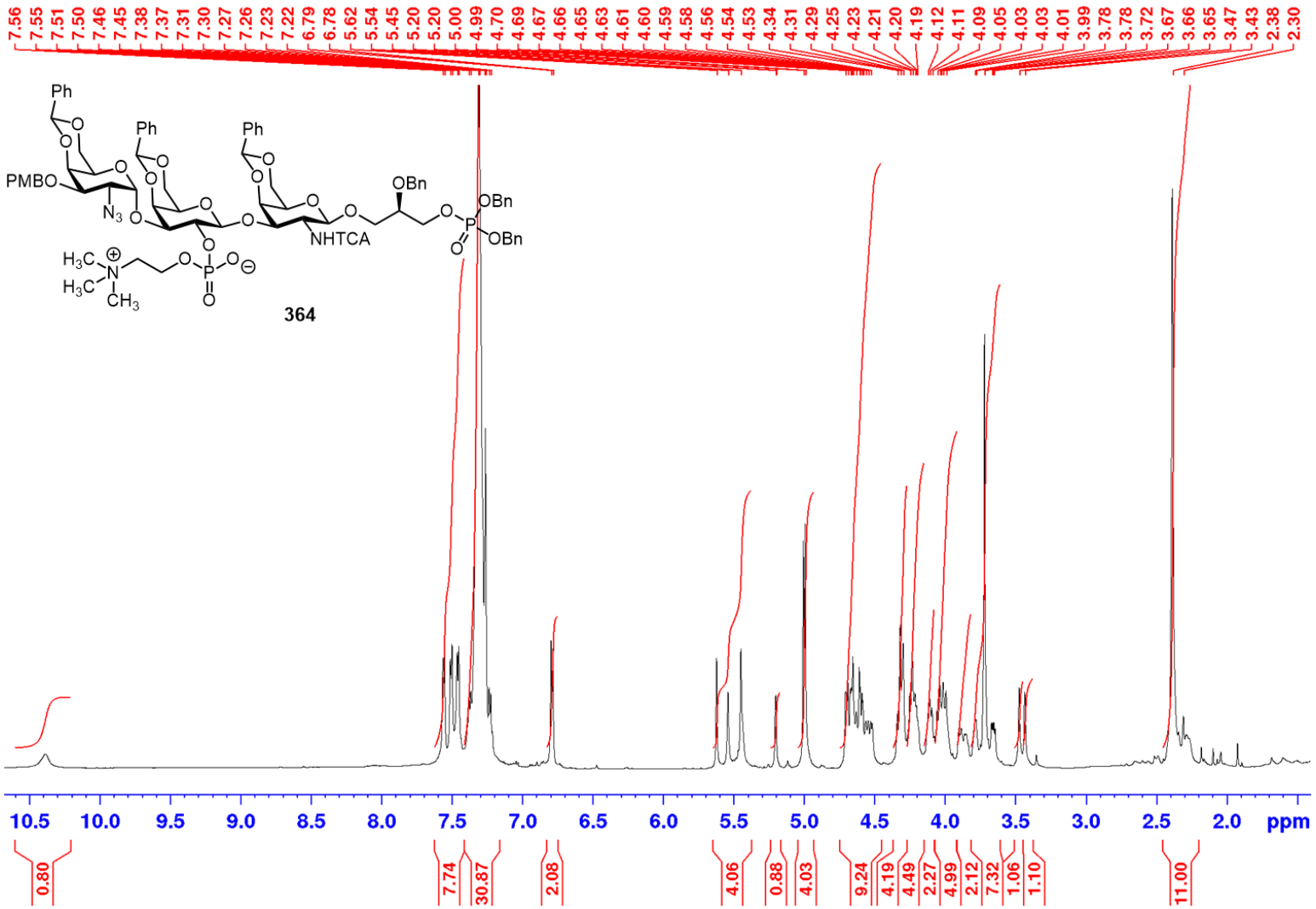


In a round bottom flask, under an argon atmosphere, trisaccharide (*R*)-dibenzyl (2-(benzyloxy)-3-hydroxypropyl) phosphate 4,6-*O*-benzylidene acetal-2-deoxy-2-azido-3-*O*-*para*-methoxy benzyl-α-(1→3)-D-galactopyranosyl-4,6-*O*-benzylidene acetal-β-(1→3)-D-galactopyranosyl-4,6-*O*-benzylidene acetal-2-deoxy-2-trichloroacetamido-β-D-galactopyranoside **360** (1.0 equiv., 1.225 g, 0.826 mmol) and phosphoramidite 2-(((2-cyanoethoxy)(diisopropylamino)phosphaneyl)oxy)-*N*,*N*,*N*-trimethylethan-1-aminium tetraphenylborate salt **171** (1.2 equiv., 0.88 g, 0.991 mmol) was added a 3% tetrazole solution in anhydrous CH_3CN (1.2 equiv., 5.0 mL, 0.991 mmol) at room temperature under an argon atmosphere. At 0.5 h, the crude reaction was filtered through a pad of Celite to remove salts. The filtrate was cooled to 0 °C then added tert-butyl hydroperoxide (2.0 equiv., 0.088 mL, 1.65 mmol). At 1 h, the crude reaction mixture was concentrated to dryness by co-evaporated with toluene (3x). The resulting solid was dissolved with anhydrous CH_2Cl_2 (1.0 mL) and added DBU (2.0 equiv., 0.25 mL, 1.65 mmol) at room temperature under argon. At 12 h, the reaction was diluted with CH_2Cl_2 (25 mL) and transferred to a separatory funnel and washed with 1 N HCl (3 x 10 mL), satd. aq. $NaHCO_3$ (1 x 10 mL), brine (1 x 10 mL), dried ($NaSO_4$) and filtered to remove drying agent. The

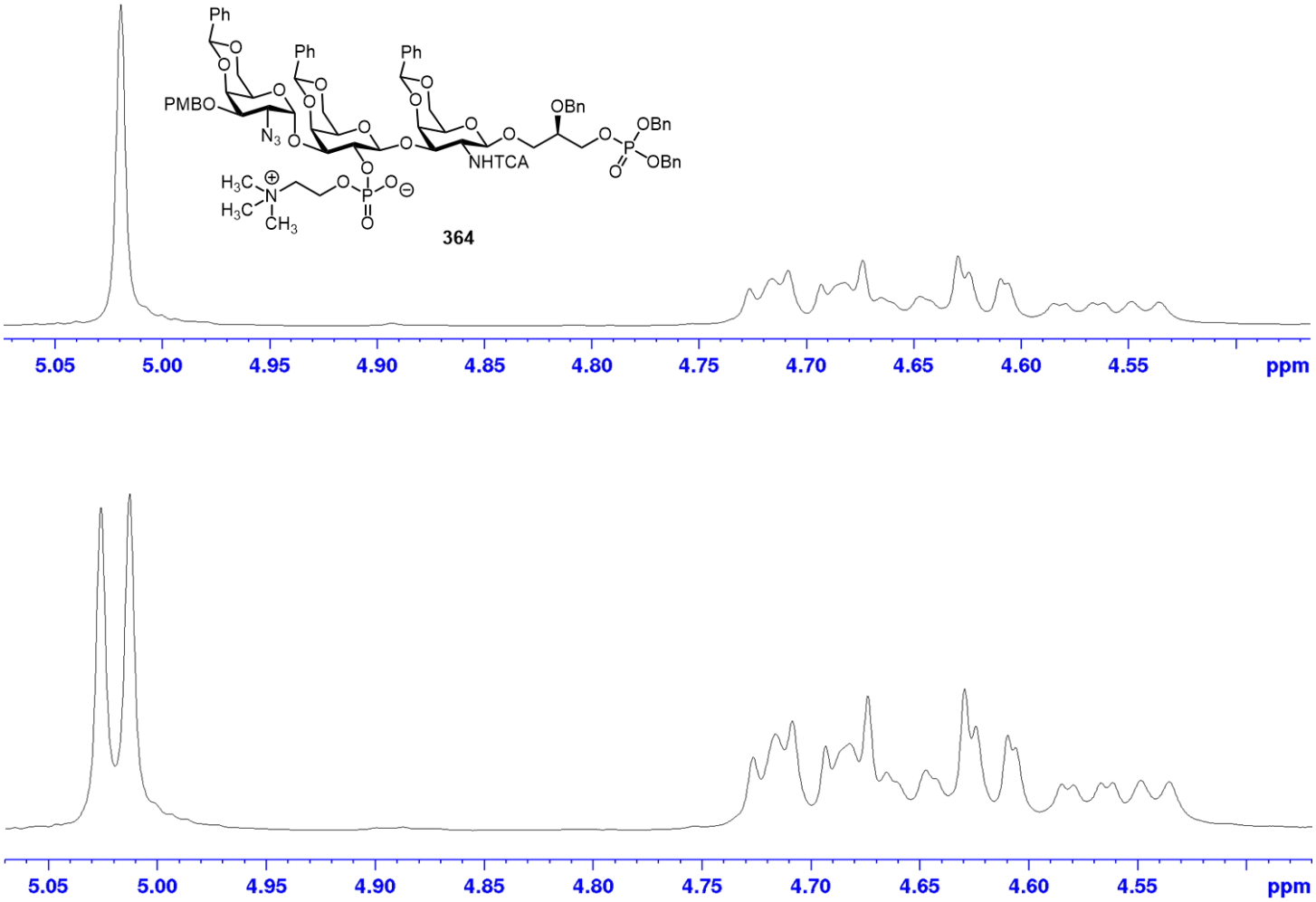
filtrate was concentrated to a crude solid. The crude material was then dissolved in hot CH_2Cl_2 and allowed to cool to room temperature. At 12 h, the residual amine-phosphate salts were crystalized into thin needles and were removed from the crude reaction mixture. The mother liquor, containing the desired product) was concentrated down and purified by silica gel flash column chromatography (10:90 to 50:50 CH_3OH/CH_2Cl_2) to give fully protected trisaccharide (*R*)-dibenzyl (2-(benzyloxy)-3-hydroxypropyl) phosphate 4,6-*O*-benzylidene acetal-2-deoxy-2-azido-3-*O*-*para*-methoxy benzyl-α-(1→3)-D-galactopyranosyl-4,6-*O*-benzylidene acetal-2-*O*-phosphocholine-β-(1→3)-D-galactopyranosyl-4,6-*O*-benzylidene acetal-2-deoxy-2-trichloroacetamido-β-D-galactopyranoside **364** (1.026 g, 0.662 mmol, 75%) as a white amorphous solid. R_f = 0.30 (25:75 CH_3OH/CH_2Cl_2) visualized with ceric ammonium molybdate stain; $[\alpha]D^{25}$ = +4.88° (c = 1.0, $CHCl_3$); IR ν_{max} (film) 3943.44, 3409.69, 3054.01, 2986.53, 2685.07, 2410.41, 2305.26, 2112.42, 1638.98, 1421.56, 1265.14, 1155.29, 1051.73, 895.85, 739.23, 705.28 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.55 (d, *J* = 7.0 Hz, 2H), 7.50 (d, *J* = 6.8 Hz, 2H), 7.45 (d, *J* = 7.3 Hz, 2H), 7.38-7.22 (m, 24H), 6.78 (d, *J* = 8.4 Hz, 2H), 5.61 (s, 1H), 5.53 (s, 1H), 5.44 (s, 2H), 5.19 (d, *J* = 3.2 Hz, 1H), 4.99 (s, 4H), 4.70-4.68 (m, 2H), 4.67-4.57 (m, 6H), 4.56-5.40 (m, 3H), 4.34-4.27 (m, 5H), 4.25-4.18 (m, 6H), 4.12-4.08 (m, 3H), 4.05-3.97 (m, 6H), 3.90-3.82 (m, 3H), 3.79-3.76 (m, 2H), 3.73-3.70 (m, 4H), 3.66-3.63 (m, 2H), 3.47 (s, 1H), 3.43 (s, 1H), 3.38 (s, 11H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 162.9, 158.9, 138.7, 138.2, 138.1, 138.0, 135.8, 130.7, 129.7, 129.5, 129.4, 128.6, 128.6, 128.5, 128.5, 128.4, 128.4, 128.3, 128.2, 128.0, 128.0, 127.9, 127.9, 127.9, 127.8, 127.7, 127.7, 127.5, 127.3, 126.1, 125.9, 113.6, 103.6, 101.3, 100.3, 99.7, 98.8, 94.6, 92.5, 76.3, 75.9, 74.9, 73.1, 72.8, 72.5, 72.3, 71.6, 69.8, 69.7, 69.2, 69.1, 69.0, 68.8, 67.1, 66.4, 66.4, 66.3, 63.0,

59.7, 58.0, 56.0, 55.2, 54.2; ^{31}P NMR (151 MHz, $CDCl_3$) δ -1.04, -3.98; HR-ESI-MS (m/z): calcd for $C_{78}H_{88}Cl_3N_5O_{24}P_2Na^+$ $(M+Na)^+$ 1668.425218, found 1668.4192.

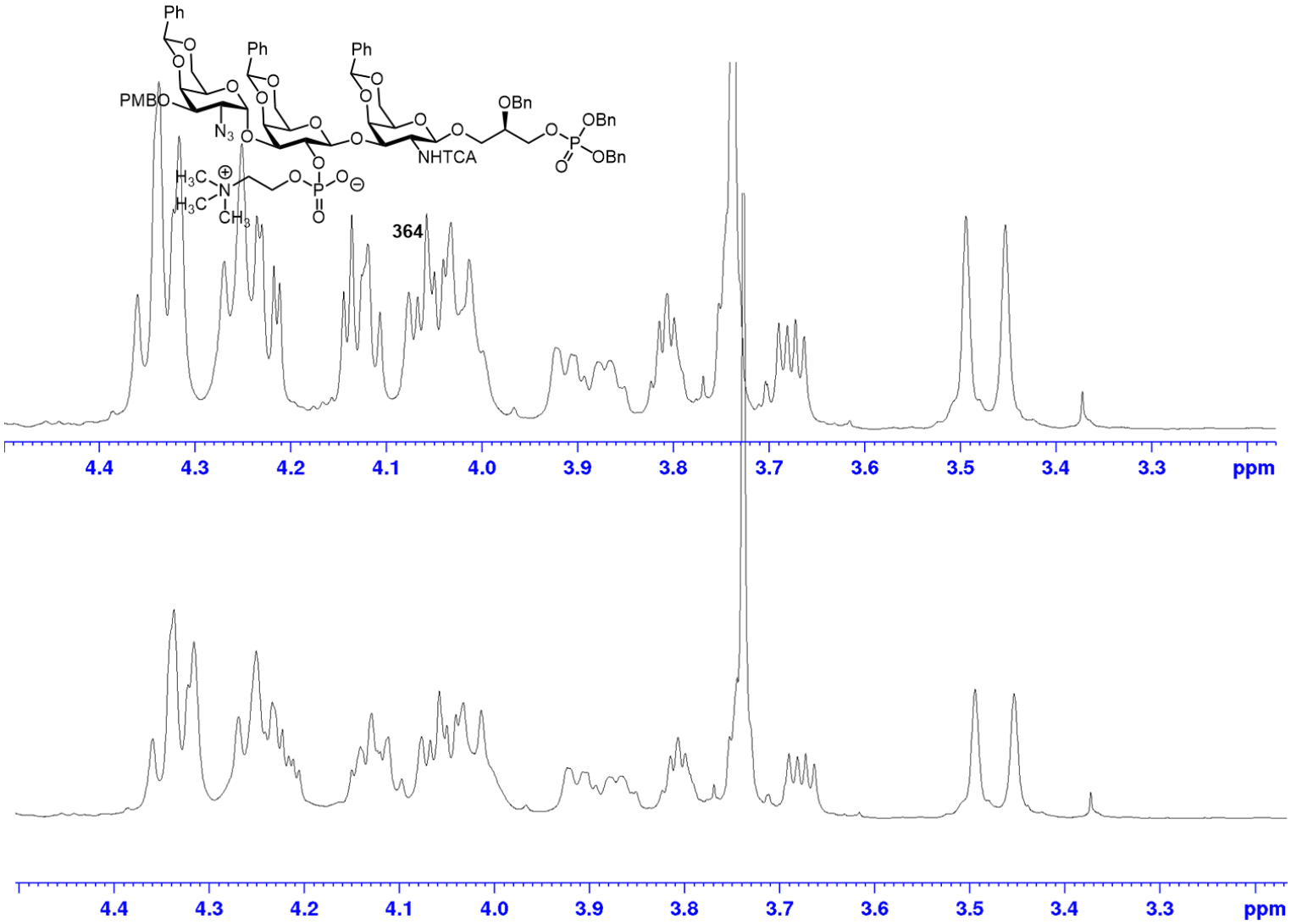
$^{1}H\{^{31}P\}$ NMR ($CDCl_3$, 400 MHz) of compound 364



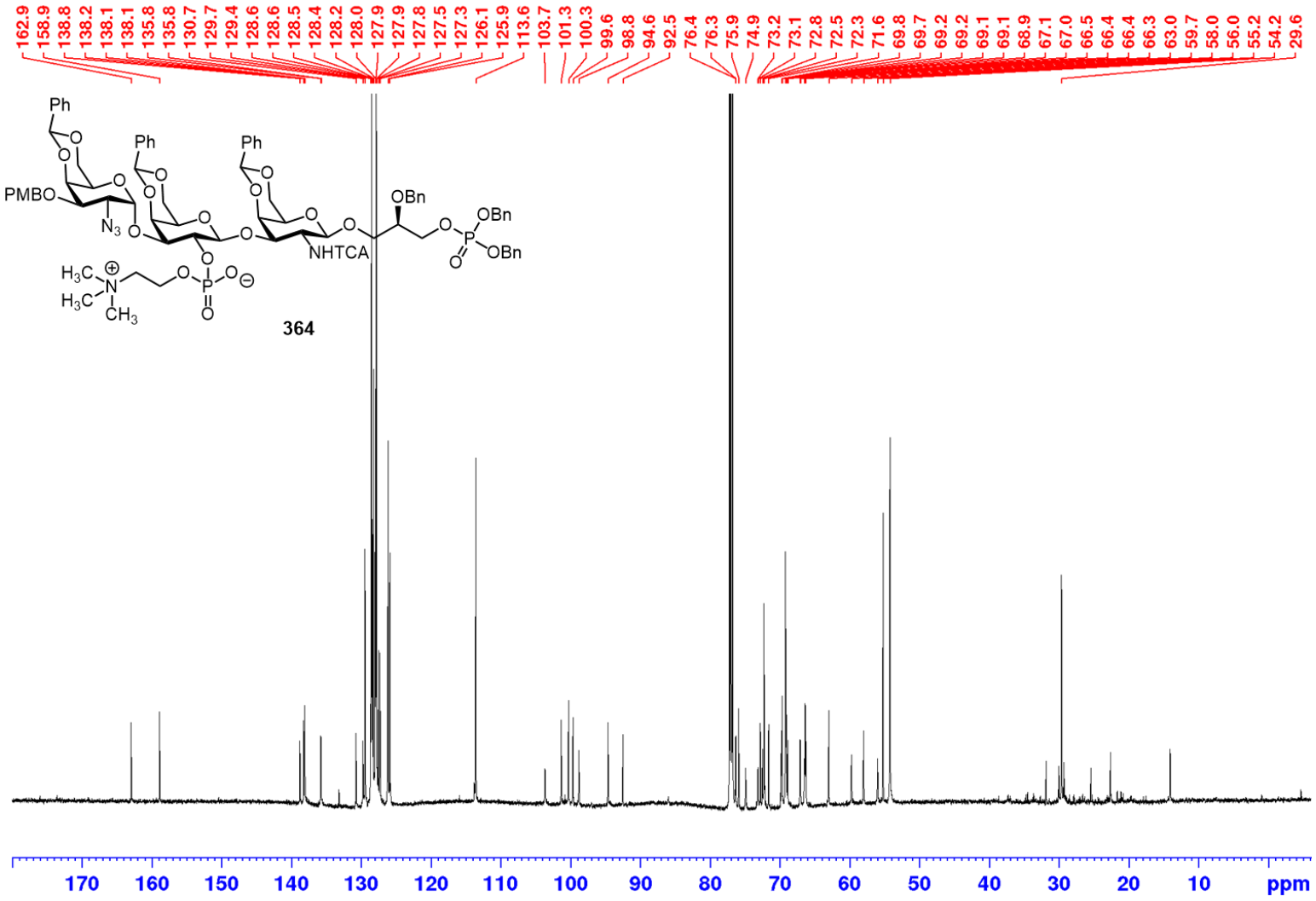
$^{1}H\{^{31}P\}$ NMR (top) vs. ^{1}H NMR (bottom) ($CDCl_3$, 400 MHz) zoomed in region of compound 364



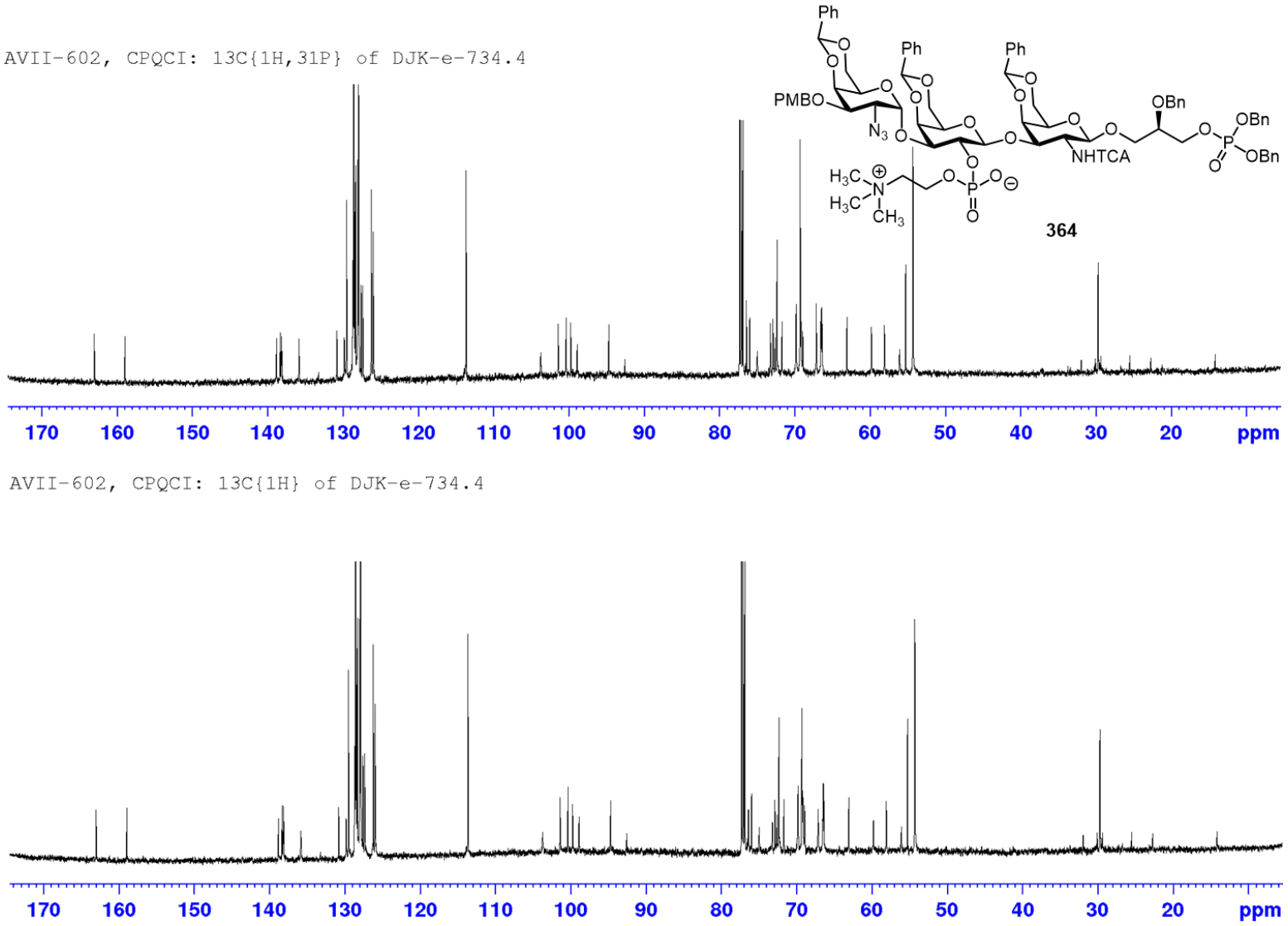
$^{1}H\{^{31}P\}$ NMR (top) vs. ^{1}H NMR (bottom) ($CDCl_3$, 400 MHz) zoomed in region of compound 364



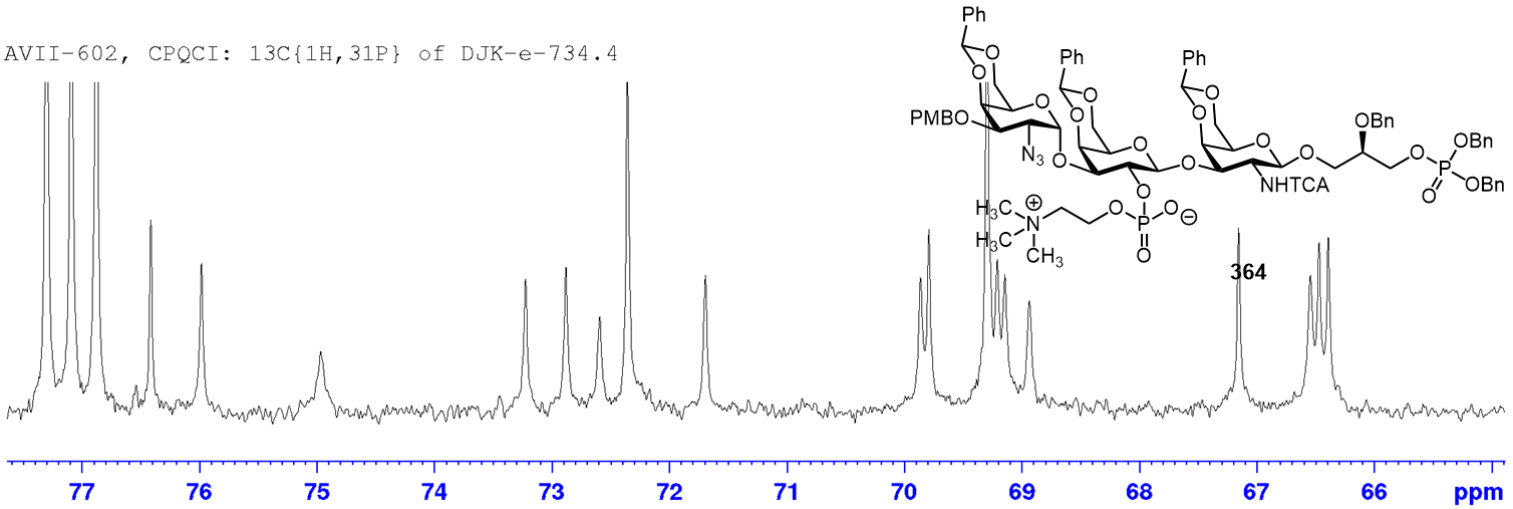
$^{13}C\{^{1}H, ^{31}P\}$ NMR ($CDCl_3$, 100 MHz) of compound 364

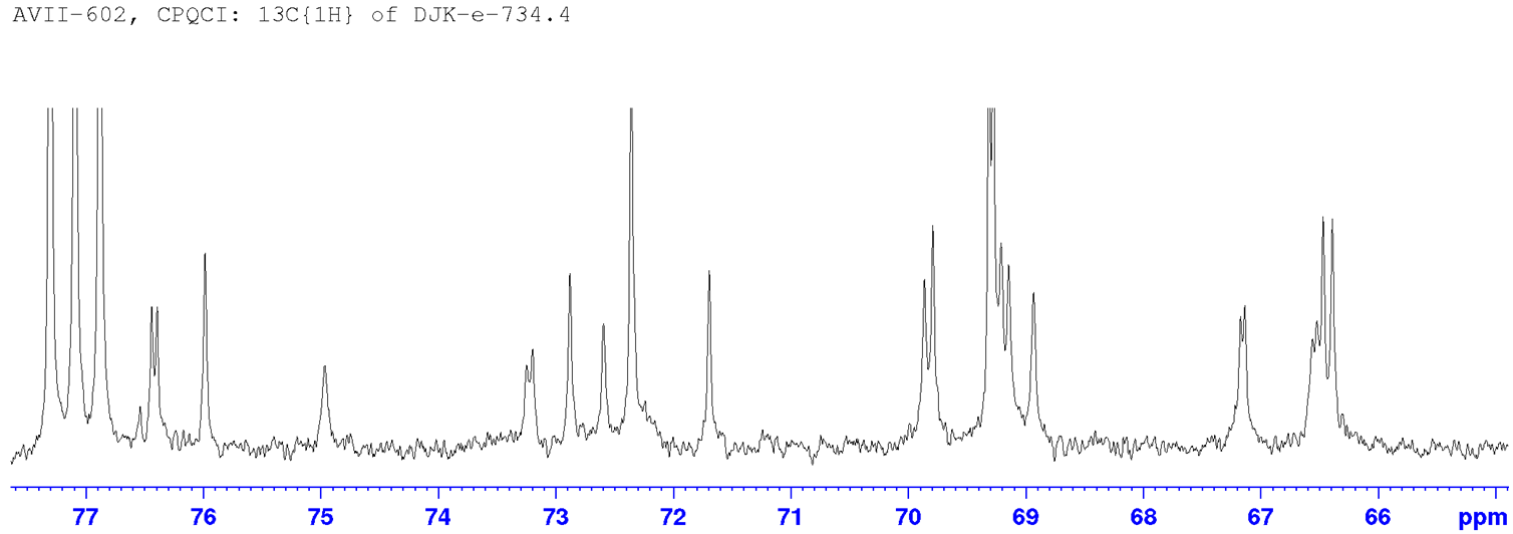


$^{13}C\{^{1}H, ^{31}P\}$ (top) vs. $^{13}C\{^{1}H\}$ (bottom) NMR ($CDCl_3$, 100 MHz) of compound 364

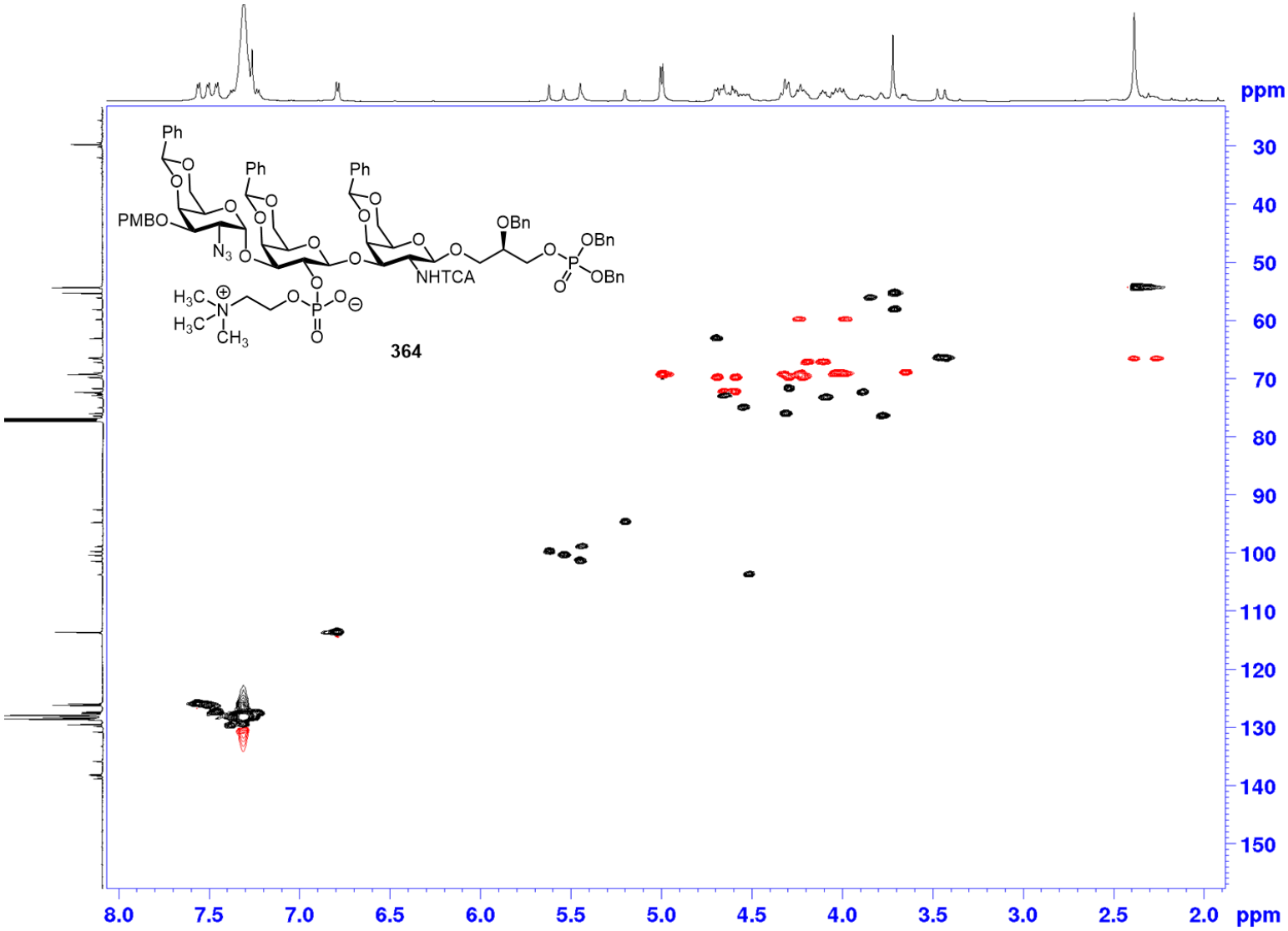


$^{13}C\{^{1}H, ^{31}P\}$ (top) vs. $^{13}C\{^{1}H\}$ (bottom) NMR ($CDCl_3$, 100 MHz) zoomed in region of compound 364

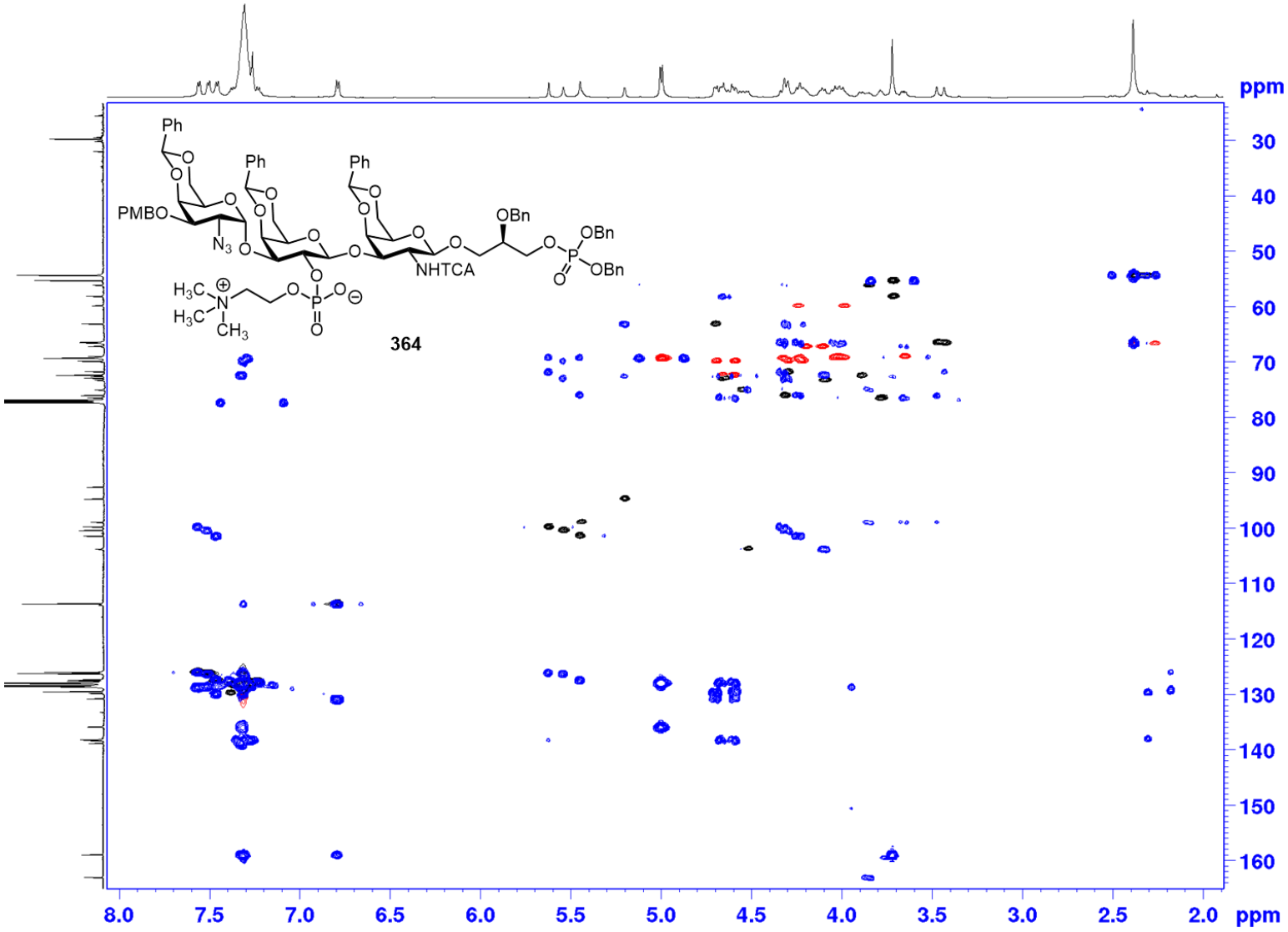




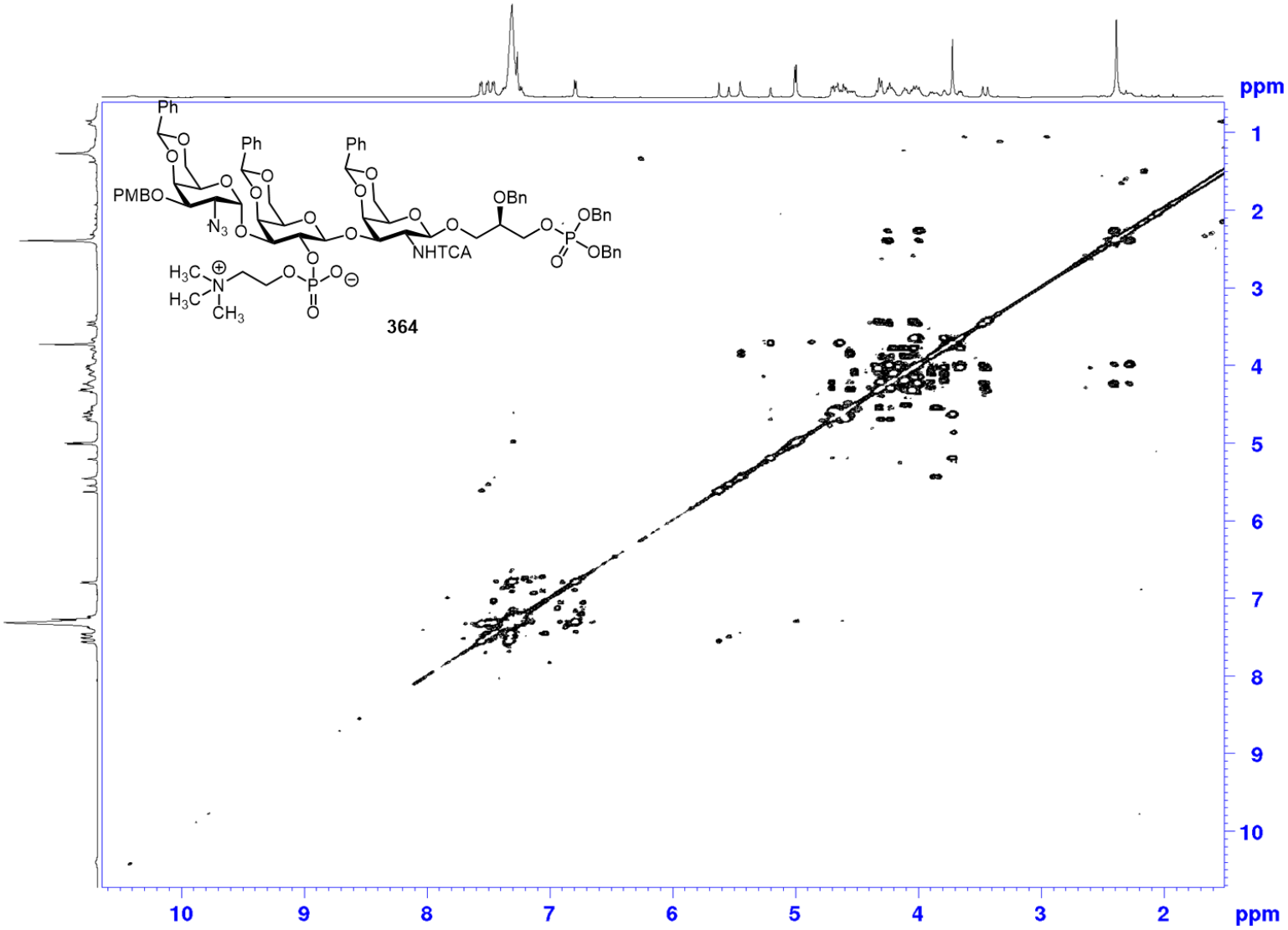
^{1}H-^{13}C HSCQ ($CDCl_3$) zoomed in region of compound 364



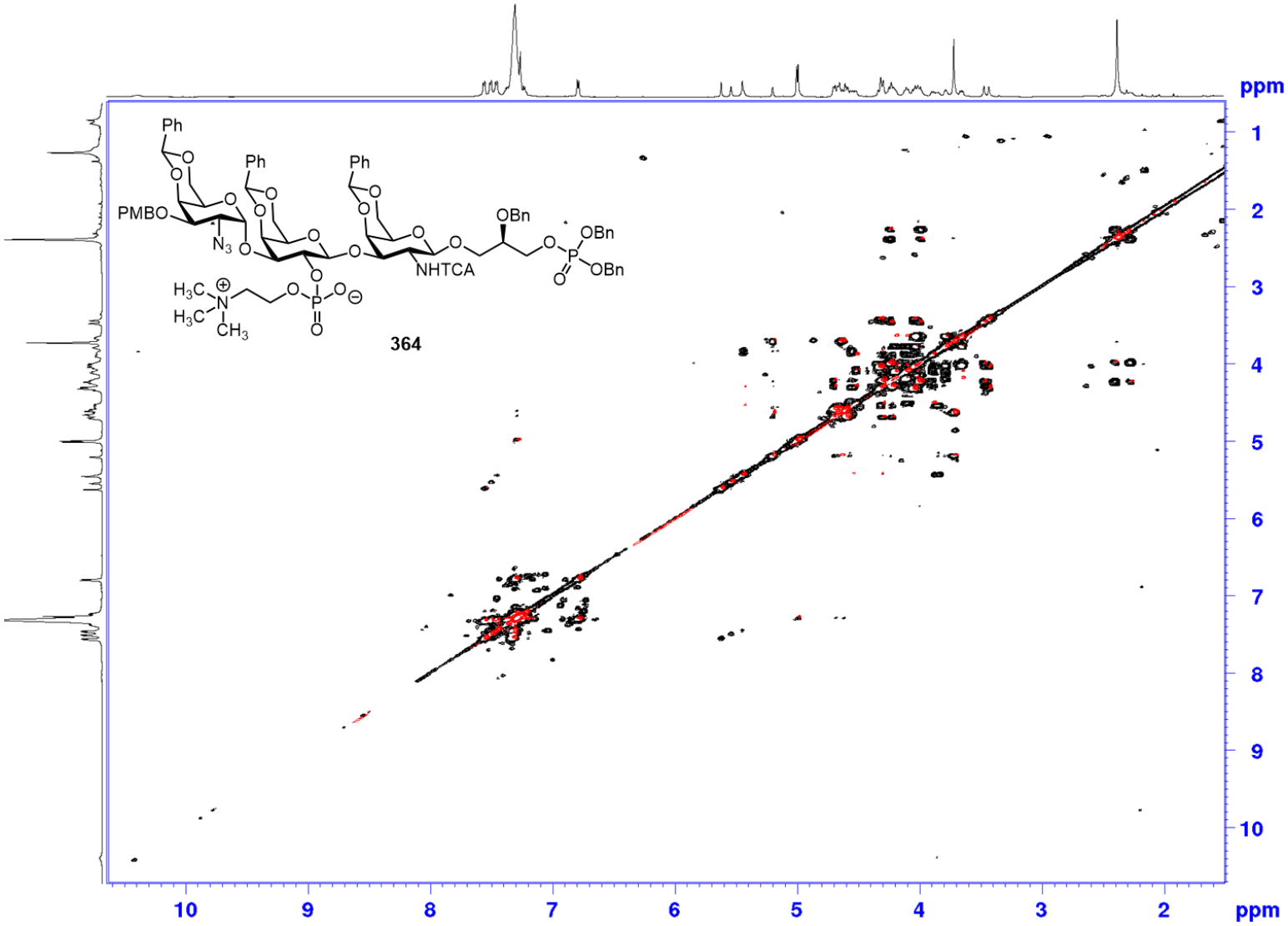
^{1}H-^{13}C HSCQ with ^{1}H-^{13}C HMBC overlay ($CDCl_3$) of compound 364



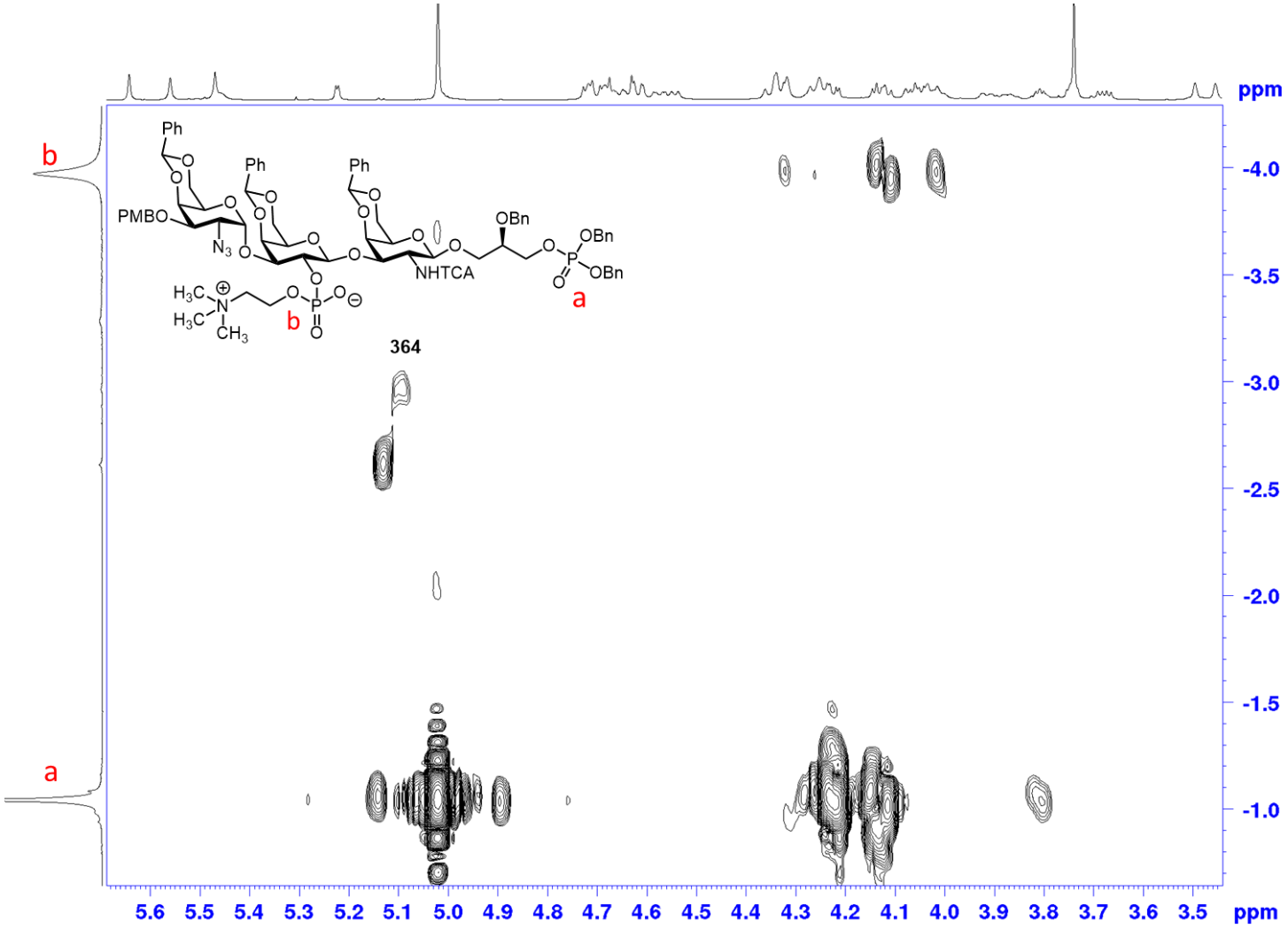
^{1}H-^{1}H COSY ($CDCl_3$) of compound 364



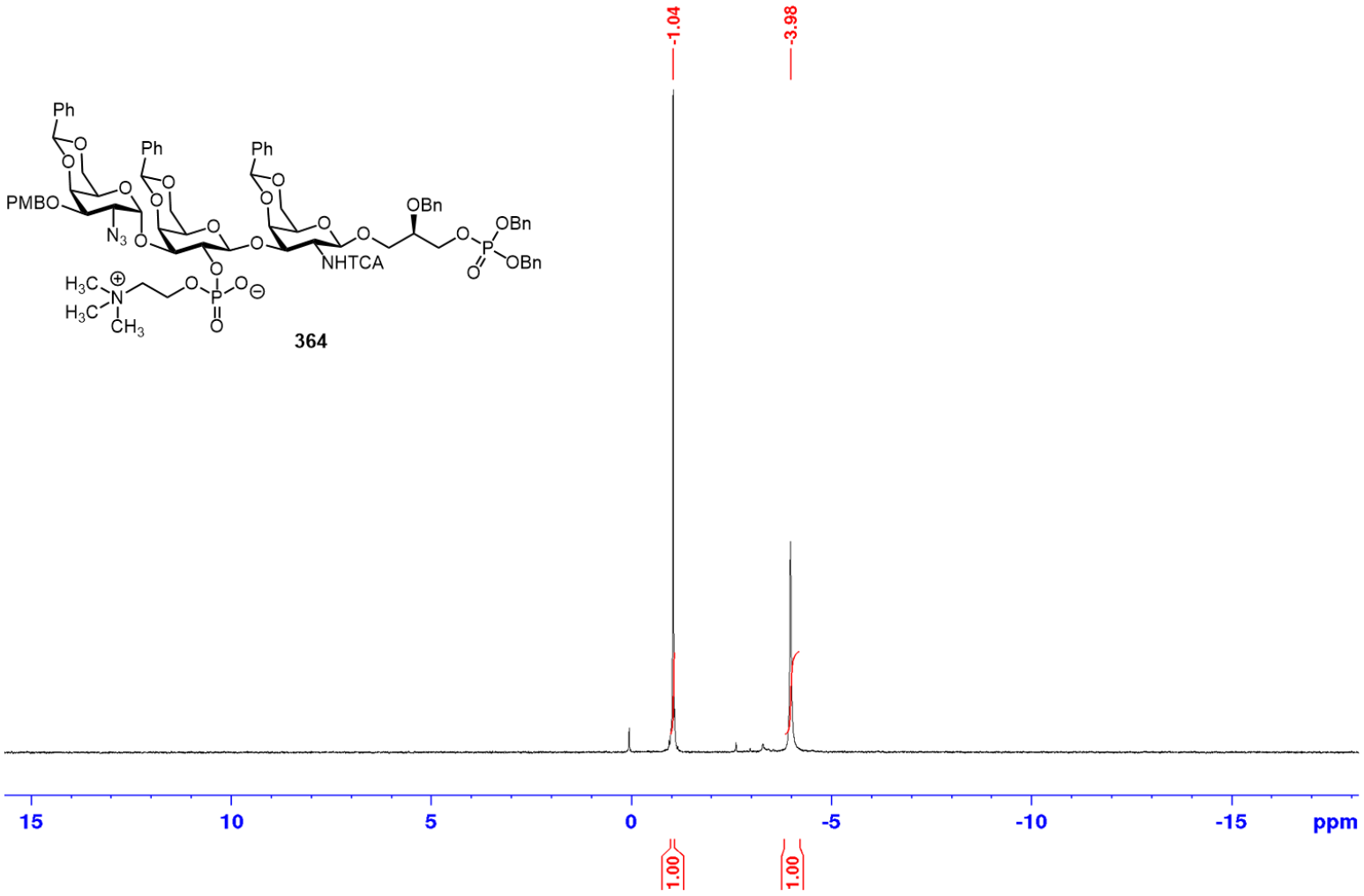
^{1}H-^{1}H COSY with ^{1}H-^{1}H TOSY overlay ($CDCl_3$) of compound 364

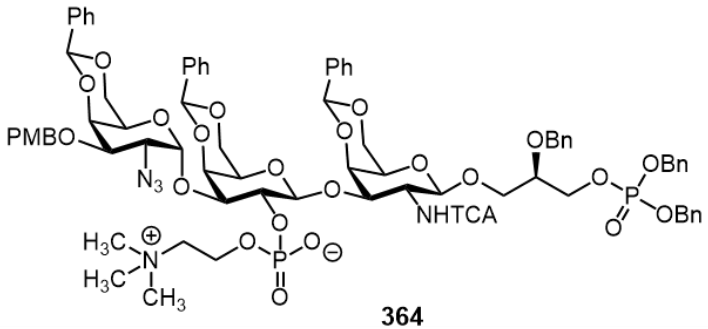


^{1}H-^{31}P HSCQ ($CDCl_3$) zoomed in region of compound 364

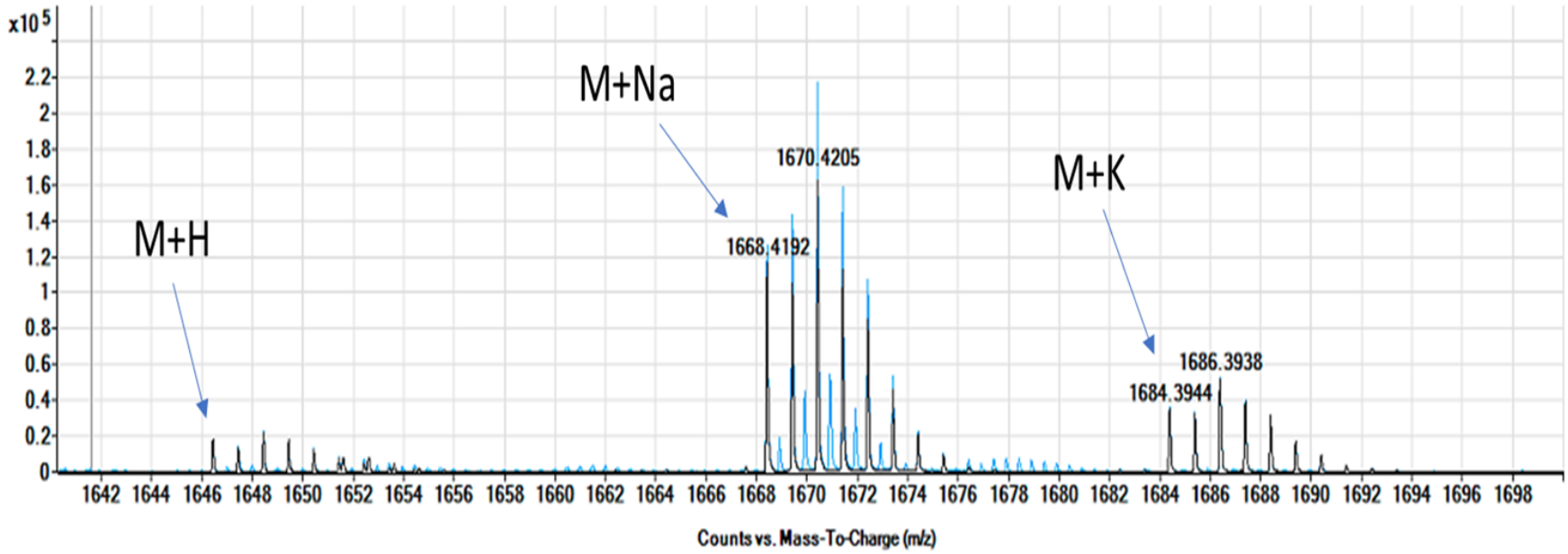


^{31}P NMR ($CDCl_3$, 162 MHz) of compound 364

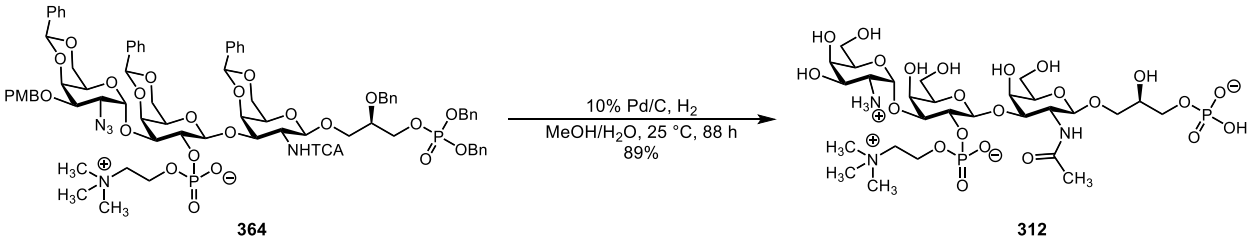



Ph
Ph
Ph
PMBO
N3
NHTCA
OBn
OBn
OBn
H3C
H3C
CH3

364


x10 5
2.2
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
M+H
M+Na
M+K
1670.4205
1668.4192
1686.3938
1684.3944
1642 1644 1646 1648 1650 1652 1654 1656 1658 1660 1662 1664 1666 1668 1670 1672 1674 1676 1678 1680 1682 1684 1686 1688 1690 1692 1694 1696 1698
Counts vs. Mass-To-Charge (m/z)

(*R*)-2-hydroxypropyl phosphate 2-deoxy-2-amino-α-(1→3)-D-galactopyranosyl-2-O-phosphocholine-β-(1→3)-D-galactopyranosyl-2-deoxy-2-acetamido-β-D-galactopyranoside (312)

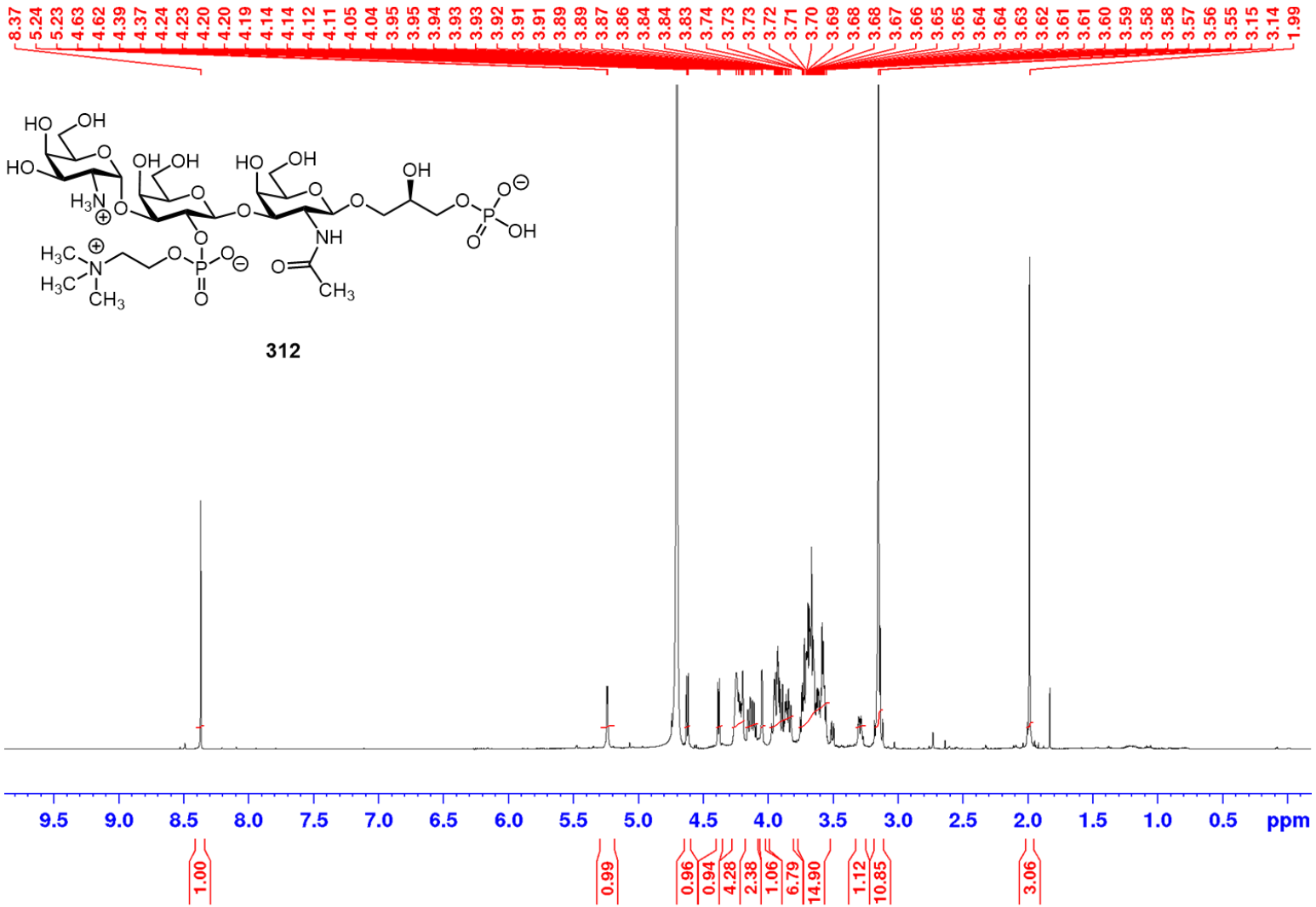


364 312

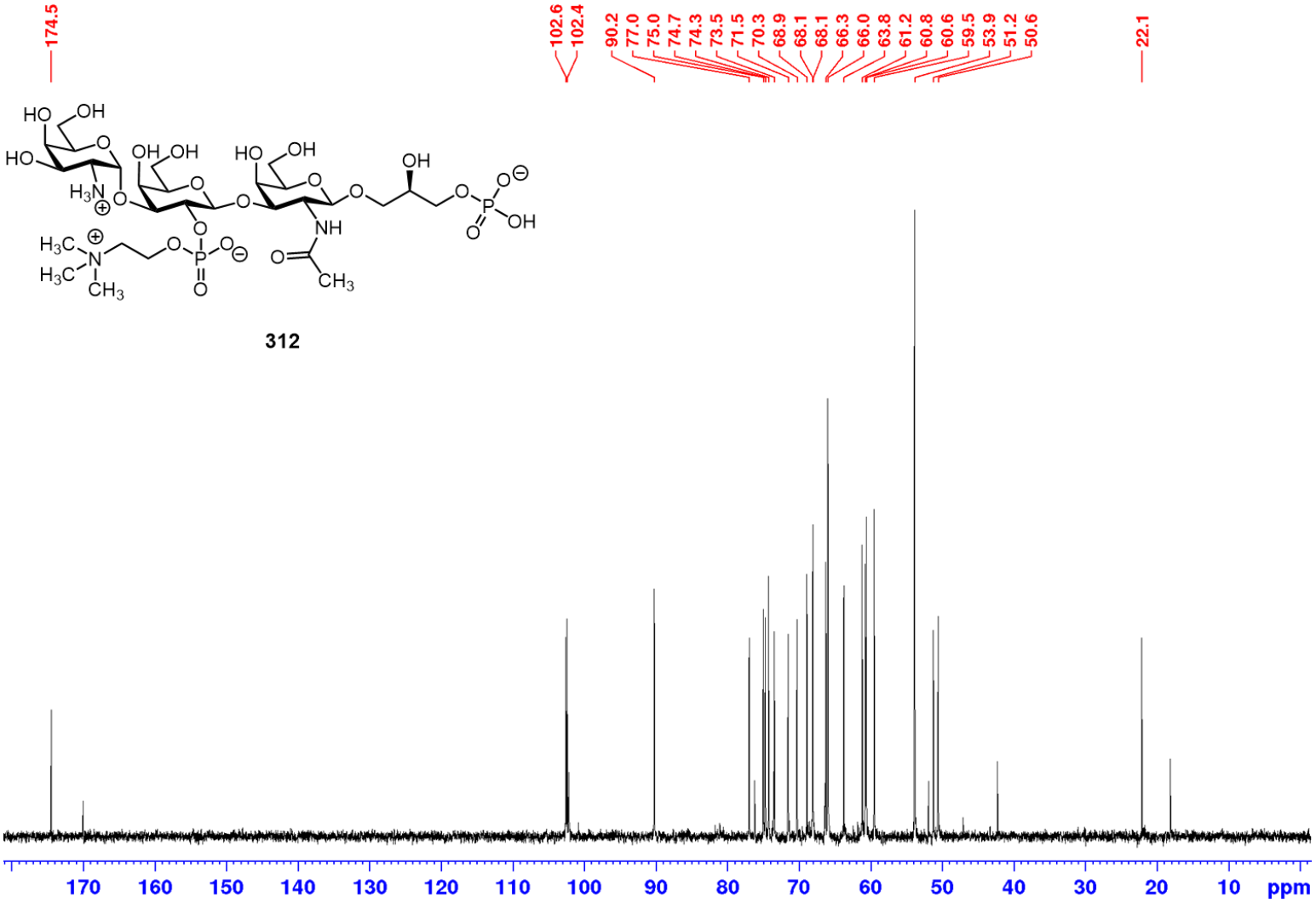
A solution of (*R*)-dibenzyl (2-(benzyloxy)-3-hydroxypropyl) phosphate 4,6-*O*-benzylidene acetal-2-deoxy-2-azido-3-*O*-*para*-methoxy benzyl-α-(1→3)-D-galactopyranosyl-4,6-*O*-benzylidene acetal-2-*O*-phosphocholine-β-(1→3)-D-galactopyranosyl-4,6-*O*-benzylidene acetal-2-deoxy-2-trichloroacetamido-β-D-galactopyranoside **364** (1.0 equiv., 0.33 g, 0.2 mmol) in anhydrous CH_3OH (5.0 mL) was purged with argon, then added 10% Pd/C (0.5 g). The reaction mixture was purged with H_2 (g) for 15 min, then stirred under a H_2 atmosphere. At 88 h, the reaction was purged with an argon atmosphere, filtered through a glass fritted vacuum funnel equipped with a pad of Celite. The filtrate was concentrated to a white solid. The material was purified by size exclusion chromatography (Bio-Gel P-2 gel) using deionized water as an eluant. Fractions containing the desired product (determined from MS) were combined and lyophilized to give (*R*)-2-hydroxypropyl phosphate 2-deoxy-2-amino-α-(1→3)-D-galactopyranosyl-2-O-phosphocholine-β-(1→3)-D-galactopyranosyl-2-deoxy-2-acetamido-β-D-galactopyranoside **312** (0.156 g, 0.172 mmol, 89%) as a white powder. Our spectroscopic data strongly matched previously reported data.[8] $[\alpha]D^{25}$ = +14.48° (c = 1.0, $CHCl_3$); IR ν_{max} (film) 3410.43, 2927.86, 2112.85, 1648.25, 1496.69, 1454.35, 1359.06, 1251.93, 1101.95, 1034.53, 837.55,

779.22, 735.50, 697.33 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.45 (s, -OH, , J = Hz, 1H), 5.31 (d, J = 3.6 Hz, 1H), 4.72 (d, J = 7.9 Hz, 1H), 4.48 (d, J = 7.6 Hz, 1H), 4.34-4.28 (m, 4H), 4.23 (dd, J = 2.9, 11.2 Hz, 1H), 4.20 (dd, J = 8.7, 9.8 Hz, 1H), 4.14 (d, J = 2 Hz, 1H), 4.04-3.93 (m, 5H), 3.92 (dd, J = 3.1, 9.8 Hz, 1H), 3.84-3.74 (m, 15H), 3.60 (dd, J = 3.1, 10.6 Hz, 1H), 3.38 (ddd, J = 1.8, 5.90, 12.8 Hz, 1H), 3.25 (s, 9H), 2.09 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 174.45, 102.6, 102.4, 90.2, 77.0, 75.0, 74.7, 74.3, 73.5, 71.6, 70.3, 68.9, 68.1, 68.1, 66.3, 66.0, 63.8, 61.2, 60.8, 60.6, 59.5, 53.9, 51.2, 50.6, 22.1; HR-ESI-MS (m/z): calcd for $C_{28}H_{56}N_3O_{23}P_2^+$ $(M+H)^+$ 864.277476, found 864.27724.

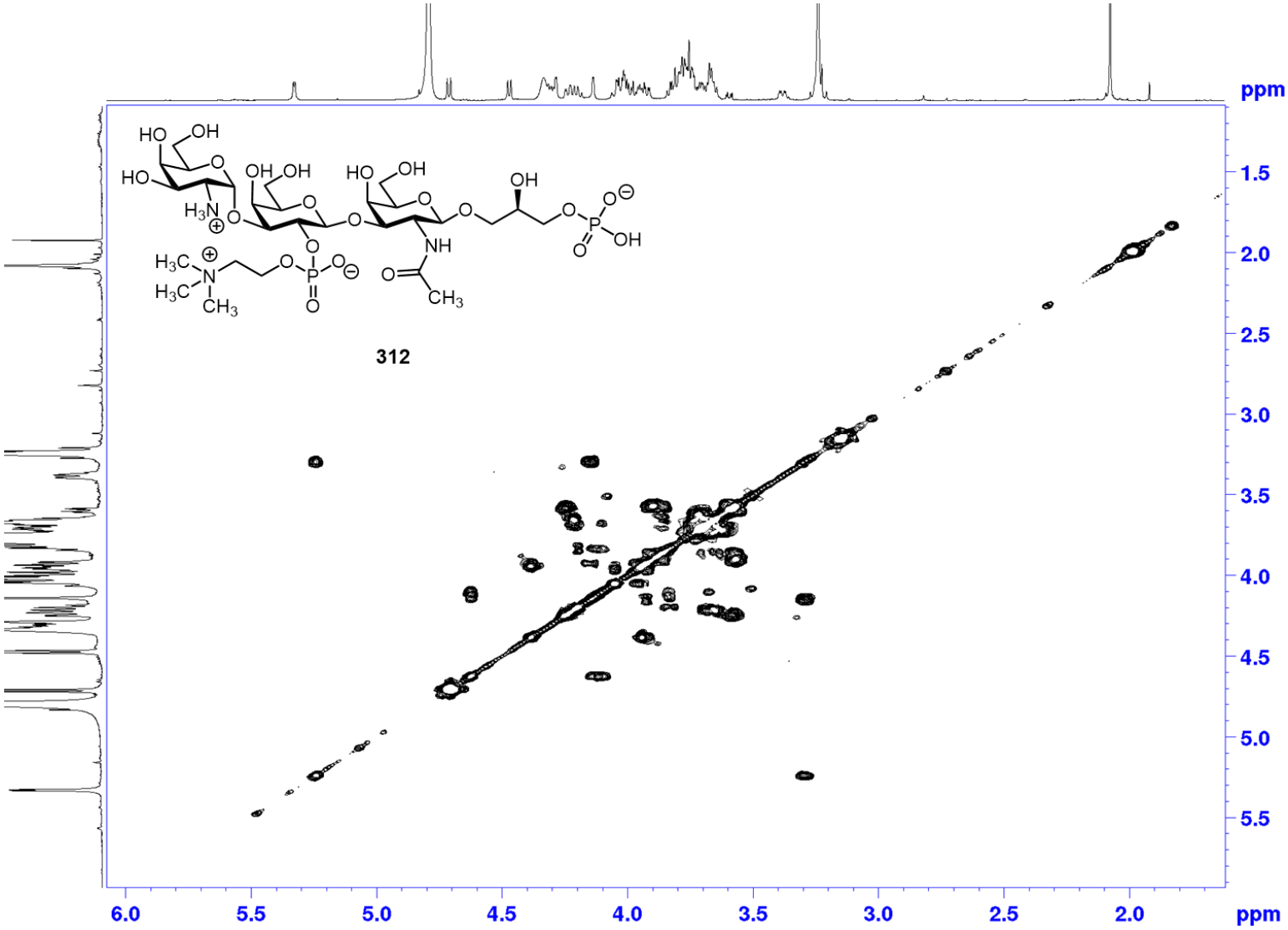
$^{1}H\{^{31}P\}$ NMR ($CDCl_3$, 400 MHz) of compound 312



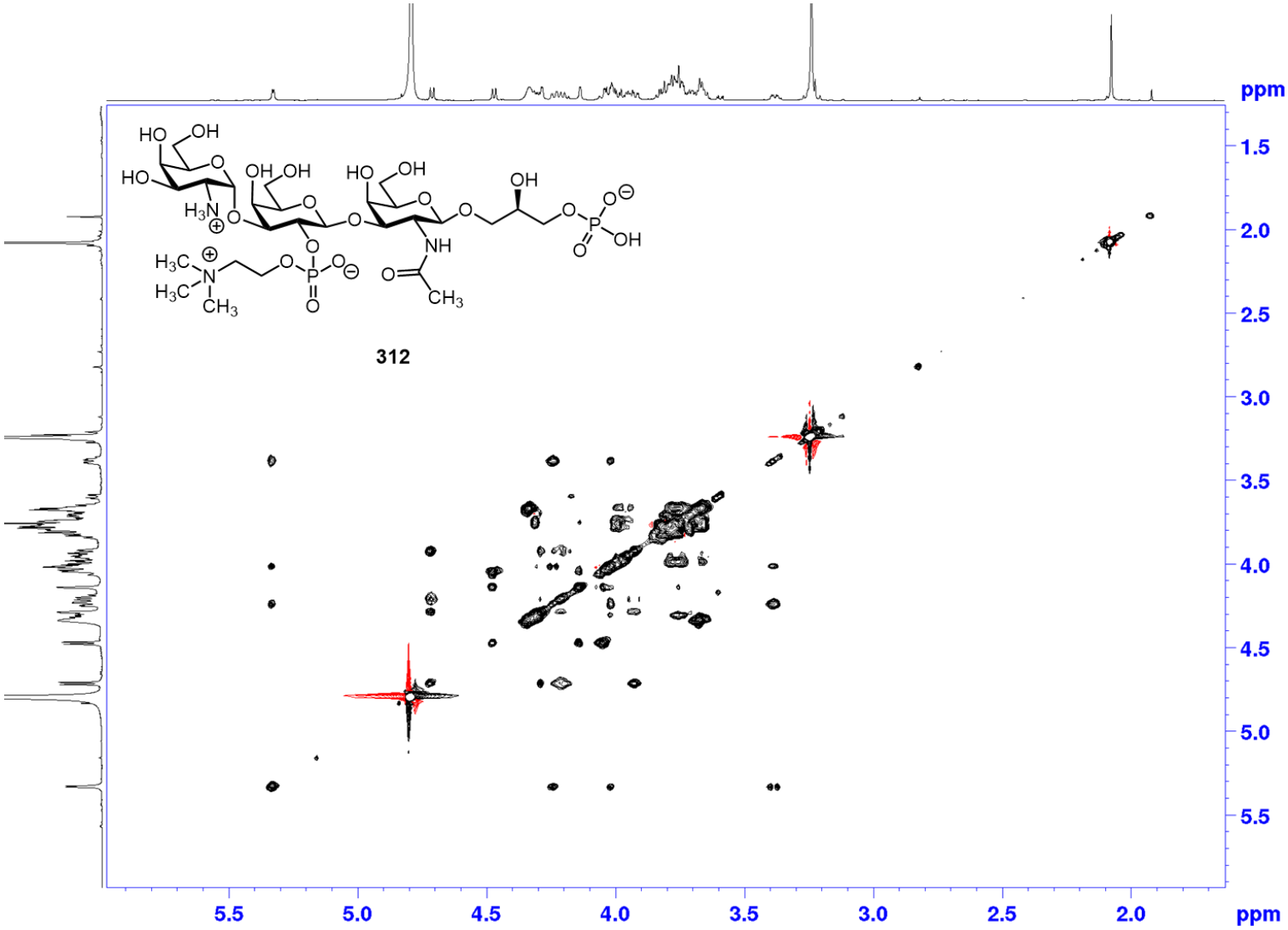
$^{13}C\{^{1}H, ^{31}P\}$ NMR ($CDCl_3$, 100 MHz) of compound 312



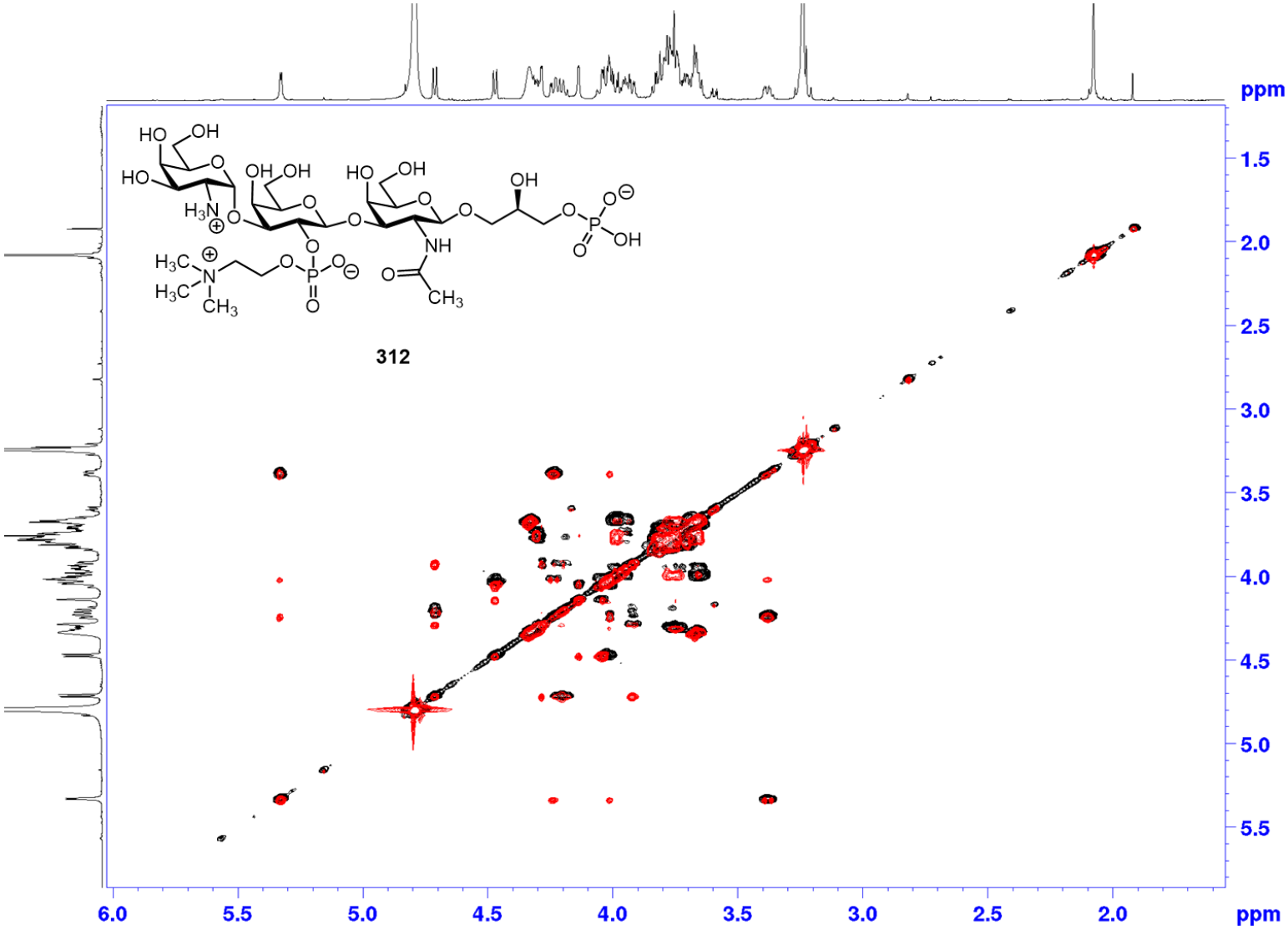
^{1}H-^{1}H COSY ($CDCl_3$) of compound 312



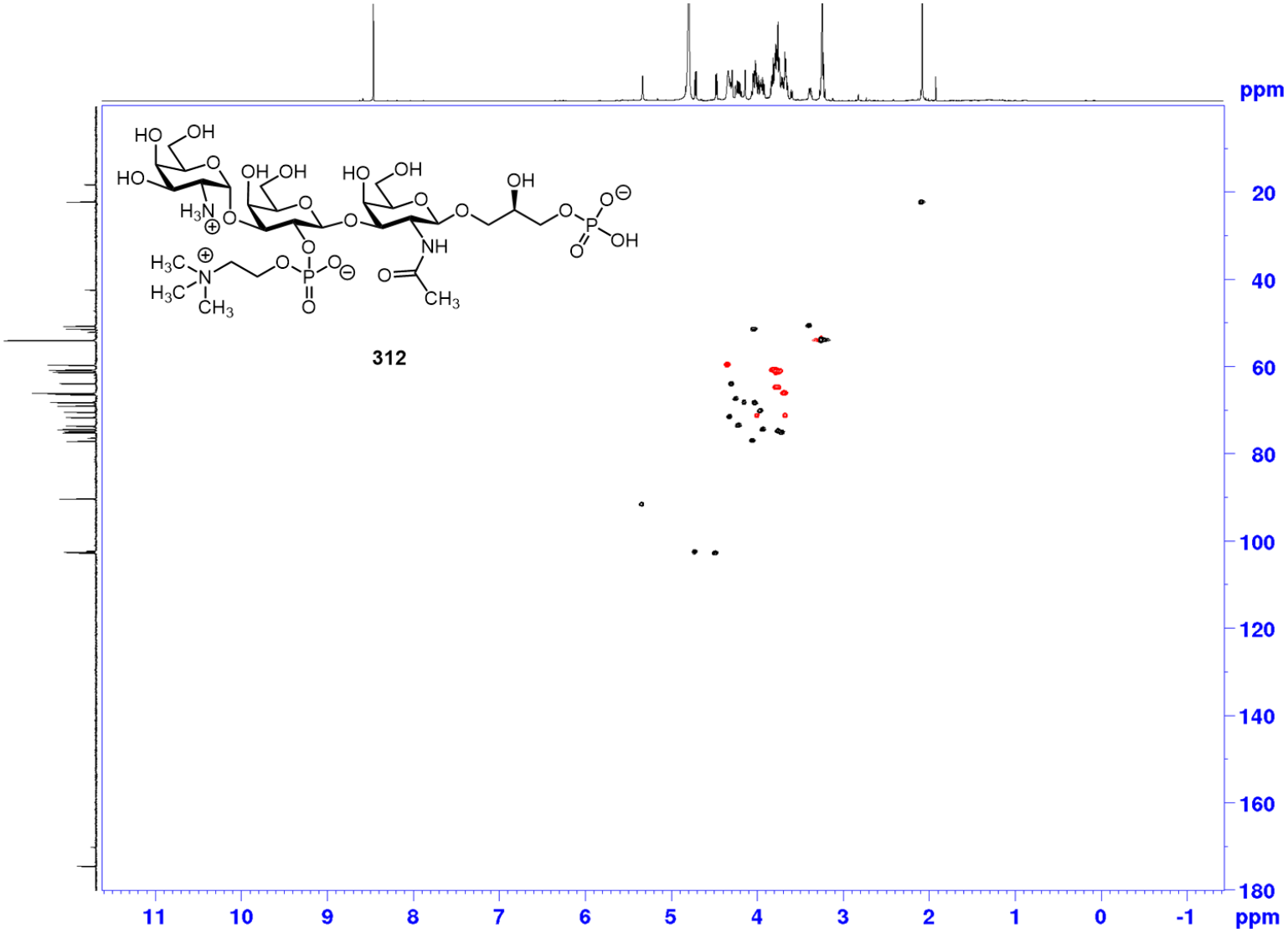
^{1}H-^{1}H TOSY ($CDCl_3$) of compound 312



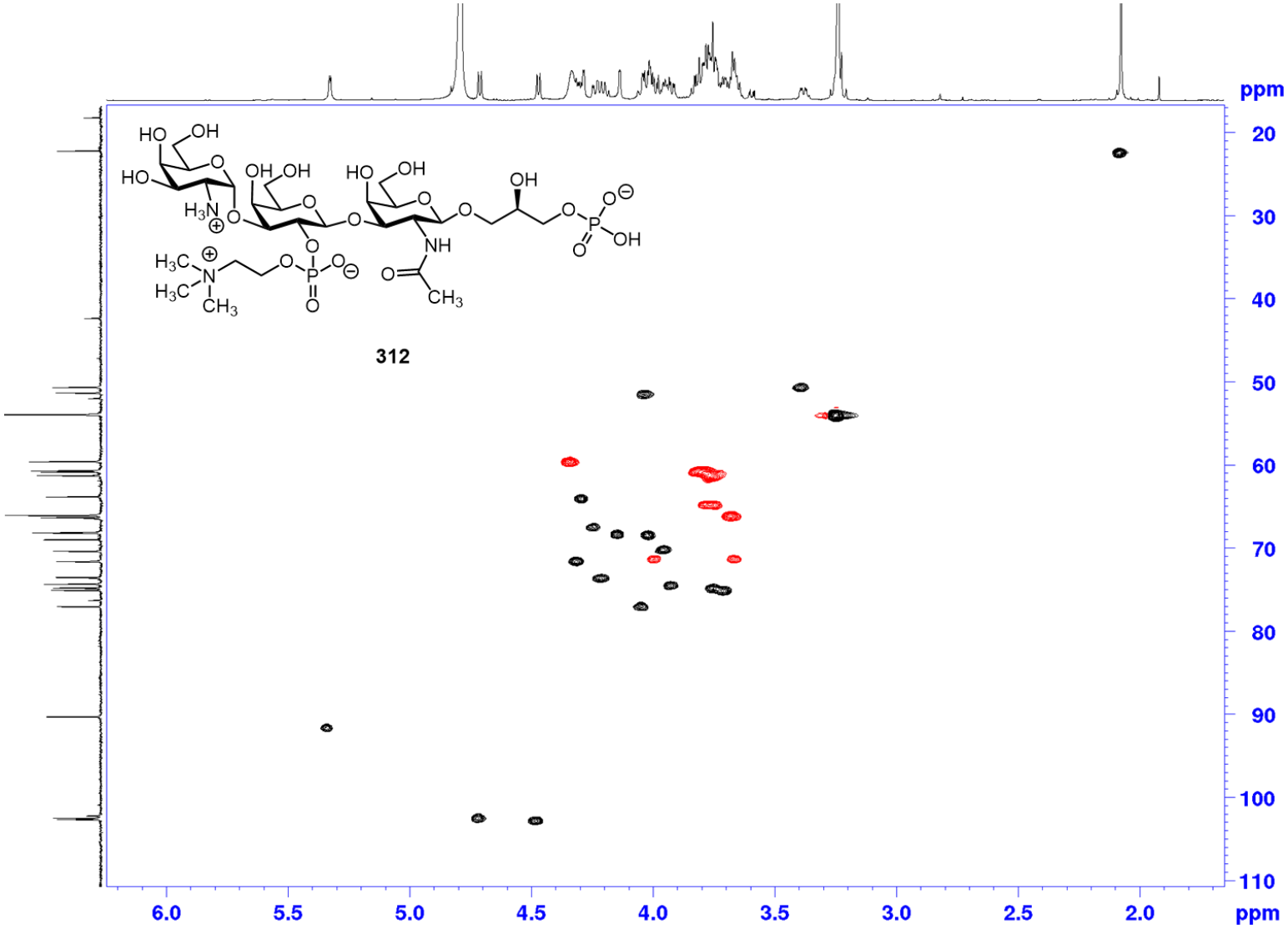
^{1}H-^{1}H COSY with ^{1}H-^{1}H TOSY overlay ($CDCl_3$) of compound 312



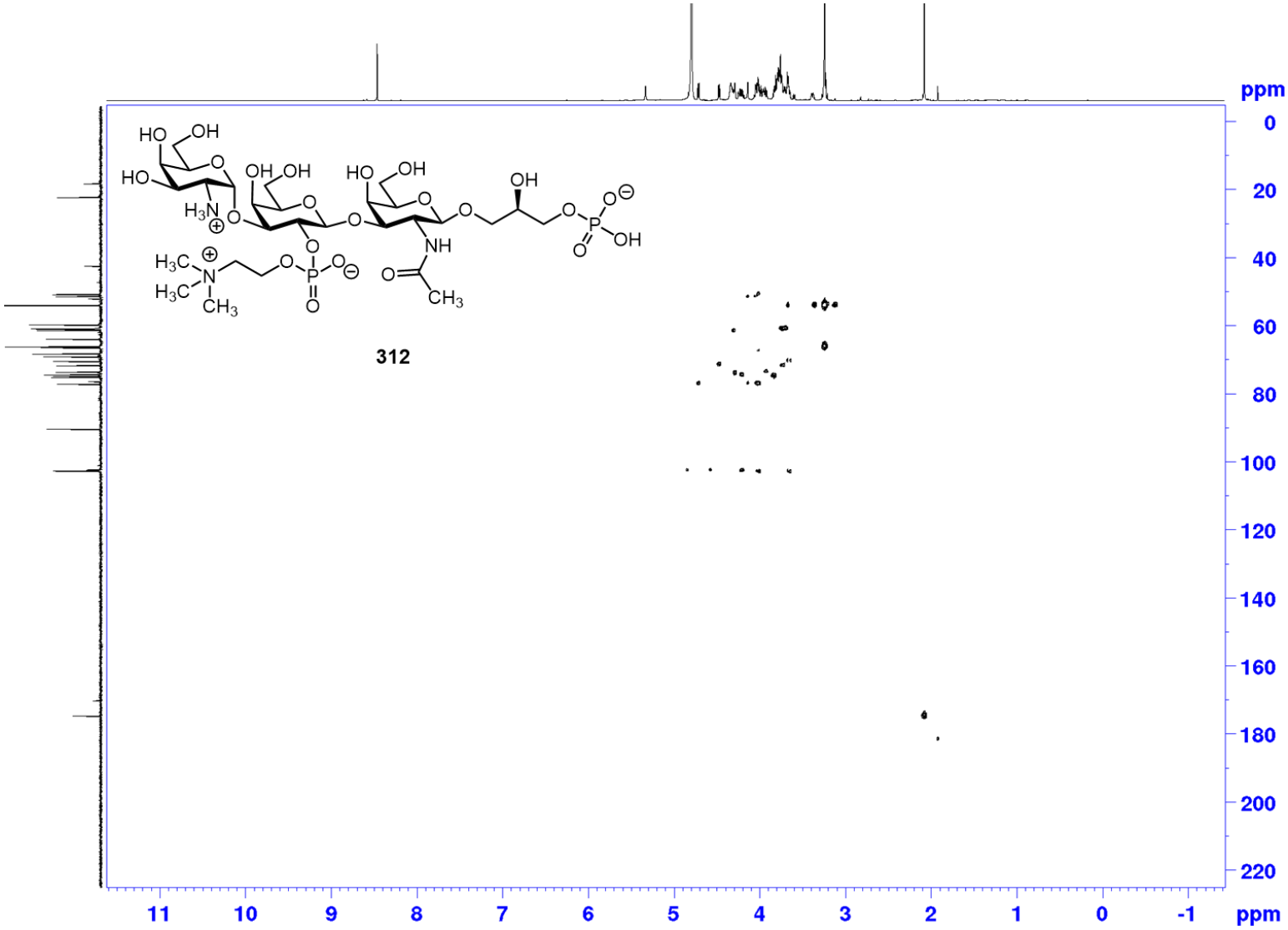
^{1}H-^{13}C HSCQ ($CDCl_3$) of compound 312



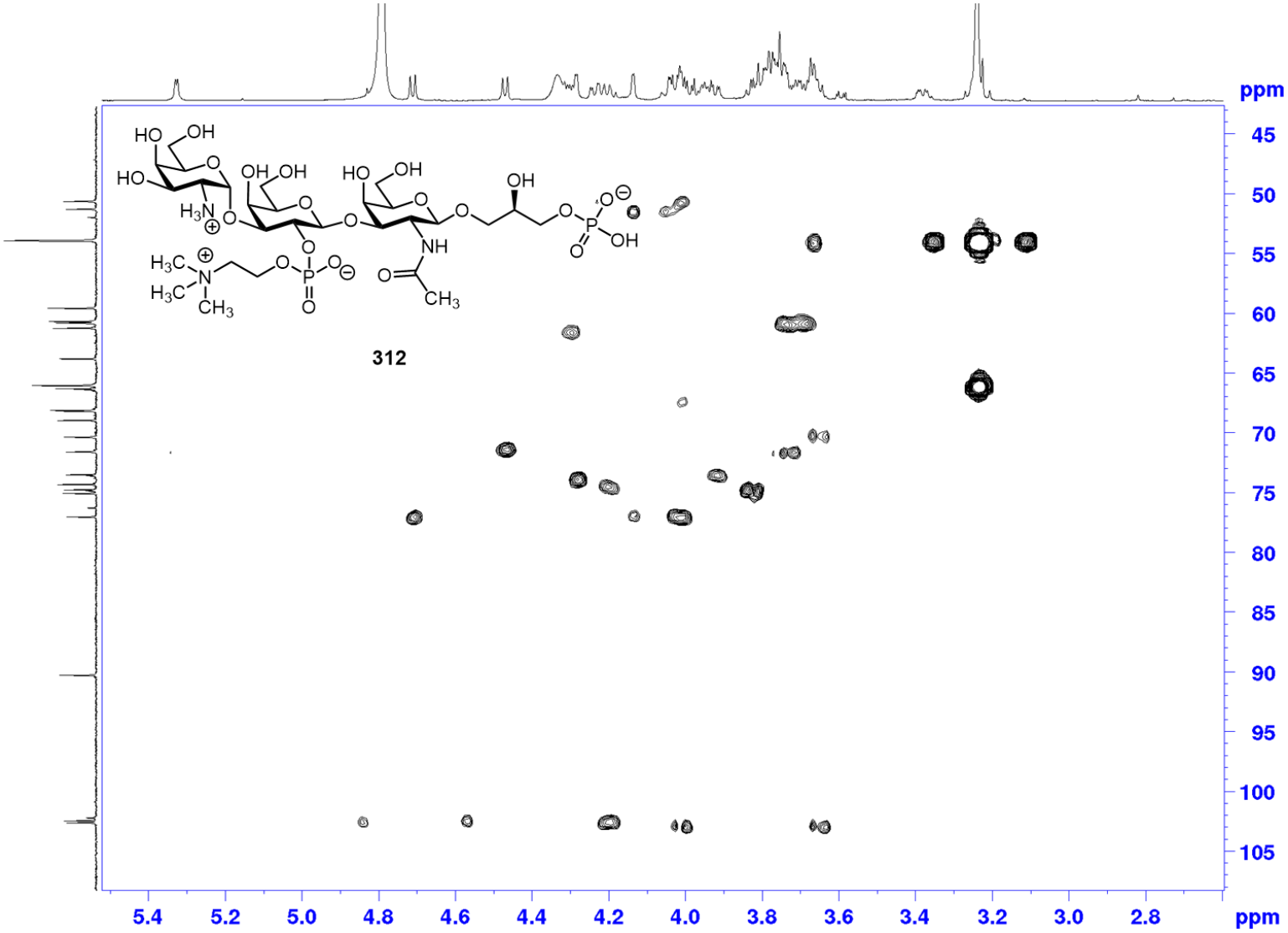
^{1}H-^{13}C HSQC ($CDCl_3$) zoomed in region of compound 312



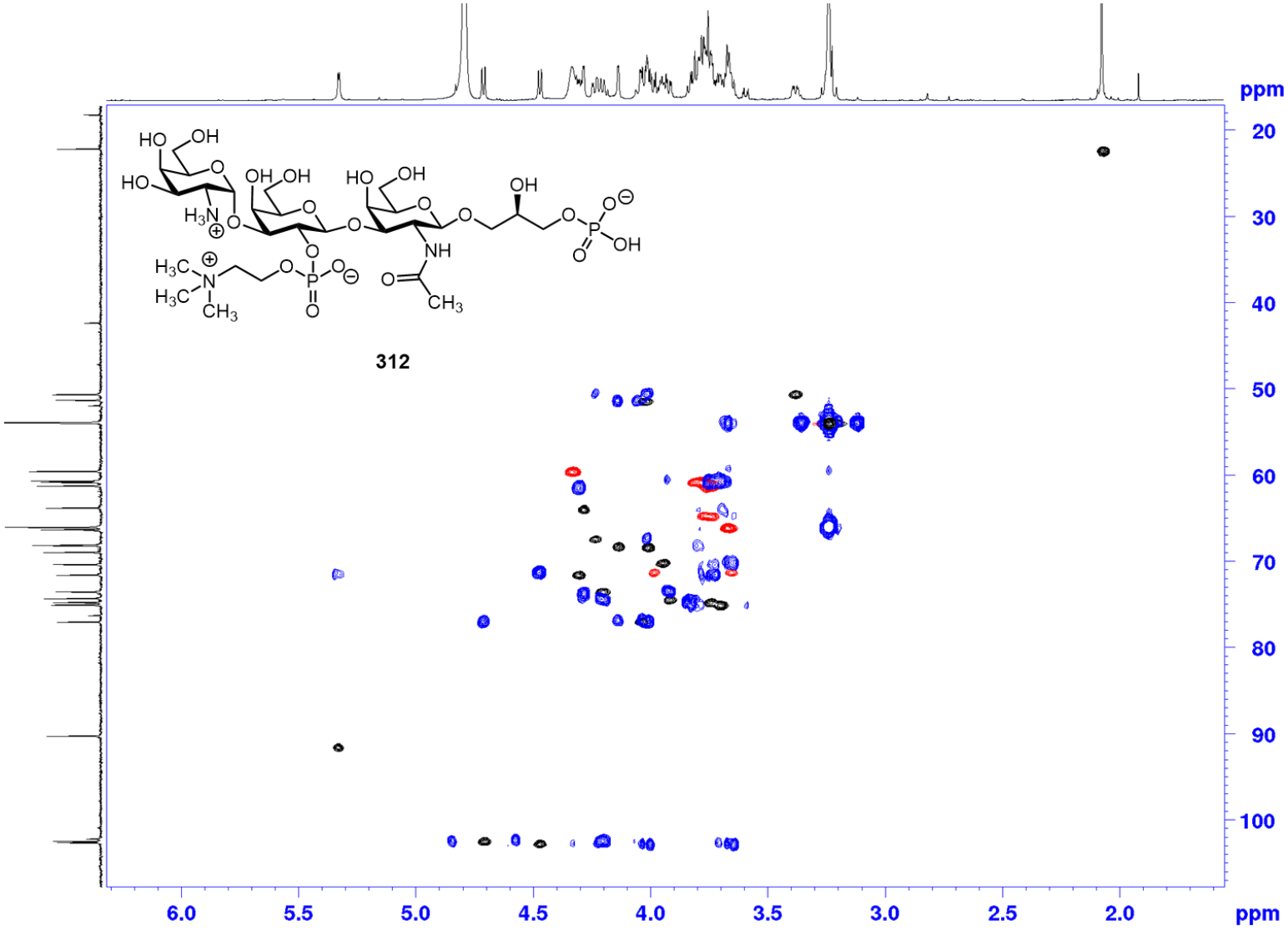
^{1}H-^{13}C HMBC ($CDCl_3$) of compound 312



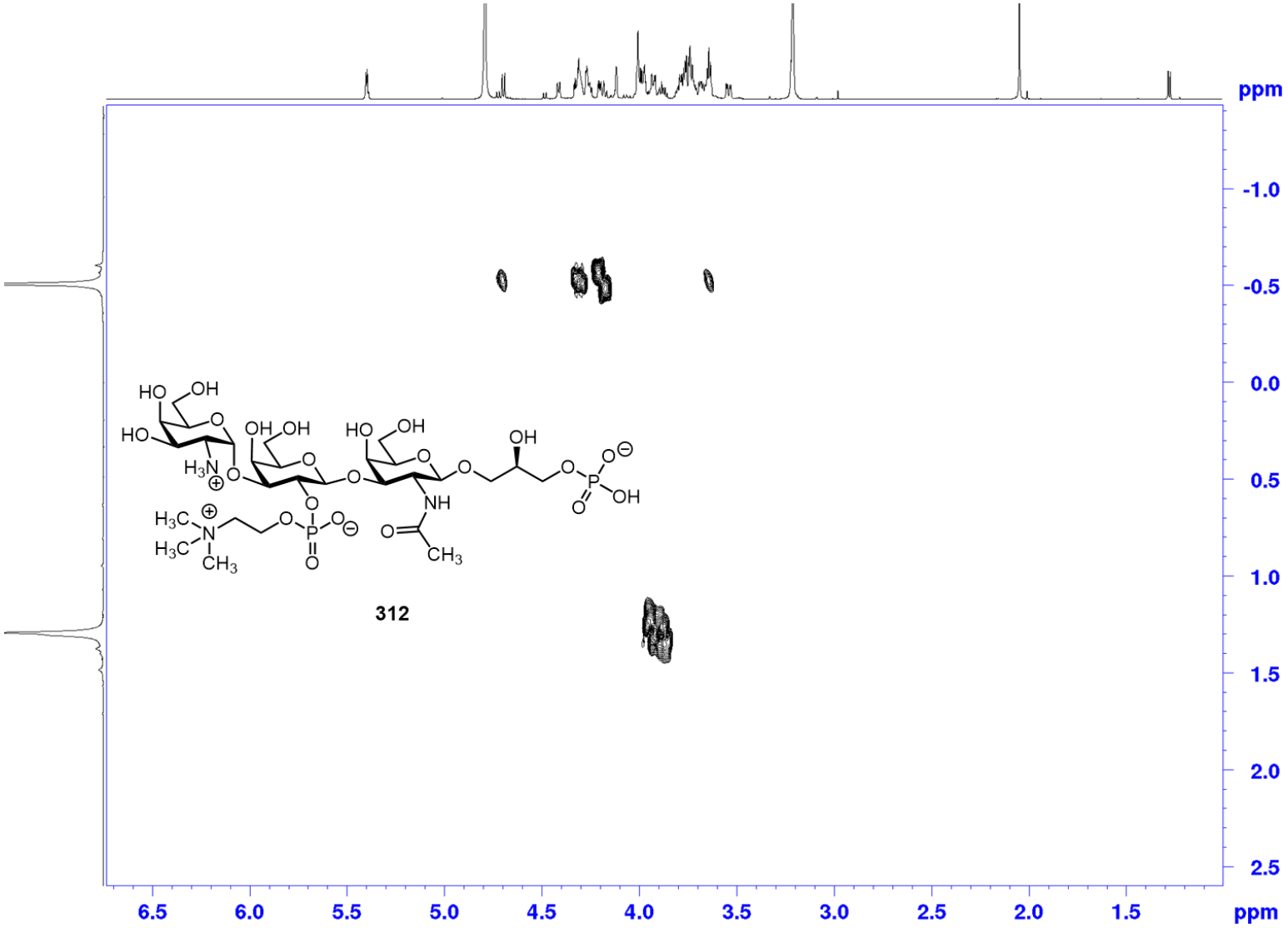
^{1}H-^{13}C HMBC ($CDCl_3$) zoomed in region of compound 312



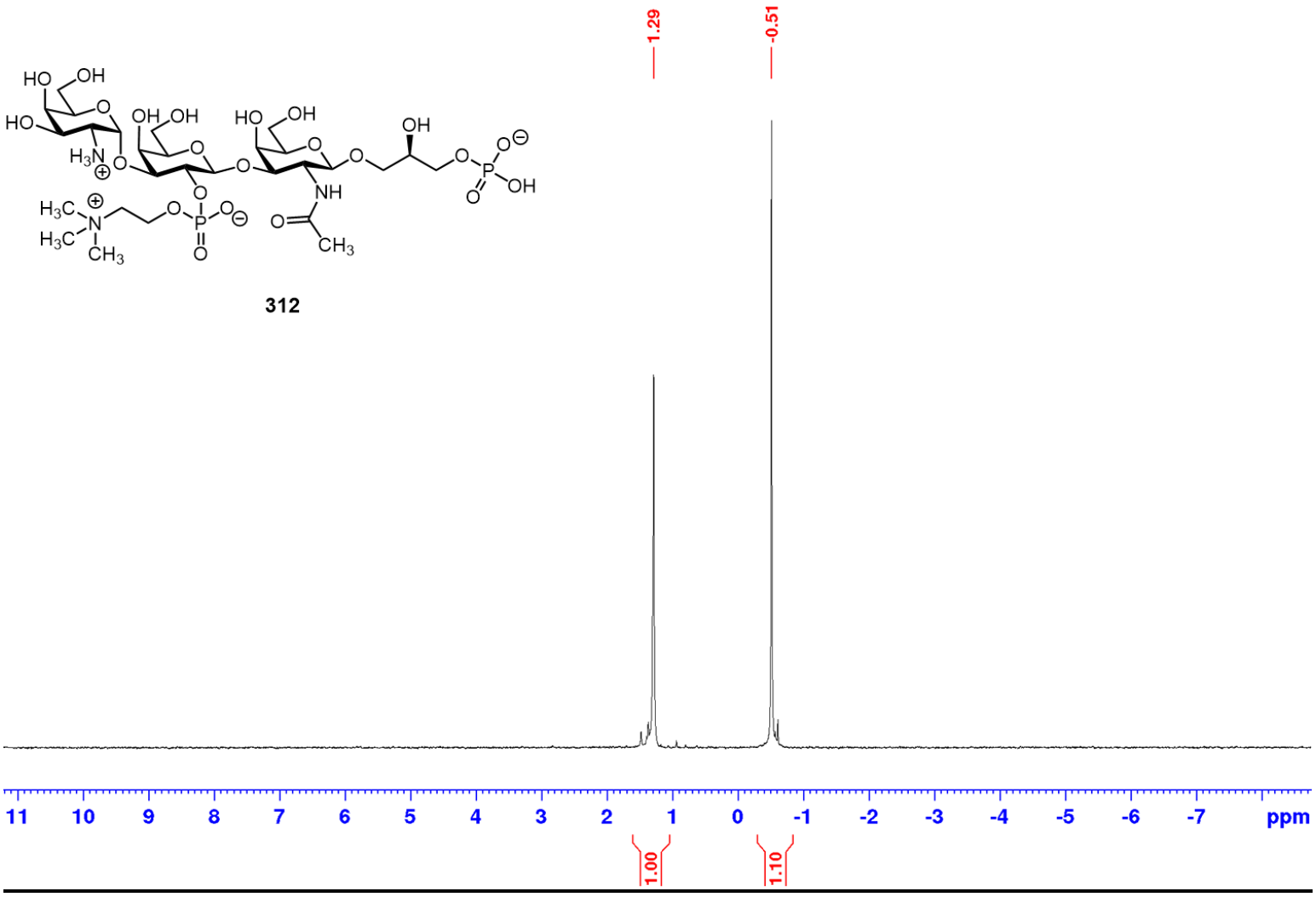
^{1}H-^{13}C HSCQ with ^{1}H-^{13}C HMBC overlay ($CDCl_3$) of compound 312



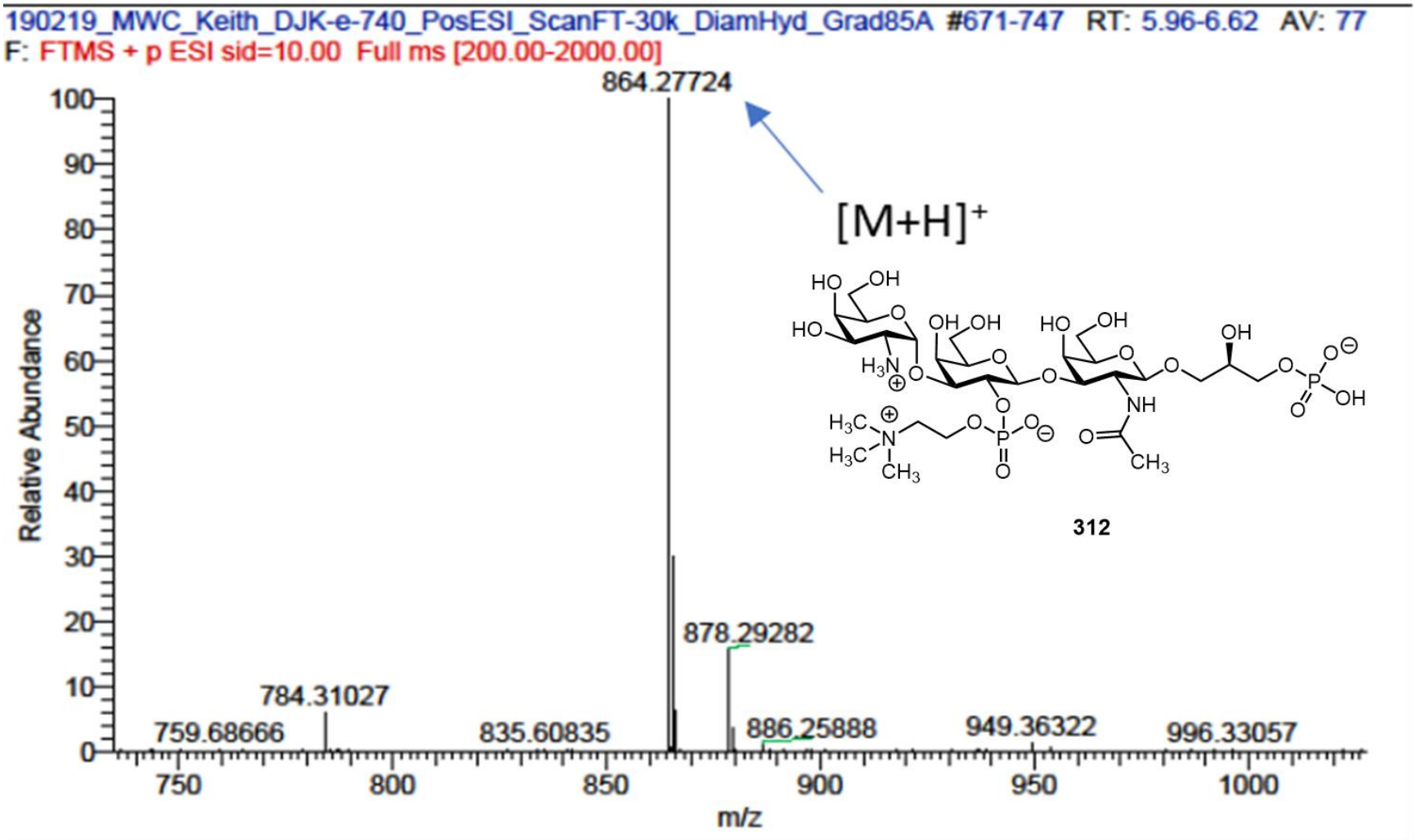
^{1}H-^{31}P HSCQ ($CDCl_3$) zoomed in region of compound 312



$^{31}P\{^{1}H\}$ NMR ($CDCl_3$, 162 MHz) of compound 312



312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F: FTMS + p ESI sid=10.00 Full ms [200.00-2000.00]
864.27724
[M+H]+
312
878.29282
784.31027
759.68666
835.60835
886.25888
949.36322
996.33057
Relative Abundance
m/z
100
90
80
70
60
50
40
30
20
10
0
750
800
850
900
950
1000

Table 12. Comparison of ^{1}H and ^{13}C NMR chemical shifts (ppm) between isolated and synthetic ZPS

Residue		Chemical shifts (ppm)							
		H1/C1	H2/C2	H3/C3	H4/C4	H5/C5	H6/C6	NAc	C=O
→3)-b-D-Gal*p* NAc-(1→	isolated[a]	4.46	4.01	4.05	4.15	3.73	3.83	2.07	175.40
		103.67	52.47	78.12	69.23	75.85	61.92	23.35	
	synthetic	4.46	4.01	4.03	4.13	3.74	3.74	2.04	174.60
		102.80	81.54	76.90	68.30	74.90	60.99	22.20	
→3)-β-D-Gal*p* -(1→	isolated[a]	4.75	4.24	4.04	4.77	3.76	3.77		
		103.57	74.34	74.34	70.32	75.67	62.38		
	synthetic	4.70	4.19	3.91	4.28	3.69	3.76		
		102.62	73.62	74.58	64.08	75.35	61.10		
α-D-Gal*p* N-(1→	isolated[a]	5.55	3.56	4.35	4.04	4.34	3.77		
		91.80	51.18	67.64	69.37	72.47	61.69		
	synthetic	5.33	3.38	4.23	4.00	4.30	3.79		
		91.60	50.68	67.55	68.41	71.59	60.99		
phospho glycerol residue	isolated[a]	3.99, 3.89	4.01	3.67, 3.99					
		71.67	70.31	67.52					
	synthetic	3.98, 3.65	3.94	3.77, 3.73					
		71.30	70.24	64.85					
phosphocholine residue	isolated[a]	4.34	3.66	3.24[b]					
		67.19	60.58	55.05[b]					
	synthetic	4.32	3.65	3.21[b]					
		59.64	66.10	53.95[b]					

[a]Data from isolation ref: Glycobiology vol. 32 no. 20 pp. 2266-1276, 2011

[b]phosphocholine -$N(CH_3)_3$ moiety

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