Progress Towards the Synthesis of Saccharomicins

A dissertation submitted by

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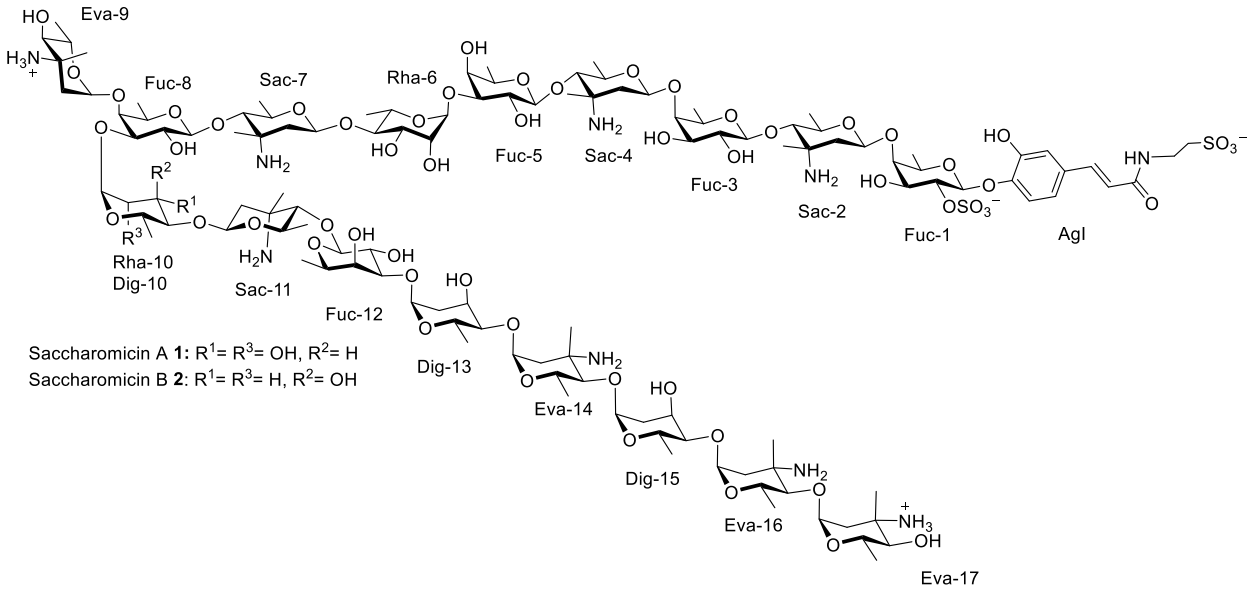
Abstract

The number of deaths caused by antimicrobial resistance is increasing. Antibacterial treatments that were once effective against dangerous bacteria are becoming less effective. New antibiotics are needed to fight against antibiotic resistance. Saccharomicins are heptadeccasaccharide natural products proven to be effective against Gram-positive and Gram-negative bacteria. The toxicity and narrow therapeutic window of the saccharomicins has prevented their use as antibiotics. However, the chemical synthesis of saccharomicins will provide an avenue to synthesize analog substrates that could become the next generation of antibiotics.



Chapter 1 highlights the need to develop saccharomicins into effective antibiotics and the progress that has been made towards their chemical synthesis. First, the McDonald, Rhee, and Wans lab's efforts towards the synthesis of saccharomicins is examined. Next, the Bennett lab's retrosynthetic analysis of the saccharomicins as well as the progress that the Bennett lab has made towards the synthesis of saccharomicins is discussed. Then, the Bennett lab's retrosynthetic analysis is reexamined and the remaining fragments of saccharomicins that need to be synthesized to complete the Bennett lab's synthetic plan are presented.

Chapters 2 will outline the synthesis of the branched tetrasaccharide fragment of saccharomicin A. The synthesis of the D-saccharosamine-L-rhamnose and 4-*epi*-L-vancosamine-D-fucose disaccharides as well as their use in a [2+2] strategy to synthesize the tetrasaccharide will be discussed in detail. Next, Chapter 3 will outline the synthesis of the branched tetrasaccharide of saccharomicin B. The synthesis of the D-saccharosamine-L-digitoxose disaccharide as well as the [2+2] strategy for the synthesis of the branched tetrasaccharide of saccharomicin B will be discussed in detail. Finally, Chapter 4 will provide an examination of glycosylations using saccharosamine donors.

Dedication

This thesis is dedicated to my parents. You are both amazing! I also want to thank all my family members and friends for supporting me over the years.

Acknowledgments

I want to thank Professor Clay S. Bennett for his mentorship throughout my PhD journey. The guidance provided to me by Professor Bennett was essential for the completion of the work outlined in this thesis and the progress that I have made as a chemist. I have had enormous growth as a scientist since joining your lab and I am forever grateful for the experiences we have shared. Thank you, Professor Bennett, for being my supervisor during my PhD.

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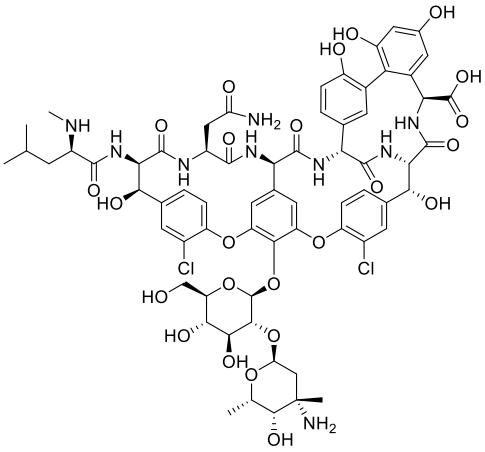
Chapter 1. The Synthesis of the Saccharomicins

1.1 The Urgent Need for New Antibiotics

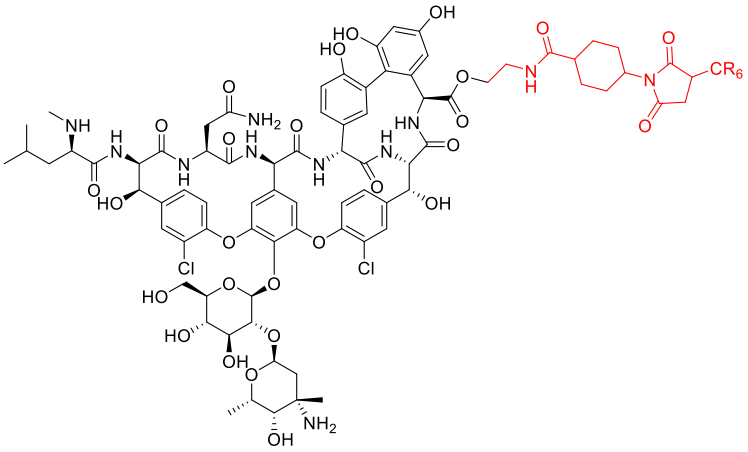
Antimicrobial resistance is a world-wide threat causing 1.27 million deaths in 2019.[1] A 2016 review on microbial resistance stated that the number of deaths caused by resistant bacteria could grow to 10 million per year by 2050 and cost the world's economy USD 100 trillion by that same year. [2,3] The CDC, WHO, and UN have each released plans to combat antimicrobial resistance.[4–6] The WHO proposed that antibiotic development is desperately needed to help fight antimicrobial resistance.[7] New antibiotics are always in the development pipeline and each year more antibiotics become available for use by patients around the word.[7] However, antimicrobial resistance will occur after using these new drugs, as exposure will trigger the pathogens to adapt and mutate to become resistant.[4,8,9] As a result, antimicrobial resistance appears endless, and therefore antibiotic development will continue to be essential for the fight against these pathogens.

Natural products and chemically synthesized analogs can serve as viable options for discovering potential antibiotics.[10–15] Many of the former, such as the discovery of penicillin, have been vital for human survival. Chemical synthesis on the other hand, allows for infinite possibilities of varying structural motifs to be accessed and provides the instrument for which current and potential antibiotics can be improved. The development of new antibiotics through the chemical modification of current therapeutics or other known molecules has proven to be a successful strategy.[10–15] Analog structures can address problems with potency and toxicity in current antibiotics and therefore increase the lifetime of a particular class of antimicrobials. For example, Umstatter *et al.* modified vancomycin **3** by installing a heterobifunctional cross linker which reacted with a cysteine residue of a peptide (Scheme 1.1).[16] This allowed them to synthesize a substrate **4** with increased potency against vancomycin-resistant bacteria.[16] In another example, Surivet *et al.* modified tetrahydropyran-based antibiotics which are effective against Gram-positive bacteria and were able to produce antibiotics effective against both Gram-positive and Gram-negative bacterial strains (Scheme 1.2).[17] Surivet *et al.* showed that they could improve the

potency of antibiotic **5** by replacing a hydroxyl with an amine affording **6**, which resulted in an 8-fold increase in potency of the synthesized analog against *P. aeruginosa*.[17] These two examples demonstrate the utility that chemical synthesis adds to the development of more effective antibiotics and proves that reinvestigation into known antibiotics can lead to analogs with improved antibacterial properties.

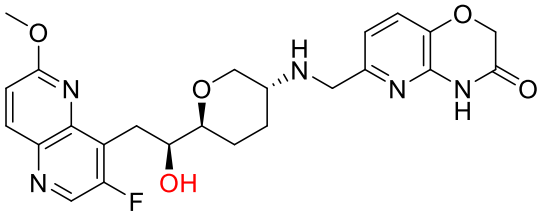


3: Vancomycin
MIC: 4.42 x 10^{-7} mol/mL

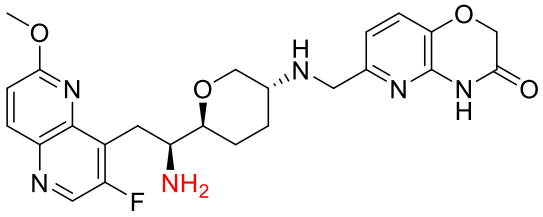


4: Vancomycin FU013 (Vc)
MIC: 1.01 x 10^{-9} mol/mL

Scheme 1.1 MIC of **3** and **4** against *E. faecium* UL602570*.[16] Modified portion highlighted in red.



5
IC_{50}: Topo IV 0.125 μM
MIC: 8 μg/mL

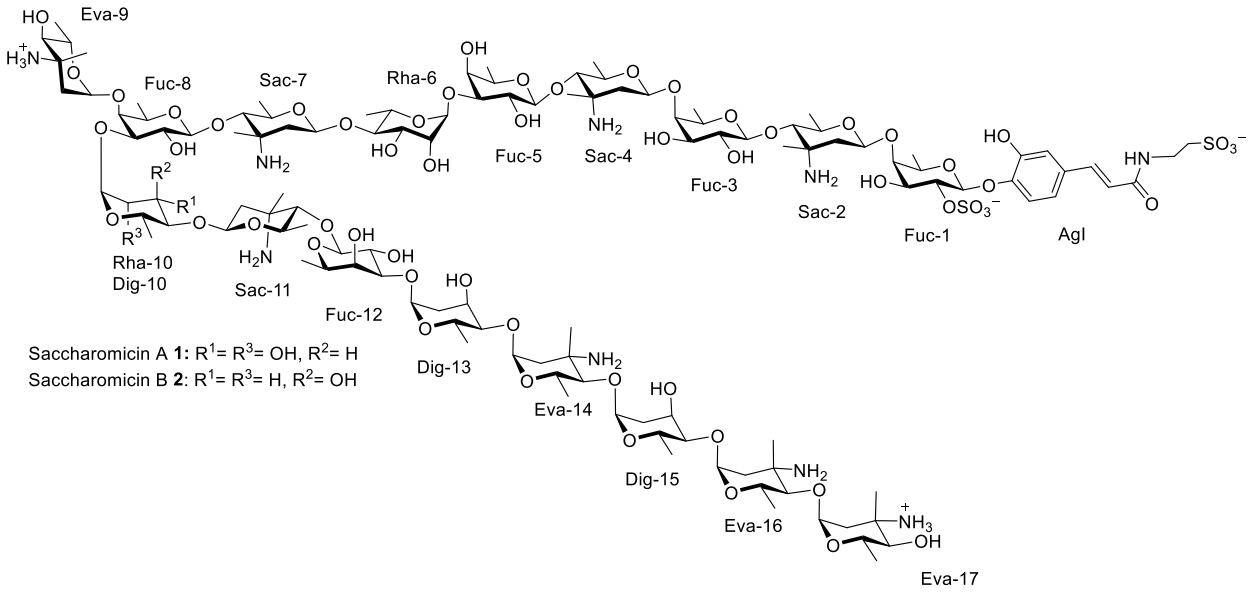


6
IC_{50}: Topo IV 0.03 μM
MIC: 1 μg/mL

Scheme 1.2 IC_{50} of **5** and **6** against Topo IV *P. aeruginosa*. MIC of **3** and **4** against *P. aeruginosa*.[17] Modified portion highlighted in red.

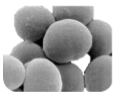
1.2 Saccharomicins: Novel antibacterial agents

Saccharomicins **1** and **2** are heptadeccasaccharide natural products that have been proven to be effective against both Gram-positive and Gram-negative bacteria (Scheme 1.3).[18–20] Saccharomicins were isolated from an antibacterial complex that is produced by the Bacteria actinomycete *Saccharothrix espanaensis* found in a soil sample from Puertollano, Spain.[18,19] Notably, saccharomicins displayed more potent antibacterial properties against *E. Coli* GC 2203 compared to vancomycin (Table 1.1).[20] However, when tested in mice infected with *S. aureus Smith*, saccharomicins displayed toxic effects with an LD_{50} of 21 mg/kg when administered subcutaneously.[20] The Bennett lab believes that chemical modification of saccharomicins could decrease this toxicity and improve the effectiveness of saccharomicins as antibiotics. The total synthesis of saccharomicins will provide each of the molecules at the milligram scale so that they can be studied to understand how they work as antibiotics. Then, analogs of the saccharomicins can be synthesized to develop more potent antibiotics. In addition, the total synthesis of the saccharomicins will rely on the development of novel chemistries to synthesize the many unique glycosyl linkages throughout the molecules.



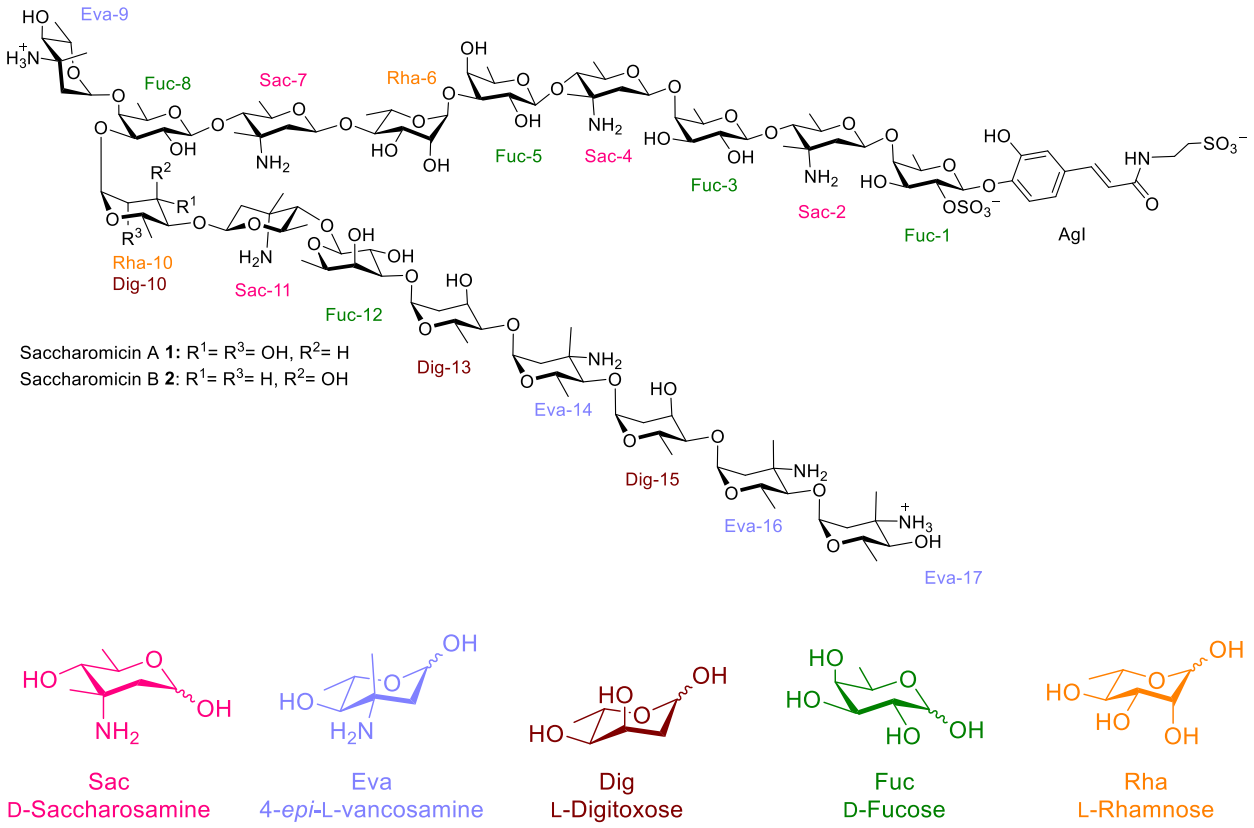
Scheme 1.3 Structures of saccharomicin A **1** and B **2**.[18,19]

Table 1.1 Activities of saccharomicin A **5** vs. vancomycin **3** against *E. coli GC 2203*.[20]



Bacteria	Antibiotic	MIC
E. coli GC 2203	vancomycin	>128 μg/mL
E. coli GC 2203	**saccharomicin A**	4 μg/mL

The saccharomicins differ at the 10th-residue, where in saccharomicin A **1** there is a rhamnose monosaccharide and in saccharomicin B **2** there is a digitoxose monosaccharide.[18,19] Each saccharomicin contains five different monosaccharides and an aglycone attached to the nonreducing end of the molecule (Scheme 1.4).[18,19] Two of the monosaccharides, D-fucose and L-rhamnose, are 6-deoxy sugars which are commercially available. The other three monosaccharides, 4-*epi*-L-vancosamine, L-digitoxose, and D-saccharosamine, are 2,6-di-deoxysugars which are not commercially available. 4-*epi*-L-vancosamine and D-saccharosamine are tri-deoxy amino sugars and D-saccharosamine has only been found in the saccharomicins (Scheme 1.4).



Scheme 1.4 Structure of **1** and **2**. Five monosaccharides found in saccharomicins.[18,19]

Saccharomicins were isolated starting with 41 kg of soil that was extracted providing 1.25 g of the antibiotic LL-C19004 complex.[18] Next, 31 g of the antibiotic complex were combined and after performing chromatography and extractions, 5.0 g of saccharomicin A and 5.5 g of saccharomicin B were isolated.[18] The isolation required roughly a thousand kilograms of soil and twenty thousand liters of bacterial fermentation broth.[18] Consequently, alternative methods such as biosynthetic strategies and chemical synthesis have also been explored as a means to access saccharomicins.

Investigation into the biosynthesis of the saccharomicins has led to the elucidation of each of the glycotransferases that are involved in the assembly of saccharomicin A **1**.[21–23] First, the Bechthold lab reported the genes associated with the synthesis of the aglycone of saccharomicins and traced its synthesis to L-tyrosine and taurine.[21] Then, the Ruckert lab was able to sequence the genome of *Saccharothrix espanaensis* DSM 44229T.[22] With the newly established genome,

the Zhang lab employed gene-knockout strategies to realize the biosynthetic pathway to assemble saccharomicin A **1**.[23] Together, these studies have uncovered the biosynthesis of saccharomicins, however this investigation is ongoing and saccharomicins have only ever been isolated from nature. Furthermore, saccharomicins will need to be modified to be used as antibiotics and late-stage chemical modifications to decrease the toxicity of the antibiotics could pose a challenge. Therefore, chemical synthesis remains the most attractive strategy to access saccharomicins and analogs.

1.3 Saccharomicins: Synthetic challenges

The total synthesis of saccharomicins is a daunting task. Chemical syntheses are needed to access the monosaccharides, 4-*epi*-L-vancosamine, L-digitoxose, and D-saccharosamine. In addition, monosaccharides D-fucose and L-rhamnose will need to be purchased and orthogonally protected or chemically synthesized. After synthesizing the monosaccharides new chemistries will need to be developed to couple them together. A major challenge for the synthesis of saccharomicins is controlling the anomeric selectivity of the glycosylation reactions that will be used to link the sugars. The selective synthesis of glycosyl linkages using 2-deoxy sugar donors is challenging because they lack C-2 functionality. C-2 neighboring group participation strategies from the protecting group on the C-2 hydroxyl of the donor sugar are commonly used in the selective synthesis of glycosides.[24]

The Bennett lab has developed methods for the selective synthesis of glycosides using 2-deoxy sugar donors and has demonstrated that it is possible to control the selectivity in glycosylation reactions through changing the promoter (Scheme 1.5).[25–30] The dehydrative glycosylation approach developed by the Bennett lab has been used for the α-selective synthesis of 2-deoxy sugar glycosides.[27,29] In the reaction the donor species reacts with 2,3-bis(2,3,4-trimethoxyphenyl)cyclopropene-1-thione which has been activated by reaction with oxalyl bromide. An intermediate donor-cyclopropene species can form and be displaced after the

addition of a nucleophile species, which can lead to the selective synthesis of α-linked glycosides. A second method developed by the Bennett lab is the reagent-controlled glycosylation method which has been used for the synthesis of both α-and β-linked glycosides.[25,26,28,30,31] In the reaction, the donor species is deprotonated using potassium bis(trimethylsilyl)amide (KHMDS) forming an alkoxide which can react with the sulphonate promoter and generate a glycosyl sulphonate species. Then, addition of an acceptor nucleophile can facilitate an S_N2 reaction resulting in the synthesis of glycosides. Selectivity of the reaction is thought to be controlled by the α or β-position of the glycosyl sulphonate.[30] Using ^{1}H-^{13}C heteronuclear single-quantum correlation (HSQC) variable temperature NMR experiments (VT-NMR), the Bennett lab demonstrated the formation of a glycosyl sulphonate intermediate.[25] This was done by reacting a glycosyl potassium alkoxide donor with *p*-toluenesulfonic anhydride (Ts_2O) at -78 °C within a sealed NMR tube.[25] The formation of a peak at δ 6.11 ppm within the ^{1}H NMR spectrum and a peak at δ 102.3 ppm within the ^{13}C spectrum demonstrated that a glycosyl sulphonate was formed.[25,32,33] Then, the Bennett lab concluded that because only one anomeric carbon existed that a single glycosyl sulphonate anomer was formed in the reaction.[25] The Bennett lab noted that the glycosyl sulphonate intermediate was stable between -5 °C to -78 °C and degraded at warmer temperatures.[25] In another study using variable temperature NMR experiments, the Bennett lab again demonstrated the formation of a α-glycosyl sulfonate which was stable at temperatures up to -50 °C.[26] The formation of a α-glycosyl sulfonate could be indicative of the glycosylation proceeding through an S_N2-like pathway.[26] If the reaction was occurring through an S_N2-like pathway then the formation of an α-glycosyl sulfonate intermediate would result in the synthesis of β-linked glycosides which is consistent with studies from the Bennett lab.[25,26] The key to the reagent-controlled method is choosing a sulphonate that will react to form a glycosyl sulfonate intermediate that is also labile enough to be a good leaving group in the presence of a nucleophile acceptor.[30,34] Choosing the sulphonate to match the donor species allowed the Bennett lab to tune the reagent-controlled

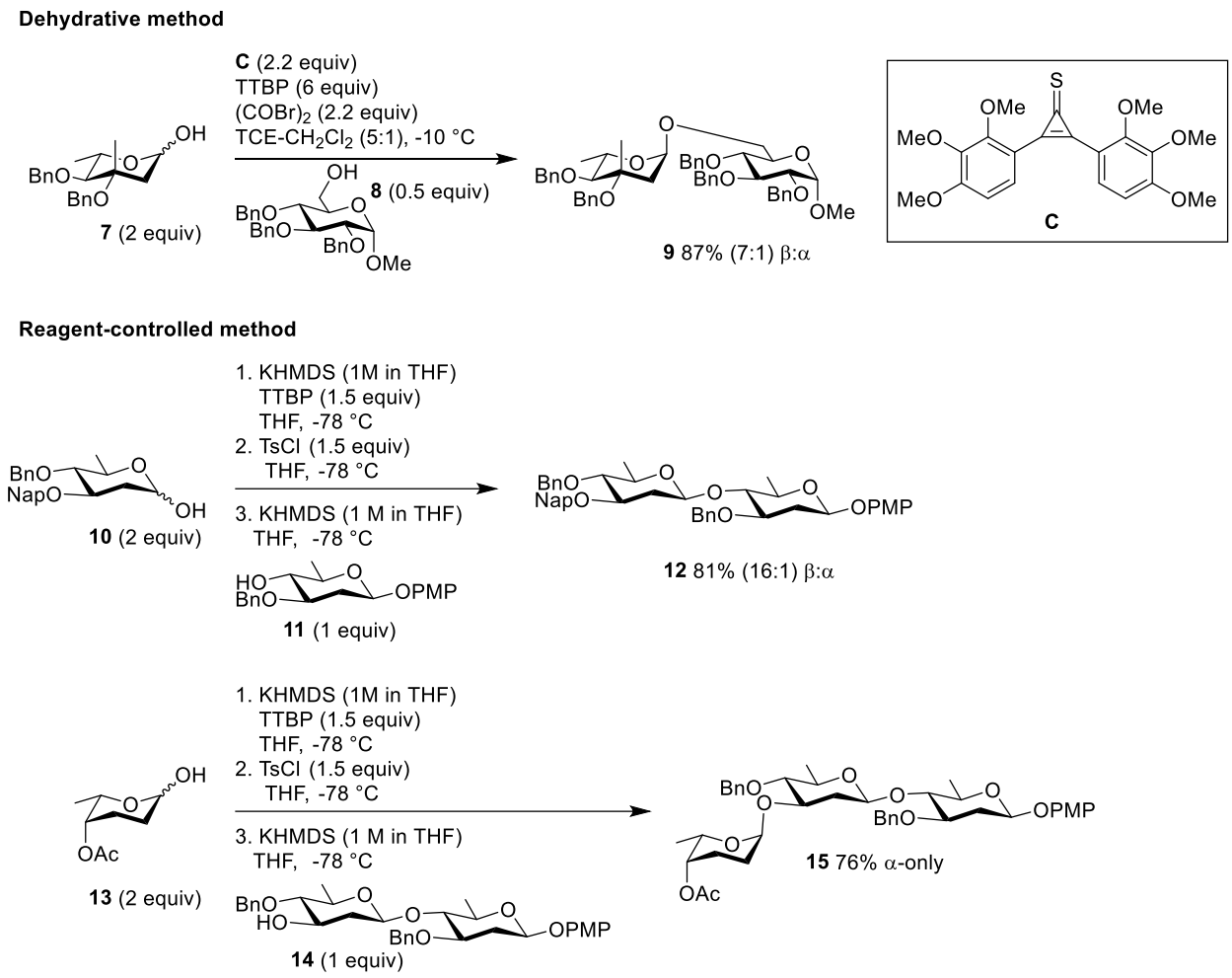
glycosylation method so that it could be used to selectively synthesize glycosides.[30] In addition to variable temperature NMR experiments, the Bennett lab used $^{12}C/^{13}C$ kinetic isotope effects (KIEs) studies and density functional theory (DFT) calculations to determine that the reaction was indeed occurring through an S_N2-like pathway.[30]

Interestingly, on route towards the synthesis of the hexasaccharide fragment of landomycin A the Bennett lab's reagent-controlled method resulted in the synthesis of β-linked glycosides when using D-olivose donors and in the synthesis of α-linked glycosides when using L-rhodinose donors (Scheme 1.5).[31] The promoter-directed glycosylation strategies developed by the Bennett lab provide a means to use the same donor and acceptor species to synthesize α and β-linkages.[25–30]

Alternatively, approaches such as *de novo* synthetic methods could be developed to synthesize glycoside derivatives which can be functionalized following the glycosylation step. *De novo* syntheses are linear approaches and can require more synthetic steps than convergent strategies making them less efficient.

The α-β-selectivity of a chemical glycosylation reaction can be determined by various techniques. A common strategy is to use NMR spectroscopy to study each of the positions on a pyranose ring. ^1H NMR experiments can provide information such as coupling constants, chemical shifts, and peak splitting which can help determine the anomeric selectivity of a glycosylation reaction. Protons on a pyranose ring will have chemical shifts between 3 and 6 ppm and the anomeric proton can be found between 4.5 and 5.5 ppm.[35–39] Anomeric protons can be split into a doublet or doublet of doublets and a coupling constant can be calculated from the doublet peaks. Coupling constants that range from 7 to 9 Hz are indicative of an axial proton and therefore a β-linked glycoside.[35–39] Coupling constants that range from 2 to 4 Hz are indicative of an equatorial linked proton and therefore an α-linked glycoside.[35–39] This rationale is a simple

approach to understanding the selectivity of glycosylation reactions and is used in this thesis along with literature references to assign anomers.



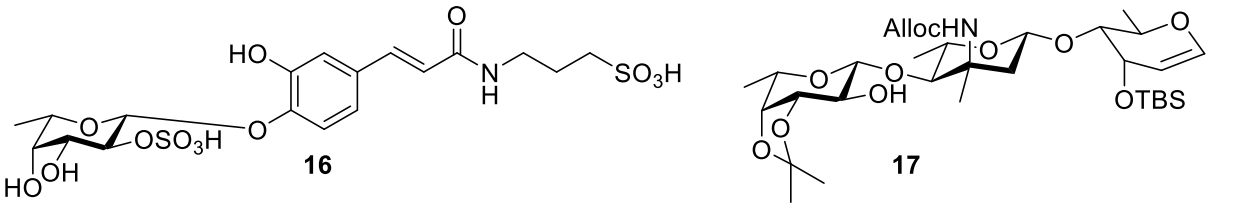
Scheme 1.5 Bennett lab's glycosylation methods. Dehydrative method.[27] Reagent-controlled glycosylation method.[25,26,31]

1.4 Progress Towards the Total Synthesis of Saccharomicins: Other Labs

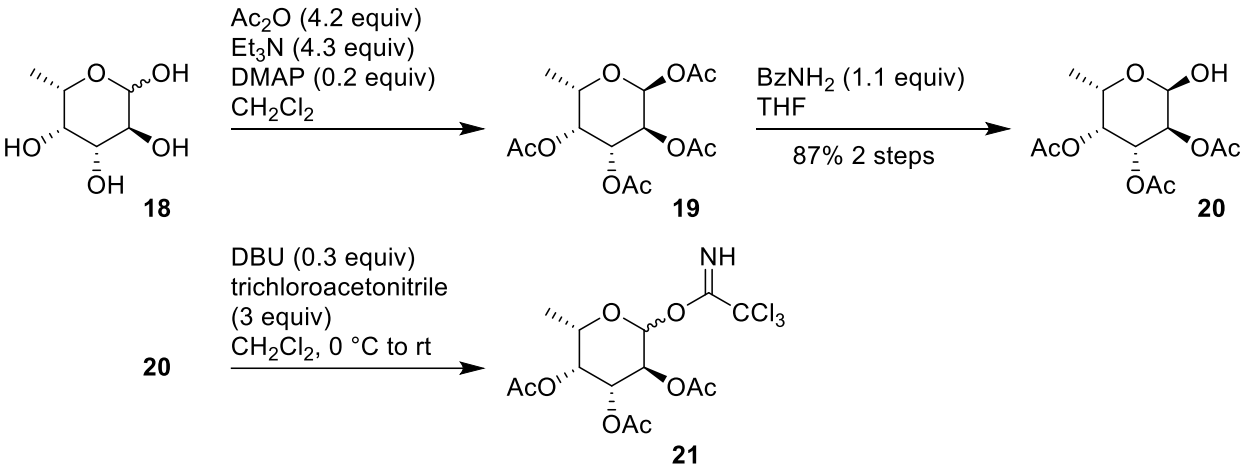
The McDonald lab was the first to synthesize fragments of the saccharomicins.[40,41] They synthesized the L-fucosyl aglycone fragment **16** of both saccharomicins and the L-fucose-L-saccharosamine-D-digitoxose trisaccharide derivative of saccharomicin B **17** (Scheme 1.6).[40,41] Both fragments synthesized by the McDonald lab contain the wrong "handed" sugars.

Saccharomicins contain the D-fucosyl aglycone moiety and the trisaccharide fragment found in saccharomicin B **2** consists of D-fucose, L-digitoxose, and D-saccharosamine.[40]

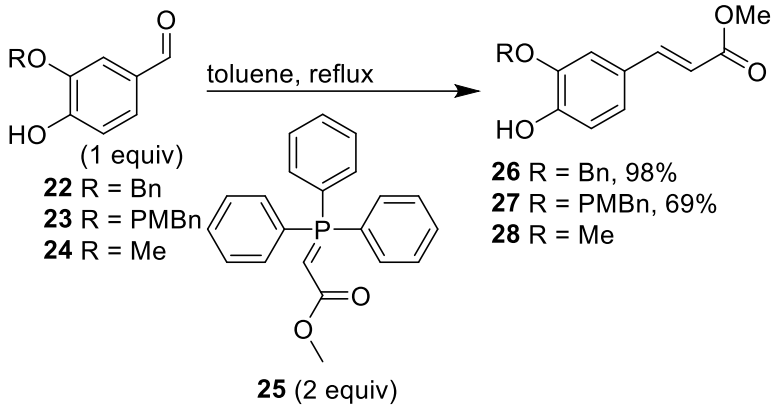
Initially, the McDonald lab synthesized L-fucosyl trichloroacetimidate donor **21** which was used as a precursor for the synthesis of both fragments **16** and **17**. To this end, commercially available L-fucose **18** was acetylated prior to selective removal of the anomeric acetate using benzamide ($BzNH_2$) in tetrahydrofuran (THF) to afford L-fucose hemiacetal **20** in 87% yield over two steps (Scheme 1.7).[40] Next, 1,8-Diazabicyclo(5.4.0)undec-7-ene (DBU) and trichloroacetonitrile (Cl_3CCN) were used to synthesize L-fucosyl trichloroacetimidate donor **21**. A yield was not reported for this step.



Scheme 1.6 Fragments of saccharomicins synthesized by the McDonald lab. The L-fucose aglycone **16**.[41] The L-fucose-L-saccharosamine-D-digitoxose glycal **17**.[40]



Scheme 1.7 McDonald lab's synthesis of L-fucosyl trichloroacetimidate donor **21**.[40]

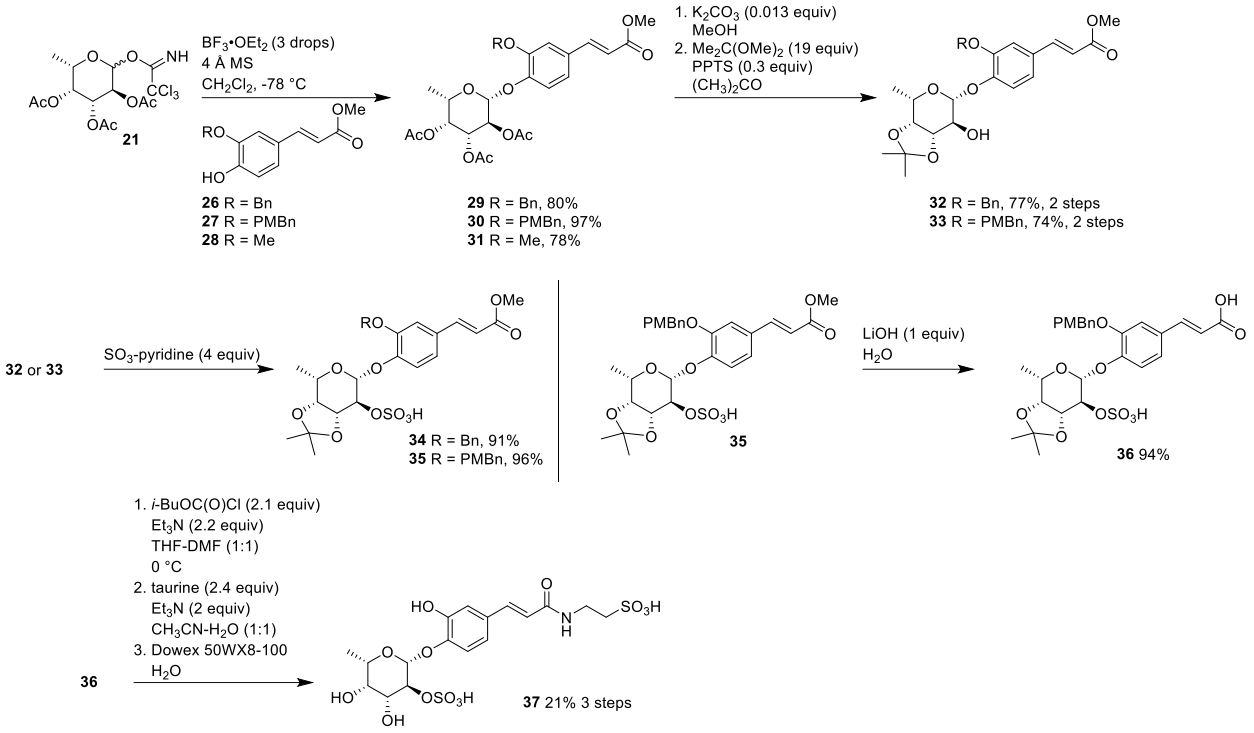


Scheme 1.8 McDonald lab's synthesis of aglycone derivatives **26 and 27**.[41]

With the protected fucose donor **21** in hand, the McDonald lab sought to synthesize the aglycone of the saccharomicins. Starting from either 3-benzyloxy-4-methoxybenzaldehyde **22** or 4-hydroxy-3-(4-methoxybenzyloxy)benzaldehyde **23**, compounds **26** and **27** were synthesized in 98% yield and in 69% yield respectfully using a lithium-free Wittig reaction (Scheme 1.8).[41] 4-hydroxy-3-(4-methoxybenzyloxy)benzaldehyde **23** was synthesized in one step from 3,4-dihydroxy-benzaldehyde and 4-methoxybenzyl chloride (PMBCl).[42,43]

Having synthesized both coupling partners, the McDonald lab could attempt to unite them. To this end, the 3,4-tri-O-acetyl L-fucosyl trichloroacetimidate **21** was glycosylated with either **26** or **27** using the Lewis acid boron trifluoride-tetrahydrofuran (BF_3·THF) to afford **29** in 80% yield and **30** in 97% yield respectfully (Scheme 1.9).[41] Moving forward, the acetate protecting groups were removed from **29** and **30** using potassium carbonate (K_2CO_3) in methanol (MeOH) prior to installing the C-3 and C-4 acetonide protection using 2,2-dimethoxypropane ($Me_2C(OMe)_2$) and pyridinium *p*-toluenesulfonate (PPTS) in acetone ($(CH_3)_2CO$) affording **32** in 77% yield and **33** in 74% yield over two steps.[41] Then, a sulfur trioxide pyridine complex (SO_3·Py) was used to synthesize the sulfonate moiety at the C-2 position of the sugar for both **32** and **33** providing **34** in 91% yield and **35** in 96% yield.[41] Unfortunately, all attempts to debenzylate **34** failed, prompting the McDonald lab to continue forward using only **35**. Next, compound **35** was subjected to deesterification using lithium hydroxide (LiOH) in water (H_2O) which afforded **36** in 94% yield.

Next, the amide was installed in a two-step process using *iso*-butyl chloroformate (*i*-BuOC(O)Cl), followed by taurine and triethylamine (Et_3N) which afforded an instable intermediate species. The acetonide and PMBn protecting groups were removed using Dowex 50WX8-100 ion-exchange resin in H_2O provided **37** in 21% yield over three steps (Scheme 1.9).[41]

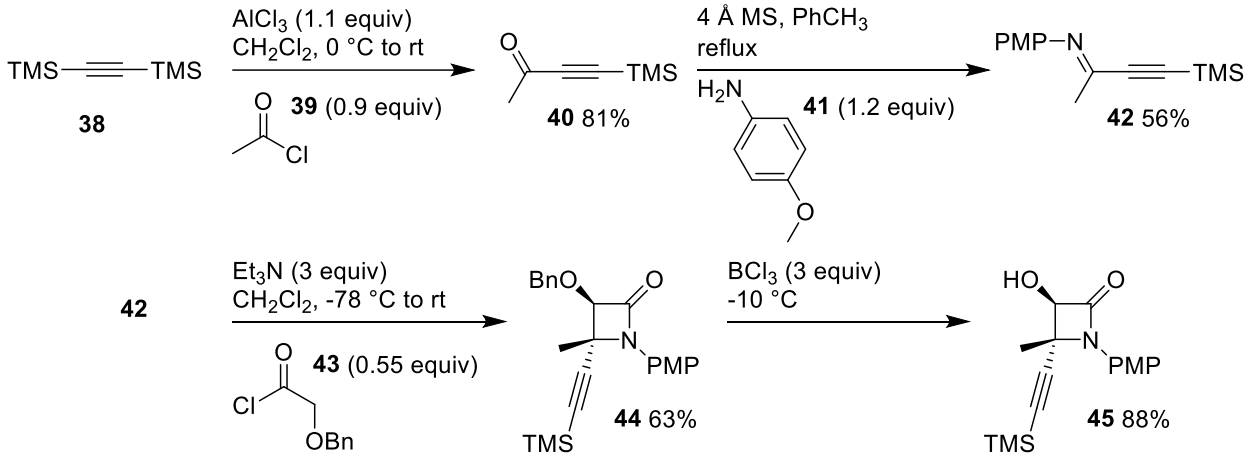


Scheme 1.9 McDonald lab's synthesis of the L-fucose aglycone **37**.[41]

In summary, the L-fucosyl aglycone fragment **37** was synthesized in eight steps using the wrong 'handed" fucose monosaccharide.[41] The key innovation made by the McDonald lab was to activate L-fucosyl trichloroacetimidate donor **21** using BF_3·THF to synthesize β-linked fucose glycoside **30** in 97% yield (Scheme 1.9).[41] The synthesis of **37** provided access to the fully deprotected substrate with a sulfonate moiety at the C-2 position of L-fucose and a sulfonate at the terminal end of the aglycone. Additionally, the amide linkage and alkene of the aglycone were also synthesized.

The McDonald lab again utilized the BF_3·THF glycosylation strategy for their synthesis of the L-fucose-L-saccharosamine disaccharide glycal **51** of the trisaccharide derivative **17** of

saccharomicin B.[40] Initially, the McDonald lab synthesized racemic β-lactam **45** starting from bis-trimethylsilylacetylene **38** and acetyl chloride **39** which were treated with aluminum trichloride ($AlCl_3$) in dichloromethane (CH_2Cl_2) to afford **40** in 81% yield (Scheme 1.10).[40] Next, **40** was reacted with *p*-anisidine **41** in toluene ($PhCH_3$) to afford oxime **42** in 56% yield.[40] Then, **42** was treated benzyloxyacetyl chloride **43** in CH_2Cl_2 which synthesized **44** in 63%. The addition of boron trichloride (BCl_3) in CH_2Cl_2 removed the benzyl ether protection and finished the synthesis of racemic β-lactam **45** in 88% yield (Scheme 1.10).[40]



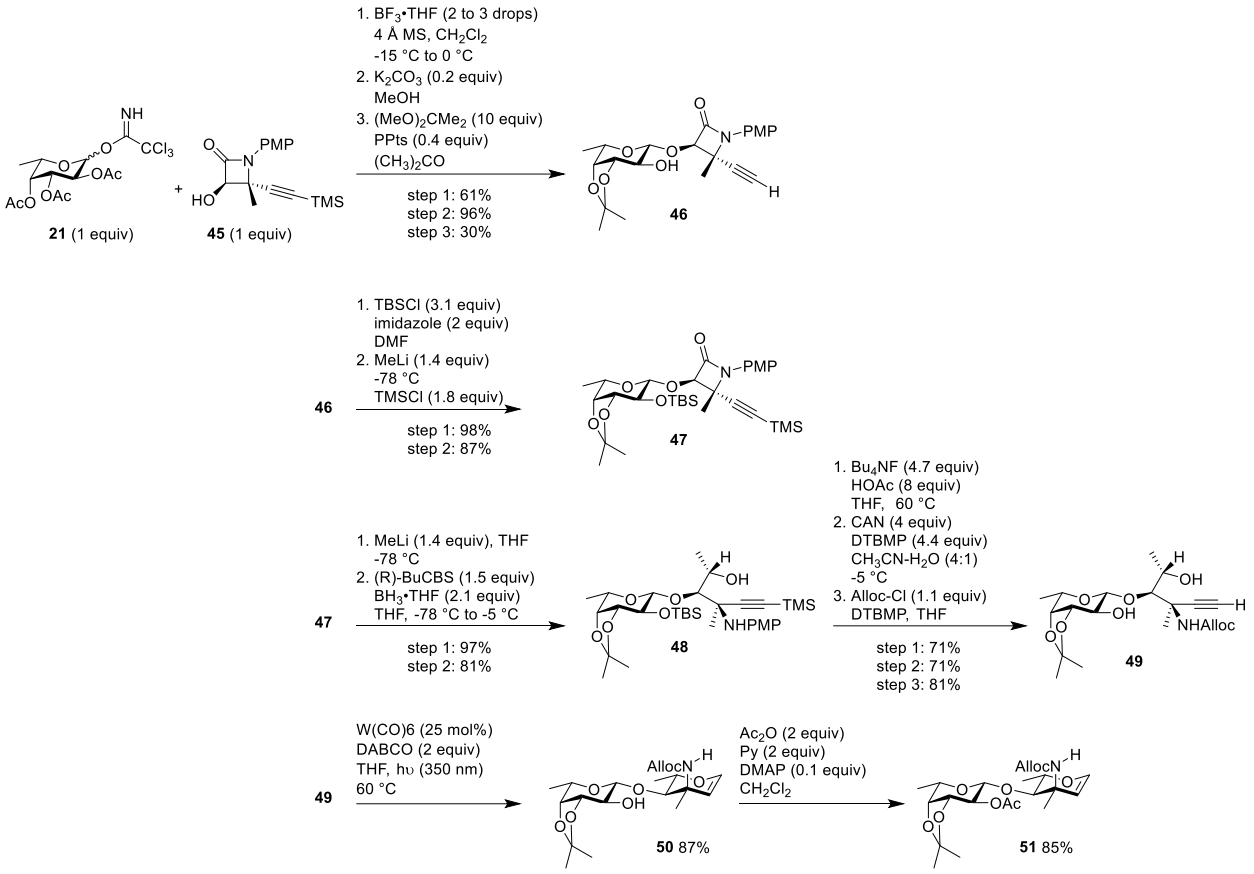
Scheme 1.10 McDonald lab's synthesis of racemic β-lactam **45**.[40]

With the newly synthesized acceptor **45** in hand, the McDonald lab turned their attention to the synthesis of β-linked glycoside **46**. To this end, L-fucosyl trichloroacetimidate donor **21** was subjected to glycosylation with acceptor **42** using $BF_3 \cdot THF$, affording an inseparable mixture of β-linked diastereomers (Scheme 1.11).[40] After acetate deprotection and silyl either removal using K_2CO_3 in MeOH followed by C-3 and C-4 acetonide protection using $Me_2C(OMe)_2$ and PPTS in $(CH_3)_2CO$, **46** was synthesized in 18% yield over three steps.[40] Next, *tert*-butyldimethylsilyl chloride (TBSCl) and imidazole in DMF were used to install a silyl ether at the C-2 position of glycoside **46**. Then, trimethylsilyl chloride (TMSCl) and methyl lithium (MeLi) in THF were used to install a TMS group afforded **47** in 85% yield over two steps.[40] Addition of a methyl to the ketone of **47** using MeLi in THF opened the four-membered ring and after subsequent stereoselective

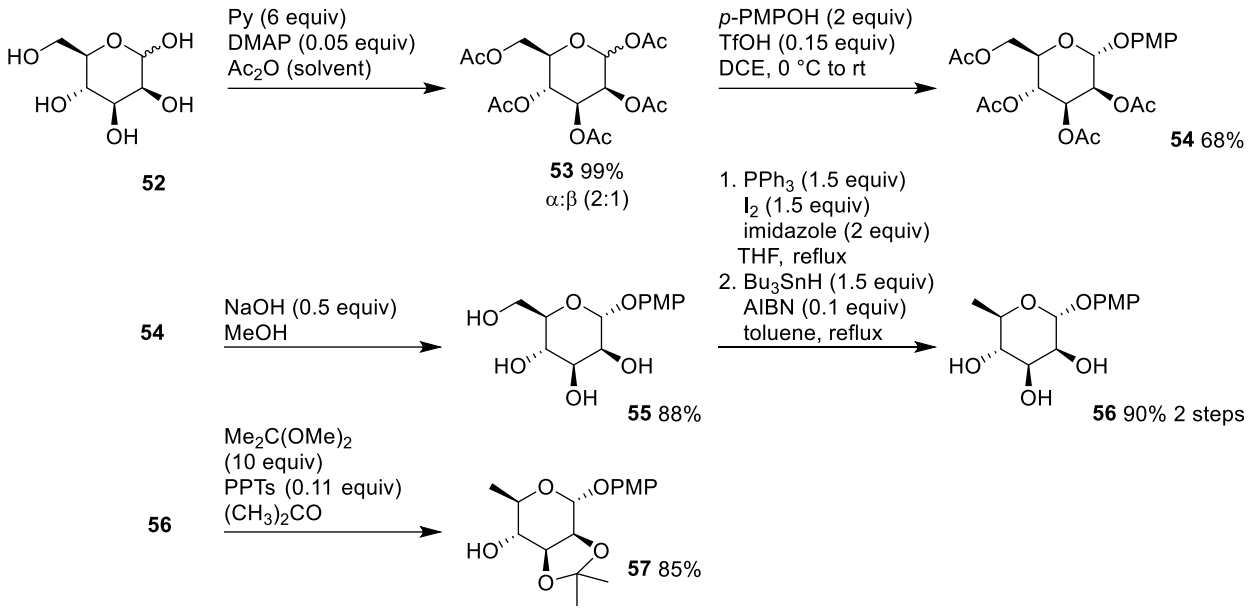
hydride addition, alkynyl alcohol **48** was synthesized in 79% yield over two steps.[40] The Corey–Bakshi–Shibata asymmetric catalyst (R-BuCBS) and BF_3·THF in THF were used to selectively install the hydride nucleophile. The resulting alkyne **48** is the precursor to the saccharosamine glycal which McDonald will use as a glycosyl donor. Alkynyl alcohol **48** was then subjected to tetra-*n*-butylammonium fluoride (TBAF or Bu_4NF) and acetic acid (AcOH) in THF which removed both the TBS and TMS silyl ether protecting groups.[40] Then the *p*-methoxyphenyl (PMP) was oxidatively removed using ceric ammonium nitrate (CAN).[40] Next, allyl chloroformate (Alloc-Cl) and the non-nucleophilic base 2,6-di-*tert*-butyl-4-methylpyridine (DTBMP) were used to synthesize protected amine **49** in 41% yield over three steps.[40] Disaccharide glycal **50** was synthesized from **49** in 87% yield via a tungsten-catalyzed alkynol cycloisomerization using tungsten hexacarbonyl ($W(CO)_6$) and 1,4-diazabicyclo[2.2.2]octane (DABCO) in THF and under ultra violet radiation (hv 350 nm).[40] Subsequent acetylation of the C-2 position of the fucose moiety afforded **51** in 85% yield (Scheme 1.11).

With the fully-protected disaccharide glycal **51** in hand, the McDonald lab could synthesize both the L-fucose-L-saccharosamine-L-rhamnose **58** and the L-fucose-L-saccharosamine-D-digitoxose-derivative glycal **17** trisaccharides.[40] To this end, the McDonald lab began their synthesis of fully protected L-rhamnose acceptor **57** with the acetylation of commercially available D-mannose **52** to afford **53** in 99% yield with (2:1) alpha to beta (α:β) selectivity (Scheme 1.12). Next, PMP was installed to the anomeric C-1 position using 4-methoxyphenol (PMP) and trifluoromethanesulfonic acid (TfOH) in 1,2-dichloroethane (DCE) to afford the α-linked anomer **54** in 68% yield.[40] Deacetylation using sodium methoxide (NaOMe) in MeOH removed all acetate protecting groups affording **55** in 88% yield.[40] Then, iodination of the C-6 position using iodine (I_2), triphenylphosphine (PPh_3), and imidazole in THF followed by subsequent dehalogenation using tributyltin oxide (Bu_3SnH) and azobisisobutyronitrile (AIBN) in toluene provided triol **56** in 90% yield over two steps. The C-3 and C-4 hydroxyls were protected with an acetonide group using

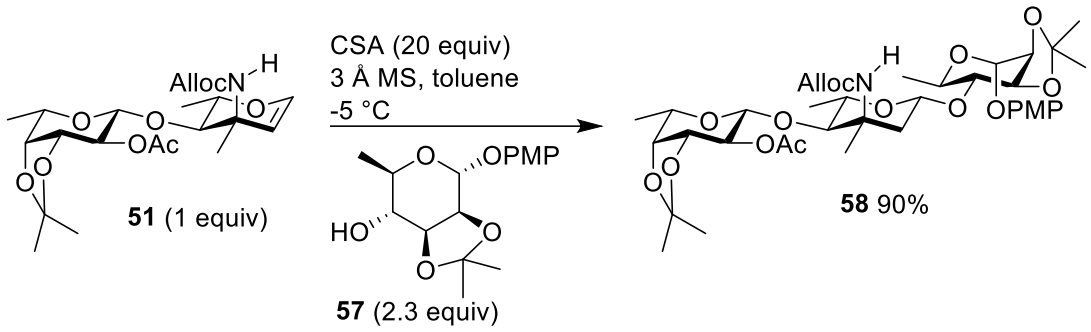
$Me_2C(OMe)_2$ and PPTS in $(CH_3)_2CO$ affording L-rhamnose acceptor **57** in 85% yield (Scheme 1.12). Having synthesized the new acceptor **57**, the McDonald lab focused on the synthesis of the L-fucose-L-saccharosamine-L-rhamnose trisaccharide **58**. Moving forward, disaccharide glycal **51** was glycosylated with acceptor **57** using camphorsulfonic acid (CSA) in toluene to afford trisaccharide **58** in 90% yield as a single β-anomer (Scheme 1.13).



Scheme 1.11 McDonald lab's synthesis of L-fucose-L-saccharosamine glycal **51**.[40]



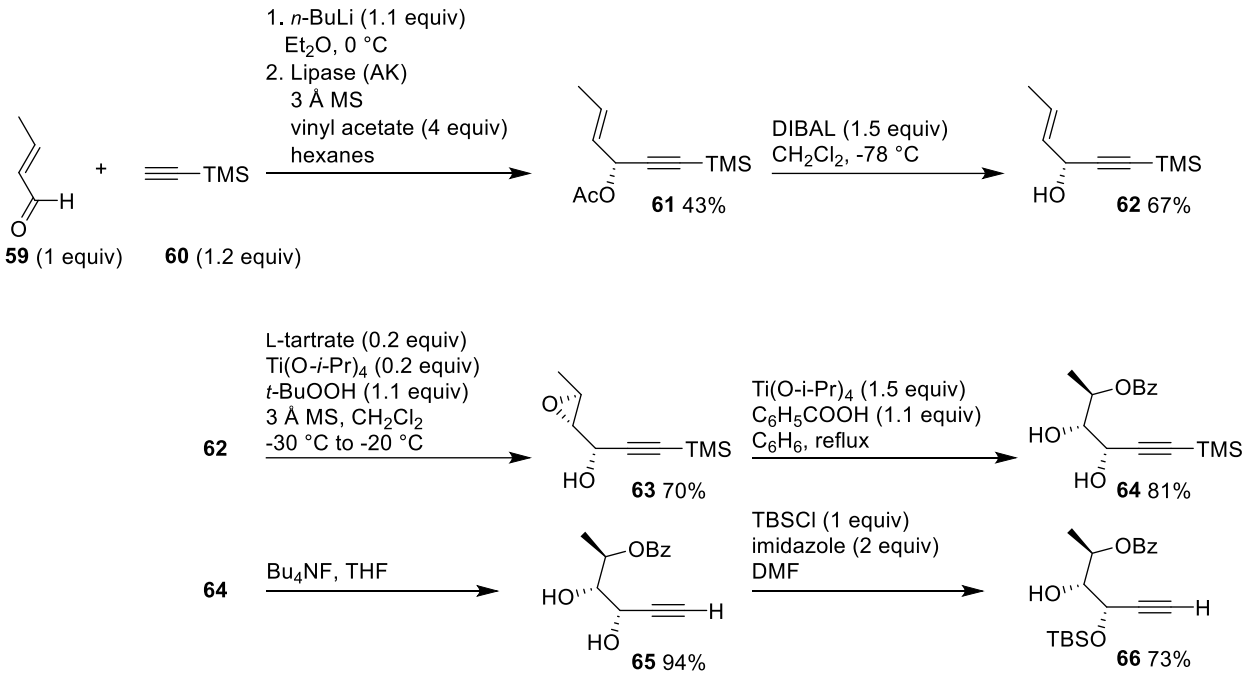
Scheme 1.12 McDonald lab's synthesis of L-rhamnose **57**.[40]



Scheme 1.13 McDonald lab's synthesis of trisaccharide **58.** Bronsted acid-promoted glycosylation using CSA.[40]

Having proven the utility of the Bronsted acid-promoted glycosylation using CSA, the McDonald lab sought to synthesize new acceptor **66** which could be used to synthesize the L-fucose-L-saccharosamine-D-digitoxose glycal trisaccharide **17**. To do so, the McDonald lab synthesized the digitoxose glycal moiety through a cyclization of an alkynyl species, similar to what they used for the synthesis of the saccharosamine glycal moiety in disaccharide **51**. The synthesis of alkynyl acceptor **66** began with crotonaldehyde **59** and trimethylsilylacetylene **60** which were reacted with using *n*-butyllithium (*n*-BuLi) in diethyl ether prior to being subjected to

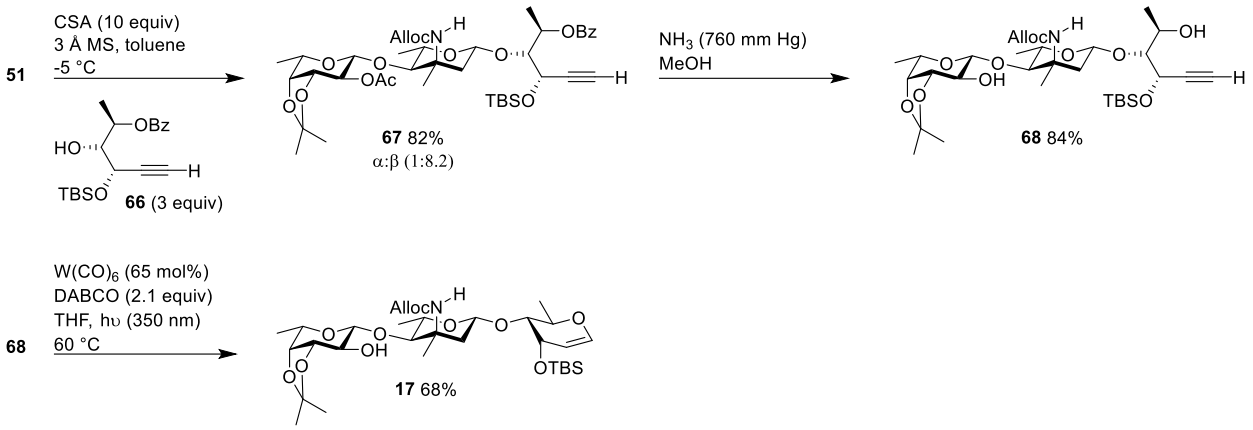
transesterification using Lipase (AK) and vinyl acetate which synthesized **61** in 43% yield over two steps. Diisobutylaluminium hydride (DIBAL) was used to remove the acetate protection which afforded **62** in 67% yield (Scheme 1.14).[40] Next, oxidative epoxidation using *tert*-butyl hydroperoxide (*t*-BuOOH), titanium isopropoxide (Ti(O-*i*-Pr)$_4$, and L-diisopropyl tartrate in CH_2Cl_2 followed by subsequent benzyl alcohol addition using Ti(O-*i*-Pr)$_4$, benzoic acid (C_6H_5COOH) in benzene (C_6H_6) synthesized **64** in 57% yield over two steps. Then, the silyl group was removed using Bu_4NF in THF to afford **65** in 94% yield. Finally, TBS silyl ether protection using TBSCl and imidazole in DMF afforded alkynyl acceptor **66** in 73% yield (Scheme 1.14).[40]



Scheme 1.14 McDonald lab's synthesis of alkynyl acceptor **66**.[40]

With the newly synthesized acceptor **66** in hand, the McDonald lab could test the Bronsted acid-promoted glycosylation using CSA for the synthesis of the L-fucose-L-saccharosamine-D-digitoxose glycal trisaccharide **17**. To this end, disaccharide glycal **51** was glycosylated with acceptor **66** using CSA in toluene to afford glycoside **67** in 82% yield with (1:8.2) α:β selectivity (Scheme 1.15).[40] The acetate protection was then removed using ammonia (NH_3) gas at atmospheric pressure (760 mm Hg) in MeOH to afford alcohol **68** in 84% yield. Finally, a tungsten-

catalyzed alkynyl cycloisomerization of the alkyne to a glycal using $W(CO)_6$ and DABCO in THF and under ultraviolet radiation (hv 350 nm) provided the L-fucose-L-saccharosamine-D-digitoxose glycal trisaccharide **17** in 68% yield (Scheme 1.15).

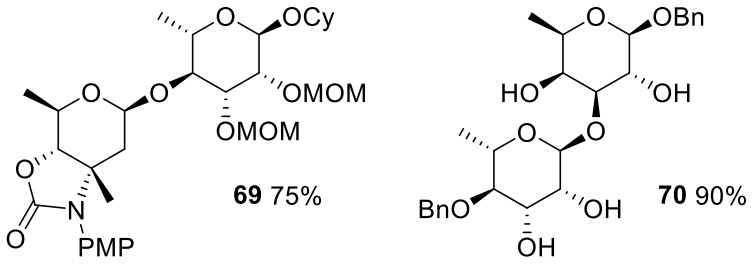


Scheme 1.15 McDonald lab's synthesis of L-fucose-L-saccharosamine-D-digitoxose glycal trisaccharide **17**.[40]

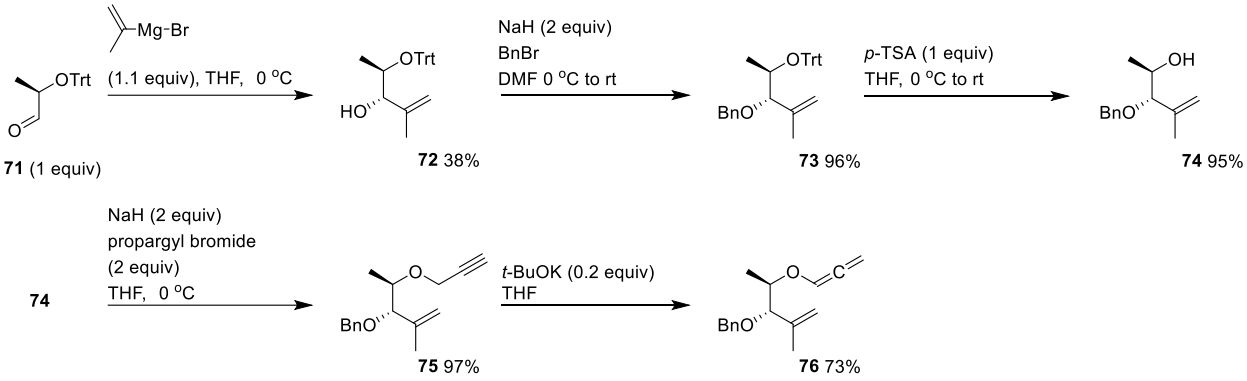
In summary, the McDonald lab synthesized the L-fucose-L-saccharosamine-D-digitoxose glycal trisaccharide **17** in twenty-nine steps. The key steps in this synthesis are the glycosylations using either BF_3·THF or CSA. The McDonald lab was able to use the Lewis acid promoted glycosylation to synthesize a β (1-4) linkage between fucose and a saccharosamine derivative. This method provides a route to access all the β-linked D-fucose-D-saccharosamine moieties within the saccharomcins. The [2+1] Bronsted acid-promoted glycosylation strategy provided access to the β (1-4) linkage between a saccharosamine donor glycal and a digitoxose derivative.[40] The McDonald lab has proven that Bronsted acid-promoted glycosylations have potential to be used to synthesize 2-deoxy sugar glycosides.

Although the glycosylations performed by the McDonald lab to access the trisaccharide fragment of saccharomicin B are both high yielding and highly stereoselective, the need for additional chemical manipulations to synthesize the monosaccharide moieties after these glycosylations makes the McDonald lab's syntheses less attractive to be used to access saccharomcins. The *de-novo* approach to the synthesis of the saccharosamine and digitoxose

glycals is unique, but synthesizing functional sugars from glycals on a larger polysaccharide fragment could pose a problem if this approach was taken to synthesize the saccharomicins.



Scheme 1.16 Fragments of saccharomicins synthesized by the Rhee lab. Disaccharides D-saccharosamine-L-rhamnose **69** and L-rhamnose-D-fucose **70**.[44]

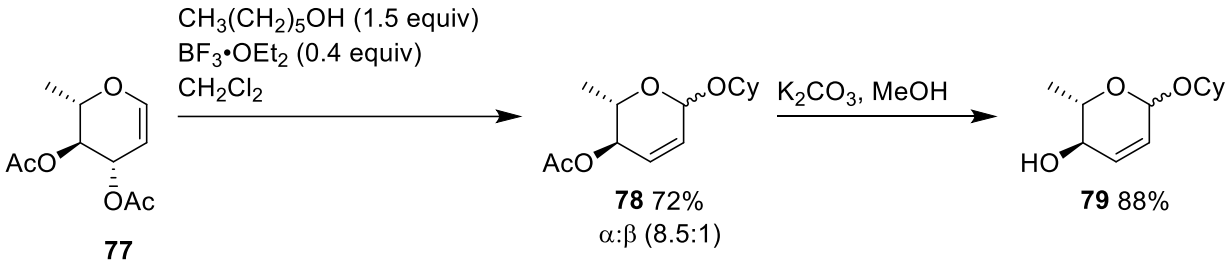


Scheme 1.17 Rhee lab's synthesis of allene **76**.[44–48]

Recently, the Rhee lab has developed their own *de novo* approach to the synthesis of the saccharomicins.[44] The key to Rhee's method is the Pd-catalyzed asymmetric coupling of ene-alkoxyallenes with alcohol species which subsequently undergo ring closing metathesis to synthesize 2,3,6-trideoxy sugar glycosides.[44] Using this approach, the Rhee lab synthesized both the D-saccharosamine-L-rhamnose **69** and L-rhamnose-D-fucose disaccharides **70** (Scheme 1.16).

The synthesis of the D-saccharosamine-L-rhamnose disaccharide **69** began with the synthesis of allene **76**, which is the derivative to the D-saccharosamine moiety. To this end, protection of the hydroxyl of ethyl (S)-lactate using triphenylmethyl (Tr) followed by reduction afforded aldehyde **71**.[44–48] Next, a 1,2-addition of the vinyl magnesium bromide Grignard reagent

afforded alkene **72** in 38% yield (Scheme 1.17).[44,46] The remaining alcohol was benzyl protected using benzyl bromide (BnBr) and sodium hydride (NaH) in DMF and after subsequent acidic removal of the Tr protection using *p*-toluenesulfonic acid (*p*-TSA) in THF, **74** was synthesized in 91% yield over two steps.[44] Then, alkyne **75** was synthesized in 97% yield using propargyl bromide and NaH in THF. Allene **76** was synthesized in 73% yield using potassium *tert*-butoxide (*t*-BuOK) in THF (Scheme 1.17).[44]

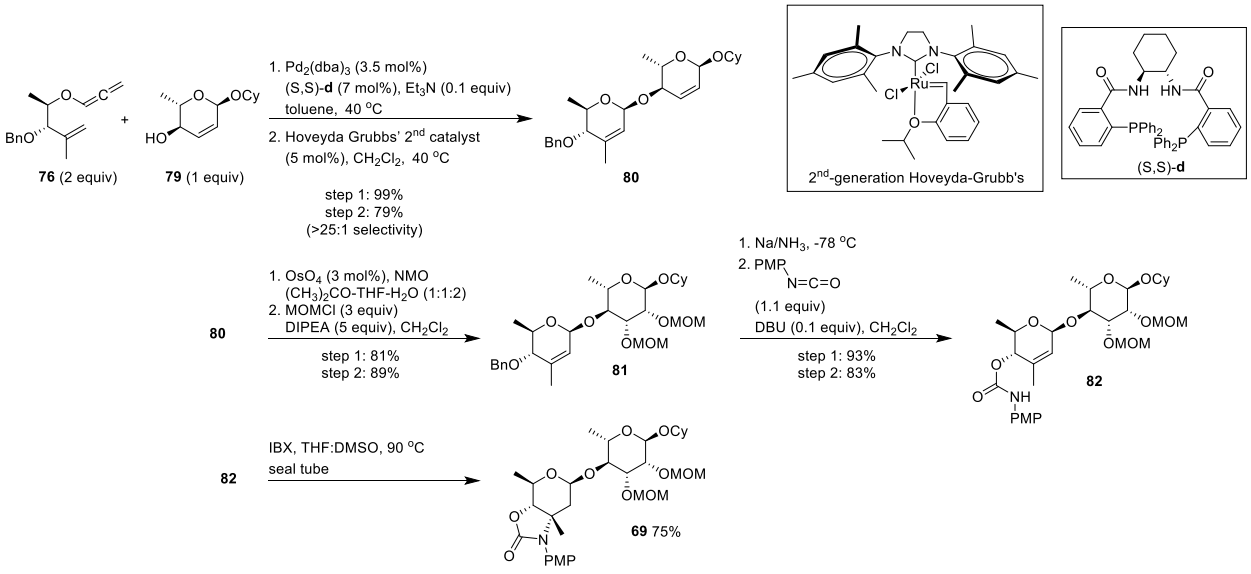


Scheme 1.18 Rhee lab's synthesis of L-rhamnose derivative **79**.[44,46]

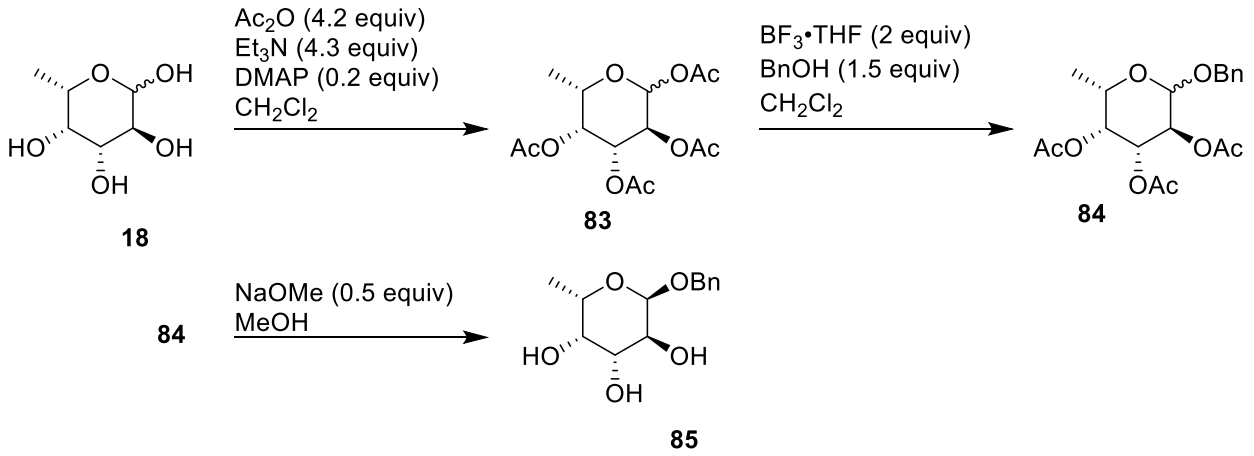
With allene **76** in hand, the Rhee lab sought to synthesize the L-rhamnose derivative **79**. With this objective in mind, commercially available 3,4-di-O-acetyl-L-rhamnal **77** was subjected to Ferrier rearrangement using hexanol ($CH_3(CH_2)_5OH$) and boron trifluoride diethyl etherate ($BF_3{\cdot}Et_2O$) in CH_2Cl_2 to afford **78** in 72% yield with (8.5:1) (α:β) selectivity (Scheme 1.18).[46] Next, the alcohol acceptor **79** was synthesized in 88% yield by removal of the acetate protecting groups using K_2CO_3 in MeOH.[46]

With both the allene **76** and alcohol acceptor **79** in hand, the Rhee lab was ready to couple them using their metal-catalyzed cross-coupling reaction. Coupling of **76** and **79** using the $Pd_2(dba)_3$ metal (3.5 mol %) and the (S,S)-**d** ligand (7 mol %) with Et_3N in toluene provided the desired compound in quantitative yield as a single diastereomer. Ring closing metathesis catalyzed by the second-generation Hoveyda−Grubb's catalyst (5 mol %) provided **80** in 78% yield over two steps (Scheme 1.19).[44] The stereoselectivity for the coupling step favors the equatorial-linked species more than (25:1) over the axial-linked compound. It is worth noting that the authors tested the coupling reaction using the (R,R)-catalyst which synthesized the axial-

linked species with an diastereomeric ratio of (25:1) over the equatorial-linked species. In these coupling reactions, the ligand controls the stereochemical outcome and thus, this stereochemistry can be changed by switching from the (S,S) to the (R,R) ligand. Moving forward, the C-2 and C-3 hydroxyls of L-rhamnose were introduced to the glycoside using a chemo-selective Upjohn dihydroxylation with osmium tetroxide (OsO_4), *N*-methylmorpholine *N*-oxide in an acetone-THF-H_2O (1:1:2) mixture. Then, protection using chloromethyl methyl ether (MOMCl) with *N*,*N*-diisopropylethylamine (DIPEA) in CH_2Cl_2 provided **81** in 72% yield over two steps.[44] Next, the Rhee lab sought to functionalize the saccharosamine moiety. Thus, the C-4 benzyl ether was removed using sodium metal and ammonia gas (NH_3) in THF followed by treatment with *p*-methoxyphenyl isocyanate and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in CH_2Cl_2 to afford carbamate **82** in 77% yield over two steps.[44] The synthesis of the D-saccharosamine-L-rhamnose disaccharide **69** was completed through an oxidative cyclization using 2-iodoxybenzoic acid (IBX) with sodium bicarbonate ($NaHCO_3$) in a mixture of dimethyl sulfoxide (DMSO) and THF (10:1) to afford disaccharide **69** in 75% yield (Scheme 1.19).[44]

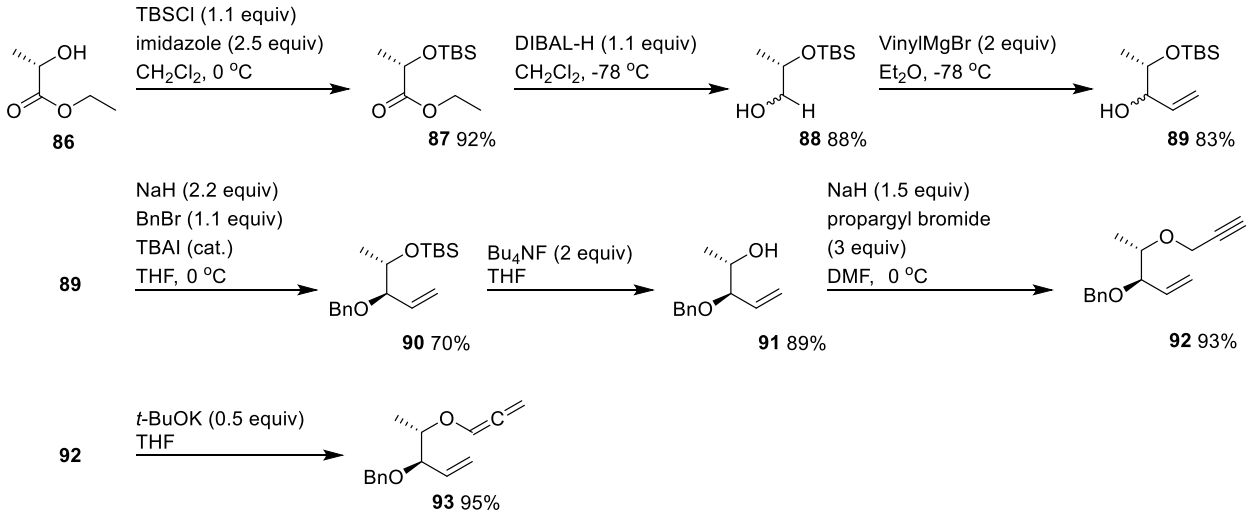


Scheme 1.19 Rhee lab's synthesis of the D-saccharosamine-L-rhamnose disaccharide **69**.[44]



Scheme 1.20 Rhee lab's synthesis of L-rhamnose acceptor **85**.[44,49]

With the D-saccharosamine-L-rhamnose disaccharide **69** in hand, the Rhee lab turned their attention to the synthesis of the L-rhamnose-D-fucose disaccharide **70**. The synthesis of disaccharide **70** began with the synthesis of L-rhamnose acceptor **85** which started from commercially available L-rhamnose **18**. One approach to the synthesis of acceptor **85** is to acetylate L-rhamnose **18** prior to glycosylation with benzyl alcohol (BnOH) and $BF_3 \cdot THF$ in CH_2Cl_2 (Scheme 1.20).[49] Next, deacetylation with sodium methoxide (NaOMe) in MeOH can provide acceptor **85**.



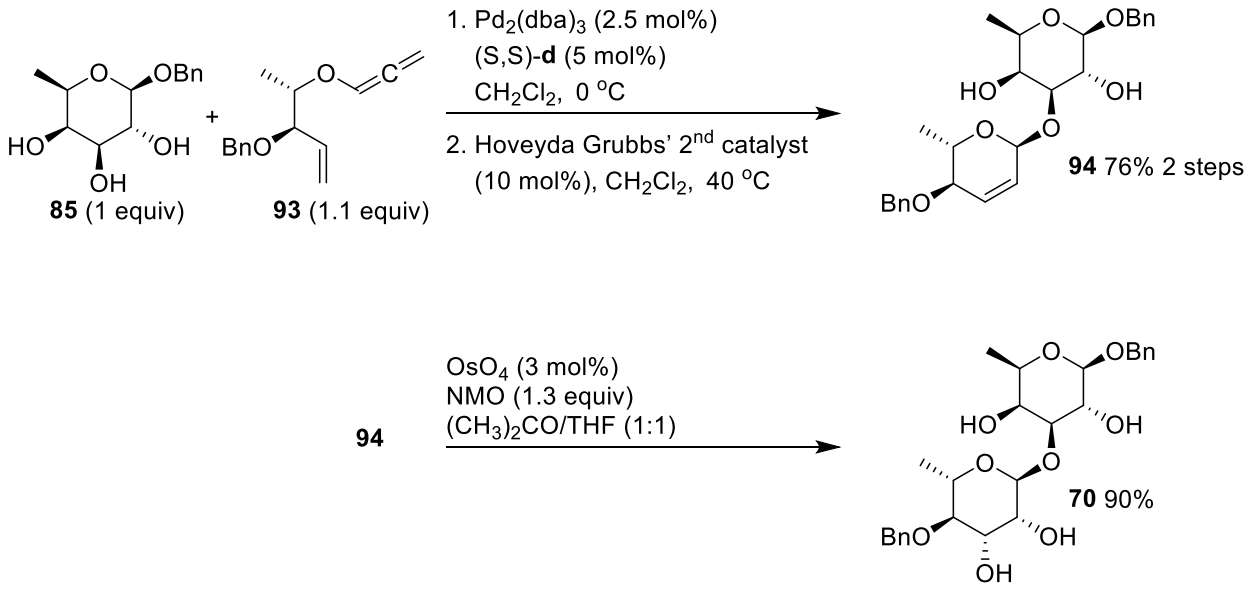
Scheme 1.21 Rhee lab's synthesis of allene **93**.[44,46,50]

The D-fucose moiety of disaccharide **70** was synthesized from allene **93** which can be traced back to ethyl (R) lactate. In the forward direction, the hydroxyl of ethyl (R) lactate **86** was TBS protected providing **87** in 92% yield (Scheme 1.21).[50] Reduction of the ester to an aldehyde using DIBAL-H in CH_2Cl_2 afforded **88** in 88% yield.[50] Next, the alkene **89** was synthesized in 83% yield using vinyl magnesium bromide Grignard reagent in diethyl ether (Et_2O).[50] The remaining alcohol was then protected using BnBr and NaH in THF affording **90** in 70% yield prior to the removal of the silyl ether using Bu_4NF in THF to provide **91** in 89% yield.[50] Similar to the synthesis of allene **76**, allene **93** was synthesized in two steps by first installing the alkyne using propargyl bromide and NaH in DMF followed by treatment with *t*-BuOK in THF to afford allene **93** in 88% yield (Scheme 1.21).[46]

Having synthesized fucose derivative allene **93**, the Rhee lab was ready to use their Pd-catalyzed asymmetric coupling to synthesize the L-rhamnose-D-fucose disaccharide **70**. Coupling of allene **93** with acceptor **85** using the $Pd_2(dba)_3$ metal (2.5 mol %) and the (S,S)-**d** ligand (5 mol %) with Et_3N in toluene synthesized the desired compound and after subsequent ring closing metathesis catalyzed by the second-generation Hoveyda−Grubb's catalyst (10 mol %), **94** was synthesized in 76% yield over two steps (Scheme 1.22).[44] Symmetric dihydroxylation using OsO4 and NMO in a mixture of acetone and THF (1:1) afforded disaccharide **70** in 90% yield.[44]

In summary, the innovation made by the Rhee lab towards the synthesis of saccharomicins is their two-step approach to the synthesis of the D-saccharosamine-L-rhamnose **69** and D-fucose-L-rhamnose **70** disaccharides of the saccharomicins. Diasaccharide **69** was synthesized in fourteen steps and disaccharide **70** was synthesized in thirteen steps. After synthesizing the allene and alcohol coupling partners, the Rhee lab used their Pd-catalyzed asymmetric coupling to synthesize the glycosidic bonds and after ring closing metathesis the disaccharides were synthesized. An advantage of this approach is that the allene can be modified both prior to the glycosylation step as well as after the glycosylation to provide alternative glycoside structures.

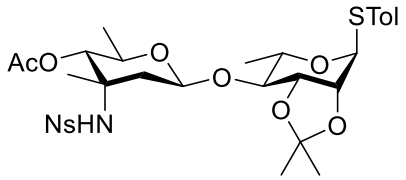
Additionally, the Rhee lab's glycosylation strategy could be used to synthesize the remaining glycosidic linkages within saccharomcins which include deoxy sugars such as L-digitoxose and 4-*epi*-L-vancosamine.



Scheme 1.22 Rhee lab's synthesis of the D-fucose-L-rhamnose disaccharide **70**.[44,46,50]

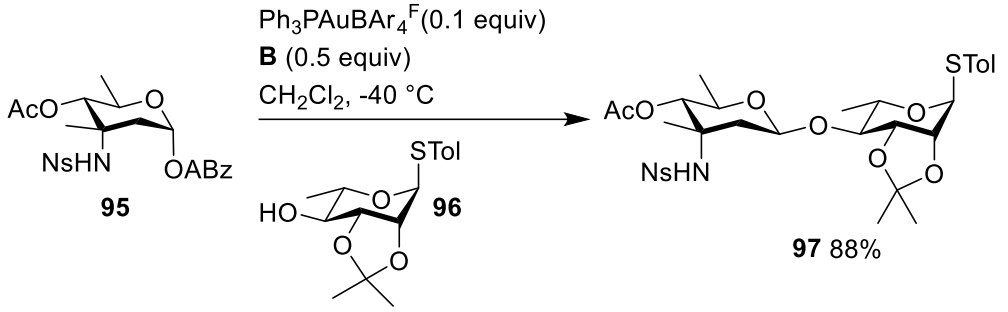
The Rhee lab focused on using a *de novo* approach to the synthesis of the saccharomicins. In contrast to the McDonald lab's synthesis of the glycosidic linkages, the Rhee lab's synthesis avoids harsh glycosylation conditions such as using Lewis acids, and instead relies on a palladium metal catalyst. A disadvantage of the Rhee lab's two-step strategy for the synthesis of each disaccharide **69** and **70** is the need for additional steps after the glycosylation step to functionalize the sugar. Another disadvantage of this synthetic approach is that applying it to the synthesis of larger fragments of saccharomicins would necessitate a linear synthesis which is less efficient than a convergent strategy. In addition to the greater number of steps that the Rhee lab's method requires to functionalize the sugar following the coupling and ring closing metathesis steps, extra synthetic steps would be needed to orthogonally protect the remaining hydroxyls of the D-fucose-L-rhamnose disaccharide **70** for it to be used in subsequent coupling reactions. An alternative approach to the Rhee lab's method could be to synthesize disaccharide

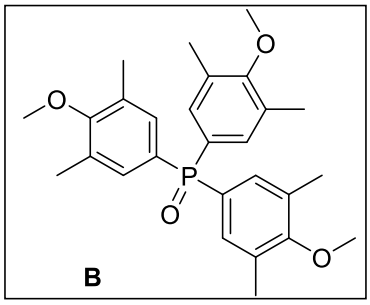
acceptors with only one unmasked hydroxyl so that it can be used directly in a coupling reaction with an allene to afford a larger polysaccharide species without the need for additional protecting group manipulation steps.

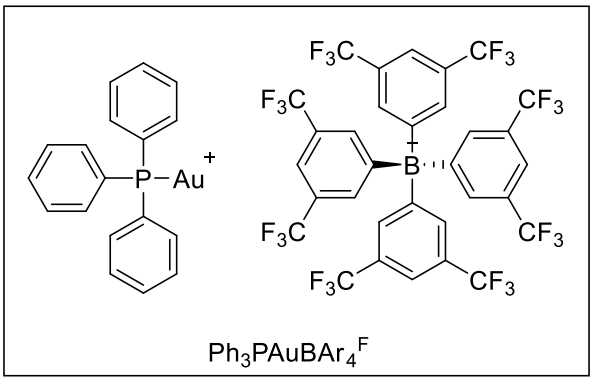


97

Scheme 1.23 D-saccharosamine-L-rhamnose fragment **97** of saccharomicins synthesized by the Wan lab.[51]



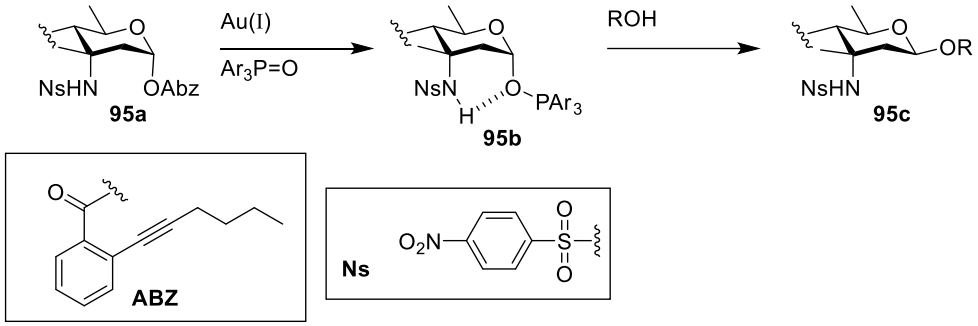




Scheme 1.24 Wan lab's synthesis of the D-saccharosamine-L-rhamnose disaccharide **97**.[51]

Prior to the Rhee lab's progress towards the synthesis of saccharomcins, the Wan lab successfully synthesized the D-saccharosamine-L-rhamnose disaccharide **97** of the saccharomicins in 88% yield (Scheme 1.23, Scheme 1.24).[51] At the time, the Wan lab was developing a methodology which they adapted from Biao Yu's Gold (I) catalyzed activation of alkynes for glycosylation reactions.[51–59] The advancement made by the Wan lab is the added

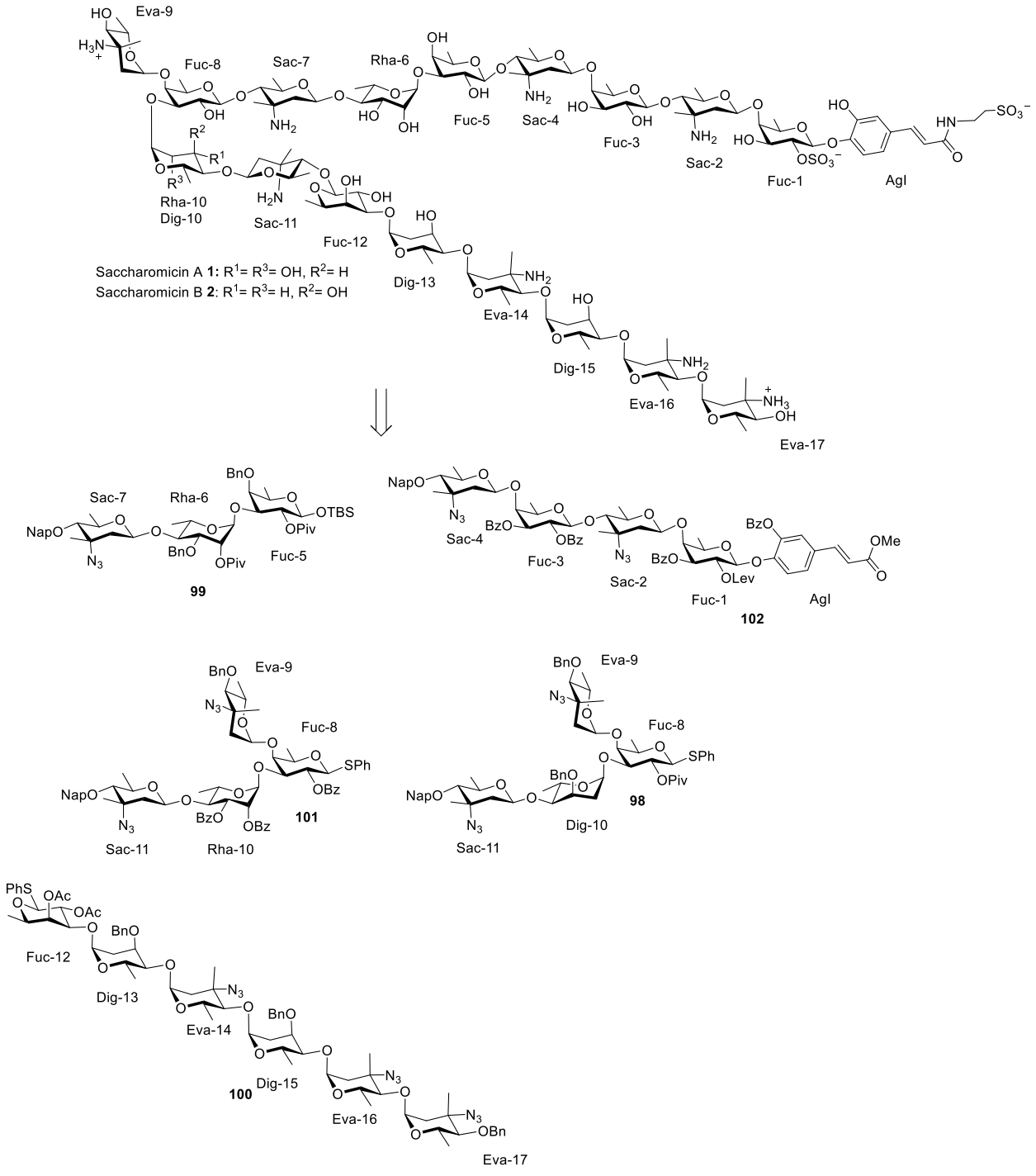
diastereoselective control that is achieved through C-3 neighboring group participation for the selective synthesis of deoxy amino sugar glycosides. The Wan lab's reaction begins with the activation of an *ortho*-alkynyl benzoate saccharosamine donor **95** using tetrakis[3,5-bis(trifluoromethyl)phenyl]borate) triphenylphosphine gold (I) ($Ph_3PAuBAr_4F$) (Scheme 1.25).[51] Then after activation, a triaryl phosphine additive attacks the C-1 carbon and adopts the axial orientation. The Wan lab has proposed that a hydrogen bonding interaction can occur between the C-3 amine and the axial-linked phosphine oxide resulting in an α-oxyphosphonium intermediate saccharosamine species **95b**.[51] After the addition of an alcohol acceptor such as L-rhamnose **96**, the phosphine oxide is displaced in what the Wan lab believes to be an S_N2 reaction. The β-selectivity is a result of the triaryl phosphine blocking the α-face of the donor so that incoming nucleophiles must approach from the β-face.[51]



Scheme 1.25 Proposed mechanism for the Wan lab's glycosylation reaction.[51]

Although the Wan lab only synthesized the D-saccharosamine-L-rhamnose disaccharide **97** of saccharomicins, this method could be applied to the synthesis of the D-saccharosamine-D-fucose and D-saccharosamine-L-digitoxose β-linkages within the saccharomicins. Additionally, the gold (I) catalyzed activation is a much milder method than the Lewis acid activation which the McDonald lab uses. The milder approach could be advantageous when synthesizing larger polysaccharide fragments of saccharomicins as some of the glycosidic linkages might not be stable to harsher conditions.

1.5 Progress Towards the Total Synthesis of Saccharomicins: Bennett Lab

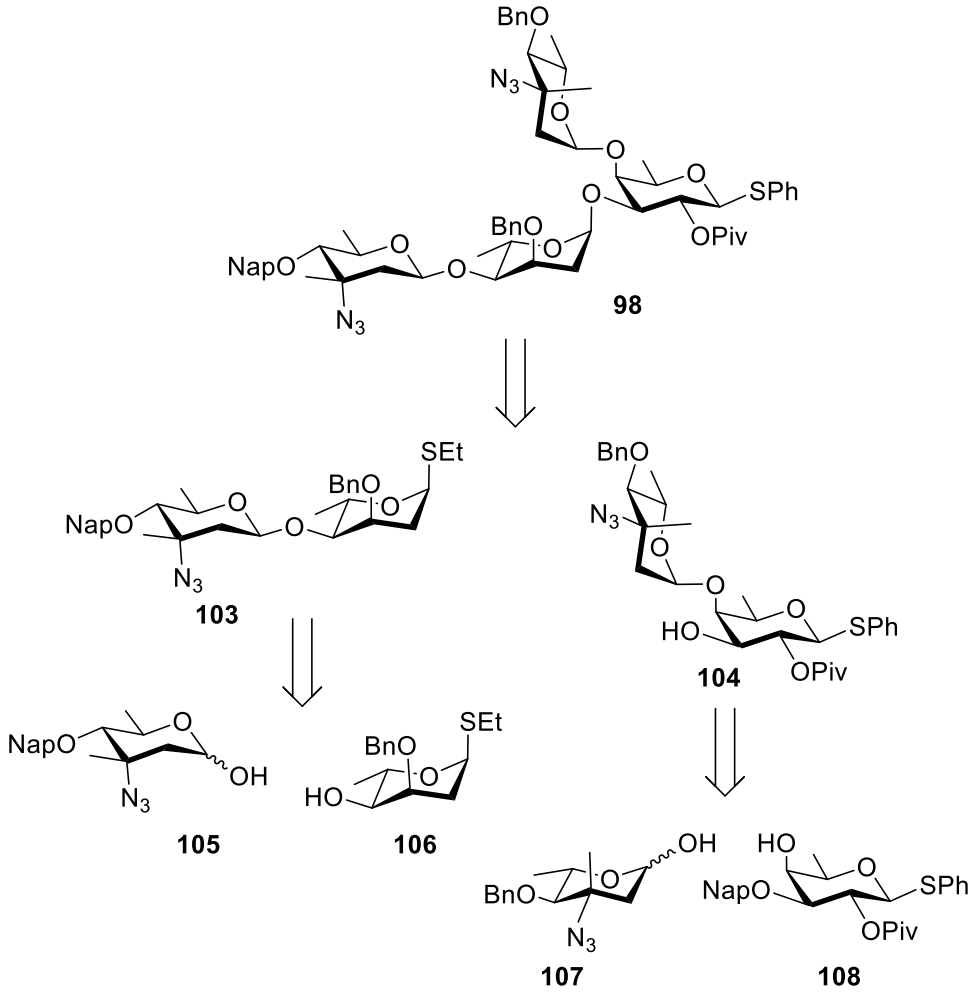


Scheme 1.26 Retrosynthesis of saccharomicins proposed by the Bennett lab.

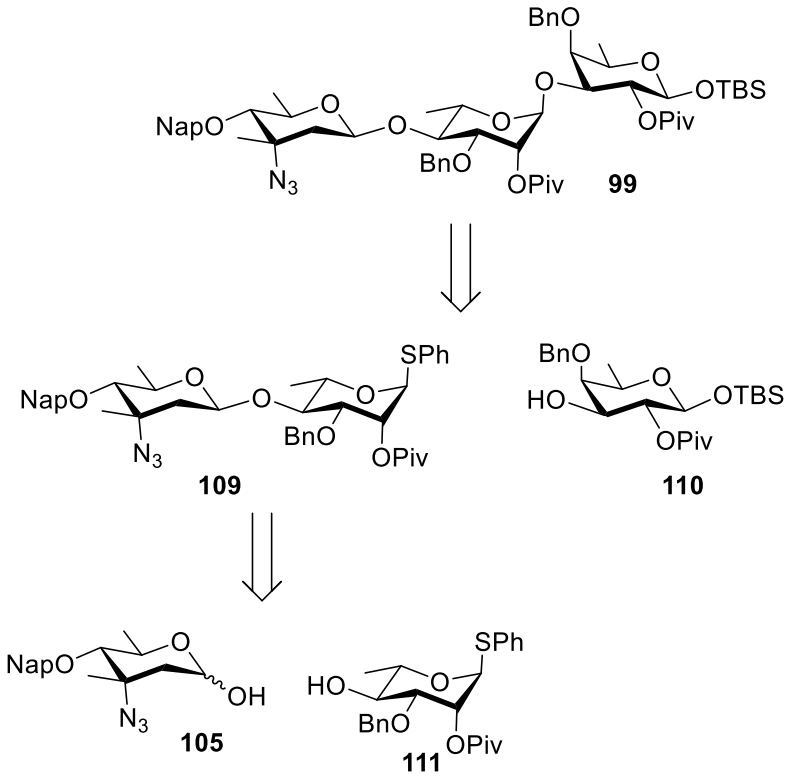
The Bennett lab has made significant progress towards the total synthesis of saccharomicins. Unlike the Rhee lab, the Bennett lab focused on a convergent strategy in which

they planned to synthesize large polysaccharide fragments that could be linked together towards the end of the synthesis. Retrosynthetically, the Bennett lab envisioned that saccharomicins **1** and **2** could be synthesized starting from five large fragments, including **98**, **99**, **100**, **101**, and **102**, which each possess a D-fucose moiety at the terminal end (Scheme 1.26). Glycosylation strategies such as the Lewis acid activation of a D-fucosyl trichloroacetimidate donor as seen by the McDonald lab could be used to link the fragments to the D-saccharosamine moiety of the corresponding acceptor species.[40,41] The branched tetrasaccharide fragment of saccharomicin B **98** could be synthesized from a [2+2] coupling of D-saccharosamine-L-digitoxose donor **103** and a 4-*epi*-L-vancosamine-D-fucose acceptor **104** (Scheme 1.27).[60] Disaccharide **103** could be synthesized from the union of monosaccharides D-saccharosamine **105** and L-digitoxose **106** while disaccharide **104** could be synthesized by joining monosaccharides 4-*epi*-L-vancosamine **107** and D-fucose **108**.[60] The trisaccharide fragment **99** could be synthesized from a [2+1] coupling of D-saccharosamine-L-rhamnose donor **109** and D-fucose acceptor **110** (Scheme 1.28).[61] Disaccharide **109** could then be synthesized through a glycosylation between D-saccharosamine donor **105** and L-rhamnose acceptor **111**.[61] The hexasaccharide fragment **100** could be synthesized from a [2+4] glycosylation of disaccharide donor **112** and tetrasaccharide acceptor **113** (Scheme 1.29).[62] Disaccharide **112** could be synthesized from the coupling of two 4-*epi*-L-vancosamine monosaccharides **116** and **119**.[62] Tetrasaccharide **113** could be synthesized using a [1+1+1+1] linear approach with L-digitoxose **115**, 4-*epi*-L-vancosamine **116**, and D-fucose **118**.[62] Tetrasaccharide **101** of saccharomicin A, could be synthesized through a [2+2] glycosylation using disaccharides donor **120** and acceptor **121** (Scheme 1.30). Disaccharide **120** could be synthesized from monosaccharides D-saccharosamine **122** and L-rhamnose **123** similar to the synthesis of disaccharide **109**. Disaccharide **121** could be synthesized from monosaccharides 4-*epi*-L-vancosamine **107** and D-fucose **124** similar to the synthesis of disaccharide **104**. Lastly, tetrasaccharide **102** could be synthesized from a [1+3] of coupling of D-

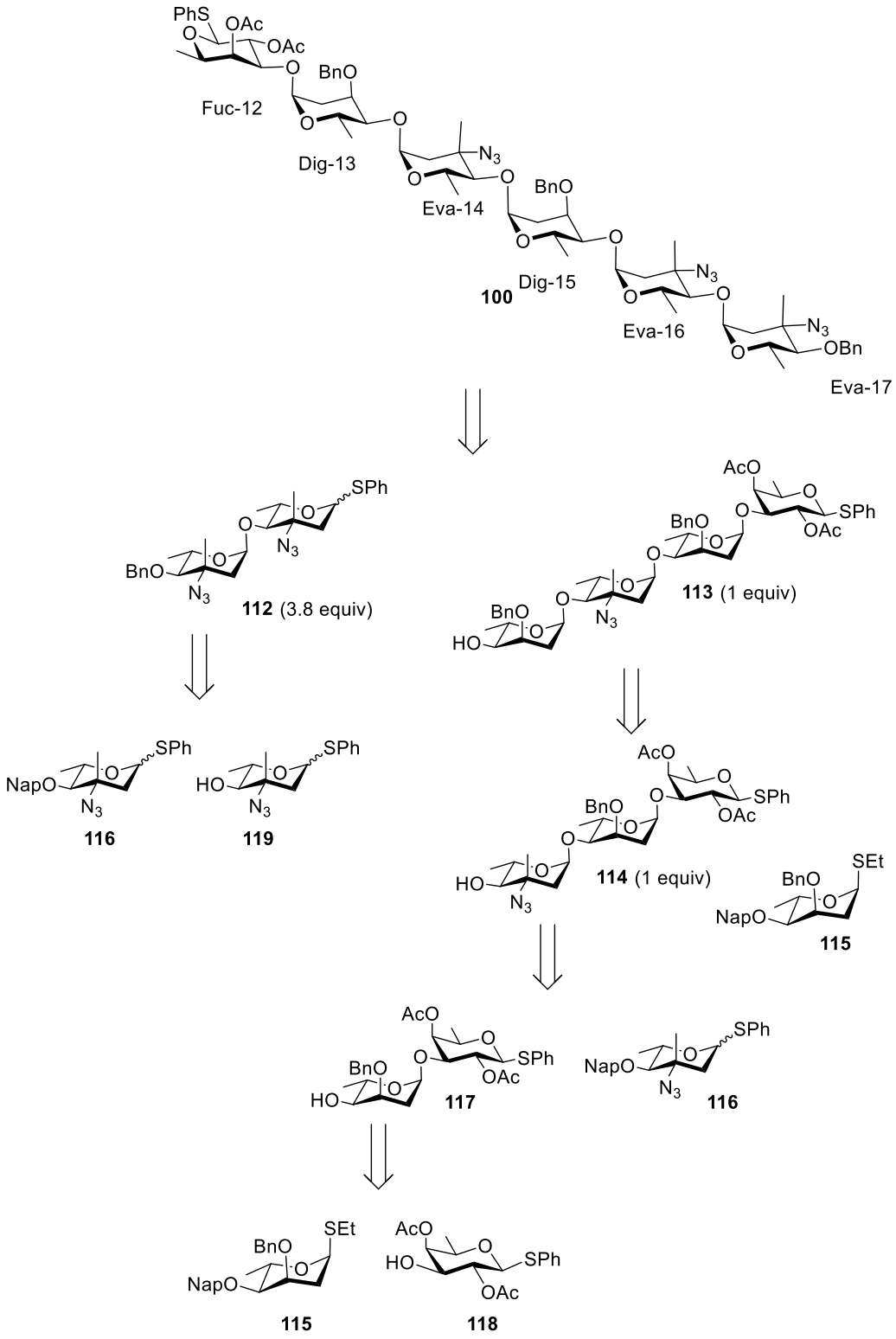
saccharosamine **125** and trisaccharide **126**. Trisaccharide **126**, could be obtained from the [1+2] coupling of D-fucose **127** and disaccharide **128** (Scheme 1.31). Disaccharide **128** can be synthesized from the union of D-saccharosamine **129** and D-fucose **130** monosaccharides. D-fucose monosaccharide **130** can be synthesized from the coupling of D-fucose monosaccharide **131** and alcohol acceptor **132**.



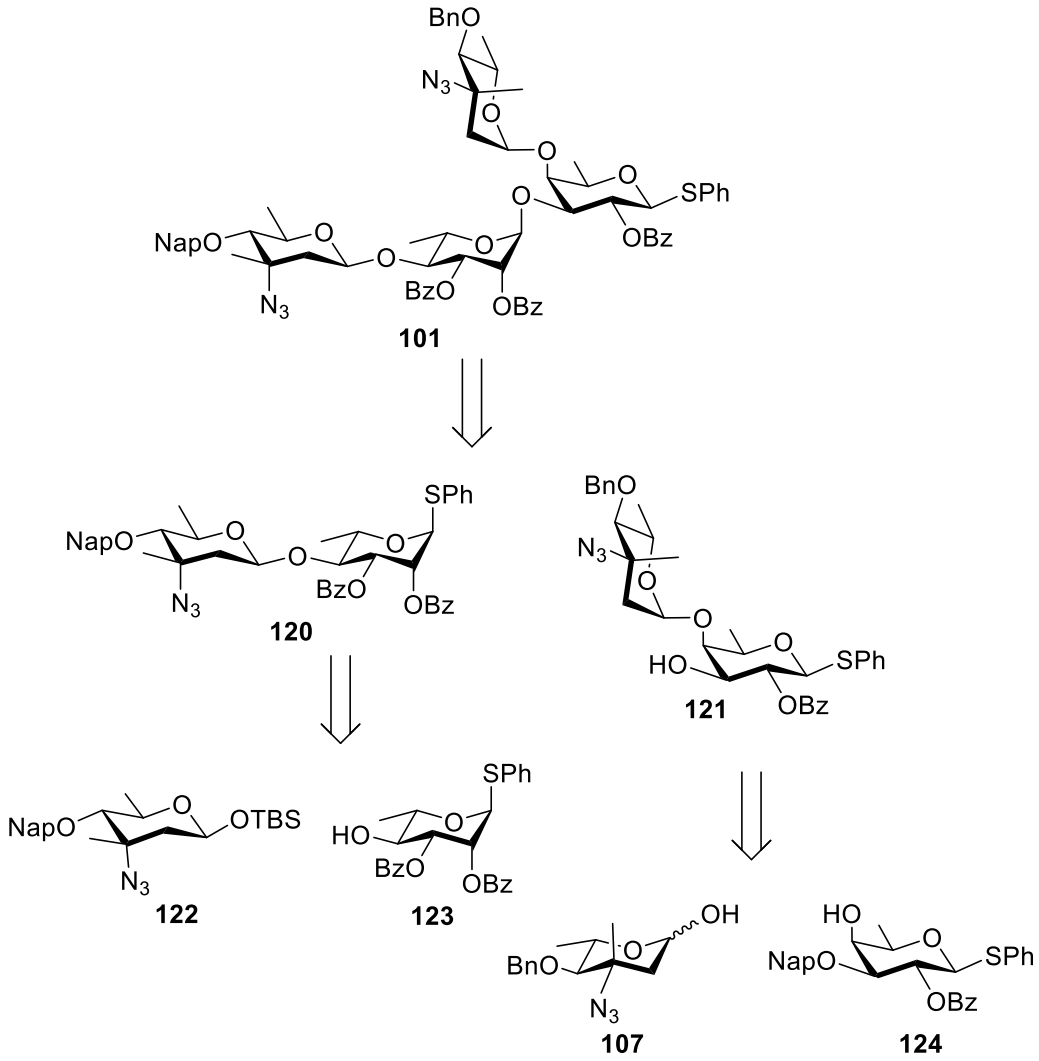
Scheme 1.27 Retrosynthesis of fragment **98** of saccharomicin B proposed by the Bennett lab.



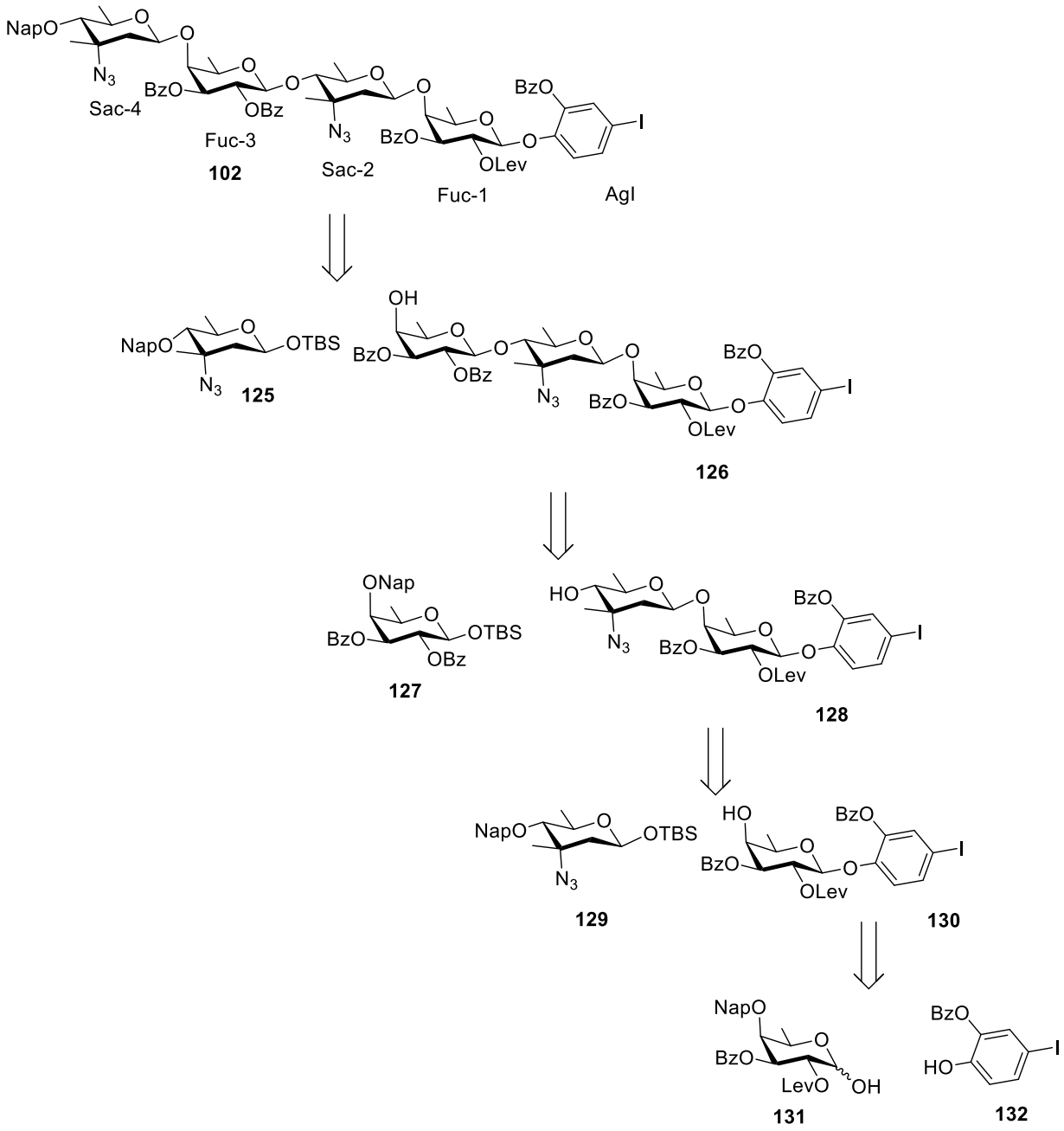
Scheme 1.28 Retrosynthesis of fragment **99** of saccharomicins proposed by the Bennett lab.



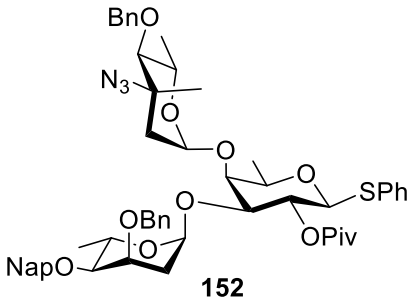
Scheme 1.29 Retrosynthesis of fragment **100** of saccharomicins proposed by the Bennett lab.



Scheme 1.30 Retrosynthesis of fragment **101** of saccharomicin A proposed by the Bennett lab.



Scheme 1.31 Retrosynthesis of fragment **102** of saccharomicins proposed by the Bennett lab.

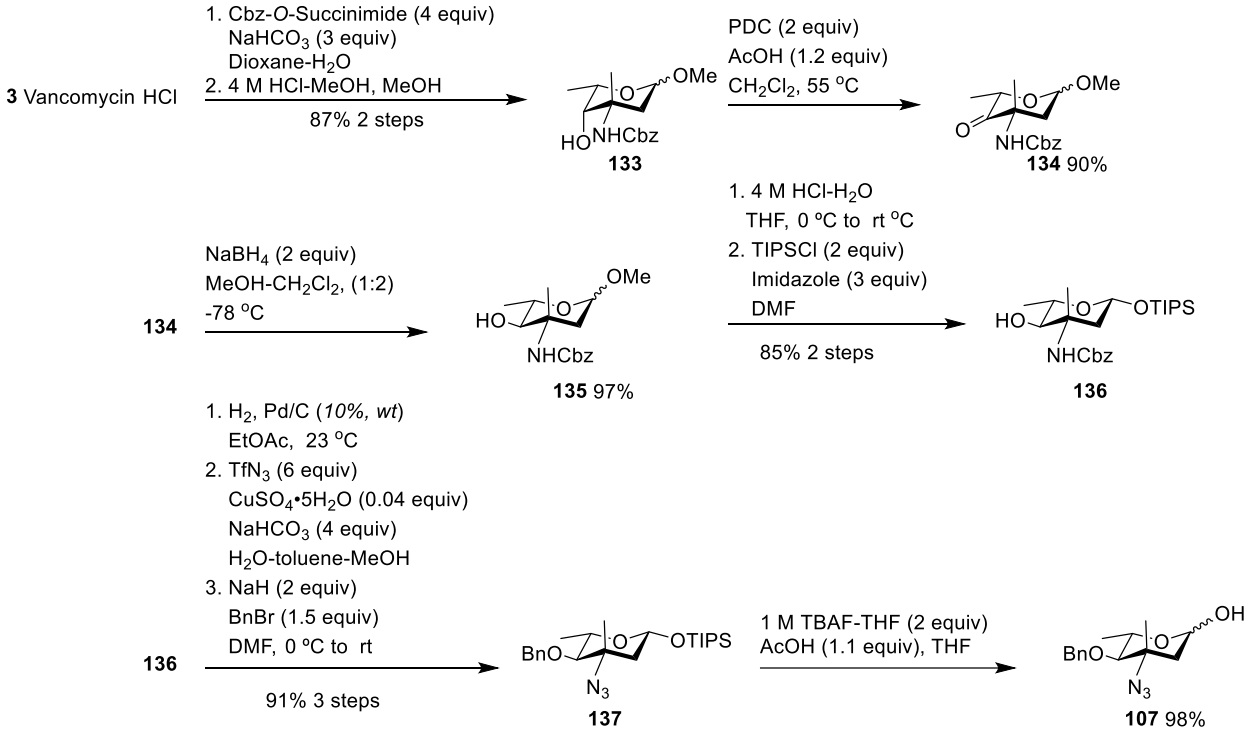


152

Scheme 1.32 Trisaccharide fragment **152** of saccharomicin B. The first fragment of saccharomicins synthesized by the Bennett lab.[60]

The first fragment synthesized by the Bennett lab was the branched trisaccharide fragment **152** of saccharomicin B (Scheme 1.32).[60] Initially, the Bennett lab sought to synthesize the tetrasaccharide fragment **98** which includes a saccharosamine moiety, however at that time, they were only able to synthesize the trisaccharide **152**. The synthesis of **152** began with the synthesis of 4-*epi*-L-vancosamine **107** from vancomycin-HCl **3** while following a modified procedure (Scheme 1.33).[60,63] To this end, the amine of vancomycin-HCl **3** was protected using *N*-(benzyloxycarbonyloxy)succinimide (Cbz-O-succinimide) and $NaHCO_3$ in a mixture of dioxane and H_2O (1:1). Next acid mediated methanolysis using methanolic-hydrochloric acid (HCl-MeOH) removed the vancosamine monosaccharide from the aglycone affording methyl glycoside **133** in 87% yield over two steps.[60,63,64] Then compound **133** was isomerized at the C-4 position by first oxidation using pyridinium dichromate (PDC) with AcOH in CH_2Cl_2.[60] Subsequent hydride addition to the C-4 carbon synthesized the equatorial hydroxyl using sodium borohydride ($NaBH_4$) in MeOH-CH_2Cl_2 (1:2) and afforded the C-4 epimer of vancosamine **135** in 87% yield over two steps.[60,65] The methoxy was then hydrolyzed from the anomeric position using HCl in THF followed by the regioselective synthesis of the silyl ether using triisopropylsilyl chloride (TIPSCl) and imidazole in DMF to provide β-linked **136** in 85% yield over two steps.[60] Next, the carbamate protection was removed via reductive hydrogenolysis using palladium on carbon (Pd/C) and hydrogen gas (H_2). The azide was synthesized by diazo transfer using trifluoromethanesulfonyl

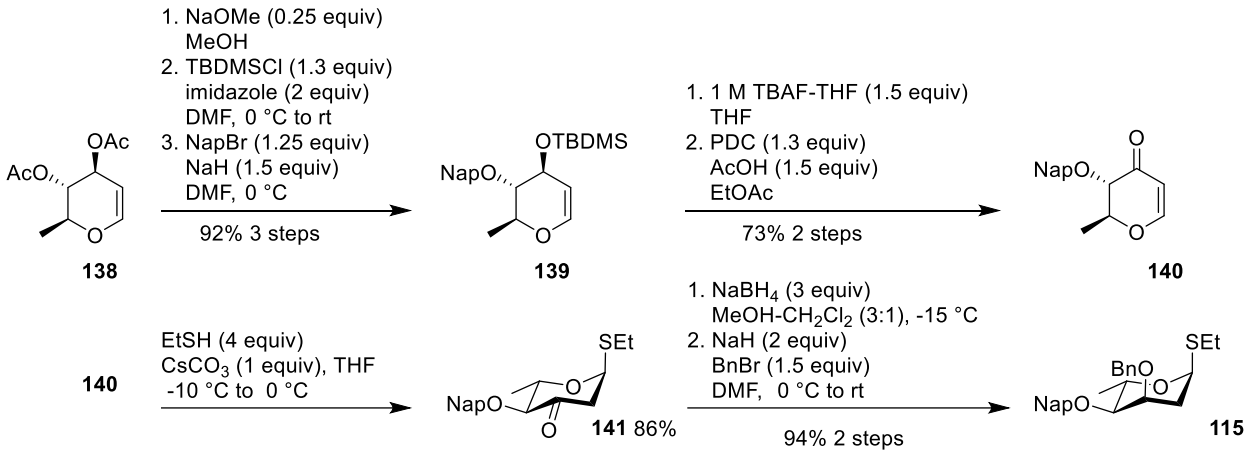
azide (TfN_3), copper (II) sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$), and $NaHCO_3$ in a mixture of H_2O-toluene-MeOH.[60,66,67] Then, the C-4 hydroxyl was benzyl protected using BnBr and NaH in DMF to afford **137** in 91% over three steps.[60] Hemiacetal **107** was synthesized in 98% yield by removing the silyl ether using Bu_4NF in THF (Scheme 1.33).[60]



Scheme 1.33 Bennett lab's synthesis of 4-*epi*-L-vancosamine **107**.[60]

Having synthesized the 4-*epi*-L-vancosamine monosaccharide **107**, the Bennett lab turned their attention to the synthesis of the L-digitoxose species **115** which can be traced back to commercially available 3,4-di-O-acetyl-L-rhamnal. Therefore, 3,4-di-O-acetyl-L-rhamnal **138** was subjected to a three-step transformation sequence starting with deacetylation using sodium methoxide (NaOMe) in methanol.[60] Next selective C-3 silyl ether protection using *tert*-butyldimethylsilyl chloride (TBDMSCl) and imidazole in DMF followed by installation of the C-4 naphthol methyl ether protection using 2-(bromomethyl)naphthalene (NapBr) and NaH in DMF afforded compound **139** in 92% yield over three steps (Scheme 1.34).[60,68] Removal of the C-3 silyl ether protection using Bu_4NF in THF followed by subsequent oxidation using PDC and AcOH in

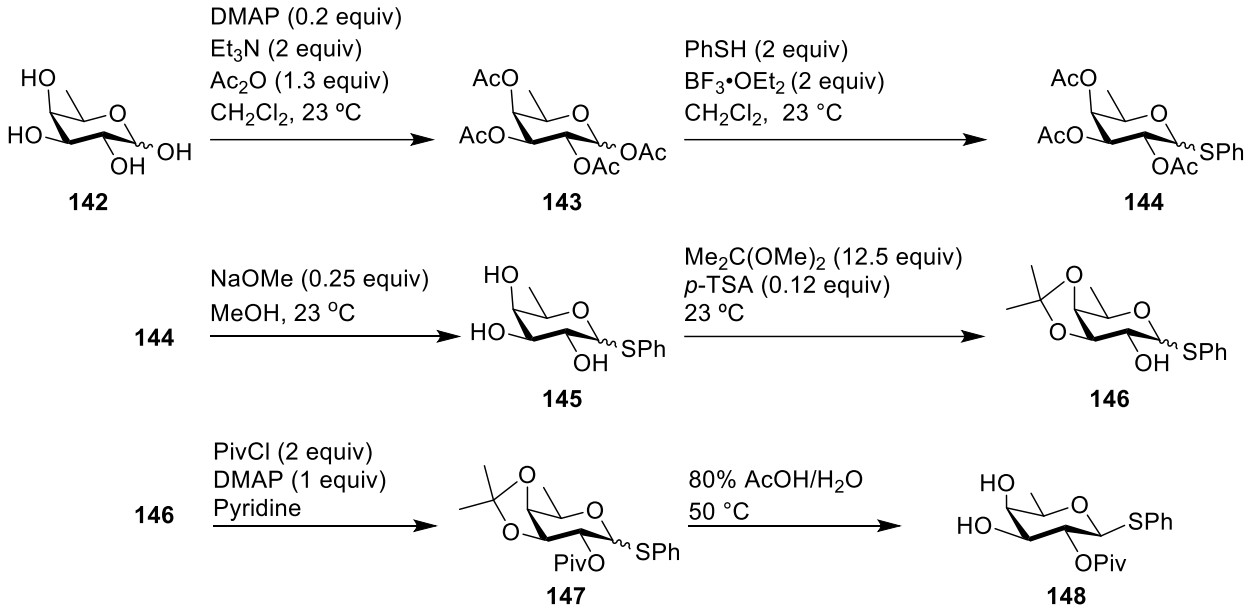
ethyl acetate (EtOAc) provided keto glycal **140** in 73% yield over two steps.[60,76,77] Next, a Michael-type addition reaction of ethanethiol to anomeric position (EtSH) using cesium carbonate ($CsCO_3$) in THF selectively synthesized the α-linked thioglycoside **141** in 86% yield.[60,69] The ketone was then selectively reduced to the axial orientation using $NaBH_4$ in a mixture of MeOH-CH_2Cl_2 (3:1) prior to the installation of the C-3 benzyl ether protection using BnBr and NaH in DMF to afford L-digitoxose **115** in 94% yield over two steps (Scheme 1.34).[60]



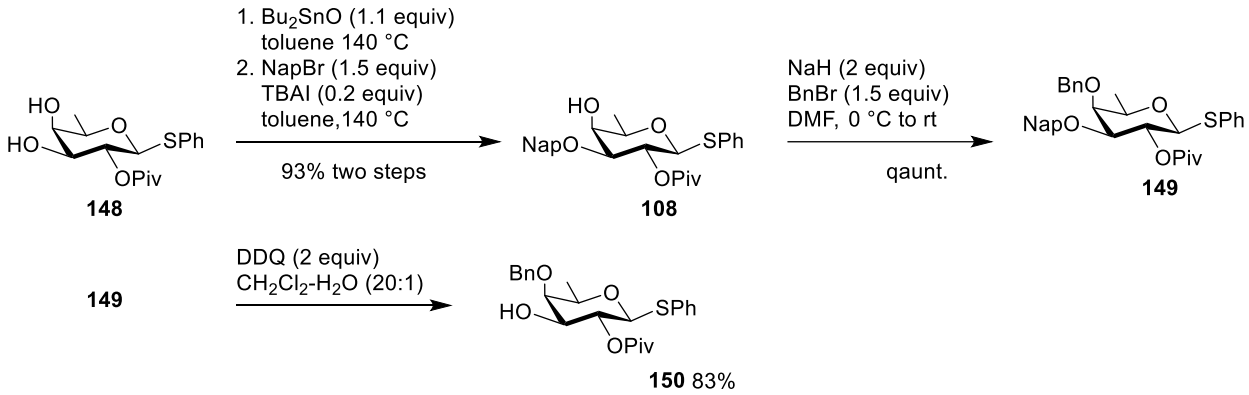
Scheme 1.34 Bennett lab's synthesis of L-digitoxose **115**.[60]

Following the synthesis of both the 4-*epi*-L-vancosamine **107** and L-digitoxose **115** monosaccharide moieties, the Bennett lab focused on the synthesis of D-fucose **150**. The Bennett lab started their synthesis of D-fucose **150** with D-fucose **148** which they obtained in six-steps from D-fucose **142** (Scheme 1.35).[60,70–72] These steps include the acetylation, glycosylation with thiophenol, deacetylation, acetonide installation, esterification using pivaloyl chloride (PivCl), and acetonide removal to provide D-fucose **148**.[60,70–72] Next, selective protection of the C-3 hydroxyl by first dibutylstannylene acetal formation using dibutyltin oxide (Bu_2SnO) in toluene prior to the addition of NapBr and tetra-n-butylammonium iodide (TBAI) synthesized the naphthol methyl either species **108** in 93% yield over two steps (Scheme 1.36).[60] The C-4 hydroxyl was benzyl ether protected using BnBr and NaH in DMF to afford **149** in quantitative yield.[60] Next, the naphthol methyl ether protection at C-3 was oxidatively removed using 2,3-dichloro-5,6-dicyano-

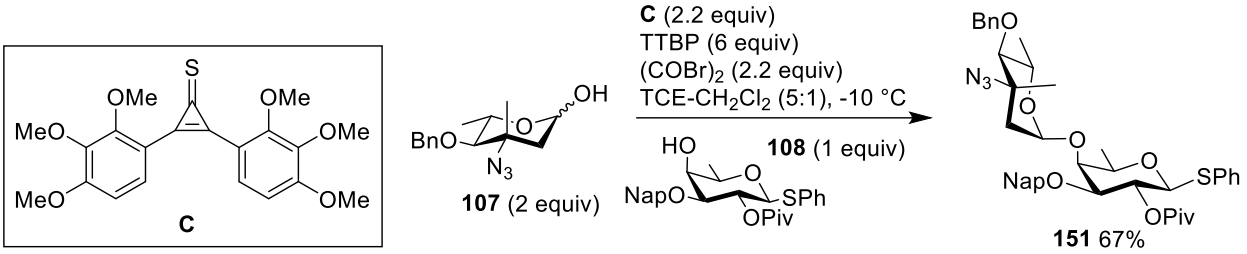
1,4-benzoquinone (DDQ) in a mixture of CH_2Cl_2 and H_2O (20:1) providing the D-fucose C-3 acceptor **150** in 83% yield (Scheme 1.36).[60]



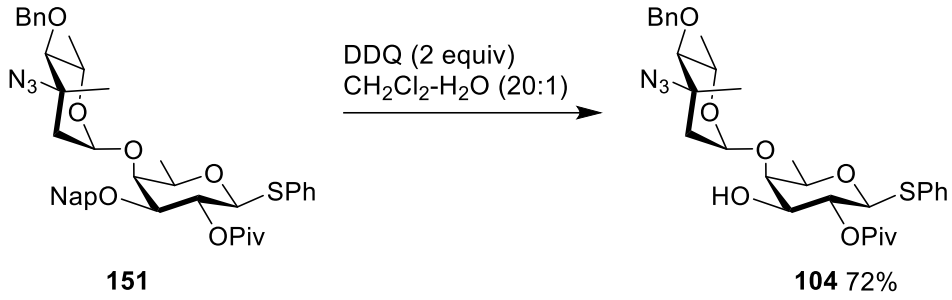
Scheme 1.35 Bennett lab's synthesis of D-fucose **149**.[60,70–72]



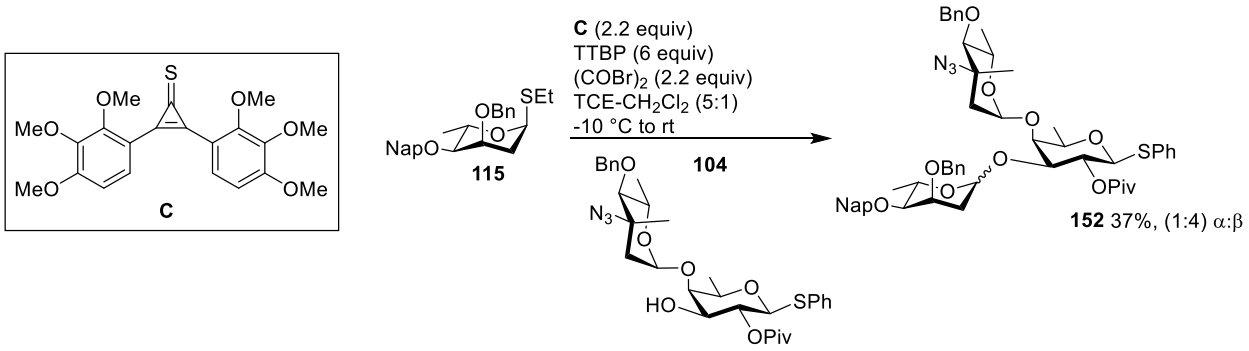
Scheme 1.36 Bennett lab's synthesis of L-digitoxose **150**.[60]



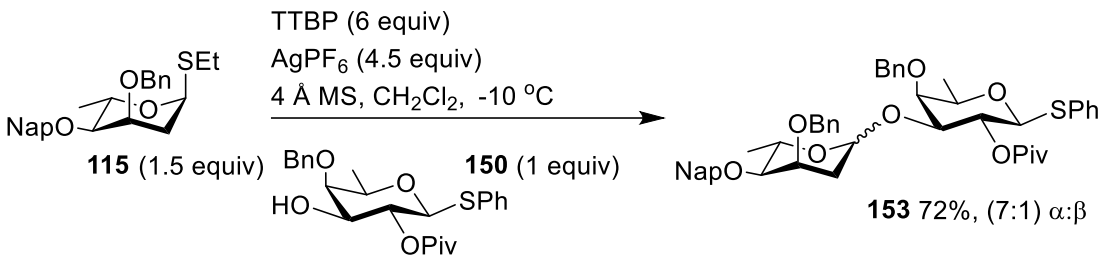
Scheme 1.37 Bennett lab's synthesis of disaccharide **151** using the dehydrative glycosylation strategy.[60]



Scheme 1.38 Bennett lab's synthesis of disaccharide **104**.[60]



Scheme 1.39 Bennett lab's synthesis of trisaccharide **152**. Initial attempt using the dehydrative glycosylation strategy.[60]

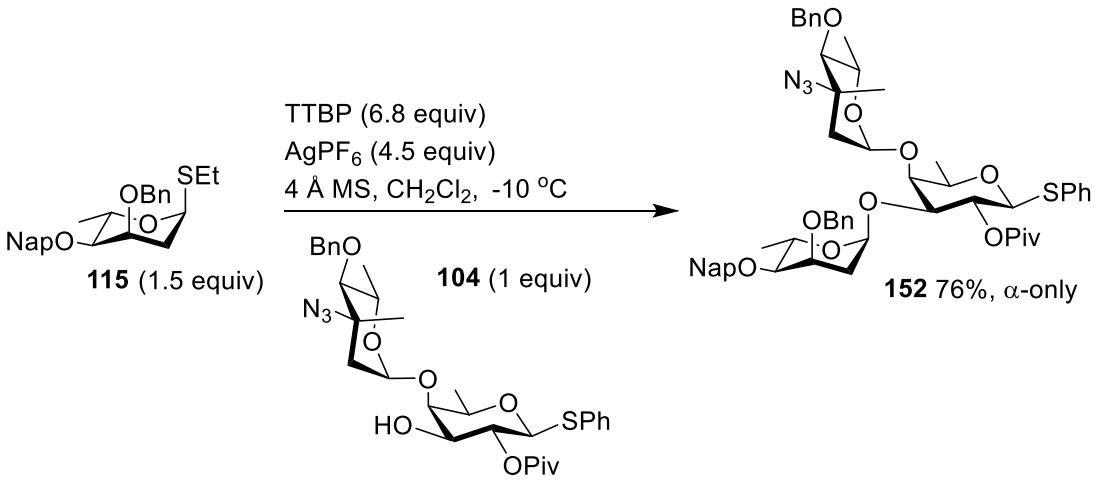


Scheme 1.40 Bennett lab's synthesis of disaccharide **153** using the Hirama lab's glycosylation method.[73,74] Model reaction for the synthesis of the α-linkage between L-digitoxose and D-fucose.[60]

With each of the three monosaccharides needed for the synthesis of trisaccharide fragment **152** in hand, the Bennett lab could attempt to unite them. Previously, the Bennett lab had developed a dehydrative glycosylation strategy for the selective synthesis of α-linked glycosides with deoxy sugar donors.[27,29] They sought to use this chemistry for the synthesis of the α-linkage between 4-*epi*-L-vancosamine **107** and D-fucose **108**. To this end, donor **107** was subjected to reaction with activated 2,3-bis(2,3,4-trimethoxyphenyl)-cyclopropene-1-thione (**C**) and 2,4,6-tri-tert-butylpyrimidine (TTBP) in a mixture of 1,1,2-trichloroethylene (TCE) and CH_2Cl_2 (5:1) prior to the addition of acceptor **108** to selectively synthesize α-linked disaccharide **151** in 67% yield (Scheme 1.37).[60] Reagent **C** was activated by treatment with oxalyl bromide $(COBr)_2$ within a separate vessel before donor **107** was added. Next, the naphthol methyl ether protecting group was oxidatively removed using DDQ in a mixture of CH_2Cl_2-H_2O (20:1) to afford **104** in 72% yield (Scheme 1.38).[60]

Moving forward, the Bennett lab attempted the same dehydrative glycosylation for the [1+2] synthesis of trisaccharide **152** using donor **115** and disaccharide acceptor **104** which provided trisaccharide **152** in 37% yield with (1:4) α:β selectivity (Scheme 1.39).[60] The low yield and β-favored selectivity of the dehydrative glycosylation prompted the Bennett lab to look for alternative approaches to the synthesis of α-linked trisaccharide **152**. The Bennett lab were inspired by the Hirama lab who had used a silver hexafluorophosphate ($AgPF_6$)-mediated

activation of a 2-deoxy sugar thioglycoside donor in the presence of a C-2 functionalized thioglycoside acceptor for the synthesis of α-linked glycosides.[73,74] One possible explanation for why 2-deoxy sugar donors are activated instead of C-2 hydroxyl functionalized sugars is that the C-2 hydroxyl is able to coordinate to the $AgPF_6$ reagent. C-2 hydroxyl coordination could prevent the electrons of the sulfur at the C-1 position from attacking the reagent, thus preventing activation of the anomeric thiol. The Bennett lab decided to test this chemistry for the synthesis of trisaccharide **152**.[73,74] Compounds **115** and **150** were coupled using $AgPF_6$ and either TTBP or 2,6-di-tert-butyl-4-methylpyridine (DTBMP) in CH_2Cl_2 and after optimization, disaccharide **153** was synthesized in 72% yield with (7:1) α:β selectivity (Scheme 1.40).[60] Being satisfied with the α-favored selectivity of the model reaction, donor **115** and acceptor **104** were subjected to reaction with $AgPF_6$ and TTBP in CH_2Cl_2 to selectively afford α-linked trisaccharide **152** in 76% yield (Scheme 1.41).[60]

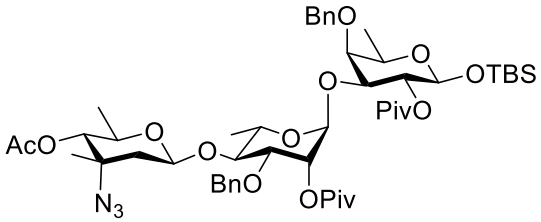


Scheme 1.41 Bennett lab's synthesis of trisaccharide **152**.[60]

In summary, the synthesis of **152** began with commercially available building blocks which were used to synthesize the donor and acceptor monosaccharides. Synthetic routes to 4-*epi*-L-vancosamine **107**, D-fucose **108**, and L-digitoxose **115** were developed. The Bennett lab was able to use their own dehydrative glycosylation strategy for the union of **107** and **108**, but they were

unable to use this chemistry for the synthesis of the trisaccharide **152**.[60] Fortunately, a method described by the Hirama lab provided the desired trisaccharide **152**.[60]

The Bennett lab used a convergent strategy to synthesize **152**. By focusing on monosaccharide synthesis prior to synthesizing disaccharides they were able to synthesize trisaccharide **152** with protected hydroxyls and amine in only three steps from **107**, **108**, and **115**. An advantage of this strategy is that trisaccharide **152** is suitable for synthesis as either a donor or after naphthol methyl ether removal as an acceptor. This limits the number of times that the glycosyl linkages need to be exposed to reagents for the synthesis of the desired glycoside following the glycosylation step.

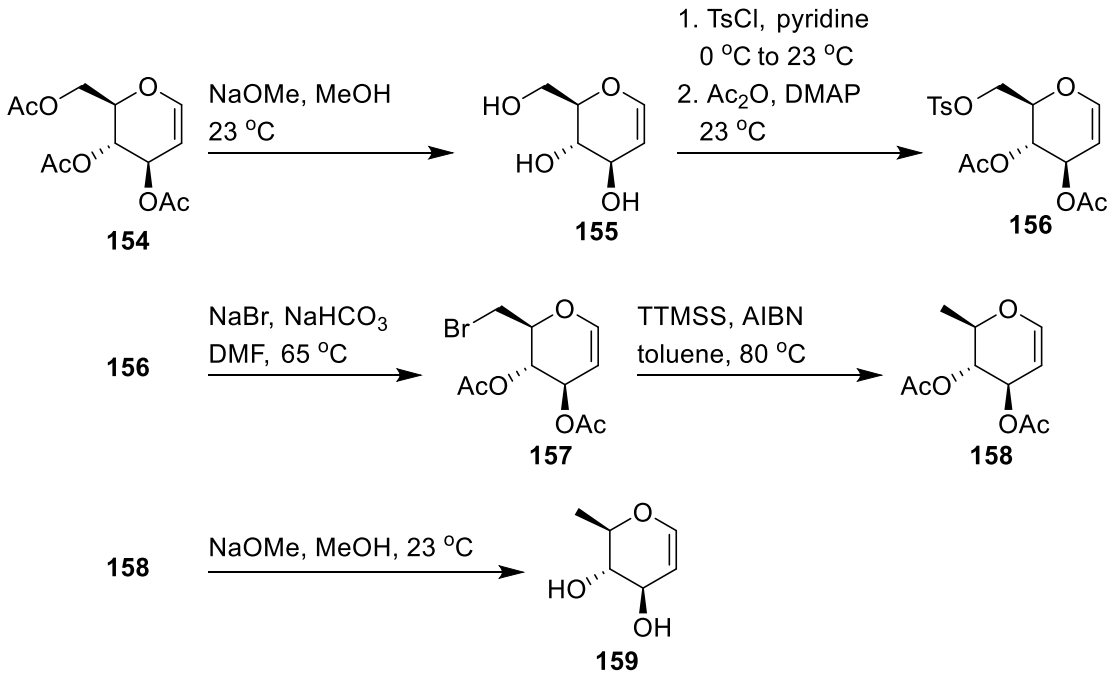


181

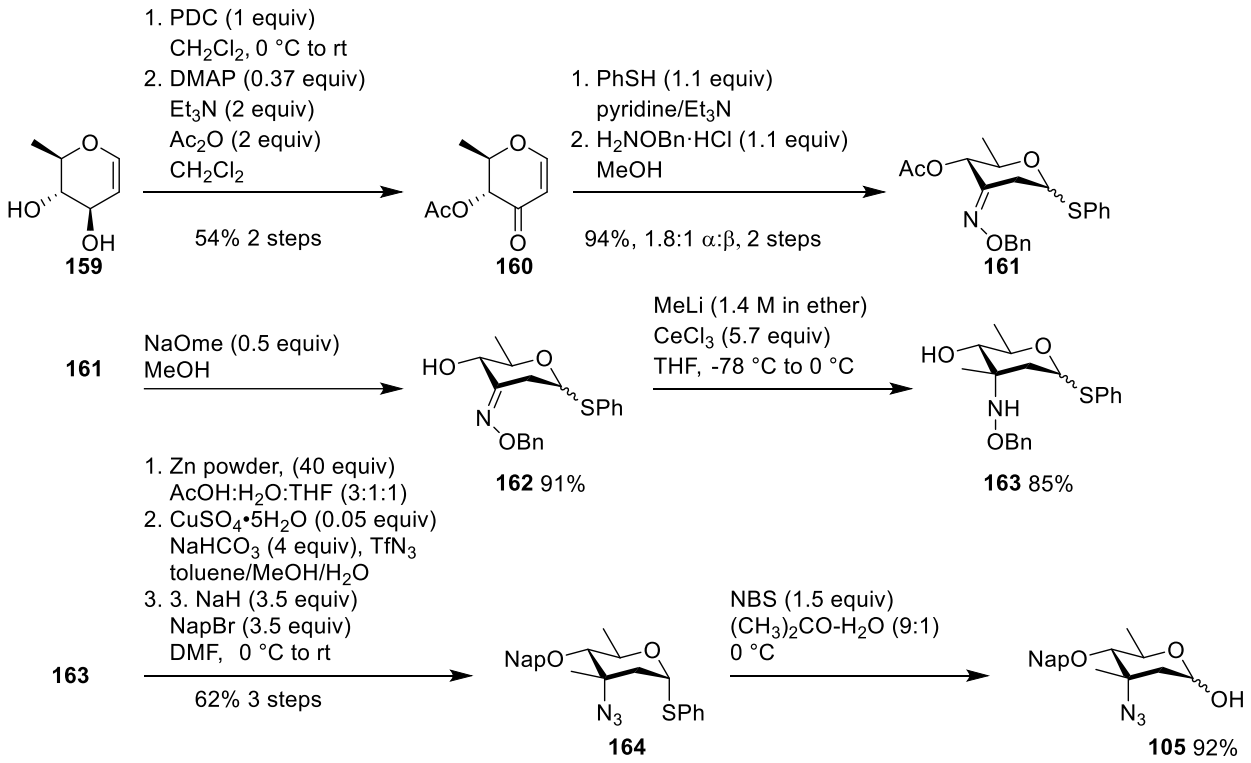
Scheme 1.42 Trisaccharide fragment **181** of saccharomicins. The second fragment of saccharomicins synthesized by the Bennett lab.[61]

The next fragment of saccharomicins that was synthesized by the Bennett lab was the trisaccharide fragment **181** (Scheme 1.42).[61] The synthesis of **181** began with the synthesis of monosaccharide D-saccharosamine **105** from D-rhamnal **159**.[61] D-rhamnal **159** was synthesized from commercially available 3,4,6-tri-O-acetyl-D-glucal **154** in six steps involving deacetylation, C-6 tosylation, acetylation, C-6 halogenation, C-6 dehalogenation, and finally deacetylation (Scheme 1.43).[61,75] Starting from D-rhamnal **159**, the C-3 hydroxyl was selectively oxidized using PDC in CH_2Cl_2 prior to the synthesis of the C-4 acetate protection using Ac_2O, DMAP, and Et_3N in CH_2Cl_2 to afford ketone **160** in 54% yield over two steps (Scheme 1.44).[61,76,77] Next, the C-1 thiol was introduced through a Michael-type addition reaction using thiophenol in pyridine with a few drops of Et_3N followed by the synthesis of the C-3 nitrogen through the conversion of the

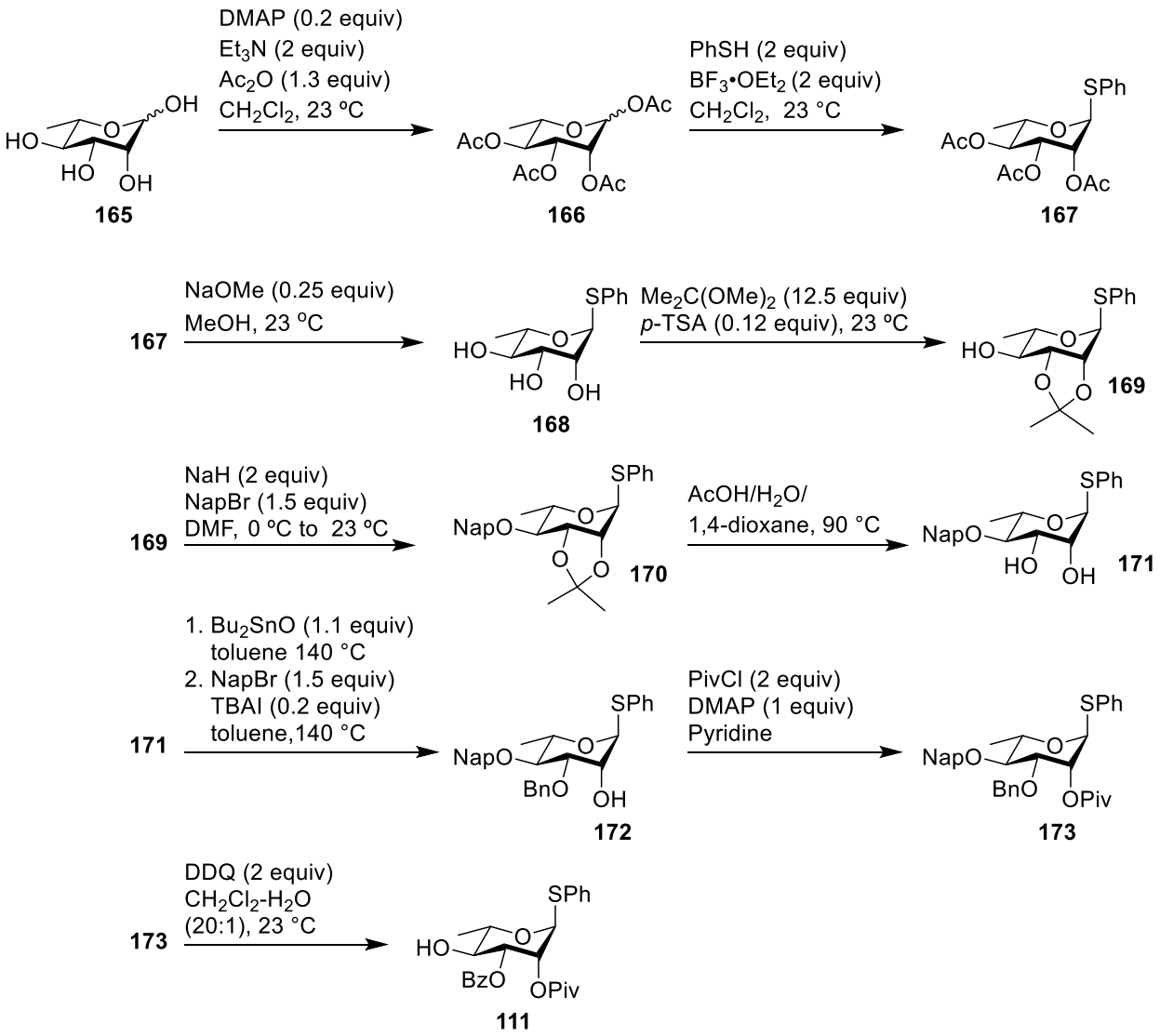
ketone to an oxime using benzylamine hydrochloride ($H_2NOBn \cdot HCl$) in MeOH providing **161** in 94% yield over two steps with (1.8:1) α:β selectivity.[61,78] Then after deacetylation using NaOMe in MeOH, the C-3 methyl group was selectively synthesized using methyllithium (MeLi) and cerium (III) chloride ($CeCl_3$) in THF affording **163** in 77% yield over two steps.[61,79] Reduction of the benzyl ether to an amine using zinc (Zn) in AcOH-H_2O-THF (3:1:1) was followed by diazo transfer using TfN_3, $CuSO_4 \cdot 5H_2O$, and $NaHCO_3$ in a mixture of H_2O-toluene-MeOH.[61,66,80] Subsequent synthesis of the C-4 naphthol methyl ether using NapBr and NaH in DMF provided **164** in 62% yield over three steps.[61,70] Then, the anomeric thiophenol was hydrolyzed using NBS in acetone-H_2O (9:1) afforded hemiacetal **105** in 92% yield (Scheme 1.44).



Scheme 1.43 Synthesis of D-rhamnal **159**.[61,75]



Scheme 1.44 Bennett lab's synthesis of D-saccharosamine **105**.[61,66,70,76,77,79–81]

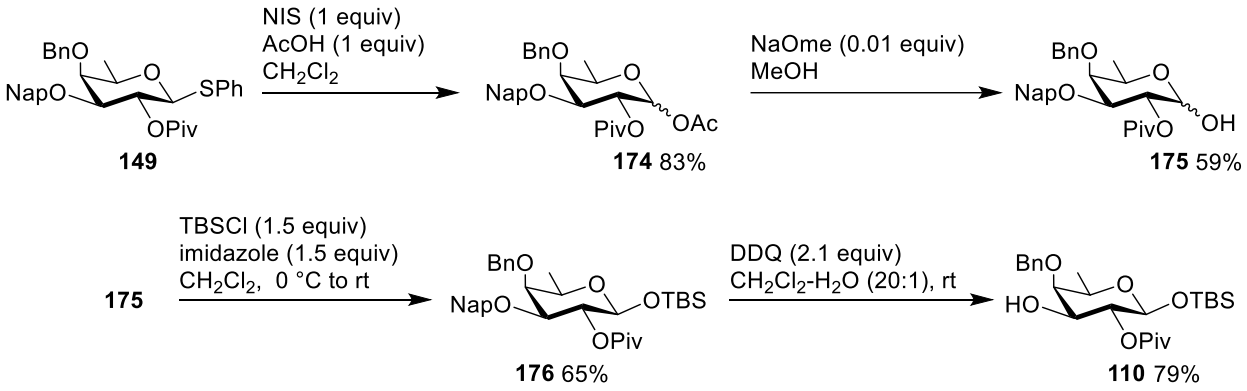


Scheme 1.45 Synthesis of L-rhamnose **111**.[61,70,82–85]

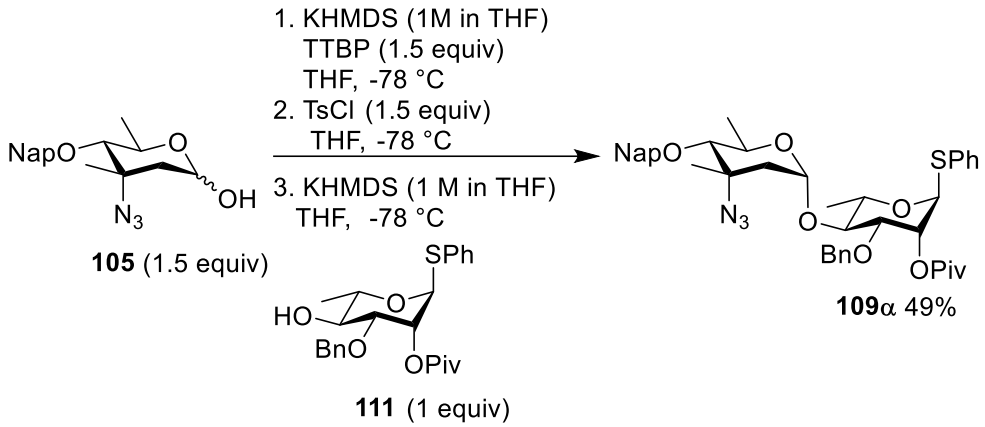
Having synthesized saccharosamine donor **105**, the Bennett lab turned their attention to the synthesis of L-rhamnose **111** and D-fucose **110**. Thus, L-rhamnose acceptor **111** was synthesized in eight steps from commercially available L-rhamnose **165** using a procedure that was found in the literature (Scheme 1.45).[61,70,82–85] L-rhamnose **165** was subjected to acetylation, thioglycoside formation, deacetylation, C-2 and C-3 acetonide synthesis, C-4 naphthol methyl

ether installation, acetonide removal, selective C-3 benzylation, C-2 piv-ester synthesis, and finally C-4 naphthol methyl ether removal.

The last monosaccharide needed for the synthesis of trisaccharide **181**, is D-fucose **110**. The synthesis of **110** begins with D-fucose **150** which was previously synthesized by the Bennett lab for the synthesis of trisaccharide **152** of saccharomicin B. Starting from **150**, the anomeric thiophenol was hydrolyzed using *N*-iodosuccinimide (NIS) and AcOH in CH_2Cl_2 to provide **174** in 83% yield (Scheme 1.46).[61] Next, deacetylation using NaOMe in THF followed by selective silyl ether synthesis using TBSCl and imidazole in CH_2Cl_2 afforded **176** in 38% yield over two steps.[61] D-fucose acceptor **110** was synthesized in 79% yield after the oxidative naphthol methyl ether removal using DDQ in CH_2Cl_2-H_2O (20:1).[61,86,87]



Scheme 1.46 Bennett lab's synthesis of D-fucose **110**.[61]

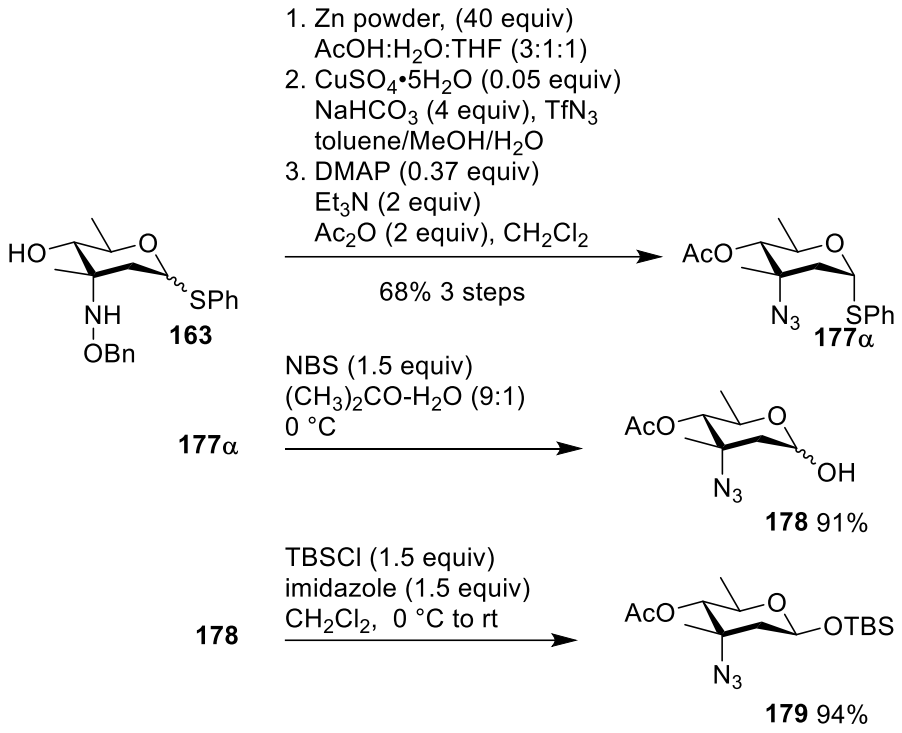


Scheme 1.47 Bennett lab's synthesis of the α-linked D-saccharosamine-L-rhamnose disaccharide **109α** using the reagent-controlled glycosylation method.[61]

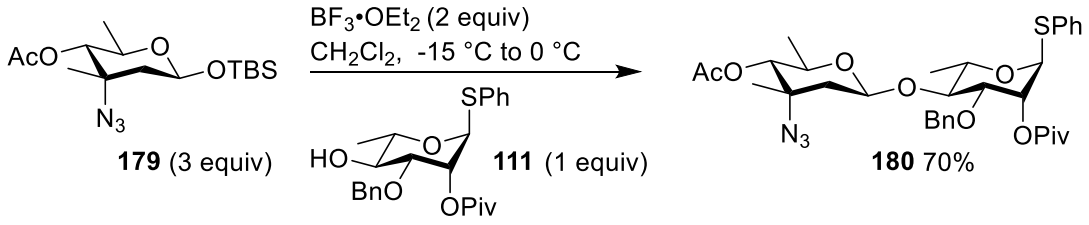
With each of the desired monosaccharides in hand, the Bennett lab sought to synthesize the D-saccharosamine-L-rhamnose disaccharide **109**. Initially, coupling of **105** and **111** was attempted using the Bennett lab's reagent-controlled glycosylation strategy which was previously used for the selective synthesis of β-linked glycosides.[25,26,28,31] The reagent-controlled glycosylation begins with the deprotection of a hemiacetal donor with potassium bis(trimethylsilyl)amide (KHMDS) before the addition of a 4-toluenesulfonyl chloride (TsCl). The reaction between the donor alkoxide and the TsCl can result in glycosyl-tosylate intermediate species which can undergo an S_N2 reaction with a nucleophile to selectively synthesize glycosides.[25,28,30] However, this method resulted in the selective synthesis of α-linked disaccharide **109α** in 49% yield (Scheme 1.47).[61] Having failed to synthesize the β-linked disaccharide, the Bennett lab turned their attention towards a method used by the Monneret lab for the selective synthesis of β-linked glycosides.[88] The Monneret lab's method used a 1-OTBS 2,3-dideoxy sugar donor with an azide at the C-3 position which after activation with $BF_3{\cdot}OEt_2$ or trimethylsilyl trifluoromethanesulfonate (TMSOTf) glycosides were synthesized. The observed α-β-selectivity of the glycosylation changes depending on the position of the C-3 azide. Examples provided by the Monneret lab show that when the C-3 azide is in the equatorial orientation α-linked glycosides are synthesized and when the azide is in the axial orientation β-linked glycosides are

synthesized.[88] The selectivity of the Monneret lab's glycosylation reaction could be derived from the position of the azide which can form an antiparallel dipole with the incoming nucleophile and thus, when the azide is in the axial orientation the dipole between the azide and incoming nucleophile favors the nucleophile to approach from the equatorial position, resulting in the synthesis of β-linked glycosides.[88] The Bennett lab decided to test the Monneret lab's approach for the synthesis of D-saccharosamine-L-rhamnose disaccharide **180**. To this end, compound **163** was subjected to reduction of the benzyl ether to an amine using zinc (Zn) in AcOH-H_2O-THF (3:1:1) and was followed by diazo transfer using TfN_3, $CuSO_4 \cdot 5H_2O$, and $NaHCO_3$ in a mixture of H_2O-toluene-MeOH.[61,66,80] Subsequent synthesis of the C-4 acetate using Ac_2O, Et_3N, and DMAP in and CH_2Cl_2 provided **177α** in 68% yield over three steps.[61,70] Next, the anomeric thiophenol of **177α** was hydrolyzed using NBS in acetone-H_2O (9:1) followed by the synthesis of the anomeric silyl ether using TBSCl and imidazole in CH_2Cl_2 to provide **179** in 81% yield over two steps (Scheme 1.48).[61]

Having synthesized the new D-saccharosamine donor **179**, the Bennett lab could test the Monneret lab's method for the synthesis of disaccharide **180**. Donor **179** and acceptor **111** were glycosylated using $BF_3 \cdot OEt_2$ in CH_2Cl_2 to selectively afford the β-linked disaccharide **180** in 70% yield (Scheme 1.49). Interestingly, there is a change in the selectivity of the glycosylation reaction with saccharosamine donors when using the Bennett lab's reagent-controlled approach as compared with the 1-OTBS donor and $BF_3 \cdot OEt_2$ method. Investigation into the mechanisms for each reaction could provide valuable insight into the origins of these selectivity's.



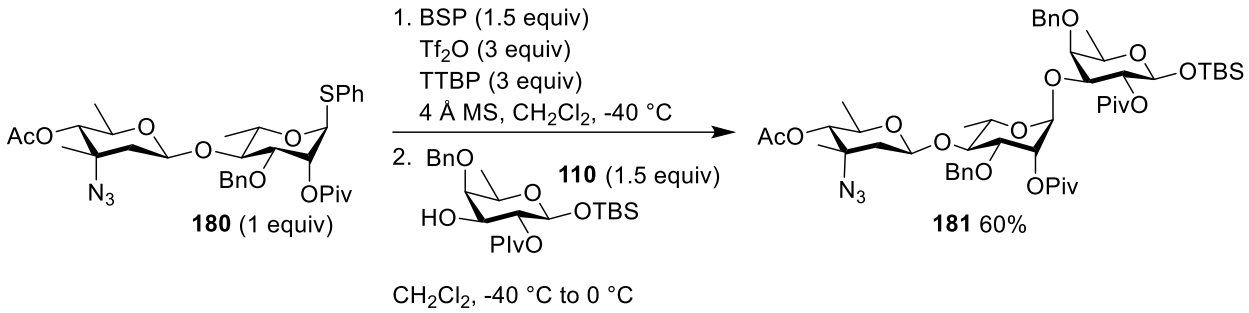
Scheme 1.48 Bennett lab's synthesis of D-saccharosamine **179**.[61]



Scheme 1.49 Bennett lab's synthesis of the β-linked D-saccharosamine-L-rhamnose disaccharide **180** using the Monneret lab's 1-OTBS donor $BF_3 \cdot OEt_2$ glycosylation method.[61,88]

Having synthesized disaccharide **180**, the Bennett lab could direct their efforts to the synthesis of trisaccharide **181**. The glycosylation method developed by the Crich lab was used for the [2+1] synthesis of **181**.[89,90] The reaction starts with the activation of a thioglycoside donor with 1-(phenylsulfinyl)piperidine (BSP) and trifluoromethanesulfonic anhydride (Tf_2O) and then the addition of a nucleophile acceptor promotes the synthesis of glycosides.[89,90] Initially, the Bennett lab attempted the [2+1] glycosylation with BSP, Tf_2O, and TTBP in CH_2Cl_2 using **150**, however degradation of both the donor and acceptor species was observed. D-fucose acceptor

110 was synthesized because the 1-OTBS group was thought to be more stable than the 1-thiophenol to the BSP/Tf_2O reaction conditions. Consequently, glycosylation of **180** and **110** using BSP, Tf_2O, and TTBP in CH_2Cl_2 selectively synthesized trisaccharide **181** in 60% yield (Scheme 1.50).[61]

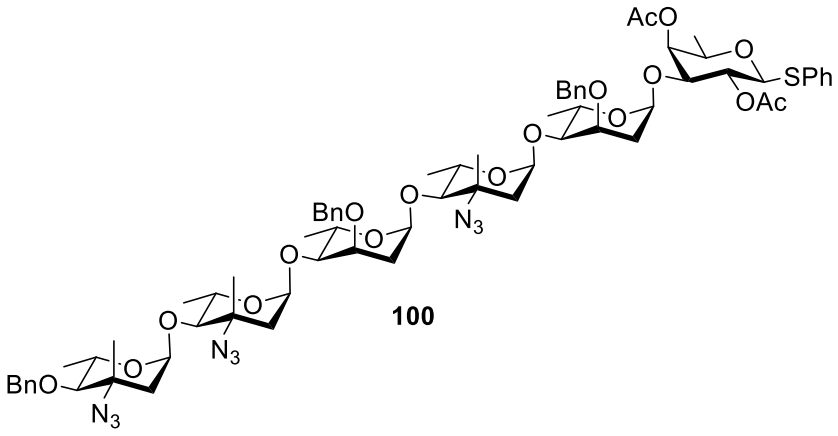


Scheme 1.50 Bennett lab's synthesis of the D-saccharosamine-L-rhamnose-D-fucose trisaccharide **181** using the BSP/Tf_2O glycosylation method.[61]

In summary, trisaccharide **181** was synthesized using a [2+1] glycosylation strategy. The three monosaccharides needed for the synthesis of **181** were synthesized prior to glycosylation reactions. The rare tri-deoxy D-saccharosamine sugar **179** was synthesized from D-rhamnal **159**. Both L-rhamnose **111** and D-fucose **110** were synthesized by orthogonally protecting commercially available L-rhamnose **165** and D-fucose **142** respectively. The Bennett lab's reagent-controlled glycosylation method provided α-linked disaccharide **109α**. Fortunately, the Monneret lab's Lewis acid mediated glycosylation of a 1-OTBS donor was used to selectively synthesize disaccharide **180**. The mechanisms for the two glycosylation reactions which afforded **109α** and **180** will be examined in Chapter four of this thesis to understand the origins for the differences in the selectivity's for the two methods. Finally, trisaccharide **181** was selectively synthesized using the BSP/ Tf_2O method.

The Bennett lab again used a convergent strategy for the synthesis of **181**. Additionally, trisaccharide **181** is suitable to be used as a donor or after chemical modification, as an acceptor species. Interestingly, the Lewis acid mediated glycosylation step used for the synthesis of

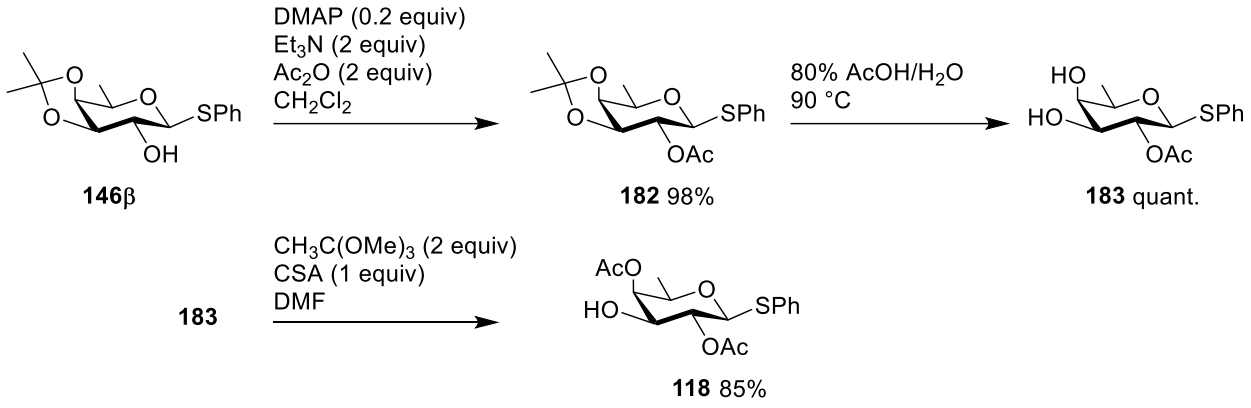
disaccharide **180** resembles the method that was used by the McDonald lab for the synthesis of the L-fucose-L-saccharosamine derivative glycoside **46**.[40,61]



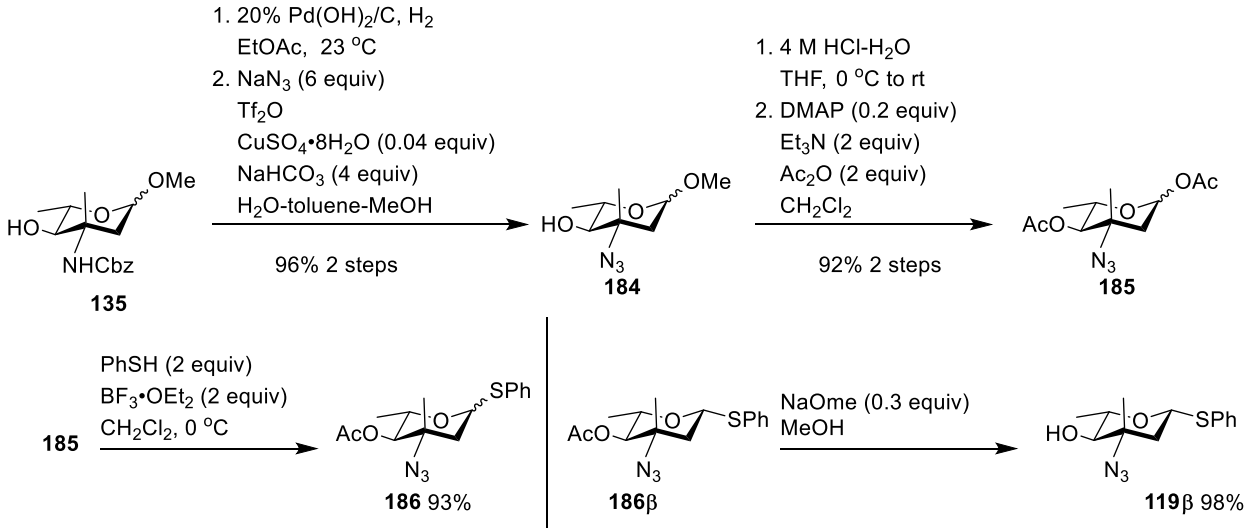
100

Scheme 1.51 Hexasaccharide fragment **100** of saccharomicins. The third fragment of saccharomicins synthesized by the Bennett lab.[62]

The third fragment of saccharomicins synthesized by the Bennett lab was the hexasaccharide fragment **100** (Scheme 1.51).[62] Similar to the syntheses of **152** and **181**, the Bennett lab started the synthesis of **100** by first synthesizing the monosaccharides **118**, **115, 116,** and **119β**. The Bennett lab starts their synthesis of D-fucose **118** with the acetylation of the C-2 hydroxyl of **146β** using Ac_2O, Et_3N, and DMAP in CH_2Cl_2 to afford **182** in 98% yield (Scheme 1.52).[62] Next, the C-3 and C-4 acetonide protection was removed using 80% AcOH in H_2O to provide **183** in quantitative yield.[62] Selective synthesis of the C-4 acetate using trimethyl orthoacetate $CH_3(OMe)_3$ and CSA in DMF afforded **118** in 85% yield.[62]



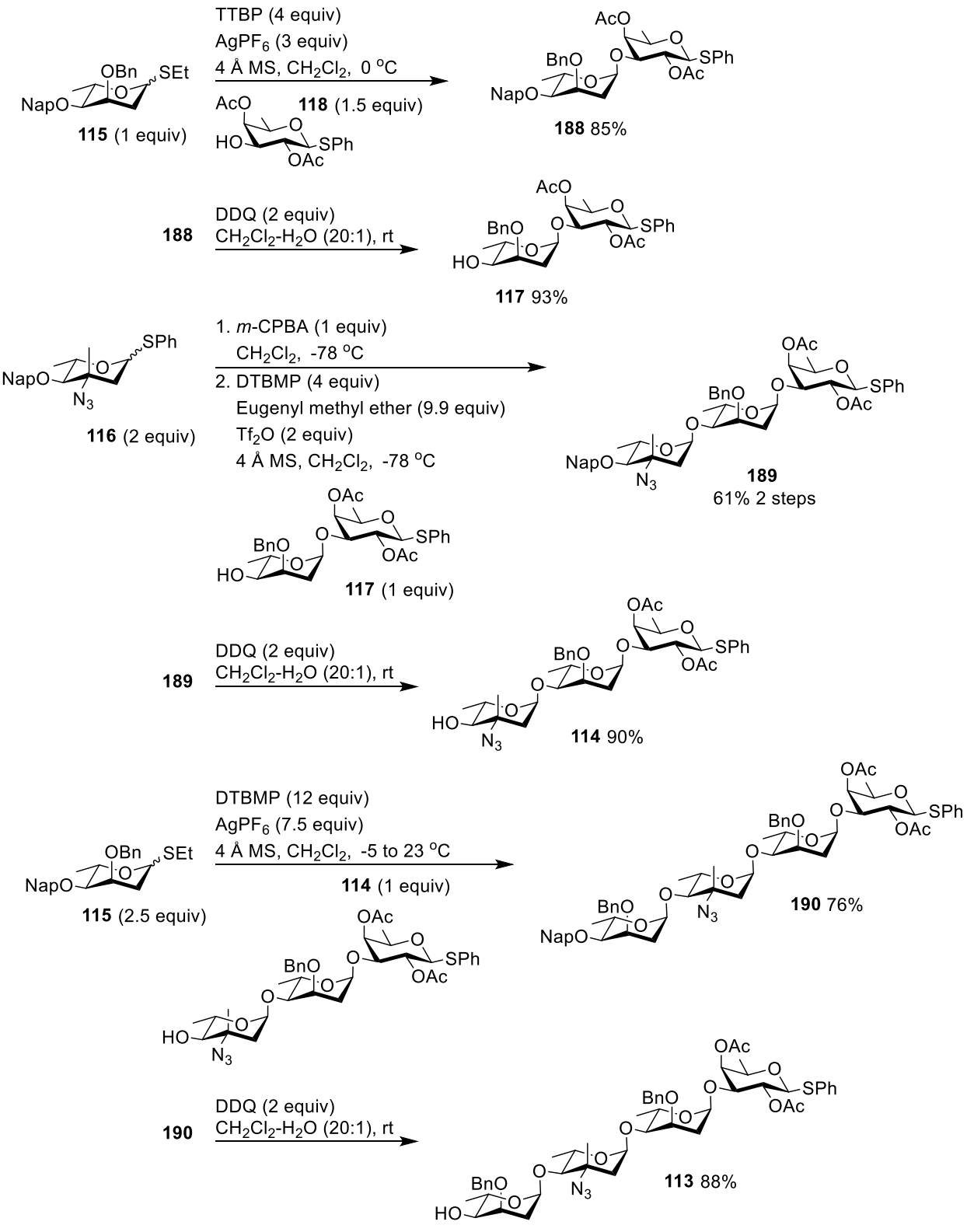
Scheme 1.52 Bennett lab's synthesis of D-fucose **118** from D-fucose **146β**.[62]



Scheme 1.53 Bennett lab's synthesis of 4-*epi*-L-vancosamine **119β** from 4-*epi*-L-vancosamine **135**.[62]

With D-fucose **118** in hand, the Bennett lab sought to synthesize 4-*epi*-L-vancosamines **116** and **119β**. Previously, the Bennett lab developed a route to synthesize **135** from vancomycin HCl **3**. Starting from **135**, the carbamate was reductively removed using palladium hydroxide on carbon ($Pd(OH)_2/C$) under a hydrogen gas atmosphere (H_2) in EtOAc, followed by diazo transfer using TfN_3, $CuSO_4 \cdot 5H_2O$, and $NaHCO_3$ in a mixture of H_2O-toluene-MeOH to provide **184** in 96% yield over two steps (Scheme 1.53).[62] Next, HCl in H_2O and THF worked to hydrolyze the anomeric methoxy and subsequent acetylation using Ac_2O, Et_3N, and DMAP in CH_2Cl_2 afforded

185 in 92% yield over two steps.[62] Glycosylation with thiophenol (PhSH) and $BF_3 \cdot Et_2O$ in CH_2Cl_2 was used to synthesize thioglycoside **186** in 93% yield.[62] Following subsequent separation of the anomers of **186**, the β-anomer **186β** was subjected to deacetylation using NaOMe and MeOH prior to the synthesis of the C-4 naphthol methyl ether using NapBr and NaH in DMF to provide **119β** in 98% yield.[62] The final monosaccharide needed for the synthesis of the hexasacccharide fragment **100** is L-digitoxose **115** which the Bennett lab had previously synthesized while on route to **152** (Scheme 1.53).[60,62]

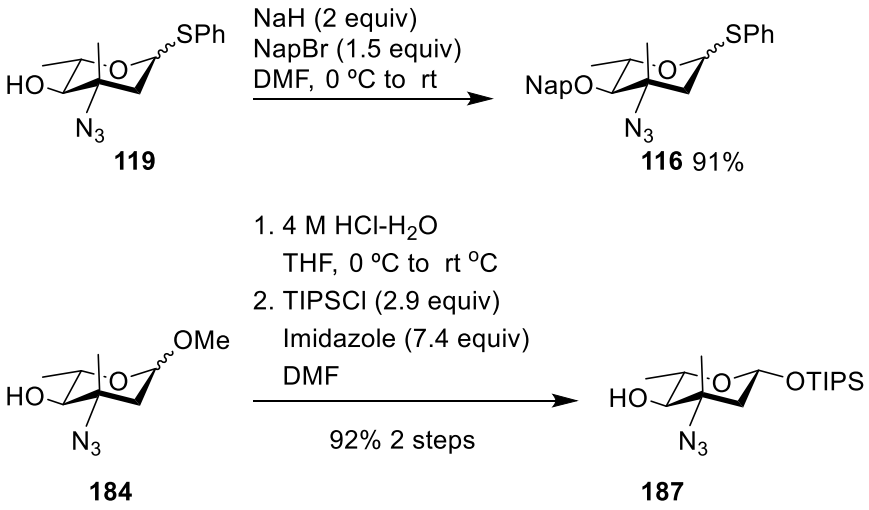


Scheme 1.54 Bennett lab's synthesis of the tetrasaccharide fragment **113**.[62]

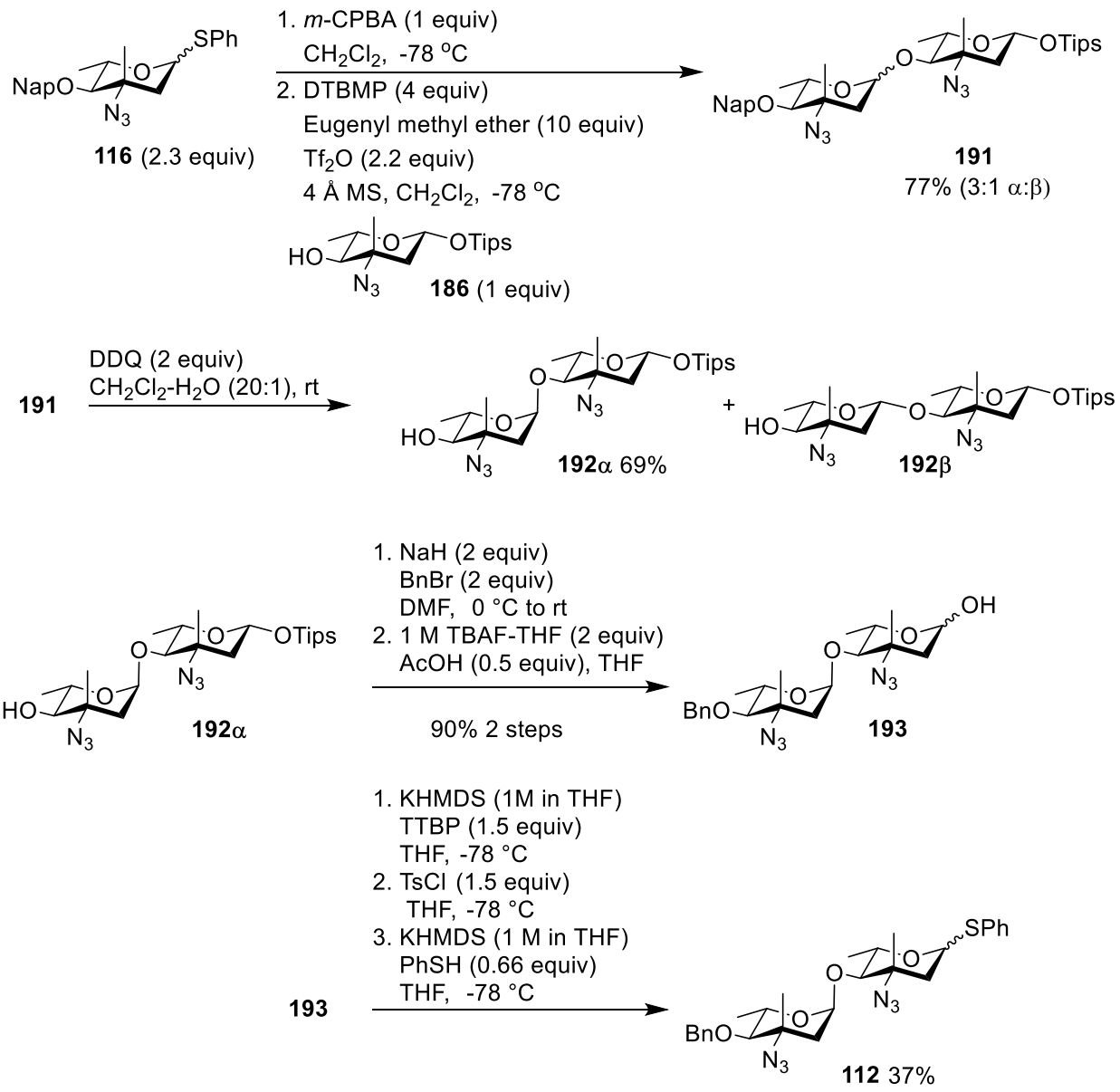
Having synthesized the four monosaccharides needed for the synthesis of **100**, the Bennett lab focused on the synthesis of disaccharide **117**. Previously, the Bennett lab had success synthesizing α-linkages with an L-digitoxose donor bearing an ethanethiol moiety at the anomeric position using the Hirama lab's silver hexafluorophosphate ($AgPF_6$)-mediated glycosylation reaction.[60,73,74] Reasoning that the α-linked L-digitoxose-D-fucose disaccharide **188** could be synthesized using a similar approach, the Bennett lab moved forward to test the $AgPF_6$-mediated reaction for the coupling of **115** and **118** using $AgPF_6$ and DTBMP in CH_2Cl_2 to afford **188** in 43% yield with (4:1) α:β selectivity.[46] Changing the base to TTBP and increasing the temperature of the reaction provided **188** in 85% yield as a single α-anomer (Scheme 1.54).[62] Deprotection of the naphthyl methyl ether using DDQ in a mixture of CH_2Cl_2-H_2O (20:1) was used to synthesize disaccharide acceptor **117** in 93% yield.[62]

With the newly synthesized disaccharide acceptor **117** in hand, the Bennett lab could attempt to couple **116** and **117** for the synthesis of **189**. Previously, the Bennett lab had utilized their dehydrative glycosylation method for the synthesis of the α-linkage between 4-*epi*-L-vancosamine **107** and D-fucose **108** and attempted to use this method for the synthesis of the α-linkage between 4-*epi*-L-vancosamine **116** and disaccharide **117**.[27,29,60,62] However, the Bennett lab's dehydrative glycosylation method failed to provide the desired trisaccharide and therefore the two-step glycosylation strategy developed by the Kahne lab for the synthesis of an α-linked L-vancosamine glycoside was chosen to be a promising strategy for the synthesis of **189**.[62,63,65] The Kahne lab's glycosylation starts with the oxidation of a thioglycoside glycosyl donor to a reactive sulfoxide which after addition trifluoromethanesulfonic anhydride (T_2O) and a nucleophile, can react to synthesize glycosides. Fortunately, using the Kahne lab's method, **189** was synthesized in 61% yield over two steps by first oxidation of **116** using *meta*-chloroperoxybenzoic acid (*m*-CPBA) in CH_2Cl_2 followed by reaction with Tf_2O and DTBMP in CH_2Cl_2 and the addition of **117** (Scheme 1.54).[62] Deprotection of the naphthol methyl ether at the C-4 position of the 4-*epi*-L-

vancosamine moiety of **189** using DDQ in a mixture of CH_2Cl_2 and H_2O (20:1) provided **114** in 90% yield.[62] Next, the α-linkage between L-digitoxose **115** and trisaccharide **114** was synthesized using $AgPF_6$ and DTBMP in CH_2Cl_2 to afford tetrasaccharide **190** in 76% yield as a single α-anomer.[62] Subsequent deprotection of the naphthol methyl ether at the C-4 position of the L-digitoxose moiety using DDQ in a mixture of CH_2Cl_2 and H_2O (20:1) was used to synthesize acceptor **113** in 88% yield (Scheme 1.54).[62]



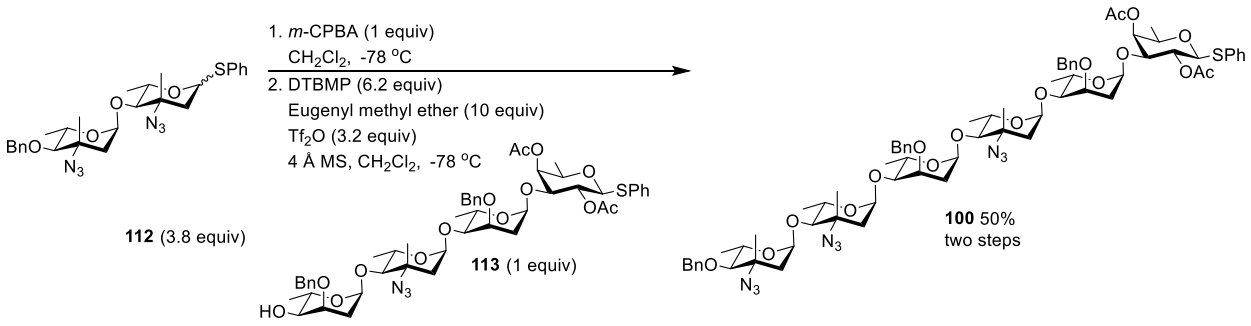
Scheme 1.55 Bennett lab's synthesis of 4-*epi*-L-vancosamine monosaccharides **116** and **187**.[62]



Scheme 1.56 Bennett lab's synthesis of 4-*epi*-L-vancosamine-4-*epi*-L-vancosamine disaccharide **112**.[62]

Initially, the Bennett lab attempted to couple 4-*epi*-L-vancosamine **116** to tetrasaccharide **113** using the Kahne lab's glycosylation strategy which was used to synthesize the α-linkage between **116** and **117**. After multiple unsuccessful attempts to couple **116** and **113** using the Kahne lab's method, the Bennett lab turned their attention to the [1+1] synthesis of disaccharide **191α**, which could then be used in a [2+4] strategy to synthesize hexasaccharide **100**.[62] To this

end, 4-*epi*-L-vancosamine acceptor **187** was synthesized in 92% yield over two steps starting from **184** by first acidic hydrolysis of the anomeric methoxy using HCl in H_2O and THF followed by the synthesis of the anomeric silyl either using triisopropylsilyl chloride (TIPSCl) and imidazole in DMF (Scheme 1.55).[62] Next, **116** and **186** were coupled using the Kahne lab's two step method to afford **191** in 77% yield with as a (3:1) mixture of α-and β-anomers over two steps (Scheme 1.56).[62] The optimized synthesis of **191** used additive 4-allyl 1,2-dimethoxybenzene as an additional proton scavenger.[62] Deprotection of the naphthol methyl ether using DDQ in a mixture of CH_2Cl_2 and H_2O (20:1) followed by separation of the glycosyl anomers provided **192α** in 69% yield as a single α-anomer.[62] Next, the hydroxyl of **192α** was protected with a benzyl ether using BnBr and NaH in DMF followed by the removal of the silyl ether using Bu_4NF and AcOH in THF synthesized **193** in 90% yield over two steps.[62] The thioglycoside **112** was synthesized using the Bennett lab's reagent-controlled glycosylation strategy with thiophenol to afford **112** in 37% yield as a mixture of α-and β-anomers (Scheme 1.56).[62] Then, **112** was used in a [2+4] glycosylation with **113** under the Kahne lab's glycosylation conditions to provide hexasaccharide **100** in 50% yield over two steps (Scheme 1.57).[62,63,65]



Scheme 1.57 Bennett lab's [2+4] synthesis of hexasaccharide **100** using the two-step Kahne glycosylation method.[62,63]

In summary, the synthesis of the hexasaccharide fragment **100** of the saccharomicins began with the synthesis of 4-*epi*-L-vancosamines **116** and **119β**, D-fucose **118**, and L-digitoxose **115**.[62] After synthesizing the monosaccharides, the AgPF6-mediated glycosylation was used to

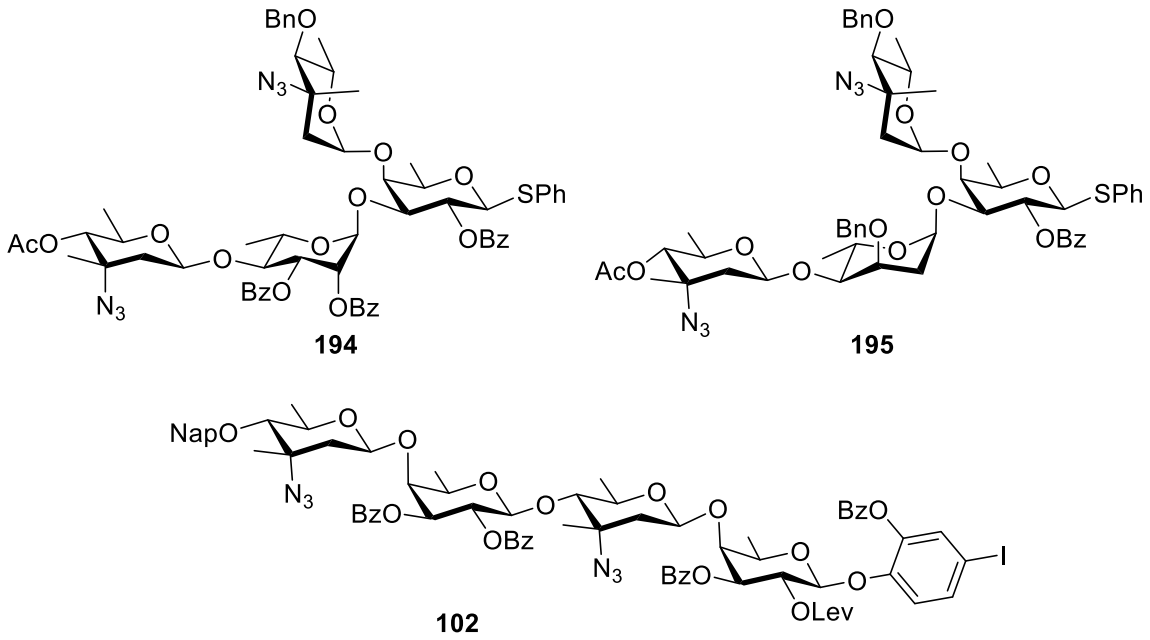
synthesize **188**. The Kahne method was used for the [1+2] synthesis of **189** and then the $AgPF_6$-mediated glycosylation was used for the [1+3] synthesis of **190**.[62] Disaccharide **191** was synthesized using the Kahne glycosylation method and hexasaccharide **100** was synthesized via a [2+4] reaction of **112** and **113** also using the Kahne method. Naphthol methyl ether deprotections were used after each glycosylation reaction to synthesize the corresponding acceptor species.[62]

The approach used by the Bennett lab for the synthesis of **100** relies on the ability to selectively remove the naphthol methyl ether protection in between glycosylation reactions. This limits the number of chemical transformations needed following the glycosylation step to synthesize the next coupling partner. Interestingly, tetrasaccharide **113** was synthesized in a linear fashion and hexasaccharide **100** was synthesized in a convergent [2+4] approach. Hexasaccharide **100** is suitable to be used as a donor species to synthesize a larger fragment of saccharomicins.

The Bennett lab has synthesized three fragments of the saccharomicins which account for twelve of the seventeen sugars within the saccharomicins. Each of the fragments synthesized by the Bennett lab is suitable to be used in a subsequent coupling reaction to synthesize larger fragments of saccharomicins. The syntheses of **152**, **181**, and **100** have provided a means to synthesize almost all the linkages within the saccharomicins. The remaining linkages within the saccharomicins which the Bennett lab needs to synthesize are the D-saccharosamine-D-fucose β-linkages and the D-fucose-D-saccharosamine β-linkages found in both saccharomicins, as well as the D-saccharosamine-L-digitoxose β-linkage found in saccharomicin B. Furthermore, the Bennett lab has developed syntheses to access 4-*epi*-L-vancosamine, D-saccharosamine, and L-digitoxose monosaccharides. Remarkably, the synthetic route to the rare D-saccharosamine monosaccharide developed by the Bennett lab is the most efficient method to synthesize this sugar.

1.6 Synthesis of Saccharomicins: What Remains and Outline of Chapters 2, 3, and 4

The immense progress made by the Bennett lab towards the total synthesis of saccharomicins has encouraged further investigation into the synthesis of the remaining fragments. The retrosynthetic analysis envisioned by the Bennett lab has proven so far to be a success and to complete the synthesis of saccharomicins, three fragments will need to be synthesized. The synthesis of the branched tetrasaccharide **194** is needed to access the backbone of saccharomicin A (Scheme 1.58). Then, coupling of a D-saccharosamine monosaccharide to **152** will provide tetrasaccharide **195** which is the missing piece to link hexasaccharide **100** and trisaccharide **181** to complete the synthesis of the backbone of saccharomicin B. The synthesis of tetrasaccharide **102**, which contains a twice-repeating D-saccharosamine-D-fucose glycoside would provide the last fragment needed to synthesize the heptadecasaccharide core of saccharomicins. Finally, the aglycone **Agl** will have to be synthesized and coupled to D-fucose-1 prior to global deprotection of all masked functional groups to complete the total synthesis of the saccharomcins.



Scheme 1.58 Remaining fragments of saccharomicins that need to be synthesized to complete the Bennett lab synthesis.

The remaining large fragments of saccharomicins that still need to be synthesized are the branched tetrasaccharide fragments of each saccharomicin A **194** and saccharomicin B **195** as well as the reducing tetrasaccharide fragment **102** (Schemes 1.58). The linkages within the branched tetrasaccharide fragment of saccharomicin A **194** that need to be synthesized are the β (1-4) linkage between sac-11 and rha-10 and the α (1-3) linkage between rha-10 and fuc-8. The synthesis of the branched tetrasaccharide of saccharomicin A **194** will be discussed in Chapter 2 of this thesis. The linkages within the branched tetrasaccharide fragment of saccharomicin B **195** that need to be synthesized are the β (1-4) linkage between sac-11 and dig-10 and the α (1-3) linkage between dig-10 and fuc-8. The β-linked D-saccharosamine-L-digitoxose moiety has yet to be synthesized. The synthesis of the branched tetrasaccharide of saccharomicin B **195** will be discussed in Chapter 3 of this thesis. The linkages within the tetrasaccharide fragment **102** that need to be synthesized are the repeating β (1-4) linkages between sac-4 and fuc-3 and sac-2 and fuc-1. In addition, the β (1-4) linkage between fuc-3 and sac-2 and the β (1-4) linkage between

fuc-1 and the aglycone will need to be synthesized. A synthesis to access the aglycone will also need to be developed. The synthesis of the β (1-4) linkage between D-fucose and D-saccharosamine will provide a method to couple each of the large fragments that the Bennett lab has previously synthesized to synthesize the saccharomicins. Chapter 4 of this thesis will discuss a mechanistic study performed in collaboration with computational chemists to better understand the origins of the selectivity of glycosylation reactions using D-saccharosamine donors. With the help of experimental and computational data, the Bennett lab's reagent-controlled glycosylation and the 1-OTBS donor $BF_3 \cdot Et_2O$ glycosylation methods will be analyzed.

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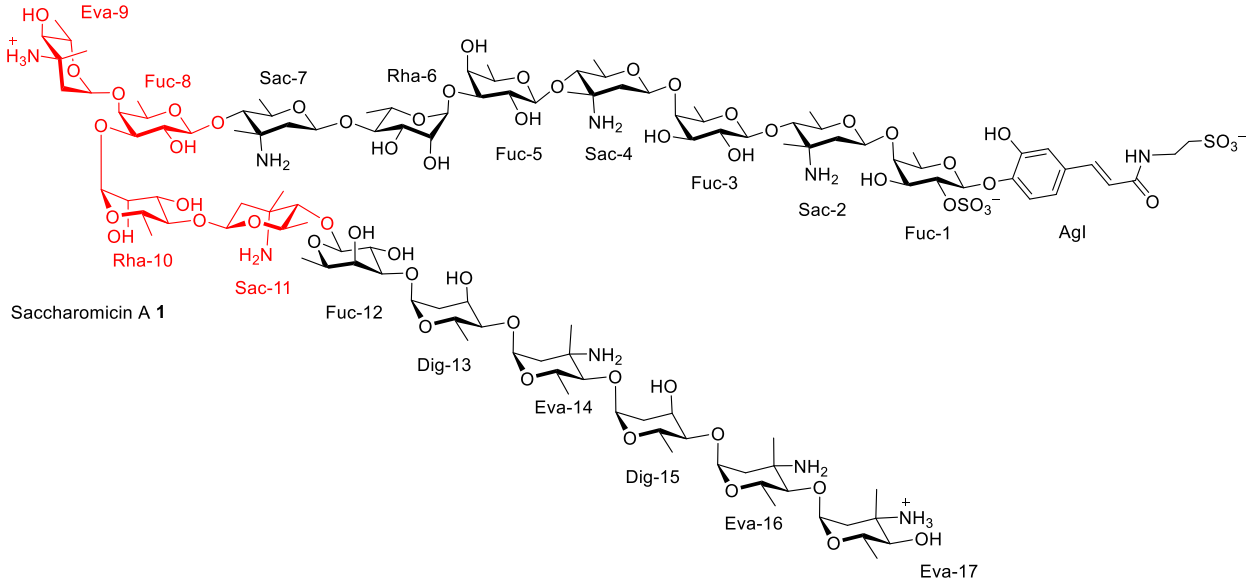
Chapter 2. Synthesis of the Branched Tetrasacccharide of Saccharomicin A

Adapted in part with permission from:

Garreffi, B. P.; Maney, A. P.; Bennett, C. S. Synthesis of the Branched Tetrasaccharide Fragment of Saccharomicin A. *Org. Lett.* **2023**, *25* (2), 369–372.

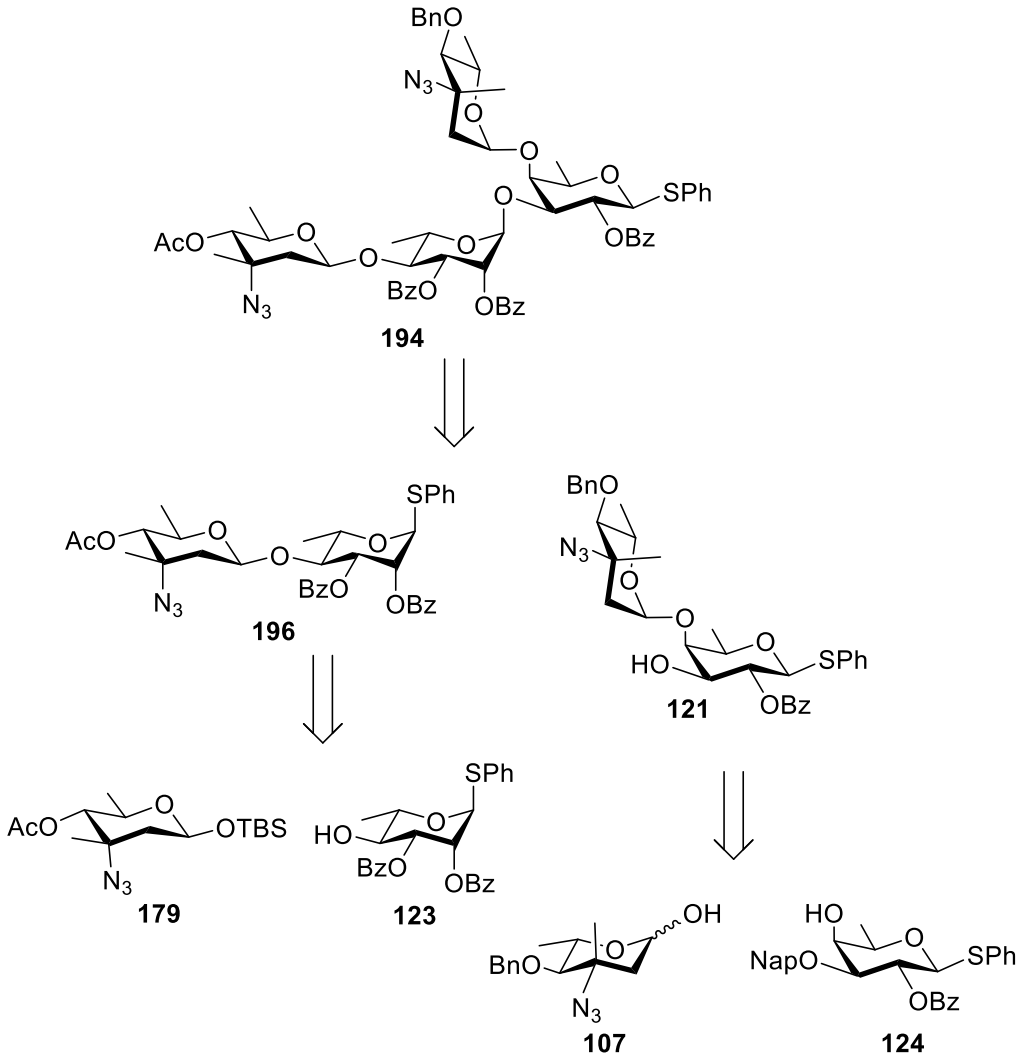
2.1 Retrosynthetic Analysis for the Synthesis of the Branched Tetrasaccharide Fragment of Saccharomicin A



Scheme 2.1 Structure of saccharomicin A **1**. Tetrasaccharide **194** is shown in red.

The next fragment of saccharomicins that the Bennett lab wanted to synthesize is the branched tetrasaccharide fragment saccharomicin A **194** (Scheme 2.1).[1] Following the Bennett lab's retrosynthesis of the saccharomicins, tetrasaccharide **194** could be synthesized from the union of D-saccharosamine-L-rhamnose disaccharide **196** and 4-*epi*-L-vancosamine-D-fucose disaccharide **121** (Scheme 2.2).[1] The Bennett lab has chosen to alter the original protecting group scheme used for the L-rhamnose and D-fucose monosaccharides for the synthesis of **194** from what was used for the syntheses of trisaccharides **152** and **181** (Scheme 2.2).[2,3] Benzoyl esters will replace the C-2 piv ester and C-3 benzyl ether which was used for the L-rhamnose moiety of **181** (Scheme 2.2, Scheme 1.45).[1,2] Benzoyl esters will also replace the C-2 piv ester which was used for the D-fucose moieties found in both **152** and **181** (Scheme 2.2, Scheme 1.46, Scheme 1.36).[1–3] Changing to benzoyl esters will streamline the final deprotections that will be used to access saccharomicins **1** and **2**. The α-linkage between the L-rhamnose and D-fucose moieties could be synthesized using the BSP/ Tf_2O method which was used for the [2+1] synthesis of

trisaccharide **181** of the saccharomicins (Scheme 2.2, Scheme 1.50).[1,2] Disaccharide **196** could be synthesized from the coupling of D-saccharosamine **179** and L-rhamnose **123** (Scheme 2.2). The β-linkage between the D-saccharosamine and L-rhamnose moieties could be synthesized using the $BF_3{\cdot}OEt_2$ activation of a 1-OTBS saccharosamine donor which was used for the synthesis of disaccharide **180** (Scheme 2.2, Scheme 1.49).[1,2] Disaccharide **121** could be synthesized from the coupling of 4-*epi*-L-vancosamine **107** and D-fucose **124** and subsequent deprotection of the naphthyl methyl ether (Scheme 2.2). The α-linkage between the 4-*epi*-L-vancosamine and D-fucose moieties could be synthesized using the Bennett lab's dehydrative glycosylation method which was used for the synthesis of disaccharide **151** of saccharomicin B (Scheme 2.2, Scheme 1.37).[1,3] D-saccharosamine monosaccharide **179** could be synthesized from D-rhamnal **159** which can be traced back to commercially available 3,4,6-tri-O-acetyl-D-glucal **154** (Scheme 2.2, Scheme 1.48, Scheme 1.44, Scheme 1.43).[1,2,4–10] The C-4 position of D-saccharosamine can be protected with an acetate instead of a naphthol methyl ether which was the protecting group used in the retrosynthesis of **101** in Chapter 1 (Scheme 2.2, Scheme 1.30). The yield of the Lewis acid mediated glycosylation reaction used for the synthesis of D-saccharosamine-L-rhamnose disaccharide **109** was much higher when an acetate protection was used at the C-4 position instead of a naphthol methyl ether.[2] Selective removal of the C-4 acetate in the presence of benzoates could be used to synthesize a tetrasaccharide acceptor when it is time to couple the branched tetrasaccharide of saccharomicin A to hexasaccharide **100**. 4-*epi*-L-vancosamine **107** could be synthesized from vancomycin HCl **3** (Scheme 2.2, Scheme 1.53, Scheme 1.33).[1,3] L-rhamnose **123** and D-fucose **124** could be synthesized from commercially available L-rhamnose **165** and D-fucose **142** respectively (Scheme 2.2, Scheme 1.52, Scheme 1.46, Scheme 1.45, Scheme 1.36, Scheme 1.35).[1–3,11–14]

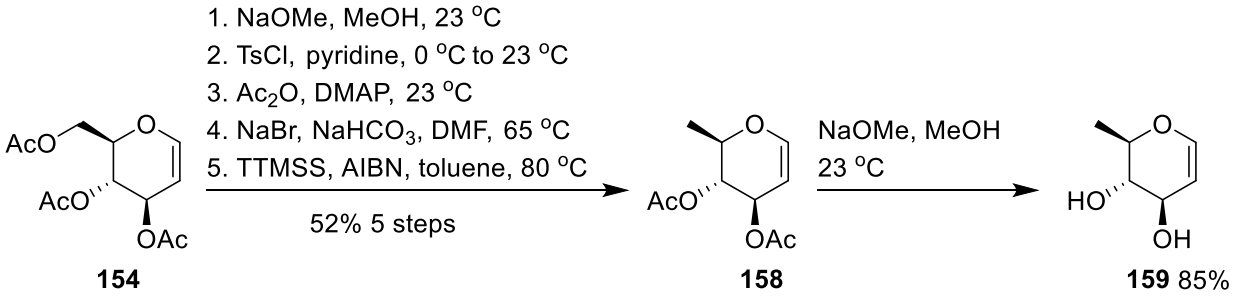


Scheme 2.2 Retrosynthesis of the Branched Tetrasaccharide Fragment **194**.[1]

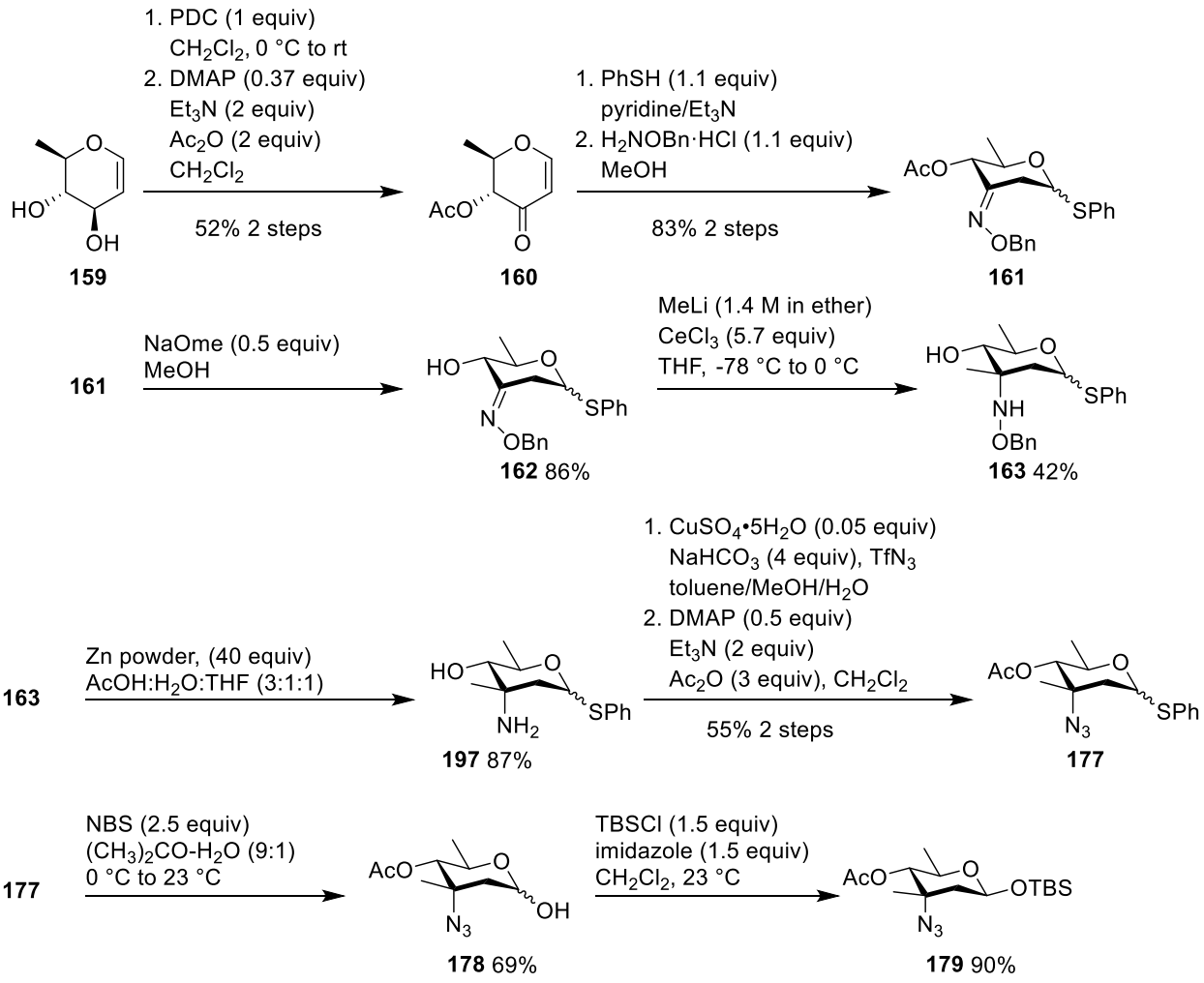
2.2 Monosaccharide Synthesis

The synthesis of tetrasaccharide **194** began with the synthesis D-saccharosamine **179**. To this end, 3,4,6-tri-O-acetyl-D-glucal **154** was subjected to deacetylation using NaOMe in MeOH (Scheme 2.3).[2,4,5] Next, tosylation of the C-6 hydroxyl using TsCl in pyridine followed by acetylation of the C-2 and C-3 hydroxyls using Ac_2O and DMAP in CH_2Cl_2 (Scheme 2.3).[2,5] Then halogenation of the C-6 position using sodium bromide (NaBr) and $NaHCO_3$ followed by dehalogenation using tris(trimethylsilyl)silane (TTMSS) and azobisisobutyronitrile (AIBN) afforded **159** in 52% yield over five steps (Scheme 2.3).[4,5] The synthesis of D-rhamnal **159** was completed

in 85% yield with the deacetylation of **158** using NaOMe in MeOH (Scheme 2.3). Selective oxidation of the C-3 hydroxyl using PDC in CH_2Cl_2 followed by subsequent acetylation of the C-4 hydroxyl using Ac_2O in pyridine provided ketone **160** in 52% yield over two steps (Scheme 2.4).[2,6,7] Addition of thiophenol to C-1 was completed using PhSH with Et_3N in pyridine prior to the synthesis of the C-3 oxime using $H_2NOBn{\cdot}HCl$ in MeOH afforded **161** in 83% yield over two steps (Scheme 2.4).[2,5,6,15] Deacetylation of the C-4 hydroxyl using NaOMe in MeOH provided **162** in 86% yield (Scheme 2.4). D-saccharosamine **163** was synthesized in 42% yield by methylation of the C-3 position using $CeCl_3$ in THF (Scheme 2.4).[2,6–8] Then, the benzyl ether was removed using Zn in a mixture of AcOH-H_2O-THF (3:1:1) to provide **197** in 87% yield (Scheme 2.4). Diazo transfer using TfN_3, $CuSO_4{\cdot}5H_2O$, and $NaHCO_3$ in a mixture of H_2O-toluene-MeOH followed by subsequent acetylation of the C-4 hydroxyl provided **177** in 55% yield over two steps (Scheme 2.4).[2,9,10] Hydrolysis of the anomeric thiophenol using NBS in acetone-H_2O (9:1) afforded **178** in 69% yield.[2,16,17] The synthesis 1-OTBS D-saccharosamine **179** was completed in 90% yield after treatment with TBSCl and imidazole in CH_2Cl_2 (Scheme 2.4).[1,2]



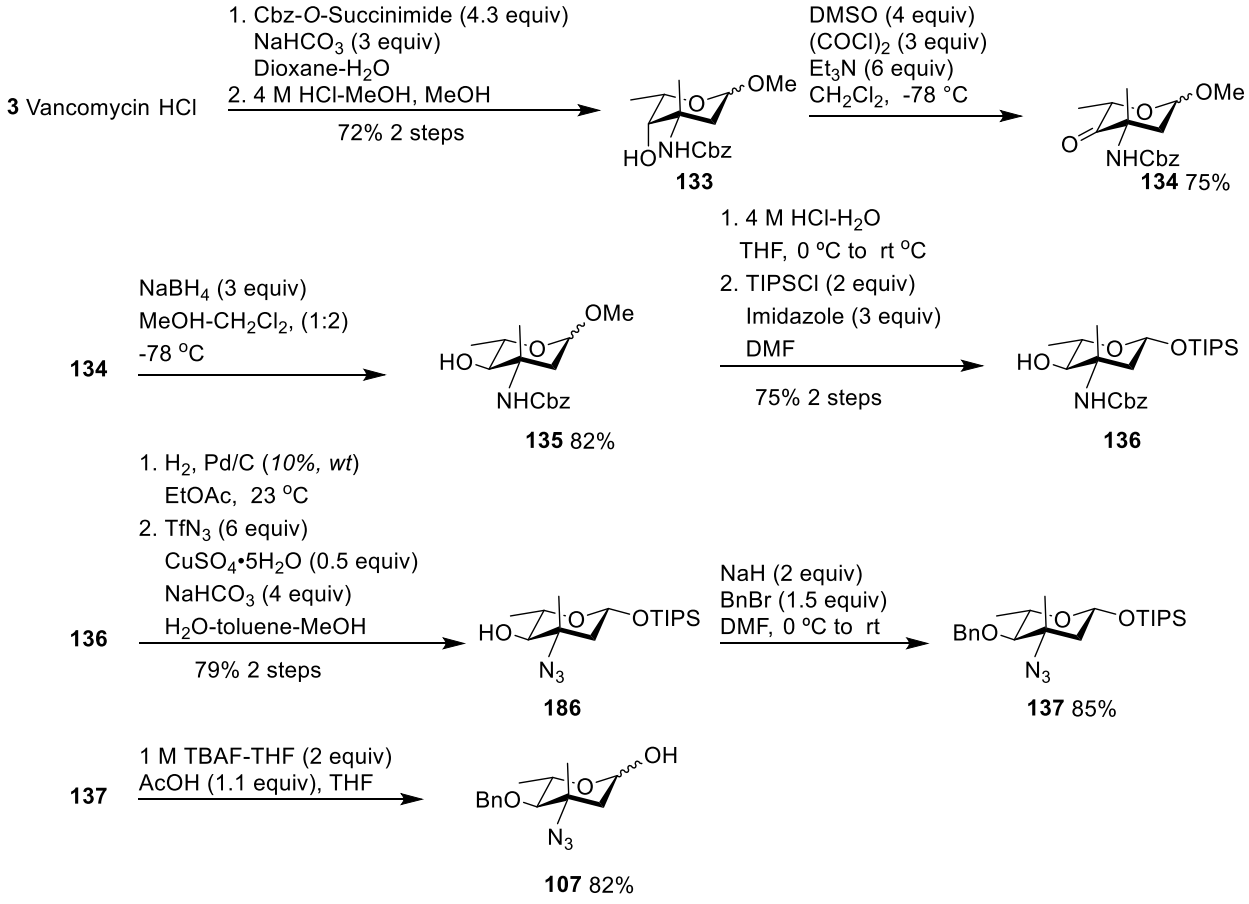
Scheme 2.3 Synthesis of D-rhamnal **159** from 3,4,6-tri-O-acetyl-D-glucal **154**.[2,4,5]



Scheme 2.4 Synthesis of D-saccharosamine **179** from D-rhamnal **159**.[1,2,4–10,15]

The next monosaccharide needed for the synthesis of tetrasaccharide **194** is 4-*epi*-L-vancosamine **107**.[1] To this end, commercially available vancomycin-HCl **3** was subjected to treatment with Cbz-O-succinimide and $NaHCO_3$ in a mixture of dioxane and H2O (1:1) followed by subsequent acid mediated methanolysis using HCl-MeOH to afford vancosamine **133** in 72% yield over two steps (Scheme 2.5).[3,18,19] Next, oxidation of the C-4 hydroxyl was completed using Swern oxidation conditions to provide **134** in 75% yield (Scheme 2.5).[20] Reduction of the C-4 position using $NaBH_4$ in a mixture of MeOH-CH_2Cl_2 (1:2) afforded 4-*epi*-L-vancosamine **135** in 82% yield (Scheme 2.5).[3,21] Acid mediated hydrolysis of the anomeric methoxy using HCl in THF and then synthesis of the anomeric silyl ether using TIPSCl and imidazole in DMF provided **136**

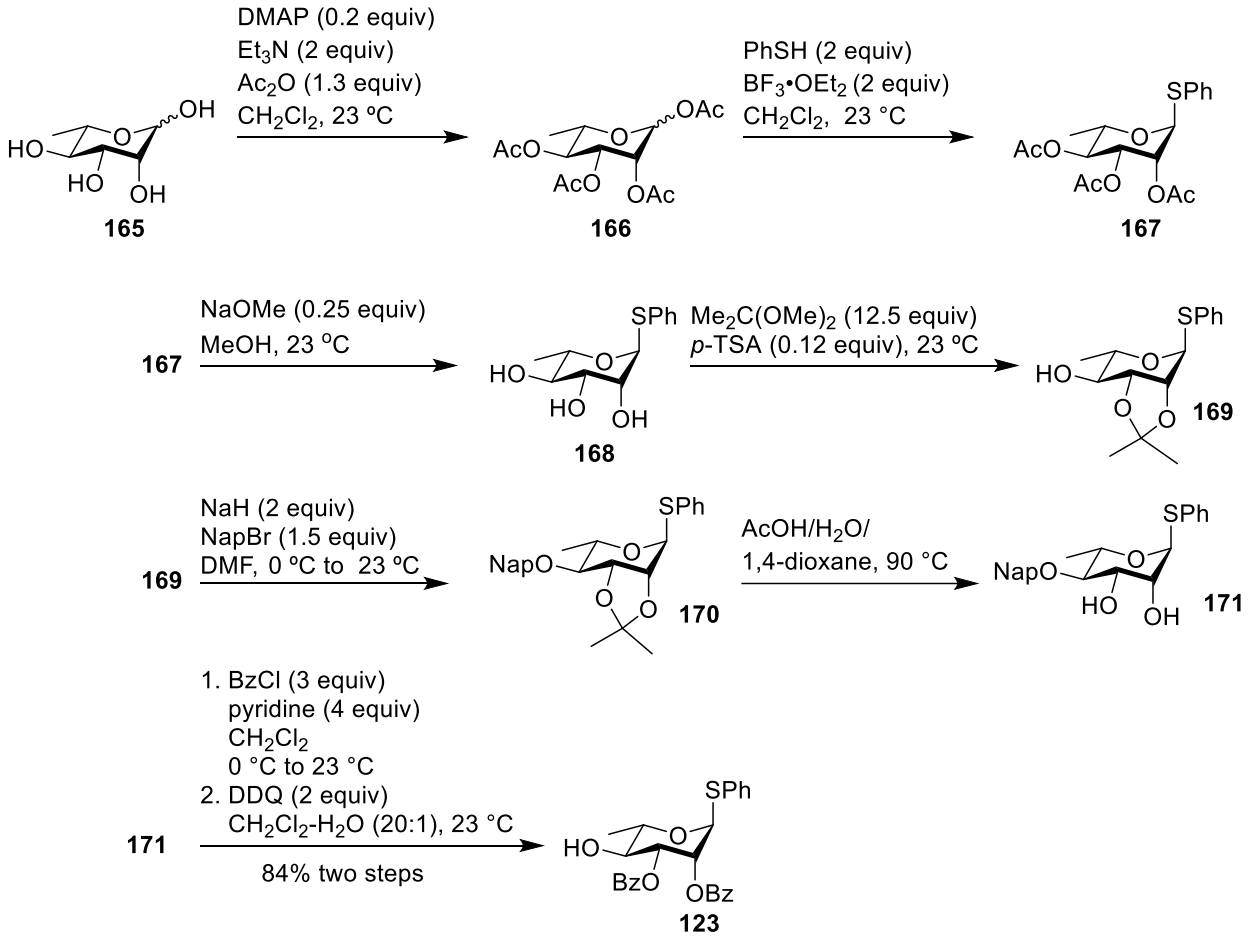
in 75% yield over two steps (Scheme 2.5).[3] Next, a two-step process starting with the removal of the Cbz protection from the C-3 nitrogen using Pd/C and H_2 gas in THF followed by the synthesis of the C-3 azide using TfN_3, $CuSO_4 \cdot 5H_2O$, and $NaHCO_3$ in a mixture of H_2O-toluene-MeOH afforded **187** in 79% yield over two steps (Scheme 2.5).[3,9,22] Then, the C-4 hydroxyl was benzyl protected using BnBr and NaH in DMF to provide **137** in 85% yield (Scheme 2.5). The synthesis of 4-*epi*-L-vancosamine **107** was completed in 82% yield with the hydrolysis of the anomeric silyl ether using Bu_4NF in THF (Scheme 2.5).[3]



Scheme 2.5 Synthesis of 4-*epi*-L-vancosamine **107** from vancomycin HCl **3**.[1,3,9,18–22]

The third monosaccharide needed for the synthesis of tetrasaccharide **194** is L-rhamnose **123**. To this end, a published procedure was used to synthesize **171** in six steps from commercially available L-rhamnose **165** (Scheme 2.6).[12,23,24] These steps include acetylation, thioglycoside formation, deacetylation, C-2 and C-3 acetonide synthesis, C-4 naphthol methyl

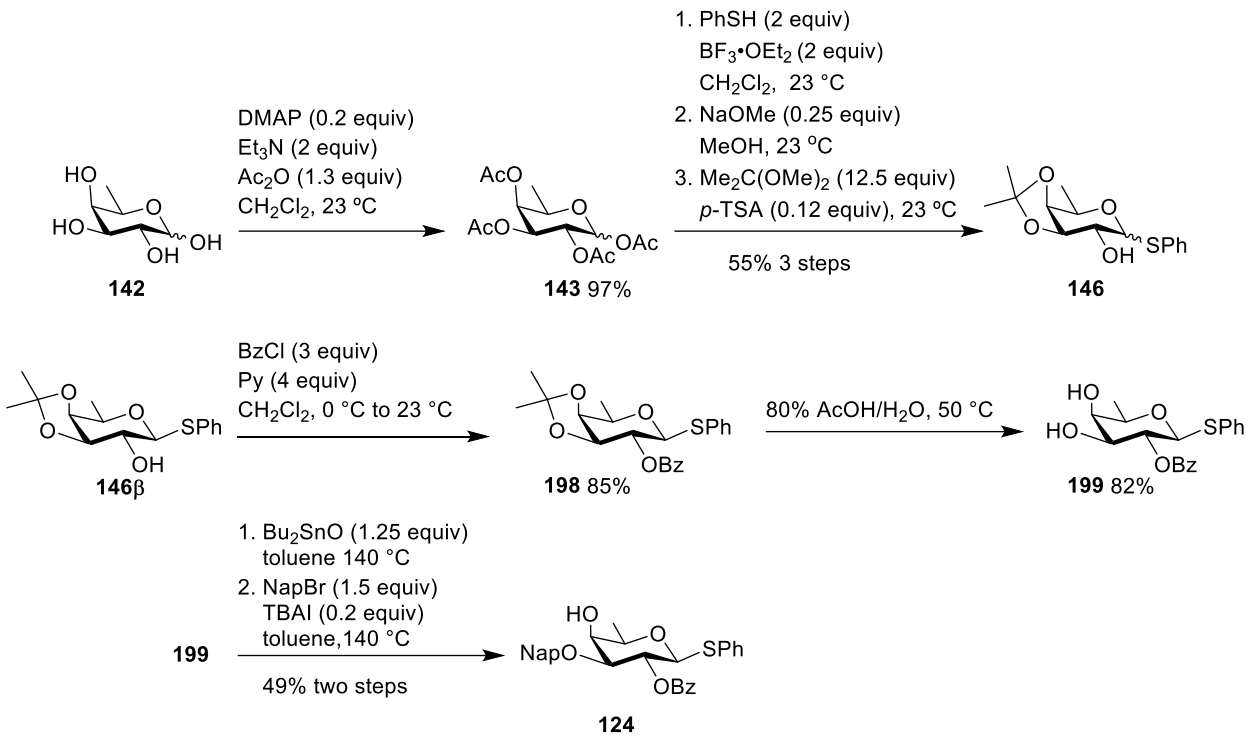
ether installation, and acetonide removal (Scheme 2.6). Starting from **171**, the C-2 and C-3 hydroxyls were benzoyl protected using benzoyl chloride (BzCl) and pyridine in CH_2Cl_2 which was subsequently treated with DDQ in a mixture of CH_2Cl_2 and H_2O (20:1) to provide **123** in 84% yield over two steps (Scheme 2.6).[1,3,13]



Scheme 2.6 Synthesis of L-rhamnose **123** from L-rhamnose **165**.[1,12,23,24]

The final monosaccharide needed for the synthesis of tetrasaccharide **194** is D-fucose **124** which can be traced back to commercially available D-fucose **142**.[3,12–14] To this end, each of the hydroxyls of D-fucose **142** were acetylated using Ac_2O, Et_3N, and DMAP in CH_2Cl_2 to afford **143** in 97% yield (Scheme 2.7). Next, glycosylation with PhSH and $BF_3 \cdot OEt_2$ in CH_2Cl_2 was used to install the anomeric thiol prior to deacetylation using NaOMe in MeOH and C-3 and C-4 acetonide protection using 2,2-dimethoxypropane and *p*-toluenesulfonic acid (*p*-TSA) in CH_2Cl_2 providing

146 in 55% yield over three steps (Scheme 2.7). Next, the β-anomer of **146β** was subjected to treatment with BzCl and pyridine in CH_2Cl_2 which installed the C-2 ester protection to afford **198** in 85% yield (Scheme 2.7).[1,14] Then, the C-3 and C-4 acetonide protection was removed using 80% AcOH in H_2O providing **199** in 82% yield (Scheme 2.7).[11–13] Lastly, D-Fucose **124** was synthesized in 49% yield by a two-step selective protection of the C-3 hydroxyl using Bu_2SnO in toluene prior to the addition of NapBr and TBAI (Scheme 2.7).[1–3,13]



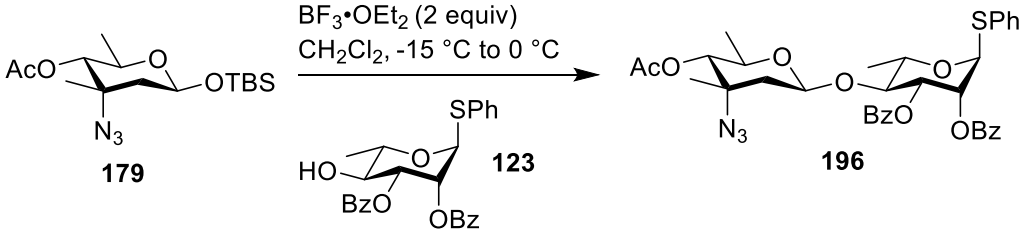
Scheme 2.7 Synthesis of D-fucose **124** from D-fucose **142**.[1–3,11–14]

2.3 Disaccharide Synthesis

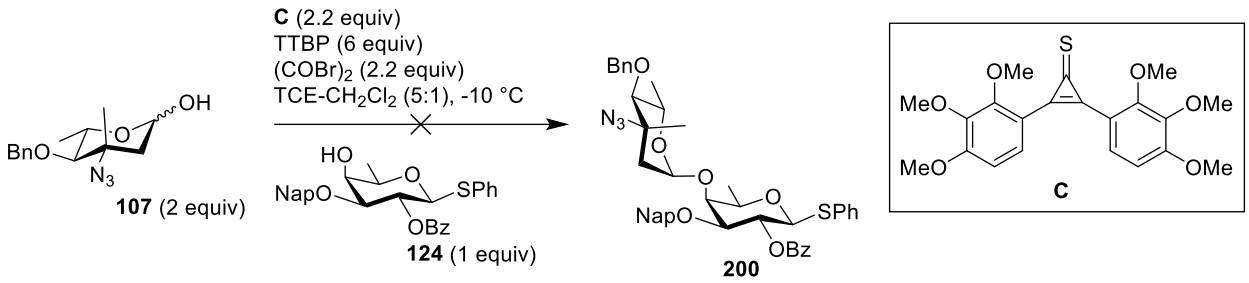
Having synthesized the four monosaccharides needed for the synthesis of tetrasaccharide **194**, the Bennett lab's focus was shifted to the synthesis of D-saccharosamine-L-rhamnose disaccharide **196**. The Lewis acid mediated glycosylation conditions that were used for the synthesis of D-saccharosamine-L-rhamnose disaccharide **180** were used as the initial starting point (Scheme 1.49). [1,2] The conditions used for the synthesis of **180** required the donor species **179** to be in excess of three equivalents to one equivalent of acceptor species **123** (Scheme

1.49).[2] These conditions provided the desired disaccharide **196**, but in only 46% yield (Table 2.1, entry 1).[1,2] The coupling constant for the anomeric proton of the D-saccharosamine moiety of disaccharide **196** is 9.2 Hz confirming the β-linkage was synthesized.[1] The Wan lab reported a coupling constant of 9.2 Hz for the anomeric proton of the D-saccharosamine moiety of D-saccharosamine-L-rhamnose disaccharide **97**.[25] The Bennett lab reported a coupling constant of 9.5 Hz for the anomeric proton of the D-saccharosamine moiety of D-saccharosamine-L-rhamnose disaccharide **180**.[2,25] In an attempt to improve the yield of the glycosylation reaction, the equivalence of donor species **179** was increased to four equivalents to one equivalent of acceptor species **123** (Table 2.1, entry 2).[1] Unfortunately, the increase in donor equivalence led to a decrease in the yield of **196** to 31% yield (Table 2.1, entry 2). Therefore, the stoichiometry from the first attempt was switched so that the acceptor species **123** was in three equivalents excess to one equivalent of donor species **179**. Changing the stoichiometry afforded disaccharide **196** in 75% yield (Table 2.1, entry 3).[1]

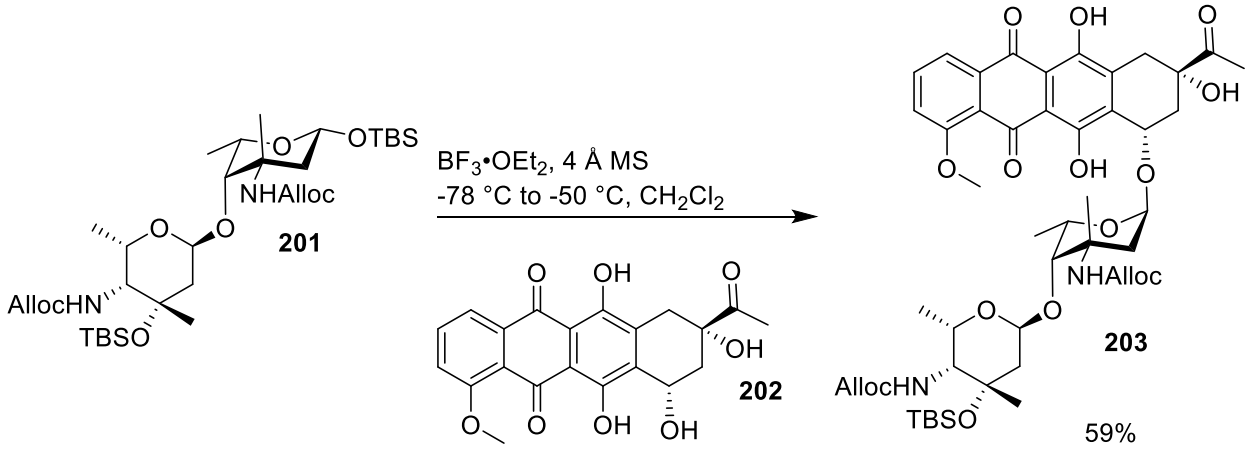
Table 2.1 D-Saccharosamine-L-Rhamnose Disaccharide **196** Synthesis and Optimizations[1]



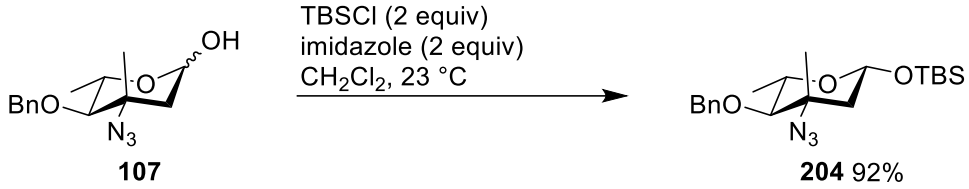
entry	**179**:**123** ratio	yield %, **196** (**α**:**β**)
1	3:1	46 β-only
2	4:1	31 β-only
3	1:3	75 β-only



Scheme 2.8 Attempt at the Synthesis of 4-*epi*-L-Vancosamine-D-Fucose Disaccharide **200** from 4-*epi*-L-vancosamine **107** and D-fucose **124**.[1,3]



Scheme 2.9 Townsend lab's synthesis of L-vancosamine glycoside **203** from 1-OTBS L-vancosamine donor **201**.[26]

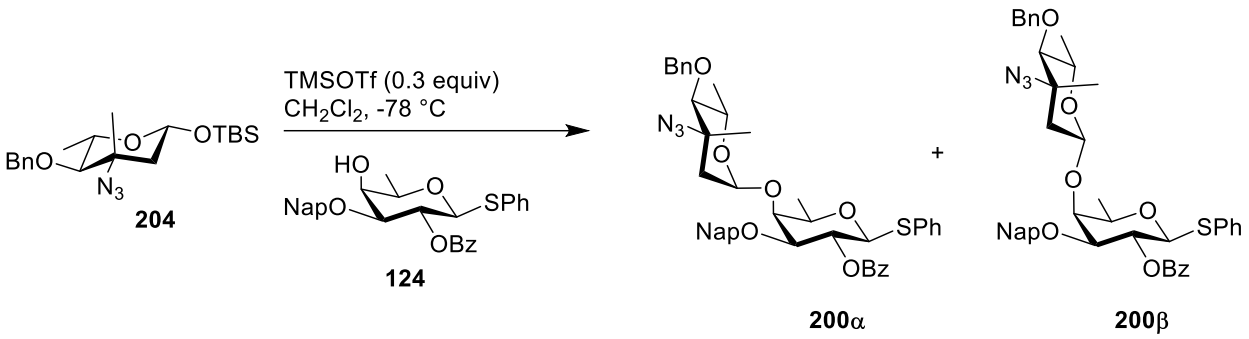


Scheme 2.10 Synthesis of 4-*epi*-L-Vancosamine **204** from 4-*epi*-L-vancosamine **107**.[1,2]

The final piece needed for the synthesis of tetrasaccharide **193** is 4-*epi*-L-vancosamine-D-fucose disaccharide **200**. The original plan was to use the Bennett lab's dehydrative glycosylation method for the synthesis of disaccharide **200** (Scheme 2.8, Scheme 1.5, Scheme 1.37). However, after an initial attempt to synthesize **200** using the dehydrative method failed, an alternative method used by the Townsend lab for the synthesis of an α-linked glycoside **203** with

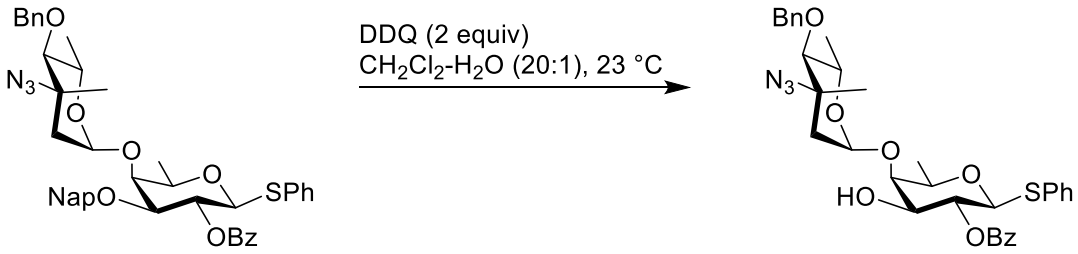
1-OTBS vancosamine glycoside donor **201** was chosen to be a promising approach (Scheme 2.9, Scheme 2.8).[1,26] To this end, 1-OTBS silyl ether 4-*epi*-L-vancosamine **204** was synthesized in 92% yield by treatment of **107** with TBSCl and imidazole in CH_2Cl_2 (Scheme 2.10).[1] Next, **204** and D-fucose **124** were reacted with TMSOTf in CH_2Cl_2 to afford the desired disaccharide **200** in 70% yield with (4:1) α:β selectivity (Table 2.2, entry 1).[1] The coupling constant for the anomeric proton of the 4-*epi*-L-vancosamine moiety of disaccharide **200α** is 4.2 Hz confirming the α-linkage was synthesized.[1] The Bennett lab reported a coupling constant of 4.3 Hz for anomeric proton of the 4-*epi*-L-vancosamine moiety of disaccharide **151**.[3] Reasoning that the α-selectivity of the glycosylation could be improved by exploiting the "ether effect", the glycosylation was run with a one to one solvent ratio of CH_2Cl_2 and THF, which unfortunately led to no product formation (Table 2.2, entry 2).[1,27–30] Having failed to improve the α-selectivity of the reaction by changing the solvent, it was decided that the selectivity could be a result of *in-situ* anomerization caused by the acidic nature of the reaction.[1,31–34] The nonnucleophilic base 2,4,6-tri-tert-butylpyridine (TTBP) had been used in glycosylation reactions as a proton scavenger and therefore the reaction was repeated with the addition of TTBP.[1,35–38] Unfortunately, this again led to no product formation (Table 2.2, entry 3). It was then hypothesized that over longer reaction times *in-situ* anomerization could occur, which would lead to formation of the β-linked disaccharide.[32–34,39,40] To test this hypothesis, the glycosylation was run for eight hours which provided **200** in slightly lower yield, but with increased α-selectivity (Table 2.2, entry 4). Additionally, reducing the reaction time to only 5 hours led to the synthesis of **200** in 93% yield with good α-selectivity (8:1) (Table 2.2, entry 5).[1] Decreasing the equivalence of acceptor species had no effect on selectivity or yield (Table 2.2, entry 6). Deprotection of **200α** using DDQ in a mixture of CH_2Cl_2 and H_2O (20:1) provided **121** in 69% yield (Scheme 2.11).[1,3,13]

Table 2.2 4-*epi*-L-Vancosamine-D-Fucose Disaccharide Synthesis and Optimizations[1]



entry	time (h)	**204**:**124**	yield %, **200** (**α**:**β**)
1	16	1:2	70, 4:1
2[c]	16	1:2	NR [b]
3[a]	16	1:2	NR [b]
4	8	1:2	64, 16:1
5	5	1:2	93, 8:1
6	5	1:1.5	92, 8:1

[a]Reaction is run with the addition of TTBP. [b]NR: no reaction. [c]Reaction run with a mixture of CH_2Cl_2:THF (1:1).[1]

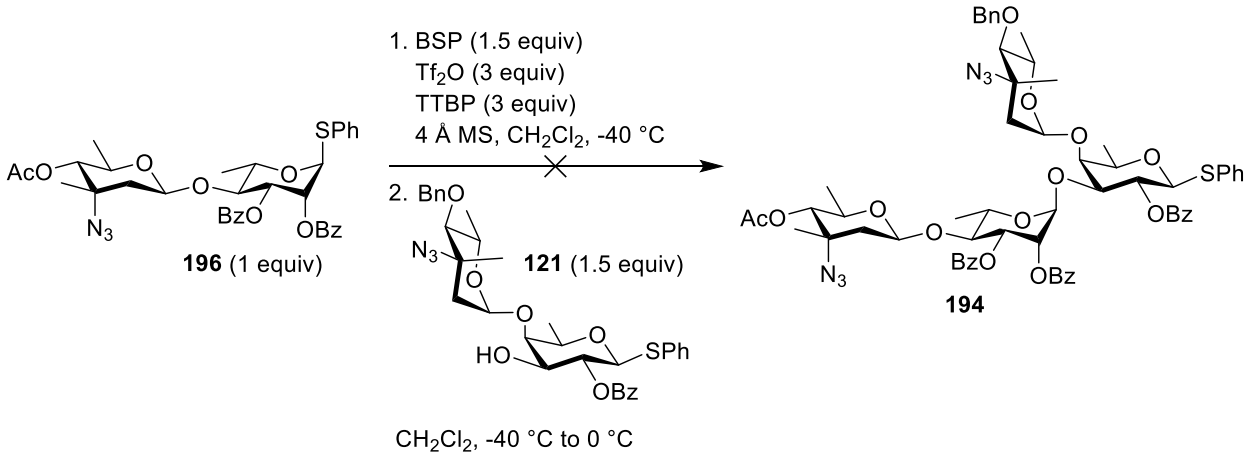


Scheme 2.11 Synthesis of 4-*epi*-L-Vancosamine-D-Fucose Disaccharide **121** from 4-*epi*-L-Vancosamine-D-Fucose Disaccharide **200α**.[1]

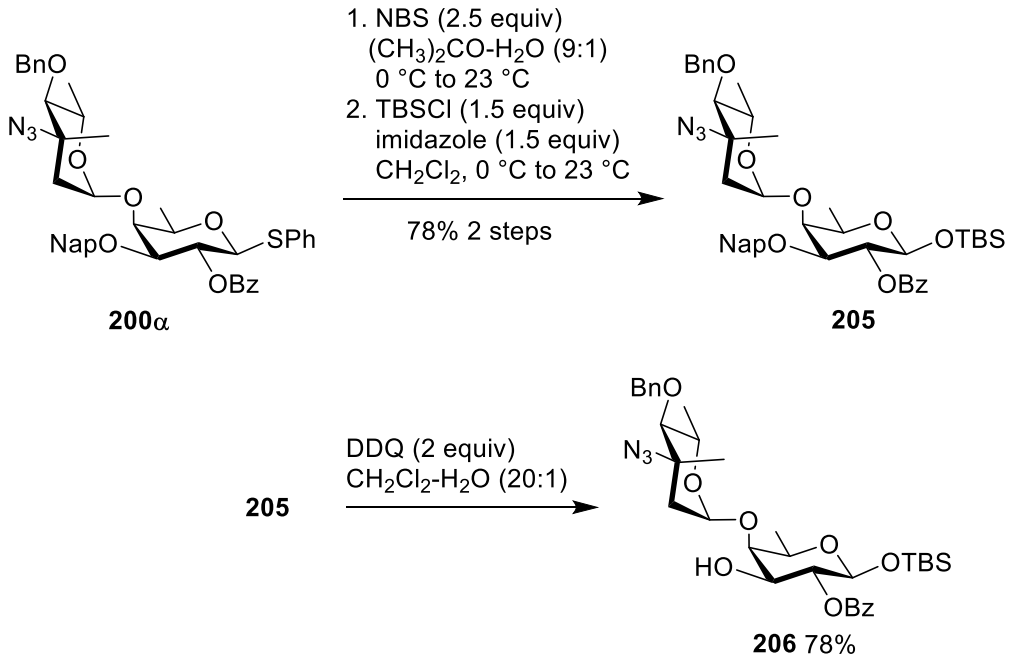
2.4 Synthesis of the Branched Tetrasaccharide of Saccharomicin A

With both coupling partners in hand, the Bennett lad sought to synthesize tetrasaccharide **194**. Having had success using the BSP/Tf_2O method for the [2+1] synthesis of trisaccharide **181**, this approach seemed promising for the synthesis of the α-linkage between disaccharides **196** and **121** (Scheme 1.49).[1,2] To this end, disaccharides **196** and **121** were reacted with BSP, Tf_2O, and TTBP in CH_2Cl_2, but unfortunately the desired tetrasaccharide was not synthesized (Scheme 2.12). The BSP/Tf_2O reaction conditions caused disaccharide **121** to degrade through intermolecular aglycone transfer from **121** to the activated donor.[41] In an attempt to couple disaccharides **196** and **121** using alternative chemistries, **196** and **121** were reacted in the presence of N-iodosuccinimide (NIS) and trifluoromethanesulfonic acid (TfOH), which again failed to provide the desired product. Reasoning that the intermolecular aglycone transfer could be mitigated by using an aglycone that would better withstand the BSP/Tf_2O reaction conditions, the synthesis of 1-OTBS disaccharide **206** from disaccharide **200α** became the new objective. With this aim in mind, the anomeric thiophenol of disaccharide **200α** was hydrolyzed using NBS in acetone-H_2O (9:1) and subsequently treated with TBSCl and imidazole in CH_2Cl_2 to afford **205** in 78% yield over two steps (Scheme 2.13).[1,16,17,26] Deprotection of the C-4 naphthyl methyl ether of the D-fucose moiety of **205** provided **206** in 78% yield (Scheme 2.13).[1,3,13,42]

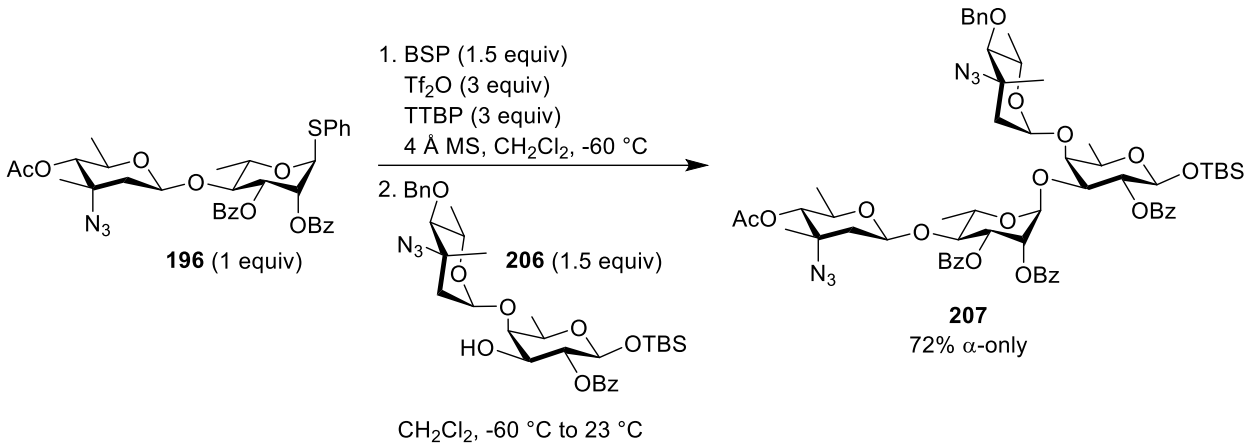
Having synthesized the 1-OTBS disaccharide **206**, the Bennett lab could attempt the [2+2] coupling using the BSP/Tf_2O method for the synthesis of tetrasaccharide **207**. Fortunately, coupling of **196** and **206** using the BSP/Tf_2O method provided the desired tetrasaccharide **207** in 72% yield (Scheme 2.14).[1]



Scheme 2.12 Attempt at the [2+2] Synthesis of Tetrasaccharide **193** from disaccharides **195** and **196**.[1]



Scheme 2.13 Synthesis of 4-*epi*-L-Vancosamine-D-Fucose Disaccharide **206** from 4-*epi*-L-Vancosamine-D-Fucose Disaccharide **200α**.[1–3,13]



Scheme 2.14 Synthesis of the Branched Tetrasaccharide of Saccharomicin A **207**.[1,2]

2.5 Conclusion

In conclusion, the branched tetrasaccharide fragment of saccharomicin A **207** was synthesized through the union of disaccharides **196** and **206**. Disaccharides **195** and **200α** were synthesized using the Lewis acid 1-OTBS chemistry.[1] After optimization, the Lewis acid promoted couplings resulted in good yields with high selectivity. Although they are similar, donor **179** and donor **204** provide the opposite anomer when promoted by Lewis acids. The underlying cause of the different selectivity's is unclear and further investigation is needed to better understand the reaction mechanisms. The synthesis of tetrasaccharide **207** has provided the missing piece needed to synthesize the backbone of saccharomicin A. Importantly, only one tetrasaccharide fragment and the aglycone remain to be synthesized for all the fragments of saccharomicin A to be synthesized.

2.6 General Experimental Details

All reactions were performed under an argon atmosphere unless otherwise stated. Cryogenic temperatures were obtained using a cryostat with 2-propanol bath. Column chromatography was performed using a Yamazan Smart Flash W-Prep 2XY automated purification system equipped with universal silica gel columns unless otherwise specified. When used, flash column chromatography was performed on silica gel, 230-400 Mesh. Analytical and preparative thin layer chromatography was carried out on silica gel F254 plates. Products were visualized using UV or by staining with 7.5% aqueous sulfuric acid. NMR spectra were recorded on a Bruker Avance III NMR spectrometer at 500 MHz for ^{1}H-NMR and 125 MHz for ^{13}C-NMR. Chemical shifts are reported in ppm relative to C_6D_6 or $CDCl_3$ (for ^{1}H-NMR and ^{13}C-NMR). For ^{1}H NMR spectra, data are reported as follows: δ shift, multiplicity [s = singlet, m = multiplet, t = triplet, d = doublet, q = quartet, brs = broad singlet, dt = doublet of triplets, dq = doublet of quartets, and dd = doublet of doublets], coupling constants are reported in Hz. Low resolution mass spectra (LRMS) were recorded using a Finnigan LTQ ESI-MS with an additional APCI source. High resolution mass spectra (HRMS) were obtained on Electro Spray Ionization (ESI) on a Agelent TOF instrument in the positive mode. Optical rotations were measured on a Rudolph Research Analysis AUTOPUL IV polarimeter @ 589 nm in a 5 cm cell at 23 °C.

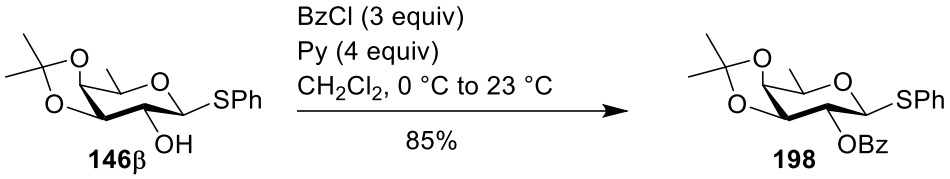
2.7 Materials

Prior to running all glycosylation reactions, donors and acceptors were dried by azeotropic removal of water using anhydrous toluene followed by anhydrous dichloromethane on a rotary evaporator before stirring overnight under vacuum. Solvents for reactions were dried using an Innovative Technologies PureSolv 400 system. Dichloromethane (DCM) used for glycosylations was stored over 4 Å molecular sieves beads that had been microwaved in a conventional microwave ten times for 15 seconds and flame dried under vacuum three times. Trifluoromethanesulfonic anhydride (Tf_2O) and trimethylsilyl trifluoromethanesulfonate (TMSOTf)

were distilled using a short path apparatus equipped with a cow adapter directly before use. Borontriflouride diethyl etherate ($BF_3 \cdot OEt_2$) was distilled over calcium hydride (CaH_2) using a short path apparatus equipped with a cow adapter directly before use. Only portions of the distilled reagents that were collected at their exact boiling temperatures were used in glycosylation reactions. 4 Å molecular sieves powder were activated by flame drying under vacuum three times, cooled to room temperature, and directly used in glycosylation. All other chemicals were purchased at the highest possible quality and used as received.

2.8 Experimental Procedures

Phenyl 2-O-benzoyl-6-deoxy-3,4-O-isopropylidene-1-thio-β-D-fucopyranoside (**198**)



A round bottom flask with stir bar was flame dried prior to the addition of thioglycoside **146β** (0.50 g, 1.689 mmol). The substrate was then dissolved in anhydrous CH_2Cl_2 (10 mL) before being cooled to 0 °C using an ice/water bath. After stirring for 15 minutes, anhydrous pyridine (0.55 mL, 6.827 mmol) followed by BzCl (0.59 mL, 5.079 mmol) were added and the reaction was allowed to warm to 23 °C. After 23 h, the reaction was quenched with a saturated aqueous solution of $NaHCO_3$ (4 mL), then diluted with CH_2Cl_2 and H_2O. The mixture was extracted 5 times using CH_2Cl_2 and the combined organic layers were dried over Na_2SO_4, filtered, and concentrated in *vacuo*. The crude residue was purified using a Yamazan Smart Flash W-Prep 2XY automated purification system equipped with universal silica gel column (100% toluene to 10% EtOAc in toluene) to afford **198** (0.572 g, 85%) as a white solid.

$[\alpha]_D^{23}$ = +42.4 (*c* 0.42, CH_2Cl_2).

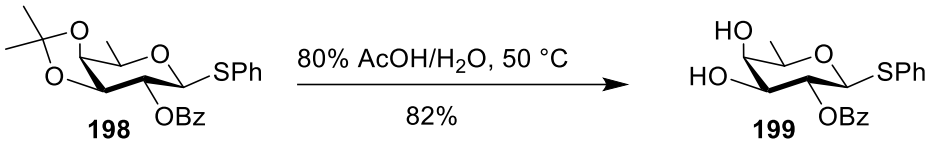
^{1}H NMR (500 MHz, C_6D_6) δ = 8.20 (d, J = 7.4 Hz, 2H), 7.54 (d, J = 7.3 Hz, 2H), 7.14 – 6.93 (m, 6H), 5.70 – 5.66 (m, 1H), 4.63 (d, J = 10.1 Hz, 1H), 4.09 – 4.06 (m, 1H), 3.58 (d, 3.5 Hz, 1H), 3.33 – 3.32 (m, 1H), 1.55 (s, 3H), 1.35 (d, J = 6.4 Hz, 3H), 1.20 (s, 3H).

^{13}C NMR (125 MHz, C_6D_6) δ = 165.4, 134.2, 133.0, 132.9, 130.9, 130.2, 129.0, 128.6, 128.3, 128.2, 128.0,127.8, 110.3, 85.8, 77.7, 76.7, 72.7, 72.5, 28.0, 26.6, 17.1.

LRMS (ESI) *m/z* $[M+Na]^+$ Calcd for $C_{22}H_{24}NaO_5S$ 423.12; Found 423.18.

HRMS (ESI) *m/z* $[M+Na]^+$ Calcd for $C_{22}H_{24}NaO_5S$ 423.1242; Found 423.1243.

Phenyl 2-O-benzoyl-6-deoxy-1-thio-β-D-fucopyranoside (**199**)



To a round bottom flask with stir bar was added thioglycoside **198** (0.431 g, 1.077 mmol) and 80% AcOH/H_2O. A condenser was attached to the round bottom flask and the reaction was heated under an argon atmosphere to 50 °C using an oil bath. After 16 h, the reaction was allowed to cool to 23 °C before being concentrated in *vacuo*. AcOH and H_2O were removed by azeotrope three times with toluene. The crude residue was purified using a Yamazan Smart Flash W-Prep 2XY automated purification system equipped with universal silica gel column (100% toluene to 80% EtOAc in toluene) to afford **199** (0.319 g, 82%) as a white solid.

$[\alpha]_D^{23}$ = -18.8 (*c* 0.26, CH_2Cl_2).

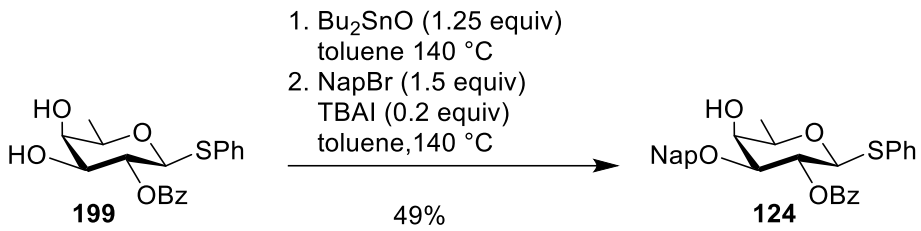
^{1}H NMR (500 MHz, C_6D_6) δ = 8.22 (d, J = 7.4 Hz, 2H), 7.57 (d, J = 7.3 Hz, 2H), 7.12 – 7.05 (m, 3H), 7.00 – 6.91 (m, 3H), 5.36 – 5.32 (m, 1H), 4.55 (d, J = 10.0 Hz, 1H), 3.46 – 3.42 (m, 1H), 3.20 (Brs, 1H), 2.99 – 2.96 (m, 2H), 1.69 – 1.67 (m, 1H), 1.12 (d, J = 6.4 Hz, 3H).

^{13}C NMR (125 MHz, $CDCl_3$) δ = 167.2, 133.6, 132.81, 132.75, 130.2, 129.6, 129.0, 128.6, 128.1, 86.0, 74.9, 74.4, 72.4, 72.1, 16.8.

LRMS (ESI) *m/z* $[M+Na]^+$ Calcd for $C_{19}H_{20}NaO_5S$ 383.09; Found 383.18.

HRMS (ESI) *m/z* $[M+Na]^+$ Calcd for $C_{19}H_{20}NaO_5S$ 383.0929; Found 383.0927.

Phenyl 6-deoxy-3-O-(2-naphthyl)methyl-2-O-benzoyl-1-thio-β-D-fucopyranoside (**124**)



A round bottom flask with stir bar was flame dried before the addition of diol **199** (0.210 g, 5.832 mmol) and Bu_2SnO (0.182 g, 0.7312 mmol). Anhydrous toluene (25 mL) was added to the round bottom flask which was then equipped with a Dean-Stark apparatus filled with anhydrous toluene and a condenser. The reaction was heated to 140 °C using an oil bath for 6 h and then cooled to 23 °C before the addition of NapBr (0.194 g, 0.8775 mmol) and TBAI (0.043 g, 0.1164 mmol). The reaction was then heated to 140 °C using an oil bath with the Dean-Stark apparatus and condenser for 18.5 h. Then, the reaction was allowed to cool to 23 °C before being concentrated in *vacuo*. The crude residue was purified using a Yamazan Smart Flash W-Prep 2XY automated purification system equipped with universal silica gel column (100% hexanes to 30% acetone in hexanes) to afford **124** (0.143 g, 49%) as a white solid.

$[\alpha]_D^{23}$ = +43.8 (*c* 0.32, CH_2Cl_2).

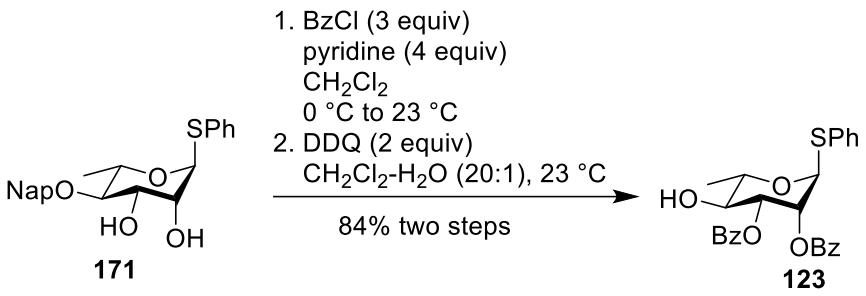
^{1}H NMR (500 MHz, C_6D_6) δ = 8.18 (d, J = 7.4 Hz, 2H), 7.62 (d, J = 7.5 Hz, 2H), 7.57 – 7.55 (m, 1H), 7.52 – 7.50 (m, 2H), 7.45 (d, J = 8.4 Hz, 1H), 7.24 – 7.19 (m, 3H), 7.14 (s, 1H), 7.09 – 7.06 (m, 2H), 7.02 – 6.99 (m, 2H), 6.95 – 6.92 (m, 1H), 5.89 (t, J = 9.7 Hz, 1H), 4.58 (d, J = 10.1 Hz, 1H), 4.43 (d, J = 12.6 Hz, 1H), 4.29 – 4.26 (m, 1H), 3.48 (Brs, 1H), 3.39 (dd, J = 9.4, 3.1 Hz, 1H), 3.03 – 2.99 (m, 1H), 2.15 (s, 1H), 1.32 (d, J = 6.4 Hz, 3H).

^{13}C NMR (125 MHz, C_6D_6) δ = 165.3, 135.5, 133.8, 133.7, 133.6, 133.3, 133.0, 131.1, 130.2, 129.0, 128.6, 128.4, 128.2, 128.0, 126.8, 126.4, 126.3, 126.0, 86.4, 80.1, 74.7, 71.0, 69.9, 68.9, 17.0.

LRMS (ESI) *m/z* $[M+Na]^+$ Calcd for $C_{30}H_{28}NaO_5S$ 523.16; Found 523.45.

HRMS (ESI) *m/z* $[M+Na]^+$ Calcd for $C_{30}H_{28}NaO_5S$ 523.1555; Found 523.1561.

Phenyl 6-deoxy- 2,3-di-O-benzoyl-1-thio-α-L-mannopyranoside (**123**)



A round bottom flask with stir bar was flame dried before the addition of diol **171** (1.00 g, 2.524 mmol). Anhydrous CH_2Cl_2 (20 mL) was added before cooling the mixture to 0 °C using an ice/water bath. After stirring for 20 minutes, anhydrous pyridine (0.82 mL, 10.10 mmol) followed by BzCl (0.88 mL, 7.575 mmol) were added and the reaction was allowed to warm to 23 °C for 22 h. The reaction was quenched with a saturated aqueous solution of $NaHCO_3$ (5 mL), then diluted with CH_2Cl_2 and H_2O. The mixture was extracted 5 times using CH_2Cl_2 and the combined organic layers were dried over Na_2SO_4, filtered, and concentrated in *vacuo*. The crude residue was purified by flash column chromatography on silica gel (5% to 50% EtOAc in hexanes) to afford intermediate L-rhamnose (1.506 g) as a white solid which was used directly in the next step. To a round bottom flask with stir bar was added intermediate L-rhamnose (1.506 g, 2.493 mmol), CH_2Cl_2 (150 mL), and H_2O (7.5 mL). Then, DDQ (1.132 g, 4.987 mmol) was added and the resulting reaction was stirred under an argon atmosphere at 23 °C. After 4.5 h, the reaction was diluted with CH_2Cl_2. The mixture was washed twice with NaOH (2 M). The aqueous portion was then extracted 5 times using CH_2Cl_2 and the combined organic layers were dried over Na_2SO_4, filtered, and concentrated in *vacuo*. The crude residue was purified by flash column chromatography on silica gel (100% toluene to 5% EtOAc in hexanes) to afford **123** (0.989 g, 84%) as a white solid over two steps.

$[\alpha]_D^{23}$ = -59.5 (*c* 0.19, CH_2Cl_2).

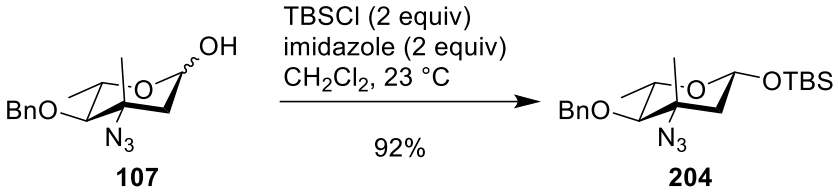
^{1}H NMR (500 MHz, C_6D_6) δ = 8.11 (d, J = 7.6 Hz, 2H), 8.02 (d, J = 7.6 Hz, 2H), 7.42 (d, J = 7.4 Hz, 2H), 7.11 – 7.08 (m, 1H), 7.03 – 6.89 (m, 8H), 6.19 (s, 1H), 5.78 (dd, J = 9.9, 3.1 Hz, 1H), 5.62 (s, 1H), 4.43 – 4.38 (m, 1H), 4.02 – 3.97 (m, 1H), 2.08 (d, J = 6.0 Hz, 1H), 1.41 (d, J = 6.1 Hz, 3H).

^{13}C NMR (125 MHz, C_6D_6) δ = 166.7, 165.4, 134.3, 133.3, 133.2, 132.1, 130.3, 130.2, 130.1, 129.3, 128.7, 128.5, 128.3, 128.2, 128.0,127.7, 86.2, 74.0, 72.9, 72.4, 70.6, 17.9.

LRMS (ESI) *m/z* $[M+Na]^+$ Calcd for $C_{26}H_{24}NaO_6S$ 487.12; Found 487.36.

HRMS (ESI) *m/z* $[M+Na]^+$ Calcd for $C_{26}H_{24}NaO_6S$ 487.1192; Found 487.1201.

Triisopropylsilyl 2,3,6-trideoxy-3-C-methyl-3-azido-4-O-benzyl-β-L-*arabino*-hexopyranoside (**204**)



107 **204**

To a round bottom flask with stir bar was added hemiacetal **107** (1.00 g, 3.608 mmol) and CH_2Cl_2 (40 mL). Then, TBSCl (1.087 g, 7.212 mmol) and imidazole (0.50 g, 7.345 mmol) were added, and the reaction was stirred at 23 °C. After 17 h, the reaction was diluted with CH_2Cl_2 and H_2O. The mixture was extracted 5 times using CH_2Cl_2 and the combined organic layers were dried over Na_2SO_4, filtered, and concentrated in *vacuo*. The crude residue was purified using a Yamazan Smart Flash W-Prep 2XY automated purification system equipped with universal silica gel column (100% toluene to 5% EtOAc in toluene) to afford **204** (1.296 g, 92%) as a colorless oil.

$[\alpha]_D^{23}$ = -16.04 (*c* 0.18, CH_2Cl_2).

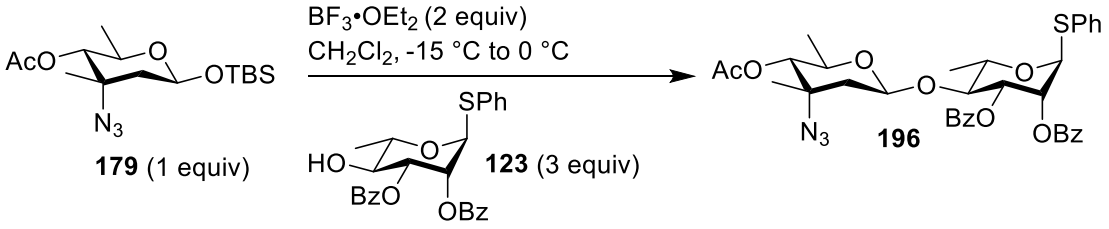
^{1}H NMR (500 MHz, C_6D_6) δ = 7.27 (d, J = 7.5 Hz, 2H), 7.18 (s, 1H), 7.10 – 7.08 (m, 1H), 4.74 – 4.71 (m, 1H), 4.67 (d, J = 11.0, 1H), 4.30 (d, J = 11.0, 1H), 3.23 – 3.18 (m, 1H), 2.96 (d, J = 9.5 Hz, 1H), 1.91 – 1.84 (m, 2H), 1.18 (d, J = 6.1 Hz, 3H), 1.15 (s, 3H), 1.00 (s, 9H), 0.20 (s, 3H), 0.14 (s, 3H).

^{13}C NMR (125 MHz, C_6D_6) δ = 138.6, 128.6, 128.3, 128.2,127.9, 93.7, 85.1, 75.2, 70.7, 63.2, 45.6, 26.0, 18.7, 18.2, 17.3, -3.7, -4.7.

LRMS (ESI) *m/z* $[M+Na]^+$ Calcd for $C_{20}H_{33}N_3NaO_3Si$ 414.22; Found 414.36.

HRMS (ESI) *m/z* $[M+Na]^+$ Calcd for $C_{20}H_{33}N_3NaO_3Si$ 414.2189; Found 414.2207.

Phenyl 4-(6'-deoxy-4'-O-acetyl-3'-azido-3'-C-methyl-1'-β-D-ribo-hexopyranosyl)-6-deoxy-2,3-di-O-benzoyl-1-thio-α-L-mannopyranoside (**196**)



To a flame dried round bottom flask with stir bar were added compounds **179** (0.095 g, 0.2768 mmol) and **123** (0.386 g, 0.8317 mmol). The mixture underwent azeotrope with anhydrous toluene (3 times) followed by co-evaporation with anhydrous CH_2Cl_2 (3 times) before being left to stir under high vacuum overnight. $BF_3{\cdot}OEt_2$ was distilled over CaH_2 immediately before the reaction. Compounds **179** and **123** were dissolved in anhydrous CH_2Cl_2 (14.9 mL) and cooled to -15 °C using a cryostat. After stirring for 15 minutes, $BF_3{\cdot}OEt_2$ (0.07 mL, 0.5672 mmol) was added, and the reaction stirred for 5 h. The cryostat was then turned off and the reaction was allowed to warm to 0 °C over 1 h. The reaction was moved into a 0 °C ice/water bath and stirred for 1 h before being quenched with a saturated aqueous solution of $NaHCO_3$ (20 mL), then diluted with CH_2Cl_2 and H_2O. The mixture was extracted 5 times using CH_2Cl_2 and the combined organic layers were dried over Na_2SO_4, filtered, and concentrated in *vacuo*. The crude residue was purified using a Yamazan Smart Flash W-Prep 2XY automated purification system equipped with universal silica gel column (100% hexanes to 20% EtOAc in hexanes) to afford **196** (0.140 g, 75%) as a white solid.

$[\alpha]_D^{23}$ = -43.8 (*c* 0.21, CH_2Cl_2).

^{1}H NMR (500 MHz, C_6D_6) δ = 8.24 (d, J = 7.3 Hz, 2H), 8.11 (d, J = 7.7 Hz, 2H), 7.44 (d, J = 7.7 Hz, 2H), 7.10 – 6.91 (m, 9H), 6.40 (d, J = 1.3 Hz, 1H), 6.12 (dd, J = 9.8, 3.2 Hz, 1H), 5.68 (s, 1H), 5.16 (d, J = 9.2 Hz, 1H), 4.75 – 4.70 (m, 1H), 4.58 (d, J = 9.6 Hz, 1H), 4.41 (t, J = 9.6 Hz, 1H),

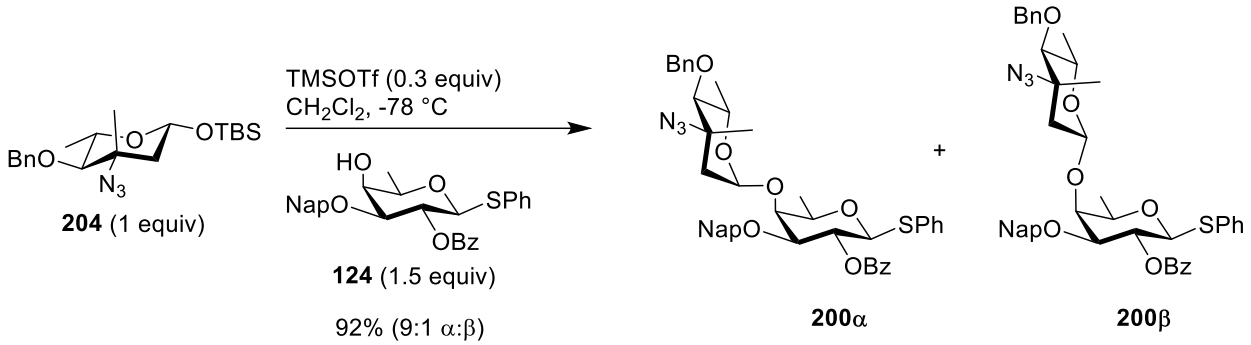
3.67 – 3.62 (m, 1H), 1.72 (d, J = 6.2 Hz, 3H), 1.59 (s, 3H), 1.57 (d, J = 13.7, 1H), 1.30 – 1.26 (m, 1H), 1.04 (d, J = 6.2 Hz, 3H), 0.72 (s, 3H).

^{13}C NMR (125 MHz, C_6D_6) δ = 169.3, 165.8, 165.4, 134.4, 133.4, 133.3, 132.1, 130.3, 130.2, 130.1, 129.3, 128.7, 128.6, 128.3, 128.2, 128.0, 127.7, 99.6, 86.3, 78.7, 78.0, 73.8, 73.0, 69.5, 69.2, 62.0, 42.7, 21.5, 19.7, 18.3, 17.8.

LRMS (ESI) *m/z* $[M+Na]^+$ Calcd for $C_{35}H_{37}N_3NaO_9S$ 698.21; Found 698.36.

HRMS (ESI) *m/z* $[M+Na]^+$ Calcd for $C_{35}H_{37}N_3NaO_9S$ 698.2149; Found 698.2144.

Phenyl 4-(6'-deoxy-4'-O-benzyl-3'-azido-3'-C-methyl-1'-α-L-*arabino*-hexopyranosyl)-6-deoxy-3-O-(2-naphthyl)methyl-2-O-benzoyl-1-thio-β-D-galactopyranoside **200α** and Phenyl 4-(6'-deoxy-4'-O-benzyl-3'-azido-3'-C-methyl-1'-β-L-*arabino*-hexopyranosyl)-6-deoxy-3-O-(2-naphthyl)methyl-2-O-benzoyl-1-thio-β-D-galactopyranoside **200β**



To a flame dried round bottom flask with stir bar were added compounds **204** (0.058 g, 0.1483 mmol) and **124** (0.111 g, 0.2219 mmol). The mixture underwent azeotrope with anhydrous toluene (3 times) followed by co-evaporation with anhydrous CH_2Cl_2 (3 times) before being left to stir under high vacuum overnight. TMSOTf was distilled immediately before the reaction. Compounds **204** and **124** were dissolved in anhydrous CH_2Cl_2 (9.6 mL) and cooled to -78 °C using a cryostat. After stirring for 15 minutes, TMSOTf (0.2 mL from stock, 0.04428 mmol) was added and the reaction stirred at -78 °C. After 5 h, the reaction was quenched with Et_3N (5 mL) and saturated aqueous solution of $NaHCO_3$ (5 mL), then diluted with CH_2Cl_2 and H_2O. The mixture was extracted 5 times using CH_2Cl_2 and the combined organic layers were dried over Na_2SO_4, filtered, and concentrated in *vacuo*. The crude residue was purified using a Yamazan Smart Flash W-Prep 2XY automated purification system equipped with universal silica gel column (100% hexanes to 10% acetone in hexanes) to afford **200α** (0.091 g, 81%) and **200β** (0.012 g, 11%) both as a white solids.

For α-anomer:

$[\alpha]_D^{23}$ = -21.2 (*c* 0.42, CH_2Cl_2).

^{1}H NMR (500 MHz, C_6D_6) δ = 8.19 (d, J = 7.7 Hz, 2H), 7.68 (d, J = 7.4 Hz, 2H), 7.56 – 7.53 (m, 3H), 7.46 (d, J = 8.4 Hz, 1H), 7.35 (d, J = 7.7 Hz, 2H), 7.25 – 7.19 (m, 5H), 7.13 – 6.97 (m, 7H),

5.85 – 5.81 (m, 1H), 5.24 (d, J = 4.2 Hz, 1H), 4.79 (d, J = 11.0 Hz, 1H), 4.53 (d, J = 9.8 Hz, 1H), 4.47 (d, J = 12.3 Hz, 1H), 4.41 (d, J = 11.0 Hz, 1H), 4.36 (d, J = 12.3 Hz, 1H), 4.00 – 3.94 (m, 1H), 3.62 (s, 1H), 3.40 (d, J = 9.8 Hz, 1H), 3.05 – 2.99 (m, 2H), 2.17 (d, J = 13.8 Hz, 1H), 1.67 – 1.64 (m, 1H), 1.62 (s, 3H), 1.29 (d, J = 6.2 Hz, 3H), 1.19 (d, J = 6.3 Hz, 3H).

^{13}C NMR (125 MHz, C_6D_6) δ = 165.3, 138.8, 135.5, 134.6, 133.7, 133.6, 133.0, 132.1, 131.2, 130.2, 128.9, 128.7, 128.6, 128.3, 128.2, 128.0, 126.7,126.5, 126.3, 125.8, 98.2, 85.6, 81.9, 75.4, 75.2, 74.7, 72.2, 70.2, 66.7, 63.1, 41.1, 18.9, 18.5, 17.7.

LRMS (ESI) *m/z* $[M+Na]^+$ Calcd for $C_{44}H_{45}N_3NaO_7S$ 782.29; Found 782.45.

HRMS (ESI) *m/z* $[M+Na]^+$ Calcd for $C_{44}H_{45}N_3NaO_7S$ 782.2876; Found 782.2890.

For β-anomer:

$[\alpha]_D^{23}$ = +81.8 (*c* 0.22, CH_2Cl_2).

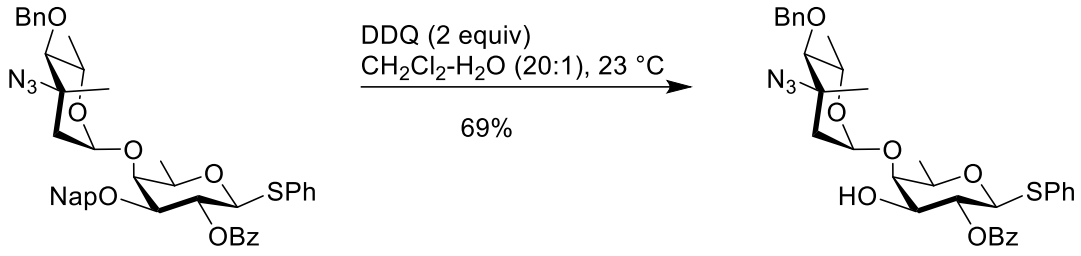
^{1}H NMR (500 MHz, C_6D_6) δ = 8.20 (d, J = 7.5 Hz, 2H), 7.77 (s, 1H), 7.64 (d, J = 7.4 Hz, 2H), 7.61 – 7.60 (m, 1H), 7.55 (d, J = 8.2 Hz, 2H), 7.42 (d, J = 8.4 Hz, 1H), 7.28 (d, J = 7.5 Hz, 2H), 7.25 – 7.23 (m, 2H), 7.12 – 6.98 (m, 9H), 6.07 – 6.03 (m, 1H), 4.91 (d, J = 12.7 Hz, 1H), 4.75 (d, J = 10 Hz, 1H), 4.69 (d, J = 11 Hz, 1H), 4.47 (d, J = 12.8 Hz, 1H), 4.33 – 4.29 (m, 2H), 3.68 (d, J = 2.6 Hz, 1H), 3.51 – 3.49 (m, 1H), 3.21 – 3.16 (m, 1H), 3.06 – 3.02 (m, 1H), 2.87 (d, J = 9.4 Hz, 1H), 1.86 – 1.76 (m, 2H), 1.15 – 1.13 (m, 6H), 1.10 (d, J = 6.3 Hz, 3H).

^{13}C NMR (125 MHz, C_6D_6) δ = 165.3, 138.6, 136.5, 134.2, 133.9, 133.5, 133.14, 133.13, 132.9, 131.3, 130.3, 128.9, 128.6, 128.4, 128.2, 128.0, 127.8, 126.4, 126.3, 126.02, 126.00, 99.3, 86.7, 84.7, 79.4, 75.3, 74.6, 73.8, 70.7, 70.4, 70.1, 63.0, 42.3, 18.6, 17.5, 17.3.

LRMS (ESI) *m/z* $[M+Na]^+$ Calcd for $C_{44}H_{45}N_3NaO_7S$ 782.29; Found 782.36.

HRMS (ESI) *m/z* $[M+Na]^+$ Calcd for $C_{44}H_{45}N_3NaO_7S$ 782.2876; Found 782.2891.

Phenyl 4-O-(6'-deoxy-4'-O-benzyl-3'-azido-3'-C-methyl-1'-α-L-*arabino*-hexopyranosyl)-6-deoxy-2-O-benzoyl-1-thio-β-D-galactopyranoside **121**



200α **121**

To a round bottom flask with stir bar were added disaccharide **200α** (0.226 g, 0.2976 mmol), CH_2Cl_2 (30 mL), and H_2O (1.5 mL). After the sugar was fully dissolved, DDQ (0.135 g, 0.5947 mmol) was added and the reaction stirred at 23 °C. After 23 h, the reaction was diluted with saturated aqueous solution of $NaHCO_3$ (30 mL) and CH_2Cl_2, then further diluted with H_2O. The mixture was extracted 5 times using CH_2Cl_2 and the combined organic layers were dried over Na_2SO_4, filtered, and concentrated in *vacuo*. The crude residue was purified using a Yamazan Smart Flash W-Prep 2XY automated purification system equipped with universal silica gel column (100% hexanes to 30% EtOAc in hexanes) to afford **121** (0.126 g, 69%) as a white solid.

$[\alpha]_D^{23}$ = -85.5 (*c* 0.22, CH_2Cl_2).

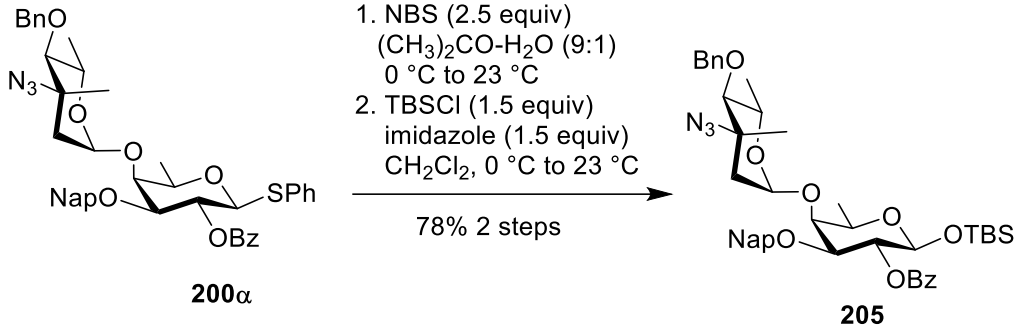
^{1}H NMR (500 MHz, C_6D_6) δ = 8.23 (d, J = 7.3 Hz, 2H), 7.57 – 7.55 (m, 2H), 7.37 (d, J = 7.3 Hz, 2H), 7.21 – 7.18 (m, 2H), 7.13 (d, 6.0 Hz, 1H), 7.12 – 7.06 (m, 3H), 6.92 (t, J = 3.1 Hz, 3H), 5.22 – 5.18 (m, 1H), 5.11 (d, J = 4.2 Hz, 1H), 4.78 (d, J = 10.9 Hz, 2H), 4.45 (d, J = 9.7 Hz, 1H), 4.41 (d, J = 10.9 Hz, 1H), 3.99 – 3.94 (m, 1H), 3.50 – 3.46 (m, 1H), 3.33 (d, J = 2.3 Hz), 3.03 – 2.99 (m, 2H), 2.89 (d, J = 5.9 Hz, 1H), 2.00 (d, J = 13.8 Hz, 1H), 1.68 – 1.64 (m, 1H), 1.53 (s, 3H), 1.25 (d, J = 6.2 Hz, 3H), 1.17 (d, J = 6.4 Hz, 3H).

^{13}C NMR (125 MHz, C_6D_6) δ = 167.9, 138.7, 135.0, 133.6, 130.8, 130.4, 130.2, 129.1, 128.8, 128.7, 128.6, 128.4, 128.2, 128.0, 99.5, 85.6, 84.5, 80.1, 75.8, 75.5, 74.7, 72.7, 66.7, 63.1, 41.4, 19.0, 18.3, 17.4.

LRMS (ESI) *m/z* $[M+Na]^+$ Calcd for $C_{33}H_{37}N_3NaO_7S$ 642.22; Found 642.55.

HRMS (ESI) *m/z* $[M+Na]^+$ Calcd for $C_{33}H_{37}N_3NaO_7S$ 642.2250; Found 642.2276.

Tert-butyldimethylsilyl 4-(6'-deoxy-4'-O-acetyl-3'-azido-3'-C-methyl-1'-α-L-arabino -hexopyranosyl)-6-deoxy-3-O-(2-naphthyl)methyl-2-O-benzoyl-1-thio-β-D-galactopyranoside **205**

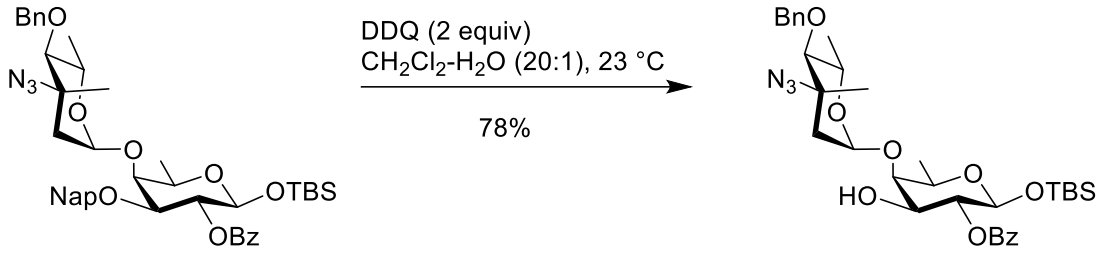


To a round bottom flask with stir bar were added **200α** (0.211 g, 0.2779 mmol), $(CH_3)_2CO$ (18 mL), and H_2O (2 mL). The mixture was cooled to 0 °C using an ice/water bath. After stirring for 10 minutes, NBS (0.124 g, 0.6967 mmol) was added and the reaction was stirred as the ice/water bath warmed to 23 °C over 2.5 h. After 0.75 h the reaction was quenched with a saturated aqueous solution of $NaHCO_3$ (2 mL), then diluted with CH_2Cl_2 and H_2O. The mixture was extracted 5 times using CH_2Cl_2 and the combined organic layers were dried over Na_2SO_4, filtered, and concentrated in *vacuo*. The crude residue was purified using a Yamazan Smart Flash W-Prep 2XY automated purification system equipped with universal silica gel column (100% hexanes to 30% acetone in hexanes) to afford crude 4-*epi*-L-vancosamine-D-fucose disaccharide intermediate as a white solid which was used directly in the next step.

To a round bottom flask with stir bar were added crude 4-*epi*-L-vancosamine-D-fucose disaccharide intermediate and CH_2Cl_2 (10 mL). The mixture was cooled to 0 °C using an ice/water bath. After stirring for 15 minutes, imidazole (0.028 g, 0.4170 mmol) followed by TBSCl (0.063 g, 0.4180 mmol) were added and the reaction stirred from 0 °C to 23 °C. After 23 h, the reaction was diluted with CH_2Cl_2 and H_2O. The mixture was extracted 5 times using CH_2Cl_2 and the combined organic layers were dried over Na_2SO_4, filtered, and concentrated in *vacuo*. The crude residue was purified using a Yamazan Smart Flash W-Prep 2XY automated purification system equipped

with universal silica gel column (100% hexanes to 10% acetone in hexanes) to afford **205** (0.171 g, 78% two steps) as a white solid. $[\alpha]_D^{23}$ = -41.1 (*c* 0.19, CH_2Cl_2). ^{1}H NMR (500 MHz, C_6D_6) δ = 8.19 (d, J = 7.25 Hz, 2H), 7.57 – 7.53 (m, 3H), 7.48 (d, J = 8.4 Hz, 1H), 7.33 (d, J = 7.7 Hz, 2H), 7.27 (d, J = 8.5 Hz, 1H), 7.23 (t, J = 3.8 Hz, 2H), 7.19 (s, 1H), 7.14 – 7.06 (m, 5H), 5.89 (dd, J = 10.0, 7.8 Hz, 1H), 5.37 (d, J = 4.3 Hz, 1H), 4.78 (d, J = 11.0 Hz, 1H), 4.71 (d, J = 7.6 Hz, 1H), 4.54 (d, J = 12.4 Hz, 1H), 4.44 – 4.39 (m, 2H), 4.03 – 3.97 (m, 1H), 3.69 (s, 1H), 3.41 (dd, J = 10.2, 2.3 Hz, 1H), 3.07 – 3.02 (m, 2H), 2.29 (d, J = 13.8 Hz, 1H), 1.75 (s, 3H), 1.73 – 1.70 (m, 1H), 1.30 (d, J = 6.2 Hz, 3H), 1.20 (d, J = 6.3 Hz, 3H), 0.90 (s, 9H), 0.24 (s, 3H), 0.14 (s, 3H). ^{13}C NMR (125 MHz, C_6D_6) δ = 165.4, 138.7, 135.7, 133.8, 133.6, 132.9, 131.3, 130.0, 128.7, 128.6, 128.34, 128.30, 128.2, 128.0, 126.6, 126.5, 126.2, 125.8, 98.0, 97.0, 85.7, 80.5, 75.4, 74.8, 74.1, 72.3, 70.6, 66.6, 63.1, 41.1, 25.9, 18.8, 18.5, 18.1, 17.5, -3.7, -4.4. LRMS (ESI) *m/z* $[M+Na]^+$ Calcd for $C_{44}H_{55}N_3NaO_8Si$ 804.37; Found 804.45. HRMS (ESI) *m/z* $[M+Na]^+$ Calcd for $C_{44}H_{55}N_3NaO_8Si$ 804.3656; Found 804.3679.

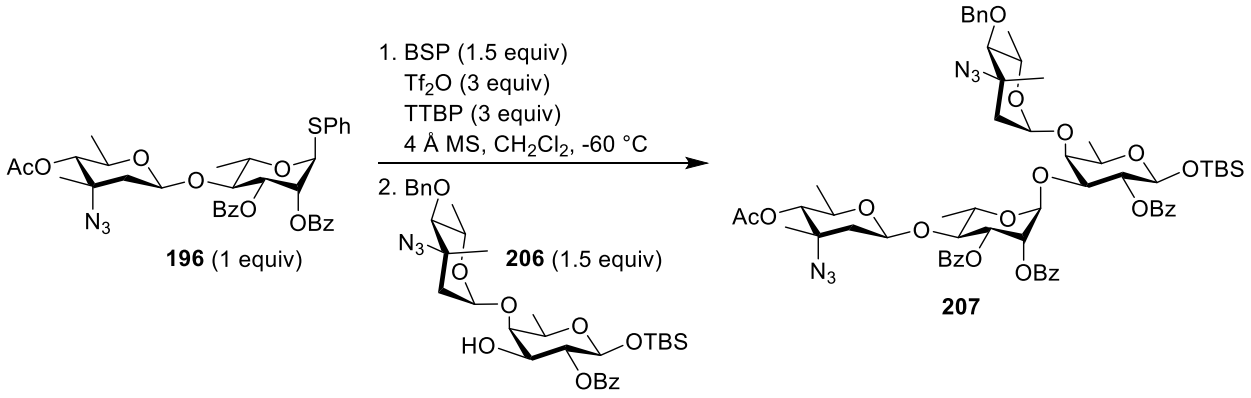
Tert-butyldimethylsilyl 4-(6'-deoxy-4'-O-acetyl-3'-azido-3'-C-methyl-1'-α-L-*arabino*-hexopyranosyl)-6-deoxy-2-O-benzoyl-1-thio-β-D-galactopyranoside **206**



205 **206**

To a round bottom flask with stir bar were added **205** (0.115 g, 0.1473 mmol), CH_2Cl_2 (30 mL), and H_2O (1.5 mL). After the sugar was fully dissolved, DDQ (0.067 g, 0.2952 mmol) was added and the reaction stirred at 23 °C. After 21 h, the reaction was diluted with CH_2Cl_2 and H_2O. The mixture was extracted 5 times using CH_2Cl_2 and the combined organic layers were dried over Na_2SO_4, filtered, and concentrated in *vacuo*. The crude residue was purified using a Yamazan Smart Flash W-Prep 2XY automated purification system equipped with universal silica gel column (100% hexanes to 20% acetone in hexanes) to afford **206** (0.074 g, 78%) as a white solid. $[\alpha]_D^{23}$ = -25.9 (*c* 0.34, CH_2Cl_2). ^{1}H NMR (500 MHz, C_6D_6) δ = 8.18 (d, J = 7.4 Hz, 2H), 7.32 (d, J = 7.4 Hz, 2H), 7.19 – 7.18 (m, 2H), 7.13 – 7.05 (m, 4H), 5.41 – 5.38 (m, 1H), 5.25 (d, J = 4.3 Hz, 1H), 4.77 (d, J = 11.0 Hz, 1H), 4.72 (d, J = 7.5 Hz, 1H), 4.39 (d, J = 11.0 Hz, 1H), 4.02 – 3.97 (m, 1H), 3.51 – 3.48 (m, 1H), 3.41 – 3.40 (m, 1H), 3.06 – 3.01 (m, 2H), 2.64 (d, J = 5.9 Hz, 1H), 2.15 (d, J = 13.8 Hz, 1H), 1.75 – 1.72 (m, 1H), 1.70 (s, 3H), 1.24 (d, J = 6.2 Hz, 3H), 1.16 (d, J = 6.4 Hz, 3H), 0.89 (s, 9H), 0.20 (s, 3H), 0.15 (s, 3H). ^{13}C NMR (125 MHz, C_6D_6) δ = 167.8, 138.7, 133.4, 130.4, 130.2, 128.63, 128.59, 128.3, 128.2, 128.0, 99.2, 96.4, 85.7, 79.3, 77.1, 75.4, 74.3, 70.5, 66.7, 63.1, 41.4, 25.8, 18.7, 18.3, 18.1, 17.2, -3.6, -4.3. LRMS (ESI) *m/z* $[M+Na]^+$ Calcd for $C_{33}H_{47}N_3NaO_8Si$ 664.30; Found 664.55. HRMS (ESI) *m/z* $[M+Na]^+$ Calcd for $C_{33}H_{47}N_3NaO_8Si$ 664.3030; Found 664.3043.

Tert-butyldimethylsilyl 3-(4-(6'''-deoxy-4'''-O-acetyl-3'''-azido-3'''-C-methyl-1'''-α-L-*arabino*-hexopyranosyl)-(4-(6''-deoxy-4''-O-acetate-3''-azido-3''-C-methyl-1''-β-D-ribo-hexopyranosyl)-6'-deoxy-2,3-di-O-benzoyl-1'-thio-α-L-mannopyranoside) 6-deoxy-2-O-benzoyl-1-thio-β-D-galactopyranoside **207**



To a flame dried round bottom flask with stir bar were added compounds **196** (0.042 g, 0.06220 mmol) and **206** (0.060 g, 0.09356 mmol). The mixture underwent azeotrope with anhydrous toluene (3 times) followed by co-evaporation with anhydrous CH_2Cl_2 (3 times) before being left to stir under high vacuum overnight. Tf_2O was distilled immediately before the reaction. 4 Å MS (0.20 g) were added to the flask which contained compounds **196** and **206** and the mixture was dissolved in anhydrous CH_2Cl_2 (3.05 mL) and cooled to -60 °C using a cryostat. After stirring for 15 minutes, BSP (0.020 g, 0.09563 mmol) and TTBP (0.046 g, 0.1852 mmol) were added and the reaction stirred at -60 °C. After 5 minutes, Tf_2O (0.30 mL from stock, 0.1866 mmol) was added and the reaction was left to stir at -60 °C. After 2 h, the cryostat was turned off and the reaction was warmed to 0 °C over 3.5 h. Once at 0 °C, the reaction was removed from the cryostat bath and allowed to warm to 23 °C over 0.5 h before being filtered through a pad of celite using CH_2Cl_2. The mixture was then diluted with CH_2Cl_2, saturated aqueous solution of $NaHCO_3$ (15 mL), and further diluted with H_2O. The mixture was extracted 5 times using CH_2Cl_2 and the combined organic layers were dried over Na_2SO_4, filtered, and concentrated in *vacuo*. The crude residue

was purified using a Yamazan Smart Flash W-Prep 2XY automated purification system equipped with universal silica gel column (100% hexanes to 30% EtOAc in hexanes) to afford **207** (0.054 g, 72%) as a white solid.

$[\alpha]_D^{23}$ = +82.5 (*c* 0.16, CH_2Cl_2).

^{1}H NMR (500 MHz, C_6D_6) δ = 8.20 – 8.18 (m, 2H), 8.13 (d, J = 7.1 Hz, 2H), 7.88 (d, J = 7.3 Hz, 2H), 7.33 (d, J = 7.3 Hz, 2H), 7.19 – 7.17 (m, 1H), 7.12 – 6.87 (m, 11H), 6.00 – 5.95 (m, 2H), 5.91 (s, 1H), 5.71 (d, J = 4.1 Hz, 1H), 5.51 (s, 1H), 5.08 (d, J = 9.2 Hz, 1H), 4.80 (d, J = 11 Hz, 1H), 4.73 (d, J = 7.6 Hz, 1H), 4.58 (d, J = 9.6 Hz, 1H), 4.40 (d, J = 11.1 Hz, 1H), 4.38 – 4.29 (m, 2H), 4.09 – 4.04 (m, 2H), 3.80 (s, 1H), 3.65 – 3.59 (m, 1H), 3.09 (d, J = 9.3 Hz, 2H), 2.58 (d, J = 14.0, 1H), 2.33 (dd, J = 14.0, 4.4 Hz, 1H), 1.81 (s, 6H), 1.58 (s, 3H), 1.50 (d, J = 13.8 Hz, 1H), 1.31 (d, J = 6.2 Hz, 3H), 1.26 (d, J = 9.5 Hz, 1H), 1.23 (d, J = 6.2 Hz, 3H), 1.06 (d, J = 6.2 Hz, 3H), 0.90 (s, 9H), 0.69 (s, 3H), 0.24 (s, 3H), 0.15 (s, 3H).

^{13}C NMR (125 MHz, C_6D_6) δ = 169.4, 165.32, 165.28, 164.8, 138.9, 133.2, 133.1, 132.6, 130.7, 130.23, 130.19, 130.15, 130.00, 129.9, 128.6, 128.47, 128.45, 128.35, 128.2, 128.0, 99.5, 99.1, 96.9, 85.6, 78.5, 78.2, 78.1, 78.0, 75.4, 74.8, 72.2, 71.5, 70.7, 69.4, 69.2, 66.9, 63.1, 61.9, 42.7, 41.4, 25.9, 21.5, 19.7, 18.9, 18.8, 18.4, 18.2, 17.8, 17.3, -3.7, -4.3.

LRMS (ESI) *m/z* $[M+Na]^+$ Calcd for $C_{62}H_{78}N_6NaO_{17}Si$ 1229.51; Found 1229.64.

HRMS (ESI) *m/z* $[M+Na]^+$ Calcd for $C_{62}H_{78}N_6NaO_{17}Si$ 1229.5091; Found 1229.5106.

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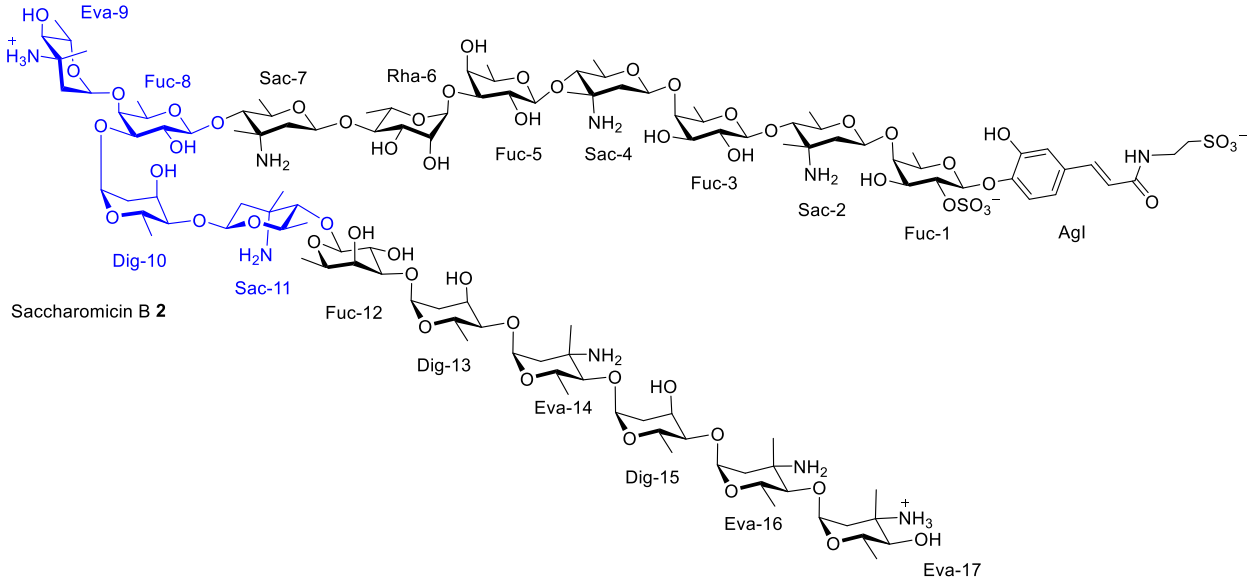
Chapter 3. Synthesis of the Branched Tetrasaccharide of Saccharomicin B

Adapted in part with permission from:

Garreffi, B. P.; Maney, A. P.; Bennett, C. S. Synthesis of the Branched Tetrasaccharide Fragment of Saccharomicin B. *Tetrahedron Letters* **2023**, *125*, 154615.

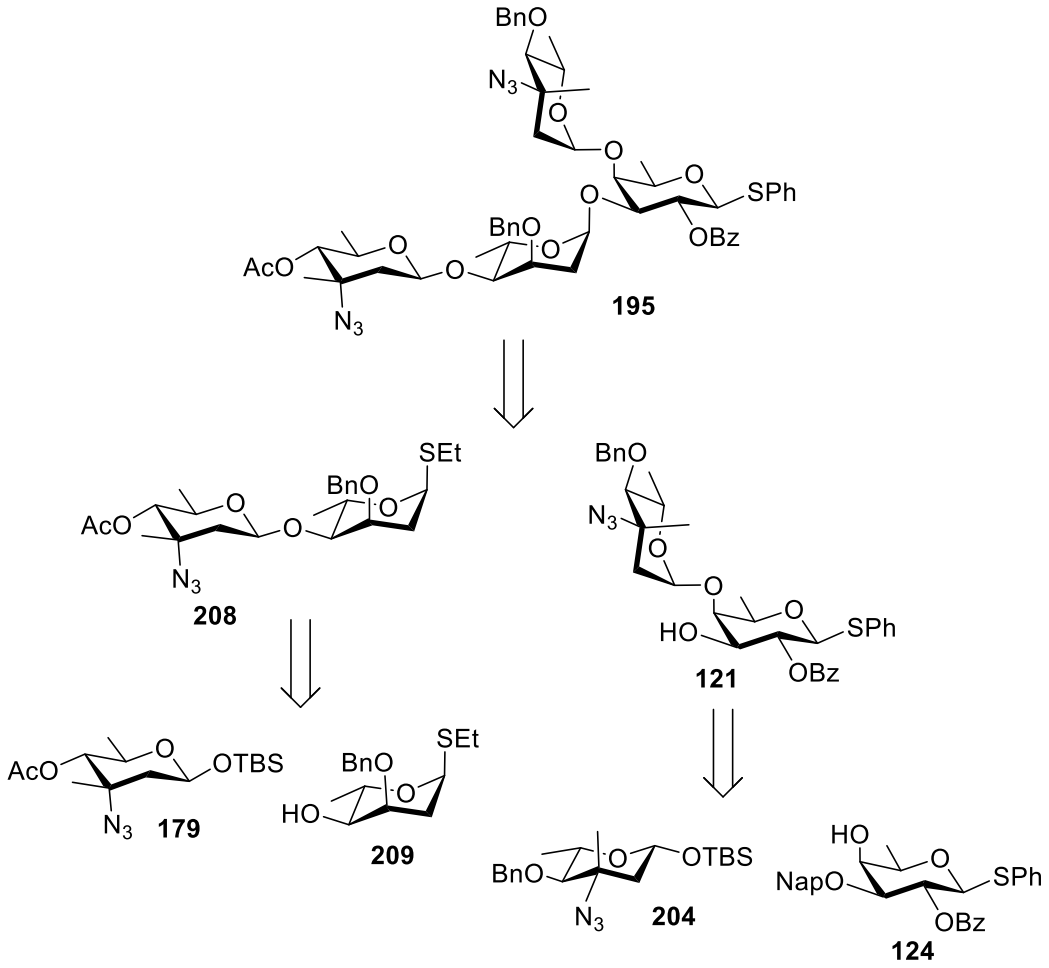
3.1 Retrosynthetic Analysis for the Synthesis of the Branched Tetrasaccharide Fragment of Saccharomicin B



Scheme 3.1 Structure of saccharomicin B **2**. Tetrasaccharide **195** shown in blue.

Having synthesized tetrasaccharide **207** of saccharomicin A, the Bennett lab sought to synthesize the branched tetrasaccharide of saccharomicin B **195** (Scheme 3.1).[1,2] Continuing the Bennett lab's retrosynthesis, tetrasaccharide **195** could be synthesized from the coupling of D-saccharosamine-L-digitoxose disaccharide **208** and 4-*epi*-L-vancosamine-D-fucose disaccharide **121** (Scheme 3.2).[2–5] The same protecting group scheme that was used for the synthesis of tetrasaccharide **207** was chosen for the D-saccharosamine and D-fucose moieties of **195** (Scheme 3.2, Scheme 2.6, Scheme 2.4).[1,2] The Bennett lab has also chosen to keep same protecting group scheme for the L-digitoxose moiety of **195** as for what was used in the synthesis of trisaccharide fragment **152** (Scheme 3.2, Scheme 134, Scheme 132).[2,3] The α-linkage between the L-digitoxose and D-fucose moieties could be synthesized using the Hirama lab's glycosylation method which was used for the [1+2] synthesis of trisaccharide **152** of saccharomicin B (Scheme 3.2, Scheme 1.41).[2–5] Disaccharide **208** could be synthesized from the union of D-saccharosamine **179** and L-digitoxose **209** (Scheme 3.2).[1,2,6] The β-linkage between the D-

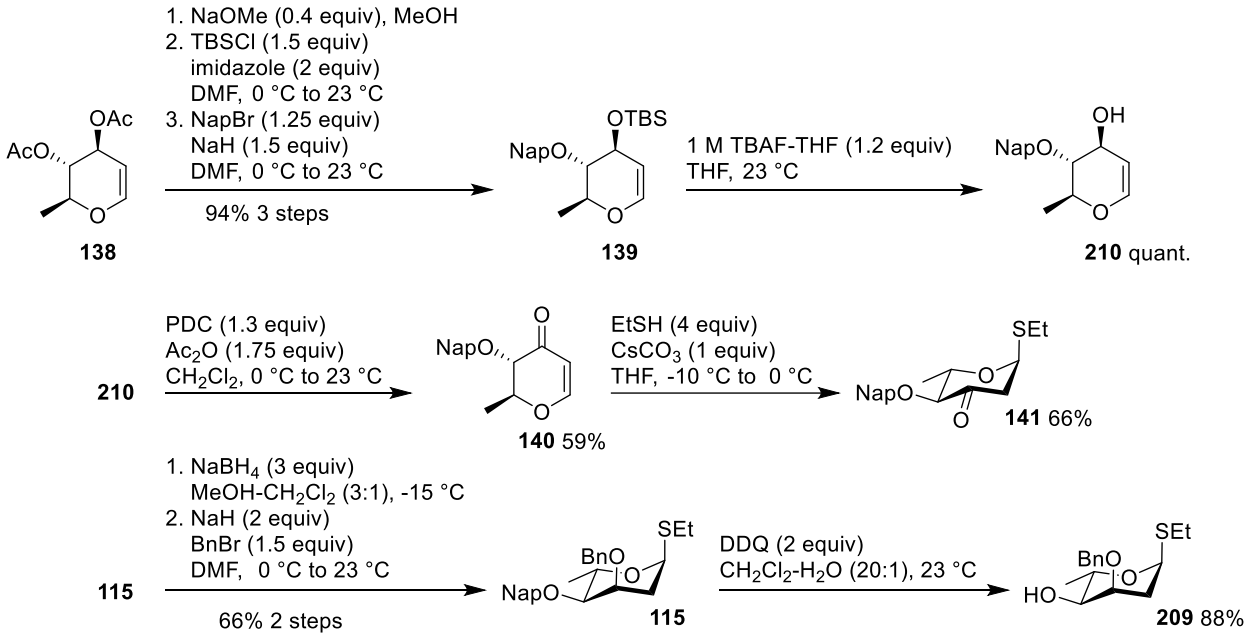
saccharosamine and L-digitoxose moieties could be synthesized using the $BF_3 \cdot OEt_2$ activation of a 1-OTBS saccharosamine donor which was used for the synthesis of D-saccharosamine-L-rhamnose disaccharide **196** (Table 2.1).[1,2,6] Disaccharide **121** could be synthesized from the coupling of 4-*epi*-L-vancosamine **204** and D-fucose **124** and subsequent deprotection of the naphthyl methyl ether (Scheme 3.2, Scheme 2.2).[1–3,7] The α-linkage between the 4-*epi*-L-vancosamine and D-fucose moieties could be synthesized using the Lewis acid activation of a 1-OTBS 4-*epi*-L-vancosamine donor glycosylation method which was used for the synthesis of disaccharide **200α** (Scheme 3.2, Scheme 2.13, Table 2.2).[1,2] D-saccharosamine monosaccharide **179** could be synthesized from D-rhamnal **159** which can be traced back to commercially available 3,4,6-tri-O-acetyl-D-glucal **154** (Scheme 3.2, Scheme 2.2, Scheme 1.48, Scheme 1.44, Scheme 1.43).[1,2,6,8–13] L-digitoxose **209** could be synthesized from commercially available 3,4-di-O-acetyl-L-rhamnal **138** (Scheme 3.2, Scheme 1.34).[2,3] 4-*epi*-L-vancosamine **204** could be synthesized from vancomycin HCl **3** (Scheme 3.2, Scheme 2.2, Scheme 1.53, Scheme 1.33).[1,3,14,15] D-fucose **124** can be synthesized from commercially available D-fucose **142** (Scheme 3.2, Scheme 2.2, Scheme 1.52, Scheme 1.46, Scheme 1.36, Scheme 1.35).[1–3,6,7,14,16,17]



Scheme 3.2 Retrosynthesis of the Branched Tetrasaccharide Fragment **195**.[2]

The synthesis of tetrasaccharide **194** began with the synthesis of D-saccharosamine **179**, 4-*epi*-L-vancosamine **204**, D-fucose **124**, and 4-*epi*-L-vancosamine-D-fucose disaccharide **196**.[1–3,6–14,16,18–22] A synthetic route has been developed by the Bennett lab to synthesize **179**, **204**, **124**, and **121** (Table 2.2, Table 2.1, Scheme 2.11, Scheme 2.7, Scheme 2.5, Scheme 2.4, Scheme 2.3, Scheme 2.1, Scheme 1.53, Scheme 1.52, Scheme 1.48, Scheme 1.46, Scheme 1.44, Scheme 1.43, Scheme 1.36, Scheme 1.35, Scheme 1.34, Scheme 1.33).[1–3,6,14] Therefore, L-digitoxose **209** was the remaining monosaccharide needed for the synthesis of tetrasaccharide **194**. To this end, commercially available 3,4-di-O-acetyl-L-rhamnal **138** was subjected to deacetylation using NaOMe in MeOH.[3] Next, the C-3 silyl ether protecting was selectively synthesized using TBSCl

and imidazole followed by the subsequent synthesis of the C-4 protection using NapBr and NaH in DMF to afford **139** in 94% yield over three steps (Scheme 3.3).[3,23] Deprotection of the C-3 silyl ether using *n*-Bu_4NF in THF provided **210** in quantitive yield (Scheme 3.3).[3] Oxidation of the C-3 hydroxyl using PDC and AcOH in CH_2Cl_2 afforded **140** in 59% yield (Scheme 3.3).[3,8,18] Then, the α-linked thioglycoside **141** was selectively synthesized in 66% yield by a Michael-type addition reaction using EtSH and $CsCO_3$ in THF (Scheme 3.3).[3,9] Next, the C-3 ketone was selectively reduced to an axial-linked hydroxyl using $NaBH_4$ in a mixture of MeOH-CH_2Cl_2 (3:1) followed by the synthesis of the C-3 benzyl ether using BnBr and NaH in DMF to provide **115** in 66% yield over two steps.[3] L-digitoxose **209** was synthesized in 88% yield by removing the C-4 naphthol methyl ether protection using DDQ in a mixture of CH_2Cl_2-H_2O (20:1) (Scheme 3.3).[2,3,7,24]

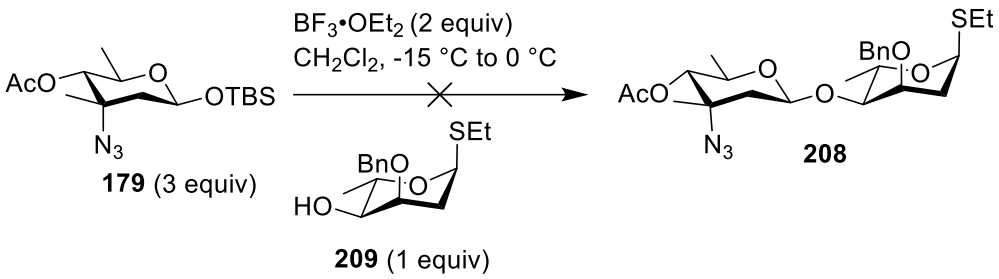


Scheme 3.3 Synthesis of L-digitoxose acceptor **209** from 3,4-di-O-acetyl-L-rhamnal **138**.[1–3,7–9,18,23,24]

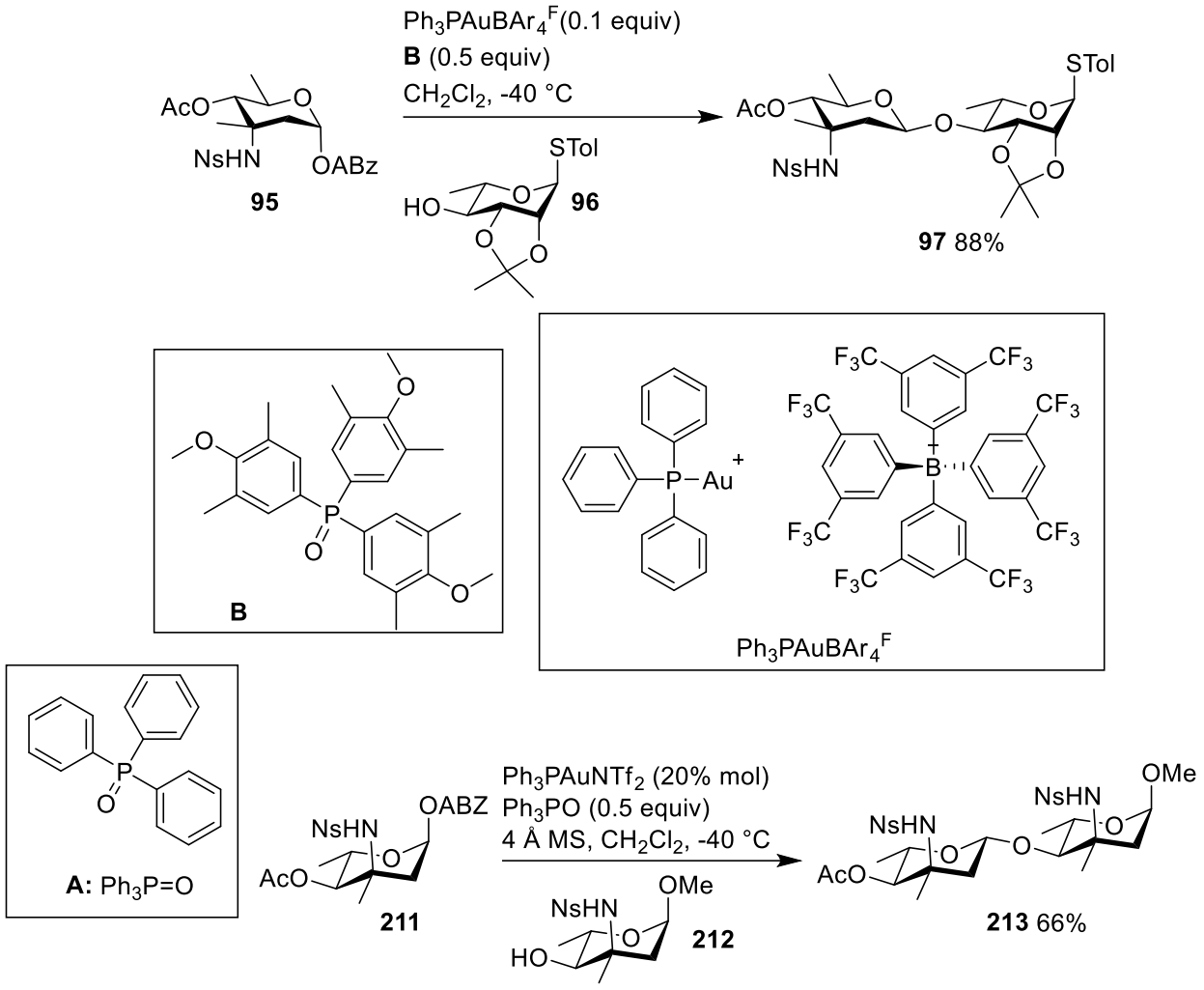
3.2 Synthesis of the D-saccharosamine-L-digitoxose Disaccharide

With each of the monosaccharides needed for the synthesis of tetrasaccharide **195** in hand, the Bennett lab sought to synthesize D-saccharosamine-L-digitoxose disaccharide **208**. The

original plan was to synthesize disaccharide **208** using the Lewis acid activation of a 1-OTBS saccharosamine donor.[1,2,6] However, the Lewis acid mediated glycosylation of **179** and **209** caused acceptor **209** to degrade through intermolecular aglycone transfer from **209** to **179** (Scheme 3.4).[2,25] The instability of L-digitoxose **209** to the Lewis acid glycosylation conditions prompted the Bennett lab to find an alternative method to synthesize the D-saccharosamine-L-digitoxose disaccharide.



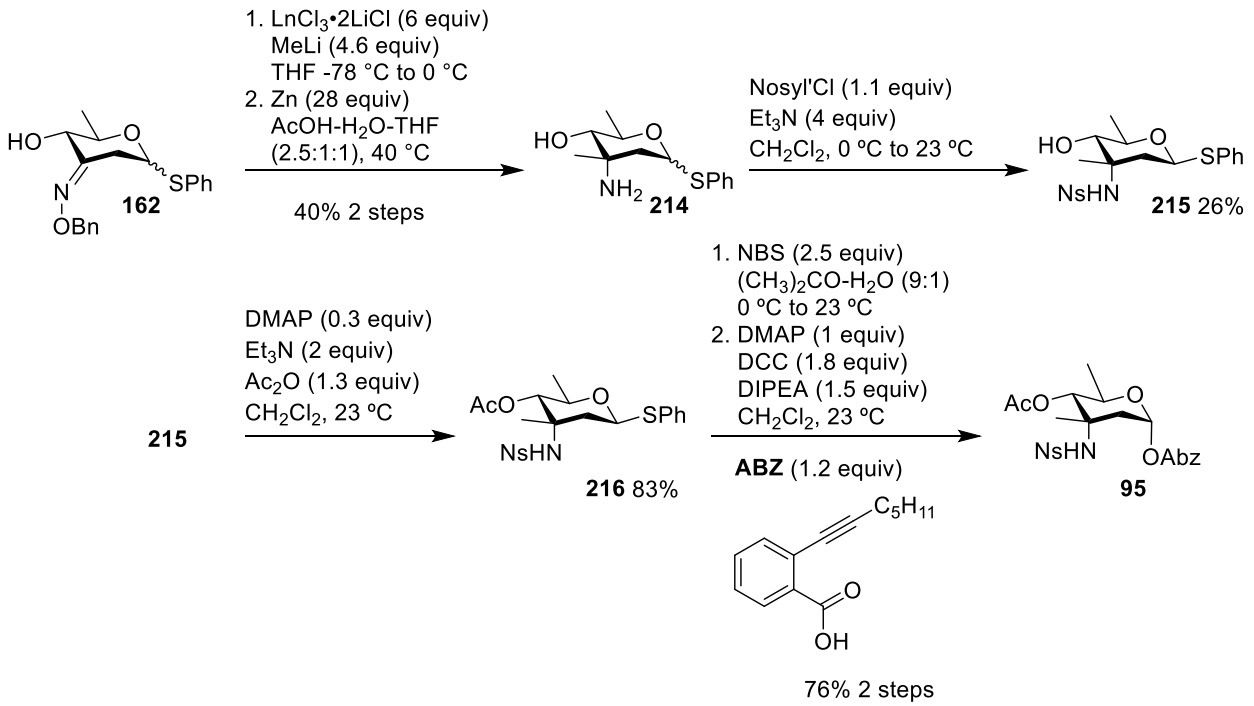
Scheme 3.4 Attempt at using the Lewis acid 1-OTBS method for the synthesis of D-saccharosamine-L-digitoxose disaccharide **208**.



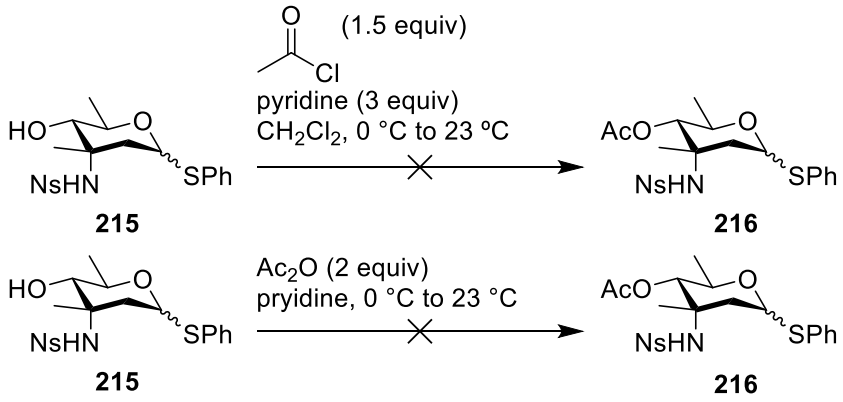
Scheme 3.5 Wan lab's synthesis of disaccharides **97** and **213** using gold (I) catalyzed activation of saccharosamine *ortho*-alkynyl benzoate donors.[26]

Previously, the Wan lab had developed a method which was used to selectively synthesize the β-linked D-saccharosamine-L-rhamnose disaccharide **97** and β-linked L-saccharosamine-L-saccharosamine disaccharide **213** (Scheme 3.5, Scheme 1.24).[26] The Wan Lab's method relied on the activation of a D-saccharosamine *ortho*-alkynyl benzoate donor **95** or **211** by tetrakis[3,5-bis(trifluoromethyl)phenyl]borate) triphenylphosphine gold (I) ($Ph_3PAuBAr_4F$) or triphenylphosphine gold (I) bis(trifluoromethanesulfonyl)imidate ($Ph_3PAuNTf_2$).[26] In addition to the high yield and β-selectivity of the Wan Lab's method, the approach is also milder than the Lewis acid method. Therefore, the Bennett lab sought to use the Wan lab's gold (I) catalyzed method

for the synthesis of D-saccharosamine-L-digitoxose disaccharide **217**.[2] Thus, the synthesis of D-saccharosamine ABZ **95** became the next target for the Bennett lab. To this end, D-saccharosamine oxime **162** was subjected to methylation at the C-3 position using MeLi and lanthanum (III) chloride bis(lithium chloride) complex solution ($LnCl_3 \cdot 2LiCl$) in THF which selectively synthesized the C-3 equatorial methyl (Scheme 3.6).[2,12,26,27] Next, subsequent removal of the benzyl ether protection from the C-3 nitrogen by reduction using Zn in a mixture of AcOH-H_2O-THF (2.5:1:1) provided amino sugar **214** in 40% yield over two steps (Scheme 3.6).[2,6] Then, the amine was protected using 4-nitrobenzenesulfonyl chloride (NsCl) to afford **215β** in 26% yield (Scheme 3.6).[2,12,26] The α-anomer of **215** was not isolated due to purification difficulties and therefore, the synthesis was continued with the β-anomer. Protection of the C-4 hydroxyl was with an acetate proved to be challenging. Careful control of the reaction conditions were needed to install the C-4 acetate and prevent acetate migration to the C-3 amine (Table 3.1, Scheme 3.7, Scheme 3.6).[2,26,28–34] Acetyl chloride and pyridine failed to provide **216** (Scheme 3.7).[2] Running the reaction in pyridine as opposed to CH_2Cl_2 did not afford **216** (Scheme 3.7).[2] Increasing the equivalence of DMAP 0.1, 0.2, 0.3, and to 0.4 equivalents did not provide **216** consistently (Table 3.1 entries 2-7). In addition, increasing the temperature of the reaction to 40 °C also did not afford **216** consistently (Table 3.1 entries 3-7).[2] Furthermore, starting the reaction at 0 °C and allowing it to warm to 23 °C did not provide **216** consistently (Table 3.1 entries 8 and 9).[2] Stock solutions of Et_3N (0.36 M) and Ac_2O (0.22 M) in addition to increasing the reaction temperature to 40 °C failed to afford **216** (Table 3.1 entry 5). However, when the concentration of the stock solution of Ac_2O was increased to Ac_2O (0.53 M) and the reaction temperature was held at 23 °C, **216β** was consistently synthesized in yields around 83% (Table 3.1 entry 10, Scheme 3.6).[2] Finally, the anomeric thiol was hydrolyzed using NBS in a mixture of in acetone H_2O (9:1) (Scheme 3.6).[2,12,26,35,36] Then, subsequent esterification of the hemiacetal afforded **95** in 76% yield over two steps (Scheme 3.6).[2,3,26]

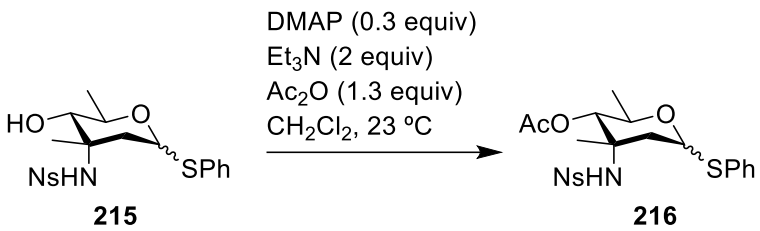


Scheme 3.6 Synthesis of D-saccharosamine ABZ **95**.[1–3,6,12,26,27,35,36]



Scheme 3.7 Attempts at the synthesis of D-saccharosamine **216**.

Table 3.1 Synthesis and Optimization of D-saccharosamine **216**.



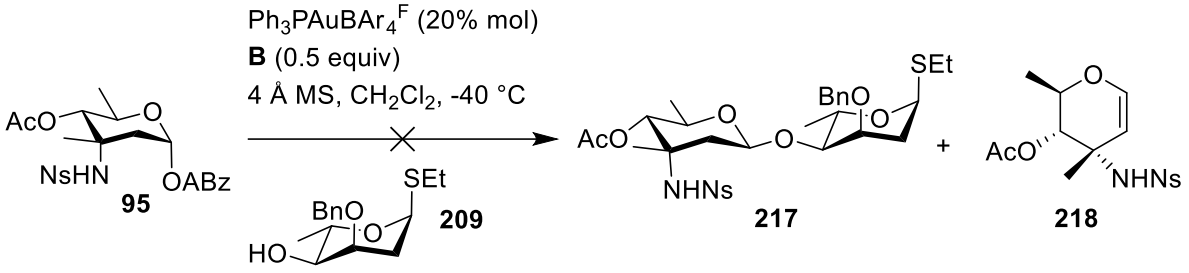
215 **216**

Entry	DMAP (equiv.)	Ac_2O (equiv.)	Temperature	Yield
1	0.3	1.2	23 °C	NR
2	0.1	1.2	23 °C	0% to 25%
3	0.2	1.3	40 °C	NR
4	0.3	1.2	40 °C	NR
5[a]	0.2	1.2	40 °C	NR
6	0.4	1.2	40 °C	NR
7	0.15	1.2	40 °C	15% to quant.
8	0.2	1.3	0 °C to 23 °C	NR
9	0.3	1.2	0 °C to 23 °C	0% to 25%
10[b]	0.3	1.2	23 °C	83%

All reactions were run in CH_2Cl_2 using Et_3N (2 equiv). [a]Stock solutions of Et_3N (0.36 M) Ac_2O (0.22 M). [b]Stock solutions of Et_3N (0.22 M) and Ac_2O (0.53 M). Reactions that produced **216** in a range of yields were not consistent. NR = no reaction.

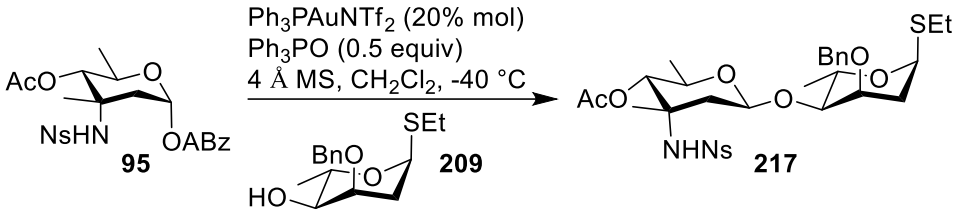
Having synthesized D-saccharosamine ABZ **95**, the Bennett lab could attempt to synthesize D-saccharosamine-L-digitoxose disaccharide **217** using the Wan lab's method.[2,26] To this end, **95** and **209** were reacted using $Ph_3PAuBAr_4F$ and additive tris(3,5-dimethyl-4-methoxyphenyl)phosphine oxide (**B**) (Scheme 3.8).[2,26] Unfortunately, these reaction conditions did not provide the desired disaccharide. Interestingly, the major product isolated from the reaction

was the D-saccharosamine glycal **218** which is the result of an elimination reaction occurring at the C-2 position of **95** (Scheme 3.8).[2,26] In an effort to utilize donor **95** and change the reaction conditions, the glycosylation was attempted using commercially available triphenylphosphine oxide (Ph_3PO) and commercially available triphenylphosphine gold (I) bis(trifluoromethanesulfonyl)imidate ($Ph_3PAuNTf_2$) (Table 3.2).[26] Fortunately, changing the additive and the catalyst provided the desired disaccharide **217** in 50% yield (Table 3.2, entry 1).[2] The coupling constant for the anomeric proton of the D-saccharosamine moiety of disaccharide **217** is 8.7 Hz confirming the β-linkage was synthesized.[1] The Wan lab reported a coupling constant of 9.2 Hz for the anomeric proton of the D-saccharosamine moiety of D-saccharosamine-L-rhamnose disaccharide **97**.[26] The Bennett lab reported coupling constants of 9.5 Hz and 9.2 Hz for the anomeric proton of the D-saccharosamine moiety of disaccharides **180** and **196**.[1,6] The yield of this glycosylation decreased when **209** was used in excess relative to **95** (Table 3.2, entry 2).[2] Furthermore, warming the reaction to 23 °C did not result in a significant difference in the yield of the **217** (Table 3.2, entry 3).[2] This could be because the reaction was complete prior to reaching warmer temperatures or because the donor species degrades at warmer temperatures under the reaction conditions.



Scheme 3.8 Attempt at the synthesis of D-saccharosamine-L-digitoxose disaccharide **217** using the Wan lab's glycosylation method with additive **B** and the gold (I) catalyst $Ph_3PAuBAr_4^F$.[2,26]

Table 3.2 Synthesis of D-saccharosamine-L-digitoxose disaccharide **217** and Optimizations.[2,26]

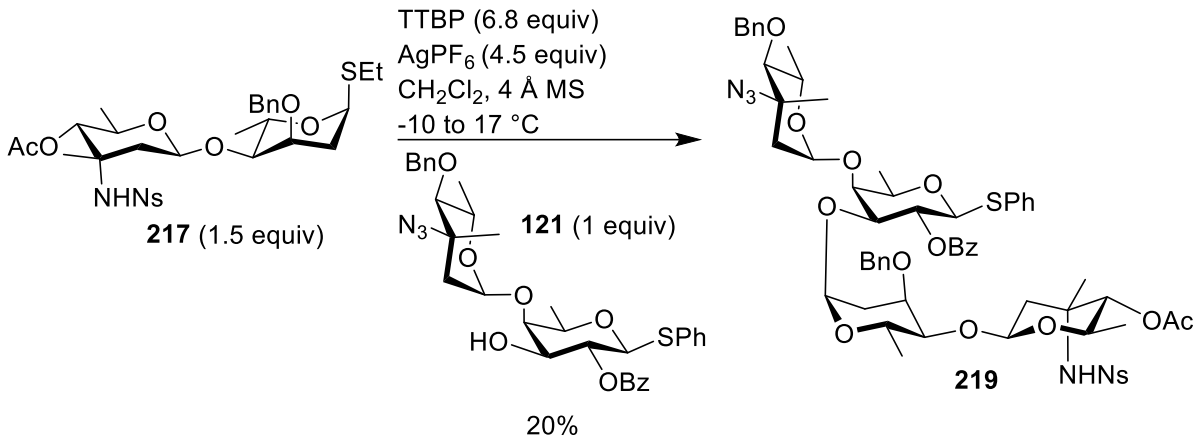


Entry	**95**:**209** ratio	yield %, **217**
1	1.5:1	50, *β*-only
2	1:1.5	34, *β*-only
3[a]	1.5:1	46, *β*-only

[a]Reaction was stirred for 6 h at -40 °C before being warmed to 23 °C over 18 h.

3.3 Synthesis of the Branched Tetrasaccharide Fragment of Saccharomicin B

Having synthesized D-saccharosamine-L-digitoxose disaccharide **217**, the Bennet lab was ready to attempt the [2+2] synthesis of the branched tetrasaccharide of saccharomicin B **219**.[2–5,37,38] The Bennett lab's plan was to use the Hirama lab's silver hexafluorophosphate ($AgPF_6$)-mediated activation for the synthesis of tetrasaccharide **219**.[3–5,37,38] The Bennett lab used this chemistry to activate ethanethiol in the presence of thiophenol for the synthesis of the branched trisaccharide of saccharomicin B **152** (Scheme 1.41).[3] To this end, disaccharides **217** and **121** were coupled using $AgPF_6$ and TTBP in CH_2Cl_2 and unfortunately the desired tetrasaccharide degraded when being purified on silica gel (Scheme 3.9).[2] A second purification using reverse phase chromatography was used afford the branched tetrasaccharide of saccharomicin B **219** in 20% yield (Scheme 3.9).[2] The coupling constant for the anomeric proton of the L-digitoxose moiety of tetrasaccharide **219** is 4.5 Hz confirming the α-linkage was synthesized.[2] The Bennett lab reported a coupling constant of 4.6 Hz for anomeric proton of the L-digitoxose moiety of trisaccharide **152**.[3]



Scheme 3.9 Synthesis of the Branched Tetrasaccharide Fragment of Saccharomicin B **219**.[2–5,37,38]

3.4 Conclusion

In conclusion, the branched tetrasaccharide fragment of saccharomicin B **219** was synthesized through the union of disaccharides **217** and **121**. The Lewis acid mediated method caused L-digitoxose **209** to degrade and therefore the Wan lab's gold (I) catalyzed activation of an *ortho*-alkynyl benzoate donor was used to selectively synthesize disaccharide **217**.[2,26] Tetrasaccharide **219** was not stable to silica gel and therefore the desired tetrasaccharide was isolated in low yield. The synthesis of tetrasaccharide **219** has provided the missing piece needed to synthesize the backbone of saccharomicin B. It is worth noting that only fragment **102** and the aglycone need to be synthesized to access saccharomicins A and B.

3.5 General Experimental Details

All reactions were performed under an argon atmosphere unless otherwise stated. Cryogenic temperatures were obtained using a cryostat with 2-propanol bath. Column chromatography was performed using a Yamazan Smart Flash W-Prep 2XY automated purification system equipped with universal silica gel columns unless otherwise specified. When used, flash column chromatography was performed on silica gel, 230-400 Mesh. Analytical and preparative thin layer chromatography was carried out on silica gel F254 plates. Products were visualized using UV or by staining with 7.5% aqueous sulfuric acid. NMR spectra were recorded on a Bruker Avance III NMR spectrometer at 500 MHz for ^{1}H-NMR and 125 MHz for ^{13}C-NMR. Chemical shifts are reported in ppm relative to C_6D_6, $CDCl_3$, $(CH_3)_2CO$ (for ^{1}H-NMR and ^{13}C-NMR). For ^{1}H NMR spectra, data are reported as follows: δ shift, multiplicity [s = singlet, m = multiplet, t = triplet, d = doublet, q = quartet, brs = broad singlet, dt = doublet of triplets, dq = doublet of quartets, and dd = doublet of doublets], coupling constants are reported in Hz. Low resolution mass spectra (LRMS) were recorded using a Finnigan LTQ ESI-MS with an additional APCI source. High resolution mass spectra (HRMS) were obtained on Electro Spray Ionization (ESI) on an Agelent TOF instrument in the positive mode. Optical rotations were measured on a Rudolph Research Analysis AUTOPUL IV polarimeter @ 589 nm in a 5 cm cell at 23 °C or 24 °C.

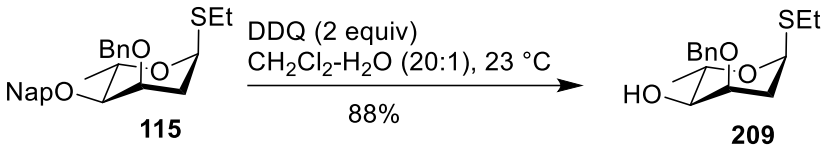
3.6 Materials

Prior to running all glycosylation reactions, donors and acceptors were dried by azeotropic removal of water using anhydrous toluene three times followed by anhydrous dichloromethane three times on a rotary evaporator before stirring overnight under vacuum. Solvents for reactions were dried using an Innovative Technologies PureSolv 400 system. Dichloromethane (CH_2Cl_2) used for glycosylations was stored over 4 Å molecular sieves beads that had been microwaved in a conventional microwave oven ten times for 10 seconds and then flame dried under vacuum three times. *N,N*-Diisopropylethylamine (DIPEA) was distilled over ninhydrin using a short path

apparatus before being distilled over potassium hydroxide (KOH) using a short path apparatus equipped with a cow adapter directly before use. 4 Å molecular sieves powder were activated by flame drying under vacuum three times, cooled to room temperature, and directly used in glycosylation. All other chemicals were purchased at the highest possible quality and used as received.

3.7 Experimental Procedures

Ethyl 2,3 6-trideoxy-3-O-benzyl-1-thio-α-L-mannopyranoside **209**



To a round bottom flask with stir bar were added **115** (0.145 g, 0.3435 mmol), CH_2Cl_2 (45 mL), and H_2O (2.25 mL). After the sugar was fully dissolved, DDQ (0.156 g, 0.6870 mmol) was added and the reaction stirred at 23 °C. After 2.5 h, the reaction was quenched with a saturated aqueous solution of $NaHCO_3$ (5 mL), then diluted with CH_2Cl_2 and H_2O. The mixture was extracted 5 times using CH_2Cl_2 and the combined organic layers were dried over Na_2SO_4, filtered, and concentrated in *vacuo*. The crude residue was purified using a Yamazan Smart Flash W-Prep 2XY automated purification system equipped with universal silica gel column (100% toluene to 10% EtOAc in toluene) to afford **209** (0.085 g, 88%) as a colorless oil.

[α] D ^{23} = -234.2 (*c* 0.38, CH_2Cl_2).

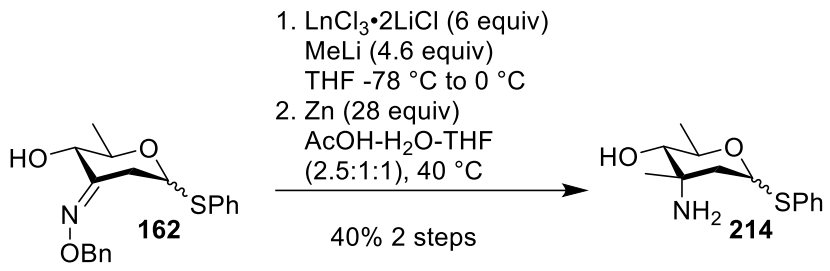
^{1}H NMR (500 MHz, C6D6) δ = 7.41 (d, J = 7.2 Hz, 2H), 7.37 – 7.34 (m, 2H), 7.32 – 7.29 (m, 1H), 5.20 (d, J = 6.2 Hz, 1H), 4.81 (d, J = 11.4 Hz, 1H), 4.36 (d, J = 11.3 Hz, 1H), 4.25 – 4.19 (m, 1H), 3.83 (d, J = 3.1 Hz, 1H), 3.25 – 3.20 (m, 1H), 2.68 – 2.55 (m, 2H), 2.45 (d, J = 10.6 Hz, 1H), 2.39 – 2.35 (m, 1H), 2.13 (qd, J = 8.6 Hz, 2.8 Hz, 1H), 1.30 – 1.26 (m, 6H).

^{13}C NMR (125 MHz, C6D6) δ = 137.7, 128.6, 128.3, 128.0, 79.4, 77.4, 77.2, 76.9, 73.7, 72.5, 71.2, 65.5, 33.2, 26.8, 17.8, 15.3.

LRMS (ESI) *m/z* $[M+Na]^+$ Calcd for $C_{15}H_{22}NaO_3S$ 305.12; Found 305.09.

HRMS (ESI) *m/z* $[M+Na]^+$ Calcd for $C_{15}H_{22}NaO_3S$ 305.1188; Found 305.1180.

Phenyl 2,3,6-trideoxy-3-amino-3-C-methyl-1-thio-α-β-D-ribo-hexopyranosyl **214**



To a flame dried round bottom flask with stir bar were added $LnCl_3 \cdot 2LiCl$ (200 mL, 120.1 mmol) and anhydrous THF (150 mL). The solution was then cooled to -78 °C using a 2-propanol dry ice bath before the dropwise addition of MeLi (3.1 M in diethoxymethane) (30 mL, 93 mmol). To a second flame dried round bottom flask was added compound **162** (6.87 g, 20.02 mmol) before dissolving in anhydrous THF (150 mL). After stirring for 1.5 h at -78 °C, compound **162** was added dropwise to the $LnCl_3 \cdot 2LiCl$ and MeLi complex over 30 minutes. After stirring for 2 h at -78 °C, the reaction was warmed to 0 °C by moving the reaction to an ice/water bath. After 1.25 h, the reaction was quenched with a saturated aqueous solution of NH_4Cl (15 mL) and then with H_2O (40 mL). The mixture was then concentrated in *vacuo* at 20 °C before being diluted with Et_2O and H_2O. The mixture was extracted 6 times using Et_2O and the combined organic layers were dried over Na_2SO_4, filtered, and concentrated in *vacuo*. The crude residue was purified using a Yamazan Smart Flash W-Prep 2XY automated purification system equipped with universal silica gel column (100% hexanes to 30% EtOAc in hexanes) to afford crude D-saccharosamine colorless oil which was used directly in the next step.

To a round bottom flask were added crude D-saccharosamine, zinc metal (36 g, 550.5 mmol), THF (21 mL), H_2O (21 mL), and AcOH (52 mL). The round bottom flask was equipped with a condenser and the mixture was warmed to 40 °C using an oil bath. After stirring for 21.5 h, the mixture was concentrated in *vacuo*, before undergoing azeotrope with toluene (3 times). Then the resulting crude material was filtered through a celite pad using CH_2Cl_2 (500 mL) before being

concentrated in *vacuo*. The crude residue was purified using a Yamazan Smart Flash W-Prep 2XY automated purification system equipped with universal silica gel column (100% CH_2Cl_2 with 5% Et_3N to 30% EtOAc with 5% Et_3N in CH_2Cl_2) to afford **214** (2.002 g, 40%) as a white solid over two steps.

[α] D ^{24} = +208.8 (*c* 0.34, CH_2Cl_2).

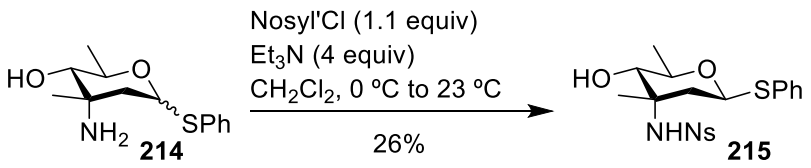
^{1}H NMR (500 MHz, C6D6) δ = 7.64 (d, J = 7.4 Hz, 1H), 7.49 (d, J = 7.3 Hz, 2H), 7.25 – 7.23 (m, 2H), 7.18 (s, 1H), 7.09 – 7.04 (m, 3H), 6.99 – 6.96 (m, 1H), 5.36 (d, J = 6.2 Hz, 1H), 4.96 (d, J = 11.6 Hz, 1H), 4.43 (s, 2H), 4.03 – 4.00 (m, 1H), 3.31 – 3.28 (m, 1H), 2.84 (d, J = 9.7 Hz, 1H), 2.76 (d, J = 9.4 Hz, 1H), 1.75 (dd, J = 14.7 Hz, J = 6.5 Hz, 1H), 1.69 (s, 1H), 1.68 (d, J = 12.7 Hz, 1H), 1.58 (d, J = 13.0 Hz, 1H), 1.35 (d, J = 6.0 Hz, 3H), 1.32 (s, 3H), 0.84 (s, 3H), 0.80 (s, 3H).

^{13}C NMR (125 MHz, C6D6) δ = 142.1, 136.5, 135.8, 131.6, 131.5, 129.2, 129.1, 128.6, 128.3, 127.4, 127.3, 127.2, 127.1, 83.2, 80.7, 76.6, 74.1, 66.5, 64.8, 51.6, 50.5, 45.5, 44.0, 29.0, 28.4, 18.9, 18.4.

LRMS (ESI) *m/z* $[M+H]^+$ Calcd for $C_{13}H_{20}NO_2S$ 254.12; Found 254.18.

HRMS (ESI) *m/z* $[M+H]^+$ Calcd for $C_{13}H_{20}NO_2S$ 254.1214; Found 254.1226.

Phenyl 2,3,6-trideoxy-3-amine-Nos'l-3-C-methyl-1-thio-α-D-ribo-hexopyranosyl **215β**



Compound **214** was dissolved into CH_2Cl_2 and washed with an aqueous sodium hydroxide solution (1 M) once and then washed with a saturated aqueous solution of $NaHCO_3$ once before being dried over Na_2SO_4, filtered, and concentrated in *vacuo*. To a flame dried round bottom flask with stir bar were added compound **214** (1.2 g, 4.741 mmol) and anhydrous CH_2Cl_2 (20 mL). The mixture was cooled to 0 °C using an ice/water bath and after stirring for 15 minutes Et_3N (2.65 mL, 19.0 mmol) followed by Ns'Cl (1.2 g, 5.415 mmol) were added. The reaction was allowed to warm from 0 °C to 23 °C for 16.75 h before being diluted with CH_2Cl_2 and H_2O. The mixture was extracted 5 times using CH_2Cl_2 and the combined organic layers were dried over Na_2SO_4, filtered, and concentrated in *vacuo*. The crude residue was purified by flash column chromatography on silica gel (100% hexanes to 70% EtOAc in hexanes) to afford the β-anomer **215β** (0.546 g, 26%) as a white solid.

[α] D ^{23} = +224.3 (*c* 0.23, CH_2Cl_2).

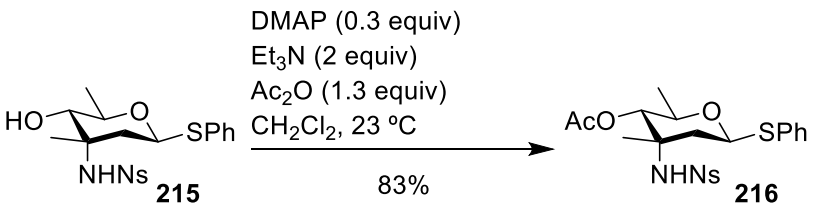
^{1}H NMR (500 MHz, C6D6) δ = 8.37 (d, J = 8.9 Hz, 2H), 8.15 (d, J = 8.9 Hz, 2H), 7.48 (d, J = 6.9 Hz, 2H), 7.34 – 7.28 (m, 3H), 5.91 (s, 1H), 5.48 (d, J = 6.0 Hz, 1H), 4.26 – 4.21 (m, 1H), 3.14 – 3.11 (m , 1H), 2.60 (d, J = 15.1 Hz, 1H), 2.38 (d, J = 6.7 Hz, 1H), 2.09 (dd, J = 15.1 Hz, J = 6.5 Hz, 1H), 1.29 – 1.28 (m, 6H).

^{13}C NMR (125 MHz, C6D6) δ = 150.1, 148.7, 135.0, 131.8, 129.2, 128.5, 127.8, 124.5, 82.3, 78.2, 65.2, 58.4, 41.6, 24.4, 17.9.

LRMS (ESI) *m/z* $[M+H]^+$ Calcd for $C_{19}H_{23}N_2O_6S_2$ 439.10; Found 439.36.

HRMS (ESI) *m/z* $[M+H]^+$ Calcd for $C_{19}H_{23}N_2O_6S_2$ 439.0997; Found 439.1063.

Phenyl 2,3,6-trideoxy-4-O-acetyl-3-amine-Nos'l-3-C-methyl-1-thio-α-D-ribo-hexopyranosyl **216β**



To a round bottom flask were added compound **215β** (0.250 g, 0.5707 mmol), DMAP (0.021 g, 0.1719 mmol), and CH_2Cl_2 (20.5 mL). Stock solutions for Et_3N (0.5 mL Et_3N in 10 mL CH_2Cl_2, 0.3414 M) and Ac_2O (0.5 mL Ac_2O in 10 mL CH_2Cl_2, 0.5037 M) were prepared. After stirring for 15 minutes, Et_3N (3.4 mL from stock, 1.141 mmol) and Ac_2O (1.4 mL from stock, 0.7052 mmol) were added. After another 15 minutes, the reaction was quenched with a saturated aqueous solution of $NaHCO_3$ (10 mL), then diluted with CH_2Cl_2 and H_2O. The mixture was extracted 5 times using CH_2Cl_2 and the combined organic layers were dried over Na_2SO_4, filtered, and concentrated in *vacuo*. The crude residue was purified twice using a Yamazan Smart Flash W-Prep 2XY automated purification system equipped with universal silica gel column (100% hexanes to 40% EtOAc in hexanes) to afford **216β** (0.227 g, 83%) as a white solid.

[α] D ^{24} = +209.3 (*c* 0.28, CH_2Cl_2).

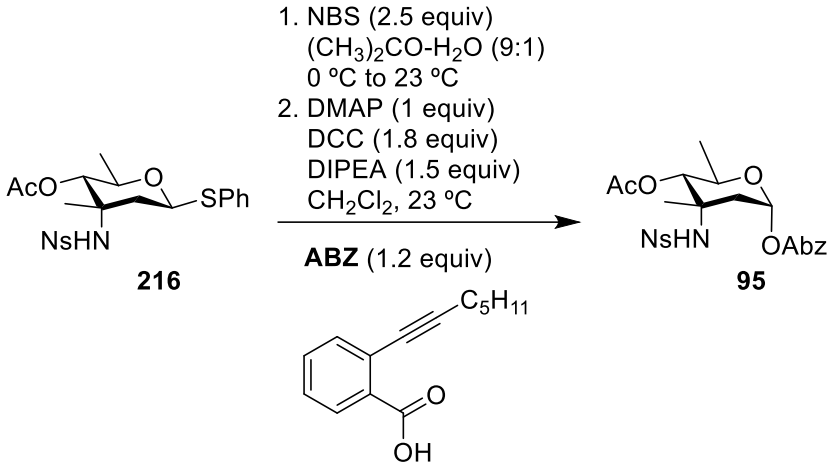
^{1}H NMR (500 MHz, C6D6) δ = 8.33 (d, J = 8.8 Hz, 2H), 8.14 (d, J = 8.8 Hz, 2H), 7.46 (d, J = 6.8 Hz, 2H), 7.32 – 7.27 (m, 3H), 5.72 (s, 1H), 5.50 (d, J = 6.2 Hz, 1H), 4.60 (d, J = 10.0 Hz, 1H), 4.44 – 4.38 (m, 1H), 2.72 (d, J = 15.0 Hz, 1H), 2.18 – 2.13 (m, 1H), 2.10 (s, 3H), 1.15 (s, 3H), 1.11 (d, J = 6.2 Hz, 3H).

^{13}C NMR (125 MHz, C6D6) δ = 169.7, 150.0, 149.1, 135.2, 131.6, 129.1, 128.3, 127.7, 124.3, 82.5, 77.3, 63.0, 57.7, 42.0, 24.3, 20.8, 17.5.

LRMS (ESI) *m/z* $[M+Na]^+$ Calcd for $C_{21}H_{24}N_2NaO_7S_2$ 503.09; Found 503.18.

HRMS (ESI) *m/z* $[M+Na]^+$ Calcd for $C_{21}H_{24}N_2NaO_7S_2$ 503.0923; Found 503.0926.

Ortho-alkynyl benzoate 2,3,6-trideoxy-4-O-acetyl-3-amine-Nos'l-3-C-methyl-1-α-D-ribo-hexopyranosyl **95**



76% 2 steps

To a round bottom flask with stir bar were added **216β** (0.358 g, 0.7457 mmol), $(CH_3)_2CO$ (22.5 mL), and H_2O (2.5 mL). The mixture was cooled to 0 °C using an ice/water bath. After stirring for 20 minutes, NBS (0.332 g, 1.865 mmol) was added and the reaction was stirred at 0 °C. After 0.75 h the reaction was quenched with a saturated aqueous solution of $NaHCO_3$ (10 mL), then diluted with CH_2Cl_2 and H_2O. The mixture was extracted 5 times using CH_2Cl_2 and the combined organic layers were dried over Na_22SO_4, filtered, and concentrated in *vacuo*. The crude residue was purified using a Yamazan Smart Flash W-Prep 2XY automated purification system equipped with universal silica gel column (100% hexanes to 40% EtOAc in hexanes) to afford crude hemiacetal D-saccharosamine (0.279 g) as a white solid which was used directly in the next step.

To a round bottom flask was added crude hemiacetal D-saccharosamine (0.279 g) and underwent azeotrope with anhydrous toluene (3 times) followed by co-evaporation with anhydrous CH_2Cl_2 (3 times) before being left to stir under vacuum overnight. To a flame dried round bottom flask with stir bar was added crude hemiacetal D-saccharosamine (0.269 g) and anhydrous CH_2Cl_2 (30 mL). Then, DMAP (0.085 g, 0.6958 mmol), **ABZ** (0.168 g, 0.8313 mmol), DCC (0.257 g, 1.246 mmol),

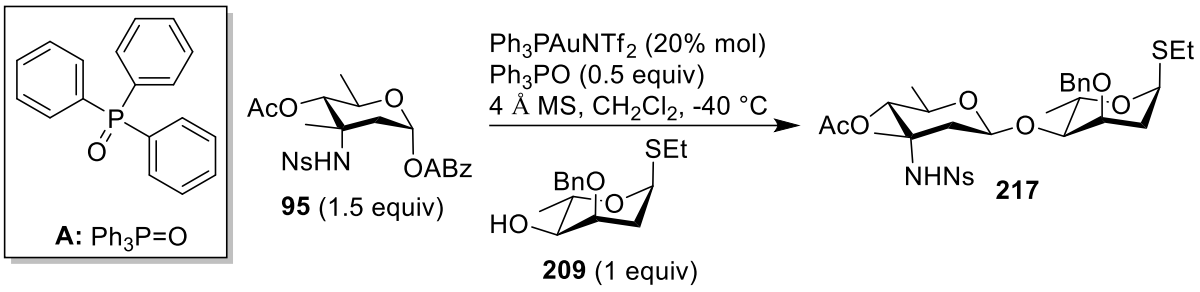
and DIPEA (0.2 mL, 1.157 mmol) were added and the reaction was left to stir at 23 °C. After stirring for 22.25 h, the reaction was quenched with a saturated aqueous solution of $NaHCO_3$ (20 mL), then diluted with CH_2Cl_2 and H_2O. The mixture was extracted 5 times using CH_2Cl_2 and the combined organic layers were dried over Na_22SO_4, filtered, and concentrated in *vacuo*. The crude residue was purified using a Yamazan Smart Flash W-Prep 2XY automated purification system equipped with universal silica gel column (100% hexanes to 30% EtOAc in hexanes) to afford **95** (0.323 g, 76%) as a white solid over two steps.

[α] D ^{24} = +107.1 (*c* 0.28, CH_2Cl_2).

LRMS (ESI) *m/z* $[M+Na]^+$ Calcd for $C_{28}H_{32}N_2NaO_9S$ 595.17; Found 595.27.

HRMS (ESI) *m/z* $[M+Na]^+$ Calcd for $C_{28}H_{32}N_2NaO_9S$ 595.1727; Found 595.1744.

Ethyl 4-(2'',3'',6''-trideoxy-4''-O-acetyl-3''-amine-Nos'l-3''-C-methyl-1''-β-D-ribo-hexopyranosyl)-2',6'-dideoxy, 3-O-benzyl-1'-thio-α-L-mannopyranoside **217**



To a flame dried round bottom flask with stir bar were added compounds **95** (0.152 g, 0.2657 mmol) and **209** (0.05 g, 0.1772 mmol). The mixture underwent azeotrope with anhydrous toluene (3 times) followed by co-evaporation with anhydrous CH_2Cl_2 (3 times) before being left to stir under vacuum overnight. 4 Å MS (0.2 g) were added to the flask which contained compounds **95** and **209** and the mixture was dissolved in anhydrous CH_2Cl_2 (1.6 mL) and stirred for 1.25 h before being covered in aluminum foil, turning off the hood light, and being cooled to -40 °C using a cryostat 2-propanol bath. A stock solution of $PPh_3PAuNTf_2$ (0.052 g) in anhydrous CH_2Cl_2 (0.6 mL) was prepared and covered in aluminum foil. After stirring the mixture for 25 minutes at -40 °C, $PPh_3PAuNTf_2$ (0.3 mL from stock, 0.03543 mmol) was added dropwise over 1.5 minutes to the reaction. After stirring for 21 h at -40 °C, the reaction was filtered through a pad of celite using CH_2Cl_2 and then diluted with CH_2Cl_2 and a saturated aqueous solution of $NaHCO_3$ (20 mL). The mixture was extracted 5 times using CH_2Cl_2 and the combined organic layers were dried over Na_2SO_4, filtered, and concentrated in *vacuo*. The crude residue was purified using a Yamazan Smart Flash W-Prep 2XY automated purification system equipped with universal silica gel column (100% toluene to 20% EtOAc in toluene) to afford **217** (0.058 g, 50%) as a white solid.

$[\alpha]_D^{23}$ = -101.1 (*c* 0.18, CH_2Cl_2).

^{1}H NMR (500 MHz, C6D6) δ = 7.71 (d, J = 7.4 Hz, 2H), 7.52 (d, J = 8.3 Hz, 2H), 7.36 – 7.31 (m, 4H), 7.18 (s, 1H), 5.07 (d, J = 6.2 Hz, 1H), 5.03 (d, J = 8.7 Hz, 1H), 4.97 – 4.94 (m, 1H), 4.85 (d,

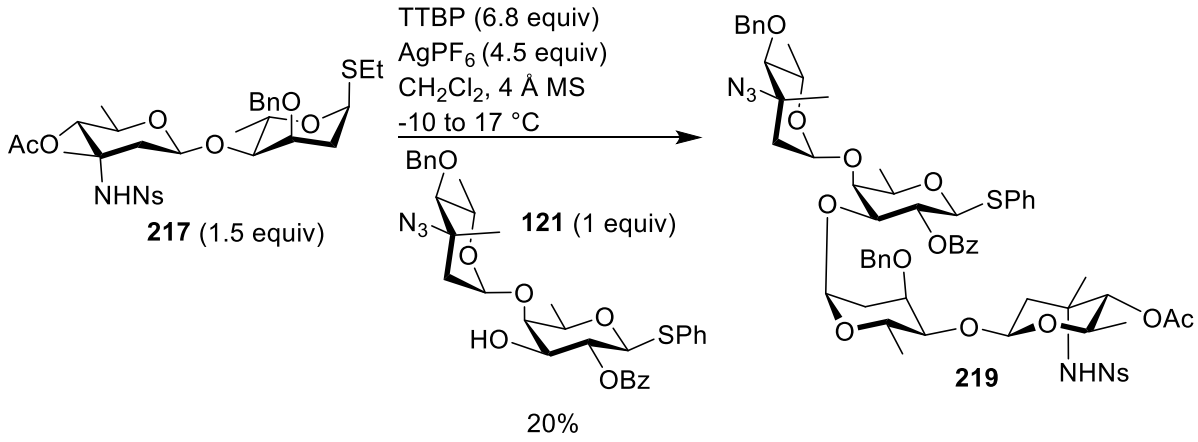
J = 12.4 Hz, 1H), 4.74 (d, J = 12.4 Hz, 1H), 4.56 (d, J = 9.6 Hz, 1H), 3.73 – 3.71 (m, 2H), 3.56 (dd, J = 9.1 Hz, 2.7 Hz, 1H), 2.65 (d, J = 14.4 Hz, 1H), 2.55 – 2.44 (m, 2H), 2.23 – 2.20 (m, 1H), 1.81 – 1.76 (m, 1H), 1.57 (d, J = 6.3 Hz, 3H), 1.53 (d, J = 5.7 Hz, 3H), 1.47 – 1.42 (m, 1H), 1.17 (t, J = 7.4 Hz, 3H), 1.09 (d, J = 6.2 Hz, 3H), 0.57 (s, 3H).

^{13}C NMR (125 MHz, C6D6) δ = 168.7, 149.8, 148.1, 140.1, 128.7, 128.3, 128.1, 128.0, 127.6, 127.5, 124.3, 95.6, 80.1, 79.4, 78.2, 72.1, 71.9, 68.1, 64.4, 59.0, 40.4, 34.8, 27.1, 26.2, 20.0, 18.4, 17.8, 15.6.

LRMS (ESI) *m/z* $[M+Na]^+$ Calcd for $C_{30}H_{40}N_2NaO_{10}S_2$ 675.20; Found 675.27.

HRMS (ESI) *m/z* $[M+Na]^+$ Calcd for $C_{30}H_{40}N_2NaO_{10}S_2$ 675.2022; Found 675.2020.

Phenyl 3-(4-(2‴,3‴,6‴-trideoxy-4‴-O-benzyl-3‴-azido-3‴-C-methyl-1‴-α-L-*arabino*-hexopyranosyl)-O-(2″,3″,6″-trideoxy-4″-O-acetyl-3″-amine-Nos'l-3″-C-methyl-1″-β-D-ribo-hexopyranosyl)-2',6'-dideoxy, 3-O-benzyl-1'-α-L-mannopyranoside) 6-deoxy-2-O-benzoyl-1-thio-β-D-galactopyranoside **219**



To a flame dried round bottom flask with stir bar were added compounds **217** (0.038 g, 0.05826 mmol) and **121** (0.024 g, 0.03876 mmol). The mixture underwent azeotrope with anhydrous toluene (3 times) followed by co-evaporation with anhydrous CH_2Cl_2 (3 times) before being left to stir under vacuum overnight. To the flask which contained compounds **217** and **121** were added TTBP (0.066 g, 0.2657 mmol) and 4 Å MS (0.2 g) were added and the mixture was dissolved in anhydrous CH_2Cl_2 (2.2 mL) and stirred for 1.5 h before being covered in aluminum foil, turning off the hood light, and being cooled to -10 °C using a cryostat 2-propanol bath. A stock solution of $AgPF_6$ (0.10 g in anhydrous CH_2Cl_2 (1.5 mL) was prepared and covered in aluminum foil. After stirring for 0.5 h at -10 °C, $AgPF_6$ (0.66 mL from stock, 0.1740 mmol) was added dropwise over 2 minutes to the reaction. After stirring for 0.5 h at -10 °C, the cryostat bath was turned off and the reaction was allowed to warm from -10 °C. After 4 h, the reaction had reached 17 °C, and was then quenched with pyridine (0.5 mL) before being filtered through a pad of celite using CH_2Cl_2. The mixture was concentrated in *vacuo* before undergoing azeotrope with anhydrous toluene twice. The crude residue was purified using a Yamazan Smart Flash W-Prep 2XY automated

purification system equipped with universal silica gel column (100% hexanes to 30% EtOAc in hexanes) to afford impure **219** (0.0175 g) as a white solid. The impure compound was purified using a Yamazan Smart Flash W-Prep 2XY automated purification system equipped with universal ODS column (100% H_2O to 100% MeCN) to afford impure **219** (0.0102 g) as a white solid. The impure compound was dissolved into HPLC grade MeCN and washed once with HPLC grade hexanes before being concentrated in *vacuo*. The compound was redissolved in HPLC grade benzene and washed once with a saturated aqueous solution of $NaHCO_3$ before being concentrated in *vacuo*, to afford compound **219** (0.0096 g, 20%) as a white solid.

[α] D ^{24} = -19.5 (*c* 0.38, CH_2Cl_2).

^{1}H NMR (500 MHz, C_6D_6) δ = 8.24 (d, J = 7.1 Hz, 2H), 7.74 – 7.70 (m, 4H), 7.52 – 7.49 (m, 4H), 7.35 – 7.33 (m, 3H), 7.28 (d, J = 8.7 Hz, 2H), 7.22 – 7.17 (m, 4H), 7.11 – 7.04 (m, 5H), 5.88 (t, J = 9.8 Hz, 1H), 5.80 (d, J = 4.2 Hz, 1H), 5.06 (d, J = 8.9 Hz, 1H), 4.88 (d, J = 4.5 Hz, 1H), 4.81 – 4.77 (m, 2H), 4.68 – 4.59 (m, 4H), 4.54 (d, J = 9.6 Hz, 1H), 4.39 (d, J = 11.0 Hz, 1H), 3.91 (s, 1H), 3.88 – 3.82 (m, 1H), 3.77 (d, J = 9.8 Hz, 1H), 3.69 – 3.65 (m, 1H), 3.63 (d, J = 2.5 Hz, 1H), 3.47 (dd, J = 9.3 Hz, 2.2 Hz, 1H), 3.26 – 3.22 (m, 1H), 2.75 (d, J = 9.6 Hz, 1H), 2.70 (d, J = 14.2 Hz, 1H), 2.03 – 1.99 (m, 1H), 1.93 (d, J = 13.9 Hz, 1H), 1.61 – 1.60 (m, 6H), 1.47 (s, 3H), 1.45 – 1.42 (m, 1H), 1.30 – 1.27 (m, 1H), 1.21 – 1.18 (m, 6H), 1.08 (d, J = 6.2 Hz, 3H), 1.06 (Brs, 1H), 0.50 (s, 3H).

^{13}C NMR (125 MHz, C6D6) δ = 168.6, 165.2, 149.9, 148.1, 139.9, 139.0, 134.5, 133.2, 132.2, 131.0, 130.0, 129.0, 128.93, 128.87, 128.59, 128.56, 128.2, 128.0, 127.5, 124.3, 99.6, 96.6, 95.3, 85.5, 85.1, 82.9, 78.7, 78.3, 76.2, 75.4, 72.0, 71.3, 70.7, 68.1, 66.2, 64.1, 63.4, 58.8, 40.6, 40.5, 33.1, 26.1, 19.9, 18.9, 18.7, 18.3, 18.0, 17.8.

LRMS (ESI) *m/z* $[M+Na]^+$ Calcd for $C_{61}H_{71}N_5NaO_{17}S_2$ 1232.42; Found 1232.36.

HRMS (ESI) *m/z* $[M+Na]^+$ Calcd for $C_{61}H_{71}N_5NaO_{17}S_2$ 1232.4184; Found 1232.4180.

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Chapter 4. Origins of the Selectivity for Glycosylation Reactions with Saccharosamine Donors

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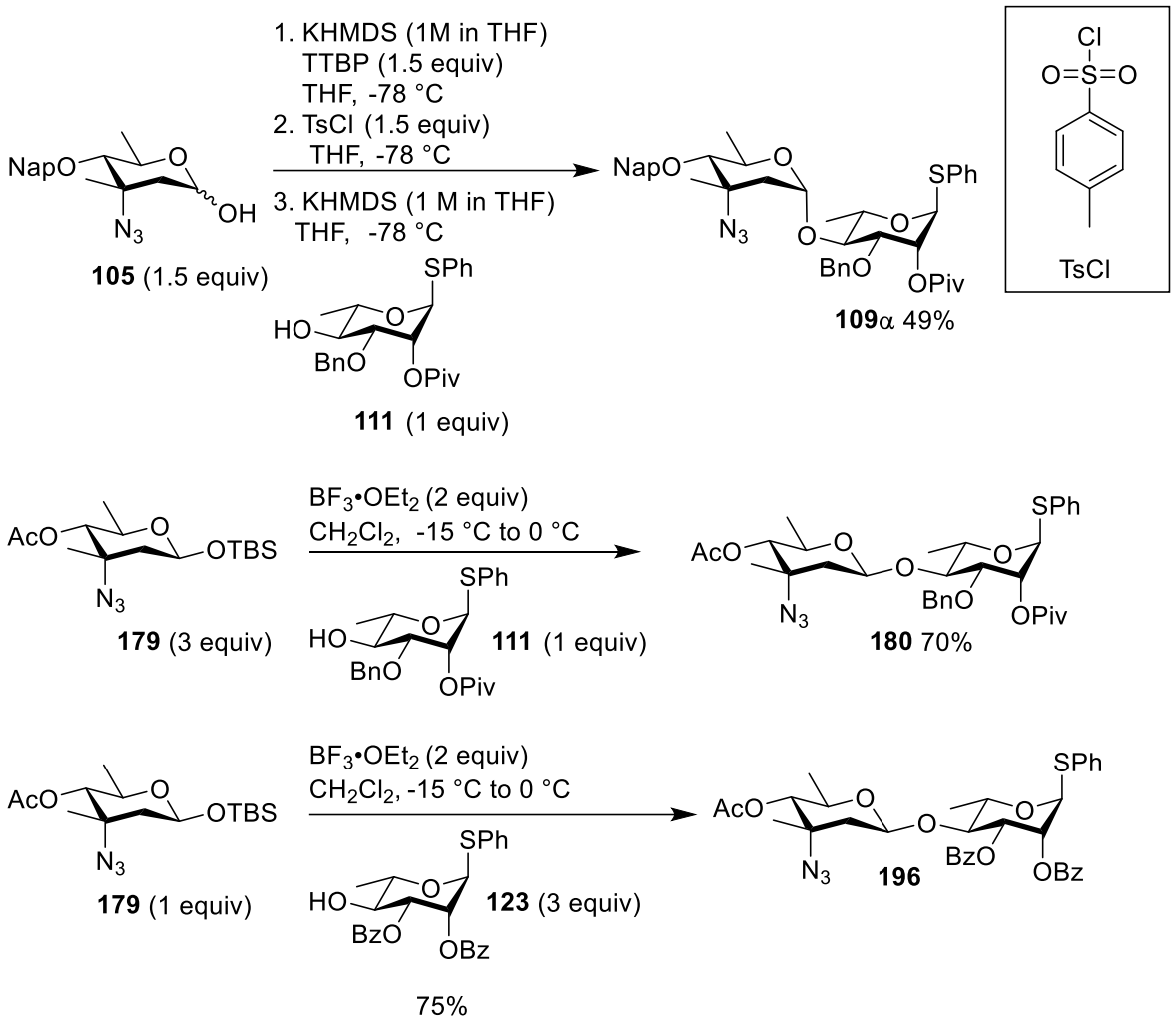
Garreffi, B. P.; Kwok, R. W.; Marianski, M.; Bennett, C. S. Origins of Selectivity in Glycosylation Reactions with Saccharosamine Donors. *Org. Lett.* **2023**, *25* (49), 8856–8860.

4.1 Investigating Saccharosamine Selectivity in Glycosylation Reactions

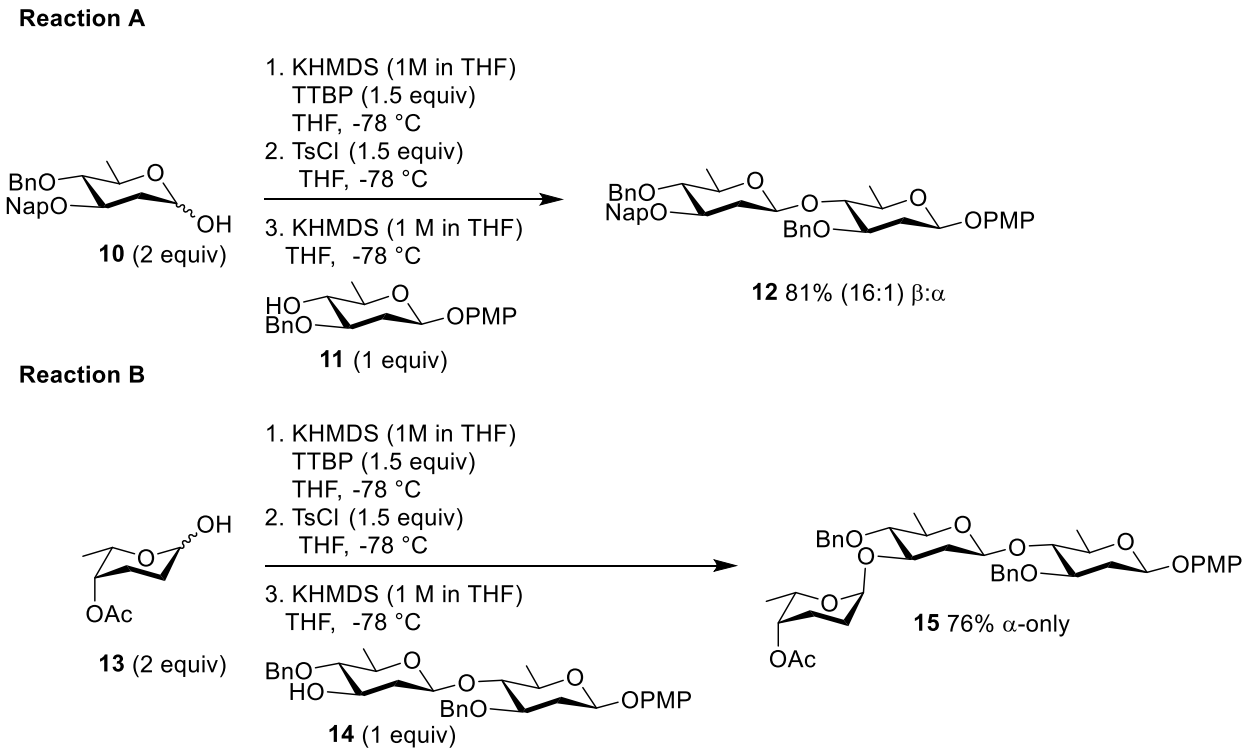
To date, the major obstacle preventing the synthesis of saccharomicins is controlling the selectivity of glycosylations for the synthesis of each of the 2-deoxy sugar linkages within the molecules. Many natural products which contain 2-deoxy sugars share this challenge with saccharomicins. As mentioned in chapter 1, the Bennett lab has demonstrated that D-saccharosamine donors can be used to synthesize either α or β-linked glycosides by changing the glycosylation reaction promoter.[1–3] The Bennett lab's reagent-controlled glycosylation method was used to synthesize the α-linked D-saccharosamine-L-rhamnose disaccharide **109α**. (Scheme 4.1, Scheme 1.47).[1,3] In contrast, the Lewis acid glycosylation method was used to synthesize the β-linked D-saccharosamine-L-rhamnose disaccharides **180** and **196** of the saccharomicins (Scheme 4.1, Table 2.1, Scheme 1.49).[1–3] Together, the synthesis of disaccharides **109α** and **180** have provided a strategy to synthesize α and β-linked glycosides using saccharosamine donors. However, the chemical mechanisms for each the reagent-controlled method and the Lewis acid method with saccharosamine donors is not fully understood. Therefore, further investigation into the origins of the selectivity's for the two glycosylation reactions is needed to understand how the chemical mechanisms work to synthesize glycosides.



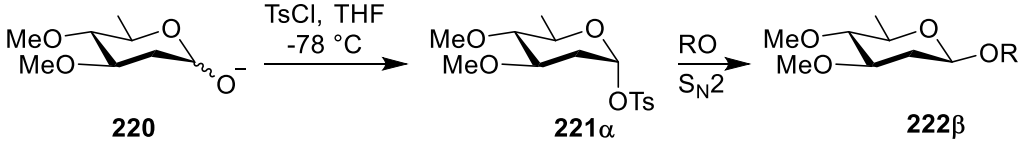
Scheme 4.1 Reagent-controlled glycosylation method for the synthesis of α-linked D-saccharosamine-L-rhamnose disaccharide **109α**.[1] Lewis acid glycosylation method for the synthesis β-linked D-saccharosamine-L-rhamnose disaccharides **180** and **196**.[1–3]

Previously, the Bennett lab demonstrated that either α or β-linkages can be synthesized with 2-deoxy sugar donors using the reagent-controlled glycosylation strategy (Scheme 4.2).[4–6] While on route to synthesizing the hexasaccharide of landomycin A, the Bennett lab selectively synthesized the β-linked disaccharide **12** in 81% yield using the reagent-controlled method (Scheme 4.2).[4] In this study, α-linked trisaccharide **15** was selectively synthesized in 76% yield using the same conditions used for the synthesis of the β-linkage within disaccharide **12** (Scheme 4.2).[4] The major difference between **Reaction A** and **Reaction B** is the donor species (Scheme

4.2).[4] In earlier studies, the Bennett lab used variable-temperature NMR (VT-NMR) experiments to demonstrate that the reaction of 2-deoxy sugar alkoxide **220** with TsCl produces a single α-glycosyl sulfonate **221α** (Scheme 4.3).[5,6] Addition of a sugar nucleophile to **221α** can initiate an S_N2-like pathway to afford β-linked disaccharides (Scheme 4.3).[5,6] The S_N2-like pathway was further confirmed by the Bennet lab using a combination of VT-NMR, KIE experiments, and DFT calculations.[7]

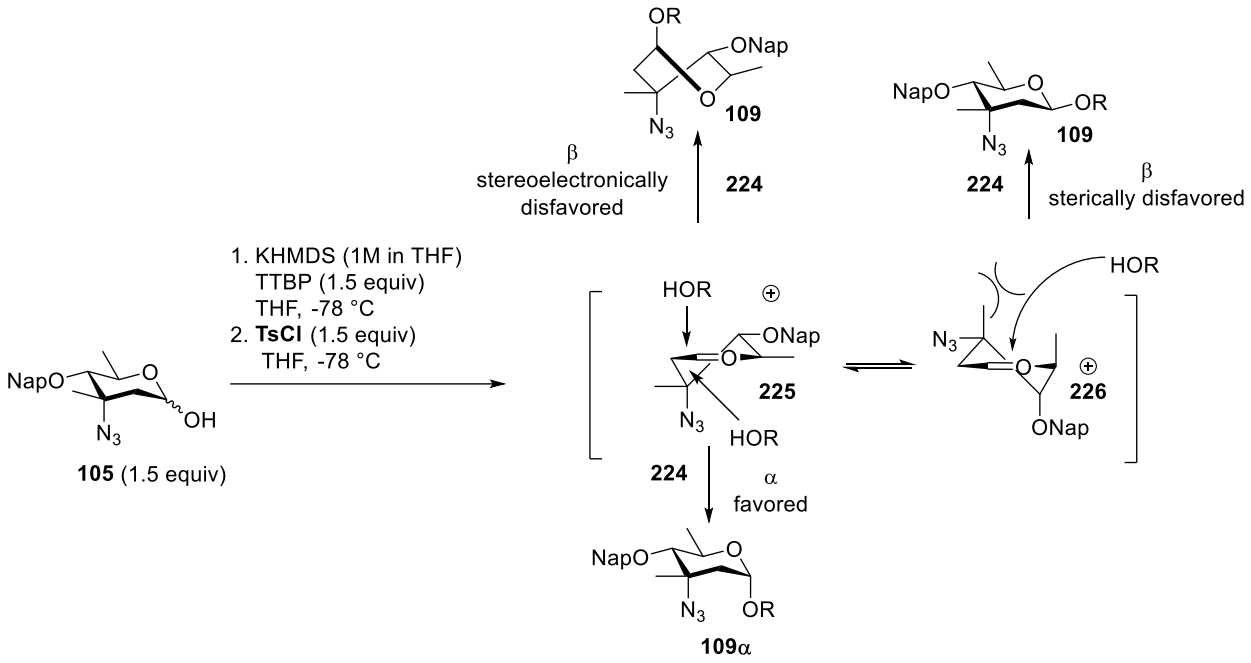


Scheme 4.2 **Reaction A** is an example of the reagent-controlled method affording β-linkages.[4,6] **Reaction B** is an example of the reagent-controlled method affording α-linkages.[4,6]

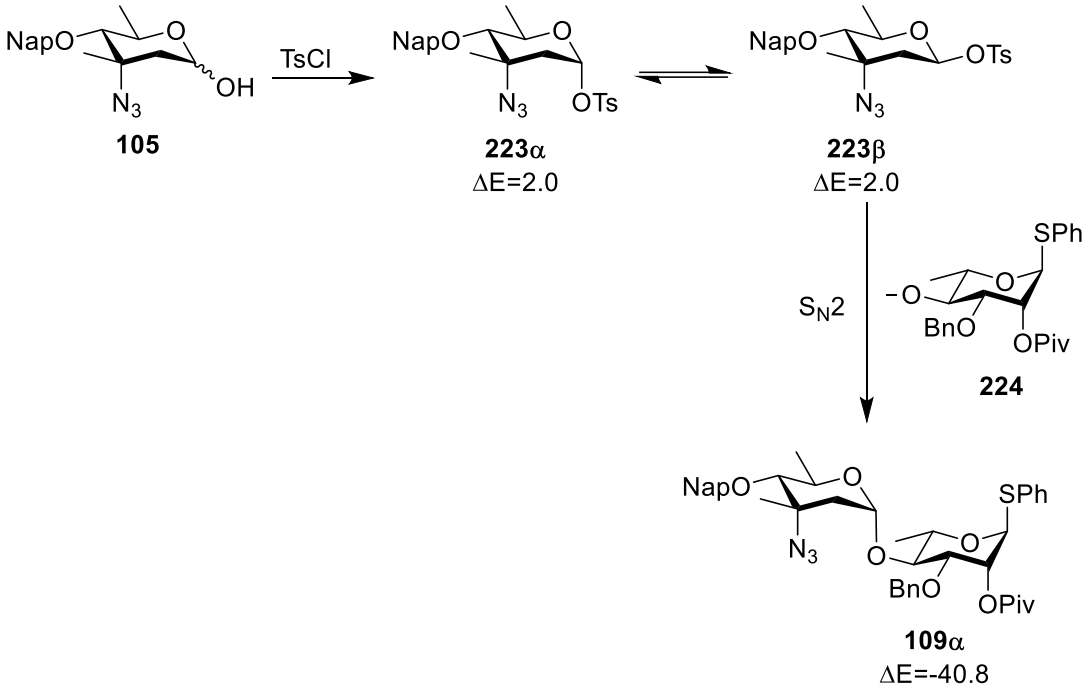


Scheme 4.3 VT-NMR experiment that demonstrates the formation of an α-glycosyl sulfonate.[6,8]

Reaction A affords a β-linked product and is consistent with the S_N2-like nature of the reagent-controlled glycosylation method and with the formation of an α-glycosyl sulfonate intermediate (Scheme 4.2).[4–7] In contrast, **Reaction B** affords an α-linked product and thus if the reaction was occurring through an S_N2-like pathway then a β-glycosyl sulfonate would need to form before the attack by the acceptor nucleophile (Scheme 4.2). The formation of a β-glycosyl sulfonate would provide an explanation for why the reagent-controlled method afforded α-linked disaccharide **109α** when using saccharosamine donor **105**. However, the selectivity is reversed in favor of the synthesis of β-linkages when saccharosamine donor **179** is used under the Lewis acid glycosylation conditions. Although unlikely due to the selective nature of the reaction, the Lewis acid glycosylation with saccharosamine could occur through the formation a glycosyl cation intermediate (Scheme 4.4).[3,9] With the help of the Marianski lab's computational expertise, it was demonstrated that for the reagent-controlled method with saccharosamine donors, the β-glycosyl sulfonate **223β** was favored over the α-glycosyl sulfonate **223α** by a ΔE value of 2.0 kcal mol^{-1} (Scheme 4.5).[3] The more stable β-glycosyl sulfonate then leads to the synthesis of α-linked glycoside **109α** through an S_N2-like pathway.[3] Furthermore, the Marianski lab used DFT calculations to demonstrate that non-half-chair intermediates which would be the result of glycosyl cation formation were unstable (Scheme 4.4).[3] With these results in mind, the Bennett and Marianski lab's sought to uncover the explanation for the β-selectivity of the Lewis acid glycosylation method with saccharosamine donors.

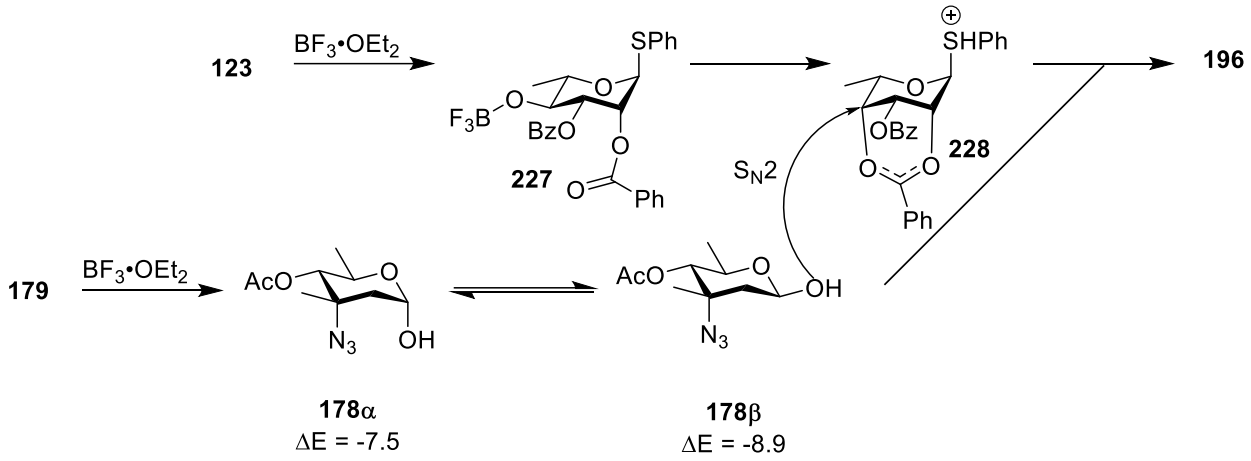


Scheme 4.4 Addition to oxocarbenium cations do not account for the selectivity of the Lewis acid glycosylation method.[3,9]



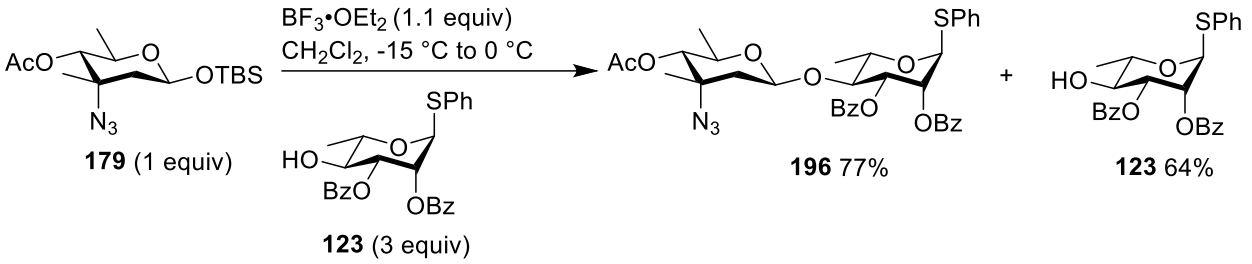
Scheme 4.5 Proposed mechanism of the reagent-controlled methods synthesis of disaccharide **109α**.[1,3] Relative energies, ΔE, in kcal mol −1 obtained through DFT and the PCM solvation model.

4.2 Proposed mechanisms: Experimental and Computational Study



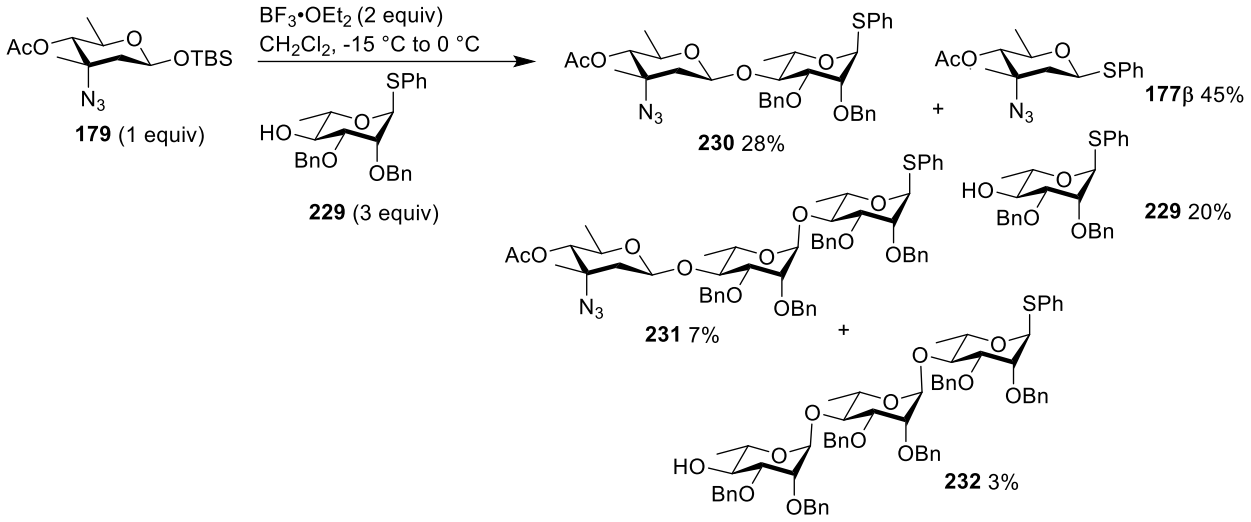
Scheme 4.6 Proposed mechanism for the synthesis of β-linkages with D-saccharosamine **179** using the Lewis acid conditions. Once equivalent of $BF_3{\cdot}OEt_2$ activates **179** and the other equivalent activates the acceptor **123**. Relative energies, ΔE, in kcal mol −1 obtained through DFT and the PCM solvation model.[3]

Having established that a glycosyl cation was not forming, the Bennet lab turned to chemical experiments to gain a better understanding of the types of intermediates that might form from the Lewis acid activation of saccharosamine. To this end, the Bennett lab began by varying the stoichiometry of the promoter. Initially, it was thought that when two equivalents of $BF_3{\cdot}OEt_2$ were used the first equivalent would activate the 1-OTBS as a leaving group and the second equivalent could react with the acceptor species (Scheme 4.6). Lowering the equivalence of $BF_3{\cdot}OEt_2$ from 2 to 1.1 equivalents did not have any effect on the yield or selectivity of the reaction and **196** was synthesized in 77% yield (Scheme 4.7).[3]

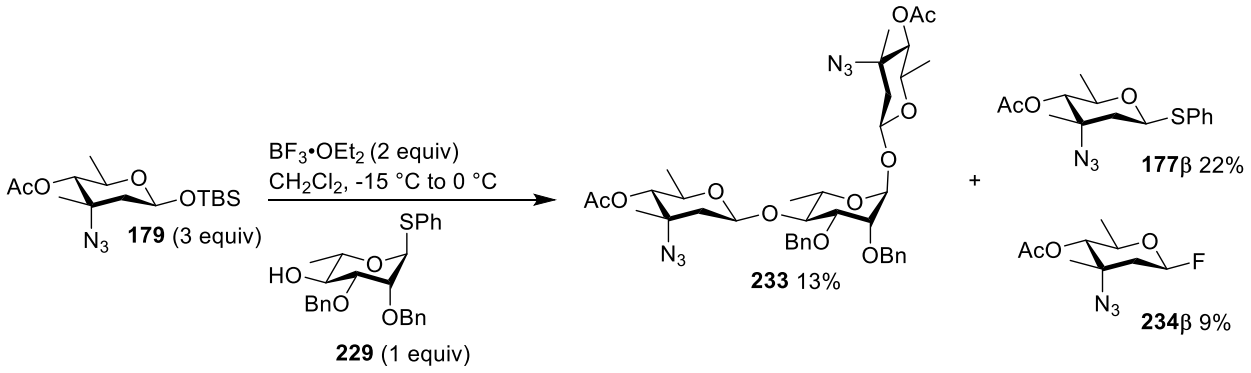


Scheme 4.7 Synthesis of disaccharide **196** using only 1.1 equivalents of $BF_3{\cdot}OEt_2$.[3]

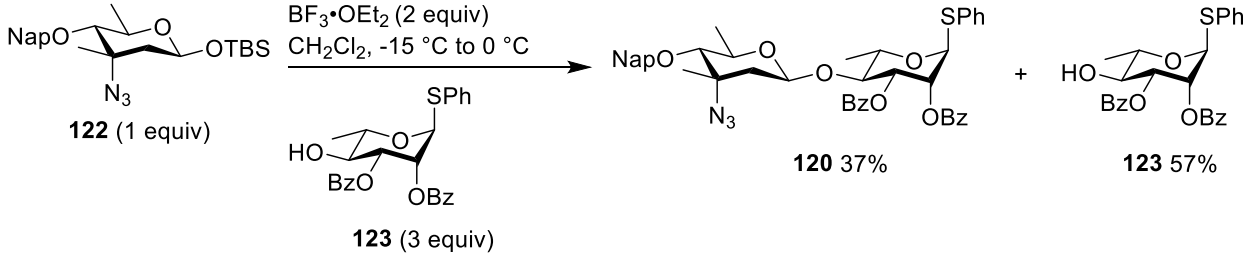
Additionally, the proposed reaction would not be possible if the C-2 and C-3 benzoyl esters of the acceptor were replaced with benzyl ethers. To test this hypothesis, the Bennett lab conducted the Lewis acid glycosylation with **179** and C-2 and C-3 di-O-benzyl **229** which provided the β-linked disaccharide **230** in 28% yield (Scheme 4.8).[3] To ensure the lower yield of the reaction was due to the arming-nature of the benzyl ether protecting groups and not because of the donor and acceptor stoichiometry the reaction was repeated with donor **179** in excess of 3 to 1 equivalents of acceptor **229** (Scheme 4.9). Again, the reaction afforded β-linked products exclusively (Scheme 4.9). In addition to the synthesis of the desired disaccharide when using di-O-benzyl **229**, these reactions afforded trisaccharides **231**, **232**, and **233** (Scheme 4.9, Scheme 4.8). β-linked **177β** was also isolated from the reactions with **229** and is the result of the degradation of **229** (Scheme 4.9, Scheme 4.8).[10] Furthermore, glycosyl fluoride **234β** was isolated when **179** was used in excess (Scheme 4.9). To ensure the disarming effect of the C-4 acetate protection of **179** was not contributing to the selectivity of the Lewis acid glycosylation, **122** which bears a C-4 naphthol methyl ether was tested (Scheme 4.10).[3,11–14] The coupling of **122** and **123** provided disaccharide **120** in 37% yield, thus proving the selectivity's demonstrated within this study are not effected by arming/disarming electronic characteristics (Scheme 4.10).[3] With the results of the experiments mentioned above, the Bennett lab concluded that the acceptor was not being activated by $BF_3{\cdot}OEt_2$.



Scheme 4.8 Synthesis of disaccharide **230** using armed C-2 and C-3 di-O-benzyl **229**.[3] 3 equivalents of acceptor **229** to 1 equivalent of donor **179** were used in the reaction.



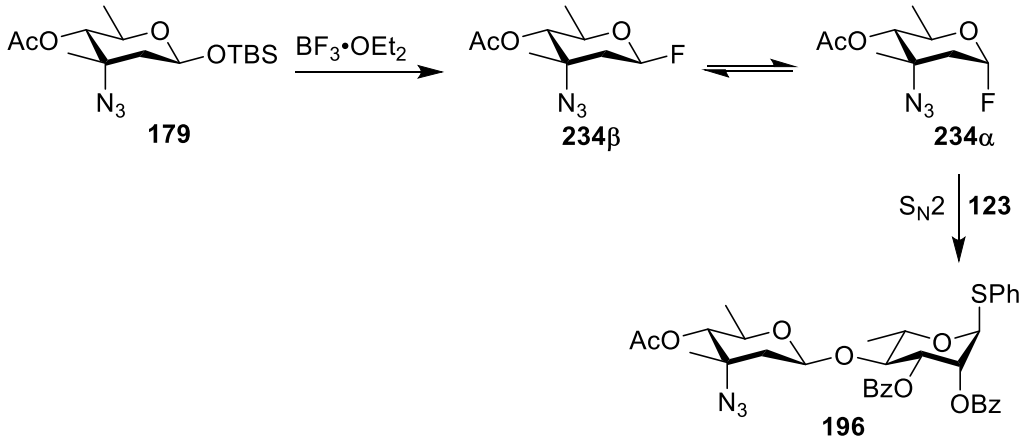
Scheme 4.9 Glycosylation of donor **179** and armed C-2 and C-3 di-O-benzyl **229**. 3 equivalents of donor **179** to 1 equivalent of acceptor **229** were used in the reaction.



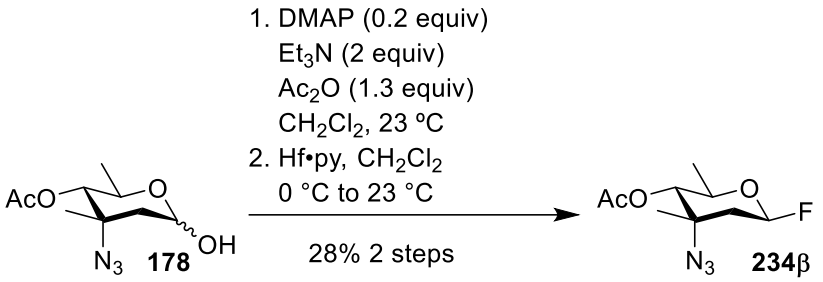
Scheme 4.10 Synthesis of disaccharide **120** using armed donor **122**.[3]

In an effort to understand the results from the glycosylations with **179** and di-O-benzyl **229**, the Bennett lab examined the synthesis glycosyl fluoride **234β** (Scheme 4.9). A possible

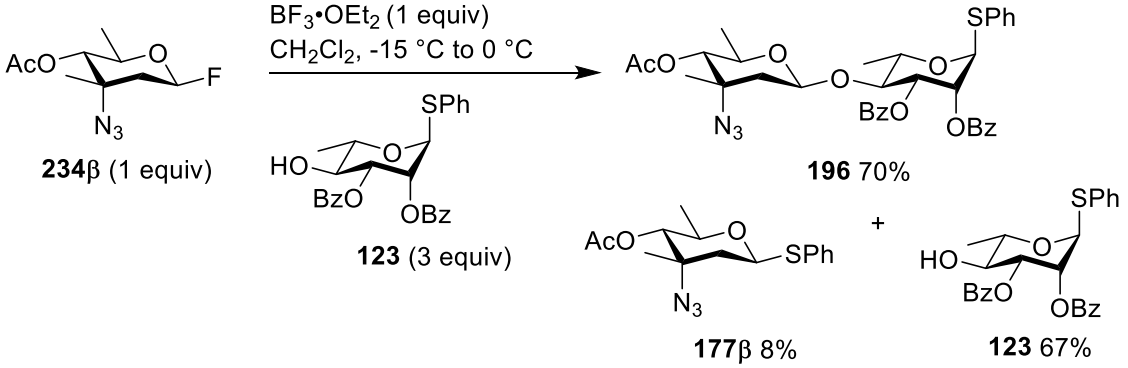
explanation for why **234β** was isolated could be that an α-glycosyl fluoride intermediate **234α** is formed and displaced by an acceptor in an S_N2-like pathway to afford β-linkages. In theory, anomerization of this proposed α-glycosyl fluoride intermediate **234α** could provide the potentially more stable β-linked glycosyl fluoride **234β** which was synthesized in 9% yield (Scheme 4.11, Scheme 4.9). To test this hypothesis, the Bennett lab sought to synthesize glycosyl fluoride **234** and react it under the Lewis acid conditions.[15–17] To this end, hemiacetal **178** was subjected to acetylation using Ac_2O, Et_3N, and DMAP in CH_2Cl_2 followed by treatment with a 30% hydrogen fluoride and pyridine solution in CH_2Cl_2 to afford **233β** in 28% yield over two steps (Scheme 4.12). Next, the Bennett lab coupled **234β** and **123** using the Lewis acid conditions to provide disaccharide **196** in 70% yield (Scheme 4.13). Having synthesized **196** using glycosyl fluoride **234β**, the Bennett lab looked to the Marianski lab to validate the possibility of an α-glycosyl fluoride as an intermediate in the reaction (Scheme 4.11). However, using DFT calculations the Marianski lab was able to demonstrate that the α-glycosyl fluoride **234α** was too high in energy to be an intermediate in the reaction.



Scheme 4.11 Proposed mechanism where a glycosyl fluoride intermediate is formed and then leads to the synthesis of **196**.



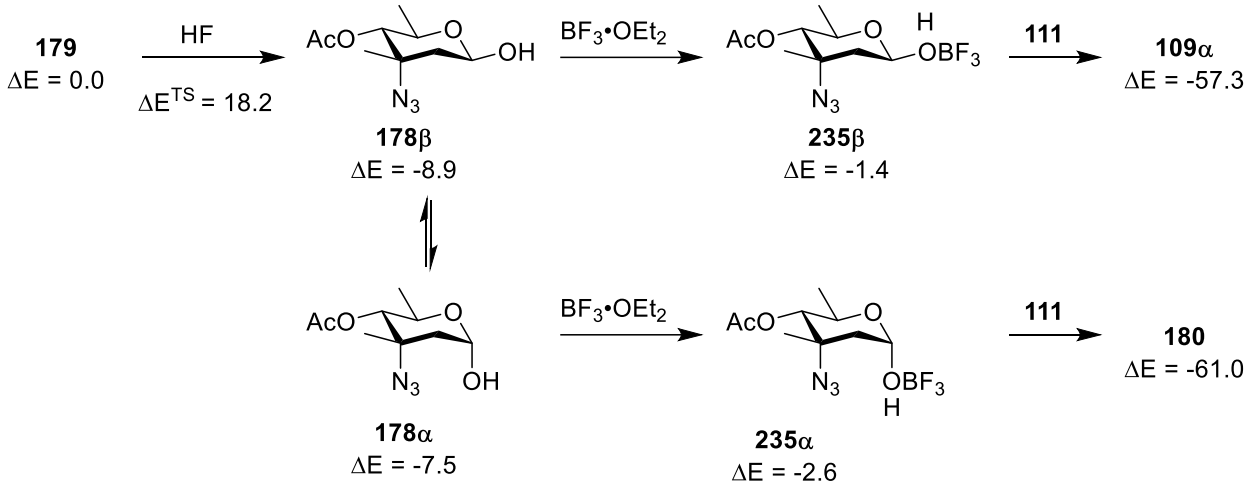
Scheme 4.12 Synthesis of D-saccharosamine glycosyl fluoride **234β**.



Scheme 4.13 Glycosylation using **234β** which provided β-linked disaccharide **196** in 70% yield. Only 1 equivalent of $BF_3{\cdot}OEt_2$ was used in the reaction.

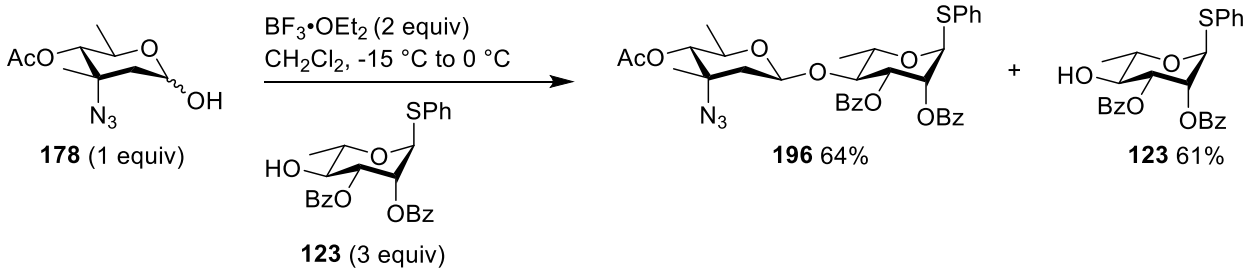
4.3 Promoter Driven Mechanism for the Lewis Acid mediated Glycosylation with Saccharosamine Donors

With the aim of uncovering the origin for the selectivity of the Lewis acid glycosylation with saccharosamine donors, the Bennett and Marianski labs proposed the following mechanism (Scheme 4.14). The activation of D-saccharosamine donor **179** with $BF_3{\cdot}OEt_2$ can result in hemiacetal intermediate species **178β** and **178α** (Scheme 4.14).[3] Either **178β** or **178α** can then react with another equivalent of $BF_3{\cdot}OEt_2$ to form $HOBF_3$-saccharosamine intermediates **235β** or **235α** respectively (Scheme 4.14).[3] Intermediates **235β** and **178β** could react with L-rhamnose acceptor **111** to synthesize α-linked disaccharide **109α**. In contrast, intermediates **235α** and **178α** could react with L-rhamnose acceptor **111** to synthesize β-linked disaccharide **180** (Scheme 4.14).[3] Using DFT calculations, the Marianski lab demonstrated that intermediate **235α** is lower in energy than **235β** and that β-linked **180** is lower in energy than α-linked **109α** (Scheme 4.14).



Scheme 4.14 Proposed mechanism for the synthesis of β-linkages using D-saccharosamine donor **179** under the Lewis acid conditions. A hemiacetal intermediate species is formed which can interconvert between α and β-anomers and lead to another 1-OBF_3 intermediate which can undergo displacement by a nucleophile for the selective synthesis of glycosides **109α** and **180**.[3] Relative energies, ΔE, in kcal mol −1 obtained through DFT and the PCM solvation model.[3]

Next, the Bennett lab sought to test whether the proposed intermediates would lead to the synthesis of the β-linked disaccharide **196** under the Lewis acid conditions (Scheme 414). To this end, hemiacetal D-saccharosamine **178** was coupled with **123** under the Lewis acid conditions to afford disaccharide **196** in 64% yield (Scheme 4.15).[3] With this result, the Bennett and Marianski lab's concluded that it is possible for intermediate **235α** to form in the reaction and that through an S_N2-like pathway β-linked glycosides are synthesized.



Scheme 4.15 Synthesis of disaccharide **196** using hemiacetal donor **178**.[3]

4.4 Conclusion

In conclusion, with the help of the Marianski lab, the Bennett lab was able to uncover the reaction mechanism for the synthesis of β-linkages using saccharosamine donors. This study adds to the promoter driven glycosylation strategy that the Bennett lab has been developing over the past decade.[3,5–7,18,19] With the methods provided by the Bennett lab it is possible to use the same D-saccharosamine hemiacetal donor to synthesize both α and β-anomers of a glycoside by changing the promoter.[3] Furthermore, this study has provided a means to examine the α-selectivity that occurs when 4-*epi*-L-vancosamine donors are reacted under the Lewis acid conditions.[2]

4.5 General Experimental Details

All reactions were performed under an argon atmosphere unless otherwise stated. Cryogenic temperatures were obtained using a cryostat with 2-propanol bath. Column chromatography was performed using a Yamazan Smart Flash W-Prep 2XY automated purification system equipped with universal silica gel columns unless otherwise specified. Analytical and preparative thin layer chromatography was carried out on silica gel F254 plates or RP plates. Products were visualized using UV or by staining with 7.5% aqueous sulfuric acid. NMR spectra were recorded on a Bruker Avance III NMR spectrometer at 500 MHz for ^{1}H-NMR, 125 MHz for ^{13}C-NMR, and 125 MHz ^{19}F decoupled ^{1}H NMR. Chemical shifts are reported in ppm relative to C_6D_6 (for ^{1}H-NMR and ^{13}C-NMR). For ^{1}H NMR spectra, data are reported as follows: δ shift, multiplicity [s = singlet, m = multiplet, t = triplet, d = doublet, brs = broad singlet, and dd = doublet of doublets], coupling constants are reported in Hz. Low resolution mass spectra (LRMS) were recorded using a Finnigan LTQ ESI-MS with an additional APCI source. High resolution mass spectra (HRMS) were obtained on Electro Spray Ionization (ESI) on an Agelent TOF instrument in the positive mode. Optical rotations were measured on a Rudolph Research Analysis AUTOPUL IV polarimeter @ 589 nm in a 5 cm cell at 23 °C.

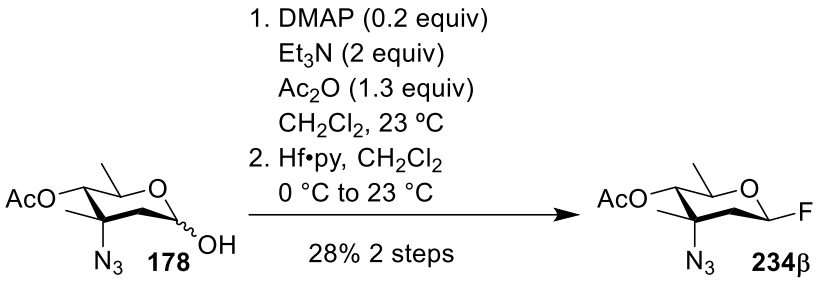
4.6 Materials

Prior to running all glycosylation reactions, donors and acceptors were dried by azeotropic removal of water using anhydrous toluene followed by anhydrous dichloromethane on a rotary evaporator before stirring overnight under vacuum. Solvents for reactions were dried using an Innovative Technologies PureSolv 400 system. Dichloromethane (CH_2Cl_2) used for glycosylations was stored over 4 Å molecular sieves beads that had been microwaved in a conventional microwave ten times for ten seconds and flame dried under vacuum three times. Borontriflouride diethyl etherate ($BF_3{\cdot}OEt_2$) was distilled over calcium hydride (CaH_2) using a short path apparatus equipped with a cow adapter directly before use. Only portions of the distilled reagent that were

collected at their exact boiling temperatures were used in glycosylation reactions. All other chemicals were purchased at the highest possible quality and used as received.

4.7 Experimental Procedures

Fluoro 2,3,6-trideoxy-4-O-acetyl-3-azido-Nos'l-3-C-methyl-1-F-β-D-ribo-hexopyranosyl **234β**



To a round bottom flask were added compound **178** (0.15 g, 0.6547 mmol), DMAP (0.016 g, 0.1309 mmol), and CH_2Cl_2 (5 mL). Then Et_3N (0.18 mL, 0.1291 x 10^{-1} mmol, and Ac_2O (0.08 mL, 0.8463 mmol) were added, and the reaction was stirred at 23 °C. After stirring for 1.25 h, the reaction was quenched with a saturated aqueous solution of $NaHCO_3$ (5 mL), then diluted with CH_2Cl_2 and H_2O. The mixture was extracted 5 times using CH_2Cl_2 and the combined organic layers were dried over Na_2SO_4, filtered, and concentrated in *vacuo*. The crude residue was purified using a Yamazan Smart Flash W-Prep 2XY automated purification system equipped with universal silica gel column (100% hexanes to 20% EtOAc in hexanes) to afford crude D-saccharosamine 1-OAc as a colorless oil which was used directly in the next step.

To a round bottom flask were added crude D-saccharosamine 1-OAc (0.6547 mmol) which was dissolved into anhydrous CH_2Cl_2 (2 mL) and cooled to 0 °C using an ice/water bath. After stirring for 15 minutes at 0 °C, a solution of HF·py (1 mL) was added and the reaction was allowed to warm to 23 °C over 16 h. Then, the reaction was cooled to 0 °C using an ice/water bath, before being quenched with a saturated aqueous solution of $NaHCO_3$ (15 mL). The mixture was diluted with CH_2Cl_2 and H_2O before being extracted 5 times using CH_2Cl_2. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated in *vacuo*. The crude residue was purified using a Yamazan Smart Flash W-Prep 2XY automated purification system equipped with universal silica gel column (100% hexanes to 10% EtOAc in hexanes) to afford **234β** (0.043 g, 28%) as a white solid over two steps.

[α] D ^{23} = +15.9 (c 0.54, CH_2Cl_2).

^{1}H NMR (500 MHz, C_6D_6) δ = 5.40 (dd, J = 9.5, 1.9 Hz, 1H), 4.73 (d, J = 9.6 Hz, 1H), 3.90 – 3.85 (m, 1H), 1.86 – 1.83 (m, 1H), 1.61 (s, 3H), 1.52 – 1.48 (m, 1H), 1.14 (d, J = 6.2 Hz, 3H), 0.90 (s, 3H).

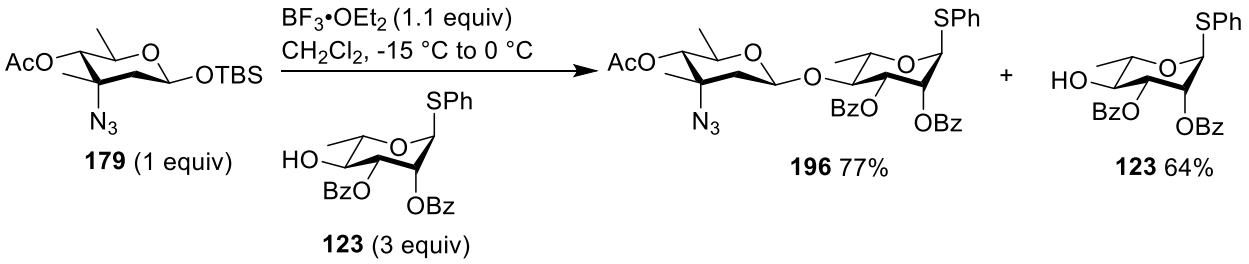
^{13}C NMR (125 MHz, C_6D_6) δ = 169.5, 128.3, 128.0, 94.5, 78.2, 69.3, 62.1, 42.9, 21.9, 19.8, 17.9.

^{19}F NMR (470 MHz, C_6D_6) δ = -163.7.

LRMS (ESI) *m/z* dimer $[M+H]^+$ Calcd for $C_{18}H_{29}F_2N_6O_6$ 463.21; Found 463.36.

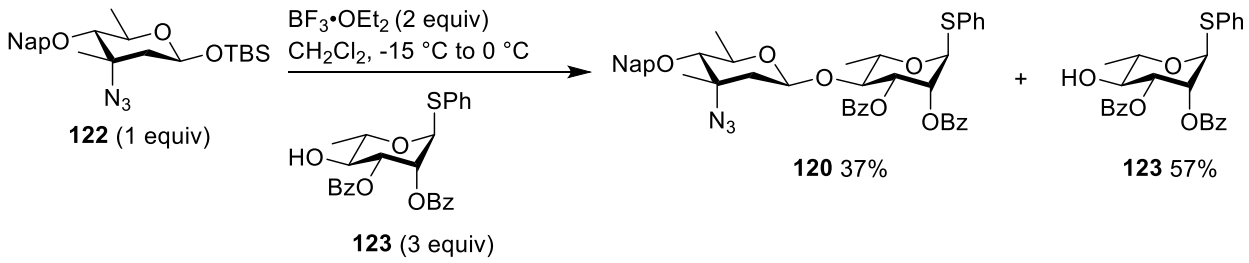
HRMS (ESI) *m/z* dimer $[M+H]^+$ Calcd for $C_{18}H_{29}F_2N_6O_6$ 463.2116; Found 463.1922.

$BF_3{\cdot}OEt_2$ 1.1 equivalents. Acceptor in excess 3 equivalents to 1 equivalent of donor. Disarmed Donor and Acceptor



To a flame dried round bottom flask with stir bar were added compounds **179** (0.050 g, 0.1457 mmol) and **123** (0.203 g, 0.4374 mmol). The mixture underwent azeotrope with anhydrous toluene (3 times) followed by co-evaporation with anhydrous CH_2Cl_2 (3 times) before being left to stir under high vacuum overnight. $BF_3{\cdot}OEt_2$ was distilled over CaH_2 immediately before the reaction. Compounds **179** and **123** were dissolved in anhydrous CH_2Cl_2 (7.8 mL) and cooled to -15 °C using a cryostat. After stirring for 15 minutes, $BF_3{\cdot}OEt_2$ (0.02 mL, 0.1603 mmol) was added, and the reaction stirred for 5 h. The cryostat was then turned off and the reaction was allowed to warm to 0 °C over 1.5 h. Then, the reaction was moved into a 0 °C ice/water bath and stirred for 1.5 h before being quenched with a saturated aqueous solution of $NaHCO_3$ (10 mL), then diluted with CH_2Cl_2 and H_2O. The mixture was extracted 5 times using CH_2Cl_2 and the combined organic layers were dried over Na_2SO_4, filtered, and concentrated in *vacuo*. The crude residue was purified using a Yamazan Smart Flash W-Prep 2XY automated purification system equipped with universal silica gel column (100% hexanes to 30% EtOAc in hexanes) to afford **196** (0.076 g, 77%) and **123** (0.130 g, 64%) both as white solids.

Acceptor in excess 3 equivalents to 1 equivalent of donor. Armed Donor and Disarmed Acceptor



To a flame dried round bottom flask with stir bar were added compounds **122** (0.035 g, 0.07932 mmol) and **123** (0.110 g, 0.2370 mmol). The mixture underwent azeotrope with anhydrous toluene (3 times) followed by co-evaporation with anhydrous CH_2Cl_2 (3 times) before being left to stir under high vacuum overnight. $BF_3{\cdot}OEt_2$ was distilled over CaH_2 immediately before the reaction. Compounds **122** and **123** were dissolved in anhydrous CH_2Cl_2 (4.3 mL) and cooled to -15 °C using a cryostat. After stirring for 15 minutes, $BF_3{\cdot}OEt_2$ (0.02 mL, 0.1586 mmol) was added, and the reaction stirred for 5 h. The cryostat was then turned off and the reaction was allowed to warm to 0 °C over 1.5 h. Then, the reaction was moved into a 0 °C ice/water bath and stirred for 1.5 h before being quenched with a saturated aqueous solution of $NaHCO_3$ (10 mL), then diluted with CH_2Cl_2 and H_2O. The mixture was extracted 5 times using CH_2Cl_2 and the combined organic layers were dried over Na_2SO_4, filtered, and concentrated in *vacuo*. The crude residue was purified using a Yamazan Smart Flash W-Prep 2XY automated purification system equipped with universal silica gel column (100% hexanes to 30% EtOAc in hexanes) to afford **120** (0.023 g, 37%) and 3 (0.063 g, 57%) both as white solids.

Compound **120**

[α] D ^{23} = -49.6 (c 0.50, CH_2Cl_2).

^{1}H NMR (500 MHz, C_6D_6) δ = 8.27 – 8.26 (m, 2H), 8.11 (d, J = 7.4 Hz, 2H), 7.73 (d, J = 7.7 Hz, 2H), 7.70 (s, 1H), 7.63 – 7.61 (m, 2H), 7.44 (d, J = 7.2 Hz, 2H), 7.32 (d, J = 8.3 Hz, 1H), 7.28 – 7.23 (m, 2H), 7.06 – 7.03 (m, 3H), 7.01 – 6.97 (m, 3H), 6.95 – 6.91 (m, 3H), 6.42 (d, J = 1.5 Hz, 1H), 6.14 (dd, J = 9.8, 3.2 Hz, 1H), 5.70 (s, 1H), 5.23 (d, J = 9.1 Hz, 1H), 4.78 – 4.73 (m, 1H),

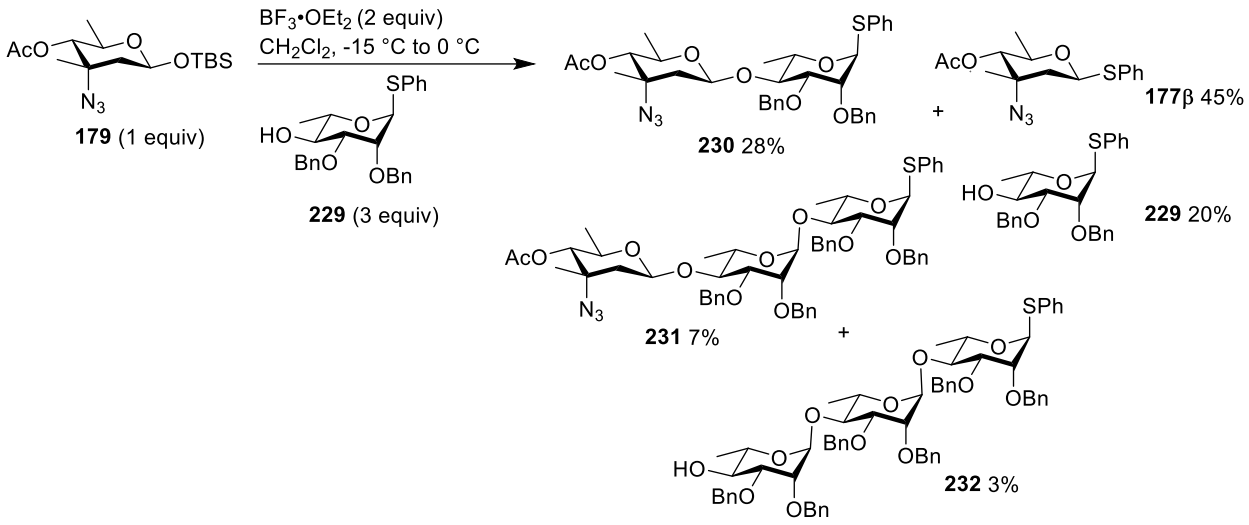
4.47 – 4.40 (m, 2H), 4.31 – 4.28 (m, 1H), 3.78 – 3.73 (m, 1H), 2.60 (d, J = 9.2 Hz, 1H), 1.76 (d, J = 6.1 Hz, 3H), 1.63 (d, J = 13.4 Hz, 1H), 1.31 (dd, J = 13.4, 9.4 Hz, 1H), 1.19 (d, J = 6.1 Hz, 3H), 0.82 (s, 3H).

^{13}C NMR (125 MHz, C_6D_6) δ = 165.9, 165.5, 135.7, 134.4, 133.9, 133.5, 133.4, 133.3, 132.1, 130.3, 130.2, 130.10, 130.08, 129.3, 128.7, 128.6, 128.5, 128.3, 128.0, 127.6, 126.5, 126.3, 126.2, 125.5, 99.9, 86.9, 86.3, 78.8, 76.0, 73.8, 73.0, 70.8, 69.5, 62.8, 43.2, 22.2, 18.6, 18.4.

LRMS (ESI) *m/z* $[M+Na]^+$ Calcd for $C_{44}H_{43}N_3NaO_8S$ 796.27; Found 796.36.

HRMS (ESI) *m/z* $[M+K]^+$ Calcd for $C_{44}H_{43}KN_3O_8S$ 812.2408; Found 812.2624.

Acceptor in excess 3 equivalents to 1 equivalent of donor. Disarmed Donor and Armed Acceptor



To a flame dried round bottom flask with stir bar were added compounds **179** (0.050 g, 0.1457 mmol) and **229** (0.191 g, 0.4379 mmol). The mixture underwent azeotrope with anhydrous toluene (3 times) followed by co-evaporation with anhydrous CH_2Cl_2 (3 times) before being left to stir under high vacuum overnight. $BF_3{\cdot}OEt_2$ was distilled over CaH_2 immediately before the reaction. Compounds **179** and **229** were dissolved in anhydrous CH_2Cl_2 (7.8 mL) and cooled to -15 °C using a cryostat. After stirring for 15 minutes, $BF_3{\cdot}OEt_2$ (0.04 mL, 0.3241 mmol) was added, and the reaction stirred for 5 h. The cryostat was then turned off and the reaction was allowed to warm to 0 °C over 1.5 h. Then, the reaction was moved into a 0 °C ice/water bath and stirred for 1.5 h before being quenched with a saturated aqueous solution of $NaHCO_3$ (10 mL), then diluted with CH_2Cl_2 and H_2O. The mixture was extracted 5 times using CH_2Cl_2 and the combined organic layers were dried over Na_2SO_4, filtered, and concentrated in *vacuo*. The crude residue was purified using a Yamazan Smart Flash W-Prep 2XY automated purification system equipped with universal silica gel column (100% hexanes to 30% EtOAc in hexanes) to afford crude **230** (0.036 g), **231** (0.0096 g, 7%) as a colorless oil, **177β** (0.021 g, 45%) as a white solid, **229** (0.039 g, 20%) as a colorless oil, and **232** (0.013 g, 3%) as a colorless oil. Crude **230** (0.036 g) was purified using a Yamazan Smart Flash W-Prep 2XY automated purification system equipped with

universal silica gel column (100% hexanes to 5% acetone in hexanes) to afford **230** (0.026 g, 28%) as a white solid.

Compound **230**

[α] D ^{23} = -54.2 (c 0.24, CH_2Cl_2).

^{1}H NMR (500 MHz, C_6D_6) δ = 7.54 (d, J = 7.3 Hz, 2H), 7.42 (d, J = 7.4 Hz, 2H), 7.32 – 7.29 (m, 2H), 7.23 (d, J = 6.9 Hz, 2H), 7.18 (d, J = 7.4 Hz, 1H), 7.06 – 6.96 (m, 6H), 5.79 (s, 1H), 5.31 (d, J = 9.2 Hz, 1H), 4.66 (d, J = 9.6 Hz, 1H), 4.52 – 4.45 (m, 2H), 4.38 (d, J = 11.4 Hz, 1H), 4.34 – 4.31 (m, 3H), 4.10 (s, 1H), 3.98 – 3.96 (m, 1H), 3.53 – 3.47 (m, 1H), 1.86 (d, J = 13.5 Hz, 1H), 1.70 (d, J = 6.1 Hz, 3H), 1.62 (s, 3H), 1.44 – 1.39 (m, 1H), 0.97 – 0.96 (m, 6H).

^{13}C NMR (125 MHz, C_6D_6) δ = 169.5, 138.6, 138.5, 135.8, 131.6, 129.4, 128.7, 128.6, 128.3, 128.2, 128.0, 127.4, 99.0, 86.3, 80.7, 78.4, 78.2, 76.9, 72.2, 71.9, 69.7, 68.8, 62.3, 43.1, 22.1, 19.8, 18.4, 17.7.

LRMS (ESI) *m/z* $[M+Na]^+$ Calcd for $C_{35}H_{41}N_3NaO_7S$ 670.26; Found 670.27.

HRMS (ESI) *m/z* $[M+Na]^+$ Calcd for $C_{35}H_{41}N_3NaO_7S$ 670.2563; Found 670.2557.

Compound **231**

[α] D ^{23} = -31.1 (c 0.75, CH_2Cl_2).

^{1}H NMR (500 MHz, C_6D_6) δ = 7.54 (d, J = 7.3 Hz, 2H), 7.49 (d, J = 7.4 Hz, 2H), 7.32 (t, J = 7.6 Hz, 2H), 7.25 – 7.23 (m, 6H), 7.18 (d, J = 7.7 Hz, 1H), 7.11 – 6.98 (m, 12H), 5.75 (s, 1H), 5.68 (s, 1H), 5.34 (d, J = 8.8 Hz, 1H), 4.66 (d, J = 9.6 Hz, 1H), 4.56 (d, J = 11.4 Hz, 1H), 4.49 (d, J = 11.5 Hz, 1H), 4.45 – 4.39 (m, 3H), 4.36 – 4.24 (m, 6H), 4.21 – 4.16 (m, 2H), 4.08 – 4.05 (m, 3H), 3.97 – 3.94 (m, 1H), 3.52 – 3.47 (m, 1H), 1.89 (d, J = 13.1 Hz, 1H), 1.69 (d, J = 6.1 Hz, 3H), 1.61 (s, 3H), 1.55 (d, J = 6.1 Hz, 3H), 1.46 – 1.41 (m, 1H), 0.97 (d, J = 6.2 Hz, 3H), 0.95 (s, 3H).

^{13}C NMR (125 MHz, C_6D_6) δ = 169.5, 139.3, 139.1, 138.6, 138.4, 135.5, 131.7, 129.4, 128.8, 128.62, 128.55, 128.4, 128.2, 128.0, 127.7, 127.6, 127.52, 127.47, 100.4, 99.0, 86.0, 81.0, 80.6,

79.7, 78.5, 78.3, 76.4, 76.0, 72.4, 72.03, 71.96, 71.0, 69.5, 69.3, 68.8, 62.3, 43.2, 22.1, 19.8, 18.7, 18.4, 17.7.

LRMS (ESI) *m/z* $[M+Na]^+$ Calcd for $C_{55}H_{63}N_3NaO_{11}S$ 996.41; Found 996.45.

HRMS (ESI) *m/z* $[M+K]^+$ Calcd for $C_{55}H_{63}KN_3O_{11}S$ 1012.3820; Found 1012.4028.

Compound **232**

$[\alpha]$ D ^{23} = -17.0 (c 0.92, CH_2Cl_2).

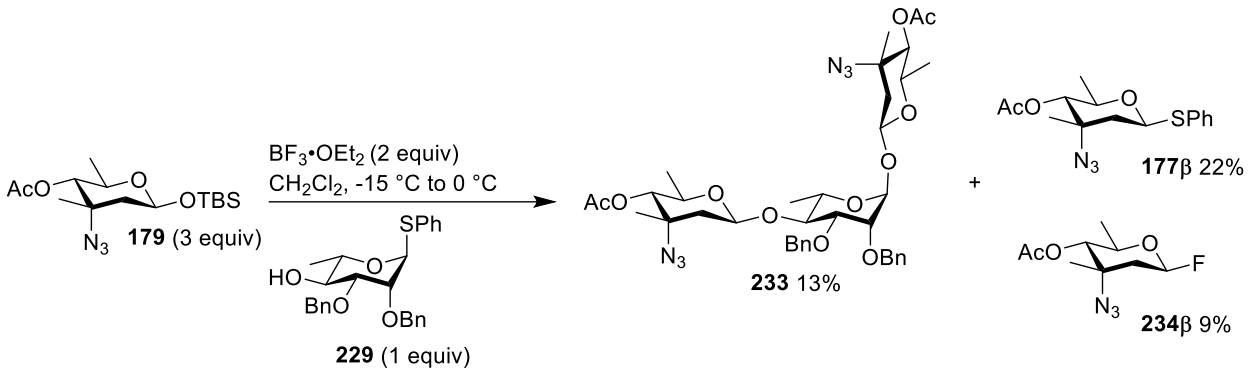
^{1}H NMR (500 MHz, C_6D_6) δ = 7.54 (d, J = 7.7Hz, 2H), 7.29 (d, J = 7.5 Hz, 2H), 7.26 – 7.22 (m, 10H), 7.13 (d, J = 9.2 Hz, 2H), 7.10 – 7.04 (m, 15H), 7.02 – 7.00 (m, 4H), 5.76 (s, 1H), 5.68 (s, 1H), 5.62 (s, 1H), 4.53 (d, J = 11.6 Hz, 1H), 4.49 – 4.47 (m, 1H), 4.46 – 4.43 (m, 1H), 4.40 (d, J = 5.4 Hz, 1H), 4.37 – 4.32 (m, 4H), 4.30 – 4.22 (m, 6H), 4.16 (d, J = 12.1 Hz, 1H), 4.13 – 4.12 (m, 1H), 4.09 – 4.05 (m, 3H), 4.03 – 3.95 (m, 4H), 3.89 – 3.87 (m, 1H), 2.09 (brs, 1H), 1.54 (d, J = 6.1 Hz, 3H), 1.51 (d, J = 6.1 Hz, 3H), 1.44 (d, J = 6.0 Hz, 3H).

^{13}C NMR (125 MHz, C_6D_6) δ = 139.5, 139.3, 139.2, 139.0, 138.5, 138.4, 135.4, 131.7, 129.4, 128.8, 128.7, 128.64, 128.59, 128.44, 128.35, 128.0, 127.7, 127.63, 127.55, 127.4, 100.5, 100.3, 86.0, 80.9, 80.8, 80.7, 80.0, 79.4, 76.4, 76.1, 75.7, 72.4, 72.3, 72.1, 71.6, 71.1, 71.0, 70.1, 69.4, 69.2, 18.70, 18.68, 18.0.

LRMS (ESI) *m/z* $[M+Na]^+$ Calcd for $C_{66}H_{72}NaO_{12}S$ 1111.4642; Found 1111.4650.

HRMS (ESI) *m/z* $[M+Na]^+$ Calcd for $C_{66}H_{72}N_aO_{12}S$ 1111.46; Found 1111.64.

Glycosyl Donor in excess 3 equivalents to 1 equivalent of acceptor. Disarmed Donor and Armed Accepter



To a flame dried round bottom flask with stir bar were added compounds **179** (0.224 g, 0.6527 mmol) and **229** (0.095 g, 0.2178 mmol). The mixture underwent azeotrope with anhydrous toluene (3 times) followed by co-evaporation with anhydrous CH_2Cl_2 (3 times) before being left to stir under high vacuum overnight. $BF_3{\cdot}OEt_2$ was distilled over CaH_2 immediately before the reaction. Compounds **179** and **229** were dissolved in anhydrous CH_2Cl_2 (11 mL) and cooled to -15 °C using a cryostat. After stirring for 30 minutes, $BF_3{\cdot}OEt_2$ (0.06 mL, 0.4862 mmol) was added, and the reaction stirred for 5 h. The cryostat was then turned off and the reaction was allowed to warm to 0 °C over 1 h. Then, the reaction was moved into a 0 °C ice/water bath and stirred for 1 h before being quenched with a saturated aqueous solution of $NaHCO_3$ (15 mL), then diluted with CH_2Cl_2 and H_2O. The mixture was extracted 5 times using CH_2Cl_2 and the combined organic layers were dried over Na_2SO_4, filtered, and concentrated in *vacuo*. The crude residue was purified using a Yamazan Smart Flash W-Prep 2XY automated purification system equipped with universal silica gel column (100% hexanes to 20% EtOAc in hexanes) to afford **177β** (0.046 g, 22%) as a white solid, crude **234β** (0.050 g) as a white solid, and crude **233** (0.048 g) as a white solid. Crude **233** (0.048 g) was purified using a Yamazan Smart Flash W-Prep 2XY automated purification system equipped with universal silica gel column (100% toluene to 5% EtOAc in toluene) to afford **233** (0.021 g, 13%) as a colorless oil and **234β** (0.014 g, 9%) as a white solid.

Compound **233**

[α] D ^{23} = +6.5 (c 0.68, CH_2Cl_2).

^{1}H NMR (500 MHz, C_6D_6) δ = 7.59 (d, J = 7.2 Hz, 2H), 7.06 – 7.03 (m, 2H), 6.97 (t, J = 7.4 Hz, 1H), 5.07 (dd, J = 11.5, 1.8 Hz, 1H), 4.65 (d, J = 9.6 Hz, 1H), 3.70 – 3.64 (m, 1H), 1.73 – 1.70 (m, 1H), 1.61 (s, 3H), 1.46 (dd, J = 13.7, 11.7 Hz, 1H), 1.08 (d, J = 6.2 Hz, 3H), 0.85 (s, 3H).

^{13}C NMR (125 MHz, C_6D_6) δ = 169.4, 134.7, 132.2, 129.1, 128.3, 128.0, 127.7, 80.6, 77.9, 71.9, 62.4, 43.2, 21.7, 19.7, 18.0.

LRMS (ESI) *m/z* $[M+K]^+$ Calcd for $C_{15}H_{19}KN_3O_3S$ 360.08; Found 360.18.

HRMS (ESI) *m/z* $[M+K]^+$ Calcd for $C_{15}H_{19}KN_3O_3S$ 360.0784; Found 359.9825.

Compound **177β**

[α] D ^{23} = -25.2 (c 0.53, CH_2Cl_2).

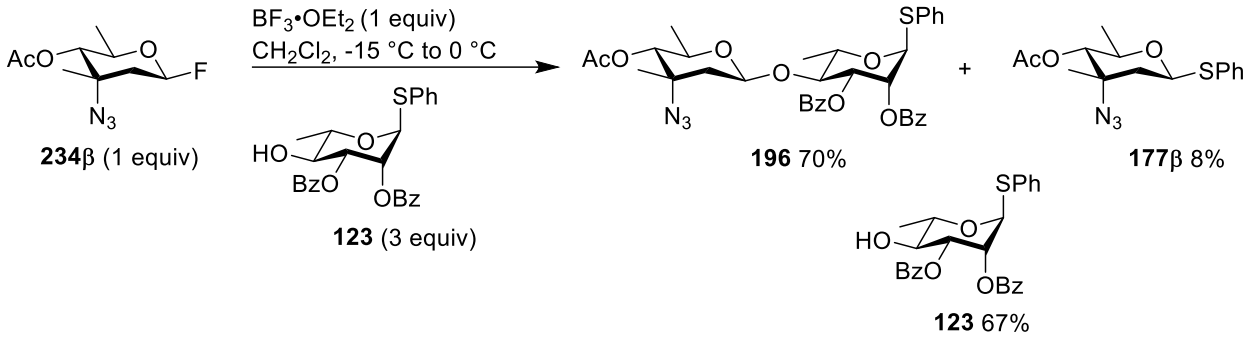
^{1}H NMR (500 MHz, C_6D_6) δ = 7.46 (d, J = 7.5, 2H), 7.34 – 7.28 (m, 4H), 7.18 (d, J = 7.5 Hz, 1H), 7.09 – 7.01 (m, 3H), 5.62 (s, 1H), 5.35 (d, J = 8.3 Hz, 1H), 5.23 (d, J = 9.3 Hz, 1H), 4.71 (d, J = 9.6 Hz, 1H), 4.66 (d, J = 9.6 Hz, 1H), 4.54 (s, 2H), 4.46 (d, J = 11.6 Hz, 1H), 4.40 (d, J = 11.6 Hz, 1H), 4.36 – 4.32 (m, 1H), 4.07 – 4.00 (m, 3H), 3.81 – 3.76 (m, 1H), 3.54 – 3.48 (m, 1H), 1.89 (d, J = 13.4 Hz, 1H), 1.75 (d, J = 6.2 Hz, 3H), 1.65 (s, 3H), 1.62 (s, 3H), 1.59 (d. J = 13.8 Hz, 1H), 1.46 – 1.40 (m, 2H), 1.11 (d, J = 6.2 Hz, 3H), 0.99 – 0.95 (m, 9H).

^{13}C NMR (125 MHz, C_6D_6) δ = 169.5, 138.9, 138.8, 128.64, 128.59, 128.5, 128.2, 128.0, 127.8, 99.0, 95.0, 93.3, 80.2, 78.4, 78.2, 78.1, 74.9, 73.0, 71.7, 69.4, 69.2, 68.8, 62.3, 62.0, 43.2, 42.7, 22.1, 22.0, 19.8, 18.6, 17.8, 17.7.

LRMS (ESI) *m/z* $[M+Na]^+$ Calcd for $C_{38}H_{50}N_6NaO_{11}$ 789.34; Found 789.45.

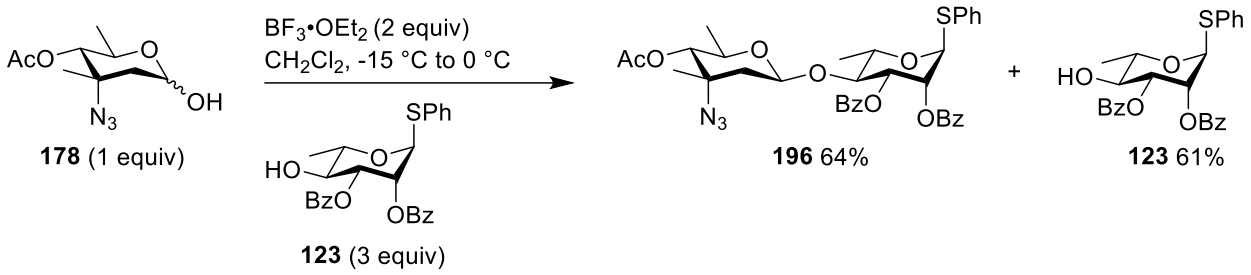
HRMS (ESI) *m/z* $[M+Na]^+$ Calcd for $C_{38}H_{50}N_6NaO_{11}$ 789.3436 Found 789.3429.

Fluoride Donor. Acceptor in excess 3 equivalents to 1 equivalent of donor. Disarmed Donor and Acceptor



To a flame dried round bottom flask with stir bar were added compounds **234β** (0.050 g, 0.2164 mmol) and **123** (0.301 g, 0.6485 mmol). The mixture underwent azeotrope with anhydrous toluene (3 times) followed by co-evaporation with anhydrous CH_2Cl_2 (3 times) before being left to stir under high vacuum overnight. $BF_3{\cdot}OEt_2$ was distilled over CaH_2 immediately before the reaction. Compounds **234β** and **123** were dissolved in anhydrous CH_2Cl_2 (11.6 mL) and cooled to -15 °C using a cryostat. After stirring for 15 minutes, $BF_3{\cdot}OEt_2$ (0.03 mL, 0.2431 mmol) was added, and the reaction stirred for 6 h. The cryostat was then turned off and the reaction was allowed to warm to 0 °C over 1 h. Then, the reaction was moved into a 0 °C ice/water bath and stirred for 1 h before being quenched with a saturated aqueous solution of $NaHCO_3$ (15 mL), then diluted with CH_2Cl_2 and H_2O. The mixture was extracted 5 times using CH_2Cl_2 and the combined organic layers were dried over Na_2SO_4, filtered, and concentrated in *vacuo*. The crude residue was purified using a Yamazan Smart Flash W-Prep 2XY automated purification system equipped with universal silica gel column (100% hexanes to 20% EtOAc in hexanes) to afford **196** (0.103 g, 70%), **123** (0.203 g, 67%), and **177β** (0.0052 g, 8%), all as white solids.

Hemiacetal Donor. Acceptor in excess 3 equivalents to 1 equivalent of donor. Disarmed Donor and Acceptor



To a flame dried round bottom flask with stir bar were added compounds **178** (0.029 g, 0.1266 mmol) and **123** (0.176 g, 0.3792 mmol). The mixture underwent azeotrope with anhydrous toluene (3 times) followed by co-evaporation with anhydrous CH_2Cl_2 (3 times) before being left to stir under high vacuum overnight. $BF_3{\cdot}OEt_2$ was distilled over CaH_2 immediately before the reaction. Compounds **178** and **123** were dissolved in anhydrous CH_2Cl_2 (6.8 mL) and cooled to -15 °C using a cryostat. After stirring for 15 minutes, $BF_3{\cdot}OEt_2$ (0.03 mL, 0.2532 mmol) was added, and the reaction stirred for 5 h. The cryostat was then turned off and the reaction was allowed to warm to 0 °C over 1 h. Then, the reaction was moved into a 0 °C ice/water bath and stirred for 1.25 h before being quenched with a saturated aqueous solution of $NaHCO_3$ (10 mL), then diluted with CH_2Cl_2 and H_2O. The mixture was extracted 5 times using CH_2Cl_2 and the combined organic layers were dried over Na_2SO_4, filtered, and concentrated in *vacuo*. The crude residue was purified using a Yamazan Smart Flash W-Prep 2XY automated purification system equipped with universal silica gel column (100% hexanes to 30% EtOAc in hexanes) to afford **178** (0.055 g, 64%) and **123** (0.107 g, 61%) both as a white solids.

4.8 References

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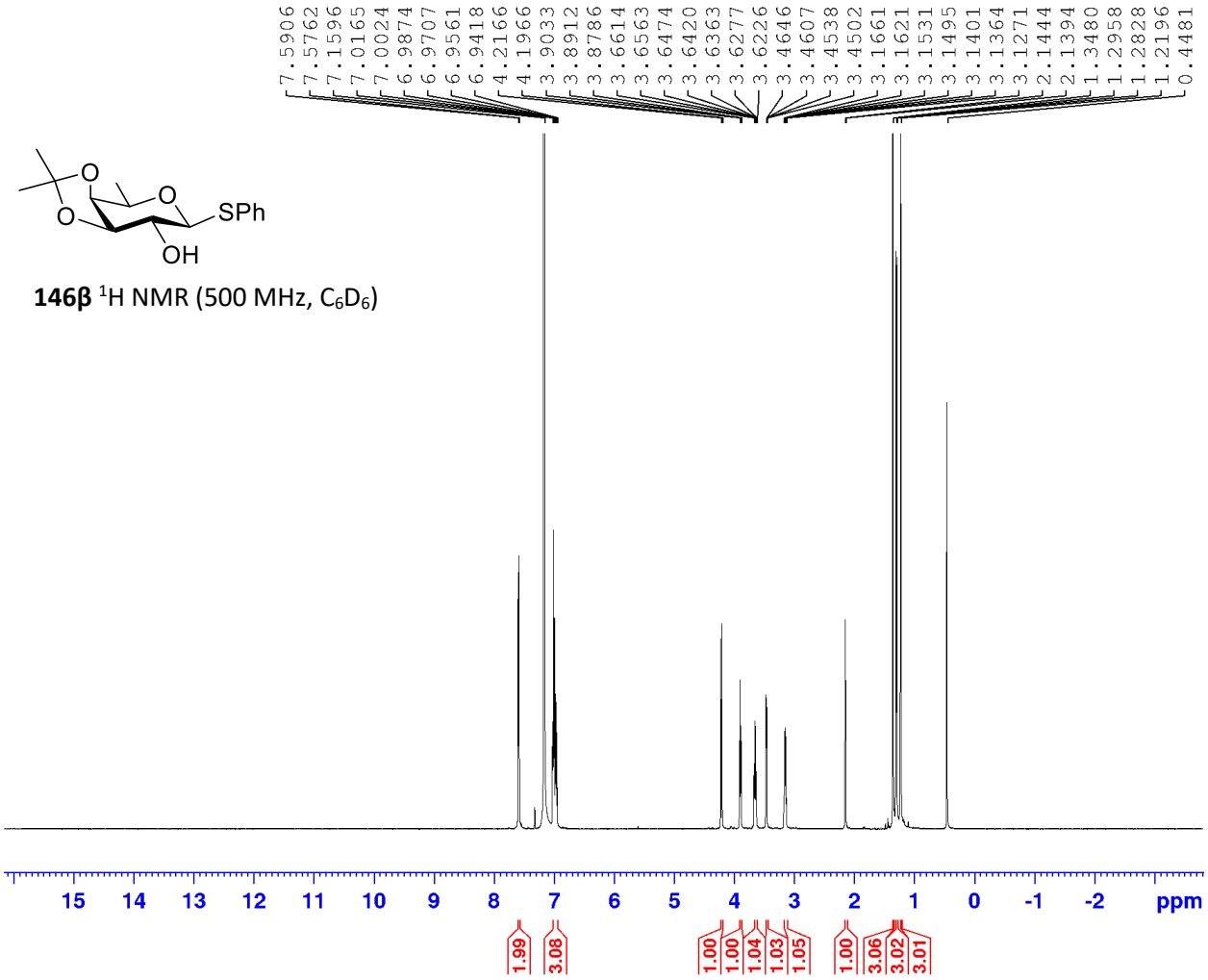
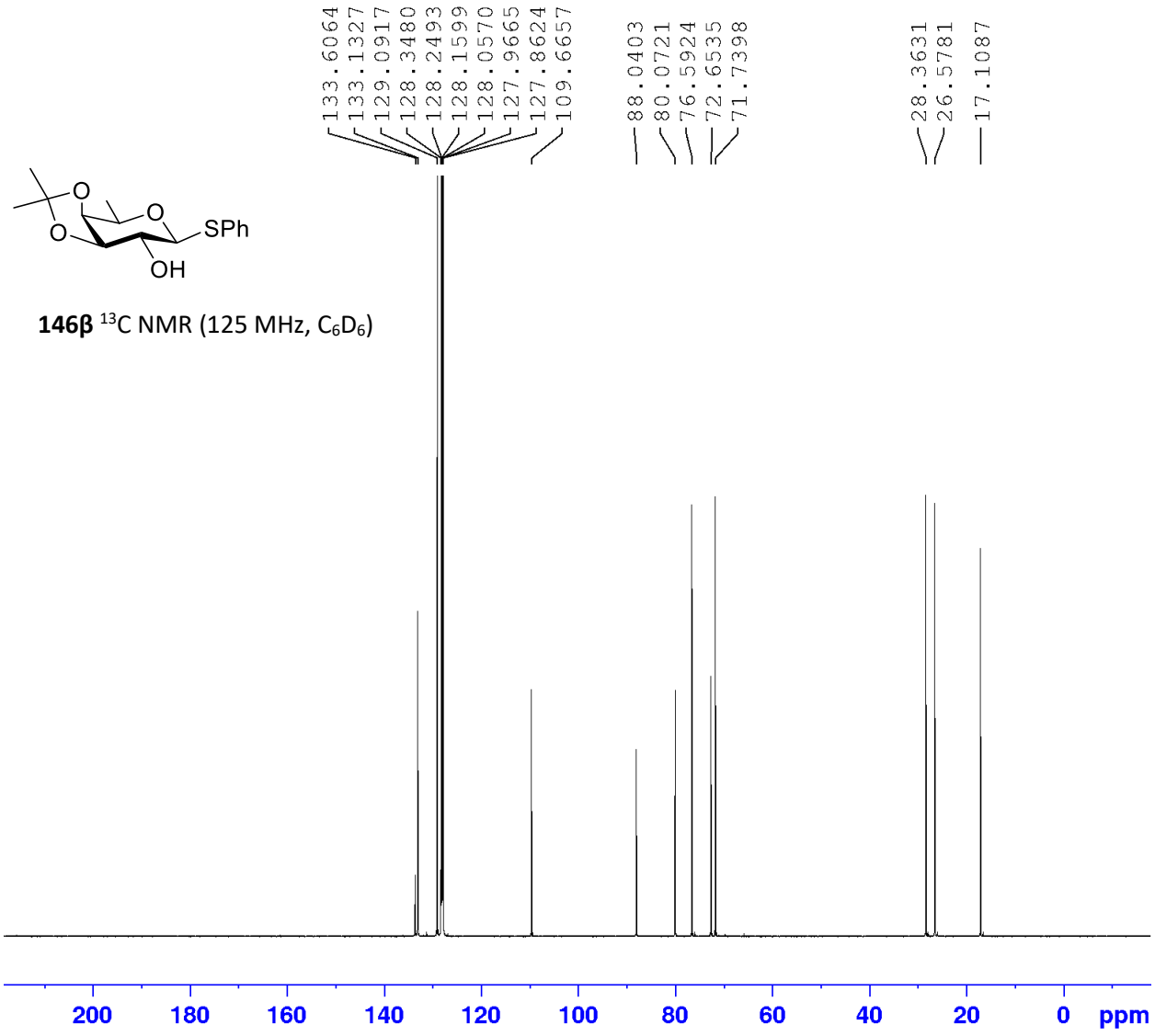
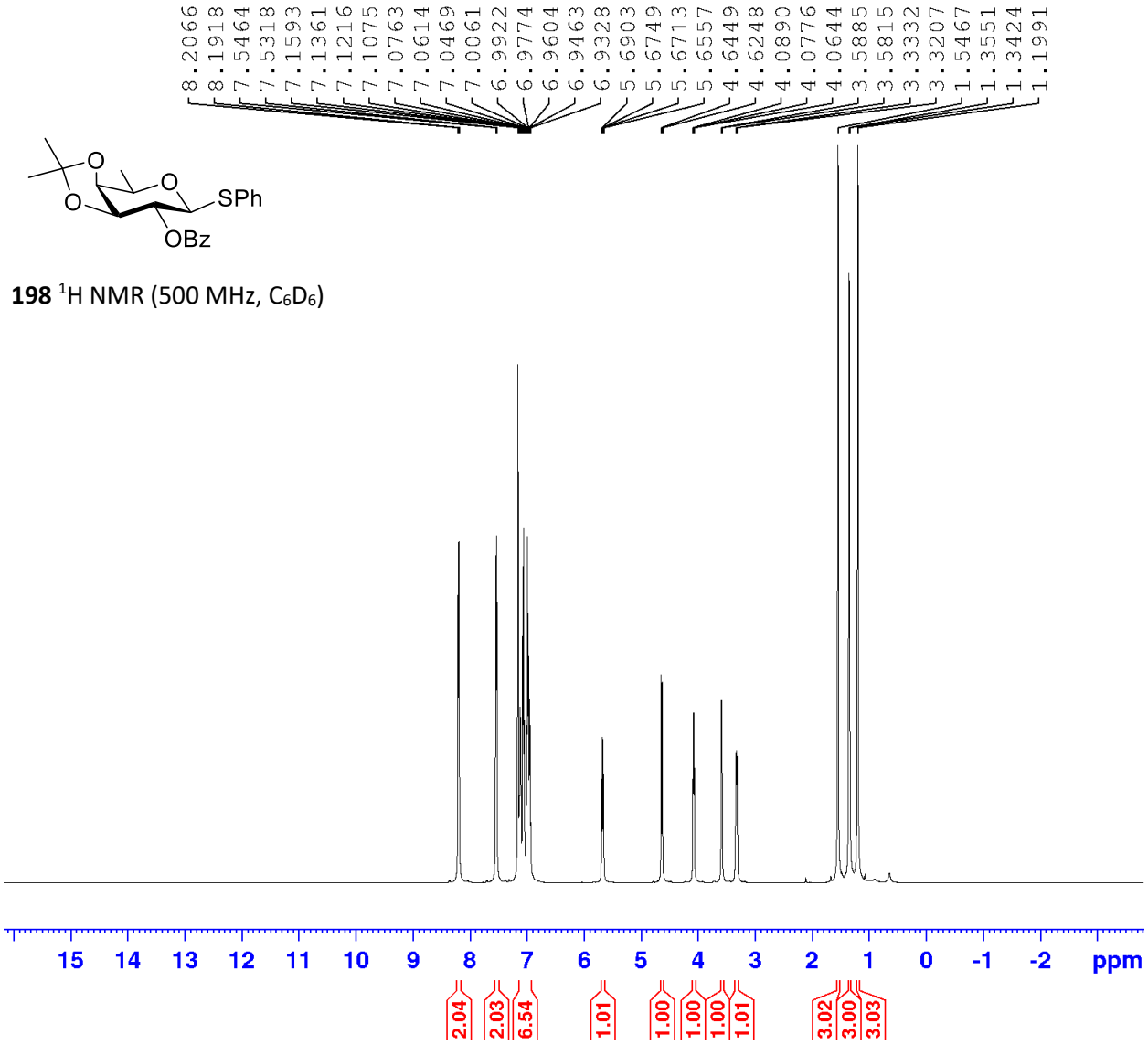
Appendix: Spectra for New Compounds in Chapters 2, 3, and 4



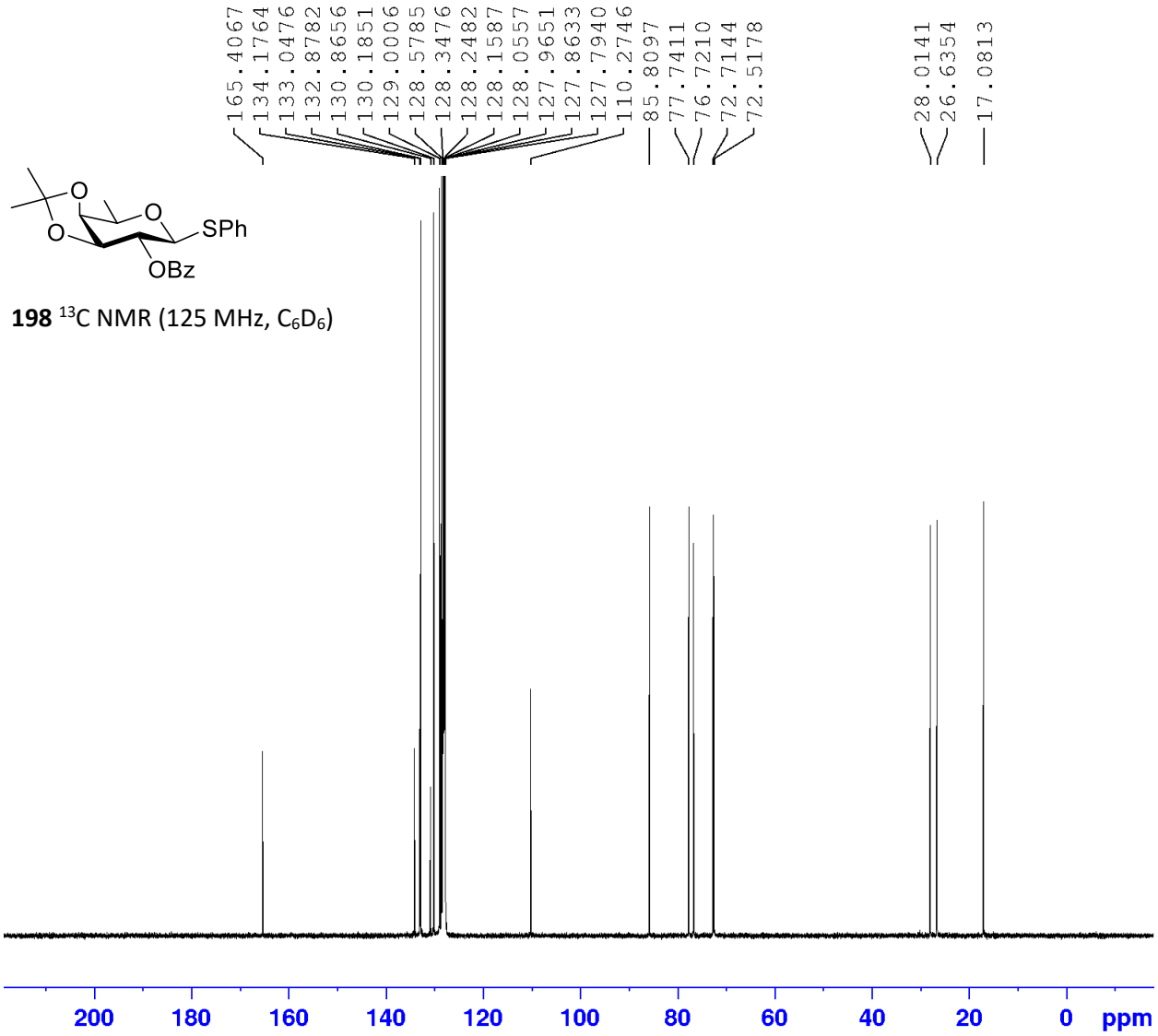
146β ^{1}H NMR (500 MHz, C_6D_6)



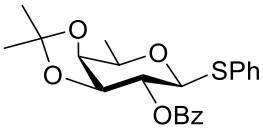
146β ^{13}C NMR (125 MHz, C_6D_6)



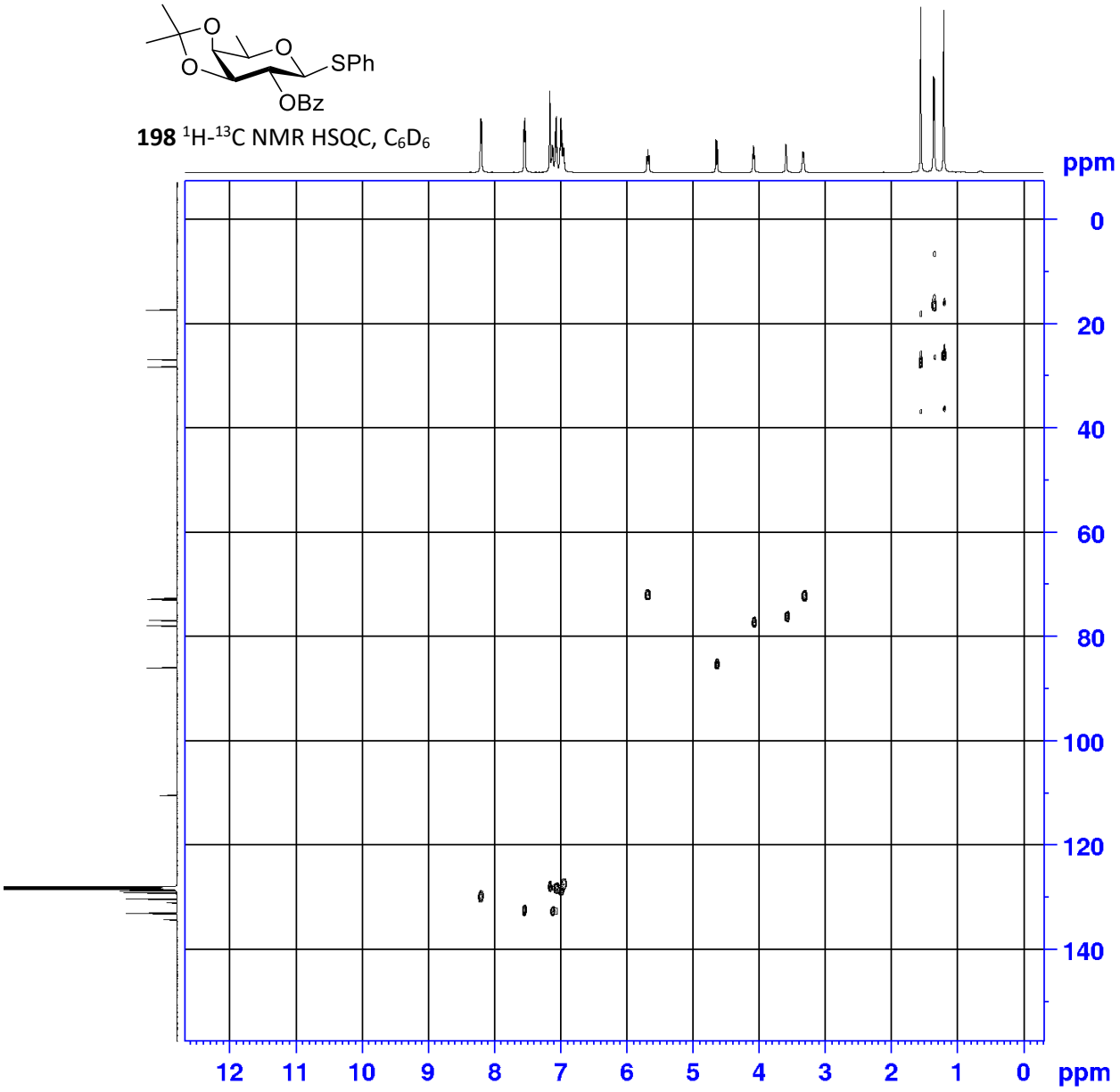
198 ^{1}H NMR (500 MHz, C_6D_6)



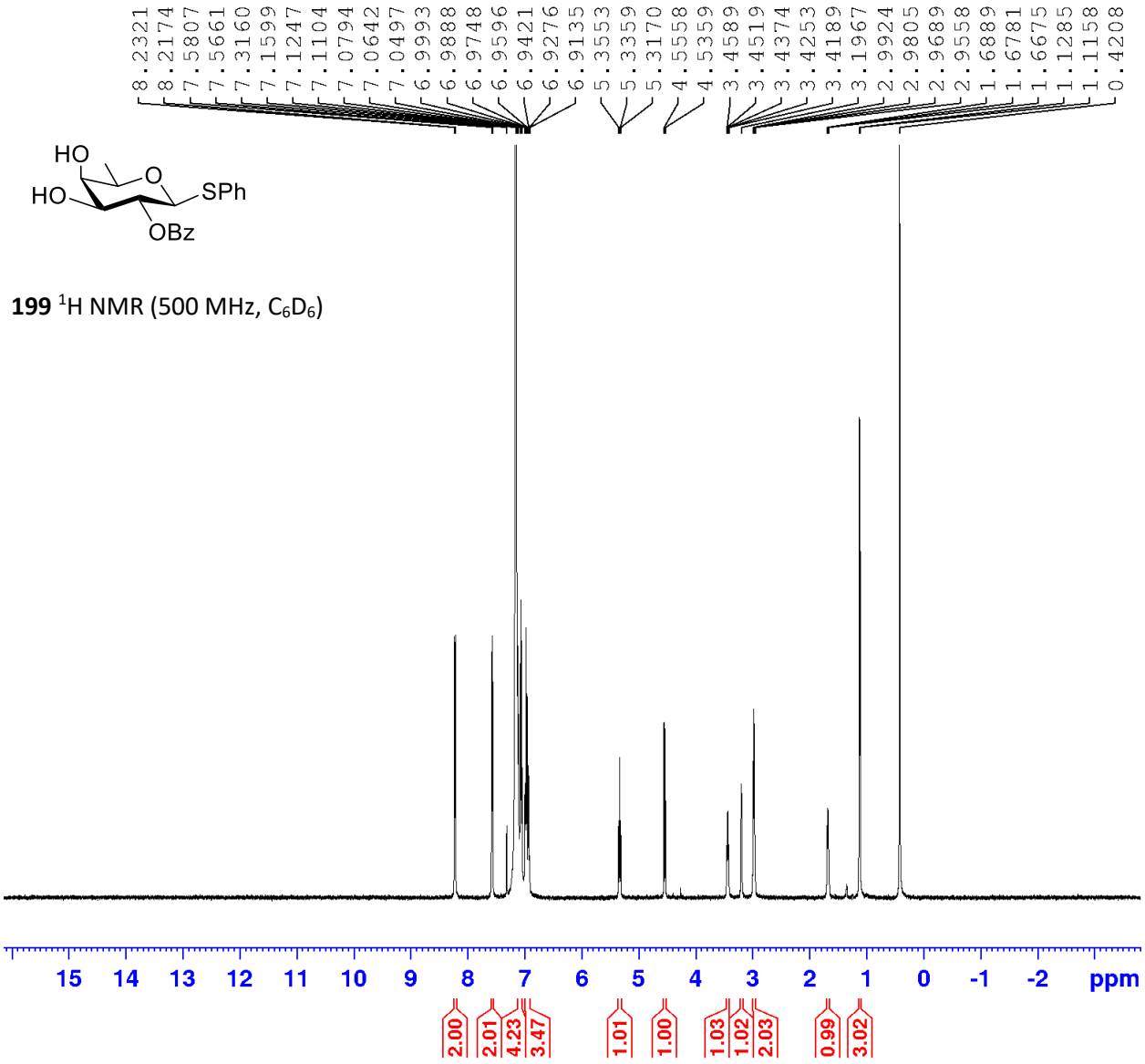
198 ^{13}C NMR (125 MHz, C_6D_6)



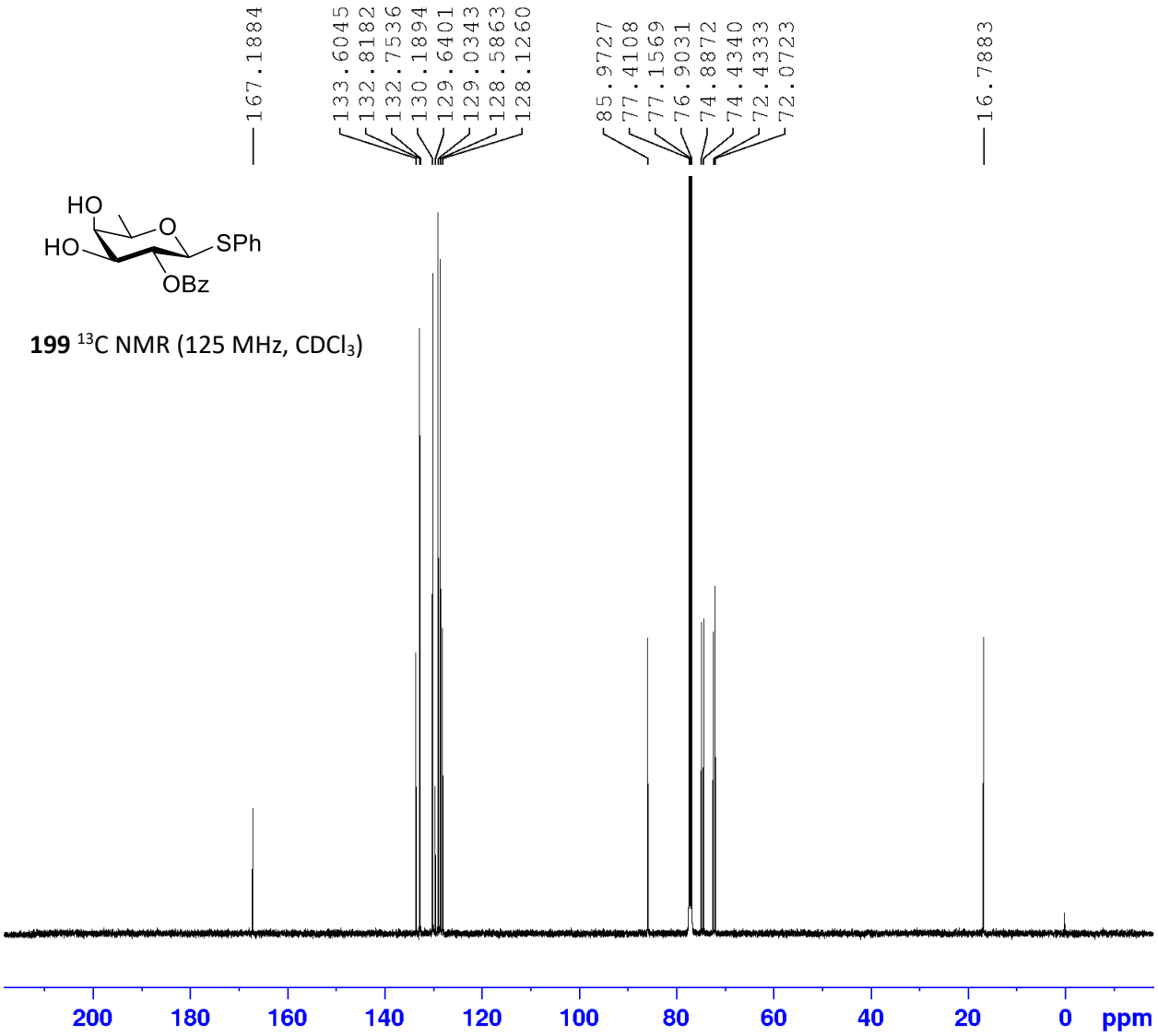
198 ^{1}H-^{1}H NMR COSY, C_6D_6



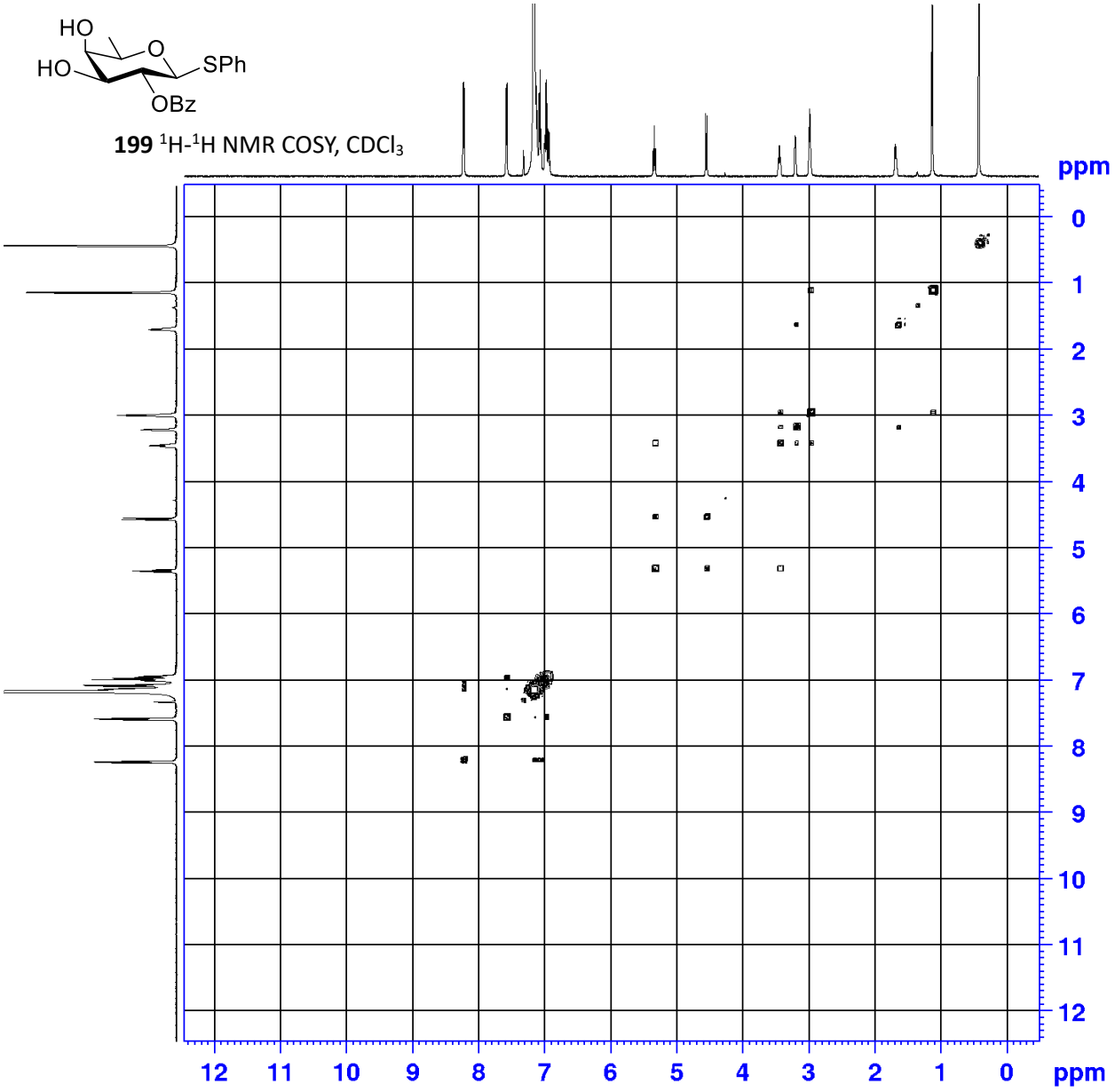
198 ^{1}H-^{13}C NMR HSQC, C_6D_6



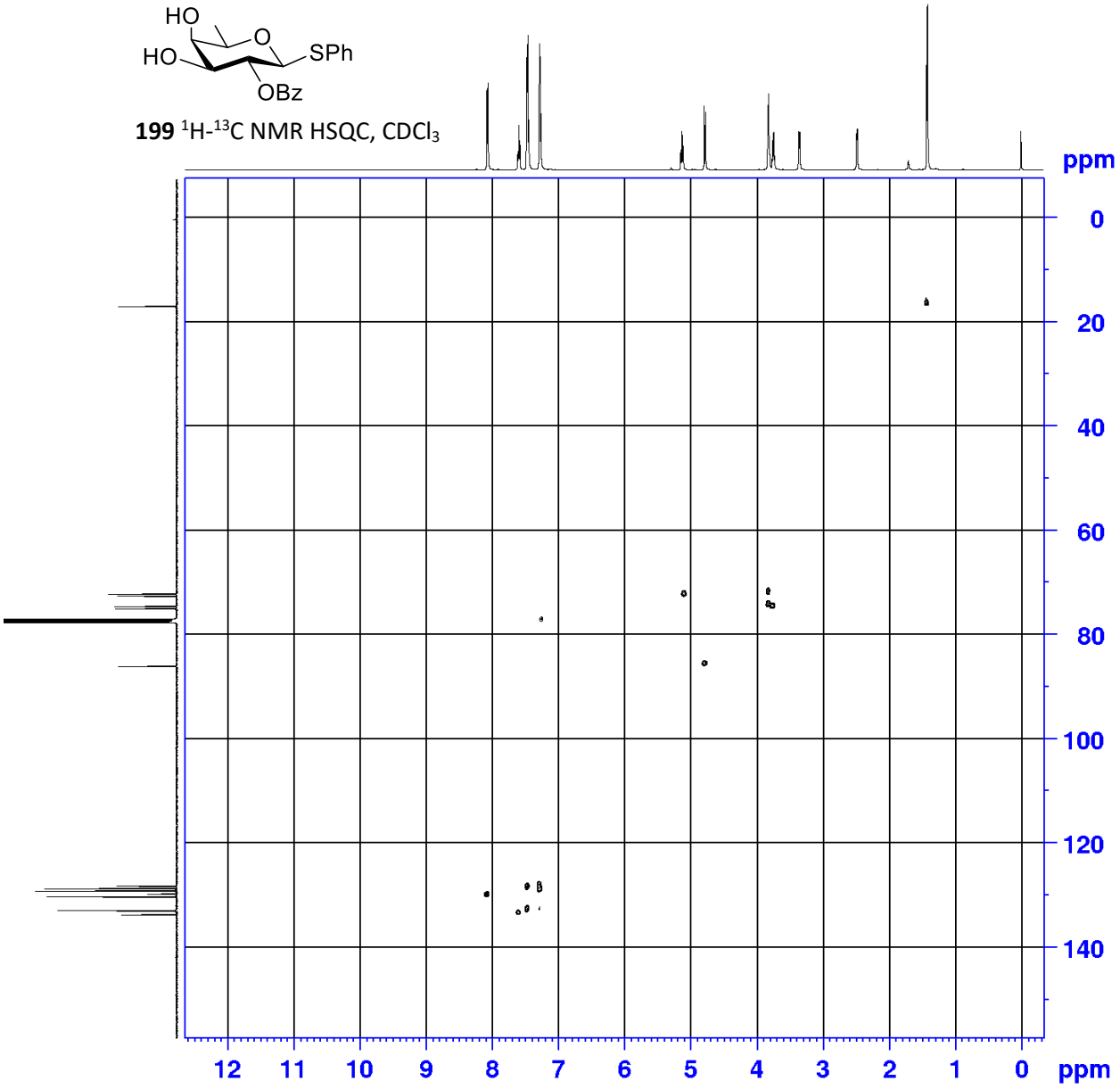
199 ^{1}H NMR (500 MHz, C_6D_6)



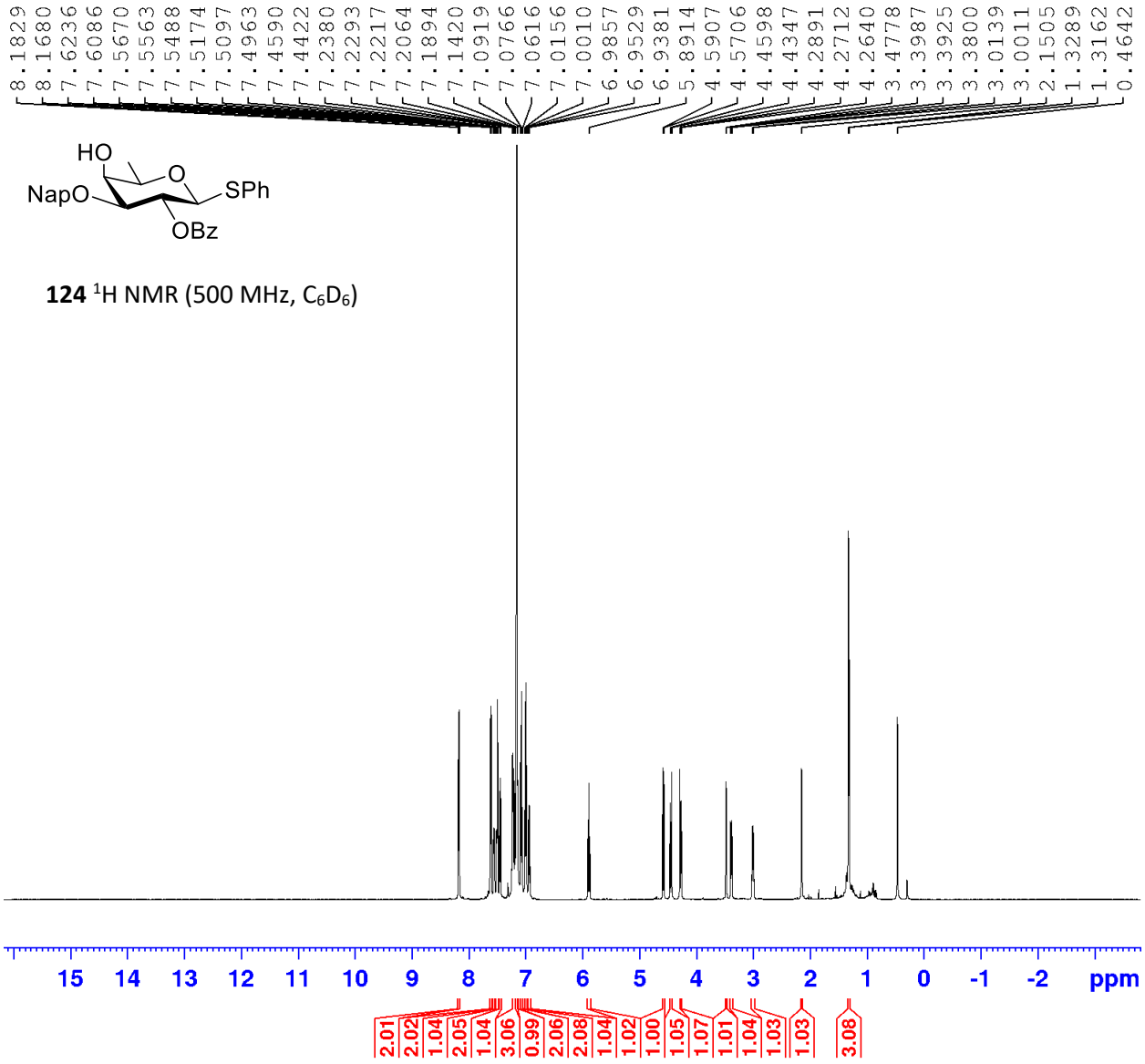
199 ^{13}C NMR (125 MHz, $CDCl_3$)



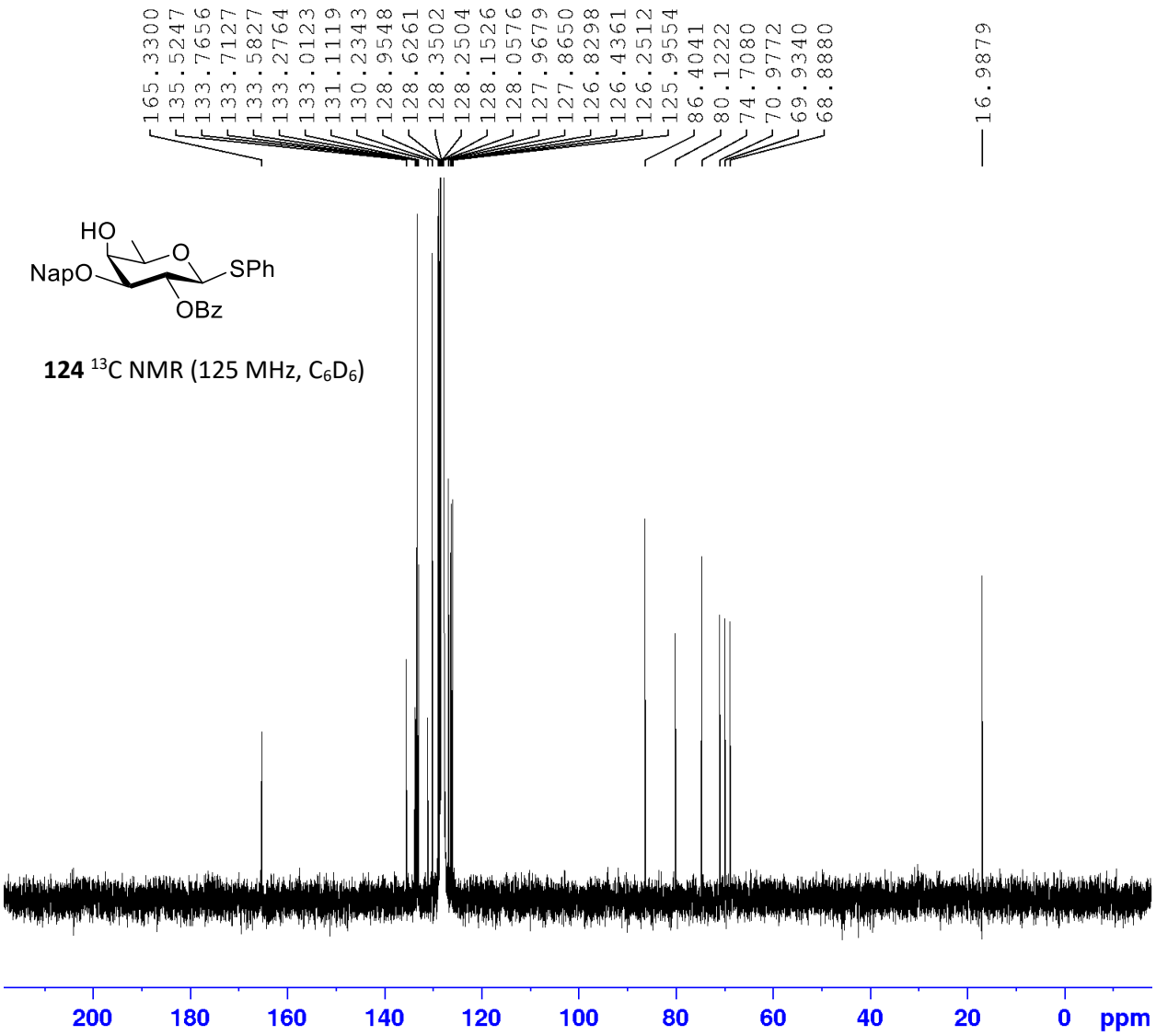
199 ^{1}H-^{1}H NMR COSY, $CDCl_3$



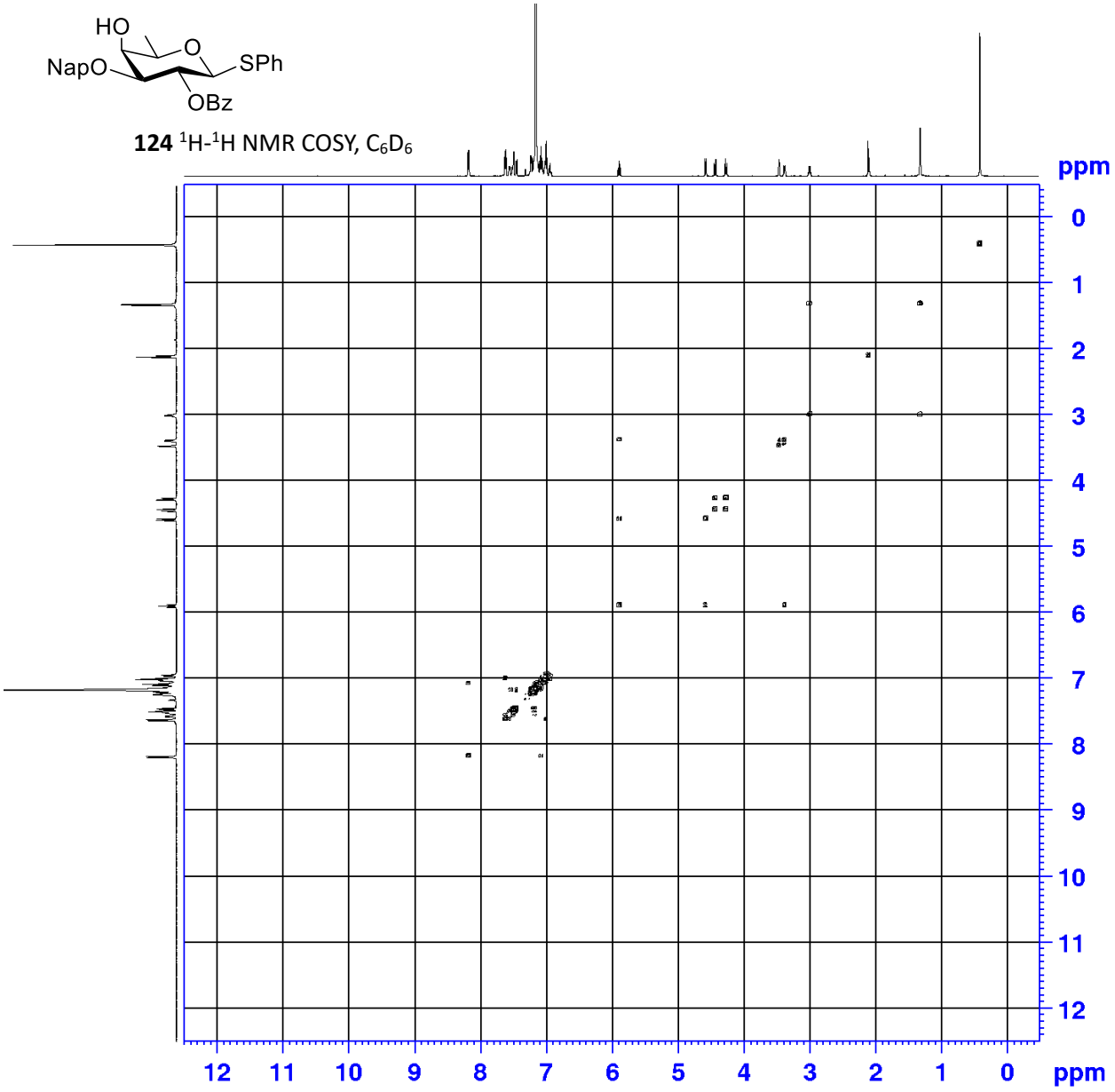
199 ^{1}H-^{13}C NMR HSQC, $CDCl_3$



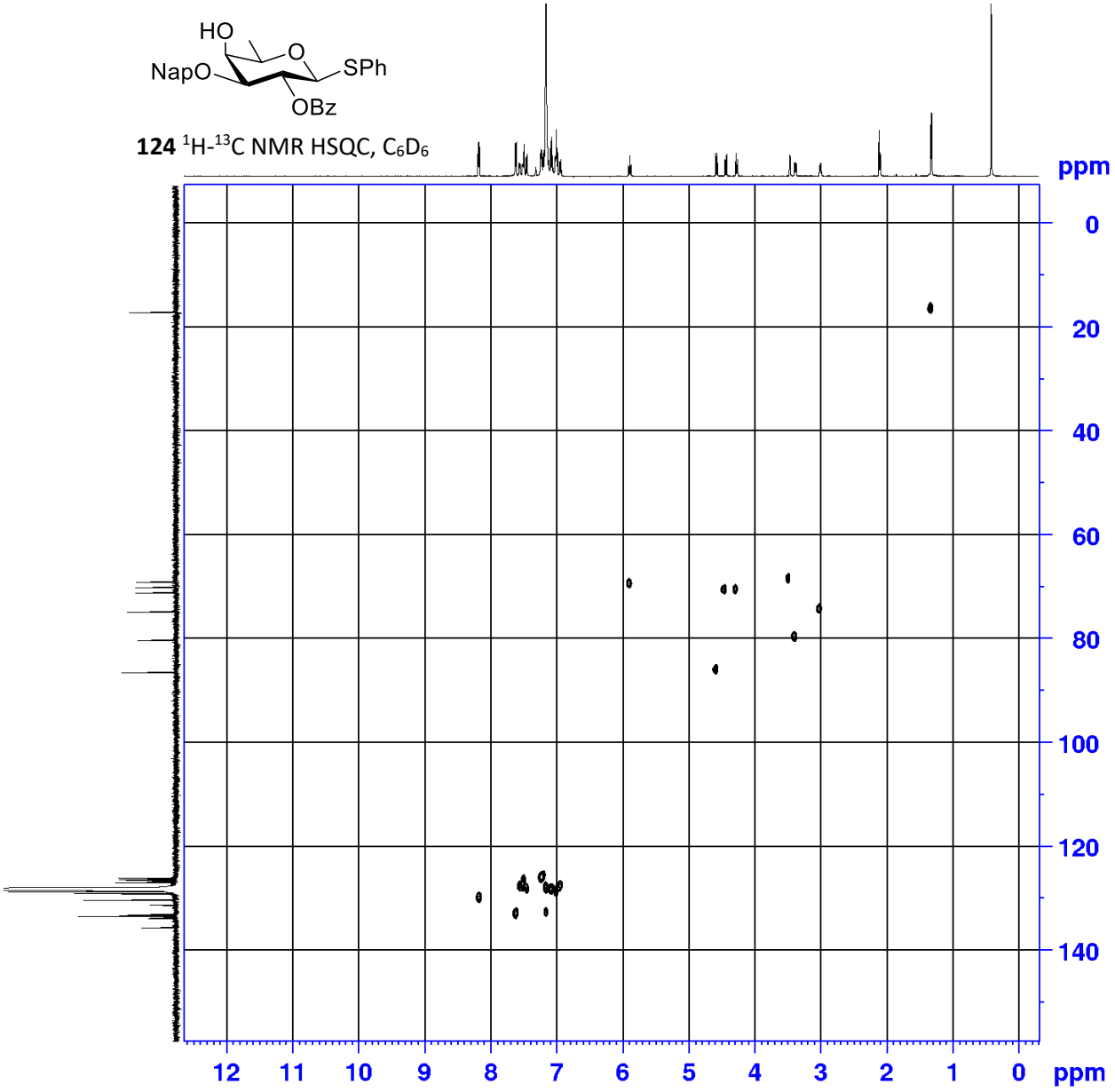
124 ^{1}H NMR (500 MHz, C_6D_6)



124 ^{13}C NMR (125 MHz, C_6D_6)



124 ^{1}H-^{1}H NMR COSY, C_6D_6


HO
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NapO
SPh
OBz
124 ^{1}H-^{13}C NMR HSQC, C_6D_6
ppm
0
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120
140
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11
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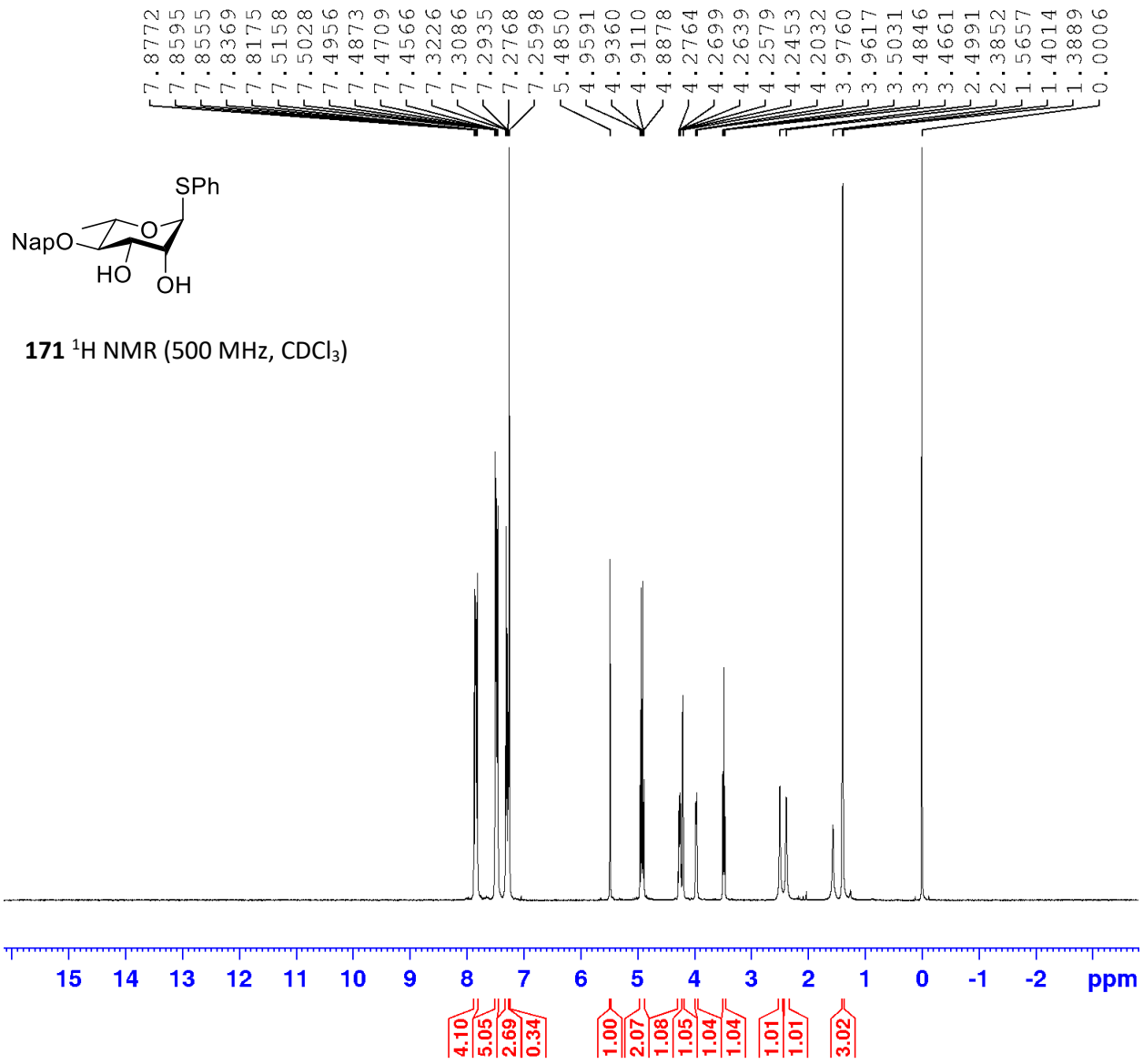
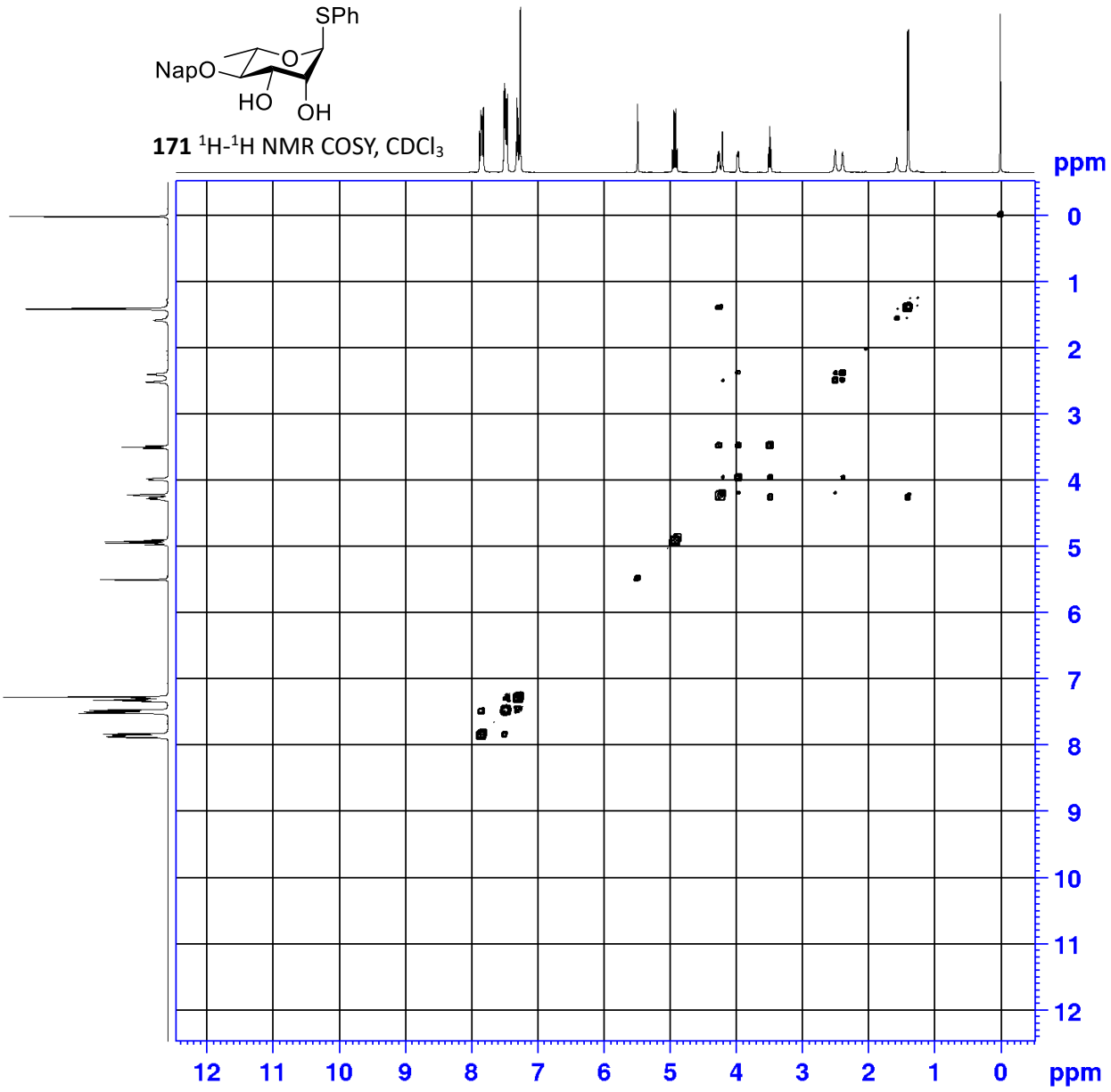
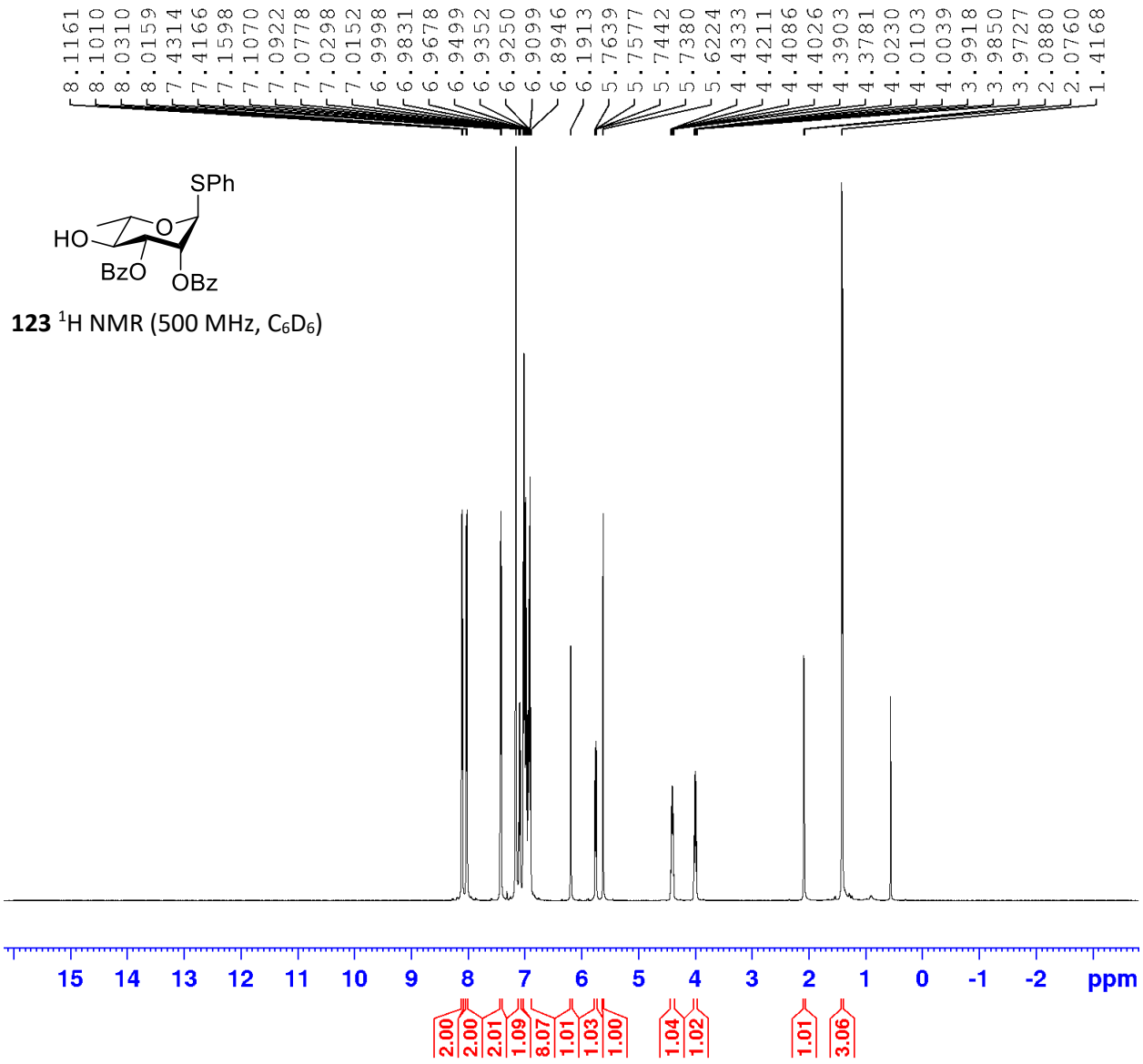
ppm



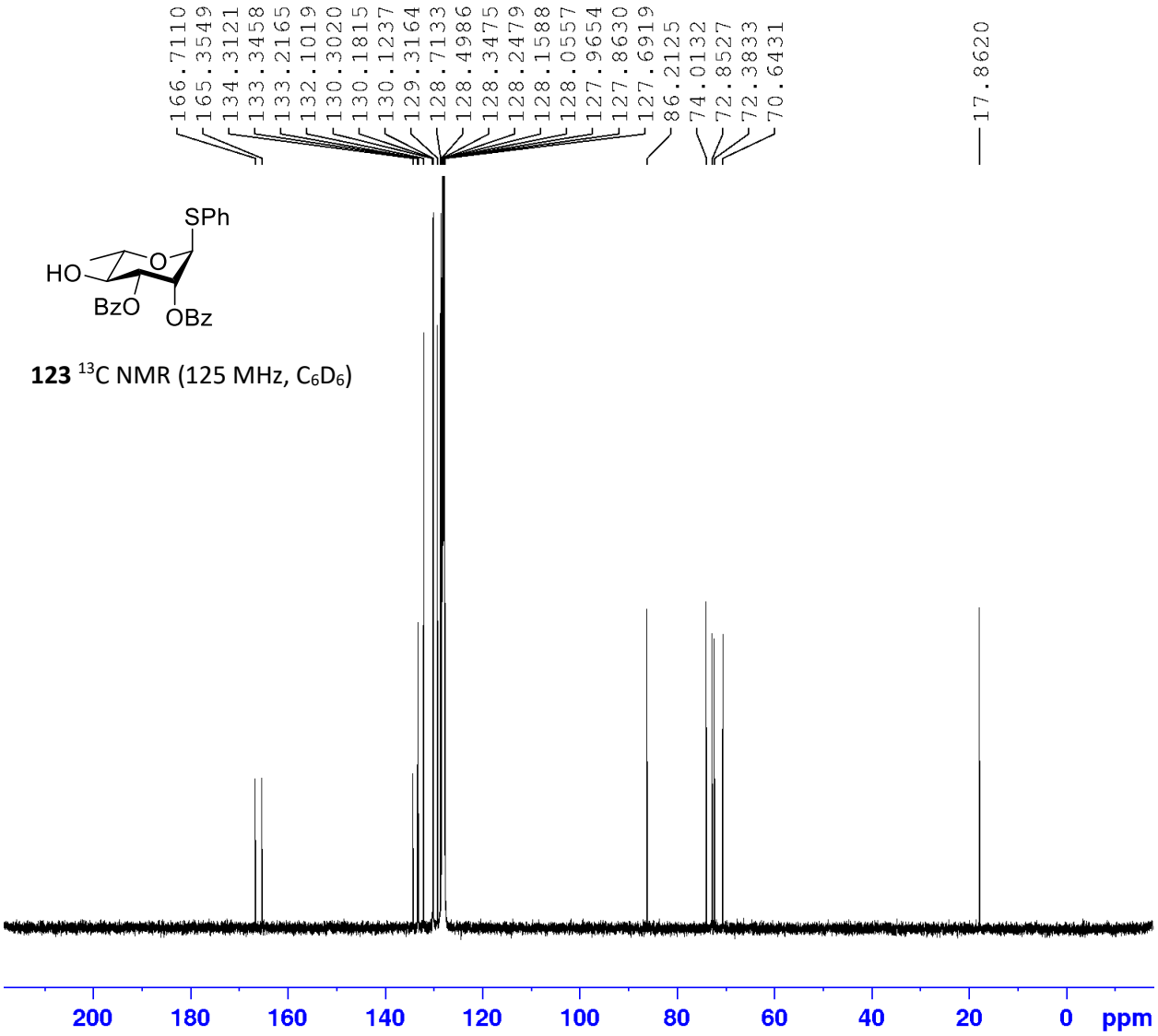
171 ^{1}H NMR (500 MHz, $CDCl_3$)



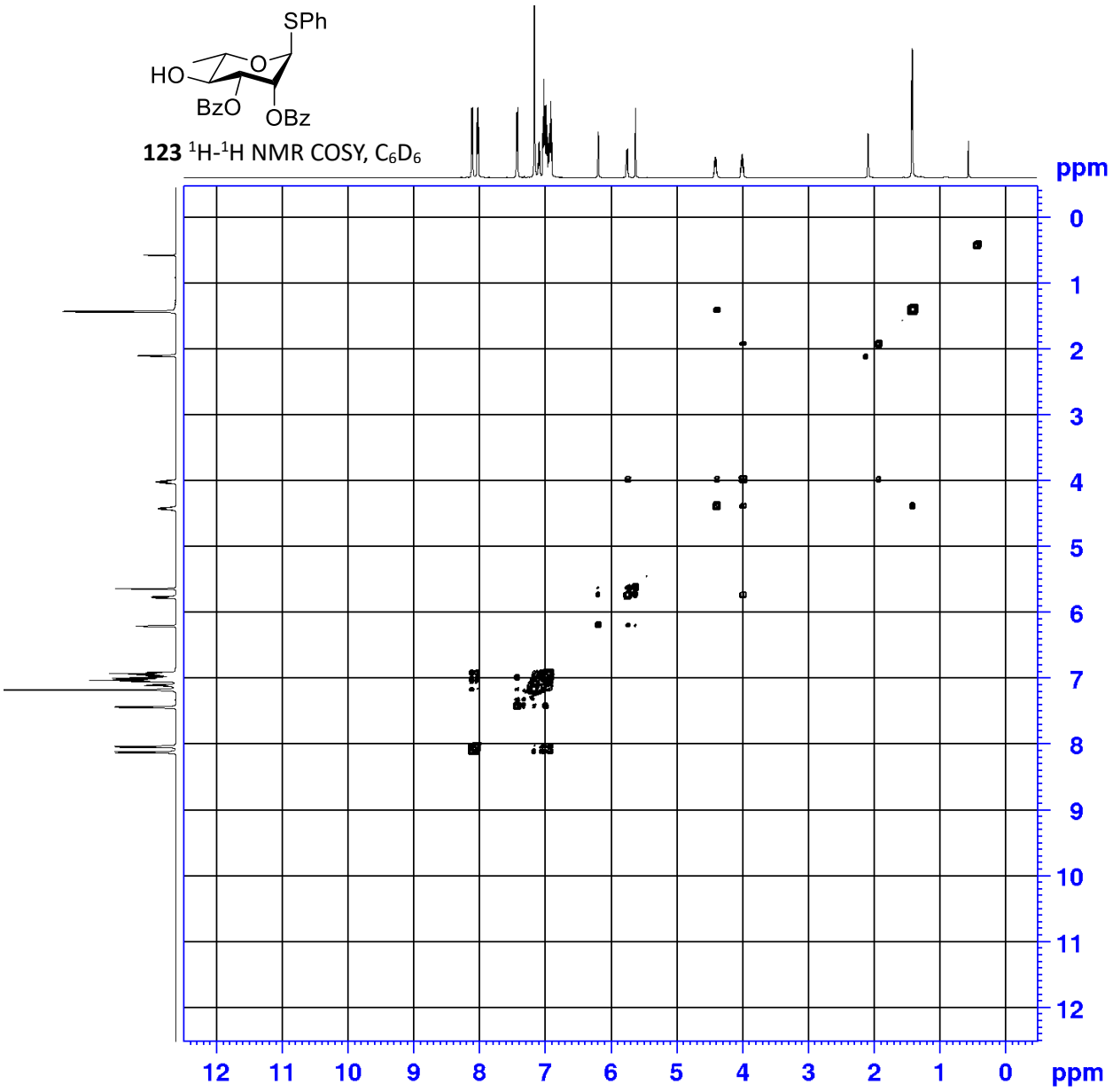
171 ^{1}H-^{1}H NMR COSY, $CDCl_3$



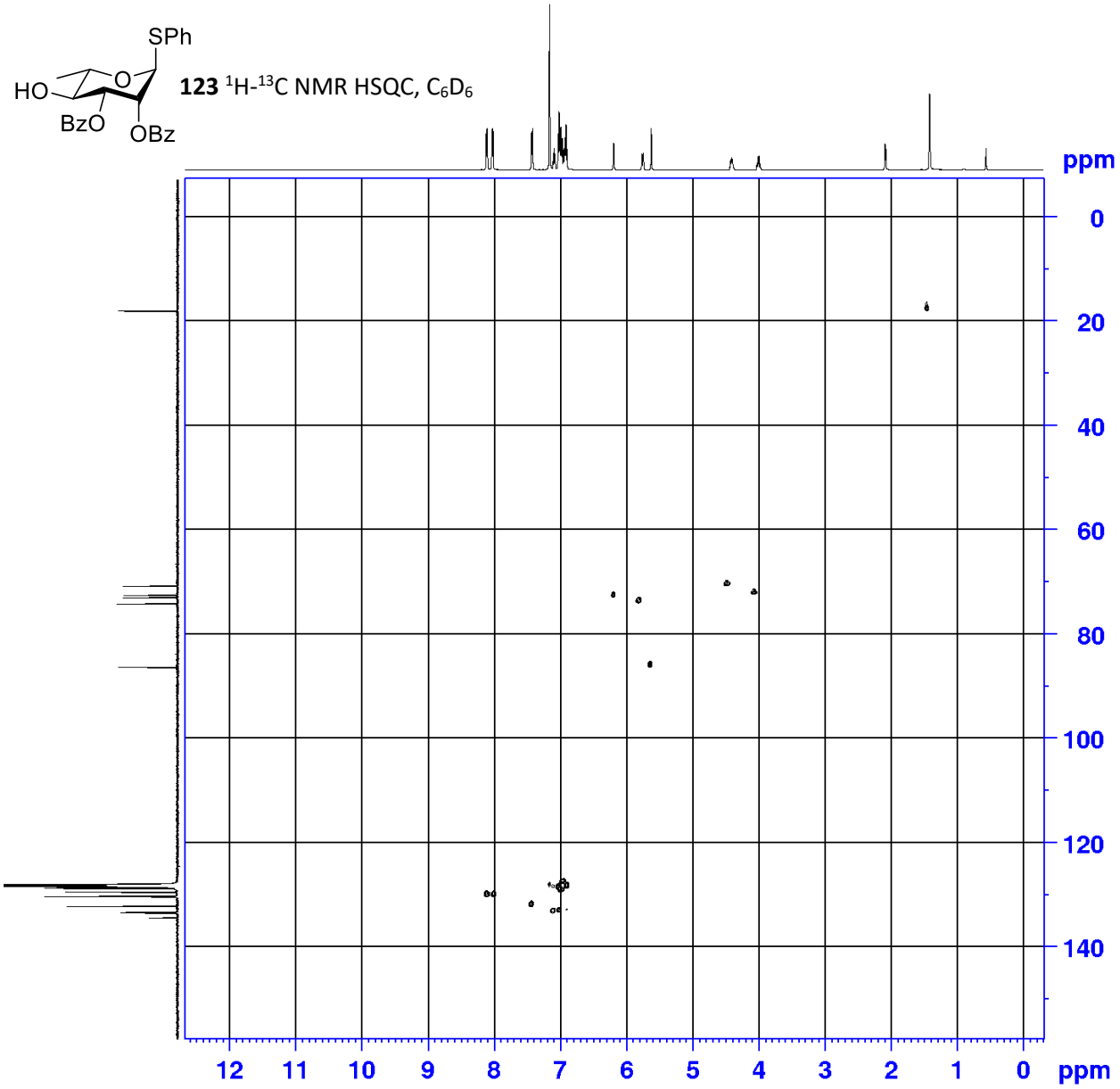
123 ^{1}H NMR (500 MHz, C_6D_6)

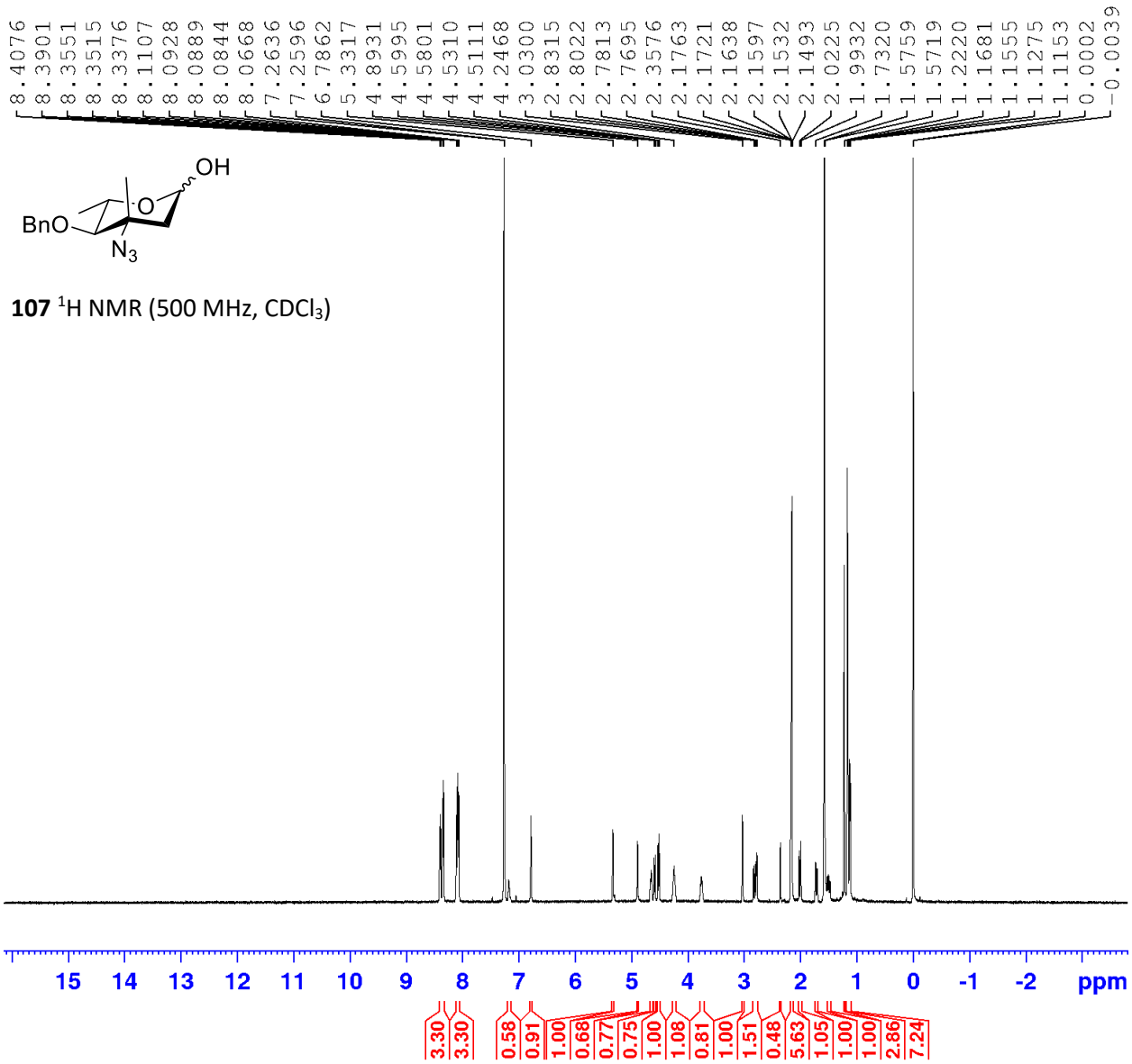


123 ^{13}C NMR (125 MHz, C_6D_6)

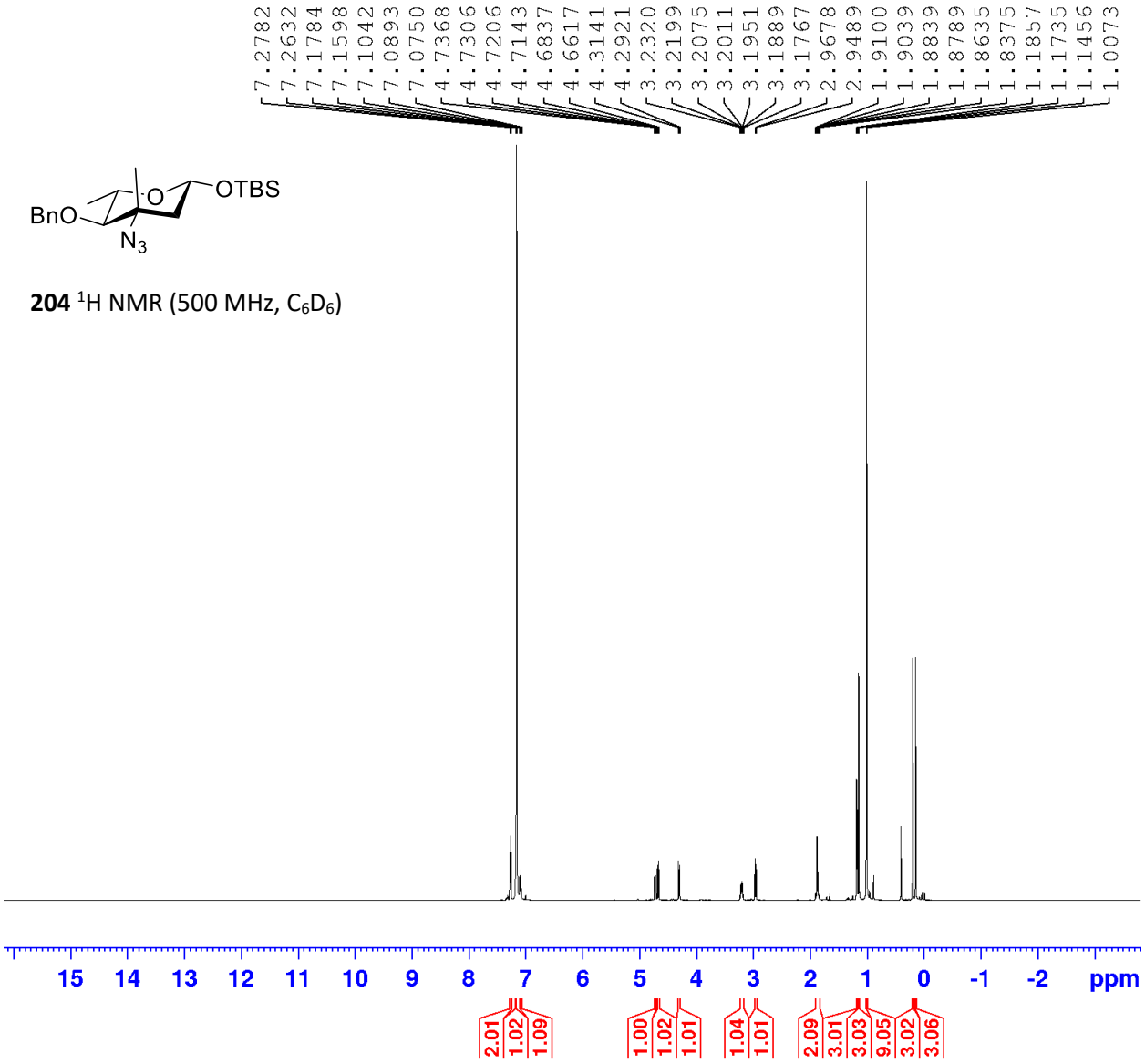


123 ^{1}H-^{1}H NMR COSY, C_6D_6

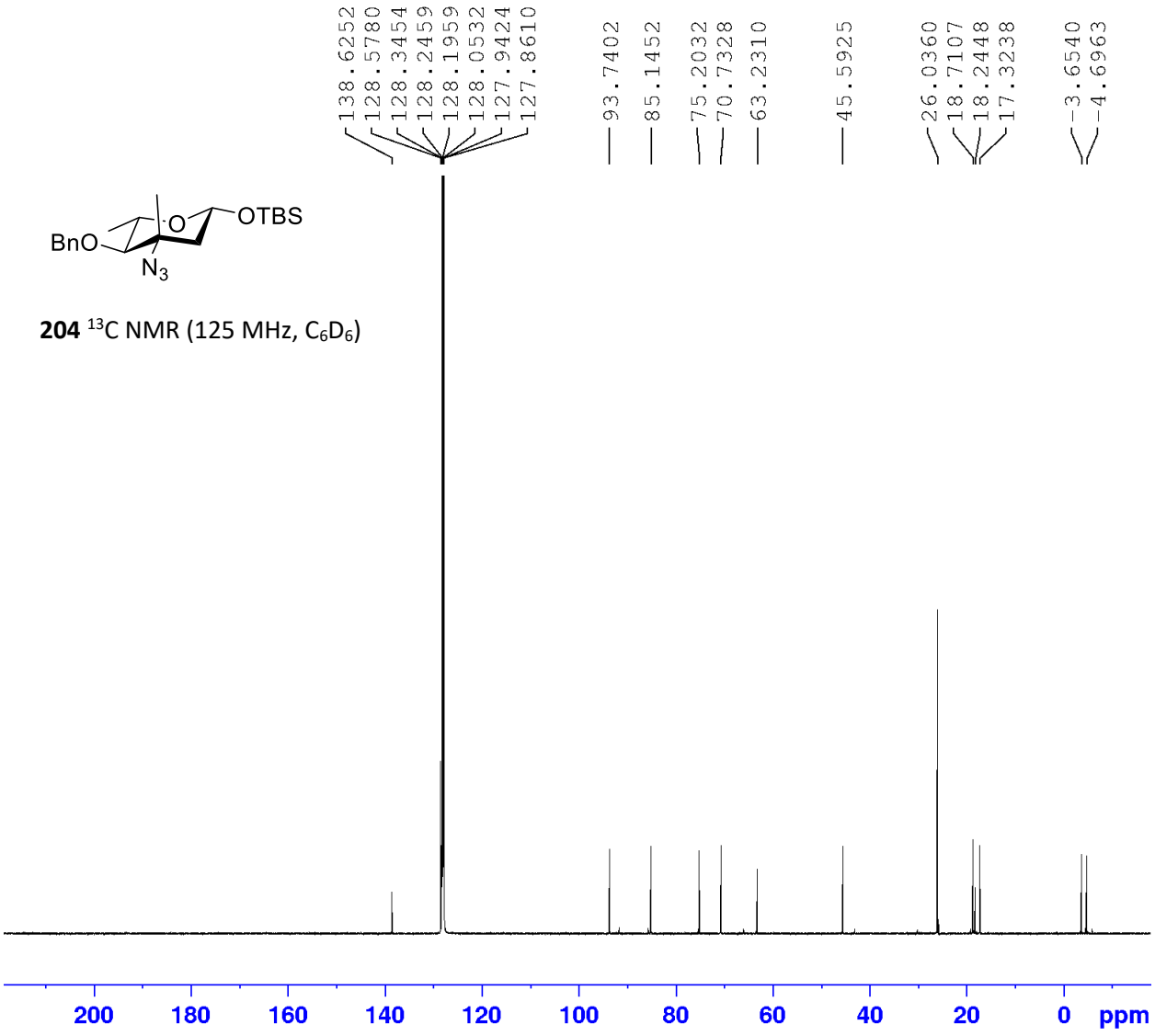

SPh
HO
O
BzO
OBz
123 ^{1}H-^{13}C NMR HSQC, C_6D_6
ppm
0
20
40
60
80
100
120
140
12
11
10
9
8
7
6
5
4
3
2
1
0
ppm



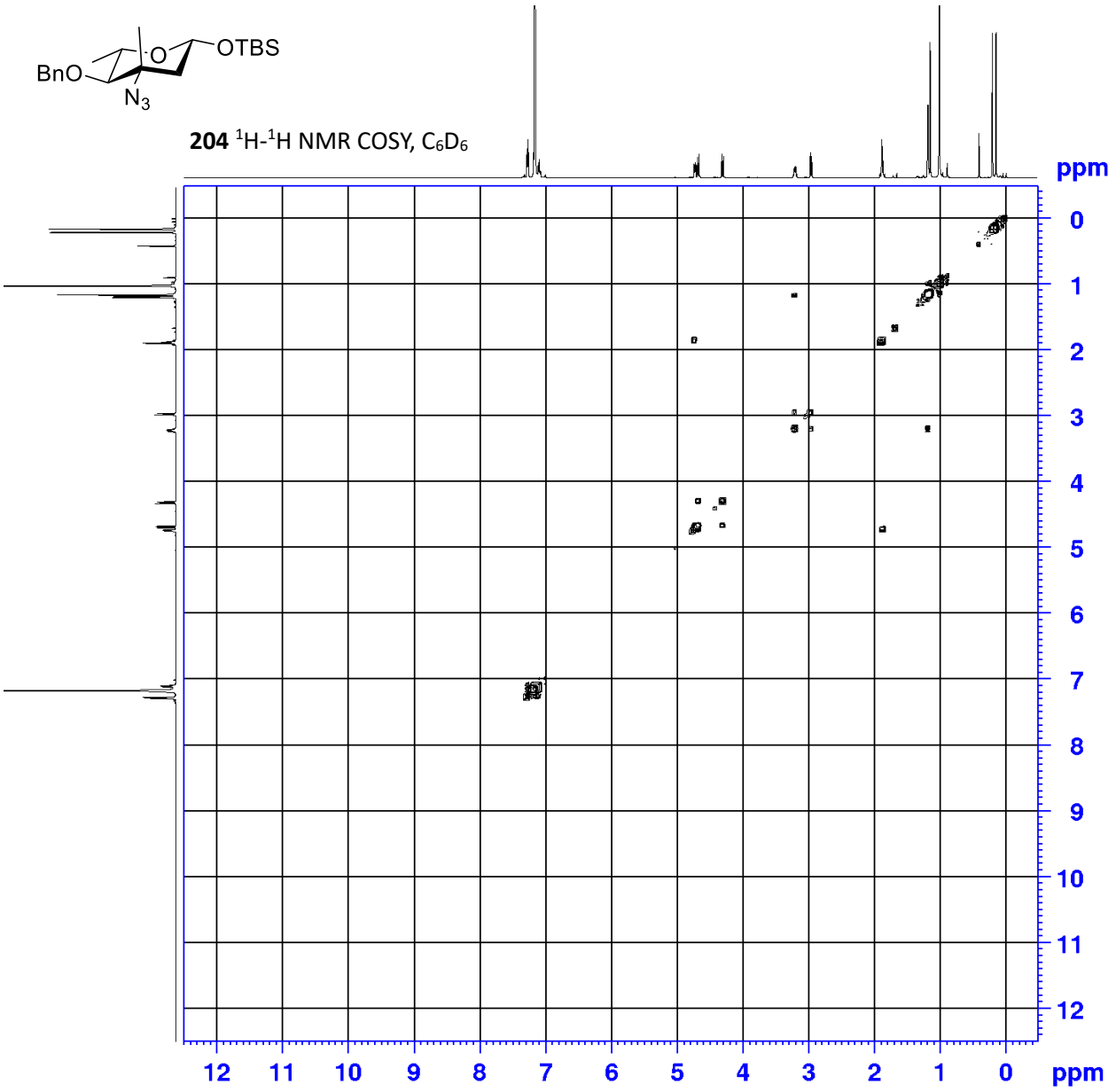
107 ^{1}H NMR (500 MHz, $CDCl_3$)



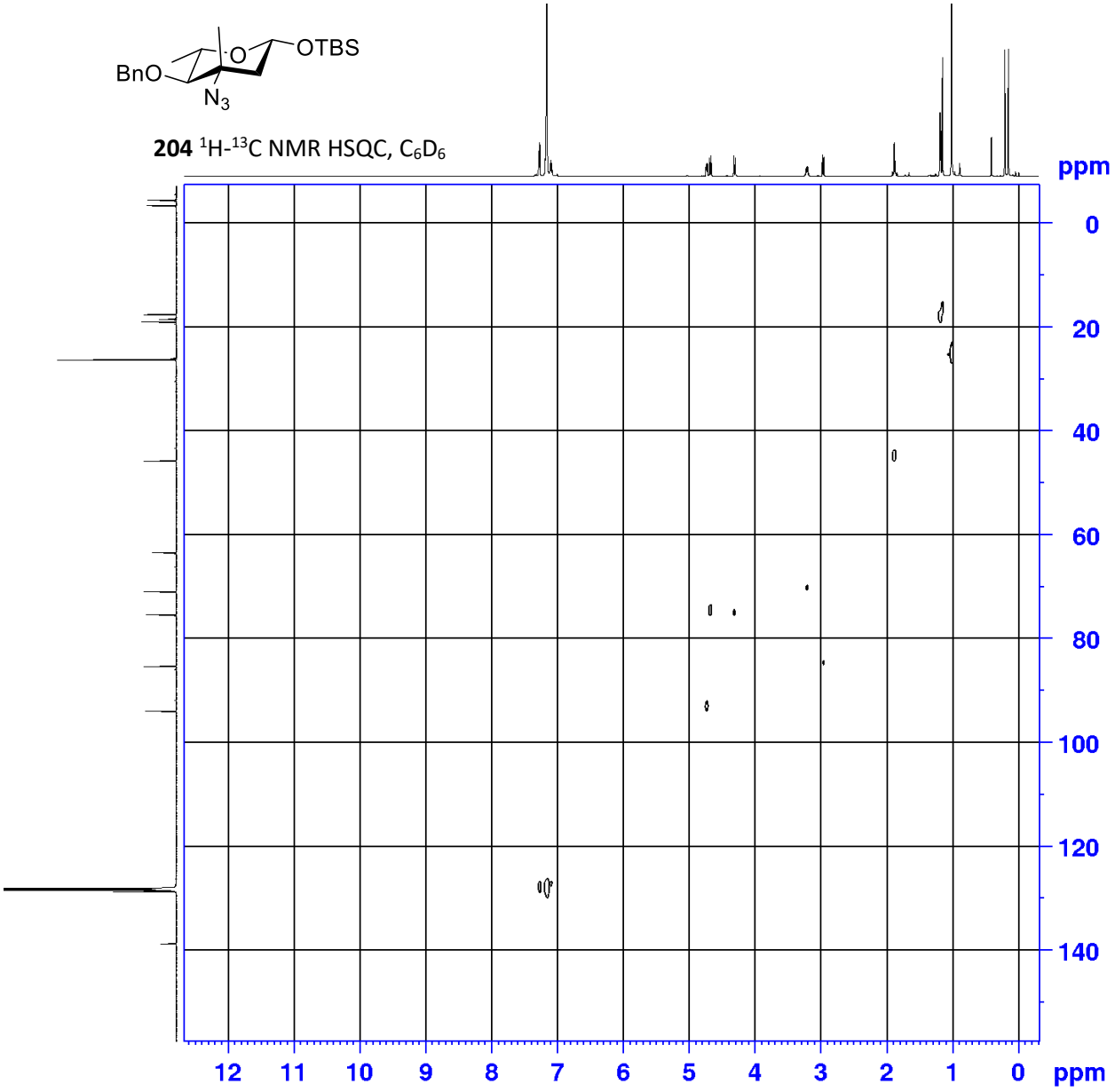
204 ^{1}H NMR (500 MHz, C_6D_6)

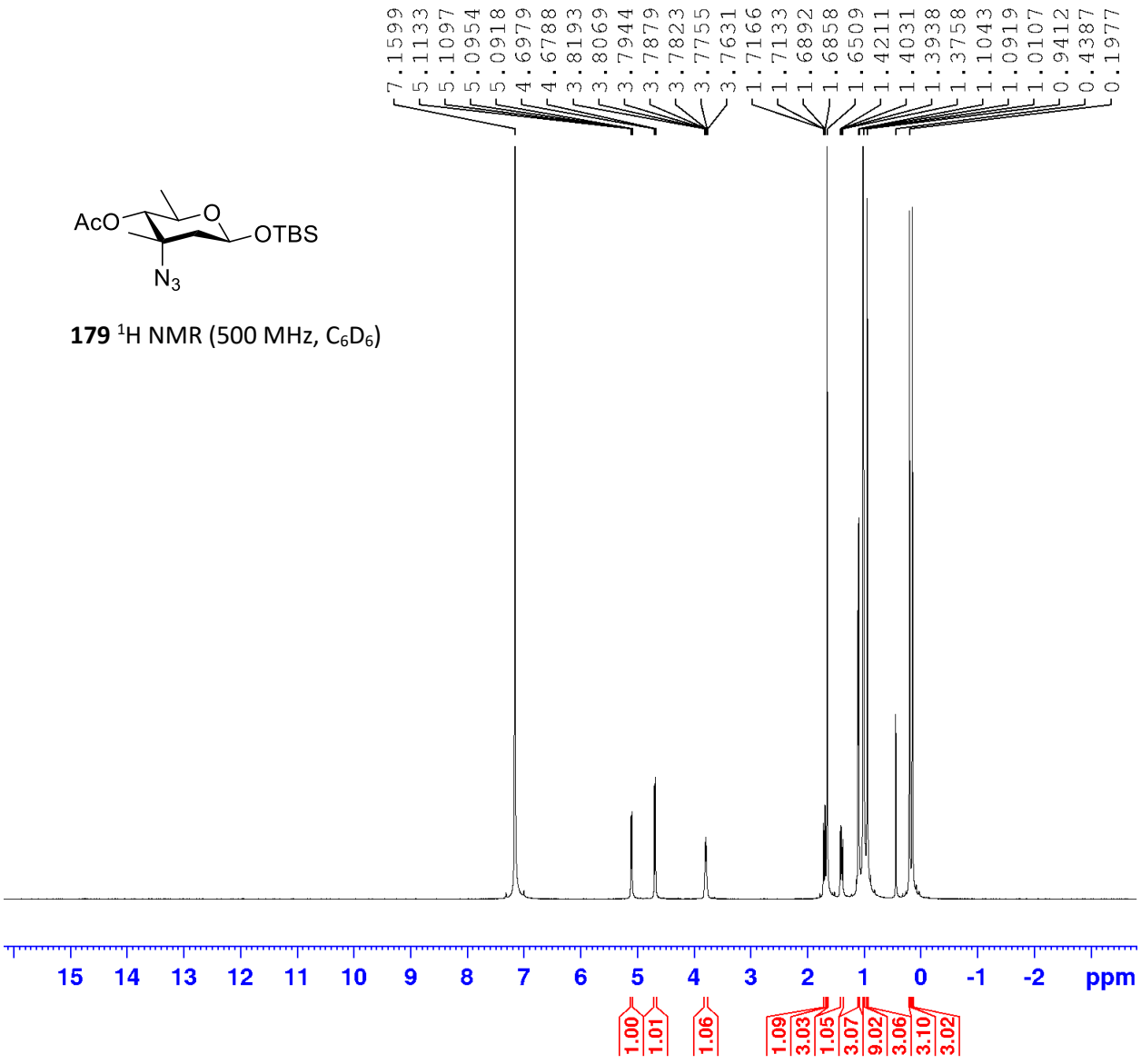


204 ^{13}C NMR (125 MHz, C_6D_6)

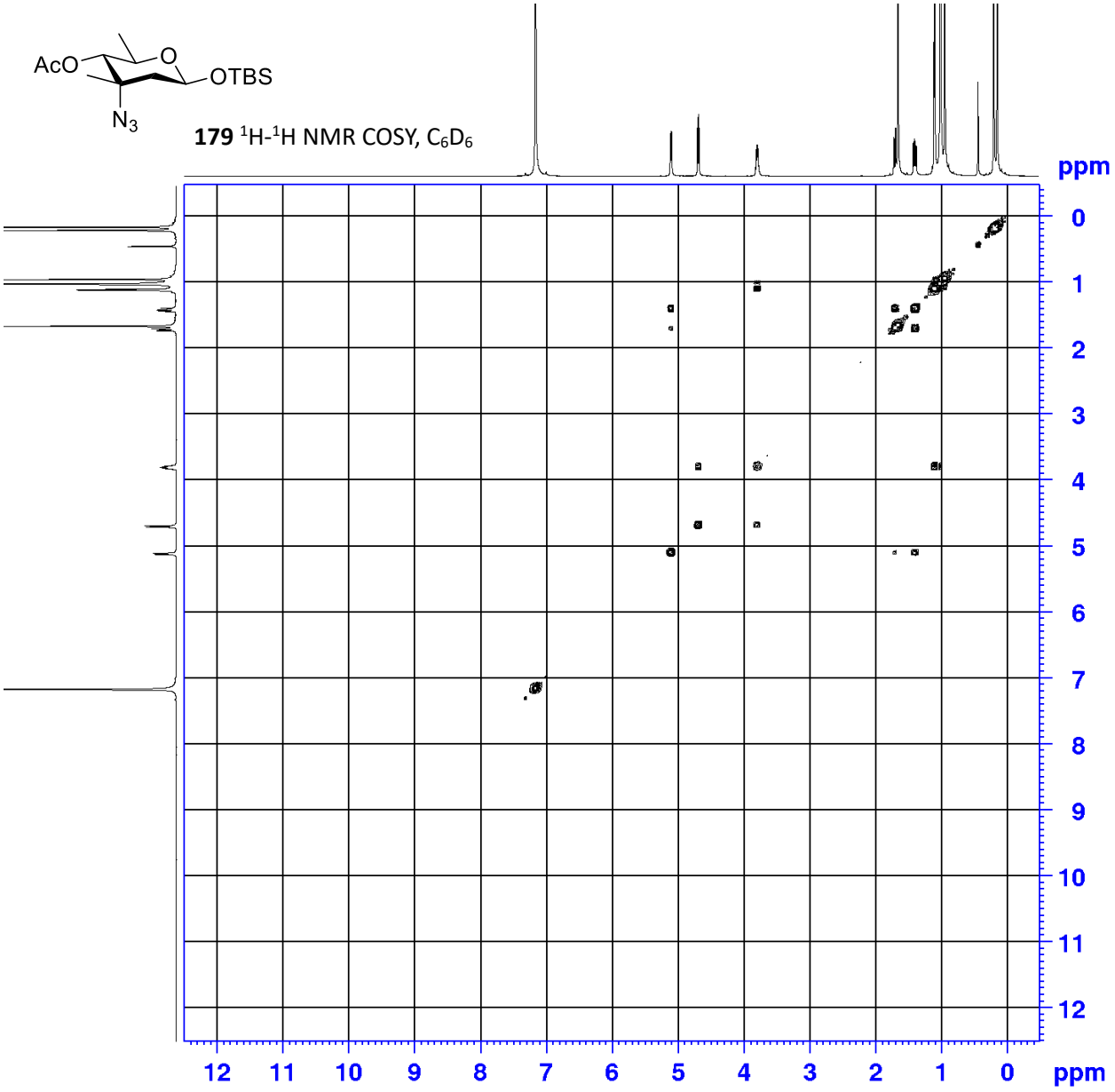


204 ^{1}H-^{1}H NMR COSY, C_6D_6

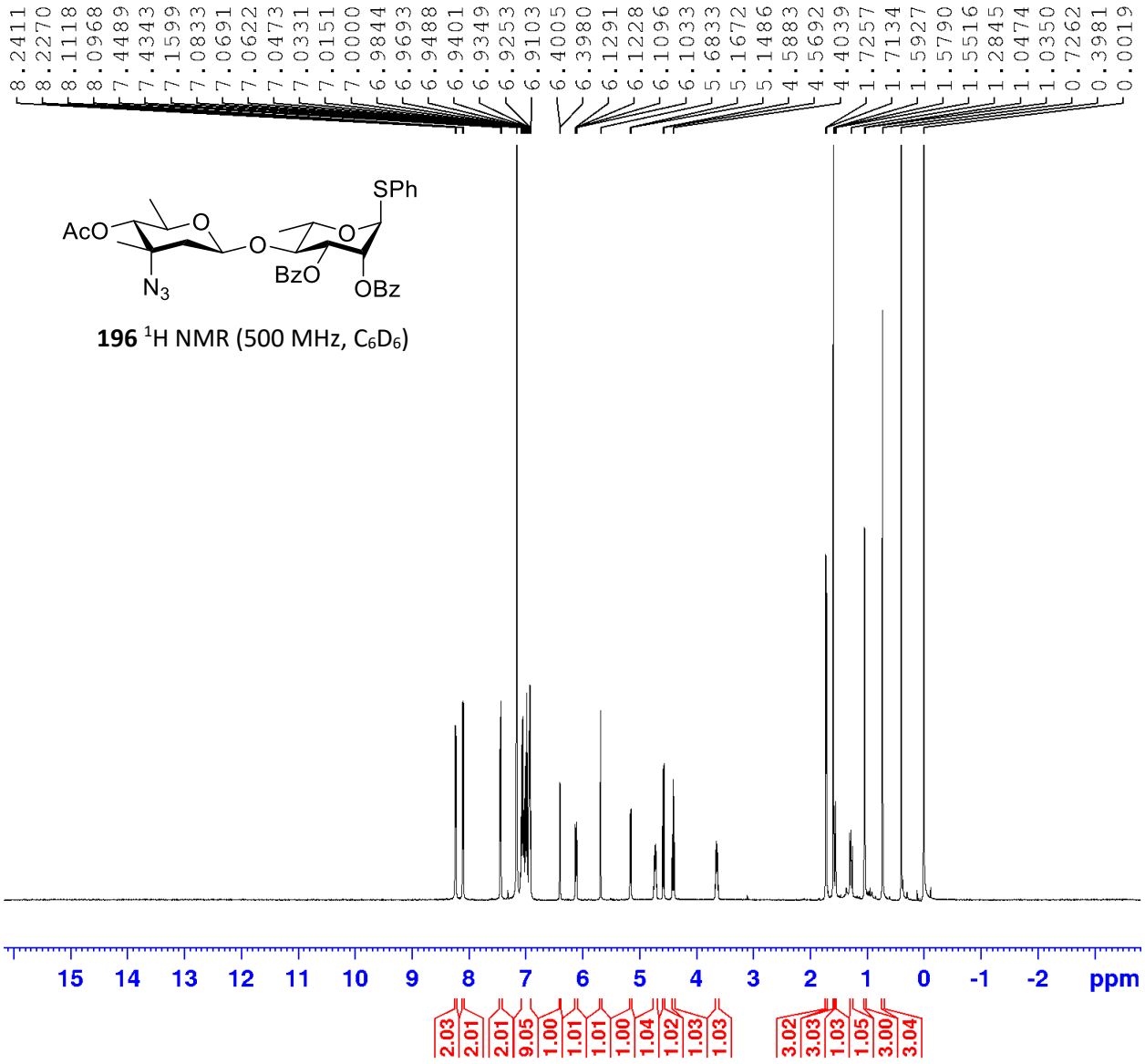

BnO
O
OTBS
N3
204 1H-13C NMR HSQC, C6D6
ppm
0
20
40
60
80
100
120
140
12
11
10
9
8
7
6
5
4
3
2
1
0
ppm



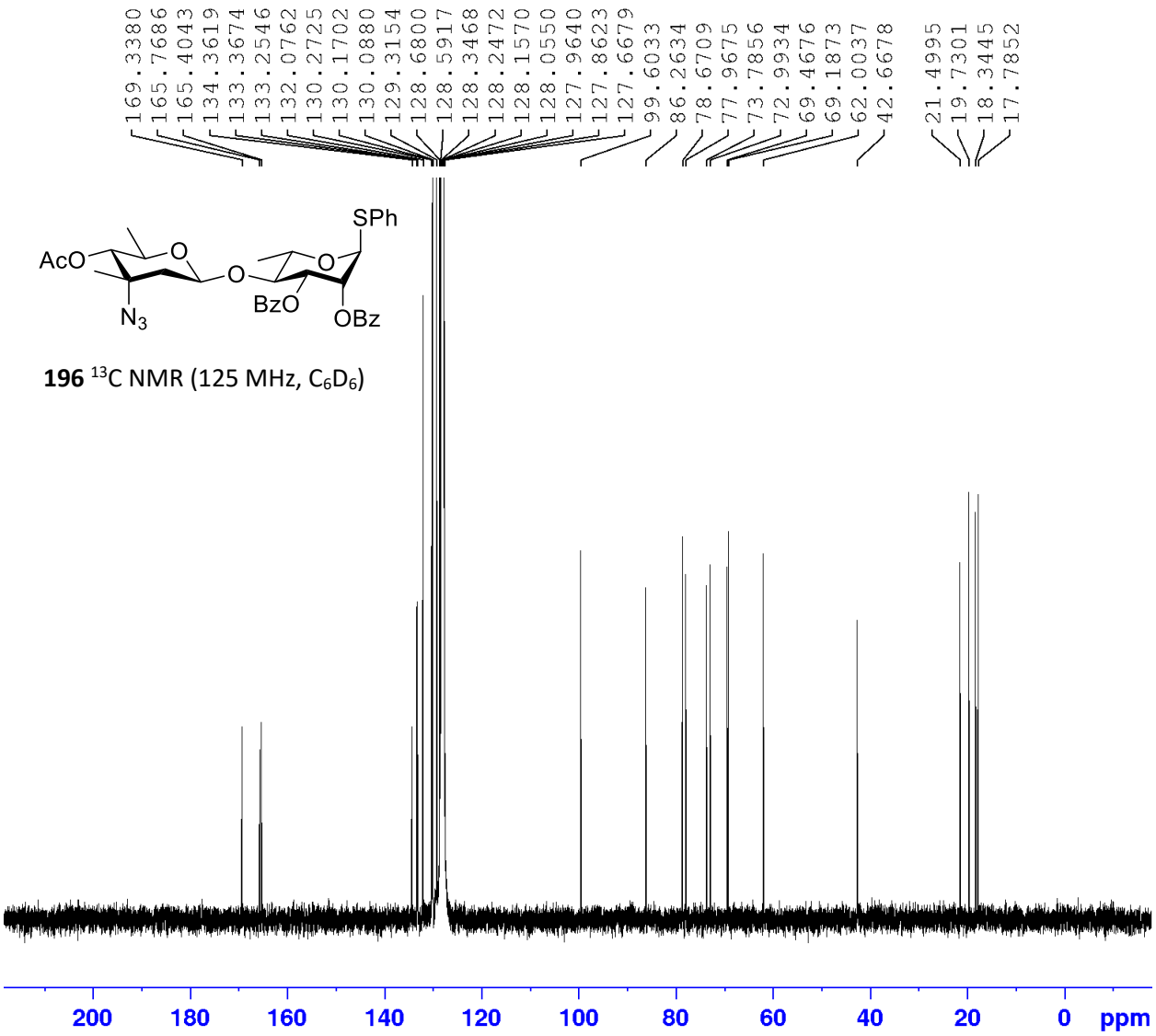
179 ^{1}H NMR (500 MHz, C_6D_6)



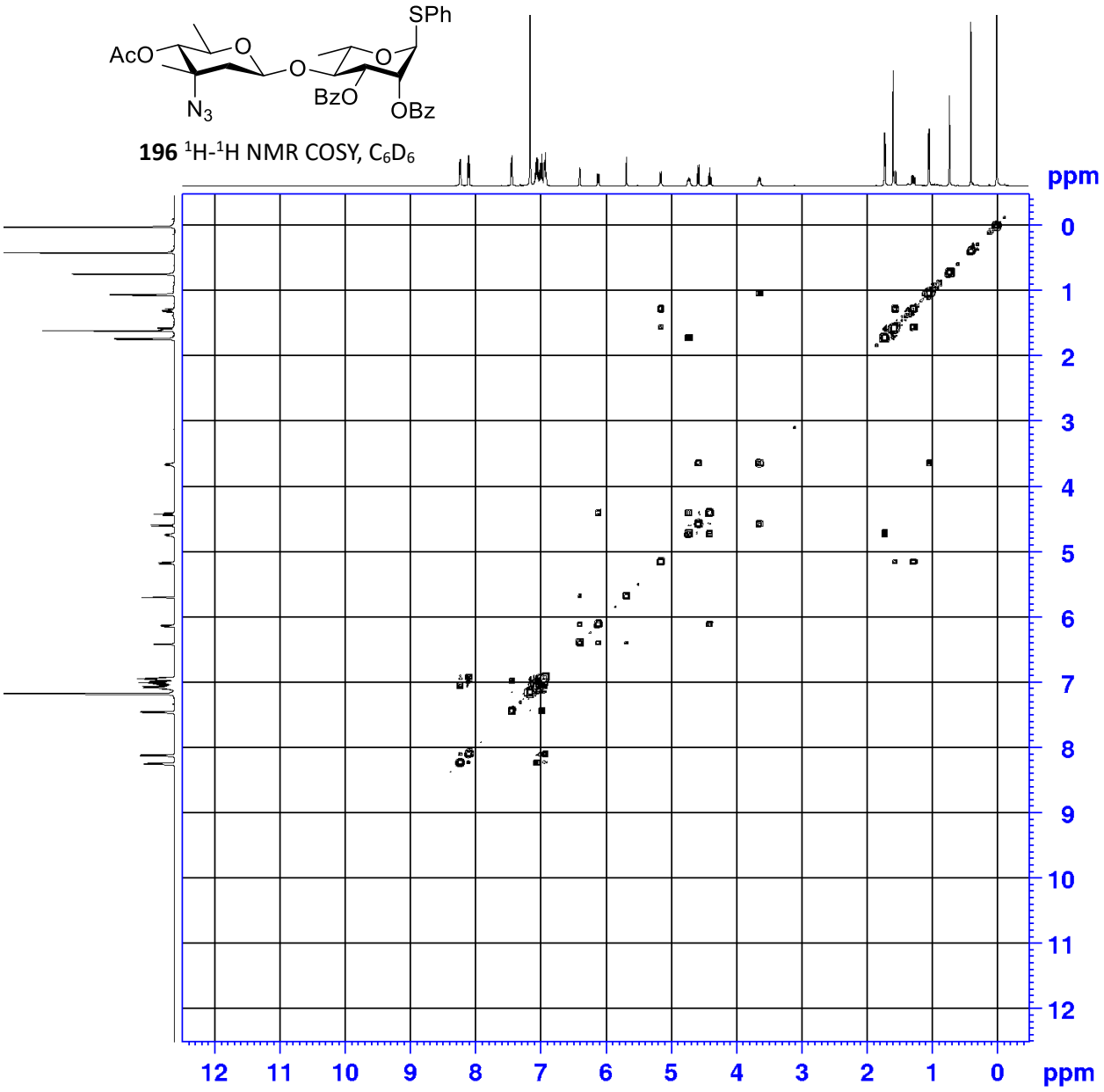
179 ^{1}H-^{1}H NMR COSY, C_6D_6



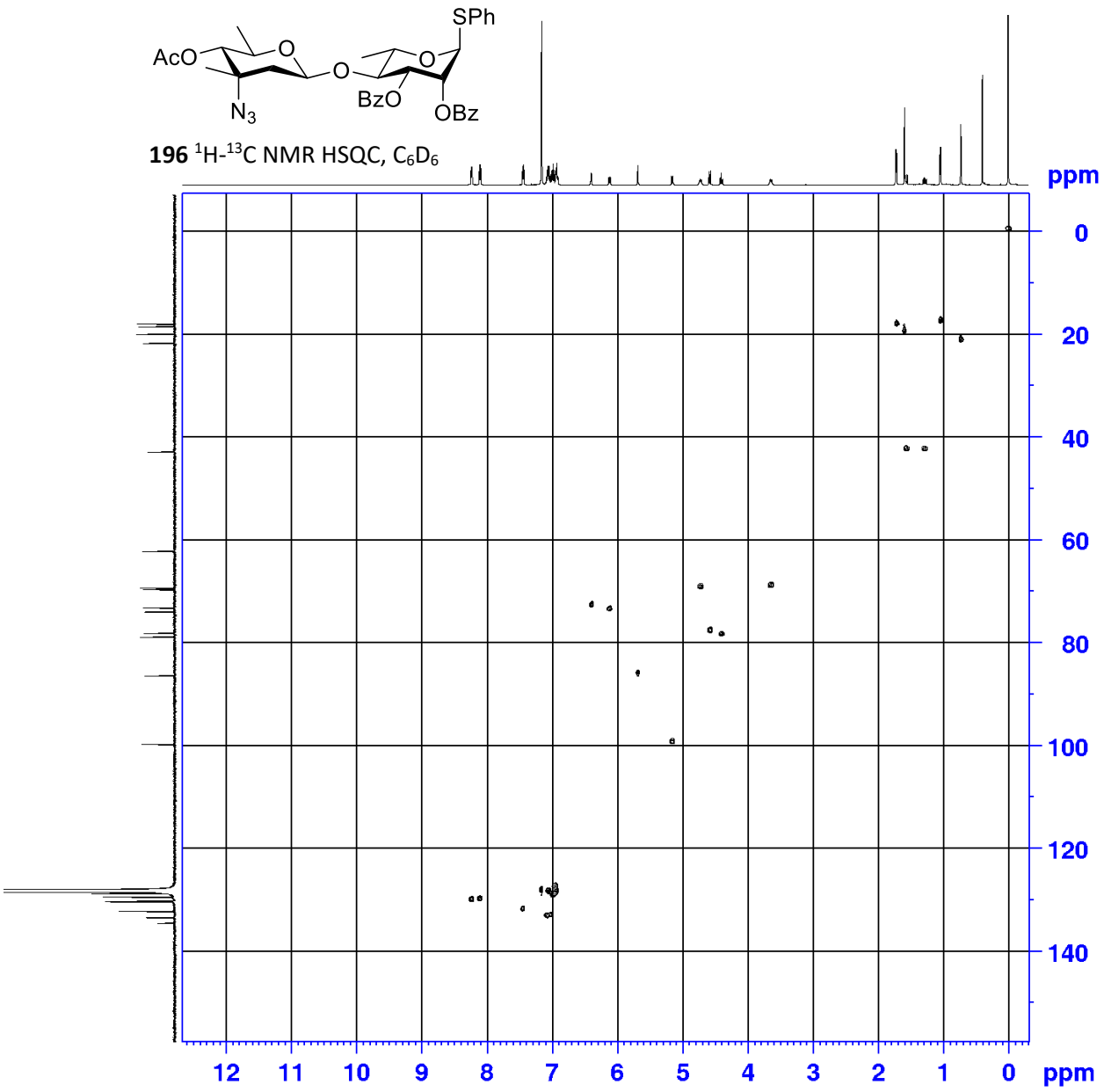
196 ^{1}H NMR (500 MHz, C_6D_6)



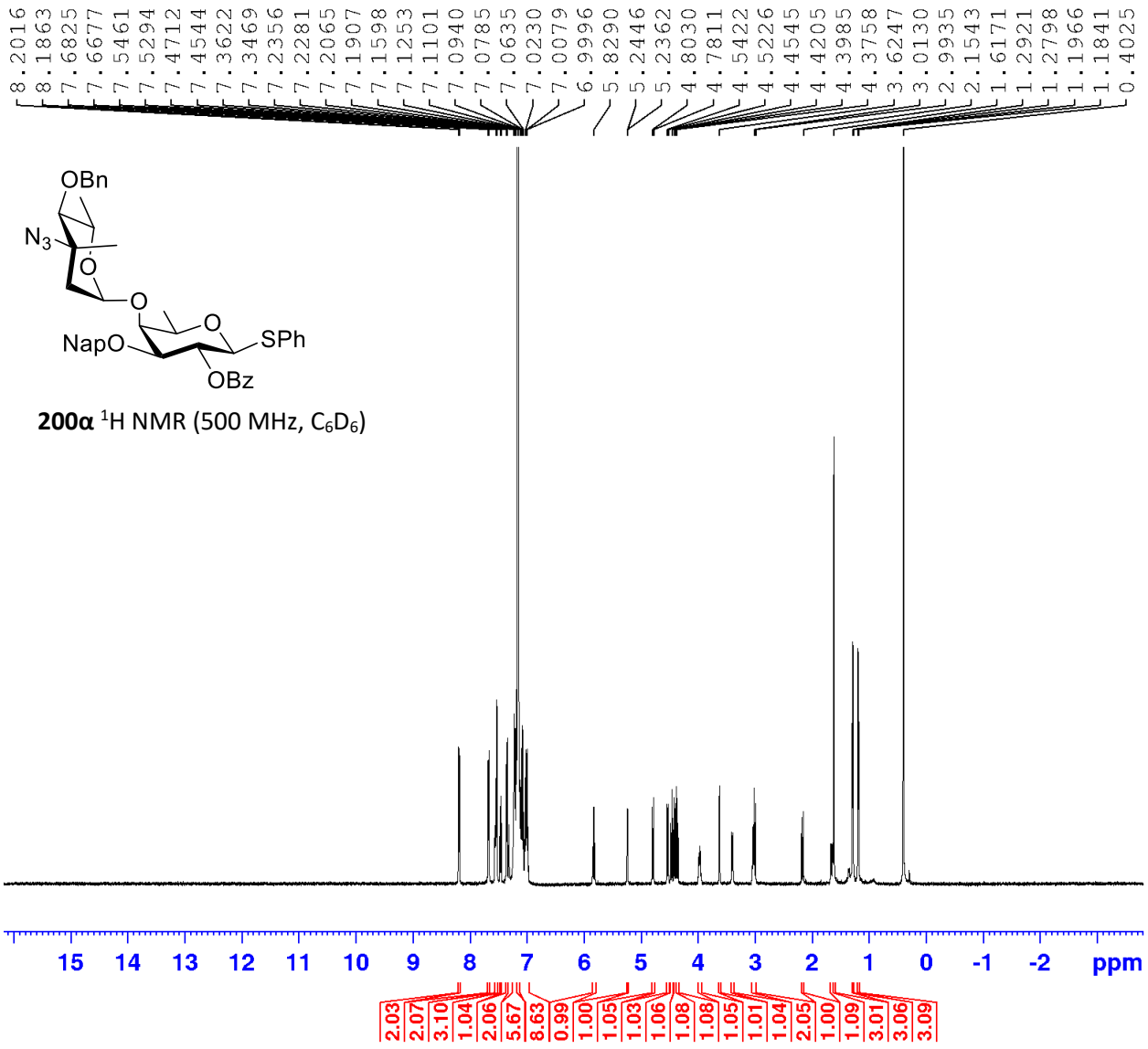
196 ^{13}C NMR (125 MHz, C_6D_6)



196 ^{1}H-^{1}H NMR COSY, C_6D_6

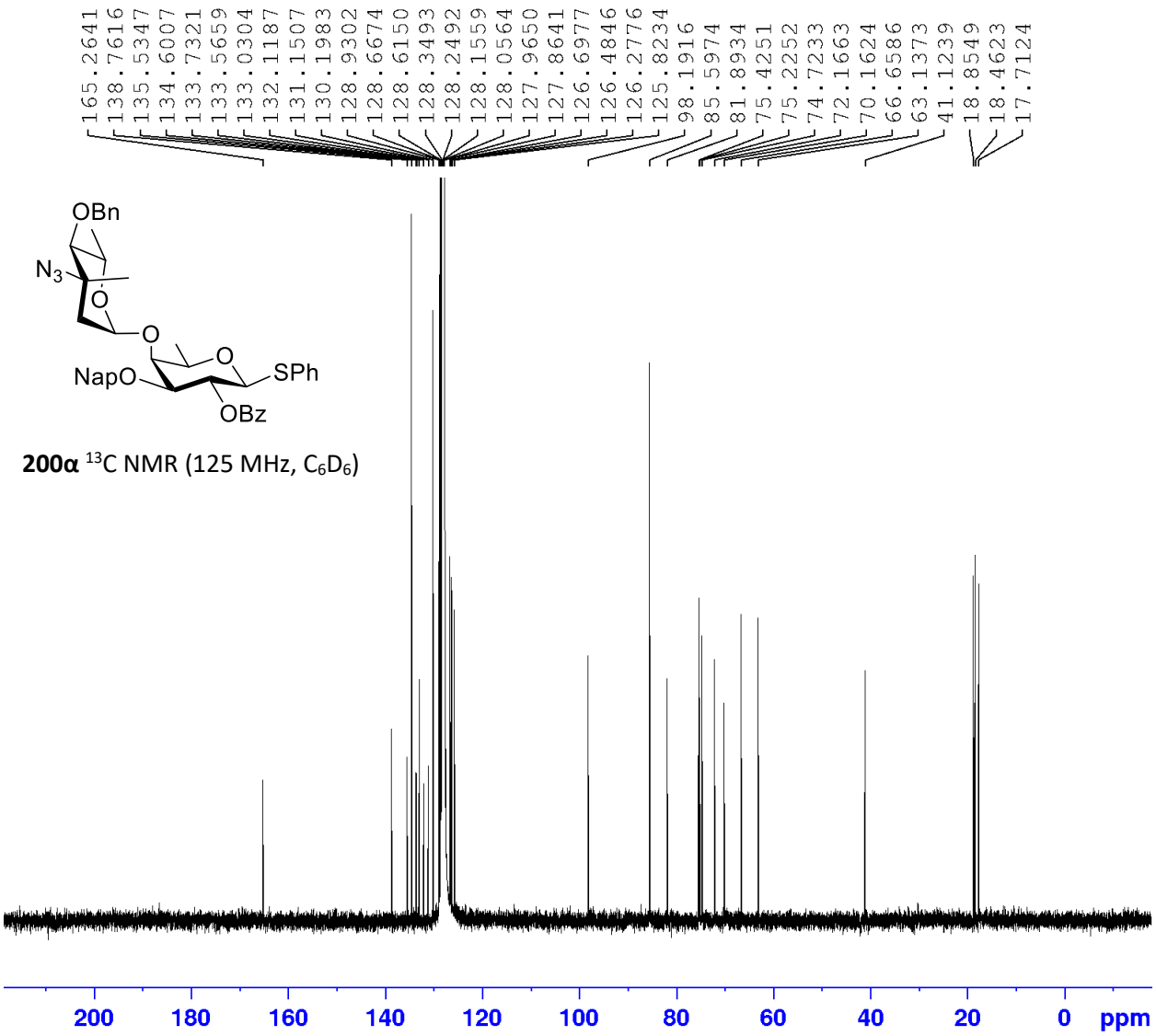


196 ^{1}H-^{13}C NMR HSQC, C_6D_6

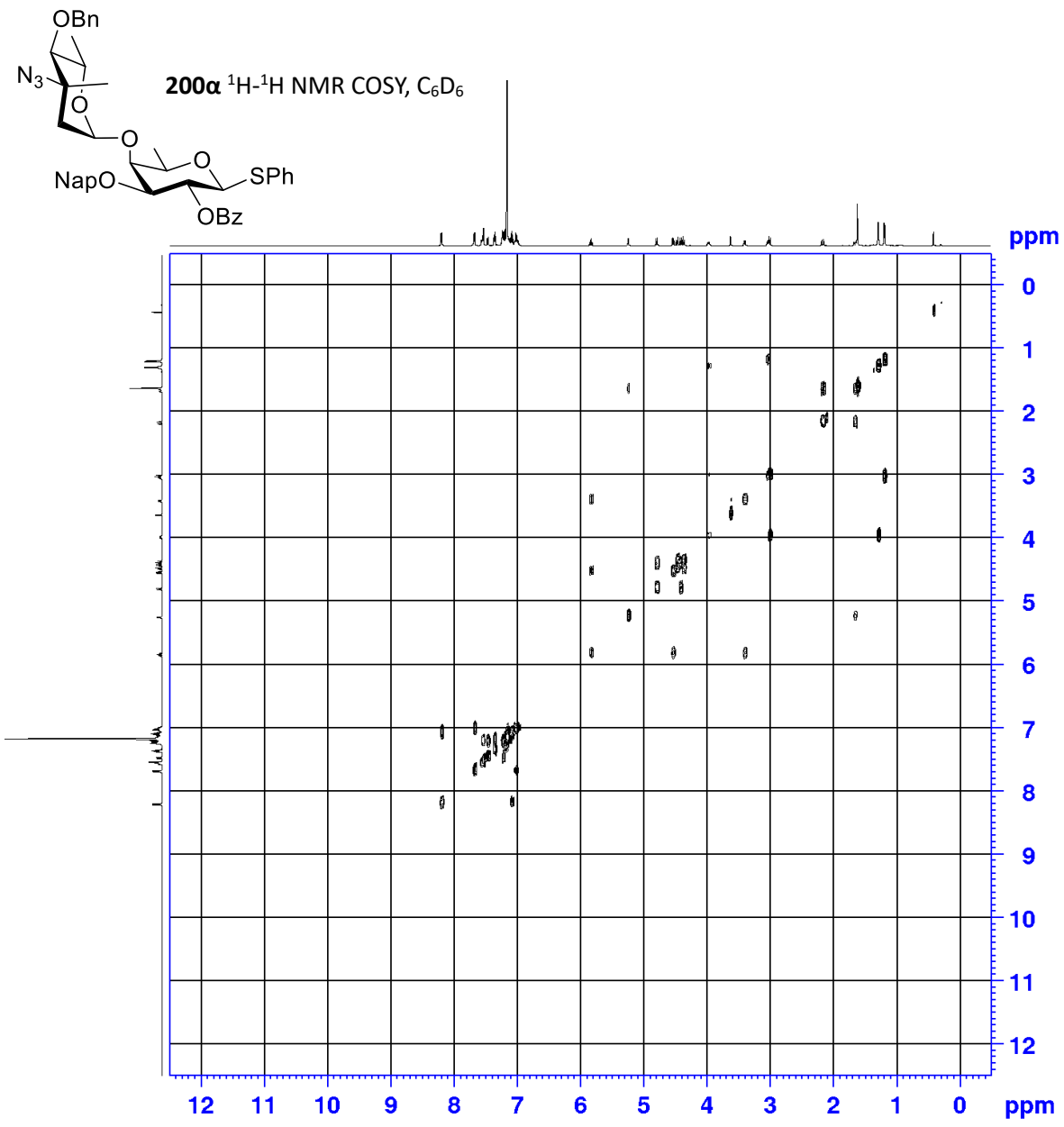


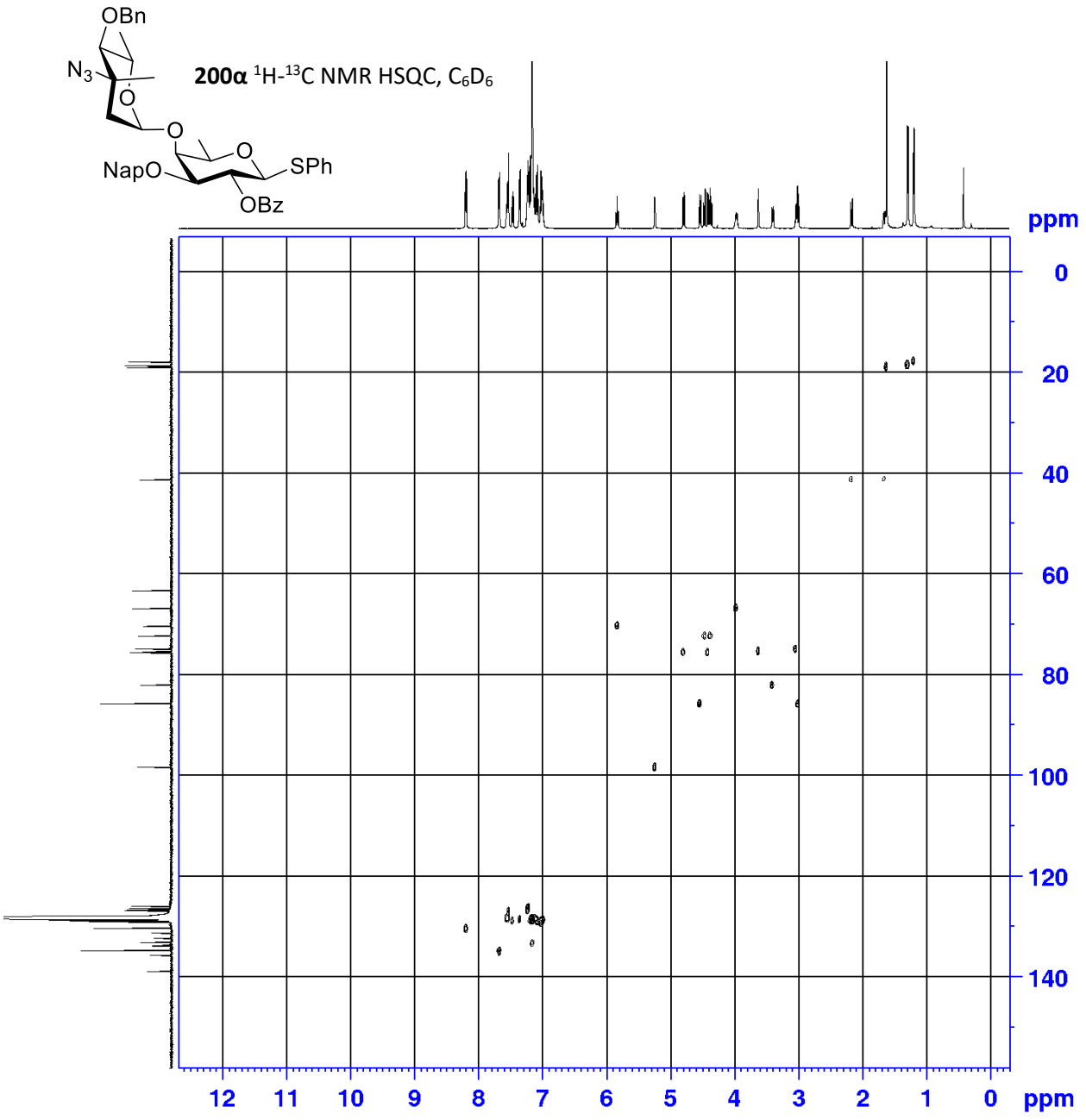
200α ^{1}H NMR (500 MHz, C_6D_6)

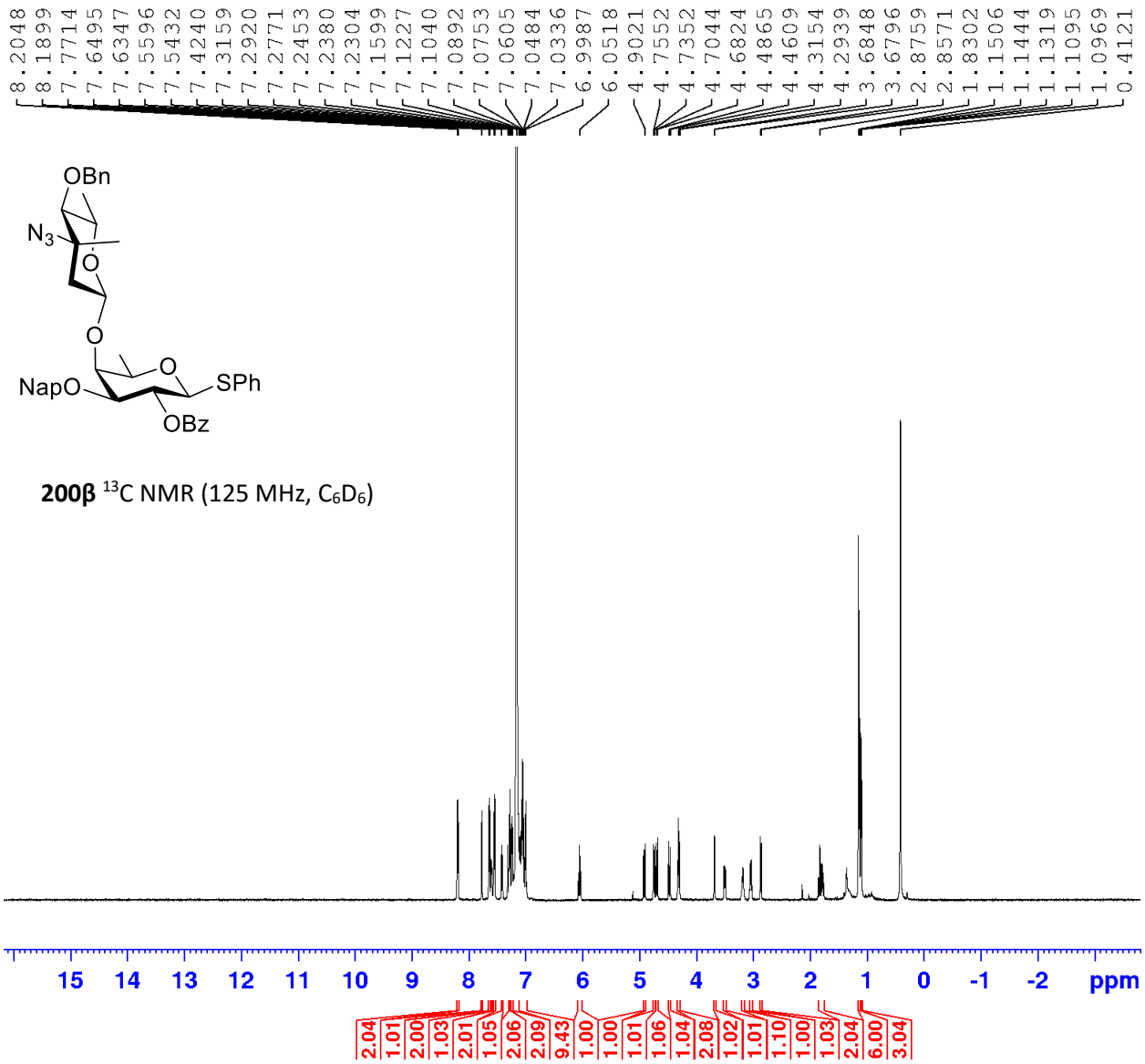
;



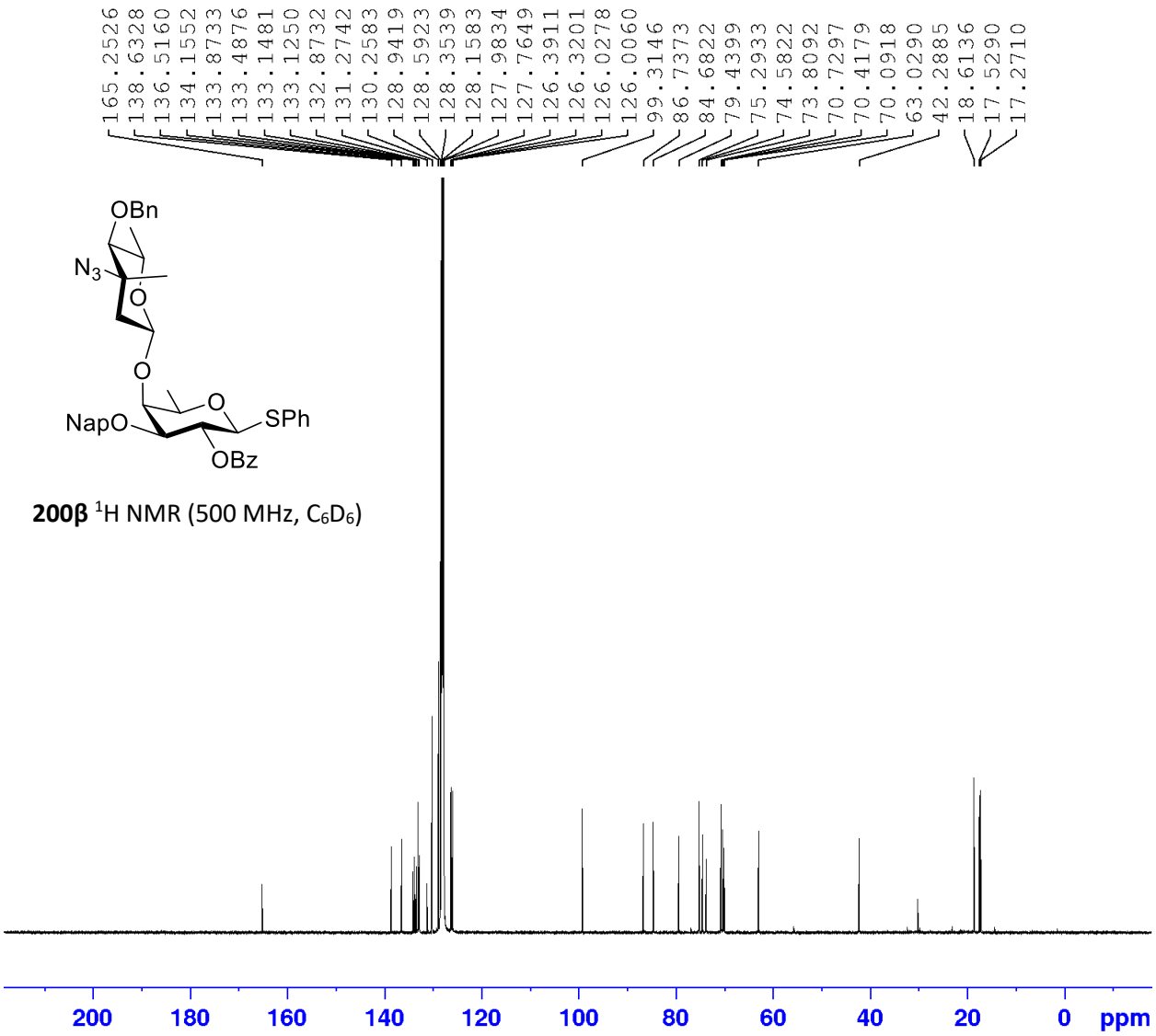
200α ^{13}C NMR (125 MHz, C_6D_6)


OBn
N3
O
O
NapO
O
SPh
OBz
200α 1H-1H NMR COSY, C6D6
ppm
0
1
2
3
4
5
6
7
8
9
10
11
12
12
11
10
9
8
7
6
5
4
3
2
1
0
ppm

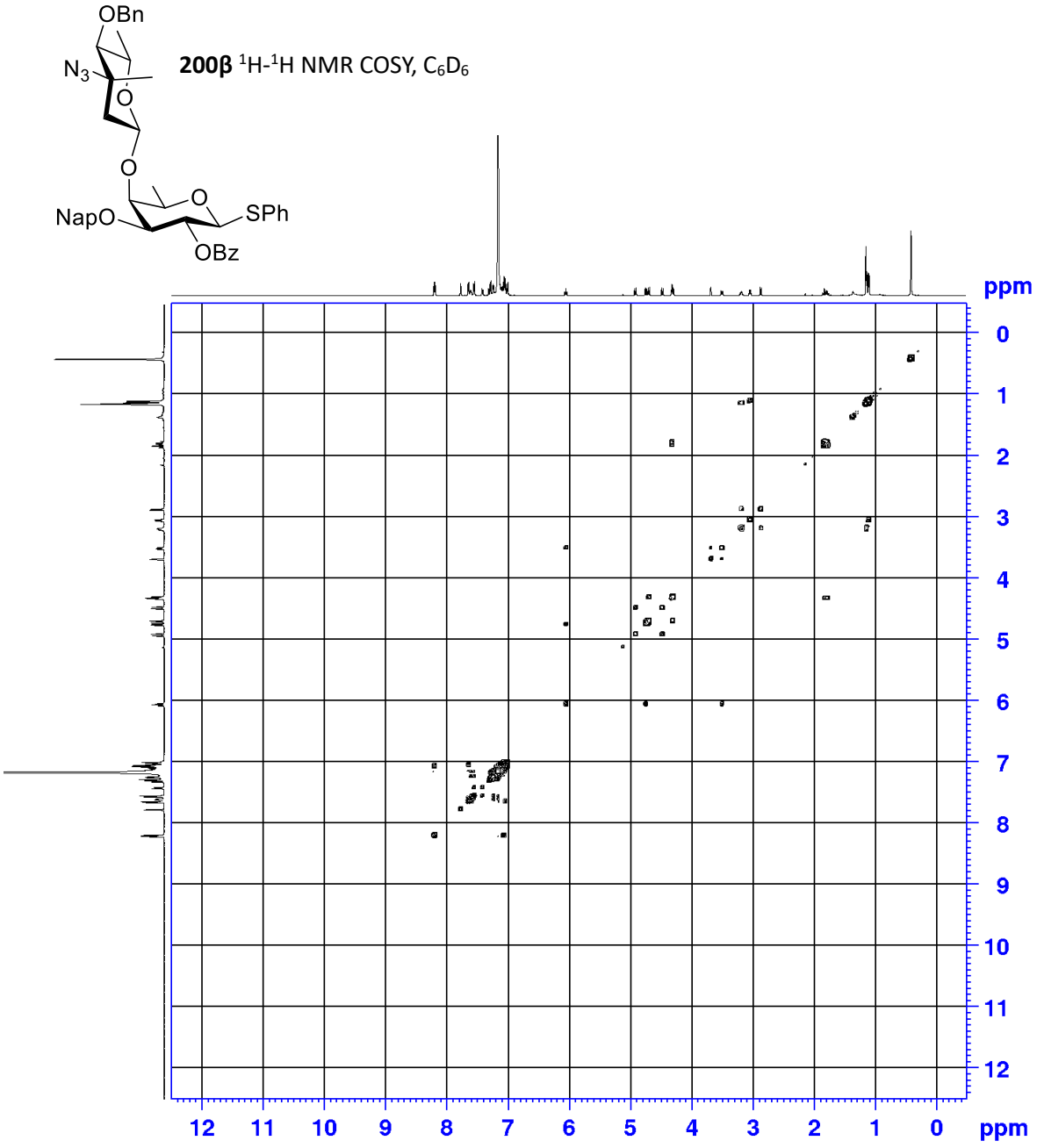

OBn
N3
O
O
O
NapO
SPh
OBz
200α 1H-13C NMR HSQC, C6D6
ppm
0
20
40
60
80
100
120
140
12
11
10
9
8
7
6
5
4
3
2
1
0
ppm

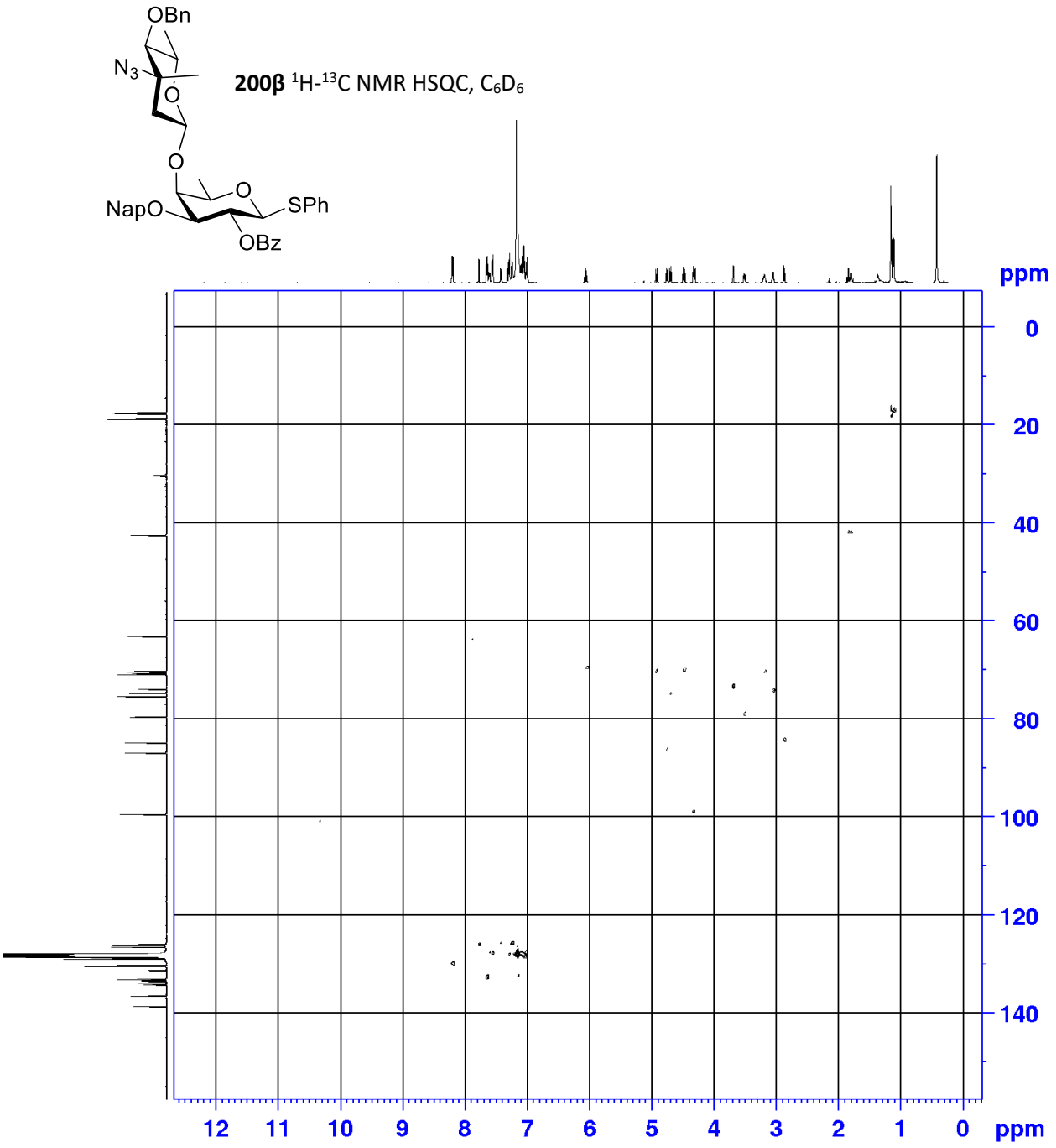


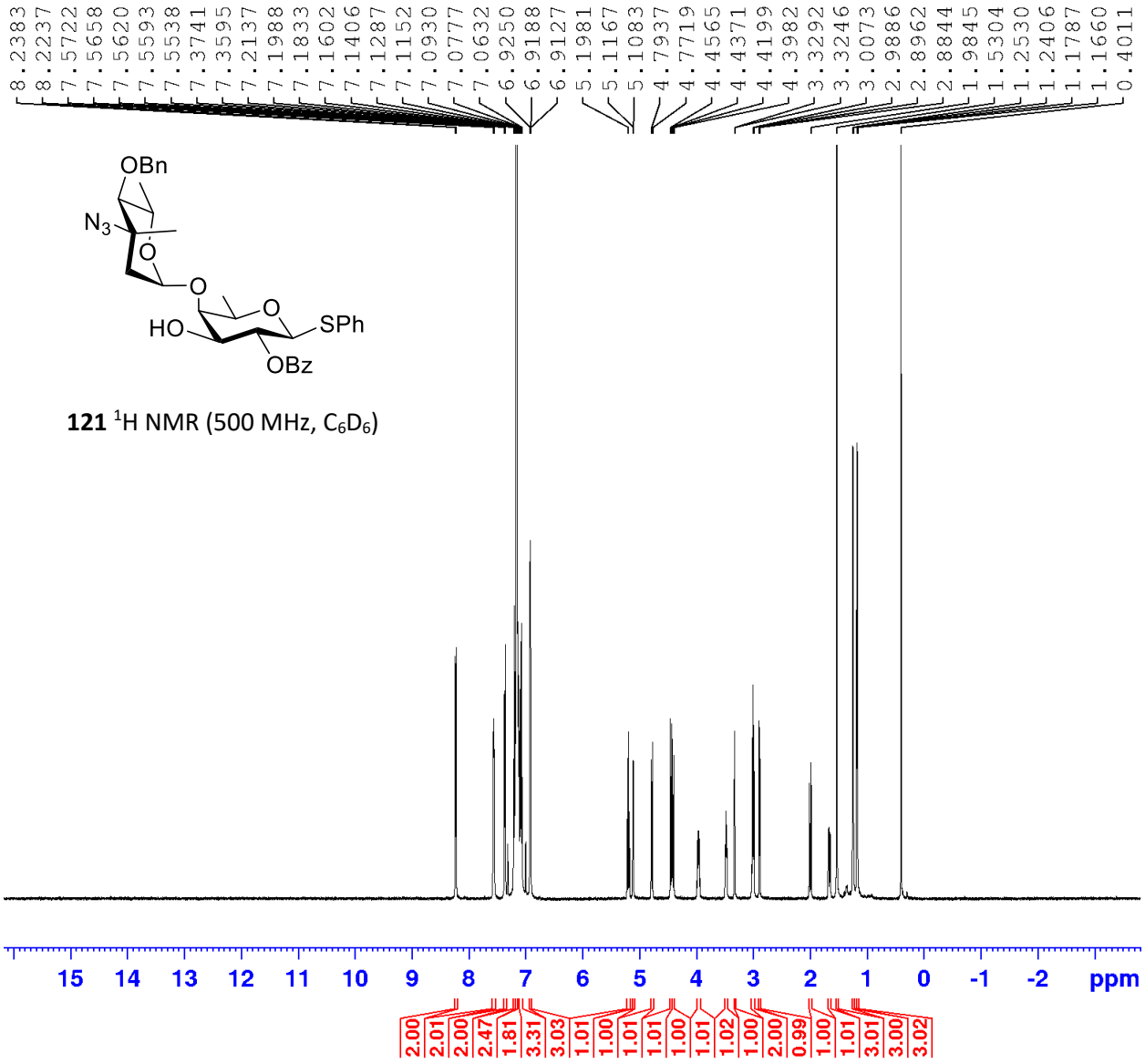
200β ^{13}C NMR (125 MHz, C_6D_6)



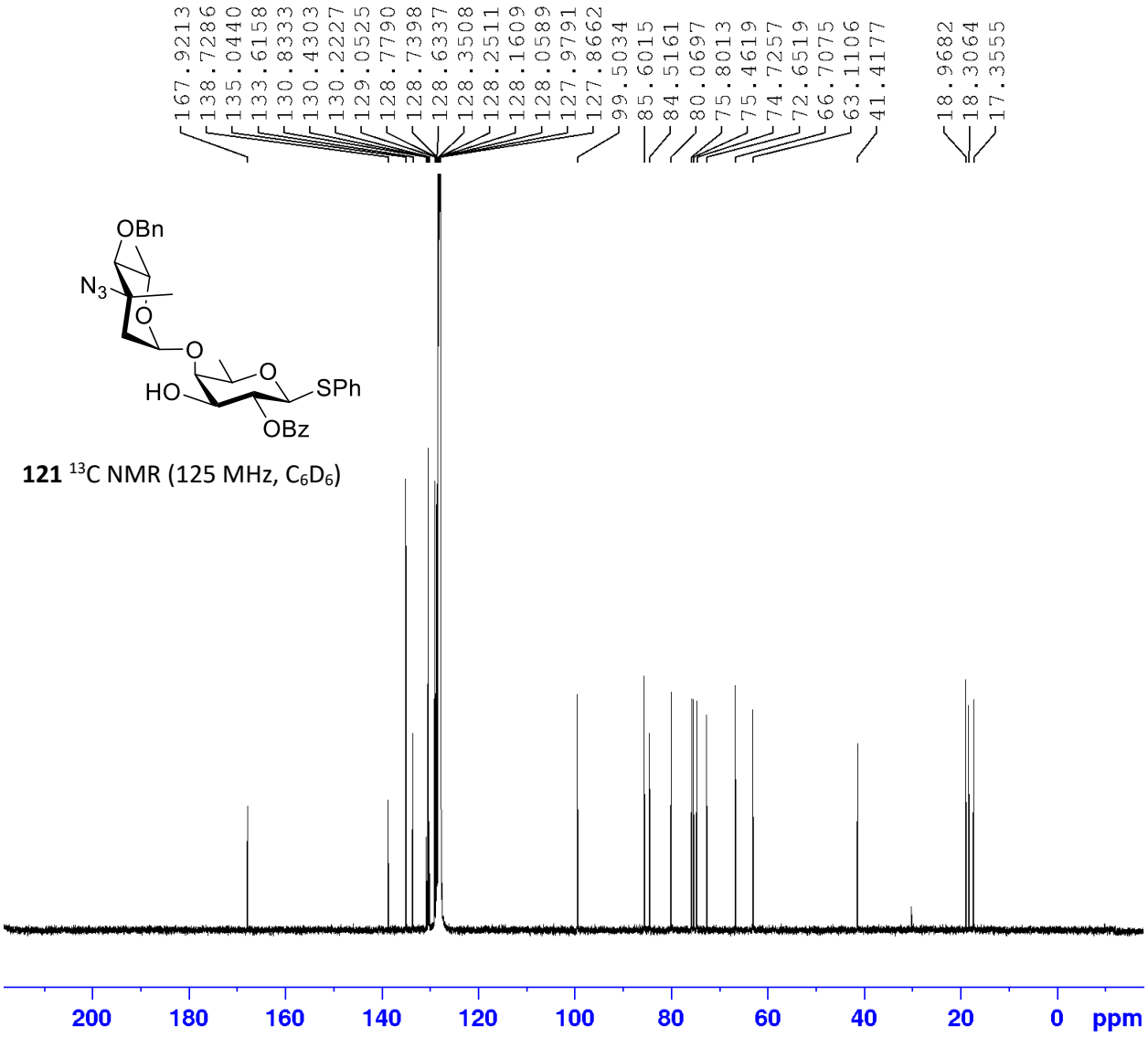
200β ^{1}H NMR (500 MHz, C_6D_6)


OBn
N3
O
O
NapO
O
SPh
OBz
200β 1H-1H NMR COSY, C6D6
ppm
0
1
2
3
4
5
6
7
8
9
10
11
12
12
11
10
9
8
7
6
5
4
3
2
1
0
ppm

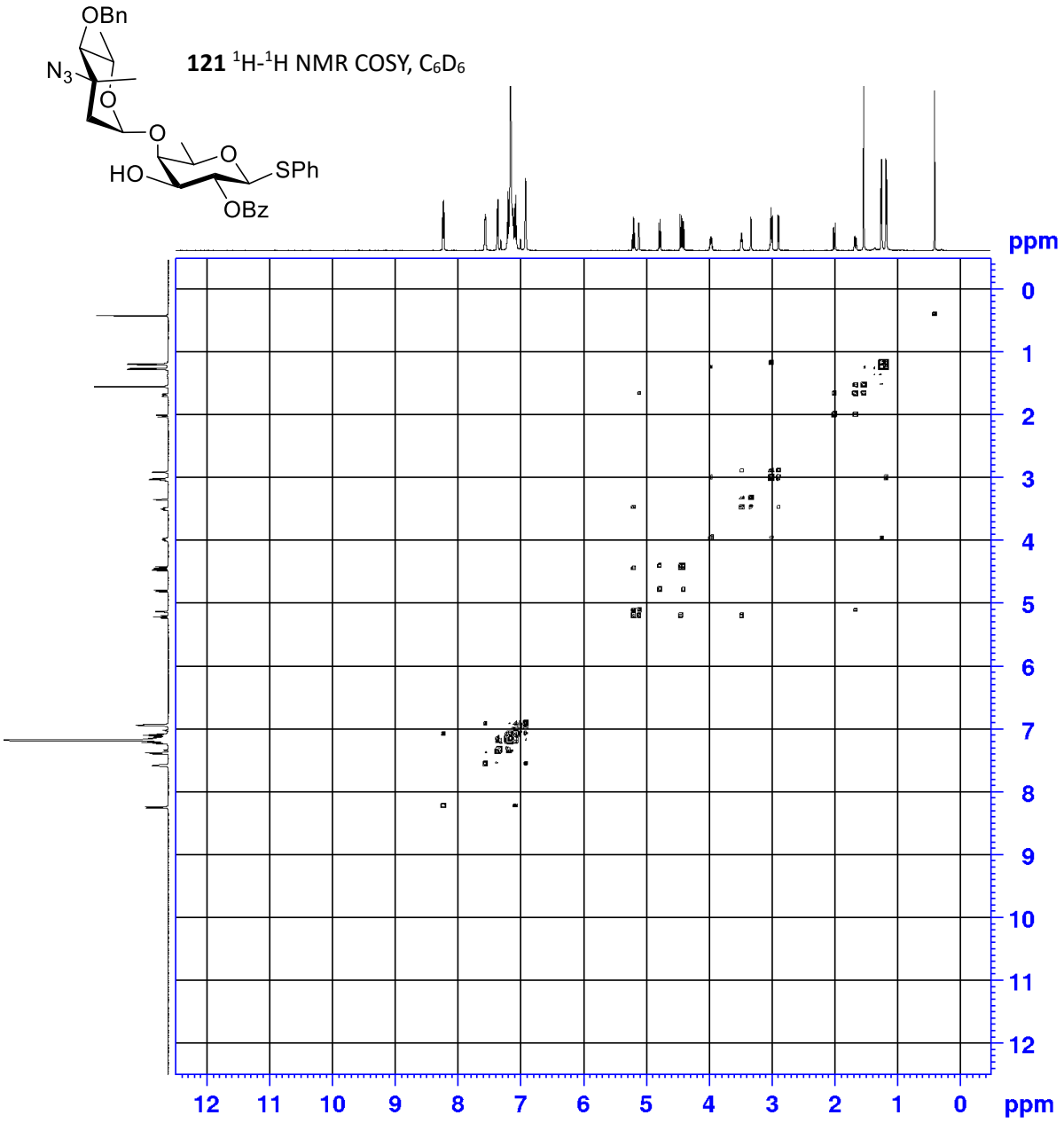

OBn
N3
O
O
NapO
O
SPh
OBz
200β 1H-13C NMR HSQC, C6D6
ppm
0
20
40
60
80
100
120
140
12
11
10
9
8
7
6
5
4
3
2
1
0
ppm

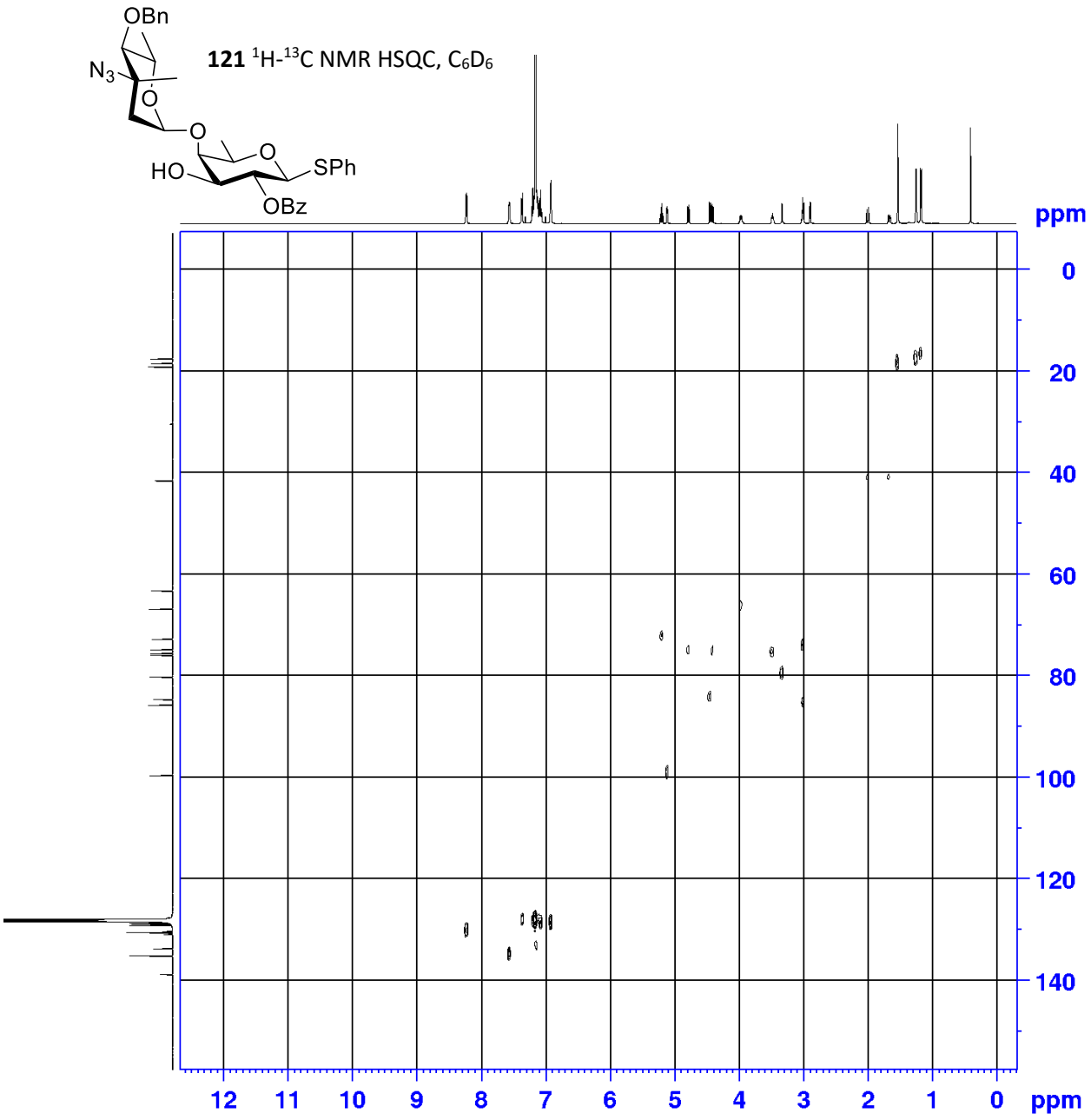


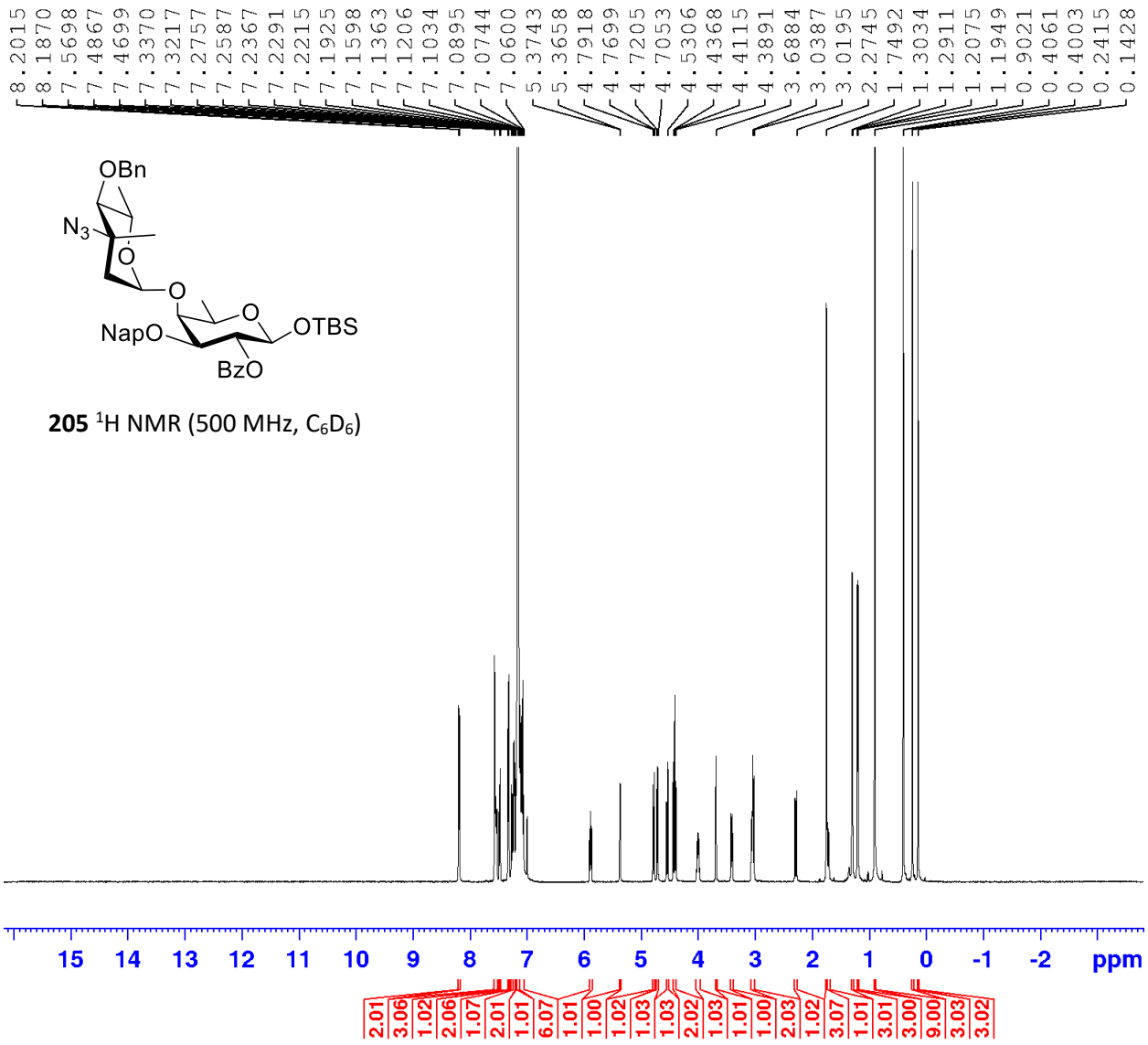
121 ^{1}H NMR (500 MHz, C_6D_6)



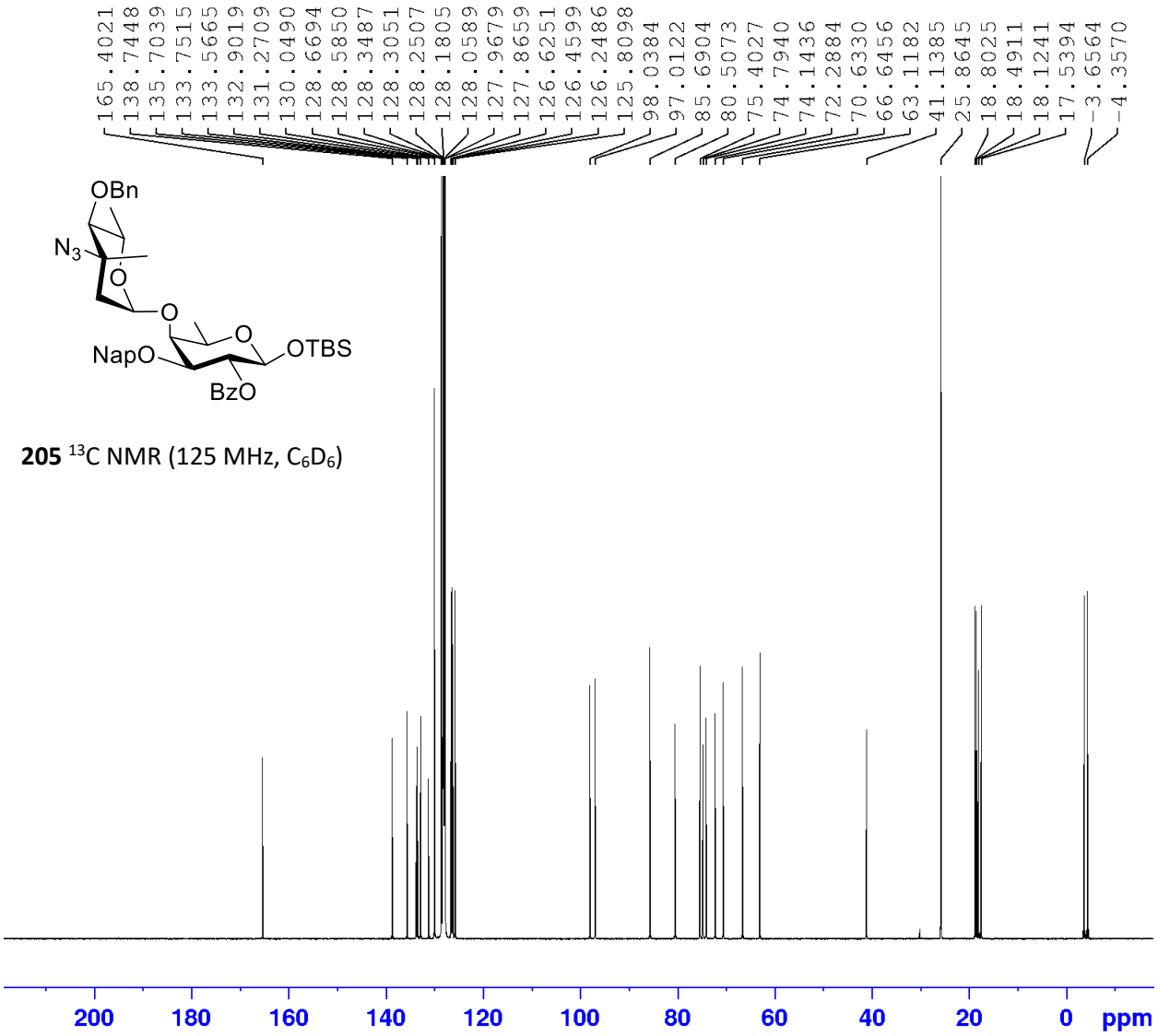
121 ^{13}C NMR (125 MHz, C_6D_6)


OBn
N3
O
O
HO
O
SPh
OBz
121 1H-1H NMR COSY, C6D6
ppm
0
1
2
3
4
5
6
7
8
9
10
11
12
12 11 10 9 8 7 6 5 4 3 2 1 0 ppm

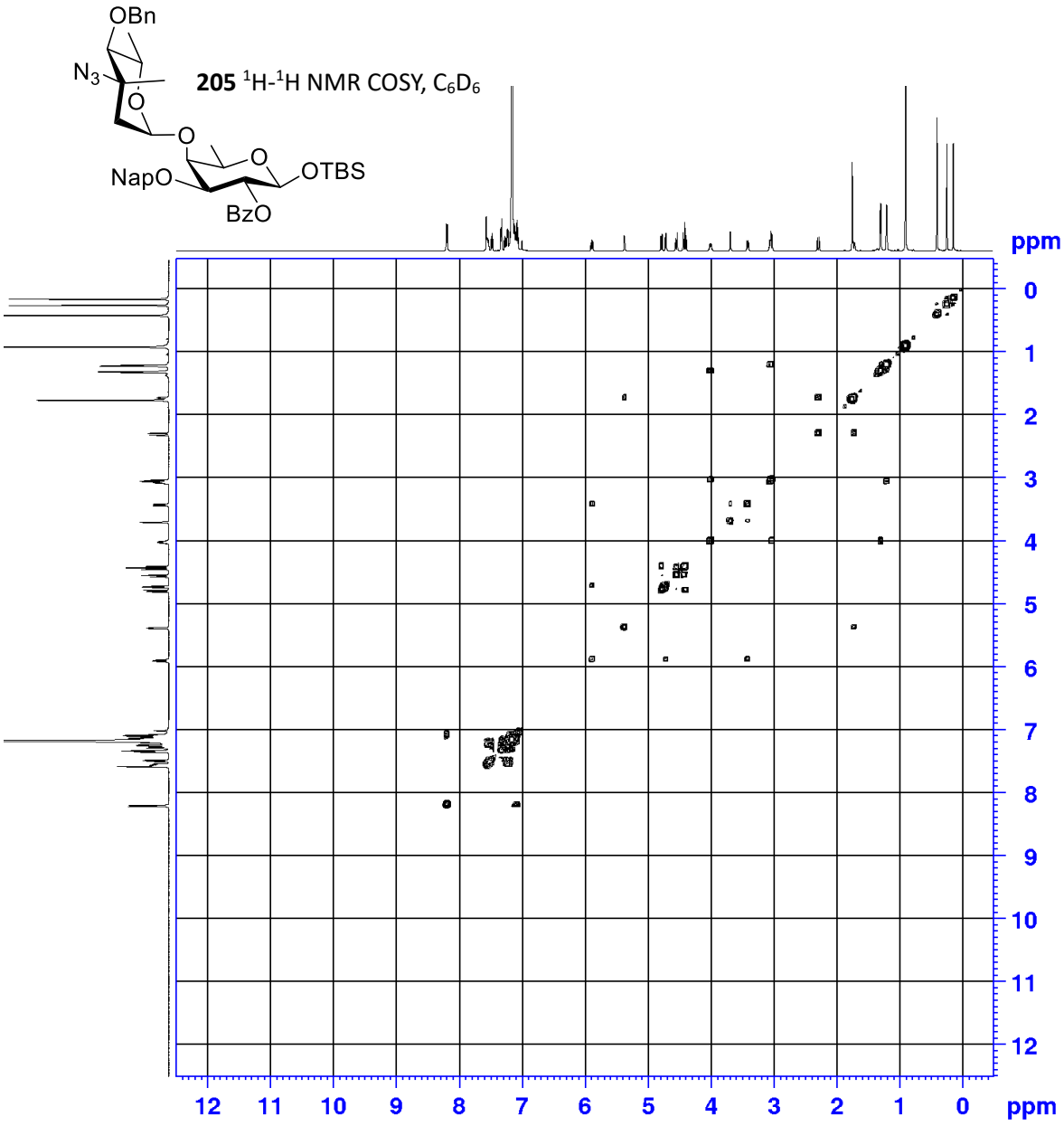

OBn
N3
O
O
HO
O
SPh
OBz
121 1H-13C NMR HSQC, C6D6
ppm
0
20
40
60
80
100
120
140
12
11
10
9
8
7
6
5
4
3
2
1
0
ppm

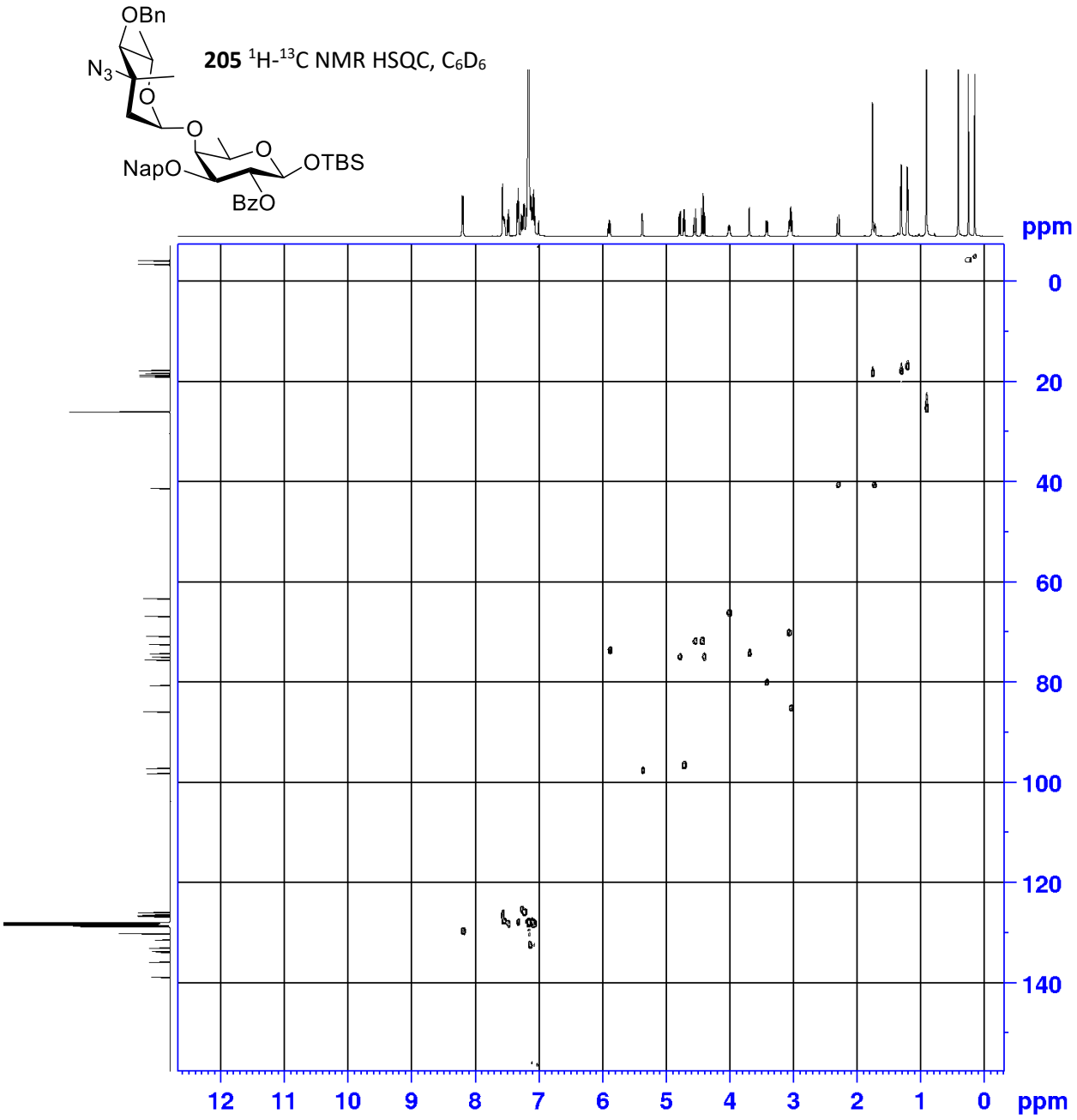


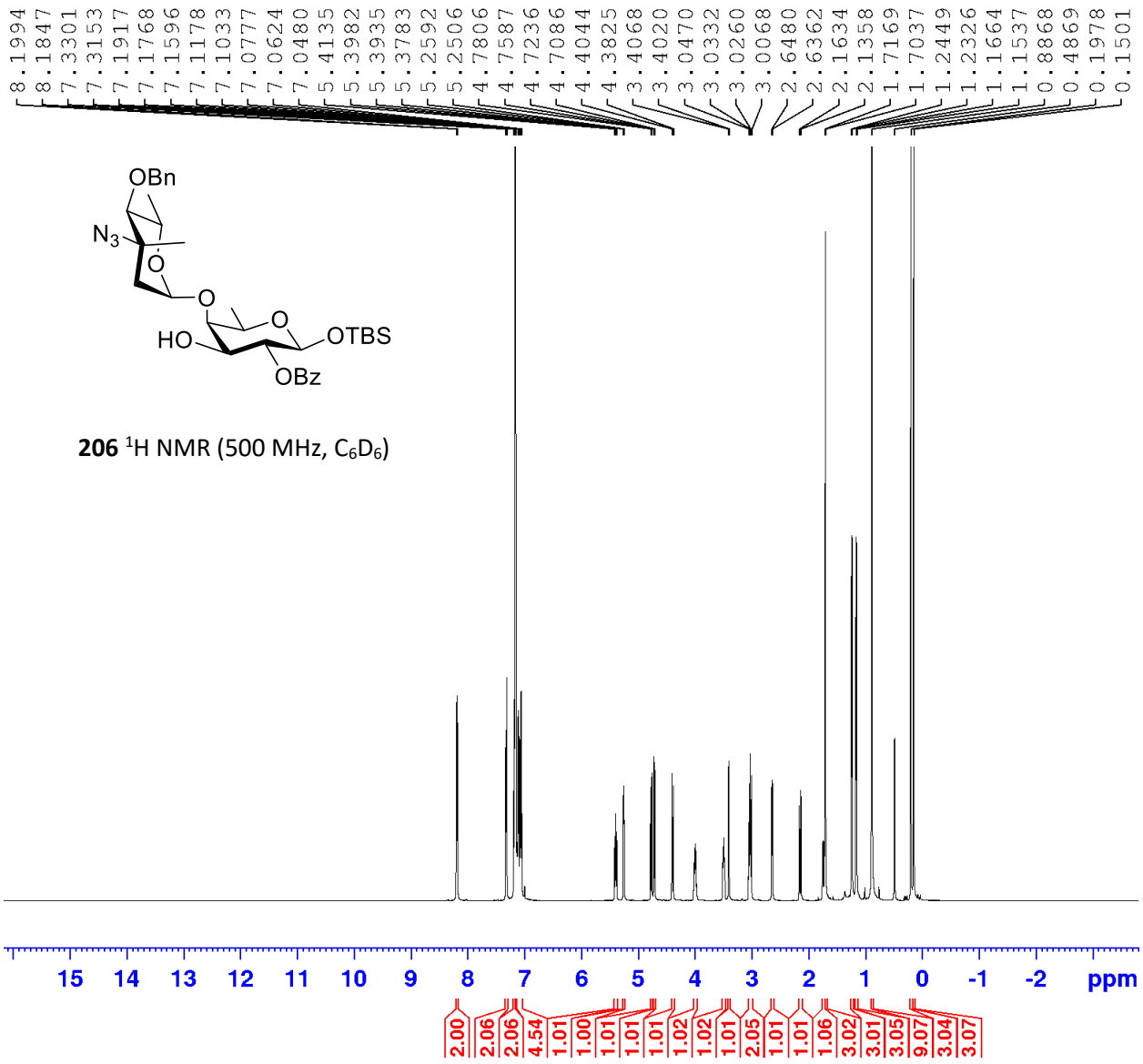
205 ^{1}H NMR (500 MHz, C_6D_6)



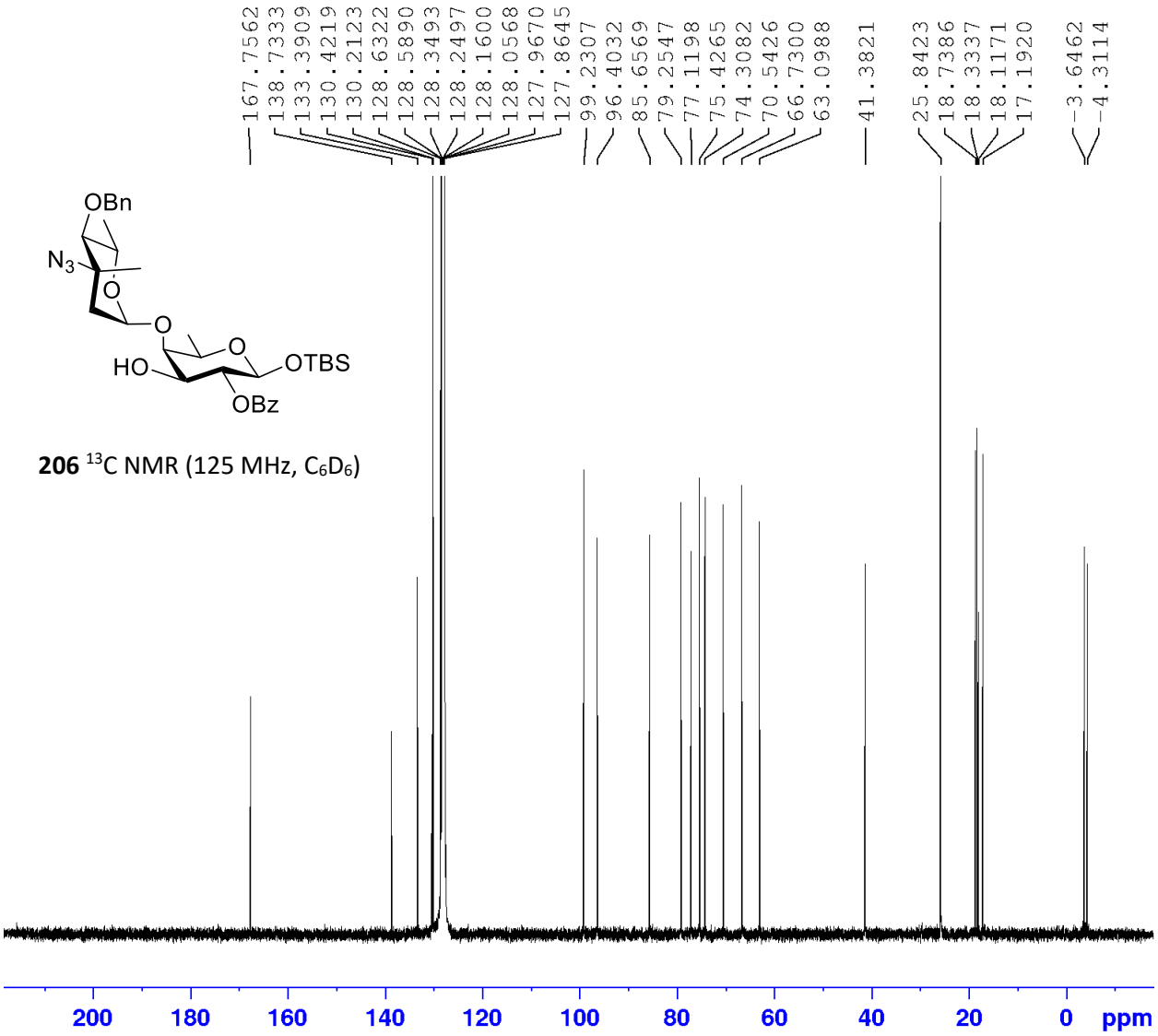
205 ^{13}C NMR (125 MHz, C_6D_6)


OBn
N3
O
O
NapO
O
OTBS
BzO
205 1H-1H NMR COSY, C6D6
ppm
0
1
2
3
4
5
6
7
8
9
10
11
12
12 11 10 9 8 7 6 5 4 3 2 1 0 ppm

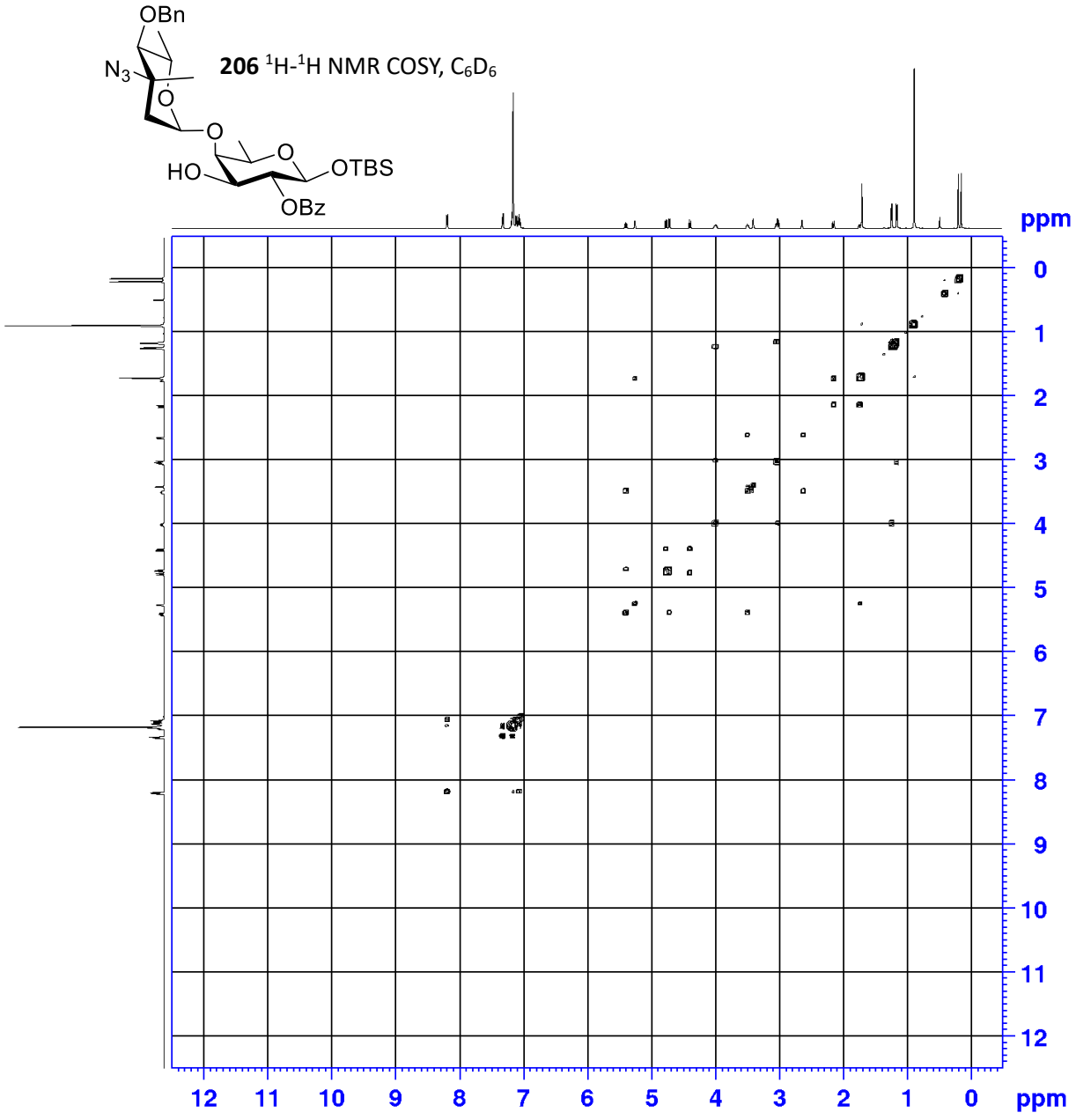

OBn
N3
O
O
O
NapO
OTBS
BzO
205 1H-13C NMR HSQC, C6D6
ppm
0
20
40
60
80
100
120
140
12
11
10
9
8
7
6
5
4
3
2
1
0
ppm

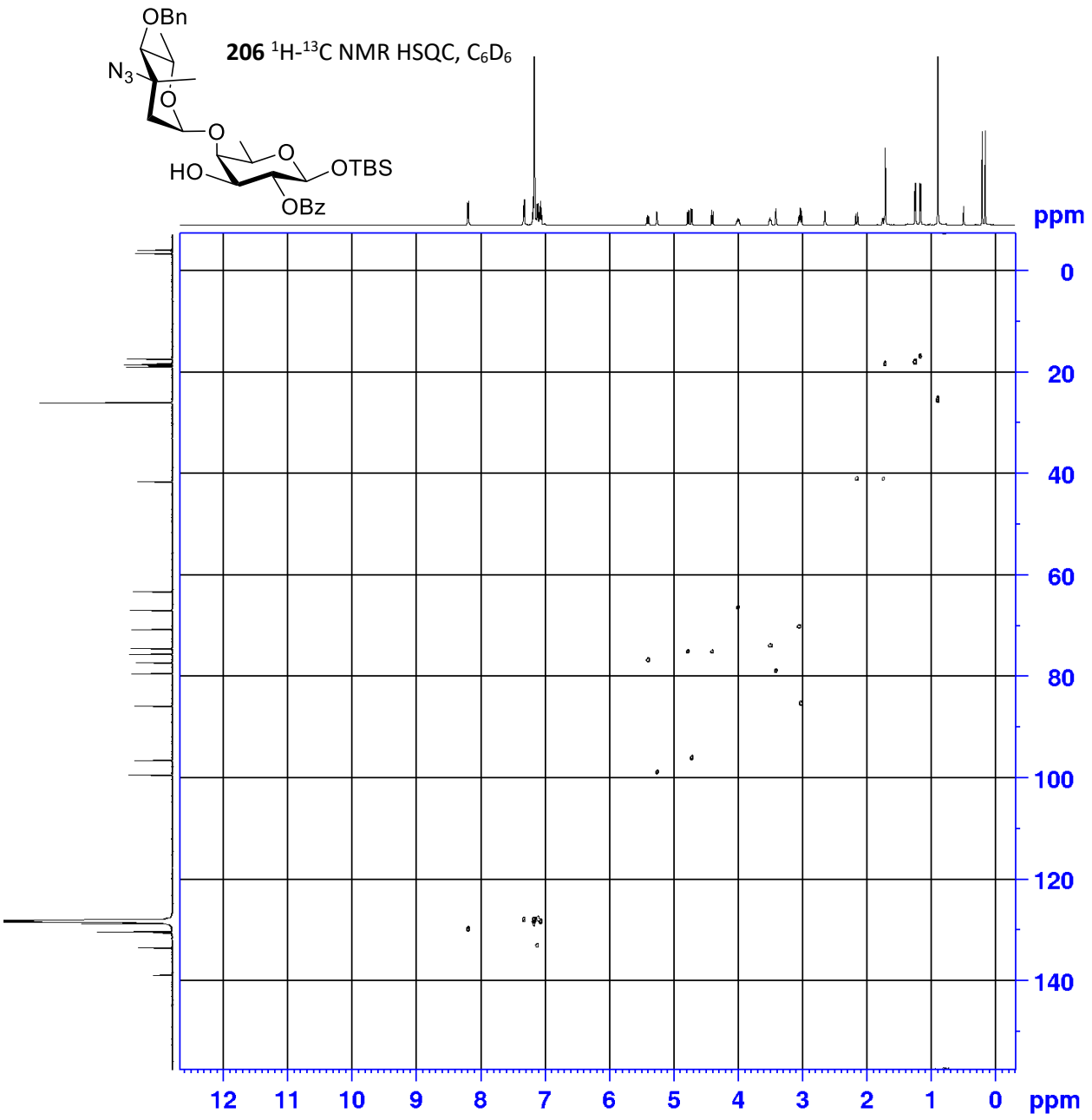


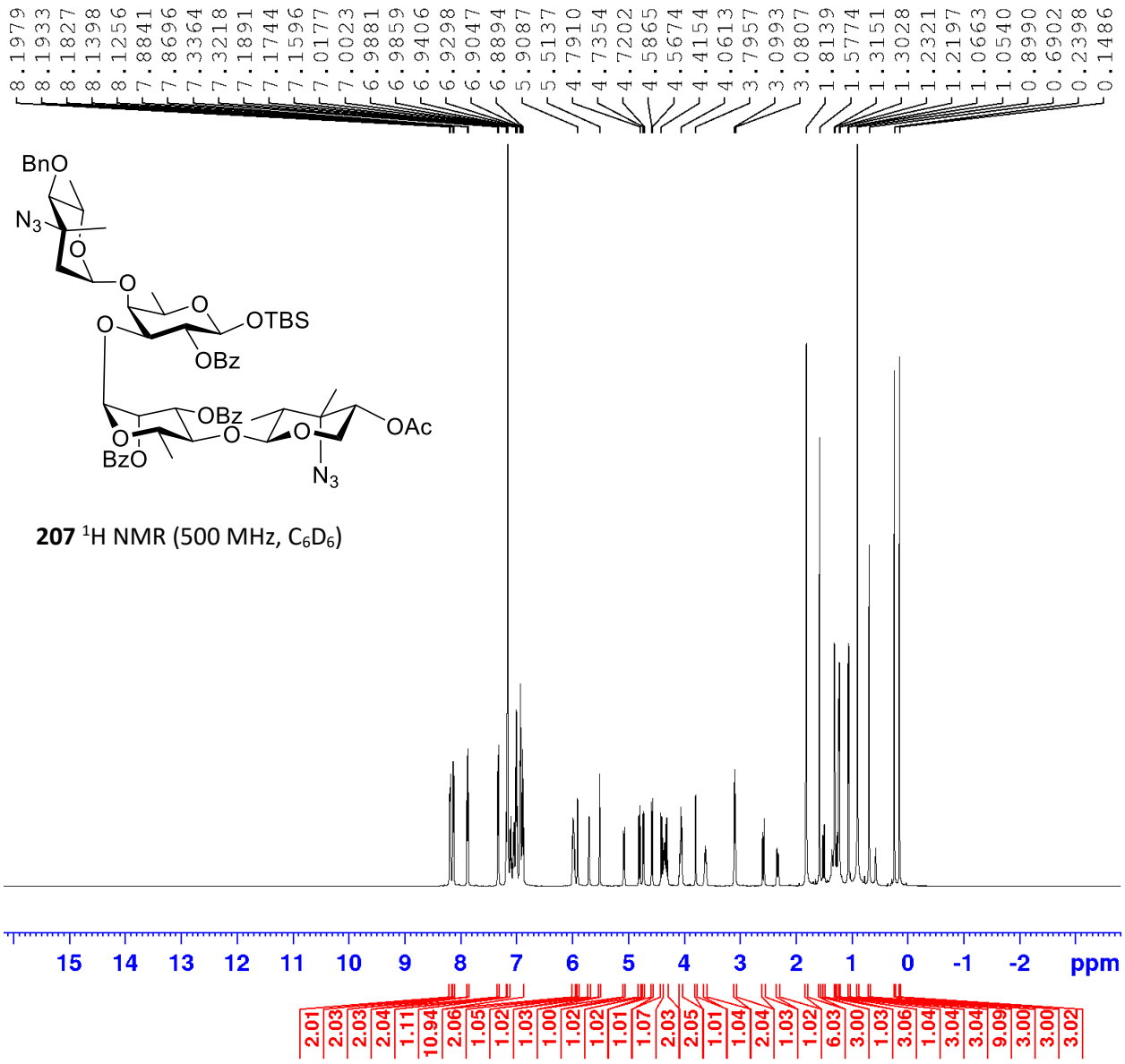
206 ^{1}H NMR (500 MHz, C_6D_6)



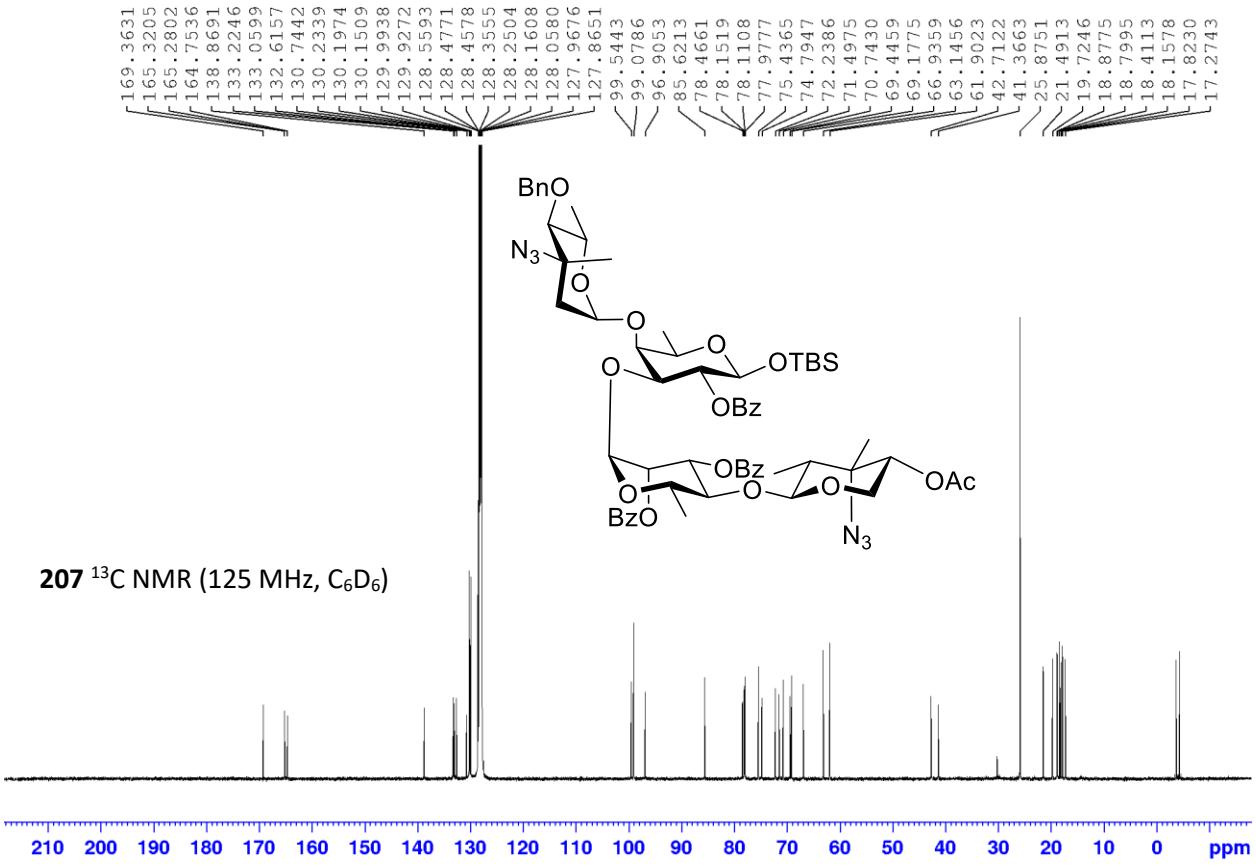
206 ^{13}C NMR (125 MHz, C_6D_6)


OBn
N3
O
O
O
HO
OTBS
OBz
206 1H-1H NMR COSY, C6D6
ppm
0
1
2
3
4
5
6
7
8
9
10
11
12
12
11
10
9
8
7
6
5
4
3
2
1
0
ppm

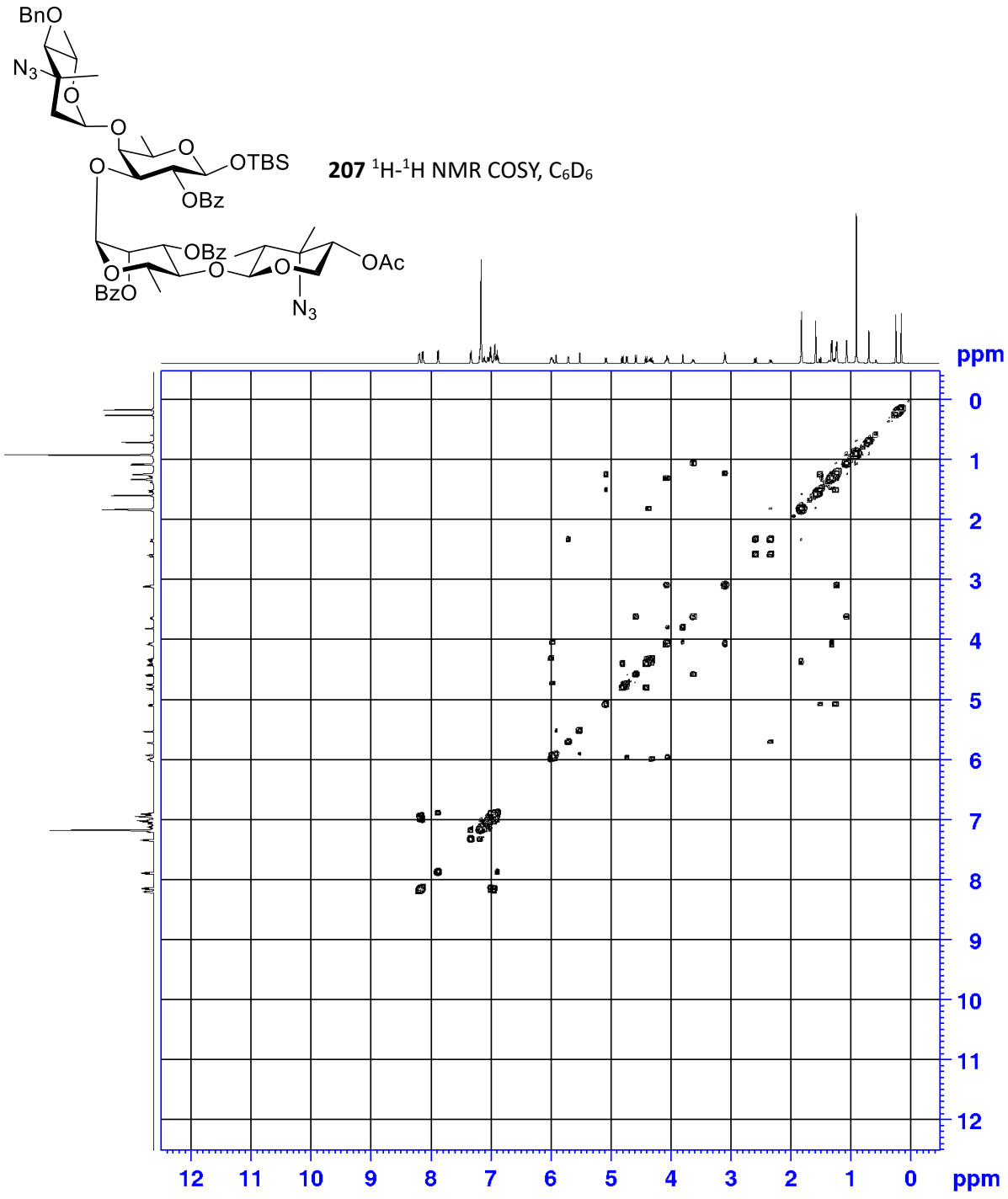

OBn
N3
O
O
HO
O
OTBS
OBz
206 1H-13C NMR HSQC, C6D6
ppm
0
20
40
60
80
100
120
140
12
11
10
9
8
7
6
5
4
3
2
1
0
ppm



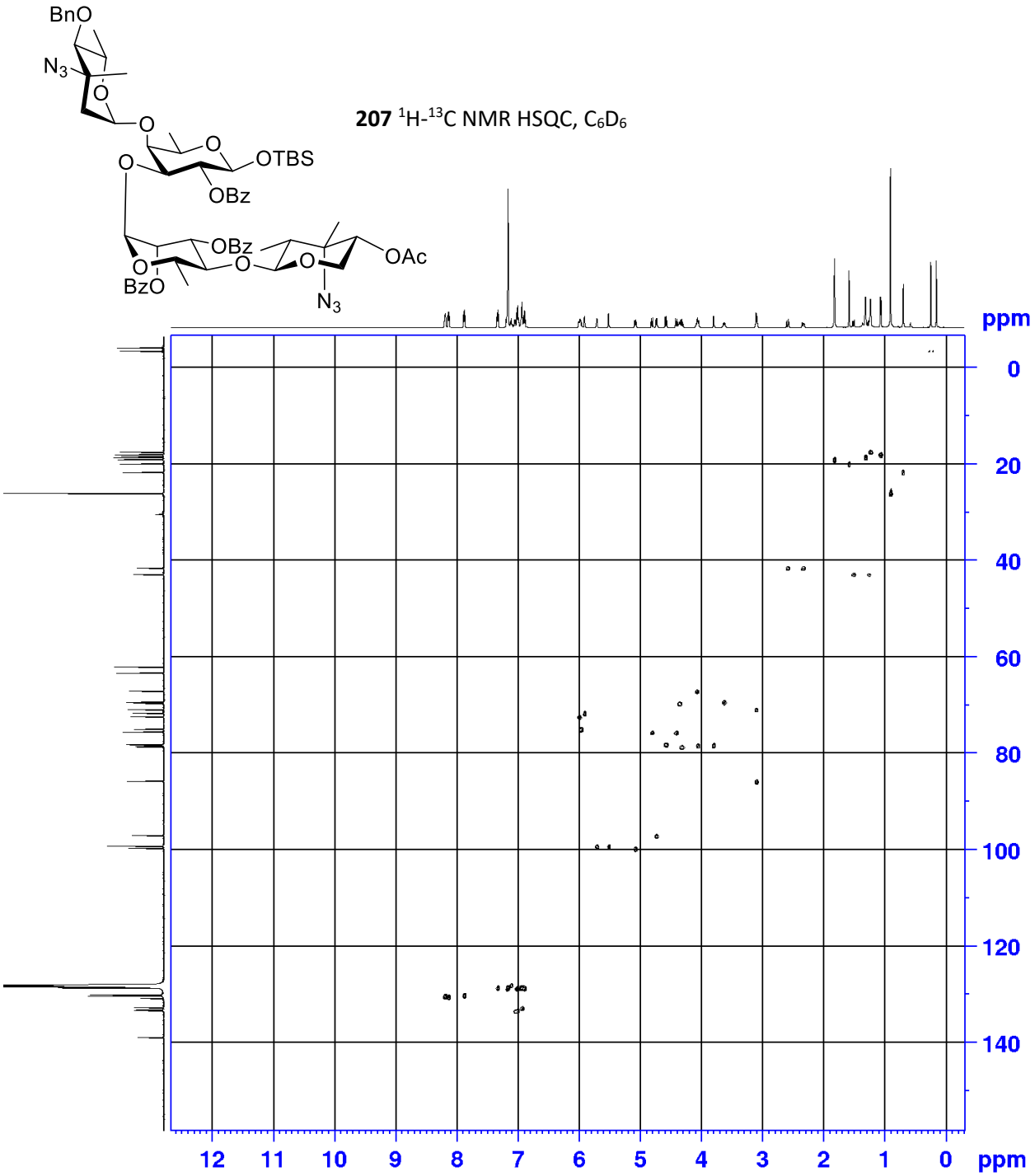
207 ^{1}H NMR (500 MHz, C_6D_6)



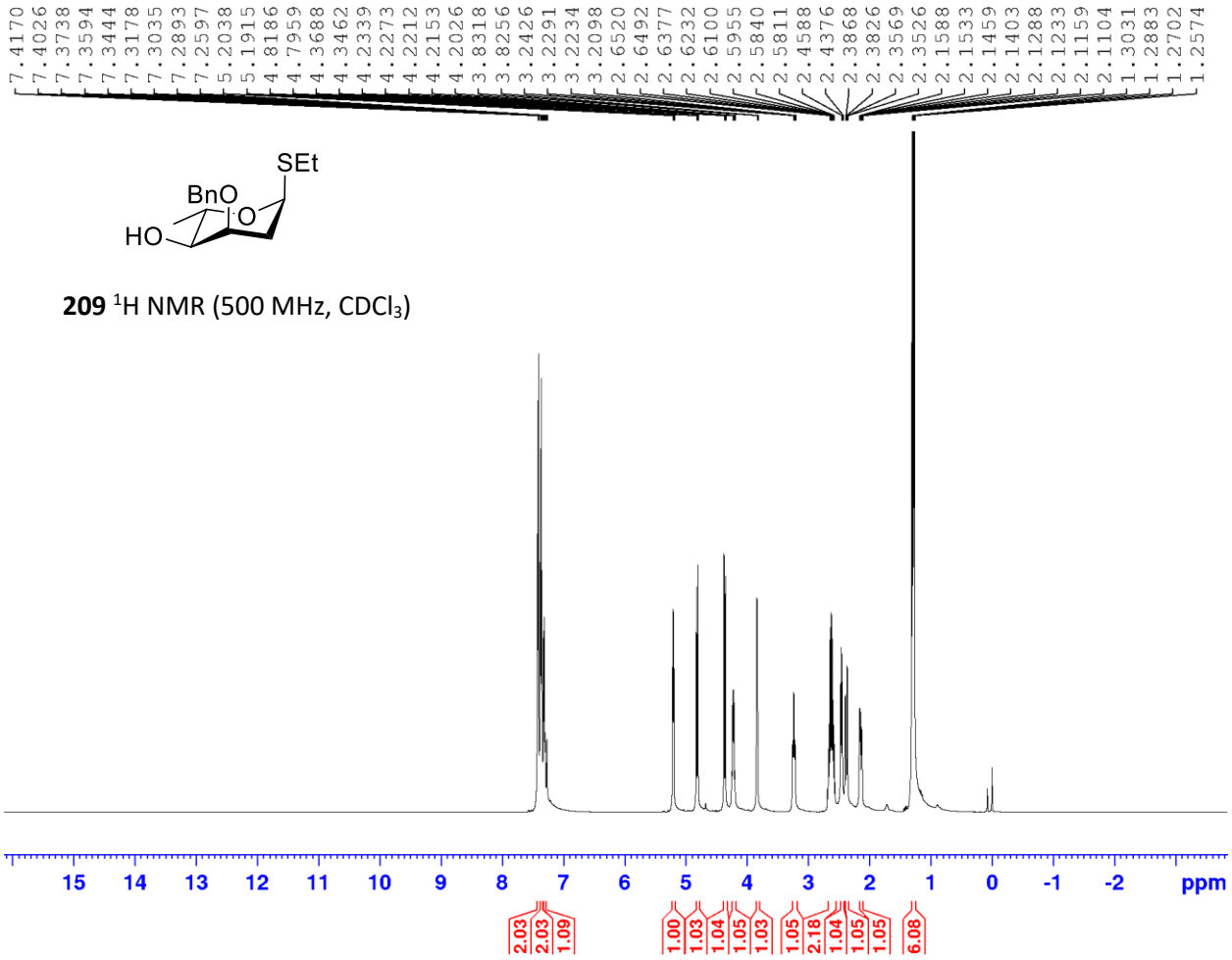
207 ^{13}C NMR (125 MHz, C_6D_6)



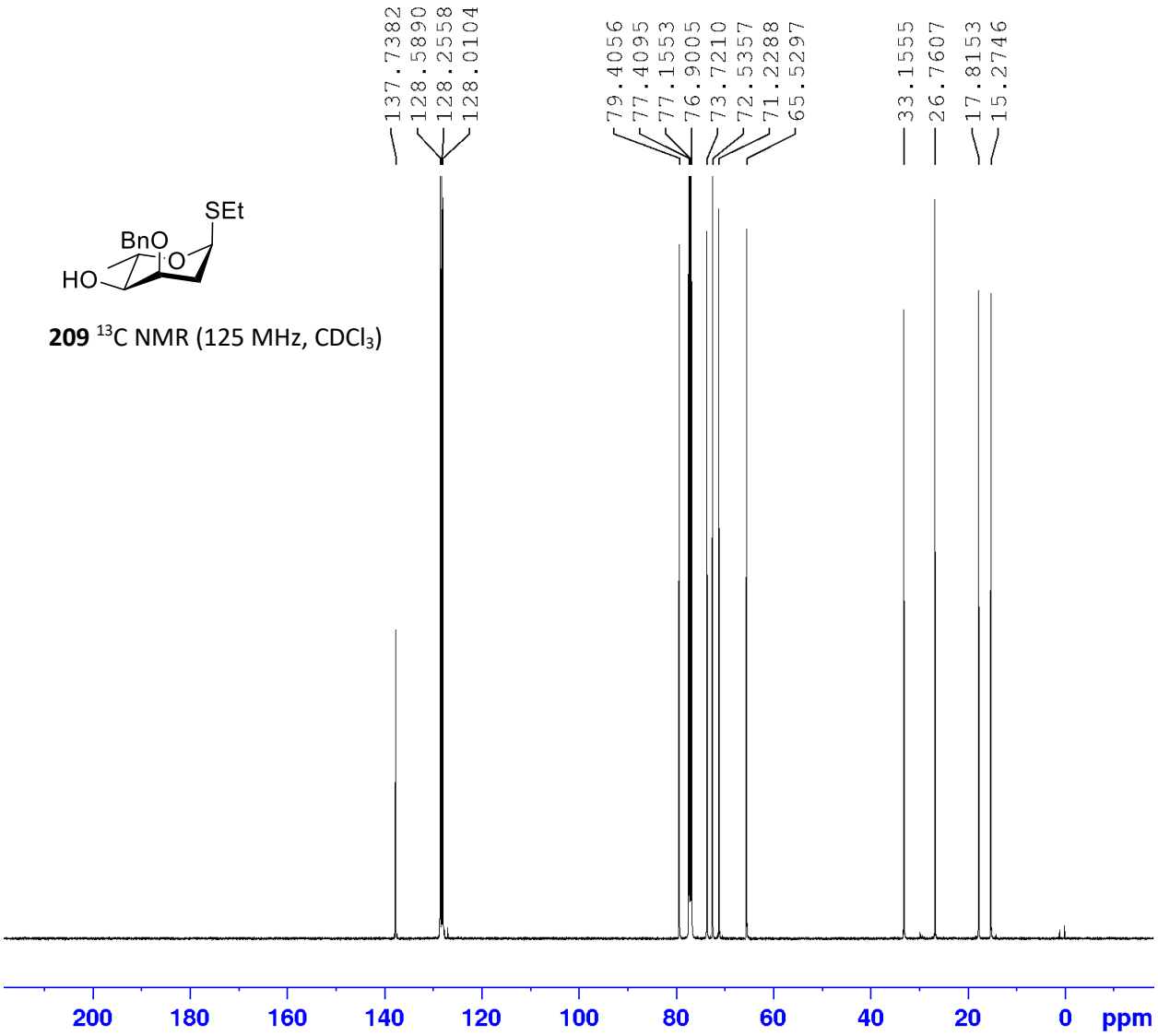
207 ^{1}H-^{1}H NMR COSY, C_6D_6



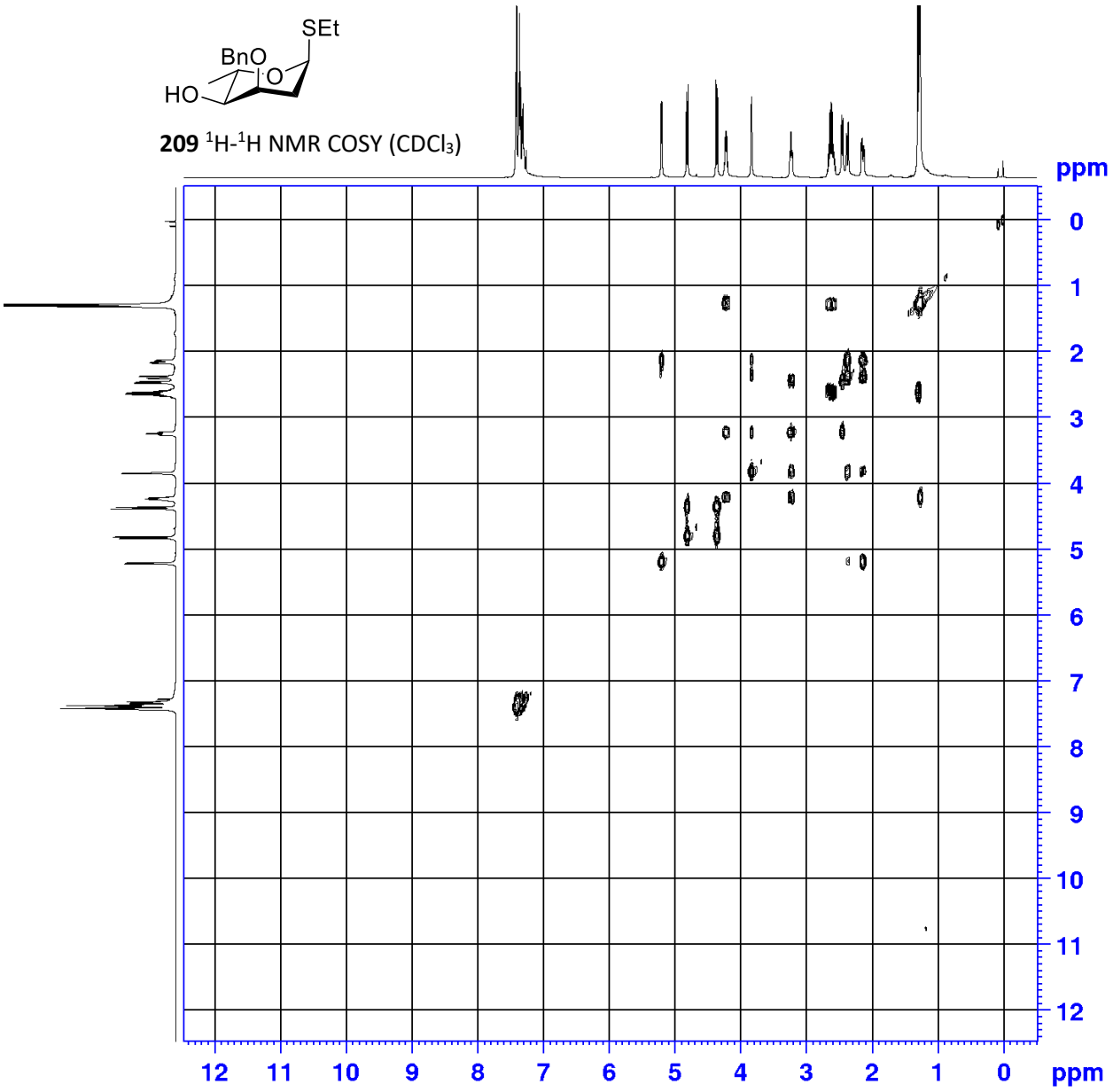
207 ^{1}H-^{13}C NMR HSQC, C_6D_6

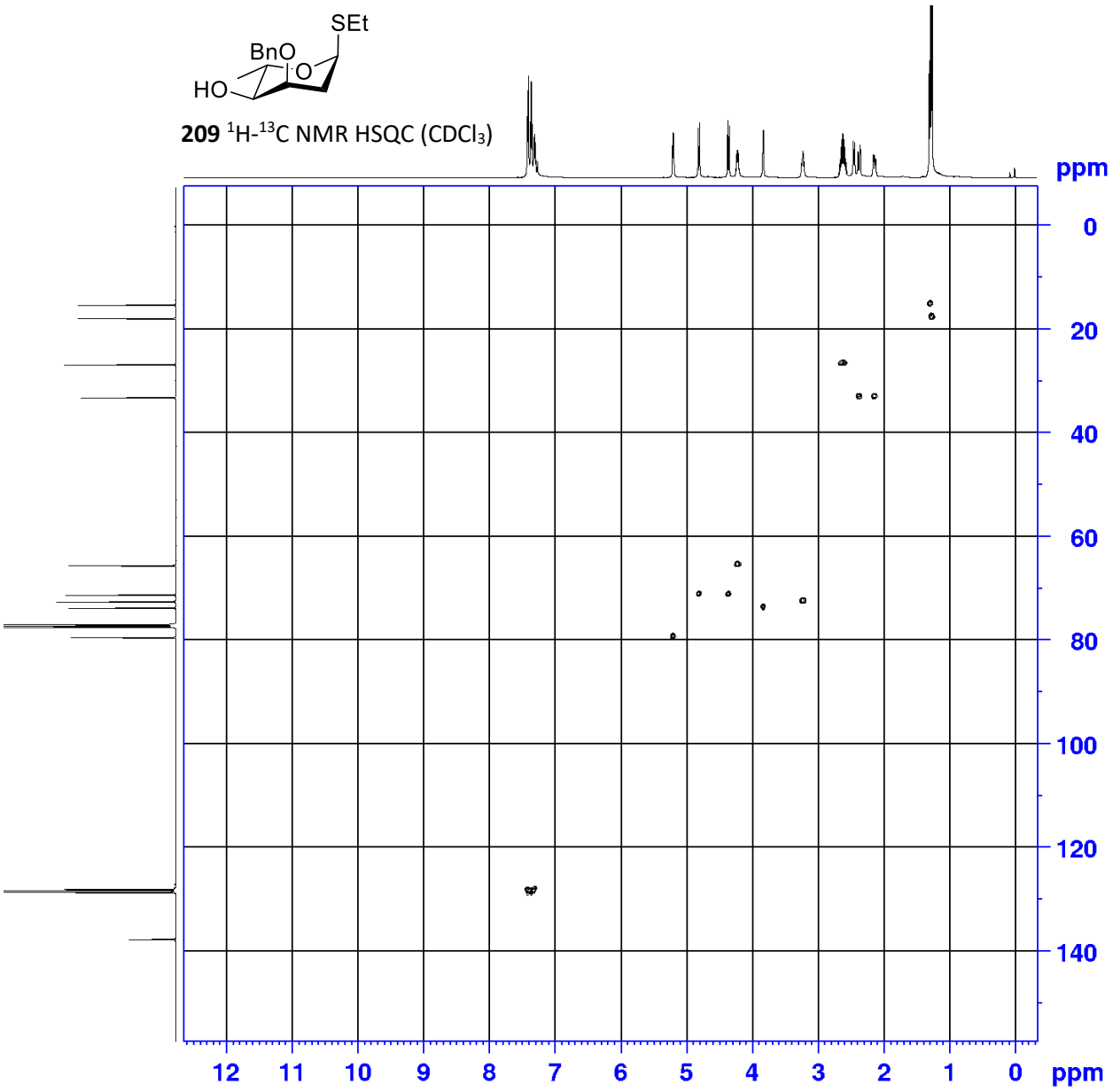


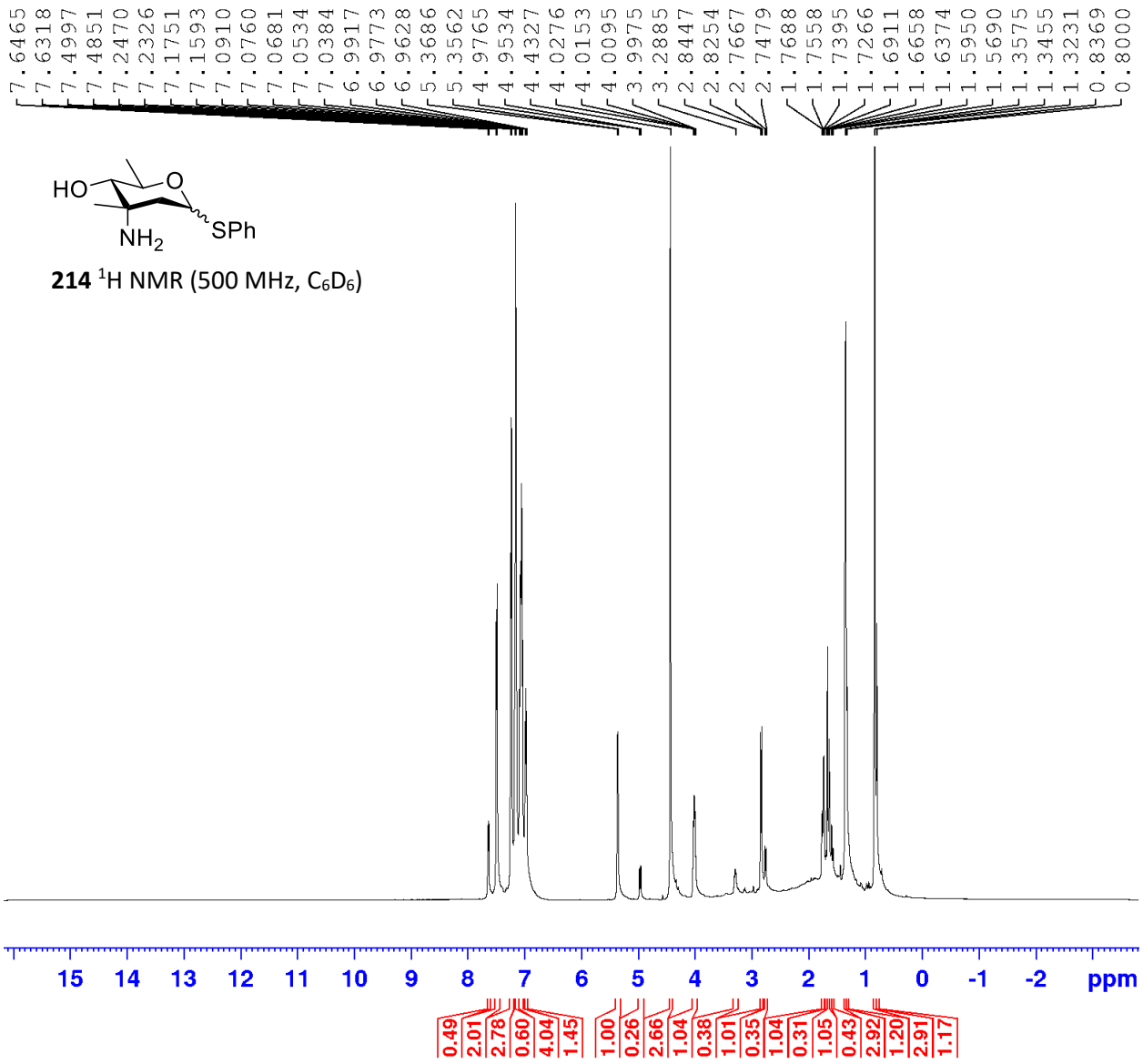
209 ^{1}H NMR (500 MHz, $CDCl_3$)



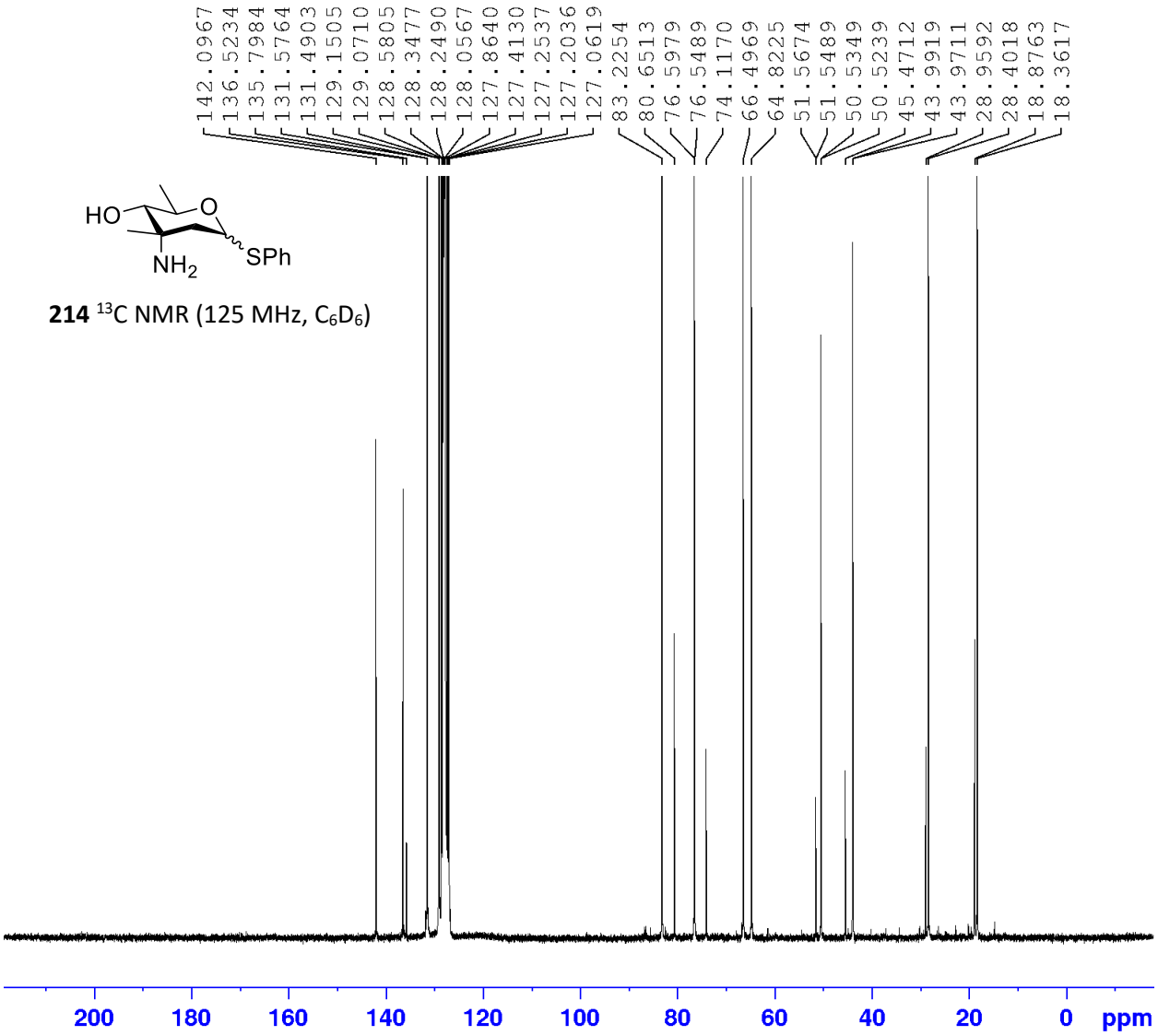
209 ^{13}C NMR (125 MHz, $CDCl_3$)


SEt
BnO
O
HO
209 ^{1}H-^{1}H NMR COSY ($CDCl_3$)
ppm
0
1
2
3
4
5
6
7
8
9
10
11
12
12 11 10 9 8 7 6 5 4 3 2 1 0 ppm

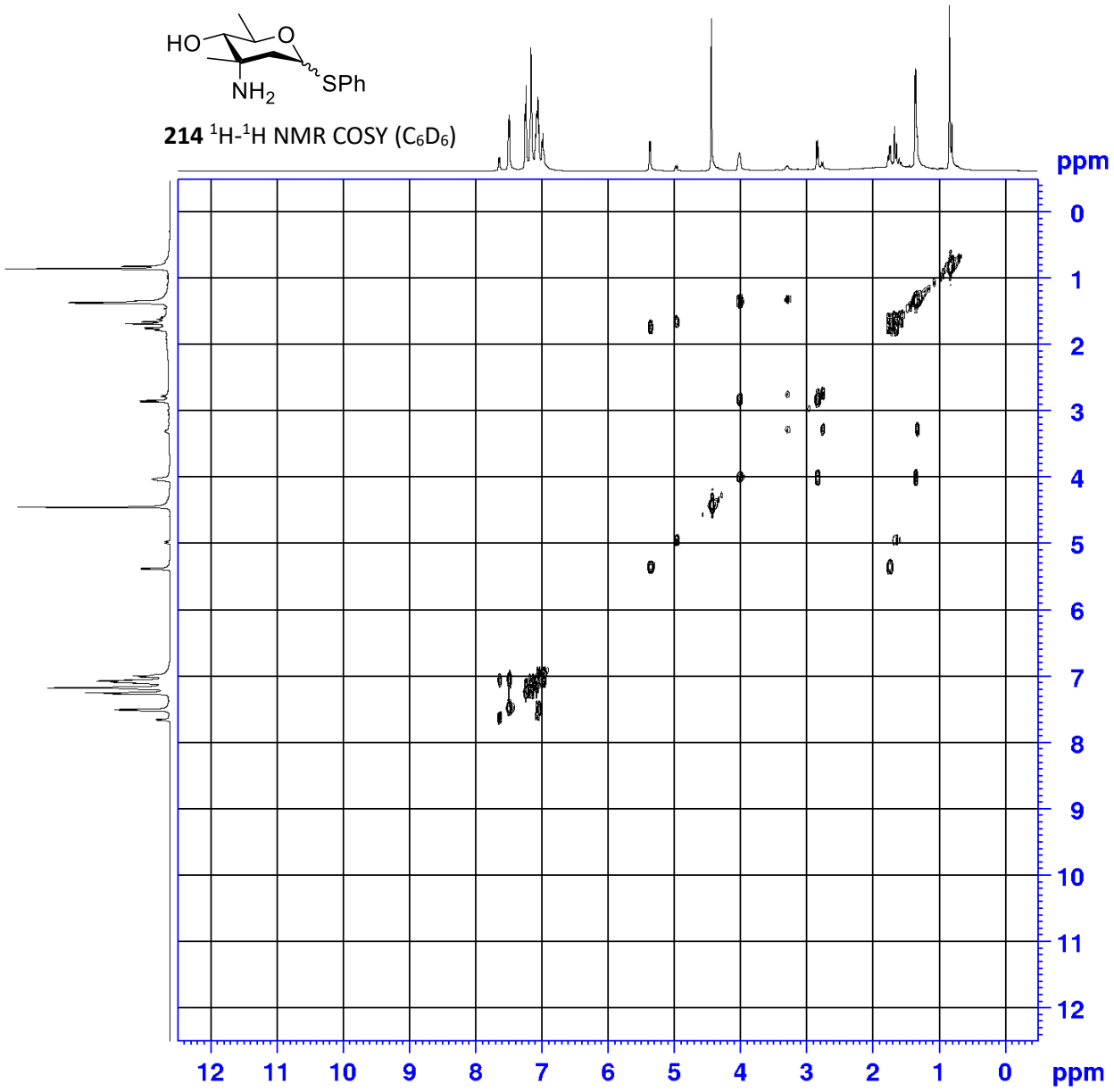

SEt
BnO
O
HO
209 ^{1}H-^{13}C NMR HSQC ($CDCl_3$)
ppm
0
20
40
60
80
100
120
140
12
11
10
9
8
7
6
5
4
3
2
1
0
ppm



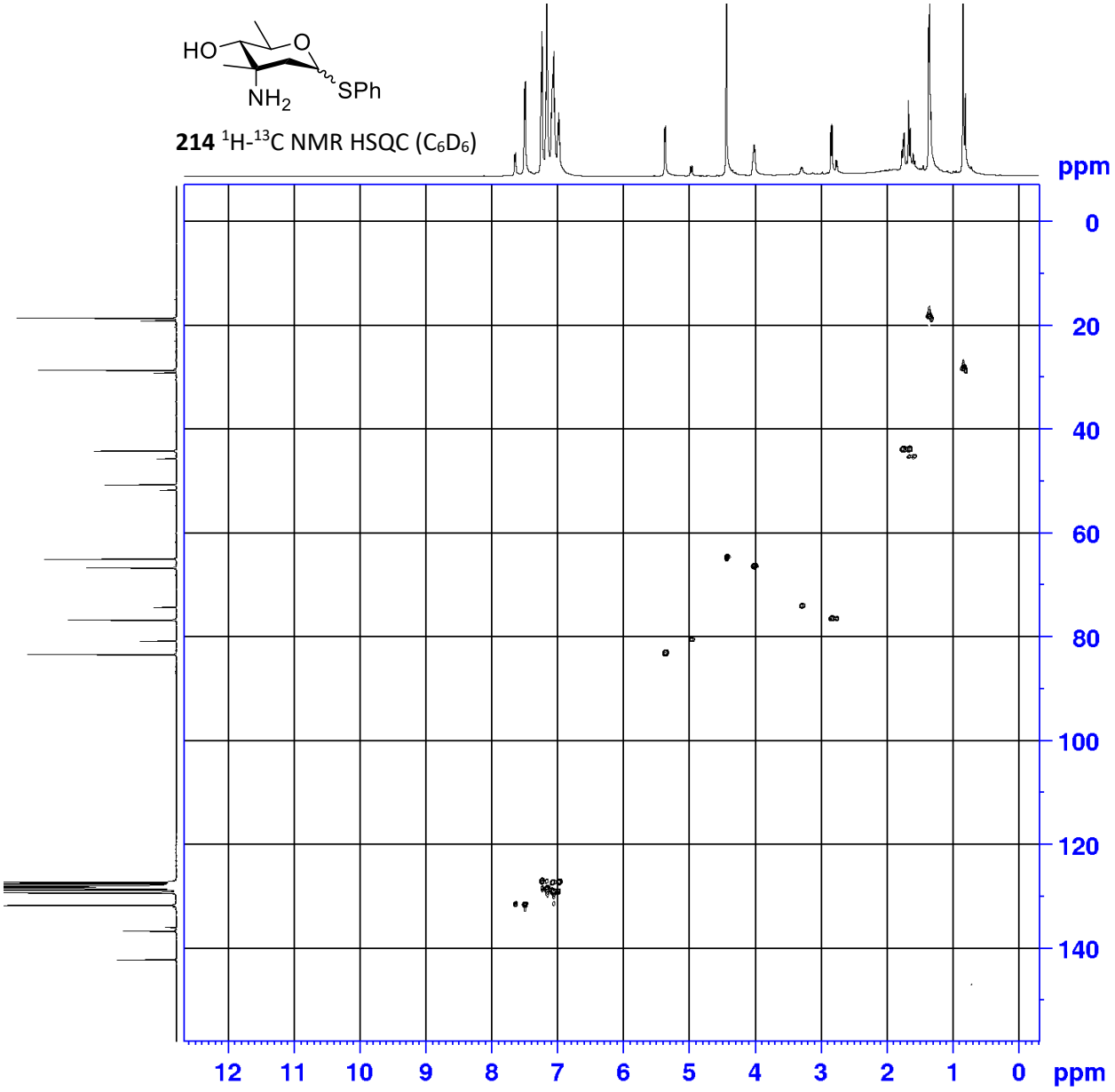
214 ^{1}H NMR (500 MHz, C_6D_6)



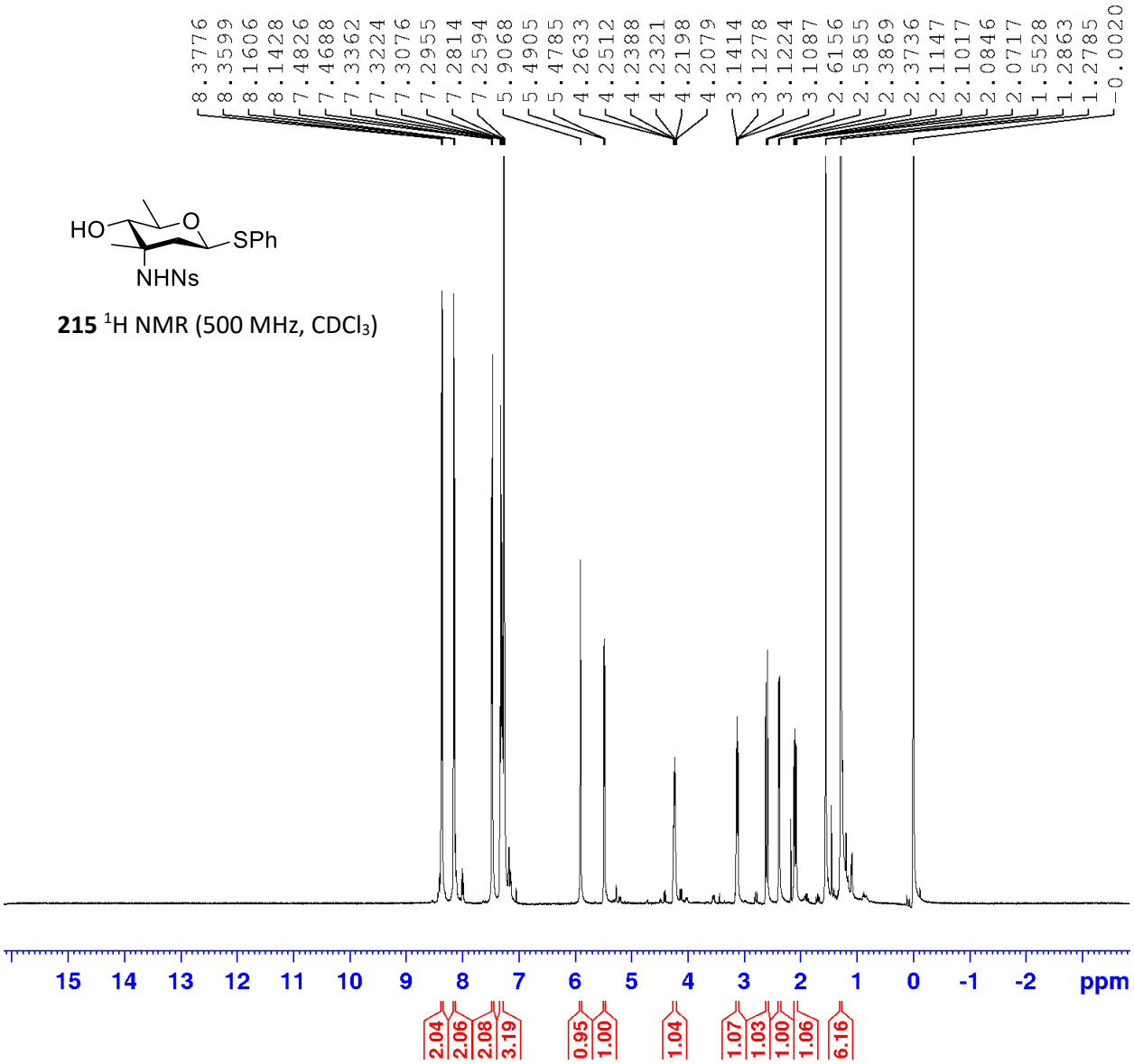
214 ^{13}C NMR (125 MHz, C_6D_6)



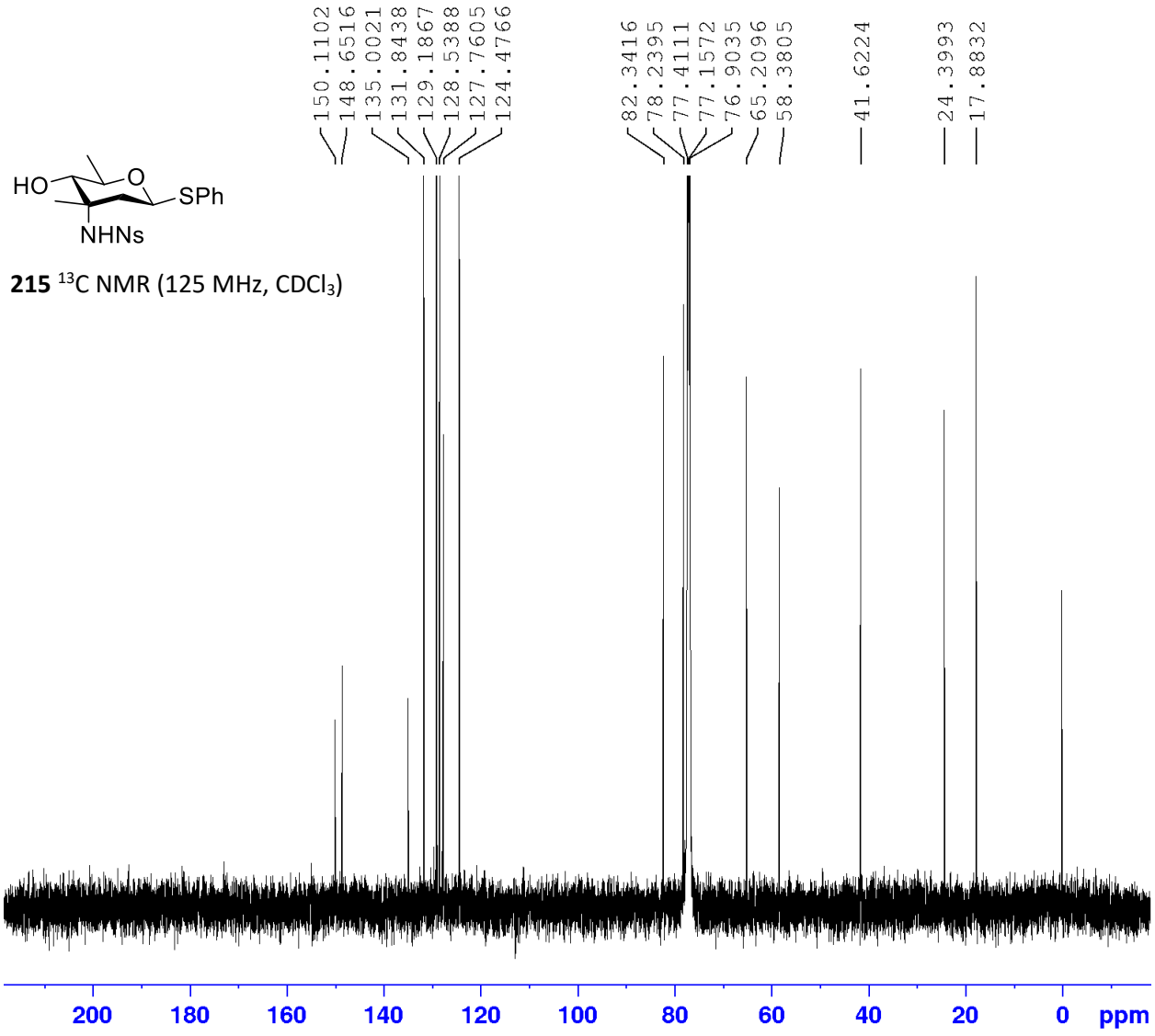
214 ^{1}H-^{1}H NMR COSY (C_6D_6)



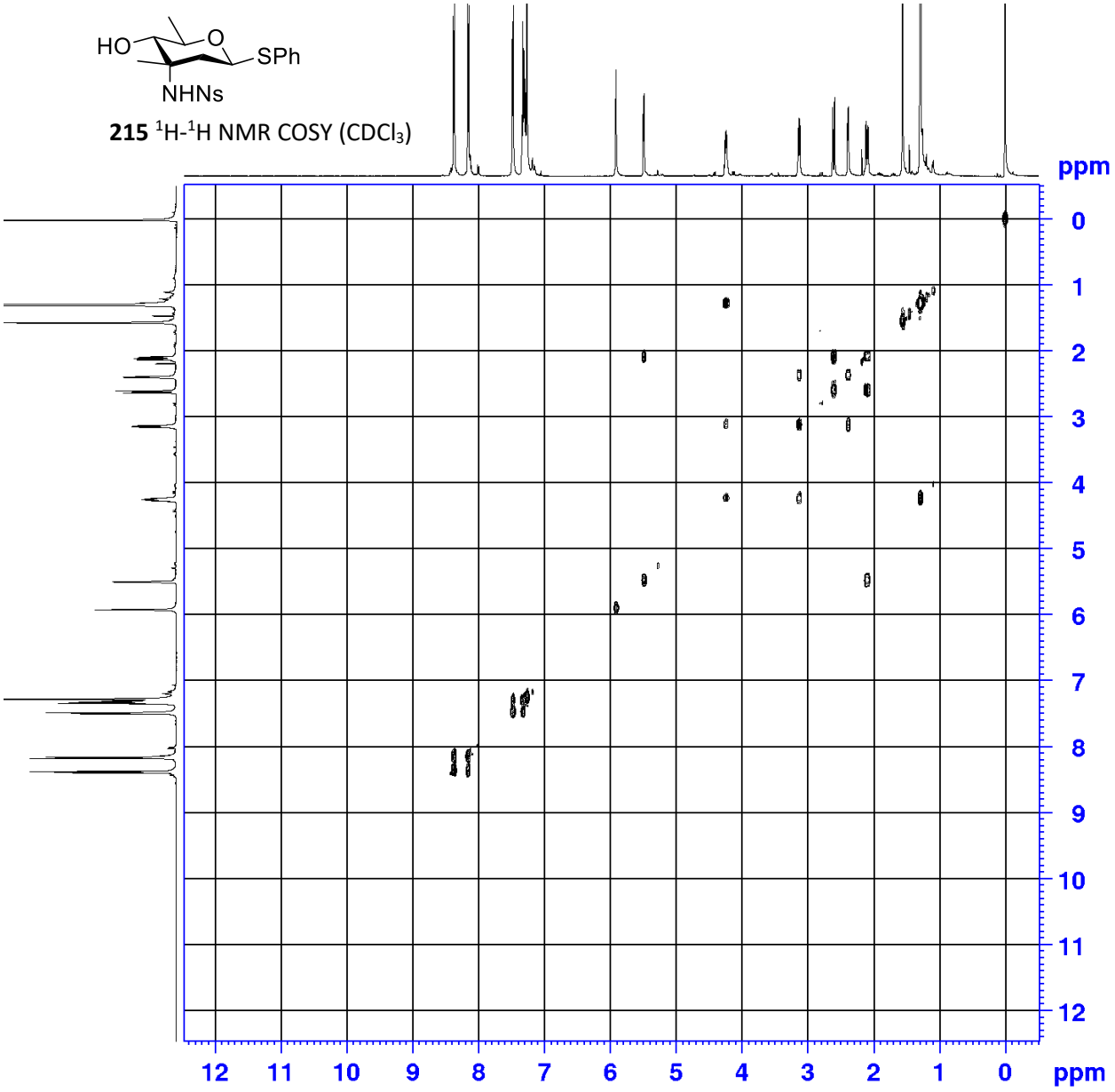
214 ^{1}H-^{13}C NMR HSQC (C_6D_6)



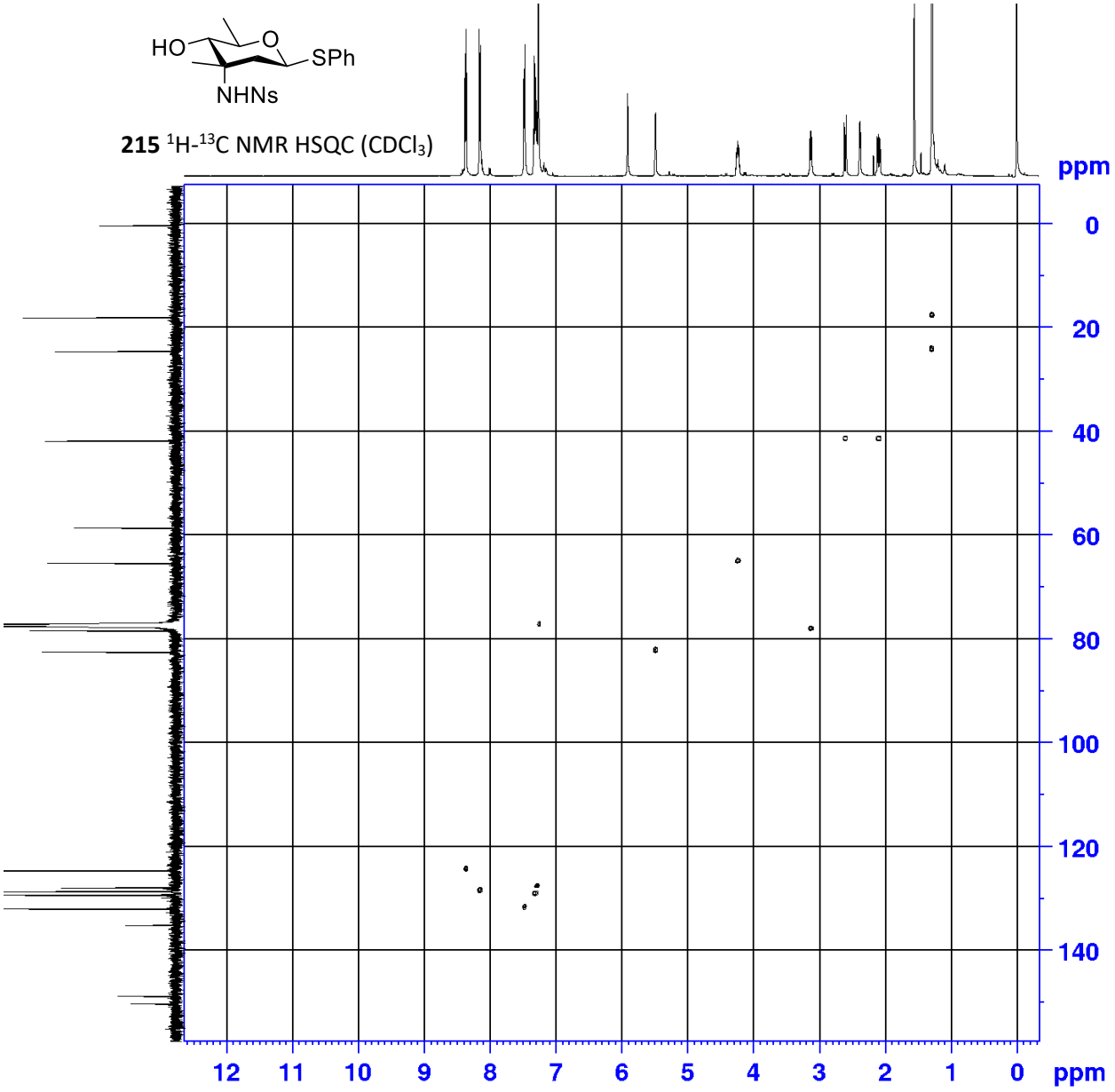
215 ^{1}H NMR (500 MHz, $CDCl_3$)



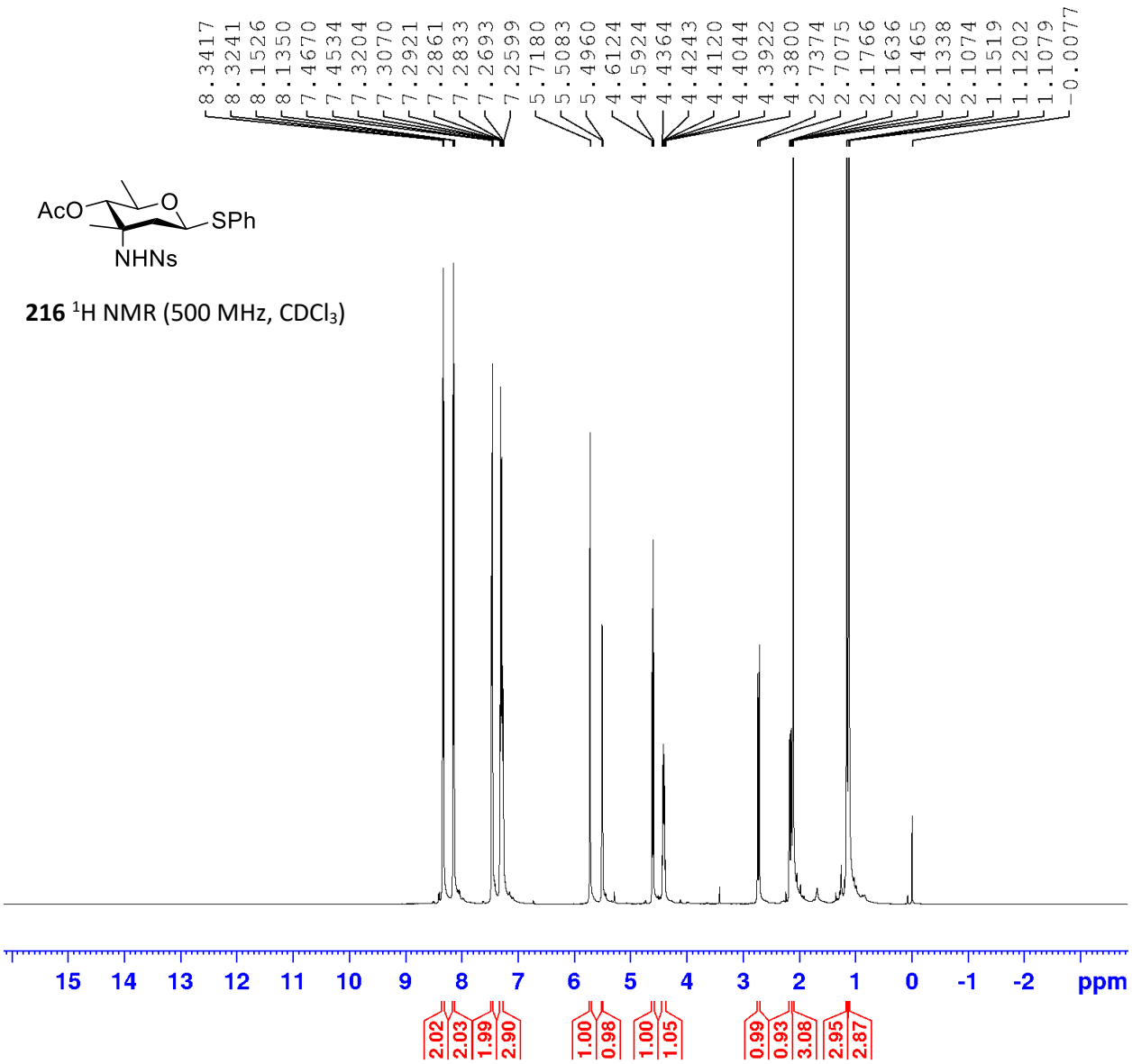
215 ^{13}C NMR (125 MHz, $CDCl_3$)



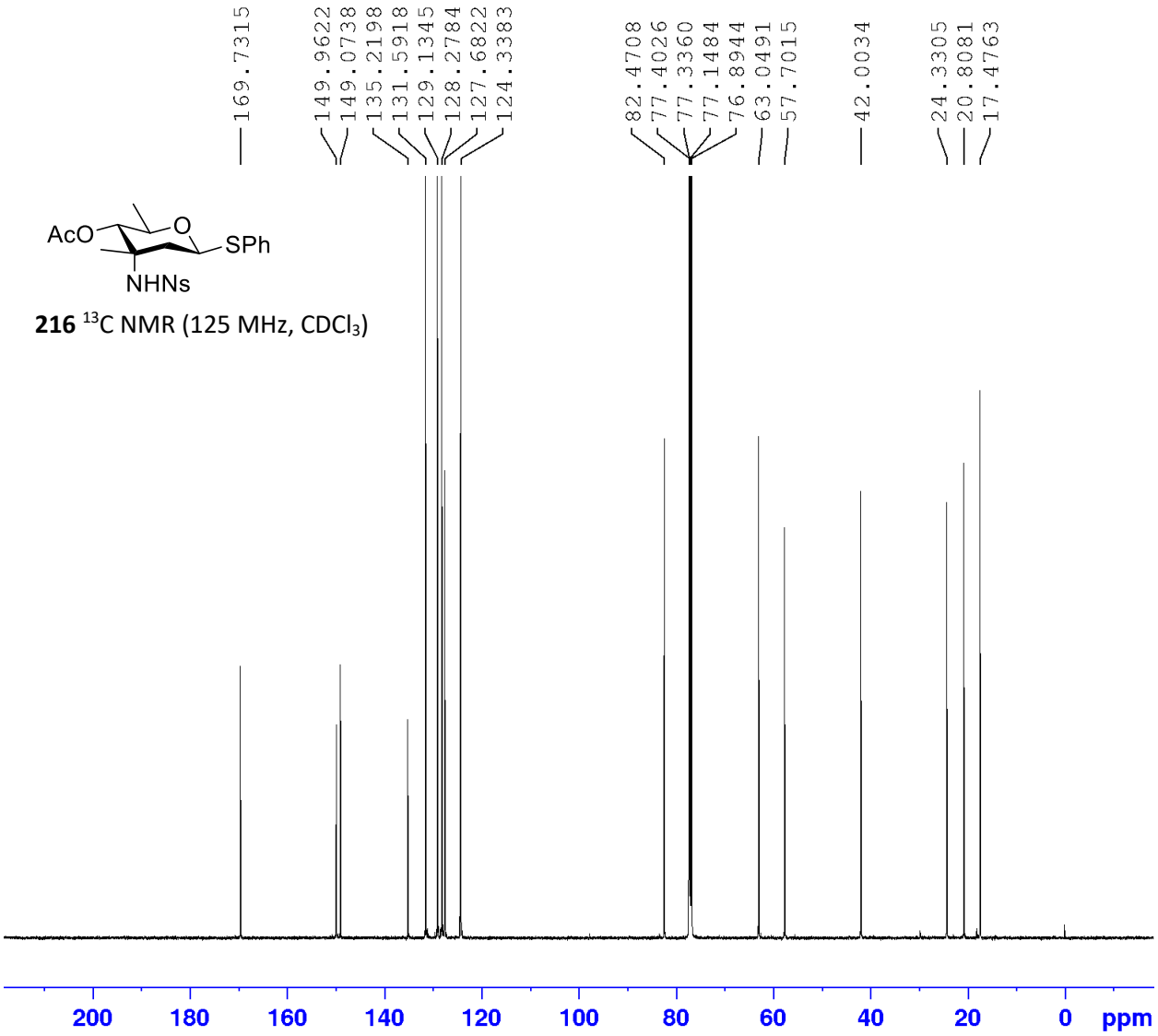
215 ^{1}H-^{1}H NMR COSY ($CDCl_3$)



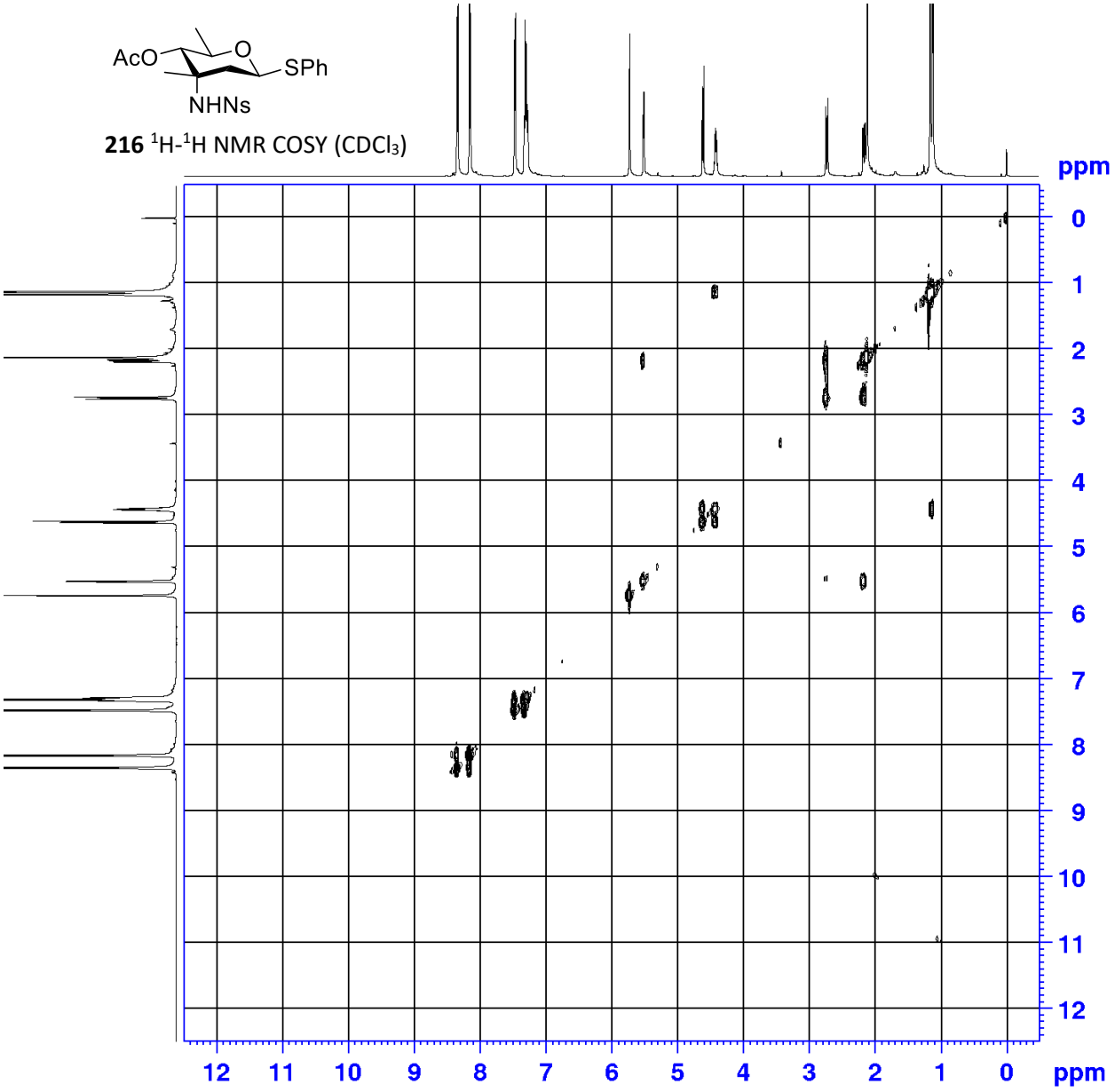
215 ^{1}H-^{13}C NMR HSQC ($CDCl_3$)



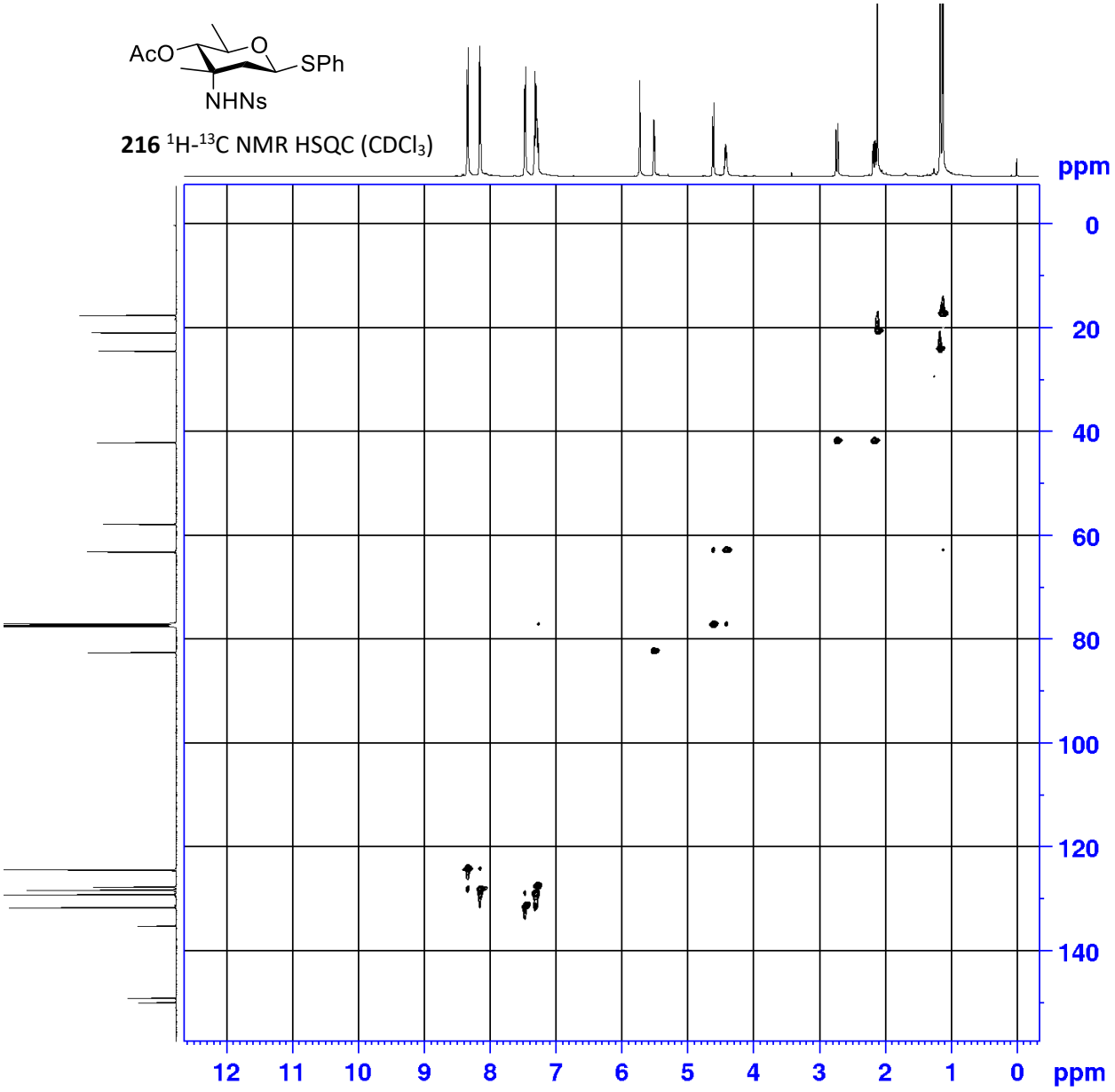
216 ^{1}H NMR (500 MHz, $CDCl_3$)



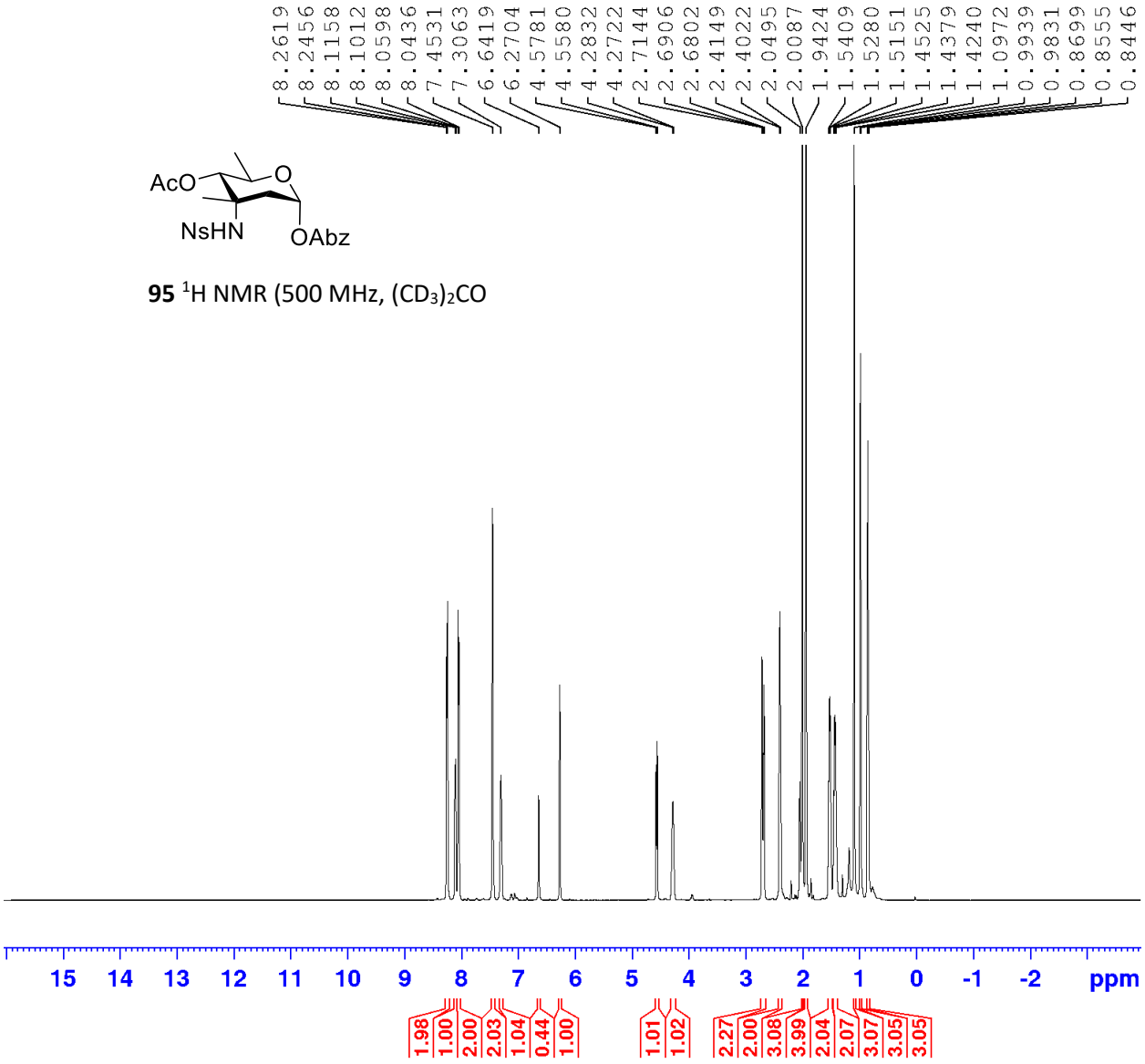
216 ^{13}C NMR (125 MHz, $CDCl_3$)



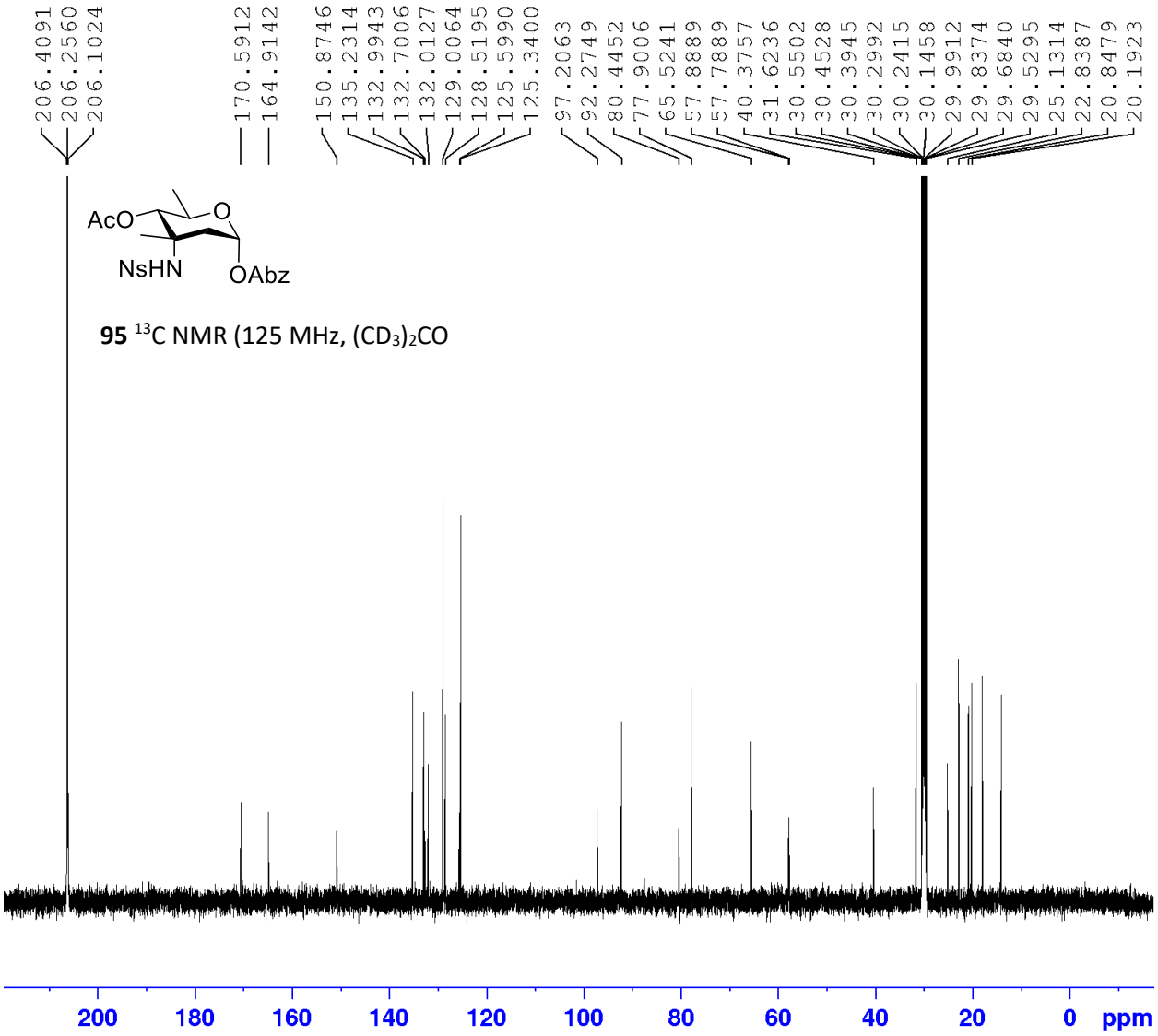
216 ^{1}H-^{1}H NMR COSY ($CDCl_3$)



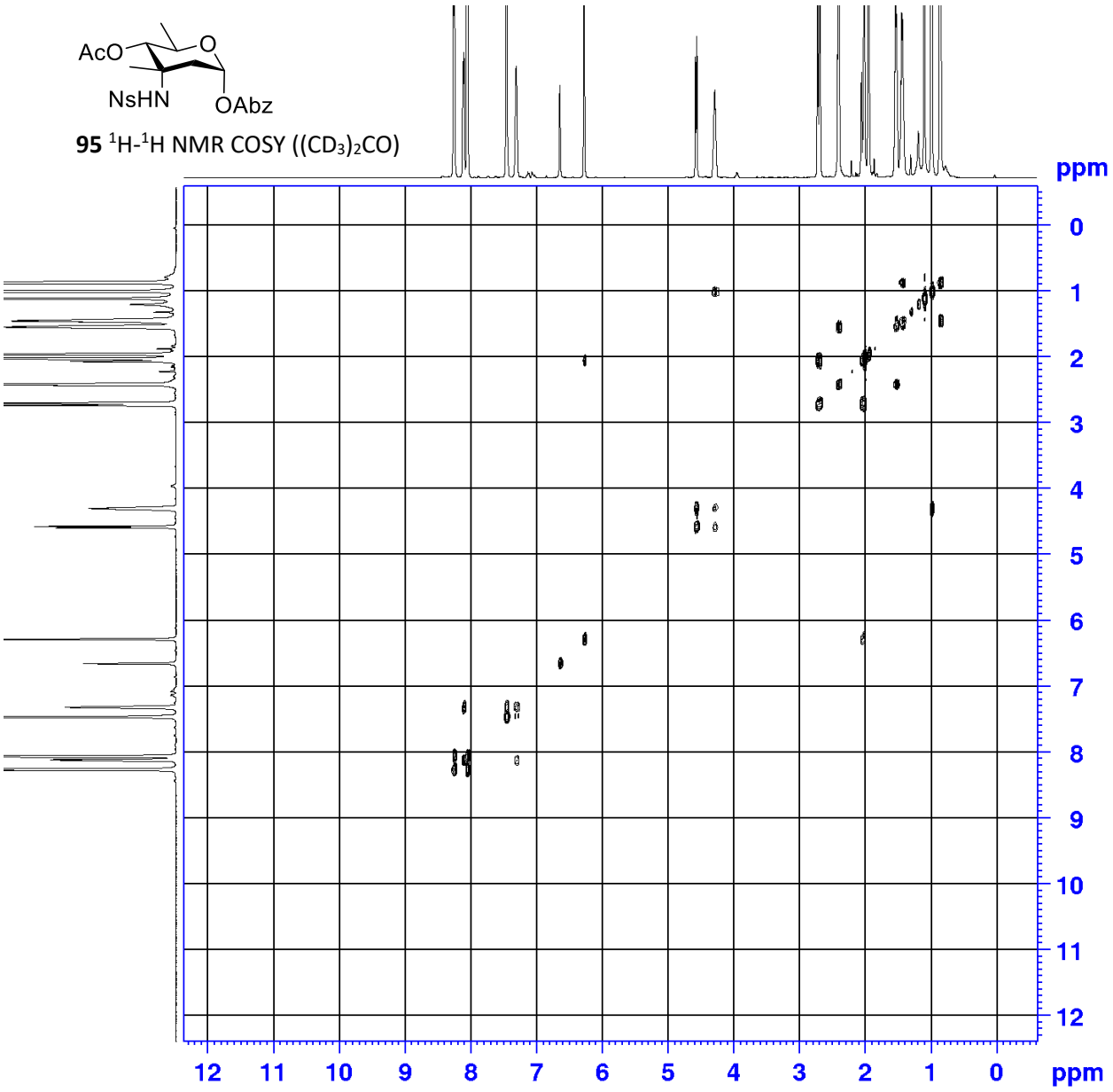
216 ^{1}H-^{13}C NMR HSQC ($CDCl_3$)



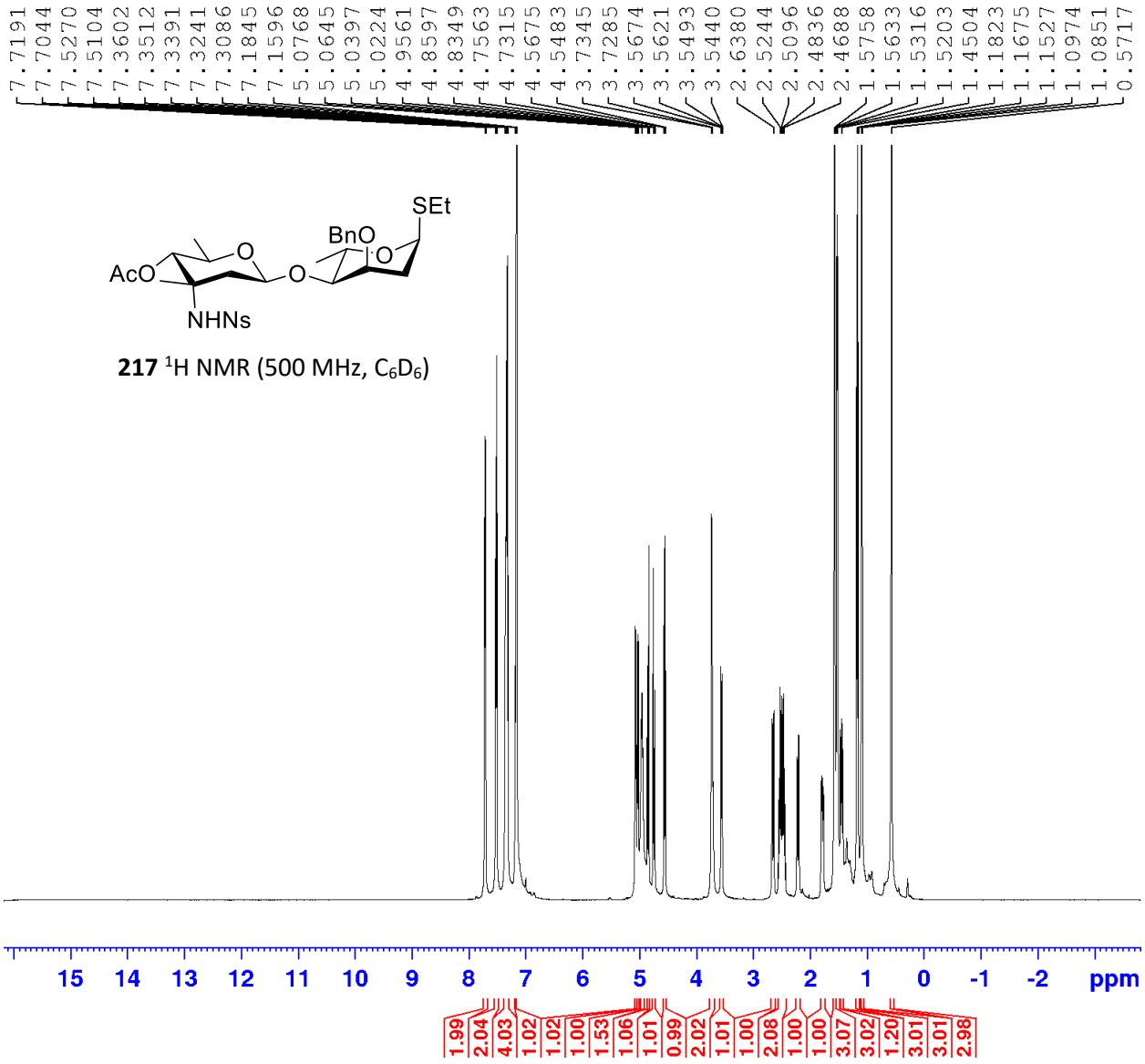
95 ^{1}H NMR (500 MHz, $(CD_3)_2CO$



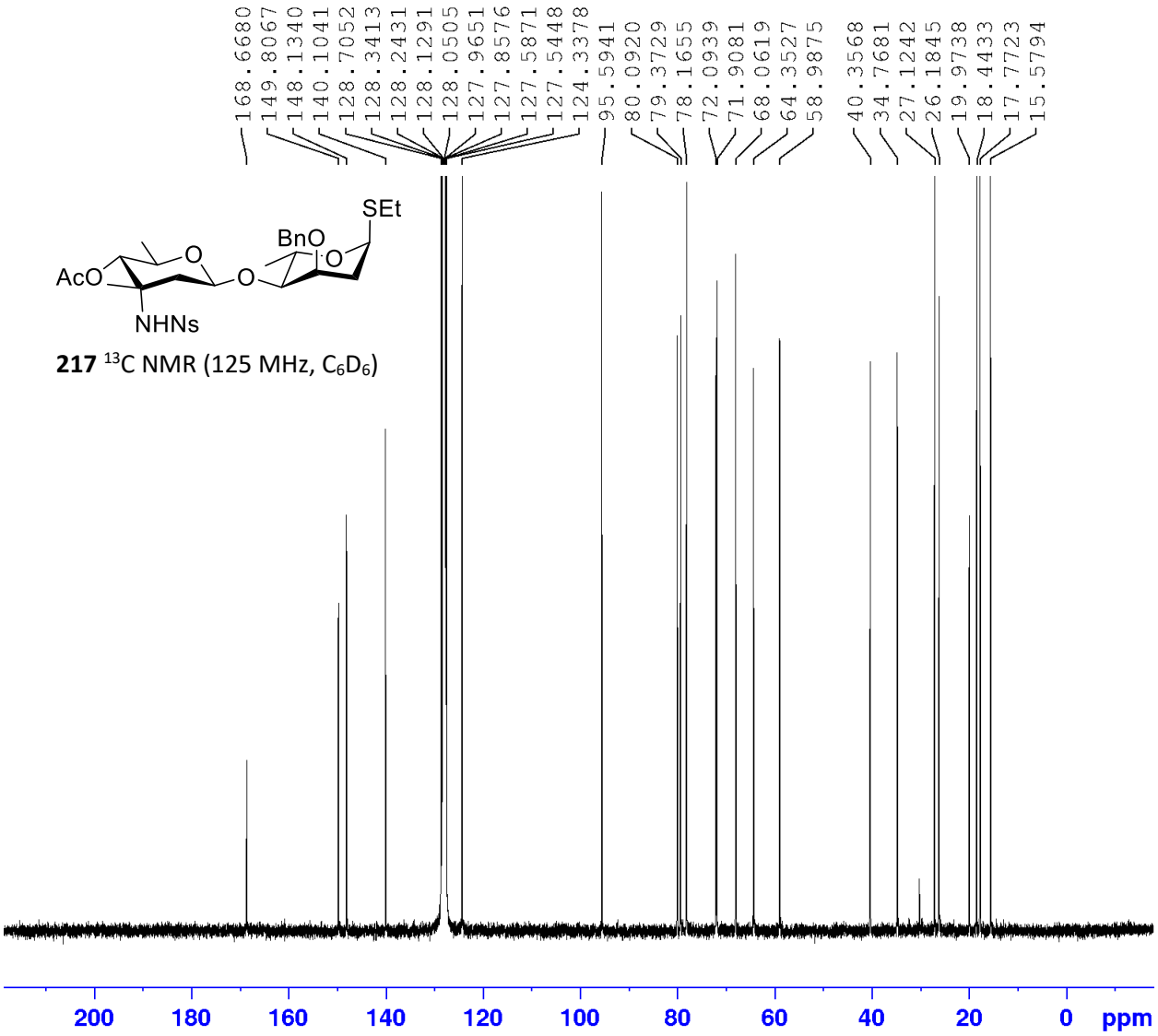
95 ^{13}C NMR (125 MHz, $(CD_3)_2CO$



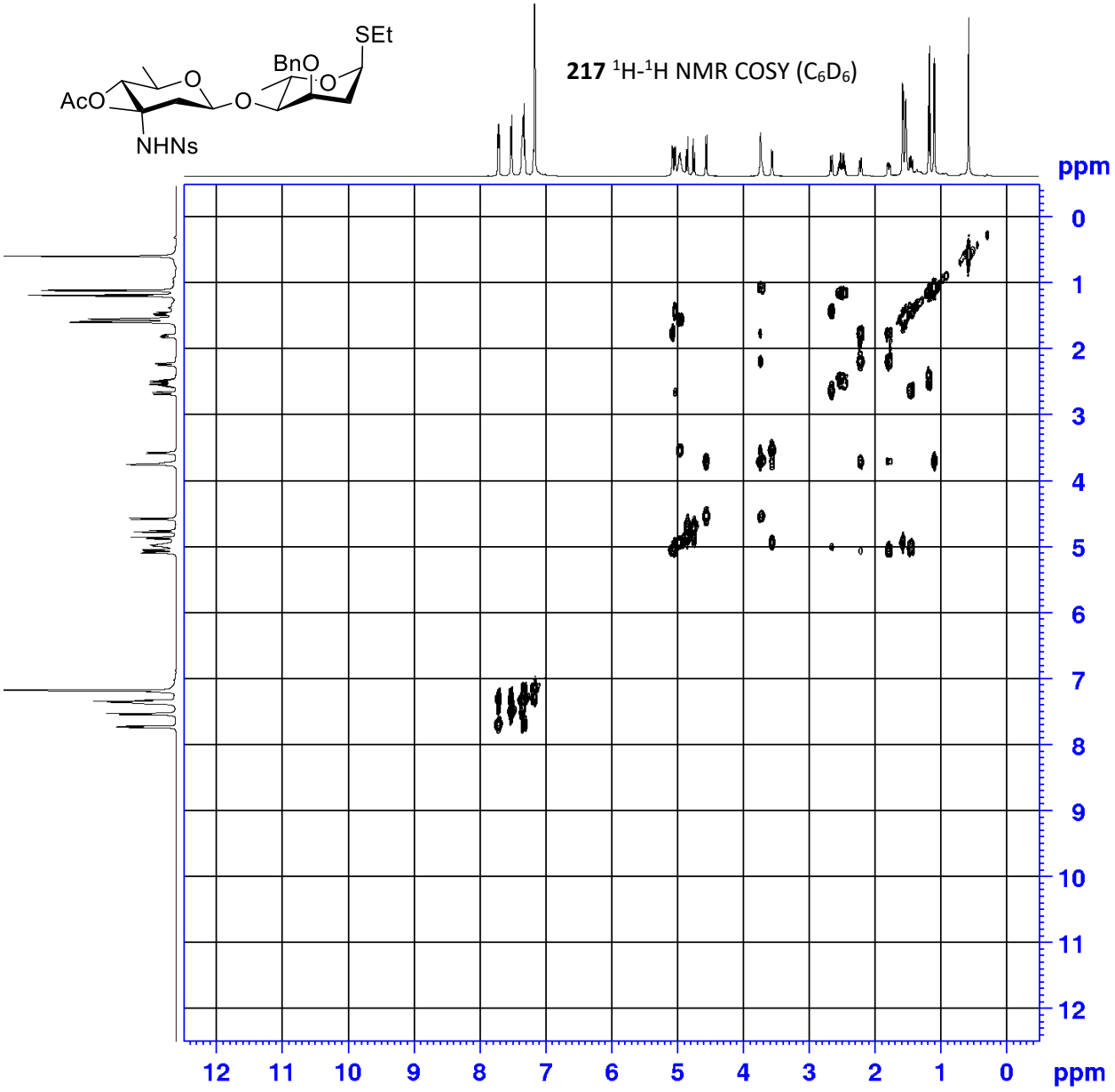
95 ^{1}H-^{1}H NMR COSY ($(CD_3)_2CO$)

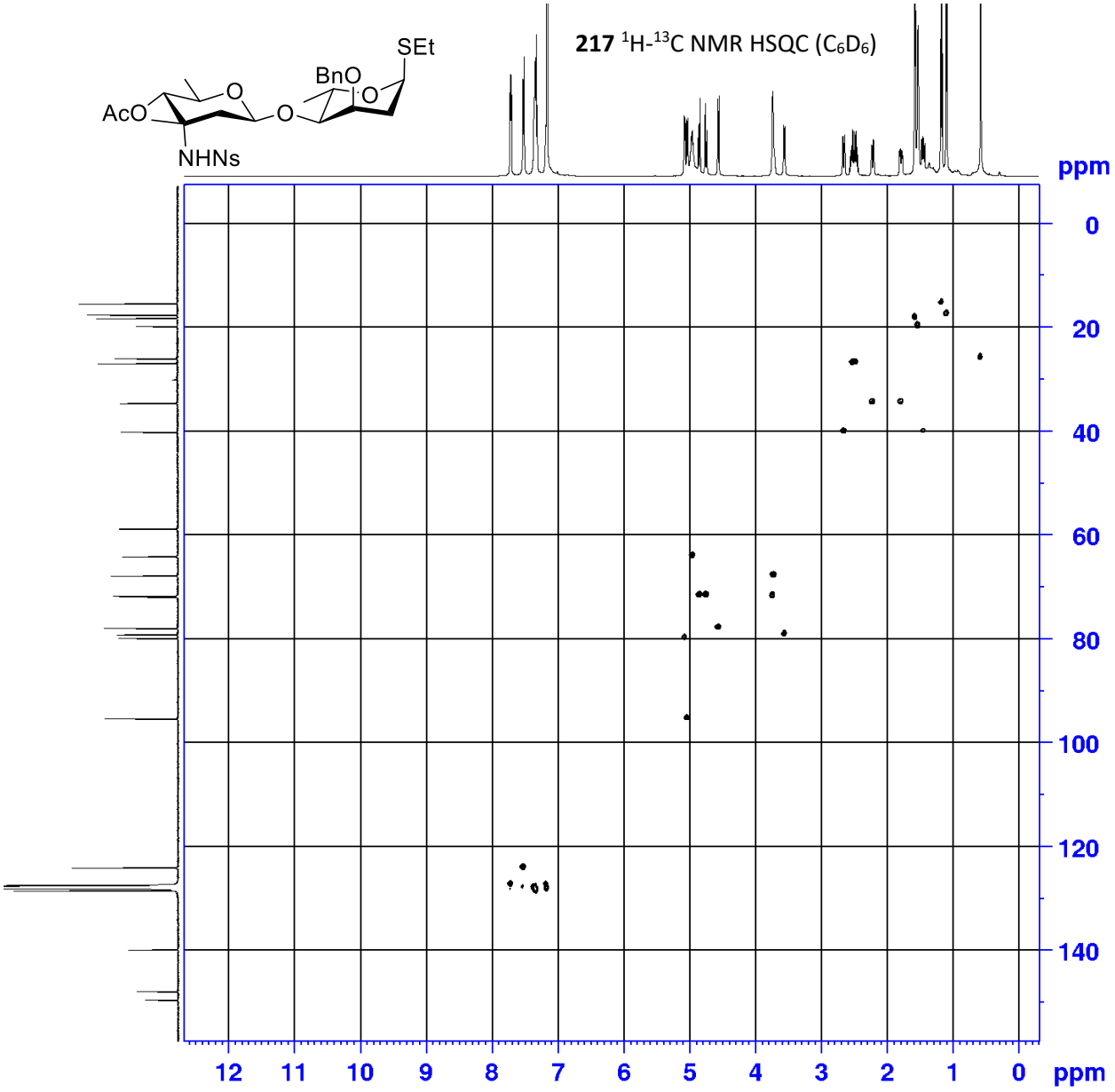


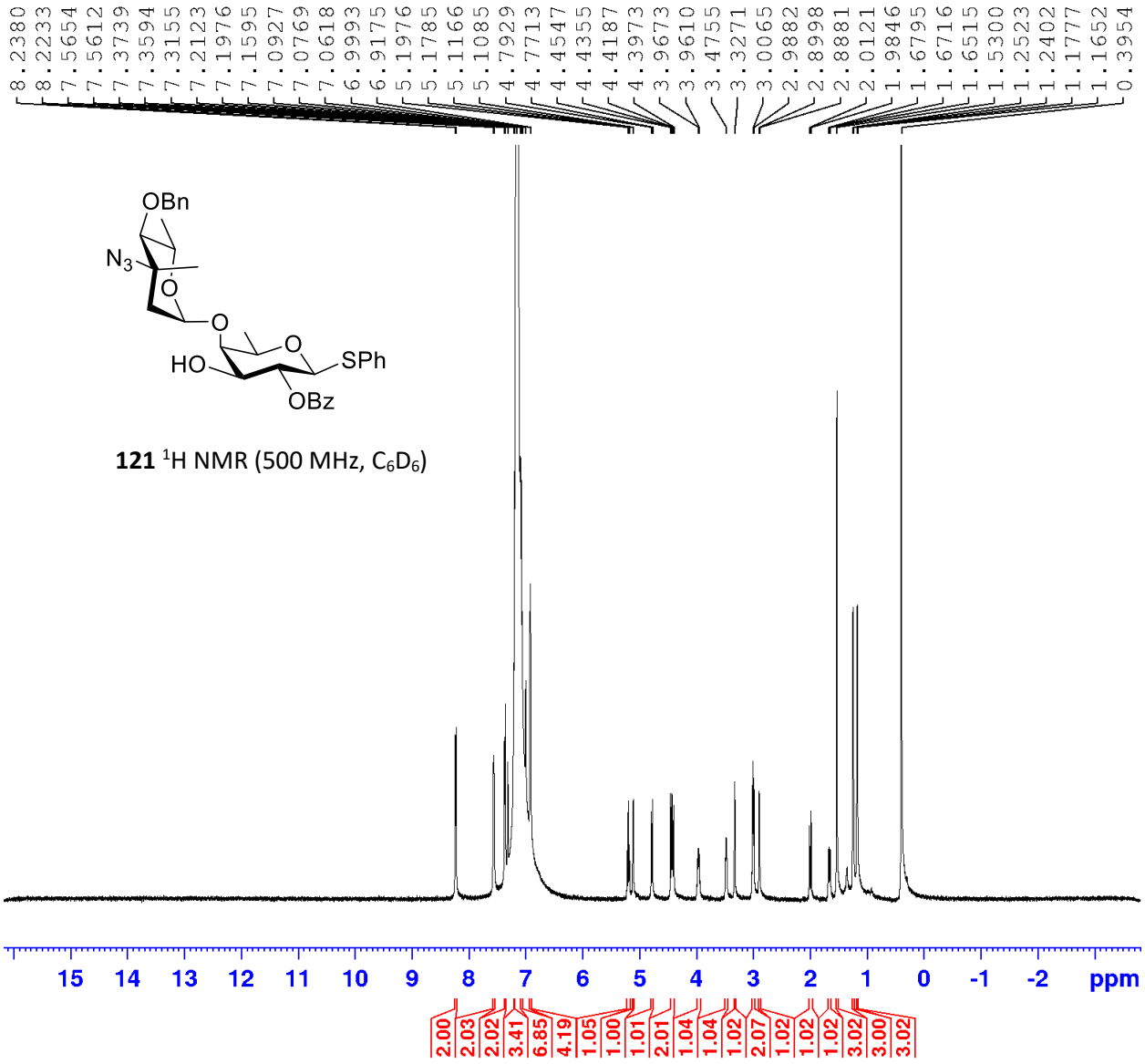
217 ^{1}H NMR (500 MHz, C_6D_6)



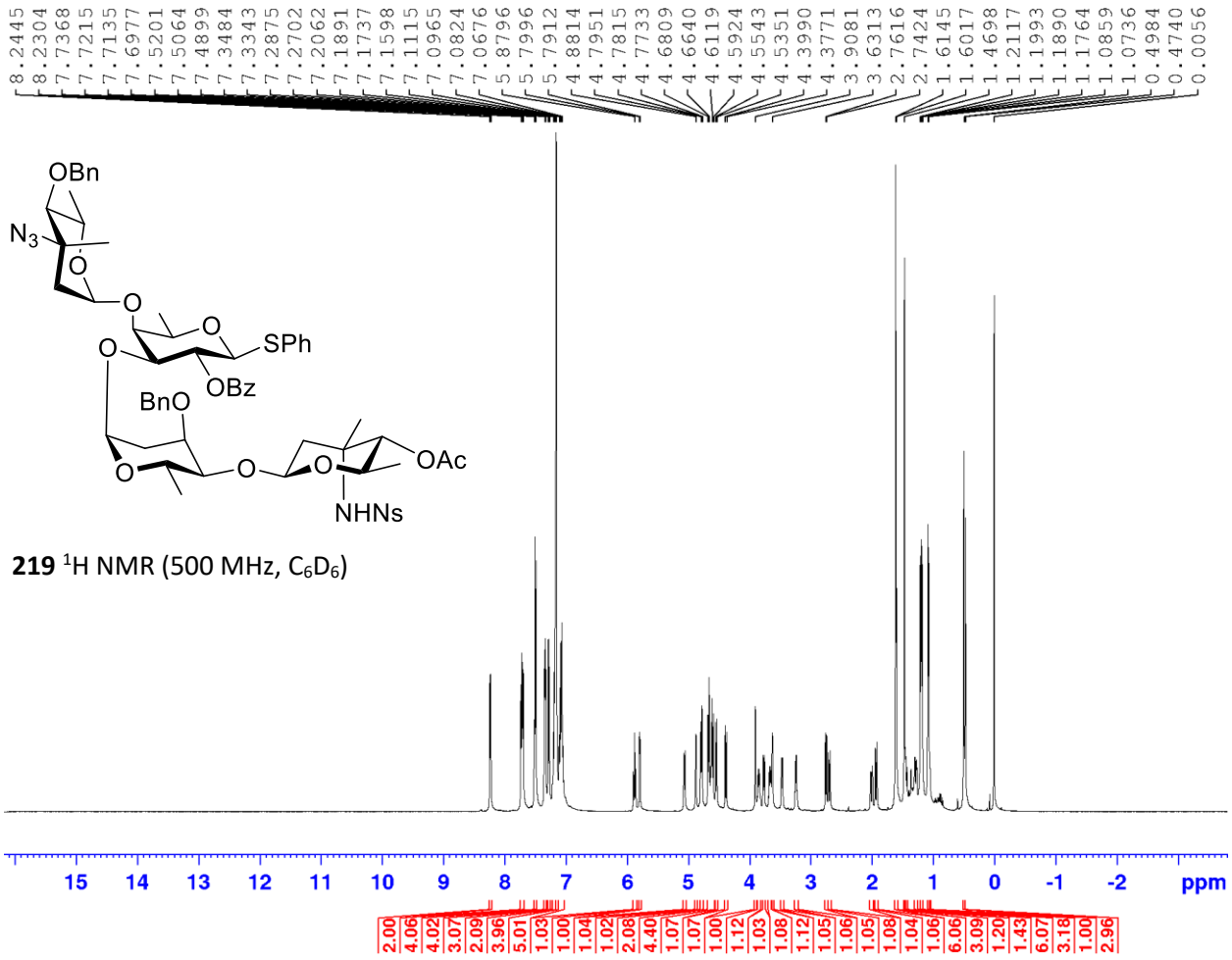
217 ^{13}C NMR (125 MHz, C_6D_6)


SEt
BnO
O
AcO
O
O
NHNs
217 ^{1}H-^{1}H NMR COSY (C_6D_6)
ppm
0
1
2
3
4
5
6
7
8
9
10
11
12
12
11
10
9
8
7
6
5
4
3
2
1
0
ppm

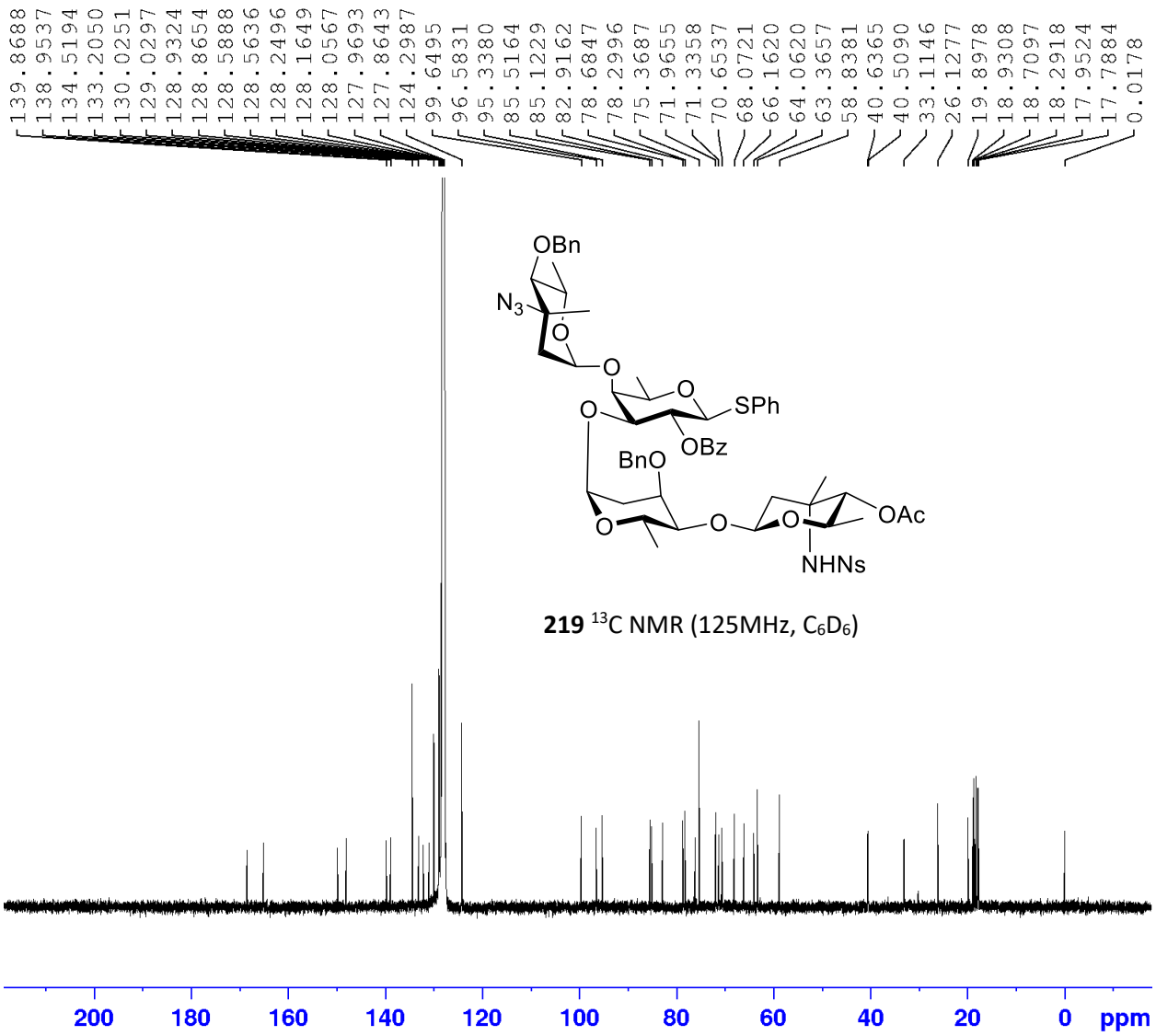

SEt
BnO
AcO
O
O
O
NHNs
217 ^{1}H-^{13}C NMR HSQC (C_6D_6)
ppm
0
20
40
60
80
100
120
140
12
11
10
9
8
7
6
5
4
3
2
1
0
ppm



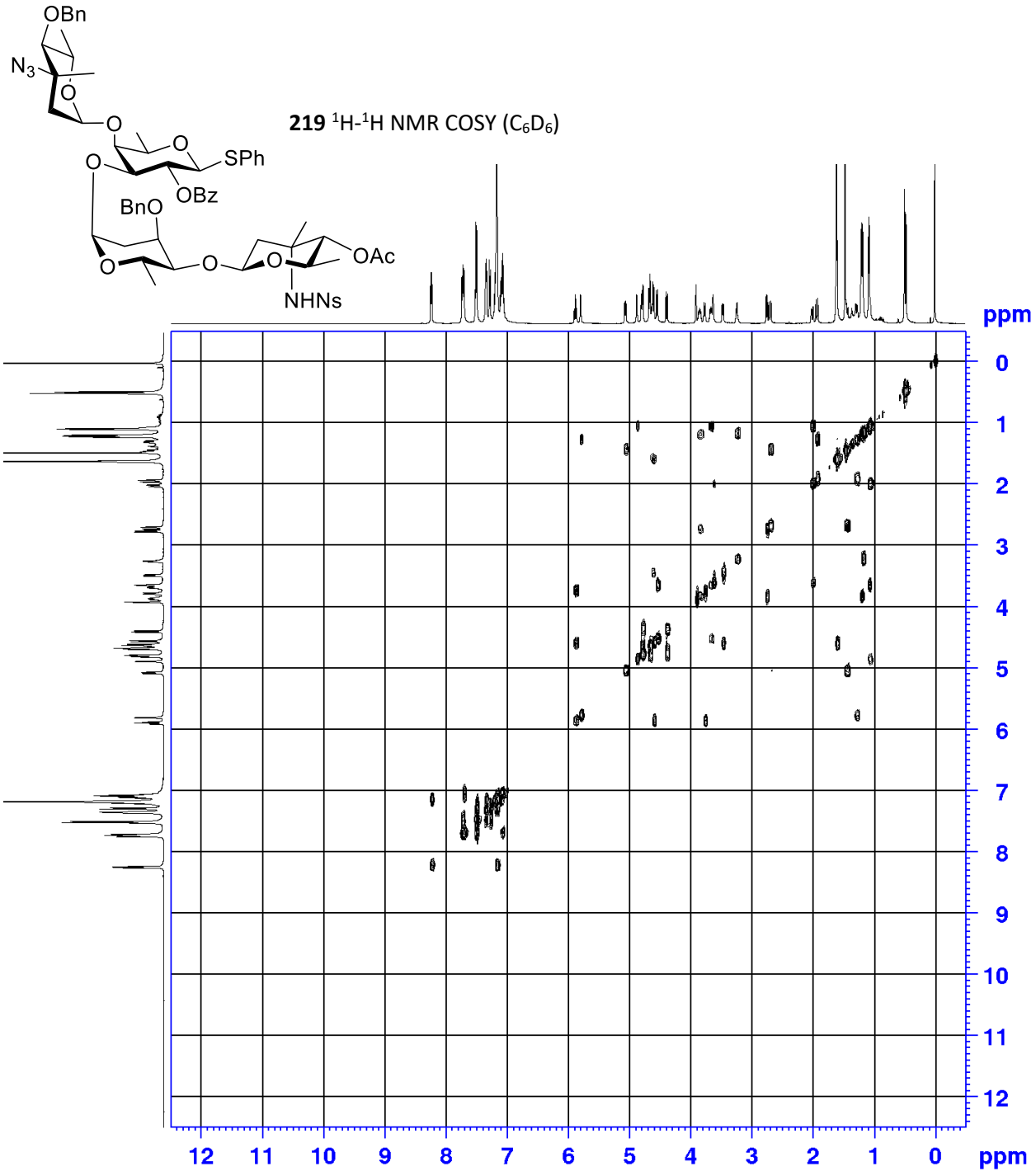
121 ^{1}H NMR (500 MHz, C_6D_6)



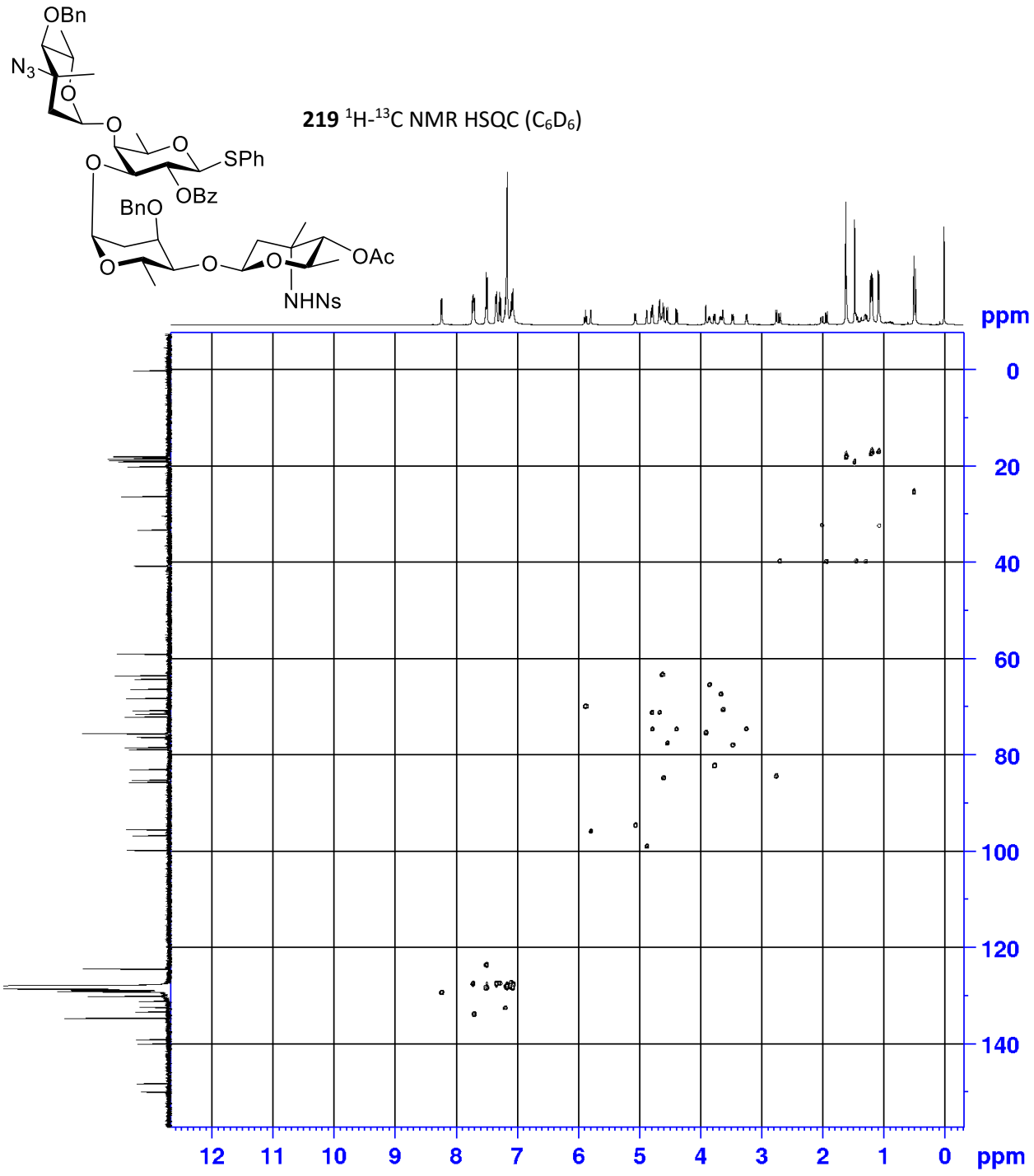
219 ^{1}H NMR (500 MHz, C_6D_6)



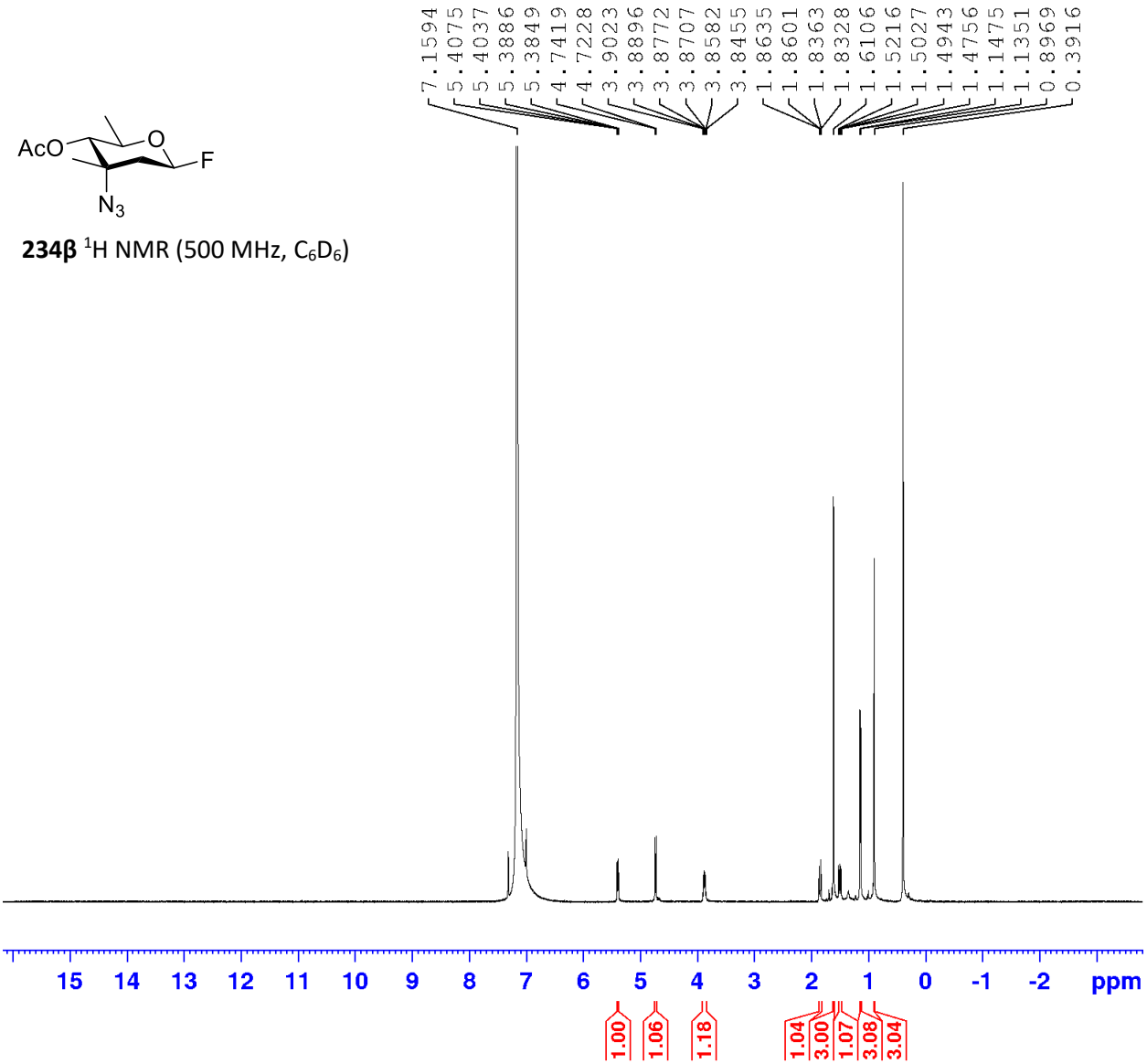
219 ^{13}C NMR (125MHz, C_6D_6)



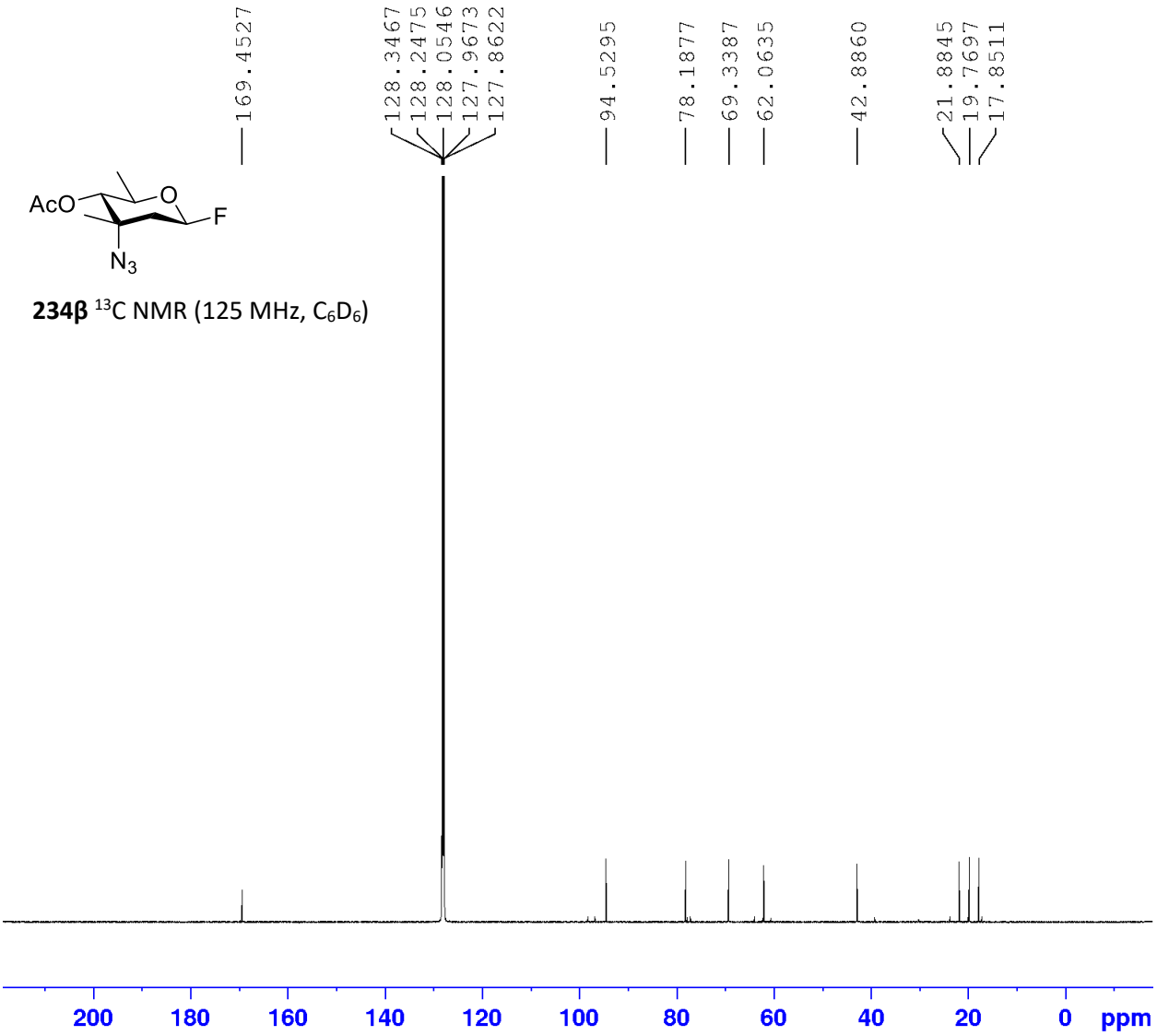
219 ^{1}H-^{1}H NMR COSY (C_6D_6)



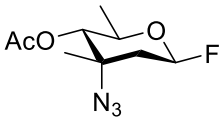
219 ^{1}H-^{13}C NMR HSQC (C_6D_6)



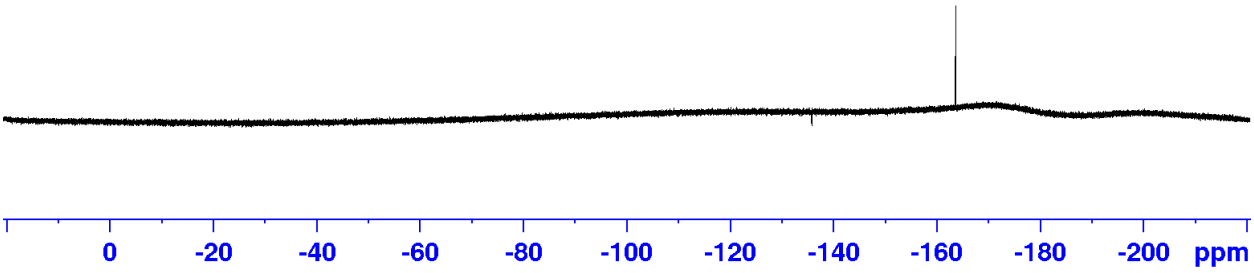
234β ^{1}H NMR (500 MHz, C_6D_6)

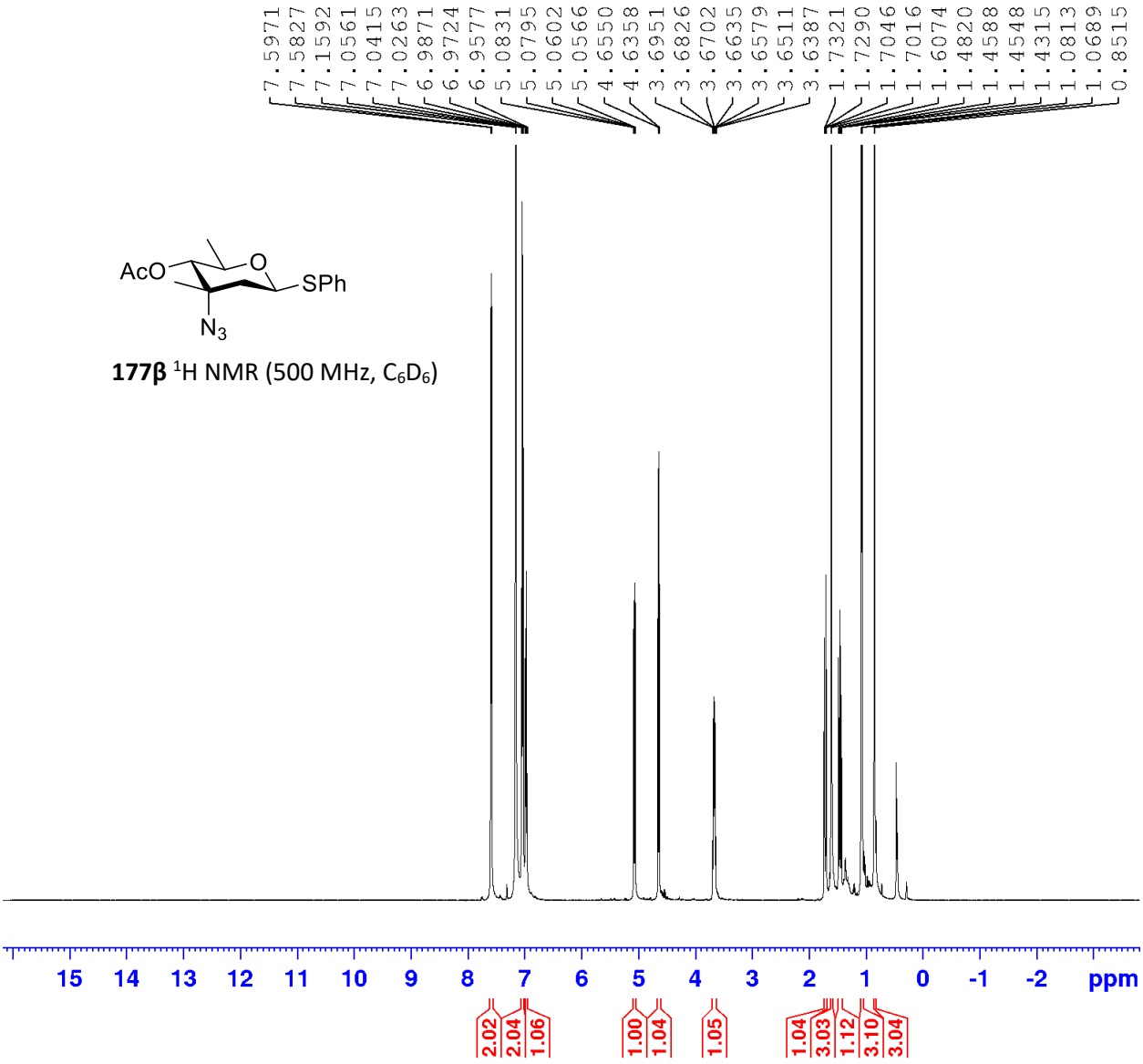


234β ^{13}C NMR (125 MHz, C_6D_6)

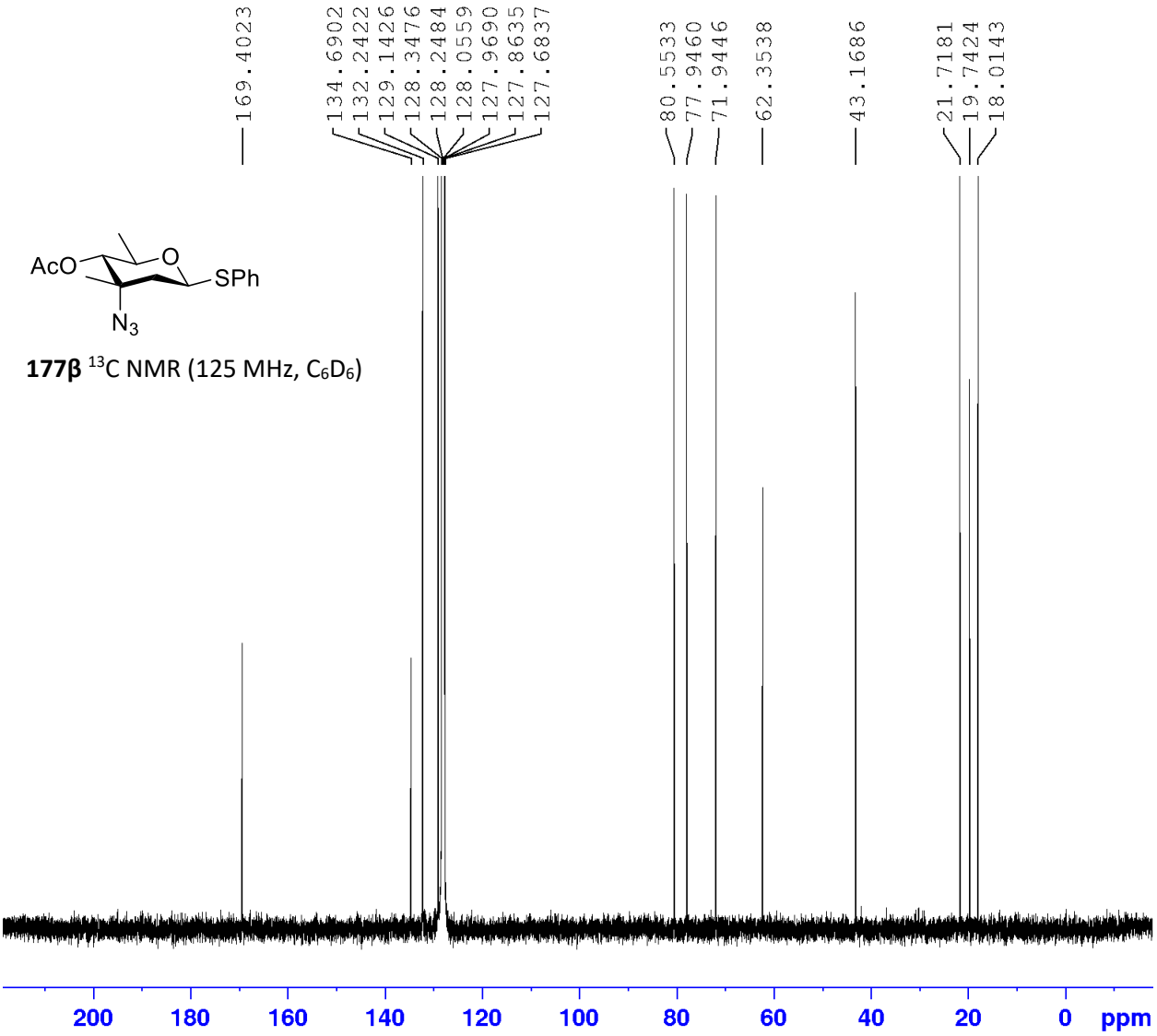


234β ^{19}F decoupled ^{1}H NMR (125 MHz, C_6D_6)

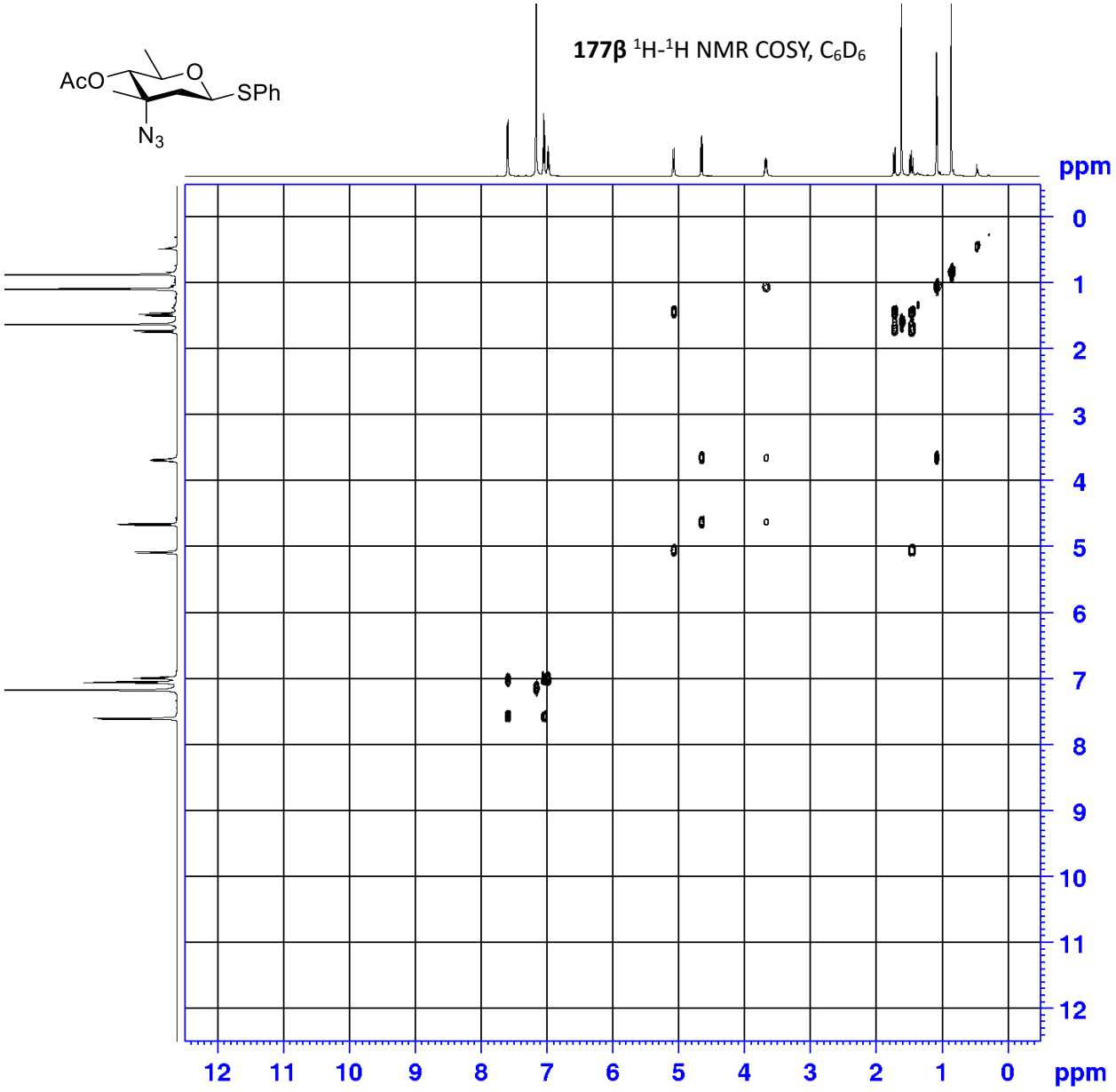




177β ^{1}H NMR (500 MHz, C_6D_6)



177β ^{13}C NMR (125 MHz, C_6D_6)


AcO
O
SPh
N3
177β 1H-1H NMR COSY, C6D6
ppm
0
1
2
3
4
5
6
7
8
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10
11
12
12
11
10
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8
7
6
5
4
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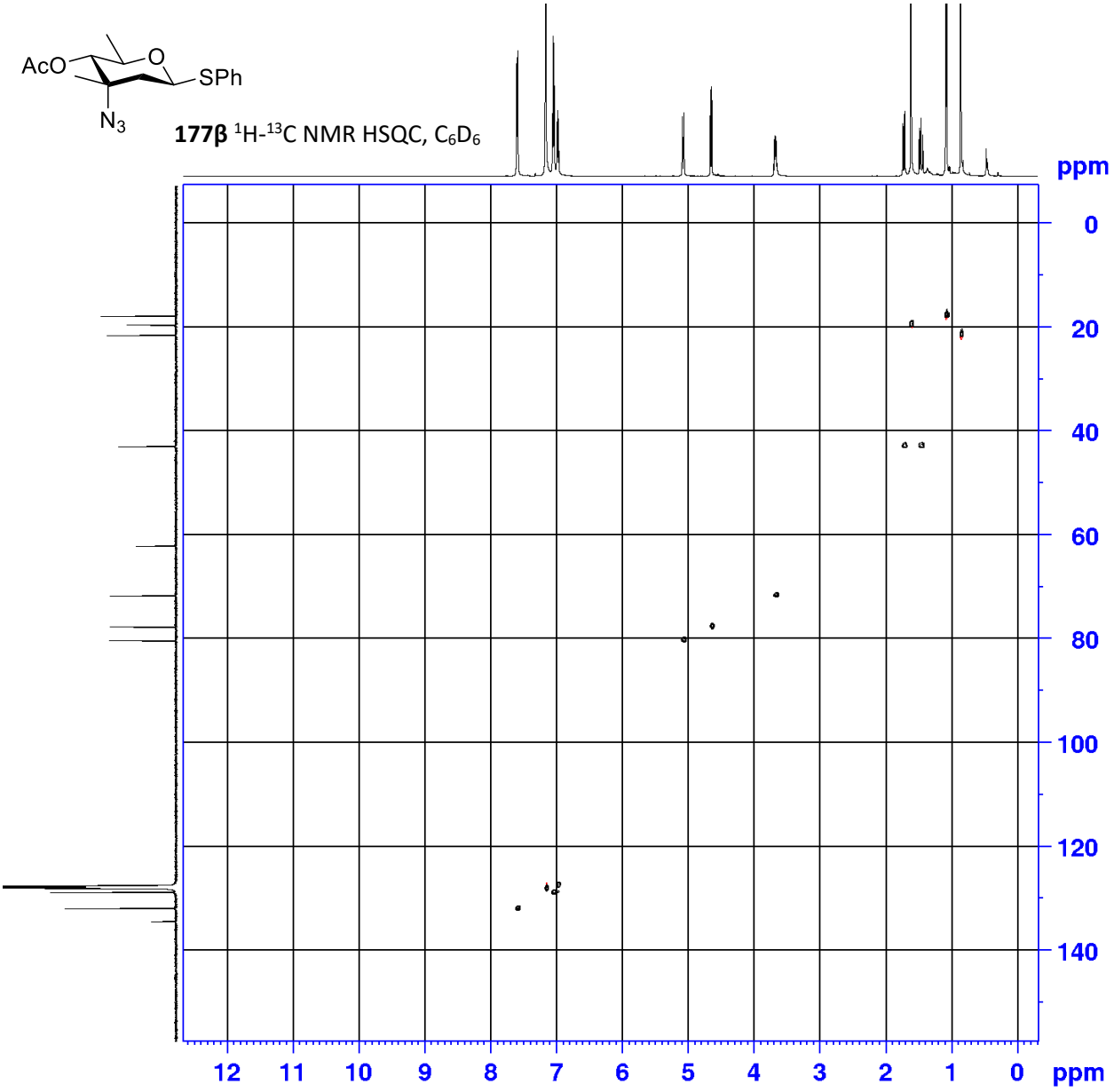
2
1
0
ppm


AcO
O
SPh
N3
177β 1H-13C NMR HSQC, C6D6
ppm
0
20
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60
80
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120
140
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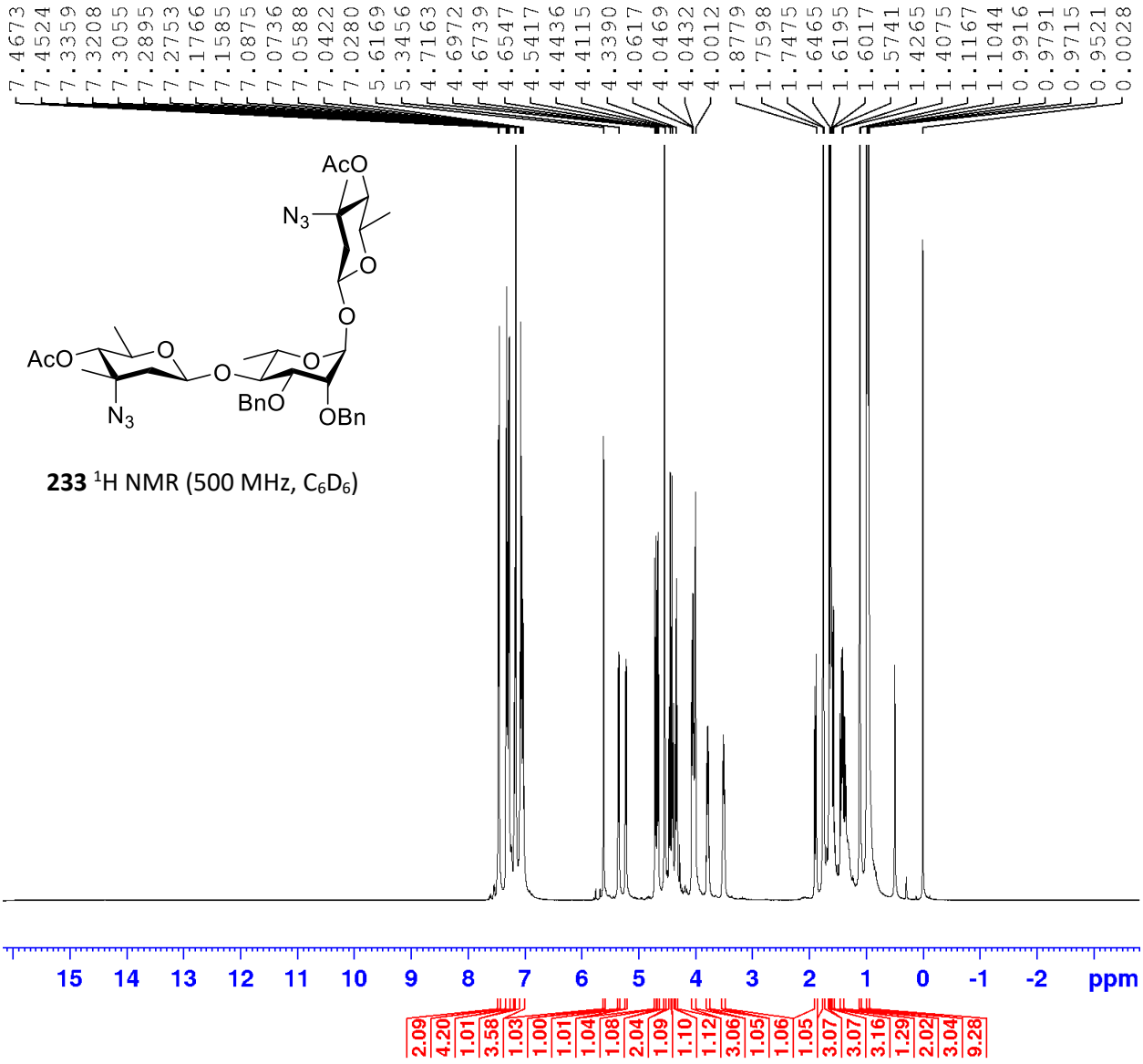
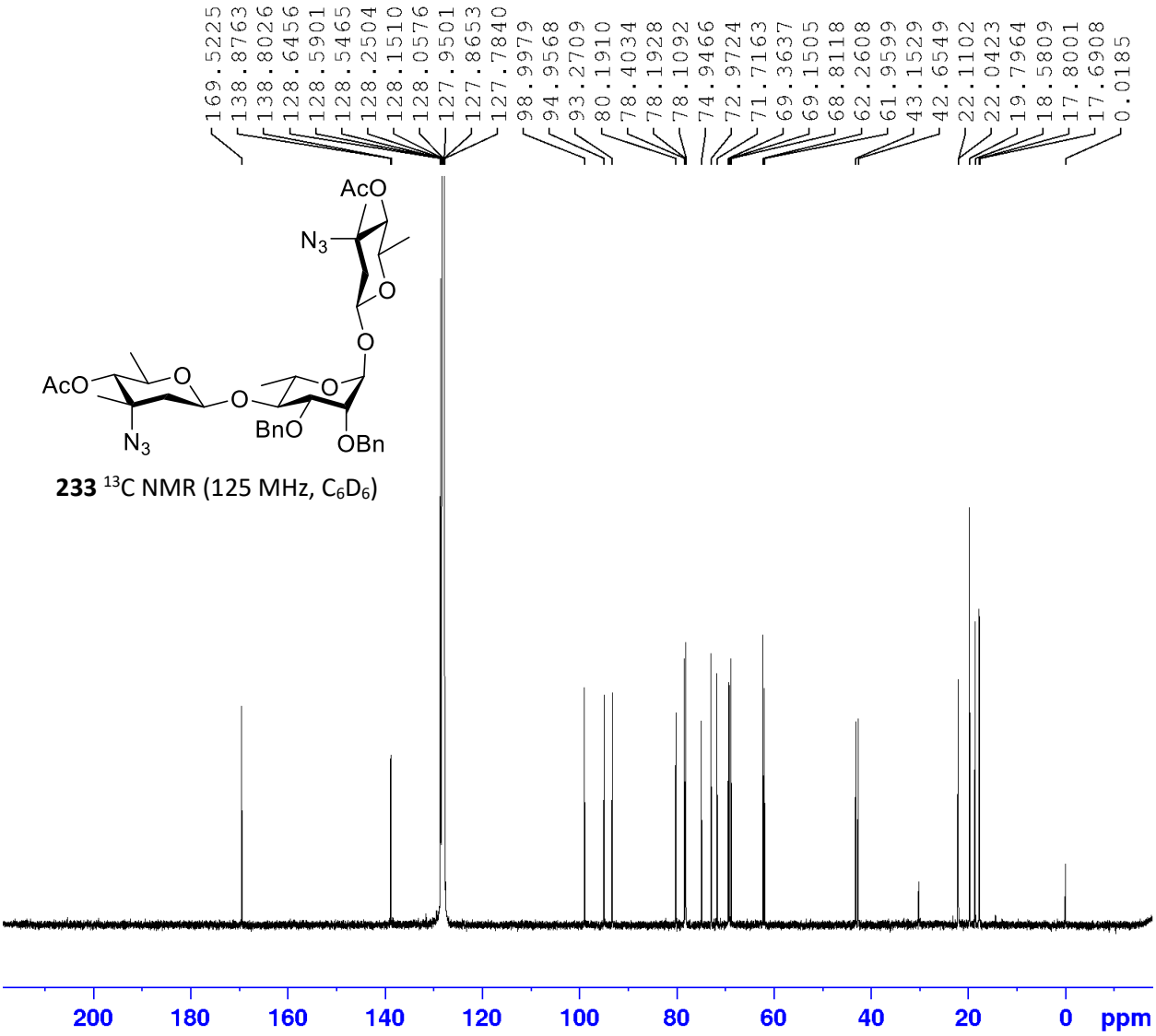
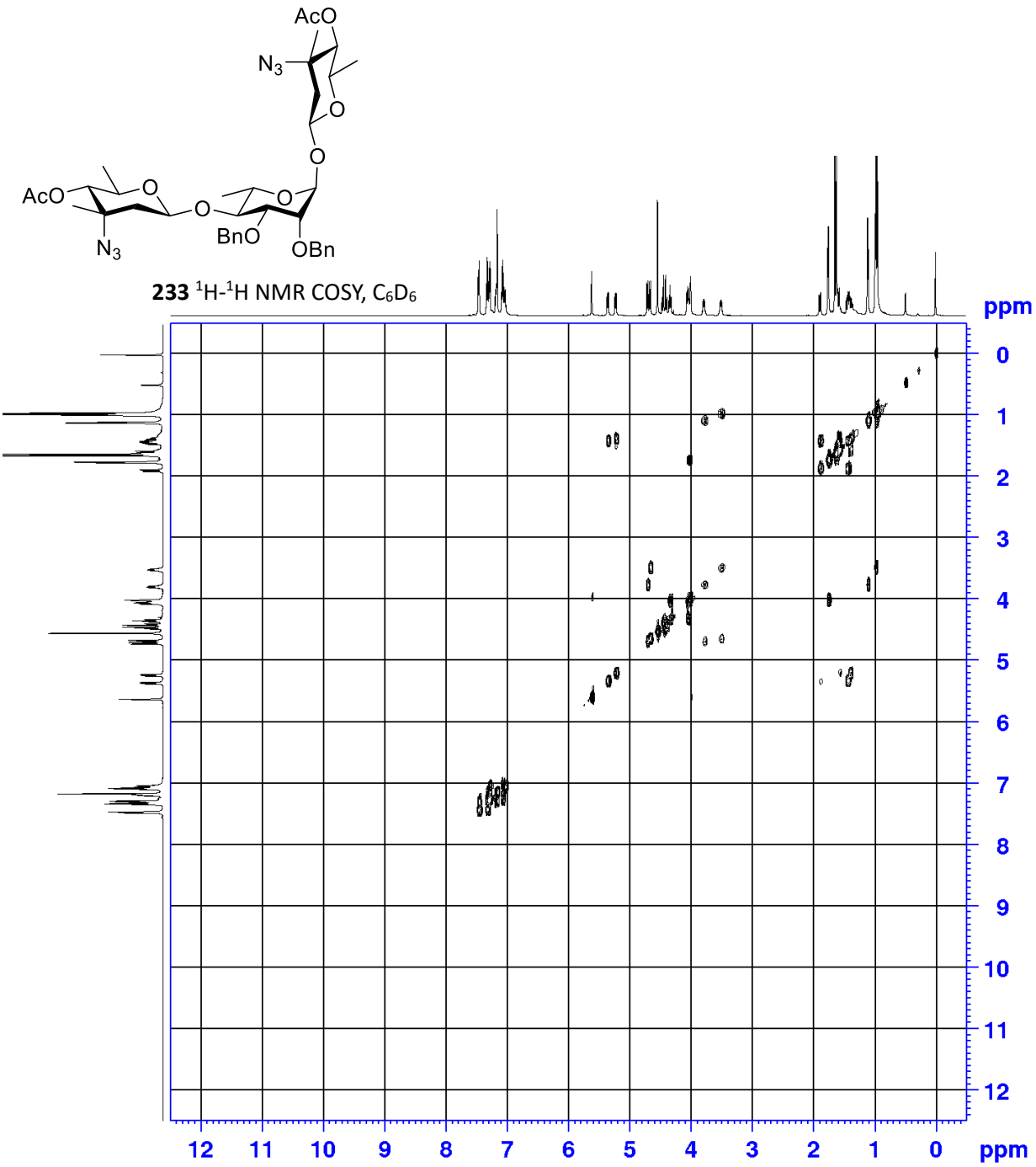
10
9
8
7
6
5
4
3
2
1
0
ppm



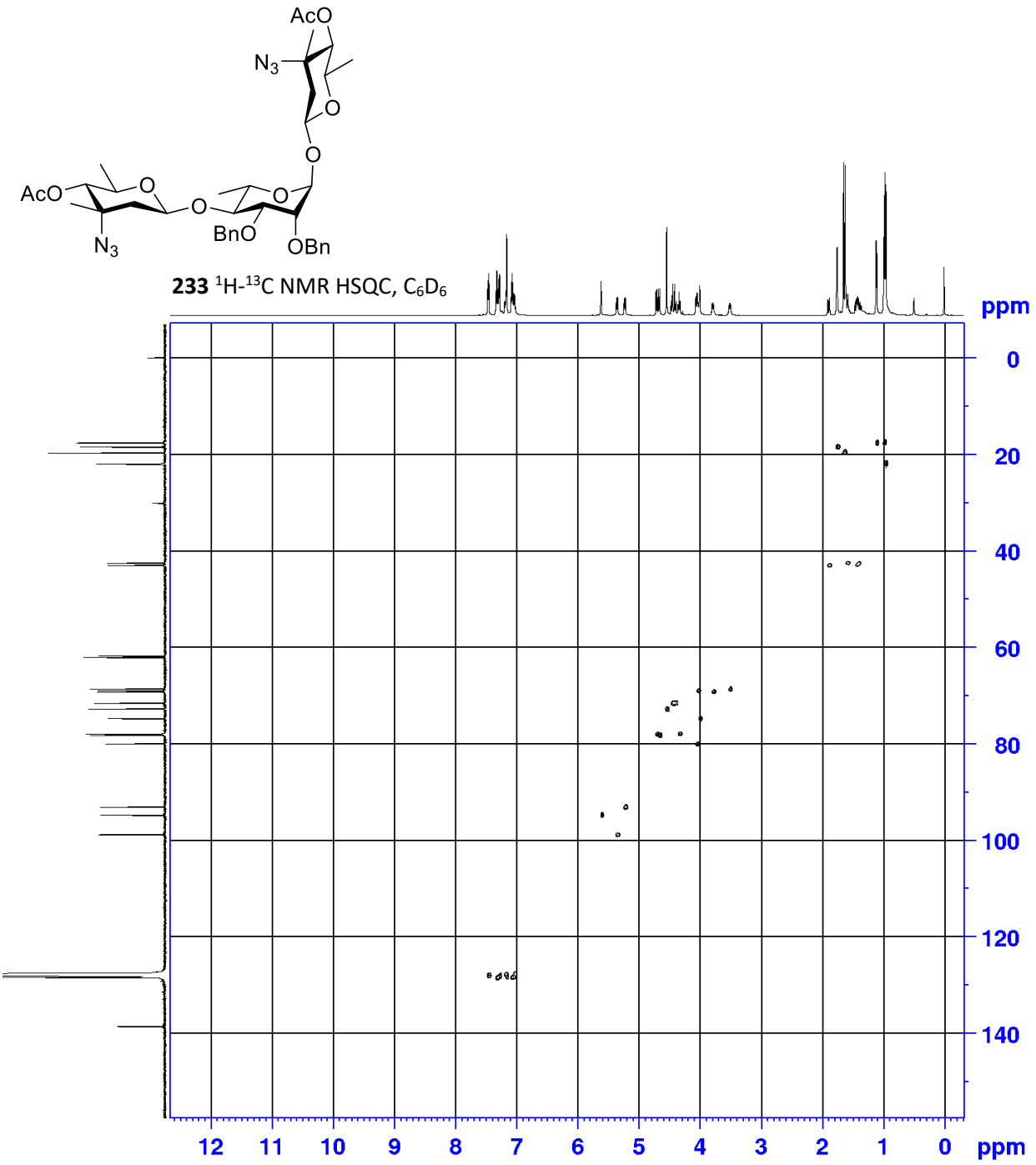
233 ^{1}H NMR (500 MHz, C_6D_6)



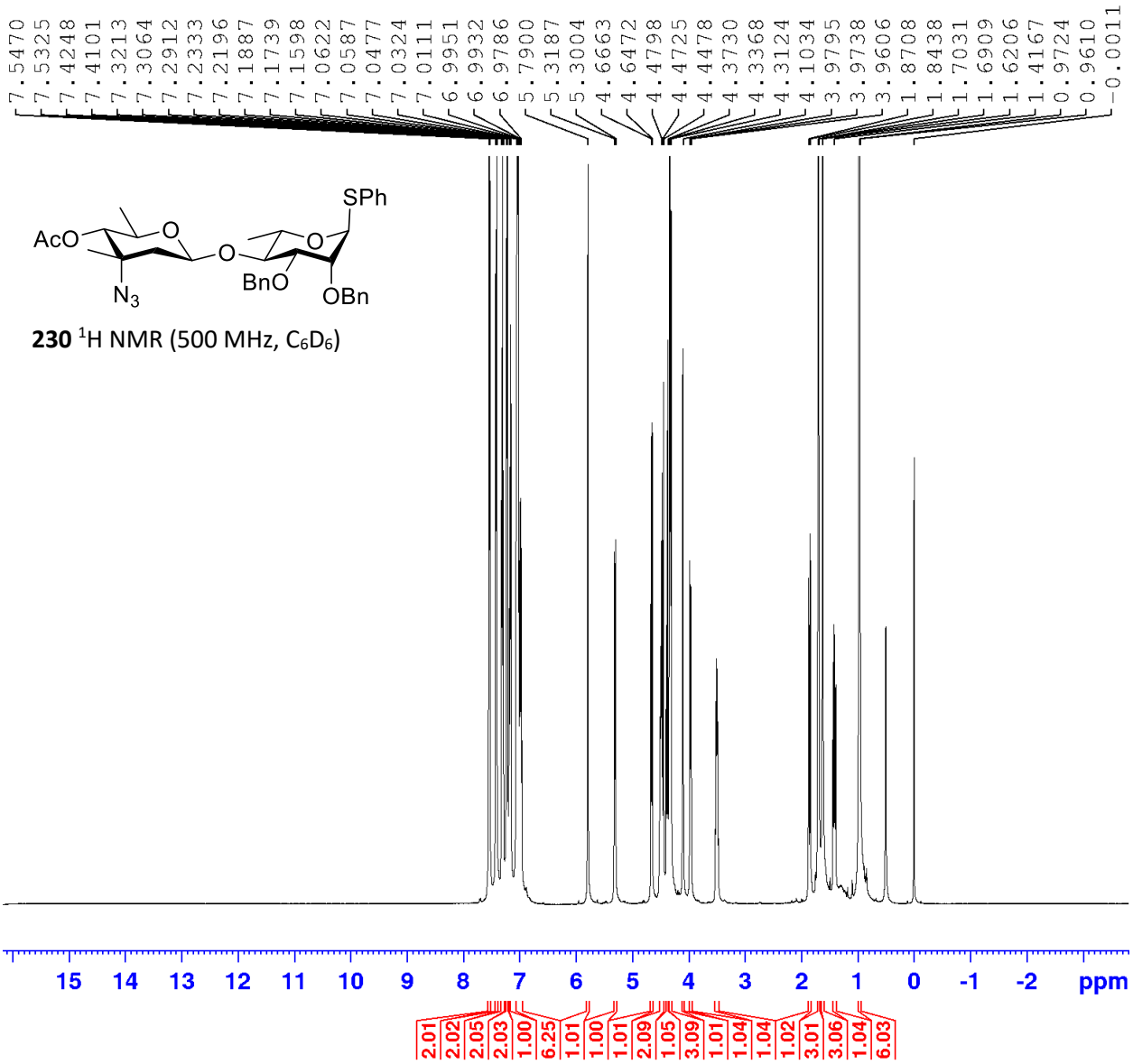
233 ^{13}C NMR (125 MHz, C_6D_6)



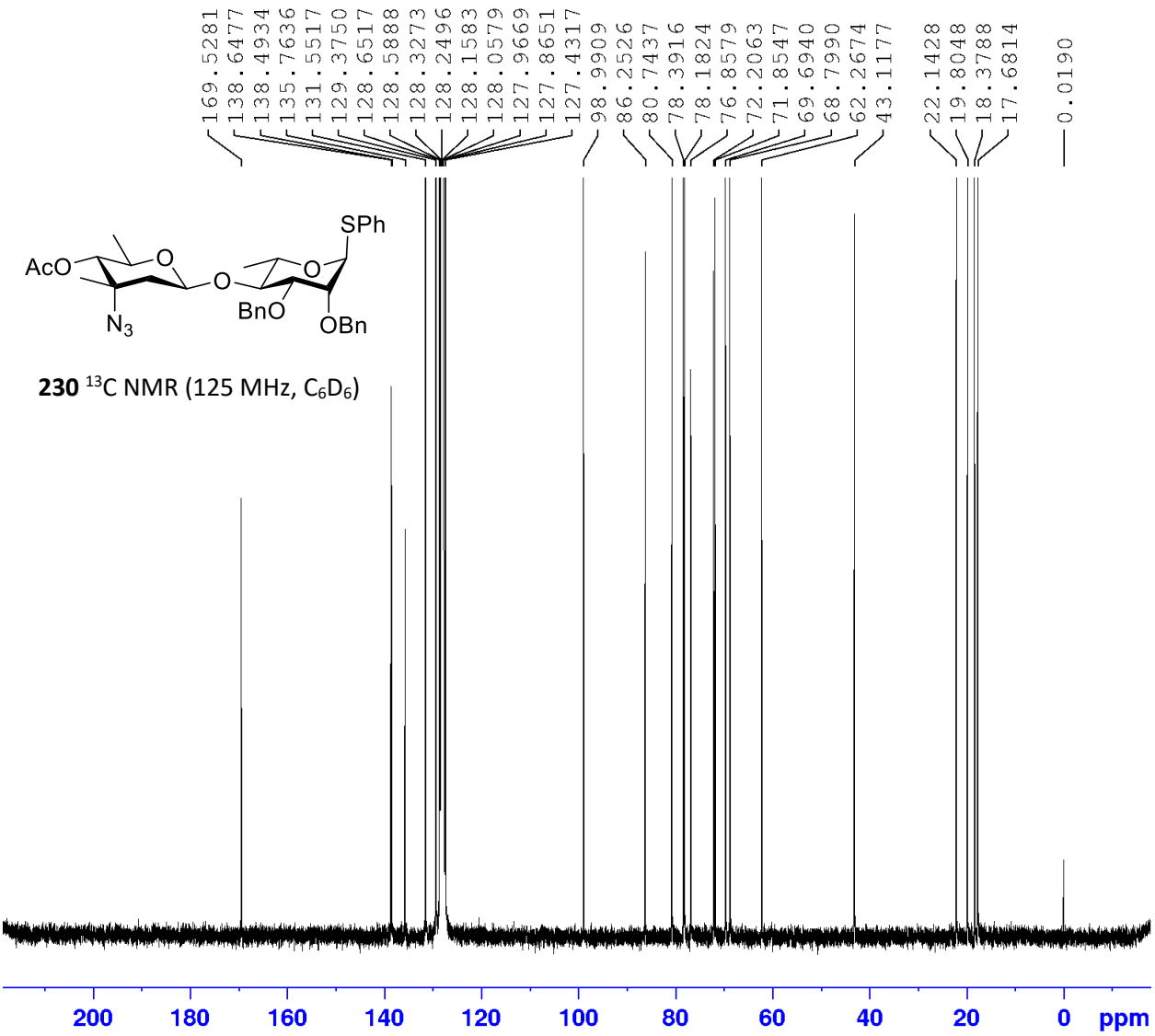
233 ^{1}H-^{1}H NMR COSY, C_6D_6



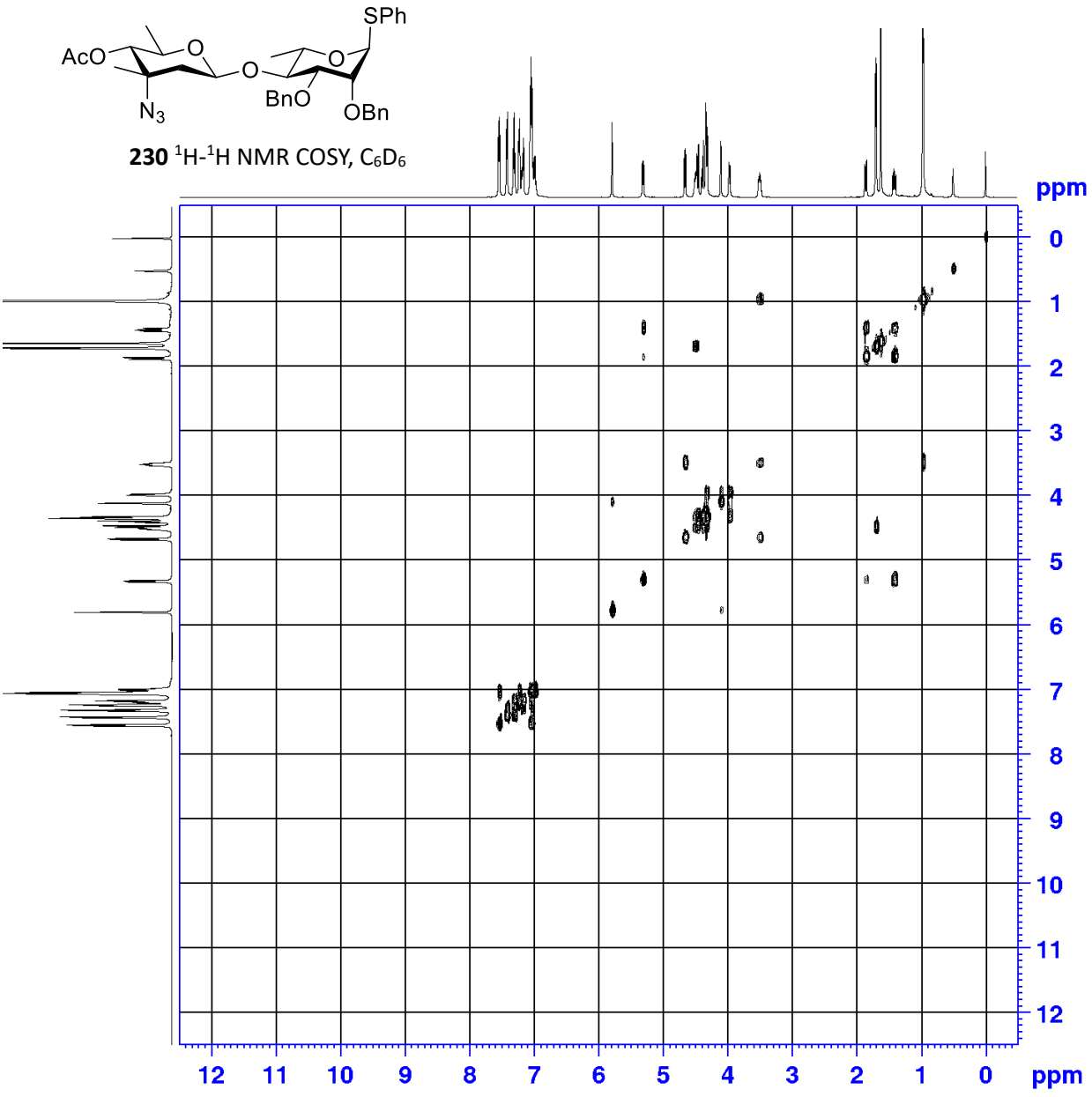
233 ^{1}H-^{13}C NMR HSQC, C_6D_6



230 ^{1}H NMR (500 MHz, C_6D_6)



230 ^{13}C NMR (125 MHz, C_6D_6)


SPh
AcO
O
O
O
BnO
OBn
N3
230 1H-1H NMR COSY, C6D6
ppm
0
1
2
3
4
5
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12
12
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10
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6
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2
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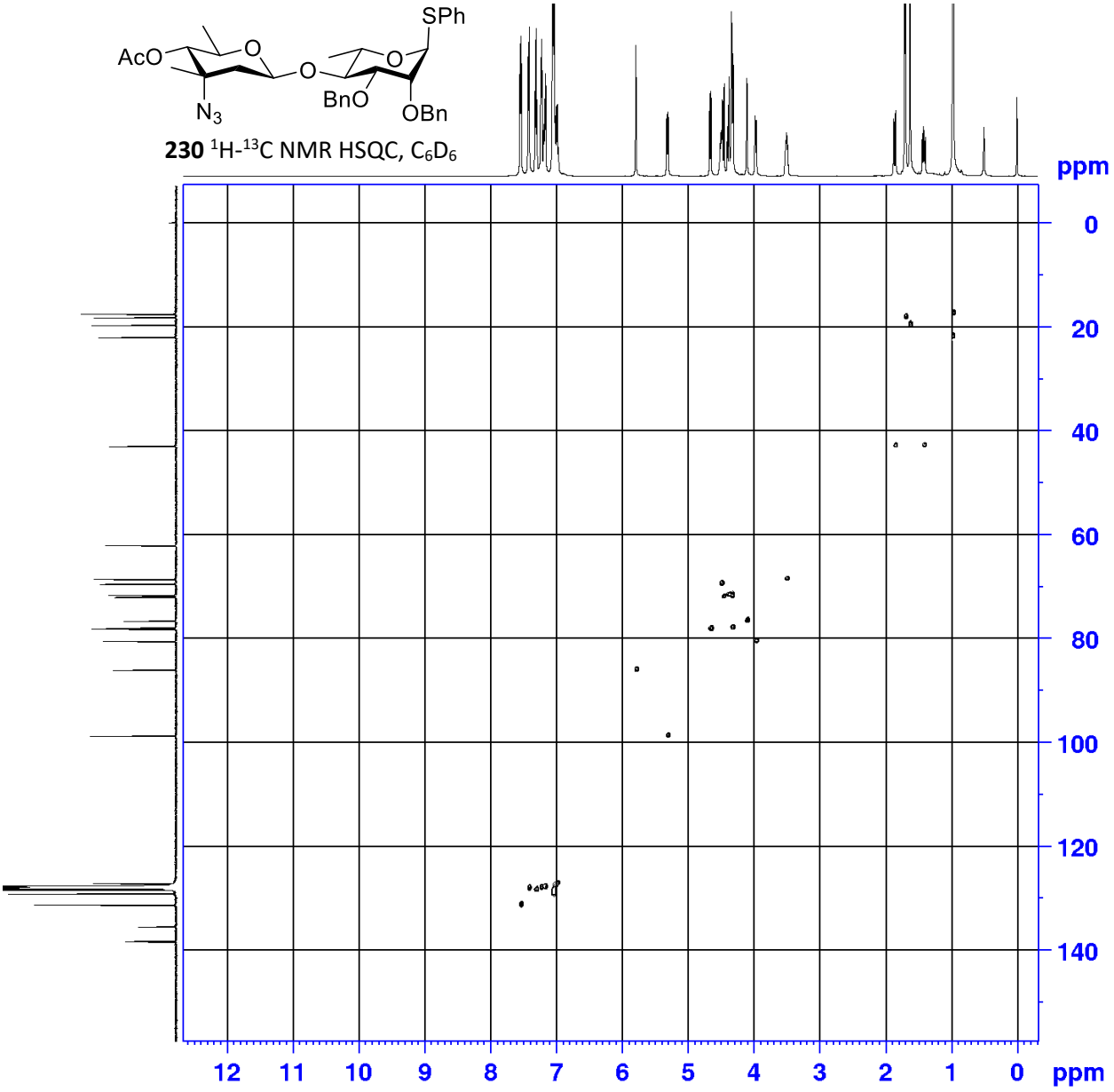
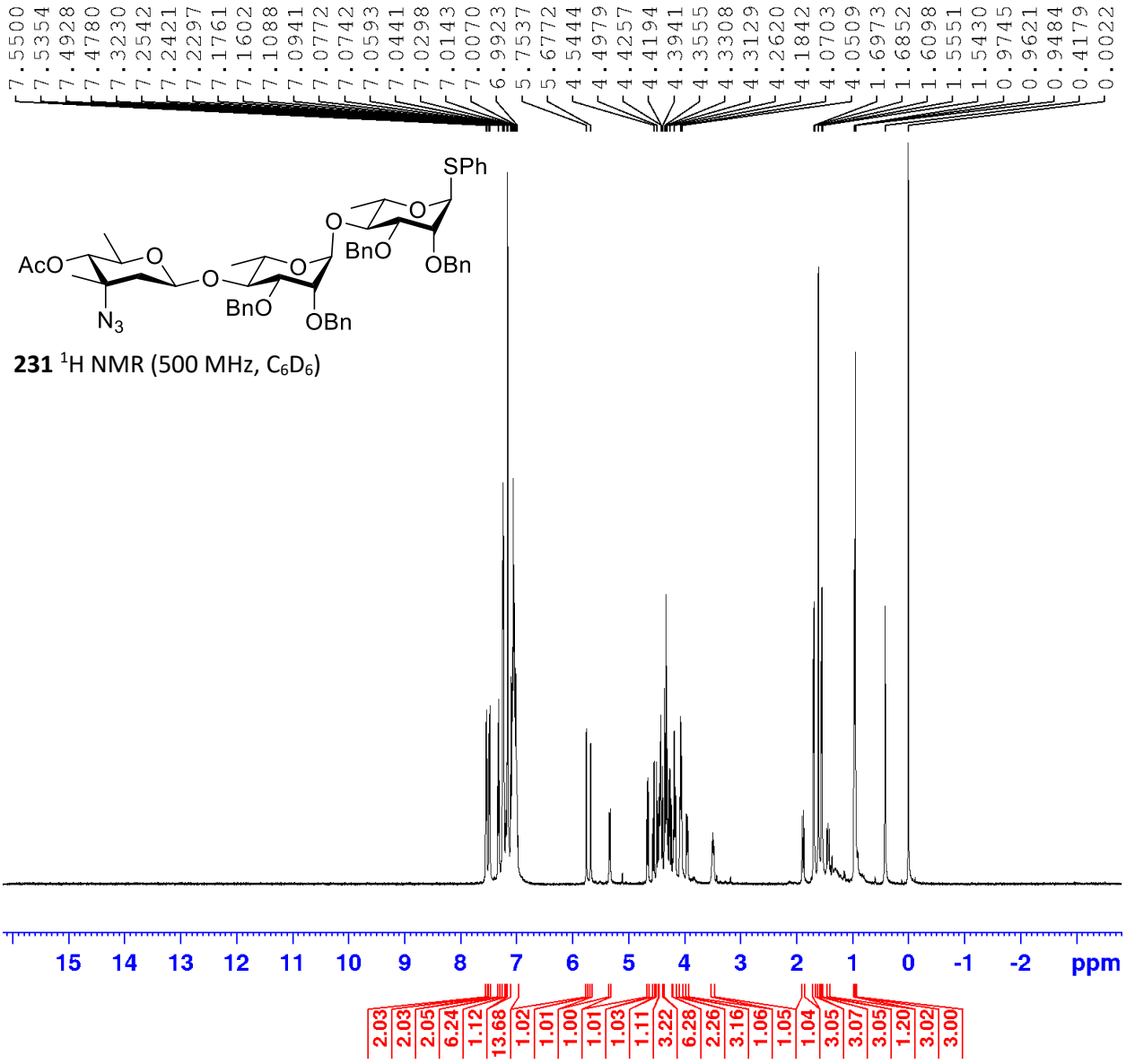
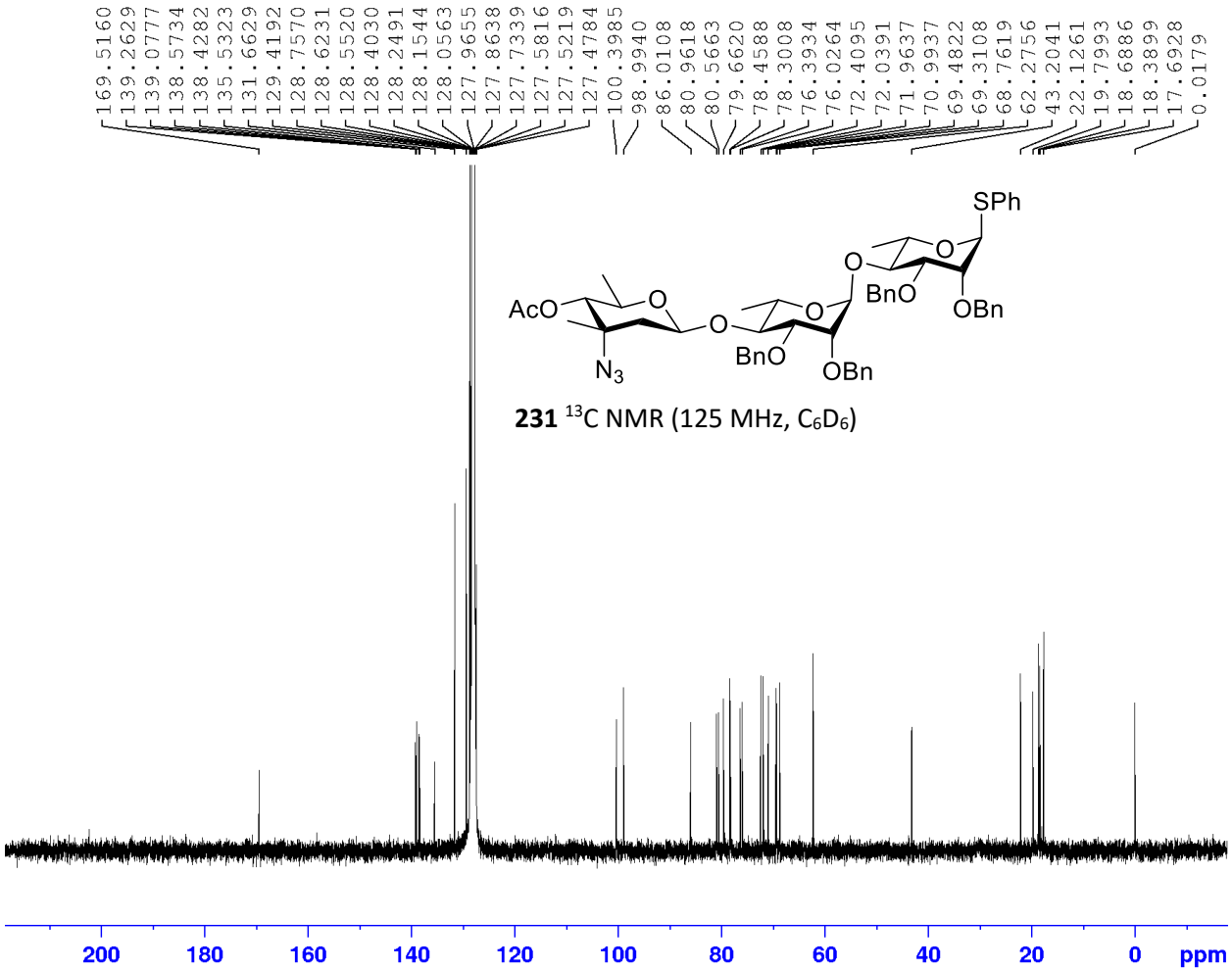
0
ppm



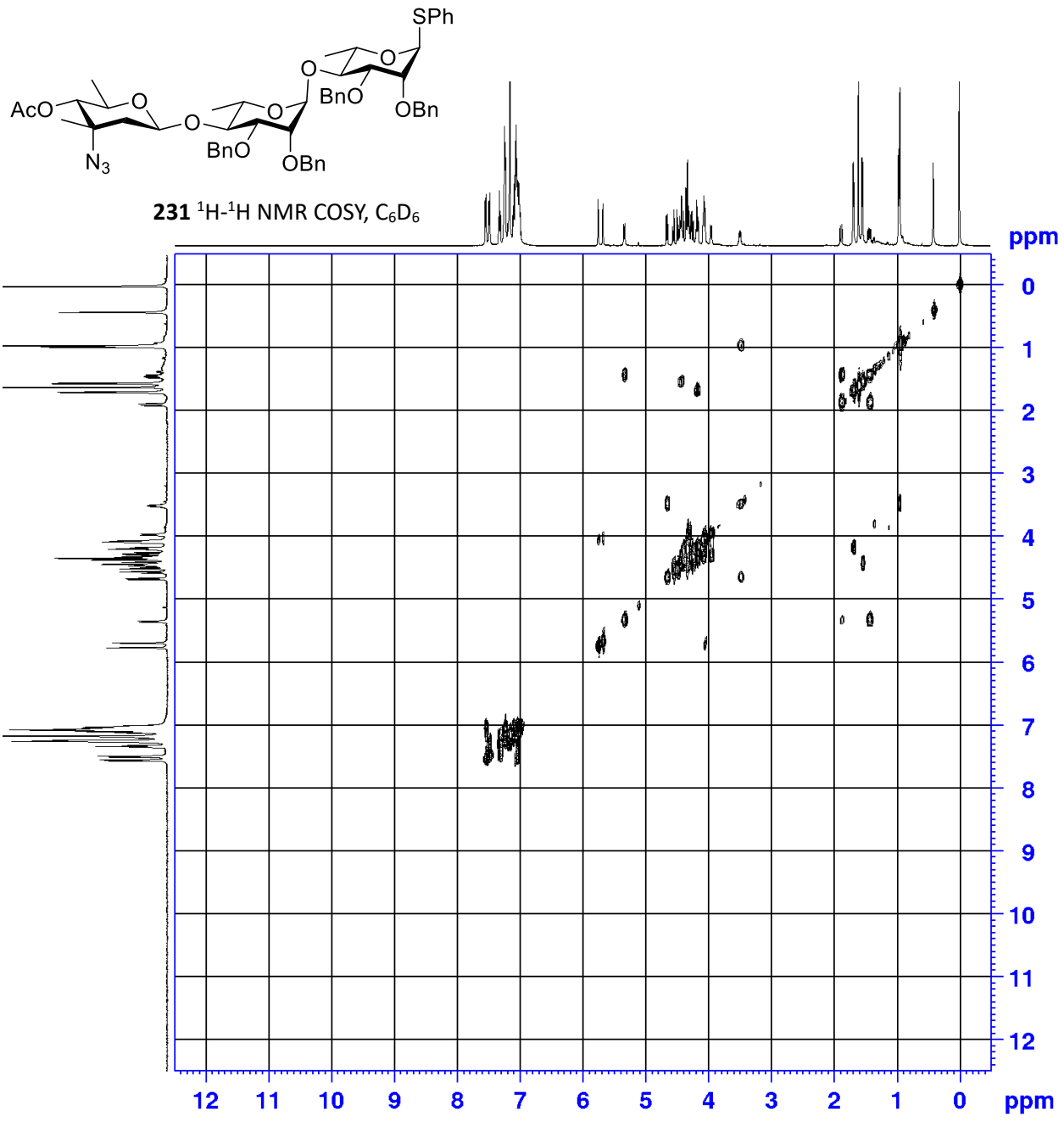
230 ^{1}H-^{13}C NMR HSQC, C_6D_6



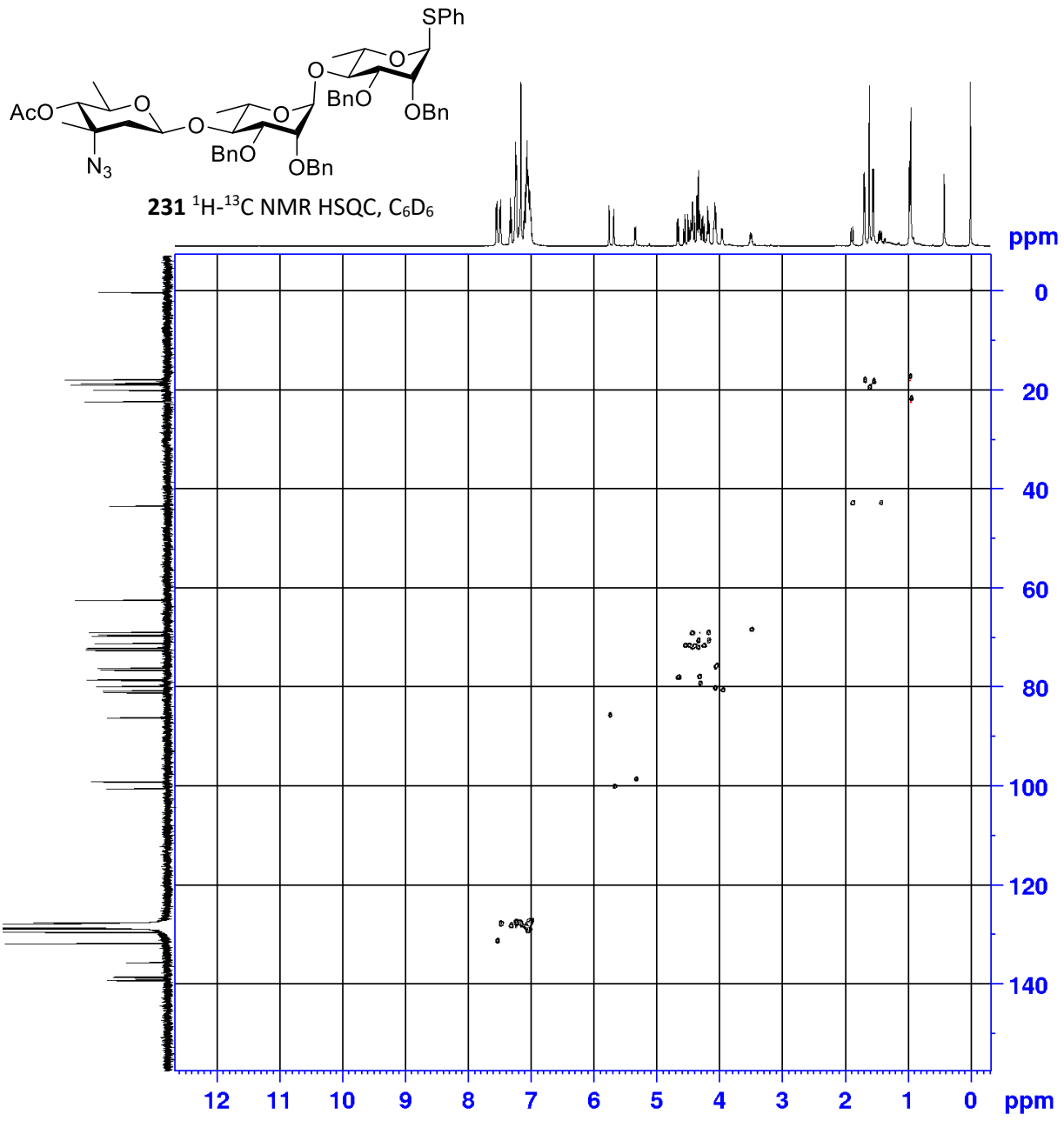
231 ^{1}H NMR (500 MHz, C_6D_6)



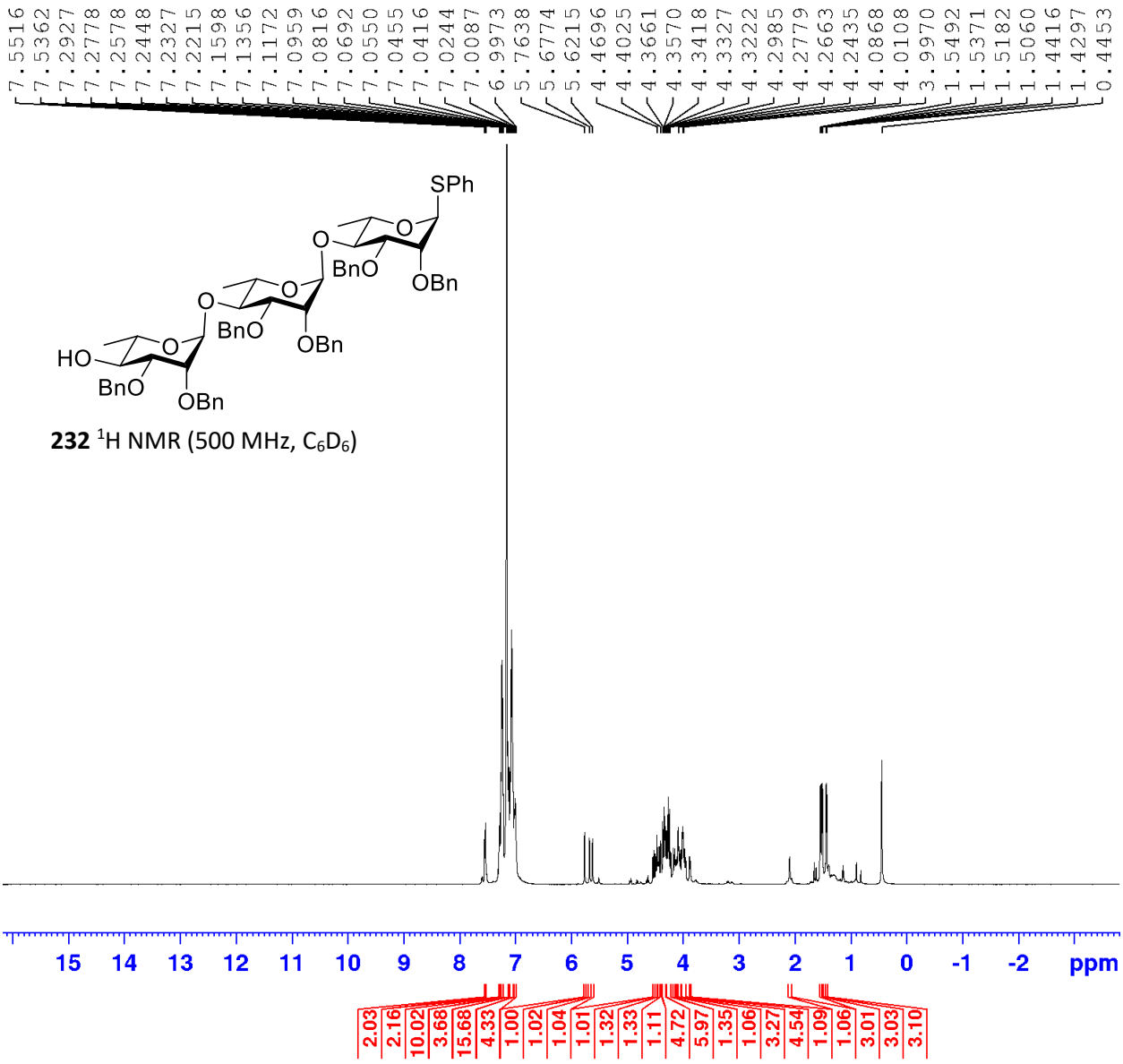
231 ^{13}C NMR (125 MHz, C_6D_6)



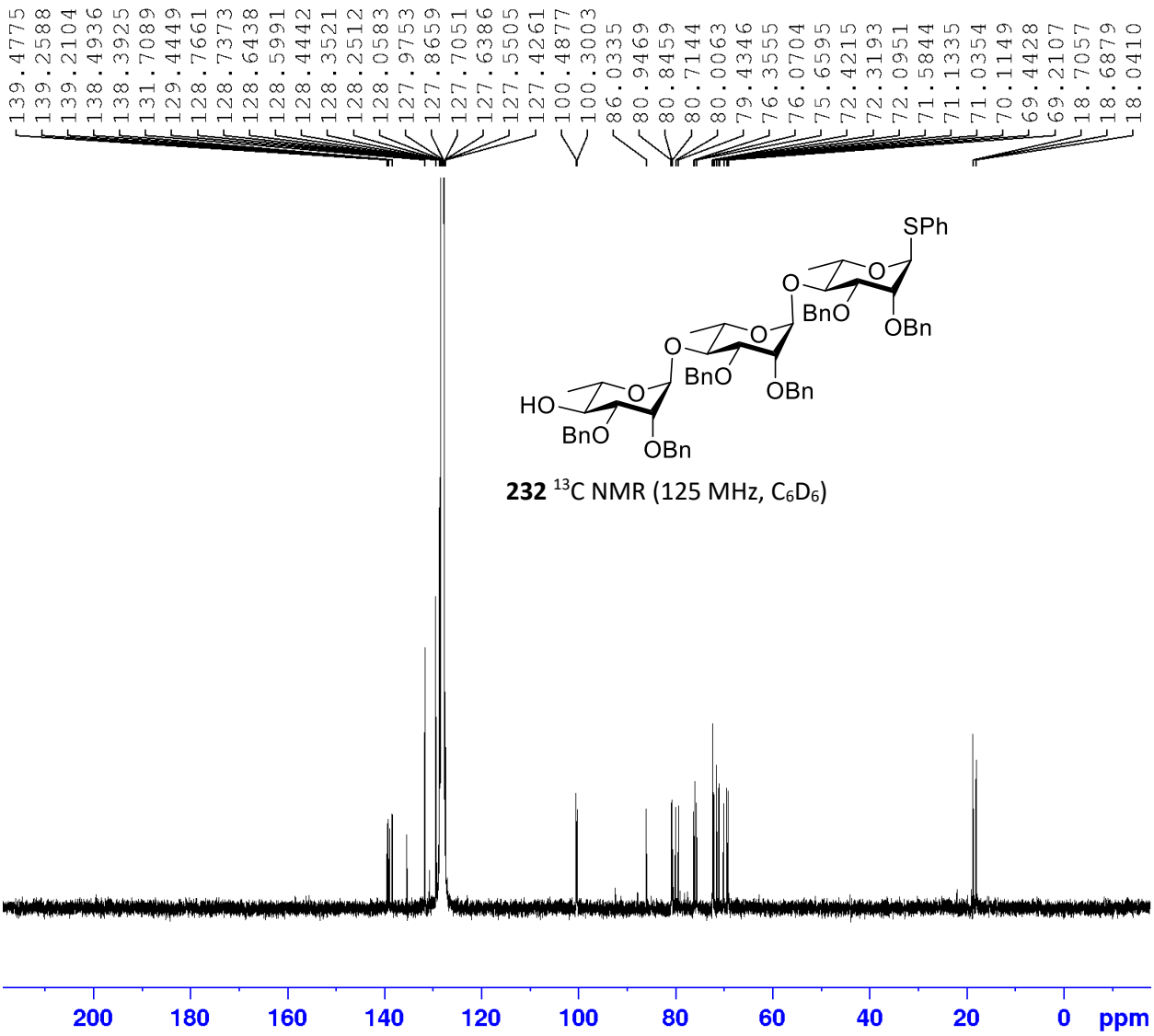
231 ^{1}H-^{1}H NMR COSY, C_6D_6



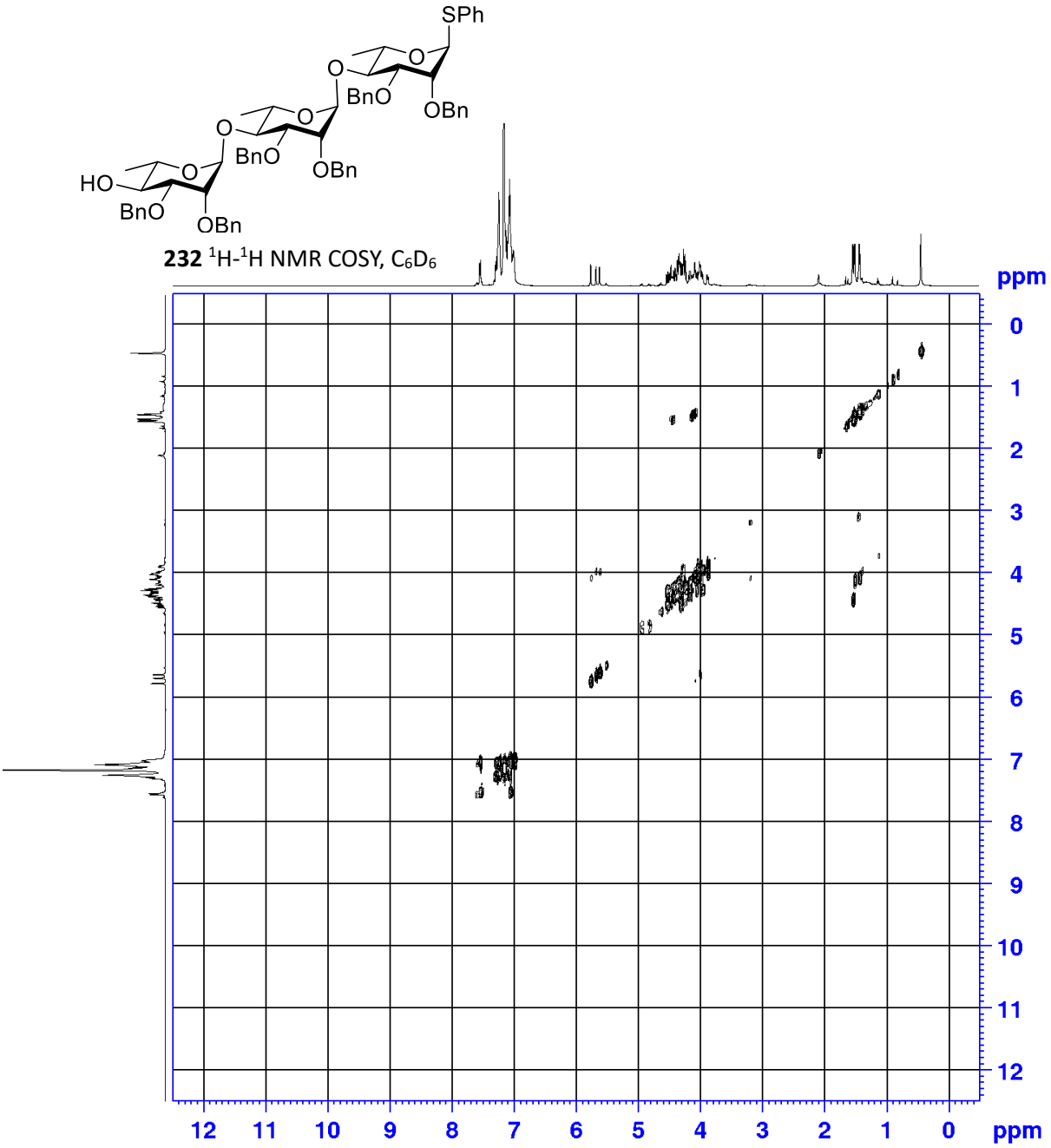
231 ^{1}H-^{13}C NMR HSQC, C_6D_6



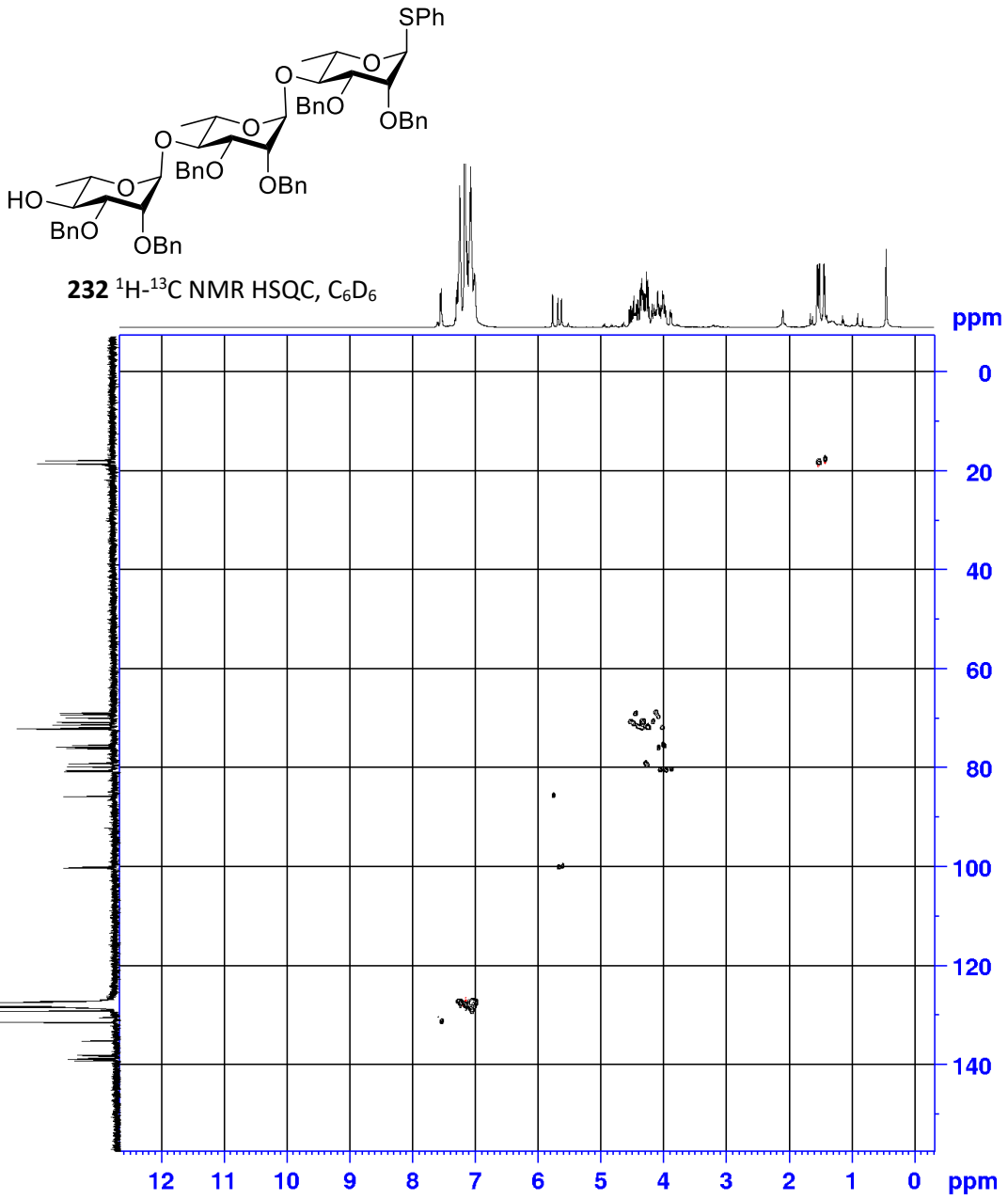
232 ^{1}H NMR (500 MHz, C_6D_6)



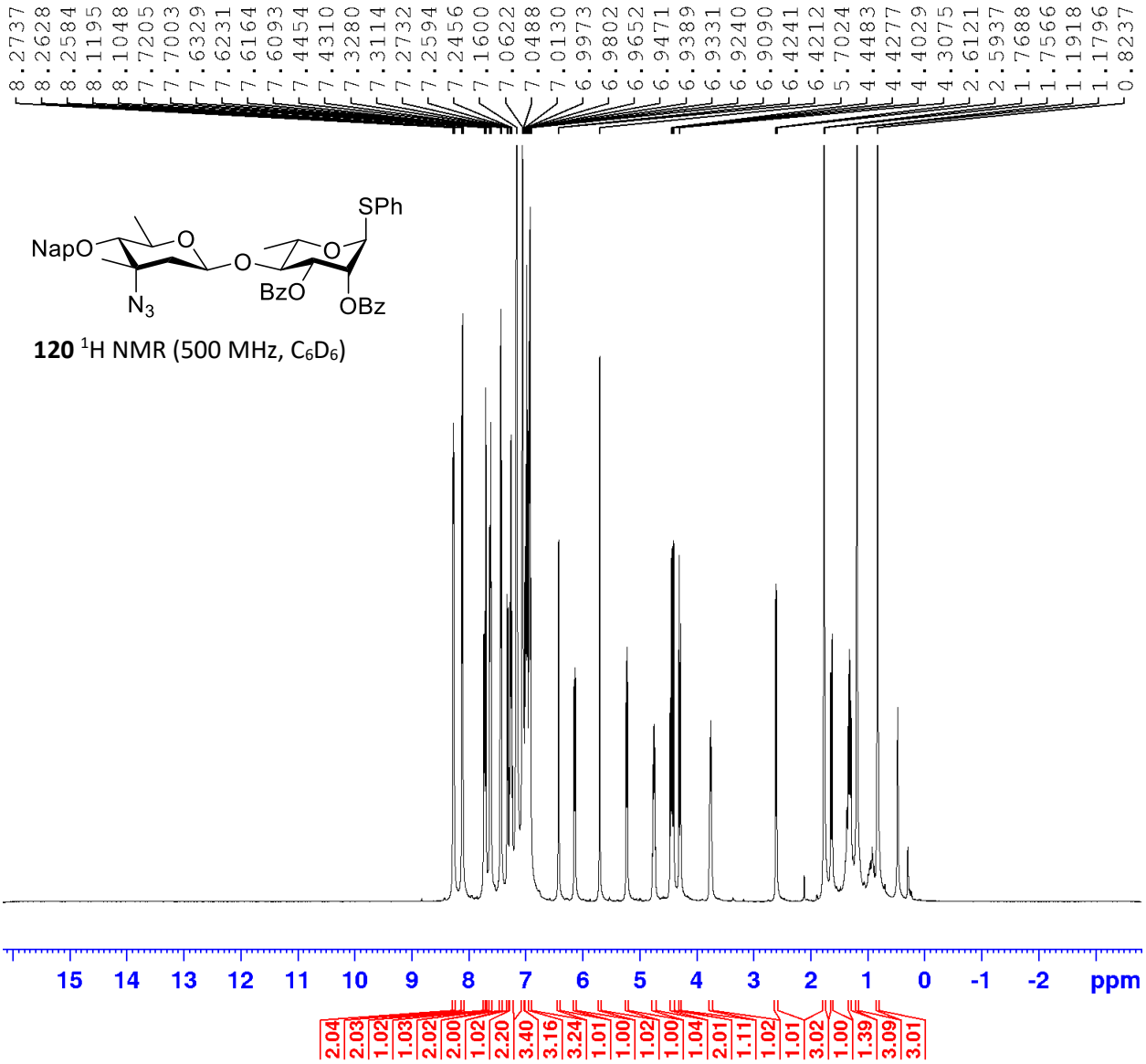
232 ^{13}C NMR (125 MHz, C_6D_6)



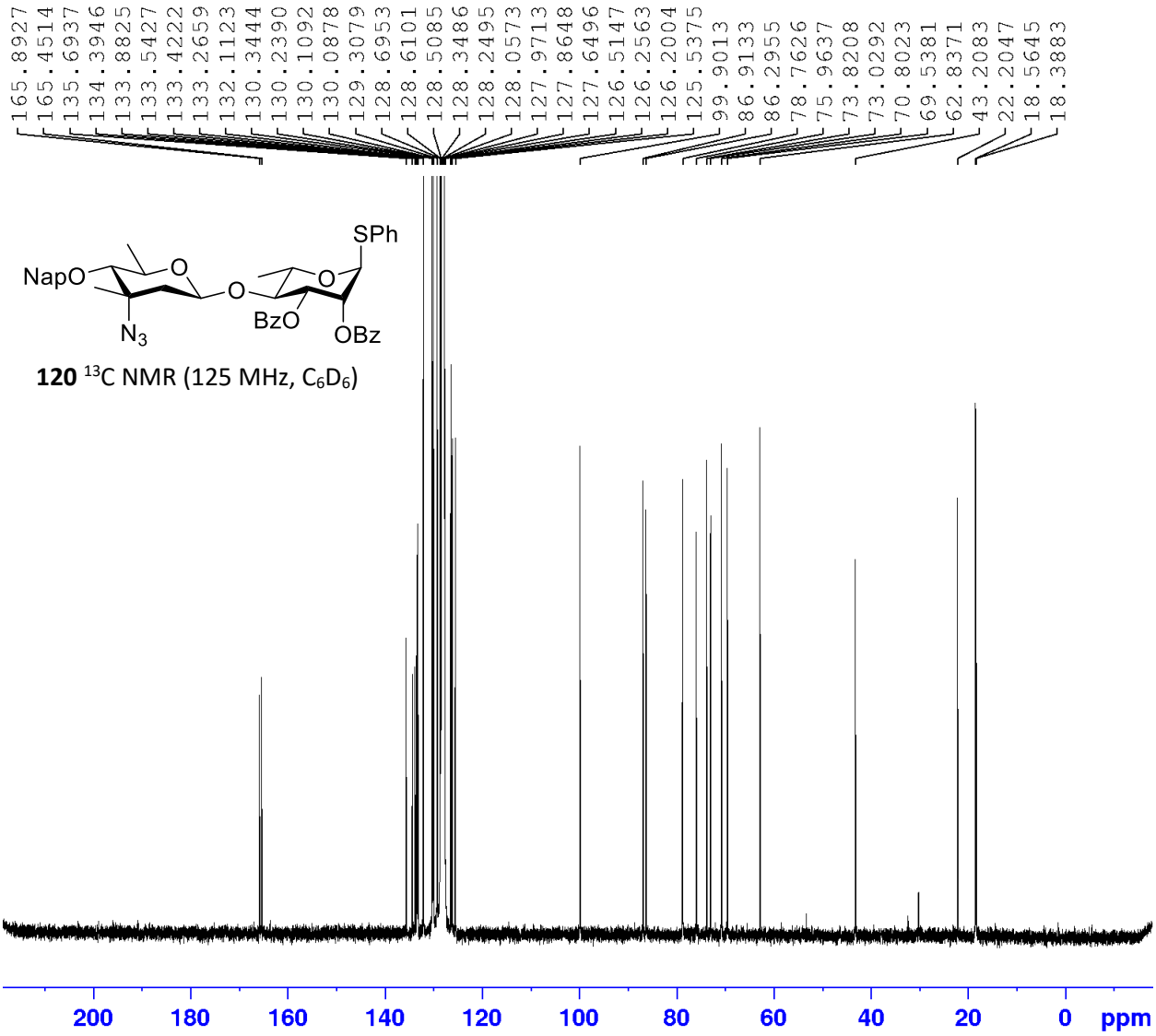
232 ^{1}H-^{1}H NMR COSY, C_6D_6



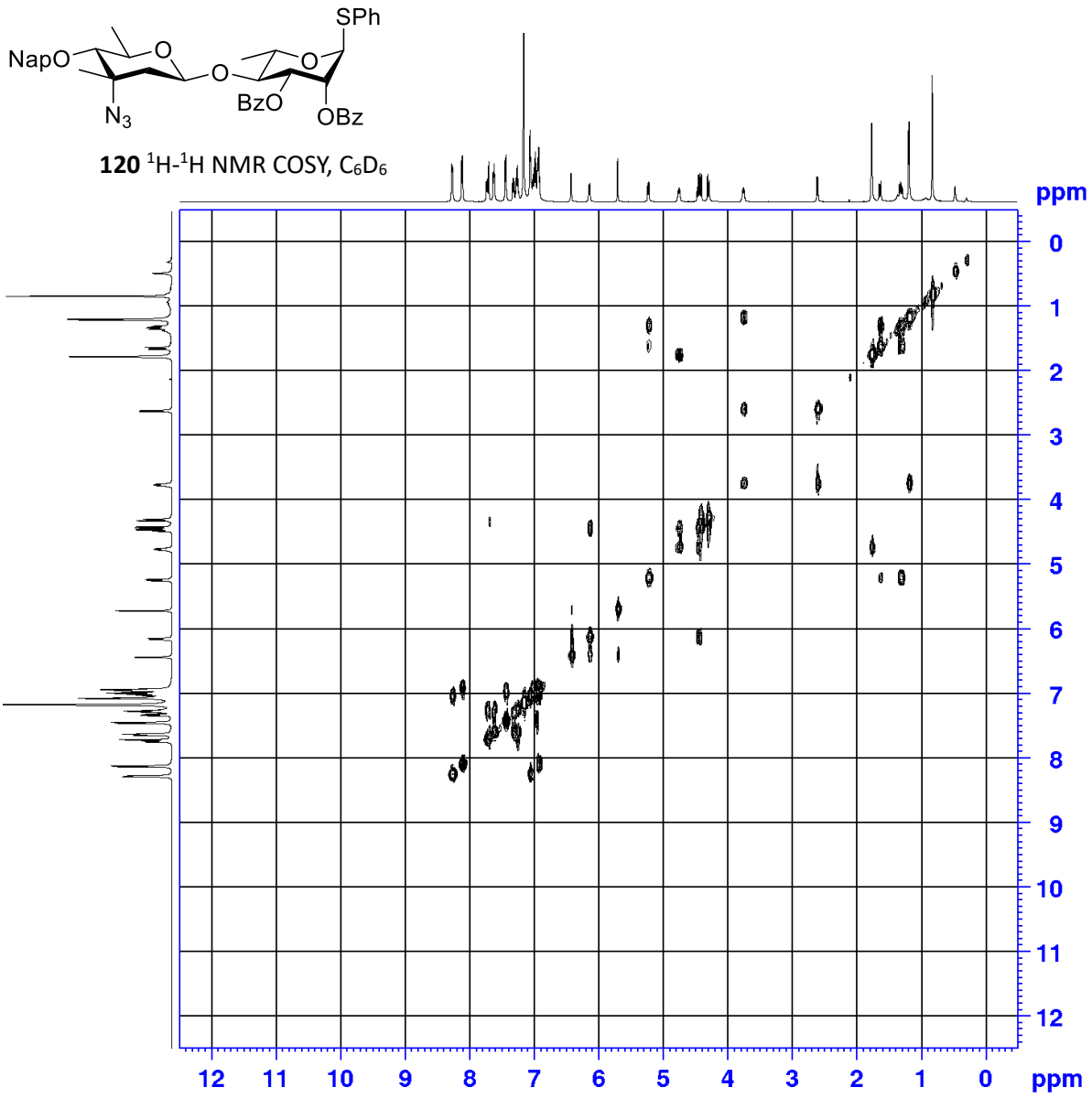
232 ^{1}H-^{13}C NMR HSQC, C_6D_6



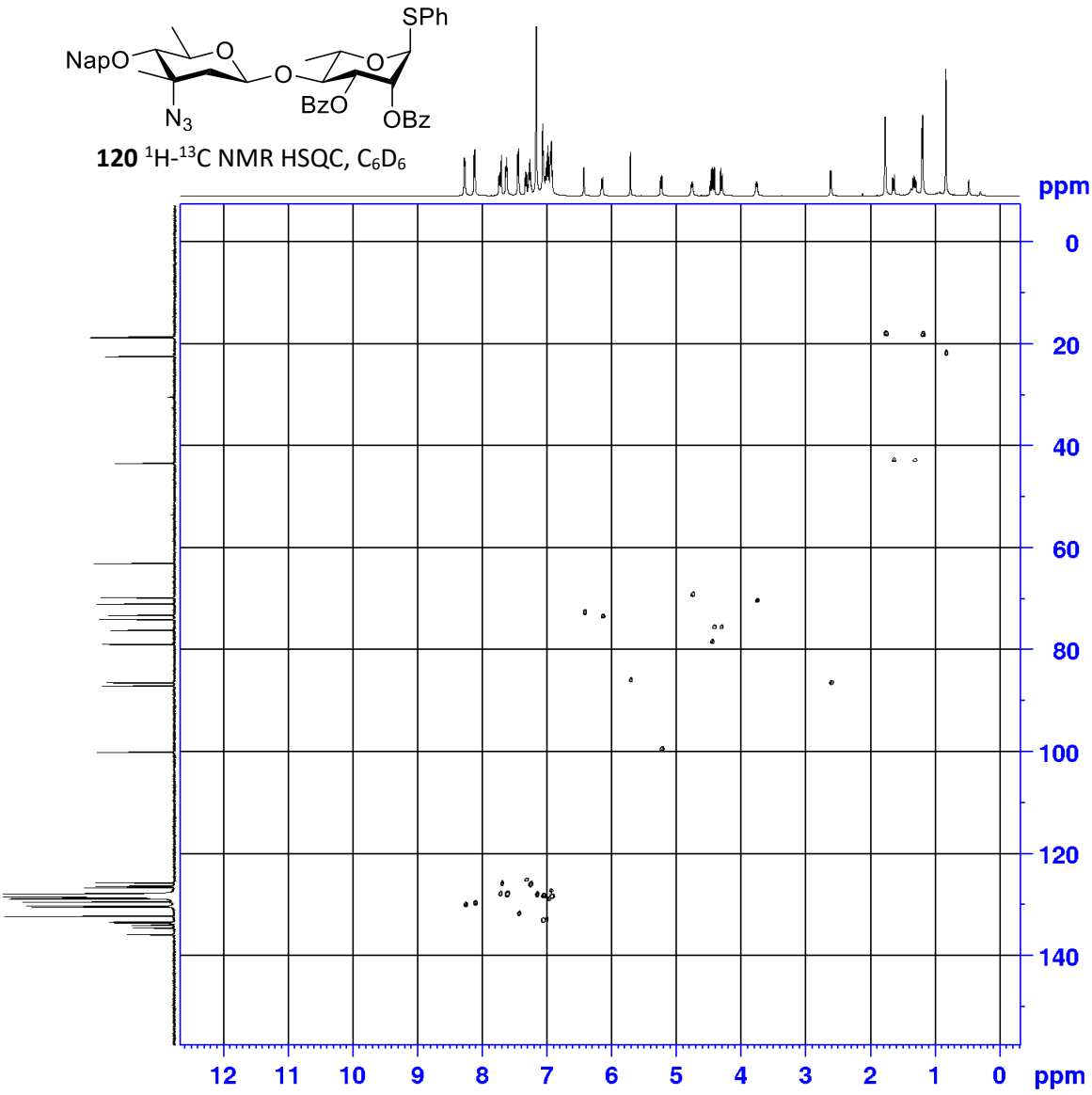
120 ^{1}H NMR (500 MHz, C_6D_6)



120 ^{13}C NMR (125 MHz, C_6D_6)



120 ^{1}H-^{1}H NMR COSY, C_6D_6



120 ^{1}H-^{13}C NMR HSQC, C_6D_6