Towards the Directing Group-Free Synthesis of the *S. pneumoniae* Serotype 6B Capsular Polysaccharide Minimum Repeat

An Honors Thesis for the Department of Chemistry

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Schemes and Figures

Chapter 1 – Introduction

The Global Burden of Pneumonia

Despite medical advances in the modern era, pneumonia remains a potent threat. A recent *Lancet* article by Walker and colleagues estimated that 120 million episodes of pneumonia occurred worldwide in 2010 in a population particularly vulnerable to pneumonia, children under 5 years. 14 million of these occurrences progressed to severe episodes. Furthermore, it has been estimated by the same group that 1.3 million deaths globally could be attributed to pneumonia in 2011, 81% of which occurred in children younger than 2 years.[1] This makes pneumonia the leading infectious cause of death in children younger than 5 years worldwide.[2]

It is certainly true that medical advances have contributed to substantial decreases in childhood mortality in recent years. The global mortality of children younger than 5 from all causes has fallen from 12 million in 1990 to 6.9 million in 2011. However, the progress has been disproportionate across geographic regions, with half of deaths in children under 5 occurring in 5 countries, where vaccination rates remain lower and living conditions are conducive to the spread of *Streptococcus pneumoniae*: China, Pakistan, India, Nigeria, and the Democratic Republic of the Congo.[2] However, in view of the facts that 18.3% of pneumonia infections are caused by *Streptococcus pneumoniae*[1] and are vaccine-preventable, more can be done to alleviate the global disease burden of pneumonia.

Vaccination is an effective strategy for reducing the impact of pneumonia globally. A *Lancet* study suggested that increasing the levels of intervention in pneumonia cases to 80% and increasing vaccination rates to 90% would eliminate in excess of two-thirds of deaths due to pneumonia by 2025.[3] Vaccination, known as an effective means of disease reduction, should play a prominent role in a complete solution to the global problem of pneumonia, and the creation of a cost-effective and comprehensive vaccine therapy for pneumonia is thus of high importance.

S. pneumoniae Serotype 6B

The most significant virulence factor of *Streptococcus pneumoniae* has been identified to be the capsular polysaccharide, which surrounds the bacterium. To date, 94 distinct serotypes have been identified, and this number continues to grow.[4]

Within the capsular polysaccharide, serogroup 6, comprising the 4 serotypes 6A, 6B, 6C, and 6D, constitutes a disproportionately large number of isolates, at 9.5% of serotyped isolates from children, and 6.7% of adults. In children, 6B was the most common serotype, at 7.5% of the total serotypes observed. Serotype 6B is also notable in showing especially high levels of antibiotic resistance.[4]

Given the clear prevalence of serotype 6B, it has been included in previous vaccines against *S. pneumoniae*. In 2001, a pneumonia conjugate vaccine, PCV7, was released, which provided immunity against the seven most common serotypes found in the *S. pneumoniae* capsular polysaccharide, 4, 6B, 9V, 14, 18C, 19F, and 23F.[5] Fortuitously, serotype 6A decreased in prevalence after the release of the vaccine, indicating cross-reactivity of the PCV7 vaccine to serotype 6A.[6] Furthermore, 6B has been shown to elicit antibody response against 6C and 19 as well.[7] Since the release of the PCV7 vaccine, two other vaccines have been released against *S. pneumoniae*: the 10-valent PCV10 vaccine, which adds serotypes 1, 5, and 7F, and the 13-valent PCV13, which further adds serotypes 3, 6A, and 19A.[4]

The Significance of Glycoconjugate Vaccines

In many cases, vaccines are produced from digests of the extracellular matrix of the pathogen. These consist largely of proteins, which are commonly used in vaccine therapies owing to their relative ease of isolation, as well as to the robust immune response they generate in humans. However, due to the fact that these proteins are encoded by genes which face constant mutation, the protein antigen profile of a pathogen is subject to frequent alteration. This renders previously produced immune memory obsolete in the face of infection by mutated pathogen.[8]

A possible solution to this problem is glycoconjugate vaccines, which are composed of complex carbohydrates. These polysaccharides are also found in the extracellular matrix of pathogens, and are also antigenic. Because they are synthesized and expressed by multiple enzymes, they are less susceptible to change due to mutations. Small genetic variations in the genes encoding the enzymes responsible for constructing these polysaccharides result in either no change in enzymatic function, or else a non-functional enzyme. Though this does not completely eliminate mutation as an obstacle in glycoconjugate vaccines, it does greatly reduce the rate at which it occurs.[8]

However, glycoconjugate vaccines are not without difficulties. Polysaccharide antigens are not as active at stimulating human immune systems as are protein antigens. Little to no immune memory response is generated against polysaccharides by themselves, and the immune response is inadequate in young children. To remedy these problems, the carbohydrates are frequently conjugated to some immunogenic carrier protein.[9] Also, isolating homogenous polysaccharides from natural sources is difficult.[8] This makes the laboratory synthesis of carbohydrate antigens an attractive route to developing glycoconjugate vaccines. Efficient laboratory syntheses of these carbohydrate antigens would also provide a batch-to-batch homogenous production of antigen, which would eliminate the natural variation inherent in isolation from biological sources. This would serve to streamline immunological analysis of vaccine candidates derived from purely synthetic sources, accelerating the process of drug discovery.

Mechanism of Glyconjugate Vaccine-Based Immunology

Before discussing the challenges present in glycosylation chemistry, it would be worthwhile to examine the mechanism by which immunity can result from glycoconjugate vaccines. The presence of sugar molecules on the surface of microbial pathogens is considered essential to their ability to colonize a host. Each serotype of a pathogen possesses its own pathogen-associated molecular pattern (PAMP), which consists of, among other molecules, the extracellular polysaccharide. This PAMP is crucial to ensuring efficient cell-to-cell adhesion and infection.[10]

This same PAMP is also used by the host organism to detect the presence of pathogens, as toll-like receptors (TLR) recognize the presence of certain PAMPs, and initiate an immune response to counter the invasive pathogen.[11] It is important to note that a TLR will not recognize all PAMPs.[9]

A crucial observation is that pathogens present large clusters of individual repeating units of oligosaccharides to the host pathogen-associated molecular pattern receptor. This ensures effective adhesion for the pathogen, but also ensures robust immune response by the host. The adhesion of a single oligosaccharide to a protein has a weak dissociation constant, often in the mM range, whereas clustering of glycan molecules, as is present on pathogen cell surfaces, results in stronger adhesion, often with dissociation constants in the nM range.[12] Effective vaccine therapies seek to mimic this clustered effect by displaying multivalent arrays of oligosaccharides bound to a single carrier protein.

Carbohydrate vaccines containing only purified oligosaccharides generate poor immune response. This is commonly attributed to the fact that oligosaccharides elicit a T cell-independent response. The repetitive motif of oligosaccharides (a minimum repeat oligosaccharide contained in a polysaccharide) results in activation of B cells without the assistance of $CD4^+$ helper T cells. This occurs when B cells are activated after binding of the oligosaccharide to B-lymphocyte receptors[12]. Following this binding, cross-linking of the Ig proteins produces short-lived immunoglobulin M (IgM) antibodies, which do not result in long-term immune memory.[13]

However, coupling the B cell to a helper T cell induces a T cell-dependent immune response. T cells are activated with the aid of antigen-presenting cells. An immune response involving T cells creates IgG antibodies,[12] which are known to result in the generation of memory B cells, which confer long-lasting immunity. This allows efficient immune response upon presentation of another instance of the antigen which generated the memory B cell.[9] Coupling the oligosaccharide of the vaccine to an immunogenic carrier protein known to elicit T cell-response thus results in a T cell-dependent immune response, generation of IgG antibodies and memory B cells, and long-lasting immune memory.

Challenges in Glycosylation Chemistry

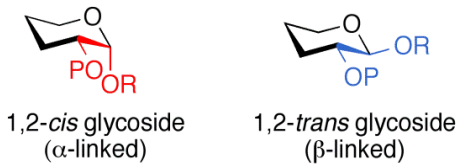


Figure 1. The difference between a 1,2-*cis* glycoside and a 1,2-*trans* glycoside.

Constructing the carbohydrate antigen is a key process in the synthesis of carbohydrate vaccines. Within this process, the stereospecific synthesis of the correct glycosidic linkage between monosaccharides is crucial (**Figure 1**). One important challenging example is the construction of 1,2-*cis*-*O* glycosidic linkages, a common motif in bacterial antigens.[14]

Given the biological significance of 1,2-*cis*-*O*-linked glycosides, considerable effort has gone into developing methods for creation of these key bonds.[15] One common technique has been using directing groups and/or chiral auxiliaries that bias the reaction towards selective α-glycoside formation. Examples of this include Boons use of a pre-installed anomeric glycosyl sulfonium species,[16] and Turnbull's oxathiane spiroketal-activated donors that selectively block the β face of a molecule and permit S_N2 attack at the anomeric position, resulting in α-selectivity.[17]

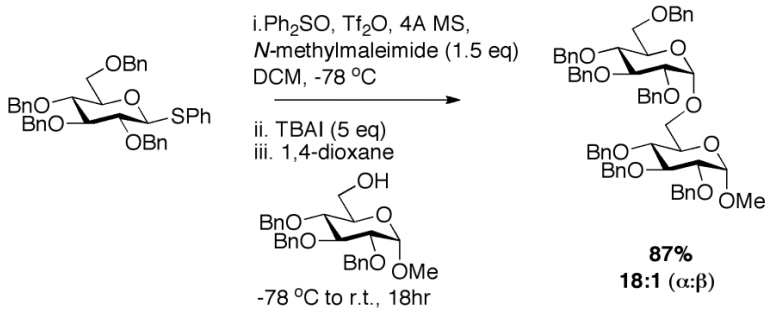
More distant effects have been shown to induce α selectivity, for example the Crich group demonstrated that installation of a benzylidene acetal on C-4 and C-6 of glucopyranoside-derived donors conformationally restricts the activated donors to favor the formation of α-linked products.[18] However, this approach necessitates extensive protecting group manipulation during oligosaccharide synthesis. This both lengthens the synthesis and limits the scope of these methods to those donor and acceptor pairs which are compatible with these methods. A more general method, which is tolerant of a broad range of substrates and involves minimal group manipulations, is therefore necessary.

One method which has been shown to be effective with a large scope of substrates is the use of glycosyl iodides as glycosyl donors,[19] a method pioneered by Lemieux in 1974.[20] Glycosyl iodides react to preferentially form 1,2-*cis*-*O*-glycosides with a broad range of nucleophiles. In the presence of excess iodide ion, the glycosyl iodide equilibrates between the more stable α-glycosyl iodide and the more reactive β-glycosyl iodide. Nucleophilic displacement of the leaving group in the β-glycosyl iodide then results in the desired α glycosylation product.[21] However, previous methods of glycosyl iodide preparation involve harsh reagents that are potentially damaging to an assembling oligosaccharide such as trimethylsilyl (TMS) iodide or HI.[22,23] More mild conditions are warranted for the creation of labile oligosaccharide linkages.

A similar approach using dimethylformamide (DMF) as a modulator has been used by Mong and colleagues. In this approach, excess DMF intercepts an oxocarbenium cation intermediate, and sets up an equilibrium between an α-glycosyl imidate and a β-glycosyl imidate. This latter species is more reactive and result in preferential formation of an α glycosylation

product.[24] The approach is powerful, however it can be less selective with more reactive coupling partners.

New Technology for Constructing 1,2-*cis* Glycosidic Linkages

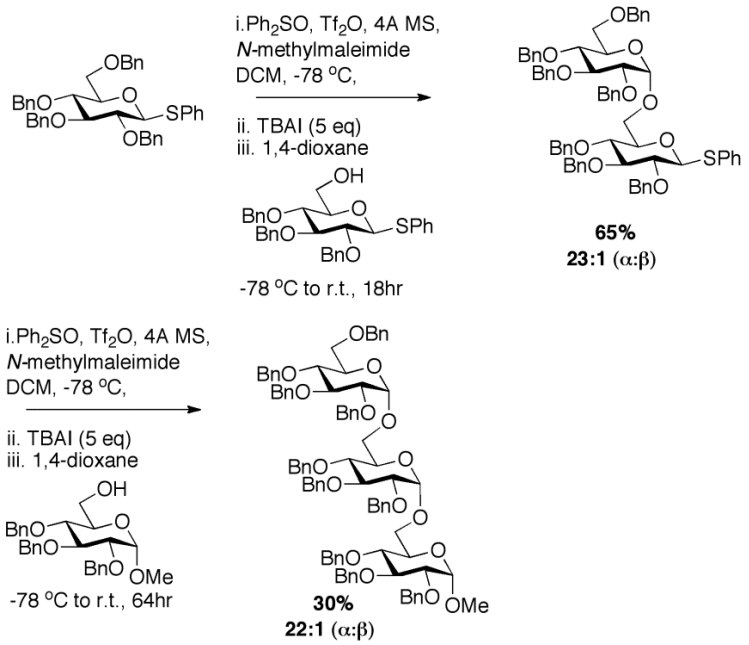


Scheme 1. Novel α-selective glycosylation method.

One possible solution to this problem is a method for the mild and *in situ* generation of glycosyl iodides for α-selective glycosylations recently developed in the Bennett lab (**Scheme 1**). This is done by treating pre-formed phenylthioglycoside donor with a mixture of diphenyl sulfoxide and tirfluoromethanesulfonic (triflic) anhydride to generate a highly-reactive glycosyl triflate species. In the presence of tetrabutylammonium iodide (TBAI), a glycosyl iodide is generated *in situ*, which acts as a glycosyl donor upon addition of a suitable acceptor. Equilibration occurs between the more thermodynamically-stable α-glycosyl iodide, and the less stable and more reactive β-glycosyl iodide. Reaction of the β-glycosyl iodide with a nucleophile then generates the 1,2-*cis*-O-linked glycoside.[21]

This method has the advantage over previous methods that it relies on halide ion-mediated glycosylation, which had been shown previously to give high levels of α-selectivity.[19,20,22,23] However, unlike previous glycosyl iodide-based methods, this method provides mild conditions for the *in situ* generation of the iodide species, removing the necessity for harsh conditions, and uses shelf-stable thioglycoside donors. One potential downfall of this method is the generation of phenylthiolate ions through the reaction of TBAI with side products of the thioglycoside activation using the Ph_2SO/Tf_2O activating system.[25] This was found to irreversibly regenerate the starting phenyl thioglycoside donor by acting as a competitive nucleophile for glycosylation. This problem was averted with the use of a thiol scavenger, N-methylmaleimide.[21]

The observation that glycosylation still readily occurred in the face of regenerated starting donor was serendipitous, and led to the prediction that this glycosylation method would still work with glycosyl acceptors which contained both a free hydroxyl function and an anomeric phenyl thioglycoside. Excitingly, this prediction was experimentally supported **(Scheme 2)**.[21]



Scheme 2. Preliminary study on iterative synthesis with new α-selective glycosylation technique.

The conclusion that glycosyl acceptors could contain the necessary phenyl thioglycoside function to act as glycosyl donors in subsequent reactions allows consideration of this method for iterative synthesis. Iterative synthesis involves taking the product of one glycosylation reaction directly as the glycosyl donor for a subsequent glycosylation reaction. This would eliminate the need for directing group installation between glycosylations, creating a more efficient synthesis with fewer chemical steps, a smaller time burden, and a higher overall yield. Firstly, an improved method for α-glycosylation permits a wider library of antigenic material to be synthesized. In addition, an iterative method would greatly accelerate the synthesis of carbohydrate antigens for vaccine developments. The combined impact of both of these effects would have a substantially beneficial impact on human health as the rate of vaccine development is increased. The focus of this current work has been directed with this end goal in mind. However, as the monosaccharide building blocks required for this synthesis are not commercially available, it has been necessary to synthesize them from commercially available sugars.

Chapter 2 – Retrosynthesis

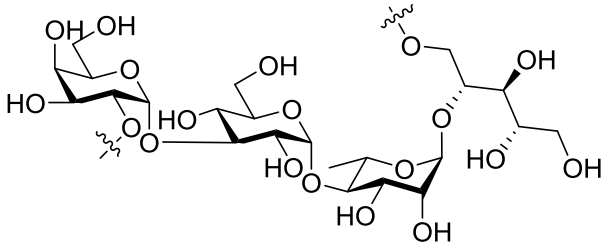
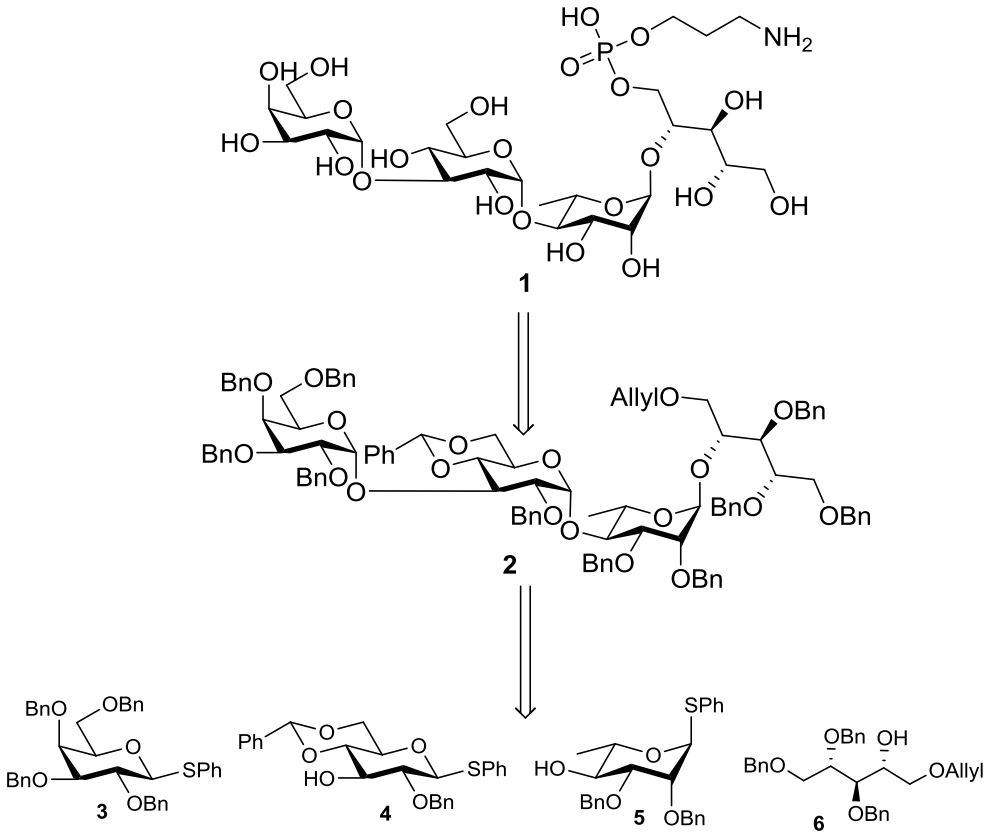


Figure 2. Minimum repeating unit of the *S. pneumoniae* serotype 6B capsular polysaccharide.



Scheme 3. Retrosynthetic analysis of a proposed antigenic *S. pneumoniae* serotype 6B capsular polysaccharide minimum repeating unit analogue.

It was envisioned that the minimum repeat of the *Streptococcus pneumoniae* capsular polysaccharide serotype 6B (**Figure 2**) could be synthesized as analogue **1** (**Scheme 3**). Compound **1** contains the minimum repeat tetrasaccharide attached to an aminopropyl linker through a phosphate group. The amino group on the linker will be used for conjugation of the

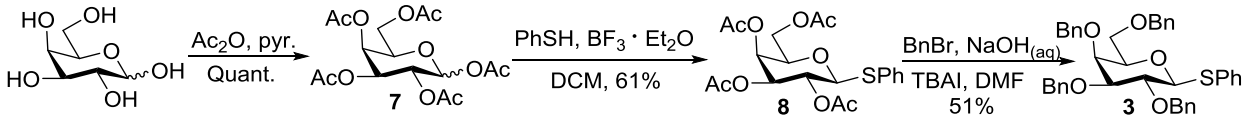
tetrasaccharide to a suitable carrier protein to enhance T cell-dependent immune response to the tetrasaccharide.

Compound **1** would arise through a series of protecting group manipulations on compound **2**. Envisioned in the synthetic direction, this would involve first removal of the allyl protecting group of **2**, followed by introduction of the linker to the free hydroxyl function, and then hydrogenation of all benzyl ether protecting groups.[26] Compound **2**, in turn, is seen as the product of three successive glycosylation reactions. In order in the synthetic direction, galactose donor **3** (three steps from commercially available D-galactopyranose[27]) must be glycosylated to glucose donor **4** (5 steps from commercially available D-glucopyranose[28,29]), then the disaccharide product must be glycosylated to rhamnose donor **5** (8 steps from commercially available L-rhamnopyranoside[30,31,32]), and the resulting trisaccharide glycosylated to final ribotol acceptor **6** (7 steps from commercially available methyl β-D ribofuranoside[33]). The 3 1,2-*cis* glycosidic linkages all could conveniently arise from this novel method involving the *in situ* conversion of the donor sugar to a glycosyl iodide prior to addition of the appropriate acceptor sugar[21]. There are thus a total of 26 synthetic steps envisioned between 4 commercially available sugars and compound **2**, and 4 additional steps between compound **2** and compound **1**, making a total of 30 steps to synthesize compound **1** from starting materials.

Two previous syntheses of the *S. pneumoniae* serotype 6B capsular polysaccharide minimum repeating unit have been reported. The 31-step Kamerling synthesis, in 1998[26], used older glycosylative methods that gave mixtures of α and β glycosides. The 29-step Demchenko synthesis, in 2007[34], involved multiple steps between each glycosylation, slowing down the synthesis and lowering the overall yield. It is envisioned that this proposed method would provide a more efficient synthesis of the *S. pneumoniae* serotype 6B capsular polysaccharide minimum repeating unit.

Chapter 3 – Synthesis

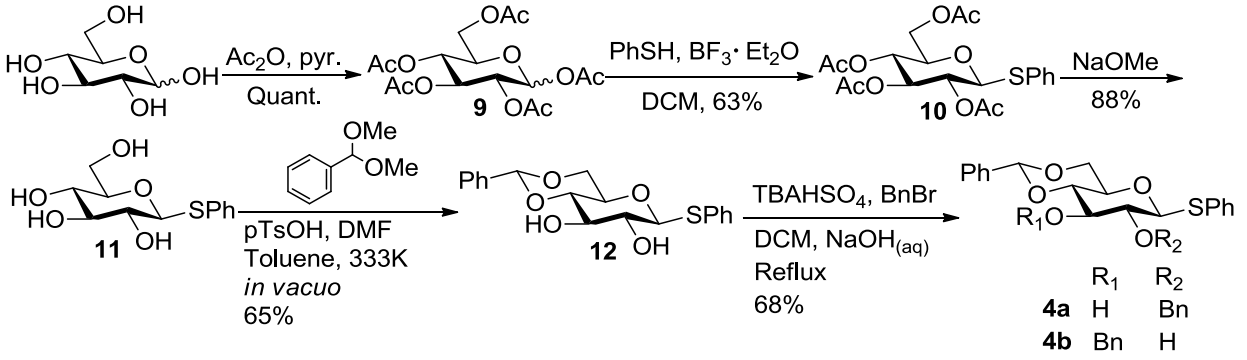
Synthesis of Galactose Building Block



Scheme 4. Synthesis of the galactose monosaccharide building block **3.**

The first step in the synthesis of galactose donor **3** (**Scheme 4**) involved the global acetylation of commercially available D-galactopyranose using acetic anhydride in pyridine solvent to yield galactose derivative **7** in quantitative yield. Compound **7** underwent glycoside formation with thiophenol and catalytic boron trifluoride diethyl etherate in methylene chloride to yield a mixture of α- and β- phenylthiogalactosides. β-phenylthiogalactoside **8** was isolated by flash column chromatography in 61% yield. Compound **8** then underwent one-pot basic hydrolysis of all acetyl ester groups and benzyl protection of all hydroxyl functions to yield galactose donor **3** in 51% yield.[27]

Synthesis of Glucose Building Block



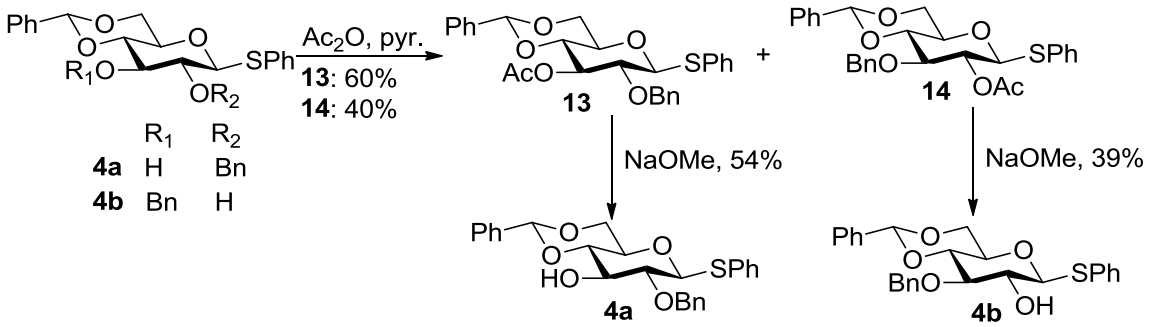
Scheme 5. Synthesis of a mixture of **4a** and **4b**, of which **4a** is the desired glucose monosaccharide building block.

The synthesis of the glucose building block **4a** (**Scheme 5**) began with the quantitative peracetylation of commercially available D-glucopyranose in acetic anhydride and pyridine solvent to yield compound **9**. Compound **9** then underwent glycoside formation with thiophenol and catalytic boron trifluoride diethyl etherate to yield a mixture of α- and β-phenylthioglucosides, where the β-phenylthioglucoside **10** was isolated in 63% yield. Initial reactions were observed to proceed very slowly, and were accelerated by sonication over the

course of three days. Basic methanol was then used to remove all acetyl esters from **10** to yield compound **11** in 88% yield.

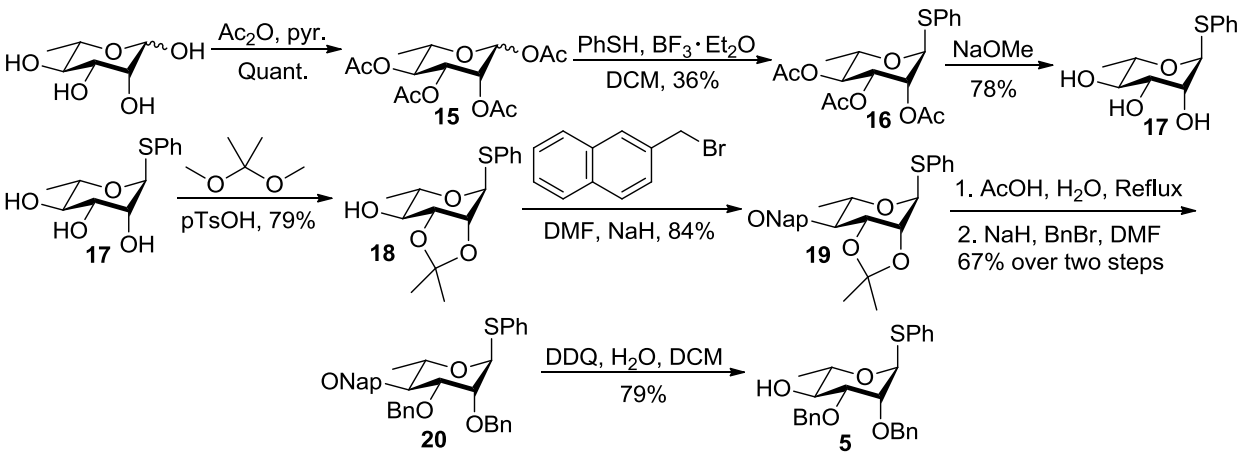
The 4,6-O-benzylidene protection of compound **11** was then performed with the dimethyl acetal of benzaldehyde and a catalytic quantity of *para*-toluenesulfonic acid in N,N'-dimethylformamide. Toluene was added to abstract the methanol product of the reaction, which was then evaporated *in vacuo* at 60°C on the rotary evaporator to drive the reaction forward, yielding benzylidene acetal **12** in 65% yield.[28] Compound **12** was then subjected to phase transfer benzylation conditions with refluxing benzyl bromide and a catalytic quantity of tetrabutylammonium hydrogensulfate, with both methylene chloride and a 50% solution of sodium hydroxide.[29] This provide a mixture of both benzyl regioisomers **4a** and **4b** in 68% yield.

Separation conditions for **4a** and **4b** could not be found, and so a mixture of the two compounds was acetylated using acetic anhydride and pyridine to give a mixture of the two compounds **13** (60% of the mixture) and **14** (40% of the mixture) (**Scheme 6**). These compounds were separated by flash column chromatography, and all proton and carbon NMR peaks were assigned with HSQC and COSY. It was observed that the faster eluting compound was compound **14**, and the slower eluting compound was **13**. This assignment was made based on the downfield shift of the C-2 proton in **14** relative to the C-2 in **13**, as well as the downfield shift of the C-3 proton in **13** relative to **14**. Both **13** and **14** were then subjected to basic methanol to removal acetyl protecting groups, yielding isolated compounds **4a** in 54% yield, and **4b** in 39% yield.



Scheme 6. Resolution of the desired glucose monosaccharide building block **4a** from a mixture of **4a** and **4b**.

Synthesis of Rhamnose Building Block



Scheme 7. Synthesis of the rhamnose monosaccharide building block **5**.

Synthesis of rhamnose monosaccharide building block **5** (**Scheme 7**) began with the peracetylation of commercially available L-rhamnose using acetic anhydride and pyridine to yield compound **15** in quantitative yield. This was then converted into phenyl thiorhamnopyranoside **16** using thiophenol with catalytic boron trifluoride diethyl etherate in 36% yield. Removal of the acetyl protecting groups using basic methanol yielded compound **17** in 78% yield.[30]

2,2-dimethoxypropane and *para*-toluenesulfonic acid was then used to selectively protect the C-2 and C-3 hydroxyls of **17** in 79% yield.[31] The remaining free hydroxyl at C-3 was then protected with napthylmethylbromide in dimethylformamide with sodium hydride to yield compound **19** in 84% yield. Refluxing **19** in acetic acid and water effected the hydrolysis of the isopropylidene group, and then treatment with benzyl bromide in dimethylformamide and sodium hydride protected the free hydroxyls to yield compound **20** in 67% yield over two steps.

All that remained in the synthesis of **5** was now the removal of the napthylmethyl protecting group. The oxidative removal of a napthylmethyl ether in the presence of benzyl ethers had been shown possible with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ, **Figure 3**) with a range of substrates.[32] Gratifyingly, using DDQ, the napthylmethyl ether was removed from **20** with DDQ to yield **5** in 79% yield.

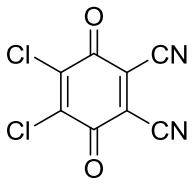
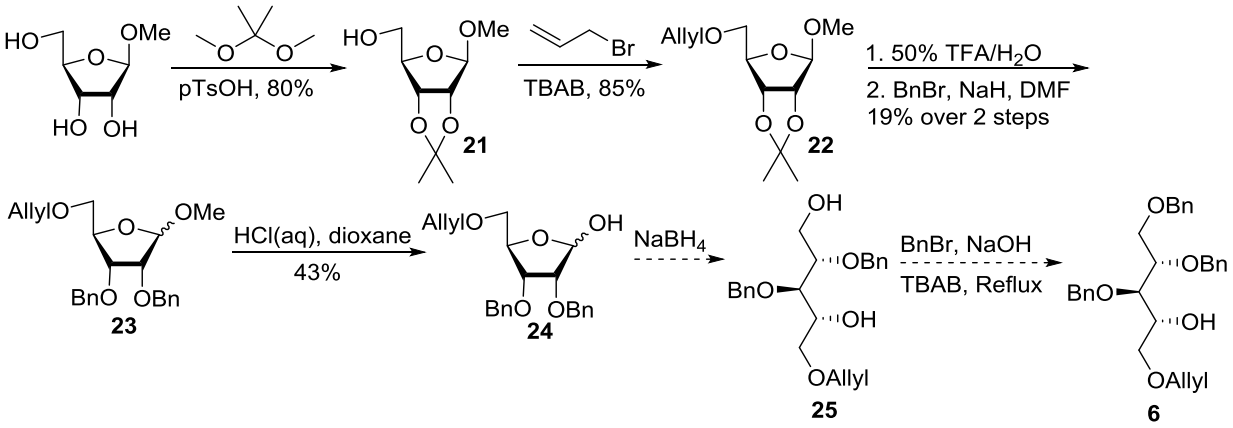


Figure 3. DDQ: 2,3-dichloro-5,6-dicyano-1,4-benzoquinone.

Synthesis of Ribotol Building Block

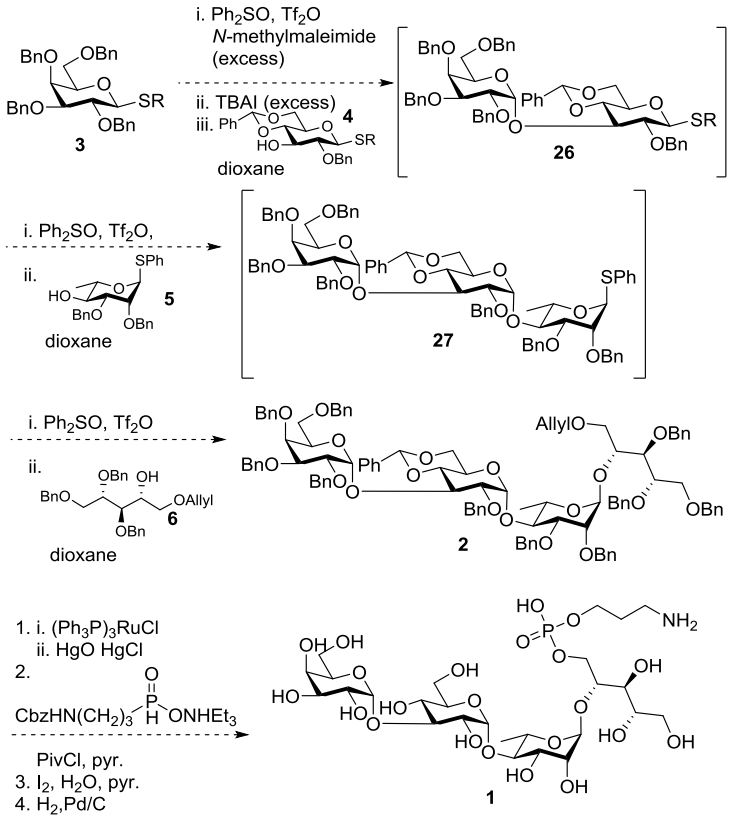


Scheme 8. Synthesis of the ribotol monosaccharide building block **6**.

The synthesis of the ribotol monosaccharide building block (**Scheme 8**) began with the protection of the *cis*-diol found in commercially available methyl β-D ribofuranoside. Once again, 2,2-dimethoxypropane was chosen and installed with *para*-toluenesulfonic acid. It was found that initially, 2 compounds were formed, but upon isolation, it was seen that one was the desired compound **21**, and the other was a second, acyclic acetal formed between a second equivalent of 2,2-dimethoxypropane and the C-5 hydroxyl. Subjection of the bis-protected compound to 2N HCl for one hour was found to be sufficient to hydrolyze the errant acetal to a hydroxyl group, but to leave the cyclic acetal between C-2 and C-3 untouched. This allowed the production of compound **21** in 80% yield. Treatment of **21** with neat allyl bromide and tetrabutylammonium bromide effected the desired protection of the C-5 hydroxyl to the allyl protecting group that will be crucial for the linker installation later in the synthesis, giving compound **22** in 85% yield.[33]

Refluxing compound **22** in 50% trifluoroacetic acid in water hydrolyzed the isopropylidene protecting group, yielding the 2,3-diol, which was then benzylated using benzyl bromide and sodium hydride in dimethylformamide. This yielded compound **23** in 19% yield over two steps. Compound **23** was then refluxed with 2N hydrochloric acid and dioxane to dissolve starting compound **23** to yield hemiacetal **24** in 43% yield. Hemiacetal **24** will then be reduced using sodium borohydride to yield diol **25**. Diol **25** will then be subjected to phase transfer benzylation conditions in a mixture of methylene chloride and sodium hydroxide solution with benzyl bromide and tetrabutylammonium bromide. This monobenzylation is expected to take advantage of the inherent reactivity of a primary alcohol compared to a secondary to generate predominately compound **6**.[33]

Chapter 4 – Conclusions and Future Directions



Scheme 9. Synthesis of **1**, the *S. pneumoniae* serotype 6B capsular polysaccharide minimum repeating unit with linker.

With compounds **3**, **4**, **5**, and **6** in hand, they can be assembled together to afford compound **1**, the *S. pneumoniae* serotype 6B capsular polysaccharide minimum repeating unit with linker. Glycosylation of **3** to **4** is anticipated to give compound **26**, which will then be glycosylated to **5** to give compound **27**. Finally, attachment of 27 to 6 will give compound **2**. Compound **2** then only requires the 4-step sequence to attach the linker and remove all benzyl ether protecting groups and the benzylidene acetal.

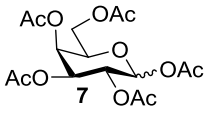
Once 1 is synthesized and conjugated to a T cell-inducing compound, it can be used in biological testing for its immunogenic activity as an isolated oligosaccharide. It can also be assembled onto a multivalent scaffold to potentially increase its ability to raise suitable levels of T-cell dependent immune response. Overall, however, this synthesis will serve as a powerful means to test the prospects of a novel and promising technology for the formation of a synthetically difficult class of glycosides.

Appendix I – Experimental Information

General Experimental Details

All reactions were performed under inert argon atmosphere. Flash column chromatography was performed on SiliCycle P-60 silica gel, 230-400 mesh. Analytical thin-layer chromatography was carried out on EMD silica gel 60 F-254 plates, and products were visualized using UV or by staining with 5% aqueous sulfuric acid. NMR spectra were recorded on a Bruker Avance III NMR spectrometer at 500 MHz or 300 MHz as indicated for ^{1}H-NMR and 125 MHz or 75 MHz as indicated for ^{13}C-NMR. Chemical shifts are reported in ppm relative to TMS (for ^{1}H-NMR in $CDCl_3$), H_2O (for ^{1}H-NMR in D_2O), or $CDCl_3$ (for ^{13}C-NMR in $CDCl_3$). For 1H NMR spectra, data are reported as δ shift, then multiplicity (s = singlet, d = doublet, dd = doublet of doublets, t = triplet, m = multiplet). Coupling constants are given in Hz. NMR solvents were purchased from Cambridge Isotope Labs. Methyl β-D-ribofuranoside was purchased from CarboSynth, and all other chemicals were purchased from Sigma-Aldrich at the highest possible purity and used without further purification.

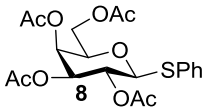
Experimental Data



1,2,3,4,6-penta-*O*-acetyl-D-galactopyranose (7):

D-galactopyranose (1 equiv., 28.05 mmol, 5.053 g) was dissolved in pyridine (90 mL) and treated with acetic anhydride (6.045 equiv., 169.6 mmol, 16 mL). The reaction was followed by thin-layer chromatography (5% MeOH/95% DCM). After 22 hours, the reaction was condensed. Residual pyridine was co-evaporated 3X with toluene, affording compound **7** (28.5 mmol, 11.12 g, quantitative yield).

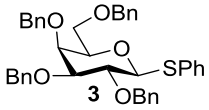
^{1}H NMR (300 MHz, $CDCl_3$): δ 6.38 (s, 1H), 5.51 (s, 1H), 5.31 (s, 2H), 4.35 (t, J = 6.2 Hz, 1H), 4.11 (m, 2H), 2.17 – 2.01 (m, 16H).



***S*-phenyl 2,3,4,6-tetra-*O*-acetyl-β-D-thiogalactopyranoside (8):**

Compound **7** (1 equiv., 27.75 mmol, 10.95 g) was dissolved in dry dichloromethane (80 mL) and cooled to 0°C in an ice bath. Boron trifluoride diethyl etherate (2.7 equiv., 75.65 mmol, 9.5 mL) was added dropwise to the stirring solution, followed by thiophenol (2.27 equiv., 63.57 mmol, 6.5 mL). After 5 minutes, the solution was removed from the ice bath and allowed to warm to room temperature. The reaction was monitored by thin-layer chromatography (40% EtOAc/60% Hex). After 53 hours, the reaction was condensed to remove all volatiles. The residue was dissolved in dichloromethane and neutralized with sodium bicarbonate, and then extracted 3X with brine, dried with sodium sulfate, filtered, and condensed. The product was purified by silica gel flash column chromatography (40% ethyl acetate in hexanes) to yield product **8** (17.02 mmol, 7.49 g, 61% yield).

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.53 – 7.51 (m, 2H), 7.31 – 7.30 (m, 3H), 5.42 (d, J = 3 Hz, 1H), 5.24 (t, J = 10 Hz, 1H), 5.06 (dd, J = 5, 3.5 Hz, 1H), 4.73 (d, J = 10 Hz, 1H), 4.21 – 4.10 (m, 2H), 3.95 (t, J = 7 Hz, 1H), 2.11 (s, 3H), 2.09 (s, 3H), 2.04 (s, 3H), 1.97 (s, 3H).

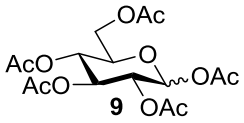


***S*-phenyl 2,3,4,6-tetra-*O*-benzyl-β-D-thiogalactopyranoside (3):**

In a flame-dried flask, finely-ground sodium hydroxide (26.07 equiv., 59.15 mmol, 2.368 g) and tetrabutylammonium iodide (0.112 equiv., 0.254 mmol, 0.0936 g) were dissolved in dimethylformamide (7.5 mL). Compound **8** (1 equiv., 2.27 mmol, 1.00 g) was separately dissolved in dimethylformamide (7.5 mL) and added dropwise. The sides of the flask were rinsed with dimethylformamide (5 mL). The reaction was followed by thin-layer chromatography (15% EtOAc/85% Hex). After 17 hours, the reaction was diluted with 20 mL of water and extracted 4X with diethyl ether. The ether layers were combined and extracted 2X with brine, dried, filtered, and concentrated. The product was purified by silica gel flash column chromatography (15% ethyl acetate in hexanes) to yield product **3** (1.17 mmol, 740 mg, 51% yield).

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.57 – 7.55 (m, 2H), 7.39 – 7.26 (m, 20H), 7.18 – 7.15 (m, 3H), 4.96 (d, J = 11.5 Hz, 1H), 4.79 – 4.69 (m, 4H), 4.63 (t, J = 9.5 Hz, 1H), 4.59 (s, 1H), 4.49 (dd, J = 14, 12 Hz, 2H), 3.98 (d, J = 2.5 Hz, 1H), 3.93 (t, J = 9.5 Hz, 1 H), 3.66 – 3.64 (m, 2H), 3.63 – 3.60 (m, 2H); **^{13}C NMR** (125 MHz, $CDCl_3$): δ 138.9, 138.4, 138.3, 138.0, 134.3, 131.6, 128.9,

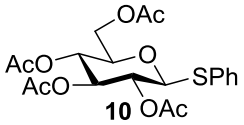
128.6, 128.5, 128.3, 128.0, 127.9, 127.8, 127.7, 127.6, 127.2, 87.8, 84.3, 75.8, 74.6, 73.7, 72.8, 68.9.



1,2,3,4,6-penta-*O*-acetyl-D-glucopyranose (9):

D-glucopyranose (1 equiv., 27.97 mmol, 5.04 g) was dissolved in pyridine (90 mL) and treated with acetic anhydride (6.045 equiv., 169.6 mmol, 16 mL). The reaction was followed by thin-layer chromatography (5% MeOH/95% DCM). After 20 hours, the reaction was condensed. Residual pyridine was co-evaporated 3X with toluene, affording compound **9** (28 mmol, 10.93 g, quantitative yield).

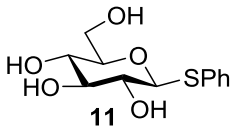
^{1}H NMR (500 MHz, $CDCl_3$): δ 6.34 (d, J = 3.5 Hz, 1H), 5.48 (t, J = 10 Hz, 1H), 5.17 – 5.09 (m, 2H), 4.27 (dd, J = 8, 3.5 Hz, 1H), 4.14 – 4.08 (m, 2H), 2.19 (s, 3H), 2.10 (s, 3H), 2.05 (s, 3H), 2.03 (s, 3H), 2.02 (s, 3H).



***S*-phenyl 2,3,4,6-tetra-*O*-acetyl-β-D-thioglucopyranoside (10):**

Compound **9** (1 equiv., 28 mmol, 10.93 g) was dissolved in dry dichloromethane (55 mL) and cooled to 0°C in an ice bath. Boron trifluoride diethyl etherate (1.49 equiv., 42.13 mmol, 5.2 mL) was added dropwise to the stirring solution, followed by thiophenol (1.21 equiv., 34.11 mmol, 3.5 mL). After 5 minutes, the solution was removed from the ice bath and allowed to warm to room temperature. The reaction was monitored by thin-layer chromatography (40% EtOAc/60% Hex). After 65 hours, the reaction was condensed to remove all volatiles. The residue was dissolved in dichloromethane and neutralized with sodium bicarbonate, and then extracted 2X with brine, dried with sodium sulfate, filtered, and condensed. The product was purified by silica gel flash column chromatography (25% ethyl acetate in hexanes) to yield product **10** (17.77 mmol, 7.82 g, 63% yield).

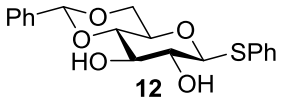
^{1}H NMR (300 MHz, $CDCl_3$): δ 7.51 – 7.48 (m, 2H), 7.33 – 7.30 (m, 3H), 5.23 (t, J = 9.3 Hz, 1H), 5.08 – 4.95 (m, 2H), 4.71 (d, J = 10.2 Hz, 1H), 4.26 – 4.16 (m, 2H), 3.76 – 3.70 (m, 1H), 2.091 (s, 3H), 2.085 (s, 3H), 2.02 (s, 3H), 1.99 (s, 3H).



***S*-phenyl β-D-thioglucopyranoside (11):**

Compound **10** (1 equiv., 17.77 mmol, 7.82 g) was dissolved in a prepared solution of sodium methoxide (0.5480 g Na dissolved in 180 mL of methanol). The reaction was monitored by thin-layer chromatography (40% EtOAc/60% Hex). After 1 hour, the reaction was neutralized with Amberlite IR-120 resin, filtered, and condensed, affording compound **11** (15.61 mmol, 4.25 g, 88% yield).

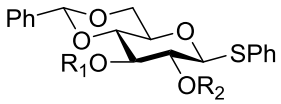
^{1}H NMR (500 MHz, D_2O): δ 7.57 – 7.56 (m, 2H), 7.41 – 7.37 (m, 3H), 3.88 (dd, J = 6.25, 1.5 Hz, 1H), 3.70 (dd, J = 6.25, 5.5 Hz, 1H), 3.51 (t, J = 9 Hz, 1H), 3.48 – 3.45 (m, 1H), 3.39 (t, J = 9.5 Hz, 1H), 3.34 (t, J = 9.5 Hz, 1H).



***S*-phenyl 4,6-*O*-benzylidene-β-D-thioglucopyranoside (12):**

Compound **11** (1 equiv., 3.75 mmol, 1.02 g) was dissolved in dry dimethylformamide (8 mL) and dry toluene (8 mL) and treated with *para*-toluenesulfonic acid monohydrate (17 mol%, 0.638 mmol, 0.12 g) and benzaldehyde dimethyl acetal (1.51 equiv., 5.68 mmol, 1.7 mL). The reaction was placed under the reduced pressure on the rotary evaporator at 60°C and 100 rpm. After 2 hours, the reaction was found to be complete by thin-layer chromatography (5% MeOH/95% DCM), and neutralized with saturated aqueous sodium bicarbonate, followed by dilution with DCM and water. The organic layer was collected, and the aqueous layer was extracted 2X with DCM. All organic layers were combined and extracted 3X with brine, then dried, filtered, and concentrated. The product was purified by silica gel flash column chromatography (5% methanol in methylene chloride) to yield **12** (2.39 mmol, 860 mg, 65% yield).

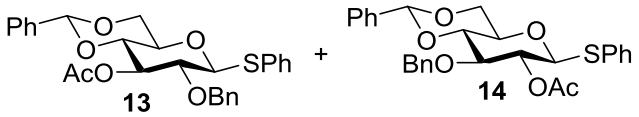
^{1}H NMR (500 MHz, $CDCl_3$): δ 8.11 – 8.10 (m, 2H), 7.61 – 7.58 (m, 1H), 7.55 – 7.44 (m, 6H), 7.37 – 7.25 (m, 6H), 5.51 (s, 1H), 4.62 (d, J = 10 Hz, 1H), 4.38 – 4.35 (m, 1H), 3.83 (t, J = 8.5 Hz, 1H), 3.76 (t, J = 9.5 Hz, 1H), 3.53 – 3.44 (m, 4H), 3.20 (s, 1H), 2.16 (s, 1H).



	R_1	R_2
4a	H	Bn
4b	Bn	H

_S_-phenyl 2-_O_-benzyl-4,6-_O_-benzylidene-β-D-thioglucopyranoside (4a) and _S_-phenyl 3-_O_-benzyl-4,6-_O_-benzylidene-β-D-thioglucopyranoside (4b):

Compound **12** (1 equiv., 6.102 mmol, 2.2 g) and tetrabutylammonium hydrogen sulfate (23.2 mol%, 1.42 mmol, 0.4802 g) were dissolved in methylene chloride (100 mL) and treated with benzyl bromide (1.72 equiv., 10.51 mmol, 1.25 mL) and an aqueous solution of sodium hydroxide (0.4209 g of sodium hydroxide in 8.7 mL of water, 4.84% solution). The resulting solution was refluxed at 50°C for 66 hours and followed by thin-layer chromatography (20% ethyl acetate in hexanes). The organic layer was then separated and washed 1X with 100 mL of water, dried with sodium sulfate, filtered, and concentrated. Silica gel flash column chromatography (15% ethyl acetate in hexanes) resulted in a mixture of compounds **4a** and **4b** (4.12 mmol, 1.85 g, 68% yield).



_S_-phenyl 3-_O_-acetyl-2-_O_-benzyl-4,6-_O_-benzylidene-β-D-thioglucopyranoside (13) and _S_-phenyl 2-_O_-acetyl-3-_O_-benzyl-4,6-_O_-benzylidene-β-D-thioglucopyranoside (14):

A mixture of compounds 4a and 4b (1 equiv., 1.11 mmol, 517.8 mg) was dissolved in pyridine (3.6 mL) and treated with acetic anhydride (2.90 equiv., 3.22 mmol, 0.3 mL). The reaction was monitored by thin-layer chromatography (10% ethyl acetate in hexanes). After 16 hours, the reaction was condensed and co-evaporated 3X with toluene. The products were separated by silica gel flash column chromatography (hexanes → 10% ethyl acetate in hexanes) to afford compounds **13** (0.67 mmol, 302 mg, 60% yield) and **14** (0.44 mmol, 198 mg, 40% yield).

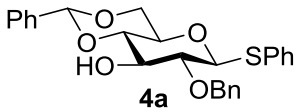
Compound **13**:

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.54 – 7.51 (m, 2H, H-Ar), 7.42 – 7.39 (m, 2H, H-Ar), 7.35 – 7.23 (m, 12H, H-Ar), 5.44 (s, 1H, CHPhO-), 5.38 (t, J = 9 Hz, 1H, H-3), 4.89 (d, J = 11 Hz, 1H, OCH_2Ph), 4.80 (d, J = 10 Hz, 1H, H-1), 4.60 (d, J = 10.5 Hz, 1H, OCH_2Ph), 4.32 (dd, J = 5.25, 5 Hz, 1H, H-6), 3.73 (t, J = 10.5 Hz, 1H, H-6'), 3.58 – 3.51 (m, 2H, H-2, H-4), 3.50 – 3.47 (m, 1H, H-5), 1.92 (s, 3H, $OC(O)CH_3$) ;**^{13}C NMR** (125 MHz, $CDCl_3$): δ 169.6 (OC(O)), 137.6 (C-Ar), 136.9 (C-Ar), 132.9 (C-Ar), 132.2 (C-Ar), 129.1 (C-Ar), 129.0 (C-Ar), 128.4 (C-Ar), 128.2 (C-

Ar), 128.1 (C-Ar), 128.0 (C-Ar), 127.97 (C-Ar), 127.9 (C-Ar), 126.1 (C-Ar), 101.2 (CHPh), 88.3 (C-1), 79.1 (C-2), 78.5 (C-4), 75.1 (OCH_2Ph), 74.2 (C-3), 70.3 (C-5), 88.5 (C-6), 20.8 (CH_3).

Compound **14**:

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.49 – 7.43 (m, 4H, H-Ar), 7.42 – 7.35 (m, 3H, H-Ar), 7.35 – 7.23 (m, 8H, H-Ar), 5.56 (s, 1H, CHPhO-), 5.03 (t, J = 9.5 Hz, 1H, H-2), 4.86 (d, J = 12 Hz, 1H, OCH_2Ph), 4.71 – 4.64 (m, 2H, H-1, OCH_2Ph), 4.37 (dd, J = 5.25, 4.5 Hz, H-6), 3.81 – 3.70 (m, 3H, H-6, H-3, H-4), 3.52 – 3.49 (m, 1H, H-5), 2.02 (s, 3H, $OC(O)CH_3$) ;**^{13}C NMR** (125 MHz, $CDCl_3$): δ 169.4 (OC(O)), 138.2 (C-Ar), 137.2 (C-Ar), 132.7 (C-Ar), 132.4 (C-Ar), 129.0 (C-Ar), 128.4 (C-Ar), 128.37 (C-Ar), 128.0 (C-Ar), 126.1 (C-Ar), 101.3 (CHPh), 86.9 (C-1), 81.4 and 79.8 (C-3 and C-4), 74.4 (OCH_2Ph), 71.4 (C-2), 70.6 (C-5), 70.3 (C-5), 68.6 (C-6), 21.1 (CH_3).

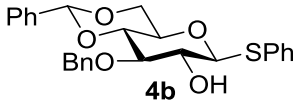


4a

***S*-phenyl 2-*O*-benzyl-4,6-*O*-benzylidene-β-D-thioglucopyranoside (4a):**

Compound **13** (1 equiv., 1.035 mmol, 510 mg) was dissolved in dry methanol (9 mL) and treated with 0.8 mL of a prepared sodium methoxide solution (260 mg Na in 10 mL of methanol). The reaction was monitored by thin-layer chromatography (10% ethyl acetate in hexanes). After 3 hours, the reaction was neutralized with IR-Amberlite resin, filtered, and condensed, yielding compound **4a** (0.556 mmol, 200.5 mg, 54% yield).

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.54 – 7.51 (m, 2H, H-Ar), 7.48 – 7.43 (m, 2H, H-Ar), 7.43 – 7.38 (m, 2H, H-Ar), 7.38 – 7.26 (m, 10H, H-Ar), 5.48 (s, 1H, CHPhO-), 4.91 (d, J = 11 Hz, 1H, OCH_2Ph), 4.79 (d, J = 11 Hz, 1H, OCH_2Ph), 4.71 (d, J = 10 Hz, 1H, H-1), 4.33 (dd, J = 5.25, 5 Hz, 1H, H-6), 3.85 (t, J = 9 Hz, 1H, H-3), 3.73 (t, J = 10 Hz, 1H, H-6'), 3.48 (t, J = 9.5 Hz, 1H, H-4), 3.42 – 3.38 (m, 2H, H-2, H-5), 2.68 (s, 1H, OH) ;**^{13}C NMR** (125 MHz, $CDCl_3$): δ 138.1 (C-Ar), 137.0 (C-Ar), 133.2 (C-Ar), 132.3 (C-Ar), 129.4 (C-Ar), 129.1 (C-Ar), 128.6 (C-Ar), 128.4 (C-Ar), 128.3 (C-Ar), 128.1 (C-Ar), 128.0 (C-Ar), 126.4 (C-Ar), 101.9 (C-7), 88.0 (C-1), 80.8 (C-2), 80.3 (C-4), 75.6 (C-3), 75.4 (OCH_2Ph), 70.1 (C-5), 68.7 (C-6).

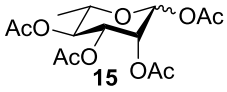


4b

***S*-phenyl 3-*O*-benzyl-4,6-*O*-benzylidene-β-D-thioglucopyranoside (4b):**

Compound **14** (1 equiv., 0.122 mmol, 60 mg) was dissolved in dry methanol (1 mL) and treated with 0.1 mL of a prepared sodium methoxide solution (260 mg Na in 10 mL of methanol). The reaction was monitored by thin-layer chromatography (10% ethyl acetate in hexanes). After 3 hours, the reaction was neutralized with IR-Amberlite resin, filtered, and condensed, yielding compound **4b** (0.0472 mmol, 17 mg, 39% yield).

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.55 – 7.52 (m, 2H, H-Ar), 7.49 – 7.46 (m, 2H, H-Ar), 7.41 – 7.26 (m, 11H, H-Ar), 5.57 (s, 1H, CHPhO-), 4.95 (d, J = 11.5 Hz, 1H, OCH_2Ph), 4.79 (d, J = 11.5 Hz, 1H, OCH_2Ph), 4.63 (d, J = 10 Hz, 1H, H-1), 4.39 (dd, J = 5.25, 5 Hz, 1H, H-6), 3.80 (t, J = 10.5 Hz, 1H, H-6'), 3.72 – 3.64 (m, 2H, H-3, H-4), 3.55 – 3.49 (m, 2H, H-5, H-2), 2.55 (d, J = 2 Hz, 1H, OH) ;**^{13}C NMR** (125 MHz, $CDCl_3$): δ 138.3 (C-Ar), 137.3 (C-Ar), 133.4 (C-Ar), 131.4 (C-Ar), 129.2 (C-Ar), 129.17 (C-Ar), 128.6 (C-Ar), 128.5 (C-Ar), 128.4 (C-Ar), 128.3 (C-Ar), 128.1 (C-Ar), 126.2 (C-Ar), 101.4 (CHPhO-), 88.6 (C-1), 81.8 and 81.3 (C-3 and C-4), 75.0 (OCH_2Ph), 72.4 (C-2), 70.9 (C-5), 68.8 (C-6).

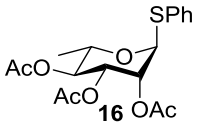


15

1,2,3,4-tetra-*O*-acetyl-L-rhamnopyranose (15):

L-rhamnopyranose (1 equiv., 5.85 mmol, 1.07 g) was dissolved in pyridine (17 mL) and treated with acetic anhydride (5.80 equiv., 33.92 mmol, 3.2 mL). The reaction was followed by thin-layer chromatography (5% MeOH/95% DCM). After 20 hours, the reaction was condensed. Residual pyridine was co-evaporated 3X with toluene, affording compound **15** (5.85 mmol, 2.05 g, quantitative yield).

^{1}H NMR (500 MHz, $CDCl_3$): δ 6.00 (s, 1H), 5.32 – 5.23 (m, 2H), 5.15 – 5.03 (m, 1H), 4.00 – 3.90 (m, 1H), 2.20 – 2.16 (m, 6H), 2.07 (s, 3H), 2.00 (s, 3H), 1.23 (d, J = 10.5 Hz, 3H).



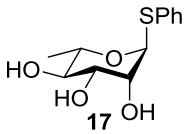
16

***S*-phenyl 2,3,4-tri-*O*-acetyl-α-L-thiorhamnopyranoside (16):**

Compound **15** (1 equiv., 33.68 mmol, 11.80 g) was dissolved in dry dichloromethane (150 mL) and cooled to 0°C in an ice bath. Boron trifluoride diethyl etherate (1.49 equiv., 50.24 mmol, 6.2 mL) was added dropwise to the stirring solution, followed by thiophenol (1.22 equiv., 41.04 mmol, 4.2 mL). After 5 minutes, the solution was removed from the ice bath and allowed to

warm to room temperature. The reaction was monitored by thin-layer chromatography (25% EtOAc/75% Hex). After 4 hours, the reaction was condensed to remove all volatiles. The residue was dissolved in dichloromethane and neutralized with sodium bicarbonate, and then extracted 3X with brine, dried with sodium sulfate, filtered, and condensed. The product was purified by silica gel flash column chromatography (25% ethyl acetate in hexanes) to yield product **16** (12.19 mmol, 4.66 g, 36% yield).

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.51 – 7.46 (m, 2H), 7.33 – 7.26 (m, 3H), 5.50 (dd, J = 1.75, 1.5 Hz, 1H), 5.41 (s, 1H), 5.29 (dd, J = 5, 3.5 Hz, 1H), 5.17 – 5.12 (m, 1H), 4.39 – 4.33 (m, 1H), 2.13 (s, 3H), 2.07 (s, 3H), 2.01 (s, 3H), 1.24 (d, J = 6 Hz, 3H).

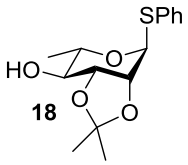


17

***S*-phenyl α-L-thiorhamnopyranoside (17):**

Compound **16** (1 equiv., 12.19 mmol, 4.66 g) was dissolved in methanol (40 mL) and treated with a prepared solution of sodium methoxide (10 mL of 0.1N in methanol). The reaction was monitored by thin-layer chromatography (25% EtOAc/75% Hex). After 3 hours, the reaction was neutralized with Amberlite IR-120 resin, filtered, and condensed, affording compound **17** (9.48 mmol, 2.43 g, 78% yield).

^{1}H NMR (500 MHz, D_2O): δ 7.60 – 7.58 (m, 2H), 7.48 – 7.42 (m, 3H), 5.47 (s, 1H), 4.26 – 4.21 (m, 2H), 3.87 (dd, J = 5, 3 Hz, 1H), 3.55 (t, J = 10 Hz, 1H), 1.29 (d, J = 6 Hz, 3H).

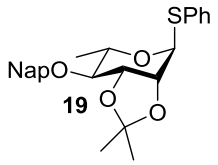


18

***S*-phenyl 2,3-*O*-isopropylidene-α-L-thiorhamnopyranoside (18):**

Compound **17** (1 equiv., 9.48 mmol, 2.43 g) was dissolved in 2,2-dimethoxypropane (12.91 equiv., 122.4 mmol, 15 mL) and treated with *para*-toluenesulfonic acid monohydrate (1.67 mol%, 0.158 mmol, 30 mg). The reaction was monitored by thin-layer chromatography (40% ethyl acetate in hexanes). After 18 hours, the reaction was neutralized with triethylamine and condensed to afford compound **18** (7.46 mmol, 2.21 g, 79% yield).

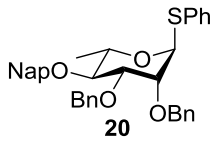
^{1}H NMR (500 MHz, $CDCl_3$): δ 7.47 – 7.45 (m, 2H), 7.29 – 7.21 (m, 3H), 5.78 (s, 1H), 4.34 (d, J = 5 Hz, 1H), 4.13 (t, J = 7.5 Hz, 1H), 4.11 – 4.07 (m, 1H), 3.78 (d, J = 4.5 Hz, 1H), 3.47 – 3.44 (m, 1H), 1.53 (s, 3H), 1.35 (s, 3H), 1.24 (d, J = 6.5 Hz, 3H).



***S*-phenyl 2,3-*O*-isopropylidene-4-*O*-(2-naphthyl-methyl)-α-L-thiorhamnopyranoside (19):**

Compound **18** (1 equiv., 7.46 mmol, 2.21 g) was dissolved in dimethylformamide (2.5 mL) and cooled to 0°C in an ice bath. The solution was treated with sodium hydride (95% in mineral oil, 3.07 equiv., 22.91 mmol, 0.5789 g) followed by 2-(bromomethyl)naphthalene (2.06 equiv., 15.35 mmol, 3.395 g). The reaction was then removed from the ice bath and allowed to warm to room temperature. The reaction was monitored by thin-layer chromatography (15% ethyl acetate in hexanes). After 21 hours, the reaction was diluted with ethyl acetate and extracted with saturated aqueous ammonium chloride, dried with sodium sulfate, filtered, and concentrated. The product was purified by silica gel flash column chromatography (15% ethyl acetate in hexanes) to afford compound **19** (6.28 mmol, 2.74 g, 84% yield).

^{1}H NMR (300 MHz, $CDCl_3$): δ 7.87 (m, 8H), 7.54 (m, 8H), 7.32 – 7.27 (m, 2H), 5.74 (s, 1H), 5.06 (d, J = 11.7 Hz, 1H), 4.78 (d, J = 11.7 Hz, 1H), 4.35 (s, 2H), 4.22 – 4.16 (m, 1H), 3.37 – 3.33 (m, 1H), 1.48 (s, 3H), 1.35 (s, 3H), 1.25 (d, J = 6.3 Hz).

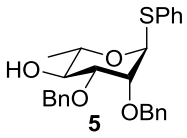


***S*-phenyl 2,3-di-*O*-benzyl-4-*O*-(2-naphthyl-methyl)-α-L-thiorhamnopyranoside (20):**

Compound **19** (1 equiv., 6.28 mmol, 2.74 g) was refluxed in acetic acid/water/1,4-dioxane (58 mL/6.5 mL/32 mL) for 8 hours. The reaction was followed by thin-layer chromatography (15% ethyl acetate in hexanes). The reaction was then condensed, and co-evaporated 3X with toluene. The residue was then dissolved in dimethylformamide (48 mL) and cooled to 0°C in an ice bath. The solution was then treated with sodium hydride (95% in mineral oil, 4.23 equiv., 26.58 mmol, 671 mg) and benzyl bromide (4.02 equiv., 25.22 mmol, 3 mL). The reaction was then removed from the ice bath and allowed to warm to room temperature. The reaction was monitored by thin-layer chromatography (40% ethyl acetate in hexanes). After 4 hours, the reaction was diluted

with methylene chloride, washed with saturated aqueous ammonium chloride, dried with sodium sulfate, filtered, and condensed. The product was purified by silica gel flash column chromatography (15% ethyl acetate in hexanes) to yield compound **20** (4.21 mmol, 2.43 g, 67% yield).

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.80 – 7.75 (m, 4H), 7.46 – 7.17 (m, 19H), 5.52 (s, 1H), 5.11 (d, J = 11 Hz, 1H), 4.80 (d, J = 11 Hz, 1H), 4.67 (dd, J = 18, 12.5 Hz, 2H), 4.60 (s, 2H), 4.22 – 4.16 (m, 1H), 4.011 (t, J = 2.5 Hz, 1H), 3.88 (dd, J = 4.75, 3 Hz, 1H), 3.753 (t, J = 10 Hz, 1H), 1.37 (d, J = 6 Hz).

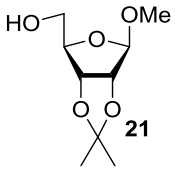


5

***S*-phenyl 2,3-di-*O*-benzyl-α-L-thiorhamnopyranoside (5):**

Compound **20** (1 equiv., 2.54 mmol, 1.47 g) was dissolved in methylene chloride (82 mL) and water (4.80 mL) and treated with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (2.29 equiv., 5.67 mmol, 0.8587 g) at 0°C in an ice bath. After 30 minutes, the reaction was removed from the ice bath and allowed to warm to room temperature. The reaction was followed by thin-layer chromatography (40% ethyl acetate in hexanes). 1.5 hours after removing from the ice bath, the reaction was diluted with methylene chloride and extracted with saturated aqueous sodium bicarbonate 2X, dried with sodium sulfate, filtered, and condensed. The product was purified by silica gel flash column chromatography (hexanes → 50% ethyl acetate in hexanes) to afford compound **5** (1.62 mmol, 707.6 mg, 79% yield).

^{1}H NMR (300 MHz, $CDCl_3$): δ 7.44 – 7.38 (m, 2H), 7.36 – 7.20 (m, 13H), 5.55 (d, J = 1.2 Hz, 1H), 4.68 (d, J = 12.3 Hz, 1H), 4.52 (dd, J = 5.85, 3.3 Hz, 2H), 4.41 (d, J = 11.7 Hz, 1H), 4.16 – 4. 06 (m 1H), 4.01 – 3.99 (m, 1H), 3.80 (t, J = 9.3 Hz, 1H), 3.62 (dd, J = 4.65, 3 Hz, 1H), 2.56 (s, 1H), 1.34 (d, J = 6 Hz, 3H); **^{13}C NMR** (75 MHz, $CDCl_3$): δ 137.8, 134.7, 131.4, 129.1, 128.7, 128.5, 128.1, 128.07, 128.04, 127.9, 127.4, 85.9, 79.7, 75.8, 72.1, 71.9, 71.6, 69.8, 17.8.

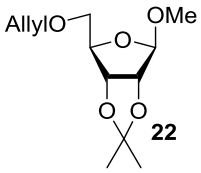


21

***O*-Methyl 2,3-*O*-isopropylidene-β-D-ribofuranoside (21):**

Methyl β-D-ribofuranoside (1 equiv., 41.4 mmol, 6.8 g) was dissolved in 2,2-dimethoxypropane (12.61 equiv., 0.523 mol, 64 mL) and treated with *para*-toluenesulfonic acid monohydrate (2.13 mol%, 0.882 mmol, 0.1672 g). The reaction was monitored by thin-layer chromatography (15% ethyl acetate in hexanes). After 18 hours, 2N HCl (10 mL) was added to the solution. After a further 1 hour, the reaction was neutralized with saturated aqueous sodium bicarbonate and diluted with diethyl ether. The organic layer was extracted with saturated sodium chloride 1X and water 1X, then dried with sodium sulfate, filtered, and condensed to afford compound **21** (33.14 mmol, 8.07 g, 80% yield).

^{1}H NMR (500 MHz, $CDCl_3$): δ 4.71 (s, 1H), 4.52 (d, J = 6 Hz, 1H), 4.33 (d, J = 6 Hz, 1H), 4.07 (s, 1H), 3.35 – 3.30 (m, 3H), 3.13 (s, 3H), 1.22 (s, 3H), 1.06 (s, 1H).

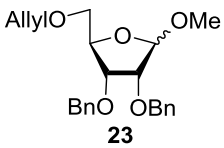


22

***O*-Methyl 5-*O*-allyl-2,3-*O*-isopropylidene-β-D-ribofuranoside (22):**

Compound **21** (1 equiv., 33.14 mmol, 8.07 g) was suspended in allyl bromide (1.81 equiv., 60.11 mmol, 5.2 mL) and treated with a 52% solution of sodium hydroxide (6.2366 g of sodium hydroxide in 12 mL of water) and tetrabutylammonium bromide (1.67 mol%, 0.553 mmol, 0.1775 g). The reaction was followed by thin-layer chromatography (15% ethyl acetate in hexanes). After 16 hours, the reaction was diluted in methylene chloride and water. The organic layer was dried with sodium sulfate, filtered, and condensed. The product was purified by silica gel flash column chromatography (15% ethyl acetate in hexanes) to afford compound **22** (28.13 mmol, 6.85 g, 85% yield).

^{1}H NMR (500 MHz, $CDCl_3$): δ 5.74 – 5.71 (m, 1H), 5.15 (d, J = 1.5 Hz, 1H), 5.03 (dd, J = 5.25, 1.5 Hz, 1H), 4.80 (s, 1H), 4.52 (d, J = 6 Hz, 1H), 4.42 (d, J = 6 Hz, 1H), 4.17 (t, J = 8 Hz, 1H), 3.87 (dd, J = 3, 1.5 Hz, 2H), 3.35 – 3.24 (m, 2H), 3.16 (s, 3H), 1.32 (s, 3H), 1.16 (s, 3H).

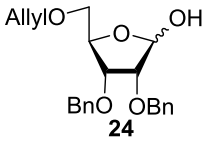


23

***O*-Methyl 5-*O*-allyl-2,3-di-*O*-benzyl-β-D-ribofuranoside (23):**

Compound **22** (1 equiv., 3.022 mmol, 0.7358 g) was dissolved in methanol (5 mL) and refluxed in 2 mL of 50% trifluoroacetic acid in water for 4 hours. The reaction was followed by thin-layer chromatography (15% ethyl acetate in hexanes). The reaction was then neutralized with Amberlite IRA-402(OH) ion exchange resin, filtered, and condensed. The residue was then dissolved in dimethylformamide (23 mL) and cooled to 0°C in an ice bath. The solution was then treated with sodium hydride (95% in mineral oil, 4.13 equiv., 12.48 mmol, 315 mg) and benzyl bromide (4.17 equiv., 12.60 mmol, 1.5 mL). The reaction was then removed from the ice bath and allowed to warm to room temperature. The reaction was monitored by thin-layer chromatography (15% ethyl acetate in hexanes). After 17 hours, the reaction was diluted diethyl ether and water. The organic layer was extracted, and washed 4X with water, washed, dried with sodium sulfate, filtered, and condensed. The product was purified by silica gel flash column chromatography (15% ethyl acetate in hexanes) to yield compound **23** (0.571 mmol, 220 mg, 19% yield over 2 steps).

^{1}H NMR (300 MHz, $CDCl_3$): δ 7.35 – 7.25 (m, 12H), 5.91 – 5.80 (m, 1H), 5.29 – 5.13 (m, 2H), 4.91 (s, 1H), 4.68 – 4.43 (m, 4H), 4.34 – 4.28 (m, 1H), 4.02 – 3.97 (m, 3H), 3.84 – 3.82 (d, J = 4.5 Hz, 1H), 3.59 – 3.44 (m, 2H), 3.32 (s, 3H).



24

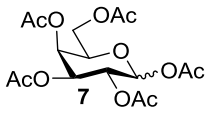
5-*O*-allyl-2,3-di-*O*-benzyl-D-ribofuranose (24):

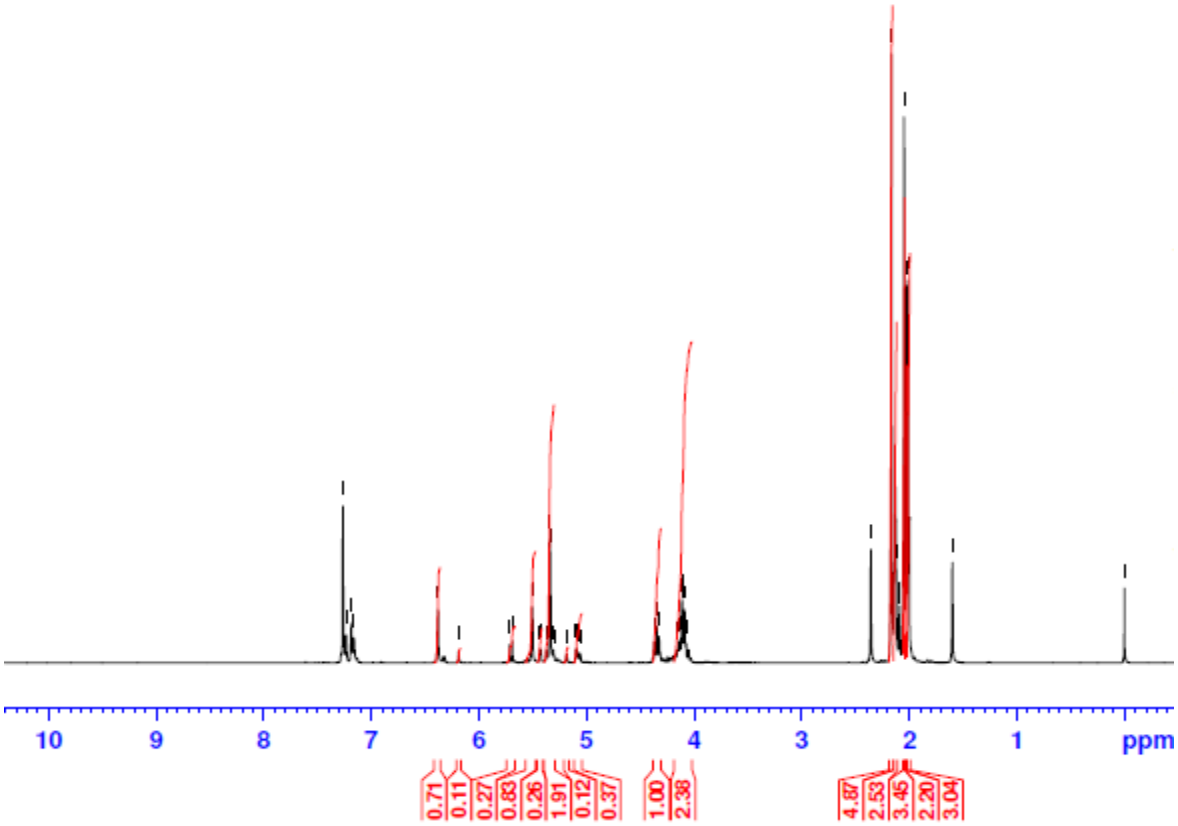
Compound **23** (1 equiv., 0.571 mmol, 220 mg) was dissolved in 1,4-dioxane (2 mL) and treated with 2N HCl (2 mL). The reaction was followed by thin-layer chromatography (10% ethyl acetate in hexanes) for two hours. The reaction was then neutralized with Amberlite IRA-402(OH) ion exchange resin. The resin was filtered out and the product was concentrated. The product was purified by silica gel flash column chromatography (15% ethyl acetate in hexanes) to yield compound **24** (0.246 mmol, 90.4 mg, 43% yield).

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.39 – 7.24 (m, 10H), 5.85 – 5.75 (m, 1H), 5.32 – 5.30 (m, 1H), 5.21 – 5.10 (m, 2H), 4.75 – 4.58 (m, 4H), 4.22 – 4.19 (m, 1H), 4.10 – 3.86 (m, 3H), 3.55 – 3.35 (m, 2H).

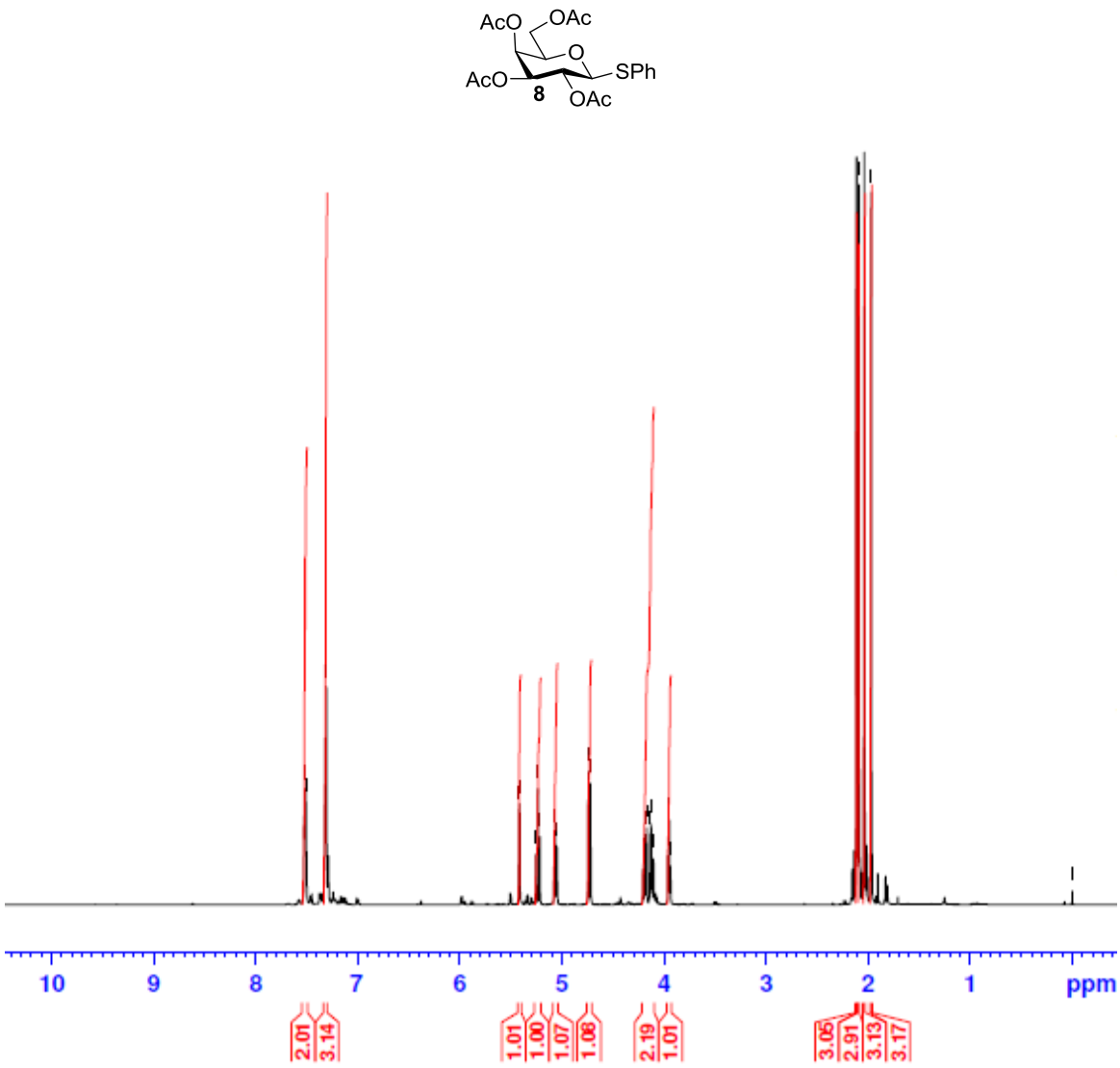
Appendix II – NMR Spectra

^{1}H NMR (300 MHz, $CDCl_3$)

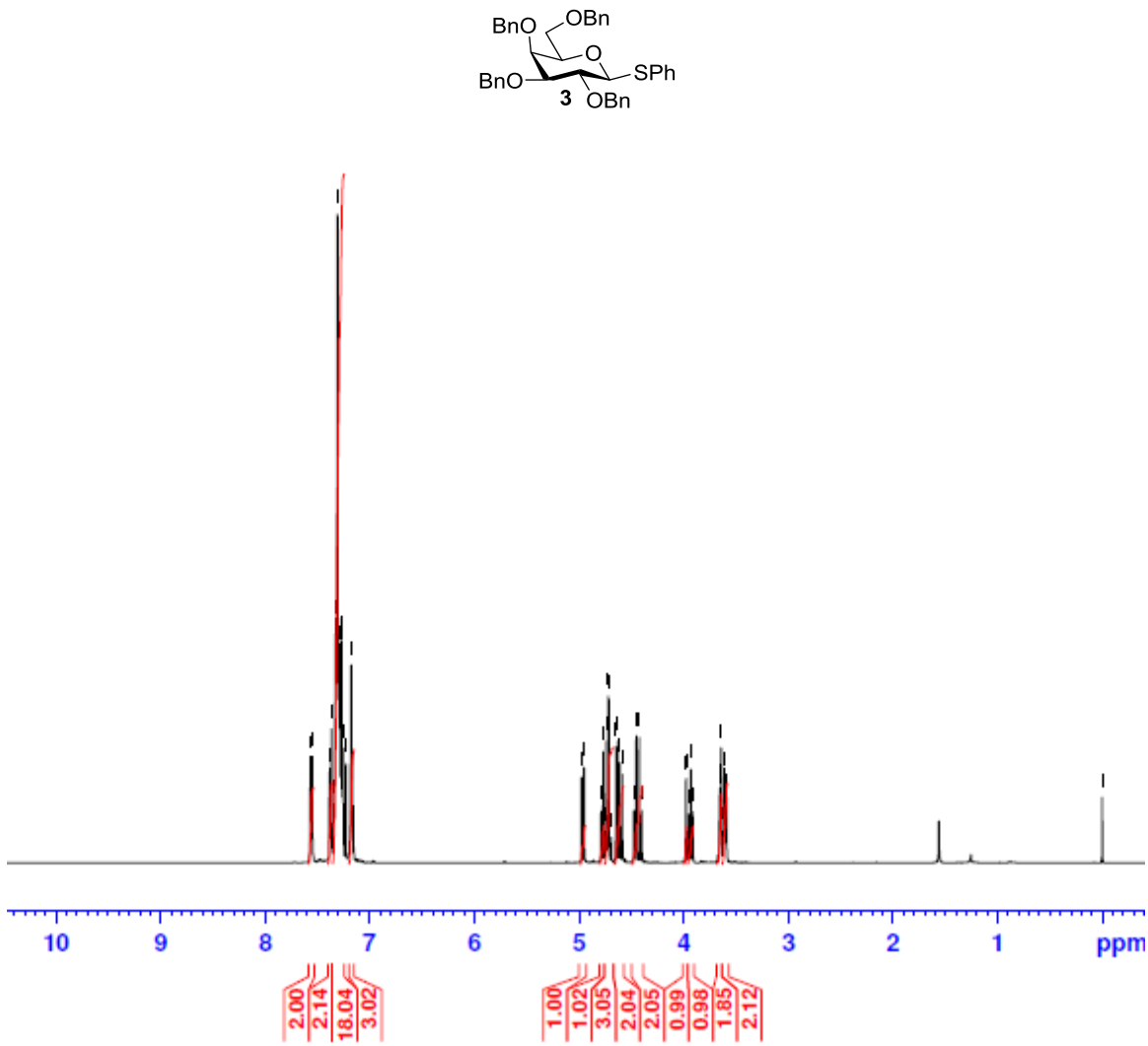




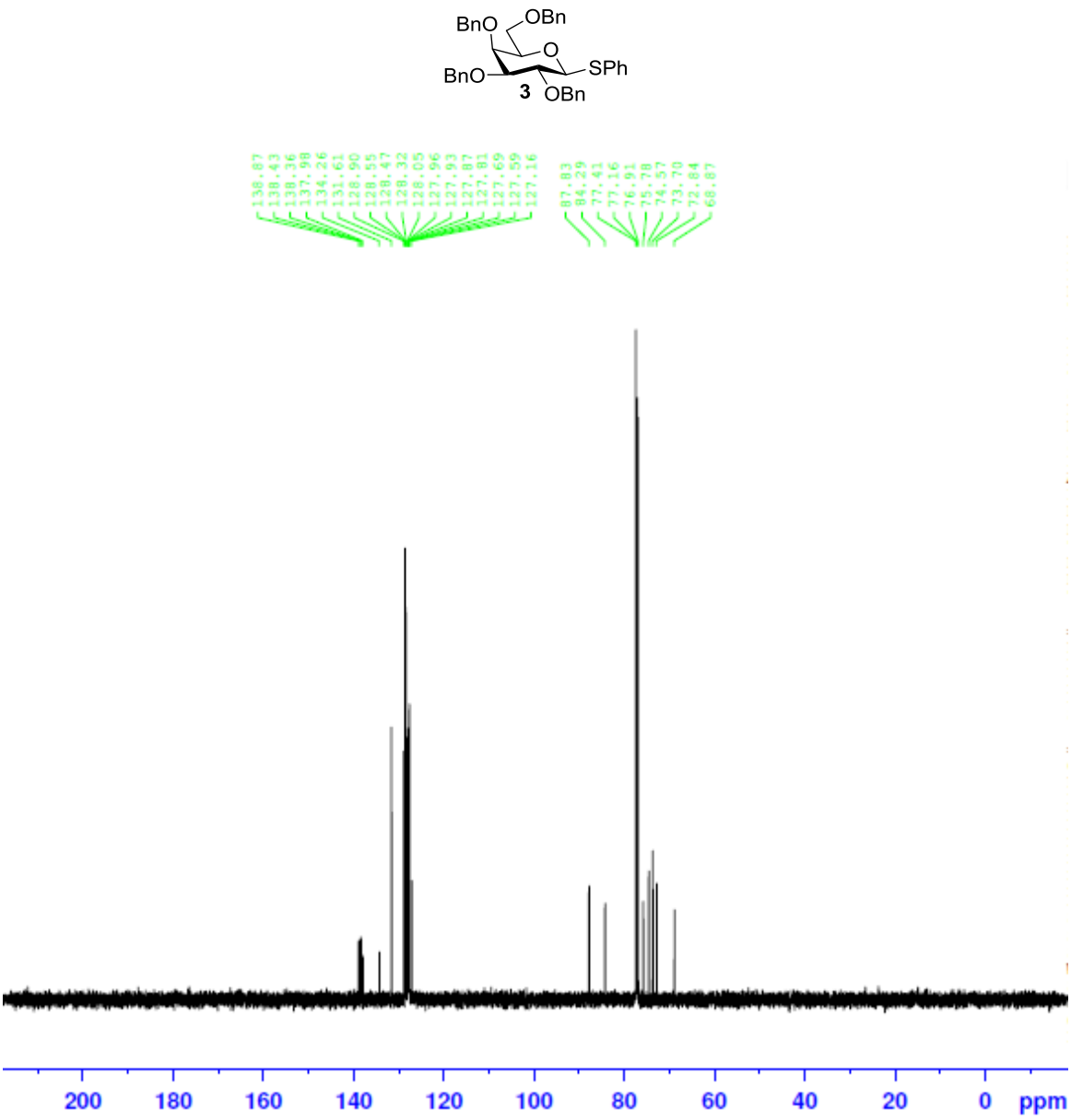
^{1}H NMR (500 MHz, $CDCl_3$)



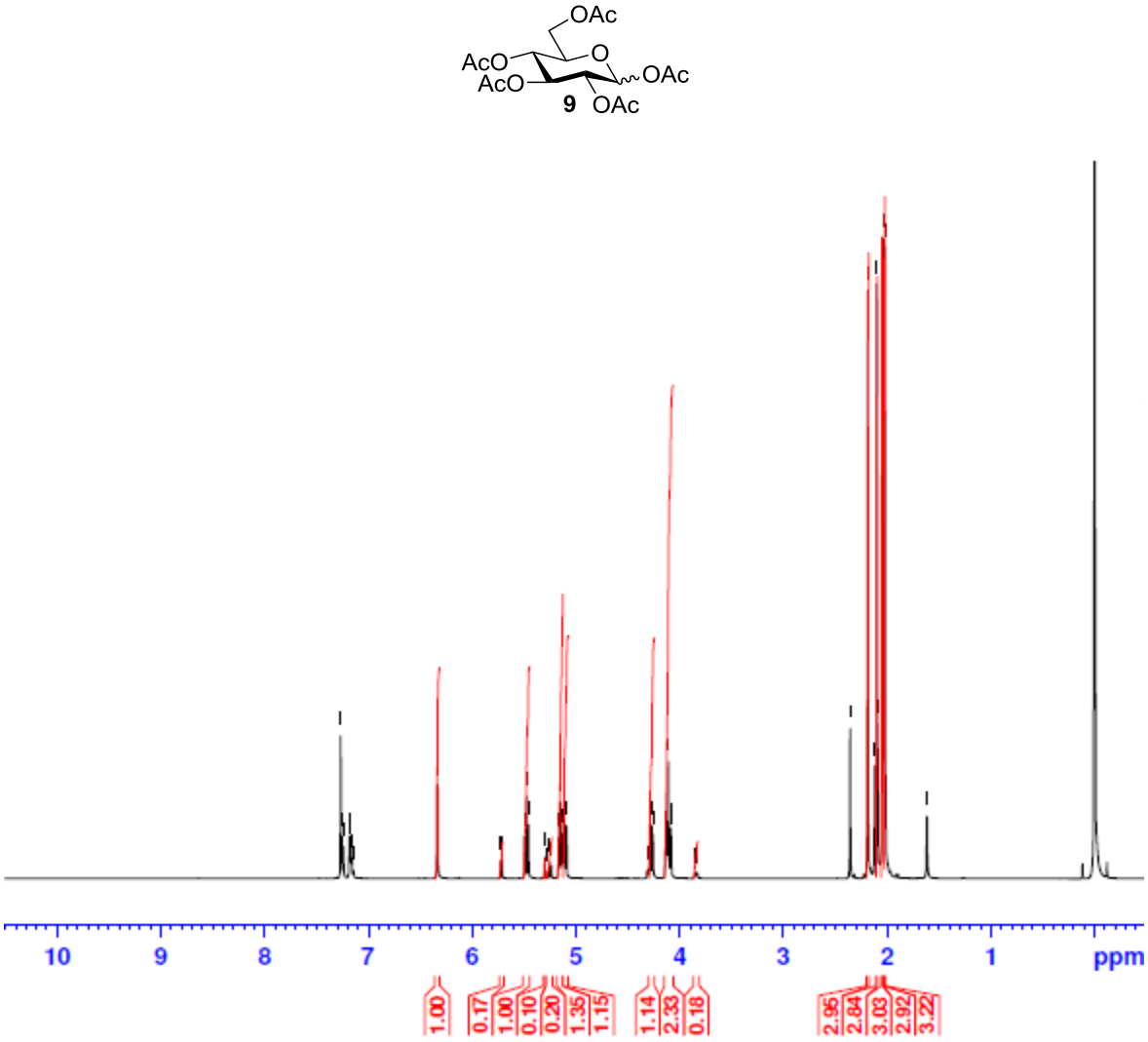
^{1}H NMR (500 MHz, $CDCl_3$)



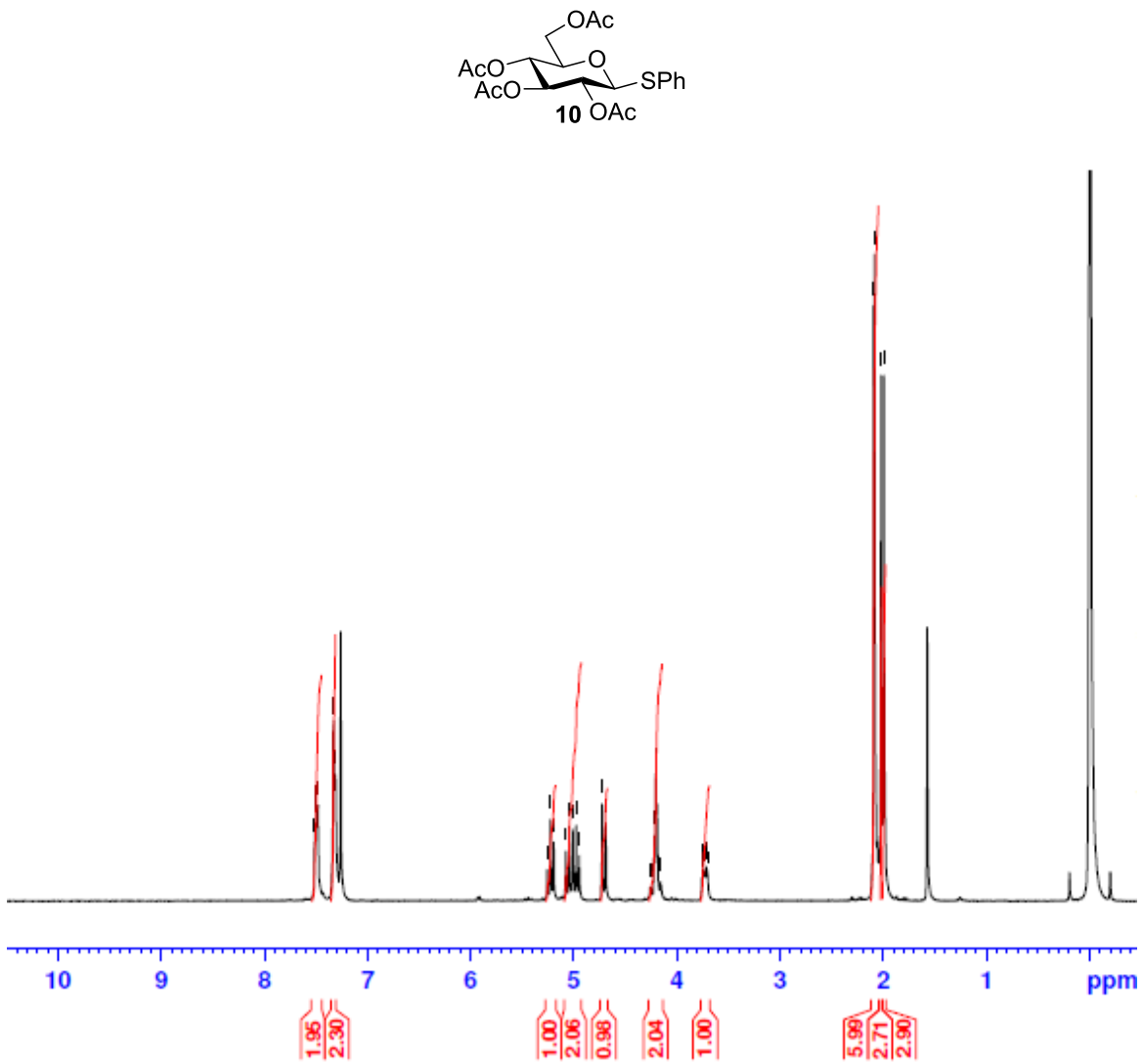
^{13}C NMR (125 MHz, $CDCl_3$)



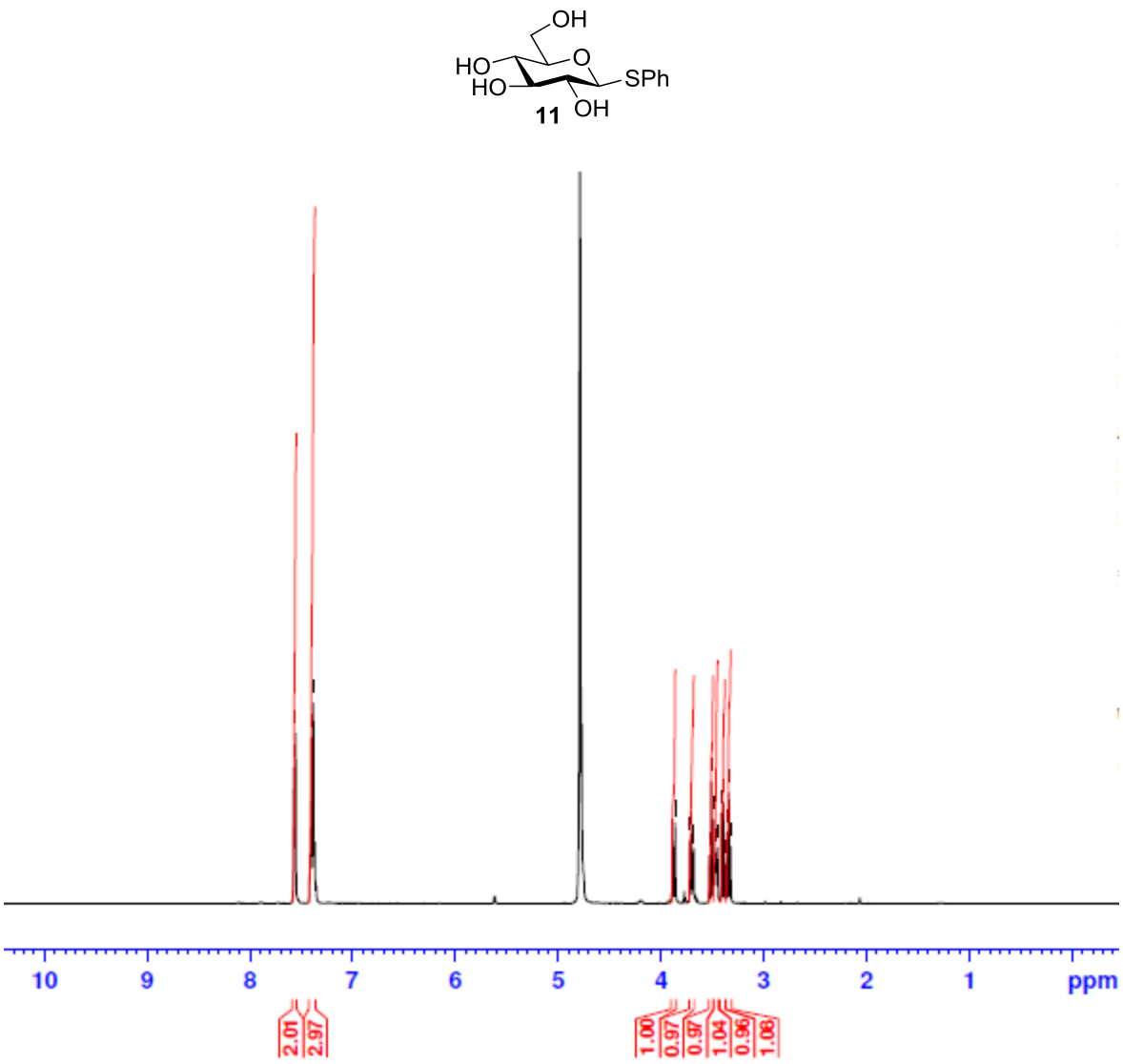
^{1}H NMR (500 MHz, $CDCl_3$)



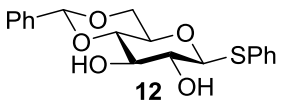
^{1}H NMR (300 MHz, $CDCl_3$)



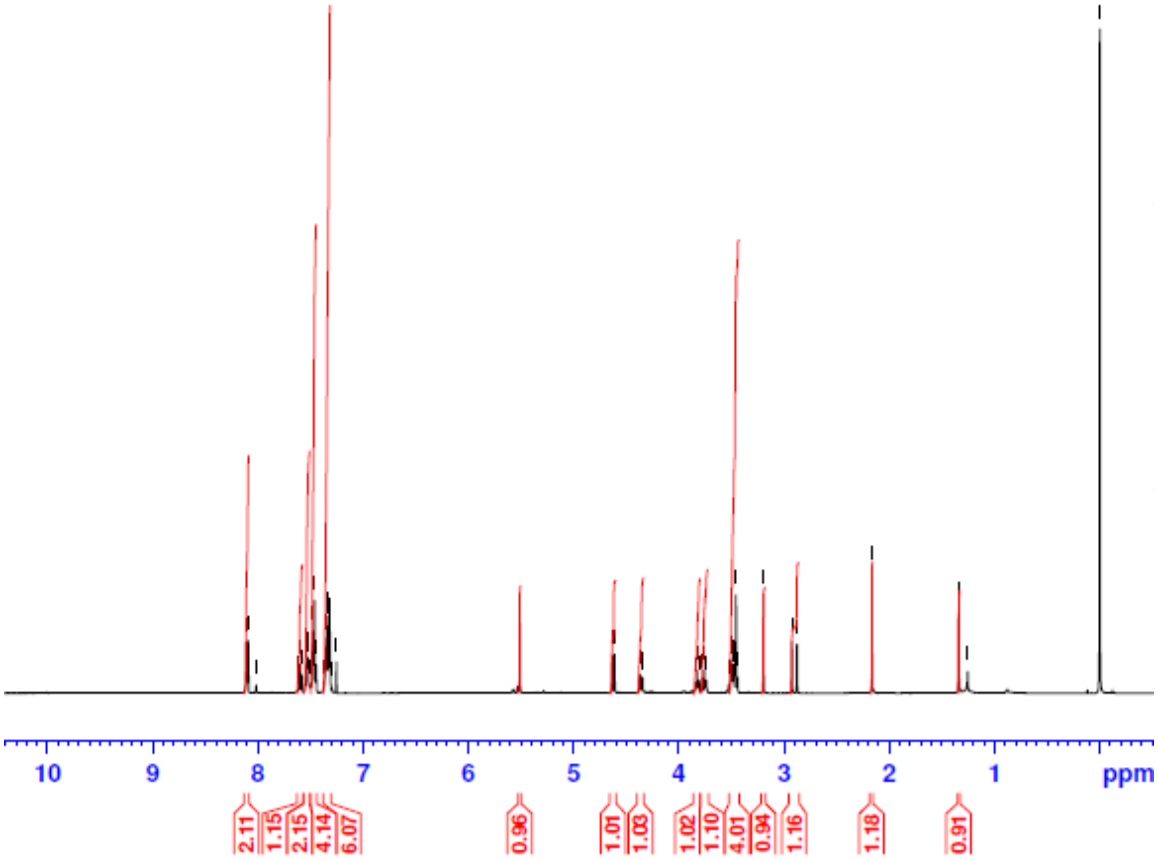
^{1}H NMR (500 MHz, D_2O)



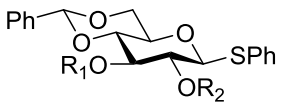
^{1}H NMR (500 MHz, $CDCl_3$)



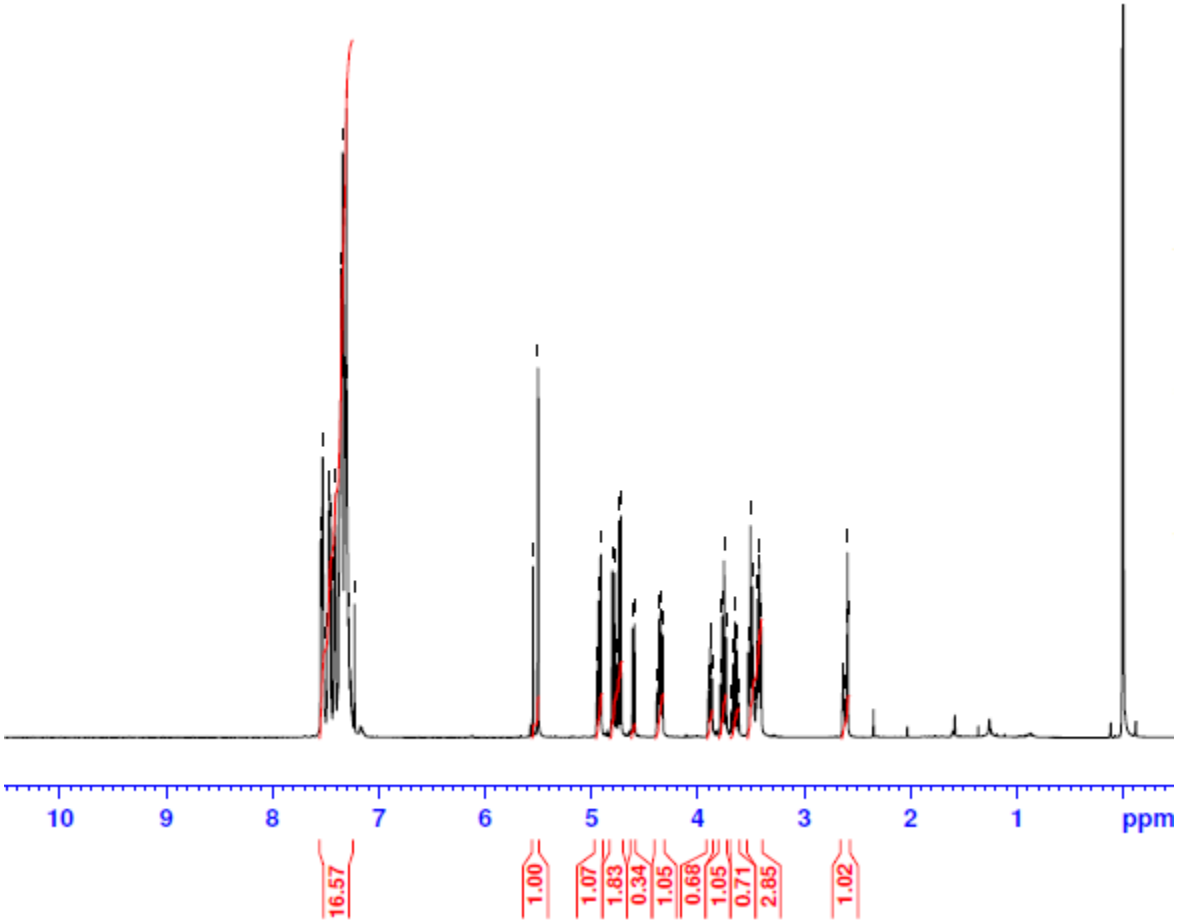
12



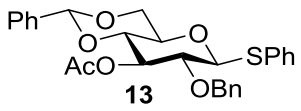
^{1}H NMR (500 MHz, $CDCl_3$)



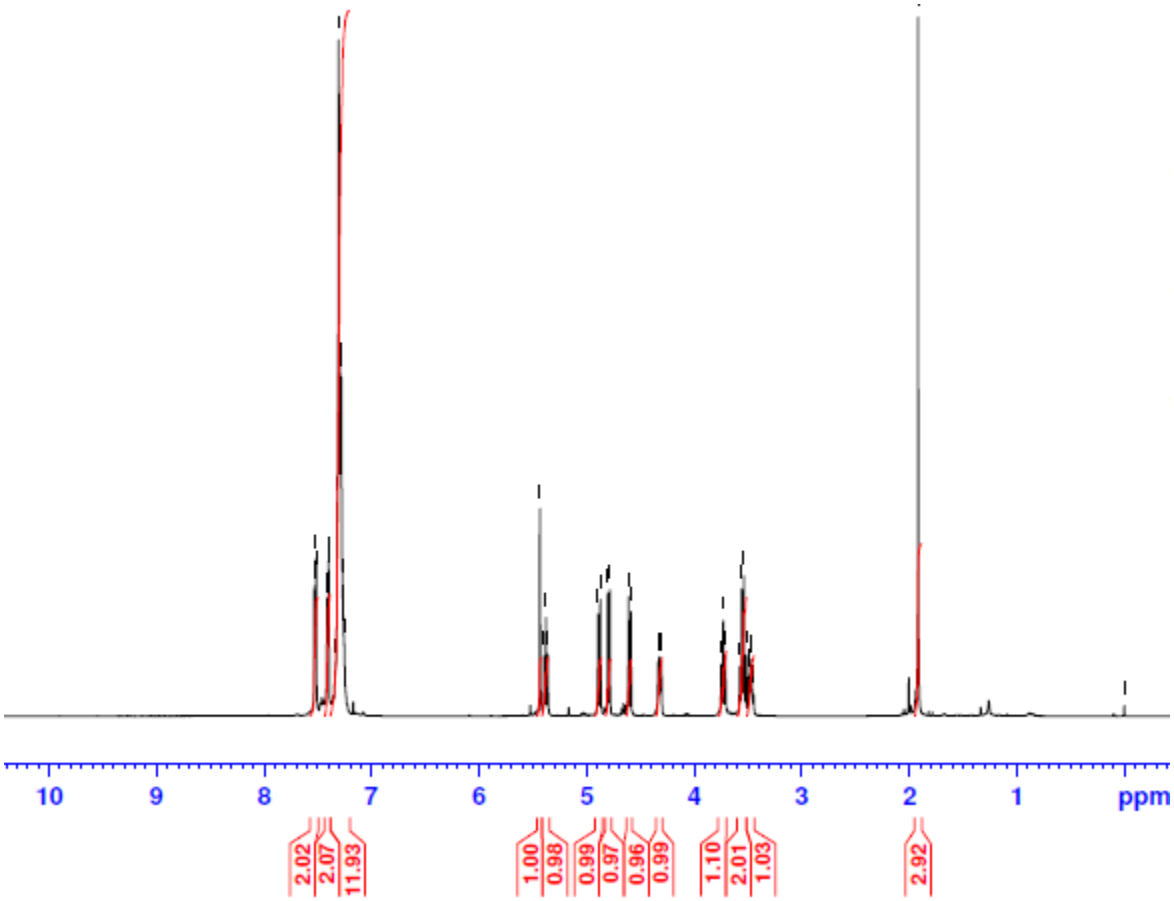
	R_1	R_2
4a	H	Bn
4b	Bn	H



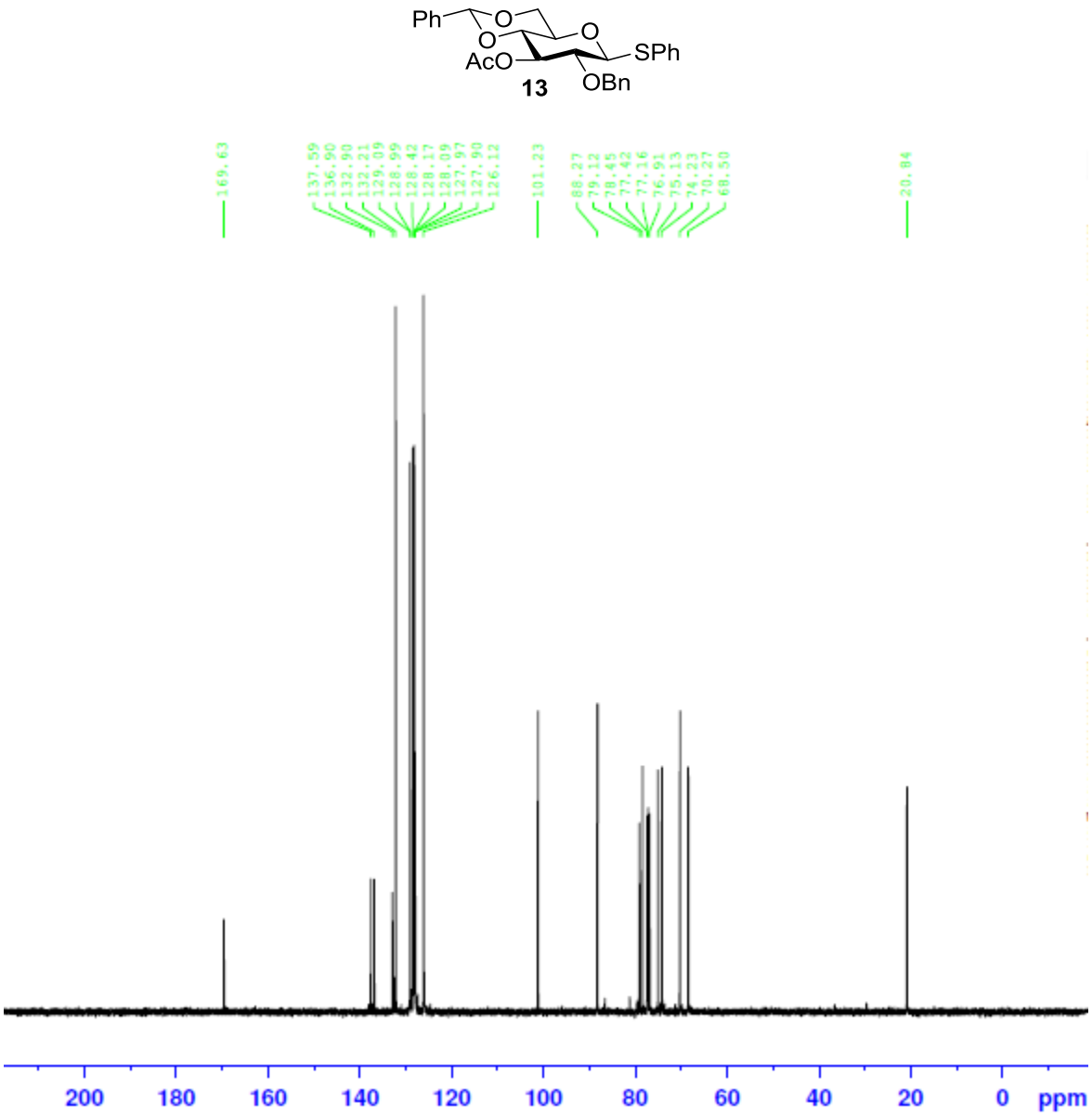
^{1}H NMR (500 MHz, $CDCl_3$)



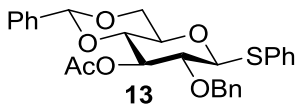
13



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



^{1}H-^{1}H Gr. COSY NMR (500 MHz, $CDCl_3$)



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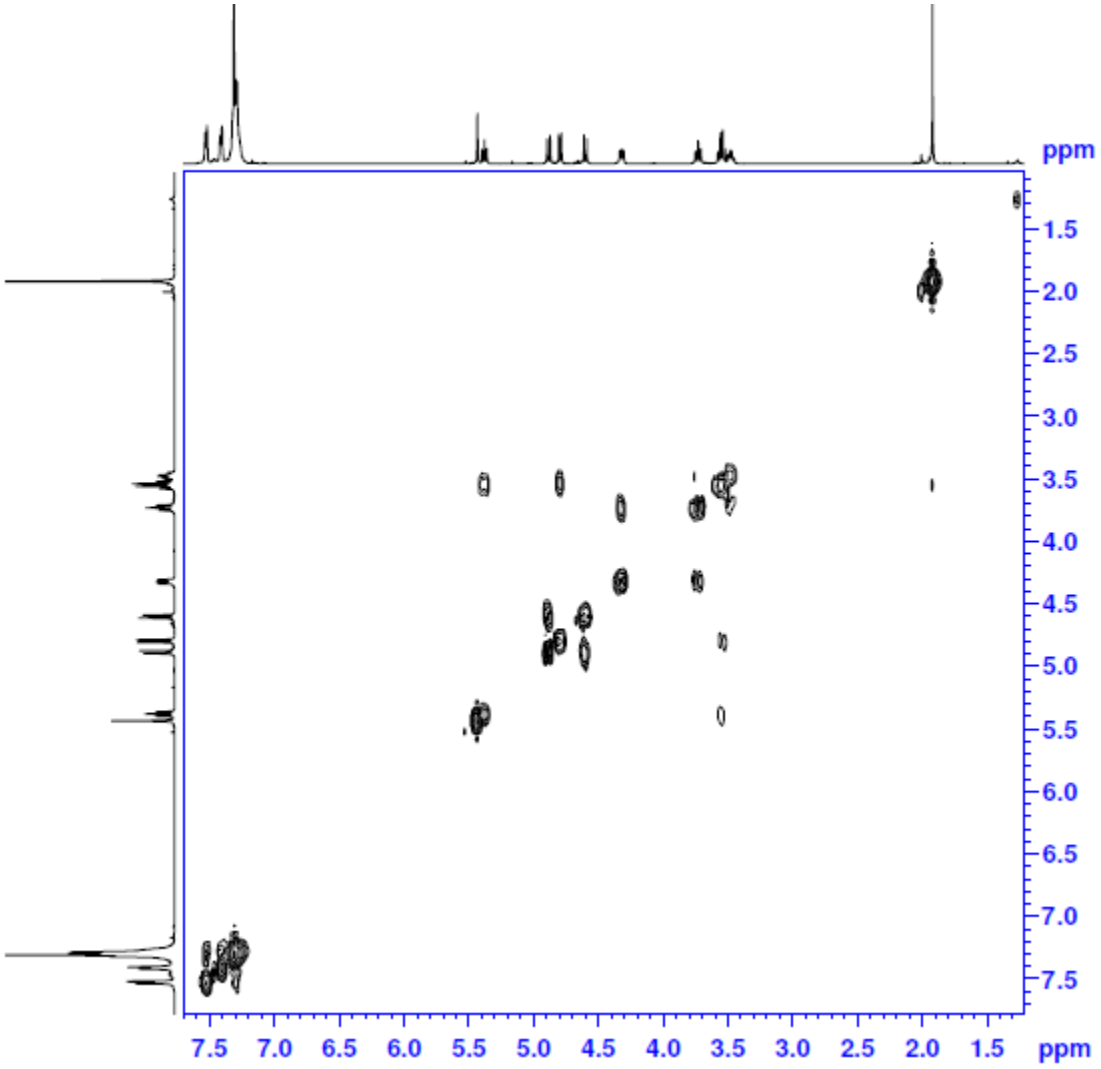
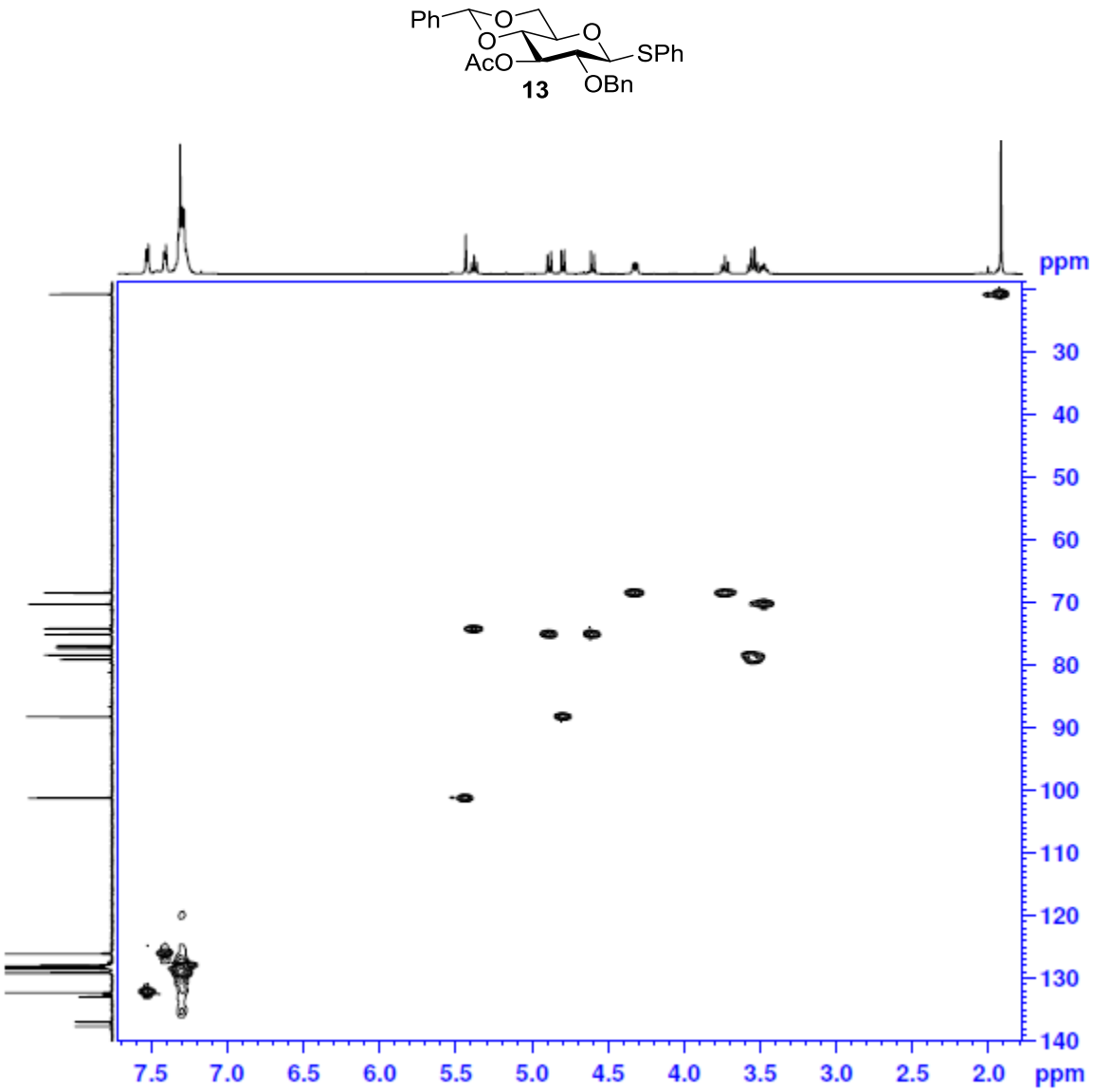
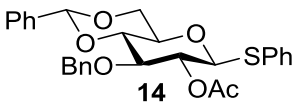


^{1}H-^{13}C Gr. HSQC NMR (125 MHz, $CDCl_3$)



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



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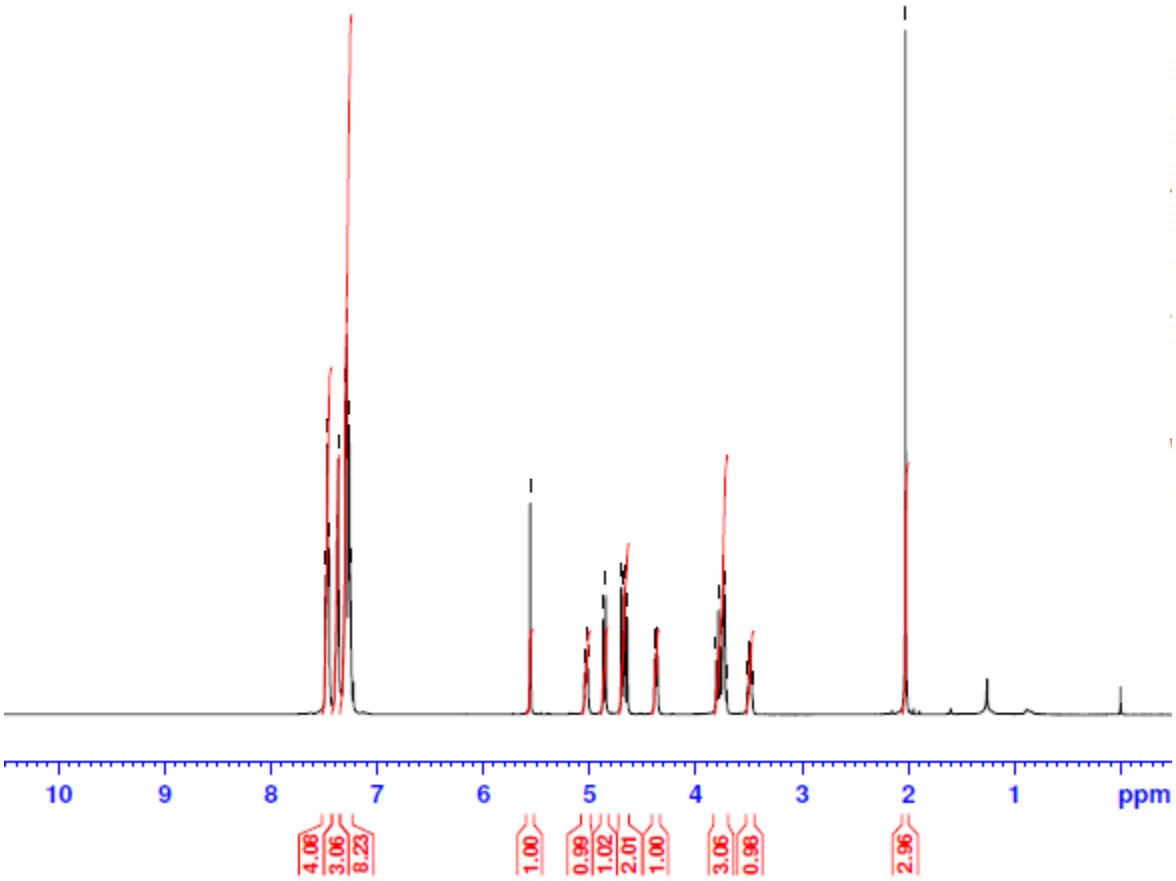
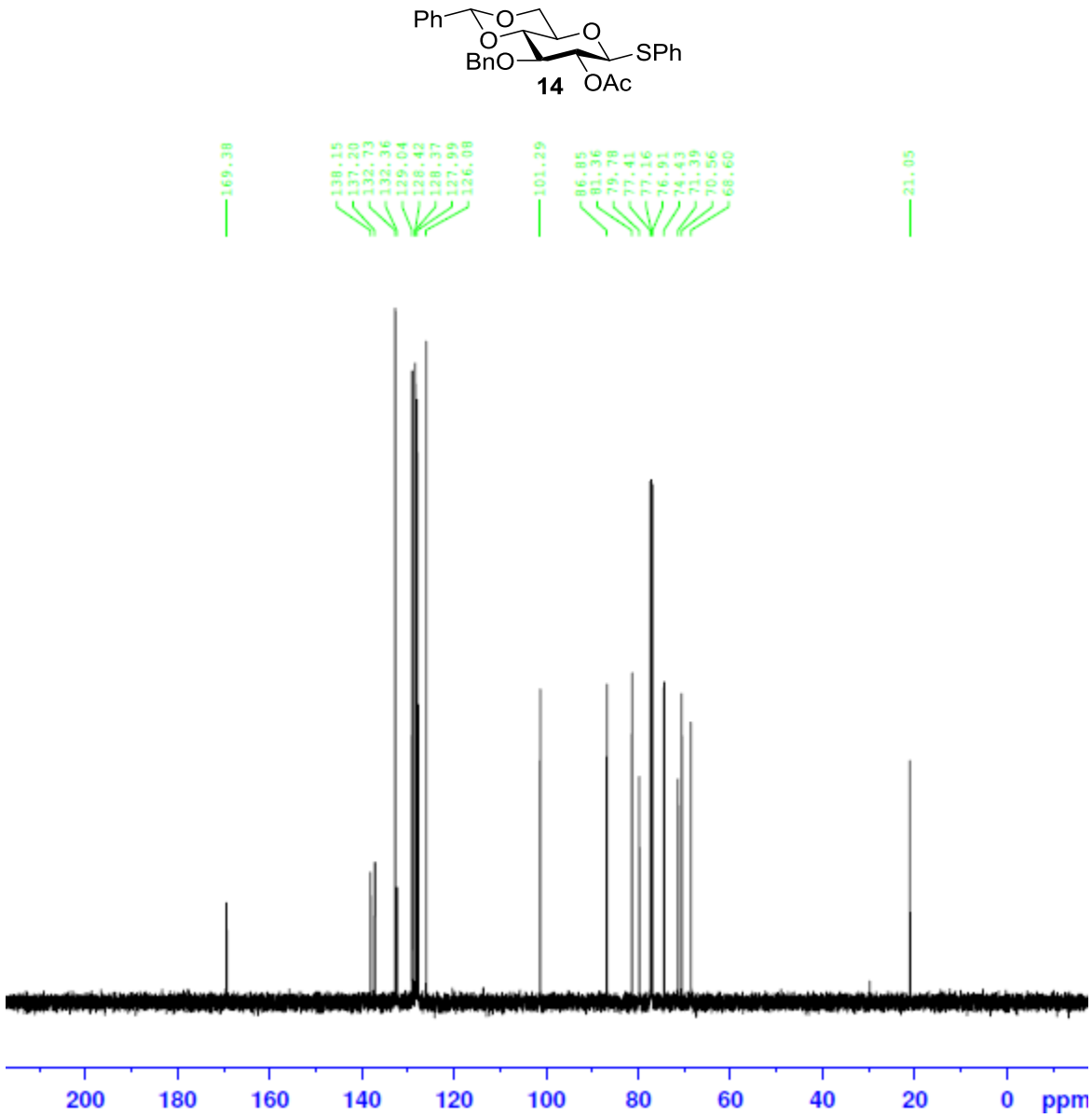
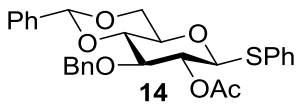


^{13}C NMR (125 MHz, $CDCl_3$)



^{1}H-^{1}H Gr. COSY NMR (500 MHz, $CDCl_3$)



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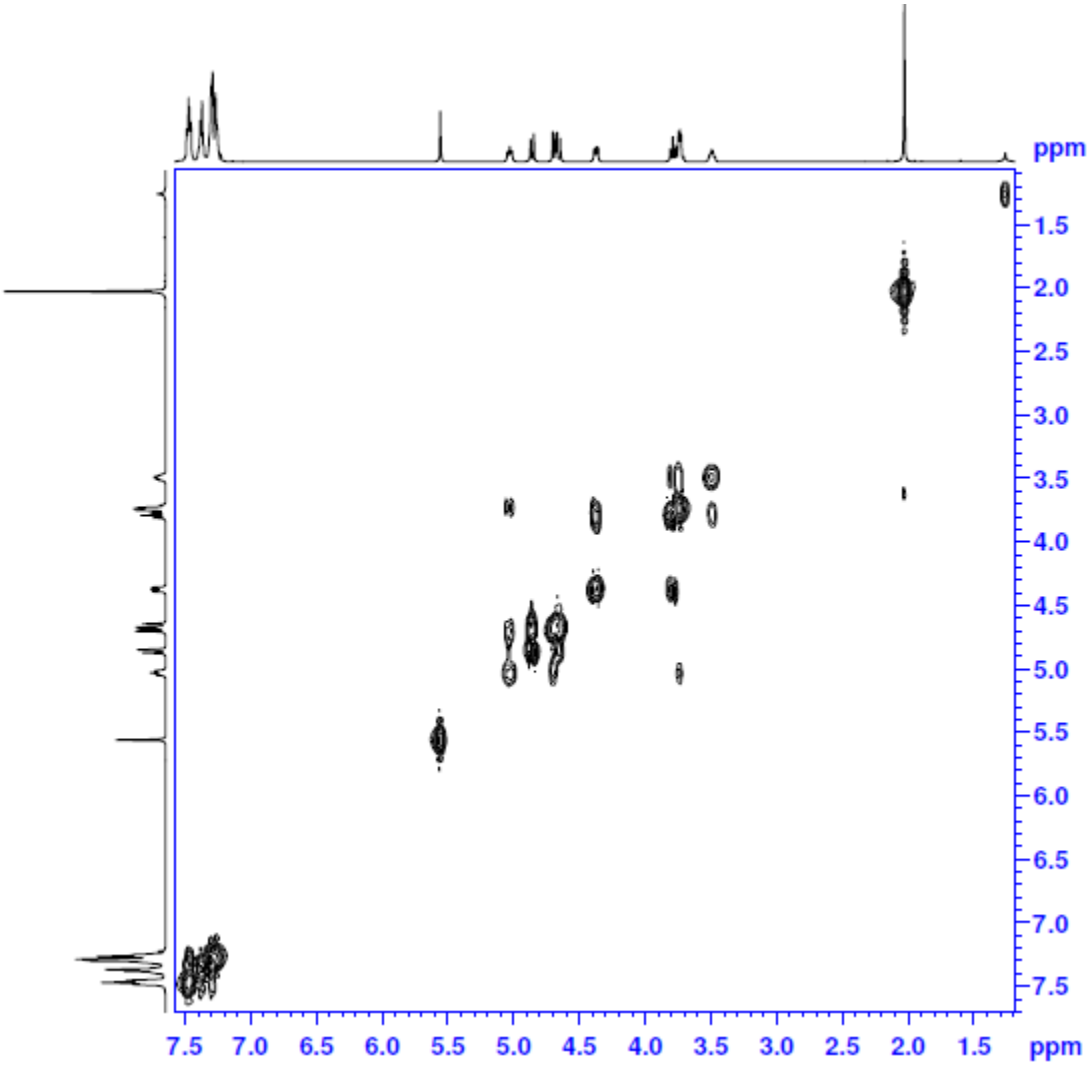
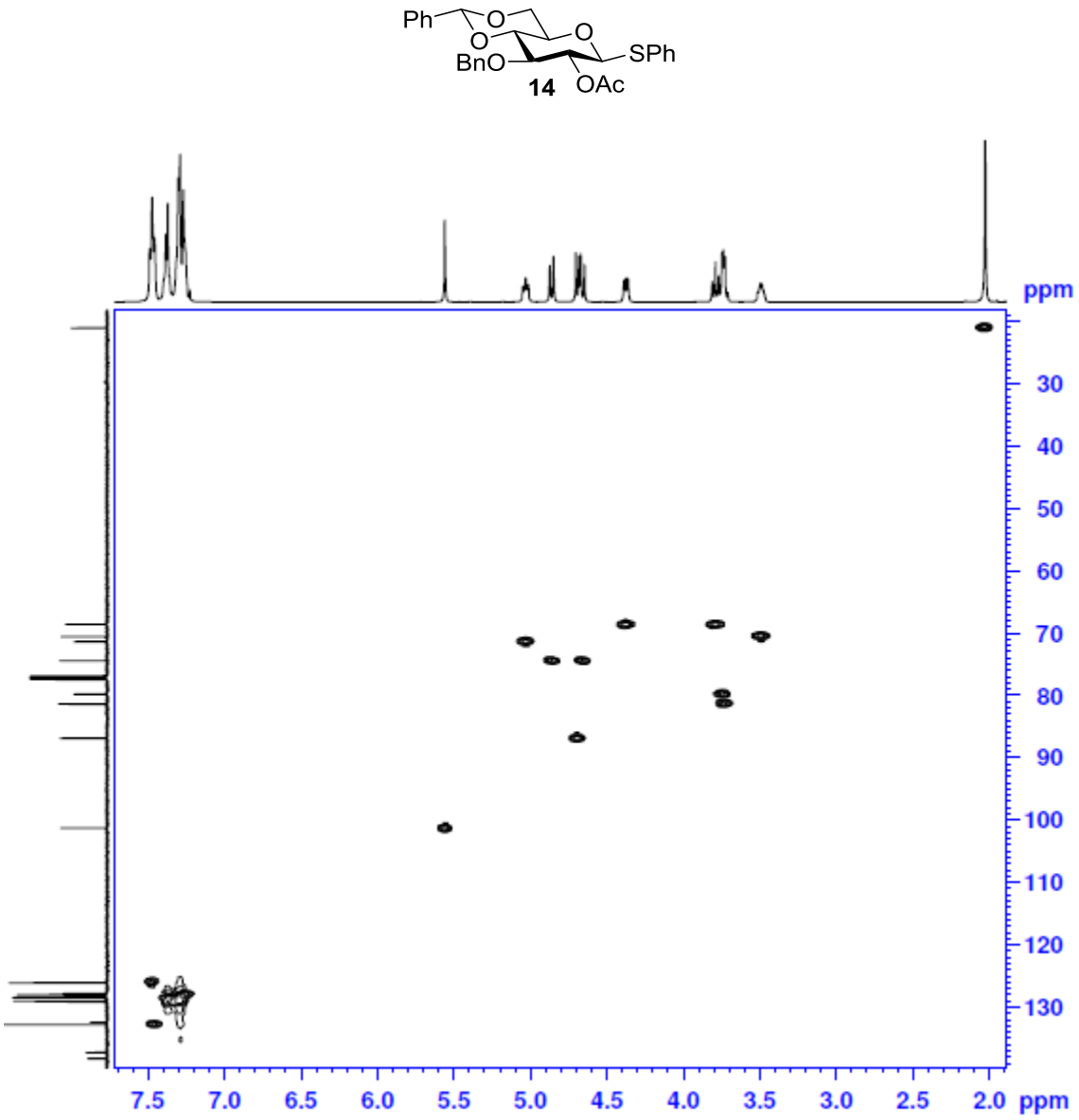
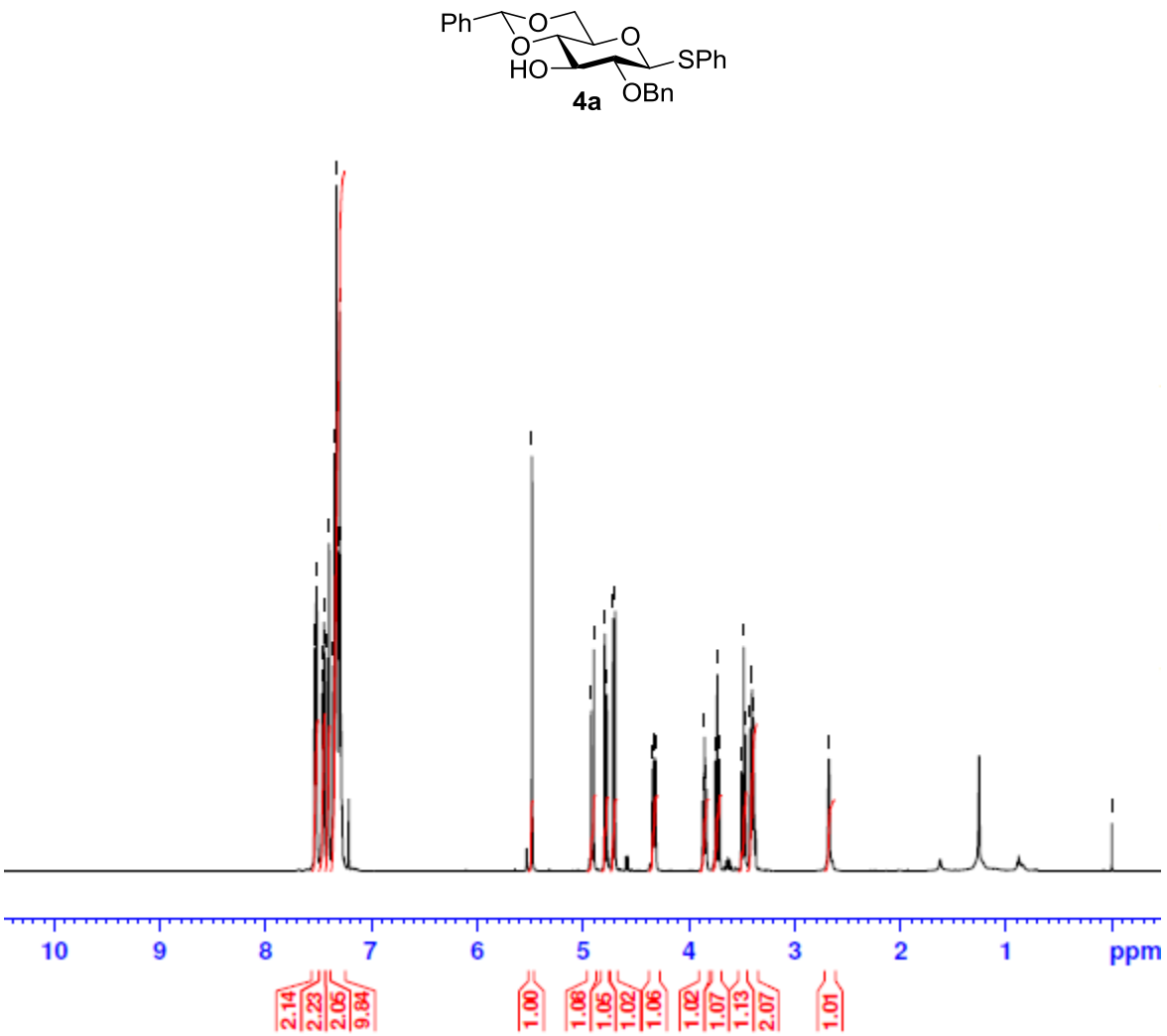


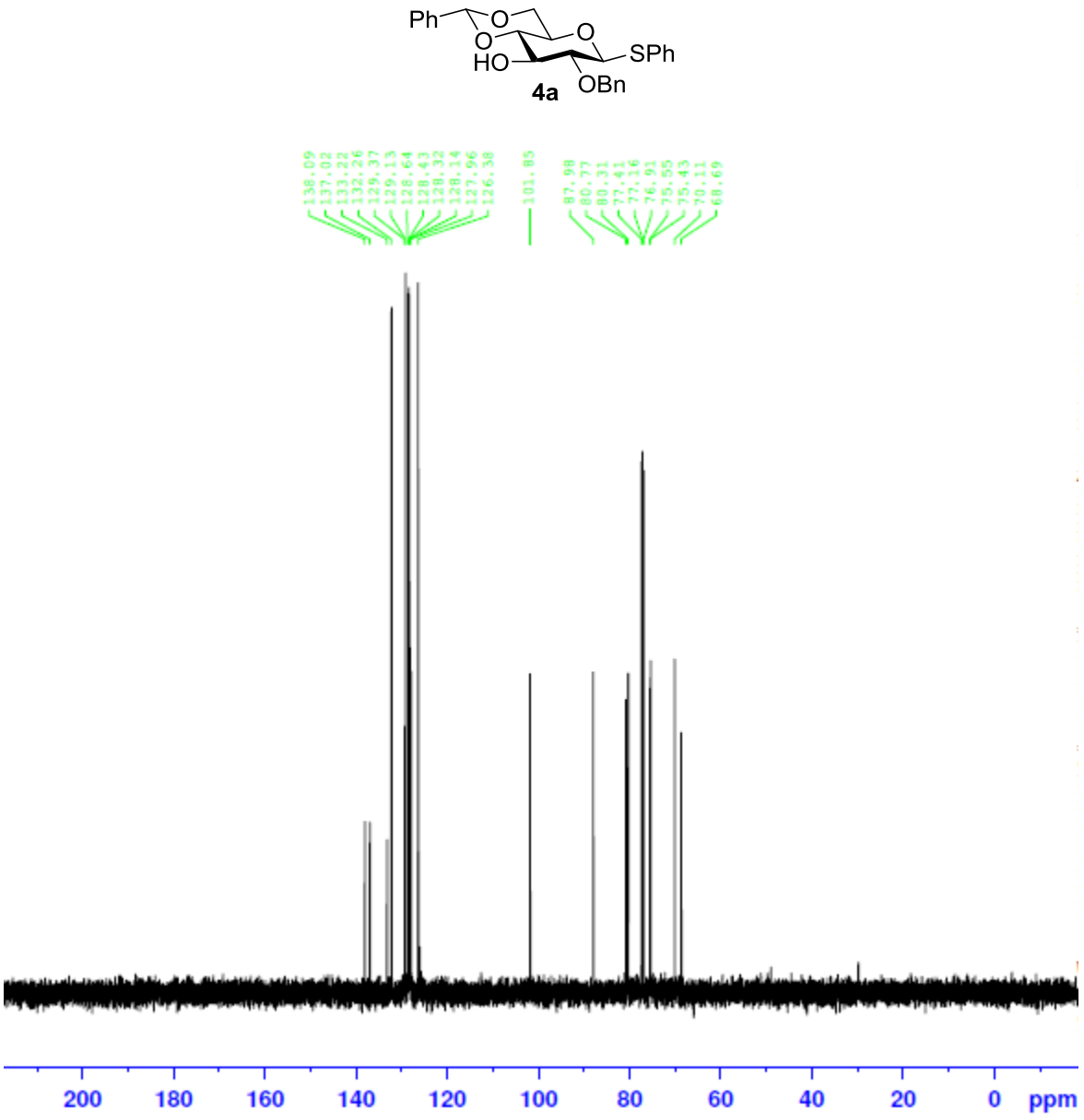
^{1}H-^{13}C Gr. HSQC NMR (125 MHz, $CDCl_3$)



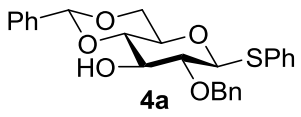
^{1}H NMR (500 MHz, $CDCl_3$)



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



^{1}H-^{1}H Gr. COSY NMR (500 MHz, $CDCl_3$)



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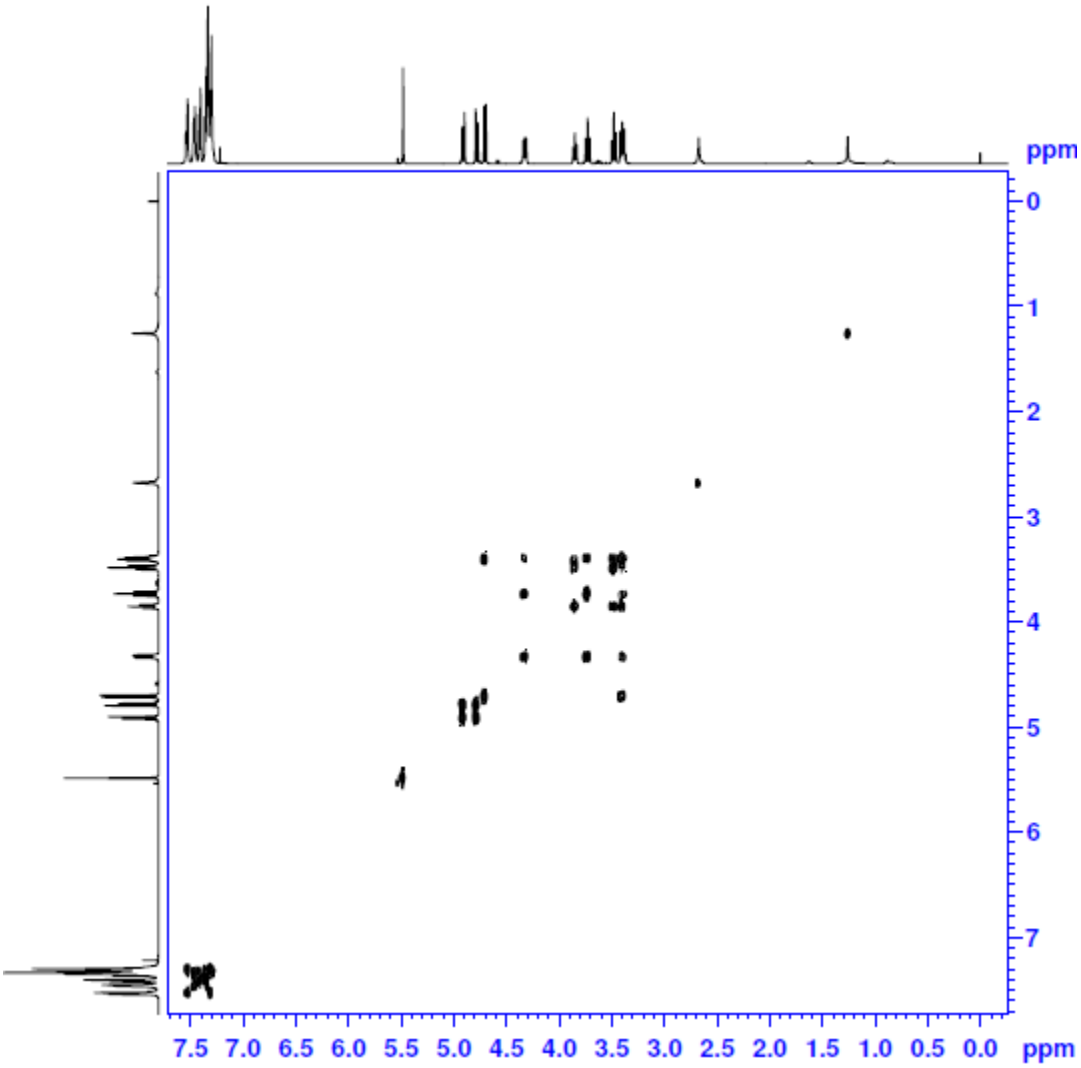
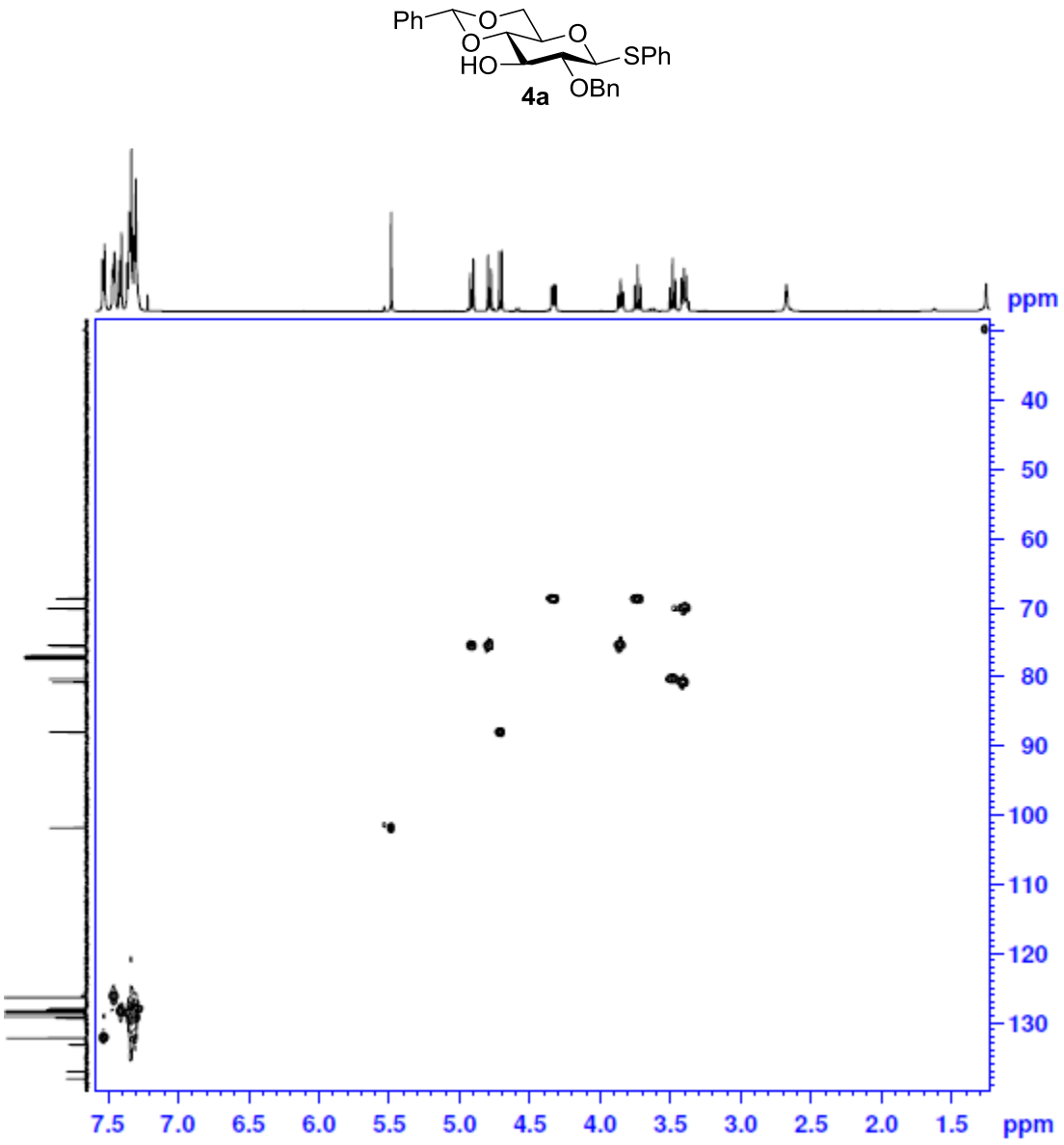


^{1}H-^{13}C Gr. HSQC NMR (125 MHz, $CDCl_3$)



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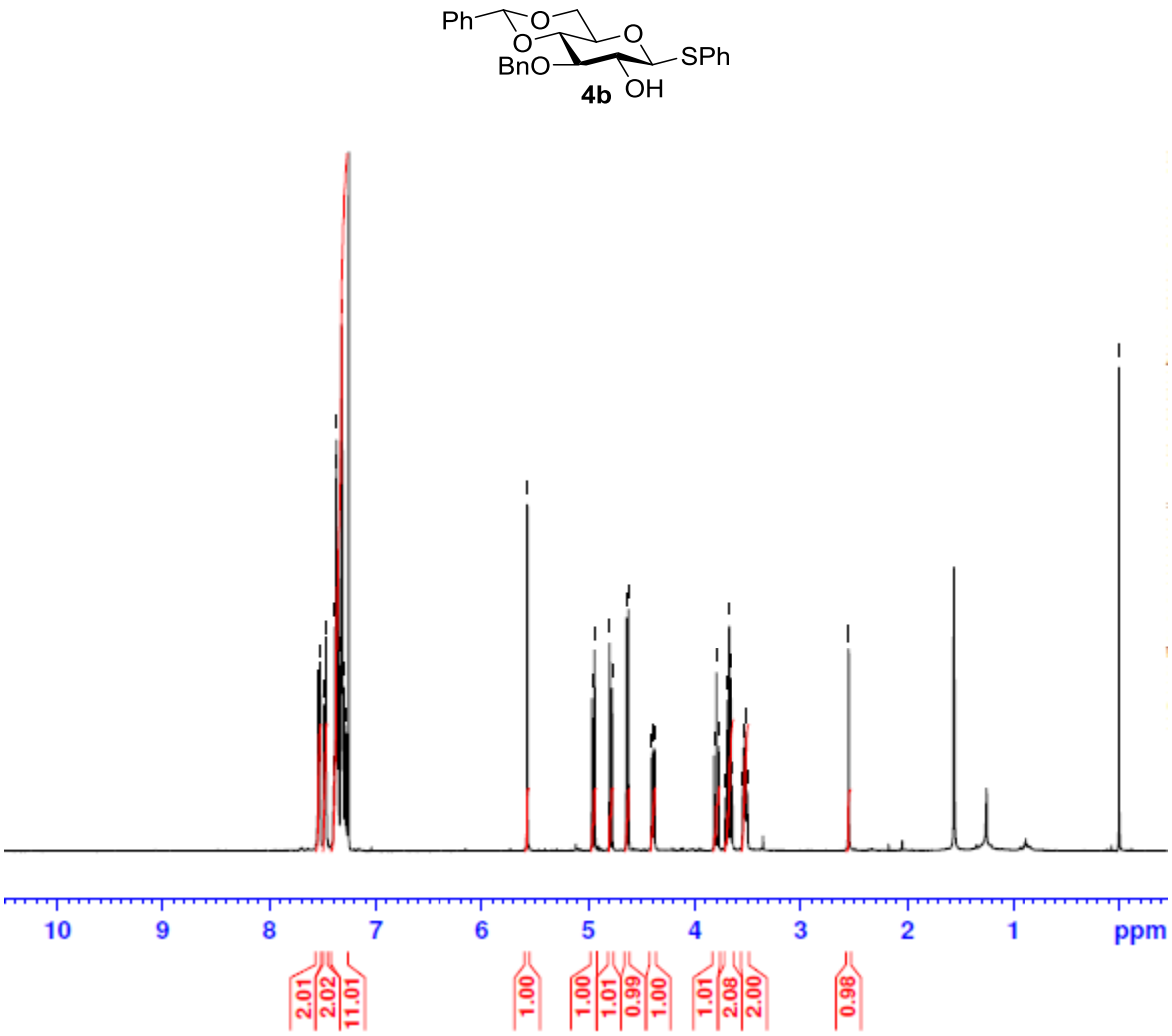

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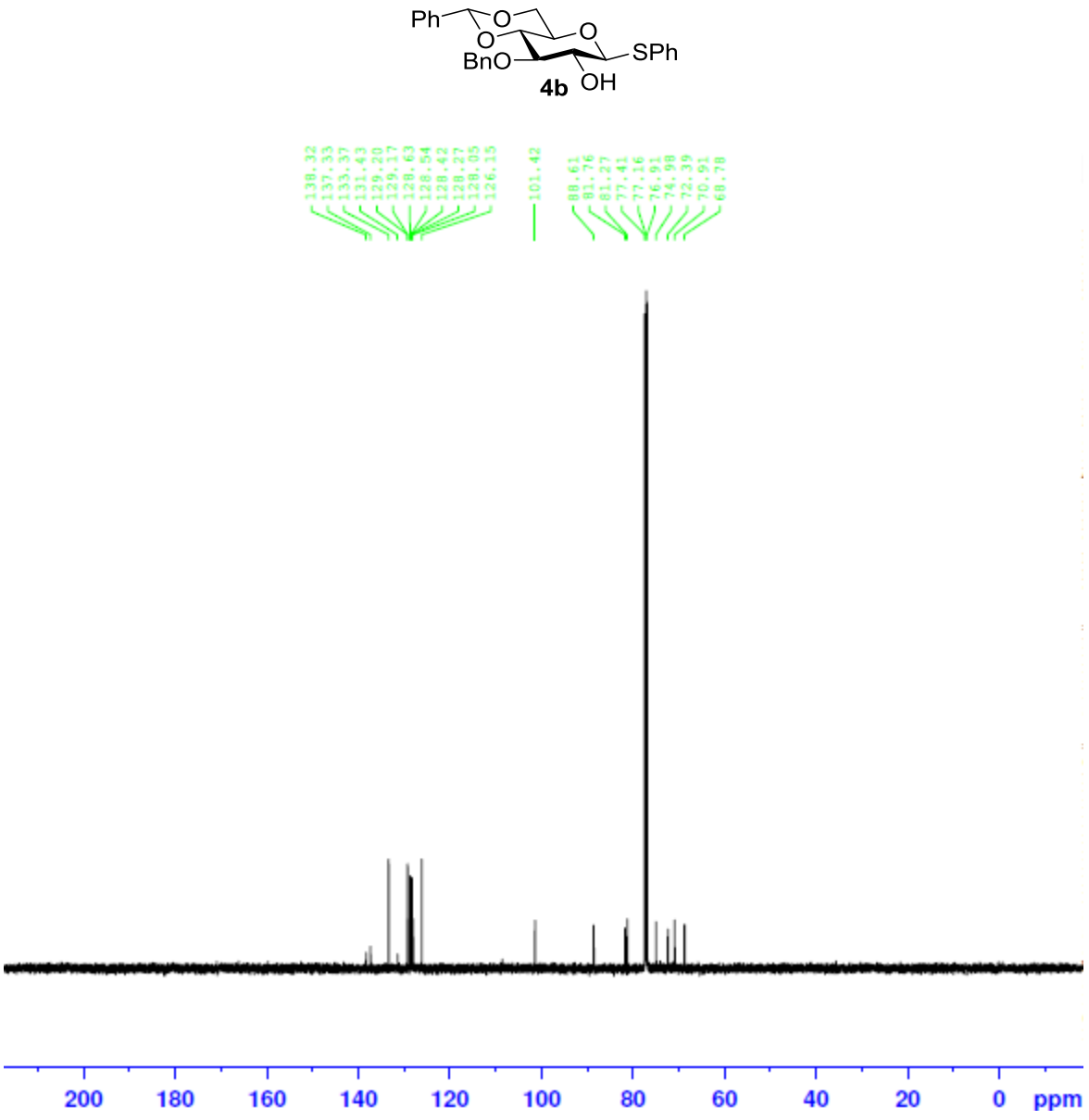

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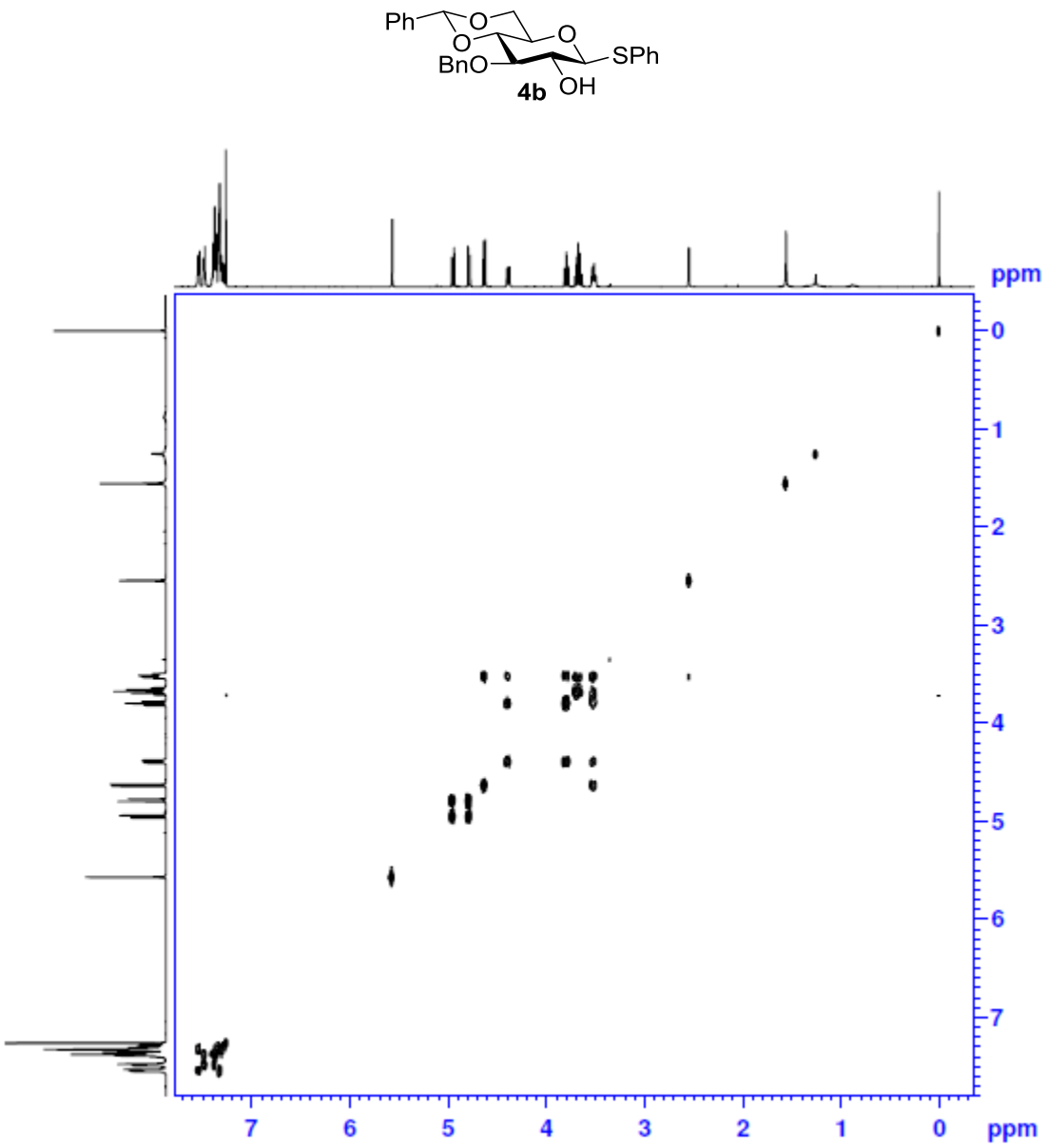

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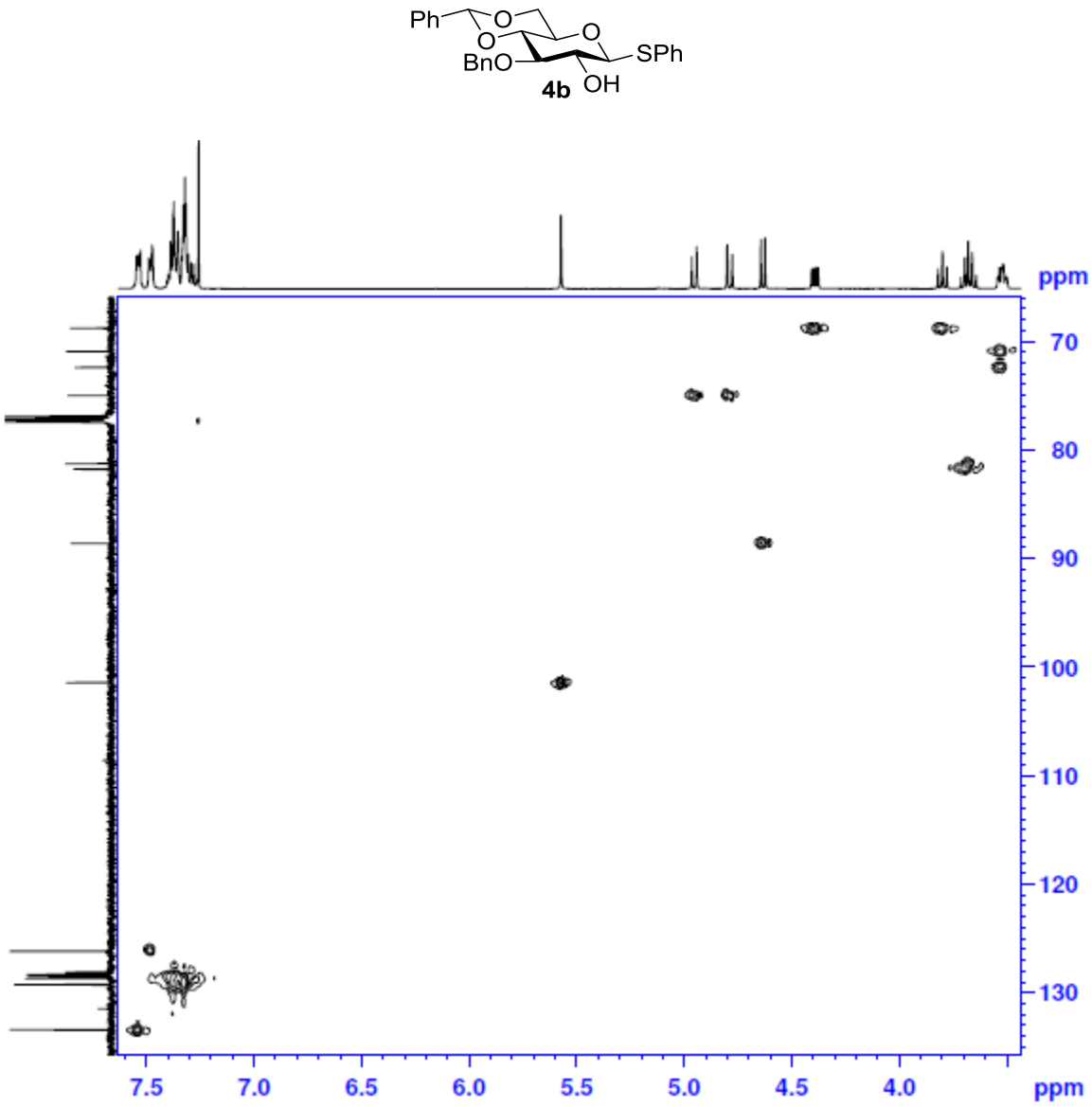

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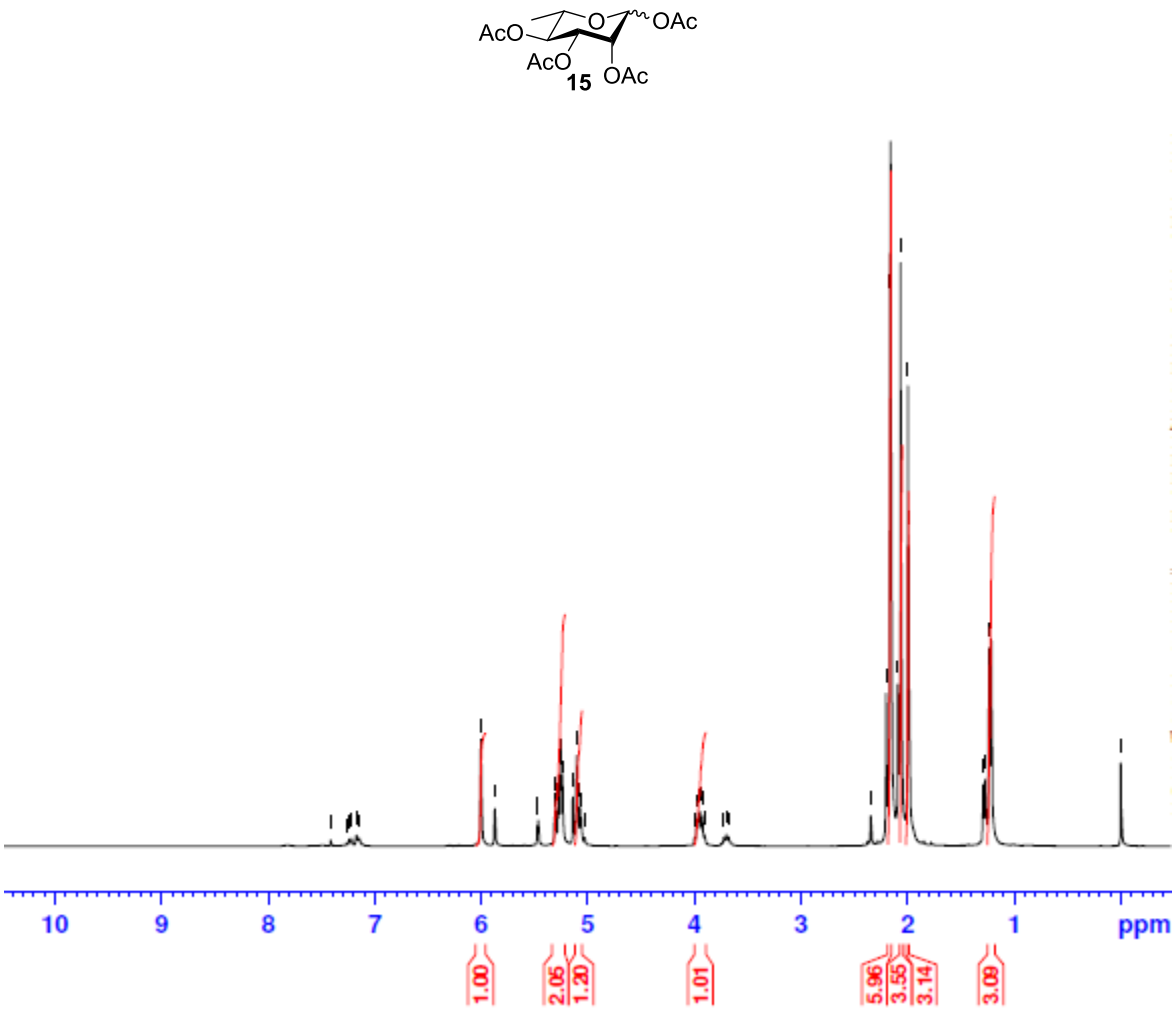

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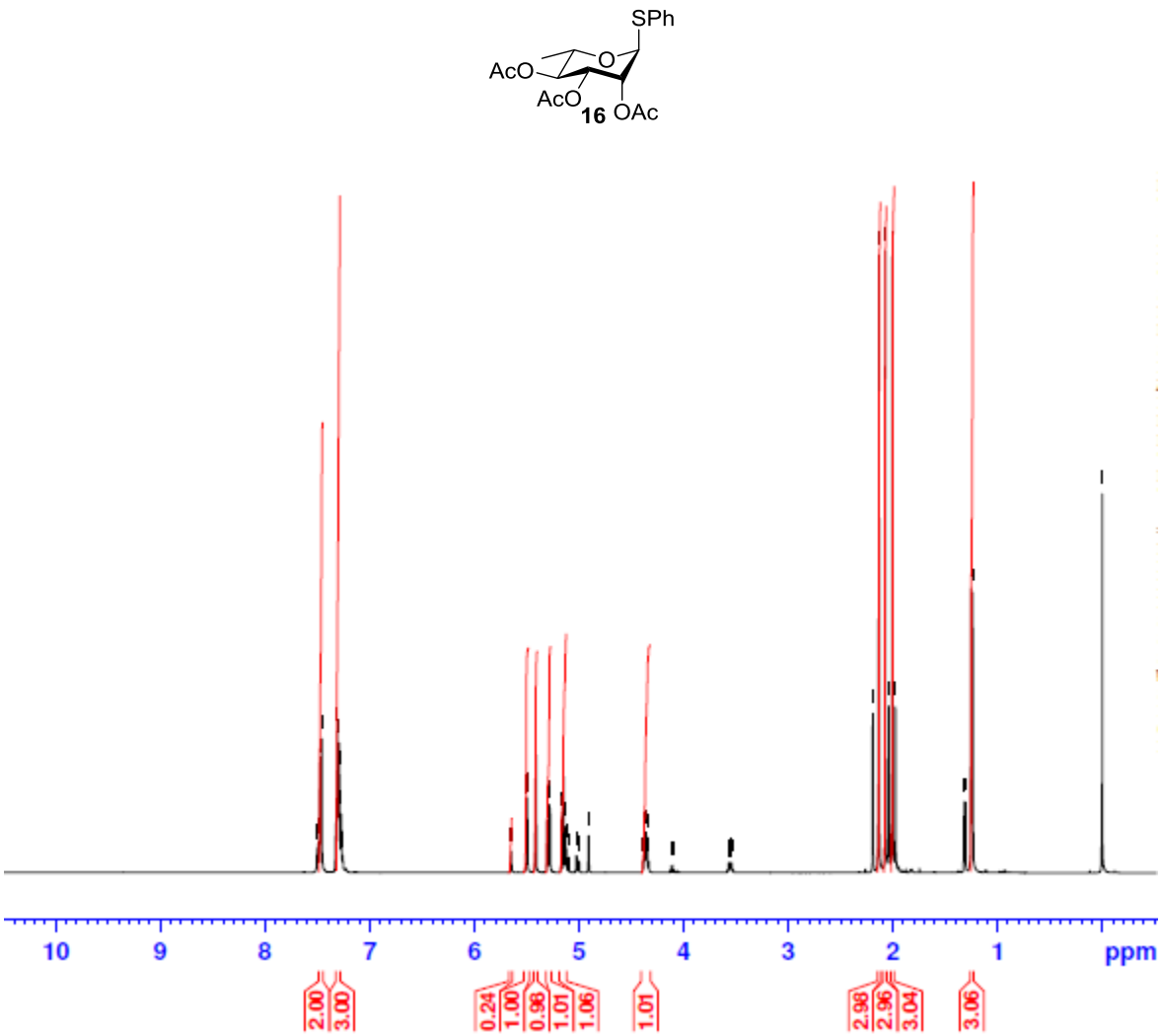

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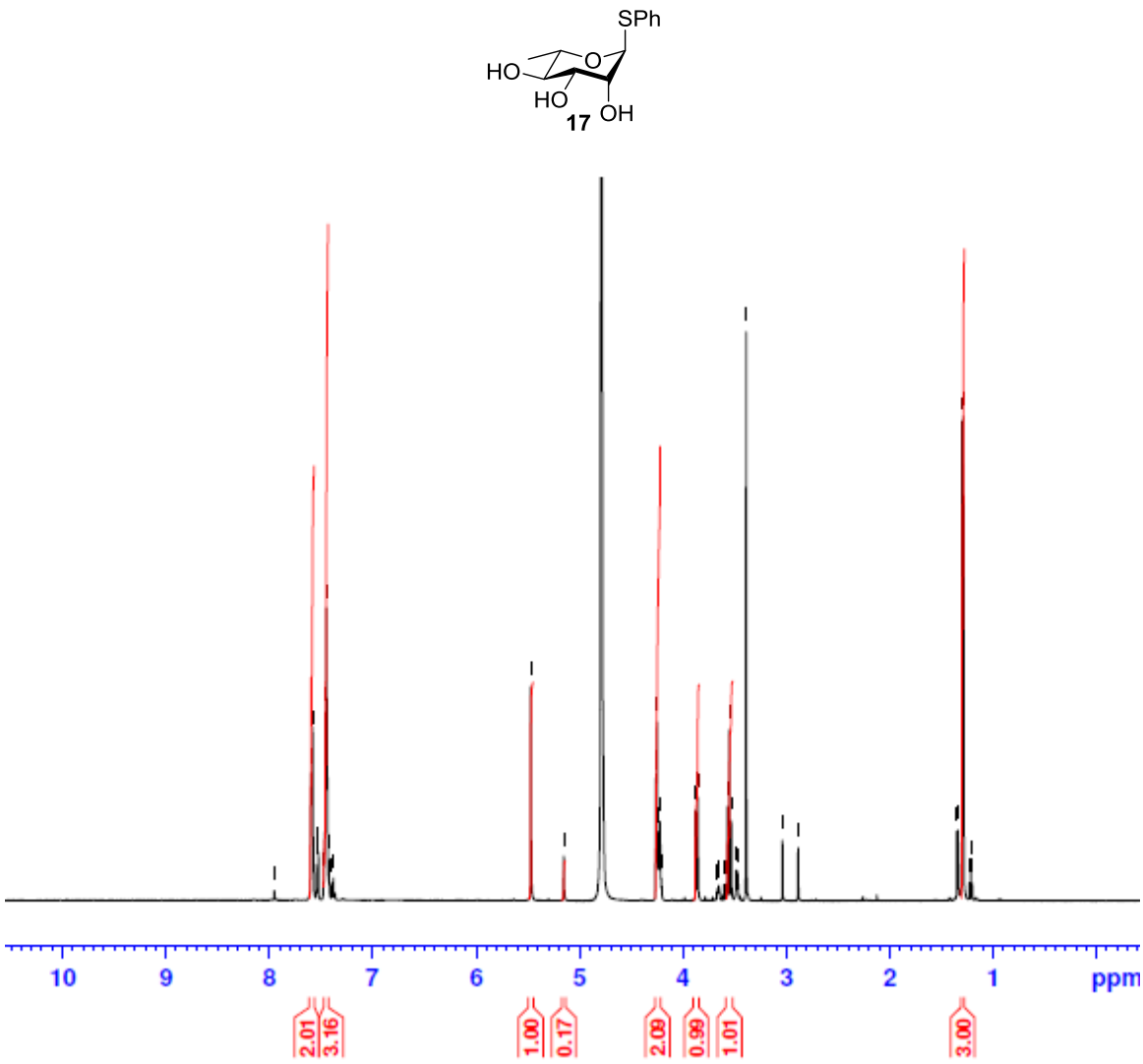
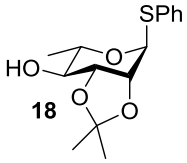
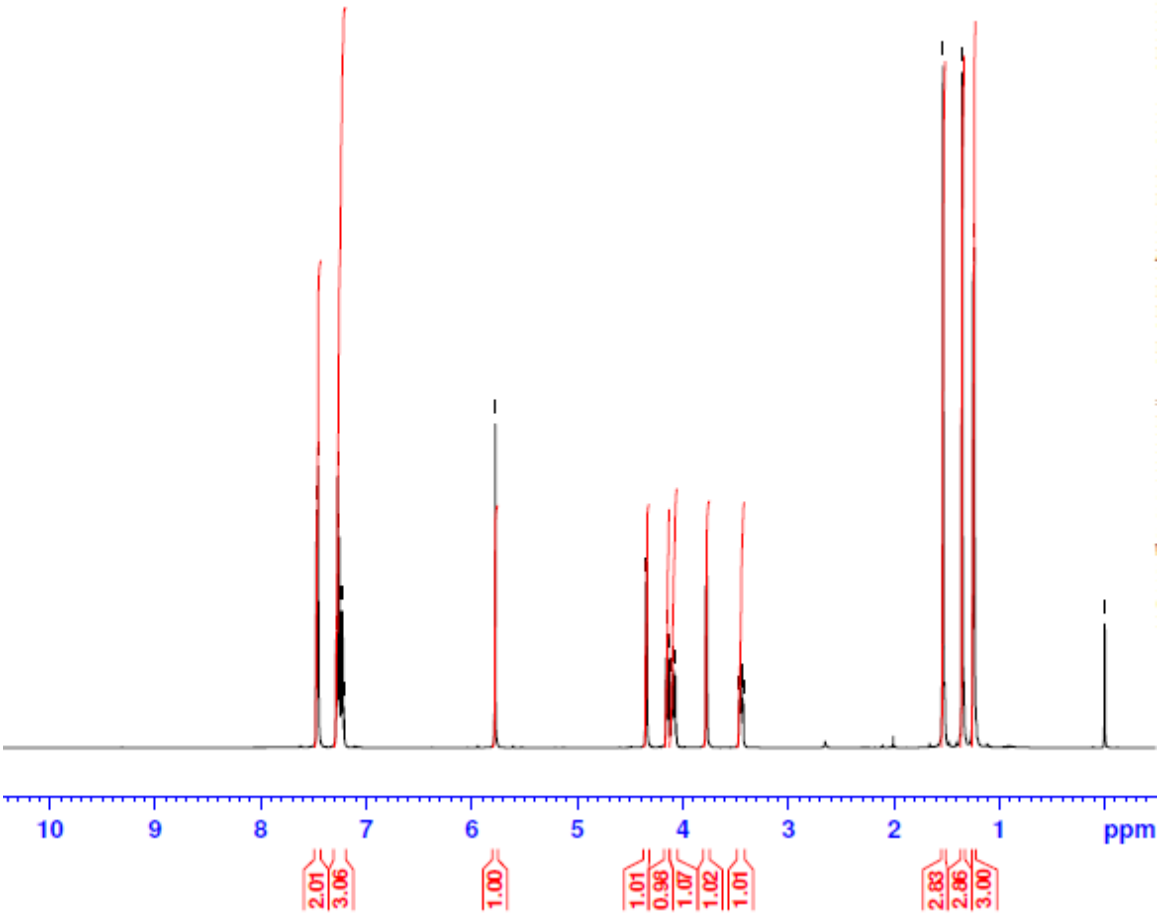
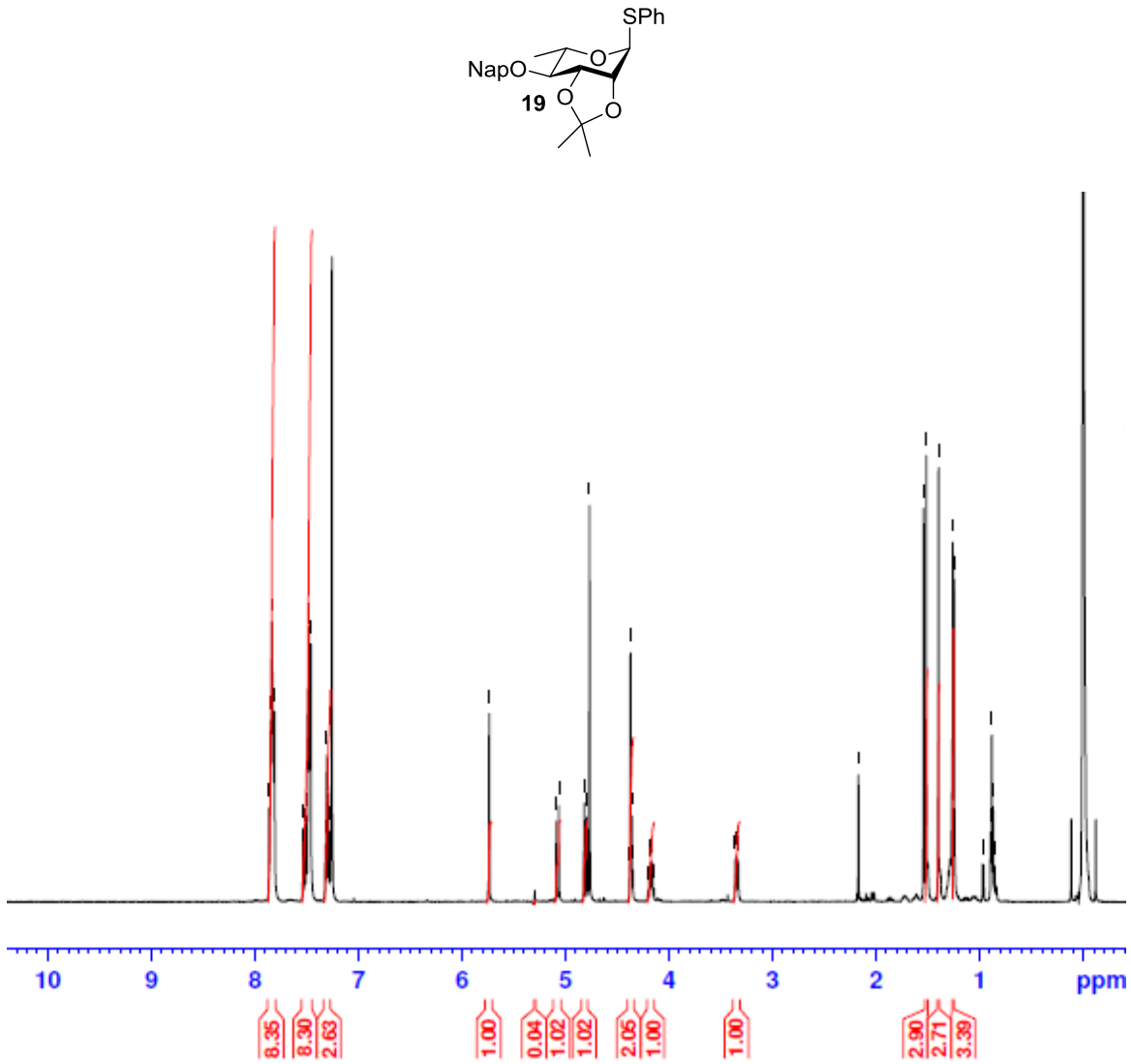


^{1}H NMR (500 MHz, $CDCl_3$)

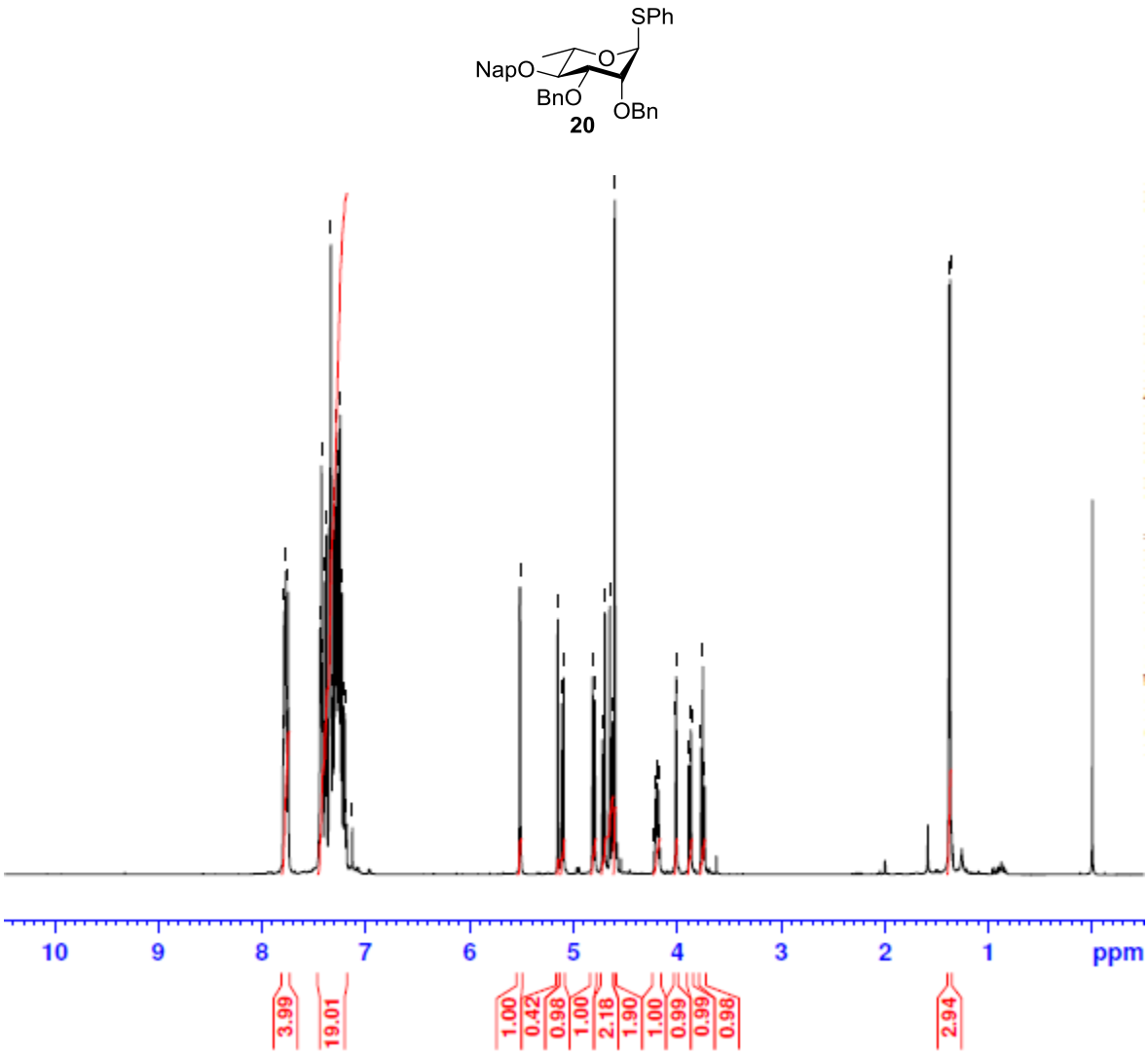




^{1}H NMR (500 MHz, $CDCl_3$)



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



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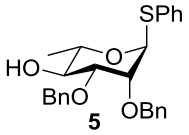

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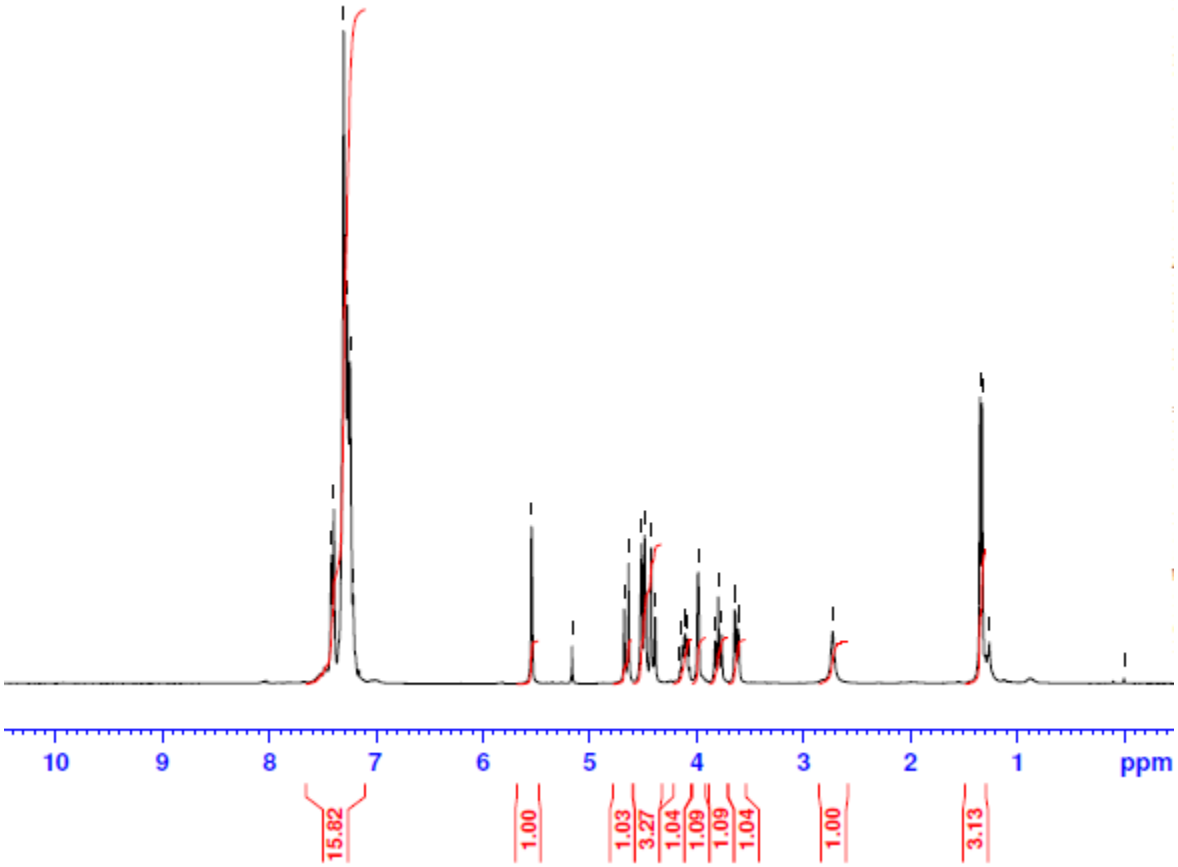

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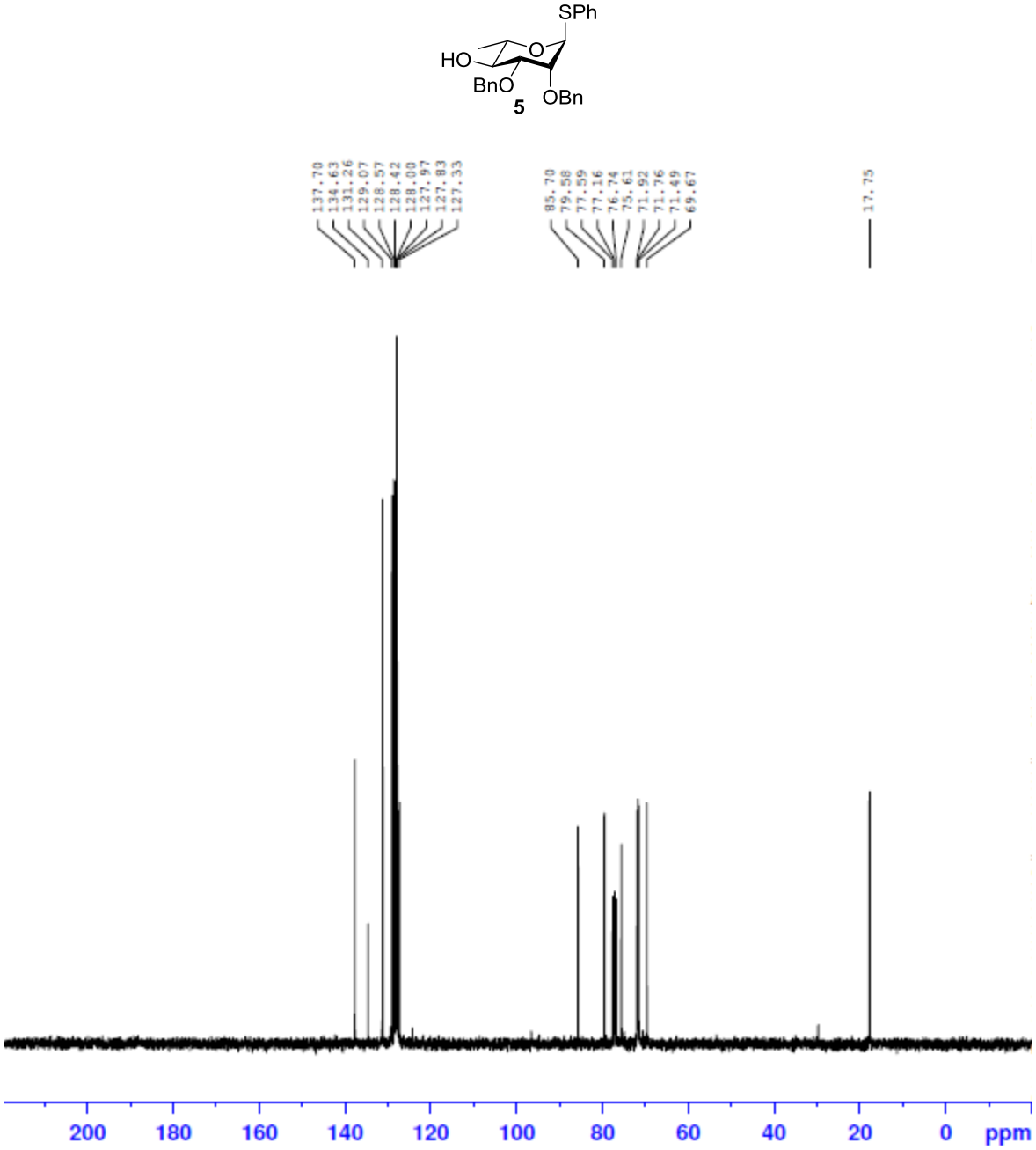
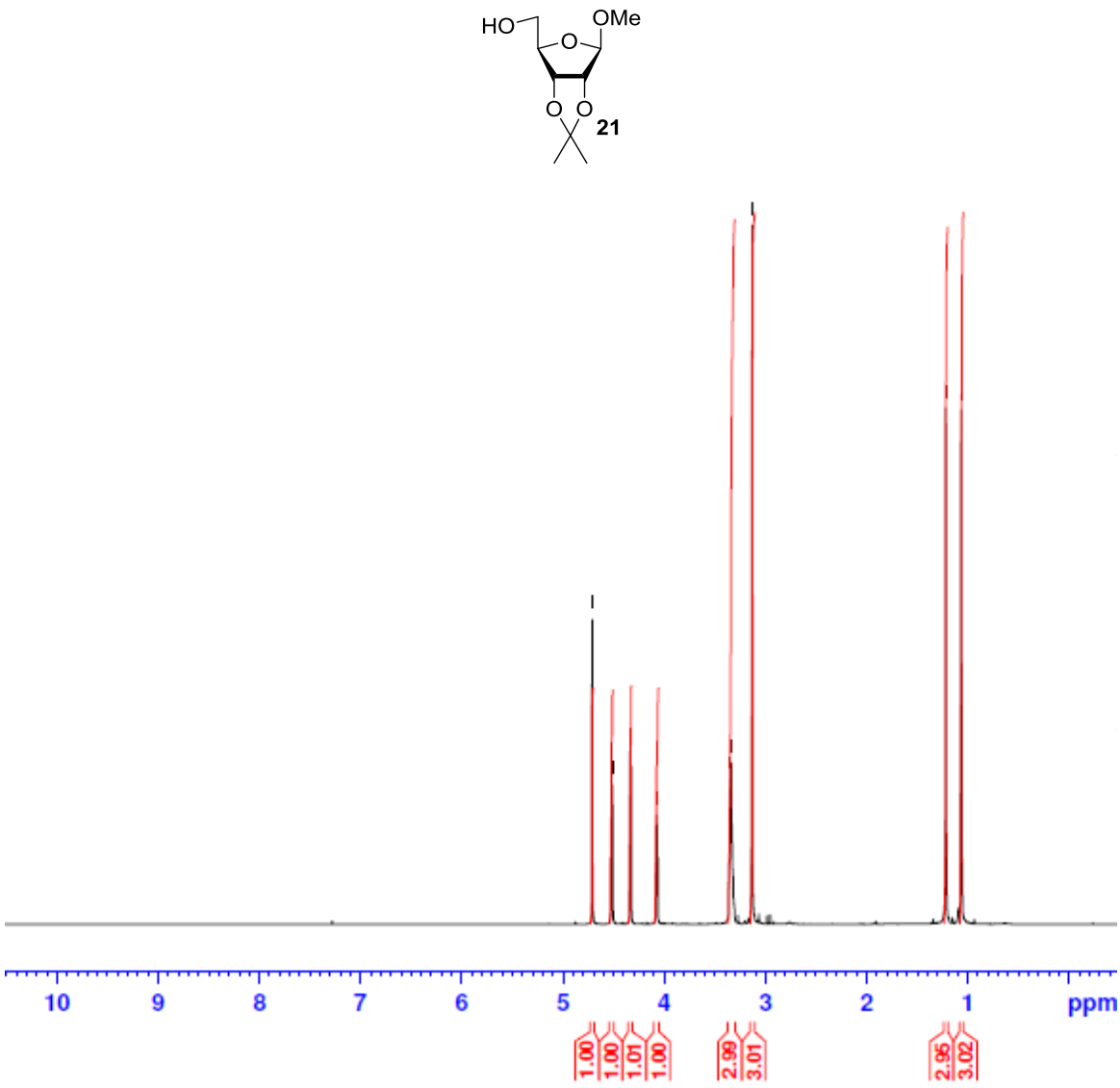


^{1}H NMR (500 MHz, $CDCl_3$)



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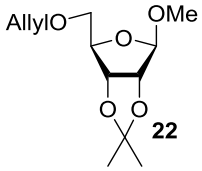

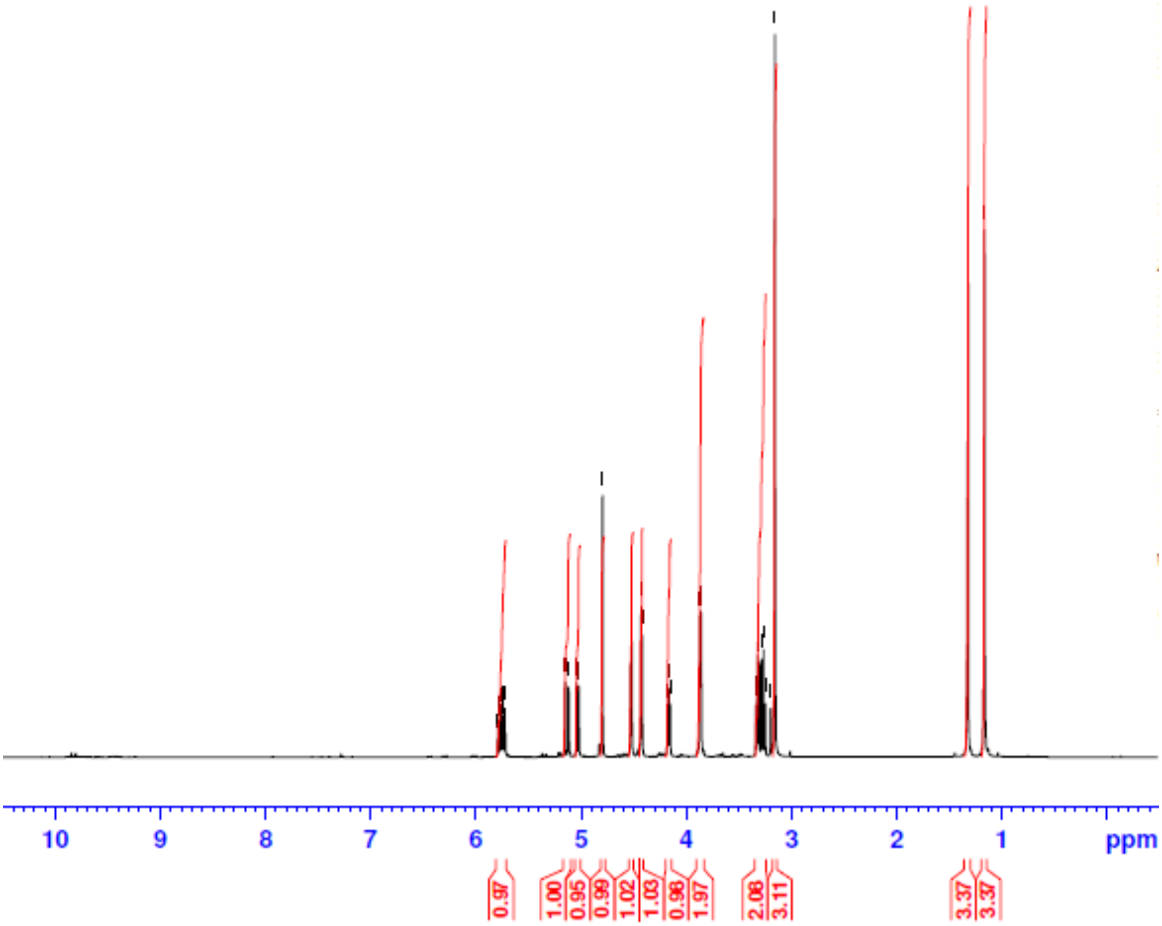

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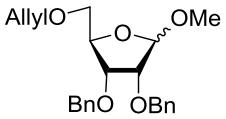

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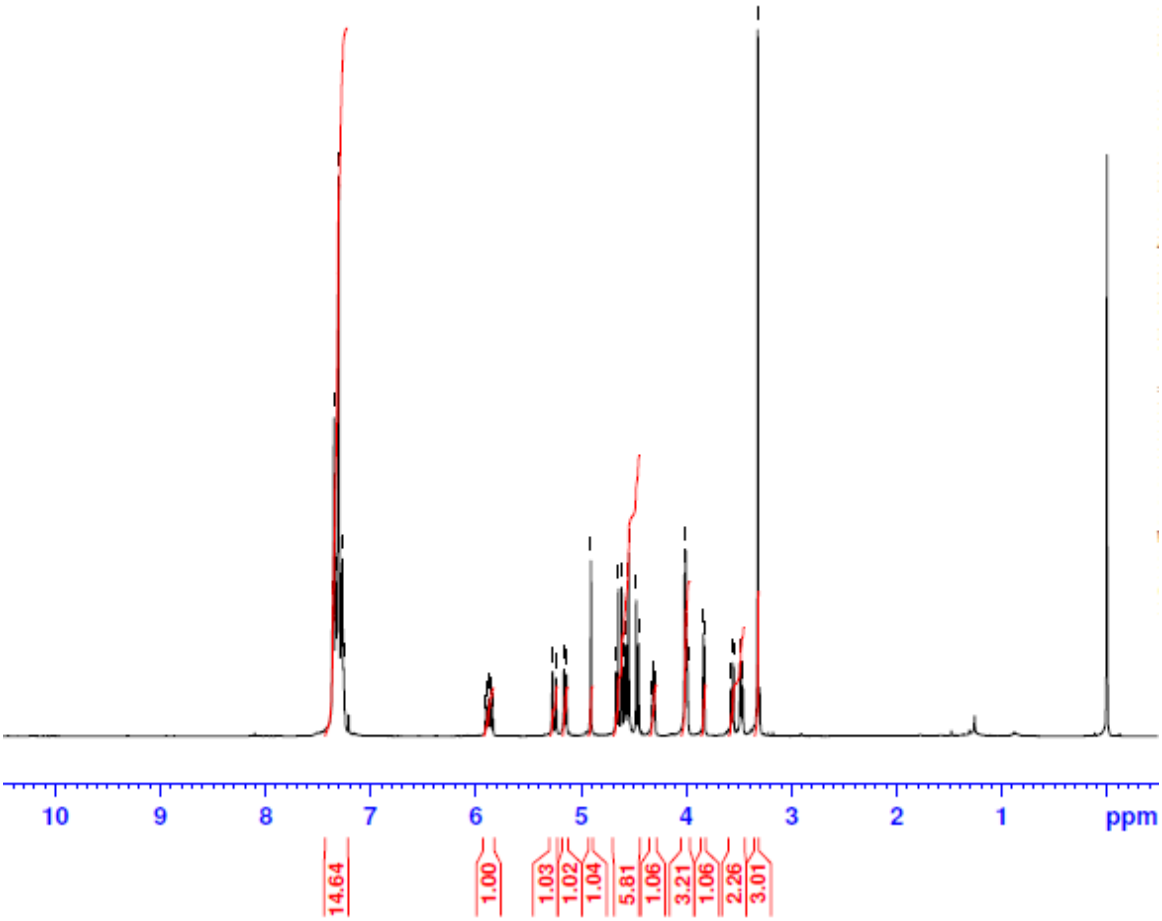

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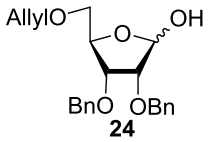

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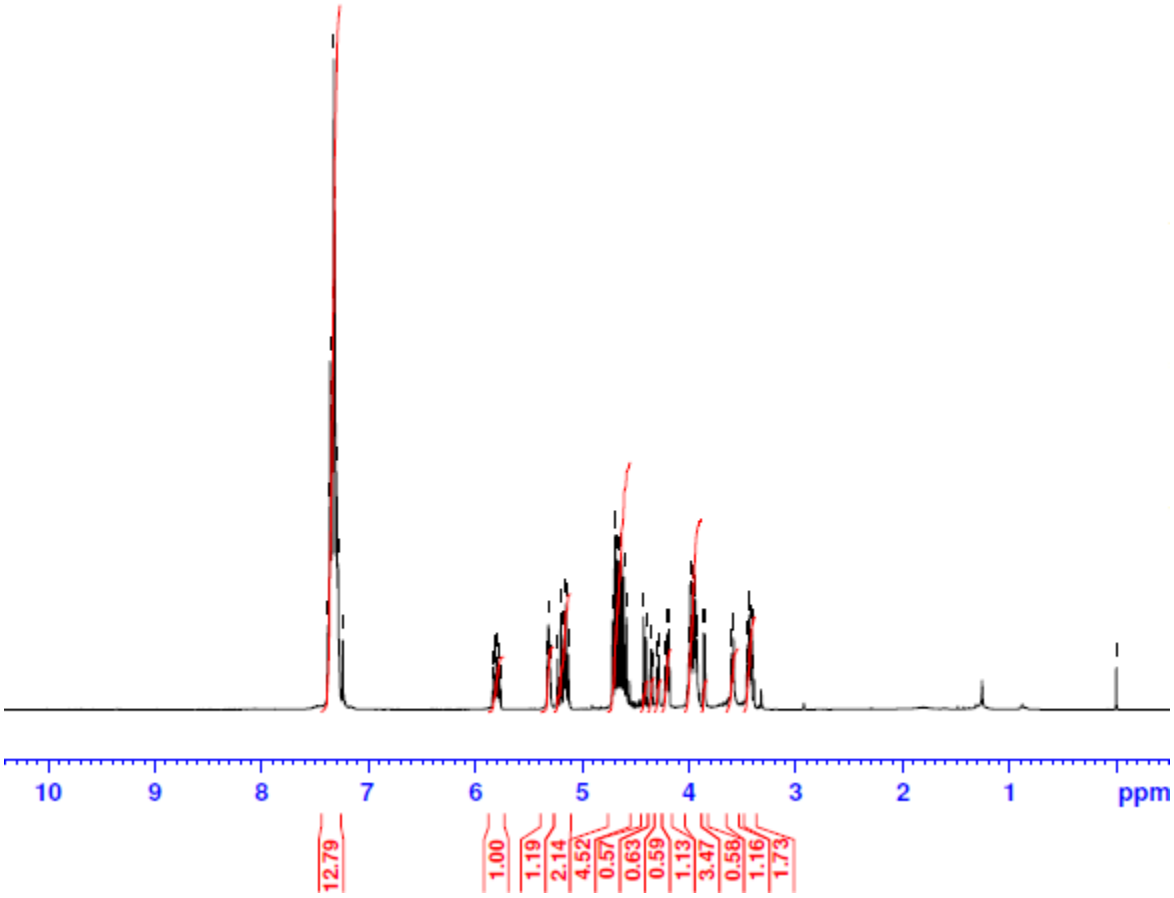

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