

THE SYNTHESIS OF DISACCHARIDES FOR THE FUNCTIONALIZATION OF
PAMAM DENDRIMERS

by

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DEDICATION

To my God and Saviour through whom all things are possible; to my husband for his undying support; to my beautiful baby boy for bringing so many smiles to my face; and to my parents for molding me into the person I am.

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ABSTRACT

An increase in the number of incidences of fungal disease among immunocompromised patients has developed in recent years. While a patient with an intact immune system would be able to fight the pathogen, an immunocompromised patient is not able to do so. The mortality rate of disseminated candidiasis can be as high as 30-40%. Although there have been great advances in the understanding of how the immune system detects pathogens, there is still much to be learned.

PAMAM (poly(amidoamine)) dendrimers have been chosen as scaffolds on which to display disaccharides that are found on the surface of *Candida albicans*. Preliminary results from immunostimulation assays using $\alpha(1,2)$ -dimannose functionalized PAMAM dendrimers showed that disaccharide functionalized dendrimers can stimulate cytokine production. In light of these results, several disaccharides - phenyl 2-O-benzyl-4,6-O-benzylidene-3-O-(2,3,4,6-tetra-O-acetyl- α -D-mannopyranosyl)-1-thio- α -D-mannopyranoside, 1-O-2-(2-azidoethoxy)ethyl-3-O-benzyl-4,6-O-benzylidene-2-O-(2,3-di-O-benzyl-4,6-O-benzylidene- β -D-mannopyranosyl)- α -D-mannopyranoside, 1-O-2-(2-azidoethoxy)ethyl-3-O-benzyl-4,6-O-benzylidene-2-O-(2,3-di-O-benzyl-4,6-O-benzylidene- β -D-glucopyranosyl)- β -D-glucopyranoside, 1-O-2-(2-azidoethoxy)ethyl-2-O-benzyl-4,6-O-benzylidene-3-O-(2,3-di-O-benzyl-4,6-O-benzylidene- β -D-glucopyranosyl)- β -D-glucopyranoside - have been synthesized for the functionalization of G(3.5) PAMAM dendrimers.

CHAPTER ONE

INTRODUCTION

Candida albicans

In recent years, there has been a worldwide increase in the occurrence of fungal disease.¹ This is largely due to the continued success of implantation of foreign bodies into humans for therapeutic purposes. When receiving an organ transplant, for example, the patient's immune system must be suppressed to ensure acceptance of the new organ, resulting in high risk of infection. If an immunocompromised patient is infected with *Candida albicans*, this patient will suffer from candidiasis. The mortality rate of disseminated candidiasis is approximately 30-40%.² Therefore, selective activation of the immune system so that the fungal pathogen can be eliminated without posing a threat to the newly implanted organ would be very beneficial. The design of synthetic systems that will facilitate a more complete understanding of the immunological processes involved in fungal adhesion and fungal pathogenesis is the primary goal of this research project.

The fungal pathogen to be targeted is *Candida albicans*. *C. albicans* is the most common of the *Candida* species. The cell wall of *C. albicans* is comprised of two layers: a mannoprotein layer and a chitin layer. The mannoprotein layer is made up of both *N*- and *O*-linked mannose residues. As seen in Figure 1.1, the *N*-linked mannose residues form a highly branched oligosaccharide that contains a variety of linkages. The *O*-linked mannose residues form a straight chain consisting of $\alpha(1,2)$ -linkages. The chitin layer is

composed of a polymer of $\beta(1,4)$ -*N*-acetylglucosamine. There is strong evidence that the immune response is due to recognition of both layers.²

Netea et. al. have performed experiments probing the relationship between cytokine production and several cell wall mutations of *C. albicans*.² The *pmr1* mutant (Figure 1.1), severely lacking mannosyl residues, resulted in strong reduction of cytokine production. The mannosylphosphate deficient mutant (*mnn4*), however, caused normal cytokine production. A 70% reduction in cytokine production was observed when *N*-linked mannans (*och1*) were not present, and a 30% reduction was observed with truncated *O*-linked mannan (*mnt1mnt2*). Furthermore, the pattern-recognition receptors (PRRs) for both the *N*- and *O*-linked mannosyl residues were identified. TLR4 (toll-like receptor) was found to recognize *O*-linked residues, whereas MR (mannose receptor) recognizes *N*-linked residues (Figure 1.2). These results suggest that both the *N*- and *O*-linked mannosyl residues play an important role in pathogen recognition.

As stated earlier, *N*-linked mannan and *O*-linked mannan possess different mannosyl linkages. *O*-linked mannan is composed of straight chain $\alpha(1,2)$ -mannose linkages. The *N*-linked mannan is more complicated, containing an $\alpha(1,6)$ -linked mannoside backbone from which the other sugars branch. As seen in Figure 1.1, the *N*-linked mannan contains mostly $\alpha(1,2)$ -dimannoside and $\beta(1,3)$ -dimannoside terminal disaccharides. However, a few terminal $\alpha(1,3)$ -dimannose residues are also present. It is proposed that the different mannose linkages are recognized by PRRs, thus initiating the immune response.

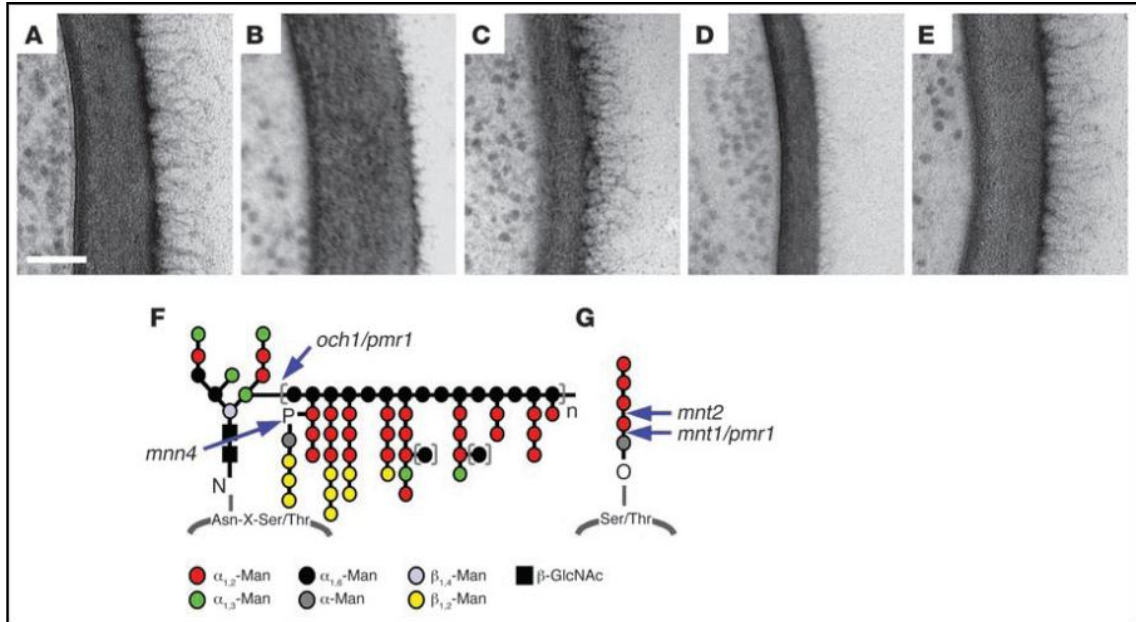


Figure 1.1 This figure is reproduced from reference 2. Cell wall morphology in the *C. albicans* strains. (A–E) TEM micrographs. (A) Wild-type strain. (B) *och1* null or doxycycline-regulated conditional mutants, which are defective in the branched outer *N*-linked mannosyl chains. (C) *mnt1 mnt2* mutant, which lacks 4 terminal *O*-linked α(1,2)-mannosyl residues. (D) *pmr1* mutant, which has gross defects in mannosylation, characterized by absence of phosphomannan and reduced *O*-linked and *N*-linked glycans. (E) *mnn4* mutant, which lacks phosphomannan. Scale bar: 100 nm. (F and G) Structure of the *N*- (F) and *O*-linked (G) glycans and the site of action of deleted gene products. Man, mannosyl; β-GlcNAc, β *N*-acetylglucosamine.²

PAMAM Dendrimers

Carbohydrate functionalized PAMAM (poly(amidoamine)) dendrimers that are used in our labs to study protein-carbohydrate interactions were chosen as frameworks on which to display disaccharides to mimic the mannosyl residues on the surface of *C. albicans*. PAMAM dendrimers are highly branched macromolecules that consist of a central core from which many terminal groups emanate (Figure 1.3). More specifically, PAMAM dendrimers have primary amines or carboxylates on their surface.

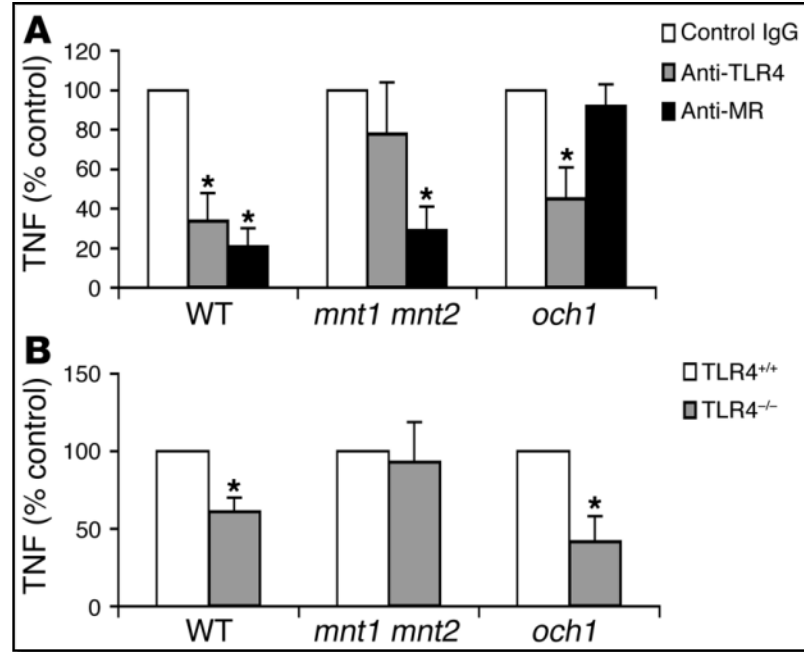


Figure 1.2 This figure is reproduced from reference 2. Differential recognition of O- and N-linked mannosyl residues by TLR4 and MR. (A) Human MNCs were stimulated with the various *C. albicans* strains — the parent NGY152 strain; the *och1* mutant, defective in N-linked mannosylation; and the *mnt1 mnt2* mutant, defective in O-linked mannosylation — in the presence of monoclonal antibodies against TLR4 or MR or a isotypematched control antibody. After 24 hours' stimulation at 37°C, supernatants were collected, and TNF concentration was measured by RIA. Results are pooled triplicate data from 2 separate experiments with a total of 8 volunteers per group. (B) Murine peritoneal macrophages from TLR4^{+/+} C57BL/10J and TLR4^{-/-} ScCr mice were stimulated with the various *C. albicans* strains: NGY152, the *och1* mutant, and the *mnt1 mnt2* mutant. After 24 hours' stimulation at 37°C, supernatants were collected, and TNF levels were determined by RIA. Results (mean ± SD) are pooled data from 2 separate experiments with a total of 10 mice per group. **P* < 0.05 versus stimulation in the presence of control antibodies (A) or versus TLR4^{+/+} mice (B).²

PAMAM dendrimers are synthesized via a 1,4-addition of ethylene diamine to methyl acrylate. The result is a half generation dendrimer having four carboxylates on its surface. Addition of ethylene diamine to the tetraester results in a generation 0 dendrimer having four terminal amines. Higher generations are achieved by repeating the above synthetic sequence.

The size and the number of branches of the dendrimer increase uniformly as the generation of the dendrimer increases. With each increasing generation, the number of terminal amines is doubled. A G(0) (generation 0) dendrimer, for example, has four terminal amines whereas a G(1) dendrimer has eight. Dendrimers having terminal carboxylates are half-generations, but those having terminal amines are whole generations. For instance, a G(4) dendrimer has 64 terminal amines, whereas a G(3.5) dendrimer has 64 terminal carboxylates. Unlike many polymers, PAMAM dendrimers have a polydispersity close to one. Due to their relative homogeneity and the variety of sizes available by choosing the appropriate generation, PAMAM dendrimers are a useful scaffold on which to display carbohydrates for studying the immune processes.³

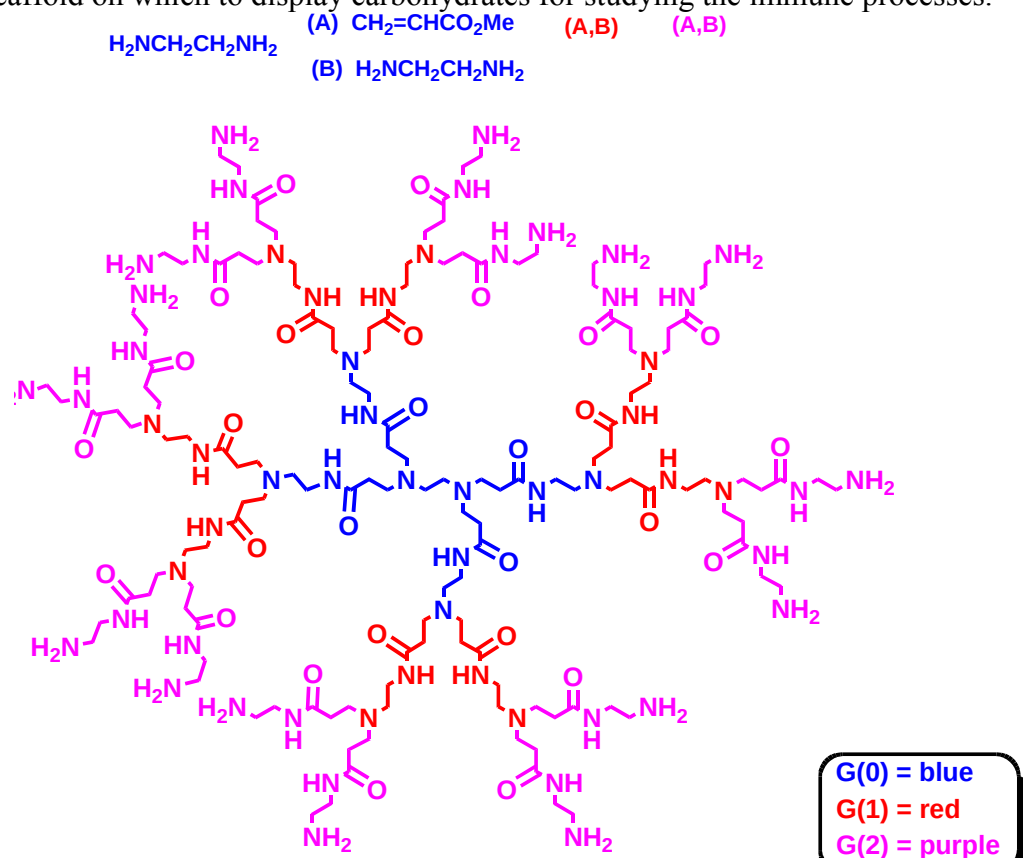


Figure 1.3 Generation 2 PAMAM (poly(amidoamine)) dendrimer.

Preliminary Results

The carbohydrates on the surface of *C. albicans* are extremely complex. Rather than try to synthesize an intricate oligosaccharide for the study of the immune response, simpler disaccharides were targeted. If the PRRs primarily recognize the outermost sugars, then the disaccharides will be an excellent imitation of the cell wall carbohydrates.

Immunostimulation assays were performed by Dr. Neil Gow and co-workers using $\alpha(1,2)$ -dimannose functionalized G(3) and G(4) PAMAM dendrimers previously synthesized in our lab with differing amounts of dimannosides on the periphery. G(3) dendrimers functionalized with $\alpha(1,2)$ -dimannosides did not stimulate cytokine production (data not shown). Neither hydroxyl nor galactose functionalized dendrimers were active in the assay. However, G(4) dimannose dendrimers did stimulate cytokine production. As shown in Figure 1.4, dendrimers that presented a larger number of dimannose residues (29 dimannosides) induced more cytokine production than dendrimers that had less dimannose units. Likewise, a higher concentration of glycodendrimer led to increased cytokine production.

While these results are promising, it is important to note that $\alpha(1,2)$ -mannosides are found not only on the surface of *C. albicans*, but also on the surface of other fungi. Specifically, *Saccharomyces cerevisiae*, a harmless fungi, has many $\alpha(1,2)$ -mannosides expressed on its surface.⁴ However, the $\beta(1,2)$ -linkage is specific to *C. albicans*.⁵ Therefore, identifying the saccharide unit that will selectively elicit cytokine production

for targeting of *C. albicans* necessitates the synthesis of disaccharides other than $\alpha(1,2)$ -dimannose.

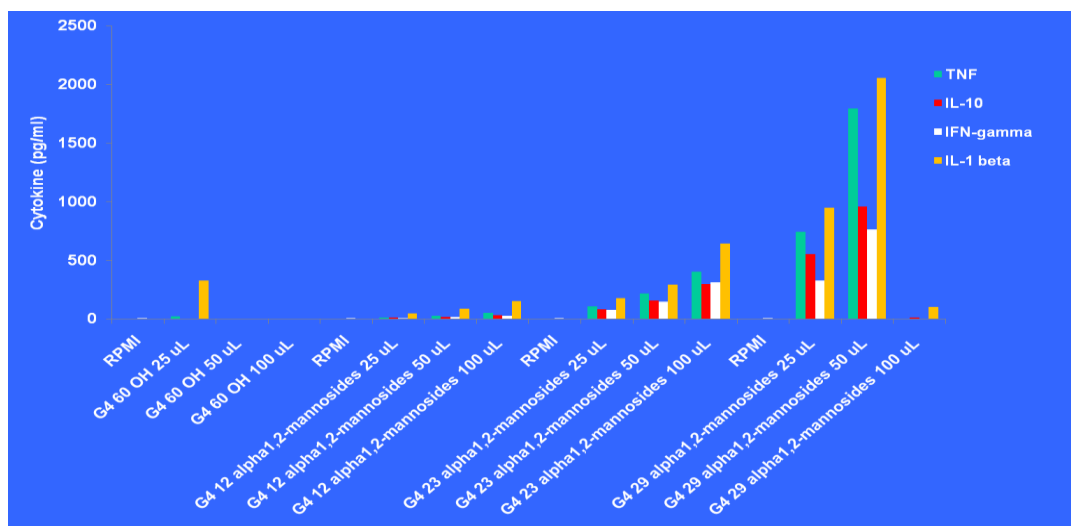


Figure 1.4 Preliminary results of immunostimulation assay with $\alpha(1,2)$ -dimannose functionalized G(4) PAMAM dendrimers. RPMI = media. TNF, IL-10, IFN- γ , and IL-1 β = cytokines

Project Goals

To further probe cytokine production, additional disaccharide-functionalized dendrimers are required. Specifically, the sugars of interest are $\alpha(1,3)$ -dimannose 1, $\beta(1,2)$ -dimannose 2, $\beta(1,3)$ -dimannose 3, $\beta(1,2)$ -diglucose 4, and $\beta(1,3)$ -diglucose 5. Before functionalizing the dendrimers with the disaccharides, they will be derivatized with an amine-terminated spacer to form 6-10. The amines 6-10 will react with the terminal carboxylates of the dendrimer to create an amide linkage between the PAMAM framework and the disaccharide. Since previous results were negative with G(3) dendrimers, target disaccharide derivatives 6-10 will be appended to G(3.5) PAMAM dendrimers (Figure 1.5). Also, the dendrimers will be fully functionalized with

disaccharides, as the preliminary results indicate that lower functionalization results in reduced cytokine production. The work reported here will focus on the synthesis of the disaccharide-functionalized dendrimers.

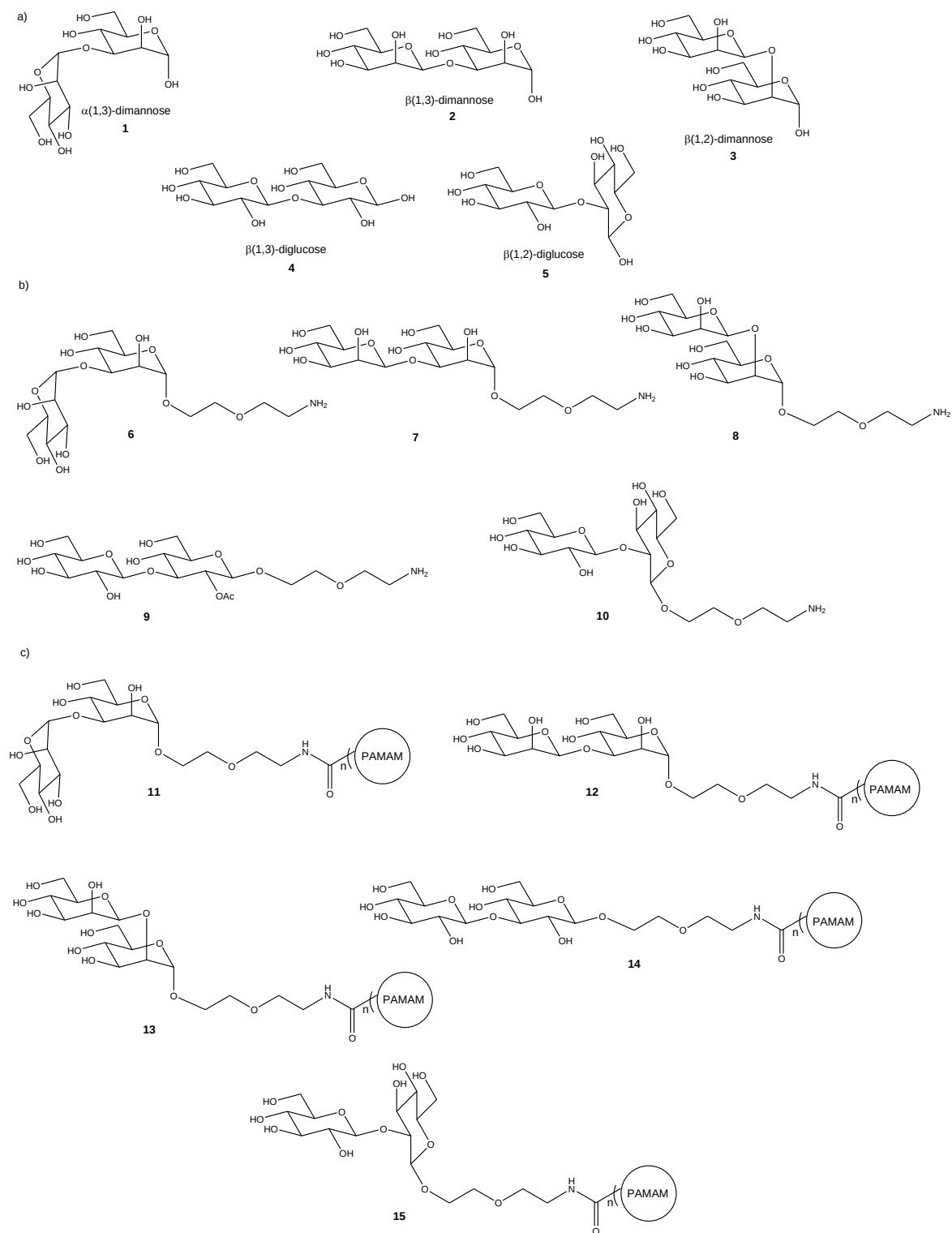


Figure 1.5 a) 1-5: Disaccharides of interest. b) 6-10: Disaccharides with isothiocyanate linker. c) 11-15: Disaccharide-functionalized PAMAM dendrimers with thiourea linkage.

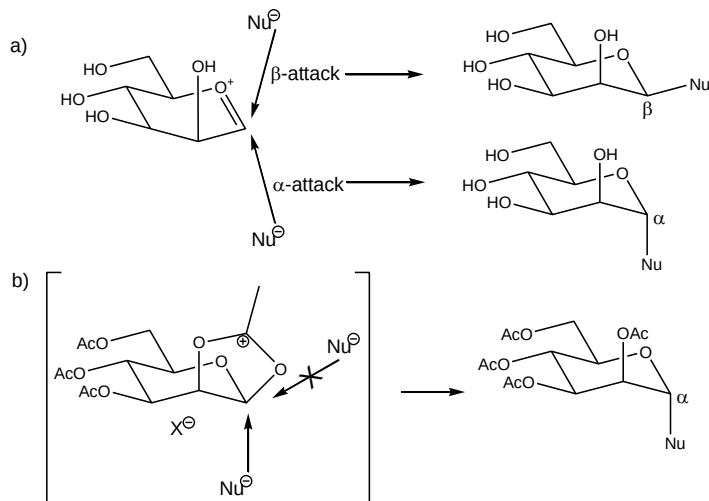
CHAPTER TWO

SYNTHESIS OF DIMANNOSIDES

 β -Mannosides

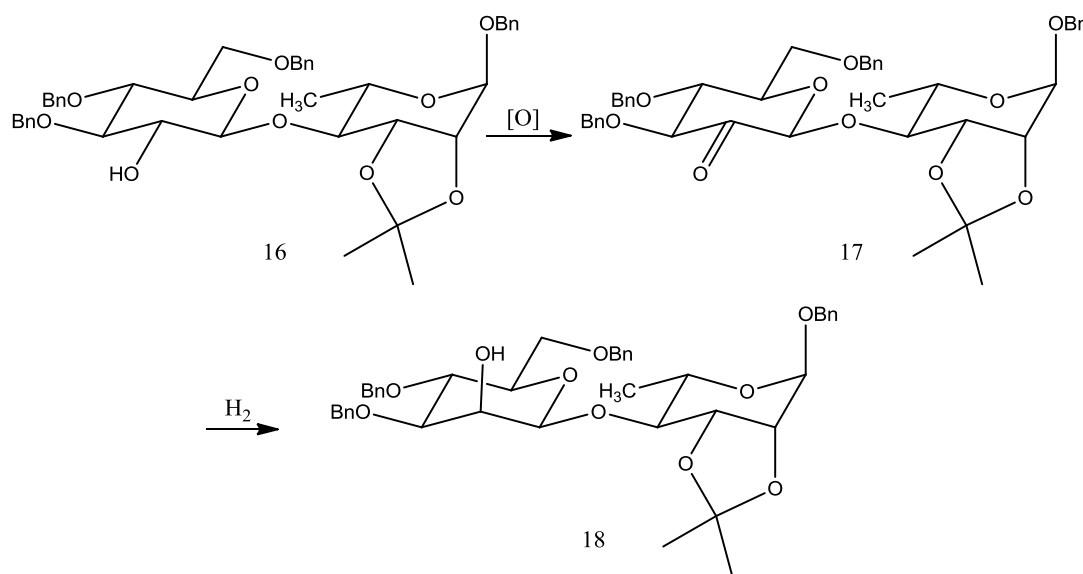
Formation of β -mannosides has been one of the most challenging glycosidic bonds to make.⁶ Mannose residues have an overwhelming preference for the α configuration due to stereoelectronic effects. In general, β -mannosides are difficult to prepare because the axial hydroxyl at C-2 hinders nucleophilic attack from the β face. This is exacerbated if there is a large protecting group at the C-2 position. If there is an ester protecting group at C-2, the carbonyl can actively block the β face through neighboring group participation (Scheme 2.1).⁶ In addition, the anomeric effect further promotes formation of the α -mannoside.

Scheme 2.1 Nucleophilic attack on mannose: a) Nucleophilic attack on the oxocarbenium ion to yield β and α products. b) Nucleophilic attack with a participating group at C2; the α product is formed.

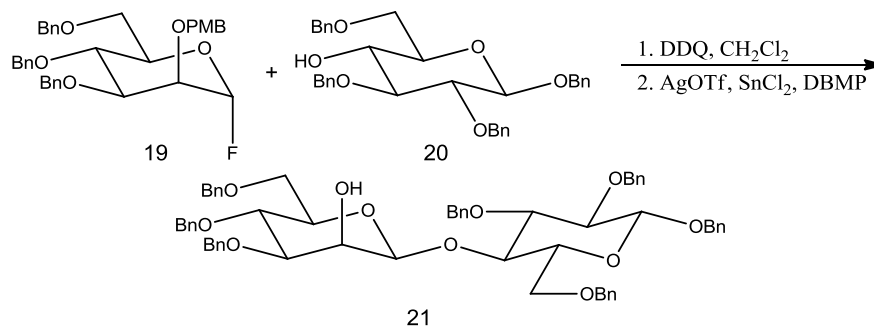


Several methods have been utilized to overcome the obstacles to synthesize β -mannosides. In one method, glucose was used to form the β glycosidic bond, followed by conversion of glucose to mannose⁷ (Scheme 2.2). This was achieved by oxidation of the C-2 hydroxyl in 16 to form ketone 17. Selective reduction of 17 led to β -mannoside 18. Although this route provides the desired β product, it is not suitable to a wide variety of functional groups that are often present during carbohydrate synthesis.

Scheme 2.2 Formation of β -mannoside via oxidation and selective reduction of C-2 OH of glucose.



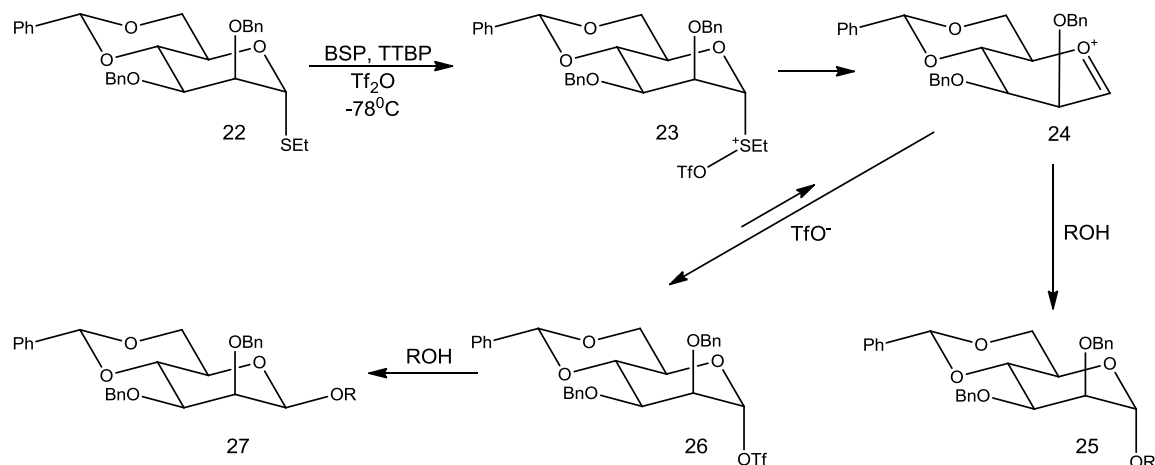
A different approach to forming β -mannoside linkages is the aglycon delivery method.⁸ In this case, advantage is taken of the axial OH on C-2. The acceptor 20 (aglycon) is covalently linked to the protecting group on C-2 of 19. Upon activation at the anomeric position with AgOTf, the aglycon is delivered to the β face to afford 21 (Scheme 2.3).

Scheme 2.3 Aglycon delivery to form β -mannoside.

Finally, Crich and coworkers have developed a sulfoxide method for synthesizing β -mannosides.⁹ According to the mechanism proposed by Crich (Scheme 2.4), premixing of donor 22 with benzene sulfinyl piperidine and base followed by triflic anhydride at -78 °C gives activated thiomannoside 23. Once oxocarbenium ion 24 is formed, triflate can add giving the α -triflate 26. The acceptor alcohol can then add in an S_N2-like mechanism to give the β -mannoside 27. However, if the donor is not activated prior to addition of the alcohol, the alcohol will add to 24, giving the α -mannoside 25 rather than the desired β product. If the alcohol is bulky, it may be slower to displace Tf₂O⁻, and react instead with 24 to give 25. It should also be noted that the 4,6-*O*-benzylidene substituent has proven necessary for acceptable α : β ratios. The equilibrium between 24 and 26 is expected to favor triflate 26 due to constraints of the *trans*-fused ring system. When the benzylidene is absent, the oxocarbenium ion 24 is no longer disfavored, and readily reacts with acceptor to produce α -mannoside 25. This methodology has proven to be useful for a wide range of alcohols,¹⁰ but lower selectivity is observed when bulky

substituents are present at C-2.¹⁰ The mechanism described above is in accordance with the products observed under the given conditions.

Scheme 2.4 Proposed mechanism of β -mannoside formation.¹¹ ROH includes: MeOH and various 1° and 2° alcohols. For a detailed list of alcohols, see reference 11.



Woerpel et. al. have probed the effect of electrostatic interactions of substituted oxocarbenium ions.¹² They observed that monosubstituted tetrahydropyran oxocarbenium ions prefer a half-chair with a pseudoaxial orientation. As more substituents are added to the ring, the analysis becomes more complex. However, it is worth looking at the possible half-chair conformations of the proposed donor 22. As is evident from Figure 2.1, only one half-chair is possible due to the trans-fused ring system. Nucleophilic attack from the β -face would be disfavored because of sterics and it leads to a twist boat transition state. Therefore, α attack is preferred, leading to intermediate triflate 26 or to α -mannoside 25.

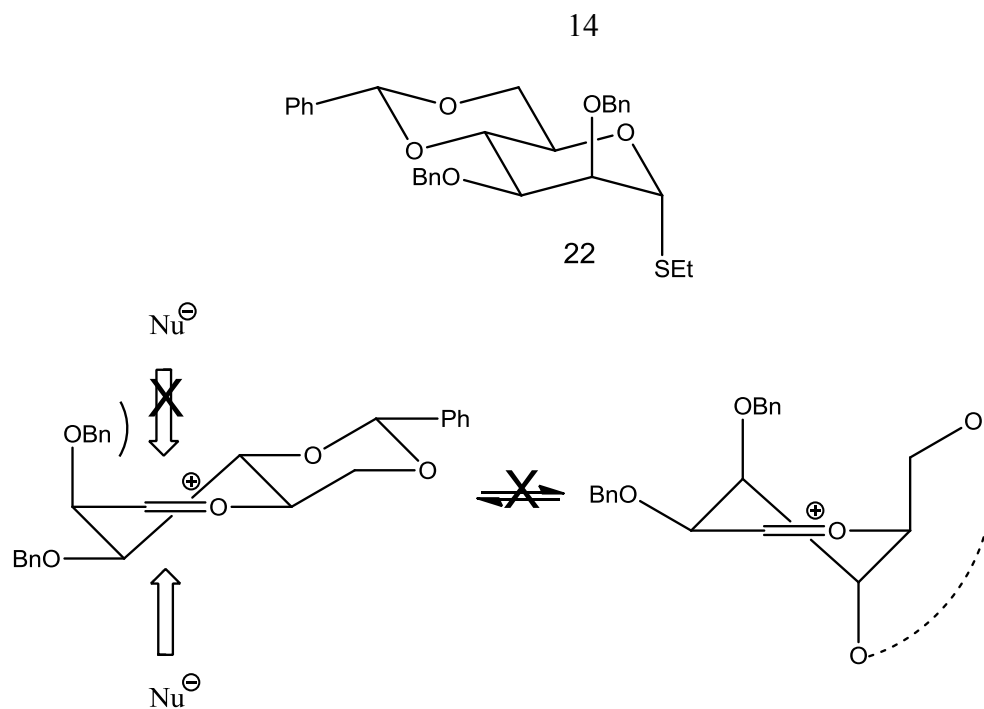
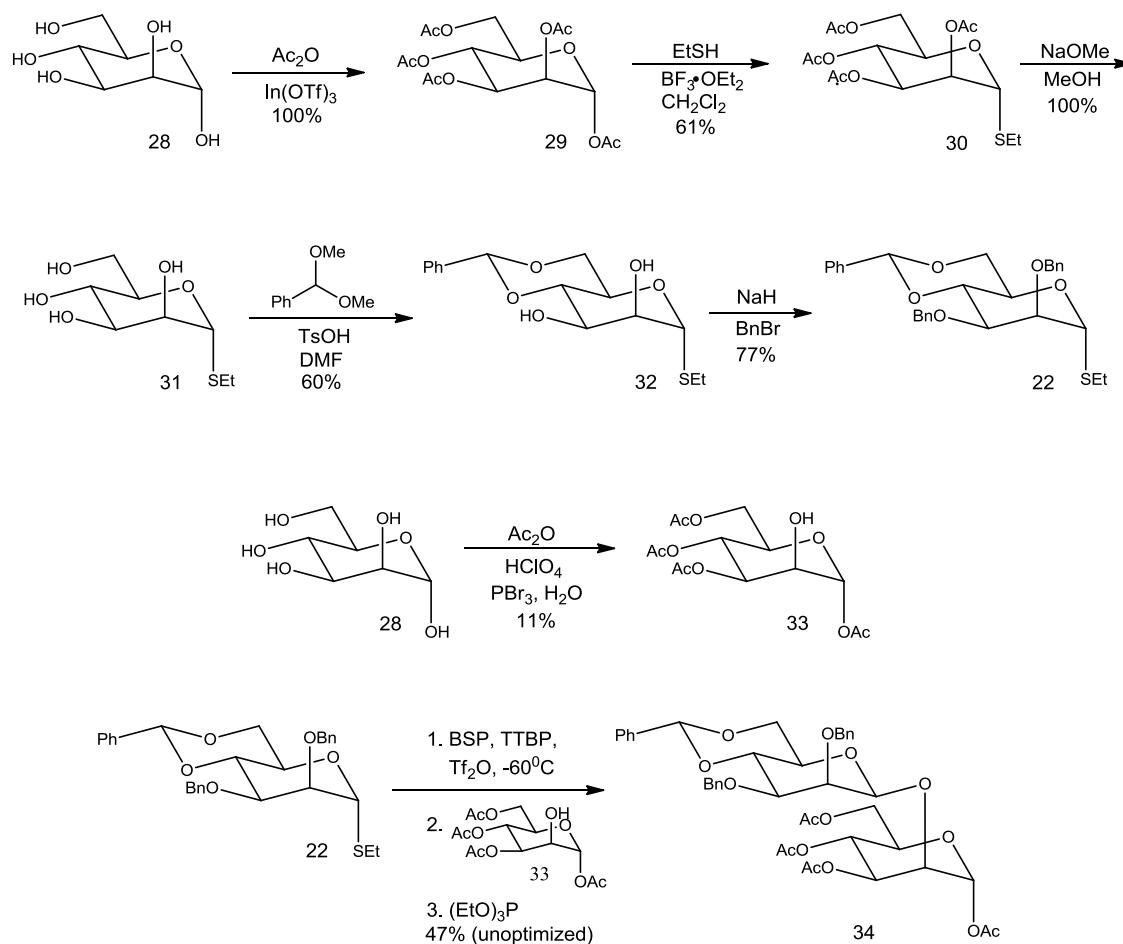


Figure 2.1 Nucleophilic addition to oxocarbenium ion

Synthesis of $\beta(1,2)$ -Dimannose

To utilize Crich's methodology, donor 22 was synthesized. Universal protection of α -D-mannose using acetic anhydride with indium triflate¹³ as a catalyst afforded peracetylated mannoside 29 in quantitative yield (Scheme 2.5). Treatment of 29 with ethanethiol and boron trifluoride etherate gave thiomannoside 30.¹⁴ Deprotection with sodium methoxide in methanol¹⁵ followed by transacetalation with benzaldehyde dimethylacetal¹⁶ provided diol 32. Benzyl protection of the diol to afford 22 was achieved in 77% yield with sodium hydride and benzyl bromide.¹⁶

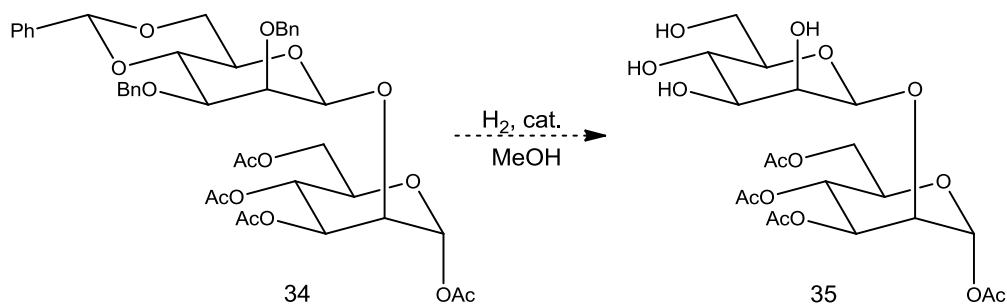
Acceptor 33 was readily synthesized from mannose using acetic anhydride and perchloric acid.¹⁷ Applying Crich's sulfoxide method, activation of donor 22 with triflic anhydride and base followed by addition of acceptor 33 led to disaccharide 34 in 47% yield.

Scheme 2.5 Formation of β -mannoside 34

Because thioureas are often used to link the end groups to the dendrimers, removal of the benzyl protecting groups at this point was desired to avoid poisoning of the palladium catalyst used for hydrogenolysis. By all accounts, the deprotection should proceed smoothly.¹⁰ However, many attempts to deprotect the disaccharide proved unsuccessful. As seen in Table 2.1, hydrogenolysis with 10% Pd/C at various pressures led to decomposition (entries 1-3). Promising results were achieved using Pearlman's catalyst (Pd(OH)₂, (entry 4), but the benzylidene acetal was left intact and the acetyl

groups were removed. Since removal of the benzylidene acetal under acidic conditions to give $\beta(1,2)$ -dimannose 3 should be straightforward, entry 4 with $\text{Pd}(\text{OH})_2$ indicates an acceptable strategy for deprotection of this dimannoside.

Table 2.1 Deprotection conditions

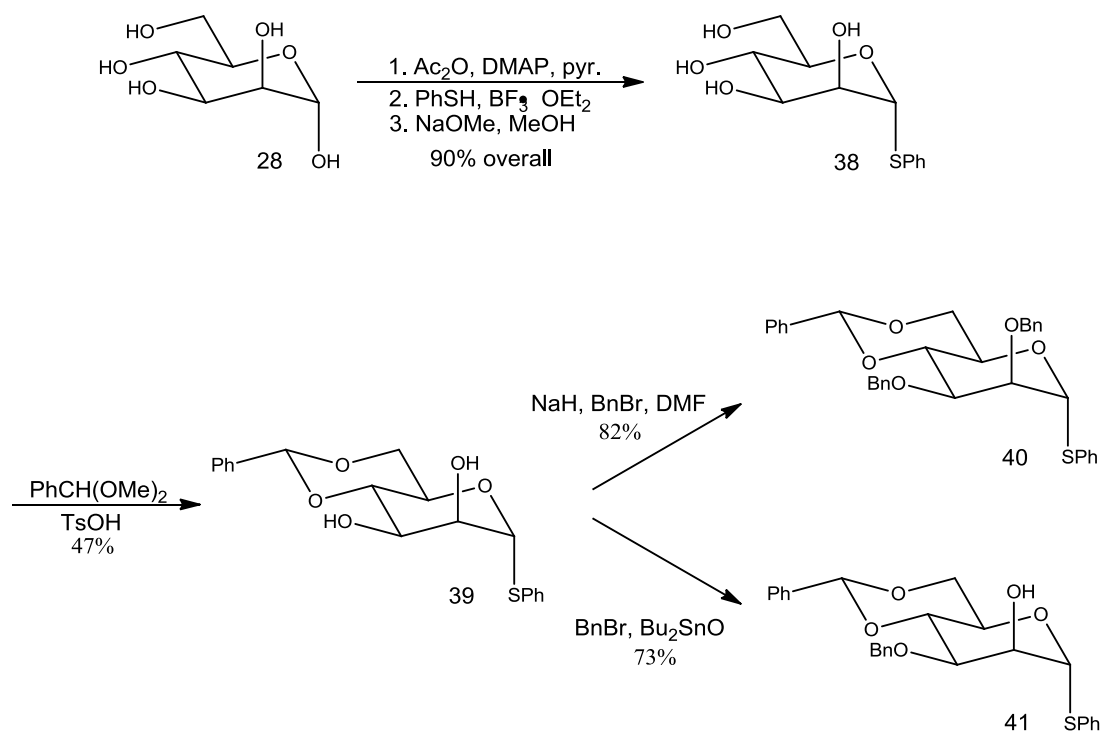


	catalyst	psi	result
1	10% Pd/C	balloon	decomposition
2	10% Pd/C	50	decomposition
3	10% Pd/C	100	decomposition
4	$\text{Pd}(\text{OH})_2$	100	acetyl and benzyl groups removed, benzylidene acetal intact

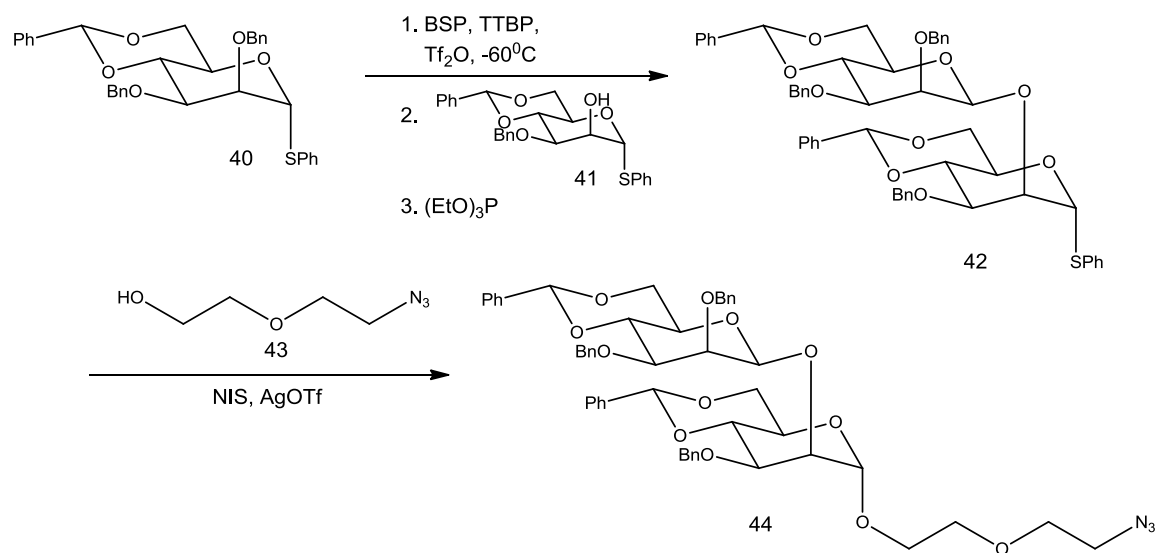
Due to the difficulties of deprotecting disaccharide 34, an alternative route to the $\beta(1,2)$ -dimannose via donor 40 and acceptor 41 was pursued. The synthesis of phenyl 1-thio- α -D-mannopyranoside 38 is analogous to that of ethyl 1-thio- α -D-mannopyranoside 31 and is shown in Scheme 2.6. Thiomannoside 38 was synthesized from mannose in 90% yield in 3 steps. Benzylidene acetal formation was achieved by reaction of 38 with benzaldehyde dimethylacetal and tosic acid. Reaction of diol 39 with sodium hydride and benzyl bromide gave donor 40 in 82% yield. Monobenylation of the same diol 39 - via a stannylene acetal - using dibutyltin oxide¹⁸ and benzyl bromide led to acceptor 41.

Thus, both donor 40 and acceptor 41 for glycosylation were readily available from diol 39.

Scheme 2.6 Synthesis of donor 40 and acceptor 41

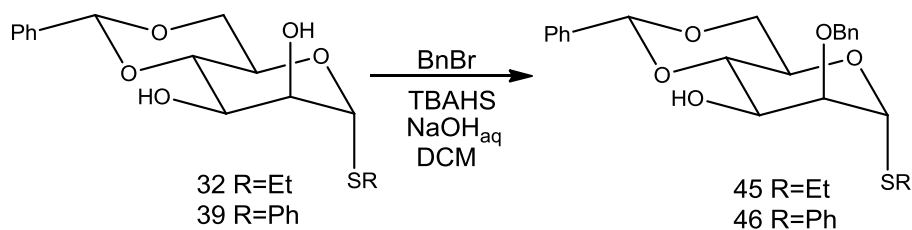


Alcohol acceptor 41 was allowed to react with activated donor 40 to give phenyl 3-*O*-benzyl-4,6-*O*-benzylidene-2-*O*-(2,3-di-*O*-benzyl-4,6-*O*-benzylidene- β -D-mannopyranosyl)-1-thio- α -D-mannopyranoside 42 (Scheme 2.7). The azido alcohol 43 was tethered to the disaccharide with *N*-iodosuccinimide (NIS) and silver triflate (AgOTf).¹⁹ Although only trace amounts of 44 were made, its presence was confirmed by HRMS.

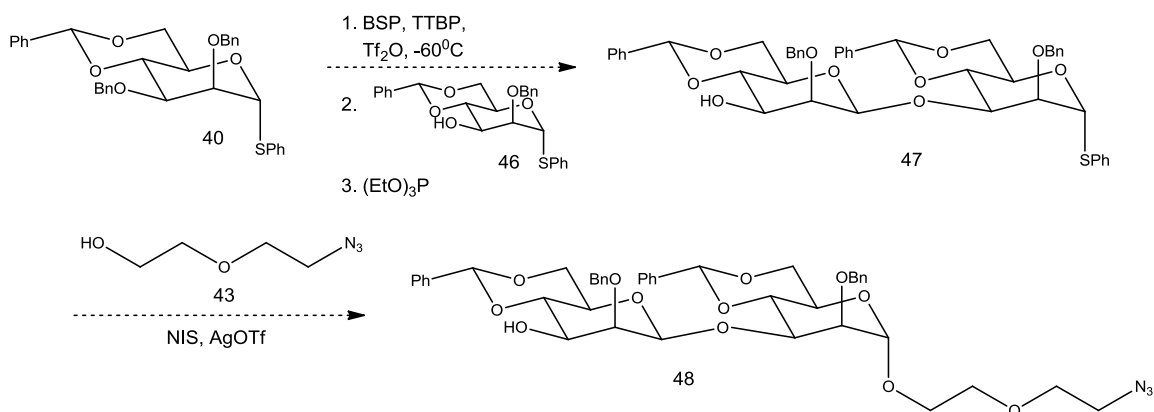
Scheme 2.7 Formation of β -1,2-dimannose 44Synthesis of β -1,3-Dimannose

Acceptor 45 for the formation of (1,3)-dimannoside was synthesized from benzylidene acetal 32 using phase transfer catalysis (Scheme 2.8).²⁰ The fact that diol 32 is more water soluble than the monobenzylated product 45 is the basis for phase transfer catalysis. Since the base is in the aqueous layer and the monobenzylated product is in the organic layer, good yields of the monobenzylated product can be achieved.^{10, 20} Reaction of the diol with benzyl bromide, tetrabutylammonium hydrogen sulfate, and aqueous sodium hydroxide in methylene chloride gave a mixture of monobenzylated product and the starting diol. Due to poor yield of the monobenylation and issues with solubility, it was decided to proceed through the phenyl 1-thio- α -D-mannopyranoside instead of the ethyl 1-thio- α -D-mannopyranoside. The added phenyl group was hypothesized to be likely to improve the solubility of the mannose derivative in organic solvents.

Scheme 2.8 Formation of monobenzylated product via phase transfer catalysis



Therefore, acceptor alcohol 46 was prepared similar to that of the 1,2-dimannose acceptor 41. Diol 39 was transformed to monobenzylated acceptor 46 in 30% yield using phase transfer catalysis as described above (Scheme 2.8). Reaction of donor 40 with acceptor 46 should yield β -dimannoside 47 (Scheme 2.9). Azido mannoside 48 will be synthesized using NIS, AgOTf and azido alcohol 43.

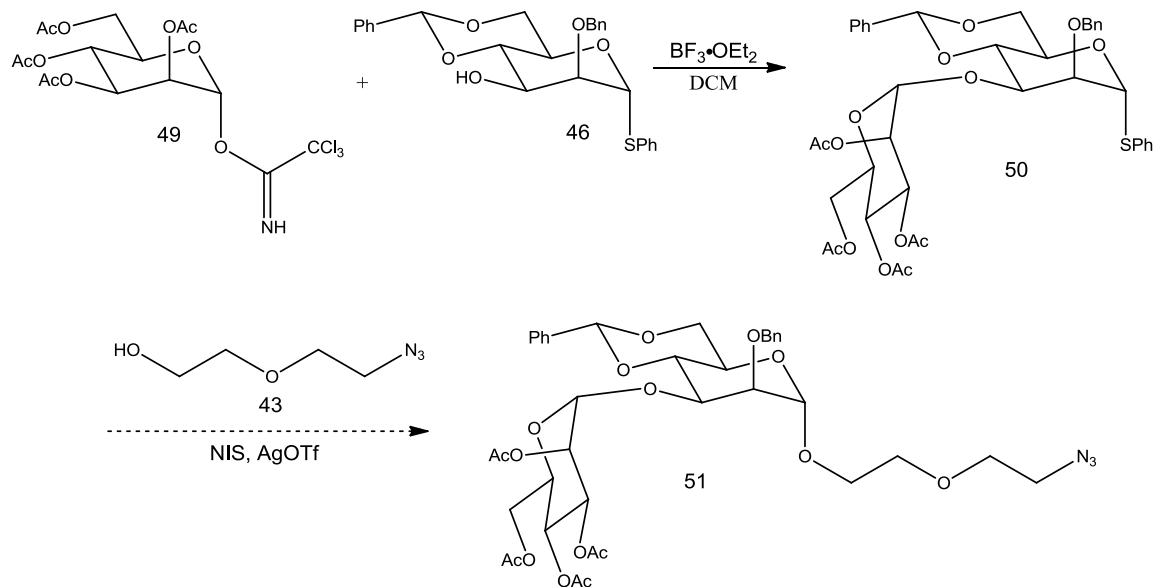
Scheme 2.9 Formation of β (1,3)-dimannose 48

Synthesis of α (1,3)-Dimannose

The synthesis of α (1,3)-dimannose will be achieved with readily available starting materials. As seen in Scheme 2.10, coupling of acceptor alcohol 46 and

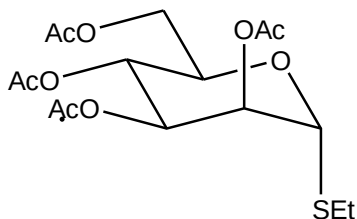
trichloroacetimidate 49 with $\text{BF}_3 \cdot \text{OEt}_2$ yielded $\alpha(1,3)$ -dimannoside 50 in %. Reaction of 50 with azido alcohol 43 utilizing NIS and AgOTf should afford α -mannoside 51.

Scheme 2.10 Formation of $\alpha(1,3)$ -dimannoside 51

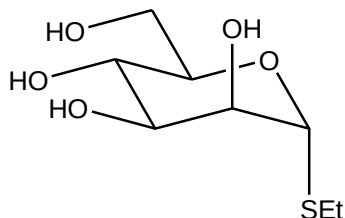


Summary

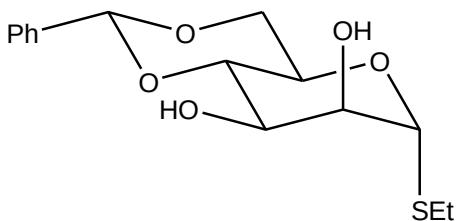
In summary, $\beta(1,2)$ -dimannoside 34 was synthesized using Crich's methodology. Deprotection of 34 did not proceed satisfactorily under several different conditions, so an alternate acceptor 41 and $\beta(1,2)$ -dimannoside 44 were synthesized. $\alpha(1,3)$ -Dimannoside 50 was prepared from alcohol acceptor 46 and trichloroacetimidate 49. The precursors to $\beta(1,3)$ -dimannoside 47 have also been prepared. Structures have been confirmed using ^1H and ^{13}C NMR as well as HRMS.

Experimental Procedures

Ethyl 2,3,4,6-tetra-O-acetyl-1-thio- α -D-mannopyranoside (30).¹⁴ 1,2,3,4,6-penta-O-acetyl- α -D-mannopyranose (29) (2.15 g, 5.51 mmol) was dissolved in 20 mL of dry CH_2Cl_2 . The flask was flushed with argon and cooled to 0 °C. Ethane thiol (1.22 mL, 16.5 mmol) followed by boron trifluoride etherate (2.10 mL, 16.5 mmol) were added to the flask via syringe. The solution was stirred for 2 h at 0 °C, warmed to room temperature, and stirred for 16 h. Aqueous saturated NaHCO_3 solution was added, and after stirring for 2 h, the organics were separated, dried over MgSO_4 , and concentrated. The residue was purified by column chromatography on silica gel (3:1 toluene : EtOAc) to give 30 (1.69 g, 78% yield). ^1H NMR (500 MHz, CDCl_3) δ 5.32 (dd, J = 3.2, 1.5 Hz, 1H), 5.31 – 5.22 (m, 3H), 4.38 (ddd, J = 9.5, 5.3, 2.3 Hz, 1H), 4.30 (dd, J = 12.2, 5.3 Hz, 1H), 4.07 (dd, J = 12.2, 2.3 Hz, 1H), 2.71 – 2.53 (m, 2H), 2.15 (s, 3H), 2.08 (s, 3H), 2.03 (s, 3H), 1.97 (s, 3H), 1.29 (t, J = 7.4 Hz, 3H) ppm; ^{13}C NMR (126 MHz, CDCl_3) δ 82.27, 71.17, 69.46, 68.89, 66.36, 62.41, 25.46, 20.92, 20.70, 20.69, 20.62, 14.73 ppm.

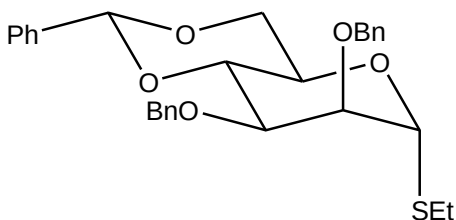


Ethyl 1-thio- α -D-mannopyranoside (31).¹⁴ To a solution of tetraacetate 30 (1.69 g, 4.32 mmol) in distilled methanol (10 mL) sodium metal (0.20 g, 8.64 mmol) was added at room temperature. The reaction mixture was stirred overnight. The solution was neutralized with Amberlite IR-120 (acidic), filtered over celite and concentrated to give 0.97 g of 31 in quantitative yield. This product was used without further purification. ^1H NMR (300 MHz D_2O), δ 5.17 (s, 1H), 3.89 (dd, $J = 3.2, 1.4$ Hz, 1H), 3.87 – 3.81 (m, 1H), 3.73 (dd, $J = 12.2, 2.2$ Hz, 1H), 3.67 – 3.56 (m, 2H), 3.51 (t, $J = 9.7$ Hz, 1H), 2.65 – 2.40 (m, 2H), 1.12 (t, $J = 7.4$ Hz, 3H); ^{13}C NMR (126 MHz, D_2O) δ 84.14, 72.95, 71.73, 70.98, 67.01, 60.75, 24.70, 13.98 ppm.



Ethyl 4,6-O-benzylidene-1-thio- α -D-mannopyranoside (32). To a solution of ethyl 1-thio- α -D-mannopyranoside (31) (2.22 g, 9.9 mmol) and p-TsOH (0.56 g, 3.0 mmol) in 15 mL DMF was added benzaldehyde dimethyl acetal (1.5 mL, 9.9 mmol) at room temperature. The reaction mixture was heated to 50 °C with evacuation by rotary evaporation for 3 h. Triethyl amine (1.1 mL) was added, and the mixture was poured into

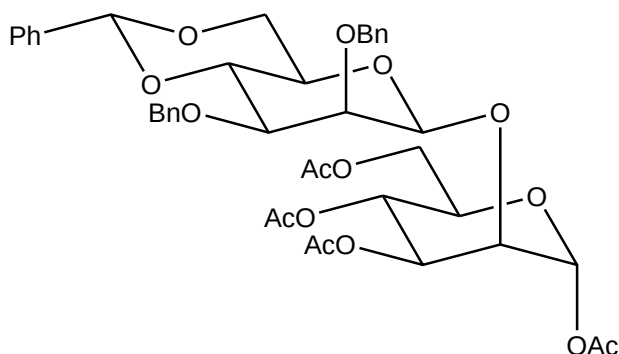
ice water (67 mL). The precipitate was filtered, washed with cold water and dried under vacuum to afford 32 (1.8 g, 60%) as fluffy white crystals. ^1H NMR (300 MHz, CDCl_3) δ 7.63 – 7.42 (m, 2H), 7.36 (m, 3H), 5.55 (s, 1H), 5.36 (s, 1H), 4.31 – 4.17 (m, 2H), 4.14 (m, 1H), 4.06 (dt, $J = 9.6, 3.0$ Hz, 1H), 4.00 – 3.91 (t, $J = 9.2$ Hz, 1H), 3.84 (t, $J = 11.7$ Hz, 1H), 2.73 (d, $J = 1.9$ Hz, 1H), 2.72 – 2.53 (m, 2H), 2.57 (d, $J = 2.9$ Hz, 1H), 1.29 (t, $J = 7.4$ Hz, 3H) ppm; HRMS (micro-TOF) calc. for $\text{C}_{15}\text{H}_{20}\text{O}_5\text{S} + \text{H} = 313.1109$, found 313.1083.



Ethyl 2,3-di-O-benzyl-4,6-O-benzylidene-1-thio- α -D-mannopyranoside (22).

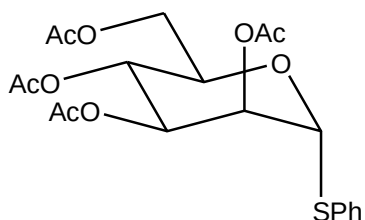
A solution of 32 (0.81 g, 2.6 mmol) in 4 mL of DMF was added to a solution of sodium hydride (0.42 g, 10.4 mmol) in DMF (4 mL) at 0 °C. After stirring for 30 min, benzyl bromide (935 μL , 7.8 mmol) was added to the reaction mixture and stirred for 8 h at room temperature. Methanol was added, and the mixture was poured into water. The product was extracted with EtOAc, and the organics were washed with brine, dried over Na_2SO_4 , and concentrated. The residue was purified by column chromatography on silica gel (9:1 hexanes : EtOAc) to give 22 (0.99 g, 77% yield) as a colorless oil. ^1H NMR (600 MHz, CDCl_3) δ 7.55 (d, $J = 7.5$ Hz, 2H), 7.48 – 7.22 (m, 10H), 5.67 (s, 1H), 5.34 (s, 1H), 4.83 (d, $J = 12.1$ Hz, 1H), 4.77 (q, $J = 12.2$ Hz, 2H), 4.65 (d, $J = 12.1$ Hz, 1H), 4.32 (t, $J = 9.5$ Hz, 1H), 4.28 – 4.19 (m, 2H), 3.99 – 3.88 (m, 3H), 2.80 – 2.43 (m, 2H), 1.26 (t, $J =$

7.4 Hz, 3H) ppm; ^{13}C NMR (151 MHz, CDCl_3) δ 138.62, 138.12, 137.85, 128.96, 128.56, 128.45, 128.30, 128.21, 127.95, 127.75, 127.69, 126.25, 101.62, 83.76, 79.44, 78.41, 76.64, 73.27, 73.20, 68.78, 64.82, 25.49, 15.09; HRMS (micro-TOF) calc. for $\text{C}_{29}\text{H}_{32}\text{O}_5\text{S}$ + H = 493.2048, found 493.2047.



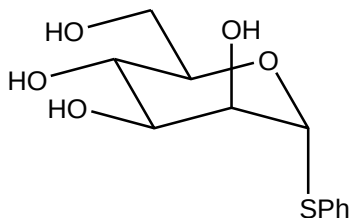
1,3,4,6-tetra-*O*-acetyl-2-*O*-(2,3-di-*O*-benzyl-4,6-*O*-benzylidene- β -D-mannopyranosyl) - α -D-mannopyranoside (34). A solution of donor 22 (0.10 g, 0.2 mmol), 1-(phenylsulfinyl)-piperidine (0.02 g, 0.1 mmol), 2,4,6-tri-*tert*-butylpyrimidine (0.05 g, 0.2 mmol) and powdered 4 Å molecular sieves in dry CH_2Cl_2 (2 mL) was stirred under argon at -50°C for 30 min. Triflic anhydride (34 μL , 0.2mmol) was added to the flask, and the solution was stirred for 10 min. A solution of acceptor 33 (0.10 g, 0.3 mmol) in dry CH_2Cl_2 (2 mL) was added, and the reaction mixture was allowed to stir for 3 h. The reaction was quenched upon the addition of triethylphosphite (70 μL , 0.4 mmol). After stirring for another 10 min at -50°C , the mixture was warmed to room temperature. The solids were removed by filtration, and the product was washed with saturated aqueous NaHCO_3 solution, brine, and dried over MgSO_4 . The solvent was removed, and the product was purified by column chromatography on silica gel (2:1

hexanes : EtOAc) to give 75 mg (47% yield) of the disaccharide 34. ^1H NMR (300 MHz, CDCl_3) δ 7.69 – 7.05 (m, 15H), 5.75 (s, 1H), 5.57 (s, 1H), 5.49 (t, $J = 10.0$ Hz, 1H), 5.28 (s, 1H), 5.10 (d, $J = 12.0$ Hz, 1H), 4.92 (d, $J = 12.0$ Hz, 1H), 4.82 (dd, $J = 10.0, 3.2$ Hz, 1H), 4.65 – 4.53 (m, 2H), 4.41 (d, $J = 3.2$ Hz, 1H), 4.34 – 4.19 (m, 2H), 4.19 – 4.10 (m, 2H), 4.10 – 4.00 (m, 1H), 3.83 (t, $J = 9.8$ Hz, 1H), 3.74 (ddd, $J = 9.8, 4.4, 2.1$ Hz, 1H), 3.50 (dd, $J = 9.8, 3.1$ Hz, 1H), 3.23 (td, $J = 9.8, 4.4$ Hz, 1H), 2.05 (s, 3H), 2.03 (s, 3H), 1.98 (s, 3H), 1.88 (s, 3H) ppm; ^{13}C NMR (75 MHz, CDCl_3) δ 170.71, 170.63, 169.12, 168.20, 138.55, 138.29, 137.49, 128.93, 128.85, 128.49, 128.33, 128.25, 128.24, 128.03, 127.59, 126.07, 103.21, 101.50, 91.75, 78.34, 77.02, 75.44, 74.74, 73.84, 73.53, 73.19, 72.08, 68.45, 67.79, 64.82, 61.79, 21.03, 20.83, 20.72, 20.62 ppm; HRMS (micro-TOF) calc. for $\text{C}_{41}\text{H}_{46}\text{O}_{15} + \text{Na} = 801.2734$, found 801.2723.

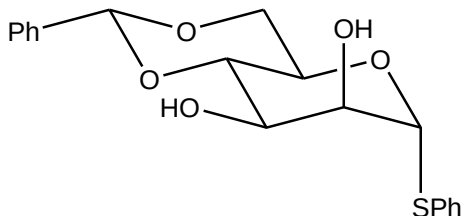


Phenyl 2,3,4,6-tetra-O-acetyl-1-thio- α -D-mannopyranoside (37). 1,2,3,4,6-penta-O-acetyl- α -D-mannopyranose (36) (4.1, 10.5 mmol) was dissolved in 15 mL of dry CH_2Cl_2 . The flask was flushed with argon and cooled to 0 °C. Thiophenol (3.47 mL, 31.5 mmol) and boron trifluoride etherate (3.99 mL, 31.5 mmol) were added to the flask via syringe. The solution was stirred for 2 h at 0 °C, warmed to room temperature, and stirred for 16 h. Aqueous saturated NaHCO_3 solution was added, and after stirring for 2 h, the organics were separated, dried over MgSO_4 , and concentrated. The residue was

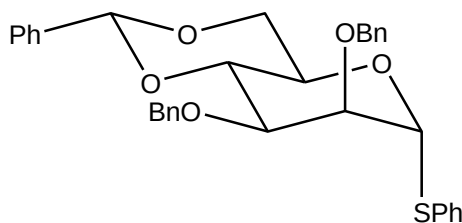
purified by column chromatography on silica gel (3:1 hexanes : EtOAc) to give **37** (4.23 g, 92% yield). ^1H NMR (300 MHz, CDCl_3) δ 7.68 – 7.02 (m, 5H), 5.48 (s, 2H), 5.37 – 5.26 (m, 2H), 4.53 (ddd, $J = 9.3, 5.8, 2.3$ Hz, 1H), 4.29 (dd, $J = 12.2, 5.8$ Hz, 1H), 4.08 (dd, $J = 12.2, 2.3$ Hz, 1H), 2.13 (s, 3H), 2.06 (s, 3H), 2.03 (s, 3H), 2.00 (s, 3H) ppm; ^{13}C NMR (75 MHz, CDCl_3) δ 170.56, 169.94, 169.84, 169.77, 132.08, 129.22, 128.14, 85.71, 70.92, 69.53, 69.39, 66.39, 62.46, 20.89, 20.71, 20.66 ppm.



Phenyl 1-thio- α -D-mannopyranoside (38**).** To a solution of tetraacetate **37** (4.23 g, 9.6 mmol) in distilled methanol (40 mL) sodium metal (0.44 g, 19.2 mmol) was added at room temperature. The reaction mixture was stirred overnight. The solution was neutralized with Amberlite IR-120 (acidic), filtered over celite and concentrated to give 2.62 g of **38** in quantitative yield. This product was used without further purification. ^1H NMR (D_2O , 300 MHz) δ 7.60 – 7.11 (m, 5H), 5.36 (d, $J = 1.3$ Hz, 1H), 4.07 (dd, $J = 3.2, 1.3$ Hz, 1H), 4.01 (ddd, $J = 9.5, 5.7, 2.3$ Hz, 1H), 3.76 – 3.65 (m, 2H), 3.65 – 3.54 (m, 2H). ^{13}C (75 MHz, D_2O) δ 132.62, 129.43, 128.35, 88.20, 73.60, 71.49, 71.03, 67.02, 60.71 ppm.



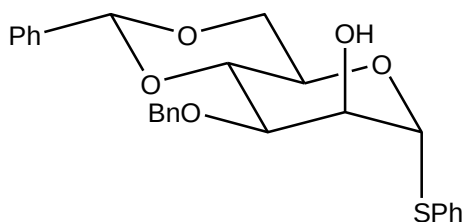
Phenyl 4,6-O-benzylidene-1-thio- α -D-mannopyranoside (39).⁹ To a solution of phenyl 1-thio- α -D-mannopyranoside 38 (0.1 g, 0.37 mmol) and p-TsOH (0.07 g, 37 μ mol) in 1 mL DMF was added benzaldehyde dimethyl acetal (55 μ L, 0.37 mmol) at room temperature. The reaction mixture was heated to 60 °C with evacuation by rotary evaporation for 2 h. The solvent was removed in vacuo, and the product was crystallized from ethyl acetate to give 62.3 mg (47%) of 39 as white crystals. ^1H NMR (300 MHz, CDCl_3) δ 7.79 – 6.96 (m, 10H), 5.59 (s, 1H), 5.57 (s, 1H), 4.33 (m, 2H), 4.21 (dd, J = 10.3, 5.0 Hz, 1H), 4.13 (dt, J = 9.5, 3.0 Hz, 1H), 4.00 (t, J = 9.5 Hz, 1H), 3.82 (t, J = 10.2 Hz, 1H), 2.75 (d, J = 1.9 Hz, 1H), 2.62 (d, J = 3.0 Hz, 1H) ppm; HRMS (micro-TOF) calc. for $\text{C}_{19}\text{H}_{20}\text{O}_5\text{S} + \text{Na}$ = 383.0929, found 383.0938.



Phenyl 2,3-Di-O-benzyl-4,6-O-benzylidene-1-thio- α -D-mannopyranoside (40).

A solution of 39 (1.0 g, 2.8 mmol) in 25 mL of DMF was added to a solution of sodium hydride (0.44 g, 11.1 mmol) in DMF (25 mL) at 0 °C. After stirring for 30 min, benzyl bromide (996 μ L, 7.8 mmol) was added to the reaction mixture and stirred for 8 hours at

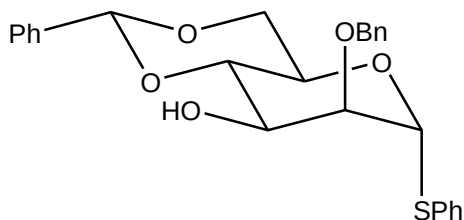
room temperature. Methanol was added, and the mixture was poured into water. The product was extracted with EtOAc, and the organics were washed with brine, dried over Na_2SO_4 , and concentrated. The residue was purified by column chromatography on silica gel (9:1 hexanes : EtOAc) to give 40 (1.22 g, 82% yield) as a colorless oil. ^1H NMR (500 MHz, CDCl_3) δ 7.61-7.26 (m, 20H), 5.68 (s, 1H), 5.55 (s, 1H), 4.85 (d, $J = 12.2$ Hz, 1H), 4.75 (s, 2H), 4.68 (d, $J = 12.2$ Hz, 1H), 4.38 – 4.29 (m, 2H), 4.25 (dd, $J = 10.2, 4.1$ Hz, 1H), 4.08 (d, $J = 3.0$ Hz, 1H), 4.00 (dd, $J = 9.2, 3.0$ Hz, 1H), 3.92 (t, $J = 9.8$ Hz, 1H) ppm; HRMS (micro-TOF) calc. for $\text{C}_{33}\text{H}_{32}\text{O}_5\text{S} + \text{Na} = 563.1863$, found 563.1889.



Phenyl 3-O-Benzyl-4,6-O-benzylidene-1-thio- α -D-mannopyranoside (41).

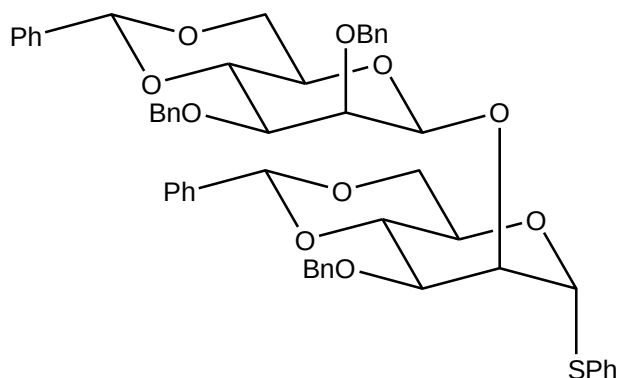
Bu_2SnO (1.38 g, 5.6 mmol) was added to a solution of diol 39 (1.0 g, 2.8 mmol) in toluene (25 mL). The resulting solution was stirred under reflux for 3 h. After cooling to room temperature, CsF (0.84 g, 5.6 mmol) and BnBr (1.33 mL, 11.1 mmol) were added to the reaction mixture. The contents of the flask were then stirred under reflux for an additional 24 h. Upon cooling, the mixture was diluted with EtOAc and quenched with aqueous saturated NaHCO_3 solution. The organic layer was separated, and the aqueous layer was extracted with EtOAc (2x). The combined organic layers were dried over Na_2SO_4 and filtered over celite to yield a yellow oil. Purification by column chromatography on silica gel (10% EtOAc in toluene) yielded 0.91 g (73%) of 41. ^1H

NMR (500 MHz, CDCl_3) δ 7.76 – 7.11 (m, 15H), 5.69 (s, 1H), 5.64 (s, 1H), 4.95 (d, J = 11.7 Hz, 1H), 4.79 (d, J = 11.7 Hz, 1H), 4.43 (td, J = 9.8, 4.9 Hz, 1H), 4.34 – 4.23 (m, 3H), 4.03 (dd, J = 9.5, 3.0 Hz, 1H), 3.93 (t, J = 10.2 Hz, 1H), 3.26 (s, 1H); ^{13}C NMR (75 MHz, CDCl_3) δ 137.80, 137.55, 133.49, 131.66, 129.24, 129.09, 128.64, 128.35, 128.18, 128.09, 127.72, 126.20, 101.67, 88.04, 79.09, 75.93, 73.34, 71.40, 68.58, 64.76. HRMS (micro-TOF) calc. for $\text{C}_{26}\text{H}_{26}\text{O}_5\text{S} + \text{Na}$ = 473.1399, found 473.1367.



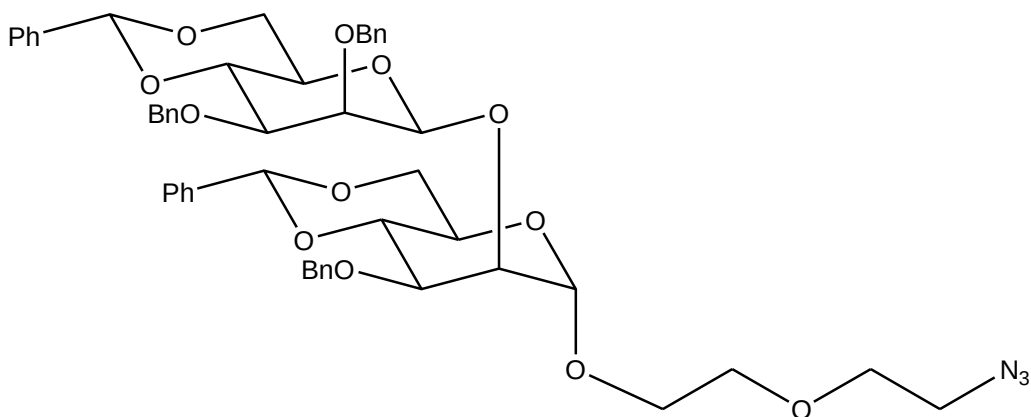
Phenyl 2-O-Benzyl-4,6-O-benzylidene-1-thio- α -D-mannopyranoside (46). To a solution of diol 39 (0.37 g, 1.0 mmol) in CH_2Cl_2 (30 mL) was added tetrabutylammonium hydrogensulfate (0.07 g, 0.2 mmol) and BnBr (150 μL , 1.2 mmol). A solution of 1 M NaOH (5 mL) was then added to the flask. The reaction mixture was stirred under reflux for 20 h, cooled to room temperature, and diluted with CH_2Cl_2 . The organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2 (2x). The combined organic layers were washed with saturated aqueous NaHCO_3 solution, brine, and dried over MgSO_4 . Column chromatography on silica gel (5% EtOAc in toluene) yielded 0.14 g (30%) of pure alcohol. ^1H NMR (500 MHz, CDCl_3) δ 7.88 – 7.03 (m, 15H), 5.59 (d, J = 10.2 Hz, 2H), 4.74 (d, J = 11.6 Hz, 1H), 4.65 (d, J = 11.6 Hz, 1H), 4.34 (td, J = 9.8, 4.9 Hz, 1H), 4.24 (dd, J = 10.2, 4.9 Hz, 1H), 4.15–4.10 (m, 2H), 4.02 (t, J = 9.5 Hz, 1H), 3.85 (t, J = 10.2 Hz, 1H), 2.65 (d, J = 7.7 Hz, 1H). ^{13}C NMR (126 MHz,

CDCl₃) δ 137.34, 137.29, 133.71, 131.80, 129.25, 128.70, 128.38, 128.25, 128.11, 127.81, 126.42, 102.20, 86.31, 80.08, 79.58, 73.22, 69.05, 68.48, 64.76 ppm; HRMS (micro-TOF) calc. for C₂₆H₂₆O₅S + Na = 473.1399, found 473.1391.



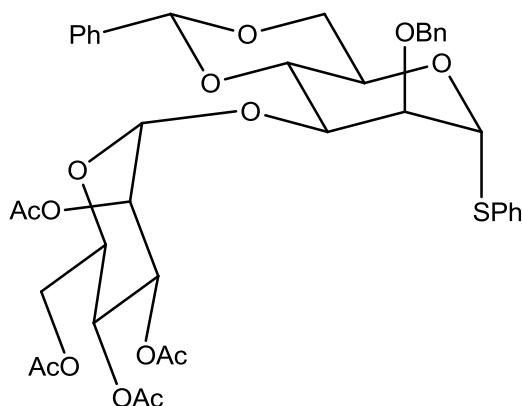
Phenyl 3-O-Benzyl-4,6-O-benzylidene-2-O-(2,3-di-O-benzyl-4,6-O-benzylidene-β-D-mannopyranosyl)-1-thio-α-D-mannopyranoside (42). A solution of donor 40 (0.20 g, 0.37 mmol), BSP (0.039 g, 0.19 mmol), TTBP (0.092 g, 0.37 mmol), and 4 Å molecular sieves in CH₂Cl₂ was stirred at -50 °C for 30 min. Tf₂O (61.4 mL, 0.37 mmol) was added, and the resulting solution was stirred for 10 min. A solution of acceptor 41 (0.25 g, 0.55 mmol) in CH₂Cl₂ was then added to the reaction mixture via syringe. After 6 h, P(OEt)₃ was added, and the contents of the flask were warmed to room temperature. The solids were filtered off, and the filtrate was washed with saturated aqueous NaHCO₃ solution, brine, and dried over Na₂SO₄. The product was purified by column chromatography on silica gel (10:1 Hex : EtOAc, then 5:1 Hex : EtOAc) to give 0.03 g (10%) of disaccharide 42. ¹H NMR (500 MHz, CDCl₃) δ 7.70 – 7.08 (m, 30H), 5.59 (s, 1H), 5.50 (s, 1H), 5.47 (s, 1H), 5.03 (d, *J* = 12.3 Hz, 1H), 4.95 (d, *J* = 12.2 Hz, 1H), 4.77 (q, *J* = 12.1 Hz, 2H), 4.67 (d, *J* = 12.6 Hz, 1H), 4.59 (d, *J* = 13.2

Hz, 2H), 4.49 (s, 1H), 4.32 (td, $J = 9.8, 4.7$ Hz, 1H), 4.28 – 4.19 (m, $J = 16.2, 6.7$ Hz, 3H), 4.16 (t, $J = 9.7$ Hz, 1H), 4.03 – 3.92 (m, $J = 8.1, 4.5$ Hz, 2H), 3.86 (t, $J = 10.2$ Hz, 1H), 3.78 (t, $J = 10.2$ Hz, 1H), 3.57 (dd, $J = 9.9, 2.9$ Hz, 1H), 3.29 (td, $J = 9.6, 4.8$ Hz, 1H); ^{13}C NMR (126 MHz, CDCl_3) δ 138.48, 138.45, 137.50, 137.41, 133.56, 131.86, 129.23, 128.93, 128.87, 128.52, 128.31, 128.19, 127.96, 127.68, 127.55, 127.52, 127.48, 127.45, 126.12, 126.02, 101.66, 101.40, 99.82, 86.37, 78.70, 78.40, 77.52, 76.19, 76.01, 74.63, 74.26, 72.24, 71.47, 68.55, 68.47, 67.77, 65.42 ppm; HRMS (micro-TOF) calc. for $\text{C}_{53}\text{H}_{52}\text{O}_{10}\text{S} + \text{Na} = 903.3179$, found 903.3149.



1-O-2-(2-azidoethoxy)ethyl-3-O-benzyl-4,6-O-benzylidene-2-O-(2,3-di-O-benzyl-4,6-O-benzylidene- β -D-mannopyranosyl)- α -D-mannopyranoside (44). N-iodosuccinimide (0.013 g, 58 μmol) and 4 Å molecular sieves were added to a solution of disaccharide 42 (0.034 g, 39 μmol) in CH_2Cl_2 (5 mL). The resulting mixture was stirred under an atmosphere of argon for 6 h. After addition of azido alcohol 43 (0.005 g, 39 μmol), enough AgOTf was added to turn the solution dark pink. The contents of the flask were immediately filtered over celite, concentrated, and coevaporated with toluene

several times. The product was purified by column chromatography on silica gel (3:5 EtOAc : Hex) to yield 13 mg (41%) of disaccharide 44. ^1H NMR (500 MHz, CDCl_3) δ 7.88 – 7.18 (m, 25 H), 5.60 (s, 1H), 5.42 (s, 1H), 5.07 (d, J = 12.3 Hz, 1H), 4.99 (d, J = 12.3 Hz, 1H), 4.82 (s, 1H), 4.76 (q, J = 12.8 Hz, 4H), 4.64 (d, J = 12.8 Hz, 1H), 4.57 (d, J = 12.8 Hz, 1H), 4.55 (s, 1H), 4.34 – 4.25 (m, 3H), 4.22 (t, J = 9.6 Hz, 1H), 4.15 (d, J = 3.1 Hz, 1H), 4.04 (t, J = 9.5 Hz, 1H), 3.98 – 3.87 (m, 2H), 3.79 – 3.69 (m, 2H), 3.60 (dd, J = 10.0, 3.1 Hz, 1H), 3.58 – 3.53 (m, 2H), 3.53 – 3.40 (m, 3H), 3.35 – 3.28 (m, 2H), 3.28 – 3.21 (m, 1H), 3.21 – 3.13 (m, 1H) ppm; HRMS (micro-TOF) calc. for $\text{C}_{51}\text{H}_{55}\text{N}_3\text{O}_{12} + \text{Na}$ = 924.3683, found 924.3657.



Phenyl 2-O-Benzyl-4,6-O-benzylidene-3-O-(2,3,4,6-tetra-O-acetyl- α -D-mannopyranosyl)-1-thio- α -D-mannopyranoside (50). To a solution of trichloroacetimidate 49 (50.3 mg, 0.1 mmol) and 4 Å molecular sieves in 3 mL of dry methylene chloride was added alcohol 46 (46 mg, 0.1 mmol) and boron trifluoride etherate (10 μL , 0.08 mmol) at -78°C . The resulting mixture was warmed to room temperature and stirred for 24 h. Solid sodium bicarbonate was added, and the mixture

was stirred for 30 min. The solids were removed by filtration over celite, and the product was concentrated and purified by column chromatography on silica gel (5:1 toluene : EtOAc) to afford 35 mg of disaccharide 50. ^1H NMR (500 MHz, CDCl_3) δ 7.57 – 7.36 (m, 8H), 7.36 – 7.28 (m, 7H), 5.60 (s, 1H), 5.58 (d, $J = 1.5$ Hz, 1H), 5.45 (dd, $J = 3.5, 1.5$ Hz, 1H), 5.39 (dd, $J = 10.0, 3.5$ Hz, 1H), 5.27 (d, $J = 1.5$ Hz, 1H), 5.20 (t, $J = 10.0$ Hz, 1H), 4.80 (d, $J = 12.0$ Hz, 1H), 4.68 (d, $J = 12.0$ Hz, 1H), 4.38 – 4.25 (m, 2H), 4.25 – 4.20 (m, 2H), 4.16 (dd, $J = 12.0, 6.4$ Hz, 1H), 4.05 – 3.97 (m, 2H), 3.86 (t, $J = 9.9$ Hz, 1H), 3.78 (ddd, $J = 9.9, 6.4, 2.0$ Hz, 1H), 2.11 (s, 3H), 2.07 (s, 3H), 2.06 (s, 3H), 1.99 (s, 3H); ^{13}C NMR (126 MHz, CDCl_3) δ 170.68, 169.74, 169.60, 169.55, 137.24, 137.12, 133.46, 131.63, 129.23, 128.78, 128.68, 128.19, 128.08, 128.01, 127.83, 125.95, 101.24, 98.60, 86.63, 78.81, 78.78, 73.63, 72.82, 69.19, 68.94, 68.79, 68.29, 66.32, 65.23, 62.64, 20.86, 20.70, 20.64 ppm; HRMS (micro-TOF) calc. for $\text{C}_{40}\text{H}_{44}\text{O}_{14}\text{S} + \text{Na} = 803.2349$, found 803.2369.

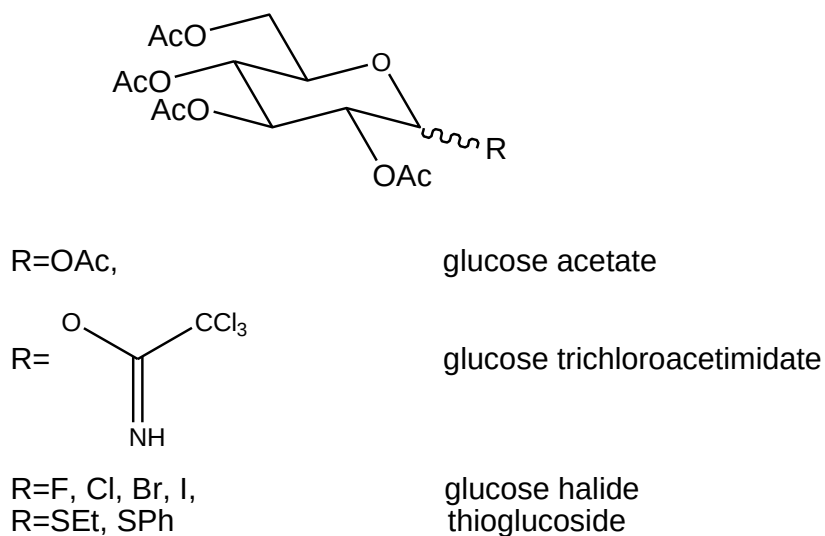
CHAPTER THREE

SYNTHESIS OF DIGLUCOSIDES

Retrosynthesis

There are several widely used methods for the glycosylation of glucose. Glucose acetates²¹, trichloroacetimidates²², halides²³, and thioglucosides²² have all been successfully used as donors for glycosylation (Figure 3.1). Each of the above mentioned donors have their advantages and disadvantages. All of the donors require a promoter, often a lewis acid. Thioglucosides were chosen for this work because of precedence²² and stability.

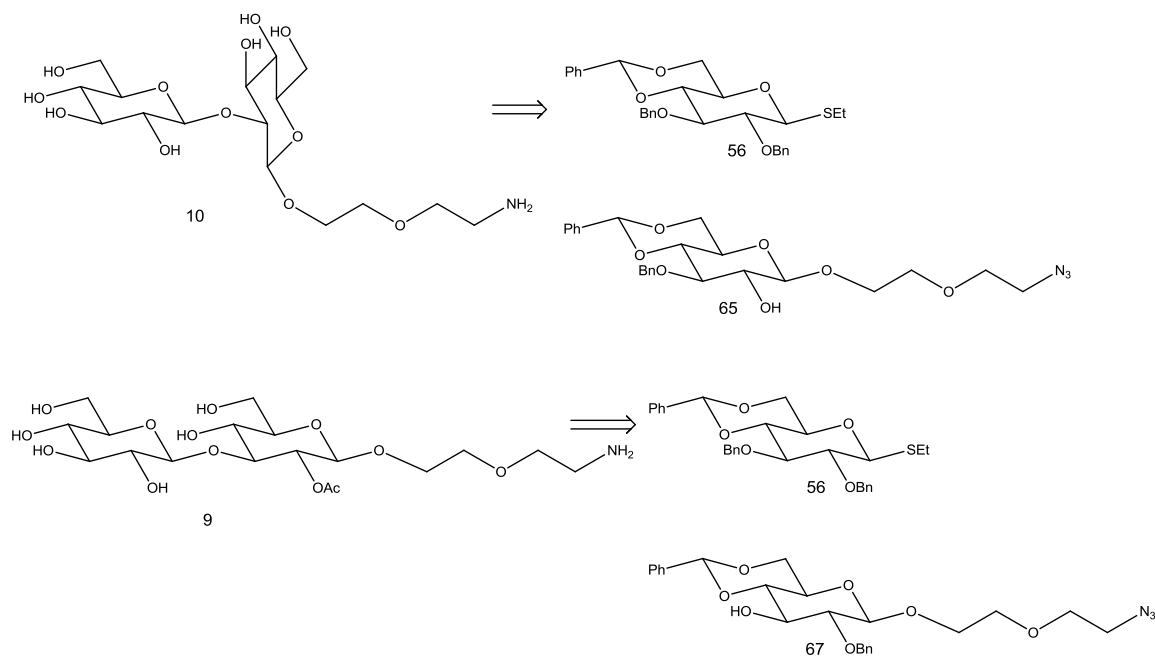
Figure 3.1 Examples of glucose donors



The desired diglucosides are 1,2-diglucose 10 and 1,3-diglucose 9. As seen in Scheme 3.1, both diglucosides share a common thioglucoside donor (56). 1,2-Diglucose

10 should be attainable from acceptor 56 and donor 65, and 1,3-diglucose 9 from acceptor 67 and donor 56. Thus, donor 56, and acceptors 65 and 67 will be synthesized.

Scheme 3.1 Retrosynthesis of diglucosides 9 and 10

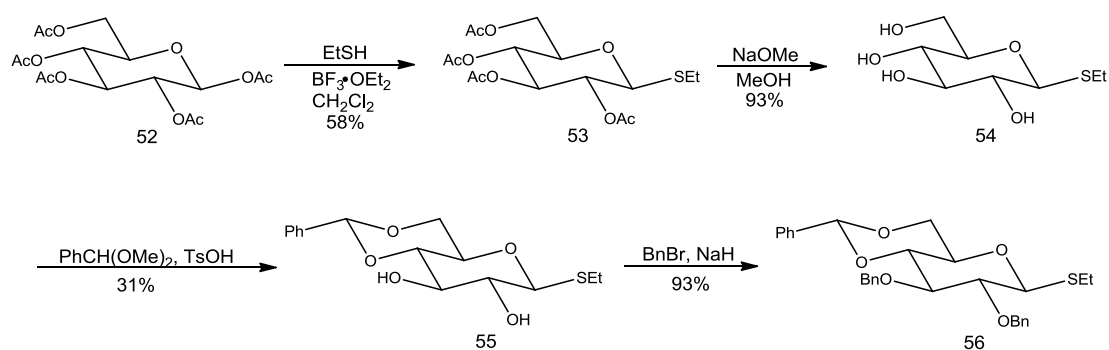


Synthesis of 1,2-Diglucose

Glucose donor 56 was synthesized as shown in Scheme 3.2. Peracetylated β -glucose 52 was allowed to react with ethanethiol and boron trifluoride etherate to give thioglucose 53.²⁴ After deprotection, 54 was treated with benzaldehyde dimethylacetal to give diol 55.²¹ Benzylation of 55 led to donor 56 in 93% yield.

A slightly different route used to obtain diol 64 is shown in Scheme 3.3. In the glucose series, azido alcohol 43 was tethered to the sugar before the coupling of the

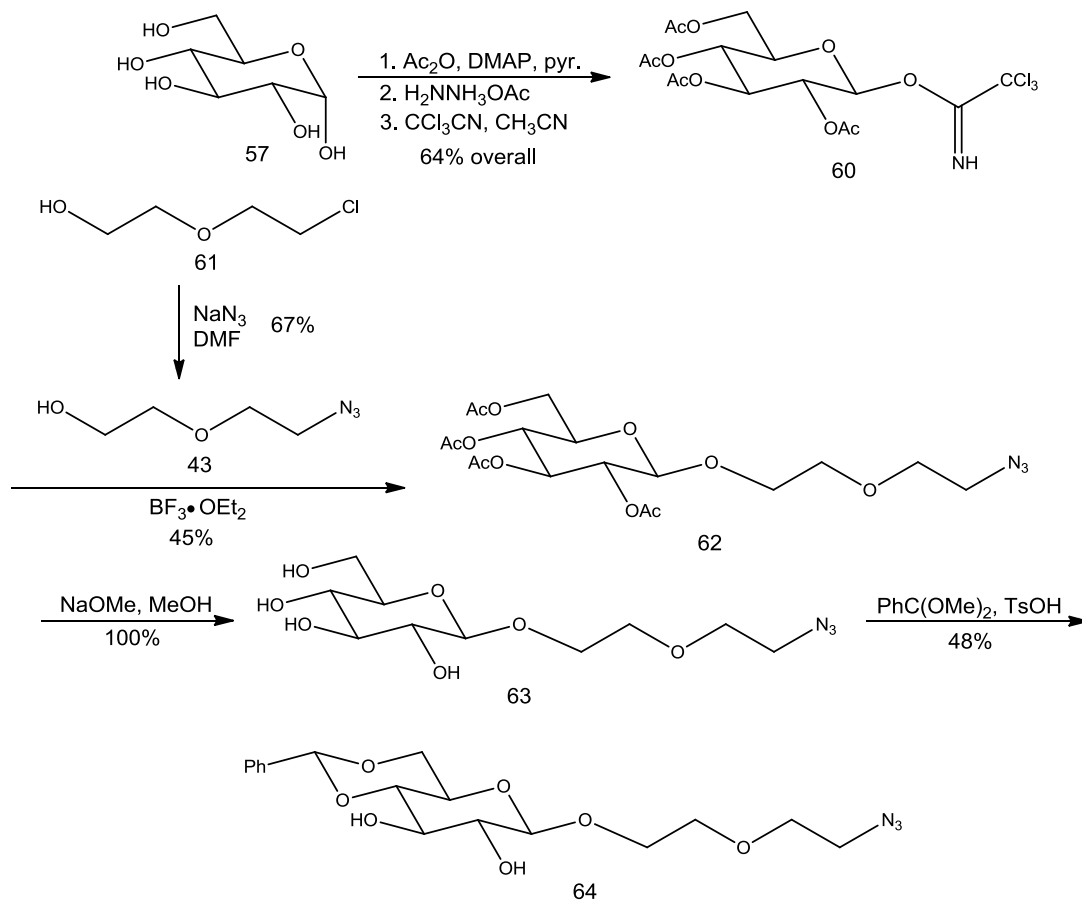
Scheme 3.2 Formation of donor 56



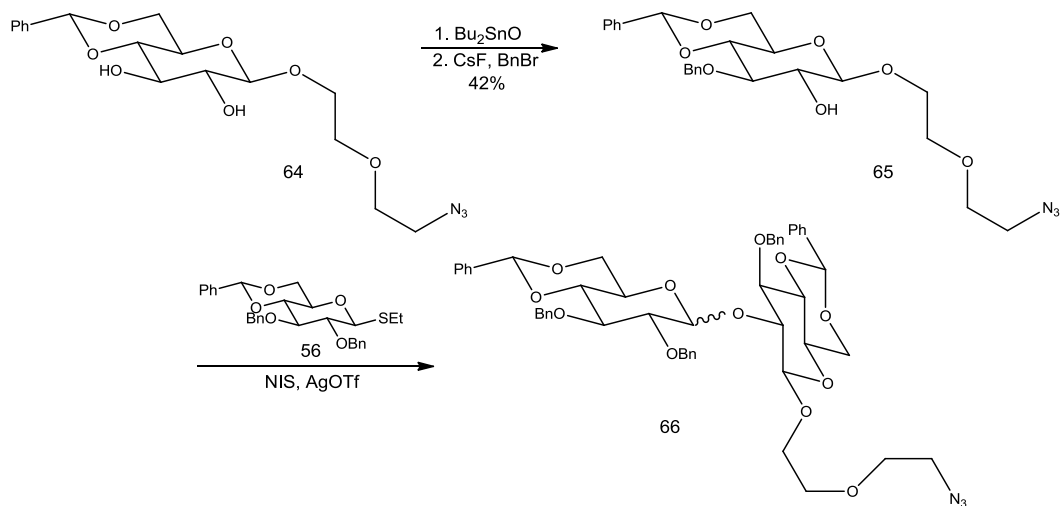
saccharides. Chloro alcohol **61** was allowed to react with sodium azide to give the $\text{S}_{\text{N}}2$ product **43** in 67% yield. Glucose was peracetylated using acetic anhydride in pyridine, and after selective deprotection at the anomeric position, treatment with trichloroacetonitrile gave trichloroacetimidate **60**.²⁵ Glycosylation of **60** and azido alcohol **43** with boron trifluoride etherate yielded **62**. Deprotection with sodium methoxide in methanol gave glucoside **63** which was transformed to diol **64** in 48% yield.

To complete the synthesis of 1,2-diglucose, acceptor **65** was synthesized via a stannylene acetal intermediate (Scheme 3.4). Treatment of diol **64** with Bu_2SnO in toluene, followed by addition of CsF and BnBr afforded monobenzylated acceptor **65** in 42% yield. Reaction of **65** with donor **56** in the presence of NIS and AgOTf yielded a mixture of both α - and β -diglucose **66** as indicated by NMR. The presence of diglucose was confirmed by HRMS.

Scheme 3.3 Formation of diol 64



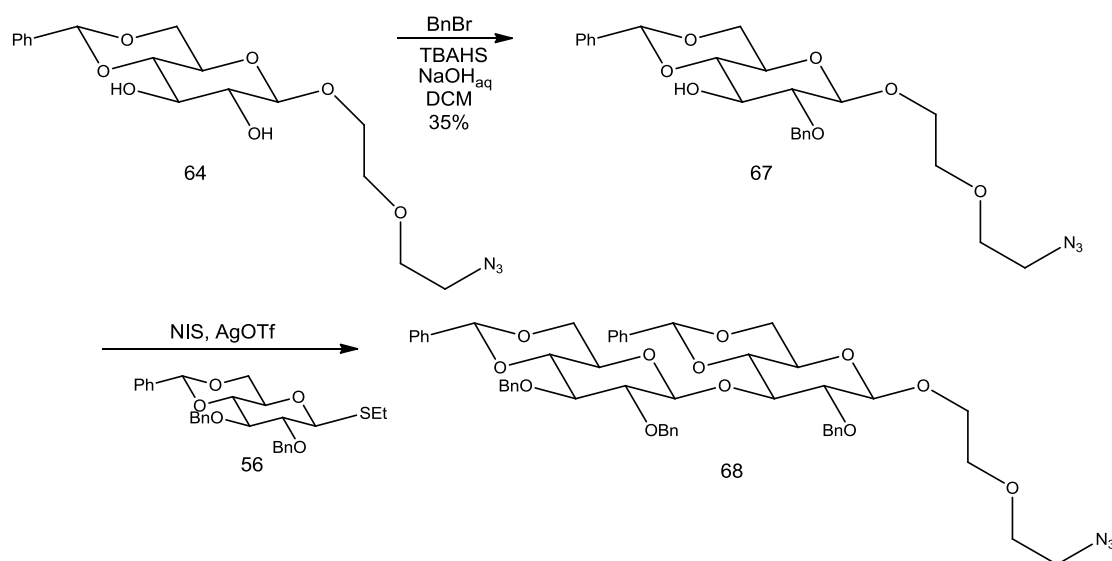
Scheme 3.4 Formation of diglucoside 66



Synthesis of 1,3-Diglucose

The synthesis of 1,3-diglucose began with formation of acceptor 67 under phase transfer catalysis (Scheme 3.5). In light of the difficulties encountered with the ethyl thiomannoside series, it was not expected that this reaction would give the desired product due to its polarity. However, alcohol acceptor 67 was obtained in 35% yield. Treatment of 67 and acceptor 56 with NIS and AgOTf yielded diglucoside 68 in %.

Scheme 3.5 Formation of diglucoside 68



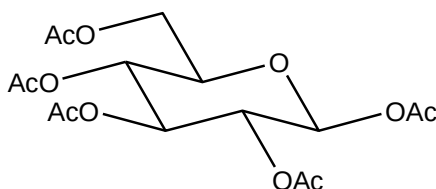
Summary

In summary, 1,2-diglucoside 66 was prepared as a mixture of α - and β - anomers using NIS and AgOTf. 1,3-Diglucoside 68 was also prepared using NIS and AgOTf. Structures have been confirmed with ^1H and ^{13}C NMR and HRMS.

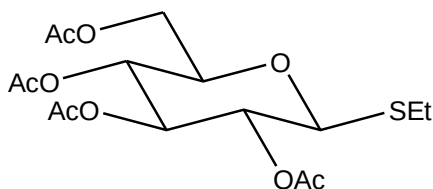
Experimental Procedures

General Procedure for Glycosylation.

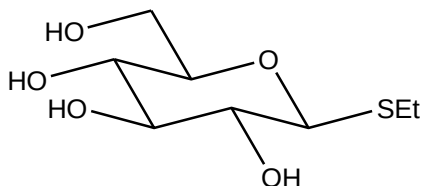
Donor (1 equiv.) and NIS (1 equiv.) were dissolved in CH_2Cl_2 . After purging the solution with argon, 4 Å molecular sieves were added to the flask. The resulting mixture was stirred under an argon atmosphere for 1-3 h before the acceptor (1 equiv.) and enough AgOTf to turn the solution pink were added. The reaction mixture was immediately filtered over celite, concentrated, and co-evaporated with toluene several times.



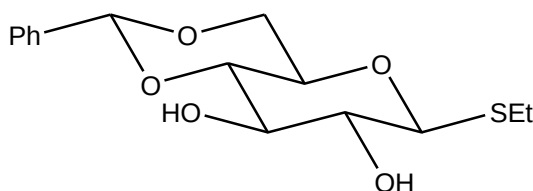
1,2,3,4,6-penta-O-acetyl- β -D-glucopyranoside (52). To a solution of α -D-glucose (10.0 g, 55.5 mmol) in toluene (85 mL) was added NaOAc (1.67 g, 20.4 mmol) and acetic anhydride (33 mL, 350 mmol). The resulting mixture was stirred at reflux for 3 h, cooled to room temperature, and neutralized with 3% aqueous NaOH solution. EtOAc was added, and the organic layer was separated and dried over MgSO_4 . The solvent was removed, and the solid was recrystallized from EtOH to yield 16.76 g (77%) of the β product 52 as white crystals. ^1H NMR (500 MHz, CDCl_3) δ 5.72 (d, J = 8.3 Hz, 1H), 5.26 (t, J = 9.4 Hz, 1H), 5.21 – 5.00 (m, 2H), 4.30 (dd, J = 12.5, 4.5 Hz, 1H), 4.12 (dd, J = 12.5, 2.1 Hz, 1H), 3.85 (ddd, J = 10.0, 4.4, 2.1 Hz, 1H), 2.13 (s, 3H), 2.10 (s, 3H), 2.04 (s, 3H), 2.04 (s, 3H), 2.02 (s, 3H).



Ethyl 2,3,4,6-tetra-*O*-acetyl-1-thio- β -D-glucopyranoside (53).²⁴ To a solution of 1,2,3,4,6-penta-*O*-acetyl- β -D-glucopyranoside 52 (2.5 g, 6.4 mmol) and $\text{In}(\text{OTf})_3$ (36 mg, 64 μmol) in dry methylene chloride (10 mL) was added ethane thiol (474 μL , 6.4 mmol). The resulting orange solution was stirred overnight. Aqueous saturated NaHCO_3 solution was added, and the reaction mixture was stirred for 2 h. The organics were separated, dried over MgSO_4 , and concentrated. The product was purified by column chromatography on silica gel (7:3 hexanes : ethyl acetate) to give 1.4 g of 53 in 58% yield. ^1H NMR (300 MHz, CDCl_3) δ 5.15 (t, $J = 9.3$ Hz, 1H), 5.01 (t, $J = 9.7$ Hz, 1H), 4.96 (t, $J = 9.7$ Hz, 1H), 4.43 (d, $J = 10.0$ Hz, 1H), 4.17 (dd, $J = 12.4, 4.9$ Hz, 1H), 4.06 (dd, $J = 12.4, 2.2$ Hz, 1H), 3.64 (ddd, $J = 10.0, 4.9, 2.2$ Hz, 1H), 2.81 – 2.45 (m, 2H), 2.00 (s, 3H), 1.98 (s, 3H), 1.95 (s, 3H), 1.93 (s, 3H), 1.20 (t, $J = 7.4$ Hz, 3H) ppm; ^{13}C (75 MHz, CDCl_3) δ 170.5, 170.1, 169.3, 169.2, 83.4, 75.8, 73.8, 69.8, 68.3, 62.1, 24.1, 20.7, 20.5, 14.8 ppm

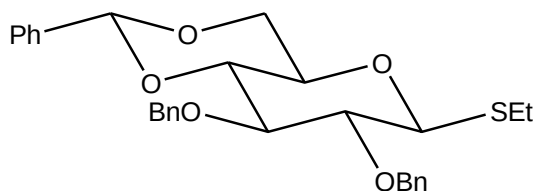


Ethyl 1-thio- β -D-glucopyranoside (54).²⁴ To a solution of tetraacetate 53 (1.4 g, 3.7 mmol) in distilled methanol (10 mL) sodium metal (0.17 g, 7.4 mmol) was added at room temperature. The reaction mixture was stirred overnight. The solution was then neutralized with Amberlite IR-120 (acidic), filtered over celite and concentrated to give 0.77 g of 54 in 93% yield. This product was used without further purification. ¹H NMR (300 MHz, D₂O) δ 4.39 (d, J = 9.9 Hz, 1H), 3.74 (dd, J = 12.4, 1.8 Hz, 1H), 3.54 (dd, J = 12.4, 5.6 Hz, 1H), 3.38 – 3.11 (m, 4H), 2.76 – 2.45 (m, 2H), 1.12 (t, J = 7.4 Hz, 3H) ppm; ¹³C NMR (75 MHz, D₂O) δ 170.22, 169.43, 83.50, 75.82, 73.86, 69.77, 68.26, 62.13, 24.18, 20.74, 20.62, 20.61, 14.80.



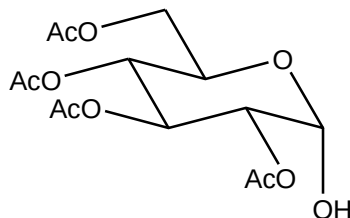
Ethyl 4,6-O-benzylidene-1-thio- β -D-glucopyranoside (55).²⁴ To a solution of ethyl 1-thio- β -D-glucopyranoside (54) (0.77 g, 3.43 mmol) and TsOH (65 mg, 34 μ mol) in 5 mL DMF was added benzaldehyde dimethyl acetal (1.0 mL, 6.9 mmol) at room temperature. The reaction mixture was heated to 60 °C with evacuation by rotary evaporation for 2 h. The solvent was removed in vacuo, and the product was purified by column chromatography on silica gel (1:1 toluene : ethyl acetate) to afford 55 (0.33 g, 31%). ¹H NMR (500 MHz, CDCl₃) δ 7.73 – 7.31 (m, 5H), 5.53 (s, 1H), 4.46 (d, J = 9.8 Hz, 1H), 4.34 (dd, J = 10.5, 4.9 Hz, 1H), 3.84 (td, J = 9.0, 2.1 Hz, 1H), 3.76 (t, J = 10.2

Hz, 1H), 3.58 (t, $J = 9.3$ Hz, 1H), 3.55 – 3.48 (m, 2H), 2.76 (qd, $J = 7.4, 2.4$ Hz, 2H), 2.71 (d, $J = 2.2$ Hz, 1H), 2.55 (d, $J = 2.0$ Hz, 1H), 1.32 (t, $J = 7.4$ Hz, 3H) ppm.

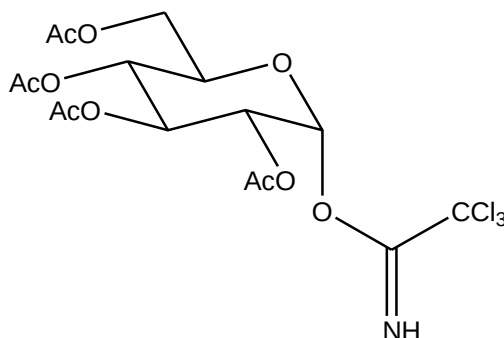


Ethyl 2,3-di-O-benzyl-4,6-O-benzylidene-1-thio- β -D-glucopyranoside (56). A

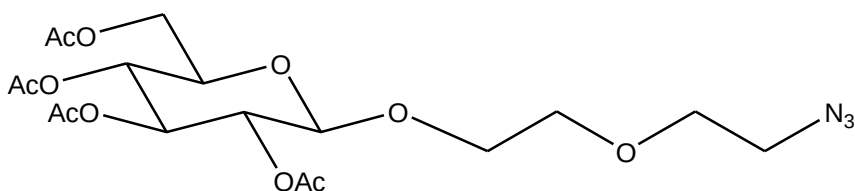
solution of 55 (0.33 g, 1.1 mmol) in 2 mL of DMF was added to a solution of sodium hydride (0.17 g, 4.28 mmol) in DMF (2 mL) at 0 °C. After stirring for 30 min, benzyl bromide (384 μ L, 3.2 mmol) was added, and the reaction was stirred for 8 h at room temperature. Methanol was added, and the mixture was poured into water. The product was extracted with ethyl acetate, and the organics were washed with brine, dried over Na_2SO_4 , and concentrated. The residue was purified by column chromatography on silica gel (9:1 hexanes : ethyl acetate) to give 56 (0.49 g, 93% yield). ^1H NMR (500 MHz, CDCl_3) δ 7.90 – 7.05 (m, 15H), 5.57 (s, 1H), 4.94 (d, $J = 11.2$ Hz, 1H), 4.86 (d, $J = 10.1$ Hz, 1H), 4.79 (dd, $J = 10.7, 5.2$ Hz, 2H), 4.55 (d, $J = 9.8$ Hz, 1H), 4.34 (dd, $J = 10.5, 4.9$ Hz, 1H), 3.78 (m, 2H), 3.70 (t, $J = 9.3$ Hz, 1H), 3.44 (m, 2H), 2.87 – 2.66 (m, 2H), 1.30 (t, $J = 7.4$ Hz, 3H) ppm; ^{13}C NMR (126 MHz, CDCl_3) δ 128.97, 128.38, 128.34, 128.26, 128.07, 127.89, 127.73, 125.97, 101.08, 85.83, 82.78, 81.58, 81.24, 75.99, 75.25, 70.19, 68.70, 25.18, 15.11 ppm; HRMS (micro-TOF) calc. for $\text{C}_{29}\text{H}_{32}\text{O}_5\text{S} + \text{Na} = 515.1863$, found 515.1843.



2,3,4,6-tetra-O-acetyl- α -D-glucopyranose (59). A solution of 1,2,3,4,6-penta-O-acetyl- α -D-glucopyranose (6.09 g, 15.6 mmol) in 20 mL DMF was heated to 55 °C. Hydrazine acetate (1.87 g, 20.3 mmol) was then added to the warmed solution. The resulting mixture was stirred for 30 min after which the solution turned yellow. Ethyl acetate (20 mL) was added, and the resulting solution was washed with water (20 mL). The aqueous layer was extracted with ethyl acetate (20 mL). The combined organic layers were washed with water (3 x 20 mL), saturated aqueous NaHCO₃ solution (3 x 20 mL), and brine (3 x 20 mL) and dried over MgSO₄. The solvent was removed to afford 5.18 g (95%) of 59. ¹H NMR (300 MHz, CDCl₃) δ 5.43 (t, J = 9.8 Hz, 1H), 5.35 (s, 1H), 4.98 (t, J = 9.8 Hz, 1H), 4.78 (dd, J = 10.2, 3.5 Hz, 1H), 4.51 (d, J = 2.9 Hz, 1H), 4.22 – 4.08 (m, 2H), 4.04 (m, 2H), 2.00 (s, 3H), 1.99 (s, 3H), 1.94 (s, 3H), 1.92 (s, 3H) ppm.

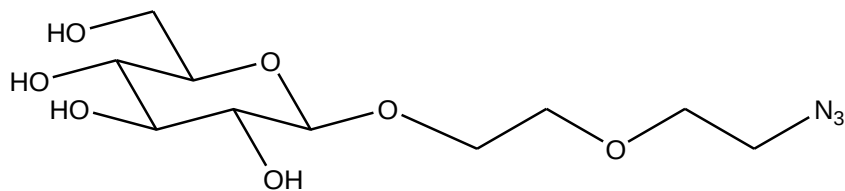


2,3,4,6-tetra-O-acetyl- α -D-glucopyranose trichloroacetimidate (60). To a solution of 59 (5.18 g, 14.9 mmol) and DBU (1,8-Diazabicycloundec-7-ene; 101 μ L, 0.7 mmol) was added trichloroacetonitrile (5.22 mL, 52.1 mmol) drop wise at 0 °C. The resulting solution was stirred for 3 h, during which time it turned orange. The solvent was removed, and the residue was filtered over a silica plug (6:4 hexanes : ethyl acetate). The product was then purified by column chromatography on silica gel (6:4 hexanes : ethyl acetate) to give 60 (5.71 g, 78%) as a clear oil. ^1H NMR (500 MHz, CDCl_3) δ 8.67 (s, 1H), 6.54 (d, J = 3.7 Hz, 1H), 5.54 (t, J = 10.0 Hz, 1H), 5.16 (t, J = 10.0 Hz, 1H), 5.11 (dd, J = 10.0, 3.7 Hz, 1H), 4.25 (dd, J = 12.3, 4.1 Hz, 1H), 4.19 (ddd, J = 10.0, 4.1, 2.2 Hz, 1H), 4.11 (dd, J = 12.3, 2.2 Hz, 1H), 2.06 (s, 3H), 2.03 (s, 3H), 2.01 (s, 3H), 2.00 (s, 3H) ppm; ^{13}C NMR (126 MHz, CDCl_3) δ 170.58, 170.02, 169.87, 169.51, 160.76, 92.85, 69.95, 69.82, 69.67, 67.71, 61.32, 20.69, 20.60, 20.46 ppm.



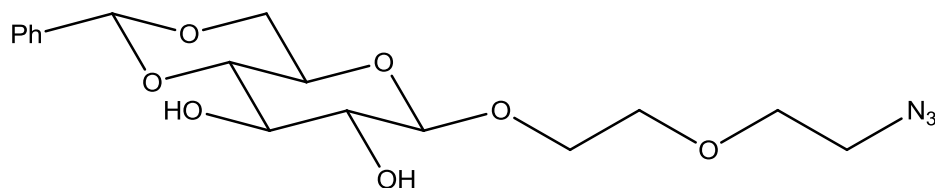
2,3,4,6-tetra-O-acetyl-1-O-2-(2-azidoethoxy)ethyl- β -D-glucopyranose (62). To a solution of 60 (5.64 g, 11.4 mmol) and 4 Å molecular sieves in 35 mL of dry methylene chloride was added azido alcohol 43 (1.5 g, 11.4 mmol) and boron trifluoride etherate (1.45 mL, 11.4 mmol). The resulting mixture was stirred at room temperature for 23 h. Solid sodium bicarbonate (6.7 g) was added, and the mixture was stirred for 30 min. The solids were removed by filtration, and the product was concentrated and purified by

column chromatography on silica gel (1:1 hexanes : EtOAc) to afford 62 (2.38 g, 45%) as a light yellow oil. ^1H NMR (500 MHz, CDCl_3) δ 5.18 (t, $J = 9.6$ Hz, 1H), 5.06 (t, $J = 9.6$ Hz, 1H), 4.98 (dd, $J = 9.6, 8.0$ Hz, 1H), 4.59 (d, $J = 8.0$ Hz, 1H), 4.23 (dd, $J = 12.3, 4.7$ Hz, 1H), 4.11 (dd, $J = 12.3, 2.5$ Hz, 1H), 3.93 (dt, $J = 11.1, 4.0$ Hz, 1H), 3.73 (ddd, $J = 11.0, 6.5, 4.3$ Hz, 1H), 3.68 (ddd, $J = 10.1, 4.7, 2.4$ Hz, 1H), 3.65 – 3.59 (m, 4H), 3.37 – 3.31 (m, 2H), 2.06 (s, 3H), 2.03 (s, 3H), 2.00 (s, 3H), 1.98 (s, 3H) ppm; ^{13}C NMR (126 MHz, CDCl_3) δ 170.68, 170.26, 169.42, 169.39, 100.81, 72.78, 71.76, 71.25, 70.40, 70.20, 69.05, 68.35, 61.89, 50.74, 20.73, 20.66, 20.60 ppm.

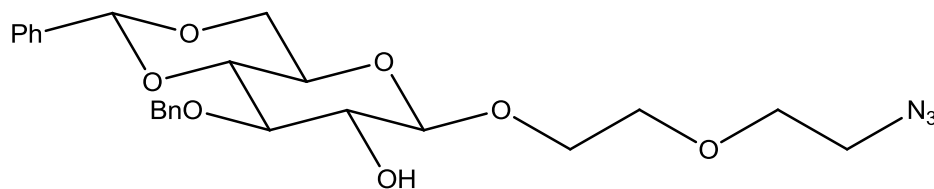


1-O-2-(2-azidoethoxy)ethyl- β -D-glucopyranose (63). To a solution of tetraacetate 62 (2.17 g, 4.7 mmol) in distilled methanol (10 mL) sodium metal (0.22 g, 9.4 mmol) was added at room temperature. The reaction mixture was stirred overnight. The solution was then neutralized with Amberlite IR-120 (acidic), filtered over celite and concentrated to give 1.38 g of 63 in quantitative yield. This product was used without further purification. ^1H NMR (500 MHz, D_2O) δ 4.30 (d, $J = 7.9$ Hz, 1H), 3.87 (dt, $J = 7.3, 3.3$ Hz, 1H), 3.72 (d, $J = 12.0$ Hz, 1H), 3.68 – 3.62 (m, 1H), 3.58 (m, 2H), 3.56 – 3.49 (m, 3H), 3.37 – 3.31 (m, 2H), 3.27 (m, 2H), 3.18 (m, 1H), 3.10 (t, $J = 8.6$ Hz, 1H) ppm; ^{13}C NMR (126 MHz, D_2O) δ 102.15, 75.80, 75.56, 73.01, 69.58, 69.53, 69.07,

68.56, 60.64, 50.06 ppm; HRMS (micro-TOF) calc. for $C_{10}H_{19}N_3O_7 + Na = 316.1121$, found 316.1094.

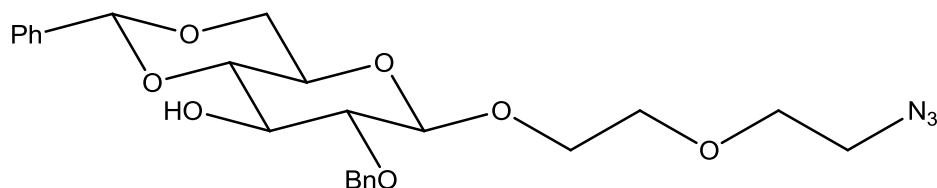


1-O-2-(2-azidoethoxy)ethyl-4,6-O-benzylidene- β -D-glucopyranose (64). To a solution of 63 (1.05 g, 3.58 mmol) and TsOH (0.14 g, 0.72 mmol) in 15 mL DMF was added benzaldehyde dimethyl acetal (0.8 mL, 5.37 mmol) at room temperature. The reaction mixture was heated to 60 °C with evacuation by rotary evaporation for 3 h. After the addition of TEA, the reaction mixture was washed with H_2O . The organic layer was collected, dried over Na_2SO_4 , and concentrated. Column chromatography on silica gel (8:2 EtOAc : Hexanes) yielded 0.66 g (48%) of pure 64. 1H NMR (500 MHz, $CDCl_3$) δ 7.52 – 7.38 (m, 2H), 7.35 – 7.22 (m, 3H), 5.42 (s, 1H), 4.33 (d, $J = 7.8$ Hz, 1H), 4.23 (dd, $J = 10.4, 4.9$ Hz, 1H), 3.94 – 3.85 (m, 1H), 3.76 – 3.38 (m, 10H), 3.37 – 3.28 (m, 1H), 3.26 (t, $J = 5.0$ Hz, 2H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 137.16, 129.18, 128.27, 126.41, 103.43, 101.75, 80.45, 74.39, 72.93, 70.16, 69.79, 68.94, 68.57, 66.30, 50.47 ppm; HRMS (micro-TOF) calc. for $C_{17}H_{23}N_3O_7 + H = 382.1608$, found 382.1589.



1-O-2-(2-azidoethoxy)ethyl-3-O-benzyl-4,6-O-benzylidene- β -D-glucopyranose

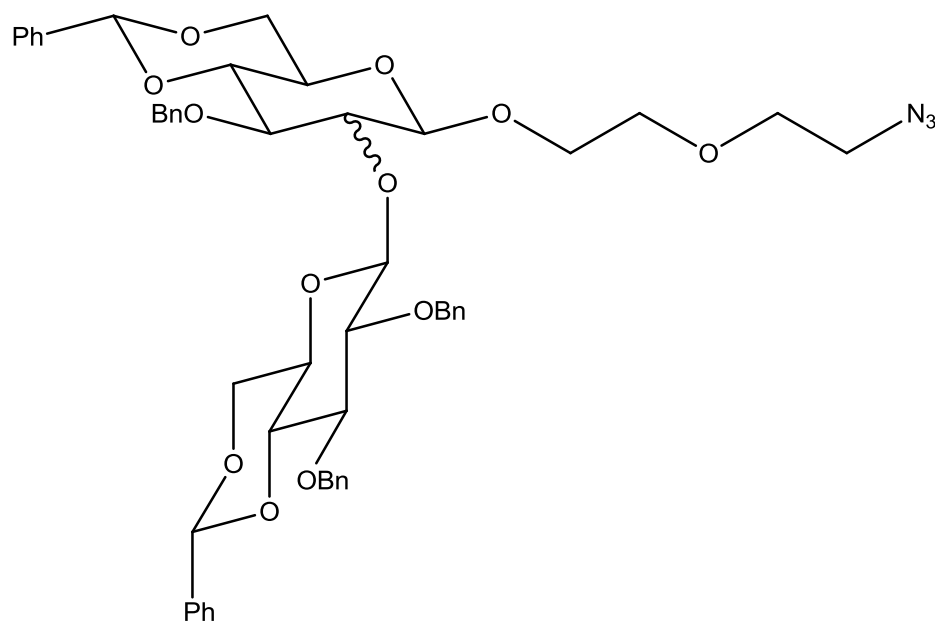
(65). A solution of diol 64 (0.47 g, 1.23 mmol) and Bu₂SnO (0.61 g, 2.46 mmol) in 25 mL of toluene was stirred under reflux for 3 h. Upon cooling to room temperature, CsF (0.37 g, 2.46 mol) and BnBr (0.59 mL, 4.92 mmol) were added, and the resulting mixture was stirred under reflux for 24 h, and then cooled to room temperature, diluted with EtOAc and quenched with saturated aqueous NaHCO₃ solution. The aqueous layer was extracted twice with EtOAc, and the organic layers were combined, dried over Na₂SO₄, and filtered over celite. Purification by column chromatography on silica gel (2:1 toluene : EtOAc) afforded 0.24 g (42%) of acceptor 65. ¹H NMR (500 MHz, CDCl₃) δ 7.72 – 7.03 (m, 10H), 5.60 (s, 1H), 5.00 (d, *J* = 11.7 Hz, 1H), 4.90 (d, *J* = 11.7 Hz, 1H), 4.49 (d, *J* = 7.6 Hz, 1H), 4.38 (dd, *J* = 10.4, 4.9 Hz, 1H), 4.06 (dt, *J* = 7.9, 3.7 Hz, 1H), 3.88 – 3.79 (m, 2H), 3.77 – 3.63 (m, 7H), 3.53 – 3.44 (m, 1H), 3.40 (t, *J* = 4.8 Hz, 2H), 3.11 (s, 1H); ¹³C NMR (126 MHz, CDCl₃) δ 138.58, 137.36, 129.11, 129.04, 128.41, 128.30, 128.06, 127.74, 126.11, 125.38, 103.84, 101.28, 81.28, 80.24, 74.63, 74.50, 70.30, 70.10, 69.35, 68.74, 66.50, 50.66 ppm; HRMS (micro-TOF) calc. for C₂₄H₂₉N₃O₇ + H = 472.2078, found 472.2091.



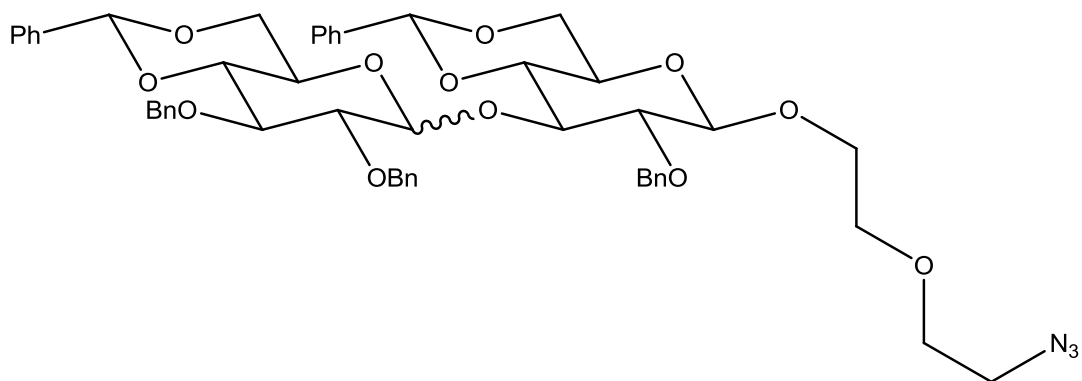
1-O-2-(2-azidoethoxy)ethyl-2-O-benzyl-4,6-O-benzylidene- β -D-glucopyranose

(67). To a solution of diol 64 (0.23 g, 0.8 mmol) in CH₂Cl₂ (9 mL) was added

tetrabutylammonium hydrogensulfate (0.05 g, 0.2 mmol) and BnBr (114 μ L, 1 mmol). A solution of 1 M NaOH (3.9 mL) was then added to the flask. The reaction mixture was stirred under reflux for 18 h, cooled to room temperature, and diluted with CH_2Cl_2 . The organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2 (2x). The combined organic layers were washed with saturated aqueous NaHCO_3 solution, brine, and dried over MgSO_4 . Column chromatography on silica gel (3:1 toluene : EtOAc) yielded 0.13 g (35%) of pure alcohol. ^1H NMR (500 MHz, CDCl_3) δ 7.72 – 6.97 (m, 10H), 5.51 (s, 1H), 4.99 (d, J = 11.0 Hz, 1H), 4.77 (d, J = 11.0 Hz, 1H), 4.57 (d, J = 7.7 Hz, 1H), 4.34 (dd, J = 11.0, 5.0 Hz, 1H), 4.04 (ddd, J = 11.0, 5.0, 3.7 Hz, 1H), 3.80 (m, 3 H), 3.73 – 3.67 (m, 2H), 3.64 (t, J = 5.1 Hz, 2H), 3.54 (t, J = 9.4 Hz, 2H), 3.47 – 3.35 (m, 2H), 3.31 (td, J = 4.8, 2.4 Hz, 2H); ^{13}C NMR (126 MHz, CDCl_3) δ 138.42, 137.10, 129.20, 128.51, 128.31, 128.04, 127.88, 126.34, 104.00, 101.77, 81.74, 80.42, 74.62, 73.13, 70.43, 70.01, 69.36, 68.72, 66.10, 50.67 ppm.



1-O-2-(2-azidoethoxy)ethyl-3-O-benzyl-4,6-O-benzylidene-2-O-(2,3-di-O-benzyl-4,6-O-benzylidene- β -D-glucopyranosyl)- β -D-glucopyranoside (66). Donor 56 (20 mg, 0.04 mmol) was allowed to react with acceptor 65 (19.1 mg, 0.04 mmol) in the presence of NIS (9.1 mg, 0.04 mmol) in accordance with the general glycosylation procedure above. The residue was purified by column chromatography on silica gel (5:1 toluene : EtOAc) to give 18 mg (49%) of disaccharide 66. HRMS (micro-TOF) calc. for $C_{51}H_{55}N_3O_{12} + Na = 924.3683$, found 924.3671.



1-O-2-(2-azidoethoxy)ethyl-2-O-benzyl-4,6-O-benzylidene-3-O-(2,3-di-O-benzyl-4,6-O-benzylidene- β -D-glucopyranosyl)- β -D-glucopyranoside (68). Donor 56 (50 mg, 0.10 mmol) was allowed to react with acceptor 67 (48 mg, 0.10 mmol) in the presence of NIS (23 mg, 0.10 mmol) in accordance with the general glycosylation procedure above. The residue was purified by column chromatography on silica gel (5:1 toluene : EtOAc) to give 32 mg (35%) of disaccharide 68. 1H NMR (500 MHz, $CDCl_3$) (major anomer) δ 7.70 – 6.83 (m, 25H), 5.49 (s, 1H), 5.43 (s, 1H), 4.90 (dd, $J = 10.9$, 6.1 Hz, 2H), 4.82 (d, $J = 10.9$ Hz, 2H), 4.65 – 4.58 (m, 1H), 4.57 (s, 1H), 4.40 (d, $J = 12.4$

Hz, 1H), 4.30 (m, 2H), 4.14 – 4.08 (m, 1H), 4.08 – 4.00 (m, 2H), 3.98 (t, $J = 9.4$ Hz, 1H), 3.87 – 3.76 (m, 3H), 3.69 (m, 3H), 3.61 (t, $J = 5.1$ Hz, 2H), 3.55 (m, 3H), 3.45 (dd, $J = 9.4, 3.9$ Hz, 2H), 3.28 (m, 2H). ^{13}C NMR (126 MHz, CDCl_3) δ 138.85, 137.75, 137.52, 137.03, 129.39, 129.01, 128.70, 128.44, 128.37, 128.22, 128.15, 128.04, 127.97, 127.78, 127.52, 127.47, 127.43, 127.35, 126.33, 126.18, 104.69, 101.99, 101.17, 96.98, 82.15, 82.02, 79.08, 78.33, 77.89, 75.53, 75.20, 74.70, 71.61, 70.39, 69.98, 69.36, 68.88, 68.80, 65.65, 62.30, 50.61 ppm; HRMS (micro-TOF) calc. for $\text{C}_{51}\text{H}_{55}\text{N}_3\text{O}_{12} + \text{Na} = 924.3678$, found 924.3679.

CHAPTER FOUR

CONCLUSION

Despite the growing incidences of fungal disease, not much is known about how the immune system defends against *Candida albicans*. PAMAM dendrimers can be used as a scaffold on which to display saccharides found on the surface of *C. albicans*. The disaccharide functionalized dendrimers can be used to probe the immune response to *C. albicans* in immunostimulation assays. Therefore, several disaccharides - $\alpha(1,3)$ -dimannose, $\beta(1,2)$ -dimannose, $\beta(1,3)$ -dimannose, $\beta(1,2)$ -diglucose, and $\beta(1,3)$ -diglucose - are attractive targets for the functionalization of G(3.5) PAMAM dendrimers.

The successful synthesis of several useful mannose and glucose fragments was achieved. The synthesis of $\beta(1,2)$ -dimannoside and $\alpha(1,3)$ -dimannoside was completed. The donor and acceptor for the formation of $\beta(1,3)$ -dimannoside were synthesized. Once $\beta(1,3)$ -dimannoside is in hand, it will be allowed to react with azido alcohol **43**. In addition, both 1,2-diglucose and 1,3-diglucose were synthesized as a mixture of α - and β -anomers.

Upon completion of the synthesis of the disaccharides, they will be universally deprotected with $H_2/Pd(OH)_2$ in the presence of acid. In addition to removing the benzyl and benzylidene protecting groups, these reaction conditions should reduce the azide to an amine. The amino sugars will be appended to G(3.5) PAMAM dendrimers by the formation of an amide bond using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and N-hydroxysuccinimide (NHS).

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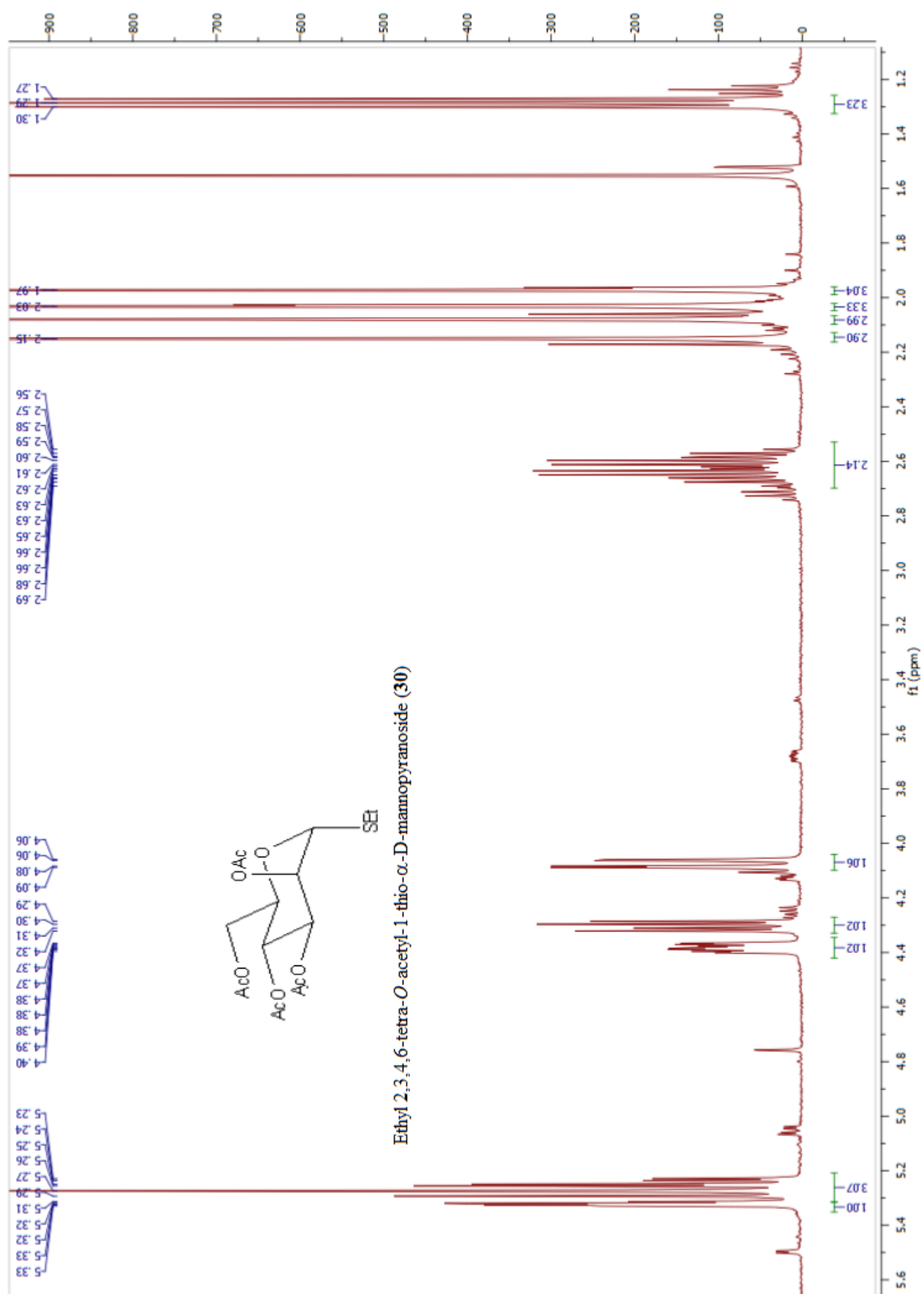
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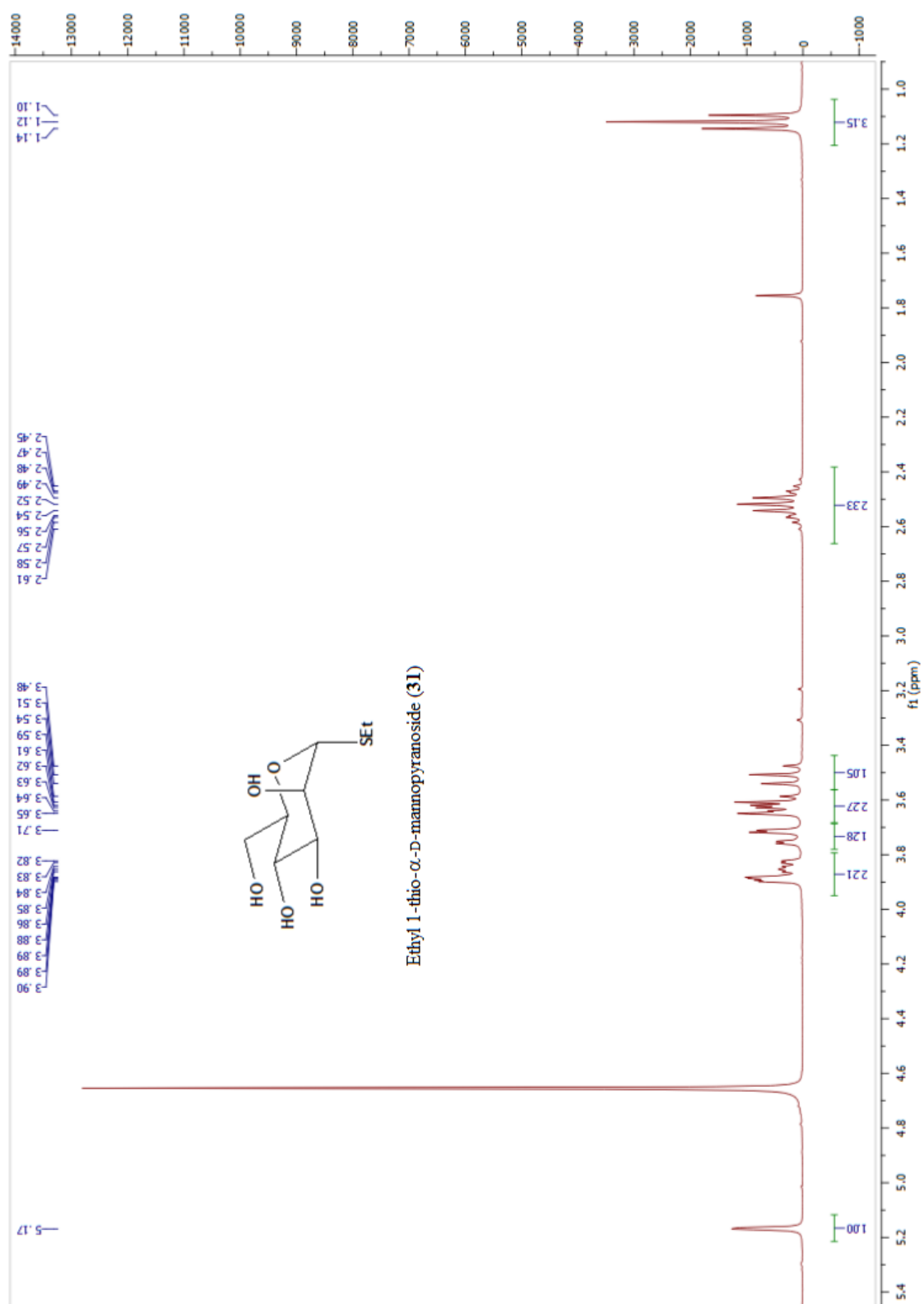
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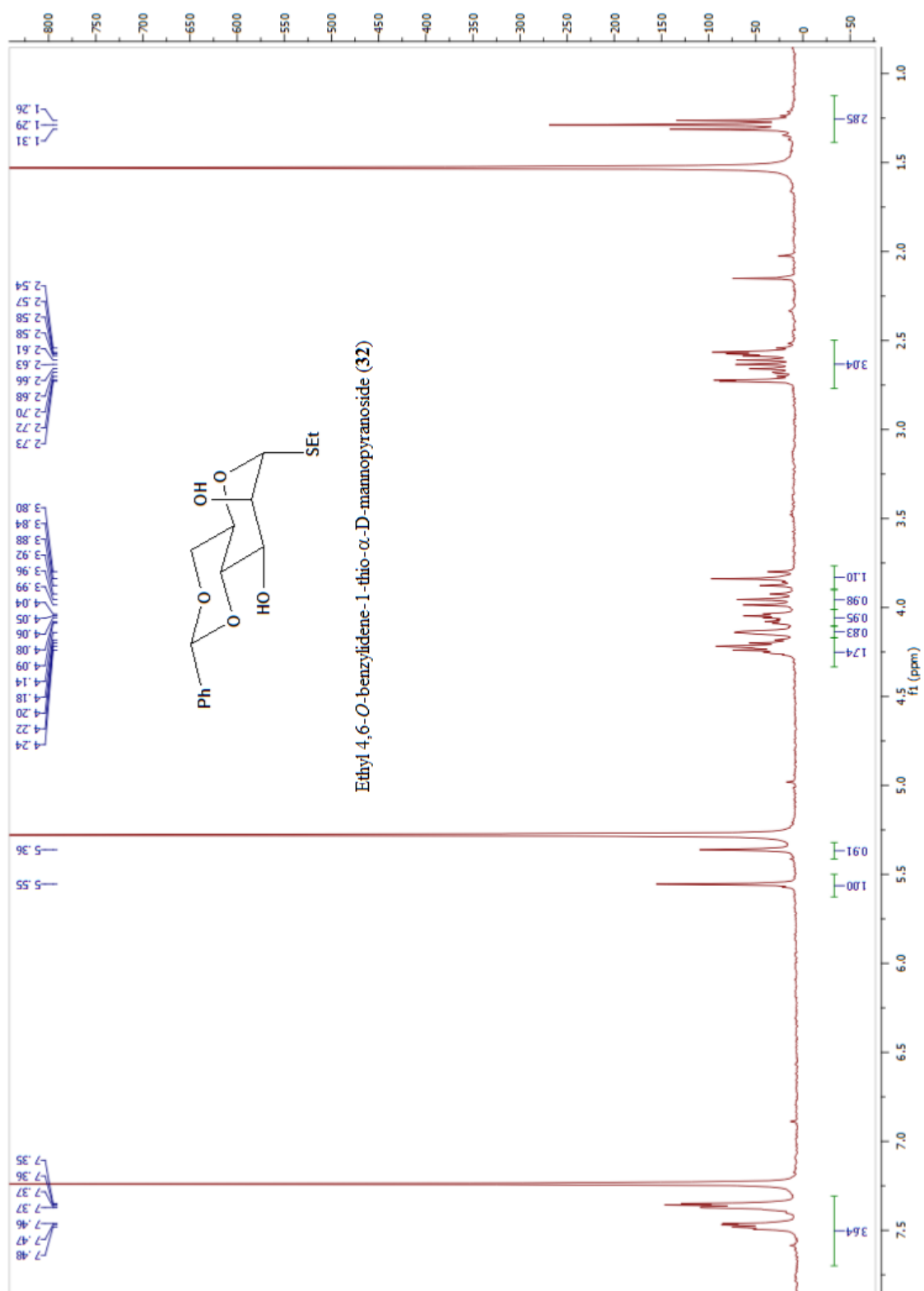
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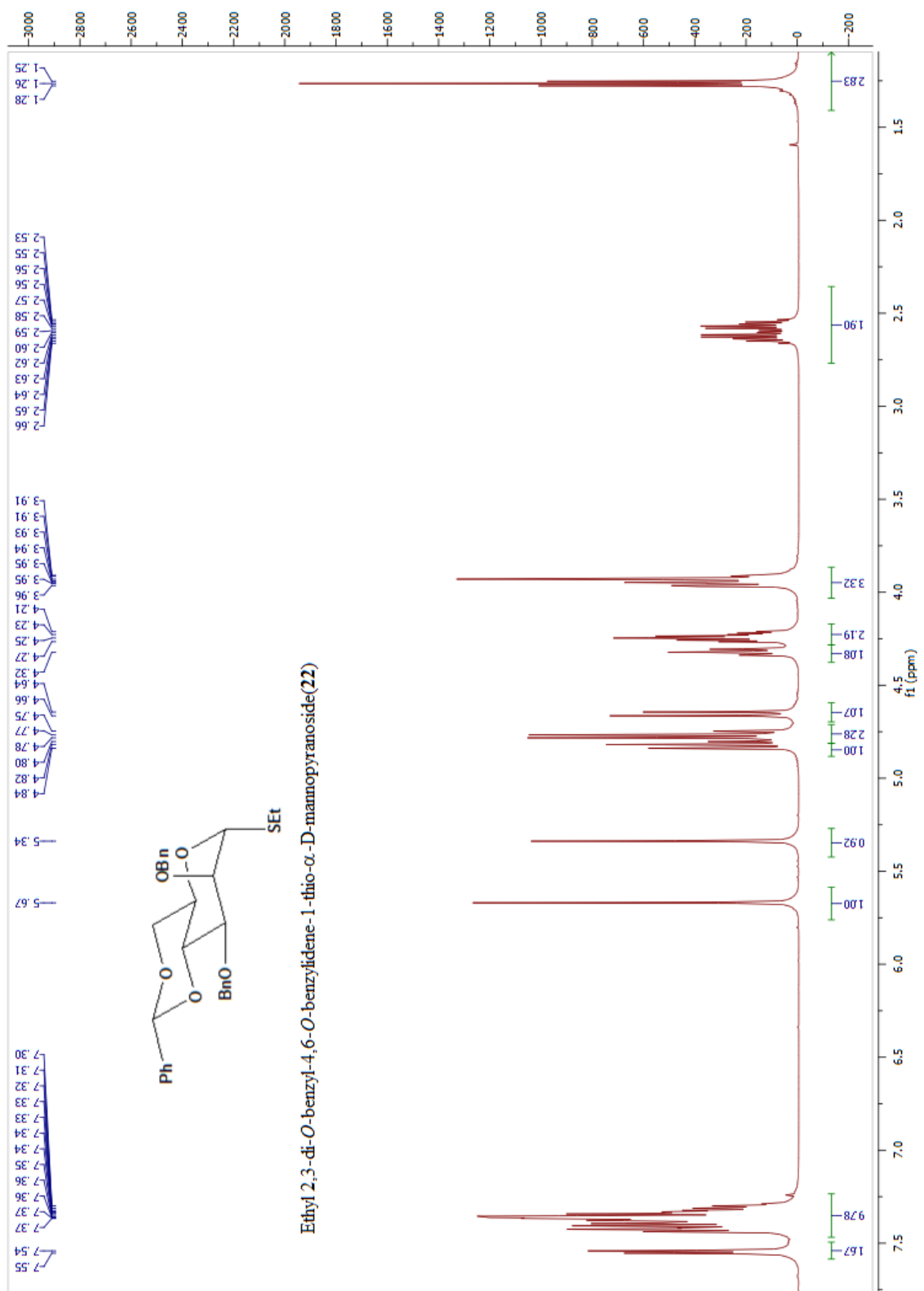
APPENDICES

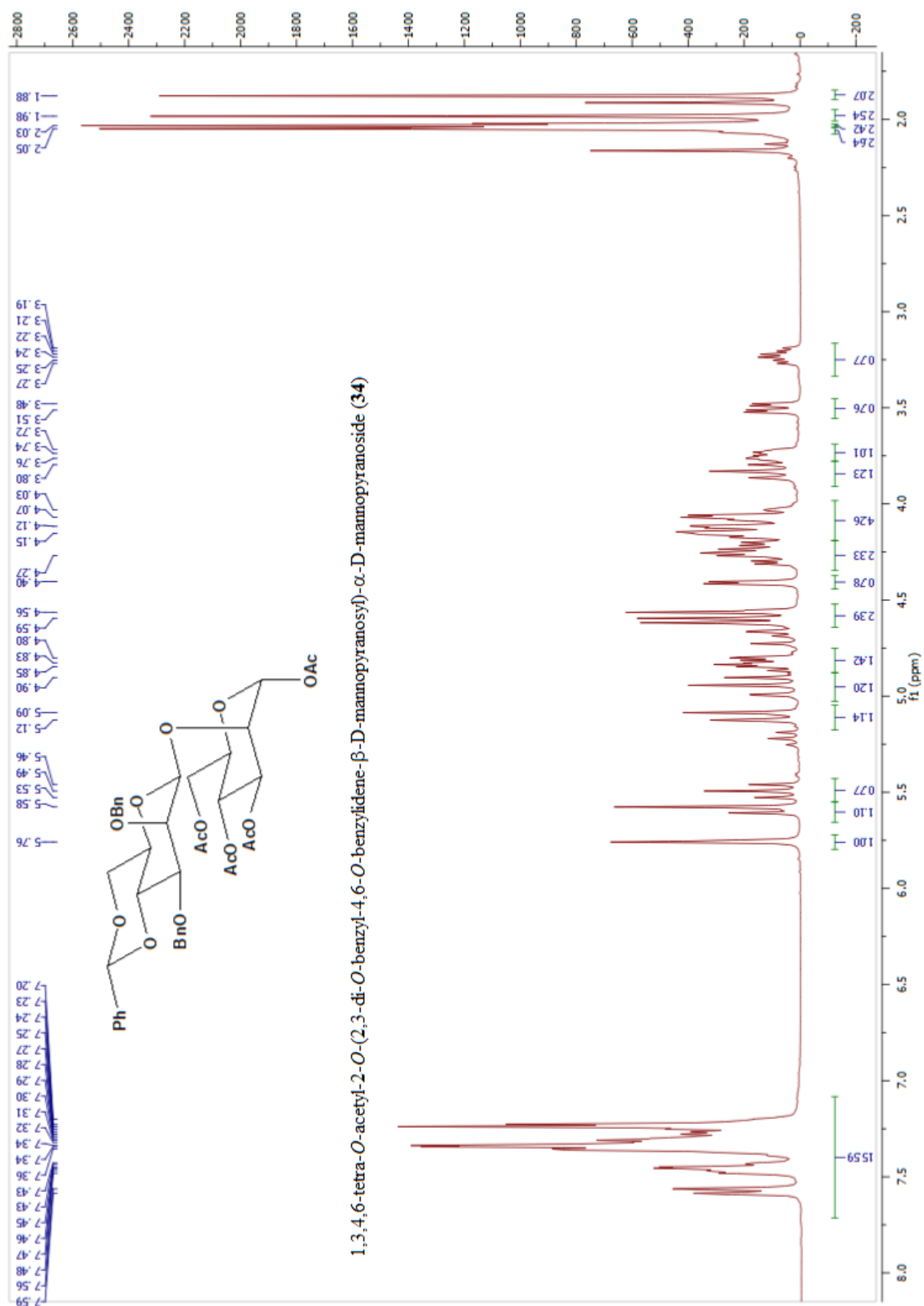
APPENDIX A ^1H NMR SPECTRA

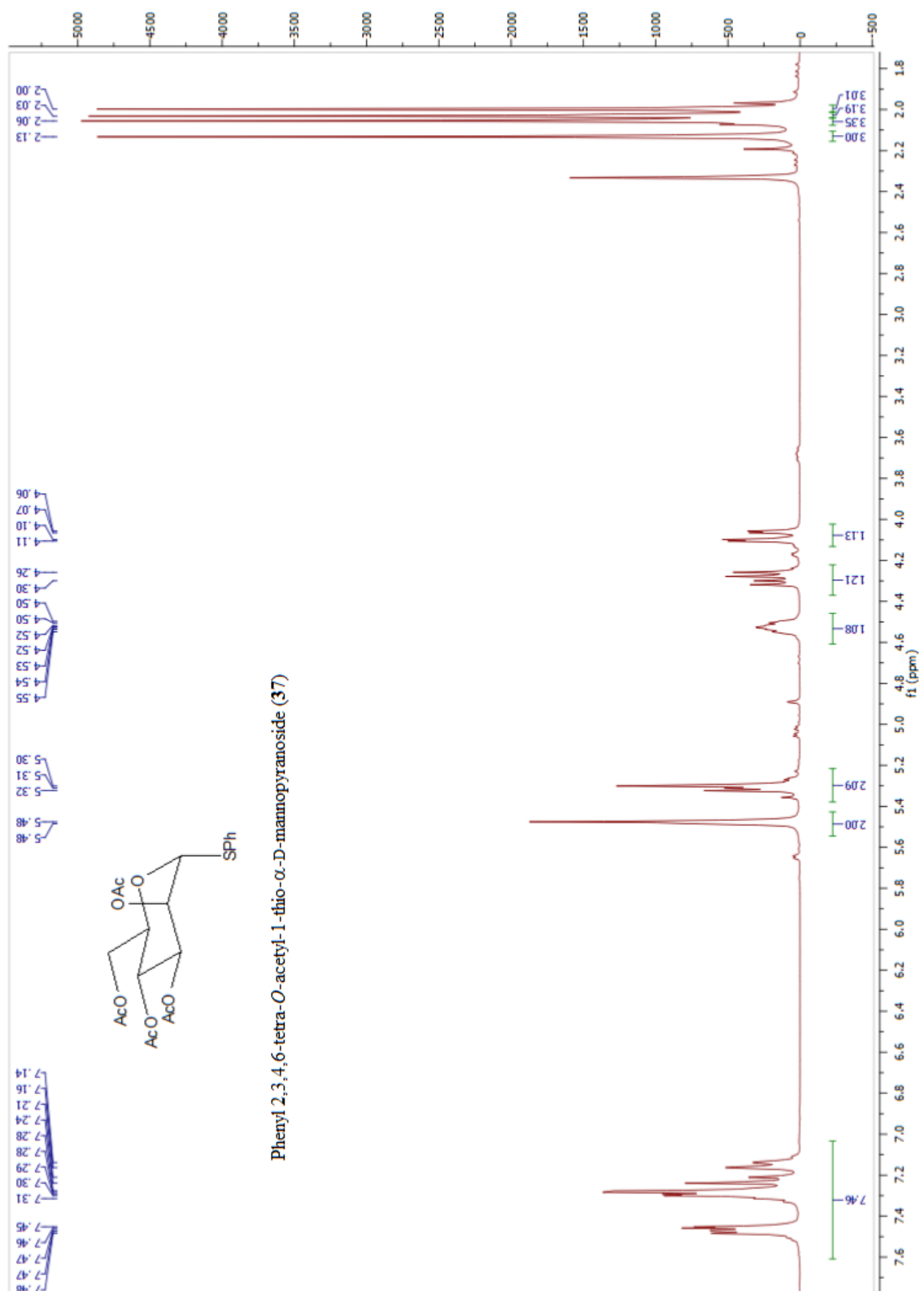


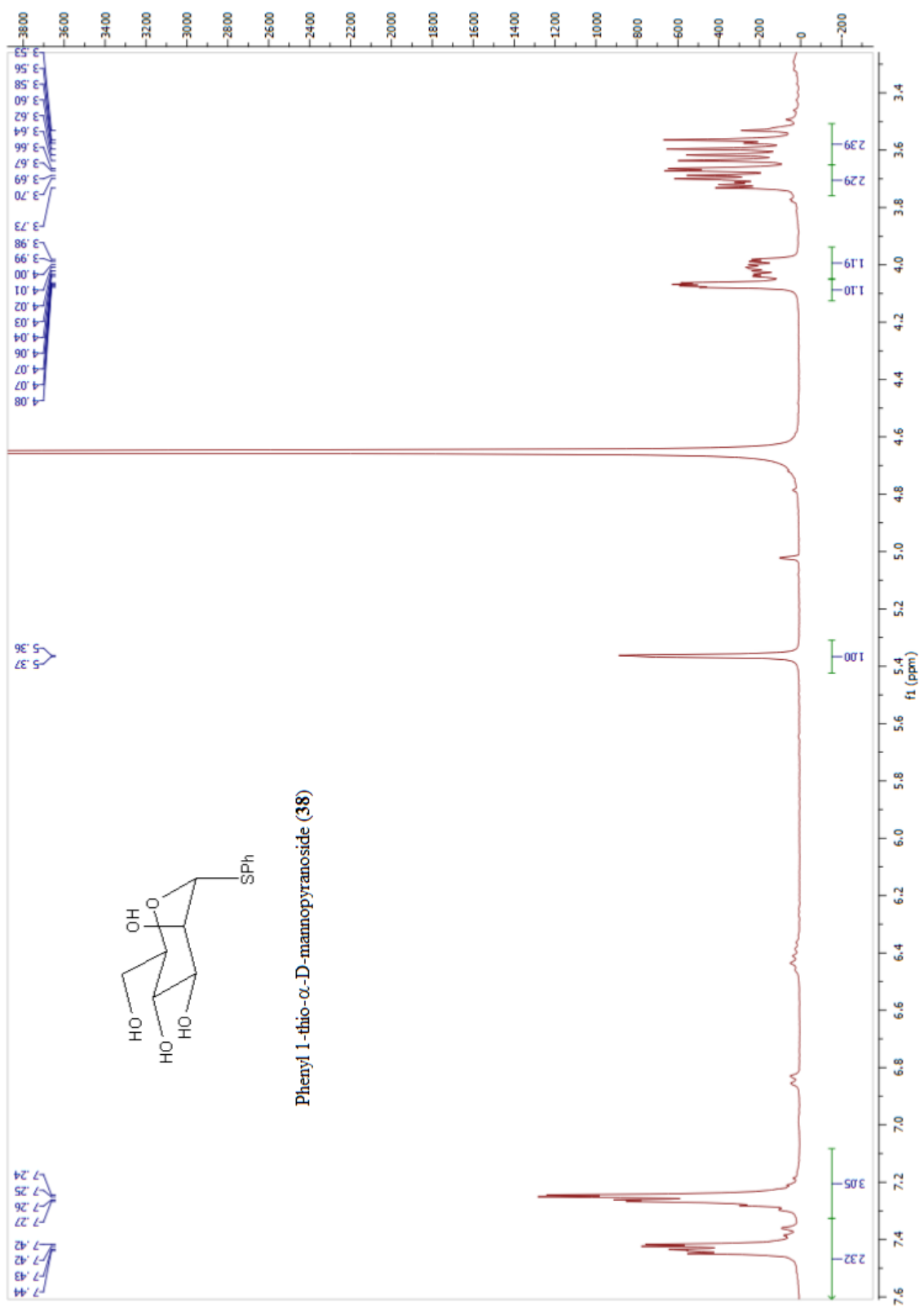


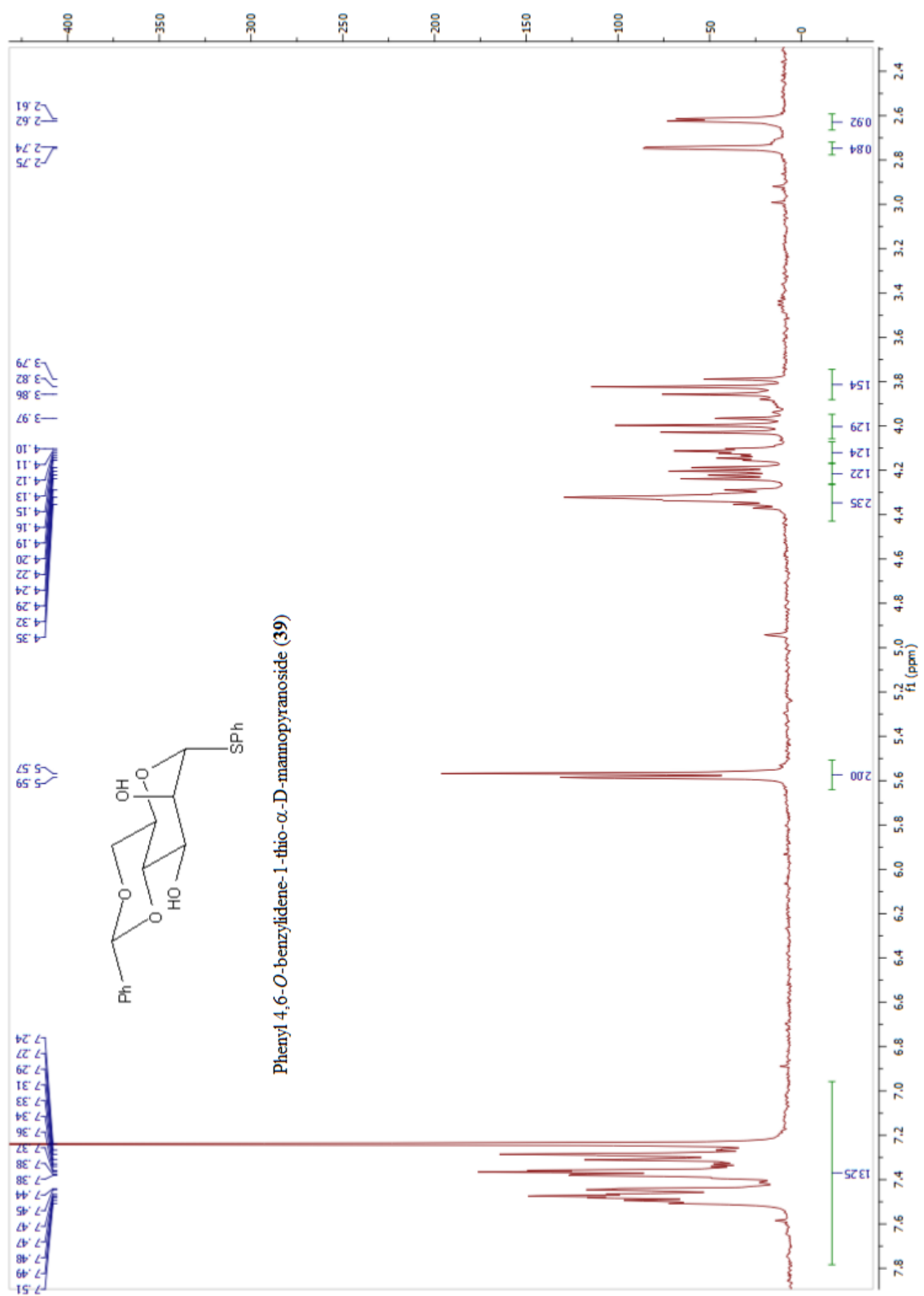


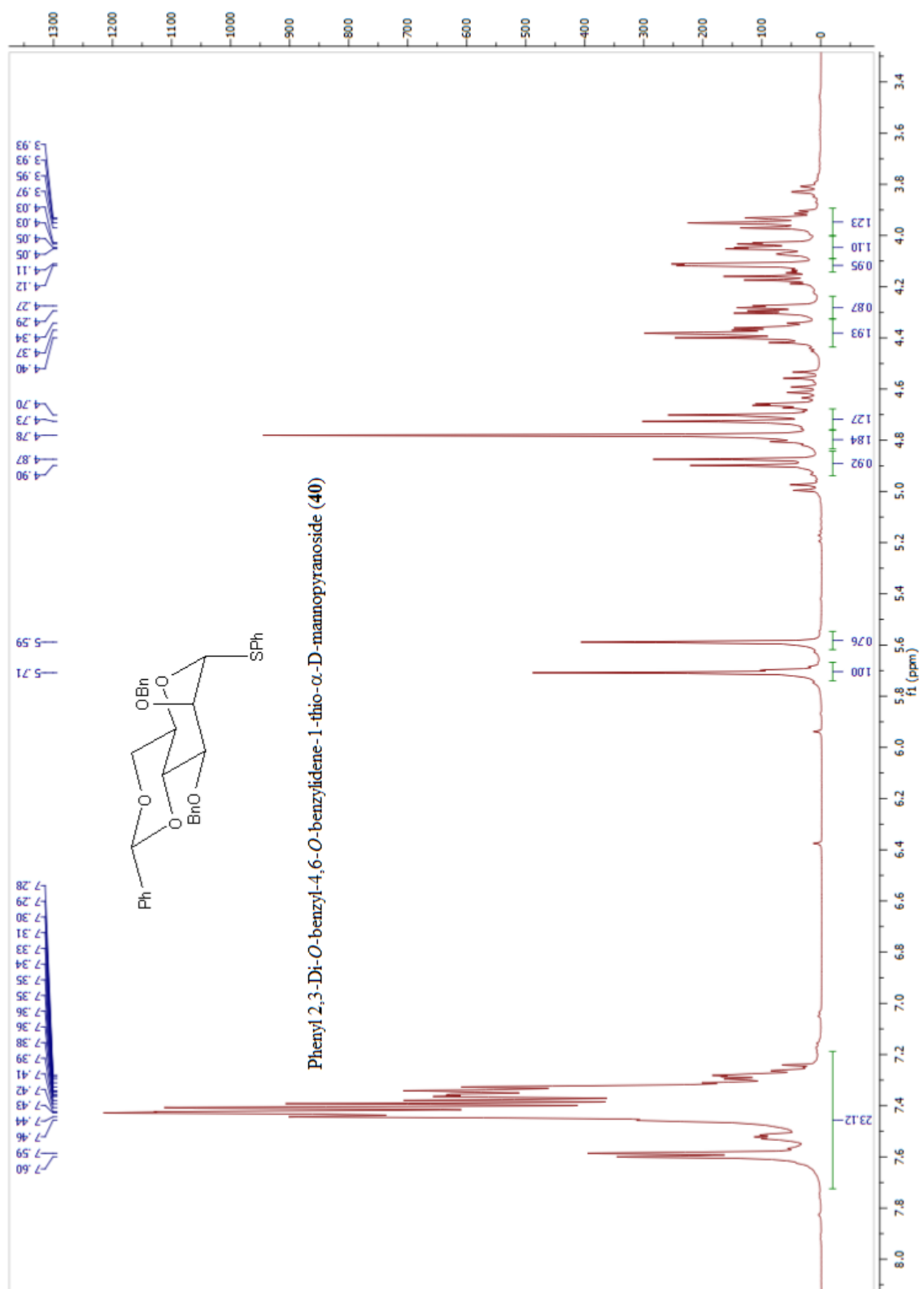


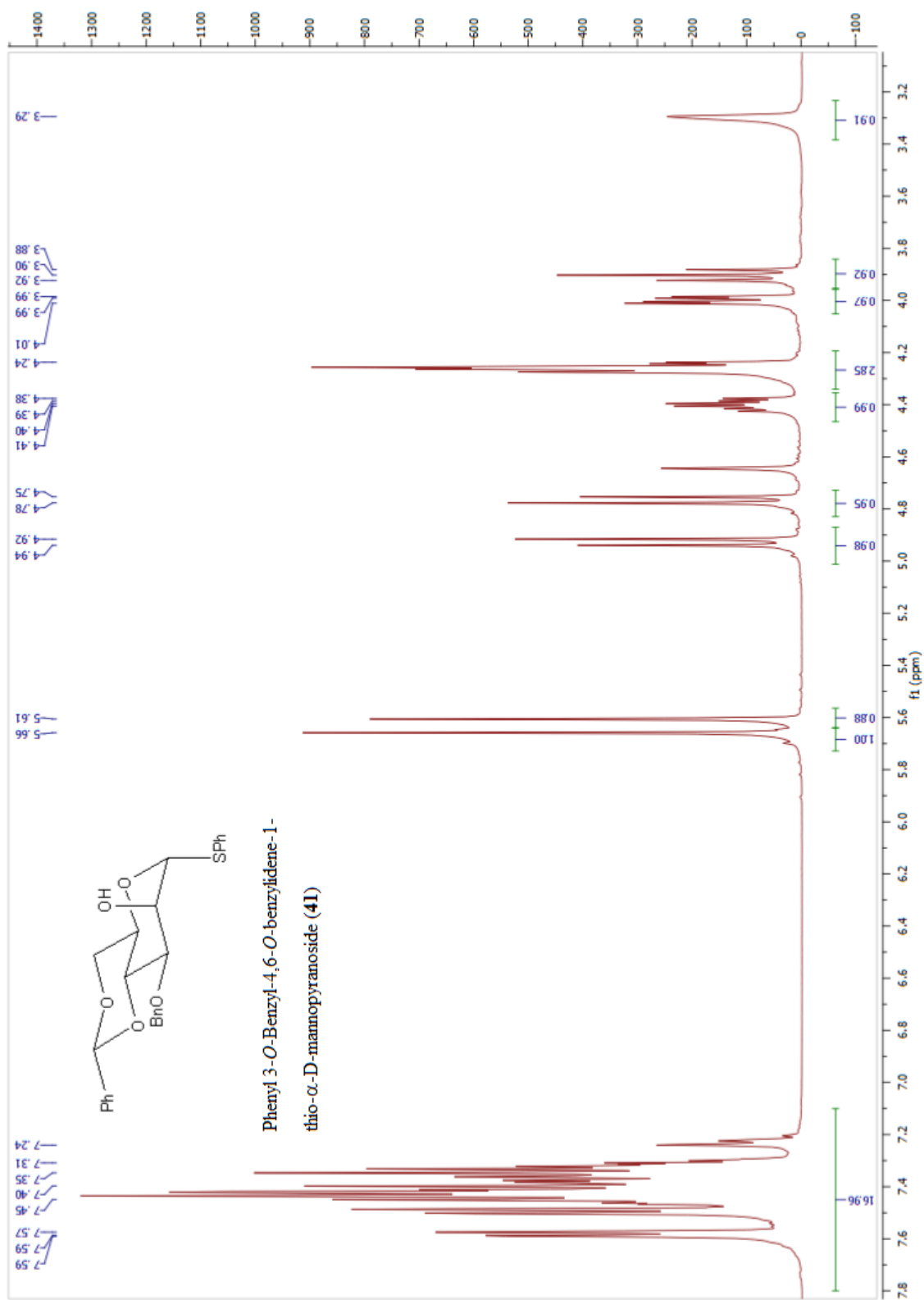


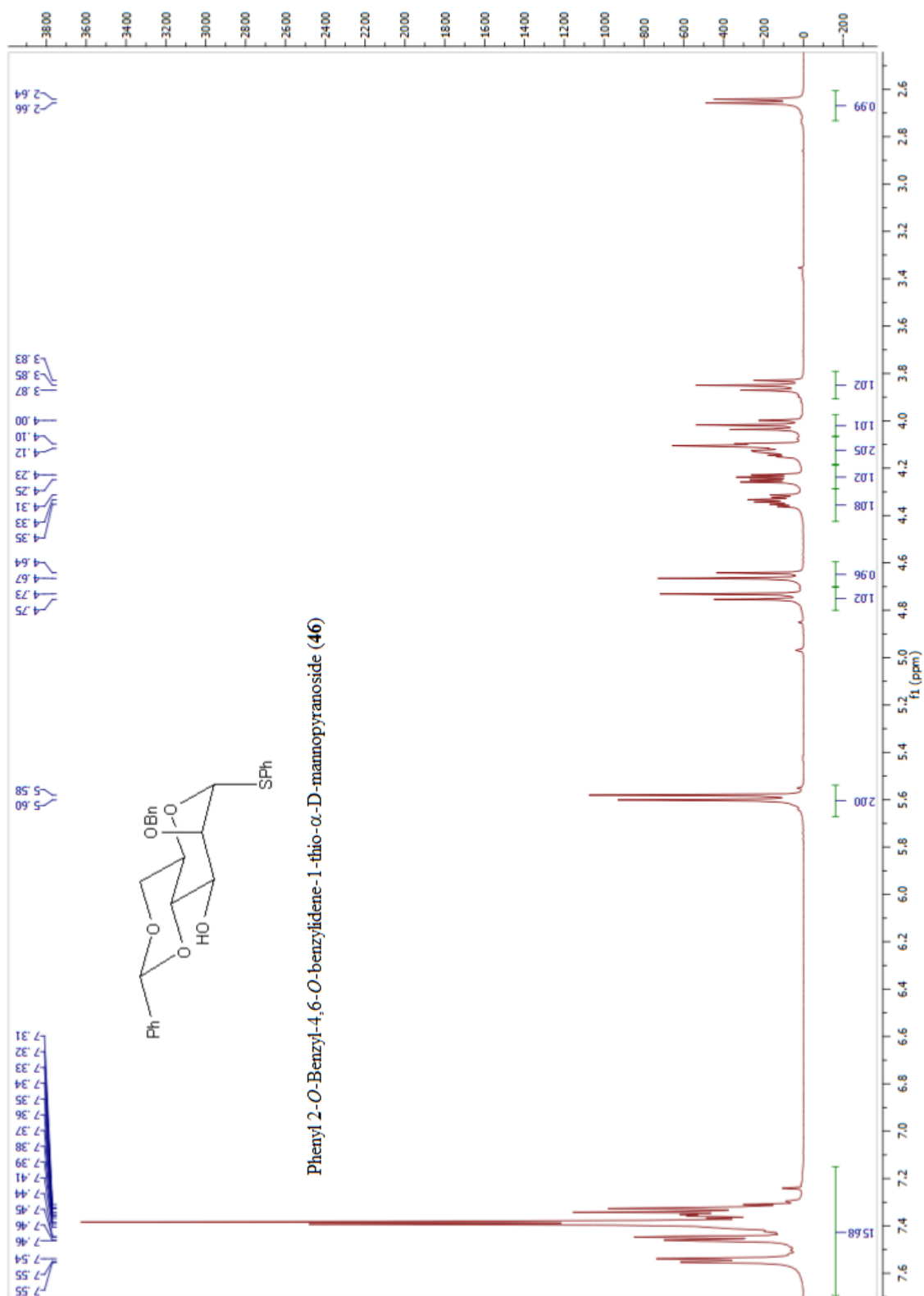


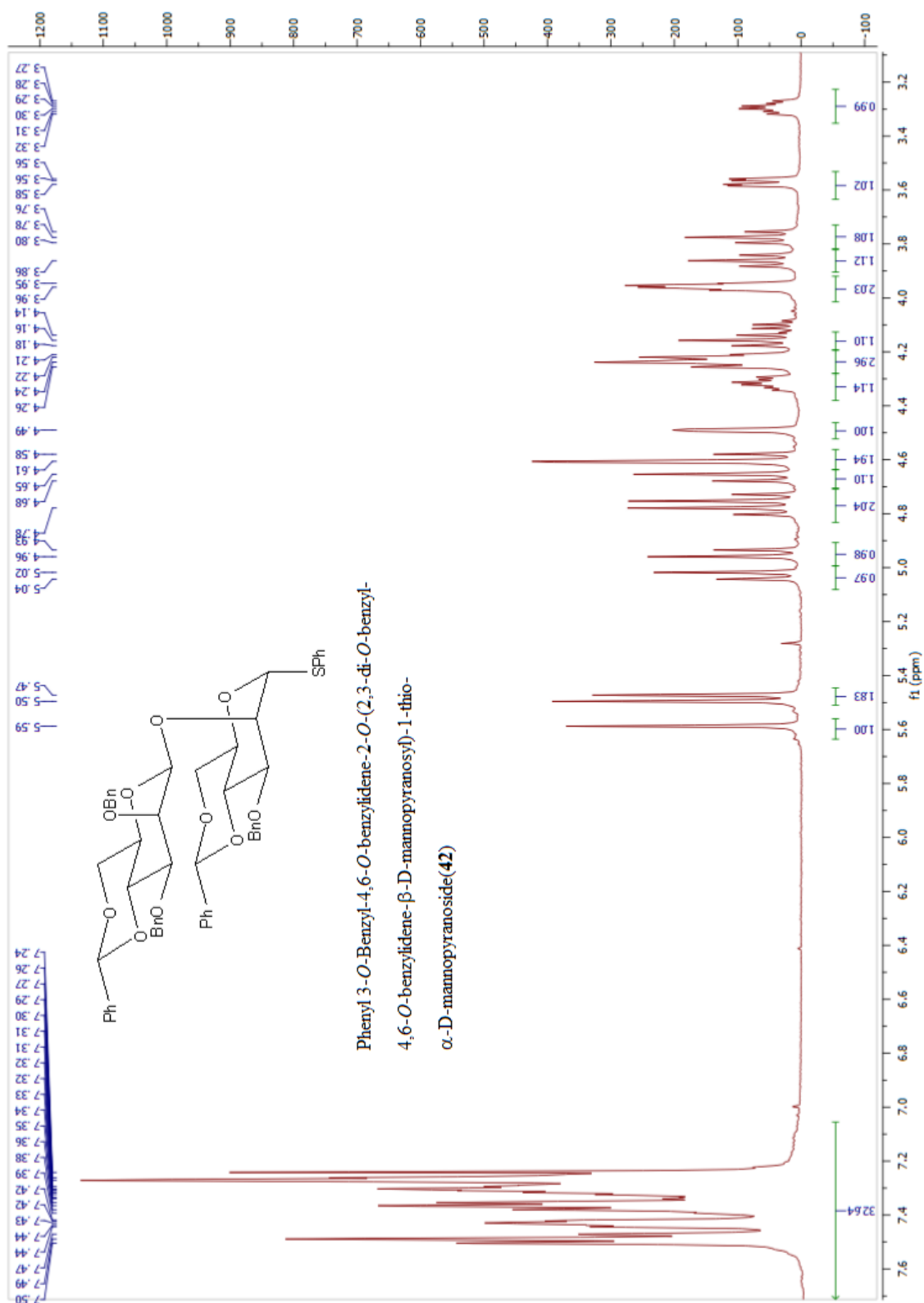


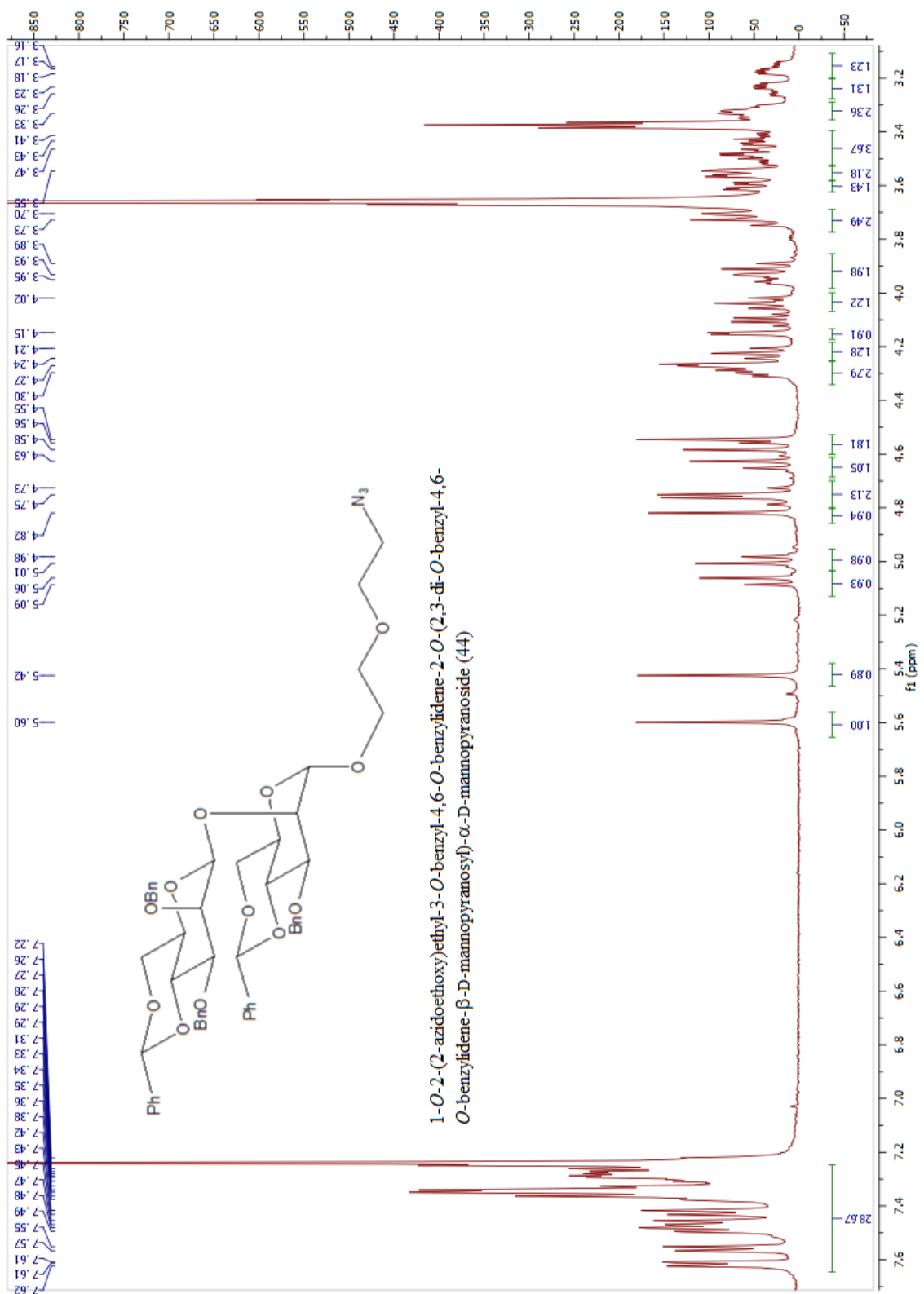


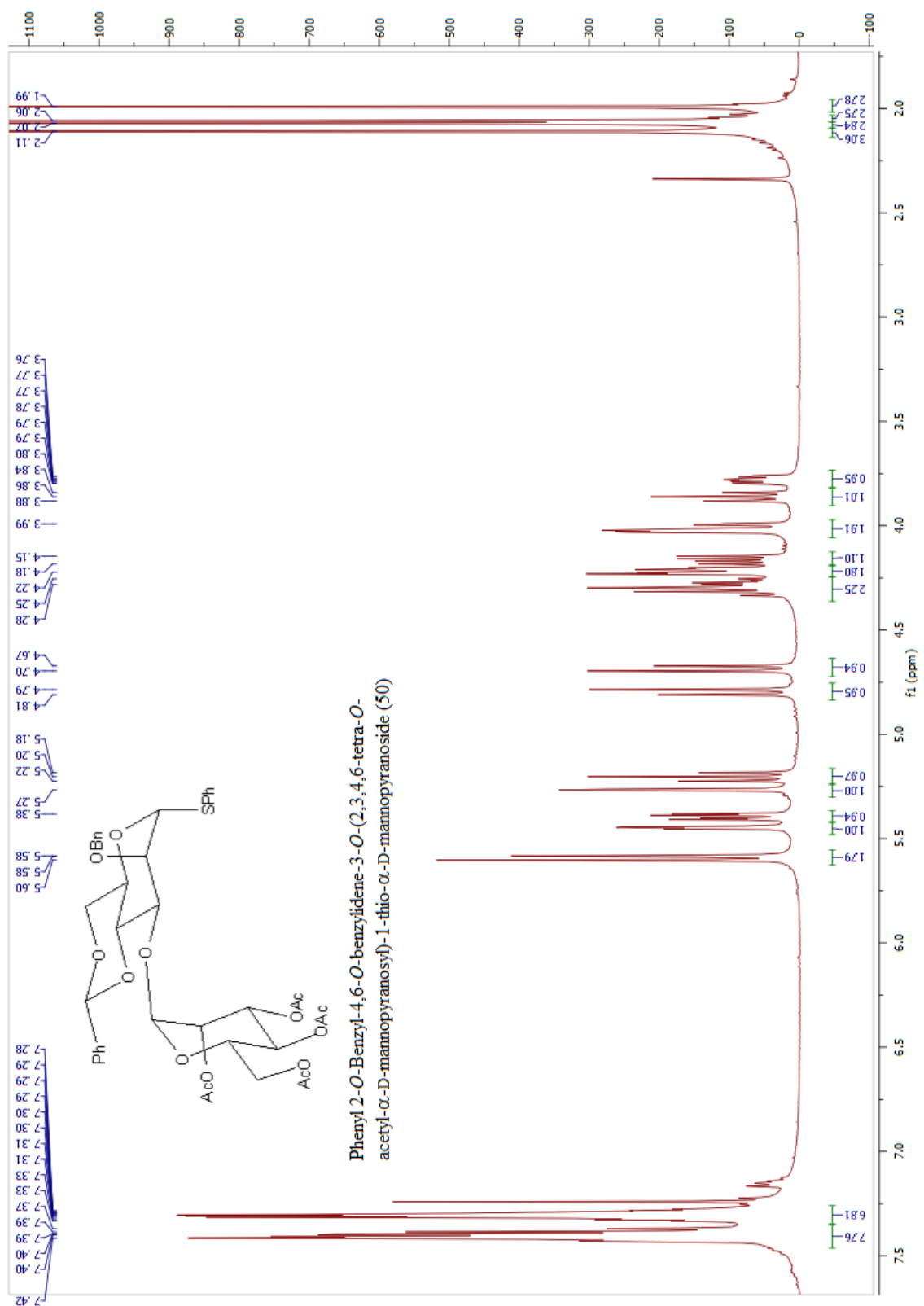


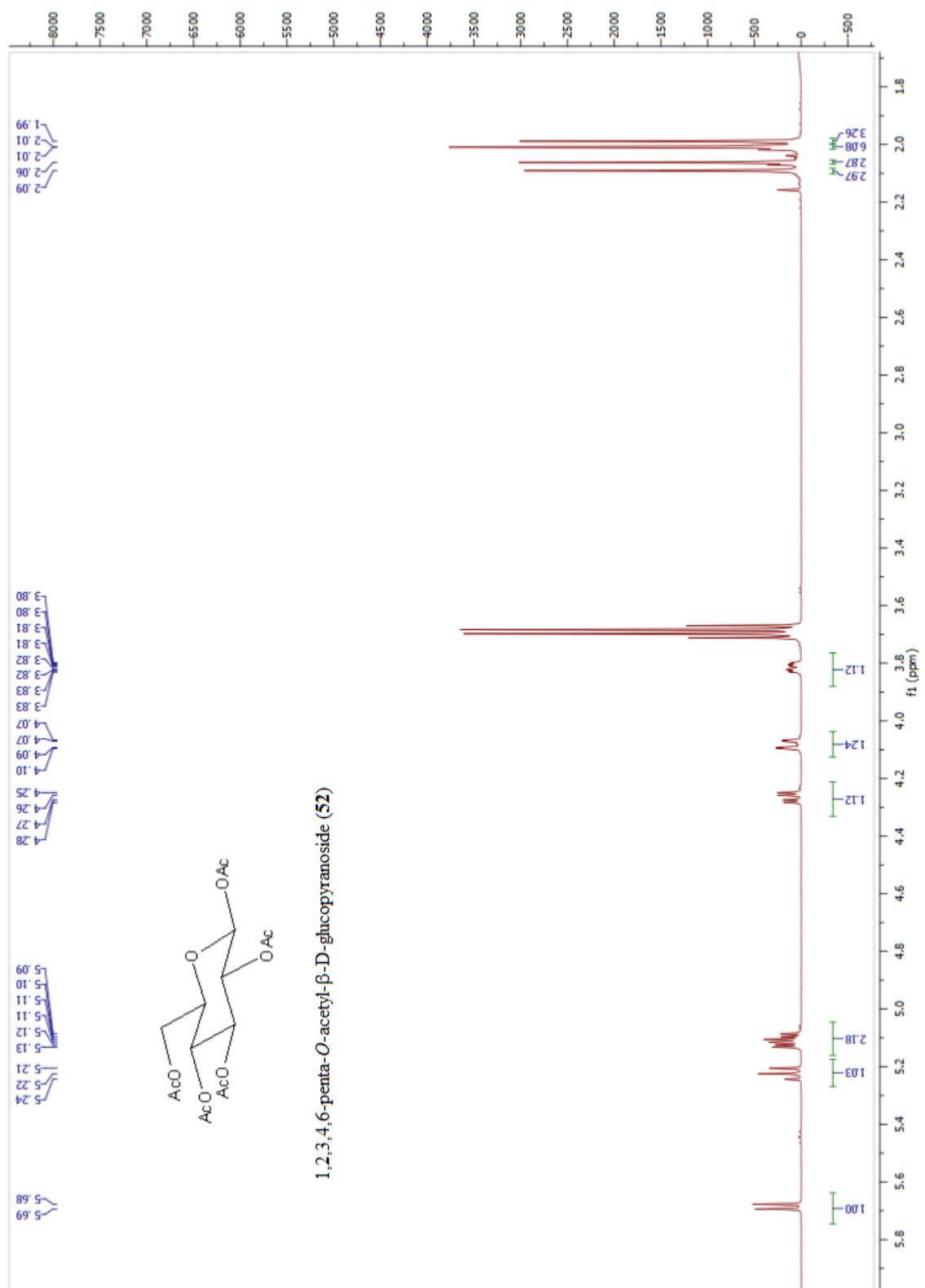


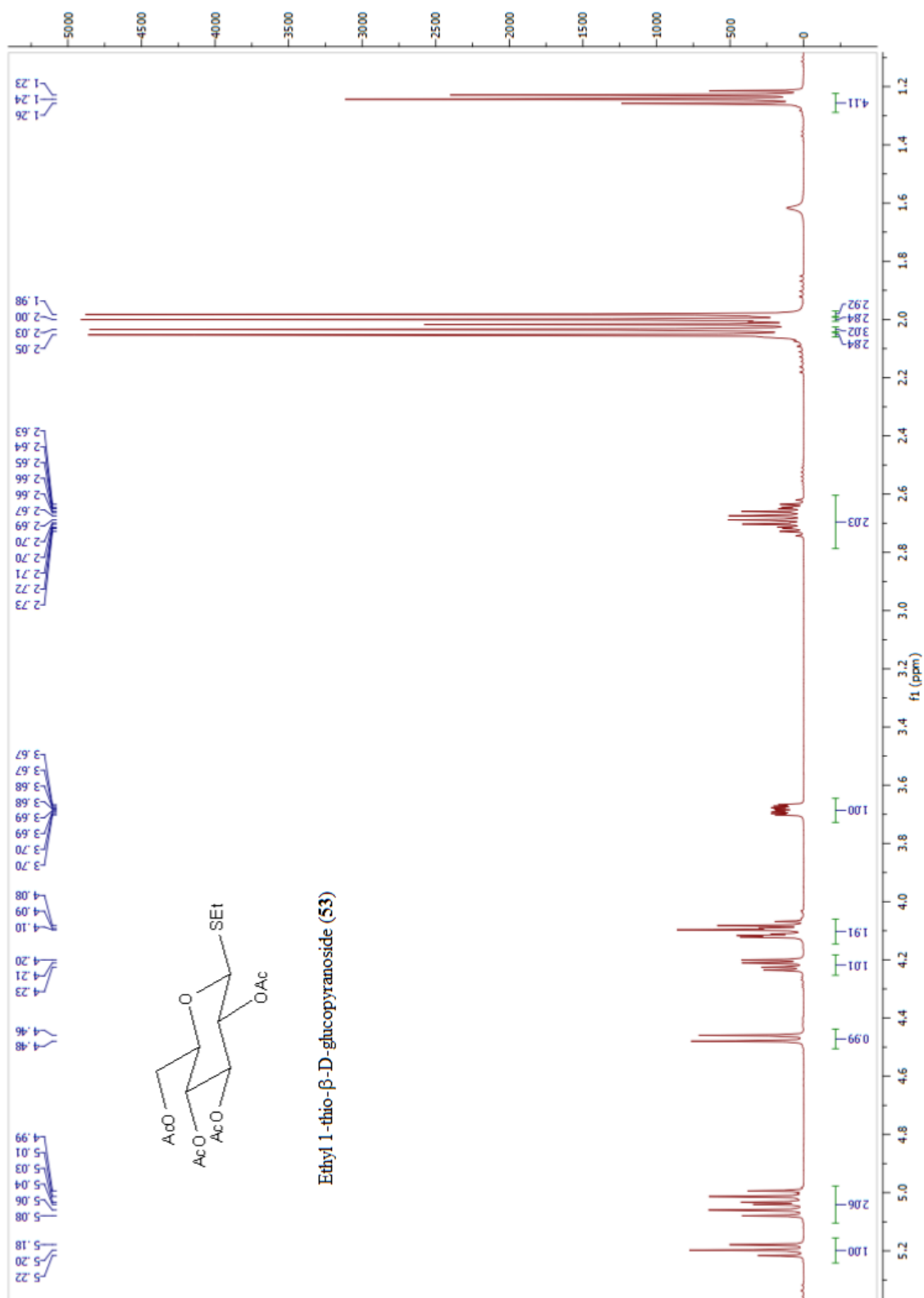


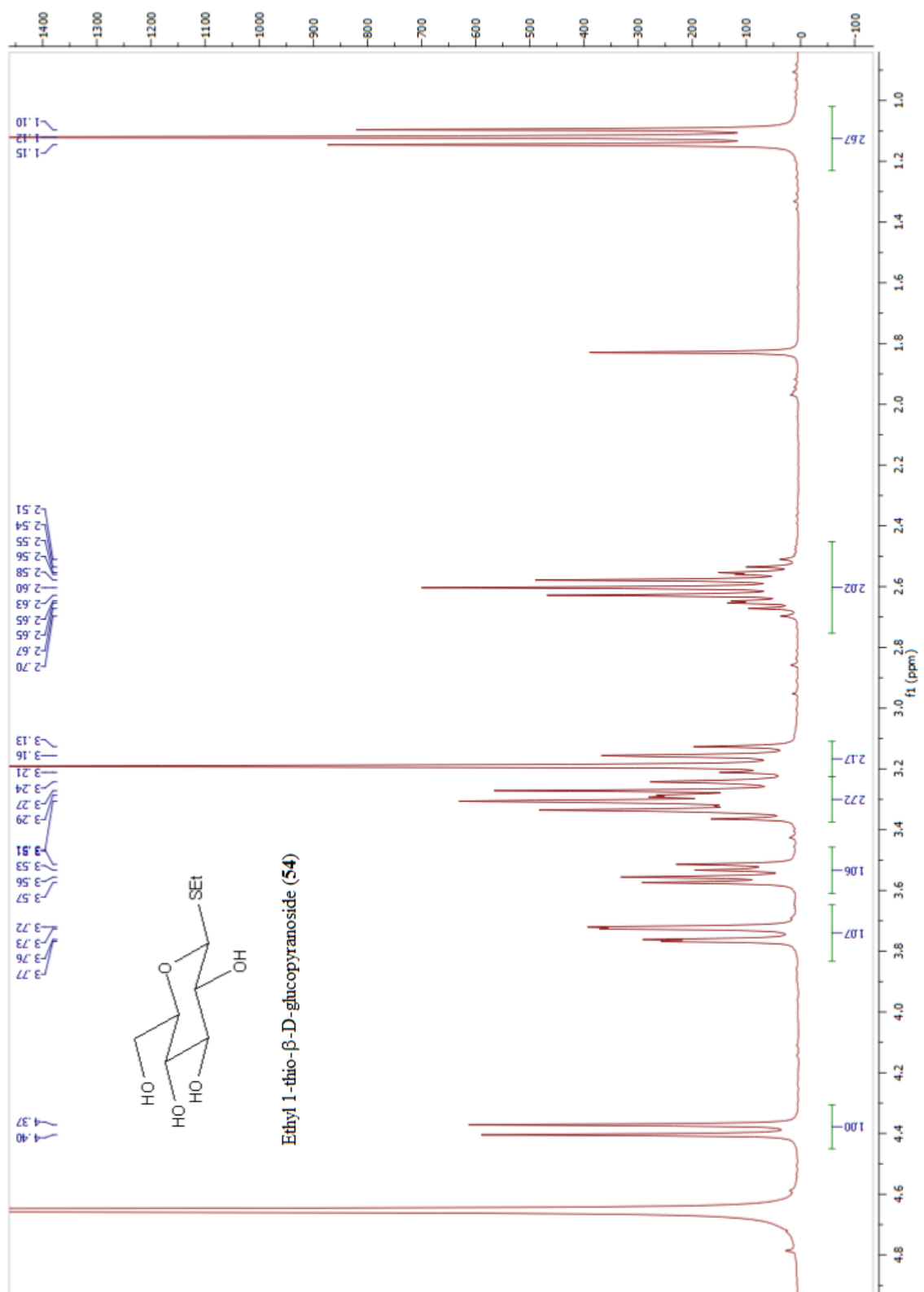


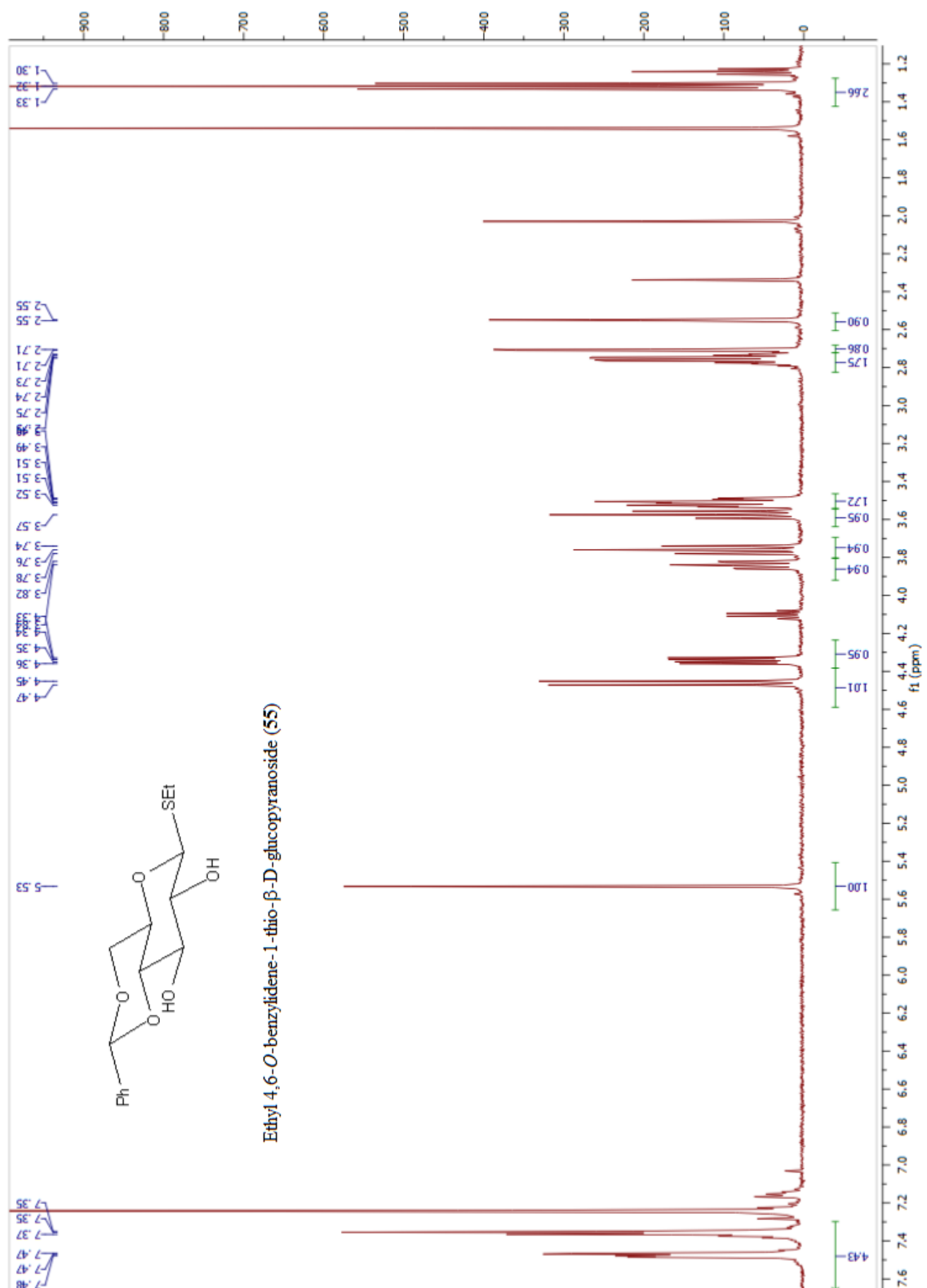


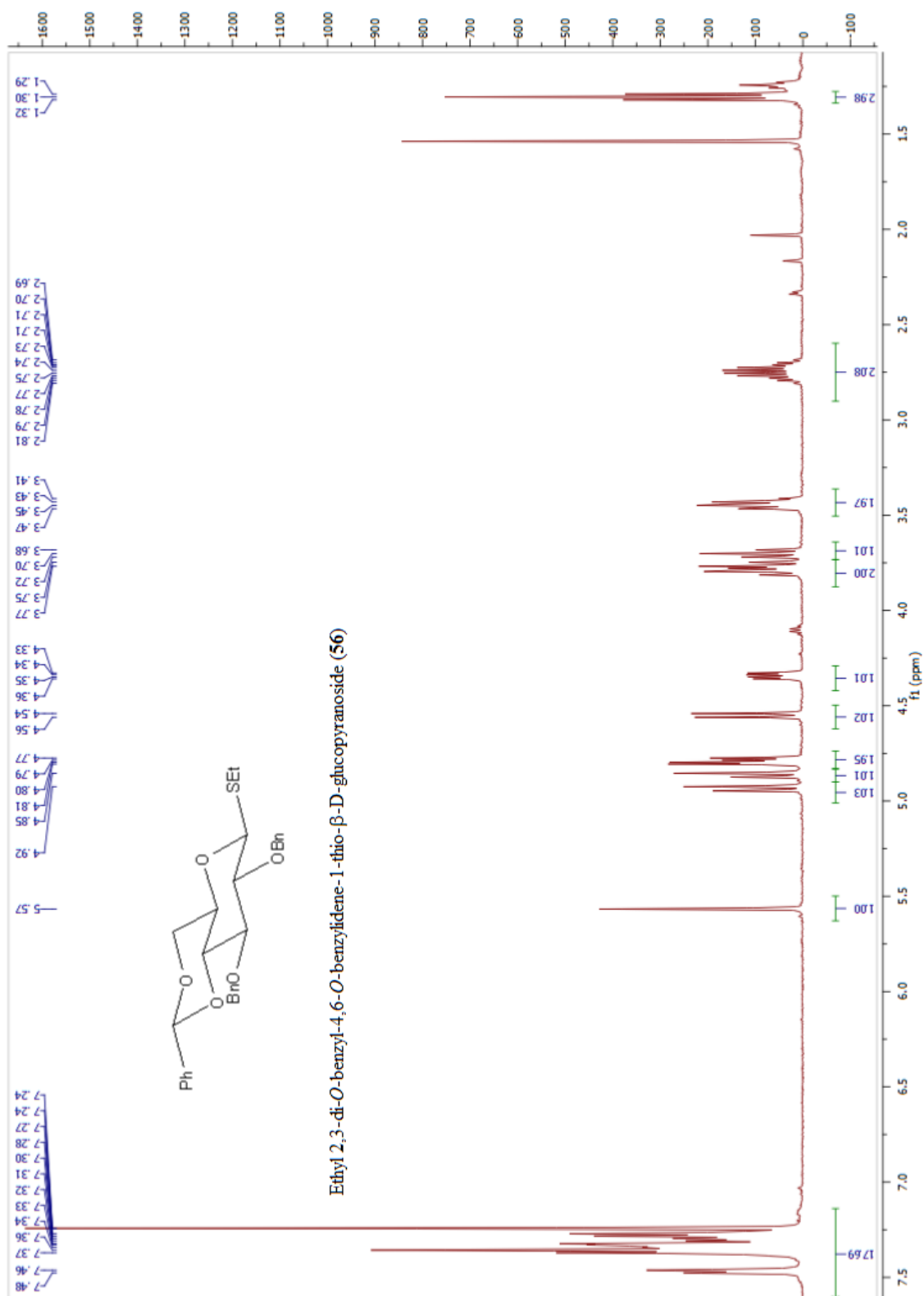


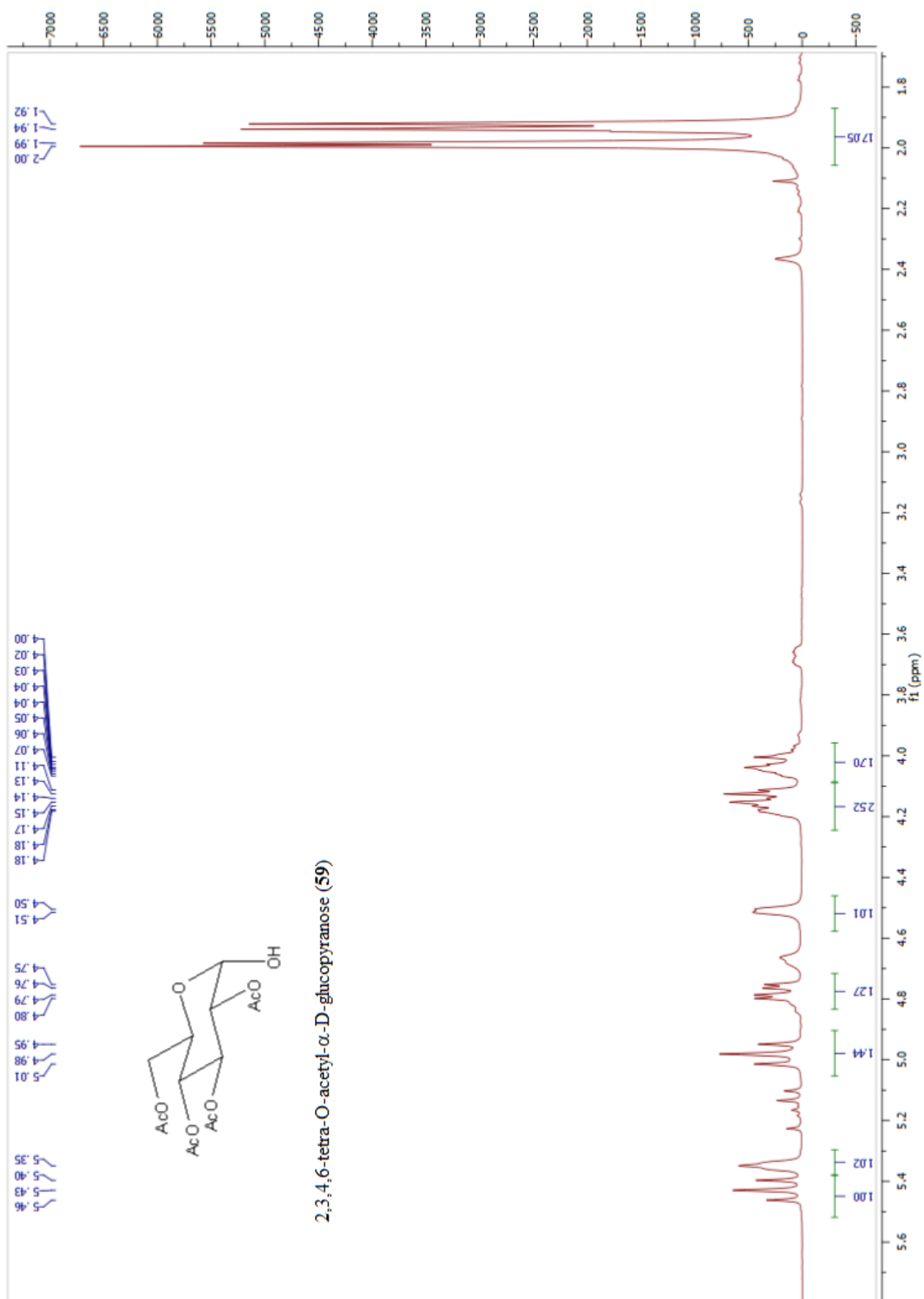


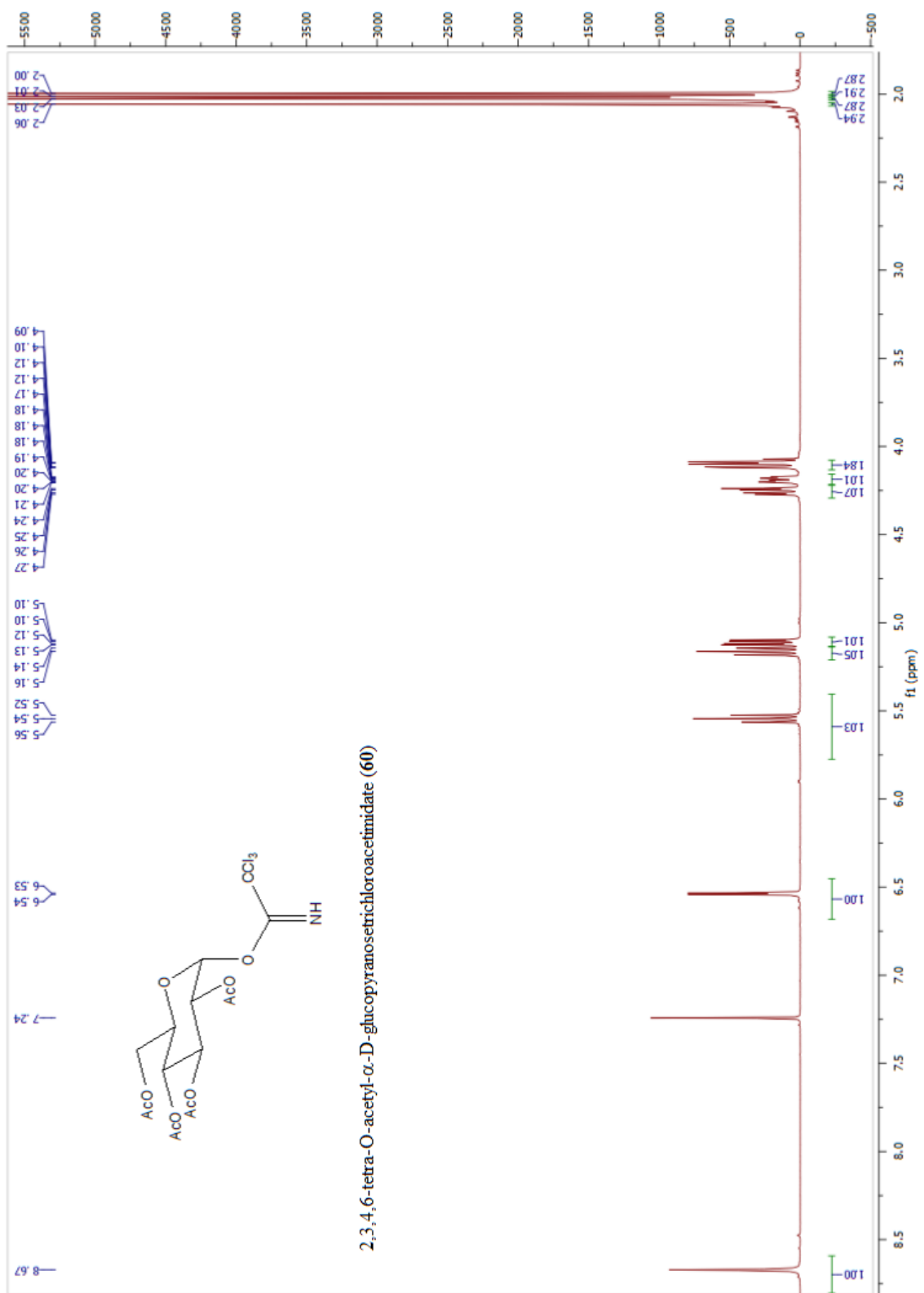


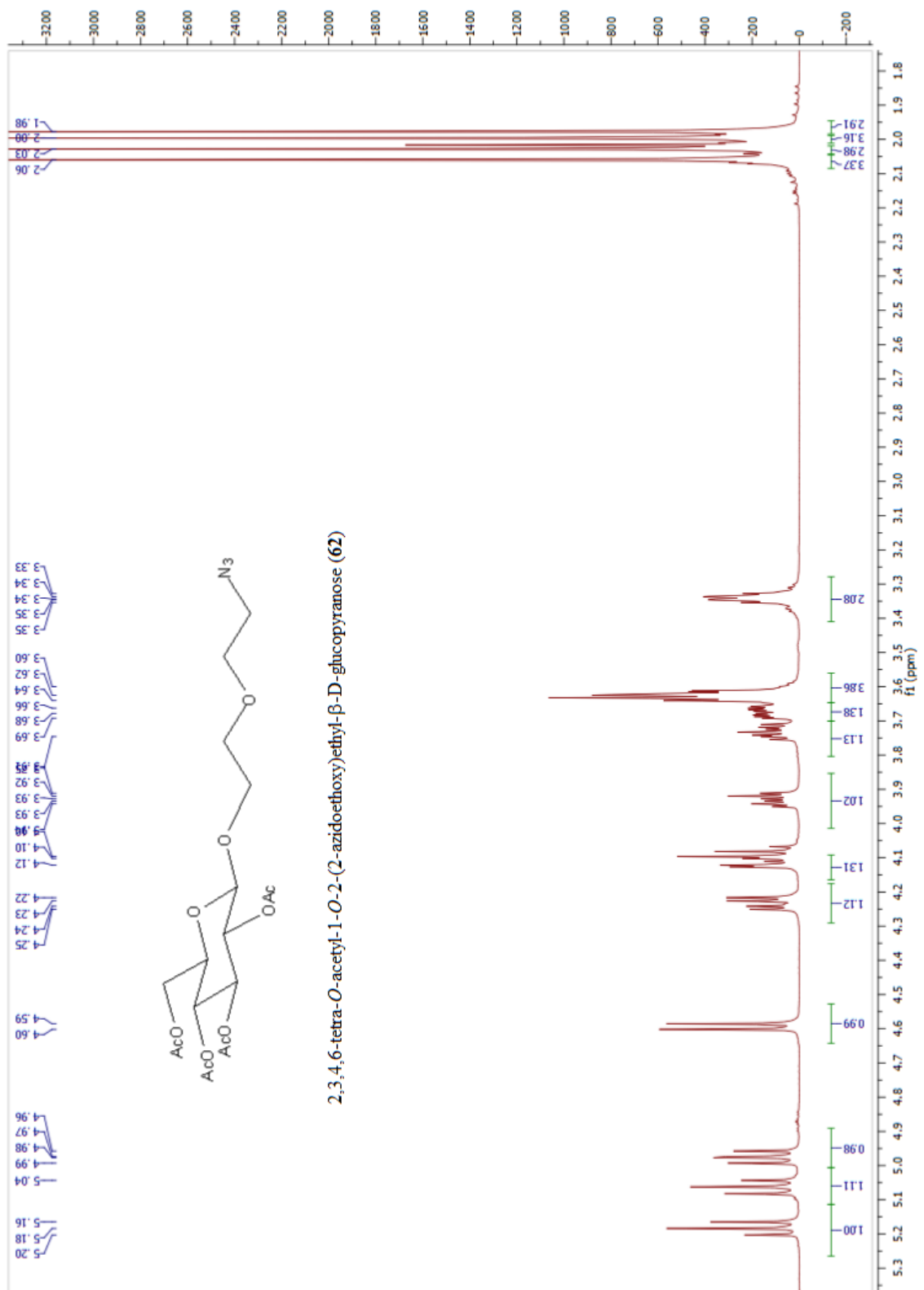


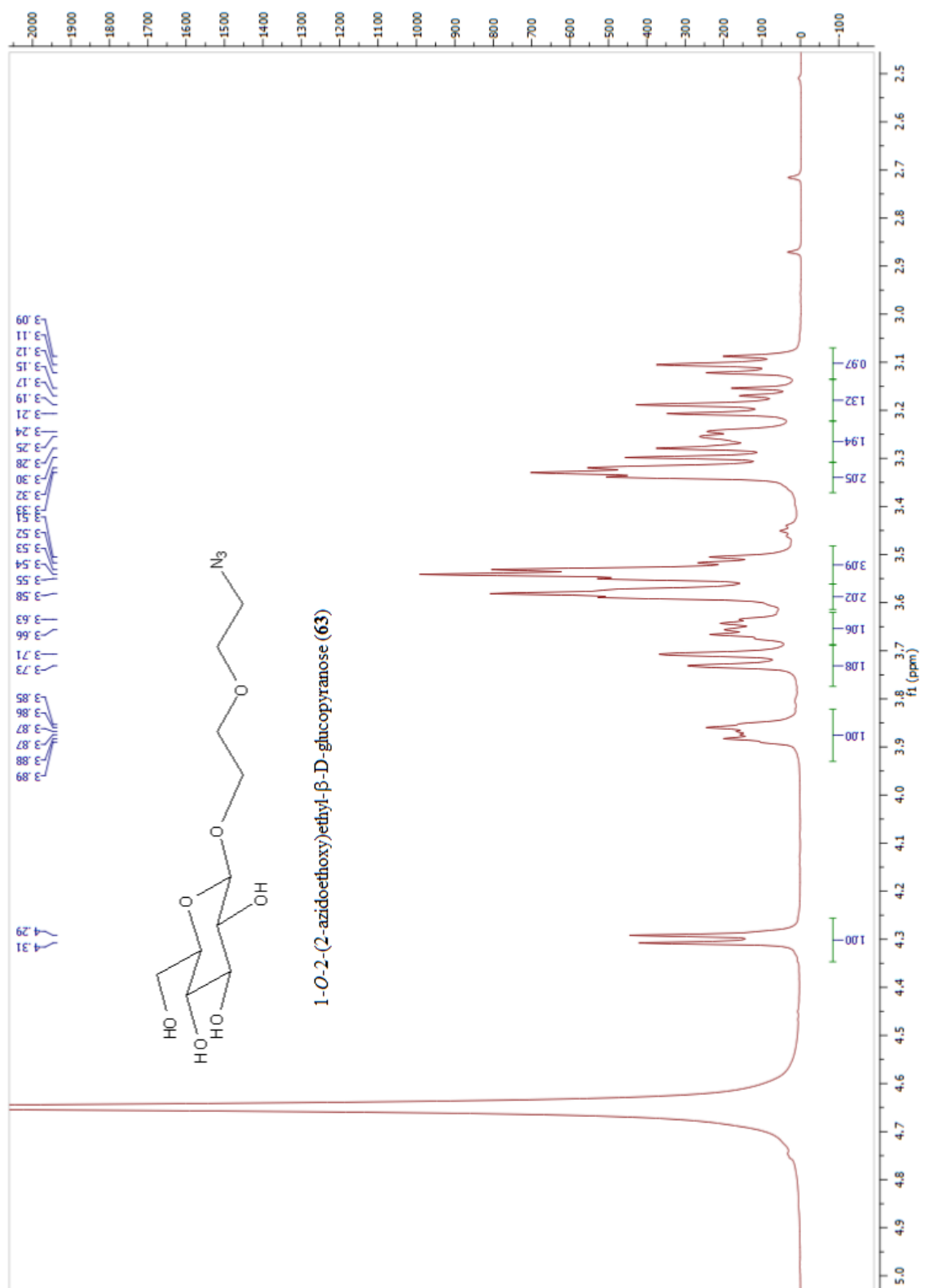


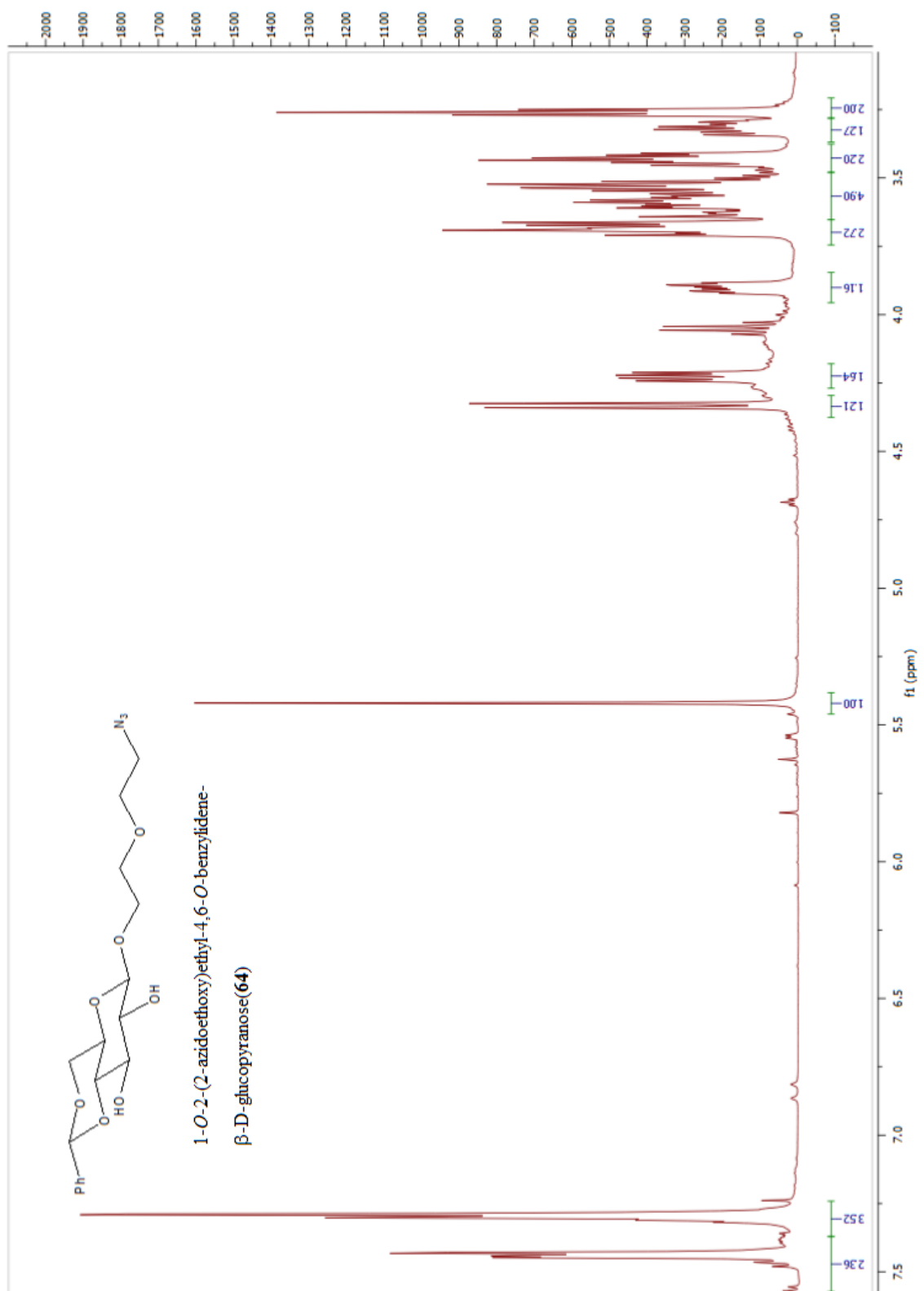


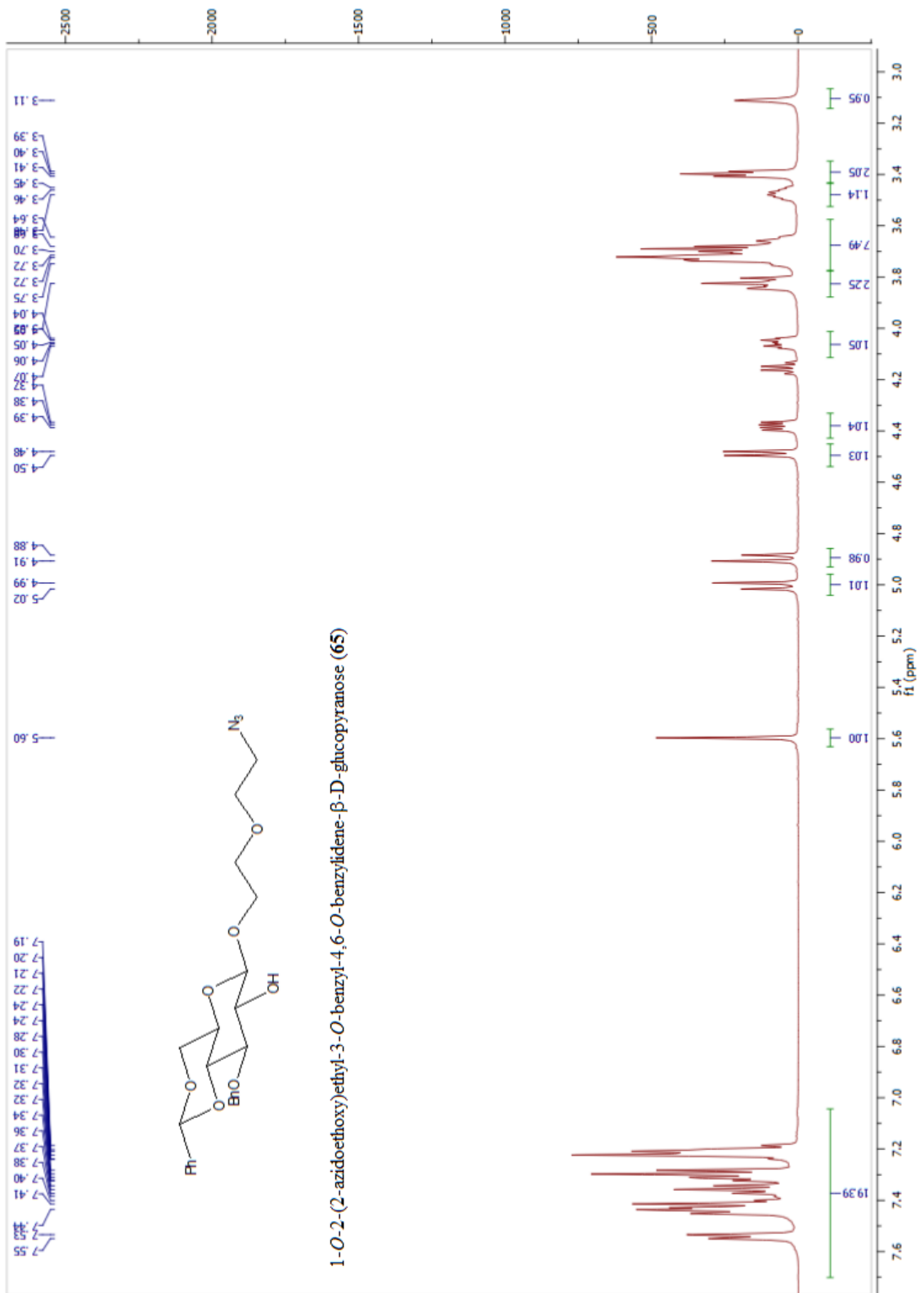


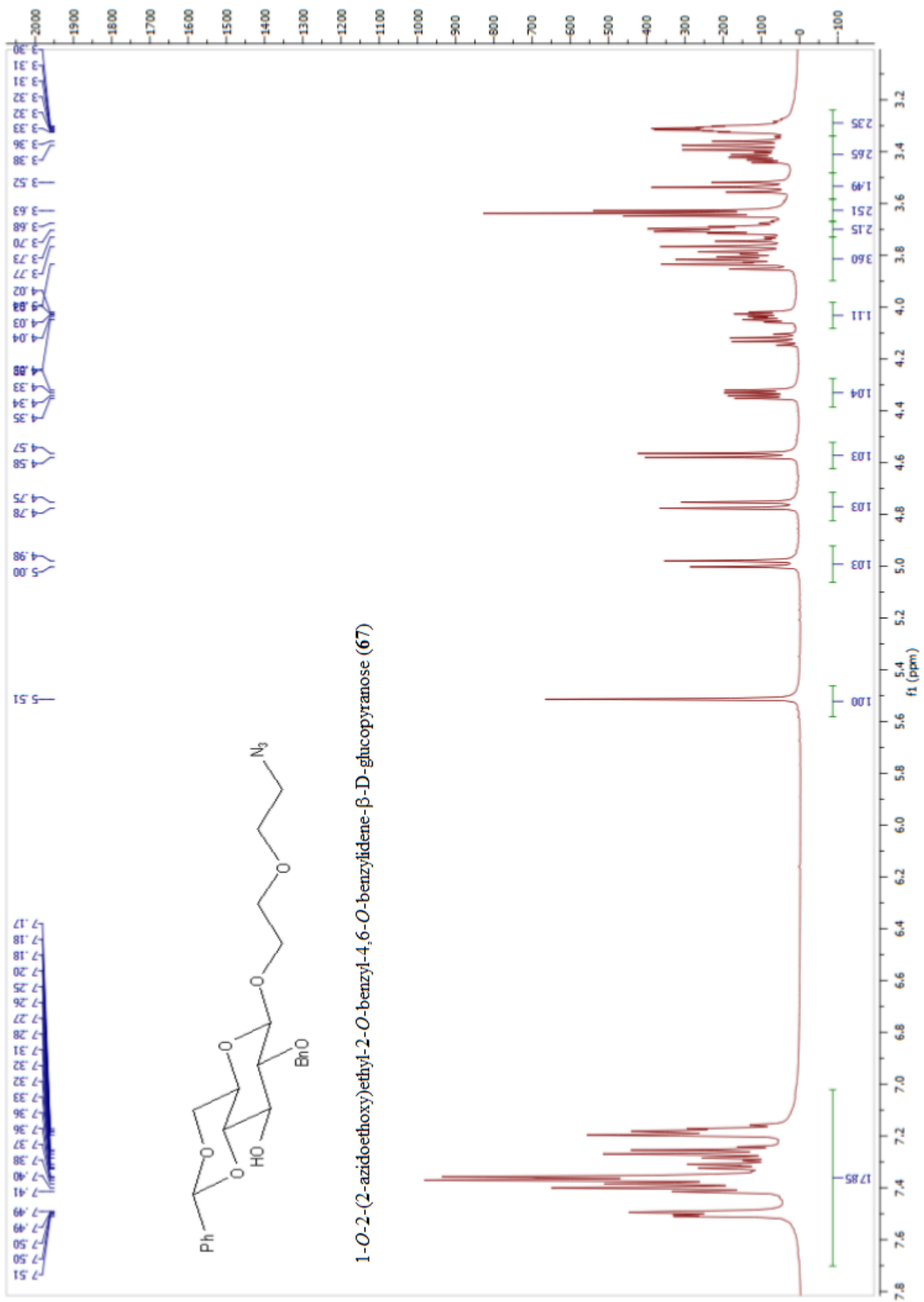


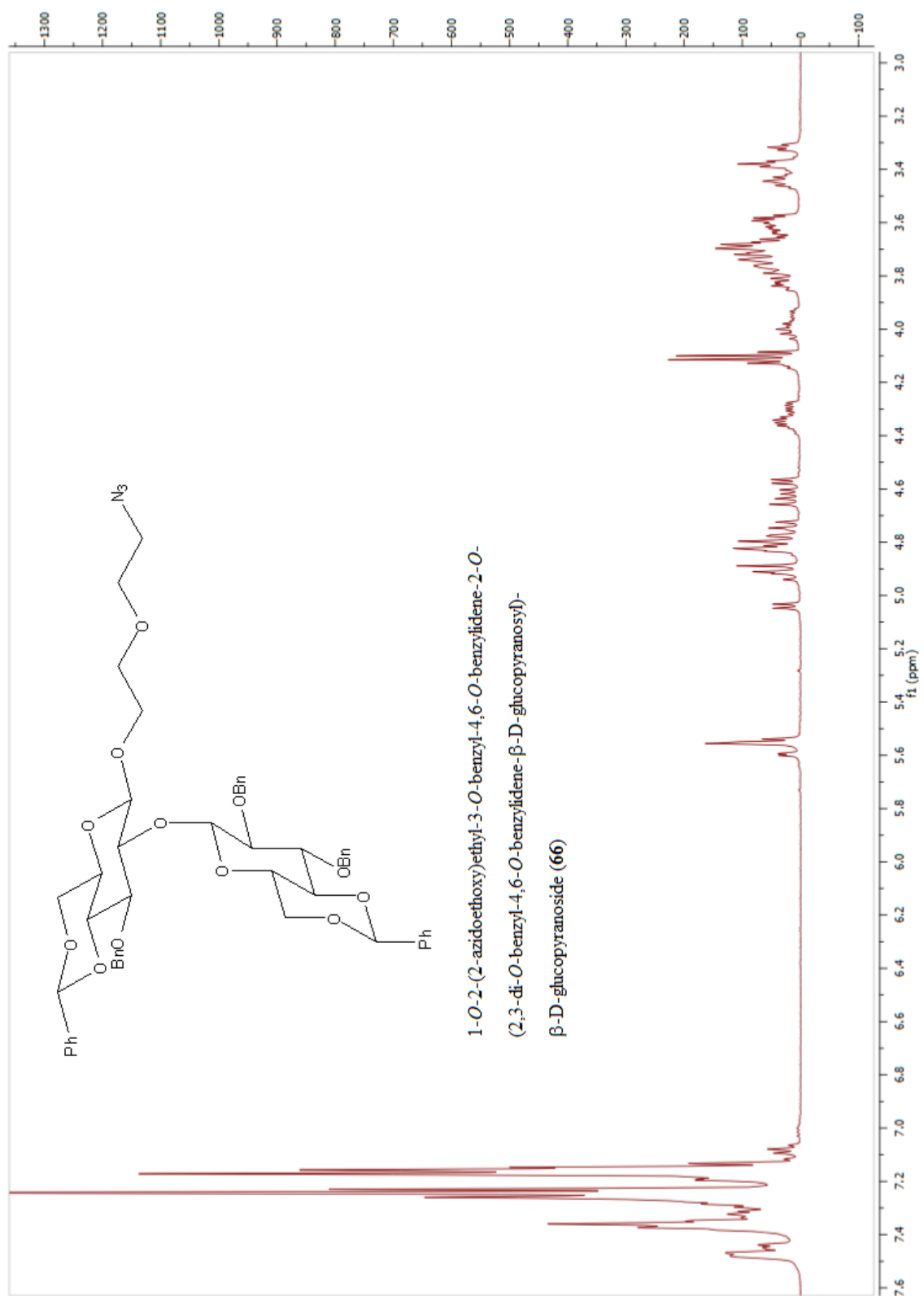


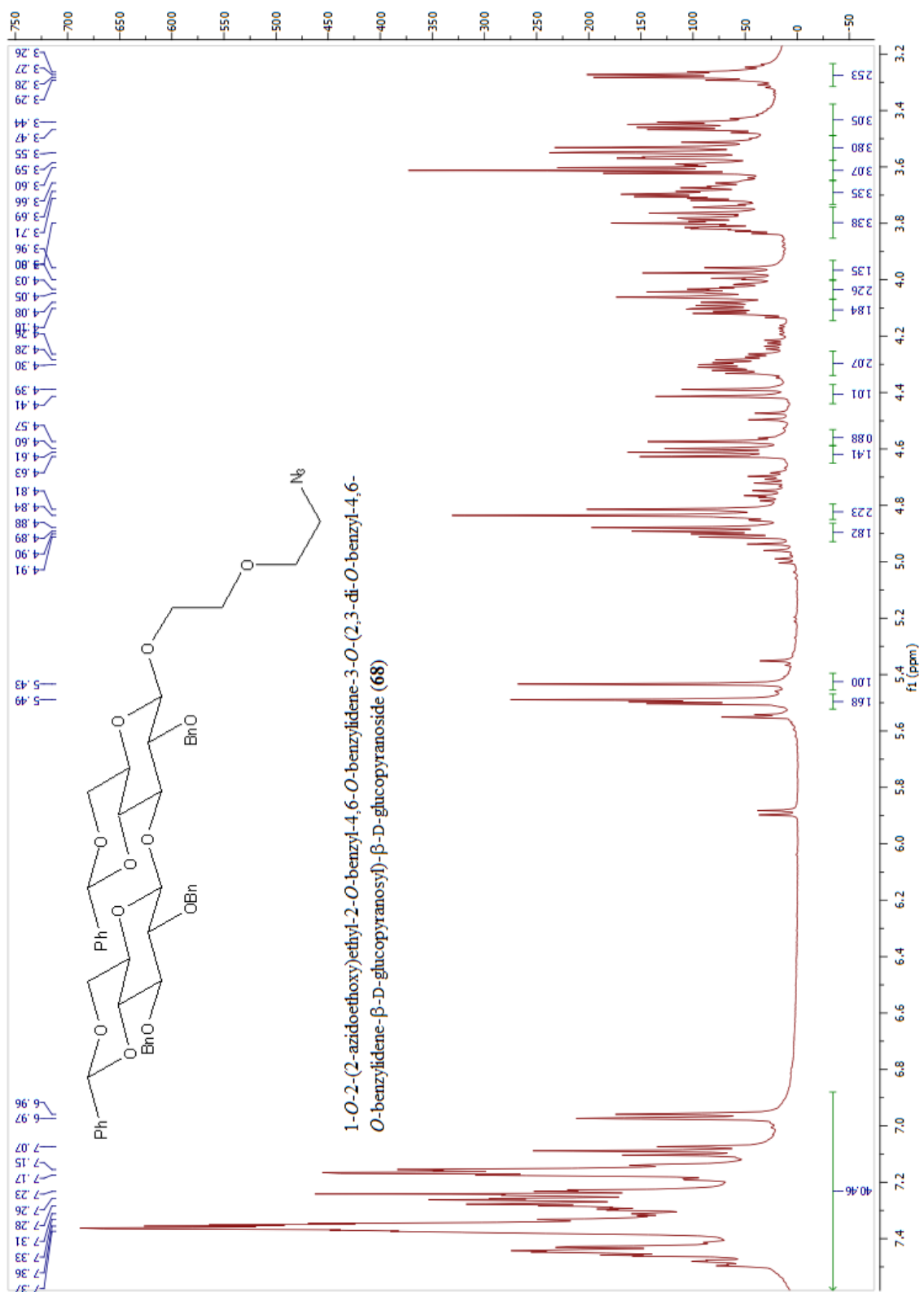












APPENDIX B ^{13}C NMR SPECTRA

