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# Glycosylation of Amino Acids and Efficient Synthesis of Glycosphingolipids

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GLYCOSYLATION OF AMINO ACIDS AND EFFICIENT SYNTHESIS OF

GLYCOSPHINGOLIPIDS

by

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Under the Direction of Peng George Wang, PhD

ABSTRACT

Glycoscience is an emerging field of science that focuses on the study of the structure, biosynthesis, biology, and evolution of saccharides (sugars). It covers a broad range of subjects including microwave-assisted synthesis as well as sphingolipid synthesis. In this field, knowledge is limited due to the complexity of carbohydrates and their derivatives. Therefore, it is all the more important that synthesis of these complex molecules occurs in order to fully understand their biological significance. The following report summarizes two aspects of glycoscience and discusses their biological applications.

INDEX WORDS: Microwave-assisted synthesis, Peracetylation, Diazotransfer reagent, Glycoconjugate

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JONATHAN VALENTINE WOOTEN


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# DEDICATION

I dedicate this thesis to my family and my friends.

# ACKNOWLEDGEMENTS

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# 1    Comparison of Conventional and Microwave-assisted Synthesis of Glycosylated Amino Acids

## 1.1 Introduction

Microwave irradiation has been a known form of energy for years, but it was not until 1986 that microwaves were introduced into the scientific laboratory.[1] Microwave chemistry is essentially the science of applying microwave radiation to chemical reactions.[2] In comparison with other frequencies such as earth radiation, UV, and even infrared, microwave radiation is much lower. With the influence of microwave irradiation, reactions that would normally take many hours or days to complete can be run in a considerable shorter time of several minutes or even seconds. Microwave heating results in the superheating of substances—particularly those that respond to dipole rotation or ionic conductivity.[3] Microwaves will only cause bonds to rotate, not break. With microwaves, energy is transferred to the reaction components within the solution.[1] More specifically, thermally driven organic transformations can take place by conventional heating or microwave-accelerated heating, but in the microwave system, microwave energy is directed into a defined area leading to a rapid rise in temperature thus decreasing reaction time and increasing product yield and purity.[4] Reaction components at the center of the reaction are heated at the same rate the as reactants near the walls of the vessel.[1] In other words, with microwave heating, only the reaction vessels contents are heated rather than the vessel itself.

There are numerous advantages to microwave heating. These advantages include: rapid reactions, high purity of products, few by-products, improved yields, simplified and improved, synthetic procedure, wider temperature range, higher energy efficiency, sophisticated measurement and safety technology.[2-5] There are few disadvantages to microwave heating. One

disadvantage in particular to microwave heating lies in the potential for a microwave vial to burst during the superheating synthesis process. To elaborate, because microwave heating heats the contents of the vessel rather than the vessel itself, any solid that is stuck on the sides of the vessel and not flushed into the solution before microwave heating is applied has the potential to become too hot and crack the glass of the vessel; consequently the vessel will rupture under the pressure applied during the microwave heating.

Before the introduction of microwave assisted reactions, conventional heating was the only method used to heat a reaction. Conventional heating normally involves using a furnace or an oil bath to heat the walls of the reactor.[2] In this process, the core of the sample takes much longer to achieve the target temperature. With microwave heating, the target compounds are able to be heated without having to heat the entire furnace or oil bath, saving time and energy.[2]

Compared to conventional heating, microwave heating causes more extensive heating inside materials rather than the outer layers of materials while also utilizing the ability of some compounds to transform electromagnetic energy into heat.[6] With microwave heating, energy transmission is produced by dielectric losses while conventional heating uses conduction and convection processes. Furthermore, microwave irradiation is rapid and volumetric and quite dependent on the properties of the material whereas conventional heating is slow and superficial and less dependent on the properties of the material.[6]

Carbohydrates are large biological molecules consisting of carbon hydrogen and oxygen atoms and have a basic composition of $(CH_2O)n$. Carbohydrates come in many forms but all types of carbohydrates are essentially chains of very simple sugar molecules: glucose (Glc), galactose (Gal), mannose (Man), fucose (Fuc), xylose (Xly), N-Acetyl-D-glucosamine (glcNAc), N-Acetylgalactosamine (GalNAc), glucuronic acid (GlcA), and N-Acetylneuraminic acid

(NeuAc) (**Figure 1**). There are four distinctive types of carbohydrates: monosaccharides, disaccharides, oligosaccharides, and polysaccharides. Monosaccharides are sugars with multiple hydroxyl (OH) groups and are named based on the number of carbons they possess.[7] For example, a monosaccharide with three carbons is called a triose whereas a monosaccharide containing six carbons is called a hexose. Disaccharides are simply two monosaccharides that are covalently linked. Oligosaccharides consist of more than two but less than 10 monosaccharides that are covalently linked. Finally, polysaccharides (glycans) are polymers that consist of chains of monosaccharides (more than 10) and sometimes form glycoconugates with proteins or lipids.[7]

Glycosylation is a post-translational modification (PTM) that is primarily associated with the attachment of a sugar molecule to a protein. However, it is not subject to proteins alone. Glycosylation, in a more accurate sense, can be defined as the attachment of a sugar molecule to almost any organic compound/structure (i.e., peptides, lipids, amino acids, etc.). Glycosylated bonds are categorized into specific groups based on the nature of the bond and the sugar attached. There are five known types of glycosylation, N-linked, O-linked, glypiation, c-linked, and phosphoglycosylation.[8]

All nitrogen-linked carbohydrates are linked though N-acetylglucosamine (GlcNAc) and the amino acid asparagine.[9] N-linked glycoproteins are the most common type of glycosylation and often have large, extensively branched glycans. O-glycosylation occurs post-translationally on serine and threonine side chains in the Golgi apparatus. O-glycosylation differs from N-glycosylation in not only linkage but in mechanism. While a precursor glycan is transferred all together or at the same time (*en bloc*) to asparagine for N-glycosylation, sugars are added one at a time to serine or threonine residues for O-glycosylation.[8] Glypiation is a common post-

translational modification that involves the covalent attachment of glycosylphosphatidylinositol (GPI) that localizes proteins to cell membranes. C-glycosylation is commonly associated with PTMs of mannose and involves reactions that form carbon-carbon bonds rather than carbon-nitrogen or carbon-oxygen bonds. Phosphoglycosylatoin is a PTM limited to parasites and slime molds. It is characterized by the linking of glycans to serine or threonine via phosphodiester bonds.[8]

### 1.1.1  9 common sugars

**Figure 1 Nine Common Sugars**
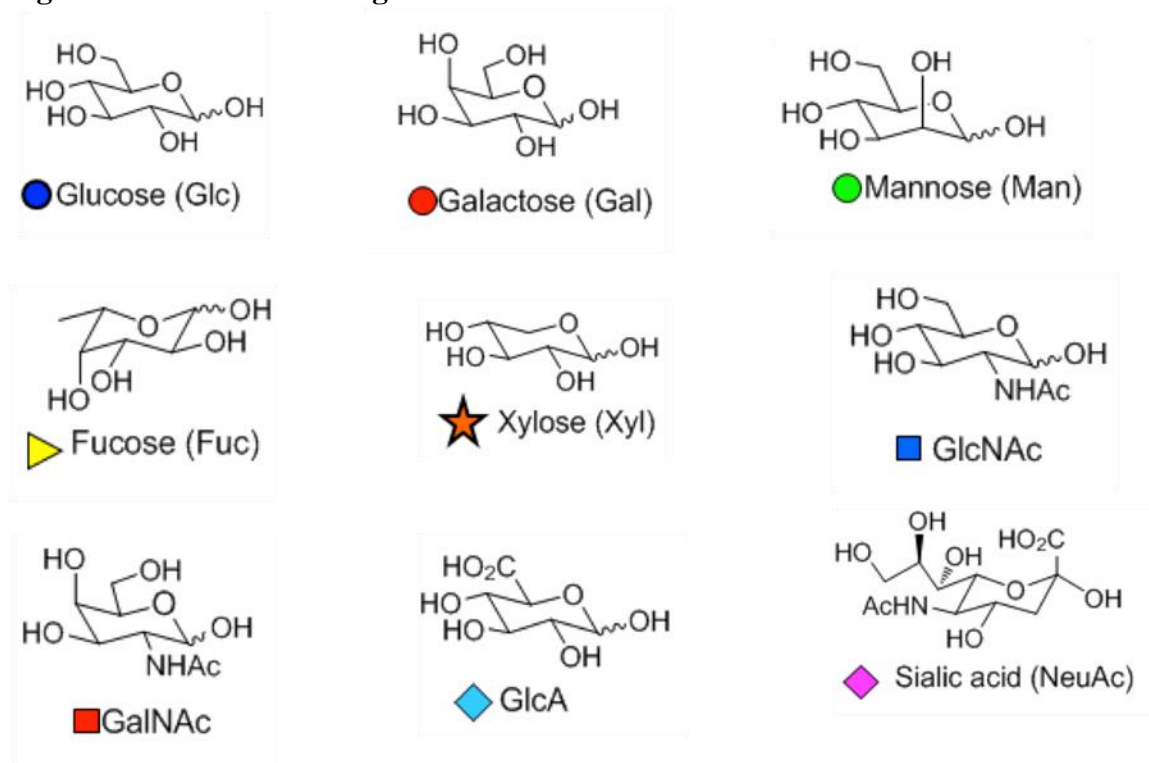


### 1.2 Purpose of the Study

Glycosylation is a crucial step in the modification of proteins or sphingolipids to form glycoproteins or glycosphingolipids. Approximately half of all proteins that are expressed in a

cell undergo glycosylation and involves the covalent addition of sugar moieties to specific amino acids.[8] Carbohydrates are important components of glycoproteins, having key roles in many biological processes such as cell adhesion, inflammation, the immune system, and tumor metastasis.[10-11] Therefore, there is great interest in synthesizing glycopeptides.

There are two approaches to synthesize glycopeptides: the convergent approach[12] and the building block approach.[13] The convergent approach is based on the glycosylation of a peptide in solution or on solid-phase. This direct O-glycosylation of a peptide approach often results in low yields and therefore, the building block approach is most commonly used where a glycosylated amino acid building block is easily introduced into a solid-phase peptide synthesizer.[14]

Previous approaches to glycosylated amino acids involve the use of glycosyl donors and amino acids that are protected on the α-carboxyl and α-amino groups. The Kihlberg group previously reported that Fmoc amino acids with unprotected carboxyl groups could be directly glycosylated with commercial peracetylated carbohydrates.[15] Unfortunately, these conventional methods suffered from low yields or long reaction times.

Microwave irradiation has been proven to dramatically improve the yields, purity, and conversion in synthetic chemistry.[16] The Seibel group recently reported on the glycosylation of Fmoc amino acids with protected carboxyl groups using microwave methodology.[17] Unfortunately these building blocks cannot be directly used for the solid-phase synthesis of glycopeptides. Therefore, this study employs the combined methodologies of the Kihlberg group's method of using Fmoc amino acids with unprotected carboxyl groups with the Seibel group's method of using microwave irradiation to efficiently synthesize amino acid building blocks.

A report by the Lam group demonstrates the validity that using microwave methodology is an efficient synthetic route for glycosylating Fmoc amino acid building blocks.[16] In their report, Fmoc-Ser, Thr, and Tyr-OH were glycosylated using a variety of sugar donors under the influence of a Lewis acid. The sugar donors included galactose, glucose, xylose, maltose, and lactose pentaacetates and had yields ranging from ~40-70% yields. Although there are several different types of sugar molecules one can use as a glycosylated amino acid building block, this study primarily focuses on the use of a mannose sugar donor for the preparation of glycosylated Fmoc amino acid building blocks.

In recent years, O-linked mannose (O-Man) glycans have been demonstrated to play critical roles in cellular interaction-based pathologies, including congenital muscular dystrophies (CMDs) [18-21] and cancers.[22-24] In particular, defects in the biosynthesis of O-Man glycans often result in the hypoglycosylation of α-dystroglycan (α-DG), the most well characterized O-mannosylated mammalian protein. O-Man glycans account for up to 30% of all glycans O-linked to proteins in mammalian brain tissue.[25,26] It is therefore understandable that O-Man glycans have been shown to be essential for normal nervous system development that is dependent on neuron migration,[27,28] and axon path finding,[29] and to play a role in remyelination following myelin sheath damage.[30] O-Man glycans are initiated by covalent linkage of mannose to the hydroxyl oxygen of a serine or threonine amino acid residue. O-Man may then be extended by the addition of other monosaccharides and functional groups to form a variety of glycan structures.

Based on the aforementioned information, it is clear as to the importance of the applications of O-mannose glycosylated Fmoc amino acids. Herein, its synthetic approach using microwave methodology and conventional methods are reported.

## 1.3 Expected Results

One of the keys to successful preparation of glycopeptides is the acquirement of well-defined and suitable quantities of glycosylated Fmoc amino acid building blocks. Given the advantages microwave methodology has over conventional methods—rapid reactions, high purity of products, few by-products, improved yields, etc.—it is expected that the microwave-assisted glycosylation of serine and threonine will provide greater results (higher purity and greater yields) than the conventional method. The microwave methodology was to be tested first followed by the conventional method. A synthetic plan was drawn for the microwave-assisted glycosylation of serine (**Figure 2**) and threonine (**Figure 3**) and for the conventional glycosylation of serine (**Figure 4**) and theronine (**Figure 5**).

### *1.3.1   Scheme of O-Mannose attached Fmoc-serine (microwave)*

**Figure 2 Fmoc-Serine (Microwave)**
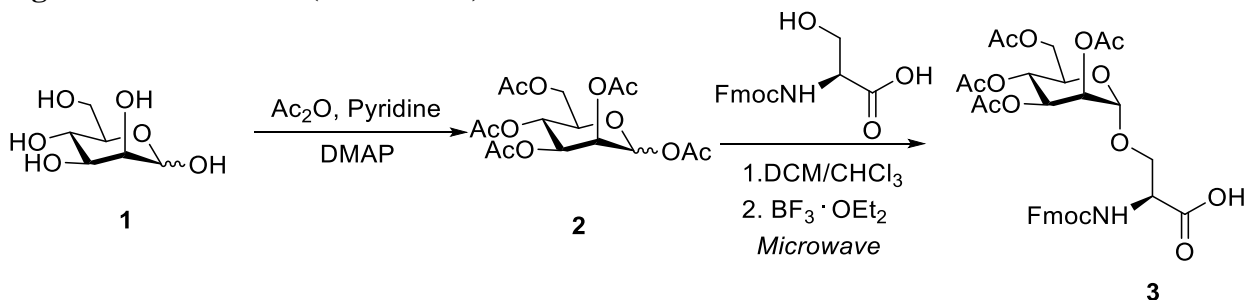


### *1.3.3   Scheme O-Mannose attached Fmoc-Threonine (microwave)*

**Figure 3 Fmoc-Threonine (Microwave)**
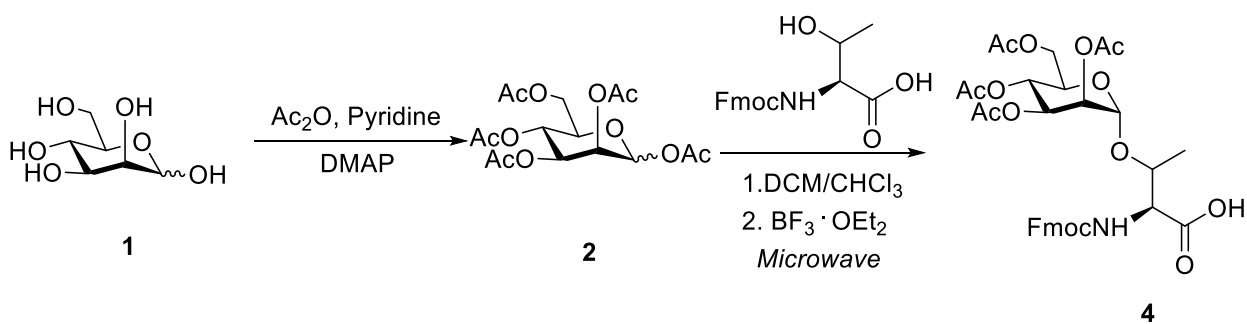
### 1.3.4 Scheme of O-Mannose attached Fmoc-serine (conventional)

**Figure 4  Fmoc-serine (conventional)**
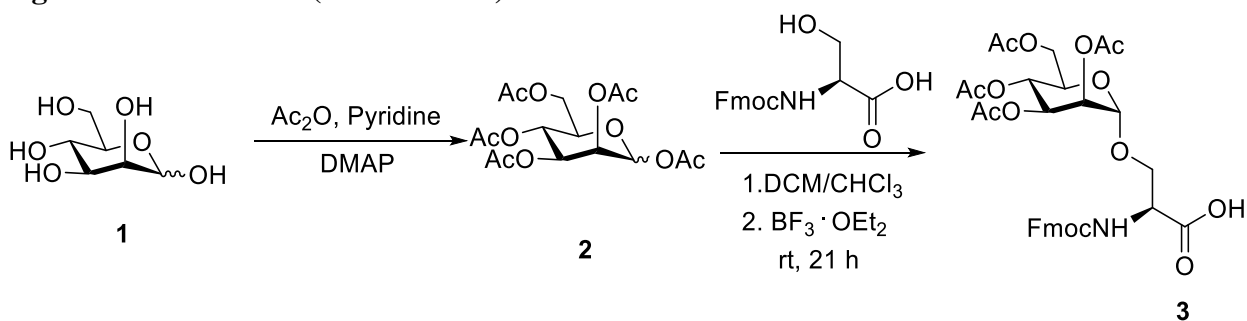


### 1.3.5 Scheme of O-Mannose attached Fmoc-Threonine (conventional)

**Figure 5 Fmoc-Threonine (conventional)**
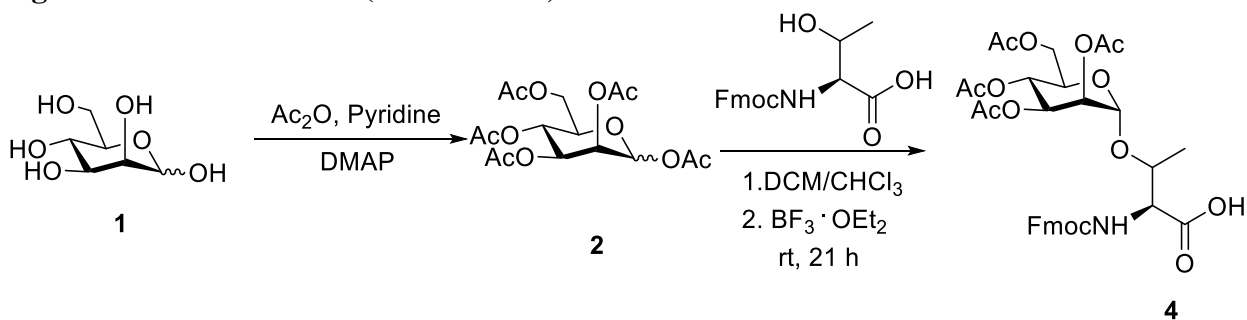
## 1.4   EXPERIMENT

### 1.4.1   *Microwave-assisted synthesis of Glycosylated Serine and Theronine.*

After the plan was drawn for the microwave-assisted synthesis of serine, the protected mannose sugar needed to be synthesized. Mannose's unique individuality compared to the other sugars lies in its structure. As illustrated in **Figure 1**, the second position hydroxyl group is in an axial conformation. All other sugars have the second position hydroxyl group in an equatorial conformation. Mannose is therefore unique in that all reactions predominately produce alpha-configuration products. However due to its neighboring participation effect, mannose needs to be protected in order to prevent side products from forming. One of the most commonly used techniques involves the acetylation of the entire molecule (peracetylation). According to the mechanism illustrated in **Figure 6**, commercially available mannose **1** is protected using acetic anhydride in pyridine in the presence of a catalyst. The overnight reaction in room temperature affords the protected mannose **2** in approximately 95% yield. Dichloromethane is introduced to **2**, dissolving the sugar. Portions of the previous solution are introduced into microwave vials and added Fmoc-serine and Boron trifluoride diethyl etherate (BF$_3$ Et$_2$O). The sealed vial was heated to a variety of degrees over a variety of minutes—to find the optimal reaction conditions—in a microwave reactor to give the desired product in a low yield of less than 30%.

The synthetic steps for the microwave-assisted synthesis of glycosylated threonine are the same for serine except that the amino acid Fmoc-theronine was substituted for Fmoc- serine during the addition to the microwave vial. The results were similar however with low yields of less than 30%.

### 1.4.2   *Mechanism of Fmoc-serine synthesis*

**Figure 6 Mechanism of Fmoc-serine Synthesis**
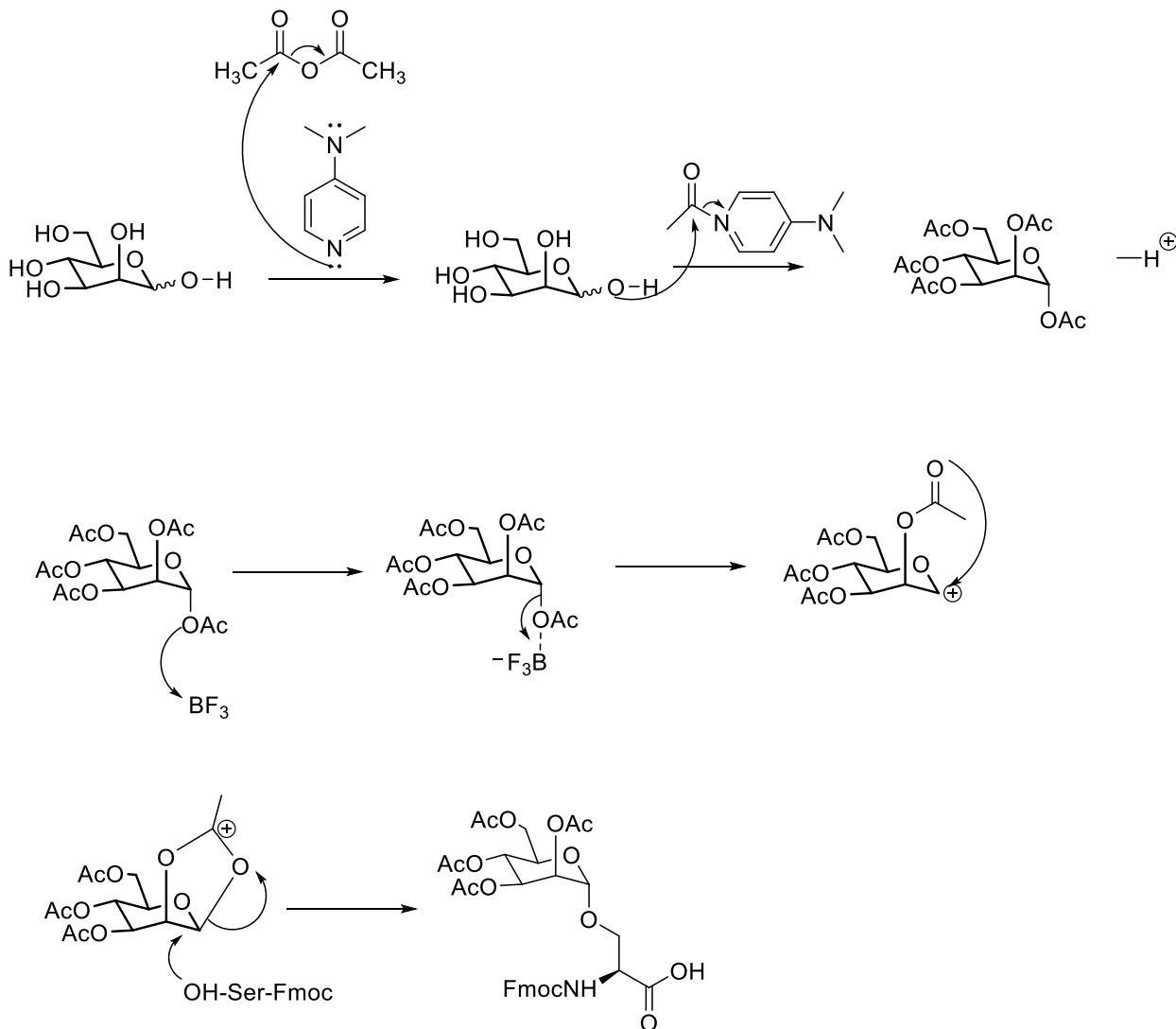
### 1.4.3  Conventional synthesis of Glycosylated Serine and Threonine

Conventional synthesis of glycosylated serine and threonine, much like the microwave-assisted synthesis, begins with the peracetylation of mannose. The peracetylated mannose was dissolved in DCM and added Fmoc-serine and $BF_3$ $Et_2O$. This mixture was allowed to stir at room temperature for 21 hours yielding the desired product at an improved 60% yield.

## 1.5 RESULTS

Utilizing identical methods the Lam group previously reported, the glycosylated Fmoc amino acid serine was successfully synthesized. Unfortunately, however, the yields reported were far from satisfactory. During the microwave-assisted glycosylation, a variety of temperatures and reaction times were used (**Table 1**). Thin Layer Chromatography (TLC) was performed after each reaction. TLC analysis showed similar results with each trial yielding an average of 28% of the desired product. The first trial had the highest yield of 30%. The resulting glycosylated amino acids (conventional and microwave) were analyzed and characterized using NMR and mass spectrometry for their purity and confirmation of structure (**Figures 7-11**). Furthermore, the conventional synthesis NMR spectrums appear more defined and pure compared to the microwave-assisted synthesis NMR spectrums.

### *1.5.1 Proton NMR Glycosylated Serine-microwave*

**Figure 7 Proton NMR Glycosylated Serine-microwave**
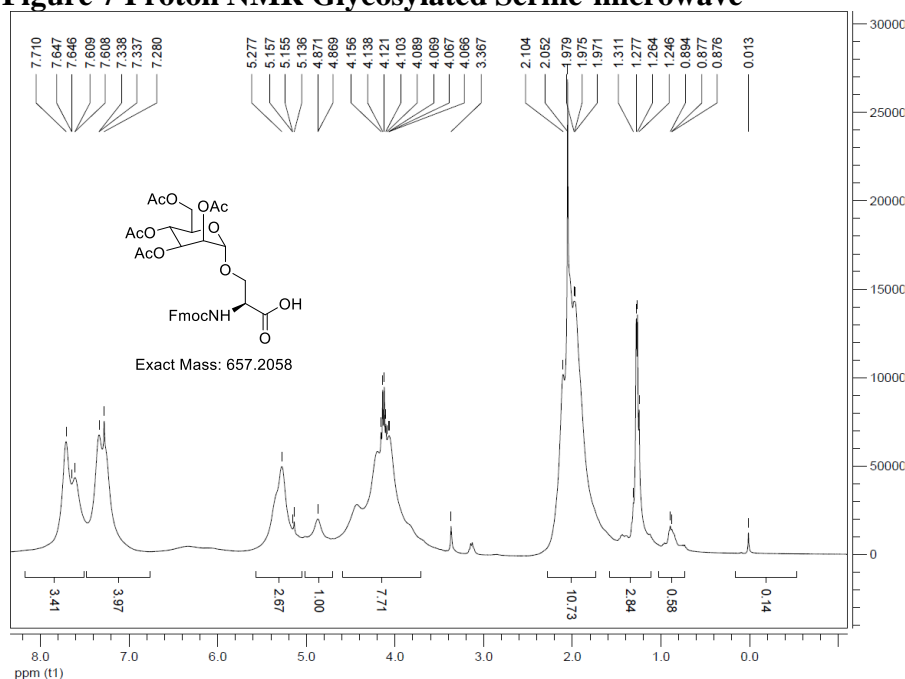
## 1.5.2 Carbon NMR Glycosylated Serine-microwave

**Figure 8 Carbon NMR Glycosylated Serine-microwave**
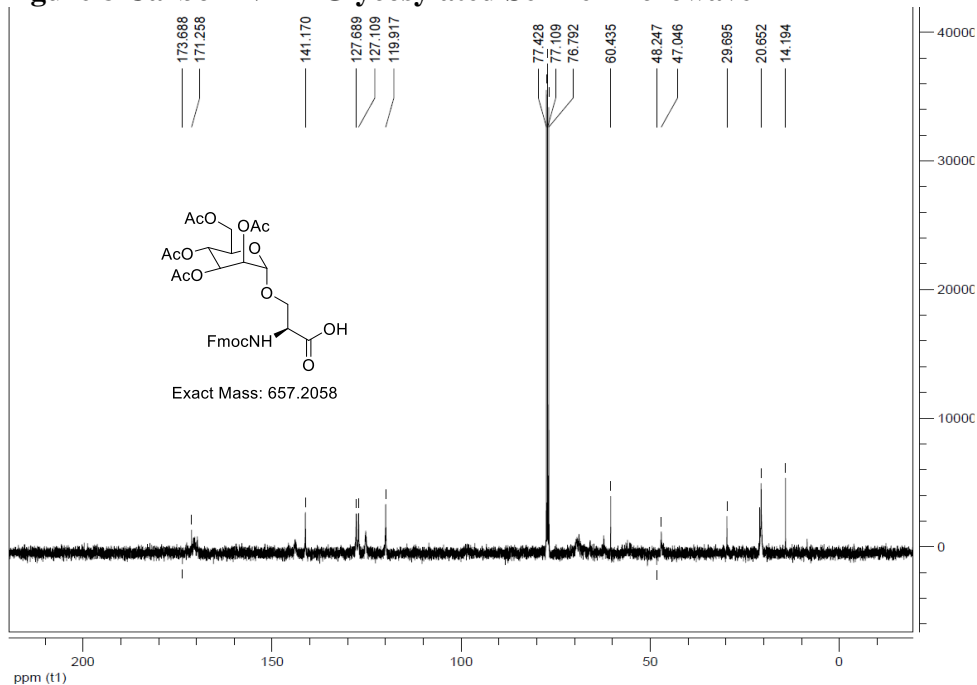


## 1.5.3 Mass Spectrometry (ESI) Glycosylated Serine-microwave

**Figure 9 Mass Spectrometry (ESI) Glycosylated Serine-microwave**
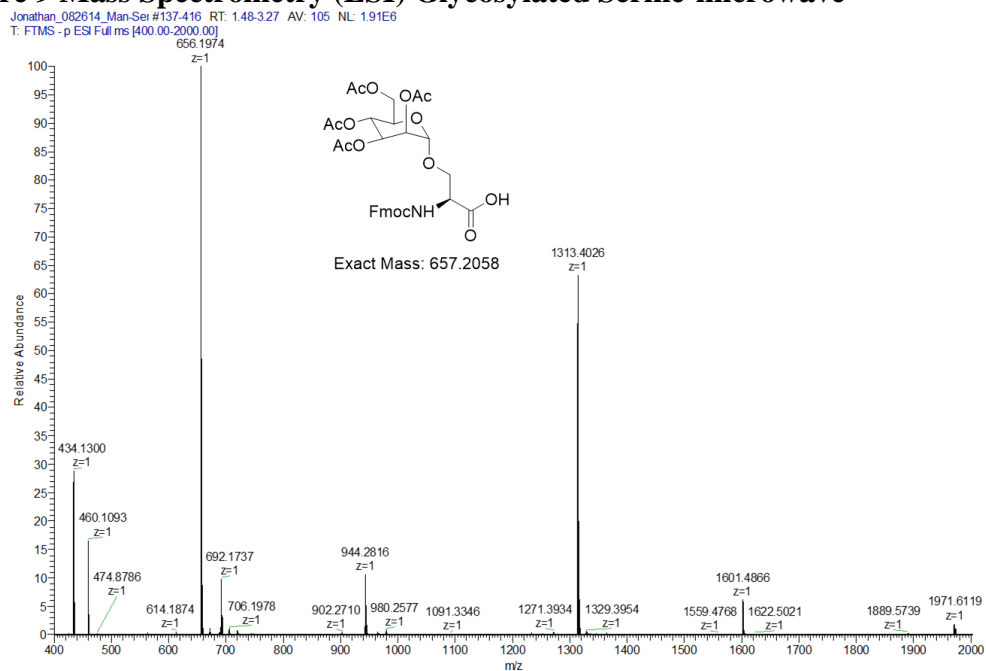
## 1.5.4 *Proton NMR Glycosylated Serine-conventional*

**Figure 10 Proton NMR Gyycosylated Serine-conventional**
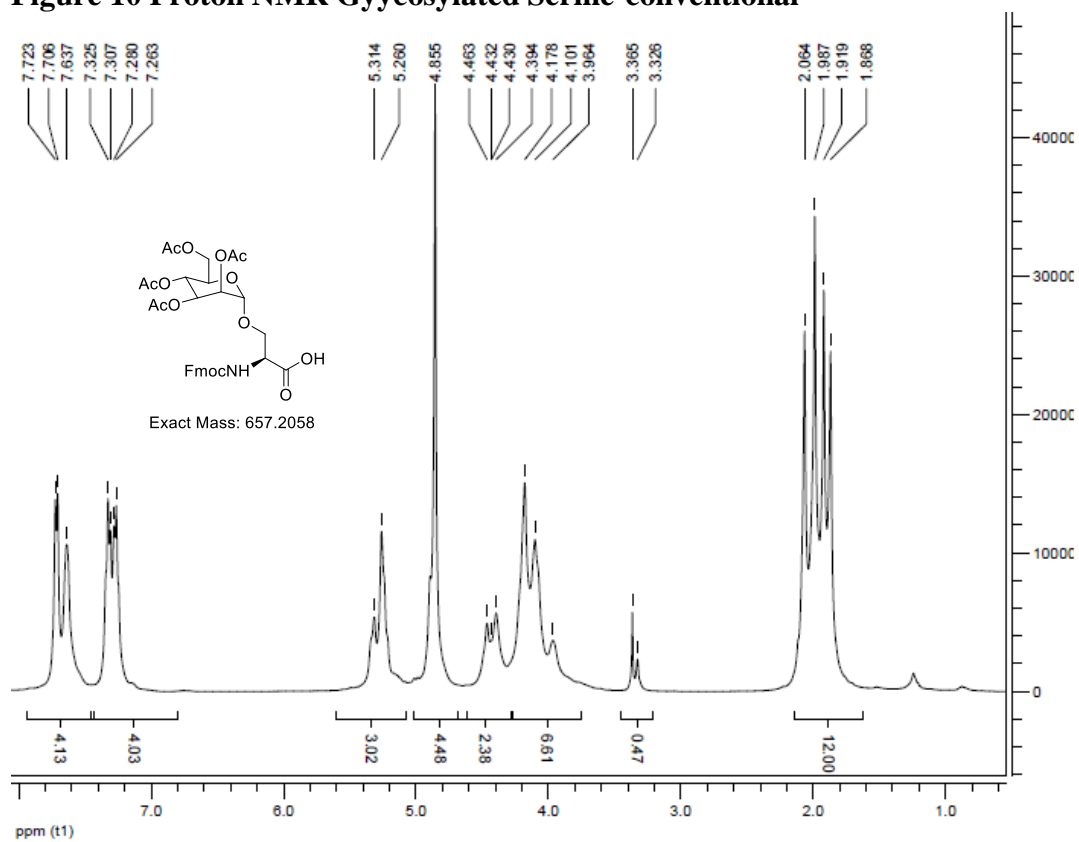


## 1.5.5 *Carbon NMR Glycosylated Serine-conventional*

**Figure 11 Carbon NMR Glycosylated Serine-conventional**

AcO OAc
AcO
AcO
FmocNH OH
O

Exact Mass: 657.2058
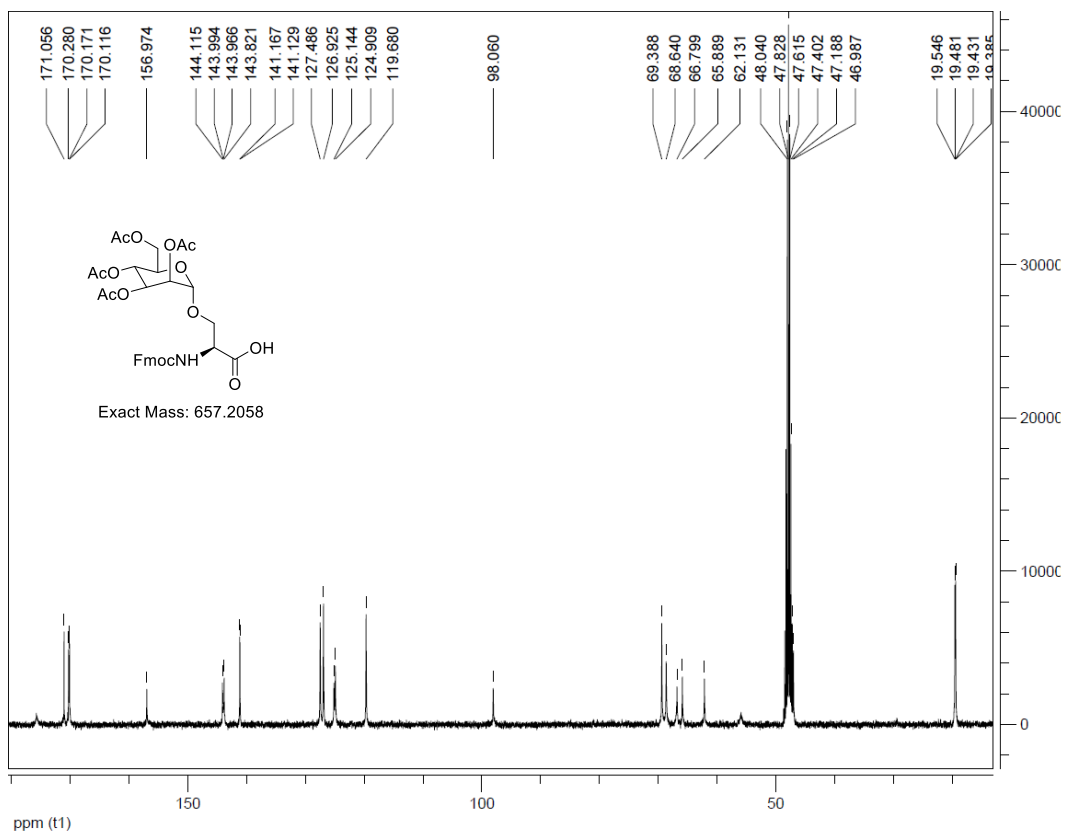
**Table 1Microwave Reaction Conditions**

*1.5.6   Table.  Microwave Reaction conditions*

| Reaction | Time | Temperature | Yield |
|---|---|---|---|
| 1 | 5 mins | 100°C | 30% |
| 2 | 10 mins | 100°C | 29% |
| 3 | 5 mins | 90°C | No reaction |
| 4 | 10 mins | 90°C | No reaction |

| | | | |
|---|---|---|---|
| 5 | 5 mins | 120 $^{\circ}$C | 28% |
| 6 | 10 mins | 120 $^{\circ}$C | 28% |

## 1.6 DISCUSSION

With microwaves, superheating is performed in closed vessels under high pressure and reactions that may have taken several hours under conventional conditions can be completed within minutes with microwaves. High-pressure chemistry should only be conducted in special reactors with a microwave oven that is specifically designed for this purpose. Without microwave irradiation, many chemists could only obtain by-products. The Shimizu group could not have obtained trisaccharide at any temperature without microwave irradiation; however, at low temperatures with microwave irradiation, their hydroxyl groups reacted and gave the Lewis X derivative (**Figure 10**) they desired.[31] In a study by the Bornaghi group, remarkable acceleration of the glyocosylation reactions (minutes compared to hours) over conventional reflux heating was observed with good yields and production of the α-glycoside as the dominant product.[4]

Although the Lam group's previous report claimed to have improved yields when using microwave methodology to glycosylate Fmoc amino acids using several sugar donors, this study does not support the notion that microwave methodology is a more efficient means of synthesizing glycosylated Fmoc amino acids using mannose as the sugar donor. Looking back at **Figure 6**, it is possible to hypothesize why using mannose, as a sugar donor was unsuccessful. The introduction of the Lewis acid allows for a neighboring group participation effect that forms a "barrier" that prevents any attack from above from the Fmoc amino acid to form beta-products.

However, it is hypothesized that the use of the microwave reactor—due to the high temperatures and fast reaction time—somehow skips the protective "barrier" step and ultimately allows for more attacks from above, provides a greater mix of alpha- and beta-products.

## 1.7   CONCLUSION

In this study, glycosylated Fmoc amino acid serine was successfully synthesized using microwave methodology and conventional methods. Microwave methodology is a new technique that utilizes superheating of substances within a sealed vial.  Although it has been proven to increase purer and greater yields than conventional methods in many other studies, in this study, microwave methodology was proven not to be a universal technique for improving yield. The microwave-assisted synthesis of glycosylated amino acids resulted in a poor yield less than 30% while conventional methods yielded a moderate yield of approximately 60%.

## 2 Efficient synthesis of glycosphingolipids using N-phenyltrifluoroacetimidates as a leaving group

### 2.1 INTRODUCTION

Sphingolipids are a class of lipids that contain a backbone of sphingoid bases. There are many different types and derivatives of sphingolipids but they all share a common sphingoid base. The sphingosine backbone was named by J. L. W. Thudichum in 1884 for its enigmatic, "Sphinx-like" properties.[32] The term "sphingolipid" was later introduced by Herbert Carter—an American biochemist and educator—and colleagues in 1947.[33] Sphingoid bases are long-chain aliphatic compounds typically possessing a 2-amino-1,3-diol functionality.[34]  The long-chain sphingoid bases are defined by their foundation of 18 carbon atoms.

The biosynthesis of sphingolipids occurs in the endoplasmic reticulum (ER) (**Figure 12**). Ceramides are considered to be the core of sphingolipid metabolism. Generally, the *de novo* pathway of its synthesis begins with the condensation of the amino acid serine and palmitoyl-CoA catalyzed by the enzyme palmitoyl transferase to generate 3-keto-dihydrosphingosine (KDS).[35] KDS is then reduced to form dihydrosphingosine (DHS) which is N-acylated to form dihydroceramide. A double bond is introduced to the DHS base that converts dihydroceramide into ceramide. The newly synthesized ceramide is then transported to the Golgi apparatus where it is converted to sphingomyelin (SM) and glucosylceramide (GluCer).[35] Sphingomyelin is an ever-present component of animal cell membranes, comprising about 50% of the lipids in certain tissues and makes up about 10% of the lipids of the brain.[36] Sphingomyelin is therefore the most abundant sphingolipid. Glucosylceramide belongs to a class of sphingolipids called glycosphingolipids that display variations in their carbohydrate head groups and are organized in

signaling domains on the cell surface.[37] Ceramides can also be synthesized and deacylated to give sphingosine through the recycling of higher-order sphingolipids in the plasma membrane.[35]

### 2.1.1 Figure 12: Biosynthesis of a sphingolipid

**Figure 12 Biosynthesis of a sphingolipid**
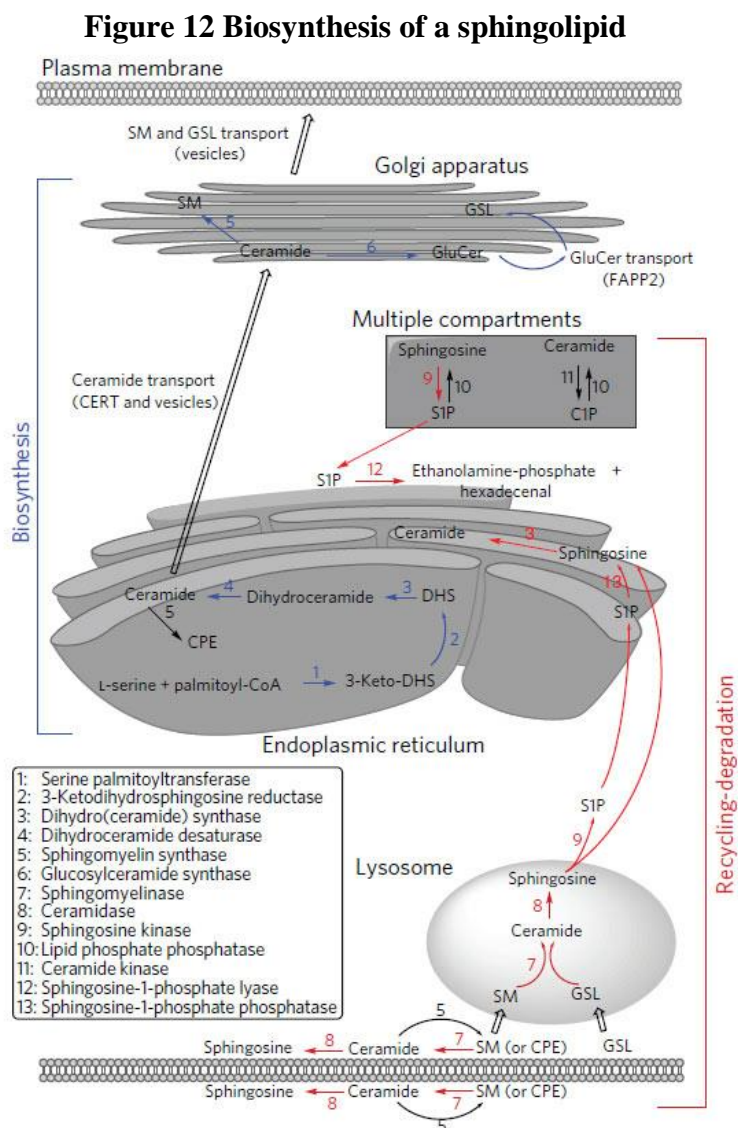


Sphingolipids are important structural and functional components of essentially all eukaryotic cells. They have critical roles in many physiological processes including cell recognition, adhesion, and signaling.[33] Ceramides—a class of sphingoid bases containing an N-

acetyl group—along with the sphingolipid derivative sphingosine-1-phosphate (S1P), have also been implicated as modulators of autophagy, angiogenesis, intracellular trafficking, stress, and inflammatory responses.[38] Additionally, ceramide and S1P often exert opposing functions in the cell. For example, ceramide has been shown to mediate cell cycle arrest and apoptosis and S1P has been shown to advance cell survival and proliferation.[33,38] Current researchers' understanding of the sphingoid base signaling pathways(s) of exactly which molecules affect which targets is in the early stage. A more thorough understanding of these pathways will provide important insight into how sphingolipids are involved in cell regulation, and improvements in targeting effective pathways for therapeutics and chemoprevention.[39]

Glycosphingolipids (GSLs) are a subclass of glycolipids composed of a long chain amino alcohol, known as a sphingoid base, a fatty acid residue linked to its amino group; together, the resulting amide is called a ceramide.[40] A carbohydrate chain is attached to the primary hydroxy group of the ceramide **Figure 13**.

*Figure 13: structure of glycosphingolipid*

**Figure 13 Structure of Glycosphingolipid**
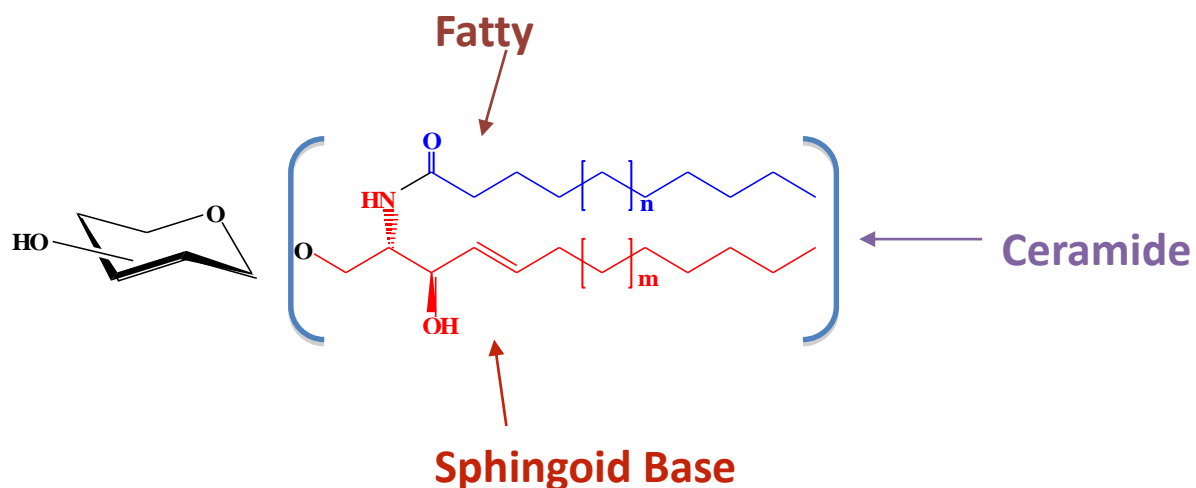
Glycosphingolipids are found in the plasma membrane of all cells in "higher" animals and comprise from less than 5% to more than 20% of the membrane lipids.[41] GSLs have been known for many years to function in animal cells as antigens, receptors for microbial toxins, and many other biological functions including mediators of cell adhesion and modulators of signal transduction.[42] Additionally, specific events are associated with specific glycosphingolipids. For example, β-galactosyl ceramide (β-GalCer) is known to act as a ligand for the HIV-1 viral glycoprotein gp120,[43] mediating viral entry into epithelial cells. Glycosphingoipid storage diseases are rare diseases that lead to the accumulation of glycosphingolipids in lysosomes. They are generally a result from mutations in glycosidases and from mutations in activator proteins. The most common glycosphingolipid storage disease, the Gaucher's disease, is caused by mutations in the enzyme β-glucocerebrosidase that results in the accumulation of GlcCer—a glycosphingolipid—in the liver, spleen, and other tissues.[41]

Several factors contribute to the dilemma sourrounding glycosphingolipids and their importance to research. Firstly, they are complex in structure. Their structural variety and complex patterns present challenges for their elucidation and quantification by mass spectrometric techniques.[44] Second, because they are complex in structure, they are rather difficult and expensive to extract from natural sources. Furthermore, GSLs extracted from natural sources are limited in quantity and quality; glycosphingolipids extracted from natural sources are impure. As a solution, scientists have turned to chemical synthesis for the acquirement of well-defined and pure compounds.

## 2.2 Purpose of Study

The retrosynthetic analysis of glycosphingolipids requires three parts: a sphingoid base, a fatty acid, and a carbohydrate donor (**Figure 14**).

**Figure 14 Retrosynthetic Analysis**
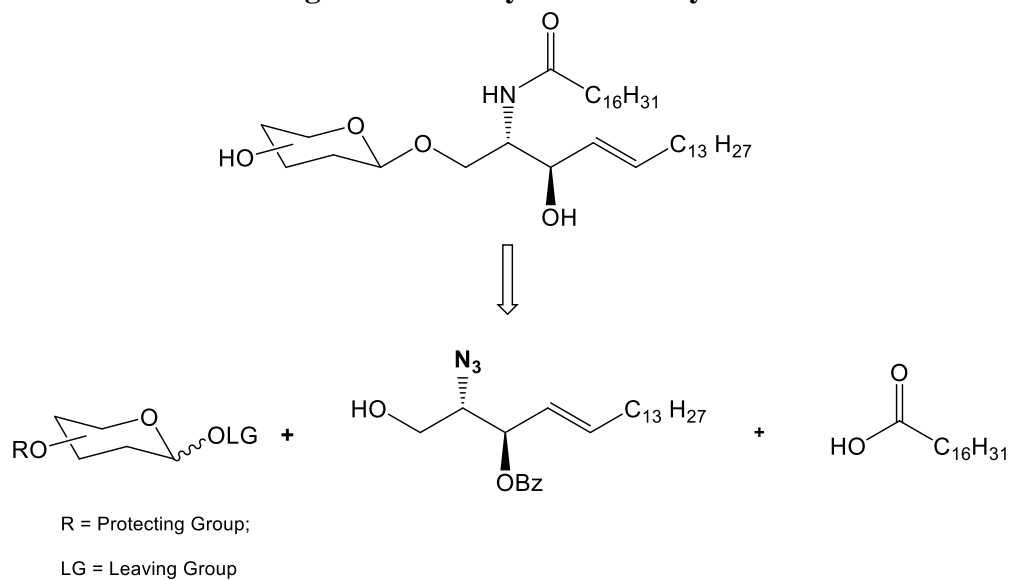


*Figure 14: Retrosynthetic analysis*

The key component of the glycosphingolipid is the sugar donor. There are numerous glycosylation methods that involve different glycosyl donors. **Figure 15** contains a list of sugar donors commonly used in carbohydrate synthesis. Unfortunately, these donors are not ideal, as they have been reported to produce by-products, to be easily decomposed, to produce poor yields, or to have slow reaction times.

**Figure 15  Common Sugar Donors**
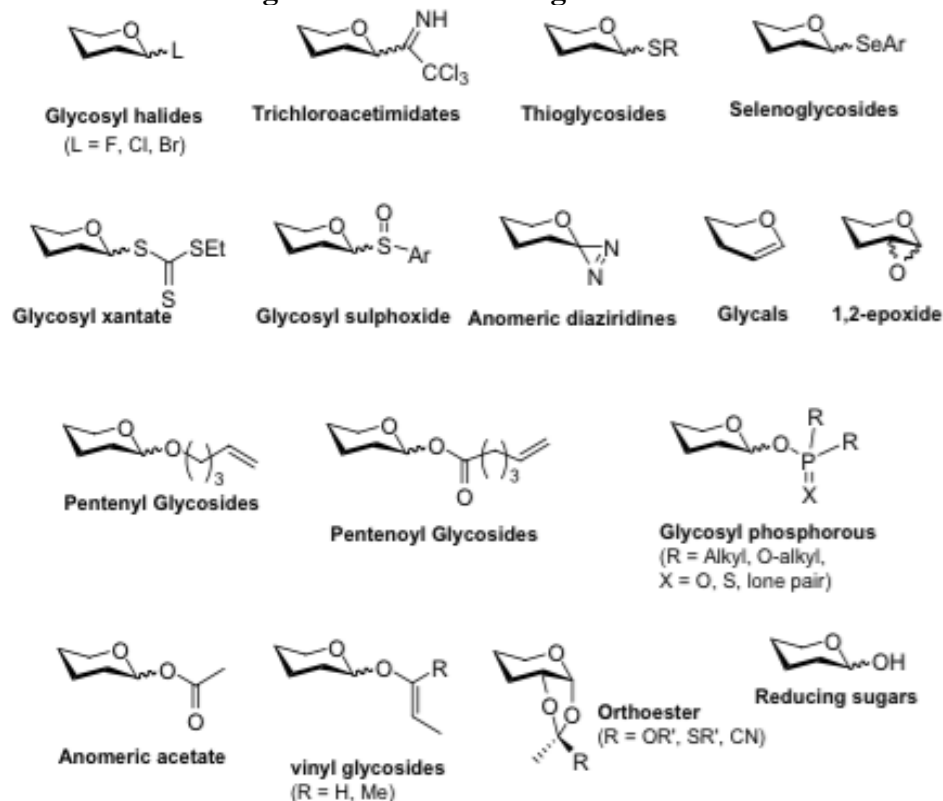


*Figure 15: Common sugar donors*

In order to efficiently synthesize a glycosphingolipid, an alternate glycosyl donor is needed. A previous study by the Liu group has reported on a donor that is able to effectively synthesize a glycosphingolipid.[45] The proposed glycosyl donor used in this study is a benzoylated lactopyranosyl trichloroacetimidate (**Figure 16**) that utilizes an N-phenyltrifluoroacetimidate as a leaving group. Glycosyl trifluoroacetimidates' accessibility, stability, and activity have been proven to be comparable with those of the corresponding glycosyl trichloroacetimidate donors.[46] In this study, the glycosyl trifluoroacetimidate is readily prepared and is used as a glycosyl donor. Herein, the sugar donor synthesis, the sphingolipid synthesis, and its glycosylation are reported.

**Figure 16  Benzoylated lactospyranosyl trichloroacetimidate**
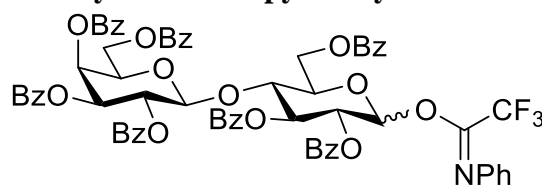


*Figure 16: Benzoylated lactospyranosyl trichloroacetimidate*

## 2.3 Expected Results

It is expected that through the addition of a diazotransfer reagent, the target sphingolipid (azidosphingolipid) will be synthesized followed by its glycosylation to the benzoylated lactopyranosyl trichloroacetimidate (N-phenyltrifluoroacetimidate) sugar donor, to effectively synthesize the desired glycosphingolipid.

## 2.4 Experiment

### 2.4.1  *Synthesis of azidosphingosine*

One of the keys to successful preparation of sphingolipids is the acquirement of appropriate sphingoid bases (**Figure 17**). The strategy to achieve the desired product is based on the use of a cyclic sulfate for the regio- and stereoselective transformation of the 4-position hydroxyl group of phytosphingosine into the characteristic 4,5-trans double bond of sphingosine. The advantage of this strategy is the elimination of a need for selective activation of only one vicinal hydroxyl group. Synthesis begins with the conversion of an amine from inexpensive and commercially available phytosphingosine to an azide through the addition of trifluoromethanesulfonyl azide ($TfN_3$) with potassium carbonate ($K_2CO_3$). This step is also considered to be a protection reaction of the amino function and primary hydroxyl group of phytosphingosine. The next step is to selectively protect its silyl ether. This is carried out through

the addition of tert-butyl(chloro)diphenylsilane (TBDPSCl) in the presence of a catalyst and

triethylamine (Et$_3$N). Then the 3,4-vicinal diol is converted though its cyclic sulfite with thionyl

chloride (SOCl2) in the presence of Et$_3$N. The reaction is then followed by oxidation with

ruthenium chloride (RuCl$_3$) and sodium periodate (NaIO$_4$) to provide the cyclic sulfate. The

cyclic sulfate is then treated with tetra-n-butylammonium iodide (Bu$_4$NI) in tetrahydrofuran

(THF) which opens the ring. 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU) is added and the

reaction temperature is raised to reflux. This treatment is followed by acidic hydrolysis which

successfully yields the desired product. The final step involves the removal of the silyl group by

treatment with tetra-n-butylammonium fluoride (TBAF) in THF.

### 2.4.2   *Figure 17: Synthesis of azidosphingosine*

**Figure 17 Synthesis of azidosphingosine**
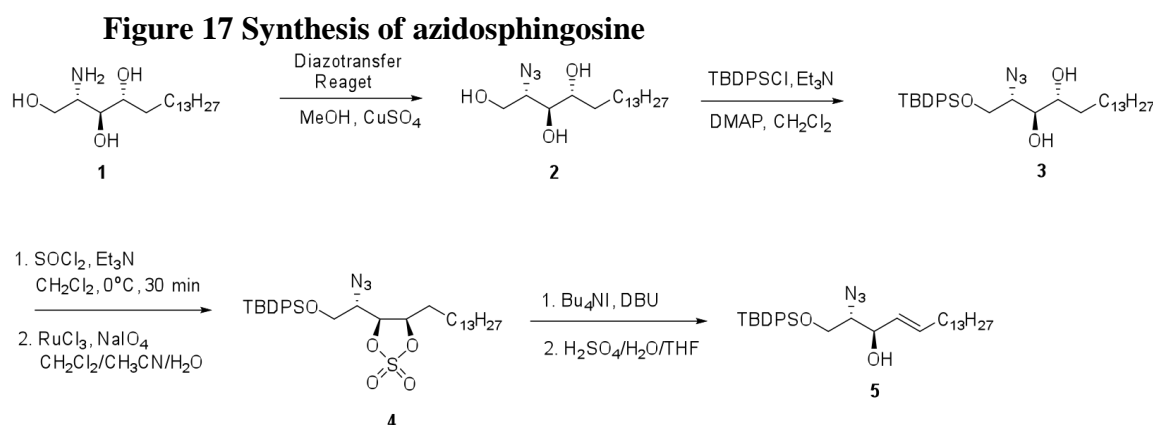


### 2.4.3   *Synthesis of Diazotransfer reagent 1—Trifluoromethanesulfonyl azide (TfN$_3$)*

Perhaps the most essential step in the sphingolipid synthesis lies in the addition

diazotransfer reagent. This reagent allows for the conversion of an amine to an azide. TfN$_3$ is not

commercial available and thus needs to be synthesized. Fortunately the preparation is fairly

simple. Sodium azide (NaN$_3$) in water is cooled to 0 °C and treated with dichloromethane (DCM)

and the resulting biphasic solution is treated with Trifluoromethanesulfonic anhydride (Tf$_2$O). The solution is then extracted with DCM. Tf$_2$O is a dangerous reagent in that it is highly explosive and needs to be kept in solution.

### 2.4.4   Synthesis of Diazotrasnfer reagent 2—Imidazole-1-sulfonyl Azide

Because of the explosive potential of TfN$_3$,a new diazotransfer reagent was sought out and synthesized—one that is significantly less dangerous but still as effective to use.  Imidazole-1-sulfonyl Azide is such a reagent. Synthesis begins with the addition of sulfuryl chloride to imidazole in DCM. After the reaction is completed it is filtered, concentrated, and purified through recrystallization. The resulting solid in DCM is added methyl triflate. After the reaction completes it is filered and dried to give a triflate salt. The salt is then dissolved and added NaN$_3$. When the reaction completes, the organic phase is extracted and can be used directly in the azide conversion reaction.

### 2.4.5   Synthesis of a Glycosphingolipid

The glycosylation of azidosphingosine is illustrated in **Figure 18-(3)**. The secondary hydroxyl group of azidosphingosine is protected using benzoyl chloride (BzCl); after which, the primary hydroxyl group is deprotected. This is because the glycosylation with occur on the primary hydroxyl group. Lactose is then perbenzoylated followed by the selective deprotection of the primary benzoyl group. The newly synthesized sugar **10** reacts with 2,2,2-Trifluoro-N-phenylacetimidate to yield compound **11**, which is then combined with the sphingoid base **7** to afford the protected glycosphingosine **12**. The sugar is then to be deprotected yielding the desired product **13**.

### 2.4.6 Figure 18: Final synthesis of azidosphingosine (1), preparation of the glycosyl donor (2). Glycosylation of azidosphingosine (3)

**Figure 18 Final synthesis and Glycosylation of azidosphingosine**
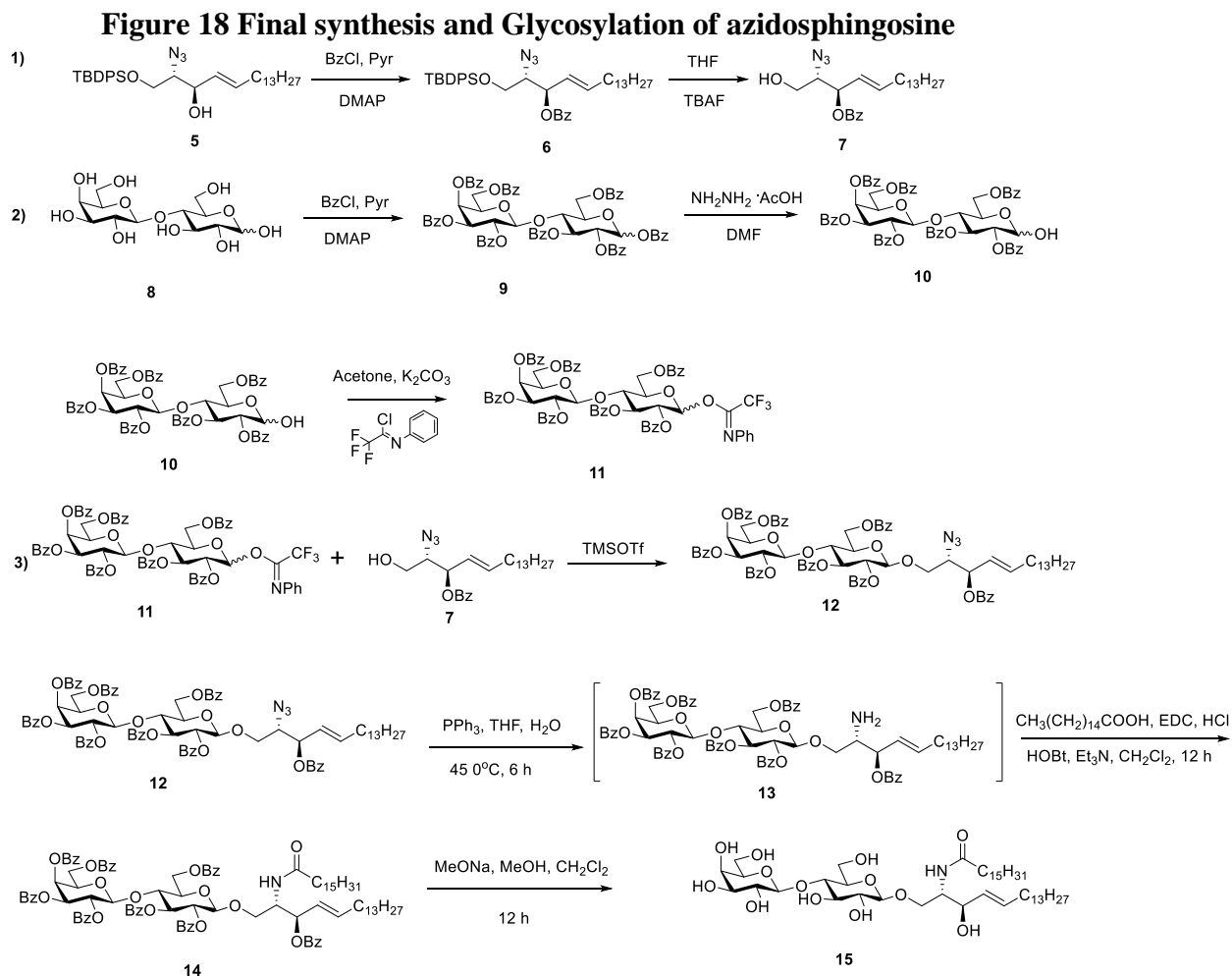


## 2.5 Results

The synthesis of azidosphingosine proved to be somewhat of a challenging experience. Several small-scale reactions were attempted in its synthesis. The majority of the reactions stemmed from the TfN$_3$ route. The Imidazole-1-sulfonyl Azide route proved to be a safer alternative, however, it was more time consuming and was much more depended on the

homogeneous mixture of ethyl acetate and water to successfully convert the amine to an azide. Therefore the TfN$_3$ route was the preferred route to synthesize the azidosphingosine.

Once appropriate amounts of azidosphingosine were synthesized, preparation of the sugar donor was successfully achieved at 99% yield. The glycosylation of the azidosphingolipid that followed resulted in 80% of the desired product proving that the proposed donor is an effective donor for glycosylation. Characterization of the glycosphingolipid was carried out through NMR analysis.


## 2.6 DISCUSSION

Each step was analyzed through NMR to confirm the existence of the desired product.

In order to achieve the final compound **11** shown in **Figure 18-(3)**, sufficient amounts of the starting material **7** needed to be acquired. However, this was a difficult task to accomplish due to the first and last reactions. The first reaction is the conversion of an amine from phytosphingosine to an azide through the addition of a diazotransfer reagent. This is quite possibly the most important step in the entire reaction. Without the appropriate amount or the appropriate concentration of the diazotrasnfer reagent, the resulting azide will not form. As stated in the experimental section, there were two individual diazotransfer reagents I have attempted to use for this step. The first is TfN$_3$ (trifluoromethanesulfonyl azide) and the other is an imidazole azide. Both are effective as diazotransfer reagents but they each differ significantly in the amount of danger they each pose.

Azides are not the safest reagents to use. TfN$_3$ is especially dangerous because it is explosive when condensed or gets too dry, however, I was still able to successfully synthesize it on a small-scale. **Figure 19** illustrates the comparison of the two diazotransfer reagents used. As

you can see, TfN₃'s synthesis is extremely simple. Triflouromethanesulfonyl anhydride reacts with sodium azide in DCM to yield the desired product. However, keep in mind that the drawback is that it is pretty explosive when condensed.

### 2.6.1   *Figure 19: Comparison of TfN₃ and Imidazole-1-sulfonyl Azide*

**Figure 19 Comparison of TfN3 and Imidazole-1-sulfonyl Azide**
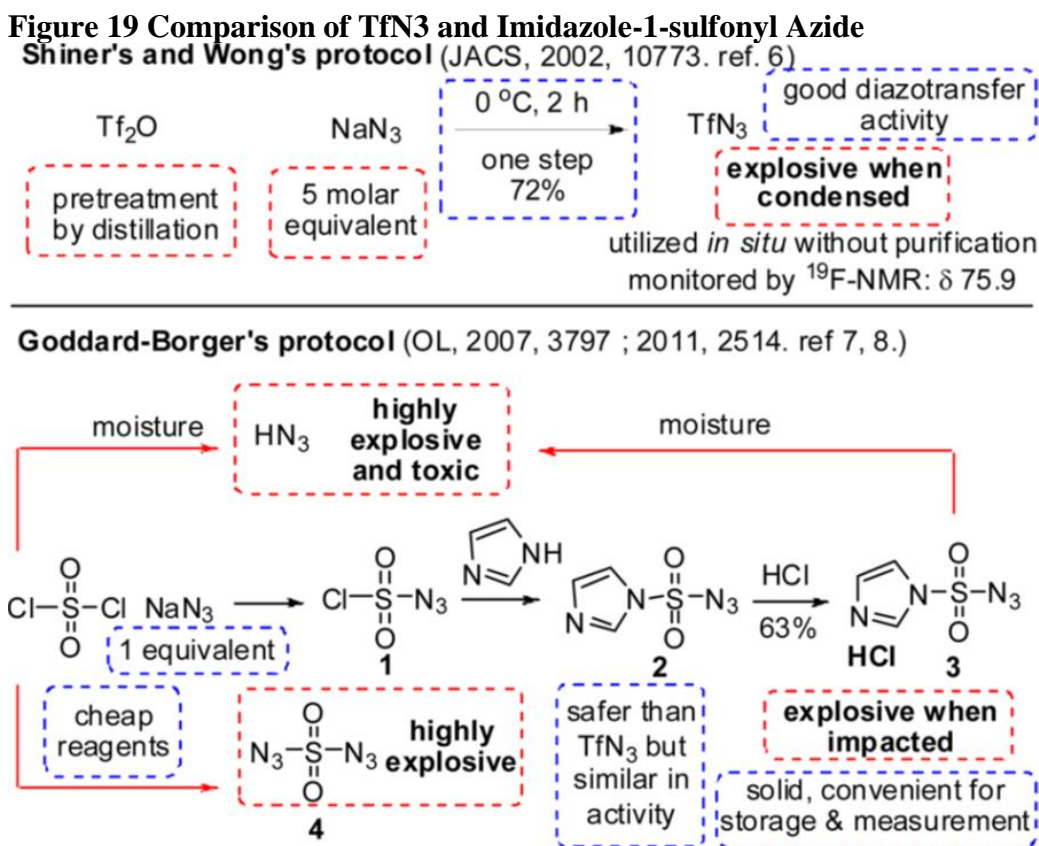


The small-scale synthesis continued without issue but afforded little product in the end to continue to the next phase of the experiment. For the second attempt at synthesizing more starting material, I was primarily concerned with a safer alternative to using a diazotransfer reagent and that's when I was led to the imidazole-1-sulfonyl azide. **Figure 4** is an old diagram and has long since been updated and improved to the effect that there is no highly explosive and

toxic by-products; however, it is the general scheme for the synthesis of the diazotransfer reagent.

**Figure 20** illustrates the updated and improved synthetic route developed by Dr. Peng George Wang.[42] In this scheme imidazole reacts with sulfuryl chloride in DCM to yield this product **2** (sulfuryldiimidazole) which then reacts with methyl triflate to form this triflate salt intermediate **3**. The triflate salt is dissolved in water and added ethyl acetate and sodium azide to yield the desired product. The reaction was just as successful as $TfN_3$ but took longer to achieve. Another small-scale reaction was successful but too little of the desired product was produced and the process had to be repeated once more.

### 2.6.2 *Figure 20: Synthetic Scheme of Imidazole-1-sulfonyl Azide*

**Figure 20 Synthesis Scheme of Imidazole-1-sulfonyl Azide**
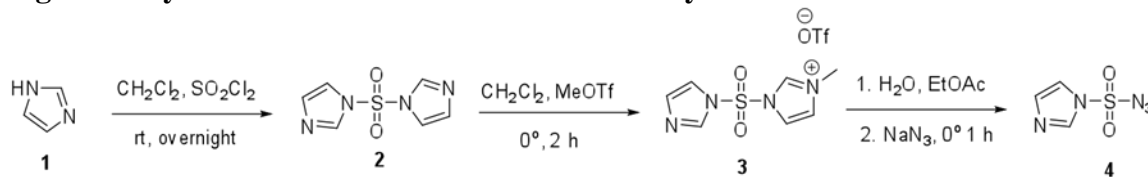


Further attempts were conducted on a large-scale reaction using $TfN_3$ as the diazotransfer reagent. Similarly in my experiment, the large-scale reactions proved to be just as successful as the small-scale reactions until the final step—removing the cyclic sulfate ring. $TfN_3$, known to be unstable is also potentially explosive and difficult to isolate, which hinders its use in high purity.[43]

After purifying the last step of the first attempted large-scale reaction, what had in previous small-scale reactions been an 85% yield for the desired product, became a 30% yield. The sudden degeneration of product was perplexing and another large-scale reaction was attempted to achieve a greater yield. Unfortunately, the same result was yielded. The proper

procedure for conducting reactions, especially those that are done for the first time or have a degree of uncertainty about, is to use a portion of what was synthesized before and to use TLC to check and determine the appropriate eluting system. Because the small-scale reactions had been successful, additional experiments were attempted using larger amounts of reagents without checking TLC for choosing an eluting system for purification. Up until the final step, this method had served well and so the problem was sure to be associated with the purification method.

After another failed attempt at producing high yields of the desired product, the reaction was attempted once more but included checked the TLC before purifying the last reaction. It was determined that the problem did not lie in the purification but in the reaction itself. TLC analysis showed that the product existed; however, there was a major product other than the desired product that was identified, confirming that the previous reaction had failed.

At the time of this discovery, time did not allow for a recreation of the experiment. Therefore, the acceptor sphingolipid previously synthesized by a fellow lab member was used in the glycosylation procedure.

## 2.7 CONCLUSION

Azidosphingosine was successfully synthesized and NMR analysis was conducted to prove the existence and purity of the final product. Diazotransfer reagents are important components in the azidosphingosine synthesis and if synthesized and handled with care, will provide the desired products. The glycosylated sphingosine (glycosphingolipid) was successfully achieved with good yields of 80%, proving that the proposed donor is an effective donor in the glycosylation of sphingolipids.

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**APPENDICES**

**Appendix A**

**General Procedures**

All reagents and solvents used were purchased from commercial sources and used without further purification. Analytical TLC was carried out on silica gel 60 F254 aluminum-backed plates (E. Merck). The 200–400 mesh size of the same absorbent was utilized for all chromatographic purifications. Unless noted, all compounds isolated by chromatography were sufficiently pure by ^{1}H NMR analysis for use in subsequent reactions. Proton nuclear magnetic resonance (^{1}H NMR) spectra and carbon-13 (^{13}C NMR) spectra were recorded on a Bruker DPX 400 spectrometer at 400 MHz and 100 MHz respectively. Chemical shifts and coupling constants were reported in ppm and Hz respectively.

**Peracetylation of Mannose (2)**

To a solution of mannose **1** (1.00 g) and pyridine (10 mL) was added $Ac_2O$ (5 mL). As a catalyst, DMAP (0.067 g) was added and the reaction mixture was stirred, under nitrogen, overnight.After TLC confirmed the starting material was consumed, the mixture was neutralized with HCl (10 ml HCl in 50 ml DI water) and extracted with DCM. The DCM layer was dried over $Na_2SO_4$, concentrated and purified to afford the desired product **2** (2.1 g, 95%) as a slight-yellow oil: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.02 (s, 3H), 2.07 (s, 3H), 2.12 (d, J=3.6 Hz, 3H), 2.19 (d, J=3.2 Hz, 3H), 2.23 (s, 1H), 3.82 (m, 1H), 4.04-4.13 (m, 1H), 4.17 (dd, J=2.6 Hz, 1H), 4.28-4.34 (m, 1H), 5.14 (dd, J=3.4 Hz, 1H), 5.28 (d, J=2.0 Hz, 1H), 5.33-5.37 (m, 1H), 5.50 (d, J=2.4 Hz, 1H), 5.87 (s, 1H) 6.10 (d, J=2.0 Hz, 1H), 7.28 (s, 1H).

**Microwave Assisted Synthesis of Glycosylated Serine**

To make the microwave synthesis more convenient, **2** was dissolved in DCM (64 ml). Every 3 ml extraction represented 100 mg of compound. To a 3 ml extraction of solution in a 5 ml microwave vial was added Fmoc-serine (65 mg) and 3 ml of DMC and $BF_3$ Et2O (0.285 ml). The vial was sealed and heated to 100 °C for 5 mins in a microwave reactor. TLC confirmed the existence of the desired product, however, there were other major products present as well. The mixture was filtered and concentrated under reduced pressure and purified to yield the desired product **3** (39 mg, 30%) as a slightly-yellow oil: $^1$H NMR (MeOH-$d_4$, 400 MHz) δ 0.87 (t, J=4.0 Hz, 1H), 1.27 (s, 3H), 1.97 (s, 11H), 4.06-4.15 (m, 8H), 4.87 (s, 1H), 5.15 (s, 3H), 7.60 (dd, J=12.6, 7H). $^{13}$C NMR (MeOH-$d_4$, 100 MHz) δ 14.1, 20.6, 29.7, 47.0, 48.2, 60.4, 119.9, 127.1, 127.6, 141.2, 171.2, 173.7; ESI FTMS: m/z calcd for $C_{32}H_{35}NO14$ [M +NH$_4$]$^+$ 657.2058, found 656.1974.

### Conventional Synthesis of Glycosylated Serine

To a mixture of Fmoc-serine (130 mg) and 2 (0.200 g) in DMC (8 ml) was added BF3(OEt2) (0.57 mL) and the mixture was stirred for 21 h under nitrogen at room temperature. The reaction was quenched with sodium bicarbonate (NaHCO3). The resulting solution was extracted with DCM, dried with sodium sulfate (Na2SO4), and concentrated under reduced pressure. The resulting residue was purified by chromatography (gradient elution DCM/methanol 5%). NMR and mass-spec analysis were done to confirm the desired product 3 (156 mg, 60%) as a slightly-yellow oil: 1H NMR (MeOH-d4, 400 MHz) δ 1.86-2.06 (m, 12H), 3.96-4.46 (m, 9H), 4.85 (s, 4H), 5.27 (d, J=4.0 Hz, 3H), 7.28 (dd, J=7.0 Hz, 4H), 7.70 (d, J=6.8 Hz, 4H); 13C NMR (MeOH-d4, 100 MHz), δ 19.3, 19.4, 19.5, 62.1, 65.8, 66.7, 68.6, 69.3, 98.0, 119.6, 124.9, 125.1, 126.9, 127.4, 141.1, 143.8, 143.9, 144.1, 156.9, 170.1, 170.2, 171.0.

**Appendix B**

**General Procedures**

All reagents and solvents used were purchased from commercial sources and used without further purification. Analytical TLC was carried out on silica gel 60 F254 aluminum-backed plates (E. Merck). The 200–400 mesh size of the same absorbent was utilized for all chromatographic purifications. Unless noted, all compounds isolated by chromatography were sufficiently pure by ^{1}H NMR analysis for use in subsequent reactions. Proton nuclear magnetic resonance (^{1}H NMR) spectra and carbon-13 (^{13}C NMR) spectra were recorded on a Bruker DPX 400 spectrometer at 400 MHz and 100 MHz respectively. Chemical shifts and coupling constants were reported in ppm and Hz respectively.

**Preparation of Trifluoromethanesulfonyl azide (TfN$_3$)—solution**

A solution of NaN3 (2.011 g, 3.38 mmol) in 5 mL H2O was cooled in an ice bath and treated with 8.5 mL DCM. The resulting biphasic solution was stirred vigorously in the ice bath and treated with Tf2O (1 mL, 3.38 mmol) over a period of 5 min. The reaction continued to stir for 2 hours and was separated. The aqueous phase was extracted twice with DCM. The collected organic layers were washed with Na2Co3 and dried with sodium sulfate yielding approximately 20 mL of solution. The solution was used directly without further purification.

**Preparation of Imidazole-1-sulfonyl Azide**

To the solution of imidazole **1** (20 g, 0.294 mol) in DCM (160 mL) was added sulfuryl choride (5.00 mL) in DCM (30 mL) at 0 ºC dropwise. The mixture was stirred at room temperature overnight. TLC confirmed the reaction was complete and the mixture was filtered. The filtrate was evaporated under reduced pressure. The crude product (N,N'-Sulfuryldiimidazole **2**) was then recrystallized in 40 mL isopropanol to yield the desired product

**2** (7 g 58%),  as a white solid: $^1$H NMR (d$_6$-DMSO, 400 MHz), δ 7.23 (s, 2H), 7.91 (s, 2H), 8.50 (s, 2H); $^{13}$C NMR (d$_6$-DMSO, 100 MHz), δ 121.1, 134.5, 140.3.

To a solution of **2** (1 g) in DCM (10 mL) at 0 °C was added methyl triflate (0.67 mL) dropwise for about 15 min. After 2h at 0 °C, the solid was filtered and dried under high vacuum to give triflate salt **3** (1.63 g, quantitatively) as a white solid: $^1$H NMR (D$_2$O, 400 MHz), δ 3.93 (s, 3H), 7.19 (s, 1H), 7.63 (s, 1H), 7.71 (s, 1H), 8.42 (s, 1H).

**3** (1.63 g) was dissolved in H$_2$O (5.4 mL) at 0 °C. An equal volume of ethyl acetate (EtOAc) (5.4 mL) was added and stirred for 30 min. NaN$_3$ (0.46 g) was added slowly and the mixture was stirred at 0 °C for 1h. The EtOAc phase was extracted and dried and used for the diazotransfer reaction directly.

**Amine to Azide (using TfN$_3$ diazotransfer reagent)—SL 2**

Phytosphingosine **1** (500 mg, 3.38 mmol) was dissolved in 10 mL H$_2$O and treated with potassium carbonate (650 mg, 3.38) and CuSO$_4$ hydrate (4.7 mg). To the solution was added MeOH (20 mL) and the TfN$_3$ solution (20 mL). More MeOH was added to homogeneity and the reaction was stirred for 18 hrs. TLC confirmed starting material had been consumed (eluting system: Hexane/EA 1:1). The solution was then concentrated under reduced pressure, diluted with ethyl acetate and washed twice with H$_2$O. Solution was dried and concentrated again

NOTE: When TfN$_3$ was used as the diazotransfer reagent, after being washed twice with H2O and concentrating again, the resulting blue-white solid would only dissolve in a mixture of DCM and MeOH. It was determined that purification was to be voided and the reaction proceeded to the next step.

**Protection of Primary Alcohol—SL 3**

To a solution of **2** (500 mg, 1.51 mmol) in DCM was added 4-DMAP (9 mg) and TBDPS-Cl (0.457 mL) at 0°C. The reaction was allowed to stir for 24 hours. TLC confirmed the reaction works however the starting material had not been fully consumed. The reaction was allowed to stir under nitrogen for another 24 hours. TLC confirmed with starting material was fully consumed and the reaction was diluted with EtOAc (about 20 mL) and in a separatory funnel, was washed with brine, then dried over sodium sulfate, concentrated and purified to yield the desired product **3** (719 mg, 85%) as a colorless oil: ^{1}H NMR (CDCl$_3$, 400 MHz) δ 0.89 (t, J = 6.9 Hz, 3H), 1.09 (s, 9H), 1.27 (s, 22H), 1.41-1.60 (m, 2H), 2.17 (br s, 2H), 3.57 (m, 1H), 3.65-3.71 (m, 2H), 3.92 (dd, J = 5.7, 10.8 Hz, 1H), 4.04 (dd, J = 3.9, 10.8 Hz, 1H), 7.39-7.50 (m, 6H), 7.69-7.72 (m, 4H); ^{13}C NMR (CDCl$_3$, 100 MHz) δ 14.1, 19.0, 22.6, 25.6, 26.7, 29.3, 29.5, 29.6, 29.7, 31.6, 31.9, 63.4, 64.2, 72.3, 74.0, 127.8, 129.9, 132.45, 132.53, 135.5, 135.6.

## Generation of a cyclic sulfate—SL 4

To a solution of **3** (150 mg) in DCM (6 mL) were added triethylamine (107.95 uL) and thionyl chloride (22.45 uL) at 0°C. After 30 min, the solution was diluted in EtOAc and washed with brine. The organic layer was dried over sodium sulfate and concentrated. The product was then dried in vacuo for 3 h and dissolved in DCM;CH$_3$CN;H$_2$O (6 mL, 1:1:1). To the resulting solution was added RuCl$_3$ (2.5 mg) and NaIO4 (165.6 mg) and was stirred at RT for 2 h. The solution was diluted with EtOAc and washed with sodium thiosulfate. The organic layer was dried, concentrated and purified by column chromatography to yield the desired product **4** (148 mg, 90%) as a colorless oil: ^{1}H NMR (CDCl$_3$, 400 MHz) δ 0.90 (t, J= 6.6 Hz, 3H), 1.11 (s, 9H), 1.29 (s, 22H), 1.47-1.63 (m, 2H), 1.71-1.80 (m, 1H), 1.88-2.00 (m 1H), 3.70 (ddd, J ) 2.4, 5.1, 9.9 Hz, 1H), 3.91 (dd, J ) 5.1, 11.4 Hz, 1H), 4.05 (dd, J ) 2.4, 11.4 Hz, 1H), 4.91- 5.03 (m, 2H), 7.41-7.51 (m, 6H), 7.68-7.72 (m, 4H); ^{13}C NMR (CDCl$_3$, 100 MHz) δ 14.1, 19.1, 22.6, 25.1,

26.6, 28.0, 28.9, 29.2, 29.3, 29.4, 29.5, 29.59, 29.61, 29.63, 31.9, 59.1, 63.5, 79.8, 86.4, 127.90, 127.92, 130.1, 131.9, 132.1, 135.47, 135.50.

**Final Synthesis of Azidosphingosine—SL 5**

To a solution of **4** (148 mg) in toluene (5 mL) were added Bu4NI (86.5 mg) and DBU (50 uL). The mixture was heated to reflux for 2h. It was cooled to room temperature and to it were added H2SO4 (10 uL), H2O (10 uL) and THF (150 uL). The mixture was stirred overnight at room temperature and diluted with EtOAc. The mixture was washed with sodium bicarbonate and brine. The collected organic layers were dried, concentrated and purified to yield the desired product (8 mg, 6%) as a colorless oil. $^1$H NMR (CDCl$_3$, 400 MHz) δ 0.90 (t, J = 6.9 Hz, 3H), 1.09 (s, 11H), 1.28 (s, 17H), 1.31-1.33 (m, 2H), 2.02-2.07 (m, 2H), 3.52 (td, J = 1.2, 5.1 Hz, 1H), 3.8 (dd, J = 4.5, 11.1 Hz, 1H), 4.1 (dd, J = 6.6, 11.1 Hz, 1H), 4.24 (t, J = 6.0 Hz, 1H), 5.43 (tdd, J = 1.2, 6.9, 15.3 Hz, 1H), 5.75 (dtd, J = 0.9, 7.8, 15.3 Hz, 1H), 7.40-7.47 (m, 6H), 7.68-7.75 (m, 4H).

**Protection of 3-position hydroxyl group—SL 6**

To a solution of **5** (84 mg) in pyridine (10 mL) were added Benzoyl Chloride (45 uL) and DMAP (3mg). The mixture was allowed to stir overnight at rt and was diluted with ethyl acetate, washed with HCl, sodium bicarbonate, and brine and dried over Na$_2$SO$_4$. The resulting liquid was concentrated under reduced pressure and purified to yield the desired product **6** (72 mg, 72%) as a colorless oil: $^1$H NMR (CDCl$_3$, 400 MHz),  δ 0.91 (t, J=8.0 Hz, 3H), 1.11 (s, 8H), 1.28 (s, 22H), 2.03-2.08 (m, 2H), 3.78 (d, J=4.8 Hz, 1H), 3.82-3.88 (m, 1H), 5.50-5.56 (m, 1H), 5.70 (t, J=8.0 Hz, 1H), 5.89-5.96 (m, 1H), 7.33-7.48 (m, 6H), 7.58-7.72 (m, 4H), 8.04 (d, J=7.6 Hz, 1H).

**Deprotection of TBDPSO—SL7**

To a solution of **6** (72 mg) in THF (3 mL) was added TBAF (1.5 eq, 162 uL) and was allowed to stir overnight. After TLC confirmed the starting material had been consumed, the solution was diluted with ethyl acetate, washed with HCl, sodium bicarbonate, and brine and dried over $Na_2SO_4$. The resulting liquid was concentrated under reduced pressure and purified to yield the desired product **7** (13 mg, 28%) as a colorless oil: ^{1}H NMR (CDCl$_3$, 400 MHz), δ 0.90 (t, J=6.4 Hz, 3H), 1.27 (s, 20H), 1.40 (t, J=6.8 Hz, 2H), 2.07-2.12 (m, 2H), 3.81-3.85 (m, 1H), 2.45-4.29 (m, 1H), 4.41-4.46 (m, 1H), 4.55 (dd, J=3.8 Hz, 1H), 5.55-5.61 (m, 1H), 5.82-5.89 (m, 1H), 7.42 (td, J= 1.6, 8.0 Hz, 3H), 7.61 (t, J=7.6 Hz, 1H), 7.74 (dd, J=1.6 Hz, 1H), 8.08 (d, J=8.8 Hz, 2H); ^{13}C NMR (CDCl$_3$, 100 MHz), δ 26.5, 29.1, 29.3, 31.9, 32.3, 64.2,64.8, 72.6, 127.2, 128.5, 129.8, 133.3, 132.8, 136.4, 166.3.

## Perbenzoylation of Lactose—SL 9

To a solution of lactose **8** (2g, 1 eq) in pyridine (15 mL) were added BzCl (7.8 mL, 12 eq) and DMAP (71 mg, 0.1 eq). The reaction was allowed to stir overnight and was diluted with ethyl acetate, washed with HCl, sodium bicarbonate, and brine and dried over $Na_2SO_4$. The solution was concentrated under reduced pressure and purified to yield the desired product **9** (5.2 g, 73%) as a white solid. ^{1}H NMR (CDCl$_3$, 400 MHz), δ 3.76-3.78 (m, 1H), 3.80-3.91 (m, 1H), 4.14, (q, J=7.2 Hz, 1H), 4.30-4.26 (m, 1H), 4.56 (s, 1H), 4.91 (dd, J=8.0 Hz, 1H), 5.37-5.40 (m, 1H), 5.61-5.64 (m, 1H), 5.75-5.80 (m, 1H), 6.14-6.24 (m, 1H), 7.20-7.65 (m, 16H), 7.75 (d, J=7.6 Hz, 1H), 7.88-7.92 (m, 1H), 7.99-8.06 (m, 3H).

## Deprotection of Primary Alcohol—SL 10

To a solution of **9** (5.2 g, 1 eq) in DMF (20 mL) was added hydrazine (618 mg, 1.5 eq). The reaction was allowed to stir overnight at rt and washed with water and brine. The organic layer was collected and dried over $Na_2SO_4$, concentrated, and purified to yield the desired product **10** (3.7 g, 78%) as a white solid: ^{1}H NMR (CDCl$_3$, 400 MHz), δ 3.81-3.94 (m, 3H),

4.11-4.17 (m, 1H), 4.24-4.29 (m, 1H), 4.39-4.42 (m, 1H), 4.51-4.63 (m, 2H), 4.93 (dd, J=8.0 Hz, 1H), 5.26 (dd, J=3.6 Hz, 1H), 5.40-5.44 (m, 1H), 5.73-5.78 (m, 2H), 6.16 (t, J=9.6 Hz, 1H), 7.18-7.25 (m, 2H), 7.28-7.50 (m, 8H), 7.57-7.64 (m, 3H), 7.75 (d, J=7.2 Hz, 1H); 13C NMR (CDCl3, 100 MHz), δ 14.2, 21.1, 60.4, 61.1, 62.2, 67.5, 68.5, 69.9, 71.3, 71.8, 72.0, 76.0, 90.3, 101.0, 128.2, 128.4, 129.6, 129.7, 130.0, 133.3, 133.4, 164.8, 165.3, 165.6.

## Synthesis of SL-11

To a solution of **10** (500 mg, 1 eq) in acetone (10 mL) were added $K_2CO_3$ (193 mg, 3 eq) and 2,2,2-Trifluoro-N-phenylacetimidate ( 0.4 mL, 6 eq). The reaction was allowed to stir overnight at rt. The solution was filtered, concentrated, and purified to yield the desired product **11** (516 mg, 99%) as a white solid: 1H NMR (CDCl3, 400 MHz) δ 3.85-3.88 (m, 3H), 4.02-4.05 (m, 3H), 4.32-4.39 (m, 3H), 4.58 (s, 3H), 5.01-5.13 (m, 2H), 5.35 (dd, J=3.2 Hz, 1H), 5.49-5.53 (m, 2H), 5.68 (d, J=3.2 Hz, 1H), 5.80-5.85 (m, 4H), 6.25 (t, J=9.6 Hz, 1H).

NOTE: Because there is a mixture of alpha and beta products in the NMR spectrum, it is difficult to identify individual chemical shifts.

## Glycosylation of a sphingolipid

To a solution of **11** (100 mg, 1.5 eq) and **7** (23 mg, 1 eq) in DCM (10 mL) was added molecular sieves. The mixture was allowed to stir for 5 mins and then cooled in an acetone/dry ice bath to -20 °C. TMSOTf (4 uL) was then added dropwise and the solution was allowed to stir overnight. The mixture was filtered, concentrated and purified to yield the desired product **12** (63 mg, 80%) as a clear liquid; $^1$H NMR (CDCl$_3$, 400 MHz): δ 7.15–8.01 (40 H), 5.82 (t, J=9.1 Hz, 1H), 5.69–5.75 (m, 2 H), 5.69 (dt, J =15.2, 6.8 Hz, 1H), 5.49–5.53 (m, 3H), 5.39–5.44 (m, 2H), 4.89 (d, J=7.8 Hz, 1H), 4.75 (d, J=7.8 Hz, 1H), 4.58 (dd, J=12.3, 1.8 Hz, 1H), 4.48 (dd, J=12.3, 4.1 Hz, 1H), 4.29 (t, J=9.7 Hz, 1H), 3.90–3.92 (m, 2 H), 3.85–3.87 (m, 2 H), 3.70–3.75 (m, 2H), 3.54–3.57 (m, 1H), 1.87–1.90 (m, 2H), 1.32 – 1.34 (m, 2H), 1.20 – 1.25 (m, 20H), 0.88 (t, J= 6.8

Hz, 3H); $^{13}$C NMR (CDCl$_3$): δ 165.8, 165.5, 165.4, 165.3, 165.2, 165.0, 164.9, 164.8, 138.9, 133.5, 133.4, 133.3, 133.2, 133.0, 128.2–129.9, 122.3, 100.9, 100.7, 75.8, 74.7, 73.0, 72.8, 71.7, 71.5, 71.3, 69.8, 68.2, 67.5, 63.3, 62.2, 61.0, 46.3, 32.2, 31.9, 22.5–29.6, 22.6, 14.1.

## $^1$H and $^{13}$C NMR

### $^1$H NMR S2-1
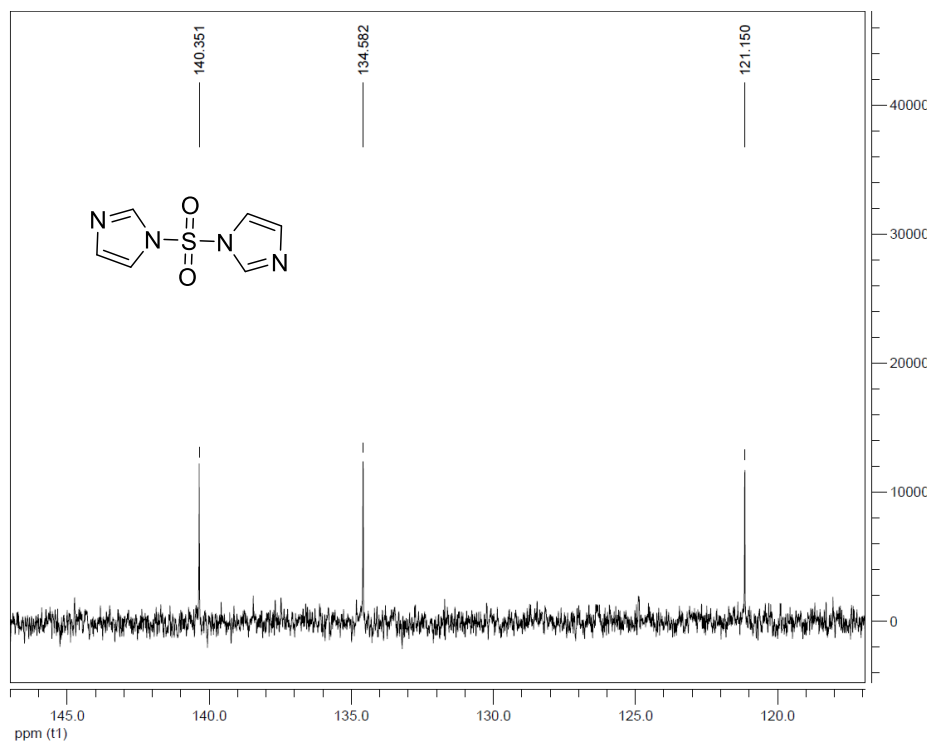
**Figure 21 Proton NMR S2-1**
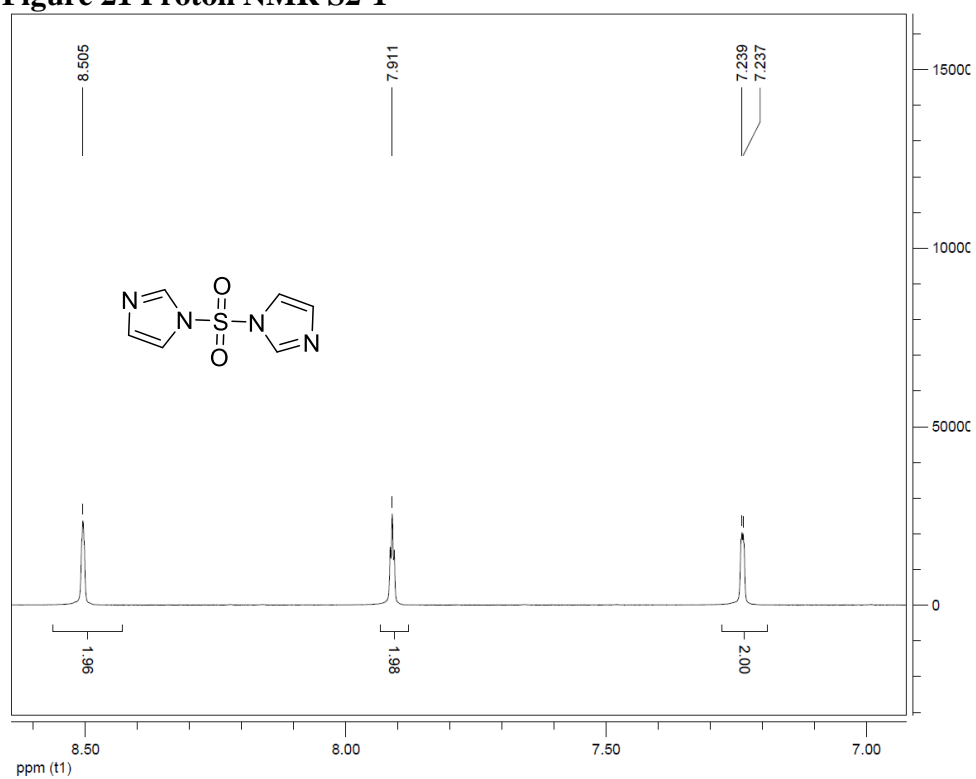


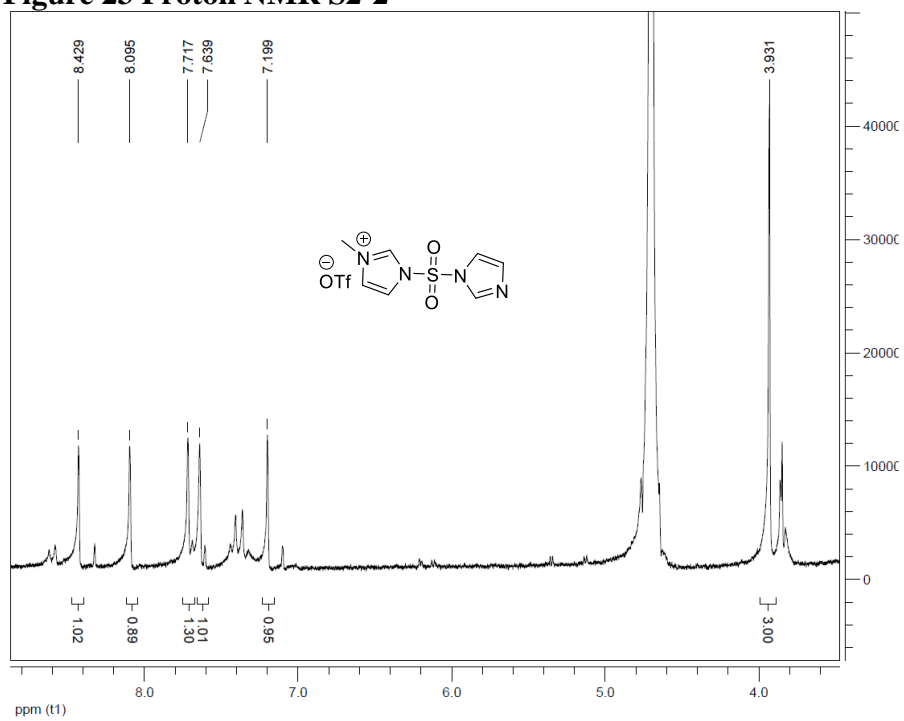### $^{13}$C NMR S2-1

**Figure 22 Carbon NMR S2-1**

**¹H NMR S2-2**

**Figure 23 Proton NMR S2-2**

### <sup>13</sup>C NMR S2-2

**Figure 24 Carbon NMR S2-2**



### <sup>1</sup>J NMR SL-3

**Figure 25 Proton NMR SL-3**

# ¹H NMR SL-4

## Figure 26 Proton NMR SL-4



# ¹³C NMR SL-4

## Figure 27 Carbon NMR SL-4

**¹H NMR SL-5**

**Figure 28 Proton NMR SL-5**
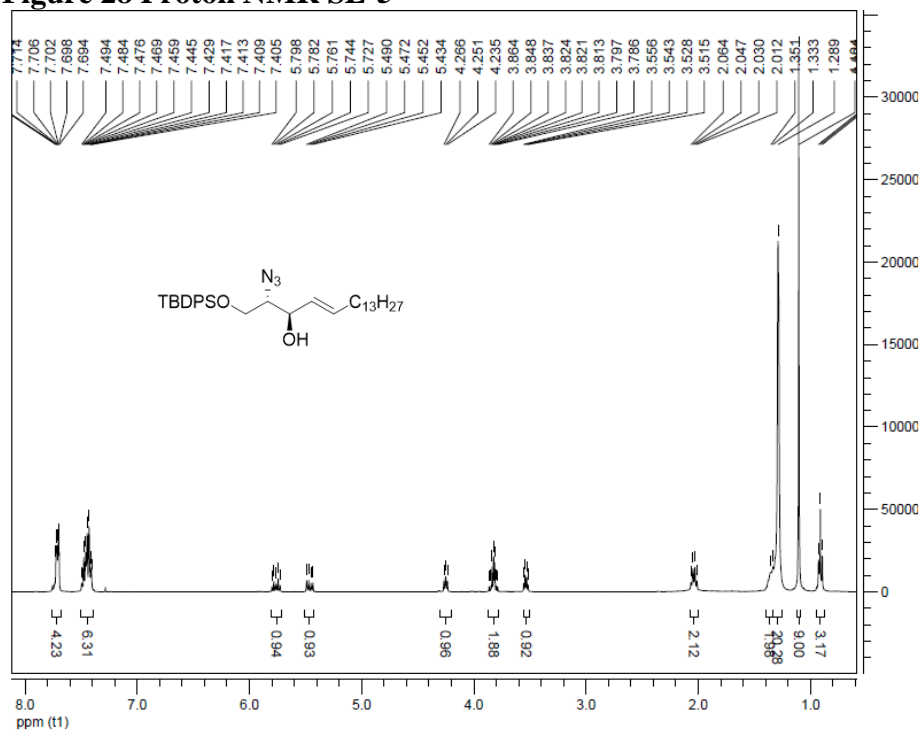


**¹³C NMR SL-5**

**Figure 29 Carbon NMR SL-5**

# ¹H NMR SL-6

## Figure 30 Proton NMR SL-6



# ¹³C NMR SL-6

## Figure 31 Carbon NMR SL-6
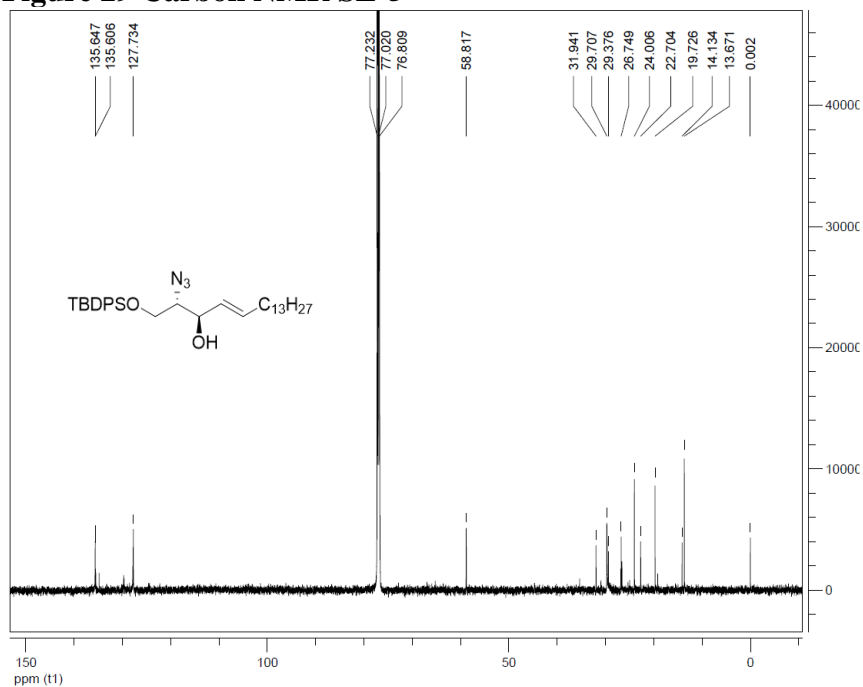
## ¹H NMR SL-7

### Figure 32 Proton NMR SL-7
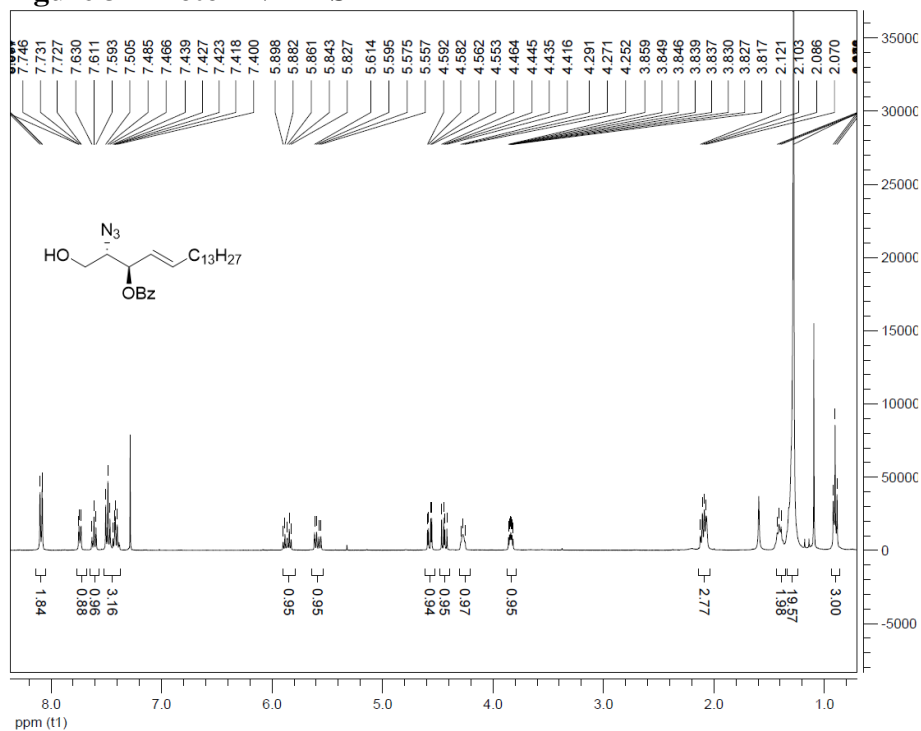


## ¹³C NMR SL-7

### Figure 33 Carbon NMR SL-7
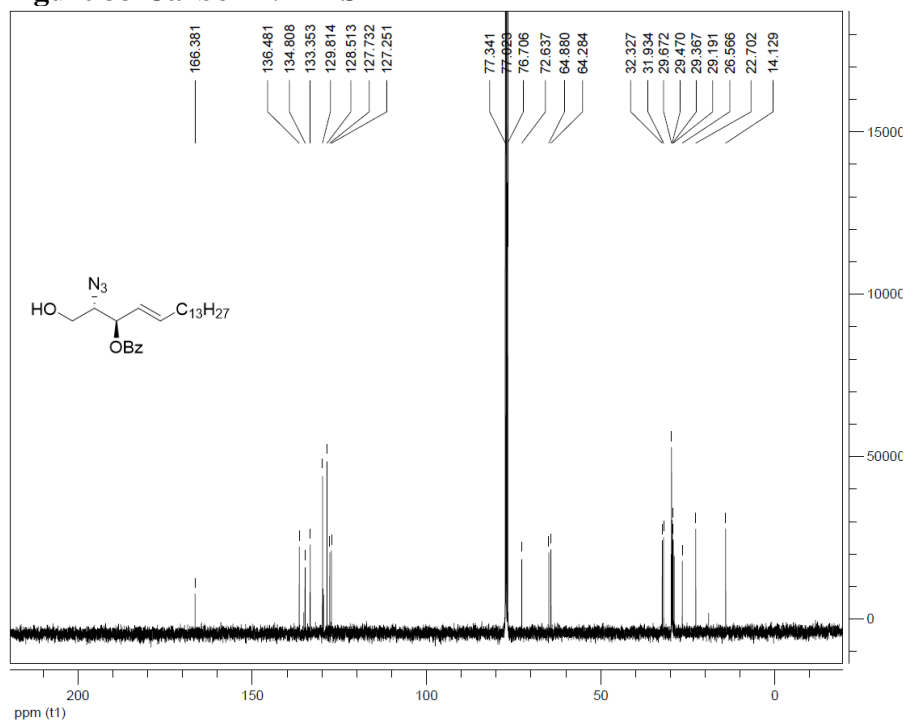
## ¹H NMR SL-9

**Figure 34 Proton NMR SL-9**
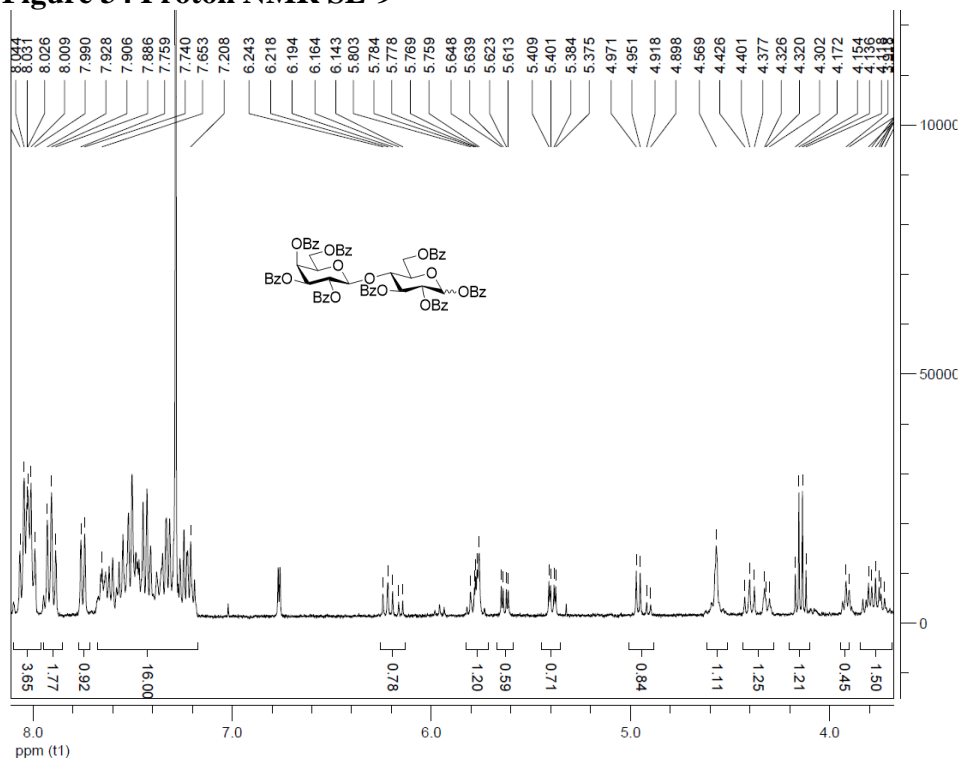


## ¹³C NMR SL-9

**Figure 35 Carbon NMR SL-9**
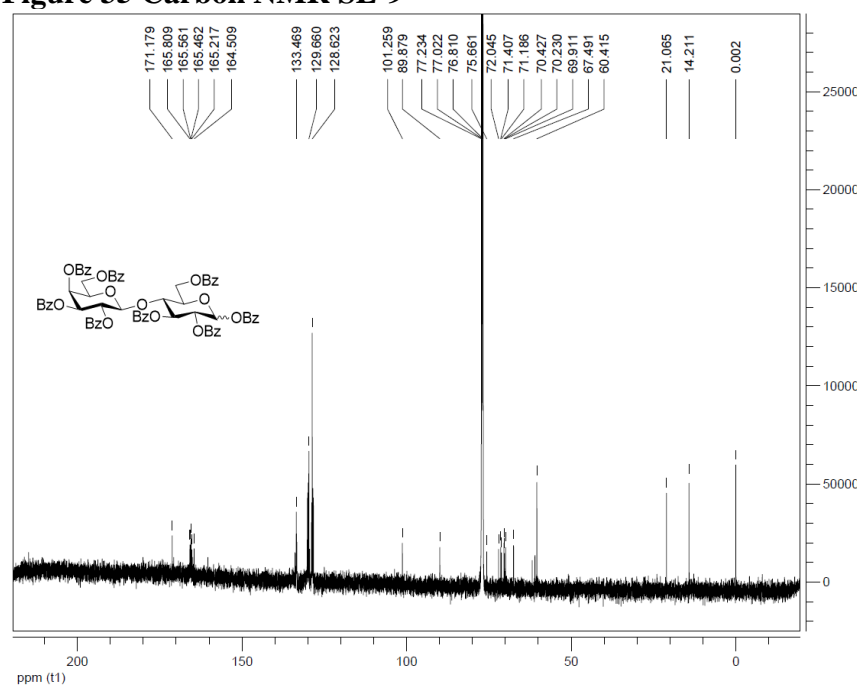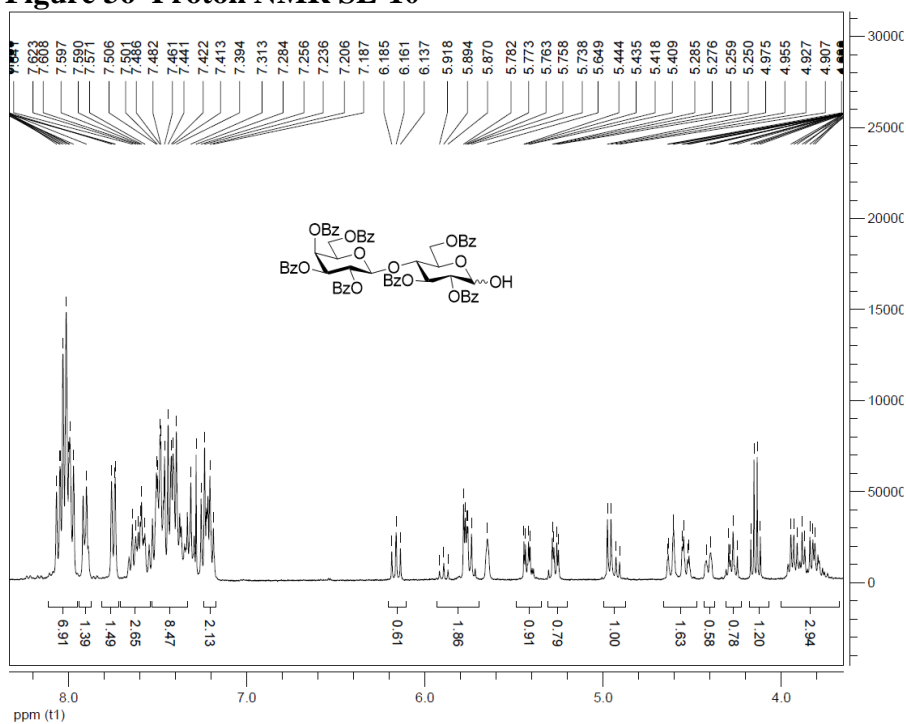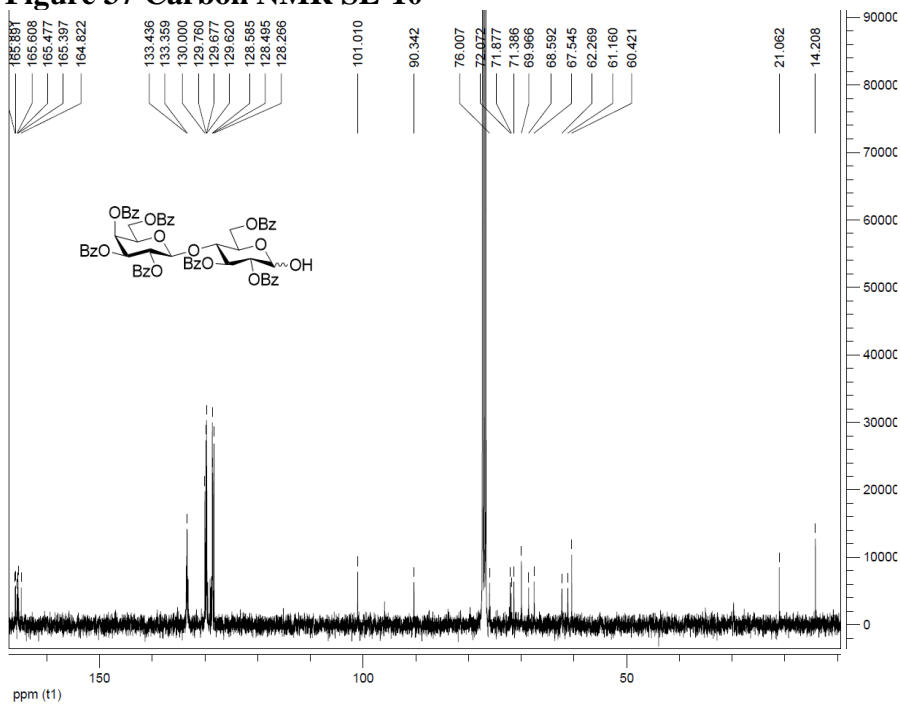
# ¹H NMR SL-10

## Figure 36  Proton NMR SL-10



# ¹³C NMR SL-10

## Figure 37 Carbon NMR SL-10

# ¹H NMR SL-11

## Figure 38  Proton NMR SL-11



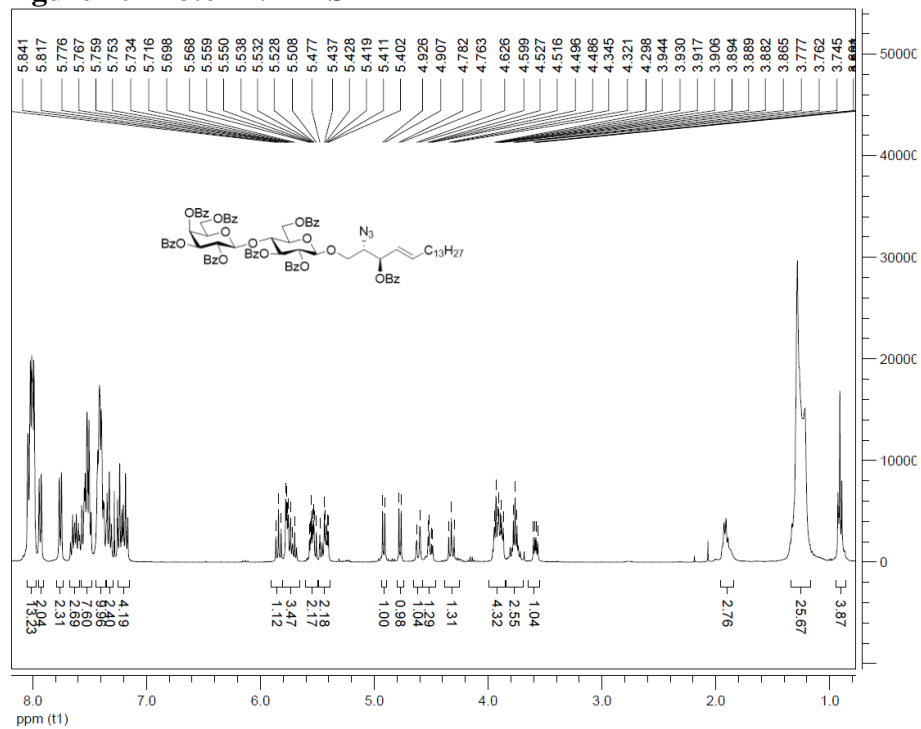# ¹³C NMR SL-11

## Figure 39 Carbon NMR SL-11

## ¹H NMR SL-12

**Figure 40 Proton NMR SL-12**



## ¹³C NMR SL-12

**Figure 41 Carbon NMR SL-12**