

# **Gram-Scale Synthesis of Type-A and Type-B Blood Antigens**

by

**Thomas Hugh Tugwell**

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This thesis/dissertation was presented

by

**Thomas Hugh Tugwell**

It was defended on

July 9, 2018

and approved by

Dr. Seth Horne, PhD, Associate Professor

Dr. Peng Liu, PhD, Associate Professor

Thesis Advisor/Dissertation Director: Dr. Xinyu Liu, PhD, Assistant Professor

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Thomas Hugh Tugwell, M.S.

University of Pittsburgh, 2018

Described in the thesis is the design and implementation of novel synthetic procedures that allow for the gram-scale preparation of Type-B blood antigen as well as efforts towards the gram-scale preparation of Type-A blood antigen. The prepared synthetic Type-A and –B blood antigens are equipped with an amino linker at their reducing ends that allow for ready conjugations to biocompatible solid supports and surfaces.

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## 1.0 Chapter 1

### 1.1 Introduction

The ABO histo-blood group is of biological significance for the study of medical sciences and is especially significant in modern blood transfusion practices<sup>1</sup>. After the discovery of ABO blood groups in 1900 by Dr. Karl Landsteiner (later awarded a Nobel Prize for this work in 1930), interest in the study of these blood groups increased. Blood antigens are present on the surface of red blood cells (RBCs) as O-linked glycans. In tandem with antibodies present in the blood plasma, they are responsible for the rejection of transfusions observed when going across incompatible blood types. This reaction, called agglutination, is observed when antigens, classified as either A or B in human blood, on the cell membrane recognize and bind their respective antibodies, also classified as A or B in plasma. Agglutination can easily result in death. Below, in **Table 1-1** is a summary of the antigens and antibodies present for each blood group.

**Table 1-1 Antigens present on RBCs and antibodies present in plasma for each of the blood groups.**

	<b>A</b>	<b>B</b>	<b>AB</b>	<b>O</b>
<b>Antigens on RBC</b>	A-antigen	B-antigen	A and B-antigens	none
<b>Antibodies in Plasma</b>	anti-B	anti-A	none	anti-A and anti-B

From the table, you can determine compatibilities between blood and plasma across type. For instance, the absence of antigens on the surface of RBCs of type O candidates allows for the

donation of type O blood into all other groups. This is why type O blood donors are termed ‘Universal blood donors’. Alternatively, type AB plasma lacks any antibodies, making type AB plasma universal for donations across all blood groups. It is interesting to note that there exists no anti-O antibody with which the O-antigen would bind, making it a less interesting target to study.

The simplified structural features of the antigenic determinants of blood antigens A, B and O are shown below in **Figure 1-1**.

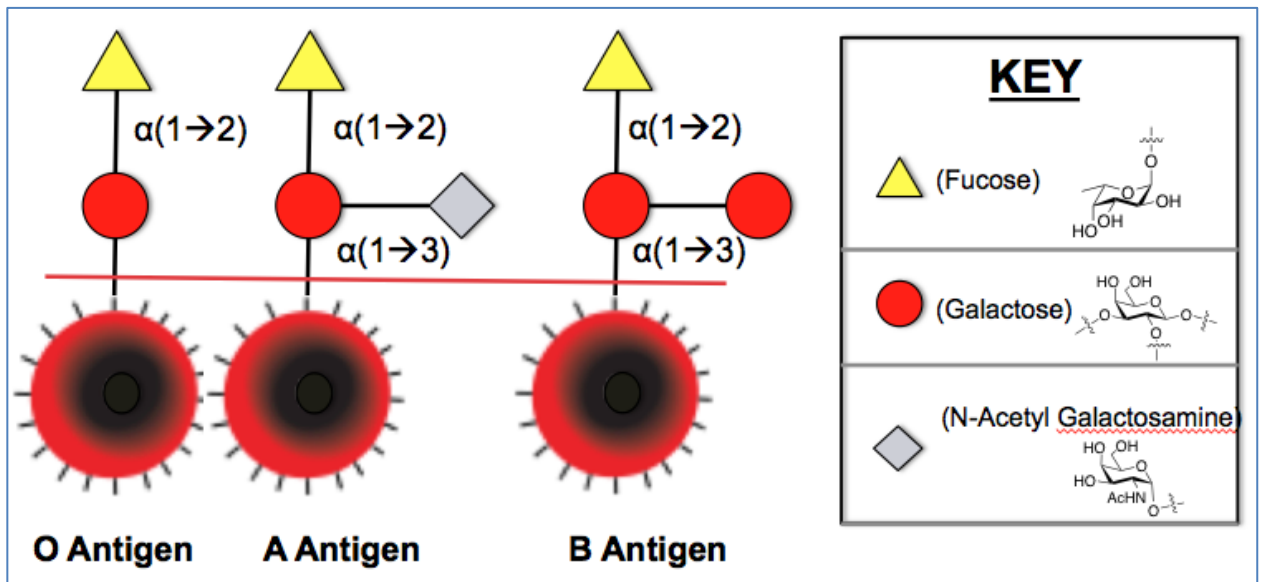


Figure 1-1 Simplified explanation of blood antigen structural features.

The blood antigens are oligosaccharides all sharing a common disaccharide core, 2-O-( $\alpha$ -L-fucopyranosyl)-D-galactose. For the A and B antigens, there is an additional monosaccharide residue branching from the 3-O position of the central galactose, also exhibiting  $\alpha$ -connectivity in each case. For the A-antigen, this residue is galactosamine, and for B-antigen this residue is galactose. Each of these three blood-antigens can also be divided into six sub-types depending on the residue and connectivity exhibited at the anomeric position on the central galactose residue. Although six sub-types do exist on human RBCs, the structural components responsible for

binding the respective antibodies are contained to just trisaccharide determinant aforementioned in **Figure 1-1**.

### 1.2 First reported synthesis – Lemieux (1975)

Dr. Raymond Lemieux, a pioneer in carbohydrate chemistry, reported the first synthesis of a synthetic ABO blood antigen in 1975<sup>3</sup>. In his 1975 paper, he describes the design and synthesis of the O and B antigenic determinants. Dr. Lemieux chose the B and O antigenic determinants as fittingly complex targets for testing a budding methodology being developed in his lab at the time; the halide ion catalyzed glycosylation for the creation of  $\alpha$ -glycopyranosides<sup>3</sup>. The targets of this paper, **1-1** and **1-2** are show in **Figure 1-2**.

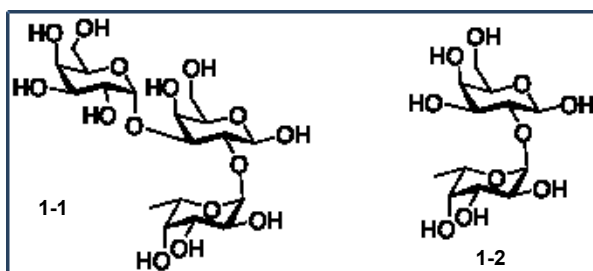
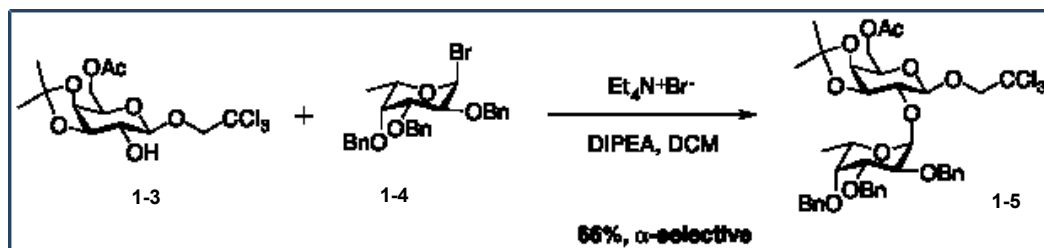


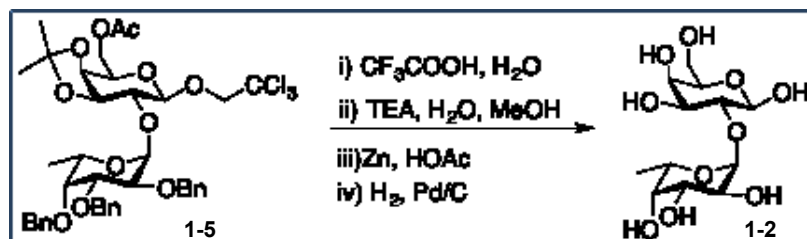
Figure 1-2 B antigenic determinant (left) and O antigenic determinant (right) targets.

The building blocks used and the glycosylation conditions for the first key transformation are shown in **Scheme 1-1**.



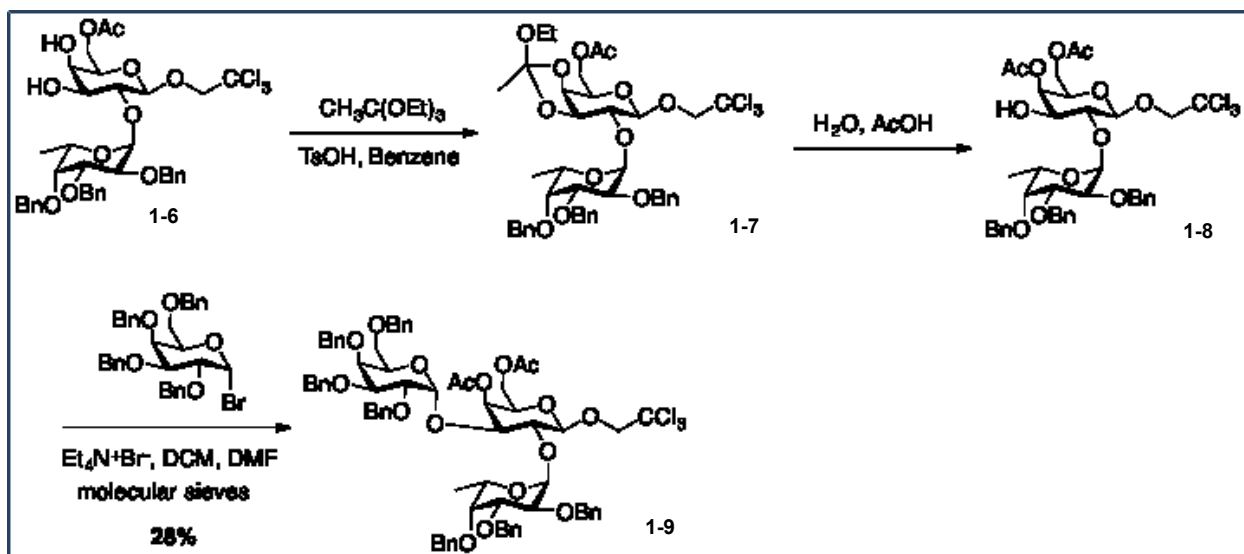
Scheme 1-1 Glycosylation to obtain core disaccharide.

The halide ion catalyzed method gave **1-5** in 66% yield with the  $\alpha$ -anomer exclusively. To obtain the O antigenic determinant, a few deprotection procedures were utilized to remove the 2,2,2-trichloroethyl, isopropylidene, acetyl and benzyl protecting groups. This 4-step deprotection strategy is shown in **Scheme 1-2**.



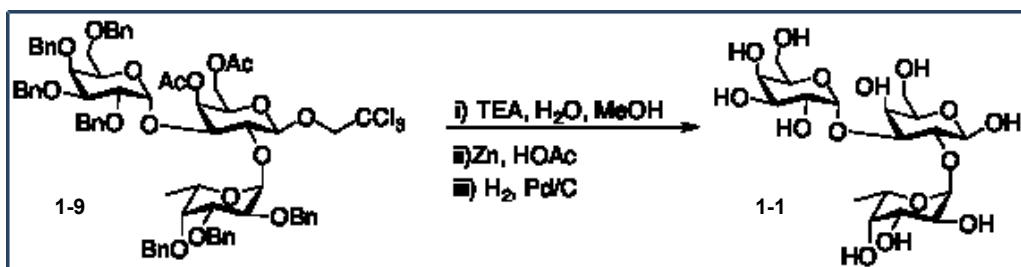
Scheme 1-2 Deprotection sequence to obtain the O antigenic determinant.

Unfortunately, Lemieux did not report the yields for the deprotection sequence or the scale of material obtained for the O-antigen in this paper. Instead, Lemieux's focus shifted to the synthesis of the B antigenic determinant. In **Scheme 1-2**, the deblocking of the isopropylidene (step i) gave the starting point for synthesis of the B antigen, **1-6**, which is shown in **Scheme 1-3**.



Scheme 1-3 Assembly of protected B-antigen trisaccharide.

Lemieux uses a clever installation and selective hydrolysis to obtain the preferably blocked O-4 position, leaving the O-3 position as a free hydroxyl acceptor, **1-8**. In fear of an acetyl group migration to the O-3 position during an interaction on a silica column, no attempt was made to purify this compound and it was used crude as the acceptor in the next glycosylation step. Using their halide ion catalyzed methods once more and adding molecular sieves as a hydrogen bromide acceptor yielded the protected B-antigen trisaccharide, **1-9**, in a low 28% yield. Following the deprotection strategy shown in **Scheme 1-4**, Lemieux was able to obtain the pure B antigenic determinant, **1-1**, on a scale of 14 mg.

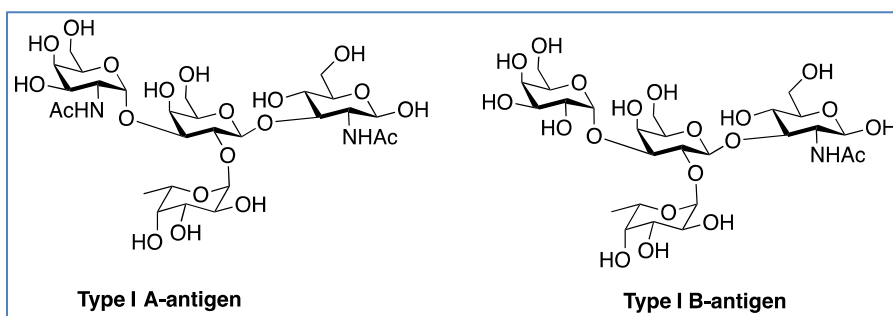


Scheme 1-4 Deprotection strategy to obtain B antigenic determinant.

Although the successful synthesis of these compounds was achieved, there are some disadvantages to the techniques used by Lemieux. Most notably, the limitation of the glycosyl halides relied upon throughout the paper. These glycosylation reactions typically have very long reaction times (~4 days) and also require the use of armed protecting groups to proceed effectively.

### 1.3 Paulsen and Kolar – Type I A/B-antigens (1978)

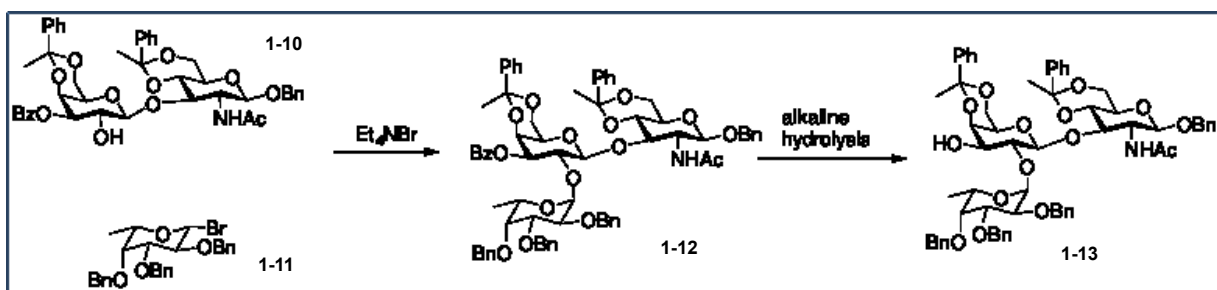
Shortly after Lemieux's seminal synthesis on the B-antigenic determinant, Paulsen and Kolar sought to expand on Lemieux's work by achieving a synthesis of the A-antigen as well as the B-antigen<sup>4</sup>. The work of Paulsen and Kolar extend their work to target the Type I antigens, thus the complexity is heightened since this is now a tetrasaccharide synthesis as opposed to the trisaccharide synthesized by Lemieux. The two target compounds can be seen in **Figure 1-3**.



**Figure 1-3 Type I A and B antigen targets.**

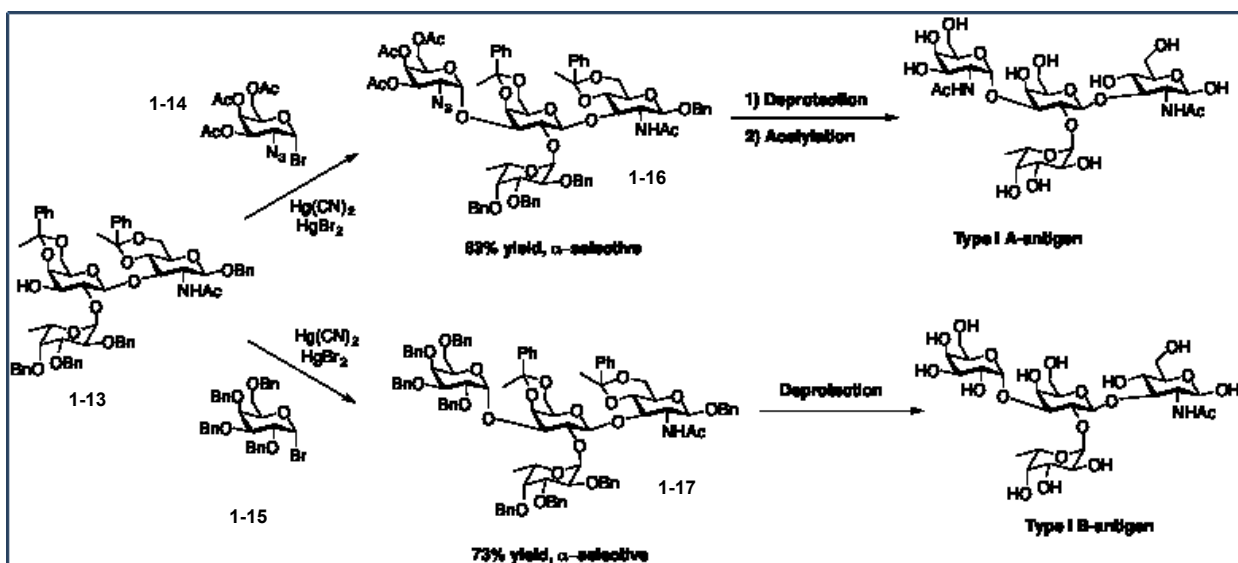
The synthesis begins from the disaccharide, **1-10**, shown in **Scheme 1-5**. This building block was reported previously through the work of another group<sup>5</sup>.





Scheme 1-5 Assembly of the core trisaccharide unit.

Electing to use the same glycosylation strategy as Lemieux had previously used (halide ion catalyzed), they were able to achieve the trisaccharide, **1-13**, shown in **Scheme 1-5** in 82% yield with exclusive  $\alpha$ -selectivity. The benzoyl group is selectively removed through an alkaline hydrolysis and the core trisaccharide, **1-13**, was obtained. This trisaccharide was used in both the synthesis of the Type I A and B-antigens as shown in **Scheme 1-6**.



Scheme 1-6 Route to Type I A and B antigens.

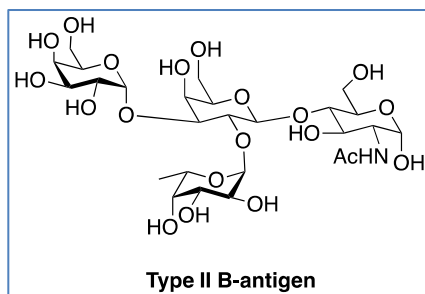
Again the same halide ion catalyzed method is used to obtain the protected tetrasaccharides, **1-16** and **1-17**, in good yield with complete  $\alpha$ -selectivity. The deprotection strategy to obtain the Type I A-antigen involves a hydrogenolysis to reduce the azide and to cleave all protecting groups with the exception of the acetyl groups. This is followed by an

acetylation on the newly freed amine. Deacetylation is achieved by subjecting the tetrasaccharide to sodium methoxide in methanol to give the final Type I A-antigen. The Type I B-antigen is obtained with a simple hydrogenolysis with Pd/C. Unfortunately, no mention of the scale of either of these routes were made in the paper and supplemental information couldn't be found.

Again, though the syntheses reported in this paper were successful, there are still disadvantages to using the chosen methodologies as before. As was the case in Lemieux's paper, the halide ion catalyzed reactions tend to be very sluggish. Additionally, the use of mercury (II) cyanide is certainly not ideal as this is quite a toxic substance capable of absorption through skin and is also toxic through inhalation.

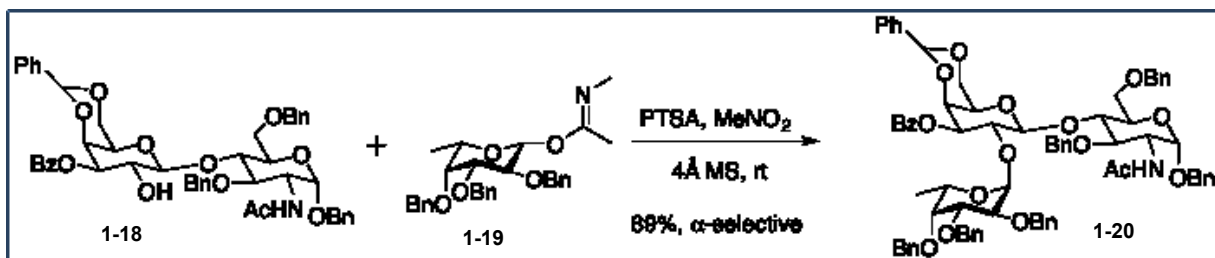
#### 1.4 Milat & Sinay - Type II B antigen (1981)

In 1981, Marie-Louis Milat and Pierre Sinay approached the synthesis of the Type II B antigen using a different tactic than those previously mentioned<sup>6</sup>. The target molecule is shown in **Figure 1-4**.



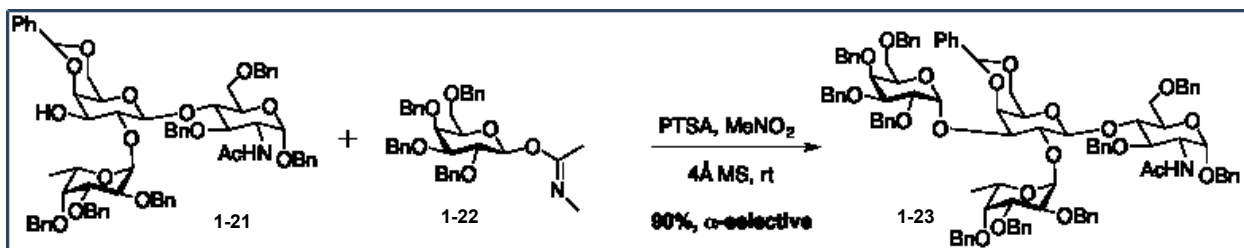
**Figure 1-4 Type II B-antigen target molecule.**

Starting from a previously synthesized disaccharide building block<sup>7</sup>, **1-18**, shown in **Scheme 1-7**, Milat and Sinay envisioned a route to the target relying on an imidate donor for key glycosylation steps. The method used had been established and explored previously in their lab<sup>8</sup>. The activation was achieved through the use of para-toluenesulfonic acid (PTSA) in dry nitromethane. The activation was achieved through the use of para-toluenesulfonic acid (PTSA) in dry nitromethane.



Scheme 1-7 initial glycosylation using imidate donor

The trisaccharide **1-20** was debenzoylated selectively using sodium methoxide in methanol to obtain acceptor **1-21**. With the acceptor in hand the second key glycosylation was performed as shown in **Scheme 1-8** using the same conditions as before with much the same results (good yield/high selectivity). A hydrogenolysis in acetic acid in the presence of palladium on carbon afforded the globally deprotected Type II B-antigen (**Figure 1-4**). The scale used afforded 113 mg of this antigen.



Scheme 1-8 second key glycosylation to obtain protected Type II B-antigen.

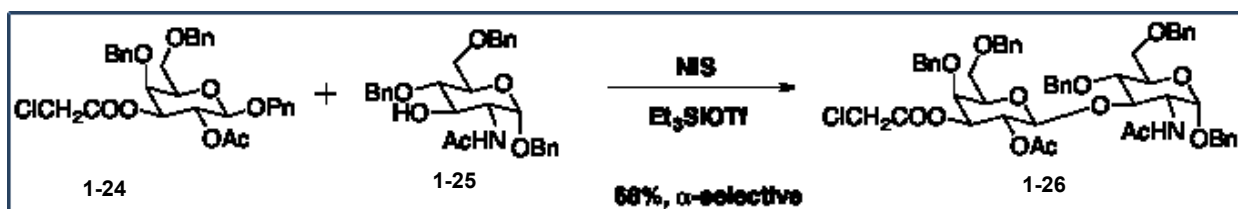
Although good yield and high selectivity were achieved through this imidate procedure, long reaction times are again a considerable drawback as this glycosylation strategy took up to 4

days to complete. This iteration in imidate glycosylation strategies could have been improved through the use of an easier to activate trichloroacetimidate leaving group and likely would have shortened these reaction times.

### 1.5 Fraser-Reid Type I B-antigen (1992)

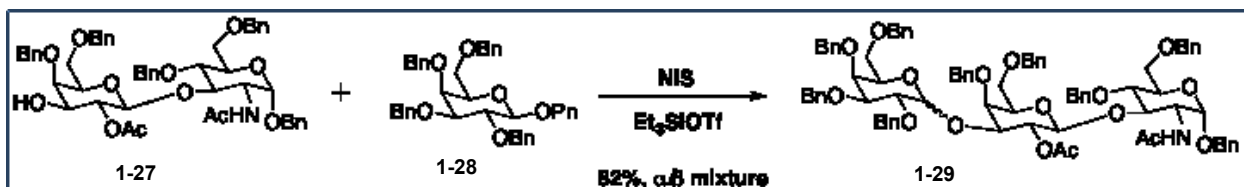
The Type I B-antigen previously reported by Paulsen and Kolar 14 years earlier (**Figure 1-3**) had relied heavily upon a halide ion catalyzed glycosylation procedure for key steps. In Fraser-Reid's lab, the approach to the same target used n-pentenyl glycoside (nPG) donors in key steps<sup>9</sup>. These nPG's have their advantages and disadvantages in the assembly of oligosaccharides. For one, the leaving group is stable under a myriad of reaction conditions, making it ideal for early installation as opposed to installation just before a glycosylation as would be necessary with a less stable leaving group. To selectively activate the nPG a source of I<sup>+</sup> and suitable Lewis acid is necessary to commence the glycosylation. In this case, Fraser-Reid uses a combination of N-Iodosuccinimide (NIS), a source of I<sup>+</sup>, and triethylsilyl triflate (TESOTf), a Lewis acid, for activation in dried DCM as the solvent. To the disadvantage of these nPG's is the fact that anomeric selectivity is not always guaranteed and the yields are hard to optimize.

The first key glycosylation is shown in **Scheme 1-9** between a galactose nPG donor and glucosamine acceptor.



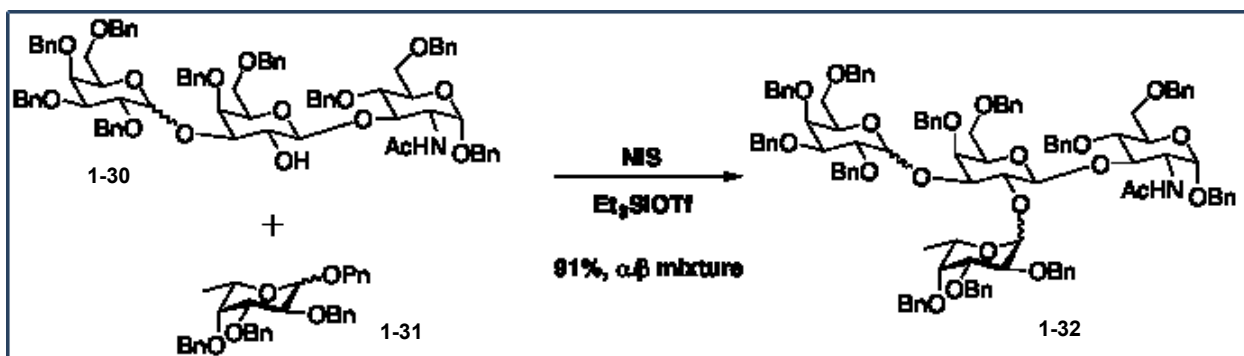
Scheme 1-9 glycosylation between nPG donor and glucosamine acceptor.

This step gave a 68% yield with complete  $\alpha$ -selectivity. Freeing of the chloroacetate at the 3-O position on the galactose residue to open the hydroxyl was followed by a subsequent glycosylation shown in **Scheme 1-10**.



**Scheme 1-10** Second key glycosylation toward Type I B-antigen using nPG approach.

The yield for this second glycosylation was higher than before, however the anomeric selectivity was not as good and a  $\alpha/\beta$ -mixture was obtained. Deblocking of the 2-O position on the central galactose residue was achieved using a standard Zemplen deacetylation. With this trisaccharide acceptor in hand, the fucose residue was ready for installation using the same glycosylation conditions as before and is shown in **Scheme 1-11**.



**Scheme 1-11** Fucose installation

Again, although yield was high, the stereoselectivity was not complete and a  $\alpha/\beta$ -mixture was obtained. The scale of the reaction was relatively small, as only 25.3 mg of the fully deprotected Type I B-antigen was obtained.

## 1.6 Lowary Types I & II A/B/O-antigens (2010)

In Todd Lowary's 2010 paper, he details the synthesis of Type I and II A, B and O antigens using trichloroacetimidate donors for the key glycosylation reactions<sup>2</sup>. Since the strategy to obtain each of these antigens (Type I and II A/B/O antigens) uses the same basic key transformation strategies and features similar yields and stereoselectivities, only the construction of the Type I A-antigen shown in **Figure 1-5** will be outlined to illustrate the efficacy of the trichloroacetimidate methodology chosen.

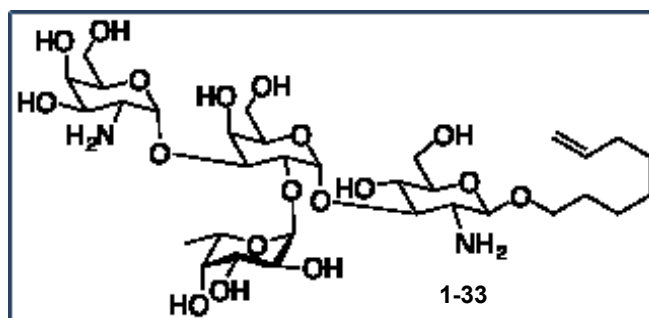
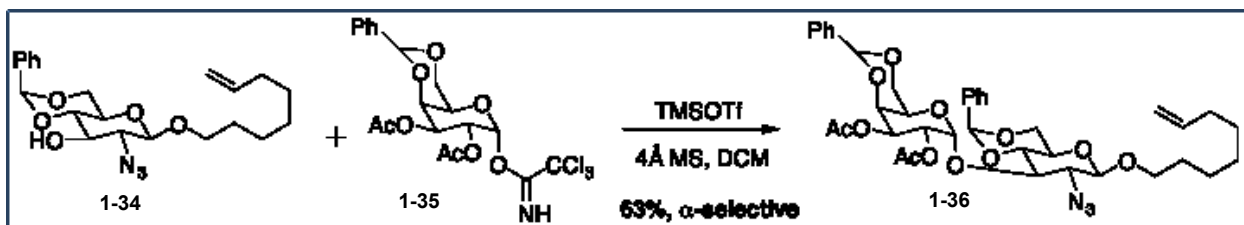


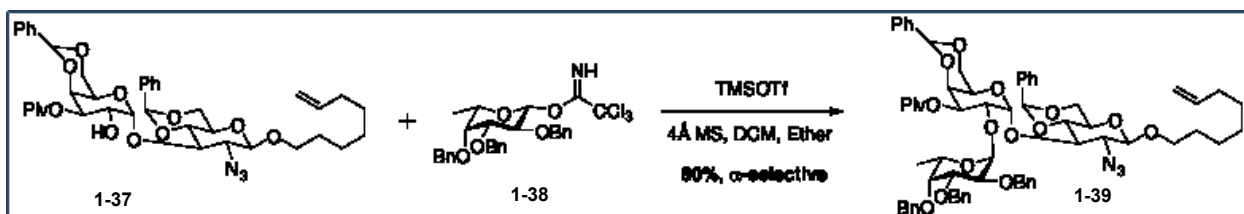
Figure 1-5 Type I A-antigen target with linker.

The synthesis started with a glycosylation between a glucosamine donor with an azide protected amine as well as benzylidene protection on the 4,6-O positions. This glucosamine residue was also equipped with an octenyl aglycone that could be used as a linker later on for further practical applications of the antigen. This acceptor is used in the glycosylation reaction with the galactose trichloroacetimidate donor as shown in **Scheme 1-12**.



Scheme 1-12 First glycosylation toward preparation of Type I A-antigen

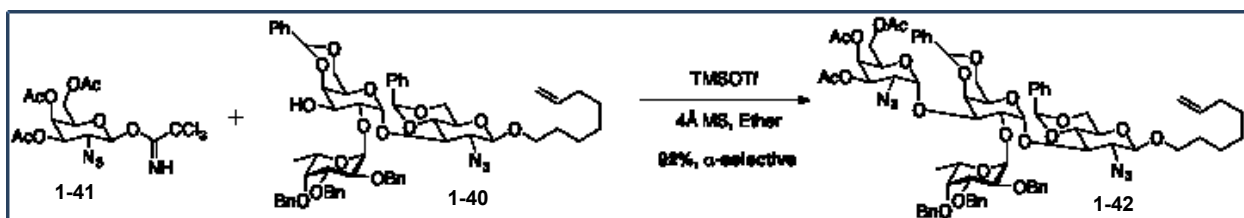
The imidate is activated with TMSOTf under dry conditions (dry solvent and molecular sieves). The selectivity for this glycosylation gave exclusively the  $\alpha$ -anomer with a yield of 63% to form the disaccharide shown. In order to form the trisaccharide, a deacetylation followed by selective 3-O pivaloylation on the galactose residue gave the acceptor shown in **Scheme 1-13**.



Scheme 1-13 Imidate glycosylation to obtain the trisaccharide.

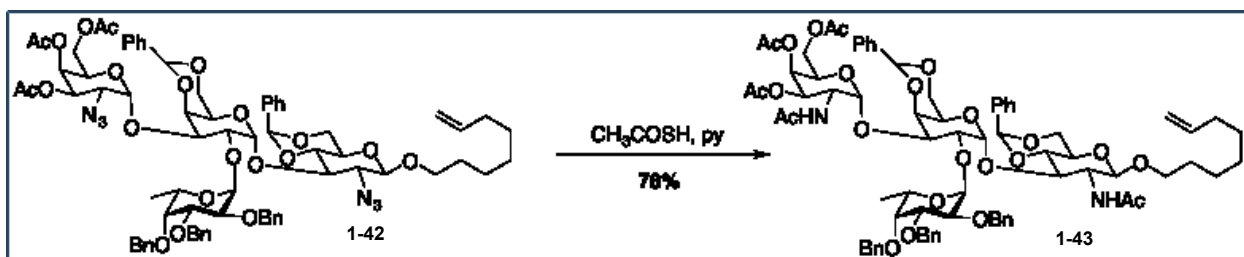
This acceptor was used in the presence of a fucose trichloroacetimidate donor under the same glycosylation conditions used previously with the exception of a small amount of DCM to help fully dissolve the starting materials as complete solubility in one solvent can sometimes pose a problem in carbohydrate chemistry. The reaction gave a  $\alpha$ -selective product in 80% yield. This trisaccharide was used as a core unit to obtain both the Type I A, B and O antigens. The path to obtain the Type II antigens core trisaccharide was very similar with the exception of the first key glycosylation having the glucosamine acceptor's free hydroxyl on the C-4 position instead of the C-3 position. Removal of the pivaloyl protecting group on the central galactose

was achieved using lithium methoxide in methanol to obtain the acceptor shown in **Scheme 1-14**.



Scheme 1-14 Final key glycosylation to obtain the protected Type I A-antigen.

The trichloroacetimidate strategy was again used for the final glycosylation step using an azide protected galactosamine donor. As was the theme with the other conversions, this reaction also gave complete  $\alpha$ -stereoselectivity with a high yield of 92%. An efficient and interesting formation of the carboxamides (N-Acetyl) featured in the final compound directly from the azides present is achieved using thioacetic acid in pyridine<sup>10</sup> as shown in **Scheme 1-15**.



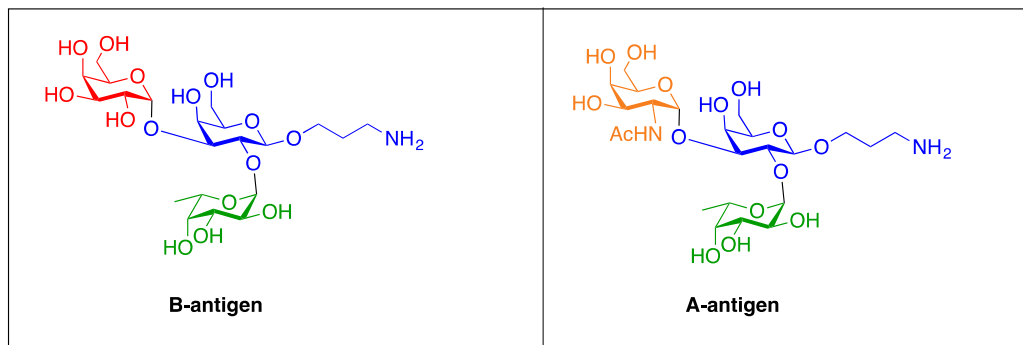
Scheme 1-15 Acetyl group installation

After a deprotection strategy consisting of a Zemplen deacetylation followed by removal of the benzylidene and benzyl groups using distilled liquid ammonia treated with metallic sodium in a methanol/THF mixture, the Type I A-antigen with an octenyl linker was obtained. The group was able to produce this antigen on a scale of 33.5 mg.



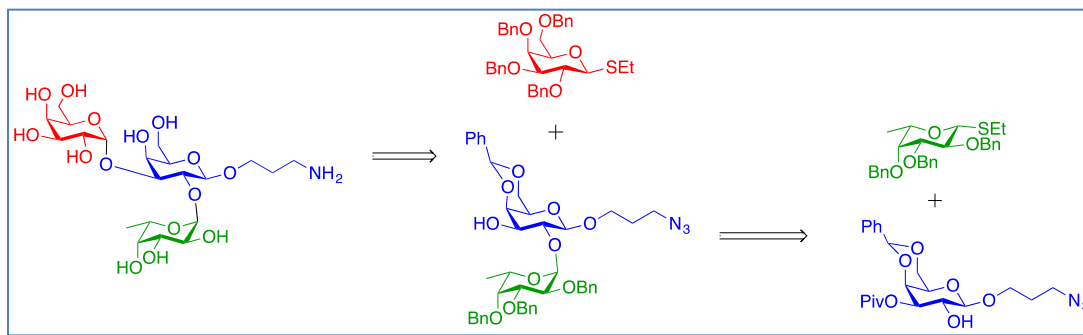
## 1.7 Retrosynthesis of proposed B-antigen with amine linker

Our goal on this project was to make the A and B antigens with functionality allowing these antigens to be fixed onto a solid support. For this we proposed the structures in **Figure 1-6**.



**Figure 1-6** Structure of desired A and B-antigen targets with amine linker on the central galactose residue.

The two proposed structures include the trisaccharide portion of the A and B-antigens that are responsible for binding to their respective antigens, and additionally we have a propylamine amine linker that is located on the central galactose residue that is capable of joining the antigen to a solid support. The linker also functions as a spacer, leaving enough distance as to not interfere with the binding affinity of the antigens to their antibodies once fixed onto the solid support surface. A first generation retrosynthetic route to the B-antigen was laid out at the beginning of this project and is shown in **Scheme 1-16**.

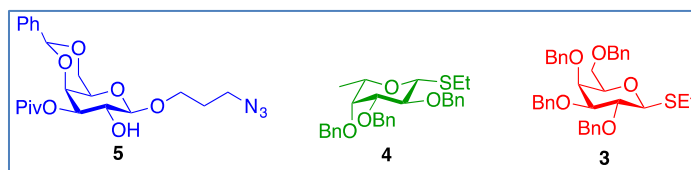


**Scheme 1-16** 1<sup>st</sup> generation retrosynthetic pathway to obtain B-antigen

## 2.0 Chapter 2

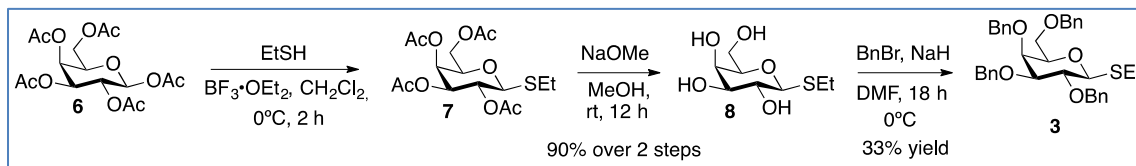
### 2.1 1<sup>st</sup> Generation B-antigen Synthesis

En route to the synthesis of the desired B-antigen with amine linker (**Figure 1-6**, left) we went through a 1<sup>st</sup> generation retrosynthetic route (**Scheme 1-16**) that eventually proved to yield stereochemical impurities (anomeric mixture) in the final glycosylation step. However, due to time constraints defined by the DOD grant proposal we are working on, this 1<sup>st</sup>-generation B-antigen proved acceptable for use as a proof of concept for the stage one deadline at the time even with an anomeric mixture present. In the first generation of the B-antigen synthesis there were three monosaccharide building blocks that needed to be synthesized for the construction of our B-antigen trisaccharide featuring a long amine linker, compounds **3**, **4** and **5** (**Figure 2-1**).



**Figure 2-1** Three monosaccharide building blocks involved in the first generation synthesis of the B-antigen.

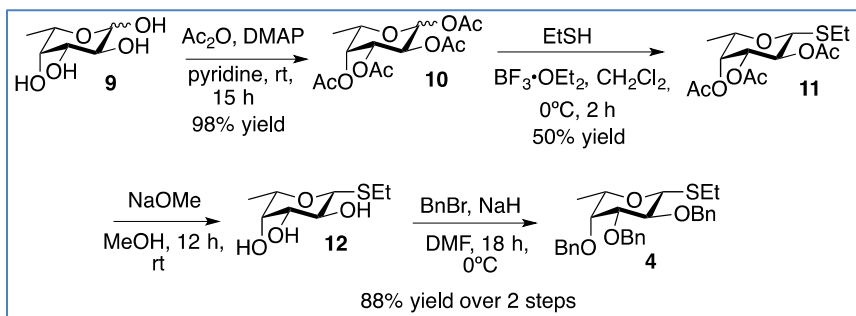
Compound **3** was synthesized starting from  $\beta$ -D-Galactose pentaacetate **6**, which is commercially available and economically a very viable material to commence from (**Scheme 2-1**).



**Scheme 2-1** Synthesis of galactose thioglycoside donor **3**.

The route began with glycosylation of ethanethiol on the anomeric center to yield compound **7**<sup>11</sup>. Compound **7** was then used directly in the deacetylation step using a solution of sodium methoxide generated in situ to yield compound **8**<sup>11</sup>. After purification by flash column chromatography (Hexane/EtOAc), pure thioglycoside **8** was obtained in high yield (90%) over two steps. Benzylation of all four hydroxyl groups was necessary to obtain a suitable donor (armed and fully protected, no C-2 participating groups present), and was achieved using benzyl bromide in basic conditions. After purification, compound **3**<sup>11</sup> was achieved with a 33% yield.

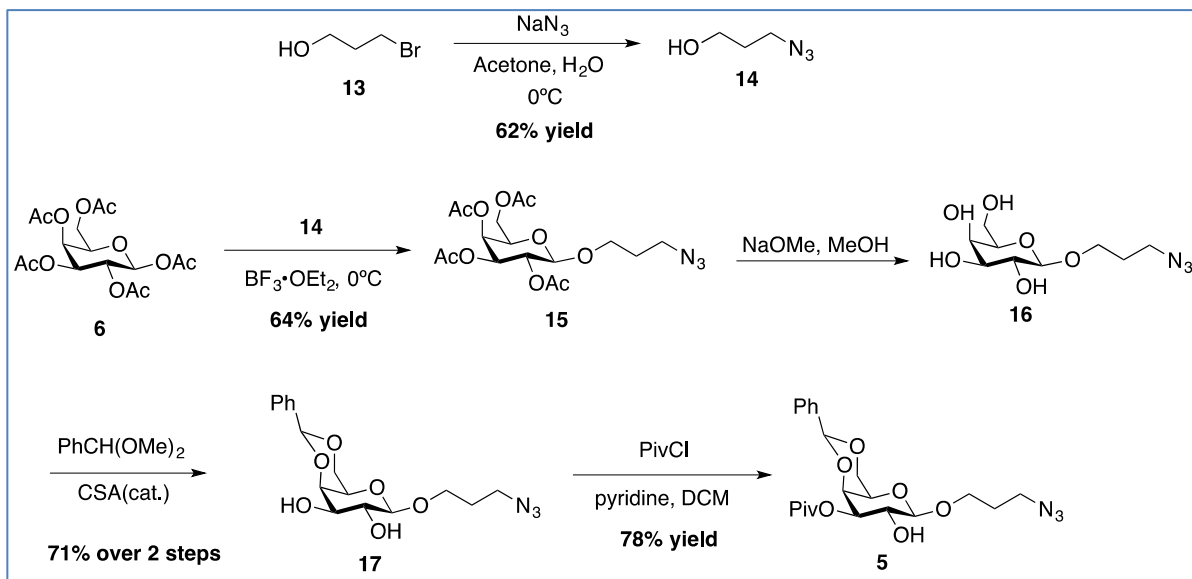
The next monosaccharide building block to be made was the fucose donor **4** (Scheme 2-2) and started from the commercially available L-fucose **9**.



Scheme 2-2 Synthesis of fucose thioglycoside donor **4**.

Acetylation of **9** in basic conditions using acetic anhydride yielded compound **10**<sup>12</sup> in near quantitative yield. Transformation to the thioglycoside was achieved by activating the anomeric O-acetyl group with  $\text{BF}_3 \cdot \text{OEt}_2$  in the presence of ethanethiol. Donor **11**<sup>12</sup> was obtained with a 50% yield after purification by flash column chromatography. A deacetylation using sodium methoxide gave compound **12** which was used directly in the benzylation step to give compound **4**<sup>12</sup> in 88% yield over two steps.

The last building block for the 1<sup>st</sup> generation synthesis was the central galactose residue, which features an amine linker at the anomeric position. Therefore, preparation of building block **5** proceeded with the synthesis of the amine linker **14** (Scheme 2-3), a simple azide substitution.



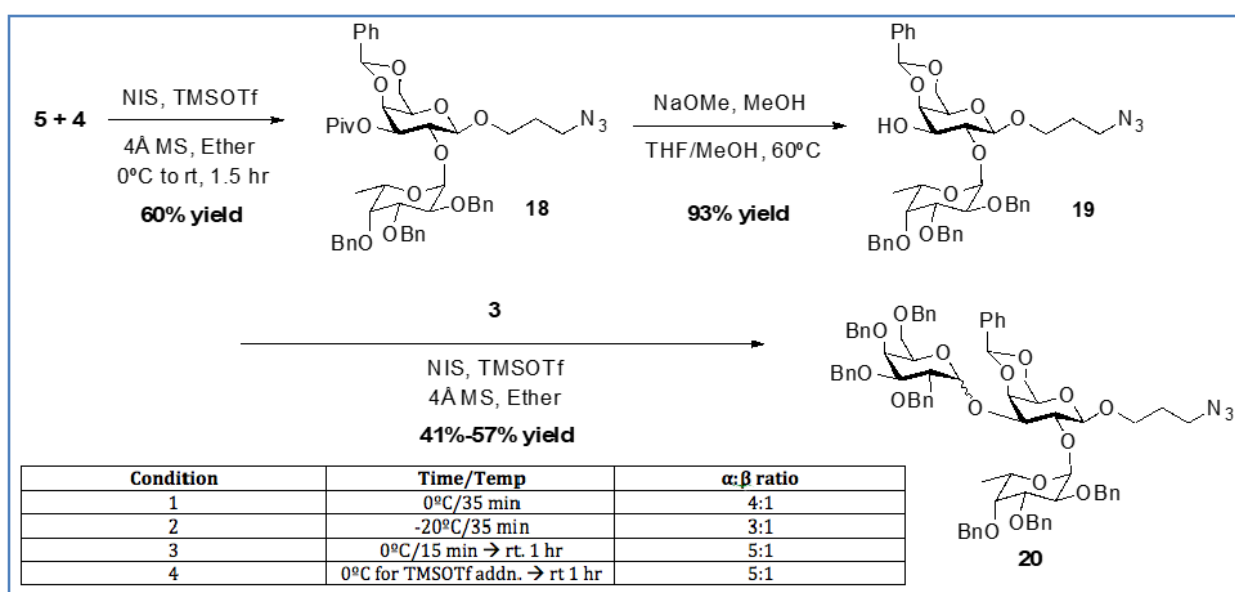
Scheme 2-3 Synthesis of linker and central galactose building block **5**.

In order to stifle possible reactions of a reactive free amine during the route to the final trisaccharide, it was envisioned that the linker would be protected as an azide throughout and later be reduced to the primary amine. Commercially available **13** was refluxed in presence of sodium azide to obtain **14**. This linker was then installed at the anomeric center of the same per-acetylated galactose starting material, **6**, as was used previously for the synthesis of **3**. Galactose **15**<sup>13</sup> with linker installed was obtained in 64% yield with complete selectivity for the  $\beta$ -anomer.

In order for this central galactose building block to be useful in our synthetic strategy, we needed a way to keep the C-2 position free for glycosylation while also allowing for a selective deprotection of the C-3 position and keeping the C-4 and C-6 positions capped. This was achieved first by deacetylating compound **15** in sodium methoxide and subsequent installation of 4,6-O-

benzylidene to obtain compound **17**<sup>13</sup> in 71% yield over these two steps. For the selective protection of the C-3 position, a pivaloyl protecting group was chosen since this would also remain an orthogonal protecting group with the 4,6-O-benzylidene already installed. Pivaloylation to obtain compound **5** proceeded smoothly, obtaining 78% of the finished central galactose building block. The selectivity for the C-3 protection instead of the C-2 protection is most likely attributed to a higher nucleophilicity. The C-2 position is adjacent to the anomeric center and thus is less rich in electron density as a result. The synthesis of compound **5** was previously unknown and therefore was fully characterized (<sup>1</sup>H NMR, <sup>13</sup>C NMR, HRMS, [α]<sub>D</sub>, COSY and HSQC).

With all three building blocks (**3**, **4** and **5**) now in hand, assembly of the trisaccharide commenced (**Scheme 2-4**).



**Scheme 2-4** Assembly of the 1<sup>st</sup> generation B-antigen from the previously synthesized monosaccharide building blocks **3**, **4** and **5**.

The key steps in our assembly include two glycosylation reactions utilizing thioglycoside donors. These thioglycosides contain sulfur, a soft nucleophile, at the anomeric position and are

thus capable of being activated by soft electrophiles. In these cases, activation is carried out in the presence of TMSOTf (Lewis acid) and NIS, a source of I<sup>+</sup> (our soft electrophile). Additionally, very dry conditions need to be utilized in order to prevent hydrolysis at the anomeric position. Therefore, molecular sieves and freshly distilled solvents are used to maximize product formation in each glycosylation. Synthesis of the core disaccharide unit **19** (common to both A and B-antigens [1<sup>st</sup> & 2<sup>nd</sup> generations]) began with the glycosylation of building blocks **4** (1.3 eq) and **5** (1.0 eq). Optimizations of this reaction showed the best result when the donor was used in excess in the presence of a catalytic amount of TMSOTf and 1.5 equivalents of NIS. Selective deprotection of the pivaloyl group at the C-3 position using sodium methoxide in a solvent mixture of methanol and tetrahydrofuran gave the disaccharide acceptor **19**. Again using thioglycoside donor **3** in excess under the same conditions used in the previous glycosylation yielded the expected trisaccharide **20** in 57% yield. The problem that arose in optimizing this glycosylation was that complete  $\alpha$ -selectivity was not obtainable with this specific galactose building block. The highest anomeric purity able to be obtained from the 1<sup>st</sup> generation synthesis of the B-antigen was a 5:1( $\alpha/\beta$ ) ratio using the conditions 3 & 4 shown in **Scheme 2-4**. The proton NMR (**Figure 2-2**) and proton coupled HSQC (**Figure 2-3**) of compound **20** clearly show the stereochemical impure nature of the product and helped determine the ratios for each condition used.

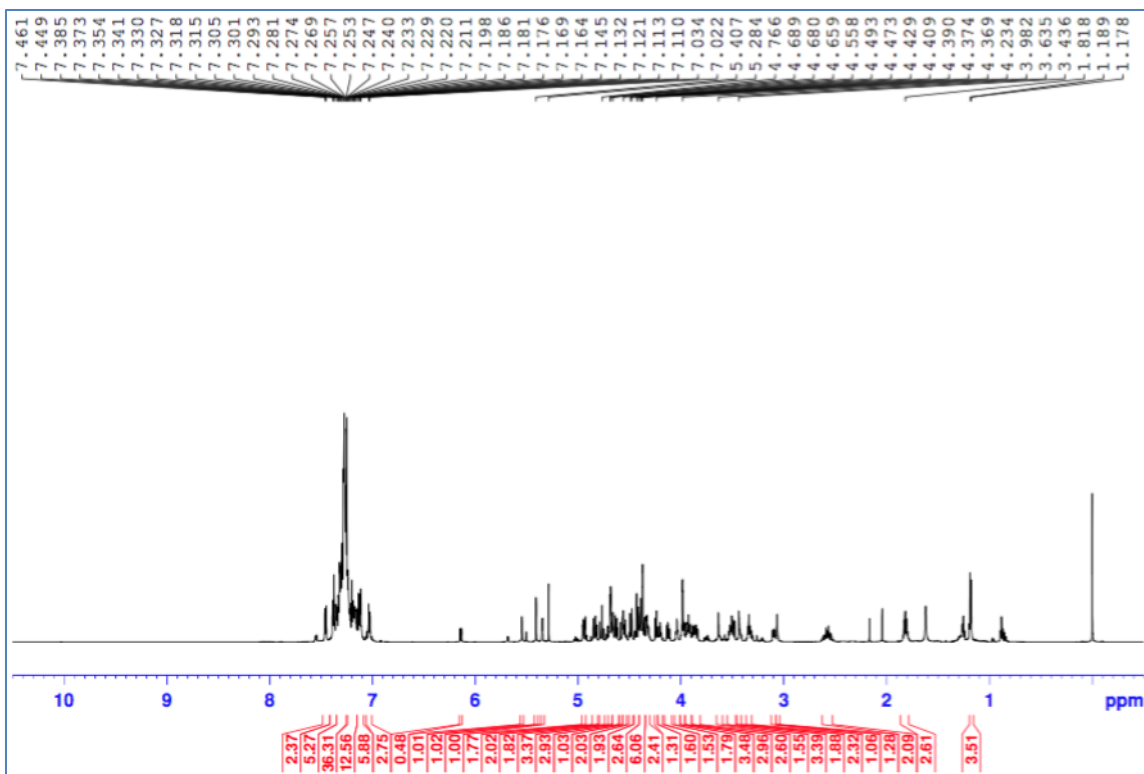


Figure 2-2 <sup>1</sup>H NMR spectra of compound 20

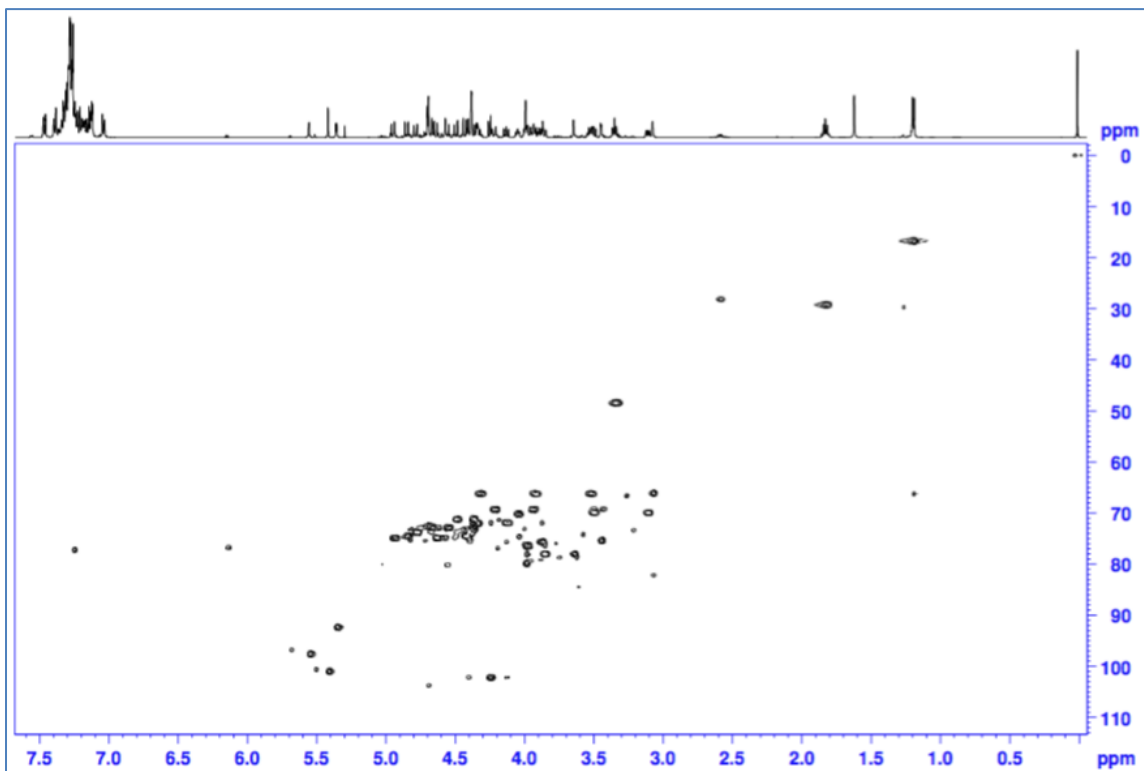
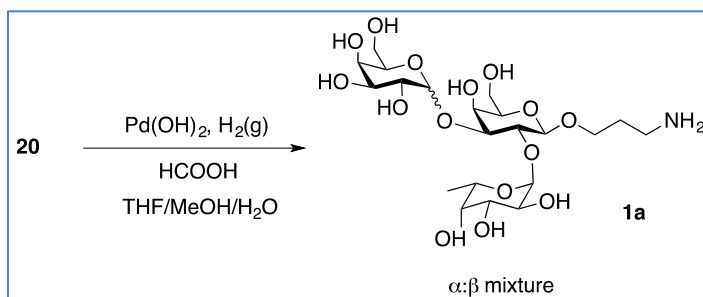


Figure 2-3 <sup>1</sup>H coupled HSQC spectra of compound 20

Thus, it was concluded that modifying the terminal galactose residue building block was necessary for complete  $\alpha$ -selectivity. However, since anomeric purity was not the goal at this phase of the project, completion of this route proceeded. Global deprotection of this anomeric mixture with palladium hydroxide under hydrogen gas gave B-antigen **1** in quantitative yield (75 mg) as shown in **Scheme 2-5**.



**Scheme 2-5** Final deprotection to yield anomeric mixture of B-antigen

## 2.2 Second generation synthesis of B-antigen

Again, targeting the B-antigen equipped with an amine linker (**Figure 1-6**, left), the added challenge of obtaining anomeric purity from the final glycosylation step was now the primary goal. Considering the core disaccharide **19** had already been synthesized with good yield and stereoselectivity, we shifted focus onto a galactose building block superior to the galactose donor **3** used in the 1<sup>st</sup> generation synthesis. For this purpose, galactose donor **24** (**Figure 2-8**) was suggested.



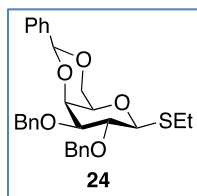
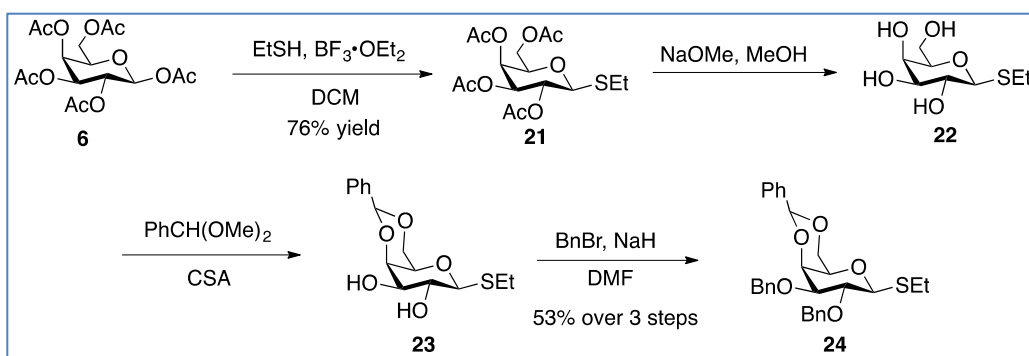


Figure 2-8 Proposed galactose donor to enhance  $\alpha$ -selectivity in the final glycosylation step of the B-antigen synthesis.

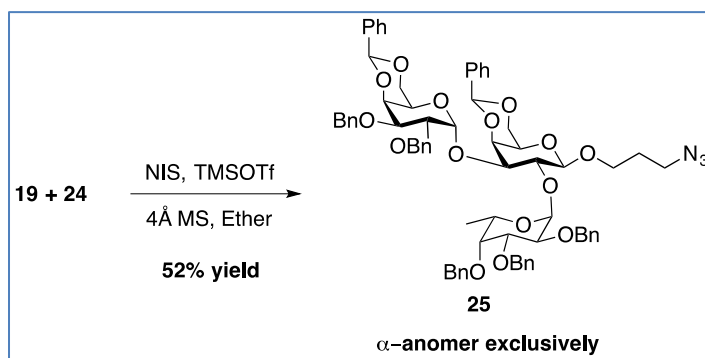
With the newly proposed building block in mind, a synthetic strategy was identified as shown in **Scheme 2-8**.



Scheme 2-8 Synthesis of improved terminal galactose building block.

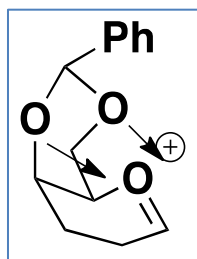
Again starting from the commercially available peracetylated galactose **6**, thioglycoside **21** was achieved with activation of the anomeric acetyl group using  $\text{BF}_3 \cdot \text{OEt}_2$  in the presence of Ethanethiol in 76% yield. Deacetylation followed by the direct installation of a 4,6-O-benzylidene protecting group gave compound **23**. This was then exposed to benzyl bromide under basic conditions to yield the final galactose donor **24** in 53% over three steps.

With the modified terminal galactose building block **24** and the core disaccharide **19** in hand, we then proceeded in the hopes of increasing the anomeric purity for the final glycosylation step (**Scheme 2-9**)



**Scheme 2-9** Improved glycosylation using benzylidene protected terminal galactose building block **24**.

To our delight, this modification proved to have a big impact on the stereoselectivity of the glycosylation, producing only the desired  $\alpha$ -anomer in 52% yield. Although the exact mechanism leading to this stereochemical outcome is not completely understood, there are some arguments one can propose to explain this phenomenon. With the benzylidene installed, free rotation around the C-6 position is now hindered. Upon formation of the oxocarbenium ion, the oxygen atoms at positions C-4 and C-6 are now in a better position to donate electron density toward the anomeric position than was possible in the previous case, effectively sterically blocking an equatorial attack from the acceptor hydroxyl with the added bulk of a phenyl group (**Figure 2-9**).



**Figure 2-9** Argument for the increased stereoselectivity due to presence of benzylidene protection.

The purity and anomeric selectivity were verified by  $^1\text{H}$  NMR (Figure 2-10) and  $^1\text{H}$  coupled HSQC (Figure 2-11) spectroscopy.

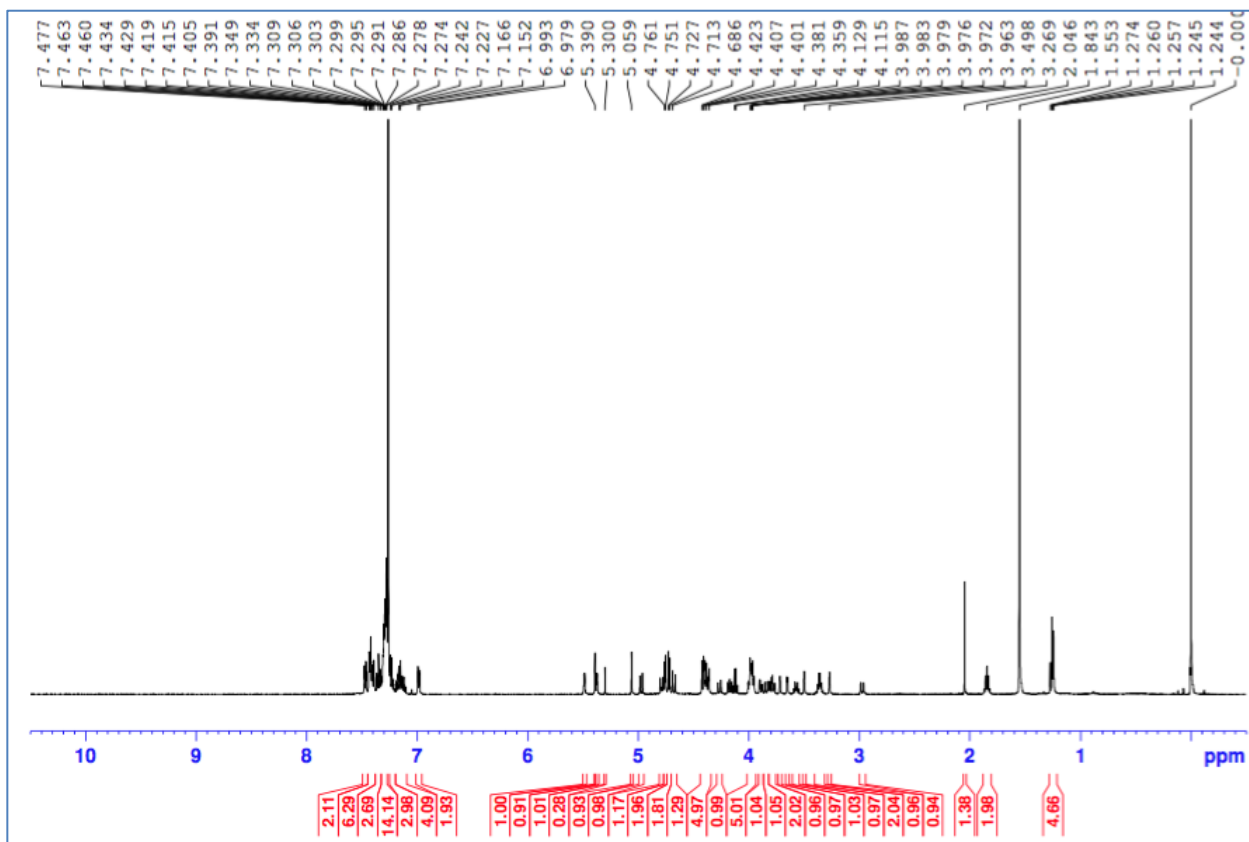


Figure 2-10  $^1\text{H}$  NMR spectra of compound 25

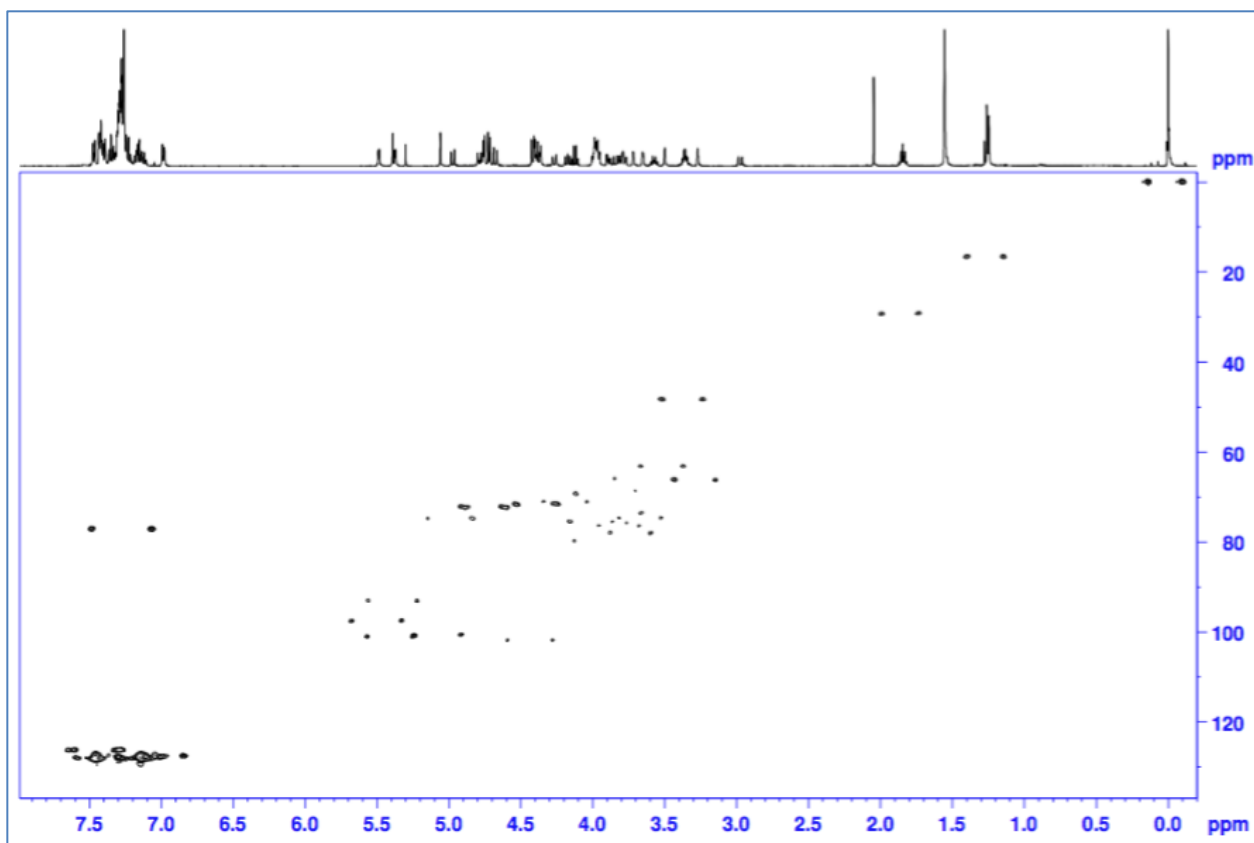
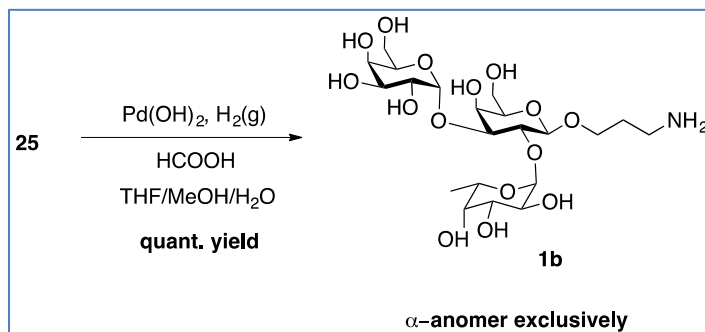


Figure 2-11  $^1\text{H}$  coupled HSQC spectra of compound 25

Global deprotection again provided the target B-antigen trisaccharide **1b** in quantitative yield (23 mg) as shown in **Scheme 2-10**.



Scheme 2-10 Final deprotection to obtain purely  $\alpha$ -anomer of the B-antigen, **1b**.

Upon the success of this anomerically pure B-antigen, we then focused our efforts on scaling up the synthesis to provide the first known gram-scale quantity of a man-made B-antigen.

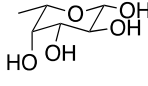
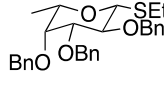
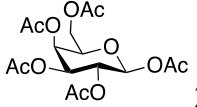
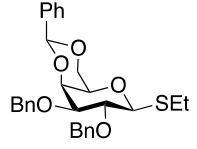
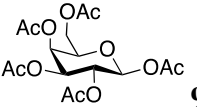
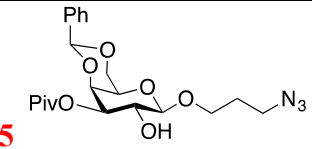
### 2.3 Gram-scale stereoselective synthesis of B-antigen

The first goal that we had in this project was to synthesize our B-antigen under strict time constraints according to a timeline defined by the Department of Defense (DoD). Because of this, scale and stereoselectivity were not an issue in our Phase I priorities. However, one of the goals of Phase II defined by the DoD proposal stipulated that we were to produce 2-3g in total of the B-antigen for our collaborators at Lynntech Inc. This achievement would mark the first successful gram-scale synthesis of a manmade B-antigen.

The scale-up began with a back calculation to determine the quantities of starting materials we would need to begin large-scale trials of the synthetic routes done on small-scale before. 3-10 g of each starting material (**4**, **5** and **24**) was found to be a sufficient amount of each to begin. Our synthetic design turned out to be very scalable and modifications to procedures were only needed in late stage glycosylation reactions. Yields were also very consistent with what was observed during the small-scale production.

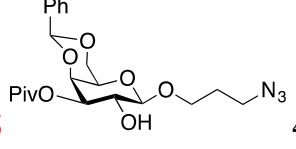
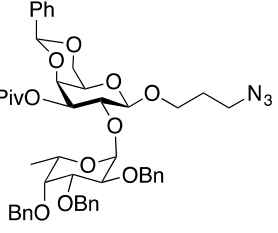
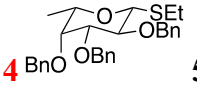
The gram-scale preparations of our monosaccharide building blocks follow the exact procedures as before. The amounts of each building block are described in **Table 2-1**.

**Table 2-1 Gram-scale preparation of monosaccharide building blocks from commercially available materials**

Starting Material/Amount	Steps	Building Block/Amount
 <b>10 g</b>	<b>4</b>	 <b>4</b> <b>10.6 g</b>
 <b>20 g</b>	<b>4</b>	 <b>24</b> <b>10.2 g</b>
 <b>9.9 g</b>	<b>5</b>	 <b>5</b> <b>3.3 g</b>

Following the same route, the next step was the large-scale glycosylation of donor **4** and acceptor **5**. The amounts of each used and the amount of material obtained and the yield are shown in **Table 2-2**.

**Table 2-2 Gram-scale preparation of core disaccharide**

Starting Material/Amount	Product/Amount
 <b>5</b> <b>4.0 g</b>	 <b>18</b> <b>3.8 g (51%)</b>
 <b>4</b> <b>5.72 g</b>	

With the removal of the pivaloyl group from compound **18**, We then had 2.7g total of the acceptor **19** that would all be used in the final glycosylation as shown in **Table 2-3**.

Table 2-3 Gram-scale preparation of protected B-antigen

Starting Material/Amount	Product/Amount
<p><b>19</b> 2.7 g</p>	<p><b>25</b> 2.18 g(52%)</p>
<p><b>24</b> 2.08 g</p>	

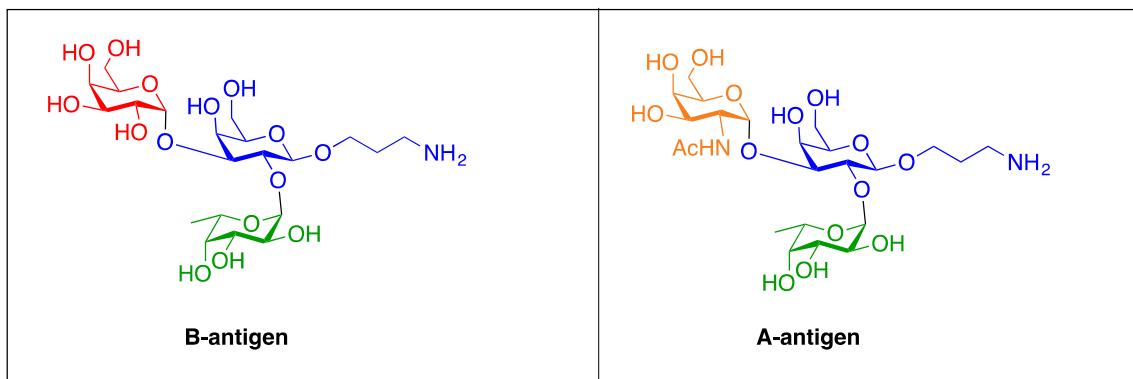
This afforded us 2.18 g of the protected trisaccharide in 52% yield. With this material in hand, the only thing left was a global deprotection using palladium hydroxide as before. On this larger scale, the deprotection took almost twice as long to accomplish. Once the deprotection was finished, we had obtained the first known gram-scale quantity of man-made B-antigen. This is summed up in **Table 2-4**.

Table 2-4 Gram-scale global deprotection to obtain B-antigen

Starting Material/Amount	Product/Amount
<p><b>25</b> 2.18 g</p>	<p><b>1b</b> 1.17g(&gt;99%)</p>

## 2.4 Progress toward A-antigen

The structural similarities between the two target compounds (A and B-antigens) are readily apparent (**Figure 2-12**), as the only difference is one sugar residue.



**Figure 2-12** Side by side comparison of A and B-antigens.

There are unique challenges that arise from the presence of an N-acetyl galactosamine residue, however, that did not pose a problem for the B-antigen synthesis. First, since the acetyl group at the C-2 position on the galactosamine residue would act as a participating group as shown in **Figure 2-13** (giving undesired  $\beta$ -selectivity upon glycosylation), this needed to be installed late-stage.

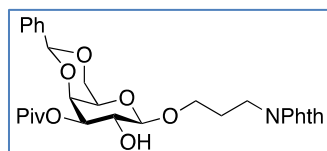


**Figure 2-13** Acetyl group acting to inhibit alpha selectivity in glycosylation reaction



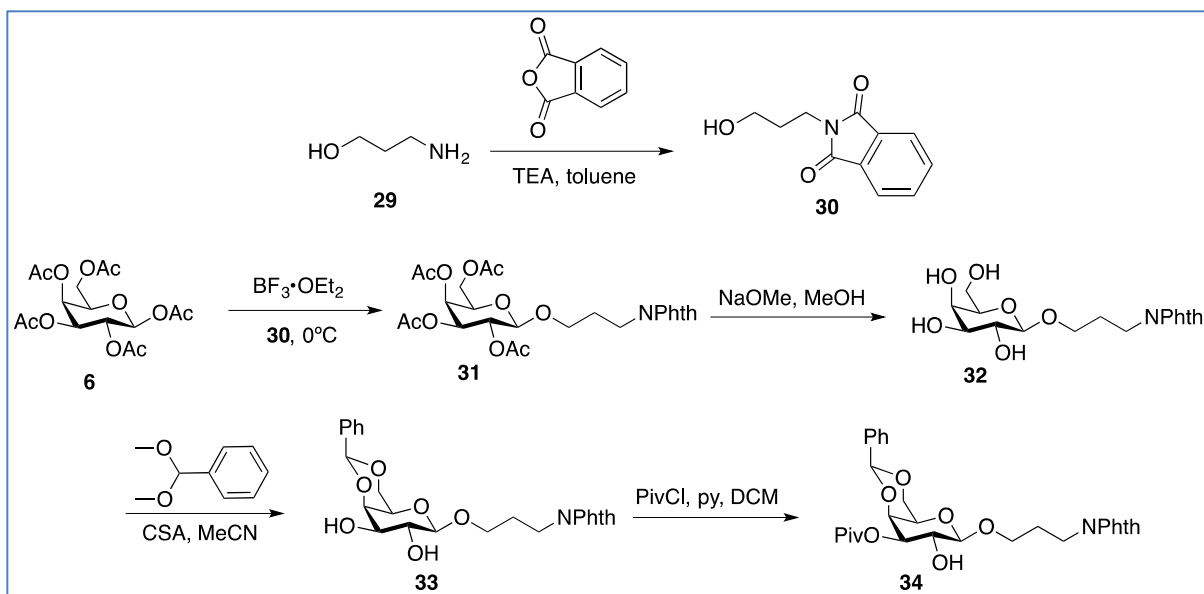
This brings up an issue of assigning orthogonally protected amines throughout the synthesis since the linker needs to be a free amine group upon completion of the synthesis. This chapter is devoted to an array of efforts we've taken, in chronological order, to achieve a concise synthesis of the A-antigen for use in our universal plasma device.

The first synthetic strategy that was imagined involved modifying the central galactose building block as shown in **Figure 2-14**.



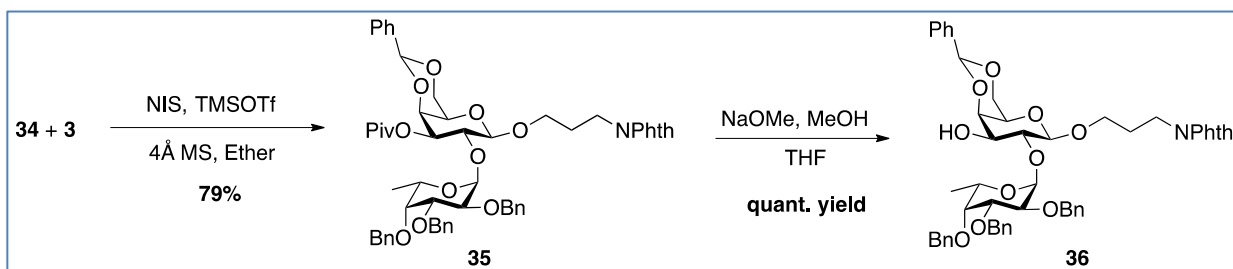
**Figure 2-14 Modified central galactose building block.**

The shift from an azide-protected linker to a phthalamide protection would allow for orthogonality in the presence of an azide protected galactosamine building block. The synthetic strategy to make this building block was very similar to that of compound **5** and is outlined in **Scheme 2-11**.



**Scheme 2-11** Synthesis of modified central galactose building block possessing phthalamide linker.

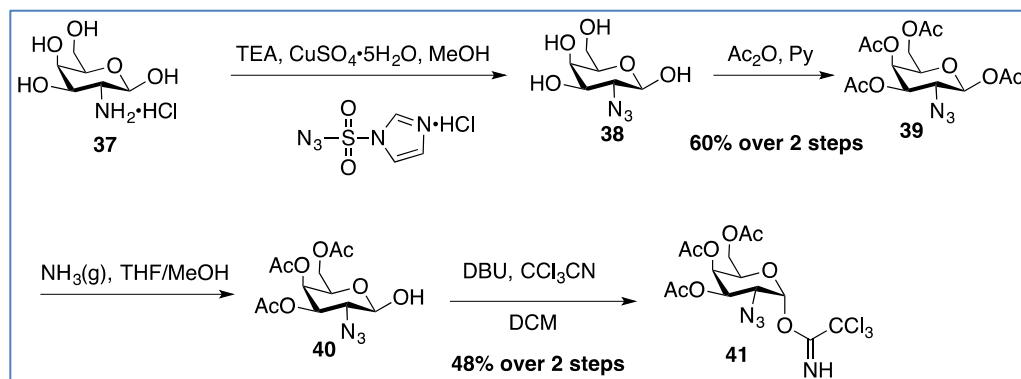
The linker was first made prior to installation by exposing 3-aminopropanol to phthalic anhydride in the presence of base. Compound **30** was recovered in high yield. Galactose **6** was then O-glycosylated with the linker in the presence of  $\text{BF}_3 \cdot \text{OEt}_2$ . Deacetylation and installation of the benzylidene and pivaloyl protecting groups gave compound **34**. Assembly of the disaccharide **35** was achieved in good yield through the glycosylation of previously made donor **3** and **34** as shown in **Scheme 2-12**.



**Scheme 2-12** Synthesis of disaccharide **35** with phthalamide linker installed.

Deblocking of the 3-O position of the galactose residue yielded acceptor **36** quantitatively.

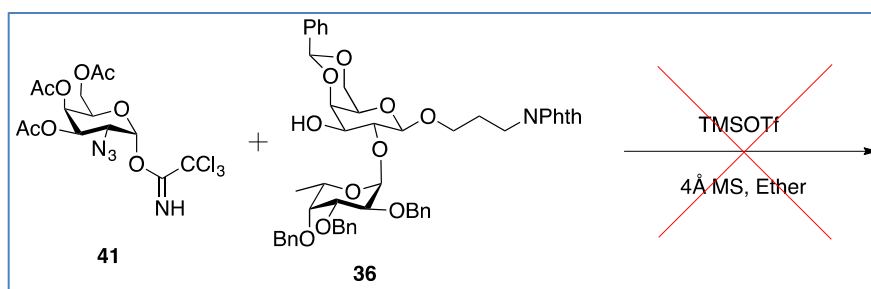
The donor for the glycosylation with acceptor **36** needed to incorporate an amine protecting group; it was therefore decided to mask the amine as an azide. The route to producing the trichloroacetimidate donor used is shown in **Scheme 2-13**.



**Scheme 2-13** Synthesis of donor **41**

Commercially available galactosamine hydrochloride **37** was treated with an azide transfer reagent<sup>25</sup> yield **38**. This material was used crude in an acetylation reaction using a pyridine base and acetic anhydride to obtain compound **39** in 60% over 2 steps. Liberation of the anomeric hydroxyl group using ammonia in a MeOH/THF solvent mix followed by subsequent installation of the trichloroacetimidate leaving group gave the desired donor **41** in 48% yield over 2 steps.

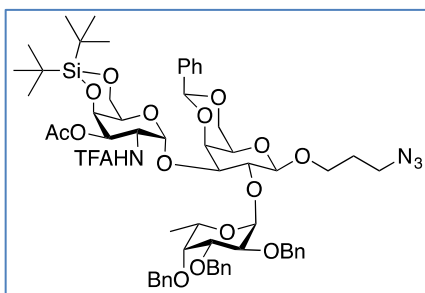
Unfortunately, the acceptor **36** proved to be unreactive with the trichloroacetimidate donor **41** as shown in **Scheme 2-14**.



**Scheme 2-14** Attempted glycosylation with acceptor **36**

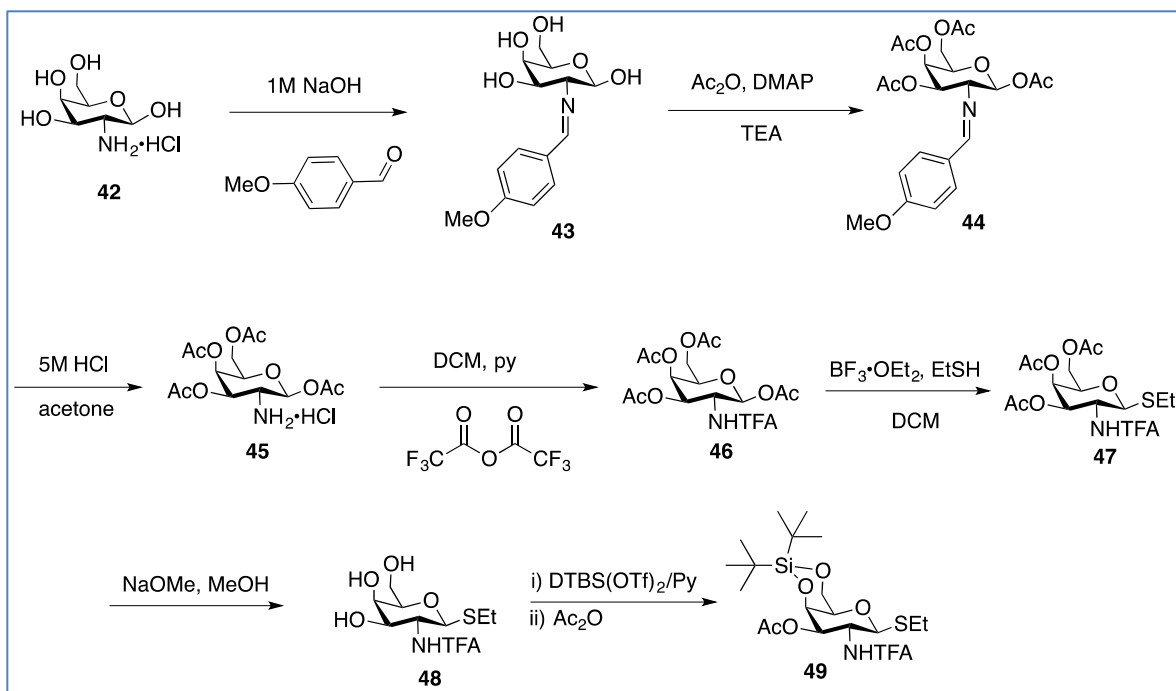
It is possible that the phthalamide group present on the amine linker hindered reactivity of the acceptor. One possibility could be due to  $\pi$ -stacking of the aromatic portion of the phthalamide functionality, sterically hindering approach of the donor.

At this point a new strategy needed to be devised to obtain the A-antigen. A new target was imagined and is shown in **Figure 2-15**.



**Figure 2-15** Second proposed intermediate to the A-antigen

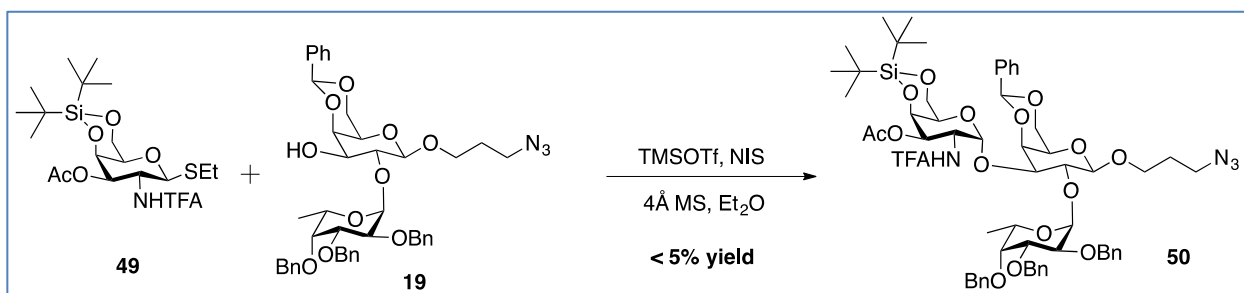
This new route made use of a thioglycoside galactosamine donor, **49** (shown in **Scheme 2-15**), and had the advantage of using the same core disaccharide acceptor **19** as used before in the production of both the first and second generation syntheses of the B-antigen. The thioglycoside donor features a 4,6-O-di-tert-butylsilyl (DTBS) protecting group as well as a trifluoroacetyl (TFA) protection of the amine. The synthesis of this donor is shown below in **Scheme 2-15**.



**Scheme 2-15 Synthesis of the galactosamine donor 49**

This donor had previously shown to give exclusive  $\alpha$ -anomeric stereoselectivity despite the participating group (TFA) adjacent to the anomeric center<sup>22</sup>, and was deemed suitable for our needs as an orthogonal donor to the acceptor **19**.

Once obtaining the donor and already having the acceptor made previously, we ran the glycosylation as shown in **Scheme 2-16**.

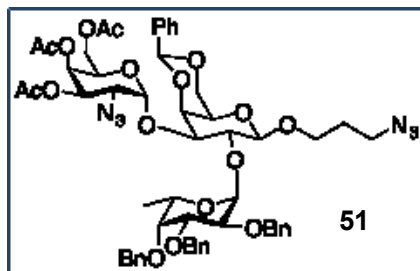


**Scheme 2-16 Glycosylation between donor 49 and disaccharide acceptor 19.**

Although some trisaccharide product formation was observed by HRMS, the yield was much too low to isolate via column chromatography and optimization did not improve these yields.

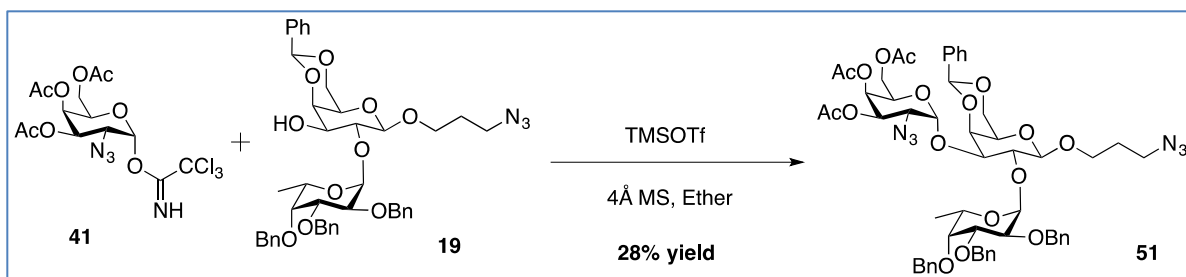
Additionally, the reaction was very hard to monitor as the donor **49** ran at the same  $R_f$  value as the product **50**. This also meant that unless the entire quantity of donor introduced into the system was consumed during the reaction, which it was not, the purification would be very hard to perform. For these reasons, this strategy to make the A-antigen was abandoned and another route was proposed instead.

After coming across a paper from the Chi-Huey Wong's lab detailing a method for a regioselective azide reduction<sup>23</sup>, we imagined a route to the A-antigen utilizing this novel method. The proposed trisaccharide intermediate to achieve this goal is shown in **Figure 2-16**.



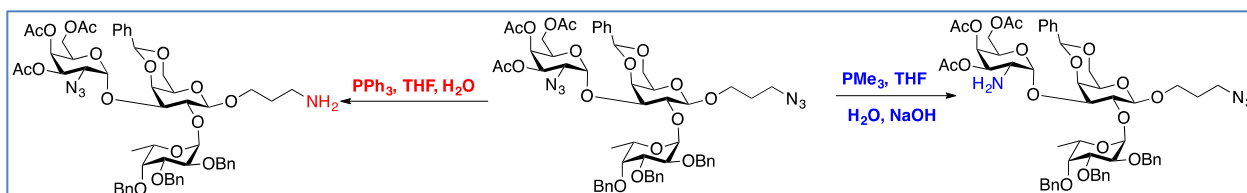
**Figure 2-16** Intermediate for selective azide reduction towards synthesis of the A-antigen

To arrive at this intermediate, the previously synthesized donor **41** was activated with TMSOTf in the presence of the previously synthesized acceptor **19** as shown in **Scheme 2-17** to give the trisaccharide **51** in a low 28% yield on a scale of 27 mg.



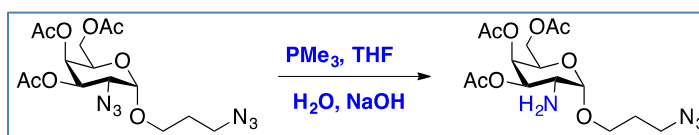
**Scheme 2-17 Synthesis of trisaccharide diazide 51**

The idea behind creating this diazide was that the two azide groups are unique in both electronic properties and steric environments. The primary azide is less sterically hindered and in theory could be selectively reduced with the use of a bulky phosphine reagent like triphenylphosphine. Alternatively, the secondary azide is adjacent to an anomeric center, making this azide electronically less dense and a better electrophile in the presence of a smaller phosphine reagent like trimethylphosphine. This strategy is illustrated in **Figure 2-17**.

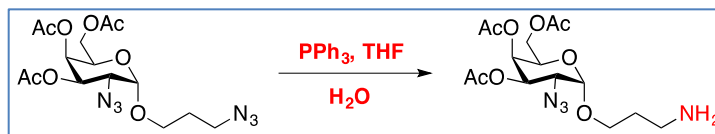


**Figure 2-17 Site selective azide reduction using different phosphine reagents.**

Using test substrates, the efficacy of this method is shown in **Schemes 2-18 & 2-19** and it was determined that this could indeed be a viable route. However the low yield of trisaccharide **51** did not stop us from developing alternative methods in parallel.

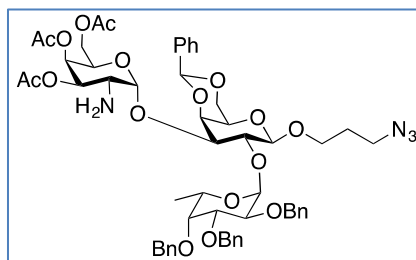


**Scheme 2-18 Selective reduction of the secondary azide using trimethylphosphine**



**Scheme 2-19** Selective reduction of the primary azide using triphenylphosphine

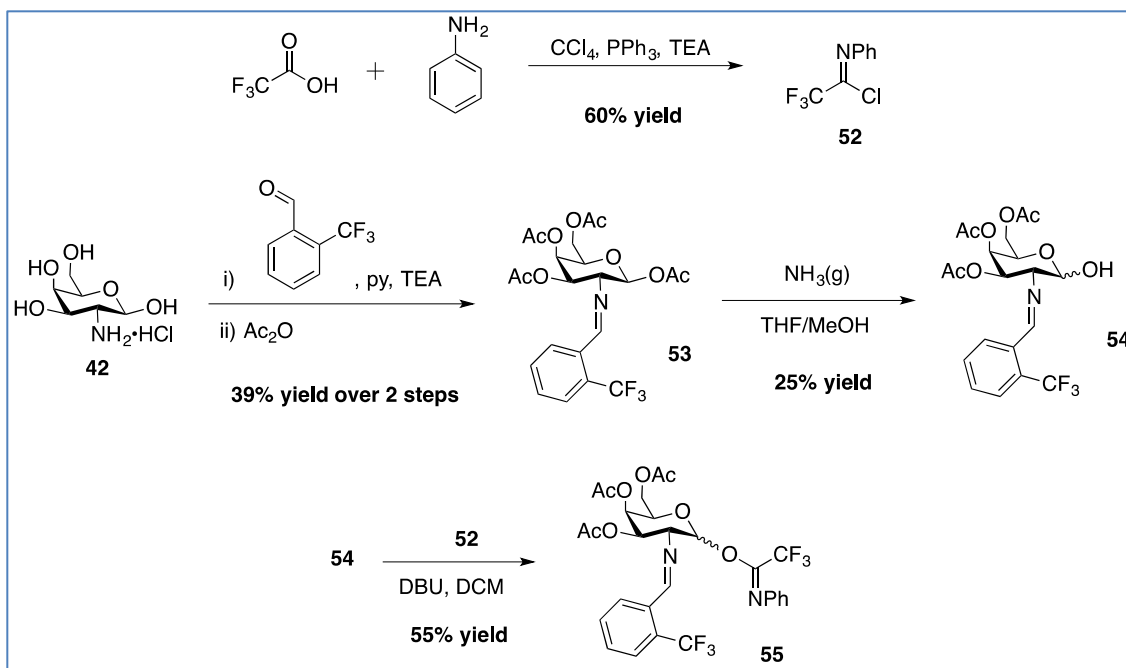
Alongside the development of this selective azide reduction strategy we were also developing another strategy that proved more fruitful. The trisaccharide intermediate to obtain the A-antigen for this strategy can be seen in **Figure 2-18**.



**Figure 2-18** Trisaccharide intermediate with free secondary amine.

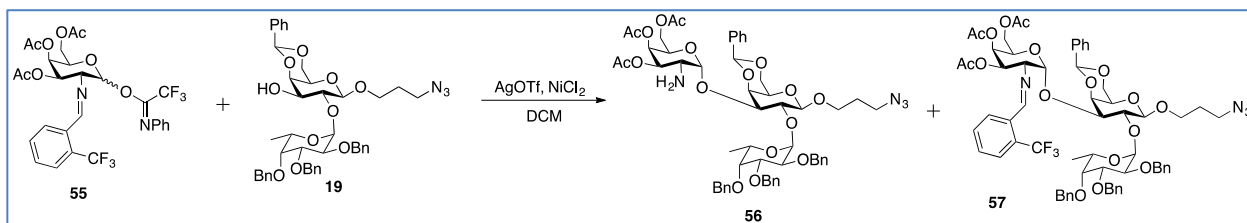
The simplicity of this intermediate was achieved because of an unintended result of the final glycosylation between the disaccharide acceptor **19** and the trifluoroacetimidate donor **55**, whose synthesis is shown in **Scheme 2-20**<sup>26,27</sup>.





**Scheme 2-20 Production of trifluoroacetimidate donor 55.**

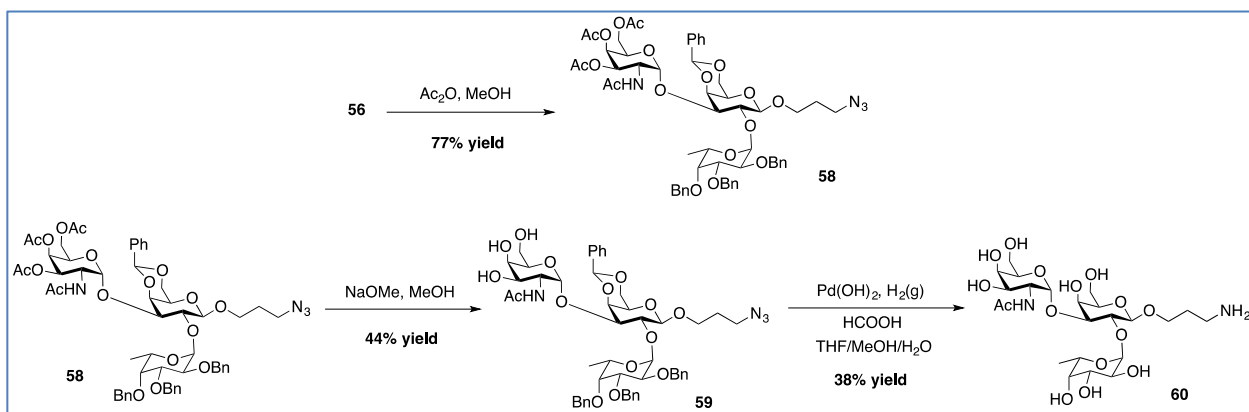
With the trifluoroacetimidate donor in hand and the acceptor made previously, we ran the glycosylation reaction as shown in **Scheme 2-21**.



**Scheme 2-21 Preparation of the trisaccharide intermediate 56.**

The expected product **57** as well as the unexpected cleavage of the benzylideneamino protecting group to give compound **56** was both obtained. Of course, compound **57** can be further converted to trisaccharide **56** with an additional deprotection step. However, with prolonged exposure time to the nickel(II) catalyst, it was observed that a larger ratio of the deprotected compound **56** is obtained. This could likely be optimized to obtain the free amine exclusively in

high yield. After obtaining compound **56**, the rest of the synthetic route proved to be trivial and is illustrated in **Scheme 2-22**.



**Scheme 2-22 Acetylation followed by deprotection to obtain A-antigen.**

Compound **56** was acetylated using acetic anhydride in methanol to obtain compound **58** in 77% yield. Next was the deprotection sequence that consisted of a deacetylation and a palladium reduction to obtain the A-antigen **60** equipped with an amine linker. So far, only small-scale trials of this method have been achieved (5 mg of A-antigen). Plans on scaling up this route in the near future as per the requirements of the DoD grant are underway.

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## Experimental

### **Ethyl $\beta$ -D-galactothiopyranoside (8):**

To a solution of galactose 6 (5g, 12.809 mmol) dissolved in  $\text{CH}_2\text{Cl}_2$  (15 mL) under inert nitrogen atmosphere was added ethanethiol (1.3 mL, 17.933 mmol). The mixture was cooled to  $0^\circ\text{C}$  and  $\text{BF}_3\cdot\text{OEt}_2$  (2.68 mL, 21.264 mmol) was added dropwise. The mixture was allowed to stir for 2 h. A saturated solution of  $\text{NaHCO}_3$  was added to the mixture and the aqueous layer was extracted with  $\text{CH}_2\text{Cl}_2$  (3 x 50 mL). The combined organic layers were washed with a brine solution and dried over  $\text{Na}_2\text{SO}_4$ . The crude material, galactose 7, was used directly without purification in the next step. To a solution of galactose 7 (12.809 mmol) dissolved in MeOH (80 mL) under a nitrogen atmosphere. NaOMe was generated in situ by adding metallic sodium (31.65 mg, 0.1 mmol). The mixture was allowed to stir for 12 h. The solvent was evaporated off under reduced atmosphere and the crude material was purified by a short column filtration (starting with 10% MeOH in  $\text{CH}_2\text{Cl}_2$ ). Pure galactose 8 (5.4g, 90% over 2 steps) was obtained.  $^1\text{H}$  NMR (400 MHz, MeOD):  $\delta$  4.34 (d, 1H,  $J = 9.6$  Hz), 3.90 (dd, 1H,  $J = 0.4$  Hz, 3.2 Hz), 3.78-3.68 (m, 2H), 3.59-3.52 (m, 2H), 3.47 (dd, 1H,  $J = 3.2, 9.2$ ), 3.33 (m, 2H), 2.84-2.73 (m, 2H), 1.3 (t, 3H).

The spectroscopic data is in agreement with literature data [11].

### **Ethyl 2,3,4,6-tetra-benzyl- $\beta$ -D-galactothiopyranoside (3):**

A solution of galactose 8 (5.4 g, 24.099 mmol) dissolved in DMF (25 mL) was cooled down to  $0^\circ\text{C}$ . NaH (5.784 g, 144.6 mmol) was added to the reaction in a solution of DMF (20 mL). The mixture was allowed to stir at  $0^\circ\text{C}$  for 30 minutes. Benzyl Bromide (16.65 mL, 108.448 mmol) was added and the mixture was allowed to stir for 18 h. To the reaction flask was added some ice,

and then water. The mixture was extracted with diethyl ether (3 x 50 mL) and the combined organic layers were washed with water. Flash column chromatography (starting at 5% EtOAc in hexanes) was used to purify the crude material. Pure galactose donor 3 (4.7g, 33% yield) was obtained. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 7.40-7.25 (m, 22H), 4.94 (d, 1H, *J* = 11.6), 4.85 (d, 1H, *J* = 10), 4.78 (d, 1H, *J* = 10), 4.72 (s, 2H), 4.61 (d, 1H, *J* = 12), 4.47-4.39 (m, 3H), 3.95 (d, 1H, *J* = 2.8), 3.82 (t, 1H), 3.60-3.55 (m, 4H), 2.81-2.72 (m, 2H), 1.31-1.24 (m, 7H), 0.97-0.86 (m, 3H).

The spectroscopic data is in agreement with literature data [11].

#### **1,2,3,4-tetra-*O*-acetyl-L-fucopyranoside (10):**

Fucose 9 (5 g, 30.46 mmol) and DMAP (371 mg, 3.04 mmol) were dissolved in pyridine (30 mL). Ac<sub>2</sub>O (18 mL, 182.75 mmol) was added dropwise over 15 min. The mixture was allowed to stir for 20 h. The mixture was concentrated under vacuum and diluted with ethyl acetate. The organic layer was washed with water, aqueous NaHCO<sub>3</sub>, 1M HCl and a brine solution. The organic layer was concentrated and the crude material was purified by flash column chromatography. Pure Fucose 10 (9.9 g, 98% yield) was obtained. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 6.34 (d, 1H, *J* = 2.4 Hz), 5.52-5.27 (m, 4H), 5.20-5.06 (m, 1H), 4.3-4.22 (m, 1H), 4.12 (q, 1H), 4.00-3.95 (m, 1H), 2.19-2.00 (m, 21H), 1.60 (d, 1H, *J* = 2.4 Hz), 1.32-1.22 (m, 4H), 1.16 (d, 3H, *J* = 2.4 Hz).

The spectroscopic data is in agreement with literature data [12].

#### **Ethyl 2,3,4-tri-acetyl-β-L-fucothiopyranoside (11):**

Fucose 10 (9.9 g, 29.79 mmol) was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (150 mL, 0.2M). Ethanethiol (3 mL, 41.708 mmol) was added to the flask and the solution was cooled to 0°C. BF<sub>3</sub>•OEt<sub>2</sub> (6.24 mL, 49.45 mmol) was added to the mixture dropwise. The mixture was allowed to stir for 18 h. The

reaction was quenched with aqueous NaHCO<sub>3</sub>. The aqueous layer was extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 x 100 mL). Pure Fucose 11 (5 g, 50% yield) was obtained by flash column chromatography. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 5.28 (d, 1H, *J* = 0.8 Hz), 5.23 (t, 1H, *J* = 10 Hz), 5.05 (dd, 1H, *J* = 3.2 Hz, 10 Hz), 4.45 (d, 1H, *J* = 9.6 Hz), 4.12 (q, 0.2H), 3.85-3.80 (m, 1H), 2.79-2.66 (m, 2H), 2.18 (s, 3H), 2.07 (s, 3H), 1.99 (s, 3H), 1.60 (s, 1H), 1.28 (t, 3H), 1.22 (d, 3H, *J* = 6.4 Hz).

The spectroscopic data is in agreement with literature data [12].

#### **Ethyl 2,3,4-tri-benzyl-β-L-fucothiopyranoside (4):**

Fucose 11 (5g, 14.953 mmol) was dissolved in MeOH (75 mL, 0.2M). NaOMe was generated in situ by adding metallic sodium (34.4 mg, 1.495 mmol). The reaction was allowed to stir for 18 h. Amberlite H form resin (3.5 g) was added to the solution and allowed to stir for 15 min. The resin was filtered out and the solution was coevaporated with toluene twice. The crude material, Fucose 12 (3.3 g) was used directly in the next step.

Crude Fucose 12 was dissolved in DMF (30 mL) and the solution was cooled to 0°C. NaH (3.59 g, 89.72 mmol) was added to the mixture and was allowed to stir for 30 min. Benzyl bromide (10.33 mL, 67.29 mmol) was added to the solution and was allowed to stir at 0°C for 1 h. The mixture was cooled to rt and was allowed to stir for 15 h. Ice was added to the reaction, followed by water. The mixture was extracted with diethyl ether (3 x 100 mL). The crude material was purified by flash column chromatography (using Hexanes/EtOAc mixture) to obtain a pure sample of building block donor 4 (6.3 g, 88% yield over 2 steps). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 7.41-7.25 (m, 18H), 5.0 (d, 1H, *J* = 12 Hz), 4.89 (d, 1H, *J* = 10.4 Hz), 4.79 (d, 1H, *J* = 10.4 Hz), 4.75 (d, 2H, *J* = 3.6 Hz), 4.69 (d, 1H, *J* = 12 Hz), 4.39 (d, 1H, *J* = 9.6 Hz), 3.82 (t, 1H, *J* = 9.6 Hz, 9.4



Hz), 3.61 (d, 1H,  $J = 2$  Hz), 3.56 (dd, 1H,  $J = 2.8$  Hz, 9.2 Hz), 3.47 (q, 1H), 2.82-2.71 (m, 2H), 1.57 (s, 1H), 1.30 (t, 3H), 1.20 (d, 3H)

The spectroscopic data is in agreement with literature data [12].

### **3-azidopropanol (14):**

Starting material 13 (6.5 mL, 71.95 mmol) was dissolved in a mixture of acetone (120 mL) and water (20 mL). To this solution was added  $\text{NaN}_3$  (7.67 g, 118 mmol). This solution was allowed to reflux for 18 h. then was cooled to rt. The acetone was evaporated off and the aqueous layer was further diluted with 100 mL of water. This aqueous solution was extracted with diethyl ether (3 x 100 mL) and the resulting organic layer was concentrated and purified by flash column chromatography (using a solvent system of hexanes/EtOAc). Pure azide linker 14 (4.2 g, 57% yield) was obtained.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  3.78 (s, 2H), 3.46 (t, 2H), 1.87-1.81 (m, 2H).

### **3-azidopropyl**

### **2,3,4,6-tetra-O-acetyl- $\beta$ -D-galactopyranoside (15):**

Galactose 6 (5.35 g, 13.709 mmol) and linker 14 (4.2 g, 41.128 mmol) were dissolved in  $\text{CH}_2\text{Cl}_2$  (140 mL, 0.1M). The solution was cooled to  $0^\circ\text{C}$  and  $\text{BF}_3 \cdot \text{OEt}_2$  (5.07 mL, 41.128 mmol) was added dropwise. The mixture was allowed to stir at  $0^\circ\text{C}$  for 2 h and was then warmed to rt and stirred for 14 h. The reaction was quenched with aqueous  $\text{NaHCO}_3$  and was washed with a brine solution. The aqueous layer was extracted with  $\text{CH}_2\text{Cl}_2$  (3 x 75 mL) and the resulting organic layer was concentrated under vacuum. The crude material was purified by flash column chromatography (using a solvent system of hexanes/EtOAc). Pure galactose 15 (3.8 g, 64% yield) was obtained.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  5.39 (dd, 1H,  $J = 1.2$  Hz, 3.6 Hz), 5.20 (dd, 1H,  $J = 8$  Hz, 10.4 Hz),

5.02 (dd, 1H,  $J = 3.6$  Hz, 10.8 Hz), 4.47 (d, 1H,  $J = 8$  Hz), 4.18 (dd, 1H,  $J = 6.4$  Hz, 11.2 Hz), 4.12 (dd, 1H,  $J = 6.8$  Hz, 11.2 Hz), 3.97 (ddd, 1H,  $J = 5.2$  Hz, 5.2 Hz, 10 Hz), 3.91 (ddd, 1H, 6.8 Hz, 6.8 Hz, 0.8 Hz), 3.76 (dd, 1H,  $J = 6$  Hz, 11.2 Hz), 3.64-3.59 (m, 1H), 3.46 (t, 1H), 3.40-3.36 (m, 2H), 2.16 (s, 3H), 2.07 (s, 3H), 2.06 (s, 3H), 1.99 (s, 3H), 1.95-1.78 (m, 3H), 1.60 (d, 3H,  $J = 0.8$  Hz).

The spectroscopic data is in agreement with literature data [13].

### **3-azidopropyl 4,6-*O*-benzylidene- $\beta$ -D-galactopyranoside (17):**

Galactose 15 (3.8 g, 8.809 mmol) was dissolved in MeOH (45 mL, 0.2M). NaOMe was generated in situ by adding metallic sodium (60 mg, 2.643 mmol) to the solution. This mixture was heated to 35°C and was allowed to stir for 2 h. The reaction was cooled to rt and Amberlite H form resin (1.5 g) was added to the mixture and allowed to stir for 15 min. The resin was filtered off and the solution was coevaporated with toluene twice. This crude material was used directly in the next step of the synthesis.

Crude galactose 16 (8.809 mmol) was dissolved in acetonitrile (35 mL, 0.25M). To this solution was added benzaldehyde dimethyl acetal (2 mL, 13.105 mmol) and CSA (200 mg, 0.8737 mmol). The reaction was allowed to stir for 10 h. The reaction was quenched with triethylamine (4 mL) and the mixture was evaporated with toluene. Flash column chromatography (Hexanes/EtOAc) was used to purify the crude material. Pure galactose 17 (2.187 g, 70.7% yield over 2 steps) was obtained.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.52-7.14 (m, 25H), 5.56 (s, 1H), 3.34 (dd, 1H,  $J = 1.6$  Hz, 12.6 Hz), 4.29 (d, 1H,  $J = 7.6$  Hz), 4.21 (dd, 1H,  $J = 0.8$  Hz, 4 Hz), 4.09 (dd, 1H,  $J = 2$  Hz, 12.6 Hz), 4.07-4.02 (m, 1H), 3.79-3.67 (m, 3H), 3.51-3.44 (m, 3H), 2.56 (s, 1H), 2.51 (d, 1H,  $J = 8.8$  Hz), 1.99-1.88 (m, 2H), 1.58 (s, 5H).

The spectroscopic data is in agreement with literature data [13].

**3-azidopropyl 4,6-*O*-benzylidene-3-pivaloyl- $\beta$ -D-galactopyranoside (5):**

Galactose 17 (2.187 g, 6.22 mmol) was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (70 mL). To this solution was added pyridine (1.729 mL, 3.6M) and PivCl (1.15 mL, 9.337 mmol). This mixture was allowed to stir for 12 h. The reaction was quenched with MeOH and the mixture was coevaporated with toluene twice. Pure galactose acceptor 5 (2.1178, 78% yield) was obtained by flash column chromatography. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  7.49-7.16 (m, 11H), 5.51 (s, 1H), 4.82 (dd, 1H,  $J = 4$  Hz, 10 Hz), 4.39-4.32 (m, 3H), 4.09-4.0 (m, 3H), 3.68-3.62 (m, 1H), 3.51 (d, 1H,  $J = .8$  Hz), 3.45-3.42 (m, 2H), 2.35 (s, 4H), 1.94-1.87 (m, 2H), 1.61 (s, 2H).

**3-azidopropyl-2,3,4-tri-*O*-benzyl- $\alpha$ -l-fucopyranosyl-(1 $\rightarrow$ 2)-4,6-*O*-benzylidene-3-*O*-pivaloyl- $\beta$ -d-galactopyranoside (18):**

To a solution of fucose donor 5 (0.5 g, 1.04 mmol) and galactose acceptor 4 (0.55 g, 1.25 mmol) in 14 mL of dry ether was added 4 Å molecular sieves and the mixture was stirred at ambient temperature for 45 min. The mixture was then cooled to 0°C and was added *N*-iodosuccinimide (0.3 g, 1.35 mmol) followed by drop-wise addition of a solution of trimethylsilyl triflate (19  $\mu$ L, 0.104 mmol) in ether (1 mL). The mixture was further stirred for 30 min at the same temperature and slowly warmed up to room temperature. After 1 h the reaction mixture was diluted with ether, filtered thru a bed of Celite, and quenched with 10% aqueous solution of Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>. The organic layer was separated and washed with saturated NaHCO<sub>3</sub> solution and brine. Organic layer was dried with Na<sub>2</sub>SO<sub>4</sub>, filtered, and evaporated. Crude residue was purified by flash chromatography to yield disaccharide 18 as pale-yellow solid (0.53 g, 60% yield). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$

7.49-7.47 (m, 2H), 7.37-7.24 (m, 18H), 5.47 (s, 1H), 5.42 (d, 1H,  $J=3.6$  Hz), 5.07 (dd, 1H,  $J=9.6$  Hz, 4 Hz), 4.99 (d, 1H,  $J=12$  Hz), 4.78 (d, 1H,  $J=12$  Hz), 4.77 (d, 1H,  $J=11.6$  Hz), 4.70 (d, 1H,  $J=12.4$  Hz), 4.67 (d, 1H,  $J=12$  Hz), 4.61 (d, 1H,  $J=11.6$  Hz), 4.53 (d, 1H,  $J=8$  Hz), 4.44 (d, 1H,  $J=2.4$  Hz), 4.31 (dd, 1H,  $J=10.8$  Hz, 4.8 Hz), 4.25 (dd, 1H,  $J=9.6$  Hz, 7.8 Hz), 4.09 (dd, 1H,  $J=10.2$  Hz, 4.4 Hz), 4.04 (dd, 1H,  $J=11.2$  Hz, 1.6 Hz), 3.99-3.92 (m, 2H), 3.69 (d, 1H,  $J=1.6$  Hz), 3.55 (ddd, 1H,  $J=9.8$  Hz, 5.8 Hz, 5.8 Hz), 3.47 (app s, 1H), 3.32 (t, 2H,  $J=6.8$  Hz), 1.81 (quin, 2H,  $J=6.5$  Hz), 1.13 (d, 3H,  $J=6.8$  Hz), 1.11 (s, 9H).  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  178.0, 138.7, 138.6, 138.3, 137.6, 128.8, 128.4, 128.3, 128.2, 128.1, 127.6, 127.5(2), 126.0, 101.8, 100.6, 96.9, 79.6, 77.7, 77.2, 76.5, 75.9, 74.8, 73.6, 72.7, 70.4, 69.0, 66.4, 66.2, 66.1, 48.3, 38.9, 29.2, 27.0, 16.6. HRMS-ESI:  $m/z$   $\text{C}_{48}\text{H}_{61}\text{N}_4\text{O}_{11}$   $[\text{M}+\text{NH}_4]^+$  calcd 869.4331, found 869.4336.

**3-azidopropyl-2,3,4-tri-*O*-benzyl- $\alpha$ -l-fucopyranosyl-(1 $\rightarrow$ 2)-4,6-*O*-benzylidene- $\beta$ -d-galactopyranoside (19):**

To a solution of disaccharide 18 (0.5 g, 0.59 mmol) in 16 mL of THF/MeOH (5:3 v/v) was added sodium methoxide (0.32 g, 5.9 mmol). The mixture was heated at 40°C and stirred for 16 h. The solution was then cooled down at room temperature, quenched with Amberlite H<sup>+</sup> resin, and filtered. After evaporating solvent the crude residue was purified by short column filtration to afford 19 as white solid (0.42 g, 93% yield).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.55-7.53 (m, 2H), 7.40-7.18 (m, 18H), 5.56 (s, 1H), 5.17 (d, 1H,  $J=3.6$  Hz), 4.97 (d, 1H,  $J=11.6$  Hz), 4.82 (d, 1H,  $J=11.6$  Hz), 4.79 (d, 1H,  $J=12$  Hz), 4.76 (d, 1H,  $J=12$  Hz), 4.74 (d, 1H,  $J=11.8$  Hz), 4.65 (d, 1H,  $J=11.6$  Hz), 4.34 (d, 1H,  $J=7.6$  Hz), 4.30 (dd, 1H,  $J=12.4$  Hz, 2 Hz), 4.21 (d, 1H,  $J=1.6$  Hz), 4.07 (dd, 2H,  $J=10.2$  Hz, 4.4 Hz), 3.98-3.93 (m, 2H), 3.83 (d, 2H,  $J=5.2$  Hz), 3.67 (d, 1H,  $J=$

2.0 Hz), 3.55 (ddd, 1H,  $J = 10.2$  Hz, 7.2 Hz, 6.4 Hz), 3.44-3.35 (m, 3H), 1.83 (quin, 2H,  $J = 6.0$  Hz), 1.12 (d, 3H,  $J = 7.2$  Hz).  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  138.6, 138.5, 137.7, 137.6, 129.0, 128.5, 128.4(3), 128.3, 128.2, 128.1, 127.9, 127.6, 127.5, 127.4, 126.5, 102.0 101.3, 99.6, 79.5, 78.2, 77.3, 75.4, 74.8, 73.9, 73.2, 72.9, 69.2, 67.1, 66.6, 66.1, 48.2, 29.2, 27.0, 16.7. HRMS-ESI:  $m/z$   $\text{C}_{43}\text{H}_{53}\text{N}_4\text{O}_{10}$   $[\text{M}+\text{NH}_4]^+$  calcd 785.3756, found 785.3761.

**3-azidopropyl-2-*O*-(2,3,4-tri-*O*-benzyl- $\alpha$ -l-fucopyranosyl)-3-*O*-(2,3,4,6-tetra-*O*-benzyl- $\alpha$ -d-galactopyranosyl)-4,6-*O*-benzylidene- $\beta$ -d-galactopyranoside (20):**

To a solution of galactose donor 3 (0.356 g, 0.61 mmol) and disaccharide acceptor 19 (0.39 g, 0.508 mmol) in 12 mL of dry ether was added 4 Å molecular sieves and the mixture was stirred at ambient temperature for 1 h. The mixture was then cooled at 0°C and to it was added *N*-iodosuccinimide (0.16 g, 0.71 mmol) followed by drop-wise addition of a solution of trimethylsilyl triflate (9  $\mu\text{L}$ , 0.051 mmol) in ether (0.5 mL). The mixture was further stirred for 35 min at the same temperature and slowly warmed up to room temperature. After 10 min, the reaction mixture was diluted with ether, filtered thru a bed of Celite, and quenched with 10% aqueous solution of  $\text{Na}_2\text{S}_2\text{O}_3$ . The organic layer was separated and washed with saturated  $\text{NaHCO}_3$  solution and brine. Organic layer was dried with  $\text{Na}_2\text{SO}_4$ , filtered, and evaporated. Crude residue was purified by flash chromatography to yield trisaccharide 20 as white solid (0.37 g, 57% yield).  $^1\text{H}$  NMR (600 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.45 (d, 2H,  $J = 7.2$  Hz), 7.37 (d, 2H,  $J = 7.2$  Hz), 7.35-7.10 (m, 34H), 7.02 (d, 2H,  $J = 7.2$  Hz), 5.54 (d, 1H,  $J = 1.6$  Hz), 5.40 (s, 1H), 5.34 (d, 1H,  $J = 3.6$  Hz), 4.93 (d, 1H,  $J = 11.4$  Hz), 4.84 (d, 1H,  $J = 11.4$  Hz), 4.77 (d, 1H,  $J = 12$  Hz), 4.69 (d, 1H,  $J = 11.4$  Hz), 4.67 (d, 1H,  $J = 11.4$  Hz), 4.66 (d, 1H,  $J = 12$  Hz), 4.63 (d, 1H,  $J = 11.4$  Hz), 4.54 (d, 1H,  $J = 12$  Hz), 4.48 (d, 1H,  $J = 12$  Hz), 4.41 (d, 1H,  $J = 12$  Hz), 4.38-4.31 (m, 7H), 4.23 (d, 1H,  $J = 7.8$  Hz), 4.20 (dd, 1H,  $J = 12$  Hz, 1.3

Hz), 4.12 (dd, 1H,  $J = 9.8$  Hz, 7.8 Hz), 4.03 (dd, 1H,  $J = 7.8$  Hz, 4.8 Hz), 3.98-3.95 (m, 3H), 3.93 (dd, 1H,  $J = 10.8$  Hz, 2.1 Hz), 3.91 (ddd, 1H,  $J = 10.8$  Hz, 6 Hz, 6 Hz), 3.87 (dd, 1H,  $J = 10.2$  Hz, 4 Hz), 3.83 (dd, 1H,  $J = 10.2$  Hz, 3.4 Hz), 3.63 (app s, 1H), 3.51 (ddd, 1H,  $J = 10.2$  Hz, 6 Hz, 6 Hz), 3.48 (dd, 1H,  $J = 10.2$  Hz, 7.8 Hz), 3.43 (d, 1H, 2.2 Hz), 3.34 (t, 2H,  $J = 6.8$  Hz), 3.08 (dd, 1H,  $J = 10.2$  Hz, 4.8 Hz), 3.06 (s, 1H), 1.81 (quin, 2H,  $J = 6.0$  Hz), 1.18 (d, 3H,  $J = 6.6$  Hz).  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ ):  $\delta$  139.0, 138.9, 138.8, 138.7, 138.6(2), 138.4, 137.5, 128.4(2), 128.3(2), 128.2(2), 128.1(3), 127.9, 127.8, 127.7, 127.6, 127.5(2), 127.4(4), 127.3(2), 127.1(2), 126.2, 102.1, 100.9, 97.6, 92.3, 79.8, 78.0(2), 76.5, 76.2, 75.6, 75.3, 74.8, 74.4, 73.7, 73.0, 72.8, 72.5, 71.9, 71.8, 71.2, 70.1, 69.9, 69.3, 66.2(2), 66.0, 48.4, 29.2, 27.0, 16.7. HRMS-ESI:  $m/z$   $\text{C}_{77}\text{H}_{87}\text{N}_4\text{O}_{15}$   $[\text{M}+\text{NH}_4]^+$  calcd 1307.6162, found 1307.6166.

**3-aminopropyl-2-*O*-( $\alpha$ -l-fucopyranosyl)-3-*O*-( $\alpha$ -d-galactopyranosyl)- $\beta$ -d-galactopyranoside (1a):**

A solution of trisaccharide 20 (0.22 g, 0.17 mmol) was prepared in a mixture of THF-MeOH-H<sub>2</sub>O (25 mL, 3:3:1 v/v) and to it was added formic acid (0.5 mL, 2 vol%). Three cycles of evacuation-nitrogen purge were applied followed by addition of Pd(OH)<sub>2</sub>/C. The mixture was then evacuated and purged with H<sub>2</sub> balloon and stirred for 18 h at ambient temperature. The reaction mixture was then filtered thru a bed of Celite and solvent was evaporated. After lyophilization trisaccharide 1a was obtained as off-white solid (0.091 g, quant. yield).  $^1\text{H}$  NMR (700 MHz, D<sub>2</sub>O):  $\delta$  5.25 (d, 1H,  $J = 2.8$  Hz), 5.23 (d, 1H,  $J = 2.8$  Hz), 4.57 (d, 1H,  $J = 7.7$  Hz), 4.36-4.33 (m, 1H), 4.26 (app s, 1H), 4.22 (dd, 1H,  $J = 5.6$  Hz, 5.6 Hz), 3.96-3.13 (m, 24 H), 3.13 (t, 2H), 2.01 (quin, 2H,  $J = 6.3$  Hz), 1.21 (d, 3H,  $J = 6.3$  Hz).  $^{13}\text{C}$  NMR (175 MHz,  $\text{CDCl}_3$ ):  $\delta$  101.5, 98.7, 93.0, 75.9,

74.5, 73.1, 71.7, 71.0, 69.6, 69.3, 69.2, 68.0, 67.7, 67.2, 66.7, 63.2, 61.2, 60.9(2), 37.1, 26.8, 15.2.

HRMS-ESI:  $m/z$   $C_{21}H_{40}NO_{15}$   $[M+H]^+$  calcd 546.2392, found 546.2404.

#### **4-nitrophenyl acrylate (27):**

Dry  $K_2CO_3$  (2 g, 14.38 mmol) was suspended in a mixture of water (2 mL) and acetone (7 mL) and was cooled to 0°C. Acryloyl Chloride was added, followed by 4-nitrophenol in a mixture of water (2 mL) and acetone (7 mL). This mixture was allowed to stir at 0°C for 1 h. The  $K_2CO_3$  was filtered off and the acetone was evaporated off. The remaining aqueous solution was extracted with EtOAc (3x50 mL). The crude material was purified by flash column chromatography using a hexane/EtOAc solvent system. Pure acrylate 27 (1.387g, 50%) was obtained.  $^1H$  NMR (400 MHz,  $CDCl_3$ ):  $\delta$  8.29 (d, 2H,  $J = 9.2$  Hz), 7.34 (t, 2H), 6.66 (dd, 1H,  $J = 0.8$  Hz, 17.2 Hz), 6.33 (dd, 1H,  $J = 10.4$  Hz, 17.2 Hz), 6.12 (dd, 1H,  $J = 0.8$  Hz, 10.4 Hz).

#### **Poly(4-nitrophenyl)acrylate (28):**

Monomer 27 (1.39 g, 7.185 mmol) was dissolved in anhydrous benzene. AIBN (40 mg, 3%w/w) was added to the solution and the mixture was heated to 70°C and gently stirred for 70 h. under nitrogen. The mixture was cooled to room temperature and the benzene was filtered away from the solid that had formed. The white solid was washed with methanol several times. Polymer 28 (0.7125 g) was obtained. Method adapted from work done in another lab [24].

#### **Ethyl 4,6-*O*-benzylidene- $\beta$ -D-galactothiopyranoside (23):**

Galactose pentaacetate 6 (5g, 12.81 mmol) was dissolved in dry DCM (65 mL, 0.2M). To this solution was added EtSH (1.29 mL, 17.93 mmol). The solution was cooled to 0°C and

BF<sub>3</sub>•OEt<sub>2</sub> (2.68 mL, 21.26 mmol) was added dropwise. The mixture was allowed to stir for 4 hours. The reaction was quenched with triethylamine and concentrated under vacuum. The crude material was used directly in the next step.

Crude 21 (12.81 mmol) was dissolved in MeOH (80 mL). To this solution was added metallic sodium (30 mg, 1.281 mmol). This solution was stirred 12 hr. and was quenched by adding Dowex H-form resin and allowing to stir for 15 minutes. The solvent was evaporated and the crude material was used directly in the next step.

Crude 22 (12.81 mmol) was dissolved in dry acetonitrile (50 mL, 0.25M). To this solution was added PhCH(OMe)<sub>2</sub> (2.9 mL, 19.215 mmol) and CSA (300 mg, 1.281 mmol). This mixture was allowed to stir for 10 hours. The reaction was quenched with triethylamine (4 mL) and the solvent was evaporated. This crude material was purified using column chromatography (hexanes/EtOAc). Pure 23 (1.9 g, 48% over three steps) was obtained.

**Ethyl 2,3-benzyl-4,6-*O*-benzylidene-β-D-galactothiopyranoside (24):**

Galactose 23 (1.9 g, 3.86 mol) was dissolved in DMF (20 mL, 0.3M) and cooled to 0°C. To this solution was added 60 % NaH in oil (620 mg, 15.44 mmol) and the mixture was allowed to stir for 30 minutes. To this was added benzyl bromide (1.8 mL, 11.581 mmol) and the mixture was allowed to stir overnight. The reaction was quenched with ice water and was extracted with EtOAc (3 x 30 mL). The organic layer was separated and washed with water and brine (100 mL each). The solvent was evaporated and the crude material was purified by column chromatography (hexanes/EtOAc). Pure 24 (2.175 g, 73% yield) was obtained. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 7.55-7.28 (m, 15H), 5.49 (s, 1H), 4.86 (dd, 2H, *J* = 10, 24 Hz), 4.75 (d, 2H, *J* = 2 Hz), 4.43 (d, 1H,



9.6 Hz), 4.31 (dd, 1H,  $J = 1.6, 12.4$  Hz), 4.15 (d, 1H,  $J = 3.2$  Hz), 3.96 (dd, 1H,  $J = 1.6, 12.4$  Hz), 3.891 (dd, 1H,  $J = 9.6, 9.6$  Hz), 3.59 (dd, 1H,  $J = 3.6, 9.2$  Hz), 3.36 (s, 1H), 2.90-2.70 (m, 2H).

**3-azidopropyl-2-*O*-( $\alpha$ -l-fucopyranosyl)-3-*O*-( $\alpha$ -d-galactopyranosyl)- $\beta$ -d-galactopyranoside (25):**

Compound 24 (77 mg, 0.156 mmol) and 19 (100 mg, 0.130 mmol) were dissolved in dry diethyl ether (4 mL). 4Å MS (200 mg) were added to this solution and this mixture was allowed to stir under nitrogen for 1 hour. The mixture was cooled to 0°C and NIS (41 mg, 0.182 mmol) was added. This mixture was stirred for 15 minutes at which point TMSOTf (2.4  $\mu$ L, 0.013 mmol) was added dropwise. The reaction was stirred at 0°C for 15 minutes and warmed to room temperature to stir for another hour. The mixture was diluted with ether and filtered through a celite cake. The solution was washed with aqueous sodium thiosulfate, aqueous sodium bicarbonate and brine. The solvent was evaporate and the crude material was purified by column chromatography (hexanes/EtOAc). Pure 25 (77mg, 50% yield) was obtained.  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.56-7.27 (m, 30H), 5.48 (d, 1H,  $J = 3.2$  Hz), 5.38 (s, 1H), 5.36 (d, 1H,  $J = 2.4$  Hz), 5.06 (s, 1H), 4.97 (d, 1H,  $J = 9.2$  Hz), 4.80-4.65 (m, 8H), 4.42-4.36 (m, 5H), 4.26 (d, 1H, 10 Hz), 4.17 (dd, 1H,  $J = 7.5, 9.5$  Hz), 4.12 (dd, 1H,  $J = 7, 14.5$  Hz), 4.00-3.95 (m, 6H), 3.89 (dd, 1H,  $J = 3.5, 10$  Hz), 3.78 (ddd, 2H,  $J = 3.5, 9, 14.5$  Hz), 3.72 (s, 1H), 3.65 (d, 1H,  $J = 3.5$  Hz), 3.59-3.55 (m, 1H), 3.50 (s, 1H), 3.86-3.34 (m, 2H), 3.28 (s, 1H), 2.98 (d, 1H,  $J = 12$  Hz).

**3-aminopropyl-2-*O*-( $\alpha$ -l-fucopyranosyl)-3-*O*-( $\alpha$ -d-galactopyranosyl)- $\beta$ -d-galactopyranoside (1b):**

A solution of trisaccharide 25 (2.2 g, 1.83 mmol) was prepared in a mixture of THF-MeOH-H<sub>2</sub>O (120 mL, 3:3:1 v/v) and to it was added formic acid (4 mL). Three cycles of evacuation-nitrogen purge were applied followed by addition of Pd(OH)<sub>2</sub>/C (30% w/w). The mixture was then evacuated and purged with H<sub>2</sub> balloon and stirred for 18 h at ambient temperature. The reaction mixture was then filtered thru a bed of Celite and solvent was evaporated. After lyophilization trisaccharide 1b was obtained as off-white solid (1.02 g, quant. yield). <sup>1</sup>H NMR (500 MHz, MeOD):  $\delta$  5.22 (d, 1H,  $J$  = 1.5 Hz), 5.15 (d, 1H,  $J$  = 3 Hz), 4.23 (d, 1H,  $J$  = 7.5 Hz), 4.29-4.12 (m, 3H), 3.95-3.68 (m, 23H), 3.57 (p, 2H), 3.13-3.09 (m, 3H), 1.97-1.96 (m, 3H)

**3-phthallicpropyl  $\beta$ -D-galactopyranoside (32):**

3-aminopropanol 29 (5 mL, 67 mmol) and phthalic anhydride (9.9 g, 67 mmol) were dissolved in toluene (100 ml). To this solution was added triethylamine (9.3 mL, 67 mmol) and the reaction was refluxed (125°C) for 4 hours. The reaction was cooled to room temperature. The toluene was evaporated and the crude material was used directly in the next step without purification.

Crude linker 30 (67 mmol) and commercially available peracetylated galactose 6 (8.7 g, 22.33 mol) were dissolved in DCM (200 mL, 0.1M) and this solution was cooled to 0°C. BF<sub>3</sub>•OEt<sub>2</sub> (9.6 mL, 78.17 mmol) was added to the solution dropwise and the reaction was allowed to stir at 0°C for 2 hours. The reaction was then warmed to room temperature and stirred an additional 12 hours. The reaction was quenched with triethylamine and washed with water and brine. The DCM

was evaporated under vacuum and the crude material was used directly in the next step without purification.

Crude galactose 31 (22.33 mmol) was dissolved in methanol (100 mL) and metallic sodium (200 mg, 6.70 mmol) was added to the solution. This reaction was allowed to stir for 3 hours. The reaction was worked up by addition of H-Form resin (3.5 g) and allowing this to stir for 15 minutes. The resin was filtered from solution and the filtrate was concentrated under vacuum. This crude material was purified through flash column chromatography (DCM/MeOH) to obtain pure compound 32 (3.48 g, 71% over 2 steps).

**3-phthalamidopropyl 3-O-pivaloyl-4,6-O-benzylidene- $\beta$ -D-galactopyranoside (34):**

Galactose 32 (3.48 g, 9.48 mmol) was dissolved in acetonitrile (40 mL, 0.25 M). To this solution was added benzaldehyde dimethyl acetal (2.13 mL, 14.218 mmol) and camphorsulfonic acid (220 mg, 0.948 mmol). This reaction was allowed to stir for 2 hours and was subsequently quenched with triethylamine. The solution was concentrated under vacuum and the crude material was used directly in the next step.

Crude 33 (9.48 mmol) was dissolved in DCM (100 mL, 0.1 M) and pyridine (2.63 mL, 3.6 M) and pivaloyl chloride (1.75 mL, 14.218 mmol) were added. This reaction was allowed to stir overnight at room temperature. The reaction was quenched with methanol. The solution was then concentrated un vacuum and the crude material was purified by flash column chromatography (hexanes/EtOAc) to obtain pure acceptor 34 (4.38 g, 85% over 2 steps).

**3-phthallicpropyl-2-O-( $\alpha$ -l-fucopyranosyl)-3-O-pivaloyl-4,6-O-benzylidene- $\beta$ -D-galactopyranoside (35):**

Acceptor 34 (50 mg, 0.093 mmol) and donor 4 (58 mg, 0.120 mmol) were dissolved in dry diethyl ether (3 mL) and dry DCM (0.5 mL). To this solution were added 4Å molecular sieves (110 mg) and the mixture was allowed to stir under nitrogen for 1 hour. The solution was cooled to 0°C and NIS (31 mg, 0.139 mmol) was added followed by TMSOTf (2.5  $\mu$ L, 0.0139 mmol). The reaction was allowed to stir at 0°C for 30 minutes, then at room temperature for an hour. The reaction was diluted with diethyl ether and was filtered through celite. The filtrate was washed with Na<sub>2</sub>S<sub>2</sub>O<sub>4</sub> and the aqueous layer was extracted with diethyl ether (3 x 10 mL). The organic layer was washed with a saturated sodium bicarbonate solution and brine and then was dried with sodium sulfate. The solvent was concentrated under vacuum and the crude material was purified through flash column chromatography to obtain pure 35 (66 mg, 75% yield).

**2-azido-2-deoxy-1,3,4,6-O-acetyl-galactopyranoside (39):**

Galactosamine 37 (2 g, 9.28 mmol) was dissolved in methanol (40 mL) and triethylamine (3.24 mL, 23.2 mmol). To this solution was added the imidazole azide transfer reagent (2.33 g, 11.13 mmol) and copper sulfate pentahydrate (23 mg, 0.11 mmol). This mixture was allowed to stir at room temperature for 2 hr. The solvent was evaporated off and this crude material was used directly in the next step.

Crude 38 (9.28 mmol) was dissolved in pyridine (40 mL, 0.4M) and acetic anhydride (5.25 mL, 55.68 mmol) was added dropwise. This mixture was allowed to stir for 12 hours. The solvent was evaporated and the crude residue was dissolved in ethyl acetate (20 mL). The solution was washed with 1M HCl (40 mL), aqueous sodium bicarbonate (40 mL) and a brine solution (40 mL).

Flash column chromatography (hexanes/EtOAc) was used to purify the crude product. Pure 39 (2.086 g, 60% over two steps) was obtained.

**Trichloroacetimidate-2-azido-2-deoxy-3,4,6-O-acetyl-galactopyranoside (41):**

Compound 39 (7.3 g, 19.55 mmol) was dissolved in methanol (75 mL) and THF (175 mL) and the solution was cooled to 0°C. ammonia gas was then bubbled into the solution for 20 minutes at 0°C then warmed to room temperature. The reaction was allowed to stir for 20 minutes at room temperature. Air was then bubbled into the solution for one hour. The solvent was evaporated and this crude material was used directly in the next step.

Crude compound 40 (19.554 mmol) was dissolved in DCM (70 mL). CCl<sub>3</sub>CN (9.8 mL, 97.77 mmol) and DBU (1.5 mL, 9.78 mmol) were then added to the solution and the reaction was stirred for 45 minutes. The solvent was evaporated and the crude material was purified by flash column chromatography (hexanes/EtOAc). The pure donor 41 (7.17 g) was obtained in 77% yield over two steps.

**3-azidopropyl-2-O-(2,3,4-tri-O-benzyl- $\alpha$ -l-fucopyranosyl)-3-O-(2-azido-2-deoxy-3,4,6-tetra-O-benzyl- $\alpha$ -d-galactopyranosyl)-4,6-O-benzylidene- $\beta$ -d-galactopyranoside (51):**

41 (100 mg, 0.130 mmol) and 16 (130 mg, 0.110 mmol) were dissolved in dry diethyl ether (4 mL). 4Å MS (250 mg) were added to this solution and this mixture was allowed to stir under nitrogen for 1 hour. The mixture was cooled to 0°C and TMSOTf (7  $\mu$ L, 0.016 mmol) was added dropwise. This mixture was stirred at 0°C for 30 minutes. The reaction was quenched with triethylamine (0.5 mL) and was then filtered through a celite cake and concentrated. The crude

residue was purified by column chromatography (hexanes/EtOAc). Pure 51 (146 mg, 80% yield) was obtained.

**2,2,2-trifluoro-N-phenyl-ethanimidoyl (52):**

A flask was charged with triphenylphosphine (26.2 g, 99.9 mmol), triethylamine (5.5 mL, 39.43 mmol) and carbon tetrachloride (16 mL) and this solution was cooled to 0°C. Trifluoroacetic acid (2.5 mL, 32.7 mmol) was added to the solution dropwise and this mixture was allowed to stir at 0°C for 30 minutes. Aniline (3.7 mL, 40.5 mmol) dissolved in carbon tetrachloride (16 mL) was added to the reaction mixture and this solution was warmed to room temperature. The reaction was then refluxed (95°C) while stirring for 3 hours. The solution was cooled back to room temperature and was diluted with hexanes and filtered. The solid was washed several times with hexanes and the filtrate was concentrated under vacuum. This crude material was purified by flash column chromatography (hexanes/EtOAc) to obtain pure chloride 52 (3.46 g, 60% yield). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>): δ 7.45 (t, 2H), 7.31 (t, 1H), 7.10 (d, 2H, *J* = 7.5 Hz). The spectroscopic data is in agreement with literature data [26].

**C(2)-N-ortho-Trifluoromethylbenzylideneamino-1,3,4,6-O-triacetyl-D-Galactosaminepyranoside (53):**

Commercially available galactosamine 42 (8 g, 37.1 mmol) and 2-(trifluoromethyl)-benzaldehyde (14.7 mL, 111.3 mmol) were dissolved in pyridine (75 mL, 0.5M). To this solutions was added triethylamine (7.75 mL, 55.65 mmol) and the reaction was heated to 60°C and stirred for 24 hours. The reaction was cooled to room temperature and acetic anhydride (28 mL, 296.8 mmol) was added slowly and this was allowed to stir overnight. The reaction was then diluted

extracted with DCM and the organic phase washed with water (4 x 50 mL) and brine (4 x 50 mL). This wash evaporated under vacuum and purified by flash column chromatography to obtain pure 53 (1.2 g, 40% yield). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>): δ 8.65 (d, 1H, *J* = 1.5 Hz), 8.06 (d, 1H, *J* = 8.5 Hz), 7.69-7.67 (m, 1H), 7.60-7.52 (m, 2H), 5.97 (d, 1H, *J* = 8 Hz), 5.45 (d, 1H, *J* = 3.5 Hz), 5.29 (dd, 1H, *J* = 3.5, 10.5 Hz), 4.24-4.13 (m, 3H), 3.71 (dd, 1H, *J* = 8.5, 10 Hz), 2.19 (s, 3H), 2.07 (s, 3H), 2.04 (s, 3H), 1.90 (s, 3H). The spectroscopic data is in agreement with literature data [27].

**C(2)-N-ortho-Trifluoromethylbenzylideneamino-3,4,6-O-triacetyl-D-Galactosaminepyranoside (54):**

Galactosamine 53 (1.2 g, 2.39 mmol) was dissolved in a 7:3 THF/MeOH (30 mL, 0.08M) solvent system and NH<sub>3</sub> gas was bubbled through solution for 30 minutes at 0°C. The reaction was warmed and stirred for another 30 minutes while being monitored on TLC for completion. Once the reaction was complete, a stream of air was bubbled through the solution for 1 hour. The solvent was evaporated under vacuum and the crude material was purified by flash column chromatography to give an anomeric mixture of galactosamine 54 (0.518 g, 25% yield).

**Galactosaminepyranosyl N-Phenyl Trifluoroacetimidate (55):**

Galactosamine 54 (2 g, 4.34 mmol), chloride 1 (0.77 mL, 4.77 mmol) and DBU (0.324 mL, 2.17 mmol) were dissolved in DCM (12 mL) and this mixture was allowed to stir for 12 hours. The solvent was evaporated and the Crude material was purified by flash column chromatography to give donor 55 (1.66 g, 61% yield). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 8.71 (s, 1H), 8.10 (d, 1H, *J* = 7.6 Hz), 7.71 (d, 1H, *J* = 7.2 Hz), 7.64-7.55 (m, 2H), 7.29 (t, 2H, *J* = 7.6 Hz), 7.10 (t, 1H, *J* = 7.6 Hz), 6.80 (d, 2H, *J* = 7.6 Hz), 6.02 (bs, 0.6H), 5.44 (s, 1H), 5.26 (bs, 1H), 4.20 (d, 2H, *J* = 6.8

Hz), 3.83 (t, 1H,  $J = 7.2$  Hz), 2.21 (s, 3H), 2.04 (s, 3H), 1.92 (s, 3H). The spectroscopic data is in agreement with literature data [27].

**3-azidopropyl 2-*O*-(2,3,4-tri-*O*-benzyl- $\alpha$ -L-fucopyranosyl)-3-*O*-(2-amino-2-deoxy-3,4,6-tri-*O*-acetyl- $\alpha$ -D-galactopyranosyl)-4,6-*O*-benzylidene- $\beta$ -D-galactopyranoside (56):**

NiCl<sub>2</sub> (15 mg, 15 mol%) and Ag(OTf)<sub>2</sub> (61 mg, 30 mol%) were suspended in dry DCM (1 mL) and was shielded from the light with tin foil. This flask was stirred for 30 minutes to generate Ni(OTf)<sub>2</sub> catalyst in situ. In a separate flask Trifluoroacetimidate donor 55 (500 mg, 0.791 mmol) and disaccharide acceptor 19 (300 mg, 0.395 mmol) were dissolved in dry DCM (1 mL). The catalyst from the first flask was transferred to the reaction flask and was shielded from light with tin foil. The reaction mixture was heated to 35°C and was allowed to stir overnight. The solvent was evaporated and the crude material was purified by flash column chromatography (hexanes/EtOAc) to obtain pure 56 (112 mg, 27% yield). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>):  $\delta$  7.56-7.54 (m, 2H), 7.42-7.40 (m, 2H), 7.34-7.28 (m, 12H), 7.19-7.17 (m, 3H), 5.55 (s, 1H), 5.41 (d, 1H,  $J = 3.5$  Hz), 5.20 (d, 1H,  $J = 3.5$  Hz), 5.14-5.10 (m, 2H), 4.97-4.92 (dd, 2H,  $J = 12, 13.5$  Hz), 4.83-4.80 (m, 2H), 4.72 (d, 1H,  $J = 11.5$  Hz), 4.63 (d, 1H,  $J = 12$  Hz), 4.43-4.39 (m, 2H), 4.36-4.23 (m, 2H), 4.22 (dd, 1H,  $J = 4, 7.5$  Hz), 4.17 (dd, 1H,  $J = 4, 10.5$  Hz), 4.14-4.08 (m, 3H), 3.99-3.95 (m, 2H), 3.91 (dd, 1H,  $J = 3, 9.5$  Hz), 3.75 (dd, 1H,  $J = 7.5, 11$  Hz), 3.70 (s, 1H), 3.60-3.56 (m, 1H), 3.41 (s, 1H), 3.36-3.33 (m, 3H), 3.19-3.15 (m, 2H), 2.04 (s, 3H), 1.99 (s, 3H), 1.96 (s, 3H), 1.83 (p, 2H), 1.25 (t, 3H,  $J = 7$  Hz), 1.12 (d, 3H,  $J = 6.5$  Hz).



**3-azidopropyl-2-*O*-(2,3,4-tri-*O*-benzyl- $\alpha$ -L-fucopyranosyl)-3-*O*-(2-acetamido-2-deoxy-3,4,6-tetra-*O*-acetyl- $\alpha$ -D-galactopyranosyl)-4,6-*O*-benzylidene- $\beta$ -D-galactopyranoside (58):**

Trisaccharide 56 (50 mg, 0.047 mmol) was dissolved in methanol (1 mL) and acetic anhydride (50  $\mu$ L, 0.474 mmol) in methanol (0.5 mL) was added to the solution dropwise. This reaction was allowed to stir for 1 hour and was subsequently quenched with triethylamine (until neutral pH). The solvent was evaporated and the crude material was purified by a short column filtration (hexanes/EtOAc  $\rightarrow$  EtOAc/MeOH) to obtain acetylated trisaccharide 58 (50 mg, 99% yield).  $^1\text{H NMR}$  (500 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.56-7.54 (m, 2H), 7.42-7.40 (m, 2H), 7.34-7.28 (m, 12H), 7.19-7.17 (m, 3H), 5.55 (s, 1H), 5.41 (d, 1H,  $J = 3.5$  Hz), 5.20 (d, 1H,  $J = 3.5$  Hz), 5.14-5.10 (m, 2H), 4.97-4.92 (dd, 2H,  $J = 12, 13.5$  Hz), 4.83-4.80 (m, 2H), 4.72 (d, 1H,  $J = 11.5$  Hz), 4.63 (d, 1H,  $J = 12$  Hz), 4.43-4.39 (m, 2H), 4.36-4.23 (m, 2H), 4.22 (dd, 1H,  $J = 4, 7.5$  Hz), 4.17 (dd, 1H,  $J = 4, 10.5$  Hz), 4.14-4.08 (m, 3H), 3.99-3.95 (m, 2H), 3.91 (dd, 1H,  $J = 3, 9.5$  Hz), 3.75 (dd, 1H,  $J = 7.5, 11$  Hz), 3.70 (s, 1H), 3.60-3.56 (m, 1H), 3.41 (s, 1H), 3.36-3.33 (m, 3H), 3.19-3.15 (m, 2H), 2.04 (s, 3H), 1.99 (s, 3H), 1.96 (s, 3H), 1.83 (p, 2H), 1.25 (t, 3H,  $J = 7$  Hz), 1.12 (d, 3H,  $J = 6.5$  Hz).

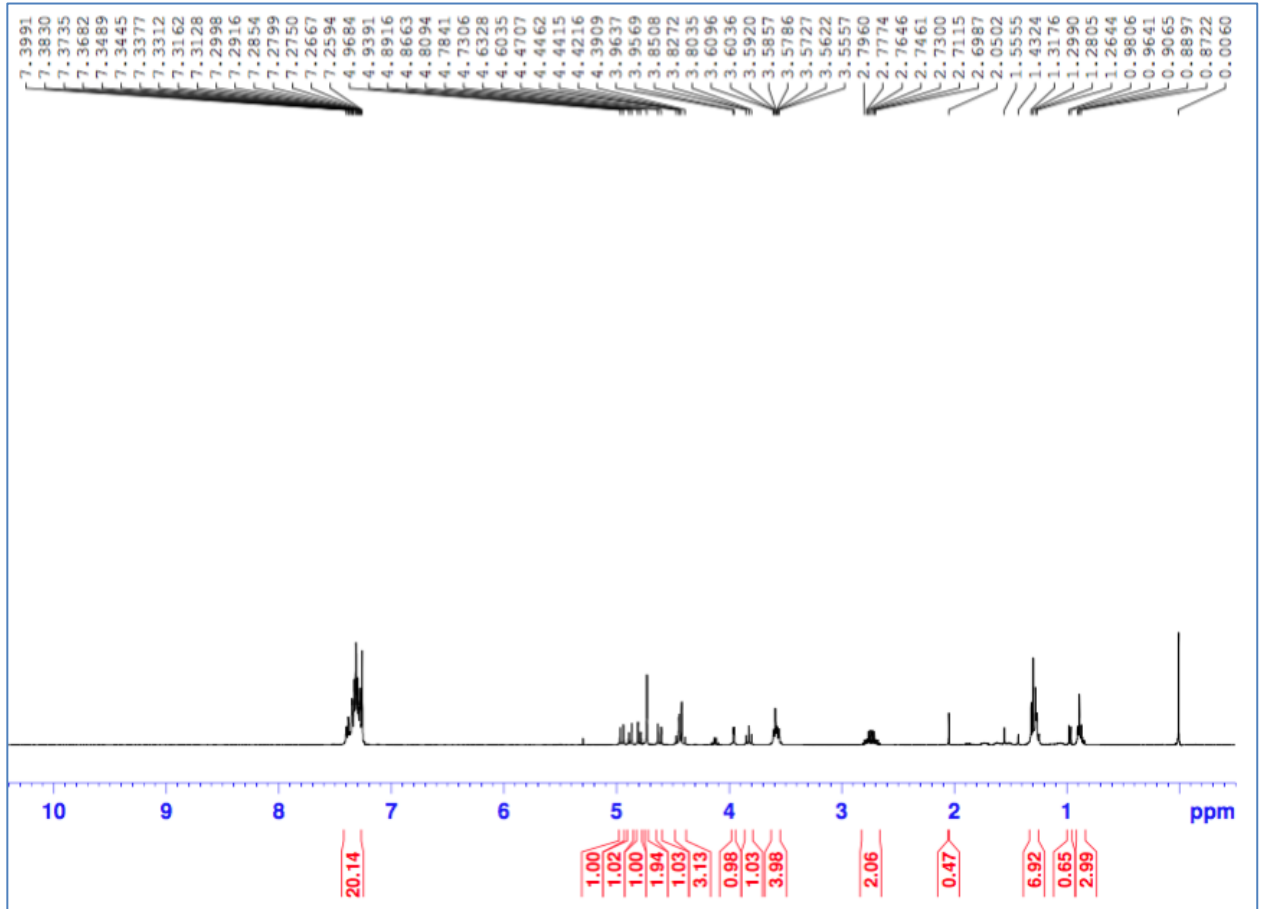
**3-aminopropyl-2-*O*-( $\alpha$ -L-fucopyranosyl)-3-*O*-(2-acetamido-2-deoxy- $\alpha$ -D-galactopyranosyl)- $\beta$ -D-galactopyranoside (60):**

Trisaccharide 58 (61 mg, 0.0556 mmol) was dissolved in MeOH (1 mL) and metallic sodium was added to the solution (2 mg, 0.0056 mmol). The solution was allowed to stir for one hour and was quenched with H-form resin. The resin was filtered and the solvent was evaporated under vacuum to obtain compound 59. This material was used crude in the next step.

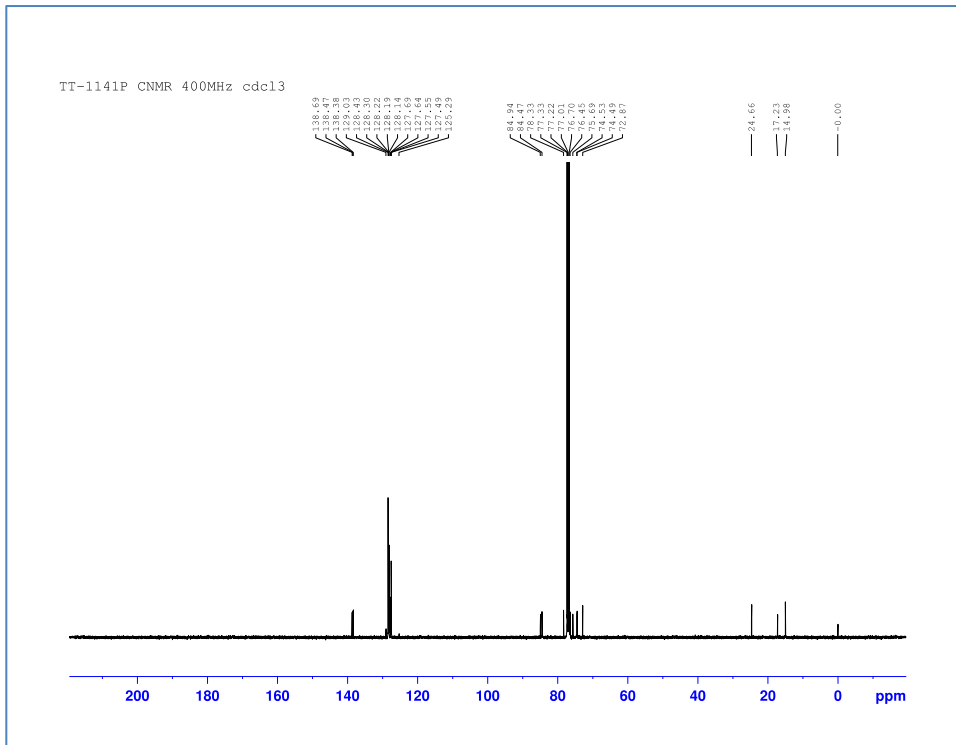
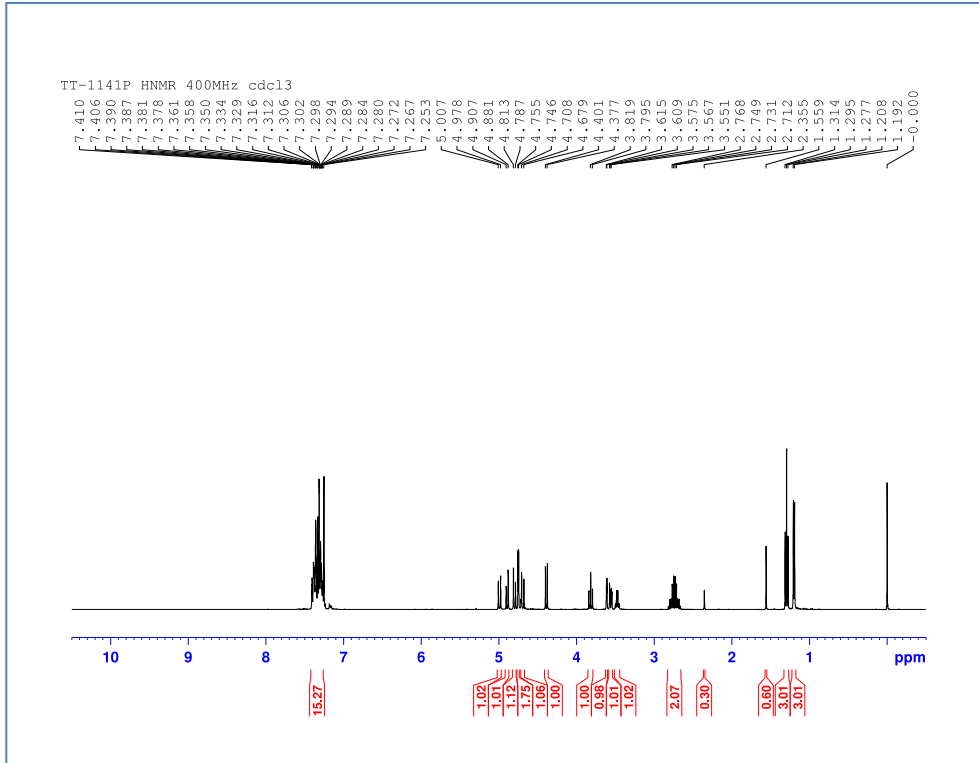
A solution of crude trisaccharide 59 (24 mg, 0.0257 mmol) was prepared in a mixture of THF-MeOH-H<sub>2</sub>O (7 mL, 3:3:1 v/v) and to it was added formic acid (0.4 mL). Three cycles of evacuation-nitrogen purge were applied followed by addition of Pd(OH)<sub>2</sub>/C (30% w/w). The mixture was then evacuated and purged with H<sub>2</sub> balloon and stirred for 24 h at ambient temperature. The reaction mixture was then filtered thru a bed of Celite and solvent was evaporated. After lyophilization trisaccharide 60 was obtained as off-white solid (5.5 mg). <sup>1</sup>H NMR (500 MHz, D<sub>2</sub>O): δ 5.32 (s, 1H), 5.21 (s, 1H), 4.61 (s, 1H), 4.40 (s, 1H), 4.27 (bs, 3H), 4.04-3.71 (m, 14H), 3.18 (s, 2H), 2.08 (bs, 4H), 1.30 (s, 3H).

# NMR Spectra

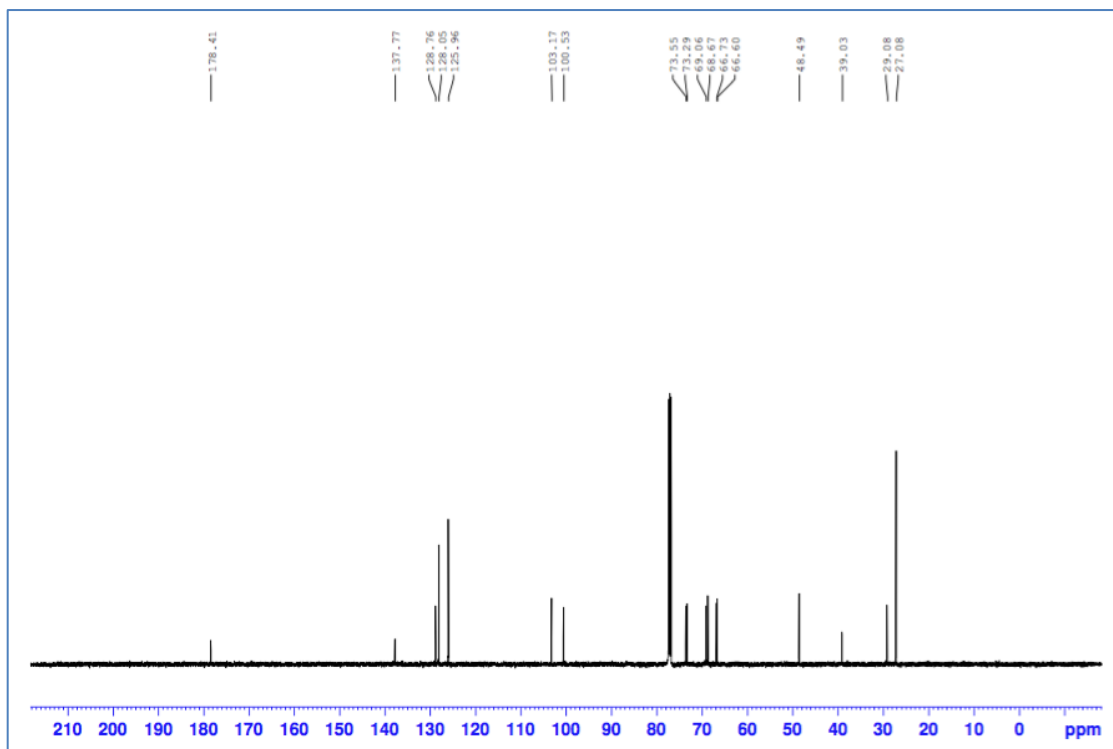
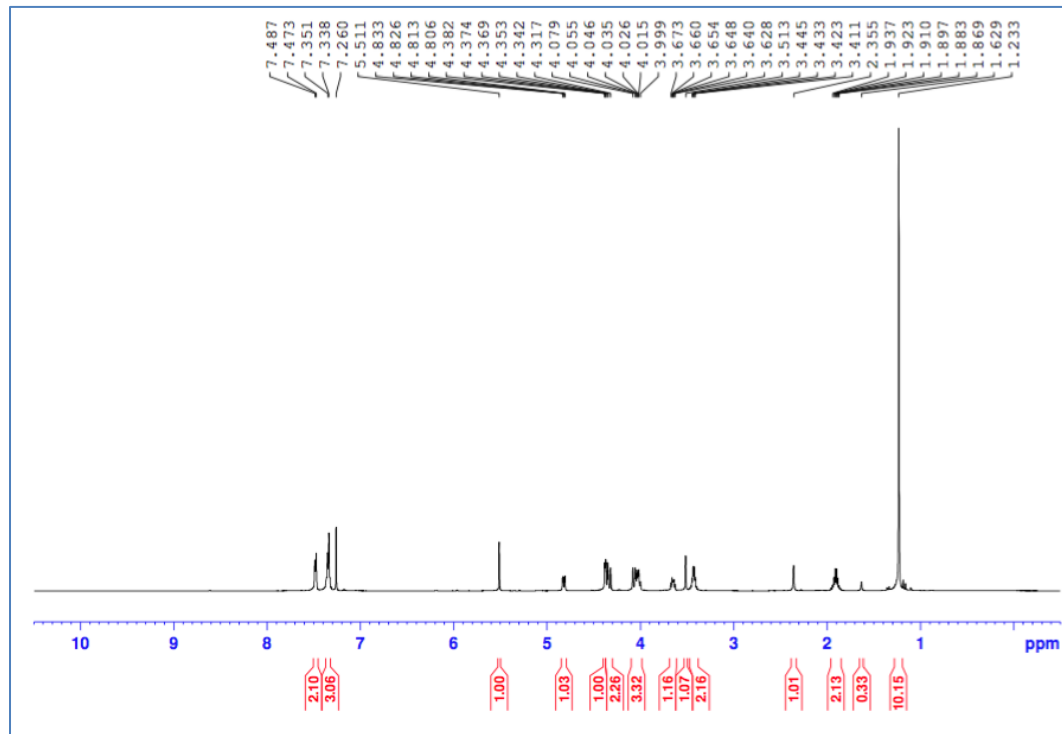
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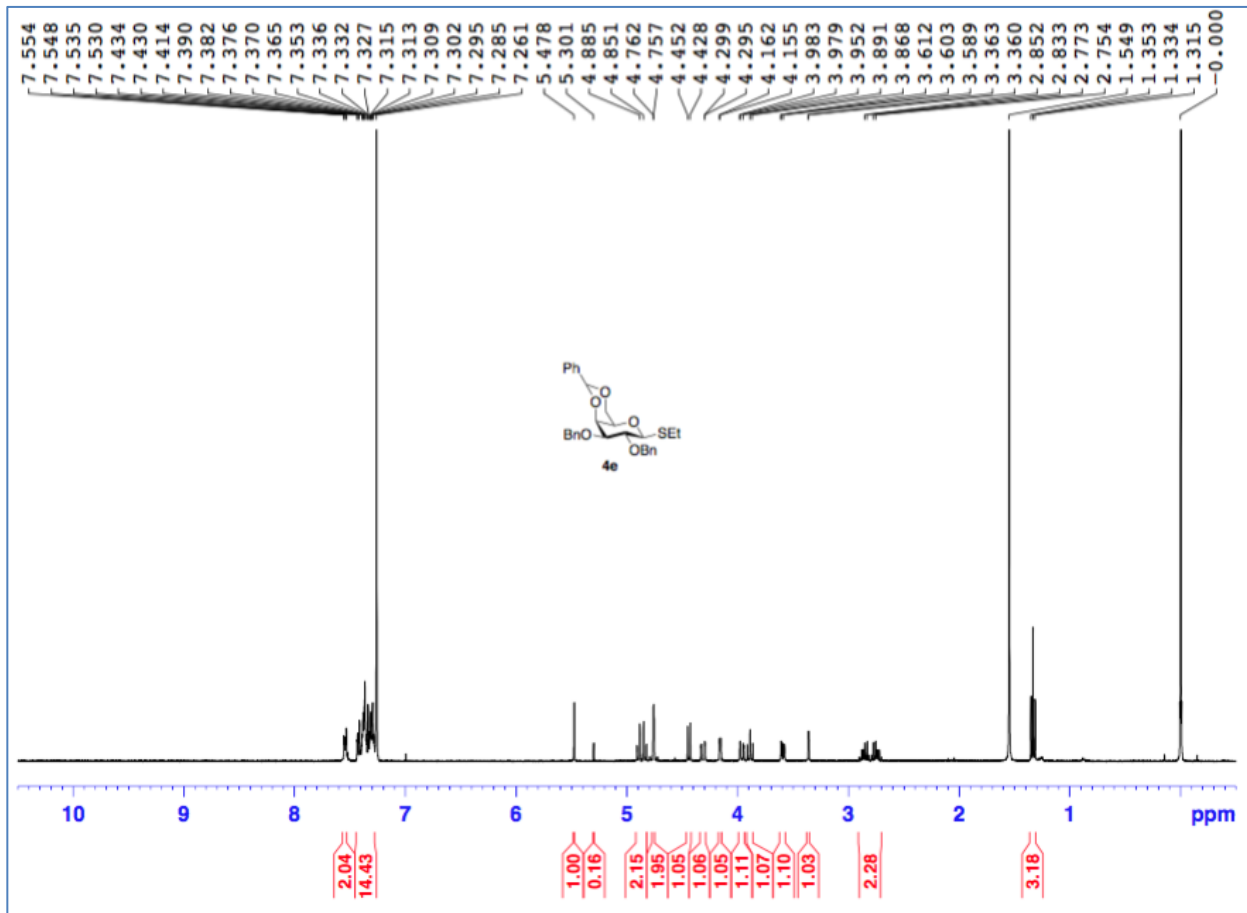
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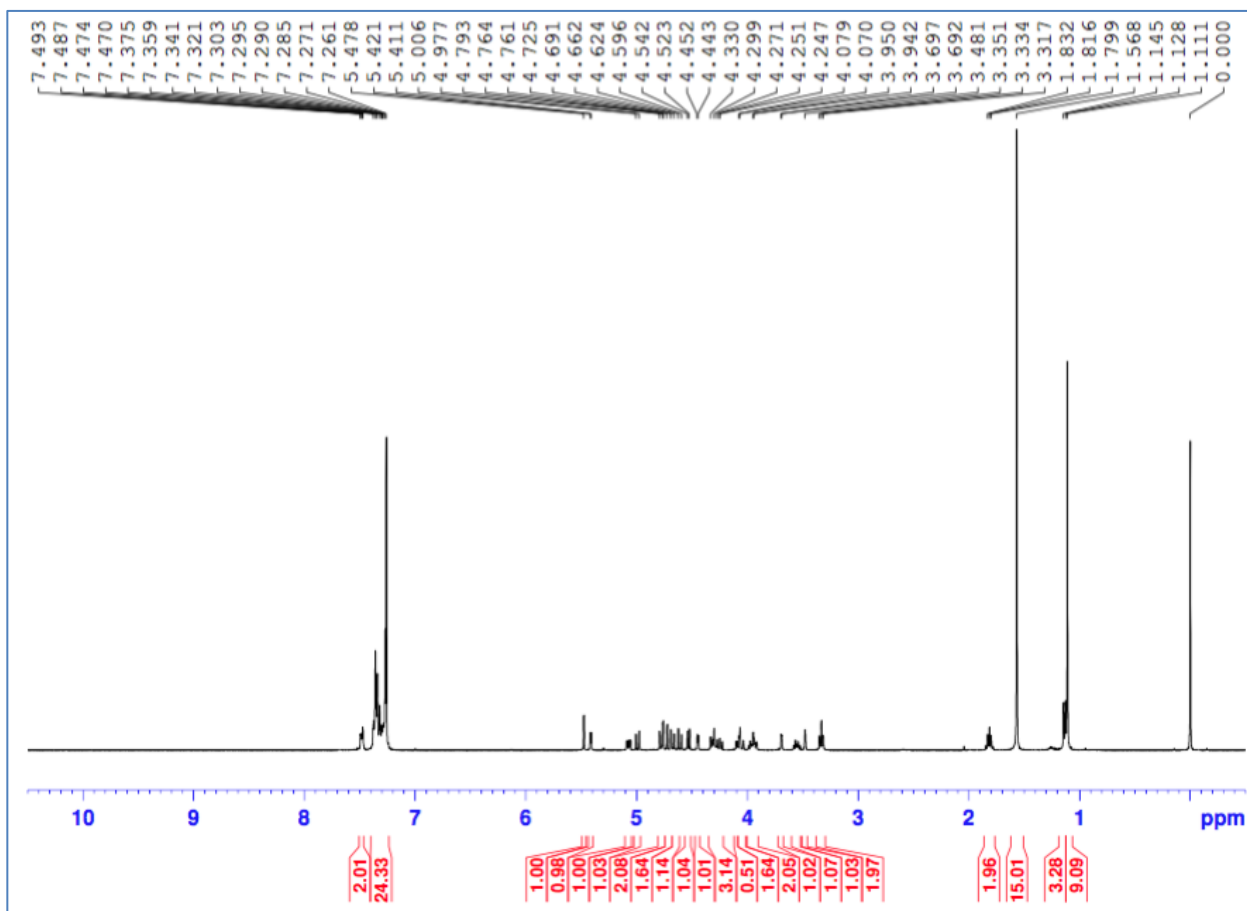
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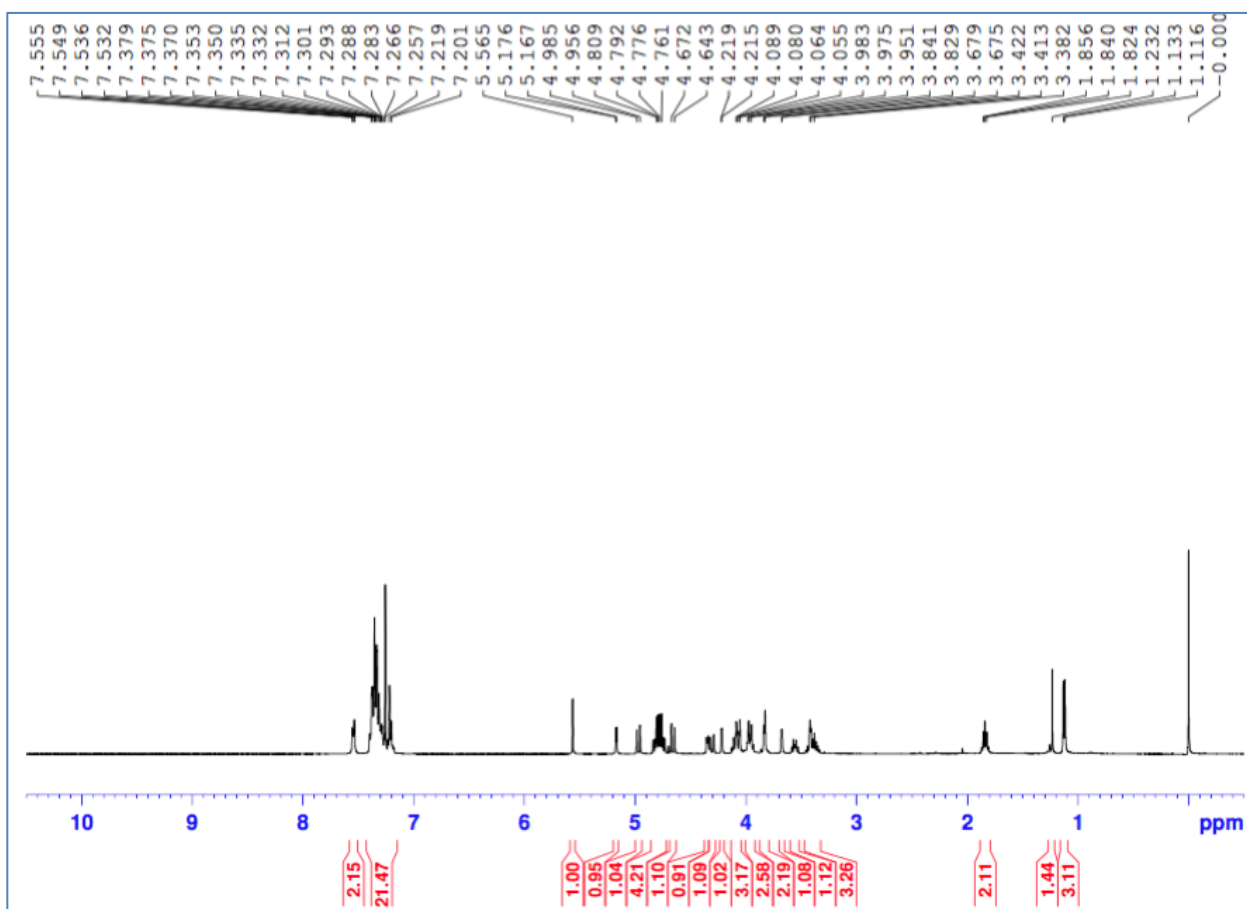
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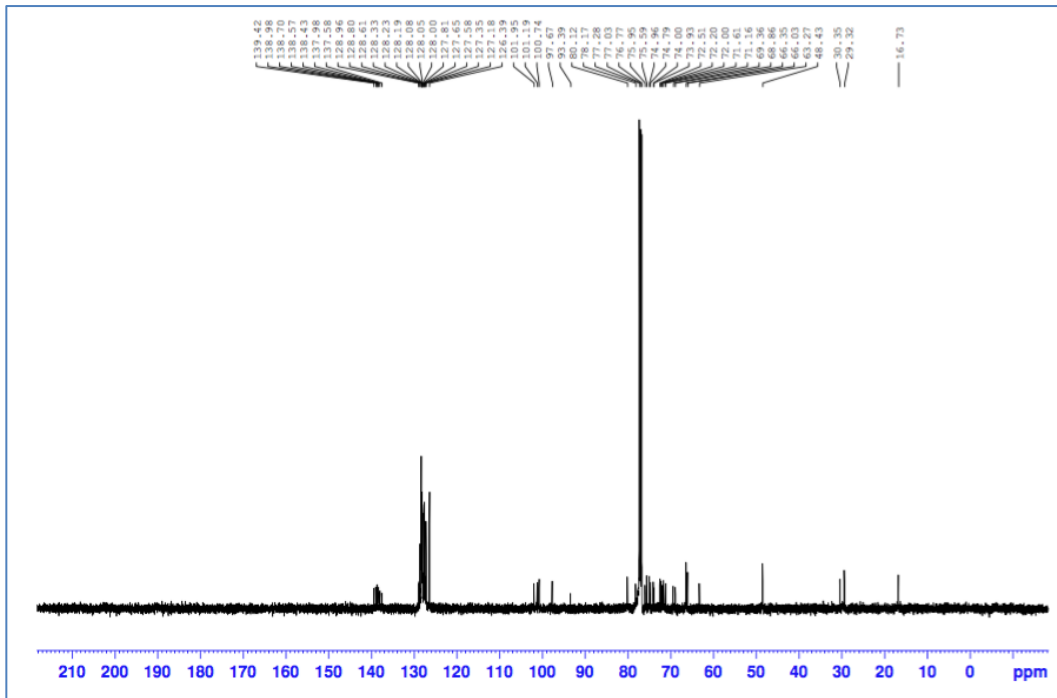
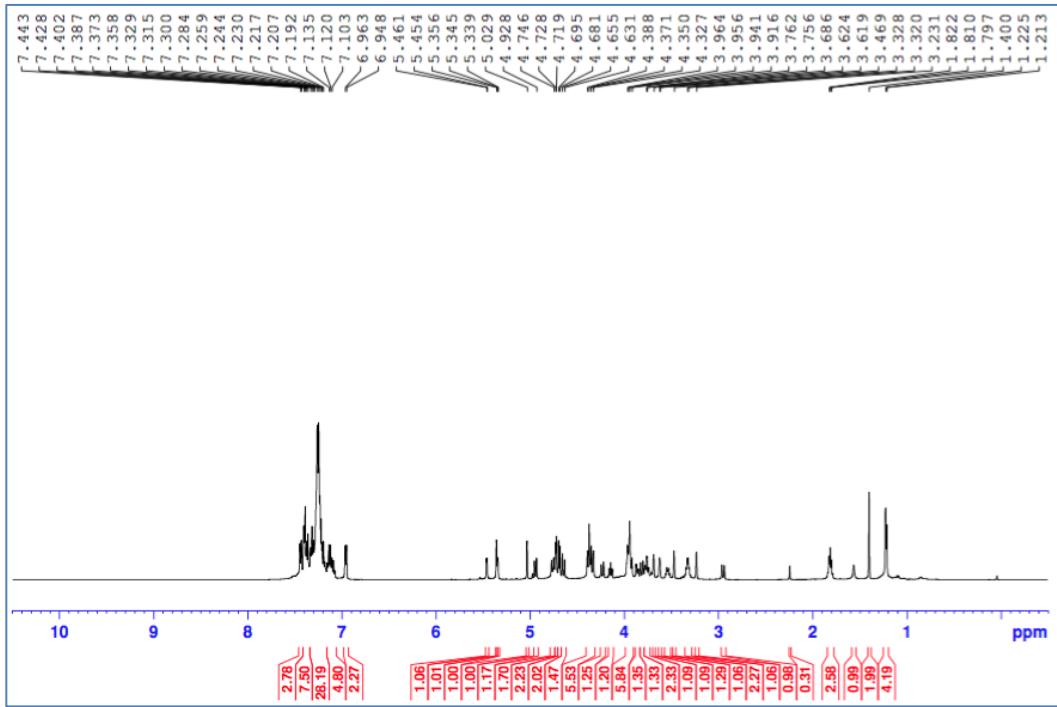


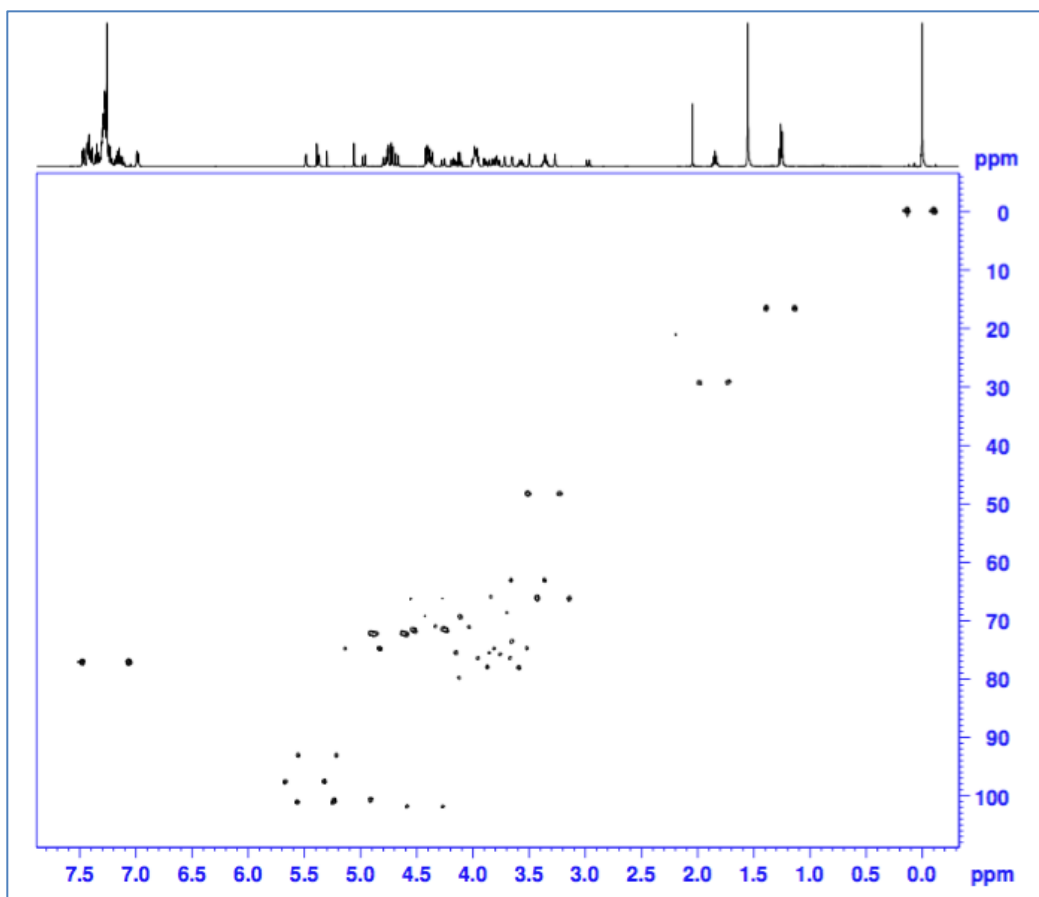
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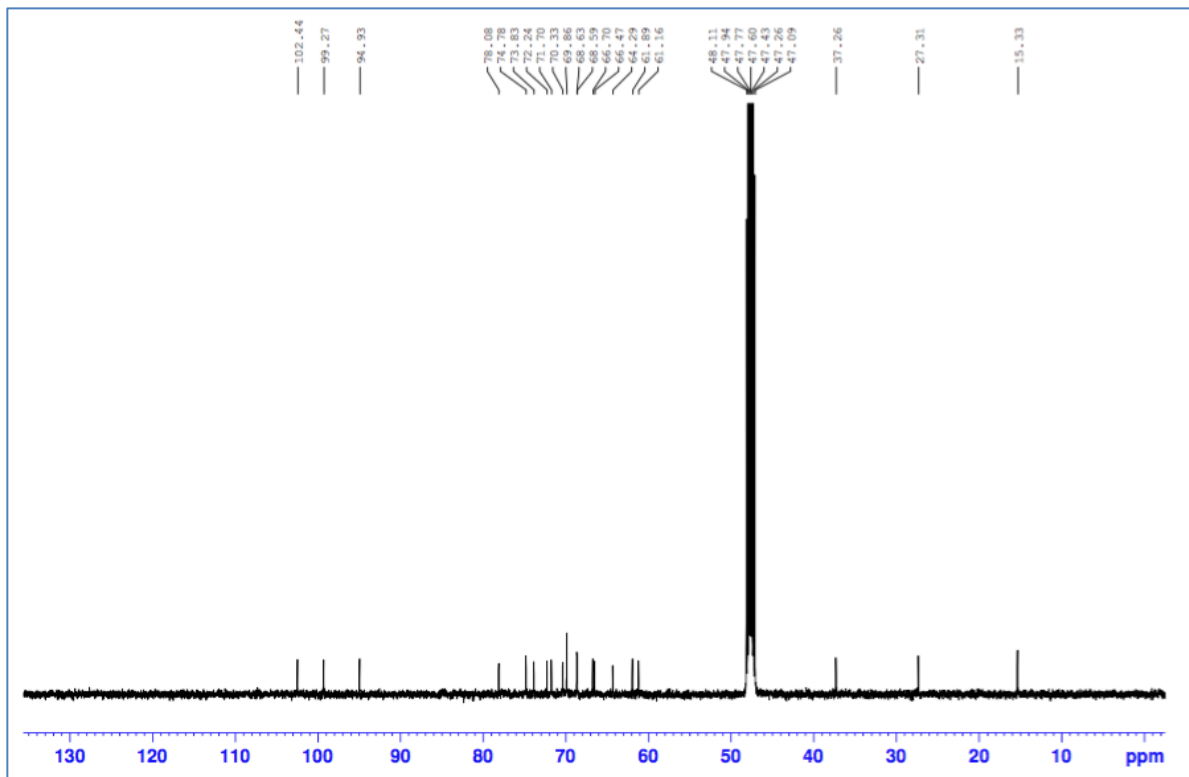
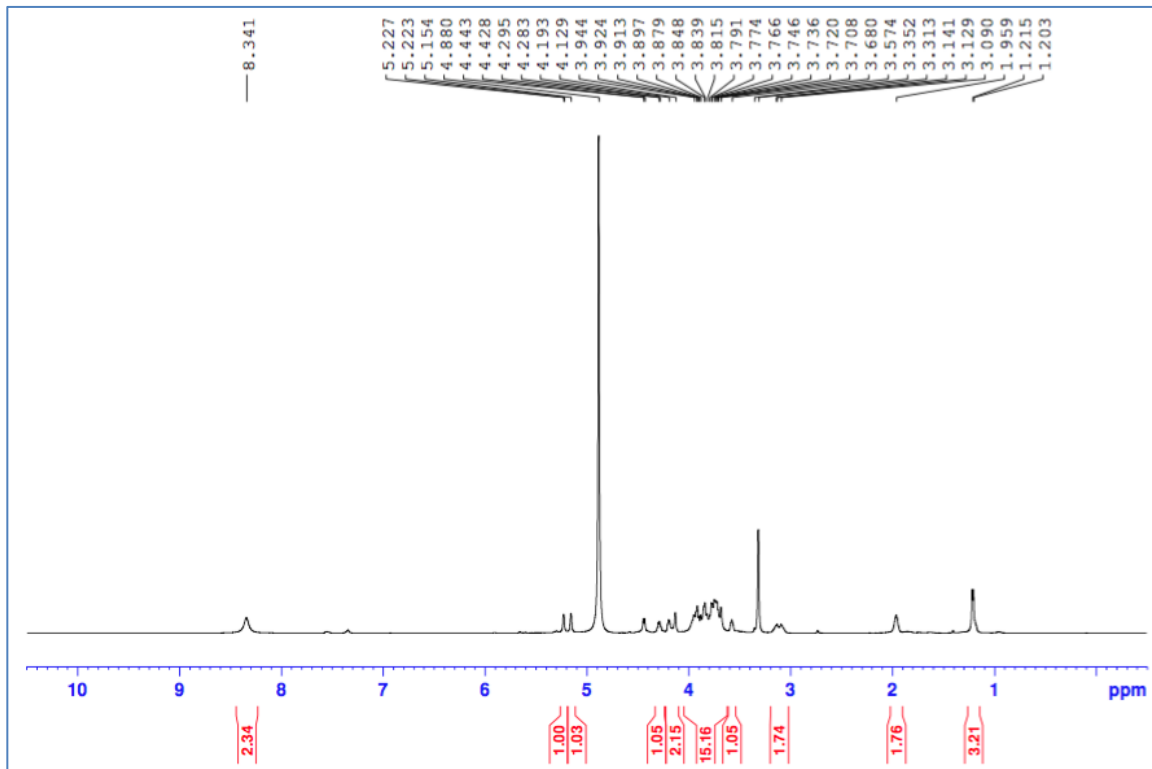


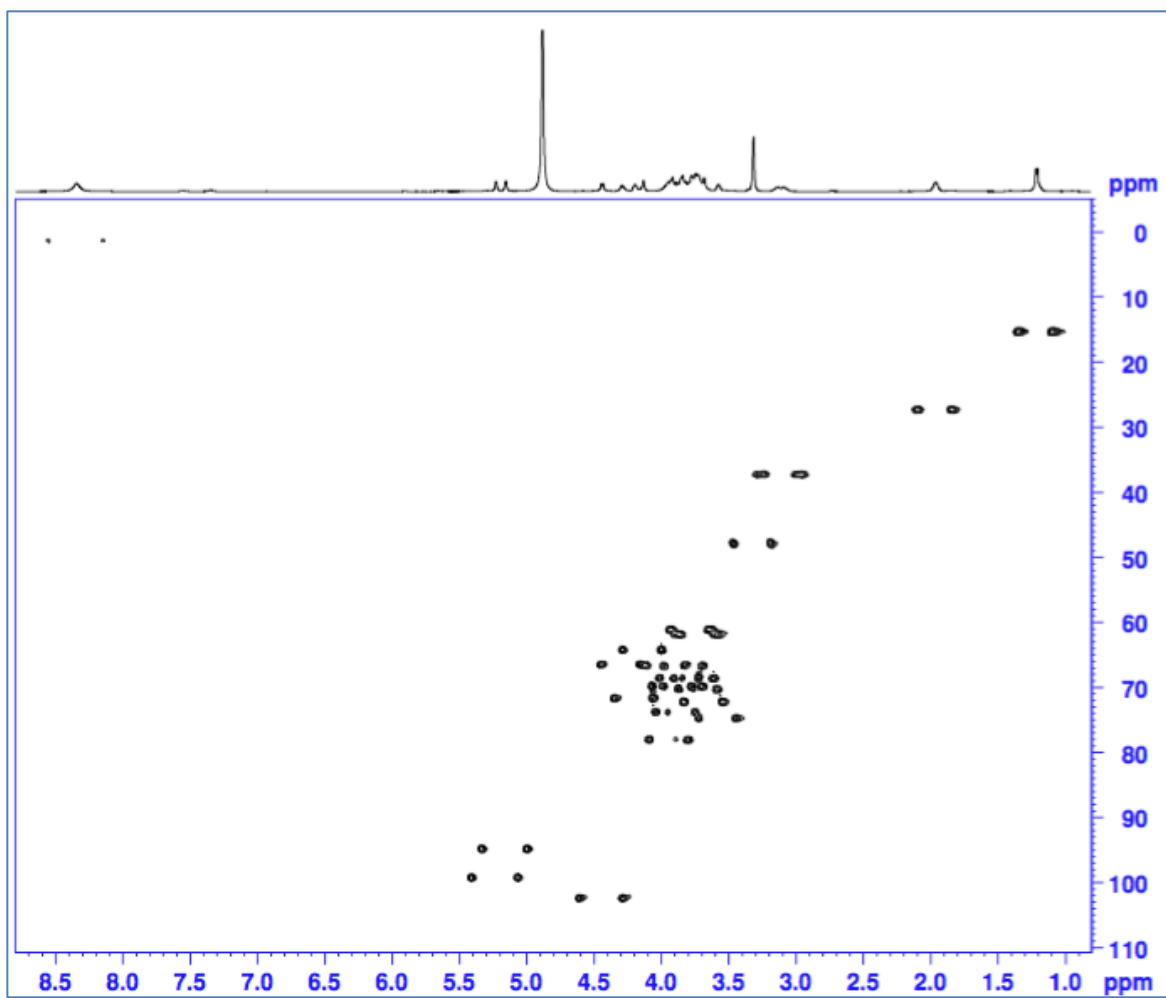
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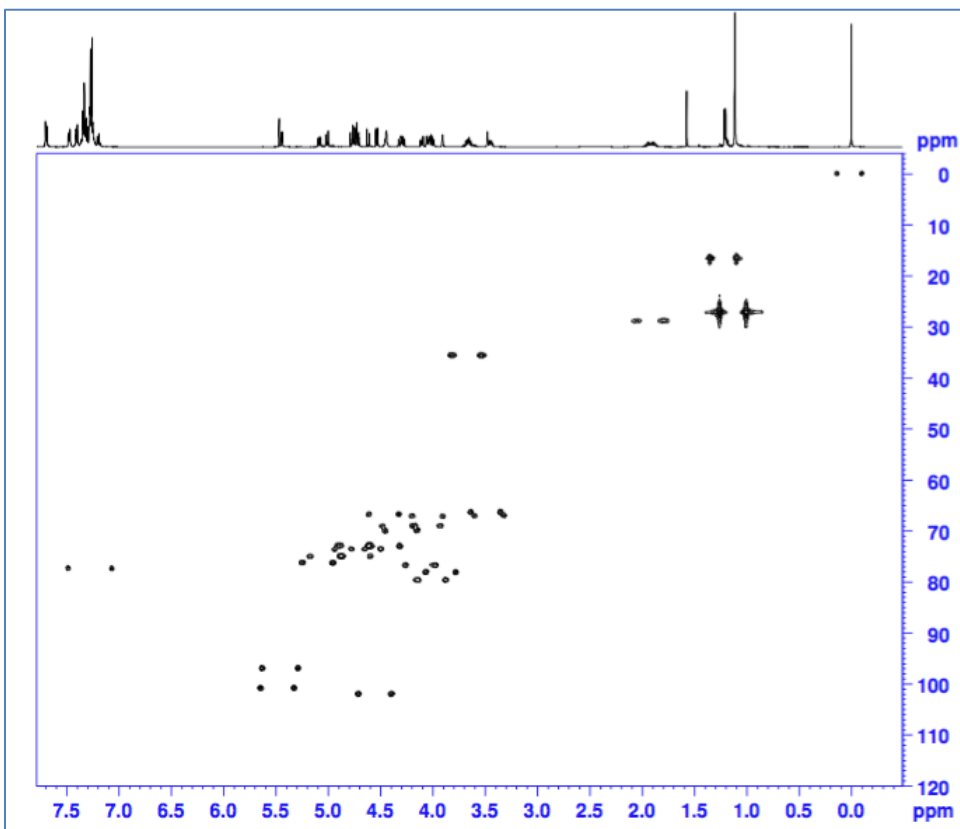
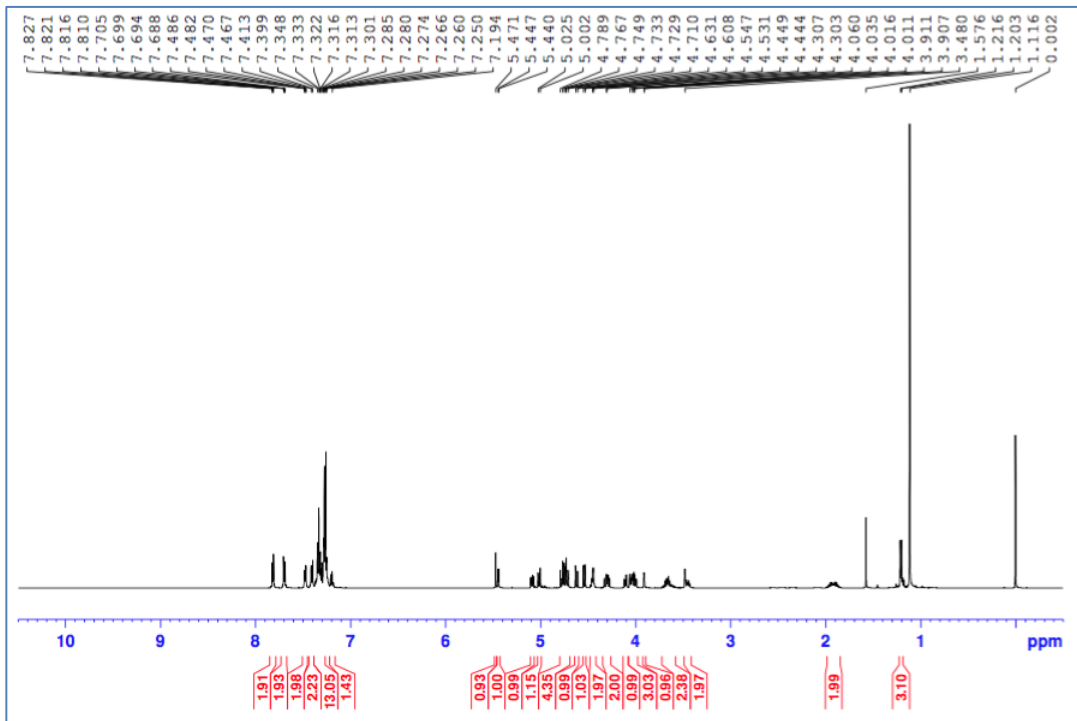


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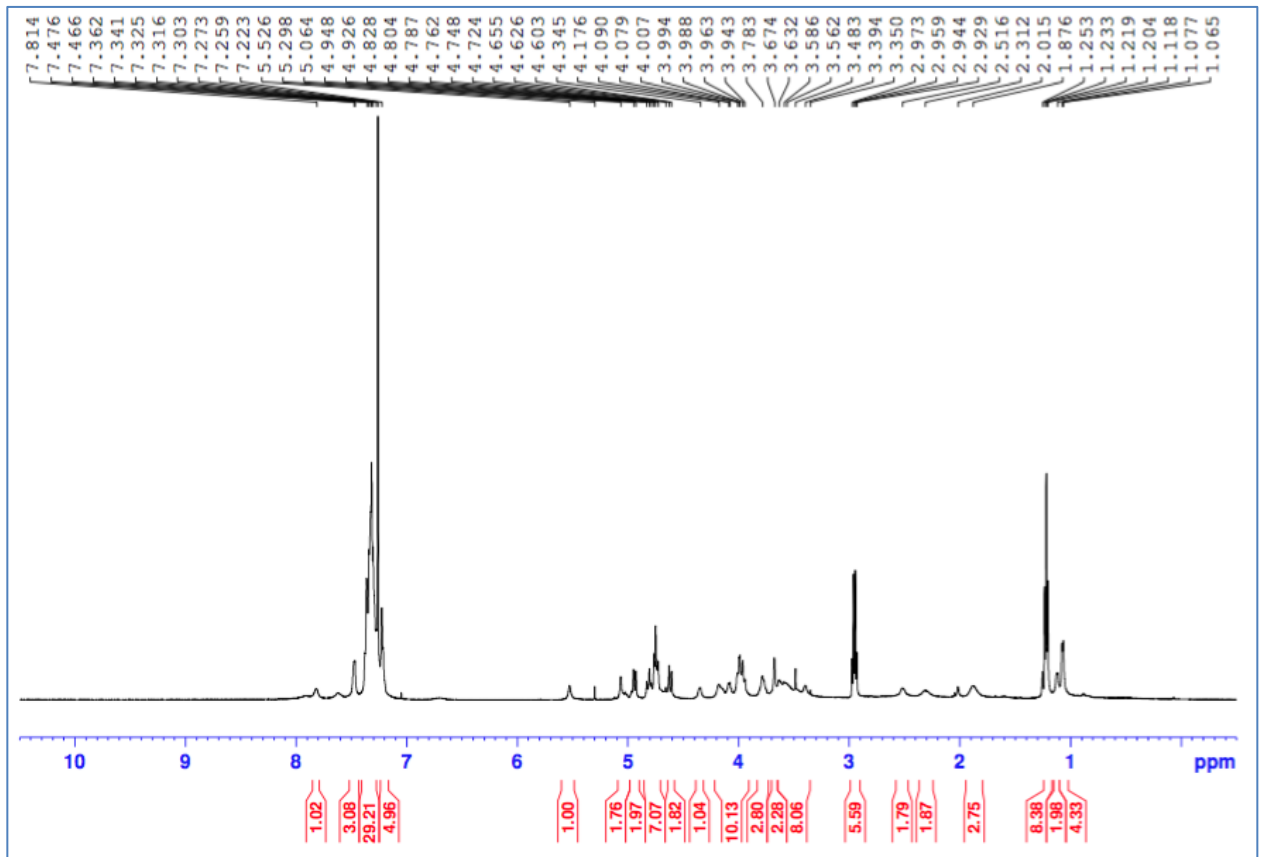




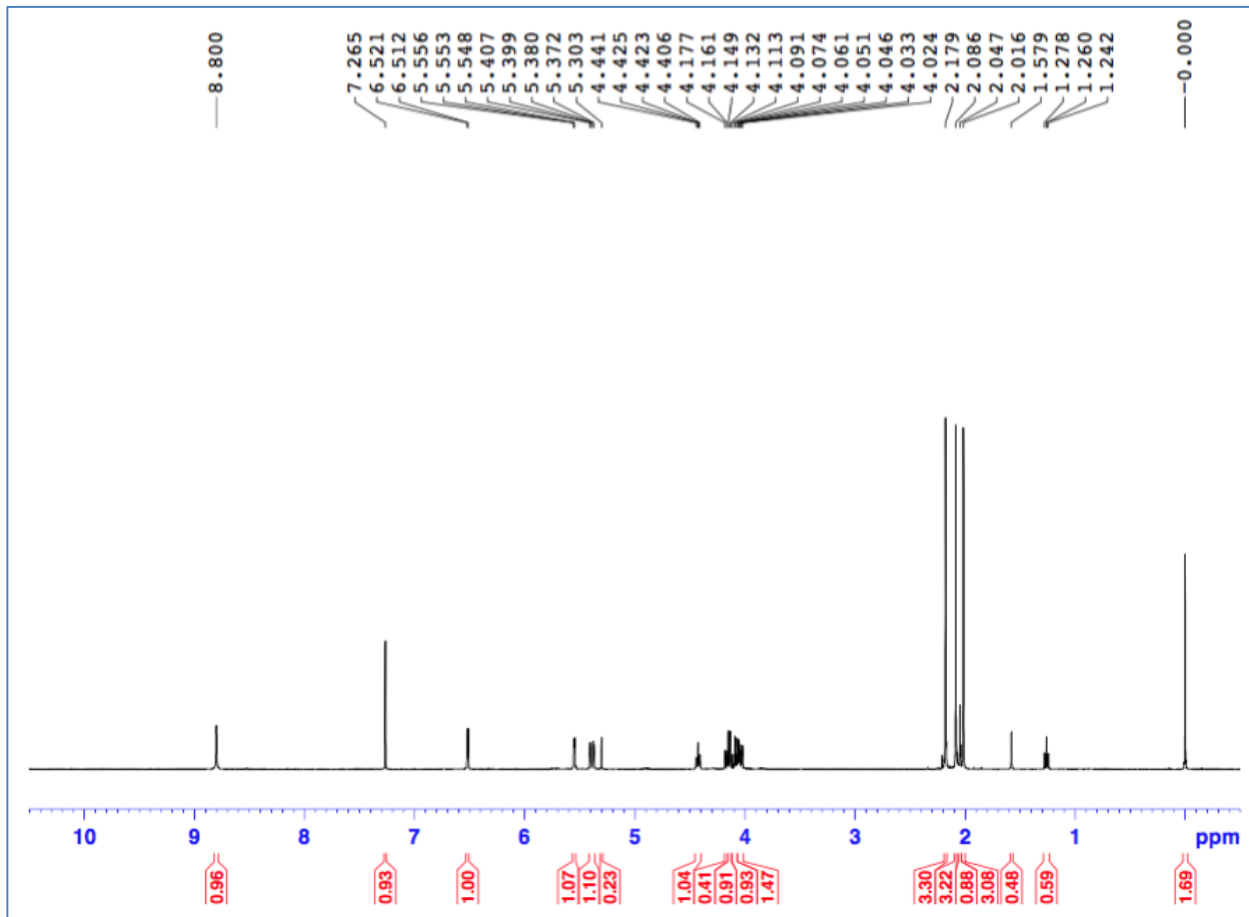
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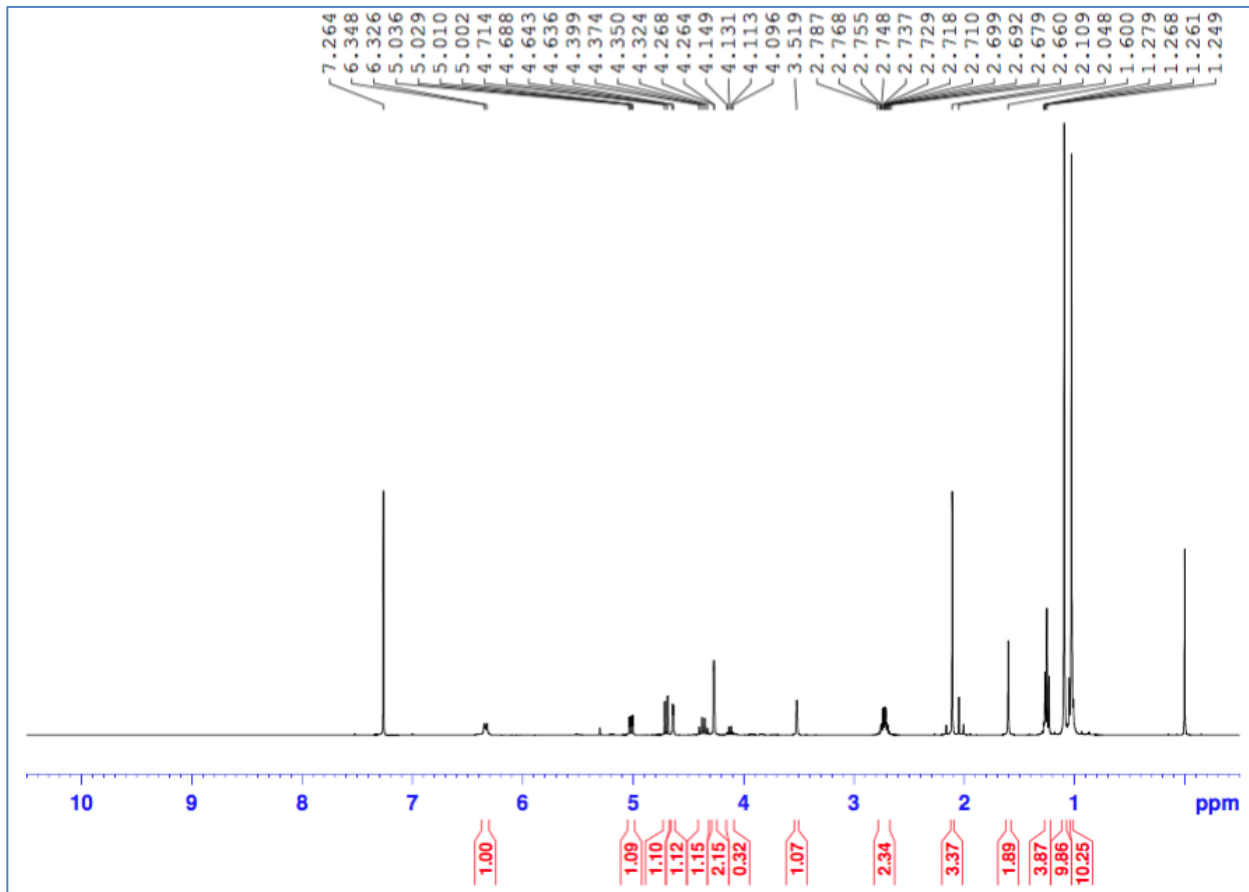
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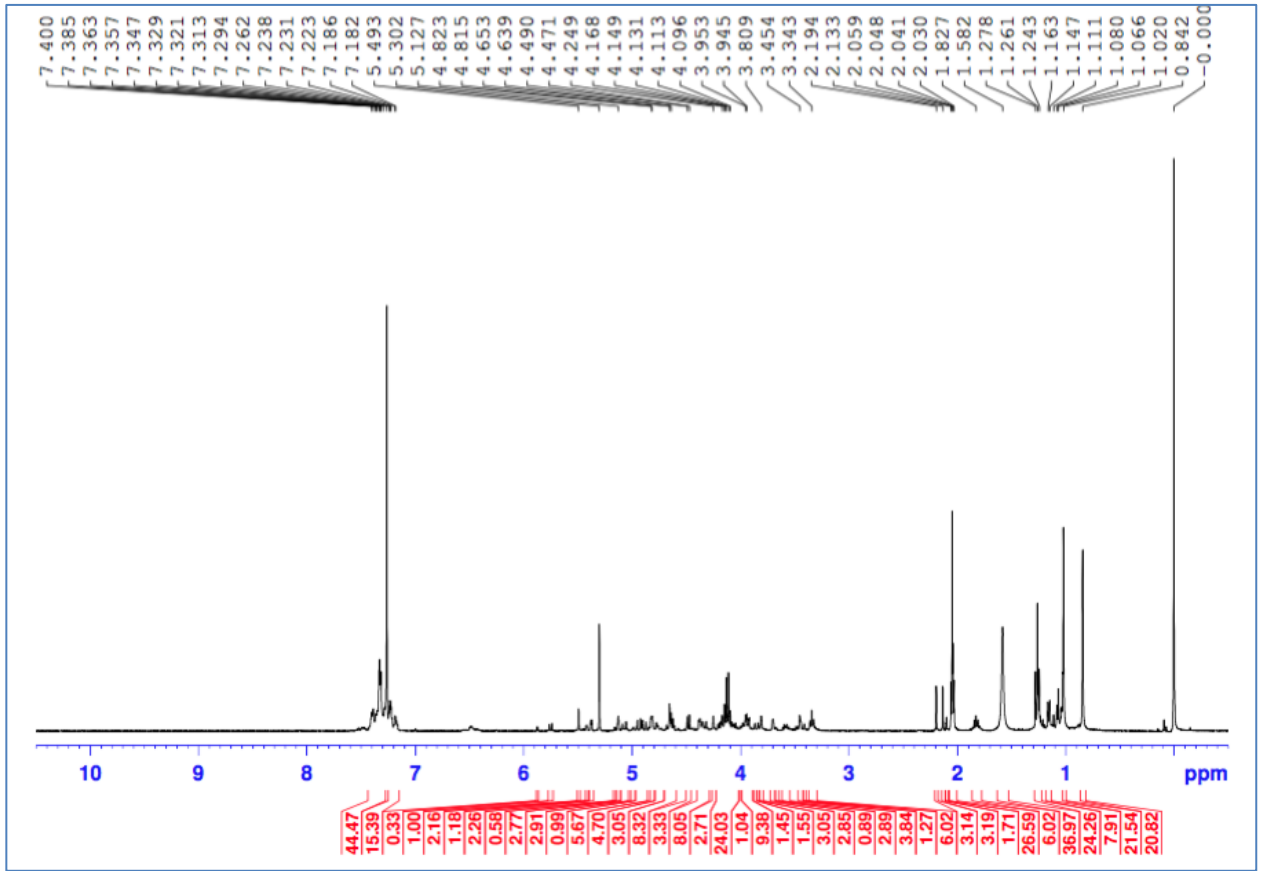


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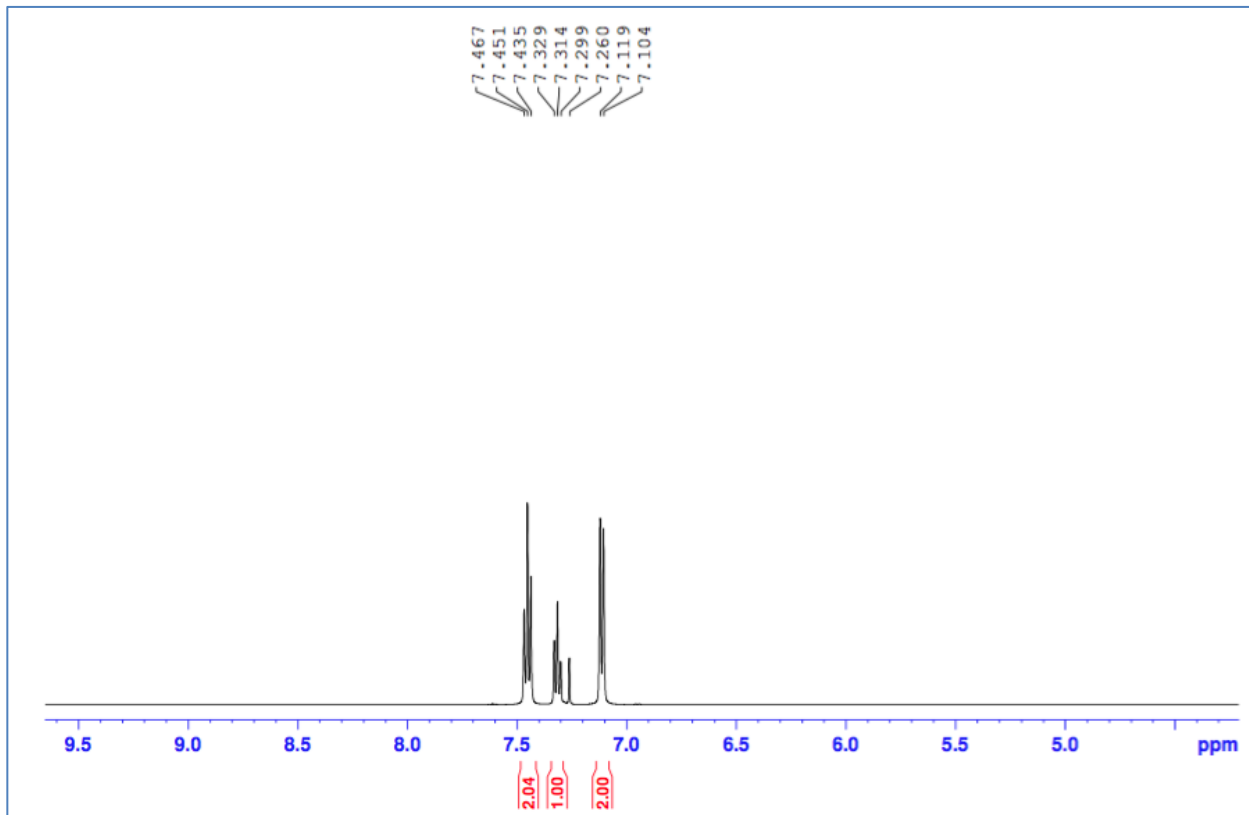




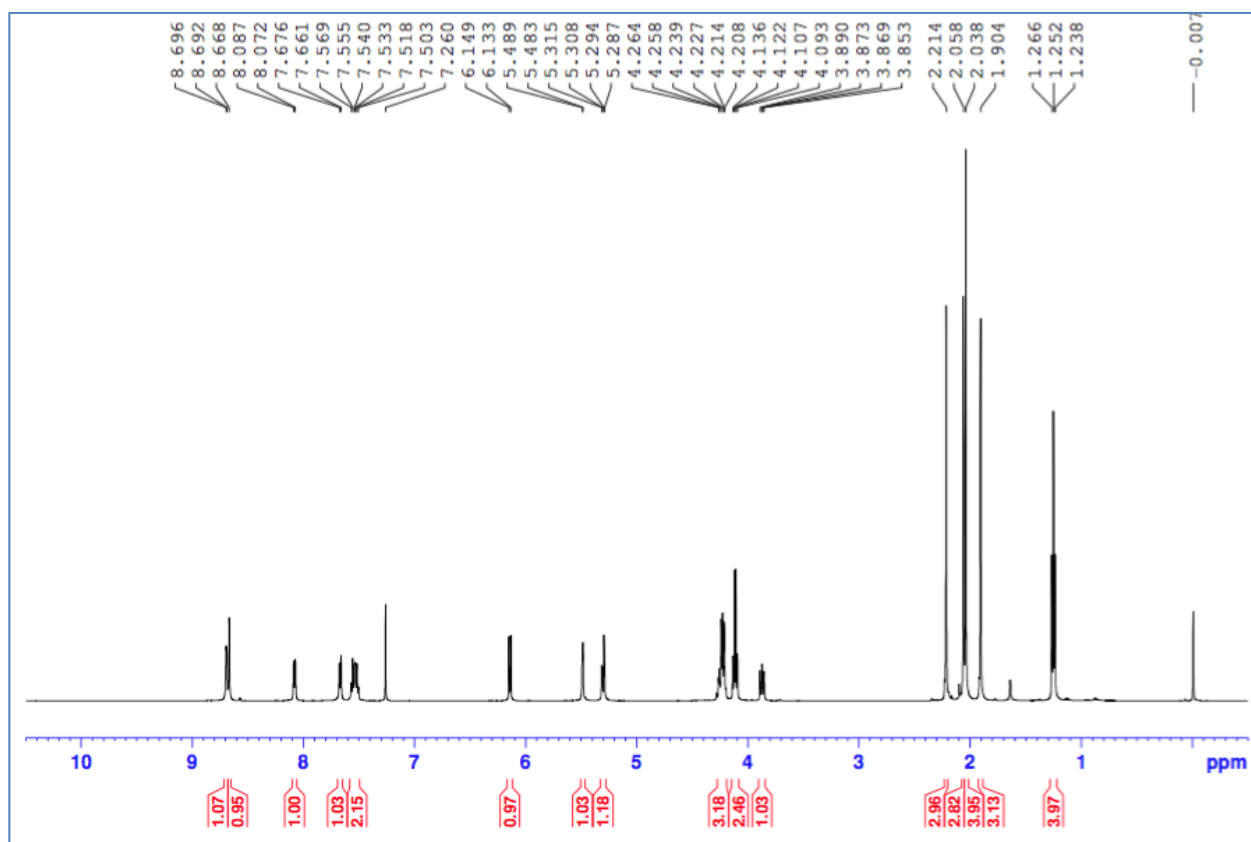
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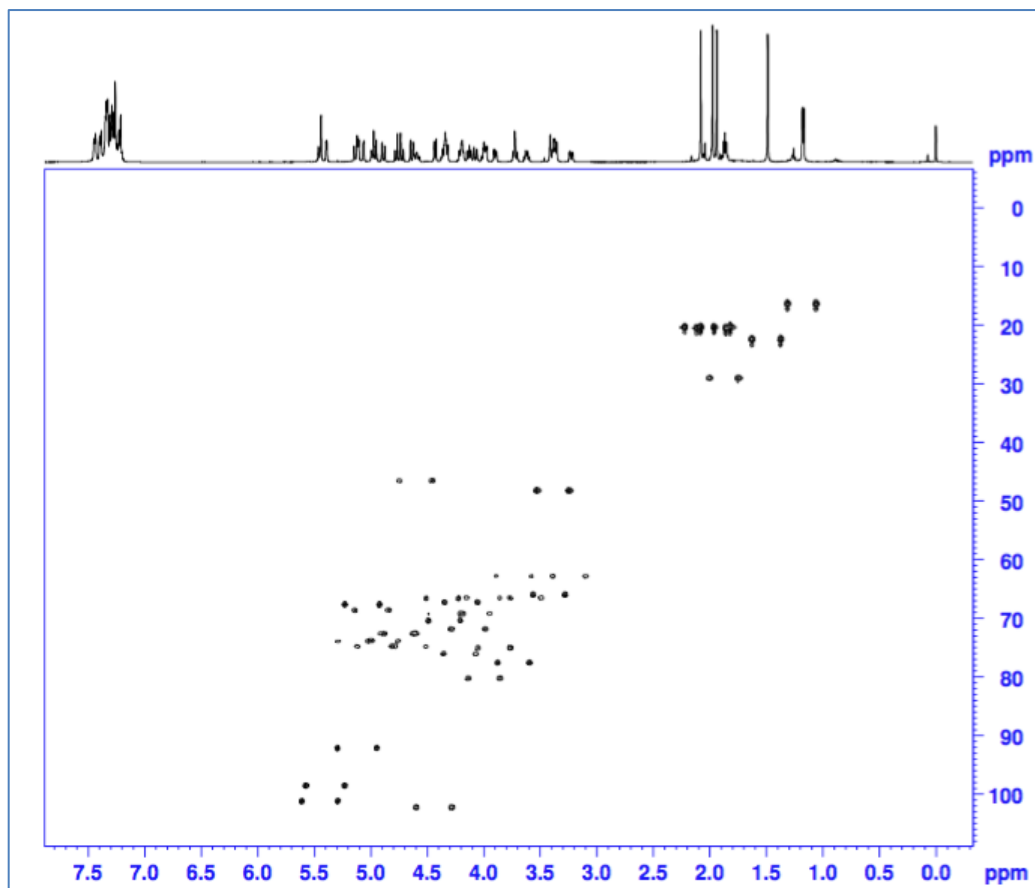
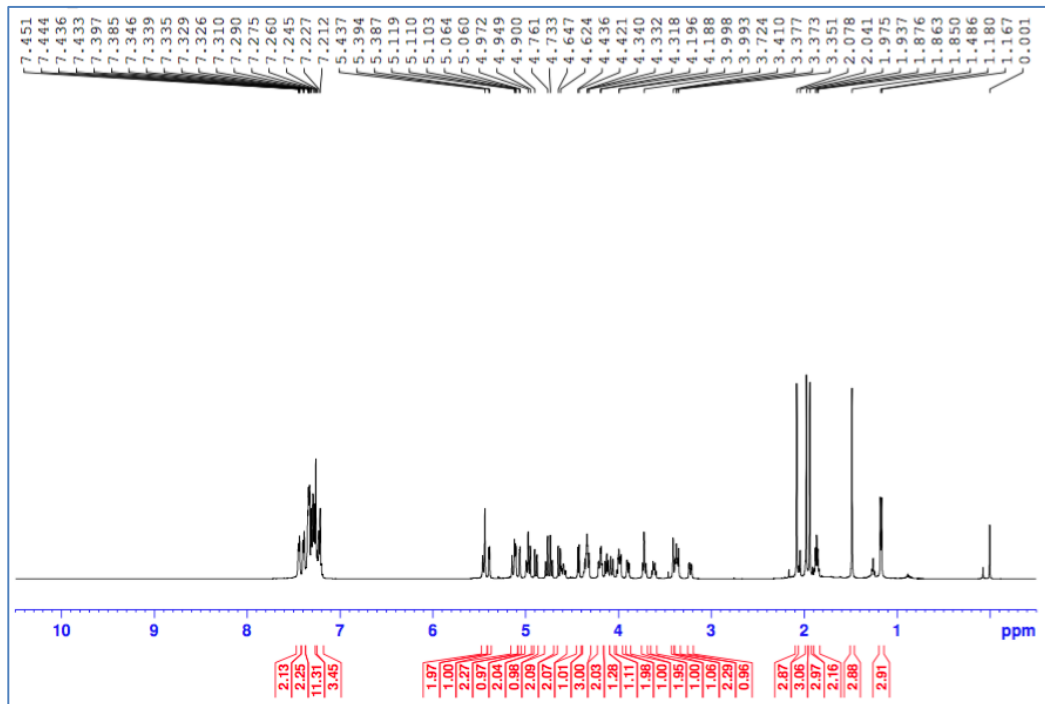
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56:



60:

