Synthesis of fragments of a polysaccharide from *Nocardia nova* for structural and biological studies

by

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Master of Science

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Abstract

Nocardia are gram-positive bacteria. *Nocardia nova* is one of the *Nocardia* species and can cause serious disease, particularly in immunocompromised patients. Rapid diagnosis of *N. nova* can be lifesaving. The cell wall of *N. nova* contains an unusual glucose-modified arabinogalactan polysaccharide with a unique structure that may be a possible target for diagnostic development.

In this thesis I describe the synthesis of oligosaccharide fragments of the glucosyl-galactan domain of *N. nova* arabinogalactan. My targets are structures that contain one or two tetrasaccharide repeating units with an 8-azido-octyl linker at their reducing end. The approach I developed was to synthesize a key tetrasaccharide building block and then use it to glycosylate 8-azido-octanol. The same building block was used to carry out 4+4 glycosylation to obtain the octasaccharide. These molecules will be useful in confirming the structure of the polysaccharide, to generate monoclonal antibodies that could be used in a diagnostic, and to understand the immunological activity of this unique bacterial glycan.

Preface

This thesis is submitted for the degree of Master of Science at the University of Alberta. The research described herein was conducted under the supervision of Professor Todd L. Lowary in the Department of Chemistry, University of Alberta, between September 2018 and December 2019.

This thesis is original, unpublished, independent work by the author, Fazheng Han.

At the end, may this thesis be useful for all the readers and for future related research.

December 2019

Fazheng Han

Acknowledgments

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List of Schemes

List of Abbreviations

$[\alpha]_D$	Specific rotation (sodium D line)
Ac	Acetyl
AG	Arabinogalactan
AgOTf	Silver trifluoromethanesulfonate
aq.	Aqueous
Ar	Aromatic
Bn	Benzyl
br s	Broad singlet (NMR spectra)
Bu	Butyl
Bz	Benzoyl
°C	Degrees Celsius
calcd	Calculated
CDC	Centers for Disease Control and Prevention
COSY	Correlation spectroscopy
Cp_2ZrCl_2	Bis(cyclopentadienyl)zirconium(IV) dichloride
d	Doublet (NMR spectra)

DAST	Diethylaminosulfur trifluoride
DBU	Diazabicyclo[5.4.0]undec-7-ene
DCM	Dichloromethane
DMAP	4-Dimethylaminopyridine
DMF	*N,N*-Dimethylformamide
EDC·HCl	*N*-(3-Dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride
ELISA	Enzyme-linked immunosorbent assay
equiv.	Equivalent
Et	Ethyl
ESI	Electrospray Ionization
Et_3N	Triethylamine
FDG	2-^{18}F-fluoro-2-deoxy-D-glucose
FMNPs	Fluorescent magnetic nanoparticles
Gal	Galactose
Gal*f*	Galactofuranose
Glc*p*	Glucopyranose
GPI	Glycosylphosphatidylinositol

HF	Hydrogen fluoride
HMBC	Heteronuclear Multiple Bond Correlation
HSQC	Heteronuclear Single Quantum Coherence
HRMS	High resolution mass spectrometry
Hz	Hertz
J	Coupling constant
LAM	lipoarabinomannan
Lev	Levulinic
m	Multiplet (NMR spectra)
m-AuNP	Encapsulated gold nanoparticles
M	Molar
mAbs	Monoclonal antibodies
Me	Methyl
mg	Milligram(s)
min	Minute(s)
mL	Millilitre(s)
MOE	Metabolic oligosaccharide engineering
mol	Mole(s)

MRI	Magnetic resonance imaging
NBS	*N*-Bromosuccinimide
NIS	*N*-Iodosuccinimide
NMR	Nuclear Magnetic Resonance
PET	Positron emission tomography
Ph	Phenyl
PMB	*para*-Methoxybenzyl
PMP	*para*-Methoxy phenol
PSA	Prostate-specific antigen
Pyr	Pyridine
q	Quartet (NMR spectra)
quant	Quantitative
R_f	Retention factor
rt	Room temperature
s	Singlet (NMR spectra)
satd	Saturated
SELEX	Systemic Evolution of Ligands by EXponential enrichment

STol	*para*-Methyl thiophenyl
t	Triplet (NMR spectra)
TBAF	Tetrabutylammonium fluoride
TBDPS	*tert*-Butyldiphenylsilyl
TBDPSCl	*tert*-Butyl(chloro)diphenylsilane
TEM	Transmission electron microscopy
Tf	Triflate, trifluoromethanesulfonate
TfOH	Trifluoromethanesulfonic acid
THF	Tetrahydrofuran
TLC	Thin layer chromatography
p-TsOH	*p*-Toluenesulfonic acid

Chapter 1： Introduction

1.1 Carbohydrate-based diagnostic methods

Carbohydrates can be found ubiquitously in biological systems and they are involved in a variety of biological processes, especially recognition processes.[1] Carbohydrates present on the cell surface have highly diversified structure as they can be formed from many different kinds of monosaccharides and they can have many different ways of linkages. The unique structure of glycans gives them high selectivity when targeted by carbohydrate-binding proteins in cell–cell recognition processes. The recognition of cell-surface glycans by proteins plays an essential role in infection, inflammatory responses and immune regulation processes, and they have large potential value in diagnosis of diseases.[2]

It has been found that many diseases cause changes in the abundance and/or types of glycans in cells and bodily fluids. This includes changes in host glycans and appearance of pathogen glycans. For example, many kinds of fungal infections lead to fungal cell-wall (1→3)-β-D-glucans showing up in patient serum. In another example, cancer cells have very different glycan expression patterns from normal cells.[3] Many diagnostic methods have been developed using interactions between carbohydrates and proteins (e.g., antibodies) or carbohydrates and aptamers (nucleic acids that can bind to small molecules) to diagnose diseases. The following section discusses some of representative methods and examples.

1.1.1 Carbohydrate–protein interactions

Antibodies have an important role in the human immune system and they can specifically recognize glycans[4]. Although the binding between carbohydrate-targeted antibodies and their corresponding glycan ligands is weaker than the binding between peptide antigens and their corresponding antibodies, the affinity can be enhanced by using a multivalent display of the antigen. Carbohydrate–antibody recognition and its usage in detecting carbohydrate-based biomarkers has been extensively exploited in the past few years.[5] Many monoclonal antibodies (mAbs) that recognize carbohydrates have been discovered. For example, our research group has made contributions to understanding how one mAb, CS-35,[6,7] binds to lipoarabinomannan (LAM) in mycobacteria. X-ray crystallography of LAM fragments and the CS-35 antibody revealed the structure of the binding site (Figure 1.1), which contains suitable space for this Y-shape sugar epitope, so that they can specifically bind to each other. This discovery confirms this specific carbohydrate–antibody recognition.[7]

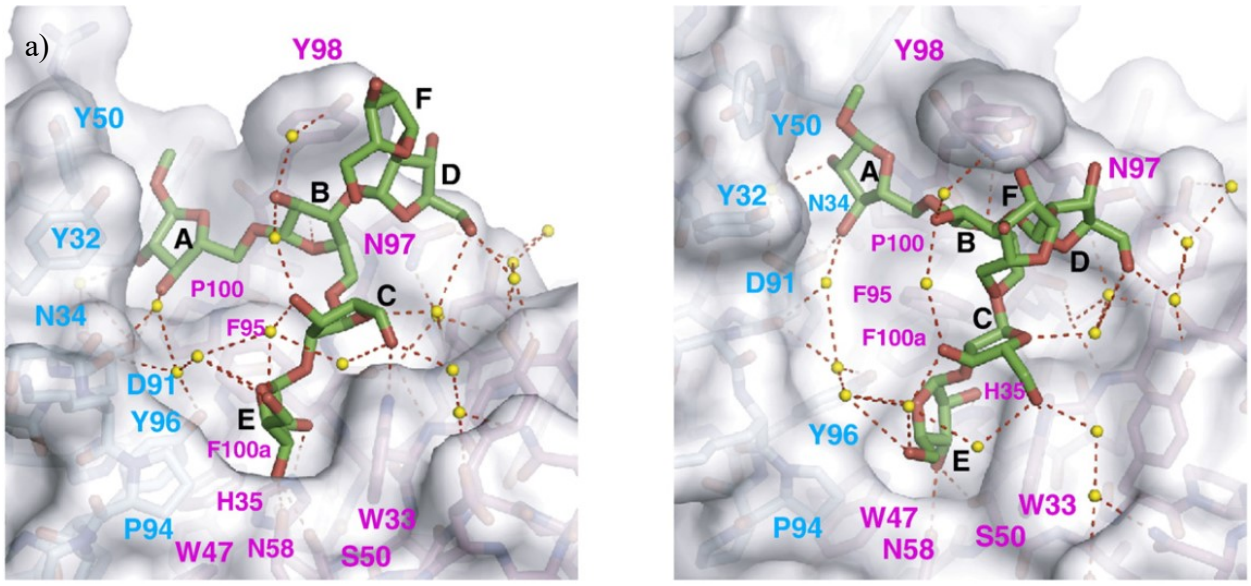


Figure 1.1: Antigen bonding site structures of CS-35Fab bound to arabinofuranoside, a) look from front of the front, b) look from the top. Reprinted with permission from Murase, T.; Zheng, R. B.; Joe, M.; Bai, Y.; Marcus, S. L.; Lowary, T. L.; Ng, K. K. S. *Journal of Molecular Biology* **2009**, *392*, 381–392. [7]

1.1.2 Diagnostics based upon carbohydrate–protein interactions

Many diagnostic methods have been developed using carbohydrate–antibody interactions. There are two general types of methods. In the first type, pathogen glycans are immobilized on the diagnostic device and they recognize the presence of a specific antibody in the patient (e.g. in serum); the presence of the antibody indicates the presence of the pathogen. In the other type of diagnostic method, an antibody is immobilized on the device and it detects the presence of pathogen glycans in the patient (Figure 1.2).

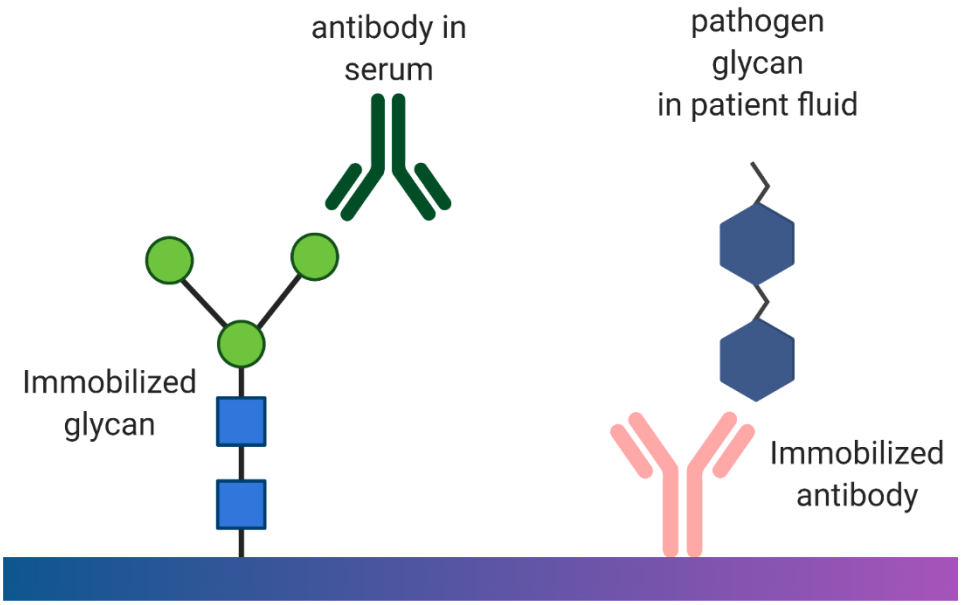


Figure 1.2: Two types of antibody–glycan recognition methods.

I provide two examples of using immobilized glycans to recognize antibodies to diagnose diseases. First, in 2008, Blixt et al. published a study in which a microarray containing *Salmonella* O-antigen oligosaccharide fragments (Figure 1.3) specific for *Salmonella enterica* sv. Paratyphi (group A), Typhimurium (group B) and Enteritidis (group D) were prepared. The array could detect antibodies in patient serum, allowing a rapid diagnostic method to be developed.[8]

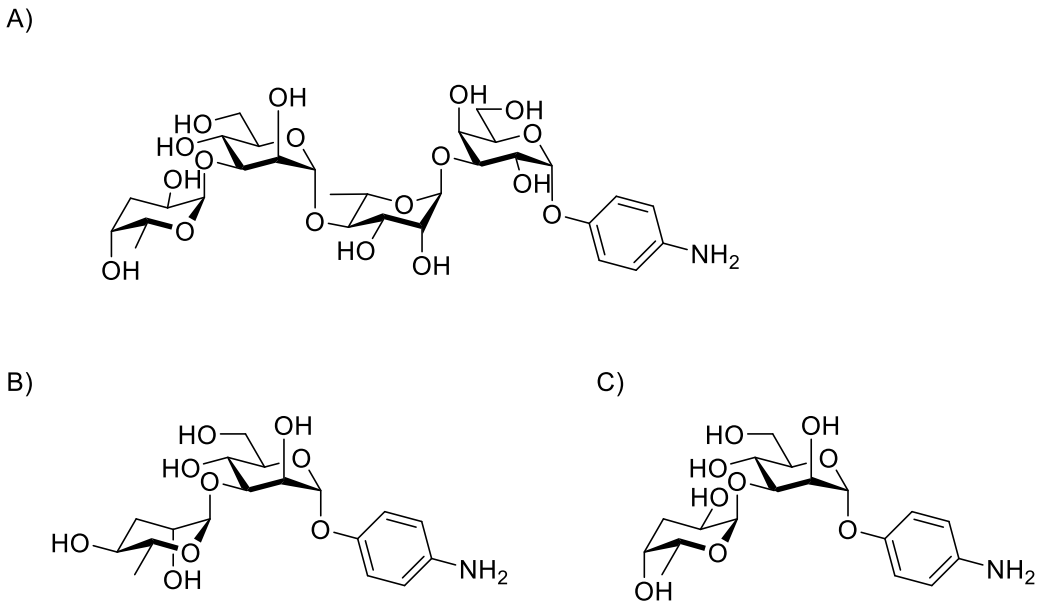


Figure 1.3: Structures of O-antigen (with amine linker) from *S.enterica sv.* Parathyphi (A), O-antigen from *S. enterica sv.* Enteritidis (B), and O-antigen from *S. enterica sv.* Typhimurium (C).

Also, in 2008, Seeberger's group developed a glycan array based on parasitic glycosylphosphatidylinositol (GPI) from malaria (Figure 1.4A) and the *Toxoplasma gondii* parasite (Figure 1.4B). Like the work of Blixt et al., this GPI array could work as a diagnostic method because it can detect the corresponding anti-carbohydrate antibodies in patient serum, indicating the presence of the parasite.[9,10]

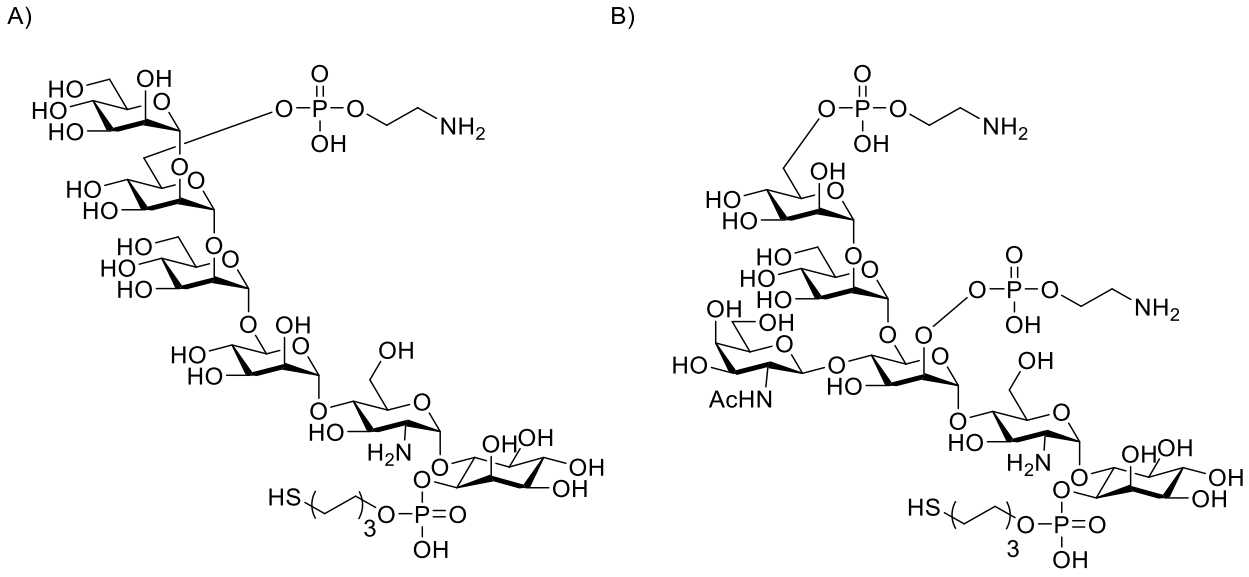


Figure 1.4: Structures of synthetic GPI glycans for microarray construction from malaria (A) and *T. gondii* (B) antigens.

Having presented two examples of using glycans to detect antibodies, I will now provide examples of using immobilized antibodies to detect glycans. One example is a sandwich type enzyme-linked immunosorbent assay (ELISA) (Figure 1.5), which has been used to diagnose invasive aspergillosis[11], by detecting the presence of galactomannan in patient sera.

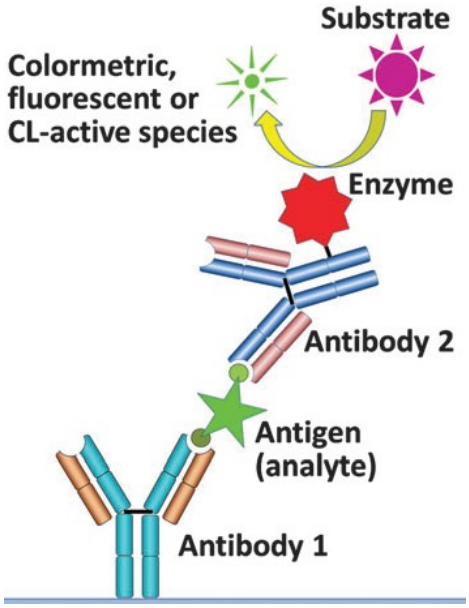


Figure 1.5: Sandwich type enzyme-linked immunosorbent assay. An antibody is attached to the surface to recognize the antigen. Then a detecting antibody, which also binds to the antigen is added. Then, a substrate for the enzyme conjugated to detecting antibody is added to generate the signal. Reprinted with permission from Wang, S.-K.; Cheng, C.-M. *Chem. Commun.* **2015**, *51*, 16750–16762.[12]

Lectins are another kind of protein that can specifically bind to glycans.[13,14] Many of them have been well studied and there are diagnostic methods developed based on carbohydrate–lectin recognition. Like for antibodies, there are two ways of using this detection method. The lectins can be attached to the device (a surface or a particle) to detect the corresponding pathogen glycans. Alternatively, the glycans can be attached to the device for detecting lectins on pathogens (Figure 1.6).[15,16]

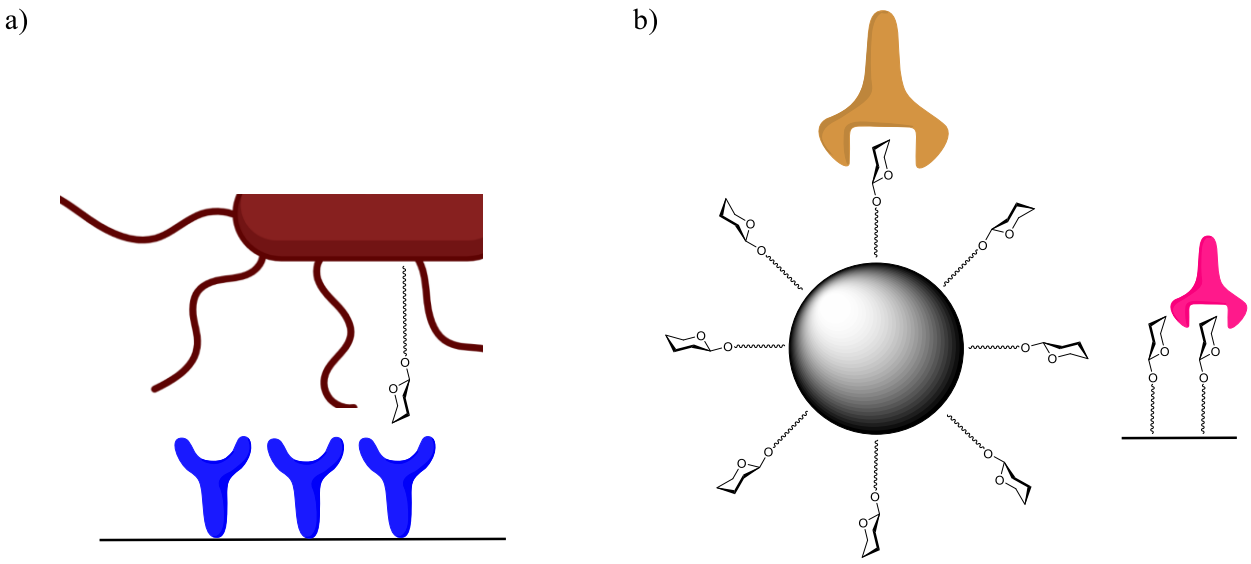


Figure 1.6: a) lectins are immobilized to detect a pathogen glycan. b) Glycans are attached to nanoparticles or surface to detect pathogen lectins.

Here we have an example of using a lectin to recognize glycans: the mannose binding FimH adhesin protein is a popular target lectin for this approach, detection of this protein can indicate the presence of pathogens, for example *Escherichia coli* (*E. coli)* ORN178. In this method, the mannose residues are attached to encapsulated gold nanoparticles (m-AuNP) and examined by transmission electron microscopy (TEM) to detect bacteria that have the FimH adhesin protein (Figure 1.7).[17]

Another example of this kind of method is the detection of *Helicobacter pylori* by recognizing its BabA adhesion protein by the Lewis a (Le^a), Lewis b (Le^b) or the blood group H type 1 (H1) antigen (Figure 1.8).[18] The authors of this study prepared oligosaccharide-conjugated dual-modal fluorescent magnetic nanoparticles (FMNPs), which can be detected by TEM and

confocal microscopy.

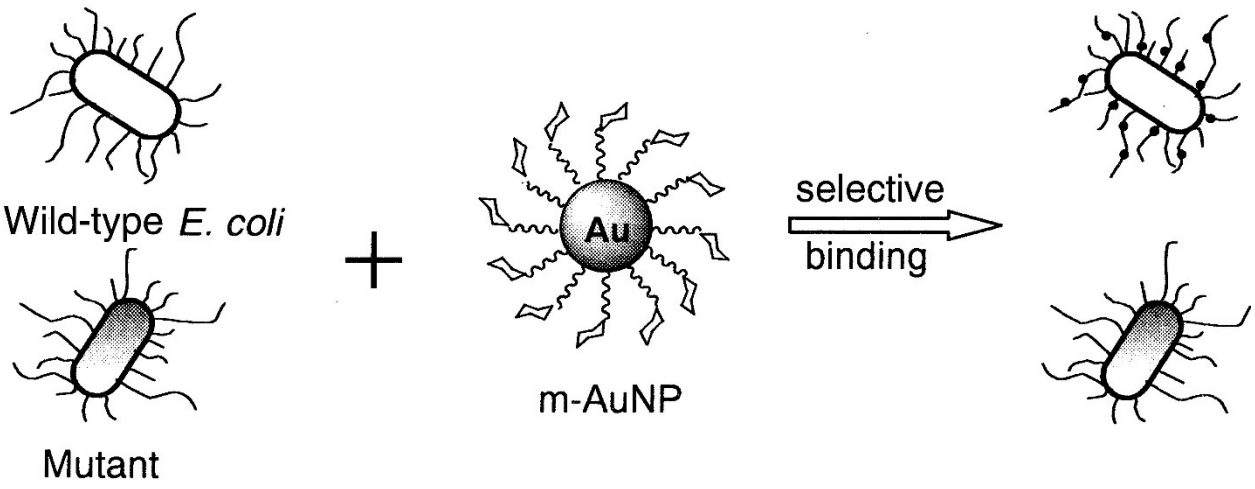


Figure 1.7: m-AuNP selectively bind to *E. coli* (ORN178 strain) that have receptor of type 1 pili, but not the ORN208 strain, which has the receptor mutant. Reprinted with permission from Lin, C.-C.; Yeh, Y.-C.; Yang, C.-Y.; Chen, C.-L.; Chen, G.-F.; Chen, C.-C.; Wu, Y.-C. *J. Am. Chem. Soc.* **2002**, *124*, 3508–3509.[17]

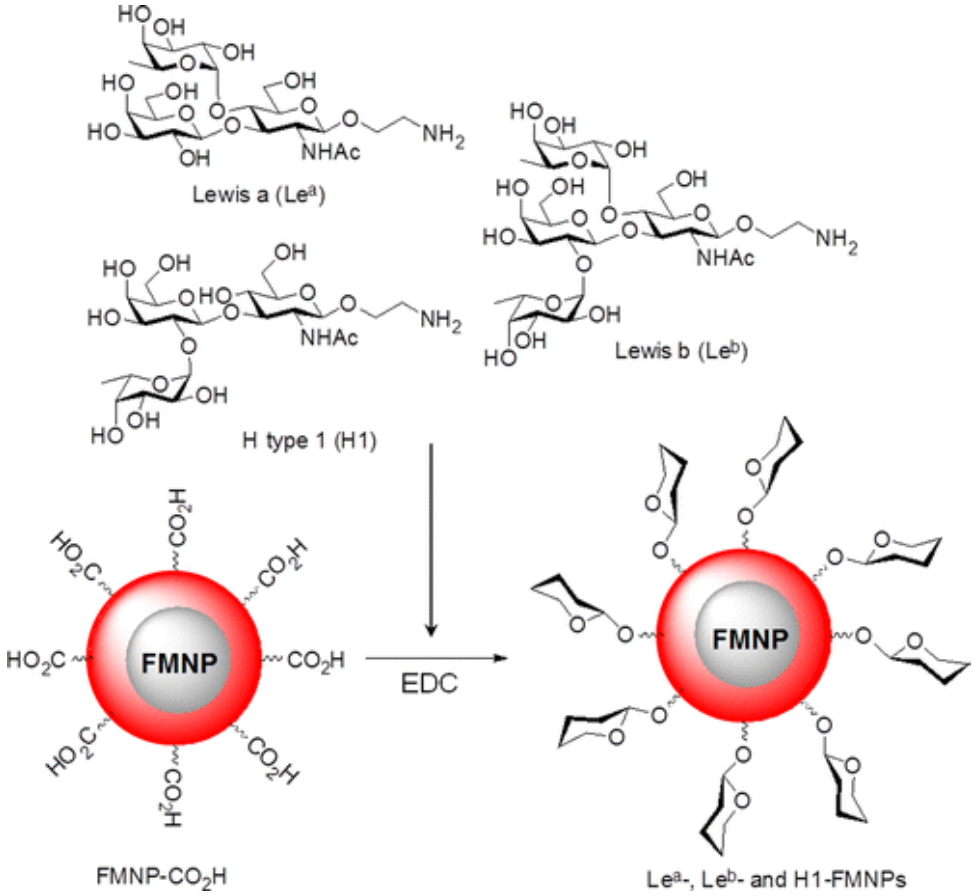


Figure 1.8: Glycan-conjugated nanoparticles that bind to BabA displaying *Helicobacter pylori*. Reprinted with permission from Park, S.; Kim, G.-H.; Park, S.-H.; Pai, J.; Rathwell, D.; Park, J.-Y.; Kang, Y.-S.; Shin, I. *J. Am. Chem. Soc.* **2015**, *137*, 5961–5968.[18]

In the other way around, biosensors with immobilized lectins can detect pathogen glycans to diagnose diseases.[19–22] For example, the lectin concanavalin A (Con A) was used for the detection of *E. coli* W1485.[23]

1.1.3 Carbohydrate–aptamer interactions

Aptamers are oligonucleotides that bind to a specific target molecules.[24] In the early 1990s, a method called "Systemic Evolution of Ligands by EXponential enrichment" (SELEX) was developed by the Gold and Szostak groups.[25,26] By selection and evolution, aptamers that have strong interaction with the selected glycans can be developed (Figure 1.9).

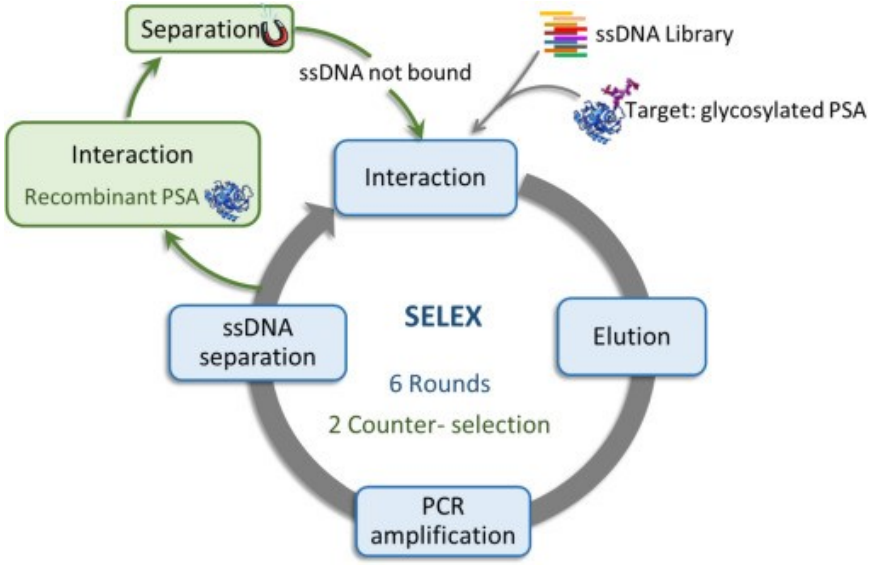


Figure 1.9: Scheme for the SELEX enrichment process. Reprinted with permission from Díaz-Fernández, A.; Miranda-Castro, R.; de-los-Santos-Álvarez, N.; Rodríguez, E. F.; Lobo-Castañón, M. J. *Biosensors and Bioelectronics* **2019**, *128*, 83–90.[27]

Good binding affinity and selectivity in binding make it possible to use aptamers as biosensors.[28] There are some recent studies that show that glycan-recognizing aptamers have potential to work as diagnostic methods. In 2012, an RNA aptamer was developed to detect one of the most dangerous *E. coli* strains, *E. coli* O157:H7.[29] The aptamer specifically binds to the lipopolysaccharide on the surface of the bacteria. In this way, the aptamer can distinguish between

the virulent strain O157:H7 from other *E. coil* strains. In 2013, Shi-Ying Lu's group reported six different aptamers that bind to *N*-glycolylneuraminic acid (Neu5Gc), which occurs in many human tumors.[30] DNA aptamers that target glycans been also been developed. For instance, an aptamer that recognizes the glycan moiety of the prostate-specific antigen (PSA), a glycoprotein, has been identified and an aptamer-based biosensor developed (Figure 1.10).[27]

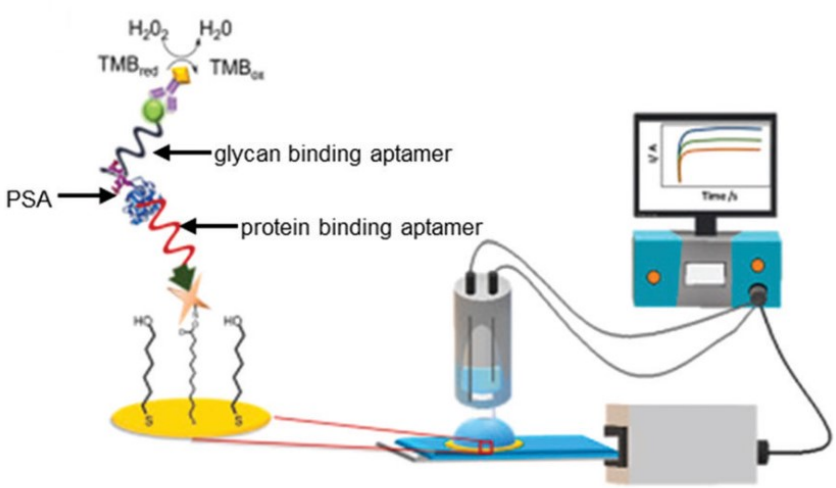


Figure 1.10: Sandwich-like PSA biosensor using both glycan and protein binding aptamers, similar to the sandwich-ELISA, the detecting antibody was added to visualize the presence of PSA. Reprinted with permission from Díaz-Fernández, A.; Miranda-Castro, R.; de-los-Santos-Álvarez, N.; Rodríguez, E. F.; Lobo-Castañón, M. J. *Biosensors and Bioelectronics* **2019**, *128*, 83–90.[27].

1.2 Carbohydrate-based Diagnostic devices

Based on variant of recognition methods of glycans, many devices/techniques have been developed to detect human diseases. Examples of these techniques are non-invasive magnetic resonance imaging (MRI), positron emission tomography (PET) or metabolic oligosaccharide

engineering (MOE) to visualize glycans *in vivo*. In 2009, Davis and coworkers in Oxford reported their studies on using MRI-visible carbohydrate-functionalized nanoparticles (Figure 1.11) to detect the inflammatory markers E-/P-selectin.[31] Another example is the detection of 2-^{18}F-fluoro-2-deoxy-D-glucose (FDG) separation in the human body by PET. This can lead to early cancer diagnosis, because in cancer cells FDG has a higher concentration than in normal cells (Figure 1-11).[32] In 2004, Bertozzi and coworkers published a paper in Nature reporting their research on using MOE, to selectively modify glycans on cell surface *in vivo*. The use of MOE may lead to therapeutic targeting and diagnostic method development (Figure 1.12).[33]

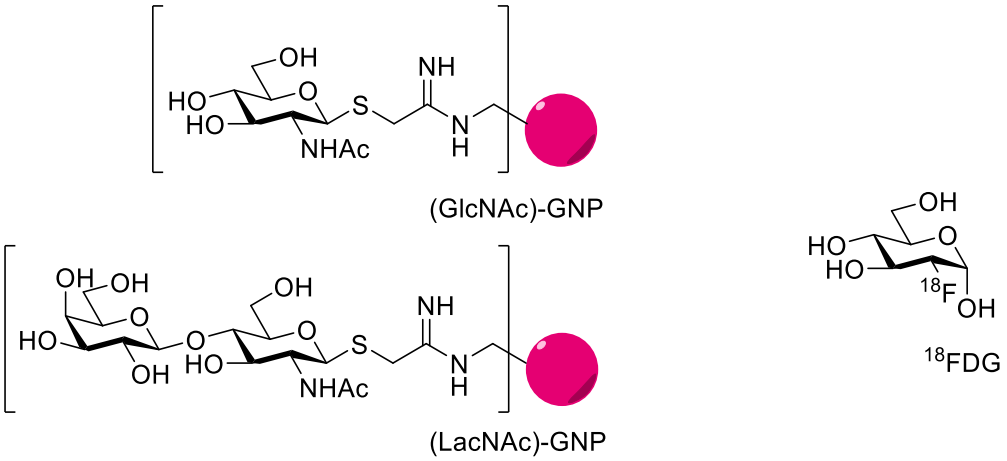


Figure 1.11: Structure of MRI-viable nanoparticles and ^{18}FDG.

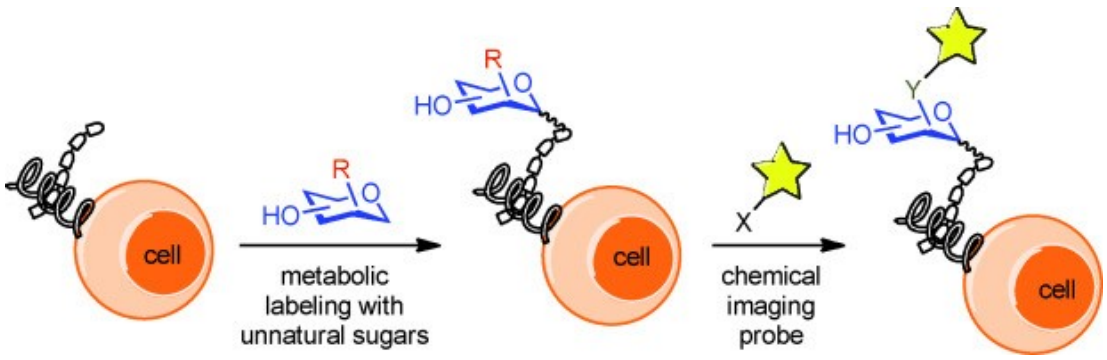


Figure 1.12: Metabolic oligosaccharide engineering for in vivo molecular imaging of glycans. Reprinted with permission from Fernández-Tejada, A.; Cañada, F. J.; Jiménez-Barbero, J. *Chem. Eur. J.* **2015**, *21*, 10616–10628.[2]

To develop diagnostic methods based on glycan–receptor interactions, one needs to understand the structure of the glycan and also have an appropriate receptor. Synthetic glycans are useful for both things. Carbohydrates obtained from synthesis can confirm the structure proposed based on structural studies of materials isolated from nature. In addition, synthetic glycans can also be used to generate appropriate receptors (e.g., antibodies). In this thesis, I describe the synthesis of oligosaccharides related to the arabinogalactan of Nocardia, an organism that is a concern particularly in immunocompromised patients. In the next section, I discuss this organism and the arabinogalactan it produces.

1.3 *Nocardia Nova* and *N. nova* arabinogalactan

The genus *Nocardia* is a gram-positive bacterium. More than 100 species of the genus have been characterized[34,35], and at least 16 can cause human infections.[36] *Nocardia* species are found

in the soil and organic matter, as well as in fresh or salt water. *Nocardia* infection usually occurs as an opportunistic infection in immunocompromised people (e.g., HIV+ individuals), the elderly, and small children. Most *Nocardia* infections are caused by *Nocardia brasiliensis*, *Nocardia cyriacigeorgica*, *Nocardia farcinica*, and *Nocardia nova*. The most common site of *Nocardia* infection is the lungs and it leads to fever and cough. *N. nova* has an aggressive pattern of causing disseminated nocardiosis, which means the infection can spread to different locations within the body. For example, the infection can spread from the lungs to brain and other organs and if *Nocardia* gets into open wounds or cuts, skin infections can also happen. According to CDC's (Centers for Disease Control and Prevention) report: "In the United States, it has been estimated that 500–1,000 new cases of nocardiosis infection occur every year. Approximately 60% of nocardiosis cases are associated with pre-existing immune compromise."[37] Disseminated nocardiosis results in a high risk of death.[38] It is difficult to diagnose; therefore, rapid diagnostic methods are desired.

As outlined above, glycans can be used to diagnose disease. The cell wall of *Nocardia* is believed to be similar to that of mycobacteria (Figure 1.13A) and glycans in this structure could be a suitable target for glycan-based diagnostics. Recent unpublished work from our collaborator, Dr. Yann Guerardel at the University of Lille in France, has studied the structure of the arabinogalactan polysaccharide in *N. nova*'s cell wall. They proposed the structure of the galactan

portion of the polysaccharide (Figure 1-13B), which is distinctive enough for developing a diagnostic method based on recognition of this glycan.

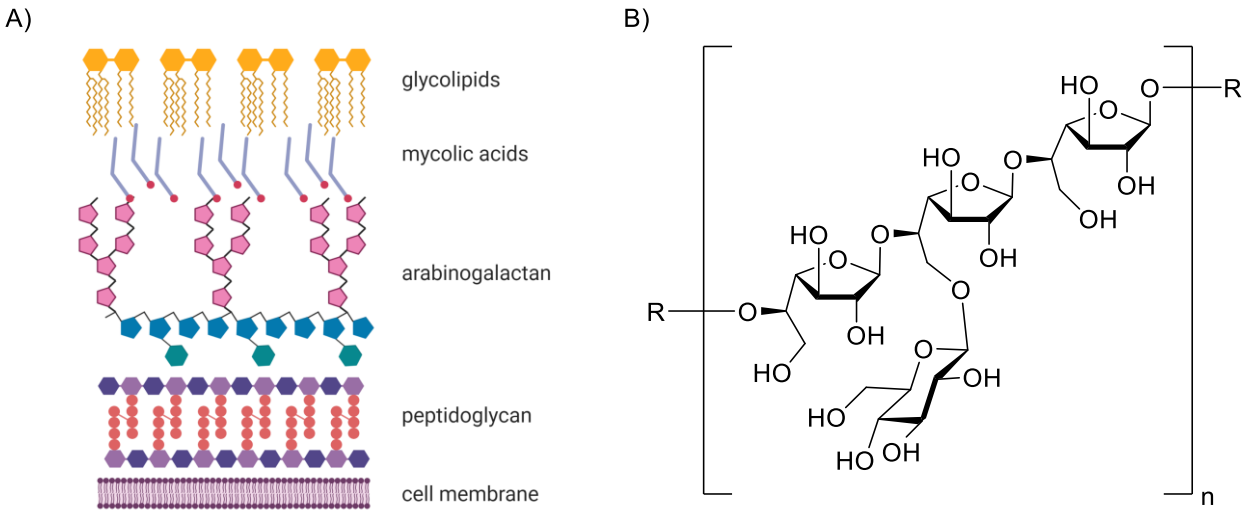


Figure 1.13: Structure cell wall of *N. nova* (A) and glucose-modified-galactan of *N. nova* (B).

In this project, I am focused on the chemical synthesis of fragments of the galactan part of the *N. nova* arabinogalactan. Based on Dr. Guerardel's work, the galactan is modified by β-D-glucose residue, which is different from the galactan portion of mycobacterial arabinogalactan.[39] The polysaccharide has a tetrasaccharide repeating unit with three β-(1→5)-linked galactofuranoside (Gal*f*) residues; the second Gal*f* in the repeating unit has a glucopyranoside (Glc*p*) connected to it in a β-(1→6)-linkage (Figure 1.13B).

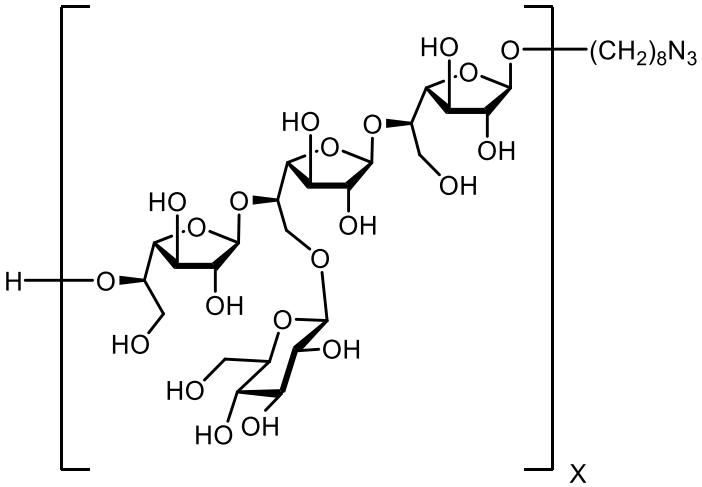
The purpose of the synthesis of these compounds is the following. **First**, the chemically-synthesized galactan fragments can serve as standards for confirming the NMR data and structure

of the natural polysaccharide as determined by Dr. Guerardel. This can be done by comparing the NMR or GC-MS data of the oligosaccharides with that of the natural glycan. **Second**, as I introduced in the previous section, the compounds could be used as an antigen to generate monoclonal antibodies that could be used in the detection of the polysaccharide, maybe eventually leading to a diagnostic method. **Third**, the glycans can be used to study the ability of these molecules to give a positive result in the Fungitell® Assay, which allows diagnosing fungal disease by detecting (1→3)-linked β-glucans. This assay is usually used to diagnose fungi infections. However, there are few reported cases that this assay also gives a positive result for patients that only have *Nocardia* (a bacteria) infections.[40,41] Our collaborator Dr. Guerardel found out that the bacteria itself is not active in the Fungitell® Assay; however, after degradation by the macrophage, the unmasked *N. nova* arabinogalactan shows cross-reactivity in the assay, but the reasons for now are unclear. We would like to find out if it is the β-glucose residue attached to the galactan core, or an impurity in the preparation, that gives the positive result. Having access to pure oligosaccharides would allow us to examine this.

Chapter 2: Synthesis of galactan fragments of the arabinogalactan from *Nocardia nova*

2.1 Introduction

As I outlined in Chapter 1, my target molecules (**1** and **2**, Scheme 2.1) are galactan fragments of the arabinogalactan from *Nocardia nova*. The goal of my project was to use chemical methods to synthesize these oligosaccharides to provide materials that will help confirm the structure of the natural polysaccharide and also explore its biological activity. The natural polysaccharide is a large structure that contains multiple repeating units. Oligosaccharides **1** and **2** contain one and two repeating units, respectively. In developing an approach, we wanted to develop a synthetic method that could be used to synthesize larger oligosaccharides by iteration of the glycosylation reactions. In this chapter, I will describe the retrosynthesis analysis of **1** and **2** and their synthesis.



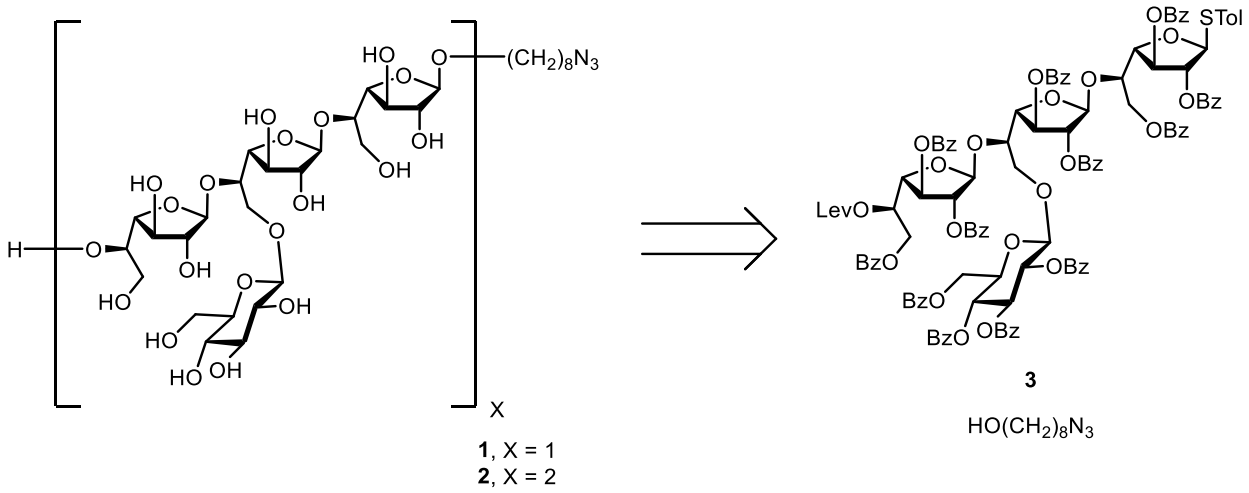
1, X = 1
2, X = 2

Scheme 2.1: Structure of target molecules **1** and **2**

2.2 Retrosynthetic Analysis of Target Molecules

2.2.1 Overall Strategy of Retrosynthesis

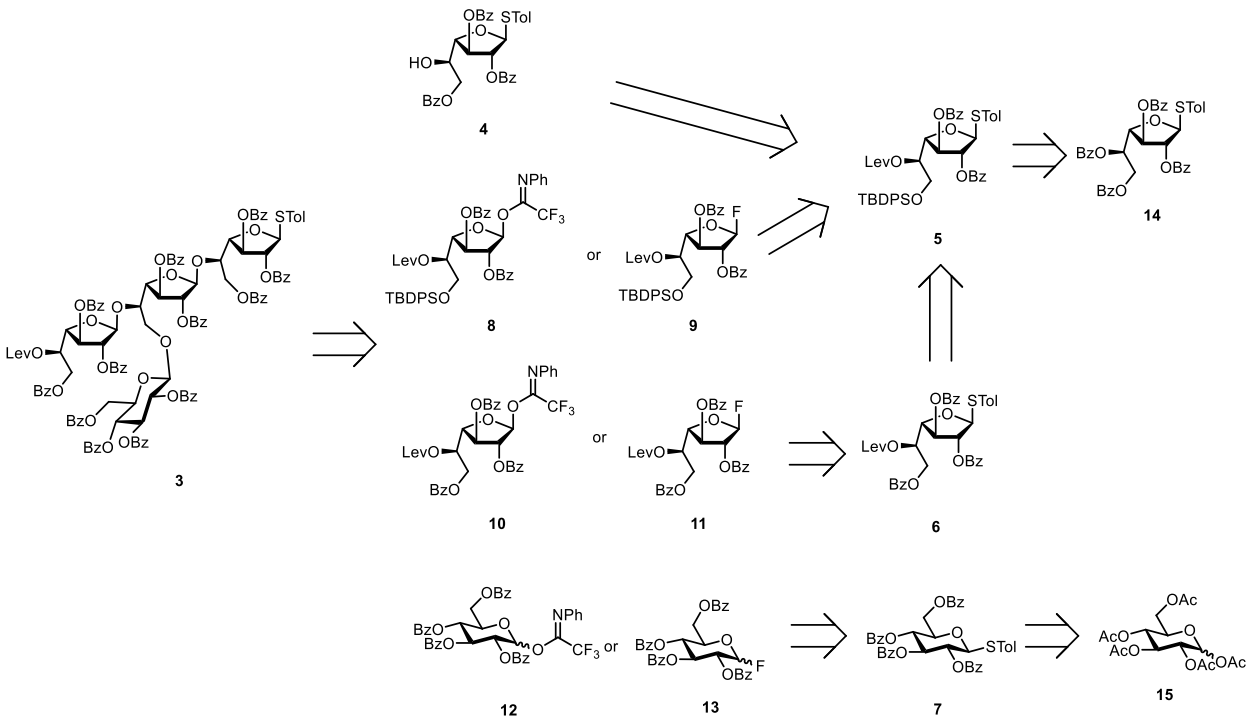
The retrosynthesis analysis of an oligosaccharide is based primarily on the type of sugar residues and the type of glycosidic linkages present in the target molecules. Compounds **1** and **2** have a tetrasaccharide repeating unit with three β-(1→5)-linked galactofuranoside (Gal*f*) residues; the second Gal*f* in the repeating unit has a glucopyranoside (Glc*p*) connected to it in a β-(1→6)-linkage, and a azido octanol linker will be at the reducing end of those compounds, because we need to couple the compound to a solid phase for future need. There are well established synthetic methods to access linear fragments that contain alternating β-(1→5)-linked and β-(1→6)-linked D-Gal*f* residues.[42,43] However, structures containing a linear Gal*f* core and a branching β-(1→6)-linked Glc*p* residue have not been reported before. We envisioned that the most convenient way to synthesize **1** and **2** was to prepare a tetrasaccharide building block, followed by its oligomerization to make compounds that contain more than one repeating unit (Scheme 2.2).



Scheme 2.2: Retrosynthesis of target molecules

2.2.2 Retrosynthetic Analysis of Tetrasaccharide Building Block

The tetrasaccharide building block can be further cut into four monosaccharide building blocks. The first is the "primer acceptor" **4**. The next is the "centerpiece" Gal*f* residue, which could be either a glycosyl *N*-phenyl trifluoroacetimidate donor **8** or glycosyl fluoride donor **9**. Another is the "tailpiece" Gal*f* residue, which could be introduced from either *N*-phenyl trifluoroacetimidate donor **10** or glycosyl fluoride donor **11**. Finally, is the branching Glc*p* residue, which could be introduced by either *N*-phenyl trifluoroacetimidate donor **12** or glycosyl fluoride donor **13**. The modifications on the sugar determines the reactivity and selectivity in glycosylation reactions. I will next explain why those monosaccharide derivatives were chosen to assemble the tetrasaccharide building block.



Scheme 2.3: Retrosynthesis of tetrasaccharide building block

As shown in Scheme 2-3, there are only 1,2-*trans* glycosidic linkages in the target molecules. Therefore, to control the selectivity of the glycosylation reactions, the C-2 hydroxyl group on all of the donors should be protected by an acyl group, which can participate during the glycosylation leading to 1,2-*trans* linkages.[44,45,46] As the acyl group, I chose benzoate esters because they have less chance to form orthoesters during glycosylation compared to acetate esters.[47] However, for the introduction of the Glc*p* residues I also explored donors with acetate esters.

The "primer acceptor" **4** needs to have a free hydroxyl group on C-5. Moreover, because I need to elongate the tetrasaccharide into an octasaccharide by a 4+4 glycosylation later on, it

should have a latent leaving group (e.g., a methyl thiophenyl group) on its C-1 position. Upon activation, this group can serve as leaving group on the tetrasaccharide donor when doing the 4+4 glycosylation. Another choice is to orthogonally protect the C-1 hydroxy group, e.g., with a paramethoxyphenyl (PMP) group. Cleavage of the PMP group would provide a reducing sugar that could be converted into a donor for the 4+4 glycosylation. After reading through the literature and consideration, I decided using another orthogonal protection group was going to make the approach too complicated. Besides, based on literature reports, deprotection of the PMP group and conversion into a donor usually results in a loss of around 40% of the product (in my case a precious tetrasaccharide), which is unfavored in the later stage of a synthesis. Therefore, I decided to install the methyl thiophenyl (STol) group on C-1, and use other types of donors that can be orthogonally activated to couple with the "primer acceptor."

For the "centerpiece" building block, during assembly of the tetrasaccharide this compound needs to serve as donor to couple with the "primer acceptor" with the β-(1→5)-linkage. To prevent self-coupling of the "primer acceptor", the "centerpiece" must be some other type of donor that can be orthogonally activated. There are plenty of viable choices; I chose to explore the glycosyl *N*-phenyl trifluoroacetimidate donor **8** and glycosyl fluoride donor **9** (synthesized from reported[45] precursor **5**). For the same reason, the "tailpiece" and glucose building block also need to be converted into *N*-phenyl trifluoroacetimidate donor **10** and fluoride donor **11** (synthesized from

reported[48] precursor **6**). On the "centerpiece" building block, the C-5 and C-6 hydroxyl groups must be orthogonally protected because they are going to be coupled with different sugar residues in later steps. I chose a levulinate (Lev) ester to protect the C-5 hydroxyl group and a *tert*-butyldiphenylsilyl (TBDPS) ether to protect the C-6 hydroxyl group.

For the "tailpiece", it is used to form a β-(1→5)-linkage with the "centerpiece". In addition, the protecting group on the C-5 hydroxyl group must be capable of being selectively deprotected after the assembly of tetrasaccharide so it can serve as accepter in a 4+4 glycosylation. Therefore. the C-5 hydroxyl group was protected by a Lev ester.

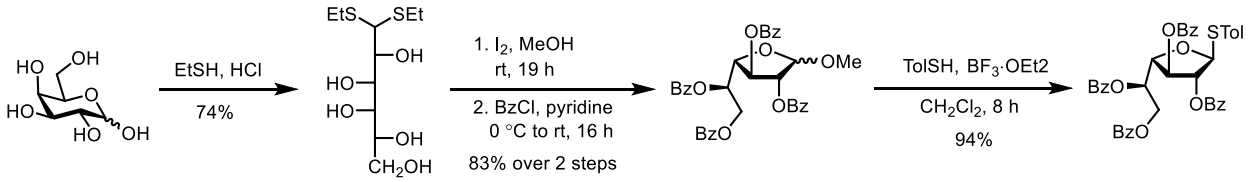
The other hydroxyl groups on these monosaccharide building blocks were protected with benzoate esters. Having a consistent protecting group will minimize the number of steps for global deprotection at the end of the synthesis.

For all the Gal*f* building blocks discussed above, they all come from the key intermediate **5**, which could be synthesized from **14**.[49] The Glc*p* building blocks **12** and **13** could synthesized[50] from commercially available **15**.

2.3 Synthesis of Target

2.3.1 Synthesis of Gal*f* Building Blocks

D-Gal*f* (five-membered ring) is thermodynamically disfavored compared to the pyranose form of the monosaccharide. Therefore, the first challenge in accessing these compounds is making a furanose derivative from the natural pyranoside.[51] Luckily, there are many procedures developed to perform this transformation. One of them, which uses the cyclization of galactose dithioacetal cyclization, a method first reported by Szarek and co-workers,[52] has been frequently used in our group. The method starts with the transformation of the reducing sugar D-galactose (**16**) to the dithioacetal **17** (Scheme 2.4).[53] Using 2% iodine in methanol, compound **17** is cyclized to the unprotected methyl glycoside, which is then benzoylated to give **18**. Methyl glycoside **18** can be converted to thioglycoside **14** by reaction with TolSH in the presence of $BF_3{\cdot}OEt_2$.[49]

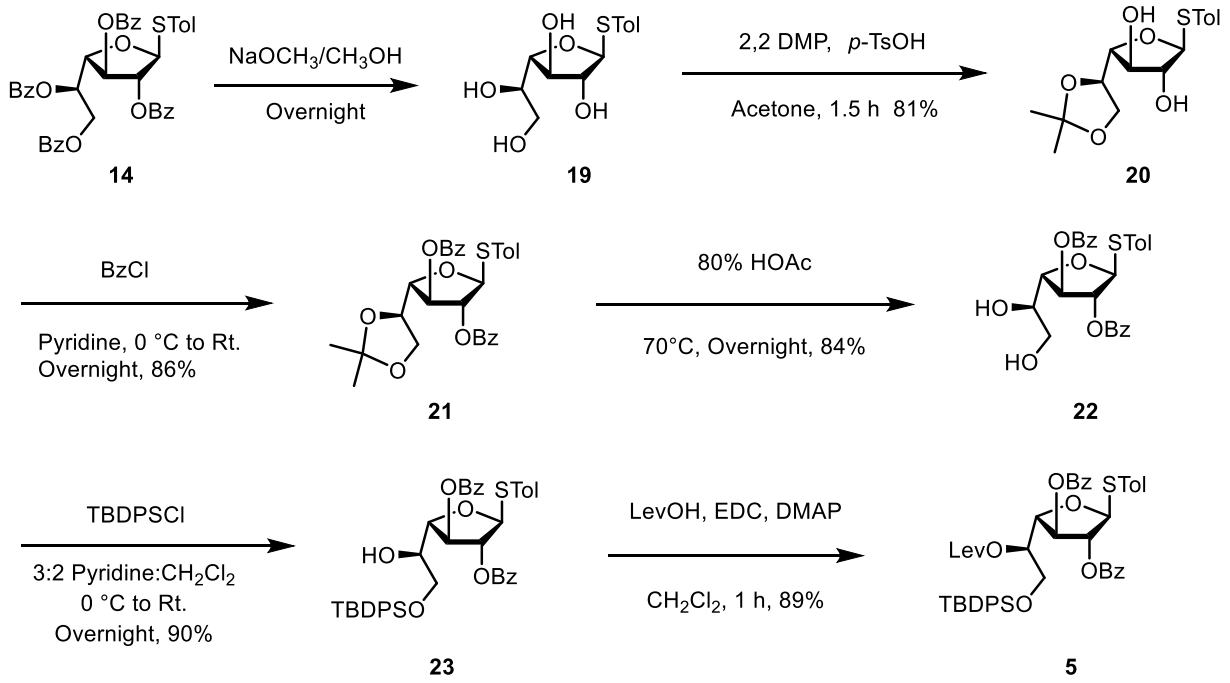


Scheme 2.4: Synthesis of tetra-benzoylated galactofuranoside **14**

2.3.1.1 Synthesis of Key Intermediate 5.

Starting from **14**, the compound could be fully deprotected using sodium methoxide in methanol to give tetrol **19** (Scheme 2.5). Protection of the C-5 and C-6 hydroxyl groups as an isopropylidene acetal was achieved by treatment of **19** with dimethoxypropane in dry acetone with

p-TsOH as the catalyst. The product, **20**, was obtained in 81% yield over two steps.[54] There are minor byproducts observed on TLC. In experience of a previous group member, Dr. Gladys Completo, these compounds could be acyclic acetals formed on O-2 and O-3.[55] The free hydroxyl groups in **20**, at C-2 and C-3, were then protected by reaction with benzoyl chloride in pyridine to give an 86% yield of compound **21**. The structure of the product was confirmed from the ^{1}H NMR data. Compared to its parent diol **20**, benzoylation resulted in a downfield shift of the resonances for H-2 and H-3 in **21**. In addition, there were 10 more aromatic hydrogens in the spectra. Subsequent hydrolysis of the isopropylidene acetal was carried out in 4:1 AcOH–water at 80 °C leading to a compound (**22**) with the C-5 and C-6 hydroxyl groups liberated. The next step was to selectively protect the primary alcohol by treating **22** with *t*-butylchlorodiphenylsilane (TBDPSCl) in pyridine and CH_2Cl_2. Because the TBPDS is a bulky group, the formation of the silyl ether occurred only on O-6 but not O-5. In this way, the primary alcohol was selectively protected affording **23** in 90% yield. The remaining hydroxyl group at C-5 was then protected as a Lev ester upon exposure of **23** to levulinic acid and EDC·HCl promoted by DMAP in CH_2Cl_2. The ^{1}H NMR data for **23** matched that previously reported.[45] To be specific, there was a 3 H singlet peak arising from the Lev ester at 2.08 ppm and 9 H singlet arising from the TBDPS ether at 1.00 ppm. In addition, the benzoate esters were present based on chemical shifts of H-2 (5.64 ppm) and H-3 (5.53 ppm). This reaction sequence gave the first key intermediate, **5**, which could be modified into the other Gal*f* building blocks needed in the assembly of the tetrasaccharide building block.

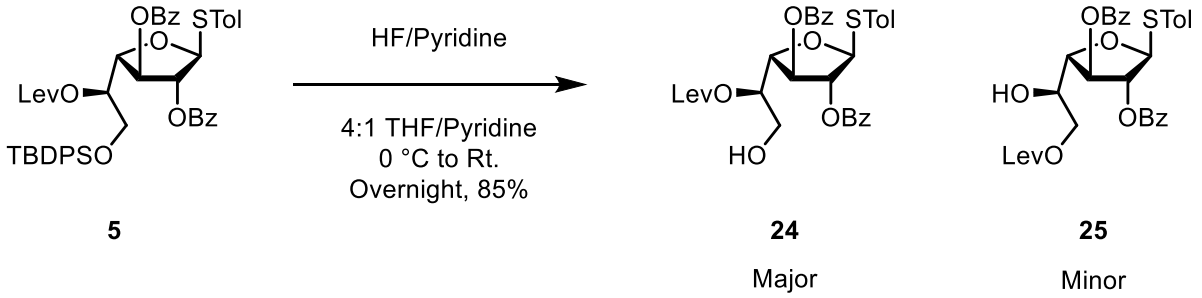


Scheme 2.5: Synthesis of key monosaccharide **5**

2.3.1.2 Synthesis of "Primer Acceptor" 4.

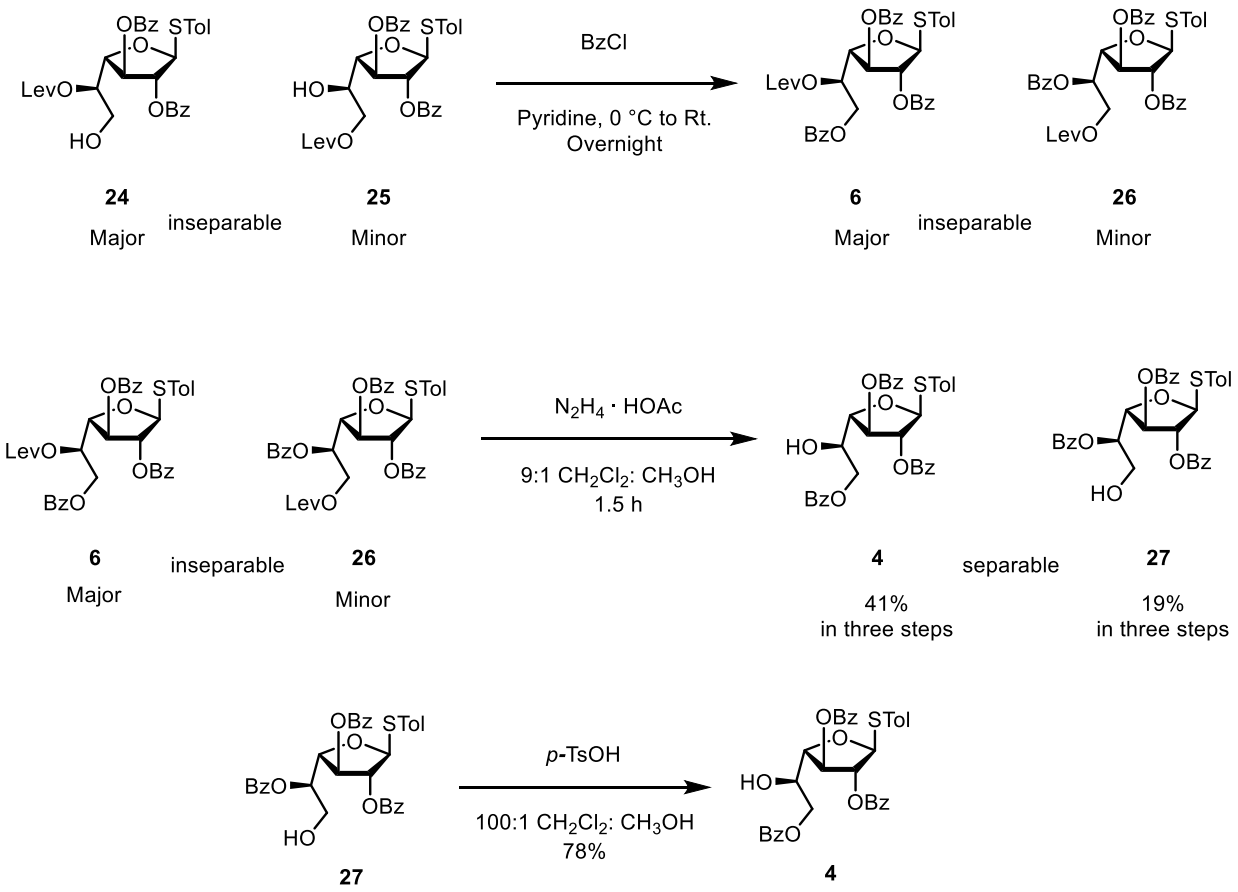
With key intermediate **5** in hand, I worked towards making the three key building blocks needed for the assembly of the tetrasaccharide. I focused first on the route to "primer acceptor" **4**. Doing so required exchanging the TBDPS group for a benzoate ester and then removal of the Lev ester. Thus, the next step was to deprotect the silyl ether on O-6 (Scheme 2.6). Usually, a silyl ether can be removed by treatment with either tetra-*n*-butyl ammonium fluoride (TBAF) or 70% HF in pyridine. Considering that I have benzoate ester protecting groups in the molecule, I chose HF in pyridine instead of TBAF, because I was worried that the TBAF would remove the benzoyl groups as well.[56] However, when I explored this reaction using HF in pyridine, I observed Lev migration

from O-5 to O-6. The reaction gave a mixture of two compounds, **24** and **25**, in a combined 85% yield in a 69:31 ratio.



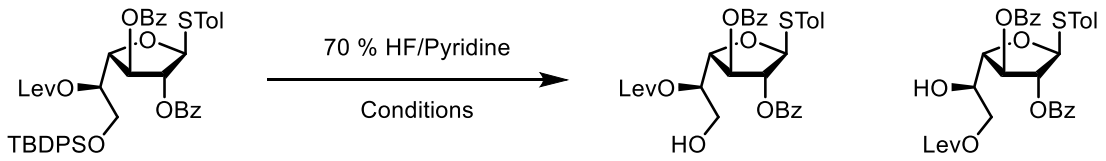
Scheme 2.6: Deprotection of TBDPS group

The major product, compound **24**, was the desired one; however, because the byproduct **25** has a very close R_f value, purification was very difficult. Thus, the mixture was submitted to the next two steps, benzoylation and deprotection of the levulinate group (Scheme 2.7). This resulted in the mixture being turned into two separable compounds – "primer acceptor" **4** (41% yield) and the O-5 benzoylated compound **27** (19% yield) over the three steps. These compounds can be differentiated by analyzing their ^{1}H NMR spectra, by comparing the signals for H-5. This signal in compound **4** is more upfield (4.54 ppm) than in compound **27** (5.65 ppm), indicating that **4** has a free hydroxyl group on C-5, The data also matches that reported previously.[55] The undesired regioisomer **27** could be converted into **4** by a method discovered by another coworker in the group upon treating it with *p*-TsOH in a relatively non-polar solvent system. In this case, I used dichloromethane as the solvent and 1% methanol was added to dissolve the *p*-TsOH. After stirring for three hours at room temperature, the desired compound **4** was obtained in 78% yield.



Scheme 2.7: Separation and recovery of **4** from mixture of regioisomers formed upon TBPDS deprotection of **5**

To optimize the deprotection step, I tried increasing the amount of pyridine relative to tetrahydrofuran (THF), from the initial 4:1 THF–pyridine ratio (Table 2.1). By changing the ratio to 1:1 to decrease the acidity of the reaction system, and more frequently monitoring the reaction progress, I could stop the reaction when the TBPDS deprotection was complete, but before migration had occurred (from 30 hours to ~15 hours). In doing this, I could perform the reaction such that only a trace amount of migration product was formed. The migration product was barely detectable by TLC and the ^{1}H NMR spectrum showed only **25** after purification.



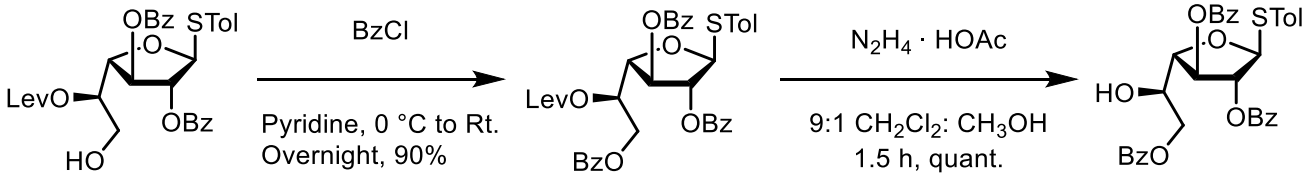
5 **24** **25**

Entry	THF–pyridine ratio	Reaction time	Ratio of **24**/**25**[a]
1	4:1	30 h	2:1
2	1:1	20 h	10:1
3	1:1	15 h	**25** not observed

[a] determined by NMR analysis of crude reaction mixture

Table 2.1: Optimization of the deprotection of the TBDPS group

Using **24** prepared using this optimized approach, the hydroxyl group on C-6 was protected (Scheme 2.8) as a benzoate ester by treatment with benzoyl chloride in pyridine, which gave thioglycoside **6** in 90% yield. As detailed in Scheme 2-5, getting this compound in pure form was not possible without minimizing the Lev migration. Compound **6** could then be converted quantitively to the primer acceptor **4** upon treatment with hydrazine and acetic acid.

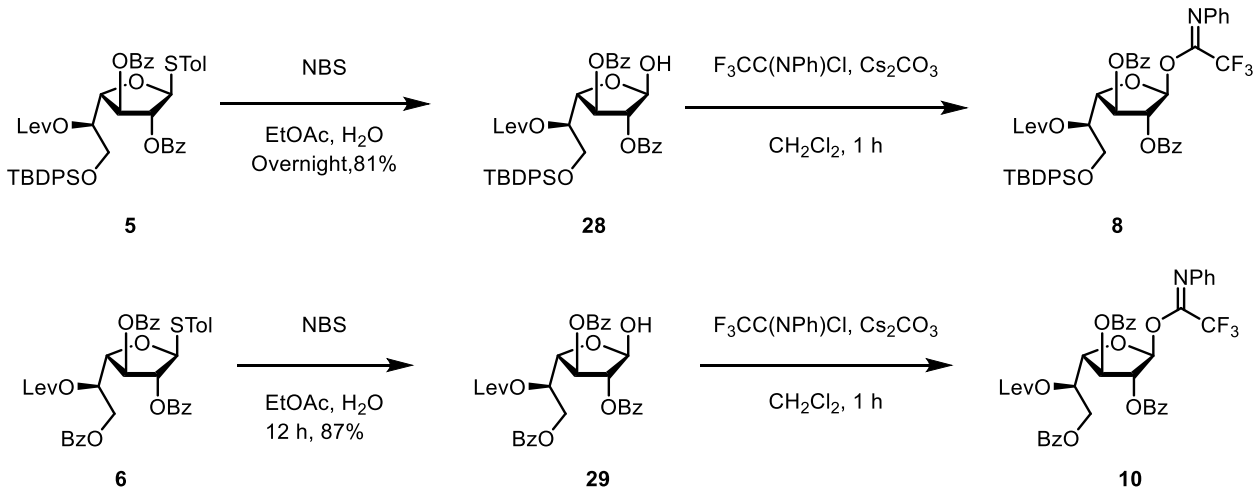


24 **6** **4**

Scheme 2.8: Synthesis of the "primer acceptor" building block **4**

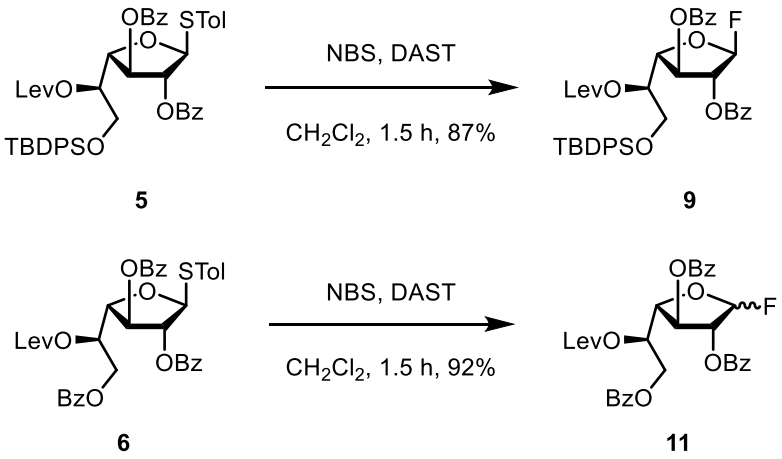
2.3.1.3 Synthesis of 8 and 10, donors needed for "centerpiece" and "tailpiece" Gal*f* residues

As I mentioned before, thioglycosides **5** and **6** must be converted into either the glycosyl *N*-phenyl trifluoroacetimidate or glycosyl fluoride glycosyl donor to install either the "centerpiece" or "tailpiece" Gal*f* residues into the target molecules. The synthesis of the glycosyl *N*-phenyl trifluoroacetimidate donor needs two steps. First is the hydrolysis of the thioglycoside and the second is the reaction of the resulting hemiacetal with 2,2,2-trifluoro-*N*-phenylacetimidyl chloride. Treatment of either **5** or **6** with *N*-bromosuccinimide (NBS) and water in ethyl acetate (Scheme 2.9) resulted in the formation of **28** and **29**, respectively. In both cases the yields were good 81% (**28**) and 87% (**29**). Because of the instability of imidate donors **8** and **10**, the synthesis from **28** and **29**, respectively, was usually done right before the glycosylation reaction.



Scheme 2.9: Synthesis of galactose *N*-phenyl trifluoroacetimidate donors **8** and **10**

The synthesis of the glycosyl fluoride donors is more straightforward. It requires a one-pot reaction of the thioglycoside with NBS and diethylaminosulfur trifluoride (DAST). Treatment of **5** or **6** under these conditions (Scheme 2.10) produced glycosyl fluorides **9** and **11**, respectively, in good yield. In both cases, the R_f value of the glycosyl fluoride and thioglycoside on TLC is almost the same so it is a bit difficult to monitor the reaction. However, it is a relatively clean and quick reaction; it always finishes before 1.5 hours and in good yield (>85%). The ^{1}H NMR spectra of the glycosyl fluorides show a large one-bond H-1–F coupling constant (e.g. 58.8 Hz for **9**). The ^{13}C NMR spectrum shows a large one-bond C-1–F coupling constant (e.g. 226.2 Hz for **9**) and two bond C-2–F coupling constants (e.g. 39.9 Hz for **9**). These coupling constants indicate the successful fluorination of **5** and **6**.



5 **9**

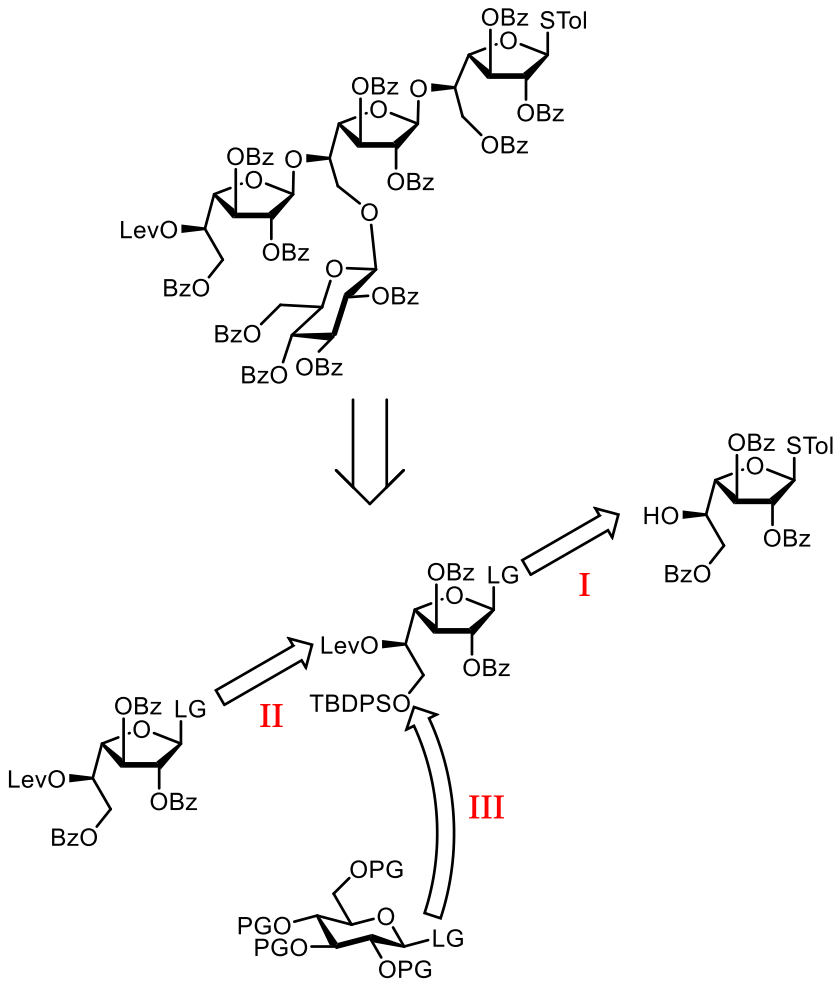
6 **11**

Scheme 2.10: Synthesis of galactosyl fluoride donors **9** and **11**

2.3.2 Synthesis of Glucose Building Block

Because the Glc*p* residue is located on the side chain of our target oligosaccharides, linked to a Gal*f* residue in a β-(1→6)-linkage, no orthogonal protecting groups on this building block are needed. The preparation of the required donor was thus envisioned to be straightforward. To ensure the formation of a β-linkage in the glycosylation step, O-2 needs to be protected by an acyl group which can participate during the glycosylation. Based on my strategy (Scheme 2.11), the glycosylation between a trisaccharide acceptor and the glucose donor will be the last glycosylation step in forming the essential tetrasaccharide building block. Thus, during that glycosylation I expected the steric hindrance around the reaction site to be large. Based on this reason I first proposed using the least bulky acyl protecting group, an acetyl ester, to protect the Glc*p* donor. However, I also wanted to prepare the corresponding benzoyl protected Glc*p* donor to compare their reactivity in the 3+1 glycosylation reaction. As was done for the centerpiece and tailpiece

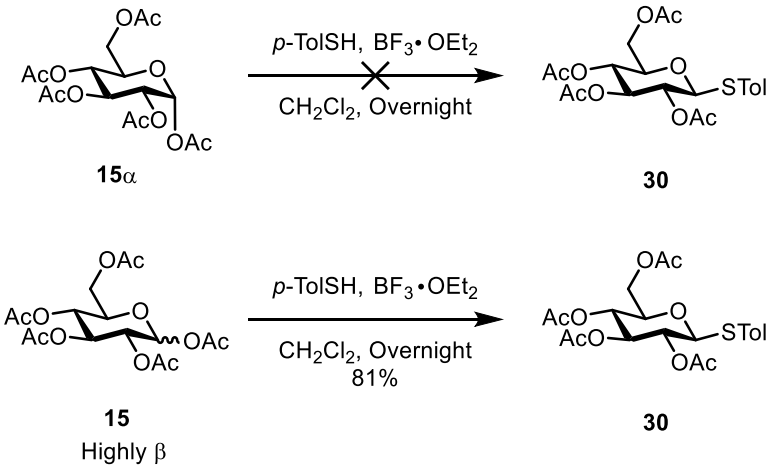
Gal*f* building blocks, I planned to make both the glycosyl *N*-phenyl trifluoroacetimidate and glycosyl fluoride donors.



Scheme 2.11: Three planned glycosylation to afford the tetrasaccharide

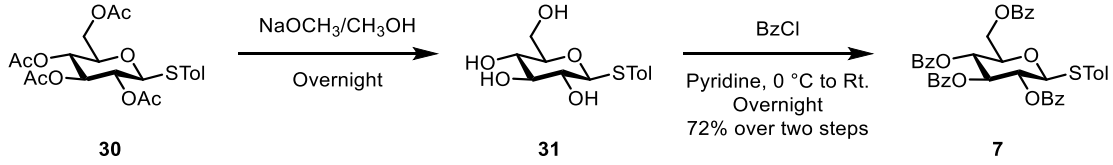
The synthesis of the Glc*p* donors started with the 1,2,3,4,6-penta-*O*-acetyl-glucopyranose (**15**, Scheme 2.12). For the first step of the synthesis, **15** was reacted with 4-methylbenzenethiol in the presence of $BF_3{\cdot}OEt_2$ to yield thioglycoside **30**. At this step I faced a small problem. The first time I ran this reaction, pure α-glucose pentaacetate (**15α**) was used; however, the reaction was

extremely slow and less than 10% of **30** was formed after an overnight reaction. When I switched to using the α,β mixture, which consisted primarily of the β isomer (**15**), the reaction was finished after one night and gave good yield (81%).



Scheme 2.12: Synthesis of thioglucoside **30**

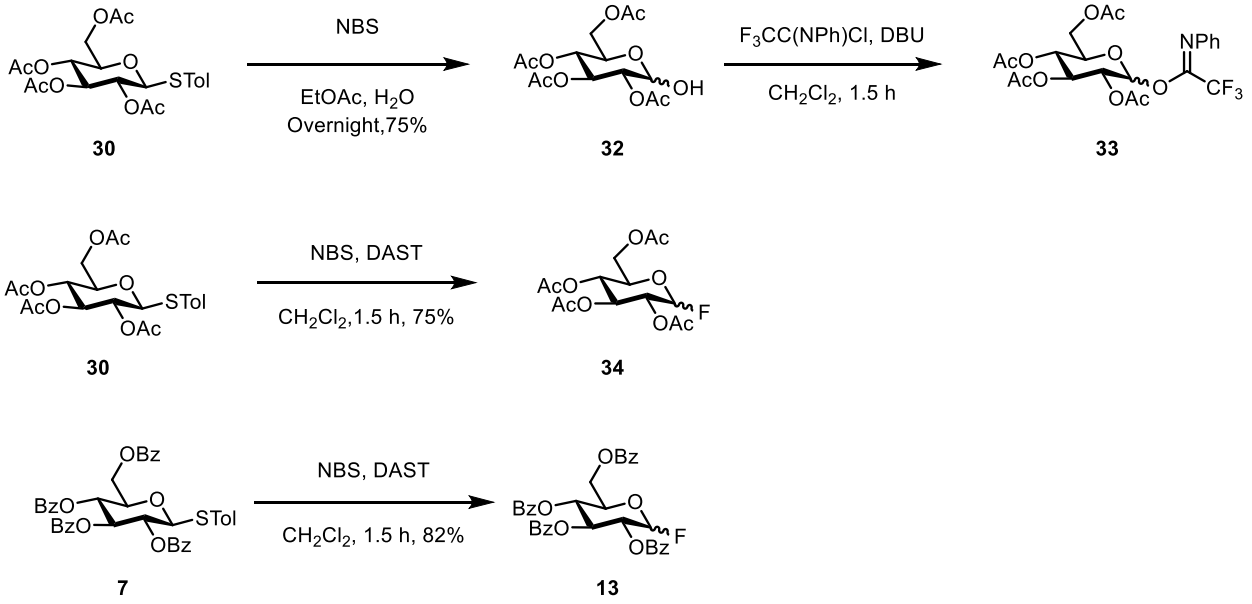
Because I also wanted the corresponding benzoylated thioglycoside, I deacetylated half of **30** using sodium methoxide and then benzoylated the product by treatment with benzoyl chloride in pyridine (Scheme 2.13). This reaction sequence gave the tetra-*O*-benzoylated thioglycoside **7** in 72% yield over the two steps.



Scheme 2.13: Synthesis of 2,3,4,6-tetra-*O*-benzoylated thioglucoside **7**

Similar to the Gal*f* donors, thioglucosides **7** and **30** needed to be converted into the glycosyl

N-phenyl trifluoroacetimidate or glycosyl fluoride donors and this was done as described above. Therefore, I treated the thioglycoside **30** with NBS and water to produce the reducing sugar **32** in 75% overall yield (Scheme 2.14). This could be converted to the glycosyl *N*-phenyl trifluoroacetimidate donor **30** immediately before use (see below). Alternatively, reaction of thioglycoside **30** with NBS/DAST gave glycosyl fluoride **34** in 75% yield.[57] Similar treatment of **7** gave and 82% yield of **13**.[58]



Scheme 2.14: Synthesis of glycosyl fluoride/N-phenyl trifluoroacetimidate Glc*p* donors **13**, **33** and **34**

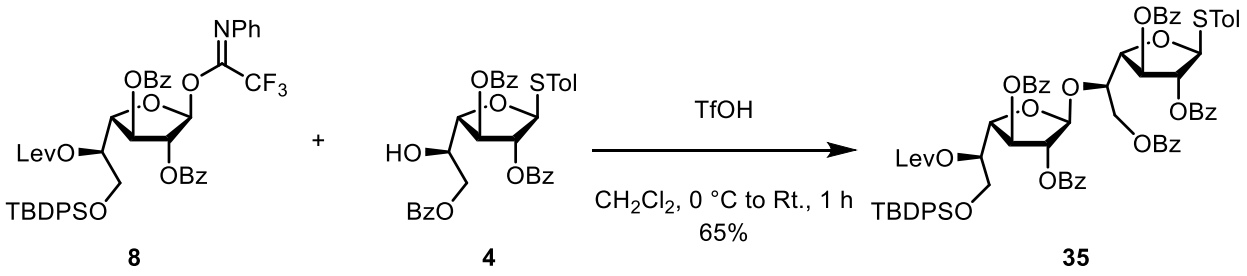
2.3.3 Assembly of the tetrasaccharide

With "primer acceptor" **4**, the "centerpiece" **8** and **9**, the "tailpiece" **10** and **11**, and the Glc*p* building block **13**, **33** and **34** available, the assembly of tetrasaccharide was attempted. As mentioned previously, the first glycoslylation involved the "primer acceptor" **4**, which has a free

hydroxyl group on C-5, and one of two possible donors: either the glycosyl *N*-phenyl trifluoroacetimidate donor **8** or glycosyl fluoride donor **9**, which can be activated either by TfOH or Cp_2ZrCl_2–AgOTf, respectively. I decided to try the glycosyl *N*-phenyl trifluoroacetimidate donor first and the fluoride donor next on small scale to compare if there is any reactivity difference and compare the overall yield.

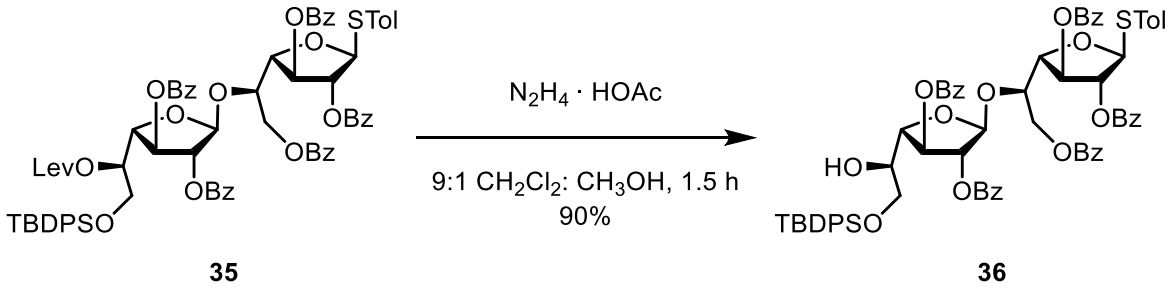
2.3.3.1 Attempts to assemble the tetrasaccharide using glycosyl *N*-phenyl trifluoroacetimidate donors

The first target was disaccharide **35**, which could be obtained from the coupling of donor **8** and acceptor **4** upon activation with TfOH, to give the disaccharide **35** in 65% yield (Scheme 2.15). The ^{1}H NMR spectrum of **35** showed the correct numbers of hydrogens and H-1 of the newly-introduced residue (H-1′) appeared as a singlet, which matches with previous reports for β-galactofuranosides.[49,59] A Heteronuclear Multiple Bond Correlation (HMBC) experiment showed a correlation between H-1′ and C-5, which indicates the desired (1→5)-linkage was formed. The ^{13}C NMR spectrum shows the anomeric carbons at 105.7 ppm (C-1′) and 91.1 ppm (C-1), which are consistent with Gal*f* O-glycosides and thioglycoside linkages, respectively.[49]



Scheme 2.15: Synthesis of disaccharide **35** by glycosylation of **4** with glycosyl *N*-phenyl triflouroacetimidate donor **8**

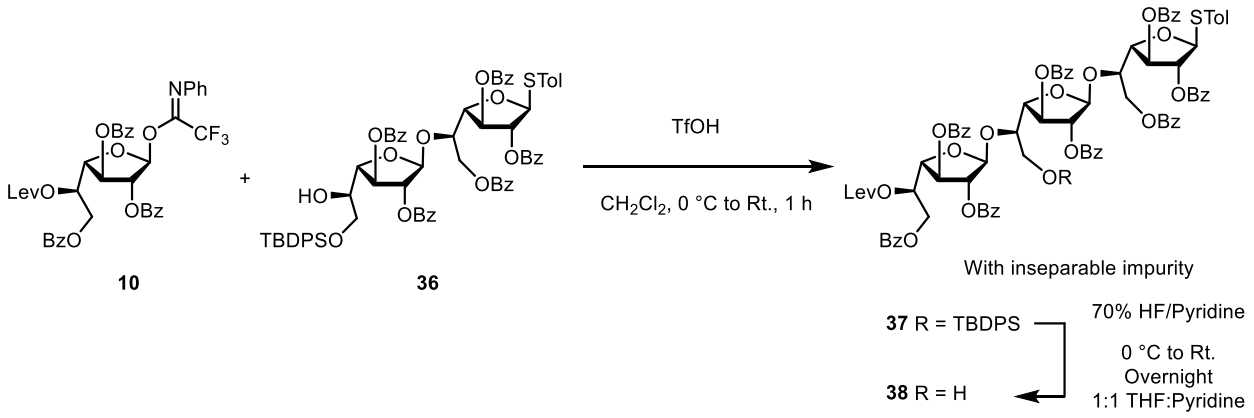
Treatment of **35** with hydrazine acetate in dichloromethane selectively deprotected the Lev ester in the presence of the benzoyl groups giving disaccharide **36** in 90% yield. The deprotection was confirmed from the ^{1}H NMR spectrum of the product – the 3 H singlet and two 2 H multiplets characteristic of the Lev ester disappeared from the spectrum. Moreover, the signal for H-5′ moved upfield (from 5.49 ppm in **35** to 4.06 in **36**) as would be expected.



Scheme 2.16: Deprotection of Lev group in **32** with hydrazine hydrate giving disaccharide **36**

With **36** in hand, the next step was coupling between **36** and **10** under the same conditions (Scheme 2.17) as described above. However, this time the reaction looked very messy by TLC. There seemed to be one major spot and I isolated it. Unfortunately, the ^{1}H NMR spectrum showed it was a mixture that was difficult to separate. Low resolution mass spectrometric analysis

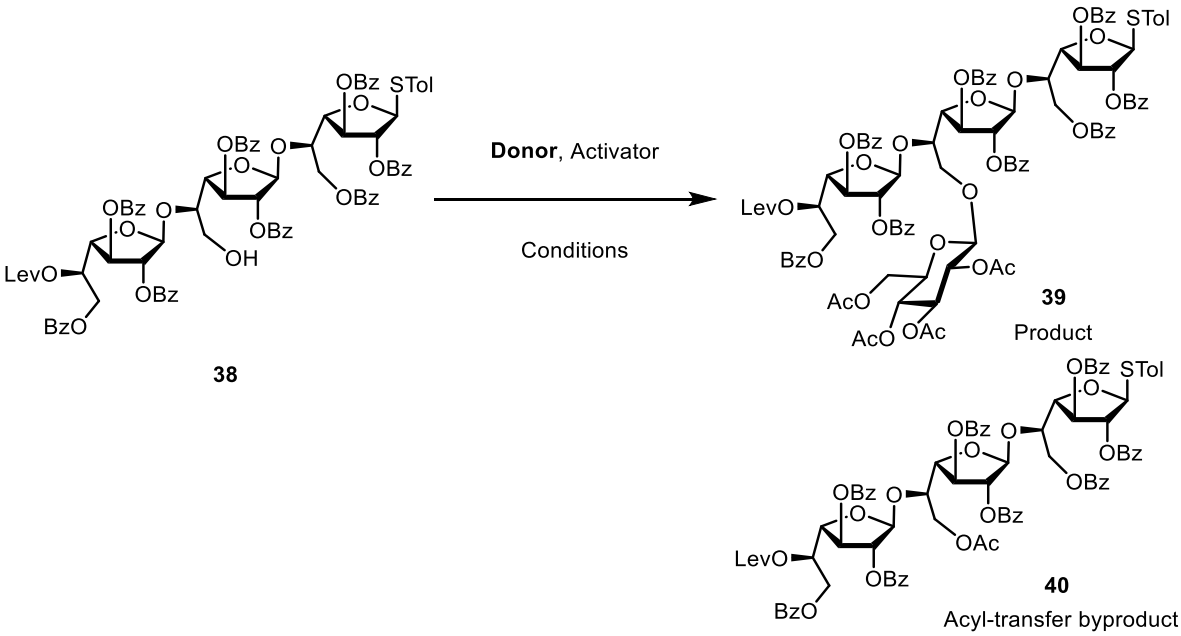
indicated that the desired product was in the mixture, but it was impossible to separate it from the other impurities. I then tried to manipulate the protecting groups on the molecule to make the purification possible. I first tried to remove the TBDPS group; however, I still got a mixture. At this point, I decided to move on to the next step with the hope that the tetrasaccharide product following the next coupling could be purified.

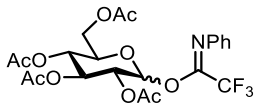
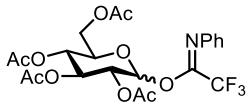
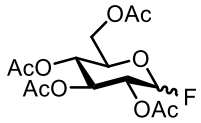


Scheme 2.17: Glycosylation of **36** with **10**, leading to trisaccharide **37** and subsequent deprotection of the TBDPS group

The next step towards the tetrasaccharide was a 3+1 glycosylation between trisaccharide acceptor **38** and a Glc*p* donor, either **33** or **34**. However, the trisaccharide acceptor **38** I had available contained impurities. I tried several different donors and conditions (Table 2.2) and all of them looked extremely messy by TLC. Analysis of the crude reaction mixture by low resolution mass spectrometric analysis indicated there might be a trace amount of the desired tetrasaccharide **39** formed; however, the spectrum also showed many other byproducts. The spectrum also

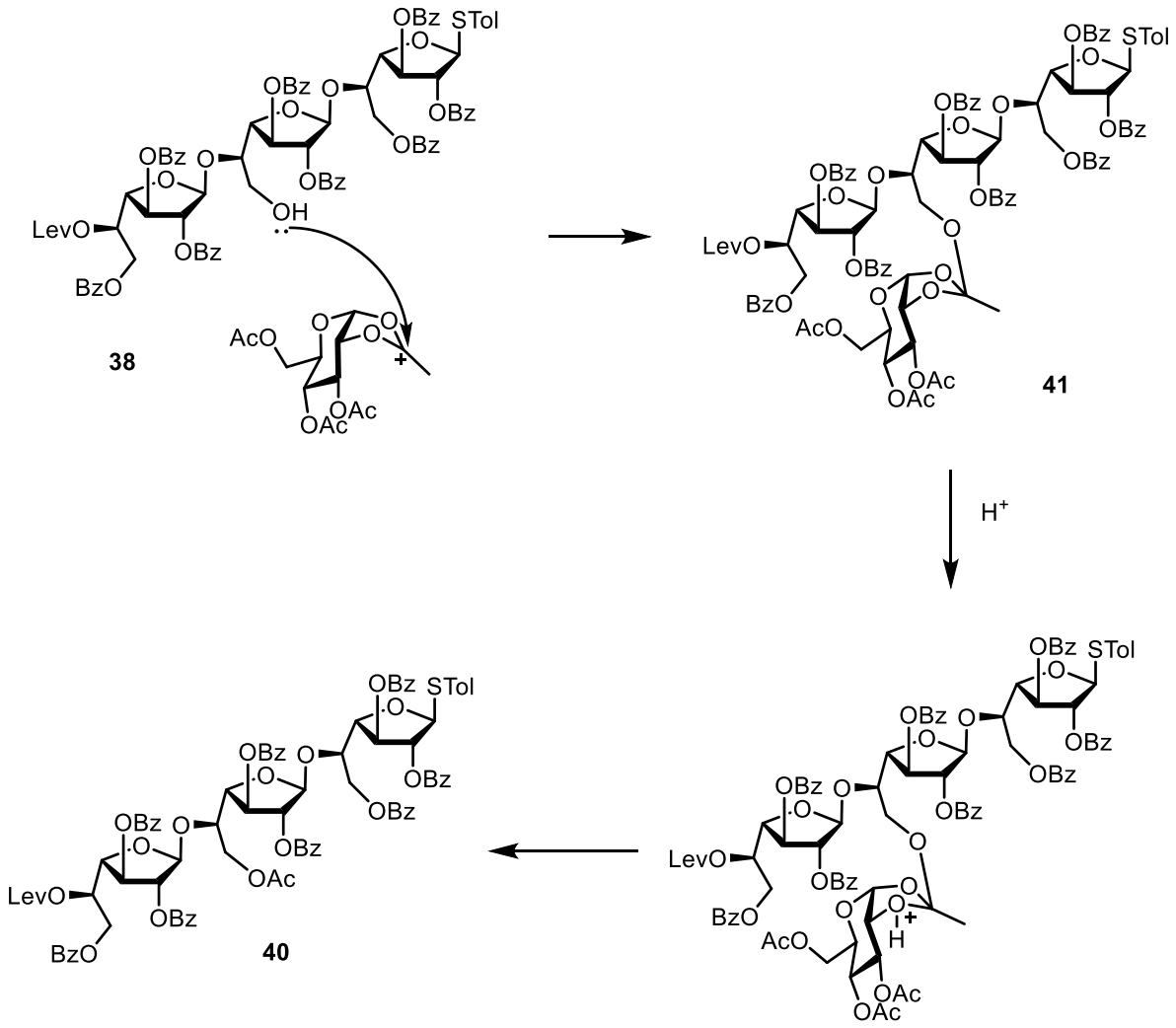
indicated that the main byproduct was the fully acylated trisaccharide **40**, resulting from acyl group transfer to the C-6’ hydroxyl group, during orthoester break down (Scheme 2.18).



Entry	Donor	Activator	Conditions	Result
1	 **30**	TfOH	CH_2Cl_2 0 °C to rt,1 h	Very messy by TLC
2	 **30**	TBSOTf	CH_2Cl_2 –78 °C to rt, 1 h	Acyl-transfer byproduct **40** may be the major product[a]
3	 **31**	Cp_2ZrCl_2/AgOTf	CH_2Cl_2, 0 °C to rt, 1 h	

[a] determined by mass spectrometric analysis

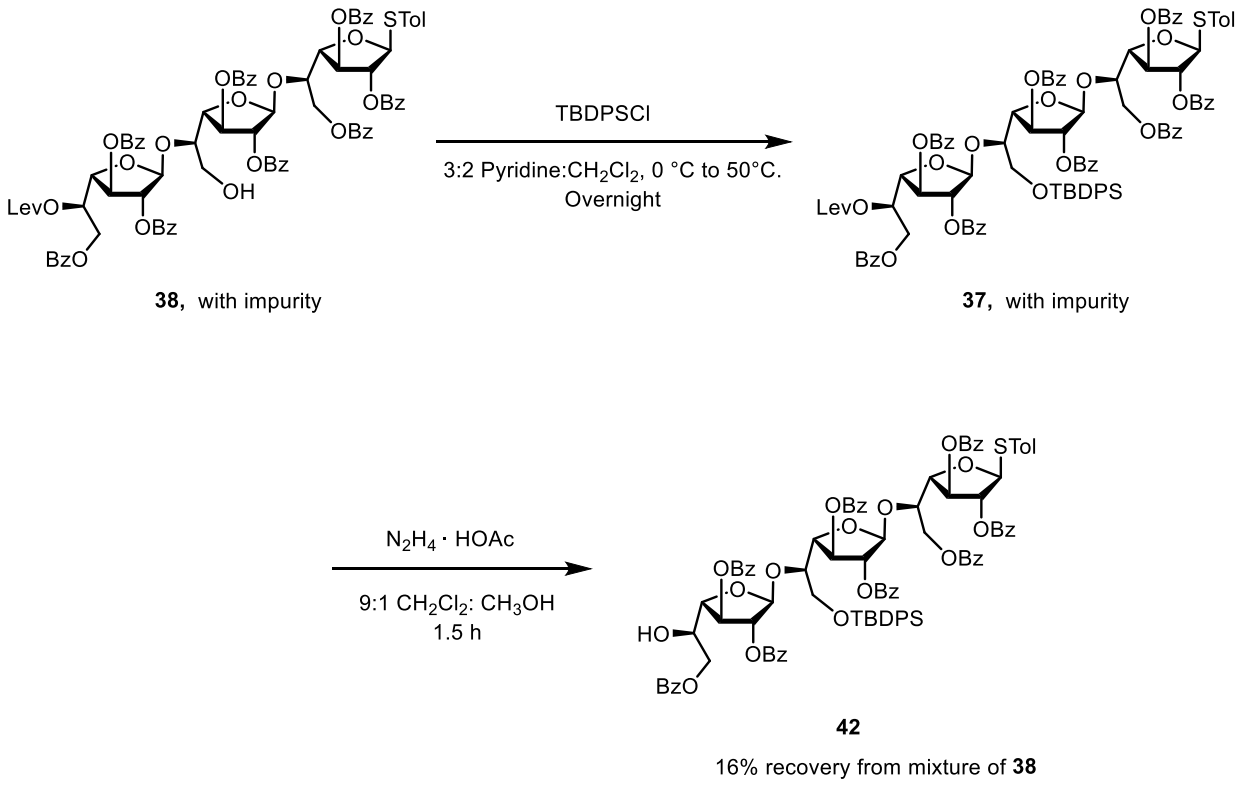
Table 2.2: Attempts to synthesize tetrasaccharide **39**



Scheme 2.18: Formation of orthoester **41** from **38** and the dioxolenium ion derived from **33** or **34** and decomposition of **41** leading to acyl-transfer by product **40**

Because I was unable to obtain tetrasaccharide **39** by glycosylation of impure **38** with either **33** or **34**, I chose to purify trisaccharide acceptor **38** again (Scheme 2.19). I attempted this by trying to protect the primary hydroxyl group by TBDPSCl, and removal of the O-5′′ Lev group. Doing this, I reasoned would reduce the polarity of the molecule, and make the mixture easier to separate. This approach was successful and pure trisaccharide **42** could be obtained, but the overall yield

suffered from the back-and-forth manipulation of the protecting groups. Therefore, I decided to stop using this method in the further assembly of the tetrasaccharide.



38, with impurity

37, with impurity

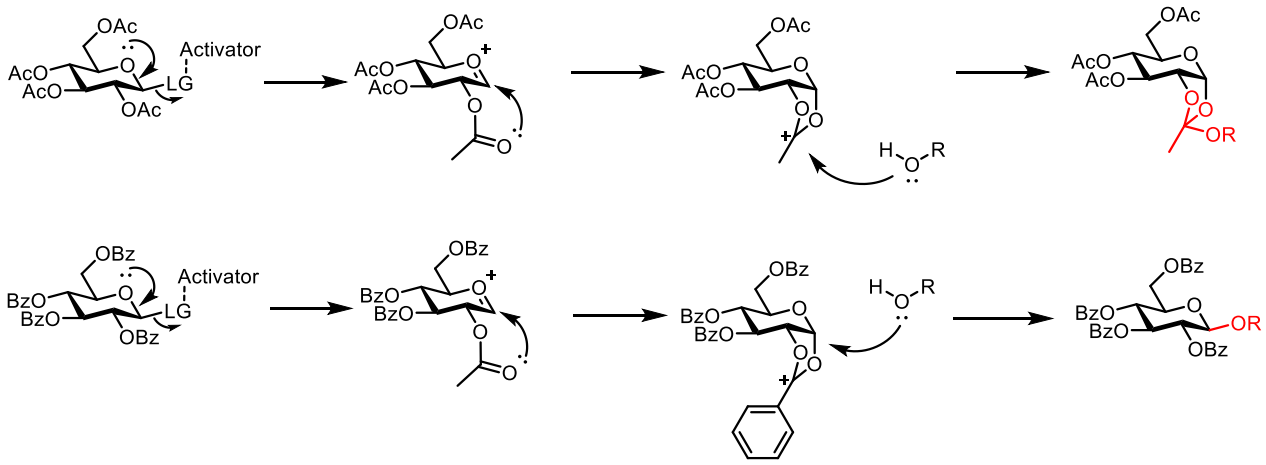
42

16% recovery from mixture of **38**

Scheme 2.19: Purification of trisaccharide **38** via protecting group manipulation

Being unable to synthesize tetrasaccharide **39** by using the approach discussed above, I tried to figure out a solution to the problems I faced. When using the glycosyl *N*-phenyl trifluoroacetimidate donor, I observed a large amount of an inseparable byproduct in the 2+1 glycosylation. In addition, the first glycosylation, the reaction of **4** and **8**, gave the product **35** in modest yields. To solve this problem, I proposed to switch to using glycosyl fluoride donors, which are easier to prepare and more stable compared to glycosyl *N*-phenyl trifluoroacetimidate donors.

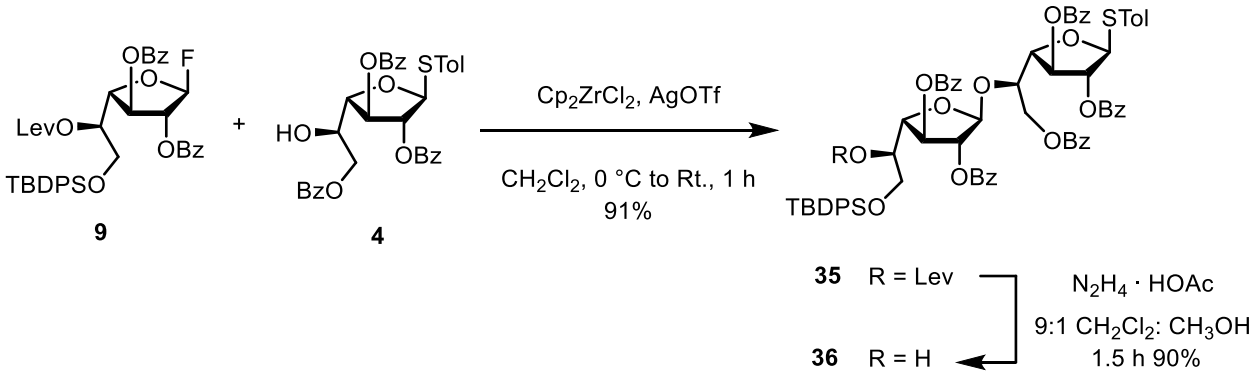
Furthermore, to solve the acyl transfer problem in the 3+1 glycosylation, I decided to switch to a tetra-*O*-benzoylated Glc*p* donor. Given the larger size of the phenyl group compared to a methyl group, the dioxolenium ion formed from a benzoylated donor would be less prone to orthoester formation and hence acyl transfer (Scheme 2.20).



Scheme 2.20: Proposed inhibition of an orthoester by using a benzoylated donor, which has larger steric hindrance

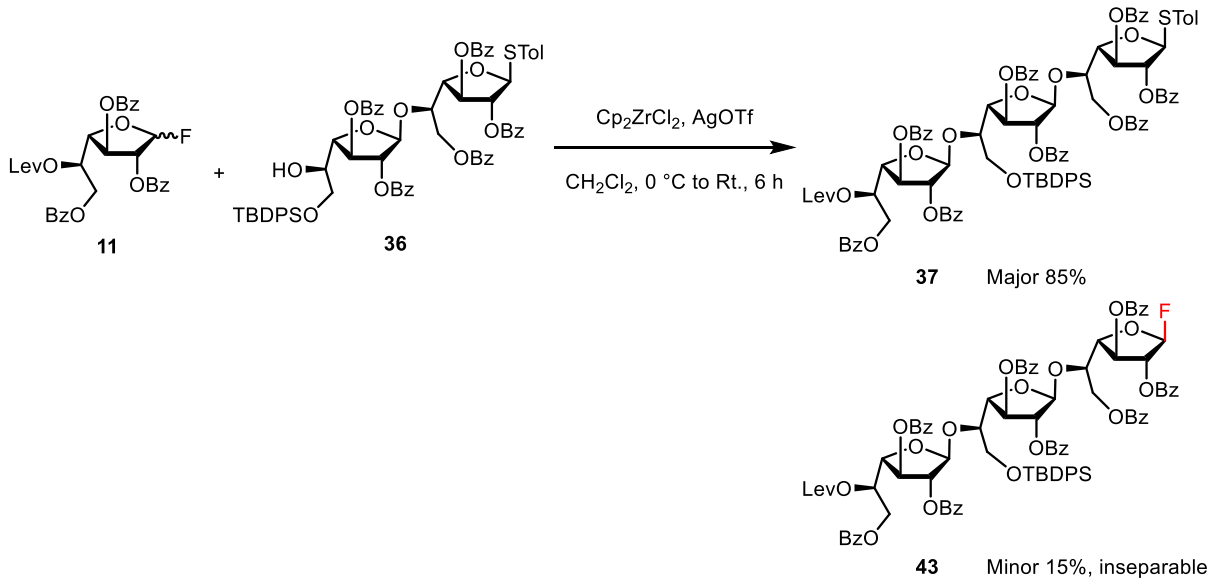
2.3.3.2 Attempts to assemble the tetrasaccharide using glycosyl fluoride donors

My second attempt to assemble the tetrasaccharide started with the coupling between acceptor **4** and glucosyl fluoride donor **9** in the presence of bis(cyclopentadienyl)zirconium(IV) dichloride (Cp_2ZrCl_2) and silver triflate (AgOTf) as the activators (Scheme 2.21). The reaction gave disaccharide **35** in a higher yield (91%) than the coupling between the glycosyl *N*-phenyl trifluoroacetimidate donor and same acceptor. Cleavage of the Lev ester by treatment of **35** with hydrazine acetate provided acceptor **36** in 90% yield.



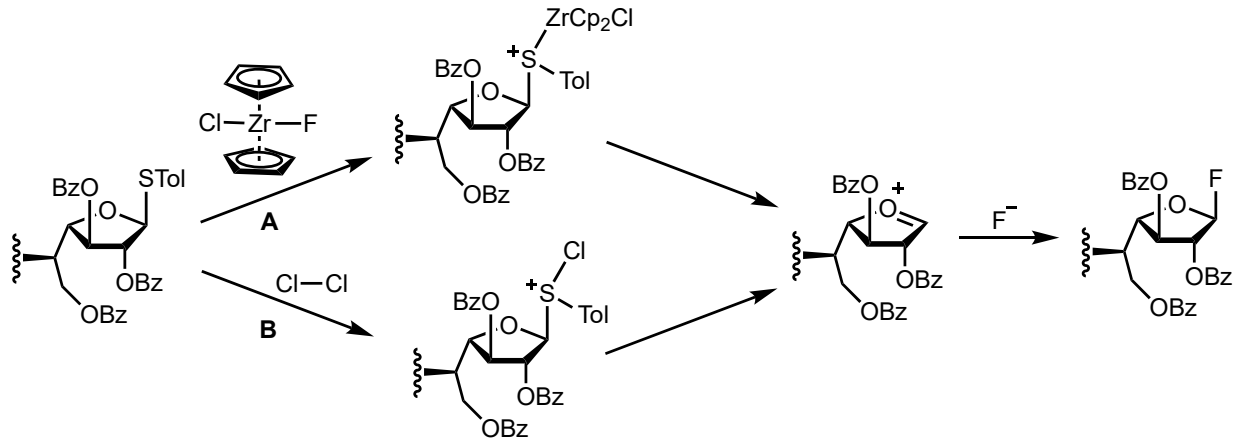
Scheme 2.21: Synthesis of disaccharide **35** by glycosylation of **4** with glycosyl fluoride **9** and subsequent cleavage of the Lev ester group

The next step is the 2+1 coupling between acceptor **36** and donor **11** (Scheme 2.22), which was also promoted by Cp_2ZrCl_2 (0.25 equiv) and AgOTf (0.5 equiv). However, this time the reaction was much slower than the 1+1 glycosylation. Increasing the number of equivalents of donor and the activators (to 0.5 equiv Cp_2ZrCl_2 and 1.0 equiv AgOTf) greatly accelerated the process; however, a byproduct that was inseparable from my desired product was formed. In the mass spectrum of the partially-purified material the *m/z* of byproduct (1693.5664) was consistent with either one benzoyl group being cleaved with the trisaccharide or the STol group on the reducing end being replaced by fluorine. By measuring the ^{19}F NMR spectrum of the mixture, I could confirm that product was the one resulting from the replacement of thioglyoside moiety in **36** with fluorine, producing trisaccharide glycosyl fluoride **43**. In the ^{1}H NMR spectrum of the mixture, the H-1 signal for **43** appeared as a doublet with a large coupling constant ($^{2}J_{H\text{-}F}$ = 58.4 Hz), indicative of a fluorine atom on H-1.



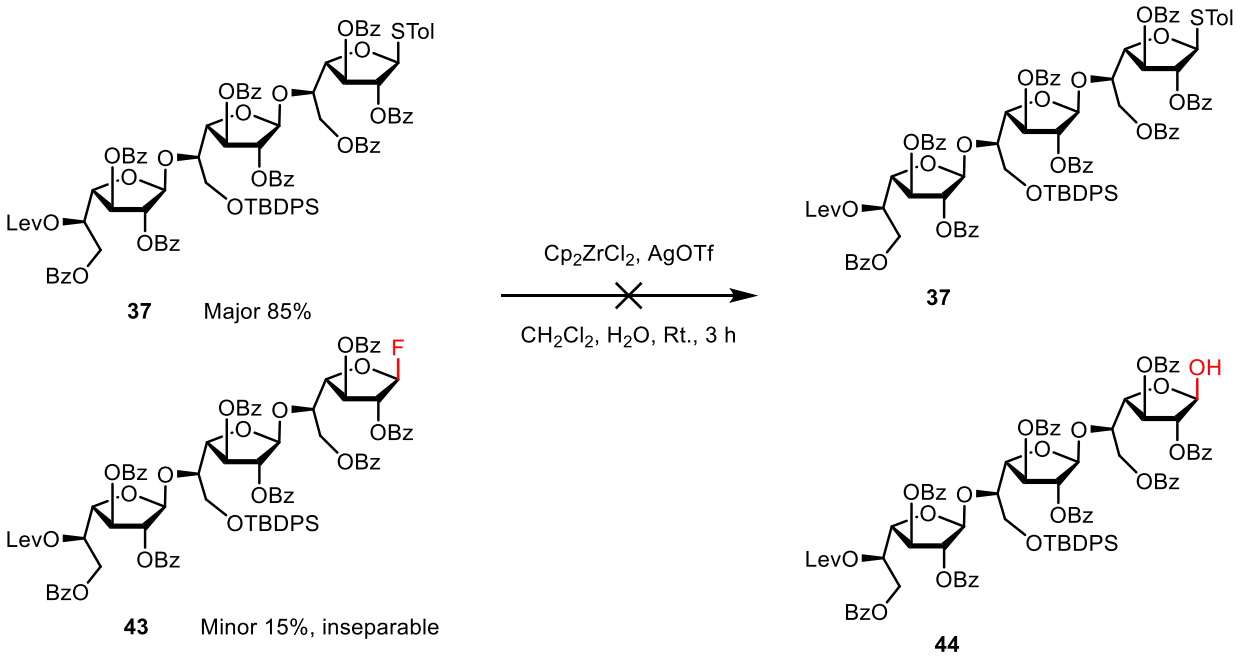
Scheme 2.22: Glycosylation of **37** with **11** and formation of inseparable byproduct **43**

The formation of glycosyl fluorides from thioglycosides under these conditions appears not to have been reported before. We postulate that at longer reaction times the byproduct of the promotor (Cp_2ZrClF) can activate the thioglycoside and in turn liberate fluoride ion, which then reacts with the electrophilic carbohydrate intermediate formed to generate the glycosyl fluoride. Alternatively, it could be in the presence of a large excess of the promotors and the byproducts formed from the glycosylation, that redox processes leading to chlorine (Cl_2) occur. Similar to bromine (Br_2), chlorine could activate the thioglycoside leading to glycosyl fluoride formation. (Scheme 2.23)



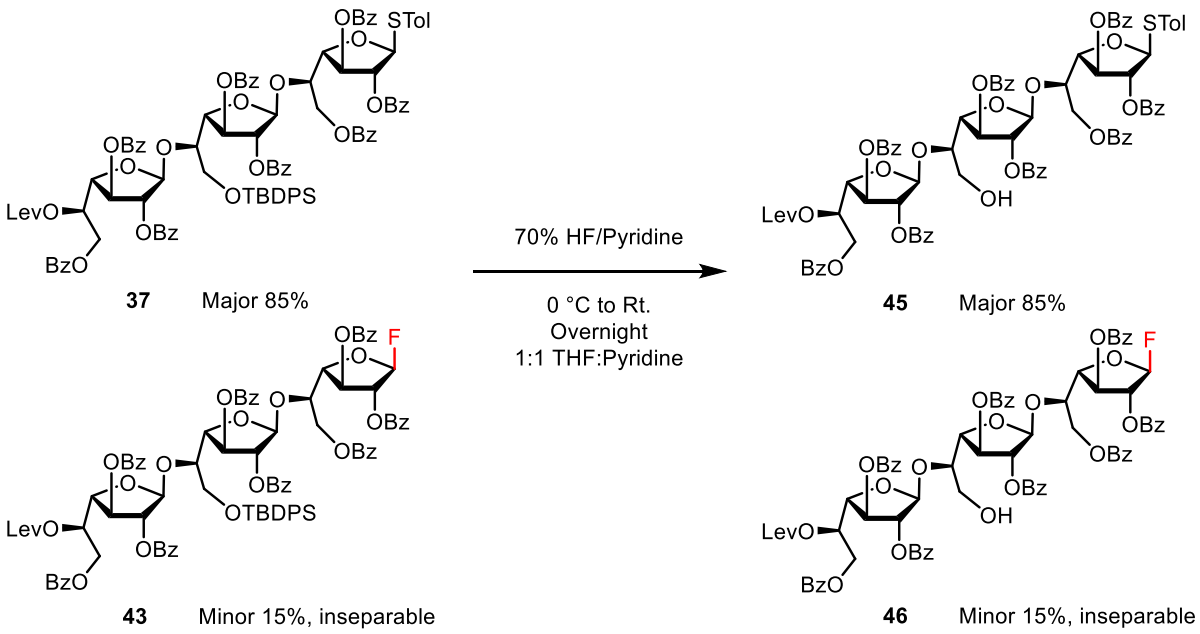
Scheme 2.23: Possible formation pathway of trisaccharide glycosyl fluoride **43**.A) Activated by Cp_2ZrClF, B) Activated by chlorine

At this point, purification of the trisaccharide **37** was attempted (Scheme 2.24). The first method I tried was to hydrolyze the fluorine on the reducing end of **43** by Cp_2ZrCl_2 and AgOTf. This would form hemiacetal **44**, which I anticipated would be more polar than **37** and which thus could be separated from the main desired product. Unfortunately, the reaction was extremely slow and over time, multiple spots appeared by TLC.



Scheme 2.24: Attempt to remove glycosyl fluoride byproduct (**43**) from trisaccharide **37** by hydrolysis

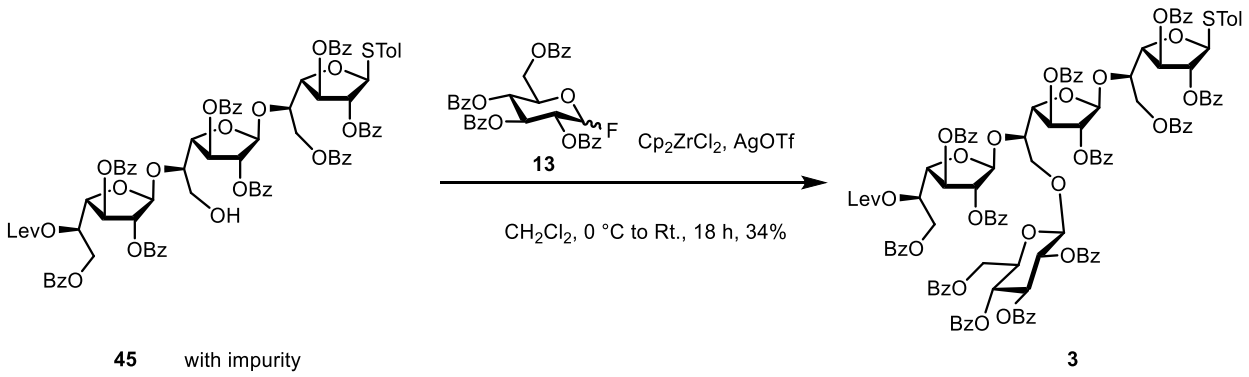
Being unable to purify the trisaccharide **37**, I had no choice but to carry it forward with the ~15% of byproduct **43**. Deprotection of the TBDPS group using HF·pyridine, gave the trisaccharide acceptor **45**, which was unfortunately still contaminated with the glycosyl fluoride by-product **46** (Scheme 2.25).



Scheme 2.25: Deprotection of TBDPS group in the mixture of **45** and **46**

The 3+1 coupling was performed between the trisaccharide acceptor **45** and Glc*p* fluoride donor **13** (Scheme 2.26). My major concern for this reaction was that the acceptor was contaminated with glycosyl fluoride **46**, which could also be activated and glycosylate with alcohol **45** or doing self-coupling. However, this was my only choice. The reaction between the mixture of **45**/**46** and **13** was activated by Cp_2ZrCl_2 and AgOTf. By TLC all of the acceptor and donor were fully consumed and, after the reaction, two major spots that have R_f's lower than the acceptor and donor were formed. Using 1:1 hexane–ethyl acetate as the eluent, one spot is at R_f 0.15 and the other is around R_f 0.05. The less polar of these compounds was my desired tetrasaccharide product **3**, which was obtained in 34% yield following purification. The lower spot was a mixture of several byproducts. In the ^{1}H NMR spectrum of **3**, the Glc*p* anomeric hydrogen appears as a doublet at

4.90 ppm ($J_{1,2}$ = 7.8 Hz) consistent with the β-stereochemistry. Two singlet peaks at 5.66 and 5.55 ppm indicate the two anomeric hydrogens on the Gal*f* O-glycoside residues; the anomeric hydrogen on reducing end Gal*f* residue, which is attached to the STol group, appears at 5.72 ppm ($J_{1,2}$ = 2.1 Hz). The HMBC spectrum of **3** also shows a correlation between the C-1 of the Glc*p* residue (C-1‴) and H-6ab′ as well as between H-1″ and C-5′, indicating the correct linkages (Scheme 2.27)**.**



Scheme 2.26: Synthesis of tetrasaccharide building block **3** from the glycosylation of **45** with **13**

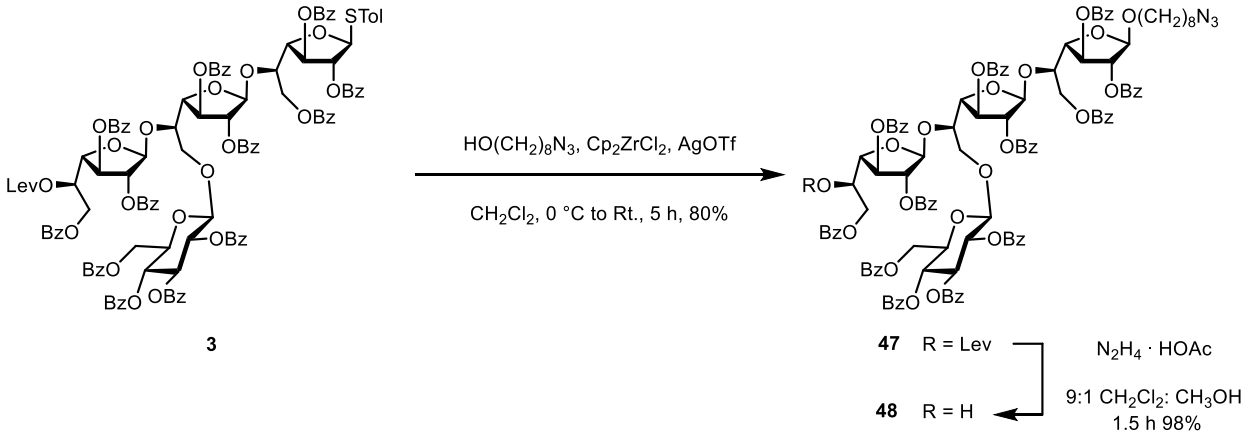
Scheme 2.27: HBMC spectrum of tetrasaccharide 3

2.3.4 Chain elongation and deprotections

Having established the route to tetrasaccharide thioglycoside **3**, the next step was to couple it to 8-azido-octanol and then either deprotect it to give tetrasaccharide **1** or elongate it and then deprotect it to give octasaccharide **2**. These glycosylation reactions are relativity well established and I anticipated they would be more straightforward than the previous steps.

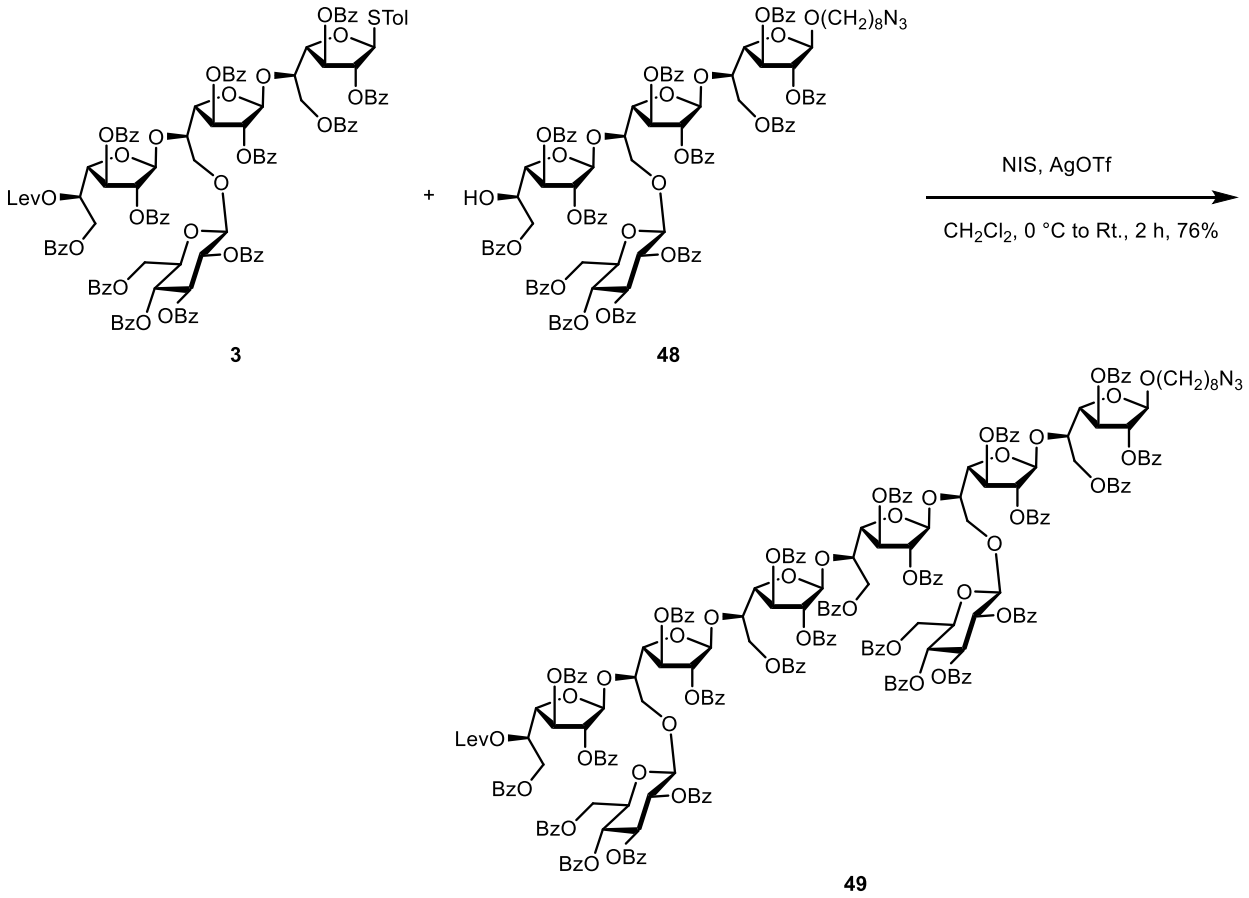
The first step was the glycosylation between **3** and 8-azido-octanol activated by N-iodosuccinimide (NIS) and AgOTf (Scheme 2.28). After five hours, the reaction was complete;

however, the product was very close to the excess azido-octanol, which could be a potential problem for purification. It turned out that if I used a lower polarity eluent (4:1 hexane–ethyl acetate) the R_f value of product decreases dramatically but the R_f for the azido octanol barely changes. Thus, by using a gradient elution (4:1 to 1:1 hexane–ethyl acetate) the product **43** could be isolated pure in 80% yield. By inspecting the ^{1}H NMR spectrum, I can clearly see the alkyl peaks for the azido-octanol from 3.69 to 1.16 ppm with the disappearance of 3 H singlet representing the methyl group on STol. A new anomeric hydrogen signal (5.21 ppm, singlet) appeared as did a new anomeric carbon resonance (105.5 ppm) in the ^{13}C NMR spectrum. After that by treating **47** with hydrazine acetate in dichloromethane and methanol the Lev ester on O-5″ was removed affording acceptor **48** in 98% yield. On the ^{1}H NMR spectrum, we can see the disappearance for the 3 H singlet, indicating the removal of the Lev group.



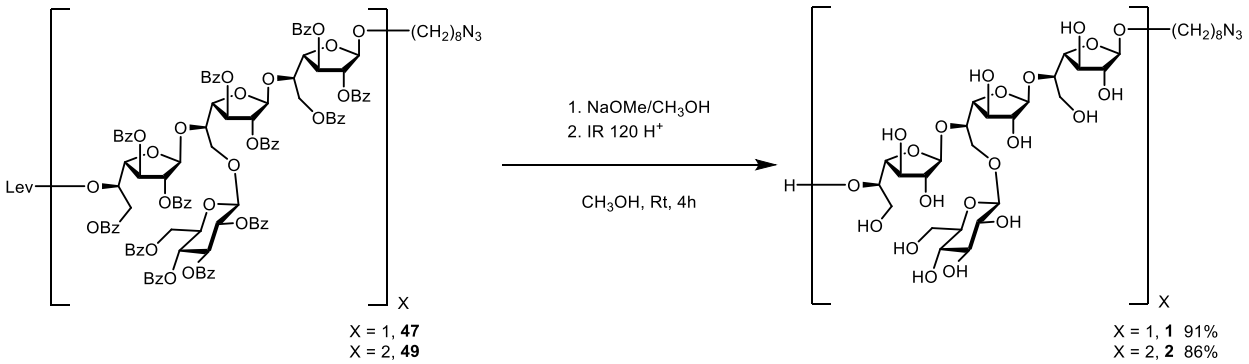
Scheme 2.28: Glycosylation between tetrasaccharide building block **3** and 8-azido-octanol and subsequent cleavage of the Lev ester in the product.

With the route to **48** in place the final coupling was a 4+4 glycosylation to give an octasaccharide. Thus, the glycosylation of acceptor **48** with thioglycoside **3** was achieved upon treatment with NIS and AgOTf to provide octasaccharide **49** in 76% yield. The ^{1}H NMR is a bit complicated to assign, but at 4.92 and 4.82 ppm I can clearly observe the two doublet peaks for two anomeric hydrogen representing the two β-Glc*p* residues in the molecule, as well as alkyl peaks for the azido-octanol and 3 H singlet which represents the Lev group on the non-reducing end sugar residue. The ^{13}C NMR also shows there are six Gal*f* anomeric carbons between 105.7 and 105.5 and two Glc*p* anomeric carbons at 101.1 and 100.7. This indicated to me that I had a successful coupling between two tetrasaccharides. The mass of the product (*m/z* $[M+2Na]^{+2}$ = 2054.1006) was also consistent with an octasaccharide.



Scheme 2.29: 4+4 glycosylation leading to octasaccharide **49**

Having the protected tetrasaccharide (**47**) and octasaccharide (**49**) assembled, treatment of them with sodium methoxide in methanol for six hours removed all of the acyl protecting groups on the compounds. After the reaction, the reaction mixtures were neutralized by the addition of IR-120 (H+) resin. Purification of the final products by C-18 column chromatography gave **1** in 91% yield from **47** and **2** in 96% yield from **49**.



Scheme 2.30: Debenzoylation of **47** and **49** leading to **1** and **2**, respectively

2.4 Conclusion

In summary, two approaches for the synthesis of fragments of the arabinogalactan from *Nocardia nova* were explored with one giving the two desired products. The first target, the tetrasaccharide **1**, contains one repeating unit including three Gal*f* residues and one Glc*p* residue. The second target, the octasaccharide **2**, is a dimer of two repeating units.

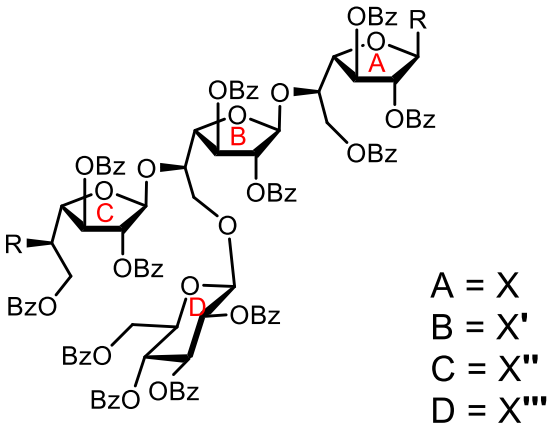
The successful approach begins with the synthesis of Gal*f* (**4**, **9** and **11**) and Glc*p* (**13**) building blocks from *p*-tolyl 2,3,5,6-tetra-*O*-benzoyl-1-thio-D-galactofuranoside (**14**)[44,60,61] and *p*-tolyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranoside (**30**).[62] With monosaccharide building blocks **4**, **9**, **11** and **13** in hand, Cp_2ZrCl_2–AgOTf-promoted glycosylation and orthogonal deprotections of TBPDS ethers or levulinate esters were used to assemble a key tetrasaccharide thioglycoside building block **3**. After the coupling of **3** with 8-azido-octanol, tetrasaccharide **47** was obtained, which could be deprotected to give target tetrasaccharide **1**. Alternatively, **47** could

be converted into an octasaccharide (**49**) and then deprotected to give **2**. Although both targets could be obtained, a couple of the glycosylations require future optimization to reduce the formation of byproducts that complicated the purifications.

2.5 Experimental section

General Methods: Reactions were carried out in oven-dried glassware. All reagents used were purchased from commercial sources and were used without further purification unless noted. Solvents used in reactions were purified by successive passage through columns of alumina and copper under nitrogen. Unless stated otherwise, all reactions were carried out at room temperature (rt). under a positive pressure of argon and were monitored by TLC on silica gel 60 F_{254} (0.25 mm, E. Merck). Spots were detected under UV light or by charring with a solution of ammonium molybdate (12 g), ceric ammonium nitrate (0.42 g) and concentrated sulfuric acid (15 mL) in H_2O (235 mL) or by charring with acidified anisaldehyde solution in ethanol. Unless otherwise indicated, all column chromatography was performed on silica gel 60 (40–60 μM). The ratio between silica gel and crude product ranged from 100 to 50:1 (w/w). Optical rotations were measured at 22 ± 2 °C at the sodium D line (589 nm) and are in units of $deg \cdot mL(dm \cdot g)^{-1}$. ^1H NMR spectra were recorded at 500 or 700 MHz, and chemical shifts are referenced to either residual $CHCl_3$ (7.26 ppm, $CDCl_3$), CHD_2OD (3.30 ppm, CD_3OD). ^{13}C NMR spectra were recorded at 176 MHz, and ^{13}C chemical shifts were referenced to internal $CDCl_3$ (77.06 ppm, $CDCl_3$), CD_3OD

(49.0 ppm, CD_3OD). In the processing of reaction mixtures, solutions of organic solvents were washed with equal amounts of aqueous solutions. Organic solutions were concentrated under vacuum at < 40˚C (bath). Electrospray mass spectra (time-of-light analyzer) were recorded on samples suspended in mixtures of THF with CH_3OH and added NaCl. MALDI mass spectrum were obtained in the linear positive mode of ionization on a MALDI TOF/TOF mass spectrometer using sinapinic acid as the matrix.



Scheme 2.31: Order when assigning hydrogens and carbons on carbohydrate rings

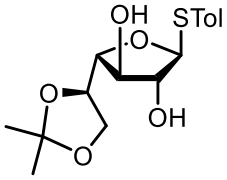
General procedure A. Synthesis of glycosyl fluoride donors from thioglycosides

To a solution of the thioglycoside (1 equiv) in CH_2Cl_2 at 0 °C was added *N*,*N*-diethylaminosulfur trifluoride (2 equiv, 1 M in CH_2Cl_2) followed by *N*-bromosuccinimide (2 equiv). The reaction mixture was stirred at 0 °C for 2 h and then methanol was added. The reaction mixture was diluted with CH_2Cl_2, and washed with satd aq $NaHCO_3$ soln before the organic layer was separated. The

aqueous layer was extracted again with CH_2Cl_2 and the combined organic layers were dried over Na_2SO_4. The solution was filtered, concentrated and the resulting residue was purified by chromatography to afford the glycosyl fluoride donor for use in the glycosylation reactions.

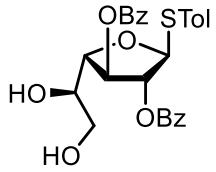
General procedure B. Synthesis of glycosyl *N*-phenyl trifluoroacetimidate donors from thioglycosides

To a solution of the thioglycoside (1 equiv) in 5:1 EtOAc–H_2O was added *N*-bromosuccinimide (5 equiv.) at rt. The mixture was vigorously stirred overnight and then a satd aq $Na_2S_2O_3$ soln of was added. The organic layer was then washed with water, brine, and dried with $NaSO_4$, before being filtered. The solution was then concentrated, and the resulting residue was purified by chromatography to afford the hemiacetal. Then, to a solution of the hemiacetal (1 equiv) in dry CH_2Cl_2 at 0 °C was added 2,2,2-trifluoro-*N*-phenylacetimidoyl chloride (3 equiv) and Cs_2CO_3 (3 equiv). The mixture was stirred at rt for 1 h, concentrated, and the resulting residue was purified by chromatography to afford the glycosyl *N*-phenyl trifluoroacetimidate donor that was used immediately in the glycosylation reactions.



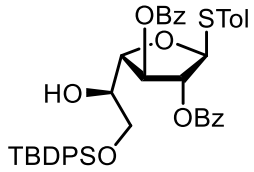
***p*-Tolyl 5,6-*O*-isopropylidene-1-thio-β-D-galactofuranoside (20)**

To a solution of **14** (30.00 g, 42.68 mmol) in 3:1 CH_3OH–CH_2Cl_2 was added sodium methoxide in methanol (0.1 M) dropwise until the pH of the reaction mixture was 12. The solution was stirred at rt overnight and neutralized by the addition of glacial acetic acid. Concentration gave a yellow syrup that was purified by recrystallization (hexane–EtOAc, 1:1) to afford **19** as a yellow solid. This material was then dissolved in acetone (50 mL) and 2,2-dimethoxypropane (7.0 mL, 56.8 mmol) and to the solution was then added *p*-toluenesulfonic acid (10 mg) at rt. The solution was stirred for 1.5 h and then neutralized by the addition of Et_3N. The reaction mixture was then concentrated and then diluted with CH_2Cl_2 (50 ml), washed with satd aq $NaHCO_3$ soln and the organic layer was separated. The organic layer was dried over Na_2SO_4, filtered, concentrated, and the resulting residue was purified by chromatography (hexane–EtOAc, 1:1) to afford **20** (11.3 g, 81% yield over two steps) as a pale-yellow syrup. The data for this material matched that previously reported.[63,64]



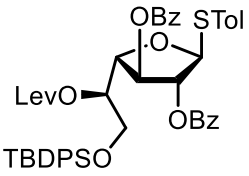
***p*-Tolyl 2,3-di-*O*-benzoyl-1-thio-β-D-galactofuranoside (22)**

Compound **20** (9.75 g, 31.35 mmol) was dissolved in pyridine (20 mL) and cooled to 0 °C followed by the addition of benzoyl chloride (5.8 mL, 50.2 mmol) dropwise. The reaction mixture was warmed to rt and stirred overnight. Excess benzoyl chloride was quenched by the addition of chilled water (50 mL) and the mixture was extracted with CH_2Cl_2 (60 mL). The CH_2Cl_2 layer was washed with 10% aq copper sulfate soln (30 mL × 4) and water (40 mL). The separated CH_2Cl_2 layer was dried over Na_2SO_4 and concentrated. The syrupy residue obtained was dissolved in 80% acetic acid (50 mL) and the solution was heated at 65–70 °C overnight. After cooling to rt, the reaction mixture was concentrated and the residue was purified by column chromatography (hexanes–EtOAc, 3:2) to yield **22** (10.7 g, 72% yield over two steps) as a thick syrup. The data for this material matched that previously reported.[45]



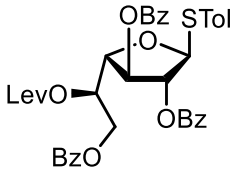
***p*-Tolyl 2,3-di-*O*-benzoyl-6-*O*-t-butyldiphenylsilyl-1-thio-β-D-galactofuranoside (23)**

To a solution of **22** (8.71 g, 17.6 mmol) in pyridine (30 mL) and CH_2Cl_2 (20 mL) at 0 °C was added t-butyldiphenylsilyl chloride (7.6 mL, 30.0 mmol) dropwise. The solution was then stirred overnight with warming to room temperature before CH_3OH (8 mL) was added. After stirring for 30 min, the solution was poured into a satd aq $NaHCO_3$ soln (50 mL) and then extracted with CH_2Cl_2 (50 mL). The organic layer was washed with brine (15 mL), dried over Na_2SO_4, filtered and concentrated to a residue that was purified by chromatography (hexanes–EtOAc, 85:15) to yield **23** (11.6 g, 90% yield) as a thick syrup. The data for this material matched that previously reported.[45]



***p*-Tolyl 2,3-di-*O*-benzoyl-5-*O*-levulinoyl-6-*O*-t-butyldiphenylsilyl-1-thio-β-D-galactofuranoside (5)**

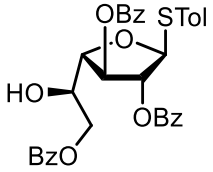
A mixture of **23** (10.5 g, 14.5 mmol), levulinic acid (2.5 g, 22 mmol), *N*-(3-dimethylaminopropyl)-*N′*-ethylcarbodiimide hydrochloride (4.2 g, 22 mmol), and 4-(dimethylamino)pyridine (0.36 g, 3 mmol) in CH_2Cl_2 (50 mL) was stirred for 1 h. The reaction mixture washed with a satd aq $NaHCO_3$ soln (70 mL) and brine (15 mL). The organic layer was then dried over Na_2SO_4, filtered and concentrated to give a residue that was purified by column chromatography (hexanes–EtOAc, 4:1) to afford **5** (1.42 g, 89% yield) as a white foam. The data for this material matched that previously reported.[45]



***p*-Tolyl 2,3,6-tri-*O*-benzoyl-5-*O*-levulinoyl-1-thio-β-D-galactofuranoside (6)**

Compound **5** (4.6 g, 5.5 mmol) was dissolved in a solution of pyridine–THF (1:1, 20 mL) at 0 °C and 70% HF·pyridine (2 mL) was added dropwise. The reaction mixture was stirred for 15 h while

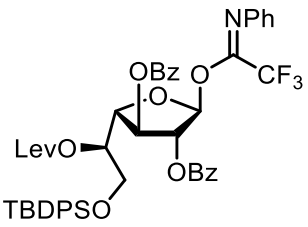
warming to rt before being diluted with EtOAc, poured into a satd aq $NaHCO_3$ soln (40 mL) and extracted with EtOAc (40 mL). The organic layer was washed with water, dried over Na_2SO_4, filtered and concentrated to give crude **24** as a yellow syrup. Then, crude **24** was dissolved in pyridine (10 mL) and cooled to 0 °C followed by the addition of benzoyl chloride (1.1 mL, 10.1 mmol) dropwise. The reaction mixture was warmed to rt and stirred overnight. Excess benzoyl chloride was quenched by the addition of chilled water (30 mL) and the solution was extracted with CH_2Cl_2 (20 mL). The CH_2Cl_2 layer was washed with 10% aq copper sulfate soln (20 mL × 3) and then water (20 mL). The separated CH_2Cl_2 layer was dried over Na_2SO_4, filtered and concentrated. The resulting crude residue was purified by chromatography (hexane–EtOAc, 3:1) to afford alcohol **6** (2.6 g, 68% yield over two steps) as a white foam. The data for this material matched that previously reported.[48]



***p*-Tolyl 2,3,6-tri-*O*-benzoyl-1-thio-β-D-galactofuranoside (4)**

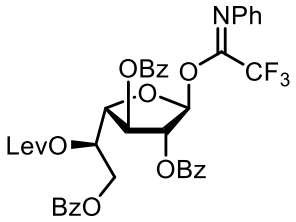
To a stirred solution of **6** (2.1 g, 3.01 mmol) in a mixture of CH_2Cl_2 (27 mL) and CH_3OH (3 mL) at rt was added hydrazine acetate (417.2 mg, 4.53 mmol) in one portion. The resulting reaction mixture was stirred at rt for 1.5 h and then the solvent was removed under vacuum. The resulting

crude residue was purified by chromatography (hexane–EtOAc, 4:1) to afford alcohol **4** (1.79 g, 99% yield) as a white foam. The data for this material matched that previously reported.[48]



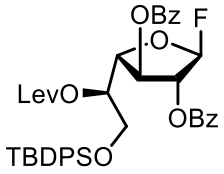
2,3-di-*O*-benzoyl-5-*O*-levulinoyl-6-*O*-*tert*-butyldiphenylsilyl-β-D-galactofuranosyl 2,2,2-trifluoro-*N*-phenylacetimidate (8)

The conversion of thioglycoside 5 to glycosyl *N*-phenyl trifluoroacetimidate donor 8 was performed following general procedure B described above. The product 8 was not characterized but instead was directly used in glycosylation.



2,3,6-tri-*O*-benzoyl-5-*O*-levulinoyl-1-β-D-galactofuranosyl 2,2,2-trifluoro-*N*-phenylacetimidate (10)

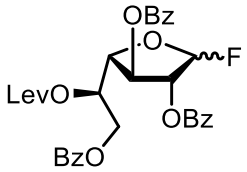
The conversion of thioglycoside 6 to glycosyl *N*-phenyl trifluoroacetimidate donor 10 was performed following general procedure B described above. The product 10 was not characterized but instead was directly used in glycosylation.



2,3-di-*O*-benzoyl-5-*O*-levulinoyl-6-*O*-*tert*-butyldiphenylsilyl-β-D-galactofuranosyl fluoride (9)

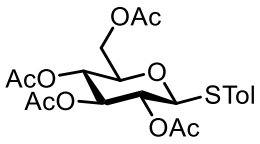
The conversion of thioglycoside 30 to glycosyl fluoride donor 9 was performed following general procedure A described above. R_f = 0.46 (hexane–EtOAc, 4:1); $[\alpha]_D$ = 21.5 (*c* = 2.5, $CHCl_3$); ^1H NMR (700 MHz, $CDCl_3$): δ 8.08 – 8.05 (m, 4H, ArH), 7.65 – 7.57 (m, 6H, ArH), 7.47 – 7.32 (m, 10H, ArH), 5.94 (d, J_{H-F} = 58.8 Hz, 1H, H-1), 5.64 (d, *J* = 6.5 Hz, 1H, H-2), 5.52 (d, *J* = 4.4 Hz, 1H, H-3), 5.48 (q, *J* = 5.6 Hz, 1H, H-5), 4.91 – 4.88 (m, 1H, H-4), 3.94 (dd, J = 10.9, 5.6 Hz, 1H, H-6a), 3.89 (dd, *J* = 10.9, 5.6 Hz, 1H, H-6b), 2.67 – 2.64 (td, J = 6.9, 2.4 Hz, 2H,

OC(O)C<u>H</u>$_2$CH$_2$C(O)), 2.55 – 2.53 (td, J = 6.9, 2.4 Hz, 2H, OC(O)CH$_2$C<u>H</u>$_2$C(O)), 2.10 (s, 3H, C(O)CH$_3$), 0.99 (s, 9H, 9 × SiC(C<u>H</u>$_3$)$_3$); ^{13}C NMR (176 MHz, CDCl$_3$): δ 205.9 (C=O), 171.9 (C=O), 165.4 (C=O), 165.1 (C=O), 135.6(Ar), 135.5 (Ar), 133.8 (Ar), 133.6 (Ar), 132.9 (Ar), 132.8 (Ar), 130.0 (Ar), 129.8 (Ar), 128.9 (Ar), 128.7 (Ar), 128.6 (Ar), 128.5 (Ar), 127.8 (Ar), 127.7 (Ar), 112.3 (d, $J_{C–F}$ = 226.2 Hz, C-1), 84.0 (C-4), 80.8 (d, $J_{C–F}$ = 39.9 Hz, C-2), 76.1 (C-3), 72.4 (C-5), 62.1 (C-6), 37.9 (OC(O)<u>C</u>H$_2$CH$_2$C(O)), 29.6 (C(O)<u>C</u>H$_3$), 27.9 (OC(O)CH$_2$<u>C</u>H$_2$C(O)), 26.6 (3C, 3 × SiC(<u>C</u>H$_3$)$_3$), 19.1 (Si<u>C</u>(CH$_3$)$_3$); HRMS (ESI): *m/z* [M + Na]$^+$ calcd for C$_{41}$H$_{43}$FNaO$_9$Si: 749.2553, found: 749.2550.



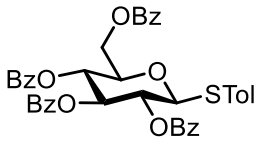
2,3,6-tri-*O*-benzoyl-5-*O*-levulinoyl-D-galactofuranosyl fluoride (11)

The conversion of thioglycoside 30 to glycosyl fluoride donor 34 was performed following general procedure A described above. The product 34 was directly used in glycosylation as a α/β mixture. The data for this material matched that previously reported.[60]



***p*-Tolyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranoside (30)**

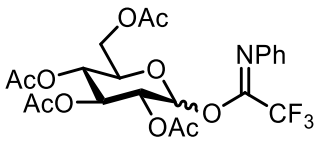
To a mixture of **15** (5.91 g, 15.2 mmol) in CH_2Cl_2 (70 mL) at 0 °C was added $BF_3{\cdot}Et_2O$ (12.7 mL, 106.1 mmol) and 4-methoxybenzenethiol (12.1 g, 98.9 mmol) under. The temperature was gradually raised to rt over 2 h and the mixture was stirred overnight. Satd aq $NaHCO_3$ soln (150 mL) was added and the mixture was extracted with CH_2Cl_2 (80 mL × 3). The combined organic extracts were washed with brine (40 mL) and then dried over Na_2SO_4 filtered and concentrated. The crude product was recrystallized from hexane–CH_2Cl_2 to give **30** (5.5 g. 81% yield). The data for this material matched that previously reported.[65]



***p*-Tolyl 2,3,4,6-tetra-*O*-benzoyl-1-thio-β-D-glucopyranoside (7)**

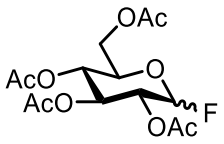
To a solution of **30** (3.08 g, 6.78 mmol) in 3:1 CH_3OH–CH_2Cl_2 was added sodium methoxide in methanol (0.1 M) dropwise until the pH of the reaction mixture was 12. The solution was stirred at rt overnight and neutralized by the addition of glacial acetic acid. Concentration of the solution gave crude **31** as a yellow solid, which was then dissolved in pyridine (10 mL) and cooled to 0 °C

followed by the addition of benzoyl chloride (3.5 mL, 31.1 mmol) dropwise. The reaction mixture was warmed to rt and stirred overnight. Excess benzoyl chloride was quenched by the addition of chilled water (50 mL) and the solution was extracted with CH_2Cl_2 (30 mL). The CH_2Cl_2 layer was washed with 10% aq copper sulfate soln (20 mL × 3) and water (20 mL). The separated CH_2Cl_2 layer was dried over Na_2SO_4, filtered and concentrated. The residue was recrystallized from EtOAc to give **7** (3.42 g, 72% yield) as white crystals. The data for this material matched that previously reported.[66]



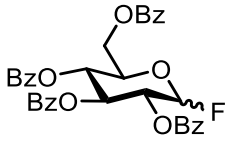
2,3,4,6-tetra-O-acetyl-D-glucopyranoside 2,2,2-trifluoro-*N*-phenylacetimidate (33)

The conversion of thioglycoside 30 to glycosyl *N*-phenyl trifluoroacetimidate donor 33 was performed following general procedure B described above. The product 33 was not characterized but instead was directly used in glycosylation.



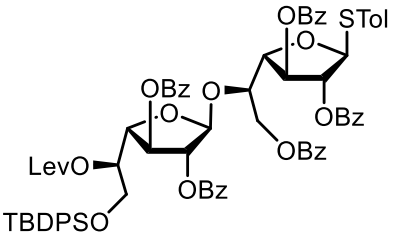
2,3,4,6-tetra-*O*-acetyl-D-glucopyranosyl fluoride (34)

The conversion of thioglycoside 30 to glycosyl fluoride donor 34 was performed following general procedure A described above. The product 34 was directly used in glycosylation as a α/β mixture. The data for this material matched that previously reported.[57]



2,3,4,6-tetra-O-benzoyl-D-glucopyranosyl fluoride (13)

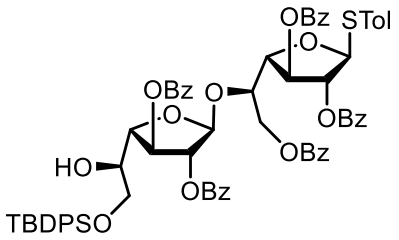
The conversion of thioglycoside 7 to glycosyl fluoride donor 13 was performed following general procedure A described above. The product 13 was directly used in glycosylation as a α/β mixture. The data for this material matched that previously reported.[58]



***p*-Tolyl 2,3-di-*O*-benzoyl-6-*O*-*tert*-butyldiphenylsilyl-5-*O*-levulinyl-β-D-galactofuranosyl-(1→5)-2,3,6-tri-*O*-benzoyl-1-thio-β-D-galactofuranoside (35)**

A mixture of donor **9** (1.44 g, 1.98 mmol), acceptor **4** (0.99 g, 1.65 mmol) and molecular sieves (4 g, 4 Å, powder) was suspended in anhydrous CH_2Cl_2 (40 mL) and the solution was stirred at rt for 30 min. The reaction mixture was then cooled to 0 °C, and then bis(cyclopentadienyl)zirconium(IV) dichloride (120 mg, 412 μmol) and silver trifluoromethanesulfonate (211 mg, 825 μmol) were added successively. The solution was slowly warmed to rt and stirred for 1 h before Et_3N was added and the mixture was filtered. The filtrate was diluted with CH_2Cl_2 (40 mL), washed with satd aq $NaHCO_3$ soln and the organic layer was separated. The aqueous layer was extracted again with CH_2Cl_2 (15 mL × 3) and the combined organic layers were dried over Na_2SO_4, filtered, concentrated, and the resulting residue was purified by chromatography (gradient 6:1→4:1 hexane–EtOAc) to afford **35** (1.95 g, 91% yield) as a white foam. R_f = 0.21 (hexane–EtOAc, 4:1); $[\alpha]_D$ = –36.5 (c = 0.7, $CHCl_3$); ^{1}H NMR (700 MHz, $CDCl_3$): δ 8.00 – 7.84 (m, 10H, ArH), 7.57 – 7.53 (m, 6H, ArH), 7.44 – 7.26 (m, 16H, ArH), 7.25 – 7.19 (m, 5H, ArH), 7.03 (d, J = 8.0 Hz, 2H, ArH), 5.81 (dd, J = 5.1, 2.1 Hz, 1H, H-3), 5.63 (s, 1H, H-1′), 5.62 (t, J = 2.2 Hz, 1H, H-2), 5.60 –

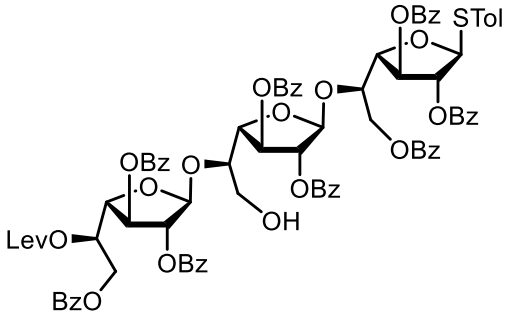
5.58 (m, 2H, H-2′, H-1), 5.49 (dt, J = 7.3, 4.5 Hz, 1H, H-5′), 5.44 (dd, J = 5.1, 1.8 Hz, 1H, H-3′), 4.72 (dd, J = 5.1, 3.4 Hz, 1H, H-4), 4.70 – 4.60 (m, 4H, H-4′, H-6a, H-6b, H-5), 3.87 – 3.83 (m, 1H, H-6a′), 3.80 – 3.78 (m, 1H, H-6b′), 2.61 – 2.57 (m, 2H, OC(O)C$\underline{H}_2$CH$_2$C(O)), 2.50 – 2.48 (m, 2H, OC(O)CH$_2$C$\underline{H}_2$C(O)), 2.27 (s, 3H, ArCH$_3$), 2.02 (s, 3H, C(O)CH$_3$), 0.92 (s, 9H, 9 × SiC(C$\underline{H}_3$)$_3$); ^{13}C NMR (176 MHz, CDCl$_3$): δ 206.0 (C=O), 166.1 (C=O), 165.3 (2C, 2 × C=O), 165.2 (C=O), 165.1 (C=O), 138.1(Ar), 135.5 (Ar), 133.5 (Ar), 133.4 (Ar), 133.2 (Ar), 133.1 (Ar), 133.0 (Ar), 132.7 (Ar), 130.0 (Ar), 129.9 (Ar), 129.8 (Ar), 129.6 (Ar), 129.5 (Ar), 129.1 (Ar), 129.0(Ar), 128.8 (Ar), 128.5 (Ar), 128.4 (Ar), 128.3 (Ar), 128.2 (Ar), 127.7 (Ar), 127.6 (Ar), 105.6 (C-1′), 91.2 (C-1), 82.3 (C-4), 81.9 (C-2′), 81.6 (C-4′), 81.5 (C-2), 77.3 (2C, C-3, C-3′), 73.7 (C-5), 73.0 (C-5′), 64.4 (C-6), 63.0 (C-6′), 38.0 (OC(O)$\underline{C}$H$_2$CH$_2$C(O)), 29.7 (C(O)$\underline{C}$H$_3$), 28.0 (OC(O)CH$_2$$\underline{C}H_2$C(O)), 26.6 (3C, 3 × SiC($\underline{C}$H$_3$)$_3$) 21.1 (Ar$\underline{C}$H$_3$), 19.1 (Si$\underline{C}$(CH$_3$)$_3$); HRMS (ESI): *m/z* [M + NH$_4$]$^+$ calcd for C$_{75}$H$_{76}$NO$_{17}$SSi:1322.4598, found: 1322.4598.



***p*-Tolyl 2,3-di-*O*-benzoyl-6-*O*-*tert*-butyldiphenylsilyl-β-D-galactofuranosyl-(1→5)-2,3,6-tri-*O*-benzoyl-1-thio-β-D-galactofuranoside (36)**

To a stirred solution of **35** (1.67 g, 1.29 mmol) in a mixture of CH_2Cl_2 (27 mL) and CH_3OH (3 mL) at rt was added hydrazine acetate (178 mg, 1.94 mmol) in one portion. The resulting reaction mixture was stirred at rt for 1.5 h and then the solvent was removed under vacuum. The resulting crude residue was purified by chromatography (hexane–EtOAc, 4:1) to afford alcohol **36** (1.4 g, 90%) as a white foam. R_f = 0.35 (hexane–EtOAc, 4:1); $[\alpha]_D$ = –51.3 (*c* = 0.2, $CHCl_3$); ^{1}H NMR (700 MHz, $CDCl_3$): δ 8.02 – 7.88 (m, 8H, ArH), 7.88 – 7.83 (m, 2H, ArH), 7.62 – 7.51 (m, 6H, ArH), 7.47 – 7.43 (m, 3H, ArH), 7.43 – 7.37 (m, 2H, ArH), 7.37 – 7.33 (m, 4H, ArH), 7.33 – 7.24 (m, 9H, ArH), 7.24 – 7.21 (m, 3H, ArH), 7.03 – 6.99 (m, 2H, ArH), 5.82 (ddd, *J* = 5.1, 2.2, 0.8 Hz, 1H, H-3), 5.70 (dd, *J* = 5.4, 2.0 Hz, 1H, H-3′), 5.66 (s, 1H, H-1′), 5.64 (d, *J* = 2.0 Hz, 1H, H-2′), 5.63 (t, *J* = 2.2 Hz, 1H, H-2), 5.59 (d, *J* = 2.3 Hz, 1H, H-1), 4.72 (dd, *J* = 5.1, 3.6 Hz, 1H, H-4), 4.70 – 4.58 (m, 4H, H-5, H-6a, H-6b, H-4′), 4.06 (br s, 1H, H-5′), 3.81 – 3.72 (m, 2H, H-6a′, H-6b′), 2.61 (s, 1H, 5′-OH), 2.26 (s, 3H, $ArCH_3$), 0.97 (s, 9H, 9 × $SiC(C\underline{H}_3)_3$). ^{13}C NMR (176 MHz, $CDCl_3$): δ 166.0 (C=O), 165.6 (C=O), 165.4 (C=O), 165.3 (C=O), 165.2 (C=O), 138.0 (Ar), 135.5

(Ar), 133.4 (Ar), 133.2 (Ar), 133.1 (Ar), 133.0 (Ar), 132.7 (Ar), 130.0 (Ar), 129.9 (Ar), 129.8 (Ar), 129.6 (Ar), 129.4 (Ar), 129.1 (Ar), 129.0 (Ar), 128.9 (Ar), 128.8 (Ar), 128.5 (Ar), 128.4 (Ar), 128.3 (Ar), 128.2 (Ar), 127.7 (Ar), 127.6 (Ar), 105.7 (C-1′), 91.1 (C-1), 83.0 (C-4′), 82.2 (C-4), 81.9 (C-2′), 81.5 (C-2), 77.7 (C-3), 77.3 (C-3′), 73.6 (C-5), 71.4 (C-5′), 65.3 (C-6′), 64.5 (C-6), 26.8 (3C, 3 × SiC($\underline{C}H_3)_3$), 21.1 (Ar$\underline{C}H_3$), 19.1 (Si$\underline{C}(CH_3)_3$); HRMS (ESI): *m/z* $[M + NH_4]^+$ calcd for $C_{70}H_{70}NO_{15}SSi$: 1224.4230, found: 1224.4218.

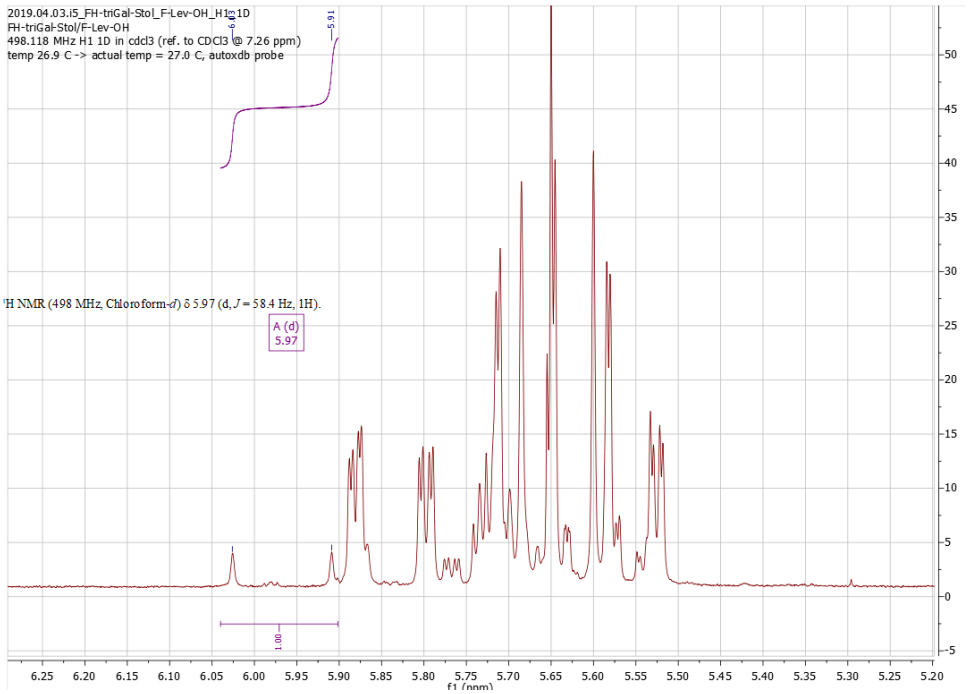


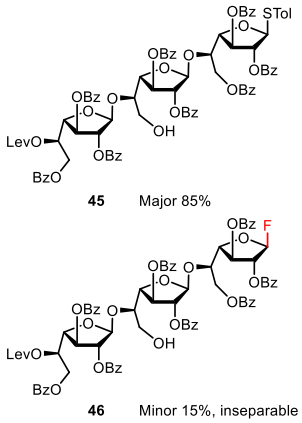
_p_-Tolyl 2,3,6-tri-_O_-benzoyl-5-_O_-levulinyl-β-D-galactofuranosyl-(1→5)-2,3-di-_O_-benzoyl-β-D-galactofuranosyl-(1→5)-2,3,6-tri-_O_-benzoyl-1-thio-β-D-galactofuranoside (45)

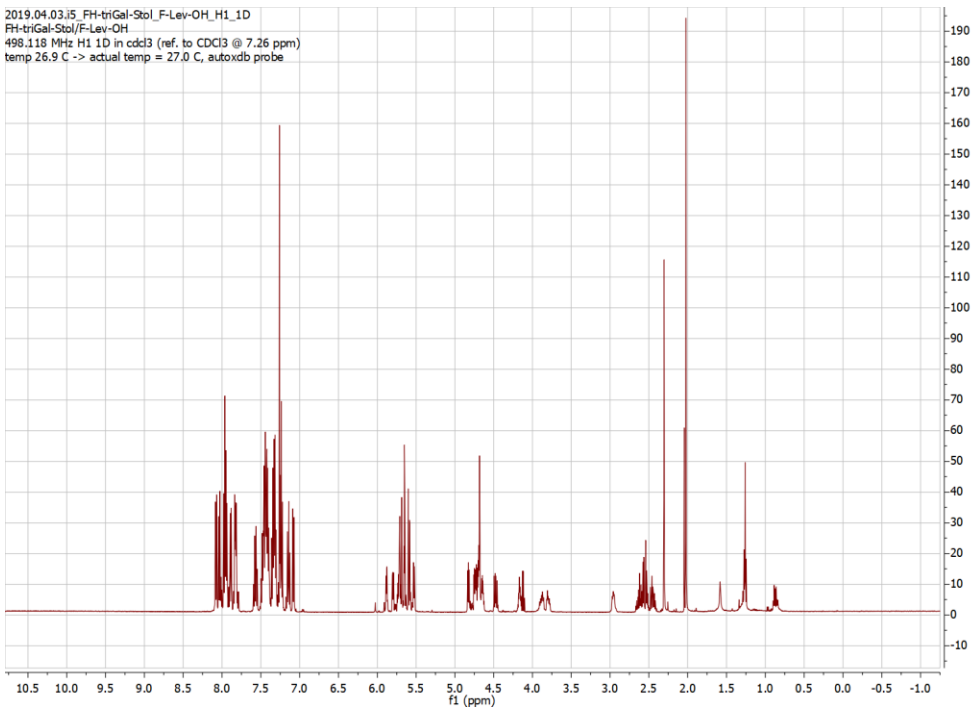
A mixture of donor **11** (685 mg, 1.15 mmol), acceptor **36** (925 mg, 0.76 mmol) and molecular sieves (3 g, 4 Å, powder) was suspended in anhydrous CH_2Cl_2 (30 mL) and stirred at rt for 30 min. The solution was then cooled to 0 °C, and then bis(cyclopentadienyl)zirconium(IV) dichloride (110 mg, 0.38 mmol) and silver trifluoromethanesulfonate (194 mg, 0.76 mmol) were added successively. The solution was slowly warmed to rt and stirred for 6 h before Et_3N was added and the mixture was filtered. The filtrate was diluted with CH_2Cl_2 (40 mL), washed with satd aq

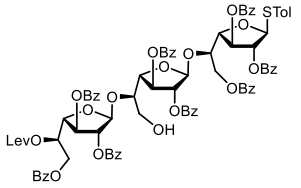
$NaHCO_3$ soln, the organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2 (15 mL × 3). The combined organic layer was dried over Na_2SO_4, filtered and concentrated. The crude residue (**crude 37**) was dissolved in a solution of pyridine–THF (1:1, 15 mL) at 0 °C and 70% HF–pyridine (0.6 mL) was added dropwise. The reaction mixture was stirred overnight while warming to rt before being diluted with EtOAc. The solution was poured into satd aq $NaHCO_3$ (25 mL) soln and extracted with EtOAc (40 mL). The organic layer was washed with water, dried over Na_2SO_4, filtered and concentrated to give **crude 45** (222 mg) as colorless syrup. It was difficult to assign data for this compound mixture, but the raw data is provided below.

^{1}H NMR spentrum of compound 45/46 mixture

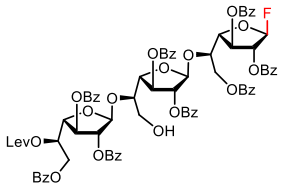




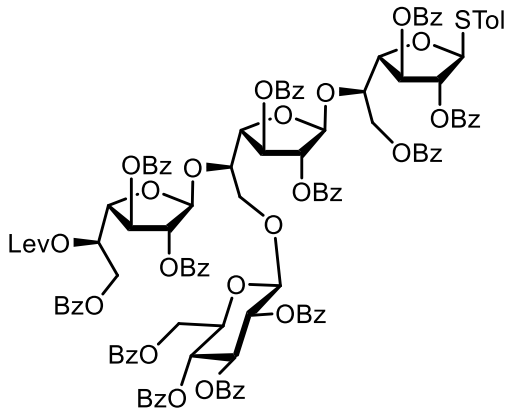




45 Major 85%



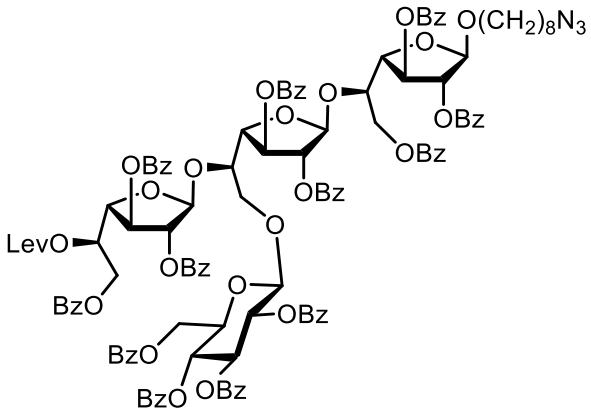
46 Minor 15%, inseparable



***p*-Tolyl 2,3,6-tri-*O*-benzoyl-5-*O*-levulinyl-β-D-galactofuranosyl-(1→5)-[2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→6)]-2,3-di-*O*-benzoyl-β-D-galactofuranosyl-(1→5)-2,3,6-tri-*O*-benzoyl-1-thio-β-D-galactofuranoside (3)**

A mixture of donor **13** (222 mg, 368 μmol), acceptor **45** (241 mg, crude) and molecular sieves (600 mg, 4 Å, powder) was suspended in anhydrous CH_2Cl_2 (6 mL) and stirred at rt for 30 min. The solution was then cooled to 0 °C, and then bis(cyclopentadienyl)zirconium(IV) dichloride (123 mg, 421 μmol) and silver trifluoromethanesulfonate (239 mg, 933 μmol) were added successively. The solution was slowly warmed to rt and stirred for 18 h. The filtrate was diluted with CH_2Cl_2 (15 mL), washed with satd aq $NaHCO_3$ soln, the organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2 (10 mL × 3). The combined organic layer was dried over Na_2SO_4, filtered, concentrated, and the resulting residue was purified by chromatography (gradient 2:1→4:3 hexane–EtOAc) to afford **3** (389 mg, 16% yield over three steps) as a colorless syrup. R_f = 0.15 (hexane–EtOAc, 2:1); $[\alpha]_D$ = –34.5 (c = 0.3, $CHCl_3$); ^{1}H NMR (700 MHz, $CHCl_3$): δ 8.13–

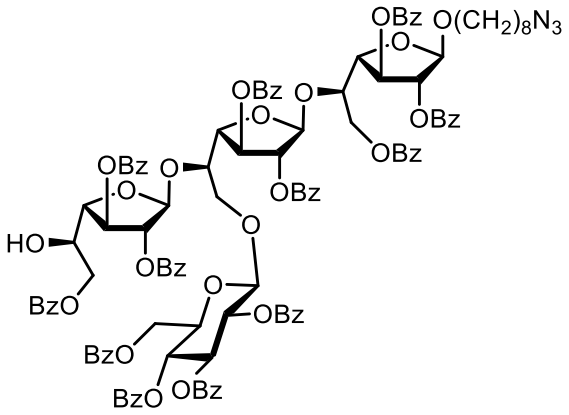
7.03 (m, 64H, Ar), 5.84 (dd, J = 4.5, 1.2 Hz, 1H, H-3), 5.80 (t, J = 9.6 Hz, 1H, H-3‴), 5.72 (d, J = 2.1 Hz, 1H, H-1), 5.71 (dd, J = 5.8, 2.2 Hz, 1H, H-3′), 5.66 (s, 1H, H-1″), 5.64 (t, J = 1.9 Hz, 1H, H-2), 5.62 – 5.51 (m, 5H, H-4‴, H-5″, H-1′, H-2′, H-2‴), 5.50 (d, J = 1.8 Hz, 1H, H-2″), 5.38 (dd, J = 5.0, 1.6 Hz, 1H, H-3″), 4.90 (d, J = 7.8 Hz, 1H, H-1‴), 4.78 (dd, J = 4.8, 3.1 Hz, 1H, H-4), 4.73 (t, J = 4.7 Hz, 1H, H-4″), 4.69–4.57 (m, 5H, H-6a, H-6b, H-5, H-4′, H-6a″), 4.45–4.36 (m, 3H, H-6a‴, H-5′, H-6b″), 4.32 (dd, J = 12.0, 4.6 Hz, 1H, H-6b‴), 4.23 (dd, J = 11.0, 3.3 Hz, 1H, H-6a′), 4.15 – 4.08 (m, 1H, H-6b′), 4.00 (ddd, J = 9.9, 4.6, 3.5 Hz, 1H, H-5‴), 2.61–2.50 (m, 2H, OC(O)C$\underline{H}_2$CH$_2$C(O)), 2.47–2.38 (m, 2H, OC(O)CH$_2$C$\underline{H}_2$C(O)), 2.29 (s, 3H, ArC$\underline{H}_3$), 1.96 (s, 3H, C(O)C$\underline{H}_3$). ^{13}C NMR (176 MHz, $CDCl_3$): δ 205.9 (C=O), 171.9 (C=O), 166.1 (C=O), 165.9 (2C, C=O), 165.6 (C=O), 165.5 (C=O), 165.4 (C=O), 165.3 (2C, C=O), 165.2 (C=O), 165.1 (C=O), 165.0 (C=O), 164.8 (C=O), 138.1 (Ar), 133.5 (Ar), 133.4 (Ar), 133.3 (Ar), 133.2 (Ar), 133.1 (Ar), 133.0, (Ar) 132.9 (Ar), 132.8 (Ar), 132.7 (Ar), 130.1 (Ar), 129.9 (Ar), 129.8 (Ar), 129.7 (Ar), 129.6 (Ar), 129.5 (Ar), 129.3 (Ar), 129.0 (Ar), 128.9 (Ar), 128.8 (Ar), 128.6 (Ar), 128.5 (Ar), 128.4 (Ar), 128.3 (Ar), 128.2 (Ar), 128.1 (Ar), 105.6 (C-1′), 105.4 (C-1″), 100.9 (C-1‴), 91.3 (C-1), 82.5 (C-4′), 82.4 (C-4), 81.9 (C-2), 81.7 (2C, C-2″, C-2′), 81.6 (C-4″), 77.6 (C-3), 77.1 (C-3″), 76.6 (C-3′), 73.9 (C-5), 73.6 (C-5′), 73.0 (C-3‴), 72.3 (C-5‴), 71.6 (C-2‴), 70.2 (C-6′), 70.1 (C-4‴), 69.7 (C-5″), 64.3 (C-6), 63.7 (C-6″), 63.0 (C-6‴), 37.9 (OC(O)$\underline{C}$H$_2$CH$_2$C(O)), 29.6(C(O)$\underline{C}$H$_3$), 27.9 (OC(O)CH$_2\underline{C}$H$_2$C(O)), 21.1 (Ar$\underline{C}$H$_3$); HRMS (ESI): *m/z* $[M + NH_4]^+$ calcd for $C_{120}H_{106}NO_{34}S$: 2136.6311, found 2136.6326.



8-Azidooctyl 2,3,6-tri-*O*-benzoyl-5-*O*-levulinyl-β-D-galactofuranosyl-(1→5)-[2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→6)]-2,3-di-*O*-benzoyl-β-D-galactofuranosyl-(1→5)-2,3,6-tri-*O*-benzoyl-β-D-galactofuranoside (47)

Thioglycoside **3** (6 mg, 2.89 μmol) and 8-azido-octanol (0.5 mg, 3.3 μmol) were dried under vacuum for 6 h and then dissolved in CH_2Cl_2 (1 mL) and the resulting solution was cooled to 0 °C. Powdered 4 Å molecular sieves (40 mg) were added and the suspension was stirred for 30 min at 0 °C before *N*-iodosuccinimide (1 mg, 4.1 μmol) and silver triflate (1 mg, 3.8 μmol) were added. The reaction mixture was stirred for 5 h, neutralized with Et_3N, diluted with CH_2Cl_2 (5 mL) and filtered. The filtrate was diluted with CH_2Cl_2 (30 mL), washed with satd aq $NaHCO_3$ soln, the organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2 (10 mL × 3). The combined organic layer was dried over Na_2SO_4, filtered, concentrated, and the resulting residue was purified by chromatography (gradient 4:1→4:3 hexane–EtOAc) to afford **47** (5 mg, 80% yield) as a colorless syrup. R_f = 0.21 (hexane–EtOAc, 2:1); $[\alpha]_D$ = –10.3 (*c* = 0.4, $CHCl_3$); ^{1}H NMR (700

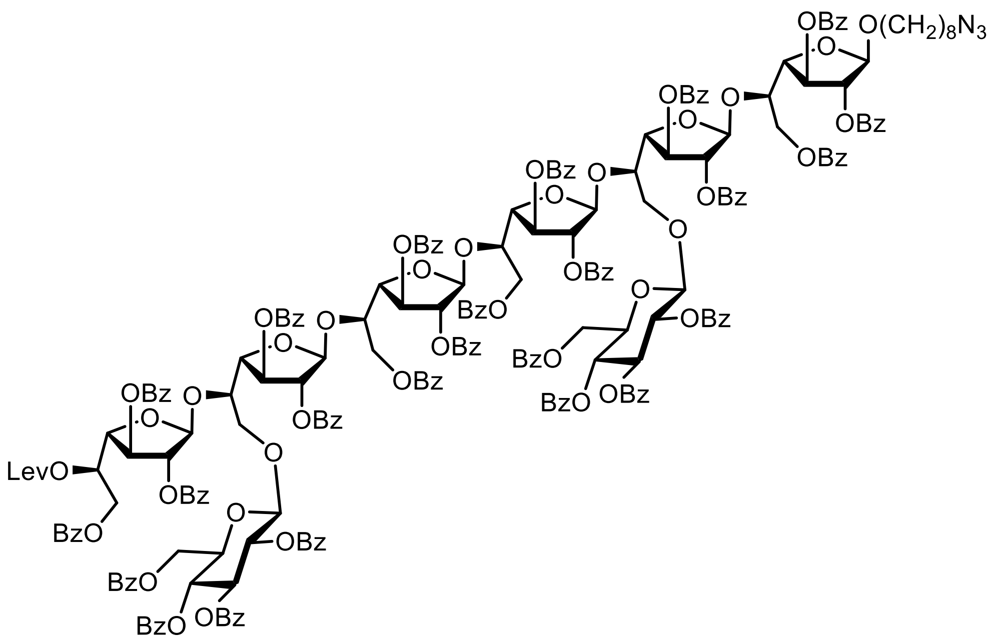
MHz, $CHCl_3$): δ 8.11 – 7.67 (m, 26H, ArH), 7.58 – 7.27 (m, 26H, ArH), 7.25 – 7.19 (m, 7H, ArH), 7.15 – 7.06 (m, 4H, ArH), 5.82 (t, J = 9.6 Hz, 1H, H-3‴), 5.74 (d, J = 4.9 Hz, 1H, H-3), 5.70 (dd, J = 5.9, 2.3 Hz, 1H, H-3′), 5.65 (s, 1H, H-1″) 5.62 – 5.57 (m, 4H, H-1′**,** H-4‴, H-2″**,** H-5″**),** 5.52 – 5.46 (m, 3H, H-2‴**,** H-2′, H-2), 5.39 (dd, J = 5.0, 1.6 Hz, 1H, H-3″), 5.21 (s, 1H, H-1), 4.89 (d, J = 7.9 Hz, 1H, H-1‴), 4.72 (t, J = 4.7 Hz, 1H, H-4″), 4.70 – 4.56 (m, 5H, H-6a, H-6b, H-5, H-4′, H-6a″), 4.50 (dd, J = 4.9, 3.6 Hz, 1H, H-4), 4.44 (dq, J = 7.6, 3.7 Hz, 2H, H-5′, H-6a‴), 4.39 – 4.31 (m, 2H, H-6b″**,** H-6b‴), 4.24 (dd, J = 11.0, 3.3 Hz, 1H, H-6a′), 4.11 – 4.08 (m, 1H, H-6b′), 4.01 (dt, J = 9.9, 4.0 Hz, 1H, H-5‴), 3.69 (dt, J = 9.6, 6.7 Hz, 1H, octyl OC$\underline{H}_2$), 3.47 (dt, J = 9.6, 6.3 Hz, 1H, octyl OC$\underline{H}_2$), 3.19 (t, J = 7.0 Hz, 2H, C$\underline{H}_2N_3$), 2.58 – 2.50 (m, 1H, OC(O)C$\underline{H}_2CH_2C$(O)), 2.47 – 2.37 (m, 2H, OC(O)C$\underline{H}_2CH_2C$(O)), 2.30 – 2.23 (m, 1H, OC(O)C$\underline{H}_2CH_2C$(O)), 2.04 (s, 3H, s, 3H, C(O)C$\underline{H}_3$), 1.58 – 1.50 (m, 4H, CH_2 x 2), 1.37 – 1.16 (m, 4H, CH_2 x 2); ^{13}C NMR (176 MHz, $CDCl_3$): δ 205.9 (C=O), 165.9 (C=O), 165.7 (C=O), 165.3 (C=O), 165.2 (C=O), 165.2 (C=O), 133.5 (Ar), 133.0(Ar), 130.1(Ar), 129.9(Ar), 129.8(Ar), 129.7(Ar), 129.6(Ar), 129.5(Ar), 129.0(Ar), 128.9(Ar), 128.6(Ar), 128.5(Ar), 128.4(Ar), 128.3(Ar), 128.2(Ar), 128.1(Ar), 105.5 (2C, C-1″, C-1), 105.3 (C-1′), 101.0 (C-1‴), 82.5, 81.9, 81.7, 81.6, 77.2, 77.0, 76.8, 73.7, 73.0, 72.2, 71.7, 69.7, 67.5, 63.7, 63.0, 51.4, 37.9, 29.6, 29.5, 29.3, 29.1, 28.8, 27.9, 26.7, 26.1; HRMS (ESI): *m/z* $[M+NH_4]^+$ calcd for $C_{141}H_{115}N_4O_{35}$: 2183.7336, found 2183.7363



8-Azidooctyl 2,3,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→5)-[2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→6)]-2,3-di-*O*-benzoyl-β-D-galactofuranosyl-(1→5)-2,3,6-tri-*O*-benzoyl-β-D-galactofuranoside (48)

To a stirred solution of **47** (22 mg, 10 μmol) in a mixture of CH_2Cl_2 (3 mL) and CH_3OH (1 mL) was added hydrazine acetate (1.3 mg, 14 μmol) in one portion at rt. The resulting reaction mixture was stirred at rt for 1.5 h, then the solvent was removed under vacuum, and the resulting crude residue was purified by chromatography (hexane–EtOAc, 2:1) to afford alcohol **48** (21.5 mg, 98%) as a white foam. R_f = 0.29 (hexane–EtOAc, 2:1); $[\alpha]_D$ = –67.0 (c = 0.1, $CHCl_3$); ^{1}H NMR (700 MHz, $CDCl_3$): δ 8.05 – 7.94 (m, 10H, ArH), 7.91 – 7.83 (m, 6H, ArH), 7.76 – 7.70 (m, 8H,ArH), 7.58 (tt, J = 7.3, 1.3 Hz, 1H, ArH), 7.53 – 7.26 (m, 25H, ArH), 7.26 – 7.16 (m, 6H, ArH), 7.12 – 7.07 (m, 4H, ArH), 5.85 (t, J = 9.6 Hz, 1H, H-3‴), 5.75 – 5.72 (m, 2H, H-3, H-3′), 5.67 – 5.62 (m, 2H, H-1″, H-4‴), 5.60 – 5.58 (m, 2H, H-1′, H-2″), 5.56 – 5.51 (m, 2H, H-3″, H-2‴), 5.49 (dd, J = 5.1, 2.0 Hz, 1H, H-2′), 5.47 (d, J = 1.7 Hz, 1H, H-2), 5.20 (s, 1H, H-1), 4.91 (d, J = 7.8 Hz, 1H,

H-1‴), 4.70 – 4.59 (m, 5H, H-6a, H-6b, H-5, H-4′, H-6a″), 4.50 – 4.45 (m, 3H, H-4, H-4″, H-5′), 4.40 (dd, J = 11.7, 3.9 Hz, 1H, H-6a‴), 4.37 – 4.32 (m, 2H, H-6b″, H-6b‴), 4.27 (dd, J = 11.4, 2.6 Hz, 1H, H-6a′), 4.17 (br, 1H, H-5″), 4.13 – 4.08 (m, 1H, H-6a′), 4.05 – 3.99 (m, 1H, H-5‴), 3.68 (dt, J = 9.7, 6.7 Hz, 1H, octyl OC$\underline{H}_2$), 3.46 (dt, J = 9.6, 6.3 Hz, 1H, octyl OC$\underline{H}_2$), 3.20 (t, J = 7.0 Hz, 2H, C$\underline{H}_2N_3$), 2.66 (d, *J* = 8.4 Hz, 1H, 5″-OH), 1.70 – 1.47 (m, 4H, CH_2 x 2), 1.41 – 1.16 (m, 4H, CH_2 x 2); ^{13}C NMR (176 MHz, $CDCl_3$): δ 166.3 (C=O), 166.1 (C=O), 165.9 (C=O), 165.7 (C=O), 165.7 (C=O), 165.5 (C=O), 165.4 (C=O), 165.3 (C=O), 165.2 (C=O), 165.1 (C=O), 164.9 (C=O), 133.4 (Ar), 133.4 (Ar), 133.3 (Ar), 133.2 (Ar), 133.1 (Ar), 133.0 (Ar), 132.9 (Ar), 132.8 (Ar), 130.1 (Ar), 130.0 (Ar), 129.9 (Ar), 129.8 (Ar), 129.7 (Ar), 129.6 (Ar), 129.5 (Ar), 129.2 (Ar), 129.1 (Ar), 129.0 (Ar), 128.9 (Ar), 128.8 (Ar), 128.7 (Ar), 128.6 (Ar), 128.5 (Ar), 128.4 (Ar), 128.3 (Ar), 128.2 (Ar), 128.1 (Ar), 105.5 (2C, C-1″, C-1), 105.3 (C-1′), 100.9 (C-1‴), 83.3, 82.8, 82.5, 82.0, 81.8, 81.6, 78.0, 77.2, 77.0, 76.8, 73.6, 73.4, 72.9, 72.2, 71.8, 70.8, 69.7, 69.5, 66.4, 64.6, 62.9, 51.4, 31.9, 29.7, 29.5, 29.3, 29.2, 29.1, 28.8, 26.7, 26.0; HRMS (ESI): *m/z* $[M+NH_4]^+$ calcd for $C_{116}H_{109}N_4O_{33}$: 2085.7009, found 2085.6986.

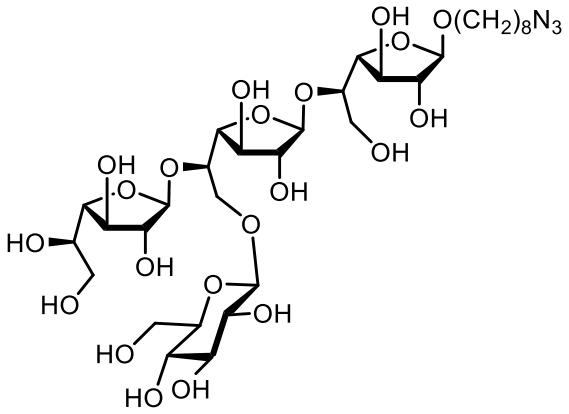


8-Azidooctyl 2,3,6-tri-*O*-benzoyl-5-*O*-levulinyl-β-D-galactofuranosyl-(1→5)-[2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→6)]-2,3-di-*O*-benzoyl-β-D-galactofuranosyl-(1→5)-2,3,6-tri-*O*-benzoyl-β-D-galactofuranoside-(1→5)-2,3,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→5)-[2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→6)]-2,3-di-*O*-benzoyl-β-D-galactofuranosyl-(1→5)-2,3,6-tri-*O*-benzoyl-β-D-galactofuranoside (49)

Thioglycoside **3** (24 mg, 11.6 μmol) and alcohol **48** (20 mg, 9.7 μmol) were dried under vacuum for 6 h and then dissolved in CH_2Cl_2 (2 mL) and the resulting solution was cooled to 0 °C. Powdered 4 Å molecular sieves (80 mg) were added and the suspension was stirred for 30 min at 0 °C before *N*-iodosuccinimide (4.5 mg, 19.4 μmol) and silver triflate (1 mg, 3.8 μmol) were added. The reaction mixture was stirred for 2 h, neutralized with Et_3N, diluted with CH_2Cl_2 (5 mL) and filtered. The filtrate was diluted with CH_2Cl_2 (5 mL), washed with satd aq $NaHCO_3$ soln, the

organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2 (5 mL × 3). The combined organic layer was dried over Na_2SO_4, filtered, concentrated, and the resulting residue was purified by chromatography (gradient 2:1→1:1 hexane–EtOAc) to afford **49** (30 mg, 78% yield) as a white foam. R_f = 0.36 (hexane–EtOAc, 1:1); $[\alpha]_D$ = –63.0 (*c* = 0.1, $CHCl_3$); ^{1}H NMR (700 MHz, $CDCl_3$): δ 8.12 – 7.61 (m, 57H), 7.58 – 7.27 (m, 28H), 7.25 – 6.93 (m, 33H), 5.82 (t, *J* = 9.5 Hz, 1H), 5.80 – 5.73 (m, 4H), 5.72 – 5.70 (m, 1H), 5.68 (s, 1H), 5.66 – 5.60 (m, 4H), 5.60 – 5.54 (m, 7H), 5.51 (ddd, *J* = 7.8, 6.4, 4.0 Hz, 2H), 5.47 – 5.43 (m, 2H), 5.41 (d, *J* = 1.9 Hz, 1H), 5.34 (dd, *J* = 4.7, 1.8 Hz, 1H), 4.92 (d, *J* = 7.8 Hz, 1H), 4.82 (d, *J* = 7.8 Hz, 1H), 4.77 (q, *J* = 3.3, 2.8 Hz, 2H), 4.69 – 4.65 (m, 2H), 4.65 – 4.48 (m, 9H), 4.46 (dt, *J* = 7.6, 3.8 Hz, 1H), 4.43 – 4.37 (m, 2H), 4.34 – 4.21 (m, 5H), 4.12 (td, *J* = 11.1, 4.8 Hz, 2H), 4.08 – 4.00 (m, 2H), 3.92 (dt, *J* = 9.7, 3.8 Hz, 1H), 3.67 (dt, *J* = 9.6, 6.7 Hz, 1H), 3.49 (s, 1H), 3.44 (dt, *J* = 9.6, 6.3 Hz, 1H), 3.17 (t, *J* = 7.0 Hz, 2H), 2.53 – 2.45 (m, 1H), 2.40 – 2.28 (m, 3H), 2.24 – 2.14 (m, 1H), 1.58 – 1.46 (m, 4H), 1.38 – 1.12 (m, 4H); ^{13}C NMR (176 MHz, $CDCl_3$): δ 205.9, 171.8, 166.1, 165.9, 165.8, 165.7, 165.6, 165.5, 165.4, 165.3, 165.2, 165.1, 165.0, 164.8, 133.5, 133.3, 132.9, 132.8, 132.7, 130.1, 130.0, 129.8, 129.7, 129.6, 129.5, 129.3, 129.1, 129.0, 128.9, 128.8, 128.6, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 105.8, 105.6, 105.5, 105.3, 105.2, 101.1, 100.8, 83.4, 83.2, 83.0, 82.5, 82.2, 82.0, 81.8, 81.7, 81.6, 81.5, 78.0, 77.2, 77.0, 76.8, 74.0, 73.7, 73.4, 73.0, 72.7, 72.2, 72.1, 71.8, 71.6, 71.4, 70.1, 69.8, 69.6, 69.3, 67.5, 65.5, 64.6, 63.6, 63.1, 62.8, 51.4, 50.9, 37.9, 31.9, 29.7,

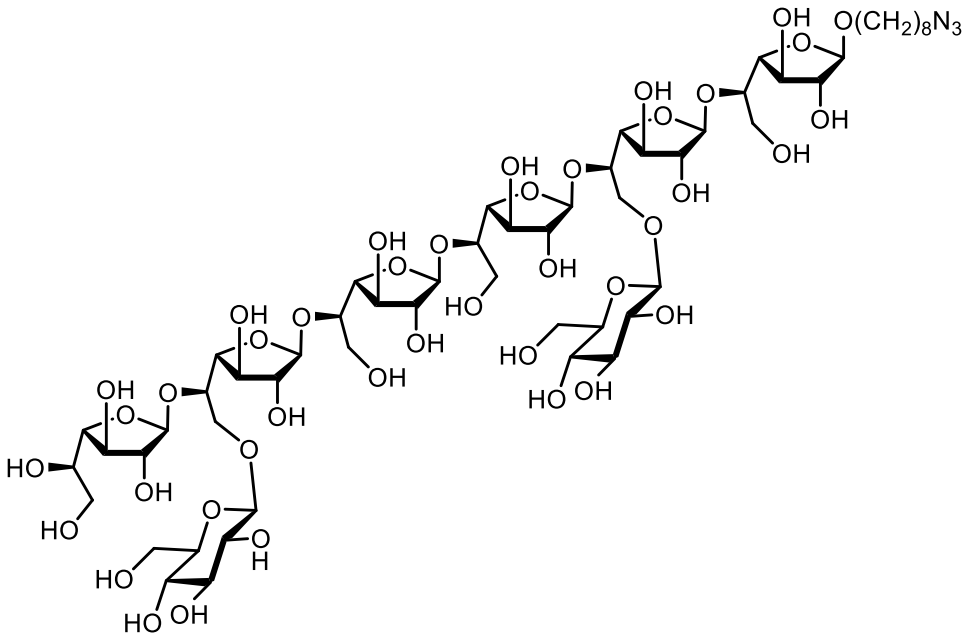
29.6, 29.5, 29.4, 29.3, 29.0, 28.8, 27.8, 26.6, 26.0; HRMS (ESI): *m/z* $[M+2Na]^{+2}$ calcd for $C_{229}H_{199}N_3Na_2O_{67}$: 2054.1021, found 2054.1006.



8-Azidooctyl β-D-galactofuranosyl-(1→5)-[β-D-glucopyranosyl-(1→6)]-β-D-galactofuranosyl-(1→5)-β-D-galactofuranoside (1)

The fully protected tetrasaccharide **47** (44 mg, 54.3 μmol) was dissolved in methanol (4 mL) followed by the dropwise addition of sodium methoxide in methanol (0.1 M) until the pH of the solution was 12. The reaction mixture was then stirred at rt for 4 h and was neutralized by the addition of Amberlyst-15 (H+) cation exchange resin. The solution was filtered, and the filtrate concentrated to give a syrupy residue. The residue was re-dissolved in water before filtration through a C-18 Seppak cartridge; fractions containing the pure product were combined and concentrated and then lyophilized to afford **2** (15 mg, 91% yield) as a white foam. R_f = 0.26 (CH_2Cl_2–CH_3OH–H_2O, 5:5:1); $[\alpha]_D$ = –60.9 (*c* = 0.1, H_2O); ^1H NMR (700 MHz, CD_3OD): δ 5.26 (d, *J* = 1.8 Hz, 1H, Gal*f* H-1), 5.15 (d, *J* = 1.8 Hz, 1H, Gal*f* H-1), 4.34 (d, *J* = 7.8 Hz, 1H, Glu*p* H-

1), 4.15 (dd, *J* = 6.1, 3.6 Hz, 1H), 4.13 – 4.08 (m, 4H), 4.05 (dd, *J* = 6.7, 3.8 Hz, 1H), 4.04 – 3.99 (m, 3H), 3.96 (dd, *J* = 6.7, 3.7 Hz, 1H), 3.93 (dd, *J* = 3.7, 1.9 Hz, 1H), 3.89 (d, *J* = 2.0 Hz, 0H), 3.88 – 3.85 (m, 2H), 3.84 – 3.79 (m, 1H), 3.42 (dt, *J* = 9.6, 6.5 Hz, 1H), 3.38 – 3.34 (m, 1H), 3.30 – 3.26 (m, 4H), 3.23 (dd, *J* = 9.3, 7.8 Hz, 1H), 1.64 – 1.52 (m, 4H), 1.43 – 1.32 (m, 4H); ^{13}C NMR (176 MHz, CD_3OD): δ 109.3 (Gal*f* C-1), 109.0 (Gal*f* C-1), 108.8 (Gal*f* C-1), 104.8 (Glu*p* C-1), 84.6, 84.3, 83.7, 83.5, 83.1, 83.0, 78.7, 78.7, 78.4, 78.0, 77.9, 77.3, 75.7, 75.0, 72.2, 71.5, 71.0, 68.9, 64.2, 62.7, 52.4, 30.6, 30.3, 30.2, 29.9, 27.7, 27.1; HRMS (ESI): *m/z* $[M+Na]^+$ calcd for $C_{32}H_{57}N_3NaO_{21}$: 842.3377, found 842.3378.

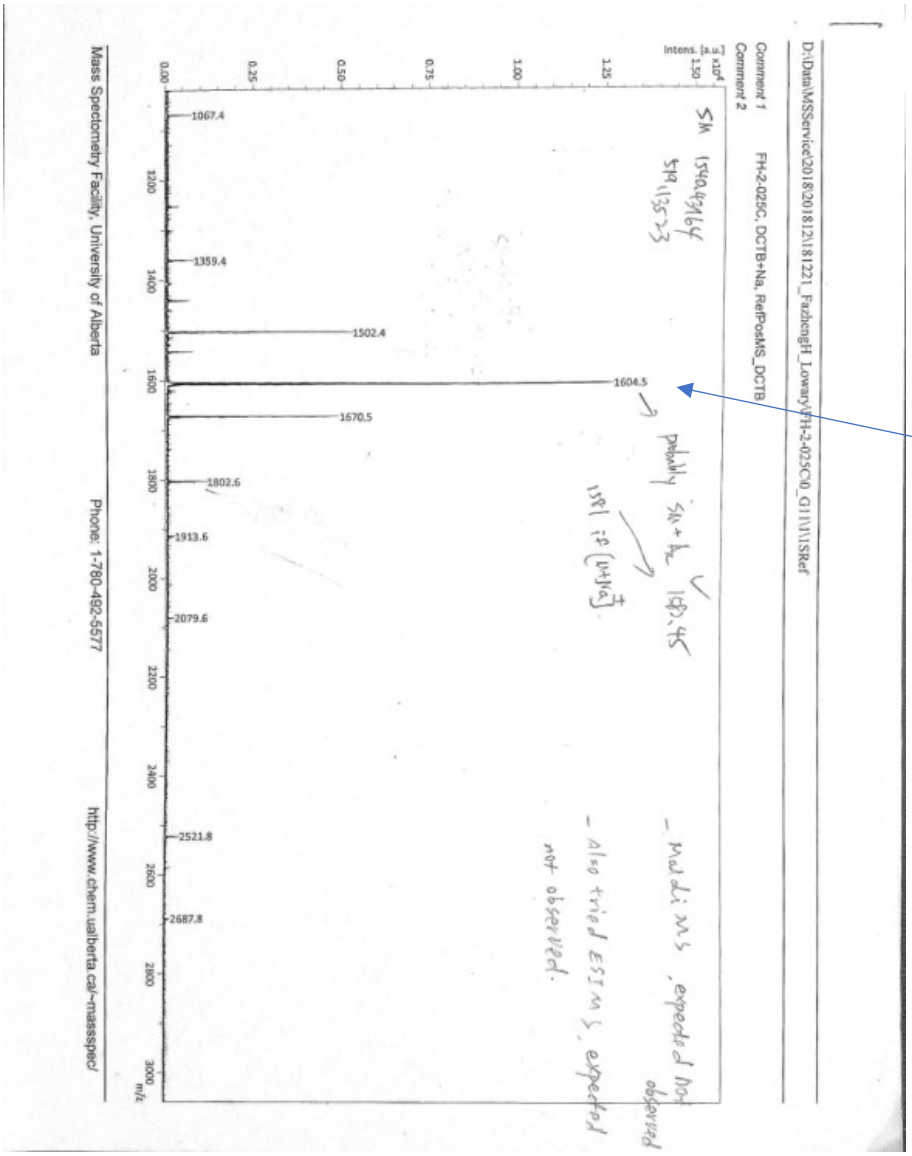


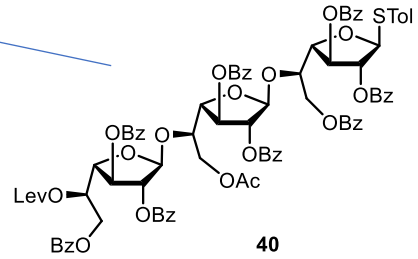
8-Azido-octyl β-D-galactofuranosyl-(1→5)-[β-D-glucopyranosyl-(1→6)]-β-D-galactofuranosyl-(1→5)-β-D-galactofuranosyl-(1→5)-β-D-galactofuranosyl-(1→5)-[β-D-glucopyranosyl-(1→6)]-β-D-galactofuranosyl-(1→5)-β-D-galactofuranoside (2)

The fully protected octasaccharide **49** (42 mg, 10.3 μmol) was dissolved in methanol (4 mL) followed by the dropwise addition of sodium methoxide in methanol (0.1 M) until the pH of the solution was 12. The reaction mixture was then stirred at rt for 4 h and was neutralized by the addition of Amberlyst-15 (H^+) cation exchange resin. The solution was filtered, and the filtrate concentrated to give a syrupy residue. The residue was re-dissolved in water before filtration through a C-18 Seppak cartridge, the fractions containing the pure product were combined and concentrated and then lyophilized to afford **2** (13 mg, 86% yield) as a white foam. R_f = 0.19 (CH_2Cl_2–CH_3OH–H_2O, 10:10:3); $[\alpha]_D$ = –70.6 (*c* = 0.1, H_2O); ^1H NMR (700 MHz, CD_3OD): δ

5.26 (d, J = 1.8 Hz, 1H), 5.25 (d, J = 1.9 Hz, 1H), 5.15 (d, J = 1.8 Hz, 1H), 5.13 (d, J = 1.7 Hz, 1H), 5.12 (d, J = 1.8 Hz, 1H), 4.35 (d, J = 6.3 Hz, 1H), 4.33 (d, J = 6.3 Hz, 1H), 4.21 – 4.07 (m, 9H), 4.06 – 3.98 (m, 8H), 3.96 (dd, J = 6.7, 3.7 Hz, 1H), 3.92 (dd, J = 3.8, 1.9 Hz, 1H), 3.90 – 3.84 (m, 4H), 3.81 (tt, J = 8.3, 4.4 Hz, 2H), 3.78 – 3.60 (m, 11H), 3.60 – 3.54 (m, 1H), 3.47 (dt, J = 12.4, 6.8 Hz, 1H), 3.41 (dt, J = 9.7, 6.7 Hz, 1H), 3.36 (ddt, J = 11.8, 6.9, 3.5 Hz, 2H), 3.27 (t, J = 6.9 Hz, 2H), 3.23 (ddd, J = 9.2, 7.8, 3.2 Hz, 2H), 1.67 – 1.48 (m, 4H), 1.43 – 1.26 (m, 4H); ^{13}C NMR (176 MHz, CD_3OD): δ 109.31, 109.05, 108.87, 108.83, 108.68, 108.65, 104.81, 84.58, 84.31, 84.09, 83.70, 83.56, 83.53, 83.33, 83.20, 83.14, 83.03, 78.70, 78.55, 78.52, 78.02, 78.00, 77.95, 77.32, 77.15, 76.99, 75.72, 75.09, 73.68, 72.21, 72.13, 71.54, 71.51, 71.41, 71.21, 71.17, 71.12, 68.93, 64.27, 62.78, 62.72, 62.70, 62.37, 62.29, 62.24, 52.47, 49.53, 49.36, 49.24, 49.12, 49.00, 48.88, 48.76, 48.63, 30.67, 30.39, 30.23, 29.91, 27.78, 27.15. HRMS (ESI): *m/z* $[M+Na]^+$ calcd for $C_{56}H_{97}N_3NaO_{41}$: 1490.5490, found 1490.5493.

Low resoultution MALDI spectrum of acyl-tranfered product 40





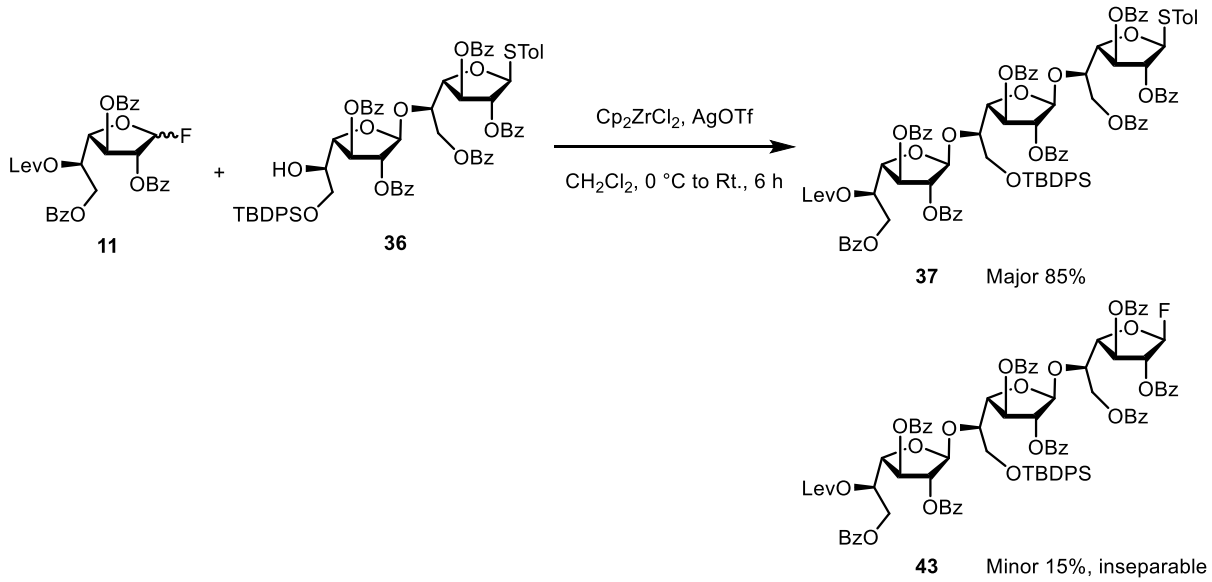
40

Acyl-transfer byproduct

Chapter 3: Summary and future work

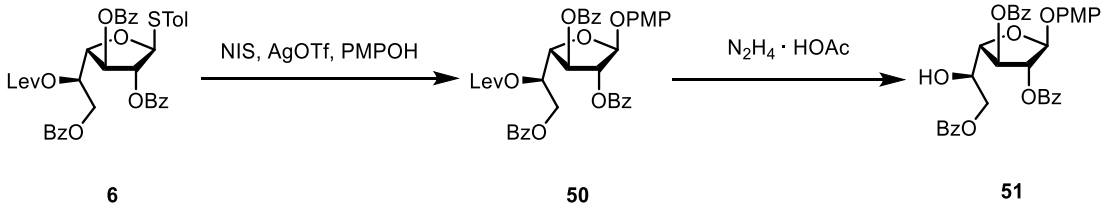
Up to now, I have already synthesized tetrasaccharide and octasaccharide fragments of the *N. nova* arabinogalactan. The NMR spectrum of those chemically-synthesized oligosaccharides are matched with the natural polysaccharides[67] suggesting that previous speculation about *N. nova* possessing a glucose-modified arabinogalactan is correct. To do further studies on the biological side, longer glycans with more repeating units might be needed. Therefore, an improved synthetic plan with higher overall yield is desirable.

The first problem during the assembly of the oligosaccharides is when doing the 2+1 glycosylation there was an inseparable byproduct formed with my desired product. From mass spectrometric and NMR spectroscopic analysis, I found that the byproduct results from the replacement of the STol group with a fluorine (Scheme 3.1). I tried several different conditions for this step; however, it seems the formation of byproduct **43** is inevitable.



Scheme 3.1: Glycosylation of **37** with **11** and formation of inseparable byproduct **43**

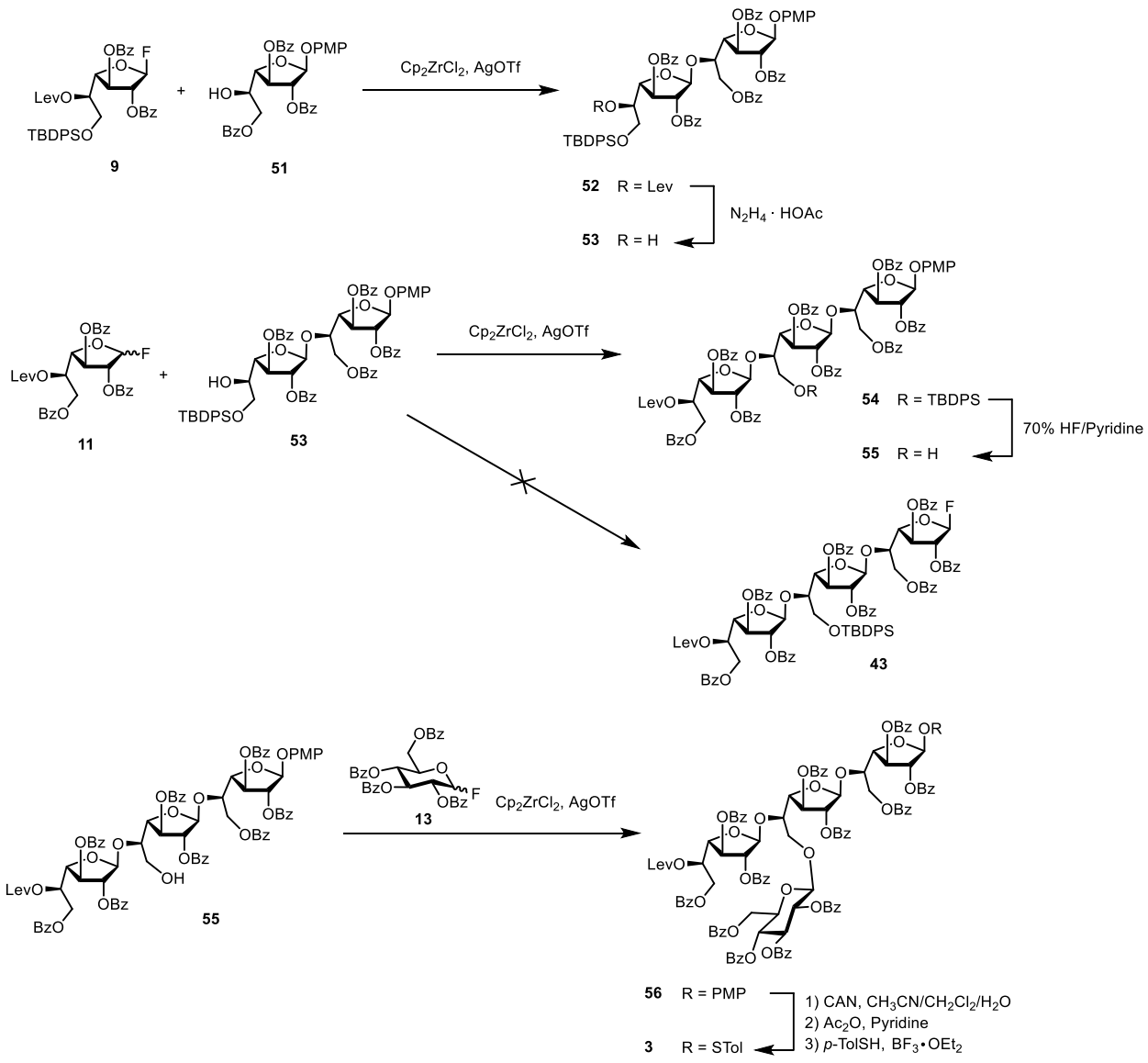
Because I cannot avoid the formation of inseparable byproduct **43**, protecting the reducing end of the acceptor oligosaccharide with *p*-methoxyphenyl (PMP) group might get rid of this problem and increase the overall yield.[68] Based on this idea, the "primer acceptor" would be **51** (Scheme 3.2), which could be prepared in two steps from compound **6**.



6 **50** **51**

Scheme 3.2: Proposed synthesis for PMP protected "primer acceptor" **51**

Another problem is the low yield of the 3 +1 glycosylation. This problem could be partially solved by preventing the fluorine transfer when doing 2+1 reaction (Scheme 3.3). In theory, byproduct **43** will not form in this method, therefore preventing the acceptor self-coupling when doing the 3+1 glycosylation. With tetrasaccharide **56** in hand, the next step is the deprotection of the PMP protecting group to form the hemi-acetal, followed by protecting the anomeric hydroxyl group by acetylation. Glycoside **3** can be obtained by the treating glycosyl acetate with TolSH in the presence of $BF_3 \cdot OEt_2$. Ideally, using this alternative method will give tetrasaccharide building block **3** with higher yield. With a larger amount of **3** in hand, a longer polysaccharide can be synthesized by 4+4, 8+4, 12+4 etc. glycosylation later.



Scheme 3.3: Proposed improved assembly pathway for tetrasaccharide

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