1-1-2018

# Design And Synthesis Of Oligo-(3,5-Dithio-B-D-Glucopyranoside) As B-(1→3)-Glucan Mimetics

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# DESIGN AND SYNTHESIS OF OLIGO-(3,5-DITHIO-*β*-D-GLUCOPYRANOSIDE) AS *β*-(1→3)-GLUCAN MIMETICS

by

**XIAOXIAO LIAO**

**DISSERTATION**

Submitted to the Graduate School

of Wayne State University

Detroit, Michigan

in partial fulfillment of the requirements

for the degree of

**DOCTOR OF PHILOSOPHY**

2018

Major: Chemistry (Organic)

Approved By:

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Advisor Date

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## DEDICATION

*I dedicate my PhD work to my parents Mr. Kaijun Liao and Mrs. Hong Li, my cousins and friends for their endless love, encouragement and support.*

**ACKNOWLEDGEMENT**

First of all, I would like to express the gratitude to my Ph.D advisor Prof. David Crich for giving me this opportunity to study in the field of carbohydrate chemistry. In the 5 years Ph.D. life, teach me everything from the beginning. He taught me how to do organic chemistry research. I remember it was an April afternoon in 2015 when we sit down in front of a table to analyze the spectrum of rearrangement product and figured out that I synthesized a compound with new structure! His education is not only about chemistry but also about how to work. He also edited my dissertation and taught me scientific writing skills. I appreciate him for his guidance and encouragement throughout the Ph.D.

Thanks to our collaborator Prof. Václav Větvička lab for their help in the biological testing of $\beta$-(1→3)-D-glucan mimetics. Thanks to Prof. Peter Andreana lab for their help in the microwave deprotection. Many thanks to my committee member Prof. Stockdill for her teaching since my entering this department at 2013 and throughout the Ph.D. She gave me the knowledge of chemistry as well as scientific writing and literature search. These knowledges helped me throughout the Ph.D and will help me in the future as well. Many thanks to my committee members Prof. SantaLucia and Prof. Dutta for their precious advice in the thesis dissertation. Thanks to Prof. Kodanko and Prof. Pflum for their teaching in the first year and suggestions in my thesis dissertation. Many thanks to Dr. Bashar who gave me NMR training. Many thanks to Melissa who helped me throughout Ph.D. from orientation to the graduation. Many thanks to Jackie who maintained the function of chemistry building. Many thanks

to Nestor who maintained the computers and email system of the Chemistry Department.

I would like to give great gratitude to my past and present lab mates. Frist, I would like to thank the postdoc members: Dr. Takayuki Furukawa, Dr. Takayuki Kato, Dr. Takahiko Matzushita. Dr. Szyman Buda, Dr. Suresh Dharuman, Dr. Oskar Popik, Dr. Parasuraman, Dr. Vikram Sarpe and Dr. Govind. They taught me techniques in the lab and much chemistry knowledge. Many thanks to senior lab members: Dr. Appi Reddy Mandhapati, Dr. Amr Sonusi, Dr. Peng Wen, Dr. Girish Sati, Dr. Philip Adero and Dr. Harsha Amarasekhara. Without their encouragement and support I would not be able to finish this Ph.D. Many thanks to my lab mates: Sandeep Dhanju, Bibek Dhakal, Guanyu Yang, Michael Pirrone, Jonny Quirke, Dean Jarios, Tim Mcmillan, Philemon Ngoje, Mohammad Hawsawi, Nuwan Kondasinghe, Sameera Jayanath, Onobun Emmanuel, Fathima Rukshana, Kondor Courtney and Samarbakhsh Amirreza. They are amazing people and very responsible to maintain the operation of the Crich lab. Especially thanks to Mike Pirrone who maintained the Mass Spec and the NMR operation and serve as mechanics in the lab. Finally, I would like to thank A. Paul. Schapp who sponsored renovating this Chemistry Building.

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## LIST OF ABBREVIATIONS

| | |
|---|---|
| TBAI | Tetrabutyl ammonium iodide |
| cm | centimeter(s) |
| DDQ | 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone |
| DBU | 1,8-Diazabicyclo[5.4.0]undec-7-ene |
| DTBMP | 2,6-Di-(*tert*-butyl)-4-methylpyridine |
| DTT | Dithiothreitol |
| DMAP | 4-(*N*,*N*-Dimethylamino)pyridine |
| DMP | Dess-Marin periodinane |
| NAP | 2-Napthylmethyl |
| KLH | keyhole limpet hemocyanin |
| UV-Vis | Ultraviolet and visible |
| PRR | Pattern recognition receptors |
| PAMP | Pathogen-associated molecular patterns |
| APC | Antigen-presenting cell |
| $Ac_2O$ | Acetic anhydride |
| TLRs | Toll like receptors |
| CRD | Carbohydrate recognition domain |
| CR3 | Complement receptor 3 |
| Trp | Tryptophan |
| His | Histidine |
| STD-NMR | Saturation transfer difference nuclear magnetic resonance |

| | |
|---|---|
| SPOS | Solid phase oligosaccharide synthesis |
| DMF | *N*,*N*-Dimethyl formamide |
| NMO | *N*-Methylmorpholine-*N*-Oxide |
| 2,4-DNP | 2,4-Dinitrophenol |
| Boc | *tert*-Butyloxycarbonyl protecting group |
| $BCl_3$ | Boron trichloride |
| Bz | Benzoyl |
| Bn | Benzyl |
| THF | Tetrahydrofuran |
| *m*-CPBA | *meta*-Chloroperoxybenzoic acid |
| *t*-Bu | *tert*-Butyl |
| $Tf_2O$ | Trifluoromethanesulfonic anhydride |
| TMSOTf | Trimethylsilyl trifluoromethanesulfonate |
| TESOTf | Triethylsilyl trifluoromethanesulfonate |
| FITC | Fluorescein isothiocyanate |
| RNA | Ribonucleic acid |
| PMB | *para*-Methoxybenzyl protecting group |
| DMB | 3,4-Dimethoxybenzyl protecting group |
| SET | Single electron transfer |
| IR | Infrared |
| PDMAB | *para*-*N*,*N*-Dimethylamino benzyl protecting group |
| PEMAB | *para*-*N*,*N*-Diethylamino benzyl protecting group |
| NIS | *N*-Iodo-succinimide |
| NK Cell | Natural Killer cell |

| | |
|---|---|
| CSC | Cancer stem cell |
| NaH | Sodium hydride |
| DCM | Dichloromethane |
| COSY | Correlation spectroscopy |
| IL-2 | Interleukin 2 |
| IL-1β | Interleukin 1β |
| TNF-α | Tumor necrosis factor α |
| $SO_2Cl_2$ | Sulfonyl chloride |
| $Na_2HPO_4$ | Sodium phosphate dibasic |

# CHAPTER 1: INTRODUCTION

## 1.00 Glucose

Natural glucose has the D-configuration (derived from *D*-glyceraldehyde). With a chemical formula $C_6H_{12}O_6$, D-glucose is a six-carbon aldehyde attached to five hydroxyl groups in the open chain form. The intramolecular nucleophilic addition of the C-5 hydroxyl group and the C-1 aldehyde forms a 6-membered hemiacetal ring, which is called D-gluco-pyranose. D-gluco-furanose is the 5-membered hemiacetal ring generated when the C-4 hydroxyl group attacks at C-1 aldehyde (**Figure 1**). The ring closure generates two stereoisomers at C-1 known as the α and β anomers. When drawn as Fischer's projection, the α isomer has anomeric hydroxyl group on the same side as the hydroxyl group of the C-5 stereogenic center, whereas the β anomer places the anomeric hydroxyl at the opposite side to the hydroxyl at the C-5 stereogenic center (**Figure 1**).

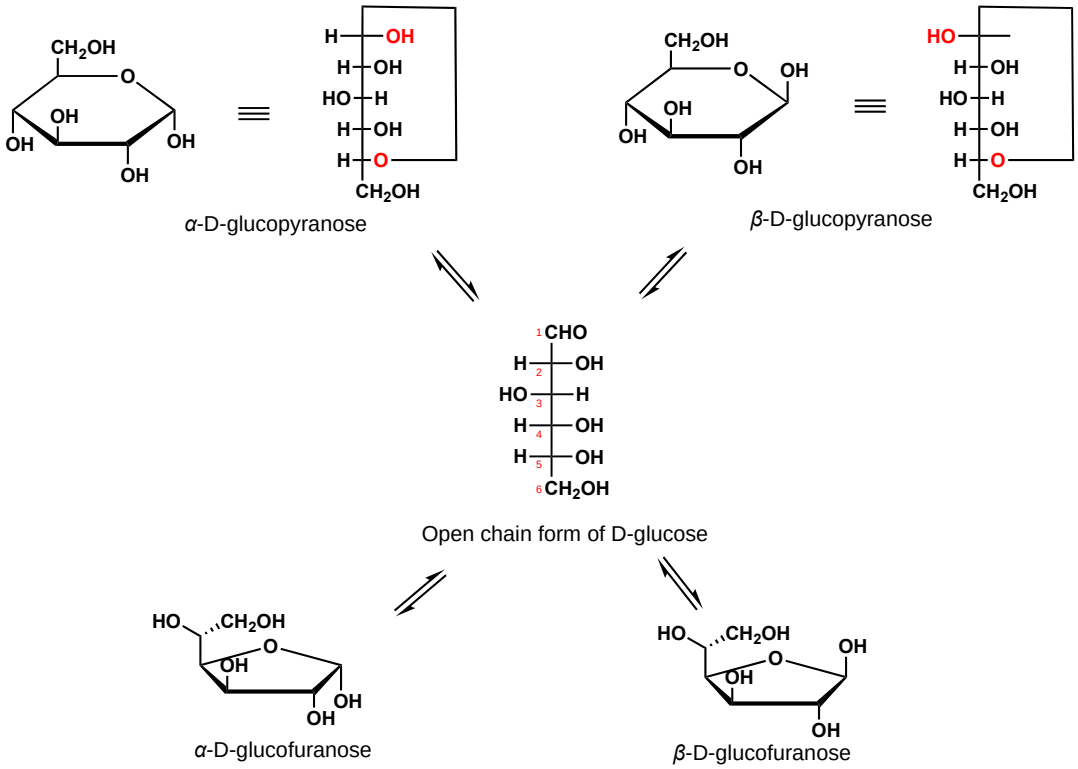



Figure 1**.** Structures of α,β-D-glucopyranose and α,β-D-glucofuranose

## 1.10 *β*-D-Glucans

## 1.11 Structure and origin

Glucans are the polysaccharides consisting of multiple glucose units. Starch, the most common carbohydrate in human diet, is a mixture of glucans consisting mainly of *α* (1 → 4) linked D-glucose units. Cellulose, the most abundant organic polymer on earth,[1] is a glucan consisting of a large number of *β* (1 → 4) linked D-glucopyranose units. *β*-(1 → 3)-D-glucans are natural polysaccharides consisting of *β* (1 → 3)-linked D-glucopyranose units and are the major constituents of many fungal and yeast cell walls.[2] *β*-D-Glucans are also abundant in cereals and bacteria.[3] Their structures vary according to their origin; *β*-(1 → 3)-D-glucans can be linear, as in the case of curdlan (produced by bacteria *Alcaligenes faecalis*), laminarin (polysaccharide found in brown algae). Others can be branched, as in the case of schyzophillan (extracellular polysaccharide of the fungus *Schizophyllum*), and lentinan (a component of the cell wall of the Japanese fungus *Lentinula edodes*). These glucans differ from each other in the number and position of branches (positions 2, 4 or 6), which depends on their origin (**Figure 2**). [3]

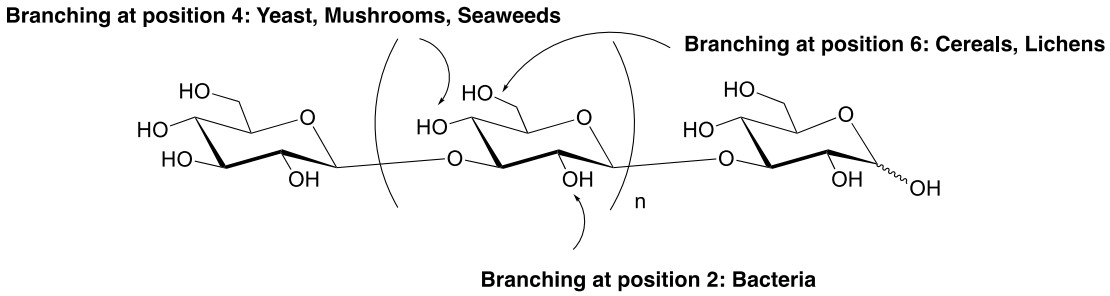



Figure 2. Structure variability of *β*-(1→3)-D-glucans according to their origin

## 1.20 Biological activity

## 1.21 Introduction of the immune system

When the body encounters an invading pathogen, the innate immune system is the first line of defense.[4] Phagocytic cells can kill invading pathogens nonspecifically. Monocytes and

macrophages together make up one of the three types of phagocytes in the immune system. The others being the granulocytes (neutrophils, eosinophils and basophils) and dendritic cells. Macrophages perform several different functions in the innate immune responses. An important function is to engulf and kill invading microorganisms; This process is known as phagocytosis. Pattern recognition receptors (**PRR**) on the surface of phagocytic cells recognize pathogen-associated molecular patterns (**PAMP**) of microorganisms. After recognition, a microorganism is trapped in a phagosome which then fuses with a lysosome to form a phagolysosome, within which enzymes and toxic peroxides digest the microorganism. The recognition and interaction of **PAMPs** by **PRRs** is a critical step in the immune response. It allows the innate immune system to distinguish self (the body) and nonself (pathogen). After phagocytosis, macrophage will activate T lymphocyte cell by presenting antigen derived from pathogen. For this reason, macrophage is also known as antigen-presenting cell (**APC**). Activated **APC** bearing pathogen antigens are delivered to the lymphoid tissues to activate the adaptive immune response. For example, immature dendritic cells are stimulated by recognition of the pathogen and migrate through the lymphatics to regional lymph nodes. They arrive as fully mature non-phagocytic dendritic cells that express antigen and co-stimulatory molecules to activate naive T cell thus initiating the adaptive immune response.

### 1.22 Immunostimulating effect of *β*-(1→3)-D-glucans

The immunostimulating properties of *β*-(1→3)-D-glucans were first discovered in the 1960's and extensive studies on them have continued ever since.[5] Binding of *β*-(1→3)-D-glucans to the PRR of macrophages will activate phagocytosis and several other process including increased chemokinesis, chemotaxis and migration of macrophages to pathogen.[6]

There are many medicinal applications of *β*-(1→3)-D-glucans and some of them have reached Phase I/II in clinical trials. [7]

**1.221 Effect of *β*-(1→3)-D-glucans on cancer**

Over the last 25 years, Japan has used several mushroom-derived *β*-glucans in cancer patients. For example, lentinan[8] is used in the treatment of colorectal and gastro-intestinal cancers, whereas schizophyllan[9] is used for the treatment of stomach and uterine cancers. Commercially available *β*-glucans have been applied to patients receiving chemotherapy. Clinical studies of *β*-glucans have shown that they prolong patients' lives and improve their quality of life.[7] Indeed, the administration of these glucans allows a better recovery of the immune system, after damage from exposure to radiation. In addition, the stimulated production of macrophages and therefore of phagocytosis by glucans, is an important factor in oncology, since macrophage limit the growth of tumors.

**1.222 Effect of *β*-(1→3)-D-glucans on infections**

In 1994, Alpha-Beta Technologies conducted a series of trials, which showed that surgical patients who received *β*-D-glucan had significantly reduced infections and a decrease in the use of antibiotics.[10] Many β-D-glucans are also effective against bacterial infections. The lentinans reduce infections in rats caused by *Mycobacterium tuberculosis* by increasing the rate and effectiveness macrophages *in vivo*.[11] PGG-glucan, a homopolymer of glucose derived from the cell wall of the yeast *Saccharomyces cerevisiae* has a *β* - (1 → 3) backbone and side chain branching at C-6. It increases the anti-infectious activity of leukocytes *in vitro* and *in vivo*, and effectively suppresses infections caused by *Staphylococcus aureus*,[12] including cell lines resistant to certain antibiotics such as β-lactams (including methicillin), improving patient

survival by 80%.[13] Overall, there is abundant evidence to demonstrate that the immune system can be stimulated by $\beta$ - (1 → 3)-D-glucans.

### 1.23 $\beta$-(1→3)-D-glucan receptors

Several receptors of $\beta$-glucans have been identified: scavenger receptors,[14] lactosylceramide,[12] Toll-like receptors (TLRs),[15] complement receptors 3 (**CR3**) [16-17]and Dectin-1.[18] Among these receptors, CR3 and Dectin-1 are the most important receptors.

#### 1.231 Complement receptor 3 (CR3)

In 1987, the Ross group identified Complement Receptor 3 (**CR3**) as a receptor for $\beta$-D-glucans.[19] Complement Receptor 3 is widely expressed on immune cells including leukocytes, macrophages and NK cells. CR3 is also known as $\alpha_M\beta_2$-integrin because it is made up of two protein subunits: the $\alpha_M$ unit CD11b and the $\beta_2$ unit CD18.[20] β-glucans can bind with high affinity to the lectin site and the overlapping I-domain of CD11b. However, β-glucan binding alone cannot activate the immune response. A simultaneous binding of iC3b-opsonized molecules and β-glucan on CR3 is required to trigger the immune system.[21]

#### 1.232 Dectin-1

Dectin-1 is a C-type lectin widely expressed on macrophages, neutrophils and dendritic cell surface membranes. It has been found to be the major receptor for $\beta$-(1 → 3)-D-glucans. [18, 22-23] The high affinity to $\beta$-(1 → 3)-D-glucan comes from the carbohydrate binding domain (CRD) on Dectin-1.[24] To understand the interaction between $\beta$-glucans and Dectin-1, Ohno and co-workers prepared 32 point mutants with mutations in the CRD of Dectin-1 and analyzed their binding with SPG (a 1,6-branched 1,3-β-glucan from *S. commune*). They found that mutations at Trp 221 and His 223 resulted in a decrease in β-glucan binding.[24] In 2007, Brown

and co-workers acquired the crystal structure of murine Dectin-1.[25] The crystal structure reveals a shallow surface groove between Trp 221 and His 223. Further analysis of the electrostatic potential surface reveals the binding groove between Trp 221 and His 223 doesn't have any imbalance of charge. This result indicating that β-glucan binding is driven mainly by vander waals interactions.

**1.24 Saturation transfer difference NMR (STD-NMR) study**

Saturation transfer difference NMR is a spectropic technique to study the interactions between the large molecule (receptor) and small molecule (ligand). The protein was selectively saturated and the saturation is transferred to the ligand via spin diffusion through the intermolecular nuclear overhauser effect.[26]

To precisely identify the binding epitope of $\beta$-(1 → 3)-D-glucan with its receptors, saturation transfer difference (STD) NMR experiments were performed on laminarin (oligo- $\beta$-(1 → 3)-D-glucan found in brown algae) in the presence of Dectin-1(**Figure 3**). In this study, the H-1 was selected as internal standard and the STD-effect was set as 100%. The α-face protons H-3, H-5 display a 142% STD-effect, whereas H-2 and H-4 protons only display 50% STD-effect. These results indicate that the binding affinity is mainly from the hydrophobic interactions between the α face of glucan and the hydrophobic groove of Dectin-1.[27]

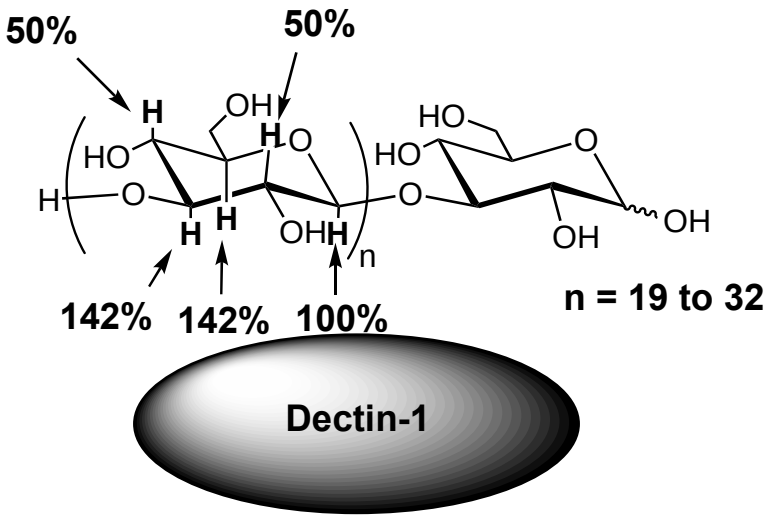


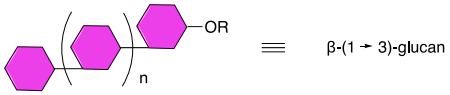

Figure 3. Schematic representation of STD effects between *β*-(1 → 3)-D-glucan and Dectin-1

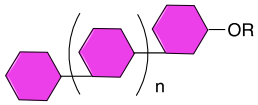
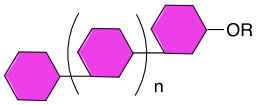
**1.30 Synthesis of *β*-(1 → 3)-D-glucans**

*β*-glucans isolated from nature show great structural variability. For example, the Vetvicka laboratory has tested the immunological effect of more than 110 β-glucans from nine countries and has found considerable biological variability. [28-29] This variability arises because, first, the structure of the cell walls from which the glucans are isolated varies with growth conditions; second, many different isolation procedures and extensive chromatographic purification give a variety of β-glucans.[30] Thus obtain reliable and reproducible results, the biological study of *β*-glucans must be performed with homogeneous synthetic *β*-glucans.

Since 1993, several strategies have been developed to synthesize the linear *β*-(1 → 3)-D-glucans (**Table 1**). [31] Two strategies to synthesize pure *β*-glucan oligosaccharides are used: a) Linear approach using monosaccharides as building blocks where chain length increases one unit at one time. b) Convergent approach using short oligosaccharides as building blocks where each glycosylation doubles the chain length.

Table 1. Linear *β*-(1→3)-D-glucans synthesized to date.

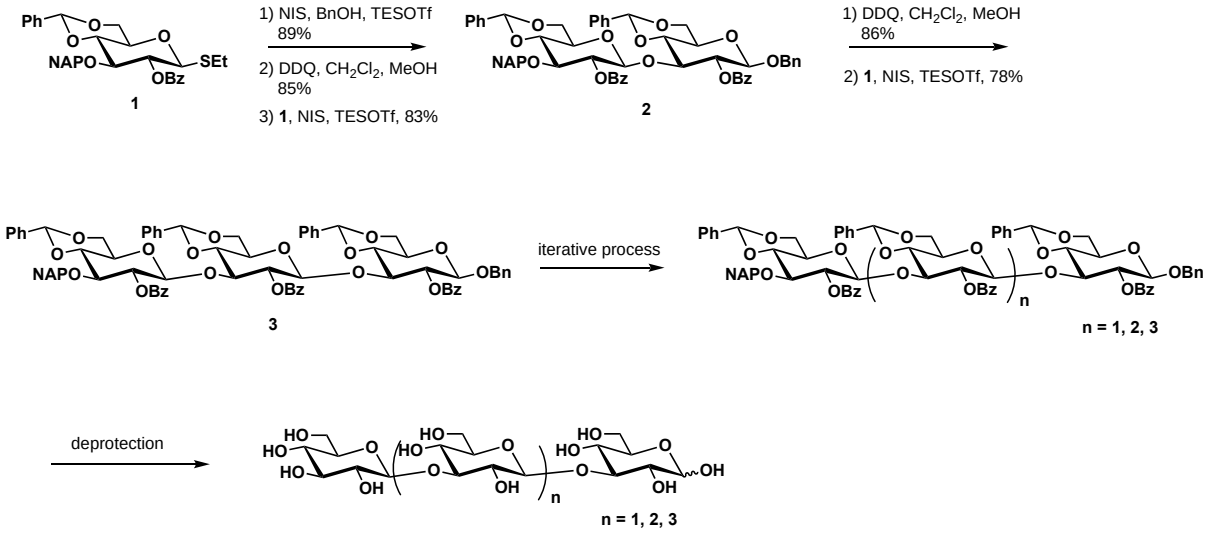


|  | Reference |  | Reference |
|---|---|---|---|
| n = 1 | 1993, Takeo *et al.*[32]<br>2005, Jamois *et al.*[33]<br>2011, Adamo *et al.*[34]<br>2013, Jong *et al.*[35] | n = 6 | 1993, Takeo *et al.*[32]<br>2010, Tanaka *et al.*[36]<br>2015, Liao *et al.*[37] |
| n = 2 | 1993, Takeo *et al.*[32]<br>2003, Zeng *et al.*[38]<br>2005, Jamois *et al.*[33] | n = 8 | 2015, Liao *et al.*[37] |
| n = 3 | 1993, Takeo *et al.*[32]<br>2005, Huang *et al.*[39]<br>2005, Jamois *et al.*[33] | n = 10 | 2010, Tanaka *et al.*[36]<br>2013, Weishaupt *et al.*[40]<br>2015, Liao *et al.*[37] |
| n = 4 | 1993, Takeo *et al.*[32]<br>2009, Mo *et al.*[41]<br>2011, Adamo *et al.*[34]<br>2015, Liao *et al.*[37]<br>2015, Elsaidi *et al.*[42] | n = 12 | 2010, Tanaka *et al.*[36] |
| n = 5 | 1993, Takeo *et al.*[32] | n = 14 | 2010, Tanaka *et al.*[36] |

### 1.31 Linear approach

In a typical example of the linear approach, Vetvicka and co-workers synthesized tri, tetra and pentasaccharides using an iterative deprotection-glycosylation process (**Scheme 1**).[33] In this process monosaccharide **1** acted as both donor and acceptor to elongate the chain. The

3-ONAP group of monosaccharide **1** was selectively deprotected by DDQ to serve as acceptor. The donor **1** was activated by NIS / TESOTf for glycosylation to give the disaccharide. The Benzoyl group at C-2 was installed for the neighboring group participation leading to β-selectivity. After glycosylation, the oligosaccharides were deprotected using Zemplén deacetylation and catalytic hydrogenolysis to afford the corresponding free oligosaccharides. It was found that the synthesized laminaritetraose and laminaripentaose have similar immunostimlatory effects to the natural *β*-(1→3)-glucan phycarine. [33]

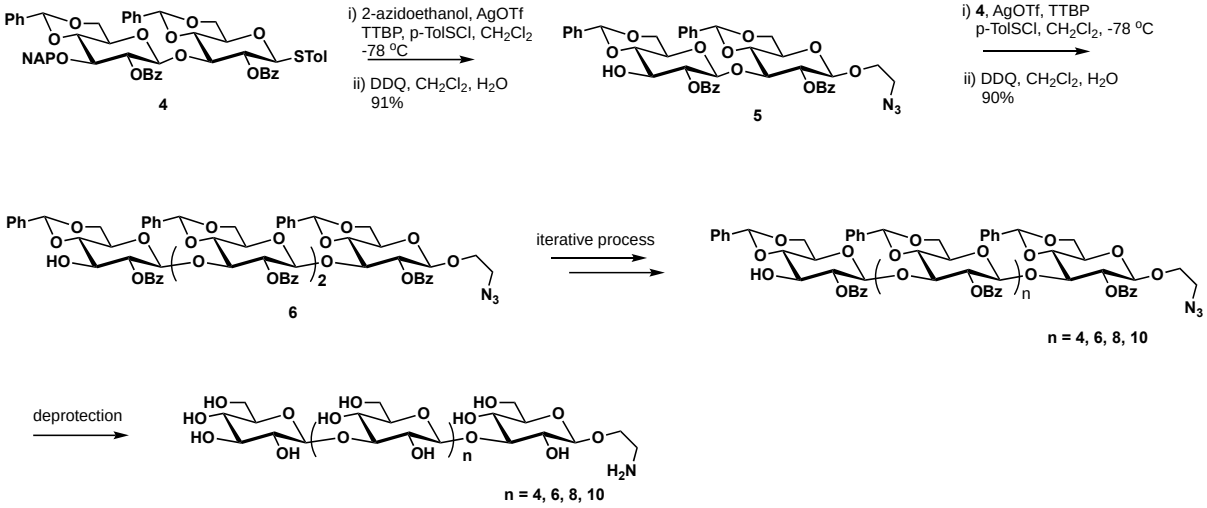



Scheme 1. *β*-(1 → 3)-D-glucan synthesis by iterative glycosylation process

### 1.32 Convergent approach

The convergent strategy improves the "n+1" elongation process into an "n+n" process by employing short oligosaccharides as building blocks. In 2012, Takahashi and co-workers synthesized linear hexadecasaccharides (16 units) employing a tetra-saccharide as key building block.[43] In 2015, the Guo lab accomplished the convergent synthesis of octa-, deca-, and dodeca- *β*-(1→3)-D-glucans. The synthesis was accomplished with preactivation-based iterative glycosylation using *p*-tolylthioglycosides as donors and disaccharide **4** as key building

block (**Scheme 2**). The synthesized oligosaccharides were coupled with a carrier protein keyhole limpet hemocyanin (**KLH**) to form a new glycoconjugate vaccine. These conjugates successfully provoked protective immunity against *Candida albicans* infections. [37]

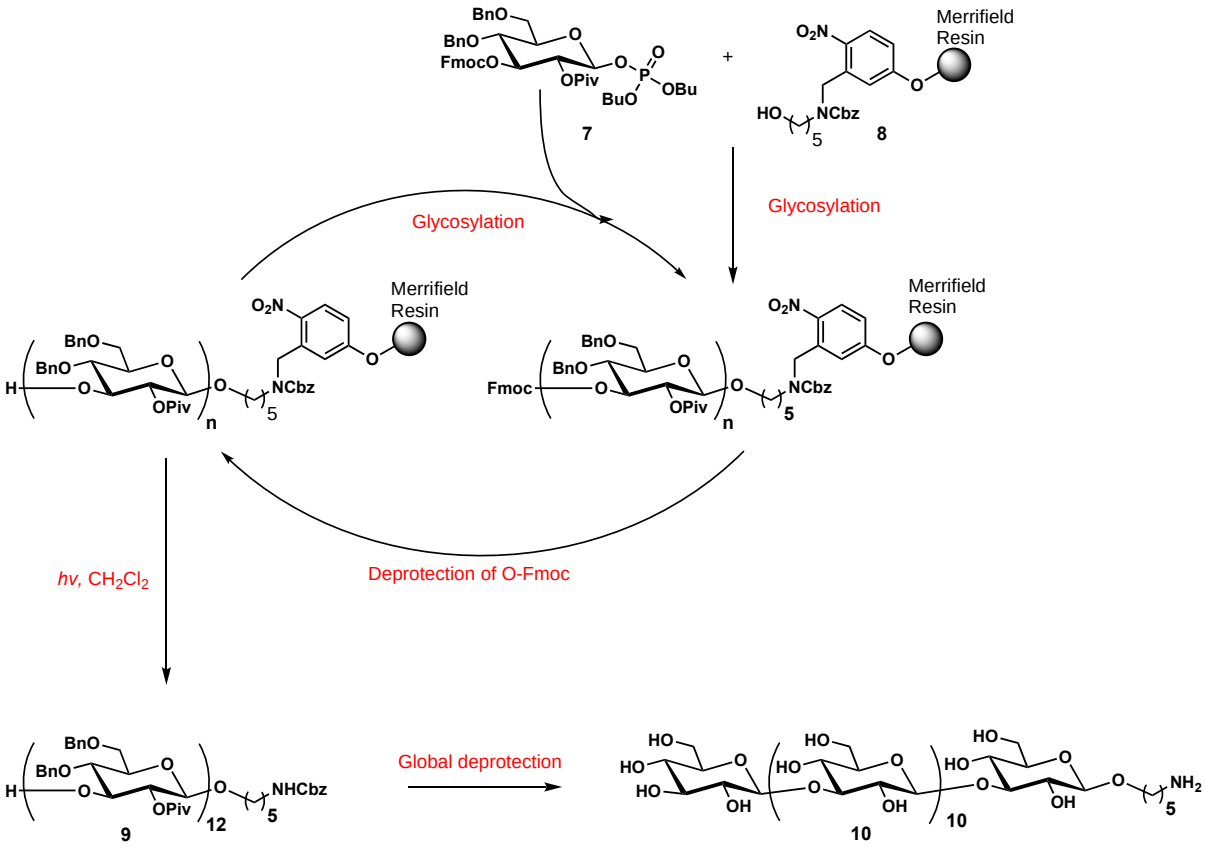



Scheme 2. Guo and co-workers' convergent synthesis of linear $\beta$-(1→3)-D-glucans

### 1.33 Solid phase oligosaccharide synthesis (SPOS)

Traditional solution phase oligosaccharide synthesis has several limitations for longer saccharide synthesis. For example, the need for separation of side products after each glycosylation, the poor solubility of longer oligosaccharides, low stepwise yields and time-consuming protecting group manipulation. Solid phase oligosaccharide synthesis provides a more efficient way of oligosaccharide synthesis. Automated solid phase synthesis of oligopeptides and oligonucleotides have made great contributions to the progress in proteomics and genomics research. [44-45] The automated oligosaccharide synthesis, however, still leaves much to be accomplished.[46] Many laboratories have realized the solid phase oligosaccharide synthesis.[47] In 2013, Seeberger's group developed the automated solid phase synthesis of a linear $\beta$-(1→3)-D-glucan with 12 glucose units.[40] The glycosylation method employs a

glucosyl phosphate as donor with a pivaloyl group in the C-2 hydroxyl group and an orthogonal protecting group at C-3 (Fmoc) (**7**). The first glycosylation connected **7** to the Merrifield resin photolabile linker **8**. After glycosylation, the Fmoc group at C-3 was selectively cleaved by piperidine in DMF and ready for the next glycosylation. This two-step process was repeated 12 times for elongation before the Merrisfield resin linkage was cleaved under UV irradiation to give the protected dodecasaccharide **9**. Global deprotection gave the final product **10** in 4.6% overall yield after 25 steps, with an average yield of 88% per step (**Scheme 3**).




Scheme 3. Seeberger's automated solid phase synthesis of oligo-$\beta$-(1→3)-D-glucan

## 2.00 Glycan mimetics

### 2.10 The challenge of oligosaccharide synthesis

The other two major biomolecules, oligopeptides and oligonucleotides, are linear biopolymers connected with amide and phosphodiester bonds. Unlike templated peptide and

nucleotide synthesis, oligosaccharide synthesis encounters two major challenges: A) Regioselectivity of different hydroxyl groups. B) Stereoselectivity of glycosidic bond formation, also branched structures. Furthermore, the complexity of oligosaccharides is enormously greater than in the oligonucleotides and oligopeptides. For example, in the case of a hexanucleotide there are a total of $4^6$ (=4096) different structures possible and $20^6$(=64 million) for hexapeptide. In the case of hexasaccharides, based on the 10 mammalian monosaccharides, regioisomers and two different stereoisomers, it was calculated that 192 billion different structures are theoretically possible.[48] On one hand, oligosaccharide synthesis is still challenging by conventional organic synthesis, and only few laboratories in the world could accomplish long oligo-$\beta$-(1→3)-D-glucans synthesis (**Table 1**). On the other hand, as the hydrophilic nature of oligosaccharides make them poor ligands to receptors, and carbohydrate protein interactions are dependent on multivalent interactions.[49] In addition, many natural oligosaccharides are easily degraded by glycosidase, which makes them poor drug candidates. Our projects are designed to provide solutions to these problems by modifying the original $\beta$-(1→3)-D-glucan structure to achieve 3 goals. A) Higher synthetic efficiency: Modification of the structure could allow development of a simple and efficient methods for connecting the different units and would allow access to long-chain polymers, ideally on solid support. B) Higher binding affinity to receptors: Rather than working on synthesizing long oligosaccharide, we seek to redefine the problem and utilize new approaches. Thus, by preparing glycan mimetics with short chain lengths we hope to reproduce the biological activity of natural long oligosaccharides. For example, natural glucans are hydrophilic and through modification we could increase the

hydrophobicity of the oligosaccharide thus increasing the binding affinity to receptors. C) Higher stability: Natural glucans are labile to enzymatic degradation. Consequently, by modifying both the exocyclic and endocyclic oxygen of glycosidic bonds we could improve glycosidase inhibition by increasing the stability of oligosaccharides (**Figure 4**). In summary, our goal is to design and synthesize *β*-(1→3)-D-glucan mimetics that have increased interactions with receptors, improved biological activities and simplified oligomer synthesis.

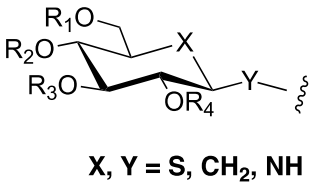



Figure 4. Glycan mimetics with exocyclic and endocyclic oxygen modification

**2.20 Precedent *β*-(1→3)-D-glucan mimetics**

**2.21 Hydroxylamine based *β*-(1→3)-D-glucan mimetics**

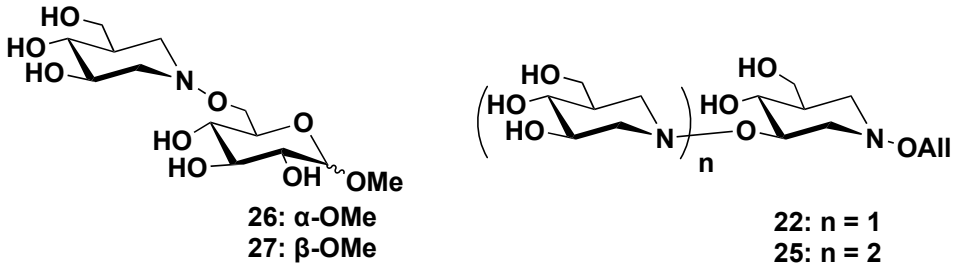
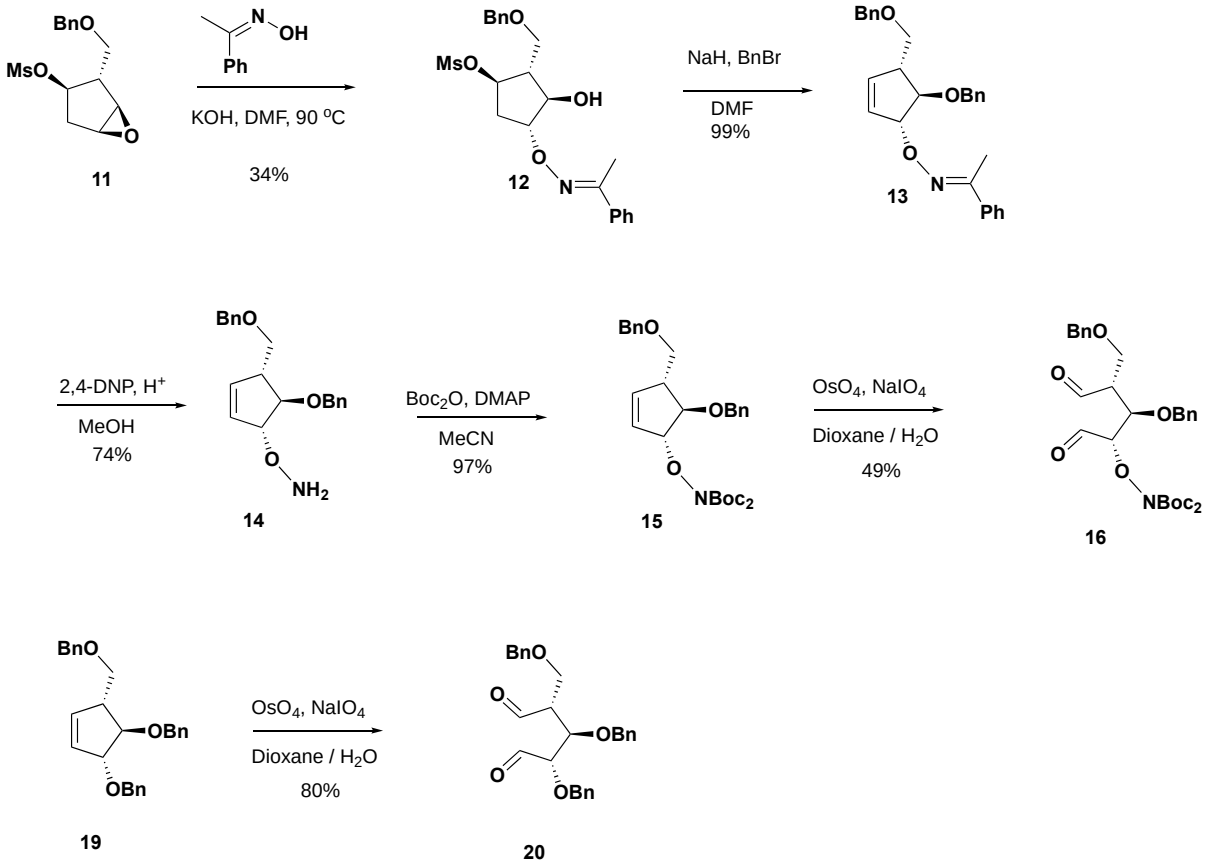



Figure 5. Chemical structure of oligomeric hydroxylamine-linked β-(1 → 3)-D-glucan mimetics

The Crich laboratory designed and synthesized *β*-(1→3)-D-glucan mimetics based on imino sugars linked through a hydroxylamine N-O bond (**Figure 5**).[50] It is known the barrier to inversion at nitrogen atom in trialkyl hydroxylamines is lower than that in simple protonated amines at approximately 15 kcal/mol. Thus, hydroxylamines lack barriers hence is not sufficient to prevent rapid inversion at room temperature.[51-53] Therefore, as an analogue of anomeric C-O bond, the hydroxylamine N-O bond doesn't have a preferential configuration.

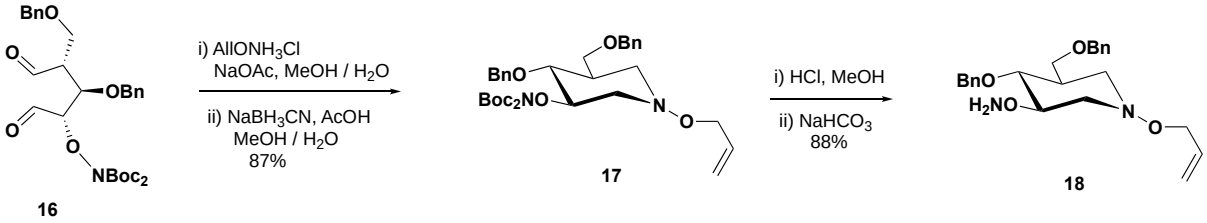
They developed a ring-closing double reductive amination method to prepare the hydroxylamine mimetics.[54]

The enantiomerically pure cyclopentadiene-derived mesyloxy epoxide **11** was subjected to ring opening with potassium hydroxide and acetophenone oxime in hot DMF to give the desired o-cyclopentenyl oxime **12** in 34% yield. Subsequent benzylation and elimination was completed in one pot to give cyclopentene derivative **13** in 99% yield. Cleavage of the oxime with 2,4-DNP catalyzed by sulfuric acid gave hydroxylamine **14** in 74% yield (**Scheme 4**). The hydroxylamine **14** was protected as its *N*, *N*-diBoc derivative **15** by a standard carbamate forming reaction in 97% yield. Ring opening of *N*, *N*-diBoc cyclopentenyl hydroxylamine **15** using catalytic osmium tetroxide and sodium metaperiodate to give a dialdehyde **16** with a protected $ONH_2$ in 49% yield. Treatment of compound **19** with catalytic amount of osmium tetroxide and NMO gave a diol intermediate which was immediately cleaved by sodium metaperiodate to give the dialdehyde **20** in 80% yield (**Scheme 4**).




Scheme 4. Dialdehyde synthesis by oxidative cleavage of cyclopentene derivatives

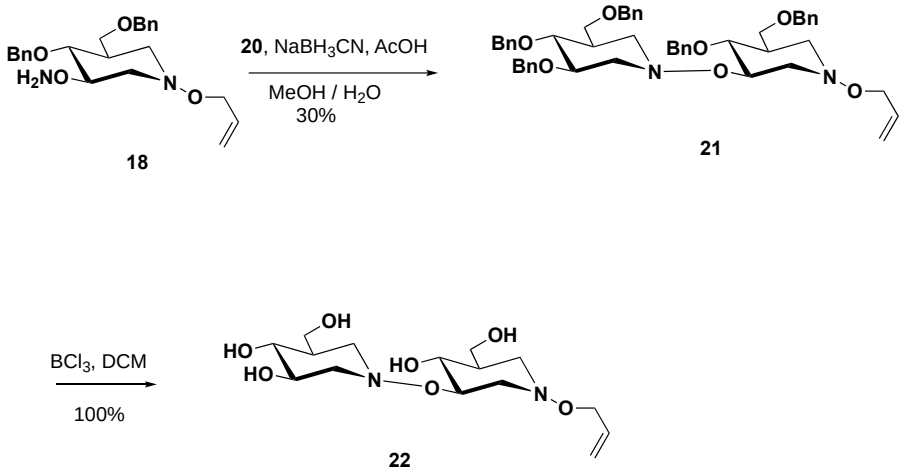
Dialdehyde **16** was subjected to ring-closing double reductive amination with allyl hydroxylamine HCl salt to give *N*,*N*-diBoc protected hydroxylamine intermediate **17**. *N*,*N*-diBoc protecting group was removed under acidic condition to give free hydroxylamine **18** in 88% yield (**Scheme** 5).




Scheme 5. Monomer synthesis by oxidative cleavage and double ring closing reductive amination

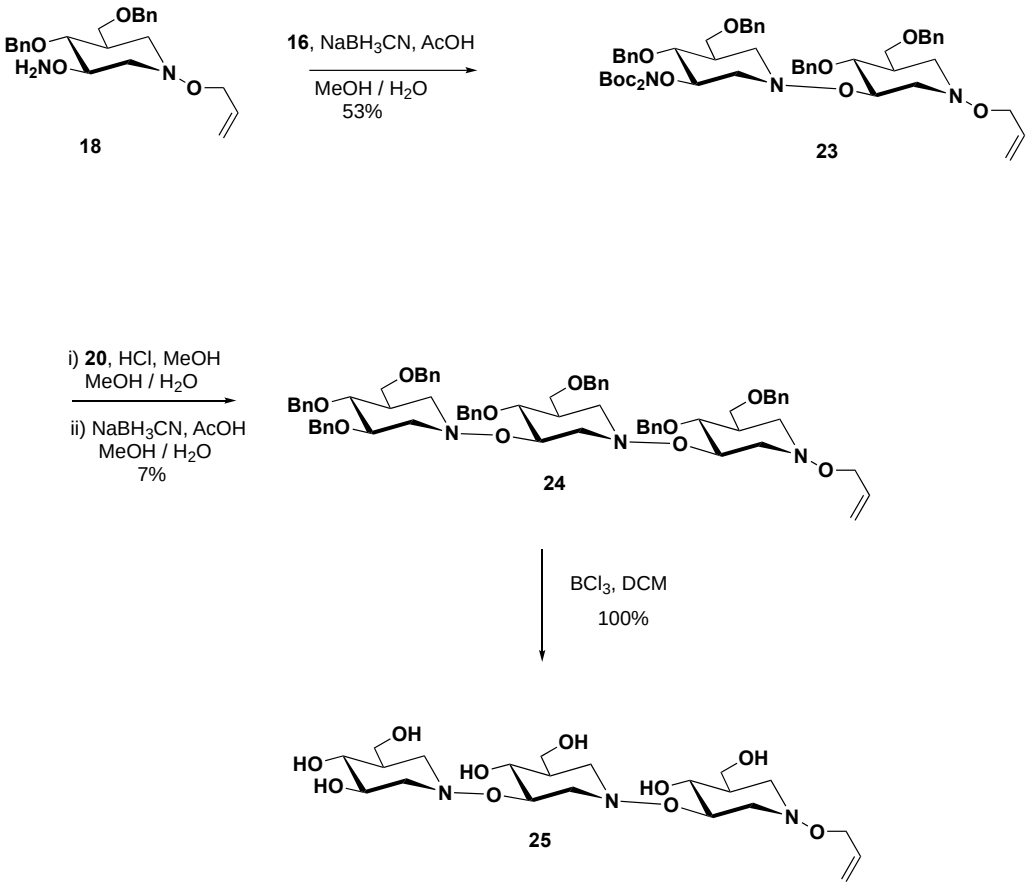
Hydroxylamine intermediate **18** was subjected to another reductive amination with dialdehyde **20** to give benzyl protected dimeric mimetic **21** in 30% yield. The benzyl group

was deprotected by $BCl_3$ to give the hydroxylamine based disaccharide mimetic **22** (**Scheme 6**).




Scheme 6. Synthesis of dimeric hydroxylamine based β-(1 → 3)-D-glucan mimetics

The Trisaccharide mimetic **25** was also synthesized from compound **18**. The *N*,*N*-diBoc protecting group of **18** was removed under acidic condition and the intermediate was subjected to double reductive amination with dialdehyde **16** to give the dimeric intermediate **23** in 53% yield. The *N*,*N*-diBoc protecting group were deprotected under acidic condition to give free hydroxylamine, which was subjected to double reductive amination with dialdehyde **20** to give benzyl protected trimeric hydroxylamine **24** in 7% yield. The benzyl protecting group was removed by $BCl_3$ in DCM to give the hydroxylamine based trisaccharide mimetic **25** (**Scheme 7**).




Scheme 7. Synthesis of trimeric hydroxylamine based β-(1 → 3)-D-glucan mimetics

To evaluate the binding affinity of the hydroxylamine to CR3 and Dectin-1, the Vetvicka laboratory tested the mimetics' ability to inhibit anti-CR3 and anti-Dectin-1 fluorescein isothiocyanate (**FITC**) conjugated antibody staining of human neutrophils and mouse macrophages. For comparison purposes, monohydroxylamines **26** and **27** were also screened. In terms of CR3 binding affinity, incubation of a 0.1μg / ml solution of β-(1→3)-dimer mimetic **22** and β-(1→3)-trimer mimetic **25** caused 26% and 34% decreases in inhibition of staining human neutrophils, while the anomeric β-(1→6)-dimer mimetic **26** and **27** decreases were 19% and 22% (**Table 2**). In terms of Dectin-1 binding affinity, incubation of a 0.1μg/ml solution of β-(1→3)-trimer mimetic **25** with mouse macrophage led to 43% decrease in the inhibition of anti-dectin-1-FITC staining of mouse neutrophils. Under the same

conditions, the β-(1→3)-dimeric mimetic **22** caused 28% decrease in inhibition of antibody staining whereas the β-(1→6)-dimer mimetics **26** and **27** caused 29% and 21% decreases respectively (**Table 2**). These results indicate that binding of the hydroxylamines to the lectin domains of both CR3 and Dectin-1 is correlate to length and linkage.

Table 2**.** Percentage inhibition of anti-CR3 and anti-Dectin-1-FITC antibody staining of neutrophils, macrophages by 0.1μg/mL substrate. a Mean ± SD

| Compound | Linkage-mimicked | Oligomer NO. | % inhibition of anti-CR3-FITC staining human neutrophils[a] | % inhibition of anti-dectin-1-FITC staining of mouse neutrophils[a] |
|---|---|---|---|---|
| **26** | 1→ 6 | dimer | 19.7±1.7 | 29.3±2.2 |
| **27** | 1→ 6 | dimer | 22.3±1.9 | 20.6±2.1 |
| **22** | 1→ 3 | dimer | 26.4±2.7 | 28.2±2.9 |
| **25** | 1→ 3 | trimer | 34.2±3.3 | 43.1±3.5 |

Hydroxylamine based β-glucan mimetics **26, 27, 22, 25** (10 μg / mL) were also tested for their ability to stimulate phagocytosis of synthetic polymeric 2-hydroxyethyl methacrylate microspheres by human macrophage-like RAW 264 cells.[55] Commercial yeast derived insoluble whole glucan particles **WGP** (hollow spheres of long polymers of primarily β-(1→ 3)-D-glucan) were used as reference (**Table 3**). The result indicates that the β-(1→3)-trimer mimetic **25** could stimulate 16% of phagocytosis, which is more effective than the β-(1→6)-dimer mimetic **26, 27** and **22.** It is notable that the level of phagocytosis stimulated by the β-(1→3)-trimer mimetic **25** was more than 50% of that induced by **WGP**. These results indicate

that the hydroxylamine glucan mimetics have good immunostimulating ability even at short length.

Table 3**.** Percentage stimulation of phagocytosis. a Mean ± SD

| Compound | Linkage mimicked | Oligomer NO. | % stimulation of phagocytosis (RAW 264 macrophages, 10µg / mL, 24h) [a] |
|---|---|---|---|
| **26** | 1→ 6 | dimer | 6.7±0.9 |
| **27** | 1→ 6 | dimer | 4.1±0.5 |
| **22** | 1→ 3 | dimer | 7.8±1.1 |
| **25** | 1→ 3 | trimer | 16.6±2.0 |
| **WGP** | 1→ 3 | Insol polymer | 30.1±2.8 |

**2.22 *β*-(1→3)-D-Glucan with thiolinkage**

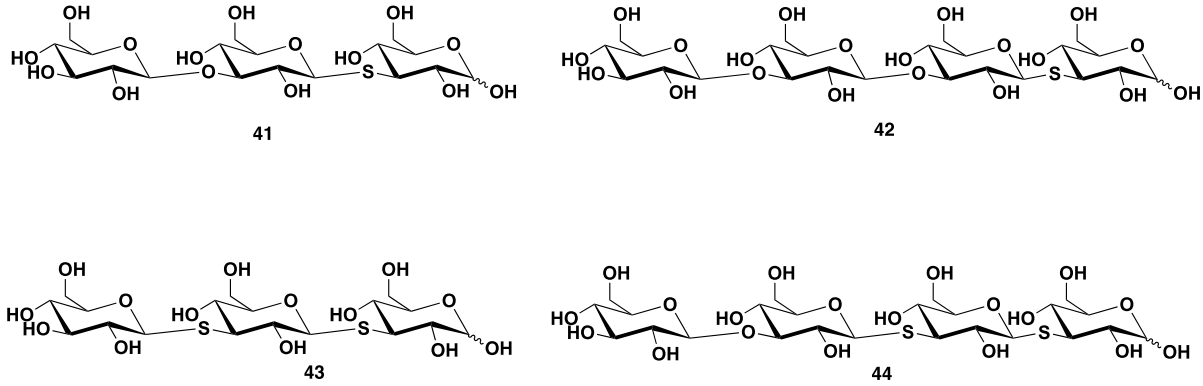
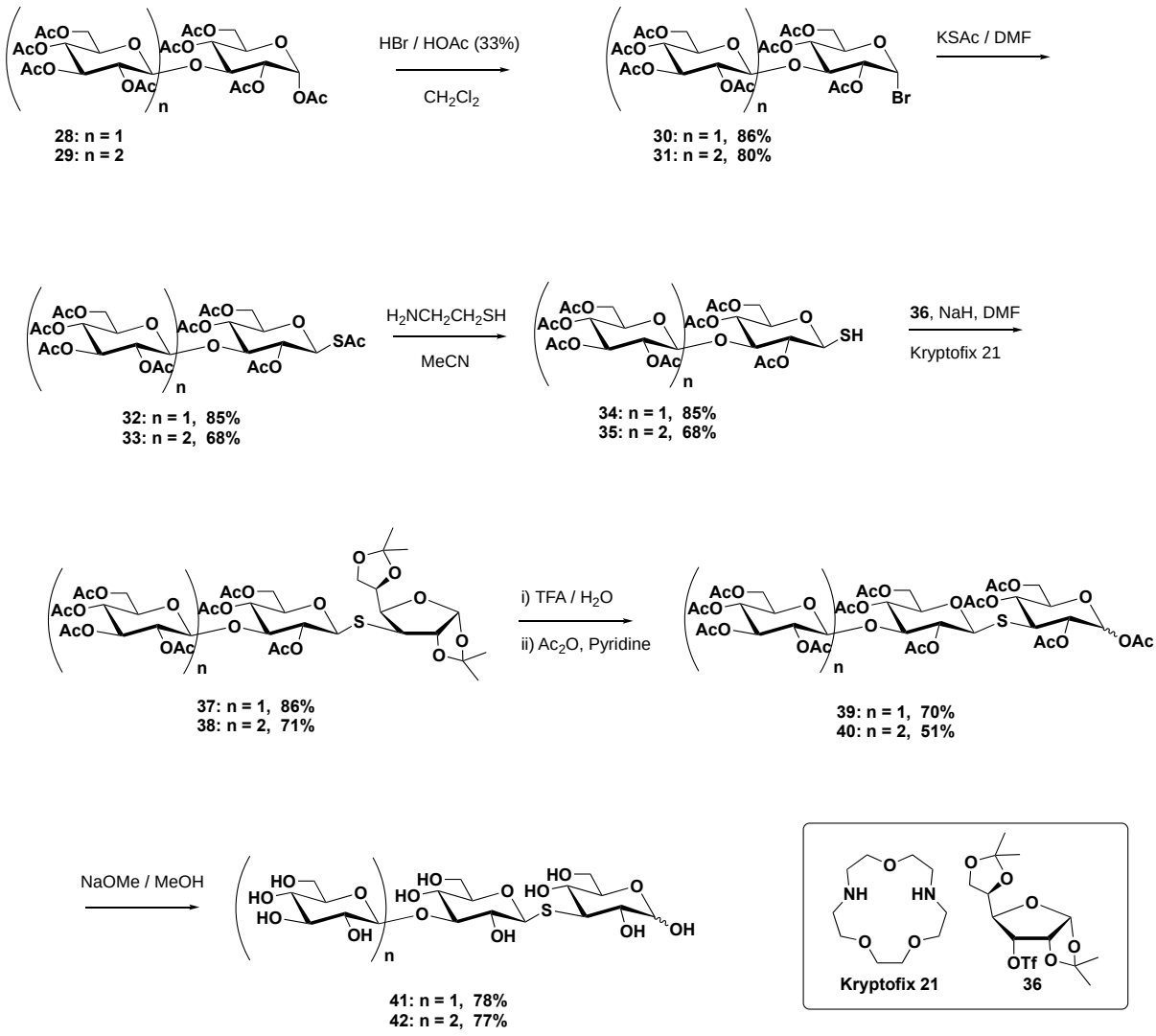



Figure 6. Chemical structure of oligo-β-(1 → 3)-D-glucans with thiolinkage

Thioglycoside, in which the glycosidic oxygen atom is replaced by sulfur, is known to be more stable to acidic or enzymatic hydrolysis. Moreover, this modulation has only minor impact on overall conformation. Based on these facts, the Vetvicka laboratory designed thioglycosidic-linked oligo-*β*-(1→3)-glucans families (**Figure 6**).[56-57] The synthesis started from peracetylated laminaribiose **28** and laminaritriose **29**. Treatment of the compound **28** and **29** with 33% HBr / HOAc afforded the anomeric bromide **30** and **31** in 86% and 80% yield

respectively. The anomeric bromide was substituted by thioacetate anion to give the β-glucopyranosyl thioacetate **32** and **33** which were subjected to selective deacylation to give the anomeric thiol **34** and **35** in 85% and 68% respectively. In the presence of the crown ether Kryptofix 21 and sodium hydride, anomeric thiol was activated to replace the triflate group of 3-*O*-trifluoromethanesulfonyl-1,2;5,6-di-*O*-isopropylidene-α-D-allofuranose. Compound **34** and **35** were coupled with triflate **36** to give the disaccharide intermediates **37** and **38** in 86% and 71% respectively. After acidic hydrolysis, acetylation and Zemplén deacetylation, the intermediates **37** and **38** were deprotected and acetylated to give trisaccharide **39** and tetrasaccharide **40** in 70% and 51% respectively. Final Zemplén deacetylation and Sephadex G-25 gel purification gave the thio-linked β-(1 → 3)-D-glucan mimetics **41** and **42** (**Scheme 8**).




Scheme 8. Synthesis of oligo-β-(1 → 3)-D-glucans with thio-linkage

The same protocol was applied to synthesize trimeric mimetic **43** and tetrameric mimetic **44**. These four compounds were evaluated by Vetvicka laboratory. First, the phagocytic abilities were tested, including their effect on stimulation of peritoneal macrophages and peritoneal blood neutrophils and monocytes. A strong immunostimlating effect was observed from compound **42, 43** and **44**. To determine if the samples influence cytokine production, they tested the production of IL-2 by splenocytes and the levels of IL-1β and TNF-α in peripheral blood. The compounds **41, 43** and **44** stimulated the production of tested cytokines and **44** is the most active mimetic. Finally, theses samples were assessed for

their potency in inhibiting colon CSC-mediated tumor formation and/or metastasis. It is noteworthy that the β-(1 → 3)-thioglucan **43** which has two thioglycosidic linkages, demonstrated good anti-cancer activity. Compound 43 significantly suppressed spheroid formation and proliferation of colon cancer stem-like cells from human colon adenocarcinoma, more effective than the natural laminarin. These promising results indicate the presence of sulfur atom is beneficial for biological activity.

**3.00 Sulfur in medicinal chemistry**

Sulfur is the 5$^{th}$ most abundant element on earth. Our body consists 0.25% of sulfur, which is crucial for many biological processes.[58] Two amino acids, cysteine and methionine and two vitamins (biotin and thiamine) are organosulfur compounds. The disulfide bond is a common linkage in proteins that is crucial to the protein structures. Sulfur-aromatic interactions were critical in chemical and biological process with many examples in the context of protein-protein and protein-ligand interactions.[59] The preferential conformation of a bridged oxathiolane compound gave the evidence for sulfur-aromatic interactions.[60] Sulfur's history in drugs dates to ancient Egypt, where people used sulfur as an antiseptic. Today, the organosulfur compounds have extraordinary impact on medicinal chemistry. In the U.S. 40% and 25% of the top 20 drugs by retail sales and prescription respectively contain sulfur.[61] In addition, a survey of the top 200 brand name drugs by total U.S. prescriptions in 2011 revealed that 24.8% of drugs contain sulfur[61]. Accoridng to statistics (**Figure 7**), over the 12 major diseases categories, sulfur containing drugs is 50% more than fluorine in the Anti-infectives category. In Cardiovascular and Musculoskeletal categories, sulfur containing drugs comprises 60% more than the fluorine containing drugs.[61]

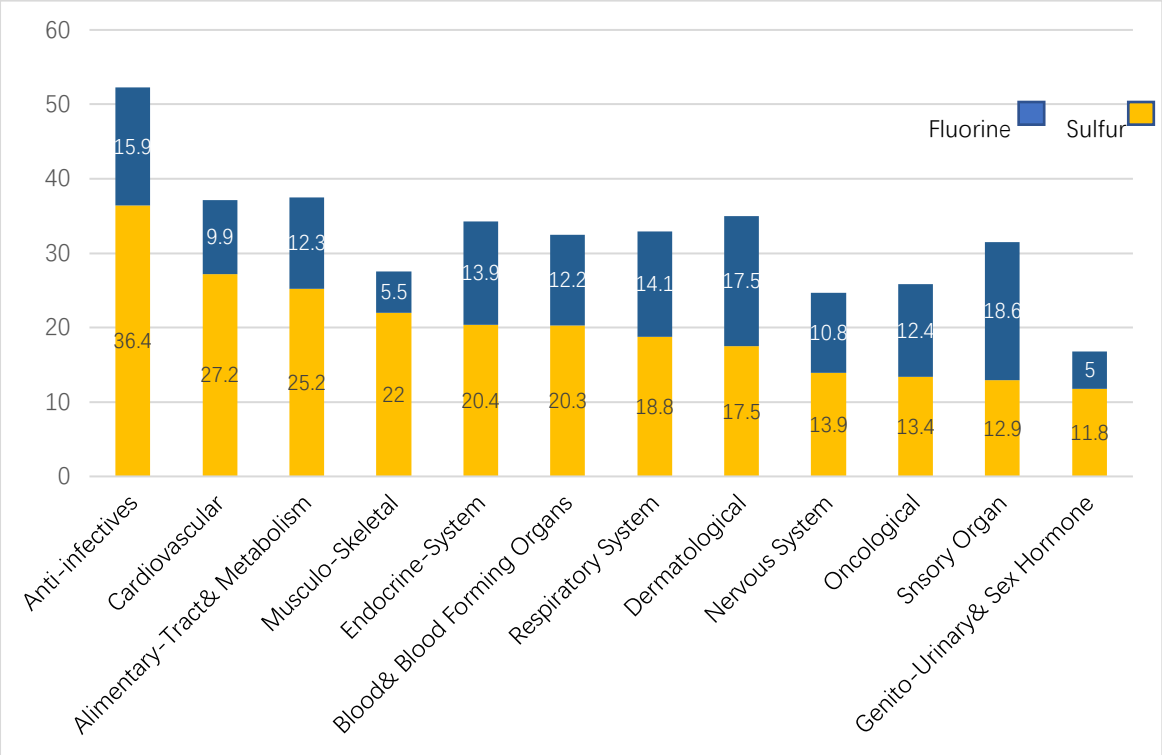



Figure 7. Percentage composition of sulfur-containg and fluorine-containing pharmaceuticals that comprise each of the 12 representative disease categories

**4.00 Conclusion**

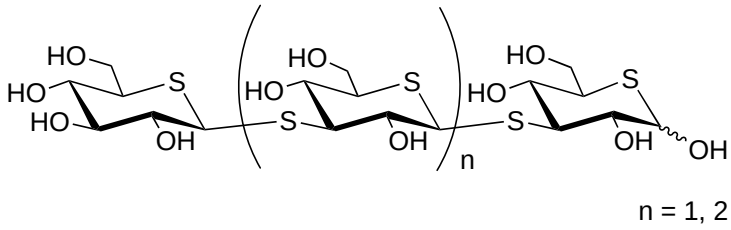



Figure 8. Chemical structure of oligomeric $\beta$-(3 → 5)-dithio-D-glucan mimetics

Over the past few years, great effort was spent on understanding and improving the immunostimulating effect of β-(1 → 3)-D-glucan.[62] It was demonstrated that laminaripentaose, a five-membered oligo-β-(1→3)-D-glucan, could also trigger immunostimulating activity equivalent to that of laminarine.[33] X-Ray crystallographic studies of recombinant Dectin-1 have revealed a hydrophobic pocket lined by the side chains of Trp 221 and His 223.[25] STD-NMR experiments revealed that laminarin binding through vander waals interaction of the α-face of terminal pyranose rings (at both the reducing and nonreducing ends) with the Trp 221

and His 223 of the Dectin-1 binding domain.[27, 63] Crich and coworkers prepared hydroxylamine analogues of β-glucan. Interestingly, although the oligomer is short, they have very good biological activity. The good activity of these small analogues is possibly because of the increased hydrophobicity of the synthetic 2-deoxy hydroxylamine mimetics. These studies and preliminary results proved the concept of principle that the increased hydrophobicity of the β-glucans increased their binding affinity to CR3 and Dectin-1. More importantly, hydroxylamine analogues of β-glucan provide an unprecedented example using hydroxylamine N-O bond mimic glycosidic C-O bond, the fact that low barrier to inversion of hydroxylamine nitrogen lone pair helps it mimic both α and β glycosidic bond. Based on these studies, we hypothesized that replacement of both the exocyclic oxygen and endocyclic oxygen atom by more lipophilic sulfur can increase the noncovalent interactions between β-(1 → 3)-D-glucan mimetics and the receptors. Second, the modification with endocyclic and exocyclic sulfur can resist the acidic and enzymatic hydrolysis to the largest extent. It is very likely that these modifications will give excellent β-(1 → 3)-D-glucan mimetics. Therefore, we designed the oligo-3,5-dithio-*β*-D-glucopyranoside as β-(1 → 3)-D-glucan mimetics (**Figure 8**).

## CHAPTER 2: DESIGN AND SYNTHESIS OF OLIGO-(3,5-DITHIO-*β*-D-GLUCOPYRANOSIDES) AS *β*-(1 → 3)-D-GLUCAN MIMETICS

### 1.00 Introduction

R. Schmidt's review[64] about thioglycoside synthesis provide many examples of thiooligoglycoside synthesis. There are three different methods to synthesize thioglycosides: 1) Base promoted thioglycosylation; 2) Anomeric thiol alkylation; 3) Acid catalyzed thioglycosylation.

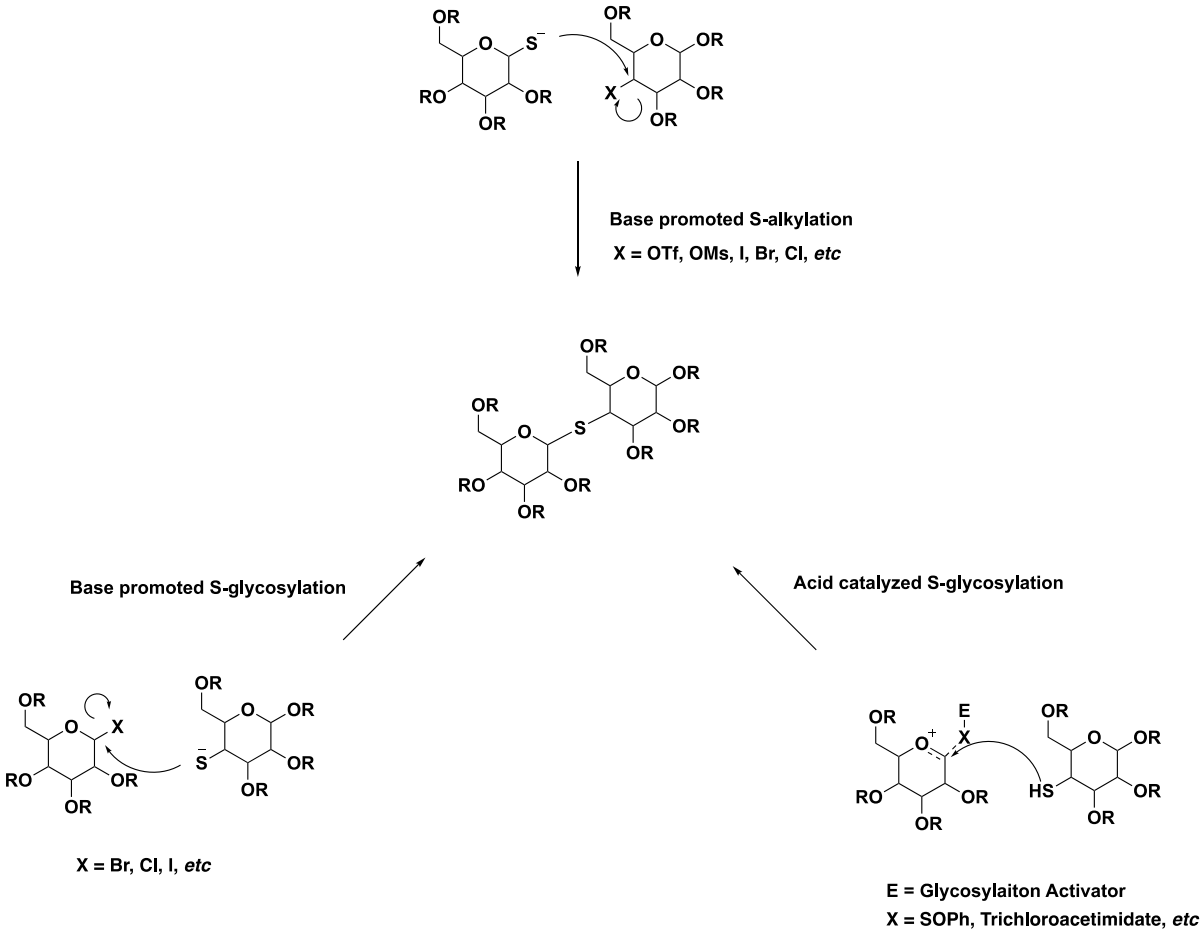
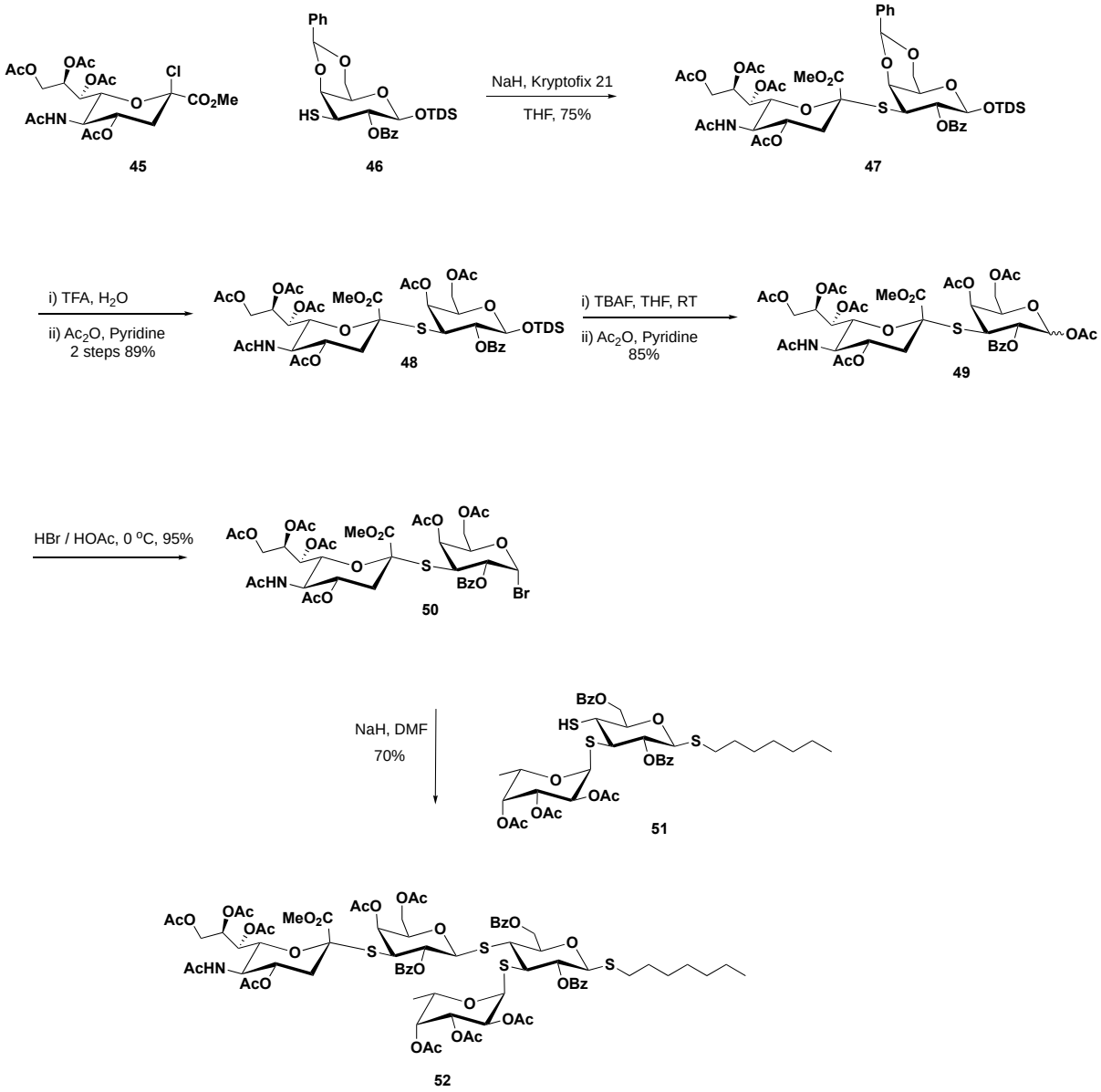



Figure 9. Illustration of 3 methods to build up thio-linked oligosaccharide

### 1.10 Example of base promoted S-glycosylation: S-analogue of Sialyl Lewis X synthesis

Sialyl Lewis X is a tetrasaccharide carbohydrate that is usually attached to *O*-glycans on the cell surface. Schmidt and co-workers[65] performed base promoted S-glycosylation to

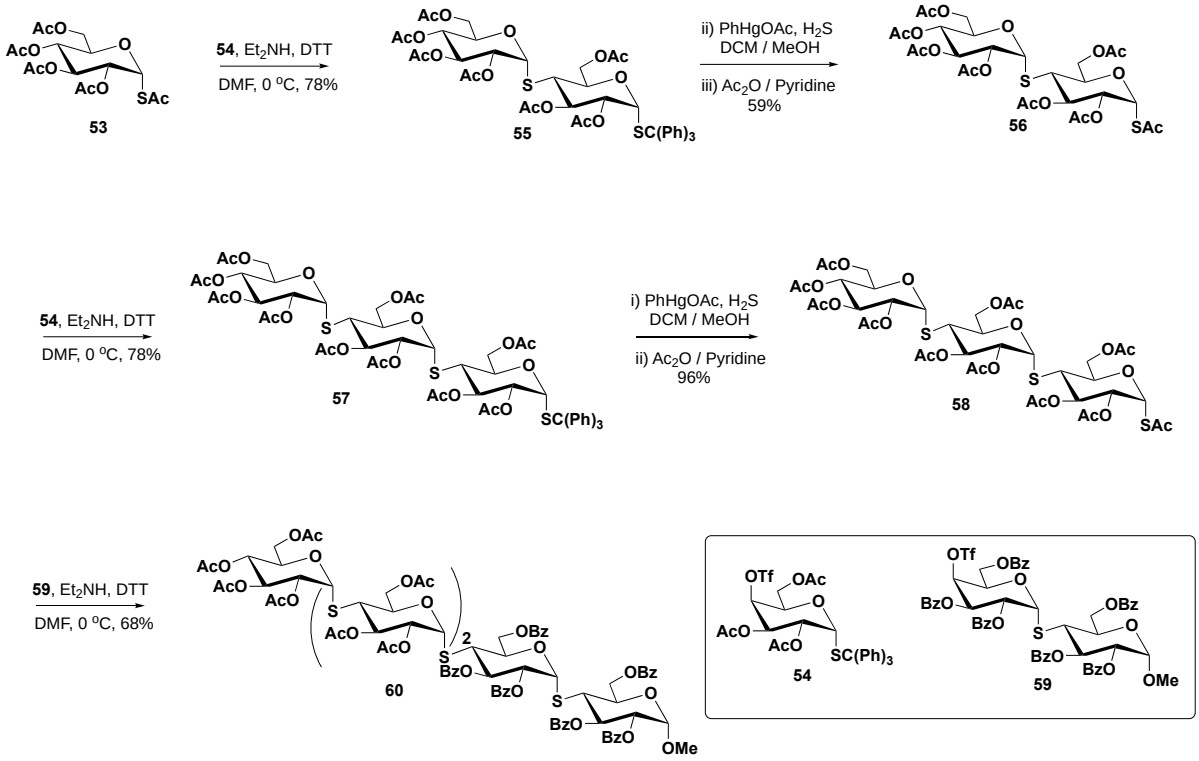
synthesize Sialyl Lewis X thio-analogue. In the presence of Kryptofix 21 in THF, the thiol of **46** substituted the anomeric chloride of compound **45** to give thioglycoside **47** in 75% yield. The anomeric siloxyl group was deprotected and acetylated to give compound **49** in 85% yield and the anomeric carbon of disaccharide **49** was brominated to give compound **50** in 95% yield. The anomeric bromide of **50** was substituted by thiol of **51** to give tetrasaccharide **52** in 70% yield.




Scheme 9. Synthesis of the S-analogue of Sialyl Lewis X via base promoted S-glycosylation

**1.20 Example of base promoted anomeric thiol alkylation: synthesis of 4-**

**thiomaltooligosaccharide**



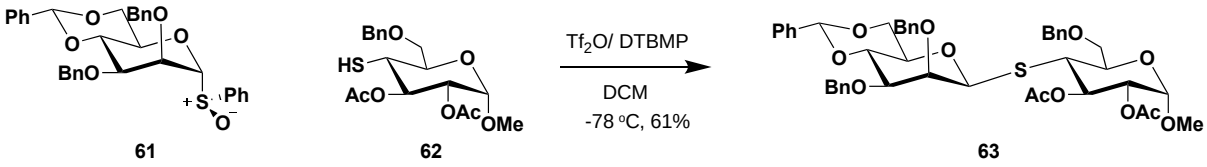

Scheme 10. The convergent synthesis of 4-thiomaltooligosaccharide

Driguez and co-workers[66]synthesized the 4-thiomaltopentaoside by anomeric thiol alkylation. The synthetic route is an iterative process and the α-(1→4)-S-linkage was built up by $SN_2$ substitution of C-4 triflate from anomeric thiol. Coupling of compound **53** with compound **54** afforded the triphenylmethyl 1,4-dithio-α-maltoside **55** in 78% yield. The 1-S-triphenylmethyl group was deprotected in a two steps process. First, S-trityl group was cleaved by PhHgOAc in the presence of hydrogen sulfide. Then, the anomeric thiol was converted to S-acetyl to give compound **56** in 59% yield. Compound **57** was coupled with triflate **54** to give the trisaccharide **57** in 78% yield. The same two steps procedure to converted the S-triphenylmethyl into S-acetyl group was applied to **57** afforded **58** in 96% yield. In a convergent approach, coupling of **58** with disaccharide triflate **59** afforded thio-malto-pentasaccharide **60** in 68% yield.

### 1.30 Examples of acid catalyzed S-glycosylation

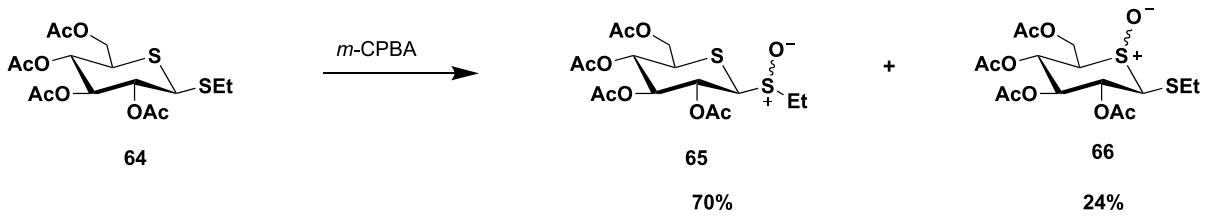
### 1.31 $Tf_2O$ / DTBMP promoted S-glycosylation using glucosyl sulfoxide donor

Crich lab[67] developed the β-selective glycosylation in which anomeric S-phenyl sulfoxide acts as glycosylation donor and $Tf_2O$ / DTBMP as the activator. They applied this method in S-glycosylation. The sulfoxide donor **61** was activated by $Tf_2O$ / DTBMP at -78 °C to 0 °C, the generated mannosyl triflate intermediate was substituted by thiol acceptor **62** to give disaccharide **63** with β thio-glycosidic linkage (**Scheme 11**).




Scheme 11. β-selective S-glycosylation of mannosyl sulfoxide **61** and thiol acceptor **62**.

Application of this method to 5-thio-glucose was not reported because of the lack of selectivity in the oxidization of exocyclic and endocyclic sulfur (**Scheme 12**). [68]

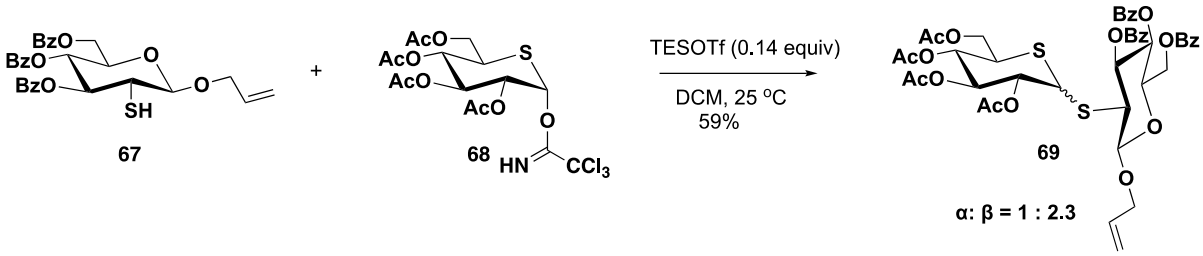



Scheme 12. Oxidation of ethyl β-1,5-dithioglucopyranosides with *m*-CPBA.

### 1.32 TESOTf promoted S-glycosylation using glucopyranosyl trichloroacetimidate donor

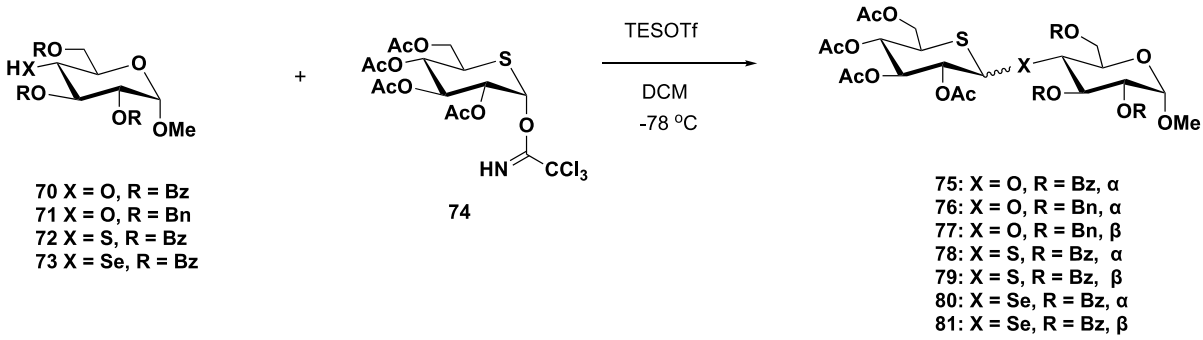
S-glycosylation using acid catalyzed trichloroacetimidate donor and thiol acceptor was studied by Pinto and co-workers.[69] In the glycosylation of the thiol **67** with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl trichloroacetimidate **68**. When 0.14 equivalent of the catalyst

TESOTf were added, both glycosylation product **69** were formed in 59% yield with an α and β ratio of 1: 2.3 (**Scheme 13**).




Scheme 13. TESOTf catalyzed S-glycosylation with 5-thio-glucopyranosyl trichloroacetimidate **68** as the donor compound **67** as the acceptor.

They also studied the TESOTf promoted glycosylation of 5-thioglucopyranosyl trichloroacetmidate **74** with 4-OH, 4-SH and 4-SeH groups of glucopyranoside acceptors **70-73** (**Scheme 14**) . [70]




Scheme 14. TESOTf promoted S-glycosylation using 5-thio-glucopyranosyl trichloroacetimidate **74** as the donor with glucose with 4-OH, 4-SH and 4-SeH (**70-73**) as the acceptors.

The glycosylation of acceptor **70** with glucopyranosyl trichloroacetimidate **74** activated by triethylsilyl triflate (donor : acceptor : promotor = 1:2:0.1) afforded exclusively α-disaccharide **75** in 87% yield (**Table 4, Entry 1**). When glycosylation was performed with the same substrate but different ratio (donor : acceptor : promotor = 2:1:0.2) at -50 °C, the glycosylation gave the orthoester in 88% yield (**Table 4, Entry 2**). The glycosylation with more reactive benzylated acceptor **71** gave a 1:1 mixture of α and β disaccharide **76** and **77**

(**Table 4, Entry 3**). The glycosylation with the acceptor 4-SH-glucopyranoside **72** afforded mainly α-disaccharide **78** in 53% and minor β-disaccharide **79** was isolated in only 1.5% yield (**Table 4, Entry 4**). The glycosylation with the acceptor 4-selenol-glucopyranoside **73** gave α-disaccharide **80** in 46% and β-disaccharide **81** in 11% yield (**Table 4, Entry 5**).

Table 4. Glycosylation reaction of 5-thio-glucopyranosyl trichloroacetimidate

[a] Donor: Acceptor: TESOTf

| Entry | Acceptor | Molar ratio[a] | Reaction condition | Products and yield |
|---|---|---|---|---|
| 1 | 70 | 1:2:0.1 | -78 °C 1 h then RT 1 h | 75, 87% |
| 2 | 70 | 2:1:0.2 | -78 °C 1 h then -50°C 1.5 h | orthoester, 88% |
| 3 | 71 | 1:2:0.1 | -78 °C 1 h then RT 1 h | 76, 45%<br>77, 45% |
| 4 | 72 | 1:2:0.25 | -78 °C to -50 °C 1 h | 78, 53%<br>79, 1.5% |
| 5 | 73 | 1:2:0.2 | -78 °C 10 min then RT 1h | 80, 46%<br>81, 11% |

**2.00 Synthesis of 3,5-dithio-glucopyranose**

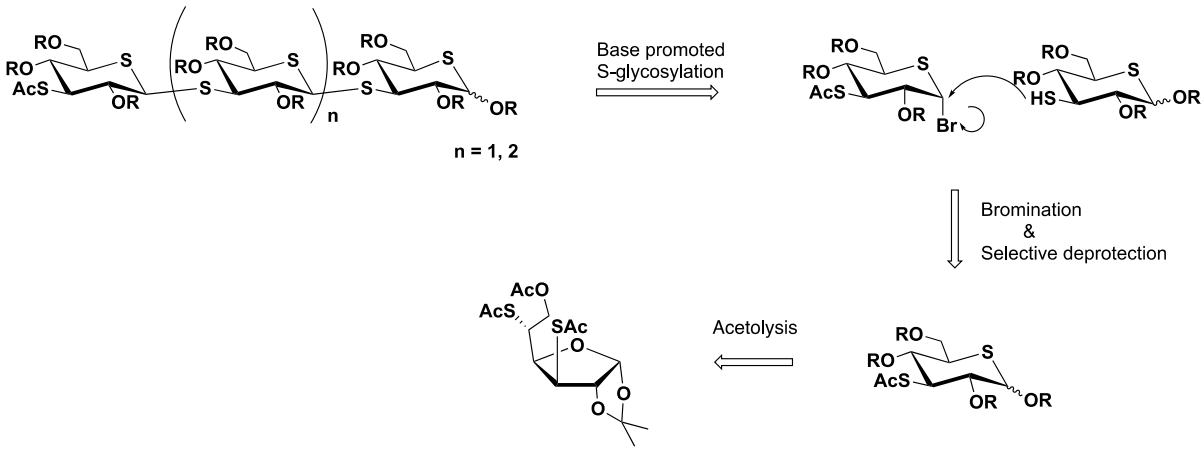
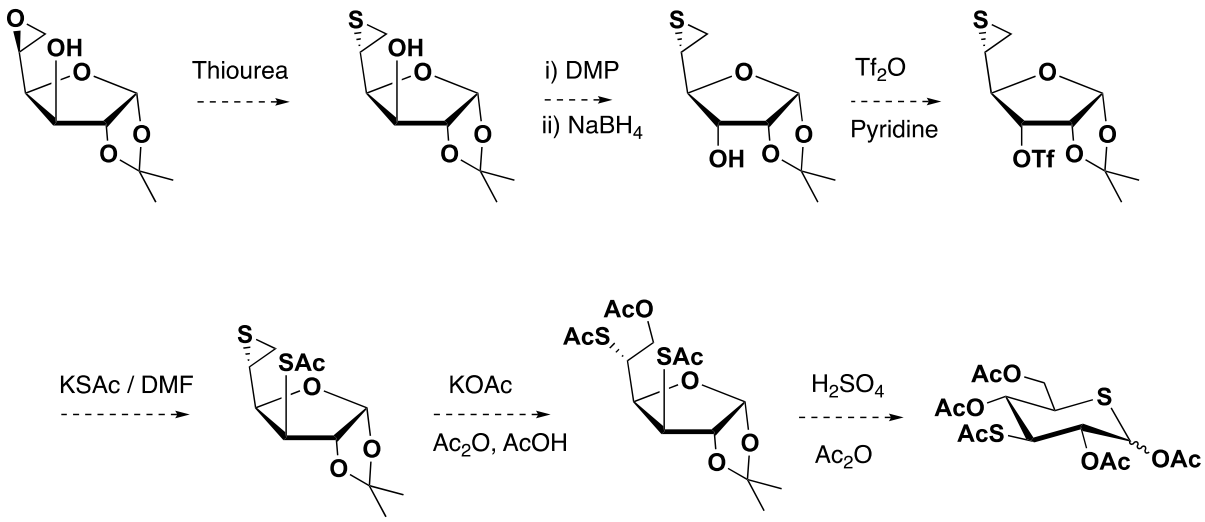



Figure 10. Retrosynthetic analysis of oligo-3,5-dithio-β-D-glucopyranoside.

At first, we planned to synthesize the oligo-3,5-dithio-β-D-glucopyranoside by base promoted S-glycosylation between the acceptor 3,5-dithioglucopyranoside with free 3-SH and 3,5-dithio-glucopyranosyl bromide as the donor (**Figure 10**). According to Whistler *et al*,[71] 5-thio-glucopyranose can be prepared from acetolysis of 5-thio-glucofuranose. Therefore, we decided to synthesize 3,5-dithio-1,2-*O*-isopropylidene-glucofuranose as the starting material. The proposed synthesis started from 1,2-*O*-isopropylidene-5,6-didexoy-5,6-epithio-α-D-allofuranose, which can be prepared in large scale from 1,2;5,6-*O*-diisopropylidene-α-D-glucofuranose.[72] S-acetyl group could be introduced at C-3 via nucleophlic substitution of 3-*O*-trifluoromethanesulfonyl of compound 1,2-*O*-isopropylidene-5,6-didexoy-5,6-epithio-α-D-allofuranose to give 3-*S*-acetyl-1,2-*O*-isopropylidene-5,6-dideoxy-5,6-dithio-α-D-glucofuranose. Finally, after ring opening of episulfide by acetate, the acidic acetolysis would give the 3,5-dithio-glucopyranose (**Scheme 15**).

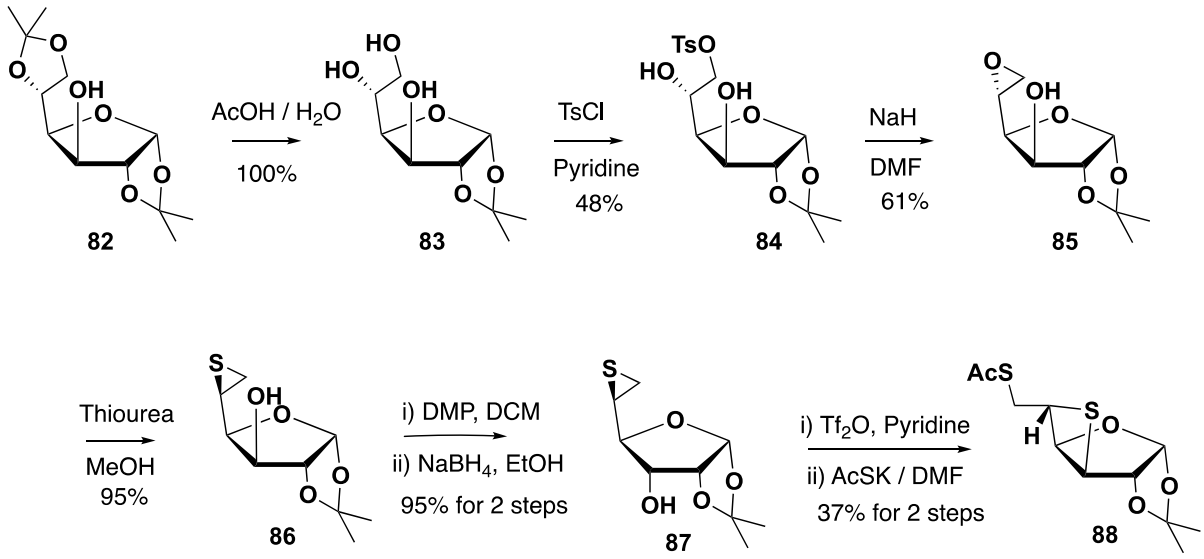
### 2.10 Synthesis of 3,5-dithio-α-D-glucofuranose

### 2.11 Synthesis of 3,5-dithio-α-D-glucofuranose by nucleophilic substitution on 3-*O*-trifluoromethanesulfonyl group of 1,2-*O*-isopropylidene-5,6-dideoxy-5,6-epithio-α-L-talofuranose




Scheme 15**.** Proposed synthesis of 3,5-dithio-glucopyranose

For the shorter synthetic route, we decided to synthesize 1,2-*O*-isopropylidene-5,6-dideoxy-5,6-epithio-β-L-talofuranose **87** as a practice. Starting from 1,2;5,6-*O*-diisopropylidene-α-D-glucofuranose **82**. The 5,6-O-isopropylidene group was selectively removed by acidic hydrolysis and the 6-OH was selectively tosylated to give 6-*O*-*p*-toluenesulfonyl-1,2-*O*-isopropylidene-glucofuranose in 48% yield. Subsequent deprotonation by sodium hydride initiated intramolecular substitution generated the 5,6-anhydro-1,2-*O*-isopropylidene-α-D-glucofuranose **85** in 61% yield. The 5,6-epoxide of compound **85** was converted to the 5,6-epithio compound **86** by treatment with thiourea. Conversion of the 3-hydroxyl group by Dess-Martin oxidation and sodium borohydride reduction gave 1,2-*O*-isopropylidene-5,6-epithio-α-L-talofuranose **88** in 95% yield.




Scheme 16. Attempted synthesis of 3,5-dithio-glucofuranose from compound **87**

With the compound **87** in hand, we tried to perform a nucleophilic substitution the triflate intermediate. Unexpectedly, the reaction afforded the rearrangement product **88** (**Scheme 16**). The possible mechanism for the formation of compound **88** is the intramolecular nucleophilic replacement generated the episulfonium intermediate, which was substituted by the thioacetate at C-6 to give the rearrangement product. (**Figure 11**).

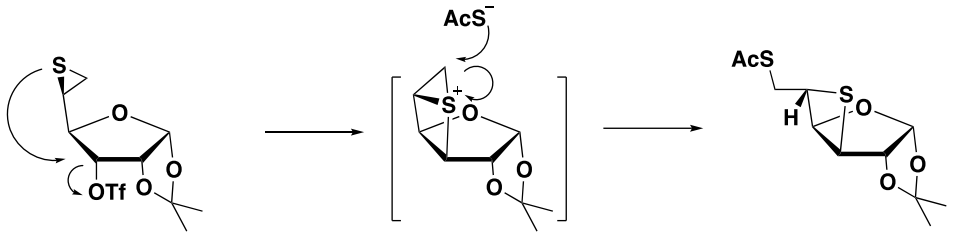
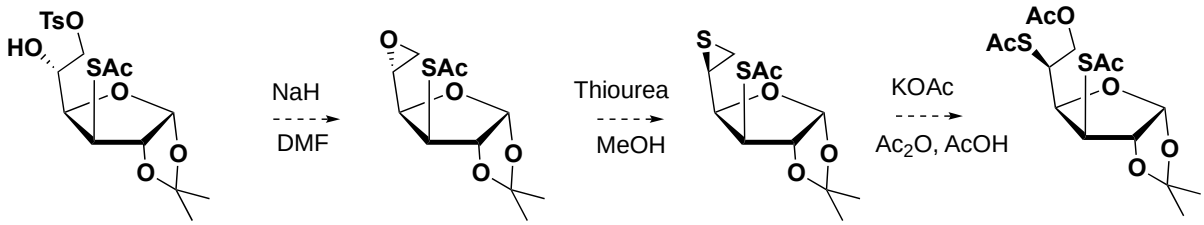


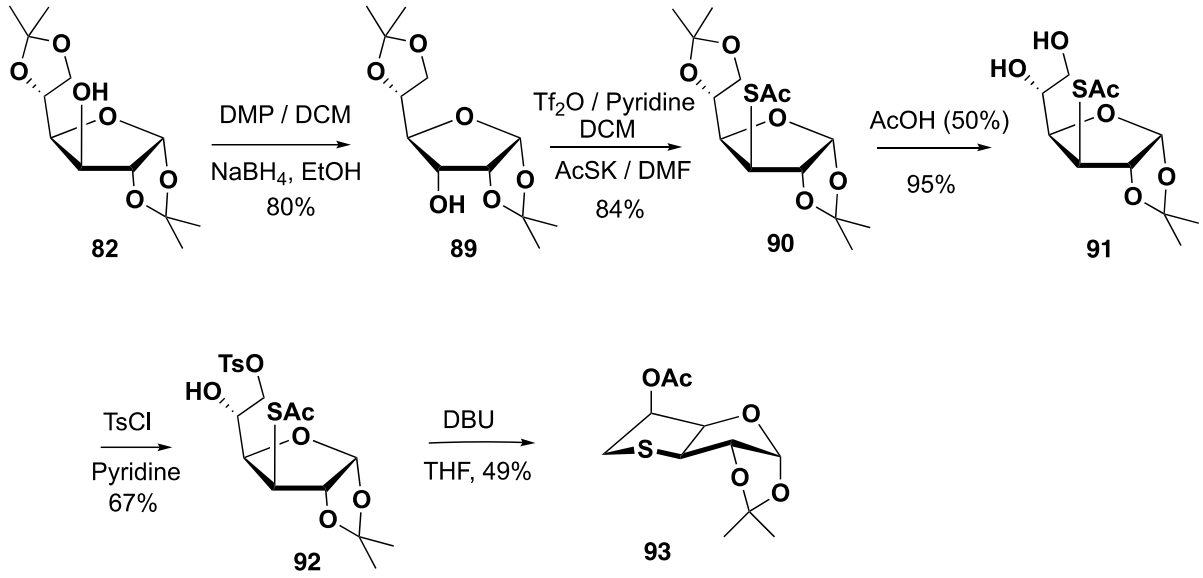
Figure 11. Proposed mechanism for rearrangement product **88** formation.

**2.12 Synthesis of 3,5-dithio-glucofuranose by epoxide-episulfide transformation of 3-*S*-acetyl-1, 2-*O*-isopropylidene-5, 6-anhydro-α-D-glucofuranose.**



Scheme 17. Proposed synthesis of 3,5-dithio-glucofuranose by epoxide-episulfide transformation

The first rearrangement product **88** taught us a lesson about the good nucleophilicity of epislfide sulfur. To avoid intramolecular nucleophilic substitution, in another synthetic route, we decided to install the first thioacetate at C-3 before the second thioacetate was introduced at C-5 (**Scheme 17**).




Scheme 18. The attempted epoxide formation of compound **92**.

The synthesis started from 1,2;5,6-di-*O*-isopropylidene-α-D-glucofuranose. After Dess-Martin oxidation and sodium borohydride reduction, the 3-OH underwent inversion to give 1,2;5,6-di-*O*-isopropylidene-α-D-allofuranose **89** in 80% yield. 3-OH of compound **89** was triflated followed by thioacetate displacement to give 3-*S*-acetyl-1,2;5,6-di-*O*-isopropylidene-α-D-glucofuranose **90** in 84% yield. After the 5,6-isopropylidene group was selectively removed with 50% acetic acid, 6-OH was tosylated to give compound **92** in 67% yield. When we were treating compound **92** with DBU to prepare 5,6-epoxide, the rearrangement product **93** was generated (**Scheme 18**). The structure was confirmed by comparing the spectral data with that of the known compound.[73] The possible mechanism is

that the acetyl migrated from S-acetyl to the neighboring hydroxyl and the thiolate cyclized to give the rearrangment product **93** (**Figure 12**).

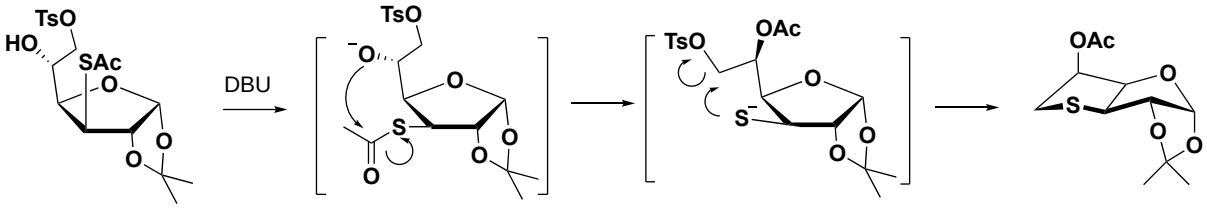
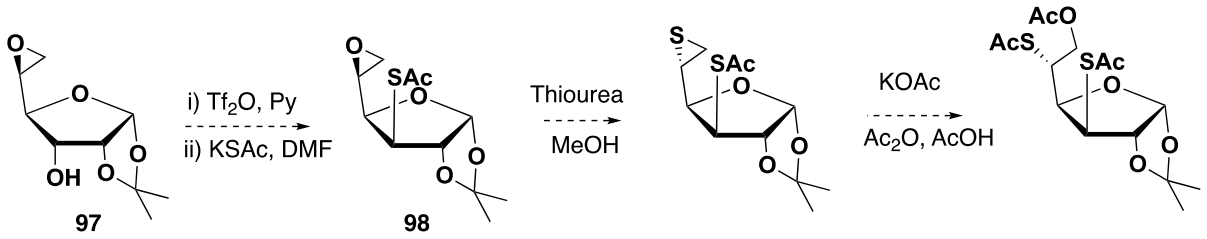



Figure 12. Proposed mechanism for the rearrangement product **93** formation

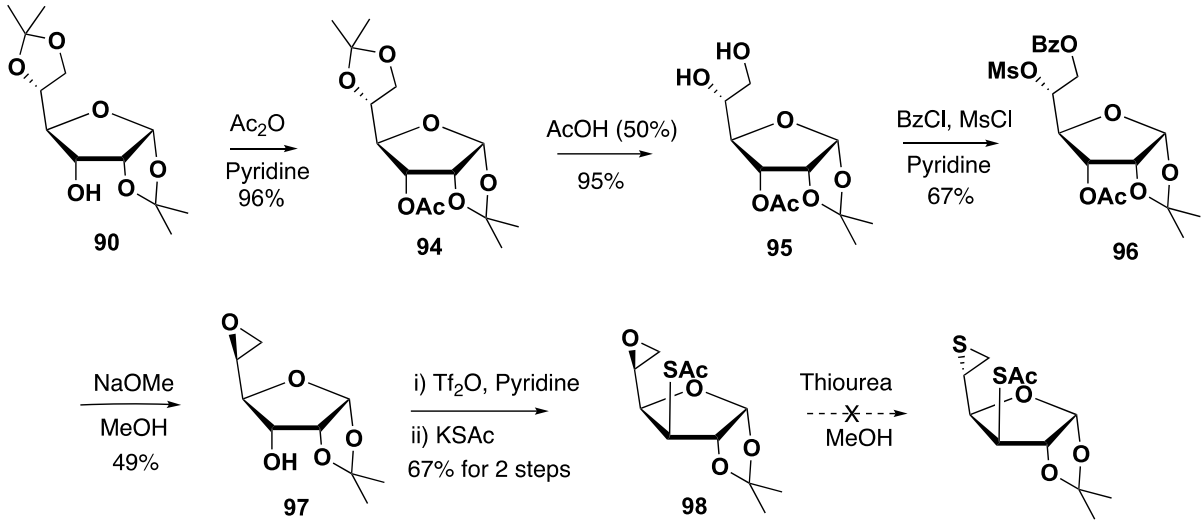
## 2.13 Attempted synthesis of 3,5-dithio-glucofuranose by nucleophilic substitution of the trifluoromethanesulfonyl group of 3-*O*-trifluoromethanesulfonyl-1,2-*O*-isopropylidene-5,6-anhydro-α-D-allofuranose




Scheme 19. Proposed synthesis of compound **98** by nucleophilic substitution of 3-*O*-trifluoromethanesulfonyl group of compound **97**.

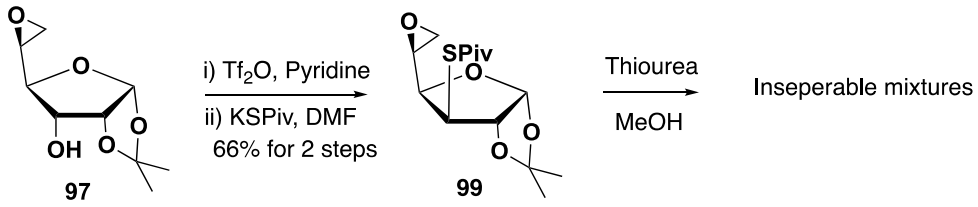
To avoid the rearrangement, we proposed a new synthetic route in which the thioacetate was introduced after the epoxide formation (**Scheme 19**). We started the synthesis from 1,2;5,6-di-*O*-isopropylidene-α-D-allofuranose. After acetylation of the 3-OH and selectively acidic hydrolysis of 5,6-*O*-isopropylidene, we prepared compound **94** in 95% yield. Primary alcohol benzoylationg and secondary alcohol sulfonylation gave the desired product **95** in 67% yield. After treatment with sodium methoxide, the epoxide formation afforded the 5,6-anhydro-1,2-*O*-isopropylidene-α-D-glucofuranose **96** in 49% yield. After the 3-OH was converted to the triflate, thioacetate was introduced by slowly adding thioacetate / DMF solution at 0°C to give the desired product 3-S-acetyl-5,6-anhydro-1,2-*O*-isopropylidene-α-D-glucofuranose **98** in 67% yield. When we performed the epoxide episulfide transformation on compound **98**, the

reaction gave complex mixtures from which no anticipated product was identified (**Scheme 20**).




Scheme 20. The attempted epoxide episulfide transformation of compound **98**

We installed the S-pivalyl group to prevent the transesterification of the S-acetyl group. The S-pivalyl group was installed by a $SN_2$ substitution of triflate by potassium S-pivalate to give 3-*S*-pivalyl-1,2-*O*-isopropylidene-5,6-anhydro-α-D-glucofuranose **99** in 66% yield. When we performed the epoxide-episulfide transformation of the compound **99**, the reaction gave the product together with inseparable rearrangement prodcut**.** This result indicated that the S-pivalyl was not able to completely prevent transesterification (**Scheme 21**).




Scheme 21. The attempted epoxide episulfide transformation of compound **99**

**2.2 Synthesis of the 3,5-dithio-glucopyranose by nucleophilic substitution of the C-3 triflate of 5-thio-allopyranose**

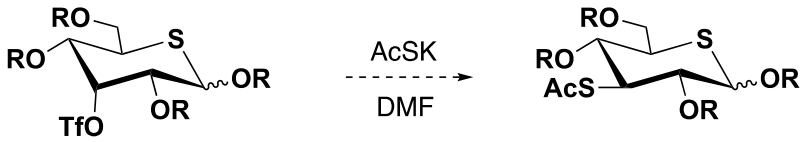
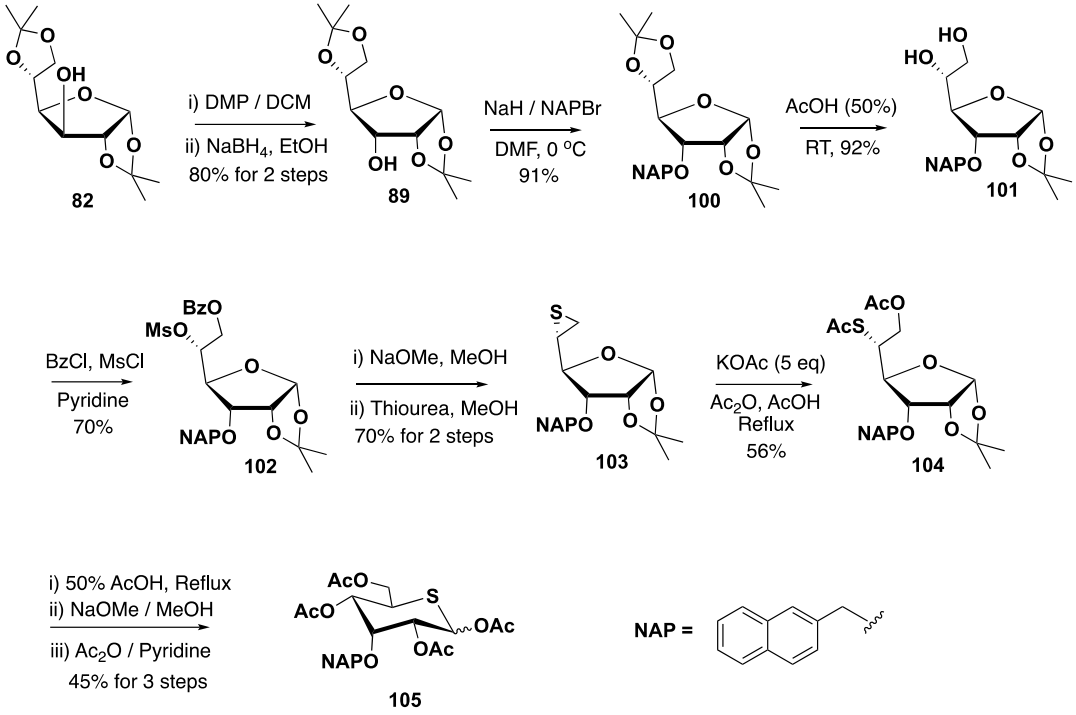


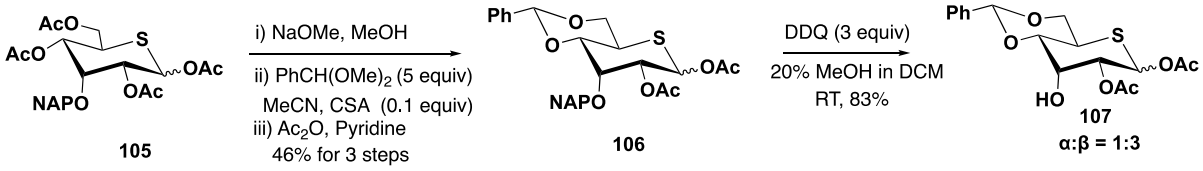

Figure 13. Proposed synthesis of the 3,5-dithio-glucopyranose by nucleophilic substitution of the C-3 triflate of 5-thio-pyranose

We decided to synthesize the 5-thio-allopyranose with an axial triflate at C-3 to install the thioacetate (**Figure 13**). The synthesis started from 1,2;5,6-di-*O*-isopropylidene-α-D-allofuranose. The installation of (2-naphthyl)methyl protecting group at 3-OH gave the product **100** in 91% yield. After acidic hydrolysis of the 5,6-*O*-isopropylidene group, the primary hydroxyl was benzoylated and secondary hydroxyl was mesylated to give the product **102** in 70% yield. Treatment with sodium methoxide in methanol, neutralization with Amberlyst IR-120 resin, and subsequent heating with thiourea afforded the 5,6-dideoxy-5,6-epithio product **103** in 70% yield. Compound **103** was refluxed with potassium acetate in the mixture of acetic anhydride and acetic acid to give the ring opening product **104** in 56% yield. With compound **104**, treatment with 60% acqueous trifluoroacetic acid gave a mixture of deprotection products including small amount of cyclized product. We followed a two steps protocol of Driguez.[74] First, compound **103** was refluxed in 50% aqueous acetic acid to remove the 1,2-*O*-isopropylidene group. Then, the hemiacetal intermediate was treated with sodium methoxide and cyclized to give 3-*O*-(2-naphthyl)methyl-5-thio-α,β-D-allopyranose. The crude mixture was subjected to acetylation to give the desired product **105** in 45% yield (**Scheme 22**).



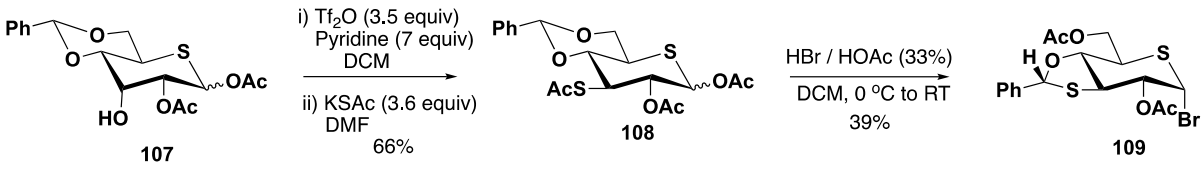

Scheme 22. Synthesis of compound **105**.

With the compound **105**, Zemplén deacetylation follwed by 4,6-*O*-benzylidene protection and acetylation give the 4,6-*O*-benzylidene protected product **106** in 46% yield. Removal of the 3-ONAP protecting group gave the product **107** as an anomeric mixture (α: β = 1: 3) (**Scheme 23**).



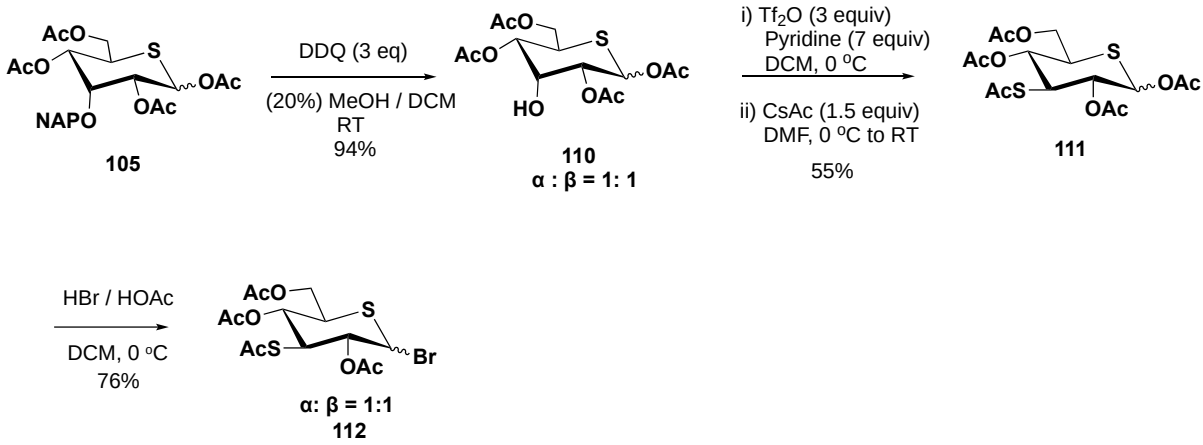

Scheme 23. The 4,6-*O*-benzylidene protection of compound **105** and selective deprotection of the NAP group.

With compound **107**, the installation of triflate group at C-3 followed by $SN_2$ substitution of the triflate using potassium thioacetate provided 3,5-dithio-glucopyranose product **108** in 66% yield (**Scheme 24**). Reaction of compound **108** with 33% hydrogen bromide in acetic acid gave the rearrangement product **109** in 39% (**Scheme 24**).




Scheme 24. The bromination reaction of compound **108**

Alternatively, we decided to use acetyl protecting group to proceed the synthesis. Oxidative deprotection of the 3-ONAP group of **105** afforded the product **110** as anomeric mixture (α: β = 1: 1.3). The axial 3-OH of compound **110** was triflated and substituted by thioacetate anion to give the acetyl protected 3,5-dithio-glucopyranose derivative **111** in 55% yield. The bromination reaction provided the desired glucopyranosyl bromide **112** as an anomeric mixture (α: β = 1:1) in 76% yield (**Scheme 25**).

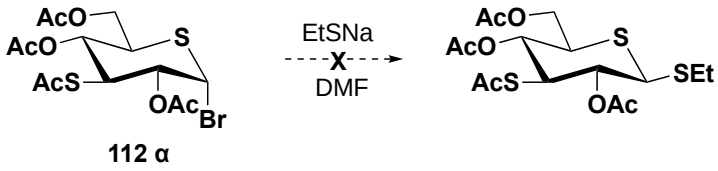



Scheme 25. Preparation of 3,5-dithio-glucopyranosyl bromide.

### 3.00 S-Glycosylation study

### 3.10 Base promoted $SN_2$ substitution of anomeric bromide by sodium ethanethiolate

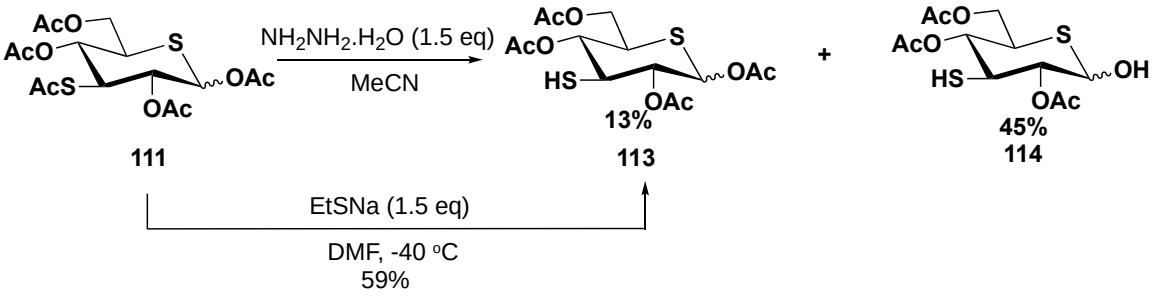
With the 3,5-dithio-glucosyl bromide **112** in hand, we performed the $SN_2$ substitution of anomeric bromide by ethane thiolate in DMF. The reaction did not give the desired product. (**Scheme 26**).




Scheme 26. Attempted sodium ethane thiolate substitution of 5-thio-glucopyranosyl bromide

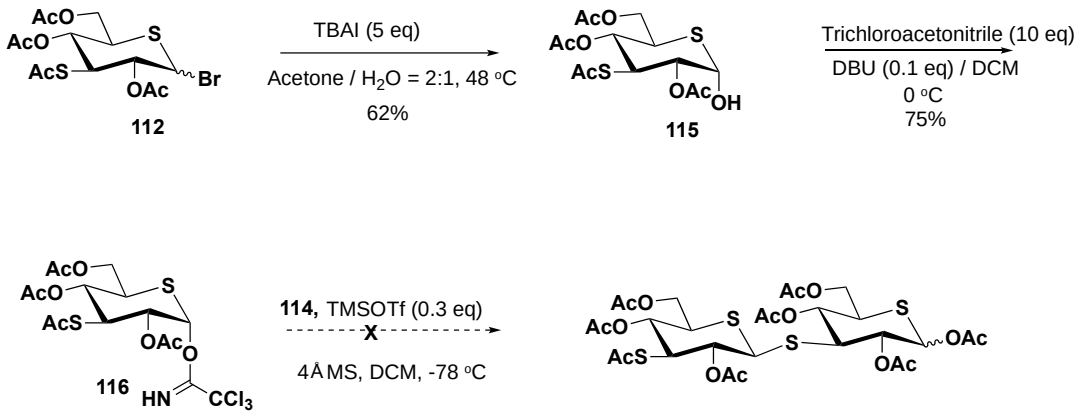
**3.20 Acid catalyzed S-glycosylation of 3,5-dithio-glucopyranosyl trichloroacetimidate**

Alternatively, we decided to try the acid catalyzed S-glycosylation to build up the thio-linked disaccharide. Selective S-acetyl deprotection was performed to prepare the glycosylation acceptor. However, when the compound **111** was treated with hydrazine monohydrate, the reaction gave both 3-*S*-acetyl deprotection product **113** and anomeric O-acetyl deprotection product **114** in 13% and 45% respectively. To improve the yield, the sodium ethanethiolate was added in DMF at -40 °C and 3-thioacetate was selectively removed to give 3-S-acetyl deprotected product **113** in 59% yield[75] (**Scheme 27**).




Scheme 27. Preparation of compound **113** as S-glycosylation acceptor

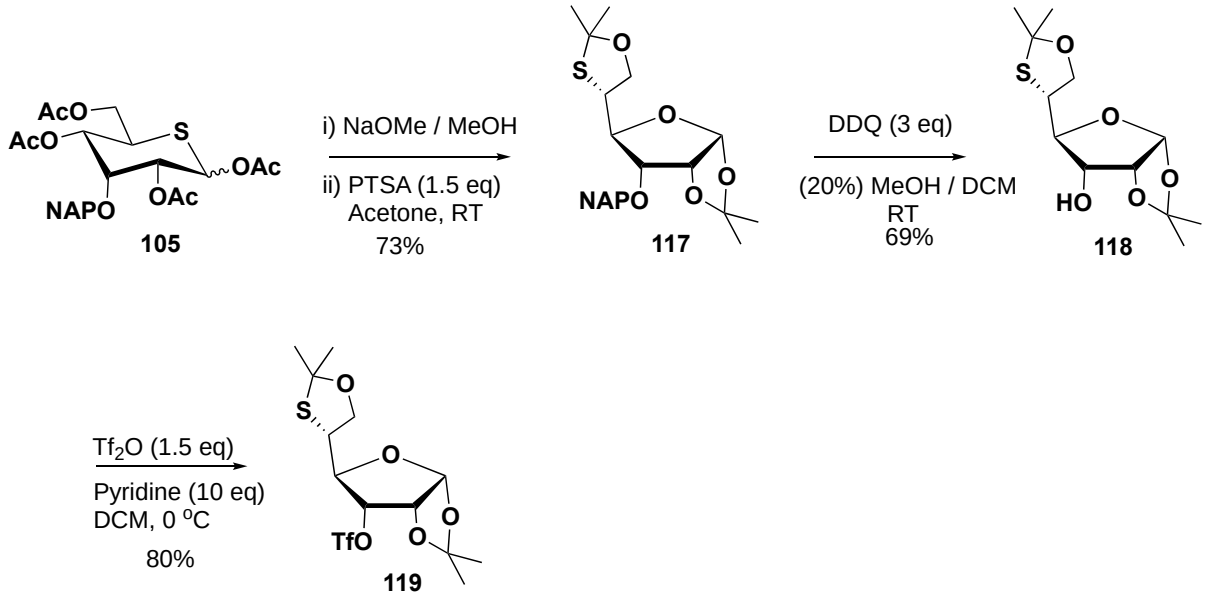
To avoid 3-S-acetyl deprotection, a two steps protocol was applied to prepare the glycosylation donor. First, the anomeric bromide of **112** was hydrolyzed in the presence of TBAI to give compound **115** in 62% yield. With compound **115**, the anomeric hydroxyl was treated with trichloroacetonitrile and catalytic amount of DBU to give the glucopyranosyl trichloroacetimidate **116** in 75% yield. With both donor and acceptor in hand, we performed the TMSOTf promoted glycosylation at -78 °C and it was not successful (**Scheme 28**).




Scheme 28**.** Synthesis of donor **116** and attempted TMSOTf catalyzed S-glycosylation

## 4.00 Oligo-(3,5-dithio-β-D-glucopyranosides) synthesis

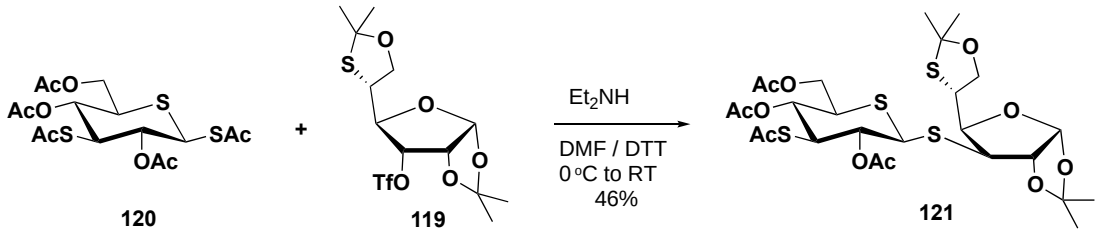
### 4.10 Synthesis of 3-*O*-trifluoromethanesulfonyl-1,2-*O*-isopropylidene-5-*S*,6-*O*-isopropylidene-5-thio-α-D-allofuranose and its application in the disaccharide synthesis




Scheme 29. Synthesis of compound **119**

Based on Ferrières's synthesis[57] and Hindsgaul's work[76] to synthesize thio-linked oligosaccharide, we designed the 3-*O*-trifluoromethanesulfonyl-1,2-*O*-isopropylidene-5-*S*,6-*O*-isopropylidene-5-thio-α-D-allofuranose **119** as the key building block for the oligomer synthesis. Compound **105** was deacetylated and stirred with *p*-toluenesulfonic acid in anhydrous acetone to give the diisopropylidene glucofuranose derivative **117** in 73% yield.[77]

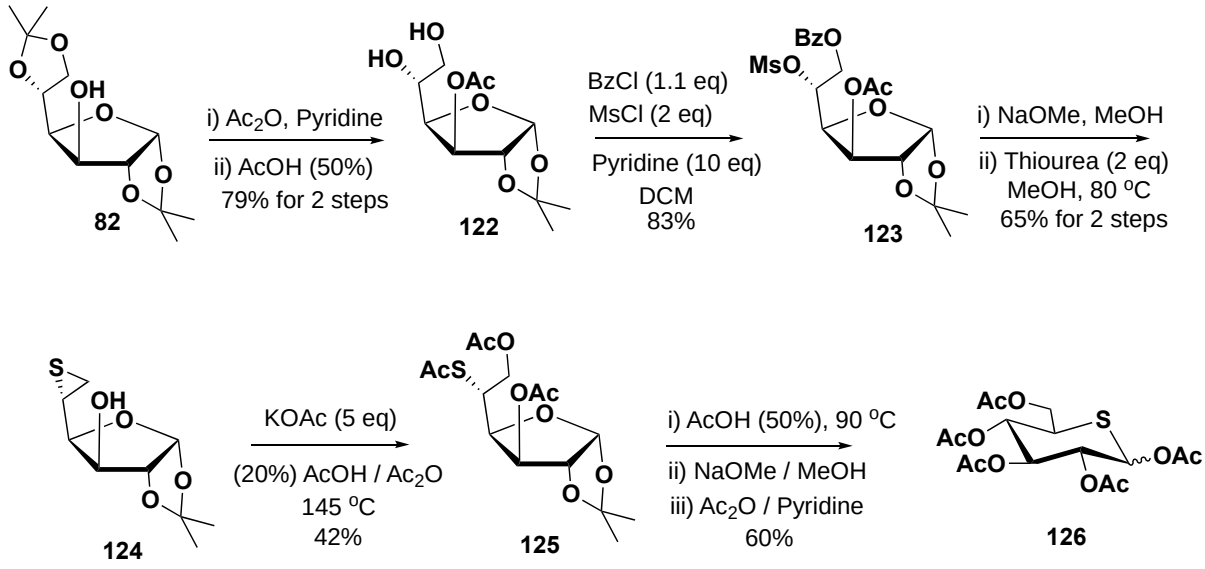
The 3-*O*-(2-naphthyl)methyl protecting group was removed by DDQ and the 3-OH was converted to the triflate to give the building block **119** in 80% yield (**Scheme 29**).




Scheme 30. The disaccharide synthesis by coupling reaction.

With the compound **119**, we performed the coupling reaction with the glucopyranosyl thioacetate **120** to give the disaccharide mimetic **121** in 46% yield. After the silica gel chromatography, extra triflate compound **119** were recycled without any rearrangement or elimination reaction. This result demonstrated that the triflate compound **119** can serve as the building block for oligomerization (**Scheme 30**).

### 4.20 Large-scale synthesis of penta-*O*-acetyl-5-thio-α,β-D-glucopyranose

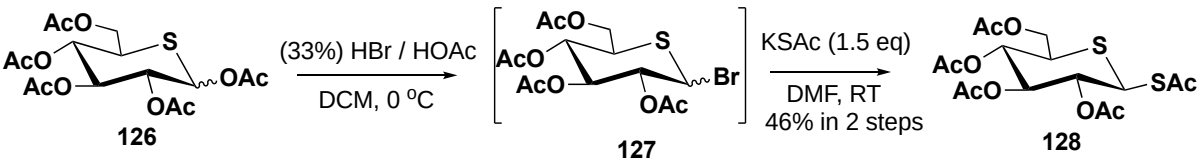



Scheme 31.The large-scale synthesis of penta-*O*-acetyl-5-thio-a,β-D-glucopyranose

The success in thiol-triflate coupling reaction helps us to proceed the oligomer synthesis. The linear synthetic route requires a large amount of monosaccharide 5-thio-glucopyranose as

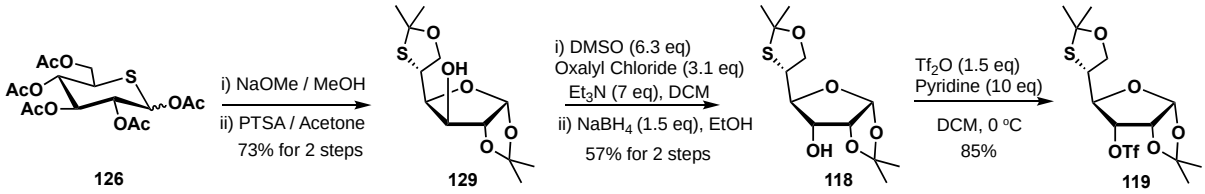
starting material. We followed the synthetic route of Whistler[78] to scale up 5-thio-glucopyranose synthesis. 3-*O*-acetyl-1,2-*O*-isopropylidene-α-D-glucofuranose **122** was synthesized after acetylation and the selective isopropylidene deprotection of 5,6-O-isopropylidene.[79] The primary alcohol was selectively benzoylated followed by sulfonylation of secondary alcohol to give fully functionalized glucofuranose derivative **123** in 83% yield. Treatment of sodium methoxide and neutralization by Amberlyst IR 120 resin gave 1,2-*O*-isopropylidene-5,6-anhydro-α-D-glucofuranose, which was converted to 1,2-*O*-isopropylidene-5,6-didexoy-5,6-epithio-α-D-glucofuranose **124** in 65% yield. The ring opening of episulfide by potassium acetate gave the 5-*S*-acetyl-3,6-di-*O*-acetyl-1,2-*O*-isopropylidene-α-D-glucofuranose **125** in 42% yield. Adapting the protocol of Driguez[74], the 5-thio-glucofuranose was rearranged to 5-thio-glucopyranose penta-acetate **126** in 60% yield (**Scheme 31**).

With large amount of penta-*O*-acetyl-5-thio-α,β-D-glucopyranose in hand. We synthesized the building block 1-*S*-Acetyl-2,3,4,6-tetra-*O*-acetyl-1,5-dithio-β-D-glucopyranose **128**. The bromination of penta-*O*-acetyl-5-thio-glucopyranose gave a mixture of anomeric bromide **127**, which was substituted by potassium thioacetate to give 1-*S*-acetyl-2,3,4,6-tetra-*O*-acetyl-1,5-dithio-β-D-glucopyranose **128** in 46% yield (**Scheme 32**).



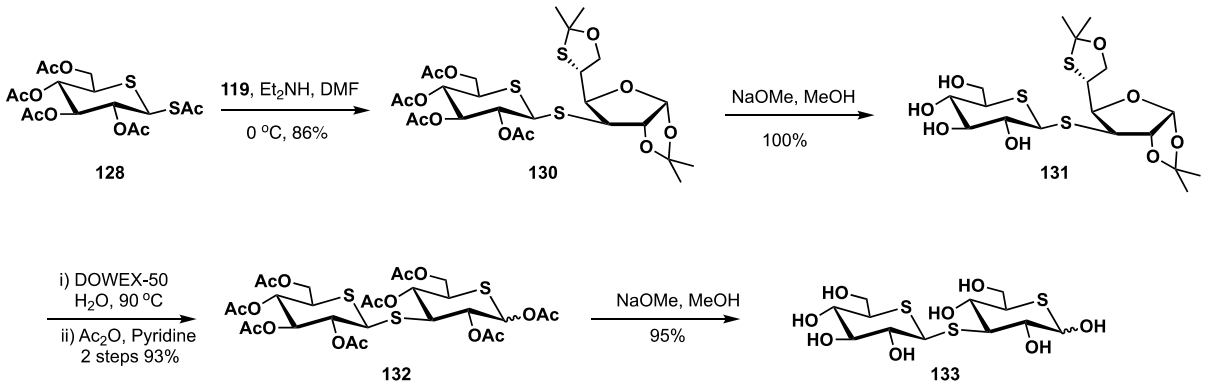

Scheme 32. The Large-scale synthesis of compound **128**

Another building block **119** was also synthesized in gram scale from penta-*O*-acetyl-5-thio-glucopyranose. First, Zemplén deacetylation of the penta-*O*-acetyl-5-thio-a,β-D-glucopyranose gave a crude preparation of 5-thio-glucose which was stirred in anhydrous acetone with *p*-toluenesulfonic acid to give the 1,2-*O*-5-*S*,6-*O*-di-isopropylidene glucofuranose **129** in 73% yield[77]. Swern Oxidation was performed on substrate **129** to give a ketone intermediate which was immediately subjected to sodium borohydride reduction to give the 1,2-*O*-5-*S*,6-*O*-di-isopropylidene-5-thio-α-D-allofuranose **118** in 57% yield. Finally, the 3-OH was converted to triflate to give the building block **119** in 85% yield (**Scheme 33**).




Scheme 33 The Large-scale synthesis of 3-*O*-trifluoromethanesulfonyl-1,2-*O*-isopropylidene-5-*S*,6-*O*-isopropylidene-5-thio-α-D-allofuranose

**4.30 Synthesis of the disaccharide mimetic**




Scheme 34. Synthesis of disaccharide mimetic **133**

With grams of the building blocks **128** and **119**. The first coupling reaction was performed under Von Itzstein condition to give the disaccharide intermediate **130** in 86% yield. The deprotection was based on a 3 steps procedure. First, Zemplén deacetylation gave free-OH

disaccharide intermediate **131**, which was soluble in water. In the aqueous solution of intermediate **131** was added DOWEX-50 and heat up to 90 °C to remove the isopropylidene groups. In the acidic hydrolysis procedure, the furanose ring opened to give the open chain form with the protonated aldehyde. Cyclization of the aldehyde intermediate gave the pyranose-form product as the thermodynamic product (**Figure 14**). The crude mixture was acetylated to give the per-*O*-acetyl disaccharide **132** in 93% yield. Finally, Zemplén deacetylation and Sephadex G-25 gel purification gave the free-OH disaccharide mimetic **133** in 95% yield (**Scheme 34**).

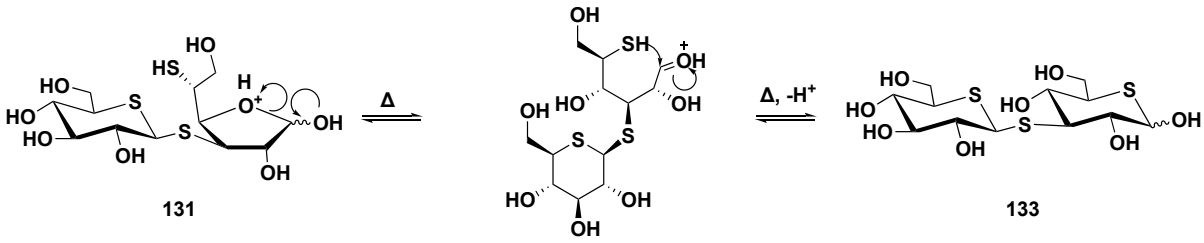
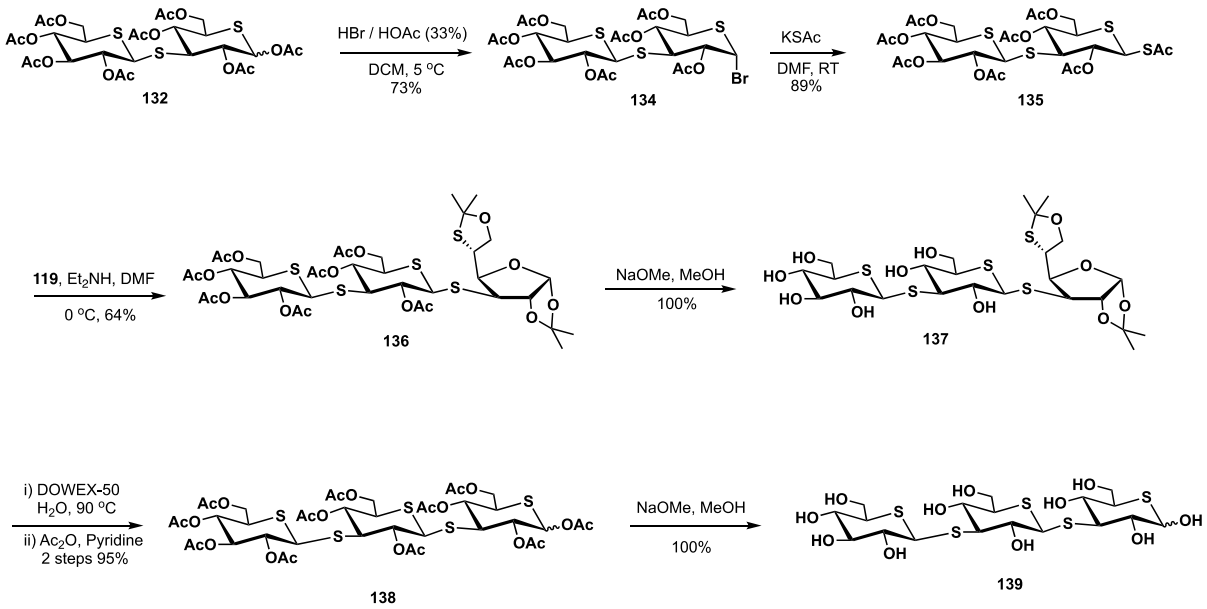



Figure 14. The rearrangement from compound **131** to compound **133**

**4.40 Synthesis of the trisaccharide and tetrasaccharide mimetic**

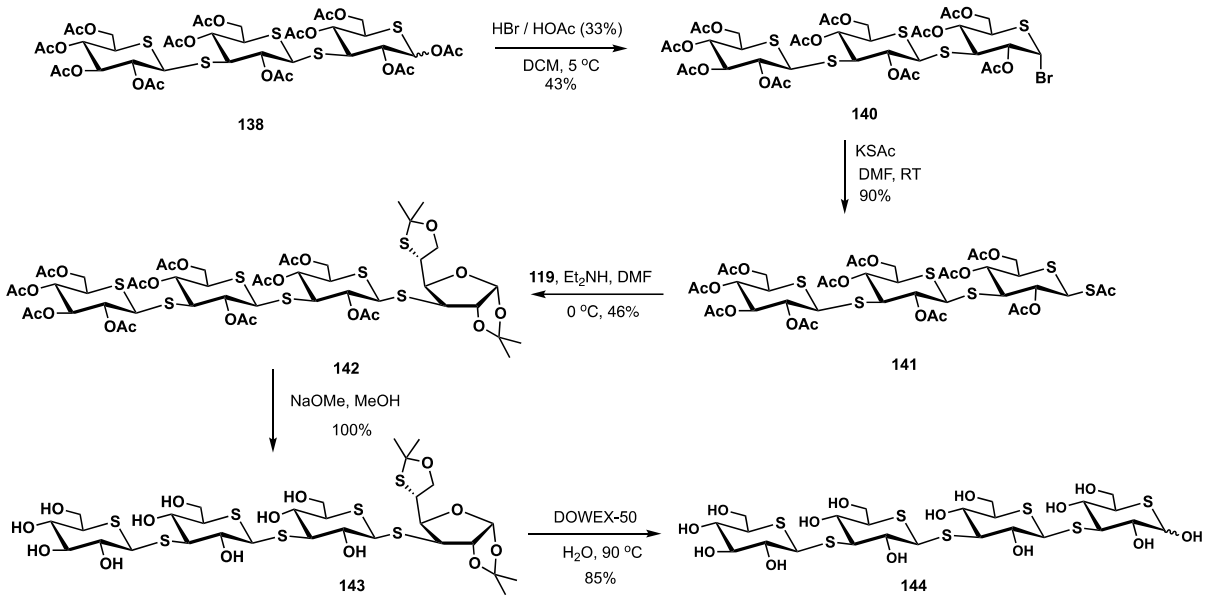
The elongation of disaccharide was based on a 3 steps procedure. First, bromination reaction of the anomeric carbon gave the anomeric α-bromide at the reducing end. Second, the anomeric bromide was substituted by potassium thioacetate in a $SN_2$ fashion to give the anomeric thioacetate with β-configuration. Finally, under Von Itzstein condition, the terminal β-thioacetate was deprotected and substituted the triflate of compound **119** to give the coupling product. The synthesis started from 2,3,4,6-tetra-*O*-acetyl-5-thio-β-D-glucopyranosyl-(1 → 3)-2,4,6-tri-*O*-acetyl-3,5-dideoxy-3,5-dithio-α,β-D-glucopyranose **132**, bromination reaction gave the glucopyranosyl bromide intermediate **134** in 73% yield. The anomeric bromide was

substituted by thioacetate anion to give the glucopyranosyl thioacetate intermediate **135** in 89% yield. Under Von Itzstein condition, compound **135** was coupled with triflate **119** to give the trisaccharide intermediate **136** in 64% yield. Adapting the same deprotection procedure applied in disaccharide synthesis, compound **136** was deprotected and purified by Sephadex G-25 gel chromatography to give the trisaccharide mimetic **139** (**Scheme 35**).




Scheme 35. Synthesis of the trisaccharide mimetic **139**

The synthesis of tetrasaccharide started from per-*O*-acetyl trisaccharide **138**. Bromination reaction gave the glucopyranosyl bromide intermediate **140** in 43% yield, the bromide was substituted by thioacetate anion to give the glucopyranosyl thioacetate intermediate **141** in 90% yield. The compound **141** was coupled with triflate **119** to give the tetrasaccharide intermediate **142** in 46% yield. Application of the deprotection procedure to the tetrasaccharide intermediate **142** gave the tetrasaccharide mimetic **144** in 85% yield (**Scheme 36**).




Scheme 36**.** Synthesis of the tetrasaccharide mimetic **144**

**5.00 Biological evaluation of the oligo-(3,5-dithio-β-D-glucopyranosides)**

To evaluate the binding affinity of the oligo-(3,5-dithio-β-D-glucopyranosides) to CR3 and Dectin-1, the Vetvicka laboratory tested the mimetics' ability to inhibit anti-CR3 and anti-Dectin-1 fluorescein isothiocyanate (**FITC**) conjugated antibody staining of human neutrophils and mouse macrophages. In terms of CR3 binding affinity, incubation of a 0.1μg / ml solution of β-(1→3)-dimer mimetic **133** caused 20% decreases in inhibition of staining human neutrophils and β-(1→3)-trimer mimetic **139** caused 37% decreases in inhibition of staining human neutrophils, while the β-(1→3)-tetramer mimetic **144** caused 21% decreases (**Table 5**). In terms of Dectin-1 binding affinity, incubation of a 0.1μg/ml solution of β-(1→3)-dimer mimetic **133** caused 31% decreases in inhibition of staining human neutrophils and β-(1→3)-trimer mimetic **139** caused 42% decreases in inhibition of staining human neutrophils, while the β-(1→3)-tetramer mimetic **144** caused 33% decreases (**Table 5**).

Table 5**.** Percentage inhibition of anti-CR3 and anti-Dectin-1-FITC antibody staining of neutrophils, macrophages by 0.1μg/mL substrate. a Mean ± SD

| Compound | Oligomer NO. | % inhibition of anti-CR3-FITC staining human neutrophils[a] | % inhibition of anti-dectin-1-FITC staining of mouse neutrophils[a] |
|---|---|---|---|
| **133** | Dimer | 20.2 ± 1.7 | 30.9 ± 3.4 |
| **139** | Trimer | 37.3 ± 3.2 | 42.2 ± 3.8 |
| **144** | Tetramer | 21.5 ± 1.4 | 33.1 ± 2.9 |

Oligo-(3,5-dithio-β-D-glucopyranosides) **133, 139, 144** (10 μg / mL) were also tested for their ability to stimulate phagocytosis of human macrophage-like RAW 264 cells.[55] Commercial available β-glucan **Glucan #300** were used as reference (**Table 6**). The result indicates that the β-(1→3)-trimer mimetic **139** could stimulate 16% of phagocytosis, which is more effective than the β-(1→3)-dimer **133** and β-(1→3) tetramer mimetic **144.** It is notable that the level of phagocytosis stimulated by the β-(1→3)-trimer mimetic **139** was about 50% of that induced by **Glucan #300**.

Table 6**.** Percentage stimulation of phagocytosis. a Mean ± SD

| Compound | Oligomer NO. | % stimulation of phagocytosis (RAW 264 macrophages, 10μg / mL, 24h) [a] |
|---|---|---|
| **133** | Dimer | 8.7 ± 0.7 |
| **139** | Timer | 16.3 ± 0.9 |
| **144** | Tetramer | 6.9 ± 0.3 |
| **Glucan #300** | Polymer | 37.5 ± 2.5 |
| **PBS** | | 2.1 ± 0.2 |

Oligo-(3,5-dithio-β-D-glucopyranosides) **133, 139, 144** (10 μg / mL) were also tested for their ability to stimulate pinocytosis of human macrophage-like RAW 264 cells.[55] Commercial available **Glucan #300** was used as reference. The result indicates that the β-(1→3)-trimer mimetic **139** could stimulate 13% of pinocytosis, which is more effective than the β-(1→3)-dimer mimetic **144** and β-(1→3)-tetramer mimetic **145** (**Table 7).**

Table 7. Percentage stimulation of pinocytosis. a Mean ± SD*

| Compound | Oligomer NO. | % stimulation of pinoocytosis (RAW 264 macrophages, 10μg / mL, 24h) [a] |
|---|---|---|
| **133** | Dimer | 7.77 ± 1.61 |
| **139** | Timer | 12.8 ± 1.72 |
| **144** | Tetramer | 3.37 ± 0.36 |
| **Glucan #300** | Polymer | 38.9 ± 2.81 |
| **PBS** | | 1.77 ± 0.11 |

*Pinocytosis was assayed by spectrophotometric measurement of neutral red dye accumulation by mouse macrophages after 2 hr incubation with 40 μg/ml of neutral red.

**6.00 Conclusion**

As already demonstrated with the di- and trimeric hydroxylamines **22** and **25**,[54] the present study demonstrates that small molecule mimetics of β-(1→3)-glucans can be designed that display significant activity in the inhibition of staining of human neutrophils and mouse macrophages by fluorescent anti-CR3 and anti-Dectin-1 antibodies suggestive of binding to the carbohydrate binding domains of the respective proteins. The affinity for the carbohydrate binding domains of CR3 and Dectin-1 is reflected in the stimulation of phagocytosis and of pinocytosis by compounds **133, 139** and **144**. Unexpectedly, the trimer **139** is more active than

either the dimer **133** or the tetramer **144** in each of the three assays conducted suggesting that, at least for the present series of glucan mimetics, there is little to be gained by preparing higher oligomers. This maximization of activity in the trimer **144** might be accounted for by a tradeoff between the greater affinity for the CR3 and Dectin-1 carbohydrate binding sites arising from the presence of the multiple thioethers on the positive side, and the accumulation of multiple long C-S bonds eventually causing a mismatch with the binding site on the negative side.

# CHAPTER 3: DEVELOPMENT OF A MICROWAVE CLEAVABLE PROTECTING GROUP AND ITS APPLICATION IN GLYCOSYLATION.

## 1.00 Introduction

Protecting groups are integral for advancing the art of organic synthesis.[80-81] They are employed in the construction of complex natural products, oligosaccharides, polyketides, RNA, and peptides. Despite reports on protecting group-free complex molecule synthesis.[82] In organic synthesis, especially carbohydrate synthesis, it is necessary to manipulate different functional groups, which lead to the concept of orthogonal protecting groups. The benzyl protecting group, as well as modified benzyl groups, have been significantly employed in hydroxyl group protection. The electronic tuning of the benzyl group has generated different orthogonal protecting groups: a) Chemical cleavable protecting groups; b) Photocleavable protecting groups; c) Enzyme cleavable protecting groups. We have expanded this boundary to a new type of protecting group: Microwave cleavable protecting groups.

## 1.10 Chemical cleavable benzyl protecting groups

## 1.11 Benzyl protecting groups cleaved by hydrogenolysis

Benzyl groups are most frequently used for hydroxyl group protection. They are stable under acidic and basic conditions, and they are only hardly cleavable under mild oxidizing agents (Dess-Martin Reagent, *etc*) or metal hydrides (Lithium Aluminum Hydride, *etc*). The deprotection method is usually hydrogenolysis in the presence of Palladium catalyst on charcoal, this process is mild and compatible with most functional groups.[83]

## 1.12 Benzyl based protecting groups cleaved by oxidation

*p*-Methoxy benzyl (PMB) as well as 3,4-dimethoxybenzyl (DMB) ethers were developed by Yonemitsu group.[84] They are more labile under acidic conditions than benzyl

ether. However, they undergo oxidative cleavage, a process where single electron transfer (SET) to 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ), generating an oxonium ion which can be captured by water. This characteristic provides an orthogonal set which helps to selectively remove PMB ethers in the presence of benzyl ether.

### 1.20 Microwave

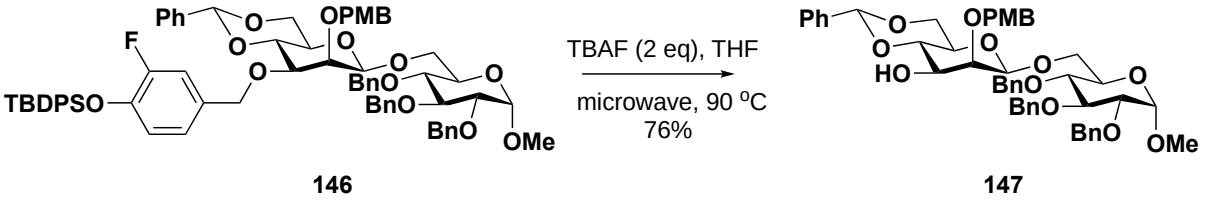
Mirowave is a form of electromagnetic energy with wavelength range from one meter to one millimeter. The development of microwave technology was initiated by World War II as the RADAR devices. Accidentally, Percy LeBaron Spencer of the Raytheon Company discovered that microwave energy could heat up food. This discovery gave birth to commercial microwave oven in the 1950s. Microwave was applied in Organic synthesis in 1980s, the first two papers on microwave-enhanced organic chemistry were published and many in 1986. Organic chemists have since discovered the benefits of using microwave energy to drive synthetic reactions.[85-86] Microwave heating is much more quickly than conductive heating since heat doesn't need to pass through the walls of vessel to heat up the reactant. Microwave heating has been proved to be significantly beneficial in accelerating many conventional heated reactions. The ease of manipulation, milder reaction conditions and formation of cleaner products are the benefits come with improved reaction rates.[87]

### 1.30 Microwave cleavable benzyl-based protecting groups

### 1.31 Microwave cleavage of 4-*O*-siloxyl benzyl ether

The Crich lab developed a microwave assisted cleavage of 4-(*tert*-Butyldiphenylsiloxy)-3-fluorobenzyl group. [88] The deprotection was performed with TBAF under microwave condition. Orthogonality was tested with PMB ether and it was proved that the 4-(*tert*-

Butyldiphenylsiloxy)-3-fluorobenzyl ether was deprotected in the presence of PMB ether (**Scheme 37**).

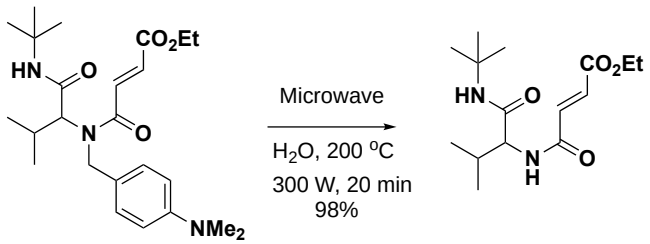



**146** **147**

Scheme 37. Microwave cleavage of 4-(*tert*-Butyldiphenylsiloxy)-3-fluorobenzyl group

### 1.32 Microwave cleavage of PDMAB protecting group

Andreana group discovered PDMAB protecting group in a microwave reaction of compound **148**. Under the cleavage of *p-N,N*-dimethylamine benzyl group in 98% yield. This discovery led us to look for appropriate protection reagents to help to extent PDMAB group into organic synthesis (**Scheme 38**).




**148** **149**

Scheme 38. Microwave cleavage of PDMAB group of compound **148**

### 2.00 *p-N,N*-Dimethylamino benzyl group protection

### 2.10 Installation of PDMAB group by nucleophilic substitution of PDMAB chloride or PDMAB tosylate

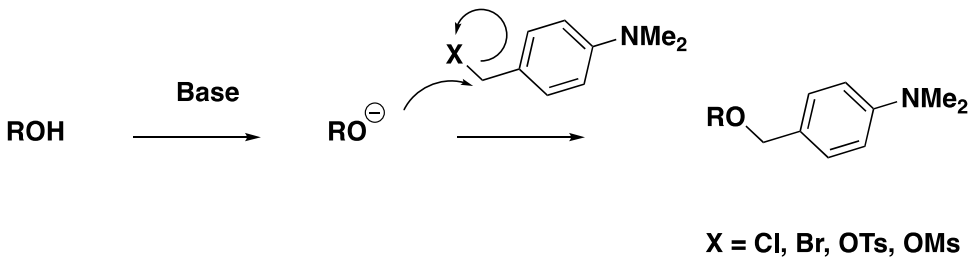
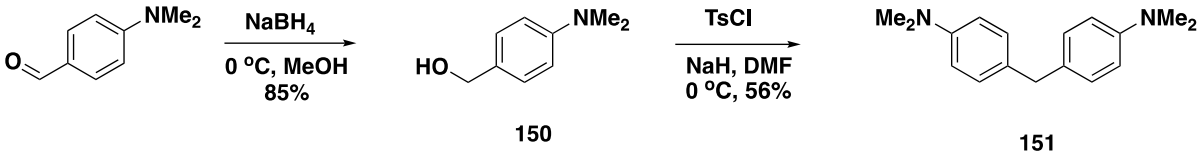


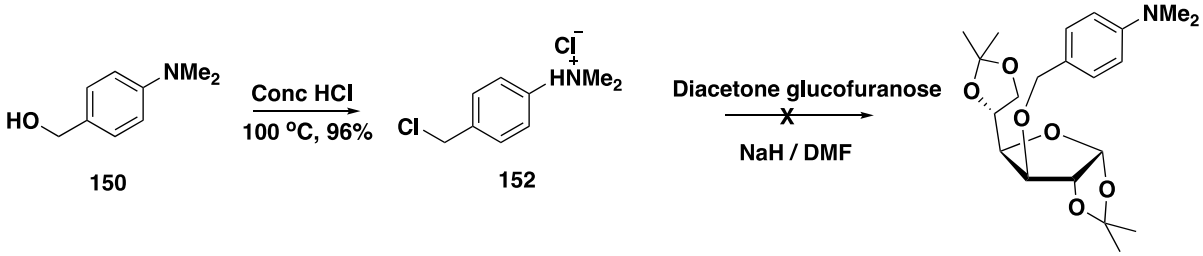

Figure 15. Installation of PDMAB group by nucleophilic substitution

In benzyl ether synthesis, the most common method is the alkylation of alkoxides with benzyl bromide or chloride. We wanted to apply this method to PDMAB group installation (**Figure 15**). With the commercially available *p-N,N*-dimethylamino benzaldehyde, the reduction with sodium borohydride gave *p-N,N*-dimethylamino benzyl alcohol **150** in 85% yield. Tosylation of the PDMAB alcohol in DMF gave the 4,4'-methylenebis-(*N,N*-dimethylaniline) **151** as the major product (**Scheme 39**).




Scheme 39. Attempted synthesis of PDMAB tosylate

The PDMAB aocohol was refluxed in concentrated hydrogen chloride at 100 °C overnight to give the *p-N,N*-dimethylamino benzyl chloride hydrochloride salt **152** in 96% yield. When applying this reagent in hydroxyl group protection, no protection product was isolated and the major product was 4,4'-methylenebis-(*N,N*-dimethylaniline) **151**. This result indicates that *p-N,N*-dimethylamino benzyl chloride hydrochloride salt decompose under basic condition (**Scheme 40**).




Scheme 40. Synthesis of PDMAB chloride hydrochloride and attempted PDMAB protection

**2.20 Buchwald amination of 4-halobenzyl ether.**

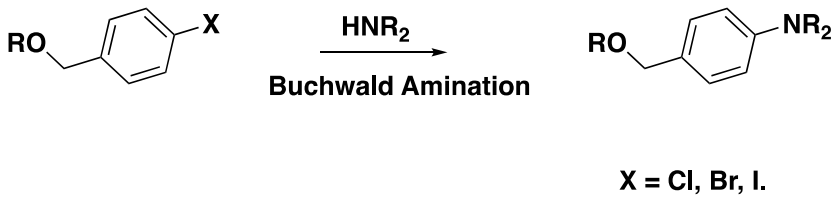
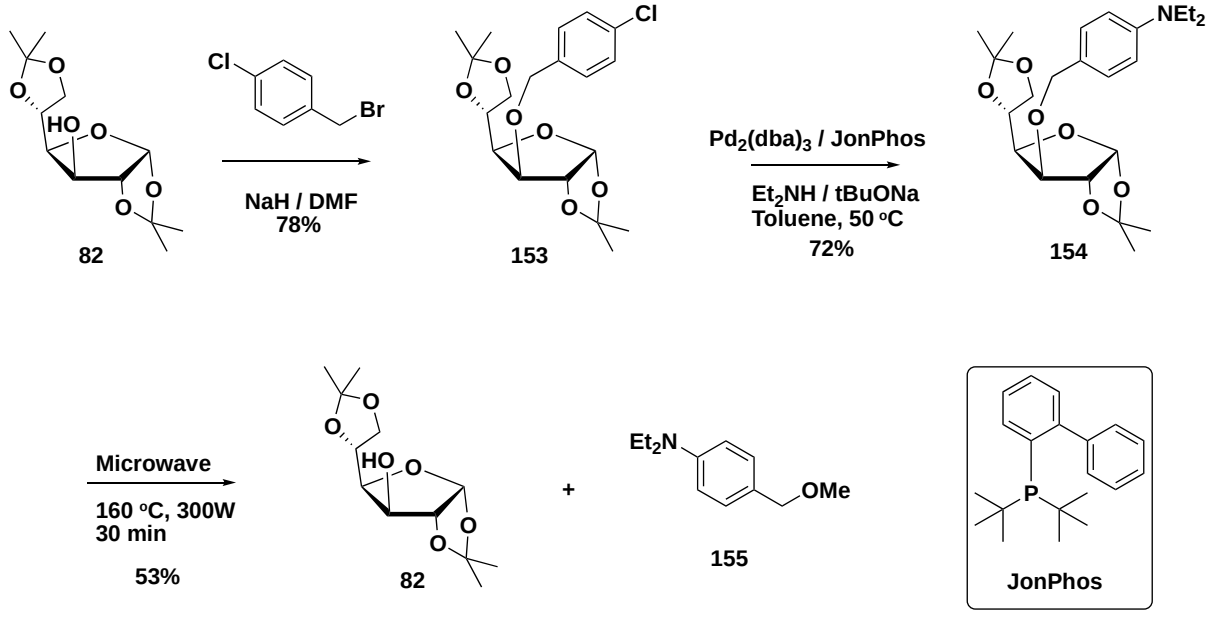


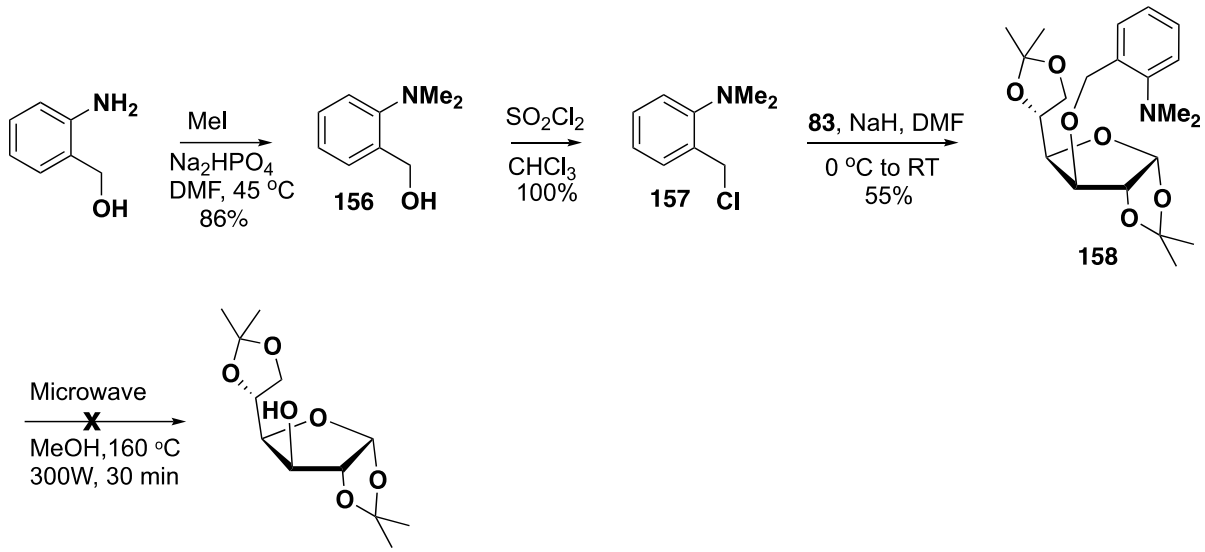

Figure 16. Installation of PDMAB protecting group by Buchwald amination of 4-halobenzyl ether

Obadiah *et al*[89] reported that *p-N,N*-dimethylamino benzyl protecting group could be installed by Buchwald amination of 4-halobenzyl ether. We successfully installed PEMAB group on the 1,2;5,6-di-*O*-isopropylidene-α-D-glucofuranose by applying this protocol. First, the 3-OH of compound **82** was protected with 4-chlorobenzyl group to give 3-*O*-(4-Chlorobenzyl)-1,2;5,6-di-*O*-isopropylidene-α-D-glucofuranose **153** in 78% yield. Buchwald amination was performed with compound **153** using diethyl amine to give PEMAB protected 1,2;5,6-di-*O*-isopropylidene-α-D-glucofuranose **154**. We tested the deprotection of PEMAB group under microwave condition and the PEMAB group was successfully cleaved under microwave irradiation to give compound **82** in 53% yield together with the *p-N,N*-diethylamino benzyl methyl ether **159** as the byproduct (**Scheme 41**).



Scheme 41. The installation of *p*-*N*,*N*-diethylamino benzyl ether and microwave deprotection

Out of curiosity, we also tested microwave deprotection of the *ortho*-*N*,*N*-dimethylamino benzyl group. First, the 3-OH of compound **82** was protected with *ortho*-*N*,*N*-dimethylamino benzyl group using *ortho*-*N*,*N*-dimethylamino benzyl chloride. The reaction gave the desired product **158** in 55% yield. When compound **158** was subjected to microwave irradiation in methanol, the *ortho*-*N*,*N*-dimethylamino benzyl group was not cleaved (**Scheme 42**).

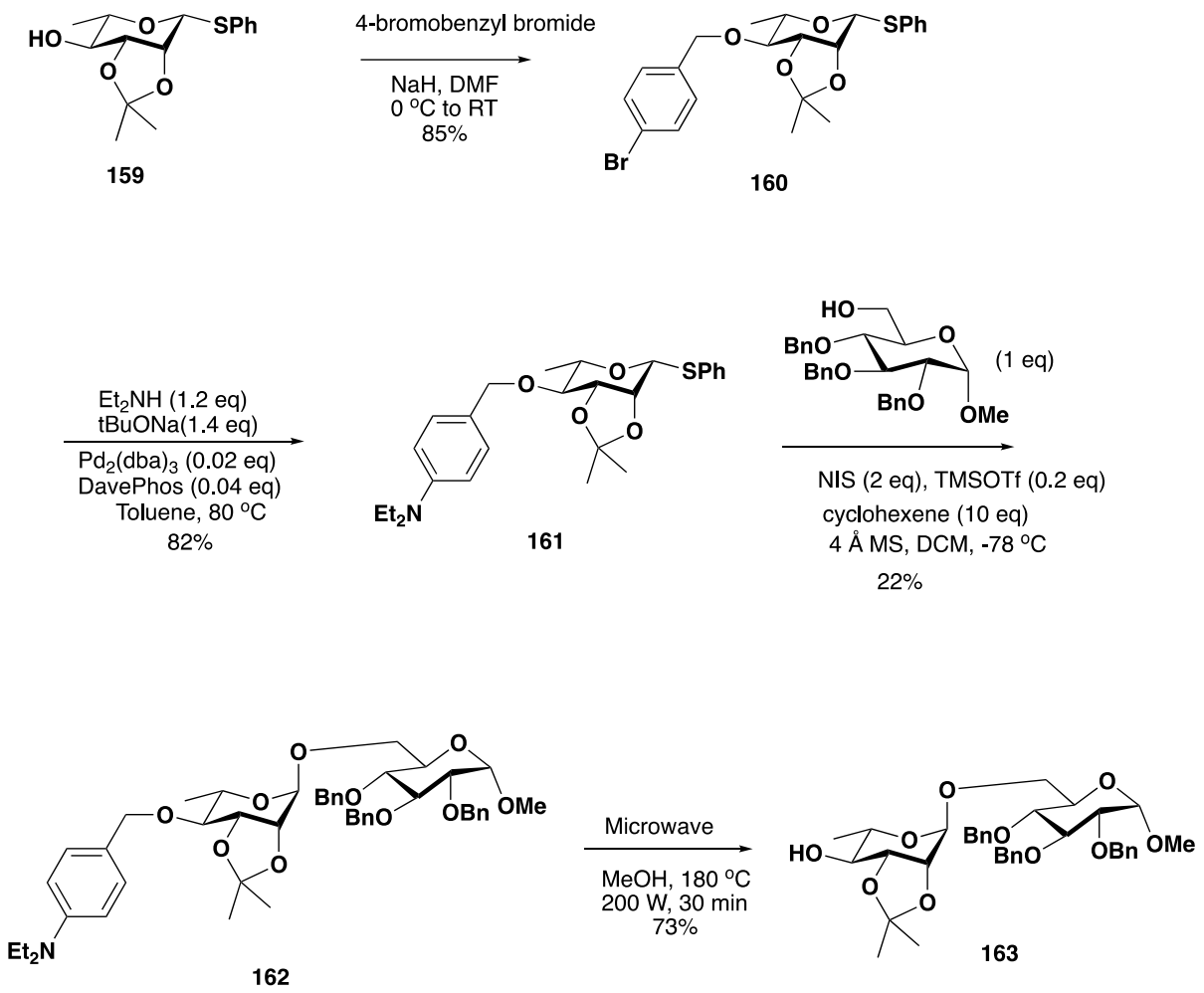



Scheme 42. Installation of *ortho*-*N*,*N*-dimethylamino benzyl ether and attempted microwave deprotection

**2.30 Application of PEMAB group in glycosylation**

The task of manipulating different hydroxyl groups becomes particularly important in oligosaccharide synthesis. To accomplish this task, orthogonal protection and deprotection is required in the complex molecule synthesis. Different protecting groups are employed, for example, 4-*O*-methoxy benzyl ethers, which is labile under oxidative condition and acidic condition, can be selectively cleaved in the presence of benzyl groups. More importantly, the compatibility of protecting groups with the glycosylation reaction will help to diversify the

oligosaccharide synthesis. To test the compatibility of PEMAB group in glycosylation, we synthesized the phenyl 4-*O*-(*p*-*N*,*N*-diethylamino benzyl)-2,3-*O*-isopropylidene-1-thio-β-L-rhamnopyranoside **161** and it was subjected to NIS / TMSOTf activated glycosylation. The synthesis started from Phenyl 2,3-*O*-isopropylidene-1-thio-β-L-rhamnopyranoside **161**, the 4-OH was protected by 4-bromobenzyl ether give compound Phenyl 4-*O*-(4-bromobenzyl)-2,3-*O*-isopropylidene-1-thio-β-L-rhamnopyranoside **162** in 85% yield. The Buchwald amination converted the 4-bromobenzyl ether protected rhamnose **162** into *p*-*N*,*N*-diethylamino benzyl protected rhamnose **163** in 82% yield. With glycosylation donor **163** in hand, the glycosylation with methyl 2,3,4-tri-O-benzyl-α-D-glucopyranoside was undertaken. The reaction was activated by NIS / TMSOTf at -78 $^{o}$C and stirred at -78 $^{o}$C for 12 h to give the glycosylation product **162** in 22% yield (**Scheme 43**). With glycosylation product **162**, we performed the deprotection under microwave irradiation. The PEMAB group was cleaved to give the product **163** in 73% yield (**Scheme 43**).




Scheme 43. Glycosylation with PEMAB protected donor **161** and deprotection of PEMAB group after glycosylation reaction

### 3.00 Conclusion

In conclusion, other than traditional orthogonal protecting groups: the chemical labile, photo labile and enzymatic labile protecting groups. A new type of protecting group: microwave labile protecting group, has been developed.) To the best of oue knowledge, this is the first example where microwave irradiation has been used to deprotect benzyl ether protecting groups. It was convinced that the PEMAB group is compatible with glycosylation conditions. The PEMAB group can be selectively deprotected in the presence of benzyl ether, isopropilydene, ester as well as benzylidene acetals under microwave irradiation at neutral conditions. As for the protection, the effective procedure requires a two steps synthesis:

installation of *p*-halo-benzyl ether and Buchwald amination. The one step protection is limited by the unstability of PEMAB chloride or tosylate.

# CHAPTER 4: EXPERIMENTAL SECTION

## General information

All reagents and solvents were purchased from commercial suppliers and were used without further purification unless otherwise stated. All reactions were performed under Argon atmosphere unless otherwise stated. Reactions were monitored by analytical thin-layer chromatography with pre-coated glass backed plates (w/UV 254) and visualized by UV absorption (254 nm) or by staining with a 5% solution of $H_2SO_4$ in MeOH or ceric ammonium molybdate solution (4.0 g ceric sulfate; 10 g ammonium molybdate; 40 mL $H_2SO_4$; 360 mL $H_2O$) followed by heating. Optical rotations were measured with an Autopol III polarimeter (Rudolph Research Analytical, Hackettstown, NJ) in the solvent specified at 589 nm at 23 °C, the path length is 10 cm. $^{1}H$, $^{13}C$, HSQC, HMBC, COSY, TOCSY NMR spectra were recorded with Agilent 600 MHz and Varian 400 MHz instruments. High resolution mass spectra were recorded with a Walters LC/ MS with an electrospray source coupled to a time-of-flight mass analyzer. Melting points were recorded with a Barnstead International electrothermal melting point apparatus.

## General Procedure A: Coupling Reactions

To a solution of 1-*S*-Acetyl-5-thio-β-D-glucopyranose (1.0 eq) and 1,2-*O*-5-*S*,6-*O*-di-isopropylidene-5-thio-α-D-allofuranose (1.1 eq) in DMF (1.0 M) was added diethylamine (2.5 eq) dropwise at 0 °C. The reaction mixture was stirred at room temperature until completion. The reaction mixture was diluted with ethyl acetate and washed with water, brine, dried over $MgSO_4$ and concentrated in *vacuo*. The residue was purified by silica gel chromatography.

**General Procedure B: Deacetylation**

At 0 °C, sodium methoxide (0.2 eq) was added to the substrate (1.0 eq) in anhydrous methanol (0.1M) and stirred until the completion of the reaction (observed by mass spectrometry). Amberlyst IR120 resin was added to neutralize the reaction. When the pH was neutral (monitored by pH paper), the resin was filtered off and the solution was concentrated in vacuo. Furanosyl systems were subjected to acidic hydrolysis according to general procedure C, and the pyranose forms were purified by Sephadex G-25 gel eluting with water.

**General procedure C: Hydrolysis and Acetylation**

The substrate (1.0 eq) was dissolved in deionized water (0.05 M). DOWEX-50WX2 hydrogen form resin (400 mg per 100 mg substrate) was added to the reaction. The reaction mixture was stirred at 90 °C until the completion of the reaction (monitored by mass spectrometry). The resin was filtered off and the solution was concentrated in *vacuo*. The crude mixture was dissolved in pyridine (0.5 M) and acetic anhydride (2 eq per OH) 4-(dimethylamino)-pyridine (0.1 eq) was added, the reaction mixture was stirred at room temperature until the completion of reaction (monitored by mass spectrometry). The solution was concentrated in *vacuo* and purified by silica gel chromatography.

**General Procedure D: Bromination**

To a solution of penta-*O*-acetyl-5-thio-D-glucopyranose (1.0 eq) in anhydrous DCM (0.5 M) was added 33% HBr in acetic acid (7.0 eq) at 0 °C. The reaction mixture was kept at 5 °C for 12 h. The reaction mixture was diluted by DCM and quenched with ice cold aqueous

$NaHCO_3$. The organic layer was washed with water, brine, dried with $MgSO_4$, and concentrated in *vacuo*. The residue was purified by silica gel chromatography.

**General procedure E: Thioacylation**

At 0 °C, potassium thioacetate (1.5 eq) was added to a solution of penta-*O*-acetyl-5-thio-D-glucopyranosyl bromide (1.0 eq) in *N*,*N*-dimethyl formamide (1.0 M). The reaction mixture was stirred 12 h at room temperature. After completion, the reaction mixture was diluted with ethyl acetate and washed by water, brine, and dried with $MgSO_4$. The reaction mixture was concentrated in *vacuo* and purified by silica gel chromatography.

**5,6-Dideoxy-5,6-epithio-1,2-*O*-isopropylidene-β-L-talofuranose (87)**

To a solution of compound **86** (100 mg, 0.46 mmol) in 10 mL DCM was added Dess Martin reagent (390 mg, 0.92 mmol). The reaction was stirred at room temperature 24h and saturated $NaS_2O_3$ aqueous solution was added to quench the reaction. The reaction mixture was extracted into DCM and washed with $NaHCO_3$ aqueous solution, water, brine and dried over $MgSO_4$. The crude mixture was concentrated in *vacuo* and re-dissolved in 20 mL EtOH. At 0 °C, sodium borohydride (26 mg, 0.69 mmol) was added into reaction mixture and it was stirred at 0 °C for 1h. The reaction was quenched with aqueous $NH_4Cl$ and concentrated in *vacuo* to remove EtOH. After the EtOH was removed, the crude mixture was diluted in ethyl acetate, the organic layer was separated and washed with water, brine and dried over $MgSO_4$. The crude mixture was concentrated in *vacuo* and purified by silica gel chromatography to give product **87** (85 mg, 85%) as a colorless oil. $[\alpha]^{23}_D$ = +57.2° (c 0.6, $CHCl_3$). $^1$H NMR (400 MHz, $CDCl_3$) δ 5.81 (d, *J* = 3.9 Hz, 1H, $H_1$), 4.60 (dd, *J* = 5.2, 3.9 Hz, 1H, $H_2$), 3.91 – 3.79 (m, 1H,

$H_4$), 3.58 (dd, *J* = 8.5, 6.4 Hz, 1H, $H_3$), 3.06 (td, *J* = 6.5, 5.4 Hz, 1H, $H_5$), 2.54 (dd, *J* = 6.6, 1.4 Hz, 1H, $H_{6'}$), 2.48 (dd, *J* = 5.4, 1.4 Hz, 1H, $H_6$), 2.43 (d, *J* = 10.6 Hz, 1H, OH), 1.54 (s, 3H), 1.36 (s, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 112.9 (O,O-isopropylidene), 103.9 ($C_1$), 83.2 ($C_2$), 78.7 ($C_4$), 75.8 ($C_3$), 34.4 ($C_5$), 26.6, 26.5 (isopropylidene), 21.1 ($C_6$). HRMS *m/z* $[M+Na]^+$ calcd for $C_9H_{14}O_4SNa$ 241.0510, found 241.05010

**6-*S*-Acetyl-3,5-dideoxy-3,5-epithio-1,2-*O*-isopropylidene-β-L-idofuranose (88)**

At 0°C, to a solution of compound **87** (107 mg, 0.49 mmol) in pyridine (1.5 mL) was added trifluoromethanesulfonic anhydride (0.1 mL, 0.6 mmol). The reaction was stirred at 0 °C for 25 min before it was diluted with diethyl ether 20 mL. The reaction mixture was quenched with aqueous $NaHCO_3$ and the organic layer was separated and washed with water, brine and dried over $MgSO_4$. The reaction mixture was concentrated in *vacuo* and re-dissolved in 2 mL DMF. At room temperature, potassium thioacetate (171 mg, 1.5 mmol) was added to the solution. The reaction was stirred at room temperature for 1h before it was diluted in diethyl ether 20 mL and washed with water, brine, dried over $MgSO_4$ and concentrated in *vacuo*. The crude mixture was purified on silica gel chromatography with eluent (hexane : ethyl acetate = 10 : 1) to give product **88** (50 mg, 37%) as a colorless oil. $[\alpha]^{23}_D$ = +81.9° (c 0.4, $CHCl_3$). $^1$H NMR (400 MHz, $CDCl_3$) δ 6.35 (d, *J* = 3.4 Hz, 1H, $H_1$), 5.24 (t, *J* = 5.0 Hz, 1H, $H_3$), 4.63 (d, *J* = 3.4 Hz, 1H, $H_2$), 3.97 (d, *J* = 5.0 Hz, 1H, $H_4$), 3.91 (td, *J* = 7.8, 5.0 Hz, 1H, $H_5$), 3.13 (d, *J* = 7.8 Hz, 2H, $H_6$), 2.32 (s, 3H, S-Acetyl), 1.45 (s, 3H), 1.36 (s, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 195.2 (S-Acetyl), 114.3 (Isopropylidene C), 108.7 ($C_1$), 85.9 ($C_2$), 83.4 ($C_3$), 42.6

($C_5$), 42.0 ($C_4$), 30.6 (S-Acetyl), 30.1 ($C_6$), 27.8 (Isopropylidene), 27.3 (Isopropylidene). HRMS *m/z* $[M+Na]^+$ calcd for $C_{11}H_{16}O_4S_2Na$ 299.0388, found 299.0384.

**3-*S*-Acetyl-6-*O*-*p*-toluenesulfonyl-1,2-*O*-isopropylidene-α-D-glucofuranose (92).**

To a solution of 3-*S*-acetyl-1,2-*O*-isopropylidene-α-D-glucofuranose in pyridine 1 mL was added *p*-toluenesulfonyl chloride (56 mg, 0.30 mmol). The reaction mixture was kept in 5 °C for 24 h before it was diluted in 20 mL DCM and quenched with aqueous $NaHCO_3$. The organic layer was separated and washed with water, brine and dried over $MgSO_4$. The crude mixture was concentrated in *vacuo* and purified by silica gel chromatography to give product **92** (85 mg, 67%) as a colorless oil. $[\alpha]^{23}_D$ = -2.6° (c 0.4, $CHCl_3$). $^1$H NMR (400 MHz, $CDCl_3$) δ 7.85 – 7.70 (m, 2H), 7.33 (d, *J* = 8.1 Hz, 2H), 5.72 (d, *J* = 3.5 Hz, 1H, $H_1$), 4.56 (d, *J* = 3.5 Hz, 1H, $H_2$), 4.30 (dd, *J* = 10.4, 2.2 Hz, 1H, $H_6$), 4.25 (dd, *J* = 9.1, 3.6 Hz, 1H, $H_4$), 4.17 (d, *J* = 3.5 Hz, 1H, $H_3$), 4.03 (dd, *J* = 10.4, 7.0 Hz, 1H, $H_{6'}$), 3.93 (ddd, *J* = 9.2, 7.0, 2.2 Hz, 1H, $H_5$), 2.44 (s, 6H), 2.39 (s, 3H), 1.48 (s, 3H), 1.28 (s, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 194.5, 145.0, 132.5, 129.9, 128.0, 112.4, 104.6 ($C_1$), 85.6 ($C_2$), 77.3 ($C_4$), 71.9 ($C_6$), 68.8 ($C_5$), 50.3 ($C_3$), 31.0, 26.4, 26.2, 21.7. HRMS *m/z* $[M+Na]^+$ calcd for $C_{18}H_{24}O_8S_2Na$ 455.0810, found 455.0814

**3-*O*-Acetyl-5-*O*-methanesulfonyl-6-*O*-benzoyl-1,2-*O*-isopropyliene-α-D-allofuranose (96).**

At 0 °C, to a solution of compound **95** (460 mg, 1.75 mmol) in DCM 18 mLwas added pyridine (0.35 mL, 4.38 mmol). At -30 °C, benzoyl chloride (0.22 mL, 1.93 mmol) was added into the reaction, after the reaction was stirred at -30 °C for 1h, methanesulfonyl chloride (0.27 mL, 3.5 mmol) was injected into the reaction mixture. The reaction was stirred at room

temperature overnight before aqueous $NaHCO_3$ was added to quench the reaction. The DCM layer was separated and washed with water, brine, dried over $MgSO_4$ and concentrated in *vacuo*. The crude mixture was purified by silica gel chromatography to give product **96** (660 mg, 85%) as a colorless crystal. m.p. 128 - 130 °C. $[\alpha]^{23}_D$ = +73.6° (c 0.5, $CHCl_3$). $^1$H NMR (400 MHz, $CDCl_3$) δ 8.06 (dt, *J* = 7.2, 1.4 Hz, 2H), 7.65 – 7.52 (m, 1H), 7.45 (dd, *J* = 8.4, 7.1 Hz, 2H), 5.83 (d, *J* = 3.3 Hz, 1H), 5.22 (dt, *J* = 7.5, 3.6 Hz, 1H), 4.97 – 4.80 (m, 2H), 4.65 (dd, *J* = 12.4, 3.5 Hz, 1H), 4.41 (ddd, *J* = 7.8, 6.8, 4.2 Hz, 2H), 3.05 (s, 3H), 2.11 (s, 3H), 1.55 (s, 3H), 1.34 (s, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 170.0, 165.9, 133.5, 129.8, 129.2, 128.6, 113.6 (O,O-isopropylidene), 104.1 ($C_1$), 78.1 ($C_2$), 76.0 ($C_4$), 72.4 ($C_3$), 62.6 ($C_6$), 38.8 (OMs), 26.7, 26.6, 20.6 (OAc). HRMS *m/z* $[M+Na]^+$ calcd for $C_{19}H_{24}O_{10}SNa$ 467.0988, found 467.0993.

**5,6-Anhydro-1,2-*O*-isopropylidene-β-L-talofuranose (97)**

To a solution of comound **96** (288 mg, 0.65 mmol) in 11 mL MeOH was added sodium methoxide (12 mg , 0.22 mmol). The reaction mixture was stirred at room temperature for 1h before it was neutralized with Amberlyst IR 120. The crude mixture was concentrated and it was purified by sílica gel chromatography to give product **97** (64 mg, 49%) as a colorless oil. $[\alpha]^{23}_D$ = +30.2° (c 0.5, $CHCl_3$). $^1$H NMR (400 MHz, $CDCl_3$) δ 5.77 (d, *J* = 3.8 Hz, 1H, $H_1$), 4.55 (dd, *J* = 5.1, 3.8 Hz, 1H, $H_2$), 3.96 (ddd, *J* = 10.4, 8.8, 5.1 Hz, 1H, $H_3$), 3.67 (dd, *J* = 8.8, 4.3 Hz, 1H, $H_4$), 3.14 (td, *J* = 4.2, 2.8 Hz, 1H, $H_5$), 2.90 – 2.74 (m, 2H, $H_6$ & $H_{6'}$), 2.57 (d, *J* = 10.4 Hz, 1H, OH), 1.53 (s, 3H), 1.34 (s, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 112.9 (O,O-isopropylidene), 104.1 ($C_1$), 79.7 ($C_2$), 78.5 ($C_4$), 72.9 ($C_3$), 51.0 ($C_5$), 43.9 ($C_6$), 26.5 (O,O-isopropylidene). HRMS *m/z* $[2M+Na]^+$ calcd for $C_{18}H_{28}O_{10}Na$ 427.1580, found 427.1586.

**3-*S*-Acetyl-5,6-anydro-1,2-*O*-isopropylidene-β-L-idofuranose (98).**

To a solution of compound **97** (74 mg, 0.37 mmol) in DCM 7.5 mL was added pyridine (0.12 mL, 1.42 mmol). At 0 °C, trifluoromethanesulfonic anhydride (0.12 mL, 0.71 mmol) was added dropwise. The reaction mixture was stirred at 0 °C for 30 min before it was poured into 8 mL ice-cold water. The mixture was extracted by 3 x 4 mL DCM. The DCM layer was washed by brine, dried over $MgSO_4$ and concentrated in *vacuo*. The crude mixture was dissolved in 4 mL DMF, at 0 °C, the solution of potassium thioacetate (84 mg, 0.73 mmol) in 4 mL DMF was slowly injected. After 1h, the reaction mixture was poured into 50 mL ice water and extracted by 50 mL ethyl acetate. The organic layer was washed with brine, dried over $MgSO_4$ and concentrated in *vacuo*. The crude mixture was purified by silica gel chromatography to give product **98** (67 mg, 67%) as a colorless oil. $[\alpha]^{23}_D$ = -5.7° (c 0.4, $CHCl_3$). $^1$H NMR (400 MHz, $CDCl_3$) δ 5.85 (d, *J* = 3.6 Hz, 1H, $H_1$), 4.56 (d, *J* = 3.5 Hz, 1H, $H_2$), 4.22 (t, *J* = 4.6 Hz, 1H, $H_4$), 4.14 (dt, *J* = 4.1, 0.7 Hz, 1H, $H_3$), 3.09 (ddd, *J* = 5.0, 4.2, 2.7 Hz, 1H, $H_5$), 2.77 (dd, *J* = 5.0, 4.2 Hz, 1H, $H_6$), 2.69 (dd, *J* = 4.9, 2.7 Hz, 1H, $H_{6'}$), 2.40 (s, 3H, S-acetyl), 1.51 (s, 3H), 1.31 (s, 3H) (isopropylidene). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 193.5 (S-acetyl), 112.3 (O,O-isopropylidene), 104.7 ($C_1$), 86.0 ($C_2$), 79.1 ($C_4$), 50.7 ($C_5$), 50.3 ($C_3$), 43.5 ($C_6$), 30.8 (S-acetyl), 26.6, 26.3 (isopropylidene). HRMS *m/z* $[M+Na]^+$ calcd for $C_{11}H_{16}O_5NaS$ 283.0616, found 283.0615.

**3-*S*-Pivalyl-5,6-anydro-1,2-*O*-isopropylidene-β-L-idofuranose (99)**

Compound **97** (110 mg, 0.54 mmol) and potassium thiopivalate (170 mg, 1.1 mmol) were applied to same procedure as the preparation of **98** to give product **99** (108 mg, 67%) as

a colorless oil. $[\alpha]^{23}_D$ = -6.7° (c 0.6, $CHCl_3$). $^1H$ NMR (400 MHz, $CDCl_3$) δ 5.85 (d, *J* = 3.6 Hz, 1H, $H_1$), 4.50 (d, *J* = 3.6 Hz, 1H, $H_2$), 4.17 (dd, *J* = 5.3, 4.3 Hz, 1H ,$H_4$), 4.05 (dt, *J* = 4.2, 0.7 Hz, 1H, $H_3$), 3.06 (dddd, *J* = 5.2, 4.3, 2.7, 0.8 Hz, 1H, $H_5$), 2.72 (td, *J* = 4.6, 0.8 Hz, 1H, $H_6$), 2.65 (ddd, *J* = 4.9, 2.7, 0.8 Hz, 1H, $H_{6'}$), 1.49 (s, 3H), 1.29 (s, 3H), 1.23 (s, 9H). $^{13}C$ NMR (101 MHz, $CDCl_3$) δ 204.43 (S-pivalyl) 112.2 (O,O-isopropylidene), 104.8 ($C_1$), 86.1 ($C_2$), 79.5 ($C_4$), 50.7 ($C_5$), 49.7 ($C_3$), 43.5 ($C_6$), 27.2 (S-pivalyl), 26.6, 26.3 (isopropylidene). HRMS *m/z* $[M+Na]^+$ calcd for $C_{14}H_{22}O_5NaS$ 325.1086, found 325.1090.

**3-*O*-(2-Napthyl)methyl-5-*O*-methanesulfonyl-6-*O*-benzoyl-1,2-*O*-isopropyliene-α-D-allofuranose (102)**

To a stirred solution of compound **101** (1.01 g, 2.8 mmol) in 20 mL DCM was added pyridine (1.2 mL, 4.66 mol) and DMAP (0.017 g, 0.14 mmol). At -30 °C benzoyl chloride (0.36 mL, 3.1 mmol) was added dropwise and the reaction mixture was stirred at -30 °C for 1 h. After completion (monitored by TLC), methanesulfonyl chloride (0.31 mL, 4.1 mmol) was added to the reaction mixture at -30 °C. The reaction mixture was stirred at room temperature for 10 min before it was concentrated at 40 °C to remove DCM. After DCM was removed, the reaction mixture was stirred at room temperature until the completion (monitored by TLC). The reaction was diluted with 40 mL DCM and quenched with aqueous $NaHCO_3$. The solution was washed with water, brine and dried over $MgSO_4$. The reaction mixture concentrated in *vacuo* and purified by silica gel chromatography to give product **102** (1.02 g, 70%) as a colorless oil. $[\alpha]^{23}_D$ = +60.0° (c 0.8, $CHCl_3$). $^1H$ NMR (400 MHz, $CDCl_3$) δ 8.14 – 7.99 (m, 2H), 7.93 – 7.79 (m, 4H), 7.62 – 7.36 (m, 6H), 5.74 (d, *J* = 3.5 Hz, 1H, $H_1$), 5.28 (dt, *J* = 8.7,

3.1 Hz, 1H, $H_5$), 4.94 (d, *J* = 11.6 Hz, 1H, NAP $CH_2$), 4.78 (d, *J* = 11.7 Hz, 1H, NAP $CH_2$), 4.65 – 4.57 (m, 2H, $H_2$ & $H_{6'}$), 4.52 (dd, *J* = 12.6, 8.6 Hz, 1H, $H_6$), 4.37 (dd, *J* = 8.9, 2.8 Hz, 1H, $H_4$), 4.05 (dd, *J* = 8.9, 4.2 Hz, 1H, $H_3$), 2.95 (s, 3H), 1.62 (s, 3H), 1.37 (s, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 170.0, 166.0, 134.3, 133.3, 133.2, 129.8, 128.5, 128.0, 127.7, 127.2, 126.3, 126.2, 125.9, 113.6 (O,O-isopropylidene), 104.1 ($C_1$), 78.1 ($C_2$), 76.0 ($C_3$), 72.5 (NAP $CH_2$), 62.7 ($C_6$), 38.8 ($C_5$), 26.7, 26.6 (isopropylidene), 20.6 (Acetyl). HRMS *m/z* $[M+Na]^+$ calcd for $C_{28}H_{30}O_9NaS$ 565.1508, found 565.1494.

**3-*O*-(2-Napthyl)methyl-5,6-dideoxy-5,6-epithio-1,2-*O*-isopropyliene-α-D-allofuranose (103)**

At 0 °C, to a stirred solution of compound **102** (0.54 g, 1.0 mmol) in anhydrous methanol (50 mL, 0.02 M) was added NaOMe (64 mg, 1.2 mmol). The reaction was stirred at 0 °C until the completion of the reaction mixture (monitored by TLC). Amberlyst IR 120 was added portionwise to neutralize the reaction mixture (monitored by pH paper). The resin was filtered off and thiourea (76 mg, 1 mmol) was added to the reaction mixture. The reaction was stirred at 80 °C for 3 h before it was concentrated in *vacuo*. The residue was dissolved in DCM and washed by water, brine and dried over $MgSO_4$ and concentrated in *vacuo*. The crude mixture was concentrated in *vacuo* and purified by silica gel chromatography to give product **103** (0.25 g, 70%) as a colorless oil. $[\alpha]^{23}_D$ = +74.2° (c 1.2, $CHCl_3$). $^1$H NMR (400 MHz, $CDCl_3$) δ 7.94 – 7.78 (m, 4H), 7.60 – 7.42 (m, 3H), 5.72 (d, *J* = 3.7 Hz, 1H, $H_1$), 4.95 (d, *J* = 12.1 Hz, 1H, NAP $CH_2$), 4.76 (d, *J* = 12.1 Hz, 1H, NAP $CH_2$), 4.59 (t, *J* = 4.1 Hz, 1H, $H_2$), 4.01 (dd, *J* = 8.6, 6.2 Hz, 1H, $H_4$), 3.72 (dd, *J* = 8.6, 4.4 Hz, 1H, $H_3$), 3.04 – 2.93 (m, 1H, $H_5$), 2.47 (dd, *J*

= 6.5, 1.4 Hz, 1H, $H_{6'}$), 2.39 (dd, *J* = 5.4, 1.4 Hz, 1H, $H_6$), 1.60 (s, 3H), 1.36 (s, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 134.8, 133.2, 133.1, 128.4, 127.9, 127.8, 126.9, 126.4, 126.3, 126.1, 125.9, 113.1 (O,O-isopropylidene), 103.8 ($C_1$), 80.7 ($C_2$), 77.6 ($C_3$), 72.3 (NAP $CH_2$), 43.6 ($C_4$), 30.5 ($C_5$), 26.8, 26.6 (isopropylidene), 20.7 ($C_6$). HRMS *m/z* $[M+Na]^+$ calcd for $C_{20}H_{22}O_4SNa$ 381.1137, found 381.1138.

**3-*O*-(2-Napthyl)methyl-5-*S*-acetyl-6-*O*-acetyl-1,2-*O*-isopropyliene-α-D-allofuranose (104)**

To a stirred solution of compound **103** (0.36 g, 1.0 mmol) in mixture of acetic anhydride (20 mL) and glacial acetic acid (4 mL) was added anhydrous potassium acetate (0.49 g, 5.0 mmol) and refluxed at 145 °C for 12 h. After the reaction mixture was cooled, it was poured into ice water and extracted with chloroform (3×10 mL). The choloroform was collected and washed with aqueous $NaHCO_3$, brine, dried over $MgSO_4$, and concentrated in *vacuo*. The residue was purified over silica gel chromatography to give **104** (0.26 g, 56%) as a colorless oil. $[\alpha]^{23}_D$ = +45.5° (c 0.5, $CHCl_3$). $^1$H NMR (400 MHz, $CDCl_3$) δ 7.90 – 7.79 (m, 4H), 7.57 – 7.40 (m, 3H), 5.69 (d, *J* = 3.7 Hz, 1H), 4.91 (d, *J* = 11.9 Hz, 1H), 4.68 (d, *J* = 11.9 Hz, 1H), 4.57 (t, *J* = 4.1 Hz, 1H), 4.34 – 4.25 (m, 2H), 4.20 (dd, *J* = 11.4, 5.9 Hz, 1H), 4.08 (q, *J* = 5.6 Hz, 1H), 3.78 (dd, *J* = 8.8, 4.4 Hz, 1H), 2.25 (s, 3H), 1.96 (s, 3H), 1.59 (s, 3H), 1.35 (s, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 193.9, 170.5, 134.7, 133.2, 133.1, 128.3, 127.9, 127.7, 126.9, 126.2, 126.1, 125.9, 113.2 (O,O-isopropylidene), 103.8 ($C_1$), 80.2 ($C_2$), 77.9 ($C_4$), 77.5 ($C_3$), 72.4 (NAP $CH_2$), 63.3 ($C_6$), 43.6 ($C_5$), 30.5 (S-acetyl), 26.9, 26.6 (isopropylidene), 20.8 (Acetyl). HRMS *m/z* $[M+Na]^+$ calcd for $C_{24}H_{28}O_7SNa$ 483.1453, found 483.1460.

**3-*O*-(2-Napthyl)methyl-1,2,4,6-tetra-*O*-acetyl-5-thio-α,β-D-allopyranose (105)**

A solution of compound **104** (460 mg, 1.0 mmol) in 20 mL aqueous acetic acid (50%) was stirred at 90 °C for 12 h. The reaction mixture was concentrated in *vacuo* and co-evaporated with toluene (3×10 mL) to remove acetic acid. The crude mixture was dissolved in anhydrous methanol (300 mL) and NaOMe (3.2 mg, 0.06 mmol) was added at 0 °C. The reaction was stirred at 0 °C for 4 h before Amberlyst IR120 resin was added to quench the reaction (monitored by pH paper). After reaction mixture was neutralized, the resin was filtered off and reaction was concentrated in *vacuo*. The residue was dissolved in pyridine (10 mL) and acetic anhydride (4.6 mL, 0.05 mol) was added at 0 °C. The reaction mixture was stirred at room temperature for 12 h before it was concentrated in *vacuo*. The residue was purified by silica gel chromatography to give **105** (227 mg, 45%) as a colorless oil. $^{1}$H NMR (400 MHz, $CDCl_3$) δ 7.90 – 7.77 (m, 5H), 7.48 (ddt, *J* = 8.9, 6.8, 1.7 Hz, 5H), 6.30 (d, *J* = 9.3 Hz, 1H, $H_{1\beta}$), 6.14 (d, *J* = 3.7 Hz, 1H, $H_{1\alpha}$), 5.30 – 5.21 (m, 2H, $H_{4\alpha}$ &$H_{4\beta}$), 5.16 (td, *J* = 10.8, 10.4, 2.3 Hz, 2H, $H_{2\alpha}$ &$H_{2\beta}$), 4.90 – 4.75 (m, 4H, Nap $CH_2$), 4.45 (dd, *J* = 12.1, 5.1 Hz, 1H, $H_{6\alpha'}$), 4.32 (dd, *J* = 12.0, 5.5 Hz, 1H, $H_{6\beta}$), 4.21 – 4.09 (m, 2H, $H_{6\alpha}$& $H_{6\beta}$), 3.99 (ddd, *J* = 10.9, 5.1, 2.7 Hz, 1H, $H_{5\alpha}$), 3.85 (ddd, *J* = 10.2, 5.4, 3.3 Hz, 1H, $H_{5\beta}$), 2.08 (s, 3H), 2.06 (s, 3H), 2.05 (s, 3H), 2.03 (s, 3H), 2.01 (s, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 170.6, 169.9, 169.6, 169.4, 169.1, 135.8, 134.9, 133.1, 133.0, 128.2, 128.0, 127.9, 127.7, 126.8, 126.3, 126.2, 126.0, 125.6, 75.6 ($C_{1\alpha}$), 75.4 ($C_{1\beta}$), 74.0 ($C_2$), 72.6 (NAP $CH_2$), 72.5 (NAP $CH_2$), 71.9, 70.6 ($C_{4\alpha}$), 70.1 ($C_{4\beta}$), 61.7 ($C_{6\alpha}$), 61.2 ($C_{6\beta}$), 40.0 ($C_{5\alpha}$), 35.6 ($C_{5\beta}$), 21.1, 20.7. HRMS *m/z* $[M+Na]^+$ calcd for $C_{25}H_{28}O_9SNa$ 527.1352, found 527.1354.

**4,6-*O*-Benzylidene-3-*O*-(2-napthyl)methyl-1,2-di-*O*-acetyl-5-thio-α,β-D-allopyranose (106)**

To a solution of compound **105** (171 mg, 0.34 mmol) in 10 mL anhydrous MeOH was added NaOMe (3.2 mg, 0.06 mmol). The reaction mixture was stirred at room temperature until the completion and Amberlyst IR 120 was added to neutralize the reaction mixture. The reaction mixture was filtered and concentrated in *vacuo* and was re-dissolved in 4 mL anhydrous MeCN. To the reaction mixture was added camphorsulfonic acid (8.0 mg, 0.03 mmol) and benzaldehyde dimethyl acetal (258 mg, 1.70 mmol). The reaction mixture was stirred at room temperature for 25 min before aqueous $NaHCO_3$ was added. The reaction mixture was extracted by 3 x 10 mL ethyl acetate, the organic layer was washed with brine, dried over $MgSO_4$ and concentrated in *vacuo*. The crude mixture was acetylated and purified by silica gel chromatography to give product **106** (66 mg, 46%) as a colorless oil. $^1$H NMR (400 MHz, $CDCl_3$) δ 7.90 – 7.70 (m, 6H), 7.60 – 7.34 (m, 11H), 6.36 (d, *J* = 9.8 Hz, 1H, $H_{1\beta}$), 6.12 (d, *J* = 3.6 Hz, 1H, $H_{1\alpha}$), 5.66 (s, 1H, α isomer benzylidene), 5.60 (s, 1H, β isomer benzylidene), 5.22 – 5.15 (m, 2H, $H_{2\alpha}$ & $H_{2\beta}$), 5.12 (d, *J* = 12.2 Hz, 1H, α isomer NAP $CH_2$), 5.02 (d, *J* = 12.0 Hz, 1H, β isomer NAP $CH_2$), 4.83 (dd, *J* = 12.1, 6.3 Hz, 2H, α,β isomers NAP $CH_2$), 4.31 (ddd, *J* = 11.6, 7.3, 4.6 Hz, 1H, $H_{6\alpha}$), 4.26 – 3.99 (m, 4H, $H_3$ &$H_4$ &$H_{6\beta}$), 3.94 – 3.87 (m, 1H, $H_{5\beta}$), 3.76 (td, *J* = 11.1, 4.5 Hz, 1H, $H_{5\alpha}$), 2.13 (s, 3H), 2.07 (s, 3H, β isomer acetate), 1.97 (s, 3H), 1.87 (s, 3H, β isomer acetate). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 170.1, 169.8, 169.0, 137.6, 136.5, 133.2, 132.8, 129.3, 128.4, 128.0, 127.9, 127.7, 127.0, 126.3, 126.2, 126.1, 126.0, 125.8, 125.7, 102.1 (Benzylidene), 82.4 ($C_{1\alpha}$), 82.3 ($C_{1\beta}$), 75.1 ($C_4$), 74.9($C_{2\alpha}$),

74.5 ($C_{2\beta}$), 72.1 (NAP $CH_2$), 70.7 ($C_{3\alpha}$), 70.1 ($C_{3\beta}$), 68.5 ($C_6$), 35.2, 31.8($C_{5\alpha}$), 31.6($C_{5\beta}$), 22.7, 21.2, 20.8. HRMS *m/z* $[M+Na]^+$ calcd for $C_{28}H_{28}O_7SNa$ 531.1453, found 531.1454.

**4,6-*O*-Benzylidene-1,2-di-*O*-acetyl-5-thio-α,β-D-allopyranose (107)**

To a solution of compound **106** (68 mg, 0.13 mmol) in 10 mL DCM and 2.5 mL MeOH was added DDQ (92 mg, 0.40 mmol). The reaction was stirred at room temperature for overnight before aqueous $NaHCO_3$ was added to quench the reaction. The reaction mixture was extracted into DCM and washed by water, brine and dried over $MgSO_4$. The crude mixture was concentrated in *vacuo* and purified by silica gel chromatography to give product **107** (41 mg, 83%) as a colorless oil. **α:** $^1$H NMR (400 MHz, $CDCl_3$) δ 7.49 (dd, *J* = 6.8, 2.9 Hz, 2H), 7.38 (dd, *J* = 5.2, 2.0 Hz, 3H), 6.08 (d, *J* = 3.6 Hz, 1H, $H_1$), 5.70 (s, 1H, benzylidene), 5.21 (t, *J* = 3.3 Hz, 1H, $H_2$), 4.38 – 4.30 (m, 2H, $H_3$ & $H_6$), 4.02 (dd, *J* = 9.8, 2.3 Hz, 1H, $H_4$), 3.86 (td, *J* = 10.5, 9.9, 4.3 Hz, 1H, $H_5$), 3.77 (t, *J* = 11.0 Hz, 1H, $H_6$), 2.51 (s, 1H, OH), 2.18 (s, 3H), 2.13 (s, 3H). **β:** $^1$H NMR (400 MHz, $CDCl_3$) δ 7.52 – 7.44 (m, 2H), 7.42 – 7.33 (m, 3H), 6.33 (d, *J* = 9.8 Hz, 1H, $H_1$), 5.65 (s, 1H, benzylidene), 5.26 (dd, *J* = 9.8, 2.6 Hz, 1H, $H_2$), 4.43 – 4.36 (m, 1H, $H_3$), 4.35 – 4.26 (m, 1H, $H_4$), 3.98 (dd, *J* = 9.4, 2.1 Hz, 1H, $H_{6'}$), 3.82 – 3.69 (m, 2H, $H_5$&$H_6$), 2.30 (bs, 1H, OH), 2.12 (s, 3H), 2.08 (s, 3H). **α:** $^{13}$C NMR (101 MHz, $CDCl_3$) δ 169.7, 169.6, 137.1, 129.4, 128.4, 126.2, 102.0 (Benzylidene), 81.3 ($C_1$), 71.3 ($C_2$), 71.2 ($C_4$), 69.4 ($C_3$), 68.3 ($C_6$), 30.8 ($C_5$), 21.2, 20.9. **β:** $^{13}$C NMR (101 MHz, $CDCl_3$) δ 129.3, 128.4, 126.0, 101.8 (Benzylidene), 81.2 ($C_1$), 73.9 ($C_2$), 70.3 ($C_4$), 69.5 ($C_3$), 68.4 ($C_6$), 34.6 ($C_5$), 20.9, 20.7. HRMS *m/z* $[M+Na]^+$ calcd for $C_{17}H_{20}NaO_7S$ 391.0827 found 391.0823

**4,6-*O*-Benzylidene-3-*S*-acetyl-1,2-di-*O*-acetyl-3,5-dithio-α,β-D-glucopyranose (108)**

To a solution of compound **107** (73 mg, 0.20 mmol) in DCM 10 mL was added pyridine (0.12 mL, 1.42 mmol). At 0 °C, trifluoromethanesulfonic anhydride (0.12 mL, 0.71 mmol) was added dropwise. The reaction mixture was stirred at 0 °C for 30 min before it was poured into 8 mL ice-cold water. The mixture was extracted by 3 x 4 mL DCM. The DCM layer was washed by brine, dried over $MgSO_4$ and concentrated in *vacuo*. The crude mixture was dissolved in 4 mL DMF, at 0 °C, the solution of potassium thioacetate (84 mg, 0.73 mmol) in 4 mL DMF was slowly injected. After 1h, the reaction mixture was poured into 50 mL ice water and extracted by 50 mL ethyl acetate. The organic layer was washed with brine, dried over $MgSO_4$ and concentrated in *vacuo*. The crude mixture was purified by silica gel chromatography to give product **108** (56 mg, 66%) as a colorless oil. **β:** $^1$H NMR (400 MHz, $CDCl_3$) δ 7.48 – 7.29 (m, 5H), 5.97 (d, *J* = 8.2 Hz, 1H, $H_1$), 5.58 (s, 1H, Benzylidene), 5.36 (t, *J* = 8.2 Hz, 1H, $H_2$), 4.27 (dd, *J* = 11.1, 4.6 Hz, 1H, $H_{6\ equatorial}$), 3.94 (t, *J* = 10.1 Hz, 1H, $H_4$), 3.84 (dd, *J* = 10.5, 8.3 Hz, 1H, $H_3$), 3.73 (t, *J* = 11.0 Hz, 1H, $H_{6\ axial}$), 3.33 (ddd, *J* = 11.0, 9.7, 4.6 Hz, 1H, $H_5$), 2.33 (s, 3H, S-acetyl), 2.09 (s, 3H, O-acetyl), 2.06 (s, 3H, O-acetyl). **α:**$^1$H NMR (400 MHz, $CDCl_3$) δ 7.52 – 7.31 (m, 5H), 6.10 (d, *J* = 3.0 Hz, 1H, $H_1$), 5.61 (s, 1H, Benzylidene), 5.31 – 5.22 (m, 1H, $H_2$), 4.25 (dd, *J* = 10.9, 4.5 Hz, 1H, $H_{6\ equatorial}$), 4.21 – 4.12 (m, 1H, $H_3$), 3.93 (dd, *J* = 10.9, 9.6 Hz, 1H, $H_4$), 3.73 (t, *J* = 11.2 Hz, 1H, $H_{6\ axial}$), 3.61 – 3.50 (m, 1H, $H_5$), 2.33 (s, 3H, S-acetyl), 2.21 (s, 3H, O-acetyl), 2.01 (s, 3H, O-acetyl). **β:**$^{13}$C NMR (101 MHz, $CDCl_3$) δ 193.1, 169.4, 168.7, 137.1, 129.1, 128.2, 126.0, 101.7 (Benzylidene), 80.3 ($C_1$), 73.1 ($C_2$), 73.0 ($C_4$), 68.6 ($C_6$), 48.4 ($C_3$), 40.1 ($C_5$), 30.5 (S-acetyl), 20.7, 20.6. **α:**$^{13}$C NMR (101 MHz, $CDCl_3$) δ 193.5, 169.7, 169.2, 137.2, 129.0, 128.2, 125.9, 101.7

(Benzylidene), 81.0 ($C_1$), 71.9 ($C_2$), 70.8 ($C_4$), 68.3 ($C_6$), 46.0 ($C_3$), 38.1 ($C_5$), 30.8 (S-acetyl), 21.0, 20.6. HRMS *m/z* $[M+Na]^+$ calcd for $C_{19}H_{22}NaO_7S_2$ 449.0705, found 449.0710.

**2,6-Di-*O*-acetyl-3-*S*,4-*O*-benzylidene-3,5-dithio-α-D-glucopyranosyl bromide (109)**

At 0°C, to a stirred solution of compound **108** (28.5 mg, 0.07 mmol) in DCM (2 mL) was added 33% HBr / AcOH (0.08 mL). The reaction was stirred at room temperature overnight and concentrated in *vacuo*. The crude mixture was purified by silica gel chromatography to give product **109** (11.4 mg, 39%) as a colorless oil. $^1$H NMR (400 MHz, $CDCl_3$) δ 7.51 – 7.31 (m, 5H), 6.18 (s, 1H, O,S-benzylidene), 5.72 (d, *J* = 3.4 Hz, 1H, $H_1$), 4.80 (dd, *J* = 10.7, 3.3 Hz, 1H, $H_2$), 4.60 (dd, *J* = 12.0, 3.5 Hz, 1H, $H_6$), 4.29 (dd, *J* = 12.0, 6.7 Hz, 1H, $H_{6'}$), 4.18 – 3.91 (m, 2H, $H_3$ & $H_4$), 3.76 (ddd, *J* = 10.0, 6.7, 3.5 Hz, 1H, $H_5$), 2.10 (S, 3H), 2.08 (s, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 170.4, 169.7, 138.8, 129.2, 128.6, 126.9, 85.5 ($C_4$), 85.3 (S,O-benzylidene), 76.2 ($C_2$), 62.1 ($C_6$), 54.5 ($C_1$), 51.8 ($C_3$), 42.7 ($C_5$), 20.7 (O-Acetyl). HRMS *m/z* $[M+Na]^+$ calcd for $C_{17}H_{19}BrNaO_5S_2$ 468.9755, found 468.9757.

**1,2,4,6-Tetra-*O*-acetyl-5-thio-α,β-D-allopyranose (110)**

To a solution of compound **105** (115 mg, 0.23 mmol) in 20 mL DCM and 5 mL MeOH was added DDQ (92 mg, 0.40 mmol). The reaction was stirred at room temperature for overnight before aqueous $NaHCO_3$ was added to quench the reaction. The reaction mixture was extracted into DCM and washed by water, brine and dried over $MgSO_4$. The crude mixture was concentrated in *vacuo* and purified by silica gel chromatography to give product **110** (69 mg, 83%) as a colorless oil.**α:** $^1$H NMR (400 MHz, $CDCl_3$) δ 6.09 (d, *J* = 3.6 Hz, 1H), 5.23 – 5.09 (m, 2H), 4.43 (dd, *J* = 12.1, 5.0 Hz, 1H), 4.29 (s, 1H), 4.14 (dd, *J* = 12.1, 3.0 Hz, 1H), 3.83

(ddd, *J* = 11.0, 4.9, 2.9 Hz, 1H), 2.65 (s, 1H), 2.14 (s, 3H), 2.11 (s, 3H), 2.07 (s, 3H), 2.04 (s, 3H). **β:** $^{1}$H NMR (400 MHz, $CDCl_3$) δ 6.23 (d, *J* = 9.0 Hz, 1H), 5.24 (dd, *J* = 9.0, 2.5 Hz, 1H), 5.17 (dd, *J* = 9.9, 2.4 Hz, 1H), 4.39 – 4.26 (m, 2H), 4.17 (dd, *J* = 11.9, 3.8 Hz, 1H), 3.78 (ddd, *J* = 9.6, 5.5, 3.8 Hz, 1H), 2.45 (s, 1H), 2.11 (s, 3H), 2.09 (s, 3H), 2.07 – 2.05 (m, 6H). **α:**$^{13}$C NMR (101 MHz, $CDCl_3$) δ 170.57, 169.5, 169.2, 168.9, 72.1, 71.6, 71.1, 69.9, 61.1, 34.6, 21.1, 20.8, 20.7, 20.6. **β:**$^{13}$C NMR (101 MHz, $CDCl_3$) δ 170.6, 169.6, 169.4, 169.0, 73.5, 71.5, 69.7, 69.2, 61.8, 39.5, 20.8, 20.8, 20.7, 20.6. HRMS *m/z* $[M+Na]^+$ calcd for $C_{14}H_{20}NaO_9S$ 387.0726, found 387.0728.

**3-*S*-Acetyl-1,2,4,6-tetra-*O*-acetyl-3,5-dithio-α,β-D-glucopyranose (111)**

To a solution of compound **110** (39 mg, 0.11 mmol) in 5 mL DCM was added pyridine (0.06 mL, 0.75 mmol). At 0 °C, trifluoromethanesulfonic anhydride (0.05 mL, 0.32 mmol) was added dropwise. The reaction mixture was stirred at 0 °C for 30 min before it was poured into 8 mL ice-cold water. The mixture was extracted by 3 x 4 mL DCM. The DCM layer was washed by brine, dried over $MgSO_4$ and concentrated in *vacuo*. The crude mixture was dissolved in 3 mL DMF, at 0 °C, cesium thioacetate (34 mg, 0.17mmol) added and the reaction mixture was stirred at room temperature. After 1h, the reaction mixture was poured into 20 mL ice water and extracted by 50 mL ethyl acetate. The organic layer was washed with brine, dried over $MgSO_4$ and concentrated in *vacuo*. The crude mixture was purified by silica gel chromatography to give product **111** (25 mg, 55%) as a colorless oil. **β:** $^{1}$H NMR (400 MHz, $CDCl_3$) δ 5.90 (d, *J* = 8.0 Hz, 1H, $H_1$), 5.31 (dd, *J* = 9.3, 8.0 Hz, 1H, $H_2$), 5.25 (t, *J* = 10.0 Hz, 1H, $H_4$), 4.27 (dd, *J* = 11.9, 5.8 Hz, 1H, $H_{6'}$), 4.17 – 4.07 (m, 1H, $H_6$), 3.81 (dd,

*J* = 10.3, 9.3 Hz, 1H, $H_3$), 3.38 (ddd, *J* = 9.7, 5.7, 4.0 Hz, 1H, $H_5$), 2.33 (s, 3H, S-acetyl), 2.08 (s, 3H, O-acetyl), 2.06 (s, 3H, O-acetyl), 2.02 (d, *J* = 1.2 Hz, 6H, O-acetyl). **α:** $^1$H NMR (400 MHz, $CDCl_3$) δ 6.09 (d, *J* = 3.6 Hz, 1H, $H_1$), 5.19 (dd, *J* = 3.7, 2.8 Hz, 1H, $H_2$), 5.15 (dd, *J* = 11.0, 2.4 Hz, 1H, $H_4$), 4.43 (dd, *J* = 12.1, 5.0 Hz, 1H, $H_6$), 4.29 (s, 1H), 4.14 (dd, *J* = 12.1, 3.0 Hz, 1H, $H_{6'}$), 3.83 (ddd, *J* = 11.0, 4.9, 2.9 Hz, 1H), 2.65 (s, 1H). 2.14 (s, 3H), 2.11 (s, 3H), 2.07 (s, 3H), 2.04 (s, 3H). **β:** $^{13}$C NMR (101 MHz, $CDCl_3$) δ 192.8, 170.5, 169.4, 169.2, 168.7, 72.8 ($C_2$ & $C_1$), 70.7 ($C_4$), 62.2 ($C_6$), 48.6 ($C_3$), 43.8 ($C_5$), 30.5 (S-acetyl), 20.7 (O-acetyl), 20.6 (O-acetyl). **α:**$^{13}$C NMR (101 MHz, $CDCl_3$) δ 170.5, 169.5, 169.4, 168.9, 72.1 ($C_1$), 71.6 ($C_2$), 70.0 ($C_4$), 61.1 ($C_6$), 47.6 ($C_3$), 42.5 ($C_5$), 30.6 (S-acetyl), 20.7 (O-acetyl), 20.6 (O-acetyl), 20.5 (O-acetyl). HRMS *m/z* $[M+Na]^+$ calcd for $C_{14}H_{19}NaO_7S_2Br$ 464.9653, found 464.9653.

**3-*S*-Acetyl-1,2,4,6-tetra-*O*-acetyl-3,5-dithio-α,β-D-glucopyranosyl Bromide (112)**

Compound **111** (25 mg, 0.06 mmol) was subjected to bromination according to general procedure D to give the product **112** (20 mg, 76%) as a colorless oil. **α:** $^1$H NMR (400 MHz, Chloroform-*d*) δ 5.53 (d, *J* = 3.5 Hz, 1H, $H_1$), 5.30 (t, *J* = 10.7 Hz, 1H, $H_4$), 4.94 (dd, *J* = 11.2, 3.5 Hz, 1H, $H_2$), 4.41 (dd, *J* = 12.2, 4.9 Hz, 1H, $H_6$), 4.23 – 4.04 (m, 2H, $H_3$ & $H_{6'}$), 3.67 (dd, *J* = 10.4, 1.9 Hz, 1H, $H_5$), 2.34 (s, 3H, S-acetyl), 2.06 (s, 6H), 2.04 (s, 3H). **β:** $[\alpha]^{23}_D$ = -6.3°(c 1.1, $CHCl_3$).$^1$H NMR (400 MHz, $CDCl_3$) δ 5.36 – 5.21 (m, 2H, $H_2$ & $H_4$), 4.75 (d, *J* = 9.7 Hz, 1H, $H_1$), 4.22 (dd, *J* = 12.1, 5.7 Hz, 1H, $H_6$), 4.17 – 4.08 (m, 1H, $H_{6'}$), 3.71 (t, *J* = 10.9 Hz, 1H, $H_3$), 3.32 (ddd, *J* = 10.3, 5.7, 3.3 Hz, 1H, $H_5$), 2.33 (s, 3H), 2.07 (s, 3H), 2.05 (s, 3H), 2.00 (s, 3H). **α:** $^{13}$C NMR (101 MHz, $CDCl_3$) δ 192.8, 170.5, 169.5, 169.4, 73.3 ($C_2$), 70.4 ($C_4$), 61.1

($C_6$), 54.6 ($C_3$), 47.6 ($C_1$), 42.5 ($C_5$), 30.6 (S-acetyl), 20.7, 20.6, 20.5. **β:** $^{13}$C NMR (101 MHz, $CDCl_3$) δ 192.9, 170.5, 169.2, 169.0, 75.0 ($C_2$), 70.7 ($C_4$), 61.3 ($C_6$), 51.0 ($C_3$), 48.2 ($C_1$), 45.5 ($C_5$), 30.5 (S-acetyl), 20.6, 20.5, 20.5. HRMS *m/z* $[M+Na]^+$ calcd for $C_{14}H_{19}NaO_7S_2Br$ 464.9653, found 464.9649.

**3-Deoxy-1,2,4,6-tetra-*O*-acetyl-3,5-dithio-α,β-D-glucopyranose (113)**

At 0 °C, to a solution of **111** (52 mg, 0.13 mmol) in 5 mL MeCN was added hydrazine monohydrate (8 mg, 0.25 mmol). The reaction mixture was stirred at 0 °C for 1h and quenched with acetone. The crude mixture was concentrated in *vacuo* and purifided by silica gel chromatography to give **113** (6.5 mg, 13%) and **114** (20 mg, 45%) as a colorless oil. Compound **113**: $^1$H NMR (400 MHz, $CDCl_3$) δ 5.82 (d, *J* = 8.7 Hz, 1H, $H_1$), 5.23 (dd, *J* = 10.3, 8.7 Hz, 1H, $H_2$), 5.12 (t, *J* = 10.2 Hz, 1H, $H_4$), 4.26 (dd, *J* = 11.9, 5.8 Hz, 1H, $H_6$), 4.13 (dd, *J* = 11.8, 3.7 Hz, 1H, $H_{6'}$), 3.26 (ddd, *J* = 9.6, 5.7, 3.6 Hz, 1H, $H_5$), 2.86 (q, *J* = 10.1 Hz, 1H, $H_3$), 2.13 (s, 3H), 2.10 (s, 3H), 2.07 (d, *J* = 3.2 Hz, 6H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 170.5, 169.7, 169.5, 168.9, 75.9 ($C_1$), 73.8 ($C_2$), 72.7 ($C_4$), 62.1 ($C_6$), 46.9 ($C_5$), 44.3 ($C_3$), 20.7, 20.7. HRMS *m/z* $[M+Na]^+$ calcd for $C_{14}H_{20}NaO_8S_2$ 403.0497, found 403.0495.

**3-Deoxy-2,4,6-tri-*O*-acetyl-3,5-dithio-α,β-D-glucopyranose (114)**

Compound **114** was prepared in the previous experiment: $^1$H NMR (400 MHz, $CDCl_3$) δ 5.20 – 5.04 (m, 3H, $H_1$ & $H_2$ & $H_4$), 4.32 (dd, *J* = 12.0, 5.1 Hz, 1H, $H_6$), 4.07 (dd, *J* = 12.0, 3.3 Hz, 1H, $H_{6'}$), 3.58 (ddd, *J* = 10.5, 5.2, 3.3 Hz, 1H, $H_5$), 3.44 – 3.31 (m, 1H, $H_3$), 2.16 (s, 3H), 2.13 (s, 3H), 2.07 (s, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 170.7, 170.0, 169.9, 74.4 ($C_2$),

71.3 ($C_1$ & $C_4$), 61.9 ($C_6$), 43.1 ($C_3$), 39.5 ($C_5$), 20.9, 20.7. HRMS *m/z* $[M+Na]^+$ calcd for $C_{12}H_{28}NaO_7S_2$ 361.0392, found 361.0395.

**3-*S*-Acetyl-2,4,6-tri-*O*-acetyl-3,5-dithio-α-D-glucopyranose (115)**

To a solution of compound **112** (16 mg, 0.04 mmol) in 10 mL aqueous acetone (50%) was added TBAI (66 mg, 0.18 mmol). After the reaction was stirred at room temperature overnight, it was diluted with 10 mL ethyl acetate and washed with water, brine and dried over $MgSO_4$. The reaction mixture was concentrated in *vacuo* and purified by silica gel chromatography to give product **115** (8.4 mg, 62%) as a colorless oil. $[\alpha]^{23}_D$ = +80.7°(c 0.3, $CHCl_3$). $^1$H NMR (400 MHz, $CDCl_3$) δ 5.27 (t, *J* = 10.7 Hz, 1H, $H_2$), 5.21 (dd, *J* = 11.6, 2.8 Hz, 1H, $H_4$), 5.09 (d, *J* = 2.8 Hz, 1H, $H_1$), 4.34 (dd, *J* = 12.0, 5.1 Hz, 1H, $H_6$), 4.21 (t, *J* = 11.2 Hz, 1H, $H_3$), 4.06 (dd, *J* = 12.0, 3.3 Hz, 1H, $H_{6'}$), 3.69 (ddd, *J* = 10.6, 5.1, 3.3 Hz, 1H, $H_5$), 2.49 (s, 1H, OH), 2.33 (s, 3H, S-acetyl), 2.06 (S, 6H), 2.02 (s, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 193.2, 170.6, 169.6, 169.5, 74.1 ($C_1$ & $C_2$), 71.3 ($C_4$), 61.8 ($C_6$), 46.2 ($C_3$), 39.5 ($C_5$), 30.6 (S-acetyl), 20.8, 20.7, 20.5. HRMS *m/z* $[M+Na]^+$ calcd for $C_{14}H_{20}NaO_8S_2$ 403.0497, found 403.0497.

**3-*S*-Acetyl-2,4,6-tri-*O*-acetyl-3,5-dithio-α-D-glucopyranosyl Trichloroacetimidate (116)**

To a solution of compound **115** (16 mg, 0.04 mmol) in 2 mL DCM was added trichloroacetonitrile (6 mg, 0.4 mmol). At 0 °C, DBU (6 mg, 0.04 mmol) was added into reaction mixture and the reaction mixture was stirred for 30 min before it was concentrated in *vacuo*. The crude mixture was purified by basified silica gel chromatography to give product **119** as a colorless oil. $^1$H NMR (400 MHz, $CDCl_3$) δ 8.68 (s, 1H, trichloroacetimidate NH),

6.31 (d, *J* = 3.0 Hz, 1H, $H_1$), 5.42 – 5.25 (m, 2H, $H_2$ & $H_4$), 4.35 (dd, *J* = 12.1, 4.9 Hz, 1H, $H_{6'}$), 4.23 (t, *J* = 11.3 Hz, 1H, $H_3$), 4.06 (dd, *J* = 12.1, 3.2 Hz, 1H, $H_6$), 3.66 – 3.58 (m, 1H, $H_5$), 2.33 (s, 3H, S-acetyl), 2.06 (s, 3H), 2.04 (s, 3H), 1.98 (s, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 192.8 (trichloroacetimidate), 170.5, 169.5, 169.4, 160.8, 75.5 ($C_1$), 72.7 ($C_2$), 70.6 ($C_4$), 61.4 ($C_6$), 46.8 ($C_3$), 41.1 ($C_5$), 30.6 (S-acetyl), 20.6, 20.5. HRMS *m/z* $[M+Na]^+$ calcd for $C_{16}H_{20}NCl_3NaO_8S_2$ 545.9594, found 545.9597.

**3-*O*-(2-Naphthyl)methyl-1,2-*O*-isopropylidene-5-*S*,6-*O*-isopropylidene-α-D-allofuranose (117)**

To a solution of compound **105** (13 mg, 0.04 mmol) in anhydrous acetone was added *p*-toluenesulfonic acid (9 mg, 0.05 mmol). The reaction was stirred at room temperature overnight before $NaHCO_3$ was added to quench the reaction. The reaction mixture was extracted into ethyl acetate and washed with water, brine and dried over $MgSO_4$. The crude mixture was concentrated in *vacuo* and purified by silica gel chromatography to give product **117** (12 mg, 73%) as a colorless oil. $^1$H NMR (400 MHz, $CDCl_3$) δ 7.88 – 7.74 (m, 4H), 7.53 – 7.40 (m, 3H), 5.94 (d, *J* = 3.8 Hz, 1H, $H_1$), 4.78 (d, *J* = 11.5 Hz, 1H, NAP $CH_2$), 4.69 (d, *J* = 11.5 Hz, 1H, NAP $CH_2$), 4.63 (d, *J* = 3.8 Hz, 1H, $H_2$), 4.41 (dd, *J* = 9.9, 2.5 Hz, 1H, $H_6$), 4.27 (dd, *J* = 10.4, 3.2 Hz, 1H), 4.16 (dd, *J* = 9.9, 5.0 Hz, 1H, $H_{6'}$), 4.02 (d, *J* = 3.2 Hz, 1H, $H_3$), 3.87 (ddd, *J* = 10.4, 4.9, 2.4 Hz, 1H, $H_5$), 1.68 (s, 3H), 1.62 (s, 3H), 1.52 (s, 3H), 1.32 (s, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 155.2, 133.2, 128.7, 128.2, 127.9, 127.7, 127.0, 126.2, 113.1(O,O-isopropylidene), 103.9 ($C_1$), 92.6 (S,O-isopropylidene), 80.0 ($C_2$), 79.4 ($C_3$), 77.8 ($C_4$), 72.6 (NAP $CH_2$), 71.1 ($C_6$), 52.7 ($C_5$), 31.0, 30.6, 26.9, 26.6 (isopropylidene).

**3-*O*-Trifluoromethanesulfonyl-1,2-*O*-5-*S*,6-*O*-di-isopropylidene-5-thio-α-D-allofuranose (119)**

At 0 °C, to a stirred solution of compound **118** (2.0 g, 7.2 mmol) and pyridine (5.8 mL, 72 mmol) was added trifluoromethanesulfonic anhydride (1.8 mL, 10.8 mmol) dropwise. The reaction mixture was stirred at 0 °C for 30min before ice cold aqueous $NaHCO_3$ was added. The reaction mixture was diluted in DCM, washed by water, brine and dried over $MgSO_4$. The reaction mixture was concentrated in *vacuo* and purified by silica gel chromatography with eluent (10 : 1 = Hexane : Ethyl Acetate) to give product **119** as a white solid (2.5 g, 85%). m.p. 79-80 °C. $[\alpha]^{23}_D$ = +15.0° (c 0.6, $CHCl_3$). $^1$H NMR (600 MHz, $CDCl_3$) δ 5.78 (d, $J_{1,2}$ = 3.9 Hz, 1H, $H_1$), 4.87 (dd, $J_{3,4}$ = 7.6 Hz, $J_{3,2}$ = 5.3 Hz, 1H, $H_3$), 4.73 (t, $J$ = 4.6 Hz, 1H, $H_2$), 4.32 (t, $J$ = 7.2 Hz, 1H, $H_4$), 4.25 (dd, $J_{6,6'}$ = 10.3 Hz, $J_{6,5}$ = 2.5 Hz, 1H, $H_6$), 4.13 (dd, $J_{6,6'}$ = 10.3 Hz, $J_{6,5}$ = 5.8 Hz, 1H, $H_{6'}$), 3.79 (m, 1H, $H_5$), 1.69 (s, 3H), 1.60 (s, 3H), 1.56 (s, 3H), 1.36 (s, 3H). $^{13}$C NMR (151 MHz, $CDCl_3$) δ 118.3 ($CF_3$), 114.2 (isopropylidene C), 103.8 ($C_1$), 93.2 (isopropylidene C), 83.2 ($C_3$), 79.0 ($C_4$), 77.6 ($C_2$), 70.6 ($C_6$), 51.7 ($C_5$), 30.7, 30.4, 26.8, 26.5 (isopropylidene $CH_3$). HRMS *m/z* $[M+Na]^+$ calcd for $C_{13}H_{19}O_7F_3NaS_2$ 431.0422, found 431.0424.

**1,3-Di-*S*-acetyl-2,4,6-tri-*O*-acetyl-1,3,5-trithio-β-D-glucopyranose (120)**

Compound **112** (161 mg, 0.37 mmol) was applied to general procedure E to give product **124** (80 mg, 50%) as a colorless oil. $[\alpha]^{23}_D$ = +157.2°(c 0.9, $CHCl_3$).$^1$H NMR (400 MHz, $CDCl_3$) δ 5.21 (dt, $J$ = 14.7, 10.7 Hz, 2H, $H_1$ & $H_2$), 4.68 (d, $J$ = 10.7 Hz, 1H, $H_4$), 4.24 (dd, $J$ = 12.0, 5.4 Hz, 1H, $H_6$), 4.10 (dd, $J$ = 12.1, 3.3 Hz, 1H, $H_{6'}$), 3.76 (t, $J$ = 10.9 Hz, 1H,

$H_3$), 3.40 (ddd, *J* = 9.2, 5.4, 3.2 Hz, 1H, $H_5$), 2.36 (s, 3H), 2.32 (s, 3H), 2.07 – 2.04 (m, 3H), 2.01 (s, 3H), 1.98 (s, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 192.8, 191.5, 170.5, 169.4, 169.3, 72.1, 71.9, 70.8, 61.5, 51.8, 47.1, 46.0, 30.5, 30.5, 20.6, 20.5, 20.4. HRMS *m/z* $[M+Na]^+$ calcd for $C_{16}H_{22}NaO_8S_3$ 461.0375, found 461.0374.

**3-*S*-Acetyl-2,4,6-tri-*O*-acetyl-3,5-dithio-β-D-glucopyranosyl-(1 → 3)-1,2-*O*-5-*S*,6-*O*-di-isopropylidene-3,5-dideoxy-3,5-dithio-α-D-glucofuranose (121)**

Compound **120** (4.0 mg, 9.0 μmol) and compound **119** (6.5 mg, 16 μmol) was subjected to coupling reaction according to general procedure A to give product **121** (2.7 mg, 46%) as a colorless oil. $[\alpha]^{23}_D$ = -22.8°(c 1.0, $CHCl_3$). $^1$H NMR (400 MHz, $CDCl_3$) δ 5.85 (d, *J* = 3.6 Hz, 1H), 5.22 (t, *J* = 10.7 Hz, 1H), 5.07 (t, *J* = 10.7 Hz, 1H), 4.70 (d, *J* = 3.6 Hz, 1H), 4.39 (dd, *J* = 10.5, 3.9 Hz, 1H), 4.32 (dd, *J* = 10.0, 2.3 Hz, 1H), 4.25 (dd, *J* = 12.0, 5.1 Hz, 1H), 4.15 – 4.02 (m, 2H), 3.96 (d, *J* = 10.5 Hz, 1H), 3.74 (t, *J* = 10.9 Hz, 1H), 3.62 (ddd, *J* = 10.4, 4.9, 2.3 Hz, 1H), 3.58 (d, *J* = 4.0 Hz, 1H), 3.22 (ddd, *J* = 10.2, 5.1, 3.3 Hz, 1H), 2.32 (s, 3H), 2.06 (s, 3H), 2.03 (d, *J* = 2.0 Hz, 4H), 1.98 (s, 3H), 1.66 (s, 3H), 1.58 (s, 3H), 1.52 (s, 3H), 1.34 (s, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 193.2, 170.5, 169.3, 169.2, 112.3, 105.3, 92.6, 86.0, 81.4, 73.1, 72.0, 71.0, 61.6, 53.5, 51.8, 50.6, 50.0, 47.0, 31.6, 31.6, 30.7, 30.6, 26.7, 26.4, 20.7, 20.6, 20.5.

**1-*S*-Acetyl-2,3,4,6-tetra-*O*-acetyl-1,5-dithio-β-D-glucopyranose (128)**

Compound **126** (4.95 g, 12.2 mmol) was subjected to bromination according to general procedure D to give an α,β mixture of anomeric bromides. The crude product was subjected to thioacylation according to general procedure E to give the α,β mixture of anomeric thioacetate, the β isomer is isolated by silica gel chromatography with eluent (Hexane : Ethyl Acetate =

3:1) to give **128** (2.36 g, 46%) as a colorless solid. m.p. 121-122 °C. $[\alpha]^{23}_D$ = +66.1° (c 0.6, $CHCl_3$). $^1H$ NMR (600 MHz, $CDCl_3$) δ 5.26 (dd, $J_{4,5}$ = 10.7 Hz, $J_{4,3}$ = 9.6 Hz, 1H, $H_4$), 5.22 (dd, $J_{2,1}$ = 11.0 Hz, $J_{2,3}$ = 9.4 Hz, 1H, $H_2$), 5.08 (t, $J$ = 9.5 Hz, 1H, $H_3$), 4.66 (d, $J_{1,2}$ = 11.0 Hz, 1H, $H_1$), 4.25 (dd, $J_{6,6'}$ = 12.1 Hz, $J_{6,5}$ = 5.4 Hz, 1H, $H_6$), 4.09 (dd, $J_{6',6}$ = 12.1 Hz, $J_{6',5}$ = 3.2 Hz, 1H, $H_{6'}$), 3.41 – 3.35 (m, 1H, $H_5$), 2.35 (s, 3H, $SCOCH_3$), 2.05 (s, 3H), 2.01 (s, 3H), 1.98 (s, 3H), 1.97 (s, 3H).$^{13}C$ NMR (151 MHz, $CDCl_3$) δ 191.4 ($SCOCH_3$), 170.5, 169.5, 169.3 ($OCOCH_3$), 74.4 ($C_3$), 72.4 ($C_2$), 71.5 ($C_4$), 61.0 ($C_6$), 44.9 ($C_1$), 44.0 ($C_5$), 30.5 ($SCOCH_3$), 20.6, 20.5, 20.4, 20.4 ($OCOCH_3$). HRMS *m/z* $[M+Na]^+$ calcd for $C_{16}H_{22}O_9NaS_2$ 445.0603, found 445.0605.

**2,3,4,6-Tetra-*O*-acetyl-5-thio-β-D-glucopyranosyl-(1 → 3)-1,2-*O*-5-*S*,6-*O*-di-isopropylidene-3,5-dideoxy-3,5-dithio-α-D-glucofuranose (130)**

Compound **128** (0.66 g, 1.5 mmol) and **119** (0.73 g, 1.8 mmol) was subjected to coupling according to general procedure A. The crude mixture was purified by silica gel chromatography with eluent (1 : 4 = Ethyl Acetate : Hexane) to give product **130** (0.75 g, 78%) as a colorless solid. m.p. 161-162 °C. $[\alpha]^{23}_D$ = +20.0° (c 0.6, $CHCl_3$). $^1H$ NMR (400 MHz, $CDCl_3$) δ 5.85 (d, $J_{1b, 2b}$ = 3.6 Hz, 1H, $H_{1b}$), 5.29 (dd, $J_{4a, 5a}$ = 10.7 Hz, $J_{4a, 3a}$ = 9.3 Hz, 1H, $H_{4a}$), 5.13 (dd, $J_{2a, 1a}$ = 10.6 Hz, $J_{2a, 3a}$ = 9.4 Hz, 1H, $H_{2a}$), 5.05 (t, $J$ = 9.4 Hz, 1H, $H_{3a}$), 4.69 (d, $J_{2b, 1b}$ = 3.6 Hz, 1H, $H_{2b}$), 4.39 (dd, $J_{4b, 5b}$ = 10.5 Hz, $J_{4b, 3b}$ = 4.0 Hz, 1H, $H_{4b}$), 4.34 – 4.26 (m, 2H, $H_{6a\&6b}$), 4.15 – 4.06 (m, 2H, $H_{6'a\&6'b}$), 3.98 (d, $J_{1a, 2a}$ = 10.6 Hz, 1H, $H_{1a}$), 3.65– 3.60 (m, 1H, $H_{5b}$), 3.57 (d, $J_{3b, 4b}$ = 4.0 Hz, 1H, $H_{3b}$), 3.23 – 3.18 (m, 1H, $H_{5a}$), 2.07 (s, 3H), 2.06 (s, 3H), 2.01 (s, 3H), 1.99 (s, 3H), 1.66 (s, 3H), 1.59 (s, 3H), 1.53 (s, 3H), 1.34 (s, 3H). $^{13}C$ NMR (101

MHz, $CDCl_3$) δ 170.5, 169.7, 169.3, 169.2 ($OCOCH_3$), 112.3 (isopropylidene C), 105.3 ($C_{1b}$), 92.7 (isopropylidene C), 86.0 ($C_{2b}$), 81.4 ($C_{4b}$), 74.5 ($C_{3a}$), 73.7 ($C_{2a}$), 72.0 ($C_{6b}$), 71.6 ($C_{4a}$), 61.0 ($C_{6a}$), 53.4 ($C_{3b}$), 50.6 ($C_{5b}$), 47.9 ($C_{1a}$), 44.6 ($C_{5a}$), 31.6, 30.7, 26.7, 26.4 (isopropylidene $CH_3$), 20.6, 20.6, 20.5, 20.4 ($OCOCH_3$). HRMS *m/z* $[M+Na]^+$ calcd for $C_{26}H_{38}O_{12}NaS_3$ 661.1423, found 661.1423.

**5-Thio-β-D-glucopyranosyl-(1 → 3)-1,2-*O*-5-*S*,6-*O*-di-isopropylidene-3,5-dideoxy-3,5-dithio-α-D-glucofuranose (131)**

Compound **130** (800 mg, 1.2 mmol) was subjected to deacylation according to general procedure B to give the product **131** (590 mg, quantitative) as a colorless solid. $[\alpha]^{23}_D$ = +43.7° (c 1.4, MeOH).$^1$H NMR (600 MHz, $CD_3OD$) δ 5.87 (d, $J_{1b,\ 2b}$ = 3.6 Hz, 1H, $H_{1b}$), 4.89 (d, $J_{2b,\ 1b}$ = 3.6 Hz, 1H, $H_{2b}$), 4.36 (dd, $J_{4b,\ 5b}$ = 10.5 Hz, $J_{4b,\ 3b}$ = 3.9 Hz, 1H, $H_{4b}$), 4.25 (dd, $J_{6b,\ 6'b}$ = 9.9 Hz, $J_{6b,\ 5b}$ = 2.2 Hz, 1H, $H_{6b}$), 4.09 (dd, $J_{6'b,\ 6b}$ = 9.9 Hz, $J_{6'b,\ 5b}$ = 4.8 Hz, 1H, $H_{6'b}$), 3.89 (dd, $J_{6a,6'a}$ = 11.4 Hz, $J_{6a,\ 5a}$ = 4.3 Hz, 1H, $H_{6a}$), 3.82 (d, $J_{1a,\ 2a}$ = 10.2 Hz, 1H, $H_{1a}$), 3.75 (dd, $J_{6'a,\ 6a}$ = 11.4 Hz, $J_{6'a,\ 5a}$ = 6.0 Hz, 1H, $H_{6'a}$), 3.63 (d, $J_{3b,\ 2b}$ = 3.9 Hz, 1H, $H_{3b}$), 3.62 – 3.60 (m, 1H, $H_{5b}$) 3.53 (t, *J* = 9.5 Hz, 1H, $H_{4a}$), 3.35 (dd, $J_{2a,\ 1a}$ = 10.3 Hz, $J_{2a,\ 3a}$ = 8.7 Hz, 1H, 3H, $H_{2a}$), 3.16 (t, *J* = 8.8 Hz, 1H, $H_{3a}$) 2.90 – 2.86 (m, 1H, $H_{5a}$) 1.62 (s, 3H), 1.55 (s, 3H), 1.46 (s, 3H), 1.30 (s, 3H). $^{13}$C NMR (151 MHz, $CD_3OD$) δ 111.6 (isopropylidene C), 105.4 ($C_{1b}$), 91.9 (isopropylidene C), 85.6 ($C_{2b}$), 81.1 ($C_{4b}$), 79.4 ($C_{3a}$), 76.3 ($C_{2a}$), 74.8 ($C_{4a}$), 71.6 ($C_{6b}$), 61.5 ($C_{6a}$), 52.2 ($C_{3b}$), 50.5 ($C_{5b}$), 49.6 ($C_{5a}$), 49.2 ($C_{1a}$), 30.7, 29.6, 25.5, 25.1 (isopropylidene $CH_3$). HRMS *m/z* $[M+Na]^+$ calcd for $C_{18}H_{30}O_8S_3Na$ 493.1000, found 493.0999.

**2,3,4,6-Tetra-*O*-acetyl-5-thio-β-D-glucopyranosyl-(1 → 3)-2,4,6-tri-*O*-acetyl-3,5-dideoxy-3,5-dithio-α,β-D-glucopyranose (132)**

Compound **130** (590 mg, 1.2 mmol) was deprotected according to general procedure C to give the product **132** (830 mg, 91%) as a colorless oil. $[\alpha]^{23}_{D}$ = +94.6° (c 0.1, $CHCl_3$). α:β = 3:1 from NMR integration of $H_{1b}$ signals. $^1$H NMR (600 MHz, $CDCl_3$) δ 6.02 (d, *J* = 3.1 Hz, 1H, $H_{1b\alpha}$), 5.84 (d, *J* = 8.4Hz, 1H, $H_{1b\beta}$), 5.37 (dd, $J_{1,2}$ = 9.6 Hz, $J_{2,3}$ = 8.4 Hz, 1H, $H_{2b\beta}$), 5.31 – 5.21 (m, 2H, $H_{4a}$& $H_{2b\alpha}$), 5.15 – 4.94 (m, 3H, $H_{2a}$& $H_{3a}$& $H_{4b}$), 4.36 – 4.01 (m, 5H, $H_{6a,6'a}$& $H_{6b,6'b}$& $H_{1a}$), 3.54 – 3.49 (m, 1H, $H_{5b\alpha}$), 3.36 – 3.22 (m, 2H, $H_{5a}$ & $H_{3b\alpha}$), 3.20 – 3.17 (m, 1H, $H_{5b\beta}$), 3.05 (t, *J* = 10.1 Hz, 1H, $H_{3b\beta}$), 2.20 (s, 3H), 2.15 (s, 3H), 2.12 (s, H), 2.08 (s, 3H), 2.05 (s, 3H), 2.00 (s, 3H), 1.98 (s, 3H). 1.97 (s, 3H). $^{13}$C NMR (151 MHz, $CDCl_3$) δ 170.5, 169.7, 169.2, 169.1, 168.9 (Carbonyl C), 75.8 ($C_{2b}$), 74.2 ($C_{3a}$), 73.2 ($C_{2a}$), 71.8 ($C_{4a}$), 70.5 ($C_{4b}$& $C_{1b}$), 61.5 ($C_{6b}$), 61.1 ($C_{6a}$), 50.3 ($C_{1a}$), 49.9 ($C_{3b}$), 44.5 ($C_{5a}$), 40.6 ($C_{5b}$), 21.1, 20.8, 20.6, 20.5, 20.4, 20.3 (Acetyl $CH_3$). HRMS *m/z* $[M+Na]^+$ calcd for $C_{28}H_{38}O_{16}S_3Na$ 749.1220, found 749.1223.

**5-Thio-β-D-glucopyranosyl-(1 → 3)-3,5-dideoxy-3,5-dithio-α,β-D-glucopyranose (133)**

Compound **132** (20 mg, 0.03 mmol) was subjected to deacetylation according to general procedure B to give **133** (8 mg, 75%) as a white solid. $[\alpha]^{23}_{D}$ = +102.8° (c 1.3, $H_2O$). α:β = 4:1 from NMR integration of $C_{1b}$ signals. $^1$H NMR (600 MHz, $D_2O$) δ 4.86 (d, *J* = 3.0 Hz, 1H, $H_{1b\alpha}$), 4.60 (d, *J* = 8.9Hz, 1H, $H_{1b\beta}$), 3.95 (d, *J* = 10.4 Hz, 1H, $H_{1a}$), 3.82 – 3.62 (m, 5H, $H_{2b}$& $H_{6a}$& $H_{6b}$), 3.54 – 3.41 (m, 2H, $H_{4a}$&$H_{4b}$), 3.34 (dd, $J_{2,1}$ = 11.8 Hz, $J_{2,3}$ = 9.8 Hz, 1H, $H_{2a}$), 3.17 (t, *J* = 9.8Hz, 1H, $H_{3a}$), 3.12 – 3.09 (m, 1H, $H_{5b\alpha}$), 3.00 (t, *J* = 10.7 Hz, 1H, $H_{3b\alpha}$), 2.94–2.91 (m, 1H, $H_{5b\beta}$), 2.90–2.84 (m, 1H, $H_{5a}$), 2.67 (t, *J* = 10.4 Hz, 1H, $H_{3b\beta}$). $^{13}$C NMR (151 MHz,

$D_2O$) δ 77.8 ($C_{3a}$), 77.2 ($C_{2b\beta}$) 76.2 ($C_{2a}$), 75.0 ($C_{1b\beta}$) 74.6 ($C_{2b\alpha}$), 72.9 ($C_{4a}$), 72.4 ($C_{1b\alpha}$), 70.8 ($C_{4b}$), 60.7 ($C_{6b\beta}$), 60.5 ($C_{6b\alpha}$), 60.0 ($C_{6a}$), 57.5 ($C_{3b\beta}$) 54.4 ($C_{3b\alpha}$), 48.59 ($C_{1a}$), 48.56 ($C_{5a}$), 48.1 ($C_{5b\beta}$), 43.4 ($C_{5b\alpha}$). HRMS *m/z* $[M+Na]^+$ calcd for $C_{12}H_{22}O_8NaS_3$ 413.0374, found 413.0374.

**2,3,4,6-Tetra-*O*-acetyl-5-thio-β-D-glucopyranosyl-(1 → 3)-2,4,6-tri-*O*-acetyl-3,5-dideoxy-3,5-dithio-α-D-glucopyranosyl bromide (134)**

Compound **132** (246 mg, 0.34 mmol) was subjected to bromination according to general procedure D to give product **134** (184 mg, 73%) as a colorless oil. $[\alpha]^{23}_D$ = +112.0° (c 1.0, $CHCl_3$). $^1$H NMR (600 MHz, $CDCl_3$) δ 5.44 (d, $J_{1b,2b}$ = 3.4 Hz, 1H, $H_{1b}$), 5.26 (dd, $J_{4a,3a}$ = 10.7 Hz, $J_{4a,5a}$ = 9.6 Hz, 1H, $H_{4a}$), 5.09 (t, *J* = 10.7 Hz, 1H, $H_{3a}$), 5.05 (t, *J* = 10.9 Hz, 1H, $H_{2a}$), 5.00 (t, *J* = 9.5 Hz, 1H, $H_{4a}$), 4.92 (dd, $J_{2b,3b}$ = 10.9 Hz, $J_{2b,1b}$ = 3.5 Hz, 1H, $H_{2b}$), 4.36 (dd, $J_{6b,6'b}$ = 12.2 Hz, $J_{6b,5b}$ = 4.9 Hz, 1H, $H_{6b}$), 4.25 (dd, $J_{6a,6'a}$ = 12.0 Hz, $J_{6a,5a}$ = 5.0 Hz, 1H, $H_{6a}$), 4.13 (dd, $J_{6a,6'a}$ = 12.1 Hz, $J_{6b,5b}$ = 3.4 Hz, 1H, $H_{6'a}$), 4.10 – 4.07 (m, 2H, $H_{6'a}$ & $H_{1a}$), 3.62 – 3.59 (m, 1H, $H_{5b}$), 3.37 (t, *J* = 10.9 Hz, 1H, $H_{3b}$), 3.27– 3.34 (m, 1H, $H_{5a}$), 2.20 (s, 3H), 2.15 (s, 3H), 2.06 (s, 4H), 2.03 (s, 3H), 1.99 (s, 3H), 1.97 (s, 3H), 1.96 (s, 3H) $^{13}$C NMR (151 MHz, $CDCl_3$) δ 170.5, 169.7, 169.3, 169.2, 169.0, 76.7 ($C_{2b}$), 74.3($C_{3a}$), 73.3 ($C_{2a}$), 71.6 ($C_{4a}$), 70.1 ($C_{4b}$), 61.1 ($C_{6a}$), 61.0 ($C_{6b}$), 54.8 ($C_{3b}$), 50.7 ($C_{1a}$), 49.9 ($C_{1b}$), 44.5 ($C_{5a}$), 42.4 ($C_{5b}$), 20.9, 20.8, 20.6, 20.5, 20.4, 20.3 ($OCOCH_3$). HRMS *m/z* $[M+Na]^+$ calcd for $C_{26}H_{35}O_{14}NaS_3Br$ 769.0270, found 769.0269.

**2,3,4,6-Tetra-*O*-acetyl-5-thio-β-D-glucopyranosyl-(1 → 3)-1-*S*-acetyl-2,4,6-tri-*O*-acetyl-1,3,5-trideoxy-1,3,5-trithio-β-D-glucopyranose (135)**

Compound **134** (67 mg, 0.09 mmol) was used according to general procedure E to give **135** (60 mg, 89%) as a colorless solid. $[\alpha]^{23}_{D}$ = +43.5° (c 1.0, $CHCl_3$). $^1$H NMR (600 MHz, $CDCl_3$) δ 5.25 (dd, *J* = 10.7, 9.5 Hz, 1H, $H_{4a}$), 5.21 (t, *J* = 10.7 Hz, 1H, $H_{2b}$), 5.06 (dd, *J* = 11.0, 9.5 Hz, 1H, $H_{2a}$), 4.95 (t, *J* = 10.7 Hz, 1H, $H_{4b}$), 4.93 (t, *J* = 9.5 Hz, 1H, $H_{3a}$), 4.57 (d, *J* = 10.7 Hz, 1H, $H_{1b}$), 4.23 (dd, *J* = 12.0, 5.3 Hz, 1H, $H_{6a}$), 4.18 (dd, *J* = 12.0, 5.6 Hz, 1H, $H_{6b}$), 4.13 (dd, *J* = 12.0, 3.3 Hz, 1H, $H_{6'a}$), 4.09 (dd, *J* = 12.0, 3.2 Hz, 1H, $H_{6'b}$), 3.99 (d, *J* = 11.1 Hz, 1H, $H_{1a}$), 3.34 (m, 1H, $H_{5b}$), 3.16 (m, 1H, $H_{5a}$), 3.01 (t, *J* = 10.8 Hz, 1H, $H_{3b}$), 2.35 (s, 3H, $SCOCH_3$), 2.12 (s, 3H), 2.10 (s, 3H), 2.06 (s, 3H), 2.02 (s, 3H), 1.99 (s, 3H), 1.98 (s, 3H), 1.95 (s, 3H). $^{13}$C NMR (151 MHz, $CDCl_3$) δ 191.9 ($SCOCH_3$), 170.5, 170.4, 169.7, 169.3, 169.2, 169.1, 168.9 ($OCOCH_3$), 75.2 ($C_{2b}$), 74.4 ($C_{3a}$), 72.9 ($C_{2a}$), 71.6 ($C_{4a}$), 70.2 ($C_{4b}$), 61.6 ($C_{6a}$), 61.0 ($C_{6b}$), 55.1 ($C_{3b}$), 50.5 ($C_{1a}$), 47.2 ($C_{5b}$), 45.8 ($C_{1b}$), 44.6 ($C_{5a}$), 30.5 ($SCOCH_3$), 20.8, 20.8, 20.6, 20.6, 20.4, 20.4, 20.2 ($OCOCH_3$). HRMS *m/z* $[M+Na]^+$ calcd for $C_{28}H_{38}O_{15}NaS_4$ 765.0991, found 765.0989.

**2,3,4,6-Tetra-*O*-acetyl-5-thio-β-D-glucopyranosyl-(1 → 3)-2,4,6-tri-*O*-acetyl-3,5-dideoxy-3,5-dithio-β-D-glucopyranosyl-(1 → 3)- 1,2-*O*-5-*S*,6-*O*-di-isopropylidene-3,5-dideoxy-3,5-dithio-α-D-glucofuranose (136)**

Compound **135** (615 mg, 0.83 mmol) and **119** (507 mg, 1.24 mmol) were subjected to coupling according to general procedure A to give **136** (510 mg, 64%) as white crystals. m.p. 163-164 °C. $[\alpha]^{23}_{D}$ = +26.5° (c 0.4, $CH_2Cl_2$). $^1$H NMR (600 MHz, $CDCl_3$) δ 5.87 (d, $J_{1c,2c}$ = 3.6 Hz, 1H, $H_{1c}$), 5.26 (dd, $J_{4a,5a}$ = 10.7 Hz, $J_{4a,3a}$ = 9.6 Hz, 1H, $H_{4a}$), 5.16 (t, *J* = 10.6 Hz, 1H, $H_{2b}$), 5.08 (dd, $J_{1a,2a}$ = 11.0 Hz, $J_{2a,3a}$ = 9.5 Hz, 1H, $H_{2a}$), 5.02 – 4.94 (m, 2H, $H_{4b}$ & $H_{3a}$), 4.71 (d,

$J_{1c,2c}$ = 3.6 Hz, 1H, $H_{2c}$), 4.39 (dd, $J_{4c,5c}$ = 10.5 Hz, $J_{3c,4c}$ = 4.0 Hz, 1H, $H_{4c}$), 4.32 (dd, $J_{6c,5c}$ = 10.0 Hz, $J_{6c,6'c}$ = 2.5 Hz, 1H, $H_{6c}$), 4.25 (dd, $J_{6a,6'a}$ = 12.0 Hz, $J_{6a,5a}$ = 5.1 Hz, 1H, $H_{6a}$), 4.22 (dd, $J_{6b,6'b}$ = 12.0 Hz, $J_{6b,5b}$ = 5.4 Hz, 1H, $H_{6b}$), 4.15 – 4.11 (m, 2H, $H_{6'a}$ & $H_{6'c}$), 4.09 (dd, $J_{6b,6'b}$ = 12.0 Hz, $J_{6'b,5b}$ = 3.3 Hz, 1H, $H_{6'b}$), 4.00 (d, $J_{1a,2a}$ = 11.1 Hz, 1H, $H_{1a}$), 3.83 (d, $J_{1b,2b}$ = 10.6 Hz, 1H, $H_{1b}$), 3.64 (m, 1H, $H_{5c}$), 3.55 (d, $J_{3c,4c}$ = 4.0 Hz, 1H, $H_{3c}$), 3.25 – 3.14 (m, 2H, $H_{5a}$ & $H_{5b}$), 2.93 (t, $J$ = 10.8 Hz, 1H, $H_{3b}$), 2.20 (s, 3H), 2.16 (s, 3H), 2.13 (s, 3H), 2.08 (s, 3H), 2.05 (s, 3H), 2.00 (s, 3H), 1.97 (s, 3H), 1.66 (s, 3H), 1.60 (s, 3H), 1.53 (s, 3H), 1.35 (s, 3H). $^{13}$C NMR (151 MHz, $CDCl_3$) δ 170.6, 170.5, 169.8, 169.3, 169.2, 169.1, 168.8, 112.3 (isopropylidene C), 105.4 (C-1c), 92.7 (isopropylidene C), 86.1 ($C_{2c}$), 81.4 ($C_{4c}$), 76.2 ($C_{2b}$), 74.3 ($C_{3a}$), 72.6 ($C_{2a}$), 72.0 ($C_{6c}$), 71.9 ($C_{4a}$), 70.3 ($C_{4b}$), 61.7 ($C_{6b}$), 61.2 ($C_{6a}$), 55.3 ($C_{3b}$), 53.6 ($C_{3c}$), 50.7 ($C_{1a}$), 50.6 ($C_{5c}$), 50.1 ($C_{1b}$), 47.0 ($C_{5b}$), 44.4 ($C_{5a}$), 31.6, 30.7, 26.7, 26.5 (Isopropylidene $CH_3$), 21.0, 20.9, 20.6, 20.5, 20.4, 20.2 (Acetyl $CH_3$). HRMS *m/z* $[M+Na]^+$ calcd for $C_{38}H_{54}O_{18}NaS_5$ 981.1811, found 981.1810.

**5-Thio-β-D-glucopyranosyl-(1 → 3)-3,5-dideoxy-3,5-dithio-β-D-glucopyranosyl-(1 → 3)-1,2-*O*-5-*S*,6-*O*-di-isopropylidene-3,5-dideoxy-3,5-dithio-α-D-glucofuranose (137)**

Compound **136** (568 mg, 0.59 mmol) was deacetylated according to general procedure B to give **137** (394 mg, quantitative) as a colorless solid. $[\alpha]^{23}_D$ = +17.2° (c 2.6, MeOH). $^1$H NMR (600 MHz, $CD_3OD$) δ 5.90 (d, $J_{1c,2c}$ = 3.5 Hz, 1H, H-1c), 4.92 (d, $J_{2c,1c}$ = 3.5 Hz, 1H, $H_{2c}$), 4.36 (dd, $J_{4c,5c}$ = 10.5 Hz, $J_{4c,3c}$ = 3.9 Hz, 1H, $H_{4c}$), 4.25 (dd, $J_{6c,6'c}$ = 10.0 Hz, $J_{6c,5c}$ = 2.1 Hz, 1H, $H_{6c}$), 4.10 (dd, $J_{6c,6'c}$ = 9.9 Hz, $J_{6'c,5c}$ = 4.8 Hz, 1H, $H_{6'c}$), 3.92 (d, $J$ = 10.1 Hz, 1H, $H_{1a}$), 3.91 – 3.85 (m, 3H, H-1b, H-6a, $H_{6b}$), 3.82 (dd, $J_{6b,6'b}$ = 11.5 Hz, $J_{6'b,5b}$ = 5.6 Hz, 1H, $H_{6'b}$),

3.73 (dd, $J_{6'a,6a}$ = 11.4 Hz, $J_{6'a,5a}$ = 6.0 Hz, 1H, $H_{6'a}$), 3.69 (d, $J_{3c,4c}$ = 3.9 Hz, 1H, $H_{3c}$), 3.64 – 3.61 (m, 1H, $H_{5c}$), 3.55 – 3.47 (m, 2H, $H_{4a}$ & $H_{4b}$), 3.47 – 3.39 (m, 2H, $H_{2a}$ & $H_{2b}$), 3.19 (t, *J* = 8.9 Hz, 1H, $H_{3a}$), 2.98 – 2.95 (m, 1H, $H_{5a}$), 2.93 – 2.90 (m, 1H, $H_{5b}$), 2.74 (t, *J* = 10.1 Hz, 1H, $H_{3b}$), 1.62 (s, 3H), 1.56 (s, 3H), 1.46 (s, 3H), 1.32 (s, 3H), (isopropilidene $CH_3$). $^{13}$C NMR (151 MHz, $CD_3OD$) δ 111.6 (isopropylidene C), 105.5 ($C_{1c}$), 91.9 (isopropylidene C), 85.5 ($C_{2c}$), 81.2 ($C_{4c}$), 79.0 ($C_{3a}$), 77.9 ($C_{2a}$), 76.2 ($C_{2b}$), 74.4 ($C_{4a}$), 73.0 ($C_{4b}$), 71.6($C_{6c}$), 62.4 ($C_{3b}$), 61.8 ($C_{6b}$), 61.5 ($C_{6a}$), 52.1($C_{3c}$), 51.6 ($C_{5b}$), 51.4 ($C_{1b}$), 50.5 ($C_{5c}$), 49.9 ($C_{5a}$), 49.5 ($C_{1a}$), 30.7, 29.7, 25.6, 25.3 ($CH_3$). HRMS *m/z* $[M+Na]^+$ calcd for $C_{24}H_{40}O_{11}NaS_5$ 687.1072, found 687.1073.

**2,3,4,6-Tetra-*O*-acetyl-5-thio-β-D-glucopyranosyl-(1 → 3)-2,4,6-tri-*O*-acetyl-3,5-dideoxy-3,5-dithio-β-D-glucopyranosyl-(1 → 3)-2,4,6-tri-*O*-acetyl-3,5-dideoxy-3,5-dithio-α,β-D-glucopyranose (138)**

Compound **137** (394 mg, 0.59 mmol) was deprotected according to general procedure C to give **138** (586 mg, 95%) as a colorless solid. $[\alpha]^{23}_D$ = +55.2° (c 0.4, $CHCl_3$). $^1$H NMR (600 MHz, $CDCl_3$) α:β = 3:1 from $^1$HNMR integration of $H_{1c}$ signals, δ 6.03 (d, $J_{1c\alpha,\ 2c\alpha}$ = 3.1 Hz, 1H, $H_{1c\alpha}$), 5.87 (d, $J_{1c\beta,\ 2c\beta}$ = 7.1 Hz, 1H, $H_{1c\beta}$), 5.36 (t, *J* = 7.1Hz, 1H, $H_{2c\beta}$), 5.28 – 5.23 (m, 2H, $H_{4a}$ & $H_{2c\alpha}$), 5.14 – 5.04 (m, 3H, $H_{2b}$ & $H_{2a}$ & $H_{4c}$), 4.99 – 4.93 (m, 2H, $H_{4b}$ & $H_{3a}$), 4.30 – 4.08 (m, 5H, $H_{6a}$ & $H_{6b}$ & $H_{6c}$), 4.05 (dd, $J_{6c,\ 6'c}$ = 12.1 Hz, $J_{6'c,\ 5c}$ = 3.3 Hz, 1H, $H_{6'c}$), 4.03 – 3.91 (m, 2H, $H_{1b}$ & $H_{1a}$), 3.56 – 3.48 (m, 1H, $H_{5c\alpha}$), 3.37– 3.33 (m, 1H, $H_{5c\beta}$), 3.28 (t, *J* = 11.5 Hz, 1H, $H_{3c\alpha}$), 3.25 – 3.21 (m, 1H, $H_{5b}$), 3.17– 3.05 (m, 2H, $H_{5a}$ & $H_{3c\beta}$), 2.93 – 2.86 (m, $H_{3b}$, 1H), 2.19 (s, 3H), 2.15 (s, 3H), 2.14 (s, 3H), 2.12 (s, 3H), 2.11 (s, 6H), 2.06 (s, 3H), 2.05 (s, 3H), 2.04 (s, 3H), 2.00 (s, 3H), 1.99 (s, 3H), 1.97 (s, 3H). $^{13}$C NMR (151 MHz, $CDCl_3$) δ

170.57, 170.54, 170.42, 169.67, 169.23, 169.17, 169.14, 169.10, 168.99, 168.86, 168.72 (Carbonyl C), 76.2 ($C_{2b}$), 75.5 ($C_{2c\alpha}$), 74.8 ($C_{2c\beta}$), 74.2 ($C_{3a}$), 72.7 ($C_{1c\beta}$), 72.6 ($C_{2a}$), 71.9 ($C_{4a}$), 70.8 ($C_{4c\alpha}$), 70.5 ($C_{1c\alpha}$ & $C_{4b}$), 70.2 ($C_{4c\beta}$), 62.8 ($C_{6c\beta}$), 61.8 ($C_{6c\alpha}$), 61.5 ($C_{6b}$), 61.1 ($C_{6a}$), 51.6 ($C_{1b}$), 50.8 ($C_{1a}$), 50.0 ($C_{3c\alpha}$), 46.6 ($C_{5b}$), 44.5 ($C_{5a}$), 42.9 ($C_{5c\beta}$), 40.7 ($C_{5c\alpha}$), 21.1, 21.0, 20.9, 20.8, 20.7, 20.7, 20.6, 20.6, 20.5, 20.4, 20.2 (Acetyl $CH_3$). HRMS *m/z* $[M+Na]^+$ calcd for $C_{40}H_{54}O_{22}NaS_5$ 1069.1608, found 1069.1613.

**5-Thio-β-D-glucopyranosyl-(1 → 3)-3,5-dideoxy-3,5-dithio-β-D-glucopyranosyl-(1 → 3)-3,5-dideoxy-3,5-dithio-α,β-D-glucopyranose (139)**

Compound **138** (20 mg, 0.02 mmol) was deacetylated according to general procedure B to give **139** (11 mg, quantitative) as a colorless solid, $[\alpha]^{23}_D$ = +78.8° (c 2.6, MeOH). α:β = 4:1 from $^{13}$CNMR integration of $C_{1c}$ signals. $^1$H NMR (600 MHz, $D_2O$) δ 4.85 (d, *J* = 2.9 Hz, 1H, $H_{1c\alpha}$), 4.59 (d, *J* = 12.0 Hz, 1H, $H_{1c\beta}$) 3.98 (t, *J* = 10.9 Hz, 2H, $H_{1a}$&$H_{1b}$), 3.83 – 3.64 (m, 6H, $H_{6a,6'a}$&$H_{6b,6'b}$&$H_{6c,6'c}$), 3.58 – 3.42 (m, 4H, $H_{2b}$&$H_{4a}$&$H_{4b}$&$H_{4c}$), 3.35 (t, *J* = 9.0 Hz, 1H, $H_{2a}$), 3.17 (t, *J* = 9.0 Hz, 1H, $H_{3a}$), 3.14 – 3.10 (m, 1H, $H_{5c}$), 3.05 (t, *J* = 10.7 Hz, 1H, $H_{3c}$), 2.95– 2.91 (m, 1H, $H_{5b}$), 2.90–2.85 (m, 1H, $H_{5a}$), 2.73 (t, *J* = 10.4 Hz, 1H, $H_{3b}$). $^{13}$C NMR (151 MHz, $D_2O$) δ 77.9 ($C_{3a}$), 77.3 ($C_{2c\beta}$), 76.1 ($C_{2a}$&$C_{2b}$), 75.1 ($C_{1c\beta}$), 74.7 ($C_{2c\alpha}$), 72.8 ($C_{4a}$), 72.5 ($C_{1c\alpha}$), 70.8 ($C_{4b}$), 70.7 ($C_{4c\beta}$), 70.6 ($C_{4c\alpha}$), 60.7 ($C_{6c\beta}$), 60.5 ($C_{6c\alpha}$), 60.4 ($C_{6b}$), 60.0 ($C_{6a}$), 59.2 ($C_{3b}$), 57.6 ($C_{3c\beta}$), 54.5 ($C_{3c\alpha}$), 50.6 ($C_{5b}$), 50.4 ($C_{1b}$), 48.8 ($C_{1a}$), 48.9 ($C_{5a}$), 48.1 ($C_{5c\beta}$), 43.5 ($C_{5c\alpha}$). HRMS *m/z* $[M+Na]^+$ calcd for $C_{18}H_{32}O_{11}NaS_5$ 607.0446, found 607.0447

**2,3,4,6-Tetra-*O*-acetyl-5-thio-β-D-glucopyranosyl-(1 → 3)-2,4,6-tri-*O*-acetyl-3,5-dideoxy-3,5-dithio-β-D-glucopyranosyl-(1 → 3)-2,4,6-tri-*O*-acetyl-3,5-trideoxy-3,5-dithio-α-D-glucopyranosyl bromide (140)**

Bromination was performed on compound **138** (53 mg, 0.05 mmol) according to general procedure D to give **140** (23 mg, 43%) as a colorless oil. $[\alpha]^{23}_{D}$ = +90.0° (c 0.6, $CH_2Cl_2$). $^1$H NMR (600 MHz, $CDCl_3$) δ 5.47 (d, *J* = 3.4 Hz, 1H, $H_{1c}$), 5.17 – 5.02 (m, 1H, $H_{4a}$), 5.17 – 5.02 (m, 3H, $H_{2a}$&$H_{2b}$& $H_{3a}$), 5.01 – 4.89 (m, 3H, $H_{2c}$&$H_{4b}$&$H_{4c}$), 4.34 (dd, $J_{6a,6'a}$ = 12.2 Hz, $J_{6a,5a}$ = 5.1 Hz, 1H, $H_{6a}$), 4.29 – 4.06 (m, 5H, $H_{6'a}$&$H_{6b}$&$H_{6'b}$&$H_{6c}$&$H_{6'c}$), 4.00 (d, *J* = 10.7 Hz, 1H, $H_{1b}$), 3.95 (d, *J* = 10.9 Hz, 1H, $H_{1a}$), 3.67 – 3.58 (m, 1H, $H_{5c}$), 3.42 (t, *J* = 10.9 Hz, 1H, $H_{3c}$), 3.29 – 3.20 (m, 1H, $H_{5b}$), 3.15 – 3.07 (m, 1H, $H_{5a}$), 2.97 – 2.85 (m, 1H, $H_{3b}$). 2.23 (s, 3H), 2.16 (s, 3H), 2.14 (s, 3H), 2.12 (s, 3H), 2.07 (s, 3H), 2.05 (s, 3H), 2.04 (s, 3H), 2.00 (s, 3H), 1.99 (s, 3H), 1.97 (s, 3H). $^{13}$C NMR (151 MHz, $CDCl_3$) δ 170.5, 170.4, 169.7, 169.3, 169.2, 169.1, 169.1, 168.9, 168.6 (Carbonyl C), 76.3 ($C_{2c}$), 76.2 ($C_{2b}$), 74.3 ($C_{3a}$), 72.6 ($C_{2a}$), 71.9 ($C_{4a}$), 70.6 ($C_{4c}$), 70.4 ($C_{4b}$), 61.7 ($C_{6c}$), 61.1 ($C_{6b}$&$C_{6a}$), 55.3 ($C_{3b}$), 55.0 ($C_{3c}$), 51.6 ($C_{1b}$), 50.8 ($C_{1a}$), 50.6 ($C_{1c}$), 46.8 ($C_{5b}$), 44.5 ($C_{5a}$), 42.6 ($C_{5c}$), 20.9, 20.8, 20.6, 20.5, 20.4, 20.2 (Acetyl $CH_3$). HRMS *m/z* $[M+Na]^+$ calcd for $C_{37}H_{47}O_{25}NaS_3Br$ 1089.0650, found 1089.0653.

**2,3,4,6-Tetra-*O*-acetyl-5-thio-β-D-glucopyranosyl-(1 → 3)-2,4,6-tri-*O*-acetyl-3,5-dideoxy-3,5-dithio-β-D-glucopyranosyl-(1 → 3)-1-*S*-acetyl-2,4,6-tri-*O*-acetyl-1,3,5-trideoxy-1,3,5-trithio-β-D-glucopyranose (141)**

The anomeric bromide of **140** (34 mg, 0.03mmol) was substituted by potassium thioacetate (5mg, 1.5 equiv) according to general procedure E to give **141** (30mg, 90%) as a

colorless solid. $[\alpha]^{23}_D$ = +99.2° (c 0.6, $CHCl_3$). $^1$H NMR (600 MHz, $CDCl_3$) δ 5.25 (t, *J* = 10.9 Hz, 1H, $H_{4a}$), 5.22 (t, *J* =10.6 Hz, 1H, $H_{2c}$), 5.16 – 5.07 (m, 1H, $H_{2b}$), 5.05 (dd, $J_{2a,1a}$ = 11.0 Hz, $J_{2a,3a}$ = 9.5 Hz, 1H, $H_{2a}$), 5.00 – 4.93 (m, 4H, $H_{3a}$&$H_{4b}$&$H_{4c}$), 4.60 (d, $J_{1c,2c}$ = 10.6 Hz, 1H, $H_{1c}$), 4.28 – 4.09 (m, 6H, $H_{6a,6'a}$ & $H_{6b,6'b}$& $H_{6c,\ 6'c}$), 3.93 (d, $J_{1a,\ 2a}$ = 11.1 Hz, 1H, $H_{1a}$), 3.90 (d, $J_{1b,\ 2b}$ = 10.6 Hz, 1H, $H_{1b}$) , 3.37 – 3.34 (m, 1H, $H_{5c}$), 3.16 – 3.13 (m, 1H, $H_{5b}$), 3.11 – 3.07 (m, 1H, $H_{5a}$), 3.03 (t, *J* = 10.7 Hz, 1H, $H_{3c}$), 2.88 – 2.80 (m, 1H, $H_{3b}$), 2.38 (s, 3H), 2.13 (s, 6H), 2.12 (s, 6H), 2.06 (s, 6H), 2.04 (s, 3H), 2.00 (s, 3H), 1.99 (s, 3H), 1.97 (s, 3H). $^{13}$C NMR (151 MHz, $CDCl_3$) δ 192.0, 170.5, 170.4, 169.7, 169.3, 169.1, 168.5 (Carbonyl C), 76.1 ($C_{2c}$), 75.3 ($C_{2b}$), 74.3 ($C_{3a}$), 72.5 ($C_{2a}$), 71.8 ($C_{4a}$), 70.5 ($C_{4c}$), 70.4 ($C_{4b}$) 61.7 ($C_{6c}$), 61.7 ($C_{6b}$), 61.1 ($C_{6a}$), 55.3 ($C_{3b}$), 54.9 ($C_{3c}$), 52.4 ($C_{1b}$), 50.8 ($C_{1a}$), 47.2 ($C_{5c}$), 46.8 ($C_{5b}$), 45.7 ($C_{1c}$), 44.5 ($C_{5a}$), 30.5 (S-Acetyl $CH_3$), 20.8, 20.7, 20.60, 20.5, 20.4, 20.2 (Acetyl $CH_3$). HRMS *m/z* $[M+Na]^+$ calcd for $C_{40}H_{54}O_{21}NaS_6$ 1085.1380, found 1085.1383.

**2,3,4,6-Tetra-*O*-acetyl-5-thio-β-D-glucopyranosyl-(1 → 3)-2,4,6-tri-*O*-acetyl-3,5-dideoxy-3,5-dithio-β-D-glucopyranosyl-(1 → 3)-2,4,6-tri-*O*-acetyl-3,5-dideoxy-3,5-dithio-β-D-glucopyranosyl-(1 → 3)-1,2-*O*-5-*S*,6-*O*-di-isopropylidene-3,5-dideoxy-3,5-dithio-α-D-glucofuranose (142)**

Compound **141** (510 mg, 0.48 mmol) and compound **119** (490 mg, 0.72 mmol) were subjected to coupling reaction according to general procedure A to give product **142** (283 mg, 46%) as white crystals. mp 139-140 °C. $[\alpha]^{23}_D$ = +31.6° (c 0.3, $CHCl_3$). $^1$H NMR (600 MHz, $CDCl_3$) δ 5.88 (d, $J_{1d,2d}$ = 3.6 Hz, 1H, $H_{1d}$), 5.27 (t, *J* = 10.1 Hz, 1H, $H_{4a}$), 5.17 (t, *J* = 10.6 Hz, 1H, $H_{2c}$), 5.13 – 5.08 (m, 1H, $H_{2b}$), 5.05 (t, *J* = 10.3 Hz, 1H, $H_{2a}$), 5.01 – 4.94 (m, 3H,

$H_{3a}$&$H_{4b}$&$H_{4c}$), 4.70 (d, $J_{2d,1d}$ = 3.6 Hz, 1H, $H_{2d}$), 4.40 (dd, $J_{4d,5d}$ = 10.5 Hz, $J_{4d,3d}$ = 3.9 Hz, 1H, $H_{4d}$), 4.33 (dd, $J_{6d,6'd}$ = 10.1 Hz, $J_{6d,5d}$ = 2.4 Hz, 1H, $H_{6d}$), 4.29 – 4.18 (m, 3H, $H_{6a}$&$H_{6b}$&$H_{6c}$), 4.17 – 4.07 (m, 4H, $H_{6'a}$&$H_{6'b}$&$H_{6'c}$&$H_{6'd}$), 3.94 (d, $J_{1a,2a}$ = 11.1 Hz, 1H, $H_{1a}$), 3.90 (d, $J_{1b,2b}$ = 10.3 Hz, 1H, $H_{1b}$), 3.84 (d, $J_{1c,2c}$ = 10.5 Hz, 1H, $H_{1c}$), 3.67 – 3.62 (m, 1H, $H_{5d}$), 3.57 (d, $J_{3d,4d}$ = 4.0 Hz, 1H, $H_{3d}$), 3.20 –3.15 (m, 2H, $H_{5b}$&$H_{5c}$), 3.11 – 3.08 (m, $J$ = 10.3, 4.1 Hz, 1H, $H_{5a}$), 2.95 (t, $J$ = 10.7 Hz, 1H, $H_{3c}$), 2.88–2.82 (m, 1H, $H_{3b}$), 2.21 (s, 3H), 2.14 (s, 3H), 2.12 (s, 3H), 2.11 (s, 3H), 2.06 (s, 6H), 2.05 (s, 3H), 2.01 (s, 3H), 1.99 (s, 3H), 1.97 (s, 3H), 1.67 (s, 3H), 1.60 (s, 3H), 1.53 (s, 3H), 1.35 (s, 3H). $^{13}$C NMR (151 MHz, $CDCl_3$) δ 170.6, 170.4, 169.7, 169.2, 169.1, 168.7, 168.3, 112.3 (isopropylidene C), 105.4 ($C_{1d}$), 92.7 (isopropylidene C), 86.1 ($C_{2d}$), 81.4 ($C_{4d}$), 76.2 ($C_{2c}$), 75.7 ($C_{2b}$), 74.2 ($C_{3a}$), 72.5 ($C_{2a}$), 72.0 ($C_{6d}$), 71.8 ($C_{4a}$), 70.6 ($C_{4c}$) 70.4 ($C_{4b}$), 61.8 ($C_{6c}$), 61.7 ($C_{6b}$), 61.0 ($C_{6a}$), 55.3 ($C_{3b}$), 54.9 ($C_{3c}$), 53.5 ($C_{3d}$), 52.5 ($C_{1b}$), 50.8 ($C_{1a}$), 50.6 ($C_{5d}$), 50.0 ($C_{1c}$), 47.0 ($C_{5c}$), 46.6 ($C_{5b}$), 44.5 ($C_{5a}$), 31.6, 30.7, 26.7, 26.5 (Isopropylidene $CH_3$), 21.0, 20.9, 20.8, 20.7, 20.6, 20.5, 20.4, 20.2 (Acetyl $CH_3$). HRMS *m/z* $[M+Na]^+$ calcd for $C_{50}H_{70}O_{24}NaS_7$ 1301.2200, found 1301.2205.

**5-Thio-β-D-glucopyranosyl-(1 → 3)-3,5-dideoxy-3,5-dithio-β-D-glucopyranosyl-(1 → 3)-3,5-dideoxy-3,5-dithio-β-D-glucopyranosyl-(1 → 3)-1,2-*O*-5-*S*,6-*O*-di-isopropylidene-3,5-dideoxy-3,5-dithio-α-D-glucofuranose (143)**

Compound **142** (150 mg, 0.12 mmol) was deacetylated according to general procedure B to give **143** (100 mg, quantitative) as a colorless solid. $[\alpha]^{23}_D$ = +18.9° (c 0.5, MeOH). $^1$H NMR (600 MHz, $CD_3OD$) δ 5.88 (d, $J_{1d,2d}$ = 3.6, 1H, $H_{1d}$), 4.90 (d, $J_{2d,1d}$ = 3.6 Hz, 1H, $H_{2d}$), 4.37 (dd, $J_{4d,5d}$ = 10.5 Hz, $J_{4d,3d}$ = 4.0 Hz, 1H, $H_{4d}$), 4.25 (dd, $J$ = 10.0, 1.8 Hz, 1H, $H_{6d}$), 4.10

(dd, $J$ = 8.0, 4.9, 1.6 Hz, 1H, $H_{6'd}$), 4.00 (d, $J$ = 10.0 Hz, 1H, $H_{1b}$) 3.97 (d, $J$ = 10.3 Hz, 1H, $H_{1a}$), 3.94 – 3.86 (m, 4H, $H_{1c}$&$H_{6a}$&$H_{6b}$&$H_{6c}$), 3.81 – 3.72 (m, 2H, $H_{6'a}$&$H_{6'b}$), 3.72 – 3.67 (m, $H_{6'c}$1H), 3.66 (d, $J$ = 3.9 Hz, 1H, $H_{3d}$), 3.65 – 3.60 (m, 1H, $H_{5d}$), 3.56 – 3.43 (m, 5H, $H_{4a}$&$H_{4b}$&$H_{4c}$&$H_{2b}$&$H_{2c}$), 3.38 (t, $J$ = 10.4 Hz, 1H, $H_{2a}$), 3.17 (t, $J$ = 8.9 Hz, 1H, $H_{3a}$), 3.02 – 2.88 (m, 3H, $H_{5a}$&$H_{5b}$&$H_{5c}$), 2.85 – 2.69 (m, 2H, $H_{3b}$&$H_{3c}$), 1.62 (s, 3H), 1.56 (s, 3H), 1.46 (s, 3H), 1.31 (s, 3H). $^{13}$C NMR (151 MHz, $CD_3OD$) δ 111.6 (isopropylidene C), 105.4 ($C_{1d}$), 92.0 (isopropylidene C), 85.6 ($C_{2d}$), 81.1 ($C_{4d}$), 78.6 ($C_{3a}$), 77.5 ($C_{2a}$), 77.0 ($C_{2b}$), 75.8 ($C_{2c}$), 73.7 ($C_{4a}$), 71.9 ($C_{4b}$), 71.8 ($C_{4c}$), 71.6 ($C_{6d}$), 61.8 ($C_{3b}$), 61.2 ($C_{6c}$), 61.1 ($C_{3c}$), 60.9 ($C_{6a}$&$C_{6b}$), 52.5 ($C_{3d}$), 51.7 ($C_{5b}$), 51.4 ($C_{5c}$), 51.3 ($C_{1b}$), 51.1 ($C_{1c}$), 50.5 ($C_{5d}$), 49.6 ($C_{5a}$&$C_{1a}$), 30.7, 29.6, 25.5, 25.2 (isopropylidene-$CH_3$). HRMS *m/z* $[M+Na]^+$ calcd for $C_{30}H_{50}O_{14}NaS_7$ 881.1143, found 881.1141.

**5-Thio-β-D-glucopyranosyl-(1 → 3)-3,5-dideoxy-3,5-dithio-β-D-glucopyranosyl-(1 → 3)-3,5-dideoxy-3,5-dithio-β-D-glucopyranosyl-(1 → 3)-3,5-dideoxy-3,5-dithio-α,β-D-glucopyranose (144)**

Compound **143** (100 mg, 0.12 mmol) was deprotected according to general procedure C to give product **144** (78 mg, 85%) as a white solid. $[\alpha]^{23}_D$ = +36.6° (c 0.5, $H_2O$). α:β = 4:1 from $^{13}$CNMR integration of $C_{1d}$ signals. $^1$H NMR (600 MHz, $D_2O$) δ 4.86 (d, $J$ = 3.0 Hz, 1H, $H_{1d\alpha}$), 4.58 (d, $J$ = 8.8Hz, 1H, $H_{1d\beta}$) 4.02 – 3.90 (m, 3H, $H_{1a}$& $H_{1b}$& $H_{1c}$), 3.86 – 3.59 (m, 9H, $H_{6a,6'a}$ & $H_{6b,6'b}$ & $H_{6c,6'c}$ & $H_{6d,6'd}$ & $H_{2d}$), 3.57 – 3.37 (m, 6H, $H_{4a}$ & $H_{4b}$ & $H_{4c}$ & $H_{4d}$, $H_{2b}$ & $H_{2c}$), 3.31 (t, $J$ = 9.0 Hz 1H, $H_{2a}$), 3.13 (t, $J$ = 9.0 Hz, 1H, $H_{3a}$), 3.10 – 3.06 (m, 1H, $H_{5d}$), 3.01 (t, $J$ = 10.6 Hz, 1H, $H_{3d}$), 2.96– 2.81 (m, 3H, $H_{5a}$ & $H_{5b}$ & $H_{5c}$), 2.76 – 2.66 (m, 2H, $H_{3b}$ & $H_{3c}$).

$^{13}$C NMR (151 MHz, $D_2O$) δ 77.9 ($C_{3a}$), 77.3 ($C_{2d\beta}$), 76.2 ($C_{2a}$&$C_{2b}$&$C_{2c}$), 75.1 ($C_{1d\beta}$), 74.7 ($C_{2d\alpha}$), 72.9 ($C_{4a}$), 72.5 ($C_{1d\alpha}$), 70.9 ($C_{4b}$), 70.8 ($C_{4d\beta}$), 70.6 ($C_{4c}$&$C_{4d\alpha}$), 60.6 ($C_{6b}$ & $C_{6c}$), 60.5 ($C_{6a}$), 60.0 ($C_{3c}$), 59.3 ($C_{3b}$), 59.2 ($C_{3d\beta}$), 54.5 ($C_{3d\alpha}$), 50.7 ($C_{1b}$) 50.6 ($C_{5b}$ & $C_{5c}$), 50.4 ($C_{1c}$), 48.8 ($C_{1a}$), 48.6 ($C_{5a}$), 48.1 ($C_{5d\beta}$), 43.5 ($C_{5d\alpha}$). HRMS *m/z* $[M+Na]^+$ calcd for $C_{24}H_{42}O_{14}NaS_7$ 801.0517, found 801.0518.

***p*-*N*,*N*-Dimethylamino benzyl alcohol (150)**

4-(dimethylamino)benzaldehyde (0.5 g, 3.35 mmol) was dissolved in dry methanol (10 mL) and added sodium borohydride ($NaBH_4$, 253 mg, 6.70 mmol) at 0 °C. The reaction mixture was stirred at 0 °C for 1 h, later at room temperature for 1 h. Methanol was removed on rotavapor, added water (200 mL) and extracted with DCM (2 x 100 mL). The DCM layer was washed with water (100 mL), brine solution (50 mL) and dried over $Na_2SO_4$. The solvent was evaporated and the residue was purified by flash chromatography on silica gel with 6/4 ethyl acetate/hexane to give the product **150** (430 mg, 85 %) as a colorless oil. $^1$H NMR (500 MHz, $CDCl_3$): δ 7.25 (d, 2H, *J* = 9.5 Hz), 6.73 (d, 2H, *J* = 8.5 Hz), 4.57 (br, 2H, Benzylic $CH_2$), 2.95 (s, 6H, Me), 1.48 (bs, 1H, OH) ; $^{13}$C NMR (125 MHz, $CDCl_3$) δ: 150.6, 129.1, 128.9, 112.9, 65.6 (Benzylic $CH_2$), 40.9 (Me). HRMS *m/z* $[M+H]^+$ calculated for $C_9H_{14}NO$ is 152.1075, found 152.1072.

**4,4'-methylenebis(*N*,*N*-dimethylaniline) (151)**

At 0 °C, to a solution of compound **150** (54 mg, 0.36 mmol) in DMF was added NaH (8.6 mg, 0.36 mmol) and *p*-toluenesulfonyl chloride (103 mg, 0.54 mmol) was added into the reaction mixture. The reaction mixture was stirred at room temperature for 2 h before the

reaction mixture was quenched with aqueous $NH_4Cl$. The crude mixture was extracted into ethyl acetate and washed with water, brine and dried over $MgSO_4$. The crude mixture was concentrated in *vacuo* and purified by silica gel chromatography to give product **151** (50.4 mg, 56%) as a yellow oil. $^1$H NMR (400 MHz, $CDCl_3$): δ 7.14-7.08 (m, 4H), 6.76-6.71 (m, 4H), 3.86 (d, 2H, *J* = 4.4 Hz, Benzylic $CH_2$), 2.95 (s, 6H), 2.94 (s, 6H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 149.3, 130.6, 129.7, 113.3, 41.2, 40.2. HRMS *m/z* $(M+H)^+$ calcd for $C_{17}H_{23}N_2$ 255.1861, found 255.1861.

***p*-*N*,*N*-Dimethylamino benzyl chloride hydrochloride (152)**

The solution of *p*-*N*,*N*-dimethyl amino benzyl alcohol (0.85 g, 5.60 mmol) in 3.5 mL aqueous HCl (36.6%) was refluxed at 100 °C for 12 h. The reaction mixture was concentrated in *vacuo* and high vacuum evaporation at 45 °C for 4 h to remove water to give product **156** (0.11 g, 96%) as a colorless oil. $^1$H NMR (400 MHz, $CDCl_3$) δ 7.85 – 7.74 (m, 2H), 7.58 – 7.49 (m, 2H), 4.58 (s, 2H, benzylic $CH_2$), 3.18 (s, 6H, $CH_3$). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 142.6, 139.9, 130.6, 121.3, 46.6 (Benzylic $CH_2$), 44.6 (Me).

**3-*O*-(4-Chlorobenzyl)-1,2;5,6-di-*O*-isopropylidene-α-D-glucofuranose (153)**

At 0 °C, to a solution of 1,2;5,6-di-*O*-isopropylidene-α-D-glucofuranose (0.2 g, 0.8 mmol) in 5 mL DMF was added NaH (40 mg, 1 mmol). The reaction was stirred at 0 °C for 30 min and 4-chlorobenzyl bromide (0.2 g, 1 mmol) was added into the reaction. The reaction was stirred at 0 °C for 3 h before it was quenched with aqueous $NH_4Cl$. The crude mixture was extracted into ethyl acetate, washed with water, brine and dried over $MgSO_4$. The crude mixture was concentrated in *vacuo* and purified by sílica gel chromatography to give product **153** (0.24

g, 78%) as a white solid. $^{1}$H NMR (400 MHz, $CDCl_3$) δ 7.37 – 7.20 (m, 5H), 5.89 (d, *J* = 3.7 Hz, 1H, $H_1$), 4.70 – 4.52 (m, 3H, Benzylic $CH_2$ & $H_2$), 4.34 (dt, *J* = 8.1, 5.9 Hz, 1H, $H_4$), 4.17 – 4.06 (m, 2H, $H_6$ & $H_3$), 4.05 – 3.93 (m, 2H, $H_{6'}$ & $H_5$), 1.49 (s, 3H), 1.42 (s, 3H), 1.37 (s, 3H), 1.31 (s, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 136.1, 133.6, 129.0, 128.6, 111.9, 109.1 (isopropylidene), 105.3 ($C_1$), 82.6, 81.7, 81.3, 77.3, 77.0, 76.7, 72.4 (Benzylic $CH_2$), 71.5, 67.5, 26.8, 26.2, 25.4.

**3-*O*-(*p*-*N*,*N*-Diethylamino benzyl)-1,2;5,6-di-*O*-isopropylidene-α-D-glucofuranose (154)**

To a solution of Tris(dibenzylideneacetone)dipalladium(0) (3.3 mg, 3.6 μmol ), 2-Dicyclohexylphosphino-2′-(*N*,*N*-dimethylamino)biphenyl (2.8 mg, 7.2 μmol), potassium tert-butoxide (28 mg, 0.25 mmol) and 1,2;5,6-di-O-isopropylidene-D-glucofuranose (110 mg, 0.18 mmol) in Toluene (2 mL) was added diethyl amine (23 μL, 0.22 mmol). The reaction was stirred at 80 °C for 12 h before the reaction mixture was concentrated in *vacuo* and the crude mixture was dissolved in Ethyl Acetate (25 mL) and washed by water, brine and dried over $Na_2SO_4$. The reaction mixture was concentrated in *vacuo* and purified by silica gel chromatography with eluent (8:1 = Hexane: Ethyl acetate) to give product **154** as a colorless oil (88 mg, 82%). $[\alpha]^{23}_D$ = -18.4° (c 1.5, $CHCl_3$). $^{1}$H NMR (600 MHz, $CDCl_3$) δ 7.18 – 7.14 (m, 2H), 6.66 – 6.60 (m, 2H), 5.86 (d, *J* = 3.7 Hz, 1H), 4.54 (d, *J* = 3.8 Hz, 1H), 4.53 – 4.46 (m, 2H), 4.37 – 4.28 (m, 1H), 4.15 (dd, *J* = 7.3, 3.1 Hz, 1H), 4.12 – 4.06 (m, 1H), 4.01 – 3.96 (m, 2H), 3.33 (q, *J* = 7.1 Hz, 4H), 1.48 (S, 3H), 1.42 (s, 3H), 1.37 (s, 3H), 1.30 (s, 3H), 1.14 (t, *J* = 7.1 Hz, 6H). $^{13}$C NMR (151 MHz, $CDCl_3$) δ 129.6, 111.6, 111.5, 105.3, 82.8, 81.3, 81.3,

80.9, 77.2, 77.0, 76.8, 72.7, 72.6, 72.2, 67.4, 67.2, 44.4, 26.9, 26.8, 26.8, 26.7, 26.3, 26.2, 25.5, 25.4, 12.5. HRMS *m/z* $[M+H]^+$ calcd for $C_{23}H_{36}NO_6$ 422.2543, found 422.2544.

**4-(Methoxymethyl)-*N*,*N*-diethyl aniline (155)**

The compound **154** (53 mg, 0.13 mmol) was dissolved in 2 mL methanol and was transferred into a 10 mL vial equipped with a magnetic stir bar. The vial was capped properly and placed in the microwave (CEM Discover SP Microwave). The microwave was run at 300 W, 160 °C and 300 psi for 30 min. The vial was cooled to room temperature and the reaction mixture was transferred into round bottom flask and concentrated in *vacuo*. The residue was purified by silica gel column chromatography to give product **155** (24 mg, 98%) as a colorless oil. $^1$H NMR (400 MHz, $CDCl_3$) δ 7.24 – 7.11 (m, 1H), 6.66 (d, *J* = 8.6 Hz, 1H), 4.34 (s, 1H), 3.35 (d, *J* = 7.5 Hz, 4H), 1.16 (t, *J* = 7.0 Hz, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 147.5, 129.6, 124.6, 111.7, 74.7, 57.5, 44.4, 12.5. HRMS *m/z* $[M+H]^+$ calcd for $C_{12}H_{20}NO$ 194.1545, found 194.1541.

***ortho*-*N*,*N*-Dimethylamino benzyl alcohol (156)**

To a solution of *ortho*-amino benzyl alcohol (1.23 g, 10 mmol) in 25 mL DMF was added $Na_2HPO_4$ (5.11 g, 36 mmol) and methyl iodide (3.4 g, 24 mmol). The reaction mixture was stirred at 45 °C for 10 h before it was poured into 200 mL water. The crude mixture was extracted into diethyl ether, the organic layer was washed with water, brine and dried over $MgSO_4$. The crude mixture was concentrated in *vacuo* and purified by sílica gel chromatography to give product **156** (1.3 g, 86%) as a colorless oil. $^1$H NMR (400 MHz, $CDCl_3$) δ 7.29 – 7.19 (m, 2H), 7.19 – 7.13 (m, 1H), 7.08 (tt, *J* = 7.3, 1.2 Hz, 1H), 5.60 (s, 1H),

4.82 (s, 2H), 2.72 (s, 6H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 151.9, 135.2, 128.4, 128.2, 124.4, 120.1, 64.9, 44.6. HRMS *m/z* $[M+H]^+$ calcd for $C_9H_{14}NO$ 152.1070, found 152.1071.

***ortho*-*N*,*N*-Dimethylamino benzyl chloride (157)**

At 0 °C, to a solution of compound **156** (141 mg, 0.93 mmol) in 3 mL chloroform was added $SO_2Cl_2$ (222 mg, 1.9 mmol). The reaction mixture was stirred at 0 °C for 4 h and concentrated in *vacuo* to give product **157** (157 mg, 100%) as a yellow oil. $^1$H NMR (400 MHz, $CDCl_3$) δ 7.68 – 7.64 (m, 1H), 7.57 – 7.45 (m, 3H), 5.27 (s, 2H), 3.34 (s, 6H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 140.6, 133.4, 131.2, 130.9, 120.9, 47.7, 41.9.

**3-*O*-(*ortho*-*N*,*N*-Dimethylamino benzyl)-1,2;5,6-di-*O*-isopropylidene-α-D-glucofuranose (158)**

At 0°C, to a stirred solution of 1,2;5,6-di-*O*-isopropylidene-α-D-glucofuranose (125 mg, 0.48 mmol) in DMF (1 mL) was added NaH (104 mg, 2.60 mmol) and *ortho*-*N*, *N*-dimethylamino benzyl chloride (157 mg, 0.93 mmol). The reaction was stirred overnight before aqueous $NH_4Cl$ was added to quench the reaction. The reaction mixture was diluted in ethyl acetate, washed by water, brine and dried over $MgSO_4$. The reaction mixture was concentrated in *vacuo* and purified by silica gel chromatography with eluent (10 : 1 = hexane : ethyl acetate) to give product **158** as a colorless oil (104 mg, 55%). $[\alpha]^{23}_D$ = -1.9° (c 1.8, $CHCl_3$). $^1$H NMR (400 MHz, $CDCl_3$) δ 7.45 (dd, *J* = 7.6, 1.8 Hz, 1H), 7.29 – 7.21 (m, 1H), 7.11 – 7.00 (m, 2H), 5.89 (d, *J* = 3.7 Hz, 1H, $H_1$), 4.82 (d, *J* = 11.8 Hz, 1H, benzyl $CH_2$), 4.71 – 4.63 (m, 2H, $H_2$ & benzyl $CH_2$), 4.41 (dt, *J* = 8.0, 6.1 Hz, 1H, $H_4$), 4.18 (dd, *J* = 7.7, 3.1 Hz, 1H, $H_5$), 4.14 – 4.05 (m, 2H, $H_6$ & $H_3$), 4.00 (dd, *J* = 8.6, 6.0 Hz, 1H, $H_{6'}$), 2.69 (d, *J* = 2.0 Hz, 6H, $NMe_2$), 1.51 (s,

3H), 1.43 (s, 3H), 1.33 (s, 3H), 1.36 (s, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 131.9, 129.3, 128.3, 123.0, 118.8, 111.7 (Isopropylidene), 108.9 (Isopropylidene), 105.3 ($C_1$), 82.4 ($C_2$), 82.1 ($C_3$), 81.4 ($C_5$), 72.5 ($C_4$), 68.1 (Benzyl $CH_2$), 67.4 ($C_6$), 45.1 ($NMe_2$), 26.9, 26.8, 26.3, 25.4 (Isopropylidene $CH_3$). HRMS *m/z* $[M+H]^+$ calcd for $C_{21}H_{32}NO_6$ 394.2230, found 394.2234.

**Phenyl 4-*O*-(4-bromobenzyl)-2,3-*O*-isopropylidene-1-thio-β-L-rhamnopyranoside (160)**

At 0°C, to a stirred solution of phenyl 2,3-*O*-isopropylidene-1-thio-β-L-rhamnopyranoside (512 mg, 1.73 mmol) in DMF (10mL) was added NaH (104 mg, 2.60 mmol) and 4-bromobenzyl bromide (513 mg, 2.08 mmol). The reaction was stirred overnight before aqueous $NH_4Cl$ was added to quench the reaction. The reaction mixture was diluted in ethyl acetate, washed by water, brine and dried over $MgSO_4$. The crude mixture was concentrated in *vacuo* and purified by silica gel chromatography with eluent (10 : 1 = hexane : ethyl acetate) to give product **162** as a white solid (2.5 g, 85%). mp 123 ~ 125 ℃. $[\alpha]^{23}_D$ = +84.0° (c 0.3, $CHCl_3$). $^1$H NMR (400 MHz, $CDCl_3$) δ 7.56 – 7.44 (m, 4H), 7.35 – 7.19 (m, 6H), 5.01 (d, *J* = 2.1 Hz, 1H, $H_1$), 4.82 (d, *J* = 11.8 Hz, 1H, benzyl $CH_2$), 4.59 (d, *J* = 11.8 Hz, 1H, benzyl $CH_2$), 4.44 (dd, *J* = 5.7, 2.2 Hz, 1H, $H_2$), 4.20 (t, *J* = 5.8 Hz, 1H, $H_3$), 3.33 (t, *J* = 5.3 Hz, 2H, $H_4$ & $H_5$), 1.57 (s, 3H), 1.43 (s, 3H), 1.36 (d, *J* = 5.5 Hz, 3H, $H_6$). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 137.1, 135.1, 131.4, 130.9, 129.6, 128.9, 127.4, 121.6, 110.5 (Isopropylidene C), 84.0 ($C_1$), 80.8 ($C_4$), 79.9 ($C_3$), 76.4 ($C_2$), 74.4 ($C_5$), 72.1 (Benzyl C), 28.0, 26.4, 18.5 ($C_6$). HRMS *m/z* $[M+Na]^+$ calcd for $C_{22}H_{25}BrO_4NaS$ 487.0555, found 487.0557.

**Phenyl 4-*O*-(*p*-*N*,*N*-diethylamino benzyl)-2,3-*O*-isopropylidene-1-thio-β-L-rhamnopyranoside (161)**

To a solution of Tris(dibenzylideneacetone)dipalladium(0) (3.3 mg, 3.6 μmol ), 2-dicyclohexylphosphino-2′-(*N*,*N*-dimethylamino)biphenyl (2.8 mg, 7.2 μmol), potassium tert-butoxide (28 mg, 0.25 mmol) and compound **160** (110 mg, 0.18 mmol) in Toluene (2 mL) was added diethyl amine (23 μL, 0.22 mmol). The reaction was stirred at 80 °C for 12 h before the reaction mixture was concentrated in *vacuo* and the crude mixture was dissolved in ethyl acetate (25 mL) and washed by water, brine and dried over $Na_2SO_4$. The reaction mixture was concentrated in *vacuo* and purified by silica gel chromatography with eluent (8:1 = Hexane: Ethyl acetate) to give product **161** as a colorless oil (88 mg, 82%). $[\alpha]^{23}_D$ = +14.0° (c 0.2, $CHCl_3$).$^1$H NMR (600 MHz, $CDCl_3$) δ 7.55 – 7.48 (m, 2H), 7.33 – 7.21 (m, 3H), 7.21 – 7.14 (m, 2H), 6.63 (d, *J* = 8.6 Hz, 2H), 5.00 (d, *J* = 2.1 Hz, 1H, $H_1$), 4.73 (d, *J* = 10.8 Hz, 1H, benzyl $CH_2$), 4.50 (d, *J* = 10.8 Hz, 1H, benzyl $CH_2$), 4.42 (dd, *J* = 5.8, 2.1 Hz, 1H, $H_2$), 4.22 (t, *J* = 5.9 Hz, 1H, H, $H_3$), 3.40 – 3.27 (m, 6H, $H_4$ & $H_5$ & Ethyl $CH_2$), 1.60 (s, 3H), 1.43 (s, 3H), 1.36 – 1.30 (m, 3H, $H_6$), 1.17 – 1.08 (m, 5H). $^{13}$C NMR (151 MHz, $CDCl_3$) δ 147.6, 135.3, 130.7, 130.0, 128.9, 127.2, 124.4, 111.6, 110.4 (isopropylidene C), 84.0 ($C_1$), 80.1 ($C_4$), 79.7 ($C_3$), 76.3 ($C_2$), 74.8 ($C_5$), 73.2 (Benzyl C), 44.4 (PDMAB Ethyl $CH_2$), 27.9 ($CH_3$), 26.4 ($CH_3$), 18.6 ($C_6$), 12.5 (PDMAB Ethyl $CH_3$). HRMS *m/z* $[M+H]^+$ calcd for $C_{26}H_{36}NO_4S$ 458.2360, found 458.2362

**Methyl 6-*O*-(4-*O*-(*p*-*N*,*N*-diethylamino benzyl)-2,3-*O*-isopropylidene-α-L-rhamnosyl)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (162)**

To a solution of compound **161** (50 mg, 0.11 mmol), methyl 2,3,4-tri-O-benzyl-α-D-glucopyranoside (50 mg, 0.11 mmol), NIS (50 mg, 0.22 mmol) and cyclohexene (0.11 mL, 1.1

mmol) in anhydrous DCM (1.0 mL) was added activated 4 Å molecular sieve (200 mg). The reaction mixture was stirred at room temperature overnight. The reaction temperature was cooled to -78 ℃ and TMSOTf (4.0 μL, 0.02 mmol) was injected into reaction mixture. The reaction was stirred at -78 ℃ for 12 h before it was quenched by triethylamine at-78 ℃. The reaction mixture was warmed to room temperature and filtered. After filtration, the organic layer was washed with aqueous $Na_2S_2O_3$ and brine and dried over $MgSO_4$ The crude mixture was concentrated and purified by sílica gel chromatography to give glycosylation product **162** (20 mg, 22%) as a colorless oil. $[\alpha]^{23}_D$ = +6.4° (c 0.5, $CHCl_3$).$^1$H NMR (600 MHz, $CDCl_3$) δ 7.39 – 7.22 (m, 12H), 7.18 (d, *J* = 8.1 Hz, 2H), 6.63 (d, *J* = 8.1 Hz, 2H), 4.98 (d, *J* = 10.9 Hz, 1H, Benzyl $CH_2$), 4.91 – 4.69 (m, 6H, Benzyl $CH_2$ & Rha $H_1$), 4.65 (d, *J* = 12.1Hz, 1H, Benzyl $CH_2$), 4.55 (ddd, *J* = 6.8, 3.7, 1.7 Hz, 2H, Glc $H_1$ & Benzyl $CH_2$), 4.50 – 4.42 (m, 1H, Benzyl $CH_2$), 4.20 (ddd, *J* = 7.1, 4.4, 1.5 Hz, 1H, Rha $H_2$), 4.04 (dd, *J* = 5.7, 1.6 Hz, 1H, Rha $H_3$), 4.00 – 3.94 (m, 1H, Glc $H_3$), 3.84 – 3.78 (m, 1H, Glc $H_6$), 3.72 – 3.61 (m, 2H, Glc $H_{6'}$ & Rha $H_4$), 3.53 – 3.42 (m, 3H, Glc $H_4$ & Glc $H_2$ & Glc $H_6$), 3.36 – 3.29 (m, 7H, Glc OMe & PEMAB Ethyl $CH_2$), 3.17 (ddt, *J* = 9.9, 7.2, 1.6 Hz, 1H, Rha $H_5$), 1.51 (s, 3H, isopropylidene), 1.35 (s, 3H, isopropylidene), 1.20 (dt, *J* = 6.3, 1.6 Hz, 3H, Rha $H_6$), 1.13 (tt, *J* = 7.0, 1.6 Hz, 7H, PEMAB Me). $^{13}$C NMR (151 MHz, $CDCl_3$) δ 147.5, 138.7, 138.1, 131.4, 129.9, 128.5, 128.4, 128.3, 128.1, 128.0, 127.9, 127.8, 127.6, 124.8, 112.5, 111.6, 109.0 (Isopropylidene C), 97.8 (Glc $C_1$), 97.1 (Rha $C_1$), 82.1 (Glc $C_3$), 80.5 (Rha $C_5$), 80.0 (Glc $C_2$), 78.8 (Rha $C_3$), 77.5 (Glc $C_4$), 75.9 (Rha $C_2$), 75.7 (Benzyl $CH_2$), 74.9 (Benzyl $CH_2$), 73.3 (Benzyl $CH_2$), 69.8 (Glc $C_5$), 65.8 (Glc $C_6$), 64.7 (Rha $C_4$), 55.1 (Glc OMe), 44.4 (PDEAB Ethyl $CH_2$), 28.0 (Isopropylidene

Me), 26.4 (Isopropylidene Me),17.7 (Rha $C_6$), 12.5 (PDEAB Ethyl $CH_3$). HRMS *m/z* $[M+H]^+$ calcd for $C_{48}H_{62}NO_{10}$ 812.4374, found 812.4375.

**Methyl 6-*O*-(2,3-*O*-isopropylidene-α-L-rhamnosyl)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (163)**

Compound **162** (9 mg, 0.01 mmol) was dissolved in 2 mL methanol and was transferred into a 10 mL vial equipped with a magnetic stir bar. The vial was capped properly and placed in the microwave (CEM Discover SP Microwave). The microwave was run at 300 W, 180 °C and 300 psi for 30 min. The vial was cooled to room temperature and the reaction mixture was transferred into round bottom flask and concentrated in *vacuo*. The residue was purified by silica gel column chromatography to give product **163** (5.3 mg, 73%) as a colorless oil. $[\alpha]^{23}_D$ = +9.6° (c 0.3, $CHCl_3$). $^1$H NMR (600 MHz, $CDCl_3$) δ 7.38 – 7.23 (m, 16H), 4.99 (d, *J* = 10.8 Hz, 1H, Benzyl $CH_2$), 4.88 (d, *J* = 11.0 Hz, 1H, Benzyl $CH_2$), 4.83 – 4.74 (m, 3H, Benzyl $CH_2$ and Rha $H_1$), 4.66 (d, *J* = 12.1 Hz, 1H, Benzyl $CH_2$), 4.59 (d, *J* = 3.5 Hz, 1H, Glc $H_1$), 4.56 (d, *J* = 11.0 Hz, 1H, Benzyl $CH_2$), 4.08 (t, *J* = 6.3 Hz, 1H, Rha $H_2$), 4.04 (d, *J* = 5.9 Hz, 1H, Rha $H_3$), 3.99 (t, *J* = 9.3 Hz, 1H, Glc $H_3$), 3.85 (dd, *J* = 10.7, 2.0 Hz, 1H, Glc $H_{6'}$), 3.76 – 3.65 (m, 2H, Glc $H_5$ & Rha $H_4$), 3.54 (dd, *J* = 10.8, 4.7 Hz, 1H, Glc $H_4$), 3.52 – 3.45 (m, 2H, Glc $H_2$ & Glc $H_6$), 3.39 (dd, *J* = 8.3, 6.7 Hz, 1H, Rha $H_5$), 3.36 (s, 3H, Glc OMe), 1.50 (s, 3H, isopropylidene), 1.33 (s, 3H, isopropylidene), 1.26 – 1.24 (m, 3H, Rha $H_6$ ).$^{13}$C NMR (151 MHz, $CDCl_3$) δ 138.6, 138.0, 128.5, 128.4 , 128.1, 128.0, 127.6, 109.4 (Isopropylidene C), 98.0 (Glc $C_1$), 97.3 (Rha $C_1$), 82.1 (Glc $C_3$), 80.0 (Glc $C_2$), 77.8 (Rha $C_3$), 77.3 (Glc $C_4$), 75.8 (Rha $C_2$), 75.2 (Benzyl $CH_2$), 75.0 (Benzyl $CH_2$), 73.7 (Benzyl $CH_2$), 73.4 (Benzyl $CH_2$), 69.7

(Glc $C_5$), 66.7 (Rha $C_4$), 65.9 (Glc $C_6$), 55.2 (Glc OMe), 27.7 (Isopropylidene Me), 25.9 (Isopropylidene Me), 17.8 (Rha $C_6$). HRMS *m/z* $[M+Na]^+$ calcd for $C_{37}H_{46}NaO_{10}$ 673.2989, found 673.2991.

**Inhibition of anti-CR3-FITC antibody staining of human neutrophils and of anti-Dectin 1-FITC antibody staining of mouse macrophages.**

For fluorescent staining, anti-CR3-FITC antibodies (MN-41 donated by Drs. Allison Eddy and Alfred Michael of the University of Minnesota, Minneapolis, MN, and Rat anti Mouse Dectin-1 antibody labeled with FITC (purchased from AbD Serotec, Raleigh, NC) were employed. Either human neutrophils or mouse peritoneal macrophages were incubated with 0.1 $\mu g.mL^{-1}$ of tested samples for 0.5 h on ice and washed. Subsequently, the cells were stained with antibodies on ice using standard techniques. After centrifugation of cells through a 3 mL cushion of 12% BSA in PBS, the cells were re-suspended in PBS containing 1% BSA and 10 mM sodium azide. Cell cytometry was performed with a Becton Dickinson-LSRII instrument. The inhibition of CR3 receptor and Dectin-1 receptor staining was calculated as described.[90]

**Stimulation of phagocytosis**

The technique employing phagocytosis of synthetic polymeric microspheres was described earlier.[91 9191]Human cells (cell line RAW 264) were incubated *in vitro* with 10 $\mu g.mL^{-1}$ of tested samples for 24 h at 37 $^oC$. After washing, 0.05 mL of 2-hydroxyethyl methacrylate particles (HEMA; $5x10^8$/mL) was added. The test tubes were incubated at 37 $^oC$ for 1 h, with intermittent shaking. Smears were stained with Wright stain. Cells with three or more HEMA particles were considered positive. The insoluble glucan Glucan #300 used as

comparison standard was obtained from Yeast-derived insoluble Glucan #300 (>85% dry w/w basis) was purchased from Transfer Point (Columbia, SC, USA). This glucan contains 96% carbohydrates and 2.1% proteins. Neutral sugar analysis confirmed 91.3% glucose and 8% mannose. Stimulation of pinocytosos was determined spectrophotometrically as described.[92]

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## ABSTRACT

## DESIGN AND SYNTHESIS OF OLIGO-(3,5-DITHIO-*β*-D-GLUCOPYRANOSIDE) AS *β*-(1→3)-GLUCAN MIMETICS

by

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**December 2018**

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**Major:** Chemistry

**Degree:** Doctor of Philosophy

This dissertation presents the design and synthesis of oligo-(3,5-dithio-*β*-D-glucopyranoside) as *β*-(1 → 3)-D-glucan mimetics. The synthesized dimeric, trimeric and tetrameric mimetics were tested for their binding affinities to Dectin-1 and CR3 and their abilities to activate phagocytosis and pinocytosis.

The first chapter gives an introduction of *β*-(1 → 3)-D-glucan. It gives an introduction about the structure and origin of *β*-(1 → 3)-D-glucan as well as the immunostimulating effect of natural *β*-(1 → 3)-D-glucan. The study of interaction between *β*-(1 → 3)-D-glucan and the receptors leads to a conclusion that the hydrophobicity of *β*-(1 → 3)-D-glucan is crucial to its immunostimulating effect. Based on this conclusion, we designed the oligo-(3,5-dithio-*β*-D-glucopyranoside) as *β*-(1 → 3)-D-glucan mimetics.

The second chapter presents the synthesis study of oligo-(3,5-dithio-*β*-D-glucopyranoside). After a brief introduction of the synthesis of S-linked oligosaccharide, we talked about the synthesis of the 3,5-dithio-glucopyranoside as the monosaccharide building

block. It presents the rearrangement product during the synthesis of 3,5-dithio-glucopyranoside and how to avoid the rearrangement to synthesize 3,5-dithio-glucopyranoside. After the monosaccharide synthesis, we talked about the S-glycosylation study of 3,5-dithio-glucopyranoside and how to synthesize the oligo-(3,5-dithio-$\beta$-D-glucopyranoside). Finally, the biological study of synthesized dimeric, trimeric and tetrameric mimetics was presented, which includes their binding affinities to Dectin-1 and CR3 and their abilities to stimulate the phagocytosis and pinocytosis.

Chapter three introduces *p*-*N*,*N*-dimethylamino benzyl (PDMAB) protecting group as a new protecting group. The novelty of PDMAB protecting group is that the deprotection can be achieved by microwave irradiation without any additives. We talked about the installation of PDMAB group and its application in the NIS / TMSOTf promoted glycosylation.

Finally, chapter four presents the experimental procedures of all the compound and the characterization data.

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Xiaoxiao Liao,[a] Václav Větvička,[b] and David Crich[a,]* "Synthesis and Evaluation of 1,5-Dithia-D-laminaribiose, Triose and Tetraose as Truncated β-(1→3)-Glucan Mimetics." *J. Org. Chem*. (Submitted)