**University of Alberta**

# Synthesis of the repeating unit of *Streptococcus pneumoniae* (Sp1) zwitterionic polysaccharide

by

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# Abstract

According to the traditional paradigm, carbohydrates are considered to be poorly immunogenic, T-cell independent antigens. Pure polysaccharides induce specific IgM responses, with minimal class switch to IgG. However, a series of recent investigations has found that a class of zwitterionic polysaccharides (ZPSs) induces a variety of T-cell specific responses such as cell proliferation, cytokine secretion, and regulation of antibody production. The two most studied among this family of molecules is capsular polysaccharide (PS) A1 from the *Bacteroides fragilis* and the type 1 *Streptococcus pneumoniae* polysaccharide capsule (Sp1). Active ZPSs share a common structural motif; a high density of positively charged amino and negatively charged carboxyl or phosphate groups. These features are essential for the activity of ZPSs. Since the biological repeating unit of the polysaccharides is not known and biological activity will most likely depend upon a precise sequence, synthesis of the repeating unit of these capsular polysaccharides was undertaken.

The goal of this work is to synthesize the repeating unit [→3)-α-D-Fuc*p*N2AcN4-(1→4)-α-D-Gal*p*A-(1→3)-α-D-Gal*p*A-(1→] of the type 1 capsular polysaccharide (Sp1) found in *S. pneumoniae*. 2-Acetamido-4-amino-2,4,6-trideoxy-D-galactopyranose is one of the three monosaccharides of the repeating unit of the Sp1 of *Streptococcus pneumoniae*. This rare amino sugar is also present in a number of bacterial polysaccharides such as *Bacteroids fragilis, Streptococcus mitis* and *Shigella sonnei*. We have developed a novel method to synthesize the orthogonally protected 2-acetamido-4-amino-2,4,6-trideoxy-D-

galactopyranose on a gram scale with high yield starting from readily available D-glucal. The crucial elements of this approach are the introduction of a 4 amino function via intramolecular cyclization of a 3-*O*-*N*-benzylcarbamate. The resulting *N*-benzyloxazolidinone derivative after conversion to the corresponding glycosyl trichloroacetimidate was shown to be an effective glycosyl donor.

The assembly of the trisaccharide was successfully carried out from the appropriate galactopyranosides selectively protected at *O*-6 to permit oxidation to uronic acid derivatives after successful assembly of the target trisaccharide. The trisaccharide was tested for its ability to stimulate interleukin 10 (IL-10) and Interferon-gamma (IFN-γ) in collaboration with Dr. Dennis L. Kasper (Channing Laboratory, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts, USA). Unfortunately the trisaccharide was not active.

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# List of Abbreviations

| | |
|---|---|
| Ab | antibody |
| Ag | antigen |
| Ac | acetyl |
| AIBN | 2,2'-azobis(isobutyronitrile) |
| All | allyl |
| APC | antigen presenting cell |
| aq. | aqueous |
| Ar | aryl |
| Bn | benzyl |
| BnBr | benzyl bromide |
| BSP | benzenesulfinyl piperidine |
| Bz | benzoyl |
| *c* | concentration |
| CAN | ceric ammonium nitrate |
| CSA | camphorsulfonic acid |
| COSY | correlation spectroscopy |
| δ | chemical shift |
| d | doublet |
| DBU | 1,8-diazabicyclo-[5.4.0]-undec-7-ene |
| DCM | dichloromethane |
| dd | doublet of doublets |
| DDQ | 2,3-dichloro-5,6-dicyano-1,4-benzoquinone |

| | |
|---|---|
| DIBAL-H | diisobutylaluminium hydride |
| DMTST | dimethyl(thiomethyl)sulfonium triflate |
| eq | molar equivalents |
| ER | endoplasmic reticulum |
| ESI HRMS | electrospray ionization high resolution mass spectrometry |
| GalNAc | *N*-acetylgalactosamine |
| gCOSY | gradient coupling correlated spectroscopy |
| GlcNAc | *N*-acetylglucosamine |
| HMBC | heteronuclear multiple bond correlation |
| Hz | Hertz |
| Ig | Immunoglobulin |
| IR | infrared spectroscopy |
| *J* | coupling constant |
| m | multiplet |
| M | molar |
| Me | methyl |
| MHC | major histocompatibility complex |
| Ms | mesyl |
| MS 4Å | molecular sieves 4 angström |
| MS | mass spectrometry |
| NBS | *N*-bromosuccinimide |
| NIS | *N*-iodosuccinimide |

| | |
|---|---|
| NMR | nuclear magnetic resonance |
| *p* | para |
| *p*TsOH | *p*-toluenesulfonic acid |
| PCC | pyridinium chlorochromate |
| PDC | pyridinium dichromate |
| Ph | phenyl |
| Phth | phthaloyl |
| $Ph_2SO$ | diphenyl sulphoxide |
| PMB | *p*-methoxybenzyl |
| PMBBr | *p*-methoxybenzyl bromide |
| PMBCl | *p*-methoxybenzyl Chloride |
| PMP | *p*-methoxyphenyl |
| ppm | parts per million |
| Py | pyridine |
| q | quartet |
| RT | room temperature |
| $R_f$ | retardation factor |
| s | singlet |
| *t*Bu | *tert*-butyl |
| t | triplet |
| TBAF | tetrabutylammonium fluoride |
| TBAI | tetrabutylammonium iodide |
| TBDPS | *tert*-butyldiphenylsilyl |

| | |
|---|---|
| TBDMS | *tert*-butyldimethylsilyl |
| TBS | *tert*-butyldimethylsilyl |
| TCR | T-cell receptor |
| TEA | triethylamine |
| TEMPO | 2,2,6,6-tetramethyl-1-piperidinyloxy |
| *tert* | tertiary |
| TfOH | trifluoromethanesulfonic acid |
| TFA | trifluoroacetic acid |
| TFA | trifluoroacetyl |
| THF | tetrahydrofuran |
| TLC | thin layer chromatography |
| TMS | trimethylsilyl |
| Tr | trityl |
| Troc | trichloroethoxycarbonyl |
| Ts | *p*-toluenesulfonyl |
| TTBP | 2,4,6-tri-*tert*-butylpyrimidine |

# Chapter One

# Introduction

## 1.1 The biological importance of Carbohydrates

Carbohydrates are the most abundant and widely distributed natural biopolymers on earth, even more so than the other biopolymers such as nucleic acids and proteins.[1] They are essential components of all living organisms.[2] Saccharides are known to be structural units as well as food and energy sources for life forms.[2] Structural polysaccharides are mainly cellulose and chitin. The principal structural component in plants is cellulose, which gives shape and rigidity to the plant cell wall. Cellulose is a linear homopolymer of D-glucose composed of β-1→4-linkages. Chitin is the major component of the exoskeletons of invertebrates such as insects, crustaceans and spiders. It is also found in the cell wall of fungi and algae. It help protects the creature against harm and pressure. Chitin is composed of β-1→4 linked units of the *N*-acetylglucosamine. Thus, chitin and cellulose have very similar molecular structures with the exception of the acetamido variation at the C-2 position of the monosaccharide hydroxyl.[2]

Starch is the energy storage polysaccharide of the plants, composed of two components: amylose and amylopectin. Amylose is a linear polymer, composed of α-1→4-D-glucose subunits whereas amylopectin is a branched polymer of predominantly α-1→4-D-glucose subunits with branches attaching to the 6$^{th}$ position to form α-1→6-glycosidic linkages. In animal cells, glycogen is the starch equivalent storage polymer. Structurally, glycogen is very similar to

amylopectin except that the branches tend to be shorter and more frequent in glycogen.[2] In glycogen, the reducing end of the glucose is covalently connected to the protein glycogenin.

Even though in the past decades, glycans were only regarded as energy sources and structural units, recently these molecules have been found to play critical roles in biological functions and physiological processes.[3] Oligosaccharides on the cell surface are involved in signaling and recognition events, determining human blood groups, mediating fertilization, serving as receptors for bacterial and viral infections and mediating immune response and inflammation. The unique structural complexity of carbohydrates enables them to play a wide variety of roles in biological systems, in contrast to nucleic acids and proteins.

An important characteristic of monosaccharides is the potential to be linked through different positions, which permits the molecule to be highly branched, whereas nucleic acids and proteins are predominantly linear polymers. Monosaccharides can exist in the pyranose form or in the furanose form. The glycosidic linkages can be of two different forms, $\alpha$ or $\beta$, providing two stereoisomers of the monosaccharide.[4]

In biological systems, oligosaccharides are most often conjugated with other biomolecules such as lipids or proteins (Figure 1). These glycolipids and glycoproteins are collectively known as glycoconjugates. The sugar portion of a glycoconjugate is referred to as a glycan.[1] Both glycoproteins and glycolipids are components of extracellular matrices and cellular surfaces. However, membrane

associated saccharides are primarily in the form of oligosaccharides covalently attached to proteins and to a lesser extent to lipids.

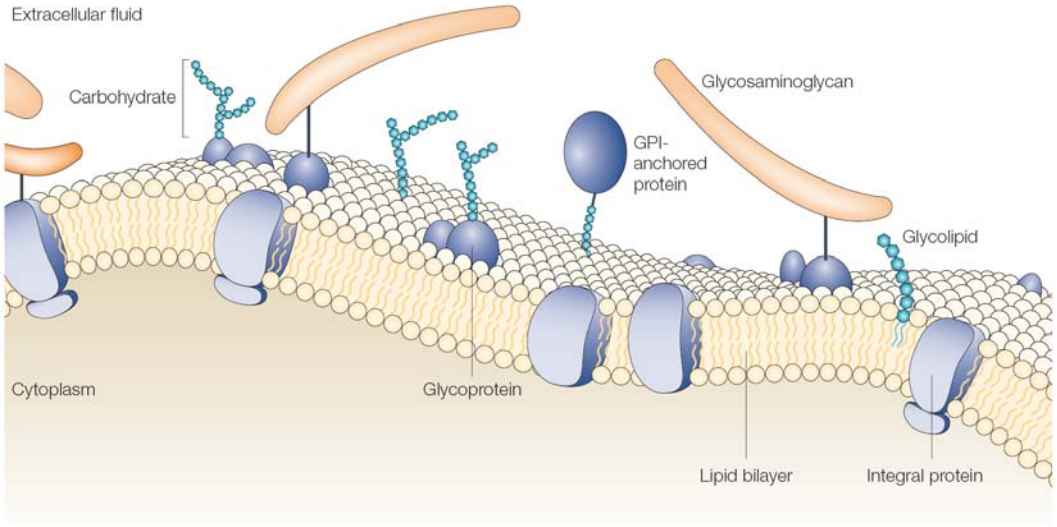



Figure 1: *Schematic view of a cell membrane with glycoconjugates*[5]

Glycoproteins can be classified into two major categories: *N*-linked and *O*-linked, depending on the type of the linkage between the reducing end of the glycan and the amino acid. The more common *N*-linked glycoproteins have their glycans attached to the amide nitrogen of an asparagine residue (Asn). Glycosylated asparagine residues frequently occur in the tripeptide sequences Asn-X-Ser or Asn-X-Thr, where X can be any amino acid except proline. In animal cells, the glycans linked to the asparagine residue is almost always β-linked *N*-acetylglucosamine (GlcNAc).[6] *N*-Linked glycans share a common pentasaccharide core structure (Figure 2).[4]

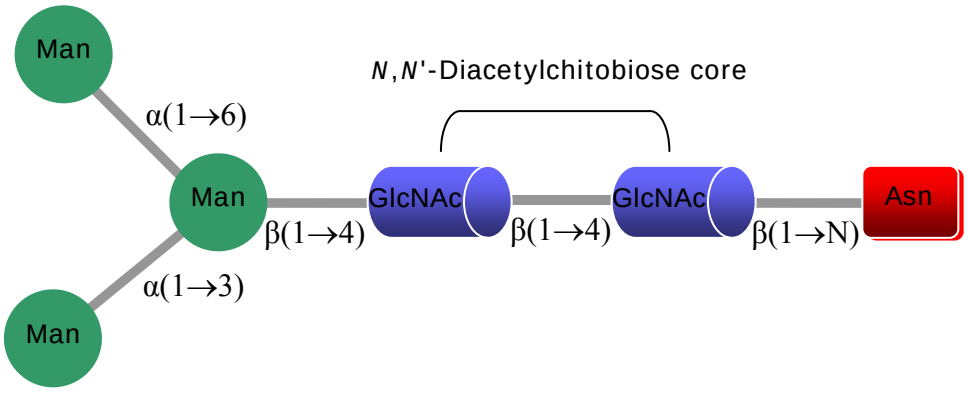



Figure 2: *Schematic diagram of the common pentasaccharide of N-linked glycan*

During biosynthesis, *N*-linked glycoproteins are assembled in three steps, which occur in the endoplasmic reticulum (ER) and continue through the Golgi apparatus. Initially, the lipid-linked oligosaccharide precursor is formed then the oligosaccharide precursor is transferred from dolichol, a long-chain unsaturated isoprenoid alcohol, to an Asn residue on a nascent polypeptide by an oligosaccharyltransferase. Processing of the oligosaccharide then occurs. Generally, *N*-linked glycoproteins include both membrane bound and circulating glycoproteins and act as signals of cell surface recognition phenomena.[4]

The *O*-linked glycans are less common, and have more varied forms. The *O*-linked glycoproteins are known as mucin type glycoproteins, since they were found in a mucus substance called 'mucin'. Glycans of *O*-linked glycoproteins confer particular physicochemical properties on proteins.[4] In *O*-linked glycoproteins, glycans are attached to the hydroxyl groups of amino acids serine (Ser) or threonine (Thr). The most common type of reducing end in the *O*-linked glycan is *N*-acetylgalactosamine (GalNAc).[7] There are other *O*-linked glycans which have α-linked *N*-acetylglucosamine, xylose, galactose, fucose, or mannose

as the first sugar bound to Ser or Thr residues. The *O*-linked glycans do not carry a common core like *N*-linked glycans, indeed, the peptide bound GalNAc is diversely glycosylated.[8] The biosynthesis of *O*-glycans differs from that of *N*-glycans: the addition of GalNAc residues to amino acids occurs post-translationally in the Golgi apparatus.

In addition to glycoproteins, a large number of glycolipids are found on the cell surface. Glycolipids are comprised of glycans attached to the polar head group of lipids. Glycolipids can be categorized into glycosphingolipids, where glycans are attached to a ceramide core structure, and glycophospholipids, which contain glycans attached to a phosphatidic acid core structure. Ceramide is composed of the long-chain amino alcohol sphingosine linked to a fatty acid via an amide bond. Glycosphingolipids are involved in cellular recognition, whereas glycophospholipids helps to anchor proteins to the cell surface.[6] The biosynthesis of glycolipids resembles that of the *N*-linked glycans. Almost all of these reactions are carried out in the Golgi or ER lumen.

Even though the extreme complexity and diversity of carbohydrates fascinate scientists, progress in carbohydrate science lags behind the science of nucleic acids and proteins,[9] primarily due to the lack of tools to probe carbohydrate function and structure. However, recent progress is beginning to correct this deficiency in the area of glycobiology, a term introduced by Raymond Dwek at the University of Oxford in 1988 to describe the structure, biosynthesis, and biology of complex glycans.

## 1.2 Carbohydrates as therapeutics

### 1.2.1 Carbohydrates and immunogenicity

Oligosaccharides are generally located on the outside of cell membranes. It is assumed that the first contact that many cells have with each other will be via the interaction of carbohydrates,[10] therefore glycoconjugates on cell surfaces serve as cellular identification tags to the surrounding world.[11] Pathogens make use of glycoproteins and glycolipids as receptors on host cell surfaces for infection and colonization.[10] Understanding the cellular function of glycans opens the door to the medical field of carbohydrates by offering exciting new therapeutic opportunities.

The discovery of heparin as an antithrombotic agent in the 1940s is the pioneer example of carbohydrate-based drugs.[5] Scientists are interested in developing medical applications of glycoconjugates to detect, prevent or treat disorders in living systems. A considerable number of drugs on the market today rely on carbohydrates for part of their therapeutic action which include anti-inflammatory, anticancer, antidiabetic, anticonvulsant, antibiotic and antiviral activities.[12]

Carbohydrate based vaccines are highly effective in providing protection against infectious pathogenic agents and combat increasing antibiotic resistance.[13] The cell surfaces of microbes have unique polysaccharide markers that are often distinct from those of their hosts. The immune system of the host recognizes these polysaccharide antigens and stimulates antibodies against them, which promotes an immune response to battle the infection.[14] Generally, the immune system

develops a memory of these antigens and more easily recognizes them on subsequent encountering and destroys these microorganisms that it later encounters. Vaccines are typically made from weakened or killed pathogens or from immunogenic proteins, glycoproteins or polysaccharides obtained from microorganisms.[15] Carbohydrate-based vaccines against several microbes have been widely used for several decades.[14] Vaccines based on purified capsular polysaccharides or neoglycoconjugates have been used to fight against *Neisseria meningitidis*, *Streptococcus pneumoniae*, *Haemophilus influenza* type b (Hib) and *Salmonella typhi*.[14]

### 1.2.2 Capsular polysaccharides: Immunogenicity and Vaccines

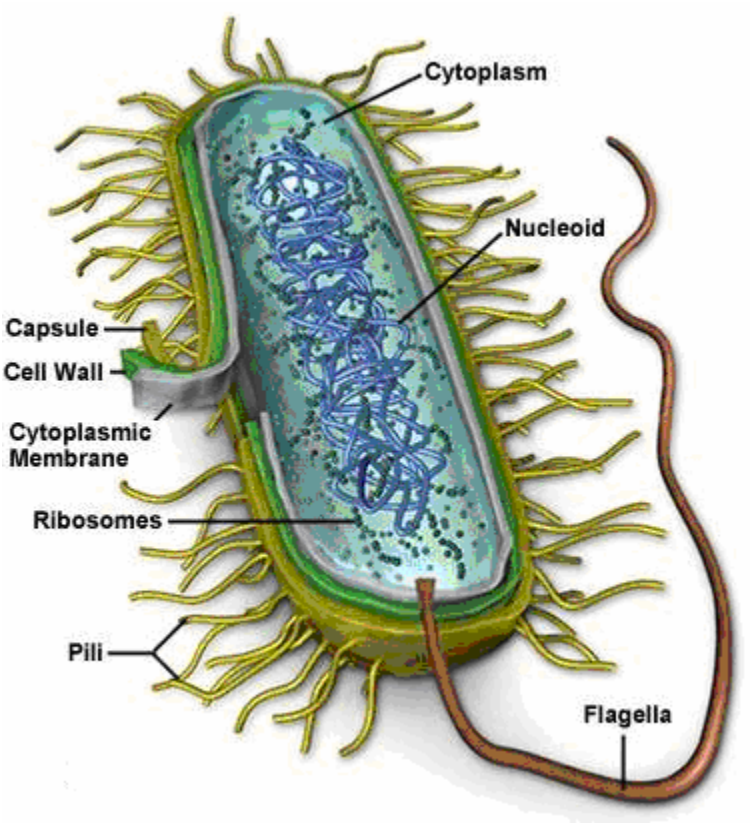



Figure 3: *Prokaryotic cell structure*[16]

Capsular polysaccharides (Figure 3) are ubiquitous structures found on the cell surface of a wide range of pathogenic bacteria.[17] They are important virulence factors in many bacteria isolated from infected people. The capsular polysaccharides exist as either regular homopolymers or heteropolymers that consist of two to six sugar residues per repeating unit. They can be found in both Gram negative and Gram positive bacteria.[18] Carbohydrate capsules can serve a number of critical roles for bacteria. Since capsular polysaccharides form the outer surface of the cell, they mediate adhesion to inert surfaces or living tissues and protect bacteria from the harmful effects of desiccation.[19] During invasive

bacterial infections, capsules have a role in virulence,[19] hiding cell surface components of the bacterium from the immune system of the host[20] that would otherwise provoke an immune response and thereby lead to the destruction of the bacterium.

Immunologically, an antigen can be classified as T-cell-independent or T-cell-dependent. T-cell-independent antigens can directly stimulate the B cells to produce an antibody without the requirement for T-cell help, whereas T-cell dependent antigens do not directly stimulate the production of an antibody without the help of T-cells. Most polysaccharides (Figure 4) fall under the former class while proteins and peptides fall in the latter class.[18] Scientists believe that the polysaccharide antigens cannot be processed by antigen presenting cells; (APC) therefore they can not utilize T-cell help. In contrast, T helper cell immune responses elicited by protein antigens stimulate long-lived cell-mediated and humoral responses. APC are capable of internalizing protein antigens, degrading them to smaller peptide units and presenting these fragments in a complex with major histocompatibility complex class II molecules (MHCII) on the cell surface. These antigens can be recognized by T-cells and are activated to perform effector functions such as providing T-cell help in the production of specific IgG antibodies.[21]

An immune response to capsular polysaccharides (Figure 4) confers protection against infection via the induction of antibodies.[18,22] This is why a number of carbohydrate-based vaccines against bacterial infections aim exclusively at the induction of antibodies against capsular polysaccharides.[22]

Pneumococcal capsular polysaccharide was the first non protein antigen to be used in a vaccine and was developed by Heidelberger and co workers.[13] Purified polysaccharide vaccines have been used for more than 40 years, however developing vaccines based on polysaccharides are difficult and several problems have to be solved.[18] One of the biggest problems is that bacterial polysaccharides, due to their T-cell-independent properties have proven to be poor immunogens in high risk groups such as infants who have immature immune systems, and immuno-compromised patients.[13,18,23]

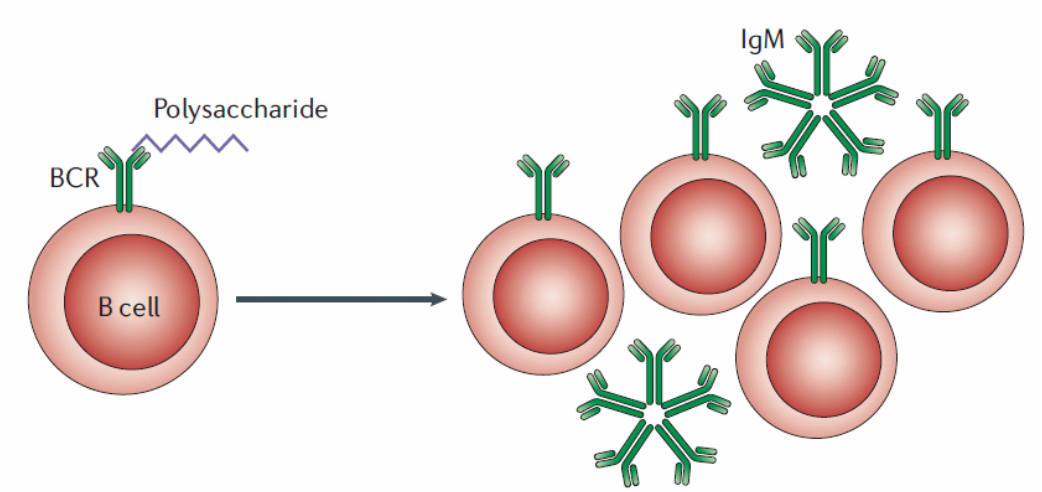



Figure 4: *Immune cell activation by bacterial polysaccharide*[24]

The T-cell independence of bacterial polysaccharides results in two other limitations such as isotype restriction. This is the failure to induce immunoglobulin class switching from IgM to IgG isotypes, a process associated with higher affinity and high antibody levels.[19,25] Additionally, for T-cell-independent antigens, antibody levels do not increase by repeated doses; therefore, only one dose is given at a time. Since antibody levels decline with time, re-vaccination is required at regular intervals. Despite their limitations,

polysaccharide vaccines are of moderate efficacy in appropriate populations, such as adolescents and adults, and are generally cheap and have an excellent safety record.[20]

In order to overcome these limitations, conjugate vaccines have been developed. In contrast to polysaccharides, glycoproteins are T-cell-dependent antigens.[13] In 1930's, Avery and Goedel reported that conjugation to a protein enhanced the immunogenicity of polysaccharides and as we now know this is achieved because it is converted to T-cell-dependent immunogen.[8,13,23] This results in an immune response against the polysaccharide with characteristics of T-cell-dependent antigens, long lasting immune memory,[25] avidity maturation and isotype switching to generate complement-activating antibody isotypes such as IgG. The most widely used carrier proteins are bacterial toxoids, which include tetanus and diphtheria toxoids. Glycoconjugate vaccines are immunogenic in young infants since both humoral and cellular arms of the immune system are involved in the response to these antigens.[20] The immune response to glycoconjugates vaccines are based on a mechanistic 'trick' played on the immune system.[24] The mechanism by which glycoconjugate vaccines elicit an immune response is significantly different from the polysaccharide vaccine itself.[20]

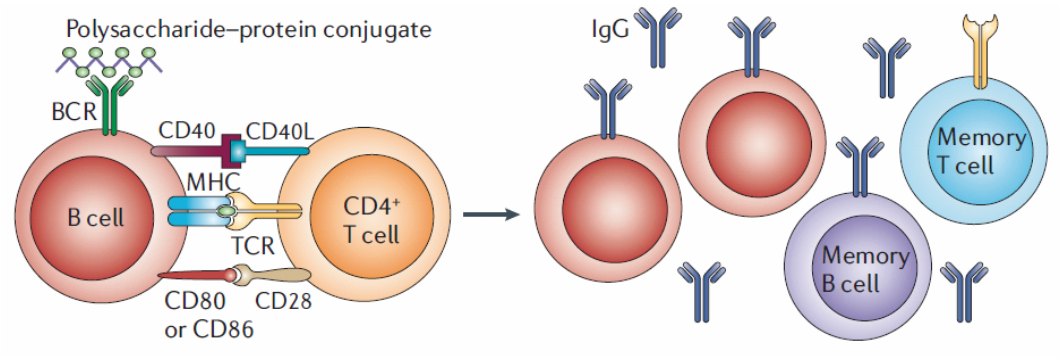



Figure 5: *Immune cell activation by polysaccharide protein conjugate*[24]

Initially, a B cell recognizes the polysaccharide epitope of the glycoconjugate and binds to the surface with appropriate specificity (Figure 5). Then, the complex is internalized and the carrier protein is degraded by proteolytic enzymes. Suitable peptides (T-cell peptides) from the attached carrier proteins are transported and displayed on MHC II proteins. T-cells recognize the peptide-loaded MHC II complex, which then provide appropriate signals to activate the B cells to produce an antibody against the polysaccharide (Figure 5).[24] The development of glycoconjugate vaccines has been one of the most important achievements in the biomedical field.[24]

## 1.3 Zwitterionic capsular polysaccharide (ZPS)

### 1.3.1 General introduction

The vast majority of bacterial polysaccharides are anionic or neutral.[22] These molecules fail to activate T-cells and antigen presenting cells,[26] and do not elicit T-cell responses such as antibody isotype switching to IgG.[27] Therefore, they are traditionally regarded as T-cell-independent antigens.[27] However, a series of recent investigations has found a structurally distinct class of polysaccharides,

those that naturally contain both positive and negative charges in their repeating units, therefore called Zwitterionic Polysaccharide (ZPS).[24,28] ZPSs are found to be especially potent T-cell activators.[27] These polysaccharides of microbial origin do, in fact, induce a variety of T-cell specific responses such as cell proliferation, cytokine secretion, and regulation of antibody production in a manner quite similar to that of proteins.[22,24,27,28] These ZPSs can induce or inhibit abscess formation.[27,29] The zwitterionic motif is critical for the distinct pathological and immunological properties of the ZPSs. It was demonstrated nearly 15 years ago that chemical neutralization of either of these charged groups results in the loss of their ability to activate T-cells.[26,30] To extend those findings, structure-function relationship of ZPSs has been investigated. In addition, recently, Andreana and coworkers showed that PS A1 can be used as a carrier for tumor-associated carbohydrate antigens (TACAs).[31]

### 1.3.2 Structure of ZPSs

Studies of both structure-function and mechanism of action of hundreds of bacterial capsular polysaccharides have been examined in detail,[21] but only a few of these contain zwitterionic charge motifs.[32] These include PS A1, PS A2, and PS B from *Bacteroides fragilis* and type 1 capsular polysaccharide (Sp1) from *Streptococcus pneumoniae*. Although they share similar biological activities, ZPSs consist of different chemical structures.[29] From chemical analysis it was found that the *B. fragilis* capsular polysaccharide complex (CPC) is composed of ionic complex components.[33-35] An isoelectric focusing technique was used to

separate the two major high molecular weight polysaccharides named polysaccharide A (PS A1) and polysaccharide B (PS B) from the *B. fragilis* CPC. High resolution NMR analysis showed that the PS A1 and PS B molecules are structurally different but are both comprised of repeating units with positively charged amino groups and negatively charged carboxyl groups.[36-38] However, immunologically, PS A1 is a significantly more potent abscess inducer compared to PS B.[39] Less than 1 µg of PS A1 is enough to induce abscess formation in rodent models.[39] The chemical structures of these ZPSs are shown in Figure 6.[29] These ZPSs differ significantly in their monosaccharide compositions, linkages and sequences.

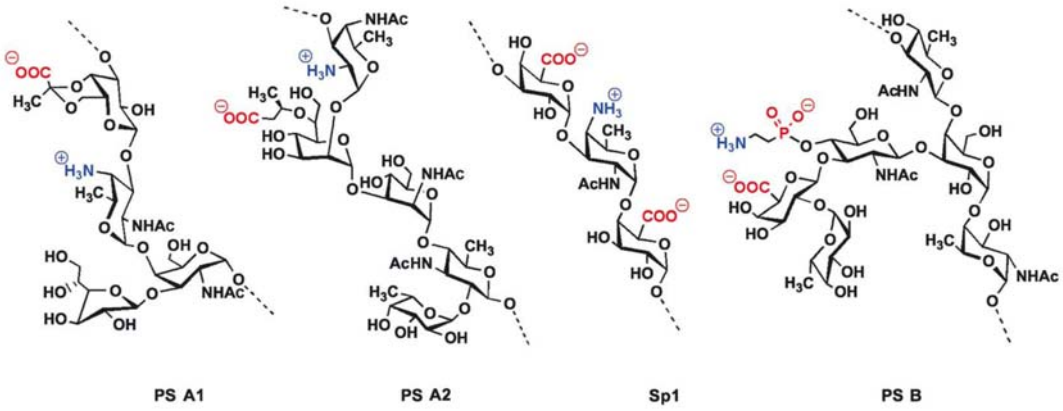



Figure 6: *Chemical structures of the repeating units of ZPSs from B. fragilis and S. pneumoniae type 1*[29]

The two most studied among this ZPS family is PS A1 from the capsular polysaccharide of *B. fragilis* and Sp1 from the *S. pneumoniae* polysaccharide capsule.[26] PS A1 is primarily composed of several hundred repeating units of a branched tetrasaccharide, which is made up of three monosaccharides in the polymer backbone and a single residue side chain.[24,29] The repeating unit of PS

A1 consists of a 4,6-pyruvate attached to D-galactopyranose, 2-acetamido-4-amino-2,4,6-trideoxygalactose, D-*N*-acetylgalactosamine and D-galactofuranose.[40] Sp1 is a linear polymer of trisaccharide repeating units, containing two galacturonic acid residues and one 2-acetamido-4-amino-2,4,6-trideoxygalactose residue which is similar to the sugar residue of PS A1.[27] Despite the difference in their chemical compositions and monosaccharide sequences, PS A1, PS A2, Sp1, and PS B all carry a high density of positive and negative charges. PS A1, PS A2, and Sp1 contain positively charged amino and negatively charged carboxylate groups whereas PS B carry positively charged amines and negatively charged carboxylate and phosphonate groups.[29]

### 1.3.3 Role of ZPS in clinical disease and essential functional groups for the immunogenic response

Kasper and coworkers have shown that ZPSs from capsular polysaccharides are potent abscess-inducing agents. Abscess formation may be initially beneficial to the host however, the development of abscess is a serious and life-threatening manifestation of persisting microbial infection in patients recovering from surgery and can lead to fatal complications.[41] By definition, an abscess is an accumulation of pus that has collected in a cavity formed by the tissue surrounding the pathogen or other foreign materials to prevent the spread of infection. Abscess can develop at a number of body sites, such as abdominal cavity, lung, skin and brain.[32]

The classic and most common type of abscess that occurs clinically is the intra-abdominal abscess,[32] which is typically encountered after surgical

procedures that involve the peritoneum. It is caused by Gram-negative anaerobic microorganism *Bacteroides fragilis* that accounts for only 0.5% of the normal human colonic microflora; however, it is the anaerobic species that are most frequently isolated from clinical infections particularly within the abdominal cavity. ZPSs of *B. fragilis* are found to be potent inducers of intra abdominal abscesses in a rat model of sepsis.[21,34] Intraperitoneal injection of rodents with PS A1 and a sterile cecal contents adjuvant (SCCA) induces abscess formation in more than 50% of the animals. SCCA is a required adjuvant, which is responsible for the concomitant secretion of proinflammatory cytokines critical to abscess formation. Depletion of $CD4^+$ T-cells from splenocytes abrogated the proliferative activity of ZPS, which indicates that T lymphocytes are required for the initial induction of host responses leading to the formation of intra-abdominal abscesses.[42] Similar to PS A1, other ZPSs also exhibit the abscess formation including Sp1 from *Streptococcus pneumoniae*. The pathogenesis of these ZPSs is attributable to the zwitterionic charge motif.

Initial research was conducted to investigate the role of the zwitterionic structure on experimental abscess formation using PS A1 and PS B.[29] Specific chemical modifications of ZPSs that neutralize or remove either of these positively or negatively charged groups on each repeating unit abrogate the abscess formation by at least two orders of magnitude.[21,32,39,43] The free amino group of the PS A1 was modified in two different experiments. Conversion of the free amino group into the uncharged *N*-acetyl group was achieved by *N*-acetylation (Figure 7, I). In another experiment, the free amino group was

converted to a tertiary amino group by treatment with formaldehyde under reducing conditions, which still preserved its positive charge (Figure 7, II).[44]

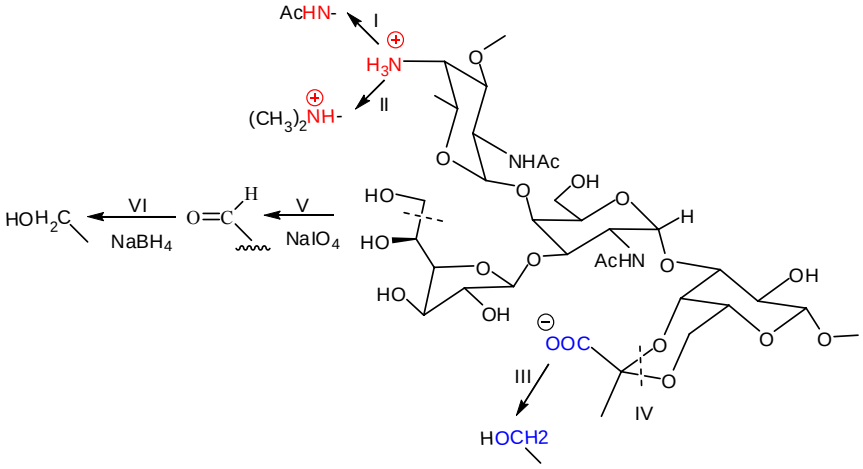



Figure 7: *Chemical modification on the PS A1*[44]

In both cases, chemically modified PS A1 results in the loss of biological activity.[29,44] Furthermore, chemical modifications of the carboxyl group associated with the pyruvate substituent on PS A1 was carried out to neutralize the carboxylate groups as follows. The negatively charged carboxyl group was converted into a hydroxymethyl group, via carbodiimide mediated reduction, thereby eliminating its negative charge (Figure 7, III). As another separate modification, the pyruvate acetal was completely removed by acid hydrolysis (Figure 7, IV). Both chemically modified polysaccharides failed to induce the abscess.[44,45] Similarly, chemical neutralization of the charged groups on the other ZPSs diminished their biological activity.[29]

Anionic polysaccharides are known to be non abscess inducers. However, addition of positively charged groups on the repeating unit converted them into zwitterionic charged polysaccharides, which conferred the ability to induce abscess formation in the animal model.[21,29] These structure-activity relationship

studies demonstrated that only ZPS containing both positive and negative charges, were able to activate the T-cell.[28] The zwitterionic charge motif is therefore a significant structural aspect associated with these capsular polysaccharides for immunogenicity.[29] Although these ZPSs possess different repeating unit structures, the presence of these charged groups is sufficient to activate the T-cell in the experimental animal model.[44]

### 1.3.4 Role of T-cells in abscess formation

T-lymphocytes play a major role in defense against intracellular pathogens and are essential in the immune response. T-cell recognition of foreign antigens is traditionally known to be the host's immune system response to proteins or peptides.[44] Twenty years ago, experimental work, first in a rat and later in a mouse model, showed that T-cells are responsible for the immune response of intra-abdominal abscesses, which are induced by zwitterionic capsular polysaccharides.[46]

ZPSs of *B. fragilis* in athymic mice or T-cell depleted mice formed significantly smaller abscesses thus indicating that T lymphocytes contribute to abscess formation in normal mice.[47] Further investigations were focused on developing *in vitro* analyses that can help to demonstrate which types of antigen presenting cells (APCs) and cytokines are associated with the induction of T-cell responses to PS A1.[48] Experimental observations indicated that the depletion of $CD4^+$ T-cells abolished PS A1 driven cellular proliferation, but not that of $CD8^+$ T-cells.

This suggests the existence of specific T-cell populations of $CD4^+$ T-cells as the PS A1 responsive cell type. The majority of polysaccharides stimulate B-cells directly, albeit weakly. B cells are themselves APCs. However, PS A1 requires the presence of MHC class II bearing APCs in order to stimulate $CD4^+$ T-cells (Figure 8). Stimulation can be inhibited by major histocompatibility complex class II antibody.[24,44] Recent studies have shown that treatment of animals with CTLA4Ig, a potent inhibitor of T-cell proliferation via the CD28-B7 costimulatory pathway, significantly reduces abscess formation when they were challenged with ZPSs. This demonstrated that activation of T-cells by ZPSs is mediated by the CD28-B7 costimulatory pathway.[44,49]

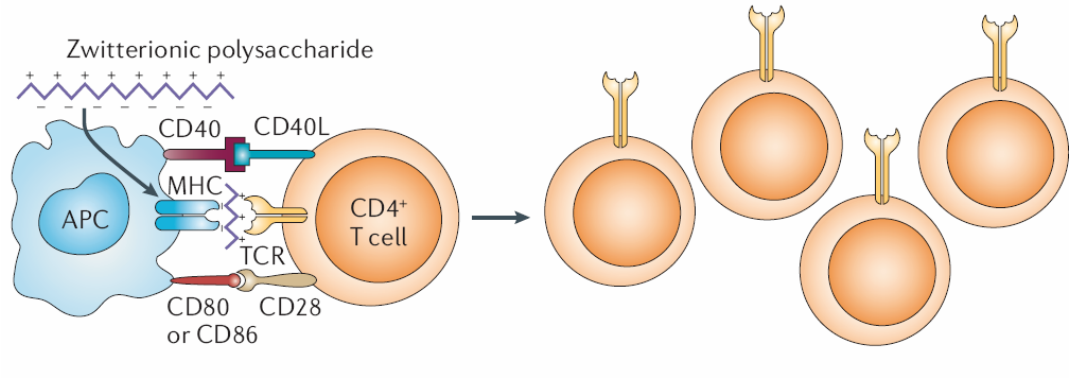



Figure 8: *Immune cell activation by ZPS*[24]

Previous investigations showed that ZPSs, which induce abscess formation necessarily contain free amino groups as the positively charged groups while different groups, such as carboxyl or phosphonate groups can serve as the source of negatively charged groups.[50] Direct interaction of these ZPSs with T-cells is therefore most likely facilitated through the amino functions on these molecules. In order to demonstrate the specificity of T-cell activation by PS A1, chemical oxidation was performed on PS A1. It was treated with sodium

metaperiodate ($NaIO_4$), which is selective for the cleavage of the C–C bond between vicinal hydroxyl groups on carbohydrates. In the case of PS A1, sodium metaperiodate removes the C-6 of the galactofuranose side chain (Figure 7, V) regioselectively and creates an aldehyde group at the C-5 position. This periodate-oxidized PSA that was tested for T-cell proliferation failed to induce the abscess formation in the animal model. The aldehyde group at C-5 of PS A1 was then converted to a hydroxymethyl group by treatment with $NaBH_4$ (Figure 7, VI), which resulted in an arabinofuranose residue. Upon reduction of the oxidized PSA with $NaBH_4$, the proliferative response to PS A1 was restored.[44]

The loss of immunological activity via periodate oxidation is likely due to the generation of an aldehyde on the PS A1 and its reaction with the amine.[44] The highly reactive nature of the amino group can readily participate in chemical reactions with other chemical structures, such as carbonyl groups via covalent bonding.[50] Perhaps free amino groups in the ZPSs participate in similar interactions that are necessary to stimulate the immunologic events that lead to abscess induction.[45,50] In the oxidized form of PS A1, free amino groups might have been involved in the Schiff base formation with intra and or intermolecular aldehydes rather than in the interaction with T-cells, which resulted in the lack of proliferation.[44]

### 1.3.4 Mechanism of antigen processing and presentation of ZPS

Until recently, it was thought that the MHC II pathway is unique to protein antigens, which are liberated from ingested pathogens.[51] $CD4^+$ T-cells recognize protein antigens derived from extracellular sources, such as pathogenic bacteria,

through presentation by MHC II proteins.[26,52] Recognition of protein antigens by the adaptive immune system is achieved through internalizing protein antigens by professional APCs, such as dendritic cells, macrophages, and B cells, antigen processing by the endocytic pathway to peptide sub units followed by MHCII loading and cell surface presentation.[52,53] However, a new addition to this class is zwitterionic capsular polysaccharides, which can activate T-cells through the traditional MHCII pathway.[26] And all three professional APCs were involved in the *in vitro* T-cell proliferation induced by PS A1.[30]

Studies have indicated that ZPSs can be processed and presented in a manner similar to that documented for conventional protein antigens. First, endocytosis of PS A1 occurs through rearrangements of the cytoskeleton that cause membrane invagination to form an endosome.[53,54] So far, the specificity of the endocytosis event and the possibility of receptor proteins are not clear.[54] In the conventional MHC II pathway, inside the endosome, the ingested organism is subjected to one of the earliest induced events, known as the oxidative or respiratory burst.[26] The oxidative burst is characterized by the production of a variety of reactive oxygen species (ROS) (eg: superoxide anions ($O_2^-$)) and reactive nitrogen species (RNS) (eg: free radical nitric oxide (NO)) (Figure 9).[26,51] The production of powerful oxidants facilitates bacterial killing and degrades the protein antigens for further processing and antigen presenting.[55]

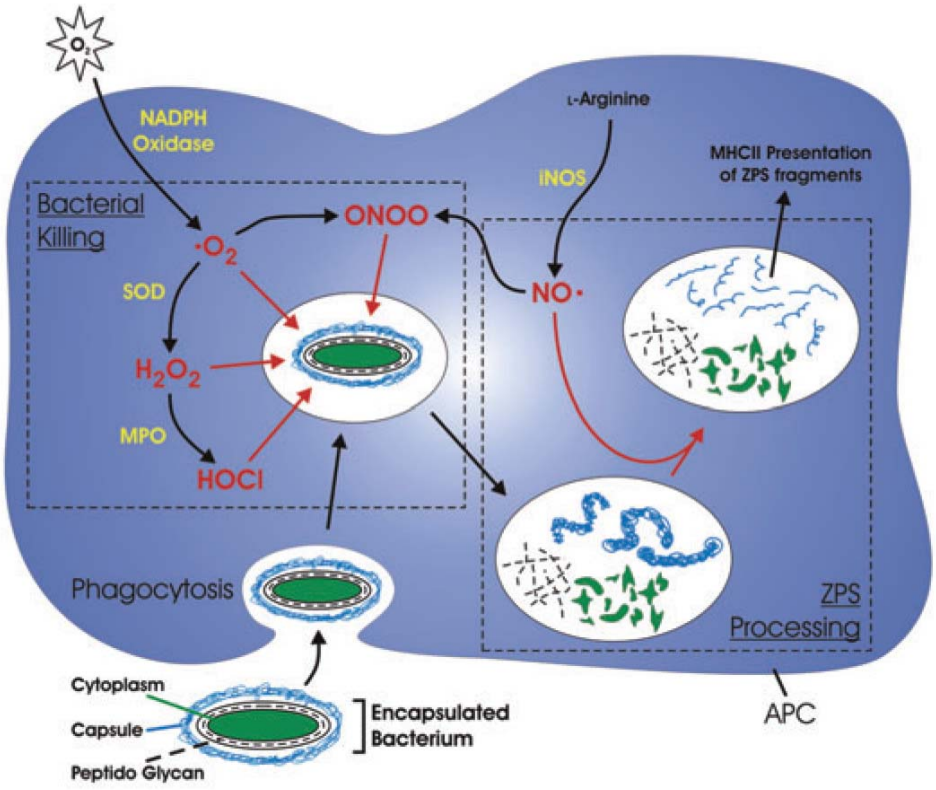



Figure 9 : *Schematic representation of the oxidative burst and its role*[26]

Experiments were conducted to determine the ROS and RNS pathway (Figure 9) for the ZPS processing using the abscess induction model in superoxide-deficient and NO-deficient mice, respectively.[54] Mice lacking nitric-oxide synthase 2 (iNOS) inhibit ZPS-mediated T-cell activation. However, "pre processed" PS A (chemically treated PS A to lower molecular weight carbohydrate antigen) was able to develop abscesses in $iNOS^{-/-}$ animals.[54] This demonstrated that NO production is important for processing of ZPS antigens into smaller fragments of approximately 12–15 kDa (≈15 repeating units) which are capable of being presented by MHCII, these fragments are larger than most peptides (< 2 kDa). In contrast, mice lacking the NADPH oxidase enzyme responsible for generating superoxide (ROS-deficient mice) do not affect ZPS

immunogenicity.[24,40,54] These findings showed that this oxidative event not only kills the bacteria but also involves the processing of ZPSs to a low molecular weight species in a NO-dependent fashion.[26,54]

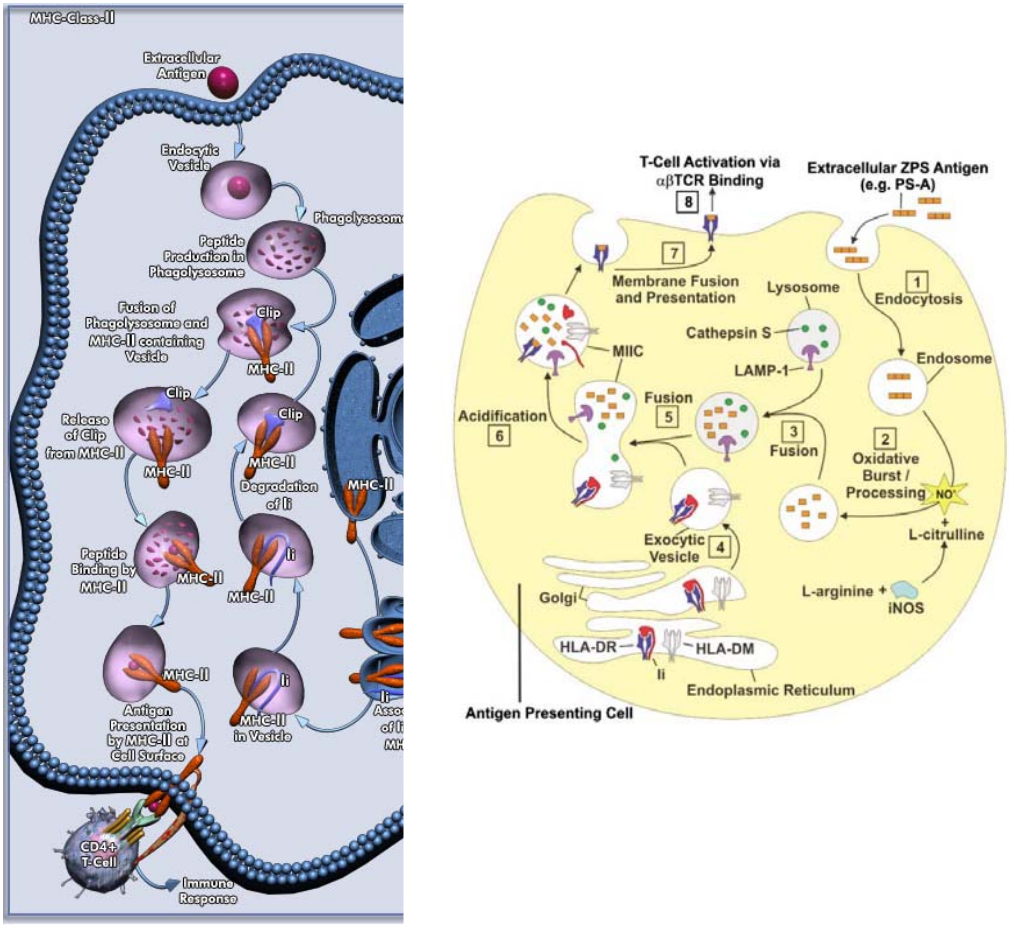



Figure 10: *MHCII Pathway Model for protein and ZPS Antigens respectively*[54,56]

After oxidative processing, ZPS antigens enter into a vesicular pathway similar to that of conventional protein antigens.[54] Endosomal vesicles containing processed ZPS antigens fuse with lysosomes to form the endo/lysosomal compartment (Figure 10). In the conventional MHCII pathway, delivery of protease enzymes from the lysosome proteolytically cleave the protein antigen into short peptides at acidic pH before the peptides are loaded onto MHCII for

presentation.[52] Interestingly, no detectable glycosidase has been identified for depolymerization of PS A1.[26,54] However, in the ZPS pathway, PS A antigens are cleaved into low molecular weight carbohydrate antigens at the earlier oxidative burst stage. The cleavage of PS A1 by oxidation is not feasible at the acidic pH and occurs at a near-neutral pH.

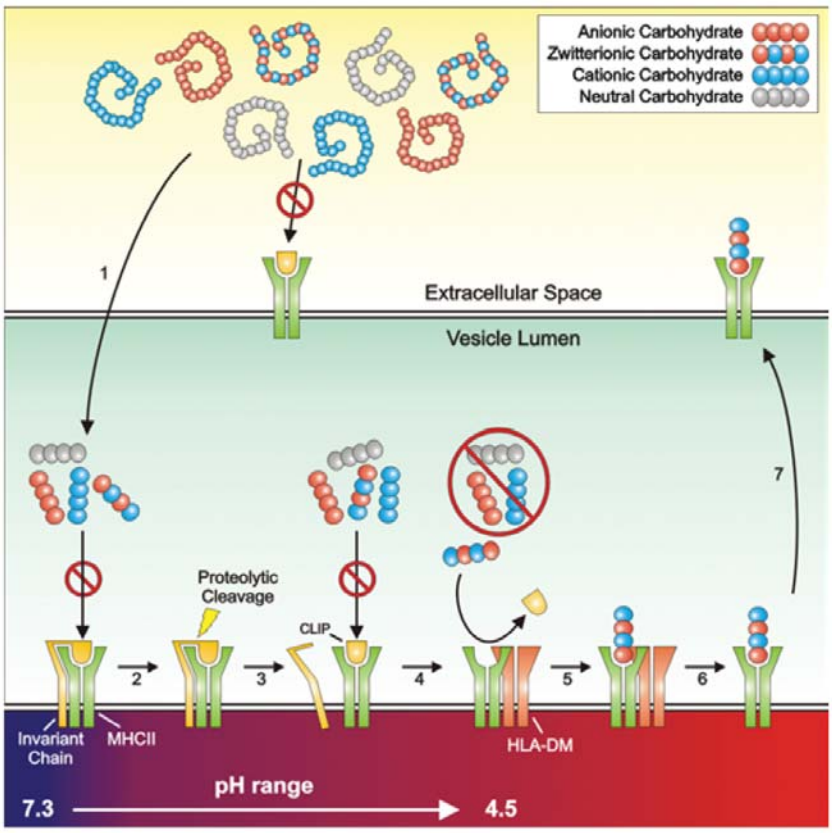



Figure 11: *Schematic presentation of ZPS antigen by MHCII*[40]

Meanwhile, the biosynthesis of MHCII takes place inside the ER by association of MHCII proteins with invariant chain protein (Ii). This newly synthesized MHCII is then transported from the ER in a exocytic vesicle through the Golgi apparatus and fuses with the endo/lysosomes compartment in order to form the MHC class II compartment (MIIC) (Figure 11).[26,52,54] The acidification (usually in the 4.5–5.0 range) of MIIC then occurs through the action of an

ATPase proton pump. The low pH activates the protease family called cathepsin, specifically cathepsin S which cleaves Ii, leaving just a small fragment called class II-associated peptide (CLIP), which still blocks the peptide-ZPS binding cleft. This cleavage event is important to allow the processed ZPS antigens to bind the class II MHC molecule for presentation. Next in the conventional MHC II pathway, antigen exchange factor HLA-DM catalyzes the exchange of CLIP for antigen peptides on MHCII. The exchange factor HLA-DM requires a low pH for its functional activity. Previous studies demonstrated that knockout mice lacking H2-M (murine HLA-DM homolog) challenged with ZPS do not develop abscesses. Further studies suggested that Ii cleavage and CLIP exchange by HLA-DM is required in order to free the necessary bind cleft for ZPS antigens.[26,54] In addition, it is also known that polysaccharide-mediated T-cell activation can be inhibited by addition of acidification blocker such as bafilomysin A1 (BFA).[26,57,58] The role played by HLA-DM in the loading of ZPS was not known until recently. Kasper and coworkers have shown HLA-DM catalyzes PSA binding to MHCII and HLA-DM is important for the cellular surface presentation of PSA.[40]

Binding studies were carried out at different pH to determine carbohydrate presentation by MHCII and they suggested that the PS A1 antigen binds to MHCII tightly in acidic conditions. The interaction between most peptides and MHCII molecules is primarily driven through hydrophobic and hydrogen bonding interactions.[59] However, the *in vitro* binding experiments carried out using PS A1 at high ionic strength suggests that even though these interactions contribute to PS

A1 binding, the highly charged zwitterionic motif on these molecules facilitates the electrostatic interactions that play a central role in anchoring the ZPS antigen to MHCII. This zwitterionic nature of these ZPS antigens is required for MHCII binding, which promotes the specific interaction between the PS A1 and MHC II molecule. The charge motif of ZPSs is essential for MHC II binding event, but on the other hand charge does not affect endocytosis, processing, or vesicular trafficking to MHCII-containing vesicles.[40] Finally, HLA-DM dissociates from the complex and the MHCII-ZPS complex is shuttled to the cell surface for T-cell recognition.[40] The ZPS antigens are then recognized by αβ TCRs on $CD4^+$ T-cells.[54] T-cells generally require two signals for complete activation. The first signal is specific, which is characterized by interaction of the TCR on T-cells and MHCII on the APC. The second signal is non specific and comes from costimulation of the B7 ligand of the APC with its receptor CD28 on the T-cell. ZPS mediated T-cell activation requires both signals and the second signal is initiated by CD28–B7-2 interactions. This induces the $CD4^+$ T-cells to secrete the cytokines that play a role in abscess formation.

## 1.36 Prevention of abscess formation by ZPS

While ZPSs are considered to be potential abscess inducers, subcutaneous or intramuscular treatment of animals with these polymers prevents the formation of intraabdominal abscesses following bacterial challenge.[60] Protection can be achieved against not only *B. fragilis* but also against different intestinal organisms capable of causing intra-abdominal abscesses including *S. aureus*.[44,60] Some

observations suggested that the protection against abscess formation is via the modulation of the immune system.[29,44]

Structure-function relationship studies were conducted using modified polysaccharides to examine whether these zwitterionic charges are essential physical properties for polysaccharides to confer protection against abscess formation. Either *N*-acetylated PS A1 or reduced PS A1 was used to vaccinate the animals and they were then challenged with the native unmodified PS A1. In each circumstance, these chemically modified single charged polysaccharides failed to protect the animals against polysaccharide induced abscess formation. From this data, it is obvious that the positively charged free amino group and negatively charged carboxyl group on PS A1 are essential for polysaccharide mediated protection against abscess formation.[50]

The cellular basis of protection against abscess formation was shown in rodent models of intraabdominal abscess in the early 1980's.[61] Passive transfer of serum antibodies from immunized rats to unimmunized recipients did not confer protection against abscess development. However, adoptive transfer of splenocytes from immunized mice to unimmunized recipients resulted in protection against abscess formation following challenge with *B. fragilis*. This indicates that-cellular immunity was crucially involved in the protection against abscess formation.[61] It was then found that protection against abscess can be adoptively transferred with immune T-cell-enriched populations, but not with immune serum, B-cells or macrophage-enriched populations.[33] Recent studies showed that $CD4^+$ T-cells stimulated with PS A1 can protect against

intraabdominal abscesses induced by viable bacterial challenge when transferred via the intracardiac route 24 hours prior to intraperitoneal challenge with bacteria.[44,48,62] The ability of the $CD4^+$ T-cells to protect or induce the abscess depends on the route and timing of the cell transfer.[29]

Studies have shown that addition of the antibody specific for cytokine Interleukin-2 **(**IL-2) neutralizes the T-cell lysates from PS A1-treated animals and abrogated the ability of these lysates to transfer protection to naive animals. This finding explains the crucial role of IL-2 produced by $CD4^+$ T-cells in protection against abscess formation. However, it was not clear how the $CD4^+$ T-cells played a role in the protection against abscesses.[29,63]

It has been proposed that ZPS causes a unique T-cell-mediated anti-inflammatory response. In response to the treatment with ZPSs, cytokine IL-10 was produced by a $CD45RB^{low}$, sub population of $CD4^+$ T-cells.[64] Cytokine IL-10 has been shown to protect against inflammation in numerous systems such as inflammatory bowel disease (IBD) and asthma. It is speculated that ZPSs can inhibit these inflammatory pathologies as well.[64,65] In order to determine the exact mechanism and regulation of the abscess formation, further investigations are necessary.

## 1.37 Conformation of ZPS structure: model for interaction

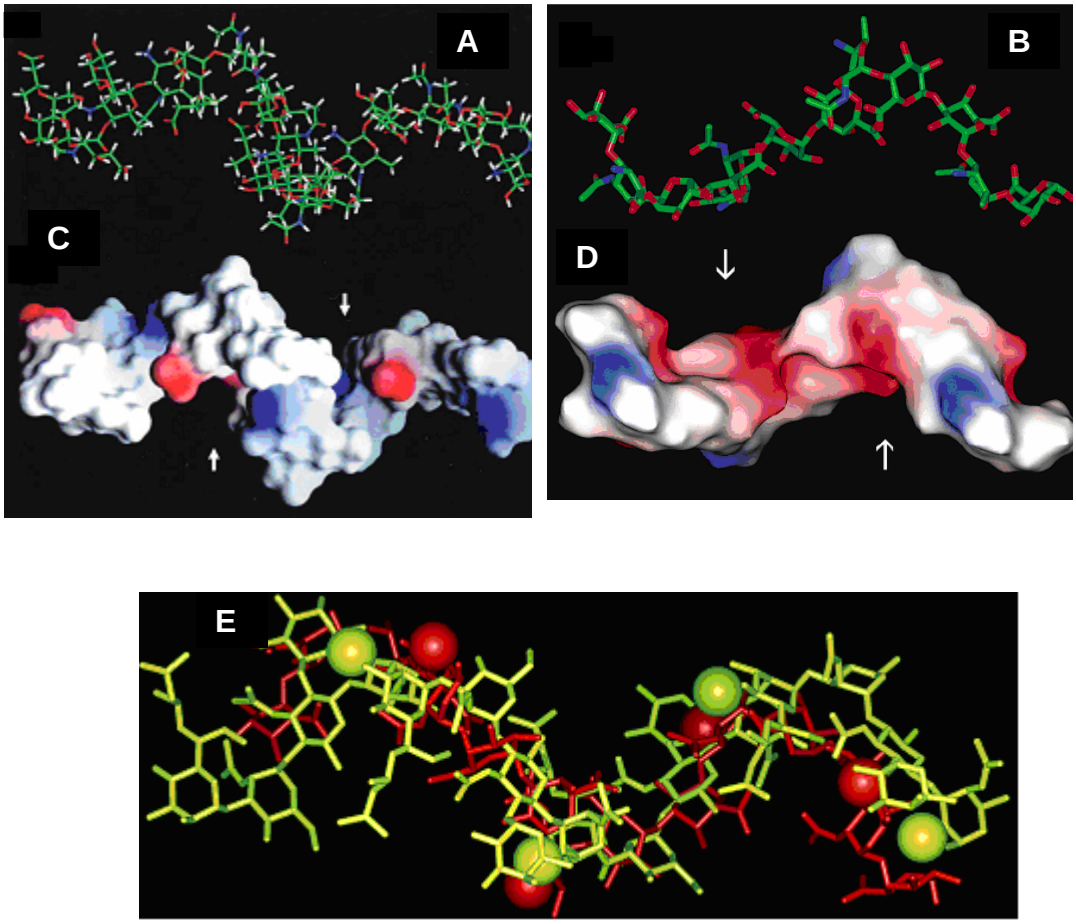



**Figure 12:** *Stick model of the 3D structures of PS A2 (A) and Sp1 (B) (Carbons are colored green, oxygens red, nitrogens blue, and hydrogens white). Electrostatic surface representation of PS A2 (C) and Sp1 (D) (Positive charges are colored blue and negative charges, red. Two grooves are indicated by arrow) E: Conformations of Sp1 (red) and PS A2 (yellow) superimposed on the basis of their amino groups. Red and yellow dots represent positive charges from Sp1 and PS A2, respectively.*[27,29,66]

Even though the primary structures of ZPSs differ significantly, they all elicit similar T-cell responses. The unique immunological properties of these ZPSs depend on the presence of the common structural feature of these ZPS antigens, the zwitterionic charge motif.[27] To determine why ZPSs comprising

different chemical structures elicit similar immunological responses, three-dimensional structures of PS A2 from *B. fragilis* and Sp1 from *S. pneumoniae* were studied by NMR spectroscopy, molecular mechanics and dynamics calculations.[27,29,66] These structural studies revealed that both PS A2 and Sp1 form extended right-handed helix with a pitch of 20 Å.[27,66] A single helical turn defines a groove. In PS A2, two repeating units per turn form the groove (Figure 12, A) while eight residues per turn form the groove in Sp1 (Figure 12, B).[27] The overall positive and negative charges are orientated in an alternate fashion and are exposed on the outer surface along the polymer chain (Figure 12, C and D). In both ZPSs, amines display very similar zigzag spatial arrangements.[27] This showed that despite the structural difference of PS A2 and Sp1, both ZPSs share a common scaffold for the presentation of the charge in a similar three dimensional conformation which accounts for their common biological property (Figure 12, E).[27]

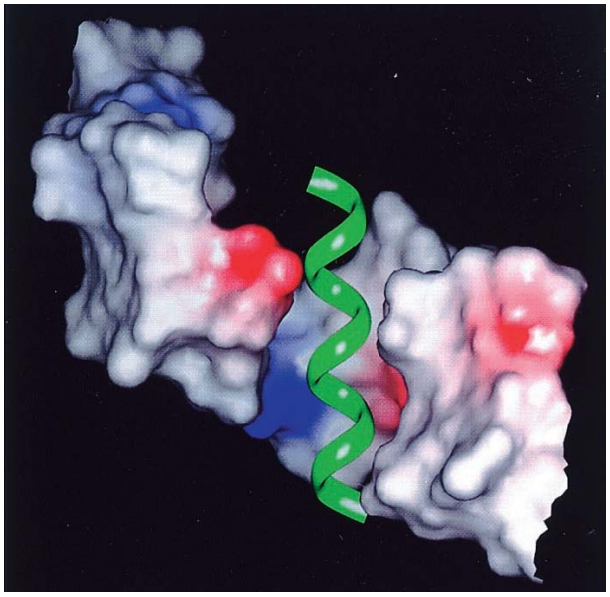


**Figure 13:** *Model of a protein a helix bound to one of the PS A2 grooves. The helix is represented by a green ribbon, and the coloring scheme for the surface is identical to that in Figure 12.*[66]

Possible mechanisms for the interaction of ZPSs with the MHCII proteins of the immune system can be explained by three dimensional models.[24,66] Kasper and coworkers proposed that PS A2 or Sp1 could bind to MHCII proteins either along its longitudinal sides or via zwitterionic grooves. The longitudinal binding site displays a high density of opposite charges in an alternate fashion, which would facilitate the binding primarily through hydrogen bonding and, to a lesser extent, van der Waals interactions. On the other hand, the geometry of each zwitterionic groove would be able to accommodate the insertion of a helix from a protein (Figure 13). The charges at the edges of the groove would help anchor the protein and strengthen the binding via multiple electrostatic salt bridges. In addition, hydrophobic interactions stabilize the protein along the inner surface of the groove. The 'groove-binding model' provided a better explanation for the

biological activity of PS A2.[66] The computer model suggested that PS A2 could complex with the MHC II protein by capturing the helix in its zwitterionic groove.

Circular dichroism (CD) is a technique used to analyze the secondary structures of the proteins. This technique was recently applied to monitor the conformational changes of the ZPSs upon binding to MHCII proteins.[67] CD studies showed that fragments smaller than three repeating units of the PSA lost the helical conformation demonstrating that it is incapable of binding to MHC II proteins.[67] PS A1 fragments between 3 and 10 kDa maintained the helical conformation and bound to MHC II better than larger ones.[67,68] The helical conformation of the ZPS is therefore important for the MHC II binding and can be stabilized by adjacent repeating units and the zwitterionic charge motif. The proposed binding mechanism is still under investigation.

## 1.4 Scope of the project

In the conventional dogma, carbohydrates are considered to be poorly immunogenic, T-cell independent antigens. However, Kasper and coworkers from Harvard Medical School have been able to demonstrate that zwitterionic polysaccharides (ZPSs) produced by bacteria can induce a variety of T-cell specific responses such as cell proliferation, cytokine secretion and regulation of antibody production in bacterial infection. Active ZPSs share a common structural motif, a high density of positively charged amino and negatively charged carboxyl or phosphate groups. These features are essential for the activity of ZPSs. A sound appreciation of structure-function relationships for ZPSs requires efficient synthesis of the repeating units. The type 1 capsular polysaccharide from the

human pathogen *Streptococcus pneumoniae* (Sp1) is a suitable target for the chemical synthesis. The project involves in the synthesis of a trisaccharide repeating unit of the Sp1. Since the biological repeating unit of ZPS is not known our group has synthesized two different repeating sequences in an effort to identify the smallest active unit. In this thesis the sequence shown in Figure 14 is the target.

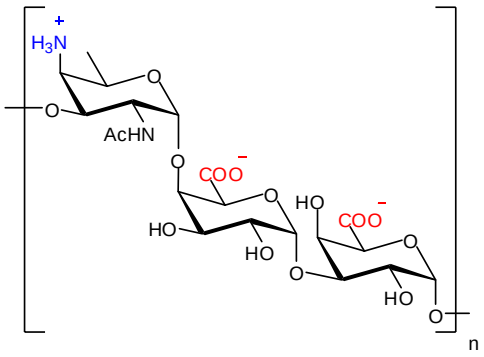



Figure 14: *The repeating unit of the Sp1 from Streptococcus pneumoniae*

At the outset of this research it was known that a PS A1 could be degraded to 3-30 kDa fragments that retain activity. However, the recent speculation regarding the correlation of helical conformation with activity had not been advanced until 2007.[67]

2-Acetamido-4-amino-2,4,6-trideoxy-D-galactopyranose is one of the three monosaccharides of the repeating unit of the Sp1 of *Streptococcus pneumoniae*. This diamino-trideoxy-hexose is also present in a number of bacterial polysaccharides such as those constituting the capsules of serotypes of *Bacteroids fragilis, Streptococcus mitis* and *Shigella sonnei*. We have developed a novel method to synthesize 2-acetamido-4-amino-2,4,6-trideoxy-D-galactopyranose, which will be described in Chapter 2. Assembly of the target

trisaccharide (Figure 14) is described in Chapter 3; this chapter is focused on the synthesis of different monomeric galactopyranose donors and acceptors that are needed for the assembly of this repeating unit. Chapter 4 describes biological activity of the Sp 1 repeating unit and conclusion, and future work.

# Chapter Two

# Novel synthesis of an oxazolidinone protected 2-azido-4-amino-2,4,6-trideoxy-D-galactopyranose

## 2.1 Introduction

Amino sugars are important constituents of many naturally occurring structural polysaccharides, bacterial capsular polysaccharides, mucoproteins, glycolipids and a number of antibiotics.[6] The first reported amino sugars, glucosamine and galactosamine, were found in the tissues of invertebrates, mammals and plants.[69] *N*-acetylglucosamine is the most abundant amino sugar, which plays a structural role in chitin, the hard exoskeleton of invertebrates. *N*-acetylgalactosamine and *N*-acetylneuraminic acid are also widely spread in nature whereas other glycosamines are much less abundant.[69] Rare amino sugars such as 2-acetamido-2-deoxy-D-gulosamine (D-GulNAc),[70] and 2,4-diamino-2,4,6-trideoxy-D-glucose (bacillosamine)[71] have also been isolated from natural sources. Amino sugars that possess distinctly functionalized amino moieties have provided synthetic carbohydrate chemists with new and challenging targets.

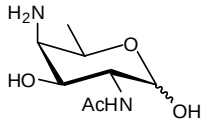



Figure 15: *Structure of AAT*

The naturally occurring rare amino sugar, 2-acetamido-4-amino-2,4,6-trideoxy-D-galactopyranose (AAT) (Figure 15), was recognized as a prominent constituent of a number of Gram-positive and Gram-negative bacterial polysaccharides either as the α- or β-pyranose form. The repeating unit of the O-specific lipopolysaccharide of the Gram-negative bacteria *Shigella sonnei* consists of β-linked AAT and α-linked 2-acetamido-2-deoxy-L-altruronic acid.[72,73] AAT was also shown to occur in α-linkages in the repeating unit of the capsular polysaccharides of *Streptococcus pneumoniae* type 1,[74,75] *Streptococcus mitis*[76] and *Bacteroides fragilis*.[37,77] The unusual AAT residue has been a synthetic target for many years and a number of chemical approaches to synthesize it have been developed.

## 2.2 Previous syntheses of the AAT residue

In 1984, Lönn and Lönngren synthesized the precursor of AAT, methyl 2-acetamido-4-azido-2,4,6-trideoxy-α-D-galactopyranoside **VII,** in six steps starting from methyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside **I** (Figure 16).[78] As described in Figure 16, the key steps for the construction of compound **VII** were achieved via selective reduction of the mesylate at the primary position of **IV** with $NaBH_4$, followed by the displacement of the mesyl group at C-4 of **V** with $NaN_3$. The overall yield of this approach from D-glucosamine to methyl 2-acetamido-4-azido-2,4,6-trideoxy-α-D-galactopyranoside **VII** was 8%.

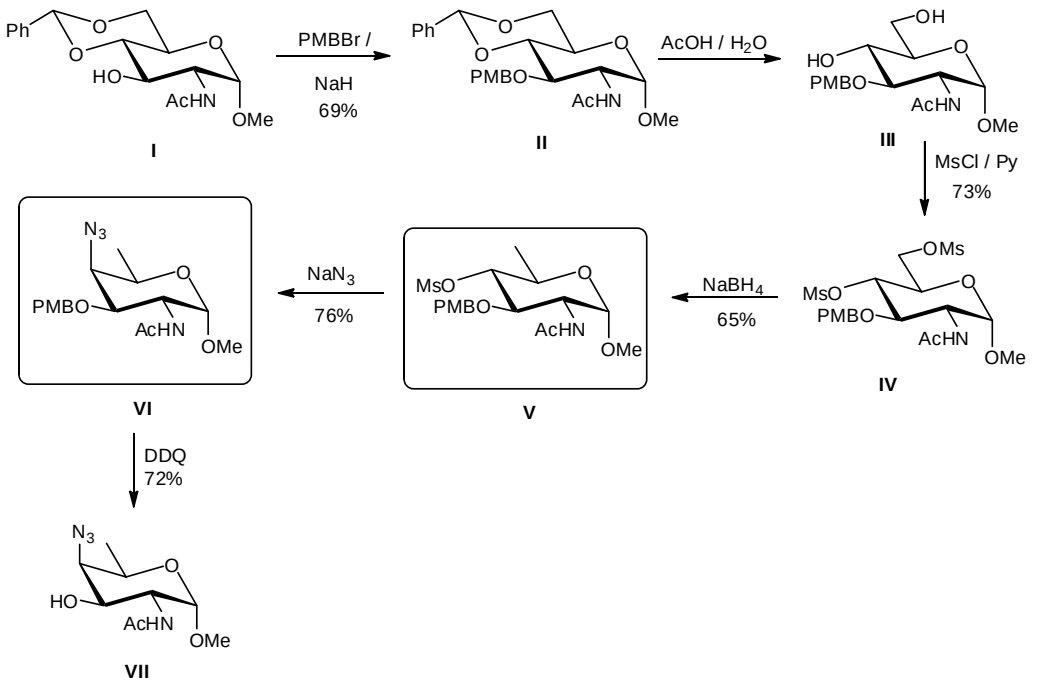



Figure 16: *Lönn and Lönngren method*[78]

In 1997, Lipták and coworkers published an alternative approach for the synthesis of AAT methyl glycoside **XVIII** in 11 steps (Figure 17).[79] They used ethyl 3-*O*-acetyl-2-deoxy-4,6-*O*-isopropylidene-2-phthalimido-1-thio-β-D-glucopyranoside **VIII** as the starting material, which itself must be synthesized from D-glucosamine in several steps.[80] The most interesting step in their synthesis is the deoxygenation at C-6. Selective displacement of the primary tosyl group of **X** via NaI followed by reduction of **XI** with Zn–AcOH gave the 6-deoxy glucose derivative **XII**. Similar to the Lönn and Lönngren approach, the azide group was introduced by inversion of configuration at C-4 via an $S_N2$ reaction. $NaN_3$ was used as a nucleophile to displace a C-4 tosyl group to give compound **XIII**. Glycosidation of the thioglycoside **XIII** with methanol in the presence of the promoter, methyl trifluoromethanesulfonate provided the β-methyl glycoside **XIV** in 63% yield. Finally, hydrogenation over Pd on charcoal readily reduced the

azido group of **XVII** to an amino group to form 2-acetamido-4-amino-2,4,6-trideoxy-D-galactopyranose **XVIII** (Figure 17). Thus, the overall yield of AAT from D-glucosamine by this approach was 2.5%.

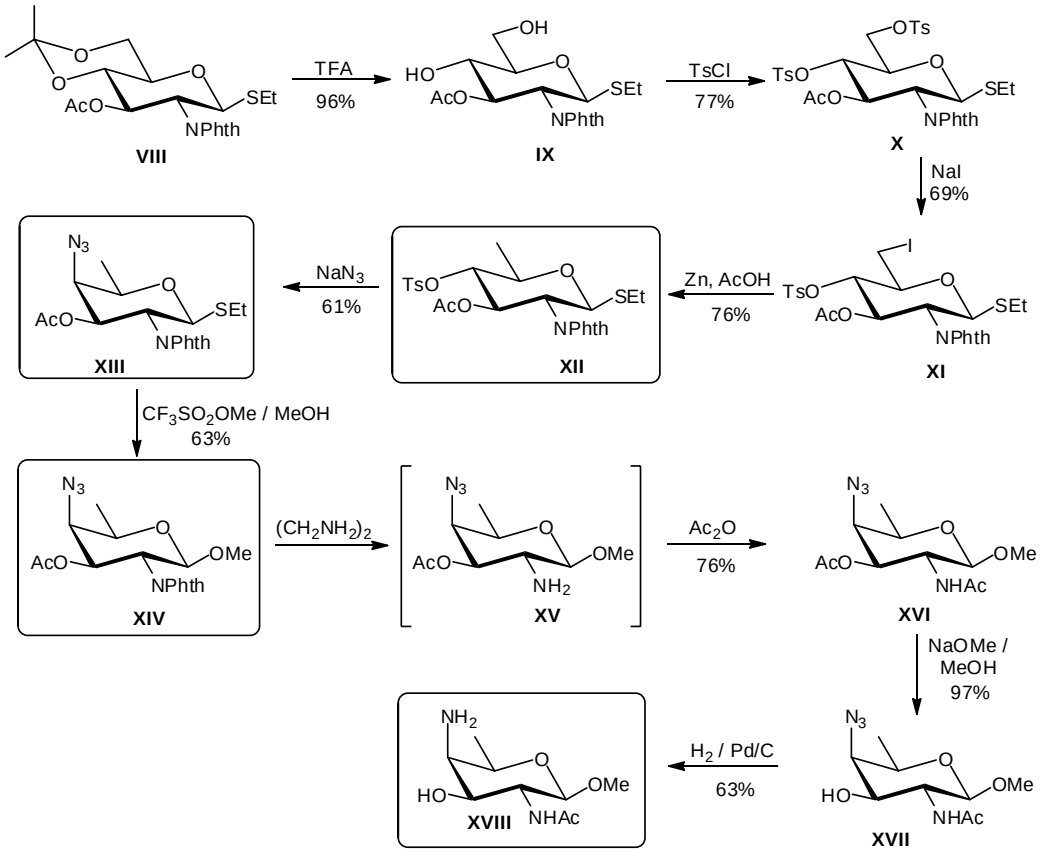



Figure 17: *Synthesis of AAT moiety by Lipták and coworkers*[79]

A few years later, in 2004, Grindley and coworkers reported a concise and efficient method to synthesize the methyl 2-acetamido-4-amino-2,4,6-trideoxy-α-D-galactopyranoside **XXVII** in eight steps from commercially available D-glucosamine hydrochloride in 31% overall yield (Figure 18).[81] D-Glucosamine hydrochloride was transformed into the corresponding methyl 2-acetamido-6-bromo-2,6-dideoxy-α-D-glucopyranoside **XXII** via an established method.[82] The regioselective bromination of **XXI** was achieved using a brominating agent based

on triphenylphosphine and carbon tetrabromide. The successive hydrogenolysis of the bromide **XXII** using 10% Pd–C afforded the 6-deoxy glucose derivative **XXIII**. Regioselective benzoylation of C-3 of **XXIII** at -35 $^{o}$C then allowed for the introduction of an azido group at C-4 of **XXIV** via the Mitsunobu type hydroxyl substitution with diphenylphosphoryl azide. Finally, the azido group of **XXVI** was transformed into the corresponding amino product 2-acetamido-4-amino-2,4,6-trideoxy-D-galactopyranose **XXVII** by hydrogenation.

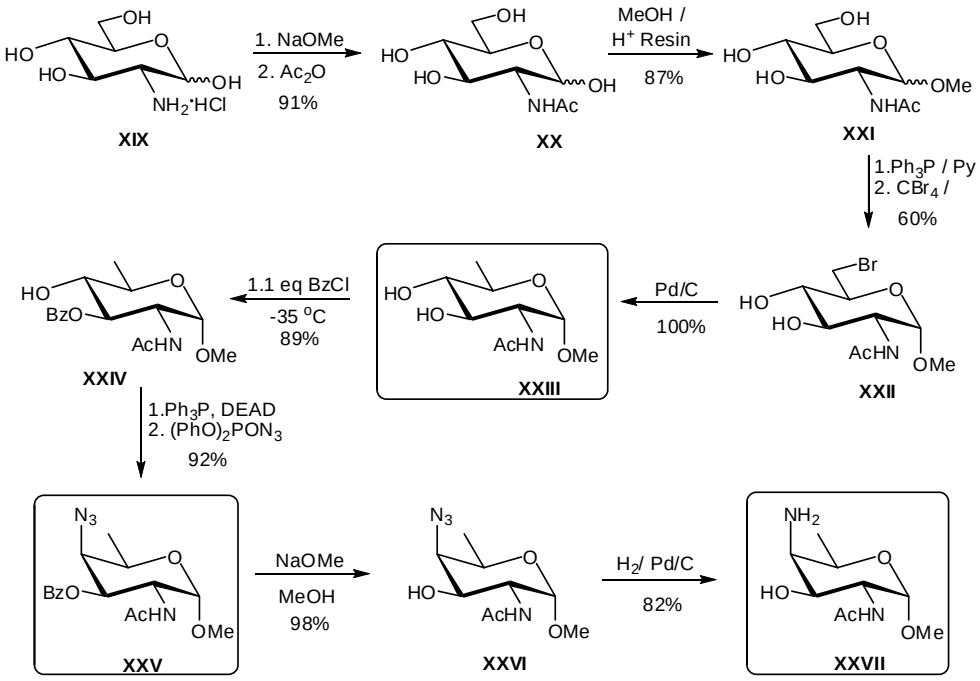



Figure 18: *Synthesis of methyl 2-acetamido-4-amino-2,4,6-trideoxy-α-D-galactopyranoside by Grindley and Liang*[81]

In 2009, our group (Cai *et al*.) published a short and efficient synthesis of methyl 2-acetamido-4-amino-2,4,6-trideoxy-β-D-galactopyranoside starting from *N*-acetylglucosamine in seven steps (Figure 19).[83] The key intermediate in this approach was the furanose oxazoline **XXIX,** which can be obtained from *N*-acetylglucosamine in dry acetone using anhydrous $FeCl_3$ as a catalyst. After

benzylation, *p*-toluenesulfonic acid-catalyzed methanolysis of **XXX** afforded the corresponding β-pyranoside **XXXI**.[84] Reduction of a 6-*O*-mesylate was performed by $NaBH_4$ to furnish 6-deoxy glucoside **XXXIII** in good yield. The 6-deoxy derivative **XXXIII** was converted to a triflate, which was displaced by azide in a one pot reaction. It was discovered that an excess of triflic anhydride could lead to a significant side reaction to produce a tetrazole functionality at C-2. Finally, hydrogenation of **XXXV** over palladium hydroxide converted the azido group to the corresponding amino functionality. This synthesis of methyl 2-acetamido-4-amino-2,4,6-trideoxy-β-D-galactopyranoside was accomplished in 15% overall yield starting from D-glucosamine.

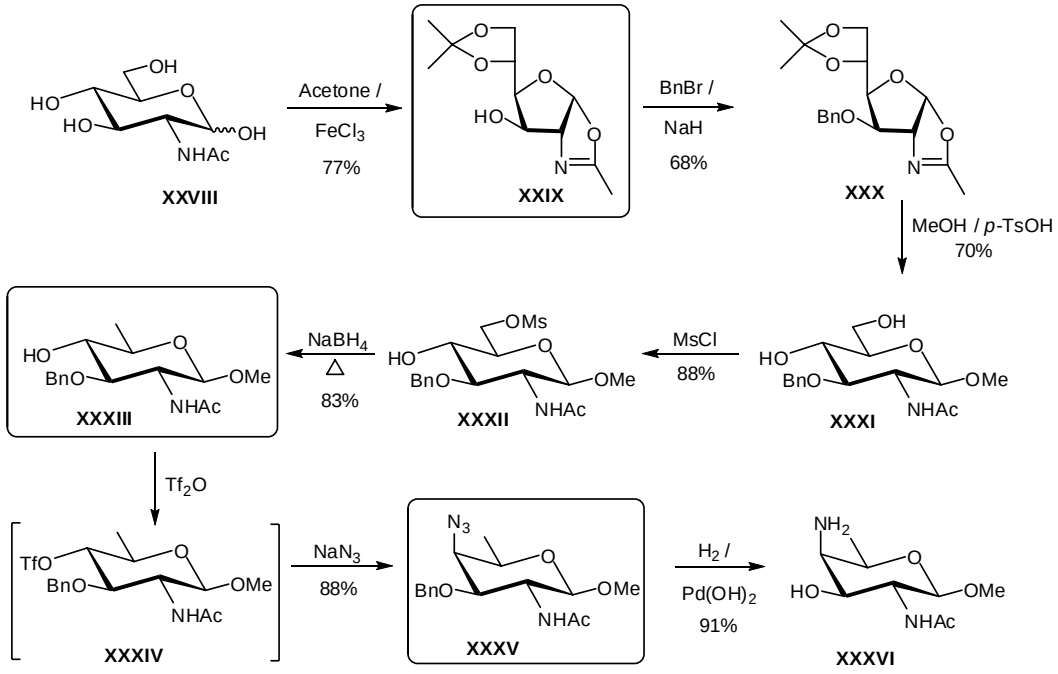



Figure 19: *Synthesis of methyl 2-acetamido-4-amino-2,4,6-trideoxy-β-D-galactopyranoside by Bundle and Cai*[83]

All these methods employed in the synthesis of orthogonally protected 2,4-diamino fucose residues had a common set of reactions: deoxygenation at C-6

followed by the installment of an amino group at C-4. The latter was achieved by nucleophilic displacement of the equatorial leaving group such as a sulfonate ester by an azide nucleophile. The presence of a participating group, such as an acetamido or phthalimido, at C-2 in these derivatives prevents their use as α-selective glycosyl donors. Therefore, a non-participating group at C-2 is required for the synthesis of an α-glycosidic bond. The most frequently employed non-participating amino protecting group is the azido group. An azido group can be introduced by azidonitration of the corresponding glycal or by nucleophilic substitution reaction of a triflate at C-2 by an azide anion or by diazotransfer of the free amino group.[85]

In 1992, the group of van Boom reported the synthesis of 3-*O*-acetyl-2-azido-4-(benzyloxycarbonyl)amino-2,4,6-trideoxy-D-galactopyranosyl trichloroacetimidate donor **LII** starting from ethyl 1-thio-α-D-mannopyranoside (Figure 20).[86] In contrast to the aforementioned approaches, they used an alternative concept to install the two distinct amino groups. The key steps involved in their synthesis were: hydride-mediated deoxygenation of the 6-*O*-tosyl group of **XXXIX** followed by reduction of an oxime **XLII** to install the first axial amino group on compound **XLIII** and finally azidonitration of a 6-deoxy glucal **XLVII** resulting in the formation of a mixture of compounds **XLVIII** and **XLIX**, which could not be purified by either column chromatography or crystallization. Subsequent removal of anomeric nitrates with thiophenol gave a mixture of **L** and **LI**. A glycosyl donor was obtained by converting **L** and **LI** into the corresponding mixture of trichloroacetimidates, which could be separated.[86]

Starting from peracetylated D-mannopyranoside, the target trichloroacetimidate 3-*O*-acetyl-2-azido-4-(benzyloxycarbonyl)amino-2,4,6-trideoxy-D-galactopyranose was obtained in 3% overall yield.

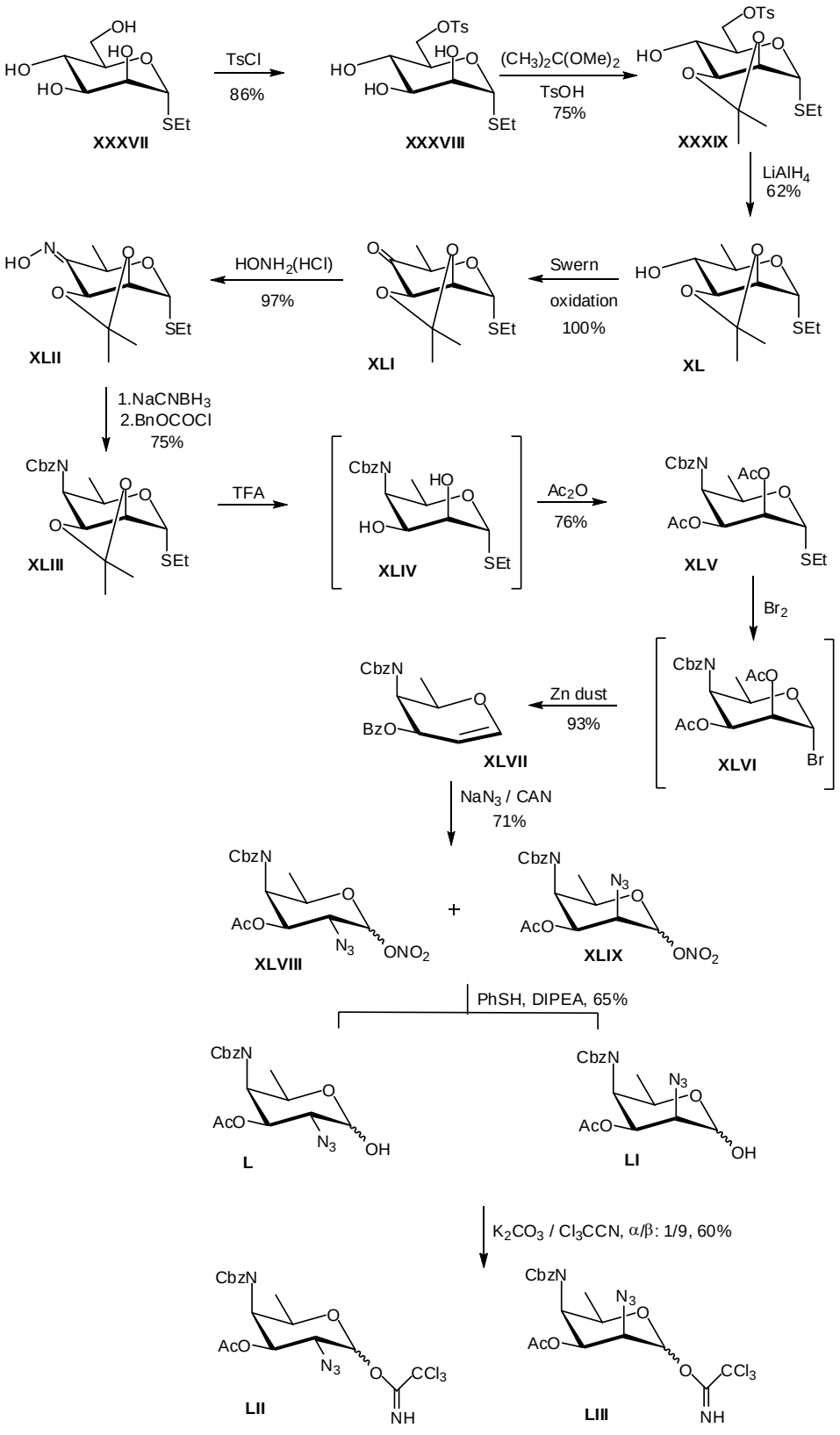



Figure 20: v*an Boom and coworkers' method*[86]

The group of van der Marel published a synthetic strategy towards the PS A1 tetrasaccharide repeating unit in 2007 and as part of this work, they synthesized the glycosyl donor 3-*O*-acetyl-2-azido-4-trichloroacetamido-2,4,6-trideoxy-β-D-galactopyranoside **LXIII** starting from *tert*-butyldimethylsilyl 2-azido-2-deoxy-β-D-glucopyranoside **LIV** in a seven step route (Figure 21). Deoxygenation at C-6 was performed similarly to Lipták and coworkers' approach but $NaBH_4$ was used to reduce the C-6 iodide **LVI**. The key element in their strategy was the introduction of the axially oriented C-4 amino group by a tethered nucleophilic inversion approach. Transformation of **LV** via a three-step one-pot procedure afforded oxazoline derivative **LX**. The oxazoline ring of **LX** was cleaved by mild acid treatment. Starting from glucosamine hydrochloride, this synthesis of 3-*O*-acetyl-2-azido-4-trichloroacetamido-2,4,6-trideoxy-β-D-galactopyranoside **LXIII** was performed in 10% overall yield. However, **LXIII** gave only a poor yield (17%) of the oligosaccharide in a glycosidation reaction using Gin's dehydrative method.[87]

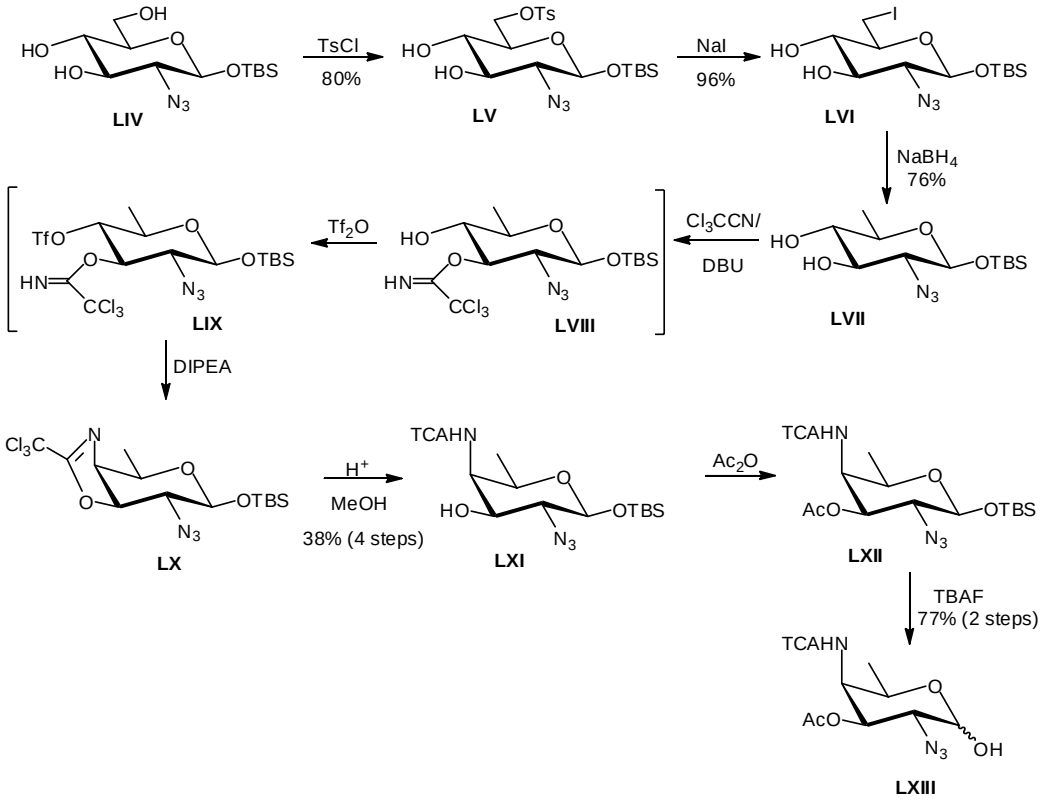



Figure 21: *Group of van der Marel approach*

The demand for a suitable 2-acetamido-4-amino-2,4,6-trideoxy-D-galactopyranose donor prompted us to search for an alternative and more straightforward approach for its synthesis. This chapter focuses on a strategy for the synthesis of the precursor **1** for AAT (Figure 22) via a novel and efficient route.

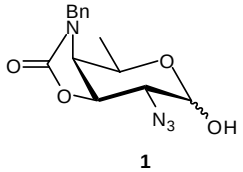
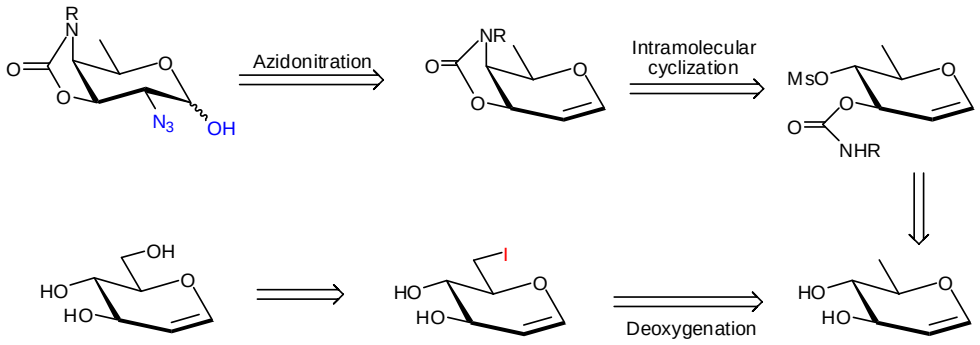



Figure 22: *The target precursor of AAT*

## 2.3 Results and discussion

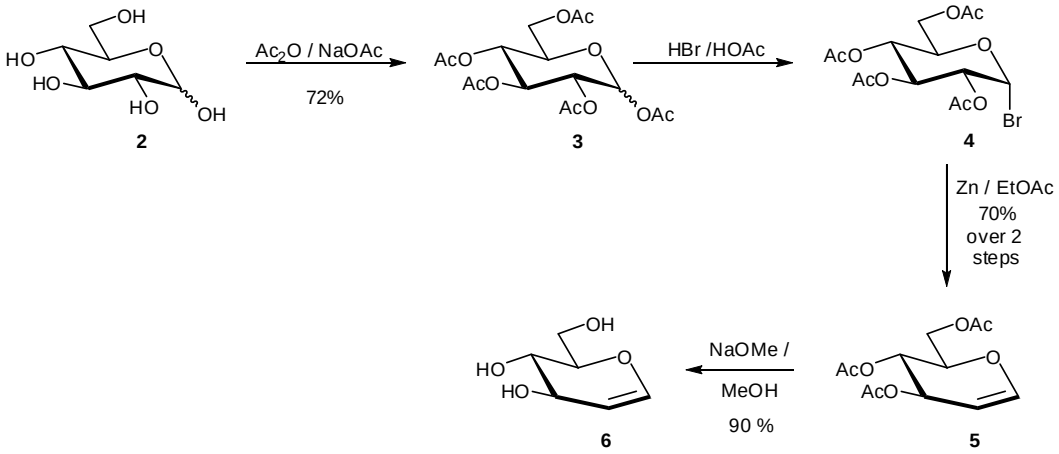
The retrosynthetic scheme (Scheme 1) shows the key steps involved in our approach. As we saw in the previous syntheses, there are several synthetic challenges to be solved in the synthesis of the AAT moiety. They are: regioselective introduction of differentiated and distinct amino groups at C-2 and C-4 and the relatively straightforward C-6 deoxygenation. D-Glucal was chosen as the staring material since our intention was to introduce the azide group at C-2 by azidonitration at a later stage. Our first goal was to introduce the C-6 deoxy functionality. Subsequently, the installment of the first amino group at C-4 was achieved via an intramolecular nucleophilic cyclization. Finally, azidonitration would introduce the requisite non-participating azido group at C-2.




Scheme 1: *Retrosynthesis of the Compound* ***1***

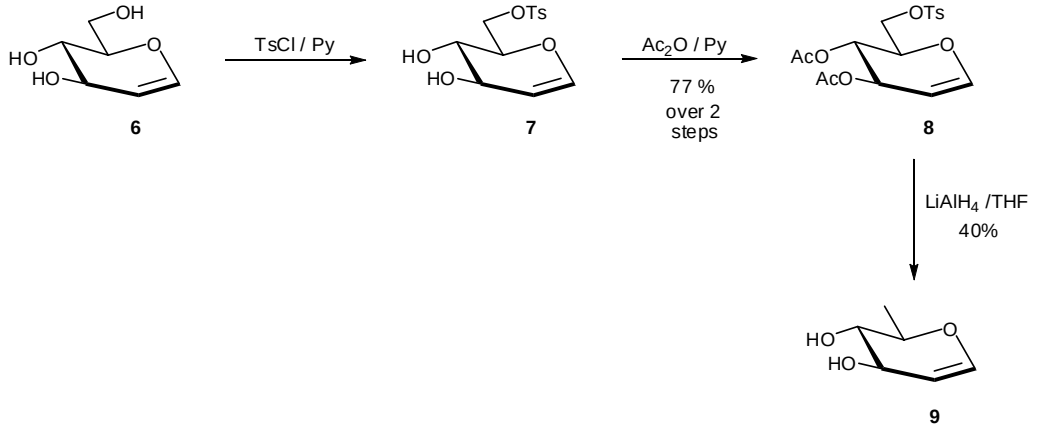
D-Glucal **6** was prepared from the inexpensive commercially available D-glucose **2** (Scheme 1).[88,89] Alternatively, D-glucal can be prepared by deacetylation of commercially available tri-*O*-acetyl-D-glucal. Acetylation of D-glucose **2** to form pentaacetate **3** followed by treatment with 30% hydrobromic acid (HBr) in acetic acid gave bromide **4** (Scheme 2). The crude bromide **4** was subjected to reductive elimination with zinc in anhydrous ethyl acetate to yield tri-

*O*-acetyl-D-glucal **5** in 70% yield over 2 steps. Removal of the acetate groups of **5** by transesterification provided D-glucal **6** in good yield.




Scheme 2: *Synthesis of D-glucal*

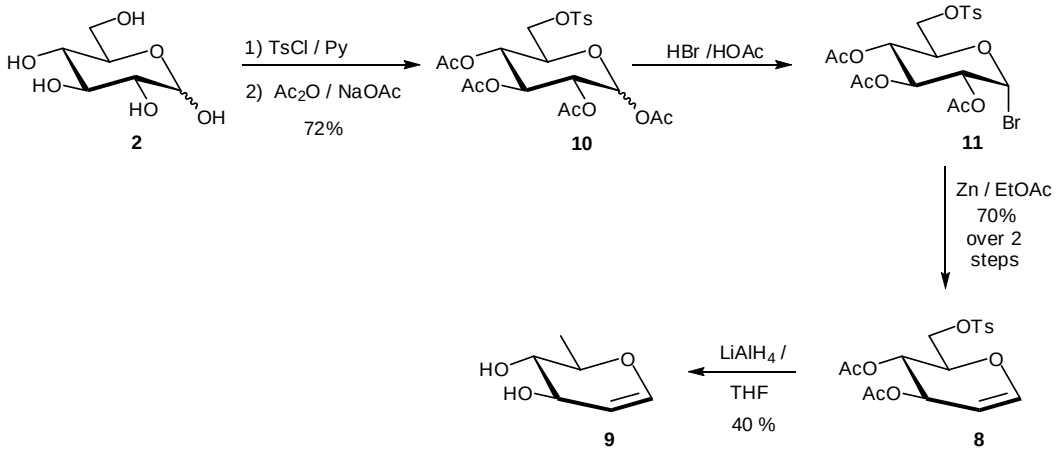
Regioselective tosylation at the C-6 position of **6** gave 6-*O*-*p*-toluenesulfonyl-D-glucal, but this compound was highly unstable. Therefore, crude **7** was directly converted to its stable di-*O*-acetyl derivative **8**.[90] Reduction of **8** with $LiAlH_4$ afforded 6-deoxy-D-glucal **9** in 40% yield (Scheme 3).




Scheme 3*: Synthesis of 6-deoxy-D-glucal*

In an alternative approach, regioselective tosylation of D-glucose **2** followed by acetylation produced compound **10** in 72% yield. Treatment with

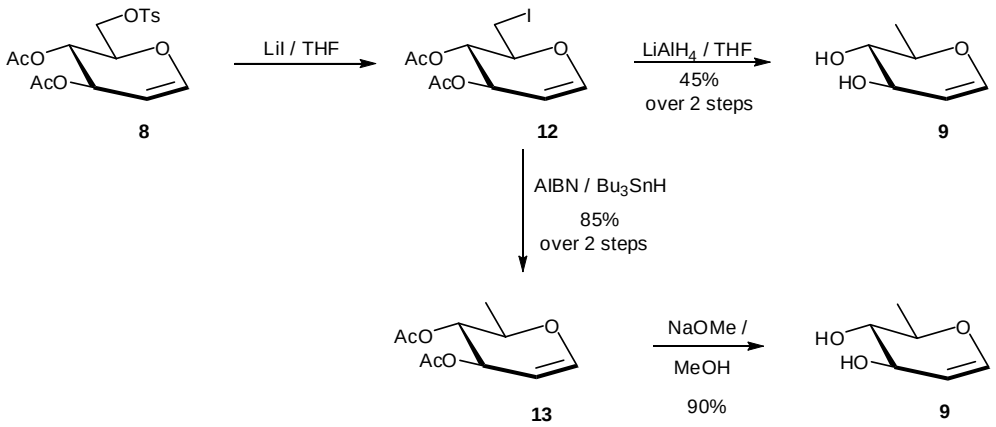
30% HBr in acetic acid, followed by reductive elimination accomplished the synthesis of 6-*O*-*p*-toluenesulfonyl-3,4,-di-*O*-acetyl-D-glucal **8** (Scheme 4).[91] This route was more efficient than the former approach. Different reducing agents such as $NaBH_4$ and DIBAL were used to reduce the tosyl group at C-6 of compound **8**. However, none of them gave good results. These results suggested that the reduction of the tosyl group of D-glucal by reducing agents is a less efficient method. We therefore turned our attention to a different approach.




Scheme 4: *Alternative approach for the synthesis of 6-deoxy-D-glucal*

Finally, it was found that deoxygenation at C-6 could be conveniently adapted to a large scale by displacement of the tosylate with iodide.[92] Treatment of **6** with LiI in THF afforded the 6-iodo derivative **12**. Decomposition of **12** during chromatographic purification suggested its direct use for the next step. Deoxyhalo sugars can be reduced to their corresponding deoxy anologs by employing radical reduction with tributylstannane or by Pd catalysed hydrogenation. Since the latter method can also reduce the double bond of the glucal we selected the first method in our synthesis. Reductive removal of the iodide using $Bu_3SnH$ and AIBN as radical initiator gave **13** in 85% yield over the

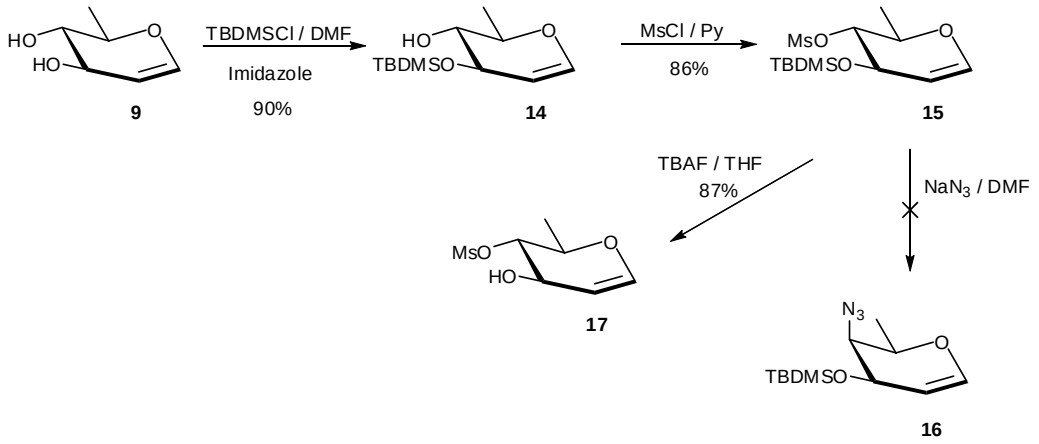
displacement and reduction steps.[92,93] The resulting compound **13** was then transesterificated under controlled conditions to obtain 6-deoxy-D-glucal **9** (Scheme 5).[92,94]




Scheme 5: *Efficient route to synthesize 6-deoxy-D-glucal*

The next objective was to address the introduction of an amino functionality at C-4 of compound **9**. A traditional strategy for the introduction of an amino group is $S_N2$ substitution of a sulfonate ester or opening of an epoxide ring with a nitrogen nucleophile, such as azide anion. In order to introduce a good leaving group, such as a mesyl group on C-4, it was necessary to selectively protect the hydroxyl group at C-3. Several approaches were attempted. Only hindered silylating reagents reacted selectively with the C-3 hydroxyl group of compound **9**. Treatment of **9** with TBDMSCl in the presence of imidazole gave the 3-OTBDMS derivative **14**.[95,96] The resulting compound was then treated with MsCl to obtain compound **15** in good yield (Scheme 6).[96] Inversion of configuration at C-4 by an azide nucleophile was unsuccessful and failed to give target **16** (Scheme 6). Several other leaving groups including triflate and tosylate were examined. However, the reaction did not proceed, instead it gave several

unidentified products. It was reasoned that this might be due to the steric hindrance by the bulky TBDMS group. The TBDMS group was therefore removed by treating **15** with TBAF;[96] however, the displacement of the mesyl group by azide did not progress. Due to the failure of the conventional azide displacement, focus was returned to the oxazolidinone approach.




Scheme 6: *Attempt to introduce azide moiety at C-4 position of 6-deoxy-D-galactal*

Cyclic carbamates (oxazolidinones) bridging vicinal amino alcohols such as those at C-2 and C-3 of 2-amino-2-deoxy-D-hexopyranoses, have been used as protecting groups in glucosamine and galactosamine. Miyai and Gross were the first to describe the cyclic carbamate group of hexosamines.[97] The Oxazolidinone can be synthesized by treating a suitable amino sugar with either *p*-nitrophenoxy carbonyl chloride (NPCC) or phenyl chloroformate (Figure 23).[97-99]

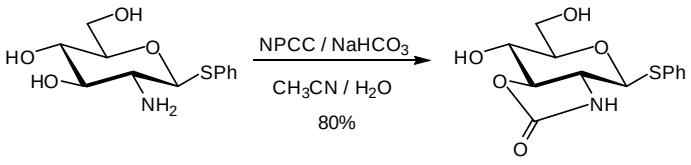



Figure 23: *Synthesis of oxazolidinone*[99]

An oxazolidinone ring may also be formed via intramolecular cyclization. For the synthesis of amino sugars, intramolecular delivery of a temporarily tethered nitrogen nucleophile in a $S_N2$ fashion to an electrophilic site offers some advantages over the intermolecular approach (Figure 24). The intramolecular cyclization method allows the synthesis of amino compounds with excellent control over the position and the stereochemistry of the amino group, while minimizing the extent of side reactions.[100] In addition, because the resulting cyclic intermediate simultaneously protects both the amino and the alcohol groups, it can be more easily manipulated towards the desired target than the corresponding free amino compound.[101,102]

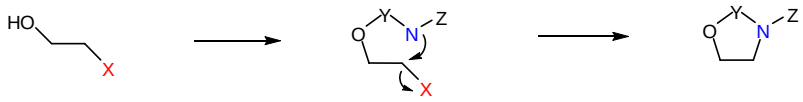



Figure 24: *Intramolecular cyclization by an attack of a tethered, internal nucleophile on an electrophilic intermediate***:** *'Y' represents the tether; 'Z' protecting group: 'X' represents the leaving group*

In this approach, a neighboring hydroxyl group can act as a handle onto which a nitrogen nucleophile can be tethered. The anion of the carbamate was chosen as the candidate for the tethered nitrogen nucleophile for the intramolecular cyclization reaction for a number of reasons.[101] Carbamate derivatives can be readily formed under neutral conditions and the anion of the carbamate can also be easily generated. Treatment with a stronger base can hydrolyze the oxazolidinone ring and lead to the amino alcohol (Figure 25).[100,102,103]

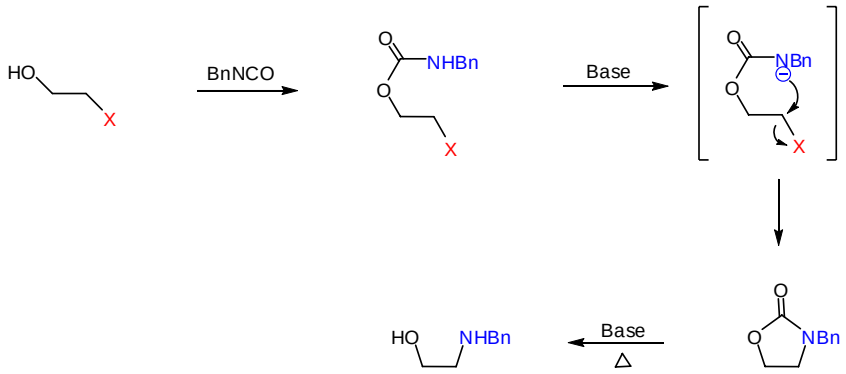
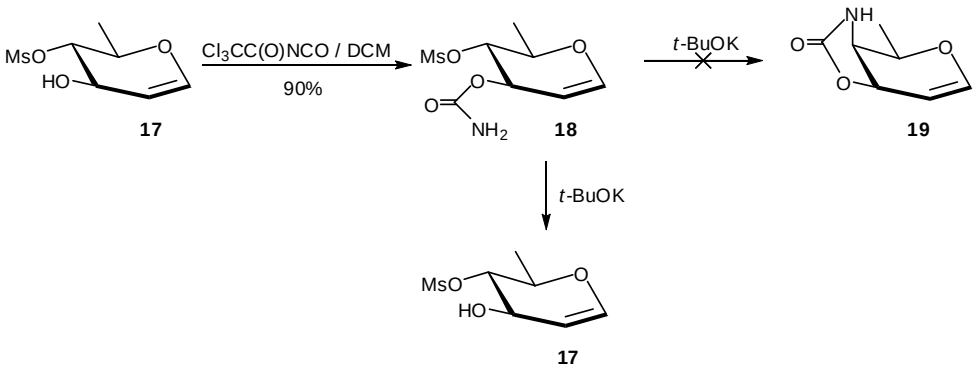



Figure 25: *Synthesis of an oxazolidinone ring by an attack of a tethered, internal nucleophile on an electrophilic intermediate*

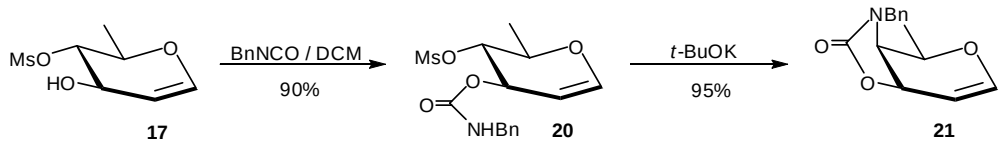
The C-3 hydroxyl group of compound **17** was converted to an unsubstituted carbamate **18** in excellent yield by carbamoylation with trichloroacetyl isocyanate to produce the corresponding trichloroacetyl carbamate *in situ* followed by hydrolysis with potassium carbonate in methanol.[104] In an attempt to prepare **19** from **18,** it was observed that **18** failed to cyclize under basic conditions, such as NaH or *t*-BuOK. Instead it hydrolyzed back to the starting material **17** (Scheme 7).




Scheme 7: *Attempt to make the oxazolidinone by unsubstituted carbamate*

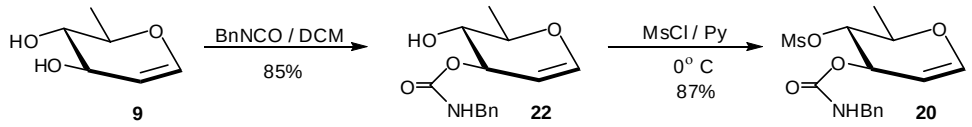
The synthetic potential of a substituted carbamate was next investigated. Condensation of compound **17** with benzyl isocyanate gave benzylcarbamate **20** in excellent yield, setting the stage for the intramolecular cyclization. Treatment

of compound **20** with *t*-BuOK generated the potassium salt of the *N*-benzylcarbamate anion, which cyclized *in situ* in an $S_N2$ fashion to afford the *N*-benzyloxazolidinone **21** (Scheme 8).[101]




Scheme 8: *Synthesis of the oxazolidinone ring via intramolecular cyclization*

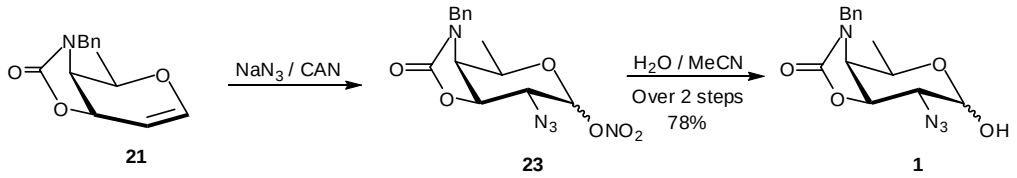
Having prepared **21,** we realized that the entire synthesis could be accomplished efficiently by avoiding two steps: the protection and deprotection of the C-3 hydroxyl by the TBDMS group was not necessary. The C-3 hydroxyl group of **9** could be regioselectively protected with benzylisocyanate in good yield (Scheme 9). The free hydroxyl group at C-4 of **22** was then activated for an intramolecular cyclization reaction by converting it to mesylate **20** under carefully monitored conditions.




Scheme 9: *Efficient and straight forward route to synthesize oxazolidinone ring*

With an efficient synthesis of **21** in hand, attention was turned towards its transformation into a glycosyl donor (Scheme 10). The pioneering work of Lemieux and Ratcliffe demonstrated that azidonitration could be performed on glycals to afford the corresponding 2-azido-2-deoxyglycosyl nitrates.[105] The stereoselectivity of this transformation depends significantly on the configuration at C-4. The reaction of oxazolidinone protected 6-deoxy-D-galactose **21** with ceric ammonium nitrate (CAN) and sodium azide resulted in the formation of the

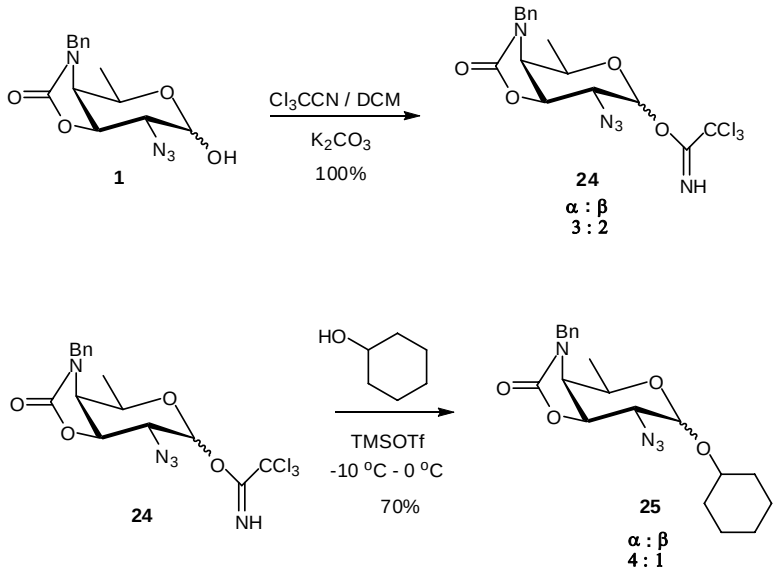
glycosyl nitrate anomers **23** with excellent stereoselectivity at C-2. The analysis of the NMR spectrum of the crude product showed that the presence of the oxazolidinone fused ring introduces rigidity onto the galactal ring and therefore increases the selectivity of the installation of an equatorial azido moiety at C-2. Conventional conditions for removal of the anomeric nitrates by halide ions,[105] thiophenoxide ion,[106] sulfide ions and acetolysis[105] gave very poor yields and, in some cases, decomposition occurred. Surprisingly, it was found that treatment of the anomeric azidonitrates **23** in acetonitrile and water gave the desired hydrolysis product **1** as an anomeric mixture in good yield (Scheme 10).




**21** **23** **1**

Scheme 10: *Azidonitration followed by denitration*

Having hemiacetal **1** in hand, several methods were attempted to investigate the preparation of glycosyl donors. Conveniently, the well established method of Schmidt *et al.*[107] using trichloroacetonitrile and $K_2CO_3$ in dichloromethane gave trichloroacetimidate donor **24** (Scheme 11). Filtration followed by concentration gave an anomeric mixture (α/β: 3:2) of compound **24** in excellent yield without the need for the chromatographic purification. The effectiveness of this glycosyl donor was accessed by reaction with cyclohexanol. In the presence of the promoter, trimethylsilyl triflate, **24** reacted with cyclohexanol to give the 2,4,6-trideoxy-galactopyranoside **25** as an α/β mixture (4:1) in 70% yield.




Scheme11: *Synthesis of cyclohexyl 6-deoxygalactopyranoside*

## 2.4 Conclusion

This chapter describes a new and efficient route for the synthesis of the precursor of 2-acetamido-4-amino-2,4,6-trideoxy-D-galactopyranose from D-Glucal in eight steps and an overall yield of 29%. The formation of the oxazolidinone ring via intramolecular cyclization is an efficient approach to install the amino group at C-4 of 6-deoxy D-glucal. It was also found that the hydrolysis of anomeric azidonitrate occurs in a water and acetonitrile mixture under mild and efficient conditions. Furthermore, imidate **24** appears to be a suitable and valuable building block for the assembly of larger oligosaccharide structures.

# Chapter Three

# Assembly of trisaccharide

## 3.1 Introduction

The chemical synthesis of oligosaccharides is much more challenging compared than the synthesis of other biopolymers, such as peptides and nucleic acids, and considerably more effort is required. The difficulties in the preparation of complex oligosaccharides are due to their multifunctionality, which gives a greater number of possibilities for the combination of monosaccharides to form oligosaccharides. In contrast to other biopolymer syntheses, a strict control of stereochemistry at the anomeric centre, usually C-1, i.e. $\alpha$ or $\beta$ configuration, is required in oligosaccharide synthesis. In addition, regioselectivity is also a prominent problem in carbohydrate chemistry, which can be achieved by choosing an orthogonal set of protecting groups and leaving a single position available on the acceptor for glycosidic bond formation.

## 3.2 Chemical synthesis of glycosides

The majority of carbohydrates found in nature exist as polysaccharides or glycoconjugates, in which monosaccharides are linked to each other or to other types of compounds (aglycones) by *O*-glycosidic bonds. Thus, *O*-glycosylation reactions play a central role in carbohydrate chemistry. However, the chemical synthesis of the glycosidic bond is one of the most challenging aspects since the first synthesis of a phenyl glucoside from sodium phenoxide and "acetobromoglucose" by Michael in 1873.[108] In 1901, Koenigs and Knorr

expanded these studies by reporting the first controlled general glycosylation procedure for oligosaccharide synthesis, using glycosyl halides as donors with a silver based promoter.[109,110] Since then, enormous progress has been made in the area of glycoside synthesis but to date, there are no general applicable methods available for oligosaccharide synthesis.

In a typical glycosylation reaction, the glycosidic bond is formed via a nucleophilic substitution, in which the anomeric carbon atom of the sugar residue to be coupled serves as the electrophile and the alcohol as the nucleophile.[111] As the reaction takes place at the secondary carbon atom with weak nucleophiles (glycosyl acceptors), it often follows an $S_N1$ mechanism. The first step involves activation of the anomeric centre of the glycosyl donor with an appropriate promoter to an electron deficient reactive intermediate, which is attacked by the hydroxyl oxygen atom of the glycosyl acceptor. In the glycosylation reaction, a promoter or activator is used in equimolar or catalytic amounts and it assists in the departure of the anomeric leaving group.

### 3.2.1 Factors influencing the stereoselectivity of glycosylation

The control of stereochemistry is absolutely essential for glycosylation reactions. The stereochemical outcome of the glycosylation reaction is often governed by the anomeric effect and the nature of the protecting group at C-2 of the glycosyl donor.

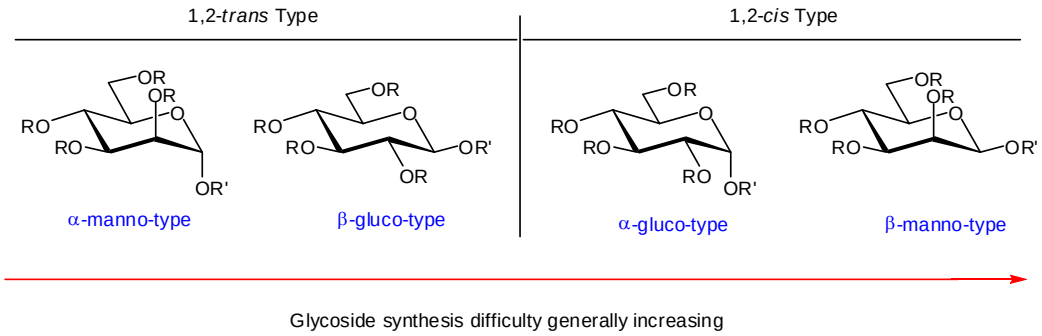



Figure 26: *Classification of glycosidic linkages from synthetic prospective*[112]

The anomeric linkages of the glycosyl donor can be classified into four different classes according to the relative and absolute configuration at the C-1 and C-2 positions: 1,2-*trans* α, 1,2-*trans* β, 1,2-*cis* α and 1,2-*cis* β (Figure 26). Generally, the preparation of 1,2-*cis* glycosides is more demanding than that of 1,2-*trans* glycosides (Figure 26). The 1,2-*trans* glycosides can be synthesized with the assistance of neighboring participating groups. In contrast, 1,2-*cis* glycosides require a sterically non-demanding and non-participating group at C-2. Since most glycosylations proceed through a common oxacarbenium ion intermediate, a non-participating group at C-2 is insufficient to guarantee stereoselective *cis* glycosylation.[111] In the case of a gluco- or galactopyranosyl donor, 1,2-*cis* glycosylation is generally favored by the anomeric effect.

### 3.2.1.1 Anomeric effect

The stereoelectronic effect known as the anomeric effect, first observed by Edward[113] and then defined by Lemieux,[114-116] is partially responsible for the stereochemical outcome of reactions at the anomeric centre of sugars. The anomeric effect demands that polar substituents, such as alkoxy and halide groups, bonded to C-1 of pyranoses (the anomeric centre) having a

thermodynamically preferred axial orientation despite the steric effect that should destabilize this conformation. This effect can be explained by dipole-dipole interaction (Figure 27) and molecular orbital theory (Figure 28).

When the electronegative substituent is in an equatorial orientation, the dipole moment of the anomeric substituent and the dipole of the ring oxygen are pointing in relatively the same direction and have repulsive interaction. This alignment of dipoles leads to a large net dipole, which is energetically unfavored. However, when the electronegative substituent is axial, the dipoles are more or less opposed to each other; therefore, the interaction is less repulsive. This relief of a net dipole stabilizes the sugar molecule (Figure 27).[117]

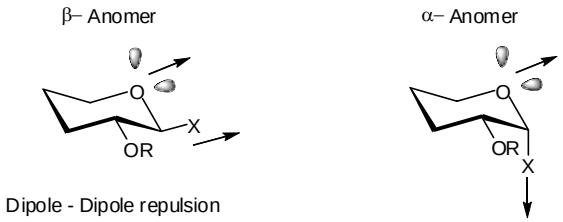



Figure 27: *The dipole moments in the $^4C_1$ conformation of the α- and β-anomers*[85]

In addition, when the electronegative substituent is in an axial orientation, it is stabilized via hyperconjugation as the lone pair of electrons on the ring oxygen is anti-periplanar to the axial C-1-X antibonding orbital σ*. This allows the delocalization of non bonding electrons by partial donation of the oxygen lone pair electrons into the σ* orbital. This orbital alignment does not occur in the β-anomer since the orbitals are in different planes (Figure 28).[117]

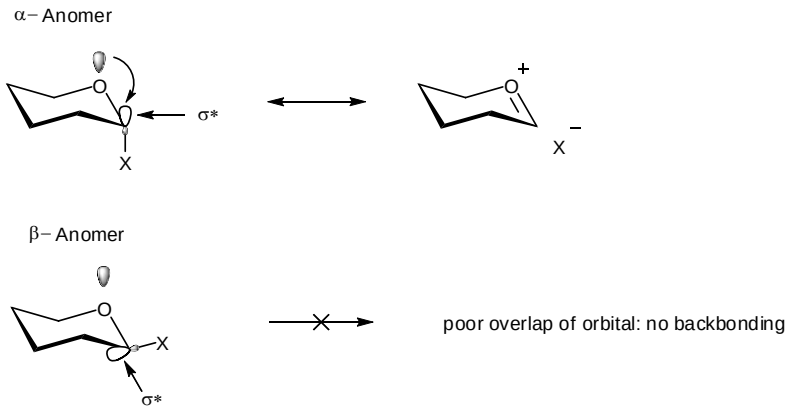



Figure 28: *Orientation of the p and σ* orbitals in the $^4C_1$ conformation of the α- and β-anomers*[85,118]

### 3.2.1.2 Neighboring group participation

Neighboring group participation is one of the most powerful and reliable tools for the construction of the 1,2-*trans* glycosidic linkages. The most commonly applied participating group is an acyl moiety, such as acetyl, benzoyl or pivaloyl for hydroxyls and phthalimido and trichloroethoxycarbonyl (Troc) for amino groups.[110] The principle of this approach is schematically illustrated in Figure 29.

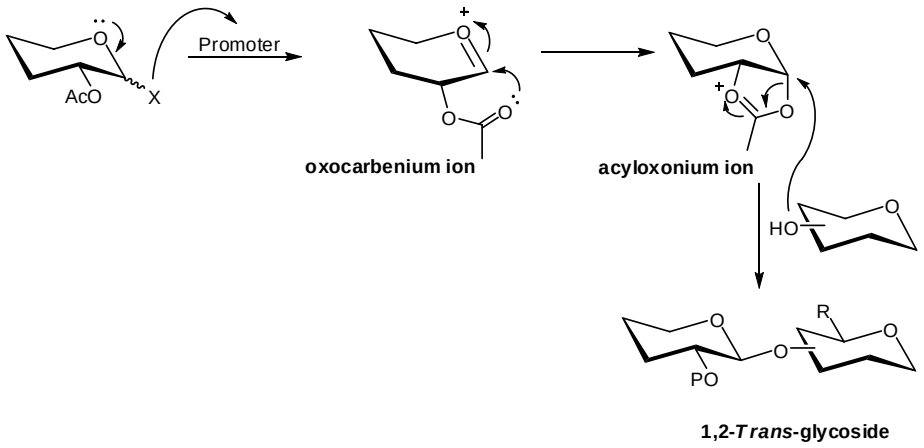



Figure 29: *Synthesis of 1,2-trans glycoside by neighboring group participation*[85,118]

Activation of a given glycosyl donor initiates the departure of the leaving group and generates the oxacarbenium intermediate. In the presence of a neighboring group substituent at C-2, stabilization of the oxacarbenium ion occurs via intramolecular cyclization, producing a more stable bicyclic acyloxonium ion. Since this cyclic intermediate shields the *cis* face, the incoming nucleophile glycosyl acceptor reacts at C-1 from the opposite site of the ring. This nucleophilic attack will open the ring and result in stereoselective formation of a 1,2-*trans* glycoside with regeneration of the acyloxy group at C-2. Glucopyranosyl type donors form β-glycosides whereas mannopyranosyl type donors form α-pyranosides. In some glycosylations, the glycosyl acceptor attacks at C-2 of the dioxolane ring resulting in the formation of a transient orthoester, which can sometimes be isolated. Under acidic conditions, this orthoester is eventually rearranged to the desired product although the orthoester may also open to yield an acylated acceptor.

**3.1.2.3. Solvent effect**

In the absence of a participating group at C-2, solvents, in particular ethers and nitriles, have been shown to play a role in the configuration of glycosylation products.[119,120] Ether type solvents, such as diethyl ether, THF and dioxirane, have a tendency to dictate the glycosylation in an α selective fashion (Figure 30) whereas nitrile solvents increase the proportion of β-glycoside in the absence of a participating group at C-2. Thus, the reaction is thought to proceed via an $S_N1$ pathway. First, an intermediate glycosyl cation is formed, which then coordinates to the ether type solvents to form an equatorial oxonium ion. Thereafter, the

glycosyl acceptor nucleophile attacks the oxonium intermediate from the α face in an $S_N2$ fashion and results in the α-glycoside.[118] Alternatively, nitrile-type solvents preferentially form the α-nitrilium ion and this leads to the β-glycoside.

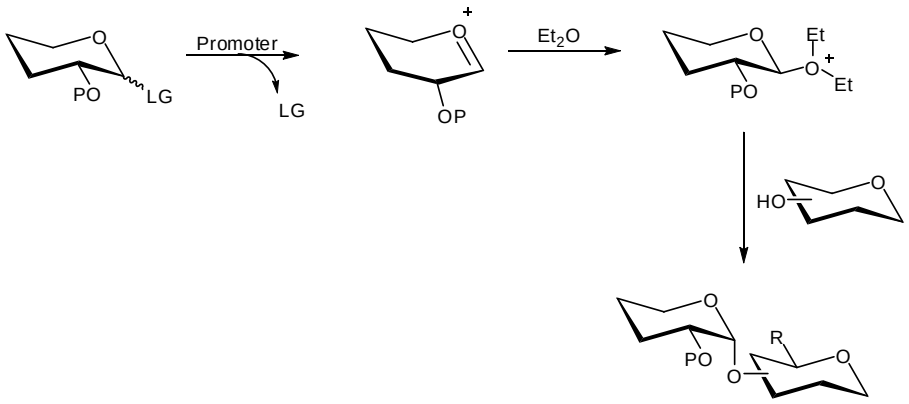



Figure 30: *Proposed mechanism for the solvent participation*[8]

The stereochemical outcome of the glycosylation not only depends on these aforementioned factors but also on several other factors such as temperature, choice of the promoter, type of glycosyl donor and acceptor, and leaving group at the anomeric centre. Some of these factors will be discussed in this chapter.

### 3.2.2 Formation of 1,2-*trans* glycosides

1,2-T*rans* linkages can be readily synthesized utilizing the participation of a neighboring group at C-2, as mentioned above (Figure 29). In addition, glycosyl donors with sterically demanding protecting groups at C-2 also lead to the formation of 1,2-*trans* pyranosides. This is the case for β-glucopyranosides, β-galactopyranosides and α-mannopyranosides.

### 3.2.3 Preparation of 1,2-*cis* glycosides

Preferential formation of the 1,2-*cis* product presents a greater challenge compared to that of a 1,2-*trans* counterpart, since the stereoselectivity of their

formation is difficult to control. These 1,2-*cis* glycosides can often be prepared by using a non-participating protecting group at C-2. The most commonly applied nonparticipating groups for neutral sugars are ether protecting groups, such as benzyl, whereas for corresponding 2-aminosugars, the azido functionality usually serves as an excellent masked form of an amino group. There are two distinct classes of *cis* glycosides: 1,2-*cis* α- (glucopyranoside or galactopyranoside) and 1,2-*cis* β- (mannopyranoside). In most cases, 1,2-*cis* pyranosides are α-anomers. The formation of a thermodynamically stable α-isomer is stereoelectronically preferred over the corresponding β-isomer due to the anomeric effect.

#### 3.2.3.1 Formation of 1,2-*cis* α-linkages

In general, glycosylation reactions follow an $S_N1$ mechanism. Therefore, attempts to make 1,2-*cis* glycosides often leads to poor stereoselectivity with mixtures of anomers. Even though an α-glycoside is thermodynamically favoured, a substantial amount of the kinetic β-linked product is often obtained.[110] In order to obtain an α-glycoside, a glycosidation reaction employing an $S_N2$ mechanism at the anomeric centre of a β-bromide would proceed with an inversion of configuration. However, reduction of this idea to practice is very difficult and has only limited applicability since the majority of the halides are reactive and easily rearrange to the thermodynamically more stable α-anomer. A major achievement in the synthesis of 1,2-*cis* glycosides was the introduction of the *in situ* anomerization concept, which was developed by Lemieux and coworkers in the 1970's. In this halide ion catalyzed glycosidation reaction, tetraethylammonium bromide ($Et_4NBr$) was used as a catalyst (Figure 31).[121]

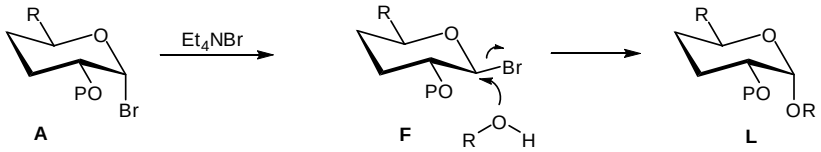



Figure 31: *Formation of α-glycoside via in situ anomerization*

In the past few years, a remote group participation strategy has been extended to control the configuration of the anomeric centre during glycosylation reactions. The effect of participating O-4 acyl functions on the anomeric stereochemistry of a glycosylation reaction was first reported by van Boeckel *et al*.[122] Boons and coworkers have shown that NIS-TMSOTf promoted glycosylation of thiogalactosyl donors exhibited exceptionally high 1,2-*cis* α-anomeric selectivity (Figure 32).[123] This effect on stereoselectivity is rationalized by the stabilization of the anomeric centre via the axially oriented acyl function at C-4. Since the β face is shielded, the glycosyl acceptor can only access the α face. In certain cases, the C-6 acyl group of galactopyranosides has also shown to be 1,2-*cis* α directing via remote group participation.[124,125] However, this long-range assistance is not a well established method for the introduction of an α-glycoside and recent work by Crich has largely discredited this idea of remote participating groups.[126]

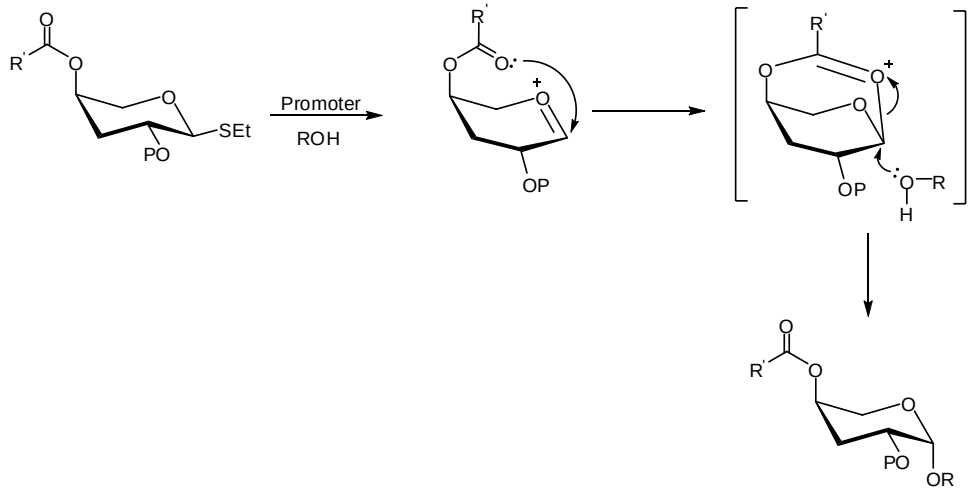



Figure 32: *Proposed long-range participation*[124]

A novel strategy for the stereoselective introduction of 1,2-*cis* glycosidic linkages was developed by Boons and coworkers, in which a chiral auxiliary at C-2 of a glycosyl donor controls the anomeric outcome of the glycosylation (Figure 33).[127] The chiral auxiliary is a C-1 substituted ethyl moiety that contains a nucleophilic group. The nucleophilic moiety of the auxiliary was either an ethoxycarbonyl[127,128] or a sulfide[129] substituent. In this approach, as mentioned earlier, the promoter activates an anomeric leaving group resulting in its departure and an oxacarbenium ion intermediate is formed. Subsequent intramolecular attack by the nucleophilic moiety of the C-2 functionality leads to the formation of an intermediate sulfonium ion as either a *trans*- or a *cis*-decalin system. An auxiliary group carrying the (*S)* stereochemistry favors the formation of a sulfonium ion as a *trans*-decalin ring system due to steric and electronic factors, where the alternate *cis* fused system will place the phenyl substituent in an axial position inducing unfavorable steric interactions. The glycosyl acceptor could

then only approach the anomeric centre of the *trans*-decalin intermediate from the bottom face, which will lead to the formation of an α 1,2-*cis* glycoside.

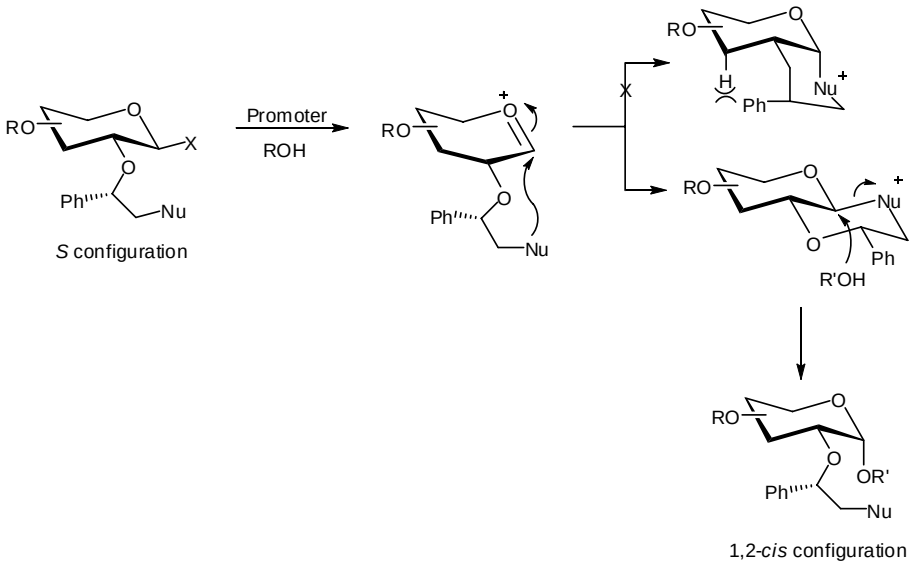



Figure 33: *Neighboring group participation by an (S) auxiliary at C-2 leading to a 1,2-cis glycoside*[127]

### 3.2.3.2 Formation of 1,2 *cis* β-linkages

In glycosylation reactions, the formation of 1,2-*cis* β-glycosidic linkages is one of the greatest challenges because it cannot take advantage of the anomeric effect, which provides stabilization of the α-anomer and it is further disfavored by the repulsive interactions that would have occurred between the axial C-2 substituent and the incoming nucleophile approaching from the β face.[130] A variety of methods have been reported for the stereoselective construction of β-D-mannopyranosides. These methods include Ag-silicate promoted glycosidation of α halides,[131] oxidation–reduction at C-2 of an α-glycoside,[132] C-2 inversion and intramolecular aglycon delivery.[133,134] Detailed discussions of the effects of all these methods are beyond the scope of this review.

Although tremendous progress has been made in the synthesis of 1,2-*cis* glycosides, a robust and general method for the synthesis of *cis* linkages compatible with a wide variety of protecting groups is still an elusive goal for carbohydrate chemists.

### 3.2.4 Glycosyl donors and synthetic strategies

Glycosidic bond formation is generally based on activation of glycosyl donors with leaving groups, such as halide or trichloroacetimidate initiated by appropriate promoters. A wide variety of anomeric substituents are used as leaving groups in glycosylation reactions. However, an important requirement for all these donors is the ability to activate the anomeric leaving group of the glycosyl donor without affecting other anomeric centres present in a complex glycosyl acceptor.[118] The most commonly employed glycosyl donors are halides, thioglycosides and trichloroacetimidates (Figure 34), each with its own advantages and disadvantages.

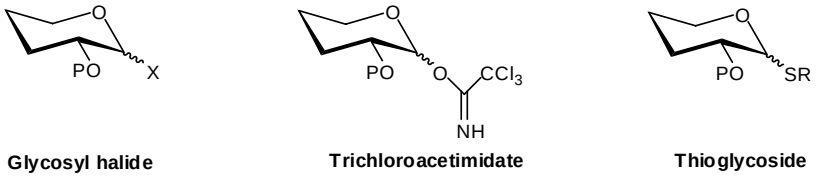



Figure 34: *Common glycosyl donors*

#### 3.2.4.1 Glycosyl halides

Glycosyl halides were the first glycosyl donors to be used for glycoside formation by Wilhelm Köenigs and Eduard Knorr in 1901.[109] Glycosyl bromides can be obtained by the reaction of the peracetylated sugars with HBr in acetic acid, while glycosyl chloride can be obtained by using the similar starting

material with tin tetrachloride. Halophilic salts, such as silver and mercury, serve as promoters in the glycosylation step. In the original form of the Koenigs–Knorr synthesis, insoluble silver salts, such as $Ag_2O$ and $Ag_2CO_3$, were used as activators.[109] Since water is produced by these catalysts, a desiccant was added to the reaction. Soluble $AgClO_4$ and AgOTf were found to be the more efficient activators compared to the insoluble salts.[119] The presence of a participating protecting group at C-2 usually results in exclusive formation of the 1,2-*trans* glycosides. A modification of the Koenigs-Knorr method was introduced by Helferich and coworkers.[135] They employed a mixture of mercury salts, particularly $HgBr_2$ and $Hg(CN)_2$. For a long time, glycosyl halides were found to be the only glycosyl donors for the synthesis of a variety of oligosaccharide targets.[8,85] However, several disadvantages, including the intrinsic lability of glycosyl halides and the need for expensive and toxic heavy metal salts in at least equimolar amounts, limit their utility as glycosyl donors.[8,85]

#### 3.2.4.2 Trichloroacetimidates

The introduction by Sinaÿ and coworkers' of the *N*-methylacetimidate approach was one of the first efficient alternative procedure to heavy metal salt promotors.[136,137] β-Imidates of this type are prepared by reacting glycosyl halides with *N*-methylacetamide (Figure 35). The acetimidates then react with glycosyl acceptors in the presence of *p*TsOH as a promoter to give 1,2-*cis* glycosides. However the glycosyl *N*-methylacetamide is very unstable.

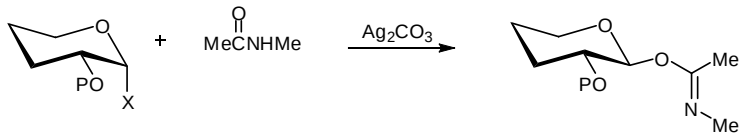



Figure 35: *Synthesis of glycosyl acetimidate*

In 1980, Schmidt and coworkers introduced the use of the sufficiently more stable trichloroacetimidate. Since these derivatives can be isolated and chromatographed, they are now the most often used glycosyl donors.[138] In glycosyl trichloroacetimidates, the anomeric oxygen is derivatized providing a suitable leaving group. The synthesis of trichloroacetimidates starts with the deprotonation of anomeric OH by treatment with a base, such as NaH, $K_2CO_3$ or DBU (Figure 36). The resulting oxyanion attacks the electron-deficient carbon atom of trichloroacetonitrile to provide the *O*-glycosyl trichloroacetimidate. Generally, a weak base, such as $K_2CO_3$, provides the β-imidate as the kinetic product, whereas strong bases, such as NaH or DBU provide the thermodynamically more stable α-imidate.

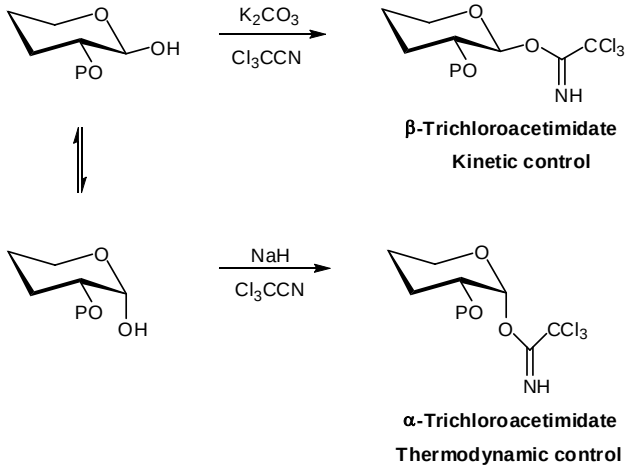



Figure 36: *Formation of trichloroacetimidates using different bases*

Activation of anomeric trichloroacetimidates is usually achieved by the treatment with catalytic amount of a Lewis acid such as $BF_3.Et_2O$ [138] or TMSOTf (Figure 37).[139]

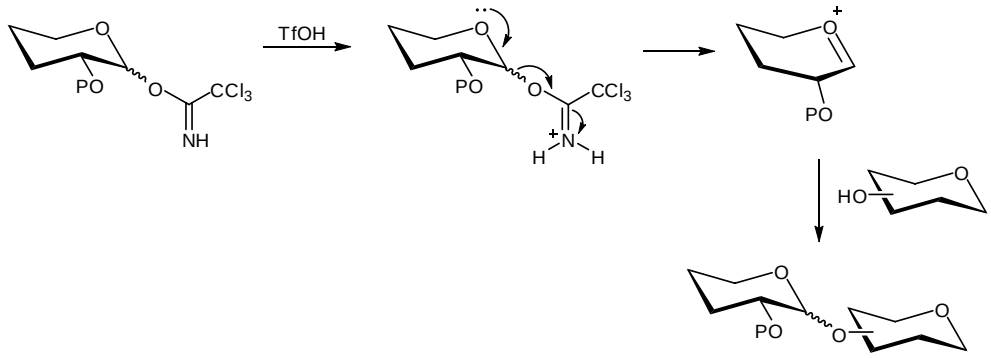



Figure 37: *Glycosylation with glycosyl trichloroacetimidates*

### 3.2.4.3 Thioglycosides

Glycosylation reactions based on thioglycoside donors were first introduced by Ferrier in 1973 and popularized by the work of Lönn.[140-142] Thioglycosides have become one of the most popular and versatile donors due to their ease of preparation and their compatibility and stability toward carbohydrate protecting group manipulation reactions. Since they are inert under several glycosylation reaction conditions, anomeric thio groups can serve as temporary protecting groups for the anomeric centre. In addition, thioglycosides can be readily activated under mild conditions, which do not affect *O*-glycosidic linkages. A great variety of methods are available for the preparation of the most commonly used thioglycosides, such as phenylthio and ethylthio derivatives.[143] Thioglycosides in so called armed and disarmed forms have been exploited in "one-pot synthesis of oligosaccharides where several glycosidic bonds are made sequentially without isolation of intermediate oligomers.

Thioglycosides are usually prepared by treating glycosyl acetates with appropriate thiols (thiophenol or thioethanol) in the presence of a Lewis acid, typically $BF_3.Et_2O$.[144,145] The thio function can be activated using a range of thiophilic promoters. Early attempts for the activation of thioglycosides employed harsh reaction conditions, such as heavy metal salts [$HgSO_4$,[140] $Cu(OTf)_2$[146] and $(PbClO_4)_2$[147]], but nowadays milder promoters have been introduced, which include MeOTf,[141,142] DMTST,[148] NBS[149,150] and NIS-TfOH.[151,152] Among those, NIS-TfOH has proven to be an efficient and frequently used promoter system (Figure 38).

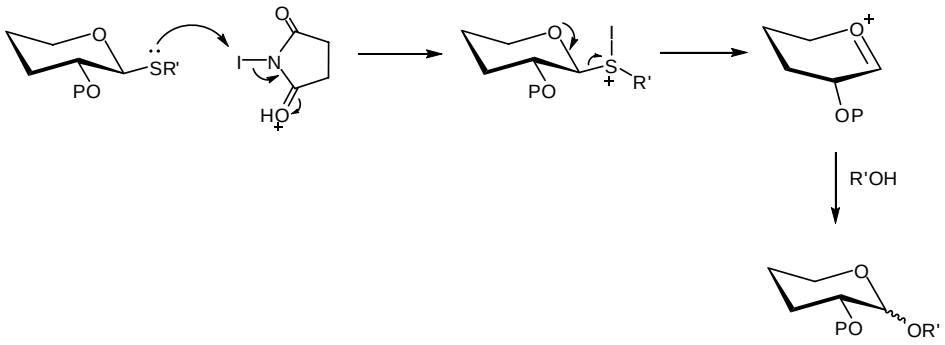



Figure 38: *Activation of thioglycosides for glycosylation by NIS-TfOH system*[118]

In our approach, thioglycosides and trichloroacetimidates were the donors of choice for the glycosylation reactions due to the aforementioned advantages.

## 3.3 Retrosynthetic analysis of trisaccharide

The repeating unit of the type 1 capsular polysaccharide from *Streptococcus pneumoniae* (Sp1) is composed of two galacturonic acid moieties and the rare orthogonally functionalized 2,4-diamino-2,4,6-trideoxygalactose residue **1** (AAT) (Figure 39). The chemical synthesis of this trisaccharide has not been reported. Due to its unique structural features and the biological properties,[27] the Bundle group has focused on developing a convenient method for the preparation of this target trisaccharide (Figure 39).

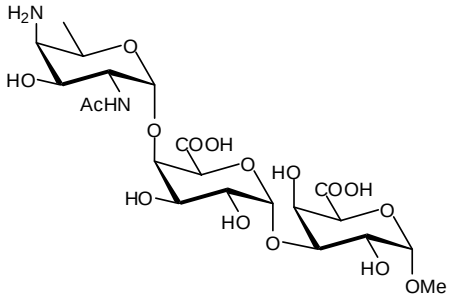



Figure 39: *Target trisaccharide*

For the synthesis of a trisaccharide, one has to overcome a number of synthetic challenges imposed by the complex zwitterionic structure:

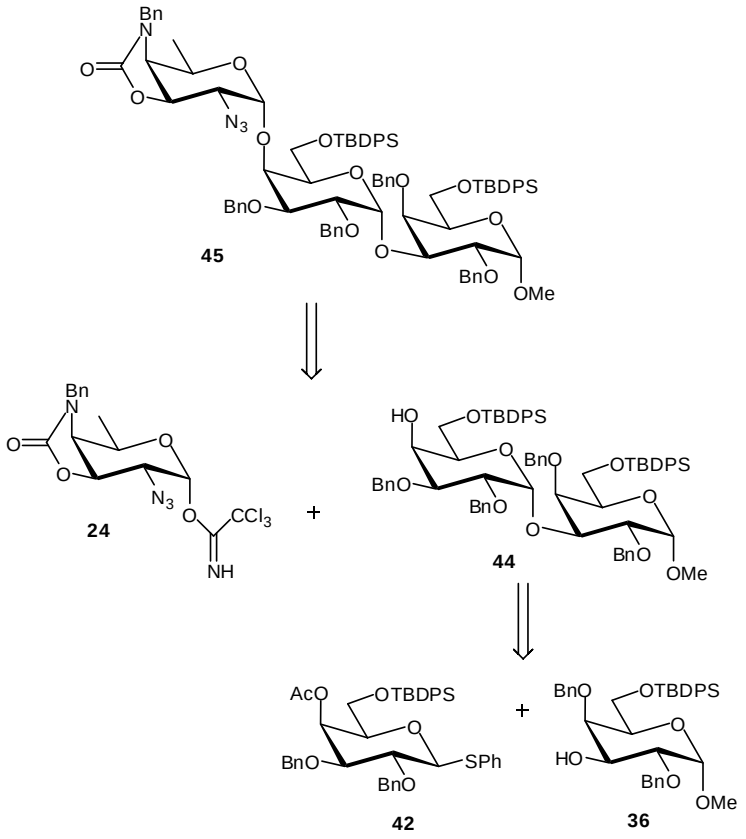
1. The introduction of two α-galacturonic acid moieties
2. Subsequent introduction of a rare amino 6-deoxygalactopyranoside with acetamido group at C-2 and amino group at C-4. This requires a careful design of protecting group strategy to enable the installation of the two distinct amino functions
3. All glycosidic linkages are α-linkages; therefore, we cannot take advantage of neighboring group participation

4. The stereoselective glycosylation between the uncommon amino sugar (donor) and relatively unreactive hydroxyl group at C-4 of galacturonic acid (glycosyl acceptor) had to be developed.

Oligosaccharides containing uronic acids are generally synthesized by one of two strategies, which differ primarily by the sequence of events. The first and most often used strategy involves using non-acidic glycosyl donors or acceptors regioselectively protected with a temporary group at the primary position.[153] Deprotection and subsequent oxidation of the primary hydroxyl groups can be performed at a later stage of synthesis. Alternatively, the second strategy involves direct glycosylation with uronic acid derivatives.[154,155] In glycosylation reactions, the use of an uronic acid building block as a glycosyl donor or acceptor is limited and often avoided because uronic acids are poor glycosyl donors or acceptors due to the low reactivity, epimerization of C-5 and complications associated with protecting group manipulations. Therefore, our synthetic approach entailed the first strategy where the carboxylic acid group was introduced at C-6 after assembly of the trisaccharide. This strategy allows the trisaccharide to be built using the more stable D-galactopyranoside as a temporary substitute for galacturonic acid and neutral trisaccharides (**45**, **49**) can be considered as precursors to the diuronic acid trisaccharide.

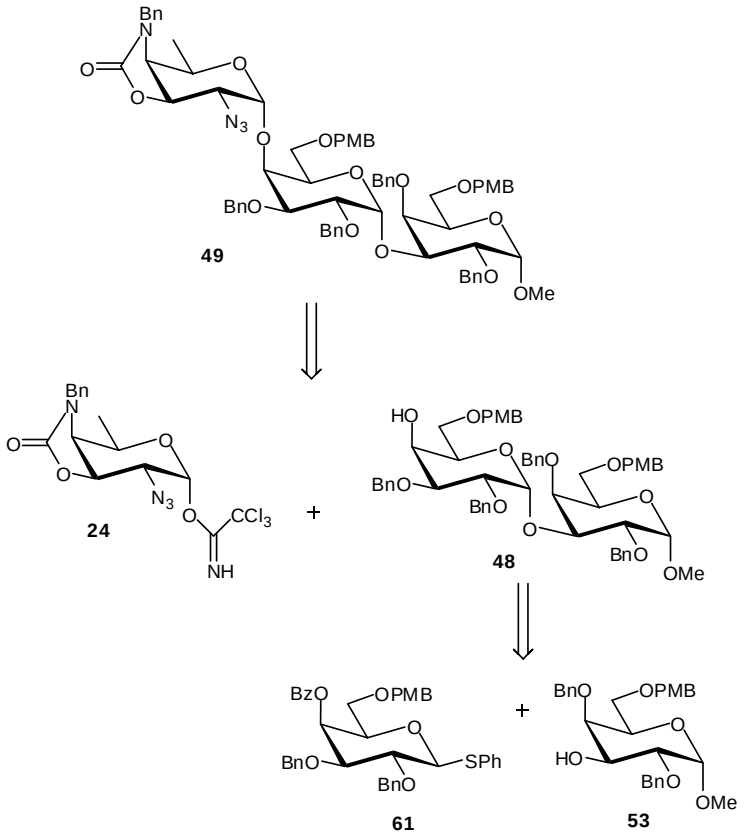
Our first attempted approach for the synthesis of protected trisaccharide **45** is outlined in the retrosynthetic analysis (Scheme 12). The strategy chosen relies on the stepwise addition of monosaccharides from the reducing end of the molecule, with the glycosidic linkage to the most valuable amino sugar, AAT,

being established last. The methyl α-D-galactopyranoside derivative **36** was targeted as a desirable building block for the reducing terminus of the trisaccharide. The benzyl group was chosen as the persistent blocking group, because it is sufficiently stable during protecting and deprotecting conditions and it was anticipated that global deprotection would be accomplished at a later stage by hydrogenolysis. The progression to trisaccharide **45** commenced with the coupling of acceptor **36** to glycosyl donor **42** using NIS-TfOH as the promoter, which gave the desired disaccharide in a good yield. However, subsequent glycosylation of disaccharide acceptor **44** by the trichloroacetimidate donor **24** gave the trisaccharide **45** only in poor yield (22%). Different glycosylation reaction conditions were attempted, but there was no improvement in yield. We suspected, that the poor yield of **45** was attributed to steric hindrance at O-4' caused by the bulky TBDPS protecting group. An alternative protecting group strategy was devised to obtain the target trisaccharide.



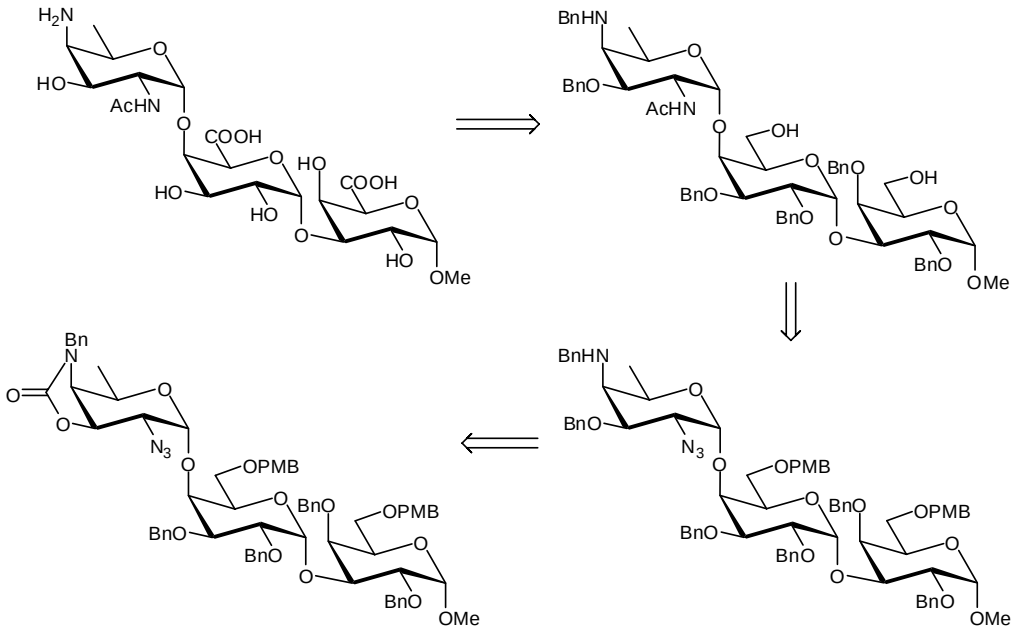

Scheme 12: *Retrosynthetic analysis*

In an alternative approach, it was envisioned that the Sp1 trisaccharide **49** could be prepared from the same donor **24**, but with galactopyranosides **61** and 5**3** bearing PMB groups of O-6 and O-6'. The sequence of glycosylation events is shown in Scheme 13. Retrosynthesis of **49** leads to the trichloroacetimidate donor **24** and disaccharide acceptor **48**. The benzoyl derivative of disaccharide **48** could be assembled from donor **61** and acceptor **53**. The trisaccharide **49** could be prepared in both high yield (70%) and α-selectivity by glycosylation of disaccharide acceptor **48** with the trichloroacetimidate donor **24**.




Scheme 13: *Retrosynthesis of trisaccharide **49***

Transformations to deprotect the amino and carboxylic acid functionalities were performed on the protected trisaccharide **49**. The oxazolidinone ring gave advantages of protection and access to the benzyl protected C-4 amino group via hydrolytic ring opening under basic conditions. Furthermore, transformation of an azide into an acetamido group was accomplished using hydrogen sulfide, followed by acetylation in methanol. Finally, oxidation of the primary hydroxyls to the corresponding diacid was achieved via TEMPO and NaOCl.



Scheme 14: *Retrosynthetic analysis of the functional group manipulation on the protected trisaccharide*

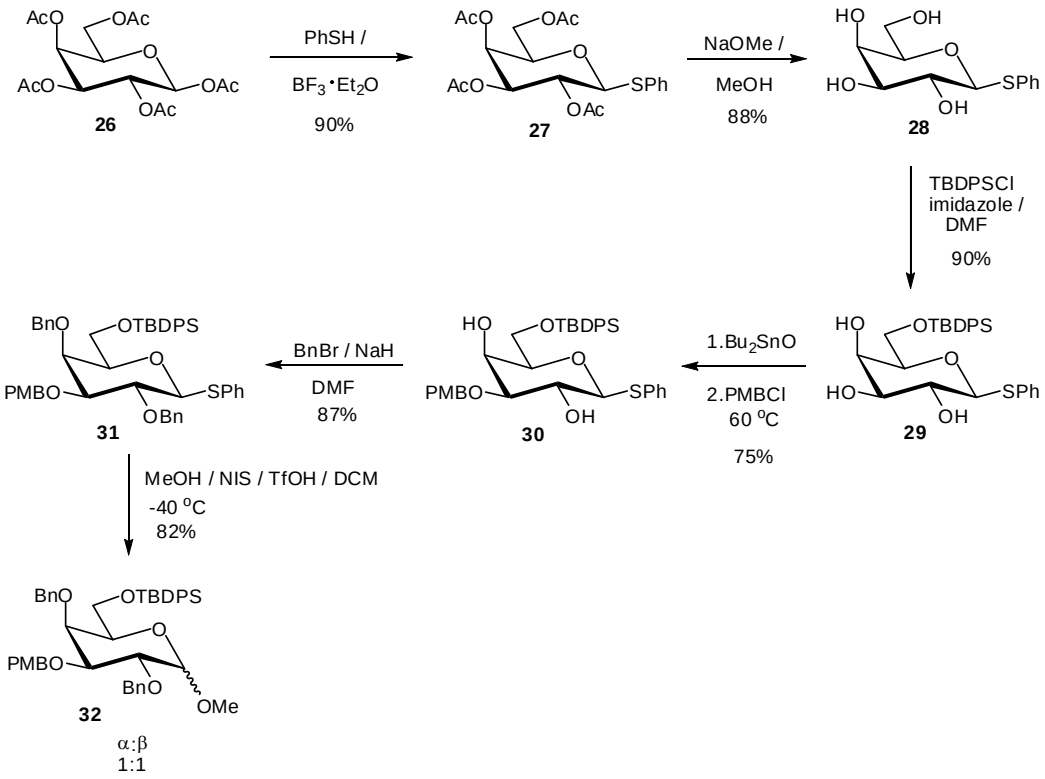
## 3.4 Synthesis of the disaccharide

### 3.4.1 Synthesis of the acceptor

The linear synthesis of trisaccharide **46** commenced with the preparation of the monosaccharide building blocks **1**, **37** and **43**. In the first attempted synthesis of glycosyl acceptor **37**, commercially available galactose pentaacetate **26** was converted into the thio β-glycoside **27** by glycosylation with thiophenol in the presence of $BF_3{\cdot}Et_2O$ (Scheme 15). Deacetylation of **27** with NaOMe in MeOH gave compound **28** in 88% yield. Subsequent regioselective silylation of the hydroxyl at C-6 was performed with TBDPSCl in the presence of imidazole in DMF with excellent yield. Considering that these protecting groups should be orthogonal, we decided to use the PMB group for protection at the C-3 hydroxyl,

since it can be more readily and selectively cleaved than the unsubstituted benzyl ethers by oxidants such as DDQ or CAN.

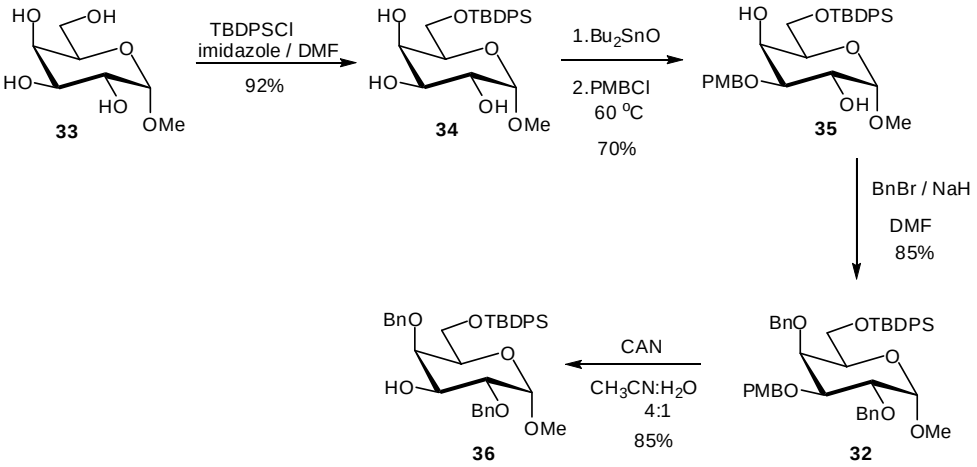
$Bu_2SnO$ is known to form five-membered cyclic dibutylstannylene acetals with carbohydrates preferably with *cis*-diol configurations and have been broadly employed to regioselectively modify the 3-OH of galactosides. In these complexes, the nucleophilicity of the equatorial hydroxyl group is enhanced towards acylation, alkylation, tosylation or silylation.[156,157] We followed this method to regioselectively protect the C-3 hydroxyl group as a PMB ether. The triol **29** was reacted with $Bu_2SnO$ in toluene at reflux with azeotropic removal of water using a Dean-Stark apparatus, and the intermediate stannylene was regioselectively alkylated by PMBCl in the presence of TBAI to afford **30** in 75% yield. Benzylation of the two remaining hydroxyls of **30** was carried out using BnBr and NaH in DMF to give compound **31**. The fully protected thioglycoside donor **31** was then glycosidated with methanol in the presence of NIS-TfOH as a promoter, which provided a 1:1 mixture of α- and β-methyl glycoside **32** in 82% yield. Since the stereochemical outcome of the glycosylation was not as good as expected, we turned our attention to an alternative approach to prepare the glycosyl acceptor.




Scheme 15: *Attempted synthetic route for the glycosyl acceptor* ***32***

The alternative synthesis of the glycosyl acceptor **36** started from commercially available methyl α-D-galactopyranoside **33** (Scheme 16). The precursor of the glycosyl acceptor **36** was prepared by a sequence of similar reactions involving regioselective silylation, tin mediated selective protection of the equatorial hydroxyl group at C-3 with PMB and benzylation via a standard procedure. It was found that the tin–mediated alkylation afforded the O-2 PMB derivative as a side-product. This can be rationalized by the complexation of the dibutylstannylene acetal between the vicinal oxygen at C-2 and the anomeric oxygen followed by reaction with PMBCl to give the O-2 derivative.[158] The equatorial oxygen atoms adjacent to axial oxygen atoms are more reactive, probably because of ease of approach of the electrophile and for acylation, where

equilibration is a factor, perhaps also because the substituents are less sterically restricted there and hence more stable. Compound **32**, containing a PMB group at C-3, allowed for selective access to the C-3 hydroxyl group upon deprotection of this PMB group. Interestingly, CAN was found to be superior to DDQ for the selective removal of the PMB at O-3 and provided the glycosyl acceptor in 85% yield.

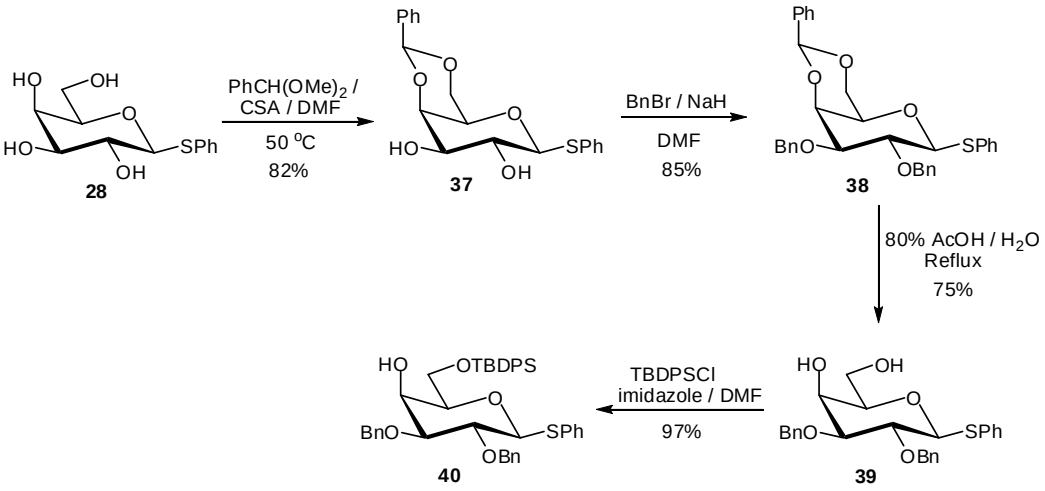



Scheme 16: *Efficient synthesis of glycosyl acceptor* ***36***

### 3.4.2 Synthesis of the donor

The synthesis of glycosyl donor **40** was started from the previously mentioned thioglycoside intermediate **28** (Scheme 17). The phenyl 1-thio-β-D-galactopyranoside **28** was treated with benzaldehyde dimethyl acetal in the presence of catalytic CSA to produce the 4,6-*O*-benzylidene acetal **37** in 82% yield.[159] The remaining hydroxyls of **37** were protected as benzyl ethers.[159] Subsequent hydrolysis of the benzylidene acetal **38** afforded the crystalline diol **39**, which after a selective silylation of the primary alcohol gave the glycosyl acceptor **40** in excellent yield. The hydroxyl at C-4 in galactose is known to be less reactive than the hydroxyl at C-3. Taking into account this differential

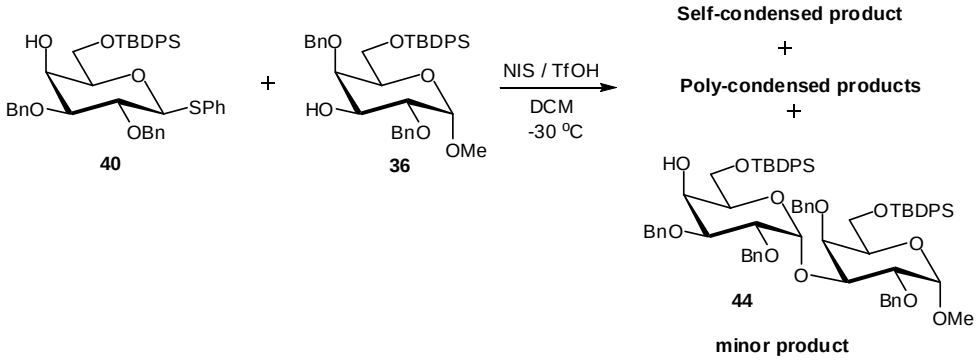
reactivity, we thought it was possible to avoid excessive manipulation of protective groups and leave the hydroxyl at C-4 in the glycosyl donor **40** unprotected during the glycosylation.




Scheme 17: *Synthesis of donor **40***

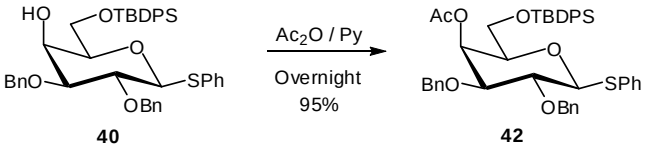
### 3.4.3 Assembly of disaccharide

With both donor **40** and acceptor **36** in hand, a glycosylation was attempted in the presence of NIS and a catalytic amount of TfOH (Scheme 18). The coupling reaction gave the desired disaccharide albeit in poor yield along with several side products that resulted from the self-condensation and poly-condensation of **40**.



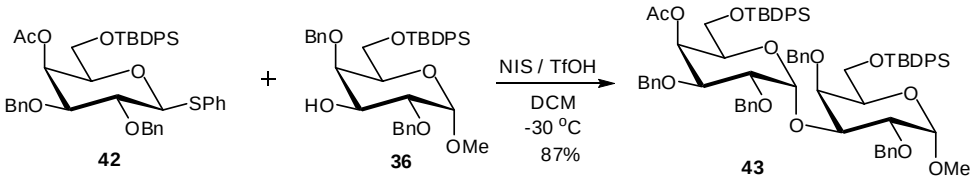

Scheme 18: *Attempted glycosylation to form disaccharide **44***

In order to improve the yield of the desired disaccharide **44**, the hydroxyl at C-4 of **40** had to be temporarily protected by a suitable protecting group, which could be selectively cleaved without affecting the silyl protecting groups at C-6. Thus, the hydroxyl group at C-4 was protected as an acetyl ester using acetic anhydride and pyridine to provide the fully protected donor **42** (Scheme 19). It was observed that acetylation at C-4 was slow and needed to be kept overnight for the completion of the reaction.




Scheme 19: *Acetylation of **40***

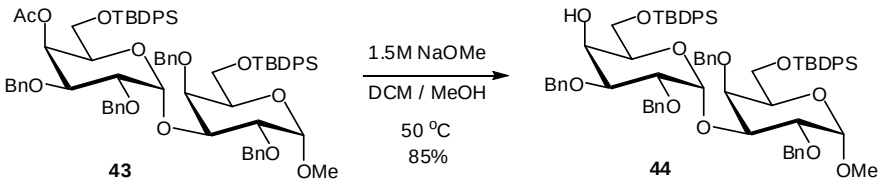
Activation of the fully protected donor **40** with NIS and a catalytic amount of TfOH in the presence of acceptor **36** gave the desired disaccharide **43** with exclusively α selectivity in excellent yield (87%) (Scheme 20).




Scheme 20: *Glycosylation to form disaccharide **43***

With substantial amounts of the disaccharide in hand, attention was turned to the synthesis of trisaccharide **45**. It was expected that the corresponding disaccharide acceptor **44** should be easily accessible by removal of the acetyl group at C-4 of **43** (Scheme 21). However, deacetylation of **43** proved somewhat problematic due to silyl migration. After several conditions were examined, the optimal conditions were found to be the treatment of **43** with NaOMe in a mixed

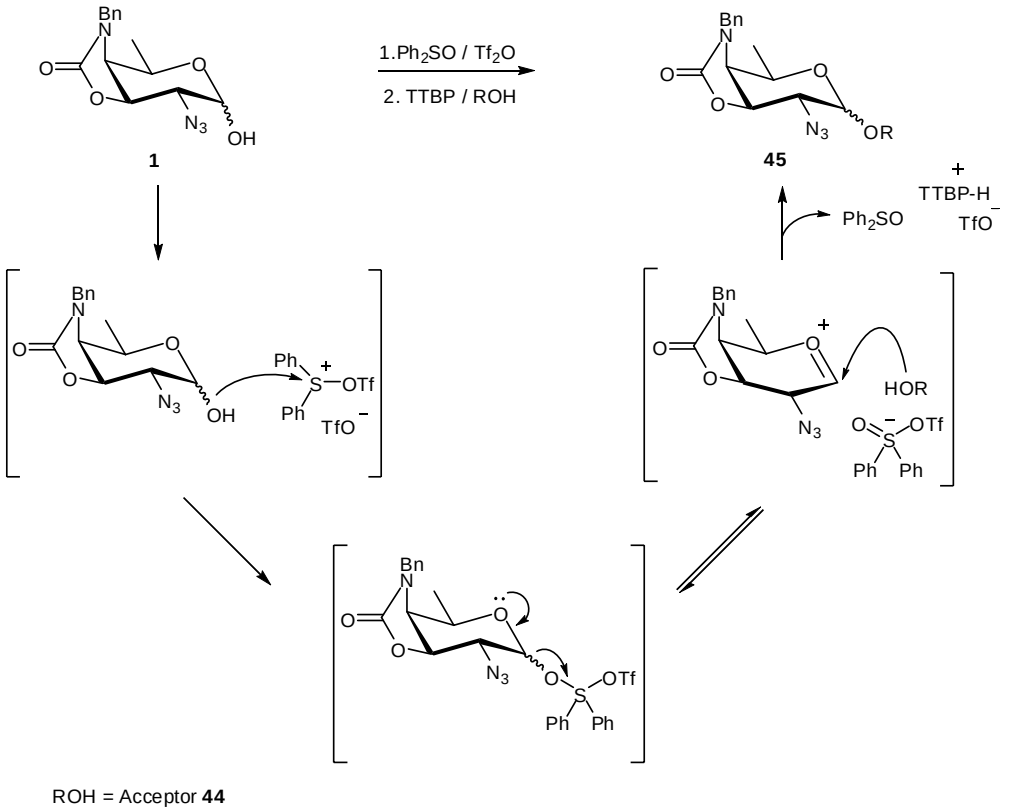
solvent of DCM and MeOH (4:1) and heating at 50 $^{o}$C, which gave the desired disaccharide in the highest yield (85%). Other conditions, such as $K_2CO_3$ in MeOH and DCM, or AcCl in methanol resulted in silyl migration and it was noticed that the silyl migration was substantial upon prolonged reaction times.




Scheme 21: *Deacetylation of disaccharide **43***

## 3.5 Synthesis of the trisaccharide

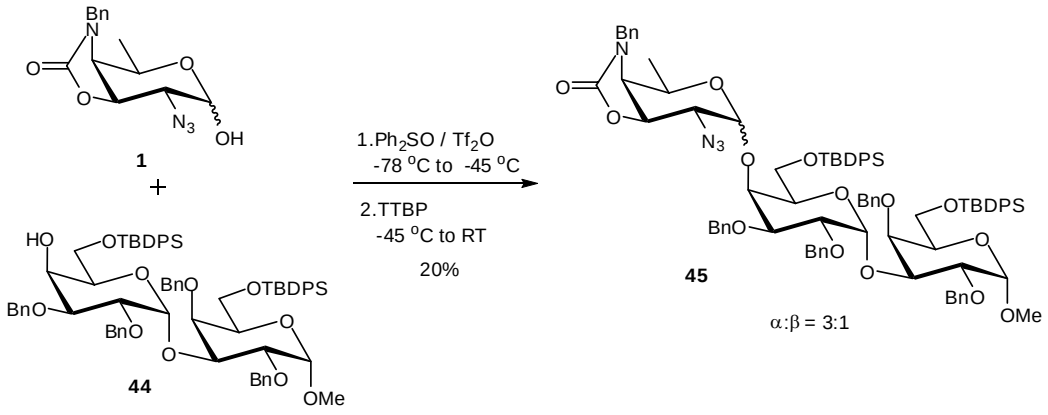
With the desired building blocks **44** and **1** (the synthesis of **1** was described in Chapter 2) in hand, assembly of the trisaccharide was attempted by the application of appropriate glycosylation protocols. In the first attempt to synthesize the protected trisaccharide **45**, attention was focused on Gin's dehydrative glycosylation method using sulfonium–based electrophilic activation of a hemiacetal, which does not require anomeric protecting group manipulation (Scheme 22).




Scheme 22: *Hypothetical mechanism of dehydrative glycosylation*

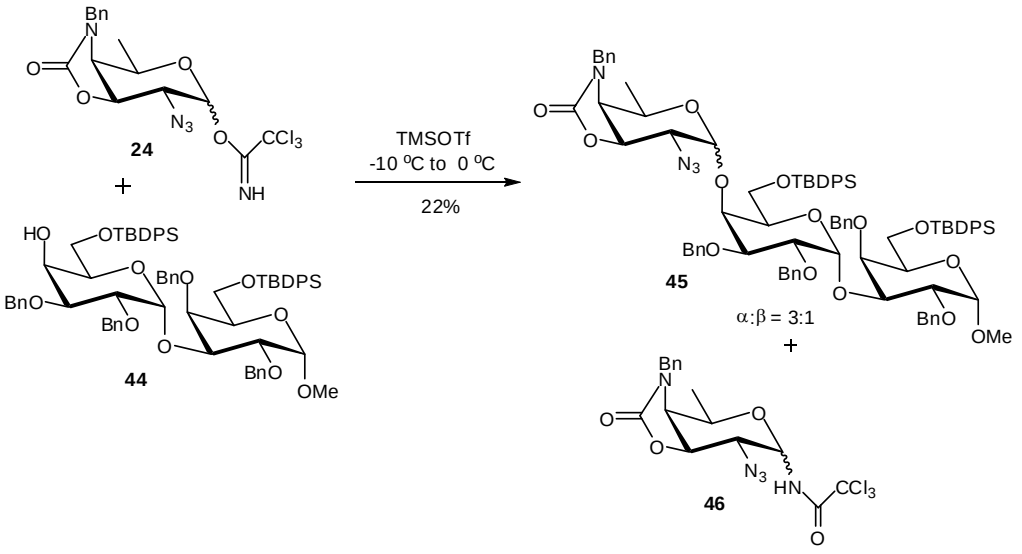
Treatment of $Ph_2SO$ with $Tf_2O$ allows for the *in situ* formation of the highly reactive diphenylsulfonium bistriflate, which in turn reacts with the hemiacetal donor **1** at -78 $^oC$ to provide the activated anomeric oxosulfonium intermediate. Subsequent addition of disaccharide **44** and the hindered base TTBP at -45 $^oC$ followed by warming the reaction mixture to room temperature afforded trisaccharide **45** in only 20% yield (α:β 3:1) (Scheme 23). The unreacted disaccharide **44** was recovered, whereas excess donor **1** completely degraded to unidentified polar products. The desired α-linked trisaccharide could not be obtained in reasonable yield, even by inverting the order of reagent addition (activating $Ph_2SO$ with $Tf_2O$ prior to the addition of glycosyl donor) and keeping the reaction temperature below -45 °C. A number of conditions, such as variable

temperatures and the order of the addition of the reagents were explored in an effort to increase the conversion of the glycosyl acceptor into the trisaccharide product. Unfortunately, attempts to optimize the glycosylation using the dehydrative method lead to unsatisfactory results.




Scheme 23: *Glycosylation by Gin's dehydrative method to form the trisaccharide*

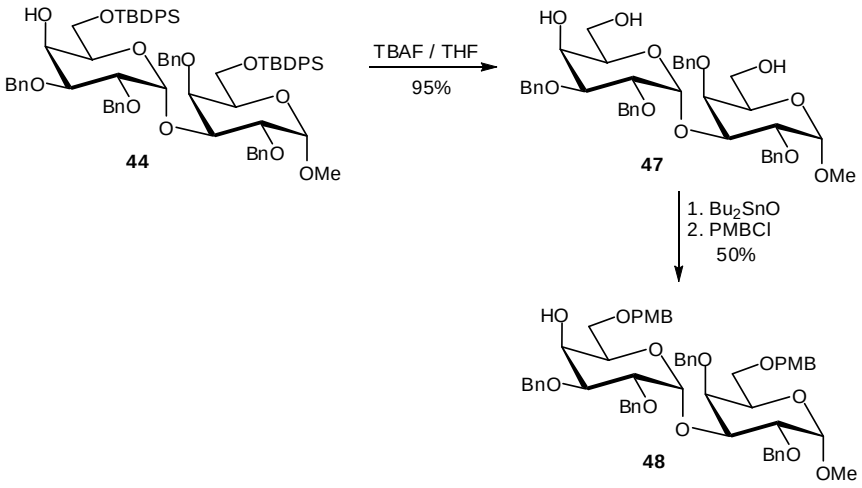
As an alternative, the imidate **24** was coupled with the same acceptor **44** in the presence of TMSOTf to furnish the trisaccharide **45** (Scheme 24). The yield and stereoselectivity of the glycosylation of the imidate turned out to be similar to that of the dehydrative glycosylation. An important side-product of this reaction was a compound having a slightly higher $R_f$ value than that of the starting glycosyl imidate. It was identified as *N*-trichloroacetamide **46**, which presumably formed from reaction of the anomeric centre of the activated donor with the departing trichloroacetimidate or its TMS derivative. As the 22% yield reported here for glycosyl acceptor **44** was by far the best result in a series of reactions performed, this glycosyl acceptor was judged to be a poor substrate and the glycosylation was abandoned at this point.




Scheme 24: *Glycosylation using imidate donor*

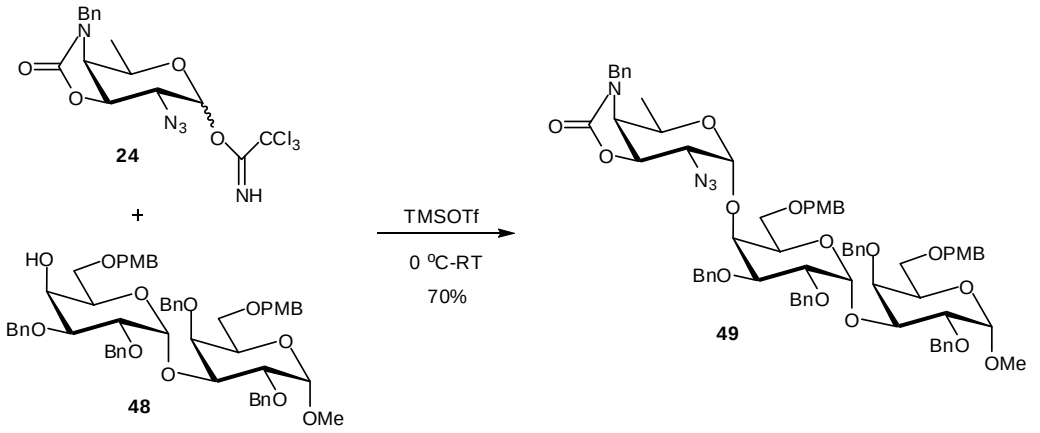
It was hypothesized that the difficulty in obtaining a satisfactory yield for the glycosylation step could be due to the steric hindrance of the bulky TBDPS group at C-6′ of **44**, thereby reducing the reactivity of the neighboring 4′ hydroxyl and making the hydroxyl less amenable to glycosylation. In the next attempt to synthesize the trisaccharide, the strategy was therefore designed to prepare the glycosyl acceptor bearing a less sterically demanding *p*-methoxybenzyl ether at C-6′.

In order to investigate the feasibility of this approach, acceptor **48**, bearing PMB groups at O-6 and O-6′, was synthesized from compound **45**. The TBDPS protecting groups of **45** were removed with TBAF in THF to afford triol **47** in excellent yield. Regioselective *p*-methoxybenzylation of **47** at O-6 and O-6′ using $Bu_2SnO$ and then PMBCl in the presence of TBAI gave **48** in 50% yield (Scheme 25).



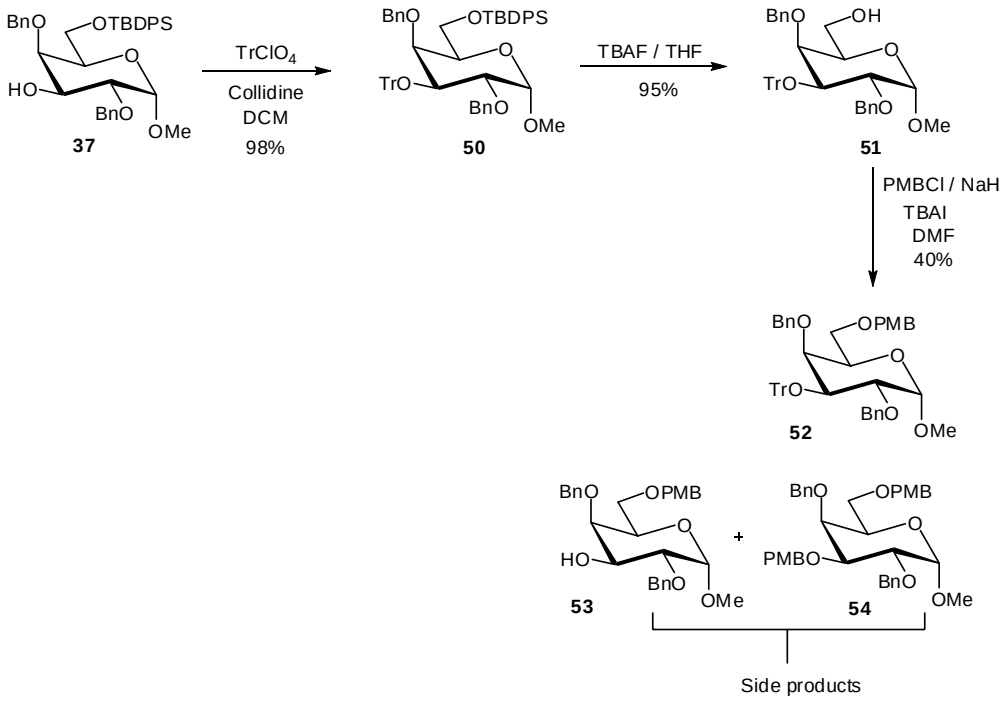

Scheme 25: *Synthesis of acceptor **48** from intermediate **44***

Having prepared the glycosyl acceptor **48**, glycosylations were performed with donors **1** and **24**. Many attempts were carried out adopting both the direct and the inverse glycosylation procedure, changing the Lewis acid and the temperature. Finally, the best conditions were found. This used a 1.5 fold excess of the imidate glycosyl donor in DCM with the glycosyl acceptor **48** in the presence of TMSOTf with raising the temperature from 0 $^{o}$C to room temperature. This method afforded the trisaccharide **49** in 70% yield with exclusively α-selectivity (Scheme 26). Having achieved the stereoselective synthesis of trisaccharide **49**, it was necessary to obtain the disaccharide **64** from the corresponding glycosyl acceptor **53** and donor **60** in reliable routes. Attention was therefore focused on the synthesis of building blocks **53** and **60**.



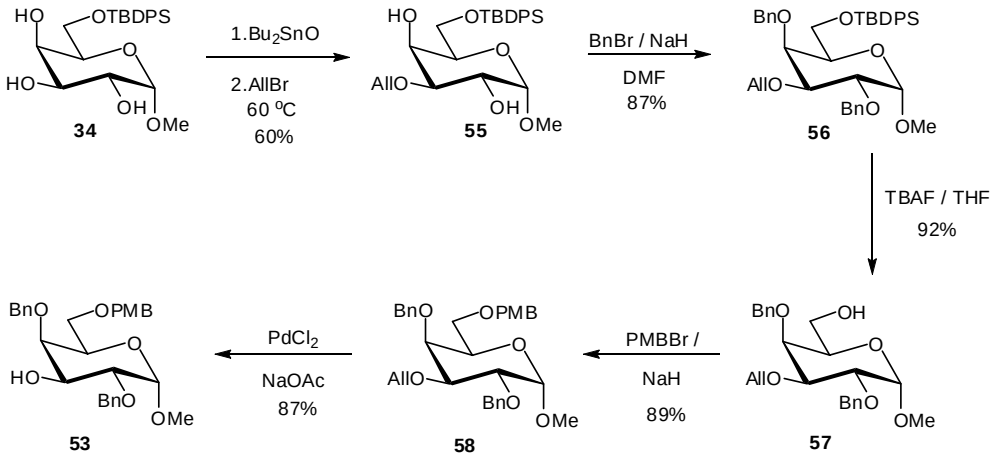

Scheme 26: *Optimized condition of glycosylation to synthesize trisaccharide* ***49***

The synthesis of acceptor **53** began with the intermediate **37** (Scheme 27). The triphenylmethyl (trityl) functionality represents an attractive protecting group for an alcohol since it is readily removed under mildly acidic conditions (1% TFA in DCM) and is also orthogonal to a variety of protecting groups. It was envisioned that tritylation of the secondary hydroxyl groups could be performed under forcing conditions, such as trityl perchlorate. Indeed, tritylation of **37** using freshly prepared trityl perchlorate in collidine and DCM furnished the 3-O-trityl compound **50**. The subsequent deprotection of the O-6 TBDPS group using TBAF in THF afforded compound **51** in excellent yield. Protection of the C-6 hydroxyl group in compound **51** with PMBCl and NaH in the presence of TBAI with heating at 65 $^{o}$C produced **52** in 30% yield. This was accompanied by side products **53** and 3,6-di-*p*-methoxybenzyl derivative **54**, which can be explained by the fact that the protection of O-6 by a PMB group was very slow and required extended time and elevated temperature. Under these conditions, it was found that the trityl group was replaced by a PMB group. At this point, to efficiently synthesize the glycosyl acceptor **53**, we decided to design a different strategy.



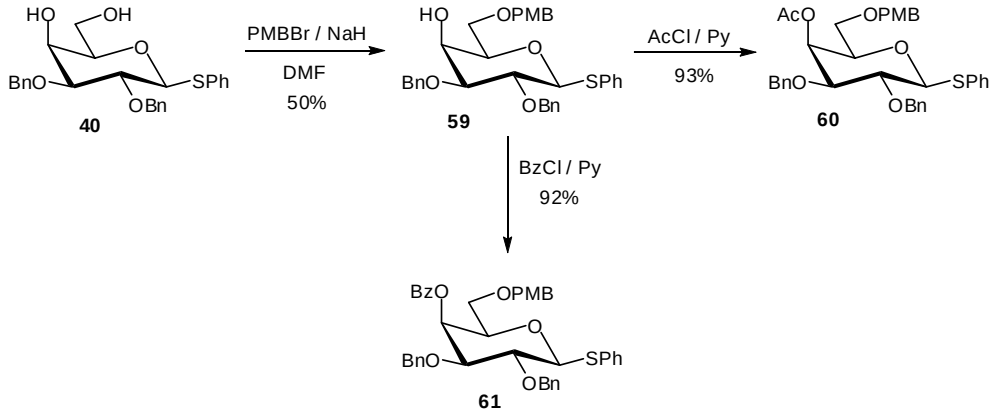

Scheme 27: *Attempted synthesis of glycosyl acceptor* ***53***

An efficient synthesis of the glycosyl acceptor **53** is illustrated in Scheme 28. The synthesis was started from the previously mentioned intermediate **34**. Regioselective introduction of the allyl group at O-3 via a transient stannylene acetal was performed by treatment with $Bu_2SnO$ and then allyl bromide in the presence of TBAI, which led to the 3-*O*-allyl protected derivative **55** in 60% yield, along with 2-*O*-allyl and 2,3-*O*-diallyl derivatives as minor products. Benzylation of the remaining hydroxyls using standard conditions of BnBr and NaH, followed by a desilylation of the O-6 TBDPS group afforded **56** in 80% yield over two steps. The O-6 position of **56** was protected as a PMB group with PMBBr and NaH in DMF to afford the fully protected **58** in 89% yield. The subsequent deallylation using $PdCl_2$ and NaOAc in aqueous AcOH secured the suitable acceptor **53** for the assembly of disaccharide **64**.




Scheme 28: *Synthesis of glycosyl acceptor* ***59*** *in an efficient route*

In an attempt to obtain the glycosyl donors, we started the synthesis from intermediate **40**. Attempted selective 6-*O*-benzylation of **40** with PMBBr and NaH gave **59** only in 50% yield and led to 4-*O*-PMB derivative and 4,6-*O*-diPMB derivatives as by-products (Scheme 29). The remaining C-4-OH of **59** was protected as an acetate or a benzoate to yield the glycosyl donors **60** and **61**, respectively.

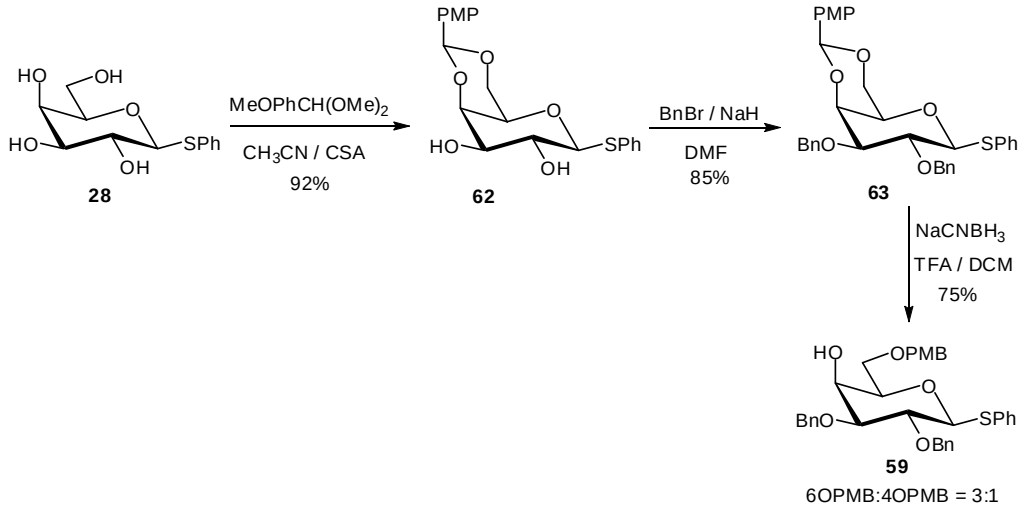



Scheme 29: *Synthesis of glycosyl donors* ***60*** *and* ***61***

Alternatively, compound **59** was reached through the sequence of transformations delineated in Scheme 30, which was found to be more amenable to scale up. The *p*-methoxybenzylidene acetal protecting group was selected for

its versatility as it could readily undergo reductive ring opening to give a regioselectively protected alcohol **59**. Thus, the synthesis of the common precursor **59** for building blocks **60** and **61** was carried out as shown in Scheme 30. Treatment of phenyl 1-thio-β-D-galactopyranoside **28** with anisaldehyde dimethyl acetal in the presence of a catalytic amount of CSA in $CH_3CN$ accomplished the selective acetal formation at C-4 and C-6 to give *p*-methoxybenzylidene acetal **62** in 92% yield.[160] Subsequent benzylation of PMP acetal **62** with sodium hydride and benzyl bromide gave dibenzyl ether **63** in 85% yield.

The regioselectivity in the reductive ring opening of 4,6-*O*-*p*-methoxybenzylidene acetals of hexopyranosides varies with reagents and solvents. For the preparation of the 6-*O*-PMB derivative **59**, $NaCNBH_3$ and TFA in THF gave the best results (Table 1, entry 1). Other reagents, such as $NaCNBH_3$–$HCl{\cdot}Et_2O$, $Et_3SiH$–TFA and $BH_3{\cdot}THF$–$Bu_2BOTf$ [161] were also effective (Table 1). However, they gave substantial amount of 4-*O*-PMB derivatives as by-products.




Scheme 30: *Synthesis of compound **59***

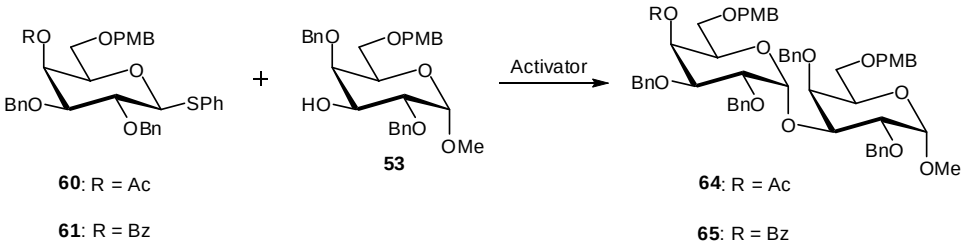
| Entry | Lewis acid | Temp ($^{o}$C) | Yield of **59** (%) | 6-OPMB:4-OPMB |
|---|---|---|---|---|
| 1 | $NaCNBH_3$–TFA | 0-RT | 75 | 3:1 |
| 2 | $NaCNBH_3$–HCl·$Et_2O$ | 0-RT | 47 | 1:1 |
| 3 | $Et_3SiH$–TFA | 0 | 60 | 2:1 |
| 4 | $BH_3$·THF–$Bu_2BOTf$ | -78 | 63 | 2:1 |

Table 1: *Reductive opening of 4,6-O-p-methoxybenzylidene acetal **63** using various reagents*

Having installed the desired PMB functionality at C-6, we then completed the synthesis of the glycosyl donors **60** and **61** by acetylation and benzoylation respectively, as previously described. This sequence provided the key glycosyl donors in an efficient manner. Having completed the synthesis of glycosyl acceptor **53** and glycosyl donors **60** and **61**, we then turned our attention to the assembly of disaccharides **64** and **65**.

Following the standard thioglycoside protocol, a series of glycosylation reactions were then conducted with glycosyl donors **60** and **61** containing

different ester groups such as acetyl and benzoyl at C-4 respectively (Scheme 31, Table 2). Initial glycosylation of thioglycoside donor **60** with the glycosyl acceptor **53** at -30 $^{o}$C, employing NIS–TfOH as the promoter gave the desired disaccharide **64** in 45% yield (entry 1). However, when the acetate group was changed to a benzoyl group, the yield of the glycosylated product improved to 57% (entry 2). Furthermore, upon isolation of the by-products, it was found that one of them was a product of the partial loss of the PMB group from the disaccharide under these reaction conditions. In a different coupling event, attempted activation of donor **61** by treatment with $Ph_2SO$–$Tf_2O$ at -78 $^{o}$C, followed by an addition of glycosyl acceptor **53** at -40 $^{o}$C in the presence of the hindered base TTBP was unproductive, resulting in a very poor yield (entries 3 and 4).[162] Fortunately, it was found that the glycosylation of **53** with donor **61** after addition of NIS–AgOTf at -30 $^{o}$C proceeded rapidly to afford the disaccharide **65** in 70% yield with exclusively α-selectivity (entry 5).

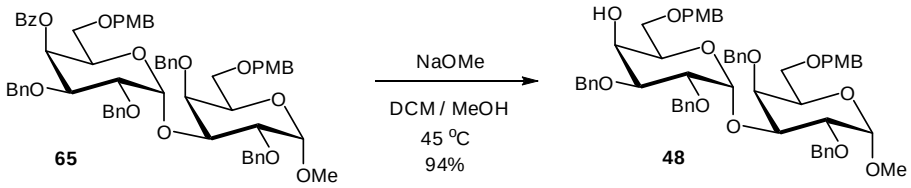



Scheme 31: *Synthesis of disaccharides*

| Entry | Donor | Activator | Temperature ($^{o}$C) | Yield (%) |
|---|---|---|---|---|
| 1 | **60** | NIS–TfOH | -30 | 45 |
| 2 | **61** | NIS–TfOH | -30 | 57 |
| 3 | **61** | $Ph_2SO$–$Tf_2O$<br>TTBP | -78<br>-40-RT | Decomposed |
| 4 | **61** | $Ph_2SO$–$Tf_2O$<br>TTBP<br>Quenched with TEA | -78<br>-40<br>-40 | 30 |
| 5 | **61** | NIS–AgOTf | -30 | 70 |

Table 2: *Glycosylation reaction of **53** using different conditions*

Liberation of the C-4 hydroxyl group was accomplished by treatment of disaccharide **65** with NaOMe in a mixture DCM and MeOH (v/v, 4:1) at 45 $^{o}$C giving the disaccharide acceptor **48** (Scheme 32).




Scheme 32: *Debenzoylation of **65***

Having constructed the requisite building blocks **48** and **24**, the stage was set for the assembly of the target trisaccharide **49** via the final glycosylation reaction. The optimization of the glycosylation to produce trisaccharide **49** was already discussed in this chapter (Scheme 26).

## 3.6 Functional group manipulation and deprotection of trisaccharide

With the trisaccharide **49** in hand, the next goal was to design a deprotection strategy. It was expected that the deprotection of the trisaccharide could be challenging due to the presence of multiple protecting groups. Therefore, the sequence for the protecting group removal had to be evaluated with great care. Since galacturonic acid esters and an *N*-acetamido group would not be compatible with the conditions known for the opening of the oxazolidinone ring, opening of the oxazolidinone ring was undertaken first.

There are several methods available in the literature for the cleavage of oxazolidinone ring.[163] Electrophilic carbon atoms in the oxazolidinone ring are the carbonyl carbon and the C-5 carbon atom. All these reported methods involved a nucleophilic attack on the electron deficient carbonyl carbon followed by an elimination of carbon dioxide to form the vicinal amino alcohol via opening the oxazolidinone ring.

Nucleophiles, such as thiophenol, open the oxazolidinone ring by attacking at C-5 of the ring (Figure 40).[164] This process, however, would destroy the target trisaccharide **49** by inversion of configuration of C-3 and installation of a new undesired group in the trisaccharide.

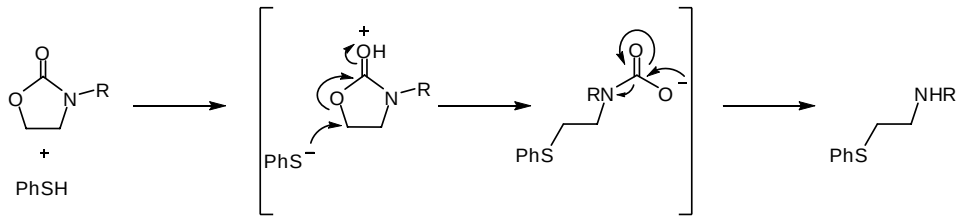



Figure 40: *Oxazolidinone ring opening by thiophenol*[164]

Therefore, we required a method that cleaves the oxazolidinone by attacking the carbonyl atom. Generally, hydrolysis of an oxazolidinone with concentrated or dilute hydrochloric acid leads to the formation of the hydrochlorides of amino alcohols.[165] However, it was found that the scope of the acidic cleavage reaction is limited to substrates containing acid–stable functional groups. Since our trisaccharide contains acid sensitive PMB groups at O-6 and O-6', we could not employ the acidic cleavage of the oxazolidinone ring.

Alternatively, but less often, trimethylsilanolate is used to cleave the oxazolidinone ring. This method failed to give the desired product (entry 6, Table 3). Oxazolidinones can also be reductively cleaved by $LiAlH_4$ to afford the corresponding *N*-methyl amino alcohols.[163,166] Nevertheless, reductive cleavage was not applicable in our case since it would lead to the formation of *N*-methyl amino alcohol instead of the desired free amino alcohol.

One of the classical methods for the cleavage of oxazolidinone is base hydrolysis (Figure 41).[163] This was our method of choice for the ring cleavage since the protecting groups on the trisaccharide **49** were stable to basic conditions.

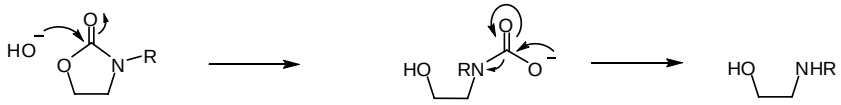
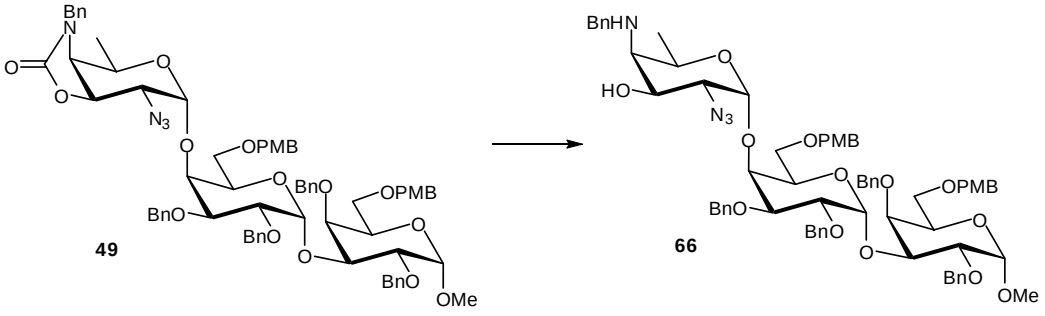



Figure 41: *Ring opening of oxazolidinone by base hydrolysis*

Base-promoted ring opening of *trans*-fused *N*-unsubstituted and *N*-acetyl subtituted 2*N*,3*O*-oxazolidinone protected *N*-acetyl-D-glucosamine has been extensively studied by Kerns[99,167] and Crich[168] by employing inorganic bases such as $Cs_2CO_3$ in a variety of alcohols,[99] NaOH–$H_2O$[167] and $Ba(OH)_2$–EtOH.[168]

After screening a series of extensive reaction conditions, such as varying the inorganic base, solvent system, temperature and reaction time, we found that the cleavage of oxazolidinone could be achieved by choosing a small and strongly Lewis acid; lithium cation (Table 3). Treatment of **49** with LiOH and catalytic amount of LiI in a mixture of THF and ethanol under reflux for four days gave the 4*N*-benzylgalatosamine derivative **66** in excellent yield (entry 8, Table 3). The ring cleavage of oxazolidinone using 1 M $Cs_2CO_3$ in THF at various temperatures did not occur (entries 1 and 2). Similar conditions using NaOH as a base also failed to open the oxazolidinone ring (entries 3 and 4). When ethanol was added as a co-solvent; however, cleavage of the oxazolidinone resulted in the desired trisaccharide **66** in 50 % yield, accompanied by a substantial amount of the 3-*O*-Et product (entry 5). An improved yield was obtained by employing 2 M LiOH in a mixture of THF and EtOH (entry 7). The addition of a catalytic amount of LiI to the aforementioned conditions further improved the yield of **66** (entry 8).

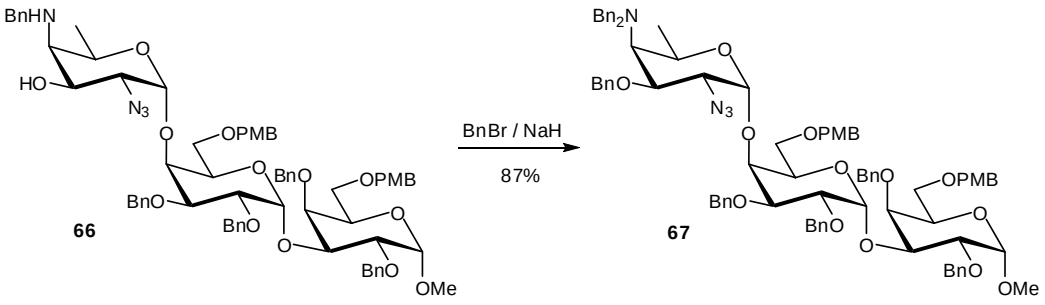



Scheme 33: *Opening of oxazolidinone ring* ***49***

| Entry | Reaction condition | Temperature ($^{o}$C) | Yield (%) |
|---|---|---|---|
| 1 | $Cs_2CO_3$–THF | RT | - |
| 2 | $Cs_2CO_3$–THF | Varying from 40 - 80 | - |
| 3 | 1 M NaOH–THF | RT | - |
| 4 | 1 M NaOH–THF | Varying from 40 - 80 | - |
| 5 | 1 M NaOH–THF–EtOH | 80 | 50 |
| 6 | KOTMS–THF | 80 | - |
| 7 | 2 M LiOH–THF–EtOH | 80 | 80 |
| 8 | 2 M LiOH–LiI–THF–EtOH | 80 | 95 |

Table 3: *Cleavage of the oxazolidinone ring of **49** under different conditions*

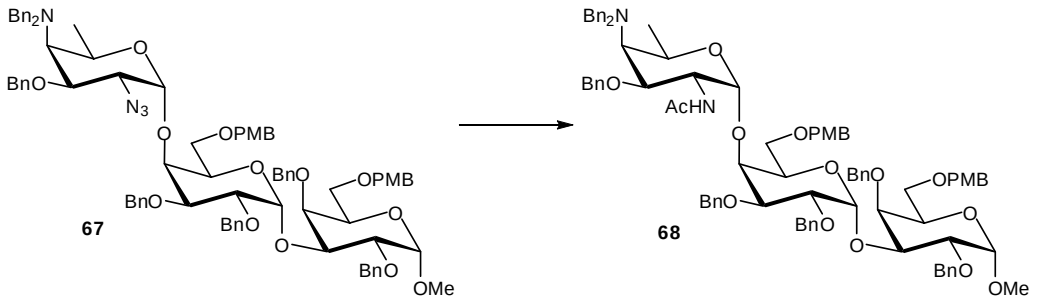
By applying excess amounts of BnBr and NaH (4 eq each), both 3-OH and 4-NHBn of **66** were benzylated to produce **67** in excellent yield (Scheme 34).




Scheme 34: *Benzylation of **66***

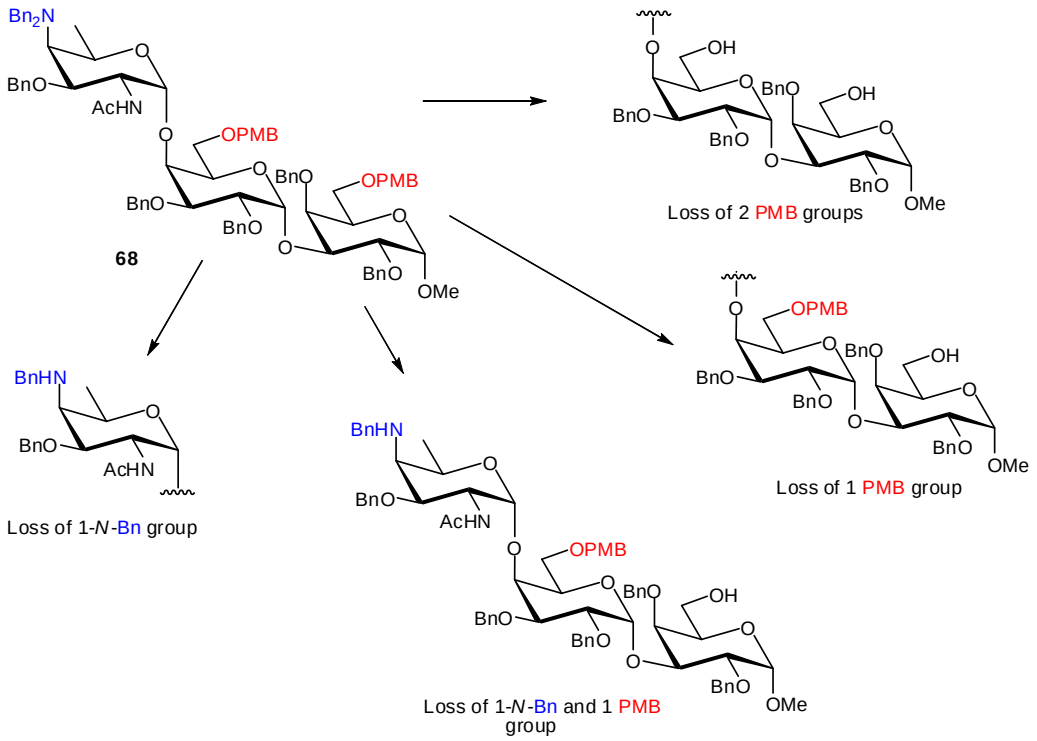
Several reductive conditions were explored to reduce the azide of **67**. The first attempt to reduce the azide functionality to amine using triphenylphosphine was unsuccessful. Then, reductive acetylation of azide **67** using thioacetic acid gave a complex mixture of products, from which acetamide derivative **68** could be isolated in only 25% yield (Scheme 35).[169] Alternatively, a one pot reduction

of this azide with hydrogen sulphide in a mixture of pyridine, water and triethylamine, followed by an acetylation of the resulting primary amine using acetic anhydride in methanol afforded the corresponding acetamide derivative **68** in 70% yield.



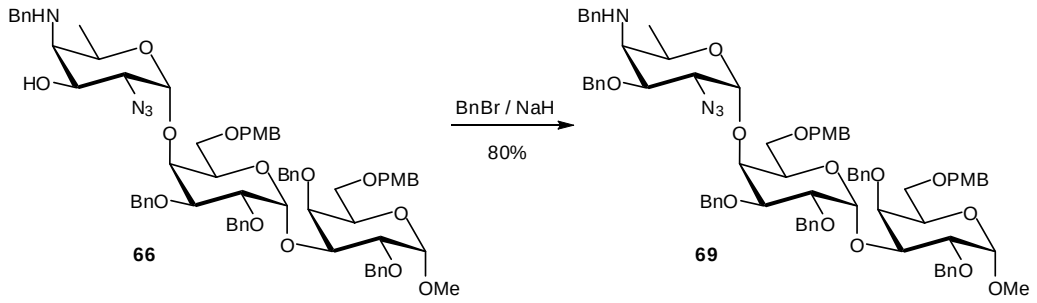

Scheme 35: *Formation of acetamide **68** from azide derivative **67***

Efforts to deprotect the PMB groups by standard oxidants, such as DDQ and CAN, were unsuccessful and resulted in a mixture of side-products. From the crude product, some of them were identified as products of the loss of one PMB group, both PMB groups, one benzyl group, or one benzyl and one PMB group (Scheme 36). It was concluded that the electron rich 4-amino benzyl protecting groups were more prone to the oxidative cleavage. We therefore sought to avoid the benzylation of the 4-amino group in **66**.



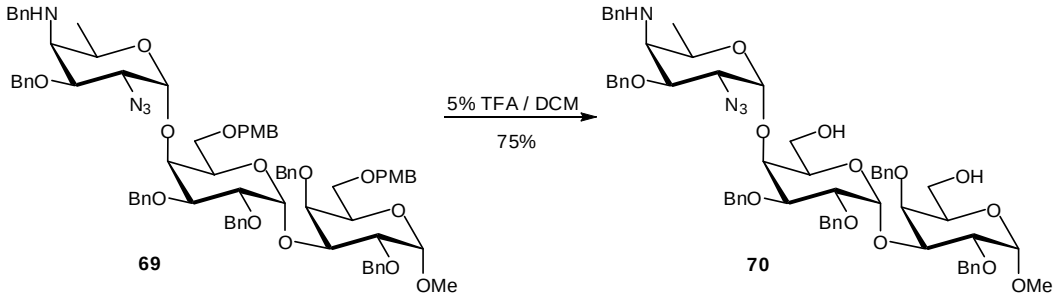

Scheme 36: *Attempted deprotection of PMB groups either by DDQ or CAN*

The regioselective benzylation of the 3-hydroxyl of **66** with the careful addition of BnBr gave the desired product **69** in 80% yield (Scheme 37).



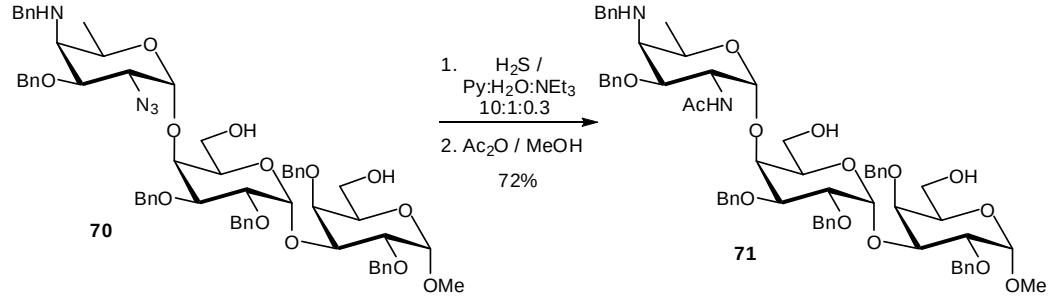

Scheme 37: *Regioselective benzylation of 3-OH*

The attempted removal of PMB groups of **69** using either DDQ or CAN gave only trace amount of the desired compound **70**. Fortunately, upon treatment of **69** with 5% TFA in DCM, the PMB groups were readily hydrolyzed without affecting the glycosidic linkages to afford **70** in excellent yield (Scheme 39).




Scheme 38: *Acidic cleavage of PMB groups*

Reduction of azide **70** by the aforementioned conditions followed by regioselective acetylation of the primary amine using acetic anhydride in methanol produced the acetamide **71** in 72% yield over two steps (Scheme 38).




Scheme 39: *Synthesis of acetamide derivative **71***

With the 6,6' dialcohol in hand, we next examined a number of oxidation conditions. Conventional oxidation conditions, including PDC or PCC, for producing a carboxylic acid from the corresponding alcohol gave a complex mixture of products. A survey of several available methods from the literature showed that, in recent decades, catalytic oxidation of carbohydrates using the TEMPO–NaOCl–KBr system has become one of the most promising procedures to convert oligosaccharides to uronic acids.[170] This method is considered to be mild and economical. Thus, we chose the TEMPO-mediated oxidation to convert the 6 and 6' hydroxyls of the trisaccharide into the corresponding carboxylic acids.

In the TEMPO mediated oxidation reaction, the oxidation reaction can be stopped either at the aldehyde or carboxylic acid stage by controlling the amount of co-oxidant added and choosing the reaction medium, either anhydrous or aqueous (Figure 42).[171] The active species in this system is the corresponding nitrosonium cation, the oxidized form of TEMPO, which is continuously regenerated from the nitroxyl radical by the co-oxidant. There have been several co-oxidants reported including *m*-chloroperbenzoic acid, copper salts, sodium bromite and sodium hypochlorite. The latter appeared to be the most efficient. The mechanistic detail is shown in Figure 43. First, the TEMPO radical is oxidized by NaOCl to the corresponding *N*-oxoammonium ion, which rapidly oxidizes the primary alcohol to the aldehyde. In aqueous medium, the aldehyde intermediate is hydrated to form a vicinal diol, which is subsequently oxidized by TEMPO–NaOCl to produce the corresponding carboxylic acid.[171]

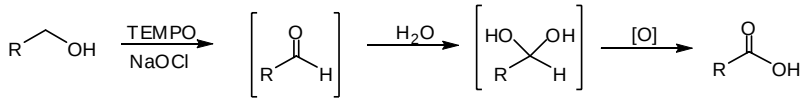



Figure 42: *Proposed intermediate for the TEMPO-mediated oxidation*[172]

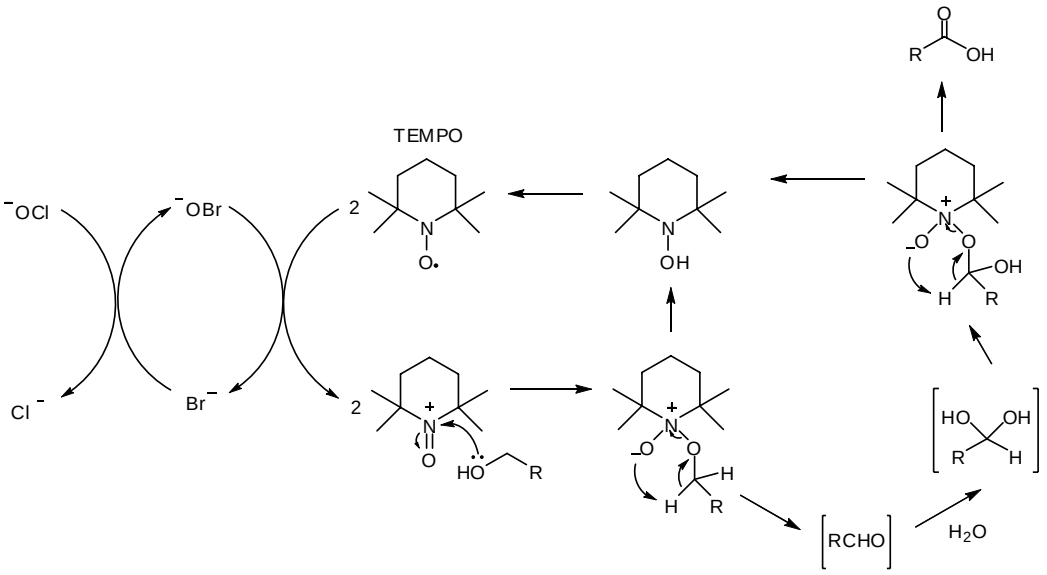
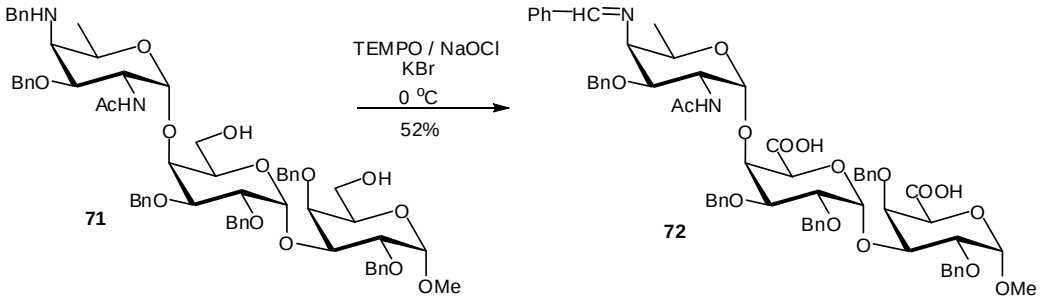


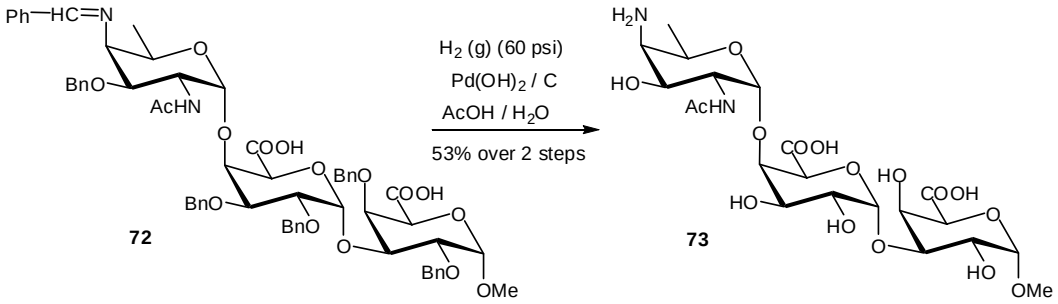

Figure 43: *The detailed mechanism of the TEMPO catalyzed alcohol oxidation*[171]

The oxidation of the primary hydroxyls of **71** to the corresponding diacid using TEMPO–NaOCl–KBr gave a very polar compound, which could not be isolated after water work up (Scheme 40). Instead, purification on Sephadex G-10 using water as eluent afforded the imine derivative of uronic acid **72** in 52% yield, which was confirmed by $^{1}$H NMR spectroscopy and mass spectrometry. Oxidation of the 4-NBn group could not be avoided. However, formation of imine does not constitute irreversible loss of amine functionality. This reaction was originally conducted on only a 10 mg scale. However, when the reaction was repeated on 15 mg scale the next step was carried out without isolation of intermediate **72**.




Scheme 40: *Oxidation of **71** using TEMPO as an oxidant*

Finally, global deprotection of **72** was successfully carried out by catalytic hydrogenolysis over $Pd(OH)_2$ / C in AcOH and MeOH at 60 psi for two days (Scheme 41). The purification was performed on a Bio-gel P2 column (1.6 x 35 cm) using $NH_4OH$ (1 mL / L) in water as eluent. A subsequent lyophilization of the purified fractions afforded the final trisaccharide **73** as a white solid.




Scheme 41: *Global deprotection of trisaccharide **72***

# Chapter Four

# Conclusions and Future Directions

## 4.1 Conclusions

The work presented in this thesis describes a novel approach to overcome the synthetic challenges in assembling the repeating unit of the type 1 capsular polysaccharide from *Streptococcus pneumoniae* (Sp1). The most intriguing feature of zwitterionic polysaccharides (ZPSs), the T-cell dependent immune response was discovered by Kasper and coworkers.[27,29] In order to understand the precise physico-chemical and structural characteristics of the interactions between ZPSs and T-cells, it is of interest to synthesize their appropriate fragments. Among immunologically active ZPSs, the *S. pneumoniae* type 1 was chosen as the target for chemical synthesis in this work.

A novel method towards the synthesis of a rare amino sugar, the orthogonally protected 2,4-diamino-2,4,6-trideoxygalatose residue was developed. A key installation of the C-4 amino functionality was achieved through the intramolecular cyclization of a carbamate moiety. It was demonstrated that the oxazolidinone trichloroacetimidate derivative **24** serves as an effective glycosyl donor for construction of oligosaccharides with high α-selectivity. Furthermore, the oxazolidinone functionality was found to be compatible with most of the protecting group manipulations. The deprotection of the oxazolidinone ring, however, required not only basic conditions but also elevated temperature.

All of the glycosidic linkages were synthesized by employing chemical methods. The assembly of the trisaccharide was successfully carried out from the appropriate monomers. Thioglycoside and trichloroacetimidate donors were the donors of choice. It appeared that the nucleophilicity of the disaccharide acceptor's 4-OH towards the activated 6-deoxygalactose donors was relatively low. This could be attributed to steric hindrance at O-4' caused by the bulky TBDPS protecting group at O-6'. An alternative protecting group strategy, having the less sterically demanding PMB group at O-6' of the disaccharide was designed to obtain the target trisaccharide in good yield. In addition, it was found that installation of the uronic acid moieties could be best performed at the trisaccharide stage by TEMPO-mediated oxidation of the C-6 and C-6' hydroxyls to their corresponding carboxylic acids. However, it was found that under the aforementioned oxidative conditions the -NBn group underwent partial oxidation to the corresponding benzylidene imine, which was cleaved by hydrogenolysis to afford the desired 4-amino derivative.

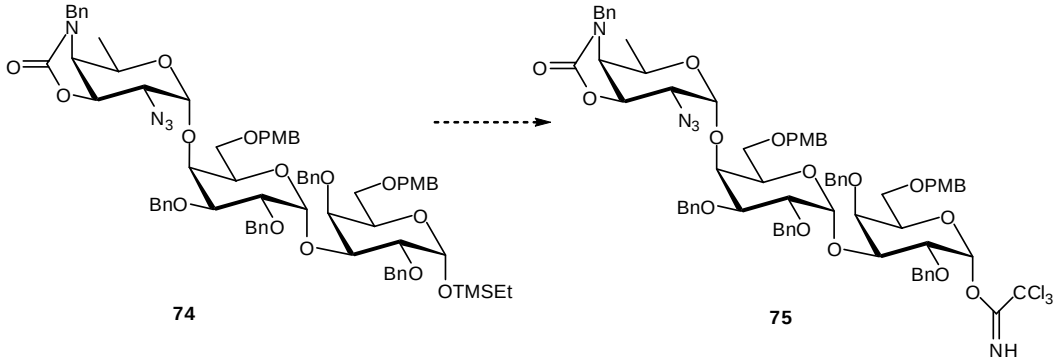
Trisaccharide **73** was tested for its ability to stimulate interleukin 10 (IL-10) and Interferon-gamma (IFN-γ) by our collaborators Kasper and coworkers at Harvard Medical School. Each of these responses is representative of the T-cell activating ability of zwitterionic antigens.[24] Unfortunately the trisaccharide was not active. Published data describing the molecular weight required for activity[68] that appeared after our work was initiated is consistant with this result. Based on this data, it appears that at least six repeating units are required for activity.

Since synthesis of oligomers of this size represents a prohibitive synthetic effort alternative approaches for the construction of oligomeric forms of **73** are presented below.

## 4.2 Future Directions

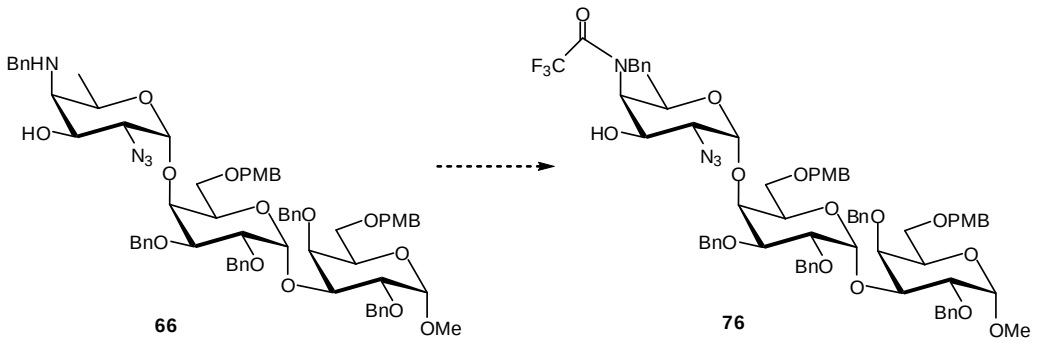
Recent reports have provided compelling evidence that PS A1 fragments of *B. fragilis* should carry at least three repeating units in order to elicit an immune response similar to that of native polysaccharide.[26,68] This observation suggests multipoint interaction between PS A1 with T-cell receptors. Therefore, future synthetic efforts should be focused on providing oligovalent forms of the trisaccharide repeat unit. Two approaches can be envisioned.

First, block assembly of the higher oligomer can be readily accomplished using the suitably protected trisaccharides **75** and **76** by similar pathways to those described in this work. Owing to an orthogonal protection group pattern, the versatile derivative **74** can serve as a precursor to the donor **75** in Scheme 42. Removal of the anomeric TMSEt group would give access to the glycosyl donor, preferably trichloroacetimidate.



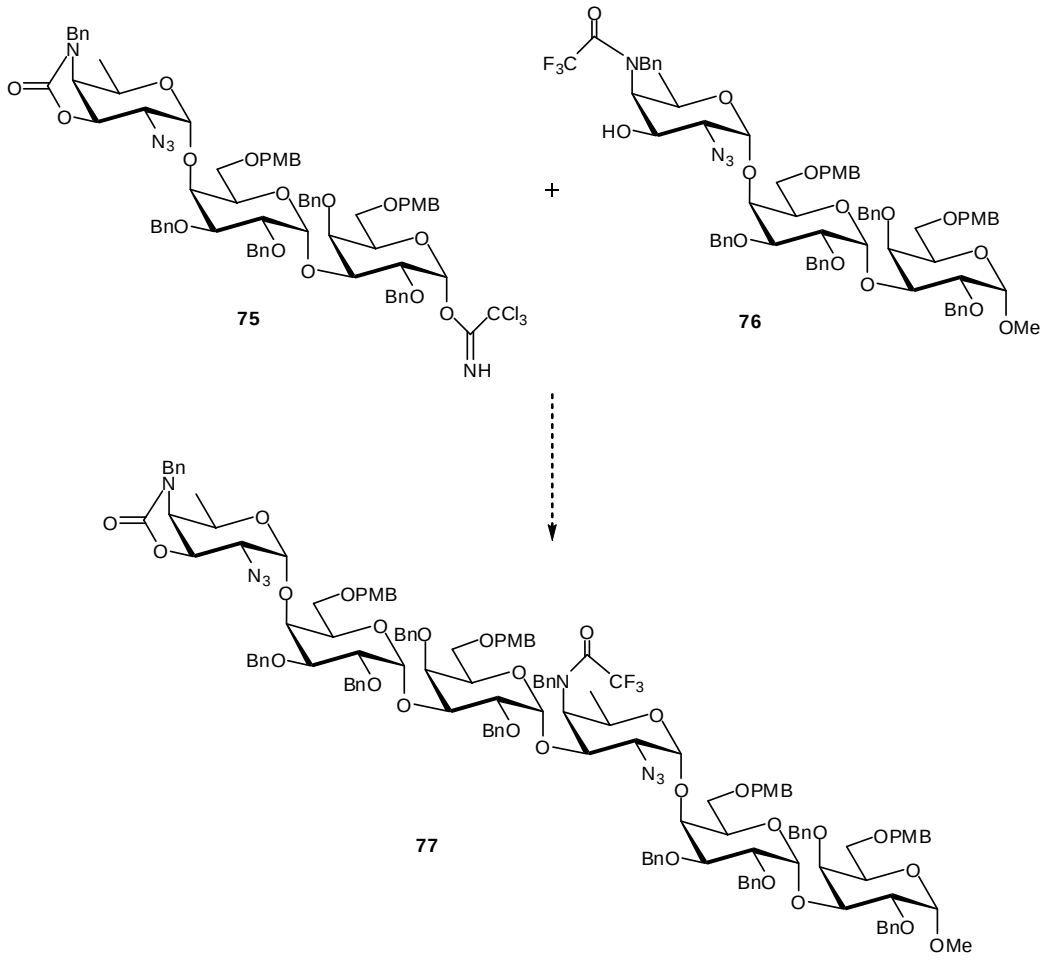

Scheme 42: *Formation of the trichloroacetimidate donor*

The amino functionality of the previously synthesized trisaccharide **66** could then be protected as a TFA amide (Scheme 43) in order to avoid undesirable glycosylation through nitrogen, which will afford the suitable acceptor **76** for the coupling.



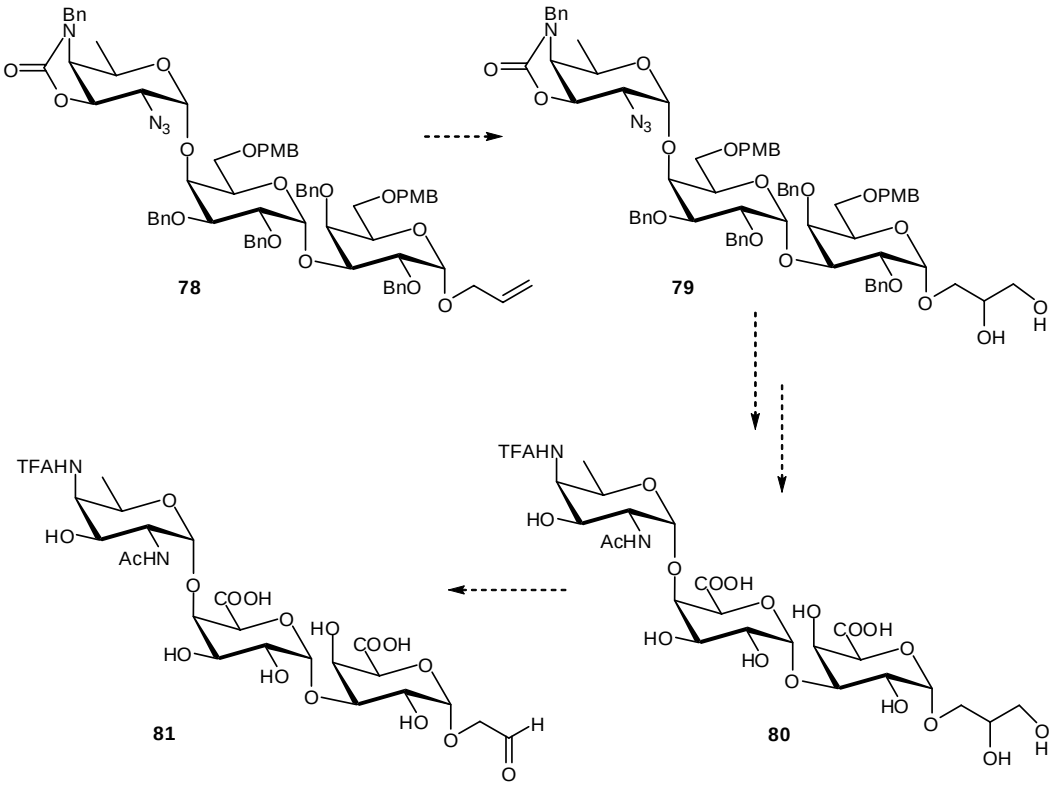

Scheme 43: *Synthesis of the glycosyl acceptor from the intermediate **66***

Subsequent coupling of the acceptor and the donor will provide the fully protected hexasaccharide (Scheme 44) and iteratively, higher oligomers. The deprotection strategy for the trisaccharide should apply to the hexasaccharide accordingly.



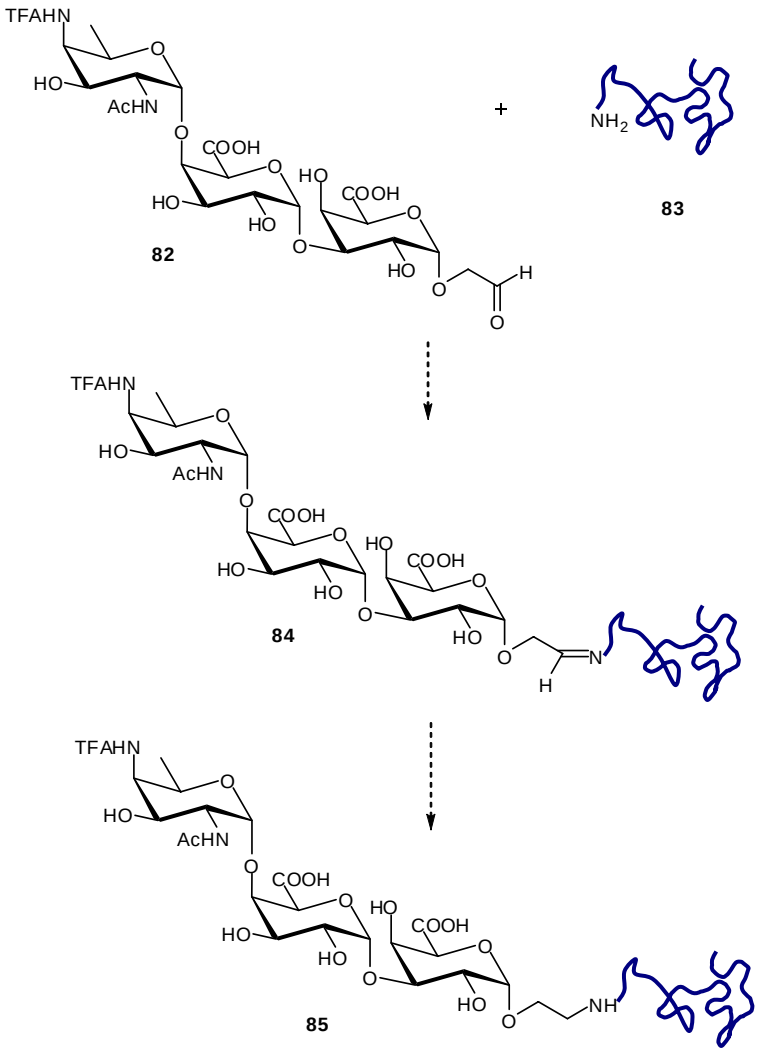

Scheme 44: *Glycosylation to afford hexasaccharide*

An alternative strategy can be conjugation of the trisaccharide to a multimeric scaffold. For this purpose, the following general scheme can be proposed (Scheme 45). A fully protected trisaccharide **78** having an allyl protecting group at the anomeric centre should be a suitable target for this strategy. The aldehyde functionality could be introduced via oxidation of acyclic vicinal diols by $NaIO_4$. It is known that acyclic polyols are more rapidly oxidized by $NaIO_4$ than secondary alcohols of hexapyranosides. Therefore oxidation of trisaccharide **80** can be avoided.



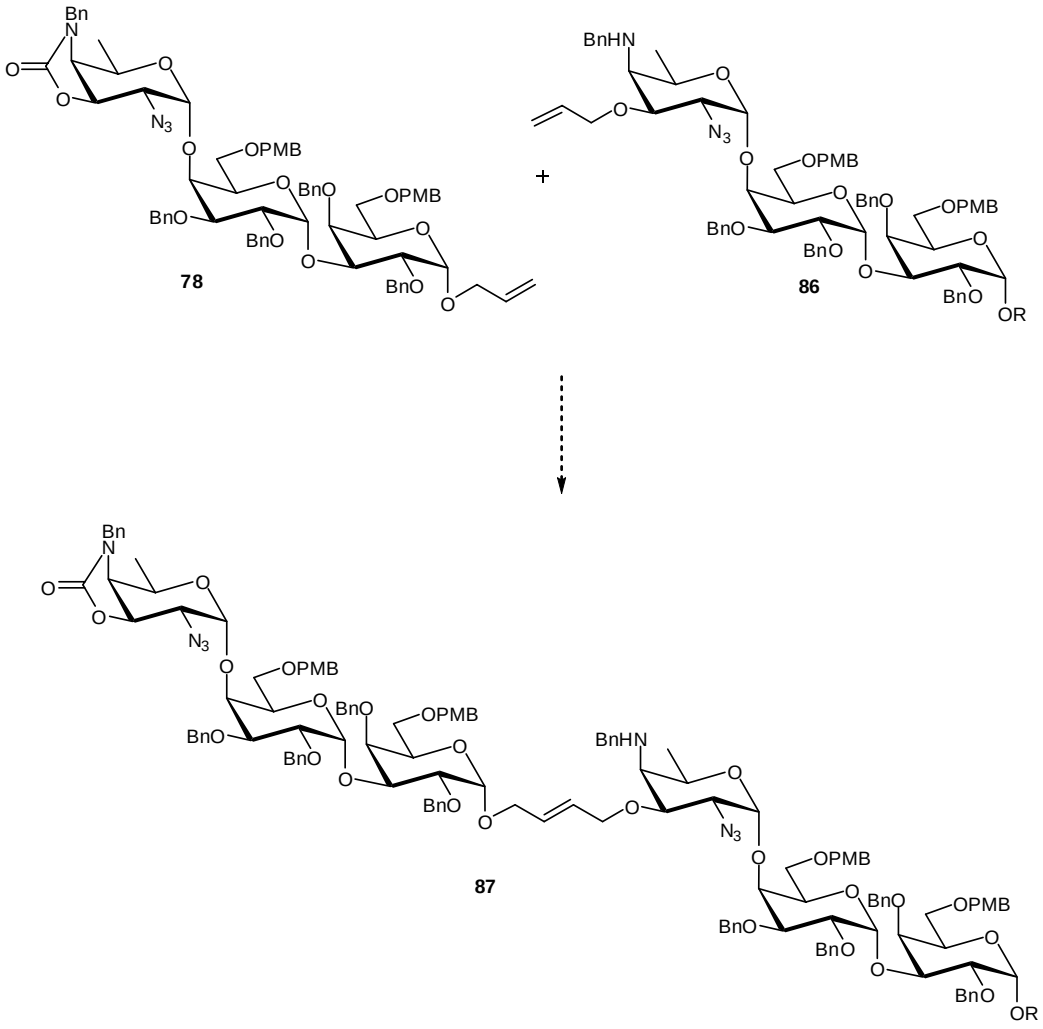

Scheme 45: *Synthesis of the precursor for the conjugation*

The aldehyde group can be conjugated to a suitable polymer **83** having primary or secondary amino groups, which can then undergo reductive amination to give the target glycoconjugates **85** (Scheme 46). It should be noted that it is necessary to keep the primary amine of trisaccharide **82** protected, preferably as a TFA amide group that is orthogonal to the required transformations.




Scheme 46: *Synthesis of the glycoconjugate* ***85***

This approach would provide comb like oligomers. To obtain linear oligomers one might consider a cross metathesis approach via a derivative of type **78** with an analogous derivative bearing a suitably active alkene containing substituent at O-3" of the diamino hexose (Scheme 47).




Scheme 47: *Synthesis of compound **87***

# Chapter Five

# Experimental Section

## General methods:

### Chemical Synthesis and purification:

All chemical reagents were of analytical grade and used as supplied from Sigma-Aldrich without further purification unless otherwise indicated. Organic solvents used in water sensitive reactions were obtained from a solvent purification system, except DCM and MeCN, which were distilled over $CaH_2$, and MeOH, which was distilled over Mg turnings under argon atmosphere. Moisture-sensitive reactions were carried out under an atmosphere of argon using oven-dried glassware. Unless otherwise noted, all the reactions were performed at room temperature. Solvents were removed under reduced pressure below 40 $^oC$ by rotary evaporation. Molecular sieves (MS) used for glycosylation reactions were 4Å which were ground into powdered form before use. Powdered MS were stored in an oven (<170 $^oC$) and flame dried in a round bottom flask under vaccum before use. After work-up, the organic phase was dried over either anhydrous $Na_2SO_4$ or $MgSO_4$.

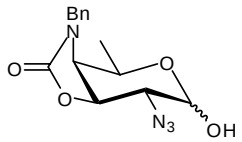
Products obtained as solids or syrups were dried under high vacuum. The progress of all reactions was monitored by analytical thin-layer chromatography (TLC) on silica gel 60-$F_{254}$ (Merck) plates. TLC plates were visualized under UV (254 nm) and / or by dipping the plates in a cerium ammonium molybdate, or in 5% $H_2SO_4$ ethanolic solution, ninhydrin, or anisaldehyde stains, followed by

heating. Column chromatography was performed on silica gel (230-400 mesh, Silicycle, Montreal) and the solvents were of reagent grade and used as supplied. After deprotection, the final trisaccharide was purified by bio-gel P2 column. Yields refer to chromatographically and spectroscopically ($^1$H NMR) homogeneous materials unless otherwise stated.

**Analytical procedures:**

$^1$H NMR spectra were recorded on Varian INOVA 500 MHz or 600 MHz spectrometers. $^{13}$C NMR spectra were recorded at 125 MHz unless otherwise stated. Chemical shifts are reported as δ values in parts per million (ppm) and coupling constants (*J*) were recorded in Hertz (Hz). Residual proton resonances $CDCl_3$ (δ 7.24), $CD_3OD$ (δ 3.31) and $C_6D_6$ (δ 7.15) were used as internal references for $^1$H NMR spectra measured in these solvents and acetone (δ 2.225) was used as an external reference for spectra measured in $D_2O$. Solvent resonances $CDCl_3$ (δ 77.0) and $CD_3OD$ (δ 49.0) were used as internal references for $^{13}$C NMR spectra measured in these solvents and acetone (δ 31.07) was used as an external reference for spectra measured in $D_2O$. Data for $^1$H NMR were reported as follows: chemical shift, multiplicity (app = apparent, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet), integration, and coupling constants. All $^{13}$C NMR spectra are recorded with complete proton decoupling. $^1$H NMR and $^{13}$C peak assignments including the identification of the residue connection were carried out with the assistance of COSY, HMBC, HMQC and TOCSY experiments where required. Electrospray ionization (ESI) under positive or negative mode was recorded on a Micromass Zabspec Hybrid Sector-TOF by

the mass spectrometry laboratory service at the University of Alberta. Optical rotations were measured by Perkin-Elmer model 241 polarimeter at 22 ± 2 °C and reported in units of $10^{-1}$ deg.mL.$g^{-1}$.$dm^{-1}$. Optical rotations and elemental analysis were performed by the analytical services facility in this department.

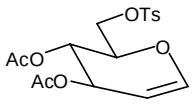



**1**

**2-Azido-4-benzylamino-4-*N*-,3-*O*-carbonyl-2,4,6-trideoxy-α/β-D-galactopyranose (1)**

A mixture of cerium (IV) ammonium nitrate (2.35 g, 4.29 mmol, 3 eq) and $NaN_3$ (0.14 g, 2.14 mmol, 1.5 eq) was added to a solution of the oxazolidinone derivative of 6-deoxy-D-galactal (0.35 g, 1.43 mmol) **21** in dry MeCN (7 mL) at -15 °C under argon. The resulting suspension was vigorously stirred at this temperature until TLC analysis (hexanes-EtOAc, 3:2) indicated complete consumption of starting material. After the reaction was complete, it was diluted with EtOAc and concentrated. The residue was redissolved in $Et_2O$, filtered through celite and concentrated under reduced pressure. The resulting residue was dissolved in MeCN (5 mL) and $H_2O$ (2 mL) was added to this solution. The reaction mixture was stirred at room temperature for 16 hours and then MeCN was evaporated. The residue was dissolved in EtOAc (7 mL) was washed with an equal volume of water, brine and then the solution was dried over $Na_2SO_4$, filtered and concentrated. Column chromatography on silica gel using hexanes and EtOAc (3:2) afforded the azido derivative **1** (α/β 2:1, 0.33 g, 78% over two

steps) as a mixture of anomers. $^{1}$H NMR (600 MHz, $CDCl_3$) δ 7.35 (m, 5H, ArH), 7.27 – 7.21 (m, 5H, ArH), α anomer: 5.41 (app t, $J_{1,2}$ = 4.6 Hz, $J_{1,OH}$ = 4.6 Hz, H-1), 5.04 (d, 1H, $J_{gem}$ = 15.6 Hz, PhC$H_2$), 4.63 (dd, 1H, $J_{2,3}$ = 4.7 Hz, $J_{3,4}$ = 8.4 Hz, H-3), 4.32 (dq, 1H, $J_{4,5}$ = 2.4 Hz, $J_{5,6}$ = 6.2 Hz, H-5), 4.18 (d, 1H, $J_{gem}$ = 15.6 Hz, PhC$H_2$), 4.11 (app t, 1H, $J_{1,2}$ = 4.5 Hz, $J_{2,3}$ = 4.5 Hz, H-2), 3.55 (dd, 1H, $J_{3,4}$ = 8.3 Hz, $J_{4,5}$ = 2.5 Hz, H-4), 3.20 (d, 1H, $J$ = 4.9 Hz, 1-OH), 1.30 (d, 3H, $J_{5,6}$ = 6.7 Hz, H-6 C$H_3$). β anomer: 4.97 (d, 1H, $J_{gem}$ = 15.9 Hz, PhC$H_2$), 4.73 (app t, $J_{1,2}$ = 6.5 Hz, $J_{1,OH}$ = 6.5 Hz, H-1), 4.36 (app t, 1H, $J_{2,3}$ = 7.5 Hz, $J_{3,4}$ = 7.5 Hz, H-3), 4.32 (d, 1H, $J_{gem}$ = 16.0 Hz, PhC$H_2$), 4.11 (dq, 1H, $J_{4,5}$ = 2.5 Hz, $J_{5,6}$ = 7.2 Hz, H-5), 3.77 – 3.63 (m, 2H, H-2, H-4), 3.44 (d, 1H, $J$ = 6.4 Hz, 1-OH), 1.39 (d, 3H, $J_{5,6}$ = 7.4 Hz, H-6 C$H_3$). $^{13}$C NMR (125 MHz, $CDCl_3$, from HMQC) δ 129.0, 129.0, 128.2, 128.0, 127.8, 127.7, 127.6, α anomer: 158.6, 135.4, 90.3, 72.3, 65.0, 57.9, 55.1, 49.3, 17.6. β anomer: 159.5, 135.1, 95.7, 74.8, 69.1, 64.5, 56.8, 49.1, 17.7. ESI HRMS calcd. for $C_{14}H_{16}O_4N_4Na$ (M+Na): 327.1064, found 327.1062.




**8**

**3,4-Di-*O*-acetyl-6-*O*-*p*-toluenesulfonyl-D-glucal (8)**[90]

To a stirred solution of D-glucal **6** (10.50 g, 71.89 mmol) in pyridine (80 mL) at 0 $^{o}$C was added TsCl (15.08 g, 79.08 mmol, 1.1 eq), and the reaction mixture was allowed to reach room temperature. After stirring for 4 hours at ambient temperature, the reaction mixture was cooled again to 0 $^{o}$C and acetic anhydride (75 mL) was added. The reaction was allowed to warm to room

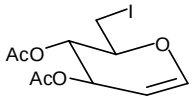
temperature and stirred at this temperature for 5 hours. When the reaction was complete, the mixture was cooled to 0 $^{o}$C, quenched with MeOH and coevaporated with toluene. The residue was dissolved in DCM (150 mL) was washed with an equal volume of water and brine, and the organic phase was dried over $Na_2SO_4$, filtered and concentrated. Column chromatography on silica gel using hexanes and EtOAc afforded acetylated tosylate **8** (21.26 g, 77% over two steps) as a white solid. $[\alpha]_D$ +11.4 (*c* 1.4, $CH_2Cl_2$); $^1$H NMR (600 MHz, $CDCl_3$) δ 7.80 (d, 2H, *J* = 8.2 Hz, ArH), 7.36 (d, 2H, *J* = 8.2 Hz, ArH), 6.36 (d, 1H, $J_{1,2}$ = 6.2 Hz, H-1), 5.28 (dd, 1H, $J_{2,3}$ = 4.4 Hz, $J_{3,4}$ = 4.3 Hz, H-3), 5.13 (dd, 1H, $J_{3,4}$ = 4.3 Hz, $J_{4,5}$ = 5.6 Hz, H-4), 4.82 (dd, 1H, $J_{2,3}$ = 3.5 Hz, $J_{1,2}$ = 6.2 Hz, H-2), 4.31 – 4.19 (m, 3H, H-5, H-6), 2.46 (s, 3H, C*H*$_3$OPh), 2.04 (s, 3H, COC*H*$_3$), 2.03 (s, 3H, COC*H*$_3$). $^{13}$C NMR (125 MHz, $CDCl_3$) δ 170.2, 169.4, 145.3, 145.1, 132.6, 129.9, 128.0, 98.9, 73.2, 67.0, 66.6, 66.4, 21.6, 20.9, 20.7. ESI HRMS calcd. for $C_{17}H_{20}O_8SNa$ (M+Na): 407.0771, found 407.0770. Anal. Calcd. for $C_{17}H_{20}O_8S$: C, 53.12; H, 5.24; S, 8.34. Found: C, 52.75; H, 5.13; S, 8.08.


**9**

**6-Deoxy-D-glucal (9)**[92,94]

To a stirred mixture of 3,4-di-*O*-acetyl-6-deoxy-D-glucal **13** (5.6 g, 26.16 mmol) in dry MeOH (50 mL) was added 1.5 M NaOMe (0.60 mL) dropwise over 10 minutes. The reaction mixture was vigorously stirred for 2 hours at room temperature. The solution was neutralized with Amberlite resin IR-120 ($H^+$),

filtered and the filtrate was concentrated under reduced pressure. The residue was applied to a silica gel column and eluted with a stepped gradient of hexanes and EtOAc (3:1 → 13:7) to afford **9** (3.06 g, 90%) as a white solid. $[\alpha]_D$ -24.2 (*c* 0.1, $CH_2Cl_2$); $^1$H NMR (600 MHz, $CDCl_3$) δ 6.32 (dd, 1H, $J_{1,2}$ = 6.0 Hz, $J_{1,3}$ = 1.7 Hz, H-1), 4.72 (dd, 1H, $J_{1,2}$ = 6.1 Hz, $J_{2,3}$ = 2.1 Hz, H-2), 4.21 (app t, 1H, *J* = 6.6 Hz, *J* = 6.0 Hz, H-3), 3.88 (dq, 1H, $J_{4,5}$ = 9.6 Hz, $J_{5,6}$ = 6.4 Hz, H-5), 3.46 – 3.39 (ddd, 1H, $J_{3,4}$ = 7.4 Hz, $J_{4,5}$ = 10.2 Hz, $J_{4,4OH}$ = 3.5 Hz, H-4), 2.34 (d, 1H, *J* = 3.7 Hz, 4-OH), 1.82 (d, 1H, *J* = 5.7 Hz, 3-OH), 1.40 (d, 3H, $J_{5,6}$ = 6.5 Hz, H-6 C$H_3$). $^{13}$C NMR (125 MHz, $CDCl_3$) δ 144.5, 102.5, 75.1, 74.3, 70.1, 17.0. ESI HRMS calcd. for $C_6H_{10}O_3Na$ (M+Na): 153.0522, found 153.0520. Anal. Calcd. for $C_6H_{10}O_3$: C, 55.37; H, 7.74. Found: C, 55.28; H, 7.75.

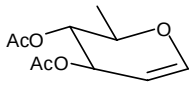



**12**

**3,4-Di-*O*-acetyl-6-deoxy-6-iodo-D-glucal (12)**[92]

A solution of 3,4-di-*O*-acetyl-6-*O*-*p*-toluenesulfonyl-D-glucal **8** (7.33 g, 19.08 mmol) in dry THF (40 mL) was treated with LiI (12.76 g, 95.34 mmol, 5 eq). The reaction mixture was protected from light and heated at reflux for 3 hours, then cooled to room temperature and quenched with 10% $Na_2S_2O_3$. The reaction mixture was diluted with EtOAc (40 mL) and washed with aqueous 10% $Na_2S_2O_3$, water and brine, dried over $MgSO_4$, filtered and the filtrate concentrated. The crude product was used in the next step without purification, due to its poor stability. However, a small amount of crude **12** was purified by

column chromatography on silica gel with 3% EtOAc in toluene. $[\alpha]_D$ -97.9 (*c* 1.7, $CH_2Cl_2$); $^1H$ NMR (498 MHz, $CDCl_3$) δ 6.50 (d, 1H, $J_{1,2}$ = 6.2 Hz, $J_{1,3}$ = 1.1 Hz, H-1), 5.35 – 5.29 (m, 1H, H-3), 5.26 (dd, 1H, $J_{3,4}$ = 5.4 Hz, $J_{4,5}$ = 6.4 Hz, H-4), 4.87 (dd, 1H, $J_{1,2}$ = 6.2 Hz, $J_{2,3}$ = 3.5 Hz, H-2), 4.10 (ddd, 1H, $J_{4,5}$ = 6.3 Hz, $J_{5,6a}$ = 5.2 Hz, $J_{5,6b}$ = 7.4 Hz, H-5), 3.44 (dd, 1H, $J_{5,6a}$ = 5.2 Hz, $J_{6a,6b}$ = 10.8 Hz, H-6a), 3.36 (dd, 1H, $J_{5,6b}$ = 7.4 Hz, $J_{6a,6b}$ = 11.1 Hz, H-6b), 2.11 (s, 3H, COC$H_3$), 2.07 (s, 3H, COC$H_3$). $^{13}C$ NMR (125 MHz, $CDCl_3$) δ 170.2,169.3, 145.1, 98.9, 75.2, 69.7, 66.8, 20.9, 20.8, 1.6. ESI HRMS calcd. for $C_{10}H_{13}O_5INa$ (M+Na): 362.9700, found 362.9699.

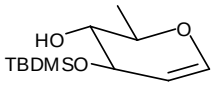



**13**

**3,4-Di-*O*-acetyl-6-deoxy-D-glucal (13)**[92]

The crude iodide **12** was dissolved in dry toluene (5.84 g, 17.18 mmol). To the resulting solution, a catalytic amount of AIBN and $Bu_3SnH$ (5.50 mL, 18.90 mmol, 1.1 eq) were added. The reaction mixture was heated at reflux for 6 hours. Then the volume of the solution was reduced and loaded on the silica gel column without any work up. Tin impurities were first eluted with hexanes. The 3,4-di-*O*-acetyl-6-deoxy-D-glucal **13** (3.12 g, 85% over two steps) was eluted with 3% EtOAc in toluene and concentrated to a colourless syrup.[173] $[\alpha]_D$ -56.0 (*c* 0.4, $CH_2Cl_2$); $^1H$ NMR (600 MHz, $CDCl_3$) δ 6.43 (dd, 1H, $J_{1,2}$ = 6.3 Hz, $J_{1,3}$ = 1.5 Hz, H-1), 5.34 (ddd, 1H, $J_{1,3}$ = 1.4 Hz, $J_{2,3}$ = 3.0 Hz, $J_{3,4}$ = 6.1 Hz, H-3), 5.03 (dd, 1H, $J_{3,4}$ = 6.0 Hz, $J_{4,5}$ = 8.1 Hz, H-4), 4.78 (dd, 1H, $J_{1,2}$ = 6.2 Hz, $J_{2,3}$ = 3.0 Hz, H-

2), 4.11 (dq, 1H, $J_{4,5}$ = 8.0 Hz, $J_{5,6}$ = 6.6 Hz, H-5), 2.09 (s, 3H, COC$H_3$), 2.03 (s, 3H, COC$H_3$), 1.31 (d, 3H, $J_{5,6}$ = 6.6 Hz, H-6 C$H_3$). ESI HRMS calcd. for $C_{10}H_{14}O_5Na$ (M+Na): 237.0733, found 237.0733.

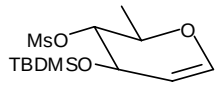



**14**

**3-*O*-(*tert*-Butyldimethylsilyl)-6-deoxy-D-glucal (14)**[96]

To a solution of 6-deoxy-D-glucal **9** (2.10 g, 16.15 mmol) in dry DMF (12 mL) was added TBDMSCl (4.88 mL, 17.76 mmol, 1.1 eq) dropwise in the presence of imidazole (1.65 g**,** 24.22 mmol, 1.5 eq) at 0 $^oC$. The resulting mixture was stirred for 6 hours at room temperature. The reaction mixture was cooled to 0 $^oC$ and quenched with methanol then solvent was removed by evaporation under reduced pressure. The residue was dissolved in DCM (25 mL) and washed with an equal volume of water and brine. The organic extract was dried over $Na_2SO_4$, filtered and concentrated. Column chromatography on silica gel using hexanes and EtOAc (10:1) afforded mono silyl derivative **14** (3.55 g, 90%) as a syrup. $^1H$ NMR (600 MHz, $CDCl_3$) δ 6.26 (dd, 1H, $J_{1,2}$ = 6.0 Hz, $J_{1,3}$ = 1.1 Hz, H-1), 4.63 (ddd, 1H, $J_{1,2}$ = 6.1 Hz, $J_{2,3}$ = 2.2 Hz, $J_{2,4}$ = 1.1 Hz, H-2), 4.25 – 4.19 (m, 1H, H-3), 3.91 (dq, $J_{4,5}$ = 8.9 Hz, $J_{5,6}$ = 6.5 Hz, H-5), 3.47 (app t, 1H, $J_{3,4}$ = 7.8 Hz, $J_{4,5}$ = 7.9 Hz, H-4), 1.39 (d, 3H, $J_{5,6}$ = 6.5 Hz, H-6 C$H_3$), 0.92 (s, 9H, C(C$H_3$)$_3$), 0.13 (s, 6H, SiC$H_3$). $^{13}C$ NMR (125 MHz, $CDCl_3$) δ 143.6, 103.4, 74.9, 74.3, 70.5, 25.8, 18.1, 17.2, -4.3, -4.5. ESI HRMS calcd. for $C_{12}H_{24}O_3SiNa$ (M+Na): 267.1387,

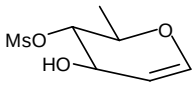
found 267.1385. Anal. Calcd. for $C_{12}H_{24}O_3Si$: C, 58.97; H, 9.90. Found: C, 55.83; H, 9.72.




**15**

**3-*O*-(*tert*-Butyldimethylsilyl)-4-*O*-mesyl-6-deoxy-D-glucal (15)**[96]

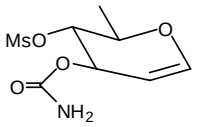
MsCl (2.25 mL, 19.66 mmol, 1.5 eq) was added to a solution of silyl derivative **14** (3.20 g, 13.11 mmol) in mixture of dry DCM and dry pyridine (4:1, v/v, 20 mL) at 0 $^oC$. The reaction mixture was warmed to room temperature while being stirred for 2.5 hours. The solution was diluted with DCM (20 mL) and washed with an equal volume of water and brine. The organic extract was dried over $Na_2SO_4$, filtered and concentrated. Chromatography on silica gel with hexanes-EtOAc (9:1) as eluent gave **15** (3.63 g, 86%). $[\alpha]_D$ -33.1 (*c* 0.9, $CH_2Cl_2$); $^1H$ NMR (600 MHz, $CDCl_3$) δ 6.33 (dd, 1H, $J_{1,2}$ = 6.2 Hz, $J_{1,3}$ = 1.2 Hz, H-1), 4.72 (dd, 1H, $J_{1,2}$ = 6.2 Hz, $J_{2,3}$ = 3.1 Hz, H-2), 4.58 (dd, 1H, $J_{3,4}$ = 5.5 Hz, $J_{4,5}$ = 7.1 Hz, H-4), 4.41 – 4.36 (m, 1H, H-3), 4.20 – 4.15 (m, 1H, H-5), 3.09 (s, 3H, C$H_3$SO$_2$), 1.45 (d, 3H, $J_{5,6}$ = 6.5 Hz, H-6 C$H_3$), 0.93 – 0.89 (s, 9H, C(C$H_3$)$_3$), 0.13 (s, 3H, SiC$H_3$), 0.13 (s, 3H, SiC$H_3$). $^{13}C$ NMR (125 MHz, $CDCl_3$) δ 143.7, 102.1, 82.1, 72.6, 66.5, 39.0, 25.8, 18.0, 16.9, -4.3, -4.4. ESI HRMS calcd. for $C_{13}H_{26}O_5SiSNa$ (M+Na): 345.1162, found 345.1162. Anal. Calcd. for $C_{13}H_{26}O_5SiS$: C, 48.42; H, 8.13; S, 9.94. Found: C, 48.63; H, 8.18; S, 9.51.




**17**

**4-*O*-Mesyl-6-deoxy-D-glucal (17)**[96]

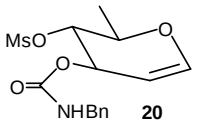
A solution of silyl ether **15** (2.70 g, 8.38 mmol) in dry THF (20 mL) was treated with TBAF (1.0 M in THF, 2.63 mL, 10.06 mmol, 1.2 eq) at 0 $^{o}$C. The reaction mixture was warmed to room temperature and stirred at this temperature for 3 hours. The solution was then diluted with EtOAc (20 mL), washed with equal volume of water and brine then dried over $Na_2SO_4$, filtered, and concentrated *in vacuo.* The residue was purified by column chromatography (hexanes-EtOAc, 6:4) to afford the title compound **17** (1.52 g, 87%) as a white solid. $[\alpha]_D$ +18.5 (*c* 0.5, $CH_2Cl_2$); $^1$H NMR (600 MHz, $CDCl_3$) δ 6.36 (d, 1H, $J_{1,2}$ = 6.9 Hz, H-1), 4.77 (dd, 1H, $J_{1,2}$ = 6.0 Hz, $J_{2,3}$ = 2.3 Hz, H-2), 4.48 (m, 2H, H-4, H-3), 3.99 (dq, 1H, $J_{4,5}$ = 10.1 Hz, $J_{5,6}$ = 6.4 Hz, H-5), 3.20 (s, 3H, C$H_3$SO$_2$), 2.43 (s, 1H, 2-OH), 1.43 (d, 3H, $J_{5,6}$ = 6.4 Hz, H-6 C$H_3$). $^{13}$C NMR (125 MHz, $CDCl_3$) δ 145.0, 102.4, 84.0, 72.5, 68.2, 38.8, 17.1. ESI HRMS calcd. for $C_7H_{12}O_5SNa$ (M+Na): 231.0298, found 231.0289. Anal. Calcd. for $C_7H_{12}O_5S$: C, 40.38; H, 5.81; S, 15.40. Found: C, 40.54; H, 5.85; S, 15.54.




**18**

**3-*O*-Carbamoyl-4-*O*-mesyl-6-deoxy-D-glucal (18)**

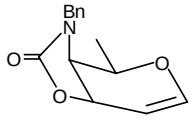
To a solution of mesylate **17** (0.04 g, 0.19 mmol) in DCM (2 mL) was added trichloroacetyl isocyanate (0.1 mL, 0.53 mmol, 2.8 eq) at 0 °C. After 10 minutes the reaction mixture was warmed to room temperature and stirring was continued at this temperature for a further 30 minutes. The mixture was cooled to 0 °C and MeOH (5.0 mL) was added, followed by $K_2CO_3$ (465.2 mg, 3.35 mmol). After 30 minutes at 0 °C the reaction mixture was stirred an additional 2.5 hours at room temperature and poured into saturated aqueous $NH_4Cl$ (5 mL). The mixture was extracted with DCM (5 mL) and the combined organic layers were washed with brine, dried over $Na_2SO_4$, filtered, and concentrated. Chromatography over silica gel using hexanes-EtOAc (3:2) as eluent gave carbamate **18** (0.043 g, 90%) as a white solid.[104] $[\alpha]_D$ -32.9 (*c* 0.2, $CH_2Cl_2$); $^1$H NMR (498 MHz, $CDCl_3$) δ 6.43 (dd, 1H, $J_{1,2}$ = 6.1 Hz, $J_{1,3}$ = 1.5 Hz, H-1), 5.41 (ddd, 1H, $J_{2,3}$ = 2.5 Hz, $J_{3,4}$ = 6.5 Hz, $J$ = 1.3 Hz, H-3), 4.82 (dd, 1H, $J_{1,2}$ = 6.1 Hz, $J_{2,3}$ = 2.8 Hz, H-2), 4.71 (dd, 1H, $J_{3,4}$ = 6.6 Hz, $J_{4,5}$ = 8.6 Hz, H-4), 4.15 (dq, 1H, $J_{4,5}$ = 8.7 Hz, $J_{5,6}$ = 6.6 Hz, H-5), 3.10 (s, 3H, C*H*$_3$SO$_2$), 1.45 (d, 3H, $J_{5,6}$ = 6.6 Hz, H-6 C*H*$_3$). $^{13}$C NMR (125 MHz, $CDCl_3$) δ 155.6, 146.1, 98.8, 79.2, 72.8, 69.5, 38.9, 16.9. ESI HRMS calcd. for $C_8H_{13}O_6NSNa$ (M+Na): 274.0356, found 274.0354.



**20**

**3-*O*-(*N*-Benzyl)-carbamoyl-4-*O*-mesyl-6-deoxy-D-glucal (20)**

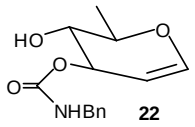
A solution of mesylate **17** (1.05 g, 5.05 mmol) in DCM (12 mL) was treated with benzylisocyanate (1.00 mL, 7.57 mmol, 1.5 eq) in the presence of TEA (1.3 mL, 12.57 mmol, 2.5 eq) at room temperature for 16 hours. The solvent was concentrated under reduced pressure. The residue was subjected to silica gel column chromatography using hexanes-EtOAc (3:1) as the eluent to obtain the carbamate **20** as a white crystalline solid (1.48 g, 90%). $[\alpha]_D$ +66.9 (*c* 0.3, $CH_2Cl_2$); $^1$H NMR (498 MHz, $CDCl_3$) δ 7.39 – 7.24 (m, 5H, ArH), 6.41 (dd, 1H, $J_{1,2}$ = 6.0 Hz, $J_{2,3}$ = 1.3 Hz, H-1), 5.43 (ddd, 1H, *J* = 6.5 Hz, *J* = 2.9 Hz, *J* = 1.6 Hz, H-3), 5.23 (s, 1H, OCON*H*Bn), 4.81 (dd, 1H, $J_{1,2}$ = 6.1 Hz, $J_{2,3}$ = 2.9 Hz, H-2), 4.68 (dd, 1H, $J_{3,4}$ = 6.7 Hz, $J_{4,5}$ = 8.4 Hz, H-4), 4.36 (d, 2H, *J* = 6.0 Hz, PhC$H_2$), 4.12 (q, 1H, $J_{4,5}$ = 10.1 Hz, $J_{5,6}$ = 6.4 Hz, H-5), 3.01 (s, 3H, C$H_3SO_2$), 1.41 (d, 3H, $J_{5,6}$ = 6.4 Hz, H-6 C$H_3$). $^{13}$C NMR (125 MHz, $CDCl_3$) δ 155.6, 146.0, 138.0, 128.7, 127.7, 127.5, 99.0, 79.3, 72.7, 69.2, 45.2, 38.8, 16.8. ESI HRMS calcd. for $C_{15}H_{19}O_6NSNa$ (M+Na): 364.0825, found 364.0824.




21

**4-Benzylamino-4-*N*-,3-*O*-carbonyl-4,6-dideoxy-D-galactal (21)**

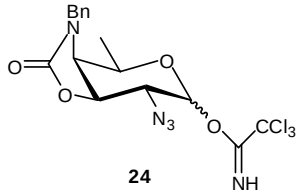
The carbamate derivative **20** (0.34 g, 1.00 mmol) was dissolved in dry THF (15 mL) and treated with *t*-BuOK (0.13 g, 0.12 mmol, 1.2 eq) in portions. The reaction mixture was stirred at room temperature for 20 hours. The reaction mixture was quenched with saturated aqueous $NH_4Cl$, then it was diluted with EtOAc (10 mL). The organic phase was washed with water (25 mL) and brine (25 mL), dried over $MgSO_4$, filtered and concentrated. Chromatography on silica gel (hexanes-EtOAc, 4:1) yielded oxazolidinone derivative **21** (0.23 g, 95%) as a white solid. $[\alpha]_D$ +69.0 (*c* 0.2, $CH_2Cl_2$); $^1$H NMR (498 MHz, $CDCl_3$) δ 7.56 – 7.24 (m, 5H, ArH), 6.56 (d, 1H, $J_{1,2}$ = 6.2 Hz, H-1), 5.03 (dd, 1H, $J_{1,2}$ = 6.2 Hz, $J_{2,3}$ = 3.7 Hz, H-2), 4.90 (d, 1H, $J_{gem}$ = 15.8 Hz, PhC$H_2$), 4.87 (dd, 1H, $J_{2,3}$ = 3.7 Hz, $J_{3,4}$ = 7.8 Hz, H-3), 4.22 (d, 1H, $J_{gem}$ = 15.8 Hz, PhC$H_2$), 4.15 (dq, 1H, $J_{4,5}$ = 3.5 Hz, $J_{5,6}$ = 6.9 Hz, H-5), 3.82 (dd, 1H, $J_{3,4}$ = 7.9 Hz, $J_{4,5}$ = 3.5 Hz, H-4), 1.35 (d, 3H, $J_{5,6}$ = 6.4 Hz, H-6 C$H_3$). $^{13}$C NMR (125 MHz, $CDCl_3$) δ 159.1, 147.6, 135.6, 128.9, 128.0, 127.9, 99.0, 70.5, 67.2, 55.3, 47.8, 15.2. ESI HRMS calcd. for $C_{14}H_{16}O_3NNa$ (M+Na): 246.1125, found 246.1125. Anal. Calcd. for $C_{14}H_{16}O_3N$: C, 68.56; H, 6.16; N, 5.71. Found: C, 68.22; H, 6.58; N, 5.61.



22

**3-*O*-(*N*-Benzyl)-carbamoyl-6-deoxy-D-glucal (22)**

A solution of 6-deoxy-D-glucal **9** (0.06 g, 0.46 mmol) in DCM (2 mL) was treated with benzylisocyanate (0.07 mL, 0.55 mmol, 1.2 eq) in the presence of TEA (0.07 mL, 0.69 mmol, 1.5 eq) at 0 $^{o}$C for 6 hours. The solvent was removed under reduced pressure. The residue was subjected to silica gel column chromatography using hexanes-EtOAc (3:1) as eluent to obtain the mono carbamate **22** as a white crystalline solid (0.10 g, 85%). $[\alpha]_D$ -14.2 (*c* 0.8, $CH_2Cl_2$); $^1$H NMR (600 MHz, $CDCl_3$) δ 7.42 – 7.27 (m, 5H, ArH), 6.43 (dd, 1H, $J_{1,2}$ = 6.2 Hz, $J_{1,3}$ = 1.4 Hz, H-1), 5.20 (br t, 1H, OCON*H*Bn), 5.15 (ddd, 1H, $J_{1,3}$ = 1.8 Hz, $J_{2,3}$ = 2.5 Hz, $J_{3,4}$ = 6.7 Hz, H-3), 4.65 (dd, 1H, $J_{1,2}$ = 6.1 Hz, $J_{2,3}$ = 2.4 Hz, H-2), 4.37 (d, 2H, *J* = 6.0 Hz, PhC$H_2$), 3.88 (dq, 1H, $J_{4,5}$ = 9.7 Hz, $J_{5,6}$ = 6.4 Hz, H-5), 3.59 (ddd, 1H, $J_{3,4}$ = 6.8 Hz, $J_{4,5}$ = 8.6 Hz, $J_{4,4OH}$ = 1.2 Hz, H-4), 1.41 (d, 3H, $J_{5,6}$ = 6.4 Hz, H-6 C$H_3$). $^{13}$C NMR (125 MHz, $CDCl_3$) δ 158.0, 146.7, 137.8, 128.8, 128.7, 127.5, 99.0, 75.2, 74.9, 73.3, 45.3, 17.2. ESI HRMS calcd. for $C_{14}H_{17}O_4NNa$ (M+Na): 286.1050, found 286.1053. Anal. Calcd. for $C_{14}H_{17}O_4N$: C, 63.87; H, 6.51; N, 5.32. Found: C, 63.88; H, 6.48; N, 5.41.

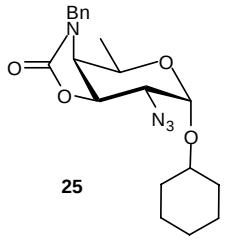



24

**2-Azido-4-benzylamino-4-*N*-,3-*O*-carbonyl-2,4,6-trideoxy-α/β-D-galactopyranosyl trichloroacetimidate (24)**

A mixture of α and β 6-deoxy-galactopyranoses **1α**,**β** (2.0 g, 6.58 mmol) was dissolved in freshly distilled DCM (15 mL). $CCl_3CN$ (4.75 mL, 32.88 mmol, 5 eq) and $K_2CO_3$ (2.73 g, 19.73 mmol, 3 eq) were added to the resulting solution and vigorously stirred at room temperature under argon. After 14 hours, the mixture was concentrated. The residue was dissolved in freshly distilled DCM (10 mL) and filtered through a microfilter to remove inorganic salts. The filtrate was concentrated under reduced pressure to afford imidate **24** as an α,β anomeric mixture (α/β 3:2, 2.94 g, 100%). $^1$H NMR (600 MHz, $CDCl_3$) δ α anomer: 8.76 (s, 1H, N*H*$COCl_3$), 7.48 – 7.31 (m, 5H, ArH), 6.42 (d, $J_{1,2}$ = 4.6 Hz, H-1), 5.06 (d, 1H, $J_{gem}$ = 15.4 Hz, PhC$H_2$), 4.69 (dd, 1H, $J_{2,3}$ = 4.8 Hz, $J_{3,4}$ = 8.4 Hz, H-3), 4.40 (app t, 1H, $J_{1,2}$ = 4.5 Hz, $J_{2,3}$ = 4.5 Hz, H-2), 4.38 (qd, 1H, $J_{4,5}$ = 2.5 Hz, $J_{5,6}$ = 7.0 Hz, H-5), 4.15 (d, 1H, $J_{gem}$ = 15.4 Hz, PhC$H_2$), 3.62 (dd, 1H, $J_{3,4}$ = 8.4 Hz, $J_{4,5}$ = 2.4 Hz, H-4), 1.34 (d, 3H, $J_{5,6}$ = 6.8 Hz, H-6 C$H_3$). β anomer: 8.72 (s, 1H, N*H*$COCl_3$), 7.28 – 7.22 (m, 5H, ArH), 5.87 (d, 1H, $J_{1,2}$ = 7.2 Hz, H-1), 4.95 (d, 1H, $J_{gem}$ = 15.3 Hz, PhC$H_2$), 4.45 (dd, 1H, $J_{2,3}$ = 7.5 Hz, $J_{3,4}$ = 8.7 Hz, H-3), 4.21 (d, 1H, $J_{gem}$ = 15.3 Hz, PhC$H_2$), 4.09 (app t, 1H, $J_{1,2}$ = 7.3 Hz, $J_{2,3}$ = 7.3 Hz, H-2), 4.05 (qd, 1H, $J_{4,5}$ = 3.8 Hz, $J_{5,6}$ = 7.2 Hz, H-5), 3.81 (dd, 1H, $J_{3,4}$ = 8.7 Hz, $J_{4,5}$ = 3.8 Hz, H-4), 1.43 (d, 3H, $J_{5,6}$ = 7.2 Hz, H-6 $CH_3$). $^{13}$C NMR (125 MHz, $CDCl_3$,

from HMQC) δ 129.1, 129.0, 128.3, 128.3, 128.0, 127.9, α anomer: 160.5, 157.9, 135.3, 93.9, 72.0, 67.4, 56.7, 54.5, 49.3, 17.3. β anomer: 161.0, 158.4, 134.7, 96.4, 73.5, 69.8, 62.1, 55.3, 48.8, 17.7. ESI HRMS calcd. for $C_{16}H_{16}O_4N_5Cl_3Na$ (M+Na): 470.0160, found 470.0161.

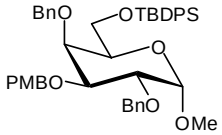



**25**

**Cyclohexyl 2-azido-4-benzylamino-4-*N*-,3-*O*-carbonyl-2,4,6-trideoxy-α-D-galactopyranoside (25)**

Cyclohexanol (0.07 mL, 0.7 mmol, 10 eq) and imidate **24** (0.03 g, 0.07 mmol) were dissolved in freshly distilled DCM (2 mL) and the mixture was stirred under argon at room temperature in the presence of powdered 4 Å molecular sieves for 30 minutes before being cooled to -10 $^{o}$C. TMSOTf (7.6 μL, 0.01 mmol, 0.5 eq) was added and the reaction mixture was stirred for a further 1 hour at -10 $^{o}$C then it was allowed to warm to 0 $^{o}$C at which point the reaction was completed. The reaction was quenched by addition of TEA and stirred for an additional 20 min, after which the mixture was diluted, filtered through celite and concentrated. Chromatography of the residue on silica gel with a stepped gradient of 15-20% EtOAc in hexanes afforded compound **25** as colourless syrup (α/β 4:1, 0.02 g, 70%). α-anomer: $[\alpha]_D$ +16.2 (*c* 0.3, $CH_2Cl_2$); $^1$H NMR (600 MHz, $CDCl_3$) δ 7.39 – 7.21 (m, 5H, ArH), 5.15 (d, 1H, $J_{1,2}$ = 3.6 Hz, H-1), 4.98 (d, 1H, $J_{gem}$ = 15.6 Hz, PhC$H_2$), 4.58 (dd, 1H, $J_{2,3}$ = 8.0 Hz, $J_{3,4}$ = 5.8 Hz, H-3), 4.28 – 4.24 (qd,

1H, $J_{4,5}$ = 2.2 Hz, $J_{5,6}$ = 6.9 Hz, H-5), 4.25-4.19 (d, 1H, $J_{gem}$ = 15.7 Hz, PhC$H_2$), 3.88 (dd, 1H, $J_{1,2}$ = 4.4 Hz, $J_{2,3}$ = 5.7 Hz, H-2), 3.65 (m, 1H, OC$H$$(CH_2)_5$), 3.58 (d, 1H, $J_{3,4}$ = 8.0 Hz, $J_{4,5}$ = 2.3 Hz, H-4), 2.03 – 1.79 (m, 4H, OCH(C$H_2$)$_2$$(CH_2)_3$), 1.77 – 1.64 (m, 6H, OCH$(CH_2)_2$(C$H_2$)$_3$), 1.55 (d, 3H, $J_{5,6}$ = 6.9 Hz, H-6 C$H_3$). $^{13}$C NMR (125 MHz, $CDCl_3$) δ 158.8, 135.6, 128.9, 128.0, 127.7, 94.7, 76.5, 72.3, 70.3, 64.3, 57.9, 55.9, 49.2, 35.6, 33.4, 31.5, 25.5, 24.1, 23.7, 17.5. ESI HRMS calcd. for $C_{20}H_{26}O_4N_4Na$ (M+Na): 409.1846, found 409.1845.

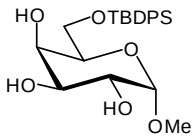


**32**

**Methyl 2,4-di-*O*-benzyl-3-*O*-*p*-methoxybenzyl-6-*O*-*tert*-butyldiphenylsilyl-α-D-galactopyranoside (32)**

Compound **35** (10.00 g, 18.11 mmol) was dissolved in dry DMF (25 mL) and argon was bubbled through the solution for 1 hour prior to cooling the solution to 0 $^{o}$C. NaH (dispersion in mineral oil; 60% by mass; 1.81 g, 45.27 mmol, 2.5 eq) was added in portions and stirring was continued until the evolution of gas had ceased. BnBr (7.75 mL, 45.27 mmol, 2.5 eq) was added and the reaction mixture was allowed to attain room temperature. The mixture was stirred for a further 4 hours, and then the reaction was quenched by addition of MeOH. The solution was diluted with DCM (100 mL) and washed with an equal volume of water and brine, the organic phase was dried over $Na_2SO_4$, filtered and the filtrate was concentrated. Column chromatography on silica gel using hexanes and EtOAc afforded benzylated galactoside **32** (11.3 g, 85%) as a white solid.

$[\alpha]_D$ +52.0 (*c* 0.8, $CH_2Cl_2$); $^1H$ NMR (498 MHz, $CDCl_3$) δ 7.72 – 7.64 (m, 4H, ArH), 7.52 – 7.21 (m, 19H, ArH), 6.99 – 6.90 (m, 2H, ArH), 5.03 (d, 1H, $J_{gem}$ = 11.4 Hz, PhC$H_2$), 4.91 (d, 1H, $J_{gem}$ = 9.7 Hz, PhC$H_2$), 4.87 (d, 1H, $J_{gem}$ = 9.7 Hz, PhC$H_2$), 4.77 (d, 1H, $J_{gem}$ = 11.8, PhC$H_2$), 4.74 (d, 1H, $J_{gem}$ = 11.8, PhC$H_2$), 4.71 (d, 1H, $J_{1,2}$ = 3.6 Hz, H-1), 4.66 (d, 1H $J_{gem}$ = 11.4 Hz, PhC$H_2$), 4.07 (dd, 1H, $J_{1,2}$ = 3.6 Hz, $J_{2,3}$ = 10.0 Hz, H-2), 3.99 (d, 1H, $J_{4,5}$ = 2.8 Hz, H-4), 3.96 (dd, 1H, $J_{2,3}$ = 10.0 Hz, $J_{3,4}$ = 2.8 Hz, H-3), 3.86 (s, 3H, $OCH_2C_6H_4OCH_3$), 3.82 – 3.71 (m, 3H, H-6a, H-5, H-6b), 3.34 (s, 3H, $OCH_3$), 1.11 (s, 9H, $C(CH_3)_3$). $^{13}C$ NMR (125 MHz, $CDCl_3$) δ 159.2, 138.8, 138.7, 135.6, 135.6, 133.4, 131.1, 129.8, 129.7, 129.2, 128.3, 128.2, 128.1, 128.1, 127.7, 127.7, 127.5, 113.8, 98.7, 79.0, 76.5, 75.2, 74.8, 73.6, 73.0, 70.7, 62.7, 55.3, 55.1, 26.9, 19.2. ESI HRMS calcd. for $C_{45}H_{52}O_7SiNa$ (M+Na): 755.3374, found 755.3379.

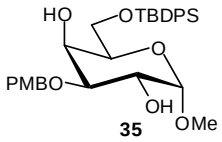



**34**

**Methyl 6-*O*-*tert*-butyldiphenylsilyl-α-D-galactopyranoside (34)**

Methyl-α-D-galactopyranoside **33** (20.00 g, 103.1 mmol) was dissolved in DMF (50 mL) with stirring under argon. Imidazole (8.41 g, 123.72 mmol, 1.2 eq) and then by *tert*-butylchlorodiphenylsilane (31.16 mL, 113.53 mmol, 1.1 eq) were added to the solution. After 5 hours the reaction was quenched by adding MeOH. Then the solvent was removed under reduced pressure. The residue was purified by column chromatography on silica gel using a mixture of hexanes / EtOAc (7:3) as eluent to afford **34** (41.00 g, 92%) as a syrup. $[\alpha]_D$ +75.7 (*c* 0.3, $CHCl_3$); $^1H$

NMR (498 MHz, $CDCl_3$) δ 7.70 (m, 4H, ArH), 7.48 – 7.35 (m, 6H, ArH), 4.79 (d, 1H, $J_{1,2}$ = 3.9 Hz, H-1), 4.11 (s, 1H, H-4), 3.93 (dd, 1H, $J_{5,6a}$ = 6.1 Hz, $J_{6a,6b}$ = 10.6 Hz, H-6a), 3.90 – 3.85 (dd, 1H, $J_{5,6b}$ = 5.3 Hz, $J_{6a,6b}$ = 10.6 Hz, H-6b), 3.85 – 3.81 (dd, 1H, $J_{1,2}$ = 3.9 Hz, $J_{2,3}$ = 9.4 Hz, H-2), 3.77 (t, 1H, $J_{5,6}$ = 5.7 Hz, H-5), 3.74 (dd, 1H, $J_{2,3}$ = 9.4 Hz, $J_{3,4}$ = 3.3 Hz, H-3), 3.37 (s, 3H, OC$H_3$), 2.52 (s, 4H, OH), 1.07 (s, 9H, C(C$H_3$)$_3$). $^{13}$C NMR (125 MHz, $CDCl_3$) δ 135.6, 135.6, 133.3, 133.2, 129.7, 127.7, 99.6, 71.0, 70.4, 69.6, 69.4, 63.4, 55.1, 26.8, 19.2. ESI HRMS calcd. for $C_{23}H_{32}O_6SiNa$ (M+Na): 455.1860, found 455.1853. Anal. Calcd. for $C_{23}H_{32}O_6Si$: C, 63.86; H, 7.46. Found: C, 63.56; H, 7.45.

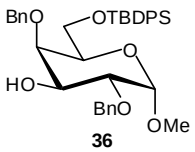


**35**

**Methyl 3-*O*-*p*-methoxybenzyl-6-*O*-*tert*-butyldiphenylsilyl-α-D-galactopyranoside (35)**

A mixture of methyl-6-*O*-*tert*-butyldiphenylsilyl-α-D-galactopyranoside (40 g, 92.55 mmol) and dibutyltin oxide (25.34 g, 101.8 mmol, 1.1 eq) in toluene (60 ml) was heated at reflux using a Dean-Stark apparatus for 5 hours until the reaction mixture became clear. Then the solution was cooled to room temperature. TBAI (32.82 g, 101.8 mmol, 1.1 eq) and PMBCl (15.94 mL, 101.8 mmol, 1.1 eq) were added respectively to the solution which was then heated at 60 $^{o}$C for 15 hours. Triethylamine was added to the mixture and the toluene was removed under reduced pressure. The residue was purified by column chromatography over silica gel (hexanes-EtOAc, 4:1) to give the product as syrup (35.78 g, 70%).

$[\alpha]_D$ +72.3 (*c* 2.8, $CH_2Cl_2$); $^1$H NMR (600 MHz, $CDCl_3$) δ 7.68 (m, 4H, ArH), 7.49 – 7.22 (m, 9H, ArH), 6.89 (m, 2H, ArH), 4.80 (d, 1H, $J_{1,2}$ = 3.9 Hz, H-1), 4.66 (d, 2H, $J_{gem}$ = 11.6 Hz, PhC$H_2$), 4.07 (d, 1H, $J_{3,4}$ = 3.1 Hz, H-4), 4.01 – 3.95 (m, 1H, H-2), 3.92 (dd, 1H, $J_{5,6a}$ = 6.3 Hz, $J_{6a,6b}$ = 10.4 Hz, H-6a), 3.84 (dd, 1H, $J_{5,6b}$ = 5.8 Hz, $J_{6a,6b}$ = 10.4 Hz, H-6b), 3.80 (s, 3H, $OCH_2C_6H_4OCH_3$), 3.74 (t, 1H, $J_{5,6}$ = 6.0 Hz, H-5), 3.59 (dd, 1H, $J_{2,3}$ = 9.6 Hz, $J_{3,4}$ = 3.1 Hz, H-3), 3.37 (s, 3H, $OCH_3$), 2.42 (s, 1H, 4-OH), 2.08 (d, 1H, *J* = 7.1 Hz, 2-OH), 1.06 (s, 9H, $C(CH_3)_3$). $^{13}$C NMR (125 MHz, $CDCl_3$) δ 159.3, 135.6, 135.6, 133.4, 133.3, 130.1, 129.7, 129.5, 127.7, 114.0, 99.4, 78.3, 71.7, 70.1, 68.6, 66.8, 63.1, 55.3, 55.2, 26.8, 19.2. ESI HRMS calcd. for $C_{31}H_{40}O_7SiNa$ (M+Na): 575.2435, found 575.2435. Anal. Calcd. for $C_{31}H_{40}O_7Si$: C, 67.36; H, 7.29. Found: C, 66.98; H, 7.22.

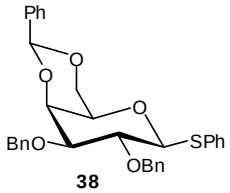


**36**

**Methyl 2,4-di-*O*-benzyl-6-*O*-*tert*-butyldiphenylsilyl-α-D-galactopyranoside (36)**

A solution of compound **32** (2.30 g, 3.14 mmol) in a mixture of $CH_3CN$ and $H_2O$ (4:1, v/v, 20 mL) containing CAN (ammonium cerium (IV) nitrate) (4.30 g, 7.85 mmol, 2.5 eq) was stirred at room temperature. This orange two phase system was vigourously stirred for 2 hours. When the starting material was consumed, the reaction was quenched with saturated aqueous $NaHCO_3$ solution followed by extraction with DCM (5 × 25 mL). The combined organic phase was

washed with brine, dried over $MgSO_4$, filtered and the filtrate was concentrated to dryness. Chromatography on silica gel (hexanes-EtOAc, 3:1) yielded **36** (1.63 g, 85%) as a syrup. $[\alpha]_D$ +62.1 (*c* 0.8, $CH_2Cl_2$); $^1$H NMR (600 MHz, $CDCl_3$) δ 7.65 (m, 4H, ArH), 7.48 – 7.25 (m, 16H, ArH), 4.86 (d, 1H, $J_{gem}$ = 11.5 Hz, PhC$H_2$), 4.72 – 4.67 (m, 3H, PhC$H_2$), 4.66 (d, 1H, $J_{1,2}$ = 3.2 Hz, H-1), 4.11 – 4.00 (m, 2H, H-3, H-4), 3.84 – 3.71 (m, 4H, H-2, H-5, H-6a, H-6b), 3.24 (s, 3H, OC$H_3$), 2.27 (s, 1H, OH), 1.08 (s, 9H, C(C$H_3$)$_3$). $^{13}$C NMR (125 MHz, $CDCl_3$) δ 138.6, 138.2, 135.6, 135.5, 133.3, 133.3, 129.9, 129.8, 128.6, 128.5, 128.4-127.7 (m), 97.9, 77.4, 76.5, 75.3, 72.9, 70.4, 70.4, 62.4, 55.1, 26.9, 19.2. ESI HRMS calcd. for $C_{37}H_{44}O_6SiNa$ (M+Na): 635.2799, found 635.2793.

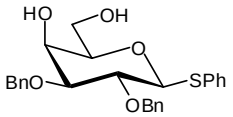



**38**

**Phenyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-1-thio-β-D-galactopyranoside (38)**[159]

To a stirred solution of phenyl thiogalactopyranoside **37** (10.4 g, 28.88 mmol) in dry DMF (50 mL), NaH (dispersion in mineral oil; 60% by mass; 3.46 g, 86.64 mmol, 3 eq) was added in portions at 0 °C and stirring was continued until the evolution of gas had ceased. After 1 hour, BnBr (14.82 mL, 86.64 mmol, 3 eq) was slowly added to the above mixture, which was then warmed to room temperature and stirred for a further 4 hours. The reaction mixture was then quenched with MeOH. The solution was diluted with DCM (100 mL) and washed

with an equal volume of water and brine. The organic extract was dried over $Na_2SO_4$, filtered and concentrated to a light yellow residue. Chromatography on silica gel with hexanes-EtOAc (3:1) as eluent gave **38** (13.26 g, 87%) as a white solid. $[\alpha]_D$ -15.4 (*c* 0.2, $CH_2Cl_2$); $^1$H NMR (498 MHz, $CDCl_3$) δ 7.72 – 7.52 (m, 4H, ArH), 7.44 – 7.18 (m, 16, ArH), 5.5 (s, 1H, PhC*H*), 4.74 – 4.69 (m, 4H, $PhCH_2$), 4.63 (d, 1H, $J_{1,2}$ = 9.6 Hz, H-1), 4.37 (d, 1H, $J_{5,6a}$ = 1.4 Hz, $J_{6a,6b}$ = 12.4 Hz, H-6a), 4.16 (d, 1H, $J_{3,4}$ = 3.2 Hz, H-4), 4.00 – 3.96 (d, 1H, $J_{5,6b}$ = 1.5 Hz, $J_{6b,6a}$ = 12.3 Hz, H-6b), 3.93 (app t, 1H, $J_{1,2}$ = 9.3 Hz, $J_{2,3}$ = 9.3 Hz, H-2), 3.64 (dd, 1H, $J_{2,3}$ = 9.3 Hz, $J_{3,4}$ = 3.4 Hz, H-3), 3.40 (s, 1H, H-5). $^{13}$C NMR (125 MHz, $CDCl_3$) δ 138.5, 138.1, 137.9, 132.8, 129.0, 128.8, 128.4, 128.3, 128.2, 128.1, 127.8, 127.7, 127.4, 126.6, 101.3, 86.6, 81.4, 75.5, 75.4, 73.7, 71.9, 69.9, 69.4. ESI HRMS calcd. for $C_{33}H_{32}O_5SNa$ (M+Na): 563.1862, found 563.1865. Anal. Calcd. for $C_{33}H_{32}O_5S$: C, 73.31; H, 5.97; S, 5.93. Found: C, 73.31; H, 6.17; S, 5.66.

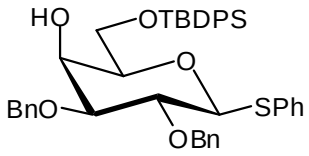



**39**

**Phenyl 2,3-di-*O*-benzyl-1-thio-β-D-galactopyranoside (39)**

Phenyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-1-thio-β-D-galactopyranoside **38** (5.2 g, 9.63 mmol) was heated at reflux with 80% acetic acid (40 mL). After stirring for 12 hours the reaction mixture was diluted with EtOAc (50 mL), washed with saturated $NaHCO_3$ solution (50 mL) followed by brine (50 mL). The organic layer was dried over $Na_2SO_4$, filtered and concentrated. The residue was

purified by chromatography on silica gel using a stepped gradient of EtOAc in hexanes (10 to 35%) to afford the title compound (3.26 g, 75%). $[\alpha]_D$ -0.5 (*c* 0.1, $CH_2Cl_2$); $^1$H NMR (600 MHz, $CDCl_3$) δ 7.57 – 7.24 (m, 2H, ArH), 7.45 – 7.39 (m, 2H, ArH), 7.38 – 7.22 (m, 11H, ArH), 4.86 (d, 1H, $J_{gem}$ = 10.3 Hz, PhC$H_2$), 4.78 (d, 1H, $J_{gem}$ = 10.3 Hz, PhC$H_2$), 4.74 (d, 1H, $J_{gem}$ = 11.6 Hz, PhC$H_2$), 4.72 (d, 1H, $J_{gem}$ = 11.6 Hz, PhC$H_2$), 4.68 (d, 1H, $J_{1,2}$ = 9.8 Hz, H-1), 4.11 (d, 1H, $J$ = 2.8 Hz, H-4), 3.97 (dd, 1H, $J_{5,6a}$ = 5.6 Hz, $J_{6a,6b}$ = 11.8 Hz, H-6a) 3.81 – 3.60 (m, 2H, H-2, H-6b), 3.58 (dd, 1H, $J_{2,3}$ = 8.9 Hz, $J_{3,4}$ = 3.3 Hz, H-3), 3.49 (t, 1H, $J_{5,6}$ = 5.6 Hz, H-5) $^{13}$C NMR (125 MHz, $CDCl_3$) δ 138.2, 137.6, 133.8, 131.7, 129.0, 128.6, 128.4, 128.3, 128.1, 127.9, 127.9, 127.5, 87.7, 82.5, 78.1, 77.0, 75.8, 72.2, 67.3, 62.6. ESI HRMS calcd. for $C_{26}H_{28}O_5SNa$ (M+Na): 475.1549, found 475.1549. Anal. Calcd. for $C_{26}H_{28}O_5S$: C, 69.00; H, 6.24; S, 7.09. Found: C, 68.64; H, 6.33; S, 6.87.

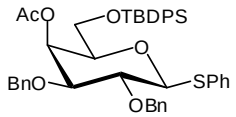



**40**

**Phenyl 2,3-di-*O*-benzyl-6-*O*-*tert*-butyldiphenylsilyl-1-thio-β-D-galactopyranoside (40)**

To a solution of phenyl thiogalactoside **39** (4.5 g, 9.95 mmol) in dry DMF (25.0 mL) were added imidazole (1.02 g, 14.93 mmol, 1.5 eq) and *tert*-butylchlorodiphenylsilane (3.3 mL, 11.94 mmol, 1.2 eq) at 0 $^o$C. After 6 hours at ambient temperature, the reaction was quenched with water and the mixture was

extracted with DCM (2 × 30 mL). The combined organic extracts were dried over $Na_2SO_4$, filtered and concentrated. The residue was purified by column chromatography to give **40** (6.70 g, 97%) as a white solid. $[\alpha]_D$ + 2.5 (*c* 1.4, $CH_2Cl_2$); $^1$H NMR (600 MHz, $CDCl_3$) δ 7.75 – 7.66 (m, 3H, ArH), 7.59 – 7.53 (m, 2H, ArH), 7.47 – 7.10 (m, 20H, ArH), 4.83 (d, 1H, $J_{gem}$ = 10.3 Hz, PhC$H_2$), 4.76 (d, 1H, $J_{gem}$ = 10.3 Hz, PhC$H_2$), 4.72 (s, 2H, PhC$H_2$), 4.61 (d, 1H, $J_{1,2}$ = 9.8 Hz, H-1), 4.11 (app t, 1H, $J_{3,4}$ = 2.4 Hz, $J_{4,5}$ = 2.4 Hz, H-4), 3.97 (dd, 1H, $J_{5,6a}$ = 5.7 Hz, $J_{6a,6b}$ = 10.6 Hz, H-6a), 3.93 (dd, 1H, $J_{5,6b}$ = 5.4 Hz, $J_{6b,6a}$ = 10.6 Hz, H-6b), 3.78 (app t, 1H, $J_{1,2}$ = 9.3 Hz, $J_{2,3}$ = 9.3 Hz, H-2 ), 3.54 (dd, 1H, $J_{2,3}$ = 8.9 Hz, $J_{3,4}$ = 3.1 Hz, H-3), 3.45 (t, 1H, $J_{5,6}$ = 5.5 Hz, H-5), 2.64 (d, 1H, $J_{4,OH}$ = 2.3 Hz, 4-OH), 1.10 – 0.82 (m, 9H, C(C$H_3$)$_3$). $^{13}$C NMR (125 MHz, $CDCl_3$) δ 138.3, 137.8, 135.7, 135.6, 134.2, 133.1, 133.0, 131.5, 129.8, 128.8, 128.5, 128.3, 128.2, 127.9, 127.9, 127.7, 127.2, 87.8, 82.7, 78.0, 75.7, 72.1, 67.0, 63.5, 26.8, 19.2. ESI HRMS calcd. for $C_{42}H_{46}O_5SiSNa$ (M+Na): 713.2727, found 713.2727.

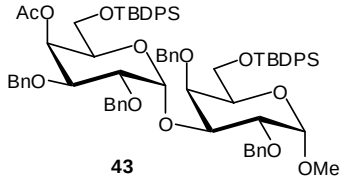



**42**

**Phenyl 4-*O*-acetyl-2,3-di-*O*-benzyl-6-*O*-*tert*-butyldiphenylsilyl-1-thio-β-D-galactopyranoside (42)**

Galactoside **40** (2.70 g, 3.91 mmol) was dissolved in dry pyridine (10 mL) with stirring under an argon atmosphere. Excess $Ac_2O$ (5 mL) was added and the reaction mixture was stirred overnight. The reaction mixture was quenched with methanol then concentrated under reduced pressure and repeatedly co-evaporated

with toluene. Chromatography of the residue on silica gel using hexanes-EtOAc (8:2) as eluent afforded the title compound **42** (2.72 g, 95%). $[\alpha]_D$ +17.8 (*c* 3.3, $CH_2Cl_2$); $^1$H NMR (498 MHz, $CDCl_3$) δ 7.69 – 7.61 (m, 4H, ArH), 7.61 – 7.17 (m, 21H), 5.68 (d, 1H, $J$ = 1.6 Hz, H-4), 4.81 (d, 1H, $J_{gem}$ = 11.1 Hz, PhC$H_2$), 4.77 – 4.70 (s, 2H, PhC$H_2$), 4.68 – 4.60 (d, 1H, $J_{1,2}$ = 9.6 Hz, H-1), 4.52 (d, 1H, $J_{gem}$ = 11.1 Hz, PhC$H_2$), 3.79 (dd, 1H, $J_{1,2}$ = 10.04 Hz, $J_{2,3}$ = 10.07 Hz, H-2), 3.72 – 3.58 (m, 4H, H-3, H-5, H-6), 2.04 (s, 3H, C$H_3$CO), 1.06 (s, 9H, C(C$H_3$)$_3$). $^{13}$C NMR (125 MHz, $CDCl_3$) δ 170.0, 138.3, 137.7, 135.7, 135.6, 133.8, 133.1, 133.0, 131.9, 129.9, 129.8, 128.8, 128.4, 128.4, 128.3, 128.2, 127.8, 127.7, 127.7, 127.4, 87.9, 81.4, 77.3, 76.8, 75.8, 72.0, 66.5, 61.9, 26.8, 20.9, 19.1. ESI HRMS calcd. for $C_{44}H_{48}O_6SiSNa$ (M+Na): 755.2833, found 755.2829.



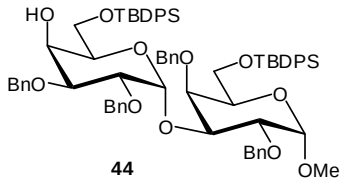
**43**

**Methyl 4-*O*-acetyl-2,3-di-*O*-benzyl-6-*O*-*tert*-butyldiphenylsilyl-α-D-galactopyranosyl-(1→3)-2,4-di-*O*-benzyl-6-*O*-*tert*-butyldiphenylsilyl-α-D-galactopyranoside (43)**

Thioglycoside **42** (1.69 g, 2.39 mmol, 1.2 eq) and the acceptor **36** (1.28 g, 2.10 mmol, 1 eq) were dissolved in freshly distilled DCM (30 mL) and the mixture was stirred at -30 $^o$C in the presence of powdered 4 Å molecular sieves for 30 minutes. To the stirred solution, NIS (707 mg, 3.14 mmol, 1.5 eq) was then added and stirring was continued for a further 30 minutes. After addition of triflic acid (63 μL, 0.42 μmol, 0.2 eq) the reaction mixture turned deep purple, and was

then stirred for a further 1 hour at -30 $^{o}$C, at which point the reaction reached completion. The mixture was quenched by addition of TEA and stirred for an additional 20 min. Then the mixture was diluted with DCM (30 mL), filtered through Celite and washed with 5% $Na_2S_2O_3$ solution and dried over anhydrous $MgSO_4$. Filtration and concentration of the filtrate gave a residue that was chromatographed on silica gel using hexanes-EtOAc (9:1) as eluent. Disaccharide **43** was obtained as an anomerically pure white crystalline solid (exclusively α, 3.22 g, 87%). $[\alpha]_D$ +72.1 (*c* 0.6, $CH_2Cl_2$); $^1$H NMR (498 MHz, $CDCl_3$) δ 7.65 – 7.59 (m, 8H, ArH), 7.43 – 7.14 (m, 30H, ArH), 7.08 (m, 2H, ArH), 5.71 (d, 1H, $J$ = 2.0 Hz, H-4′), 5.20 (d, 1H, $J_{1',2'}$ = 3.4 Hz, H-1′), 4.97 (d, 1H, $J_{gem}$ = 11.4 Hz, PhC$H_2$), 4.83 (d, 1H, $J_{gem}$ = 11.4 Hz, PhC$H_2$), 4.79 (d, 1H, $J_{gem}$ = 11.4 Hz, PhC$H_2$), 4.67 (d, 1H, $J_{gem}$ = 11.4 Hz, PhC$H_2$), 4.64 (d, 1H, $J_{gem}$ = 11.4 Hz, PhC$H_2$), 4.56 (d, 1H, $J_{1,2}$ = 3.6 Hz, H-1), 4.49 (m, 3H, H-5′, PhC$H_2$), 4.40 (d, 1H, $J_{gem}$ = 11.4 Hz, PhC$H_2$), 4.13 (dd, 1H, $J_{2,3}$ = 10.2 Hz, $J_{3,4}$ = 2.7 Hz, H-3), 4.03 (dd, 1H, $J_{2',3'}$ = 10.0 Hz, $J_{3',4'}$ = 3.2 Hz, H-3′), 3.99 (d, 1H, $J$ = 2.5 Hz, H-4), 3.95 (dd, 1H, $J_{1,2}$ = 3.6 Hz, $J_{2,3}$ = 10.2 Hz, H-2), 3.85 (dd, 1H, $J_{1',2'}$ = 3.4 Hz, $J_{2',3'}$ = 10.0 Hz, H-2′), 3.76 – 3.62 (m, 4H, H-5, H-6a′, H-6), 3.62 – 3.54 (dd, $J_{5',6a'}$ = 8.5 Hz, 1H, $J_{6a',6b'}$ = 8.9 Hz, H-6b′), 3.22 (s, 3H, OC$H_3$), 2.04 (s, 3H, C$H_3$CO), 1.05 (s, 9H, C(C$H_3$)$_3$), 1.04 (s, 9H, C(C$H_3$)$_3$). $^{13}$C NMR (125 MHz, $CDCl_3$) δ 170.0, 138.9, 138.6, 138.4, 138.3, 135.7, 135.6, 135.5, 135.5, 135.5, 133.4, 133.3, 133.2, 133.1, 129.7, 129.6, 129.5, 128.3, 128.27, 128.2, 128.2, 128.2, 128.1, 128.1, 128.0, 127.9, 127.8, 127.7, 127.7, 127.6, 127.6, 127.6, 127.5, 127.2, 98.5, 96.7, 76.7, 76.5, 76.0, 76.0, 75.9, 74.8, 74.5, 74.2, 73.3, 71.6, 70.8, 69.2, 67.6, 62.7,

61.5, 54.9, 26.8, 26.8, 20.9, 19.1, 19.1. ESI HRMS calcd. for $C_{75}H_{86}O_{12}Si_2Na$ (M+Na): 1257.5550, found 1257.5551. Anal. Calcd. for $C_{75}H_{86}O_{12}Si_2$: C, 72.90; H, 7.02. Found: C, 72.73; H, 7.04.

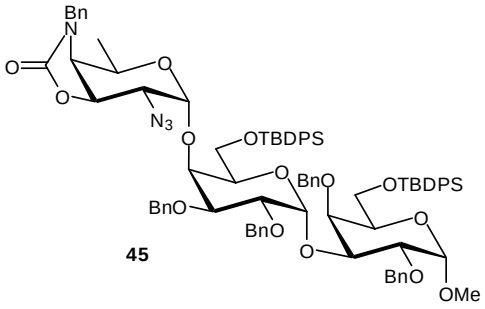


**44**

**Methyl 2,3-di-*O*-benzyl-6-*O*-*tert*-butyldiphenylsilyl-α-D-galactopyranosyl-(1→3)-2,4-di-*O*-benzyl-6-*O*-*tert*-butyldiphenylsilyl-α-D-galactopyranoside (44)**

Disaccharide **43** (1.6 g, 1.29 mmol) was dissolved in a mixture of freshly distilled DCM and MeOH (4:1; 20 mL). Then 1.5 M MeONa / MeOH (0.4 mL) was added dropwise. After heating at 50 $^{o}$C for 12 hours, the reaction was neutralized with Amberlite resin IR-120 ($H^+$), filtered and concentrated under reduced pressure. Chromatography over silica gel (hexanes-EtOAc, 9:1) gave **44** (1.30 g, 85%) as a white solid. $[\alpha]_D$ +97.2 (*c* 0.1, $CH_2Cl_2$); $^1$H NMR (600 MHz, $CDCl_3$) δ 7.72 – 7.58 (m, 8H, ArH), 7.44 – 7.14 (m, 30H, ArH), 7.07 (m, 2H, ArH), 5.22 (d, 1H, $J_{1',2'}$ = 3.4 Hz, H-1′), 5.00 (d, 1H, $J_{gem}$ = 11.3 Hz, PhC$H_2$), 4.84 (d, 1H, $J_{gem}$ = 11.6 Hz, PhC$H_2$), 4.73 – 4.68 (m, 3H, PhC$H_2$), 4.60 (d, 1H, $J_{gem}$ = 12.1 Hz, PhC$H_2$), 4.57 (d, 1H, $J_{1,2}$ = 3.6 Hz, H-1), 4.48 (d, 1H, $J_{gem}$ = 12.1 Hz, PhC$H_2$), 4.41 (d, 1H, $J_{gem}$ = 11.4 Hz, PhC$H_2$), 4.19 (app t, 1H, $J_{5',6'}$ = 5.6 Hz, H-5′), 4.12 (dd, 1H, $J_{2,3}$ = 10.2 Hz, $J_{3,4}$ = 2.7 Hz, H-3), 4.09 (app s, 1H, H-4′), 4.02 (dd, 1H, $J_{1',2'}$ = 3.5 Hz, $J_{2',3'}$ = 9.9 Hz H-2′), 4.01 (d, 1H, *J* = 2.5 Hz, H-4), 3.92

(dd, 1H, $J_{1,2}$ = 3.6 Hz, $J_{2,3}$ = 10.2 Hz, H-2), 3.91 (dd, 1H, $J_{2',3'}$ = 9.8 Hz, $J_{3',4'}$ = 3.0 Hz, H-3′), 3.85 (dd, 1H, $J_{5',6a'}$ = 6.3 Hz, $J_{6a',6b'}$ = 10.4 Hz, H-6a′), 3.74 (dd, 1H, $J_{5',6a'}$ = 4.8 Hz, $J_{6a',6b'}$ = 10.4 Hz, H-6b′), 3.68 (m, 3H, H-5, H-6), 3.20 (s, 3H, OC$H_3$), 2.90 (s, 1H, 4′-OH), 1.05 (s, 18H, C(C$H_3$)$_3$). $^{13}$C NMR (125 MHz, $CDCl_3$) δ 139.0, 138.6, 138.4, 138.3, 135.7, 135.6, 135.5, 133.4, 133.3, 133.3, 133.1, 129.6, 128.4, 128.3, 128.2, 128.0, 127.9, 127.8, 127.7, 127.6, 127.5, 127.1, 98.4, 96.4, 77.9, 76.1, 75.9, 75.7, 74.8, 74.2, 73.1, 72.0, 70.8, 69.5, 68.0, 63.6, 62.7, 54.9, 26.9, 26.8, 19.2, 19.2. ESI HRMS calcd. for $C_{73}H_{84}O_{11}Si_2Na$ (M+Na): 1215.5444, found 1215.5441. Anal. Calcd. for $C_{73}H_{84}O_{11}Si_2$: C, 73.46; H, 7.09. Found: C, 73.30; H, 7.19.



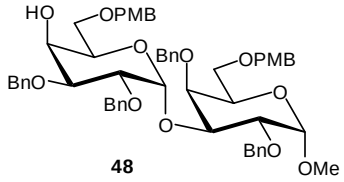

45

**Methyl 2-azido-4-benzylamino-4-*N*-,3-*O*-carbonyl-2,4,6-trideoxy-α-D-galactopyranosyl-(1→4)-2,3-di-*O*-benzyl-6-*O*-*tert*-butyldiphenylsilyl-α-D-galactopyranosyl-(1→3)-2,4-di-*O*-benzyl-6-*O*-*tert*-butyldiphenylsilyl-α-D-galactopyranoside (45)**

Acceptor **44** (0.02 g, 0.017 mmol, 1 eq) and imidate **24** (0.012 g, 0.025 mmol, 1.5 eq) were combined and concentrated from dry toluene and dried *in vacuo* overnight. The residue was dissolved in freshly distilled DCM (2 mL) and the mixture was stirred under argon at room temperature in the presence of

powdered 4 Å molecular sieves for 30 minutes before being cooled to -10 $^{o}$C. TMSOTf (0.2 μL, 0.85 μmol, 0.05 eq) was added and the reaction mixture was stirred for 1 hour at -10 $^{o}$C. The mixture was allowed to warm to 0 $^{o}$C at which point the reaction was completed. On completion of the reaction, it was quenched by addition of TEA and stirred for an additional 20 min. The mixture was diluted with DCM (3 mL), filtered through Celite and the filtrate was concentrated. Chromatography of the residue on silica gel with a gradient of 20-30% EtOAc in hexanes afforded the trisaccharide **45** as a colourless syrup (α/β 3:1, 0.005 g, 22%). α-anomer: $[\alpha]_D$ +18.3 (*c* 1.2, $CH_2Cl_2$); $^1$H NMR (600 MHz, $C_6D_6$) δ 7.95 – 7.81 (m, 2H, ArH), 7.80 – 7.70 (m, 5H, ArH), 7.50 – 7.33 (m, 5H, ArH), 7.33 – 7.12 (m, 20H, ArH), 7.12 – 6.95 (m, 13H, ArH), 5.42 (d, 1H, $J_{1",2"}$ = 4.2 Hz, H-1"), 5.38 (d, 1H, $J_{1',2'}$ = 2.0 Hz, H-1'), 5.25 (d, 1H, $J_{gem}$ = 11.1 Hz, PhC$H_2$), 4.93 – 4.78 (m, 3H, PhC$H_2$), 4.73 (d, 1H, $J_{1,2}$ = 3.5 Hz, H-1), 4.71 (d, 1H, $J_{gem}$ = 12.4 Hz, PhC$H_2$), 4.70 – 4.66 (dd, 1H, $J_{5',6b'}$ = 9.2 Hz, $J_{5',6a'}$ = 5.8 Hz, H-5'), 4.65 (app s, 1H, H-3'), 4.64 (d, 1H, $J_{gem}$ = 11.6 Hz, PhC$H_2$), 4.48 (d, 1H, $J_{gem}$ = 11.8 Hz, PhC$H_2$), 4.47 (dd, 1H, $J_{2,3}$ = 9.9 Hz, $J_{3,4}$ = 3.0 Hz, H-3), 4.43 (d, 1H, $J_{gem}$ = 11.1 Hz, PhC$H_2$), 4.37 (d, 1H, $J_{gem}$ = 12.1 Hz, PhC$H_2$), 4.30 (qd, 1H, $J_{4",5"}$ = 2.6 Hz, $J_{5",6"}$ = 6.7 Hz, H-5"), 4.28 – 4.25 (m, 2H, H-2', H-4'), 4.22 (dd, 1H, $J_{1,2}$ = 3.5 Hz, $J_{2,3}$ = 10.5 Hz, H-2), 4.16 (dd, 1H, $J_{2",3"}$ = 8.0 Hz, $J_{3",4"}$ = 5.8 Hz, H-3"), 4.09 (dd, 1H, $J_{5',6a'}$ = 5.8 Hz, $J_{6a',6b'}$ = 10.0 Hz, H-6a′), 4.07 – 4.03 (dd, 1H, $J_{4,5}$ = 6.5 Hz, $J_{5,6}$ = 6.7 Hz, H-5), 3.98 (m, 2H, H-4, H-6a), 3.93 (app t, 1H, $J_{5,6b}$ = 6.4 Hz, $J_{6a,6b}$ = 6.4 Hz, H-6b), 3.88 (app t, 1H, $J_{5',6b'}$ = 9.6 Hz, $J_{6a',6b'}$ = 9.6 Hz, H-6b'), 3.75 (d, 1H $J_{gem}$ = 15.5 Hz, PhC$H_2$), 3.49 (dd, 1H, $J_{1",2"}$ = 4.1 Hz, $J_{2",3"}$ = 5.7 Hz, H-2"), 3.23

(dd, 1H, $J_{3",4"}$ = 8.1 Hz, $J_{4",5"}$= 2.7 Hz, H-4"), 3.19 (s, 3H, OC$H_3$), 1.20 (s, 9H, C(C$H_3$)$_3$), 1.16 (s, 9H, C(C$H_3$)$_3$), 0.92 – 0.85 (d, 3H, $J_{5",6"}$ = 5.8 Hz, H-6" C$H_3$). $^{13}$C NMR (125 MHz, $CDCl_3$) δ 158.8, 138.9, 138.6, 138.1, 135.7, 135.6, 135.5, 135.4, 133.3, 132.9, 132.8, 129.9, 128.9, 128.3, 128.2, 128.0, 127.9, 127.8, 127.7, 127.5, 127.4, 127.2, 98.6, 96.5, 95.9, 76.6, 75.9, 75.8, 75.5, 74.7, 74.3, 73.8, 73.4, 73.3, 72.7, 72.3, 70.7, 70.4, 64.7, 62.6, 61.0, 58.8, 55.4, 54.9, 48.7, 26.9, 26.8, 19.1, 17.5. ESI HRMS calcd. for $C_{87}H_{98}O_{14}N_4Si_2Na$ (M+Na): 1501.6510, found 1501.6493.

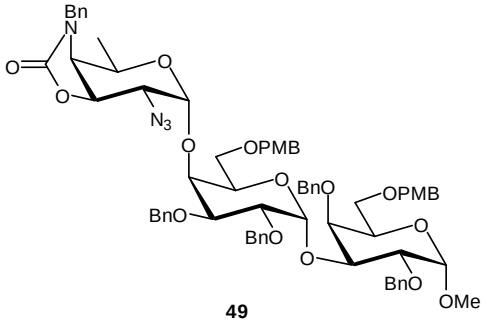



**48**

**Methyl 2,3-di-*O*-benzyl-6-*O*-*p*-methoxybenzyl-α-D-galactopyranosyl-(1→3)-2,4-di-*O*-benzyl-6-*O*-*p*-methoxybenzyl-α-D-galactopyranoside (48)**

Disaccharide **65** (0.90 g, 0.85 mmol) was dissolved in a mixture of freshly distilled DCM and MeOH (4:1; v/v, 15 mL). Then 1.5 M MeONa / MeOH (0.3 mL) was added dropwise. The solution was heated at 45 $^{o}$C for 10 hours then it was neutralized with Amberlite resin IR-120 ($H^+$), filtered and concentrated under reduced pressure. Chromatography over silica gel using a gradient eluent (25-35% EtOAc in hexanes) gave **48** (0.76 g, 94%) as a colourless syrup. $[\alpha]_D$ +64.1 (*c* 0.8, $CH_2Cl_2$); $^1$H NMR (600 MHz, $CDCl_3$) δ 7.38 – 7.33 (m, 2H, ArH), 7.33 – 7.14 (m, 22H, ArH), 7.12 (m, 2H, ArH), 6.88 – 6.81 (m, 4H, ArH), 5.21 (d, 1H, $J_{1',2'}$ = 3.5 Hz, H-1′), 4.99 (d, 1H, $J_{gem}$ = 11.4 Hz, PhC$H_2$), 4.83 (d, 1H, $J_{gem}$ = 11.5 Hz,

PhC$H_2$), 4.71 – 4.66 (m, 3H, PhC$H_2$), 4.64 (s, 1H, H-1), 4.63 (d, 1H, $J_{gem}$ = 8.7 Hz, PhC$H_2$), 4.53 (d, 1H, $J_{gem}$ = 11.9 Hz, PhC$H_2$), 4.46 (d, 1H, $J_{gem}$ = 11.6 Hz, PhC$H_2$), 4.41 (d, 1H, $J_{gem}$ = 11.5 Hz, PhC$H_2$), 4.33 (m, 3H, PhC$H_2$), 4.24 (t, 1H, $J$ = 5.2 Hz, H-5′), 4.14 (dd, 1H, $J_{2,3}$ = 10.2 Hz, $J_{3,4}$ = 2.7 Hz, H-3), 4.01 (m, 2H, H-4′, H-2′), 3.97 (dd, 1H, $J_{1,2}$ = 3.6 Hz, $J_{2,3}$ = 10.2 Hz, H-2), 3.92 (app s, 1H, H-4), 3.90 (dd, 1H, $J_{2',3'}$ = 9.9 Hz, $J_{3',4'}$ = 3.2 Hz, H-3′), 3.84 (t, 1H, $J$ = 6.8 Hz, H-5), 3.80 (s, 3H, $OCH_2C_6H_4OCH_3$), 3.78 (s, 3H, $OCH_2C_6H_4OCH_3$), 3.60 (dd, 1H, $J_{5',6a'}$ = 5.4 Hz, $J_{6a',6b'}$ = 10.3 Hz, H-6a′), 3.52 (dd, 1H, $J_{5',6b'}$ = 5.2 Hz, $J_{6a',6b'}$ = 10.3 Hz, H-6b′), 3.46 (dd, 2H, $J$ = 6.7, $J$ = 1.4 Hz, H-6), 3.30 (s, 3H, OC$H_3$), 2.90 (s, 1H, 4′-OH). $^{13}$C NMR (125 MHz, $CDCl_3$) δ 139.0, 138.5, 138.3, 138.2, 130.1, 129.4, 129.3, 128.4, 128.3, , 128.1, 128.0, 127.9, 127.7, 127.6, 127.6, 127.2, 113.8, 113.7, 98.4, 96.5, 77.7, 76.0, 75.8, 75.7, 74.8, 74.7, 74.2, 73.1, 73.0, 72.9, 71.8, 69.5, 69.2, 68.6, 68.3, 68.1, 55.3, 55.2, 55.2. ESI HRMS calcd. for $C_{57}H_{64}O_{13}Na$ (M+Na): 979.4239, found 979.4239.

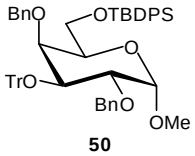



**49**

**Methyl 2-azido-4-benzylamino-4-*N*-,3-*O*-carbonyl-2,4,6-trideoxy-α-D-galactopyranosyl-(1→4)2,3-di-*O*-benzyl-6-*O*-*p*-methoxybenzyl-α-D-galactopyranosyl-(1→3)-2,4-di-*O*-benzyl-6-*O*-*p*-methoxybenzyl-α-D-galactopyranoside (49)**

The acceptor **48** (0.44 g, 0.46 mmol) and imidate **24** (0.31 g, 0.69 mmol, 1.5 eq) were combined and concentrated from dry toluene and dried *in vacuo* over night. The residue was dissolved in freshly distilled DCM (15 mL) and the mixture was stirred under argon at room temperature in the presence of powdered 4 Å molecular sieves for 30 minutes before being cooled to 0 $^{o}$C. TMSOTf (5.0 μL, 0.09 mmol, 0.05 eq) was added and the reaction mixture was stirred for 1 hour at 0 $^{o}$C and then it was allowed to warm to room temperature. The reaction mixture was quenched by addition of TEA and stirred for an additional 20 min, the mixture was diluted, filtered through Celite and the filtrate was concentrated. The residue was subjected to silica gel column chromatography using a stepped gradient eluent (20-30% EtOAc in hexanes) to obtain the trisaccharide **49** as a colourless syrup (exclusively α, 0.40 g, 70%). $[\alpha]_D$ +90.8 (*c* 1.0, $CH_2Cl_2$); $^1$H NMR (498 MHz, $CDCl_3$) δ 7.38 – 7.09 (m, 31H, ArH), 7.09 (s, 1H, ArH), 6.92 – 6.76 (m, 4H, ArH), 5.13 (s, 1H, H-1′), 5.03 (d, 1H, $J_{1",2"}$ = 4.2 Hz, H-1"), 4.93 (d, 1H, $J_{gem}$ = 11.7 Hz, PhC$H_2$) 4.91 (d, 2H, $J_{gem}$ = 15.5 Hz, PhC$H_2$), 4.75 (d, 1H, $J_{gem}$

= 8.0 Hz, PhC$H_2$), 4.72 (d, 1H, $J_{gem}$ = 7.0 Hz, PhC$H_2$), 4.64 – 4.57 (m, 4H, H-1, PhC$H_2$), 4.53 (dd, 1H, $J_{2",3"}$ = 6.3 Hz, $J_{3",4"}$ = 7.8 Hz, H-3"), 4.46 (d, 1H, $J_{gem}$ = 12.2 Hz, PhC$H_2$), 4.41 (d, 1H, $J_{gem}$ = 11.5 Hz, PhC$H_2$), 4.36 – 4.17 (m, 7H, H-5", H-4′, H-5′, H-3′, 3 × PhC$H_2$), 4.07 (dd, 1H, $J_{2,3}$ = 10.0 Hz, $J_{3,4}$ = 2.8 Hz, H-3), 4.05 (d, 1H, $J_{gem}$= 15.0 Hz, PhC$H_2$), 3.92 (dd, 1H, $J_{1,2}$ = 3.7 Hz, $J_{2,3}$ = 10.2 Hz, H-2), 3.91 – 3.88 (m, 2H, PhC$H_2$, H-2′), 3.87 (d, 1H, $J_{4,5}$ = 2.3 Hz, H-4), 3.81 – 3.77 (m, 7H, H-5, 2 OC$H_3$), 3.76 (dd, 1H, $J_{1",2"}$ = 3.9 Hz, $J_{2",3"}$ = 6.2 Hz, H-2"), 3.66 (dd, 1H, $J_{3",4"}$ = 8.0 Hz, $J_{4",5"}$ = 3.0 Hz, H-4"), 3.50 (t, 1H, $J_{6a',6b'}$ = 9.0 Hz, H-6a′), 3.47 – 3.41 (m, 3H, 2 H-6, H-6b′), 3.28 (s, 3H, OC$H_3$), 1.03 (d, 3H, $J_{5",6"}$ = 6.8 Hz, H-6" C$H_3$). $^{13}$C NMR (125 MHz, $CDCl_3$) δ 159.3, 159.2, 158.7, 139.0, 138.6, 138.5, 138.1, 135.7, 130.1 , 130.0, 129.6, 129.3, 129.0, 128.4 – 127.1 (m), 113.9, 113.8, 98.5, 96.5, 96.5, 76.6, 76.1, 75.8, 75.5, 74.9, 74.7, 73.9, 73.7, 73.1, 73.0, 72.9, 72.5, 72.0, 69.2, 68.6, 68.6, 66.7, 64.9, 58.4, 55.3, 55.2, 48.7, 17.4. ESI HRMS calcd. for $C_{71}H_{78}O_{16}N_4Na$ (M+Na): 1265.5305, found 1265.5305.



**50**

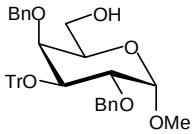
**Methyl 2,4-di-*O*-benzyl-3-*O*-trityl-6-*O*-*tert*-butyldiphenylsilyl-α-D-galactopyranoside (50)**

**Trityl perchlorate preparation**[174]

Triphenylcarbinol (4.0 g, 15.36 mmol) was dissolved in acetic anhydride (10.0 mL, 97.95 mmol) and toluene (10 mL) under argon atmosphere. 70% perchloric acid (2 mL, 13.93 mmol) was added in portions at 0 $^{o}$C. The residue

was filtered under vacuum. The crystalline residue was rinsed with dry diethylether (5 × 25 mL) to afford triphenylmethyl perchlorate as yellow crystal (4.47 g, 85%), which were further dried *in vacuo*.

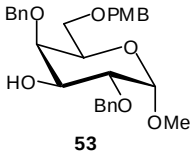
To a solution of methyl galactoside **36** (0.25 g, 0.41 mmol) and 2,4,6-collidine (0.75 mL, 0.62 mmol, 1.5 eq) in DCM (7 mL) was added freshly prepared trityl perchlorate (0.15 g, 0.45 mmol, 1.1 eq).[175] After 2 hours, the decolorized reaction mixture was diluted with DCM (20 mL) and washed with water (3 × 20 mL). The combined organic layer was dried over $Na_2SO_4$, filtered and concentrated. The residue was purified by column chromatography (hexanes-EtOAc, 3:1) to give **40** (0.34 g, 98%) as a white soild. $[\alpha]_D$ +68.1 (*c* 0.7, $CH_2Cl_2$); $^1$H NMR (498 MHz, $CDCl_3$) δ 7.74 – 7.47 (m, 8H, ArH), 7.46 – 7.14 (m, 27H, ArH), 4.91 – 4.87 (d, 1H, $J_{gem}$ = 11.9 Hz, PhC$H_2$), 4.81 – 4.77 (d, 1H, $J_{gem}$ = 11.9 Hz, PhC$H_2$), 4.67 – 4.62 (d, 1H, $J_{1,2}$ = 3.2 Hz, H-1), 4.54 (d, 1H, $J_{gem}$ = 11.7 Hz, PhC$H_2$), 4.45 – 4.41 (dd, 1H, $J_{2,3}$ = 10.0 Hz, $J_{3,4}$ = 2.4 Hz, H-3), 4.30 – 4.18 (dd, 1H, $J_{1,2}$ = 3.3 Hz, $J_{2,3}$ = 10.2 Hz, H-2), 4.06 – 3.93 (d, 1H, $J_{gem}$ = 11.7 Hz, PhC$H_2$), 3.50 – 3.39 (m, 3H, H-5, H-6), 3.24 – 3.20 (s, 3H, OC$H_3$), 2.67 – 2.55 (d, 1H, $J_{3,4}$ = 2.4 Hz, H-4), 1.02 – 0.98 (s, 9H, C(C$H_3$)$_3$). $^{13}$C NMR (125 MHz, $CDCl_3$) δ 146.9, 144.8, 139.2, 138.4, 135.5, 133.3, 133.2, 129.7, 129.6, 128.5, 128.4, 128.3, 128.1, 127.9, 127.7, 127.6, 127.6, 127.6, 127.3, 127.3, 127.2, 127.2, 127.1, 98.9, 87.4, 76.6, 75.7, 74.6, 73.6, 73.0, 70.8, 62.6, 55.0, 26.9, 19.1. ESI HRMS calcd. for $C_{56}H_{58}O_6SiNa$ (M+Na): 877.3895, found 877.3886.




**51**

**Methyl 2,4-di-*O*-benzyl-3-*O*-trityl-α-D-galactopyranoside (51)**

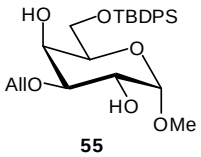
To a stirred solution of the fully protected methyl 2,4-di-*O*-benzyl-3-*O*-trityl-6-*O*-*tert*-butyldiphenylsilyl-α-D-galactopyranoside **50** (0.51 g, 0.6 mmol) in dry THF (8 mL) was added TBAF (1.0 M in THF, 0.25 mL, 0.9 mmol, 1.5 eq). After 4 hours, the reaction mixture was diluted with EtOAc (10 mL) and washed with an equal volume of saturated $NaHCO_3$ solution, water and brine. The organic phase was dried over $Na_2SO_4$, filtered and the filtrate was concentrated. Column chromatography on silica gel using hexanes and EtOAc (3:2) afforded **51** (0.35 g, 95%) as a white solid. $[\alpha]_D$ +127.5 (*c* 0.5, $CH_2Cl_2$); $^1$H NMR (600 MHz, $CDCl_3$) δ 7.67 – 7.60 (m, 4H, ArH), 7.50 – 7.46 (m, 2H, ArH), 7.46 – 6.99 (m, 19H, ArH), 4.93 (d, 1H, $J_{gem}$ = 12.1 Hz, PhC$H_2$), 4.84 (d, 1H, $J_{gem}$ = 12.1 Hz, PhC$H_2$), 4.74 (d, 1H, $J_{1,2}$ = 3.2 Hz, H-1), 4.63 (d, 1H, $J_{gem}$ = 11.2 Hz, PhC$H_2$), 4.36 (dd, 1H, $J_{2,3}$ = 10.1 Hz, $J_{3,4}$ = 2.1 Hz, H-3), 4.30 (dd, 1H, $J_{1,2}$ = 3.2 Hz, $J_{2,3}$ = 10.1 Hz, H-2), 3.93 (d, 1H, $J_{gem}$ = 11.2 Hz, PhC$H_2$), 3.56 – 3.47 (ddd, 1H, $J_{5,6a}$ = 7.1 Hz, $J_{6a,6b}$ = 11.2 Hz, $J_{6a,6OH}$ = 2.6 Hz, H-6a), 3.35 (dd, 1H, $J_{5,6a}$ = 7.1 Hz, $J_{5,6b}$ = 4.4 Hz, H-5), 3.28 (s, 3H, OC$H_3$), 3.23 (dd, 1H, $J_{5,6b}$ = 4.4 Hz, $J_{6a,6b}$ = 12.5 Hz, $J_{6b,6OH}$ = 9.2 Hz, H-6b), 2.26 (dd, 1H, $J_{3,4}$ = 2.1 Hz, $J_{4,5}$ = 1.0 Hz, H-4), 2.16 (dd, 1H, $J_{6OH-6a}$ = 3.0 Hz, $J_{6OH-6b}$ = 9.3 Hz, 6-OH). $^{13}$C NMR (125 MHz, $CDCl_3$) δ 144.7, 138.7, 138.2, 129.7, 129.6, 128.5, 128.4, 128.2, 127.8, 127.7, 127.5, 127.2, 99.0, 87.6, 77.6, 75.3, 74.6, 73.6, 73.2, 70.6, 62.9, 55.2. ESI HRMS calcd. for $C_{40}H_{40}O_6Na$ (M+Na): 639.2717, found 639.2719.




**53**

**Methyl 2,4-di-*O*-benzyl-6-*O*-*p*-methoxybenzyl-α-D-galactopyranoside (53)**

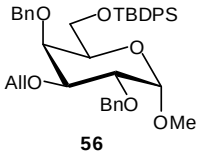
To a solution of **58** (0.48 g, 0.91 mmol) in acetic acid (10 mL) and water (10 drops) were added $PdCl_2$ (0.32 g, 1.82 mmol, 2 eq) and NaOAc (0.32 g, 3.91 mmol, 4.3 eq) at room temperature with stirring. After 7 hours the reaction mixture was filtered through Celite. The filtrate was diluted with EtOAc (15 mL) and then washed with an equal volume of saturated aqueous $NaHCO_3$ solution, brine, and water. The organic phase was dried over $Na_2SO_4$, filtered and then concentrated to dryness. The residue was chromatographed using hexanes-EtOAc (7:3) as eluent to give **19** (0.39 g, 87%) as a syrup. $[\alpha]_D$ +63.7 (*c* 1.3, $CH_2Cl_2$); $^1$H NMR (600 MHz, $CDCl_3$) δ 7.39 – 7.25 (m, 11H, ArH), 7.24 – 7.18 (m, 2H, ArH), 6.88 – 6.83 (m, 2H, ArH), 4.80 (d, 1H, $J_{gem}$ = 11.6 Hz, PhC$H_2$), 4.70 (d, 1H, $J_{gem}$ = 9.1 Hz, PhC$H_2$), 4.69 (s, 1H, H-1), 4.66 (d, 1H, $J_{gem}$ = 12.0 Hz, PhC$H_2$), 4.61 (d, 1H, $J_{gem}$ = 11.6 Hz, PhC$H_2$), 4.46 (d, 1H, $J_{gem}$ = 11.4 Hz, PhC$H_2$), 4.37 (d, 1H, $J_{gem}$ = 11.4 Hz, PhC$H_2$), 4.04 (ddd, 1H, $J_{2,3}$ = 10.0 Hz, $J_{3,3OH}$ = 4.8 Hz, $J_{3,4}$ = 3.3 Hz, H-3), 3.92 (m, 2H, H-5, H-4), 3.81 – 3.76 (dd, 1H, $J_{1,2}$ = 3.6 Hz, $J_{2,3}$ = 9.8 Hz, H-2), 3.79 (m, 3H, $OCH_2C_6H_4OCH_3$), 3.56 (dd, 1H, $J_{5,6a}$ = 6.9 Hz, $J_{6a,6b}$ = 9.4 Hz, H-6a), 3.53 (dd, 1H, $J_{5,6b}$ = 6.1 Hz, $J_{6b,6a}$ = 9.4 Hz, H-6b), 3.33 (s, 3H, OC$H_3$), 2.22 (d, 1H, $J_{3H,3OH}$ = 4.9 Hz). $^{13}$C NMR (125 MHz, $CDCl_3$) δ 159.3, 138.5, 138.1, 130.0, 129.4, 128.5, 128.4, 128.2, 128.1, 128.0, 127.7, 113.8, 113.75, 98.0, 77.4, 76.6, 75.1, 73.1, 73.0, 70.2, 69.0, 68.6, 55.4, 55.3. ESI HRMS calcd. for $C_{29}H_{34}O_7Na$ (M+Na): 517.2196, found 517.2196.




**55**

**Methyl 3-*O*-allyl-6-*O*-*tert*-butyldiphenylsilyl-α-D-galactopyranoside (55)**

A mixture of methyl 6-*O*-*tert*-butyldiphenylsilyl-α-D-galactopyranoside **34** (0.51 g, 1.18 mmol) and dibutyltin oxide (0.32 g, 1.30 mmol, 1.1 eq) in toluene (15 mL) was heated at reflux for 3 hours with azeotropic removal of water. The solution was concentrated to 10 mL by continued evaporation and was cooled to 60 $^{o}$C. TBAI (0.47 g, 1.30 mmol, 1.1 eq) and allyl bromide (0.16 mL, 1.30 mmol, 1.1 eq) were added to the solution. The reaction mixture was heated at 60 $^{o}$C overnight. It was then evaporated under reduced pressure and the residue was purified by silica gel column chromatography using a stepped gradient (20-30% EtOAc in hexanes) to give compound **55** (0.33 g, 60%). $[\alpha]_D$ +88.8 (*c* 0.4, $CH_2Cl_2$);$^1$H NMR (600 MHz, $CDCl_3$) δ 7.73 – 7.67 (m, 4H, ArH), 7.46 – 7.36 (m, 6H, ArH), 5.97 (m, 1H, $OCH_2CH$=$CH_2$), 5.37 – 5.28 (ddd, 1H, $J_{trans}$ = 17.2 Hz, $J_{gem}$ = 3.1 Hz, $^4J$ = 1.5 Hz, $OCH_2CH$=C*H*), 5.26 – 5.19 (ddd, 1H, $J_{cis}$ = 10.3 Hz, $J_{gem}$ = 2.8 Hz, $^4J$ = 1.2 Hz, $OCH_2CH$=C*H*), 4.81 (d, 1H, $J_{1,2}$ = 4.0 Hz, H-1), 4.20 (m, 2H, O*H*$_2$CH=$CH_2$), 4.15 – 4.12 (m, 1H, H-4), 3.99 – 3.92 (m, 2H, H-2, H-6), 3.87 (dd, 1H, $J_{5,6b}$ = 5.8 Hz, $J_{6a,6b}$ = 10.4 Hz, H-6b), 3.77 (app t, 1H, $J_{4,5}$ = 5.9 Hz, $J_{5,6b}$ = 5.9 Hz, H-5), 3.54 (dd, 1H, $J_{2,3}$ = 9.7 Hz, $J_{3,4}$ = 3.2 Hz, H-3), 3.39 (s, 3H, O*H*$_3$), 2.48 – 2.45 (s, 1H, 4-OH), 2.07 (s, 1H, 2-OH), 1.10 – 1.05 (s, 9H, C(C*H*$_3$)$_3$). $^{13}$C NMR (125 MHz, $CDCl_3$) δ 135.6, 135.6, 134.6, 133.3, 133.2, 129.7, 129.7, 127.8, 117.8, 99.4, 78.2, 70.9, 70.1, 68.5, 66.8, 63.3, 55.2, 26.8, 19.2. ESI HRMS calcd. for $C_{26}H_{36}O_6SiNa$ (M+Na): 495.2173, found 495.2176.

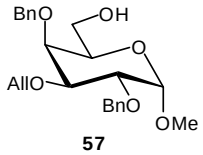



**56**

**Methyl 3-*O*-allyl-2,4-di-*O*-benzyl-6-*O*-*tert*-butyldiphenylsilyl-α-D-galactopyranoside (56)**

To a stirred solution of methyl galactopyranoside **55** (0.40 g, 0.85 mmol) in dry DMF (7 mL), NaH (dispersion in mineral oil; 60% by mass; 0.073 g, 1.78 mmol, 2.1 eq) was added in portions at 0 °C and stirring was continued until the evolution of gas had ceased. After 1 hour, BnBr (0.30 mL, 1.78 mmol, 2.1 eq) was slowly added to the above mixture, which was then warmed to room temperature and stirred for a further 4 hours. The reaction mixture was then quenched with MeOH. The solution was diluted with DCM (20 mL) and washed with an equal volume of water and brine. The organic extract was dried over $Na_2SO_4$, filtered and concentrated to a light yellow residue. Chromatography on silica gel with hexanes-EtOAc (4:1) as eluent gave **38** (0.48 g, 87%) as a white solid. $[\alpha]_D$ +76.2 (*c* 0.4, $CH_2Cl_2$); $^1$H NMR (600 MHz, $CDCl_3$) δ 7.66 – 7.60 (m, 4H, ArH), 7.47 – 7.20 (m, 16H, ArH), 6.03 – 5.95 (m, 1H, $OCH_2CH$=$CH_2$), 5.37 (ddd, 1H, $J_{trans}$ = 17.2 Hz, $J_{gem}$ = 3.5 Hz, $^4J$ = 1.7 Hz, $OCH_2CH$=C*H*), 5.20 (ddd, 1H, $J_{cis}$ = 10.6 Hz, $J_{gem}$ = 3.2 Hz, $^4J$ = 1.4 Hz, $OCH_2CH$=C*H*), 4.95 (d, 1H, $J_{gem}$ = 11.4 Hz, PhC$H_2$), 4.82 (d, 1H, $J_{gem}$ = 12.1 Hz, PhC$H_2$), 4.67 (d, 1H, $J_{gem}$ = 12.1 Hz, PhC$H_2$), 4.63 (d, 1H, $J_{1,2}$ = 3.7 Hz, H-1), 4.61 (d, 1H, $J_{gem}$ = 11.4 Hz, PhC$H_2$), 4.31 (ddt, 1H, $J_{gem}$ = 13.0 Hz, $J_{vic}$ = 5.1 Hz, $^4J$ = 1.5 Hz, OC$H_2$CH=$CH_2$), 4.23 (ddt, 1H, $J_{gem}$ = 13.0 Hz, $J_{vic}$ = 5.5 Hz, $^4J$ = 1.4 Hz, OC$H_2$CH=$CH_2$), 3.97 (d, 1H, $J_{3,4}$ = 2.9 Hz, H-4), 3.95 (dd, 1H, $J_{1,2}$ = 3.7 Hz, $J_{2,3}$ = 10.1 Hz, H-2), 3.80 (dd, 1H,

$J_{2,3}$ = 10.1 Hz, $J_{3,4}$ =2.9 Hz, H-3), 3.75 – 3.68 (m, 3H, H-6, H-5), 3.28 (s, 3H, OC$H_3$), 1.10 – 1.03 (m, 9H, C(C$H_3$)$_3$). $^{13}$C NMR (125 MHz, $CDCl_3$) δ 138.7, 138.6, 135.5, 135.3, 133.4, 129.7, 129.68, 128.3, 128.1, 128.0, 127.7, 127.68, 127.6, 127.4, 116.3, 98.7, 78.8, 76.3, 74.8, 74.7, 73.5, 71.8, 70.7, 62.7, 55.0, 26.9, 19.2. ESI HRMS calcd. for $C_{40}H_{48}O_6SiNa$ (M+Na): 675.3112, found 675.3113.

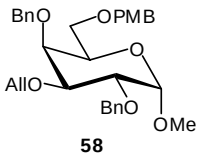


**57**

**Methyl 3-*O*-allyl-2,4-di-*O*-benzyl-α-D-galactopyranoside (57)**

A solution of silyl ether **56** (0.48 g, 0.73 mmol) in dry THF (10 mL) was treated with TBAF (1.0 M in THF; 0.23 mL, 0.88 mmol, 1.2 eq) and stirred at room temperature for 6 hours. The solution was then diluted with EtOAc (15 mL), washed with water, brine and the organic phase was dried over sodium sulfate, filtered, and the filtrate concentrated *in vacuo*. The residue was purified by silica gel column chromatography to give compound **57** (0.28 g, 92 %) as colourless oil. $[\alpha]_D$ +71.1 (*c* 2.3, $CH_2Cl_2$); $^1$H NMR (600 MHz, $CDCl_3$) δ 7.42 – 7.25 (m, 10H, ArH), 6.00 (m, 1H, $OCH_2CH$=$CH_2$), 5.37 (ddd, 1H, $J_{trans}$ = 17.2 Hz, $J_{gem}$ = 3.3 Hz, $^4J$ = 1.6 Hz, $OCH_2CH$=C*H*), 5.21 (ddd, 1H, $J_{cis}$ = 10.6 Hz, $J_{gem}$ = 3.3 Hz, $^4J$ = 1.4 Hz, $OCH_2CH$=C*H*), 4.98 (d, 1H, $J_{gem}$ = 11.2 Hz, PhC$H_2$), 4.84 (d, 1H, $J_{gem}$ = 12.1 Hz, PhC$H_2$), 4.70 (s, 1H, H-1), 4.67 (d, 1H, $J_{gem}$ = 12.1 Hz, PhC$H_2$), 4.61 (d, 1H, $J_{gem}$ = 11.2 Hz, PhC$H_2$), 4.33 (ddt, 1H, $J_{gem}$ = 13.0 Hz, $J_{vic}$ = 5.6 Hz, $^4J$ = 1.4 Hz, OC$H_2$CH=$CH_2$), 4.23 (ddt, 1H, $J_{gem}$ = 13.0 Hz, $J_{vic}$ = 5.6 Hz, $^4J$ = 1.4 Hz, OC$H_2$CH=$CH_2$), 4.00 (dd, 1H, $J_{1,2}$ = 3.7 Hz, $J_{2,3}$ = 10.1 Hz, H-2), 3.89 (d, 1H $J_{4,5}$

= 2.7 Hz, H-5), 3.83 (dd, 1H, $J_{2,3}$ = 10.1 Hz, $J_{3,4}$ = 2.9 Hz, H-3), 3.77 – 3.69 (m, 2H, H-4, H-6a), 3.55 – 3.46 (m, 1H, H-6b), 3.37 (s, 3H, OC$H_3$), 1.68 (dd, 1H, $J_{6a,6OH}$ = 9.0 Hz, $J_{6b,6OH}$ = 2.8 Hz, 2-OH). $^{13}$C NMR (125 MHz, $CDCl_3$) δ 138.5, 138.2, 135.1, 128.6, 128.5, 128.3, 128.1, 128.0, 127.7, 116.6, 98.9, 78.7, 76.2, 74.8, 74.4, 73.6, 72.1, 70.2, 62.5, 55.3. ESI HRMS calcd. for $C_{24}H_{30}O_6Na$ (M+Na): 437.1935, found 437.1935.

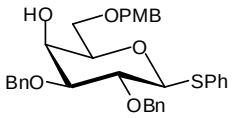



**58**

**Methyl 3-*O*-allyl-2,4-di-*O*-benzyl-6-*O*-*p*-methoxybenzyl-α-D-galactopyranoside (58)**

Alcohol **57** (0.28 g, 0.68 mmol) was dissolved in DMF (5 mL) and treated with NaH (dispersion in mineral oil; 60% by mass; 0.03 g, 0.75 mmol, 1.1 eq) in portions at 0 $^{o}$C. The reaction was stirred at room temperature for 1 hour and then PMBBr (0.15 mL, 0.75 mmol, 1.1 eq) was added dropwise and stirring was continued at room temperature for a further 5 hours. Unreacted NaH was quenched by slow addition of MeOH at 0 $^{o}$C. The reaction mixture was diluted with DCM (10 mL), and the organic phase was washed with water, brine, and the organic phase was dried over $MgSO_4$. The filtrate was concentrated under reduced pressure and the residue was purified by column chromatography (hexanes-EtOAc, 9:1) to give the product (0.31 g, 89%) as a colourless syrup. $[\alpha]_D$ +38.3 (*c* 2.2, $CH_2Cl_2$); $^1$H NMR (600 MHz, $CDCl_3$) δ 7.40 – 7.27 (m, 8H, ArH), 7.27 – 7.00 (m, 5H, ArH), 6.93 – 6.82 (m, 2H, ArH), 5.96 (m, 1H,

$OCH_2CH{=}CH_2$), 5.35 (ddd, 1H, $J_{trans}$ = 17.2 Hz, $J_{gem}$ = 3.5 Hz, $^4J$ = 1.7 Hz, $OCH_2CH{=}CH$), 5.18 (ddd, 1H, $J_{cis}$ = 10.4 Hz, $J_{gem}$ = 3.2 Hz, $^4J$ = 1.4 Hz, $OCH_2CH{=}CH$), 4.93 (d, 1H, $J_{gem}$ = 11.4 Hz, $PhCH_2$), 4.83 (d, 1H, $J_{gem}$ = 12.1 Hz, $PhCH_2$), 4.69 (d, 1H, $J_{gem}$ = 15.2 Hz, $PhCH_2$), 4.67 (s, 1H, H-1), 4.56 (d, 1H, $J_{gem}$ = 11.4 Hz, $PhCH_2$), 4.43 (d, 1H, $J_{gem}$ = 11.4 Hz, $PhCH_2$), 4.34 (d, 1H, $J_{gem}$ = 11.4 Hz, $PhCH_2$), 4.28 (ddt, 1H, $J_{gem}$ = 13.0 Hz, $J_{vic}$ = 5.1 Hz, $^4J$ = 1.6 Hz, $OCH_2CH{=}CH_2$), 4.20 (ddt, 1H, $J_{gem}$ = 13.0 Hz, $J_{vic}$ = 5.5 Hz, $^4J$ = 1.5 Hz, $OCH_2CH{=}CH_2$), 3.97 (dd, 1H, $J_{1,2}$ = 3.7 Hz, $J_{2,3}$ = 10.1 Hz, H-2), 3.94 – 3.90 (dd, 1H, $J_{3,4}$ = 3.0 Hz, $J_{4,5}$ = 1.1 Hz, H-4), 3.88 (ddd, 1H, $J_{4,5}$ = 1.1 Hz, $J_{5,6a}$ = 6.5 Hz, $J_{5,6b}$ = 6.5 Hz, H-5), 3.81 (dd, H, $J_{2,3}$ = 10.1 Hz, $J_{3,4}$ =2.9 Hz, H-3), 3.80 (s, 3H, $OCH_2C_6H_4OCH_3$), 3.53 (dd, 1H, $J_{5,6a}$ = 7.0 Hz, $J_{6a,6b}$ = 9.3 Hz, H-6a), 3.49 (dd, 1H, $J_{5,6b}$ = 5.9 Hz, $J_{6a,6b}$ = 9.3 Hz, H-6b), 3.39 – 3.31 (m, 3H, $OCH_3$). $^{13}$C NMR (125 MHz, $CDCl_3$) δ 159.3, 138.8, 138.6, 135.1, 130.1, 129.5, 128.3, 128.2, 128.2, 128.0, 127.6, 127.5, 116.3, 113.8, 98.9, 78.6, 76.2, 75.0, 74.78, 73.6, 73.2, 71.8, 69.2, 68.7, 55.3, 55.3. ESI HRMS calcd. for $C_{32}H_{38}O_7Na$ (M+Na): 557.2510, found 557.2510.

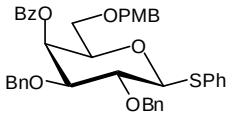



**59**

**Phenyl 2,3-di-*O*-benzyl-6-*O*-*p*-methoxybenzyl-1-thio-β-D-galactopyranoside (59)**

TFA (0.78 mL, 7.01 mmol, 5 eq) was added dropwise to a cooled (0 $^{o}$C) solution of phenyl 2,3-di-*O*-benzyl-4,6-*O*-*p*-methoxybenzylidene-1-thio-β-D-galactopyranoside **63** (0.8 g, 1.40 mmol) and $NaCNBH_3$ (0.44 g, 7.01 mmol, 5 eq) in dry THF (10 mL) containing activated 4 Å molecular sieves (0.20 g). The solution was then stirred at room temperature until TLC showed complete consumption of starting material. The reaction was quenched with TEA and then it was diluted with EtOAc (20 mL). Molecular sieves were removed by filtering through celite. The filtrate was washed with an equal volume of saturated aqueous $NaHCO_3$ solution and brine. The combined organic phase was dried over $Na_2SO_4$, filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (hexanes-EtOAc, 8:2) to give **59** (0.60 g, 75%) as a white solid. $[\alpha]_D$ +0.5 (*c* 1.5, $CH_2Cl_2$); $^1$H NMR (600 MHz, $CDCl_3$) δ 7.61 – 7.56 (m, 2H, ArH), 7.44 – 7.39 (m, 2H, ArH), 7.38 – 7.22 (m, 13H, ArH), 6.91 – 6.85 (m, 2H, ArH), 4.84 (d, 1H, $J_{gem}$ = 10.3 Hz, Ph*CH*$_2$), 4.76 (d, 1H, $J_{gem}$ = 10.3 Hz, PhC*H*$_2$), 4.73 (d, 1H, $J_{gem}$ = 11.6 Hz, PhC*H*$_2$), 4.69 (d, 1H, $J_{gem}$ = 10.3 Hz, PhC*H*$_2$), 4.64 (d, 1H, $J_{1,2}$ = 9.8 Hz, H-1), 4.51 (s, 2H, PhC*H*$_2$), 4.11 (d, 1H, *J* = 3.2 Hz, H-4), 3.81 (s, 3H, $OCH_2C_6H_4OCH_3$), 3.81 – 3.73 (m, 3H, H-2, H-6), 3.58 (m, 2H, H-5, H-3). $^{13}$C NMR (125 MHz, $CDCl_3$) δ 159.3, 138.2, 137.7, 133.9, 131.8, 130.0, 129.5, 128.9, 128.5, 128.4, 128.3, 128.0, 127.9, 127.8, 127.3, 113.8, 87.7,

82.6, 77.3, 75.8, 73.4, 72.1, 69.1, 66.9, 55.3. ESI HRMS calcd. for $C_{34}H_{36}O_6SNa$ (M+Na): 595.2125, found 595.2123. Anal. Calcd. for $C_{34}H_{36}O_6S$: C, 71.30; H, 6.34. Found: C, 70.85; H, 6.34.

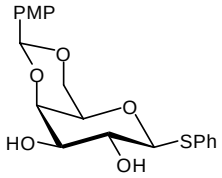



**61**

**Phenyl 4-*O*-benzoyl-2,3-di-*O*-benzyl-6-*O*-*p*-methoxybenzyl-1-thio-β-D-galactopyranoside (61)**

Compound **59** (1.71 g, 2.98 mmol) was dissolved in dry pyridine (25 mL) under an argon atmosphere. Benzoyl chloride (0.50 mL, 3.58 mmol, 1.2 eq) was added at room temperature and after 7 hours the reaction mixture was quenched by addition of MeOH. After stirring for 25 minutes the solution was concentrated under reduced pressure. The residue was dissolved in DCM, washed with saturated $NaHCO_3$ solution and brine. Then, the DCM solution was dried over $Na_2SO_4$, filtered and concentrated to dryness. Chromatography of the residue using hexanes-EtOAc (17:3) afforded the benzoylated galactoside **61** (1.86 g, 92%). $[\alpha]_D$ +7.2 (*c* 1.3, $CH_2Cl_2$); $^1$H NMR (498 MHz, $CDCl_3$) δ 8.11 (d, 1H, $J$ = 7.4 Hz, ArH), 8.02 – 7.96 (m, 2H, ArH), 7.67 – 7.55 (m, 3H, ArH), 7.53 – 7.41 (m, 3H, ArH), 7.41 – 7.18 (m, 13H, ArH), 6.81 – 6.75 (m, 2H, ArH), 5.89 (dd, 1H, $J_{3,4}$ = 2.1 Hz, $J_{4,5}$ = 0.4 Hz, H-4), 4.85 (d, 1H, $J_{gem}$ = 11.2 Hz, PhC$H_2$), 4.73 (s, 2H, PhC$H_2$), 4.70 (d, 1H, $J_{1,2}$ = 9.5 Hz, H-1), 4.52 (d, 1H, $J_{gem}$ = 11.2 Hz, PhC$H_2$), 4.45 (d, 1H, $J_{gem}$ = 11.4 Hz, PhC$H_2$), 4.37 (d, 1H, $J_{gem}$ = 11.4 Hz, PhC$H_2$), 3.88 (app t, 1H, $J_{5,6a}$ = 6.3 Hz, $J_{5,6b}$ = 6.3 Hz, H-5), 3.78 – 3.73 (dd, H,

$J_{2,3}$ = 9.0 Hz, $J_{3,4}$ = 3.2 Hz, H-3), 3.74 (s, 3H, $OCH_2C_6H_4OCH_3$), 3.71 (app t, 1H, $J_{1,2}$ = 9.3 Hz, $J_{2,3}$ = 9.3 Hz, H-2), 3.68 – 3.63 (dd, 1H, $J_{5,6a}$ = 5.9 Hz, $J_{6a,6b}$ = 9.5 Hz, H-6a), 3.55 (dd, 1H, $J_{5,6b}$ = 5.9 Hz, $J_{6a,6b}$ = 9.3 Hz, H-6b). $^{13}$C NMR (125 MHz, $CDCl_3$) δ 165.6, 159.3, 138.3, 137.6, 133.1, 132.9, 132.8, 130.0, 129.8, 129.7, 129.6, 128.8, 128.3, 128.2, 128.1, 127.7, 127.6, 113.8, 87.2, 81.5, 76.5, 76.2, 75.7, 73.3, 71.8, 67.9, 67.3, 55.2. ESI HRMS calcd. for $C_{41}H_{40}O_7SNa$ (M+Na): 699.2387, found 699.2394. Anal. Calcd. for $C_{41}H_{40}O_7S$: C, 72.76; H, 5.96; O, 16.55, S, 4.74. Found: C, 73.10; H, 6.12; O, 16.87, S, 3.91.

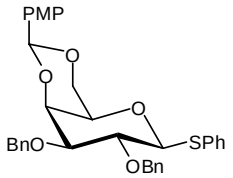



**62**

**Phenyl 4,6-*O*-*p*-methoxybenzylidene-1-thio-β-D-galactopyranoside (62)**

Phenyl-1-thio-β-D-glucopyranoside **28** (4.97 g, 18.4 mmol) was dissolved in dry $CH_3CN$ (30 mL). *p*-Anisaldehyde dimethyl acetal (4.10 mL, 22.08 mmol, 1.2 eq) and a catalytic amount of camphorsulfonic acid were added. The reaction was stirred at room temperature for 3 hours. Then the reaction mixture was quenched by addition of TEA and concentrated under reduced pressure. The yellow residue was purified by column chromatography (5% MeOH in DCM) to afford **62** (6.61 g, 92%) as a white crystalline solid. $[\alpha]_D$ -7.5 (*c* 1.5, $CHCl_3$). $^1$H NMR (600 MHz, $CDCl_3$) δ 7.72 – 7.67 (m, 2H, ArH), 7.39 – 7.25 (m, 5H, ArH), 6.92 – 6.86 (m, 2H, ArH), 5.47 (s, 1H, $CHPhOCH_3$), 4.54 – 4.49 (d, 1H, $J_{1,2}$ = 11.2 Hz, H-1), 4.38 (dd, 1H, $J_{5,6a}$ = 1.6 Hz, $J_{6a,6b}$ = 12.4 Hz, H-6a), 4.21 (dd, 1H, *J*

= 2.9, $J$ = 1.1 Hz, H-4), 4.03 (dd, 1H, $J_{5,6b}$ = 1.8 Hz, $J_{6b,6a}$ = 12.5 Hz, H-6b), 3.82 (s, 3H, CHPhOC$H_3$), 3.70 (m, 2H, H-2, H-3), 3.55 (q, 1H, $J$ = 1.5 Hz, H-5), 2.50 (s, 2H, 2-OH, 3-OH). $^{13}$C NMR (125 MHz, $CDCl_3$) δ 160.3, 133.7, 130.2, 128.9, 128.2, 127.8, 113.6, 101.3, 87.0, 75.3, 73.8, 70.0, 69.3, 68.8, 55.3. ESI HRMS calcd. for $C_{20}H_{22}O_6SNa$ (M+Na): 413.1029, found 413.1026.

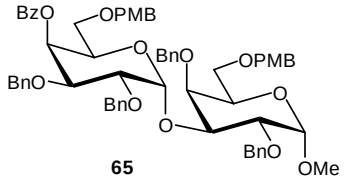



**63**

**Phenyl 2,3-di-*O*-benzyl-4,6-*O*-*p*-methoxybenzylidene-1-thio-β-D-galactopyranoside (63)**

To a solution of diol **62** (6.34 g, 16.25 mmol) in dry DMF (25 mL) at 0 $^{o}$C was added NaH (dispersion in mineral oil; 60% by mass; 1.18 g, 16.25 mmol, 3 eq) in portions under an inert argon atmosphere. After 1 hour, BnBr (8.45 mL, 16.25 mmol, 3 eq) was slowly added to the above mixture, which was then warmed to room temperature and stirred for a further 5 hours. The reaction mixture was then quenched by careful addition of MeOH. The solution was diluted with DCM (100 mL) and washed with an equal volume of water and brine. The organic extract was dried over $Na_2SO_4$, filtered and the filtrate was concentrated. Chromatography on silica gel with toluene-EtOAc (13:1) as eluent gave **38** (7.95 g, 85%). $[\alpha]_D$ 3.2 (*c* 0.6, $CH_2Cl_2$); $^1$H NMR (498 MHz, $CDCl_3$) δ 7.77 – 7.71 (m, 2H, ArH), 7.51 – 7.41 (m, 4H, ArH), 7.41 – 7.19 (m, 11H, ArH), 6.94 (t, 2H, $J$ = 5.6 Hz, ArH), 5.47 (s, 1H, C$H$PhOCH$_3$), 4.78 – 4.67 (m, 4H,

PhC$H_2$), 4.64 (d, 1H, $J_{1,2}$ = 9.6 Hz, H-1), 4.37 (d, 1H, $J_{6a,6b}$ = 12.2 Hz, H-6a), 4.16 (d, 1H, $J_{3,4}$ = 3.3 Hz, H-4), 4.00 – 3.96 (d, 1H, $J_{6a,6b}$ = 3.3 Hz, H-6b), 3.93 (app t, 1H, $J_{1,2}$ = 9.4 Hz, $J_{2,3}$ = 9.4 Hz, H-2), 3.86 (s, 3H, CHPhOC$H_3$), 3.64 (dd, 1H, $J_{2,3}$ = 9.2 Hz, $J_{3,4}$ = 3.4 Hz, H-3), 3.40 (s, 1H, H-5). $^{13}$C NMR (125 MHz, $CDCl_3$) δ 160.2, 138.6, 138.2, 132.9, 132.7, 130.6, 128.9, 128.4, 128.3, 128.2, 127.9, 127.9, 127.8, 127.8, 127.7, 127.5, 113.5, 101.3, 86.6, 81.4, 73.7, 71.8, 69.9, 69.4, 55.4. ESI HRMS calcd. for $C_{34}H_{34}O_6SNa$ (M+Na): 593.1968, found 593.1970.



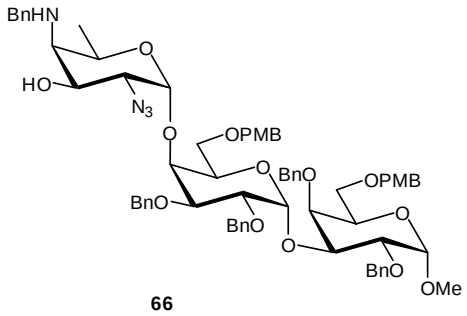
**65**

**Methyl 4-*O*-benzoyl-2,3-di-*O*-benzyl-6-*O*-*p*-methoxybenzyl-α-D-galactopyranosyl-(1→3)-2,4-di-*O*-benzyl-6-*O*-*p*-methoxybenzyl-α-D-galactopyranoside (65)**

Acceptor **53** (0.44 g, 0.88 mmol) and phenyl thiogalactoside **61** (0.77 g, 1.15 mmol, 1.3 eq) were combined and concentrated from toluene and dried *in vacuo* overnight. The residue was dissolved in freshly distilled DCM (25 mL) and the mixture was stirred under argon at room temperature in the presence of powdered 4 Å molecular sieves for 30 minutes before being cooled to -30 $^{o}$C. NIS (0.28 g, 1.01 mmol, 1.2 eq) was then added and stirring was continued for a further 30 minutes. After addition of AgOTf (0.02 g, 0.09 mmol, 0.1 eq) the reaction mixture turned deep purple, and the reaction mixture was then stirred for a further 1 hour at -30 $^{o}$C at which point the reaction was completed. The mixture was made basic by addition of TEA and stirred for an additional 20 min. The

mixture was diluted, filtered through Celite and washed with 5% $Na_2S_2O_3$ solution and dried over anhydrous $MgSO_4$. The filtrate was concentrated to a residue that was subjected to silica gel column chromatography using 8% EtOAc in toluene as eluent to obtain the disaccharide **65** as a white crystalline solid (exclusively α, 0.7 g, 70%). $[\alpha]_D$ +202.7 (*c* 0.4, $CH_2Cl_2$); $^1$H NMR (600 MHz, $CDCl_3$) δ 8.15 – 7.99 (m, 3H, ArH), 7.65 – 7.10 (m, 27H, ArH), 6.94 – 6.64 (m, 5H, ArH), 5.77 (d, 1H, $J$ = 2.0 Hz, H-4′), 5.26 (d, 1H, $J_{1',2'}$ = 3.3 Hz, H-1′), 4.99 (d, 1H, $J_{gem}$ = 11.5 Hz, PhC$H_2$), 4.83 (d, 2H, $J$ = 11.6 Hz, PhC$H_2$), 4.72 (d, 1H, $J_{gem}$ = 12.0 Hz, PhC$H_2$), 4.69 – 4.62 (m, 2H, PhC$H_2$), 4.61 – 4.50 (m, 3H, H-5′, H-1, PhC$H_2$), 4.43 (d, 1H, $J_{gem}$ = 11.5 Hz, PhC$H_2$), 4.37 (d, 1H, $J_{gem}$ = 11.5 Hz, PhC$H_2$), 4.33 (d, 1H, $J_{gem}$ = 11.5 Hz, PhC$H_2$), 4.30 (d, 1H, $J_{gem}$ = 11.5 Hz, PhC$H_2$), 4.27 (d, 1H, $J_{gem}$ = 11.5 Hz, PhC$H_2$), 4.18 (dd, 1H, $J_{2,3}$ = 10.2 Hz, $J_{3,4}$ = 2.5 Hz, H-3), 4.14 (dd, 1H, $J_{2',3'}$ = 10.0 Hz, $J_{3',4'}$ = 3.1 Hz, H-3′), 4.01 (dd, 1H, $J_{1,2}$ = 3.6 Hz, $J_{2,3}$ = 10.0 Hz, H-2), 3.99 (dd, 1H, $J_{1',2'}$ = 3.3 Hz, $J_{2',3'}$ = 8.8 Hz, H-2′), 3.95 (d, 1H, $J_{3,4}$ = 1.4 Hz, H-4), 3.85 (t, 1H, $J$ = 6.8 Hz, H-5), 3.81 (s, 3H, $OCH_2C_6H_4OCH_3$), 3.72 (s, 3H, $OCH_2C_6H_4OCH_3$), 3.50 (dd, $J_{5',6a'}$ = 6.1 Hz, 1H, $J_{6a',6b'}$ = 9.8 Hz, H-6a′), 3.47 (d, 2H, $J$ = 6.3 Hz, H-6), 3.39 (dd, $J_{5',6a'}$ = 6.8 Hz, 1H, $J_{6a',6b'}$ = 9.6 Hz, H-6b′), 3.31 (s, 3H, OC$H_3$). $^{13}$C NMR (125 MHz, $CDCl_3$) δ 165.8, 159.2, 159.0, 139.0, 138.6, 138.2, 138.2, 132.9, 130.2, 130.2, 130.1, 129.9, 129.8, 129.4, 129.4, 128.5, 128.4, 128.3, 128.3, 128.2, 128.2, 128.1, 128.1, 128.0, 128.0, 127.9, 127.8, 127.8, 127.7, 127.6, 127.4, 127.2, 113.8, 113.5, 98.5, 96.9, 76.4, 76.0, 75.8, 75.6, 74.8, 74.8, 74.3, 73.2, 73.0, 72.7, 71.3, 69.2, 68.7, 68.7,

67.9, 67.8, 55.3, 55.2, 55.1. ESI HRMS calcd. for $C_{64}H_{68}O_{14}Na$ (M+Na): 1083.4501, found 1083.4503.

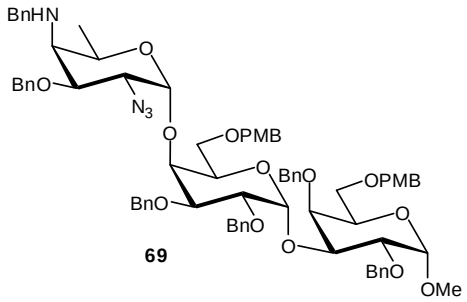



66

**Methyl 2-azido-4-benzylamino-2,4,6-trideoxy-α-D-galactopyranosyl-(1→4)-2,3-di-*O*-benzyl-6-*O*-*p*-methoxybenzyl-α-D-galactopyranosyl-(1→3)-2,4-di-*O*-benzyl-6-*O*-*p*-methoxybenzyl-α-D-galactopyranoside (66)**

To a solution of oxazolidinone derivative **49** (0.32 g, 0.26 mmol) in a mixture of THF and EtOH (2:1, v/v, 12 mL) were added 2 M LiOH (4 mL), and a catalytic amount of LiI. The mixture was heated under reflux for 4 days. It was then concentrated to dryness under reduced pressure. The residue was dissolved in DCM (15 mL) and washed with an equal volume of water and brine. The extract was dried over $Na_2SO_4$, filtered and the filtrate was concentrated. Column chromatography on silica gel using hexanes and EtOAc (3:1) afforded the title compound **66** (0.30 g, 95%) as a colourless syrup. $[\alpha]_D$ +150.1 (*c* 0.5, $CH_2Cl_2$); $^1$H NMR (600 MHz, $CDCl_3$) δ 7.49 – 7.05 (m, 31H, ArH), 6.88 – 6.79 (m, 4H, ArH), 5.22 (d, 1H, $J_{1',2'}$ = 3.3 Hz, H-1'), 4.95 (d, 1H, $J_{gem}$ = 11.3 Hz, PhC$H_2$), 4.86 – 4.79 (m, 2H, H-1", PhC$H_2$), 4.74 (d, 1H, $J_{gem}$ = 12.5 Hz, PhC$H_2$), 4.70 (d, 1H, $J_{gem}$ = 11.5 Hz, PhC$H_2$), 4.67 (d, 1H, $J_{gem}$ = 11.5 Hz, PhC$H_2$), 4.63 (d, 1H, $J_{gem}$ = 12.3 Hz, PhC$H_2$), 4.56 (d, 1H, $J_{1,2}$ = 3.6 Hz, H-1), 4.53 (q, 1H, $J_{5",6"}$ = 6.5 Hz, H-5"),

4.46 (d, 1H, $J_{gem}$ = 12.3 Hz, PhC$H_2$), 4.41 (d, 1H, $J_{gem}$ = 11.5 Hz, PhC$H_2$), 4.35 – 4.26 (m, 4H, PhC$H_2$), 4.23 (dd, 1H, $J_{5',6a'}$ = 5.8 Hz, $J_{5',6b'}$ = 9.1 Hz, H-5'), 4.15 (app s, 1H, H-4′), 4.10 (dd, 1H, $J_{2,3}$ = 9.1 Hz, $J_{3,4}$ = 5.7 Hz, H-3), 4.03 (d, 1H, $J_{gem}$ = 12.6 Hz, PhC$H_2$), 3.99 (dd, 1H, $J_{1',2'}$ = 3.3 Hz, $J_{2',3'}$ = 10.3 Hz, H-2′), 3.95 – 3.85 (m, 4H, H-3′, H-4, H-2, H-3"), 3.82 (m, 1H, H-5), 3.80 (s, 3H, $OCH_2C_6H_4OCH_3$), 3.78 – 3.68 (m, 5H, $OCH_2C_6H_4OCH_3$, PhC$H_2$, H-6a′), 3.50 (dd, 1H, $J_{5',6b'}$ = 5.7 Hz, $J_{6a',6b'}$ = 9.0 Hz, H-6b'), 3.43 (d, 2H, $J$ = 7.5 Hz, H-6), 3.27 (s, 3H, OC$H_3$), 2.90 (dd, 1H, $J_{1",2"}$ = 3.7 Hz, $J_{2",3"}$ = 10.0 Hz, H-2"), 2.78 (dd, 1H, $J$ = 4.3, 1.0 Hz, H-4"), 1.00 (d, 3H, $J_{5",6"}$ = 6.5 Hz, H-6" C$H_3$). $^{13}$C NMR (125 MHz, $CDCl_3$) δ 159.2, 140.0, 139.0, 138.6, 138.1, 130.2, 130.0, 129.7, 129.3, 128.7, 128.3, 128.0, 127.9, 127.6, 127.5, 127.4, 127.2, 127.2, 113.8, 113.7, 98.5, 98.3, 96.4, 76.9, 76.2, 76.0, 75.5, 74.9, 74.7, 73.9, 73.8, 73.1, 73.0, 72.5, 72.3, 69.2, 68.9, 68.6, 67.4, 66.5, 66.5, 62.4, 61.5, 55.5, 55.3, 55.2, 55.1, 17.3. ESI HRMS calcd. for $C_{70}H_{81}N_4O_{15}$ (M+H): 1217.5693, found 1217.5687.

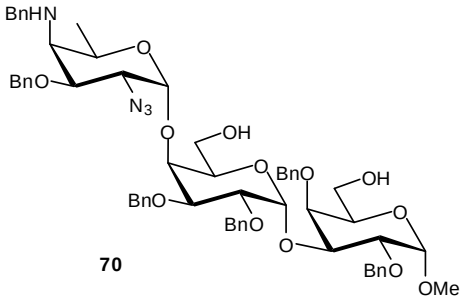



69

**Methyl 2-azido-3-benzyl-4-benzylamino-2,4,6-trideoxy-α-D-galactopyranosyl-(1→4)-2,3-di-*O*-benzyl-6-*O*-*p*-methoxybenzyl-α-D-galactopyranosyl-(1→3)-2,4-di-*O*-benzyl-6-*O*-*p*-methoxybenzyl-α-D-galactopyranoside (69)**

To a solution of **66** (0.125 g, 0.123 mmol) in dry DMF (4 mL) at 0 $^{o}$C was added NaH (dispersion in mineral oil; 60% by mass; 0.006 g, 0.18 mmol, 1.5 eq) in portions under an inert atmosphere of Argon. After 1 hour, BnBr (26.34 μL, 0.18 mmol, 1.5 eq) was slowly added to the above mixture, which was then warmed to room temperature and stirred for a further 5 hours. The reaction was then quenched by careful addition of MeOH. The solution was diluted with DCM (6 mL) and washed with an equal volume of water and brine. The organic extract was dried over $Na_2SO_4$, filtered and the filtrate was concentrated. Chromatography of the residue on silica gel with hexanes-EtOAc (17:3) as eluent gave **38** (0.107 g, 80%). $[\alpha]_D$ +47.0 (*c* 0.3, $CH_2Cl_2$); $^1$H NMR (600 MHz, $CDCl_3$) δ 7.42 – 6.96 (m, 36H, ArH), 6.88 – 6.81 (m, 4H, ArH), 5.23 (d, 1H, $J_{1',2'}$ = 3.2 Hz, H-1'), 4.95 (s, 1H, H-1''), 4.93 (s, 1H, PhC$H_2$), 4.80 (d, 1H, $J_{gem}$ = 11.7 Hz, PhC$H_2$), 4.77 (d, 1H, $J_{gem}$ = 12.7 Hz, PhC$H_2$), 4.71 (d, 1H, $J_{gem}$ = 11.6 Hz, PhC$H_2$), 4.67 (d, 1H, $J_{gem}$ = 12.7 Hz, PhC$H_2$), 4.64 (d, 1H, $J_{gem}$ = 12.3 Hz, PhC$H_2$), 4.56 (d, 1H, $J_{1,2}$ = 3.5 Hz, H-1), 4.49 – 4.40 (m, 4H, PhC$H_2$), 4.35 (q, 1H, $J_{5'',6''}$ = 6.4 Hz, H-5''), 4.33 – 4.28 (m, 4H, H-4′, PhC$H_2$), 4.22 (dd, 1H, $J_{5',6a'}$ =

6.0 Hz, $J_{5',6b'}$ = 6.1 Hz, H-5'), 4.18 (s, 1H, PhC$H_2$), 4.11 (dd, 1H, $J_{2,3}$ = 10.1 Hz, $J_{3,4}$ = 2.5 Hz, H-3), 3.97 (dd, 1H, $J_{1',2'}$ = 3.3 Hz, $J_{2',3'}$ = 10.3 Hz, H-2′), 3.92 (m, 5H, H-2, H-4, H-3′, H-3", PhC$H_2$), 3.84 – 3.74 (m, 9H, H-5, H-6a′, PhC$H_2$, 2 × $OCH_2C_6H_4OCH_3$,), 3.56 (dd, 1H, $J_{1",2"}$ = 3.7 Hz, $J_{2",3"}$ = 10.8 Hz, H-2"), 3.51 (dd, 1H, $J_{5',6b'}$ = 5.8 Hz, $J_{6a',6b'}$ = 9.1 Hz, H-6b'), 3.44 (dd, 2H, $J$ = 6.7, 1.8 Hz, H-6), 3.28 (s, 3H, OC$H_3$), 3.01 (d, 1H, $J$ = 3.4 Hz, H-4"), 1.06 (d, 3H, $J_{5",6"}$ = 6.6 Hz, H-6" C$H_3$). $^{13}$C NMR (125 MHz, $CDCl_3$) δ 159.2, 139.0, 138.6, 138.3, 137.6, 130.2, 130.0, 129.6, 129.3, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.6, 127.4, 127.3, 127.2, 127.1, 113.8, 113.7, 98.6, 98.4, 96.5, 77.0, 76.6, 76.1, 76.1, 75.5, 74.9, 74.7, 73.8, 73.6, 73.2, 73.0, 72.6, 71.1, 69.2, 68.6, 67.0, 66.7, 59.8, 57.6, 55.3, 55.2, 55.2, 55.0, 17.8. ESI HRMS calcd. for $C_{77}H_{87}N_4O_{15}$ (M+H): 1307.6162, found 1307.6161.

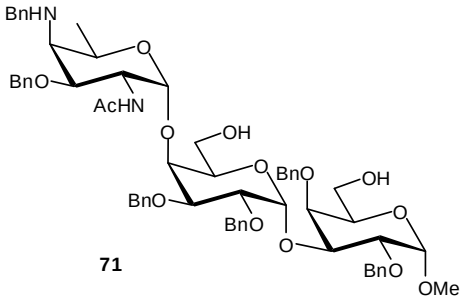



70

**Methyl 2-azido-3-benzyl-4-benzylamino-2,4,6-trideoxy-α-D-galactopyranosyl-(1→4)-2,3-di-*O*-benzyl-α-D-galactopyranosyl-(1→3)-2,4-di-*O*-benzyl-α-D-galactopyranoside (70)**

Trisaccharide **69** (0.125 g, 0.0.096 mmol) was dissolved in DCM (5 mL) stirring at room temperature. To the resulting solution 5% TFA (0.25 mL) was added dropwise and the reaction was monitored by TLC (5% MeOH in DCM). After 20 minutes the reaction was quenched by adding saturated $NaHCO_3$ solution (10 mL). The reaction mixture was diluted with DCM (10 mL), and the DCM solution was washed with water and brine. The organic phase was dried over $MgSO_4$, filtered and the filtrate was concentrated under reduced pressure. Purification of the residue via column chromatography (3% MeOH in DCM) gave the titled compound **70** (0.08 g, 75%) as a colourless syrup. $[\alpha]_D$ +91.5 (*c* 0.2, $CH_2Cl_2$); $^1$H NMR (600 MHz, $CDCl_3$) δ 7.40 – 7.09 (m, 30H, ArH), 5.19 (d, 1H, $J_{1',2'}$ = 3.3 Hz, H-1'), 5.00 (d, 1H, $J_{gem}$ = 11.5 Hz, PhC$H_2$), 4.94 (d, 1H, $J_{1'',2''}$ = 3.1 Hz, H-1''), 4.80 (d, 1H, $J_{gem}$ = 11.5 Hz, PhC$H_2$), 4.75 (d, 1H, $J_{gem}$ = 12.8 Hz, PhC$H_2$), 4.74 (s, 1H, H-1), 4.73 (d, 1H, $J_{gem}$ = 11.3 Hz, PhC$H_2$), 4.69 (d, 1H, $J_{gem}$ = 11.5 Hz, PhC$H_2$), 4.54 (s, 2H, PhC$H_2$), 4.40 (d, 1H, $J_{gem}$ = 11.5 Hz, PhC$H_2$), 4.38 – 4.20 (m, 4H, PhC$H_2$, H-5''), 4.15 (dd, 1H, $J_{2,3}$ = 10.2 Hz, $J_{3,4}$ = 2.5 Hz, H-3), 4.04 (app t, 1H, $J_{5',6'}$ = 6.6 Hz, H-5'), 3.98 (dd, 1H, $J_{1,2}$ = 3.5 Hz, $J_{2,3}$ = 10.2 Hz,

H-2), 3.96 – 3.70 (m, 6H, H-4, H-2′, H-3′, H-4′, H-3", PhC$H_2$) 3.76 (d, 1H, $J_{2",3"}$ = 10.3 Hz, H-2"), 3.72 – 3.66 (m, 2H, H-5, H-6a), 3.60 (dd, 1H, $J_{5',6a'}$ = 6.9 Hz, $J_{6a',6b'}$ = 10.7 Hz, H-6a'), 3.52 (dd, 1H, $J_{5,6b}$ = 4.6 Hz, $J_{6a,6b}$ = 10.8 Hz, H-6b), 3.47 (dd, 1H, $J_{5',6a'}$ = 6.8 Hz, $J_{6a',6b'}$ = 10.8 Hz, H-6b'), 3.34 (s, 3H, OC$H_3$), 3.13 (d, 1H, $J$ = 2.8 Hz, H-4"), 1.11 (d, 3H, $J_{5",6"}$ = 5.6 Hz, H-6" C$H_3$). $^{13}$C NMR (125 MHz, $CDCl_3$) δ 138.5, 138.3, 138.0, 138.0, 136.7, 129.5, 129.0, 128.5, 128.4, 128.3, 128.1, 128.1, 127.7, 127.4, 98.3, 98.2, 96.0, 76.3, 75.6, 75.4, 75.1, 75.0, 74.6, 74.1, 73.0, 72.5, 71.8, 70.7, 70.3, 62.4, 60.7, 59.7, 59.5, 57.2, 55.3, 38.1, 31.2, 29.7, 17.5. ESI HRMS calcd. for $C_{61}H_{71}N_4O_{13}$ (M+H): 1067.5012, found 1067.5015.

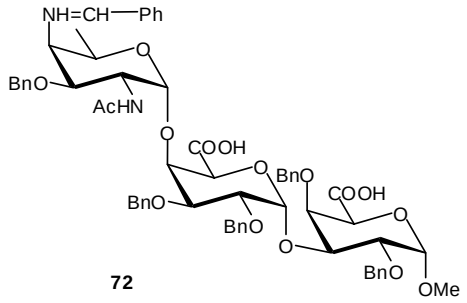



71

**Methyl 2-acetamido-3-benzyl-4-benzylamino-2,4,6-trideoxy-α-D-galactopyranosyl-(1→4)-2,3-di-*O*-benzyl-α-D-galactopyranosyl-(1→3)-2,4-di-*O*-benzyl-α-D-galactopyranoside (71)**

Trisaccharide **70** (0.09 g, 0.086 mmol) was dissolved in a solution of pyridine, water and TEA (10:1:0.3, 11.3 mL). The solution was saturated with $H_2S$ by bubbling gas into the solution for 1 hour at 0 $^oC$, followed by stirring at room temperature for 20 hours. Solvent was evaporated under reduced pressure and the residue was further dried *in vacuo* for 5 hours. The crude amine was dissolved in dry MeOH and acetic anhydride (0.1 mL) was added to the resulting mixture. After 30 minutes at room temperature the reaction mixture was concentrated. The residue was subjected to silica gel column chromatography using a stepped gradient eluent (DCM-MeOH 1→3) to obtain the acetamide derivative **71** (0.067 g, 72%). $[\alpha]_D$ +89.4 (*c* 0.1, $CH_2Cl_2$); $^1H$ NMR (600 MHz, $CDCl_3$) δ 7.41 – 7.07 (m, 30H, ArH), 5.13 (d, 1H, $J_{1',2'}$ = 3.3 Hz, H-1'), 4.98 (d, 1H, $J_{gem}$ = 12.8 Hz, PhC$H_2$), 4.89 (d, 1H, $J_{1'',2''}$ = 3.0 Hz, H-1''), 4.87 (d, 1H, $J_{gem}$ = 11.5 Hz, PhC$H_2$), 4.79 (d, 1H, $J_{gem}$ = 12.5 Hz, PhC$H_2$), 4.74 (d, 1H, $J_{1,2}$ = 3.4 Hz, H-1), 4.70 (d, 1H, $J_{gem}$ = 11.5 Hz, PhC$H_2$), 4.67 (d, 1H, $J_{gem}$ = 12.5 Hz, PhC$H_2$), 4.53 (app s, 2H, PhC$H_2$), 4.39 (m, 3H, PhC$H_2$, H-2'', H-4), 4.26 (q, 1H, $J_{5'',6''}$ = 6.4 Hz, H-5''), 4.10 (dd, 1H, $J_{2,3}$ = 10.2 Hz, $J_{3,4}$ = 2.8 Hz, H-3), 4.02 (app t,

1H, $J$ = 6.5 Hz, H-5′), 3.98 (dd, 1H, $J_{1,2}$ = 3.5 Hz, $J_{2,3}$ = 10.3 Hz, H-2), 3.91 – 3.82 (m, 5H, H-2′, H-3′, H-4′, PhC$H_2$), 3.73 – 3.64 (m, 3H, H-6, PhC$H_2$), 3.56 (dd, 1H, $J_{2",3"}$ = 10.4 Hz, $J_{3",4"}$ = 2.9 Hz, H-3"), 3.49 (d, 1H, $J$ = 9.7 Hz, H-5), 3.37 – 3.29 (m, 4H, OC$H_3$, H-6a′), 3.20 (dd, 1H, $J_{5',6a'}$ = 6.4 Hz, $J_{6a',6b'}$ = 10.7 Hz, H-6b'), 3.01 (app s, 1H, H-4"), 1.90 (s, 3H, COC$H_3$), 1.17 (d, 3H, $J_{5",6"}$ = 5.6 Hz, H-6" C$H_3$). $^{13}$C NMR (125 MHz, $CDCl_3$) δ 171.8, 138.5, 138.2, 138.1, 138.0, 128.7, 128.5, 128.4, 128.3, 128.3, 128.2, 128.1, 128.0, 127.9, 127.6, 127.6, 98.2, 96.8, 76.2, 76.1, 75.8, 75.1, 74.6, 74.3, 73.0, 72.2, 70.9, 70.3, 62.3, 60.6, 59.5, 55.3, 53.6, 49.1, 38.1, 31.2, 29.7, 23.3, 17.5. ESI HRMS calcd. for $C_{63}H_{75}N_2O_{14}Na$ (M+Na): 1083.5213, found 1083.5211.

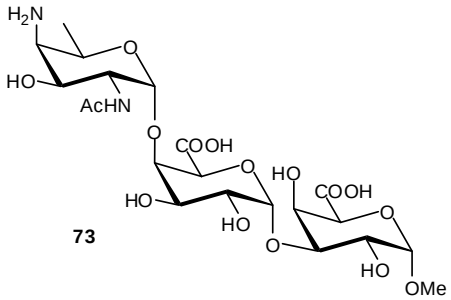



72

**Methyl 2-acetamido-3-benzyl-4-benzylideneamino-2,4,6-trideoxy-α-D-galactopyranosyl-(1→4)-2,3-di-*O*-benzyl-α-D-galactopyranosiduronate-(1→3)-2,4-di-*O*-benzyl-α-D-galactopyranosiduronate (72)**

To a stirred solution of 6,6' diol **71** (0.015 g, 13.8 μmol) in acetone (1 mL) at 0 $^{o}$C was added 5 % $NaHCO_3$ aqueous solution (0.5 mL) followed by KBr (0.003 g, 0.03 mmol, 2 eq) and TEMPO (0.003 g, 0.004 mmol, 3 eq) at 0 $^{o}$C. After 10 minutes, NaOCl (0.027 mL, 0.055 mmol, 4 eq) was slowly added to the above mixture at the same temperature and it was stirred for further 10 minutes.

The solution was concentrated under reduced pressure. The crude product was carried to the next step without purification. A small portion of crude **12** was purified by Sephadex G-10 column using $H_2O$-$NH_4OH$ as eluent monitered by Pharmacia UV detector. Subsequent lyophilization of the purified fractions afforded **72** as a white solid. $^1$H NMR of the $NH_4^+$ salt form (600 MHz, $D_2O$) δ 7.49 – 7.27 (m, 27H, ArH, PhC*H*$_2$), 7.19 (m, 2H, ArH), 5.50 (d, 1H, $J_{1',2'}$ = 3.4 Hz, H-1'), 4.93 (d, 1H, $J_{1'',2''}$ = 3.4 Hz, H-1''), 4.89 (d, 1H, $J_{gem}$ = 10.7 Hz, PhC*H*$_2$), 4.81 – 4.63 (m, 7H, H-1, H-5, PhC*H*$_2$), 4.58 – 4.49 (m, 4H, H-4, H-4′, H-5′, PhC*H*$_2$), 4.45 (m, 3H, PhC*H*$_2$), 4.22 – 4.17 (m, 3H, H-3, H-5'', PhC*H*$_2$), 4.15 (dd, 1H, $J_{1'',2''}$ = 3.7 Hz, $J_{2'',3''}$ = 10.8 Hz, H-2''), 3.93 (dd, 1H, $J_{1',2'}$ = 3.6 Hz, $J_{2',3'}$ = 11.3 Hz, H-2′) 3.88 (dd, 1H, $J_{1,2}$ = 3.7 Hz, $J_{2,3}$ = 10.3 Hz, H-2), 3.81 (dd, 1H, $J_{2',3'}$ = 10.5 Hz, $J_{3',4'}$ = 2.4 Hz, H-3′), 3.63 (dd, 1H, $J_{2'',3''}$ = 10.9 Hz, $J_{3'',4''}$ = 3.7 Hz, H-3''), 3.32 (s, 3H, OC*H*$_3$), 3.11 (app s, 1H, H-4''), 2.04 (s, 3H, COC*H*$_3$), 0.99 (d, 3H, $J_{5'',6''}$ = 7.2 Hz, H-6'' C*H*$_3$). ESI HRMS calcd. for $C_{63}H_{66}O_{16}N_2Na_3$ (M+Na): 1175.4100, found 1175.4100.




73

**Methyl 2-acetamido-4-amino-2,4,6-trideoxy-α-D-galactopyranosyl-(1→4)-α-D-galactopyranosiduronate-(1→3)-α-D-galactopyranosiduronate (73)**

Crude **72** was dissolved in a mixture of $H_2O$-AcOH (4:1, 3 mL) and 20% $Pd(OH)_2/C$ (0.015 g) was added to the resulting solution. The reaction mixture was shaken under $H_2$ (60 psi) atmosphere for 2 days. The catalyst was filtered off through cotton wool and then through a microfilter. The filtrate was concentrated (1 mL) under reduced pressure. Purification was performed on a Bio-gel P2 column (1.6 × 35 cm) using $NH_4OH$ (1 mL / L) in water as eluent and with monitoring by a Waters R403 refractive index detector. Subsequent lyophilization of the purified fractions afforded the final trisaccharide **73** (0.004 g, 53%) as a white solid. $[\alpha]_D$ +49.1 (*c* 0.2, $H_2O$); $^1H$ NMR of the $NH_4^+$ salt form (600 MHz, $D_2O$) δ 5.22 (d, 1H, $J_{1',2'}$ = 3.7 Hz, H-1'), 4.92 (d, 1H, $J_{1'',2''}$ = 3.3 Hz, H-1''), 4.86 (d, 1H, $J_{1,2}$ = 3.8 Hz, H-1), 4.64 (qd, 1H, $J_{4'',5''}$ = 1.6 Hz, $J_{5'',6''}$ = 6.5 Hz, H-5''), 4.55 (app s, 1H, H-5′), 4.49 (d, 1H, $J_{3,4}$ = 3.1 Hz, $J_{4,5}$ = 1.2 Hz, H-4), 4.33 (d, 1H, $J_{3',4'}$ = 3.1 Hz, $J_{4',5'}$ = 0.9 Hz, H-4′), 4.22 (d, 1H, $J_{4,5}$ = 1.1 Hz, H-5), 4.10 (dd, 1H, $J_{2',3'}$ = 10.9 Hz, $J_{3',4'}$ = 3.3 Hz, H-3′), 4.08 (dd, 1H, $J_{2'',3''}$ = 9.0 Hz, $J_{3'',4''}$ = 3.7 Hz, H-3''), 4.06 (dd, 1H, $J_{1'',2''}$ = 3.5 Hz, $J_{2'',3''}$ = 11.2 Hz, H-2''), 4.00 (dd, 1H, $J_{2,3}$ = 10.3 Hz, $J_{3,4}$ = 3.2 Hz, H-3), 3.94 (dd, 1H, $J_{1,2}$ = 3.8 Hz, $J_{2,3}$ = 10.2 Hz, H-2), 3.89 (dd, 1H, $J_{1',2'}$ = 3.9 Hz, $J_{2',3'}$ = 10.7 Hz, H-2′), 3.41 (s, 3H, OC$H_3$), 3.37 (dd, 1H,

$J_{3",4"}$ = 4.1 Hz, $J_{4",5"}$ = 1.5 Hz, H-4"), 2.09 (s, 3H, COC$H_3$), 1.24 (d, 3H, $J_{5",6"}$ = 6.7 Hz, H-6" C$H_3$). $^{13}$C NMR of the $NH_4^+$ salt form (125 MHz, $D_2O$) δ 176.4, 175.9, 175.5, 100.3, 99.8, 96.7, 81.0, 76.2, 72.1, 71.9, 69.6, 68.9, 68.4, 67.3, 67.0, 65.7, 56.1, 55.6, 50.1, 23.2, 16.5. ESI HRMS calcd. for $C_{21}H_{33}N_2O_{16}$ (M-H): 569.1837, found 569.1839.

# Chapter Six

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