SYNTHETIC STUDIES TOWARD PSEUDOPENTASACCHARIDE REPEATING UNIT OF STREPTOCOCCUS PNEUMONIAE ZWITTERIONIC POLYSACCHARIDE

by

SRINIVASA MURTHY KOUTHA

(Under the Direction of Geert-Jan Boons)

ABSTRACT

Streptococcus pneumoniae is one of the most common gram positive pathogens that colonizes the upper respiratory tract and causes and severe infections like otitis media, sinusitis, and more life threatening diseases like pneumonia, bacteremia and meningitis. *S. pneumoniae* has a thick polysaccharide capsule that covers the peptidoglycan cell wall. There are around 94 distinct capsular serotypes and all the 94 serotypes differ in virulence, prevalence and extent of drug resistance. All pneumococci possess two common polysaccharide antigens: C-polysaccharide and F-antigen. The C-polysaccharide is a cell-wall teichoic acid composed of tetrasaccharides attached together through ribitol phospho-diesters, and therefore it is classified as a ribitol teichoic acid. C-polysaccharide also contains phosphocholine substituents. The F-antigen is a lipoteichoic acid with a polysaccharide identical to C-polysaccharide and this part is linked to a diacylated glycerol residue via a glucose residue. The above mentioned oligosaccharides fall under the category of unique polysaccharides called as zwitteroinic polysaccharides (ZPSs).

In the last decade, several studies have shown convincing evidence that ZPSs are immunomodulatory polysaccharides and they specifically activate T cells. Research has also showed that zwitterionic charges and molecular weight around 8 kDa are essential for the biological activity of the ZPSs that were isolated from the gram-negative anaerobic *Bacteroides fragilis*. Recently, it was confirmed that a minimum chain length of 2 to 3 repeating units are required for cytokine induction from pneumococcal lipoteichoic acids isolated from two different strains (Fp 23 and R6) of *Streptococcus pneumonia.*

Because of the unique ability of zwitterionic polysaccharides to elicit T-cell immune response, few research groups have reported the syntheses of complex zwitterionic oligosaccharides. Here we report a convergent approach for the attempted synthesis of the monomer (pentasaccharide) of C-polysaccharide isolated from a noncapsulated pneumococcal strain CSR SCS2. We employed three orthogonal protecting groups (NAP, Allyl and TBS) that can be selectively removed to install phosphocholine and to oligomerize the repeating unit respectively. Access to this important ZPSs with 2 to 4 repeating units will provide an opportunity to investigate the immunological activity of these oligosaccharides, and to demonstrate the advantages of chemical synthesis for obtaining small quantities of pure complex oligosaccharides.

INDEX WORDS: *Streptococcus pneumonia,* zwitteroinic polysaccharides, C-polysaccharide, orthogonal protecting groups , NAP, Allyl TBS

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SRINIVASA MURTHY KOUTHA

B.S., NAGARJUNA UNIVERSITY, INDIA, 2000

M.S., INDIAN INSTITUTE OF TECHNOLOGY MADRAS, INDIA, 2002

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SRINIVASA MURTHY KOUTHA

Major Professor:	Geert-Jan Boons
Committee:	Robert S. Phillips
	Vladimir V. Popik

Electronic Version Approved:

Maureen Grasso
Dean of the Graduate School
The University of Georgia
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DEDICATION

This work is dedicated to my loving family.

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

1.1 *Streptococcus pneumoniae*

Streptococcus pneumoniae (*pneumococcus*) is a Gram-positive diplococcus bacterium (Figure 1).[1] It is a hemolytic, aerotolerant anaerobe and is known for its ability to undergo genetic transformation in a living host.[2] It is a common inhabitant of the upper respiratory tract of humans and is the major cause of pneumonia, an inflammatory condition in the lung. The pneumococcal cell surface is comprised of three layers- the plasma membrane, the cell wall and the capsule.

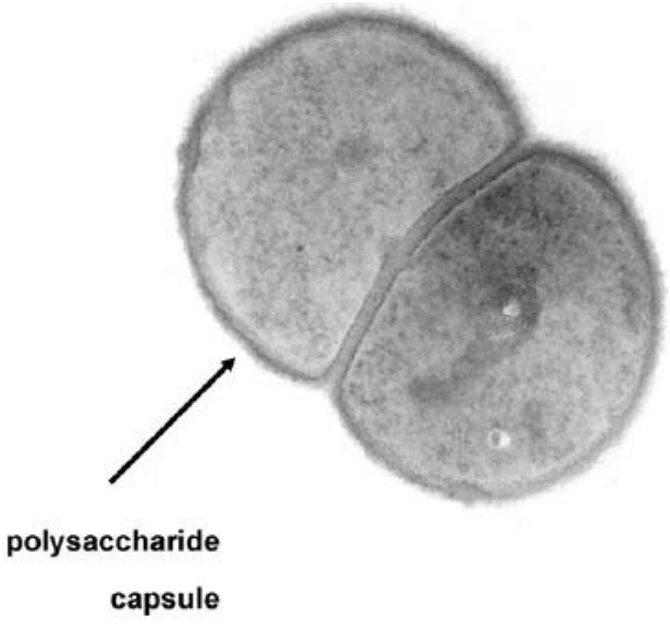


Figure 1.1: Scanning electron microscope image of *Streptococcus pneumoniae*

Bacterial cell wall gives shape and mechanical strength to the cell to withstand internal turgor pressure. The core of the pneumococcal cell wall is composed of murein (peptidoglycan) network that carries the wall techoic acid (WTA). In addition, there is lipotechoic acid (LTA) of identical repeats but with a lipid anchor on the plasma membrane.[3] 471 proteins that are secreted into the medium or anchored to the cell surface were identified on the cell wall.[4]

A thick polysaccharide (PS) capsule encompasses the cell wall. The capsule comprises of high molecular weight polymers made up of units of repeating oligosaccharides containing between two and eight monosaccharides.[5] Unlike cell wall polysaccharides, pneumococcal capsular polysaccharides vary widely in chemical structure and composition among different serotypes. Some of the components of this complex polymer capsule include sugar alcohols, amino sugars, phosphorylcholine, and neutral sugars.[6] This capsule is responsible for the overall virulence of the bacterium and shields it from phagocytosis by preventing host opsonins from coating the bacteria. 94 distinct capsular serotypes have been identified till date.[7]

1.2 Pneumococcal diseases

Streptococcus pneumoniae (*pneumococcus*) causes two kinds of pneumococcal diseases which may be classified as non-invasive pneumococcal diseases and invasive pneumococcal diseases. The non-invasive diseases are less serious infections and do not affect the major organs or the blood. Otitis media and non-bacteremic pneumonia fall under this category. Invasive pneumococcal diseases are often associated with significant morbidity and mortality worldwide.[8] These are caused when *pneumococci*

invade major organs such as lungs, liver and spleen or the blood. Invasive pneumococcal diseases include bacteremia, meningitis and bacteremic pneumonia.

Virulence effects depend on the tissue site and population density of the bacteria. Sessile *pneumococci* existing in biofilms are more likely to cause meningitis and pneumonia, whereas free living, planktonic bacteria cause bacteremia and less likely, pneumonia.[9] Incidence of invasive pneumococcal diseases varies with age, genetic background, socioeconomic status, immune status, and geographical location. General risk factors for pneumococcal diseases include age below 2 years and over 65 years, asplenia, alcoholism, smoking, crowding, poverty, recent acquisition of a virulent strain, antecedent respiratory infections, underlying lung disease, severe liver disease, influenza, immunoglobulin and complement deficiencies, and other immunocompromised states including HIV infection, and recent exposure to antibiotics.[10]

Pneumonia: Pneumonia is a general term for a wide variety of conditions that cause an inflammation of the lungs (pulmonary parenchyma). It is a common complication of a variety of underlying diseases and can lead to more complications, which can be serious and sometimes life threatening. Pneumonia is the sixth leading cause of death in the United States and 5 million people die all over the world with this disease. Pneumonia is most often caused by a bacterial or a viral infection. However, it can also be caused by a fungal infection, yeast infection, trauma, or inflammation of the lungs due to exposure to toxic substances such as poisonous gases. In addition to the classification according to the causing organism, pneumonia can also be classified

based on the part of lung that is affected and if it is community acquired or hospital acquired.

Pneumonia caused by bacterial infection is called bacterial pneumonia. *Streptococcus pneumoniae (pneumococcus)*, *Haemophilus influenzae, Legionella pneumophila, Mycoplasma pneumoniae* and *Staphylococcus aureus* account for 86% of the identified pathogens causing bacterial pneumonia, among which *pneumococcus* is the leading cause. About 175,000 hospitalizations from pneumococcal pneumonia are estimated to occur annually in the United States, with a case fatality of about 5-7%.[11]

Common symptoms of pneumococcal pneumonia include high temperature, shaking chills, a productive cough (cough and sputum), and blood in the sputum.[6] If left untreated, this toxic illness can progress to acute respiratory failure and death within several days from the onset. The general diagnosis of pneumonia involves the identification of fluid accumulation in the alveolar spaces by a chest X-ray. Diagnosis also involves lung function tests that measure how much air moved in and out of the lungs during breathing. A CT scan may help in revealing other associated underlying respiratory conditions.

Otitis media: Otitis media is an inflammatory condition of the ear, most commonly prevalent among young children. One of the causes is the colonization of *pneumococci* in the Eustachian tube of the inner ear.[12] As the bacteria begin to multiply, an inflammatory response is triggered leading to ear pain, fever and irritability.[13] More than 60 % of children are affected by this disease before reaching 1 year of age.[11]

Bacteremia: Pneumococcal bacteremia occurs when the bacteria are taken up into the lymphatic vessels of the upper respiratory tract, pass to the cervical lymph nodes and enter the venous circulation.[14] Bacteremia occurs in about 25%–30% of patients with pneumococcal pneumonia. More than 50,000 cases of pneumococcal bacteremia are reported each year. The overall case-fatality rate is about 20%, but may be as high as 60% among elderly patients.[11]

Meningitis: Meninges are a set of protective membranes covering the brain and the spinal column. Meningitis is an inflammatory condition which occurs when *pneumococci* in the blood stream attach to these membranes. When *pneumococci* from the throat enter the bloodstream and lungs, a local inflammatory response is activated which leads to a breach in the blood-brain barrier. Consequently, the bacteria and phagocytes move into the brain and spinal fluid, causing severe infection[15]. Pneumococcal meningitis is characterized by fever, irritability, and drowsiness in early stages, and seizures and coma in the later stages.[6] Bacterial meningitis is among the top 10 causes of death of children under 14 years of age in high income countries. Pneumococcal meningitis accounts for 10 % of invasive pneumococcal diseases.[16] 3,000 to 6,000 cases are estimated to occur each year in the United States. The case-fatality rate is about 30%, but may be as high as 80% among elderly persons.[11]

1.3 Progression of Pneumococcal diseases

Pneumococci begin invading the host by colonizing in the nasopharynx. They use two strategies for selection, survival and proliferation in the host. The first is by quick induction of invasive disease and efficient person-to-person transmission by coughing.

The second is long term carriage in the nasopharynx with the aid of surface adhesions, immune evasion strategies, and secretory defenses such as IgA1 protease and inhibitors of antibacterial peptides.[10]

Colonization occurs in about 10 % of healthy adults. 20-40 % of healthy children are carriers and more than 60% of infants and children in day-care settings can be carriers[10]. The bacteria colonize by adhering to the cell-surface carbohydrate (GlcNAc) receptors of the epithelial cells.[17] Adherence is mediated by cell-wall associated surface proteins, which may also facilitate adherence by non-specific physiochemical interactions.[18] The attachment can be enhanced by prior influenza virus infection[19]. Most people colonized with *pneumococci* have only one serotype at a time, although simultaneous carriage of more than one serotype is possible. Duration of pneumococcal carriage in an individual is highly serotype-specific.[10]

From the nasopharyngeal site, the bacteria gain access to the inner ear and to the lung (Figure 1.2).[6]

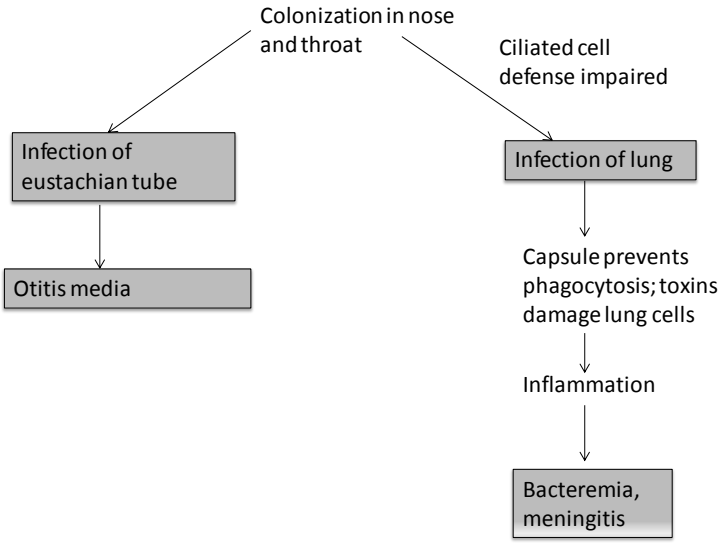


Figure 1.2: Progression of pneumococcal diseases.[6]

Ciliated cells of the upper airway of the respiratory tract prevent *pneumococci* from entering the lungs. However, the presence of influenza virus can impair these defenses by killing the ciliated cells.[20] Owing to the weakened defenses, the *pneumococci* in the throat manage to reach the lung. In the lung, the walls of the alveoli are lined up with cells called pneumocytes. These cells are of two types. Type II pneumocytes have two kinds of disaccharides on their surfaces, which could be the receptors for the pneumococcal adhesins. Once settled in the lungs, they begin to divide and colonize. The polysaccharide capsule of the bacteria shields them from phagocytes and the alveolar macrophages in the lung.[7] In due course, some bacteria undergo lysis and the bacterial fragments thus formed trigger a local inflammatory response. As a result, more and more phagocytes and lymphoid cells are attracted to the region of unsuccessful phagocytosis, causing the inflammation to grow.[21] As the infection progresses, the surface carbohydrate receptors are changed. Pneumococcal cell wall phosphorylcholine binds to one of these upregulated receptors, the platelet

activation factor (PAF), enhancing the infection process.[21] The result is lung damage and a breakdown of gas exchange mechanism of the host. *Pneumococci* also enter the bloodstream by binding to the disaccharide receptors on type II pneumocytes.[6]

Interaction with the blood brain barrier

In the body, attachment of *pneumococci* to blood vessel walls of the brain and spinal column could cause breaches in the vessel walls, which leads to disruption of the blood-brain barrier and entry of bacteria and phagocytes into the cerebrospinal fluid.[6] Alternatively, the bacteria can reach the meninges directly from the nasopharynx and cause meningitis.[22]

1.4 Virulence factors

Pneumococci have a number of virulence factors such as capsular polysaccharides, cell wall fragments, pneumolysin, and other proteins such as pneumococcal surface protein A (PspA), pneumococcal surface protein C (PspC), and pneumococcal surface adhesin A (PsaA).[7] Two distinct mechanisms of virulence have been identified during pneumococcal infection. The first is by hindering phagocytosis through complement inhibition. The second is through complement activation by pneumococcal disintegration and lysis, leading to enhanced inflammation.[5]

1.4.1 Capsular polysaccharides

The capsular polysaccharides (CPSs) have long been recognized as major virulence factors.[23] Encapsulated strains were found to be 10^5 times more virulent than uncapsulated ones.[5] The capsule prevents mucous-mediated clearance in the

respiratory tract and aids the bacteria in attaching to the epithelial cells.[24] The capsule also confers resistance against several antibiotics.[25] Different capsular types confer different levels of virulence.[26] The chemical structure of the capsular polysaccharides and, to a lesser extent, the capsule thickness determines the differential ability of host invasion by the serotype.[5] Certain structural features of serotypes such as variations in repeating unit composition, ring forms, glycosidic linkage positions, anomeric center configurations, and conformations explain the differences in immune responses generated against these polysaccharides.[27] Immune response by the serotypes may differ in terms of activation of the alternative pathway of complement, deposition and degradation of complement components on the capsule, resistance to phagocytosis, ability to induce antibody production and clearance mediated by lectin-like structures on phagocytes.[5] The serotypes which are easily cleared up by phagocytosis are poorly immunogenic. On the other hand, those which can resist phagocytosis invoke a strong inflammatory response.[28]

1.4.2 Cell wall components

Cell wall components, especially cell wall polysaccharides (CWPS), have been found to be capable of provoking a strong immune response.[29] During pneumococcal infection, the host produces antibodies to the cell wall antigens.[30] These antibodies diffuse through the cell capsule and bind to the antigens at the cell surface and activate complement by the classical pathway.[31] However, the capsule obstructs phagocytosis while the complement is degraded by the protein H bound to the capsule. Inflammation of the tissue continues to occur as invading cells are not cleared up, which results in tissue damage, fluid accumulation and disruption of gas exchange in the lungs.[6] Cell

wall components also stimulate the production of Interlukin-1 which aids inflammation.[32] Injection of purified pneumococcal cell wall or its degradation products caused pneumonia, otitis media and meningitis in mice. In addition, anti-CWPS have been demonstrated to protect animals against pneumococcal challenge.[5]

1.4.3 Pnumolysin

Pneumolysin is a cytotoxic protein present in the cytoplasm of *pneumococci*. It contains a choline binding domain and belongs to the class of thiol-activated toxins.[22] It is an important cause of virulence and is a potential candidate for anti-pneumococcal vaccine. Experimental mice models showed that it plays an important role in pneumococcal bacteremia and meningitis.[25] However, its relative importance varies from strain to strain. A protein called autolysin (LytA), which is responsible for lysis of the bacterial cell during cell division, causes the release of pneumolysin.[33]

Pneumolysin shows significant virulence effects at all concentrations. At high concentrations, pneumolysin binds to cholesterol in host cell membranes and disrupts them by forming pores.[34] At low concentrations, it has several virulence effects. It disrupts the beating of ciliated mucosal cells, thus impairing the ability of the host to clear the bacteria trapped in the mucous.[35] It also impairs bactericidal activity and migration of neutrophils[36], and inhibits phagocytosis, lymphocyte proliferation and antibody synthesis.[37] This virulence protein has been reported to cause damage to epithelial cells and blood vessels in the lung aiding bacteria to enter into the bloodstream.[38] Pneumolysin also contributes to inflammation by complement fixation. It does so either by activating the classical complement pathway by itself or by binding at

or near the Fc portion of antibodies resulting in a conformational change that causes activation of the classical pathway.[39] Inflammation may also be caused by pneumolysin stimulated production of cytokines such as interleukin-1β and tumor necrosis factor.[40]

1.4.4 Other virulence proteins

Pneumococci have a number of surface proteins that could contribute to virulence. Some of these are pneumococcal surface protein A (Psp A), pneumococcal surface adhesin A (PsaA) and pneumococcal surface protein C (PspC). PspA is a member of structurally related choline binding proteins. These proteins interfere with complement fixation, block the initiation of alternative pathway and inhibit complement receptor mediated clearance of *pneumococci*.[41] PspA aids colonization in host mucosae by binding to surface expressed lactoferrin receptors.[42] PsaA is a 37 KDa pneumococcal protein which belongs to the family of metal binding lipoproteins. This protein is involved in manganese transport process of *pneumococci*.[43]

Other virulence proteins include choline binding protein A (CbpA), putative proteinase maturation protein A (PmpA), autolysin and neuraminidase. CbpA (also known as PspC) binds to secretory immunoglobulin A and interacts with human epithelial and endothelial cells.[44] PmpA is a highly conserved surface associated virulent protein.[45] Autolysin contributes to virulence by cleaving the cells and releasing pneumolysin and other inflammatory cell components.[46] Neuraminidases, NanA and NanB, are enzymes which cleave N-acetylneuraminic acid from mucin, glycolipids, glycoproteins and oligosaccharides on host cell surfaces. These enzymes are assumed to contribute to pneumococcal virulence by aiding adhesion to the human mucosal

surfaces either by reducing the viscosity of the mucous layer or by exposing cell surface receptors.[47] The enzyme, lysozyme present in the blood of the host contributes to the lysis of bacteria in the blood by accelerating autolysin action.[48] Consequently, inflammatory cell components are released.

1.4.5 Natural transformation

Pneumococci have the ability to uptake DNA from other strains and from closely related species of *Streptococci*. This contributes to virulence and antibiotic resistance exhibited by these bacteria. It also explains the enormous strain-to-strain variability of the capsular polysaccharides which might have resulted from transformation of capsular DNA across different serotypes.[49] This action is also responsible for penicillin resistance, which may have resulted from mutations of penicillin-binding proteins (PBPs) by acquisition of DNA encoding PBPs from various sources.

1.5 Host Immune Response

The proliferation of *pneumococci* is kept under check by the immune defenses of healthy individuals. *Pneumococci* which reach the lungs are cleared by phagocytosis and intracellular killing by neutrophils and alveolar macrophages. This process occurs in the presence of type-specific immunoglobulins (IgG1, IgM and IgA) and active complement. This antibody-initiated complement-dependent opsonization, which activates the classical pathway, is the mechanism of defense in the host lungs.[17] The mechanism of clearance from the blood is dependent upon the interaction of type-specific antibodies (IgG), complement, and lectins, which are proteins present on the macrophages of liver and spleen cells.[5] Non-capsular antibodies, for example,

immunoglobulins directed against cell wall components may also play a role in the host response to pneumococcal infection as well. Certain cell surface proteins which penetrate the PS capsule are capable of generating T cell dependent immune responses.[17]

1.6 Treatment and prevention

1.6.1 Antibiotic treatment

A wide variety of antibiotics have been used for the treatment pneumococcal diseases. Some of these include β-lactams, floroquinolones, macrolides, aminoglycosides, vancomycin, rifampicin and linezolid.[50] Figure 1.3 shows the structures of a few antibiotics used in the treatment of pneumococcal diseases.

The mechanism of action of antibiotics is either killing (bactericidal) or inhibiting the growth of bacteria (bacteriostatic). The direct effects of these drugs on the bacteria depend on the pharmacokinetic parameters such as dosage, absorption and distribution, and the pharmacodynamic parameters such as the association between concentration of the drug at the infection site and its antimicrobial effect.[51] For drugs such as *β-lactams*, the main determinant for activity is achieving concentrations of free, unbound drug which are above the MIC (minimum inhibitory concentration) and last atleast 40 to 50 % of the dosing interval. However, for drugs such as floroquinolones, aminoglycosides and most macrolides, which have a prolonged post-antibiotic effect and can kill more rapidly, the determinant is the ratio of peak concentration to MIC.[52]

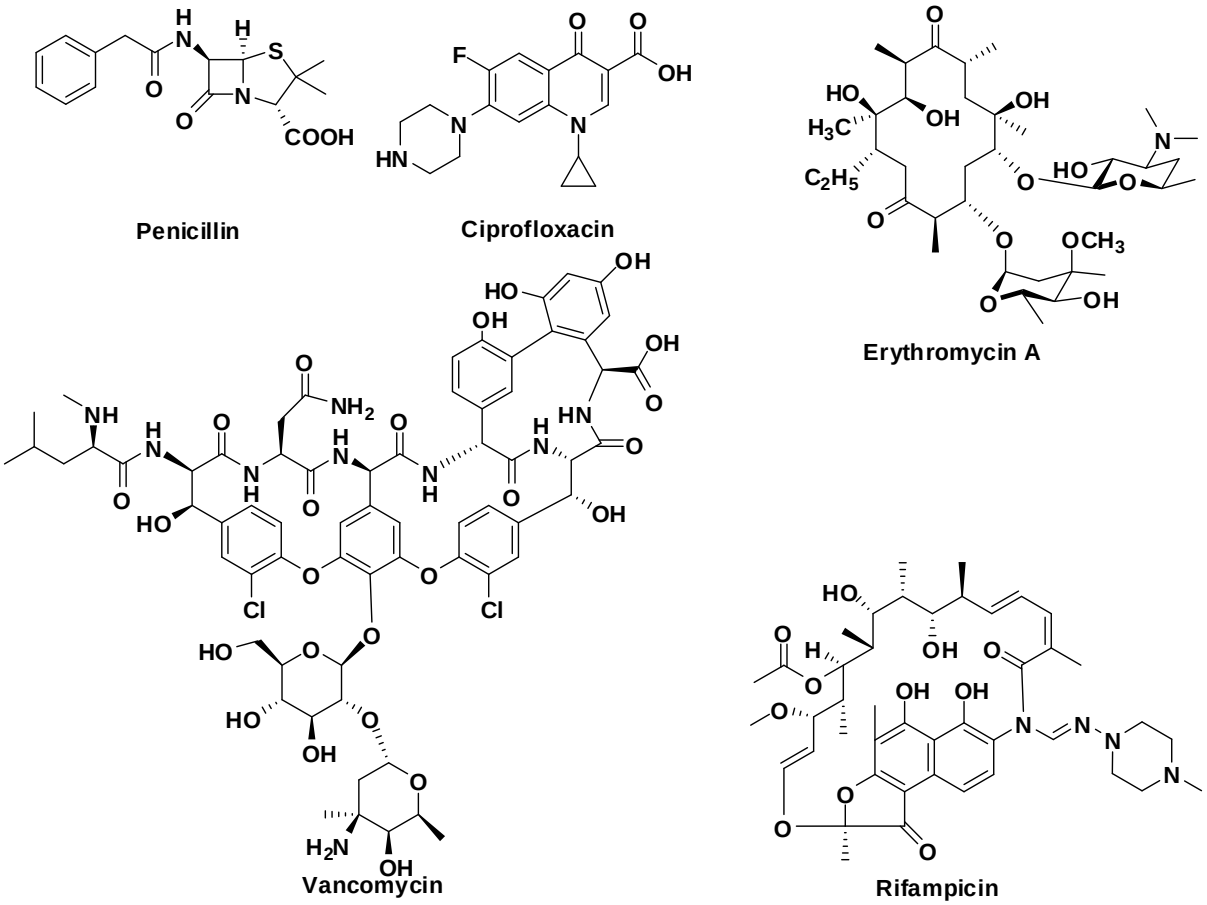


Figure 1.3: Structure of some antibiotics used for the treatment of pneumococcal diseases.

β-lactams

Penicillin was the first antibiotic used to treat pneumococcal infections.[53] In addition, other β-lactam antibiotics also proved successful in the past to treat pneumococcal diseases. β-lactam antibiotics can be classified into three categories according to their efficacy in comparison to penicillin G.[54,55] The first group consists of imipenel, meropenem, cefpirome, cefepime, cefotaxime, ceftriaxone and amoxicillin. These are highly effective drugs and are suitable for treatment against penicillin-resistant strains. The second category includes ampicillin, azlocillin, mezlocillin, piperacillin, cefdinir,

cefuroxime, cephalothin and cefamandole. These are slightly less effective than penicillin. The third category β-lactam antibiotics include oxacillin, cefixime, ceftazidime, cefaclor, cefoxitin and latamoxef. These are ineffective in treating pneumococcal diseases.

Penicillin and amoxicillin were used to treat otitis media in children for many years, until penicillin resistant strains appeared. Thereafter, therapy with high dose amoxicillin-clavulanate proved highly effective in curing resistant acute otitis media[56]. Appropriate doses of penicillin, amoxicillin, amoxicillin-clavulanate, cefuroxime, axetil or cefdinir can be used to treat pneumococcal pneumonia in children and adults.[50] Although the emergence of resistance raises serious issues, the drugs may still work if their pharmacodynamics is better understood.[57]

Non-meningeal pneumococcal diseases can usually be cured by the use of β-lactam antibiotics alone. For instance, non-meningeal bacteremia in children between 36 months and 3 years of age can be treated with broad spectrum cephalosporins.[50] However, meningitis caused by even moderately resistant strains requires the use of other agents to assure a successful outcome.[58,59] The use of β-lactam antibiotics resulted in the death of 30% of children suffering from pneumococcal meningitis. The main reason for this is the lysis of bacteria in the cerebrospinal fluid by these drugs, leading to high levels of inflammation and irreversible brain damage.[6]

Floroquinolones

Floroquinolones are broad spectrum antibiotics which target DNA gyrase and topoisomerase IV. DNA gyrase maintains the extent of DNA supercoiling to enable

unwinding during replication and transcription, and topoisomerase IV unlinks chromosomes after DNA synthesis. These compounds interact with the enzyme-DNA complexes, stimulate DNA cleavage, and inhibit relegation of the double stranded breaks, causing bacterial cell death.[60] Floroquinolones such as sparfloxacin and trovafloxacin accumulated at high concentrations in the lungs of mouse pneumonia models demonstrating higher activity than penicillin.[61,62] These drugs are recommended for the treatment of outpatient pneumonia (caused by β-lactam resistant strains) in adults but not in children.[50]

Macrolides

Macrolides are antibiotics whose chemical structure is composed of amino and/or neutral sugars attached to a 14, 15 or 16 membered lactone rings. These antibiotics are bacteriostatic and block the elongation step of protein synthesis by binding to the 50S ribosomal subunit, stimulating dissociation of the peptidyl-tRNA molecule from the ribosome and resulting in premature release of the peptide chain.[63] Macrolides alone or in combination with β-lactams are recommended for the treatment of resistant pneumococcal pneumonia in adults.[50]

Other Antibiotics

Other non-β-lactam antibiotics used for the treatment of pneumococcal diseases include tetracyclines, aminoglycosides, chloramphenicol, trimethoprimsulfamethoxazole, rifampicin and vancomycin. Tetracyclins are relatively cheap and safe drugs. They inhibit bacterial protein synthesis by blocking the attachment of aminoacyl-tRNA to the mRNA-ribosome complex.[64] Chloramphenicol inhibits protein synthesis by targeting the

enzyme peptidyl transferase, which catalyzes peptide bond formation during translation.[65] Trimethoprim-sulfamethoxazole is a low cost, broad spectrum antibiotic. This antibiotic works by inhibiting an enzyme involved in the synthesis of purines, thymidylate and certain amino acids.[66] Rifampicin is used in the treatment of penicillin-resistant pneumococcal meningitis. It causes premature termination of DNA transcription of the bacteria by binding to the β-subunit of RNA polymerase.[67] Vancomycin is a highly effective drug and can be used for the treatment of pneumococcal pneumonia and meningitis caused by highly resistant strains[50].

Challenges

Although antibiotics effectively cured most of the infections, several challenges exist with this therapy. The emergence of resistant strains in due course of time made treatment choices extremely difficult. Moreover, the products of bacterial cleavage by antibiotics could lead to inflammation and other complications, especially in the case of pneumococcal meningitis.[68] The administration of the anti-inflammatory drug dexamethasone along with the antibiotics to control inflammation proved a reasonable solution to the issue.[21] Also, since the blood-brain barrier is less permeable to antibiotic molecules even under inflammatory conditions, alternative strategies to make the antibiotics cross the barrier are to be investigated.[68] Another big disadvantage of antibiotic treatment is the risk of promoting resistant strain colonization and growth, while affecting susceptible strains.[50]

Resistance

Last two decades have seen the rapid emergence of resistance to antibiotics. Increased rates of resistance have influenced the morbidity and mortality of pneumococcal diseases in children and adults. Susceptibility of *pneumococci* to penicillin was uniformly high in the first 20-25 years of the antibiotic era. However, highly resistant strains began to appear since 1978. This was followed by increased resistance to other β-lactam and non-β-lactam drugs in the subsequent years. Factors associated with resistant pneumococcal diseases include young age, duration of hospitalization, infection with highly resistant serotypes such as 6,19,23 or 14, and previous exposure to antibiotics towards which the infecting strain is resistant.[69]

Pneumococcal resistance to antibiotics occurs by various mechanisms. Penicillin resistance (and resistance to other β-lactams) is caused by multiple alterations to the penicillin binding proteins (PBPs) which are present on the bacterial cell membrane and mediate cell wall metabolism and assembly.[70] This results in hampered binding of penicillin and other β-lactam antibiotics.[71] Alterations in the PBPs 1a, 2x and 2b confer penicillin resistance.[72] The antibiotic resistant strains are capable of synthesizing peptidoglycans despite the presence of the drug molecules.[73] This tolerance can be explained by a mechanism involving the rapid loss or disengagement of autolysin molecules from the site of infection, followed by a progressive change in cell wall structure to resist lysis by autolysin.[50] The level of resistance depends on the extent of modification of the targets.[74]

Penicillin resistant strains are also frequently resistant to other non- β-lactam antibiotics such as streptomycin, erythromycin, tetracylin, chloramphenicol and contrimoxazole that might be used for treatment of diseases caused by these organisms.[75] Increasing resistance of pneumococci to macrolides, floroquinolones, vancomycin, trimethoprim and other antimicrobial agents is being observed worldwide.[10]

Quinolone resistance originates from mutations in quinolone-resistance-determining regions of DNA gyrase and/or topoisomerase IV, or by active efflux of the antibiotic from the cell[76,77]. Prevalence of diseases caused by floroquinolone-resistant pneumococcal strains is low in most countries. It was about 1-3 % in the United States and only 0.4 % in Germany in 2003.[1] Therefore, they are promising candidates for the treatment of penicillin-resistant pneumococcal pneumonia. However, none of the floroquinolone drugs proved effective against quinolone resistant strains.[62] Resistance to macrolides for *pneumococci* can occur either by post-transcriptional modification of the 23S rRNA by methylation or mutation in 23SrRNA and two of the ribosomal proteins, and expression of Mef efflux pump.[63,78,79] About 2.9 % of pneumococcal strains were found to be macrolide resistant in 2002.[1] The mechanisms of tetracyclin resistance may include enzymatic alteration, active efflux and ribosomal protection.[69,80] Resistance to trimethoprim-sulfamethoxazole occurs when mutations occur in the gene encoding the enzyme involved in the synthesis of purines, thymidylate and certain amino acids.[81] Worldwide pneumococcal resistance to rifampicin has been low compared to other antibiotics. Resistance may occur by point mutations in RNA polymerase gene or by recombination events.[82]

The problem of resistance may be overcome in several ways. One strategy is to increase the dosage of the drugs.[10] Combination therapies of β-lactams with other drugs might prove more advantageous in treating diseases caused by resistant strains[83]. Floroquinolones and macrolides possibly limit inflammatory effects during severe pneumococcal pneumonia.[84] Thus a combination of either of these drugs with β-lactams may prove effective. For instance, treatment of postinfluenza pneumococcal pneumonia with a combination of ampicillin and inhibitors of bacterial protein synthesis such as azythromycin or clindamycin resulted in improved outcome and decreased lung inflammation[10]. A combination of β-lactams with aminoglycosides is an alternative.[85] Another strategy for controlling resistant pneumococcal diseases is to avoid changes in prescription choices owing to changes in susceptibility patterns before ruling out the possibility of curing by increased original drug dosage.[86] In addition, the use of antibiotics such as rifampicin and vancomycin with less resistance and imipenem with almost no known resistance against *pneumococci* should be strongly discouraged whenever possible.[50]

1.6.2 Pneumococcal Polysaccharide vaccines

The progressive emergence of antibiotic resistant pneumococcal strains, and the continued high morbidity and mortality of pneumococcal diseases inspite of antimicrobial therapy rekindled interest in prevention strategies. The approach of immunization with whole pneumococcal cells proved futile due to the adverse side effects of large amounts of inocula.[5]

In the 1930s the immunogenicity of purified capsular polysaccharides was demonstrated.[87,88] This led to the development of polysaccharide (PS) vaccines. This vaccine induces T-cell independent, B-cell response with minimal isotype switching from immunoglobulinM (IgM) to immunoglobulinG (IgG).[58] The mechanism of protection involves the binding of specific antibody to the capsule, resulting in opsonization and rapid clearance of invading bacteria. The first tetravalent PS vaccine which was tested during the second World war, conferred protection against the serotypes included in the vaccine.[89] The most successful and widely used pneumococcal vaccine, pneumovax, consists of 23 most virulent serotypes. This 23-valent PS vaccine was licensed in 1983. It is formulated using 25 mcg of each antigen per dose and 0.25% phenol as preservative. It is available in a single-dose vial or syringe, and in a 5-dose vial. The vaccine is administered by injection either subcutaneously or intramuscularly. It is one of the most complex subunit vaccines administered to humans and renders protection against serotypes causing invasive diseases in adults and children.[90] These serotypes were responsible for 90 % infections in the United States and 60 % in Asian countries.[91] This vaccine is about 60-75 % effective and has shown a protective effect even in risk groups such as those suffering from sickle cell disease or asplenia, and elderly with underlying conditions of chronic obstructive pulmonary disease.[17] A Spanish trial showed that the incidence of bacteremia in patients hospitalized post diagnosis of pneumococcal pneumonia was 15% in those immunized with the 23-valent PS vaccine within the previous 5 years, as opposed to 35% among unimmunized patients.[92]

Nevertheless, the effectiveness of pneumococcal PS vaccines is not unquestionable. Pneumococcal PS vaccines are poorly immunogenic in population

groups which are at high risk such as young children below 2 years of age and immunocompromised individuals.[93,94] Thus, the PS vaccine is usually administered to older children and adults and is not prescribed for children less than 2 years of age. The effectiveness of the vaccine also depends on the genetic pattern of healthy individuals making some of them hard to immunize.[95] Moreover, PS vaccines do not induce T-cell dependent immune response, which implicates the absence of memory B-cells, limiting the period of protection.[96] Thus, even in healthy adults, revaccination is often needed, although it does not result in effective subsequent responses.[5] In addition, there is also the risk of pneumococcal disease outbreak from the rest of the serotypes not covered by the vaccine.[6] These factors have led to the development of more effective vaccines.

1.6.3 Pneumococcal conjugate vaccines

Attempts to reduce the complexity of the PS vaccine and replace carbohydrate antigens with more immunogenic molecules led to the development of glycoconjugate vaccines. The improved immunogenicity achieved by conjugating a pneumococcal PS to a protein carrier was first demonstrated by Avery and Goebel in 1931. They reported that chemical conjugation of type 3 pneumococcal PS to a protein carrier resulted in high immunogenicity in rabbits.[97] This paved the way for the development of the first generation multivalent PS-protein conjugated vaccines.

The vaccine is synthesized by linking a protein carrier to capsular PS by covalent bonding.[98,99] Various techniques used to achieve this include the reaction of amino side chains on lysine or arginine residues of proteins either with the activated carboxylate groups on polysaccharide chains (i.e. carbodiimide coupling) or with aldehyde groups

generated by the oxidation of 1,2-diols on the sugar chains (i.e., reductive amination). The glycoconjugates thus obtained have highly complex, matrix-like structures that are extremely hard to characterize.[27]

Unlike PS antigens, proteins generate a T-cell dependent immunological response. Proteins are broken down into peptides that associate with class II major histocompatibility complexes (MHCs) of the cell surface and are presented to peptide-specific T cells, which stimulate antibody production by B cells. This kind of immune response is characterized by antibody isotype switching, an improved B cell response and the generation of memory B cells, which confer strong, long term protection.[100] The glycoconjugate antigens are capable of inducing immunogenicity in children as well as the elderly.

The formulation of conjugate vaccines is influenced by various parameters, which influence immunogenicity, such as the molecular size of the PS component, the nature of the carrier protein, the PS/carrier ratio and the method used to covalently link the two components.[5] The PS/carrier ratio is significant as too much carrier antigen may impair antibody response to polysaccharides by antigen competition or carrier mediated epitope suppression.[101] Moreover, due to the complexity of the vaccine and the high costs involved in the formulation, the serotypes included cannot be as high as in PS vaccines.[50] Currently available conjugated vaccines in the market comprise 7, 9, 10, 11 or 13 serotypes, use different cross-linking chemistries and incorporate different carriers. Among these, the 7-valent and the 13-valent ones have been widely used.

PCV7: The heptavalent pneumococcal vaccine (PCV7), marketed under the trade name Prevnar, is a conjugate polysaccharide vaccine which is formulated using pneumococcal serotypes 4, 6B, 9V, 14, 18C, 19F and 23F involved in pediatric infections.[1] The capsular PS are conjugated to the highly immunogenic cross-reactive material 197 (CRM197), a non-toxic diphtheria toxoid protein by reductive amination. This vaccine was licensed in the United States in the year 2000 and in many European countries thereafter. It is administered via the intramuscular route. This vaccine is safe and has been recommended for children under 2 years of age. A Californian study demonstrated a 97% reduction in pneumococcal bacteremia and 67% reduction in otitis media cases upon immunization with this vaccine.[102]

PCV7 is also reported to impart mucosal immunity in addition to systemic immunity, as demonstrated by the decline in nasopharyngeal colonization.[103] This could lead to reduced risk of person to person transmission of pneumococcal diseases and lower cases of antibiotic resistance.[104,105] Surveillance studies confirmed decreased rates of invasive pneumococcal diseases (IPD) and nasopharyngeal carriage due to vaccine-type strains in the post-PCV7 era. This was also the case in non-vaccinated human populations following universal PCV7 immunization in the United States, implying acquisition of herd immunity by this vaccination regimen.[106] Imparting of herd immunity is a big advantage of pneumococcal conjugate vaccines.

PCV13: PCV13 is a conjugate vaccine comprising of the pneumococcal serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F, individually conjugated to CRM197. Although PCV7 covered most of the disease causing serotypes in many regions of the world, it covered only about 50% of the disease causing serotypes in some countries.

Serotypes not covered in PCV7 include 1 and 5 which are a major cause of morbidity in Africa, the southern cone of South America and certain parts of Asia, 6A and 19A which have long been causes of invasive diseases, and 3 and 7F which are significant causes of invasive diseases in United States and Europe and associated with increased case fatalities.[107] Thus, PCV13 was formulated incorporating these additional serotypes. The serotypes covered in PCV-13 account for more than 80 % of pneumococcal diseases worldwide.[107]

PCV13 has the same carrier protein and is formulated in the same way as PCV7. This vaccine contains 2.2 µg of each polysaccharide (except for 4.4 µg of 6B), 5 mM succinate buffer, 0.02% polysorbate 80 and 0.125 mg of aluminum as aluminum phosphate per 0.5 ml dose.[108] It was approved in 2010 by FDA but awaits the results of clinical trials for implementation in older adults.

PCV13 has been found to be very effective in preventing invasive pneumococcal diseases (IPD), especially in young children. This was supported by a study reported by Singleton *et.al*. During 1996 to 2000, Alaska Native children aged below 5 years from Yukon Kuskokwim Delta (YKD) in Alaska suffered 10-fold higher cases of IPD compared to non-Alaska Native children. After immunization with the 7-valent pneumococcal conjugate vaccine (PCV-7), IPD rates decreased to 148 per 100,000 during 2001 to 2004, but increased to 426 per 100,000 during 2005 to 2007 due to infections caused by non-vaccine serotypes. This prompted to assess the impact and applicability of the new PCV13 vaccine. In an intervention study with PCV13 carried out on children from the YKD region in 2009, 372 subjects received this vaccine during the clinical trial and 3342 postlicensure (April 2010 to August 2011). In YKD children aged

below 5 years, 52 IPD cases occurred during 2005 to 2008 which decreased to 9 during January 2009 to August 2011. On the other hand, none of the cases were a result of serotypes included in PCV13. This suggests a protective effect of this vaccine.[108]

Table 1 summarizes the serotypes covered by current pneumococcal conjugate and polysaccharide vaccines. A few issues challenge the effectivity and long-term applicability of conjugate vaccines. Current conjugation chemistry requires polysaccharide modifications (e.g., random oxidation of the sugar chain) that alter natural epitopes, with consequent generation of low-affinity antibodies to the native polysaccharide. In addition, random conjugation between activated groups along the length of multiple repeating units of a polysaccharide and random active sites on a protein (e.g., all lysine residues) is difficult to reproduce from batch to batch in a clinical vaccine preparation. These issues have been resolved by chemically controlled coupling of polysaccharides or oligosaccharides derived from capsular PS to carriers using linker molecules.[27]

Table 1.1 Pnuemococcal serotypes covered by vaccines (Adapted from Mehr and Wood (2012).[109]

	PCV-7	*PCV-13*	*23v PSV*
Name	*Prevenar 7*	*Prevenar 13*	*Pneumovax*
Protein conjugated	+	+	-
Proteins used in conjugation	CRM197	CRM197	
1		+	+
3		+	+
4	+	+	+
5		+	+
6A		+	+
6B	+	+	+
7F		+	+
9V	+	+	+
14	+	+	+
18C	+	+	+
19A		+	+
19F	+	+	+
23F	+	+	+

Other serotypes			*(2,8,9N,10A,11A,12F,15B,17F,20,22F,33F)*

Other major concerns regarding the use of PCV7 and other pneumococcal conjugate vaccines are the limited coverage of pneumococcal serotypes and the possibility of strain replacement by non-vaccine serotypes owing to selection pressure. Some of the emerging serotypes such as 19A are attributed with increased antibiotic resistance and invasive disease causing capability.[110,111] Capsule type switching as a result of recombination events, may broaden the range of resistant strains among non-vaccine serotypes, enabling the spread of invasive disease.[111] The use of carrier proteins also carries the risk of excessive production of anti-carrier antibodies as a result of frequent immunizations in childhood, suppressing subsequent responses to antigens.[112,113] This problem may be overcome by optimizing peptide presentation to T cells by coupling PS to peptide epitopes rather than entire carrier proteins and by using recombinant protein carriers which incorporate epitopes from multiple antigens.[27] Another disadvantage of conjugate vaccines is the high cost prohibiting its use in developing countries.[17] Thus, novel techniques for the manufacture of affordable vaccines incorporating current disease causing serotypes need to be developed to sustain the applicability of conjugate vaccines.

1.6.4 Purified protein vaccines

Limited coverage of pneumococcal serotypes, complexity of manufacturing and cost are the drawbacks of glycoconjugate vaccines. These potential shortcomings necessitate the development of more reliable vaccines based on virulent proteins which are

common to all serotypes. These include pneumolysin, adhesins, invasion proteins and transport proteins.[25]

Pneumolysin is a widely studied pneumococcal virulent protein. Since pneumolysin is a cytotoxin, it is unsuitable for use as human vaccine in native form. This problem was overcome by introducing a mutation in the genes expressing toxicity, resulting in nontoxic but immunogenic pneumolysoids.[114] Although pneumolysoids proved protective against virulent pneumococci in mice, the degree of protection it can render as a human antigen is unclear.[115] The reason for this is that pneumolysin is not a surface expressed antigen and is released as a result of cell lysis. The assumed mechanism of protection is by neutralization of the toxic and inflammatory effects of pneumolysin, rather than opsonin-mediated phagocytosis.[50]

PspA is a surface expressed pneumococcal protein. Its N-terminal portion is responsible for its virulence. Thus, a fragment of the N-terminal portion of PspA used as vaccine proved effective in mice against systemic challenge.[116] Although PspA shows considerable antigenic heterogeneity among different strains, it is capable of eliciting cross-reactive antibodies which are effective against heterologous PspA molecules.[117] This makes it a suitable vaccine candidate. Active immunization with PspA has shown to protect against invasive infections and nasopharyngeal colonization[118]. The safety and immunogenicity of PspA in humans was illustrated by a phase I clinical trial. In this trial, sera from humans immunized with this antigen passively protected mice against infection by *pneumococci*.[106]

PsaA is yet another potential vaccine candidate. In the presence of strong adjuvants such as CTB, it was found to reduce nasophayngeal carriage.[119] Oral vaccination with PsaA encapsulated within microalginate microspheres provided protection against colonization and invasive disease.[120] Vaccination with PspC (Cbp A) has shown to be effective in mice. The antibodies against this protein also exhibit cross-reactivity against PspA protein.[121]

Purified protein conjugated vaccines are an interesting option for pneumococcal disease prevention. Since the virulent protein antigens are not used as vaccines in early childhood, the risk of excessive production of anti-protein antibodies leading to subsequent suppressed immune responses is relatively very low. Antibodies produced against these antigens may enhance opsonization and phagocytosis or reduce inflammation caused by release of these protein fragments.[5]

1.6.5 Combination vaccines

Although the purified-protein conjugated vaccine seems a promising alternative to glycoconjugate vaccines, the formulation of these vaccines could be quite challenging. Most virulent proteins exhibit heterogeneity across different strains. Antibodies produced against a single antigen may not recognize variants. Moreover, since almost all virulent pneumococcal proteins considered as vaccine candidates are directly or indirectly involved in pathogenesis, each protein functions independent of the other in contributing to pathogenesis and the protective capabilities of individual antigens differ towards different strains. Thus, a combination of these antigens might be a more effective vaccine than individual antigen based vaccines. Some reports have validated

this strategy. A combination of pneumulysoid and PspA administered intra-peritoneally, rendered higher protection by reducing *pneumococci* in the lungs of mice than individual antigens.[122] Similarly, mucosal immunization by a combination of PsaA and PspA proved to be more effective in reducing nasopharyngeal carriage of *pneumococci*.[123] Since nasopharyngeal colonization is the first step towards IPD, prevention of this event is of supreme importance. Moreover, prevention of colonization also leads to herd immunity by controlling the horizontal spread of the strains.

Formulation of vaccines by combining antigens requires consideration of additional factors. This was suggested by a study in which a combination of pneumolysin or PspA with PsaA, administered via the intraperitoneal route, did not result in better immunization compared to pneumolysin or pspA alone in mice. However, when administered via the mucosal route, the combination of PspA and PsaA provided higher protection compared to either protein alone or a combination of PsaA with pneumolysin. This implied that PsaA is more important in the nasopharynx than lungs and is more accessible in the former niche, while pneumolysin plays a minor role in colonization but has a major role in the lungs and in the propagation of invasive disease. It may be inferred from this study that optimum vaccine formulation must take into consideration the following factors[50]:

1) Mode of delivery: Oral or nasal delivery is as effective as systemic administration for pneumococcal vaccines. These routes are preferred in children as they already are administered a large number of vaccines by intramuscular or intraperitorial routes. Oral administration is also very helpful for HIV/AIDS patients who have intact mucosal immune system responses.[17]

2) Stage of pathogenesis being targeted: Some antigens such as PsaA are more effective in controlling colonization in the nasopharynx while others are more effective in preventing invasive infections alone. Thus, the choice of the carrier antigen is to be made depending on the target site and the stage of pathogenesis for immunophylaxis.

In conclusion, candidates for vaccine formulation must be chosen based on data from comparative studies of effectiveness against a broad range of serotypes, both singly and in combination with other proteins. Other methods to improve the effectiveness of vaccines such as addition of an adjuvant to protein based vaccines which helps in producing better immune responses and encapsulating antigenic proteins so as to protect them from degradation in the stomach are to be investigated.[120]

1.7 Zwitterionic Polysaccharides

Zwitterionic polysaccharides (ZPSs) are bacterial capsular polysaccharides which elicit T cell dependent immune responses and direct the cellular and physical maturation of the developing immune system in the host.[27] Immunomodulatory ZPSs are expressed by commensal bacteria which colonize in mammals. Some ZPSs that have been identified from different bacterial species are PS A1 and PS B from *Bacteroides fragilis* strain 9343, PS A2 from *B.fragilis* 638 and capsule polysaccharide from type 1 *Streptococcus pneumoniae* (Sp1).

The biological importance of ZPSs as immunomodulatory molecules was illustrated by the study of abscess formation in human abdomen post surgery or physical trauma. It was found that the gram negative obligate anaerobe *B.fragilis* which

is a symbiotic microorganism present in all mammals was responsible for this sepsis.[124] Studies on rodents to identify the virulence factor revealed the involvement of a high molecular weight capsular polysaccharide complex (CPC) expressed at the surface of the bacterium. Administration of this CPC alone protected animals against challenge with B.fraglis or CPC. CPC also provided protection against other encapsulated bacteria present in the caecum with which the animals were challenged.[125] This confirmed its antigenic properties. Two polymers responsible for immunogenecity, polysaccharide A1 (PS A1) and polysaccharide B (PS B) were initially purified from this CPC.[126] PSA1 is the most immunodominant constituent of CPC and was used as a prototype to investigate the properties of ZPSs.

ZPSs evoke immune responses which are unique among bacterial polysaccharides. These polysaccharides contain T cell epitopes. Activation of T cell requires antigen processing cells (APCs) and two signals characterized by the interaction of T cell receptors (TCR) on T cells with MHC class II on APCs (Signal 1), and ligation of CD28 on T cells with B7 on APCs. These ZPS-mediated T cell responses result in the production of cytokines which confer ZPS the protection ability against abscess formation (Figure 1.4).[127]

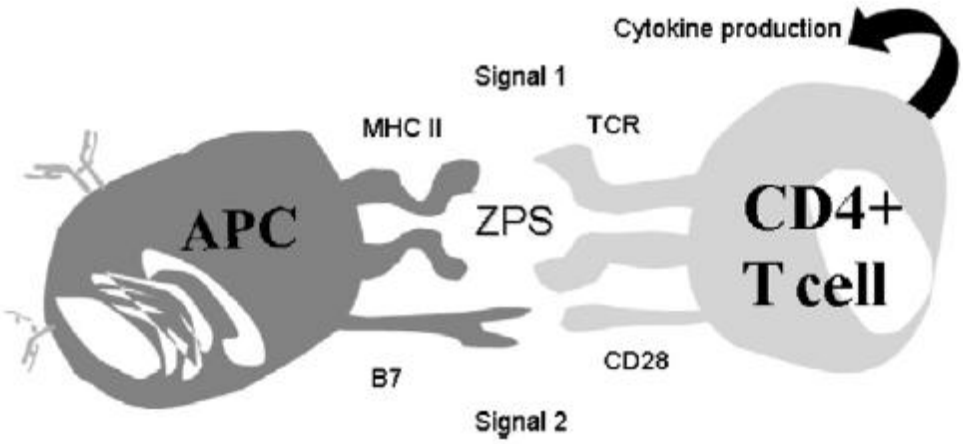


Figure **1.4:** T-cell-APC interactions during ZPS-mediated T cell activation

ZPSs differ significantly from each other in chemical structure (Figure 1.5).[127] However, their common feature is that all of them contain both a positive and negative charge in each repeating unit. This is unlike common bacterial polysaccharides which are either neutral or negatively charged. It is this feature that imparts ZPSs their unique biological functions. The zwitterionic charge motif was found to be crucial for the immunogenicity of ZPSs. This was proved by the studies of abscess formation in animals administered with modified ZPSs. Chemical neutralization of either of the charged groups destroyed the ability to induce abscess formation. Conversion of the free amino groups into N-acetyl groups or conversion of negatively charged carboxyl groups into hydroxymethyl groups via carbodiimide reduction failed to induce abscess formation. However, the conversion of a polysaccharide into a zwitterionic polymer conferred the ability to induce abscess formation.[127] These results conclude that zwitterionic charge is essential for inducing immunogenic responses. Protective activity of ZPSs was also shown to depend on the structure of the polysaccharide.

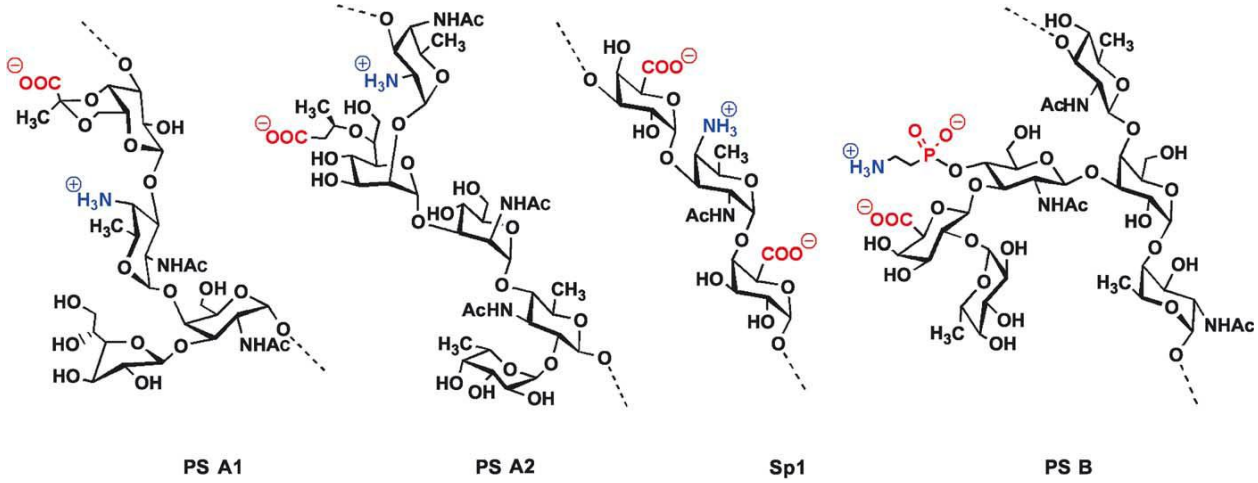


Figure 1.5: Chemical structures of the repeating units of various zwitterionic polysaccharides

Molecular size of ZPSs also plays an important role in the extent of T cell activation and hence the protective ability. This idea was substantiated by a study on the influence of molecular size of the ZPS, PS A on T cell response in vitro and in vivo. T cell proliferation in vitro and protection of rats against abscesses induced by viable B.fragilis by PS A of various chain lengths, generated by ozonolysis, was examined. It was shown that rats treated with 129 kDa, 46.9 kDa and 17.1 kDa PS A fragments were protected against abscesses whereas those treated with 5 kDa (6 repeating units) fragment were not. These results suggested that ZPS fragments comprising a minimum of 22 repeating units (88 monosaccharides) are required to elicit the required immune response.[128]

1.8 Synthesis of zwitterionic polysaccharides

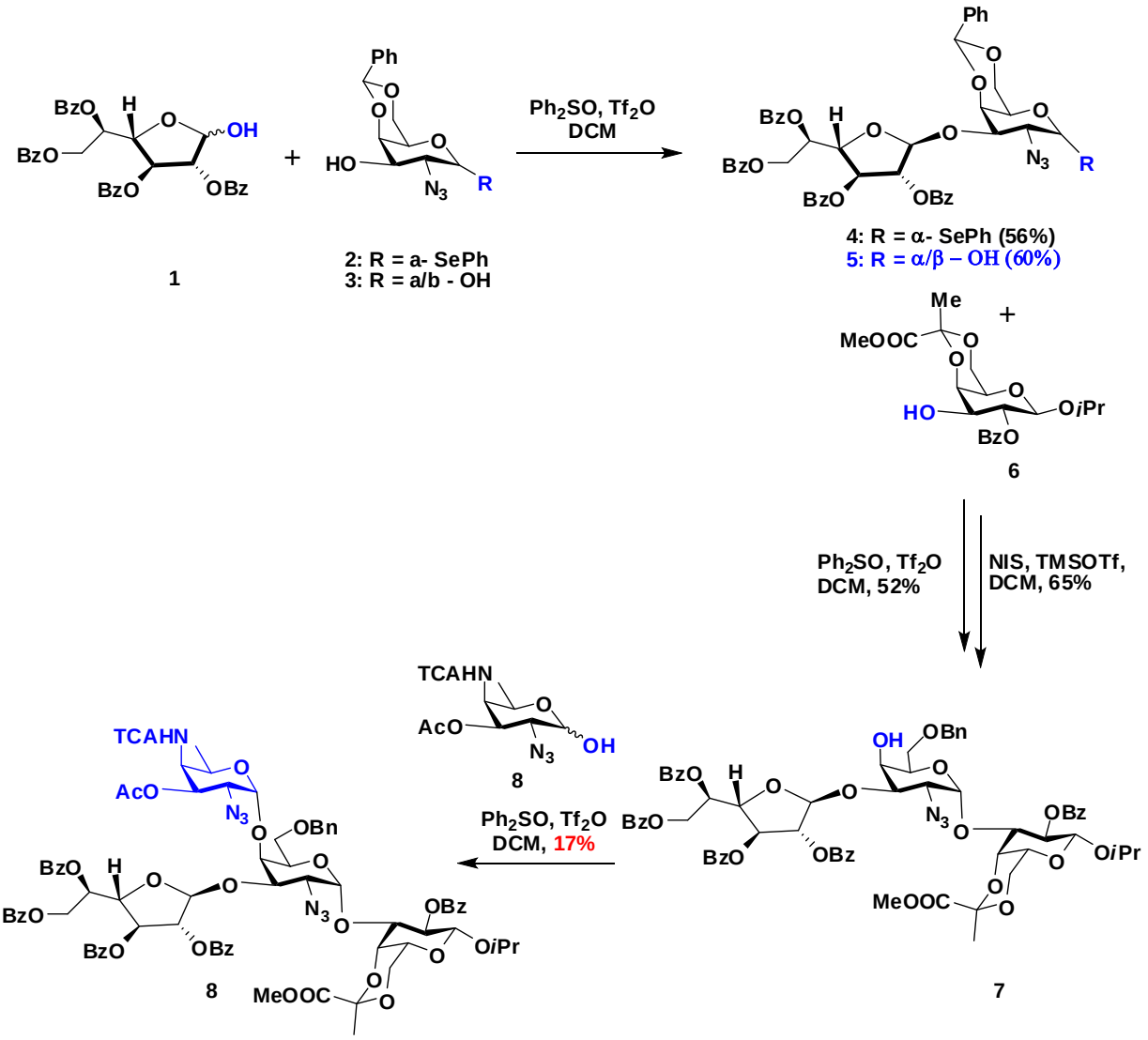
ZPSs constitute a structurally distinct category of carbohydrates and are capable of eliciting a T-cell dependent immune response. Each ZPS carries a high density of

positively charged amino group and negatively charged carboxyl or phosphate groups. Positive and negative charges are exposed on the outer surface of the polymer in a regularly spaced pattern, which renders them easily accessible to other molecules. Functional group alterations of positively charged amines to neutral acetamido groups have shown that zwitterionic charges are essential for the activity of the above mentioned molecules. Depolymerization of native oligosaccharides isolated from the gram-negative anaerobic *Bacteroides fragilis,* keeping the structure intact, has also proved that oligosaccharides of molecular weight around 8kDa possess biological activity. Hence, successful synthesis and deprotection of oligosaccharide fragments that contain the minimum structural elements (positively charged amino group and negatively charged carboxyl or phosphate groups) will provide valuable information of precise structure-function relationships. Recently there have been few reports of syntheses of zwitterionic repeating units.

1.8.1 Synthesis of PSA1 protected tetrasaccharide repeating unit

Zwitterionic polysaccharide A1 (PSA1) was isolated from the capsules of the anaerobic bacterium *Bacteriodes fragilis.* Van Der Marrel group,[129] with the aim of elucidating the interaction of bacterial polysaccharides with the immune system, synthesized the protected tetrasaccharide of the repeating unit **8** of PSA1. Because of the length and complexities involved in the synthesis of 2,4-diamino-2,4,6-trideoxygalactose (AAT) building block, they introduced AAT building block **8** toward the end of the synthesis. However, this particular retrosynthetic approach led to the poor yields in the [1+3] glycosylation because of steric hindrance on the trisaccharide acceptor. Most of the glycosylations in this synthesis utilized Gin's dehydrative conditions by pre-activating 1-

hydroxy donors (**1,5,8**) with Ph_2SO/Tf_2O followed by addition of a solution of acceptors (**2,6,7**).

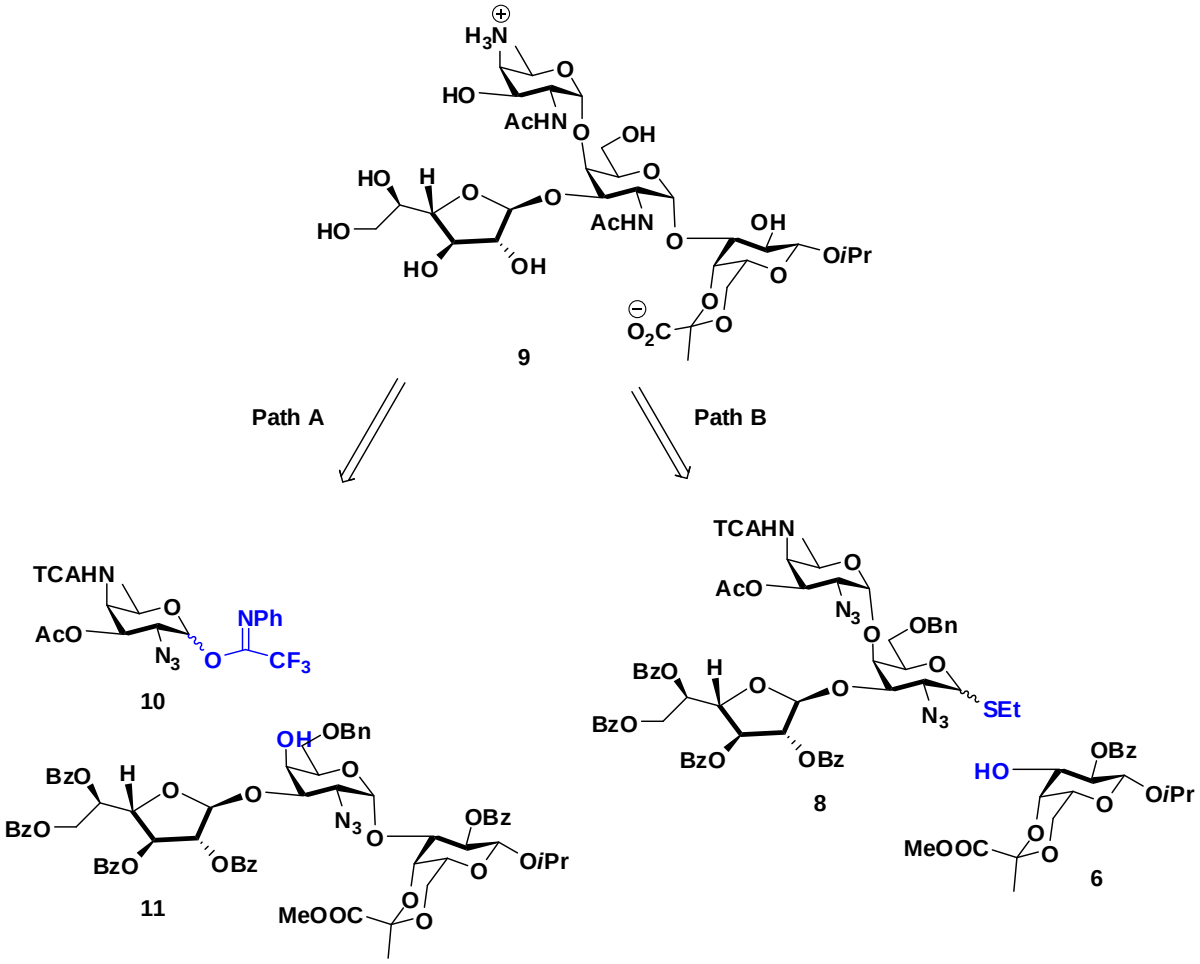


Scheme 1.1: Van Der Marrel's synthesis of PSA1 repeating unit

1.8.2 Synthesis of PSA1 deprotected tetrasaccharide repeating unit

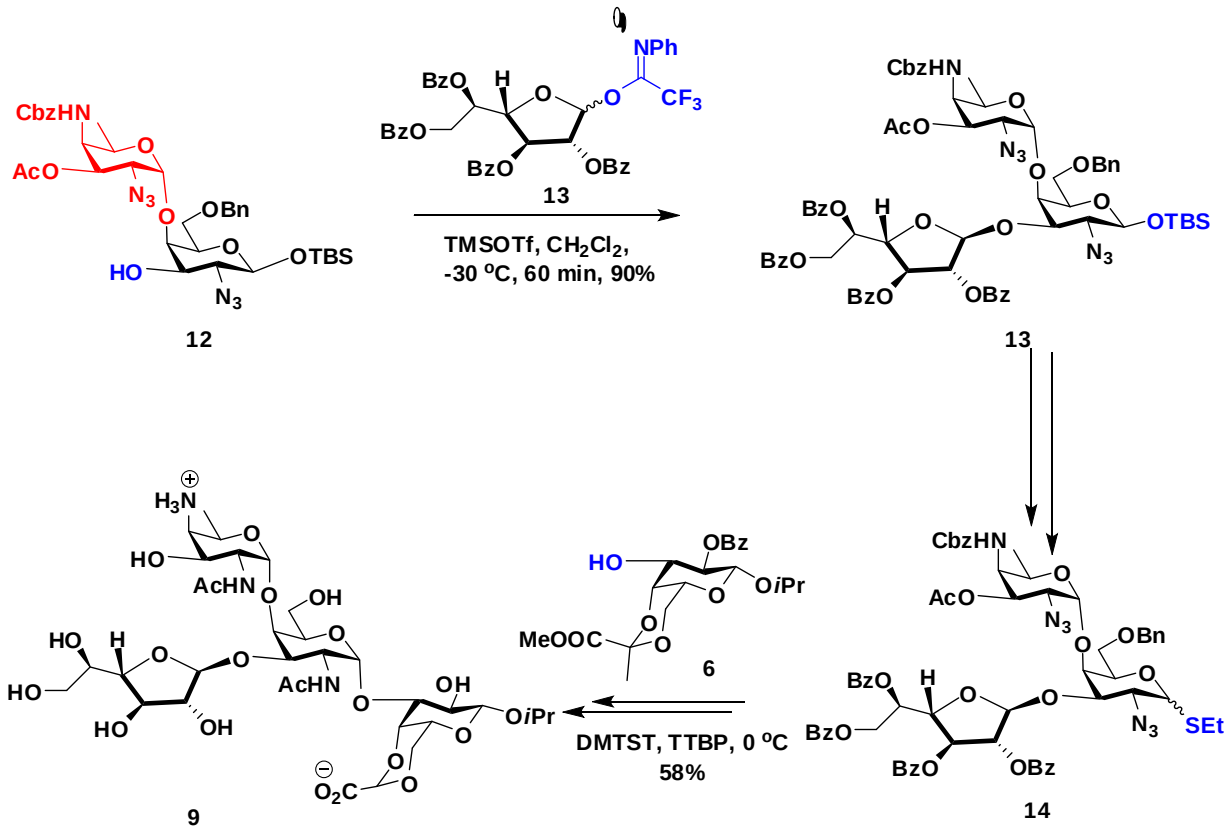
Seeberger and co-workers have addressed the challenged faced by previous synthesis by following two synthetic pathways.[130] In path A (Scheme 1.2), they attempted a [3+1]

glycosylation strategy which involved the less nucleophilic trisaccharide acceptor **11**. It was observed that they could not obtain any amounts of tetrasaccharide by coupling AAT donor **10** and trisaccharide acceptor **11** under the same conditions that yielded high amounts of disaccharides using monosaccharide C4-OH acceptor. Hence, they followed path B which involved the [3 +1] glycosylation between trisaccharide glycosylating agent **8** and pyruvalated galactose nucleophile **6.** This particular approach led to an efficient synthesis of tetrasaccharide repeating unit **9.**



Scheme 1.2: Seeberger's synthesis of PSA1 repeating unit

Initially disaccharide **12** was efficiently synthesized in 74% yields using AAT donor **10** and galactosamine acceptor. Glycosylation (Scheme 1.3) of resulting alcohol **12** with galactofuranose *N*-phenyl trifluoroacetimidate **13,** at -30 °C, generated the trisaccharide molecule **13** in 90% yield as the β-isomer. Trisaccharide **13** was converted into thioglycoside donor **14** which was coupled with pyruvalated galactose acceptor **6** under dimethyl(methylthio)sulfonium triflate (DMTST) and TTBP conditions generated the tetrasaccharide in best yields of 58%. Tetrasaccharide was efficiently deprotected to obtain the desired PSA1 repeating unit **9** in good yields.

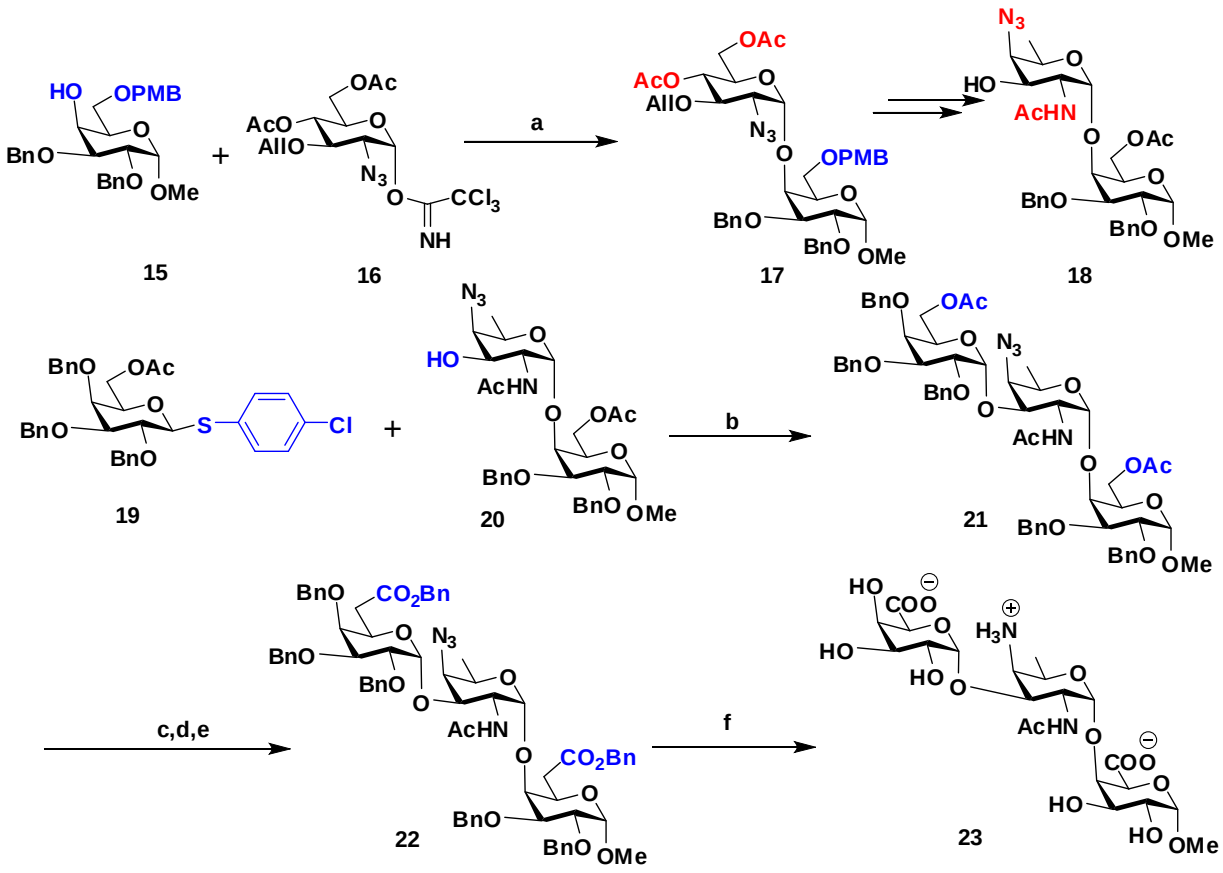


Scheme 1.3: Seeberger's synthesis of PSA1 repeating unit

1.8.3 Synthesis of Sp1 trisaccharide repeating unit

Because of the low reactivity associated with uronic acid derivatives, Bundle group chose to introduce carboxylic acids functionalities in **23** at the end of the synthesis by oxidation of the hydroxymethyl group of appropriately prepared substrate oligosaccharides.[131] 1,4-cis-*α*-galactose linkage of the diamino-dideoxyhexose residue **17** was achieved by using an azido moiety at C-2 as a nonparticipating group combined with O-6 acetate, by taking advantage of remote participation from .C6 ester functionality.

Glycosylation (Scheme 1.4) of suitably protected building blocks **15** and **16** under TMSOTf activator conditions generated the disaccharide **17** in 60% yields, which was subsequently converted into disaccharide acceptor **18**. The glycosylation of disaccharide **16** by thiogalactoside **19** in the presence of N-iodosuccinimide (NIS) and trifluoromethane sulfonic acid (TfOH) at -30 ^{o}C in dichloromethane (CH_2Cl_2) furnished the required trisaccharide **22** in 73% yield. Installation of carboxylic acids at C6 positions followed by hydrogenolysis generated the required monomeric repeating unit **23**.



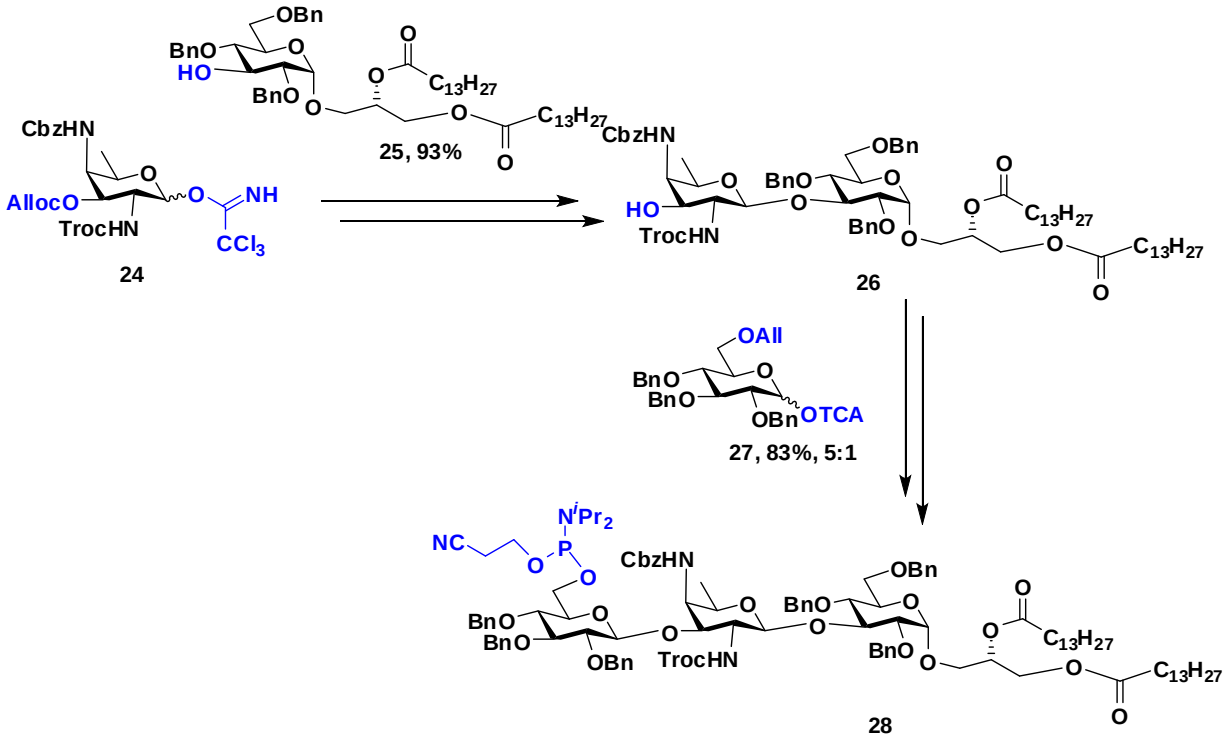
Scheme 1.4: Bundle's synthesis of Sp1 trisaccharide repeating unit

Scheme 4. (a) TMSOTf, -15 ^{0}C, rt, 60 %; (b) NIS, TfOH, CH_2Cl_2, -30 ^{0}C, rt, 73%; (c) NaOMe, MeOH, 100 %; (d) TEMPO, KBR, NaOCl; (e) BnBr, CsF, DMF, 51% over three step; (f) H_2, HOAc/H_2O, Pd/C (10 %), 58 %.

1.8.4 Synthesis of Lipoteichoic acid 40

In 2010, Schmidt and co-workers developed a complete synthesis of lipoteichoic acid (LTA) isolated from streptococcal strain R6.[132] They chose a monomeric pseudopentasaccharide repeating unit with a lipid trisaccharide as their target molecule.

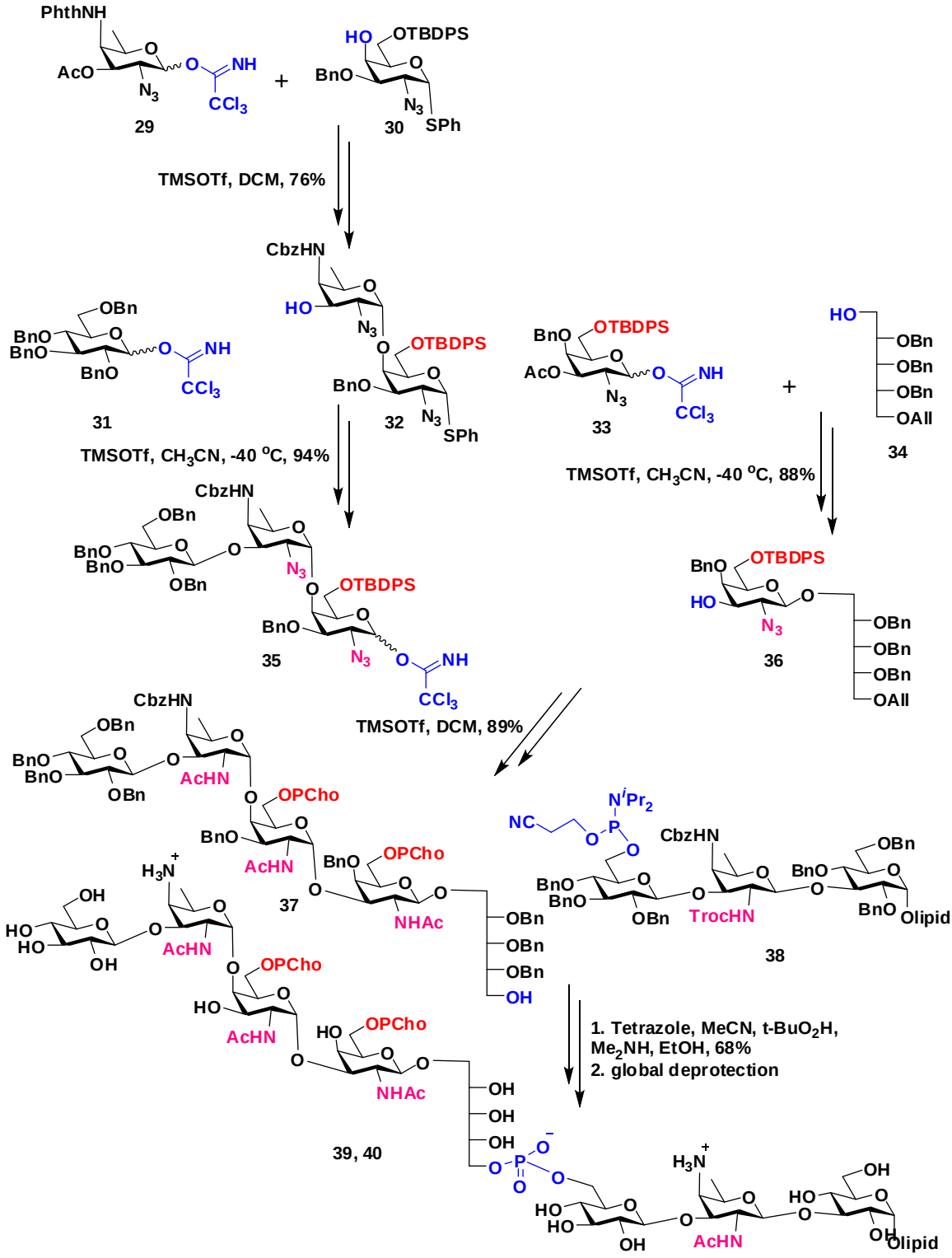
After synthesizing the AAT building blocks **24** and **29** by inversion of C4-OH with Tf_2O and potassium phthalimide, they initially attempted the synthesis of lipid containing trisaccharide **28**. After synthesizing the required lipid acceptor **25**, it was coupled to NH-Troc donor under TMSOTf conditions generated disaccharide **26** in 93% (Scheme 1.5). Cleavage of Alloc group followed by glycosylation with donor **27**, with the help of nitrile effect, yielded trisaccharide **28** in 83% yield.



Scheme 1.5: Chemical synthesis of trisaccharide **28**

The pseudopentasaccharide **37** was then constructed from trisaccharide **35** and disaccharide **36**. C4-OH acceptor **30** was glycosylated with AAT donor **29** under TMSOTf conditions followed by deacetylation at C3 position generated the disaccharide donor **32** in 76% (Scheme 1.6). Glycosylation of donor **31** with the synthesized

disaccharide acceptor **32**, with the help of nitrile effect, yielded trisaccharide in 83% yield, which was converted into a trichloroacetimidate donor **35.** Schmidt group was also able to efficiently couple trisaccharide donor **35** and disaccharide acceptor **36** in high yields using TMSOTf as an activator**.** Three azide groups were reduced to acetamido groups followed by desilylation and subsequent introduction of phosphocholines generated the challenging pseudopentasaccharide **37**. Ligation of pentasaccharide **37** with trisaccharide **38** with tetrazole followed by oxidation with *tert*-butyl hydroperoxide and dimethylamine generated the phosphodiester **39**. Global deprotection of **39** followed by purification by hydrophobic interaction chromatography generated the impressive LTA **40**.



Scheme 1.6: Complete chemical synthesis of LTA by Schmidt group

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CHAPTER 2

SYNTHESES OF THE FRAGMENTS OF PSEUDOPENTASACCHARIDE REPEATING UNIT OF *STREPTOCOCCUS PNEUMONIAE* ZWITTERIONIC POLYSACCHARIDE

2.1 Introduction

Streptococcus pneumoniae is one of the most common gram positive pathogens that colonize the upper respiratory tract and causes and severe infections like otitis media, sinusitis, and more life threatening diseases like pneumonia, bacteremia and meningitis. *Streptococcus pneumoniae* is the main cause of bacterial pneumonia, one of the leading causes of infectious disease deaths in the United States. *S. pneumoniae* has a thick polysaccharide capsule that covers the peptidoglycan cell wall.[12] There are around 94 distinct capsular serotypes and all the 94 serotypes differ in virulence, prevalence and extent of drug resistance. All pneumococci possess two common polysaccharide antigens: C-polysaccharide and F-antigen. The C-polysaccharide is a cell-wall teichoic acid composed of tetrasaccharides joined together through ribitol phospho-diesters and hence it is classified as a ribitol teichoic acid.[13] C-polysaccharide also contains phosphocholine substituents. The F-antigen is a lipoteichoic acid with a polysaccharide identical to C-polysaccharide and this part is linked to a diacylated glycerol residue via a glucose residue.[7]

Carbohydrates are poor immunogenic molecules and do not require T cells to induce immune response, and do not exhibit B cell stimulating activity. Hence, carbohydrates fall under type 2 T cell independent (TI) antigens. Also, plain polysaccharides cannot stimulate immune response in children less than two years old. However, recent studies have showed that a new class of polysaccharides, zwitterionic polysaccharides (ZPSs), are immunomodulatory polysaccharides and they specifically activate T cells.[1]

ZPSs constitute a structurally distinct category of carbohydrates and are capable of eliciting a T-cell dependent immune response. Each ZPS carries a high density of positively charged amino group and negatively charged carboxyl or phosphate groups. Positive and negative charges are exposed on the outer surface of the polymer in a regularly spaced pattern, which renders them easily accessible to other molecules.

Kasper's laboratory has shown convincing evidence that ZPSs invoke MHCII mediated T-cell response in the absence of proteins. Their research has also identified capsular polysaccharide structures that induce a CD4+ T-cell response known to modulate bacterial abscess formation. A zwitterionic polysaccharide, PS A1 (**4**), consisting of 120 repeating units of tetrasaccharide molecule carrying an electrostatic charge character on adjacent monosaccharides (because of its zwitterionic nature), has been shown to elicit an immune response similar to that for exogenous proteins.[2,3]

Functional group alterations of positively charged amines to neutral acetamido groups in ZPSs have shown that zwitterionic charges are essential for the activity of the

above mentioned molecules.[4] Depolymerization of native oligosaccharides isolated from the gram-negative anaerobic *Bacteroides fragilis,* keeping the structure intact, has also proved that oligosaccharides of molecular weight around 8 kDa possess biological activity. It was also observed that 5 kDa molecular weight oligosaccharides lost their biological activity.[5] Studies have also confirmed that a minimum chain length of 2 to 3 repeating units are required for cytokine induction from pneumococcal lipoteichoic acids (LTAs) isolated from two different strains (Fp 23 and R6) of *Streptococcus pneumonia.*[6,7] Recently, there have been few reports on the syntheses of complex zwitterionic oligosaccharides. van der Marel laboratory has published a synthetic study for the synthesis of the protected tetrasaccharide repeating unit of zwitterionic polysaccharide PSA1.[8] Very recently, Seeberger and co-workers have reported an improved complete synthesis of the same repeating unit and deprotected the resulting tetrasaccharide.[9] Bundle and Schmidt groups have also published their synthetic findings on the syntheses of zwitterionic C-polysaccharide, and lipoteichoic acid (LTA) that was isolated from different strains of *Streptococcus pneumonia*.[10,11]

Isolation and purification of pneumococcal cell wall polysaccharides from pneumococcal strains leads to micro heterogeneity with regard to substitutions on ribitol residue,[6] and different number of PCho residues on the oligosaccharide backbone. Successful synthesis of well-defined ZPS repeating units, and controlled oligomerization of the monomer resulting in polymer synthesis will provide insights into the minimum number of repeating units that are required for biological activity. Efficient chemical synthesis will also provide opportunities to synthesize specific zwitterionic oligosaccharides with different number of phosphorylcholine (PCho) residues on the

backbone, and to provide valuable information on precise structure-function relationships.

The target C-polysaccharide is isolated from a noncapsulated pneumococcal strain CSR SCS2[14] and serotype 6B, and is composed of a backbone of tetrasaccharide-ribitol repeating units that are linked to each other by a phospho-diester linkage between position 5 of a D-robitol residue and position C6 of a β-D-glucopyranosyl residue.[6,15] The polysaccharide is substituted with one residue of phosphocholine (PCho) at position C6 of the 2-acetamido-2-deoxy-α-D-galactopyranosyl residue and both galactosamine residues are *N*-acetylated. The capsular polysaccharide **1** and its repeating unit **2** are depicted in figure **1.1**. The positive charge is situated at the amino group of the unnatural 2-acetamido-2-deoxy-D-galactopyranosyl residue and the negative charge is situated on the phosphate group that connects the repeating units of psuedopentasaccharides. Our first objective is to synthesize a monomeric unit of C-polysaccharide **2** in a convergent fashion. Synthetic strategies for desired sugar building blocks, and various coupling conditions for syntheses of di- and trisaccharides will be discussed in this chapter.

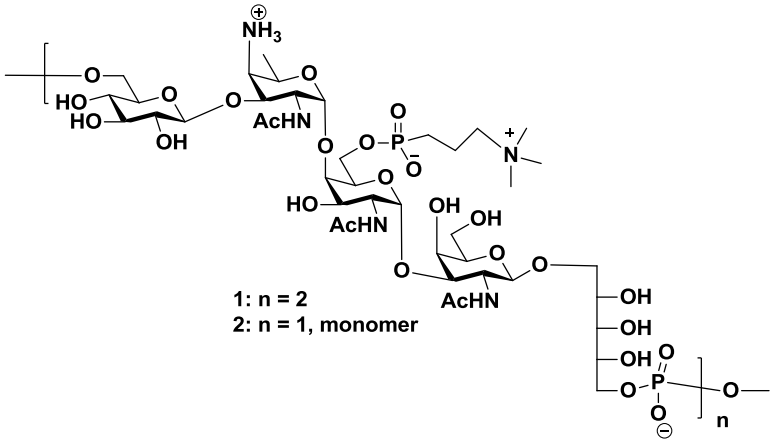


Figure 2.1: Structure of the C-polysaccharide

2.2 Results and Discussion

The C-polysaccharide target **2** is a structurally challenging and demanding molecule.[10, 11] A convergent strategy was planned for the synthesis of **2,** and it's retrosynthetic analysis is outlined in scheme **2.1**. A key issue for the synthesis of the complex oligosaccharides is the availability of high-yielding glycosylation strategies. This is especially true for the synthesis of **2** because of required gram quantities of unnatural building block, 2-acetamido-4-amino-2,4,6-trideoxy-D-galactopyranose **7** (AAT), and the low reactivity nature of C4-OH of galactose acceptor **8** or **8a**. [12, 13, 14, 15] Because of low reactivity and steric bulk on C4 hydroxyl group of galactose building block **8** or **8a**, we have planned a synthetic strategy in which the synthesis of disaccharide **5, 5a** (**BC** moiety) will be attempted initially, and then the synthesized building block **BC** will be coupled to the ribitol containing disaccharide **6** (**DE**). Finally, pentasaccharide will be achieved by coupling the tetrasaccharide **BCDE** to the suitably protected glucose building block **4** (**A** moiety). After accomplishing the synthesis of the pentasaccharide **3**,

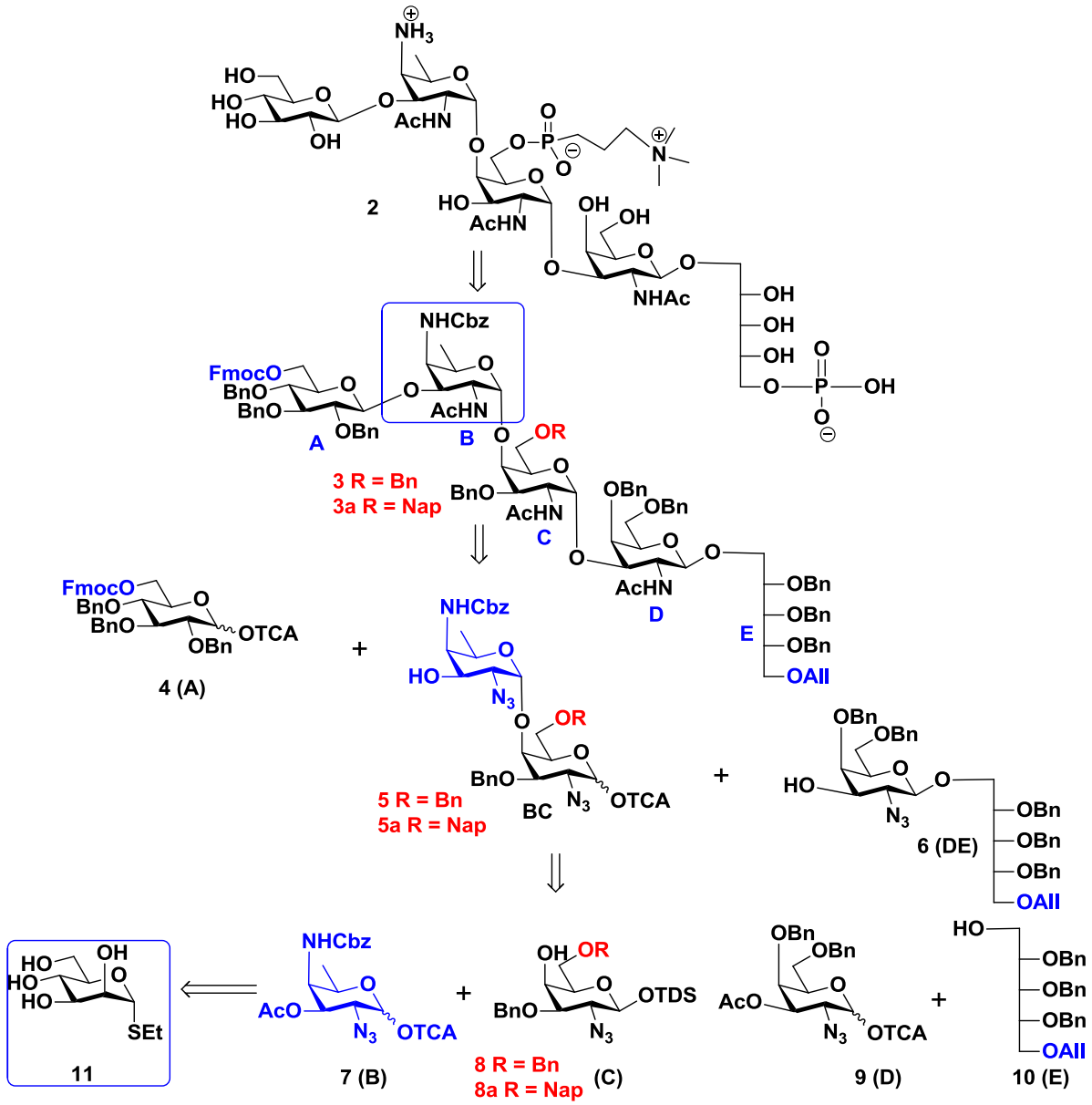
we planned to investigate reaction conditions to install phosphocholine (PCho) at C6 position of galactose in pentasaccharide **3a**.

Three temporary protective groups that are orthogonal to each other have been chosen to synthesize the oligomer **1** with repeating units**,** and to install the required phosphocholine residues. The chosen orthogonal protective groups are flourenylmethyloxycarbonate (Fmoc), 2-naphthylmethyl (Nap) and allyl (All) (Scheme **2.1**). The above mentioned protecting groups can be removed without affecting the other protecting groups to afford the required sugar moieties to oligomerize **3a** and to introduce phosphocholine toward the end of the synthesis.

The repeating pentasaccharide contains two α-linkages, and C2 position of the sugar donors (**B** and **C** moieties) contains *N*-acetamido functionality in the final target molecule. Hence, azide groups have been chosen as non-participating groups to yield stereoselective α-glycosidic linkages, and to generate an amine functionality toward the end of the synthesis. Suitable solvent conditions, taking advantage of nitrile effect, were chosen for the formation of the β-linkage in the synthesis of ribitol containing disaccharide **6**. All the non-reacting hydroxyl groups have been protected as benzyl ethers to avoid side reactions and to aid in easy global deprotection step toward the end of the synthesis. Donor **7** was synthesized through two different challenging routes starting from D-mannose and D-glucosamine.

Since the complete synthesis of the pentasaccharide was not accomplished, and we spent a significant amount of time to investigate various synthetic procedures for the syntheses of building blocks (BC, DE, B, C, D and E moieties), detailed procedures for

the syntheses of the above mentioned building blocks will be discussed in this chapter. Few less successful synthetic strategies for the desired building blocks will also be discussed.



Scheme 2.1: Retrosynthetic analysis

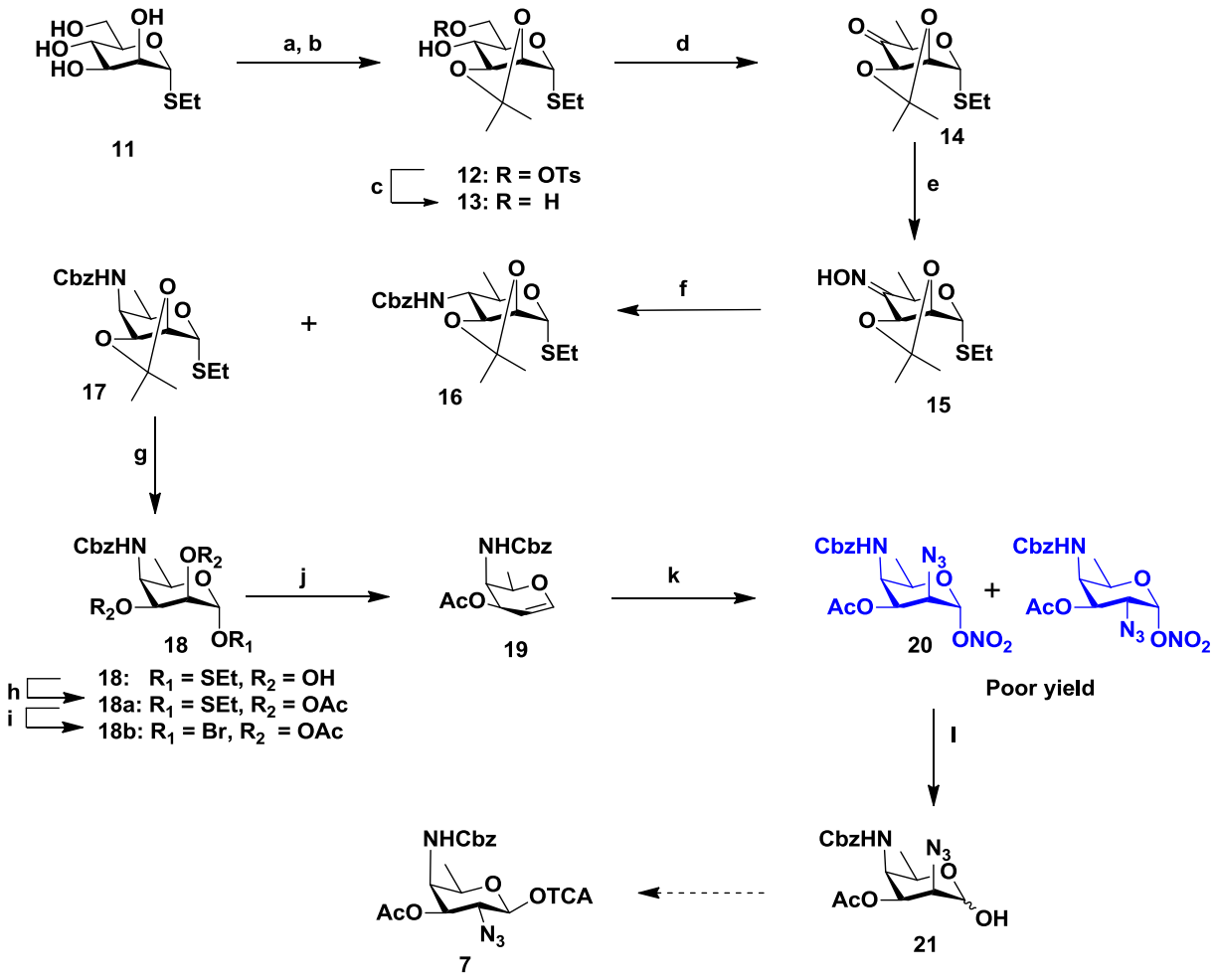
2.3 Synthesis of AAT Building Block 7

For the synthesis of C-polysaccharide **2**, synthesis of gram quantities of AAT building block **7** was important. 2-acetamido-4-amino-2,4,6-trideoxy-D-galactopyranose **7**, abbreviated as AAT, is a rare sugar residue that is present at the cell surface of a number of bacterial polysaccharides. This rare sugar residue AAT is present in many infectious bacteria - *Shigella sonnei*, *Streptococcus pneumonia*, *Bacteroides fragilis*, *Streptococcus mitis* and *Proteus vulgaris*.

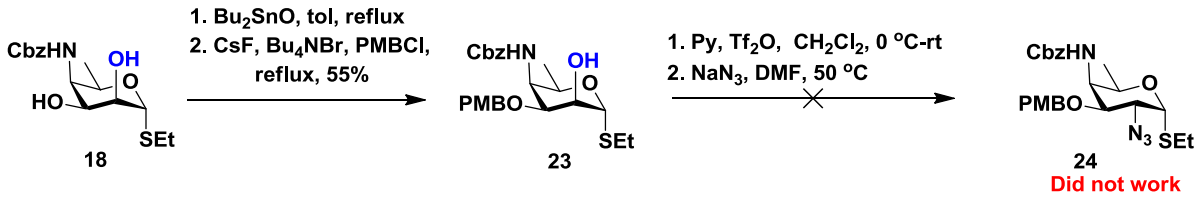
We initiated the synthesis of this demanding pentasaccharide **2** by synthesizing the building block AAT **7** by following a strategy described by van Boom and coworkers.[16] Synthesis was initiated from the easily available starting material ethyl 1-thio-α-D-mannopyranoside **11** (Scheme **2.2**). The easily obtained ethyl 1-thio-α-D-mannopyranoside **11** was regioselectively tosylated with *p*-toluenesulfonyl chloride in pyridine for 16 h to afford C6-tosylate which was converted into C2,C3 acetal **12** by treating with 2,2-dimethoxypropane and catalytic amount of *p*-toulenesulfonic acid. Reduction of C6-tosylate **12** with $LiAlH_4$ under reflux conditions in THF yielded 6-deoxy D-mannopyranoside **13** in 62% yield. C4 hydroxyl of 6-deoxy-D-mannopyranoside **13** was oxidized by Swern oxidation conditions to generate C4-ketone **14** in almost quantitative yield. Swern oxidation conditions required freshly distilled oxalyl chloride and perfect dry conditions to obtain quantitative yields of **14**. Treatment of **14** with hydroxylamine hydrochloride gave the oxime **15**. Reduction of the oxime **15** with sodium cyanoborohydride in the presence of titanium trichloride which was added in portions generated the required amine corresponding to mannose and talose derivatives. The crude amine mixtures were treated with CbzCl in a mixture of water and dioxane yielded

Cbz protected amines **16** and **17**. The required protected talose derivative **17** was separated from **16** by silica gel column chromatography. Acidic hydrolysis of acetonide functionality by treating with a mixture of trifluoroacetic acid and water afforded C3, C4-diol **18**. Diol **18** was acetylated with acetic anhydride and pyridine to generate a diacetate **18a**. Treatment of **18a** with bromine in DCM and, the resulting residue was treated with activated zinc dust and acetic acid and acetic anhydride led to poor yields of galactal **19**. The reported yields for this transformation by van Boom's procedure could not be reproduced. Having suffered many setbacks in synthesizing a significant quantity of **19,** we proceeded ahead by attempting azidonitration conditions to introduce masked amine (azide) functionality at C2 position of D-galactal **19**. However azidonitration reaction of D-galactal generated only 15% of the required derivative **20**. Repetition of this reaction also generated poor yields.

At this point, we have also tried to investigate the inversion of C2-OH in **18** by triflation and subsequent azide substitution of the generated C2 O-triflate (Scheme **2.3**). However, efforts to introduce equatorial azide functionality were unsuccessful because of the electronic effect of α-thio glycosidic linkage in **18**. Having obtained poor yields and mixtures in few key steps toward the later part of the synthetic strategy led us to change our strategy for the synthesis of AAT building block **7**.



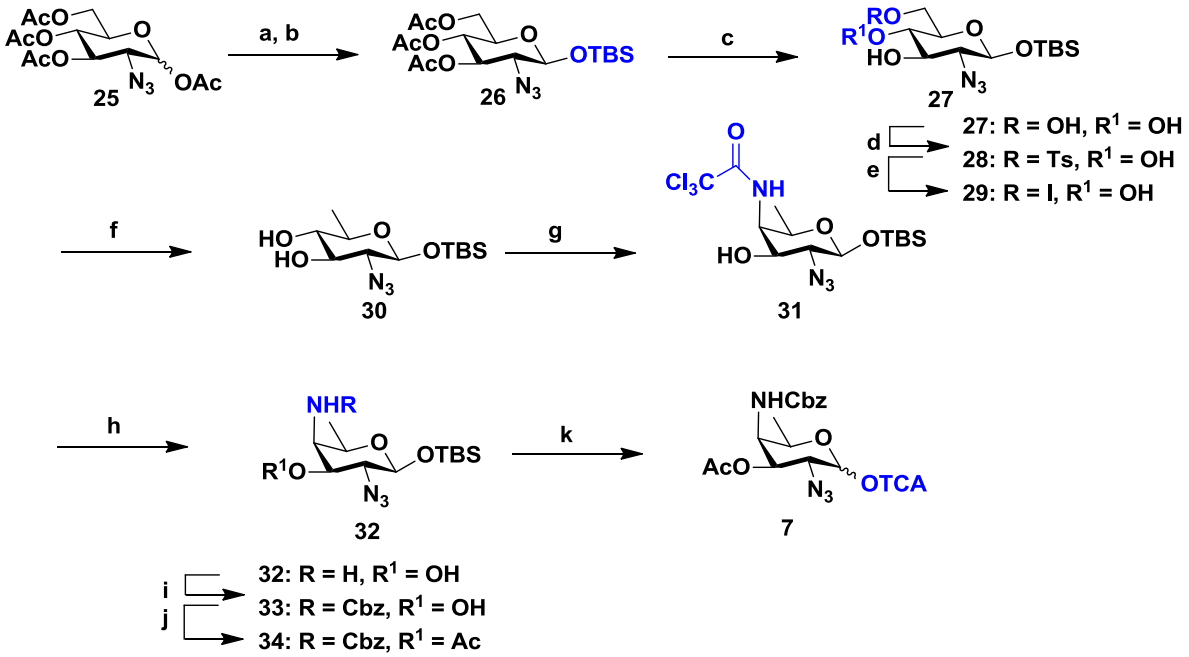
Scheme 2.2: Synthesis of AAT building block **7**. Reagent and conditions: (a) TsCl, py, 16 h, 86% (b) 2,2-dimethoxypropane, acetone, TsOH, 2 h, 75% (c) $LiAlH_4$, diethylether, reflux, 2 h, 62% (d) Swern oxidatin, 100% (e) $HONH_2(HCl)$, py, H_2O, 1 h, 97% (f) $NaCNBH_3$, NH_4OAc, $TiCl_3$ in acidified HCl, CH_3OH, 40 h, CbzCl, $NaHCO_3$, dioxane, H_2O, 1 h, 75% based on 15 (g) TFA, H_2O (9:1), 5 min (h) Ac_2O, py, 10 h, 76% (i) Br_2, CH_2Cl_2, 30 min (j) activated Zn dust, NaI, AcOH, Ac_2O, 0 ^oC, 1 h, 15% (k) NaN_3, $(NH_4)_2Ce(NO_3)_6$, CH_3CN, -15 ^oC, poor yield



Scheme 2.3: Synthesis of AAT building block **7** from D-mannose

Attention was turned to a research study in which an elegant synthesis of AAT building block **7** was described.[8] We followed a synthetic strategy by van der Marel and co-workers (Scheme **2.4**) for the synthesis of building block **7**. Synthesis was initiated from 2-azido glucose **25**. Anomeric position of 2-azido glucose **25** was selectively deacetylated by reacting with hydrazine acetate in DMF to yield anomeric alcohol in 90% yield. The resulting alcohol residue was treated with *tert*-butyldimethylsilyl chloride and imidazole in DCM to afford silyl glycoside **26** in 90% yield.[17] Deacetylation of compound **26** was performed by treating with NaOMe in MeOH by maintaining a pH of 9. Regioselective tosylation of triol **27** with *p*-toluenesulfonyl chloride in pyridine generated C6-tosylate **28** in 78%. The obtained C6-tosylate **28** was treated with NaI in 2-butanone and refluxed to generate C6-iodide **29** in excellent yields. C6-iodide **29** was reduced to 6-deoxy sugar **30** in poor yields by treating with $NaBH_4$ in DMSO. Separation of the product during the work up became cumbersome with high boiling point and water miscibility properties of DMSO. A best yield of 60% was obtained when solvent DMSO was replaced with EtOH. Reduction of C6-tosylate with $NaBH_4$ also produced poor yields of **30**. After synthesizing the 6-deoxy sugar, attention was turned to introduce the axially oriented C4 amino function in a regioselective fashion. Axially oriented C4 amino functionality was achieved by a one-pot tethered nucleophilic inversion approach.[18] Treatment of compound **30** with trichloroacetonitrile and a

catalytic amount of hindered base DBU resulted in the predominant formation of the 3-O-trichloroacetimidate intermediate. Then C4 hydroxyl group was triflated, and excess DIPEA was added to afford an intermediate oxazoline derivative which was treated with mild acid produced 4-N-trichloroacetamido-6-deoxy galactose **31** in best yields of 65%. Protective group TCA in **31** was deprotected by treating it $NaBH_4$ in DMSO. A best yield of 60% was obtained for TCA deprotection. Free amine functionality was protected with Cbz to yield **33** followed by acetylation that generated compound **34**. Then, the glycosyl donor of AAT building block **7** was achieved in good yields by deprotection of anomeric silyl protective group **34** using 70% HF/py followed by treatment of the resulting hemiacetal with trichloroacetonitrile and hindered base DBU.



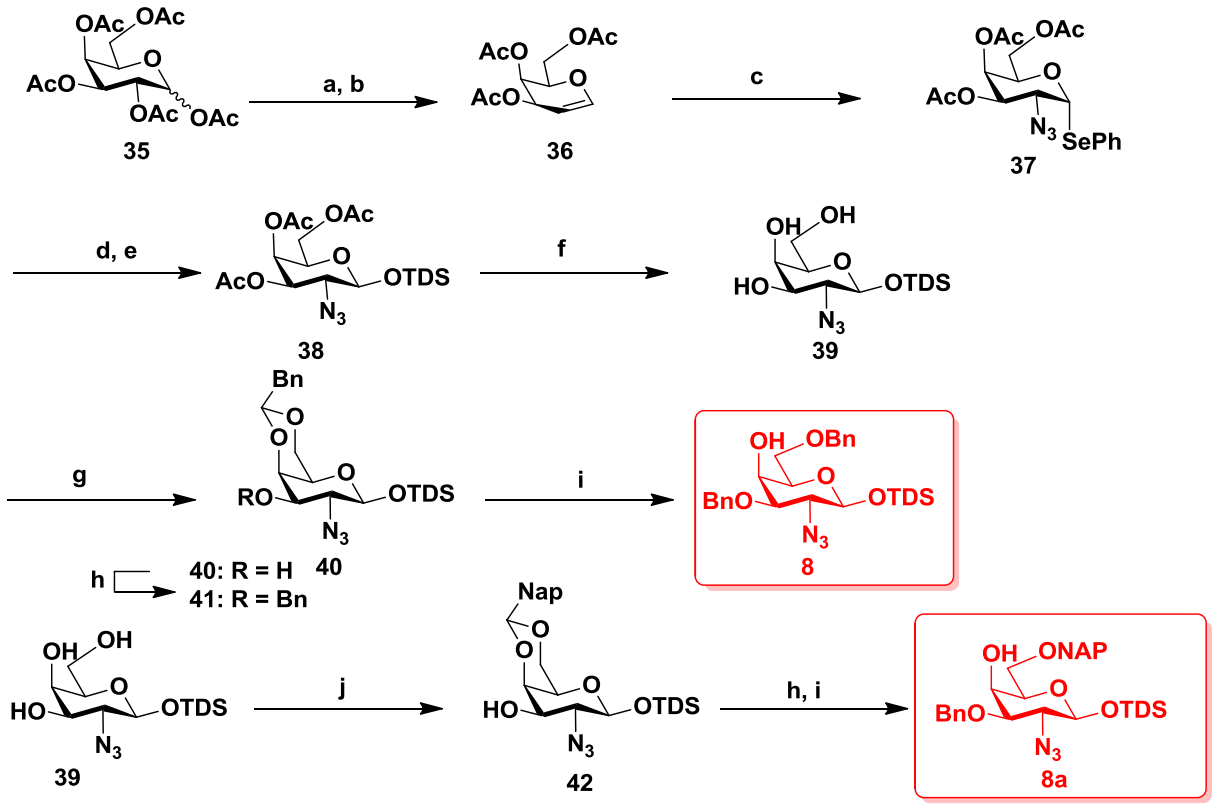
Scheme 2.4: Synthesis of AAT from 2-azido glucose **7.** Reagents and conditions: (a) NH_2NHOAc, DMF, 90%; (b) TBSCl, Imidazole,CH_2Cl_2, 90%; (c) NaOMe/ MeOH; (d) TsCl, py, 78%; (e) NaI, MEK, reflux, 96%; (f) $NaBH_4$, DMSO, 67%; (g) 1) Cl_3CCN, DBU, 3Å MS, DCM, -13 ^oC; 2) py, Tf_2O, -30 ^oC followed by DIPEA; iii) weak acidic resin,

MeOH, 65%; (h) $NaBH_4$, DMSO, 60%; (i) CbzCl, H_2O, Et_2O, $NaHCO_3$, 76%; (j) Py, Ac_2O, 90%; (k) 1) 70% HF/py, THF, 80%; 2) CCl_3CN, DBU, CH_2Cl_2, 85%.

Synthesis of C6-*O*-benzylated galactose acceptor **8** was initiated with a well-known galactose building block **37**.[19] Peracetylated galatose **35** was brominated to form glycosyl bromide which was treated with activated zinc, under reductive elimination conditions, to yield triacetylated-D-galactal **36**. Azido functional group could be efficiently introduced at C2 position of a glycal in better yields using azidophenylselenation over azidonitration. Reaction of glycal **36** with $TMSN_3$ and Ph_2Se_2 in the presence of $PhI(OAc)_2$ afforded 2-azido galactose **37** in good yields. The advantage of this reaction over azidonitration is that the scale up could be done effortlessly. Compound **37** was hydrolyzed with NBS in acetone followed by protecting the resulting alcohol with thexyldimethylsilyl chloride (TDSCl) led to generation of compound **38**. After deacetylation with sodium methoxide in methanol, the deacetylated common intermediate **39** was isolated, and it was subsequently converted into the benzylidene derivative **40** by treatment with benzaldehyde dimethyl acetal and catalytic quantity of camphorsulfonic acid.[20] Bezylation at the C3 hydroxyl group of **40** afforded the intermediate **41** in 65% yield**.** Poor yields could be due to instability of anomeric silyl group under strong basic conditions. Regioselective opening of benzylidene acetal **41** under Et_3SiH and TfOH conditions generated C6-*O*-benzylated galactose aceeptor **8** in good yields.

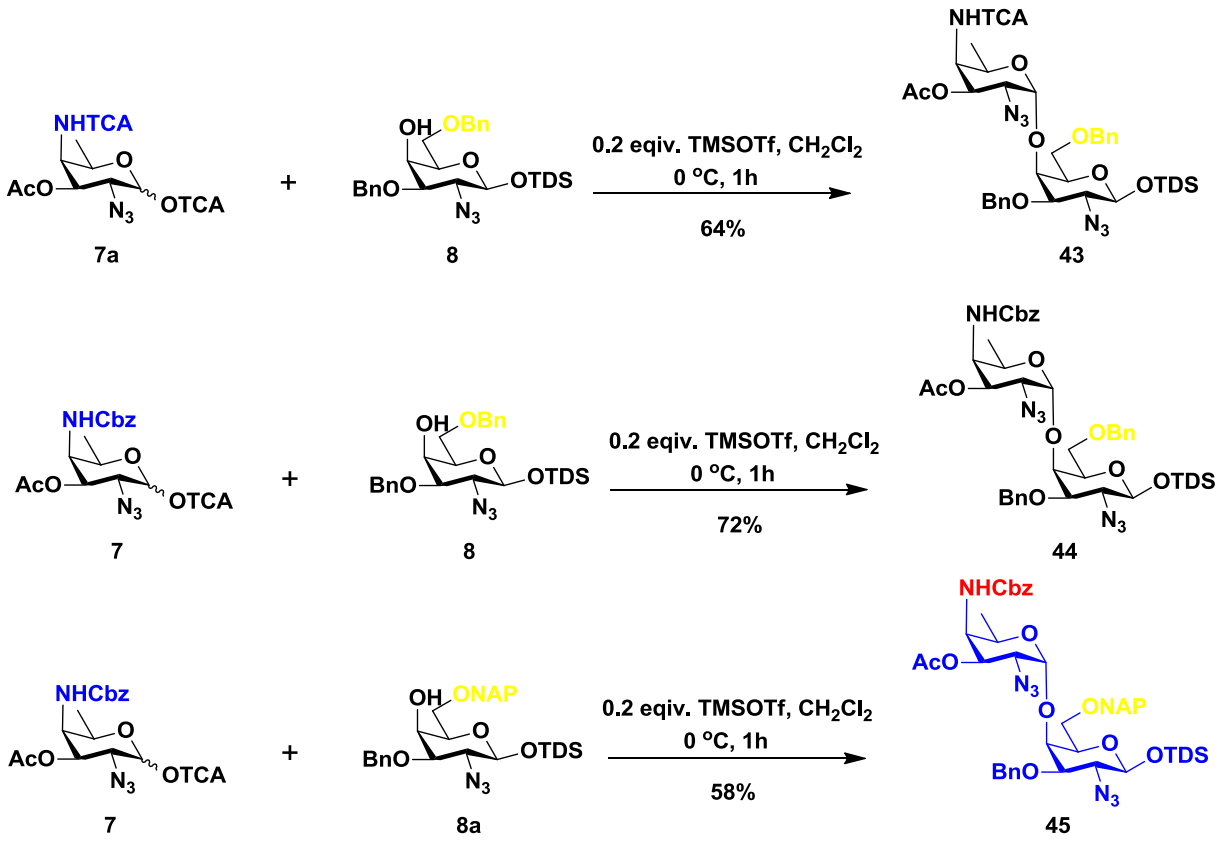
For the synthesis of C6-*O*-Nap acceptor, common intermediate **39** was transformed into 2-naphthylmethylene actetal derivative **42** with 2-naphthaldehyde and a catalytic quantity of camphorsulfonic acid. Bezylation of the compound **41** afforded C3

benzylated intermediate which was subjected to regioselective opening of 4,6-*O*-acetal under Et_3SiH and TfOH conditions to afford C6-*O*-Nap galactose aceeptor **8a** in good yields. The obtained yield for regioselective opening of Nap acetal was slightly less compared to benzylidene ring opening reaction of the same substrate.



Scheme 2.5: Synthesis of galactose acceptor building blocks **8** and **8a.** Reagents and conditions: (a) Br_2, CH_2Cl_2, 2 h; (b) Zn, AcOH, Ac_2O, 60%; (c) Ph_2Se_2, $PhI(OAc)_2$, $TMSN_3$, CH_2Cl_2, 70%; (d) NBS, acetone: water (9:1), 80%; (e) imidazole, TDSCl, DMF, 85%; (f) NaOMe, MeOH, quantitative; (g) CSA, CH_3CN, $PhCH(OMe)_2$, 75%; (h) NaH, BnBr, DMF, 65%; (i) Et_3SiH, TfOH, -78 ^oC, CH_2Cl_2, 85% (for Bn), 75% (for Nap); (j) CSA, CH_3CN, 2-naphthaldehyde, 73%.

Having synthesized glycosyl donors **7** and **7a** and galactosamine acceptors **8** and **8a,** attention was turned to explore the glycosylation conditions needed for the synthesis of various diasaccharides. The glycosylation involving glycosyl donor **7** and acceptor **8** is considered to be problematic due to the low nucleophilicity and steric bulk of the axial C4 hydroxyl group of galactose acceptors **8** and **8a**. Standard TMSOTf mediated activation procedures of trichloroacetimidates were employed in these glycosylation reactions. In first attempt, glycosylation of TCA protected ATT building block **7a** with C4 alcohol of 6-*O*-Bn protected galactosamine **8** yielded the disaccharides **43** in 64% yield and in pure α selectivity. However, the same 6-*O*-benzylated acceptor **8** with Cbz protected trichloroacetimidate donor **7** gave a better yield of 72%. It should be noted that this was the best yield that was obtained among many attempt for C4 hydroxyl acceptors. Since the original target molecule contains phosphocholine at C6 position of galactosamine building block, we synthesized C6-*O*-Nap protected acceptor **8a**. Glycosylation of sugar alcohol **8a** with Cbz protected trichloroacetimidate donor **7** furnished the desired disaccharide **45** in 58% yield**.** The disaccharide **45** will be an ideal building block for assembling the required pentasaccharide because of easy removal of Cbz group under global deprotection conditions to introduce positive charge on amine functionality, and to introduce phosphocholine by deprotecting Nap protective group.



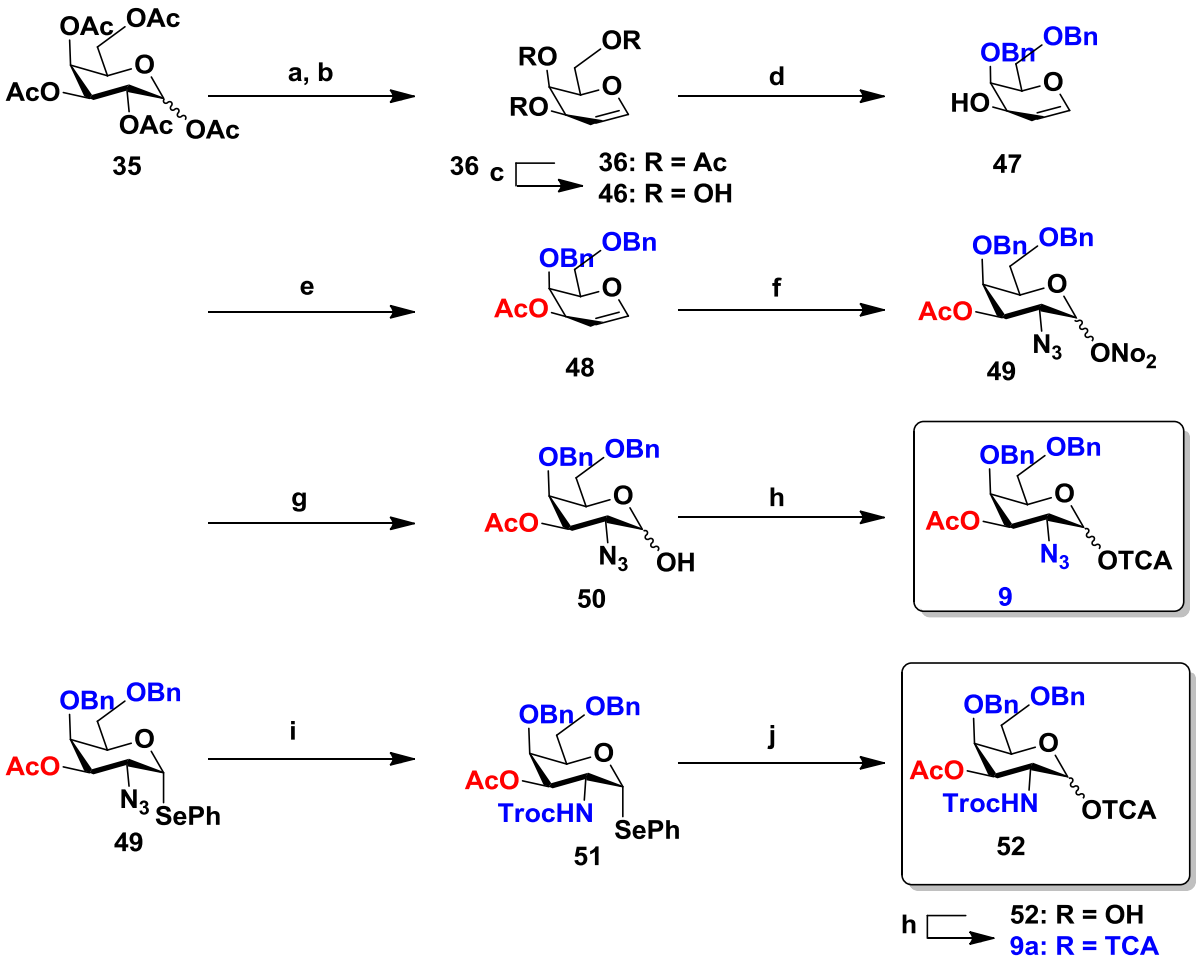
Scheme 2.6: Synthesis of disaccharides **43, 44** and **45**

After synthesizing the above mentioned three disaccharides, we came across a research work from Seeberger laboratory, which also concluded that the steric bulk around C4 hydroxyl group led to poor yields in the above mentioned glycosylations, and TMSOTf activating conditions remained the best conditions for obtaining better yields of these disaccharides.[21]

Having accomplished the synthesis of AAT-disaccharides attention was turned to the synthesis of galactosamine donors **9, 9a** which are required for the synthesis of ribitol containing disaccharide **6**. To synthesize the required galactosamine donors **9** and **9a**, we needed large quantities 4,6-di-*O*-benzyl galactal **47**.[22] Synthesis of

galactosamine donors **9** and **9a** could also be accomplished from 2-azido D-galactose.[23] Peracetylated galatose **35** was brominated to form glycosyl bromide which was treated with activated zinc, under reductive elimination conditions, to yield triacetylated D-galactal **36**. Deacetylation of **36** followed by benzylation using NaH and BnBr yielded 4,6-di-*O*-benzyl galactal **47** in poor yield of 33%. Tri-*O*-benzyl galactal (30%) is one of the side products and it was separated by column chromatography. After obtaining decent quantities of **47,** synthetic scheme **2.7** was followed by acetylating C3 hydroxyl of **47** followed by treating with $TMSN_3$ and Ph_2Se_2 in the presence of $PhI(OAc)_2$ to generate 2-azido galactose derivative **49** in good yields. Selenoglycoside was hydrolyzed with $HgCl_2$ and $CaCO_3$ in acetonitrile afforded lactol **50**. Hemiacetal **50** was converted into galactosamine donor **9** in 80% yields by reaction of trichloroacetonitrile in presence of hindered base DBU.

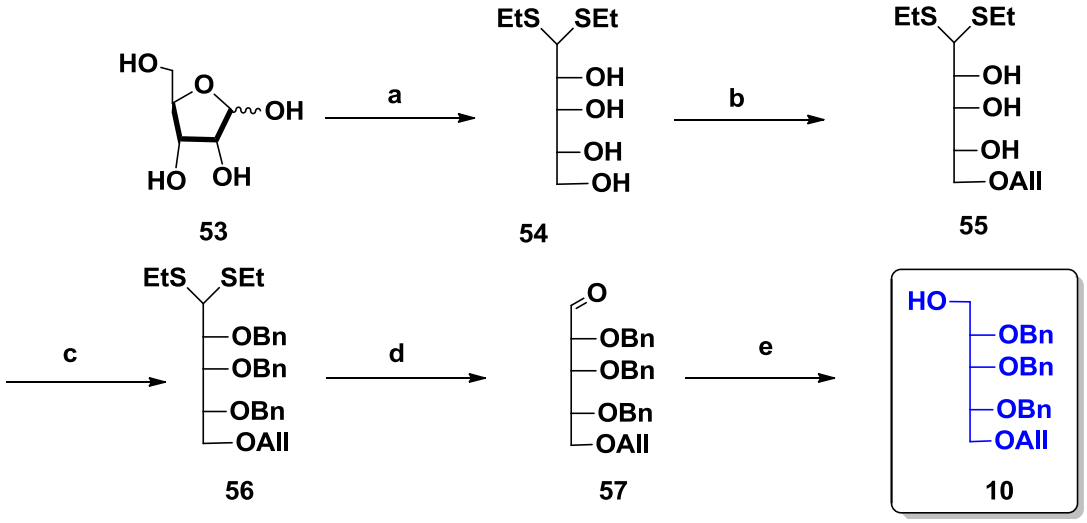
Efforts were made to synthesize a similar galactosamine building block with a participating functionality at C2 **9a (**Scheme **2.7**). Reduction of the azido group to amine functionality in 2-azido galactose intermediate **49** was accomplished by reacting with PPh_3 in THF. Crude amine residue was protected with Troc Cl in pyridene to introduce a participating functionality at C2-amine. Troc protected selenoglycoside was hydrolyzed with $HgCl_2$, $CaCO_3$ in acetonitrile afforded lactol **52**. Hemiacetal **52** was converted into galactosamine donor **9a** in 85% yields by reaction of trichloroacetonitrile in presence of hindered base DBU.



Scheme 2.7: Synthesis of galactose donors **9** and **9a.** Reactions and conditions: (a) Br_2, CH_2Cl_2, 2 h; (b) Zn, AcOH, Ac_2O, 60%; (c) NaOMe, MeOH, quantitative; (d) NaH, BnBr, DMF, 33%; (e) py, Ac_2O, 92%; (f) CAN, NaN_3, CH_3CN, 30%; (g) PhSH, DIPEA, CH_3CN, 85%; (h) CCl_3CN, DBU, CH_2Cl_2, 80% (for 9), 85% (for 9a); (i) 1) PPh_3, THF, H_2O, reflux; 2) Py, Troc Cl, 80%; (j) $HgCl_2$, $CaCO_3$, CH_3CN, H_2O, 4 h, 85% (for 50), 78% (for 52).

After synthesizing galactosamine donors **9** and **9a**, attention was paid on the synthesis of D-ribitol derivative **10**. Ribitol-5-phosphate is present in several of the capsular polysaccharides of *Streptococcus pneumoniae* as well as in the C-

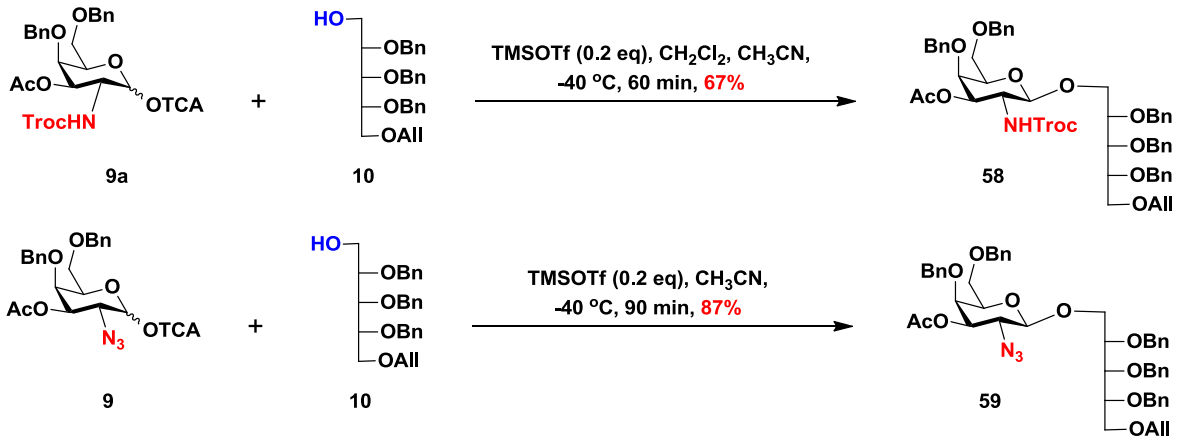
polysaccharides of other bacteria like *H. influenzae*. Ribitol acceptor **10** synthesis was initiateded from commercially available D-ribose **53**.[24] Concentrated HCl was added to D-ribose **53** followed by a dropwise addition of ethanethiol at cold temperatures generated D-ribose diethyl dithioacetal **54**. Primary hydroxyl group on C5 compound **54** was selectively allylated under dibutyltin conditions to obtain an allylated compound **55**. Tribenzylation of 2,3,4-trihydroxy compound **55** by reacting with benzyl bromide and NaH produced 5-*O*-allyl-2,3,4-tri-*O*-benzyl-D-ribose diethyl dithioacetal **56**. Diethyl dithioacetal group **56** was deprotected with $HgCl_2$ and $CaCO_3$ in acetonitrile afforded aldehyde **57**. Subsequently the resulting aldehyde **57** was reduced to ribitol acceptor derivative **10** with $NaBH_4$ in 60% yield.



Scheme 2.8: Synthesis of ribitol acceptor **10.** Reactions and Reagents: (a) HCl, EtSH, 1 h, 75%; (b)1. Bu_2SnO, tol, 2. $CsCO_3$, AllBr, 67%; (c) NaH, BnBr, DMF, 88%; (d) $HgCl_2$, $CaCO_3$, CH_3CN, H_2O, rt, 1 h, 85%; (e) $NaBH_4$, MeOH, 10 h, 60%.

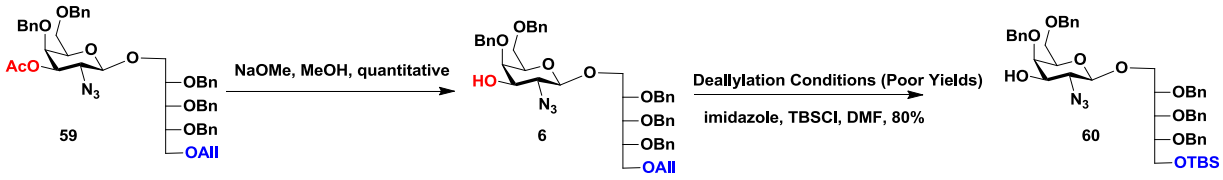
With galactosamine donors **9**, **9a** and ribitol acceptor **10** in hand, attention was paid to investigate the glycosylation conditions required for the synthesis of ribitol

containing disaccharide **6** with exclusive β-selectivity. In case of 2-azido galactosamine donor **9** which lacks neighboring group participating functionality, we took advantage of nitrile effect by using acetonitrile as solvent. Glycosylation of Troc protected galactosamine donor **9a** with ribitol acceptor **10** in acetonitrile and DCM solvent under TMSOTf activation conditions afforded a pure β-disaccharide **58** in 67% yield. The same glycosylation was also performed with donor **9a** and acceptor **10** to yield a 60% of title disaccharide **58**. With mediocre results with NHTroc donors, attention was paid to examine the effect of C2-azido functionality in glycosyl donor for the synthesis of ribitol containing disaccharide. Glycosylation of 2-azido galactosamine donor **9** with ribitol acceptor **10** in acetonitrile, and a minimum amount of DCM to solubulize acceptor and donor, with 0.2 equiv. of TMSOTf produced pure β-disaccharide **59** in 87%. As mentioned above, pure β-disaccharide was accomplished by taking advantage of nitrile effect. Our yields are also consistent with Schmidt laboratory results on a similar disaccharide synthesis.[11] Synthesis of disaccharide with C2 azide functionality **59** is particularly useful for the target pentasaccharide as three the azides could be converted into acetamido functionalities without going for an additional step to deprotect the Troc group.



Scheme 2.9: Synthesis of disaccharides **58** and **59**

After synthesizing the disaccharide **59** in significant quantities, we realized that the deprotection of allyl protective group could be challenging at penasaccharide stage. 3-*O*-Ac group was deprotected with sodium methoxide in methanol to obtain the disaccharide acceptor **6**. After synthesizing the disaccharide **6** various deallylations were attempted to replace allyl ether with TBS ether.



Scheme 2.10: Allyl group deprotection conditions

The resulting allyl ethers in compounds like **6** are stable in both acidic and basic conditions and it could be difficult to deprotect them. Transition metal compounds containing metals like Ru, Rh, Ir, or Pd catalyze the isomerization of allyl ethers to the 1-propenyl ethers, which are then converted to the alcohols under acidic or oxidative

conditions. Various reaction conditions that have been attempted are shown in table **2.1**.

Our first attempt of deallylation of compound **6** started with a reaction of **6** with $Pd(PPh_3)_4$ and morpholine in THF which did not progress and the starting material was recovered. When the solvent system was changed to DCM with 10% acetic acid we could not obtain the required product. Treatment of the disaccharide **6,** on smaller scale of 20 mg of **6** (entry 4), with palladium chloride and AcOH, NaOAc, and H_2O lead to 60% of product formation, if the recovered starting material was taken into consideration. When the reaction was scaled up (entry 5), 55% of compound **60** was obtained. Treatment of the disaccharide **6** with Wilkinson's catalyst and DABCO in mixture of EtOH and toluene did not yield significant yields of the disaccharide. After few less successful attempts with deallylation conditions, we decided to replace All with other protective groups. Hence, we searched for other protective groups and chose TBS group (*tert*-Butyldimethylsilyl) at C5 position of ribitol residue. TBS group can be used as a temporary protective group to selectively deblock to couple monomers in complex oligosaccharide synthesis.

Table 2.1 Various deallylation conditions

1	Reaction Conditions	Yield of **60**
2	$Pd(PPh_3)_4$, morpholine, THF: H_20, 12 h	No reaction. Only starting material was reclaimed.

3	$Pd(PPh_3)_4$, CH_2Cl_2 with 10% AcOH	No reaction. Only starting material was reclaimed
4	$PdCl_2$, AcOH, NaOAc, H_2O, 36 h (Small scale reaction)	60% based on recovered starting material
5	$PdCl_2$, AcOH, NaOAc, H_2O, 36 h (large scale)	**55%**
6	Wilkinson's catalyst, DABCO, EtOH: Tol: H_2O, 12 h, HgO, $HgCl_2$, acetone, H_2O	20%

2.4 Conclusions

The C-polysaccharide target **2** is a structurally challenging and demanding molecule. Successful synthesis of the repeating unit **2**, deprotection of **2**, and controlled oligomerization of the monomer leading to polymer synthesis will provide insights into the minimum number of repeating units that are required for biological activity. Unfortunately we were not able to accomplish the synthesis of the pentasaccharide **2**. However we were able to reproduce reported syntheses of the challenging unnatural building block **7,** and other required building blocks **5, 6, 8, 9** and **10**. We were able to accomplish synthesis of AAT disaccharides **5** and **5a** in decent yields and also proved that steric bulk on C4 hydroxyl of galactosamine acceptor **8a** would lead to decreased yields. It should be noted that yields (from published reports) could not be reproduced in

the conversion of 6-iodo **29** to 6-deoxy sugars **30,** deprotection of TCA functionality from compound **31** into **32,** and azido nitration of **19**. After few less successful attempts of deallylation conditions, it was decided to replace All group in disaccharide **6** with TBS functionality. The above mentioned observations should direct to an efficient synthesis of pentasaccharide **2** by following the identical synthetic strategy (Scheme **2.1**) in future.

2.5 Experimental Section

General procedures: All moisture sensitive reactions were performed under an argon atmosphere by using vacuum dried glassware. All commercial materials were used without purification, unless otherwise noted. CH_2Cl_2 was freshly distilled from calcium hydride under nitrogen prior to use. Toluene, DMF, diethylether, methanol and THF were purchased anhydrous and used without further purification. Molecular sieves (4Å) were flame activated *in vacuo* prior to use. All reactions were performed at room temperature unless specified otherwise. TLC-analysis was conducted on silica gel 60 $_{F254}$ (EMD Chemicals Inc.) with detection by UV-absorption (254 nm) were applicable, and by spraying with 20% sulfuric acid in ethanol followed by charring at ~150 °C or by spraying with a solution of $(NH_4)_6Mo_7O_{24}.H_2O$ (25 g/L) in 10% sulfuric acid in ethanol followed by charring at ~150 °C. Column chromatography was performed on silica gel G60 (Silicycle, 60-200 mm, 60 Å) or on Bondapak C-18 (Waters). ^{1}H and ^{1}3C NMR spectra were recorded on a Varian inova-300 (300/75 MHz), a Varian inova-500 (500/125 MHz) and a Varian inova-600 (600/150 MHz) spectrometer equipped with sun workstations. Chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane (TMS) as the internal standard. NMR data is presented as follows: Chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, dd = doublet of doublet, m

= multiplet and/or multiple resonances), coupling constant in Hertz (Hz), integration. All NMR signals were assigned on the basis of ^{1}HNMR, ^{13}C NMR, COSY and HSQC experiments. Optical rotations were measured using a Jasco P-1020 polarimeter. Mass spectra were recorded on an Applied Biosystems 4700 MALDI-TOF proteomics analyzer. The matrix used was 2,5-dihydroxybenzoicacid (DHB) and ultramark 1621 as the internal standard. The ESI-MS spectra were recorded on 9.4 T Bruker Apex Ultra QeFTMS (Billerica, MA) mass spectrometer.

Ethyl 1-thio-*α*-D-mannopyranoside (11): To a solution of 1,2,3,4,6-penta-*O*-acetyl-D-mannopyranose (20.6 g, 52.8 mmol) in DCM (300 mL) was added EtSH (11.02 mL, 158.5 mmol) and $BF_3.Et_2O$ (19.2 mL, 153.1 mmol) at 0 ^{o}C and the reaction mixture was stirred for 12 h. The resulting reaction mixture was quenched with sat. aq. $NaHCO_3$, diluted with DCM (100 mL), the resulting organic layer was washed with water (2 × 50 mL), dried ($MgSO_4$) and concentrated under reduced pressure. The resulting residue was purified by recrystallization, using EtOH as a solvent, to obtain ethyl 1,2,3,4-tertra-*O*-acetyl-1-thio-α-D-mannopyranoside containing a small amount of β-anomer. Flash column silica gel chromatography of the resulting residue using EtOAc/ hexanes (1/4 to 2/5, v/v) afforded pure ethyl 1,2,3,4-tetra-*O*-acetyl-1-thio-α-D-mannopyranoside (14.5 g, 70%). To a solution of ethyl 1,2,3,4-tertra-*O*-tetraacetyl-1-thio-α-D-mannopyranoside (14.5 g, 36.9 mmol) in MeOH (80 mL) was added NaOMe/ MeOH (3.5 mL, 1 M) until pH = 9 and stirred for 2 h at ambient temperature. The resulting reaction mixture was neutralized using amberlite H^{+}-resin, filtered and the filtrate was concentrated under reduced pressure which afforded title compound **11** (8 g, 95%) as an amorphous solid.

Ethyl *2,3-O*-isopropylidene-6-*O*-*p*-toluenesulfonyl-1-thio-α-D-mannopyranoside (12): To a solution of compound **11** (8 g, 35.7 mmol) in pyridine (90 mL) was added a solution of *p*-toluenesulfonyl chloride (8.14 g, 42.8 mmol) in DCM (50 mL) in drop wise fashion at -10 ^{o}C. The resulting reaction mixture was stirred for 4 h at ambient temperature and the following 12 h at room temperature. Water (10 mL) was added to the reaction mixture flask and the resulting reaction mixture was concentrated and co-evaporated with toluene under reduced pressure. The resulting residue was purified by flash column silica gel chromatography using MeOH/$CHCl_3$ (1/20 to 1/10, v/v) afforded 6-*O*-tosylate compound (10.04 g, 75%) which is a white amorphous solid. To the solution of ethyl-6-*O*-*p*-toluenesulfonyl-1-thio-α-D-mannopyranoside (10 g, 26.6 mmol) in CH_3CN (70 mL) was added 2,2-dimethoxypropane (13.09 mL, 106.6 mmol) followed by the addition of *p*-toluenesulfonic acid monohydrate (1.01 g, 5.3 mmol). The reaction mixture was stirred for 2 h and another of portion of *p*-toluenesulfonic acid monohydrate (1 g, 5.3 mmol) was added and stirred for an additional 12 h. The resulting reaction mixture was neutralized with Et_3N and concentrated under reduced pressure, diluted with $CHCl_2$ (400 mL), washed with water (30 mL), dried ($MgSO_4$), filtered and the combined organic layers were concentrated under reduced pressure. Flash column silica gel chromatography using EtOAc/ hexanes (1/5 to 1/1, v/v) of the resulting residue afforded the title compound **12** (8.91g, 80%) as an oil. The physical data were in agreement with those reported in the literature.[16]

Ethyl 6-deoxy-*2,3-O*-isopropylidene-1-thio-α-D-mannopyranoside (13): To a solution of compound **12** (8.91 g, 21.3 mmol) in diethyl ether (100 mL) at 0 ^{o}C was added lithium aluminium hydride (1.21 g, 31.97 mmol) and the reaction mixture was

warmed to 35 ^{o}C and refluxed for 4 h. The resulting reaction mixture was quenched with oxalic acid (5 mL, 1 M), diluted with diethyl ether (200 mL), washed with water (30 mL), back extracted with diethyl ether (50 mL). The combined organic layers were dried ($MgSO_4$), filtered and the resulting filtrate was concentrated under reduced pressure. Flash column silica gel chromatography of the resulting crude using EtOAc/hexanes (1/5 to 1/3, v/v) afforded the title compound **13** (3.59g, 68%). Starting material **12** (450 mg, 5%) was recovered during the purification step. The physical data were in agreement with those reported in the literature.[16]

Ethyl 6-deoxy-*2,3-O*-isopropylidene-*4*-oxo-*1*-thio-α-D- *lyxo*-hexoyranoside (14): To a solution of DMSO (1.4 mL,18.8 mmol) in CH_2Cl_2 (50 mL) at -78 ^{o}C was added freshly distilled oxalyl chloride (1.65 mL, 18.8 mmol) and the reaction mixture was stirred for 15 minutes. To the resulting reaction mixture was added a solution **13** (3.59 g, 14.47 mmol) in CH_2Cl_2 (20 mL) and the reaction mixture was stirred for 30 minutes at the same temperature. To the reaction mixture was added Et_3N (4.4 mL, 31.8 mmol) and stirred for 15 minutes and the resulting reaction mixture was brought to room temperature. The reaction mixture was diluted with CH_2Cl_2 (250 mL), washed with H_2O (50 mL), dried ($MgSO_4$), filtered and the combined organic layers were concentrated under reduced pressure. Flash column silica gel chromatography of the resulting residue using EtOAc/ hexanes (1/5 to 1/3, v/v) afforded the title compound **14** (3.38 g, 95%). The physical data were in agreement with those reported in the literature.[16]

Ethyl-4,6-dideoxy-*4*-hydroximino-*2,3-O*-isopropylidene-*1*-thio-α-D-*lyxo*-hexoyranoside (15): To a solution of compound **14** (3.4 g, 13.8 mmol) in THF (75 mL) and methanol (25 mL) was added a solution of hydroxylamine hydrochloride (4.82 g,

69.1 mmol) in a mixture of pyridine and water (1:1, 50 mL) and stirred for 1 h at ambient temperature. The resulting reaction mixture was concentrated under reduced pressure, dissolved in CH_2Cl_2, washed with H_2O, dried ($MgSO_4$), filtered and concentrated under reduced pressure. Flash column silica gel chromatography of the resulting residue using EtOAc/ hexanes (1/3, v/v) afforded the title compound **15** (2.9 g, 80%) as a mixture of *cis-trans* isomers as a light yellow color solid. The physical data were in agreement with those reported in the literature.[16]

Ethyl 4-(benzyloxycarbonyl)amino-4,6-dideoxy-*2,3-O*-isopropylidene-1-thio-α-D-talo*py*ranoside (17): To a solution of lithium aluminium hydride (1.47 g, 38.8 mmol) in THF (40 mL) at 0 ^oC was slowly added a solution of compound **15** (2.9 g, 11.1 mmol) in THF (10 mL+3 mL) and stirred at ambient temperature for 1 h and then refluxed for 4 h. After the TLC analysis indicated the completion of the reaction, excess of lithium aluminium hydride was destroyed with the addition of ice pieces, the resulting reaction mixture was diluted with EtOAc (200 mL), filtered, washed with water, back extracted with EtOAc (50 mL), dried ($MgSO_4$), filtered and the combined organic layers were concentrated under reduced pressure. To a solution of resulting crude amine (2.7 g, 11.1 mmol) in mixture of H_2O/Et_2O (1/1, 100 mL) was added benzyloxycarbonyl chloride (2.4 mL, 16.8 mmol) and solid $NaHCO_3$ (3.3 g, 44.8 mmol). The resulting reaction mixture was stirred for 2 h, diluted with diethyl ether (200 mL), separated the organic ether layer, dried ($MgSO_4$), filtered and combined organic layers were concentrated under reduced pressure. Careful flash column silica gel chromatography of the resulting residue using EtOAc/ hexanes (1/5 to 1/3, v/v) afforded title compound **17** (3.12 g, 75%) along with a small quantity of mannopyranoside **16** (200 mg). The product ratio of **17**

and **16** was found to be 95:5. The physical data were in agreement with those reported in the literature.[16]

Ethyl 4-(benzyloxycarbonyl)amino-4,6-dideoxy-1-thio-α-D-talopyranoside (18): A solution of **16** (3.12 g, 8.19 mmol) in a mixture of TFA/H_2O (9:1, 20 mL) was stirred for 1 h at ambient temperature. The resulting reaction mixture was diluted with toluene (100 mL) and concentrated under reduced pressure. Flash column silica gel chromatography of the resulting residue using EtOAc/ hexanes (1/1, v/v) afforded title compound **18** (2.37 g, 85%) as an amorphous solid. The physical data were in agreement with those reported in the literature.[16]

Ethyl 2,3-*O*-diacetyl-4-(benzyloxycarbonyl)amino-4,6-dideoxy-1-thio-α-D-talopyranoside (18a): A solution of compound **18** (220 mg, 0.64 mmol) in a mixture of pyridine/acetic anhydride (4:1, 2mL) was stirred for 2 h at ambient temperature. The resulting reaction mixture was concentrated under reduced pressure and co-evaporated with toluene. Flash column silica gel chromatography of the resulting residue using EtOAc/ hexanes (1/3, v/v) afforded title compound **18a** (241 mg, 87%). The physical data were in agreement with those reported in the literature.[16]

3-*O*-Acetyl-4-(benzyloxycarbonyl)amino-4,6-dideoxy-D-galactal (19): To a solution of compound **18a** (241 mg, 0.56 mmol) in diethyl ether (6 mL) was added Br_2 (45 µL, 0.87 mmol) and stirred for 30 minutes at ambient temperature. The resulting reaction mixture was concentrated under reduced pressure and co-evaporated with toluene to afford crude anomeric bromide **18b**. To a solution of acetic acid (50 µl, 0.85 mmol) in THF (5mL) was added NaOAc (65 mg 0.79 mmol) and cooled to 0 ^{o}C. To the resulting

reaction mixture was added a solution of **18b** (0.56 mmol) in THF (5mL) followed by Zn dust (368 mg, 5.67 mmol) and $CuSO_4$ (90 mg, 0.56 mmol) and vigorously stirred for 2 h. The resulting reaction mixture was diluted with THF (50 mL), filtered, concentrated under reduced pressure and co-evaporated with toluene. Flash column silica gel chromatography using EtOAc/ hexanes (1/5 to 1/3, v/v) afforded title compound **19** (72 mg, 40%). The physical data were in agreement with those reported in the literature.[16]

3-*O*-Acetyl-2-azido-4-(benzyloxycarbonyl)amino-4,6-dideoxy-D-galactopyranosyl nitrate (20): To a solution of compound **19** (72 mg, 0.236 mmol) in acetonitrile (3 mL) at -25 ^oC, was added sodium azide (31 mg, 0.485 mmol) and ammonium cerium nitrate (517 mg, 0.945 mmol) and the reaction mixture was stirred for 2 h at -15 ^oC. The resulting reaction mixture was diluted with diethyl ether (30 mL), filtered, washed with cold water, dried ($MgSO_4$), filtered and concentrated under reduced pressure. Careful flash column silica gel chromatography of the resulting residue using EtOAc/ hexanes (1/5 to 1/3, v/v) afforded a mixture of anomers of compound **20** and it's C2 epimer (15 mg, 15%). The physical data were in agreement with those reported in the literature.[16]

***Tert*-butyldimethylsilyl 3,4,6-tri-*O*-acyl-2-azido-2-deoxy-β-D-glucopyranoside (26):** To a solution of 1,3,4,6-tetra-*O*-acyl-2-azido-2-deoxy-*β*-D-glucopyranose (12 g, 32.1 mmol) in DMF (120 mL) was added hydrazine acetate (3.552 g, 38.57 mmol) and the reaction mixture was stirred for 30 minutes. The resulting reaction mixture was concentrated under reduced pressure and the obtained residue was diluted with EtOAc (500 mL) and washed with H_2O (2 × 50 mL). The combined organic layers were dried ($MgSO_4$), filtered and concentrated under reduced pressure. The resulting residue was co-evaporated with toluene *in vacuo* and was dissolved in DMF (100 mL). To the

resulting solution was added imidazole (2.6 g, 38.3 mmol) and *tert*-butyldimethylsilyl chloride (5.8 g, 38.3 mmol) and the reaction mixture was stirred for 12 h at ambient temperature. The resulting reaction mixture was concentrated under reduced pressure and the obtained residue was diluted with EtOAc (400 mL) and washed with H_2O (50 mL). The combined organic layers were dried ($MgSO_4$), filtered and concentrated under reduced pressure. Flash column silica gel chromatography of the resulting residue using EtOAc/ hexanes (1/5, v/v) afforded title compound **26** (11.2 g, 85%) as an amorphous solid. The physical data were in agreement with those reported in the literature.[8]

***Tert*-butyldimethylsilyl 2-azido-2-deoxy-β-D-glucopyranoside (27):** To a solution of compound **26** (11.2 g, 25.1 mmol) in methanol (100 mL) was added NaOMe in methanol (2.5 mL, 1M) and the reaction mixture was stirred for 1 h. The resulting reaction mixture was neutralized with Amberlite H+ resin, filtered and concentrated under reduced pressure to afford title compound **27 (**8 g, 30.5 mmol) as an amorphous solid. The physical data were in agreement with those reported in the literature.[8]

***Tert*-butyldimethylsilyl 2-azido-2-deoxy-6-*O*-toluenesulfonyl-β-D-glucopyranoside (28):** To an ice-cooled solution of compound **27** (6.55 g, 25 mmol) in pyridine (120 mL) was added *p*-toluenesulfonyl chloride (5.7 g, 30.4 mmol) and the reaction mixture was stirred for 2 h at 0 ^{o}C. The resulting reaction mixture was quenched with drop wise addition of MeOH. The reaction mixture was concentrated under reduced pressure and co-evaporated with toluene. Flash column silica gel chromatography of the resulting residue using EtOAc/ hexanes (1/5 to 1/2, v/v) to yield title compound **28** (9.7g, 82%) as an amorphous white solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.79 (d, 2H, *J* = 8.0 Hz, CH Aromatic), 7.33 (d, 2H, *J* = 8.0 Hz, 2H CH Aromatic), 4.53 (d, 1H, *J* = 7.6 Hz, H1), 4.27

(d, 1H, *J* = 10.0 Hz, H6), 4.21 (dd, 1H, *J* = 10.8 Hz, *J* = 4.4 Hz, H6), 3.42 – 3.50 (m, 2H, H4, H-5), 3.31 (dd, 1H, *J* = 10.0 Hz, *J* = 7.6 Hz, H3), 3.19 (dd, 1H, *J* = 10.0 Hz, *J* = 7.6 Hz, H2), 3.17 (bs, 2H, 2xOH), 2.45 (s, 3H, C*H*$_3$ Ts), 0.91 (s, 9H, *t*Bu, TBDMS), 0.15 (s, 3H, C*H*$_3$ TBDMS), 0.13 (s, 3H, CH_3 TBDMS). ^{13}C NMR (75 MHz, $CDCl_3$): δ 144.9, 132.4, 129.8, 127.8, 96.9, 74.2, 73.2, 69.6, 67.8, 68.7, 25.4, 21.5, 17.8, -4.4, -5.4. HRMS-MALDI: ($M+Na^+$) calcd. 496.1544, found 496.1241.

***Tert*-butyldimethylsilyl 2-azido-2,6-dideoxy-6-iodo-β-D-glucopyranoside** (**29**): To a solution of Compound **28** (9.7 g, 20.5 mmol) in 2-butanone (110 mL) was added NaI (6.76 g, 45.1 mmol) at ambient temperature. The reaction mixture was refluxed at 80 ^{o}C for 5 h. The reaction mixture was cooled to room temperature, diluted with EtOAc (300 mL), washed with aq. 1M $Na_2S_2O_3$ (2 × 50 mL) followed by washing with H_2O (50 mL). The combined organic layers were dried ($MgSO_4$), filtered and concentrated under reduced pressure. Flash column silica gel chromatography of the resulting residue using EtOAc/ hexanes (1/2, v/v) yielded (8.5 g, 96%) of title compound **29** as a orange yellow gummy liquid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.64 (d, 1H, *J* = 7.2 Hz, H1), 3.83 (bs, 2H, 2xOH), 3.57 (dd, 1H, *J* = 10.8 Hz, *J* = 2.0 Hz, H6), 3.34 – 3.39 (m, 2H, *J* = 8.8 Hz, *J* = 6.8 Hz, H3 and H4), 3.31 (dd, 1H, *J* = 10.8 Hz, *J* = 2.4 Hz, H6), 3.23 (t, 1H, *J* = 8.8 Hz, *J* = 8.0 Hz, H2), 3.19 (m, 1H, H5), 0.95 (s, 9H, *t*Bu TBDMS), 0.23 (s, 3H, C*H*$_3$ TBDMS), 0.21 (s, 3H, C*H*$_3$ TBDMS). ^{13}C NMR (75 MHz, $CDCl_3$): δ97.0, 74.9, 74.1, 73.8, 68.4, 25.6, 17.8, 5.0, -3.9, -5.2. HRMS-MALDI: ($M+NH_4^+$) calcd. 447.0919, found 447.0927.

***Tert*-butyldimethylsilyl 2-azido-2,6-dideoxy-β-D-glucopyranoside (30):** To a solution of compound **29** (8.7 g, 20.2 mmol) in DMSO (200 mL) was added $NaBH_4$ (14.4 g, 117.6 mmol, 5.8 equiv) in three portions. The reaction mixture was stirred for 3h at room

temperature. TLC analysis showed disappearance of most of the starting material. It must be noted that it was difficult to clearly see distinct spots on TLC because of DMSO in the reaction mixture. Reaction mixture was quenched with MeOH and separated between EtOAc (500 mL)/ H_2O (50 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated under reduced pressure. The crude oil was dissolved in EtOAc (200 mL) and filtered to remove inorganic salts and insoluble impurities. The EtOAc layer was dried ($MgSO_4$), filtered and concentrated under reduced pressure. Flash column chromatography of the resulting residue (1:2 to 1:5 EtOAc/ hexane) yielded the titled compound **30** (4.6 g, 15.18 mmol, 70%) as a colorless oil. ^{1}H NMR (75 MHz, $CDCl_3$):δ 4.56 (d, 1H, *J* = 7.0 Hz, H1), 3.22 – 3.34 (m, 4H, H2, H3, H4, H5), 2.71 (bs, 2H, 2xOH), 1.32 (d, 1H, *J* = 6.1 Hz, H6), 0.93 (s, 9H, *t*Bu TBDMS), 0.14 (s, 6H, 2xCH_3 TBDMS). ^{13}C NMR (75 MHz, $CDCl_3$): δ 96.9, 74.7, 74.3, 71.6, 69.6, 25.6, 17.9, 17.6, -4.32, -5.22. HRMS-MALDI: ($M+NH_4^+$) calcd. 321.1952, found 321.1874.

***Tert*-butyldimethylsilyl 2-azido-4-*N*-trichloroacetamido-2,4,6-trideoxy-β-D-galactopyranoside (31):** To a solution of compound **30** (900 mg, 2.97 mmol) in DCM (20 mL) was added Cl_3CCN (357 µL 3.6 mmol) and activated 3Å MS (1 g). The reaction mixture was stirred for 30 minutes at ambient temperature and cooled down to -13 °C. Subsequently a catalytic amount of DBU (89 µL) was added and the reaction mixture was allowed to stir for 1 h at that temperature and cooled down to -30°C. To the resulting reaction mixture was added pyridine (2 mL, 14.85 mmol) and triflic anhydride (600 µL, 3.6 mmol). The reaction mixture was allowed to warm to room temperature during 30 minutes and was followed by the addition of DIPEA (5.1 mL, 30 mmol). The resulting reaction mixture was stirred for 4 h, diluted with EtOAc (200 mL) and

successively washed with 1M HCl (30 mL) and saturated aq. $NaHCO_3$ (50 mL). The combined organic layers were dried ($MgSO_4$), filtered and concentrated under reduced pressure. To the resulting residue in MeOH (12 mL) was added Amberlite IR 120 H^+-resin until pH of the solution reached 5. The resulting reaction mixture was stirred for 15 minutes, filtered and concentrated under reduced pressure. Flash column silica gel chromatography of the resulting residue using EtOAc/hexane (1/5 to 1/3, v/v) afforded title compound **31** (700 mg, 52 % over 4 steps) as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.89 (d, 1H, *J* = 9.2 Hz, N*H*TCA), 4.53 (d, 1H, *J* = 8.0 Hz, H1), 4.23 (ddd, 1H, *J* = 9.4 Hz, *J* = 4.4 Hz, *J* = 1.2 Hz, H4), 3.77 (dq, 1H, *J* = 7.2 Hz, *J* = 6.4 Hz, *J* = 1.2 Hz, H5), 3.72 (dd, 1H, *J* = 10.4 Hz, *J* = 4.4 Hz, H3), 3.18 (dd, 1H, *J* = 10.6 Hz, *J* = 7.6 Hz, H2), 3.16 (bs, 1H, O*H*-3), 1.24 (d, 3H, *J* = 6.4 Hz, H6), 0.96 (s, 9H, *t*Bu TBDMS), 0.16 (s, 6H, 2xCH_3 TBDMS). ^{13}C NMR (75 MHz, $CDCl_3$): δ 163.5 (C=O TCA), 97.4 (C1), 92.4 (Cq CCl_3), 71.4 (C3), 69.3 (C5), 66.5 (C2), 55.0 (C4), 25.5 (*t*Bu TBDMS), 17.9 (Cq *t*Bu TBDMS), 16.6 (C6), -4.4 (Si(CH_3)$_2$ TBDMS), -5.1 (Si(CH_3)$_2$ TBDMS). HRMS-MALDI: ($M+H^+$) calcd. 447.0783, found 447.0812.

***Tert*-butyldimethylsilyl 2-azido-4-(benzyloxycarbonyl)amino-2,4,6-trideoxy-β-D-galactopyranoside (33):** To a solution of compound **31** (362 mg, 0.81 mmol) in EtOH (10 mL) was added $NaBH_4$ (92 mg, 2.433 mmol), and stirred at ambient temperature for 1 h. The resulting reaction mixture was diluted with $CHCl_3$ (100 mL), washed with water (20 mL) and the water layer was back-extracted with $CHCl_3$ (20 mL). The combined organic layers were dried ($MgSO_4$), filtered and concentrated under reduced pressure. Flash column silica gel chromatography of the resulting residue using $CHCl_3$/MeOH (1/20, v/v) yielded compound **32** (150 mg, 60%). To a solution of

compound **32** (180 mg, 0.49 mmol) in H_2O:Et_2O (1:1, 10 mL) was added CbzCl (90 µL, 0.63 mmol) and $NaHCO_3$ (118 mg, 1.58 mmol) and stirred at ambient temperature for 1 h. The resulting reaction mixture was diluted with diethyl ether (60 mL), separated the organic layer, dried ($MgSO_4$), filtered and concentrated under reduced pressure. Flash column silica gel chromatography of the resulting residue EtOAc/ hexane (1/5, v/v) afforded title compound **33** (220 mg, 0.479 mmol, 76%) as a white amorphous solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.28–7.60 (m, 5H), 5.16 (d, 1H, *J* = 12.4 Hz, C*H*HPh), 5.08 (d, 1H, *J* = 12.4 Hz, CH*H*Ph), 5.04 (d, 1H, *J* = 10.0 Hz, NH), 4.83 (dd, 1H, *J* = 10.4 Hz, *J* = 3.6 Hz, H3), 4.48 (d, 1H, *J* = 10.4 Hz, H1), 4.18 (dd, 1H, *J* = 10.0 Hz, *J* = 3.6 Hz, H4), 3.73 (q, 1H, *J* = 10.0 Hz, *J* = 6.0 Hz, H5), 3.45 (dd, 1H, *J* = 10.4 Hz, H-2), 1.25 (d, 3H, *J* = 6.0 Hz, H6), 0.96 (s, 9H, *t*Bu TBDMS), δ0.16 (s, 6H, 2xC*H*$_3$ TBDMS). ^{13}C NMR (75 MHz, $CDCl_3$): δ 156.3 (C=O Cbz), 136.2 (Cq Cbz), 127.6 – 136.2 (CH Aromatic), 86.4 (C1), 74.4 (C3), 73.5 (C5), 66.7 (CH_2 Cbz), 59.5 (C2), 51.8 (C4), 20.4 (CH_3 Ac), 16.6 (C6).

***Tert*-butyldimethylsilyl 2-azido-4-(benzyloxycarbonyl)amino-3-*O*-acetyl-2,4,6-trideoxy-β-D-galactopyranoside (34):** The Compound **34** (220 mg, .47 mmol) was dissolved in pyridene:acetic anhydride (3:1, 4mL) and the reaction mixture was stirred for 2 h at ambient temperature. The resulting reaction mixture was quenched with H_2O (1 mL), concentrated under reduced pressure and co-evaporated with toluene. Flash column silica gel chromatography of the resulting residue using EtOAc/ hexanes (1/5, v/v) afforded title compound **34** (220 mg, 93%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.28–7.60 (m, 5H), 5.11 (d, 1H, *J* = 12.4 Hz, C*H*HPh), 5.01 (d, 1H, *J* = 12.4 Hz, CH*H*Ph), 4.97 (d, 1H, *J* = 10.0 Hz, NH), 4.83 (dd, 1H, *J* = 10.4 Hz, *J* = 3.6 Hz, H3), 4.44 (d, 1H, *J* = 10.4

Hz, H1), 4.11 (dd, 1H, *J* = 10.0 Hz, *J* = 3.6 Hz, H4), 3.66 (q, 1H, *J* = 10.0 Hz, *J* = 6.0 Hz, H5), 3.31 (dd, 1H, *J* = 10.4 Hz, H2), 1.98 (s, 3H, C*H*$_3$ Ac), 1.18 (d, 3H, *J* = 6.0 Hz, H6), 0.86 (s, 9H, *t*Bu TBDMS), 0.13 (s, 6H, 2xC*H*$_3$ TBDMS). ^{13}C NMR (75 MHz, $CDCl_3$): δ 169.8 (C=O Ac), 156.3 (C=O Cbz), 136.2 (Cq Cbz), 127.6 – 136.2 (CH Aromatic), 87.2 (C1), 75.4 (C3), 72.1 (C5), 66.9 (CH_2 Cbz), 58.0 (C2), 51.2 (C4), 20.9 (CH_3 Ac), 16.2 (C6).

3-*O*-acetyl-2-azido-4-(benzyloxycarbonyl)amino-2,4,6-trideoxy-β-D-galactopyranosyl trichloroacetimidate (7): To a solution of compound **34** (140 mg, 0.28 mmol) in THF (3mL) was added 70% HF/py (112 µl, 5.6 mmol) at 0 ^{o}C and stirred for 12 h at room temperature. The resulting reaction mixture was diluted with EtOAc (50 mL), washed with saturated aq. $NaHCO_3$, washed with H_2O (10 mL), dried ($MgSO_4$), filtered and concentrated under reduced pressure. Flash column silica gel chromatography of the resulting residue using EtOAc/ hexanes (1/2, v/v) afforded anomeric alcohol (220 mg, 93%). To the solution of lactol (115 mg, 315 mmol) in CH_2Cl_2 (4 mL) was added Cl_3CCN (162 µL, 1.57 mmol) and DBU (8.8 µl, 0.063 mmol) and the reaction mixture was stirred for 2h at ambient temperature. The resulting reaction mixture was concentrated under reduced pressure, and the silica gel was neutralized by eluting with Et_3N/hexane (1:50, 50 mL) before loading the resulting residue. Flash column silica gel chromatography of the resulting residue EtOAc/ hexane(1/5, v/v) afforded title compound **7** (95 mg, 65%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.77 (s, 1H), 7.39-7.29 (m, 5H), 5.63 (d, 1H, *J* = 8.4 Hz), 5.18 (d, 1H, *J* = 12.0 Hz), 5.13 (d, 1H, *J* = 9.6 Hz), 5.07 (d, 1H, *J* = 12.0 Hz), 4.82 (dd, 1H, *J*= 4.0, 6.4 Hz), 4.21 (dd, 1H, *J* = 3.2, 10.0 Hz), 3.91 (q, 1H, *J* = 6.4 Hz), 3.74 (dd, 1H, *J* = 8.8, 10.8 Hz), 2.01 (s, 3H), 1.27 (d,

3H, *J*= 6.4 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.4, 161.0, 156.8, 136.5, 128.9, 128.7, 128.5, 97.3, 90.7, 73.3, 71.0, 67.5, 52.2, 21.0, 16.9, 16.8.

Phenyl 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-1-seleno-α-D-galactopyranoside (37): To a solution of compound **36** (5g, 18.37 mmol) in CH_2Cl_2 (100 mL) was added diphenyl diselenide (4.016 g, 12.87 mmol) at ambient temperature and reaction mixture was cooled down to -40 ^{o}C. To the resulting mixture was added $PhI(OAc)_2$ (5.918 g, 18.37 mmol) followed by the drop by drop addition of $TMSN_3$ (4.83 ml, 36.75 mmol). The resulting reaction mixture was stirred at -40 ^{o}C for 2 h and warmed to room temperature and stirred for 12 h. The reaction mixture was concentrated under reduced pressure, and flash column silica gel chromatography of the resulting residue using EtOAc/ hexanes (1/5 to 1/4, v/v) afforded title compound **37** (6.05 g, 70%) as a dark yellow solid. Careful silica gel chromatography separation technique was employed to isolate the pure α-anomer **35**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.60 (m, 3H), 7.30 (m, 2H), 6.01 (d, 1H, *J* = 4.9 Hz, H1), 5.47 (d, 1H, *J* =2.8 Hz, H4), 5.12 (dd, 1H, *J* =11.0, 2.8 Hz, H3), 4.67 (t, 1H, *J* =6.2 Hz, H5), 4.27 (dd, 1H, *J* = 11.0, 4.9 Hz, H2), 4.05 (m, 2H, H6a, H6b), 2.16 (s, 3H Ac), 2.07 (s, 3H Ac),1.98 (s, 3H Ac). ^{13}C NMR (75 MHz, $CDCl_3$): δ 169.7, 169.4, 168.9, 134.3, 132.6, 131.8, 127.6, 83.5, 70.6, 68.5, 66.7, 61.1, 58.1, 20.0. HRMS-MALDI: ($M+H^+$) calcd. 489.0883, found 489.1243.

Dimethylthexylsilyl 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-β-D-galactopyranoside (38): To a solution of compound **37** (5.5 g, 11.67 mmol) in CH_3CN/H_2O (9:1, 50 mL) was added $CaCO_3$ (3.85 g, 38.51 mmol) and $HgCl_2$ (35.01 mmol) and stirred for 6 h at ambient temperature. The resulting reaction mixture was diluted with EtOAc (250 mL), filtered, washed with 1 M KI solution (30 mL), washed with sat. aq.$Na_2S_2O_3$, water (30

mL), dried ($MgSO_4$), filtered and concentrated under reduced pressure. Flash column silica gel chromatography of the resulting residue EtOAc/ hexanes (1/3 to 1/2, v/v) afforded the required lactol (3.09 g, 80%). To a solution of lactol (3.09 g, 9.33 mmol) in DMF (45 mL) was added imidazole (1.9 g, 28 mmol) and dimethylthexylsilyl chloride (2.74 mL, 13.9 mmol) and the reaction mixture was stirred for 12 h at ambient temperature. The resulting reaction mixture was concentrated under reduced pressure, diluted with EtOAc (200 mL) and washed with H_2O (2 × 20 mL). The combined organic layers were dried ($MgSO_4$), filtered and concentrated under reduced pressure. Flash column silica gel chromatography of the resulting residue using EtOAc/ hexanes (1/3, v/v) afforded title compound **38** (3.97 g, 90%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.26 (dd, 1H, *J* = 3.5 Hz*, J* = 0.9 *Hz,* H4), 4.71 (dd, lH, *J* = 11.0 Hz, *J* = 3.4 Hz, H3), 4.52 (d, lH, *J* = 7.6, H1), 4.09 (dd, 1H, *J* = 11.3 Hz, *J* = 7.2 Hz*,* H6a), 4.03 (dd, 1H, *J* = 11.3 Hz*, J* = 6.0 *Hz,* H6b), 3.79 (m, lH, *J* = 7.1Hz, *J* = 7.1Hz, *J* = 0.9Hz, H5), 3,54 (dd, 1H, *J* = 7.6 Hz, *J* = 10.9 Hz, H2), 2.11 - 1.99 (3s, 9H, C*H*3 Ac), 1.63 (m, lH, -C*H*(CH_3)$_2$), 0.87 (d, 6H, *J* = 6.7 Hz)*,* 0.85 (2s, 6H), 0.16 (2s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.2, 170.0, 169.6, 97.2 (C1), 70.9 (C3), 70.8 (C5), 66.5 (C4), 63.3 (C2), 61.6 (C6), 33.8 (*C*H(CH*3*)*2*), 24.8 (C(*C*H_3)$_2$), 20.5, 20.4, 19.9, 19.8 (C(*C*H_3)$_2$), 18.4, 18.3, -2.1, -3.2.

Dimethylthexylsilyl 2-azido-4,6-*O*-benzylidene-2-deoxy-β-D-galactopyranoside (40): To a solution of compound **38** (3.97 g, 8.4 mmol) in MeOH (40 mL) was added NaOMe/ MeOH (1.5 mL, 1 M) until the reaction mixture reached pH = 9 and stirred at ambient temperature for 2 h. The resulting reaction mixture was neutralized with amberlite H^+- resin and filtered. The filtrate was concentrated to dryness, under reduced pressure, yielding the title compound **39** (2.9 g, quant.). To a solution of compound **39**

(3.9 g, 8.35 mmol) in CH_3CN (45 mL) was added benzaldehyde dimethyl acetal (3.76 mL, 25.07 mmol) and CSA (194 mg, 0.835 mmol) and stirred at ambient temperature for 12 h. The reaction mixture was concentrated under reduced pressure and the resulting residue was diluted with ethyl acetate (200 mL) and washed with saturated aq. $NaHCO_3$ (40 mL). The aqueous phase was back extracted with ethyl acetate (3 × 30 mL) and the combined organic layers were washed with water (20 mL) and brine (20 mL), dried (Mg_2SO_4), filtered and concentrated under reduced pressure. Flash column silica gel chromatography of the resulting residue using EtOAc/ hexanes (1/5 to 1/3, v/v) yielded title compound **40** (3.71 g, 76%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.34 – 7.24 (m, 1H), 7.17 (dt, *J* = 4.6, 2.9 Hz, 1H), 5.32 (s, 1H), 4.37 – 4.25 (m, 1H), 4.04 (dd, *J* = 12.5, 1.5 Hz, 1H), 3.96 – 3.74 (m, 1H), 3.35 – 3.21 (m, 1H), 3.17 (q, *J* = 1.6 Hz, 1H), 2.25 (s, 1H), 1.49 (p, *J* = 6.8 Hz, 1H), 0.78 – 0.59 (m, 6H), 0.01 (d, *J* = 5.4 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 137.6, 129.5, 128.5, 126.6 (aromatic), 101.5 (*C*H benzylidene, 97.1 (C1), 77.6, 77.2, 76.7, 74.7 (C4), 71.3 (C2), 69.3 (C6), 66.7 (C3), 66.6 (C5), 34.1 (TDS), 25.0 (TDS), 20.2 (TDS), 20.1 (TDS), 18.7 (TDS), 18.6 (TDS), -1.8 (TDS), -2.7 (TDS).

Dimethylthexylsilyl 2-azido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-β-D-galactopyranoside (41): To a solution of compound **40** (3.71 g, 8.53 mmol) in DMF (60 mL) at 0 ^{o}C was added benzyl bromide (1.63 mL, 13.6 mmol) followed by portion wise addition of 60% NaH (600 mg, 12.79 mmol). The reaction mixture was stirred for two hours at 0 ^{o}C and the excess of NaH was quenched by the addition of ice cubes. The resulting reaction mixture was concentrated under reduced pressure, diluted with EtOAc (150 mL) and washed with H_2O (2 × 20 mL). The combined organic layers were dried ($MgSO_4$), filtered and concentrated under reduced pressure. Flash column silica gel

chromatography of the resulting residue using EtOAc/ hexane (1/5, v/v) afforded title compound **41** (2.68 g, 60%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.59 – 7.49 (m, 1H), 7.44 – 7.27 (m, 4H), 5.46 (s, PhC*H*), 4.73 (s, 1H), 4.50 (dd, *J* = 7.7, 1.2 Hz, 1H), 4.24 (dd, *J* = 12.4, 1.5 Hz, 1H), 4.08 – 3.94 (m, 1H), 3.76 (dd, *J* = 10.4, 7.7 Hz, 1H), 3.36 – 3.23 (m, 1H), 1.78 – 1.60 (m, 0H), 1.03 – 0.78 (m, 5H), 0.21 (d, *J* = 5.9 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 138.0, 138.0, 129.2, 128.6, 128.4, 128.0, 127.9, 126.6, 101.4 (benzylidene), 97.3 (C-1), 77.7, 77.6, 77.2, 76.7, 72.7, 71.7, 69.5, 66.6, 64.8, 34.1, 25.0, 20.2, 20.1, 18.7, 18.6, -1.7, -2.7.

Dimethylthexylsilyl 2-azido-3,6-di-*O*-benzyl-2-deoxy-β-D-galactopyranoside (8): To a solution of compound **41** (2.68 g, 5.104 mmol) in CH_2Cl_2 (50 mL) was added 4Å molecular sieves (5g) and stirred for 1 h at ambient temperature. To the resulting reaction mixture at -78 ^{o}C was added Et_3SiH (2.44 mL,15.3 mmol) and TfOH (1.53 mL, 17.35 mmol) and stirred for 1 h at -78 ^{o}C. To the reaction mixture at -78 ^{o}C was added Et_3N (2 mL) and MeOH (2 mL) and filtered. The resulting reaction mixture was diluted with $CHCl_2$, washed with saturated aqueous $NaHCO_3$ and washed with water. The combined organic layers were dried ($MgSO_4$), filtered and concentrated under reduced pressure. Flash column silica gel chromatography of the resulting residue using EtOAc/ hexanes (1/5, v/v) yielded title compound **8** (2.28 g, 85%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.51 – 7.13 (m, 8H), 4.71 (s, 2H, CH_2), 4.57 (d, *J* = 2.2 Hz, 2H, CH_2), 4.45 (dd, *J* = 13.0, 7.7 Hz, 1H, H-1), 3.98 (t, *J* = 2.8 Hz, 1H, H-4), 3.79 (ddd, *J* = 9.9, 6.0, 2.6 Hz, 1H, H-6), 3.69 (ddd, *J* = 9.9, 5.8, 1.7 Hz, 1H, H-6), 3.62 – 3.46 (m, 2H, H-3, H-5), 3.26 (ddd, *J* = 10.2, 3.3, 1.5 Hz, 1H, H-2), 2.51 – 2.44 (m, 1H, OH), 1.75 – 1.61 (m, 1H, TDS), 1.04 – 0.94 (m, 4H, TDS), 0.90 (d, *J* = 6.6 Hz, 5H, TDS), 0.74 – 0.62 (m, 3H, TDS), 0.19 (d, *J* =

2.0 Hz, 2H, TDS). ^{13}C NMR (75 MHz, $CDCl_3$): δ 138.1, 137.5, 128.7, 128.5, 128.3, 128.1, 127.9, 127.8 (arom.), 97.3 (C-1), 79.2 (C-3) , 77.6, 77.2, 76.7, 73.8 (C-2), 73.4 (CH_2), 72.2 (CH_2), 69.4 (CH2 of C-6)), 65.9 (C-4), 65.4 (C-5), 34.0, 25.0, 20.1, 20.0, 18.6, 18.5, -1.8, -3.1.

Dimethylthexylsilyl 2-azido-3-*O*-benzyl-2-deoxy-6-*O*-(2-naphthylmethyl)-β-D-galactopyranoside (8a): Compound **41** (360 mg, 0.68 mmol) was dissolved in a mixture of AcOH/H_2O (8:2, 10 mL) and stirred at 70 ^{o}C for 3 h. Toluene (50 mL) was added to the reaction mixture and concentrated under reduced pressure. Flash column silica gel chromatography of the resulting residue using EtOAc/hexanes (1/1, v/v) yielded triol (250 mg, 83%). To a solution of triol (250 mg, 0.57 mmol) in benzene (6 mL) was added 2- naphthaldehyde (84 mg, 0.85 mmol) and PPTS (9 mg, 0.05 mmol) and stirred at 70 ^{o}C for 12 h. The resulting reaction mixture was concentrated under reduced pressure and the residue was diluted with ethyl acetate and washed with saturated aq. $NaHCO_3$. The aqueous phase was back extracted with ethyl acetate, and the combined organic layers were washed with water and brine successively, dried ($MgSO_4$), filtered and concentrated under reduced pressure. Flash column silica gel chromatography of the resulting residue EtOAc/hexane (1/5 to 1/3, v/v) yielded the required acetal compound (255 mg, 0.43 mmol, 76%). For (naphthyl)methylene acetal compound: ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.02 – 7.97 (m, 1H), 7.91 – 7.80 (m, 3H), 7.65 (dd, *J* = 8.5, 1.7 Hz, 1H), 7.49 (dd, *J* = 6.2, 3.3 Hz, 2H), 7.45 – 7.24 (m, 6H), 4.75 (s, 2H), 4.53 (d, *J* = 7.7 Hz, 1H), 4.29 (dd, *J* = 12.3, 1.6 Hz, 1H), 4.15 – 4.01 (m, 2H), 3.80 (dd, *J* = 10.4, 7.7 Hz, 1H), 3.38 – 3.27 (m, 2H), 1.78 – 1.63 (m, 1H), 0.95 – 0.88 (m, 12H), 0.22 (d, *J* = 6.9 Hz, 5H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 138.0, 135.4, 133.9, 133.1,

128.6, 128.6, 128.3, 128.0, 127.9, 127.8, 126.4, 126.1, 126.0, 124.2, 101.5, 97.3, 77.7, 77.6, 77.2, 76.7, 72.8, 71.7, 69.6, 66.7, 64.8, 55.5, 34.1, 25.1, 20.3, 20.2, 18.7, 18.6, -1.7, -2.6.

To a solution of acetal compound (255 mg, 0.43 mmol) in CH_2Cl_2 (4 mL) was added 4Å molecular sieves (500 mg) and the reaction mixture was stirred for 1 h at ambient temperature. To the reaction mixture at -78 ^oC was added Et_3SiH (207 µl, 1.30 mmol) and TfOH (130 µL, 1.47 mmol) and stirred for 1 h at the same temperature. The reaction was quenched by the addition of Et_3N (0.5 mL) and MeOH (0.5 mL), diluted with $CHCl_2$, filtered, washed with saturated aqueous $NaHCO_3$ and water. The combined organic layers were dried ($MgSO_4$), filtered and concentrated under reduced pressure. Flash column silica gel chromatography of the resulting residue using EtOAc/hexanes (1/5, v/v) yielded title compound **8a** (166 mg, 65%). ^1H NMR (300 MHz, $CDCl_3$): δ 7.90 – 7.75 (m, 4H, CH aromatic), 7.53 – 7.29 (m, 8H, CH aromatic), 4.76 – 4.68 (m, 4H, 2 CH_2), 4.44 (d, J = 7.7 Hz, 1H, H1), 4.03 – 3.96 (m, 1H, H4), 3.83 (dd, J = 9.9, 5.9 Hz, 1H, H6a), 3.74 (dd, J = 9.9, 5.9 Hz, 1H, H6b), 3.61 – 3.49 (m, 2H, H2, H5), 3.27 (dd, J = 10.1, 3.3 Hz, 1H, H3), 2.49 (dd, J = 2.3, 1.1 Hz, 1H, OH), 1.68 (p, J = 6.9 Hz, 1H, CH TDS), 0.90 (dd, J = 6.1, 1.2 Hz, 12H, TDS), 0.20 (d, J = 2.9 Hz, 6H, TDS). ^{13}C NMR (75 MHz, $CDCl_3$): δ 137.5, 135.6, 133.4, 133.2, 128.7, 128.4, 128.3, 128.1, 128.0, 127.8, 126.6, 126.3, 126.1, 125.8, 97.3 (C-1), 79.2, 77.6, 77.2, 76.7, 74.0, 73.5, 72.3, 69.4, 66.0, 65.5, 34.1, 25.0, 20.2, 20.0, 18.7, 18.6, -1.7, -3.1.

Dimethylthexylsilyl 3-*O*-acetyl-2-azido-4-trichloroacetyl-2,4,6-trideoxy-β-D-galactopyranosyl-(1→4)-2-azido-3,6-di-*O*-benzyl-2-deoxy-β-D-galactopyranoside (43): Alcohol **8** (33 mg, 0.062 mmol) and trichloroacetimidate **7a** (39 mg, 0.075 mmol)

were co-evaporated with toluene (2 x 7 mL) and placed on high vacuum for 12 h. To the solution of donor and acceptor mixture in CH_2Cl_2 (2 mL) was added 4Å molecular sieves (100 mg) and stirred for 1 h at room temperature. To the resulting reaction mixture was added TMSOTf (3 µL, 0.015 mmol) that was dissolved in CH_2Cl_2 (0.5 mL) at 0 °C. The reaction mixture was stirred for 1 h at ambient temperature, quenched with NEt_3, diluted with $CHCl_2$, filtered, washed with saturated aqueous $NaHCO_3$ and water. The combined organic layers were dried ($MgSO_4$), filtered, concentrated under reduced pressure. The resulting residue was purified by flash column silica gel chromatography using EtOAc/hexanes (1/5, v/v) to yield title compound **43** (40 mg, 66%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.5 – 7.2 (m, 8H, CH aromatic), 6.7 (d, *J* = 9.1 Hz, 1H, NH^a), 5.3 (dd, *J* = 11.3, 3.8 Hz, 1H, $H3^a$), 4.9 (d, *J* = 3.9 Hz, 1H, $H1^a$), 4.5 (q, *J* = 11.9 Hz, 2H, $H5^a$, CH_2), 4.4 (d, *J* = 7.5 Hz, 1H, $H1^b$), 4.4 (ddd, *J* = 9.2, 3.9, 1.6 Hz, 1H, $H4^a$), 4.1 (d, *J* = 3.0 Hz, 1H, $H4^b$), 3.9 (t, *J* = 9.0 Hz, 1H, $H6^b$), 3.7 – 3.4 (m, 3H, CH_2, $H2^b$, $H5^b$), 3.2 (ddd, *J* = 11.3, 6.5, 3.5 Hz, 2H, $H2^a$, $H3^b$), 2.1 (s, 3H), 1.6 (q, *J* = 6.9 Hz, 1H), 0.9 – 0.8 (m, 15H), 0.2 (d, *J* = 3.8 Hz, 5H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.3, 162.7, 137.7, 137.6, 129.2, 128.7, 128.6, 128.4, 128.3, 128.2, 128.2, 128.1, 127.8, 98.6 ($C1^a$), 97.6 ($C1^b$), 92.7 (CCl_3), 78.4, 77.6, 77.2, 76.7 ($C3^b$), 73.6, 73.2, 72.9, 72.7, 69.2, 66.9, 65.8, 64.4, 57.8, 53.3 ($C4^a$), 34.0 (C*H* of TDS), 29.8, 24.9, 20.9, 20.1 (C6 Ac), 20.0, 18.6, 18.5, 16.3, -1.8, -3.0.

Dimethylthexylsilyl 3-*O*-acetyl-2-azido-4-(benzyloxycarbonyl)amino-2,4,6-trideoxy-β-D-galactopyranosyl-(1→4)-2-azido-3,6-di-*O*-benzyl-2-deoxy-β-D-galactopyranoside (44): Alcohol **8a** (50 mg, 0.098 mmol) and trichloroacetimidate **7** (39 mg, 0.118 mmol) were co-evaporated with toluene (2 x 7 mL) and placed on high

vacuum for 12 h. To the solution of donor and acceptor mixture in CH_2Cl_2 (2 mL) was added 4Å molecular sieves (100 mg) and stirred for 1 h at room temperature. To the resulting reaction mixture was added TMSOTf (4.2 µL, 0.023 mmol) that was dissolved in CH_2Cl_2 (0.5 mL) at 0 °C. The reaction mixture was stirred for 1 h at ambient temperature, quenched with NEt_3, diluted with $CHCl_2$, filtered, washed with saturated aqueous $NaHCO_3$ and water. The combined organic layers were dried ($MgSO_4$), filtered, concentrated under reduced pressure. The resulting residue was purified by flash column silica gel chromatography using EtOAc/hexanes (1/5, v/v) to yield title compound **45** (61 mg, 72%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.9 (dd, *J* = 8.2, 4.0 Hz, 3H, Cbz), 7.8 (d, *J* = 1.6 Hz, 1H, Cbz), 7.6 – 7.2 (m, 11H, CH aromatic), 5.2 – 5.1 (m, 2H, H3^a, CH*H*Ph), 5.0 (d, *J* = 12.3 Hz, 1H, C*H*HPh), 4.7 – 4.5 (m, 7H), 4.39 (d, *J* = 7.6 Hz, 1H, H1^b), 4.05 (dd, *J* = 9.9, 3.6 Hz, 1H, H4^a), 4.0 (d, *J* = 2.9 Hz, 1H), 3.8 (t, *J* = 9.1 Hz, 1H), 3.5 (td, *J* = 8.9, 8.1, 3.0 Hz, 2H, H6a^b), 3.5 (dd, *J* = 9.0, 5.5 Hz, 1H, H6b^b), 3.0 (dd, *J* = 10.6, 3.0 Hz, 1H, H3^b), 3.0 (dd, *J* = 11.3, 3.9 Hz, 1H, H2^a), 2.1 (s, 3H, Ac), 1.7 – 1.6 (m, 1H, TDS), 0.9 – 0.8 (m, 12H, TDS), 0.1 (d, *J* = 2.9 Hz, 6H, TDS). ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.3 (Ac), 156.7, 137.7, 128.6, 128.5, 128.4, 128.1, 128.0, 128.0, 127.8, 127.7, 127.2, 126.5, 126.4, 126.2, 98.1 (C1^a), 97.3 (C1^b), 78.4, 77.6, 77.4, 77.1, 76.7, 73.6, 72.9, 72.8, 72.2, 69.7, 67.1, 67.1, 65.7, 64.5, 57.4, 52.5 (C4^a), 34.0, 29.9, 24.9, 20.7 (Ac), 20.4, 20.0, 18.1, 18.5, 16.4, -1.8, -2.8.

Dimethylthexylsilyl 3-*O*-acetyl-2-azido-4-(benzyloxycarbonyl)amino-2,4,6-trideoxy-β-D-galactopyranosyl-(1→4)-2-azido-3-*O*-benzyl-2-deoxy-6-*O*-(2-naphthylmethyl) β-D-galactopyranoside (45): Alcohol **8a** (31 mg, 0.062 mmol) and trichloroacetimidate **7** (39 mg, 0.075 mmol) were co-evaporated with toluene (2 x 7 mL) and placed on high

vacuum for 12 h. To the solution of donor and acceptor mixture in CH_2Cl_2 (2 mL) was added 4Å molecular sieves (100 mg) and stirred for 1 h at room temperature. To the resulting reaction mixture was added TMSOTf (3 µL, 0.015 mmol) that was dissolved in CH_2Cl_2 (0.5 mL) at 0 °C. The reaction mixture was stirred for 1 h at ambient temperature, quenched with NEt_3, diluted with $CHCl_2$, filtered, washed with saturated aqueous $NaHCO_3$ and water. The combined organic layers were dried ($MgSO_4$), filtered, concentrated under reduced pressure. The resulting residue was purified by flash column silica gel chromatography using EtOAc/hexanes (1/5, v/v) to yield title compound **45** (45 mg, 58%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.9 (dd, *J* = 8.2, 4.0 Hz, 3H, Cbz), 7.8 (d, *J* = 1.6 Hz, 1H, Cbz), 7.6 – 7.2 (m, 12H, CH aromatic), 5.3 – 5.1 (m, 2H, H3^a, CH*H*Ph), 5.0 (d, *J* = 12.3 Hz, 1H, C*H*HPh), 4.8 – 4.5 (m, 7H, H1^a, H-5^a, NH, 2CH_2), 4.4 (d, *J* = 7.6 Hz, 1H, H1^b), 4.1 (dd, *J* = 9.9, 3.6 Hz, 1H, H4^a), 4.1 (d, *J* = 2.9 Hz, 1H, H4^b), 3.9 (t, *J* = 9.1 Hz, 1H, H4^b), 3.6 (td, *J* = 8.9, 8.1, 3.0 Hz, 2H, H6^b, H2^b), 3.5 (dd, *J* = 9.0, 5.5 Hz, 1H, H6^b), 3.1 (dd, *J* = 10.6, 3.0 Hz, 1H, H3^b), 3.0 (dd, *J* = 11.3, 3.9 Hz, 1H, H2^a), 2.0 (s, 3H, Ac), 1.7 – 1.6 (m, 1H, TDS), 0.9 – 0.8 (m, 12H, TDS), 0.1 (d, *J* = 2.9 Hz, 7H, TDS). ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.3 (Ac), 156.7, 137.7, 136.6, 135.1, 133.4, 133.2, 128.7, 128.6, 128.5, 128.4, 128.1, 128.0, 128.0, 127.8, 127.7, 127.2, 126.5, 126.4, 126.2, 98.6 (C-1^a), 97.6 (C-1^b), 78.4, 77.6, 77.4, 77.2 (C-3^b), 76.7, 73.6, 72.9, 72.8, 72.4, 69.7, 67.1, 67.1, 65.7, 64.8 (C-5^a), 57.7 (C-2^a), 52.8 (C-4^a), 34.0, 29.9, 24.9, 21.0 (Ac), 20.1 (TDS), 20.0 (TDS), 18.6 (TDS), 18.5 (TDS), 16.3 (TDS) -1.8 (TDS), -3.0 (TDS).

D-Ribose diethyl dithioacetal (54): To a solution of D-ribose (5g, 33.30 mol) in concentrated hydrochloric acid (6 mL) was added ethanethiol (6 mL, 83.26 mmol) at 0

°C over 15 minutes and the reaction mixture was stirred for 30 minutes. To the resulting reaction mixture was added saturated aqueous sodium bicarbonate to neutralize excess acid. The resulting reaction mixture was concentrated under reduced pressure and diluted with ethyl acetate (5 × 100 mL). The insoluble inorganic salts were removed by filtration. The combined organic layer was concentrated to yield impure **54** which was recrystallized from ethanol to obtaine pure white solid (6.4 g, 75%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.2 (d, *J* = 10.2 Hz, 1H), 3.9 (d, *J* = 10.1 Hz, 1H), 3.8 (dd, *J* = 12.2, 6.0 Hz, 1H), 3.7 – 3.5 (m, 3H), 3.3 (s, 1H), 2.8 (q, *J* = 6.6 Hz, 4H), 2.2 (s, 1H), 1.2 (t, *J* = 6.6 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 74.5, 72.6, 71.7, 64.3, 55.2, 24.7, 14.5.

5-*O*-Allyl D-ribose diethyl dithioacetal (55): Dibutyltin oxide (6.22 g, 25 mmol) and compound **54** (6.4 g, 25 mmol) were refluxed in toluene (250 mL) for 4 h with the addition of a dean–stark apparatus for the azeotropic removal of water. The reaction mixture was concentrated under reduced pressure, and cesium fluoride (5.47 g, 36.25 mmol) was added. To the reaction mixture that was kept under vacuum for 1 h, a solution of allyl bromide (2 mL, 32.5 mmol) in DMF (100 mL) solution was added and the resulting mixture was stirred for 2 h. The resulting reaction mixture was concentrated to a residue that was taken up in ethyl acetate (400 mL), dried ($MgSO_4$), filtered and concentrated to a liquid residue under reduced pressure. The resulting residue was purified by flash column silica gel column chromatography using EtOAc/hexanes (1:1 to 100%, v/v) to yield title compound **55** (4.95 g, 67%). ^{1}H NMR is same as reported.[25] ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.4, 14.5 (2 × CH_3), 25.6 (2 × CH_2S), 54.4 (C1), 71.1 (C4), 71.5 (C5), 72.4 (CH_2-All), 72.8 (C3), 75.0 (C2), 117.6 (CH_2=), 133.9 (-CH=). HRMS-MALDI: (M +Na^+) calcd. 319.1014; found 319.1218.

5-*O*-Allyl-2,3,4-tri-*O*-benzyl-D-ribose diethyl dithioacetal (56): To a solution of compound **55** (4. 95 g, 16.72 mmol) in DMF (60 mL) was added excess 60% NaH (3.92 g, 13.37 mmol) at 0 °C. The resulting reaction mixture was stirred for 30 minutes and a solution of benzyl bromide (14.91 mL, 125.4 mmol) in DMF (10 mL) was added drop-wise and stirred for 3.5 h at 0 °C. Excess NaH was quenched with cold water/ice cubes (50 mL), extracted with EtOAc (5 × 100 mL), dried ($MgSO_4$), filtered and concentrated under reduced pressure. The resulting residue was purified with flash column silica gel chromatography using EtOAc/hexanes (1/8, v/v) to yield title compound **56** (6.59 g, 88%).[1]H NMR is same as reported.[25] ^{13}C NMR (75 MHz, $CDCl_3$) δ: 14.6 (2 CH_3 SEt), 24.8, 26.1 (2 CH_2 SEt), 53.8 (C-1), 69.3 (C-5), 72.0, 72.4, 73.4, 74.5 (3 CH_2Ph, 1 CH_2 All), 78.9, 79.8, 82.1 (C-2, C-3, C-4), 116.4 (CH_2=), 134.7 (-CH=), 127.2-128.7, 138.2, 138.3, 138.7 (C-Ph).

5-*O*-Allyl-2,3,4-tri-*O*-benzyl-D-ribose (57): To a solution of compound **56** (6.59 g, 11.70 mmol) in acetonitrile and water (90 mL CH_3CN + 10 mL H_2O) was added mercury(II) chloride (12.7 g, 46.82 mmol) and calcium carbonate (4.8 g, 47.97 mmol) and the resulting mixture was stirred for 1 h. The resulting reaction mixture was filtered through celite and the filtrate was concentrated to a residue that was diluted with dichloromethane (400 mL). The organic layer was subsequently washed with 1 M potassium iodide solution (3 × 50 mL), 30% sodium thiosulfate solution (2 × 50 mL), dried ($MgSO_4$) and concentrated which yielded the title compound **57** (4.52 g, 85%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.53 (dd, 1H, H-5a, *J*5a,5b= 10.7 Hz, *J*5a,4 = 4.6 Hz), 3.67 (dd, 1H, H5b, *J*5b,4 = 2.1 Hz), 3.87–4.01 (m, 4H, H3, H4, CH_2 All), 4.09 (br d, 1H, H2), 4.47–4.82 (m, 6H, 3 × CH_2Ph), 5.11–5.26 (m, 2H, CH_2=), 5.78–5.93 (m, 1H, -CH=),

7.22–7.33 (m, 15H, 3×Ph), 9.47 (br s, 1H, -CH=O). ^{1}3C NMR: (75 MHz, $CDCl_3$): δ 69.0 (C-5), 72.1 (CH_2 All), 72.5, 72.7, 79.9 (3 CH_2Ph), 76.5, 80.3 (2C C-3, C-4), 82.2 (C-2), 116.8 (CH_2=), 134.6 (-CH=), 127.4–128.3, 137.3, 137.5, 137.9, 200.9 (C-1, HC=O).

5-*O*-Allyl-2,3,4-tri-*O*-benzyl-D-ribitol (10): To a solution of compound **57** (4.52 g, 9.82 mmol) in methanol (50 mL) was added sodium borohydride (.74 g, 19.6 mmol) and stirred for 10 h. To the resulting reaction mixture water (30 mL) was added, extracted with chloroform (3 × 100 mL), dried ($MgSO_4$) and concentrated to a liquid residue. Flash column silica gel chromatography of the resulting residue using EtOAc/hexanes (1/3, v/v) afforded title compound **10** (2.81 g, 60%). ^{1}H NMR (300 $CDCl_3$): δ 7.49 – 7.18 (m, 15H), 5.92 (ddt, *J* = 17.2, 10.8, 5.5 Hz, 1H, CH=), 5.42 – 5.11 (m, 2H, CH2=), 4.85 – 4.47 (m, 8H), 4.10 – 3.50 (m, 9H), 2.33 (q, *J* = 5.1, 4.4 Hz, 1H, OH). ^{13}C NMR (75 MHz, $cdcl_3$) δ 138.4, 138.3, 138.2, 134.9, 128.5, 128.5, 128.5, 128.4, 128.4, 128.2, 128.1, 128.0, 128.0, 128.0, 127.9, 127.9, 127.9, 127.8, 127.7, 127.7, 117.0, 79.2, 79.2, 79.0, 79.0, 78.4, 78.3, 77.6, 77.2, 76.7, 74.1, 72.6, 72.4, 72.0, 69.9, 69.8, 61.5.

4,6-di-*O*-benzyl-D-galactal (47): A solution of compound **36** (1.0 g, 6.38 mmol) in DMF (10 mL) under nitrogen at 0 ^{0}C was treated with 60% NaH (600 mg, 15.05 mmol). The reaction mixture was stirred for 15 minutes at 0 ^{0}C, BnBr (1.8 mL, 15.05 mmol) was added drop-wise and stirred for 3 h at 0 ^{0}C. The reaction mixture was diluted with dichloromethane (30 mL), washed with water (20 mL), back extracted with CH_2Cl_2 (3×10 mL). The combined organic fractions were washed with brine (30 mL), dried ($MgSO_4$), filtered and concentrated under reduced pressure. Careful flash column silica gel chromatography of the resulting residue using EtOAc/hexanes (1/4, v/v) yielded the required compound **47** (2.5 g, 30%) along with dibenzylated and tribenzylated side

products. ^{1}H NMR (300 MHz, $CDCl_3$) ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.44 – 7.20 (m, 9H, arom.), 6.44 (dd, *J* = 6.2, 1.5 Hz, 1H, H1), 5.54 – 5.41 (m, 3H, H2, CH_2), 4.82 – 4.66 (m, 2H, CH_2), 4.60 – 4.39 (m, 1H, H3), 4.34 – 4.20 (m, 1H, H5), 4.03 (ddd, *J* = 4.1, 2.8, 1.0 Hz, 1H, H4), 3.77 (dd, *J* = 10.3, 7.4 Hz, 1H, H6), 3.64 (dd, *J* = 10.3, 4.8 Hz, 1H, H6); ^{13}C NMR (75 MHz, $CDCl_3$): δ 144.2, 137.7, 128.5, 127.8, 102.8 (C1), 75.1, 74.2, 73.4, 73.1, 68.1, 62.8.

3-*O*-Acetyl-4,6-di-*O*-benzyl-D-galactal (48): A solution of compound **47** (2.5 g, 7.7 mmol) in pyridine (25 mL) and acetic anhydride (15 mmol, 20 mL) was stirred at room temperature for 1 h. The resulting reaction mixture was concentrated under reduced pressure, and the residue was purified by flash column silica gel chromatography using EtOAc/hexanes (1/4, v/v) to yield title compound **48** (2.6 g, 92%). ^{1}H NMR (300 MHz, $CDC1_3$): δ 7.42 – 7.22 (m, 9H, CH aromatic), 6.44 (dd, *J* = 6.2, 1.5 Hz, 1H, H1), 5.48 (ddt, *J* = 4.6, 3.4, 1.3 Hz, 1H, H3), 4.79 – 4.68 (m, 2H, H2, H of CH_2), 4.59 – 4.39 (m, 2H, CH_2), 4.27 (dtd, *J* = 4.8, 2.3, 1.2 Hz, 1H, H5), 4.03 (ddd, *J* = 4.1, 2.8, 1.0 Hz, 1H, H4), 3.77 (dd, *J* = 10.3, 7.4 Hz, 1H, H6), 3.64 (dd, *J* = 10.3, 4.8 Hz, 1H, H6), 2.04 (s, 3H, Ac).^{13}C NMR (75 MHz, $CDCl_3$): δ 170.8, 145.7, 138.0, 137.9, 128.5, 128.1, 128.0, 127.9, 127.9, 127.8, 98.6 (C-1), 77.6, 77.2, 76.7, 75.6, 73.6, 73.5, 70.8, 67.9, 65.5, 21.2 (Ac).

***O*-(3-*O*-Acetyl-2-azido-4,6-di-*O*-benzyl-2-deoxy-α,β-D-galactopyranosyl) nitrate (49):** To a solution of compound **48** (2 g, 5.4 mmol) in acetonitrile (48 mL) was added ammonium cerium(1V) nitrate (8.9 g,16.2 mmol) and sodium azide (0.52 g, 8.0 mmol) at under nitrogen -35 ^{o}C. The reaction mixture was stirred at -35 ^{o}C for *5* h, diethyl ether (100 mL) and water (50 mL) were added. The aqueous layer was back extracted with

diethyl ether (2 X 50 mL). The combined organic layers were dried ($MgS0_4$), filtered and concentrated under reduced pressure. The resulting residue was purified by flash column silica gel chromatography using EtOAc/hexane (1/6, v/v) to yield **49** as a mixture of anomers (900 mg, 30%). α-anomer was purified and NMR data was recorded. ^{1}H NMR (300 MHz, $CDC1_3$) δ 7.41 – 7.21 (m, 11H, CH aromatic), 6.35 – 6.21 (m, 1H, H1), 5.17 (dd, *J* = 11.3, 2.9 Hz, 1H, H3), 4.68 – 4.37 (m, 5H, 2 × CH_2), 4.34 – 4.12 (m, 3H), 3.69 – 3.45 (m, 2H), 2.06 (d, *J* = 0.6 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 20.7, 56.4, 67.2, 71.2, 71.9, 73.5, 73.7, 75.4, 97.6, 127.8, 128.5, 137.4, 137.5, 170.1 (Ac).

3-*O*-Acetyl-2-azido-4,6-di-*O*-benzyl-2-deoxy-D-galactose (50): To a solution of compound **49** (139 mg, 0.294 mmol) in acetonitrile (10 mL) was added PHSH (90 µL, 0.882 mmol) and DIPEA (51 µL, 0.294 mmol) and the resulting mixture was vigorously stirred for 3 h at 0 °C. The reaction mixture was concentrated under reduced pressure and flash column silica gel chromatography of the resulting residue using EtOAc/hexanes (1/3, v/v) yielded **50** (104 mg, 85%) as a yellow oil. ^{1}H NMR (300 MHz, $CDC1_3$): δ 2.05 (s, 3H), 3.07 (br s, 1H), 3.92 (dd, 1H), 3.94 (d, 1H), 4.06 (d, 1H), 4.74 (dd, 1H), 5.32 (dd, 1H), 5.38 (d, 1H). ^{13}C NMR (75 MHz, $CDC1_3$): δ 170.3 (C=O Ac), 137.8, 137.7, 128.6, 128.6, 128.4, 128.3, 128.3, 128.1, 128.1, 128.0, 128.0, 92.6 (C1), 77.6, 77.4, 77.2, 76.7, 75.4, 74.9, 73.7, 73.6, 71.4, 69.4, 68.7, 58.7, 21.0 (Ac).

3-*O*-Acetyl-2-azido-4,6-di-*O*-benzyl-2-deoxy-α,β-D-galactopyronosyl trichloroacetimidate (9): To the solution of lactol (80 mg, 187 mmol) in CH_2Cl_2 (4 mL) was added Cl_3CCN (95 µL, 0.93 mmol) and DBU (6 µL, 0.063 mmol) and stirred for 2h at ambient temperature. The reaction mixture was concentrated under reduced pressure, and the silica gel was neutralized by running with Et_3N/hexane (1:50, 50 mL)

before loading residue that contains the compound **9**. Flash column silica gel chromatography of the resulting residue using EtOAc/hexanes (1/5, v/v) afforded the title compound **9** (83 mg, 79%). ^{1}H NMR (300 MHz, $CDC1_3$): δ 8.74 (s, 1H, NH), 7.24-7.43 (m, 10H, CH aromatic), 6.48 (d, 1H, H1), 5.36 (dd, 1H, H3), 4.40-4.75 (m, 4H, $2CH_2$), 4.31 (m, 1H, H6), 4.20-4.27 (2H, m, H6, H4), 3.67(m, 1H, H5), 3.57-3.63 (m, 1H, H2), 2.09 (s, 3H).

5-*O*-Allyl-2,3,4-tri-*O*-benzyl-1-*O*-(3-*O*-Acetyl-2-azido-4,6-di-*O*-benzyl-2-deoxy-β-D-galactopyranosyl)-D-ribitol (59): A solution of glycosyl donor **9** (83 mg, 0.145 mmol) and acceptor **10** (67 mg, 0.158 mmol) in acetonitrile (3.5 mL) and DCM (0.7 mL) was stirred with 4Å molecular sieves (200 mg) for 1 h at ambient temperature. The reaction mixture was to –42 °C and trimethylsilyl trifluoromethanesulfonate (18 µL, 0.66 mmol) was added. The reaction mixture was stirred at –42°C for 60 minutes, quenched with pyridine (1 mL), filtered through celite, diluted with DCM. The combined organic layers were washed with water (2 × 15 mL), dried ($MgSO_4$), and concentrated under reduced pressure. Flash column silica gel chromatography of the resulting residue using EtOAc/hexanes (1/5, v/v) yielded the compound **59** (107 mg, 87%). ^{1}H NMR (500 MHz, $CDC1_3$): δ 7.3 – 7.1 (m, 45H), 5.8 (ddd, *J* = 11.9, 10.5, 5.2 Hz, 1H), 5.2 (dq, *J* = 17.2, 1.7 Hz, 1H), 5.1 (dq, *J* = 10.6, 1.4 Hz, 1H), 4.9 (t, *J* = 9.4 Hz, 1H), 4.8 (dd, *J* = 10.4, 3.0 Hz, 1H), 4.7 – 4.3 (m, 24H, H3), 4.2 (dd, *J* = 8.0, 2.7 Hz, 2H, H1), 4.1 – 4.0 (m, 2H, H4), 3.9 – 3.4 (m, 2H, H2, H5), 2.0 (d, *J* = 13.9 Hz, 6H). ^{13}C NMR (75 MHz, $CDC1_3$): δ 170.3, 138.8, 138.8, 138.7, 138.7, 138.1, 137.8, 135.1, 128.7, 128.6, 128.6, 128.5, 128.5, 128.4, 128.4, 128.4, 128.4, 128.3, 128.2, 128.2, 128.1, 128.1, 128.0, 127.9, 127.9, 127.8, 127.8, 127.8, 127.6, 127.6, 127.5 (allyl), 116.8 (allyl), 102.3 (C1), 78.7, 78.5,

78.5, 77.6, 77.2, 76.7, 75.2, 73.9, 73.9, 73.6, 73.6, 73.4, 73.2, 72.5, 72.3, 70.3, 70.3, 69.3, 67.8, 61.8, 21.0.

5-*O*-allyl-2,3,4-tri-*O*-benzyl-1-*O*-[3-*O*-Acetyl-4,6-di-*O*-benzyl-2-deoxy-2-(trichloroethoxycarbonylamino)-β-D-galactopyranosyl) D-ribitol (58): A solution of glycosyl donor **9a** (86 mg, 0.119 mmol) and acceptor **10** (67 mg, 0.143 mmol) in DCM (3.5 mL) was stirred with 4Å molecular sieves (200 mg) for 1 h at ambient temperature. The resulting reaction mixture was cooled to –40 °C, trimethylsilyl trifluoromethanesulfonate (5 µL) was added and the reaction solution was stirred at –40 °C for 60 minutes after which pyridine (1 mL) was added to quench the reaction mixture and the resulting reaction mixture was filtered through celite. The resulting reaction mixture was diluted with DCM, washed with water (2 × 15 mL), dried ($MgSO_4$), and concentrated under reduced pressure. Flash column silica gel chromatography of the resulting residue using EtOAc/hexanes (1/5, v/v) yielded compound **58** (107 mg, 67%). An identical glycosylation procedure was also performed with glycosyl donor **9a** with SePh as anomeric leaving group, and acceptor **10**, yielded title disaccharide **58** (100 mg, 60%). ^{1}H NMR (500 MHz, $CDC1_3$): δ 7.4 – 7.1 (m, 33H, CH aromatic), 5.8 (dq, *J* = 16.4, 5.3 Hz, 1H), 5.2 – 5.0 (m, 2H), 5.0 – 4.7 (m, 2H), 4.7 – 4.1 (m, 18H), 4.1 – 3.3 (m, 17H), 1.9 (s, 3H). ^{13}C NMR (75 MHz, $CDC1_3$): δ 170.7, 170.7, 154.2, 138.9, 138.6, 138.6, 138.2, 137.9, 135.4, 135.1, 128.6, 128.5, 128.5, 128.5, 128.4, 128.3, 128.3, 128.3, 128.2, 128.1, 128.0, 128.0, 128.0, 127.9, 127.8, 127.8, 127.7, 117.0, 116.7, 101.5, 78.8, 78.7, 78.5, 78.2, 77.6, 77.4, 77.2, 76.7, 75.1, 74.4, 74.0, 73.6, 73.4, 73.3, 72.6, 72.4, 71.5, 70.3, 70.2, 68.6, 68.4, 68.1, 53.4, 20.9.

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CHAPTER 3

A SYNTHETIC STUDY TOWARD PSEUDOPENTASACCHARIDE REPEATING UNIT OF *STREPTOCOCCUS PNEUMONIAE* ZWITTERIONIC POLYSACCHARIDE

3.1 Introduction

Zwitterionic polysaccharides (ZPSs) present an unique class of polysaccharide molecules from both a structural and a biological perspective.[1] These bacterial polysaccharides contain both basic amino functions and acidic carboxylate groups or phosphate groups, and feature a zwitterionic character at physiological pH.[2] The ZPSs are the only known class of polysaccharides that are capable of eliciting a T-cell dependent immune response.[3,4] This type of immune response was thought to be confined to proteins and peptides.[1] Because of poor immunogenicity of regular polysaccharides, many vaccine formulations are made through conjugation to immunostimulatory carrier proteins.[5] Without the carrier proteins, capsular polysaccharides are processed by antigen presenting cells but not presented by MHC class II proteins to T-cells. This presentation of antigen fragments by MHC class II is a key step in the realization of adaptive immune responses. Unlike plain polysaccharides, ZPSs are capable of stimulating CD-4+ T-cell proliferation through presentation by MHC-II molecules.[6] ZPSs have also been shown to stimulate the innate immune system through interaction with Toll-like receptor 2 (TLR2).[7] Isolation and purification of pneumococcal cell wall polysaccharides from pneumococcal strains leads to micro

heterogeneity with regard to substitutions on ribitol residue,[8] and different number of PCho residues on the oligosaccharide backbone. To find out the mode of action of the ZPSs at the molecular level, synthetically well-defined and characterized ZPSs (monomers and repeating units of particular molecular weight) can serve as valuable tools. The rationale for the chemical synthesis of valuable ZPSs has been explained in the introduction part of chapter II.

The zwitterionic C-polysaccharide with a repeating unit pseudopentasaccharide [*β*-D-Glc*p*-(1-3)-*α*-AAT*p*-(1-4)-*α*-D-Gal*p*NAc-(1-3)-*β*-D-Gal*p*NAc-D-ribitol] was isolated from a non-capsulated pneumococcal strain CSR SCS2,[9] and also from serotype 6B. Pseudopentasaccharides are linked to each other by phosphodiester linkages between C5 of D-ribitol residue and C6 of *β*-D-glucopyranosyl residue. The polysaccharide **2** is substituted with one residue of phosphocholine (PCho) at C6 position of 4-substitued 2-acetamido-2deoxy-α-D-galactopyranosyl residue, and both galactosamine residues are *N*-acetylated. The capsular polysaccharide **1** and it's repeating unit **2** are depicted in figure **3.1**. The positive charge is situated at the amino group of the rare 2-acetamido-2-deoxy-D-galactopyranosyl residue and the negative charge is situated on the phosphate group that connects the repeating units. In this chapter, an alternative strategy that involved inversion of C4 hydroxyl group with masked amines will be explained. Various building block syntheses, and assembling building blocks into di- and trisaccharides, and an attempted pentasaccharide glycosylation will also be discussed.

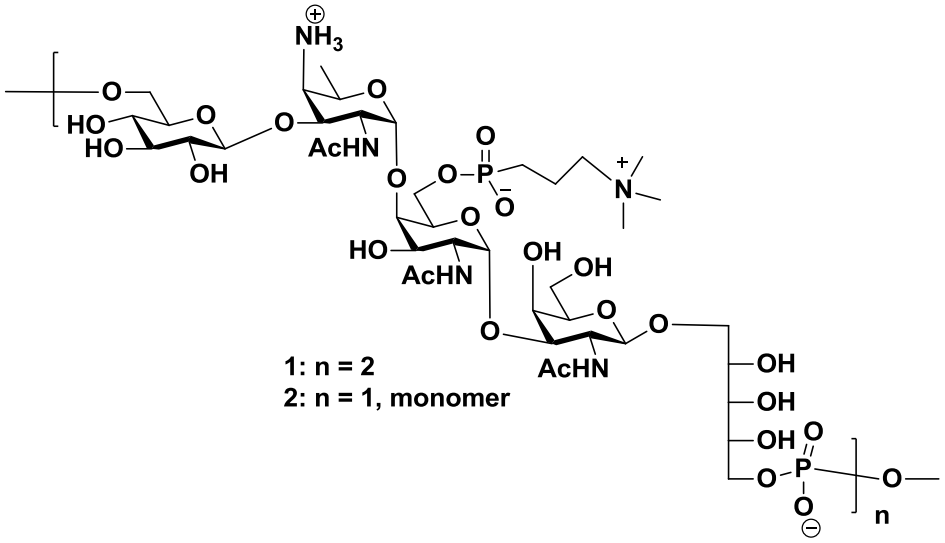
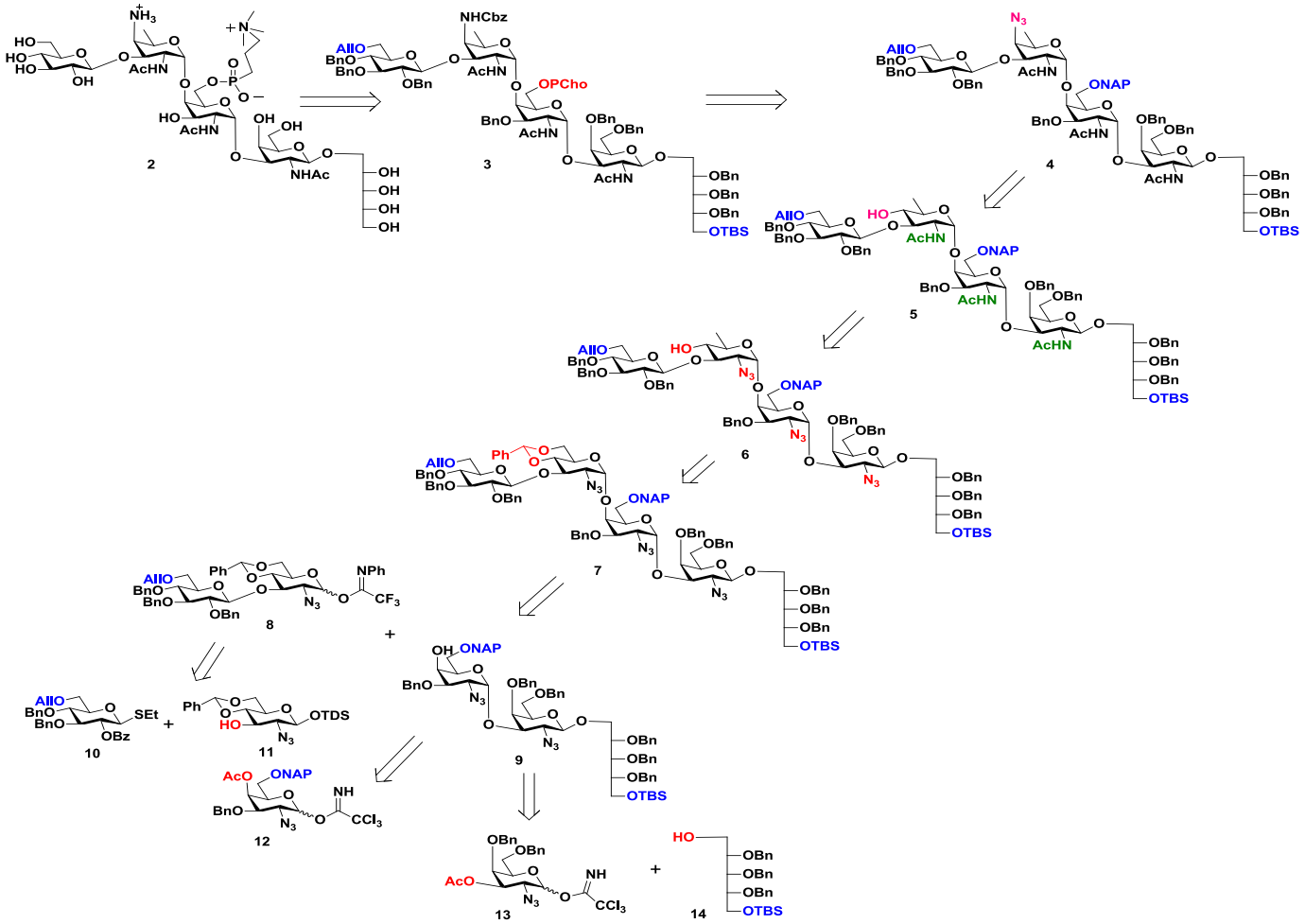


Figure 3.1: Structure of the C-polysaccharide

3.2 Results and Discussion

chapter II explained syntheses of various fragments of oligosaccharide **2**, and many challenges in reproducing reported results on the synthesis of AAT building block. Hence, we planned an alternative strategy that does not involve the synthesis of AAT monosaccharide residue. Our strategy, which is outlined in Scheme **3.1**, involved a convergent [2+3] glycosylation strategy to achieve the required pseudopentasaccharide repeating unit **2**. An important feature of this synthetic plan involves installation of axial amino functionality by inversion of C4 hydroxyl group in AAT moiety at the pentasaccharide stage. This synthetic strategy employed three orthogonal protecting groups (Nap, Allyl and TBS) that could be selectively removed to install phosphocholine residue and to oligomerize the repeating unit respectively. Target oligosaccharide with one repeating pentasaccharide unit **2** is a structurally challenging and demanding

molecule. Previous studies described in chapter II illustrate the challenges in synthesizing the pentasaccharide **2**.



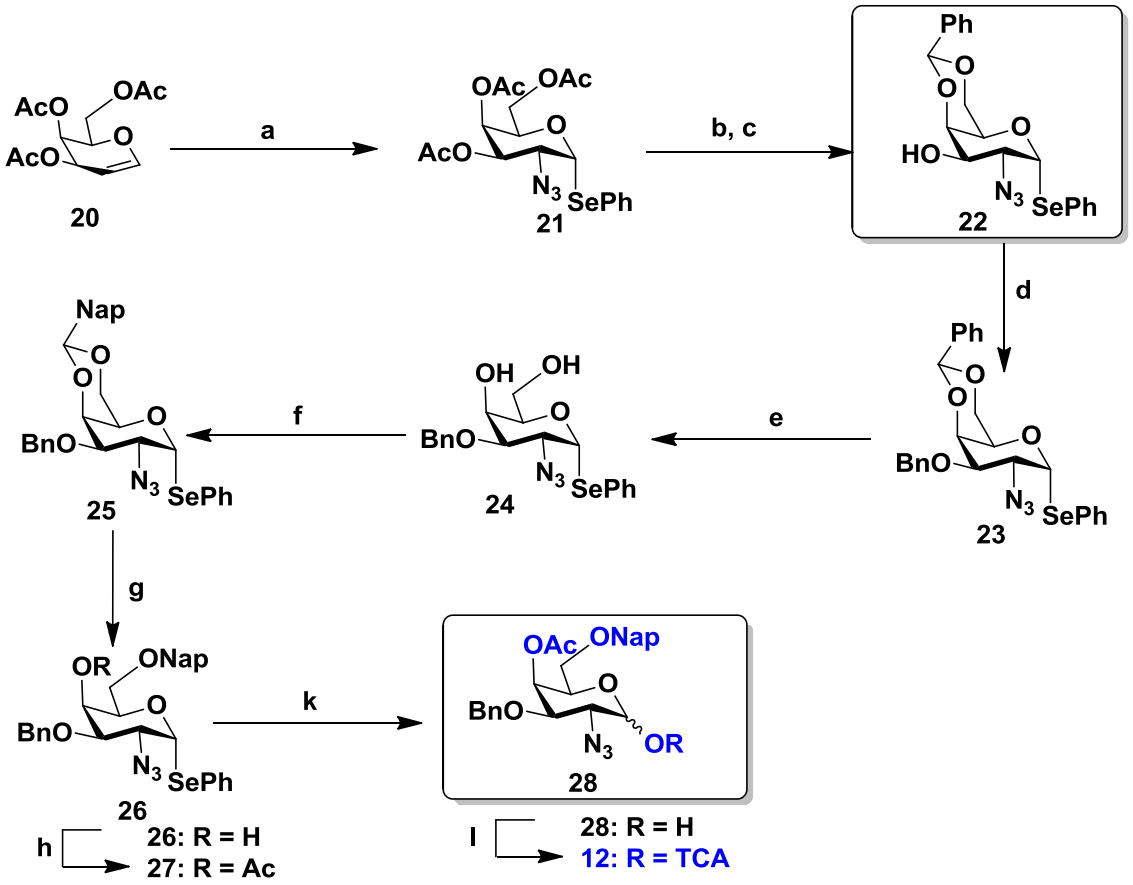
Scheme 3.1: Retrosynthetic analysis of the pentasaccharide **2**

The repeating pentasaccharide contains two α-linkages and C2 positionof galactose moieties have *N*-acetamido functionality. Hence, azide groups have been chosen as non-participating functionalities to yield stereoselective glycosidic linkages, and to reduce azides to generate *N*-acetamido functionalities toward the end of the synthesis. Replacing All protective group at C5 position of **14** with TBS required a different synthetic route for accomplishing **14**, and investigating glycosylation conditions

for the efficient synthesis of ribitol containing disaccharide **39**. Benzoyl group was chosen at C2 position of thioglucoside donor **10** to assist in neighboring group participation and to minimize orthoester formation to achieve β-linked disaccharide **8**. *N*-phenyl trifluoroacetimidate donor **12** with remote participating C4-*O*-Ac functionality was employed for achieving good α-selectivity in synthesis of trisaccharide **9.** This synthetic strategy also employed a common building block **22** for the synthesis of galactose donor **12** and galactose acceptor **13**.

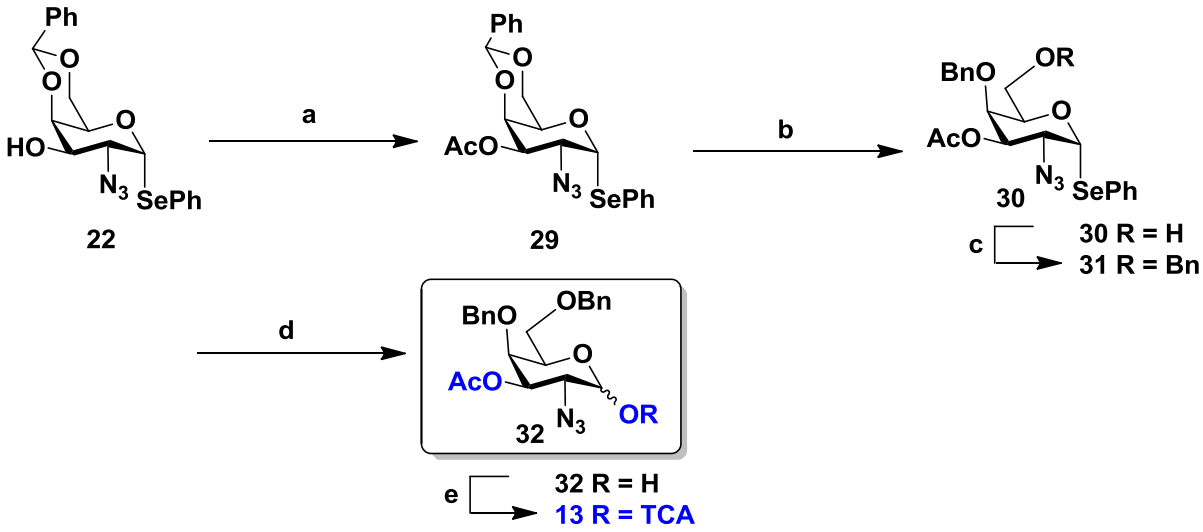
Synthesis of building block **12** was initiated from the commercially available 3,4,6-tri-*O*-acetyl-D-galactal **20** (Scheme **3.2**). Treatment of glycal **20** with $TMSN_3$ and Ph_2Se_2 in the presence of $PhI(OAc)_2$ afforded 2-azido galactose **21** in good yields. The advantage of azidophenylselenation over azidonitration is that scale up could be performed effortlessly, and higher yields of 2-azidoglycosides could be obtained. Deacetylation of **21** with sodium methoxide in methanol followed by treatment with benzaldehyde dimethyl acetal and catalytic quantity of camphorsulfonic acid yielded the 4,6-*O*-bezylidene building block **22**. This intermediate **22** is a common building block for the synthesis of compounds **12** and **13**. Benzylation of **22** employing sodium hydride (NaH) and BnBr in DMF yielded compound **23** in 87% yield. Higher yields were obtained in compounds with anomeric SePh over anomeric TDS. Benzylidene **23** was hydrolyzed by employing 70% AcOH followed by the treatment of the resulting triol with 2-naphthaldehyde and a catalytic quantity of camphorsulfonic acid generated 2-naphthylmethylene actetal derivative **25.** Regioselective ring opening of 4,6-*O*-acetal under Et_3SiH and TfOH conditions generated C6-*O*-Nap galactose moiety **26** which was acetylated to afford compound **27**. Senenoglycoside **27** was hydrolysed into hemiacetal

28 by treating with $HgCl_2$ and $CaCO_3$ followed by a reaction of resulting alcohol with trichloroacetonitrile and a hindered base DBU to afford the required 2-azido galactose donor **12**.



Scheme 3.2: Synthesis of 2-azido galactose donor **12.** Reagents and reaction conditions: (a) Ph_2Se_2, $PhI(OAc)_2$, $TMSN_3$, CH_2Cl_2, 70%; (b) NaOMe, MeOH, quantitative; (c) CSA, CH_3CN, $PhCH(OMe)_2$, 75%; (d) NaH, BnBr, DMF, 87%; (e) 70% AcOH in H_2O; (f) CSA, CH_3CN, 2-naphthaldehyde, 80%; (g) EtSiH, TfOH, -78 ^oC, CH_2Cl_2, 75%; (h) Py, Ac_2O, 90%; (i) $HgCl_2$, $CaCO_3$, CH_3CN, 85%; (j) CCl_3CN, DBU, CH_2Cl_2, 85%.

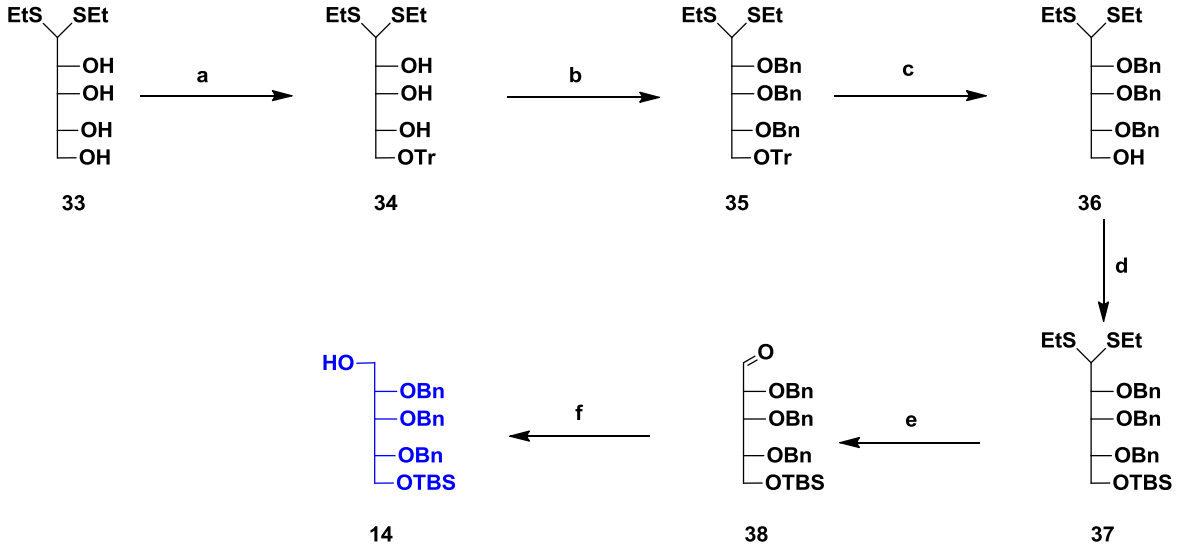
Previously synthesized intermediate **22** was acetylated followed by reductive ring opening of 4,6-*O*-benzyledene acetal under BH_3THF-Bu_2BOTf conditions in tetrahydrofuran (THF) generated C4-*O*-Bn galactose moiety **30.** Benzylation of **30** by treating with sodium hydride (NaH) and BnBr and subsequent hydrolysis of anomeric Seph functionality followed by trichloroacetimidate formation yielded glycosyl donor **13** (Scheme **3.3**).



Scheme 3.3: Synthesis of 2-azido galactose donor **13.** Reagents and reaction conditions: (a) py, Ac_2O, 80%; (b) BH_3THF, Bu_2BOTf, THF, 0 ^oC - rt, 80%; c) Ag_2O, BnBr, CH_2Cl_2, 55%; (d) $HgCl_2$, $CaCO_3$, CH_3CN, 85%; (e) CCl_3CN, DBU, CH_2Cl_2, 85%.

D-Ribitol acceptor was synthesized from commercially available D-ribose. D-Ribose was converted into the diethyl dithioacetal **33**[10] following the reported procedure and subsequently the primary hydroxy group at C5 was selectively protected with trityl chloride (TrCl) to obtain compound **34**.[11,12] Benzylation of 2,3,4-triol **34** with sodium hydride (NaH) and excess BnBr in DMF, and subsequent removal of trityl protective group generated the C1 hydroxyl compound **36**. C1 hydroxyl group in **36** was silylated

followed by hydrolysis of dithioacetal **37** by treating with NBS and 2,6- lutidene generated the aldehyde **38**. The relatively less stable primary aldehyde **38** was reduced to ribitol acceptor **14** by treating with $NaBH_4$ in 60% yield (Scheme **3.4**).



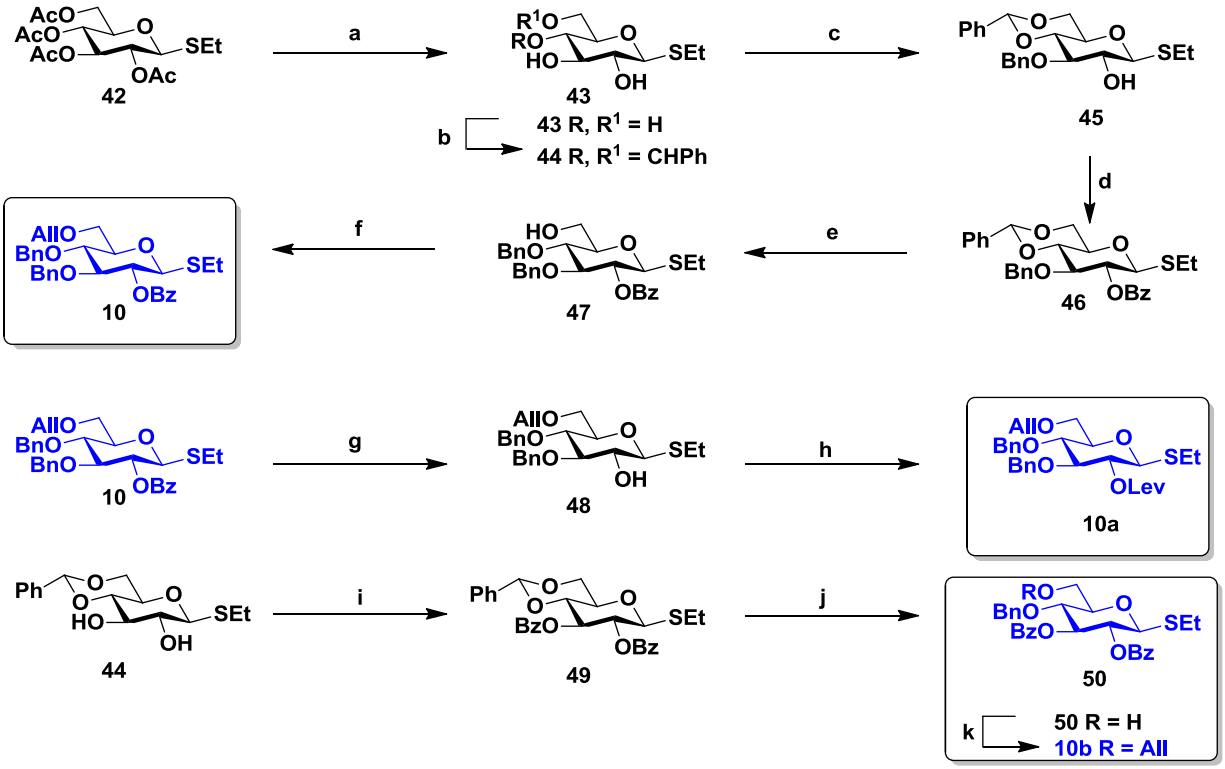
Scheme 3.4: Synthesis of ribitol acceptor **14.** Reagents and reaction conditions: (a) TrCl, DMAP, py, 80%; (b) NaH, BnBr, DMF, 80%; (c) TES, TFA, CH_2Cl_2, 60%; (d) Imidazole, TBSCl, DMF, 83%; (e) 2,6-lutidene, NBS, acetone: water (6:1), 80%; (f) $NaBH_4$, MeOH, 6 h, 60%.

Synthesis of glucose donor **10** was initiated from the known glucose derivative **44** by employing few modifications to existing scheme **3.4**.[13-16] Compound **45** was synthesized by regioselective benzylation of the stannylidene derivative of thioglucopyranoside **44.** C2 hydroxyl group in compound **44** was benzoylated using benzoyl chloride (BzCl) and catalytic DMAP in 82% yield. Reductive ring opening of benzylidene acetal **46** by treating withEt_3SiH and phenyldichloroborane ($PhBCl_2$) in CH_2Cl_2 yielded C4-*O*-Bn moiety **47** in 80%.[17] Regeoselective ring opening reaction of **46**

by employing $PhBCl_2$ generated better yields over employing BH_3THF, Bu_2BOTf mediated reaction conditions. Allylation of C6 hydroxyl group in **47** by treating with allyl bromide (AllBr) and equimolar sodium hydride (NaH) at low temperatures generated the desired glycosyl donor **10** in 70% yield.

Two hydroxyl functionalities in diol **45** were benzoylated with excess benzoyl chloride (BzCl) and catalytic DMAP, and subsequent reductive ring opening of benzylidene acetal **49** with phenyldichloroborane followed by allylation of C6 alcohol by reaction with allyl bromide generated the glycosyl donor **10b** in good yields. Glycosyl donor **10** was debenzoylated by treating with NaOMe in a mixture of MeOH and CH_2Cl_2 at 40 °C, and subsequent protection of C2 hydroxyl group in **48** with levulinic acid employing EDC.HCl as a coupling agent afforded glycosyl donor **10a** in decent yields. Donor **10b** was synthesized from the known C6 hydroxyl compound **50**[18] by treating with sodium hydride and allyl bromide.

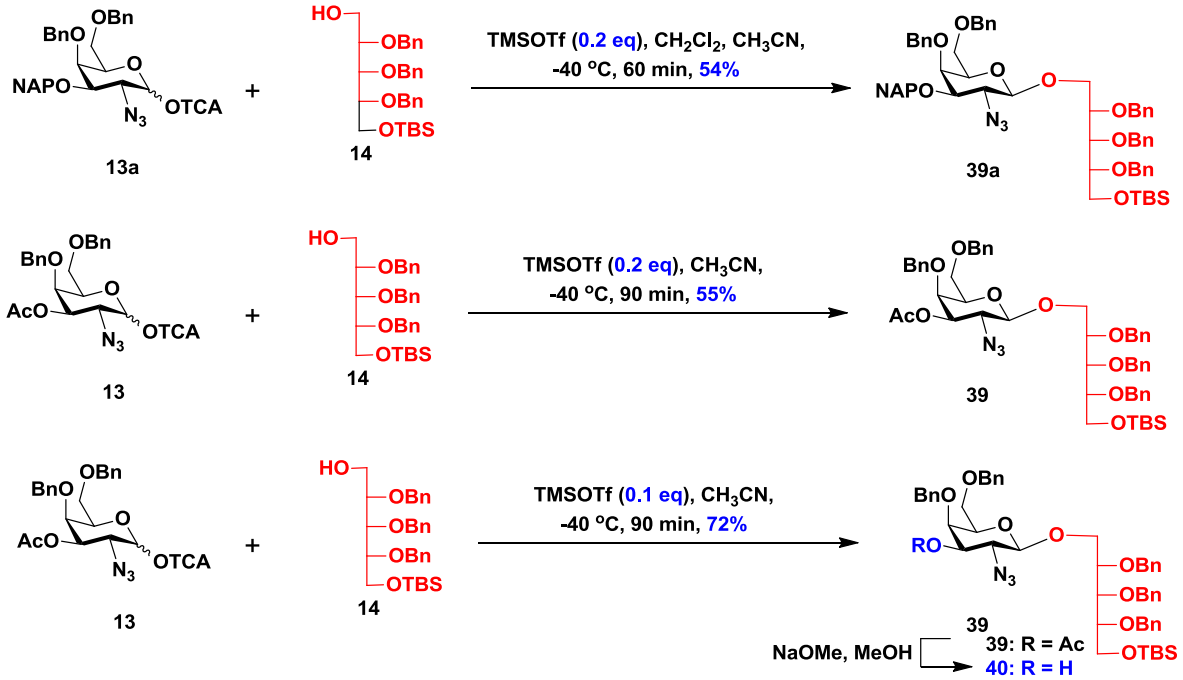
With the required building blocks in hand, assembling building blocks into disaccharides and trisaccharides was initiated by following the retrosynthetic plan (Scheme **3.1**). For the synthesis of ribitol containing disaccharides **39** and **39a,** two galactosamine donors with 3-*O*-Nap **13a** and 3-*O*-Ac **13**[19] protective groups were synthesized. This synthetic strategy took advantage of nitrile effect by employing acetonitrile as solvent and performing reactions at lower temperatures because of lack of neighboring group assisting functionality in donors. Glycosylation (Scheme **3.5**) of 3-*O*-Nap protected galactosamine donor **13a** with ribitol acceptor **14** in acetonitrile, and a minimum amount of DCM to solubulize the acceptor and donor, with 0.2 equiv. of TMSOTf activator produced the pure β-disaccharide **39a** in 54% yield. When 3-*O*-Ac-



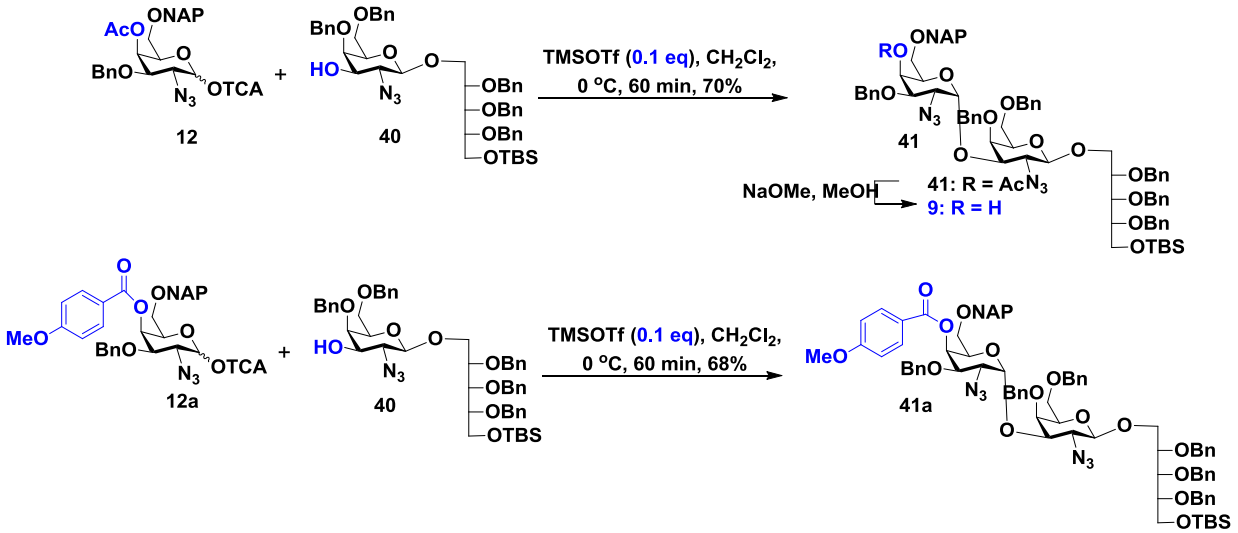
Scheme 3.5: Synthesis of glucose donors **10, 10a** and **10b.** Reagents and reaction conditions: (a) NaOMe, MeOH, 93%; (b) $PhCH(OMe)_2$, CH_3CN, CSA, 85%; (c) Bu_2SnO, MeOH, CsF, BnBr, DMF, 60%; (d) BzCl, DMAP, py, 82%; (d) BzCl, DMAP, py, 82%; (e) Et_3SiH, $PhBCl_2$, CH_2Cl_2, 80%; (f) AllBr, NaH, DMF, 0 °C, 70%; (g) NaOMe, MeOH:CH_2Cl_2, 40 °C; (h) LevOH, EDC.HCl, DMAP, CH_2Cl_2, 70%; (i) BzCl, DMAP, py, 80%; (j) Et_3SiH, $PhBCl_2$, CH_2Cl_2, 66%; (k) AllBr, NaH, DMF, 0 °C, 68%.

protected galactosamine donor **13** was employed, the disaccharide **39** yields were improved to 55%. It was also noticed that the TBS group on ribitol in disaccharide was falling off by employing 0.2 equiv. of activator. Hence, glycosylation of donor **13** and acceptor **10** was performed with 0.1 equiv. of TMSOTf. It was observed that the glycosylation yields were improved to 72%. However, it should be noted that yields in glycosylations employing TBS protected ribitol **10** are 15% less when allyl protected ribitol acceptor was used as an acceptor.

Disaccharide **39** was deacetylated with NaOMe to yield the desired disaccharide acceptor **40** in higher than 90% yields. After synthesizing the disaccharide acceptor **40** and galactosamine donor **12**, reaction conditions were investigated for the synthesis of trisaccharide **41** with high α-selectivity. Boons and coworkers have reported that α-selectivity in glycosylations employing galactosyl donors can be significantly improved by employing a neighboring group participating-functionality at C4 hydroxyl group.[20,21] It was reported that the best yields were obtained when ester functionalities bearing electron donating functionality (e.g. *P*-H_3CO-Ph-CO-OR) were employed. The glycosylation of disaccharide acceptor **40** with 4-*O*-Ac protected trochloroacetimidate donor **12** in the presence of 0.1 equiv. of TMSOTf at 0 ^oC in dichloromethane (CH_2Cl_2) furnished the required trisaccharide **41** in 70% yields (Scheme **3.6**). We have also attempted the same glycosylation with 4-*O*-anisoyl protected glycosyl donor **12a** and acceptor **40** which yielded similar results.



Scheme 3.6: Synthesis of disaccharides **39** and **39a**

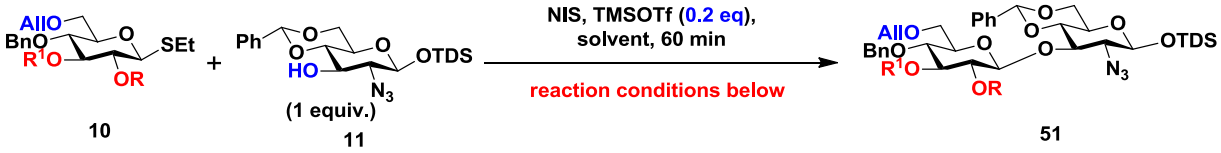


Scheme 3.7: Synthesis of trisaccharides **9, 41** and **41a**

However, it was observed that deprotection of 4-*O*-anisoyl group was cumbersome to achieve higher yields of trisaccharide acceptor **9**. Hence, 4-*O*-Ac protected trisaccharide **41** was deacetylated in excellent yields to obtain the required trisaccharide acceptor **9**.

After accomplishing the synthesis of trisaccharide acceptor **9**, various glycosylation conditions (Scheme **3.7**) and different protective groups at C2 hydroxyl group and C3 hydroxyl group were investigated for the efficient synthesis of disaccharide **51** (Table **3.1**). Glycosylation of acceptor **11** with glycosyl donor **10** with C2 benzoyl functionality in the presence of NIS and TMSOTf at -20 ^{o}C in CH_2Cl_2 furnished the required disaccharide **51** in 52% yield (Table **3.1**, entry **1**). Glycosylation yields improved up to 55% in second attempt. Glycosylation yields, under same conditions, did not improve when dibenzoyl protected donor 1**0b** was employed, and yields also went down when C2 levulinic ester donor **10a** was employed. However yields were improved

up to 65% when a large scale reaction was performed using benzoyl protected donor in the presence of NIS and TMSOTf at 0 ^{o}C.

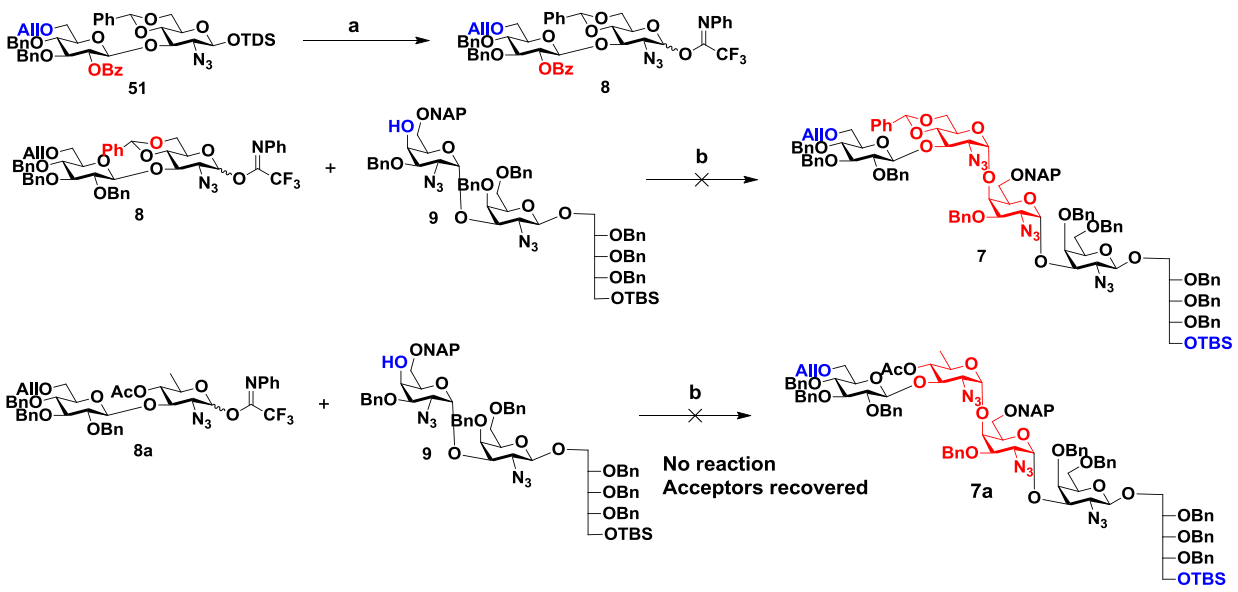


Scheme 3.8: Synthesis of disaccharide **51**

Table 3.1: Glycosylation conditions for the synthesis of disaccharide **51**

R	R^1	Donor (equiv.)	Solvent, Reaction Conditions	Temp	Yield (**51**)
Bz (**10**)	Bn	1.2	CH_2Cl_2	-20 ^{o}C	52%
Bz (second attempt, **10**)	Bn	1.2	CH_2Cl_2	-20 ^{o}C	55%
Lev (**10a**)	Bn	1.2	CH_2Cl_2	-20 ^{o}C	50%
Bz (**10b**)	Bz	1.7	CH_2Cl_2	-20 ^{o}C	60%
Bz (**10**)	Bn	1.5	CH_2Cl_2:CH_3CN (1:1)	-20 ^{o}C	55%
Bz (**10**)	Bn	1.7	CH_2Cl_2 (large scale)	0 ^{o}C	65%
Bz (**10**)	Bn		BSP, Tf_2O, DTBMP (small scale)	-60 ^{o}C	75%

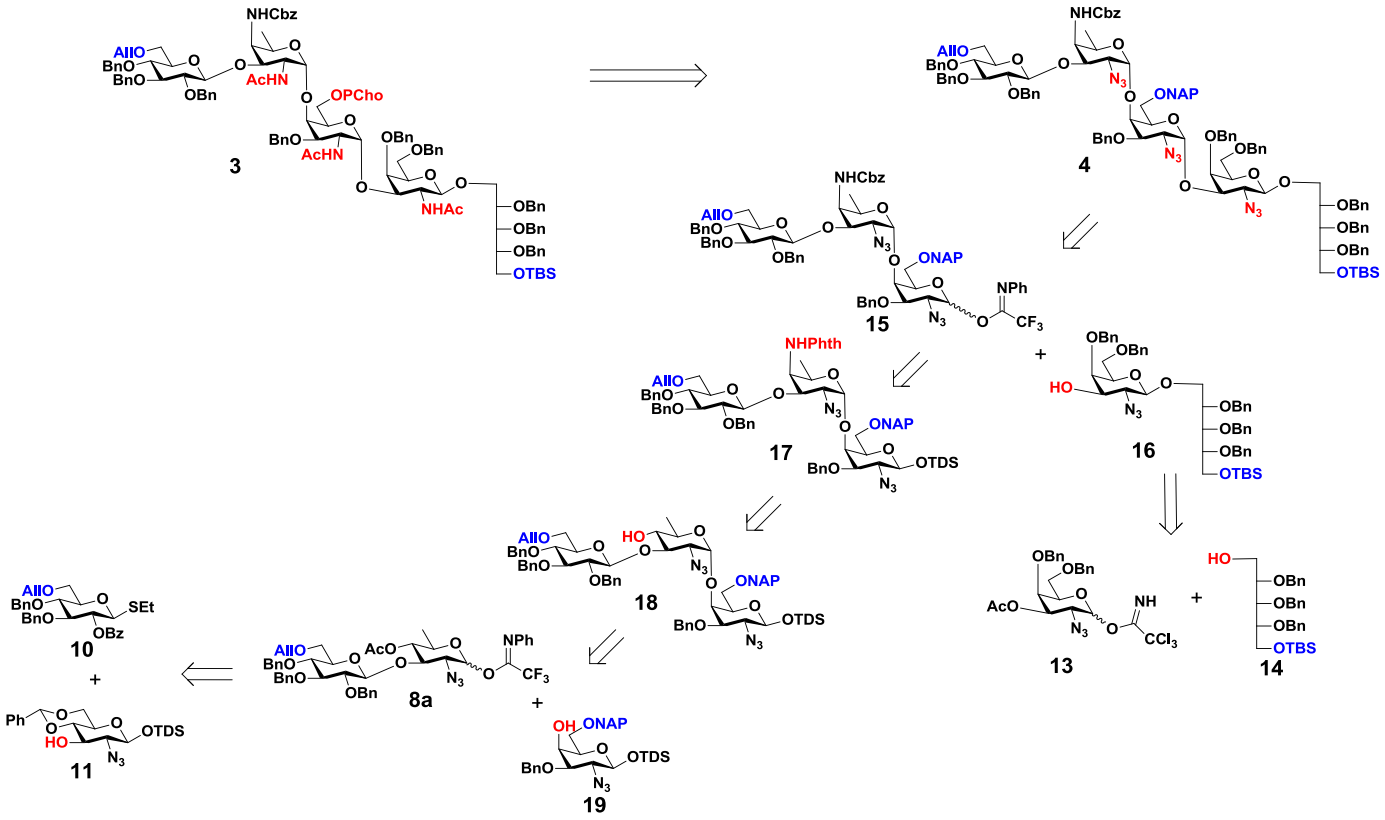
Higher yields of 70% were achieved when smaller quantities of thioglycoside **10** was preactivated with 1-benzenesulfinyl piperidine (BSP) and triflic anhydride (Tf_2O) in the presence of 2,4,6-tri-*tert*-butylpyrimidine (TTBP), and the resulting glycosyl triflate intermediate was treated with acceptor **11** at -60 ^{o}C.[22]



Scheme 3.9: Attempted synthesis of pentasaccharides **7** and **7a.** Reagents and reaction conditions: (a) 1) NaOMe, MeOH, CH_2Cl_2, 90%; 2) BnBr, NaH, DMF, 0 °C, 80% 3) 70% HF/py, THF, 80%; 4) $F_3CC(NPh)Cl$, Cs_2CO_3, CH_2Cl_2, 69%; (b) TMSOTf (0.1 equiv.), CH_2Cl_2, 0 ^{o}C, 60 min., no reaction.

In order to attempt a pentasaccharide **7** glycosylation (Scheme **3.8**), we required the disaccharide trichloroacetimidate donor **8**. Synthesis of donor **8** was accomplished by removing the anomeric silyl group with 70% HF/py and subsequent *N*-phenyl trifluoroacetimidate formation in 69%. Glycosylation of disaccharide donor **8** with trisaccharide acceptor **9** with 0.1 equiv. of promoter TMSOTf in dichloromethane at 0 ^{o}C did not yield any amount of pentasaccharide **7**.

Then we synthesized the 6-deoxy disaccharide donor **8a** (reported in the later part of this chapter) to investigate the effect of electron rich deoxy sugar moiety. It was disappointing to notice that no amount of pentasaccharide was obtained in the glycosylation of 6-deoxy donor **8a** with trisaccharide acceptor **9**. Trisaccharide acceptor **9** was mostly unreactive and was recovered. This lack of reactivity could be due to steric crowding of C4 hydroxyl group by C3 and C6 protective groups, and Nap functionalaity getting pushed onto the top face of galactosamine acceptor **9** by anomeric disaccharide on reducing end.[23]

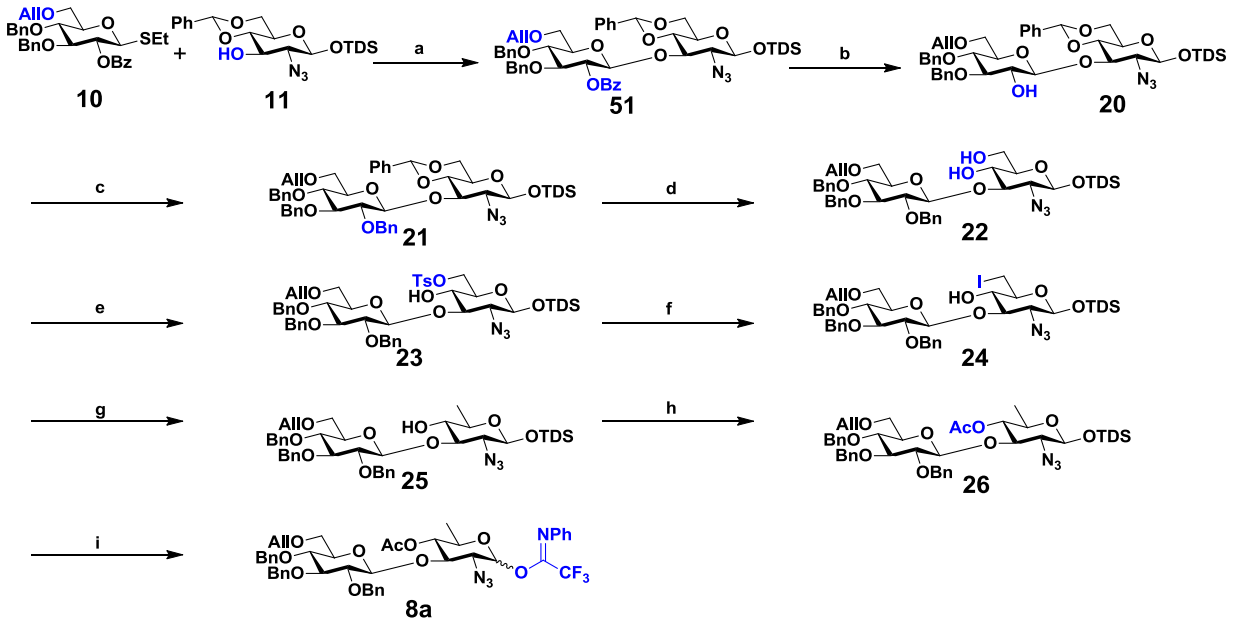


Scheme 3.10: Modified retrosynthetic analysis of pentasaccharide **3**

Due to difficulties in achieving the synthesis of the pentasaccharide **3** through a convergent [2+3] glycosylation strategy which used sterically crowded trisaccharide acceptor **9**, it was decided to employ monosaccharide acceptor **19** to afford the

synthesis of trisaccharide donor **18**. Thus, a modified synthetic strategy [2+1+2] (Scheme **3.9**) was employed to avoid the use of sterically crowded trisaccharide acceptor. Hence attention was paid to transform 4,6-*O*-benzylidene into 6-deoxy sugar at disaccharide stage **8a** instead on pentasaccharide stage to have a minimum number of chemical manipulations on the expensive pentasaccharide **7**. Since AAT building block needs an orthogonal *N*-protection at C4 position in **17**, it was chosen to carry out the inversion of C4 hydroxyl group by treating with potassium phthalimide which would subsequently be deprotected and converted into a Cbz functionality.[24] Previous synthetic efforts have already led to the synthesis of building blocks **8a**, **19**, **13**, **14** and **16**. Therefore, efforts were made to convert the disaccharide **51** into a 6-deoxy disaccharide donor **8a**. Glycosylation conditions were also examined for the synthesis of 6-deoxy trisaccharide **18**.

Debenzoylation of disaccharide **51** with sodium methoxide in a mixture of methanol and dichloromethane at 40 ^{o}C yielded C2 hydroxyl moiety **20** in 90% yield and it was subsequently benzylated to generate compound **21** (Scheme **3.10**). Hydrolysis of 4, 6-*O*-benzylidene acetal **21** with ethanethiol (EtSH) and *p*-toluenesulfonic acid (PTSA) followed by regeoselective tosylation with 1.1 equiv. of TsCl yielded C6-*O*-tosylate **23 in** high yields. C6-*O*-tosylate **23** was transformed into iodide **24** by refluxing with sodium iodide (NaI) in 2-butanone in excellent yields. Efforts to deoxygenate C6-iodo compound **24** into 6-deoxy sugar **25** by radical mediate conditions using AIBN/Bu_3SnH were largely unsuccessful and not reproducible.

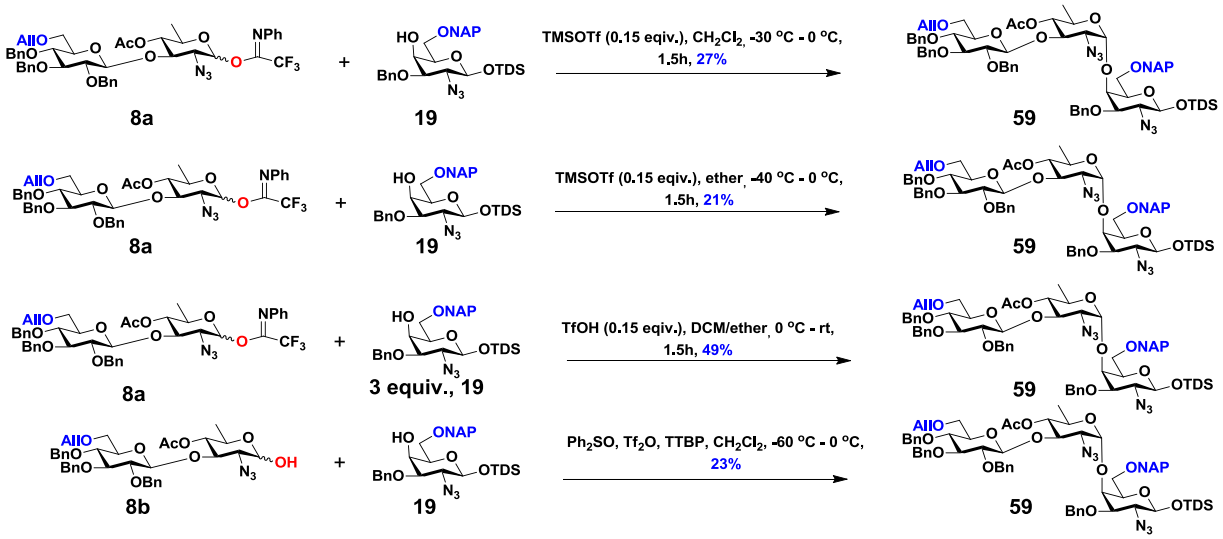


Scheme 3.11: Synthesis of disaccharide donor **8a.** Reagents and reaction conditions: (a) NIS, TMSOTf, CH_2Cl_2, 0 ^oC, 65%; (b) NaOMe, MeOH:CH_2Cl_2, 40 ^oC, 24 h, 90%; (c) NaH, BnBr, DMF, 0 ^oC, 80%; (d) EtSH, TsOH,CH_2Cl_2, 74%; (e) Py, TsCl, DMAP, Et_3N, 88%; (f) NaI, MEK, 85 ^oC, 82%; (g) $NaBH_3CN$, diethyleneglycol diethyl ether, 70%; (h) Py, Ac_2O, 2h, 89%; (i) 1) 70% HF/Py, Py, 78%; 2) $F_3CC(NPh)Cl$, Cs_2CO_3, CH_2Cl_2, 70%.

However, reduction of **24** using a milder reducing agent $NaBH_3CN$ in diethyleneglycol diethylether in refluxing conditions yielded the required compound **25** in 70% yield.[25] $NaBH_4$ was not chosen as a reducing agent to avoid partial reduction of the azide functionality. Subsequently C6-deoxy sugar was acetylated to generate compound **26** which was desilylated followed by *N*-phenyl trifluoroacetimidate formation afforded the desired disaccharide donor **8a** in 70% yields.

After synthesizing the required building blocks **8a**, **8b** and **19**, various glycosylation conditions were investigated for the efficient synthesis of trisaccharide **59**

(Scheme **3.11**). Initial glycosylation attempts of disaccharide donor **8a** with galactosamine acceptor **19** with 0.15 equiv. of TMSOTf promoter in dichloromethane at -30 ^{o}C yielded 27% of trisaccharide **59**. Changing the solvent system from dichloromethane to ether led to a decreased yield of 21%. However, increasing the number of equivalents of acceptor **19** to 3 equiv., changing the promoter to triflic acid (TfOH) and running the reaction at 0 ^{o}C afforded trisaccharide **59** in better yields of 49%.

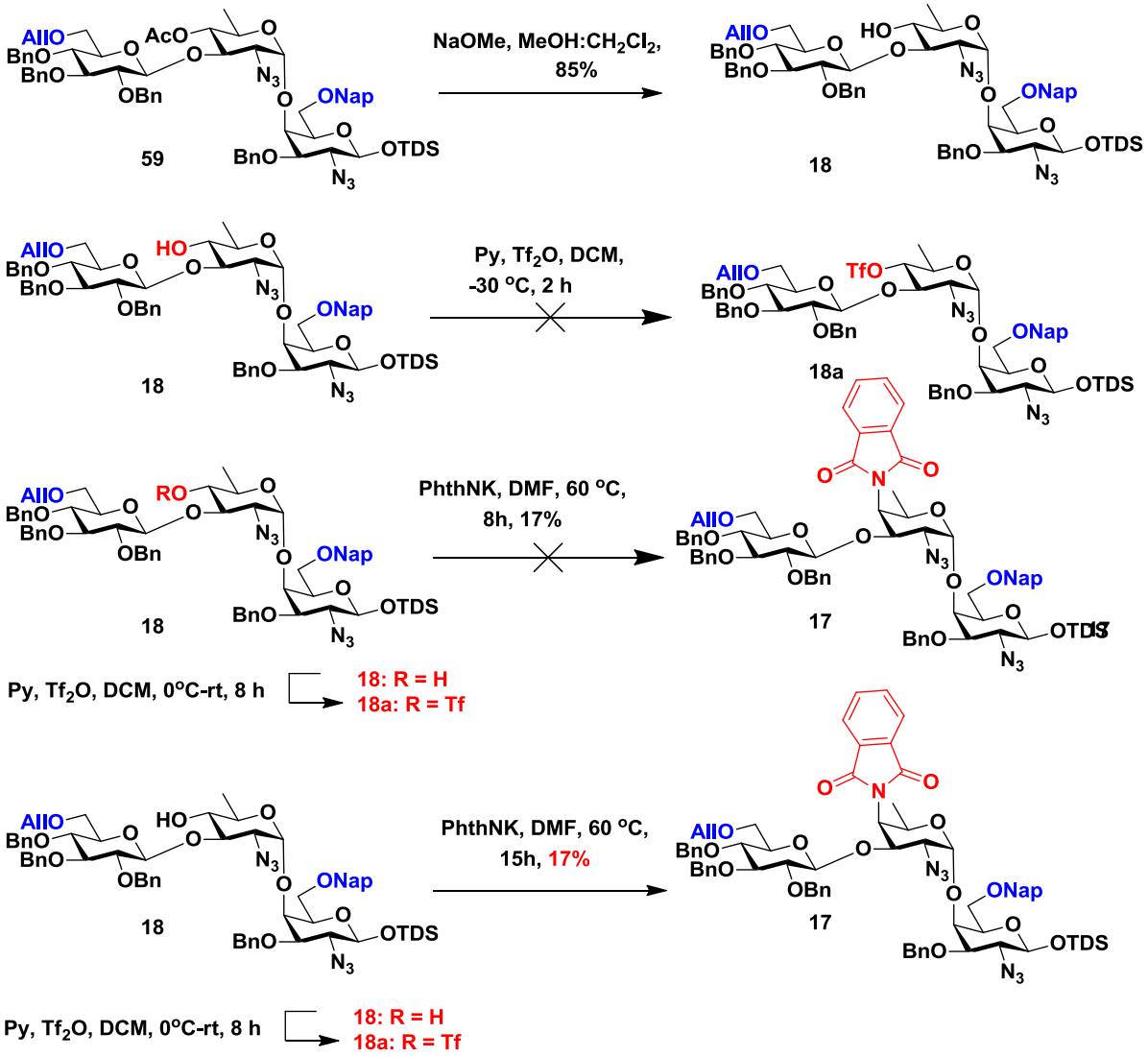


Scheme 3.12: Synthesis of trisaccharide **59**

However, it was disappointing to observe a decreased yield of 23% by following the dehydrative glycosylation conditions (donor **8b** and acceptor **19**) in the presence of diphenyl sulfoxide, triflic anhydride and tri-*tert*-butylpyrimidine (TTBP).[26-28]

We chose triflation followed by inversion of C4-OH with potassium phthalimide to introduce an orthogonal *N*-protection on trisaccharide **59** (Scheme **3.12**).[24] To introduce an orthogonal *N*-protection on trisaccharide **59,** trisaccharide **59** was deacetylated to

yield the C4 hydroxyl compound**18** in 85% yield**.** Efforts to triflate C4 hydroxylof **18** with pyridine and triflic anhydride (Tf_2O), DCM at -30 ^oC were unsuccessful**.** Hence, the same reaction was repeated by the addition of triflic anhydride (Tf_2O) in excessive pyridine and DMAP at 0 ^oC, and running the reaction at room temperature to yield the unstable triflate **18a**. Initial attempt to substitute triflate with potassium phthalimide (PhthK) were also unsuccessful. However, the third attempt of the same inversion reaction yielded the required trisaccharide **17** in 17% yield.



Scheme 3.13: Synthesis of trisaccharide **17**

3.3 Conclusions

Synthetic zwitterionic polysaccharides (ZPSs) with well characterized structures and with different number of repeating units can help immensely in furthering our knowledge on their mode of action. Unfortunately the complete synthesis of the pentasaccharide **2** was not accomplished. However, appropriate building blocks (**10**, **11**, **12**, **13**, **14** and **19**) have been synthesized in good yields. High yielding glycosylation conditions were narrowed down for synthesis of ribitol containing disaccharide **39,** glucose disaccharide **51** and trisaccharide **41.** It is disappointing to report that pentasaccharide synthesis could not be accomplished by coupling disaccharide donor **8** and sterically hindered trisaccharide acceptor **9**. Recent reports have also shown that sterically hindered C4 galactosyl acceptors are very poor nucleophiles.[23] Efficient synthetic strategy was developed for the synthesis of 6-deoxy disaccharide donor **8a** which required many synthetic manipulations at disaccharide stage.

Having obtained poor yields in the inversion of trisaccharide **18**, it would be beneficial to carry out inversion at disaccharide stage **25** and to proceed with [2+1+2] glycosylation strategy to accomplish the synthesis of **2**. It is our belief that this new strategy should lead to an efficient synthesis of pentasaccharide repeating unit **2**. Because of use of different orthogonal protective groups and anomeric leaving groups, our synthetic strategy is going to be different from the reported synthesis of similar polysaccharide.[29]

3.4 Experimental Section

General procedures: 1H and 13C NMR spectra were recorded on a 300 MHz, 500 MHz or a 600 MHz spectrometer. Chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane (TMS) as the internal standard. NMR data is presented as follows: Chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, dd = doublet of doublet, m = multiplet and/or multiple resonances), coupling constant in Hertz (Hz), integration. All NMR signals were assigned on the basis of 1H NMR, 13C NMR, GCOSY and GHSQC experiments. Mass spectra were recorded on a MALDI-TOF mass spectrometer. The matrix used was 2,5-dihydroxy-benzoic acid (DHB) and Ultramark 1621 as the internal standard. Column chromatography was performed on flash silica gel G60 (Silicycle, 60-200 μm, 60 Å). TLC-analysis was conducted on Silicagel 60 F254 (EMD Chemicals inc.) with detection by UV-absorption (254nm) were applicable, and by spraying with 20% sulfuric acid in ethanol followed by charring at ~150 ^{o}C or by spraying with a solution of $(NH4)_6Mo_7O_{24}.H_2O$ (25 g/L) in 10% sulfuric acid in ethanol followed by charring at ~150 ^{o}C. CH_2Cl_2 was freshly distilled from calcium hydride under nitrogen prior to use. Molecular sieves (4Å) were flame activated under vacuum prior to use. All other dry solvents were purchased from Aldrich and directly used in reactions. All reactions were carried out under an argon atmosphere.

Phenyl 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-1-seleno-α-D-galactopyranoside (21): To a solution of compound **20** (5g, 18.37 mmol) in CH_2Cl_2 (100 mL) was added diphenyl diselenide (4.016 g, 12.87 mmol) and $PhI(OAc)_2$ (5.918 g, 18.37 mmol) at -40 ^{o}C followed by the drop by drop addition of $TMSN_3$ (4.83 ml, 36.75 mmol) at the same temperature . The resulting reaction mixture was stirred at -40 ^{o}C for 2 h and warmed to

ambient temperature and stirred for the next 12 h. The reaction mixture was concentrated under reduced pressure and flash column silica gel chromatography of the resulting residue using EtOAc/ hexanes (1/5 to 1/4, v/v) afforded α-anomer of title compound **21** (6.05 g, 40%) as a dark yellow solid. Data matches with reported data in literature. [30]

Phenyl 3-*O*-acetyl-2-azido-4,6-benzylidiene-2-deoxy-1-seleno-α-D-galactopyranoside (22): To a solution of compound **21** (8 g, 16.9 mmol) in MeOH (70 mL) was added NaOMe/ MeOH (3 mL, 1 M solution) until the reaction mixture reached a pH = 9 and stirred for 2 h at room temperature. The resulting reaction mixture was neutralized with amberlite H^+-resin, filtered and the filtrate was concentrated under reduced pressure to yield the required triol (5.56 g, 16.1 mmol). To a solution of the resulting triol (6g, 17 mmol) in CH_3CN (50 mL) was added benzaldehyde dimethyl acetal (7.49 mL, 51 mmol) and CSA (394 mg, 1.7 mmol) and stirred for 12 h under mild vacuum. The reaction mixture was concentrated under reduced pressure and the resulting residue was diluted with ethyl acetate (250 mL) and washed with saturated $NaHCO_3$ (30 mL). The aqueous layer was back-extracted with ethyl acetate (3 × 30 mL), and the combined organic layers were washed with water (30 mL) and brine (30 mL), dried (Mg_2SO_4), filtered and the filtrate was concentrated under reduced pressure. Flash column chromatography of the resulting residue using EtOAc/ hexanes (1/5 to 1/3, v/v) yielded title compound **22** (5.52 g, 76%). ^1H NMR (300 MHz, $CDCl_3$): δ 7.59-7.57 (m, 2H, CH aromatic), 7.51-7.49 (m, 2H, CH aromatic), 7.40-7.39 (m, 3H, CH aromatic), 7.30-7.26 (m, 3H, CH aromatic), 6.03 (d, 1H, H1) 5.60 (s, 1H, C*H* benzylidene), 4.29 (d, 1H, H4), 4.18-4.12 (m, 3H, H2, H5, H6), 4.07 (dd, 2.0, 1H, H6), 3.92 (dd, *J* = 10.4, 3.6,

1H, H3), 2.41 (bs, 1H, OH). 13C NMR ($CDCl_3$, 75 MHz): δ 137.1, 133.7, 129.4, 129.2, 128.9, 128.3, 127.7, 126.2, 101.3, 85.2, 74.9, 70.6, 69.0, 65.0, 61.9.

Phenyl 2-azido-3-*O*-benzyl-4,6-*O*-benzylidiene-2-deoxy-1-seleno-α-D-galactopyranoside (23): To a solution of compound **22** (5.52 g, 12.7 mmol) in DMF (70 mL) at 0 ^oC was added benzyl bromide (2.2 mL, 17.78 mmol) and 60% NaH (15.24 mmol) successively. The resulting reaction mixture was stirred for two hours at 0 ^oC and the excess of NaH was quenched by the addition of ice cubes. The resulting reaction mixture was concentrated under reduced pressure, diluted with EtOAc (200 mL) and washed with H_2O (2 × 30 mL). The combined organic layers were dried ($MgSO_4$), filtered, concentrated under reduced pressure. Flash column silica gel chromatography of the resulting residue using EtOAc/ hexanes (1/5, v/v) afforded title compound **23** (5.76 g, 87%). ^1H NMR (300 MHz, $CDC1_3$): δ 7.20-7.60 (m, 15H, CH aromatic), 6.05 (d, lH, H1), 5.50 (s, lH, C*H* benzylidene), 4.80 (dd, 2H, C*H2* Bn), 4.40 (dd, IH, H2), 4.25 (d, 1H, H4), 3.95-415 (m, 3H, H5, H6a,H6b), 3.80 (dd, lH, H3).

Phenyl 2-azido-3-*O*-benzyl-2-deoxy-1-seleno-α-D-galactopyranoside (24): Compound **23** (5.76 g, 11.03 mmol) was dissolved in a mixture of AcOH and H_2O (7:3, 30 mL) and the reaction mixture was stirred at 70 ^oC for 4 h. The resulting reaction mixture was concentrated under reduced pressure and the resulting residue was purified with flash column silica gel chromatography using MeOH/$CHCl_3$ (1:9, v/v) to afford title compound **24** (4.75g, 95%) which is a white solid. ^1H NMR (300 MHz, $CDCl_3$): δ 7.7 – 7.5 (m, 2H, CH aromatic), 7.5 – 7.1 (m, 9H, CH aromatic), 6.0 (d, *J* = 5.4 Hz, 1H, H1), 4.7 (q, *J* = 11.3 Hz, 2H,CH_2 Bn), 4.3 – 4.2 (m, 2H, H2, H5), 4.1 (dd, *J* = 3.1, 1.4 Hz, 1H, H4), 3.9 (dd, *J* = 11.9, 5.9 Hz, 1H, H6a), 3.7 (dd, *J* = 10.1, 3.2 Hz, 2H, H3,

H6b), 2.8 (s, 1H, OH), 2.0 (s, 1H, OH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 137.0, 135.0, 129.3, 128.9, 128.6, 128.2, 128.2, 127.9, 84.7 (C1), 78.8, 77.6, 77.2, 76.7 (C3), 72.4 (C3 Bn), 72.2 (C2), 67.3 (C4), 62.9 (C6), 60.3 (C5).

Phenyl 2-azido-3-*O*-benzyl-2-deoxy-4,6-*O*-(2-naphthylmethylene)-1-seleno-α-D-galactopyranoside (25): To a solution of compound **24** (4.5 g, 10.34 mmol) in acetonitrile (90 mL) was added 2- naphthaldehyde (2.42 g, 15.5 mmol) and CSA (479 mg, 2.068 mmol) and stirred for 6 h under mild vacuum. The reaction mixture was concentrated under reduced pressure and the resulting residue was diluted with ethyl acetate (200 mL) and washed with saturated $NaHCO_3$ (40 mL). The aqueous layer was back-extracted with ethyl acetate (50 mL), and the combined organic layers were washed with water (20 mL), brine (20 mL), dried (Mg_2SO_4), filtered and concentrated under reduced pressure. Flash column silica gel chromatography of the resulting residue using EtOAc/hexanes (1/5 to 1/3, v/v) yielded compound **25** (4.74 g, 80%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.19 – 7.98 (m, 17H, C*H* aromatic), 5.71 (s, 1H, C*H* NAP acetal), 5.55 (d, 1H, J = 5.3, H1), 4.98 (d, 1H, J = 11.1), 4.83 (d, 1H, J = 11.1), 4.46 (ddd, 1H, J = 9.7, J = 4.8, 5.3), 4.25 (dd, 1H, J = 5.3, 10.1), 3.75–4.01 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 137.7,134.5, 133.7, 133.0, 132.9, 132.5, 129.2, 128.5, 128.4, 28.2,128.0,127.8, 126.6, 126.3, 125.6, 123.7, 101.7 (C1), 87.9, 82.8, 77.9, 77.3, 75.2, 68.7, 63.9, 63.6.

Phenyl 2-azido-3-*O*-benzyl-2-deoxy-6-*O*-(2-naphthylmethyl)-1-seleno-*α*-D-galactopyranoside (26): To a solution of compound **25** (1 g, 1.703 mmol) in CH_2Cl_2 (19 mL) was added 4Å molecular sieves (2 g) and stirred for 1 h at ambient temperature. The resulting reaction mixture at -78 ^{o}C was added Et_3SiH (900 µL, 5.61 mmol) and

TfOH (450 µl, 5.1 mmol) successively. The resulting reaction mixture was stirred for 1 h at -78 ^{o}C, and Et_3N (1.5 mL) and MeOH (1.5 mL) were added, reaction mixture was diluted with $CHCl_2$ (100 mL), filtered, and washed with aqueous $NaHCO_3$ (20 mL) and water (20 mL). The combined organic layers were dried ($MgSO_4$), concentrated under reduced pressure and flash column silica gel chromatography of the resulting residue using EtOAc/hexanes (1/5, v/v) yielded title compound **26** (750 mg, 80%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.8 (dt, *J* = 12.2, 5.6 Hz, 3H, CH aromatic), 7.7 (s, 1H, CH aromatic), 7.6 – 7.6 (m, 2H, CH aromatic), 7.5 – 7.5 (m, 2H, CH aromatic), 7.4 (p, *J* = 7.8 Hz, 5H, CH aromatic), 7.2 – 7.1 (m, 3H, CH aromatic), 5.9 (d, *J* = 5.3 Hz, 1H, H1), 4.8 – 4.6 (m, 5H, 2 × CH2, H1), 4.4 (t, *J* = 5.7 Hz, 1H, H5), 4.3 (dd, *J* = 10.2, 5.3 Hz, 1H, H2), 4.1 (q, *J* = 7.1 Hz, 1H, H4), 3.8 (dd, *J* = 10.3, 5.3 Hz, 1H, H6a), 3.7 – 3.6 (m, 2H, H6b, H3), 2.7 (d, *J* = 1.7 Hz, 1H, OH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 128.3, 127.3, 135.1, 126.4, 126.4, 133.4, 128.8, 128.4, 129.5, 85.4, 79.4, 74.0, 71.5, 66.6, 60.7, 60.6, 54.1.

Phenyl 4-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-6-*O*-(2-naphthylmethyl)-1-seleno-α-D-galactopyranoside (27): A solution of compound **26** (1.15 g, 1.19 mmol) in dry pyridine (10 mL) and acetic anhydride (1 mL) was stirred at room temperature for 1h. Then the mixture was concentrated in vacuo, co-evaporated with toluene and the residue was purified by flash chromatography (1:4 EtOAc/hexane) to yield compound **27** (1.04 g, 87%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.9 – 7.8 (m, 3H, CH aromatic), 7.8 (d, *J* = 1.6 Hz, 1H, CH aromatic), 7.5 – 7.2 (m, 12H, CH aromatic), 5.6 (dd, *J* = 3.4, 1.2 Hz, 1H, H4), 4.7 (dd, *J* = 17.4, 11.6 Hz, 2H, CH_2 Bn), 4.6 (d, *J* = 12.0 Hz, 1H, C*H*H Bn), 4.5 – 4.4 (m, 2H, CH*H Bn*, H1), 3.7 (ddd, *J* = 7.0, 5.8, 1.3 Hz, 1H, H5), 3.6 – 3.4 (m, 3H, H6, H6a, H2), 3.3 (dd, *J* = 10.3, 3.4 Hz, 1H, H3), 2.0 (s, 3H, CH_3 Ac). ^{13}C NMR (75 MHz, $CDCl_3$):

δ 171.0, 128.3, 127.1, 125.0, 128.6, 97.5 (C1), 77.9, 74.1, 73.5, 72.7, 72.2, 70.8, 68.5, 66.1, 65.8, 21.0.

4-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-6-*O*-(2-naphthylmethyl)-α,β-D-galactopyranoside (28): To a solution of compound **27** (210 mg, 0.34 mmol) in a mixture of acetonitrile and water (4.1 mL CH_3CN + 0.9 mL H_2O) was added mercury(II) chloride (295 mg, 1.088 mmol) and calcium carbonate (119 mg, 1.19 mmol). The resulting reaction mixture was stirred at ambient temperature for 6 h, filtered and the resulting filtrate was concentrated under reduced pressure. The resulting residue was diluted with dichloromethane (50 mL), washed with 1 M potassium iodide solution (3 × 10 mL), 30% sodium thiosulfate solution (2 × 10 mL), dried ($MgSO_4$) and concentrated to afford title compound **28** (137 mg, 85%) as a mixture of anomers. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.9 – 7.7 (m, 6H, CH aromatic), 7.5 (ddt, *J* = 9.7, 6.7, 1.5 Hz, 4H, CH aromatic), 7.4 – 7.2 (m, 8H, CH aromatic), 5.6 – 5.6 (m, 1H, H1), 5.5 (dd, *J* = 3.5, 1.0 Hz, OH), 5.3 (s, 1H), 4.8 – 4.6 (m, 4H), 4.4 (dd, *J* = 10.9, 4.2 Hz, 2H), 4.2 – 4.1 (m, 1H), 4.0 (dd, *J* = 10.5, 3.2 Hz, 1H), 3.7 – 3.6 (m, 1H), 3.6 – 3.4 (m, 3H), 3.4 – 3.3 (m, 1H), 2.1 – 2.0 (m, 5H), 1.6 (s, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.4, 137.1, 135.0, 133.3, 128.6, 128.6, 128.5, 128.5, 128.2, 128.2, 128.0, 127.8, 127.1, 127.1, 126.4, 126.4, 126.3, 126.2, 126.1, 126.0, 96.3, 92.5, 77.9, 77.6, 77.2, 76.7, 74.3, 74.0, 73.9, 72.7, 72.0, 71.8, 68.8, 68.3, 68.3, 66.9, 65.7, 63.9, 59.9, 21.0, 20.9, 0.2, 0.2.

4-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-6-*O*-(2-naphthylmethyl)-α,β-D-galactopyranosyl trichloroacetimidate (12): To a solution of compound **28** (140 mg,0.29 mmol) in CH_2Cl_2 (4 mL) was added Cl_3CCN (150 µL, 1.46 mmol) and DBU (8 µL, 0.058 mmol) and stirred for 2 h at ambient temperature. The resulting reaction

mixture was concentrated under reduced pressure. Flash column silica gel chromatography of the resulting residue using EtOAc/ hexanes (1/5, v/v) afforded title compound **12** (154 mg, 85%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.77 (s, 1H, C(N*H*)CCl_3), 7.69-7.29 (m, 12H, CH aromatic), 5.63 (d, *J*= 8.4 Hz, 1H), 5.18 (d, *J*= 12.0 Hz, 1H), 5.13 (d, *J*= 9.6 Hz, 1H), 5.07 (d, *J*= 12.0 Hz, 1H), 4.82 (dd, *J*= 4.0, 6.4 Hz, 1H), 4.21 (dd, *J*= 3.2, 10.0 Hz, 1H), 3.91 (q, *J*= 6.4 Hz, 1H), 3.74 (dd, *J*= 8.8, 10.8 Hz, 1H), 2.01 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 170.4, 161.0, 156.8, 136.5, 128.9, 128.7, 128.5, 97.3, 90.7, 73.3, 71.0, 67.5, 52.2, 21.0, 16.9, 16.8.

Phenyl 3-*O*-acetyl-2-azido-4,6-*O*-benzylidiene-2-deoxy-1-seleno-α-D-galactopyranoside (29): A solution of compound **22** (2.5 g, 5.77 mmol) in a mixture of pyridine (25 mL) and acetic anhydride (20 mL) was stirred for 1 h at ambient temperature. The resulting reaction mixture was concentrated and coevaporated with toluene under reduced pressure, and the resulting residue was purified by flash column silica gel chromatography using EtOAc/hexanes (1/4, v/v) to yield title compound **29** (2.19 g, 80%). ^{1}H NMR (300 MHz, $CDCl_3$): *δ* 7.60–7.28 (m, 10H, C*H* aromatic), 6.09 (d, 1H, *J*=5.2, H1), 5.55 (s, 1H, C*H* benzylidene), 5.07 (dd, 1H, *J*=11.0, *J*=3.4, H3), 4.53 (m, 2H, H4, H6a), 4.11 (m, 3H, H2, H5, H6b), 2.17 (s, 3H, CH_3 Ac). ^{13}C NMR (75 MHz, $CDCl_3$): *δ* 170.3 (O=C Ac), 137.3 (*C* q. benzylidene), 133.9-126.1 (*C*H aromatic), 121.1(SePh), 100.8 (*C*H benzylidene), 84.9 (C1), 72.9, 68.9, 64.7,62.4, 58.3 (C2, C3, C4, C5, C6), 20.9 (*C*H3 Ac). MALDI TOF-MS: $[M+Na]^+$ $C_{21}H_{21}N_3O_5Se$, calcd. 498.07, observed 498.18.

Phenyl 3-*O*-acetyl-2-azido-4,6-di-*O*-benzyl-2-deoxy-1-seleno-α-D-galactopyranoside (31): To a solution of compound **29** (1 g, 2.1 mmol) in CH_2Cl_2 (25

mL) was added 4Å molecular sieves (2 g) and the reaction mixture was stirred for 1 h at ambient temperature. To the reaction mixture at -78 ^{o}C was added Et_3SiH (503 µL, 3.15 mmol) and $PhBCl_2$ (463 µL, 3.57 mmol). The resulting reaction mixture was stirred for 1 h at -78 ^{o}C, and Et_3N (2 mL) and MeOH (2 mL) were added successively. The resulting reaction mixture was diluted with CH_2Cl_2 (100 mL), filtered, washed with aqueous $NaHCO_3$ (20 mL) and water (20 mL). The combined organic layers were dried ($MgSO_4$), filtered and the resulting filtrate was concentrated under reduced pressure. Flash column silica gel chromatography of the resulting residue using EtOAc/hexanes (1/5, v/v) yielded title compound **30** (803 mg, 80%). To a solution of compound **30** (800 mg, 1.67 mmol) in DCM (24 mL) was added 4Å molecular sieves, BnBr (500 µL, 4.17 mol) and freshly prepared Ag_2O (1.35 g, 5.84 mmol) at ambient temperature. The resulting reaction mixture was stirred 30 h under darkness, filtered and concentrated under reduced pressure. Flash column silica gel chromatography using EtOAc/hexanes (1/5, v/v) afforded compound **31** (522 mg, 55%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.6 – 7.5 (m, 2H, CH aromatic), 7.5 – 7.2 (m, 13H, CH aromatic), 5.9 (d, *J* = 5.4 Hz, 1H, H1), 5.1 (dd, *J* = 10.8, 3.0 Hz, 1H, H3), 4.7 – 4.3 (m, 6H, 2 × CH_2, H5, H2), 4.1 (dd, *J* = 3.0, 1.3 Hz, 1H, H4), 3.6 (dd, *J* = 9.4, 7.4 Hz, 1H, H6), 3.4 (dd, *J* = 9.4, 6.0 Hz, 1H, H6), 2.1 (s, 3H, CH_3 Ac). ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.2, 137.9, 137.8, 135.0, 129.2, 128.6, 128.5, 128.2, 128.1, 128.1, 128.0, 127.9, 127.9, 84.9, 77.6, 77.2, 76.7, 75.4, 74.3, 74.1, 73.5, 71.5, 67.9, 59.5, 21.0.

3-*O*-Acetyl-2-azido-4,6-di-*O*-benzyl-2-deoxy-α-D-galactopyranoside (32): To a solution of compound **31 (**500 mg, 0.88 mmol) in a mixture of acetonitrile and water (8.2 mL:1.8 mL) was added mercury(II) chloride (2.82 g, 2.82 mmol) and calcium carbonate

(308 mg, 3.085 mmol). The resulting reaction mixture was stirred for 6 h at ambient temperature, filtered through celite and the resulting filtrate was concentrated under reduced pressure. The resulting reaction mixture was diluted with dichloromethane (80 mL), washed with 1 M aqueous potassium iodide (3 × 15 mL), 30% aqueous sodium thiosulfate (2 × 20 mL), dried ($MgSO_4$) and concentrated under reduced pressure. The resulting residue was purified by flash column ilica gel chromatography using EtOAc/hexanes (3/10, v/v) to yield title compound **32** (320 mg, 85%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.6 – 7.1 (m, 13H, CH aromatic), 5.4 – 5.3 (m, 2H), 4.8 – 4.4 (m, 7H), 4.3 – 4.2 (m, 1H), 4.1 – 4.0 (m, 1H), 3.9 – 3.4 (m, 7H), 2.1 (d, *J* = 9.8 Hz, 3H, CH_3 Ac). ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.4, 170.3, 137.8, 137.7, 137.6, 137.5, 128.6, 128.6, 128.6, 128.4, 128.3, 128.1, 128.1, 128.1, 128.1, 128.0, 96.6, 92.5, 77.6, 77.2, 76.7, 75.3, 75.2, 74.9, 74.1, 73.7, 73.6, 73.5, 71.4, 69.3, 68.8, 68.3, 62.7, 58.6, 21.0, 21.0.

3-*O*-Acetyl-2-azido-4,6-di-*O*-benzyl-2-deoxy-D-galactopyronosyl trichloroacetimidate (13): To a solution of compound **32** (80 mg, 187 mmol) in CH_2Cl_2 (4 mL) was added Cl_3CCN (95 µL, 0.93 mmol) and DBU (6 µL, 0.063 mmol) and stirred for 2 h at ambient temperature. The resulting reaction mixture was concentrated under reduced pressure, and the flash column silica gel chromatography of the resulting residue using EtOAc/ hexanes (1/5, v/v) afforded title compound **13** (83 mg, 79%). ^{1}H NMR (300 MHz, $CDC1_3$): δ 2.09 (s, 3H), 3.57-3.63 (m, 1H), 3.67(m, 1H), 4.20-4.27 (2H, m), 4.31 (m, 1H), 4.40-4.75 (m, 4H), 5.36 (dd, 1H), 6.48 (d, 1H), 7.24-7.43 (m, 10H), 8.74 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 20.7, 57.4, 67.4,71.3, 71.4, 73.3, 73.9, 75.2, 90.8, 95.0, 127.7, 128.4, 137.5, 137. 6, 160.7, 170.0.

5-*O*-Triphenylmethyl-D-ribose diethyl dithioacetal (34): To a solution of D-ribose (5g, 33.3 mol) in concentrated hydrochloric acid (6 mL) was added ethanethiol (6 mL, 83.26 mmol) at 0 °C for 15 min and the reaction mixture was stirred for another 30 minutes. The resulting reaction mixture was neutralized with drop-wise addition of saturated aqueous sodium bicarbonate. The resulting reaction mixture was concentrated under reduced pressure, diluted with ethyl acetate (300 mL). The insoluble inorganic salts were filtered, and the resulting filtrate was concentrated to yield impure compound **33.** The pure compound **33,** a white solid, was recrystallized from ethanol (6.4 g, 75%). To a solution of D-ribose diethyldithioacetal **33** (6 g, 23.43 mmol) in pyridine (90 mL) was added trityl chloride (9.8 g, 35.15 mmol) and DMAP (286 mg, 2.34 mmol), and the reaction mixture was stirred for 3 h at 80 °C. The resulting reaction mixture was concentrated to dryness, and the resulting residue was purified by flash column silica gel chromatography EtOAc/hexanes (1:3, v/v) to afford **34** (9.9 g, 80%) which is an amorphous solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.44–7.13 (m, 15 H, CH aromatic), 4.17 (t, 1H, H3), 4.05 (d, 1H, H1), 3.94 (m, 1H, H4), 3.81 (br. d, 1H, H2), 3.70 (d, 1H, HO C4), 3.38 (dd, 1H, H5b), 3.33 (d, 1H, HO C4), 3.29 (dd, 1H, H5a), 3.08 (br. d, 1H, HO C3), 2.49–2.71(m, 4H, CH_3CH_2S), 1.19 (t, 3H, CH_3CH_2S), 1.17 (t, 3H, CH_3CH_2S). ^{13}C NMR (75 MHz, $CDCl_3$): δ 143.4, 128.4, 126.8, 127.6 (*C* aromatic), 86.6, 71.8 (C4), 70.8 (C2), 70.0 (C3), 64.8 (C5), 55.2 (C1), 25.1 (CH_3CH_2S), 23.4 (CH_3CH_2S), 14.3 (CH_3CH_2S), 14.2 (CH_3CH_2S).

2,3,4-Tri-*O*-benzyl-5-*O*-triphenylmethyl-D-ribose diethyl dithioacetal (35): To a solution of compound **34** (9 g, 17.04 mmol) in DMF (80 mL) was added 60% NaH (2.79 g, 69.8 mmol), and stirred for 30 minutes at 0 °C. To the resulting reaction mixture was

added a solution of benzyl bromide (6.72 mL, 56.23 mmol) in DMF (15 mL) and stirred for 3.5 h at 0 °C. Cold water/ice cubes (50 mL) was added to quench excessive NaH to reaction mixture. The resulting reaction mixture was extracted with EtOAc (5 × 100 mL), resulting EtOAc layer was dried ($MgSO_4$), filtered and concentrated under reduced pressure. The resulting residue was purified with flash column silica gel chromatography using EtOAc/hexanes (1/10, v/v) to yield compound **35** (12.10 g, 80%). ^{1}H NMR (300 MHz, $CDCl_3$): *δ* 7.45 – 7.03 (m, 15H, CH aromatic), 5.00 – 4.55 (m, 6H, 3×C*H*$_2$ Bn), 4.25 (d, *J* = 3.3 Hz, 1H, H1), 4.12 (dd, *J* = 7.6, 2.4 Hz, 1H, H2), 4.03 (dd, *J* = 7.5, 3.3 Hz, 1H, H2), 3.97-3.72 (m, 3H, C*H*$_2$OTBS), 2.74 – 2.51 (m, 4H, 2H, 2×C*H*$_2$ SEt), 1.19 (dt, *J* = 12.5, 7.4 Hz, 6H, 2×CH_3 SEt), 0.88 (s, 9H, 3×CH_3 TBS), 0.00 (s, 6H, 2×CH_3 TBS). ^{13}C NMR (75 MHz, $CDCl_3$): *δ* 139.2, 138.8, 138.7, 128.5, 128.4, 128.4, 128.1, 128.0, 128.0, 127.9, 127.8, 127.7, 127.6, 127.5, 82.9, 81.0, 79.9, 77.7, 77.4, 77.2, 76.8, 75.0, 73.5, 72.9, 63.7, 54.3, 26.5, 26.2, 25.2, 18.5, 14.7, 14.7, -5.1, -5.2.

2,3,4-Tri-*O*-benzyl-5-*O*-*tert*-butyldimethylsilyl-D-ribose diethyl dithioacetal (37): To a solution of compound **35** (10 g,11.26 mmol) in MeOH (200 mL) was added and CBr_4 (373 mg, 1.12 mmol) and refluxed for 3 h. On completion of the reaction, as indicated by TLC analysis, the reaction mixture was cooled to ambient temperature, diluted with water, and extracted with EtOAc (3×100 mL). The combined organic layers were washed with brine, dried ($MgSO_4$), filtered and concentrated. The resulting residue was purified by flash column silica gel chromatography using EtOAc/hexanes (1/4, v/v) to afford title compound **36** (3.8 g, 65%). To a solution of compound **36** (3.8 g, 7.22 mmol) in DMF (47 mL) was added imidazole (639 mg g, 9.39 mmol) and *tert*-butyldimethylsilyl chloride (1.305 g, 8.66 mmol), and the reaction mixture was stirred for

12 h at ambient temperature. The reaction mixture was concentrated under reduced pressure, diluted with EtOAc (400 mL) and washed twice with H_2O (50 mL). The combined organic layers were dried ($MgSO_4$) and concentrated under reduced pressure. Flash column silica gel chromatography of the resulting residue using EtOAc/hexanes (1/9, v/v) afforded title compound **37** (3.83 g, 83%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.45 – 7.03 (m, 15H, CH aromatic), 5.00 – 4.55 (m, 6H, 3×CH_2 Bn), 4.25 (d, *J* = 3.3 Hz, 1H, H1), 4.12 (dd, *J* = 7.6, 2.4 Hz, 1H, H2), 4.03 (dd, *J* = 7.5, 3.3 Hz, 1H, H2), 3.97-3.72 (m, 3H, CH_2OTBS), 2.74 – 2.51 (m, 4H, 2H, 2×CH_2 SEt), 1.19 (dt, *J* = 12.5, 7.4 Hz, 6H, 2×CH_3 SEt), 0.88 (s, 9H, 3×CH_3 TBS), 0.00 (s, 6H, 2×CH_3 TBS). ^{13}C NMR (75 MHz, $CDCl_3$): δ 139.2, 138.8, 138.7, 128.5, 128.4, 128.4, 128.1, 128.0, 128.0, 127.9, 127.8, 127.7, 127.6, 127.5, 82.9, 81.0, 79.9, 77.7, 77.4, 77.2, 76.8, 75.0, 73.5, 72.9, 63.7, 54.3, 26.5, 26.2, 25.2, 18.5, 14.7, 14.7, -5.1, -5.2.

2,3,4-Tri-*O*-benzyl-5-*O*-*tert*-butyldimethylsilyl-D-ribose (38): To a solution of **37** (3 g, 4.68 mmol) in a mixture of acetone and H_2O ((89 mL:11 mL) was added 2,6-Lutidine (8.69 mL, 75 mmol) and NBS (5.38 g, 30.42 mmol) at ambient temperature. The resulting reaction mixture was stirred for 1 h, quenched with saturated aqueous $NaHCO_3$ (50 mL) and $Na_2S_2O_3$ (50 mL). The reaction mixture was concentrated under reduced pressure and diluted with EtOAc (150 mL). The combined organic layers were dried ($MgSO_4$), filtered and the filtrate was concentrated under reduced pressure. The resulting residue was purified by flash column silica gel chromatography using EtOAc/hexanes (1/9, v/v) to afford title compound **38** (2.5 g, 80%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.73 (s, 1H, *H*C=O), 7.34-7.24 (m, 15H, CH aromatic), 4.80-4.48 (m, 6H), 3.97 (m, 1H), 3.89 (d, 1H), 3.67 (m, 2H), 3.55 (m, 1H), 0.87 (s, 9H, (CH_3)$_3$C TBS), 0.00

(d, 6H, (CH_3)$_2$Si TBS). ^{13}C NMR (75 MHz, $CDCl_3$): δ 200.8, 138.1, 137.7, 137.4, 128.5, 128.3, 128.2, 128.0, 127.7, 81.3, 78.8, 78.2, 74.2, 73.2, 73.0, 61.6, 25.9, 18.2, 5.3.

2,3,4-Tri-*O*-benzyl-5-*O*-*tert*-butyldimethylsilyl-D-ribitol (14): A solution of compound **38** (1.65 g, 3.08 mmol) in methanol (20 mL) was added sodium borohydride (250 mg, 6.16 mmol) and stirred for 4 h. To the resulting mixture water (30 mL) was added and extracted with EtOAc (3 × 50 mL). The combined organic layers were dried ($MgSO_4$), filtered and concentrated under reduced pressure. Flash column silica gel chromatography of the resulting residue using EtOAc/hexanes (1/4, v/v) to yield the title compound **14** (993 mg, 60%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.5-7.1 (m, 15H, CH aromatic), 4.9 – 4.5 (m, 6H, 3×CH_2 Bn), 4.0 – 3.6 (m, 7H, H1a, H1b, H2, H3, H4, H5a, H5b), 0.9 (d, *J* = 2.7 Hz, 9H TBS), 0.1 (d, *J* = 2.6 Hz, 7H TBS). ^{13}C NMR (75 MHz, $CDCl_3$): δ 138.7, 138.5, 138.4, 128.7, 128.6, 128.64, 128.62, 128.3, 128.1, 128.10, 128.04, 128.01, 127.88, 80.21, 79.34, 79.30, 74.18, 73.04, 72.2, 63.2, 61.8, 26.23, 18.5, -5.03, -5.08.

Ethyl 3-*O*-benzyl-4,6-*O*-benzylidene-l-thio-β-D-glucopyranoside (45): To the solution of compound **44** (10 g, 32.05 mmol) in toluene (250 mL) was added dibutyltin oxide (8.74 g, 35.25 mmol) and refluxed for 2 h. The reaction mixture was concentrated under reduced pressure, and the resulting residue was co-evaporated with toluene. To the resulting residue in DMF (120 mL) was added cesium fluoride (5.11 g, 1.05 mmol) and benzyl bromide (4.2 mL, 35.25 mmol) and stirred for 15 h at ambient temperature. The reaction mixture was concentrated, diluted with EtOAc (200 mL), and washed with 1 M aq. KF (2 × 50 mL) and water (50 mL), dried ($MgSO_4$), filtered, and concentrated under reduced pressure. Purification of the resulting residue by flash column silica gel

chromatography using EtOAc/hexanes (1/4, v/v) yielded the title compound **45** (7.7 g, 0.9 mmol), and also recovered diol **44** (3.5 g, 35%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.53-7.28 (m, 10H, CH aromatic), 5.59 (s, 1H, C*H* benzylidene), 4.99-4.82 (q, 2H, CH_2 Bn), 4.48 (d, 1H, H1), 4.37 (dd, 1H, H6a), 3.85-3.69 (m, 3H, H3, H4, H6b), 3.65-3.45 (2H, m, H2, H5), 2.76 (q, 2H, SCH_2CH$_3$), 2.52 (d, 1H, OH), 1.33 (t, 3H, SCH$_2$CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 165, 138.1, 137.0, 128.7, 128.6, 128.4, 128.0, 127.8, 127.6, 127.3, 125.7 (*C* aromatic), 100.8 (*C*H benzylidene), 86.2 (C1), 81.3, 80.7, 72.7, 70.2 (C2, C3, C4, C5), 74.2 (CH_2, Bn), 68.2 (C6), 24.1 (CH_2, SEt), 14.9 (CH_3, SEt).

Ethyl 2-*O*-benzoyl-3-*O*-benzyl-4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside (46): To a solution of **45** (3 g, 7.46 mmol) in pyridine (60 mL) was added benzoyl chloride (3.09 mL, 22.38 mmol) and the reaction mixture was stirred for 12 h at 80 ^{0}C. The reaction mixture was quenched with water (5 mL). The resulting reaction mixture was concentrated to dryness, diluted with CH_2Cl_2 (150 mL), washed with 1 M aq. HCl (30 mL), saturated aq. $NaHCO_3$ (30 mL) and water (20 mL), dried ($MgSO_4$), filtered and concentrated under reduced pressure. Purification of the resulting residue employing flash column silica gel chromatography using EtOAc/hexanes (1/4, v/v) afforded the compound **46** (3.02 g, 82%). ^{1}H NMR (300MHz, $CDCl_3$): δ 8.04-7.10 (m, 15H, CH aromatic), 5.62 (s, 1H, C*H* benzylidene), 5.34 (dd, 1H, H2), 4.83 (d, 1H, PhCH_2), 4.68 (d, 1H, PhCH_2), 4.62 (d, 1H, H1), 4.41 (dd, 1H, H6a), 3.95-3.78 (m, 3H, H3,4,6b), 3.56 (ddd, 1H, H5), 2.72 (q, 2H, SCH_2CH$_3$), 1.22 (t, 3H, SCH$_2$CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 165.3 (O=*C*Ph), 137.9, 137.3, 133.3, 130.0, 129.9, 129.2, 128.5, 128.4, 128.3, 128.2, 127.7, 126.1 (*C*H aromatic), 101.4 (*C*H benzylidene), 84.4 (C1), 81.8,

79.3 (C3, C4), 74.3 (*C*H_2 Bn), 72.5 (C2), 70.8 (C5), 68.6 (C6), 24.1 (S*C*H_2CH_3), 14.9 (SCH_2*C*H_3).

Ethyl 2-*O*-benzoyl-3,4-di-*O*-benzyl-1-thio-β-D-glucopyranoside (47): To a solution of compound **46** (1.9 g, 3.75 mmol) in CH_2Cl_2 (40 mL) was added 4Å molecular sieves (5 g) and the reaction mixture was stirred for 1 h at ambient temperature. To the resulting reaction mixture at -78 ^oC was added Et_3SiH (1.8 mL, 11.25 mmol) and $PhBCl_2$ (738 µL, 5.62 mmol) and reaction mixture was stirred for 1 h at -78 ^oC. The resulting reaction mixture was quenched with Et_3N (3 mL) and MeOH (3 mL) at the same temperature. The reaction mixture was diluted with CH_2Cl_2, filtered, washed with aqueous $NaHCO_3$ and water. The combined organic layers were dried ($MgSO_4$), filtered and concentrated under reduced pressure. Flash column silica gel chromatography of the resulting residue using EtOAc/hexanes (1/5, v/v) yielded title compound **47** (1.52 g, 80%). ^1H NMR (300 MHz, $CDCl_3$): δ 8.04-7.10 (m, 15H, aromatic), 5.28 (dd, 1H, H-2), 4.86 (d, 1H, C*H*H Bn), 4.82-4.71 (m, 2H, C*H*$_2$ Bn), 4.68 (d, 1H, J=11.0 Hz, C*H*H Bn), 4.57 (d, 1H, H1), 3.94-3.66 (m, 4H, H3, H4, H6a, H6b), 3.58 (ddd, 1H, H5), 2.70 (q, 2H, SC*H*$_2CH_3$), 2.02 (s, 1H, O*H*),1.22 (t, 3H, SC*H*$_2CH_3$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 165.3 (O=*C* Bn), 137.8, 137.7, 133.2, 129.8, 128.5, 128.4, 128.3, 128.1, 128.0, 127.7 (aromatic), 84.1 (C1), 83.8 (C3), 79.8 and 77.7 (C4, C5), 75.3 and 75.2 (2 CH_2 Bn), 72.5 (C2), 62.1 (C6), 24.1 (S*C*H_2CH_3), 14.9 (SCH_2*C*H_3).

Ethyl 6-*O*-allyl-2-*O*-benzoyl-3,4-di-*O*-benzyl-l-thio-β-D-glucopyranoside (10): To a solution of compound **47** (3.5 g, 6.88 mmol) in DMF (60 mL) was added allyl bromide (0.7 mL, 8.26 mmol) and sodium hydride (200 mg, 8.26 mmol) and stirred for 1.5 h at -20 °C. The reaction mixture was quenched with methanol (2 mL) at the same

temperature and concentrated under reduced pressure. The resulting residue was taken up in ethylacetate (200 mL), washed with water (50 mL) and aq. $NaHCO_3$ (50 mL), dried ($MgSO_4$) and filtered. The filtrate was concentrated and the resulting residue was purified by flash column silica gel chromatography (1:1:8 ethyl acetate/DCM/hexane) to afford glycosyl donor **10** (2.61 g, 70%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.19 – 7.88 (m, 2H, C*H* aromatic), 7.66 – 7.03 (m, 14H, C*H* aromatic), 6.02 – 5.80 (m, 1H, C*H* All), 5.38 – 5.23 (m, 2H, OCH_2All), 5.17 (dq, *J* = 10.4, 1.4 Hz, 1H, H2), 4.94 – 4.61 (m, 4H, 2×C*H*2 Bn), 4.52 (d, *J* = 10.0 Hz, 1H, H1), 4.15 – 3.93 (m, 2H, H3), 3.90 – 3.64 (m, 5H, H6a, H6b, H2, H4), 3.54 (ddd, *J* = 9.5, 4.4, 2.1 Hz, 1H, H5), 2.71 (qd, *J* = 7.4, 5.9 Hz, 2H, CH_2 SEt), 1.22 (t, *J* = 7.4 Hz, 3H, C*H*3 SEt). ^{13}C NMR (75 MHz, $CDCl_3$): δ 165.2 (C=O), 137.9 (qC, Bn), 137.7, 133.0, 134.6 (CH, All, Bz), 129.7, 128.2, 128.1, 127.8, 127.7, 127.5 (*C*H aromatic), 116.8 (*C*H_2 All), 84.2, 83.4, 79.5, 77.8, 72.4 (C1, C2, C3, C4, C5), 75.1, 75.0, 68.8 (C6, *C*H_2 Bn, *C*H_2 Bn All, *C*H_2 Bn), 23.7 (*C*H_2, SEt), 14.8 (*C*H_3, SEt).

Ethyl 6-*O*-allyl-3,4-di-*O*-benzyl-2-*O*-levulinoyl-l-thio-β-D-glucopyranoside (10a): A solution of EDC.HCl (219 mg, 1.14 mmol) and DMAP (7 mg, 0.05 mmol) in DCM (6 mL) was added to a solution of compound **48** (255 mg, 0.57 mmol) and levulinic acid (82 µL, 0.804 mmol) in DCM (4 mL) at 0 ^{o}C. The reaction mixture was stirred for 4 h at ambient temperature, and the mixture was filtered, diluted with DCM, washed with water and the resultant solvent was concentrated under reduced pressure. The resulting residue was purified by flash column silica gel chromatography using ethyl acetate/hexanes (1/4, v/v) to obtain title compound **10a** (200 mg, 70%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.39 – 7.20 (m, 10H, C*H* aromatic), 5.97 – 5.80 (m, 1H, C*H* All), 5.34 – 5.10 (m, 2H, OCH_2All), 5.09 – 4.94 (m, 1H, H2), 4.87 – 4.68 (m, 4H, 2×C*H*2 Bn), 4.63 (d, *J* = 10.9 Hz, 1H, H1), 4.35

(d, *J* = 10.0 Hz, 1H, H3), 4.12 – 3.92 (m, 2H, C*H*$_2$All), 3.76 – 3.58 (m, 4H, H6a, H6b, H2, H4), 3.53 – 3.39 (m, 1H, H3), 2.83 – 2.36 (m, 7H, 2 × C*H*2 Lev, C*H*$_2$ SEt), 2.16 (s, 3H, C*H*3 Lev), 1.25 (t, *J* = 7.4 Hz, 3H, C*H*3 SEt). ^{13}C NMR (75 MHz, $CDCl_3$): δ 206.3, 171.7, 138.4, 138.2, 134.8, 128.6, 128.5, 128.1, 128.0, 128.0, 127.8, 117.1, 84.4, 83.6, 79.6, 78.0, 77.6, 77.2, 76.7, 75.3, 75.2, 72.6, 72.3, 69.0, 38.0, 30.0, 28.2, 24.0, 15.0.

Ethyl 6-*O*-allyl-4-*O*-benzyl-2,3-di-*O*-benzoyl-l-thio-β-D-glucopyranoside (10b): To a solution of compound **50** (200 mg, 0.38 mmol) in DMF (4 mL) was added allyl bromide (0.039 mL, 0.45 mmol) and sodium hydride (16 mg, 0.45 mmol), and stirred for 1.5 h at -20 °C. The reaction mixture was quenched with methanol (2 mL), concentrated under reduced pressure, and the resulting residue was taken up in ethylacetate (60 mL). The resulting solution was washed with water (10 mL) and saturated aq. $NaHCO_3$ (10 mL), dried ($MgSO_4$), and filtered. The filtrate was concentrated and the resulting residue was purified by flash column silica gel chromatography using ethyl acetate/hexanes (2:8, v/v) to furnish glycosyl donor **10b** (146 mg, 68%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.19 – 7.88 (m, 2H, C*H* aromatic), 7.66 – 7.03 (m, 14H, C*H* aromatic), 6.02 – 5.80 (m, 1H, C*H* All), 5.38 – 5.23 (m, 2H, OC*H*$_2$All), 5.17 (dq, *J* = 10.3, 1.2 Hz, 1H, H2), 5.09 (dd, 1H, H3), 4.94 – 4.61 (m, 2H, C*H*2 Bn), 4.50 (d, *J* = 10.0 Hz, 1H, H1), 3.86 – 3.77 (m, 5H, H6a, H6b, H2, H4), 3.47 (ddd, *J* = 9.5, 4.4, 2.1 Hz, 1H, H5), 2.68 (qd, *J* = 7.4, 5.9 Hz, 2H, C*H*$_2$ SEt), 1.17 (t, *J* = 7.4 Hz, 3H, C*H*3 SEt).

2,3,4-Tri-*O*-benzyl-5-*O*-*tert*-butyldimethylsilyl-1-*O*-(3-*O*-acetyl-2-azido-4,6-di-*O*-benzyl-2-deoxy-β-D-galactopyranosyl)-D-ribitol (39): To solution of glycosyl donor **13** (1.26 mg, 2.10 mmol) and acceptor **14** (750 mg, 1.63 mmol) in acetonitrile (35 mL) was added activated 4Å molecular sieves (3 g) and stirred for 1 h at ambient

temperature. To the reaction mixture at –40 °C was added trimethylsilyl trifluoromethanesulfonate (58 µL, 0.316 mmol) and was stirred 60 min at the same temperature. The resulting reaction mixture was quenched with pyridine (3 mL), filtered, diluted with DCM (200 mL) and the filtrate was washed with water (2 × 50 mL), dried ($MgSO_4$), filtered and concentrated. The resulting residue was purified with flash column silica gel chromatography using EtOAc/hexanes (2/8, v/v) yielded the title compound **39** (970 mg, 71%). Disaccharide yields dropped to 54% when the above mentioned glycosylation was performed with 0.2 equiv. of promoter. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.3 (dtd, *J* = 8.7, 7.4, 6.5, 4.9 Hz, 26H, CH aromatic), 4.8 – 4.3 (m, 11H, 5 × CH_2 Bn, $H3^B$), 4.2 (d, *J* = 8.0 Hz, 1H, $H1^B$), 4.1 (dd, *J* = 10.9, 5.4 Hz, 1H, $H5^B$), 4.0 – 3.7 (m, 9H, $CH_2{}^A$, $H4^B$, $H5^B$, $H6a^B$, $H6b^B$), 3.6 – 3.4 (m, 3H, C$H_2{}^A$ CH_2OTBS), 2.0 (s, 3H, Ac), 0.9 (s, 9H, C(CH_3)$_3$ TBS), 0.0 (s, 6H, Si(CH_3)$_2$ TBS). ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.3, 139.0, 138.9, 138.8, 138.1, 137.8, 128.6, 128.5, 128.5, 128.4, 128.4, 128.4, 128.4, 128.2, 128.1, 128.0, 128.0, 127.9, 127.8, 127.8, 127.6, 127.5, 127.5, 102.3($C1^B$), 80.3, 78.8, 78.7, 77.6, 77.2, 76.7, 75.2, 73.9, 73.8, 73.6, 73.6, 73.2, 72.7, 72.5, 69.6, 67.9, 63.5, 61.8, 26.1, 21.0, 18.4, -5.2, -5.2. HRMS-MALDI: ($M+Na^+$) calcd. 945.4654, found 968.3629.

2,3,4-tri-*O*-Benzyl-5-*O*-*tert*-butyldimethylsilyl-1-*O*-[2-azido-4,6-*O*-benzyl-2-deoxy-3-*O*-(2-naphthylmethyl)-β-D-galactopyranosyl]-D-ribitol (39a): A solution of donor **13a** (190 mg, 0.28 mmol) and acceptor **14** (87 mg, 0.18 mmol, 1 equiv.) in acetonitrile (4.5 mL) was stirred with 4Å molecular sieves (500 mg) for 1 h at ambient temperature. To the reaction mixture at –40 °C was added trimethylsilyl trifluoromethanesulfonate (10 µL, 0.056 mmol) and stirred for 60 minutes at the same temperature. The resulting

reaction mixture was quenched with pyridine (1 mL), filtered, diluted with DCM (70 mL) and the filtrate was washed with water (2 × 10 mL), dried ($MgSO_4$), filtered and concentrated. The resulting residue was purified with flash column silica gel chromatography using EtOAc/hexanes (2/8, v/v) yielded the desired disaccharide compound **39a** (104 mg, 54%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.9 – 7.1 (m, 30H, CH aromatic), 5.0 – 4.3 (m, 12H, 5 × CH_2 Bn), 4.2 (d, *J* = 8.0 Hz, 1H, $H1^B$), 4.1 (dd, *J* = 11.0, 5.4 Hz, 1H, $H5^B$), 4.0 – 3.7 (m, 8H, CH_2^A, H6a ^{B}, $H6b^B$, $H4^B$, $H3^B$, $H3^A$, $H4^A$), 3.6 – 3.2 (m, 4H, CH_2^A CH_2OTBS, $H2^A$, $H2^B$), 0.9 (s, 9H, C(CH_3)$_3$ TBS), 0.0 (d, *J* = 30.9 Hz, 6H, Si(CH_3)$_2$ TBS). ^{13}C NMR (75 MHz, $CDCl_3$): δ 139.0, 138.9, 138.8, 138.5, 137.9, 135.3, 133.4, 133.2, 128.6, 128.4, 128.4, 128.3, 128.3, 128.3, 128.2, 128.1, 128.1, 128.1, 128.0, 127.9, 127.8, 127.8, 127.6, 127.4, 127.4, 126.7, 126.3, 126.2, 125.8, 102.3, 80.6, 80.3, 78.8, 78.7, 77.6, 77.4, 77.2, 76.7, 74.8, 73.8, 73.7, 73.5, 72.7, 72.6, 72.5, 72.3, 69.4, 68.4, 63.8, 63.5, 26.1, 18.4, -5.2, -5.2. HRMS-MALDI: ($M+Na^+$) calcd. 1066.5152, found 1066.7712.

2,3,4-tri-*O*-Benzyl-5-*O*-*tert*-butyldimethylsilyl-1-*O*-(2-azido-4,6-di-*O*-benzyl-2-deoxy-β-D-galactopyranosyl)-D-ribitol (40): To a solution of disaccharide **39** (340 mg, 0.35 mmol) in MeOH (9 mL) was added NaOMe/ MeOH (0.4 mL, 1 M solution) until pH = 9 and the reaction mixture was stirred for 2 h at ambient temperature. The resulting reaction mixture was neutralized with amberlite H^+-resin and filtered. The resulting iltrate was concentrated under reduced pressure, and purified with flash column silica gel chromatography using EtOAc/hexanes (2/8, v/v) to obtain the desired disaccharide **40** (305 mg, 94%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.5 – 7.1 (m, 24H, CH aromatic), 4.8 – 4.4 (m, 10H, 5 × CH_2 Bn), 4.2 (d, *J* = 7.9 Hz, 1H, $H1^B$), 4.1 (dd, *J* = 11.0, 5.5 Hz, 1H,

H5^{B}), 4.0 – 3.7 (m, 7H, CH_2^{A}, H4^{B}, H6a ^{B}, H6b^{B}), 3.6 (dd, *J* = 9.1, 8.2 Hz, 1H, H2 ^{A}), 3.6 – 3.4 (m, 3H, H2^{A}, CH_2^{A}), 3.4 (td, *J* = 7.5, 3.8 Hz, 1H, H3^{B}), 2.1 (d, *J* = 7.9 Hz, 1H, C3^{B} O*H*), 0.9 (s, 9H, C(CH_3)$_3$ TBS), -0.0 (s, 6H, Si(CH_3)$_2$ TBS). ^{13}C NMR (75 MHz, $CDCl_3$): δ 139.0, 138.9, 138.8, 138.2, 137.8, 128.7, 128.6, 128.6, 128.4, 128.3, 128.2, 128.2, 128.1, 128.0, 128.0, 127.9, 127.7, 127.6, 127.5, 102.4, 80.3, 78.8, 78.7, 77.6, 77.4, 77.2, 76.7, 75.5, 75.4, 73.8, 73.7, 73.5, 72.7, 72.7, 72.5, 69.6, 68.1, 65.4, 63.5, 26.1, 18.4, -5.2, -5.2. HRMS-MALDI: (M+Na^{+}) calcd. 926.4552, found 925.3149.

2,3,4-Tri-*O*-benzyl-5-*O*-*tert*-butyldimethylsilyl-1-*O*-[(4-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-6-*O*-(2-naphthylmethyl)-α-D-galactopyranosyl)-(1→3)-(2-azido-4,6-di-*O*-benzyl-2-deoxy-β-D-galactopyranosyl)]-D-ribitol (41): A solution of glycosyl donor **12** (360 g mg, 0.505 mmol) and disaccharide acceptor **40** (326 mg, 0.361 mmol, 1 equiv.) in DCM (4.5 mL) was stirred with 4Å molecular sieves (1 g) for 1 h at ambient temperature. To the reaction mixture at –10 °C was added trimethylsilyl trifluoromethanesulfonate (8 µL, 0.04 mmol) at the same temperature, and the reaction mixture was stirred for 60 min at 0°C. The resulting reaction mixture was quenched with pyridine (2 mL), filtered through celite, diluted with DCM (150 mL), washed with water (2 × 20 mL), dried ($MgSO_4$), and concentrated under reduced pressure. The resulting residue was purified with flash column silica gel chromatography using EtOAc/hexanes (2/8, v/v) yielded desired trisaccharide compound **41** (344 mg, 70%). Rearranged donor (30 mg, 9%) was recovered in the purification of trisaccharide **41**. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.9 – 7.1 (m, 36H, CH aromatic), 5.7 (dd, *J* = 3.2, 1.4 Hz, 1H, H4^{C}), 5.1 (d, *J* = 3.5 Hz, 1H, H1^{C}), 5.0 (d, *J* = 11.2 Hz), 4.8 – 4.3 (m, 13H, 6 × CH_2 Bn), 4.2 (dd, *J* = 18.0, 7.9 Hz, 2H, H1^{B}, H3^{C}), 4.1 (ddd, *J* = 10.6, 6.1, 4.3 Hz, 2H, H5^{B}, H6a^{C}), 4.0 – 3.7 (m,

11H, CH_2^A, $H2^A$, $H3^A$, $H4^A$, $H4^B$, $H5^B$, $H6a^B$, $H6b^B$, $H6a^C$), 3.7 – 3.3 (m, 8H, CH_2^A CH_2OTBS, $H2^B$, $H2^C$, $H3^B$), 2.0 (s, 3H, Ac^C), 0.9 (d, *J* = 1.1 Hz, 9H, C(CH_3)$_3$ TBS), 0.0 (s, 6H, Si(CH_3)$_2$ TBS). ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.3, 139.0, 138.9, 138.8, 138.5, 137.8, 137.1, 135.3, 133.3, 128.7, 128.7, 128.6, 128.6, 128.4, 128.4, 128.4, 128.3, 128.2, 128.2, 128.1, 128.1, 128.0, 128.0, 127.9, 127.9, 127.8, 127.8, 127.7, 127.6, 127.5, 127.4, 126.8, 126.3, 126.1, 125.9, 102.6, 95.1, 80.2, 78.7, 77.6, 77.4, 77.2, 76.7, 74.9, 74.7, 73.8, 73.7, 73.6, 72.7, 72.5, 71.9, 68.4, 68.2, 66.8, 63.5, 62.9, 59.4, 26.1, 21.0, 18.5, -5.2, -5.2. HRMS-MALDI: ($M+Na^+$) calcd. 926.4552, found 925.3149.

2,3,4-Tri-*O*-benzyl-5-*O*-*tert*-butyldimethylsilyl-1-*O*-[(2-azido-3-*O*-benzyl-2-deoxy-6-*O*-(2-naphthylmethyl)-α-D-galactopyranosyl)-(1→3)-(2-azido-4,6-di-*O*-benzyl-2-deoxy-β-D-galactopyranosyl)]-D-ribitol (9): To a solution of trisaccharide **41** (340 mg, 69 mmol) in MeOH (6 mL) was added NaOMe/ MeOH (0.25 mL, 1 M solution) until pH = 9 and stirred for 12 h at ambient temperature. The resulting reaction mixture was neutralized with amberlite H^+-resin, filtered and the filtrate was concentrated under reduced pressure. Flash column silica gel chromatography of the resulting residue using EtOAc/hexanes (2/8, v/v) to obtain desired trisaccharide acceptor **40** (296 mg, 90%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.9 – 7.7 (m, 5H, CH aromatic), 7.5 – 7.1 (m, 35H, CH aromatic), 5.3 (s, 1H), 5.2 (d, *J* = 3.5 Hz, 1H), 5.0 (d, *J* = 11.2 Hz, 1H), 4.8 – 4.5 (m, 13H), 4.4 – 3.6 (m, 21H), 3.6 – 3.5 (m, 2H), 3.4 (dd, *J* = 8.9, 5.2 Hz, 1H), 3.4 (dd, *J* = 8.1, 5.3 Hz, 1H), 2.7 (s, 1H), 0.9 (s, 9H, C(CH_3)$_3$ TBS), -0.1 (m, 6H, Si(CH_3)$_2$ TBS). ^{13}C NMR (75 MHz, $CDCl_3$): δ 139.0, 138.9, 138.8, 138.6, 137.8, 137.3, 135.4, 133.4, 133.2, 128.9, 128.8, 128.6, 128.5, 128.4, 128.4, 128.3, 128.3, 128.3, 128.3, 128.2, 128.1, 128.1, 128.0, 128.0, 128.0, 127.9, 127.9, 127.8, 127.8, 127.7, 127.6, 127.4, 127.4,

126.6, 126.3, 126.1, 125.7, 102.5, 95.1, 80.4, 80.3, 78.7, 77.6, 77.4, 77.2, 76.7, 76.6, 76.4, 74.9, 73.8, 73.6, 73.3, 72.7, 72.5, 72.0, 71.4, 69.8, 69.6, 69.0, 68.1, 66.9, 63.5, 63.0, 59.2, 26.1, 18.5, -5.2, -5.2.

2,3,4-Tri-*O*-benzyl-5-*O*-*tert*-butyldimethylsilyl-1-*O*-[(4-*O*-*p*-anisoyl-2-azido-3-*O*-benzyl-2-deoxy-6-*O*-(2-naphthylmethyl)-α-D-galactopyranosyl)-(1→3)-(2-azido-4,6-di-*O*-benzyl-2-deoxy-β-D-galactopyranosyl)]-D-ribitol (41a): To a solution of glycosyl donor **12a** (60 g mg, 0.083 mmol) and disaccharide acceptor **40** (63 mg, 0.069 mmol) in DCM (4 mL) was stirred with 4Å molecular sieves (300 mg) for 1 h at room temperature. To the reaction mixture at -20 °C was added trimethylsilyl trifluoromethanesulfonate (2 µL, 0.008 mmol), and stirred for 1 h at -20 °C followed by 1 h at 0 °C . The resulting reaction mixture was quenched by the addition of pyridine (2 mL), filtered through celite, diluted with DCM (100 mL), and the resulting filtrate was washed with water (2 × 20 mL), dried ($MgSO_4$), and concentrated. The resulting residue was purified with flash column silica gel chromatography using EtOAc/hexanes (2/8, v/v) yielded desired trisaccharide compound **41a** (69 mg, 68%). ^{1}H NMR (300 MHz, $cdcl_3$) δ 8.1 – 7.0 (m, 48H, CH aromatic), 7.0 – 6.7 (m, 1H, $H4^{C}$), 5.2 (d, *J* = 3.6 Hz, 1H, $H1^{C}$), 4.9 (dd, *J* = 30.8, 10.9 Hz, 2H, 1 × CH_2 Bn), 4.8 – 4.2 (m, 16H, 6 × CH_2 Bn), 4.2 – 4.0 (m, 4H, $H1^{B}$), 4.0 – 3.7 (m, 15H, $H6a^{B,}$ $H6b^{B,}$ $H2^{B,}$ $H6a^{C}$, $H6b^{C}$), 3.7 – 3.2 (m, 8H, $H2^{B}$), 1.8 (s, 3H, CH_3 anisoyl) 0.9 (d, *J* = 0.8 Hz, 10H, $C(CH_3)_3$ TBS), 0.0 (d, *J* = 17.5 Hz, 6H, $Si(CH_3)_2$ TBS). ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.4, 163.7, 139.0, 138.9, 138.8, 138.5, 137.8, 137.2, 135.2, 133.2, 133.1, 132.0, 128.7, 128.6, 128.5, 128.5, 128.4, 128.4, 128.3, 128.3, 128.2, 128.1, 128.0, 128.0, 128.0, 127.9, 127.8, 127.8, 127.8, 127.7, 127.6, 127.4, 126.6, 126.1, 125.9, 125.8, 122.2, 113.8, 102.6, 95.2, 80.3, 78.7, 77.6, 77.4, 77.2, 76.7,

74.9, 74.8, 73.8, 73.7, 73.6, 73.3, 72.7, 72.5, 71.7, 71.4, 69.7, 68.8, 68.4, 68.1, 66.9, 63.5, 62.9, 59.7, 55.6, 26.1, 18.5, 1.2, -5.2, -5.2.

Dimethylthexylsilyl 6-*O*-allyl-2-*O*-benzoyl-3,4-di-*O*-benzyl-β-D-glucopyranosyl-(1→3)-2-azido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside (51): A mixture of glycosyl donor **10** (2.05 g, 3.675 mmol), glycosyl acceptor **11** (1.07 g, 2.459 mmol), and freshly activated 4 Å molecular sieves (150 mg) in dichloromethane (50 mL) was stirred under argon for 1 h. To the reaction mixture was added NIS (1.106 g, 7.34 mmol) and TMSOTf (90 µL, 0.734 mmol) and the reaction mixture was stirred for 1 h. The resulting reaction mixture was diluted with CH_2Cl_2, the solid was filtered off, and the residue was rinsed with CH_2Cl_2. The combined organic filtrate (150 mL) was washed with 10% aq $Na_2S_2O_3$ (30 mL) and water (3 × 30 mL). The combined organic layere were dried ($MgSO_4$) and concentrated under reduced pressure. Flash column silica gel chromatography of the resulting residue was purified by flash column silica gel chromatography using EtOAc/hexanes (1/9, v/v) to afford disaccharide **51** (1.47 g, 65%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.7 – 7.1 (m, 21H, CH aromatic), 5.9 – 5.7 (m, 1H, C*H*=CH_2 All^B), 5.6 (s, 1H, C*H* benzylidene^A), 5.3 – 5.1 (m, 3H, C*H*=C*H*$_2$ All^B, H2^B), 5.0 – 4.6 (m, 5H, 2 × CH_2 Bn^B, H1^B), 4.5 (d, *J* = 7.4 Hz, 1H, H1^A), 4.3 (dd, *J* = 10.5, 5.0 Hz, 1H, H6a^A), 4.0 – 3.5 (m, 10H, H4^A, H5^A, H6b^A, H6a^B, H3^B, H4^B, H6a^B, H6b^B), 3.5 – 3.3 (m, 3H, H2^A, H3^A, H5^B), 1.7 (p, *J* = 6.9 Hz, 1H, C*H* TDS), 0.9 (d, *J* = 8.4 Hz, 12H, 4 × C*H*$_3$ TDS), 0.2 (d, *J* = 9.4 Hz, 6H, Si(C*H*$_3$)$_2$ TDS). ^{13}C NMR (75 MHz, $CDCl_3$): δ 165.4, 138.2, 138.0, 137.4, 134.9, 133.1, 130.2, 129.8, 129.2, 128.6, 128.5, 128.5, 128.4, 128.1, 128.1, 128.0, 127.7, 126.4, 116.9, 101.7, 101.3 (C1^B), 97.7 (C1^A), 82.9, 79.8, 78.7, 78.0, 77.6, 77.4, 77.2, 76.7, 75.5, 75.1, 75.1, 74.3, 72.6, 68.8, 68.7, 68.5, 66.7, 33.9, 24.9,

20.0, 19.9, 18.6, 18.5, -2.1, -3.1. HRMS-MALDI: ($M+Na^+$) calcd. 944.4232, found 944.6582.

Dimethylthexylsilyl 6-*O*-allyl-2-*O*-benzoyl-3,4-di-*O*-benzyl-β-D-glucopyranosyl-(1→3)-2-azido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside (51): To a solution of donor **10** (83 mg, 0.151 mmol), BSP (35 mg, 0.166), TTBP (56 mg, 0.226 mmol), and 4 Å molecular sieves in CH_2Cl_2 (3 mL, 0.05 M), at -60 °C, was added Tf_2O (30 µL, 0.181 mmol). The reaction mixture was stirred for 30 minutes at -60 °C, and a solution of glycosyl acceptor **11** (100 mg, 0.229 mmol) in CH_2Cl_2 (3 mL) was slowly added. The reaction mixture was stirred for further 1.5 h at -60 °C and was allowed to reach room temperature. The resulting reaction mixture was diluted with dichloromethane (50 mL), filtered through celite and washed with saturated aq. $NaHCO_3$. The combined organic layers were dried ($MgSO_4$) and concentrated under reduced pressure. Flash column silica gel chromatography of the resulting residue using EtOAc/hexanes (1/9, v/v) afforded the desired β-disaccharide **51** (97 mg, 70%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.7 – 7.1 (m, 21H, CH aromatic), 5.9 – 5.7 (m, 1H, C*H*=CH_2 All^B), 5.6 (s, 1H, C*H* benzylidene^A), 5.3 – 5.1 (m, 3H, C*H*=C*H*$_2$ All^B, H2^B), 5.0 – 4.6 (m, 5H, 2 × CH_2 Bn^B, H1^B), 4.5 (d, *J* = 7.4 Hz, 1H, H1^A), 4.3 (dd, *J* = 10.5, 5.0 Hz, 1H, H6a^A), 4.0 – 3.5 (m, 10H, H4^A, H5^A, H6b^A, H6a^B, H3^B, H4^B, H6a^B, H6b^B), 3.5 – 3.3 (m, 3H, H2^A, H3^A, H5^B), 1.7 (p, *J* = 6.9 Hz, 1H, C*H* TDS), 0.9 (d, *J* = 8.4 Hz, 12H, 4 × C*H*$_3$ TDS), 0.2 (d, *J* = 9.4 Hz, 6H, Si(C*H*$_3$)$_2$ TDS). ^{13}C NMR (75 MHz, $CDCl_3$): δ 165.4, 138.2, 138.0, 137.4, 134.9, 133.1, 130.2, 129.8, 129.2, 128.6, 128.5, 128.5, 128.4, 128.1, 128.1, 128.0, 127.7, 126.4, 116.9, 101.7, 101.3 (C1^B), 97.7 (C1^A), 82.9, 79.8, 78.7, 78.0, 77.6, 77.4, 77.2,

76.7, 75.5, 75.1, 75.1, 74.3, 72.6, 68.8, 68.7, 68.5, 66.7, 33.9, 24.9, 20.0, 19.9, 18.6, 18.5, -2.1, -3.1. HRMS-MALDI: ($M+Na^+$) calcd. 944.4232, found 944.6582.

Dimethylthexylsilyl 6-*O*-allyl-3,4-di-*O*-benzyl-β-D-glucopyranosyl-(1→3)-2-azido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside (20): To a solution of disaccharide **51** (1 g, 1.08 mmol) in a mixture of DCM and MeOH (12mL:4 mL) was added NaOMe/ MeOH (0.4 mL, 1 M solution) until pH = 9 and stirred at 55 ^oC for 12 h. The reaction mixture was neutralized with amberlite H^+-resin and filtered. The filtrate was concentrated, and flash column silica gel chromatography of the resulting residue yielded disaccharide **20** (856 mg, 92%). ^1H NMR (500 MHz, $CDCl_3$): δ 7.6 – 7.1 (m, 14H, CH aromatic), 5.8 – 5.7 (m, 1H, C*H*=CH_2 All^B), 5.5 (s, 1H, C*H* benzylidene^A), 5.2 – 5.0 (m, 2H, C*H*=CH_2 All^B), 5.0 – 4.7 (m, 3H, 1 × CH_2 $Bn^{B,}$ C*H*H Bn^B), 4.7 – 4.6 (m, 2H, C*H*H $Bn^{B,}$ $H1^A$), 4.5 (d, *J* = 7.4 Hz, 1H, $H1^B$), 4.2 (dd, *J* = 10.5, 5.0 Hz, 1H, $H6a^A$), 4.0 – 3.8 (m, 2H, OCH_2 OAll), 3.8 – 3.7 (m, 3H, $H6a^A$, $H4^{B,}$ $H5^A$), 3.7 – 3.5 (m, 5H, $H3^{A,}$ $H4^{A,}$ $H6b^B$, $H6a^B$, $H2^B$), 3.5 – 3.3 (m, 3H, $H2^{A,}$ $H3^B$, $H5^B$), 3.0 (d, *J* = 2.3 Hz, 1H, O*H*^{B}), 1.7 (p, *J* = 6.9 Hz, 1H, C*H* TDS), 0.9 (d, *J* = 8.4 Hz, 12H, 4 × CH_3 TDS), 0.2 (d, *J* = 9.4 Hz, 6H, 4 × CH_3 TDS). ^{13}C NMR (75 MHz, $cdcl_3$) δ 138.9, 138.4, 137.1, 135.0, 129.3, 128.5, 128.5, 128.4, 128.1, 128.1, 127.9, 127.7, 126.2, 117.0, 103.4, 101.7, 97.7, 84.3, 79.9, 78.8, 77.6, 77.3, 77.2, 76.7, 75.7, 75.4, 75.2, 72.5, 68.7, 68.7, 68.2, 66.6, 34.0, 25.0, 20.1, 20.0, 18.6, 18.5, -2.0, -3.0. HRMS-MALDI: ($M+Na^+$) calcd. 840.397, found 840.151.

Dimethylthexylsilyl 6-*O*-allyl-2,3,4-tri-*O*-benzyl-*β*-D-glucopyranosyl-(1→3)-2-azido--2-deoxy-*β*-D-glucopyranoside (22): To a solution of compound **20** (800 mg, 0.97 mmol) in DMF (20 mL) at 0 ^oC was added benzyl bromide (175 µL, 1.46 mmol) and 60%

NaH (47 mg, 1.175 mmol). The reaction mixture was stirred for 3 h at 0 ^{o}C and the excess NaH was quenched by the addition of ice cubes. The resulting reaction mixture was concentrated under reduced pressure, diluted with EtOAc (100 mL) and washed with H_2O (30 mL). The combined organic layers were dried ($MgSO_4$), filtered, concentrated under reduced pressure and flash column chromatography of the resulting residue using EtOAc/ hexanes (1/4, v/v) gave the title compound **21** (710 mg, 80%). Benzylidene in compound **21** was hydrolyzed using 60 AcOH in water to generate compound **22** in 74% yields. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.5 – 7.2 (m, 16H, CH aromatic), 5.9 – 5.8 (m, 1H, C*H*=CH_2 All^{B}), 5.3 – 5.1 (m, 2H, C*H*=C*H*$_2$ All^{B}), 5.1 – 4.7 (m, 5H, 2 × CH_2 Bn^{B}, C*H*H Bn^{B}), 4.6 – 4.5 (m, 3H, C*H*H Bn^{B}, H1^{A}, H1^{B}), 4.0 – 3.8 (m, 3H, OC*H*$_2$ OAll, H6a^{A}), 3.7 (ddd, *J* = 12.0, 7.2, 5.4 Hz, 1H, H6b^{A}), 3.7 – 3.6 (m, 2H, H6a^{B}, H6b^{B}), 3.6 – 3.4 (m, 6H, H3^{A}, H2^{B}, H4^{B}, H5^{B}, O*H* C4^{A}), 3.4 – 3.2 (m, 3H, H2^{A}, H5^{A}, H3^{B}) 2.1 (t, *J* = 6.7 Hz, 1H, O*H* C6^{A}), 1.7 (p, *J* = 6.8 Hz, 1H, C*H* TDS), 0.9 (d, *J* = 6.4 Hz, 12H, 4 × C*H*$_3$ TDS), 0.2 (d, *J* = 4.3 Hz, 6H, 2 × C*H*$_3$ TDS). ^{13}C NMR from GHSQC (126 MHz, $CDCl_3$): δ 131.3, 131.2, 128.2, 128.1, 128.0, 128.1, 123.3, 118.0, 104.2, 97.4, 85.5, 78.1, 76.2, 75.5, 75.3, 74.6, 74.0, 72.8, 69.6, 69.4, 69.3, 67.5, 25.0, 21.9, 20.4, 17.2, 1.1, -2.1. HRMS-MALDI: (M+Na^{+}) calcd. 842.4126, found 842.7293.

Dimethylthexylsilyl 6-*O*-allyl-2,3,4-tri-*O*-benzyl-β-D-glucopyranosyl-(1→3)-2-azido-2-deoxy-6-*O*-*p*-toluenesulfonyl-β-D-glucopyranoside (23): To a solution of compound **21** (114 mg, 0.125 mmol) in CH_2Cl_2 (2.5 mL) was added TsOH (5 mg, 0.025 mmol) and ethanethiol (52 µL, 0.75 mmol). The resulting reaction mixture was stirred for 1 h and concentrated under reduced pressure. The resulting residue was purified by flash column silica gel chromatography using EtOAc/ hexanes (1/4 to 1/1, v/v) to afford

compound **22** (77 mg, 74%). To a solution of diol **22** (155 mg, 0.18 mmol) in pyridine (2.5 mL) at 0 ^{o}C was added *p*-toluenesulfonyl chloride (47 mg, 0.246 mmol) and DMAP (12 mg, 0.09 mmol). The reaction mixture was stirred for 4 h and was concentrated under reduced pressure, diluted with DCM (100 mL), washed with water (10 mL), dried ($MgSO_4$). Flash column silica gel chromatography of the resulting residue using EtOAc/ hexanes (1/4, v/v) to afford compound **23** (162 mg, 88%). ^{1}H NMR (500 MHz, CDC_3): δ 7.8 – 7.7 (m, 2H, CH aromatic), 7.3 – 7.1 (m, 16H, CH aromatic), 5.8 (dd, *J* = 17.0, 10.6 Hz, 1H, C*H*=CH_2 All^{B}), 5.2 – 5.0 (m, 2H, C*H*=C*H*$_2$ All^{B}), 5.0 – 4.6 (m, 5H, 2 × CH_2 Bn^{B}, C*H*H Bn^{B}), 4.5 – 4.4 (m, 4H, C*H*H Bn^{B}, H1^{A}, H1^{B}), 4.3 (dd, *J* = 10.4, 1.8 Hz, 1H, H6b^{A}), 4.0 (dd, *J* = 10.4, 6.7 Hz, 1H, H6a^{A}), 3.9 (ddt, *J* = 8.2, 5.7, 1.3 Hz, 2H, OC*H*$_2$ OAll), 3.6 – 3.5 (m, 2H, H3^{A,} H4^{B}), 3.5 – 3.3 (m, 6H, H4^{A}, H5^{A}, H4^{B}, H2^{B}, H5^{B}, H6a^{B}, H6b^{B}, O*H* C4^{A}), 3.2 – 3.1 (m, 2H, H2^{A,} H3^{B}), 2.4 (s, 3H, C*H*$_3$ OTs), 1.6 (m, *J* = 6.9 Hz, 1H, C*H* TDS), 0.9 – 0.7 (m, 12H, 4 × C*H*$_3$ TDS), 0.1 (d, *J* = 11.6 Hz, 6H, 2 × C*H*$_3$ TDS). ^{13}C NMR (75 MHz, $CDCl_3$): δ 144.8, 138.5, 138.2, 137.9, 134.2, 133.1, 129.9, 128.6, 128.6, 128.5, 128.5, 128.2, 128.1, 128.1, 128.1, 128.0, 127.8, 127.8, 118.0, 104.1, 97.3, 85.3, 84.6, 82.0, 77.9, 77.6, 77.2, 76.7, 76.0, 75.2, 75.0, 74.3, 73.8, 72.6, 69.3, 69.0, 67.2, 34.0, 24.9, 21.8, 20.0, 19.9, 18.6, 18.5, 0.1, -2.0, -3.2. HRMS-MALDI: (M+Na^{+}) calcd. 996.4215, found 996.7842.

Dimethylthexylsilyl 6-*O*-allyl-2,3,4-tri-*O*-benzyl-β-D-glucopyranosyl-(1→3)-2-azido-2,6-di-deoxy-*β*-D-glucopyranoside (25): To a solution of compound **23** (162 mg, 0.166 mmol) in 2-butanone (5 mL) was added NaI (30 mg, 0.199 mmol) and the resulting mixture was stirred for 18 h at 80 °C. The resulting reaction mixture was diluted with DCM (100 mL), washed with $Na_2S_2O_3$ (20 mL), washed with water (10 mL)

dried ($MgSO_4$), filtered and concentrated under reduced pressure. Flash column silica gel chromatography of the resulting residue using EtOAc/ hexanes (2/8, v/v) to afford **24** (126 mg, 82%). To a solution of compound **24** (865 mg, 0.931 mmol) in 10 mL diethylene glycol diethyl ether was added $NaCNBH_3$ (585 mg, 9.31 mmol), and the resulting reaction mixture was refluxed for 8 h. The reaction mixture was diluted with EtOAc (100 mL), washed with water (20 mL), water layer was back extracted with EtOAc (10 mL), dried ($MgSO_4$), and concentrated under reduced pressure. Flash column chromatography of the resulting residue using EtOAc/ hexanes (2/8 to 1/1, v/v) afforded the compound **25** (485 mg, 65%). Compound **24**: ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.5 – 7.0 (m, 15H, CH aromatic), 5.9 (ddt, *J* = 17.4, 10.4, 5.7 Hz, 1H, C*H*=CH_2 All^B), 5.3 – 5.1 (m, 2H, CH=C*H*$_2$ All^B), 5.0 (d, *J* = 11.2 Hz, 1H, C*H*H Bn^B), 5.0 – 4.7 (m, 4H, 2 × CH_2 Bn^B), 4.6 – 4.4 (m, 3H, C*H*H Bn^B, H1^A, H1^B), 4.0 – 3.9 (m, 2H, OC*H*$_2$ OAll), 3.7 – 3.6 (m, 3H, H6a^A, H4^A, H4^B), 3.6 – 3.4 (m, 4H, H5^A, H6b^A, H2^A, H5^B), 3.4 – 3.1 (m, 5H, H2^A, H3^A, H3^B, H6a^B, H6b^B), 1.7 (p, *J* = 6.9 Hz, 1H, C*H* TDS), 0.9 (dd, *J* = 4.1, 2.8 Hz, 12H, 4 × C*H*$_3$ TDS), 0.3 (d, *J* = 19.8 Hz, 6H, 2 × C*H*$_3$ TDS). ^{13}C NMR (75 MHz, $CDCl_3$): δ 138.5, 138.3, 137.9, 134.3, 128.6, 128.6, 128.5, 128.2, 128.1, 128.1, 128.0, 127.8, 127.8, 117.9, 104.1, 97.2, 84.9, 84.6, 82.0, 77.9, 77.6, 77.4, 77.2, 76.7, 76.0, 75.7, 75.2, 75.1, 74.4, 72.9, 72.6, 69.1, 67.7, 34.0, 24.9, 20.1, 20.1, 18.6, 18.6, 5.6, -1.6, -3.1. HRMS-MALDI: ($M+Na^+$) calcd. 952.3144, found 952.1277. Compound 25: ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.6 – 7.1 (m, 17H, CH aromatic), 5.9 – 5.8 (m, 1H, C*H*=CH_2 All^B), 5.3 – 5.1 (m, 2H, CH=C*H*$_2$ All^B), 5.1 – 4.7 (m, 6H, 3 × CH_2 Bn^B), 4.6 – 4.5 (m, 4H), 4.3 (d, *J* = 1.3 Hz, 1H), 4.0 (qdt, *J* = 12.7, 5.7, 1.4 Hz, 2H), 3.7 – 3.6 (m, 2H), 3.6 – 3.4 (m, 5H), 3.4

– 3.2 (m, 5H), 1.7 (p, *J* = 6.8 Hz, 1H, C*H* TDS), 1.3 (d, *J* = 5.6 Hz, 3H, CH_3 C6^A), 1.0 – 0.8 (m, 12H, 4 × CH_3 TDS), 0.2 (s, 6H, 2 × CH_3 TDS).

Dimethylthexylsilyl 6-*O*-allyl-2,3,4-tri-*O*-benzyl-β-D-glucopyranosyl-(1→3)-6-*O*-acetyl-2-azido-2,6-di-deoxy-β-D-glucopyranoside (26): Compound **25** (480 mg, 0.59 mmol) was dissolved in a mixture of py/Ac_2O (10:1, 10mL) and stirred for 2 h at ambient temperature. The resulting reaction mixture was concentrated and co-evaporated with toluene under reduced pressure. Flash column chromatography of the resulting residue using EtOAc/ toluene (1/9, v/v) afforded title compound **26** (454 mg, 90%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.5 – 7.2 (m, 14H, CH aromatic), 5.9 (ddt, *J* = 17.2, 10.6, 5.4 Hz, 1H, C*H*=CH_2 All^B), 5.3 – 5.1 (m, 2H, CH=CH_2 All^B), 5.0 – 4.7 (m, 6H, 2 × CH_2 Bn^{B,} C*H*H Bn^{B,} H4^A), 4.7 (d, *J* = 7.8 Hz, 1H, H1^B), 4.6 (d, *J* = 11.0 Hz, 1H, C*H*H Bn^B), 4.5 (d, *J* = 7.7 Hz, 1H, H1^A), 3.9 (tddd, *J* = 12.8, 11.3, 4.2, 2.7 Hz, 2H, OCH_2 OAll), 3.7 – 3.5 (m, 5H, H3^B, H4^B, H6a^B, H6b^{B,} O*H* C4^A), 3.4 (dq, *J* = 9.8, 6.1 Hz, 1H, H5^A), 3.4 – 3.3 (m, 2H, H3^A, H5^B), 3.3 (dd, *J* = 10.1, 7.7 Hz, 1H, H2^A), 2.1 (s, 3H, Ac^A), 1.7 (p, *J* = 6.9 Hz, 1H, C*H* TDS), 1.2 (d, *J* = 6.1 Hz, 3H, CH_3 C6^A), 0.9 – 0.8 (m, 12H, 4 × CH_3 TDS), 0.2 – -0.1 (m, 5H, 2 × CH_3 TDS). ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.4, 138.9, 138.7, 138.5, 134.6, 128.5, 128.5, 128.4, 128.3, 128.0, 128.0, 127.8, 127.8, 127.7, 127.6, 117.0, 102.9, 97.1, 84.8, 82.8, 77.8, 77.6, 77.4, 77.2, 76.7, 75.6, 75.2, 75.1, 74.4, 73.5, 72.2, 70.0, 69.1, 68.9, 34.0, 29.8, 25.0, 21.0, 20.0, 20.0, 18.6, 18.5, 17.7, 0.1, -2.0, -3.1. HRMS-MALDI: (M+Na^+) calcd. 868.4283, found 868.2847.

Dimethylthexylsilyl 6-*O*-allyl-2,3,4-tri-*O*-benzyl-β-D-glucopyranosyl-(1→3)-6-*O*-acetyl-2-azido-2,6-di-deoxy-α-D-glucopyranosyl-(1→4)-2-azido-3-*O*-benzyl-2-deoxy-6-*O*-(2-naphthylmethyl)-β-D-galactopyranoside (59): To a solution of

compound **26** (125 mg, 0.147 mmol) in pyridine (3 mL) was added 70% HF/py (150 µl, 5.9 mmol) at 0 ^{o}C and the reaction mixture was stirred for 12 h at ambient temperature. The reaction mixture was diluted with EtOAc (50 mL), neutralized with saturated aq. $NaHCO_3$, washed with H_2O (10 mL), dried ($MgSO_4$), filtered and concentrated under reduced pressure. Flash column silica gel chromatography of the resulting residue using EtOAc/ hexanes (1/2, v/v) afforded lactol compound (81 mg, 78%). To the solution of lactol (82 mg, 0.116 mmol) in CH_2Cl_2 (3 mL) was added $F_3CC(NPH)Cl$ (73 mg, 0.34 mmol) and $CsCO_3$ (114 mg, 0.34 mmol) and stirred for 3h at ambient temperature. The reaction mixture was concentrated under reduced pressure, and the flash column silica gel chromatography of the resulting residue using EtOAc/ hexanes (1/5, v/v) afforded the title compound **8a** (70 mg, 70%). Pure title compound in higher yields could be obtained by a careful chromatography separation.

A mixture of disaccharide donor **8a** (102 mg, 0.116 mmol), acceptor **19** (48 mg, 0.083 mmol), and freshly activated 4Å molecular sieves (200 mg) in dichloromethane (3 mL) was stirred under argon for 1 h. To the reaction mixture at 0 °C was added trimethylsilyl trifluoromethanesulfonate (3 µL, 0.15 mmol,) and stirred for 1 h at ambient temperature. The resulting reaction mixture was quenched with pyridine (2 mL), filtered through celite, diluted with DCM (100 mL), filtrate was washed with water (2 × 20 mL), dried ($MgSO_4$), filtered and concentrated under reduced pressure. Flash column silica gel chromatography of the resulting residue using EtOAc/toluene (1/19, v/v) yielded the desired trisaccharide compound **59** (50 mg, 48%). Acceptor was separated from the trisaccharide by acetylating the crude residue that was obtained after the glycosylation reaction. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.9 – 7.7 (m, 4H, CH aromatic), 7.5 – 7.2 (m,

24H, CH aromatic), 5.9 (ddt, *J* = 17.3, 10.6, 5.4 Hz, 1H, C*H*=CH_2 All^B), 5.3 – 5.1 (m, 2H, CH=CH_2 All^B), 5.0 – 4.6 (m, 14H, 5 × CH_2 $Bn^{B,}$ $H1^A$, $H1^B$, $H4^B$), 4.4 (d, *J* = 7.5 Hz, 1H, $H1^C$), 4.3 (dq, *J* = 10.1, 6.2 Hz, 1H, $H5^B$), 4.2 – 4.1 (m, 2H, $H3^B$), 4.1 – 3.9 (m, 3H, OCH_2 OAll, $H6a^B$), 3.8 – 3.6 (m, 7H, $H6b^B$, $H6a^C$, $H6a^C$, $H3^A$, $H3^B$, $H5^B$, $H4^C$), 3.5 – 3.4 (m, 2H, $H5^C$, $H3^C$), 3.4 – 3.3 (m, 2H, $H2^A$, $H4^C$), 3.2 (dd, *J* = 10.6, 2.9 Hz, 1H, $H2^B$), 3.1 (dd, *J* = 10.4, 3.8 Hz, 1H, $H5^C$), 2.1 (s, 3H, Ac^B), 1.6 (p, *J* = 6.9 Hz, 1H), 1.6 (s, 4H, C*H* TDS, CH_3 $C6^B$), 1.0 – 0.8 (m, 13H, 4 × CH_3 TDS), 0.1 (d, *J* = 5.0 Hz, 6H, 2 × CH_3 TDS). ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.6, 138.9, 138.9, 138.6, 137.6, 135.1, 134.6, 133.4, 133.2, 128.6, 128.5, 128.5, 128.4, 128.4, 128.3, 128.0, 128.0, 127.9, 127.9, 127.9, 127.8, 127.7, 127.5, 127.0, 126.4, 126.3, 125.9, 117.1, 102.6, 99.1, 97.6, 84.7, 82.8, 78.2, 77.7, 77.6, 77.2, 76.7, 75.7, 75.1, 75.1, 74.6, 74.4, 73.8, 73.8, 73.0, 72.1, 72.0, 68.8, 67.4, 65.9, 65.7, 64.2, 34.1, 25.0, 21.0, 20.2, 20.0, 18.7, 18.6, 17.6, 0.2, -1.8, -2.9. HRMS-MALDI: ($M+Na^+$) calcd. 1285.5971, found 1285.5581.

Dimethylthexylsilyl 6-*O*-allyl-2,3,4-tri-*O*-benzyl-β-D-glucopyranosyl-(1→3)-2-azido-2,6-di-deoxy-α-D-glucopyranosyl-(1→4)-2-azido-3-*O*-benzyl-2-deoxy-6-*O*-(2-naphthylmethyl)-*β*-D-galactopyranoside (18): To a solution of trisaccharide **59** (50 mg, .039 mmol) in a mixture of MeOH (2 mL) and DCM (1 mL) was added NaOMe/ MeOH (0.1 mL, 1 M solution) until pH = 9 and stirred for 12 h at ambient temperature. The resulting reaction mixture was neutralized with amberlite H^+-resin and filtered. The filtrate was concentrated under reduced pressure, and purified with flash column silica gel chromatography using EtOAc/hexanes (2/8, v/v) to obtain the desired trisaccharide **18** (41 mg, 85%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.8 – 7.2 (m, 27H, CH aromatic), 5.8 (ddt, *J* = 16.1, 10.3, 4.2 Hz, 1H, C*H*=CH_2 All^B), 5.2 – 5.1 (m, 2H, CH=CH_2 All^B), 5.0 – 4.5

(m, 14H, 5 × CH_2 $Bn^{B,}$ $H1^A$, $H1^B$), 4.4 (d, *J* = 7.2 Hz, 1H, $H1^C$), 4.3 (dq, *J* = 8.1, 5.8 Hz, 1H, $H4^B$), 4.2 – 4.1 (m, 2H, $H3^{B,}$ $H5^C$), 4.1 – 3.8 (m, 3H, OCH_2 OAll, $H6a^B$), 3.8 – 3.6 (m, 7H, $H3^A$, $H6b^B$, $H3^B$, $H5^B$, $H6a^C$, $H6a^C$, $H4^C$), 3.6 – 3.4 (m, 2H, $H3^C$, $H5^C$), 3.3 – 3.0 (m, 4H, $H2^A$, $H4^C$, $H2^B$, $H5^C$), 1.6 (m, *J* = 7.9 Hz, 1H), 1.6 (s, 5H, C*H* TDS, CH_3 $C6^B$), 1.0 – 0.7 (m, 15H, 4 × CH_3 TDS), 0.1 (d, *J* = 5.0 Hz, 6H, 2 × CH_3 TDS). ^{13}C NMR (75 MHz, $CDCl_3$): δ 138.7, 138.6, 138.1, 137.4, 135.2, 134.6, 134.1, 133.2, 128.6, 128.5, 128.5, 128.4, 128.4, 128.2, 128.1, 128.0, 127.9, 127.9, 127.9, 127.8, 127.7, 127.5, 127.0, 126.4, 126.3, 125.9, 117.1, 102.4, 99.7, 97.6, 84.9, 82.1, 78.1, 77.7, 77.6, 77.2, 76.7, 75.7, 75.2, 75.1, 74.8, 74.4, 73.5, 73.2, 73.0, 72.5, 72.2, 68.7, 67.4, 65.9, 65.5, 64.1, 34.2, 20.2, 20.0, 18.7, 18.6, 17.6, -1.8, -2.9. HRMS-MALDI: ($M+Na^+$) calcd. 1243.5866, found 1243.7102.

Dimethylthexylsilyl 6-*O*-allyl-2,3,4-tri-*O*-benzyl-β-D-glucopyranosyl-(1→3)-2-azido-2,6-di-deoxy-4-phthalimido-α-D-glucopyranosyl-(1→4)-2-azido-3-*O*-benzyl-2-deoxy-6-*O*-(2-naphthylmethyl)-β-D-galactopyranoside (17): To the solution of trisaccharide compound **18** (3.188 g, 0.026 mmol) in a mixture of CH_2Cl_2 (2.25 mL) and pyridine (0.75 mL) at 0 °C was added Tf_2O (25 µL, 0.14 mmol) and DMAP (3mg,0.04 mmol) and stirred for 8 h. The resulting reaction mixture was quenched by the addition of water (1 mL), washed with water, brine, dried ($MgSO_4$) and concentrated under reduced pressure. To a solution of resulting triflate **18a** in DMF (1.5 mL) at 0 °C was added potassium phthalimide (12 mg, 0.052 mmol) and stirred for 12 h at ambient temperature. The resulting reaction mixture was diluted with water and extracted with EtOAc, washed with water and brine. The combined organic layers were dried ($MgSO_4$), filtered and concentrated under reduced pressure. Flash column silica gel

chromatography of the resulting residue using EtOAc/toluene (1/9, v/v) yielded desired trisaccharide compound **17** (12 mg, 17% over two steps). ^{1}H and ^{13}C NMR spectra were were recorded and they contained excessive amount of grease at 1 ppm in ^{1}H NMR. Further purification is required to obtain the clean NMR spectra for this molecule. HRMS-MALDI: ($M+Na^{+}$) calcd. 1372.6080, found 1372.6035.

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CHAPTER 4

CONCLUSION

Synthetic zwitterionic polysaccharides (ZPSs) with well characterized structures and with different number of repeating units can help immensely in furthering our knowledge on their mode of action. Unfortunately we were not able to accomplish the complete synthesis of the pentasaccharide molecule. However, we have synthesized the appropriate building blocks (**10**, **11**, **12**, **13**, **14** and **19**) in good yields, and narrowed down the efficient glycosylation conditions required for ribitol containing disaccharide **39,** glucose disaccharide **51** and trisaccharide **41.** It is disappointing to report that pentasaccharide synthesis could not be accomplished by coupling disaccharide donor **8** and sterically hindered trisaccharide acceptor **9**. Recent reports have also shown that sterically hindered C4-OH galactosyl acceptors are very poor nucleophiles.[23] We have also developed an efficient strategy for the synthesis of 6-deoxy disaccharide donor **8a** which required many synthetic manipulations on disaccharide.

Having obtained poor yields in the inversion of trisaccharide **18**, it would be beneficial to carry out inversion at disaccharide stage **25** and to proceed with [2+1+2] glycosylation strategy for accomplishing the synthesis of **2**. It is our belief that this new strategy should lead to an efficient synthesis of pentasaccharide repeating unit **2**. Because of use of different orthogonal protective groups and anomeric leaving groups,

our synthetic strategy is going to be different from the reported synthesis of similar polysaccharide.