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Towards development of a fully synthetic conjugate vaccine: Investigation of structural analogs of *Streptococcus pneumoniae* serogroup 6

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by

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Abstract

Serogroup 6 pneumococcal oligosaccharides as potential targets towards the development of a fully synthetic conjugate vaccine

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Doctor of Philosophy

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Involvement of complex glycostructures in a variety of damaging and healing processes has already been acknowledged by development of carbohydrate-based vaccines and therapeutics. The bacteria *Streptococcus pneumoniae* (SPn) have become one of the most frequent causes of pneumonia, bacteremia, and meningitis in the elderly, immunocompromised, and, especially, in young children. SPn has one of the largest public health and economic impacts amongst all bacterial infectious diseases. Over 2 million children die annually worldwide due to pneumonia, accounting for almost 20% of deaths under age five with more than half of these deaths attributed to SPn.

Amongst over ninety elucidated SPn serotypes, the SPn serogroup6 has been consistently ranked within the top three causes of invasive pneumococcal disease worldwide. The SPn bacterial cell is surrounded by a polysaccharide capsule and preventive vaccination is a viable tool against the bacterial invasion. Usually, serotype-specific antibodies are formed in response. Synthetic oligosaccharide components can be conjugated to a carrier

protein with conventional coupling chemistry, to obtain semi-synthetic conjugate vaccines (glycoconjugates).

This doctoral dissertation describes the application of a new glycosylation method for the synthesis of glycostructures to study immunological properties of serogroup 6. The development of an expeditious strategy to obtain synthetic saccharides of SPn6 and glycoconjugates thereof will ensure reliable and reproducible immunological studies. Consequently, by identifying the immunogenic responses of these glycoconjugates, will help target toward the development of effective vaccine candidates against SPn.

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Contents

List of abbreviations

Å.....Angstrom

Ac.....Acetyl

AgOTf..... Silver Trifluoromethanesulfonate

All ..... Allyl

Bn..... Benzyl

Bz ..... Benzoyl

$BF_3(OEt)_2$.....Boron trifluoride etherate

Bu_2SnO ..... Dibutyltinoxide

Bu_4NBr..... Tetrabutylammonium bromide

$Cu(OTf)_2$ .....Copper Trifluoromethanesulfonate

d.....Doublet

DCE..... 1,2-Dichloroethane

DCM ..... Methylene chloride

dd.....Double of doublets

DMF ..... *N,N*-Dimethylformamide

DMTST .....Dimethyl(methylthio) sulfonium trifluoromethanesulfonate

Et .....Ethyl

Et_2O.....Diethyl ether

EtAc .....Ethyl acetate

EtOH ..... Ethanol

Gal.....Galactose

Glc..... Glucose

h.....hour(s)

Hz.....Hertz

IgG and IgM..... Immunoglobulin-G and Immunoglobulin-M antibodies

HIV .....Human immunodeficiency virus

$HfCp_2Cl_2$ ..... bis (cyclopentadienyl)hafnium dichloride

HR-EI MS .....High Resolution Electron Ionization mass spectrum

HR-FAB MS .....High Resolution Fast atom bombardment mass spectrum

Hz.....Hertz

KOH.....Potassium Hydroxide

m .....Multiplet

min ..... minute

m/z.....Mass to charge ratio

Me .....Methyl

MeCN..... Acetonitrile

MeOH ..... Methanol

MS..... Molecular sieves

NaOH .....Sodium Hydroxide

NaOMe.....Sodium Methoxide

NIS .....*N*-iodosuccinimide

NMR ..... Nuclear magnetic resonance

p-TolSCl.....p-Tolylsulfenyl chloride

Pd ..... Palladium

$PdCl_2$ .....Palladium(II) chloride

Ph ..... Phenyl

Piv ..... Pivaloyl

ppm ..... Parts per million

R_f ..... Retention factor

Rha ..... Rhamnose

rt ..... Room Temperature

s ..... Singlet

sp ..... Spacer

t ..... Triplet

TBDMS ..... tert-butyldimethylsilyl

TFA ..... Trifluoroacetic acid

TfOH ..... Trifluoromethanesulfonic (triflic) acid

TLC ..... Thin layer chromatography

TMSOTf ..... Trimethylsilyl Trifluoromethanesulfonate

$Yb(OTf)_3$ ..... Ytterbium trifluoromethanesulfonate

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CHAPTER 1

Introduction

Section **1.2** to be published in:

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1 Introduction

1.1 Pneumococcal Disease: Background

The gram positive bacteria *Streptococcus pneumoniae* (SPn) have become one of the most frequent causes of pneumonia, bacteremia and meningitis in the elderly, immunocompromised, and, especially, in young children. The incidence of pneumococcal infection varies geographically, but elevated rates have been observed in both developed and developing countries. In accordance with the joint United Nations Children's Fund (UNISEF)/World Health Organization (WHO) survey, over 2 million children die annually worldwide due to pneumonia, accounting for almost 20% of deaths under age five.[1] More than half of these deaths are attributed to SPn. This situation is further complicated by the rapid increase in anti-microbial drug resistance during the last decade. SPn has one of the largest public health and economic impacts amongst all bacterial infectious diseases. Throughout the decades, SPn has become one of the most extensively studied microorganisms since its first isolation back in 1881; nevertheless, significant gaps in our knowledge remain.

1.1.1 Polysaccharide & Conjugate Vaccines

The development of resistance against antibiotics in the last decade is a serious threat for successful treatment of pneumococcal infections.[2,3] Despite the widespread use of antibiotics, the mortality and morbidity of pneumococcal disease remains high. In

addition resistant pneumococci are increasingly observed. [4,5] An alternative approach is to prevent the disease by vaccination with capsular polysaccharides (CPS) isolated from bacterial cultures, which was shown to afford protection as early as 1923.[6]

The SPn bacterial cell is surrounded by a polysaccharide capsule, which makes the organism resistant to phagocytosis. This polysaccharide capsule is the primary virulence factor of SPn and provides the basis for type-specific identification of approximately 90 different serotypes.[7] It is also the most immunogenic surface that can induce highly protective anti-CPS antibodies[8,9]; hence the CPS or saccharide-protein conjugate[10,11] vaccinations are conventional tools against the bacterial invasion; serotype-specific antibodies to the pneumococcal saccharide are formed in response.

Currently, leading SPn vaccine manufacturers, such as Wyeth, Pasteur, Merck, Aventis, and SmithKline Beecham have marketed various multi-component vaccines whose serotype composition varies. For example, vaccines containing isolated polysaccharides of 23 serotypes are commercially available as Pneumovax 23. These vaccines cover over 90% of the infections caused by SPn in the United States.[12] However polysaccharides are poorly immunogenic in high risk group patients.[13](i) infants and children less than 2 years of age. (ii) Splenectomised patients and (iii) immunocompromised patients (e.g.) HIV infected.

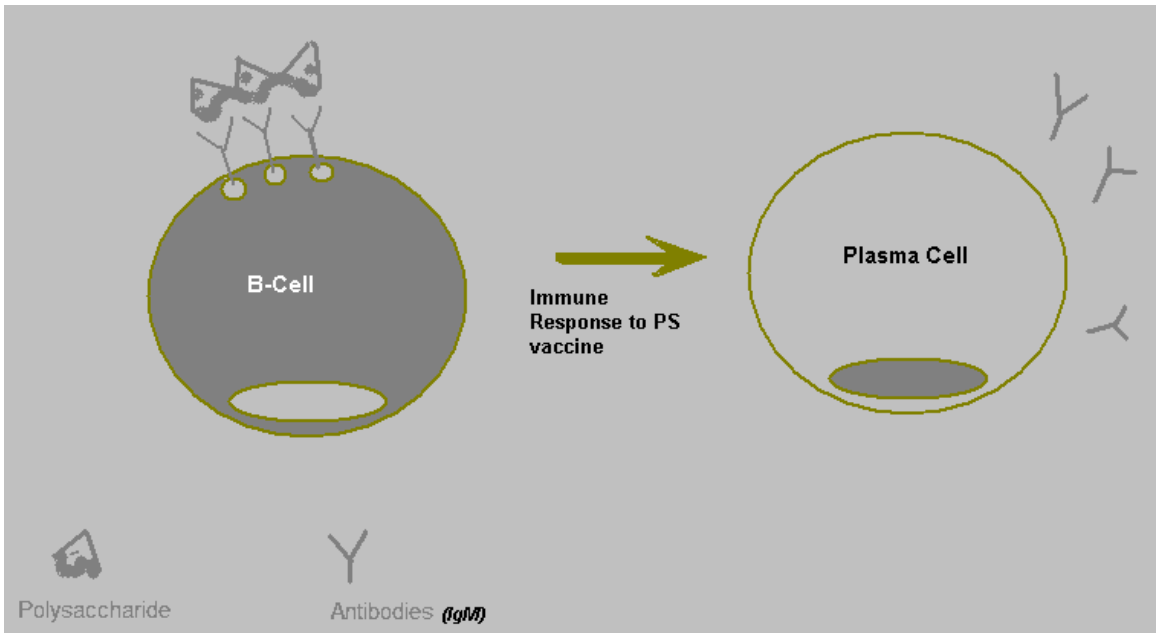

Figure 1.1 Response to polysaccharide vaccine

Polysaccharides are known to belong to a class of T-cell independent antigens; they stimulate B-cells to produce antibodies without the involvement of T-cells. Since T-cells are not involved in the process, the antibodies formed are mainly of the IgM isotype (Figure 1.1) and hence no memory B-cells are produced. To overcome the thymus independency of these antigens, PS can be conjugated to carrier proteins.

Hence, these glycoproteins belong to a class of T-cell dependent antigens. In this case the peptide-MHC complex is recognized by the T-cell receptor, thereby initiating the formation of both antibody-producing and memory B-cells and hence formation of IgG antibodies (Figure 1.2).

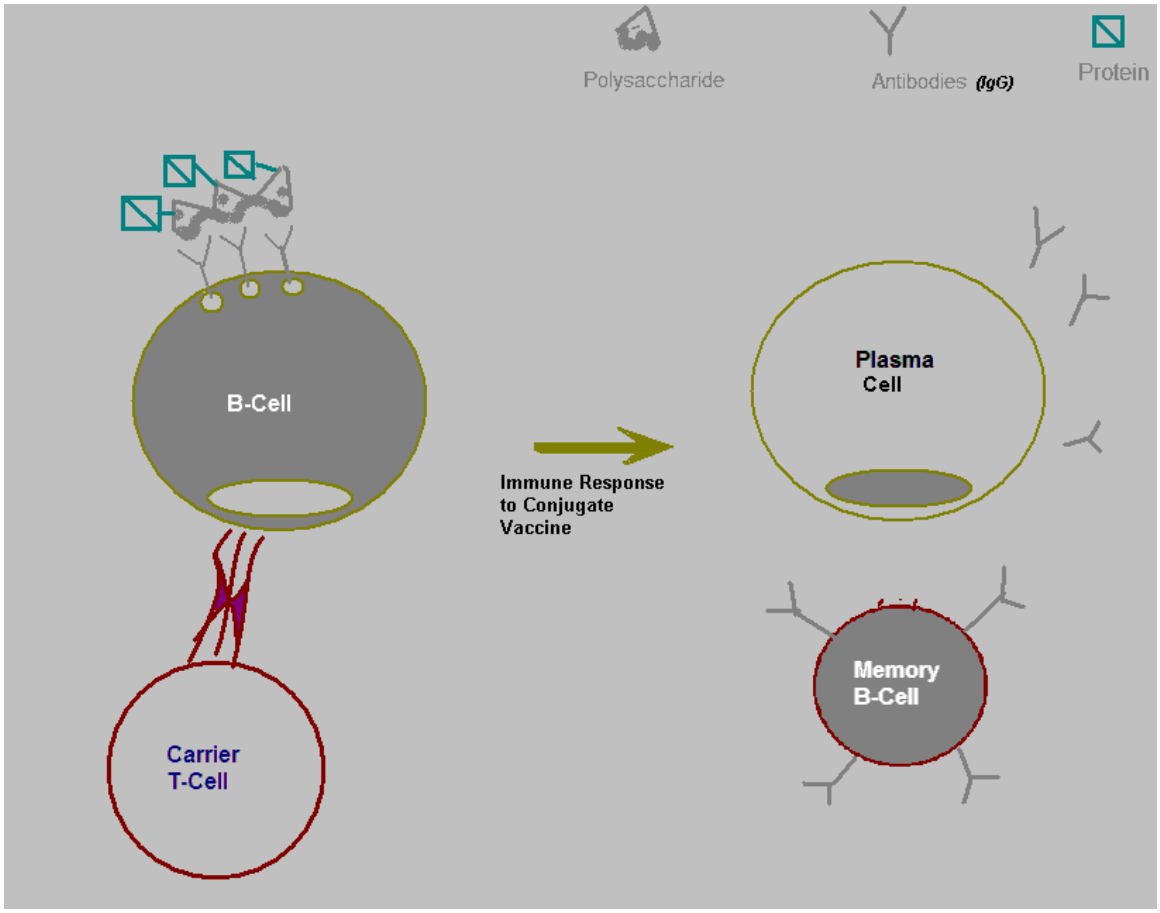

Figure 1.2 Response to conjugate vaccine

Hence by using neoglycoconjugates, long lasting immunity to the encapsulated bacteria could be achieved. Unconjugated vaccines, on the other hand, cause only an initial increase in antibody levels that soon return to the baseline levels without the induction of immunological memory. For high risk groups of patients, (i.e), infants or immunocompromised the antibodies are incapable of mounting a T-cell dependent immune response to the polysaccharides included in the current 23-valent formulation. However, by conjugation to a carrier protein a T-cell independent antigen can be converted to a T-cell dependent antigen thereby enhancing the immunogenicity of the bound molecule and/ or to elicit antibodies against the carrier which are considered

therapeutically beneficial.[13] For example, PNCRM7 or Prevnar is a pneumococcal conjugate vaccine, which is licensed for immunization of children aged 6 weeks to 9 years mainly in the US and European Union. Prevnar contains seven capsular polysaccharides of common serotypes of SPn known to cause invasive disease, each of which is conjugated to diphtheria protein (cross reactive material- CRM_{197})

1.1.2 Synthetic Oligosaccharide based bacterial vaccines

Vaccines based on isolated natural PS have been used very successfully to protect humans against various pathogens and diseases. In principle, synthetic oligosaccharides can also be conjugated to a carrier protein with conventional coupling chemistry, to obtain semi-synthetic conjugate vaccines. A major highlight in the development of the synthetic vaccines was the success of immunization with conjugates containing the purified CPS of *Haemophilus Influenza* type b to eradicate childhood meningitis, which was licensed in Cuba in 2003.[14] This vaccine (the only one currently marketed), available under the trade name Quimi-Hib, is now routinely used for immunization of infants and children.

1.1.2.1 Preparation of neoglycoconjugates- Conjugation Techniques

Conjugation of polysaccharides to a protein by random activation results in lattice-type complexes with an undefined structure. Structural factors that affect immunogenicity of the conjugates are: (i) the size of the saccharide (ii) terminal residue (iii) saccharide loading on the protein (iv) conjugation method and (v) linker geometry.

There are a number of techniques available for covalently linking PS to proteins.

Covalent linking of a molecule to a carrier confers enhanced immunogenicity and T-cell dependence.[15] Coupling methods in which saccharides are coupled to a protein via their reducing end are preferred, since the resulting products are better defined. [16]

The most commonly employed methods include:

1) Reductive amination,[17]wherein the aldehyde or ketone group on one component of the reaction reacts with the amino or hydrazide group on the other component. The C=N double bond formed is subsequently reduced to C-N single bond by a reducing agent.
2) Cyanylation conjugation,[18]wherein the polysaccharide is activated either by cyanogen bromide (CNBr) or by 1- cyano-4-dimethylammoniumpyridinium tetrafluoroborate (CDAP),[19] to introduce a cyanate group to the hydroxyl group, which forms a covalent bond to the amino or hydrazide group upon addition of the protein component.
3) Carbodiimide reaction, [20,21] wherein carbodiimide activates the carboxyl group on one component of the conjugation reaction, and the activated carbonyl group reacts with the amino or hydrazide group on the other component. These reactions are also frequently employed to activate the components of the conjugate prior to the conjugation reaction.
4) Squaric acid diester conjugation, wherein a monoamide is formed between the ligand and the squaric acid diester. The derivative is then attached to an amino group of another low molecular weight amine (e.g.) lysine via controlled formation of 1,2- bisamide of the squaric acid. [22,23,24]

1.1.2.2 Synthetic pneumococcal OS-protein conjugates: promising vaccine candidates

1.1.2.2.1 *Streptococcus pneumoniae* type 3

Oligosaccharide fragments in the spacer linked form (**1.1, 1.2**, and **1.3**, Figure 1.3) were conjugated to CRM_{197} using the squarate coupling method. The average molar ratio of the carbohydrate to protein was in the range of 3 to 12. The protective immunity was studied in inbred female mice subcutaneously immunized twice at three week interval with 2.5 μg of oligosaccharide per mouse. All mice immunized with the tri- and tetrasaccharide conjugates developed SPn3 binding IgG antibodies.[25] This study also proved that immunogenicity was not influenced by the number of saccharide chains per protein.

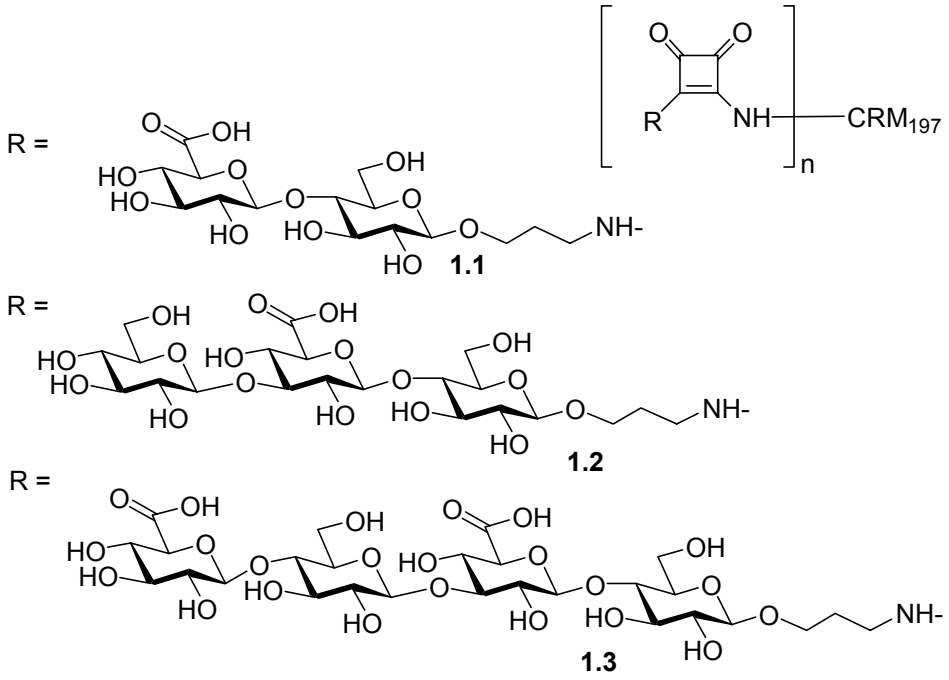

Figure 1.3 SPn3 conjugates

1.1.2.2.2 *Streptococcus pneumoniae* type 6B

Three overlapping fragments of the synthesized SPn6B saccharides (**1.4, 1.5, 1.6**, Figure 1.4) were coupled via the 3-aminopropyl spacer to carrier protein Keyhole Limpet Hemocyanin (KLH) using carbodiimide condensation method. The immunogenicity and protective capacity of these conjugates were studied in mice and rabbits.[20] In rabbits all these conjugates evoked high levels of type 6B CPS- specific IgG antibodies. The antibody levels with the tetrasaccharide conjugate were higher than they were with the KLH conjugate of the native CPS. In mice, the antibodies elicited by the tetrasaccharide conjugate **1.6** was phagocytic and protective. Also the disaccharide **1.4** contained a serotype 6B specific epitope, the trisaccharide contained a common 6A and 6B epitope, the tetrasaccharide contained both 6B specific and a common 6A and 6B epitope. These results proved that the disaccharide and tetrasaccharide conjugates already contain epitopes capable of inducing 6B- specific, fully protective antibodies in rabbits and mice, respectively.

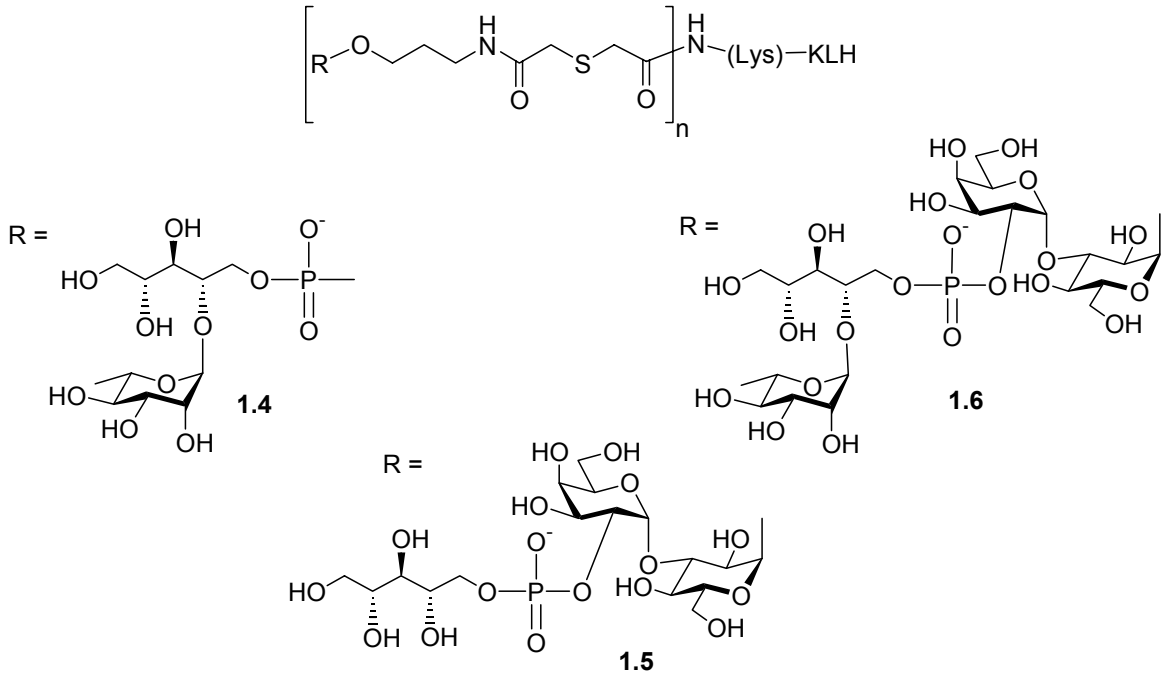

Figure 1.4 SPn6B conjugates

1.1.2.2.3 *Streptococcus pneumoniae* type 14

Synthetic oligosaccharide fragments **1.7** (tetra-), **1.8** (hexa-) and **1.9** (octa-) related to SPn14 were conjugated to CRM_{197} via the carbodiimide reaction and the squaric acid diester coupling methods. The average degree of incorporation of OS chains into CRM_{197} was in the range 4 to 24.[26] In accordance to the inhibition studies, the tetrasaccharide-CRM_{197} conjugate **1.7** with either the adipic acid or squarate linker, elicited a PS-specific IgG response after a booster injection, whereas the octasaccharide mimic **1.9** gave very low responses or even remained non-immunogenic. Hence, the OS-CRM_{197} conjugate prepared from the single repeating unit of SPn14 can be considered as a potential vaccine candidate.

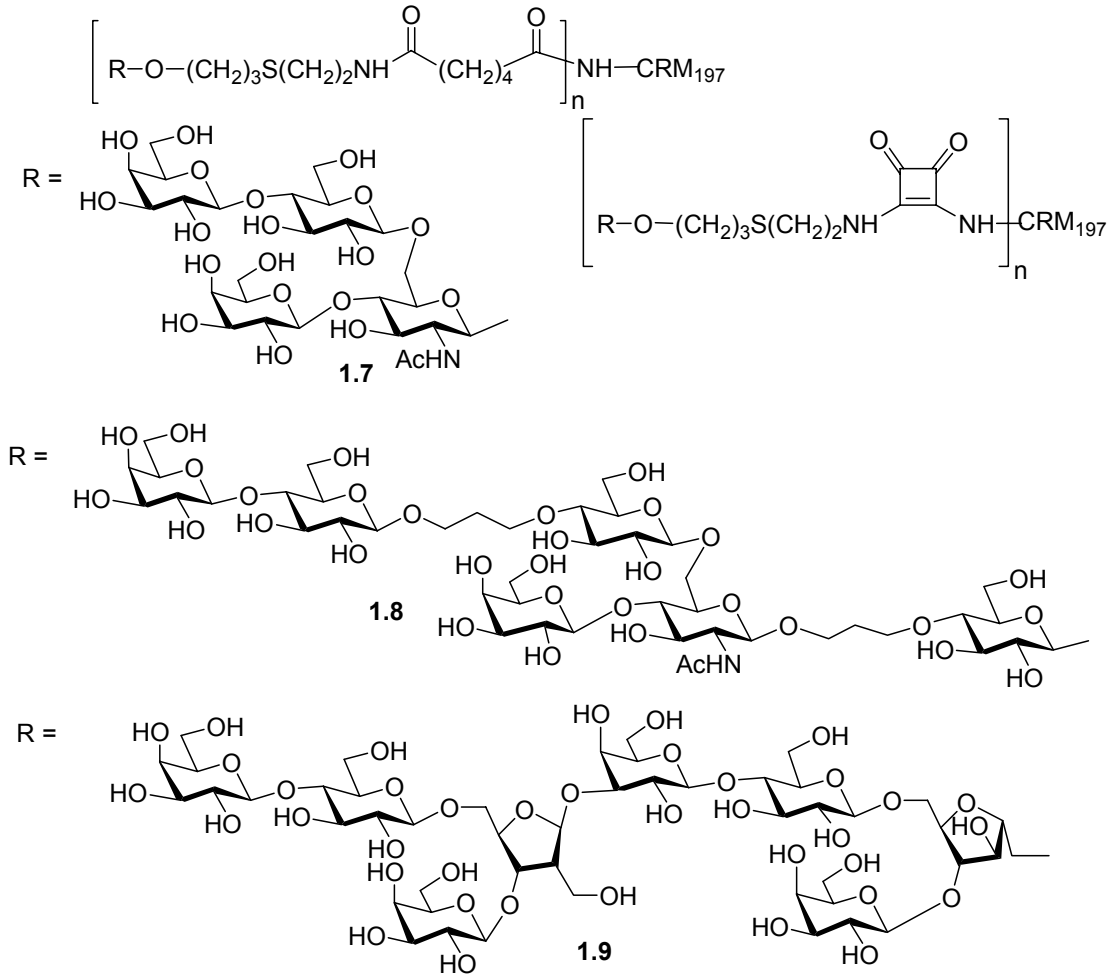

Figure 1.5 SPn14 conjugates

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1.2 Strategies for Oligosaccharide Synthesis

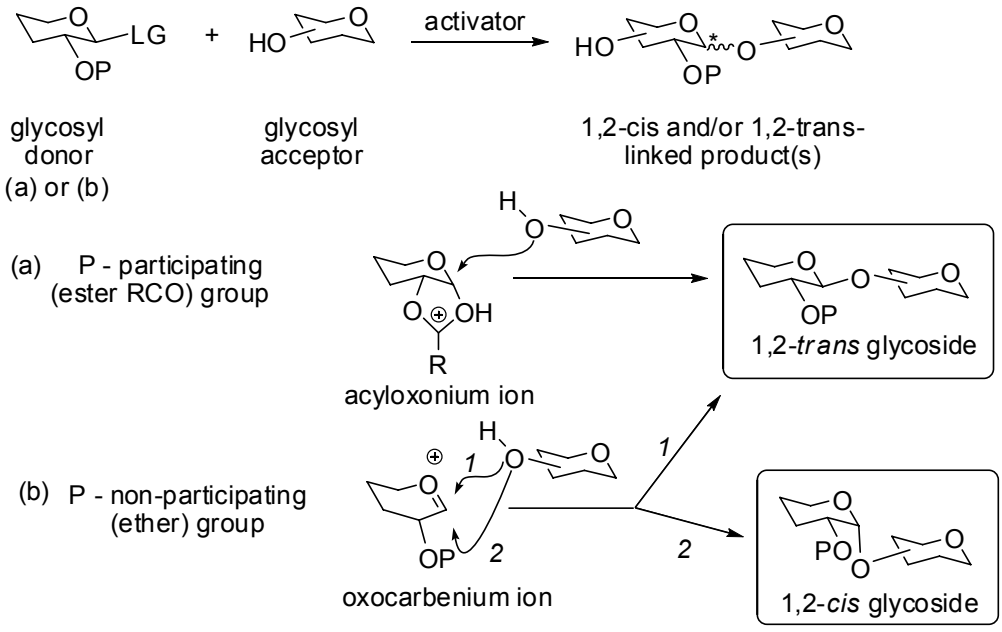
Complex carbohydrates are involved in many biological phenomena and their involvement in life-threatening processes, in particular, has associated this class of natural compounds with a tremendous diagnostic and therapeutic potential. Many synthetic laboratories have focused their research efforts towards carbohydrates associated with diseases that consistently rank among the leading causes of death worldwide, *i.e.* bacterial and viral infections, cancer, etc.[1] At the core of this phenomenal international effort is the belief that a comprehensive knowledge of the structure, conformation, and properties of these carbohydrates would aid to elucidation of the mechanisms for the pathogenesis of the disease. Consequently, this could lead to the development of effective tools for the prevention, diagnosis, and/or treatment of these diseases.

Over the years, glycoscientists have learned to isolate certain classes of naturally occurring carbohydrates. The availability of pure natural isolates, however, remained inadequate to address the challenges offered by modern glycosciences. As a result, glycoscientists have turned to chemical synthesis as a means to access complex carbohydrates. The expectations are that the efficient chemical synthesis should yield significant quantities of pure natural analogs and would be the only means to access their unnatural mimetics. Pharmaceutical and biotechnology companies have already demonstrated an interest in producing complex synthetic oligosaccharides or glycoconjugates; however, the examples including heparin and its analogs,[2] oligosaccharide antibiotics,[3] carbohydrate-based vaccines,[4] and other therapeutic agents[1,5] are scarce. Carbohydrates of even moderate complexity, however, still represent a

considerable challenge. As such, the development of efficient methods for the expeditious synthesis of complex carbohydrates stands out as an important area of research.

The glycosidic linkage is obtained by means of the glycosylation reaction, arguably the most important and challenging reaction in carbohydrate chemistry.[6] A typical chemical glycosylation is based on the nucleophilic displacement of a leaving group (LG, Scheme 1.1) on the glycosyl donor with a hydroxyl moiety of the glycosyl acceptor. The remaining hydroxyls of both units are temporarily masked with protecting groups (P). The achievement of high yields and complete stereocontrol is difficult due to complexity of the glycosylation process. The necessity to form either a 1,2-*cis* or a 1,2-*trans*-glycosidic bond with complete stereoselectivity remains the main reason chemical *O*-glycosylation remains amongst synthetic challenges.[6]

Various factors affect the stereoselectivity of glycosylation amongst which the neighboring substituent at C-2 is one of the major players. If the use of a base-labile ester-protecting groups is permitted, 1,2-*trans* glycosides can be prepared with the assistance of a neighboring participating group.[7] These glycosylations proceed primarily via a bicyclic acyloxonium ion intermediate that can only accept the top face nucleophilic attack resulting in the stereoselective formation of a 1,2-*trans* glycoside (Scheme 1.1). In case of a non-participating substituent, the flattened oxocarbenium ion is formed as the major reaction intermediate. As a result, the nucleophilic attack is nearly equally possible from either the top (*trans,* β- for the D-gluco series) or the bottom face (*cis,* α-) of the ring. Even though the α-product is thermodynamically favored (anomeric effect),[8] a substantial amount of the kinetic β-linked product is often obtained.



Scheme 1.1. **Outline of chemical glycosylation**

Another important factor affecting the outcome of glycosylations is the nature of the anomeric leaving group. As a result, a large number of glycosyl donors have been developed.[9] In addition to traditionally used chlorides or bromides,[10] more recent glycosyl donors such as Schmidt's O-trichloroacetimidates,[11] Mukaiyama's fluorides,[12,13] alkyl/aryl thioglycosides pioneered by Ferrier, Nicolaou, and Garegg,[14] Fraser-Reid's O-pentenyl glycosides,[15,16] and Danishefsky's glycal-epoxide system[17] have become valuable alternatives.

Traditional linear approaches for the synthesis of oligosaccharides involve extensive protecting group manipulations between each glycosylation step.[18] Such additional manipulations increase the number of total synthetic steps and decrease the efficiency of the oligosaccharide assembly reflected in a dramatic drop in yield. This low accessibility of medicinally relevant compounds complicates their investigation, and questions the feasibility of their application in multi-kilogram drug/vaccine development. This called

for revisiting the existing linear strategy for oligosaccharide synthesis and some significant improvements have already emerged. More recent strategies for oligosaccharide synthesis are typically based on the selective activation of one leaving group over another.[18,19] These expeditious approaches significantly shorten oligosaccharide assembly by minimizing the need for protecting group manipulations between glycosylation steps. Nicolaou's selective activation,[12] Fraser-Reid's armed-disarmed approach,[20] Danishefsky's glycal assembly,[17] Ogawa's orthogonal technique,[21,22] Roy's[23] and Boons'[24] active-latent concept are only a few of those that could be mentioned.

These excellent innovations have allowed scientists to synthesize complex oligosaccharides and glycoconjugates, which were practically inaccessible for decades. Nevertheless, those syntheses were still very far from being simple and it has become apparent that only selected sequences can be accessed by these innovative techniques: each target still required careful selection of methods, conditions, and strategies. These notable gaps stimulated additional scientific efforts that recently resulted in the development of a new pool of methods for the stereoselective synthesis of challenging glycosidic linkages and strategies for expeditious oligosaccharide synthesis. One-pot strategies,[25] including programmable strategies developed by Ley's and Wong's groups,[26-29] reactivation-based sequential couplings by Huang's[30-32] and van der Marel's groups,[33] Crich's synthesis of β-mannosides,[34] Gin's dehydrative method,[35] Seeberger's polymer-supported automated synthesis,[36] Lowary's work on furanosides,[37] Gervay's glycosyl iodide approach,[38] Hung's work of one-pot protection of sugars,[39] Boons' stereodirected synthesis of α-glycosides,[40] and fluorous-tag assisted glycosylations in

micro-reactors[41] are only few examples of recent significant breakthroughs to mention.

1.2.1 One-pot strategies, an overview

Conceptually, one-pot strategies offer the shortest pathway to oligosaccharides, as the sequential glycosylation reactions are performed in a single flask (pot) and do not require purification of the intermediates. It is very important for a successful one-pot glycosylation that there is complete consumption of the intermediate formed and a high yield. In addition, the requirement for very high or preferably complete stereoselectivity in every glycosylation step becomes particularly important. These will ensure that only the target oligosaccharide is being produced as the major product, thus providing high efficiency of this technique. Although many variations of the one-pot strategy have been developed, these protocols are based on five major concepts (Scheme 1.2) or a combination thereof:

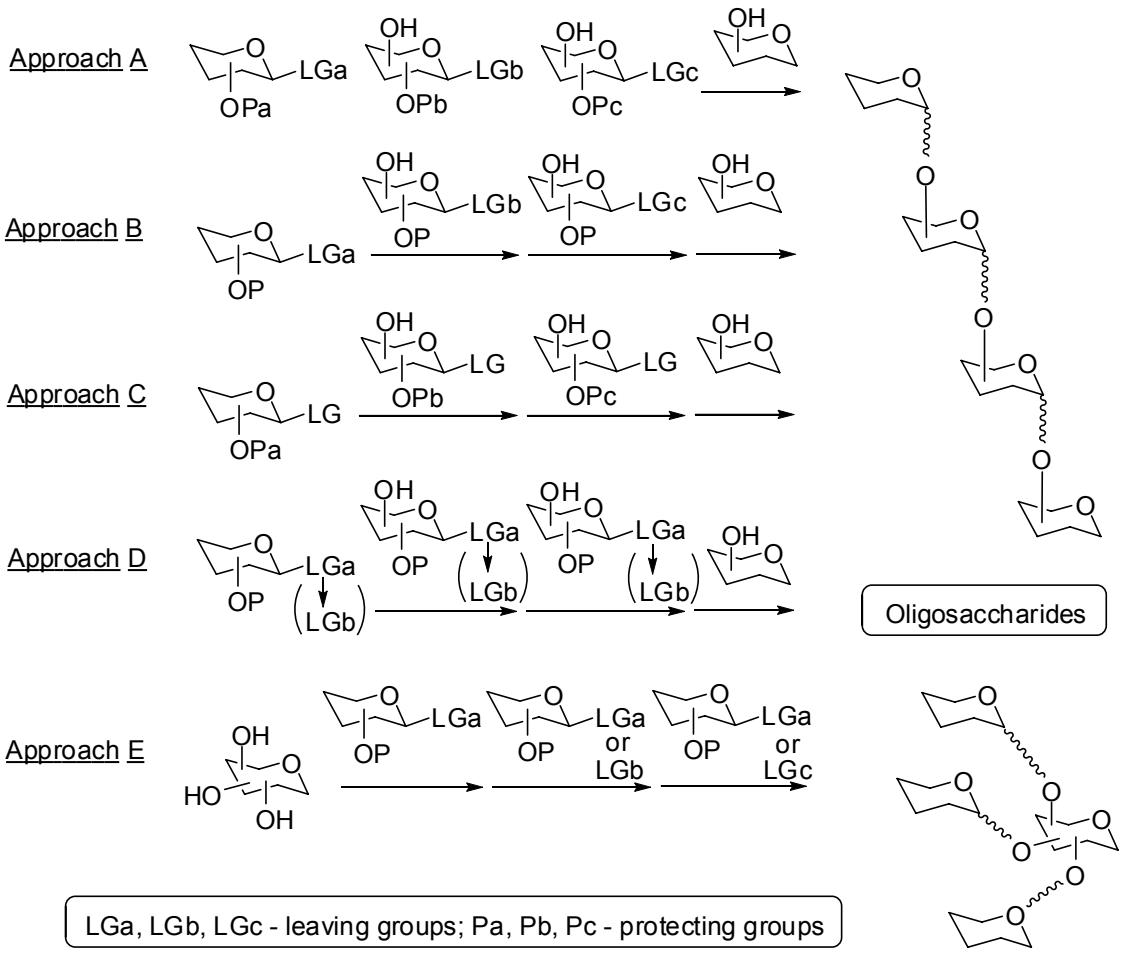
(i) Fine-tuning of all reactants or pure one-pot concept

(ii) Selective activation or leaving group based concept

(iii) Chemoselective activation or protecting group based concept

(iv) In-situ pre-activation strategy or leaving/protecting group independent concept

(v) One-pot glycosylation based on polyol acceptors or bidirectional concepts



Scheme 1.2 Major concepts for the one-pot oligosaccharide assembly

Approach A, the first ever discovered and the conceptually pure in its entirety one-pot approach is performed with all reaction components present from the very beginning. It requires fine tuning of all reaction components, according to which the most reactive leaving group reacts with the most reactive hydroxyl. Subsequent reaction takes place between the most reactive leaving group and hydroxyl remaining after the first step has been completed, etc. The Approach B is based on selective activation of one type of a leaving group over another. Since this is a leaving group based concept, one should be flexible with the protecting groups, which is an important factor for the stereoselectivity control.

The Approach C is relying on the chemoselectivity principle, according to which the reactivity difference between the glycosyl donor and the glycosyl acceptor is achieved by varying the electronic properties of the protecting groups in either or in both of the reaction components. Thus, this approach in its conventional mode requires a set of building blocks with the same type of a leaving group, the reactivity of which is differentiated by the protecting group pattern. A common feature of the approaches B and C is that both are reactivity- based, regardless whether the difference in reactivity is achieved by the protecting groups or the leaving group. Being more reactive under certain reaction conditions, the glycosyl donor unit is activated over the less reactive glycosyl acceptor. Subsequently, the saccharide obtained then serves as a glycosyl donor under adjusted (typically more harsh) reaction conditions. For this purpose, a suitable promoter, along with the glycosyl acceptor, is added for the next step.

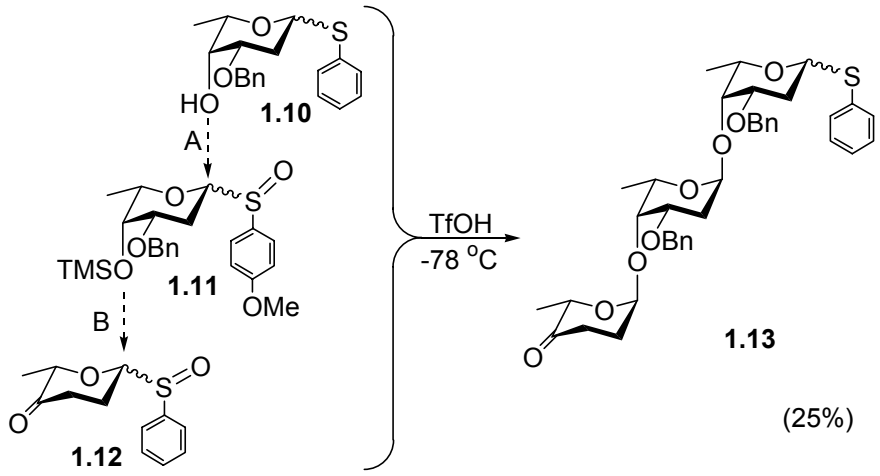
Differently, the recently established in-situ preactivation strategy (Approach D) is independent on the building block reactivity since the leaving group of the glycosyl donor is first converted into a highly reactive species (preactivation). After that, the acceptor is added to yield a coupling product bearing the identical leaving at the reducing end that can be subsequently pre-activated. On one hand, this approach involves a number of additional steps as the glycosylation is virtually a two-step reaction, it is compensated by offering more flexibility in the context of relative independency on the leaving and/or protecting groups.

As opposed to the previously describes strategies, approach E is exclusively based on the reactivity of glycosyl acceptors. The sequential regioselective glycosylations are often accomplished due to very different levels of reactivity between the hydroxyls. In this

case, the same type of leaving group can be used. Alternatively, reciprocal reactivity may take place, according to which certain hydroxyl may prefer certain class of a glycosyl donor. Apparently, if a polyol acceptor is employed, this leads to the formation of branched oligosaccharides. A variation of this approach is when other, typically less reactive hydroxyls are introduced in the glycosyl donor unit. This would result in a bidirectional one-pot glycosylations. Additional hydroxyl can be also generated during the glycosylation, such as with the use of the epoxide glycosyl donors, or liberated by the in situ removal of a certain labile substituent. A number of approaches involving mixed conventions, namely techniques combining two or more concepts, have been also developed.

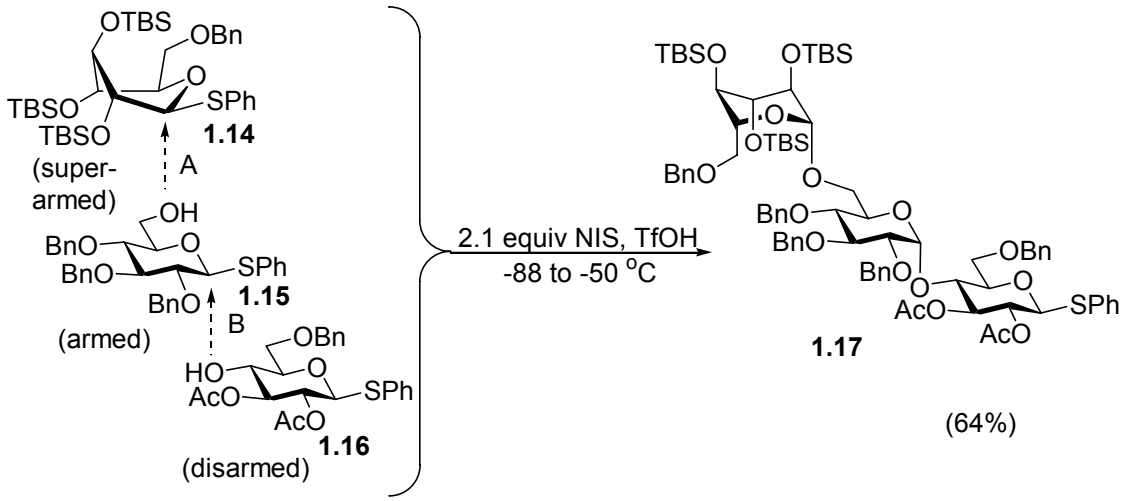
1.2.1.1 Approach A: One-pot glycosylation based on fine tuning of all reaction components

This concept was introduced by Kahne and co-workers and is illustrated herein by a representative synthesis of Ciclamycin 0 trisaccharide depicted in Scheme 1.3.[42] The strategy takes advantage of the difference in reactivities of phenyl sulfoxide donors **1.11** and **1.12**, as well as glycosyl acceptors **1.11** (masked as TMS ether) and **1.10**. All three building blocks **1.10**-**1.13** were mixed in one flask, and to ensure the successful execution of this two-step coupling, and the reaction mixture was cooled to -78 ^{o}C. The activator (TfOH) was then added and rapid coupling between **1.10** and **1.11** took place. The less reactive glycosyl donor **1.12** then slowly reacted with the TMS-protected hydroxyl of the disaccharide intermediate to provide trisaccharide **1.13** in 25% yield.



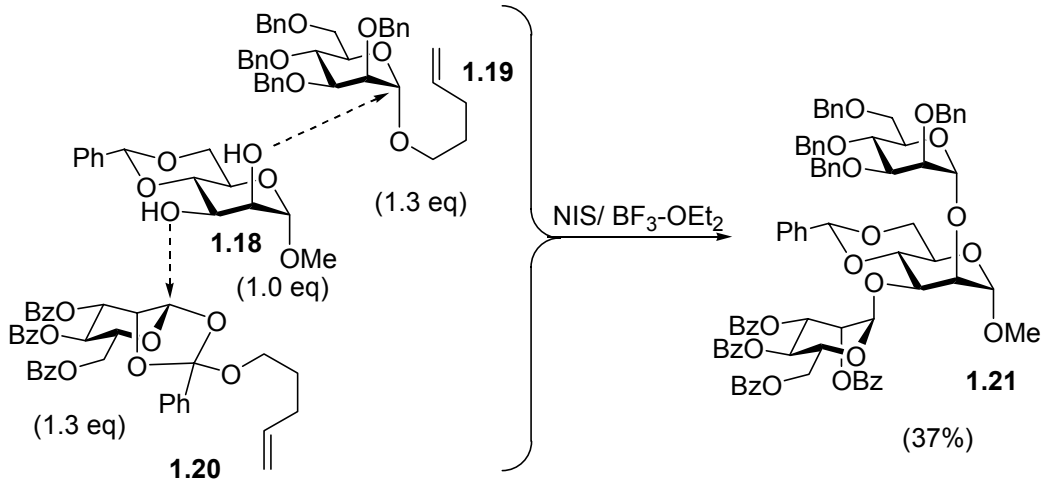
Scheme 1.3 One-pot synthesis of Ciclamycin 0 trisaccharide 1.13

Bols and co-workers discovered a new phenomenon according to which conformationally modified glycosyl donors protected with bulky TBDMS are exceptionally reactive (superarmed).[43,44] This superarmed concept was ultimately illustrated by performing the one-pot coupling with all three reaction components (thioglycosides **1.14-1.17**) mixed from the very beginning (Scheme 1.4).[44] This type of one-pot technique required the differentiation between reactivity levels of both glycosyl donors (**1.14** and **1.15**) and glycosyl acceptors (**1.15** and **1.16**) – all are bearing the same anomeric leaving group (thiophenyl). First, reaction between the super-armed glycosyl donor **1.14** and more reactive primary (and also more electron rich due to the neighboring benzyl substituents) glycosyl acceptor **1.15** took place. The resulting disaccharide derivative then reacted with the remaining glycosyl acceptor **1.16**. As a result of this one-pot coupling in the presence of NIS/TfOH, trisaccharide **1.17** was obtained in 64% yield.



Scheme 1.4 Conformationally superarmed glycosyl donors in one-pot glycosylations

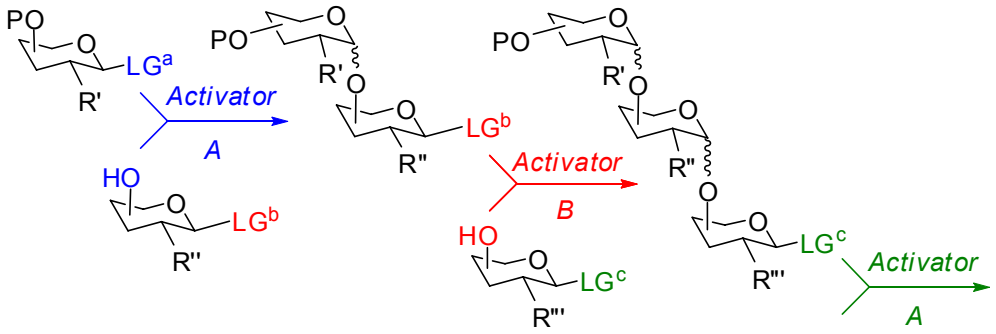
A related one-pot synthesis making use of a reciprocal donor-acceptor selectivity was reported by Fraser-Reid and co-workers.[45] Thus, when a mixture of diol **1.18**, n-pentenyl donor **1.19** and n-pentenyl orthoester donor **1.20** was treated with NIS/BF_3-OEt_2, trisaccharide **1.21** was obtained (Scheme 1.5). Since no other trisaccharide regioisomer was detected, this result clearly indicated a reciprocal choice of donor **1.20** for the 3-OH whereas the donor **1.19** preferred the 2-OH. It should be noted that this particular synthesis conceptually differs from the acceptor-based techniques classified as Approach E. Herein, all reaction components (including the activators) are present from the very beginning, whereas in Approach E, the reactants are added upon completion of the previous step.



Scheme 1.5 One-pot synthesis based on reciprocal donor-acceptor selectivity

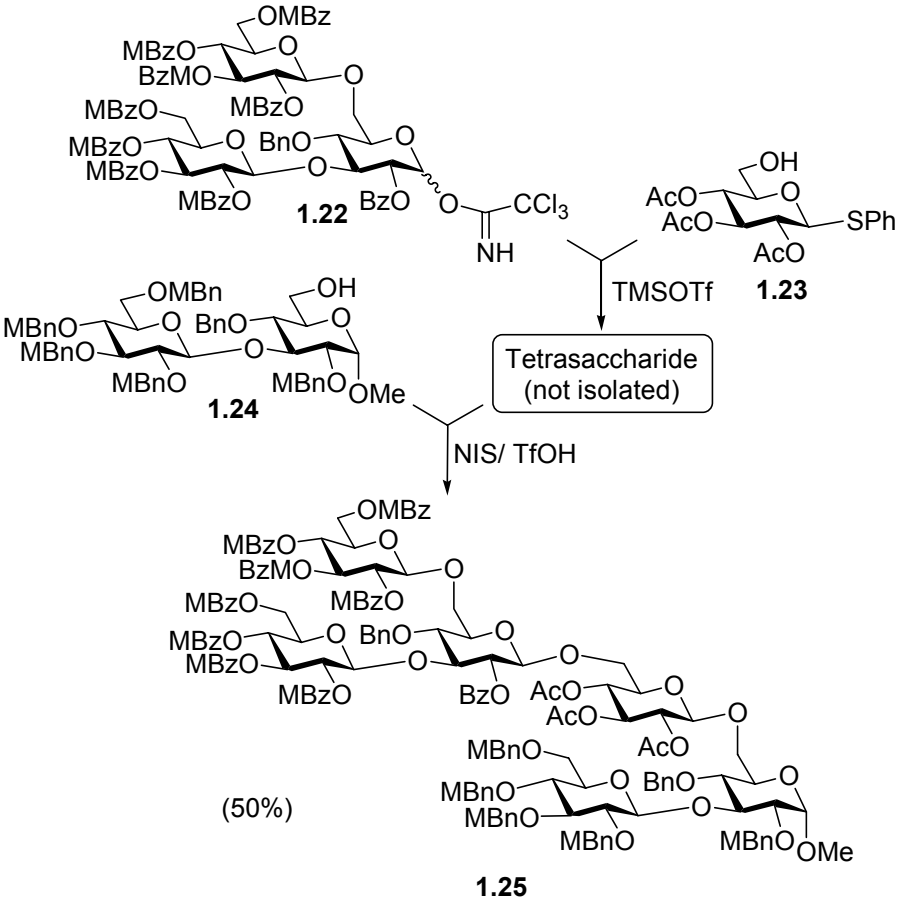
1.2.1.2 Approach B: One-pot glycosylation based on selective activation

The use of selective activation strategy for oligosaccharide sequencing is easy to envisage. As illustrated in Scheme 1.6, it requires a set of suitable leaving groups (LGa, LGb, LGc) that can be selectively activated in a sequential manner. It also requires suitable activator that would activate one leaving group over another: A (selectively activates LGa), B (selectively activates LGb), etc.. This general strategy was first popularized by Zen,[46] Nicolaou,[47,48] Lonn,[49] Garegg,[50] and others[51] for stepwise synthesis, and a large number of one-pot syntheses have been developed accordingly. Some representative examples of these one-pot syntheses are discussed below.



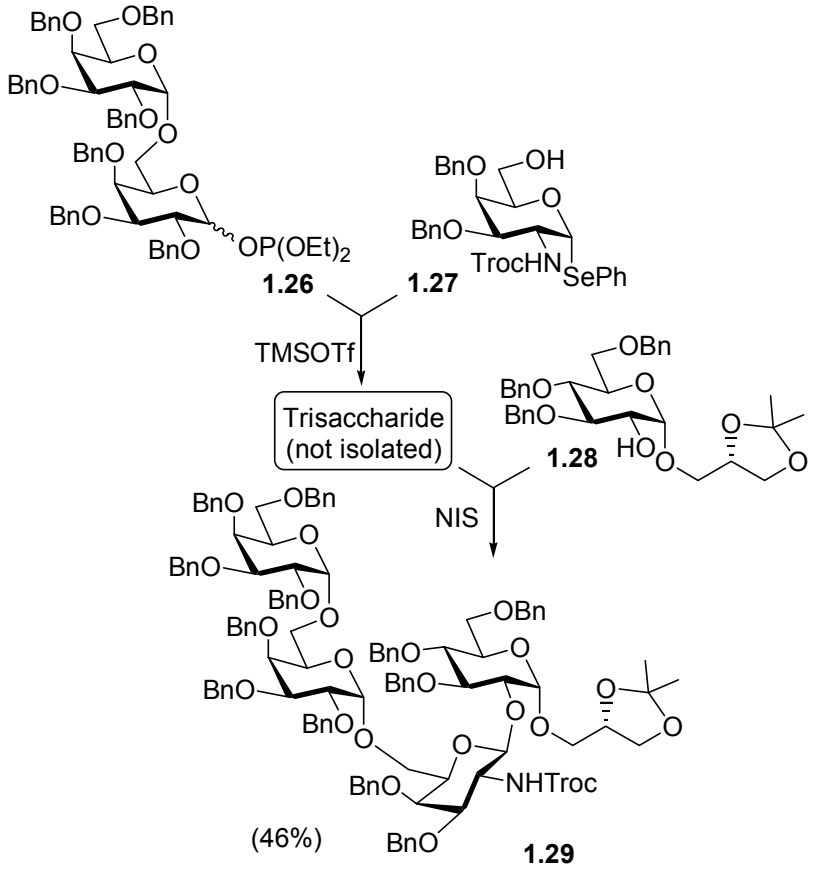
Scheme 1.6 Oligosaccharide assembly based on selective activation

The first one-pot synthesis based on the leaving group based activation concept was reported by Takahashi and co-workers.[52] The synthesis involved the use of trichloroacetimidate donor **1.22**, which was selectively activated over the thioglycoside **1.23** with TMSOTf (Scheme 1.7). The SPh moiety of the formed, but not isolated, tetrasaccharide was then activated with NIS and TfOH over the added glycosyl acceptor **1.24** to provide hexasaccharide **1.25** in 50% yield over two steps. This approach incorporates advantages of the convergent block strategy according to which pre-synthesized oligosaccharide building blocks *i.e.* **1.22** and **1.24** are converged together during the final assembly.



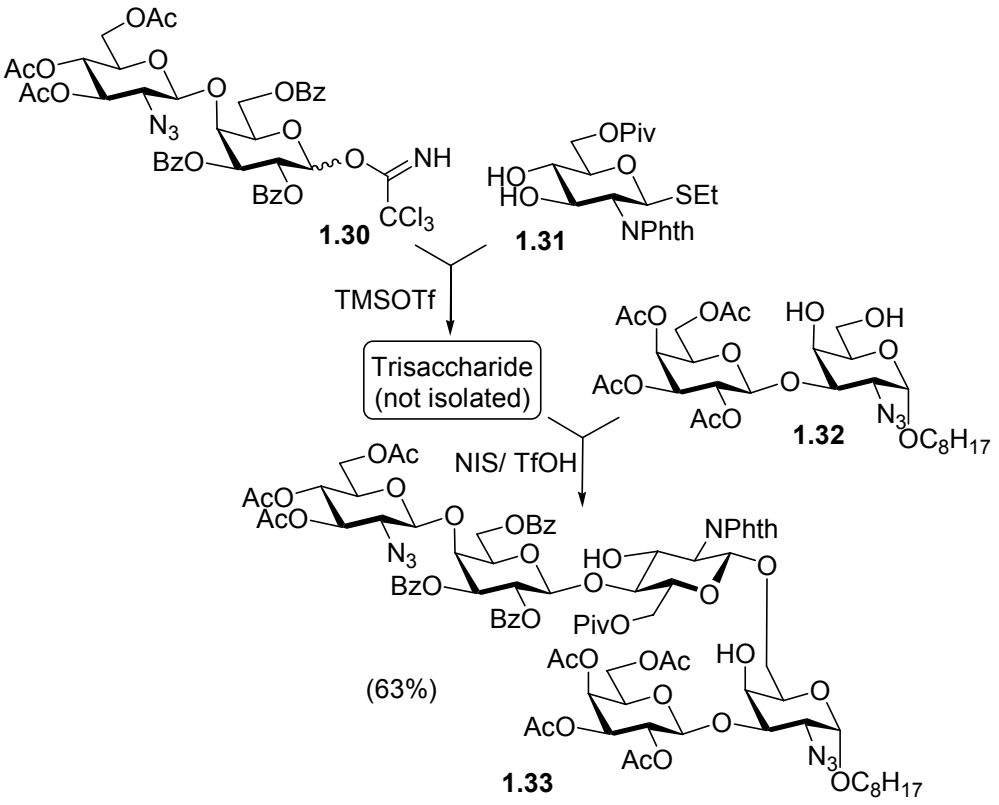
Scheme 1.7 Convergent one-pot synthesis by selective activation

In a similar fashion, a tetrasaccharide glycosyl glycerol which represents the core structure of a glycoglycerolipid of *M. taiwanesis,* based on a three-component one-pot strategy was synthesized.[53] A trisaccharide intermediate was formed from the glycosyl phosphite **1.26**, which was selectively activated over selenoglycoside acceptor **1.27** in the presence of TMSOTf. Subsequent addition of acceptor **1.28** and NIS allowed to produce the tetrasaccharide **1.29** in 46% yield over-all (Scheme 1.8).



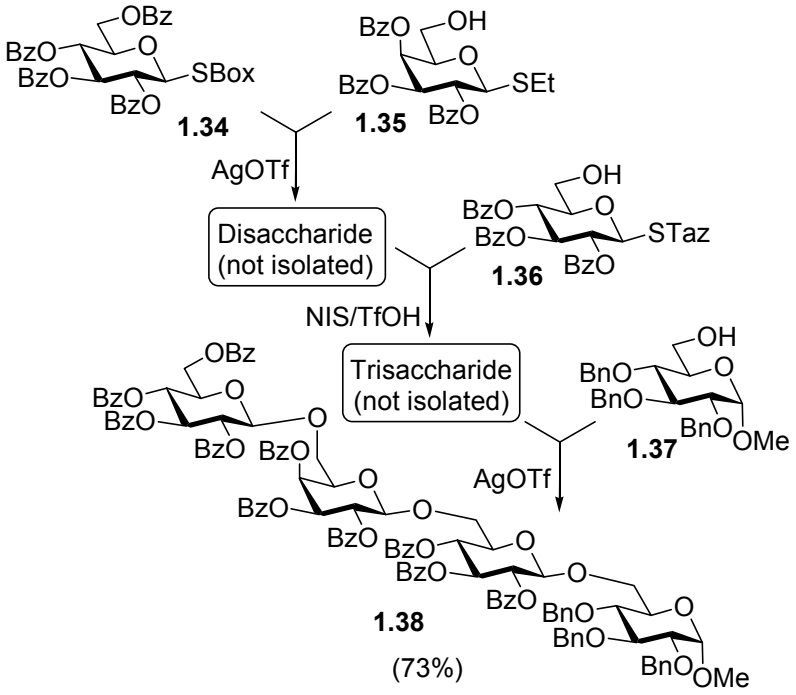
Scheme 1.8 One-pot synthesis of glycoglycerolipid analog

Seeberger and co-workers presented an efficient one-pot synthesis of a pentasaccharide implicated as a potent antibiotic against *H. pylori*.[54] Accordingly, disaccharide trichloroacetimidate **1.30** was activated with TMSOTf to react regioselectively at the C-4 hydroxyl group of the thioglycoside **1.31** (Scheme 1.9). Addition of the disaccharide acceptor **1.32** to the reaction mixture, followed by the addition of NIS/TfOH to promote the thioethyl group of the *in situ* formed trisaccharide completed the sequence. The requisite pentasaccharide **1.33** was isolated in 63% yield over-all.



Scheme 1.9 One-pot synthesis of a pentasaccharide antibiotic

A representative example involving three sequential glycosylation steps is shown in Scheme 1.10.[55] This three step activation was achieved by the stepwise activation of S-benzoxazolyl (SBox) glycoside donor **1.34** over S-ethyl glycoside acceptor **1.35** by addition of AgOTf. Thioethyl moiety of the resulting disaccharide intermediate was then activated over the added S-thiazolinyl (STaz) glycoside **1.36** by the addition of NIS and catalytic TfOH. Finally, the STaz moiety of the trisaccharide intermediate is reacted with freshly added glycosyl acceptor **1.37** in the presence of additional quantity of AgOTf. As a result, the linear tetrasaccharide **1.38** was obtained in 73% yield over three steps.

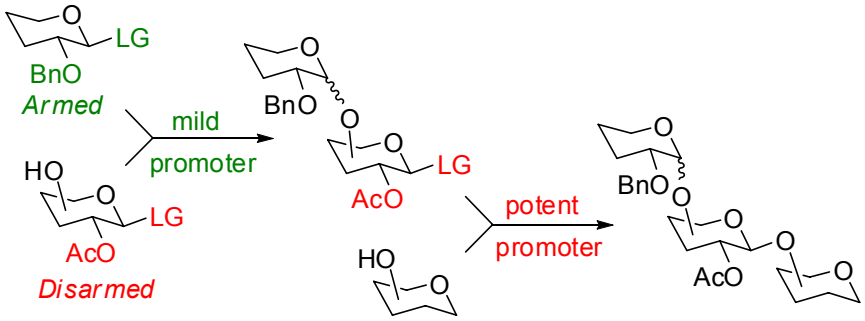


Scheme 1.10 One-pot synthesis of tetrasaccharide 1.38 by the three-step sequential selective activation.

In principle, conceptually related Ogawa's orthogonal strategy offers even more flexible oligosaccharide sequencing.[21] It only requires a set of two orthogonal leaving groups (LGa and LGb) and a pair of activators: A (selectively activates LGa over LGb) and B (selectively activates LGb over LGa). Unfortunately, this relatively simple concept is still limited to only few examples,[21,56] and to the best of our knowledge, only one one-pot synthesis involved the features of the orthogonal concept (discussed below in subsection 2.6).[57]

1.2.1.3 Approach C. One-pot glycosylation based on chemoselective activation

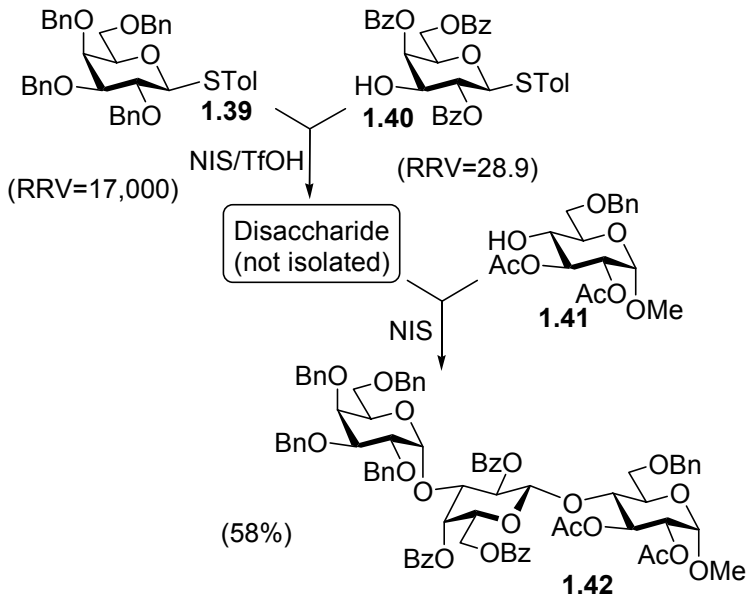
One of the most conceptually attractive and practically efficient procedures, Fraser-Reid's *armed-disarmed approach,* is based on the principle of chemoselectivity. Thus, chemoselective activation of a benzylated (electronically activated, armed) glycosyl donor can be achieved over the acylated (electronically deactivated, disarmed) glycosyl acceptor bearing the same type of leaving group (Scheme 1.11).[58,59] The availability of a suitable mild promoter is essential to effectively differentiate the reactivity levels of the armed and disarmed reactants. The obtained disaccharide can then be used for subsequent glycosylation directly, but would generally require a more potent promoter (or elevated temperature) that can activate the disarmed leaving group. Initially developed for *O*-pentenyl glycosides,[16,59] this approach works very well with many other classes of glycosyl donors.[18]



Scheme 1.11 Chemoselective armed-disarmed strategy

A significant progress in the area of chemoselective oligosaccharide synthesis has emerged with the development of programmable oligosaccharide strategy that became a logical extension of studies pioneered by Fraser-Reid's, van Boom's, Ley's, and Wong's groups. Attempts to classify and even predict the outcome of a glycosylation (or a sequence) resulted in the development of approaches to quantify the reactivity of building

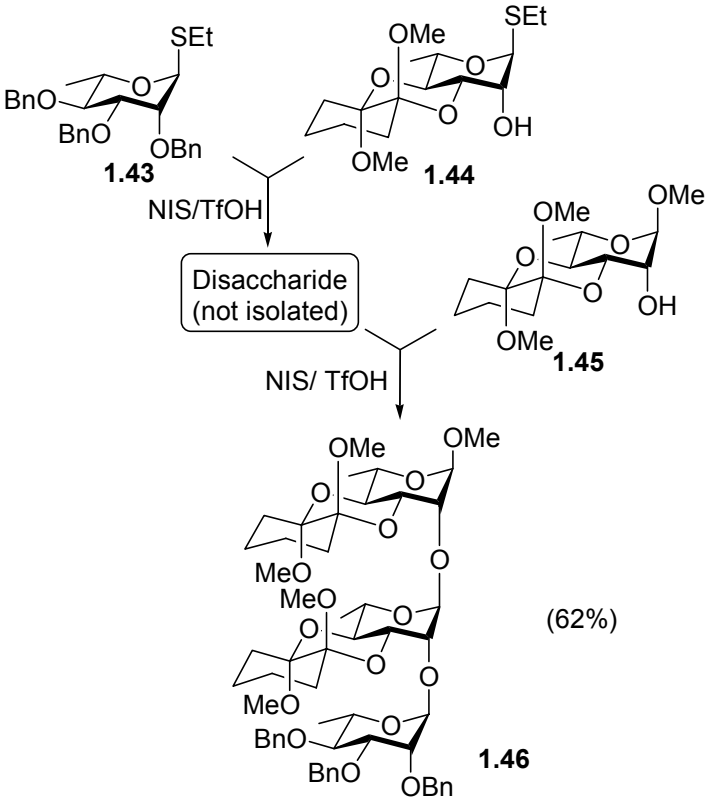
blocks.[26,27,60,61] Amongst these, a database of the relative reactivity values (RRVs) of different glycosyl donors has been generated compiled into a predictive computer program called Optimer.[27] It was noted that the optimal chemoselective couplings generally occurred when there was a large difference in the RRVs. For example: thus, selective activation of glycosyl donor **1.39** (RRV = 17,000) over glycosyl acceptor **1.40** (RRV = 28.9) in the presence of NIS/TfOH resulted in the formation of a disaccharide derivative.[27] The latter was not isolated; instead, glycosyl acceptor **1.41** and additional NIS were added to the reaction mixture. As a result of this two-step sequential activation, trisaccharide **1.42** was isolated in 58% overall yield. Further exploration of this approach by Wong's group has resulted in a well-rounded and extremely useful technology for one-pot oligosaccharide synthesis.[28,62]



Scheme 1.12 Programmable synthesis of a trisaccharide 1.42

If one would rely exclusively on the disarming effect of the neighboring substituents, these chemoselective syntheses would be limited to the synthesis of *cis*-*trans* patterned trisaccharides only. This stimulated subsequent investigation of the arming/disarming

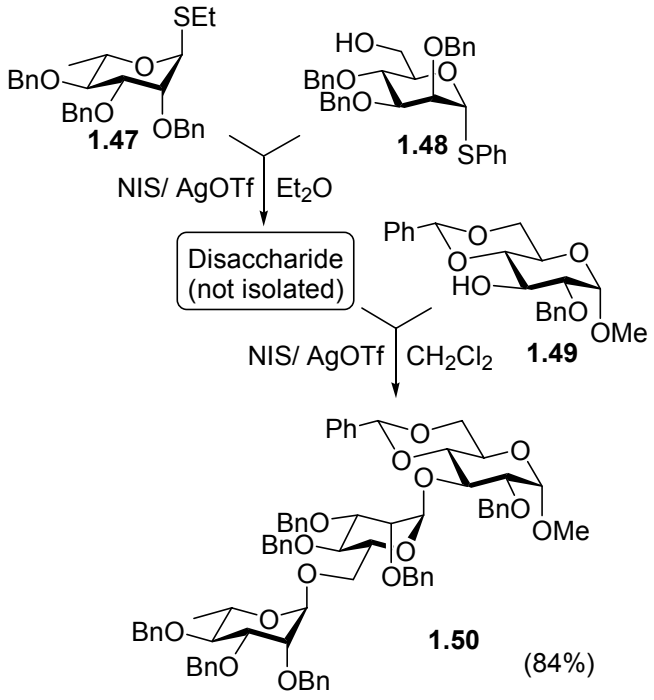
effects, and a number of useful improvements have already emerged. Amongst effects investigated,[63] torsional deactivation by cyclic substituents was arguably the most relevant to the one-pot syntheses. Fraser-Reid[60] and Ley[64] found that the anomeric deactivation can also be achieved by the torsional effect of cyclic acetal/ketal protecting groups that impart rigidity into the structure. This rigidity hinders the flattening of the ring during the formation of the oxocarbenium ion. This disarming effect was further studied by Bols,[65] who concluded that there is roughly equal deactivation from both torsional and electronic effects.



Scheme 1.13 One-pot synthesis of trisaccharide controlled by torsional deactivation

For instance, in application to one-pot synthesis, Ley and co-workers reported the synthesis of a trisaccharide unit of a group B *streptococci* polysaccharide antigen as

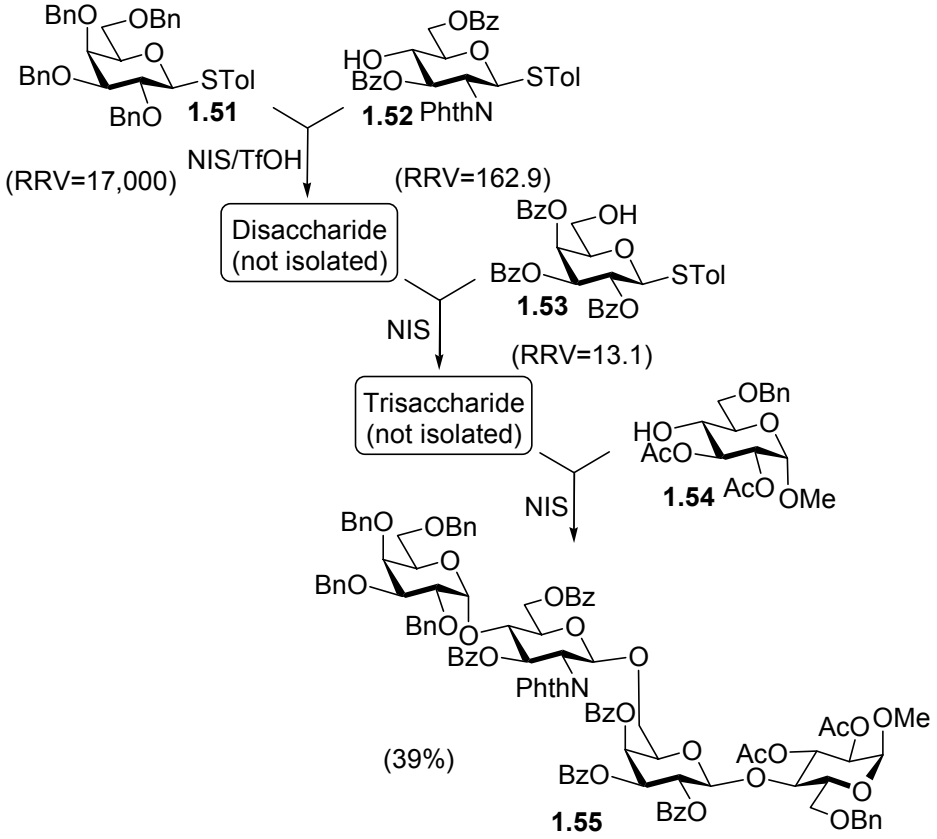
depicted in Scheme 1.13.[66] A cyclohexane-1,2-diketal protected thioglycosyl acceptor **1.44** with its reactivity tuned down via the torsional effect, this in turn would hinder the formation of the flattened cationic transition state. A similar concept was applied to the syntheses of linear and branched oligosaccharides by utilizing mixed one-pot conventions (one relevant example is discussed in section 1.2.2).[67,68]



Scheme 1.14 One-pot synthesis of trisaccharide 1.50 employing solvent effects

The reactivity difference between similarly protected sugars of different series has to be also taken into consideration. In the following example from Oscarson's group, this difference in reactivity along with an interesting solvent effect allowed an efficient one-pot oligosaccharide synthesis.[69] As shown in Scheme 1.14, the first activation could take place in Et_2O allowing for relatively low glycosylation rate due to limited solubility of NIS/ AgOTf in diethyl ether. This required the application of the highly reactive

rhamnosyl donor **1.47**, which was successfully activated over "armed" benzylated acceptor **1.48**. Subsequently, the second step is accomplished in CH_2Cl_2/Et_2O allowing for much higher glycosylation rate. As a result, trisaccharide **1.50** was synthesized in 84% yield. In accordance with the Optimer database, the reactivity ratio between per-benzylated S-tolyl glycosides of L-fuco, D-galacto, and D-gluco series was found to be 27.1/6.4/1 respectively.[27]

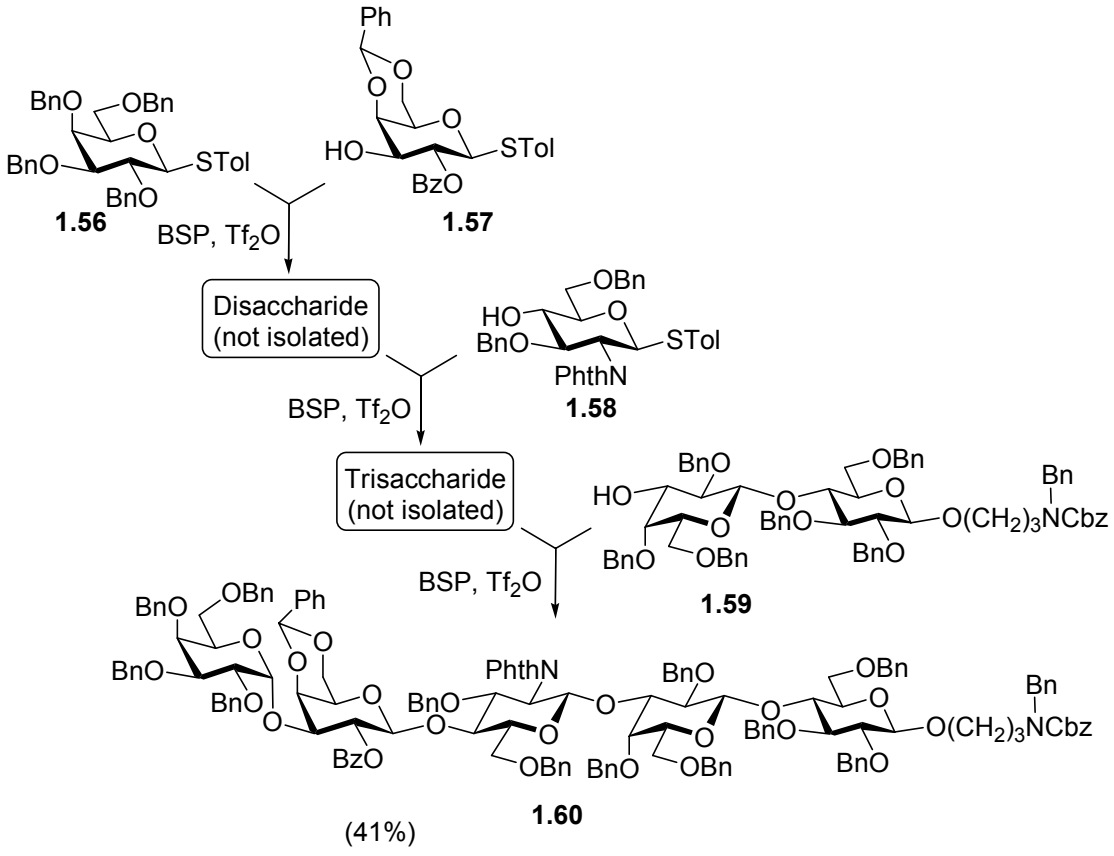


Scheme 1.15 Synthesis of tetrasaccharide 1.55 via programmable three step synthesis

A number of three-step sequential one-pot activations using this concept has also been reported. For example, sequential activation of building blocks **1.51**, **1.52** and **1.53** was anticipated based on their relative reactivity, 17000, 162.8, and 13.1, respectively

(Scheme 1.15).[27] Indeed, glycosyl donor **1.51** was activated over glycosyl acceptor **1.52** in the presence of NIS and TfOH. The resulting disaccharide intermediate was then reacted with the added glycosyl acceptor **1.53** and additional NIS. The trisaccharide intermediate was then glycosidated with added acceptor **1.54** to provide the target tetrasaccharide **1.55** in 39% yield over-all.

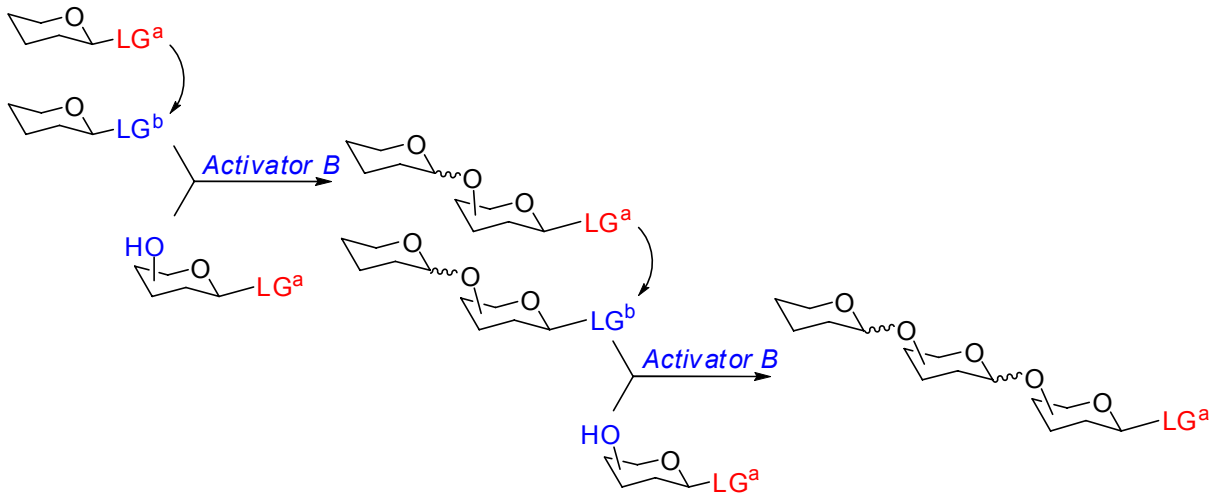
Another three-step activation sequence utilizes the activator system (BSP/ Tf_2O) to carry out the one-pot sequential synthesis of α-Gal pentasaccharide derivative **1.60** as depicted in Scheme 1.16.[70] The synthesis was carried out from the readily available building blocks **1.56-1.59**.



Scheme 1.16 Four component one-pot sequential synthesis of α-Gal pentasaccharide

1.2.1.4 Approach D: One-pot glycosylation based on in-situ preactivation

This strategy integrates the advantages of both the chemoselective and the selective activation strategy, but it does not require reactivity tuning of the building blocks. Two-step activations have been known for long time. In accordance with this concept, both glycosyl donor and glycosyl acceptor initially bear the same type of a leaving group (LG^a). In order to couple these two reactants, the LG^a of the potential glycosyl donor unit is first converted into a LG^b, which is then selectively activated over the LG^a of the glycosyl acceptor in the presence of a selective activator (Activator B, Scheme 1.17). This two-step activation sequence can be reiterated: for this purpose the leaving group LG^a of the obtained disaccharide is converted into LG^b. This will allow the coupling with the glycosyl acceptor bearing LG^a, etc.



Scheme 1.17 Two-step activation (or pre-activation) concept.

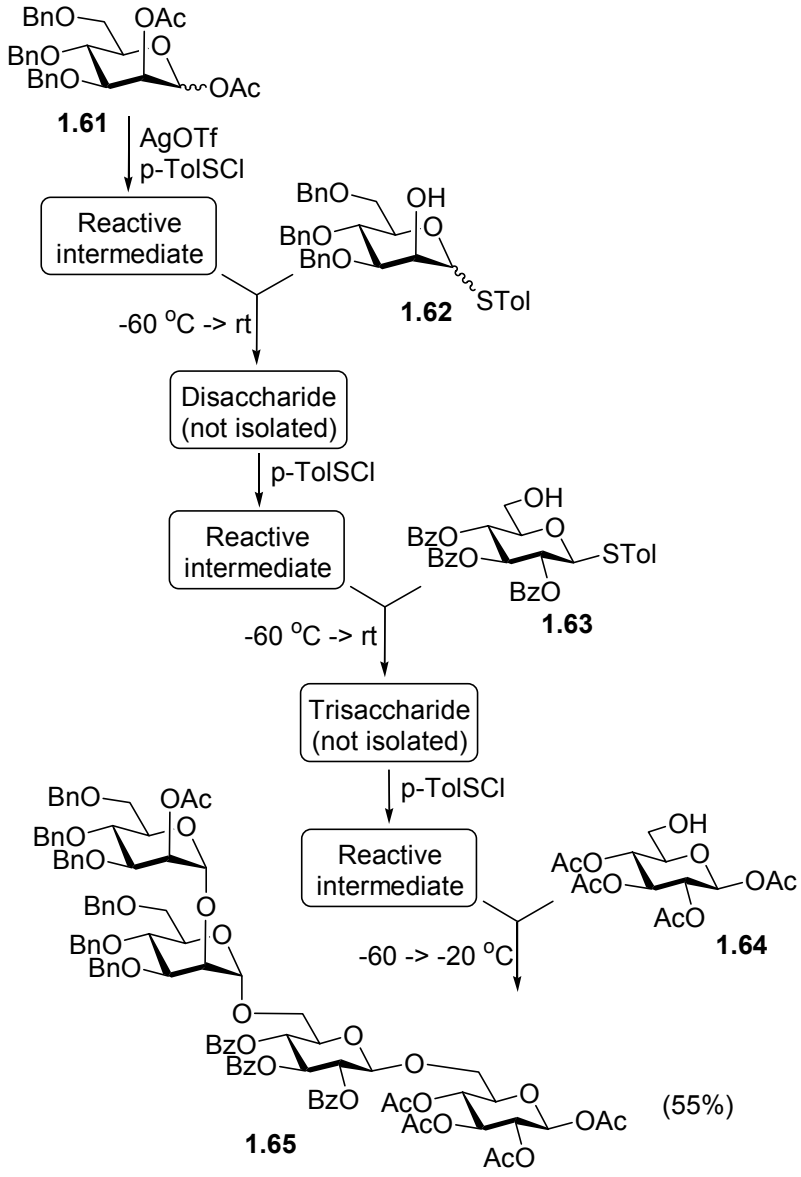
This two-step activation concept was discovered by Zen for S-ethyl (LG^a) and bromo

(LG^b) moieties.[46] Further expansion to S-phenyl (LG^a) and fluoro (LG^b) moieties by Nicolaou,[47,71] gave rise to the development of many useful approached to stereoselective glycosylation and expeditious oligosaccharide synthesis. Amongst the most versatile procedures is one-pot synthesis based on pre-activation of p-tolyl thioglycoside donor, followed by sequential addition of glycosyl acceptor.[31]

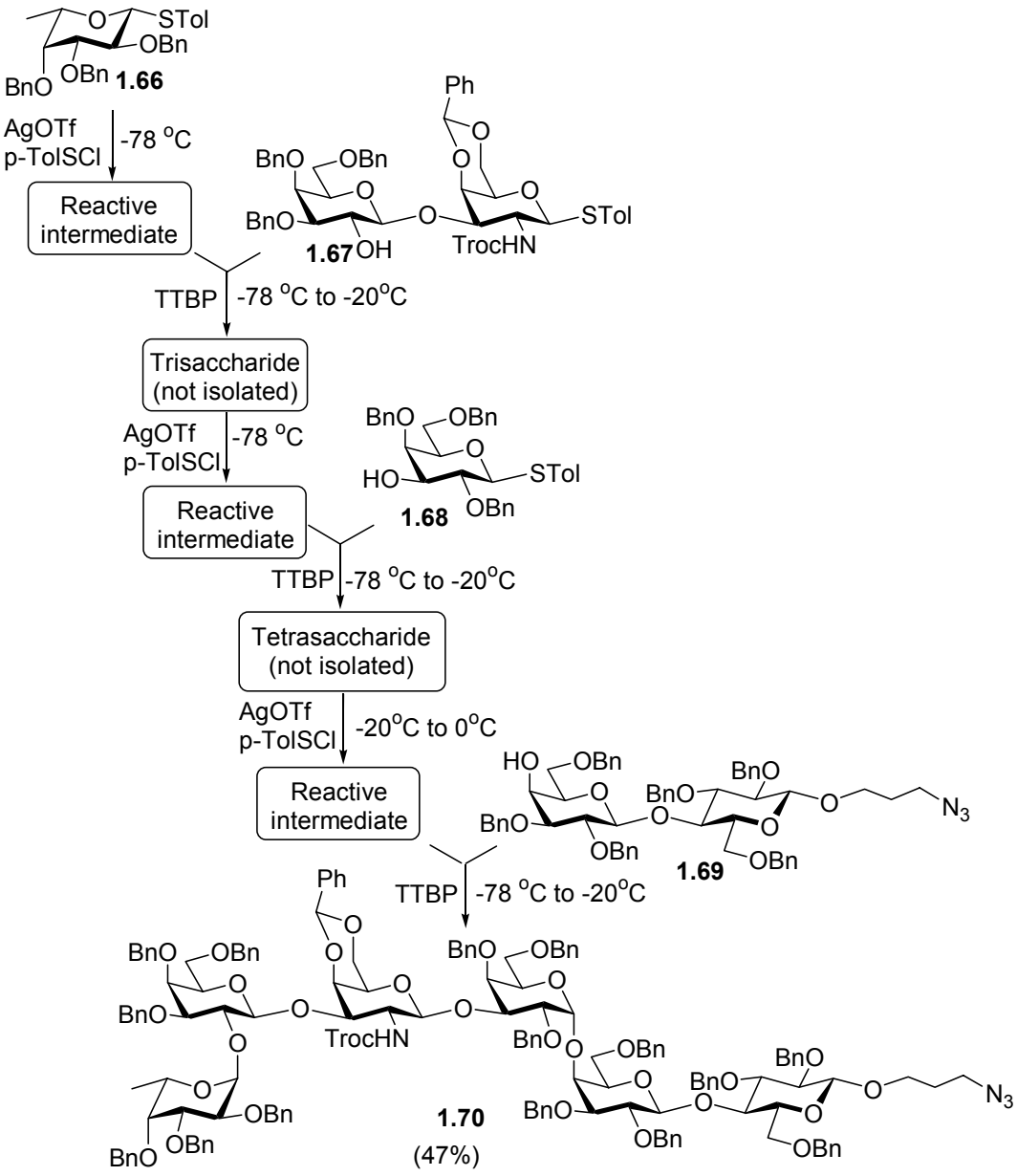
For example, glycosyl donor **1.61** was cooled to –60 ^oC and the promoter system consisting of AgOTf and p-toluenesulfenyl chloride was added (Scheme 1.18). After 5 min, glycosyl acceptor **1.62** was added and the reaction mixture was warmed to rt for 15 min to generate a disaccharide intermediate. The mixture was then again cooled to –60 ^oC and another portion of the promoter was added. After 5 min, glycosyl acceptor **1.63** was added and the reaction mixture was warmed to rt. Upon the formation of the trisaccharide intermediate, the preactivation/glycosylation sequence was repeated once again with the glycosyl acceptor **1.64** to produce the tetrasaccharide **1.65** in 55% over-all yield in only 2 h.

Another useful utilization of the preactivation concept is the synthesis of the tumor associated carbohydrate antigen Globo H hexasaccharide.[32] As illustrated in Scheme 1.19, preactivation of the fucosyl donor **1.66** at -78 ^oC with p-TolSCl/AgOTf was followed by the addition of the first acceptor **1.67** along with a sterically hindered base 2,4,6-tri-(t-butyl)-pyrimidine (TTBP). The temperature was then raised to -20 ^oC to obtain the trisaccharide intermediate. At this stage, the reaction mixture was cooled to -78 ^oC followed by the sequential addition of AgOTf, p-TolSCl, galactose acceptor **1.68**,

TTBP and then warming up the reaction to -20 °C. After complete disappearance of the acceptor **1.68**, the temperature was lowered to -78 °C and the sequence was reiterated for glycosylation of lactoside **1.69**. The resulting Globo H hexasaccharide **1.70** was thus formed in 47% overall yield based on the four-component one-pot reaction within 7 h.



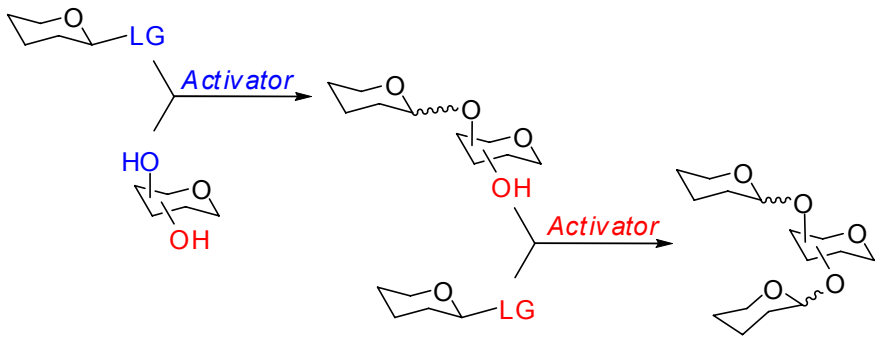
Scheme 1.18 One-pot synthesis via in-situ preactivation



Scheme 1.19 One-pot synthesis of Globo H hexasaccharide based on preactivation

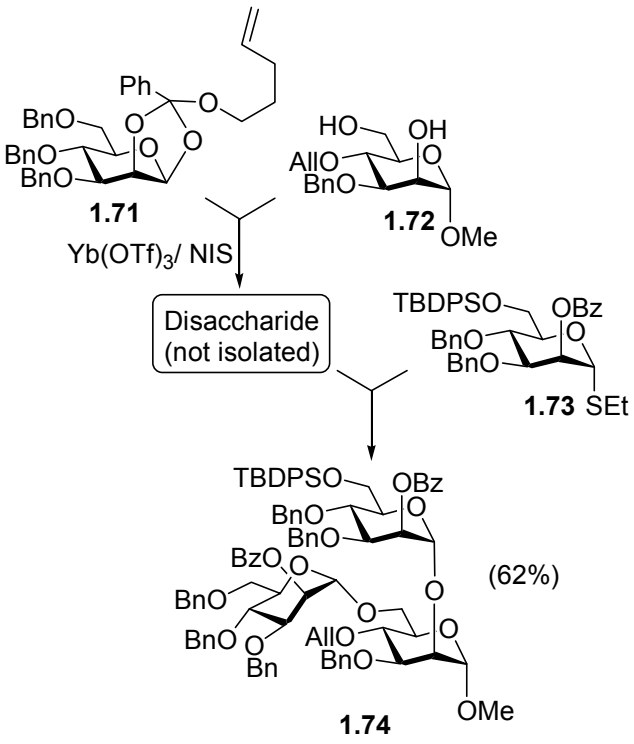
1.2.1.5 Approach E: One-pot glycosylation based on bidirectional concept

Many common chemical reactions allow for the differentiation between various hydroxyl groups, and glycosylation is no exception. Most commonly, the reactivity differences between primary / secondary or equatorial / axial alcohols have been explored. The outline of this approach that is amongst the most common techniques to obtain branched oligosaccharides is shown in Scheme 1.20.



Scheme 1.20 Reactivity-based regioselective glycosylation of polyols

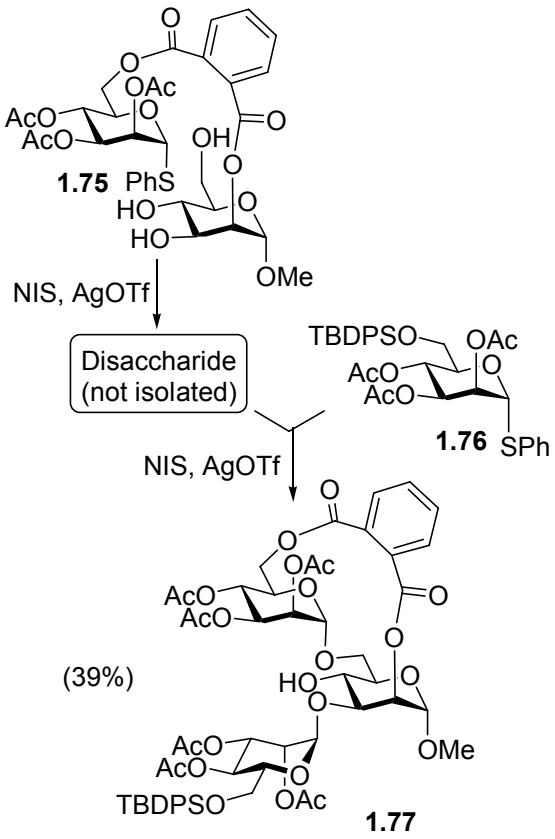
For example, as depicted in Scheme 1.21, mannose acceptor diol **1.72** was first regioselectively glycosidated at the primary position with n-pentenyl orthoester **1.71** using NIS/$Yb(OTf)_3$ promoter system. Upon completion of this step, thioglycoside donor **1.73** was added and the remaining secondary hydroxyl was glycosylated to yield the branched trisaccharide **1.74** in 62% overall yield.[72]



Scheme 1.21 Regioselective one-pot glycosylation of glycosyl acceptor 1.74

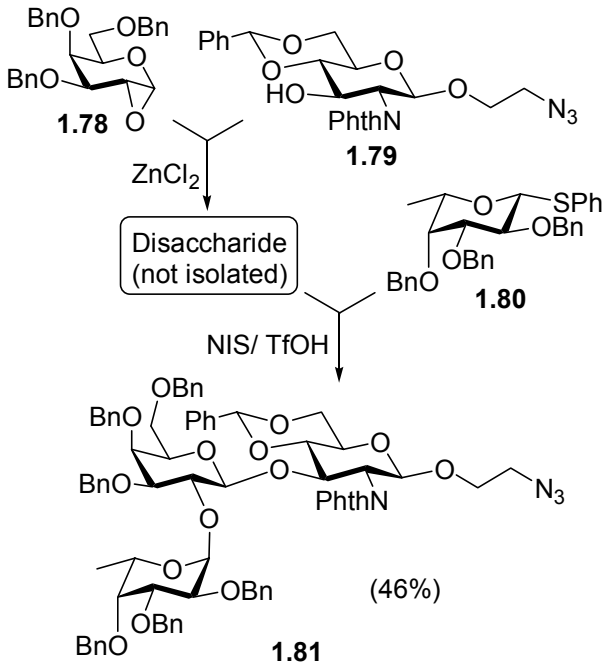
In a similar fashion, the one-pot synthesis of trisaccharide **1.77** incorporating the intramolecular glycosylation has been accomplished. As shown in Scheme 1.22, the intramolecular glycosylation of the tethered glycosyl donor / acceptor cluster **1.75** occurred regioselectively at the 6-OH. Subsequently, glycosyl donor **1.76** was added to affect the coupling on the 3-OH yielding the branched trimannoside derivative **1.77** [73]

In a series of publications, Boons developed a bidirectional strategy for oligosaccharide synthesis.[74] According to this approach, the key building block bears both a leaving group and a deactivated (or temporarily masked) hydroxyl moiety. It can first act as a glycosyl donor with a reactive glycosyl acceptor. Next, the same unit acts as the glycosyl acceptor that is glycosylated directly at the deactivated hydroxyl. Alternatively, an easily removable protecting group can be used as the temporary masking functionality that is removed prior to the next glycosylation step.



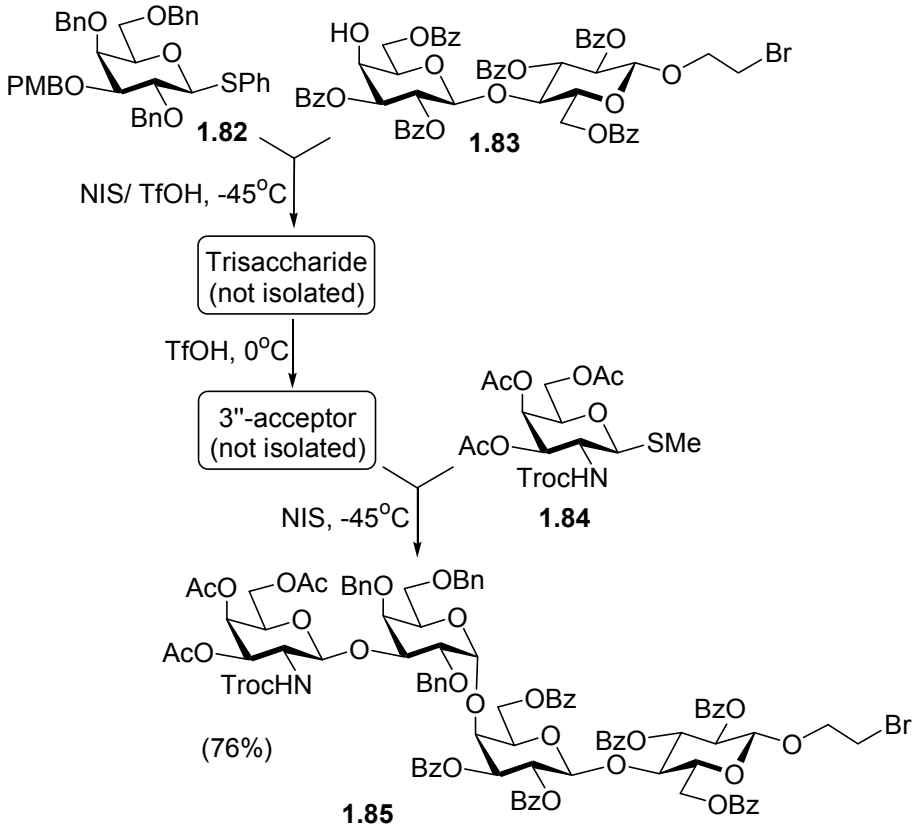
Scheme 1.22 One-pot synthesis of trimannoside 1.77.

A very effective application of the bidirectional concept to one-pot synthesis emerged with the application of 1,2-anhydro derivatives as glycosyl donors. Apparently, upon glycosidation, such epoxide donor liberates 2-OH group, which offers a useful target for the direct subsequent glycosylations.[75] As shown in Scheme 1.23, epoxide **1.78** and glucosamine **1.79** were first reacted in the presence of $ZnCl_2$. This coupling led to the formation of a disaccharide containing free hydroxyl group at C-2'. Subsequently, thiofucoside **1.80** and promoter NIS/ TfOH were added to yield the trisaccharide **1.81** in 46% overall yield.



Scheme 1.23 Bidirectional one-pot synthesis of trisaccharide 1.81

A related approach that makes use of p-methoxybenzyl ether (PMB) as an in-situ removable protecting group was developed.[76] It was observed that although PMB ether is stable toward typical electrophilic conditions for thioglycoside activation (NIS/TfOH), it can be readily cleaved with 0.2 eq. TfOH. This made possible to perform two sequential glycosylations with the intermediate deprotection, all in one-pot. When the first coupling reaction between building blocks **1.82** and **1.83** was complete, the PMB ether was cleaved by simply elevating the temperature to 0 ^{o}C and adding 0.2 eq. of TfOH to expose a hydroxyl group for the next glycosylation (Scheme 1.24). The glycosyl donor **1.84** was then added to the reaction mixture at -45 ^{o}C to carry out the second glycosylation to affect the formation of globotetraose (Gb4) tetrasaccharide **1.85** in a very high overall yield (76%). A similar approach involving reductive opening of benzylidene acetals between glycosylation steps was recently reported.[77]



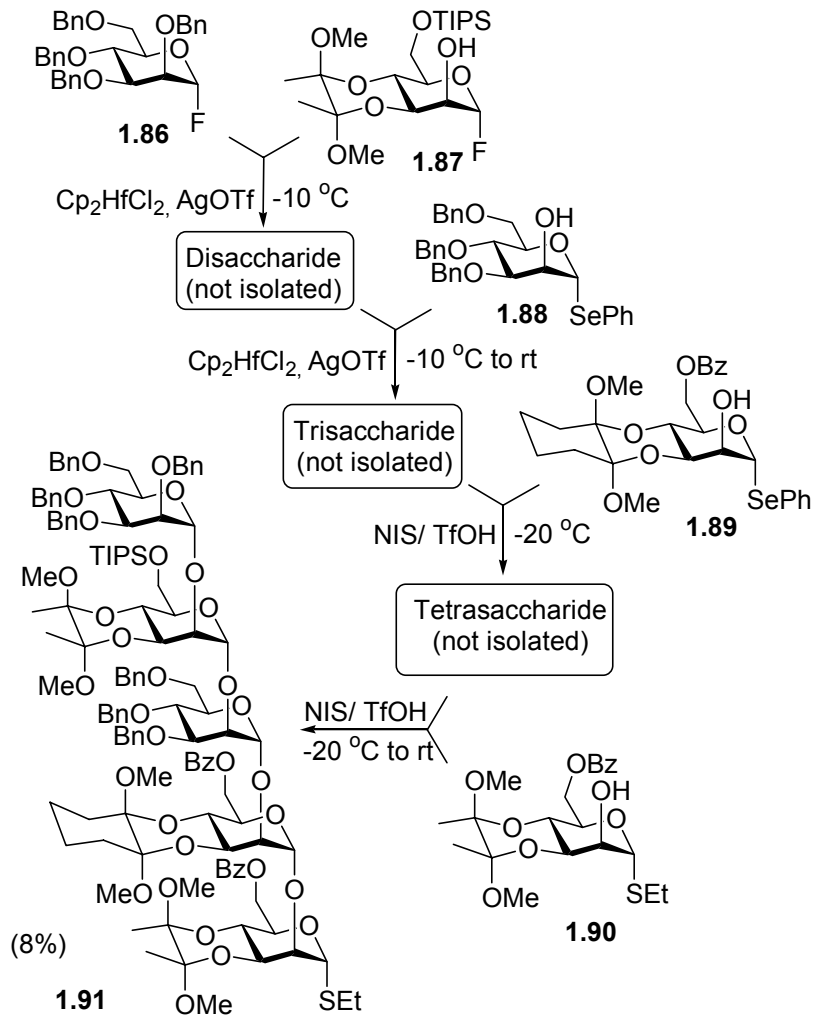
Scheme 1.24 One-pot synthesis of the Gb4 tetrasaccharide 1.85

1.2.2 Miscellaneous one-pot syntheses or approaches based on the mixed conventions.

The approaches included in this subsection cannot be classified unambiguously by the five concepts A-E discussed above. Somewhat uniquely, these syntheses combine two or more different concepts: for example the first example depicted in Scheme 1.25 incorporates both selective and chemoselective activations, Approaches B and C, respectively. The second example (Scheme 1.26) combines features of Approaches B, C, and E; in addition, it makes use of the orthogonal strategy that is not yet routinely applicable.

Ley's group reported the synthesis of a GPI anchor involving building blocks with well defined levels of reactivity (Scheme 1.25).[68] Thus, armed and disarmed glycosyl fluorides (**1.86**, **1.87**), armed and disarmed selenoglycosides (**1.88, 1.89**) and torsionally disarmed thioglycoside **1.90**, which were all sequentially activated to provide the target linear pentamannoside **1.91** in 8% overall yield in four steps.

Six-step one-pot glycosylation of heptasaccharide **1.99** was performed by Takahashi and coworkers using a manual synthesizer (Quest 210).[57] As depicted in Scheme 1.26, this one-pot synthesis required the sequential addition of seven reaction components **1.92-1.98** using four different leaving groups. Sequential activations employed herein included: activation of glycosyl bromide **1.92** (activated with AgOTf over S-ethyl acceptor), ethyl thioglycosides **1.93** and **1.94** (with MeOTf over fluoride acceptor **1.95**), tetrasaccharide fluoride (with $HfCp_2Cl_2$/ AgOTf over S-phenyl acceptor **1.96**) and phenyl thioglycosides **1.97** and hexasaccharide (with DMTST in the armed-disarmed fashion). The resulting phytoalexin elicitor heptasaccharide **1.99** was isolated in 24% yield over six steps.



Scheme 1.25 Five-step one-pot synthesis of a GPI anchor pentasaccharide 1.91

1.2.3 Conclusions and outlook

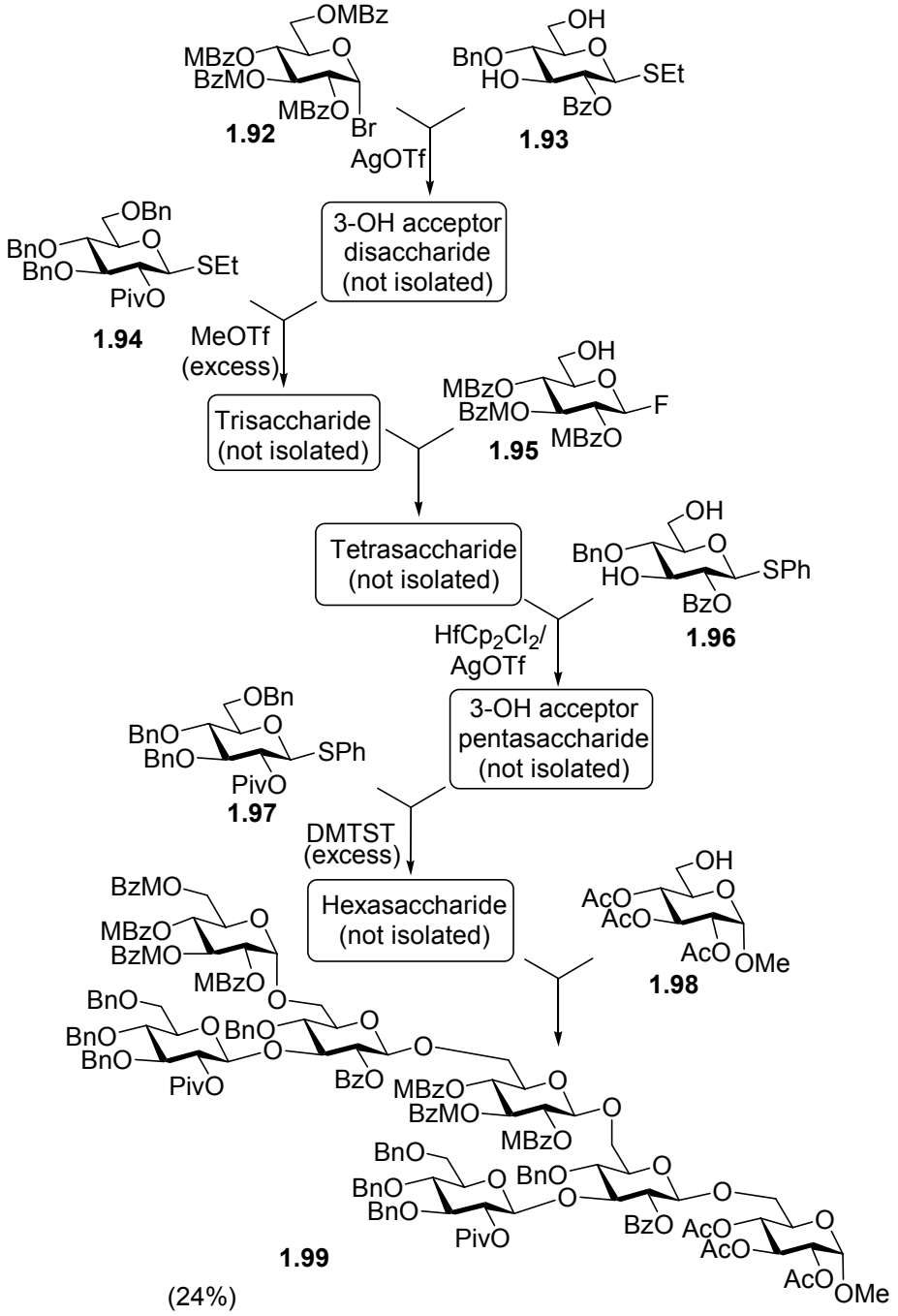
The invention of new methods and strategies for stereoselective glycoside synthesis and convergent oligosaccharide assembly has been critical for the area of glycosciences. At the heart of this chapter is the discussion of the strategies for expeditious one-pot oligosaccharide synthesis. Recent developments in this area have been categorized,

described, and supplemented with the relevant examples. The one-pot approach is arguably the simplest concept for oligosaccharide synthesis that, however, is still hindered by a number of factors that are also of the primary importance for conventional one-step glycosylations. Yield, stereoselectivity, regioselectivity, and conversion rate become increasingly important, and if not controlled would lead to complex inseparable mixtures of oligomeric isomers.

At this stage, no particular concept for one-pot synthesis has a definite advantage over other concepts. Arguably, all concepts have certain limitations, however 3 and even 4-step syntheses are becoming routinely accessible regardless of the strategy chosen. In one intends to obtain larger saccharides, this can be achieved by incorporating the convergent strategy, according to which oligosaccharides building blocks are pre-synthesized and then coupled in the one-pot fashion. An alternative approach is to make a combined use of various conventions that, if cleverly designed, could lead to longer sequences. In the last section of this chapter, two examples of such syntheses are described. Although these 5 and 6-step one-pot syntheses still remain of modest efficiency, the yields achieved were 8 and 24%, respectively, these innovative approaches clearly outline the direction, in which the one-pot synthetic strategies will be developing. It is possible that well-established one-pot synthesis can be automated, which may simplify large scale production of therapeutic agents.

As new developments in the glycosylation reaction emerge, it is expected that these basic studies will help to improve the efficiency of the one-pot strategies as well. The use of

highly reactive glycosyl donors would help to obtain quantitative (or nearly quantitative)



Scheme 1.26 Six-step one-pot synthesis of a branched heptasaccharide 1.99

yields in glycosylations. The use of stable building blocks would help to minimize side

reactions, leading to unwanted mono- and oligomeric side products. Since in some cases complex mixtures are generated, future progress in the detection and separation techniques is also expected to have a deep impact on the efficacy of the one-pot strategies. Indeed, if more advanced separation techniques were available, this would allow to fish out the target oligosaccharide even from the complex multi-component mixtures.

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CHAPTER 2

Screening of Glycosyl imidates as Glycosyl Donors for stereoselective glycosylation reaction

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Ramakrishnan (Parameswar), A.; Pornsuriyasak, P.; Demchenko, A.V."Synthesis, Glycosidation and Hydrolytic Stability of Novel Glycosyl Thioimidates", *J. Carbohydr. Chem.*, **2005**, 24, 649-663

2 Scope of Novel Glycosyl Thioimidates

2.1 Introduction

Despite significant progress in the area of synthetic carbohydrate chemistry, the necessity to form a glycosidic bond with *complete* stereoselectivity remains to be the main reason chemical *O*-glycosylation is still ranked amongst the most challenging problems of modern synthetic chemistry. The development of new glycosylation techniques persists to be of paramount importance for the rapidly developing area of glycosciences. Factors affecting the stereoselectivity of glycosylation include temperature, pressure, structure, conformation, solvent, promoter, steric hindrance, or leaving group.[1] While some of these factors influence the stereoselectivity dramatically, the leaving group is undoubtedly one of the major players in this respect. As a result, a number of glycosyl donors have been developed,[2,3] including glycosyl halides,[4,5] trichloroacetimidates,[6] and alkyl/aryl thioglycosides,[7,8]. Although these glycosyl donors are most commonly used, all of them still have significant drawbacks. For example, thioglycosides are inert under other glycosyl donor activation conditions, and therefore can fit into various orthogonal and (chemo)selective strategies for complex oligosaccharide assembly.[9,10] Unfortunately, only modest stereoselectivity is typically achieved with these stable compounds.[1] Conversely, being rather unstable glycosyl donors, trichloroacetimidates or halides (especially iodides)[11,12] often demonstrate complete stereoselectively in glycosylations.[1] Unfortunately, these compounds are not sufficiently stable to be used in block oligosaccharide synthesis via expeditious pathways.[9,10]

Previous work in our laboratory on the development of glycosylation methods has

resulted in the discovery of novel glycosyl donors with a generic thioimidoyl leaving group ($SCR_1=NR_2$, **2.1**, Figure 2.1). It has been reported that S-benzoxazolyl (**2.2**, SBox)[13,14] and, especially, S-thiazolinyl (**2.3**, STaz)[15] moieties are sufficiently stable to be used at the anomeric center; also, they can be mildly activated for glycosylation under a variety of reaction conditions

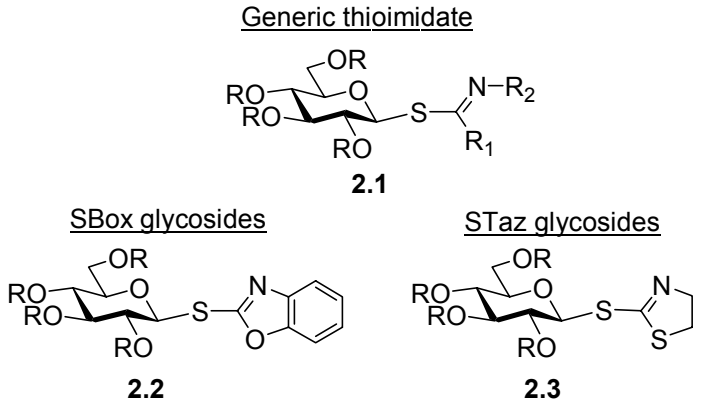


Figure 2.1 Glycosyl thioimidates ($SCR_1=NR_2$)

The SBox and STaz glycosyl donors have several useful features. First, these compounds can be efficiently synthesized from inexpensive odorless aglycones.[14,15] While this is a convenient feature for laboratory preparation, it becomes increasingly important for industrial applications. Second, glycosidation of **2.2** and **2.3** allowed very high stereoselectivity and excellent yields (often over 90%) of glycosidations.[14,15] Third, high stability of the SBox and STaz glycosides toward major protecting group manipulations and other glycosyl donor activation conditions makes it possible to use these moieties as a temporary protection of the anomeric center.[15,22] Lastly, it has been demonstrated that glycosyl thioimidates not only easily fit into existing glycosylation strategies for complex oligosaccharide synthesis, but also permit conceptually new strategies, such as temporary

deactivation approach.[23]

The high stability of both the Staz and SBox glycosides was somewhat unexpected, particularly in the context of previous reports. Thus, a fairly low stability was reported for a number of other glycosyl thioimidates: benzothiazolyl,[16] pyridin-2-yl,[17-19] pyrimidin-2-yl,[17,20] imidazolin-2-yl,[17] and 1'-phenyl-1*H*-tetrazolyl.[21] The purpose of the work described in this chapter is to clarify this disagreement.

2.2 Results and discussion

2.2.1 Synthesis of the thioimidate derivatives

We were interested in expanding our previous studies to a range of five and six-membered heterocyclic moieties, structurally related to, SBox and STaz glycosides. For this purpose we obtained novel per-acylated S-oxazolinyl (**2.4**), S-oxazinyl (**2.5**), S-benzothiazolyl (**2.6**), S-thiazinyl (**2.7**) derivatives. It should be noted that per-benzylated S-benzothiazolyl glucosides and peracetylated S-benzothiazolyl furanosides have been previously synthesized.[16,24,25]

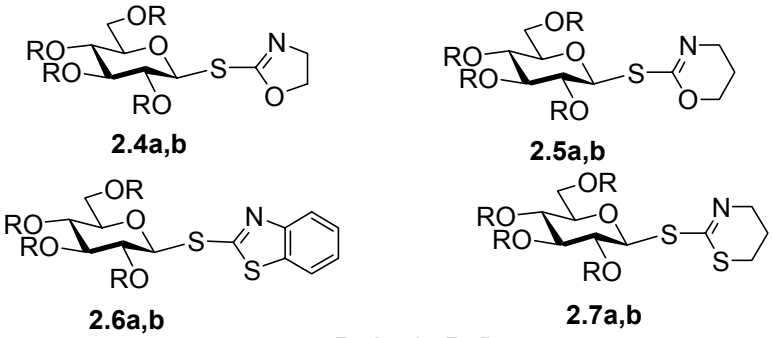


Figure 2.2 Novel glycosyl donors with a thioimidoyl leaving group

2.2.2 Synthesis of aglycones.

A general procedure to introduce the anomeric moiety (aglycone) involved the use of anomeric acetates or bromides as suitable starting compounds for these syntheses. Aglycones **2.8-2.11** for the synthesis of **2.2, 2.6, 2.3**, and **2.4**, respectively, are readily available from commercial sources, while tetrahydro-1,3-oxazine-2-thione (**2.12**)[19] and tetrahydro-1,3-thiazine-2-thione (**2.13**)[20] needed to be synthesized according to literature published procedures. For this purpose, a common precursor 3-aminopropanol was used as a staring material. The synthesis of **2.12** was achieved by the reaction of 3-aminopropanol with CS_2 in the presence of triethylamine in methanol at 0 ^oC followed by the treatment with 30 % H_2O_2.[19] Compound **2.13** was obtained by refluxing a mixture of 3-aminopropanol and CS_2 in 1M aqueous KOH.[20] The derivatives **2.12** and **2.13** were purified by crystallization.

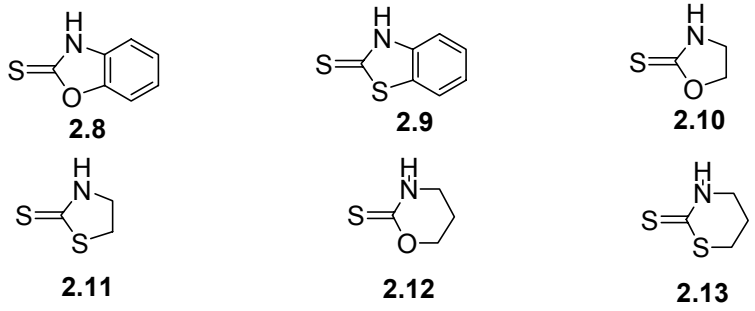


Figure 2.3 Aglycones used for the synthesis of glycosyl thioimidates

2.2.3 Synthesis of glycosyl thioimidates

Having acquired the aglycone derivatives suitable for introduction at the anomeric center, we turned our attention to the synthesis of acylated glycosyl thioimidates. These

syntheses were accomplished by slightly modified experimental procedures in comparison to those previously developed for the synthesis of SBox and STaz glycosides.[8-10] Thus, per-acetylated thioimidoyl derivatives **2.4**-**2.7** (Figure 2.2) were obtained from glucose pentaacetate **2.14** in the presence of TMSOTf at room temperature; in this context, SBox and STaz glycosides **2.2** & **2.3** were obtained in the presence of BF_3-Et_2O at low temperature (0^oC). The synthesis of acetylated thioimidates was also accomplished from acetobromoglucose **2.15**[21] by the reaction of the potassium salt of **2.10-2.13** in acetone or MeCN at room temperature. Similarly, benzobromoglucose **2.16**[21] was employed in the syntheses of per-benzoylated thioimidates. It should be noted that while the sodium salt was previously found to be advantageous in the synthesis of STaz glycosides **2.3**,[10] herein improved results were obtained with the use of potassium salts. The synthesis of benzoylated thioimidates were accomplished in the presence of 18-crown-6. The use of the crown ether was not only found to be essential for the efficient conversion but also very influential for the synthesis of the desired S-linked derivatives, as opposed to their N-linked counterparts.

While the results obtained for the synthesis of structurally similar benzoxazolyl (**2.2**) and benzothiazolyl (**2.6**) derivatives were very comparable (entries 1-3 vs 4-6, Table 2.1), other pairs of the structurally related derivatives provided very different results. Overall, the introduction of the heterocyclic moieties containing endocyclic sulfur atom at the anomeric center was significantly more simple and high yielding than that of their oxygen-containing counterparts. Thus, the syntheses of thiazolinyl **2.3a** and thiazinyl **2.7a** from pentaacetate **2.14** were achieved in high yields of 91% and 84 % (entries 10

and 16), respectively, whereas the syntheses of oxazolinyl **2.4a** and oxazinyl **2.5a** under similar reaction conditions failed (entries 4 and 13). Along with these observations, notably higher yields were obtained in the syntheses of sulfur-containing heterocyclic anomeric moieties from halides **2.15** or **2.16**

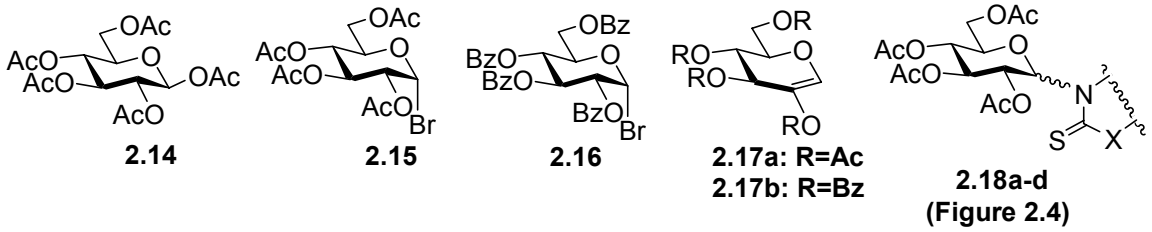


Figure 2.4 Precursors for the synthesis of glycosyl thioimidates

Table 2.1. Synthesis of Glycosyl Thioimidates **2.2-2.7**.

#	SM	Aglycone	Conditions	Product	Yield	Side products
1	**2.14**	**2.8**	BF_3-Et_2O, CH_2Cl_2, 0 $^oC \rightarrow$ rt	**2.2a**	79 %	
2	**2.15**	**2.8**	K_2CO_3, acetone, 50 ^oC	**2.2a**	96 %	
3	**2.16**	**2.8**, K salt	18-c-6, acetone, rt	**2.2b**	99 %	
4	**2.14**	**2.9**	TMSOTf, CH_2Cl_2, rt	**2.6a**	85 %	
5	**2.15**	**2.9**, K salt	acetone, rt	**2.6a**	99 %	
6	**2.16**	**2.9**, K salt	18-c-6, acetone, rt	**2.6b**	88 %	
7	**2.14**	**2.10**	TMSOTf, CH_2Cl_2, rt	**2.4a**	0 %	**2.18a** (80%)
8	**2.15**	**2.10**, K salt	MeCN, rt	**2.4a**	34 %	**2.17** (36%), **2.18a** (24%)
9	**2.16**	**2.10**, K salt	18-c-6, MeCN, rt	**2.4b**	23 %	**2.17** (49%)

10	**2.14**	**2.11**	BF_3-Et_2O, CH_2Cl_2, 0 $^oC \rightarrow$rt	**2.3a**	91 %	
11	**2.15**	**2.11**, Na salt	MeCN, rt	**2.3a**	53 %	**2.17** (13%), **18b** (11%)
12	**2.16**	**2.11**, Na salt	15-c-5, MeCN, rt	**2.3b**	50 %	**2.17** (41%)
13	**2.14**	**2.12**	TMSOTf, CH_2Cl_2, rt	**2.5a**	0 %	Complex mixture
14	**2.15**	**2.12**, K salt	MeCN, rt	**2.5a**	0 %	**2.17** (58%), **18c** (27%)
15	**2.16**	**2.12**, K salt	18-c-6, MeCN, rt	**2.5b**	12 %	**2.17** (70%)
16	**2.14**	**2.13**	TMSOTf, CH_2Cl_2, rt	**2.7a**	84 %	
17	**2.15**	**2.13**, K salt	MeCN, rt	**2.7a**	22 %	**2.17** (54%), **18d** (18%)
18	**2.16**	**2.13**, K salt	18-c-6, MeCN, rt	**2.7b**	18 %	**2.17** (55%)
19	**2.7a**	-	1. MeONa, 2. BzCl/pyridine	**2.7b**	72 %	

SM – starting material

Thus, for the synthesis of the sulfur-containing five-membered heterocyclic anomeric moieties: **2.3a** and **2.3b** was obtained in 53% and 50 % yield, respectively (entries 11 and 12), whereas the syntheses of structurally similar oxygen-containing **2.4a** and **2.4b** was achieved in only 34% and 23 %, respectively. Similar correlation, but lower yields were obtained in the syntheses of the six-membered heterocyclic leaving groups (**2.5** and **2.7**, entries 13-19).

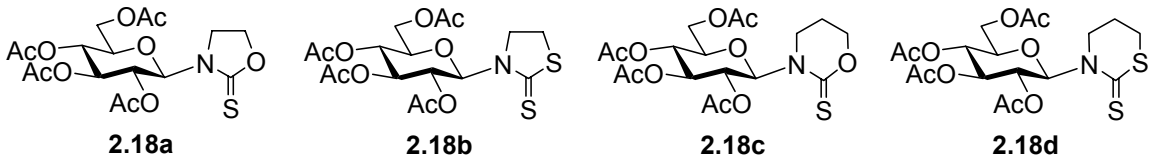
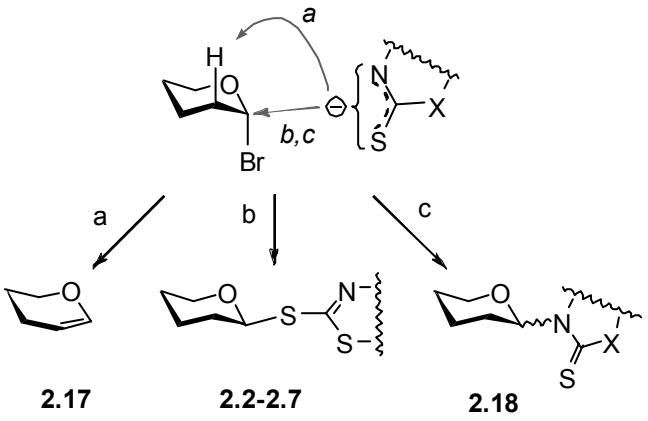


Figure 2.5 Per-acetylated N-linked derivatives

Although the reaction conditions reported here are optimized in order to favor the glycosylation at the softer sulfur atom (S-glycosylation), some of these coupling reactions were significantly compromised by competitive side processes. Among those, β-elimination resulting in the formation of a 1,2-dehydro (glycal) derivative **2.17** and/or N-glycosylation resulting in the formation of a corresponding N-linked derivative (**2.18a-d**) were found to be the most common (Scheme 2.1, Table 2.1). On a number of occassions, glycal **2.17** was obtained as a major product in the syntheses of **2.4a, 2.4b, 2.5a, 2.5b**, **2.7a** and **2.7b** with the isolated yields ranging from 36 % (entry 8) to 70 % (entry 15). Although the corresponding per-acetylated N-linked derivatives (**2.18a-d**, see Figure 2.4) were only obtained in the attempted synthesis of **2.4a** from **2.14** (entry 7) as a major product (**2.18a**), they were often detected in the reaction mixtures.

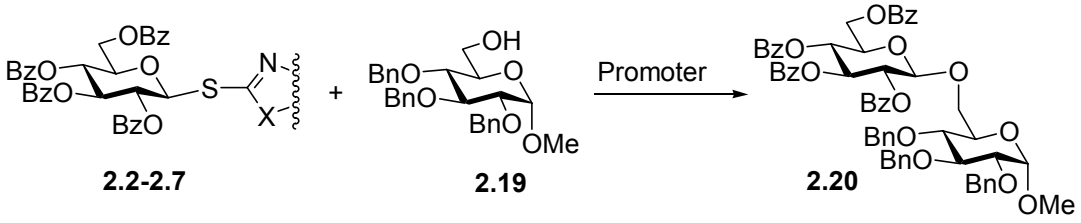


Scheme 2.1 Plausible mechanistic pathway for thioimidate activation

2.2.4 Glycosidation studies

Glycosidation of perbenzoylated thioimidates **2.4b, 2.6b**, and **2.7b** were studied under essentially the same reaction conditions as those investigated for the glycosidations of SBox (**2.2b**) and STaz (**2.3b**) glycosides.[8-10] Although these novel derivatives appear to be somewhat less efficient glycosyl donors than either **2.2b** or **2.3b**, these results are in line with many other glycosylation methods.[2] Thus, while typical yields for the synthesis of **2.20**[22] in glycosylations of **2.19**[23] with **2.2a** and **2.3a** were in the range of 94-97% (Table 2.2, entries 1-4), the yields achieved with either **2.4b, 2.6b**, or **2.7b** were considerably lower (51-81 %, entries 5-10). The use of per-acetylated glycosyl donors was found to be impractical due to a competing process of the 2-O-acetyl moiety migration from a glycosyl donor to the 6-OH of the glycosyl acceptor **2.19**.[24]

Table 2.2 Glycosylation of **2.19** with per-Benzoylated Glycosyl Donors **2.2-2.7**:



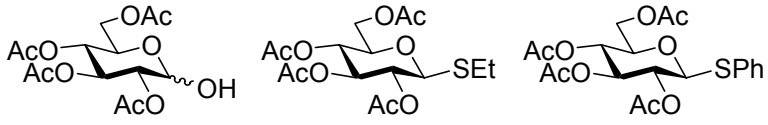
2.2-2.7 **2.19** **2.20**

Entry	Donor	Promoter	Time	Yield	Ref
1	**2.2b**	AgOTf	5 min	94 %	[9]
2	**2.2b**	MeOTf	1 h	95 %	[9]
3	**2.3b**	AgOTf	16 h	97 %	[10]
4	**2.3b**	MeOTf	2 h	97 %	[10]

5	**2.4b**	AgOTf	5 min	60 %	
6	**2.4b**	MeOTf	3.5 h	81 %	
7	**2.6b**	AgOTf	16 h	79 %	
8	**2.6b**	MeOTf	2 h	67 %	
9	**2.7b**	AgOTf	30 min	65 %	
10	**2.7b**	MeOTf	16 h	51 %	

2.2.5 Hydrolytic Stability

Per-acetylated derivatives **2.2a, 2.3a, 2.4a, 2.6a**, and **2.7a** were studied under acid hydrolysis conditions (synthesis of **2.21**, Figure 2.6). Reaction conditions selected for this purpose were essentially the same as those previously reported for the hydrolysis of S-ethyl or S-phenyl glycosides (**2.22**[25] or **2.23**[26], respectively, Figure 2.5). The first procedure involved hydrolysis in the presence of NIS (2 equiv) and TfOH (0.1-0.2 equiv) in wet CH_2Cl_2.[27] Under these reaction conditions **2.2a, 2.22**, and **2.23** were rapidly converted into the hemiacetal **2.21**;[28] these reactions required 15, 2, and 20 min, respectively, whereas the hydrolysis of **2.3a, 2.4a, 2.6a**, and **2.7a** was significantly slower and required 16, 2, 16, and 48 h, respectively.



2.21 **2.22** **2.23**

Figure 2.6 Hydrolysis product (2.21) and thioglycosides (2.22, 2.23)

The second procedure involved hydrolysis in the presence of N-bromosuccinimide (2 equiv) in aqueous acetone.[29] Under these reaction conditions **2.22** was hydrolyzed in 5 min, **2.3a, 2.7a,** and **2.23** were hydrolyzed in 3-5 h, while **2.2a** and **2.6a** were significantly more resistant: their hydrolysis was sluggish and the reaction was not completed even in 48 h. These observations clearly illustrate that S-ethyl or S-phenyl glycosides are significantly more susceptible for acid hydrolysis under the investigated reaction conditions than their thioimidoyl counterparts. This advantageous feature of thioimidates is an important factor in their application as stable building blocks for oligosaccharide synthesis.

2.3 Conclusions

We investigated a number of novel thioimidoyl glycosides, which can be obtained from anomeric acetates and halides. When glycosidation of somewhat more basic endocyclic oxygen containing heterocyclic aglycones was performed, the isolated yields were compromised due to the competing side processes, namely β-elimination and N-glycosylation. The glycosyl donor properties of these derivatives were studied in comparison to other glycosyl donors of this class, SBox and STaz glycosides. Hydrolytic stability studies have clearly demonstrated that many glycosyl thioimidates are more stable compounds overall than their S-ethyl and S-phenyl counterparts under acidic or electrophilic conditions.

2.4 Experimental part

2.4.1 General

Column chromatography was performed on silica gel 60 (EM Science, 70-230 mesh), reactions were monitored by TLC on Kieselgel 60 F_{254} (EM Science). The compounds were detected by examination under UV light and by charring with 10% sulfuric acid in methanol or $KMnO_4$ solution in EtOH. Solvents were removed under reduced pressure at <40 ^{o}C. CH_2Cl_2, $(ClCH_2)_2$, and MeCN were distilled from CaH_2 directly prior to application. MeOH was dried by refluxing with magnesium methoxide, distilled and stored under argon. Pyridine was dried by refluxing with CaH_2 and then distilled and stored over molecular sieves (3Å). Anhydrous DMF (EM Science) and ether was used as is. AgOTf (Acros) was co-evaporated with toluene (3 x 10 mL) and dried *in vacuo* for 2-3 h directly prior to application. Molecular sieves (3Å or 4 Å), used for reactions, were crushed and activated *in vacuo* at 390 °C during 8 h in the first instance and then for 2-3 h at 390 °C directly prior to application. Optical rotations were measured at Jasco P-1020 polarimeter. UV-VIS spectra were recorded at HP 8452A Diode Array Spectrophotometer. Unless noted otherwise, ^{1}H-n.m.r. spectra were recorded in $CDCl_3$ at 300 MHz (Bruker Avance), ^{13}C-NMR spectra and two-dimensional experiments were recorded in $CDCl_3$ at 75 MHz (Bruker Avance) or at 125 MHz (Bruker ARX-500). HRMS determinations were made with the use of JEOL MStation (JMS-700) Mass Spectrometer.

2.4.2 Preparation of thioimidates 2.4-2.7

2.4.2.1 Method A

*General procedure for the synthesis of **2.4a, 2.5a, 2.6a**, and **2.7a.**:* A mixture of 1,2,3,4,6-penta-O-acetyl-β-D-glucopyranose (**2.14**, 0.128 mmol), a thiol (**2.9**, **2.10,** or **2.13**, 0.192 mmol) and activated molecular sieves 3 Å (100 mg) in dry CH_2Cl_2 (1.0 mL) was stirred under an atmosphere of argon for 30 min at rt. TMSOTf (0.192 mmol) was then added dropwise and the reaction mixture was kept for 45min at rt. After that, additional amounts of thiol (0.192 mmol) and TMSOTf (0.192 mmol) were added and the reaction mixture was kept for 1 h at reflux (45 ^{o}C). Upon completion, the mixture was diluted with CH_2Cl_2, the solid was filtered off and the residue was washed with CH_2Cl_2. The combined filtrate (30 mL) was washed with aq. NaOH (2 x 15mL) and water (3 x 10 mL), the organic layer was separated, dried and concentrated in vacuo. The residue was purified by crystallization from CH_2Cl_2/ether/hexane or by column chromatography on silica gel (ethyl acetate/toluene elution) to afford the corresponding thioimidate.

2.4.2.2 Method B

General procedure for the synthesis of **2.4a, 2.5a, 2.6a,** and **2.7a**. : KOH (4.2 mmol) was added to a solution of **2.9, 2.10,** or **2.13** (4.2 mmol) in a distilled acetone. The reaction mixture was refluxed at 60 ^{o}C for 3 h, then concentrated *in vacuo* and dried to afford the corresponding potassium salts as white solids, which were sufficiently pure to be used in subsequent applications. The resulting potassium salt (4.2 mmol) was added to a stirred solution of 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl bromide (**2.15**, 3.0 mmol) in dry

acetone or MeCN (20 mL). The reaction mixture was stirred under argon for 1 h at rt. Upon completion, the mixture was diluted with toluene (20 mL) and washed with water (10 mL), 1% aq. NaOH (10 mL) and water (3 x 10 mL), the organic phase was separated, dried, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (ethyl acetate/hexane elution) to afford the corresponding thioimidate.

4,5-Dihydrooxazol-2-yl 2,3,4,6-tetra-O-acetyl-1-thio-β-D-glucopyranoside (**2.4a**) was obtained as white crystals from **2.15** (Method B) in 34 % yield. Analytical data for **2.4a**: R_f = 0.44 (acetone/ toluene, 2/3, v/v); m.p. +126-129 ^oC (recrystallised from CH_2Cl_2/ether/hexane); $[\alpha]_D^{24}$ -3.0^o (c = 0.3, $CHCl_3$); UV: λ_{max} = 272 nm; ^{1}H-n.m.r.: δ, 5.37 (d, 1H, $J_{1,2}$ = 10.4 Hz, H-1), 5.24 (dd, 1H, $J_{3,4}$ = 9.2 Hz, H-3), 5.15 (dd, 1H, $J_{2,3}$ = 9.2 Hz, H-2), 5.15 (dd, 1H, $J_{4,5}$ = 9.9 Hz, H-4), 4.40 (m, 2H, CH_2O), 4.29 (dd, 1H, $J_{5,6a}$ = 4.6 Hz, $J_{6a,6b}$ = 12.5 Hz, H-6a), 4.15 (dd, 1H, $J_{5,6b}$ = 2.2 Hz, H-6b), 3.92 (dd, 2H, CH_2N), 3.85 (m, 1H, H-5), 2.08, 2.06, 2.05, 2.03 (4s, 12H, 4 x $COCH_3$) ppm; ^{13}C-n.m.r.: δ, 171.0, 170.4, 169.7 (x 2), 163.0, 83.6, 76.8, 74.2, 74.4, 70.1, 68.2, 62.1, 55.1, 21.1 (x 2), 21.0, 20.9 ppm; HR-FAB MS $[M+Na]^+$ calcd for $C_{17}H_{23}NNaO_{10}S$ 456.0940, found 456.0947.

2,3,4,6-Tetra-O-acetyl-1-N-(4,5-dihydro-2-thione-oxazol-3-yl)-β-D-glucopyranosylamine (**2.18a**) was isolated as the sole product in the attempt of the synthesis of **2.4a** from **2.14** (80 %, Method A) and as a side product in the synthesis of **2.4a** from **2.15** (24%, Method B). Analytical data for **2.18a**: R_f = 0.41 (ethyl acetate/ hexane, 1/1, v/v); $[\alpha]_D^{26}$ +26.8^o (c = 1.13, $CHCl_3$); UV: λ_{max} = 300 nm; ^{1}H-n.m.r.: δ, 5.82 (d, 1H, $J_{1,2}$ = 9.4 Hz, H-1), 5.43 (dd, 1H, $J_{3,4}$ = 9.4 Hz, H-3), 5.08 (dd, 1H, $J_{4,5}$ = 9.4 Hz,

H-4), 5.07 (dd, 1H, $J_{2,3}$ = 9.5 Hz, H-2), 4.44-4.62 (m, 2H, CH_2O), 4.28 (dd, 1H, $J_{5,6a}$ = 4.9 Hz, $J_{6a,6b}$ = 12.5 Hz, H-6a), 4.11 (dd, 1H, $J_{5,6b}$ = 1.8 Hz, H-6b), 3.77-3.99 (m, 3H, H-5, CH_2N), 2.03, 2.05, 2.06, 2.09 (4s, 12H, 4 x $COCH_3$) ppm; ^{13}C-n.m.r.: δ, 189.4, 170.7, 170.5, 169.9, 169.7, 84.1, 74.6, 72.7, 68.7, 68.1, 67.7, 61.9, 43.0, 20.9, 20.8 (x 2), 20.7 ppm.

5,6-Dihydro-4H-1,3-oxazin-2-yl 2,3,4,6-tetra-O-acetyl-1-thio-β-D-glucopyranoside (**2.5a**) could not be obtained by either Method A or B.

2,3,4,6-Tetra-O-acetyl-1-N-(5,6-dihydro-4H-2-thione-1,3-oxazin-3-yl)-β-D-glucopyranosylamine (**2.18c**) was isolated as a colorless film in the attempt of the synthesis of **2.5a** from **2.15** (27 %, Method B). Analytical data for **2.18c**: R_f = 0.50 (acetone/ toluene, 1/3, v/v); $[\alpha]_D^{26}$ +25.7^o (c = 1.25, $CHCl_3$); UV: λ_{max} = 300 nm; ^{1}H-n.m.r.: δ, 6.77 (d, 1H, $J_{1,2}$ = 9.4 Hz, H-1), 5.43 (dd, 1H, $J_{3,4}$ = 9.6 Hz, H-3), 5.14 (dd, 1H, $J_{2,3}$ = 9.5 Hz, H-2), 5.05 (dd, 1H, $J_{4,5}$ = 9.6 Hz, H-4), 4.20-4.37 (m, 3H, H-6a, CH_2O), 4.12 (dd, 1H, $J_{5,6b}$ = 1.9 Hz, $J_{6a,6b}$ = 12.5 Hz, H-6b), 3.89-3.94 (m, 1H, H-5), 3.51 (dd, 2H, CH_2N), 2.13-2.16 (m, 2H, CCH_2C), 2.02, 2.05, 2.07, 2.09 (4s, 12H, 4 x $COCH_3$) ppm; ^{13}C-n.m.r.: δ, 188.5, 170.7, 170.6, 169.9, 169.8, 87.3, 74.5, 72.9, 68.4, 68.2, 68.1, 61.9, 40.4, 21.1, 20.9 (x 2), 20.7 (x 2) ppm; HR-FAB MS $[M+Na]^+$ calcd for $C_{18}H_{25}NNaO_{10}S$ 470.1097, found 470.1090.

Benzothiazol-2-yl 2,3,4,6-tetra-O-acetyl-1-thio-β-D-glucopyranoside (**2.6a**) was obtained as white crystals from **2.14** (Method A) or **2.15** (Method B) in 85 or 99 % yield,

respectively. Analytical data for **2.6a**: R_f = 0.38 (ethyl acetate – toluene, 3/7, v/v) ; m.p. +121-124 ^oC (CH_2Cl_2/ ether/ hexane); $[\alpha]_D^{25}$ +34.7^o (c = 1.04, $CHCl_3$); UV: λ_{max} = 276 nm; ^{1}H-n.m.r.: δ, 7.40-8.00 (m, 4H, aromatic), 5.59 (d, 1H, $J_{1,2}$ = 10.2 Hz, H-1), 5.33 (dd, 1H, $J_{3,4}$ = 9.2 Hz, H-3), 5.25 (dd, 1H, $J_{2,3}$ = 9.2 Hz, H-2), 5.19 (dd, 1H, $J_{4,5}$ = 10.0 Hz, H-4), 4.32 (dd, 1H, $J_{5,6a}$ = 4.9 Hz, $J_{6a,6b}$ = 12.5 Hz, H-6a), 4.19 (dd, 1H, $J_{5,6b}$ = 1.9 Hz, H-6b), 3.94 (m, 1H, H-5), 2.05, 2.05, 2.05, 2.03 (4s, 12H, 4 x $COCH_3$) ppm; ^{13}C-n.m.r.: δ, 170.9, 170.4, 169.7, 162.2, 153.1, 136.2, 126.7 (x 2), 125.4, 122.7 (x 2), 121.4 (x 2), 84.4, 74.1, 70.1, 68.5, 62.2, 21.0, 20.9 (x 2) ppm; HR-FAB MS $[M+Na]^+$ calcd for $C_{21}H_{23}NNaO_9S_2$ 520.0712, found 520.0717.

5,6-Dihydro-4H-1,3-thiazin-2-yl 2,3,4,6-tetra-O-acetyl-1-thio-β-D-glucopyranoside (**2.7a**) was prepared as white crystals from **2.14** (Method A) or **2.15** (Method B) in 84 or 22 % yield, respectively. Analytical data for **2.7a**: R_f = 0.53 (ethyl acetate -toluene, 3/7, v/v) ; m.p. +102-104 ^oC (CH_2Cl_2/ ether/ hexane); $[\alpha]_D^{24}$ +9.2^o (c = 1.03, $CHCl_3$); UV: λ_{max} = 274 nm; ^{1}H-n.m.r.: δ, 5.55 (d, 1H, $J_{1,2}$ = 10.5 Hz, H-1), 5.27 (dd, 1H, $J_{3,4}$ = 9.2 Hz, H-3), 5.08-5.15 (m, 2H, $J_{2,3}$ = 9.2 Hz, H-2, 4), 4.27 (dd, 1H, $J_{5,6a}$ = 4.3 Hz, $J_{6a,6b}$ = 12.4 Hz, H-6a), 4.13 (dd, 1H, $J_{5,6b}$ = 2.2 Hz, H-6b), 3.74-3.84 (m, 3H, H-5, CH_2N), 3.08 (m, 2H, CH_2S), 2.08, 2.05, 2.02, 2.02 (4s, 12H, 4 x $COCH_3$) 1.95 (m, 2H, CCH_2C), ppm; ^{13}C-n.m.r.: δ, 171.0, 170.4, 169.7 (x 2), 152.5, 81.0, 74.5, 69.4, 68.5, 62.2, 48.9, 30.0, 28.1, 20.8, 20.8, 20.7 (x 2), 20.4 ppm; HR-FAB MS$[M+Na]^+$ calcd for $C_{18}H_{25}NNaO_9S_2$ 486.0868, found 486.0876.

2,3,4,6-Tetra-O-acetyl-1-N-(5,6-dihydro-4H-2-thione-1,3-thiazin-3-yl)-β-D-glucopyranosylamine (**2.18d**) was isolated as a side product in the synthesis of **2.7a** from **2.15** (18 %, Method B). Analytical data for **2.18d**: R_f = 0.42 (ethyl acetate/ hexane, 1/1, v/v); $[\alpha]_D^{26}$ +40.6^o (c = 1.02, $CHCl_3$); UV: λ_{max} = 296 nm; ^{1}H-n.m.r.: δ, 7.08 (d, 1H, $J_{1,2}$ = 9.3 Hz, H-1), 5.41 (dd, 1H, $J_{3,4}$ = 9.5 Hz, H-3), 5.16 (dd, 1H, $J_{2,3}$ = 9.4 Hz, H-2), 5.10 (dd, 1H, H-4), 4.26 (dd, 1H, $J_{5,6a}$ = 4.9 Hz, $J_{6a,6b}$ = 12.5 Hz, H-6a), 4.13 (dd, 1H, $J_{5,6b}$ = 2.1 Hz , H- 6b), 3.87-3.92 (m, 1H, H-5), 3.41-3.66 (m, 2H, CH_2N), 2.92 (dd, 2H, CH_2S), 2.16 - 2.25 (m, 2H, CCH_2C), 2.01, 2.04, 2.05, 2.08 (4s, 12H, 4 x $COCH_3$) ppm; ^{13}C-n.m.r.: δ, 197.0, 170.7, 170.4, 169.8, 169.8, 87.7, 74.5, 72.9, 68.7, 68.3, 61.9, 44.2, 31.8, 22.7, 20.9, 20.8, 20.7 (x 2) ppm.

*2.4.2.3 General procedure for the synthesis of **2.4b, 2.5b, 2.6b,** and **2.7b**:*

KOH (4.2 mmol) was added to a solution of **2.9, 2.10, 2.12,** or **2.13** (4.2 mmol) in a distilled acetone. The reaction mixture was refluxed at 60 ^{o}C for 3 h, then concentrated *in vacuo* and dried to afford the corresponding potassium salts as white solids, which were sufficiently pure to be used in subsequent applications. A potassium salt (4.2 mmol) and 18-crown-6 (0.42 mmol) were added to a stirred solution of 2,3,4,6-tetra-O-benzoyl-α-D-glucopyranosyl bromide (**2.16**, 3.0 mmol) in dry acetone or MeCN (20 mL). The reaction mixture was stirred under argon for 1 h at rt. Upon completion, the mixture was diluted with toluene (20 mL) and washed with water (10 mL), 1% aq. NaOH (10 mL) and water (3 x 10 mL), the organic phase was separated, dried, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (ethyl acetate/toluene elution) to afford the corresponding thioimidate.

4,5-Dihydrooxazol-2-yl 2,3,4,6-tetra-O-benzoyl-1-thio-β-D-glucopyranoside (**2.4b**) was obtained as white crystals in 23 % yield. Analytical data for **2.4b**: R_f = 0.41 (acetone-toluene, 1/4, v/v); m.p. +82-84 ^oC (CH_2Cl_2/ ether/ hexane); $[\alpha]_D^{24}$ +48.1^o (c = 1.0, $CHCl_3$); UV: λ_{max} = 274 nm; ^{1}H-n.m.r.: δ, 7.20-8.00 (m, 20H, aromatic), 5.93 (dd, 1H, $J_{3,4}$ = 9.2 Hz, H-3), 5.56-5.69 (m, 3H, H-1, 3, 4), 4.56 (dd, 1H, $J_{5,6a}$ = 2.8 Hz, $J_{6a,6b}$ = 12.3 Hz, H-6a), 4.43 (dd, 1H, $J_{5,6b}$ = 5.2 Hz, H-6b), 4.13-4.28 (m, 3H, H-5, CH_2O), 3.74 (m, 2H, CH_2N) ppm; ^{13}C-n.m.r.: δ, 166.0, 165.6, 165.3, 165.0, 162.5, 133.4, 133.2, 133.0, 129.9 (x 2), 129.7 (x 4), 129.6 (x 3), 128.9, 128.6 (x 2), 128.3 (x 2), 128.3 (x 2), 128.2, 128.1 (x 2), 125.2, 85.5, 73.9, 70.2, 69.3, 69.2, 62.9, 54.5, 29.6, 21.4 ppm; HR-FAB MS $[M+Na]^+$ calcd for $C_{37}H_{31}NNaO_{10}S$ 704.1566, found 704.1573.

5,6-Dihydro-4H-1,3-oxazin-2-yl 2,3,4,6-tetra-O-benzoyl-1-thio-β-D-glucopyranoside (**2.5b**) was obtained as a colorless film in 12 % yield. Analytical data for **2.5b**: R_f = 0.40 (acetone-toluene, 1/9, v/v); $[\alpha]_D^{25}$ +25.1^o (c = 0.25, $CHCl_3$); UV: λ_{max} = 274 nm; ^{1}H-n.m.r.: δ, 7.10-8.00 (m, 20H, aromatic), 5.90 (dd, 1H, $J_{2,3}$ = 8.7 Hz, H-2), 5.55-5.64 (m, 3H, H-1, 3, 4), 4.54 (dd, 1H, $J_{5,6a}$ = 2.9 Hz, $J_{6a,6b}$ = 12.2 Hz, H-6a), 4.40 (dd, 1H, $J_{5,6b}$ = 5.3 Hz, H-6b), 4.04-4.21 (m, 3H, H-5, CH_2O), 3.33 (m, 2H, CH_2N), 1.76 (m, 2H, CCH_2C) ppm; ^{13}C-n.m.r.: δ, 166.3, 165.9, 165.4, 165.3, 133.7, 133.5, 133.3, 130.2, 130.0 (x 2), 130.5 (x 4), 130.0 (x 3), 129.6, 129.4 (x 2), 129.0 (x 2), 128.9 (x 2), 128.7, 128.6, 128.6 (x 2), 128.5, 87.7, 83.8, 75.0, 70.4 (x 2), 69.9, 63.2, 54.8, 29.9 ppm; HR-FAB MS $[M+Na]^+$ calcd for $C_{37}H_{31}NNaO_{10}S$ 704.1566, found 704.1573.

Benzothiazol-2-yl 2,3,4,6-tetra-O-benzoyl-1-thio-β-D-glucopyranoside (2.6b) was obtained as cream crystals in 88 % yield. Analytical data for **2.6b**: R_f = 0.49 (ethyl acetate-hexanes, 2/3, v/v); m.p. +212-214 ^oC (CH_2Cl_2/ ether/ hexane); $[\alpha]_D^{25}$ +58.6^o (c = 1.03, CH_2Cl_2); UV: λ_{max} = 276 nm; ^{1}H-n.m.r.: δ, 7.20-8.10 (m, 24H, aromatic), 6.08 (dd, 1H, $J_{3,4}$ = 9.4 Hz, H-3), 5.95 (d, 1H, $J_{1,2}$ = 10.2 Hz, H-1), 5.71-5.79 (m, 2H, $J_{2,3}$ = 9.4 Hz, H-2, 4), 4.71 (dd, 1H, $J_{5,6a}$ = 2.1 Hz, $J_{6a,6b}$ = 11.8 Hz, H-6a), 4.42-4.55 (m, 2H, H-5, 6b) ppm; ^{13}C-n.m.r.: δ, 166.4, 166.1, 165.6, 165.6, 62.5, 153.0, 134.0, 133.9 (x 2), 133.7 (x 2), 133.4 (x 2), 130.3 (x 2), 130.3 (x 2), 130.1 (x 2), 130.0, 129.4(x 2), 129.0 (x 2), 128.9 (x 3), 128.8 (x 2), 128.7 (x 2), 128.6, 126.6, 125.2, 122.6, 121.3, 84.6, 74.3, 70.8, 69.7, 63.6 ppm; HR-FAB MS $[M+Na]^+$ calcd for $C_{41}H_{31}NNaO_9S_2$ 768.1338, found 768.1323.

5,6-Dihydro-4H-1,3-thiazin-2-yl 2,3,4,6-tetra-O-benzoyl-1-thio-β-D-glucopyranoside (2.7b) was prepared from **2.16** in 18 % yield. Alternatively it was prepared from **2.7a** as follows: 0.1 N solution of NaOMe in MeOH was added dropwise until pH 9 to the solution of **2.7a** (0.4 g, 0.863 mmol) in MeOH (20 mL). The reaction mixture was kept for 3 h at rt and then neutralized (pH 7) by the addition of Dowex (H^+). The resin was filtered off, washed with MeOH (5 x 5 mL and the combined filtrate (35 mL) was concentrated *in vacuo* and dried. The stirring solution of the residual white solid (0.27g, 0.914 mmol) in pyridine (16 mL) was cooled to 0 ^oC and benzoyl chloride (1.05 mL, 9.146 mmol) was added dropwise. The temperature was allowed to gradually increase and the reaction mixture was left stirring for 16 h at rt. Upon completion, the reaction was quenched by slow dropwise addition of MeOH (5 mL), concentrated in vacuo and then coevaporated with toluene (3 x 10 mL). The residue was then diluted with CH_2Cl_2 (35

mL), washed with water (20 mL), the combined filtrate was then washed with $NaHCO_3$ (20 mL), water (20 mL), 1N aqueous HCl (20 mL), and water (3 x 20 mL), the organic layer was separated, dried and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate/toluene gradient elution) followed by crystallization from CH_2Cl_2/ ether/ hexane to afford **2.7b** as cream crystals in 72 % yield. Analytical data for **2.7b**: $R_f = 0.49$ (acetone-toluene, 1/9, v/v); m.p. +93-95 ^oC (CH_2Cl_2/ ether/ hexane); $[\alpha]_D^{25}$ +78.6^o (c = 1.03, $CHCl_3$); UV: λ_{max} = 276 nm; ^{1}H-n.m.r.: δ, 7.10-8.00 (m, 20H, aromatic), 5.93 (dd, 1H, $J_{3,4}$ = 9.4 Hz, H-3), 5.85 (d, 1H, $J_{1,2}$ = 10.4 Hz, H-1), 5.53-5.65 (m, 2H, $J_{2,3}$ = 9.4 Hz, H-2, 4), 4.56 (dd, 1H, $J_{5,6a}$ = 4.6 Hz, $J_{6a,6b}$ = 12.2 Hz, H-6a), 4.41 (dd, 1H, $J_{5,6b}$ = 5.6 Hz, H-6b), 4.20 (m, 1H, H-5) ppm; ^{13}C-n.m.r.: δ, 166.5, 166.1, 165.6, 152.8, 134.0, 133.9, 133.8, 133.6, 130.6 (x 2), 130.8 (x 3), 130.4 (x 3), 130.3 (x 2), 129.6, 129.4, 129.0 (x 2), 128.9 (x 3), 128.7 (x 4), 81.2, 76.8, 74.7, 70.3, 69.9, 63.6, 48.9, 28.0, 20.6 ppm; HR-FAB MS $[M+Na]^+$ calcd for $C_{38}H_{33}NNaO_9S_2$ 734.1494, found 734.1481.

2.4.3 Synthesis of the disaccharide 2.20

2.4.3.1 General AgOTf-promoted glycosylation procedure.

A mixture a glycosyl donor (0.11 mmol), **2.19** (0.10 mmol), and freshly activated molecular sieves (3Å, 200 mg) in $(ClCH_2)_2$ (2 mL) was stirred under an atmosphere of argon for 1.5 h. Freshly conditioned AgOTf (0.22 mmol) was added and the reaction mixture was stirred for 5 min - 16 h (see Table 2) at rt, then diluted with CH_2Cl_2, the solid was filtered-off and the residue was washed with CH_2Cl_2. The combined filtrate (30

mL) was washed with 20% aq. $NaHCO_3$ (15 mL) and water (3 x 10 mL), the organic phase was separated, dried and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate-hexane gradient elution) to afford **2.20**, analytical data for which were essentially the same as those previously reported.[22]

2.4.3.2 General MeOTf-promoted glycosylation procedure.

A mixture the glycosyl donor (0.11 mmol), **2.19** (0.10 mmol), and freshly activated molecular sieves (3Å, 200 mg) in $(ClCH_2)_2$ (2 mL) was stirred under an atmosphere of argon for 1.5 h. MeOTf (0.22 mmol) was added and the reaction mixture was stirred for 1 - 16 h (see Table 2) at rt, then diluted with CH_2Cl_2, the solid was filtered-off and the residue was washed with CH_2Cl_2. The combined filtrate (30 mL) was washed with 20% aq. $NaHCO_3$ (15 mL) and water (3 x 10 mL), the organic phase was separated, dried with $MgSO_4$ and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate-hexane gradient elution) to afford **2.20**

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CHAPTER 3

Synthesis of the repeating unit of *Streptococcus pneumoniae* 6A and 6B using novel thioimidate glycosylation methodology

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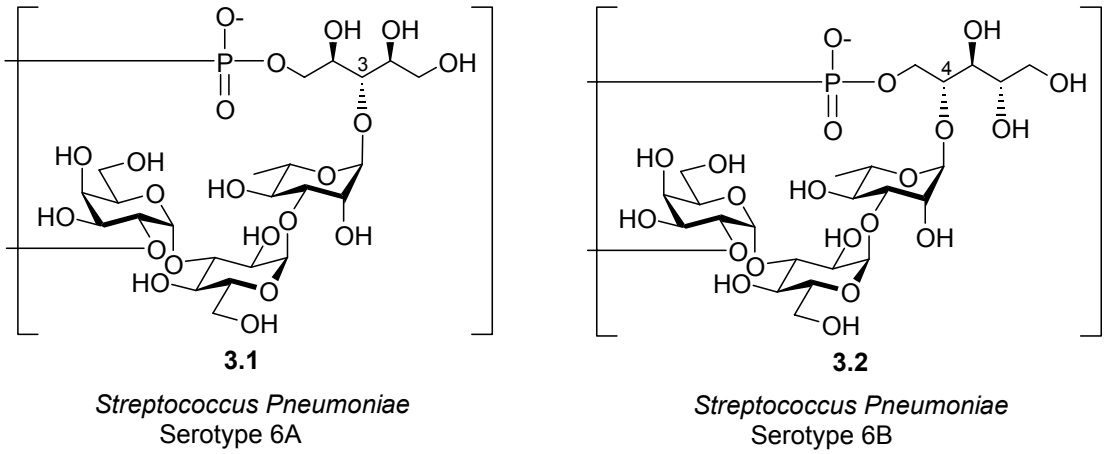
Parameswar, A. R.; Pornsuriyasak, P.; Lubanowski, N. A.; Demchenko, A. V. "Efficient stereoselective synthesis of oligosaccharides of *Streptococcus pneumoniae* 6A and 6B containing multiple 1,2- cis glycosidic linkages", *Tetrahedron,* **2007**, 63, 10083-10091.

3 Synthesis of the repeating unit of *Streptococcus pneumoniae* 6A and 6B

3.1 Introduction

Involvement of complex glycostructures in a variety of damaging and healing processes has already been acknowledged by development of carbohydrate-based vaccines[1-3] and therapeutics.[4,5] Chapter 1, section 1.1, describes introduction to this class of *Streptococcus pneumoniae* (SPn) in detail and its lethal effects

Amongst over ninety elucidated SPn serotypes[6] (Chapter 1), SPn6A and SPn6B are equally important causes of bacterial infections.[7] In addition, this serogroup has been ranked within the top three causes of invasive pneumococcal disease worldwide.[8] This fact stimulated extensive structural studies leading to establishing the structures of capsular polysaccharides of both 6A (**3.1**) and 6B (**3.2**), (Figure 3.1).[9-11] Serotype-specific antibodies are formed in response to vaccination with the pneumococcal polysaccharide or saccharide-protein conjugate, so it was believed that due to similarity in the carbohydrate core structures of SPn6A and SPn6B, the elicited antibodies would be cross-reactive against both types.[10, 12-14] As a result, only the hydrolytically more stable and, hence, mere easily accessible natural isolate of SPn6B was selected and included in all currently licensed multi-component vaccines. A number of linear chemical syntheses of SPn6B, their mimetics, and conjugates have also emerged.[15-20]



3.1

Streptococcus Pneumoniae
Serotype 6A

3.2

Streptococcus Pneumoniae
Serotype 6B

Figure 3.1 Structures of the repeating unit of SPn6A and 6B

Recent cross-reactivity studies challenged the hypothesis of the cross-reactivity by determining that SPn6B-based vaccines produce 6B-specific antibodies that cross-react with SPn6A at a much lower rate.[7,20-22] In this context, similar observations had also been made for the capsular polysaccharides types 19F and 19A.[23] Hence, the importance of including the SPn6A carbohydrate conjugates in the future generations of multi-serotype anti-SPn vaccines has been acknowledged.[24] However, the achievement of this is challenging for a number of reasons, the most important of which is low hydrolytic stability of SPn6A isolates that results in their low availability in sufficiently pure form.[25] A possible solution for this would be the chemical synthesis of the related sugar derivatives and their application as oligosaccharide-protein conjugate vaccine components. Although natural[26] and synthetic saccharides[15-17] of the SPn6A series have been investigated, no systematic studies have yet emerged. Herein, we discuss the application of the new thioimidoyl methodology developed in our laboratory to efficient stereoselective synthesis of pseudo di-, tri-, and tetrasaccharides (**3.1-3.6**, Figures 3.1 and 3.2) structurally related to SPn6A and 6B.

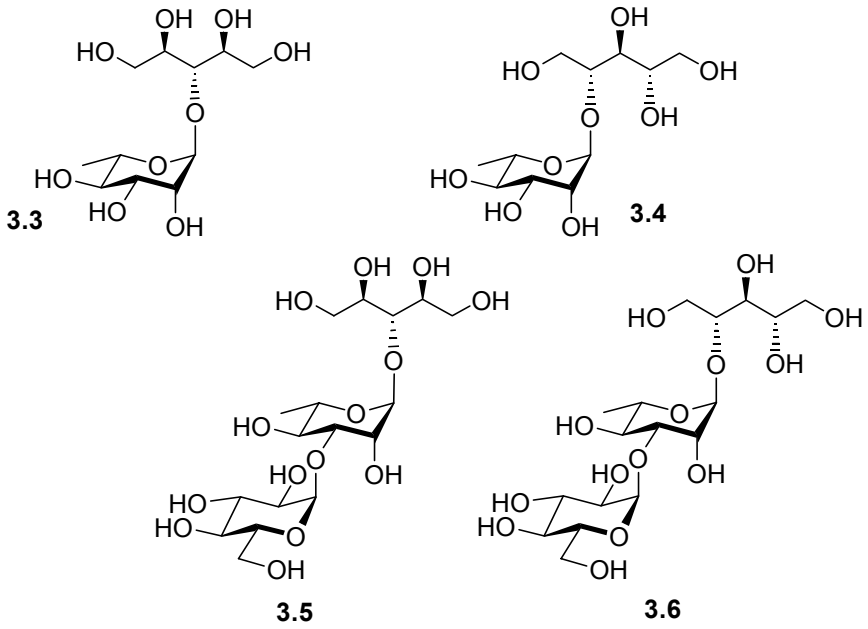


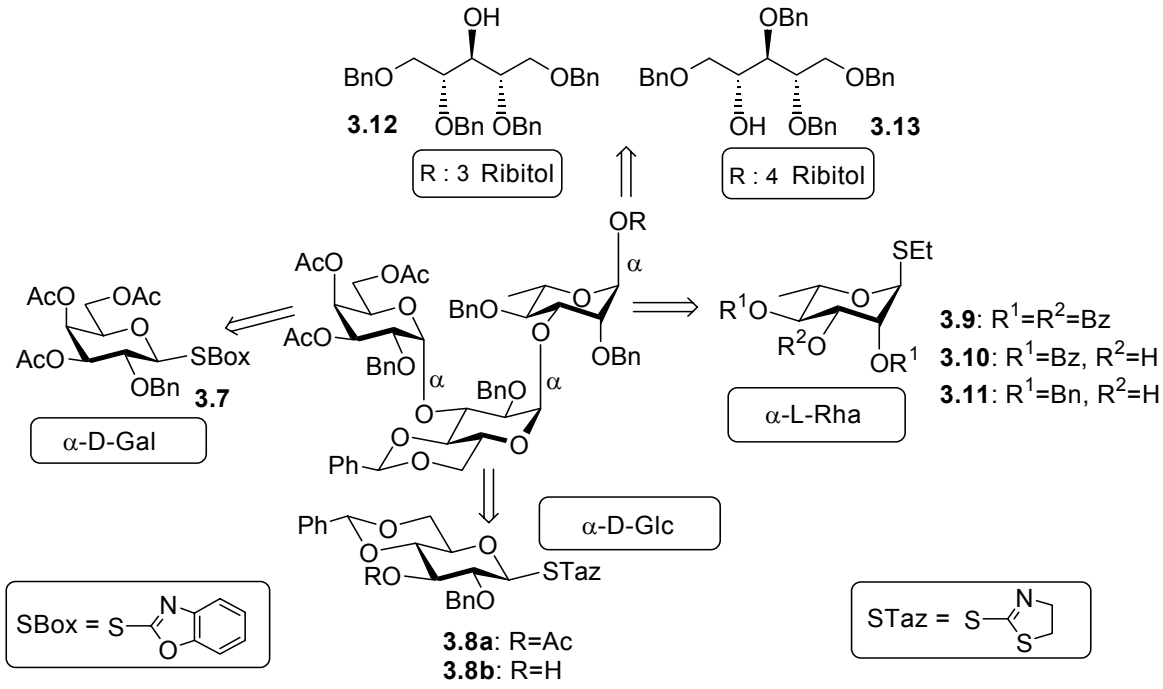
Figure 3.2 Synthetic targets of SPn6A and 6B

3.2 Retrosynthetic analysis

SPn6A and SPn6B repeating units consist of structurally similar complex pseudo tetrasaccharides **3.1** and **3.2**, respectively (Figure 3.1), which in the natural polysaccharide are connected via a phosphate (5→2”’) linkage. Both **3.1** and **3.2** bear a terminal D-galactosyl residue, which is α-glycosidically linked (1,2-cis) to the C-3 of a D-glucose unit; the latter is linked via 1,2-cis-glycosidic bond to the C-3 of L-rhamnose, which is connected via a α-glycosidic linkage (1,2-trans) to the C-3 of D-ribitol (SPn6A) or C-4 of D-ribitol (SPn6B). Efficient chemical synthesis of the repeating units of SPn6A, 6B (**3.1** and **3.2**, respectively) and structurally related glycoconjugates thereof (**3.3-3.6**, Figure 3.2) will help to quickly obtain substantial quantities of pure samples for immunological studies and subsequent fully synthetic vaccine development.

From the chemical point of view, a number of synthetic challenges could be anticipated. It should be noted that, even nowadays chemical synthesis of complex carbohydrates of this caliber, especially those containing "difficult" 1,2-cis glycosidic linkages, is still regarded as laborious and inefficient.[27] Our goal was to investigate whether the application of glycosyl thioimidates, suitable building blocks for 1,2-cis glycosylation,[28,29] would allow high 1,2-cis stereoselectivity and yields when applied to the synthesis of pneumococcal oligosaccharides. Therefore, the use of the anomeric thioimidoyl moieties, S-benzoxazolyl (SBox) or S-thiazolinyl (STaz), was projected for the introduction of 1,2-cis glycosidic linkages of oligosaccharides **3.1, 3.2, 3.5,** and **3.6**. A non-participating group at C-2, O-benzyl, is required to facilitate the introduction of 1,2-cis-linked galactose and glucose units. Since the thioimidoyl moiety can be selectively activated over conventional thioglycosides,[30] S-ethyl moiety was chosen for the introduction of the rhamnose unit.

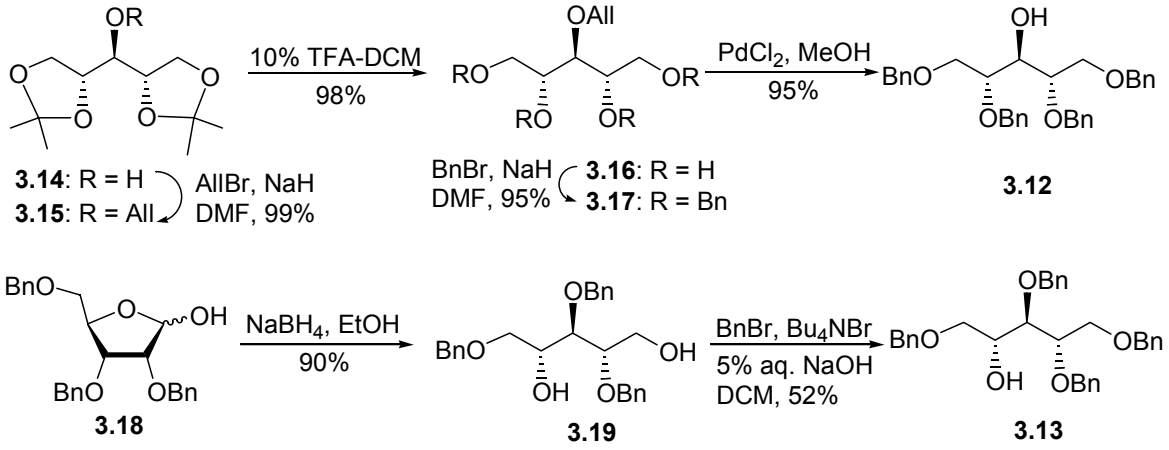
Having analyzed the target oligosaccharide structures and possible challenges associated with their synthesis, we narrowed down possible candidates for the key building blocks to the following compounds: D-galactose (**3.7**), D-glucose (**3.8a** and **b**), L-rhamnose (**3.9-3.11**) and D/L- or D-ribitol (**3.12** or **3.13**, respectively, Scheme 3.1).[31]A variety of rhamnose derivatives were to be investigated in an attempt to optimize the stereoselectivity and yield of rhamnosylation.



Scheme 3.1 Retrosynthetic analysis of oligosaccharides of SPn6A and 6B

3.3 Synthesis of building blocks

Synthesis of the 3-OH ribitol building block **3.12** was carried out from known precursor **3.14** as shown in Scheme 3.2.[32] Thus, allylation of **3.14** was accomplished in 99% yield and the resulting compound **3.15** was subjected to acetal cleavage followed by benzylation to afford fully protected intermediate **3.17** in over 90% yield for two steps. Deallylation of **3.17** using $PdCl_2$ in MeOH lead to glycosyl acceptor **3.12** in 95% yield. It is noteworthy that the synthesis of compound **3.12** has been previously accomplished using less expeditious pathway.[16] Unfortunately, the direct use of 3-OH ribitol **3.14** as glycosyl acceptor was found to be impractical. Partial loss of acetal protecting groups occurred during glycosylation and resulted in the formation of an inseparable mixture.



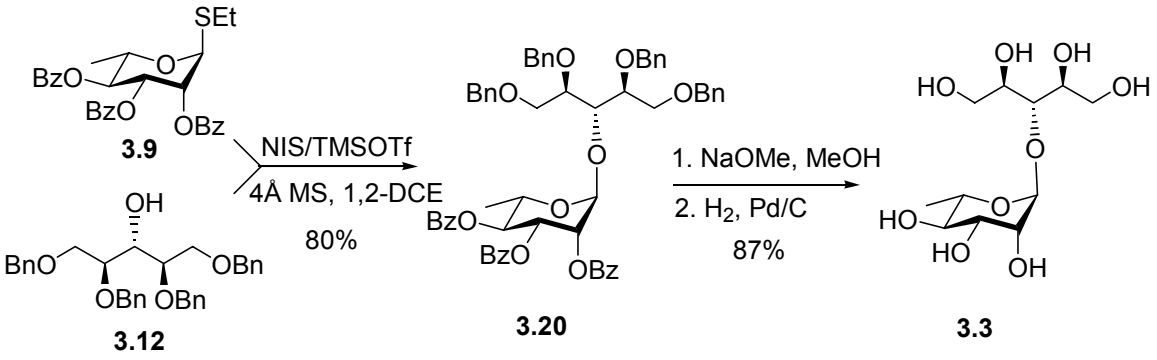
Scheme 3.2 Synthesis of ribitol acceptors **3.12** and **3.13**

Synthesis of 4-OH ribitol building block **3.13**, a component of SPn6B was achieved by the reduction of known hemiacetal **3.18**[33] to obtain **3.19**[34] in 90% yield (Scheme 3.2). The intermediate **3.19** was then regioselectively benzylated under phase transfer conditions to afford 4-OH glycosyl acceptor **3.13** as a major regioisomer in 52% yield. Other building blocks, D-galactose (**3.7**),[28] D-glucose (**3.8a** and **b**),[30] and L-rhamnose (**3.9-3.11**),[35-37] have been obtained as described previously.

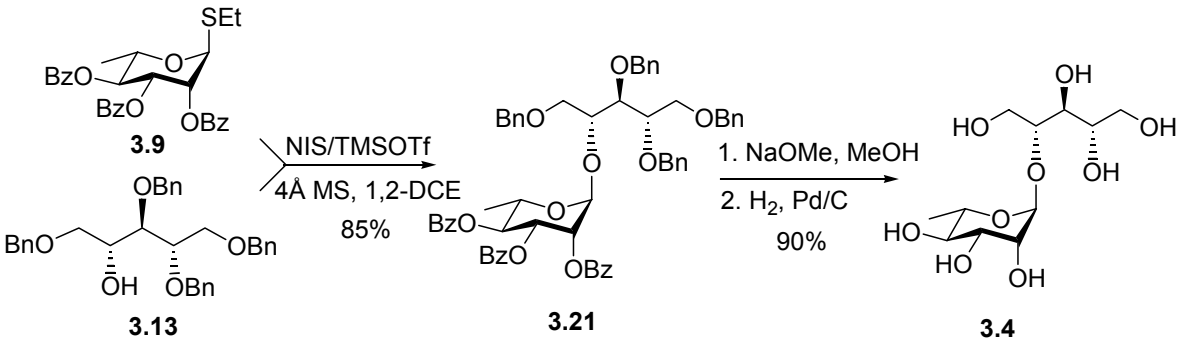
3.4 Oligosaccharide synthesis

For the synthesis of pseudo disaccharides **3.3** and **3.4**, the fully protected rhamnose building block **3.9** was used as a glycosyl donor. Coupling of **3.9** with ribitol acceptors **3.12** and **3.13** in the presence of NIS/ TMSOTf afforded glycosides **3.20** and **3.21** with complete α-selectivity in 80% and 85% yield, respectively (Schemes 3.3 and 3.4). Complete 1,2-trans stereoselectivity achieved herein is credited to the use of a neighboring participating substituent in **3.9**. The compounds obtained were then

subjected to a two-step sequential deprotection: deacylation (NaOMe in MeOH) and hydrogenation using palladium on charcoal to obtain the desired pseudo disaccharides **3.3** and **3.4** in 87% and 90% yield, respectively, over two steps.



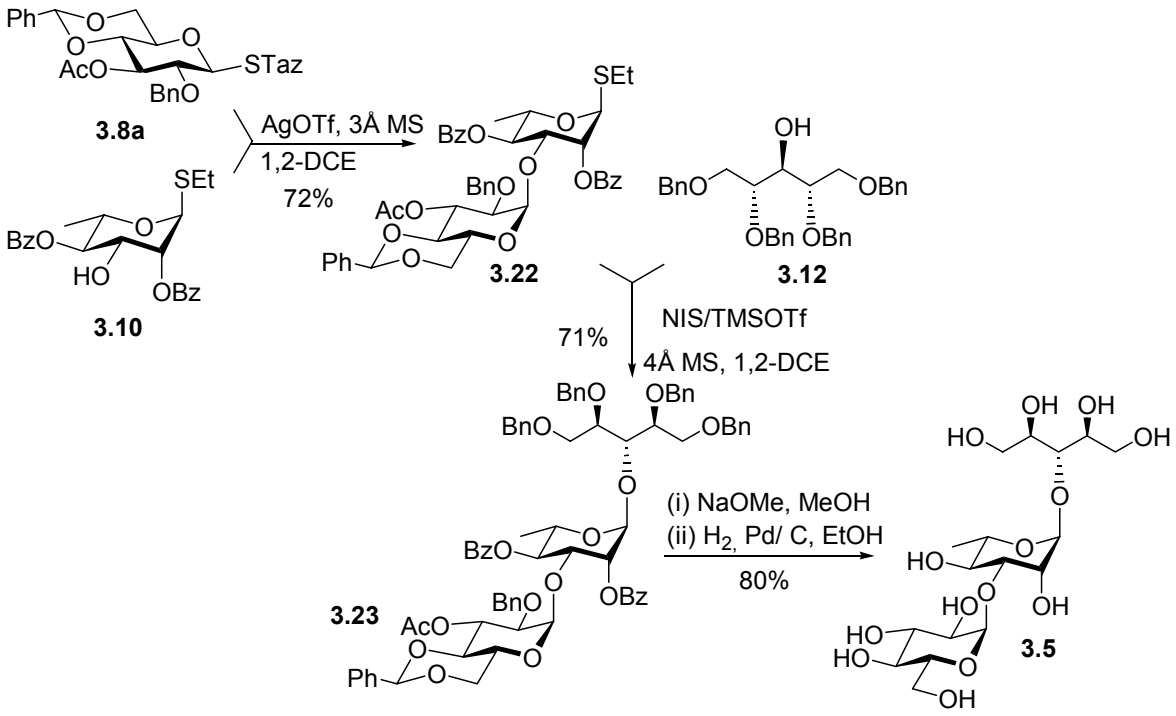
Scheme 3.3 Synthesis of Pseudo Disaccharide **3.3** of SPn6A



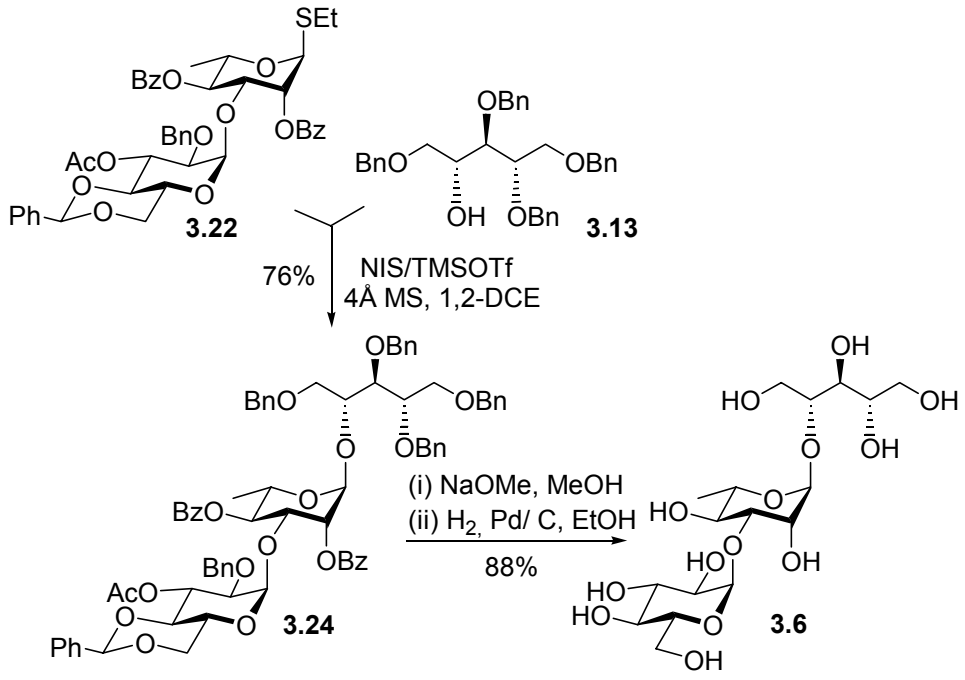
Scheme 3.4 Synthesis of Pseudo Disaccharide **3.4** of SPn6B

For the synthesis of pseudo trisaccharides **3.5**, disaccharide intermediate **3.22** was obtained in 72% yield. This was achieved by selective activation of the STaz moiety of glycosyl donor **3.8a** over the S-ethyl moiety of glycosyl acceptor **3.10** in the presence of silver(I) triflate (Scheme 3.5). This reaction proceeded with completely 1,2-cis stereoselectively as no traces of the 1,2- trans linked diastereomer were detected by analyzing the crude reaction mixture. Subsequently, the S-ethyl moiety of **3.22** was

activated with NIS and TMSOTf for glycosylation of glycosyl acceptors **3.12** and **3.13**. As a result, saccharides **3.23** (Scheme 3.5) and **3.24** (Scheme 3.6) were isolated in 71% and 76% yield, respectively. These rhamnosylations also proceeded with complete α-stereoselectivity. The protected pseudo trisaccharides **3.23** and **3.24** were subjected to standard deprotection conditions, deacylation followed by hydrogenation, to afford compounds **3.5** and **3.6** in 80% and 88% yield, respectively.



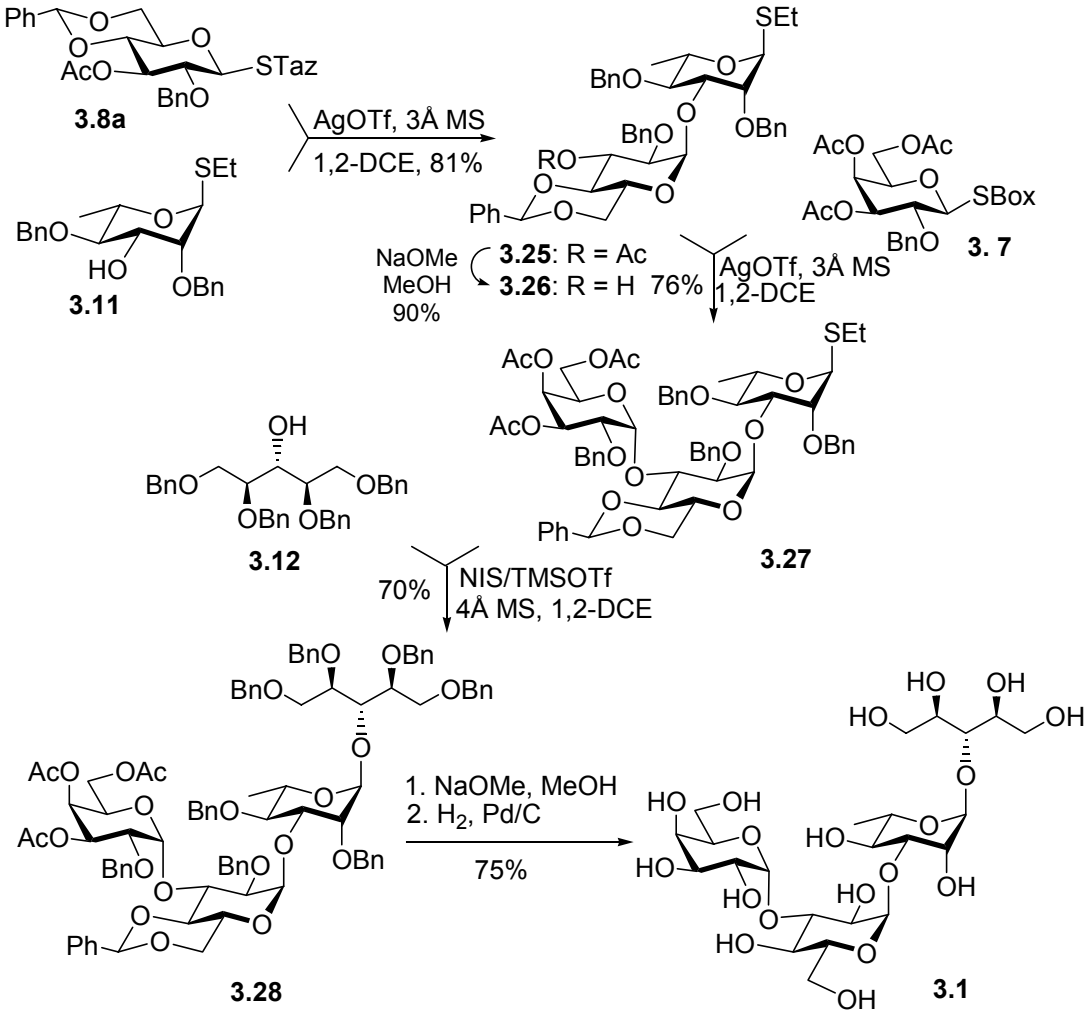
Scheme 3.5. Synthesis of Pseudo Trisaccharide **3.5** of SPn6A



Scheme 3.6 Synthesis of Pseudo Trisaccharide 3.6 of SPn6B

Additionally, we aimed for the synthesis of pseudo tetrasaccharides **3.1** and **3.2**. For this purpose, we first investigated selective activation of the SBox moiety of **3.7** over the STaz moiety of glucosyl acceptor **3.8b**. Unfortunately, this reaction resulted in the formation of an unseparable mixture of anomers (α/β = 7/1). This misfortune stimulated us to adopt a new strategy, according to which a glucosyl donor bearing the STaz leaving group (**3.8a**) was first selectively activated over the S-ethyl moiety rhamnose acceptor (**3.11**) in the presence of AgOTf. Although this reaction was performed in 1,2-dichloroethane, a solvent that does not normally favor α-glycosylation, the disaccharide **3.25** was obtained as a single α–anomer (1,2-cis) in 81% yield (Scheme 3.7). Subsequently, 3'-O-acetyl group in **3.25** was removed under Zemplen conditions and the resulting disaccharide acceptor **3.26** was coupled with glycosyl donor **3.7**. Selective activation of the SBox moiety over the S-ethyl moiety was accomplished in the presence

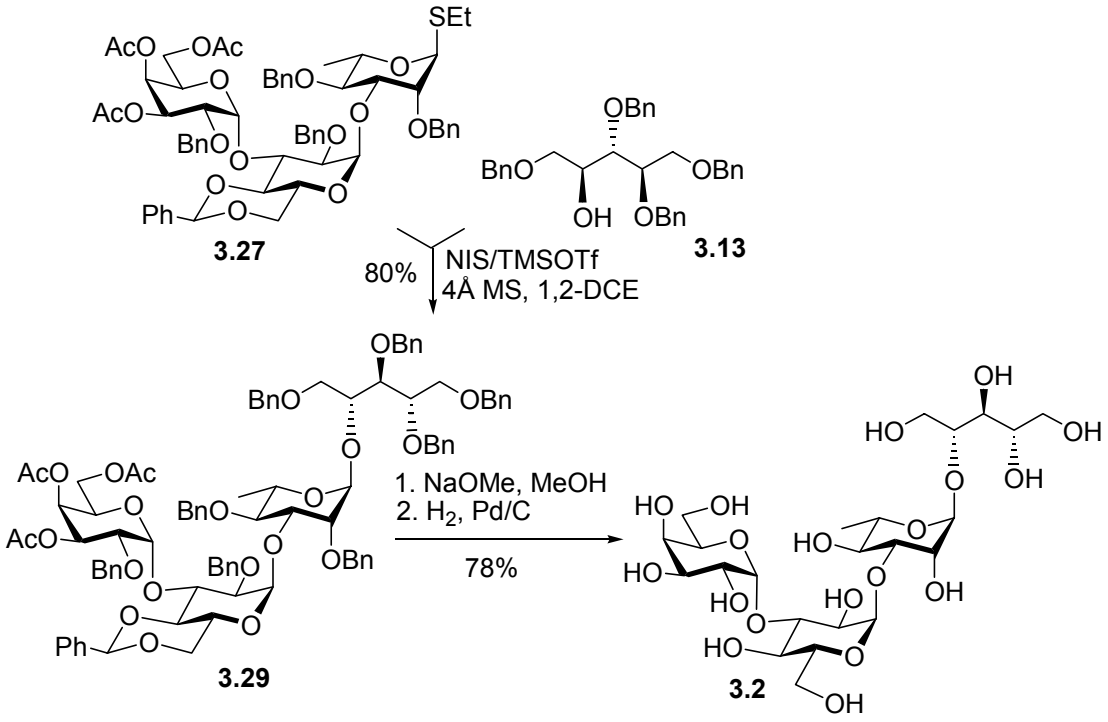
of AgOTf. In this case, trisaccharide **3.27** was obtained with complete α selectivity in 76% yield. No trace of the β-anomer could be detected by analysis of the crude reaction mixture. To complete the assembly, the trisaccharide **3.27** was coupled with ribitol acceptors **3.12** and **3.13** in the presence of NIS and TMSOTf (Schemes 3.7 and 3.8). As a result, pseudo tetrasaccharide derivatives **3.28** and **3.29** were obtained in 70% and 80% yield, respectively. Finally, the deprotection of **3.28** and **3.29** was carried out using conventional deacylation-hydrogenation sequence to afford pseudo tetrasaccharides **3.1** and **3.2** in 75% and 78% yield, respectively.



Scheme 3.7 Synthesis of Pseudo Tetrasaccharide **3.1** of SPn6A

3.5 Conclusions

An efficient convergent approach to the synthesis of pneumococcal oligosaccharides of SPn6A and 6B for model immunological studies was developed. Rapid oligosaccharide assembly was accomplished by selective activation of the STaz and SBox leaving group of glycosyl donors over the S-ethyl anomeric moiety of glycosyl acceptors. Application of the STaz and SBox methodology has also allowed completely stereoselective introduction of two challenging 1,2-cis glycosidic moieties.



Scheme 3.8 Synthesis of Pseudo Tetrasaccharide **3.2** of SPn6B

3.6 Experimental part

3.6.1 *General*

Refer to Chapter 2 (Page 81)

3-O-Allyl-1,2:4,5-di-O-isopropylidene-D/L-ribitol (3.15). Allyl bromide (0.75 mL, 8.62 mmol) was added to a stirring solution of 1,2:4,5-di-O-isopropylidene-D/L-ribitol (**3.14,** 1.0 g, 4.3 mmol) in dry DMF (10 mL) at 0 ^{o}C. Sodium hydride (60% suspension in mineral oil, 12.9 mmol) was added slowly until evolution of hydrogen gas has seized. The reaction mixture was stirred for 20 min at rt until complete disappearance of starting material as indicated by TLC. The reaction mixture was poured in crushed ice (~100 mL), stirred for 15 min and extracted with ethyl acetate/ ether (1/1, v/v, 3 x 100 mL). The combined organic phase was dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate-hexane gradient elution) to afford compound **3.15** as a syrup (1.16 g, 99% yield). Analytical data: R_f = 0.50 (ethyl acetate - hexanes, 1/5, v/v); $[\alpha]_D^{26}$ -0.7^{o} (c = 1.0, $CHCl_3$); ^{1}H-n.m.r.: δ, 1.27, 1.35 (2s, 12H, 4 x CH_3), 3.57 (dd, 1H, $J_{3,4}$ = 4.8 Hz, H-3), 3.59-3.99 (m, 4H, H-1, 5), 4.01-4.05 (m, 2H, H-2, 4), 4.17 (m, 2H, OCH_2), 5.06-5.21 (m, 2H, CH_2=), 5.80-5.91 (m, 1H, =CH-) ppm; ^{13}C-n.m.r.: δ, 25.7 (x 2), 25.8 (x 2), 66.2 (x 2), 74.2, 76.6 (x 2), 78.8, 110.0 (x 2), 117.3, 135.3 ppm, HR-EI MS $[M\text{-}CH_3]^+$ calcd for $C_{13}H_{21}O_5$ 257.1384, found 257.1389.

3-O-Allyl–D/L-ribitol (3.16). To a stirred solution of **3.15** (1.16 g, 4.6 mmol) in CH_2Cl_2 (10 mL) 90% aq. trifluoroacetic acid (1 mL) was added dropwise and the reaction was

kept for 30 min at rt until complete disappearance of the starting material (monitored by TLC). The reaction mixture was neutralized with triethylamine (~ 1 mL) and concentrated in *vacuo*. The residue was purified by column chromatography on silica gel (methanol-dichloromethane gradient elution) to afford compound **3.16** as a syrup (0.81 g, 98% yield). Analytical data: R_f = 0.46 (methanol - dichloromethane, 1/5, v/v); $[\alpha]_D^{28}$ - 1.5^o (c = 1.0, methanol); ^{1}H-n.m.r. (CD_3OD): δ, 3.47 (dd, 1H, $J_{2,3}$ = 5.7 Hz, H-3), 3.57-3.63 (m, 2H, H-5), 3.70-3.75 (m, 2H, H-1), 3.79-3.84 (m, 2H, H-2, 4), 4.13-4.16 (m, 2H, OCH_2), 5.09-5.27 (m, 2H, CH_2=), 5.84-5.98 (m, 1H, =CH-) ppm; ^{13}C-n.m.r. (CD_3OD): δ, 65.1 (x 2), 72.6 (x 2), 73.9, 82.1, 117.9, 136.7 ppm; HR-FAB MS $[M+Na]^+$ calcd for $C_8H_{16}O_5Na$ 215.0895, found 215.0895.

3-O-Allyl-1,2,4,5-tetra-O-benzyl-D/L-ribitol (3.17). Benzyl bromide (2.5 mL, 21.1 mmol) was added to a stirring solution of **3.16** (0.81g, 4.21 mmol) in dry DMF (15 mL) at 0 ^{o}C. Sodium hydride (60% suspension in mineral oil, 1.26 g, 31.6 mmol) was added slowly until evolution of hydrogen gas has seized. The reaction mixture was stirred for 30 min at rt until complete disappearance of starting material as indicated by TLC. The reaction mixture was poured in crushed ice (~50 mL), stirred for 15 min, extracted with ethyl acetate/ether (1/1, v/v, 3 x 80 mL). The combined organic extract was dried over anhydrous $MgSO_4$ and concentrated in *vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate-hexane gradient elution) to afford compound **3.17** as a syrup (2.27 g, 98% yield). Analytical data for **3.17** was essentially the same as reported previously.[16]

1,2,4,5-Tetra-O-benzyl-D/L-ribitol (3.12). To a stirring solution of **3.17** (1.5 g, 2.7 mmol) in MeOH (15 mL), $PdCl_2$ (0.6 g) was added and the reaction was stirred at rt for 12 h. The solid was then filtered off through celite, washed with methanol (3 x 10 mL) and concentrated in *vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate-hexane gradient elution) to afford compound **3.12** as a syrup (1.29 g, 93% yield). Analytical data R_f = 0.42 (ethyl acetate - hexanes, 3/7, v/v); $[\alpha]_D^{23}$ +4.3^o (c = 1.0, $CHCl_3$); ^{1}H-n.m.r.: δ, 3.54-3.60 (m, 2H, H-2,4), 3.63-3.68 (m, 4H, H-1, 5), 3.98 (dd, 1H, $J_{3,4}$ = 10.9 Hz, H-3), 4.38-4.62 (m, 8H, 4 x *CH*$_2$Ph), 7.16-7.25 (m, 20H, aromatic) ppm; ^{13}C-n.m.r. δ, 70.7 (x 2), 72.1, 72.5 (x 2), 73.9 (x 2), 78.6 (x 2), 128.1 (x 3), 128.1 (x 3), 128.2 (x 4), 128.4 (x 4), 128.8 (x 4), 128.9 (x 4), 136.6, 138.9 ppm; HR-FAB MS $[M+H]^+$ calcd for $C_{33}H_{37}O_5$ 513.2641, found 513.2642. It should be noted that partial analytical data for **3.12** was reported previously.[16]

2,3,5-Tri-O-benzyl-D-ribitol (3.19). To a stirring suspension of $NaBH_4$ (350 mg, 9.2 mmol) in absolute ethanol (17 mL) at 0 ^{o}C, was added a solution of 2,3,5-tri-O-benzyl-D-ribose (**3.18**, 2.28 g, 5.4 mmol) in absolute ethanol (19 mL) and the reaction was stirred for 15 min at rt. Upon completion, 96% aq. acetic acid was added until pH 5 (~ 1.0 mL). The resulting mixture was diluted with DCM (100 mL), washed with 1M HCl (30 mL), and water (2 x 30 mL). The organic phase was separated, dried over $MgSO_4$, and concentrated in *vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate-hexane gradient elution) to afford compound **3.19** as a syrup (2.07 g, 90% yield). Analytical data: R_f = 0.53 (ethyl acetate - hexanes, 2/3, v/v); $[\alpha]_D^{22}$ +19.9^o (c = 1.0, $CHCl_3$); ^{1}H-n.m.r.: δ, 3.55-3.58 (m, 2H, H-5), 3.69-3.76 (m, 2H, H-2, 3), 3.78-3.82

(m, 2H, H-1), 3.92-4.02 (m, 1H, H-4), 4.38-4.71 (m, 6H, 3 x *CH*$_2$Ph), 7.13-7.21 (m, 15H, aromatic) ppm; ^{13}C-n.m.r.: δ, 61.6, 71.2, 71.6, 72.6, 74.0, 74.6, 79.9, 80.0, 128.4 (x 3), 128.5 (x 2), 128.6 (x 2), 128.7 (x 2), 129.0 (x 2), 129.1 (x 4), 138.5, 138.6 (x 2) ppm; HR-FAB MS $[M+H]^+$ calcd for $C_{26}H_{31}O_5$ 423.2172, found 423.2176. It should be noted that the synthesis and partial analytical data of compound **3.19** was previously described.[34,38]

1,2,3,5-Tetra-O-benzyl-D-ribitol (3.13). To a solution of compound **3.19** (724 mg, 1.71 mmol) in CH_2Cl_2 (15 mL), BnBr (0.3mL, 1.22 mmol) was added followed by Bu_4NBr (110 mg, 0.34 mmol) and 5% aq. NaOH (4.5 mL). The reaction mixture was stirred for 16 h under reflux (50 ^oC), then cooled to rt, diluted with CH_2Cl_2 (50 mL), and washed with saturated aq. $NaHCO_3$ (15 mL) and water (3 x 15 mL). The organic phase was separated, dried over $MgSO_4$, and concentrated in *vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate - hexanes gradient elution) to afford **3.13** as white crystals (458 mg, 52% yield). Also the regioisomer (2,3,4,5-tetra-O-benzyl-D-ribitol) was isolated in 32% yield. Analytical data for **3.13**: R_f = 0.46 (ethyl acetate - toluene, 3/7, v/v); $[\alpha]_D^{22}$ +18.1^o (c = 1.0, $CHCl_3$); m.p. 40 ºC; ^{1}H-n.m.r.: δ, 3.59-3.61 (m, 2H, H-5), 3.68-3.73 (m, 1H, H-3), 3.76-3.84 (m, 2H, H-1), 3.92-3.96 (m, 1H, H-2), 3.98-4.04 (m, 1H, H-4), 4.50-4.75 (m, 8H, 4 x *CH*$_2$Ph), 7.18-7.33 (m, 20H, aromatic) ppm; ^{13}C-n.m.r.: δ, 70.3, 71.6, 71.8, 73.1, 74.0 (x 2), 74.3. 79.5, 79.7, 128.1, 128.2 (x 2), 128.3 (x 3), 128.4 (x 2), 128.5 (x 2), 128.6 (x 2), 128.9 (x 4), 129.0 (x 4), 138.7, 138.8, 138.9, 139.0 ppm; HR-FAB MS $[M+H]^+$ calcd for $C_{33}H_{37}O_5$ 513.2641, found 513.2642.

3.6.2 Preparation of disaccharides and oligosaccharides.

Method A. AgOTf-Promoted activation of the STaz and SBox glycosyl donors. A mixture the glycosyl donor (0.11 mmol), glycosyl acceptor (0.10 mmol), and freshly activated molecular sieves (3Å, 200 mg) in $ClCH_2CH_2Cl$ (2 mL) was stirred under argon for 1.5 h. Freshly conditioned AgOTf (0.22 mmol) was added and the reaction mixture was stirred for 15 min at rt, then diluted with CH_2Cl_2, the solid was filtered-off and the residue was washed with CH_2Cl_2. The combined filtrate (30 mL) was washed with 20% aq. $NaHCO_3$ (15 mL) and water (3 x 10 mL), the organic phase was separated, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate - hexane gradient elution) or Sephadex LH-20 (methanol – dichloromethane, 1/1, v/v elution) to afford a di- or a trisaccharide derivative.

Method B. NIS/TMSOTf-promoted activation of S-ethyl glycosyl donors. A mixture the glycosyl donor (0.13 mmol), glycosyl acceptor (0.10 mmol), and freshly activated molecular sieves (4Å, 200 mg) in $ClCH_2CH_2Cl$ (2 mL) was stirred under argon for 1 h. NIS (0.25 mmol) and TMSOTf (0.025 mmol) was added at 0 ^oC and the reaction mixture was stirred for 10 min. Upon completion, the solid was filtered-off and the residue was washed with CH_2Cl_2. The combined filtrate (30 mL) was washed with 20% aq. $Na_2S_2O_3$ (15 mL) and water (3 x 10 mL). The organic phase was separated, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate - hexane gradient elution) or Sephadex LH-20 (methanol – dichloromethane, 1/1, v/v elution) to afford a di- or an oligosaccharide derivative.

3-O-(2,3,4-Tri-O-benzoyl-α-L-rhamnopyranosyl)-1,2,4,5-tetra-O-benzyl-D/L-ribitol (3.20). This compound was obtained by Method B from ethyl 2,3,4-tri-O-benzoyl-1-thio-L-rhamnopyranoside **3.9** and **3.12** in 80% yield. Analytical data for **3.20**: R_f = 0.49 (ethyl acetate – hexanes, 3/7, v/v); $[\alpha]_D^{22}$ +56.3° (c =1, $CHCl_3$); ^{1}H-n.m.r.: δ, 1.14 (d, 3H, H-6'), 3.70-3.77 (m, 2H, H-1), 3.79-3.82 (m, 2H, H-5), 3.89-3.91 (m, 1H, H-2), 3.99-4.01 (m, 1H, H-4), 4.31-4.34 (m, 2H, H-3, 5'), 4.45-4.75 (m, 8H, 4 x *CH*$_2$Ph), 5.30 (s, 1H, H-1'), 5.54-5.67 (m, 2H, H-2', 4'), 5.73-5.87 (m, 1H, H-3'), 7.21-8.09 (m, 35H, aromatic) ppm; ^{13}C-n.m.r.: δ, 17.9, 67.9, 69.2, 70.3 (x 2), 71.5, 72.4, 72.7 (x 3), 73.9 (x 3), 78.5, 98.4, 28.1 (x 3), 128.2 (x 2), 128.4 (x 2), 128.5 (x 6), 128.9 (x 6), 129.0 (x 3), 129.1 (x 2), 129.2 (x 2), 130.1 (x 3) , 130.4 (x 4), 130.6 (x 2), 133.9 (x 3), 139.9 (x 4), 166.1, 166.2, 166.4 ppm; HR-FAB MS $[M+Na]^+$ calcd for $C_{60}H_{58}O_{12}Na$ 993.3826, found 993.3840.

4-O-(2,3,4-Tri-O-benzoyl-α-L-rhamnnopyranosyl)-1,2,3,5-tetra-O-benzyl-D-ribitol (3.21). This compound was obtained by Method B from **3.9** and **3.13** in 85% yield. Analytical data for **3.21**: R_f = 0.51 (ethyl acetate – hexanes, 3/7, v/v); $[\alpha]_D^{22}$ +39.6° (c = 1, $CHCl_3$); ^{1}H-n.m.r.: δ, 1.15 (d, 3H, H-6'), 3.71-3.90 (m, 6H, H-1, 2, 3, 5), 4.26-4.36 (m, 1H, H-5'), 4.42-4.81 (m, 9H, H-4, 4 x *CH*$_2$Ph), 5.48 (s, 1H, H-1'), 5.64 (dd, 1H, $J_{2',3'}$ = 9.9 Hz, H-3'), 5.78-5.87 (m, 2H, H-2', 4'), 7.19-8.12 (m, 35H, aromatic) ppm; ^{13}C-n.m.r.: δ, 18.0, 67.7, 70.6, 70.7, 71.4, 71.6, 72.5, 73.3, 73.9 (x 2), 76.4, 78.7, 79.5, 97.4, 128.1 (x 2), 128.2 (x 2), 128.3 (x 3), 128.4 (x 3), 128.5 (x 3), 128.6 (x 3), 128.7 (x 2), 128.9 (x 6), 129.1 (x 2), 129.9 (x 2), 130.0, 130.2 (x 4), 130.5 (x 2), 133.6, 133.8, 133.9, 138.7 (x 2), 138.9 (x 2), 165.8, 165.9, 166.3 ppm; HR-FAB MS $[M+Na]^+$ calcd for $C_{60}H_{58}O_{12}Na$ 993.3826, found 993.3840.

O-(α-L-Rhamnopyranosyl)-(1→3)-D/L-ribitol (3.3). To a solution of **3.20** (100 mg, 0.103 mmol) in dry methanol (1.0 mL) was added 1M NaOMe till pH = 10 (~0.1 mL). The reaction mixture was stirred for 15 h at rt, then neutralized with Dowex (H^+), filtered, and concentrated in *vacuo*. The crude residue was dissolved in ethylacetate / ethanol (1/1, v/v, 2.0 mL) and 10% Pd/C (20 mg) was added. The reaction mixture was stirred under an atmosphere of H_2 for 15 h. The catalyst was then filtered off, washed with methanol and the filtrate was concentrated under reduced pressure. The residue was purified by column chromatography on Sephadex G-15 (water elution) to afford compound **3.3** as a syrup (26.8 mg, 87% yield). Analytical data: R_f = 0.52 (methanol – dichloromethane, 1/1, v/v); $[\alpha]_D^{26}$ -45.4° (c = 0.8, H_2O); ^{1}H-n.m.r. (D_2O).: δ, 1.20 (d, 3H, H-6'), 3.37 (dd, 1H, $J_{3',4'}$ = 9.7 Hz, H-4'), 3.52-3.58 (m, 2H, H-1b, 5b), 3.66-3.74 (m, 5H, H-1a, 5a, 3, 3', 5'), 3.82-3.88 (m, 2H, H-2, 4), 3.90 (dd, 1H, $J_{2',3'}$ = 1.7 Hz, H-2'), 4.88 (d, 1H, $J_{1',2'}$ = 1.7 Hz, H-1') ppm; ^{13}C-n.m.r. (D_2O).: δ, 16.9 (C-6'), 62.7 (C-1), 63.0 (C-5), 69.7 (C-5'), 70.5 (C-3'), 70.7 (C-2'), 71.1 (C-4), 72.0 (C-2), 72.2 (C-4'), 80.2 (C-3), 100.9 (C-1') ppm; HR-FAB MS $[M+Na]^+$ calcd for $C_{11}H_{22}O_9Na$ 321.1161, found 321.1171.

O-(α-L-Rhamnopyranosyl)-(1→4)-D-ribitol (3.4). Compound **3.4** was obtained from **3.21** in 90% yield as described for the synthesis of compound **3.3**. Analytical data: R_f = 0.43 (methanol – dichloromethane, 1/1, v/v); $[\alpha]_D^{25}$ -34.3° (c =1, H_2O); ^{1}H-n.m.r. (D_2O).: δ, 1.23 (d, 3H, H-6'), 3.51 (dd, 1H, $J_{3',4'}$ = 9.6 Hz, H-4'), 3.69 (dd, 1H, $J_{1a,1b}$ = 4.9 Hz, H-1b), 3.77-3.86 (m, 4H, H-2, 3, 3', 5'), 3.90 (dd, 1H, H-1a), 3.93 (d, 1H, $J_{5a,5b}$ = 3.1 Hz, H-5b), 3.95-3.99 (m, 2H H-4, 5a), 4.07 (dd, 1H, $J_{2',3'}$ = 1.7 Hz, H-2'), 5.07 (d, 1H, $J_{1',2'}$ = 1.5 Hz, H-1') ppm; ^{13}C-n.m.r. (D_2O).: δ, 16.9 (C-6'), 59.7 (C-5), 62.9 (C-1), 69.5 (C-2),

70.6 (C-3'), 70.7 (C-2'), 71.9 (C-3), 72.3 (C-4', 5'), 78.9 (C-4), 100.5 (C-1') ppm; HR-FAB MS $[M+Na]^+$ calcd for $C_{11}H_{22}O_9Na$ 321.1161, found 321.1171.

Ethyl O-(3-O-acetyl-2-O-benzyl-4,6-O-benzylidene-α-D-glucopyranosyl)-(1→3)-2,4-di-O-benzoyl-1-thio-α-L-rhamnopyranoside (3.22). The title compound was obtained by Method A from 2-thiazolinyl 3-O-acetyl-2-O-benzyl-4,6-O-benzylidene-1-thio-β-D-glucopyranoside (**3.8a**) and ethyl 2,4-di-O-benzoyl-1-thio-α-L-rhamnopyranoside (**3.10**) in 72% yield. Analytical data: R_f = 0.48 (ethyl acetate – hexanes, 3/7, v/v); $[\alpha]_D^{22}$ +81.9° (c = 1, $CHCl_3$); m.p. 74-76 ºC; ^{1}H-n.m.r.: δ, 1.28 (t, 3H, CH_2CH_3), 1.32 (d, 3H, H-6), 1.78 (s, 3H, $COCH_3$), 2.59-2.72 (m, 2H, CH_2CH_3), 3.24 (dd, 1H, $J_{4',5'}$ = 9.6 Hz, H-4'), 3.28-3.37 (m, 2H, H-2', 6b'), 3.65 (dd, 1H, $J_{4',5'}$ = 4.8 Hz, H-5'), 3.84 (dd, 1H, $J_{5',6a'}$ = 4.8 Hz, H-6'), 4.22-4.37 (m, 4H, H-3, 5, *CH*$_2$Ph), 4.93 (d, 1H, $J_{1',2'}$= 3.6 Hz, H-1'), 5.15-5.19 (m, 2H, H-3', *CH*Ph), 5.43 (d, 1H, $J_{1,2}$ = 1.4 Hz, H-1), 5.52-5.60 (m, 2H, H-2, 4), 7.00-8.05 (m, 20H, aromatic) ppm; ^{13}C-n.m.r.: δ, 14.7, 15.6, 18.2, 21.4, 26.4, 30.3, 63.5, 68.1, 69.1, 70.9, 71.6, 73.0, 73.4, 73.9, 79.8, 82.9, 96.1, 101.7, 126.8 (x 2), 128.2, 128.3 (x 2), 128.4 (x 2), 128.8 (x 2), 129.3 (x 4), 130.0 (x 2), 130.3 (x 2), 133.7, 133.9, 137.7, 138.3, 130.6 (x 2), 166.1, 166.9, 169.8, ppm; HR-FAB MS $[M+H]^+$ calcd for $C_{44}H_{47}O_{12}S$ 799.2788, found 799.2796.

O-(3-O-Acetyl-2-O-benzyl-4,6-O-benzylidene-α-D-glucopyranosyl)-(1→3)-O-(2,4-di-O-benzoyl-α-L-rhamnnopyranosyl)-(1→3)-1,2,4,5-tetra-O-benzyl-D/L-ribitol (3.23). The title compound was obtained by Method B from **3.22** and **3.12** in 71% yield. Analytical data: R_f = 0.33 (ethyl acetate – hexanes, 3/7, v/v); $[\alpha]_D^{22}$ +48.7° (c =1,

$CHCl_3$); ^{1}H-n.m.r.: δ, 1.15 (d, 3H, H-6'), 1.88 (s, 3H, $COCH_3$), 3.32 (dd, 1H, $J_{1a,1b}$= 9.6 Hz, H-1a), 3.39-3.45 (m, 2H, H-2', 5a), 3.68-3.79 (m, 4H, H-1b, 2, 4, 5b), 3.85-3.90 (m, 2H, H-3', H-4'), 3.96-4.02 (m, 1H, H-3), 4.20-4.34 (m, 4H, H-5', 5'', 6a'', 6b''), 4.46-4.68 (m, 8H, 4 x CH_2Ph), 5.05 (d, 1H, $J_{1',2'}$= 3.5 Hz, H-1'), 5.17-5.26 (m, 3H, H-1'', 3'', C*H*Ph), 5.55-5.58 (m, 2H, H-2'', 4''), 7.00-8.07 (m, 40H, aromatic) ppm; ^{13}C-n.m.r.: δ, 18.3, 21.5, 63.4, 68.1, 69.2, 69.5, 69.6, 70.8, 71.0, 72.7, 72.8 (x 2), 72.9, 73.1, 73.8, 73.9, 77.3, 77.7, 78.2, 78.6, 79.9, 95.6, 98.4, 101.8, 127.0, 128.1, 128.2 (x 4), 128.3 (x 3), 128.5 (x 5), 128.8 (x 3), 128.9 (x 4), 129.0 (x 6), 129.1 (x 3), 129.4, 130.2 (x 2), 130.4 (x 2), 130.7 (x 2), 133.7, 133.9, 166.2, 166.9, 169.8 ppm; HR-FAB MS $[M+Na]^+$ calcd for $C_{75}H_{76}O_{17}Na$ 1271.4981, found 1271.4978.

O-(3-O-Acetyl-2-O-benzyl-4,6-O-benzylidene-α-D-glucopyranosyl)-(1→3)-O-(2,4-di-O-benzoyl-α-L-rhamnnopyranosyl)-(1→4)-1,2,3,5-tetra-O-benzyl-D-ribitol (3.24). The title compound was obtained by Method B from **3.22** and **3.13** in 76% yield. Analytical data: R_f = 0.41 (ethyl acetate – hexanes, 3/7, v/v); $[\alpha]_D^{22}$ +45.7° (c = 1, $CHCl_3$); ^{1}H-n.m.r.: δ, 1.07 (d, 3H, H-6'), 1.72 (s, 3H, $COCH_3$), 3.20 (dd, 1H, $J_{3'',4''}$ = 9.6 Hz, H-4''), 3.26-3.34 (m, 2H, H-3, 2''), 3.55-3.67 (m, 4H, H-5, 5'', 6b''), 3.70-3.79 (m, 4H, H-1, 2, 6a''), 4.12-4.17 (m, 1H, H-5'), 4.25-4.29 (m, 1H, H-4), 4.30-4.36 (m, 1H, $J_{1',2'}$ = 1.3 Hz, H-1'), 5.47 (dd, 1H, $J_{3',4'}$ = 10.9 Hz, H-4'), 5.62 (d, 1H, $J_{2',3'}$ = 2.3 Hz, H-2') 6.80-8.02 (m, 40H, aromatic) ppm; ^{13}C-n.m.r.: δ, 18.4, 21.5, 63.3, 68.1, 69.2, 70.7 (x 2), 70.9, 72.2, 72.6, 73.1, 73.3, 74.0, 74.1 (x 2), 74.2, 76.4, 77.2, 79.1, 79.7, 79.9, 94.9, 97.4, 101.7, 126.9 (x 3), 128.1, 128.2 (x 2), 128.3 (x 5), 128.4 (x 3), 128.5 (x 2), 128.6 (x 4), 128.8 (x 2), 129.0 (x 8), 129.1 (x 2), 129.3, 130.1, 130.2, 130.3 (x 2), 130.7 (x 2),

133.7, 133.9, 137.7, 138.4, 138.8 (x 2), 139.0 (x 3), 166.3, 166.8, 169.8 ppm; HR-FAB MS $[M+Na]^+$ calcd for $C_{75}H_{76}O_{17}Na$ 1271.4981, found 1271.4978.

O-(α-D-Glucopyranosyl)-(1→3)-O-(α-L-rhamnopyranosyl)-(1→3)-D/L-ribitol (3.5). The title compound was obtained from **3.23** in 80% yield as described for the synthesis of compound **3.3.** Analytical data: R_f = 0.52 (methanol – dichloromethane- water, 9/9/2, v/v); $[\alpha]_D^{26}$ +37.1° (c = 0.7, H_2O); ^{1}H-n.m.r. (D_2O).: δ, 1.36 (d, 3H, H-6'), 3.53 (dd, 1H, $J_{4'',5''}$ = 9.2 Hz, H-4''), 3.60-3.65 (m, 2H, H-4', 2''), 3.68-3.74 (m, 2H, H-1b, 5b), 3.82-3.93 (m, 8H, H-1a, 3, 5a, 3', 5', 3'', 6a'', 6b''), 3.98-4.05 (m, 3H, H-2, 4, 5''), 4.25 (dd, 1H, $J_{2',3'}$ = 2.7 Hz, H-2'), 5.07 (d, 1H, $J_{1',2'}$ = 1.8 Hz, H-1'), 5.15 (d, 1H, $J_{1'',2''}$ = 3.8 Hz, H-1'') ppm; ^{13}C-n.m.r. (D_2O): δ, 17.0 (C-6'), 60.6 (C-5'), 62.7 (C-1), 63.0 (C-5), 67.4 (C-2'), 69.7 (C-4''), 69.8 (C-6''), 70.5 (C-4'), 71.1 (C-2''), 71.8 (C-5''), 72.1 (C-2, 4), 73.3 (C-3''), 75.8 (C-3'), 80.2 (C-3), 95.9 (C-1''), 100.5 (C-1') ppm; HR-FAB MS $[M+Na]^+$ calcd for $C_{17}H_{32}O_{14}Na$ 483.1690, found 483.1684.

O-(α-D-Glucopyranosyl)-(1→3)-O-(α-L-rhamnopyranosyl)-(1→4)-D-ribitol (3.6). The title compound was obtained from **3.24** in 88% yield as described for the synthesis of compound **3.3**. Analytical data: R_f = 0.55 (methanol – dichloromethane- water, 9/9/2, v/v); $[\alpha]_D^{25}$ +13.2° (c = 1.0, H_2O); ^{1}H-n.m.r. (D_2O).: δ, 1.35 (d, 3H, H-6'), 3.51 (dd, 1H, $J_{4'',5''}$ = 9.5 Hz, H-4''), 3.60-3.64 (m, 2H, H-4, 2''), 3.68 (dd, 1H, $J_{1a,1b}$ = 6.7 Hz, H-1b), 3.78-3.94 (m, 9H, H-1a, 2, 3, 5b, 3', 5', 3'', 6a'', 6b''), 3.96-4.05 (m, 3H, H-4, 5a, 5''), 4.28 (dd, 1H, $J_{2',3'}$ = 2.4 Hz, H-2'), 5.12 (d, 1H, $J_{1',2'}$ = 1.6 Hz, H-1'), 5.14 (d, 1H, $J_{1'',2''}$ = 3.8 Hz, H-1'') ppm; ^{13}C-n.m.r. (D_2O).: δ, 17.1 (C-6'), 59.8 (C-5), 60.6 (C-6'), 62.9 (C-

1), 67.4 (C-2'), 69.7 (C-3'', 5'), 70.6 (C-4''), 71.8 (C-2''), 71.9 (C-4'), 72.0 (C-3), 72.3 (C-5''), 73.3 (C-2), 75.9 (C-3'), 79.0 (C-4), 95.9 (C-1''), 100.2 (C-1') ppm; HR-FAB MS $[M+Na]^+$ calcd for $C_{17}H_{32}O_{14}Na$ 483.1690, found 483.1684.

Ethyl O-(3-O-acetyl-2-O-benzyl-4,6-O-benzylidene-α-D-glucopyranosyl)-(1→3)-2,4-di-O-benzyl-1-thio-α-L-rhamnopyranoside (3.25). The title compound was obtained by Method A from **3.8a** and ethyl 2,4-di-O-benzyl-1-thio-α-L-rhamnopyranoside (**3.11**) in 80% yield. Analytical data for **3.25** were essentially the same as reported previously.[30]

Ethyl O-(2-O-benzyl-4,6-O-benzylidene-α-D-glucopyranosyl)-(1→3)-2,4-di-O-benzyl-1-thio-α-L-rhamnopyranoside (3.26). A solution of NaOMe in MeOH (1.0 M, 0.2 mL, 1.0 mmol) was added to a stirred suspension of **3.25** (160 mg, 0.35 mmol) in methanol (2.0 mL) and the reaction mixture was stirred for 15 h at rt. The reaction was then neutralized with Dowex (H^+), filtered, and concentrated in *vacuo* to yield crude **3.26** as a white foam 99%. Analytical data: R_f = 0.38 (ethyl acetate - hexanes, 3/7, v/v); $[\alpha]_D^{27}$ 7.5° (c = 1.0, $CHCl_3$); ^{1}H-n.m.r.: δ, 1.20 (t, 3H, CH_2CH_3), 1.29 (d, 3H, H-6), 2.54 (q, 2H, CH_2CH_3), 3.44-3.52 (m, 2H, H-2', 5'), 3.62-3.70 (m, 3H, H-3, 4', 6a'), 3.98-4.05 (m, 4H, H-2, 4, 5, 6b'), 4.06-4.21 (m, 2H, H-3', OH), 4.55-4.96 (m, 6H, 3 x CH_2Ph), 5.12 (d, 1H, $J_{1',2'}$ = 3.5 Hz, H-1'), 5.26 (d, 1H, $J_{1,2}$ = 1.8 Hz, H-1), 5.47 (s, 1H, CHPh), 7.20-7.37 (m, 20H, aromatic) ppm; ^{13}C-n.m.r.: δ, 15.2, 18.0, 25.6, 30.0, 62.9, 68.8, 69.1, 70.8, 72.7, 73.0, 79.3, 80.4, 81.6, 82.0, 95.2, 102.2, 126.7 (x 2), 127.9 (x 2), 128.0 (x 2), 128.2, 128.3 (x 2), 128.4 (x 4), 128.5 (x 2), 128.6 (x 2), 128.7 (x 2), 129.3, 137.4, 137.9, 138.2, 138.3 ppm; HR-FAB MS $[M+Na]^+$ calcd for $C_{42}H_{48}O_9SNa$ 751.2917, found 751.2929.

Ethyl O-(2-O-benzyl-3,4,6-tri-O-acetyl-α-D-galactopyranosyl)-(1→3)-O-(2-O-benzyl-4,6-O-benzylidene-α-D-glucopyranosyl)-(1→3)-2,4-di-O-benzyl-1-thio-α-L-rhamnopyranoside (3.27). The title compound was obtained by Method A from benzoxazolyl 3,4,6-tri-O-acetyl-2-O-benzyl-1-thio-β-D-galactopyranoside **3.7** and **3.26** in 76% yield. Analytical data: R_f = 0.49 (ethyl acetate - hexanes, 3/7, v/v); $[\alpha]_D^{26}$ +48.8° (c = 1.0, $CHCl_3$); ^{1}H-n.m.r.: δ, 1.25 (t, 3H, CH_2CH_3), 1.32 (d, 3H, H-6), 1.84, 1.97, 2.00 (3s, 9H, 3 x $COCH_3$), 2.56-2.66 (q, 2H, CH_2CH_3), 3.55 (dd, 1H, $J_{5'',6a''}$ = 6.3 Hz, $J_{6a'',6b''}$ = 11.1 Hz, H-6b''), 3.63-3.74 (m, 5H, H-2, 4, 2', 4', 2''), 3.76-3.87 (m, 1H, H-6a''), 4.04-4.10 (m, 4H, H-3, 5, 5', 6b'), 4.18 (dd, 1H, $J_{5',6a'}$= 5.4 Hz, $J_{6a',6b'}$ = 10.3 Hz, H-6a'), 4.25 (d, 1H, CH_2Ph), 4.39 (dd, 1H, $J_{3',4'}$ = 9.4 Hz, H-3'), 4.47-4.52 (m, 2H, H-5'', ½ CH_2Ph), 4.61-4.74 (m, 4H, 2 x CH_2Ph), 4.96 (d, 1H, ½ CH_2Ph), 5.21 (d, 1H, $J_{4'',5''}$ = 2.3 Hz, H-4''), 5.25 (d, 1H, $J_{1',2'}$ = 3.4 Hz, H-1'), 5.30-5.38 (m, 3H, H-1, 3'', CHPh), 5.57 (d, 1H, $J_{1'',2''}$ = 3.6 Hz, H-1''), 6.95-7.43 (m, 25H, aromatic) ppm; ^{13}C-n.m.r.: δ, 15.3, 18.2, 20.8, 20.9 (x 2), 21.0 (x 2), 25.7, 31.2, 61.7, 62.7, 66.2 (x 2), 68.8 (x 2), 69.2, 71.2, 72.5 (x 2), 72.9 (x 2), 75.7, 75.9, 80.3, 81.6, 82.9, 94.2, 96.6, 102.3, 126.7 (x 2), 126.8 (x 3), 127.3, 127.7 (x 3), 127.9, 128.0 (x 4), 128.3 (x 3), 128.7 (x 5), 128.8 (x 3), 129.5, 137.4, 137.8, 138.0, 138.2, 170.2, 170.3, 170.6, ppm; HR-FAB MS $[M+H]^+$ calcd for $C_{61}H_{70}O_{17}SNa$ 1129.4231, found 1129.4238.

O-(3,4,6-Tri-O-acetyl-2-O-benzyl-α-D-galactopyranosyl)-(1→3)-O-(2-O-benzyl-4,6-O-benzylidene-α-D-glucopyranosyl)-(1→3)-O-(2,4-di-O-benzyl-α-L-rhamnnopyranosyl)-(1→3)-1,2,4,5-tetra-O-benzyl-D/L-ribitol (3.28) The title compound was obtained by Method B from **3.12** and **3.27** in 70% yield. Analytical data:

R_f = 0.47 (ethyl acetate - hexanes, 2/3, v/v); $[\alpha]_D^{25}$ +55.4° (c = 1.0, $CHCl_3$); ^{1}H-n.m.r.: δ, 1.27 (d, 3H, H-6'), 1.82, 1.96, 1.98 (3s, 3H, 3 x $COCH_3$), 3.52-3.57 (m, 2H, H-4', 5b), 3.60-3.65 (m, 5H, H-1a, 4, 4'',6a'', 6a'''), 3.69-3.76 (m, 6H, H-2, 2', 2''', 4''', 6b'', 6b'''), 3.79-3.86 (m, 3H, H-5a, 3', 5'), 3.95 (dd, 1H, H-2''), 3.99 (m, 1H, H-5''), 4.05-4.13 (m, 4H, H-1b, 3, 3'', 5'''), 4.26 (d, 1H, ½ CH_2Ph), 4.32-4.68 (m, 14H, 7 x CH_2Ph), 4.99 (d, 1H, ½ CH_2Ph), 5.16 (d, 1H, $J_{1''',2'''}$ = 2.1 Hz, H-1'''), 5.18 (d, 1H, $J_{1'',2''}$ = 3.4 Hz, H-1''), 5.33 (dd, 1H, $J_{3''',4'''}$ = 3.3 Hz, H-3'''), 5.38 (s, 1H, C*H*Ph), 5.56 (d, 1H, $J_{1',2'}$ = 3.4 Hz, H-1'), 6.95-7.40 (m, 45H, aromatic) ppm; ^{13}C-n.m.r.: δ, 18.5, 20.9, 21.2 (x 2), 30.1, 62.0, 62.6, 66.4, 69.1, 69.3 (x 2), 70.3, 70.5, 71.3, 72.6, 72.8, 73.0 (x 2), 73.1 (x 2), 73.7, 73.8, 74.6, 76.0, 78.3 (x 3), 78.9, 79.9, 83.2, 93.1, 96.7, 98.8, 102.5, 126.9 (x 2), 127.5 (x 2), 127.8 (x 2), 127.9 (x 6), 128.0 (x 2), 128.1 (x 2), 128.3 (x 2), 128.4 (x 2), 128.5 (x 3), 128.6 (x 2), 128.7 (x 5), 128.7 (x 3), 128.8 (x 2), 128.9 (x 2), 129.7, 137.6 (x 2), 138.2 (x 2), 138.4 (x 2), 138.5 (x 2), 138.6 (x 2), 138.7 (x 2), 138.8 (x 2), 138.9 (x 2), 170.3, 170.5, 170.8 ppm; HR-FAB MS $[M+Na]^+$ calcd for $C_{92}H_{100}O_{22}Na$ 1579.6604, found 1579.6609.

O-(3,4,6-Tri-O-acetyl-2-O-benzyl-α-D-galactopyranosyl)-(1→3)-O-(2-O-benzyl-4,6-O-benzylidene-α-D-glucopyranosyl)-(1→3)-O-(2,4-di-O-benzyl-α-L-rhamnnopyranosyl)-(1→4)-1,2,3,5-tetra-O-benzyl-D-ribitol (3.29). The title compound was obtained by Method B from **3.13** and **3.27** in 76% yield. Analytical data: R_f = 0.49 (ethyl acetate - hexanes, 3/7, v/v); $[\alpha]_D^{27}$ +54.9° (c = 1.0, $CHCl_3$); ^{1}H-n.m.r.: δ, 1.25 (d, 3H, H-6'), 1.84, 1.97, 1.99 (3s, 3H, 3 x $COCH_3$), 3.55-3.60 (m, 2H, H-5), 3.63-3.77 (m, 10H, H-1, 2, 3, 2', 4', 2''', 4''', 6a'', 6b'''), 3.79-3.90 (m, 4H, H-4, 3', 5', 6b''),

3.96-3.99 (m, 2H, H-2'', 5''), 4.08-4.11 (m, 1H, H-3''), 4.20 (dd, 1H, $J_{5''',6a'''}$ = 2.7 Hz, $J_{6a''',6b'''}$ = 9.2 Hz, H-6a'''), 4.23 (dd, 2H, CH_2Ph), 4.29-4.32 (m, 1H, H-5'''), 4.38-4.70 (m, 12H, 6 x CH_2Ph), 5.08 (dd, 2H, CH_2Ph), 5.26 (d, 1H, $J_{1''',2'''}$ = 3.5 Hz, H-1'''), 5.31 (d, 1H, $J_{1'',2''}$ = 3.4 Hz, H-1''), 5.33 (s, 2H, H-3''', C*H*Ph), 5.56 (d, 1H, $J_{1',2'}$ = 3.5 Hz, H-1'), 7.14-7.43 (m, 45H, aromatic) ppm; ^{13}C-n.m.r.: δ, 14.6, 18.6 (x 2), 21.1 (x 2), 21.3 (x 3), 23.2, 30.2 (x 2), 32.4, 62.2, 62.6, 66.6, 69.2, 69.5, 70.4, 71.4, 72.6, 72.8, 72.9, 73.2, 73.8, 73.9, 74.2, 74.9, 76.1, 78.8, 79.8, 83.3, 96.8, 97.4, 102.5, 127.1 (x 2), 127.6 (x 3), 127.9 (x 4), 128.0, 128.1 (x 2), 128.2 (x 2), 128.3 (x 3), 128.4 (x 2), 128.5, 128.6 (x 2), 128.7 (x 3), 128.77 (x 2), 128.80 (x 6), 128.83 (x 3), 128.9 (x 2), 129.0 (x 2), 129.1 (x 2), 129.7, 137.7, 138.3, 138.6 (x 3), 138.7, 138.8, 138.9, 142.4, 142.7, 142.9, 170.4, 170.6, 170.9, ppm; HR-FAB MS $[M+Na]^+$ calcd for $C_{92}H_{100}O_{22}Na$ 1579.6604, found 1579.6609.

O-(α-D-Galactopyranosyl)-(1→3)-O-(α-D-glucopyranosyl)-(1→3)-O-(α-L-rhamnopyranosyl)-(1→3)-D/L-ribitol (3.1). The title compound was obtained from **3.28** in 75% yield as described for the synthesis of compound **3.3**. Analytical data: R_f = 0.33 (methanol – dichloromethane- water, 9/9/2, v/v); $[\alpha]_D^{26}$ +78.2° (c = 0.8, H_2O); ^{1}H-n.m.r. (D_2O): δ, 1.25 (d, 3H, H-6'), 3.53 (dd, 1H, $J_{4',5'}$ = 9.7 Hz, H-4'), 3.58-3.65 (m, 4H, H-2'', 4'', 5a, 6a''), 3.70 (dd, 2H, $J_{6a''',6b'''}$ = 11.2 Hz, H-6a''', 6b'''), 3.72-3.82 (m, 8H, H-1a, 1b, 2, 3, 5b, 3', 5', 2'''), 3.84-3.90 (m, 3H, H-4, 3'', 3'''), 3.92-3.96 (m, 3H, H-4''', 5'', 6b''), 4.15 (dd, 1H, $J_{2',3'}$ = 2.3 Hz, H-2'), 4.19 (dd, 1H, $J_{4''',5'''}$ = 6.3 Hz, H-5'''), 4.97 (d, 1H, $J_{1',2'}$ = 1.7 Hz, H-1'), 5.05 (d, 1H, $J_{1'',2''}$ = 6.0 Hz, H-1''), 5.33 (d, 1H, $J_{1''',2'''}$ = 3.9 Hz, H-1''') ppm; ^{13}C-n.m.r. (D_2O).: δ, 16.9, 60.3, 61.1, 62.5, 62.9, 67.1, 68.8, 69.3,

69.6, 69.7, 70.0, 70.3, 70.4, 70.9, 71.0, 71.7, 71.9, 75.5, 79.8, 80.1, 95.6, 99.5, 100.3 ppm; HR-FAB MS $[M+Na]^+$ calcd for $C_{23}H_{42}O_{19}Na$ 645.2218, found 645.2210.

O-(α-D-Galactopyranosyl)-(1→3)-O-(α-D-glucopyranosyl)-(1→3)-O-(α-L-rhamnopyranosyl)-(1→4)-D-ribitol (3.2). The title compound was obtained from **3.29** in 78% yield as described for the synthesis of compound **3.3**. Selected analytical data: R_f = 0.33 (methanol – dichloromethane- water, 9/9/2, v/v); $[\alpha]_D^{27}$ +70.7° (c = 1.0, H_2O); ^{1}H-n.m.r. (D_2O).: δ, 1.26 (d, 3H, H-6'), 3.53 (dd, 1H, $J_{4',5'}$ = 9.7 Hz, H-4'), 3.58-3.64 (m, 4H, H-1b, 5b, 2'', 6a''), 3.66-3.75 (m, 8H, H-1a, 2, 5', 4'', 6b'', 2''', 6a''', 6b'''), 3.78-3.81 (m, 2H, H-3, 3'''), 3.83-3.89 (m, 4H, H-4, 5a, 3', 3''), 3.94-3.96 (m, 2H, H-2', 5'''), 5.02 (broad s, 1H, H-1'), 5.06 (d, 1H, $J_{1'',2''}$ = 3.7 Hz, H-1''), 5.34 (d, 1H, $J_{1''',2'''}$ = 3.8 Hz, H-1''') ppm; ^{13}C-n.m.r. data for **2** was essentially the same as reported previously;[39] HR-FAB MS $[M+Na]^+$ calcd for $C_{23}H_{42}O_{19}Na$ 645.2218, found 645.2210.

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CHAPTER 4

Synthesis of spacer containing analogs of *Streptococcus pneumoniae* 6A and 6B

Parameswar, A. R.; Hasty, S. J.; Demchenko, A. V. "Synthesis of Spacer Containing Analogs of Serotype 6 Pneumococcal Oligosaccharides", *Carbohydr. Res.,* **2008**, 343, 1707-1717

4 Synthesis of spacer containing analogs of SPn6A & 6B

4.1 Introduction

Streptococcus pneumoniae (SPn) have become one of the most frequent causes of invasive bacterial infections, particularly in patients with weakened or immature immune system.[1] The study of the pneumococcal disease over the last 120 years has led to important scientific and clinical insights.[2] It was also established that serogroup 6 has been consistently ranked second-third most frequent cause of invasive pneumococcal disease worldwide.[3,4] This fact stimulated extensive structural[5-7] and synthetic studies[8-13] of SPn6 oligosaccharides and derivatives thereof. Type 6 pneumococci are a frequent cause of pneumococcal disease in infants together with 14, 19F and 23F which are the so-called pediatric types.[14,15]

For the most part, relatively small oligosaccharide fragments are poorly immunogenic. Hence, many synthetic targets get equipped with a spacer moiety, suitable for conjugation to the carrier protein. These conjugates were proven to significantly increase the immunoresponse and are immunogenic even in infants who are at greatest risk to infection.[16-18](see Chapter 1.1). In continuation to the study aimed towards stereoselective synthesis of pneumococcal oligosaccharides,[19] this chapter is dealing with the synthesis of spacer-containing pseudo tetrasaccharides (**4.1** and **4.2**) and derivatives thereof (**4.3-4.6)** structurally related to the repeating units of SPn6 (Figure 4.1). The spacer moiety will be essential for conjugation with a carrier protein and for subsequent immunological studies.

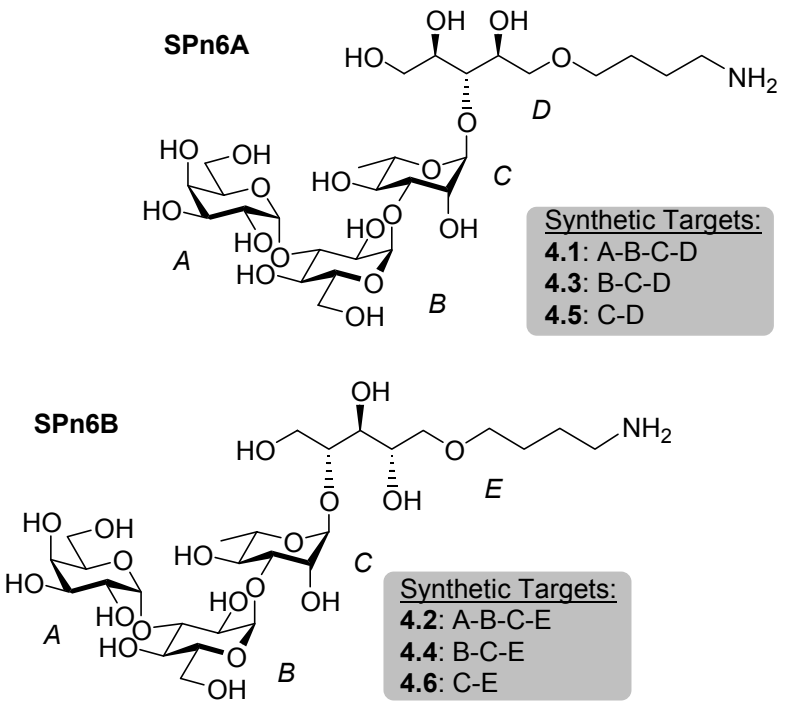


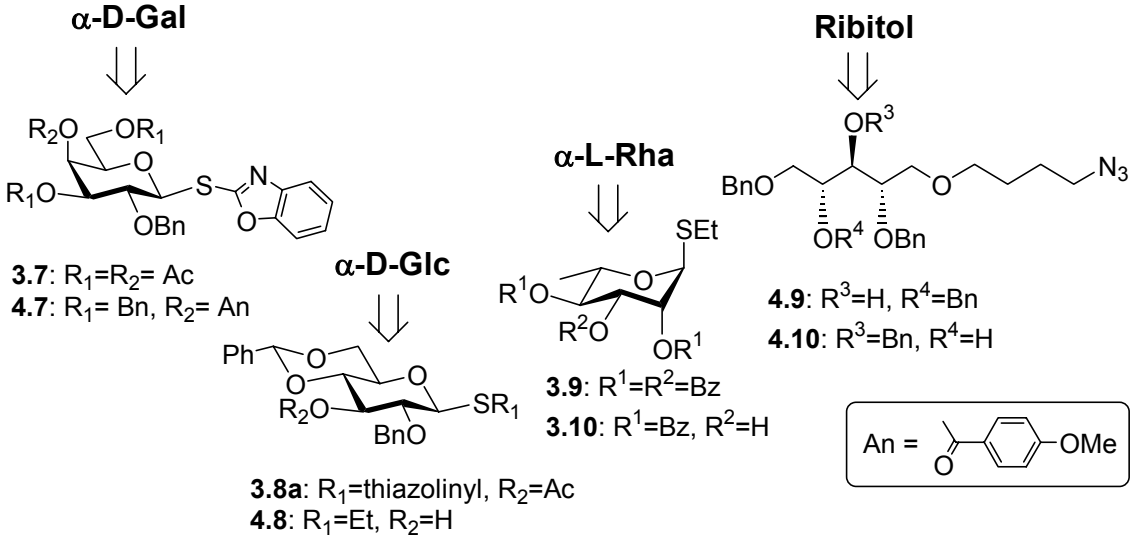
Figure 4.1 Spacer containing analogs of SPn6A & 6B

4.2 Results and discussion

Efficient chemical synthesis of the spacer-containing oligosaccharides of SPn6 and derivatives thereof will help to quickly obtain substantial quantities of pure samples for conjugation and immunological studies. In chapter 3, we have already shown that the pseudo di-, tri- and tetrasaccharides of SPn6 series could be synthesized via selective activation strategy in overall excellent yields and complete stereoselectivity.[19] This chapter describes the synthesis of spacer containing oligosaccharides (**4.1-4.6**), using a similar strategy. Nevertheless, some adjustments had been made. Recall that the synthesis of pseudo-tetrasaccharides **3.1** and **3.2** the assembly of ABCD tetrasaccharide was performed in (B+C)+A+D fashion (see Chapter 3). Herein the synthesis of spacer containing derivatives **4.1** and **4.2** was carried out in: A+B+(C+D) fashion. This strategic

adjustment required further optimization of the anomeric selectivity, and for this purpose two types of galactose building block have been investigated (**4.7a** and **b,** Scheme 4.1). Our expectation was that anisoyl group at C-4 of the galactose building block (**4.7**) would be particularly beneficial for galactosylation.[20] The D-gluco and L-rhamno units will be introduced by using simple building blocks **4.8-4.10**, whereas the synthesis of the spacer containing ribitol units **4.9** and **4.10** would require particular attention (Scheme 4.1).

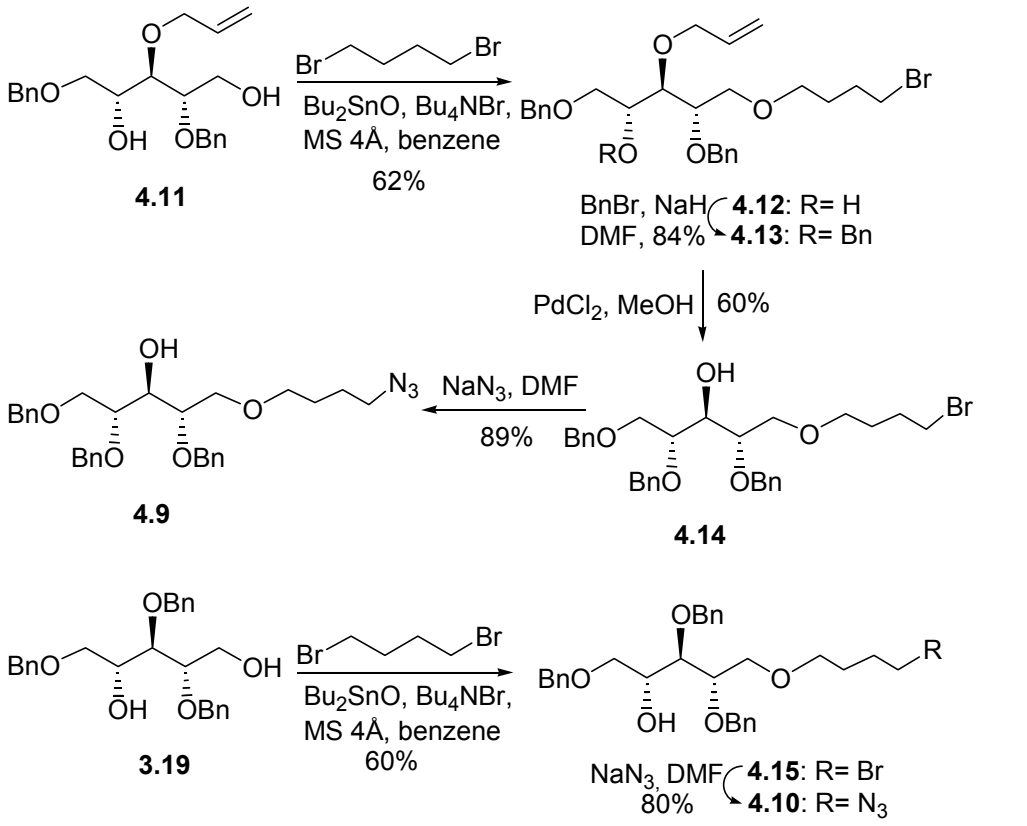
The synthesis of the SBox galactose building block (**4.7**) was carried out from the known precursor ethyl 4-O-anisoyl-2,3,6-tri-O-benzyl-1-thio-β-D-galactopyranoside (**4.7**)[20] via conventional protocol involving a bromination – thioglycosylation sequence.[21] 2-Benzyl-triacetate SBox galactoside **3.7** provided excellent results for the synthesis of spacerless oligosaccharides (see Chapter 3) and was also generated for comparison studies.[21] Known derivatives **3.9-3.10** were projected for the introduction of units B and C and their synthesis was accomplished as described previously.[22-24]



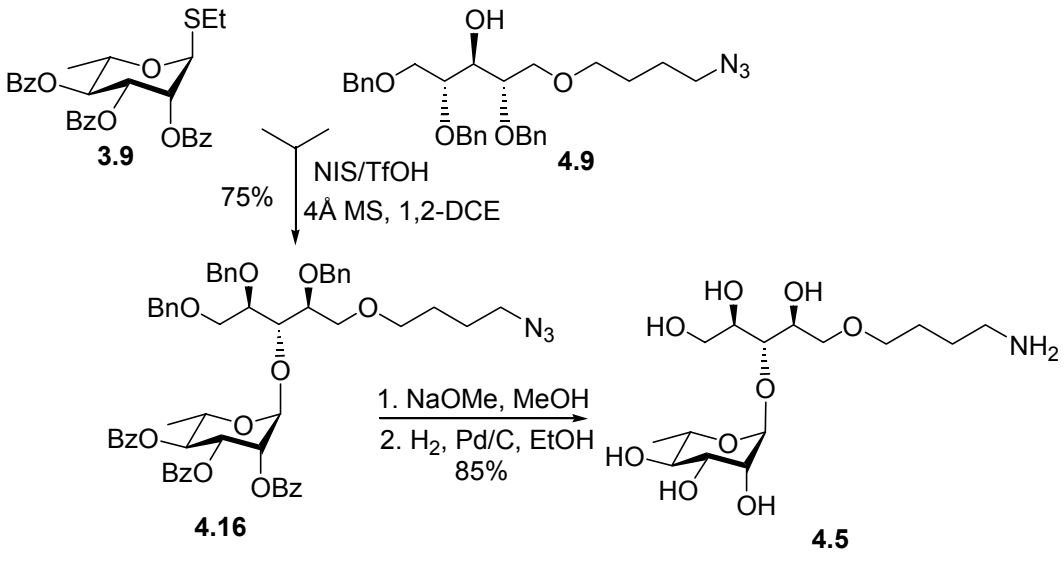
Scheme 4.1 Retrosynthetic analysis of spacer containing oligosaccharides of SPn6

Synthesis of the 3-OH ribitol building block **4.9** was carried out from known precursor **4.11**[9] as shown in Scheme 4.2. First, diol **4.11** was subjected to alkylation via dibutylstannylidene assistance that ensured excellent regioselective protection of the primary hydroxyl. The resulting compound **4.12,** obtained in 62% yield, was then subjected to benzylation to afford **4.13**, and the latter was deallylated using $PdCl_2$ in MeOH to afford compound **4.14** in 60% yield. The bromide **4.14** was then converted to the corresponding azide derivative via conventional nucleophilic displacement to allow the requisite glycosyl acceptor **4.9** in 89% yield. For the synthesis of 4-OH ribitol building block **4.10**, the previously synthesized precursor **3.19**[19] was alkylated and converted to azido derivative using similar reaction conditions as described for the synthesis of acceptor **4.9** (Scheme 4.2).

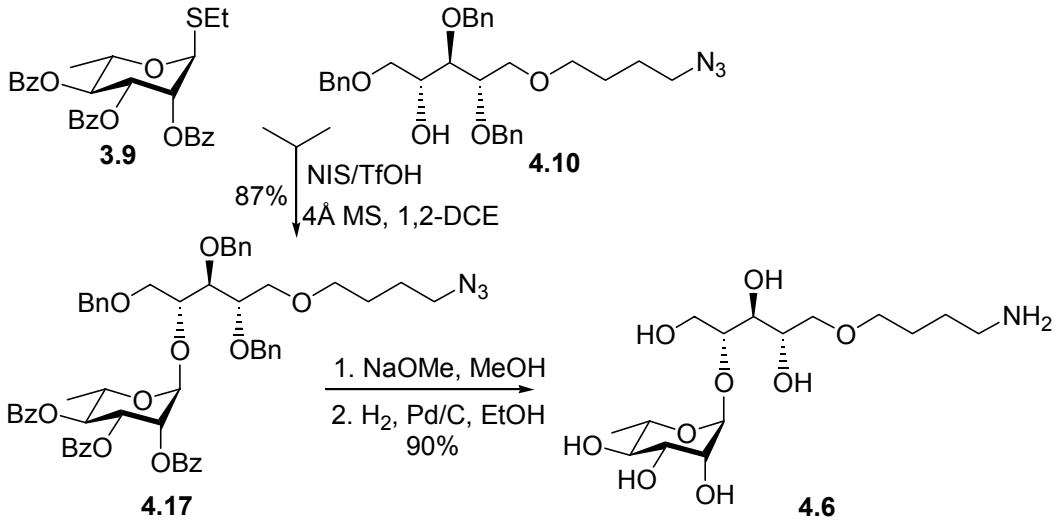
Having obtained the key monosaccharide building blocks, we turned our attention to the oligosaccharide assembly. For the synthesis of pseudo-disaccharides **4.5** and **4.6**, per-benzoylated rhamnose building block **3.9** was used as a glycosyl donor. Coupling of **3.9** with ribitol acceptors **4.9** and **4.10** in the presence of NIS/ TfOH afforded glycosides **4.16** and **4.17** with complete α-selectivity in 75% and 87% yield, respectively (Schemes 4.3 and 4.4). The compounds obtained were then subjected to a two-step sequential deprotection: deacylation (NaOMe in MeOH) and hydrogenation using palladium on charcoal in 5% HCl in EtOH to obtain pseudo disaccharides **4.5** and **4.6** in 85% and 90%, respectively.



Scheme 4.2 Synthesis of ribitol acceptors **4.9** and **4.10**

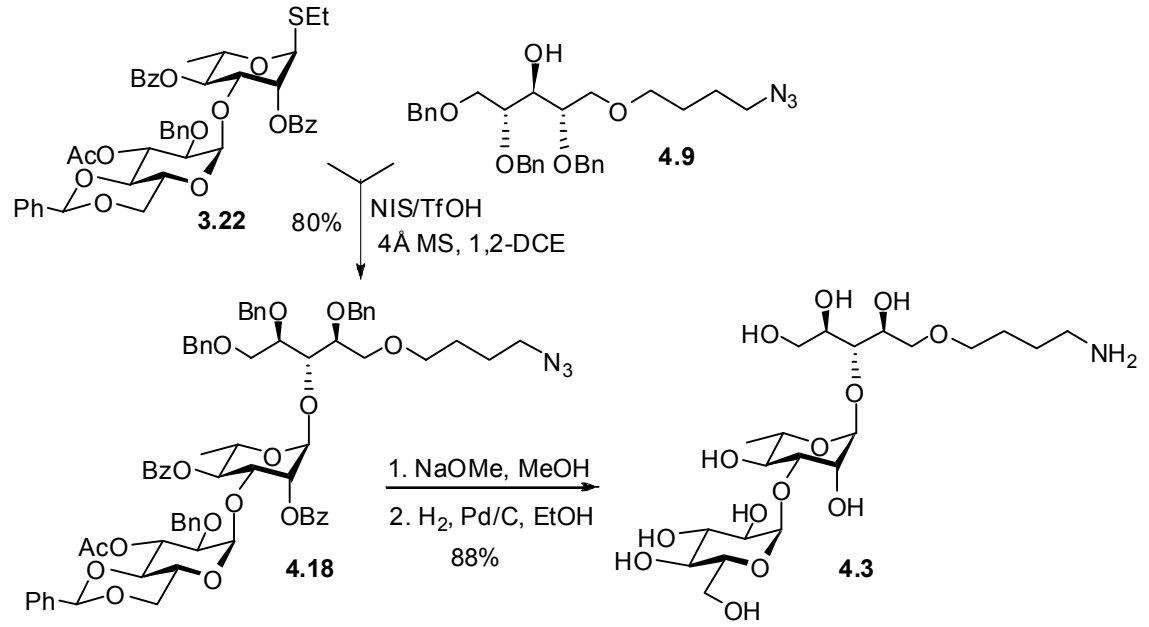


Scheme 4.3. Synthesis of spacer containing Pseudo Disaccharide **4.5** of SPn6A

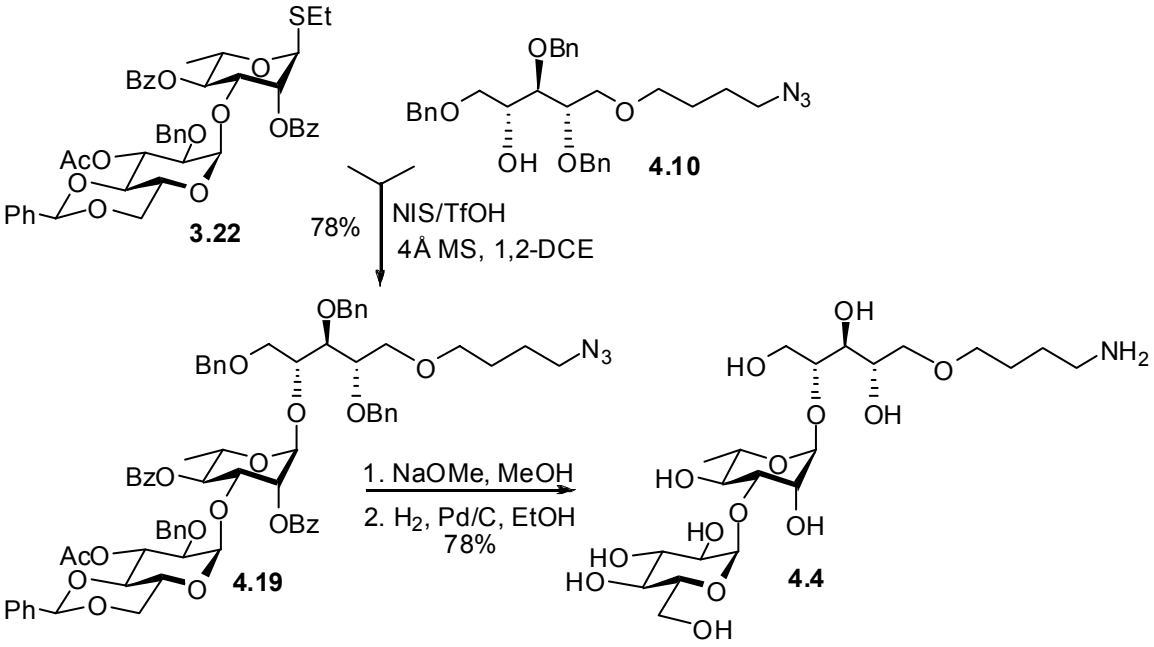


Scheme 4.4. Synthesis of spacer containing Pseudo Disaccharide **4.6** of SPn6B

For the synthesis of pseudo trisaccharides **4.3** and **4.4**, the S-ethyl anomeric moiety of disaccharide **3.22**, obtained from **3.8a** and **3.10** as described previously,[19] was activated with NIS/TfOH for glycosylation of glycosyl acceptors **4.9** and **4.10**. As a result, saccharides **4.18** and **4.19** were isolated in 80% and 78% yield, respectively. These rhamnosylations also proceeded with complete α-stereoselectivity. The protected pseudo-trisaccharides **4.18** and **4.19** were then subjected to the standard deprotection sequence, as described above for the synthesis of **4.5** and **4.6**, to afford compounds **4.3** and **4.4** in 88% and 78% yield, respectively (Schemes 4.5 and 4.6).



Scheme 4.5. Synthesis of spacer containing Pseudo Trisaccharide **4.3** of SPn6A

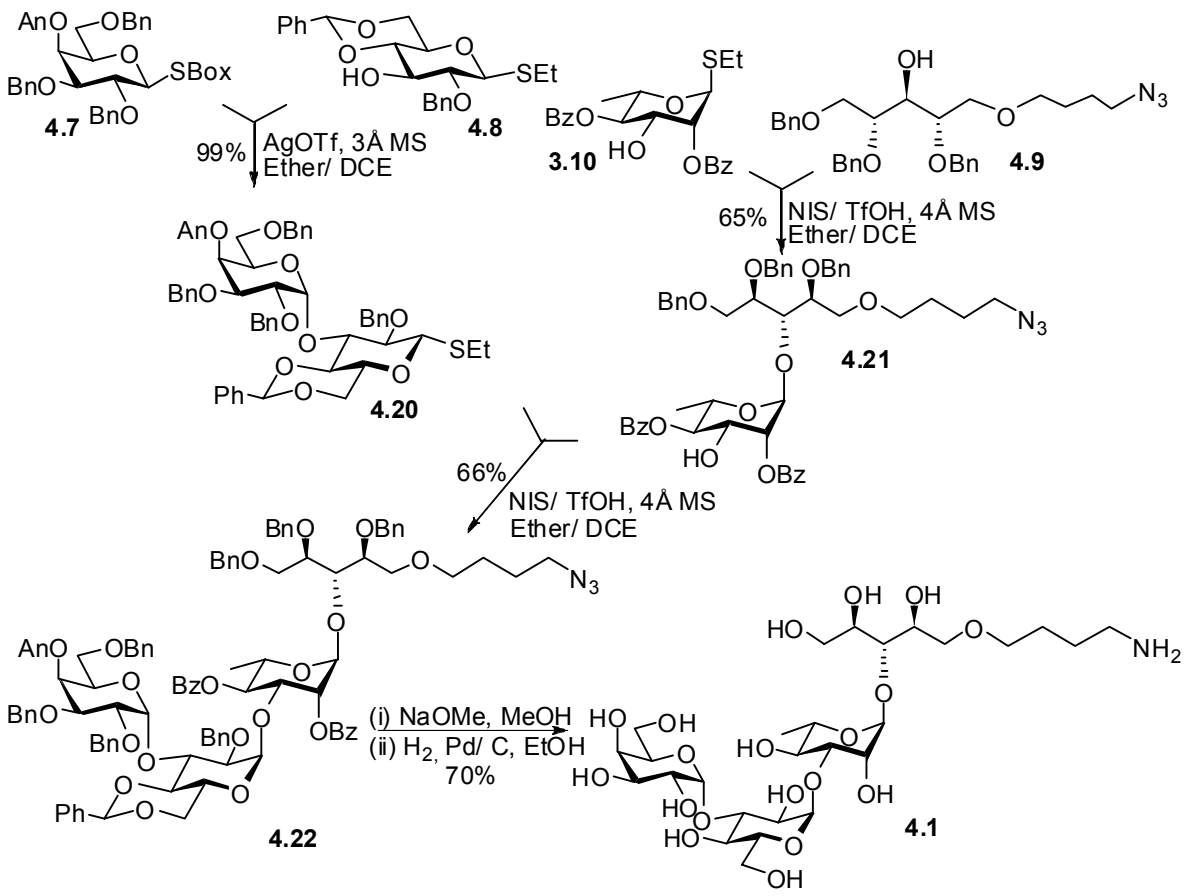


Scheme 4.6. Synthesis of spacer containing Pseudo Trisaccharide **4.4** of SPn6B

Finally, we were aiming for the synthesis of pseudo-tetrasaccharides **4.1** and **4.2**. For this

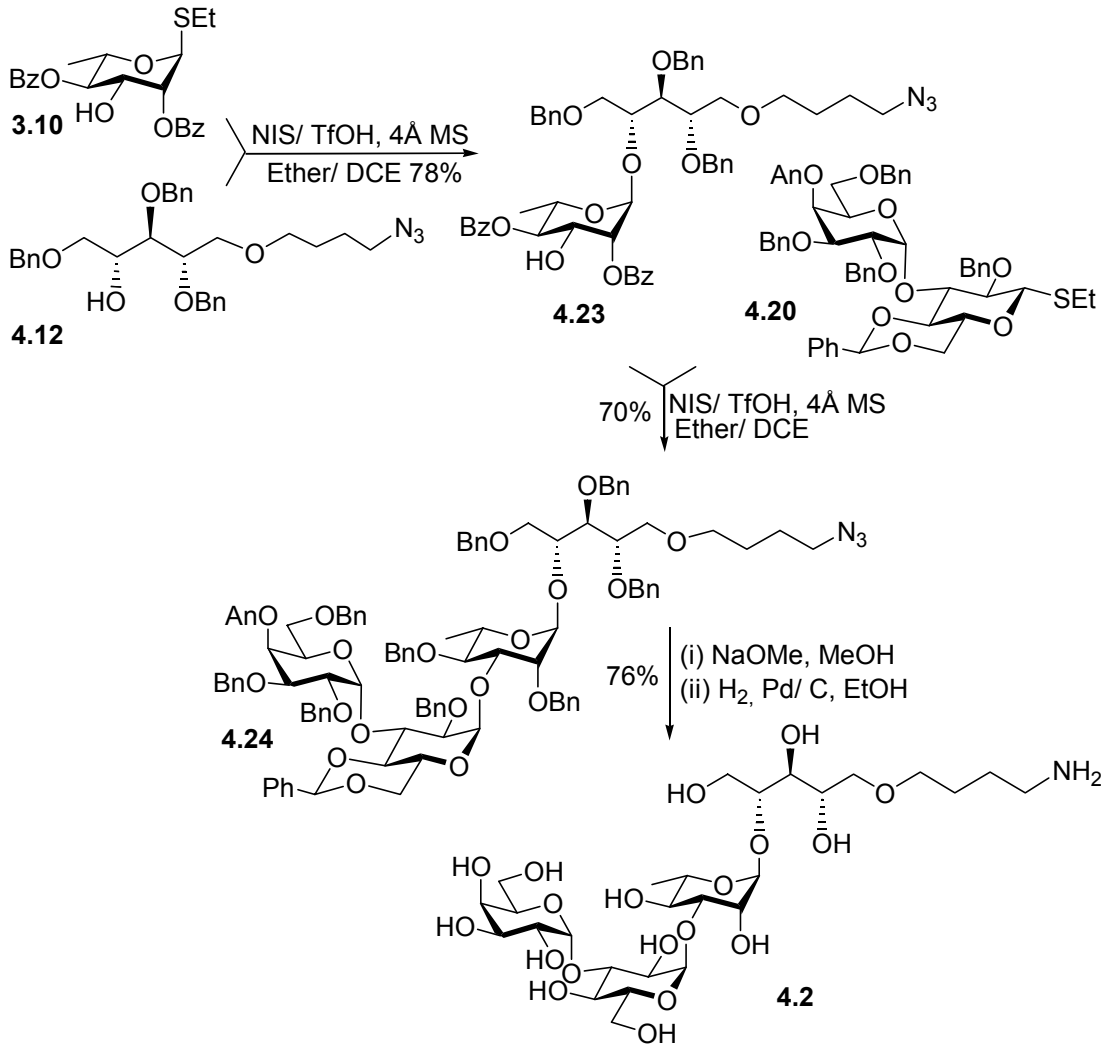
purpose, galactose derivative **3.7** was used as a glycosyl donor in a coupling with **4.8**. Unfortunately, this coupling resulted in a very poor stereoselectivity (α/β = 3/1). This was a rather unexpected result considering the success of our previous galactosylation using the A+BC strategy[19] (Chapter 3). To improve this, we investigated galactose donor **4.7**, containing anisoyl group at the C-4 position, a known concept to improve the outcome of α-galactosylations[20]. The SBox leaving group of **4.7** was then selectively activated over the SEt moiety of glucosyl acceptor **4.8**. This reaction was carried out in diethyl ether/dichloroethane (5/1, v/v) mixture of solvents to further enhance the stereoselectivity. This fine-tuning of reaction conditions using a combination of previously elaborated methodologies allowed us to obtain the disaccharide **4.20** in excellent 99% yield and very good stereoselectivity. (α/β=14/1, Scheme 4.7).

For the synthesis of the disaccharide acceptor **4.21,** rhamnose building block **3.10** was glycosidated with ribitol acceptor **4.9** to afford the requisite disaccharide **4.21** in 65% yield. To complete the assembly, the disaccharide donor **4.20** was coupled with the disaccharide acceptor **4.21** in the presence of NIS and catalytic TfOH. As a result, pseudo tetrasaccharide derivative **4.22** was obtained in 66% yield. Finally, the deprotection of **4.22** was carried out using conventional deacylation-hydrogenation sequence to afford pseudo tetrasaccharide **4.1** in 70%.



Scheme 4.7. Synthesis of spacer containing Pseudo Tetrasaccharide **4.1** of SPn6A

For the synthesis of pseudo tetrasaccharide **4.2**, corresponding to the carbohydrate part of the repeating unit of SPn6B, disaccharide acceptor **4.23** was obtained from building blocks **3.10** and **4.10** in 78% yield. Glycosylation of **4.23** with the disaccharide donor **4.20** led to the fully protected pseudo-tetrasaccharide **4.24** in 70% yield with complete α-stereoselectivity. The conventional deprotection of **4.24** afforded the target compound **4.2** in 76% yield (Scheme 4.8).



Scheme 4.8. Synthesis of spacer containing Pseudo Tetrasaccharide **4.2** of SPn6B

4.3 Conclusion

In conclusion, we have developed an efficient convergent strategy for the synthesis of a range of spacer-containing pneumococcal oligosaccharides of serogroup 6. The pseudo-tetrasaccharide assembly was carried out with excellent stereoselectivity. This convergent assembly was carried out in lesser number of steps as compared to the synthesis of spacerless tetrasaccharide (Chapter 3). Further studies related to the synthesis of other

spacer-containing oligosaccharides and their conjugation to the carrier protein and subsequent immunological studies will be discussed in the subsequent chapters.

4.4 Experimental part

4.4.1 General

Refer to Chapter 2 (p.81)

Benzoxazolyl 4-O-anisoyl-2,3,6-tri-O-benzyl-1-thio-β-D-galactopyranoside (4.7). The solution of ethyl 4-O-anisoyl-2,3,6-tri-O-benzyl-1-thio-β-D-glucopyranoside[20] (3.98 g, 6.34 mmol) and activated molecular sieves (3 Å, 3.17 g) in CH_2Cl_2 (95 mL) was stirred under argon for 1 h. Freshly prepared solution of Br_2 in CH_2Cl_2 (60 mL, 1/165, v/v) was then added and the reaction mixture was kept for 5 min at rt. After that, the solid was filtered-off and the filtrate was concentrated in vacuo at rt. Crude residue was then treated with KSBox (7.6 mmol) and 18-crown-6 (0.76 mmol) in dry acetone (12 mL) under argon for 16 h at rt. Upon completion, the mixture was diluted with dichloromethane, the solid was filtered-off and the residue was washed with dichloromethane. The combined filtrate (200 mL) was washed with 1% aq. NaOH (50 mL) and water (3 x 50 mL).The organic layer was separated, dried with $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (ethyl acetate-toluene gradient elution) to afford **4.7** as a syrup (2.2 g, 50 %). Analytical data for **4.7**: R_f = 0.46 (ethyl acetate - hexanes, 3/7, v/v); $[\alpha]_D^{29}$ +10.8^o (c = 1.0, $CHCl_3$); ^{1}H-n.m.r.: δ, 3.40-3.53 (m, 2H, H-6a, 6b), 3.69 (s, 3H, -OCH_3), 3.74 (dd, 1H, $J_{2,3}$ = 2.8 Hz, H-3), 3.89-3.97 (m, 2H, H-2, 5), 4.18-4.82 (m, 6H, 4 x *CH_2*Ph), 5.36 (dd, 1H, $J_{3,4}$ = 2.3 Hz, H-4), 6.76-7.98 (m, 25H, aromatic) ppm; ^{13}C-n.m.r.: δ, 56.2, 67.5, 68.6, 72.4, 74.3, 76.4, 81.9, 85.7, 110.7, 114.4,

119.7, 122.7, 124.9, 125.1, 128.3, 128.4, 128.5 (x 2), 128.8 (x 4), 128.9, 129.1, 132.7, 138.1, 138.2, 138.3, 142.4, 161.9, 164.2, 165.9 ppm; HR-FAB MS $[M+H]^+$ calcd for $C_{42}H_{40}NO_8$ S 718.2475, found 718.2452.

3-O-Allyl-2,5-di-O-benzyl-1-O-(4-bromobutyl)-D-ribitol (4.12). To a solution of 3-O-allyl-2,5-di-O-benzyl-D-ribitol **4.11**[9] (871 mg, 2.34 mmol) in benzene (18 mL), freshly activated molecular sieves (4Å, 4 g) was added and the mixture was stirred under argon for 1 h. Bu_2SnO (786 mg, 3.2 mmol) and Bu_4NBr (784 mg, 2.4 mmol) were then added followed by the addition of 1,4-dibromobutane (1.3 mL, 14.0 mmol). The reaction mixture was refluxed for 20 h under argon, then cooled to rt. Molecular sieves were filtered off through a pad of celite, rinsed with toluene (2 x 5 mL), and the combined filtrate was then concentrated in *vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate - hexanes gradient elution) to afford **4.12** as a colorless liquid (730 mg, 62% yield). Analytical data for **4.12**: R_f = 0.50 (ethyl acetate - hexanes, 2/3, v/v); $[\alpha]_D^{28}$ +70.1° (c = 1.0, $CHCl_3$); ^{1}H-n.m.r.: δ, 1.63-1.68 (m, 2H, CH_2sp), 1.84-1.89 (m, 2H, CH_2sp), 2.81 (d, 1H, -OH), 3.33-3.43 (m, 4H, 2 x CH_2sp), 3.54-3.63 (m, 3H, H-3,5), 3.64-3.68 (m, 2H, H-1), 3.77-3.79 (m, 1H, H-2), 3.87-3.91 (m, 1H, H-2), 3.87-3.91 (m, 1H, H-4), 4.00-4.11 (m, 2H, OCH_2), 4.44-4.66 (m, 4H, 2 x *CH_2*Ph), 5.08-5.18 (m, 2H, CH_2=), 5.72-5.87 (m, 1H, =CH-), 7.24-7.28 (m, 10H, aromatic) ppm; ^{13}C-n.m.r.: δ, 28.8, 30.2, 34.3, 70.8, 70.9, 71.4, 71.8, 73.0, 73.3, 73.9, 79.3, 79.6, 117.4, 128.1, 128.2, 128.3 (x 2), 128.4 (x 2), 128.9 (x 2), 129.0 (x 2), 135.5, 138.6, 138.9 ppm; HR-FAB MS $[M+Na]^+$ calcd for $C_{26}H_{35}BrO_5$ Na 529.1566, found 529.1542.

3-O-Allyl-2,4,5-tri-O-benzyl-1-O-(4-bromobutyl)-D-ribitol (4.13). Benzyl bromide (0.3 mL, 2.1 mmol) was added to a stirring solution of **4.12** (0.73 g, 1.44 mmol) in dry DMF (10 mL) at 0 ^{o}C. Sodium hydride (60% suspension in mineral oil, 0.12 g, 2.8 mmol) was added slowly until evolution of hydrogen gas has seized. The reaction mixture was stirred for 5 h at rt until complete disappearance of the starting material, as indicated by TLC. The reaction mixture was poured in crushed ice (~50 mL), stirred for 15 min, extracted with ethyl acetate/ether (1/1, v/v, 3 x 30 mL). The combined organic extract was dried with anhydrous $MgSO_4$, filtered and concentrated in *vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate-hexane gradient elution) to afford compound **4.13** as a colorless liquid (0.72 g, 84% yield). Analytical data for **4.13**: R_f = 0.57 (ethyl acetate - hexanes, 2/3, v/v); $[\alpha]_D^{28}$ -2.2^{o} (c = 1.0, $CHCl_3$); ^{1}H-n.m.r.: δ, 1.87-2.00 (m, 2H, CH_2sp), 2.13-2.27 (m, 2H, CH_2sp), 3.64-3.68 (m, 4H, 2 x CH_2sp), 3.85-4.10 (m, 7H, H-1, 2, 3, 4, 5), 4.38-3.41 (m, 2H, OCH_2), 4.75-5.00 (m, 6H, 3 x *CH_2*Ph), 5.36-5.50 (m, 2H, CH_2=), 6.08-6.13 (m, 1H, =CH-), 7.54-7.65 (m, 15H, aromatic) ppm; ^{13}C-n.m.r.: δ, 26.6, 28.5, 29.9, 70.3, 70.5, 70.9, 72.3, 72.5, 72.9, 73.5, 78.6, 78.7, 116.5, 127.6, 127.7, 127.8 (x 2), 127.9 (x 4), 128.4 (x 4), 128.5 (x 2), 128.6, 135.2, 135.4, 138.4, 138.6, 138.8 ppm; HR-FAB MS $[M+H]^+$ calcd for $C_{33}H_{42}BrO_5$ 597.2216, found 597.2198.

2,4,5-Tri-O-benzyl-1-O-(4-bromobutyl)-D-ribitol (4.14). To a stirring solution of **4.13** (0.7 g, 1.2 mmol) in MeOH (7 mL), $PdCl_2$ (0.25 g) was added and the reaction was stirred at rt for 3 h. The solid was then filtered off through a pad of celite, rinsed with ethyl acetate (3 x 10 mL) and the combined filtrate was concentrated in *vacuo*. The

residue was purified by column chromatography on silica gel (ethyl acetate-hexane gradient elution) to afford compound **4.14** as a yellow syrup (0.42 g, 60% yield). Analytical data for **4.14**: R_f = 0.45 (ethyl acetate - hexanes, 3/7, v/v); $[\alpha]_D^{25}$ -3.8° (c = 1.0, $CHCl_3$); ^{1}H-n.m.r.: δ, 1.69-1.76 (m, 2H, CH_2sp), 1.80-1.98 (m, 2H, CH_2sp), 2.97 (d, 1H, -OH), 3.40-3.47 (m, 4H, 2 x CH_2sp), 3.53-3.63 (m, 3H, H-4, 5), 3.68-3.80 (m, 3H, H-1,2), 4.03-4.06 (m, 1H, H-3), 4.49-4.75 (m, 6H, 3 x *CH_2*Ph), 7.26-7.33 (m, 15H, aromatic) ppm; ^{13}C-n.m.r.: δ, 27.2, 28.4, 29.6, 33.9, 70.4, 70.7, 70.9, 71.2 (x 2), 71.8, 72.2 (x 3), 73.7 (x 2), 77.6, 78.1, 127.8, 127.9 (x 2), 128.1 (x 2), 128.5 (x 4), 128.6 (x 2), 138.3, 138.6 ppm; HR-FAB MS $[M+H]^+$ calcd for $C_{30}H_{38}BrO_5$ 559.1887, found 559.1885.

1-O-(4-Azidobutyl)-2,4,5-tri-O-benzyl-D-ribitol (4.9). Sodium azide (86 mg, 1.75 mmol) was added to a stirring solution of **4.14** (0.42 g, 0.75 mmol) in dry DMF (6 mL). The reaction was heated at 60 °C for 5 h, then poured in crushed ice (~30 mL), stirred for 15 min, and extracted with ethyl acetate (3 x 20 mL). The combined organic extract was dried with anhydrous $MgSO_4$ and concentrated in *vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate-hexane gradient elution) to afford compound **4.9** as a colorless syrup (0.35 g, 89% yield). Analytical data for **4.9**: R_f = 0.48 (ethyl acetate - hexanes, 2/3, v/v); $[\alpha]_D^{29}$ -4.8° (c = 1.0, $CHCl_3$); ^{1}H-n.m.r.: δ, 1.56 (bs, 4H, 2 x CH_2sp), 2.95 (bs, 1H, -OH), 3.17-3.24 (m, 2H, CH_2sp), 3.33-3.35 (m, 2H, CH_2sp), 3.52-3.60 (m, 2H, H-2, 4), 3.62-3.73 (m, 4H, H-1, 5), 3.95 (bs, 1H, H-3), 4.40-4.67 (m, 6H, 3 x *CH_2*Ph), 7.15-7.25 (m, 15H, aromatic) ppm; ^{13}C-n.m.r.: δ, 25.8, 26.9, 51.3, 70.6, 71.3, 71.4, 72.1, 72.4, 73.9, 78.3, 78.4, 127.6 (x 2), 127.7, 127.8 (x 2), 127.9

(x 4), 128.3 (x 5), 128.4 (x 4), 138.2, 138.5 ppm; HR-FAB MS $[M+H]^+$ calcd for $C_{30}H_{38}N_3O_5$ 520.2811, found 520.2827.

2,3,5-Tri-O-benzyl-1-O-(4-bromobutyl)-D-ribitol (4.15). The title compound was obtained from **3.19**[19] as described for the synthesis of compound **4.12** in 60% yield. Analytical data for **4.15**: : R_f = 0.56 (ethyl acetate - hexanes, 2/3, v/v); $[\alpha]_D^{29}$ +16.3° (c = 1.0, $CHCl_3$); ^{1}H-n.m.r.: δ, 1.60-1.65 (m, 2H, CH_2sp), 1.81-1.88 (m, 2H, CH_2sp), 2.77 (bs, 1H, -OH), 3.30-3.39 (m, 4H, 2 x CH_2sp), 3.53-3.61 (m, 3H, H-3, 5), 3.66-3.71 (m, 2H, H-1), 3.84-3.96 (m, 1H, H-2), 4.10-4.12 (m, 1H, H-4), 4.42-4.63 (m, 6H, 3 x *CH_2*Ph), 7.21-7.29 (m, 15H, aromatic) ppm; ^{13}C-n.m.r.: δ, 28.4, 29.8, 33.9, 70.4, 70.6, 71.0, 71.3, 72.6, 73.5 (x 2), 73.8, 79.0, 79.3, 127.7, 127.8 (x 2), 127.9 (x 2), 128.0, 128.2 (x 2), 128.5 (x 4), 128.6, 138.1, 138.5, 138.6 ppm; HR-FAB MS $[M+H]^+$ calcd for $C_{30}H_{38}BrO_5$ 559.1887, found 559.1885

1-O-(4-Azidobutyl)-2,3,5-tri-O-benzyl-D-ribitol (4.10). The title compound was obtained from **4.15** as described for the preparation of compound **4.9** in 80% yield. Analytical data for **4.10**: R_f = 0.40 (ethyl acetate - hexanes, 3/7, v/v); $[\alpha]_D^{29}$ +29.0° (c = 1.0, $CHCl_3$); ^{1}H-n.m.r.: δ, 1.61 (bs, 4H, 2 x CH_2sp), 2.83 (d, 1H, -OH), 3.19-3.23 (m, 2H, CH_2sp), 3.38-3.40 (m, 2H, CH_2sp), 3.54-3.58 (m, 3H, H-3, 5), 3.66-3.72 (m, 2H, H-1), 3.82-3.87 (m, 1H, H-2), 3.93 (bs, 1H, H-4), 4.42-4.69 (m, 6H, 3 x *CH_2*Ph), 7.18-7.29 (m, 15H, aromatic) ppm; ^{13}C-n.m.r.: δ, 25.9, 27.0, 51.4, 70.4, 70.9, 71.0, 71.3, 72.6, 73.5, 73.9, 79.0, 79.3, 127.7, 127.8 (x 2), 127.9 (x 4), 128.0 (x 2), 128.1 (x 2), 128.5 (x 4), 128.6 (x 2), 138.2, 138.6 ppm; HR-FAB MS $[M+H]^+$ calcd for $C_{30}H_{38}N_3O_5$ 520.2811,

found 520.2827.

4.4.2 Preparation of pseudo-oligosaccharides

Method A. *AgOTf-Promoted activation of the SBox glycosyl donors.* A mixture of the glycosyl donor (0.11 mmol), glycosyl acceptor (0.10 mmol), and freshly activated molecular sieves (3Å, 200 mg) in $ClCH_2CH_2Cl$ (2 mL) was stirred under argon for 1.5 h. Freshly conditioned AgOTf (0.22 mmol) was added and the reaction mixture was stirred for 15 min at rt, then diluted with CH_2Cl_2, the solid was filtered-off and the residue was washed with CH_2Cl_2. The combined filtrate (30 mL) was washed with 20% aq. $NaHCO_3$ (15 mL) and water (3 x 10 mL), the organic phase was separated, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate - hexane gradient elution) to afford a pseudo-oligosaccharide derivative.

Method B. *NIS/TfOH-promoted activation of S-ethyl glycosyl donors.* A mixture the glycosyl donor (0.13 mmol), glycosyl acceptor (0.10 mmol), and freshly activated molecular sieves (4Å, 200 mg) in $ClCH_2CH_2Cl$ (2 mL) or ether/ DCE was stirred under argon for 1 h. NIS (0.25 mmol) and TfOH (0.025 mmol) were added at -30 ^oC to 0 ^oC and the reaction mixture was stirred for 10 min. Upon completion, the solid was filtered-off and the residue was washed with CH_2Cl_2. The combined filtrate (30 mL) was washed with 20% aq. $Na_2S_2O_3$ (15 mL) and water (3 x 10 mL). The organic phase was separated, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate - hexane gradient elution) or Sephadex LH-20 (methanol – dichloromethane, 1/1, v/v elution) to afford a pseudo-oligosaccharide derivative.

1-O-(4-Azidobutyl)-3-O-(2,3,4-tri-O-benzoyl-α-L-rhamnopyranosyl)-2,4,5-tri-O-benzyl-D-ribitol (4.16). The title compound was obtained by Method B from **3.9** and **4.9** in 75% yield. Analytical data for **4.16**: R_f = 0.50 (ethyl acetate – hexanes, 3/7, v/v); $[\alpha]_D^{29}$ +37.8° (c = 1, $CHCl_3$); ^{1}H-n.m.r.: δ, 1.11 (d, 3H, H-6'), 1.58 (bs, 4H, 2 x CH_2sp), 3.18 (s, 2H, CH_2sp), 3.36 (d, 2H CH_2sp), 3.66-3.70 (d, 4H, H-1, 5), 3.72-3.75 (m, 1H, H-2), 3.82-3.86 (m, 1H, H-4), 4.20-4.32 (m, 2H, H-3, 5'), 4.40-4.60 (m, 6H, 3 x *CH*$_2$Ph), 5.21 (s, 1H, H-1'), 5.52-5.59 (m, 2H, H-2', 4'), 5.67 (dd, 1H, $J_{2',3'}$ = 3.1 Hz, H-3'), 7.17-8.06 (m, 30H, aromatic) ppm; ^{13}C-n.m.r.: δ, 18.2, 26.4, 27.5, 51.9, 67.8, 69.0, 70.7, 71.2, 71.3, 71.5, 72.4, 72.6, 72.7, 73.8, 78.3, 98.3, 128.2 (x 3), 128.4 (x 3), 128.5 (x 6), 128.9 (x 6), 129.0 (x 3), 129.2 (x 3), 129.6, 129.8, 130.1, 130.3 (x 4) , 130.5 (x 2), 133.7, 133.9, 134.0, 138.8, 139.0, 166.1, 166.2, 166.4 ppm; HR-FAB MS $[M+Na]^+$ calcd for $C_{57}H_{59}N_3O_{12}Na$ 1000.3996, found 1000.3963.

1-O-(4-Aminobutyl)-3-O-(α-L-rhamnopyranosyl)-D-ribitol (4.5). To a solution of **4.16** (60 mg, 0.06 mmol) in dry methanol (1.0 mL) was added 1M NaOMe till pH = 9 (~0.1 mL). The reaction mixture was stirred for 15 h at rt, then neutralized with Dowex (H^+), filtered, and concentrated in *vacuo*. The crude residue was dissolved in a mixture of ethanol/HCl (20 mL, 12/0.03, v/v) and 10% Pd/C (130 mg) was added. The reaction mixture was stirred under an atmosphere of H_2 for 8 h. When TLC showed the formation of ninhydrin positive spot on the baseline, the catalyst was filtered off and the filtrate was neutralized with Dowex (OH^-) resin and concentrated under reduced pressure. The residue was co-evaporated with water (2 x 2 mL) and then purified by column chromatography on Sephadex G-15 (water elution) to afford compound **4.5** as a syrup (19

mg, 85% yield). Analytical data for **4.5**: $[\alpha]_D^{26}$ -24.4° (c = 0.5, H_2O); ^{1}H-n.m.r. (D_2O): δ, 1.32 (d, 3H, H-6'), 1.72-1.74 (m, 4H, 2 x CH_2sp), 2.99-3.02 (m, 2H, CH_2sp), 3.51 (dd, 1H, $J_{3',4'}$ = 9.6 Hz, H-4'), 3.62-3.65 (m, 2H, CH_2sp), 3.69-3.71 (m, 2H, H-1b, 5b), 3.75-3.90 (m, 5H, H-1a, 5a, 3, 3', 5'), 3.99-4.03 (m, 2H, H-2, 4), 4.08-4.10 (m, 1H, H-2'), 4.99 (d, 1H, $J_{1',2'}$ = 1.7 Hz, H-1') ppm; ^{13}C-n.m.r. (D_2O).: δ, 16.9, 24.8, 26.1, 39.8, 62.7, 69.7, 70.3, 70.5, 70.7, 70.8, 71.1, 71.8, 72.2, 80.4, 100.9 ppm; HR-FAB MS $[M+H]^+$ calcd for $C_{15}H_{32}O_9N$ 370.2077, found 370.2071.

1-O-(4-Azidobutyl)-4-O-(2,3,4-tri-O-benzoyl-α-L-rhamnopyranosyl)-2,3,5-tri-O-benzyl-D-ribitol (4.17). The title compound was obtained by Method B from **3.9** and **4.10** in 87% yield. Analytical data for **4.17**: R_f = 0.50 (ethyl acetate – hexanes, 3/7, v/v); $[\alpha]_D^{25}$ +61.2° (c = 1, $CHCl_3$); ^{1}H-n.m.r.: δ, 1.07 (d, 3H, H-6'), 1.59 (s, 4H, 2 x CH_2sp), 3.13 (d, 2H, CH_2sp), 3.41-3.50 (m, 2H, CH_2sp), 3.61-3.67 (m, 3H, H-3, 5), 3.71 (d, 1H, H-2), 3.74-3.80 (m, 2H, H-1), 4.18-4.23 (m, 1H, H-5'), 4.28-4.33 (m, 1H, H-4), 4.37-4.70 (m, 6H, 3 x *CH_2*Ph), 5.36 (dd, 1H, $J_{1',2'}$ = 7.4 Hz, H-1'), 5.55 (dd, 1H, $J_{3',4'}$ = 9.9 Hz, H-3'),5.66-5.77 (m, 2H, H-2', 4'), 7.11-8.02 (m, 30H, aromatic) ppm; ^{13}C-n.m.r.: δ, 18.2, 26.5, 27.6, 51.9, 67.7, 70.7, 71.3, 71.5, 71.6, 72.5, 73.4, 74.0, 74.1, 76.4, 78.8, 79.6, 97.4, 128.2, 128.3 (x 2), 128.4 (x 3), 128.5 (x 2), 128.7 (x 2), 128.9 (x 2), 129.0 (x 6), 129.2 (x 2), 129.9, 130.1, 130.2, 130.3 (x 4), 130.4 (x 2), 130.6 (x 2), 133.7, 133.9, 134.0, 138.7, 138.8, 139.1, 166.1, 166.2, 166.4 ppm; HR-FAB MS $[M+Na]^+$ calcd for $C_{57}H_{59}N_3O_{12}Na$ 1000.3996, found 1000.3963.

1-O-(4-Azidobutyl)-4-O-(α-L-rhamnopyranosyl)-D-ribitol (4.6). The title compound was obtained from **4.17** using same reaction conditions as described for the synthesis of compound **4.5** in 90% yield. Analytical data for **4.6**: $[\alpha]_D^{26}$ -81.5^o (c = 1.0, H_2O); ^{1}H-n.m.r. (D_2O): δ, 1.34 (d, 3H, H-6'), 1.71-1.82 (m, 4H, 2 x CH_2sp), 3.06-3.09 (m, 2H, CH_2sp), 3.52 (dd, 1H, $J_{3',4'}$ = 9.7 Hz, H-4'), 3.60-3.69 (m, 3H, H-1b, CH_2sp), 3.75-3.85 (m, 4H, H-2, 3, 3', 5'), 3.89-3.95 (m, 4H, H-1a, 4, 5), 4.07 (dd, 1H, $J_{2',3'}$ = 1.7 Hz, H-2'), 5.06 (dd, 1H, $J_{1',2'}$ = 1.5 Hz, H-1') ppm; ^{13}C-n.m.r. (D_2O): δ, 17.1, 24.1, 26.1, 39.7, 59.6, 69.6, 70.6, 70.7, 70.9, 71.7, 72.3, 72.4, 78.6, 100.3 ppm; HR-FAB HR-FAB MS $[M+H]^+$ calcd for $C_{15}H_{32}O_9N$ 370.2077, found 370.2071

O-(3-O-Acetyl-2-O-benzyl-4,6-O-benzylidene-α-D-glucopyranosyl)-(1→3)-O-(2,4-di-O-benzoyl-α-L-rhamnnopyranosyl)-(1→3)-1-O-(4-azidobutyl)-2,4,5-tri-O-benzyl-D-ribitol (4.18). The title compound was obtained by Method B from **3.22** and **4.9** in 80% yield. Analytical data for **4.18**: R_f = 0.38 (ethyl acetate – hexanes, 3/7, v/v); $[\alpha]_D^{22}$ +50.3^o (c = 0.8, $CHCl_3$); ^{1}H-n.m.r.: δ, 1.07 (d, 3H, H-6'), 1.55 (bs, 4H, 2 x CH_2sp), 1.73 (s, 3H, $COCH_3$), 3.13-3.19 (m, 2H, CH_2sp), 3.22 (dd, 1H, $J_{1a,1b}$= 9.6 Hz, H-1a), 3.32-3.40 (m, 3H, H-2', CH_2sp), 3.59-3.73 (m, 5H, H-1b, 2, 4, 5), 3.79-3.83 (m, 3H, H-3, 3', 4'), 4.14-4.19 (m, 3H, H-5', 5'', 6b''), 4.24-4.30 (m, 1H, H-6a''), 4.39-4.69 (m, 8H, 4 x *CH*$_2$Ph), 4.99 (dd, 1H, $J_{1',2'}$= 3.2 Hz, H-1'), 5.10-5.17 (m, 3H, H-1'', 3'', C*H*Ph), 5.45-5.51 (m, 2H, H-2'', 4''), 6.94-7.99 (m, 35H, aromatic) ppm; ^{13}C-n.m.r.: δ, 17.8, 20.9, 25.9, 27.1, 51.4, 62.9, 67.6, 68.7, 68.9, 69.0, 70.5, 70.6, 70.8, 70.9, 72.1, 72.2, 72.4, 72.6, 73., 79., 95.1, 97.8, 101.3, 126.5 (x 2), 127.7 (x 4), 127.8 (x 4), 127.9 (x 4), 128.0 (x 6), 128.3 (x 4), 128.5 (x 6), 128.7 (x 4), 129.6, 129.8, 130.2, 133.3, 133.4, 137.2, 137.9, 138.4, 138.5,

138.6, 165.7, 166.4, 169.3 ppm; HR-FAB MS $[M+Na]^+$ calcd for $C_{72}H_{77}N_3O_{17}Na$ 1278.5151, found 1278.5138.

O-(α-D-Glucopyranosyl)-(1→3)-O-(α-L-rhamnnopyranosyl)-(1→3)-1-O-(4-aminobutyl)-D-ribitol (4.3). The title compound was obtained from **4.18** as described for the synthesis of compound **4.5** in 88% yield. Analytical data for **4.3**: $[\alpha]_D^{25}$ +33.2° (c = 1.0, H_2O); ^{1}H-n.m.r. (D_2O): δ, 1.35 (d, 3H, H-6'), 1.74-1.79 (m, 4H, 2 x CH_2sp), 3.06-3.08 (m, 2H, CH_2sp), 3.51 (dd, 1H, $J_{4'',5''}$ = 9.2 Hz, H-4''), 3.58-3.65 (m, 4H, H-4', 2'',CH_2sp), 3.68-3.72 (m, 2H, H-1b, 5b), 3.77-3.93 (m, 8H, H-1a, 3, 5a, 3', 5', 3'', 6a'', 6b''), 3.99-4.03 (m, 2H, H-2, 4), 4.09-4.11 (m, 1H, H-5''), 4.23 (dd, 1H, $J_{2',3'}$ = 2.6 Hz, H-2'), 5.05 (d, 1H, $J_{1',2'}$ = 1.7 Hz, H-1'), 5.15 (d, 1H, $J_{1'',2''}$ = 3.5 Hz, H-1'') ppm; ^{13}C-n.m.r. (D_2O): δ, 17.2, 24.1, 26.1, 39.7, 60.7, 62.7, 62.9, 67.3, 69.7, 69.8 70.5, 70.7, 71.1, 71.7, 72.0, 72.5, 73.3, 75.7, 80.4, 95.8, 100.5 ppm; HR-FAB MS $[M+H]^+$ calcd for $C_{21}H_{42}NO_{14}$ 532.2605, found 532.2606

O-(3-O-Acetyl-2-O-benzyl-4,6-O-benzylidene-α-D-glucopyranosyl)-(1→3)-O-(2,4-di-O-benzoyl-α-L-rhamnnopyranosyl)-(1→4)-1-O-(4-azidobutyl)-2,3,5-tri-O-benzyl-D-ribitol (4.19). The title compound was obtained by Method B from **3.22** and **4.10** in 78% yield. Analytical data for **4.19**: R_f = 0.41 (ethyl acetate – hexanes, 3/7, v/v); $[\alpha]_D^{22}$ +58.7° (c = 1, $CHCl_3$); ^{1}H-n.m.r.: δ, 1.09 (d, 3H, H-6'), 1.59 (bs, 4H, 2 x CH_2sp), 1.76 (s, 3H, $COCH_3$), 3.15 (bs, 2H, CH_2sp), 3.26 (dd, 1H, $J_{3'',4''}$ = 9.3 Hz, H-4''), 3.38-3.45 (m, 4H, H-3, 2'', CH_2sp), 3.60-3.74 (m, 5H, H-2, 5, 5'', 6b''), 3.78-3.86 (m, 3H, H-1, 6a''), 4.12-4.15 (m, 1H, H-5'), 4.22-4.28 (m, 1H, H-4), 4.33 (s, 1H, H-3'), 4.38-4.70 (m, 8H, 4 x

CH_2Ph), 5.12-5.18 (m, 3H, H-1'', 3'', C*H*Ph), 5.36 (dd, 1H, $J_{1',2'}$ = 1.5 Hz, H-1'), 5.54 (dd, 1H, $J_{3',4'}$ = 9.8 Hz, H-4'), 5.66 (d, 1H, $J_{2',3'}$ = 2.7 Hz, H-2'), 6.95-8.11 (m, 35H, aromatic) ppm; ^{13}C-n.m.r.: δ, 17.9, 20.9, 25.9, 27.1, 51.4, 62.8, 67.6, 68.7, 70.1, 70.4, 71.0, 71.1, 71.7, 72.1, 72.6, 72.9, 73.5, 75.7, 78.6, 79.2, 79.4, 94.4, 96.8, 101.2, 126.5 (x 2), 127.6, 127.7 (x 3), 127.8 (x 2), 127.9 (x 3),128.0 (x 6), 128.1 (x 3), 128.3 (x 3), 128.5 (x 6), 128.6 (x 3), 128.8, 129.6, 129.7, 129.8, 130.2, 133.2, 133.4, 137.2, 137.9, 138.2, 138.3, 138.5, 165.7, 166.3, 169.3 ppm; HR-FAB MS $[M+Na]^+$ calcd for $C_{72}H_{77}N_3O_{17}Na$ 1278.5151, found 1278.5138

O-(α-D-Glucopyranosyl)-(1→3)-O-(α-L-rhamnnopyranosyl)-(1→4)-1-O-(4-aminobutyl)-D-ribitol (4.4). The title compound was obtained from **4.19** as described for the synthesis of compound **4.5** in 78% yield. Analytical data for **4.4**: $[\alpha]_D^{27}$ +25.4^o (c = 0.4, H_2O); ^{1}H-n.m.r. (D_2O).: δ, 1.35 (d, 3H, H-6'), 1.70-1.72 (m, 4H, 2 x CH_2sp), 2.82-2.84 (m, 2H, CH_2sp), 3.51 (dd, 1H, $J_{4'',5''}$ = 9.5 Hz, H-4''), 3.61-3.65 (m, 4H, H-4', 2'', CH_2sp), 3.75-3.87 (m, 7H, H-1, 2, 3, 5', 3'', 6a''), 3.91-3.95 (m, 5H, H-5, 3', 5'', 6b''), 4.01-4.04 (m, 1H, H-4), 4.27 (dd, 1H, $J_{2',3'}$ = 2.5 Hz, H-2'), 5.12 (d, 1H, $J_{1',2'}$ = 1.3 Hz, H-1'), 5.18 (d, 1H, $J_{1'',2''}$ = 3.2 Hz, H-1'') ppm; ^{13}C-n.m.r. (D_2O): δ, 17.1, 24.5, 26.2, 40.0, 59.6, 60.6, 67.4, 69.7 (x 2), 70.5 (x 2), 71.2, 71.6, 71.7, 72.0, 72.4, 73.2, 75.9, 78.8, 95.9, 99.9 ppm; HR-FAB MS $[M+H]^+$ calcd for $C_{21}H_{42}NO_{14}$ 532.2605, found 532.2606

Ethyl O-(4-O-anisoyl-2,3,6-tri-O-benzyl-α-D-galactopyranosyl)-(1→3)-2-O-benzyl-4,6-O-benzylidene-1-thio-β-D-glucopyranoside (4.20). The title compound was obtained by Method A from **4.7** and 4.**8** in 99% yield. Analytical data for **4.20**: R_f = 0.50

(ethyl acetate – hexanes, 3/7, v/v); $[\alpha]_D^{29}$ +42.7° (c = 1, $CHCl_3$); ^{1}H-n.m.r.: δ, 1.15 (t, 3H, CH_3), 2.77- 2.81 (m, 2H, CH_2), 3.16 (dd, 1H, $J_{5',6a'}$ = 5.2 Hz, $J_{6a',6b'}$ = 4.9 Hz, H-6a'), 3.34 (dd, 1H, $J_{5',6b'}$ = 6.9 Hz, H-6b'), 3.46-3.51 (m, 1H, H-4), 3.57 (dd, 1H, $J_{3,4}$ = 4.9 Hz, H-3), 3.81 (dd, 1H, $J_{6a,6b}$ = 10.3 Hz, H-6b), 3.85-3.91 (m, 4H, H-6a, 2', -OCH_3), 4.05 (dd, 1H, $J_{3',4'}$ = 3.2 Hz, $J_{2',3'}$ = 6.9 Hz, H-3'), 4.12-4.19 (m, 4H, 2x *CH_2Ph*), 4.33-4.37 (m, 2H, H-5, 5'), 4.52 (dd, 1H, $J_{2,3}$ = 3.2 Hz, H-2), 4.57-4.83 (m, 4H, 2 x *CH_2Ph*), 5.05 (dd, 1H, $J_{1,2}$ = 7.1 Hz, H-1), 5.45 (dd, 1H, $J_{4',5'}$ = 2.6 Hz, H-4'), 5.50 (s, 1H, CH*Ph*), 5.71 (dd, 1H, $J_{1',2'}$ = 3.5 Hz, H-1'), 6.85-7.95 (m, 30H, aromatic) ppm; ^{13}C-n.m.r.: δ, 15.3, 25.3, 53.6, 67.5, 68.9 (x 2), 70.1, 72.0, 72.3, 72.9, 74.7, 75.4, 75.8, 76.4, 80.3, 82.6, 85.2, 96.8, 102.1, 113.7 (x 2), 127.4, 127.6 (x 2), 127.7, 127.8 (x 2), 128.1 (x 2), 128.2 (x 5), 128.4 (x 5), 128.5 (x 3), 128.6 (x 3), 128.7 (x 3), 129.5, 132.1 (x 2), 137.3, 137.7, 138.3, 138.6 ppm; HR-FAB MS $[M+H]^+$ calcd for $C_{57}H_{61}O_{12}S$ 969.3883, found 969.3884.

1-O-(4-Azidobutyl)-3-O-(2,4-di-O-benzoyl-α-L-rhamnnopyranosyl)-2,4,5-tri-O-benzyl-D-ribitol (4.21). The title compound was obtained by Method B from **3.10** and **4.9** in 65% yield. Analytical data for **4.21**: R_f = 0.57 (ethyl acetate – hexanes, 2/3, v/v); $[\alpha]_D^{28}$ -14.9° (c = 1, $CHCl_3$); ^{1}H-n.m.r.: δ, 1.11 (d, 3H, H-6'), 1.59 (bs, 4H, 2 x CH_2sp), 3.18-3.22 (m, 2H, CH_2sp), 3.31-3.35 (m, 2H, CH_2sp), 3.62-3.73 (d, 4H, H-1, 5), 3.78-3.86 (m, 2H, H-2, 4), 4.15-4.24 (m, 3H, H-3, 3', 5'), 4.42-4.63 (m, 6H, 3 x *CH_2*Ph), 5.13-5.21 (m, 2H, H-1', 4'), 5.32-5.34 (m, 1H, H-2'), 7.19-8.11 (m, 25H, aromatic) ppm; ^{13}C-n.m.r.: δ, 17.7, 25.9, 27.2, 51.5, 67.0, 68.6, 69.3, 70.7, 71.0, 72.1, 72.3, 73.3, 73.5, 75.8, 77.9, 97.5, 127.7 (x 2), 127.9 (x 3), 128.0 (x 6), 128.4 (x 6), 128.7 (x 4), 129.6, 129.7, 130.1 (x 4), 133.7 (x 2) , 138.4 (x 2), 138.6, 166.1, 167.4 ppm; HR-FAB MS

$[M+Na]^+$ calcd for $C_{50}H_{55}N_3O_{11}Na$ 896.3734, found 896.3732.

O-(4-O-Anisoyl-2,3,6-tri-O-benzyl-α-D-galactopyranosyl)-(1→3)-O-(2-O-benzyl-4,6-O-benzylidene-α-D-glucopyranosyl)-(1→3)-O-(2,4-di-O-benzoyl-α-L-rhamnnopyranosyl)-(1→3)-1-O-(4-azidobutyl)-2,4,5-tri-O-benzyl-D-ribitol (4.22). The title compound was obtained by Method B from **4.20** and **4.21** in 66% yield. Analytical data for **4.22**: R_f = 0.60 (ethyl acetate - hexanes, 2/3, v/v); $[\alpha]_D^{28}$ +55.6° (c = 1.0, $CHCl_3$); ^{1}H-n.m.r.: δ, 1.19 (d, 3H, H-6'), 1.60-1.64 (m, 4H, 2 x CH_2sp), 3.24 (d, 4H, H-6a''', 6b''', CH_2sp), 3.38-3.42 (m, 2H, CH_2sp), 3.45-3.53 (m, 2H, H- 4'',6a''), 3.59 (dd, 1H, H-2''), 3.67-3.70 (m, 5H, H-1, 5, 2'''), 3.76-3.80 (m, 3H, H-5'', 6b'', 3'''), 3.85 (s, 3H, -OCH_3), 3.88-3.93 (m, 2H, H-2, 4), 3.97-4.05 (m, 1H, H-3), 4.07 (dd, 1H, $J_{3'',4''}$ = 4.8 Hz, H-3''), 4.17-4.28 (m, 4H, H-5', 5''', C*H*$_2$Ph), 4.36-4.40 (m, 3H, H-3', C*H*$_2$Ph), 4.45-4.77 (m, 10H, 5 x C*H*$_2$Ph), 5.08 (d, 1H, $J_{1'',2''}$ = 3.5 Hz, H-1''), 5.28 (s, 1H, H-1'''), 5.30 (s, 2H, H-1', C*H*Ph), 5.52 (dd, 1H, $J_{3''',4'''}$ = 3.3 Hz, H-4'''), 5.62-5.66 (m, 2H, H-2', 4'), 6.81-8.09 (m, 55H, aromatic) ppm; ^{13}C-n.m.r.: δ, 17.9, 25.9, 27.1, 36.8, 51.4, 55.6, 62.6, 67.1, 67.8, 68.0, 68.4, 68.8, 68.9, 69.4, 70.8, 70.9, 71.7, 71.9, 72.1, 72.2, 72.3, 72.4, 72.8, 72.9, 73.2, 73.4, 74.7, 75.9, 77.7, 77.8, 77.9, 82.9, 94.9, 96.8, 97.7, 101.9, 113.6, 123.1, 126.8 (x 2), 127.3, 127.4, 127.6 (x 2), 127.7 (x 2), 127.9 (x 5), 127.96 (x 3), 128.0 (x 4), 128.1 (x 6), 128.2 (x 2), 128.3 (x 4), 128.5 (x 6), 128.6 (x 3), 128.7, 128.8, 129.2, 129.7, 129.8, 129.9, 130.2, 132.0 (x 4), 133.3, 133.5, 137.4, 137.5, 138.4, 138.5 (x 2), 138.6 (x 2), 138.8, 163.3, 165.6, 165.8, 166.2 ppm; HR-FAB MS $[M+Na]^+$ calcd for $C_{105}H_{109}N_3O_{23}Na$ 1802.7344, found 1802.7366.

O-(α-D-Galactopyranosyl)-(1→3)-O-(α-D-glucopyranosyl)-(1→3)-O-(α-L-rhamnopyranosyl)-(1→3)-1-O-(4-aminobutyl)-D-ribitol (4.1). The title compound was obtained from **4.22** in 70% yield similarly to that described for the synthesis of compound **4.5**, the only difference being pH=12 to ensure rapid removal of the anisoyl moiety. Analytical data for **4.1**: $[\alpha]_D^{25}$ +80.8° (c = 1.0, H_2O); ^{1}H-n.m.r. (D_2O).: δ, 1.21 (d, 3H, H-6'), 1.71-1.76 (m, 4H, 2 x CH_2sp), 3.04-3.07 (m, 2H, CH_2sp), 3.55-3.69 (m, 16H), 3.71-3.88 (m, 14H), 3.92-4.07 (m, 8H), 4.22-4.27 (m, 2H, H-2', 5'''), 5.02 (d, 1H, $J_{1',2'}$ = 1.8 Hz, H-1'), 5.13 (d, 1H, $J_{1'',2''}$ = 6.3 Hz, H-1''), 5.40 (d, 1H, $J_{1''',2'''}$ = 3.7 Hz, H-1''') ppm; ^{13}C-n.m.r. (D_2O): δ, 17.1, 24.1, 26.1, 39.7, 60.5, 61.2, 62.7 (x 2), 67.1, 68.9, 69.5, 69.7, 69.8, 70.1, 70.2, 70.4, 70.7, 71.1, 71.8, 72.4 (x 2), 75.5, 79.9, 80.4, 90.0, 95.7, 99.7, 100.5 ppm; HR-FAB MS $[M+H]^+$ calcd for $C_{27}H_{52}NO_{19}$ 694.3134 found 694.3141.

1-O-(4-Azidobutyl)-4-O-(2,4-di-O-benzoyl-α-L-rhamnnopyranosyl)-2,3,5-tri-O-benzyl-D-ribitol (4.23). The title compound was obtained by Method B from **3.10** and **4.10** in 78% yield. Analytical data for **4.23**: R_f = 0.49 (ethyl acetate – hexanes, 3/7, v/v); $[\alpha]_D^{29}$ +3.2° (c = 0.9, $CHCl_3$); ^{1}H-n.m.r.: δ, 1.07 (d, 3H, H-6'), 1.56 (s, 4H, 2 x CH_2sp), 2.40 (d, 1H, -OH), 3.06-3.09 (m, 2H, CH_2sp), 3.38-3.40 (d, 2H, CH_2sp), 3.57-3.65 (m, 3H, H-2, 3, 5), 3.70-3.73 (m, 2H, H-1), 4.00-4.14 (m, 2H, H-3', 5'), 4.22 (bs, 1H, H-4), 4.36-4.86 (m, 6H, 3 x *CH_2*Ph), 5.14 (dd, 1H, $J_{1',2'}$ = 9.8 Hz, H-1'), 5.31-5.37 (m, 2H, H-2', 4'), 7.14-8.05 (m, 25H, aromatic) ppm; ^{13}C-n.m.r.: δ, 18.2, 26.4, 27.6, 51.8, 67.3, 67.7, 70.9, 71.3, 71.5, 73.1, 74.0, 74.1 (x 2), 76.0, 76.2, 78.5, 79.7, 97.0, 128.2, 128.3 (x 2), 128.4 (x 3), 128.5 (x 2), 128.6 (x 2), 128.9 (x 6), 129.1 (x 2), 129.2 (x 2), 130.2, 130.5 (x 2), 130.6 (x 2), 134.1 (x 2), 138.7, 138.9 (x 2), 166.6, 167.8 ppm; HR-FAB MS

$[M+Na]^+$ calcd for $C_{50}H_{55}N_3O_{11}Na$ 896.3734, found 896.3732.

O-(4-O-Anisoyl-2,3,6-tri-O-benzyl-α-D-galactopyranosyl)-(1→3)-O-(2-O-benzyl-4,6-O-benzylidene-α-D-glucopyranosyl)-(1→3)-O-(2,4-di-O-benzoyl-α-L-rhamnnopyranosyl)-(1→4)-1-O-(4-azidobutyl)-2,3,5-tri-O-benzyl-D-ribitol (4.24). The title compound was obtained by Method B from **4.20** and **4.23** in 70% yield. Analytical data for **29**: R_f = 0.56 (ethyl acetate - hexanes, 2/3, v/v); $[\alpha]_D^{29}$ +54.8 ^{o} (c = 1.0, $CHCl_3$); ^{1}H-n.m.r.: δ, 1.15 (d, 3H, H-6'), 1.64 (bs, 4H, 2 x CH_2sp), 3.21-3.23 (m, 4H, CH_2sp, H-6a''', 6b'''), 3.40-3.43 (m, 3H, CH_2sp, H-6b''), 3.44-3.47 (m, 1H, H-6a''), 3.49-3.51 (dd, 1H, H-2''), 3.57-3.61 (m, 2H, H-2''', H-4''), 3.64-3.70 (m, 5H, H-2, 3, 5, 3'''), 3.74-3.80 (m, 6H, H-1, 5'', -OCH_3), 3.86-3.90 (m, 1H, H-3), 3.96 (dd, 1H, $J_{3'',4''}$ = 9.5 Hz, H-3''), 4.17-4.21 (m, 3H, H-5', CH_2Ph), 4.24-4.26 (m, 1H, H-5'''), 4.33-4.35 (m, 1H, H-4), 4.40-4.54 (m, 9H, H-3', 4 x CH_2Ph), 4.59-4.75 (m, 4H, 4 x CH_2Ph), 5.16 (d, 1H, $J_{1'',2''}$ = 3.3 Hz, H-1''), 5.28 (s, 2H, H-1''', C*H*Ph), 5.45 (s, 1H, H-1'), 5.49 (bs, 1H, H-4'''), 5.66 (dd, 1H, $J_{4',5'}$ = 9.8 Hz, H-4'), 5.74 (bs, 1H, H-2'), 6.80-8.09 (m, 55H, aromatic) ppm; ^{13}C-n.m.r.: δ, 17.9, 26.0, 27.1, 51.4, 55.6, 62.5, 67.0, 67.7, 68.1, 68.4, 68.9, 70.2, 71.1, 71.6, 71.7, 71.8, 72.0, 72.7, 72.9, 73.0, 73.2, 73.6, 73.8, 74.7, 75.9, 76.0, 77.4 (x 3), 78.7, 79.3, 82.8, 94.2, 96.7, 96.8, 101.8, 113.5 (x 2), 123.1, 126.7 (x 2), 127.3 (x 2), 127.4, 127.6 (x 2), 127.8 (x 2), 127.9 (x 6), 128.0 (x 3), 128.1 (x 3), 128.15 (x 5), 128.19 (x 3), 128.25 (x 3), 128.3 (x 3), 128.4 (x 2), 128.6 (x 6), 128.7 (x 2), 128.8 (x 2), 129.2, 129.7, 129.9, 130.2, 131.9, 133.3, 133.5, 137.3, 137.4, 138.3 (x 2), 138.4, 138.5 (x 2), 138.8, 163.3, 165.6, 165.7, 166.1 ppm; HR-FAB MS $[M+Na]^+$ calcd for $C_{105}H_{109}N_3O_{23}Na$ 1802.7344, found 1802.7366.

O-(α-D-Galactopyranosyl)-(1→3)-O-(α-D-glucopyranosyl)-(1→3)-O-(α-L-rhamnopyranosyl)-(1→4)-1-O-(4-aminobutyl)-D-ribitol (4.2). The title compound was obtained from **4.24** as described for the synthesis of compound **4.1** in 76% yield**.** Analytical data for **4.2**: $[\alpha]_D^{25}$ +73.8^o (c = 0.8, H_2O); ^{1}H-n.m.r. (D_2O).: δ, 1.23 (d, 3H, H-6'), 1.72-1.74 (m, 4H, 2 x CH_2sp), 2.98-3.01 (m, 2H, CH_2sp), 3.57-3.63 (m, 6H), 3.63-3.70 (m, 7H), 3.71-3.84 (m, 12H), 3.87-3.99 (m, 9H), 4.01-4.05 (m, 2H), 4.28-4.31 (m, 2H, H-2', 5'''), 5.11 (d, 1H, $J_{1',2'}$ = 1.8 Hz, H-1'), 5.16 (d, 1H, $J_{1'',2''}$ = 3.3 Hz, H-1''), 5.45 (d, 1H, $J_{1''',2'''}$ = 3.7 Hz, H-1''') ppm; ^{13}C-n.m.r. (D_2O).: δ, 17.5, 24.4, 26.1, 39.8, 60.7, 61.4, 62.1, 63.9, 67.0, 68.2, 69.9, 70.4, 70.6 (x 2), 71.1, 71.7, 72.1, 72.8, 72.9, 73.3, 76.5, 79.8, 81.1, 96.8, 100.6, 101.5 ppm; HR-FAB MS $[M+H]^+$ calcd for $C_{27}H_{52}NO_{19}$ 694.3134 found 694.3141.

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CHAPTER 5

Synthesis of the newly discovered pneumococcal serotype 6C within serogroup 6 of *Streptococcus pneumoniae*

Parameswar, A. R.; Demchenko, A. V. "Stereoselective Synthesis of the newly discovered pneumococcal serotype 6C of *Streptococcus pneumoniae*", **2008**, *in preparation*

5 Synthesis of the newly discovered pneumococcal serotype SPn6C

5.1 Introduction

Streptococcus pneumoniae (SPn) is one of the most common gram-postive pathogens and causes many life-threatening diseases. These pathogenic pneumococci display one of many structurally varied carbohydrate capsules which in turn serves as a cover against external influences and renders the bacterium more or less resistant to non-specific host defense.[1] As mentioned in chapter 3, 90 different pnemococcal types have been identified with distinct serological patterns and chemical structures.[2] It is also well known that not all 90 serotypes are equally pathogenic. For example, serotypes 6A and 6B account for 4.7% and 7%, respectively, for the number of cases of invasive pneumococcal disease in the United States.[3] Since they are medically vital, the molecular natures of both SPn6A and 6B have been studied widely.

It has been shown that the 6A/ 6B polysaccharides are actually isopolymers containing galactose-glucose-rhamnose-ribitol phosphate, with 6B differing only in the rhamnose-ribitol linkage.[4] Very recently, two subtypes in pneumococci were identified by the classic quellung reactions.[5,6] Chemical studies of these capsular PS (polysaccharide's) showed that the major subtype produces capsular PS galactose-glucose-rhamnose-ribitol phosphate which matches the 6A PS, thus the major subtype retained the name as serotype 6A.[6] In comparision, the repeating unit of the minor subtype was found to be glucose-glucose-rhamnose-ribitol phosphate, hence the minor subtype was designated as serotype 6C.[6] Based on the genetic differences between serotype 6A and 6C,[5] it was

concluded that 6C should be included as the third member of serogroup 6 in view of its serological and structural relation to serotype 6A. Serotype 6C thus represents the 91st pneumococcal serotype, with 90 pneumococcal serotypes having previously been recognized. Currently available pneumococcal vaccines contain only 6B PS as it is presumed to induce cross-protection against 6A. Since there is significant structural difference of SPn6C from the currently used serotype SPn6B (Figure 5.1), the cross-protection against 6C may be inadequate, and the currently available pneumococcal vaccines may reduce the prevalence of 6A but not 6C.[5] This also makes 6C a very important and interesting model to study the genetic evolution in bacteria. Hence, it was concluded that all pneumococcal isolates should be tested for serotype 6C along with serotypes 6A and 6B

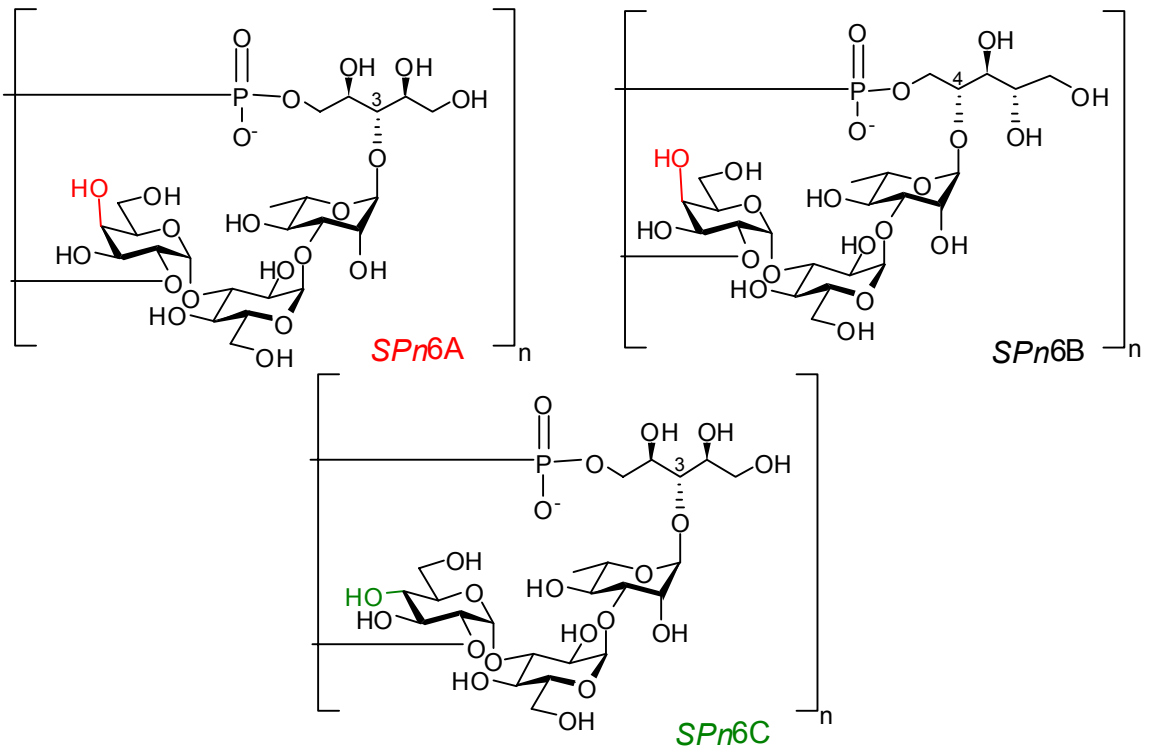


Figure 5.1 Chemical repeat units of serotypes 6A, 6B and 6C

5.2 Results and Discussions

As seen from Figure 5.1, the only structural difference between SPn6A and 6C is that the galactose unit present at the terminal end of SPn6A is replaced by the glucose unit for SPn6C. This chapter will describe the efficient chemical synthesis of both the pseudo-tetrasaccharide (**5.1**, Figure 5.2) and the spacer containing pseudo-tetrasaccharide (**5.2,** Figure 5.2) corresponding to the repeating unit of SPn6C by adopting similar techniques as described in Chapters 3 and 4.

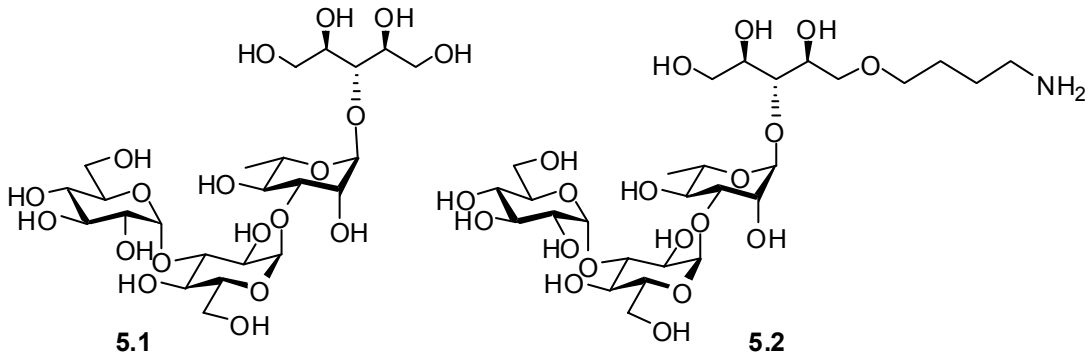
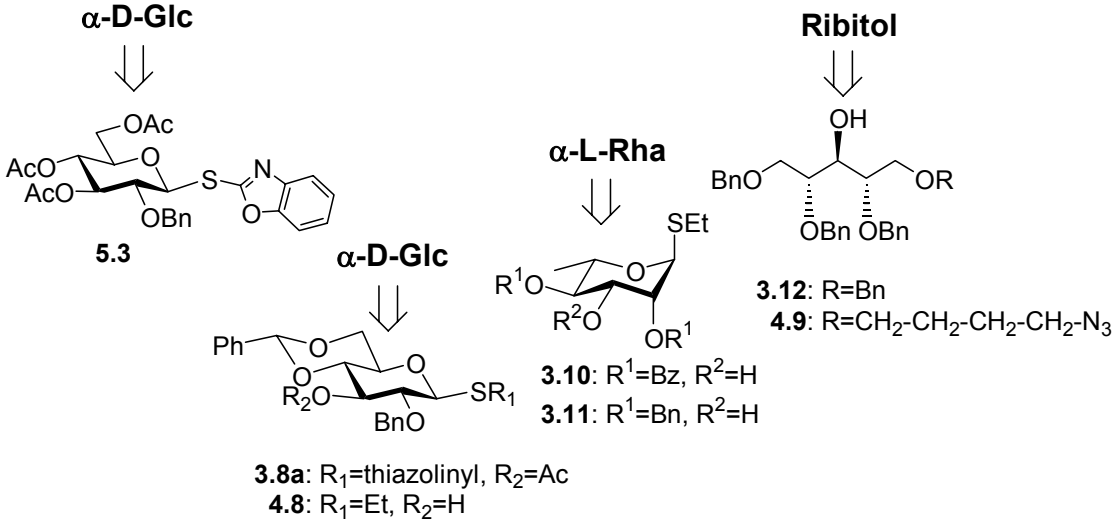
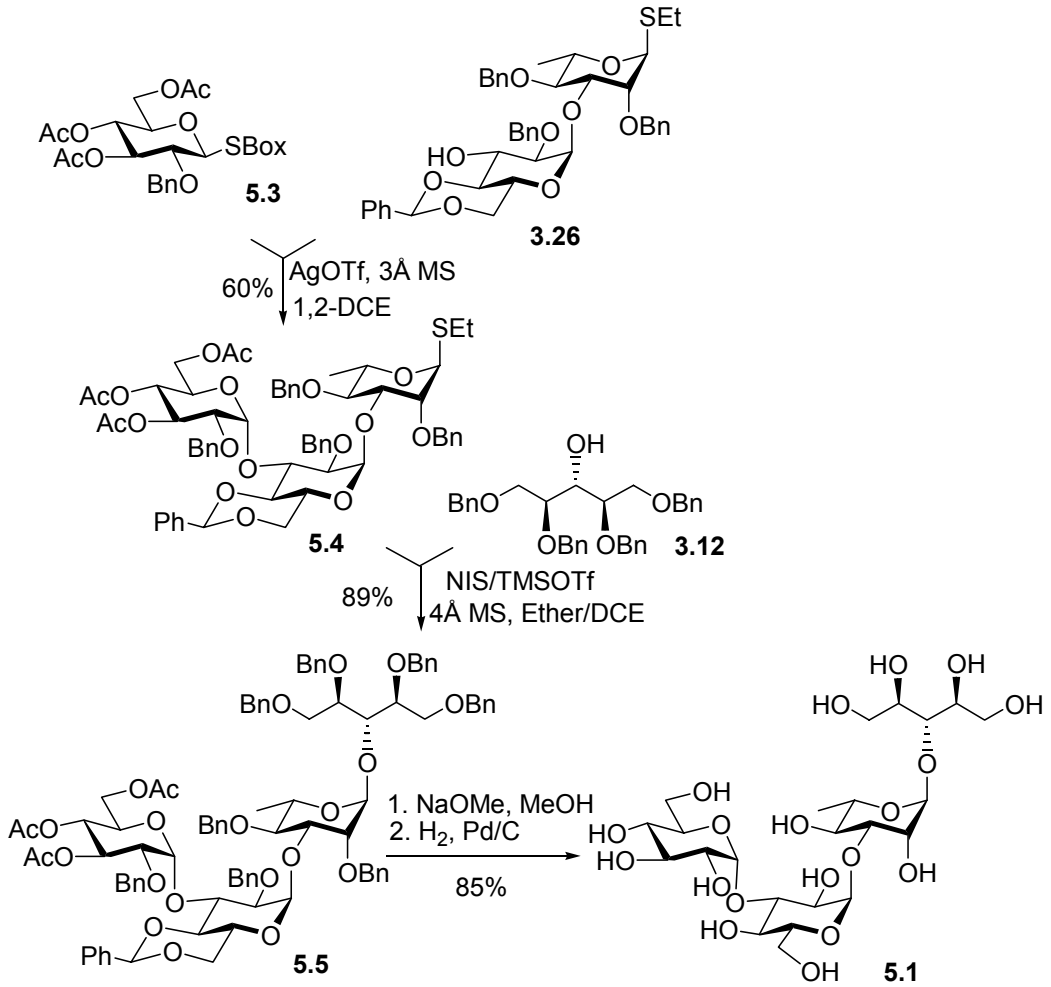


Figure 5.2 Synthetic Targets of SPn6C



Scheme 5.1 Retrosynthetic analysis of SPn6C

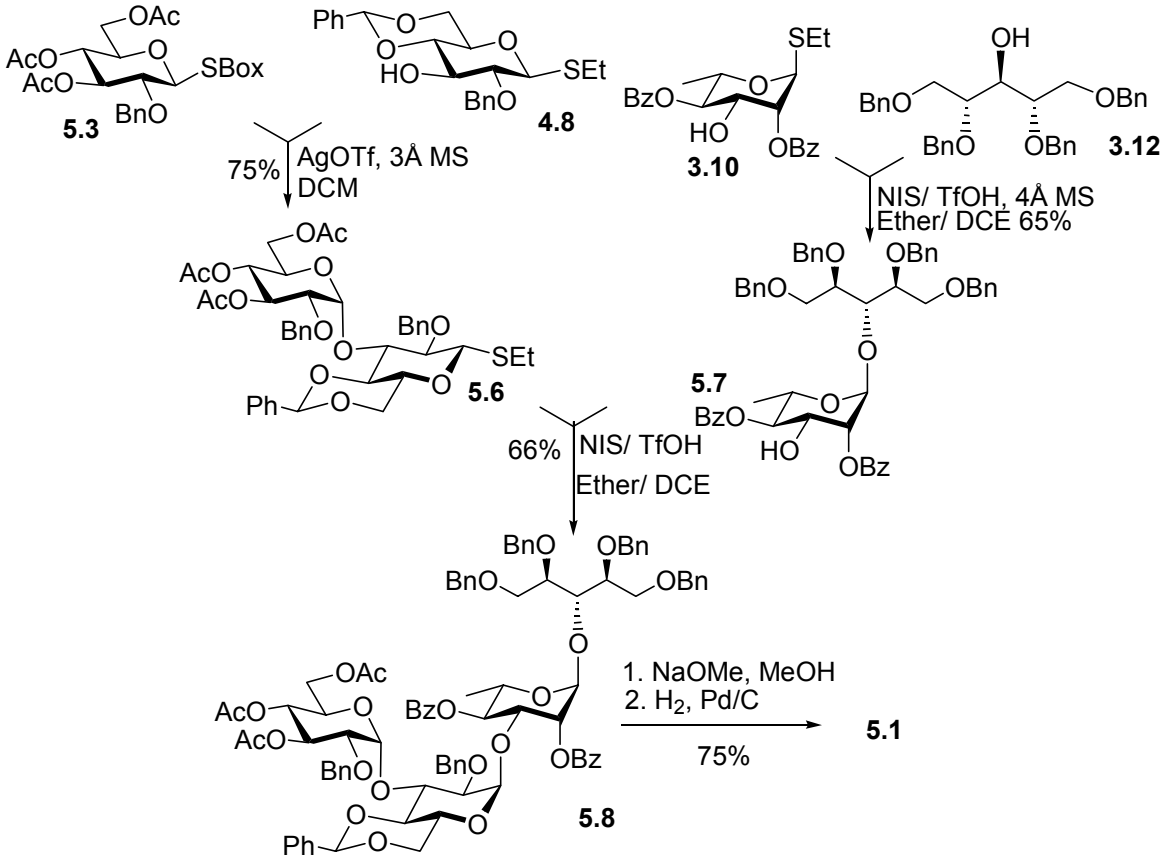
For the synthesis of pseudo-tetrasaccharide **5.1** (Scheme 5.2), two methods were adopted for the synthesis. The first method involved the synthesis of the ABCD tetrasaccharide by the (B + C) + A + D method as illustrated in Chapter 3. Building blocks of D-glucose (**3.8a** and **4.8**), L-rhamnose (**3.10** and **3.11**) and D- ribitol (**3.12** and **4.9**) required for the synthesis of SPn6C via two different approaches were synthesized as described in chapters 3 and 4. D-glucose derivative (**5.3**) was synthesized using the same method that was described previously (Scheme 5.1).[7]



Scheme 5.2 Synthesis of Pseudo Tetrasaccharide **5.1** by (B + C) + A + D method

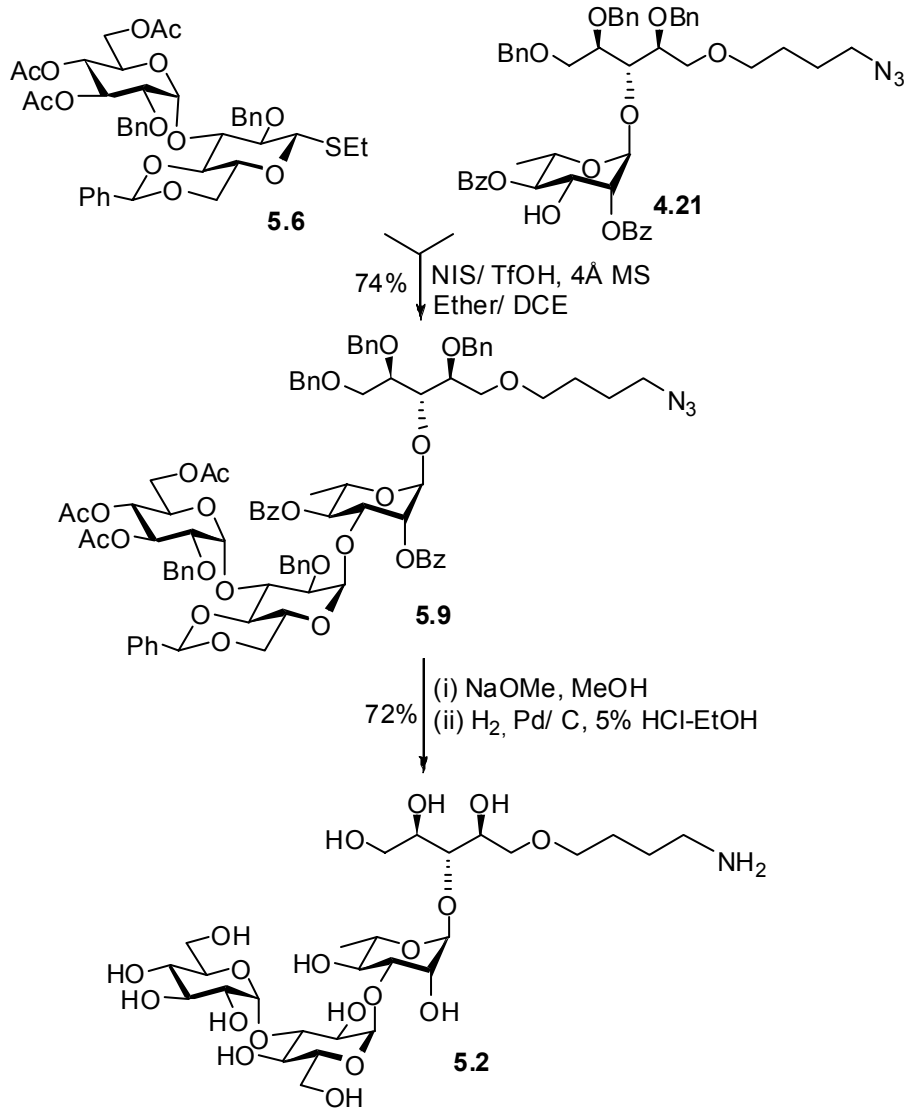
Glycosidation of the SBox glucosyl donor **5.3** with the SEt disaccharide acceptor **3.26** (see Chapter 3) was carried out to obtain the trisaccharide **5.4** in 60% yield with complete α-selectivity. To complete the assembly, the trisaccharide **5.4** was coupled with ribitol acceptor **3.12** in the presence of NIS and TMSOTf (Scheme 5.1). The resulting pseudo-tetrasaccharide derivative of SPn6C **5.5** was obtained in 89% yield with complete stereoselectivity. The tetrasaccharide was then subjected to a two-step sequential deprotection: deacylation (NaOMe in MeOH) and hydrogenation using palladium on charcoal in EtOH to obtain 85% of pseudo tetrasaccharide **5.1.**

The second method involved the synthesis of the tetrasaccharide **5.1** (Scheme 5.3) by the alternative A + B + (C + D) method in accordance with that developed in Chapter 4. Glucosyl donor **5.3** [7] bearing the SBox leaving group was selectively activated over the SEt moiety of glucosyl acceptor **4.8**. This reaction was carried out in dichloromethane using AgOTf as promoter and the disaccharide **5.6** was obtained in 75% yield and importantly with complete stereoselectivity. For the synthesis of the disaccharide acceptor **5.7,** rhamnose building block **3.10** was glycosidated with ribitol acceptor **4.11** to afford the requisite disaccharide in 65% yield. To complete the assembly, the disaccharide donor **5.6** was coupled with the disaccharide acceptor **5.7** in the presence of NIS and catalytic TfOH. As a result, pseudo-tetrasaccharide derivative **5.8** was obtained in 66% yield. Deprotection of **5.8** was carried out under the same conditions from **5.5** to **5.1** to obtain the resulting pseudo-tetrasaccharide derivative **5.1**



Scheme 5.3 Synthesis of Pseudo Tetrasaccharide **5.8** by A + B + (C + D) method

The synthesis of the spacer containing pseudo-tetrasaccharide **5.2** (Scheme 5.4) was accomplished using the same protocol as described for the synthesis of **5.8.** The disaccharide donor **5.6** was coupled to the ribitol acceptor **4.21** (refer to Chapter 4) using NIS/ TfOH as a promoter to afford the pseudo-tetrasaccharide **5.9** in 74% yield. The deprotection of **5.9** was carried out using conventional deacylation-hydrogenation sequence to afford the target compound **5.2** in 72%.



Scheme 5.4 Synthesis of spacer containing Pseudo Tetrasaccharide **5.2** of SPn6C

5.3 Conclusions

In conclusion, the first reported chemical synthesis of the newly discovered pneumococcal serotype SPn6C (discovered 2007) was accomplished using an efficient convergent approach.

5.4 Experimental part

5.4.1 General

Refer to Chapter 2 (p.81)

5.4.2 Preparation of pseudo-oligosaccharides

Method A. *AgOTf-Promoted activation of the SBox glycosyl donors.* A mixture of the glycosyl donor (0.11 mmol), glycosyl acceptor (0.10 mmol), and freshly activated molecular sieves (3Å, 200 mg) in $ClCH_2CH_2Cl$ or CH_2Cl_2 (2 mL) was stirred under argon for 1.5 h. Freshly conditioned AgOTf (0.22 mmol) was added and the reaction mixture was stirred for 15 min at rt, then diluted with CH_2Cl_2, the solid was filtered-off and the residue was washed with CH_2Cl_2. The combined filtrate (30 mL) was washed with 20% aq. $NaHCO_3$ (15 mL) and water (3 x 10 mL), the organic phase was separated, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate - hexane gradient elution) to afford a pseudo-oligosaccharide derivative.

Method B. *NIS/TfOH-promoted activation of S-ethyl glycosyl donors.* A mixture the glycosyl donor (0.13 mmol), glycosyl acceptor (0.10 mmol), and freshly activated molecular sieves (4Å, 200 mg) in $ClCH_2CH_2Cl$ (2 mL) or ether/ DCE was stirred for 1 h under argon. NIS (0.25 mmol) and TfOH (0.025 mmol) was added at -30^oC to 0^oC and the reaction mixture was stirred for 10 min. Upon completion, the solid was filtered-off and the residue was washed with CH_2Cl_2. The combined filtrate (30 mL) was washed with 20% aq. $Na_2S_2O_3$ (15 mL) and water (3 x 10 mL). The organic phase was separated,

dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate - hexane gradient elution) or Sephadex LH-20 (methanol – dichloromethane, 1/1, v/v elution) to afford a pseudo-oligosaccharide derivative.

Ethyl O-(2-O-benzyl-3,4,6-tri-O-acetyl-α-D-glucopyranosyl)-(1→3)-O-(2-O-benzyl-4,6-O-benzylidene-α-D-glucopyranosyl)-(1→3)-2,4-di-O-benzyl-1-thio-α-L-rhamnopyranoside (5.4) The title compound was obtained by Method A from benzoxazolyl 3,4,6-tri-O-acetyl-2-O-benzyl-1-thio-β-D-glucopyranoside **5.3** and **3.26** in 60% yield. Analytical data: R_f = 0.55 (ethyl acetate - hexanes, 2/3, v/v); $[\alpha]_D^{25}$ +75.0° (c = 1.0, $CHCl_3$); ^{1}H-n.m.r.: δ, 1.19 (t, 3H, CH_2CH_3), 1.36 (d, 3H, H-6), 1.90, 1.93, 1.99 (3s, 9H, 3 x $COCH_3$), 2.55-2.69 (q, 2H, CH_2CH_3), 3.35 (dd, 1H, $J_{2'',3''}$ = 3.6 Hz, H-2''), 3.57-3.79 (m, 6H, H-3, 2', 4', 6b', 6a''', 6b'''), 4.07-4.14 (m, 5H, H-2, 5, 5', 6a', ½ CH_2Ph), 4.21 (dd, 1H, $J_{4,5}$= 4.8 Hz, H-4), 4.33-4.39 (m, 2H, H-3', 5''), 4.44-4.50 (m, 2H, CH_2Ph), 4.61-4.71 (m, 4H, 2 x CH_2Ph), 4.82 (dd, 1H, $J_{4'',5''}$ = 3.8 Hz, H-4''), 4.99 (d, 1H, ½ CH_2Ph), 5.27 (d, 1H, $J_{1',2'}$ = 3.5 Hz, H-1'), 5.37-5.44 (m, 3H, H-1, 3'', CHPh), 5.49 (dd, 1H, $J_{1'',2''}$ = 3.6 Hz, H-1''), 6.79-7.45 (m, 25H, aromatic) ppm; ^{13}C-n.m.r.: δ, 15.7, 18.5, 21.3, 21.4, 21.5, 26.1, 30.3, 61.9, 63.2, 67.4, 68.7, 69.2, 69.6, 71.2, 72.1, 72.8, 72.9, 73.9, 75.7, 75.9, 76.4, 80.6, 81.8, 83.1, 94.5, 96.3, 102.9, 127.2 (x 2), 127.8 (x 2), 128.2, 128.3 (x 2), 128.4, 128.6, 128.7 (x 2), 128.8 (x 2), 128.9 (x 2), 129.0 (x 2), 129.06 (x 2), 129.08 (x 3), 129.1 (x 2), 129.3, 130.0, 137.7, 137.8, 137.9, 138.4, 138.7, 170.3, 170.7, 171.3, ppm; HR-FAB MS $[M+H]^+$ calcd for $C_{61}H_{70}O_{17}SNa$ 1129.4231, found 1129.4238.

O-(3,4,6-Tri-O-acetyl-2-O-benzyl-α-D-glucopyranosyl)-(1→3)-O-(2-O-benzyl-4,6-O-benzylidene-α-D-glucopyranosyl)-(1→3)-O-(2,4-di-O-benzyl-α-L-rhamnnopyranosyl)-(1→3)-1,2,4,5-tetra-O-benzyl-D-ribitol (5.5) The title compound was obtained by Method B from **3.12** and **5.4** in 89% yield. Analytical data: R_f = 0.46 (ethyl acetate - hexanes, 2/3, v/v); $[\alpha]_D^{25}$ +55.4° (c = 1.0, $CHCl_3$); ^{1}H-n.m.r.: δ, 1.18 (d, 3H, H-6'), 1.80, 1.84, 1.90 (3s, 3H, 3 x $COCH_3$), 3.27 (dd, $J_{2''',3'''}$ = 3.6 Hz, H-2'''), 3.50-3.70 (m, 10H, H-1, 5, 4', 2'', 6a'', 6b'', 6a''', 6b'''), 3.75-3.79 (m, 1H, H-5'), 3.85-3.87 (m, 1H, H-2'), 3.90-3.95 (m, 1H, H-4''), 4.00-4.06 (m, 5H, H-2, 3', 3'', ½ CH_2Ph), 4.21-4.33 (m, 5H, H-3, 4, 5''', CH_2Ph), 4.35-4.52 (m, 9H, 4.5 x CH_2Ph), 4.57-4.61 (m, 3H, 1.5 x CH_2Ph), 4.71 (dd, 1H, $J_{4''',5'''}$ = 10.2 Hz, H-4'''), 4.93 (d, 1H, ½ CH_2Ph), 5.12-5.14 (m, 2H, H-1',1''), 5.28-5.34 (m, 2H, H-3''',C*H*Ph), 5.40 (d, 1H, $J_{1''',2'''}$ = 3.5 Hz, H-1'''), 6.72-7.34 (m, 45H, aromatic) ppm; ^{13}C-n.m.r.: δ, 18.2, 20.8, 20.9, 21.0, 61.5, 62.5, 66.9, 68.2, 69.1, 70.1, 70.3, 70.6, 71.7, 71.9, 72.1, 72.4, 72.8, 73.5, 73.6 (x 2), 74.5, 74.6, 75.3, 75.7, 78.0, 78.5, 78.7, 79.5, 82.7, 92.9, 95.8, 98.5, 102.5, 126.8 (x 2), 127.4 (x 2), 127.5 (x 3), 127.6 (x 3), 127.7 (x 4), 127.8 (x 2), 127.9 (x 3), 128.0, 128.1 (x 2), 128.2 (x 2), 128.3 (x 3), 128.4 (x 6), 128.5 (x 6), 128.6 (x 6), 129.6, 137.3, 137.4, 137.5, 138.1, 138.3, 138.4, 138.5, 138.6, 138.7, 169.9, 170.3, 170.8 ppm; HR-FAB MS $[M+Na]^+$ calcd for $C_{92}H_{100}O_{22}Na$ 1579.6604, found 1579.6609.

O-(α-D-Glucopyranosyl)-(1→3)-O-(α-D-glucopyranosyl)-(1→3)-O-(α-L-rhamnopyranosyl)-(1→3)-D-ribitol (5.1) To a solution of **5.5** (100 mg, 0.103 mmol) in dry methanol (1.0 mL) was added 1M NaOMe till pH = 10 (~0.1 mL). The reaction mixture was stirred for 15 h at rt, then neutralized with Dowex (H^+), filtered, and

concentrated in *vacuo*. The crude residue was dissolved in ethylacetate / ethanol (1/1, v/v, 2.0 mL) and 10% Pd/C (20 mg) was added. The reaction mixture was stirred under an atmosphere of H_2 for 15 h. The catalyst was then filtered off, washed with methanol and the filtrate was concentrated under reduced pressure. The residue was purified by column chromatography on Sephadex G-15 (water elution) to afford compound **5.1** as a film (26.8 mg, 85% yield). Analytical data: R_f = 0.35 (methanol – dichloromethane- water, 9/9/2, v/v); $[\alpha]_D^{27}$ +69.2° (c = 1.0, H_2O); ^{1}H-n.m.r. (D_2O): δ, 1.35 (d, 3H, H-6'), 3.50 (dd, 1H, $J_{4''',5'''}$ = 9.7 Hz, H-4'''), 3.58-3.62 (m, 3H, H-4', 5'', 2'''), 3.66-3.95 (m, 14H, H-1, 2, 5, 3', 5', 2'', 5'', 3''', 6a'', 6b'', 6a''', 6b'''), 3.97-4.01 (m, 2H, H-4, 4''), 4.04-4.09 (m, 3H, H-3, 3'', 5'''), 4.25 (dd, 1H, $J_{2',3'}$ = 2.4 Hz, H-2'), 5.06 (d, 1H, $J_{1',2'}$ = 1.6 Hz, H-1'), 5.14 (d, 1H, $J_{1'',2''}$ = 3.8 Hz, H-1''), 5.41 (d, 1H, $J_{1''',2'''}$ = 3.8 Hz, H-1''') ppm; ^{13}C-n.m.r. (D_2O).: δ, 17.0, 60.4, 60.6, 62.7, 62.8, 63.0, 67.3, 69.7, 69.8, 69.9, 70.2, 70.4, 70.5, 71.1, 71.8, 72.0, 72.1 (x 2), 72.4, 73.3, 75.7, 80.1, 80.3, 95.9, 99.7, 100.5 ppm; HR-FAB MS $[M+Na]^+$ calcd for $C_{23}H_{42}O_{19}Na$ 645.2218, found 645.2210.

Ethyl O-(3,4,6-Tri-O-acetyl-2-O-benzyl-α-D-glucopyranosyl)-(1→3)-2-O-benzyl-4,6-O-benzylidene-1-thio-β-D-glucopyranoside (5.6) The title compound was obtained by M ethod A from Benzoxazolyl 3,4,6-tri-O-acetyl-2-O-benzyl-1-thio-β-D-glucopyranoside **5.3** and ethyl 2-O-benzyl-4,6-O-benzylidene-1-thio-β-D-glucopyranoside **4.8** in 72% yield. Analytical data: R_f = 0.5 (ethyl acetate – hexanes, 2/3, v/v); $[\alpha]_D^{25}$ +85.9° (c = 1, $CHCl_3$); m.p. 74-76 ºC; ^{1}H-n.m.r.: δ, 1.35 (t, 3H, CH_2CH_3), 1.93, 1.95, 1.99 (3s, 9H, 3 x $COCH_3$), 2.71-2.90 (m, 2H, CH_2CH_3), 3.38 (dd, 1H, $J_{2',3'}$ = 3.6 Hz, H-2'), 3.49-3.59 (m, 2H, H-2, 6a), 3.65 (dd, 2H, $J_{6a',6b'}$ = 1.9 Hz, H-6a', H-6b'), 3.73-3.87 (m, 2H, H-4, 5),

4.08-4.19 (m, 3H, H-6b, 5', ½ CH_2Ph), 4.34 (dd, 1H, $J_{3,4}$ = 4.9 Hz, H-3), 4.54 (d, 1H, ½ CH_2Ph), 4.65 (d, 1H, $J_{1,2}$ = 9.47 Hz, H-1), 4.69 (d, 1H, ½ CH_2Ph), 4.84 (dd, 1H, $J_{3',4'}$ = 9.9 Hz, H-4'), 5.13 (d, 1H, ½ CH_2Ph), 5.40-5.46 (m, 2H, H-3', *CH*Ph), 5.63 (d, 1H, $J_{1',2'}$ = 3.5 Hz, H-1'), 6.85-7.43 (m, 15H, aromatic) ppm; ^{13}C-n.m.r.: δ, 15.2, 20.7, 20.8, 21.0, 25.3, 60.5, 61.2, 67.1, 68.0, 68.9, 70.1, 70.9, 71.5, 75.3, 75.8, 79.5, 82.2, 86.1, 95.8, 102.4, 126.6 (x 2), 127.4, (x 4), 127.8 (x 3), 128.4, 128.5 (x 3), 128.6 (x 3), 129.7, 137.1, 137.4, 137.8, 169.7, 170.2, 170.7, ppm; HR-FAB MS $[M+Na]^+$ calcd for $C_{41}H_{48}O_{13}SNa$ 803.2713, found 803.2722.

3-O-(2,4-di-O-benzoyl-α-L-rhamnnopyranosyl)-1,2,4,5-tetra-O-benzyl-D-ribitol (5.7) The title compound was obtained by Method B from **3.10** and **3.12** in 65% yield. Analytical data for **4.21**: R_f = 0.42 (ethyl acetate – hexanes, 3/7, v/v); $[\alpha]_D^{25}$ -8.3° (c = 1, $CHCl_3$); ^{1}H-n.m.r.: δ, 1.04 (d, 3H, H-6'), 3.56-3.70 (d, 4H, H-1, 5), 3.76-3.79 (m, 1H, H-2), 3.88-3.91 (m, 1H, H-4), 4.08-4.20 (m, 3H, H-3, 3', 5'), 4.37-4.59 (m, 8H, 4 x *CH*$_2$Ph), 5.05-5.16 (m, 2H, H-1', 4'), 5.27-5.29 (m, 1H, H-2'), 7.04-8.03 (m, 30H, aromatic) ppm; ^{13}C-n.m.r.: δ, 18.1, 67.4, 69.0, 69.5, 70.6, 70.7, 72.5, 72.6, 72.7, 73.7 (x 2), 73.9, 74.0, 76.1, 78.4, 78.6, 97.8, 128.0 (x 2), 128.1 (x 2), 128.2, 128.3 (x 2), 128.4(x 4), 128.5 (x 2), 128.8 (x 4), 128.9 (x 3), 129.0 (x 2), 129.1 (x 2), 130.0, 130.1, 130.4 (x 2), 130.5 (x 2), 138.8 (x 2), 138.9, 139.0, 166.5, 167.7 ppm; HR-FAB MS $[M+Na]^+$ calcd for $C_{53}H_{54}O_{11}Na$ 889.3564, found 889.3577.

O-(3,4,6-Tri-O-acetyl-2-O-benzyl-α-D-glucopyranosyl)-(1→3)-O-(2-O-benzyl-4,6-O-benzylidene-α-D-glucopyranosyl)-(1→3)-O-(2,4-di-O-benzoyl-α-L-

rhamnnopyranosyl)-(1→3)-1,2,4,5-tetra-O-benzyl-D-ribitol (5.8). The title compound was obtained by Method B from **5.6** and **5.7** in 66% yield. Analytical data for **5.8**: R_f = 0.41 (ethyl acetate - hexanes, 2/3, v/v); $[\alpha]_D^{25}$ +55.6° (c = 1.0, $CHCl_3$); ^{1}H-n.m.r.: δ, 1.09 (d, 3H, H-6'), 1.80, 1.94, 1.99 (3s, 3H, 3 x $COCH_3$), 3.24 (dd, $J_{2''',3'''}$ = 3.5 Hz, H-2'''), 3.45-3.59 (m, 4H, H-2, 5, 2''), 3.67-3.82 (m, 7H, H-1, 5'', 6a'', 6b'', 6a''', 6b'''), 3.87-3.90 (m, 1H, H-4''), 3.94-4.00 (m, 3H, H-3, 4, 3''), 4.11-4.13 (m, 1H, $J_{5''', 6a'''}$ = 10.4 Hz, H-5'''), 4.24-4.31 (m, 3H, H-5',CH_2Ph), 4.35-4.40 (m, 1H, H-3'), 4.45-4.74 (m, 11H, H-4''', 5 x CH_2Ph), 5.13 (d, 1H, $J_{1'',2''}$ = 3.4 Hz, H-1''), 5.21-5.27 (m, 4H, H-1', 1''', 3''', C*H*Ph), 5.58-5.64 (m, 2H, H-2',4'), 6.70-8.12 (m, 45H, aromatic) ppm; ^{13}C-n.m.r.: δ, 17.8, 20.7, 20.9, 21.0, 29.9, 61.3, 62.6, 66.9, 67.8, 67.9, 68.9, 69.3, 70.3, 70.6, 71.6, 72.3 (x 2), 72.7, 73.4 (x 2), 75.2, 78.1, 82.5, 94.6, 95.5, 97.8, 102.2, 126.8 (x 3), 127.4 (x 3), 127.7 (x 8), 127.9 (x 3), 128.0 (x 4), 128.1 (x 8), 128.3 (x 5), 128.5 (x 8), 128.8 (x 3), 129.5, 129.6, 129.8, 129.9 (x 2), 130.2 (x 2), 133.4, 133.7, 137.2, 137.3, 137.5, 138.5 (x 2), 138.6, 163.3, 165.7, 166.1, 169.8, 170.2, 170.8 ppm; HR-FAB MS $[M+Na]^+$ calcd for $C_{92}H_{96}O_{24}Na$ 1607.6189, found 1607.6172.

O-(3,4,6-Tri-O-acetyl-2-O-benzyl-α-D-glucopyranosyl)-(1→3)-O-(2-O-benzyl-4,6-O-benzylidene-α-D-glucopyranosyl)-(1→3)-O-(2,4-di-O-benzoyl-α-L-rhamnnopyranosyl)-(1→3)-1-O-(4-azidobutyl)-2,4,5-tri-O-benzyl-D-ribitol (5.9). The title compound was obtained by Method B from **5.6** and **4.21** in 74% yield. Analytical data for **5.9**: R_f = 0.41 (ethyl acetate - hexanes, 2/3, v/v); $[\alpha]_D^{25}$ +55.6° (c = 1.0, $CHCl_3$); ^{1}H-n.m.r.: δ, 1.22 (d, 3H, H-6'), 1.64-1.68 (m, 4H, 2 x CH_2sp), 1.82, 1.96, 2.01 (3s, 3H, 3 x $COCH_3$), 3.24-3.28 (m, 3H, H-2''', CH_2sp), 3.40-3.45 (m, 2H, CH_2sp), 3.48-3.62 (m,

4H, H-2, 5, 2''), 3.70-3.85 (m, 7H, H-1, 5'', 6a'', 6b'', 6a''', 6b'''), 3.91-3.95 (m, 2H, H-4, 4''), 3.98-4.03 (m, 2H, H-3, 3''), 4.11-4.14 (m, 1H, H-5'''), 4.25-4.33 (m, 2H, H-5', ½ C*H*$_2$Ph), 4.38-4.43 (m, 2H, H-3', ½ C*H*$_2$Ph), 4.50-4.54 (m, 2H, C*H*$_2$Ph), 4.68-4.78 (m, 7H, H-4''', 3 x C*H*$_2$Ph), 5.16 (d, 1H, $J_{1'',2''}$ = 3.5 Hz, H-1''), 5.23-5.30 (m, 4H, H-1', 1''', 3''', C*H*Ph), 5.64-5.68 (m, 2H, H-2',4'), 6.75-8.16 (m, 45H, aromatic) ppm; ^{13}C-n.m.r.: δ, 17.8, 20.7, 20.9, 21.0, 26.0, 27.1, 51.4, 61.2, 62.6, 66.9, 68.8, 68.9, 68.9, 69.1, 70.6, 70.8, 70.9, 71.6, 72.1, 72.2, 72.4, 72.7, 73.4, 75.2, 77.9, 82.4, 94.5, 95.5, 97.8, 102.2, 126.8 (x 2), 127.4 (x 3), 127.7 (x 2), 127.8 (x 5), 127.9 (x 3), 128.0 (x 5), 128.1 (x 5), 128.3 (x 5), 128.5 (x 5), 128.8 (x 3), 129.5, 129.6, 129.7, 129.9 (x 2), 130.2 (x 2), 133.4, 133.7, 137.1, 137.3, 137.5, 138.4 (x 2), 138.5, 165.7, 166.1, 169.9, 170.2, 170.8 ppm; HR-FAB MS $[M+Na]^+$ calcd for $C_{89}H_{97}N_3O_{24}Na$ 1614.6360 found 1614.6372.

O-(α-D-Glucopyranosyl)-(1→3)-O-(α-D-glucopyranosyl)-(1→3)-O-(α-L-rhamnopyranosyl)-(1→3)-1-O-(4-aminobutyl)-D-ribitol (5.2). To a solution of **5.9** (60 mg, 0.06 mmol) in dry methanol (1.0 mL) was added 1M NaOMe till pH = 9 (~0.1 mL). The reaction mixture was stirred for 15 h at rt, then neutralized with Dowex (H^+), filtered, and concentrated in *vacuo*. The crude residue was dissolved in a mixture of ethanol/HCl (12/0.03, v/v) and 10% Pd/C (70 mg) was added. The reaction mixture was stirred under an atmosphere of H_2 for 8 h. When TLC showed the formation of ninhydrin positive spot on the baseline, the catalyst was filtered off and the filtrate was neutralized with Dowex (OH^-) resin and concentrated under reduced pressure. The residue was co-evaporated with water (2 x 2 mL) and then purified by column chromatography on Sephadex G-15 (water elution) to afford compound **4.5** as a film (72% yield). Analytical

data for **5.2**: $[\alpha]_D^{27}$ +69.4° (c = 1.0, H_2O); ^{1}H-n.m.r. (D_2O).: δ, 1.35 (d, 3H, H-6'), 1.73-1.83 (m, 4H, 2 x CH_2sp), 3.08-3.10 (m, 2H, CH_2sp), 3.51 (dd, 1H, $J_{4''',5'''}$ = 9.9 Hz, H-4'''), 3.60-3.68 (m, 5H, H-4', 5'', 2''', CH_2sp), 3.71-3.93 (m, 14H, H-1, 2, 5, 3', 5', 2'', 5'', 3''', 6a'', 6b'', 6a''', 6b'''), 3.95-4.02 (m, 2H, H-4, 4''), 4.06-4.12 (m, 3H, H-3, 3'', 5'''), 4.24-4.27 (m, 1H, H-2'), 5.05 (d, 1H, $J_{1',2'}$ = 1.7 Hz, H-1'), 5.15 (d, 1H, $J_{1'',2''}$ = 3.6 Hz, H-1''), 5.41 (d, 1H, $J_{1''',2'''}$ = 3.7 Hz, H-1''') ppm; ^{13}C-n.m.r. (D_2O).: δ, 17.1, 24.1, 26.1, 39.7, 60.5, 60.6, 62.7, 67.2, 69.7 (x 2), 69.8, 70.2 (x 2), 70.4 (x 2), 70.7, 71.1, 71.8, 72.1 (x 2), 73.2, 75.6, 80.1, 80.4, 95.8, 99.6, 100.5 ppm; HR-FAB MS $[M+H]^+$ calcd for $C_{27}H_{52}NO_{19}$ 694.3134 found 694.3141.

5.5 References

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CHAPTER 6

Conjugation of the spacer containing serogroup 6 pneumococcal oligosaccharides with Bovine Serum Albumin

6 Synthesis of glycoconjugates of serogroup 6

6.1 Introduction

Early in the last century it was discovered that non-immunogenic, low-molecular weight saccharides, known as haptens, which mimic determinants of bacterial polysaccharides, can be made immunogenic when linked to protein carriers.[1] Since then, this principle has been applied multiple times and a large number of neoglycoconjugates have been synthesized for use in experimental vaccines.[2,3]

One of the important characteristics of glycoconjugates is the number of oligosaccharide units incorporated in the carrier as a result of conjugation. There are many existing methods for conjugation of carbohydrates to proteins.[4,5] Chapter 1 (section 1.1) describes some of the existing methods for the synthesis of glycoconjugates.

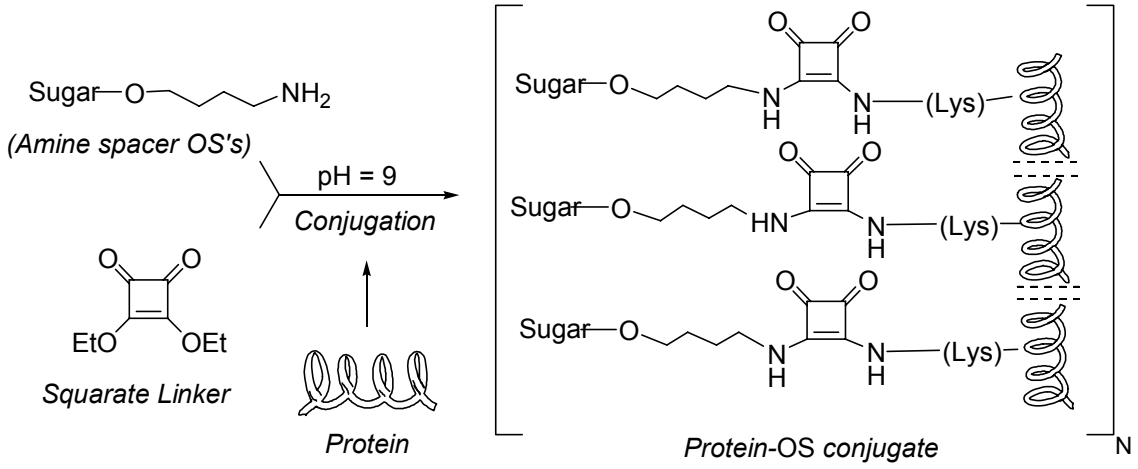


Figure 6.1 Glycoconjugation reaction using squarate linker

The method adopted for the synthesis of neoglycoconjugates of SPn6 oligosaccharides was the method originally described by Tietze, which is based on the selective reaction of

amines with squaric acid diesters.[6] The first ester function of the squaric acid diester reacts at neutral pH, to form a monoamide between the ligand and the ester. The second ester group can subsequently be coupled to another amino group in basic medium via controlled formation of 1,2- bisamide of the squaric acid. This allows for subsequent coupling of two different amine components to obtain a distinct conjugate without the formation of mixtures (Figure 6.1).

Further, the method is simple, it makes use of relatively inexpensive, commercially available reagents, it has gained popularity in synthetic vaccine development because it allows preparation of neoglycoconjugates with a wide range of hapten:carrier ratios.[7,2,8] The main advantage of this method is that it does not result in cross linked poorly defined lattice type products and that some of the excess hapten used at the onset of the reaction can be recovered.

6.2 Results & Discussion

The spacer containing oligosaccharides (**4.1-4.6** and **5.2,** Figure 6.2) were synthesized as described in Chapters 4 and 5. The free amino group present in the oligosaccharides **4.1-4.6** and **5.2** was subjected to elongation by the reaction of 3,4-diethoxy-3-cyclobutene-1,2-dione (squaric acid diethyl ester) in 50 mM potassium phosphate buffer (pH 7). When TLC indicated completion of the reaction (12 h), the reaction mixtures were purified by reverse phase chromatography to obtain the hapten-squarate adducts (**6.1-6.7**). The identity and purity of these compounds were confirmed by proton NMR and HRMS measurements.

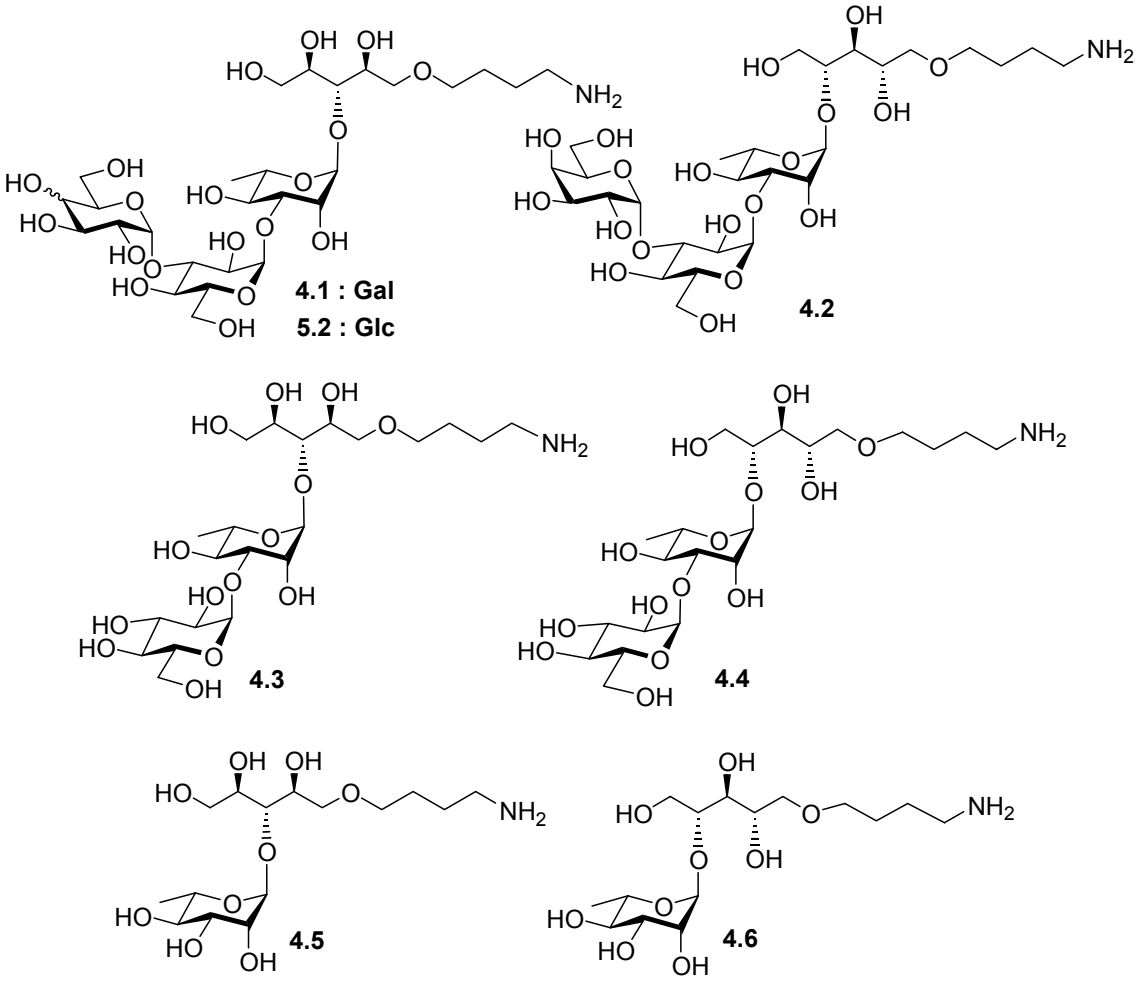
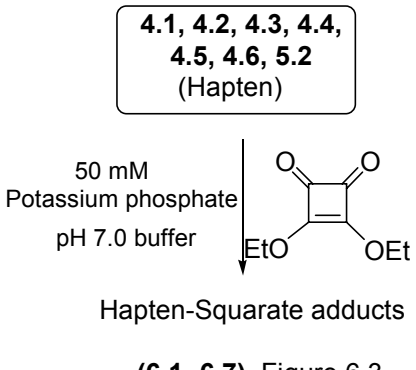


Figure 6.2 Spacer containing SPn6 oligosaccharides



Scheme 6.1 General protocol for preparation of serotype 6 squarates

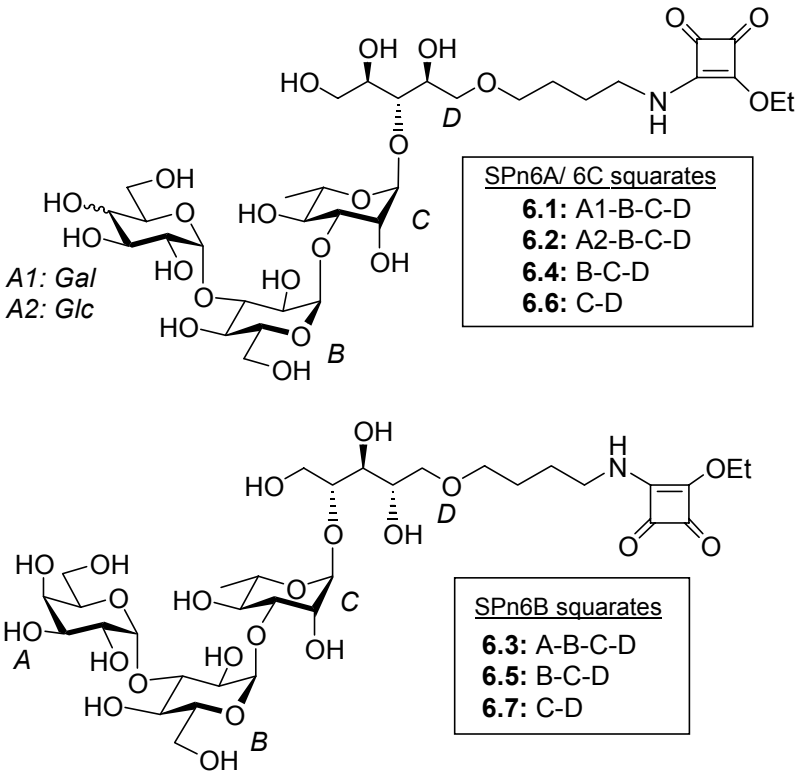
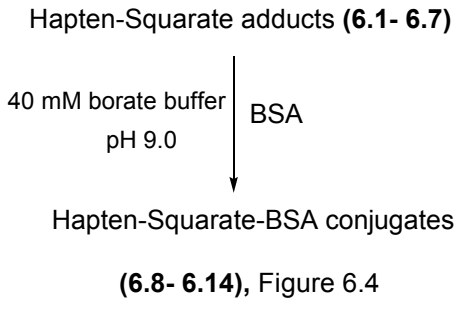


Figure 6.3 Squarates of SPn6A-6C

Glycoconjugates of the SPn6 series were prepared using a model carrier, bovine serum albumin (BSA, molecular mass 66430 Da).[9,10] BSA has 59 lysine residues[9,11] each bearing a terminal amino group of which only 30-35 have amino groups that are accessible for chemical coupling.[12] In general, BSA-neoglycoconjugates are ideal for inhibition studies or for binding to solid phases (such as agarose, ELISA plates or biosensor surfaces) useful for separation or analysis of proteins, bacteria or cells.

One of the important characteristics of glycoconjugates is the number of hapten units incorporated in the carrier protein as a result of conjugation.[10] This could in principle be determined by matrix-assisted laser desorption/ ionization-time of flight mass

spectrometry (MALDI-TOF MS). The quality of the MALDI-TOF spectra depends largely on the mode of sample preparation and the nature of the contaminants, hence this method is normally applied to characterize the mass of highly purified products. The utility of Surface Enhanced Laser Desorption Time of Flight mass spectrometry (SELDI-TOF) in combination with the protein chip system® was introduced by Kovac's group to monitor the conjugation of synthetic oligosaccharides to protein.[10] It combines solid-phase chromatography with the power of LDI-TOFMS analysis. The technique uses a variety of chemically active surfaces capable of retaining proteins or modified derivatives, depending upon their intrinsic properties.[13] After proteins have bound to the surface, contaminants may be washed out and the retained sample can be directly analyzed by the detector unit. An important point to note is that, when a conjugation reaction is monitored by SELDI-TOFMS, a portion of the reaction mixture can be withdrawn, and processed when the desired carbohydrate-protein ratio is reached, the rest of the material is allowed to react until the conjugate shows the next desired predetermined molecular weight. The obtained squarate adducts (**6.1**-**6.7**, Figure 6.3) were subjected to a conjugation reaction using BSA in pH 9 borate buffer (Scheme 6.2) and the progress of the reaction was monitored by SELDI-TOFMS



Scheme 6.2 General protocol for preparation of serotype 6 conjugates

It is well known that the number of hapten units incorporated into the protein is one of the most important factors for inducing antibodies. Since these conjugates were prepared with the objective to be tested for immunogenic activity, the ability to prepare these neoglycoconjugates with narrow, well-defined hapten-squarate: protein ratios was important. For preliminary studies, our target was to obtain the conjugates to have a hapten-squarate / BSA ratio between 5 and 10. Hence, the reactions were set up so that at the onset of the reaction, the molar **6.1**-**6.7**/ BSA ratio was 6:1 (see Experimental Part-Table 6.2).

From a chemical perspective, it should make no difference whether the conjugation involves a larger or smaller linker-equipped carbohydrate. It has been observed that squaric acid derivatives made from monosaccharides are generally more reactive than those made from larger oligosaccharides.[13] In order for a derivatized hapten to react with the carrier protein, these larger molecules have to penetrate deeper into the 3-dimensional structure of the protein, where the individual ε-amino groups present in the lysine residues all are not equally accessible.[7] Therefore, with all other reaction parameters equal, the reaction time required to achieve a certain loading with mono- or disaccharide derivative is generally shorter than the case with tetra- or higher oligosaccharides.[13] Also, increasing the concentration of the hapten increases the rate and efficiency of conjugations by squaric acid chemistry.[11] For example, to obtain the tetrasaccharide conjugate **6.9**, loading of 3 was observed within 7 h of stirring and did not change until 25 h (see Table 6.2), in which case the excess hapten-squarate (sq) **6.2** had to be added to the reaction. Target loading of 5 was then observed after 62 h of adding excess starting material. Longer reaction times and lower conjugation efficiency were more prominent in

case of the tri- and tetrasaccharide conjugates (see Tables 6.1 and 6.2).

It is also proved that pH is a very important factor for squaric acid chemistry conjugations. pH 9 or slightly more basic has been generally considered optimal for these conjugations.[14,15] Hence salts obtained from pH 9 borate buffer was added in cases where longer reaction times was observed.

When the desired loading was achieved (between 4 to 8, see table 6.2), the conjugation reactions were terminated by neutralization first and then subjected to ultrafiltration; the retained material was lyophilized to obtain the pure hapten-squarate-BSA conjugates (**6.8-6.14,** Figure 6.4)

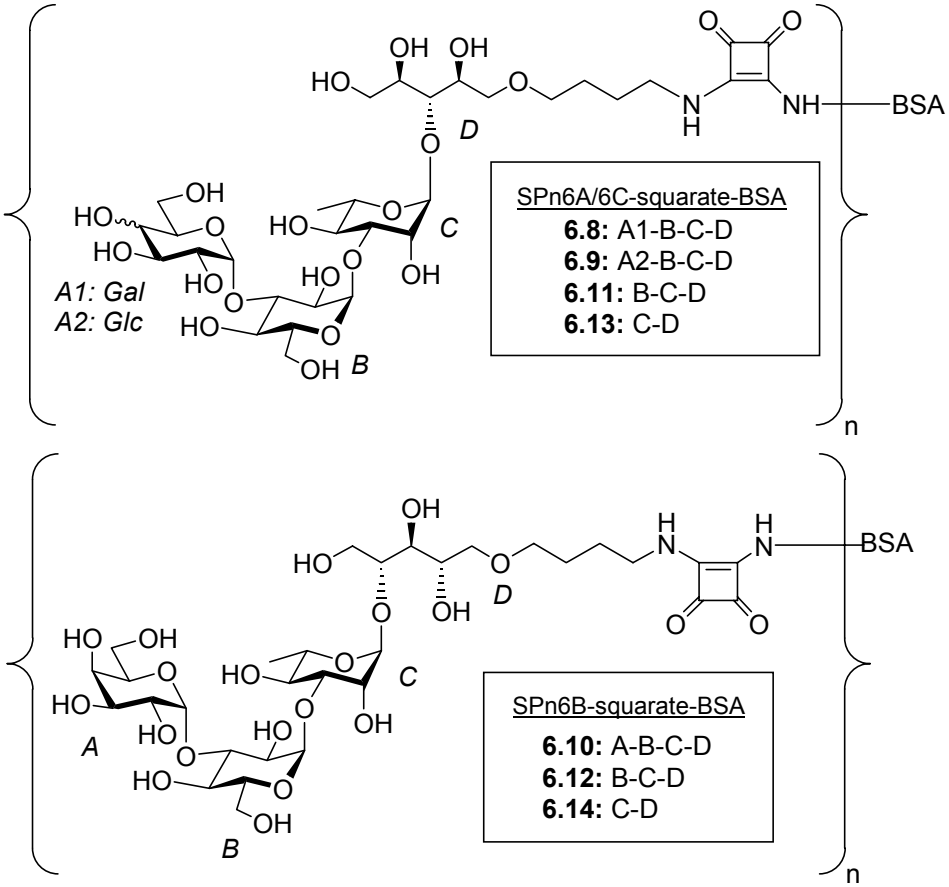


Figure 6.4 Conjugates of SPn6A- 6C

The final results of the conjugation reactions of SPn6 series with BSA have been

summarized in Table 6.1

Table 6.1 Data Sheet of SPn6 Conjugates

Hapten-sq / Conjugate	Ratio (hapten-sq:BSA)	Loading (Hapten-sq / BSA)	Conjugate Mol. Wt.	% yield	Conjugtn' efficiency
6.1 / 6.8	8 : 1	4.0	69527.50	55%	50%
6.2 / 6.9	12 : 1	5.2	70502.19	98%	42%
6.3 / 6.10	6 : 1	5.0	70344.94	93%	83%
6.4 / 6.11	6 : 1	5.0	69493.86	98%	83%
6.5 / 6.12	12 : 1	8.3	71534.90	96%	69%
6.6 / 6.13	10 : 1	7.3	69713.19	95%	75%
6.7 / 6.14	12 : 1	5.1	68734.70	84%	42%

6.3 Conclusions

In conclusion, a range of glycoconjugates of SPn6 series was obtained. The increasing mass of the glycoconjugate formation was monitored by SELDI-TOF mass spectrometry. The conjugates are being tested for immunological activity by our collaborator Dr. Moon Nahm and his coworkers at UAB (University of Alabama, Birmingham) medical school whose laboratory serves as the WHO reference laboratory for pneumococcal serology. Based on the results obtained from the immunological studies, we would be able to

identify the most active component towards the development of a fully synthetic pneumococcal conjugate vaccine.

6.4 Experimental part

6.4.1 General procedure for preparation of squarate intermediates (6.1-6.7)

Diethyl squarate (0.08 mmol) was added to a solution of the amine spacer containing oligosaccharides **4.1-4.6** and **5.2** (0.04 mmol) in a potassium phosphate pH 7.0 buffer (2 mL). The mixture was stirred overnight (15 h) at rt. When TLC on silica gel (4/3/3/2 $CHCl_3$/ MeOH/ AcOH/ H_2O, v/v/v/v) showed complete conversion to a faster moving squarate derivative, the reaction mixture was concentrated. Purification of these intermediates was carried out on strata- X reverse phase tubes from Phenomenex (33 μm polymeric reverse phase) which were conditioned by washing with MeOH (70 mL), followed by water (90 mL). Elution of the product was effected with water (3 x 10 mL), followed by stepwise gradient of MeOH in water (5%, 10 mL of eluent in each step). An aqueous solution of the pure material, thus obtained from purification, was filtered through a Sterile Millipore syringe driven filter unit (0.22 μM) and then lyophilized to give the pure white or pale yellow solids that was used for conjugation reactions.

6.4.2 General procedure for the preparation of neoglycoconjugates (6.8-6.14)

Squaric acid adducts **6.1-6.7,** (1.0 mg, 0.0018 mmol) was transferred into the glass reaction vessel containing BSA (20 mg, 0.0003 mmol) using pH 9, borate buffer (45 μL, 40 mmol). The reaction was gently stirred at rt and the progression of the conjugation was periodically monitored by SELDI-TOF MS. When the desired loading was achieved

(see Table 6.2), the reaction mixture was neutralized with pH 7 phosphate buffer. The resulting mixture was transferred into a centrifugal filter device (Amicon Ultra-15) which was subjected to centrifugation at 4000 r.p.m for 12 min. After that, the filter was again subjected to centrifugation with washings from aq. 10mM $(NH_4)_2CO_3$ (7 x 15 mL) to remove low molecular mass material. The retained material was then filtered through a Sterile Millipore syringe driven filter unit (0.22 μM) and then lyophilized to afford the conjugates as white solids in good yields.

6.4.3 General procedure for SELDI-TOFMS analysis

SELDI-TOF was measured using Bio-Rad Laboratories Inc. Protein Chip data Manager Software 3.0.7. The ProteinChip® arrays used were NP-20, Normal Phase protein chip aluminium strips that contain 8 active sites having silicon-oxide groups. Conjugation reactions were performed as described below:

6.4.3.1 Blank preparation

1 mg of BSA was diluted with water (6 μL) in a centrifuge tube (stock solution). A portion of this stock solution (2 μL) was applied on the Protein Chip®. The chip was air dried, washed twice with water (5 μL) with drying in between the washes. Finally saturated solution of sinapinic acid (2 μL, prepared by dissolving 15 mg of sinapinic acid in 1:1 mixture of acetonitrile and 1% TFA, centrifugating twice at 4000 r.p.m for 3 min) was applied and dried.

6.4.3.2 Sample preparation

A sample of the stirring crude reaction mixture (1 μL) was diluted with water (9 μL) in a

centrifuge tube. A portion of this solution (2 µL) was applied on the chip. The chip was air dried, washed with water (2 x 5 µL) with drying in between the washes. Finally saturated solution of sinapinic acid (2 µL) was applied and dried.

Table 6.2 Monitoring conjugation reactions of SPn6 Conjugates

Hapten-sq / Conjugate	Ratio (hapten:BSA)	Target loading (N)	Time (h)	Conjugate mass obs'd (SELDI-TOF)	Observed loading (N)
6.7 / 6.14	6:1 + 6:0	5	16 + 4	66860.43 68730.14	1.0 5.1
6.6 / 6.13	10:1	5	2.5	70056.96	8.0
6.5 / 6.12	6:1 + 6:0	5	7 + 25 + 62	68770.21 68771.30 70320.93	3.0 3.0 5.0
6.4 / 6.11	6:1	5	19	70436.35	6.5
6.3 / 6.10	6:1	5	19 + 5	68673.29 70325.94	2.9 5.0
6.2 / 6.9	6:1 + 6:0	5	7 + 25 + 62	68612.49 68857.41 71360.99	3.6 3.9 8.1
6.1 / 6.8	6:1 + 2:0	5	15 + 25 + 10	68708.06 69586.47 70393.60	3.2 4.2 5.1

6.5 References

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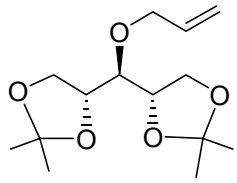
APPENDIX

(Selected NMR DATA/ SELDI-TOF MS)



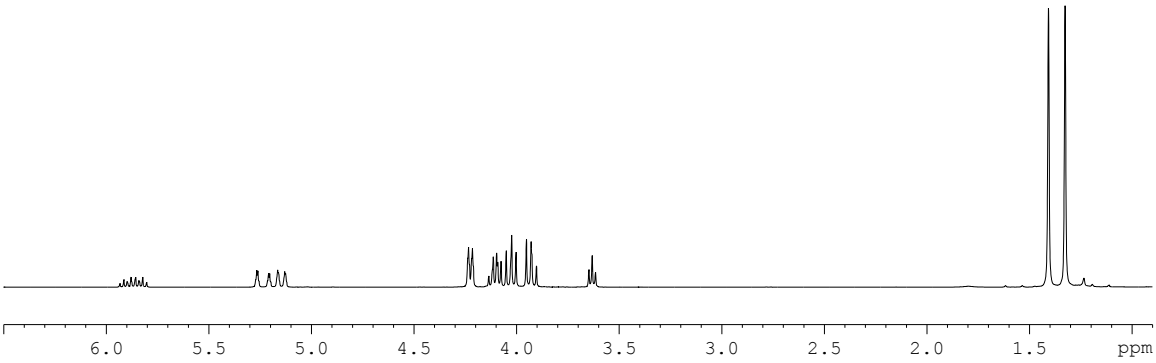


Figure A-1 ^{1}H NMR spectrum of 3-O-Allyl-1,2:4,5-di-O-isopropylidene-D/L-ribitol (**3.15**)

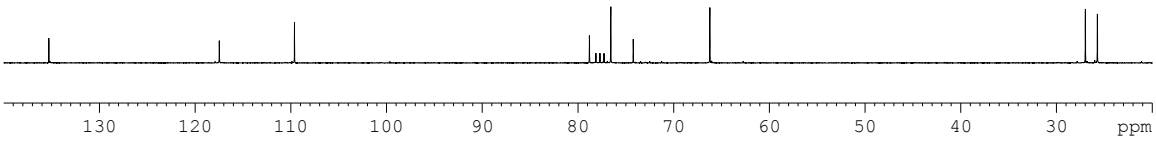
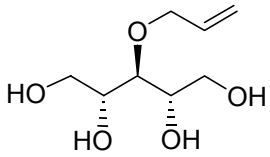


Figure A-2 ^{13}C $\{^{1}H\}$ NMR spectrum of 3-O-Allyl-1,2:4,5-di-O-isopropylidene-D/L-ribitol (**3.15**)



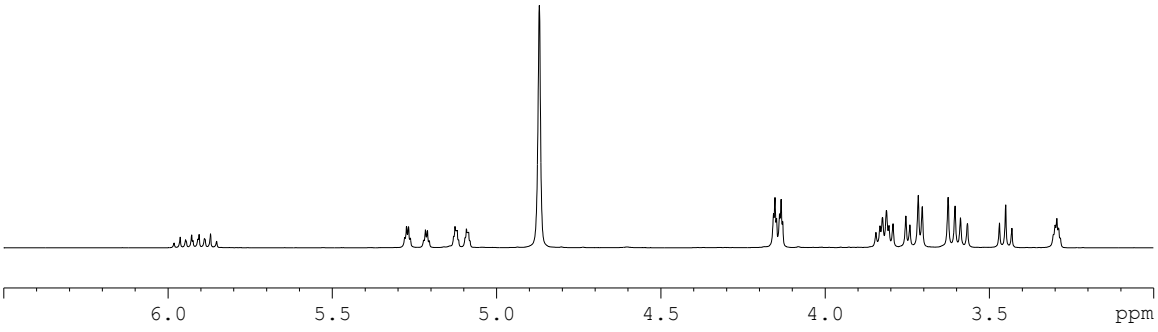


Figure A-3 ^{1}H NMR spectrum of 3-O-Allyl-D/L-ribitol (**3.16**)

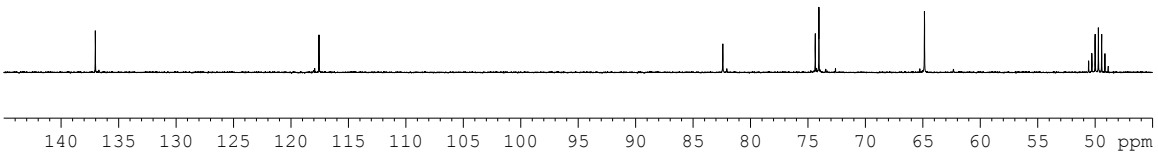
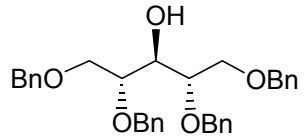


Figure A-4 ^{13}C {^{1}H} NMR spectrum of 3-O-Allyl-D/L- ribitol (**3.16**)



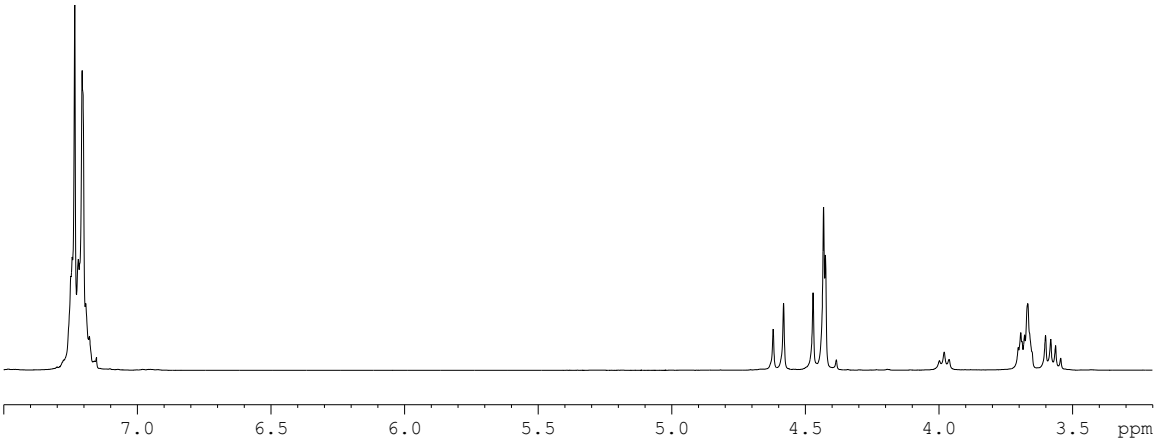


Figure A-5 ^{1}H NMR spectrum of 1, 2, 4, 5-tetra-O-benzyl- D/L- ribitol (**3.12**)

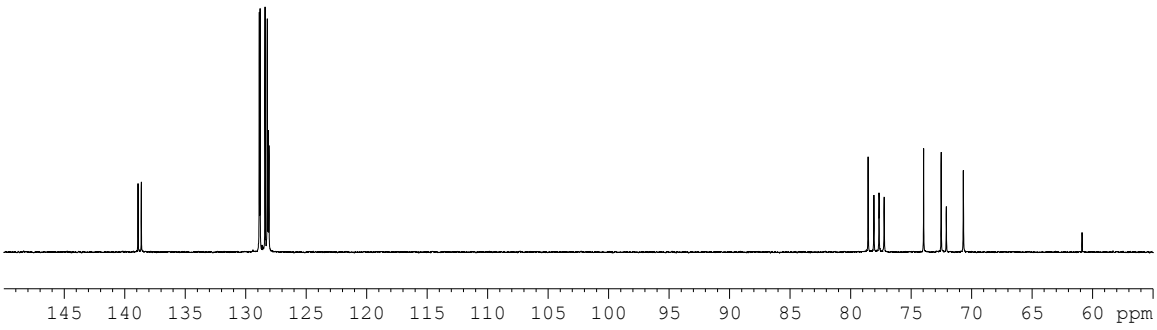
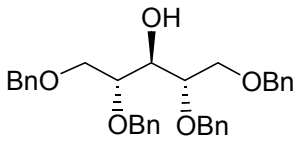


Figure A-6 ^{13}C {^{1}H} NMR spectrum of 1, 2, 4, 5-tetra-O-benzyl D/L- ribitol (**3.12**)



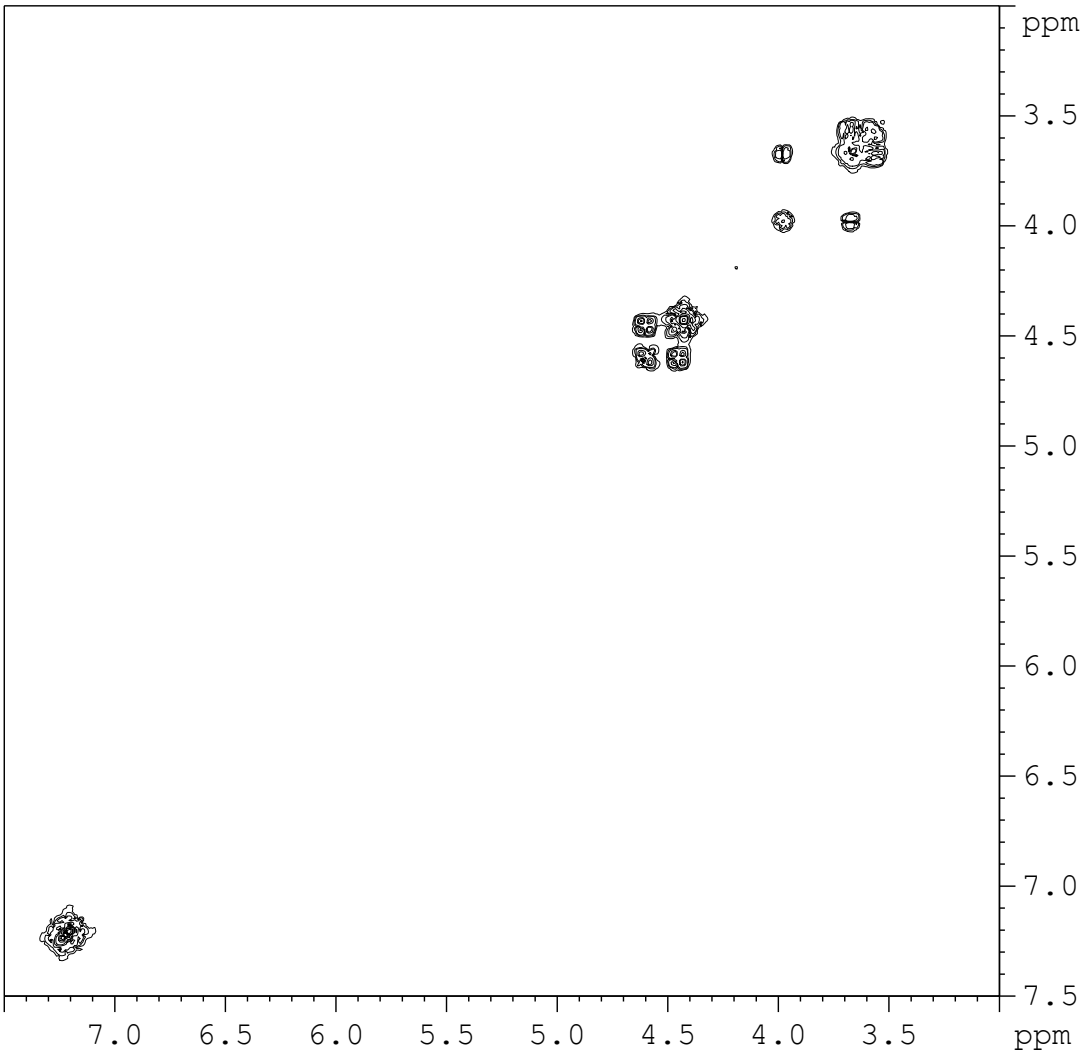


Figure A-7 2-D NMR: COSY spectrum of 1, 2, 4, 5-tetra-O-benzyl- D/L- ribitol (**3.12**)

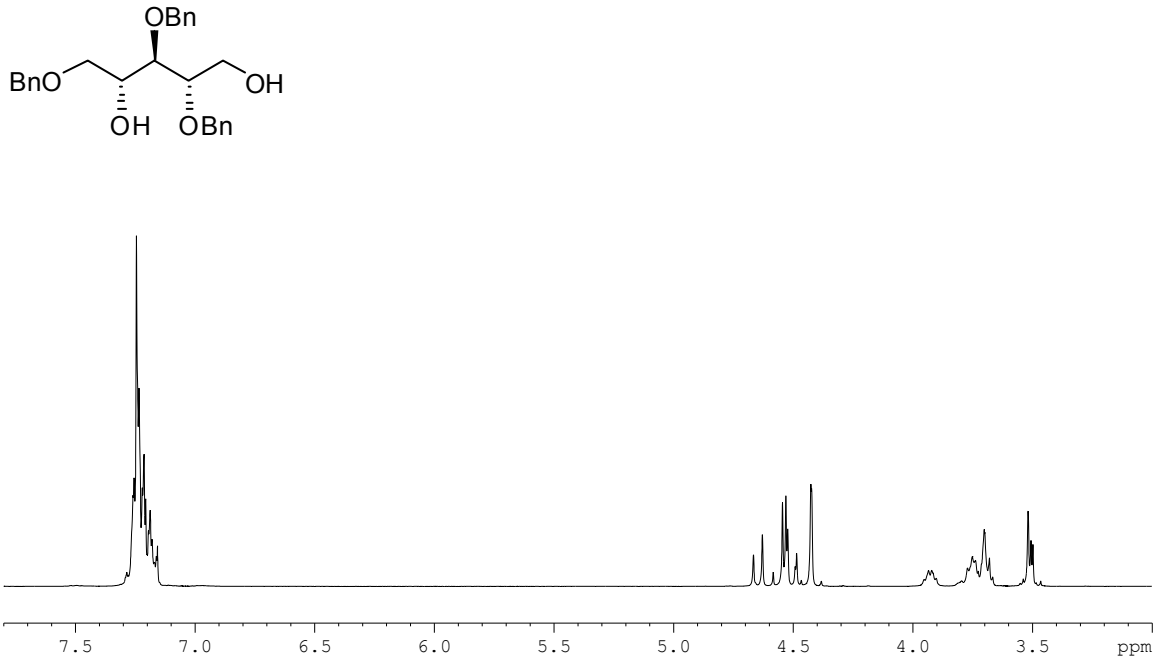


Figure A-8 ^{1}H NMR spectrum of 2, 3, 5-tri-O-benzyl-D- ribitol (**3.19**)

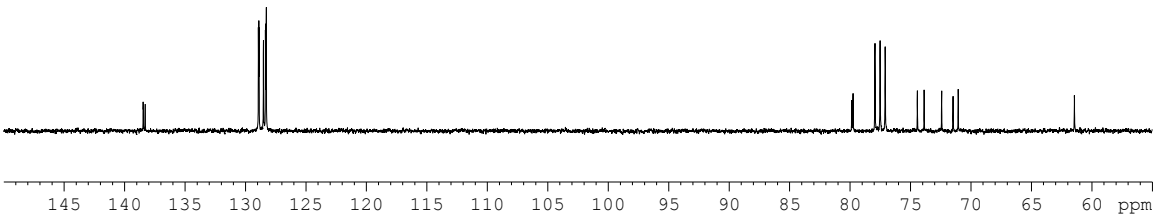


Figure A-9 ^{13}C {^{1}H} NMR spectrum of 2, 3, 5-tri-O-benzyl-D- ribitol (**3.19**)

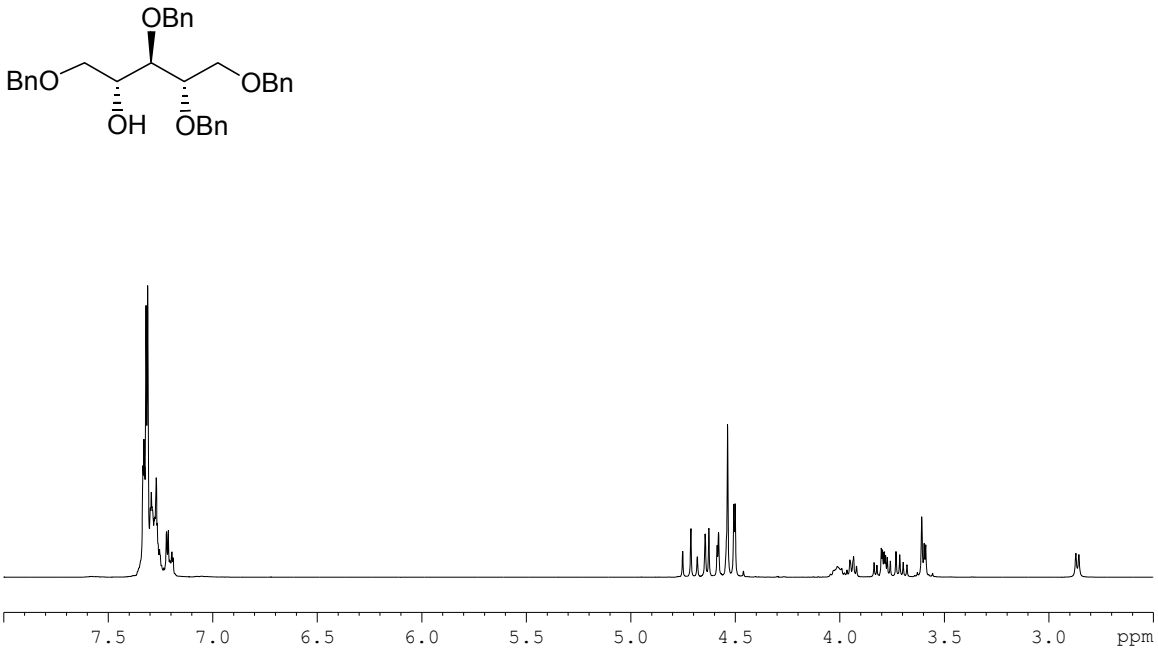


Figure A-10 ^{1}H NMR spectrum of 1, 2, 3, 5-tetra-O-benzyl-D- ribitol (**3.13**)

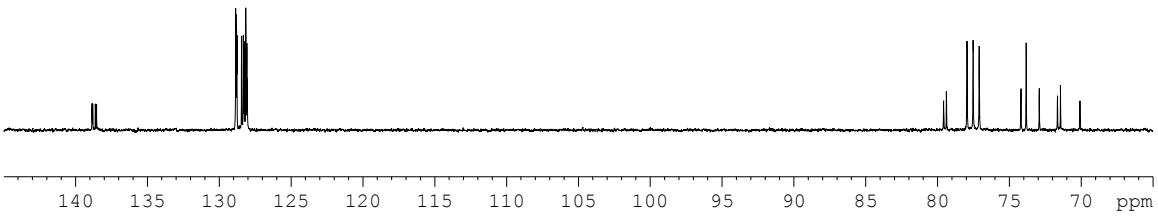
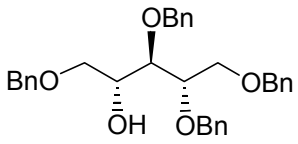


Figure A-11 ^{13}C {^{1}H} NMR spectrum of 1, 2, 3, 5-tetra-O-benzyl-D- ribitol (**3.13**)



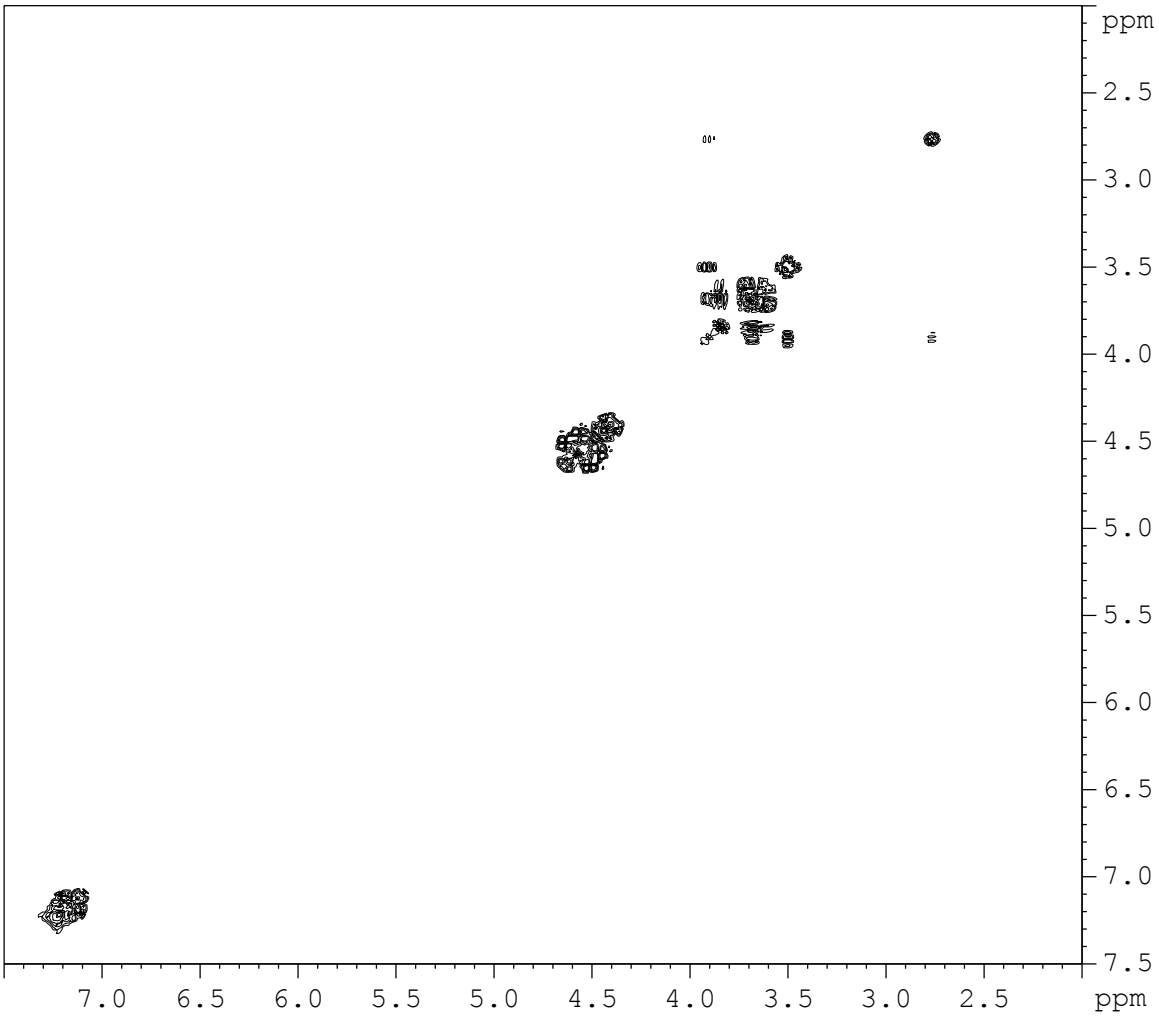
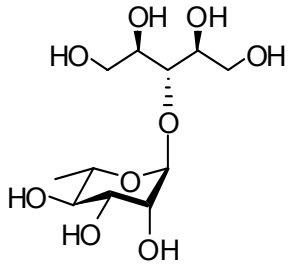


Figure A-12 2-D NMR: COSY spectrum of 1, 2, 3, 5-tetra-O-benzyl- D/L- ribitol (**3.13**)



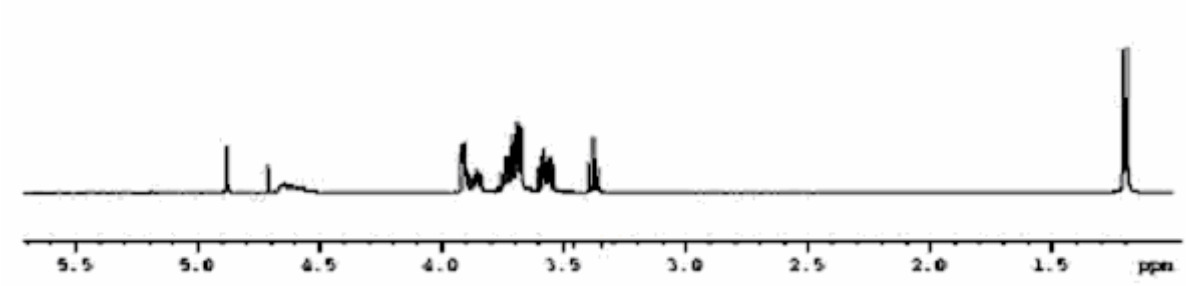


Figure A-13 ^{1}H NMR spectrum of O-(α-L-Rhamnopyranosyl)-(1→3)-D/L-ribitol (**3.3**)

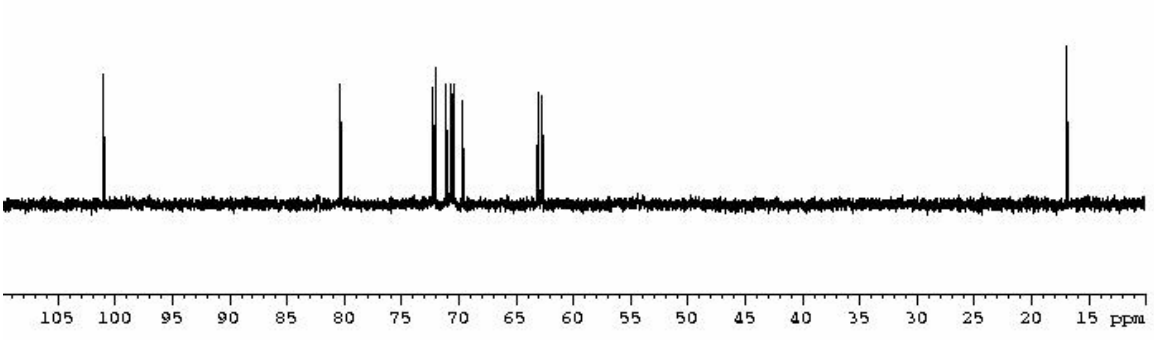
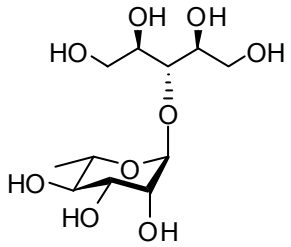


Figure A-14 ^{13}C {^{1}H} NMR spectrum of O-(α-L-Rhamnopyranosyl)-(1→3)-D/L-ribitol (**3.3**)



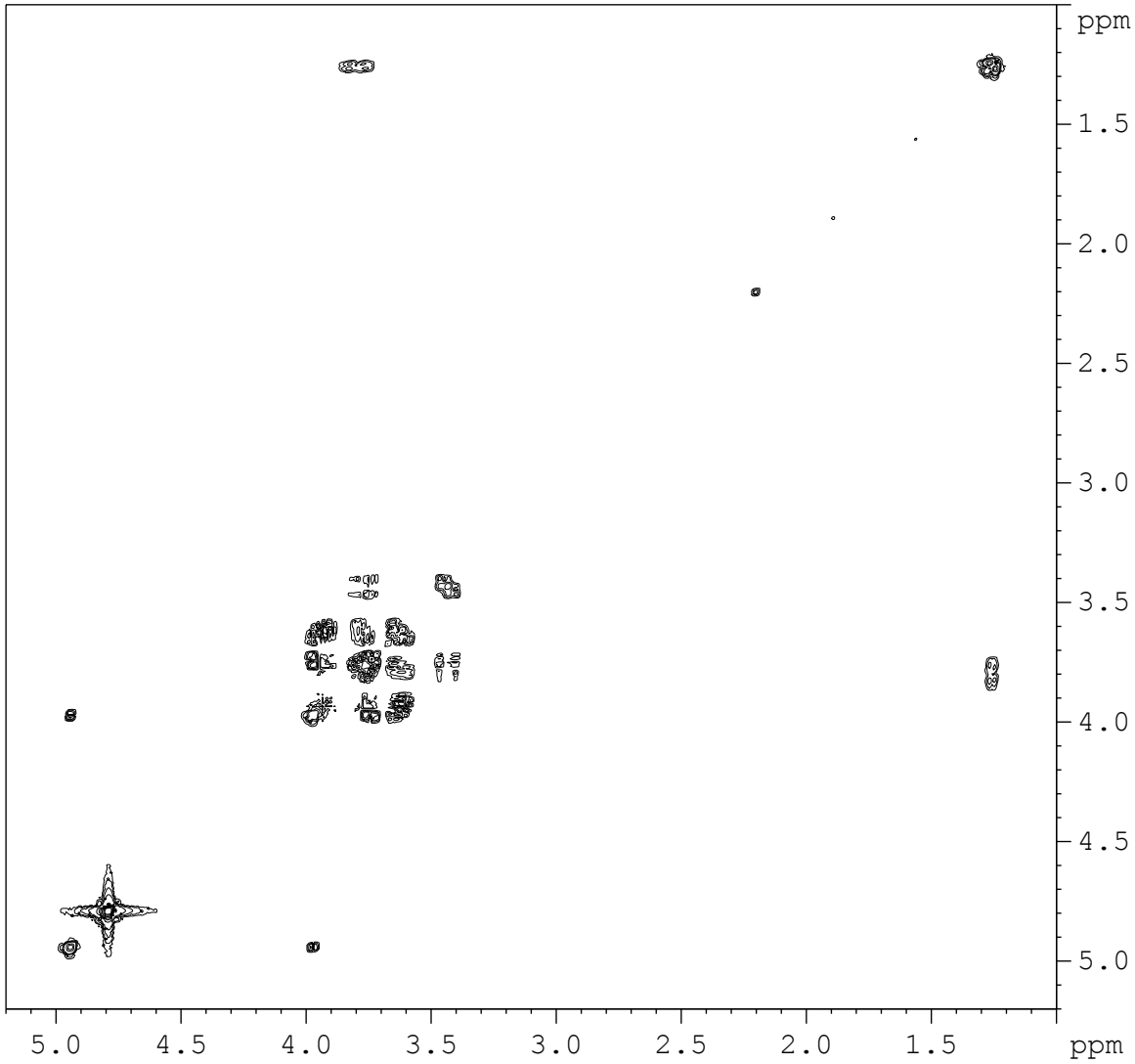
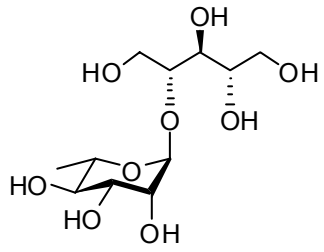


Figure A-15 2-D NMR: COSY spectrum of 1, 2, 3, 5-tetra-O-benzyl- D/L- ribitol (**3.3**)



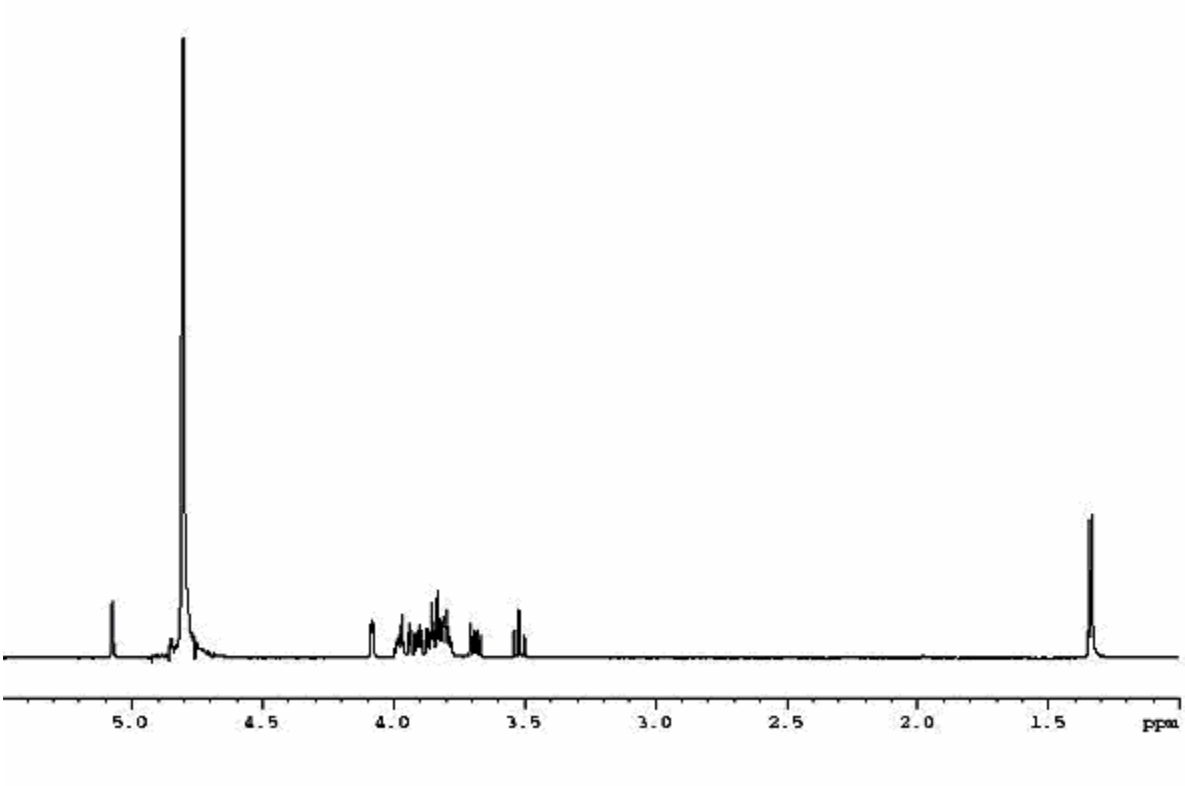


Figure A-16 ^{1}H NMR spectrum of O-(α-L-Rhamnopyranosyl)-(1→4)-D-ribitol (**3.4**)

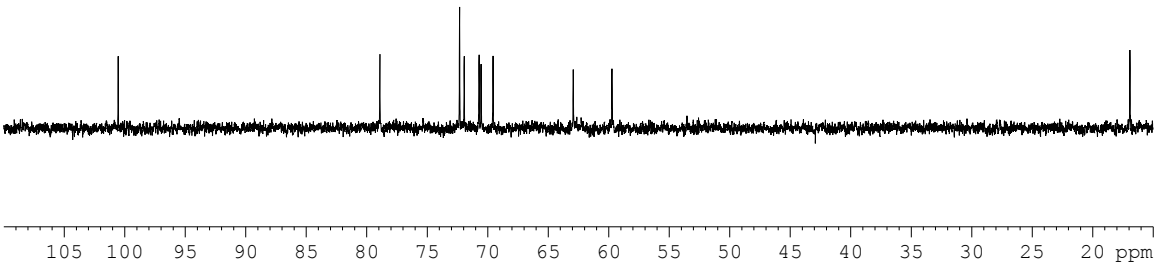
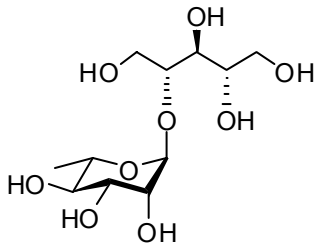


Figure A-17 ^{13}C {^{1}H} NMR spectrum of O-(α-L-Rhamnopyranosyl)-(1→4)-D-ribitol (**3.4**)



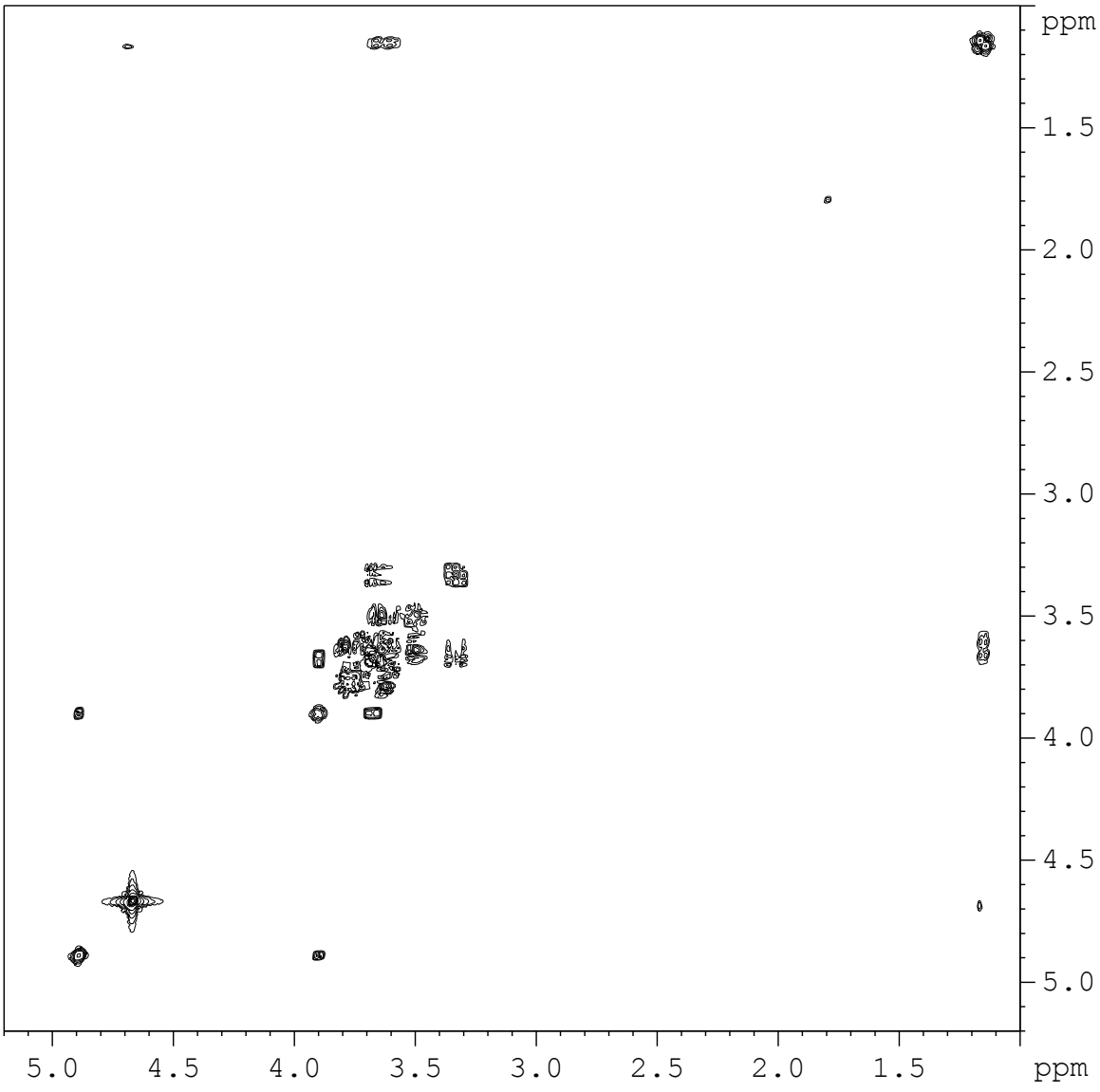
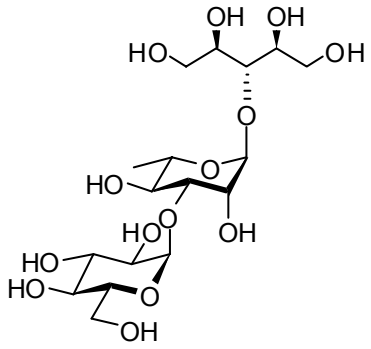


Figure A-18 2-D NMR: COSY spectrum of O-(α-L-Rhamnopyranosyl)-(1→4)-D-ribitol (**3.4**)



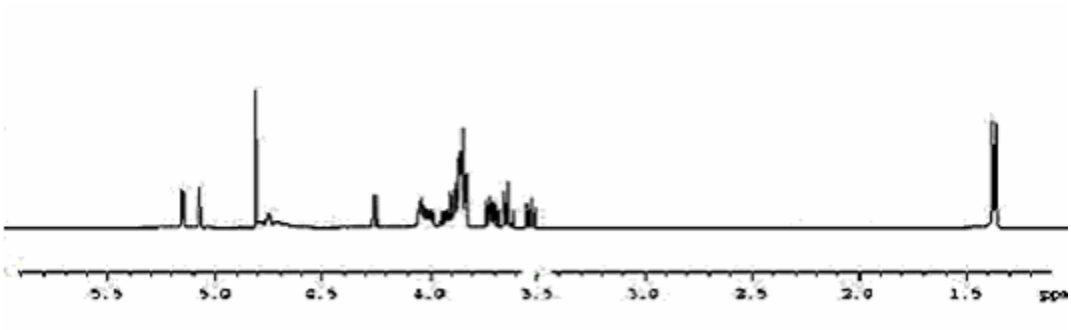


Figure A-19 ^{1}H NMR spectrum of O-(α-D-Glucopyranosyl)-(1→3)-O-(α-L-rhamnopyranosyl)-(1→3)-D/L-ribitol **(3.5)**

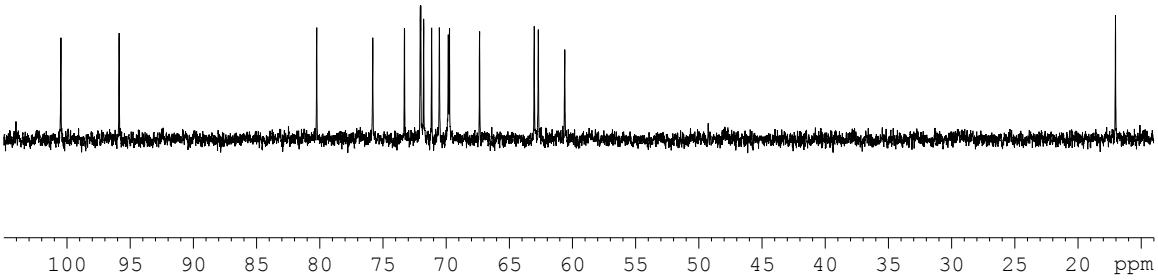
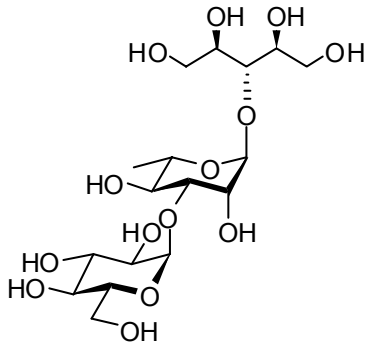


Figure A-20 $^{13}C\{^{1}H\}$ NMR spectrum of O-(α-D-Glucopyranosyl)-(1→3)-O-(α-L-rhamnopyranosyl)-(1→3)-D/L-ribitol **(3.5)**



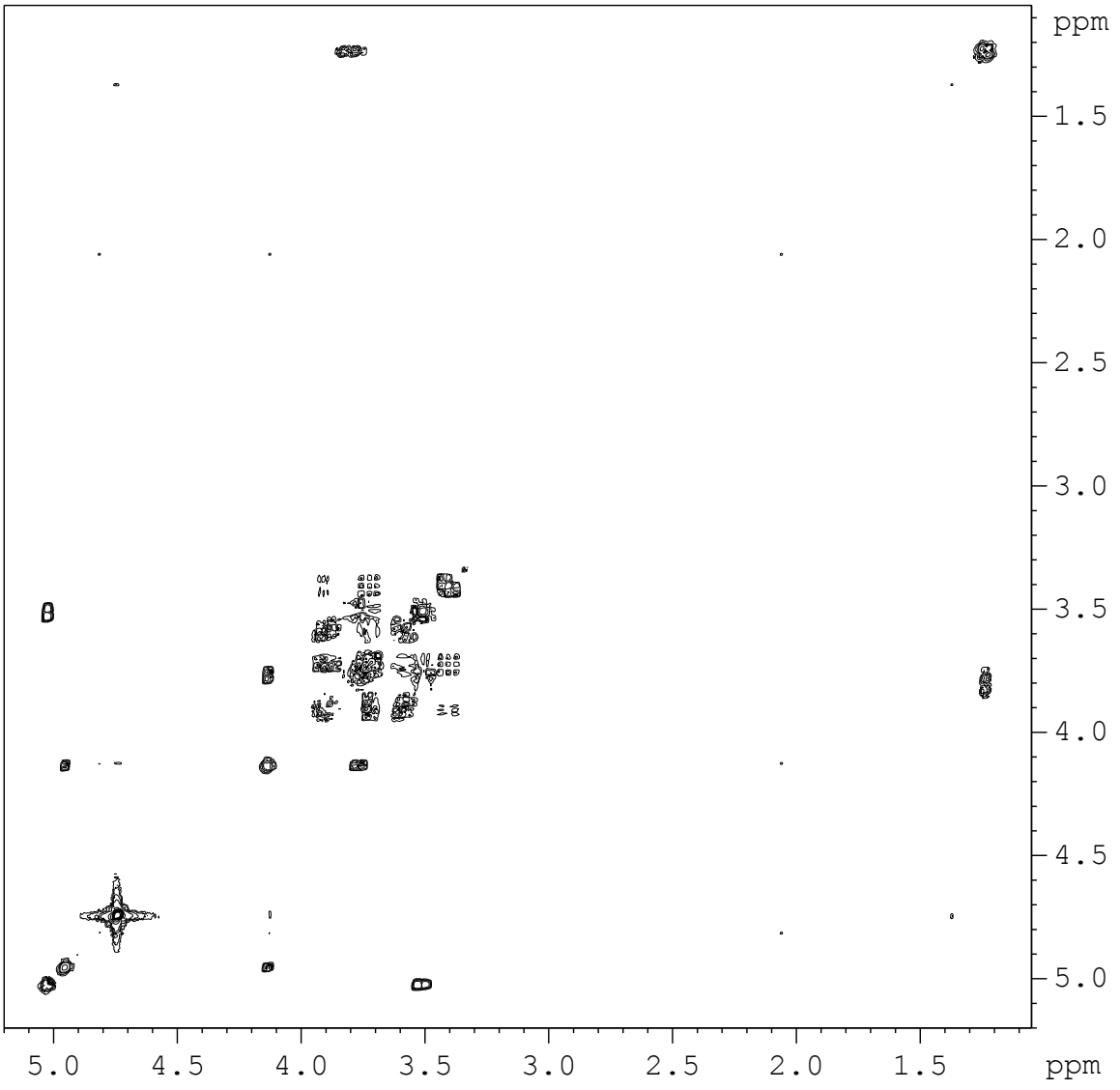


Figure A-21 2-D NMR: COSY spectrum of O-(α-D-Glucopyranosyl)-(1→3)-O-(α-L-rhamnopyranosyl)-(1→3)-D/L-ribitol **(3.5)**

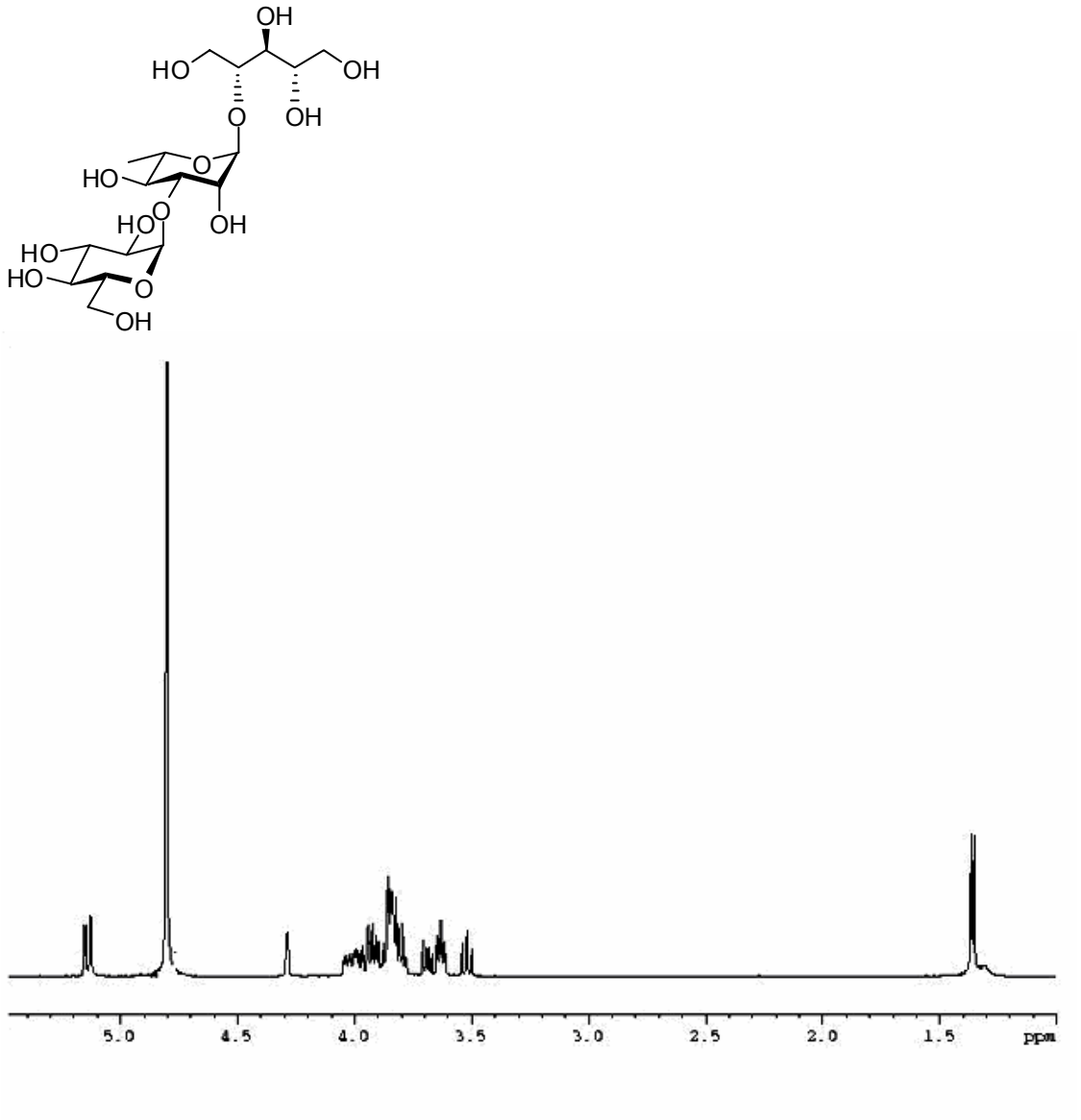


Figure A-22 ^{1}H NMR spectrum of O-(α-D-Glucopyranosyl)-(1→3)-O-(α-L-rhamnopyranosyl)-(1→4)-D-ribitol **(3.6)**

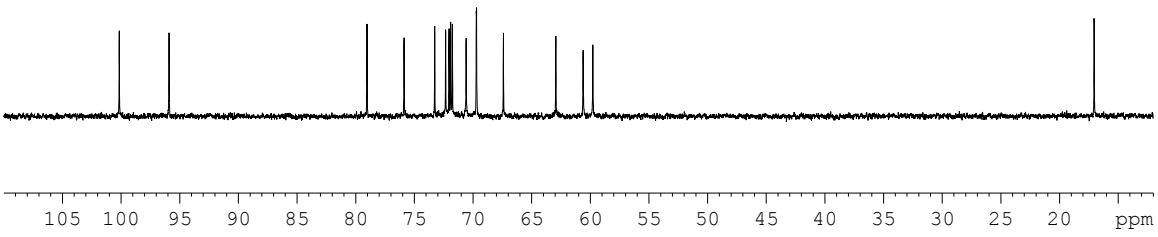
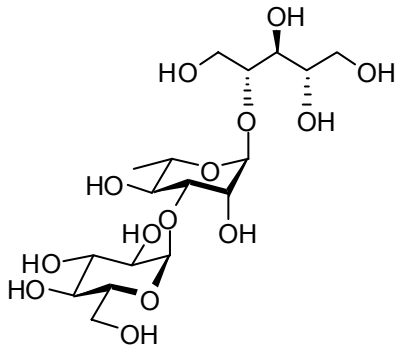


Figure A-23 $^{13}C\{^{1}H\}$ NMR spectrum of O-(α-D-Glucopyranosyl)-(1→3)-O-(α-L-rhamnopyranosyl)-(1→4)-D-ribitol **(3.6)**



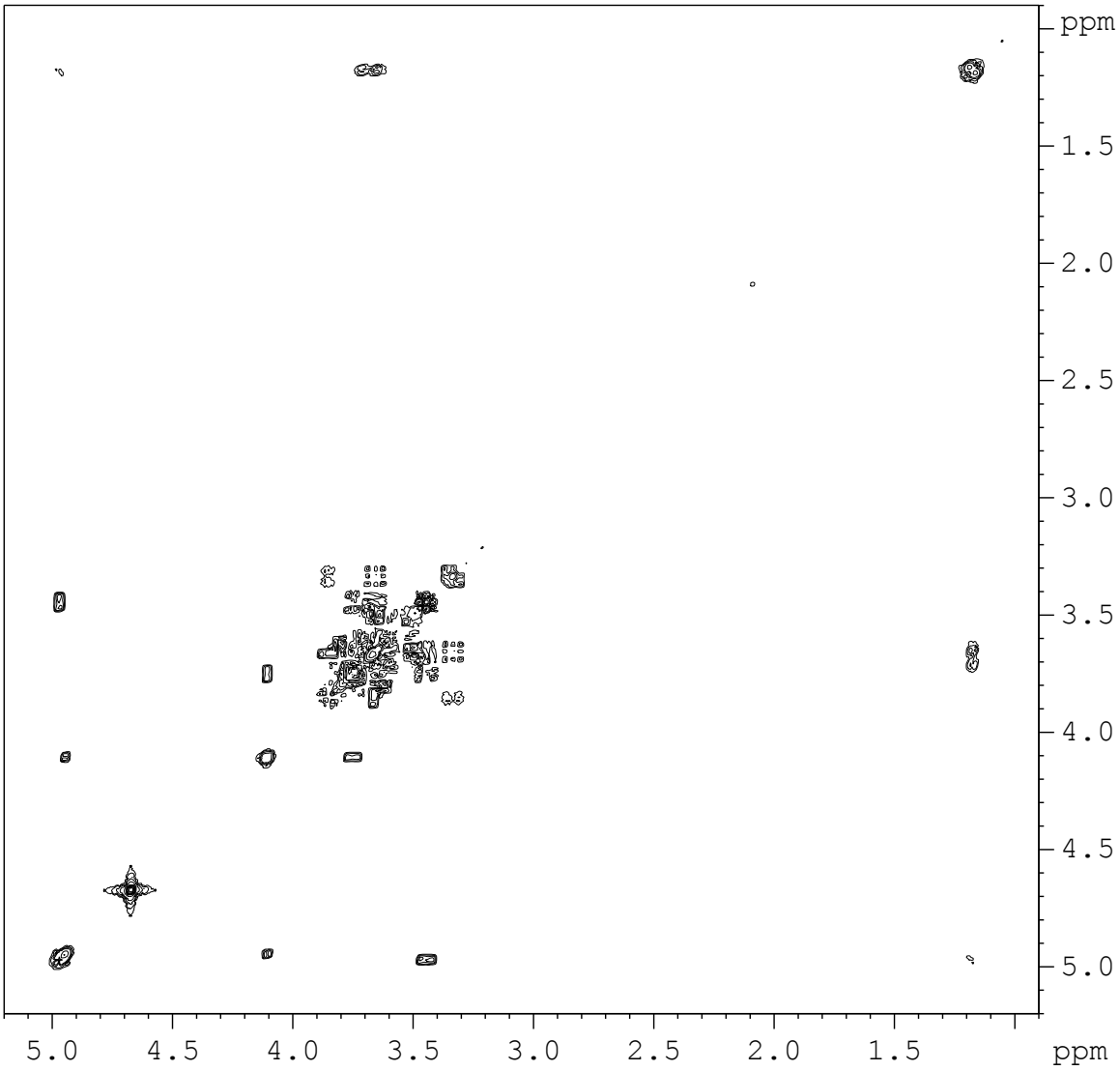
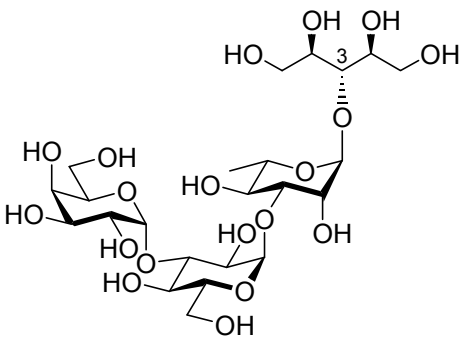


Figure A-24 2-D NMR: COSY spectrum of O-(α-D-Glucopyranosyl)-(1→3)-O-(α-L-rhamnopyranosyl)-(1→4)-D -ribitol **(3.6)**



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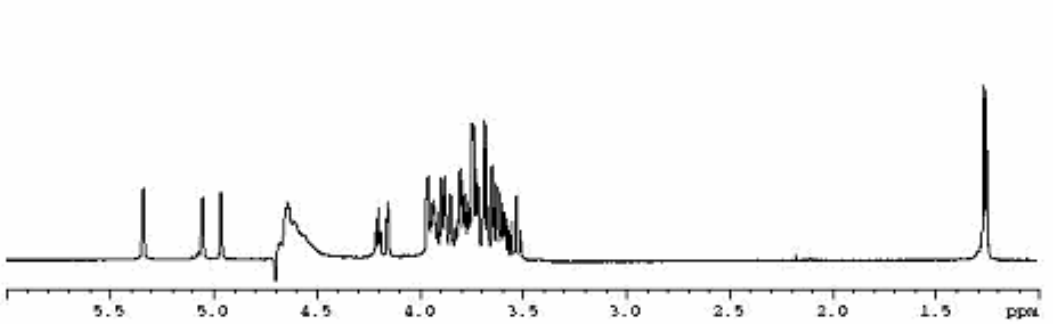


Figure A-25 ^{1}H NMR spectrum of O-(α-D-Galactopyranosyl)-(1→3)-O-(α-D-Glucopyranosyl)-(1→3)-O-(α-L-rhamnopyranosyl)-(1→3)-D/L-ribitol **(3.1)**

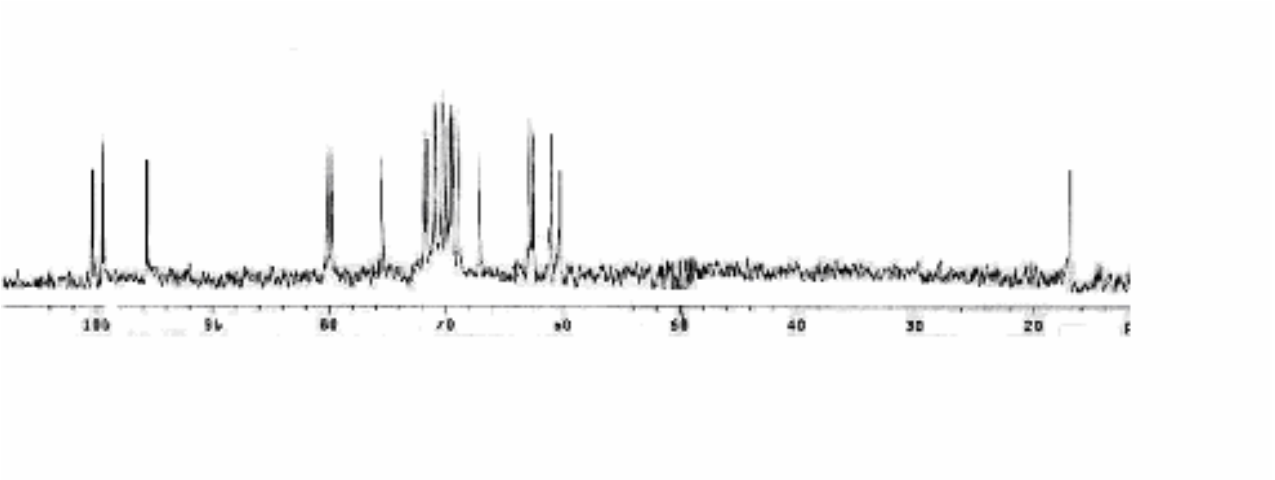
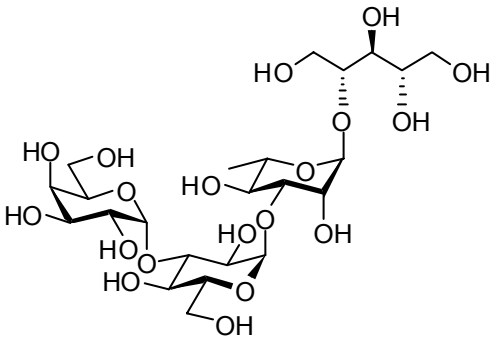


Figure A-26 ^{13}C{^{1}H} NMR spectrum of O-(α-D-Galactopyranosyl))-(1→3)-O-(α-D-Glucopyranosyl)-(1→3)-O-(α-L-rhamnopyranosyl)-(1→3)-D/L-ribitol **(3.1)**



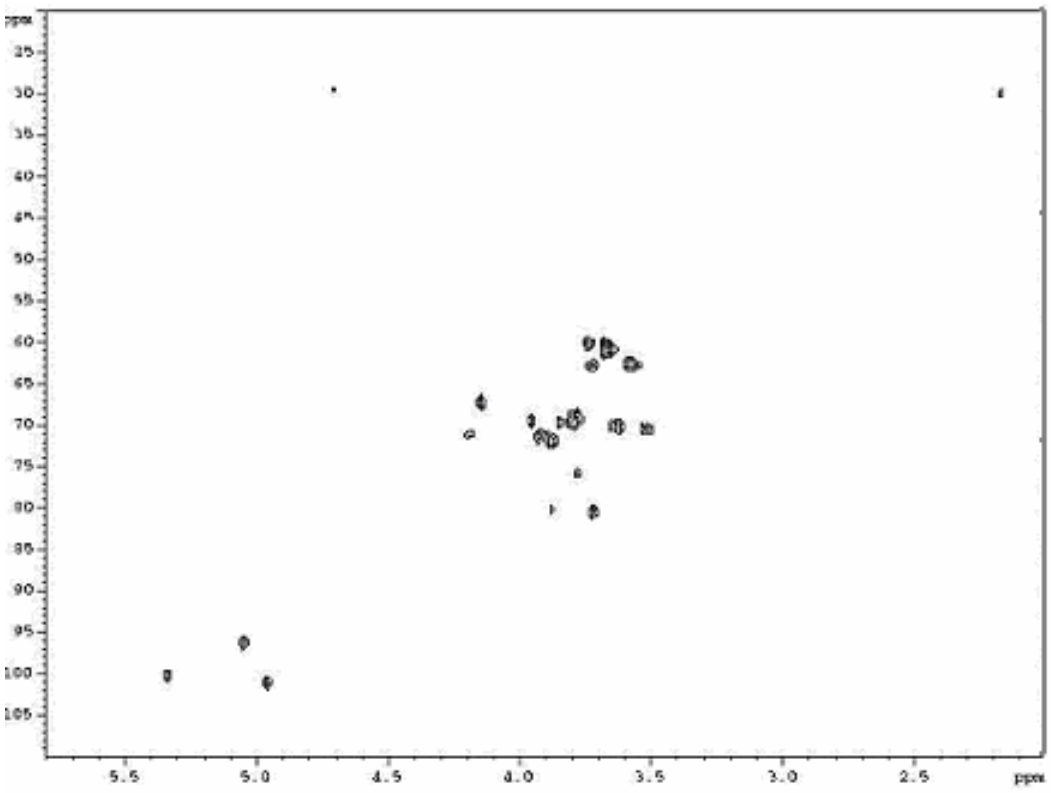


Figure A-27 2-D NMR: HMQC spectrum of O-(α-D-Galactopyranosyl)-(1→3)-O-(α-D-Glucopyranosyl)-(1→3)-O-(α-L-rhamnopyranosyl)-(1→3)-D/L-ribitol **(3.1)**

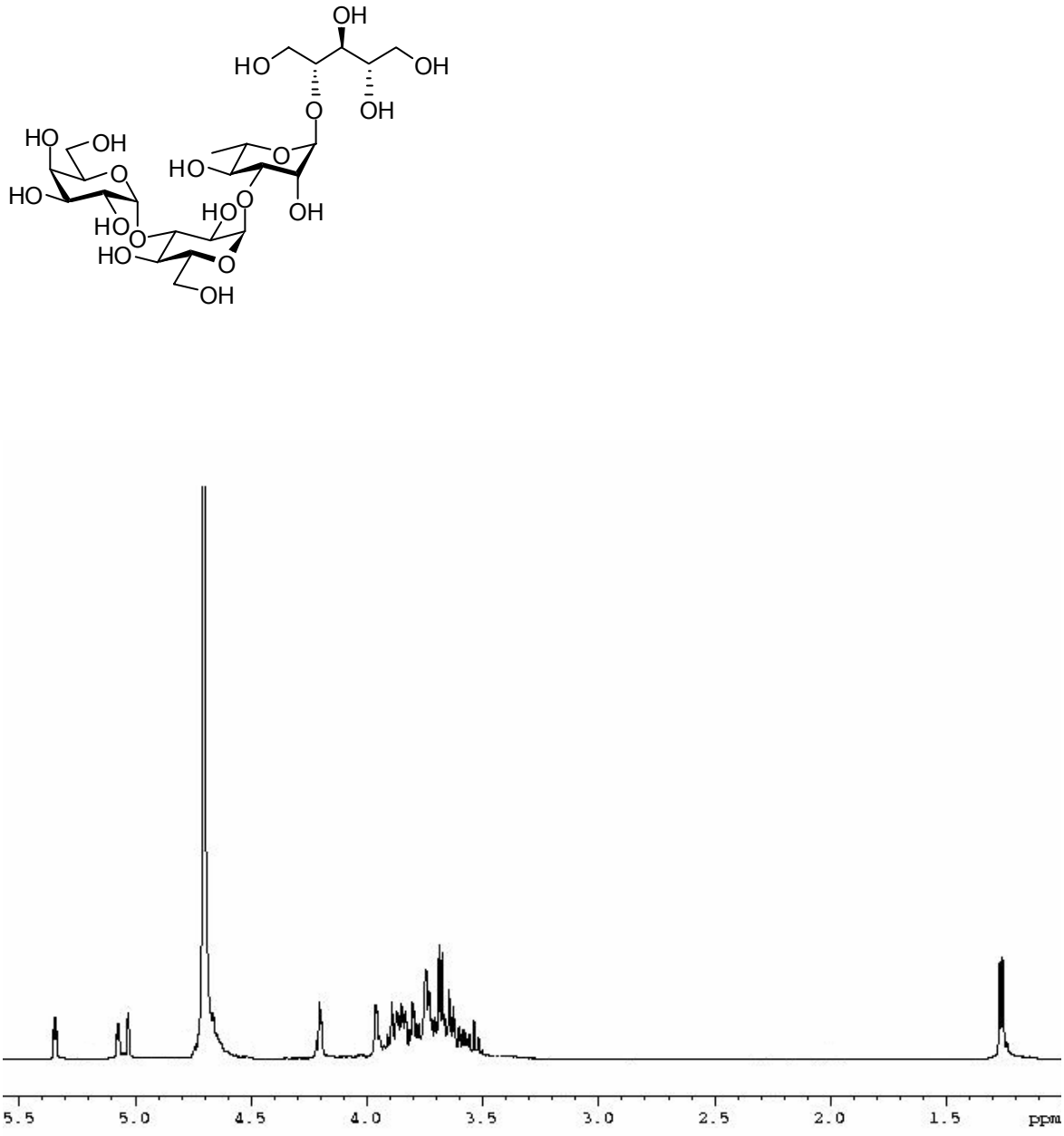
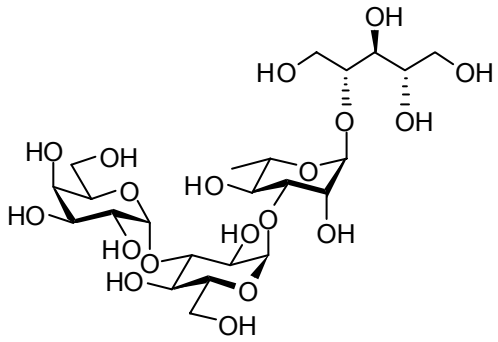


Figure A-28 ^{1}H NMR spectrum of O-(α-D-Galactopyranosyl)-(1→3)-O-(α-D-Glucopyranosyl)-(1→3)-O-(α-L-rhamnopyranosyl)-(1→4)-D-ribitol **(3.2)**



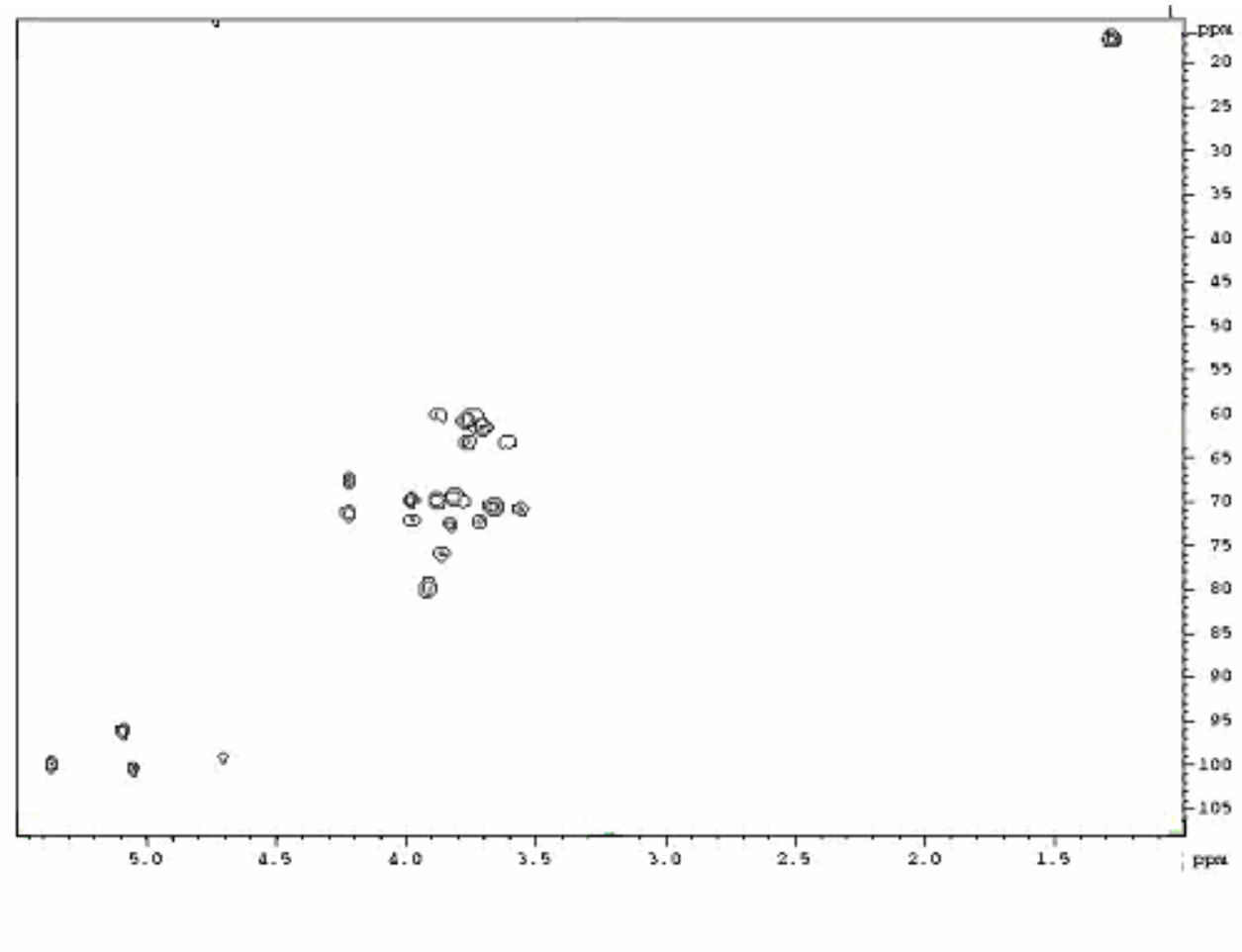


Figure A-29 2-D NMR: HMQC spectrum of O-(α-D-Galactopyranosyl)-(1→3)-O-(α-D-Glucopyranosyl)-(1→3)-O-(α-L-rhamnopyranosyl)-(1→4)-D-ribitol **(3.2)**

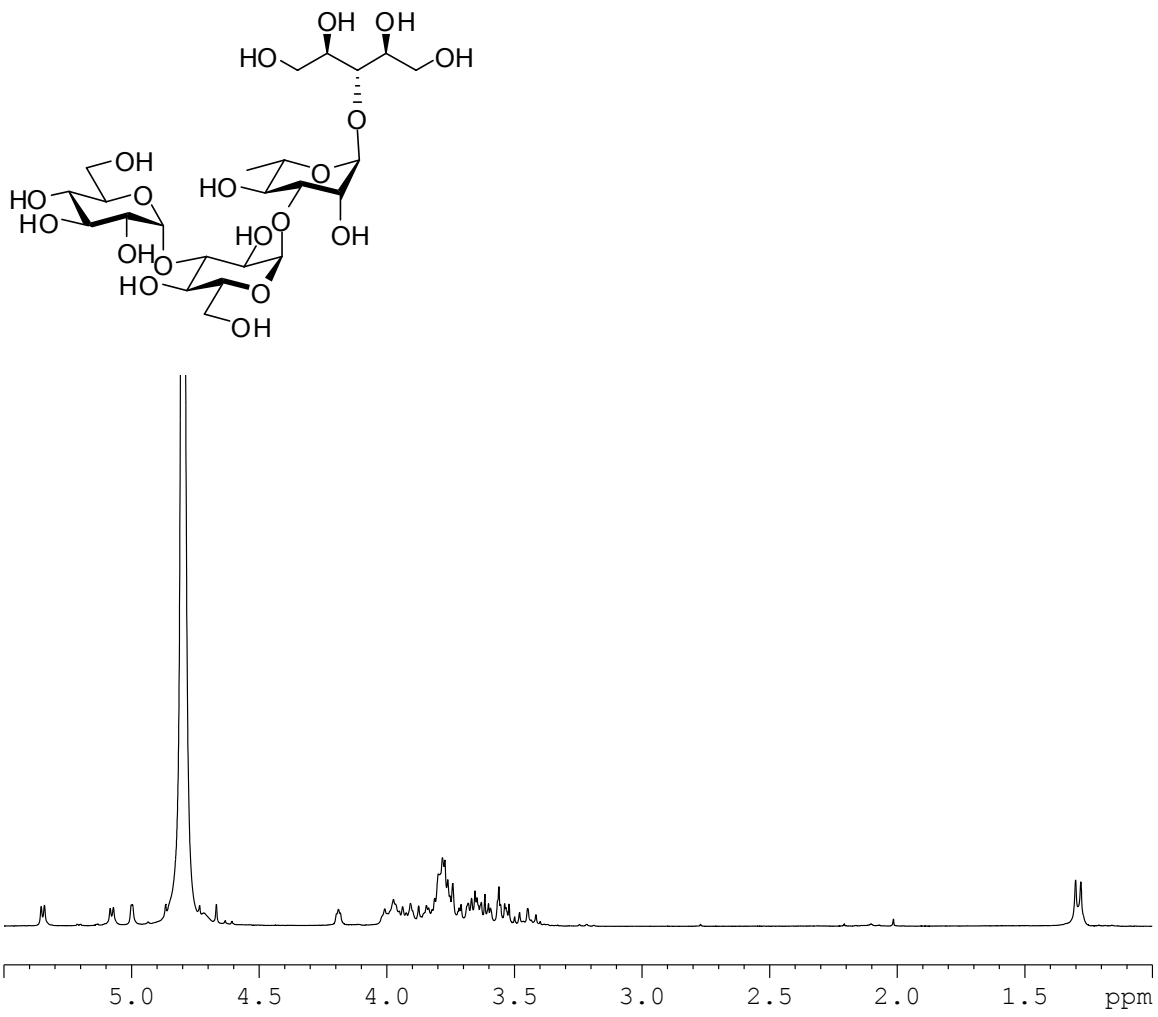


Figure A-30 ^{1}H NMR spectrum of O-(α-D-Glucopyranosyl)-(1→3)-O-(α-D-Glucopyranosyl)-(1→3)-O-(α-L-rhamnopyranosyl)-(1→3)-D/L-ribitol **(5.1)**

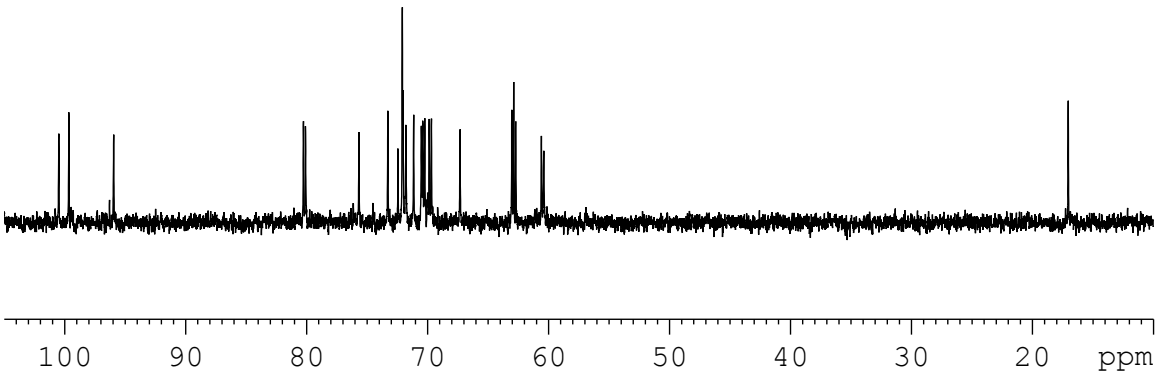


Figure A-31 ^{13}C{^{1}H} NMR spectrum of O-(α-D-Glucopyranosyl)-(1→3)-O-(α-D-Glucopyranosyl)-(1→3)-O-(α-L-rhamnopyranosyl)-(1→3)-D/L-ribitol **(5.1)**

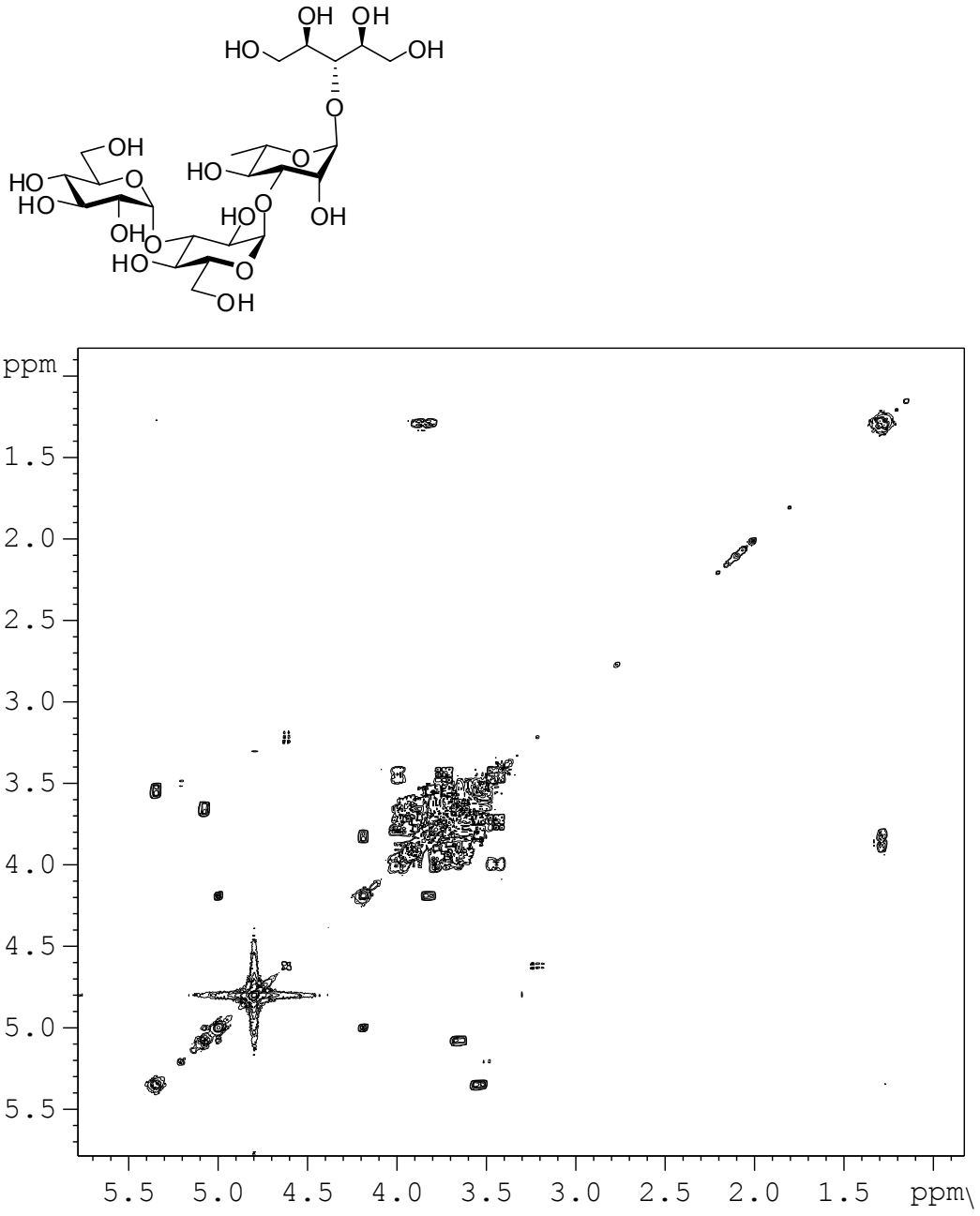


Figure A-32 2-D NMR: COSY spectrum of O-(α-D-Glucopyranosyl)-(1→3)-O-(α-D-Glucopyranosyl)-(1→3)-O-(α-L-rhamnopyranosyl)-(1→3)-D/L-ribitol **(5.1)**

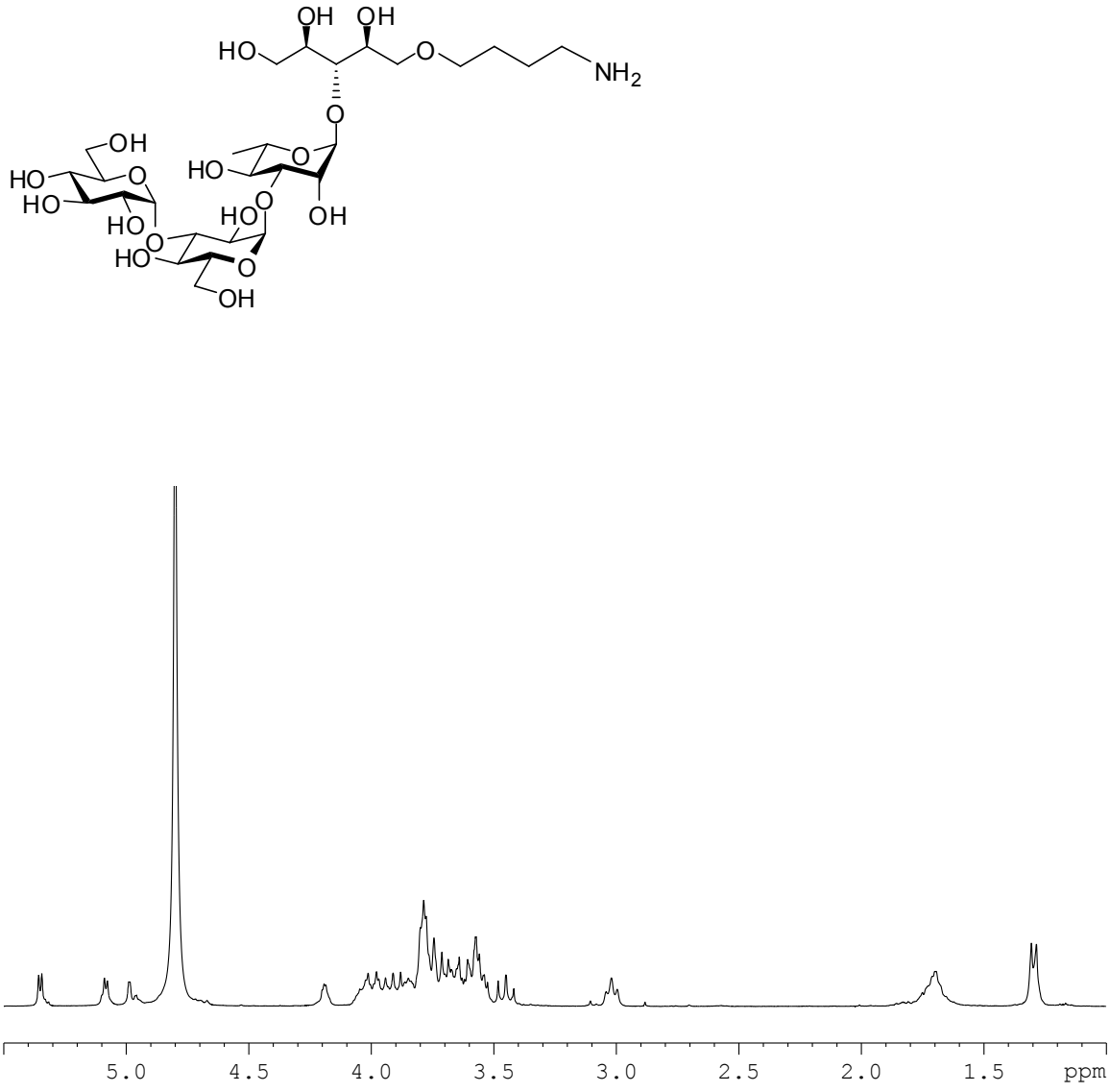
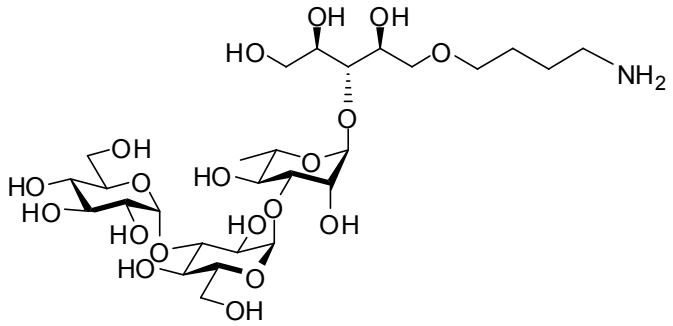


Figure A-33 ^{1}H NMR spectrum of O-(α-D-Glucopyranosyl)-(1→3)-O-(α-D-glucopyranosyl)-(1→3)-O-(α-L-rhamnopyranosyl)-(1→3)-1-O-(4-aminobutyl)-D-ribitol **(5.2)**



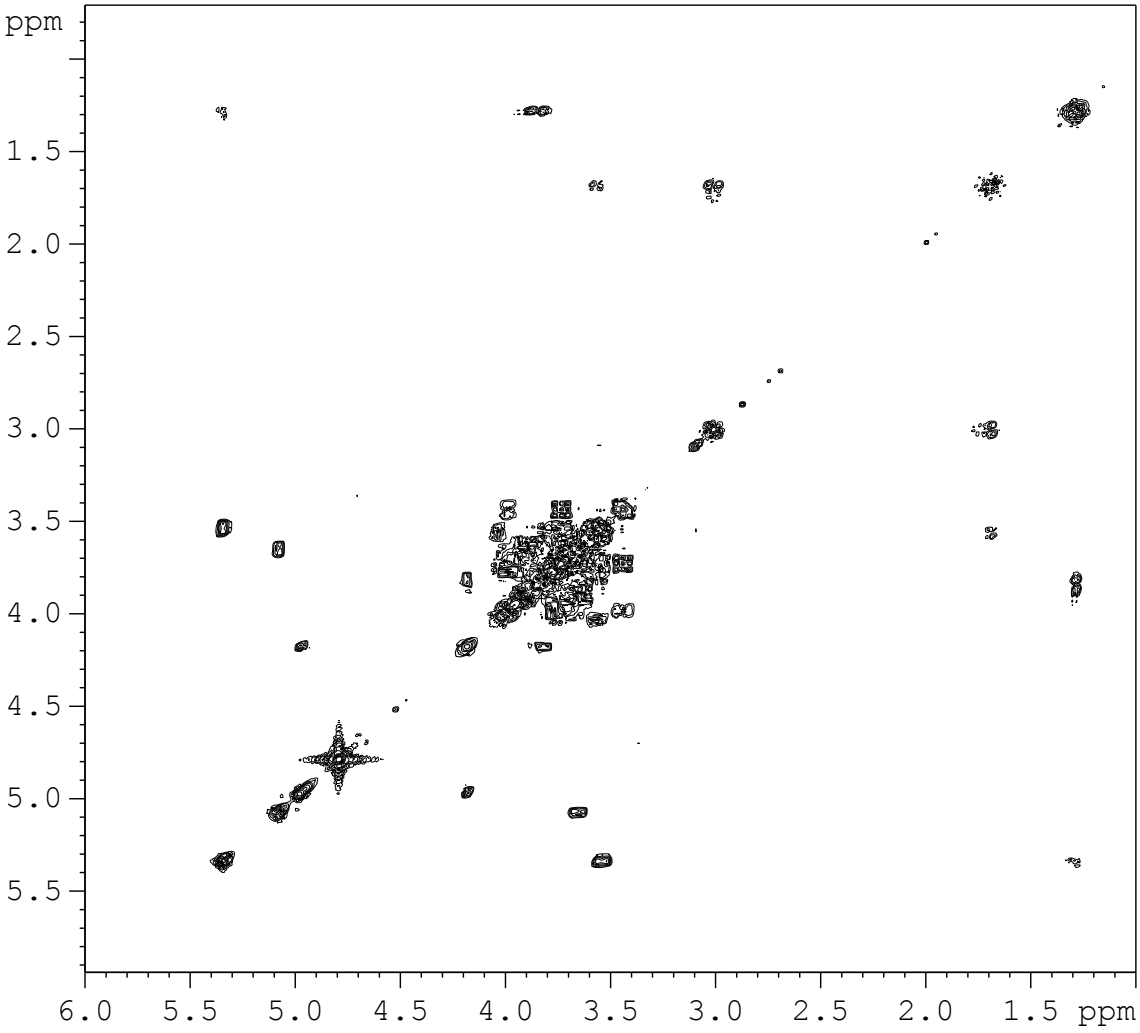
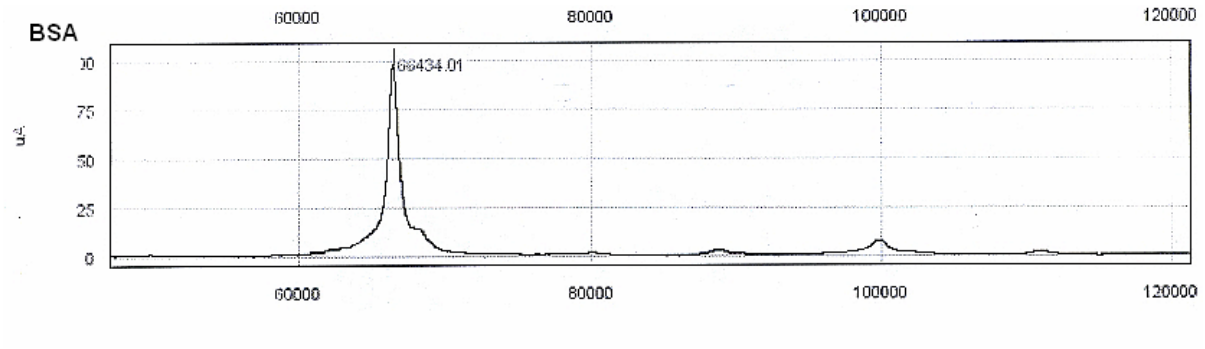
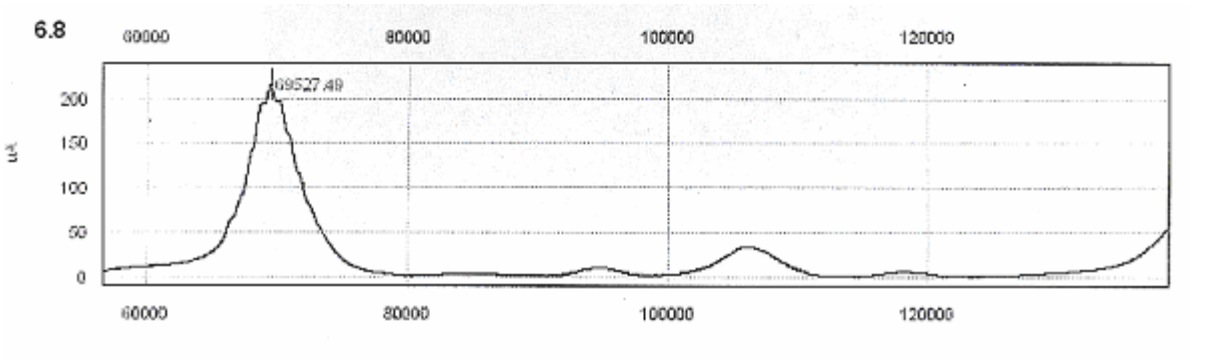
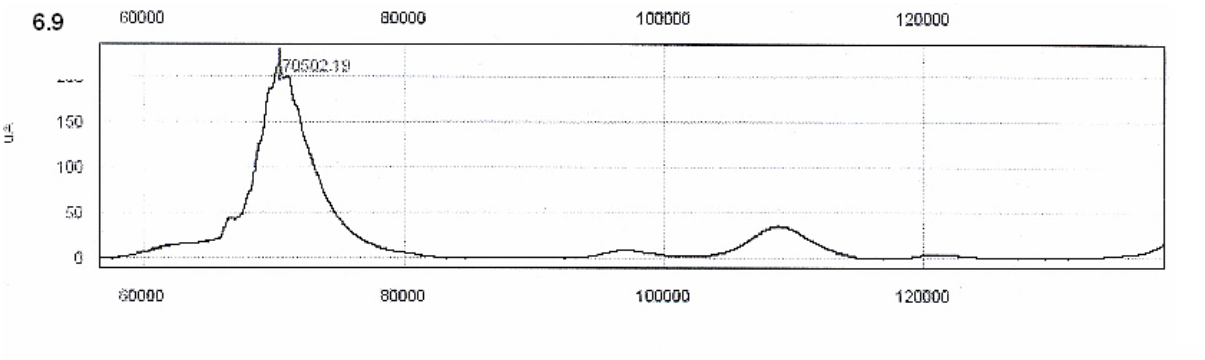


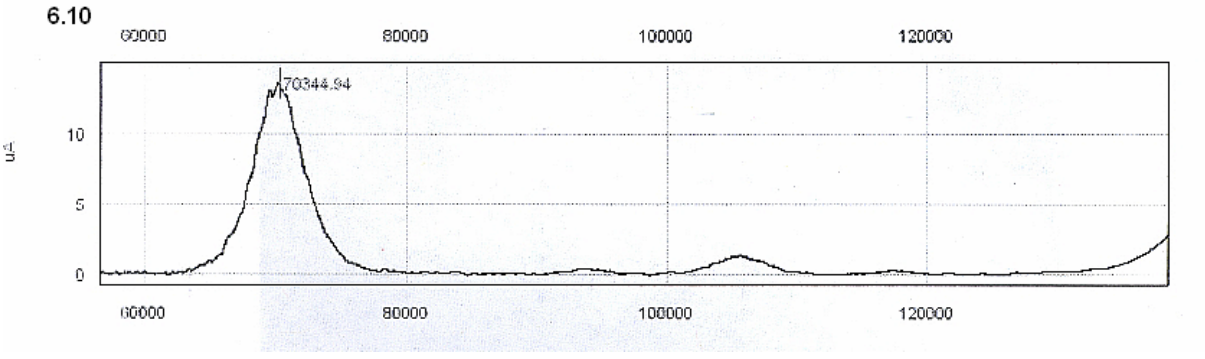
Figure A-34 2-D NMR: COSY spectrum of O-(α-D-Glucopyranosyl)-(1→3)-O-(α-D-glucopyranosyl)-(1→3)-O-(α-L-rhamnopyranosyl)-(1→3)-1-O-(4-aminobutyl)-D-ribitol **(5.2)**

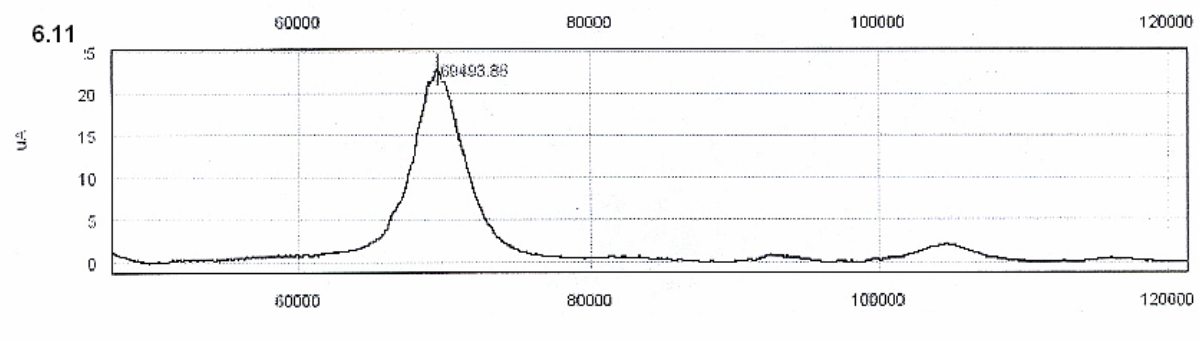
SELDI-TOF spectra of purified glycoconjugates (**6.8-6.14**)







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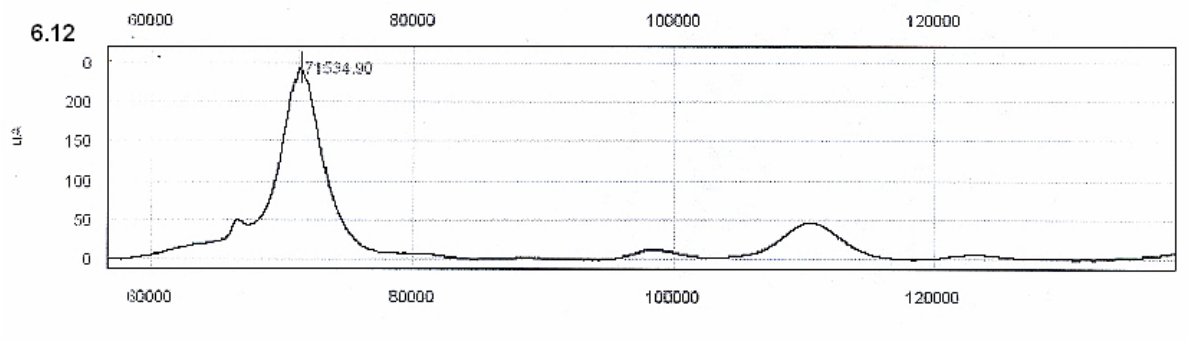
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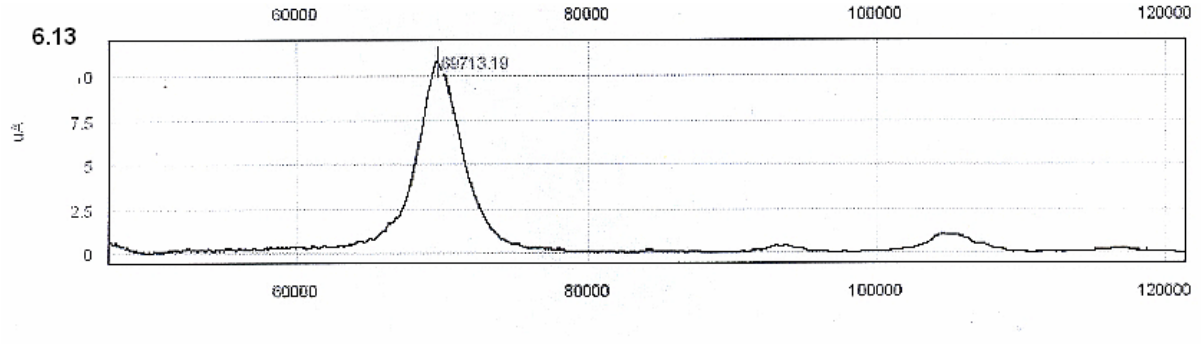
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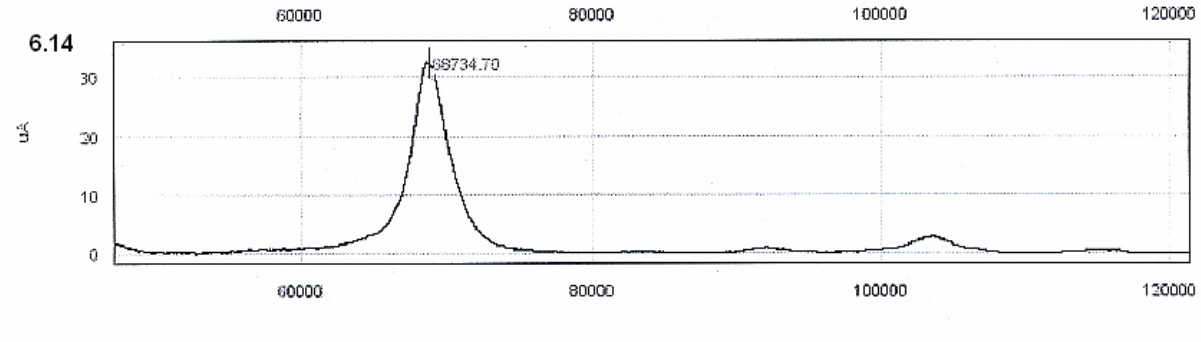
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