Chemical Synthesis and Biological Evaluation of Cell Surface Carbohydrate Antigens for Rational Vaccine Design

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Publications

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Peer-reviewed Publications

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‡These authors contributed equally

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Martin, C. E.‡; Broecker, F.‡; Oberli, M. A.; Komor, J.; Mattner, J.; Anish, C.; Seeberger, P. H.: Immunological evaluation of a synthetic *Clostridium difficile* oligosaccharide conjugate vaccine candidate and identification of a minimal epitope. *J. Am. Chem. Soc.*, **2013**, *135*, 9713. http://dx.doi.org/10.1021/ja401410y
‡These authors contributed equally

Martin, C. E.; Weishaupt, M. W.; Seeberger, P. H. (2011): Progress toward developing a carbohydrate-conjugate vaccine against *Clostridium difficile* ribotype 027: synthesis of the cell-surface polysaccharide PS-I repeating unit. *Chem. Commun.*, *47*, **2011**, 10260. http://dx.doi.org/10.1039/C1CC13614C

Patents

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Oral Presentations

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Conference Poster Presentations

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Martin, C. E.; Weishaupt, M. W.; Seeberger, P. H. Total synthesis of the *Clostridium difficile* ribotype 027 cell-surface polysaccharide PS-I repeating unit. Poster presented at the 6th Glycan Forum, *Berlin*, **2012**

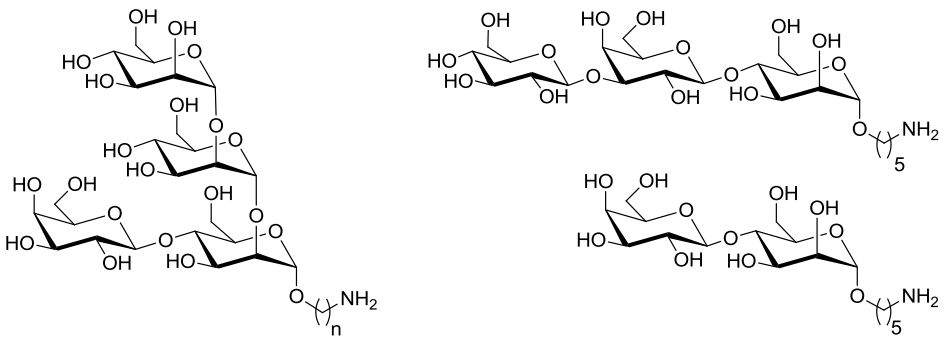
Summary

Complex carbohydrates are major components of cell surfaces and therefore play an important role in biological systems. In the case of infectious, disease-causing pathogens, cell surface carbohydrate antigens contribute to the recognition of these pathogens by the mammalian immune system, since glycans are part of the outer cell wall which is exposed to the host's immune cells. The host's immune response includes the generation of specific anti-glycan antibodies that neutralize the pathogen. Therefore, glycans found on the pathogen's cell surface can be used as components of preventative vaccines. Any improvement of current vaccines and rational design of new vaccines requires fundamental research of the glycans' interaction with the immune system, in particular, the identification of the glycan epitope structures that are recognized by the immune system. For these investigations, access to sufficient amounts of pure and well-characterized oligosaccharides is necessary. However, the availability of these molecules is still a limiting factor in the field of glycan research.

The first objective of this dissertation is to provide novel glycan antigens found on the cell surface of pathogens using chemical synthesis, since these glycans cannot be obtained from other sources in the same amounts and purity. A systematic approach is taken by the synthesis of larger penta- and tetrasaccharides, as well as comprehensive libraries of smaller mono-, di- and trisaccharide substructures.

The second objective involves the biological evaluation of the synthetic antigens with particular focus on the identification of minimal glycan epitopes by microarray technology. In doing so, samples from infected or vaccinated individuals are screened for antibodies against the libraries of synthetic glycan structures.

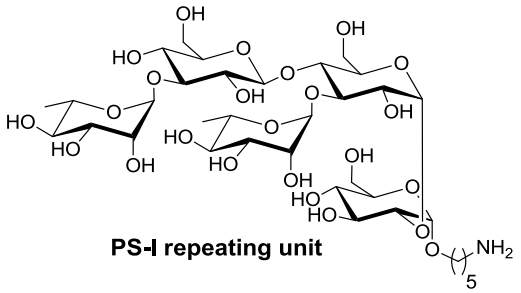
The synthesis and evaluation of the immunogenicity and diagnostic potential of oligosaccharides from *Leishmania* parasites is described in Chapter 2. Chemical synthesis gave access to several oligosaccharides containing galactose (Gal) and mannose (Man) found on the cell surface of different *Leishmania* species.

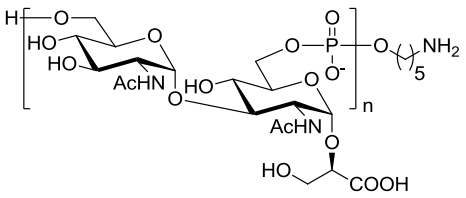


Synthetic *Leishmania* cell surface glycans

Glycan microarray screening of samples from infected dogs identified the two epitopes β-Gal-(1→4)-α-Man and α-Man-(1→2)-α-Man-(1→2)-α-Man that could play a role in canine leishmaniasis infection. In order to raise antibodies that recognize the identified epitopes, a tetrasaccharide containing both epitopes was identified as a hapten for glycoconjugate preparation and subsequent immunization experiments. This work demonstrates the feasibility of an approach that relies on synthetic glycans to identify minimal epitopes, allowing for the rational design and synthesis of glycan antigens used for immunization studies.

The same concept was also applied to the PS-I and PS-III glycans present on the surface of *Clostridium difficile* bacteria, described in Chapter 3. The first total synthesis of the PS-I pentasaccharide repeating unit confirmed the reported chemical structure. An optimized synthesis produced the PS-I pentasaccharide as well as a library of its substructures in sufficient amounts for immunological studies. Phosphodiester-bridged oligomers of the PS-III pseudodisaccharide repeating unit were synthesized by a combination of carbohydrate and phosphoramidite chemistry. For this purpose, an efficient one-pot coupling, oxidation and deprotection procedure for the pseudodisaccharide repeating units was developed.



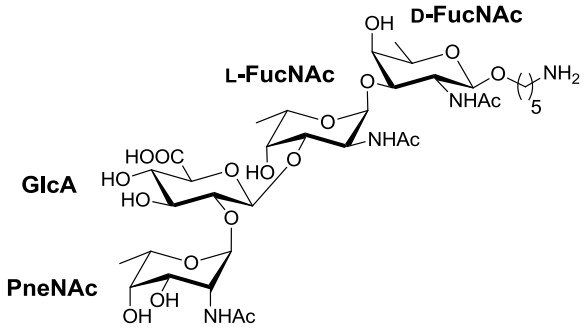


Synthetic variants of *C. difficile* cell surface glycans PS-I and PS-III

Microarray screening revealed that the sera of hospitalized patients infected with *C. difficile* contain antibodies against the synthetic PS-I and PS-III glycans, implying their immunogenicity during infection. The binding epitopes of antibodies, obtained by the immunization of mice with PS-I pentasaccharide, were studied with synthetic PS-I substructures. A disaccharide containing rhamnose (Rha) and glucose (Glc) was identified as the minimal epitope of the PSI-pentasaccharide; this was further confirmed by immunization experiments. The PS-I pentasaccharide and the minimal epitope disaccharide, as well as the PS-III oligomers were identified as possible vaccine candidates against *C. difficile*.

Chapter 4 describes the synthesis and evaluation of *Streptococcus pneumoniae* Serotype 5 (SP5) capsular polysaccharide (CPS) substructures. Synthetic substructures of the CPS repeating unit concentrated on two rare monosaccharides: firstly, the ketone-containing

4-keto-*N*-acetyl-D-fucosamine (Sug) and secondly, *N*-acetyl-L-pneumosamine (PneNAc). The synthetic strategy thus devised enabled the preparation of glycans containing Sug or PneNAc. It was found that the ketone is hydrated and unstable; therefore *N*-acetyl-D-fucosamine (D-FucNAc) was identified as a stable mimic of Sug.



Tetrasaccharide substructure of the *S. pneumoniae* serotype 5 capsular polysaccharide repeating unit FucNAc: *N*-acetyl-fucosamine; GlcA: glucuronic acid; PneNAc: *N*-acetyl-pneumosamine

Glycan microarray screenings carried out using eleven synthetic glycans and rabbit typing sera containing antibodies specifically produced to only recognize SP5 revealed that a α-PneNAc-(1→2)-GlcA disaccharide is the predominant epitope recognized by these antibodies. Screenings carried out with samples from vaccinated humans further identified a tetrasaccharide as a relevant epitope. Both the disaccharide as well as the tetrasaccharide are ideal targets for vaccine applications, identified by the rational design, synthesis and biological evaluation of cell surface carbohydrate antigens.

In conclusion, chemical synthesis and biological evaluation of cell surface carbohydrate antigens demonstrate a rational approach for the identification of glycan epitopes that can be used as components of vaccines against *Leishmania*, *C. difficile* and *S. pneumoniae*.

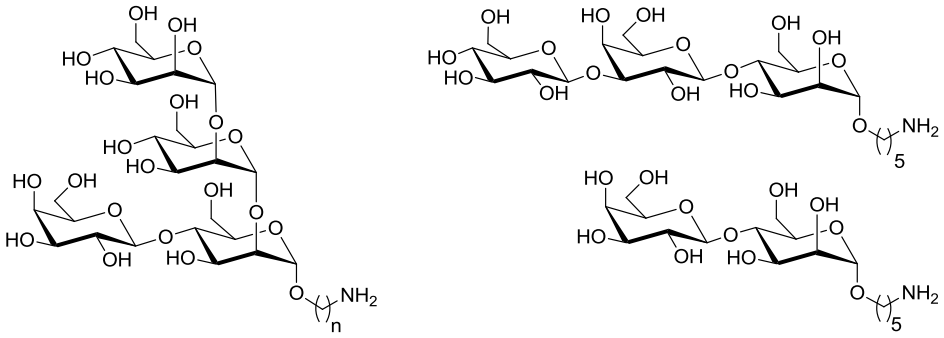
Zusammenfassung

Komplexe Kohlenhydrate gehören zu den Hauptbestandteilen von Zelloberflächen und sind daher von großer Bedeutung für biologische Systeme. Kohlenhydrate die auf der Zelloberfläche von Krankheitserregern vorkommen, spielen eine besondere Rolle für die Erkennung der Krankheitserreger durch das Immunsystem von Säugetieren. Die Immunantwort des Wirts besteht dabei in der Bildung von neutralisierenden Antikörpern, die spezifisch die Glykane binden. Daher können Glykane, die auf der Oberfläche von Krankheitserregern vorkommen, als Bestandteile von Impfstoffen verwendet werden. Grundlagenforschung zur Wechselwirkung von Glykanen mit dem Immunsystem ist eine Voraussetzung für die Verbesserung existierender und die rationale Entwicklung neuer Impfstoffe. Die Bestimmung von Glykan Epitopen, die vom Immunsystem erkannt werden, ist dabei von besonderer Bedeutung. Ausreichende Mengen von reinen, gut charakterisierten Oligosacchariden sind für diese Untersuchungen notwendig. Die Verfügbarkeit dieser Moleküle ist jedoch ein limitierender Faktor der Glykanforschung.

Die erste Zielsetzung dieser Dissertation besteht in der chemischen Synthese von Glykan Antigenen, die nicht aus anderen Quellen in derselben Menge und Reinheit gewonnen werden können. Dabei wird ein systematischer Ansatz gewählt, der den Zugang, sowohl zu größeren Penta- und Tetrasacchariden, sowie umfassende Bibliotheken deren Mono-, Di- und Trisaccharid Substrukturen durch chemische Synthese gewährt.

Die zweite Zielsetzung besteht aus der biologischen Evaluation der synthetischen Antigene, dabei liegt ein Fokus auf der Identifikation von minimalen Glykan Epitopen mittels Microarray Technologie. Hierfür werden Proben infizierter oder geimpfter Individuen auf Antikörper gegen die synthetischen Glykane untersucht.

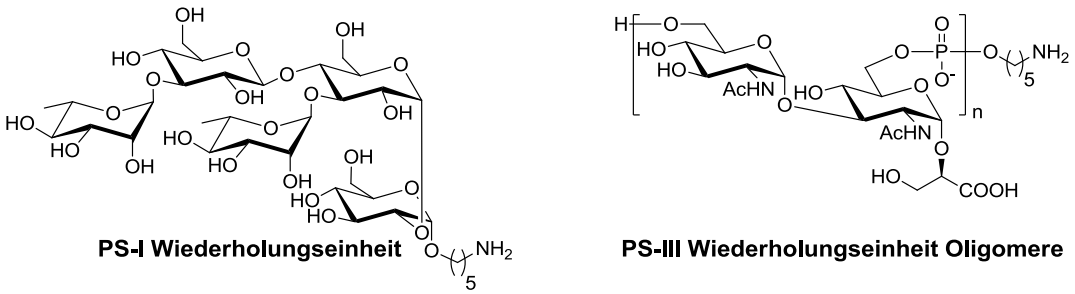
Die Synthese sowie die immunologische und diagnostische Evaluation von Oligosacchariden von *Leishmania* Parasiten wird in Kapitel 2 beschrieben. Oligosaccharide die auf der Zelloberfläche von Leishmanien vorkommen und aus Galaktose (Gal) und Mannose (Man) Bausteinen bestehen, wurden chemisch synthetisiert.



Synthetisierte *Leishmania* Zelloberflächen Glykane

Microarray Untersuchungen mit proben von infizierter Hunden ermöglichten die Identifikation zweier Bindungsepitope, nämlich Gal-(1→4)-Man und α-Man-(1→2)-α-Man-(1→2)-α-Man. Ein Tetrasaccharid, das beide Epitope enthält, wurde daraufhin verwendet um in Immunisierungsexperimenten Antikörper zu erzeugen, die diese Epitope binden. Damit wurde das Konzept, das auf synthetischen Oligosacchariden beruht um minimale Epitope und somit die rationale Entwicklung neuer Antigene für Immunisierungsstudien ermöglicht, unter Beweis gestellt.

Das selbe Konzept wurde auch auf die Glykane PS-I und PS-III angewandt, die auf dem Bakterium *Clostridium difficile* vorkommen und in Kapitel 3 beschrieben sind. Die erste Totalsynthese der PS-I Pentasaccharid Wiederholungseinheit ermöglichte zunächst die Bestätigung der publizierten chemischen Struktur von PS-I. Eine optimierte Synthese lieferte das PS-I Pentasaccharid und eine umfassende Bibliothek kleinerer Oligosaccharid Substrukturen für immunologische Studien. Phosphodiester verbundene Oligomere der PS-III Pseudodisaccharid Wiederholungseinheit wurden durch eine Kombination von Kohlenhydratchemie und Phosphoramiditchemie synthetisiert. Zu diesem Zweck wurde eine effizient Eintopfstrategie zur Kopplung, Oxidation und Entschützung der Pseudodisaccharid Wiederholungseinheiten einwickelt.

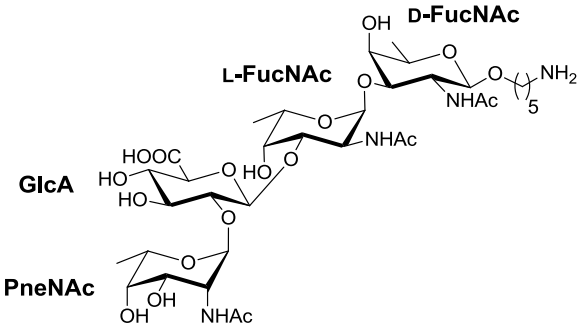


Synthetisierte *C. difficile* Zelloberflächen Glykane PS-I und PS-III

Microarray Untersuchungen ergaben, dass Blutproben *C. difficile* infizierter Patienten Antikörper gegen die synthetischen PS-I und PS-III Glykane enthalten, somit konnte davon ausgegangen werden, dass PS-I und PS-III immunogen sind. Die Bindungsepitope von

Antikörpern, die durch Immunisierung von Mäusen mit dem PS-I Pentasaccharid erzeugt wurden, wurden mittels der synthetischen PS-I Substrukturen untersucht. Ein Disaccharid, bestehend aus Rhamnose (Rha) und Glukose (Glc) wurde als minimales Epitop von PS-I identifiziert und durch Immunisierungsexperimente bestätigt. Das PS-I Pentasaccharid sowie das identifizierte Disaccharide und die synthetisierten PS-III Oligomere kommen somit als Impfstoffkandidaten gegen *C. difficile* in Betracht.

Kapitel 4 beschreibt die Synthese und Evaluation von Substrukturen des Kapsulären Polysaccharids (CPS) von *Streptococcus pneumoniae* Serotyp 5 (SP5). Dabei lag das Augenmerk auf der Synthese von Oligosacchariden, die die seltenen Zuckern 4-keto-*N*-Acetyl-D-Fucosamin (Sug) oder *N*-Acetyl-L-Pneumosamin (PneNAc) beinhalteten. Es wurde festgestellt, dass das Keton des Sug Zuckers hydratisiert und zudem chemisch labil ist. Daher wurde *N*-Acetyl-D-Fucosamin (D-FucNAc) als stabiles Imitat des Sug Zuckers identifiziert.



Tetrasaccharide Substruktur der *S. pneumoniae* Serotyp 5 Polysaccharid Wiederholungseinheit FucNAc: *N*-Acetyl-Fucosamin; GlcA: Glukuronsäure; PneNAc: *N*-Acetyl-Pneumosamin

Microarray Untersuchungen offenbarten, dass ein α-PneNAc-(1→2)-GlcA Disaccharid das minimale Epitop darstellt, dass von Antikörpern erkannt wird, die in Hasen hergestellt wurden um nur SP5 spezifisch zu binden. Weiterführende Untersuchungen, mit Blutproben geimpfter Menschen, identifizierten ein Tetrasaccharid als relevantes Epitop. Somit wurden mit dem Disaccharid und dem Tetrasaccharid zwei Zielstrukturen für Impfstoffanwendungen identifiziert.

Die chemische Synthese und biologische Evaluation von Kohlenhydrat Antigenen zeigen einen rationalen Ansatz für die Identifikation von Epitopen die als Impfstoffkomponenten gegen *Leishmania, C. difficile* und *S. pneumoniae* verwendet werden können.

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1 Introduction

1.1 Cell Surface Carbohydrate Antigens

Living cells are coated with a dense layer of various complex carbohydrates that are involved in cell-interaction and adhesion. In the case of pathogens, these glycans play an important role in recognition by the mammalian immune system, since they are part of the outer cell wall which is exposed to the host's immune cells. The structure and composition of cell surface glycans vary strongly between different species of pathogens. This dissertation focuses on carbohydrates found on the surface of Gram-positive bacteria and the protozoan parasite *Leishmania*.

The cell wall of Gram-positive bacteria, such as *Clostridium difficile* and *Streptococcus pneumoniae*, typically consists of the cytoplasmic membrane, which is covered by multiple layers of peptidoglycan (Fig. 1).[1] Many bacteria are additionally surrounded by a capsular polysaccharide (CPS) that functions as a physical barrier, shielding other cell surface components from the host's immune system and preventing dehydration. CPS is built up from oligosaccharide repeating units, linked through glycosidic bonds or phosphodiester bridges. In the early 20th century it was discovered that CPS can be used to categorize bacteria into different serotypes, due to their structural diversity between different bacterial species and subtypes.[2]

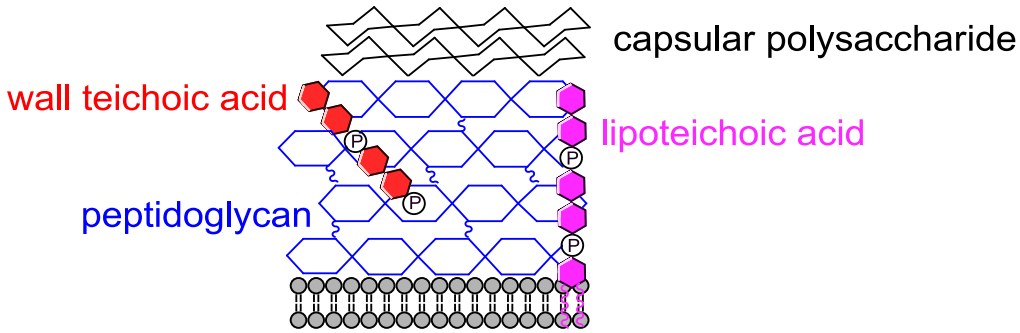


Figure 1: Schematic depiction of the carbohydrate-containing components of the Gram-positive cell wall.

The peptidoglycan consists of repeating disaccharide β-*N*-acetylglucosamine-(1→4)-β-*N*-acetylmuramic acid attached to a cross-linked peptide chain, this mesh being the main structural feature of Gram-positive cell walls.[3] A further class of cell wall carbohydrates consists of the teichoic acids that are embedded within the peptidoglycan network. Teichoic acids are comprised of sugar-phosphate repeating units and include two classes: wall teichoic

acids (WTAs) and lipoteichoic acids (LTAs). WTAs are covalently bound to the peptidoglycan matrix, whereas LTAs are anchored in the cell membrane via a glycolipid anchor.[4,5] If the bacterium is not covered by a CPS, the teichoic acids are often exposed on the cell surface.

Protozoa express a large variety of phosphoglycans in high density on their cell surface; these are predominately glycosylphosphatidylinositol (GPI) anchors that are often modified with proteins.[6] Parasites belonging to the genus of *Leishmania*, express GPI-related lipophosphoglycans (LPGs) as a major carbohydrate. LPGs are anchored in the protozoan cell membrane via a phosphatidylinositol-lipid, which is connected to a saccharide core. Phosphodiester bridged disaccharide repeating units extend from the saccharide core and are terminated by an oligosaccharide capping moiety (Fig. 2).[7] The expression of LPG on the protozoan cell surface is life-cycle and host dependent, since the LPG plays a dominant role in host-parasite interactions.[8-10]

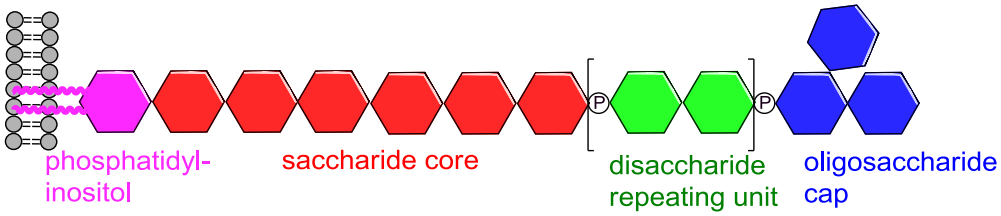


Figure 2: Schematic depiction of the components of protozoan lipophosphoglycans.

Since cell surface carbohydrates are found on the outermost layer of pathogens, these are the structures that come into first contact with cells of the host's immune system. The diversity of glycans produced by bacteria, with there being more than one hundred monosaccharide building blocks, is far greater than the nine different monosaccharides that make up the structures present on the surface of human cells (Fig. 3 & 4).[11,12]

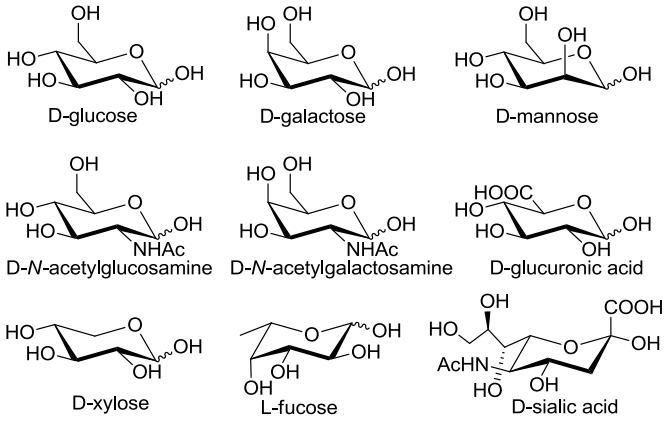


Figure 3: The nine monosaccharides that make up the glycan structures on human cell surfaces: D-glucose (Glc), D-galactose (Gal), D-mannose (Man), L-fucose (Fuc), D-*N*-acetylglucosamine (GlcNAc), D-*N*-acetylgalactosamine (GalNAc), D-xylose (Xyl), D-sialic acid (Sia) and D-glucuronic acid (GlcA)

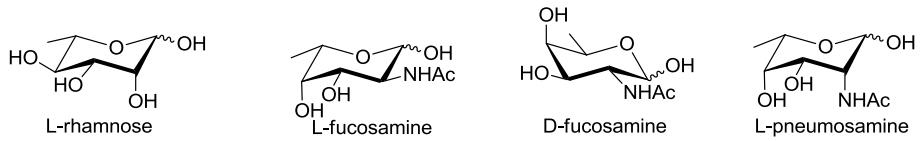


Figure 4: Four exemplary non-mammalian monosaccharides: L-rhamnose (Rha), L-and D-fucosamine (FucNAc), L-pneumosamine (PneNAc)

The mammalian immune system has evolved to distinguish between host-derived "self" and foreign "non-self" structures. Glycans containing non-mammalian monosaccharides are therefore often recognized as non-self antigens. Defined pathogen-derived cell surface glycans that are recognized as non-self antigens by the mammalian immune system are likely to trigger an immune response and could therefore be used as vaccine components targeting the respective pathogen.

1.2 Carbohydrate Vaccines

Vaccines are designed to confer immunity towards a particular pathogen. Historically, they are prepared through isolation, attenuation or inactivation of the causative pathogen. These live attenuated or killed whole cell vaccines mimic the protective immunity in people who have acquired the infection and often elicit a strong immune response and confer long lasting immunity.[13] However, not all pathogens can be grown and isolated in laboratory cultures and the efficiency of vaccines towards organisms that mutate rapidly or exist as multiple serotypes can be reduced.[13,14]

Subunit vaccines consist of components derived from the targeted pathogen such as inactivated toxins, cell surface proteins or carbohydrates. Due to the lower immunogenicity, compared to whole cell vaccines, subunit vaccines are administered in formulations containing an adjuvant. Adjuvants are substances that serve to enhance the immune response against the subunit antigens.[15 14]

1.2.1 Polysaccharide Vaccines

It has been known for over ninety years that polysaccharides are the immunodominant type-specific substance of *S. pneumoniae*.[2] Research in this field led to the development of the first CPS containing vaccines against certain pneumococcal serotypes in the first half of the 20th century.[16] It was further demonstrated that so-called glycan haptens can induce a glycan-specific immune response *in vivo* when conjugated to carrier proteins.[17-19] These advances fell into the same time-period as the discovery and development of antibiotic drugs. The success

of antibiotics for the treatment of bacterial infectious diseases in the middle of the 20th century overshadowed the development of preventative vaccines. However, the necessity for a complimentary preventative approach became apparent with the appearance and rise of antibiotic-resistant bacterial strains.[20] This led to a revival of vaccine research and the licensing of the first anti-menigococcal and anti-pneumococcal polysaccharide vaccines in the 1970s.[21] Despite their success, these first polysaccharide vaccines had several shortcomings since they did not elicit a long-lasting protection in adults and were not effective in the population most vulnerable to bacterial infections: children under two years of age.[22] These shortcomings result from the immunology of carbohydrates and were overcome by the introduction of carbohydrate-protein conjugate vaccines.

1.2.2 Immunology of Carbohydrate Vaccines

The adaptive mammalian immune system consists of a multitude of components that interact in a cascade of events against non-self antigens.[23] The main components are antigen presenting cells (APCs), such as dendritic cells, macrophages and B-cells, which display the major histocompatibility complex (MHC); T-cells, which carry T-cell receptors (TCR) on their surface and T-helper (T_H) cells, which express co-receptors, such as CD4, on their surface. In the first step of the cascade, APCs recognize the foreign antigen, internalize it and partially degrade it. The degraded antigen fragments are then presented on the MHC to the TCR of T-cells, which in turn activate B-cells.[23,24] The activated B-cells then produce the antigen-specific antibodies, also known as immunoglobulins (Ig). The B-cells can further evolve into memory B-cells able to proliferate and secrete specific antibodies when they encounter the antigen in future.[23,24]

Antigens are categorized by their immunological mechanism. Thymus-dependent (T_D) antigens, such as proteins that interact with B-cells with the immediate help of $CD4^+$ T-cells, elicit long-lasting immunological memory and furthermore allow for antibody affinity maturation and Ig class switching from IgM to IgG, resulting in high antigen binding affinity.[25] Carbohydrate antigens fall into the class of Thymus-independent (T_I) antigens because they can stimulate antibody production by B-cells without the assistance of T_H cells.[25] The antibodies produced against carbohydrates predominately belong to the IgM class, which typically have lower antigen binding affinities compared to IgG. Another feature of T_I antigens is the lack of an immunological memory. Within the class of T_I, a distinction is made

between T_I-1 and T_I-2 antigens. T_I-1 antigens, such as lipopolysaccharides (LPS), can activate immature, neonatal B-cells in infants, a task T_I-2 polysaccharides are not capable of.[26]

When confronted with the entire pathogen, the human immune system can generate a robust immune response and develop immunological memory due the combination of T_D, T_I-1 and T_I-2 antigen responses. A comparable response is not achieved with carbohydrate substructures that consist exclusively of T_I-2 antigens.[26,27] The crucial step for overcoming this inherent property of carbohydrates for their use as vaccines is the covalent attachment to a protein, thereby converting the T_I into a T_D response resulting in anti-carbohydrate antibody affinity maturation, Ig class switching and immunological memory. These glycoconjugate vaccines are effective in infants under two years of age and have almost completely replaced carbohydrate-only vaccines.[24,26,27]

1.2.3 Glycoconjugate Vaccines

Carbohydrate-protein conjugates consist of three components the carbohydrate antigen, the protein carrier and a cross-linker covalently connecting both. Each component has a profound effect that influences the immunogenicity of the glycoconjugate in a vaccine formulation.[23,28,29]

The main function of the carrier protein is to enhance the immunogenicity of glycoconjugates by recruiting T-cells and thereby inducing a strong T_D response. Therefore, highly immunogenic proteins derived from bacterial toxins are often employed. Deactivated tetanus toxin (TT), which has been used as a vaccine component since the 1930s, and the non-toxic mutant of diphtheria toxin CRM197 are frequently used carrier proteins. [30,31] Since the proteins are degraded and presented as peptide epitopes on the MHC of APC, studies have identified the peptide sequences that act as T-cell epitopes.[30-32]

The choice of cross-linker and conjugation chemistry used for linking proteins and carbohydrate antigens determines several important properties of the resulting conjugate. The ratio of glycan to protein and thereby the antigen density is one of the parameters influenced by the conjugation chemistry. [23,28,29] A further variable influenced by the length of the cross-linker is the distance between the protein surface and the attached antigen. Since any molecule connected to a carrier protein is potentially immunogenic, cross-linkers can also elicit an immune response. The anti-cross-linker immune response can compete with the immune response against the antigen and thereby suppress the desired anti-antigen response. [23,28,29]

Therefore, the immunogenicity of the cross-linker has to be taken into particular consideration when weakly immunogenic carbohydrate antigens are used.[33,34]

The carbohydrate antigen forms the focal point of this thesis. A multitude of parameters determine the antigenicity of glycans, including their monosaccharide composition, the stereochemistry of their glycosidic linkage, their sequence as well as the glycan size and its three-dimensional structure. Other factors, such as monosaccharide modification and charges introduced by anionic or cationic functional groups, also have powerful effects.

In order to be recognized and provoke a response from the immune system, the glycan needs to contain an epitope that is recognized as non-self and binds to B-cell receptors (BCR). Consequently, glycans containing monosaccharides that are not produced by the mammalian host are likely to be identified as foreign. The immunogenicity of glycans can be enhanced by the reduction of structural motifs that resemble mammalian glycans. This was shown for the example of Group B *Streptococcus* Type 5 (GBS V) that carries a terminal sialic acid (Sia) residue on its CPS. The Sia position and linkage are very similar to that of glycans found on human cells.[35] De-sialylation of the GBS V CPS resulted in glycans and glycoconjugates that share fewer structural similarities with the host and were therefore strongly immunogenic in non-human primates and mice.[36] In order to be recognized by the BCR, the B-cell epitopes on the pathogen cell surface or glycoconjugate need to be exposed. This implies that terminal epitopes are more immunogenic than internal structures.[37,38] Terminal epitopes are those sequences typically found at the non-reducing end of a polysaccharide repeating unit or side-branch. The size of a glycan antigen necessary to produce a strong immune response has been studied mostly on polysaccharides isolated from natural sources.[29] This research has led to two concepts regarding the length of glycan epitopes: sequential epitopes and conformational epitopes. Sequential epitopes, as described above, rely on the recognition of fairly small substructures and therefore can be immunogenic even on a monosaccharide level.[18] Conformational epitopes rely on larger glycan chains that adopt a rigid, often helical, conformation.[39] Over the past decades, most research has been carried out using polysaccharides isolated from natural sources. Glycoconjugates containing the polysaccharides are used in animal immunization trials and the affinity of antisera from immunized animals against the polysaccharides is assessed. The intrinsic heterogeneity in size and composition of isolated polysaccharides and the resulting glycoconjugates seriously limit the deducible information on the mode of action and structure-activity relationships of glycan antigens. Furthermore, not all pathogens can be cultured under laboratory conditions. The

understanding of the interactions that occur on a molecular level, however, is of great academic interest and necessary for progress in the field of glycoconjugate vaccines. A solution is the use of pure and well-characterized synthetic glycan antigens.

1.2.4 Synthetic Glycan Antigens

Synthetic glycan antigens are less extensively studied than their isolated counterparts. *S. pneumoniae* is one of the few pathogens of which different synthetic glycans of several serotype CPSs have been investigated. The CPS of *S. pneumoniae* serotype 3 (SP3) is composed of a [→4)-β-Glc-(1→3)-β-GlcA-(1→] repeating unit. Glycoconjugates of synthetic di-, tri- and tetrasaccharides, representing fragments of the SP3 CPS repeating unit, used in immunization studies showed that a trisaccharide-conjugate is sufficient to protect mice from a lethal dose of SP3.[40] Immunization experiments in mice were also carried out with synthetic oligosaccharides derived from the CPS of *S. pneumoniae* serotype 14 (SP14) with the branched tetrasaccharide repeating unit [→6)-[β-Gal(1→4)-]-β-GlcNAc-(1→3)-β-Gal-(1→4)-β-Glcp-(1→]. It was shown that a synthetic tetrasaccharide conjugate produced specific antibody response to the SP14 CPS. [41] Functional phagocytosis-promoting antibodies against SP14 were only obtained with glycoconjugates where the branching structure was present in the synthetic tetrasaccharide hapten.[38]

The *Haemophilus influenzae* type b (Hib) CPS structure consists of a linear polyribosylribitol phosphate (PRP) and is a further antigen that has been studied using both synthetic and isolated molecules.[42-44] Immunological studies with synthetic PRP of variable lengths revealed that the optimal anti-PRP response was achieved with three ribosylribitol phosphate repeating units.[45] This implies that comparably small synthetic oligosaccharides can be an efficient glycan antigen for glycoconjugate vaccines. This concept has been proven correct by the success of a synthetic glycoconjugate Hib vaccine produced and administered in Cuba, where the necessary infrastructure to cultivate and isolate Hib polysaccharides was not available.[43,44]

The studies conducted with different synthetic bacterial CPS-related antigens demonstrate the validity of the concept using synthetic glycans as vaccine candidates against pathogenic bacteria. Applying this concept to eukaryotic protozoan parasitic pathogens, however, is hindered by the complex protozoan life-cycle taking place in different host species and the resulting complex expression patterns of cell surface glycan antigens. Despite these challenges, glycans play key roles in the interaction of protozoan parasites with their

host's immune system and are interesting targets for glycoconjugate vaccines.[6,8,9] GPI anchors are one of the main cell surface carbohydrate components of many protozoa, such as *Plasmodium falciparum*, the causative agent of malaria.[46] Synthetic *P. falciparum* GPI containing glycoconjugates proved to be a prototype anti-toxic vaccine against malaria in mice models, since they elicited anti-GPI antibodies that neutralized the toxicity of the parasite without killing it.[47] This finding led to a deeper investigation of the anti-GPI antibody response as well as the required minimal epitope to induce an immune response by glycan microarray studies with several GPI substructures. [48-50]

Glycan microarrays are powerful tools to study the role of cell surface carbohydrate antigens and their interactions with glycan binding antibodies.

1.3 Glycan Microarrays

Glycan arrays are produced by spot-wise immobilization of glycans on a glass-slide surface; synthetic glycans that contain an amine handle are typically reacted with the *N*-hydroxysuccinimide ester derivatized surface to form a covalent amide bond (Fig. 5).[51] The slides are then incubated with samples containing anti-glycan antibodies. After washing, the bound antibodies are labeled with a fluorescent secondary antibody. The glycan spots that bind the anti-glycan antibodies are read out using a fluorescence scanner.

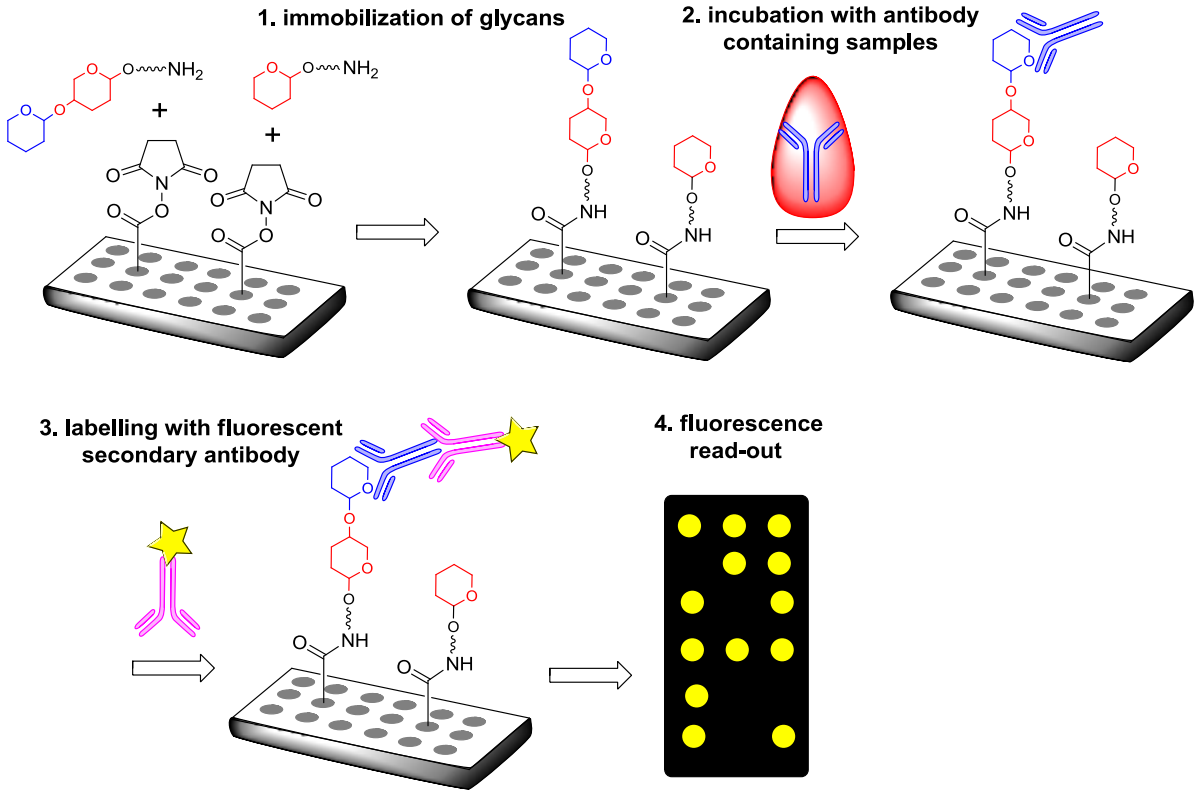


Figure 5: Work-flow of glycan microarray screening experiments.

The high sensitivity of this method allows for miniaturization that results in glycan-spot diameters of merely 200 µm. Consequently, one glass slide contains several thousand glycan spots and only requires nanomole amounts of glycan. The high sensitivity also enables the antibody-containing samples, such as sera or other body fluids, to be applied in a high dilution, thereby minimizing the amount of sample needed. Typically 64 samples are analyzed against one dozen glycans in parallel on a single slide, allowing for high-throughput screening.[49,50,52,53]

Glycan microarrays can be applied in two crucial steps of glycoconjugate vaccine design. Firstly, the identification of immunodominant cell surface glycan epitopes against which anti-glycan antibodies are elicited in infected individuals; and secondly, evaluation of the immune response of subjects immunized with a glycoconjugate. As described previously in chapter 1.1, an individual infected with a pathogen is likely to produce antibodies against certain glycan epitopes that are exposed on the pathogen's cell surface. A glycan array containing a library of glycan substructures derived from the pathogenic glycans can be used to detect antibodies binding to certain structural motifs and thereby identify the minimal immunodominant epitopes. These immunodominant epitopes should consequently be included in a glycoconjugate vaccine.

Access to large amounts of pure, well defined oligosaccharides is a prerequisite for detailed studies and currently poses the major bottle-neck in the advancement of the entire glycan research field.[54-56] One of the main objectives of this thesis is to provide access to the required oligosaccharides by chemical synthesis.

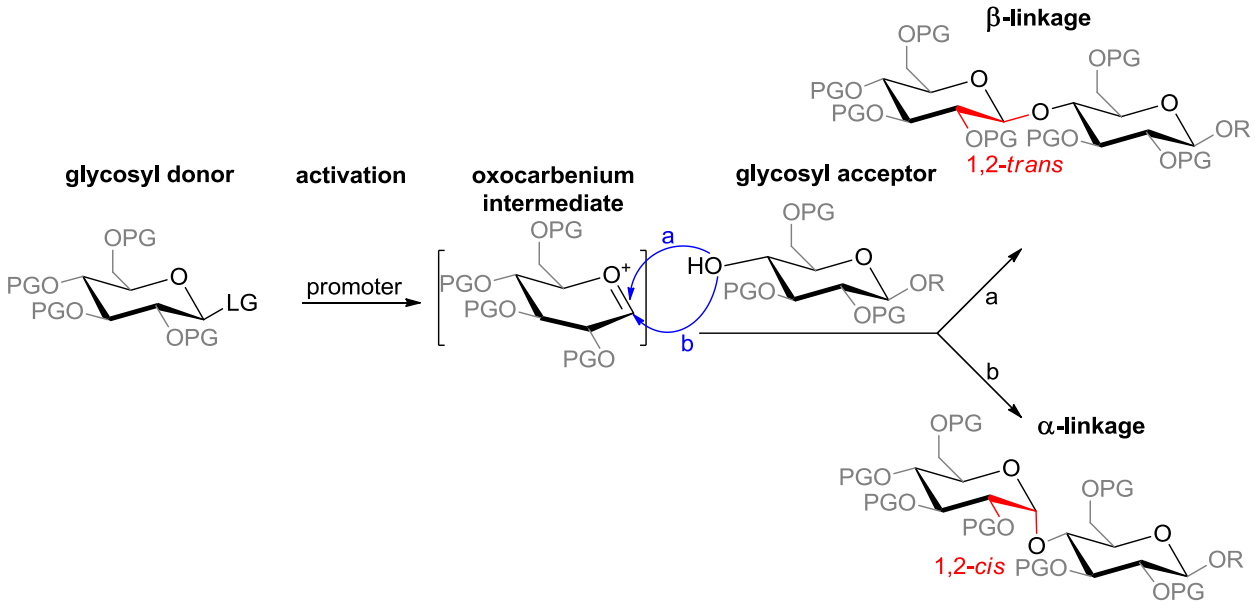
1.4 Oligosaccharide Synthesis

The chemical synthesis of oligosaccharides is positioned between the domains of bioorganic chemistry and total synthesis. On the one hand, oligosaccharide synthesis can be seen as a sequential assembly of monosaccharide building blocks through repetitive coupling and deprotection steps, similar to the syntheses of peptides and oligonucleotides. Consequently, this approach allows the assembly of oligosaccharides by automated solid phase synthesis.[57-63] The complexity of glycan structures, on the other hand, is far greater than that of linear oligonucleotide or peptide molecules, therefore synthetic approaches that resemble syntheses of natural products are often used.[64,65]

Several features determine the chemistry of oligosaccharide synthesis. A typical hexose monosaccharide forms a cyclic hemiacetal resulting in a six-membered "pyranose"

ring that contains five stereocenters. The hemiacetal position is called "anomeric" or "reducing end" and can adopt two diastereomeric forms called "anomers" that are designated as α or β. The stereoselective formation of a glycosidic linkage at the anomeric position is one of the greatest challenges of oligosaccharide synthesis. Besides the hemiacetal, the ring contains four hydroxyl-groups that are axially or equatorially oriented when the ring adopts a chair conformation. Due to the similar reactivity of the hydroxyl-groups, regioselective installation and removal of protecting groups is a further challenge of oligosaccharide synthesis.

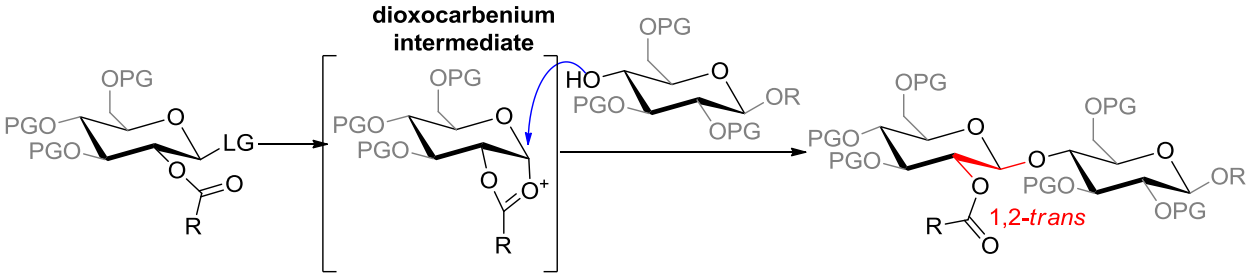
The key step in oligosaccharide synthesis is the formation of a glycosidic bond by chemical glycosylation. A chemical glycosylation is formally an irreversible nucleophilic displacement of an anomeric leaving group of a glycosylating agent, also called a glycosyl "donor", by a nucleophilic hydroxyl group of a glycosyl "acceptor" (Scheme 1). Upon "activation" of the glycosylating agent by the promoter-assisted departure of the leaving group, an oxocarbenium ion is formed. This oxocarbenium intermediate is vulnerable for nucleophilic attack from both the top or bottom face of the ring, resulting in two possible diastereomeric products.[66,67]



Scheme 1: Mechanism of a chemical glycosylation. LG: leaving group; PG: protecting group

As illustrated in Scheme 1, nucleophilic attack from the top face of the oxocarbenium ion results in the 1,2-*trans*, β-product; attack from the bottom face results in the 1,2-*cis*, α-product. The "anomeric effect" favors formation of the α-anomer, whereas the β-anomer is favored by kinetic control.[68] The orientation and protecting group of the substituent at the *C*-2 position has a major effect on the stereoselectivity of the reaction. The presence of a

"participating" group at *C*-2 strongly favors the formation of a 1,2-*trans* linkage *via* a dioxocarbenium intermediate, that allows nucleophilic attack only from one face (Scheme 2).[66,67] Non-participating groups at *C*-2, such as ethers or azides, typically promote formation of the α-glycosidic bond.



Scheme 2: Mechanism of 1,2-*trans* selective glycosylations under participation of the *C*-2 substituent.

A glycosylation reaction is affected by a number of reaction condition variables, such as the reaction solvent, the temperature and promoter.[66,67] The most important factor that determines the progress of a glycosylation, however, is the design of the monosaccharide building blocks employed.[69]

1.4.1 Building Block Design

The outcome of a glycosylation reaction is to a large extent inherently programmed into the building blocks employed for the particular reaction. A monosaccharide building block is modified with permanent protecting groups, temporary protecting groups and the anomeric leaving group. Permanent protecting groups are very stable groups that are removed from the assembled oligosaccharide targets in the very last steps of the synthesis; usually they consist of aromatic ethers that are cleaved by hydrogenation or relatively inert esters that are removed under strongly basic conditions.[66,67] Temporary protecting groups mask a particular hydroxyl-group during a glycosylation step, only to be removed afterwards to yield the free alcohol as a point of elongation for a subsequent glycosylation. A requirement for temporary protecting groups is that the conditions under which they are cleaved are orthogonal to the other protecting groups present in the molecule. A wide array of temporary protecting groups is known, amongst them are: esters, acetals, carbonates, silyl ethers and aromatic ethers.[66,67]

The nature of the anomeric leaving group is a key property of a building block that strongly affects the outcome of a glycosylation reaction. Thioglycosides are widely used because the anomeric thiol-group can be activated with a large number of electrophilic promoters under various temperature and solvent conditions.[70-73] Furthermore, thioglycosides

are stable to a large number of reaction conditions employed in protecting group pattern modifications of building blocks. A further advantage of thioglycosides is their convertibility to other leaving groups that can result in glycosyl-imidates and glycosyl-phosphates. Glycosyl-trichloroacetimidates are also widely used glycosylating agents because they can be activated with catalytic amounts of a Lewis acid promoter at low temperatures, thereby making them compatible with acid-labile protecting groups.[74] However, glycosyl-trichloroacetimidates can have two disadvantages; their stability during storage and the fact that the nitrogen atom of the leaving group can act as a nucleophile, competing with the glycosyl acceptor and leading to undesired side reactions. These disadvantages are overcome by use of the *N*-phenyltrifluoroacetimidate leaving group that gives storage-stable building blocks and minimizes side-reactions during glycosylation.[75] Glycosyl-phosphates are very reactive and therefore activated at very low temperatures with stoichiometric amounts of Lewis acid.[76-78]

The design, synthesis, evaluation and optimization of a particular building block for a specific glycosylation reaction is carried out case-by-case and is therefore the most time consuming task in oligosaccharide synthesis. Fine-tuning of the complex interplay between protecting group pattern, leaving group and reaction conditions is necessary for the successful assembly of synthetic glycan antigens.

1.5 Thesis Goal and Objectives

The overall goal of this dissertation is to contribute to prevention, therapy and diagnosis of infectious diseases worldwide. In pursuit of this goal, research was conducted on three disease-causing pathogens. Firstly, the *Leishmania* parasite that infects millions of people in developing countries. Secondly, the nosocomial infections-causing bacterium *C. difficile* that is predominantly found in healthcare settings of developed countries. Thirdly, *S. pneumoniae* that is one of the leading causes of infectious diseases in children worldwide.

The first objective of this dissertation is the chemical synthesis of glycan antigens found on the pathogens' cell surface. The second objective is the biological evaluation of the synthetic antigens with a particular focus on the identification of minimal glycan epitopes. Taken together, both objectives provide a strategy for the rational design of synthetic carbohydrate vaccines.

The synthesis and evaluation of the immunogenicity and diagnostic potential of oligosaccharides found on the cell surface of *Leishmania* parasites is described in Chapter 2. Synthesis of a small library of oligosaccharides should allow for the identification of relevant epitopes for the development of diagnostics or a vaccine.

Chapter 3 describes the research conducted on oligosaccharides derived from two *C. difficile* cell surface antigens named PS-I and PS-III. I planned to develop a synthesis that gives access to sufficient amounts of the PS-I pentasaccharide repeating unit and several substructures as a basis for the subsequent identification of the minimal PS-I epitope. For the synthesis of phosphodiester-containing PS-III oligosaccharides, a synthetic strategy had to be developed that relies on carbohydrate and phosphoramidite chemistry.

The work on the CPS repeating unit of *S. pneumoniae* serotype 5 (SP5) is covered in Chapter 4. The main focus of the chemical synthesis lay on the assembly of a library of oligosaccharides that contain two very rare monosaccharide residues. The oligosaccharide library was used to identify antigenic SP5 CPS substructures and lays the foundation for rational pneumococcal vaccine design.

2 Synthesis, Immunogenicity and Diagnostic Potential of Cell Surface Glycan Antigens of *Leishmania*

2.1 Introduction

2.1.1 *Leishmania* Parasites and Leishmaniasis

Protozoan *Leishmania* parasites are endemic in many tropical and subtropical regions of Asia, Africa, South America and the Mediterranean basin. [79] They are transmitted through the bite of an infected sand fly vector and cause the disease known as leishmaniasis. It is estimated that there are 12 million currently infected, most of them in developing countries.[80] More than twenty species of the *Leishmania* genus are known to cause disease in humans.[81,82]. Leishmaniasis is a diverse and complex disease that can cause four clinical syndromes: cutaneous leishmaniasis (CL), muco-cutaneous leishmaniasis, visceral leishmaniasis (VL, also known as kala-azar) and post-kala-azar dermal leishmaniasis (PKDL). If left untreated, VL is a serious systemic disease that is often fatal.[79] Two predominant *Leishmania* species cause VL in different regions of the world: *Leishmania donovani* in East Africa and India; *L. infantum* (also known as *L. chagasi*) in North Africa, South America and Europe. There are two types of VL, anthroponotic and zoonotic, which differ in their transmission pathway. Anthroponotic VL is transmitted from humans to humans through the sand fly vector and is mostly caused by *L. donovani*. Zoonotic VL is transmitted from animals to humans and is mostly caused by *L. infantum* (*L. chagasi*).[79,83,84] Dogs are the main reservoir host of *L. infantum* in regions where zoonotic VL is endemic; therefore prevention, diagnosis and treatment of canine leishmaniasis has a direct impact on human health in these regions.

2.1.2 *Leishmania* Vaccines

Anti-leishmanial vaccines are in different stages of development. [80,85] Whole cell vaccine approaches rely on the use of attenuated parasites or related nonpathogenic species, as well as whole killed parasites.[85] Several studies focus on DNA subunit vaccines that have recently been shown to be protective in animal models.[86,87] A different strategy employs the saliva or saliva components of the transmitting sand fly vector as vaccines.[85] Despite these

developments, no vaccine for humans is currently available. In the case of canine leishmaniasis, progress has been faster with a first subunit vaccine licensed in Brazil.[88]

2.1.3 *Leishmania* Diagnostics

A further aspect in the control of VL is the development of a diagnostic test. Ideally such a test would be fast, easy to perform as well as highly sensitive and specific. Additionally, it should be able to identify acutely diseased individuals with asymptomatic infections. These confirmatory tests are particularly important, since the treatment of leishmaniasis relies on highly toxic anti-parasitic drugs that should only be administered to acutely diseased patients.[79] Microscopic examination of tissue samples, PCR techniques and parasite cultures are currently the most widely used tests because of their high specificity. However, the sensitivity varies depending on the origin of the samples, the ability of the laboratory workers and the quality of the reagents.[89]

A different approach relies on the detection of leishmanial antigen-specific antibodies, however, these tests are biased by variations in antibody levels.[90,91]. Low anti-leishmania antibody levels are often observed in non-invasive CL infections or co-infections in immunocompromised individuals, such as HIV positive patients.[92]

Antigen-based diagnostic tests rely on direct detection of *Leishmania*-specific antigens that are presented directly on the parasitic cell surface and are therefore not biased by variations in antibody levels in individuals.[79,89] These tests require antibodies to specifically target cell surface antigens of the respective pathogen.[93,94] In the case of *Leishmania* parasites, the LPG consists of major cell surface glycoconjugates that could qualify as target antigens.

2.1.4 Leishmanial Lipophosphoglycan

As mentioned in chapter 1, LPG is expressed in high density on the parasitic cell surface and plays an important role in host-parasite interactions.[7-10] The structure of LPG varies between *Leishmania* species and the stages of their life-cycle. Its dominant structural feature is the conserved disaccharide phosphate repeating unit consisting of a β-Gal-(1→4)-α-Man-(1→*P*) backbone (Fig. 6).[7,95,96] The substituents branching from this backbone can vary between different species.

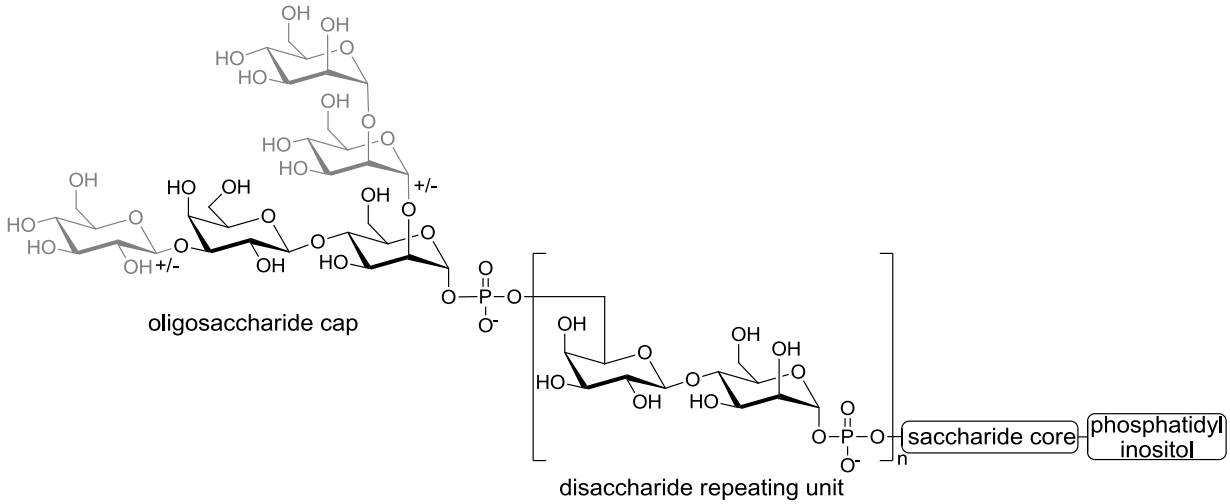


Figure 6: Structure of the disaccharide phosphate backbone and oligosaccharide caps.

The outermost LPG region is terminated by a neutral capping oligosaccharide htat also varies between different species. The oligosaccharide caps most commonly found on *L. donovani* are branched and consist of β-Gal-(1→4)-α-Man and α-Man-(1→2)-α-Man substructures.[7,96] *L. chagasi* (*L. infantum*) produces two linear oligosaccharide caps: the disaccharide β-Gal-(1→4)-α-Man and the trisaccharide β-Glc-(1→3)-β-Gal-(1→4)-α-Man.[96] The capping oligosaccharides mediate binding to the sand fly mid-gut and are vital for the parasite's transmission.[97] However, little is known about the contribution of LPG capping oligosaccharides to the immune response against the pathogen.

The capping oligosaccharides are the outermost moiety of the LPG molecule and consequently the most exposed glycans on the parasitic cell surface. Therefore, they are interesting targets for vaccine development and could also be of diagnostic relevance. In order to investigate the potential of LPG cap vaccines and diagnostics, three oligosaccharides from *L. donovani* and *L. chagasi* were synthesized.

2.2 Synthesis of Leishmanial LPG Cap Oligosaccharides

2.2.1 Synthetic Strategy

The three leishmanial lipophosphoglycan (LPG) cap oligosaccharides **1**–**3** consist of three monosaccharides: mannose, galactose and glucose (Fig. 7). All three glycans share the β-Gal-(1→4)-α-Man disaccharide motif of **2**. In the case of β-Glc-(1→3)-β-Gal-(1→4)-α-Man(1→Linker) **3**, the *C*-3 of Gal is elongated by a β-Glc residue. The branched tetrasaccharide α-Man-(1→2)-α-Man-(1→2)-[β-Gal-(1→4)]-α-Man(1→Linker) **1** consists of the disaccharide Gal-(1→4)-Man motif that carries two additional mannose residues. To investigate the influence of the linker length on the immunological properties, **1** was synthesized with two different linkers. Tetrasaccharide **1a** was equipped with the longer aminopentyl linker and **1b** with the shorter aminoethyl linker at the reducing end.

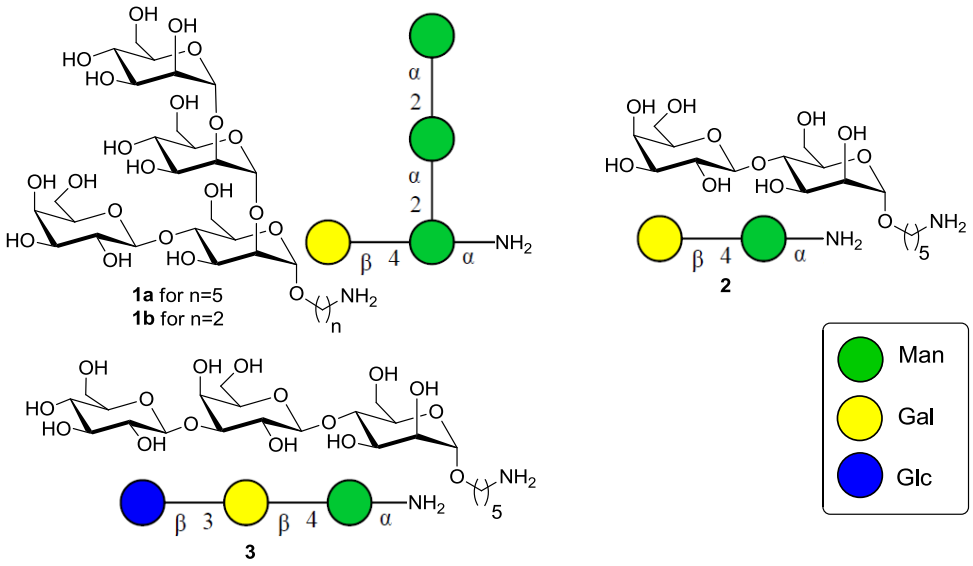


Figure 7: Synthetic LPG capping oligosaccharides **1**–**3**. Tetrasaccharide **1** is found on *L. donovani*. Disaccharide **2** and trisaccharide **3** are found on *L. chagasi* (*L. infantum*).

Syntheses of tetrasaccharide **1** equipped with different linkers, by solution and solid phase synthesis, have been reported previously.[98-100] Similar to the reported approaches, synthesis of the three oligosaccharides **1**–**3** relied on the selection of building blocks **4**–**9** that were prepared according to known procedures (Fig. 8).[77,98-101]

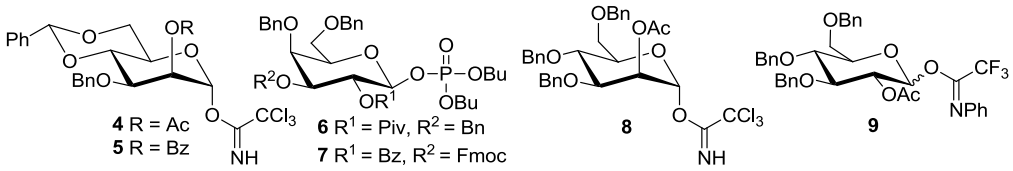
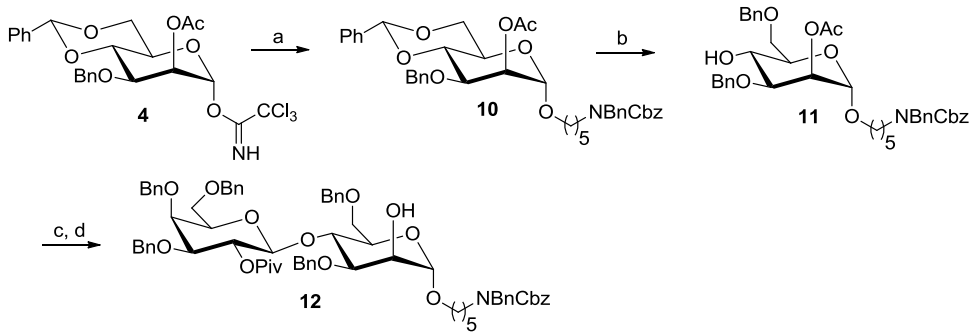


Figure 8: Monosaccharide building blocks **4**–**9** employed in the synthesis of oligosaccharides **1**–**3**.

The reducing-end mannoside residue relied on 4,6-*O*-benzylidene acetal building block **4** with an acetate or **5** with a benzoate at *C*-2.[100] Galactosyl phosphate **6** was used to introduce the terminal galactosides of **1** and **2**. The *C*-2 participating pivaloyl group in **6** was chosen in order to ensure β-selectivity.[77] Temporarily protected 3-*O*-Fmoc galactosyl phosphate **7** was synthesized by Dr. Fumiko Matsumura and used for synthesis of **3**. The benzoate at *C*-2 of **7** was designed to ensure β-selectivity. Terminal glucoside of **3** was installed using glucosyl-imidate **9**. The di-mannose motif of **1** relied on mannosyl-trichloroacetimidate **8**. [101]

2.2.2 Assembly of the Capping Oligosaccharides

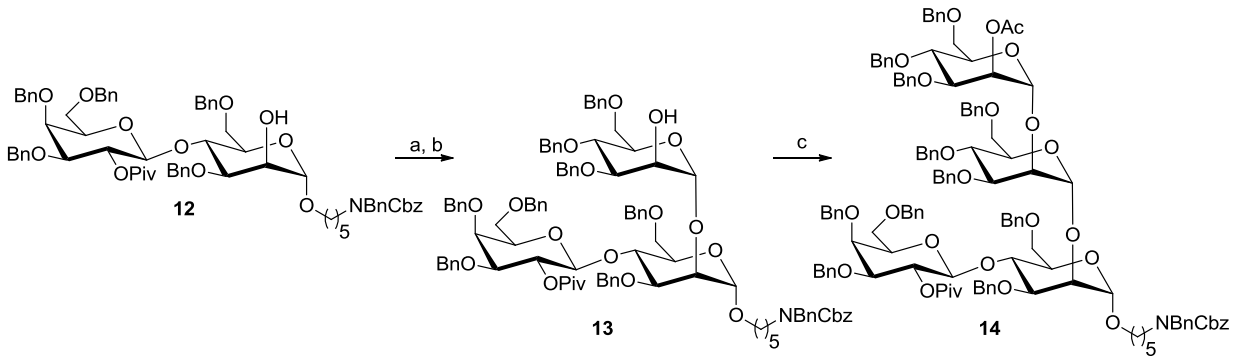
Synthesis of the disaccharide motif β-Gal-(1→4)-α-Man, common to all three glycans and bearing an aminopentyl linker at the reducing end, started with the completely α-selective glycosylation of mannosyl-imidate **4** with the *N*-BnCbz protected linker in a mixture of DCM and Et_2O at 0 °C yielding **10** (Scheme 3).



Scheme 3: Synthesis of **12**. Reagents and conditions: a) $HO(CH_2)_5NBnCbz$, TMSOTf, DCM/Et_2O, 0 °C, 90%; b) TES, TfOH, DCM, 4Å MS, –78 °C, 63%; c) **6**, TMSOTf, DCM, –40 °C; d) AcCl, THF/MeOH 0°C to rt, 86% over two steps

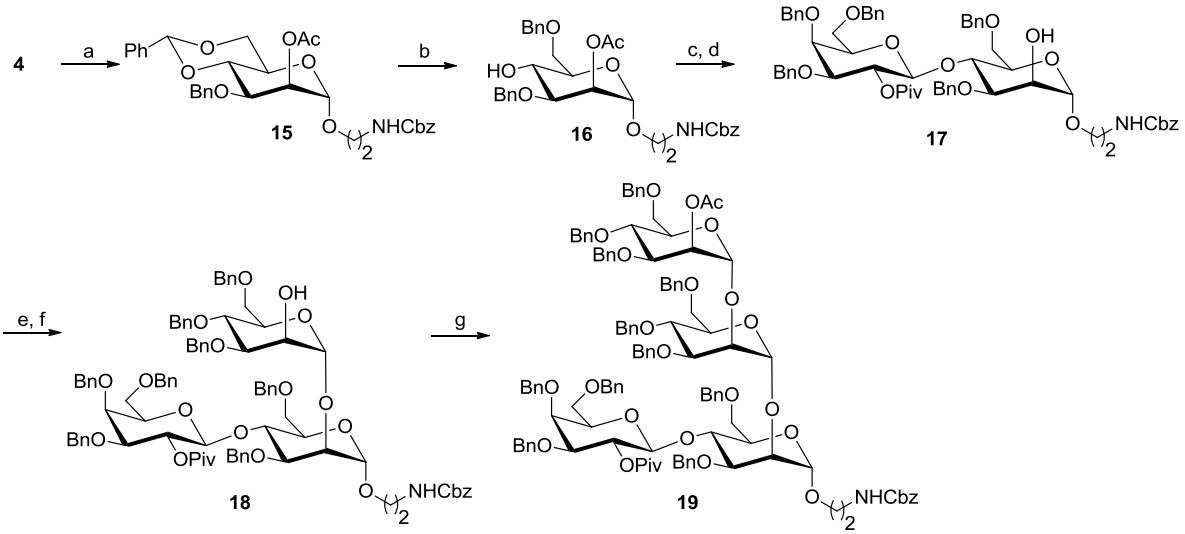
Regioselective opening of the 4,6-*O*-benzylidene acetal with TES and TfOH gave the corresponding *C*-4 hydroxyl **11** in a yield of only 63%. The low yield was attributed deacetylation at *C*-2 under the strongly acidic conditions. TMSOTf-mediated β-selective glycosylation with galactosyl-phosphate **6** gave the fully protected disaccharide which was deacetylated under acidic conditions with acetyl chloride (AcCl) in a mixture of MeOH and THF for 24 hours, yielding **12**.

Reaction of mannosyl-imidate **8** with disaccharide **12** in DCM and Et_2O at 0 °C was again followed by AcCl-mediated de-acetylation and resulted in trisaccharide **13** (Scheme 4). A further round of glycosylation with **8** finally gave fully protected tetrasaccharide **14**.



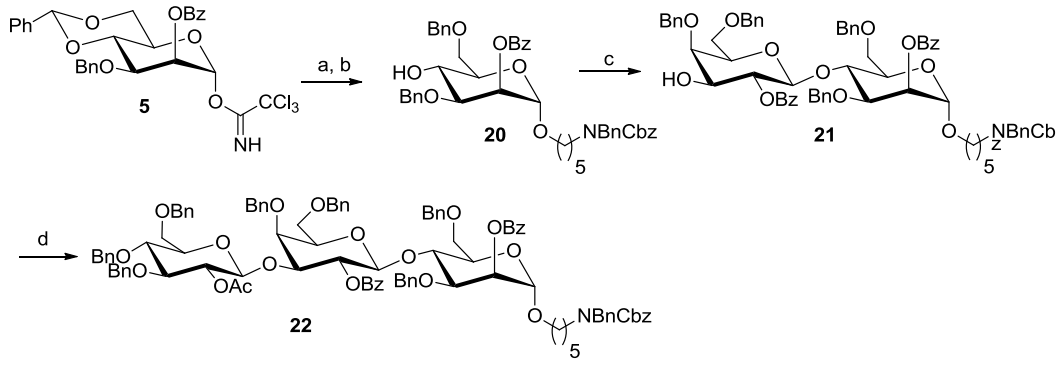
Scheme 4: Synthesis of **14**. Reagents and conditions: a) **8**, TMSOTf, DCM/Et_2O, 0 °C; b) AcCl, THF/MeOH 0 °C to rt, 73% over two steps; c) **8**, TMSOTf, DCM/Et_2O, 0 °C, 84%.

Synthesis of fully protected tetrasaccharide **19** with the shorter *N*HCbz protected aminoethyl linker was carried out very similarly to the procedure described for **14** (Scheme 5). To circumvent the long reaction times of AcCl-mediated de-acetylation, the steps resulting in **17** and **18** were carried out under Zemplén conditions in less than 1 hour.[102]



Scheme 5: Synthesis of **19**. Reagents and conditions: a) $HO(CH_2)_2NHCbz$, TMSOTf, DCM/Et_2O, 0°C, 76%; b) TES, TfOH, DCM, 4Å MS, 77%; c) **6**, TMSOTf, DCM, –40°C to –20°C; d) NaOMe, DCM/MeOH, 83% over two steps; e) **8**, TMSOTf, DCM/Et_2O, 0°C; f) NaOMe, DCM/MeOH, 69% over two steps; g) **8**, TMSOTf, DCM/Et_2O, 0°C 86%.

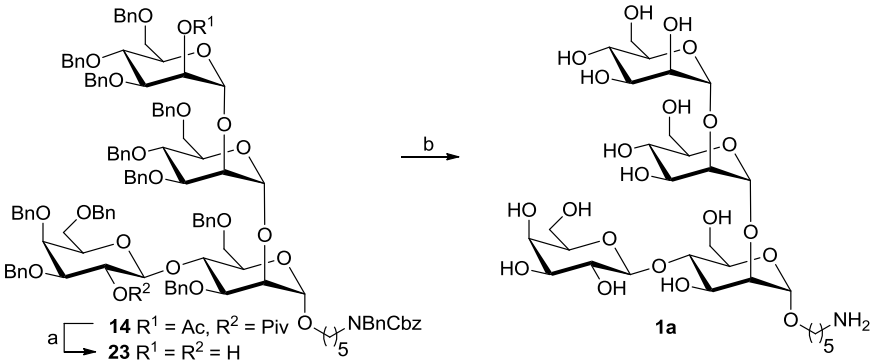
Synthesis of trisaccharide **22** relied on mannosyl-imidate **5**, functionalized with a benzoyl group at *C*-2 (Scheme 6). During regioselective 4,6-*O*-benzylidene acetal opening following glycosylation to the linker, the benzoate group proved to be more stable than the acetate used previously. The TMSOTf-mediated glycosylation of **20** with galactosyl-phosphate **7** was quenched by the addition of triethylamine, which also removed the temporary 3-*O*-Fmoc group, yielding **21**. Finally, elongation with glucosyl-imidate **9** resulted in fully protected trisaccharide **22**.



Scheme 6: Synthesis of **22**. Reagents and conditions: a) $HO(CH_2)_5NBnCbz$, TMSOTf, DCM/Et_2O, 0 °C; b) TES, TfOH, DCM, 4Å MS, –78 °C, 78% over two steps; c) **7**, TMSOTf, DCM, -40 °C to -20 °C; NEt_3, DCM 89%; d) **9**, TMSOTf, DCM, –40 °C to –20 °C, 53%.

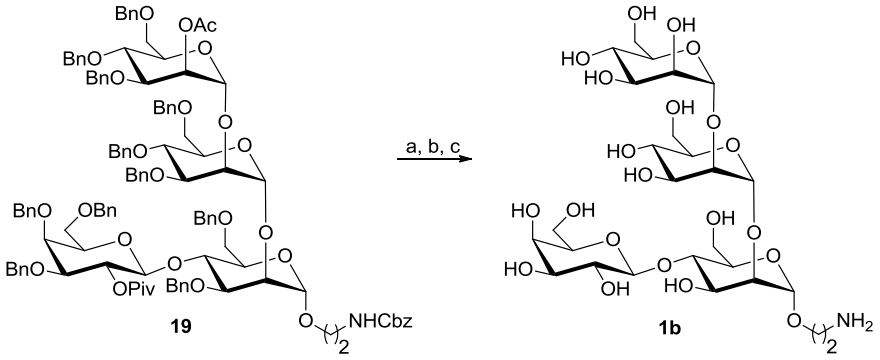
2.2.3 Deprotection of the Capping Oligosaccharides

Global deprotection of fully protected oligosaccharides **14**, **19** and **22** required two transformations: first, saponification of the ester protecting groups and secondly, Pd-catalyzed hydrogenolysis of the aromatic groups. Treatment of tetrasaccharide **14** with NaOMe or NaOH resulted in rapid de-acetylation. However, cleavage of the pivaloyl-ester proceeded sluggishly, even at elevated temperatures over several days (Scheme 7).



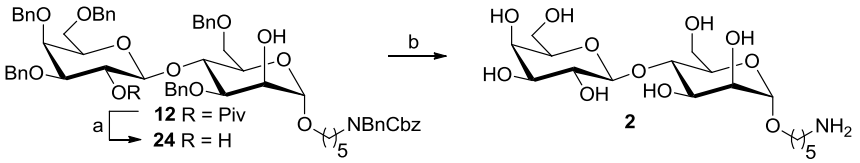
Scheme 7: Synthesis of **1a**. Reagents and conditions: a) NaOMe, MeOH/THF, microwave (3W, 100 °C, 5 bar), 92%; b) H_2, Pd/C, THF/MeOH/H_2O/AcOH, 77%.

To accelerate cleavage of the inert pivaloyl-ester, a microwave reactor was employed that reduced the reaction time to one hour, yielding **23**. The subsequent hydrogenolysis gave fully deprotected tetrasaccharide **1a**, equipped with the aminopentyl linker at the reducing end. The microwave assisted de-acylation was also applied to tetrasaccharide **19** with the shorter *N*HCbz protected linker. In this case however, cleavage of the Cbz group and formation of a methyl-carbamate was observed. Milder conditions, using KOH at room temperature, only resulted in deacetylation. Therefore, the hydrogenolysis step was performed before KOH-mediated de-pivaloylation to yield tetrasaccharide **1b** equipped with the shorter linker (Scheme 8).



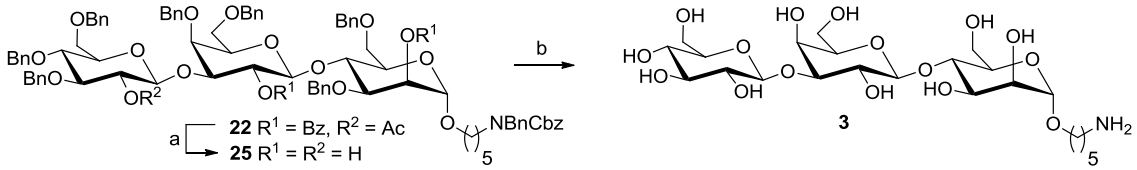
Scheme 8: Synthesis of **1b**. Reagents and conditions: a) KOH, MeOH/THF/H_2O; b) Pd/C, H_2, MeOH/THF/H_2O/AcOH; c) 0.25N KOH, H_2O, 54% over three steps.

Deprotection of disaccharide **12** was carried out in a similar way to tetrasaccharide **1a**, using a microwave reactor yielding diol **24**, followed by hydrogenolysis to give **2** (Scheme 9).



Scheme 9: Synthesis of **2**. Reagents and conditions: a) NaOMe, THF/MeOH, microwave (3W, 100°C, 5 bar) 98%; b) Pd/C, H_2, MeOH/THF/H_2O/AcOH, 80%.

De-acylation of trisaccharide **22** was carried out under comparably mild conditions; finally, hydrogenolysis gave fully deprotected trisaccharide **3** (Scheme 10).



Scheme 10: Synthesis of **3**. Reagents and conditions: a) NaOMe, THF/MeOH, 91%; b) Pd/C, H_2, MeOH/THF/H_2O/AcOH, 70%.

Access to sufficient amounts of pure synthetic leishmanial LPG cap oligosaccharides **1a**, **1b**, **2** and **3** enabled the first investigation of the contribution of capping oligosaccharides to the immune response against the parasite.

2.3 Identification of Glycan Epitopes

To assess the contribution of LPG capping oligosaccharide epitopes to the immune response and to identify diagnostically relevant glycan epitopes, a glycan array was prepared containing glycans **1**–**3**, as well as trisaccharide α-Man-(1→2)-α-Man-(1→2)-α-Man-(1→Linker) **26** that was synthesized by Dr. Dan Grünstein (Fig. 9).

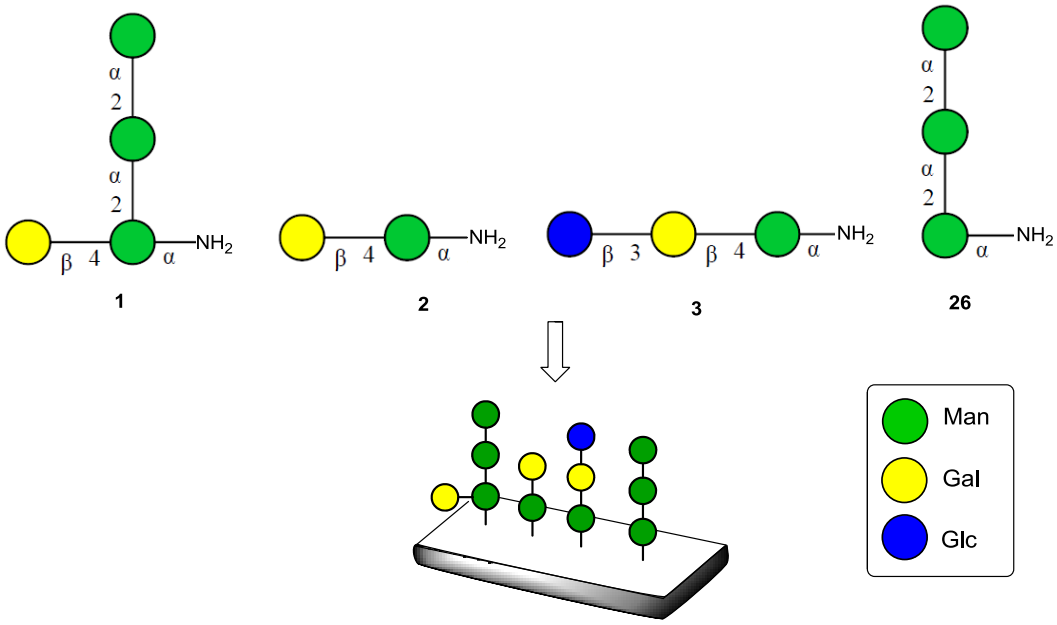


Figure 9: Schematic depiction of a leishmanial LPG capping glycan array. Tetrasaccharide **1** and trisaccharide **26** are found on *L. donovani*. Disaccharide **2** and trisaccharide **3** are found on *L. chagasi* (*L. infantum*).

The microarray was used to screen a positive anti-*L. chagasi* reference serum from a commercial immunofluorescence test kit for anti-oligosaccharide antibodies. The negative reference serum provided in the kit was used as a negative control. The antibodies that bound the oligosaccharides were then labeled with an anti-dog secondary antibody (Fig. 10).

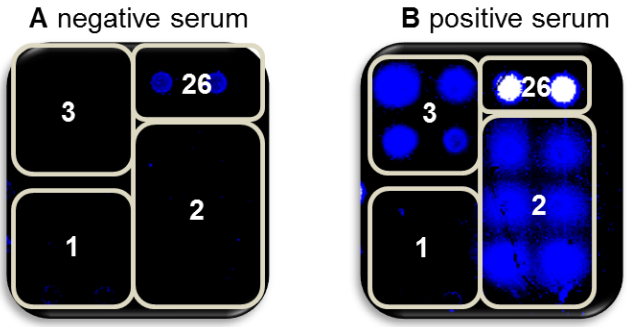


Figure 10: Glycan array screening of reference canine serum from a commercial diagnostic kit for anti-glycan antibodies. **A**) A representative well from glycan array analysis of a negative control canine serum plus the secondary anti-dog antibody; **B**) A representative well from glycan array analysis of positive canine reference serum showing reactivity of secondary anti-dog antibody. Positions of respective glycans **1**, **2**, **3** and **26** are shown with the numbers. Fluorescent spots indicate antibody binding.

Fluorescence-based readout of the microarray revealed that the negative control serum contained IgG only against tri-mannose **26** (Fig. 10A). In the positive reference serum, the IgG response against tri-mannose **26** was higher than in the negative control; additionally, IgG against *L. chagasi* disaccharide **2** and trisaccharide **3** were detected (Fig. 10B). IgG that recognize *L. donovani* tetrasaccharide **1** were neither detected in the negative, nor in the positive serum. This discovery is surprising, because tetrasaccharide **1** contains the Gal-

(1→4)-Man motif, of **2** and **3**, as well as the tri-mannose motif of **26** that are recognized by IgG in the positive serum.

These findings imply that tri-mannose **26** is not strictly *Leishmnania*-specific since anti-**26** antibodies are present in the negative control sera, the higher anti-**26** response of the positive sera, however, indicates that it could play a role in leishmanial infections. The recognition of **2** and **3** *L. chagasi* LPG caps by the positive sera only, leads to the conclusion that these are antigens that interact with the immune system of dogs during a leishmanial infection. Consequently, detection of these oligosaccharides can be of diagnostic importance in the case of zoonotic leishmaniasis. Furthermore, these initial results suggested that disaccharide **2**, which is also present in the LPG disaccharide phosphate repeating unit could be a minimal antigenic epitope of the leishmanial LPG.

To further validate these results, we conducted a broader screening with samples from 50 infected dogs, tested leishmaniasis-positive, and 25 asymptomatic dogs, tested negative, from *Leishmania* endemic areas in Greece. The samples were provided by Professor Maria Antoniou (University of Crete, Greece). No significant differences in IgG response were observed between the dogs from endemic areas that were tested positive or negative for leishmaniasis. This could be explained by the fact that even dogs tested negative have been exposed to the parasite in the past. Most distinctively, high variations of anti-glycan IgG responses were observed between individual dogs. Most samples contained IgG against tri-mannose **26**; IgG against tetrasaccharide **1** and disaccharide **2** were observed in approximately 40% of the samples and only a few samples contained anti-**3** IgG. Two exemplary samples are shown in Figure 11. Sample A contains IgG against glycans **1**, **2**, and **26**, whereas sample B contains IgG against glycans **2**, **3**, and **26**.

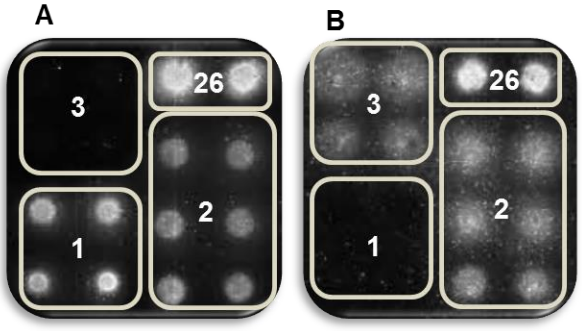


Figure 11: Glycan array screening of two exemplary canine sera from endemic areas for anti-glycan antibodies. Positions of respective glycans **1**, **2**, **3** and **26** are shown with the numbers.

The results from these screenings support the notion that the tri-mannose epitope of **26** might play a role in leishmania infections. The fact that approximately 40% of tested samples recognize tetrasaccharide **1** and the disaccharide **2** Gal-(1→4)-Man epitope, implies that these

could be weakly immunogenic *Leishmania* antigens. The low response to trisaccharide **3** suggests that this glycan is of lesser importance.

The screenings also revealed that due to the high variation of antibody-levels between individuals, none of the synthetic glycans can be used in a diagnostic test that relies on the detection of antigen-specific antibodies. Therefore, we planned a strategy that relies on the detection of capping oligosaccharides directly on the parasitic cell surface by antibodies. These antibodies would be generated by the immunization of mice with a glycoconjugate containing a synthetic glycan. Ideally, this glycan hapten would be able to elicit antibodies against the Gal-(1→4)-Man and tri-mannose motifs that were identified by the screening experiments. Therefore, tetrasaccharide **1** that contains both the Gal-(1→4)-Man and tri-mannose epitopes was selected for immunogenicity studies to raise antibodies. Since **1** does not contain any sugars foreign to the mammalian glycome, it was considered likely to be a weakly immunogenic antigen. The nature of the cross-linker between the protein and glycan is known to have a profound effect on the anti-glycan immune response, particularly when weakly immunogenic antigens are employed.[33,34] In order to study this effect with the selected tetrasaccharide, glycoconjugates were prepared by Dr. Chakkumkal Anish containing either **1a** with the longer linker or **1b** with the shorter linker.

2.4 Immunological Evaluation

The immunological evaluation was carried out by Dr. Chakkumkal Anish and therefore is only described briefly here. A full account is given in our recent publication.[103] It was found that a CRM197-**1a** conjugate only elicited a weak immune response against the glycan and a strong response against the cross-linker. In order to generate a robust immune response against the glycan, mice were first immunized with a CRM197-**1a** conjugate and boosted with a CRM197-**1b** conjugate after one week. This strategy suppressed the maturation of anti-cross-linker response and promoted a strong anti-glycan response, resulting in mice sera that contained antibodies that bound to the synthetic glycans on a microarray. More importantly, it was shown by immunolabelling studies of inactivated parasites that polyclonal sera raised against the leishmanial glycans detected the parasite with high specificity.

2.5 Conclusion and Perspectives

The identification, synthesis and evaluation of a set of *Leishmania* LPG capping oligosaccharides were pursued. Chemical synthesis relying on a set of monosaccharide building blocks gave access to pure oligosaccharides **1a**, **1b**, **2** and **3**. Microarray screenings with synthetic oligosaccharides enabled the identification of two immunologically relevant epitopes, namely β-Gal-(1→4)-α-Man and α-Man-(1→2)-α-Man-(1→2)-α-Man. Successful immunization experiments were only made possible by chemical synthesis of two variants of tetrasaccharide **1**, differing in linker length. Due to its low immunogenicity, the synthetic glycan does not qualify as a vaccine candidate. However, the fact that antibodies raised against synthetic tetrasaccharide **1** actually bind the glycan epitopes present on the cell surface of *Leishmania*, implies that these antibodies could form the basis for a diagnostic test based on the detection of the parasite through its cell surface glycans.

3 Synthesis and Evaluation of *Clostridium difficile* Cell Surface Glycan Antigens

3.1 Introduction

3.1.1 *Clostridium difficile* Infection

Clostridium difficile is a Gram-positive, spore-forming bacterium that causes *C. difficile* infection (CDI). CDI is the most commonly diagnosed cause of hospital-acquired diarrhea worldwide and can be life threatening.[104-106] *C. difficile* can colonize the gut when the normal intestinal flora is disturbed, as is the case during antibiotic treatment; therefore CDI is also classified as antibiotic-associated diarrhea (AAD).[107] In the absence of commensal bacteria, antibiotic-resistant *C. difficile* spores can germinate, leading to colonization and overgrowth of the intestinal epithelium.[105] Two toxins, called toxin A and B, cause the clinical symptoms of CDI; these range from asymptomatic colonization to various diarrhea syndromes, and in severe cases can result in colitis, sepsis, shock and even death.[107] The main risk groups for infection are elderly hospitalized patients undergoing antibiotic treatment, however, in the past decade the numbers of infections have risen drastically and CDI now also affects children, young adults and pregnant women.[107-110] The steep rise in infections is attributed to the prevalence of resistant *C. difficile* spores in healthcare facilities and the emergence of new hypervirulent *C. difficile* strains that display increased virulence, toxin production and antibiotic resistance.[111-113] Ribotype 027 is one of these hypervirulent strains found in hospitals in North America, Europe, Asia and Oceania, in addition to toxins A and B, it produces a third toxin, called toxin C.[113,114]

In addition to the burden of morbidity and the mortality, CDI significantly increases healthcare costs due to extended treatment of patients that were hospitalized for another illness.[115] These costs are especially high in cases of recurrent infection and re-hospitalization affecting up to 30% of patients.[115-118] Since antibiotic treatment of CDI is often inefficient,[119] an alternative based on preventive vaccination is highly desirable.

3.1.2 *Clostridium difficile* Vaccines

Although vaccines against *C. difficile* are presumed to be cost-effective over a wide range of infection risks, vaccine costs, and vaccine efficacies,[120] no licensed vaccine is currently available. The development of anti-toxin vaccines is furthest advanced to date.[105] Over thirty years ago, it was demonstrated that toxin-neutralizing active immunization can protect against lethal challenge with *C. difficile* in hamsters. [121-123] Currently, two vaccine candidates relying either on inactivated toxins A and B or on subunits thereof are being tested in clinical trials.[105] As an alternative to active immunization, which can be ineffective for immunodeficient patients, routes of passive administration of anti-toxin antibodies are also being explored.[105] Although anti-toxin vaccine approaches may be efficient in neutralizing the toxins and thereby alleviating the symptoms of CDI, they cannot prevent colonization of the pathogen that occurs before toxin secretion.

Vaccination approaches that can prevent colonization are directed against *C. difficile* cell surface antigens. Several cell wall proteins have been identified and tested as vaccine candidates.[105] Antibodies against the conserved cell wall protein Cwp84 were detected in CDI patients, demonstrating its immunogenicity.[124] Animal immunization experiments with Cwp84 partially protected hamsters from a lethal *C. difficile* challenge.[125,126] Cell surface *C. difficile* glycans also pose a formidable target for vaccine design.

3.1.3 *Clostridium difficile* Cell Surface Glycans as Vaccine Targets

Cell surface carbohydrates of *C. difficile* were first identified as immunogenic antigens in the early 1980s.[127,128] Due to their structural complexity, however, it took more than a quarter of a century until their chemical structure was determined. Currently, the structures of three cell wall polysaccharides, called PS-I, PS-II and PS-III are known (Fig. 12).[129,130] PS-I consists of a branched pentasaccharide phosphate repeating unit of the sequence [→4)-α-Rha*p*-(1→3)-β-Glc*p*-(1→4)-[α-Rha*p*-(1→3)]-α-Glc*p*-(1→2)-α-Glc*p*-(1→P] and was originally described as ribotype 027 specific but recently has also been identified on other *C. difficile* strains.[129,131] The polysaccharide PS-II is built up of the branched hexasaccharide phosphate repeating unit [→6)-β-D-Glc*p*-(1→3)-β-D-Gal*p*NAc-(1→4)-α-D-Glc*p*-(1→4)-[β-D-Glc*p*-(1→3)]-β-D-Gal*p*NAc-(1→3)-α-D-Man*p*-(1→] and has to date been identified on numerous strains and is believed to be a conserved antigen.[129-132] The classification of PS-I and PS-II as CPS or teichoic acid-like glycans is not clear-cut. For instance, PS-II repeating unit numbers vary from two to ten; low molecular weight glycans are anchored to fatty acids

and therefore LTA-like; high molecular weight polysaccharides are CPS-like with no connection to the peptidoglycan or fatty acids.[129,131,132] A third glycan, discovered several years later, is the LTA called PS-III, which mainly contains a polymer consisting of phosphodiester-linked repeating units with the sequence [→6)-α-D-Glc*p*NAc-(1→3)-[→*P*-6]-α-D-Glc*p*NAc-(1→2)-D-GroA], GroA being glyceric acid.[130] The first repeating unit of the polymer is connected *via* a phosphodiester to a glycolipid anchor with the structure (→6)-β-D-Glc*p*-(1→6)-β-D-Glc*p*-(1→6)-β-D-Glc*p*-(1→1)-Gro, where glycerol (Gro) is esterified with fatty acids.[130] Just like PS-II, PS-III is believed to be conserved amongst most *C. difficile* strains.[130,133]

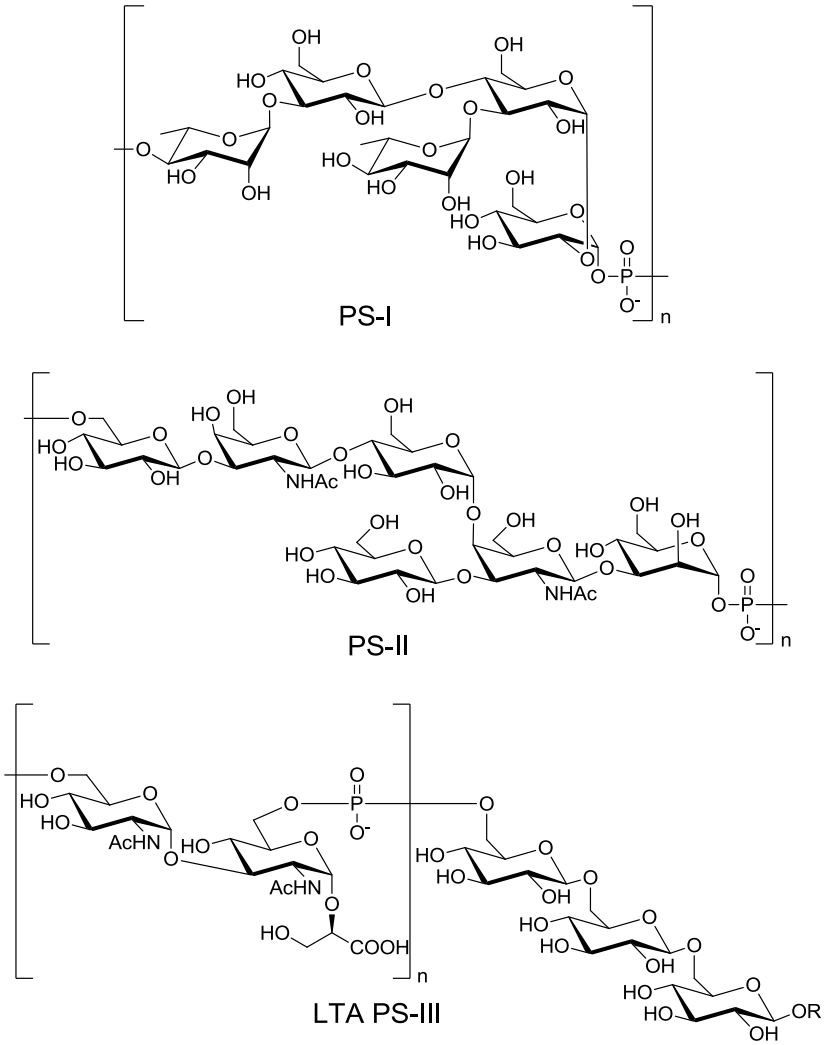


Figure 12: Chemical structure of the three known *C. difficile* cell surface polysaccharides PS-I, PS-II and PS-III.

The PS-II antigen is so far the best studied *C. difficile* cell surface glycan, in particular for its potential application as a vaccine as it was found to be abundantly expressed on most *C. difficile* strains.[129-131,134] Immunizations of mice carried out with isolated native PS-II-CRM conjugates raised high levels of anti-PS-II IgG. Furthermore, it was shown that PS-II is an exposed part of the cell wall by immunofluorescent labeling of anti-PS-II IgG binding to the

bacterial cell surface.[132] Isolated PS-II polysaccharide is also immunogenic in mice when conjugated to keyhole limpet hemocyanin (KLH) carrier protein.[134] Synthetic variants of PS-II have also been synthesized and evaluated as vaccine candidates by several groups.[132,135,136] Studies carried out by our group with the synthetic PS-II hexasaccharide repeating unit revealed that the hexasaccharide was immunogenic in mice when conjugated to CRM. These results were reproduced by another group using a synthetic hexasaccharide-CRM conjugate.[132] In the same study, a synthetic hexasaccharide that was phosphorylated at the *C*-6 position of the non-reducing end Glc residue was also synthesized and conjugated to CRM. The authors claim that although both the non-phosphorylated and the phosphorylated hexasaccharide-conjugates are immunogenic, only the antibodies generated against the phosphorylated hapten also bind to native PS-II polysaccharide and whole *C. difficile* bacteria.[132] It is also interesting to note that glycan microarray studies revealed that PS-II hexasaccharide specific IgA antibodies are present in the stool of CDI patients.[136] This implies that the immune system of the infected patients recognizes PS-II and generates antibodies against it.

In mid-2013 a publication appeared that describes the investigation of the candidacy of isolated native PS-III as a vaccine against *C. difficile*.[133] Rabbits were immunized with glycoconjugates containing PS-III; additionally, rabbits were immunized with killed whole cells of *C. difficile*. It was shown that sera of rabbits immunized with the glycoconjugate bind PS-III on the bacteria and that sera obtained from whole-cell immunizations cross-react with isolated PS-III. However, a strong immune response against the maleimide linker or bromo-acetyl linker employed was observed upon immunization with the glycoconjugates, since approximately every third repeating unit was modified with a linker.[133] A synthetic PS-III variant with a single linker-attachment point would be of advantage for reducing the undesired anti-linker response. The synthesis and preliminary biological evaluation of PS-III repeating unit oligomers is described later in this chapter.

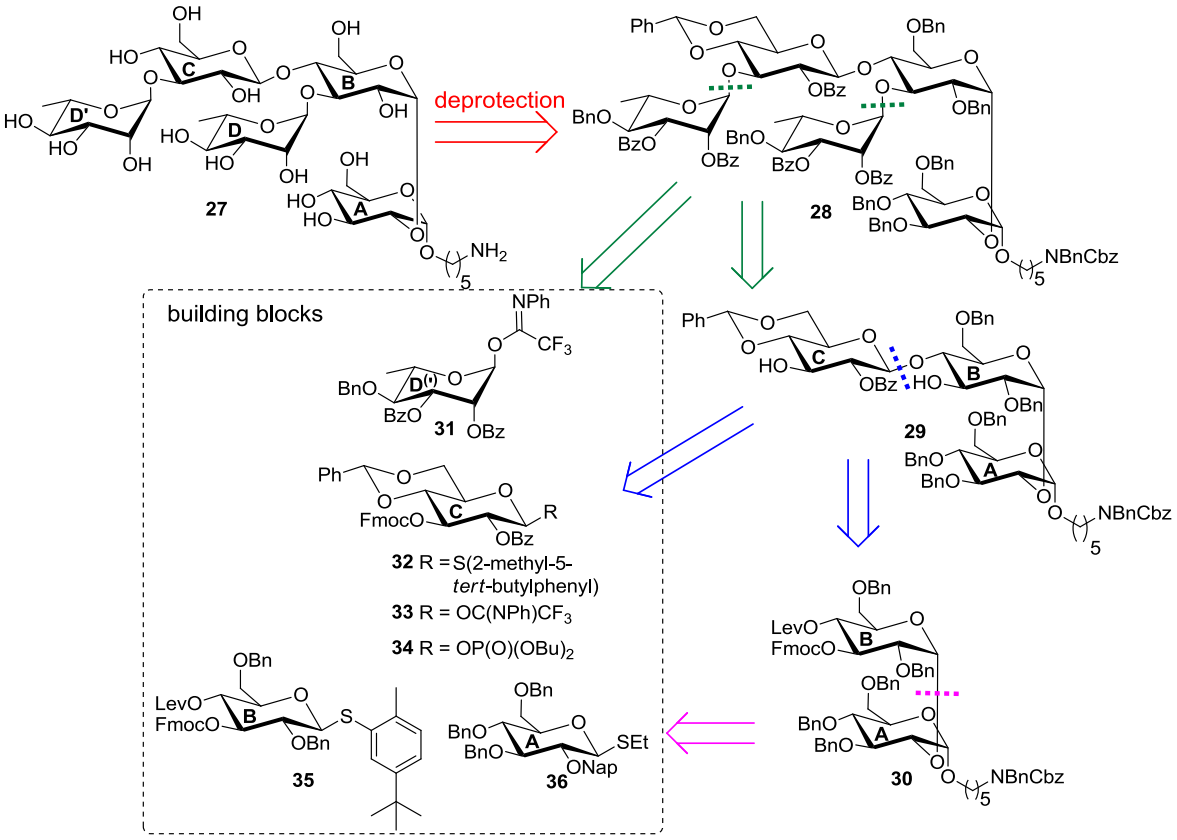
After its structural assignment in 2008, further investigation of PS-I was hampered by a lack of availability.[134] The work on the synthesis and biological evaluation of PS-I, described here, has prompted others to adopt the synthetic approach to PS-I.[137]

3.2 Synthesis of the *C. difficile* PS-I Pentasaccharide Repeating Unit

3.2.1 First Total Synthesis of the *C. difficile* PS-I Pentasaccharide Repeating Unit

3.2.1.1 Retrosynthetic Analysis

The first total synthesis of the PS-I pentasaccharide repeating unit **27** with the structure α-Rha*p*-(1→3)-β-Glc*p*-(1→4)-[α-Rha*p*-(1→3)]-α-Glc*p*-(1→2)-α-Glc*p*-(1→Linker) was approached by devising a linear strategy relying on the set of building blocks **31**–**36** (Scheme 11).



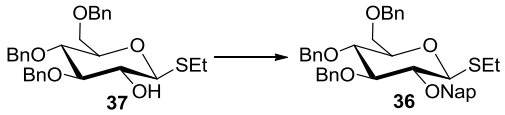
Scheme 11: Retrosynthetic analysis of pentasaccharide **27**.

The linear strategy was devised in anticipation of applying the pentasaccharide in automated oligosaccharide solid phase synthesis. Automated oligosaccharide synthesis typically relies on monosaccharide building blocks that are assembled in a linear fashion from the reducing to the non-reducing end.[57-63] In order for this strategy to succeed, high yields and stereoselectivity are required for each individual glycosylation. Pentasaccharide **27** posed two main challenges: firstly, the 1,2-*cis* glycosidic linkages of both glucosides A and B, wherein

A is equipped with the aminopentyl linker at the reducing terminus; secondly, installation of the branches extending from *C*-3 and *C*-4 of residue B. In order to overcome the first challenge, non-participating *C*-2 substituents were chosen for glucosides **35** and **36**. Hence, **36** was equipped with the temporary 2-naphthylmethyl (Nap) protecting group at *C*-2, which would be removed in order to introduce residue B with building block **35** that carries the non-participating benzyl group at *C*-2 to favor the formation of the α-(1→2) glycosidic linkage between the A and B fragments. To meet the second challenge, **35** was functionalized with the orthogonal hydroxyl-protecting groups Fmoc at *C*-3 and Lev at *C*-4; these temporary protecting groups were chosen for their compatibility with solid phase synthesis. [57-63] Selective removal of the Lev group would allow for elongation with the solid-phase compatible glycosylating agents **32**–**34**. In order to maximize the yields for this glycosylation step, thioglycoside **32**, *N*-phenyl trifluoroacetimidate **33** and glycosyl phosphate **34** would be individually evaluated for their reactivity towards the AB disaccharide in assembly of ABC trisaccharide **29**. In order to minimize the number of deprotection and glycosylation steps, both terminal Fmoc protecting groups of **28** would be cleaved in one step and Rha residues D and D' added to the trisaccharide in a single bis-glycosylation reaction with building block **31**.

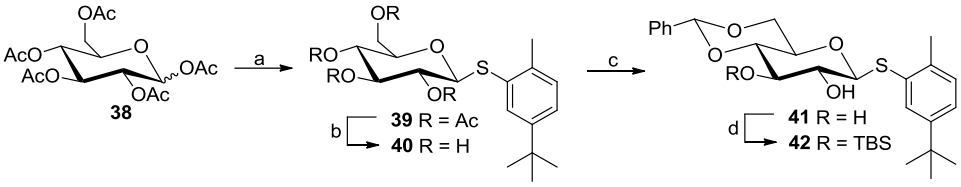
3.2.1.2 Building Block Synthesis

The linear synthetic approach relied on six monosaccharide building blocks **31**–**36**. Reducing-end building block **36** was synthesized from known intermediate **37**[138] by reaction with 2-naphthylmethyl bromide in the presence of sodium hydride (Scheme 12).



Scheme 12: Synthesis of building block **36**. Reagents and conditions: NaH, NAPBr, DMF, 0 °C to rt, 92%

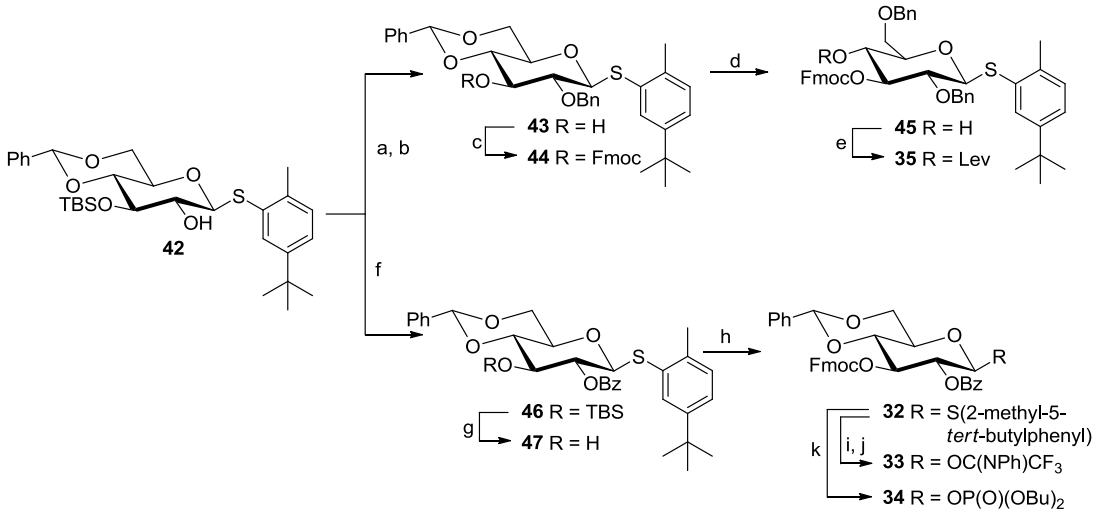
All four building blocks **32**–**35** were obtained by diversification of the single key intermediate **42**, which in turn was derived from glucose pentaacetate **38** in four steps (Scheme 13). Exclusive β-anomer formation of thioglucoside **39** was observed when 2-methyl-5-*tert*-butylthiophenol in presence of the Lewis acid boron trifluoride etherate was used. This particular aromatic thiol was chosen because it is odorless and non-toxic.[139] De-acetylation of **39** under Zemplén conditions,[102] followed regioselective formation of the 4,6-*O*-benzylidene acetal with benzaldehyde dimethyl acetal and catalytic amounts of camphorsulfonic acid resulted in 2,3-diol **41**. Key intermediate **42** was obtained by regioselective 3-*O*-TBS ether protection.



Scheme 13: Synthesis of building block **42**. Reagents and conditions: a) 2-methyl-5-*tert*-butylthiophenol, $BF_3 \cdot OEt_2$, DCM, 85%; b) NaOMe, MeOH, rt; c) benzaldehyde dimethyl acetal, CSA, MeCN, 87% over two steps; d) TBS-Cl, imidazole, DMF, 0 °C, 69%.

The orthogonal protecting group decoration of key intermediate **42** enables the further diversification of all positions of the hexose-ring. The anomeric thiol can be replaced by a variety of leaving groups known for carbohydrate synthesis; *C*-2 is readily modified by esterification or ether formation; the same is true for *C*-3 after removal of the silyl ether. Finally, regioselective opening of the 4,6-*O*-benzylidene acetal allows the further modification of either *C*-4 or *C*-6. This versatility was exploited in the synthesis of building blocks **32**–**35** (Scheme 14).

For synthesis of building block **35**, representing the branched α-Glc residue B, a benzyl ether was introduced at *C*-2 to favor the formation of the α-linkage. The branching positions *C*-3 and *C*-4 were orthogonally protected with Fmoc and Lev, respectively. Benzylation of **42** with benzyl bromide in the presence of sodium hydride was followed by de-silylation with tetrabutylammonium fluoride (TBAF), yielding alcohol **43**, 3-*O*-Fmoc-protection of the alcohol with Fmoc chloride in pyridine furnished **44**. Regioselective opening of the 4,6-*O*-benzylidene acetal with triethylsilane (TES) and triflic acid (TfOH) gave 4-hydroxyl **45**. Esterification of the free hydroxyl group was accomplished with levulinic anhydride (Lev_2O), giving building block **35**. Lev_2O, which was previously prepared from levulinic acid and dicyclohexylcarbodiimide, was chosen since it allowed esterification without the presence of a strong base which could compromise the integrity of the Fmoc group.



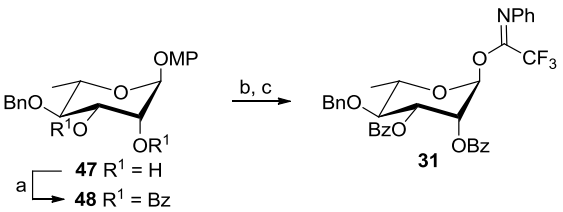
Scheme 14: Synthesis of building blocks **32–35**. Reagents and conditions: a) NaH, BnBr, DMF, 0 °C to rt; b) 1M TBAF in THF, 0 °C to rt, 93% over two steps; c) Fmoc-Cl, pyridine, DCM, 95%; d) TES, TfOH, DCM, 4 Å MS, –78 °C, 73%; e) Lev_2O, pyridine, DCM, three days, 79%; f) BzCl, DMAP, pyridine, 70 °C, 88%; g) TBAF·$3H_2O$, AcOH, DMF, 35 °C, 91%; h) Fmoc-Cl, pyridine, DCM, 96%; i) NIS, AgOTf, TTBP, MeCN, H_2O; j) $CF_3C(NPh)Cl$, Cs_2CO_3, DCM, 67% over two steps; k) $HOPO(OBu)_2$, NIS/TfOH, DCM, 4 Å MS 0°C, 81%.

Building blocks **32-34** were functionalized with a participating benzoyl group at *C*-2 to favor the formation of the anticipated β-linkage, and a Fmoc group at *C*-3. Esterification of **42** with benzoyl chloride proceeded at an elevated temperature, giving **46**. Due to the basic properties of TBAF solutions and the tendency of ester groups to migrate or cleave under basic conditions, acetic acid was added to the TBAF solution for subsequent de-silylation. At room temperature, the reaction proceeded very slowly; increasing the temperature to 50 °C resulted in migration of the benzoyl group from *C*-2 to *C*-3, yielding the 3-*O*-benzoate as the major product. Optimal results for de-silylation to produce alcohol **47** were obtained using a TBAF:AcOH ratio of 6:7 at 35 °C over nine hours. Glucoside building block **32** was completed by Fmoc protection of the free alcohol.

In order to meet the objective of identifying the best glycosylation agent for the formation of the β-Glc-(1→4)-Glc linkage, thioglycoside **32** was further diversified to the glycosylating agents glycosyl imidate **33** and glycosyl phosphate **34** (Scheme 14). Removal of the anomeric thiol group was investigated under various conditions. When *N*-iodosuccinimide (NIS) was used in combination with an acidic promoter in a solvent containing water, due to the acidity of the solution, hydrolysis of the benzylidene acetal was observed along with the formation of the free lactol. Therefore, the sterically hindered base tri-*tert*-butylpyrimidine (TTBP) was added and silver triflate was chosen as a mildly acidic promoter. The free lactol was then converted to *N*-phenyl trifluoroacetimidate **33**. Glycosyl phosphate **34** was obtained in one step by reaction of **32** with dibutyl phosphate using NIS/TfOH as the activation system.

Since anhydrous conditions were ensured by molecular sieves in the reaction mixture, no hydrolysis of the benzylidene acetal was observed despite the acidic reaction conditions.

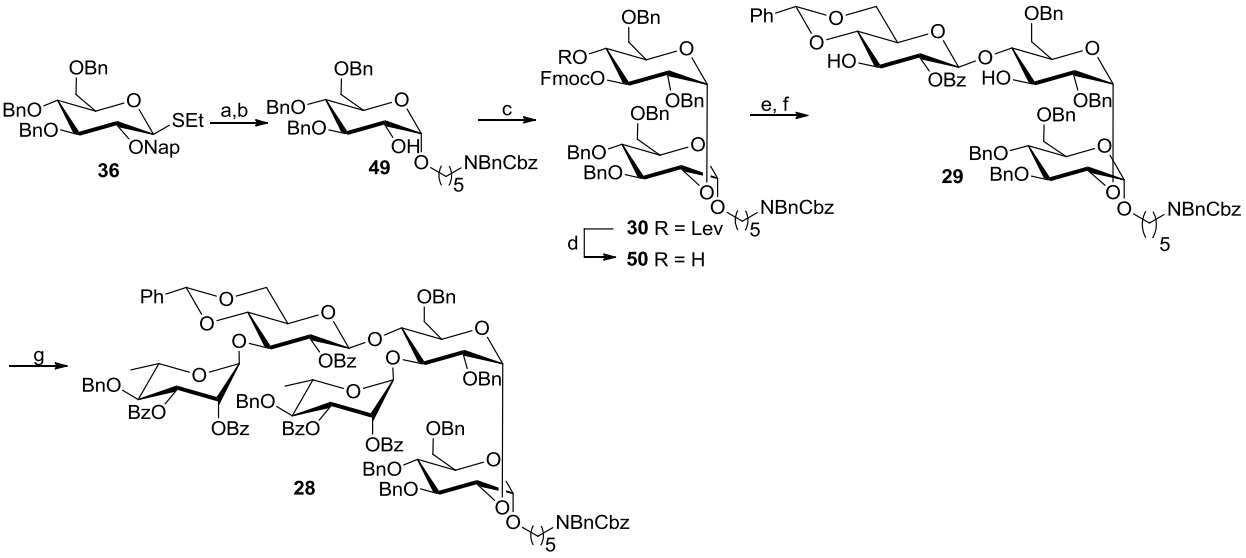
The terminal rhamnose residues D and D' of the repeating unit were provided by rhamnosyl imidate **31**, synthesized from **47** (Scheme 15).[140] Bis-benzoylation of **47** with benzoyl chloride gave rhamnoside **48**. The anomeric *para*-methoxyphenyl was removed with ceric ammonium nitrate to yield the free lactol that was immediately converted into rhamnosyl imidate **31**.[75]



Scheme 15: Synthesis of rhamnosyl building block **31**. Reagents and conditions: a) BzCl, DMAP, pyridine, DCM, 0 °C to rt, 97%; b) CAN, MeCN, H_2O; c) $CF_3C(NPh)Cl$, Cs_2CO_3, DCM, 74% over two steps.

3.2.1.3 Assembly of the PS-I Pentasaccharide Repeating Unit

Assembly of the pentasaccharide repeating unit was performed linearly, combining the building blocks in sequence. This allowed for the challenging installation of the 1,2-*cis* linkages of A and B early in the synthetic pathway. The protected amino-linker at the reducing end was introduced by glycosylation of thioglucoside **36** and the linker (Scheme 16).



Scheme 16: Assembly of fully protected pentasaccharide **28**. Reagents and conditions: a) $HO(CH_2)_5NBnCbz$, NIS, TfOH, toluene/dioxane, –40°C to –20°C; b) DDQ, DCM, H_2O, 35% over two steps; c) **35**, NIS/TfOH, Et_2O, –35 °C to –10 °C, 70%; d) $N_2H_4 \cdot H_2O$, AcOH/pyridine, DCM, 94%; e) **34**, TMSOTf, DCM, 4Å MS, –35 °C to –7 °C; f) NEt_3, DCM, rt, 38% over two steps; g) **31**, TMSOTf, DCM, 4Å MS, –30 °C to –15 °C, 81%.

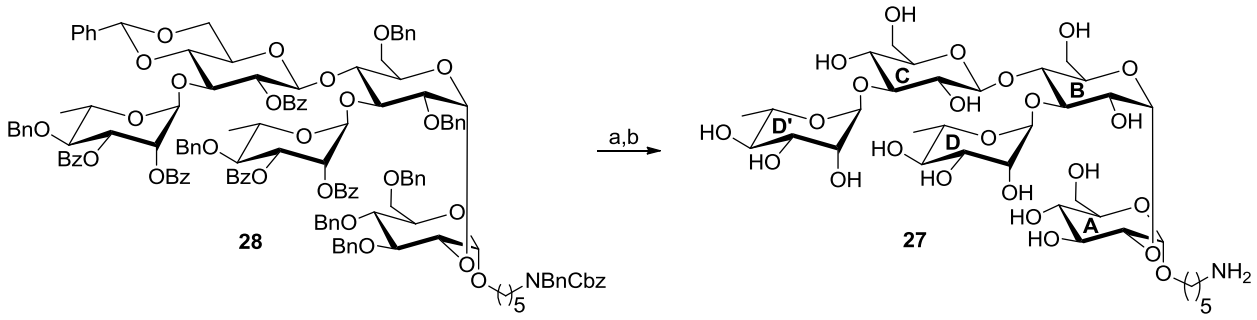
The non-participating temporary protecting group 2-naphthylmethyl at *C*-2 thereby promoted formation of the desired α-glycosidic linkage. The influence on stereoselectivity of a variety of solvent mixtures containing DCM, Et_2O, toluene, dioxane, DMF and a temperature ranging from –50 °C to room temperature were tested with NIS/TfOH as activators. In all cases, the α:β selectivity was in the range of 1:1 and yields ranged from 80% to 95%. The best selectivity of 1.3:1 was achieved with a solvent mixture of toluene and dioxane over a temperature ranging from –40 °C to –20 °C. Separation of the α and β anomers only succeeded after subsequent cleavage of the Nap ether with 3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) to give **49**. Glycosylation of **49** and thioglycoside **35** proceeded with a α:β selectivity of 7:1 when reacted with NIS/TfOH in Et_2O at –35 °C to –10 °C, giving disaccharide **30** in a high yield. Treatment of **30** with hydrazine hydrate in pyridine and acetic acid selectively cleaved the levulinic ester while keeping the Fmoc-group intact, thus yielding disaccharide **50**. Having successfully mastered the first challenge posed by the two reducing end α-glucosides, attention was turned to the second challenge: installation of the branches at *C*-3 and *C*-4 of residue C. Formation of the β-Glc-(1→4)-Glc linkage was screened using the three glycosylating agents **32**–**34**. Due to the low reactivity of thioglycoside **32**, activation with NIS/TfOH proceeded sluggishly, the acidic conditions led to hydrolysis of the benzylidene acetal, resulting in the presence of various hydroxyl-group nucleophiles in the reaction mixture. Reaction of these *in situ* generated nucleophiles with the activated thioglycoside resulted in an inseparable mixture of various oligosaccharides. It is assumed that the low reactivity of the thioglycoside, paired with the low nucleophilicity of the 4-OH of disaccharide **50**, results in a long reaction time that favors hydrolysis of the acetal over formation of the desired glycosidic bond. Reaction of glycosyl imidate **33** with disaccharide **50** using catalytic amounts of TMSOTf as an activator gave trace amounts of the desired trisaccharide and anomerically linked Glc-(1→1)-Glc as a major product. This suggests that glycosyl imidate **33** is too reactive, favoring the reaction with itself to form the di-glucose byproduct over the desired linkage. Glycosyl phosphate **34** was reacted with **50** using stoichiometric amounts of TMSOTf and molecular sieves to ensure anhydrous conditions. The same byproducts as described for the other two glycosylation agents were observed, however to a much lesser degree. Thus, the desired trisaccharide was obtained as a major product, although purification only succeeded after Fmoc cleavage to yield diol **29** in a 38% yield over two steps. The reactivity of the glycosyl phosphate appears to be balanced between that of the thioglycoside and glycosyl imidate and therefore proved to be a superior glycosylating agent for this particular linkage. The assembly of fully protected

pentasaccharide **28** was completed by a single bis-glycosylation between rhamnosyl imidate **31** and diol **29** at –30 °C to –15 °C with TMSOTf as promoter.

The devised synthetic strategy enabled the synthesis of the protected pentasaccharide repeating unit. The low selectivity for the first glycosylation of residue A (**36**) to the linker is acceptable for solution phase synthesis, since this step occurs early in the synthetic pathway and the starting materials are readily synthesized on a multi-gram scale. For solid phase synthesis that does not allow for the purification of the intermediates, this step would not be acceptable. The second glycosylation is sufficiently efficient for solution phase synthesis, however the α:β selectivity of 7:1 and the overall yield of 70% for the desired stereomer would still be too low for a successful solid phase synthesis. The same is true for the third glycosylation step; by identifying the optimal glycosylating agent for this step, the desired product was obtained, although the yields are far from what would be acceptable for solid phase synthesis. Therefore, it was concluded that the pentasaccharide is at this moments not a suitable target for automated solid phase synthesis.

3.2.1.4 Deprotection and Confirmation of the Repeating Unit Structure Reported for PS-I

Final deprotection of fully protected pentasaccharide **28** proceeded in two steps (Scheme 17). First, saponification of the benzoate esters with sodium methoxide at an elevated temperature, followed by palladium-catalyzed hydrogenolysis of the aromatic groups.



Scheme 17: Global deprotection of the pentasaccharide **28**. Reagents and conditions: a) NaOMe, THF/MeOH, 50 °C; b) H_2, 10% Pd/C, MeOH, H_2O, AcOH, 61% over two steps.

Proton NMR spectra obtained from pure pentasaccharide **27** (Fig. 13A) showed the characteristic chemical shift and ^1H-^1H coupling constants for peaks assigned to anomeric protons of one β-Glc (4.53 ppm, d, J = 8.0 Hz), two α-Glc (5.18 ppm, d, J = 3.5 Hz; 5.09

ppm, d, J = 3.8 Hz), and two α-Rha (5.24 ppm, s; 5.14 ppm, s). This assignment was confirmed by proton-carbon HSQC (Fig. 13B) and HMBC measurements. For the complete assignment of all ^{1}H and ^{13}C signals please see the Experimental Section.

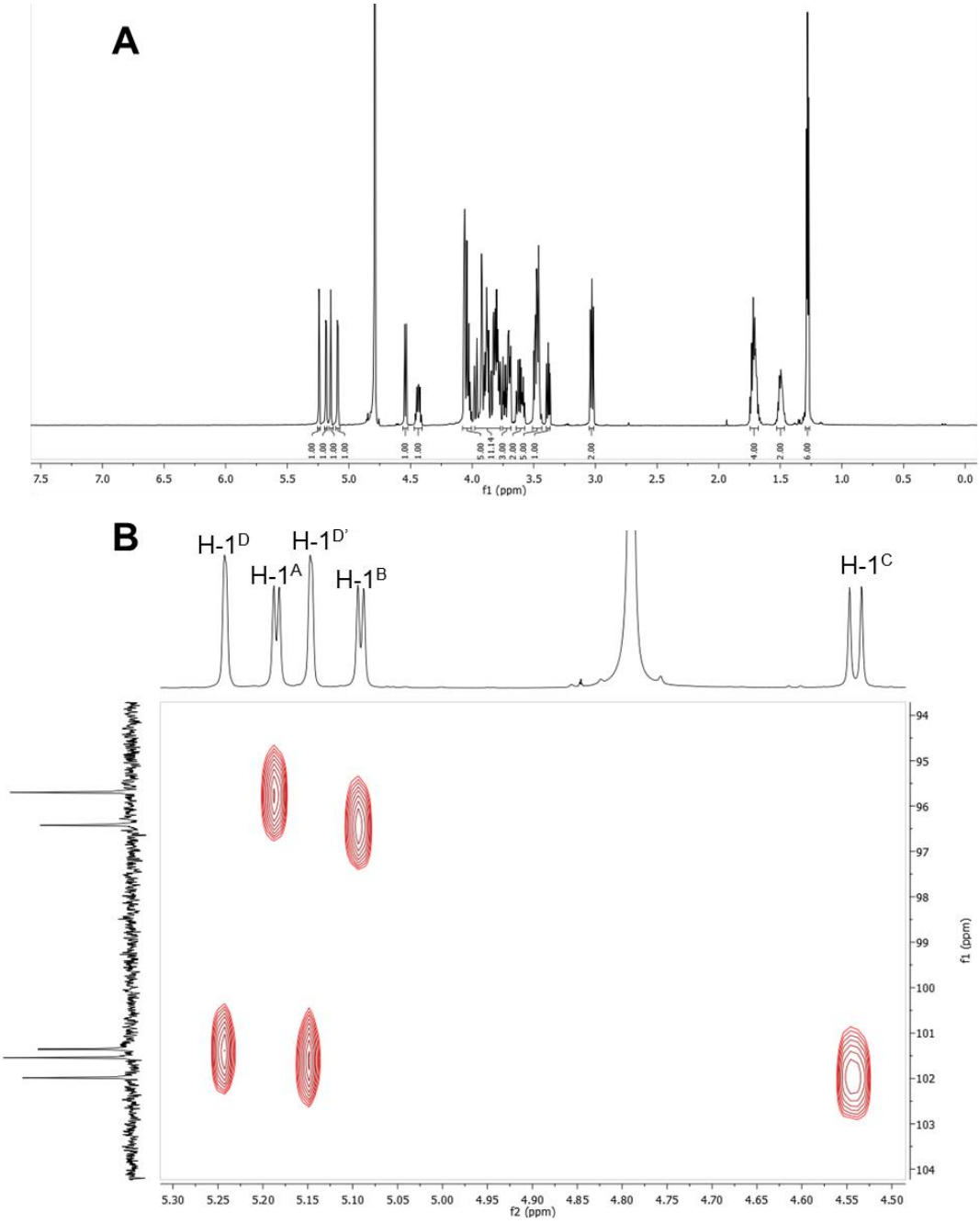


Figure 13: NMR spectra of the synthetic PS-I pentasaccharide repeating unit **27**. **A**: ^{1}H-NMR spectrum (600 MHz, D_2O). **B**: Anomeric region of ^{1}H-^{13}C HSQC spectrum.

Careful comparison of the NMR data for **27** and that reported for the native polysaccharide, isolated from bacteria,[129] showed overall good agreement (Table 1 and Table 3 of the Experimental Section). Chemical shifts of signals corresponding to residues B, C and D that are located in the center of the repeating unit were nearly identical with those reported. Deviations were observed for terminal sugars A and D' due to the phosphate linkages at *C*-1

of A and *C*-4 of D' in the native repeating units that were not present in the synthetic structure.

Table 1: Comparison of ^{1}H and ^{13}C NMR δ between **27** and the native PS-I repeating unit.

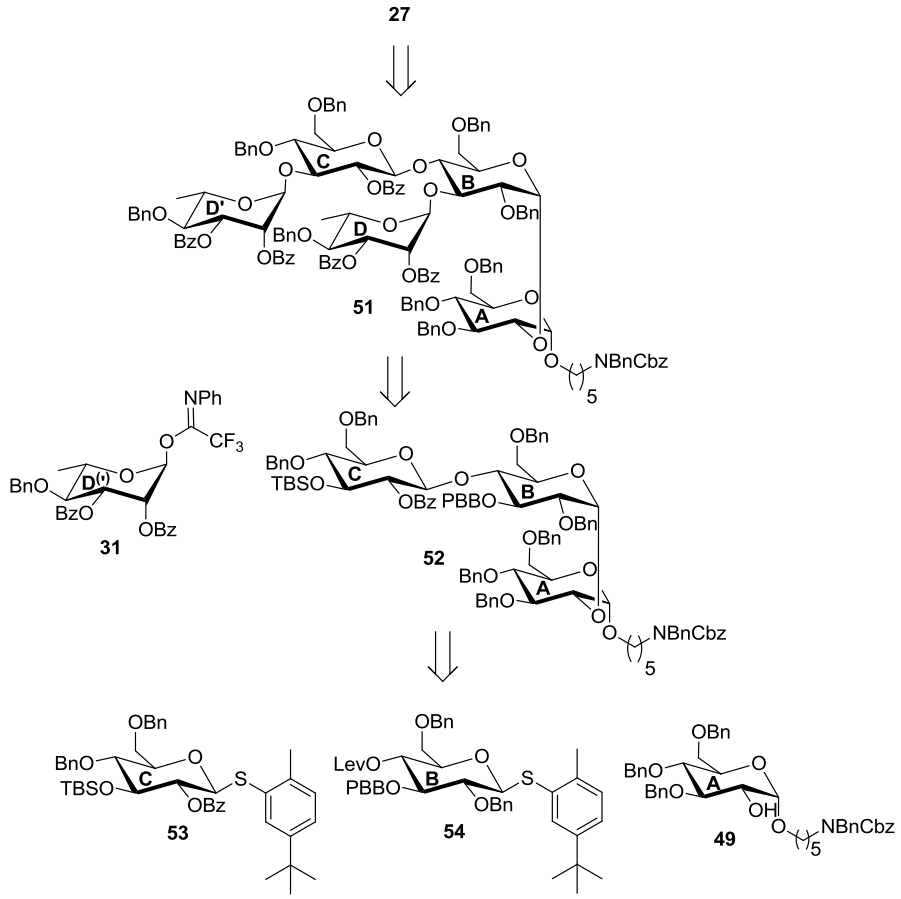
	α-Glc **(A)**	*α-Glc* **(B)**	*β-Glc* **(C)**	*α-Rha* **(D)**	*α-Rha* **(D')**
H-1	5.18	5.09	4.53	5.24	5.14
Lit[129]	*5.75*	*5.13*	*4.53*	*5.23*	*5.17*
C-1	96.1	96.8	102.4	101.8	102.0
Lit[129]	*93.5*	*98.0*	*102.4*	*101.9*	*101.4*

In conclusion, this first total synthesis confirmed the chemical structure of the repeating unit previously reported by analysis of isolates from *C. difficile* bacteria. However, it became apparent that the synthesis needed to be optimized in order to obtain the amounts of synthetic material required for the next steps in vaccine development.

3.2.2 Revised Synthesis of the PS-I Repeating Unit

3.2.2.1 Retrosynthetic Analysis

The Achilles' heel of the first PS-I pentasaccharide synthesis was the low yield obtained for the formation of the glycosidic linkage between residue C and the AB disaccharide. To increase the efficiency of this coupling, changes were made to the glycosyl donor and acceptor. The glycosyl acceptor was modified to carry an electron-donating aromatic ether at the *C*-3 position next to the glycosylation site of *C*-4. It was presumed that this would increase the nucleophilicity of the 4-OH group compared to the *C*-3 Fmoc group used previously. The glycosyl donor was freed of the cyclic benzylidene acetal that was unstable under the acidic conditions employed for thioglycoside activation. Additionally, the TBS silyl ether was used as a *C*-3 temporary protecting group; it was speculated that this electron-donating group would increase reactivity compared to the Fmoc group previously used in this position. It was envisioned, that these changes would allow the use of a thioglycoside for this glycosylation step (Scheme 18).

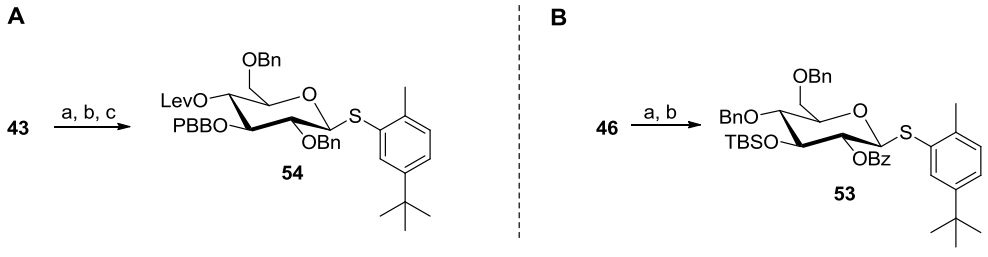


Scheme 18: Revised retrosynthetic analysis of pentasaccharide **27**.

The linear assembly strategy as well as building blocks **31** and **49** were adopted from the first synthesis. Modified building blocks **53** and **54** were used for the assembly of residues B and C, respectively.

3.2.2.2 Building Block Synthesis

Building blocks **53** and **54** were synthesized in few steps each from intermediates already used in the first synthesis (Scheme 19).

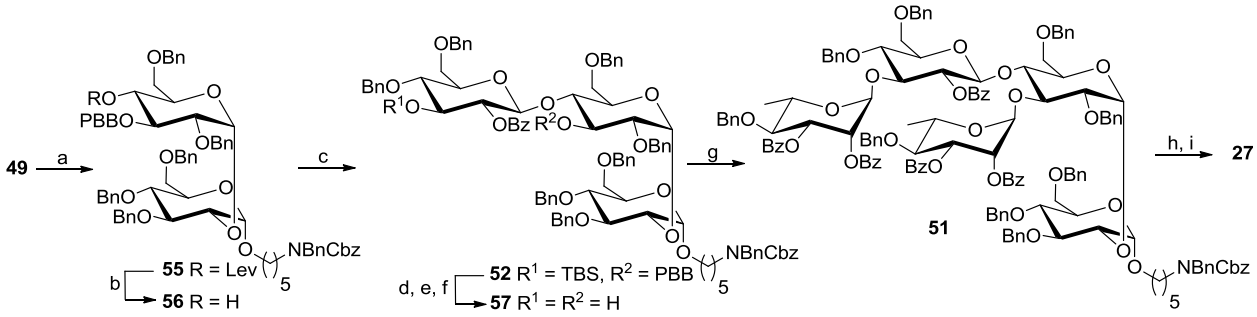


Scheme 19: Synthesis of building blocks **53** and **54**. Reagents and conditions: (**A**) a) *p*-bromobenzyl (PBB) bromide, NaH, DMF, 0 °C to rt; b) TES, TfOH, 4Å MS, DCM, –78 °C; c) LevOH, DCC, DMAP, DCM, 0 °C to rt, 81% over three steps. (**B**) a) BH_3·THF, TMSOTf, DCM, 0 °C to rt; b) BnBr, NaH, THF/DMF, 0 °C to rt, 88% over two steps.

Thioglycoside **54** was prepared from **43** by formation of the *para*-bromobenzyl ether (PBB),[141,142] followed by regioselective opening of the benzylidene acetal and levulination of *C*-4. Building block **53** was prepared in two steps from **46**.

3.2.2.3 Assembly and Deprotection of the PS-I Pentasaccharide

In a method very similar to the first synthesis, disaccharide **55** was assembled by the union of thioglucoside **54** and glucoside **49** in a high yield (Scheme 20). Cleavage of the levulinic ester resulted in glycosyl acceptor **56** that carried the aromatic PBB ether at *C*-3 position next to the glycosylation site. Coupling of thioglycoside **53** and disaccharide **56** proceeded in 92% yield to give trisaccharide **52**. The high yield obtained for this coupling compared to the first synthesis confirmed the considerations taken into account when revising the synthetic strategy. Conversion of **52** to diol **57** required a three-step deprotection sequence that was followed by a bis-glycosylation with rhamnosyl-imidate **31**. The fully protected pentasaccharide **51** thus obtained was then deprotected to give **27**.

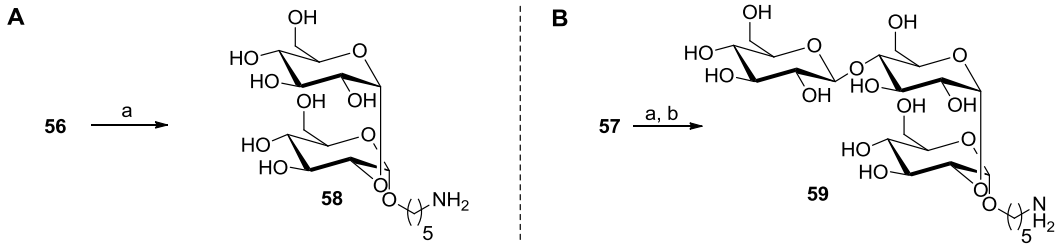


Scheme 20: Revised synthesis of pentasaccharide **27**. Reagents and conditions: a) **54**, NIS/TfOH, Et_2O, –20 °C to –10 °C, 69%; b) $N_2H_4 \cdot H_2O$, AcOH/Pyridine, DCM, 96%; c) **53**, NIS/TfOH, DCM, –30 °C to –17 °C, 92%; d) cat. $Pd(OAc)_2$, (3,4-dimethoxyphenyl)boronic acid, TBABr, K_3PO_4, EtOH; e) DDQ, aq. $NaHCO_3$, H_2O, DCM; f) $TBAF \cdot 3H_2O$, AcOH, DMF, 50 °C, 62% over 3 steps; g) **31**, TMSOTf, DCM, 4Å MS, –40 °C to –20 °C, 88%; g) NaOMe, THF/MeOH; i) H_2, 10% Pd/C, MeOH, THF, H_2O, AcOH, 60% over two steps.

This optimized synthesis of the *C. difficile* PS-I pentasaccharide repeating unit gave access to dozens of milligrams of pure **27** for its subsequent biological evaluation. The efficient synthetic strategy developed here also enabled the rapid assembly of PS-I substructures that are crucial for the identification of minimal antigenic epitopes of PS-I.

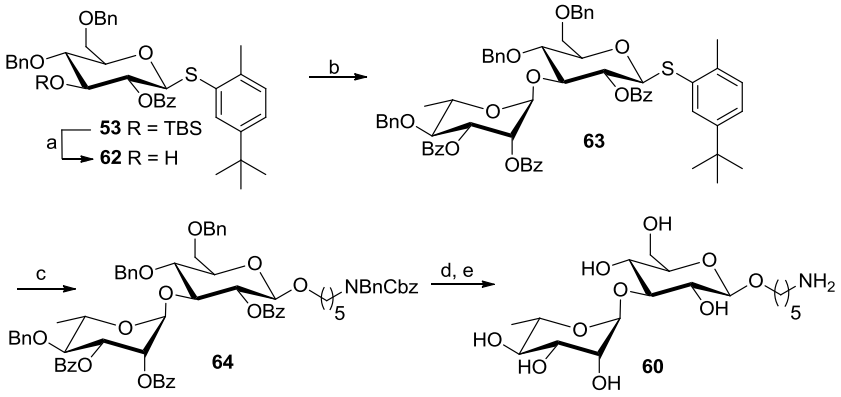
3.2.3 Synthesis of PS-I Substructures

The two oligoglucoside substructures **58** and **59** were obtained by deprotection of intermediates **56** and **57**, respectively (Scheme 21).

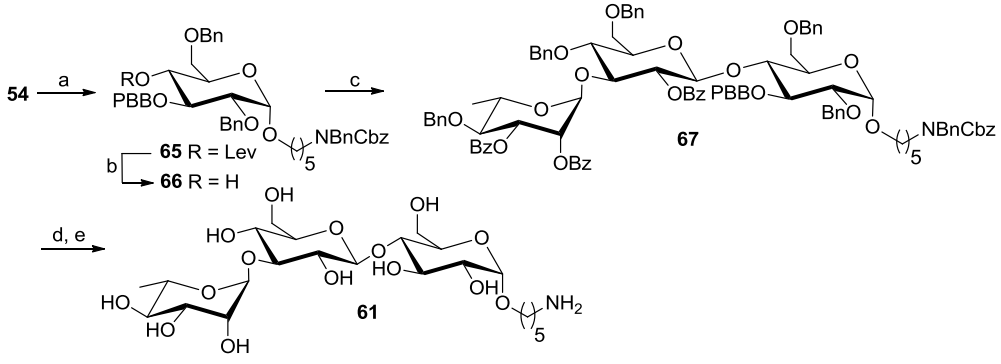


Scheme 21: Deprotection of oligoglucosides. Reagents and conditions: (**A**) a) H_2, 10% Pd/C, MeOH, THF, H_2O, AcOH, 99%. (**B**) a) NaOMe, THF/MeOH; b) H_2, 10% Pd/C, MeOH, THF, H_2O, AcOH, 49% over two steps.

Disaccharide **60** and trisaccharide **61** substructures were synthesized from disaccharide **63**, which in turn was obtained from building blocks **62** and **31** (Schemes 22 and 23).

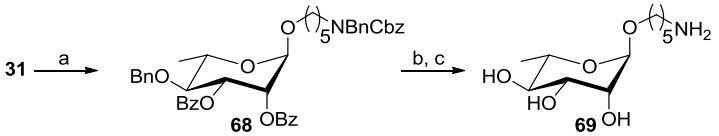


Scheme 22: Synthesis of **60**. Reagents and conditions: a) TBAF·3H_2O, AcOH, DMF, 35 °C, 92%; b) **31**, TMSOTf, DCM, 4Å MS, –40 °C to –20 °C, 86%; c) $HO(CH_2)_5NBnCbz$, NIS/TfOH, DCM, –20 °C to 0 °C, 91%; d) NaOMe, THF/MeOH; e) H_2, 10% Pd/C, MeOH, THF, H_2O, AcOH, 76% over two steps.



Scheme 23: Synthesis of **61**. Reagents and conditions: a) $HO(CH_2)_5NBnCbz$, NIS/TfOH, Et_2O, –10 °C to 0 °C, 39%; b) $N_2H_4·H_2O$, AcOH/Pyridine, DCM, 81%; c) **63**, NIS/TfOH, DCM, –20 °C to 0°C, 95%; d) NaOMe, THF/MeOH; e) H_2, 10% Pd/C, MeOH, THF, H_2O, AcOH, 77% over two steps.

Rhamnose monosaccharide **69** equipped with the linker at the reducing end resulted from glycosylation of the linker with **31**, followed by deprotection (Scheme 24).



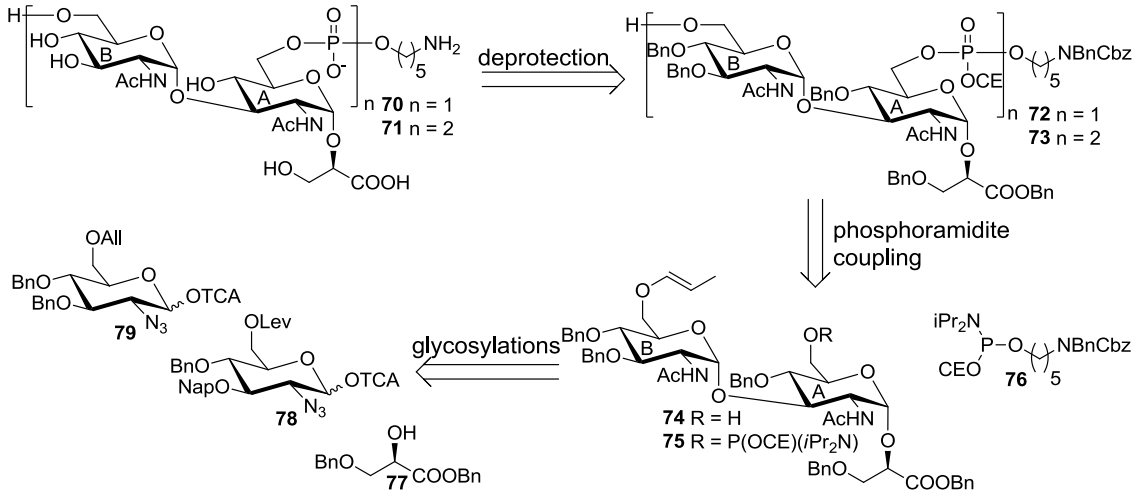
Scheme 24 Synthesis of **69**. Reagents and conditions: a) $HO(CH_2)_5NBnCbz$, TMSOTf, DCM, 4Å MS, –30 °C to –20 °C, 94%; b) NaOMe, THF/MeOH; c) H_2, 10% Pd/C, MeOH, THF, H_2O, AcOH, 94% over two steps.

Due to low expression levels under culture conditions, chemical synthesis is currently the only feasible method to access sufficient amounts of pure PS-I related glycans.[134,137] Thus, chemical synthesis relying on four monosaccharide building blocks gave access to PS-I substructures **58**, **59**, **60**, **61** and **69** to be used in subsequent biological studies together with pentasaccharide **27**.

3.3 Synthesis of PS-III Oligomers

3.3.1 Retrosynthetic Analysis

The major component of the recently identified lipoteichoic acid polysaccharide PS-III consists of phosphodiester-linked repeating units with the sequence α-GlcNAc-(1→3)-α-GlcNAc-(1→2)-GroA.[130] The repeating units are linked *via* phosphodiester bridges at the *C*-6 positions. In pursuit of the target, it was sought to develop a strategy that allows for the facile assembly of phosphate-linked oligomers **70** and **71** from one repeating unit building block (Scheme 25). Repeating unit **75** was identified as such a building block, carrying a phosphoramidite at *C*-6 of A and a vinyl-ether protecting group at *C*-6 of B. Repeating unit **75** would be synthesized from non-phosphorylated **74**, which in turn would be obtained by sequential glycosylation of glyceric acid **77** and glucosamine trichloroacetimidate building blocks **78** and **79**.

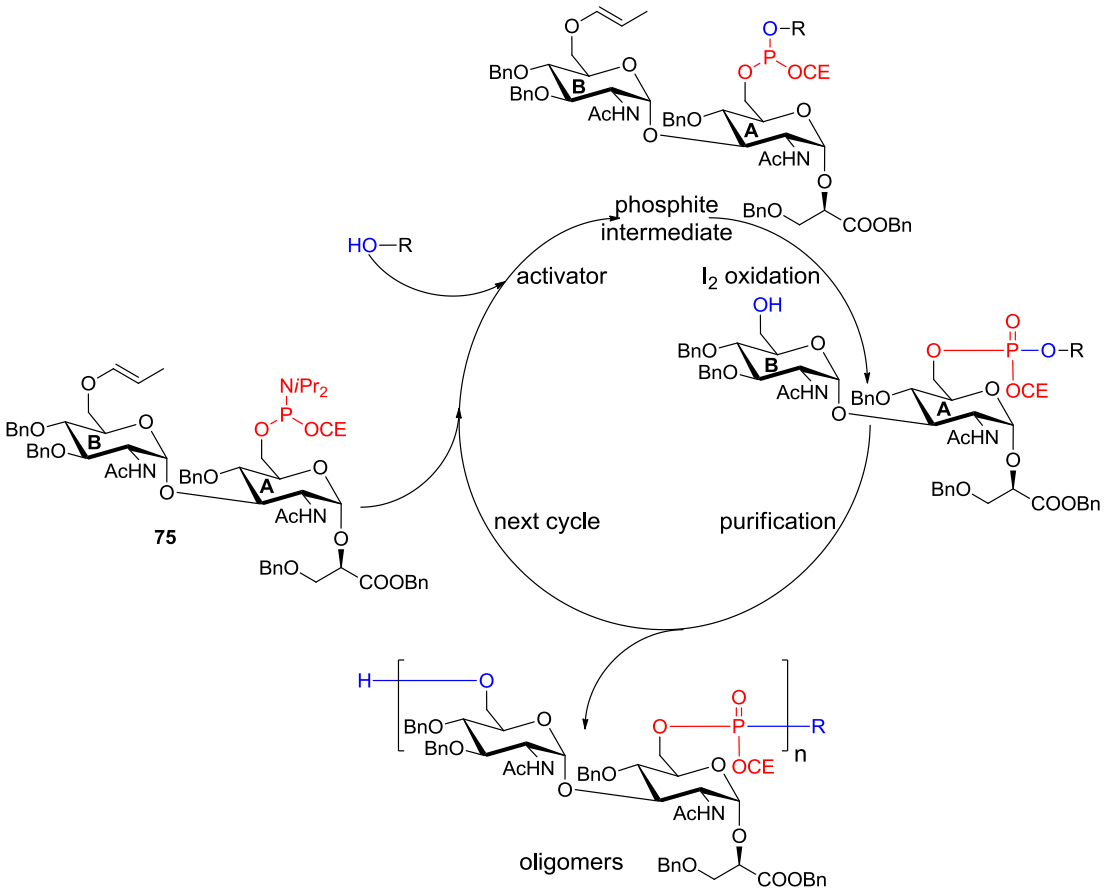


Scheme 25: Retrosynthetic analysis of PS-III repeating unit monomer **70** and dimer **71**. CE: 2-cyanoethyl, Nap: 2-naphthylmethyl, All: allyl, Lev: levulinate, TCA: trichloroacetimidate.

The oligomers **70** and **71**, carrying an amino-linker for covalent attachment to surfaces and proteins, are obtained by deprotection of **72** and **73**, respectively. The use of benzyl protecting groups, also for the carboxylic acid, is due to their removal under mild hydrogenolytic conditions that would not compromise the integrity of the phosphodiesters or glyceric acid residue. The linker is introduced using phosphoramidite **79**, which is coupled with the free alcohol of sugar A of repeating unit **74** providing monomer **72**. Elongation at the free alcohol at *C*-6 of sugar B of monomer **72** with the phosphoramidite at *C*-6 of sugar A of repeating unit **75** gives dimer **73**.

A synthetic strategy that allows for efficient coupling, oxidation and cleavage of the temporary vinyl-protecting group of the repeating units was envisioned. Reaction of the

phosphoramidite of A with a nucleophile in the presence of an activator gives a phosphite intermediate that is oxidized by iodine to the corresponding phosphate *in situ* (Scheme 26). Simultaneously, the oxidative conditions also cleave the vinyl ether of B, providing a new nucleophile for a further round of coupling.

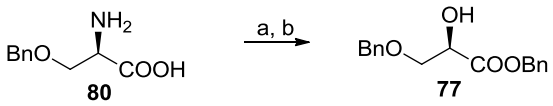


Scheme 26: Synthetic strategy for the coupling of PS-III repeating units.

This one-pot coupling, oxidation and deprotection procedure poses an advantage over the previously described syntheses of teichoic acid molecules that rely on the dimethoxytrityl (DMT) protecting group, which requires an additional acidic cleavage step.[143-145]

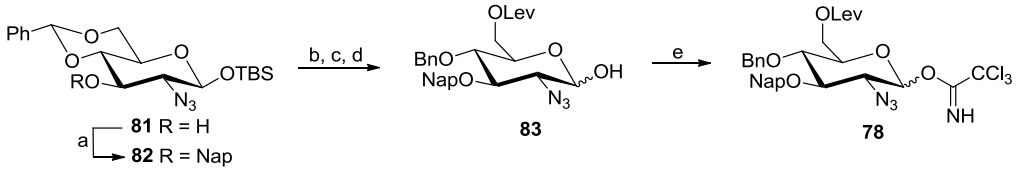
3.3.2 Building Block Synthesis

The reducing end residue of the repeating unit consists of (2*R*)-glyceric acid (GroA). In order to be used in carbohydrate synthesis the primary alcohol was protected with a benzyl ether and the carboxylic acid as a benzyl ester resulting in building block **77**. Starting from *O*-benzyl-D-serine **80**, using the procedures similar to those described for the (S) enantiomer, the amino group was converted to the alcohol under retention of the (2*R*) configuration (Scheme 27).[146,147] The benzyl ester was formed using benzyl bromide in the presence of potassium carbonate.



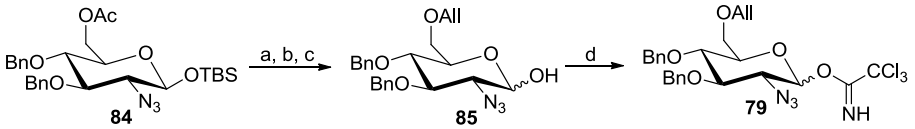
Scheme 27: Synthesis of glyceric acid building block **77**. Reagents and conditions: (a) $NaNO_2$, H_2SO_4, H_2O, 0 °C to rt; (b) BnBr, K_2CO_3, DMF, 0 °C to rt, 63% over two steps.

Glucosamine building block **78** was synthesized in five steps starting from **81** (Scheme 28).[148] The 3-OH group was temporarily protected as a 2-naphthylmethyl (Nap) ether with Nap bromide in the presence of sodium hydride to give **82**. Regioselective opening of the 4,6-*O*-benzylidene acetal with borane and TMSOTf gave the *C*-6 alcohol, which was immediately converted to the levulinic ester. Removal of the anomeric TBS group using TBAF neutralized with acetic acid gave free lactol **83** in high yields over three steps. Conversion of the lactol to trichloroacetimidate **84** with trichloroacetonitrile and potassium carbonate proceeded quantitatively.



Scheme 28: Synthesis of building block **78**. Reagents and conditions: a) NapBr, NaH, DMF, 0 °C to rt, 87%; b) BH3•THF, TMSOTf, DCM, 0 °C to 10 °C; c) LevOH, EDC, DMAP, DCM; d) TBAF, AcOH, THF, 0 °C, 84% over three steps; (e) CCl_3CN, K_2CO_3, DCM, quant.

In a similar way, the synthesis of **79** proceeded in four steps from **84** (Scheme 29).[148] After removal of the 6-*O*-acetate under Zemplén conditions,[102] an allyl ether was introduced using allyl bromide and sodium hydride. TBAF-mediated TBS cleavage gave free lactol **85** in high yields over three steps. Formation of the anomeric trichloroacetimidate resulted in **79**.

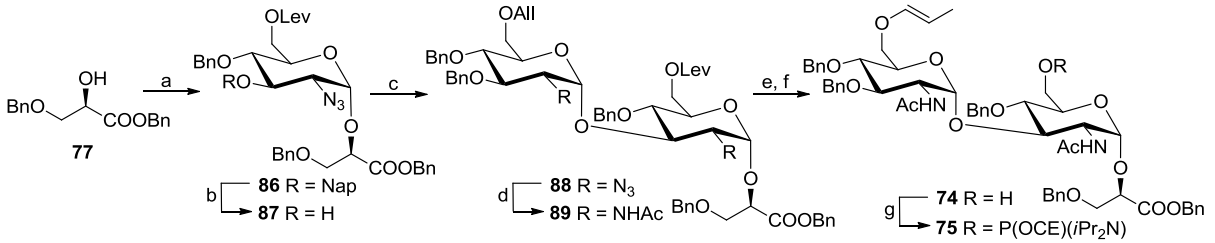


Scheme 29: Synthesis of building block **79**. Reagents and conditions: a) NaOMe, MeOH; b) AllBr, NaH, TBAI, THF, 0 °C to rt; c) TBAF, AcOH, THF, 0 °C, 75% over three steps; d) CCl_3CN, K_2CO_3, DCM, 82%.

3.3.3 Assembly of the Repeating Unit

The repeating unit α-GlcNAc-(1→3)-α-GlcNAc-(1→2)-GroA was assembled by sequential glycosylation of building blocks **77**–**79**, followed by protecting group manipulation (Scheme 3). Glycosyl-imidate **78** and glyceric acid building block **77** were reacted at –20 °C with catalytic amounts of TMSOTf, giving **86**. The solvent mixture of DCM and Et_2O and non-participating azide *C*-2 substituent thereby ensured a high α:β selectivity of 9:1.

Oxidative removal of the temporary Nap protecting group with DDQ resulted in **87**. Identical conditions as for the first glycosylations were applied in the reaction of **87** and glycosyl imidate **79**. Repeating unit **88** was thereby obtained in a high yield and good selectivity (α:β = 8:1). Following the assembly of the repeating unit's pseudodisaccharide backbone, the next steps consisted of the manipulation of the substituents. Both azides were reduced to the corresponding acetamides **89** using thioacetic acid in pyridine.[149] Modification of the *C*-6 substituents commenced with isomerization of the allyl double bond using hydrogen-preactivated $[Ir(COD)(PMePh_2)_2]^+PF_6^-$catalyst in THF. The corresponding vinyl ether was formed in 30 minutes, a longer reaction time resulted in partial cleavage of the vinyl ether. The levulinic ester was removed with hydrazine hydrate in the presence of pyridine and acetic acid giving **74**, longer reaction times again lead to loss of the vinyl ether.



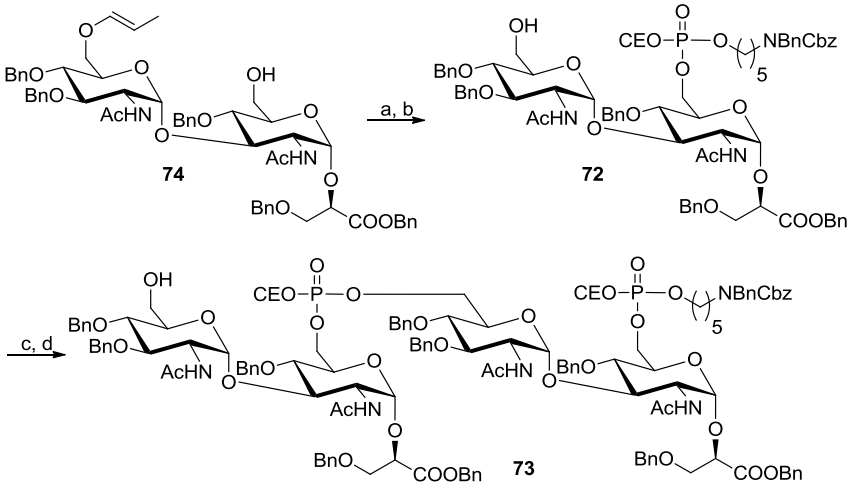
Scheme 30: Synthesis of repeating units **74** and **75**. Reagents and conditions: a) **78**, TMSOTf, DCM, Et_2O, 4Å MS, –20 °C to –10 °C, 81% (α:β = 9:1); b) DDQ, DCM, phosphate-buffer pH 7.2, 0 °C to rt, 80%; c) **79**, TMSOTf, DCM, Et_2O, 4Å MS, –20 °C to –10 °C, 69% (α:β = 8:1); d) AcSH, pyridine, 67%; e) $[Ir(COD)(PMePh_2)_2]^+PF_6^-$, THF; f) $N_2H_4 \cdot H_2O$, AcOH, Pyridine, DCM, 78% over two steps; g) $(iPr_2N)_2POCE$, tetrazole, iPr_2NH, 3Å MS, DCM, MeCN, 82%.

The free primary alcohol of **74** was converted to phosphoramidite **75** with bis(diisopropylamino)cyanoethoxyphosphine in a mixture of DCM and acetonitrile. In order to prevent dimerization of the primary alcohols, diisopropylammonium tetrazolide was used as a coupling agent.[150-152] Phosphoramidite-linker building block **76** was prepared under the same conditions, here again diisopropylammonium tetrazolide ensured that only the phosphoramidite monomer was formed (see the Experimental Section).

3.3.4 Oligomer Assembly

Assembly of oligomers proceeded in acetonitrile by condensation of phosphoramidites with the primary alcohols to the corresponding phosphite using 5-(ethylthio)tetrazole (ETT) as an activator. Coupling was then quenched by the addition of an aqueous iodine solution that oxidized the phosphite intermediate to the corresponding phosphotriester *in situ*, simultaneously cleaving the C-6 vinyl ether protecting group. Following this procedure, monomer **72** was obtained by condensation of linker phosphoramidite **76** and repeating unit

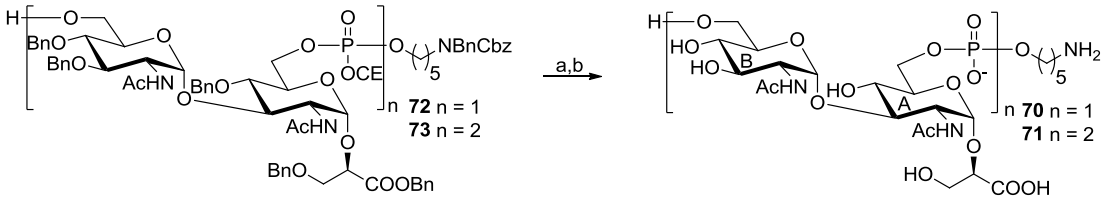
74 (Scheme 31). Nearly quantitative yields were obtained when **76** was added in three-fold excess. Elongation of **72** with phosphoramidite repeating unit **75** gave phosphotriester bridged dimer **73** in a 78% yield. The lower yield of this coupling results from the fact that only a small excess of phosphoramidite **75** was used. Thus, syntheses of protected phosphotriester bridged oligomers **72** and **73** were achieved by a combination of carbohydrate chemistry and phosphoramidite chemistry.



Scheme 31: Assembly of protected oligomers **72** and **73**. Reagents and conditions: a) **76**, 5-(ethylthio)tetrazole (ETT), 3Å MS, MeCN; b) I_2, H_2O, THF , 98% over two steps; c) **75**, ETT, 3Å MS, MeCN; d) I_2, H_2O, THF , 78% over two steps.

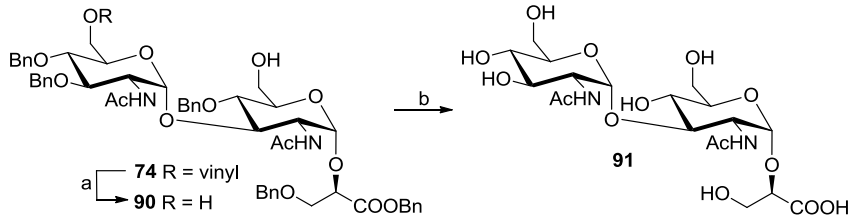
3.3.5 Deprotection and Confirmation of the Repeating Unit Structure Reported for PS-III

Final deprotection of oligomers **72** and **73** required two transformations: firstly, cleavage of the 2-cyanoethyl group under basic conditions; secondly, hydrogenation of the benzyl groups (Scheme 32). Initially, cleavage of 2-cyanoethyl was attempted using an aqueous ammonium hydroxide solution. However, these conditions led to hydrolysis of the benzyl ester and partial formation of a carboxamide. In order not to compromise the integrity of the (2*R*)-glyceric acid, anhydrous methods were chosen for 2-cyanoethyl cleavage. It was found that triethylamine efficiently removed the protecting group giving the corresponding triethylammonium salts.[153] Palladium-catalyzed hydrogenation of the triethylammonium salts did not proceed to completion under atmospheric hydrogen pressure. Reaction under an elevated hydrogen pressure of 4 bar was necessary for removal of all benzyl groups, giving fully deprotected monomer **70** and dimer **71**.



Scheme 32: Global deprotection of PS-III repeating unit oligomers. Reagents and conditions: a) Et_3N; b) H_2 (4 bar), Pd/C, EtOH, H_2O, AcOH, (**70***:* 63% over two steps; **71**: 72% over two steps).

The non-phosphorylated, fully deprotected repeating unit **91** was prepared by oxidative vinyl ether cleavage of **74** with iodine and palladium-catalyzed hydrogenation of the resulting diol **90** (Scheme 33). In case of the non-phosphorylated pseudodisaccharide, atmospheric hydrogen pressure sufficed for global de-benzylation.



Scheme 33: Global deprotection of the non-phosphorylated PS-III repeating unit **91**. Reagents and conditions: a) I_2, H_2O, THF, 89%; b) H_2, Pd/C, EtOH, H_2O, AcOH, 78%.

Proton NMR spectra obtained from **70**, **71** and **91** showed two characteristic peaks above 4.9 ppm corresponding to the anomeric α-Glc protons (Fig. 14). The peak observed at around 4.4 ppm corresponds to the α-proton of glyceric acid. For the complete assignment of all ^{1}H and ^{13}C signals see the Experimental Section.

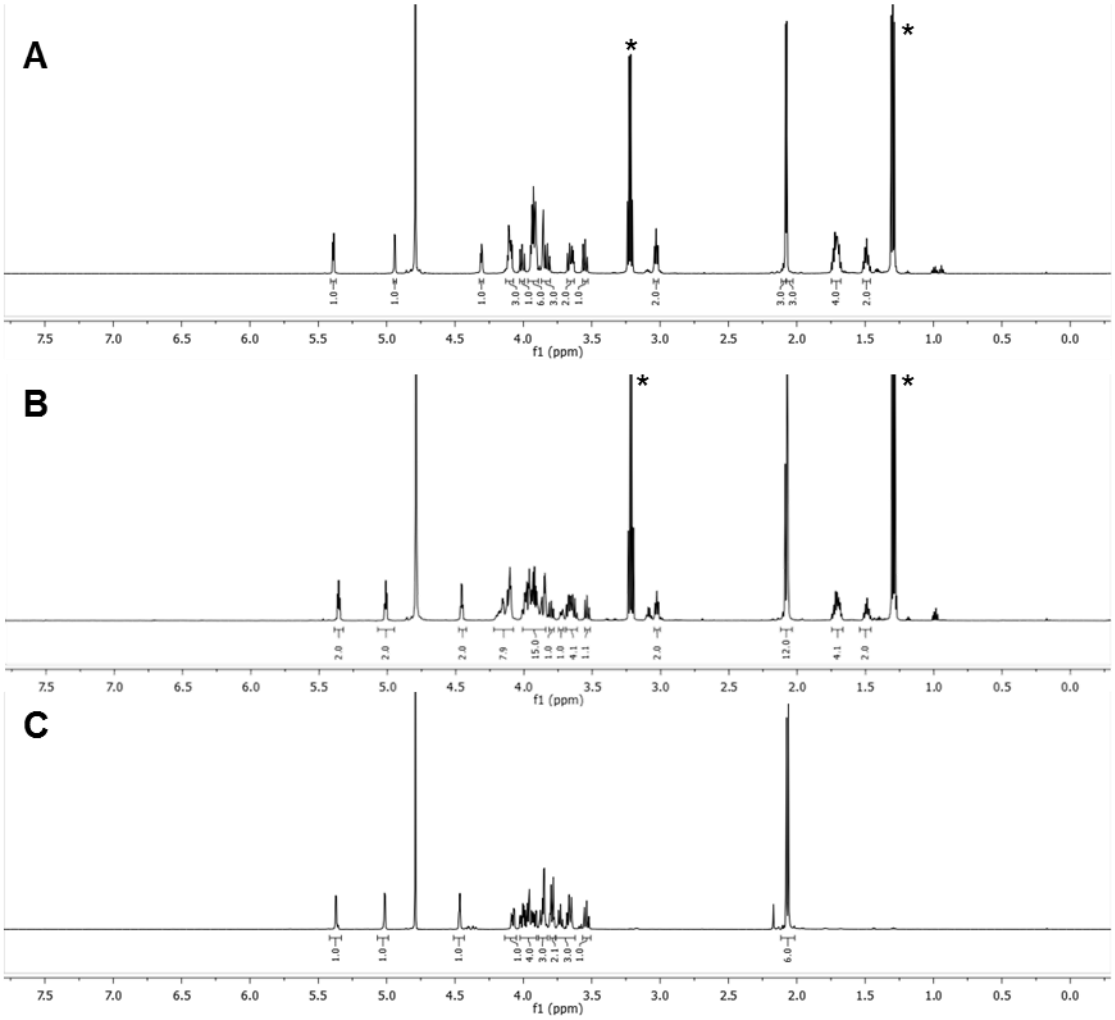


Figure 14: ^{1}NMR spectra (600 MHz, D_2O) of the synthetic PS-III structures. **A**: monomer **70**; **B**: dimer **71**; **C**: non-phosphorylated repeating unit **91**. Peaks at 3.2 ppm and 1.3 ppm labelled with asterisk* correspond to Et_3NH^+.

Careful comparison of the spectra with those reported for the isolated LTA polymer[130] showed overall excellent agreement. In particular, an overlay of the ^{1}H NMR spectra obtained for repeating unit **91** and the one published for the isolated, non-phosphorylated repeating unit[130] demonstrated that these compounds were identical. Thereby reported structure of the repeating unit was confirmed.

3.4 Immunological Evaluation of the Synthetic *C. difficile* Oligosaccharides

3.4.1 Screening of Patient Samples for Antibodies against Synthetic PS-I, PS-II and PS-III

To be able to interact with the immune system and generate an immune response, glycan antigens must be presented on the *C. difficile* surface. Different groups have shown independently that this is the case for PS-II.[130-133] The approach taken by our group relied on the identification of anti-glycan antibodies in CDI patients from glycan microarray based screenings.[136] The detection of specific anti-glycan antibodies is an indication that these are indeed immunogenic cell surface components of *C. difficile* and therefore viable candidates for vaccines or diagnostic applications.

To assess whether anti-PS-I, PS-II or PS-III IgG antibodies circulate in CDI patients' blood, twelve human sera (provided by Prof. Mattner, Universitätsklinikum Erlangen) were screened using glycan microarrays containing PS-I pentasaccharide **27**, PS-III oligomers **70** and **71** and the PS-II hexasaccharide repeating unit synthesized previously in our group by Dr. Matthias Oberli.[136] Serum IgG against the PS-I pentasaccharide was detected in eleven of the twelve screened samples and ten samples contained anti-PS-II repeating unit IgG. Anti-PS-III IgG was detected in six of the samples. Three representative samples A, B and C are shown in Figure 15.

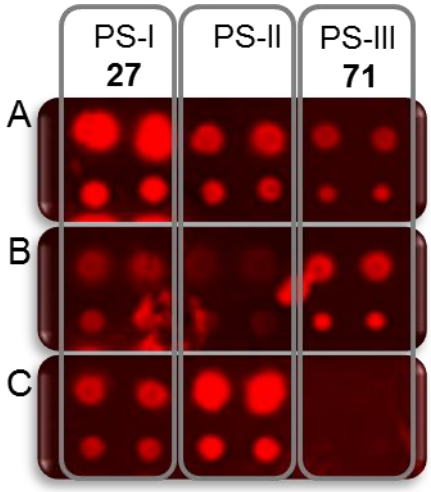


Figure 15: Detection of anti-PS-I, PS-II and PS-III IgG antibodies in the blood of CDI patients. Representative microarray scans (samples A, B and C) are shown. The intensity of red spots corresponds to the abundance of the respective anti-glycan antibodies. Anti-PS-I antibodies were detected in samples A, B and C; anti-PS-II in A and C; anti-PS-III in A and B.

Sample A contained high IgG antibody levels against PS-I and lower levels against PS-II and PS-III. Patient B generated IgG primarily against PS-III and to a lesser extent against PS-I, while anti-PS-II IgG was almost undetectable. High levels of anti-PS-II and lower levels of anti-PS-I IgG were detected for C, anti-PS-III antibodies were not present in this sample. The six samples positive for anti-PS-III antibodies displayed comparable levels of anti-monomer **70** and anti-dimer **71** IgG levels. The findings indicate that the monomeric repeating unit **70** is sufficient for recognition by anti-PS-III IgG.

The presence of anti-glycan antibodies in the blood of CDI patients suggests that all three glycans PS-I, PS-II and PS-III may well be relevant *C. difficile* antigens. Further screenings of serum and stool samples to elucidate the role of PS-I during CDI were carried out by Felix Broecker using the synthetic PS-I structures **27**, **58**, **59**, **60**, **61** and **69** and reported in our recent publication.[154] The data obtained from glycan array screens suggests that PS-I is a common antigen expressed *in vivo*. These findings prompted the evaluation of PS-I pentasaccharide **27** as a vaccine candidate antigen.[154]

3.4.2 Immunological Evaluation of Synthetic *C. difficile* Glycoconjugates

3.4.2.1 Identification of a Minimal PS-I Epitope

Immunization experiments with **27**-CRM glycoconjugates formulated with Freund's adjuvant were conducted in mice by Dr. Chakkumkal Anish and Felix Broecker. To identify the minimum size antigenic epitope of pentasaccharide **27**, sera obtained from immunized mice were screened for IgG against all synthetic PS-I substructures using a glycan microarray. Microarray analysis of pooled sera from mice before immunization and five weeks after the first immunization (three weeks after the subsequent boosting injection) are shown in Figure 16.

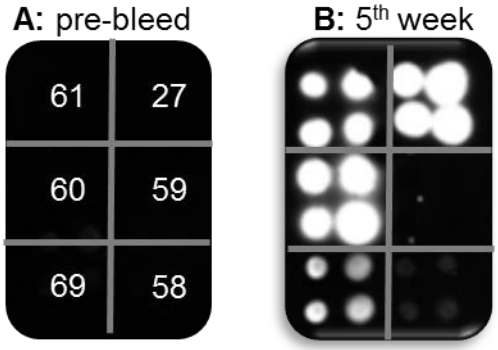


Figure 16: Serum IgG response against oligosaccharides **27**, **58**, **59**, **60**, **61** and **69** in mice immunized with **27**-CRM glycoconjugate. Representative microarray scans of: (**A**) week 0, pre-bleed; (**B**) week 5, indicating serum IgG against the PS-I derived synthetic epitopes.

No serum IgG antibodies were detected in mice before administration of the glycoconjugate (Fig. 16A). Antibodies raised against glycoconjugate **27**-CRM recognized pentasaccharide **27**, trisaccharide **61** and, more pronounced, disaccharide **60**. Response to mono-rhamnose **69** was observed to a lesser extent and no antibodies against di-glucose **58** or tri-glucose **59** were detected. These results allow the conclusion that the minimal epitope recognized by antibodies generated against PS-I pentasaccharide **27** is Rha-(1→3)-Glc disaccharide **60**. This can be explained by the fact that terminal motifs containing rare carbohydrates, such as rhamnose, are known to be immunogenic.[37] In the case of pentasaccharide **27**, the Rha-(1→3)-Glc motif is presented twice as terminal units. Therefore, disaccharide **60** could potentially qualify as a vaccine component against *C. difficile*. This notion was further confirmed by immunization experiments with a **60**-CRM glycoconjugate conducted by Felix Broecker.[154]

3.4.2.2 Further Immunological Evaluations of Synthetic *C. difficile* Glycans

The immunological evaluation of PS-III oligomers was carried out by Felix Broecker. The PS-III dimer was immunogenic in mice when formulated as a CRM glycoconjugates and the generated antibodies recognized the natural antigen present on *C. difficile* bacteria (Broecker *et al*., manuscript in preparation).

3.5 Conclusion and Perspectives

The work carried out on *C. difficile* antigens PS-I and PS-III complements previous investigations on PS-II by identifying, synthesizing and testing four novel potential vaccine candidates, namely PS-I pentasaccharide **27**, disaccharide **60** and PS-III oligomers **70** and **71**. Carbohydrate synthesis proved a powerful tool to produce pure and well defined glycans that are not accessible through other sources. The highly efficient synthetic strategy devised for the synthesis of PS-III repeating unit oligomers is also applicable to other polyphosphate carbohydrate antigens, for example the Hib CPS.

Microarray screenings carried out with samples from infected patients and immunized mice demonstrated the immunogenicity of *C. difficile* cell surface glycans and revealed minimal epitopes of the respective polysaccharide antigens. In a broader sense, the findings reported in this chapter are of relevance for the future design of synthetic carbohydrate antigens. The synthetic antigens are currently being used in multiple collaborative projects, including challenge studies to evaluate their effectiveness in preventing *C. difficile* infection.

A further indication for the significance of this work towards a *C. difficile* vaccine is the fact that it has encouraged other groups to adopt the synthetic approach to access *C. difficile* glycans.[134,137]

4 Synthesis and Evaluation of *Streptococcus pneumoniae* Serotype 5 CPS Substructures

4.1 Introduction

4.1.1 *Streptococcus pneumoniae* Vaccines

S. pneumoniae is a Gram-positive, encapsulated bacterium that is a main cause of infections of the respiratory tract and can lead to severe invasive pneumococcal disease (IPD). More than 90 different pneumococcal serotypes have been described to date, these are classified by the structure of their capsular polysaccharide (CPS), which is unique to each serotype.[155,156] Consequently, the immune response generated against the CPS varies between different serotypes. This is used to generate specific antibodies in rabbits against the antigen of each serotype.[155] Cross-reactivity between these specific antibodies and other serotypes than those they were raised against is often observed, due to structural similarities of the CPS of different serotypes. Due to its immunological properties, CPS is used as the main component of *S. pneumoniae* vaccines.

The first efficient vaccine that contained the CPS of four different serotypes was described in 1945.[16] It then took over thirty years until a vaccine was introduced that covered 14 serotypes, shortly followed by a 23-valent vaccine.[155] However, these polysaccharide vaccines had several shortcomings. They were not able to elicit a long-lasting protection and were not effective in the populations most vulnerable to infection, namely children under two years of age as well as immunodeficient and elderly patients.[22,155,157] These shortcomings result from the immunology of carbohydrates and were overcome by the introduction of carbohydrate-protein conjugate vaccines. The first pneumococcal conjugate vaccines were the seven-valent (PCV-7) and 10-valent (PCV-10) vaccine. PCV-7 was later replaced with the most recent vaccine (PCV-13), which contains the CPS-glycoconjugates of 13 different serotypes.

4.1.2 *S. pneumoniae* Serotype 5

S. pneumoniae serotype 5 (SP5) is globally the fifth most prevalent IPD-causing serotype among young children, and the second most prevalent serotype amongst children in the poorest countries of the world, eligible to the support of the Global Alliance for Vaccines and Immunisation (GAVI).[158] Furthermore, SP5 was recently identified as the causative agent of an epidemic of severe pneumonia among young Israeli army recruits and community outbreaks of invasive infection in impoverished, urban populations in Canada.[159-161] A very recent study showed that SP5 is a frequent cause of IPD outbreaks among children and adults in Spain.[162] Although most SP5 subtypes are still susceptible to antibiotics, the emergence and dissemination of antibiotic-resistant SP5 bacteria is of concern.[163]

The SP5 CPS is a component of the most recent PCV-10 and PCV-13 conjugate vaccines. The SP5 CPS consists of a branched pentasaccharide repeating unit with the sequence [→4)-β-D-Glc*p*-(1→4)-[α-L-Pne*p*NAc-(1→2)-β-D-Glc*p*A-(1→3)]-α-L-Fuc*p*NAc-(1→3)-β-D-Sug*p* (1→], where Sug*p* is 4-keto-D-FucNAc (systematic name: 2-acetamido-2,5-dideoxy-D-*xylo*-hexos-4-ulose) and PneNAc is *N*-acetyl-L-pneumosamine (Fig. 17).

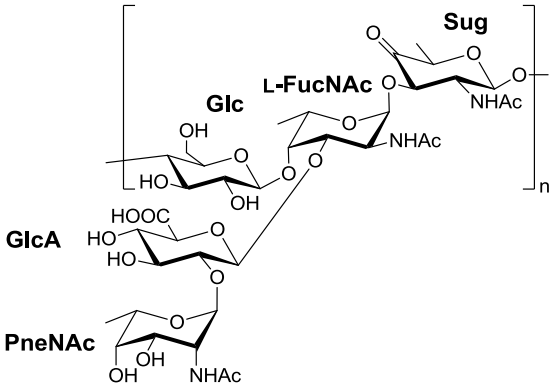


Figure 17: Chemical structure of the *S. pneumoniae* serotype 5 repeating unit.

Ketone-containing polysaccharides are comparatively sporadic within the bacterial glycome; to date only few structures containing the ketone-containing Sug moiety are known.[164,165] This is probably due to their inherent alkaline lability that is responsible for the degradation of isolated SP5 CPS under alkaline conditions.[166,167] The PneNAc monosaccharide residing at the terminus of the side branch of the repeating unit is also extremely rare, so far only very few examples of its occurrence are known.[168,169] The branching FucNAc residue is a fairly common bacterial sugar also found in the CPS of *S. pneumoniae* serotypes 4, 12A and 12F.[155]. The two remaining SP5 CPS components, Glc and GlcA, are abundant in the mammalian and bacterial glycome. An immunological relationship between serotypes 5 and 2 is tentatively attributed to the presence of GlcA residues in both CPS structures.[170,171]

Several challenging aspects for glycoconjugate vaccine preparation with SP5 CPS arise from the presence of the Sug residue. The heterogeneity of isolated CPS is associated with modifications of the Sug residue and complicates structural NMR studies.[167] Furthermore, the instability of the ketone under alkaline conditions results in degradation of the polysaccharide. [166,167] Additionally, the ketone is also susceptible to derivatization under conditions typically applied for glycoconjugate preparation.[172] Reductive amination, for instance, is frequently used for coupling of polysaccharides to carrier proteins, the reducing end aldehyde thereby forming a Schiff base with an amine on the protein surface, the Schiff base is then reduced with $NaBH_4$. However, in the case of SP5 CPS, the amination can also take place at the ketone, resulting in an undefined protein-carbohydrate connectivity.[172] Furthermore, the unreacted ketone is reduced by $NaBH_4$ to a mixture of the corresponding equatorial or axial alcohols. The polysaccharide is thus typically reduced before conjugation, so that glycoconjugate vaccines do not contain the native polysaccharide but rather a heterogeneous mixture containing the axial or equatorial hydroxyl-groups at the former position of the ketone.[171,172]

As demonstrated in the previous chapters, chemical synthesis gives rise to homogeneous and well-characterized oligosaccharides that are used to study the immunological properties of the respective cell surface antigen. In the case of SP5 chemical synthesis needs to resolve the heterogeneity of the isolated CPS.

4.2 Synthesis and Evaluation of *S. pneumoniae* Serotype 5 CPS Repeating Unit Substructures

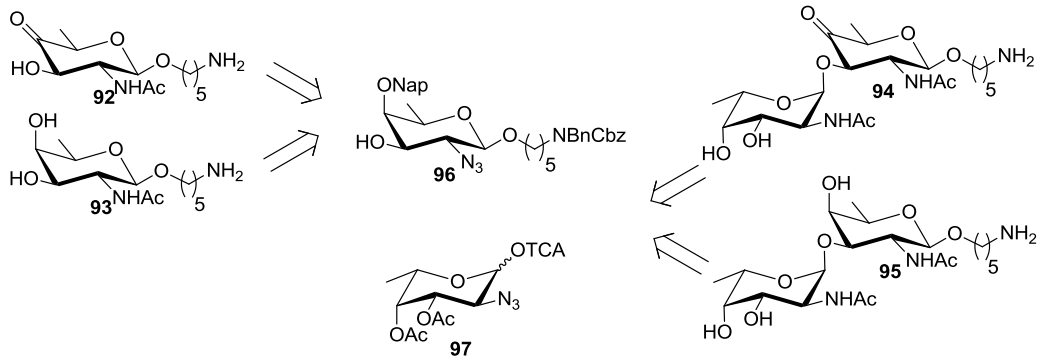
4.2.1 Identification of SP5 Oligosaccharides Targets for Synthesis

The CPS structure of SP5 represents a formidable target for chemical synthesis. The presence of the ketone, which is known to be base-labile even on a monosaccharide, poses a significant challenge.[173] The branches extending from *C*-3 and *C*-4 of the FucNAc residue are considered to be particularly difficult to install, since substituents at these position are oriented *cis* to each other and therefore lead to significant steric crowding at these sites. Introduction of the PneNAc residue is a novelty, to the best of our knowledge no glycans larger than monosaccharides that contain this rare sugar have been synthesized. [174-176]

For immunological evaluation, a glycan microarray containing defined SP 5 CPS-derived oligosaccharides would be ideal for studying the binding epitopes of antibodies generated in the immune response towards CPS antigens. In these studies two aspects are of main concern: the influence of the ketone and the unique terminal PneNAc residue on the immune response. As was demonstrated by the work on *C. difficile* PS-I, terminal motifs containing rare sugars are likely to be immunogenic. In order to investigate the influence of these components, oligosaccharide substructures were designed that either contain the reducing-end of the repeating unit or the terminus. A further, larger substructure that contains both the reducing- and non-reducing end was designed to investigate whether larger epitopes are crucial for antibody recognition.

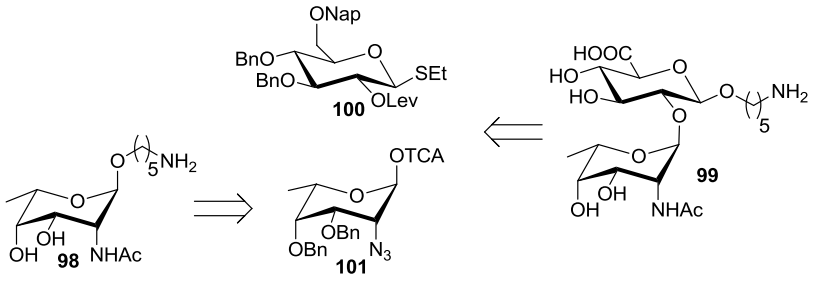
4.2.2 Retrosynthetic Analysis

Four substructures, all carrying the amine-linker, were envisioned to represent the reducing end motif of the repeating unit: the ketone-containing monosaccharides β-Sug **92** and its reduced form β-D-FucNAc **93** as well as the two disaccharides α-L-FucNAc-(1→3)-Sug **94** and α-L-FucNAc-(1→3)-β-D-FucNAc **95** (Scheme 34). These would be synthesized from D-FucNAc building blocks **96** and L-FucNAc building block **97**. The temporary 2-naphthylmethyl of **96** would be removed to enable the installation of the ketone at this position. Subsequent deprotection of the ketone-containing molecules would require a single hydrogenolytic step under comparably mild conditions.



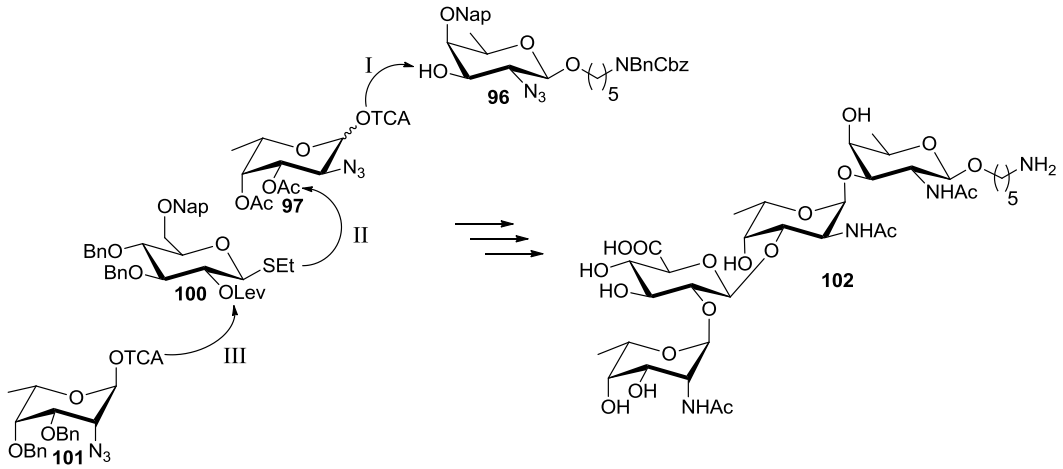
Scheme 34: Retrosynthetic analysis of monosaccharides **92**, **93** and disaccharides **94**, **95**.

The terminal motif is represented by linker-containing α-PneNAc monosaccharide **98** and α-PneNAc-(1→2)-β-GlcA disaccharide **99** (Scheme 35). The synthesis of the terminal structures relies on the novel glycosyl-trichloroacetimidate **101** and thioglucoside **100**.



Scheme 35: Retrosynthetic analysis of monosaccharide **98** and disaccharide **99**.

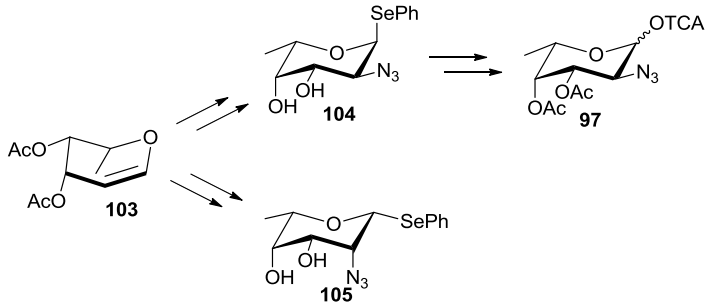
Building on the procedures optimized for synthesis of the mono- and disaccharides, larger tetrasaccharide **102** would be assembled by linear combination of building blocks **96**, **97**, **100** and **101** from the reducing- to the non-reducing end (Scheme 36).



Scheme 36: Synthetic strategy for the assembly of tetrasaccharide **102** from building blocks **96**, **97**, **100** and **101**. Roman numerals indicate the assembly sequence.

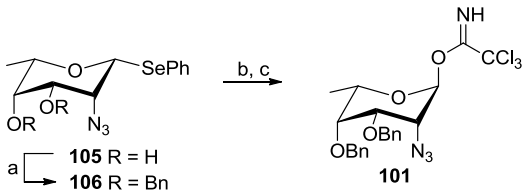
4.2.3 Building Block Synthesis

Synthesis of the L-FucNAc and PneNAc building blocks relied on azidoselenation of L-fucal **103** carried out by Dr. Claney Pereira according to known procedures (Scheme 37).[177-180] This procedure was chosen above the recently reported *de novo* synthesis[181,182] due to its ability to produce the two desired diastereomers **104** and **105** in a single reaction. Diastereomer **104** is the precursor to L-FucNAc building block **97** synthesized by Dr. Claney Pereira.



Scheme 37: Synthesis of building block **97** and impure **105** performed by Dr. Claney Pereira.

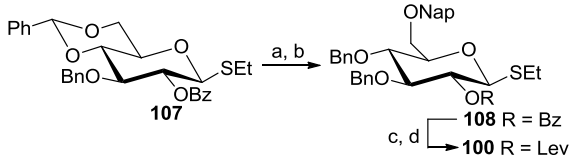
Diastereomer **105** was obtained in a mixture of compounds provided by Dr. Claney Pereira. Purification was achieved after di-benzylation producing **106** (Scheme 38), allowing confirmation of the correct pneumosamine configuration by NMR. Hydrolysis of the anomeric selenide-group was followed by trichloroacetimidate formation to produce PneNAc building block **101**. Although several syntheses of PneNAc monosaccharides were reported in the 1960s and 70s for structural elucidation of the SP5 CPS,[174-176] to the best of our knowledge **101** is the first functional PneNAc building block applicable in carbohydrate synthesis.



Scheme 38: Synthesis of PneNAc building block **101**. Reagents and conditions: a) BnBr, NaH, DMF, 0 °C, (22% based on crude starting material); b) NIS, THF/H_2O, 92%; c) trichoroacetonitrile, K_2CO_3, DCM, 62%.

D-FucNAc building block **96** was synthesized from D-fucal by Dr. Marilda Lisboa using a procedure very similar to the procedure employed in the synthesis of L-FucNAc building block **97**. Installation of the protecting group pattern of **96** was inspired by a recent publication describing the synthesis of *Staphylococcus aureus* type 5 CPS repeating unit using L- and D-FucNAc synthons.[183]

Synthesis of building block **100** was achieved in four steps starting from **107** (Scheme 39). Removal of the *C*-6 Nap-group allows oxidation of this position to the corresponding uronic acid.

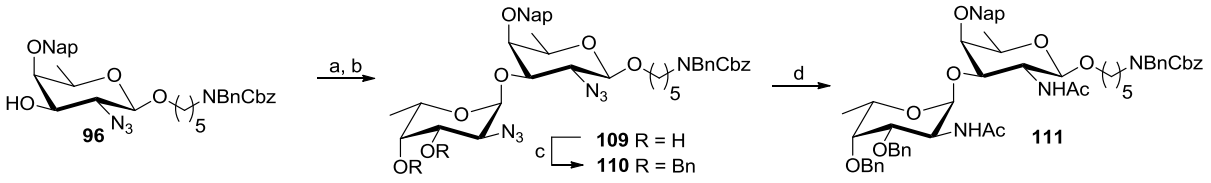


Scheme 39: Synthesis of building block **100**. Reagents and conditions: a) BH_3·THF, TMSOTf, DCM, 0 °C to rt; b) NapBr, NaH, THF/DMF, 0 °C to rt, 84% over two steps; c) NaOMe, MeOH; d) LevOH, EDC, DMAP, DCM, 75% over two steps.

After all the required building blocks had been obtained, their applicability in the synthesis of the targeted SP5 CPS oligosaccharide substructures was investigated.

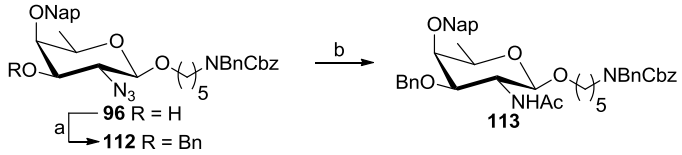
4.2.4 Assembly of SP5 CPS Oligosaccharide Substructures

Initial oligosaccharide assembly focused on the protected mono- and disaccharide substructures to test the applicability of the synthesized building blocks. TMSOTf-catalyzed glycosylation of L-FucNAc imidate **97** and D-FucNAc **96** proceeded with high efficiency under complete α-selectivity, subsequent deacetylation yielded diol **109** in an 87% yield over two steps (Scheme 40). This excellent result confirmed that **97** is a highly efficient glycosylating agent for the formation this particular linkage. Benzylation of diol **109** gave disaccharide **110** and subsequent reduction of both azido-groups produced **111** in high yield.



Scheme 40: Synthesis of **111**. Reagents and conditions: a) **97**, TMSOTf, DCM, 4Å MS, –10 °C; b) NaOMe, DCM/MeOH, 87% over two steps; c) BnBr, NaH, TBAI DMF, 0 °C to rt, 83%; d) AcSH, pyridine, 90%.

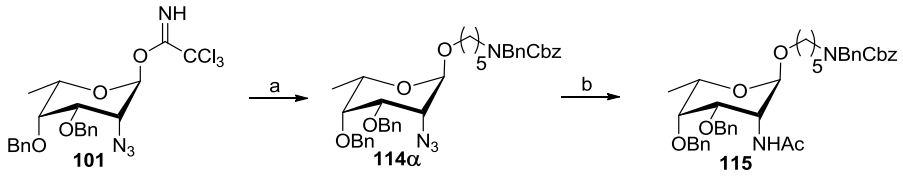
In case of the D-FucNAc monosaccharide, the *C*-3 was first masked with a benzyl group yielding **112**, subsequent treatment with thioacetic acid produced **113** (Scheme 41).



Scheme 41: Synthesis of **113**. Reagents and conditions: a) BnBr, NaH, TBAI, DMF, 0 °C to rt, 56%; b) AcSH, pyridine, 80%.

The applicability of PneNAc building block **101** was first tested by glycosylation to the linker using TMSOTf as a promoter at temperatures between –30 °C and –20 °C in DCM,

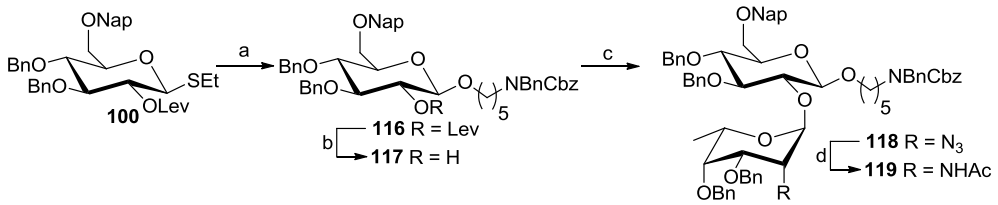
yielding pneumosaminosides **114α** and **114β** in a combined yield of 96% (**114α**: 71%, **114β**: 25%) (Scheme 42).



Scheme 42: Synthesis of **115**. Reagents and conditions:a) $HO(CH_2)_5NBnCbz$, TMSOTf, DCM, 4Å MS, –30°C to –20 °C, **114α**: 71%, **114β**: 25%; b) AcSH, pyridine, 66%.

The excellent yield and good selectivity achieved for the desired α-anomer demonstrate that **101** is an efficient building block for the introduction of terminal α-PneNAc residues. Azide reduction with thioacetic acid gave protected monosaccharide **115**, carrying the linker at the reducing end.

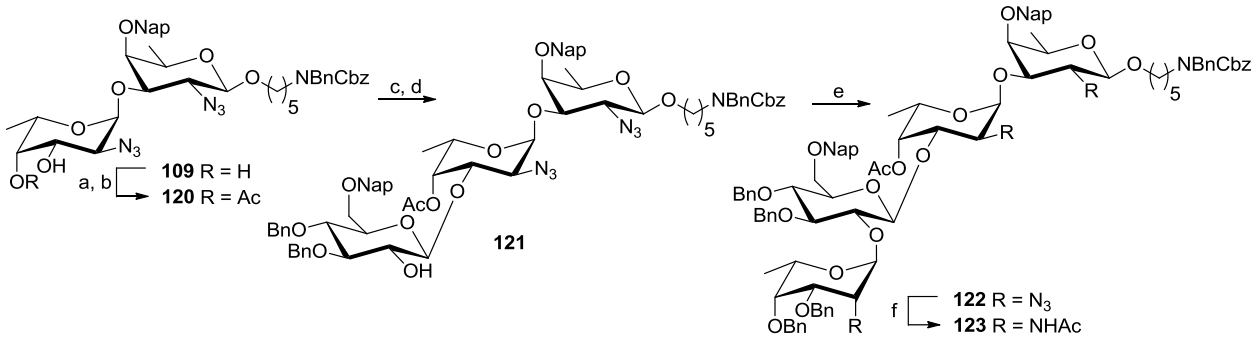
Synthesis of the terminal disaccharide started with the union of thioglycoside **100** to the linker, yielding **116** (Scheme 43). Removal of the levulinic ester gave alcohol **117** that was subjected to glycosylation with **101**, resulting in disaccharide **118** in a 91% yield. Once more, **101** proved to be an excellent glycosylating agent, providing exclusive formation of the desired α-glycosidic linkage in this case. Thioacetic acid-based conversion of the azide finally yielded **119**.



Scheme 43: Synthesis of **119**. Reagents and conditions: a) $HO(CH_2)_5NBnCbz$, NIS/TfOH, DCM, 4 Å MS, –30 °C to –20 °C, 61%; b) $N_2H_4{\cdot}H_2O$, AcOH/pyridine, DCM, 86%; c) **101**, TMSOTf, DCM, 4Å MS, –30°C to –20 °C, 91%; d) AcSH, pyridine, 76%.

After establishing the functionality of the building blocks designated for the synthesis of SP5 CPS mono- and disaccharide substructures, attention was turned to the assembly of the tetrasaccharide target. Selective mono-acetylation of diol **109** placed an acetate-group at *C*-4 of the L-FucNAc residue of **120**, allowing for glycosylation at *C*-3 with thioglucoside **100** (Scheme 44). Cleavage of the Lev-group following the glycosylation resulted in **121**, which was subjected to glycosylation with **101**. In contrast to the synthesis of PneNAc containing mono- and disaccharide, the efficiency of this reaction was low, producing tetrasaccharide **122** in just 55% yield. Formation of the β-anomer was not observed, whereas trisaccharide **121** and lactol **107** were re-isolated from the reaction mixture. This suggests that trichloroacetimidate **101** may be too reactive when confronted with sterically hindered

nucleophiles, resulting in hydrolysis of the glycosylation agent. Finally, tetrasaccharide **123** was obtained by simultaneous reduction of the three azido-groups.

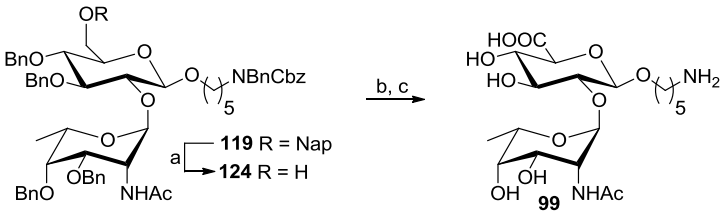


Scheme 44: Synthesis of tetrasaccharide **123**. Reagents and conditions: a) trimethyl orthoacetate, *p*-TsOH, DMF; b) 80% AcOH, 80% over two steps; c) **100**, NIS/TfOH, DCM, 4 Å MS, –20 °C to –10 °C; d) $N_2H_4 \cdot H_2O$, AcOH/pyridine, DCM, 91% over two steps; e) **101**, TMSOTf, DCM, 4Å MS, –30 °C, 55%; d) AcSH, pyridine, 75%.

With the fully protected oligosaccharides in hand, installation of the ketone and global deprotection could commence.

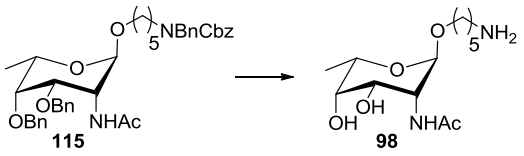
4.2.5 Deprotection and NMR Studies of Oligosaccharide Repeating Unit Substructures

Deprotection of the terminal disaccharide required three transformations: removal of the Nap-group, oxidation of the primary alcohol to the carboxylic acid and finally hydrogenolysis. The Nap-group of **119** was removed using DDQ and the corresponding alcohol **124** was oxidized using iodobenzene diacetate and catalytic amounts of TEMPO, followed by hydrogenolysis to yield fully deprotected PneNAc-(1→2)-GlcA disaccharide **99** (Scheme 45).



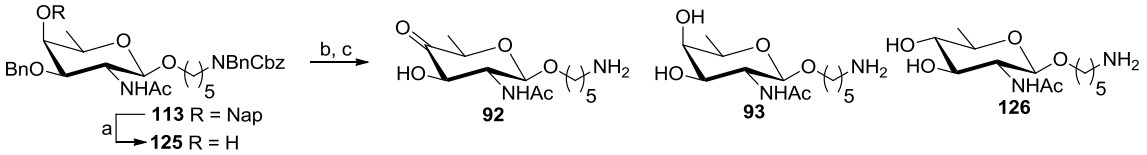
Scheme 45: Synthesis of disaccharide **99**. Reagents and conditions: a) DDQ, DCM, phosphate buffer, pH 7.2, 0 °C to rt, 79%; b) TEMPO, iodobenzene diacetate, DCM, H_2O, 0 °C to rt; c) H_2, Pd/C, EtOH/H_2O/AcOH, 59% over two steps.

Monosaccharide **115** was deprotected by hydrogenolysis yielding **98** (Scheme 46). The pneumosamine configuration of the monosaccharide was once more confirmed by NMR, as was previously the case for building block **105**.



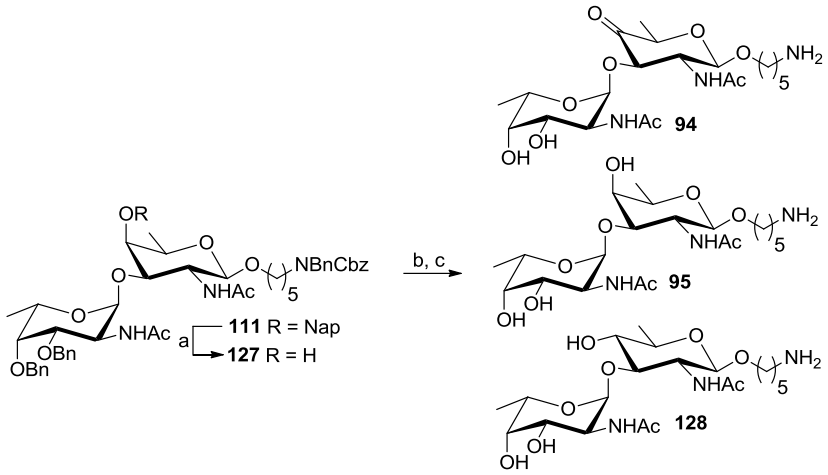
Scheme 46: Synthesis of monosaccharide **98**. Reagents and conditions: H_2, Pd/C, EtOH/EtOAc/H_2O/AcOH, 88%.

Installation of the ketone in the reducing end mono- and disaccharides required selective removal of the Nap-group. Treatment of **113** with DDQ gave **125** with the free alcohol at *C*-4. Oxidation of the secondary alcohol to the corresponding ketone was achieved using Dess-Martin periodinane (Scheme 47). However, reduction of the ketone back to the alcohol was observed in the hydrogenolysis step using palladium on charcoal as a catalyst at atmospheric hydrogen pressure. Sug monosaccharide **92** was only obtained as a minor component of a mixture containing predominantly D-FucNAc **93**, with the hydroxyl-group in axial position and traces of *N*-acetyl-D-quinovosamine (QuiNAc) **126**, with the equatorial hydroxyl-group.



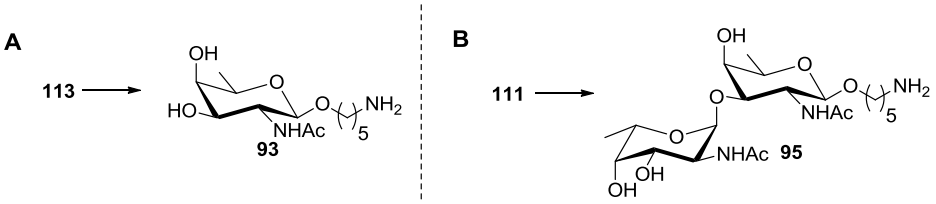
Scheme 47: Synthesis of monosaccharide **92**. Reagents and conditions: a) DDQ, DCM, H_2O, 0 °C to rt, 74%; b) Dess-Martin periodinane, DCM; c) H_2, Pd/C, EtOH/EtOAc/H_2O/AcOH.

When applied to disaccharide **111**, the identical sequence consisting of Nap-removal, Dess-Martin oxidation and Pd-catalyzed hydrogenolysis also gave a mixture of products **94**, **95** and **128** (Scheme 48). In contrast to the monosaccharides, however, ketone-containing L-FucNAc-(1→3)-β-Sug disaccharide **94** was by far the major component of this sample. It appears that the steric hindrance resulting from the bulky hexose substituent at *C*-3 of the Sug residue significantly decelerates reduction of the ketone under the applied conditions.



Scheme 48: Synthesis of disaccharide **94**. Reagents and conditions: a) DDQ, DCM, H_2O, 0 °C to rt, 76%; b) Dess-Martin periodinane, DCM; c) H_2, Pd/C, EtOH/EtOAc/H_2O/AcOH.

Reference samples of pure D-FucNAc monosaccharide **93** and pure L-FucNAc-(1→3)-D-FucNAc disaccharide **95** were obtained by deprotection of **113** and **111**, respectively (Scheme 49).



Scheme 49: Synthesis of pure monosaccharide **93** and disaccharide **95**. Reagents and conditions: H_2, Pd/C, EtOH/EtOAc/H_2O/AcOH, **93**: 92%, **95**: 83%.

Carbon NMR spectra obtained for the sample containing disaccharide **94** as the major product did not display a signal above 200 ppm that would be indicative for the presence of a ketone (Fig. 18A). Rather, a ^{13}C-peak was observed at 95.4 ppm (labeled with an arrow in Fig. 18A and B) that did not show any correlation with a proton in ^{1}H-^{13}C HSQC spectra (Fig. 18B). These findings led to the conclusion that the ketone is hydrated, an observation that was also reported for NMR studies of the natural polysaccharide.[167]

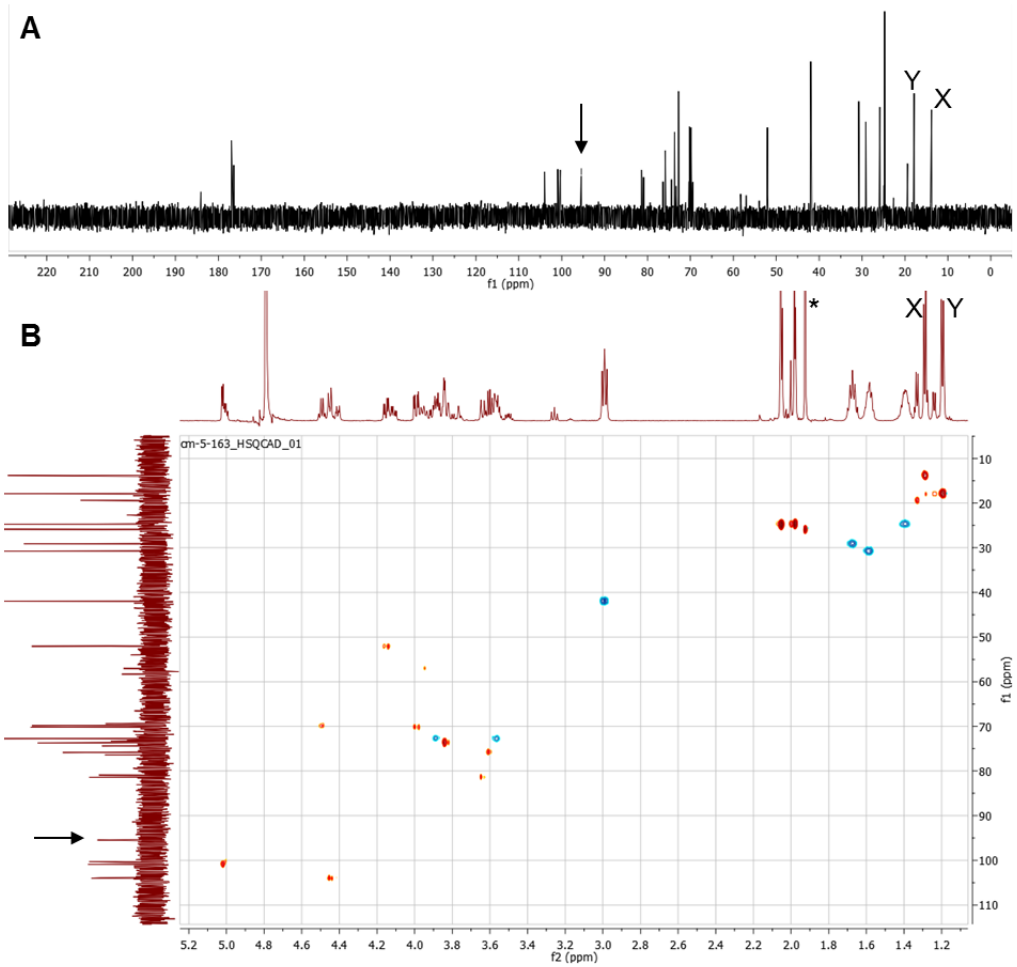
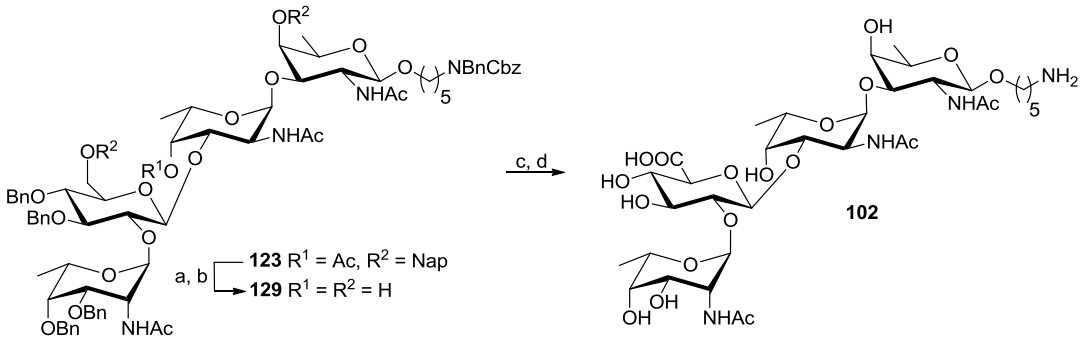


Figure 18: NMR spectra of the sample containing disaccharide **94**. **A**: ^{13}C-NMR spectrum (150 MHz, D_2O). **B**: ^{1}H- HSQC spectrum. The peak labelled with arrow (→) at 95.4 ppm corresponds to *C*-4 of Sug. The peak labelled X corresponds to the *C*-6 CH_3 of Sug. The peak labelled Y corresponds to the *C*-6 CH_3 of L-FucNAc. The peak at 1.9 ppm labelled with asterisk* corresponds to acetate.

Proton NMR spectra obtained after twelve hours in neutral D_2O at room temperature indicated the onset of degradation by the appearance of unaccounted for new peaks. This degradation may result from the lability of the ketone-containing Sug moiety. Therefore, a glycan containing a hydroxyl-group displaying D-FucNAc instead of the hydrated Sug ketone, could be a stable, well-defined mimic of the natural, hydrated structure.

The observations made on a mono- and disaccharide level were taken into account for deprotection of tetrasaccharide **123**. It was decided to synthesize the stable mimic, containing D-FucNAc at the reducing end and thereby avoid contamination of the targeted structure resulting from reduction or decomposition of the ketone. Deacetylation of **123** was followed by removal of both Nap-groups, resulting in **129** (Scheme 50). Selective TEMPO-catalyzed oxidation of the primary alcohol with iodobenzene diacetate was then directly followed by hydrogenolysis resulting in the final tetrasaccharide target **102**.



Scheme 50: Synthesis of tetrasaccharide **102**. Reagents and conditions: a) NaOMe, DCM/MeOH; b) DDQ, DCM, H_2O, 0 °C to rt, 64% over two steps; c) TEMPO, iodobenzene diacetate, DCM, H_2O, 0 °C to rt; d) H_2, Pd/C, EtOH/H_2O/AcOH, 83% over two steps.

NMR characterization of **102** confirmed the integrity of the molecule (Fig. 19), however, no comparison with the native CPS was possible due to the complexity of native CPS NMR spectra resulting from its heterogeneity.[167]

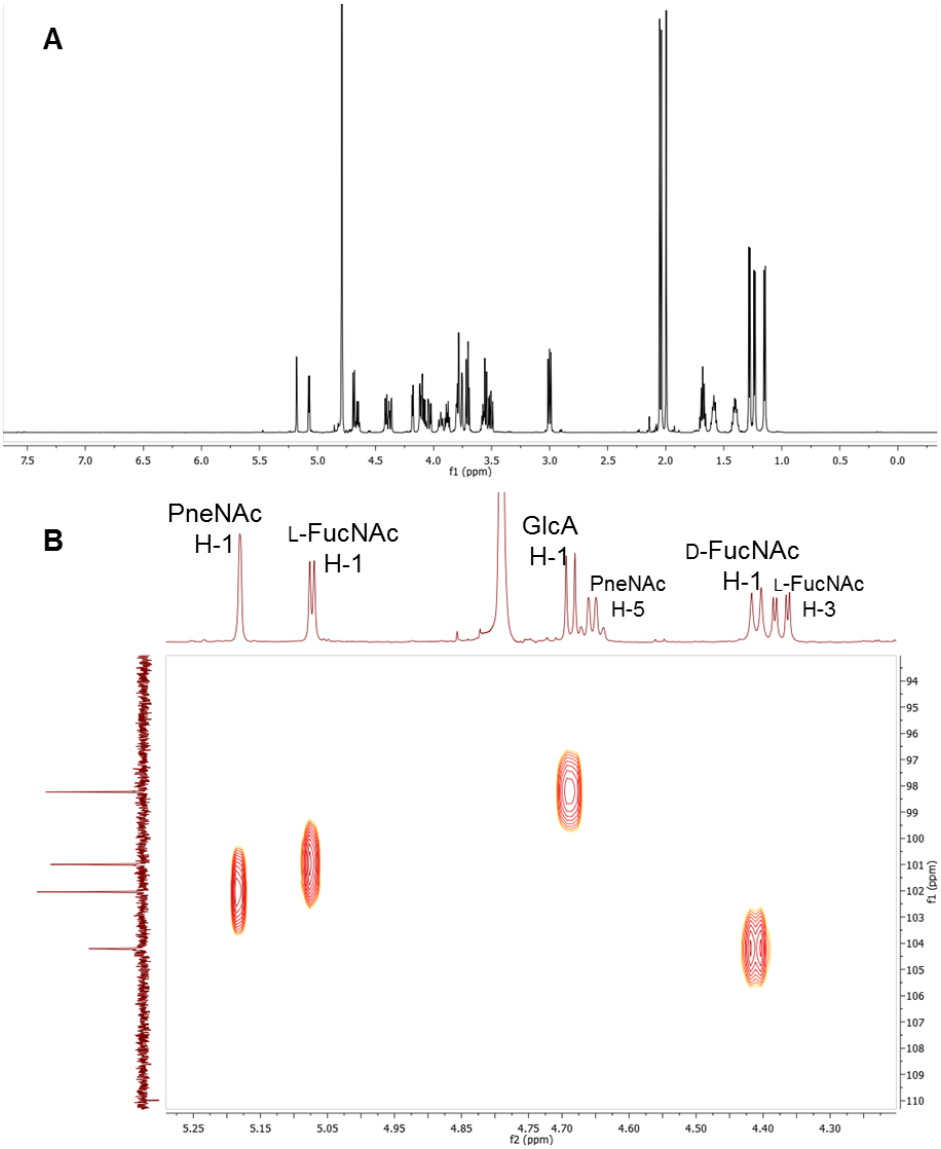


Figure 19: NMR spectra of tetrasaccharide **102**. **A**: ^{1}H-NMR spectrum (600 MHz, D_2O). **B**: Anomeric region of ^{1}H-^{13}C HSQC spectrum.

Thus, pure tetrasaccharide **102**, disaccharides **95** and **99** and monosaccharides **93** and **98** were prepared alongside the samples containing ketones **92** and **94**. For subsequent microarray screenings, four further CPS substructure-related oligosaccharides **130**–**133** were synthesized. (Fig. 20, see the Experimental Section for details)

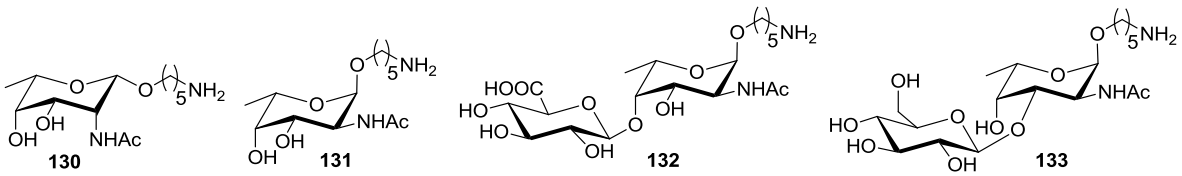
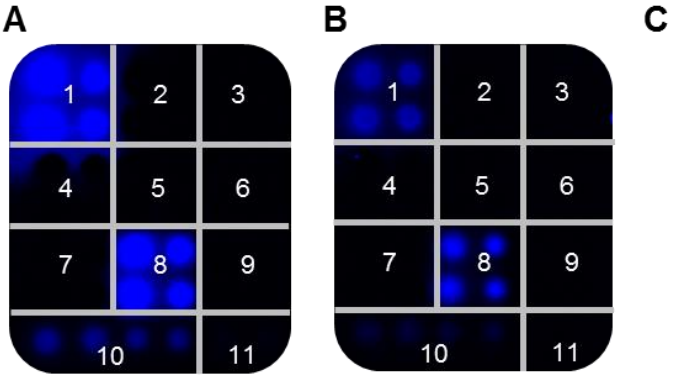


Figure 20: Further CPS substructure-related oligosaccharides **130**–**133**.

4.3 Identification of *S. pneumoniae* Serotype 5 CPS Epitopes

The immunological properties of pneumococcal CPS isolated from bacteria have been studied extensively over the past 90 years, which means a variety of reference sera containing anti-CPS antibodies are available. Specific typing sera contain antibodies that recognize the CPS of one pneumococcal serotype only. However, very little is known about the binding epitopes of these antibodies due to the lack of defined CPS epitope substructures. With the assistance of Andreas Geissner, a glycan microarray containing all eleven SP5 CPS substructures was used to identify the binding epitopes of commercial anti-SP5 specific typing sera generated in rabbits (Fig. 21).



C

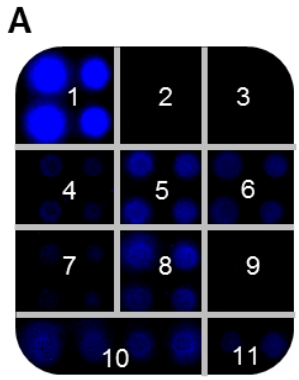
Position	Glycan	Position	Glycan
1	**102**	7	**133**
2	**92***	8	**99**
3	**93**	9	**131**
4	**94***	10	**98**
5	**95**	11	**130**
6	**132**		

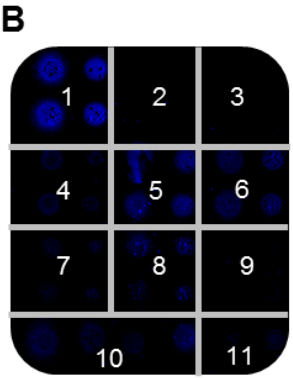
Figure 21: Glycan array screening of *S. pneumoniae* Serotype 5 rabbit typing serum. **A**) Representative well of rabbit typing serum (dilution 1:1000); **B**) Representative well of rabbit typing serum (dilution 1:1000) pre-incubated with SP5 CPS (5µg/mL); **C**) Table listing the positions 1-11 of respective glycans. Samples **92** and **94** marked with asterisk were impure.

As shown in Figure 21A, only three out of eleven glycans were bound by specific anti-SP5 IgG, namely tetrasaccharide **102** (position 1), α-PneNAc-(1→2)-GlcA disaccharide **99** (position 8) and α-PneNAc monosaccharide **98** (position 10). All three recognized oligosaccharides contain the α-PneNAc residue, demonstrating that this motif is part of the

recognized epitope. It was further observed that IgG bound tetrasaccharide **102** and disaccharide **99** in comparable levels, suggesting that α-PneNAc-(1→2)-GlcA is in fact the epitope recognized by IgG of specific SP5 typing sera. Figure 21B shows a microarray sample where the typing serum was pre-incubated with a solution of the natural SP5 CPS. The anti-glycan IgG levels are significantly reduced compared to the sample of Figure 21A. This means that antibodies bound to the natural CPS during pre-incubation are no longer available for binding to the synthetic glycans. Consequently, the binding epitopes of natural CPS and the synthetic glycans are identical.

In order to identify epitopes recognized by the human immune system, further screenings were carried out using pooled sera of humans who had received a pneumococcal CPS-based vaccine, containing the CPS of multiple different serotypes (Fig. 22).





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Position	Glycan	Position	Glycan
1	**102**	7	**133**
2	**92***	8	**99**
3	**93**	9	**131**
4	**94***	10	**98**
5	**95**	11	**130**
6	**132**		

Figure 22: Glycan array screening of human pneumococcal CPS vaccine recipients' pooled serum. **A**) Representative well of human pneumococcal serum (dilution 1:100); **B**) Representative well of human pneumococcal serum (dilution 1:100) pre-incubated with SP5 CPS (5μg/mL); **C**) Table listing the positions 1-11 of respective glycans. Samples **92** and **94** marked with an asterisk were impure.

The IgG response of immunized humans towards the synthetic glycans is shown in Figure 22A. Although the sera of immunized humans were not SP5 specific, they contained IgG predominantly against tetrasaccharide **102** (position 1). IgG binding to PneNAc-(1→2)-GlcA disaccharide **99** (position 8) and PneNAc **98** (position 10) were also observed, however at lower levels. Additionally, low levels of anti-**95** (position 5) and anti-**132** (position 6) IgG and very low levels of anti-**94** (position 4), anti-**133** (position 7), anti-**131** (position 9) and anti-**130** (position 11) IgG were detected. Antibodies against D-FucNAc **93** (position 3) and the Sug-containing sample **92** (position 2) were not detected at all. Interestingly, higher levels of anti-disaccharide L-FucNAc-(1→3)-D-FucNAc **95** antibodies were detected than for the sample containing L-FucNAc-(1→3)-β-Sug **94**. This would suggest that the presence of the ketone is indeed detrimental to antibody binding; however this result could also be an artefact resulting from the impurity of sample **94** or its decomposition during microarray preparation. Pre-incubation of the human sera with the natural SP5 CPS, shown in Figure 22B, led to a

significant reduction of anti-tetrasaccharide **102** IgG levels. This proves that the antibodies binding **102** are SP5 specific since they also bind the SP5 CPS.

In conclusion, the microarray analyses revealed that the terminal PneNAc residue is part of the epitope recognized by anti-SP5 CPS antibodies and that α-PneNAc-(1→2)-GlcA is the predominant epitope recognized by SP5 typing sera. In the case of vaccinated humans, the SP5-specific epitope seems to be larger, resembling tetrasaccharide **102**. The reducing end D-FucNAc or Sug residues do not seem to be important for the immunological response to SP5.

4.4 Conclusion and Perspectives

A library of eleven oligosaccharides was synthesized from the corresponding building blocks. The strategy and building blocks devised for this task enabled the efficient assembly of tetrasaccharide **102**. A particular highlight is the successful use of the PneNAc building block in the assembly of PneNAc-containing oligosaccharides, which are, to the best of our knowledge, the only ones of their kind to date. A promising approach to the access of ketone-containing oligosaccharide is described; however the deprotection step of these oligosaccharides still requires optimization. In the meantime, D-FucNAc was identified as a stable mimic of the ketone-containing Sug residue.

Glycan microarray screenings with the eleven synthetic glycans revealed that α-PneNAc is part of the epitope recognized by anti-SP5 IgG antibodies. It was further determined that α-PneNAc-(1→2)-GlcA is the predominant epitope recognized by antibodies that are specific for SP5. Vaccinated humans generate high levels of antibodies against tetrasaccharide **102**, making this the ideal vaccine candidate for subsequent immunization studies. Additionally, disaccharide **99** could also be a valid vaccine candidate, since SP5-specific antibodies recognize this structure with high specificity.

5 Conclusion and Perspectives

Glycans play an important role in biological systems, particularly as major components of cell surfaces. In the case of infectious, disease-causing pathogens, glycans found on the pathogen's cell surface can be used for the diagnosis of infections and their prevention. In order to study the glycans' interaction with the immune system, access to sufficient amounts of pure and well-characterized oligosaccharides is necessary. However, this is still a limiting factor in the field of glycan research.

This dissertation set out to provide novel glycans, found on the cell surface of pathogens, using chemical synthesis, since these glycans cannot be obtained from other sources in the same amounts and purity. A systematic approach was taken through the synthesis of larger penta- and tetrasaccharides, as well as comprehensive libraries of their mono-, di- and trisaccharide substructures. These libraries were used to identify the binding epitopes of anti-glycan antibodies by microarray technology.

Several oligosaccharides found on the cell surface of *Leishmania* were synthesized from their corresponding monosaccharide building blocks. Glycan microarray screening of samples from infected dogs identified the two epitopes Gal-(1→4)-Man and α-Man-(1→2)-α-Man-(1→2)-α-Man that could play a role in canine leishmaniasis infection. In order to raise antibodies that recognize the identified epitopes, a tetrasaccharide containing both epitopes was identified as a hapten for glycoconjugate preparation and subsequent immunization experiments. The tetrasaccharide proved to be weakly immunogenic in mice, possibly due to the absence of rare, non-mammalian sugars in its structure. A robust anti-tetrasaccharide immune response that generated antibodies recognizing the synthetic glycans as well as the entire *Leishmania* parasite were only successful when tetrasaccharide-conjugates were administered in two variants with different linker lengths. These two variants of the tetrasaccharide with two different defined linker lengths were only accessible through synthetic chemistry. Although the synthetic glycans are too weakly immunogenic to qualify as vaccine candidates, the antibodies obtained that specifically recognize the pathogen could form the basis for the development of a diagnostic test. This work demonstrated the feasibility of an approach that relies on synthetic glycans to identify minimal epitopes, allowing for the rational design and synthesis of glycan antigens used for immunization studies.

The same concept was also applied to the PS-I and PS-III glycans present on the surface of *C. difficile* bacteria. The first total synthesis of the PS-I pentasaccharide repeating

unit made it possible to confirm the reported chemical structure. An optimized synthesis produced the PS-I pentasaccharide as well as a library of its substructures in sufficient amounts for immunological studies. Phosphodiester-bridged oligomers of the PS-III pseudodisaccharide repeating unit were synthesized by a combination of carbohydrate and phosphoramidite chemistry. For this purpose, an efficient one-pot coupling, oxidation and deprotection procedure for the pseudodisaccharide repeating units was developed. Microarray screenings revealed that sera of CDI patients contain antibodies against the synthetic PS-I and PS-III glycans, implying their immunogenicity during infection. The binding epitopes of antibodies, obtained by the immunization of mice with PS-I pentasaccharide conjugates, were studied using glycan microarrays with the synthetic PS-I substructures. The Rha-(1→3)-Glc disaccharide was identified as the minimal epitope of the PSI-pentasaccharide; this was further confirmed by immunization experiments. The immunogenicity of the comparably small Rha-(1→3)-Glc disaccharide is explained by the presence of the rare bacterial sugar rhamnose and its location at the termini of the branched pentasaccharide repeating unit. So far, immunological studies have identified the PS-I pentasaccharide and disaccharide as well as the PS-III oligomers as possible vaccine candidates against *C. difficile*, further investigations, including challenge studies, are underway.

Synthetic substructures of the *S. pneumoniae* serotype 5 CPS repeating unit concentrated on the extremely rare ketone-containing Sug residue, found at the reducing end and the equally rare PneNAc residue, present on the terminus of the repeating unit. The synthetic strategy thus devised enabled the preparation of glycans containing Sug or PneNAc. It was found that the ketone is hydrated and unstable; therefore D-FucNAc was identified as a stable mimic of Sug. Glycan microarray screenings carried out using eleven synthetic glycans and rabbit typing sera containing antibodies produced to specifically recognize SP5 only revealed that a α-PneNAc-(1→2)-GlcA disaccharide is the predominant epitope recognized by these antibodies. Screenings carried out with samples from vaccinated humans further identified a tetrasaccharide as a relevant epitope. Both the disaccharide as well as the tetrasaccharide are ideal targets for vaccine applications, identified by the rational design, synthesis and biological evaluation of cell surface carbohydrate antigens.

As described throughout this dissertation, the assembly of large complex oligosaccharides still remains a time-consuming task reserved for specialized scientists. Small glycans with unique biological properties, similar to those of more complex structures, however, are comparatively easily produced. Therefore, the reduction of carbohydrate antigens to less complex substructures by a systematic reductionist approach is an important

step towards cost-efficient synthetic carbohydrate-based vaccines. My contribution to the rational design of synthetic glycan antigens will hopefully lead to the development of novel diagnostics and vaccines over the next decades.

6 Experimental Section

6.1 Reagents and General Procedures

Commercial grade reagents and solvents were used without further purification, except as indicated below. All batch reactions were conducted under an Ar atmosphere. ^{1}H-NMR and ^{13}C-NMR spectra were measured with a Varian 400-MR or Varian 600 spectrometer. The proton signal of residual, non-deuterated solvent (δ 7.26 ppm for $CHCl_3$; δ 4.79 ppm for H_2O, 2.84 ppm for acetone) was used as an internal reference for ^{1}H spectra. For ^{13}C spectra, the chemical shifts are reported relative to the respective solvent (δ 77.16 ppm for $CDCl_3$, δ 29.84 ppm for acetone-d6). For ^{13}C spectra in D_2O, MeOH (δ 49.50 ppm) was added as internal standard. Coupling constants are reported in Hertz (Hz). The following abbreviations are used to indicate the multiplicities: s, singlet; d, doublet; t, triplet; m multiplet. Infrared (IR) spectra were recorded as thin films on a Perkin Elmer Spectrum 100 FTIR spectrophotometer. Optical rotations (OR) were measured with a Schmidt & Haensch UniPol L 1000 at 589 nm and a concentration (c) expressed in g/100 mL. High-resolution mass spectra (HRMS) were recorded with an Agilent 6210 ESI-TOF mass spectrometer at the Freie Universität Berlin, Mass Spectrometry Core Facility. MALDI-TOF spectra were recorded on a Bruker Daltonics Autoflex Speed, using a 2,4,6-trihydroxyacetophenone (THAP) matrix.

Analytical thin layer chromatography (TLC) was performed on Kieselgel 60 F254 glass plates precoated with a 0.25 mm thickness of silica gel. The TLC plates were visualized with UV light and by staining with Hanessian solution (ceric sulfate and ammonium molybdate in aqueous sulfuric acid) or a 1:1 mixture of H_2SO_4 (2N) and resorcine monomethylether (0.2%) in ethanol. Column chromatography was performed using silica gel 60 (230–400 mesh). Size exclusion chromatography (SEC) was performed using Sephadex® LH-20 (GE Healthcare).

6.2 Synthesis of Leishmanial LPG Cap Oligosaccharides

6.2.1 Geneneral Synthetic Procedures for the Synthesis of Leishmanial LPG Cap Oligosaccharides

General procedure (A) for TMSOTf-mediated glycosylation of mannosyl-imidates.
The acceptor (1.0 equiv) and mannosyl-trichloroacetimidate (1.3 to 3.0 equiv) were coevaporated with toluene three times and dried *in vacuo*. The mixture was dissolved in DCM/Et_2O (1:1 or 1:2, reaction concentration at 30 to 150 mm), cooled to 0 °C and TMSOTf (0.3 equiv) was added. After stirring at 0 °C for 30 min to 1 h, the reaction was quenched by the addition of NEt_3 and concentrated under reduced pressure. Column chromatography (hexanes/EtOAc) afforded the pure product.

General procedure (B) for selective 4,6-*O*-benzylidene opening.
A mixture of 4,6-*O*-benzylidene mannoside (1.0 equiv) and freshly activated 4 Å molecular sieves in DCM (reaction concentration 50 to 100 mM) was cooled to −78 °C and triethylsilane (3.0 equiv) was added followed by the addition of triflic acid (3.4 equiv). The reaction was stirred at −78 °C for 1 to 2.5 h until complete conversion of the starting material (monitored by TLC) and quenched by the addition of saturated $NaHCO_3$. The phases were separated, the organic phase was washed with brine, dried over $MgSO_4$, filtered and concentrated. Column chromatography (hexanes/EtOAc) afforded the pure product.

General procedure (C) for TMSOTf-mediated glycosylation of galactosyl-phosphates.
The acceptor (1.0 equiv) and galactosyl-dibutylphosphate (1.1 to 1.3 equiv) were coevaporated with toluene three times and dried *in vacuo*. The mixture was dissolved in DCM (reaction concentration at 70 to 90 mM), cooled to −40 °C and TMSOTf (1.1 to 1.3 equiv) was added. The reaction was kept at −40 °C or warmed to −20 °C over the period of 1 h, quenched by the addition of NEt_3 and concentrated under reduced pressure. Column chromatography (hexanes/EtOAc) afforded the pure product.

General procedure (D) for AcCl-mediated acetyl-ester deprotection.
The starting material (1.0 equiv) was dissolved in THF/MeOH (3:8, reaction concentration at 30 mM) and AcCl (10 equiv) was added drop wise at 0 °C. The reaction was warmed to room temperature, stirred for 24 h and then quenched by the addition of NEt_3 (10 equiv). The

solvent was removed under reduced pressure, and the residue purified by column chromatography (hexanes/EtOAc).

General procedure (E) for NaOMe-mediated acetyl- and benzyl-ester deprotection.

To a solution of starting material (1.0 equiv) in DCM or THF (30 to 50 mM), was added a solution of NaOMe (1:8 v/v, 0.5 m in MeOH) and stirred until complete conversion of the starting material (monitored by TLC). The mixture was neutralized with Amberlite ® IR 120 (H^+) ion exchange resin, filtered and concentrated. Column chromatography (hexanes/EtOAc) afforded the pure product.

General procedure (F) for microwave assisted pivaloyl- and acetyl-ester deprotection.

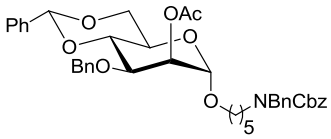
To a solution of starting material (1.0 equiv) in THF (reaction concentration at 7 to 10 mM) was added a saturated solution of NaOMe in MeOH (double the volume of THF). The mixture was heated in a microwave reactor (closed vessel, 3 to 5 W, 100 °C, 5 bar, ramp 10 min, hold 20 to 45 min), neutralized with Amberlite® IR 120 (H^+) ion exchange resin, filtered and concentrated. Column chromatography on silica gel (hexanes/EtOAc) afforded the pure product.

General hydrogenolysis procedure (G).

A solution of starting material (reaction concentration at 4 to 8 mM) in a mixture of MeOH/THF/H_2O/AcOH (10/5/4/1) was purged with Ar. After that 10% Pd/C (same weight as starting material) was added and the solution purged with H_2 for 10 min, then stirred under an H_2 atmosphere for 12 h, filtered and concentrated. The crude product was dissolved in H_2O, subjected to reversed phase solid phase extraction (RP SPE) (Waters Sep-Pak®, C18) and lyophilized. When necessary, size exclusion chromatography on Sephadex LH-20 (MeOH) was performed.

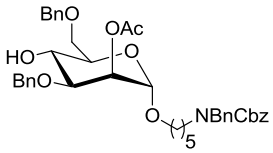
6.2.2 Assembly of Leishmanial LPG Cap Oligosaccharides

_N_-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl 2-_O_-acetyl-3-_O_-benzyl-4,6-_O_-benzylidene-α-D-mannopyranoside (10).



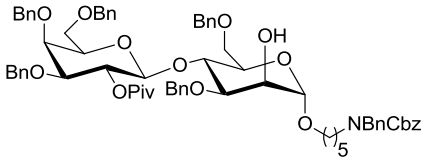
According to general procedure (A), mannosyl-imidate **4** (296 mg, 0.54 mmol) and *N*-(benzyl)benzyloxycarbonyl-5-aminopentanol (267 mg, 0.82 mmol) were dissolved in DCM/Et_2O (1:1, 4 mL) and reacted to give **10** (348 mg, 0.49 mmol, 90%). $[\alpha]_D^{20}$ = + 6.3 º (c = 1.0, $CHCl_3$), IR ν_{max} (film) 3366, 2930, 1732, 1694, 1455, 1372, 1233, 1094 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.58-7.12 (m, 20H), 5.64 (s, 1H), 5.37 (s, 1H), 5.18 (d, *J* = 13.4 Hz, 2H), 4.78-4.62 (m, 3H), 4.50 (d, *J* = 6.6 Hz, 2H), 4.30-4.19 (m, 1H), 4.11-3.94 (m, 2H), 3.90-3.75 (m, 2H), 3.69-3.51 (m, 1H), 3.42-3.13 (m, 3H), 2.16 (s, 3H), 1.68-1.45 (m, 4H), 1.37-1.16 (m, 2H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 170.4, 138.1, 129.0, 128.7, 128.5, 128.3, 128.0, 127.8, 126.2, 101.7, 98.9 (anomeric), 78.5, 74.1, 72.3, 69.9, 68.9, 67.3, 64.0, 29.2, 27.1, 23.4, 21.2; HRMS (MALDI-TOF): Calcd for $C_{42}H_{47}NO_9Na^+$ $[M+Na]^+$ 732.3143, found 732.3160.

_N_-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl 2-_O_-acetyl-3,6-di-_O_-benzyl-α-D-mannopyranoside (11).



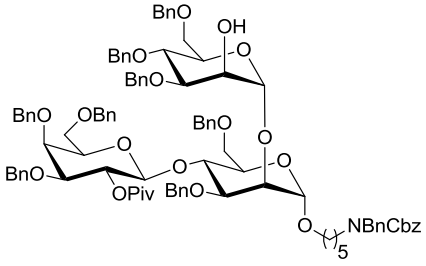
According to general procedure (B), compound **10 (**338 mg, 0.48 mmol) was reacted with triethylsilane (0.23 mL, 1.43 mmol) and triflic acid (0.14 mL, 1.62 mmol) in DCM (9 mL) at −78 °C to give **11** (214 mg, 0.30 mmol, 63%). $[\alpha]_D^{20}$ = + 2.6 º (c = 1.0 , $CHCl_3$), IR ν_{max} (film) 3472, 2929, 1744, 1698, 1454, 1423, 1371, 1236, 1139, 1090 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.56-7.08 (m, 20H), 5.32 (s, 1H), 5.24-5.11 (m, 2H), 4.79 (s, 1H), 4.72 (d, *J* = 11.2 Hz, 1H), 4.64 (d, *J* = 12.1 Hz, 1H), 4.57 (d, *J* = 12.1 Hz, 1H), 4.53-4.42 (m, 3H), 3.97-3.88 (m, 1H), 3.80-3.56 (m, 5H), 3.46-3.12 (m, 3H), 2.11 (s, 3H, acetyl), 1.60-1.47 (m, 4H), 1.37-1.21 (m, 2H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 170.6, 138.0, 128.7 (2C), 128.5, 128.2, 128.1, 128.0, 127.7, 98.0 (anomeric), 77.8, 73.4, 71.8, 70.0, 67.8, 67.5, 67.3, 29.2, 21.2; HRMS (MALDI-TOF): Calcd for $C_{42}H_{49}NO_9Na^+$ $[M+Na]^+$ 734.3300, found 734.3275.

***N*-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl 3,4,6-tri-*O*-benzyl-2-*O*-pivaloyl-β-D-galactopyranosyl (1→4)-3,6-di-*O*-benzyl-α-D-mannopyranoside (12).**



According to general procedure (C), galactosyl-phosphate **6** (271 mg, 0.37 mmol) and **11** (204 mg, 0.29 mmol) were reacted at −40 °C to give the corresponding acetylated disaccharide. The disaccharide was deacetylated according to general procedure (D) to give **12** (290 mg, 0.24 mmol, 86% over two steps). $[\alpha]_D^{20}$ = + 21.6 º (c = 1.9, $CHCl_3$), IR ν_{max} (film) 3464, 3031, 2931, 2870, 1740, 1699, 1454, 1366, 1229, 1132, 1101, 1066 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.60-6.99 (m, 35H), 5.38 (dd, *J* = 10.1, 8.0 Hz, 1H), 5.19 (d, *J* = 9.1 Hz, 2H), 4.92 (t, *J* = 11.7 Hz, 2H), 4.85-4.74 (m, 2H), 4.66 (d, *J* = 11.9 Hz, 1H), 4.58 (d, *J* = 11.2 Hz, 1H), 4.52-4.37 (m, 7H), 4.28 (d, *J* = 11.8 Hz, 1H), 4.11 (d, *J* = 9.3 Hz, 1H), 4.00-3.91 (m, 2H), 3.82-3.72 (m, 2H), 3.71-3.54 (m, 4H), 3.53-3.41 (m, 2H), 3.40-3.17 (m, 4H), 2.47 (d, *J* = 1.8 Hz, 1H, OH), 1.59-1.42 (m, 4H), 1.34-1.19 (m, 2H), 1.13 (s, 9H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 177.0, 138.8, 138.5, 138.2, 137.9, 128.7, 128.5 (2C), 128.3, 128.2, 128.1 (2C), 128.0, 127.9 (2C), 127.7, 127.5, 127.4, 127.2, 100.4 (anomeric), 99.1 (anomeric), 81.2, 78.0, 74.6, 73.7, 73.6 (2C) 73.2, 73.1, 72.8, 72.1, 71.8, 71.0, 69.5, 68.8, 68.5, 67.3, 50.6, 47.3, 46.3, 38.9, 29.2, 28.0, 27.4, 23.5; HRMS (MALDI-TOF): Calcd for $C_{72}H_{83}NO_{14}Na^+$ $[M+Na]^+$ 1208.5706, found 1208.5711.

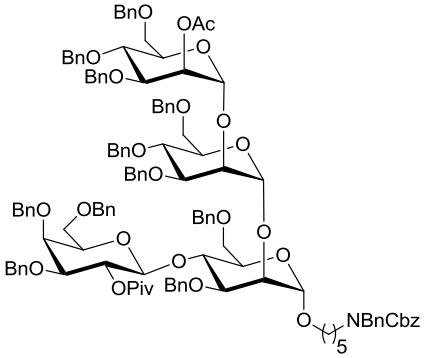
***N*-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl (3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→2)-3,6-di-*O*-benzyl-4-*O*-(3,4,6-tri-*O*-benzyl-2-*O*-pivaloyl-β-D-galactopyranosyl)-α-D-mannopyranoside (13).**



According to general procedure (A), mannosyl-imidate **8** (467 mg, 0.73 mmol) and disaccharide **12** (290 mg, 0.24 mmol) were reacted to give the corresponding acetylated trisaccharide. The trisaccharide was deacetylated according to general procedure (D) to give **13** (286 mg, 0.18 mmol, 73% over two steps). $[\alpha]_D^{20}$ = + 11.7 º (c = 1.8, $CHCl_3$), IR ν_{max} (film) 3031, 2870, 1740, 1698, 1497, 1454, 1365, 1068 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ

7.57-6.97 (m, 50H), 5.40 (dd, *J* = 10.0, 8.1 Hz, 1H), 5.25-5.07 (m, 3H), 4.89 (dd, *J* = 11.5, 4.9 Hz, 2H), 4.82-4.69 (m, 3H), 4.68-4.55 (m, 4H), 4.51-4.40 (m, 7H), 4.38-4.28 (m, 3H), 4.23 (d, *J* = 11.9 Hz, 1H), 4.20-4.11 (m, 1H), 4.04-3.94 (m, 2H), 3.91-3.60 (m, 10H), 3.55-3.38 (m, 5H), 3.28-3.09 (m, 3H), 1.56-1.32 (m, 4H), 1.25-1.03 (m, 11H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 177.0, 139.2, 138.8, 138.7, 138.5, 138.4, 138.2, 137.9, 128.8, 128.7 (2C), 128.5 (3C), 128.4, 128.3, 128.2, 128.1, 128.0 (3C), 127.9 (2C), 127.8 (2C), 127.7 (2C), 127.6 (3C), 127.5, 127.2, 101.0 (anomeric), 100.4 (anomeric), 98.9 (anomeric), 81.3, 80.2, 78.0, 77.4, 76.3, 75.7, 75.6, 75.0, 74.6, 73.9, 73.7, 73.6, 73.5 (2C), 73.3, 72.8, 72.2, 72.0, 71.9, 71.8, 71.7, 71.6, 69.3, 69.1, 68.8, 68.4, 67.7, 67.3, 66.6, 64.9, 50.6, 50.3, 47.2, 46.3, 38.9, 29.3, 27.7, 27.4, 23.5; HRMS (MALDI-TOF): Calcd for $C_{99}H_{111}NO_{19}Na^+$ $[M+Na]^+$ 1640.7643, found 1640.7611.

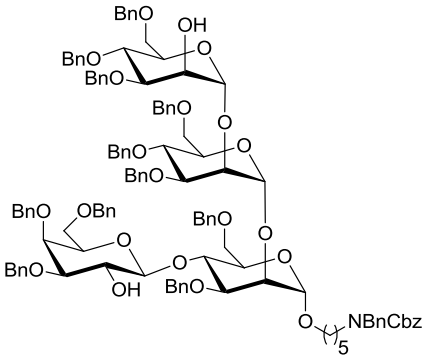
_N_-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl (2-_O_-acetyl-3,6-di-_O_-benzyl-α-D-mannopyranosyl)-(1→2)-(3,4,6-tri-_O_-benzyl-α-D-mannopyranosyl)-(1→2)-3,6-di-_O_-benzyl-4-_O_-(3,4,6-tri-_O_-benzyl-2-_O_-pivaloyl-β-D-galactopyranosyl)-α-D-mannopyranoside (14).



According to general procedure (A), mannosyl-imidate **8** (170 mg, 270 µmol) and trisaccharide **13** (144 mg, 89 µmol) were reacted to give tetrasaccharide **14** (156 mg, 75 µmol, 84%). $[\alpha]_D^{20}$ = + 12.7 º (c = 1.5, $CHCl_3$), IR ν_{max} (film) 3031, 2868, 1742, 1700, 1497, 1454, 1366, 1235, 1057 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.35-7.07 (m, 65H), 5.46 (dd, *J* = 3.1, 1.9 Hz, 1H), 5.40 (dd, *J* = 10.1, 8.0 Hz, 1H), 5.17 (d, *J* = 5.9 Hz, 2H), 5.11 (d, *J* = 1.3 Hz, 1H, anomeric), 4.92-4.85 (m, 3H), 4.83-4.71 (m, 4H), 4.68-4.59 (m, 4H), 4.56-4.37 (m, 13H), 4.23 (dd, *J* = 23.1, 11.7 Hz, 3H), 4.09 (t, *J* = 9.3 Hz, 1H), 4.03-3.99 (m, 1H), 3.95-3.89 (m, 4H), 3.87-3.82 (m, 3H), 3.70-3.67 (m, 2H), 3.65-3.55 (m, 4H), 3.47-3.35 (m, 5H), 3.24-3.11 (m, 3H), 2.08 (s, 3H), 1.50-1.35 (m, 4H), 1.20-1.08 (m, 11H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 177.0, 170.1, 139.4, 138.8 (3C), 138.6, 138.5, 138.2, 138.1, 138.0, 128.7, 128.6, 128.5 (2C), 128.4 (2C), 128.3 (3C), 128.1, 128.0 (2C), 127.9, 127.8 (2C), 127.7, 127.6 (2C), 127.5, 127.4, 127.1, 101.1 (anomeric), 100.4 (anomeric), 99.6 (anomeric), 98.7 (anomeric), 81.3, 79.9, 78.2,

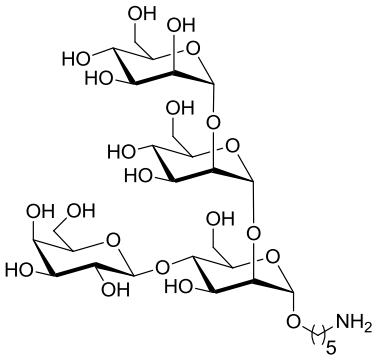
77.7, 77.4, 77.0, 75.3, 75.1, 74.8, 74.6, 74.4, 73.7, 73.4 (2C), 73.2, 72.6, 72.4, 72.3, 72.0, 71.6, 69.7, 69.2, 68.9, 68.3, 67.7, 67.3, 50.6, 50.3, 47.2, 46.3, 38.9, 29.3, 27.4, 23.5, 21.3; HRMS (MALDI-TOF): Calcd for $C_{128}H_{141}NO_{25}Na^+$ $[M+Na]^+$ 2114.9685, found 2114.9696.

_N_-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl (3,6-di-_O_-benzyl-α-D-mannopyranosyl)-(1→2)-(3,4,6-tri-_O_-benzyl-α-D-mannopyranosyl)-(1→2)-3,6-di-_O_-benzyl-4-_O_-(3,4,6-tri-_O_-benzyl-β-D-galactopyranosyl)-α-D-mannopyranoside (23).



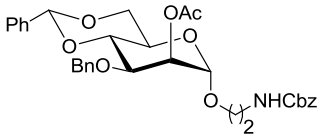
According to general procedure (F), tetrasaccharide **14** (84 mg, 41 µmol) was subjected to microwave assisted de-esterification to give **23** (74 mg, 38 µmol, 92%). $[\alpha]_D^{20}$ = + 21.8 º (c = 2.4, $CHCl_3$), IR ν_{max} (film) 3440, 3030, 2917, 2866, 1698, 1497, 1454, 1363, 1100, 1054 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.39- 7.12 (m, 65H), 5.18 (d, *J* = 9.5 Hz, 2H), 5.06 (d, *J* = 1.2 Hz, 1H, anomeric), 5.03 (d, *J* = 1.5 Hz, 1H, anomeric), 4.92 (d, *J* = 1.0 Hz, 1H, anomeric), 4.91-4.86 (m, 1H), 4.81 (dd, *J* = 10.9, 8.1 Hz, 2H), 4.76-4.66 (m, 3H), 4.64-4.46 (m, 15H), 4.32-4.21 (m, 3H), 4.18 (t, *J* = 9.4 Hz, 1H), 4.10-3.80 (m, 12H), 3.77-3.56 (m, 8H), 3.51-3.35 (m, 4H), 3.30-3.07 (m, 4H), 1.50-1.34 (m, 4H), 1.20-1.09 (m, 2H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 139.0, 138.8, 138.6, 138.4 (2C), 138.2, 138.1, 128.7, 128.6, 128.5 (3C), 128.4 (4C), 128.18, 128.1, 128.0 (3C), 127.9 (2C), 127.8 (2C), 127.7 (2C), 127.6 (2C), 127.5, 127.4 (2C), 104.4 (anomeric), 101.5 (anomeric), 101.1 (anomeric), 98.7 (anomeric), 81.7, 80.0, 79.7, 78.7, 77.4, 75.6, 75.1, 75.0, 74.7, 74.4, 73.5, 73.4, 73.4, 73.3, 73.2, 73.1, 72.6, 72.5, 72.3, 72.2, 71.7, 70.8, 69.9, 69.2, 68.6, 68.4, 67.5, 67.3, 50.6, 50.4, 47.3, 46.3, 29.4, 23.5; HRMS (MALDI-TOF): Calcd for $C_{121}H_{131}NO_{23}Na^+$ $[M+Na]^+$ 1988.9004, found 1988.9058.

5-Amino-pentanyl α-D-mannopyranosyl-(1→2)-α-D-mannopyranosyl-(1→2)-4-*O*-β-D-galactopyranosyl-α-D-mannopyranoside (1a).



According to general procedure (G), tetrasaccharide **23** (75 mg, 38 µmol) was subjected to Pd-catalyzed hydrogenation. Reversed phase solid phase extraction (Waters Sep-Pak®, C18) followed size exclusion chromatography on Sephadex LH-20 (MeOH) afforded **1a** (22 mg, 29 µmol, 77%). ^{1}H-NMR (400 MHz, D_2O) δ 5.32 (d, *J* = 1.6 Hz, 1H, anomeric), 5.13 (d, *J* = 1.3 Hz, 1H, anomeric), 5.05 (d, *J* = 1.7 Hz, 1H, anomeric), 4.47 (d, *J* = 7.8 Hz, 1H, anomeric), 4.14 (dd, *J* = 3.2, 1.8 Hz, 1H), 4.07 (dd, *J* = 3.3, 1.8 Hz, 1H), 4.06-3.52 (m, 24H), 3.02 (t, *J* = 7.6 Hz, 2H), 1.78-1.61 (m, 5H), 1.55-1.39 (m, 2H); ^{13}C-NMR (100 MHz, D_2O) δ 103.0 (anomeric), 102.2 (anomeric), 100.5 (anomeric), 97.8 (anomeric), 78.6, 78.4, 76.8, 75.2, 73.2, 72.5, 71.4, 70.9, 70.2, 69.8, 69.0, 68.5, 67.6, 67.0, 66.8, 61.1, 61.0, 60.3, 39.3, 27.9, 26.4, 22.4; HRMS (MALDI-TOF): Calcd for $C_{29}H_{53}NO_{21}Na^{+}$ $[M+Na]^{+}$ 774.3002 found 774.3953.

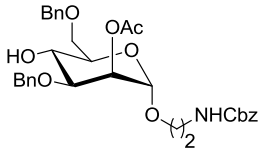
***N*-Benzyloxycarbonyl-2-amino-ethyl 2-*O*-Acetyl-3-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside (15)**



According to general procedure (A), mannosyl-imidate **4** (288 mg, 0.53 mmol) and *N*-benzyloxycarbonyl-2-aminoethanol (206 mg, 1.1 mmol) were reacted to give **15** (232 mg, 0.40 mmol, 76%). $[\alpha]_D^{20}$ = + 9.0 º (c = 5.3, $CHCl_3$), IR ν_{max} (film) 3353, 2928, 2877, 1719, 1521, 1455, 1372, 1229, 1133, 1074 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.56-7.17 (m, 15H), 5.60 (s, 1H), 5.38-5.32 (m, 1H), 5.13-5.01 (m, 3H), 4.76 (s, 1H, anomeric), 4.69-4.59 (m, 2H), 4.27-4.15 (m, 1H), 4.08-3.92 (m, 2H), 3.85- 3.68 (m, 3H), 3.50-3.23 (m, 3H), 2.13 (s, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 170.3, 156.4, 138.0, 137.4, 136.4, 129.0, 128.6, 128.4, 128.3, 127.9, 127.7, 126.2, 101.7, 99.0 (anomeric), 78.3, 73.9, 72.3, 69.7, 68.7, 67.2, 67.0, 64.2,

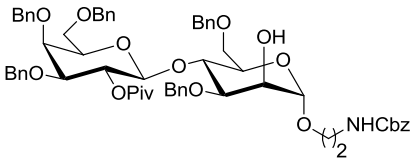
40.7, 21.1; HRMS (MALDI-TOF): Calcd for $C_{32}H_{35}NO_9Na^+$ $[M+Na]^+$ 600.2204, found 600.2314.

_N_-Benzyloxycarbonyl-2-amino-ethyl 2-_O_-acetyl-3,6-di-_O_-benzyl-α-D-mannopyranoside (16)



According to general procedure (B), compound **15 (**170 mg, 0.29 mmol) was reacted with triethylsilane (141 µL, 0.88 mmol) and triflic acid (89 µL, 1.00 mmol) in DCM (3 mL) at −78 °C to give **16** (132 mg, 0.23 mmol, 77%). $[\alpha]_D^{20}$ = − 6.0 º (c = 5.3, $CHCl_3$), IR ν_{max} (film) 3352, 2922, 1742, 1717, 1702, 1523, 1454, 1370, 1233, 1138, 1077 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.34-7.19 (m, 15H), 5.45-5.33 (m, 1H, NH), 5.28 (dd, *J* = 3.2, 1.8 Hz, 1H), 5.11-5.02 (m, 2H), 4.78 (d, *J* = 1.7 Hz, 1H, anomeric), 4.66 (d, *J* = 11.2 Hz, 1H), 4.53 (q, *J* = 12.2 Hz, 2H), 4.41 (d, *J* = 11.2 Hz, 1H), 3.86-3.55 (m, 7H), 3.41-3.29 (m, 2H), 2.06 (s, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 170.4, 156.5, 138.0, 137.6, 136.6, 128.6 (2C), 128.4 (2C), 128.3, 128.2, 128.1 (2C), 127.9, 127.8, 127.7 (2C), 98.4 (anomeric), 77.6, 73.6, 71.8, 69.8, 68.1, 68.1, 67.2, 66.8, 41.0, 21.0; HRMS (MALDI-TOF): Calcd for $C_{32}H_{37}NO_9Na^+$ $[M+Na]^+$ 602.2361, found 602.2420.

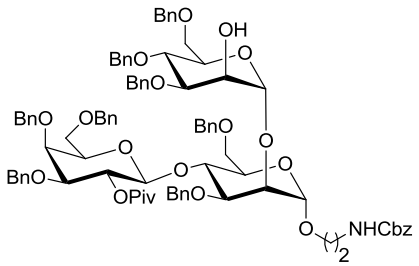
_N_-Benzyloxycarbonyl-2-amino-ethyl 3,4,6-tri-O-benzyl-2-_O_-pivaloyl-β-D-galactopyranosyl (1→4)-3,6-di-_O_-benzyl-α-D-mannopyranoside (17)



According to general procedure (C), galactosyl-phosphate **6** (126 mg, 0.17 mmol) and **16** (84 mg, 0.14 mmol) were reacted at −40 °C to −20 °C to give the corresponding acetylated disaccharide. The disaccharide was de-acetylated according to general procedure (E) to give **17** (126 mg, 0.12 mmol, 83% over two steps). $[\alpha]_D^{20}$ = + 18.6 º (c = 4.7, $CHCl_3$), IR ν_{max} (film) 3421, 3031, 2928, 2872, 1737, 1720, 1519, 1454, 1366, 1276, 1252, 1103, 1061cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.45-7.12 (m, 30H), 5.39 (dd, *J* = 10.1, 7.9 Hz, 2H), 5.14- 5.07 (m, 2H), 4.94 (d, *J* = 11.4 Hz, 1H), 4.89 (d, *J* = 11.3 Hz, 1H), 4.84 (d, *J* = 1.8 Hz, 1H), 4.73 (d, *J* = 12.1 Hz, 1H), 4.67 (d, *J* = 11.9 Hz, 1H), 4.59 (d, *J* = 11.3 Hz, 1H), 4.54-4.37 (m, 5H), 4.30 (d, *J* = 11.7 Hz, 1H), 4.07 (t, *J* = 8.7 Hz, 1H), 3.96 (dd, *J* = 3.4, 1.9 Hz, 1H), 3.94 (d, *J* =

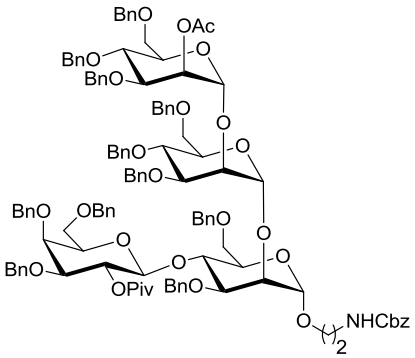
2.4 Hz, 1H), 3.80- 3.56 (m, 7H), 3.53-3.29 (m, 5H), 1.12 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.0, 138.7, 138.6, 138.2, 138.1, 137.9, 136. 7, 128.6, 128.5, 128.4 (2C), 128.3 (2C), 128.2, 128.1 (2C), 127.9 (2C), 127.8, 127.7, 127.6, 127.2, 100.4 (anomeric), 99.8 (anomeric), 81.2, 77.9, 74.6, 73.7, 73.6 (2C), 73.2, 73.1, 72.7, 72.1, 71.8, 71.3, 69.3, 68.9, 68.6, 68.1, 66.8, 41.1, 38.9, 27.4; HRMS (MALDI-TOF): Calcd for $C_{62}H_{71}NO_{14}Na^+$ $[M+Na]^+$ 1076.4767, found 1076.4584.

***N*-Benzyloxycarbonyl-2-amino-ethyl (3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→2)-3,6-di-*O*-benzyl-4-*O*-(3,4,6-tri-*O*-benzyl-2-*O*-pivaloyl-β-D-galactopyranosyl)-α-D-mannopyranoside (18).**



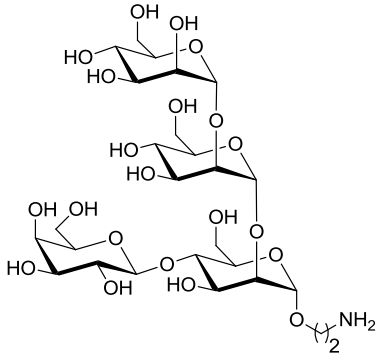
According to general procedure (A), mannosyl-imidate **8** (114 mg, 0.18 mmol) and disaccharide **17** (126 mg, 0.12 mmol) were reacted to give the corresponding acetylated trisaccharide. The trisaccharide was deacetylated according to general procedure (E) to give **18** (123 mg, 83 µmol, 69%). $[\alpha]_D^{20}$ = + 14.4º (c = 2.5, $CHCl_3$), IR ν_{max} (film) 3425, 3031, 2919, 2870, 1737, 1497, 1454, 1364, 1277, 1251, 1210, 1100, 1052 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.41-7.11 (m, 45H), 5.53-5.33 (m, 2H), 5.18-4.99 (m, 3H), 4.96-4.80 (m, 3H), 4.78-4.70 (m, 2H), 4.68-4.54 (m, 4H), 4.52-4.35 (m, 8H), 4.27 (d, *J* = 11.9 Hz, 1H), 4.09-3.82 (m, 6H), 3.80- 3.62 (m, 7H), 3.60-3.39 (m, 6H), 3.31-3.16 (m, 2H), 1.12 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.9, 139.0, 138.6, 138.4, 138.3, 138.1 (2C), 137.9, 128.6, 128.5 (3C), 128.4, 128.3, 128.2 (2C), 128.1, 128.0 (3C), 127.9, 127.8 (2C), 127.7 (3C), 127.6, 127.4, 127.2, 100.8 (anomeric), 100.6 (anomeric), 99.4 (anomeric), 81.2, 80.2, 77.8, 75.4, 75.1, 74.6, 73.8, 73.6, 73.5, 73.2, 72.7, 72.1, 72.0, 71.9, 71.8, 69.5, 69.2, 68.8, 68.4, 66.7, 38.9, 27.4; HRMS (MALDI-TOF): Calcd for $C_{89}H_{99}NO_{19}Na^+$ $[M+Na]^+$ 1508.6704, found 1508.6704.

***N*-Benzyloxycarbonyl-2-amino-ethyl (2-O-acetyl-3,6-di-O-benzyl-α-D-mannopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→2)-3,6-di-*O*-benzyl-4-*O*-(3,4,6-tri-O-benzyl-2-*O*-pivaloyl-β-D-galactopyranosyl)-α-D-mannopyranoside (19)**



According to general procedure (A), mannosyl-imidate **8** (49 mg, 77 μmol) and trisaccharide **18** (38 mg, 26 μmol) were reacted to give tetrasaccharide **19** (43 mg, 22 μmol, 86%). $[\alpha]_D^{20}$ = + 10.1 º (c = 4.4, $CHCl_3$), IR ν_{max} (film) 3031, 2912, 2868, 1740, 1497, 1454, 1497, 1454, 1365, 1235, 1051 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.40-7.09 (m, 60H), 5.51-5.43 (m, 1H), 5.40 (dd, *J* = 10.1, 7.9 Hz, 1H), 5.17-5.02 (m, 3H), 4.96-4.79 (m, 5H), 4.77- 4.39 (m, 17H), 4.33-4.23 (m, 3H), 4.05-3.39 (m, 24H), 3.28-3.15 (m, 2H), 2.11 (s, 3H), 1.11 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.9, 170.2, 139.1, 138.7, 138.5, 138.4, 138.1, 138.0, 137.9, 128.6, 128.5, 128.4 (3C), 128.3 (3C), 128.2, 128.1 (2C), 128.0, 127.9, 127.8 (3C), 127.7 (3C), 127.6, 127.5 (2C), 127.3, 127.1, 100.9 (anomeric), 100.5 (anomeric), 99.7 (anomeric), 99.2 (anomeric), 81.3, 79.8, 78.1, 75.4, 75.2, 75.2, 74.8, 74.6, 74.4, 73.5 (2C), 73.4 (2C), 73.3, 72.6, 72.4, 72.3, 72. (2C), 71.7, 69.8, 69.3, 68.9, 68.4, 68.0, 66.7, 41.1, 38.9, 27.4, 21.3; HRMS (MALDI-TOF): Calcd for $C_{118}H_{129}NO_{25}Na^+$ $[M+Na]^+$ 1982.8746, found 1982.8837.

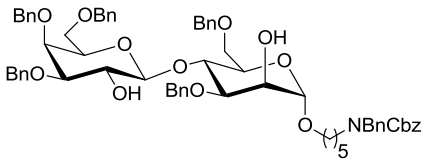
2-Amino-ethyl α-D-mannopyranosyl-(1→2)-α-D-mannopyranosyl-(1→2)-4-*O*-β-D-galactopyranosyl-α-D-mannopyranoside (1b)



To a solution of fully protected tetrasaccharide **19** (32 mg, 16 μmol) in THF (2 mL) and MeOH (2 mL), a solution KOH (0.25N, 0.2 mL) was added. After 3 h the mixture was

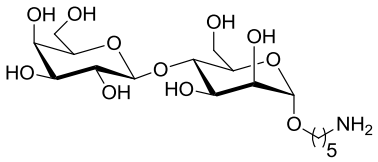
neutralized with Amberlite ® IR 120 (H^+) ion exchange resin, filtered and concentrated. Column chromatography on silica gel (toluene/acetone) afforded the corresponding de-acetylated tetrasaccharide (29 mg). The de-acetylated tetrasaccharide was divided into two equal batches for hydrogenation, each batch was dissolved in a mixture of MeOH (2 mL), H_2O (0.4 mL) and AcOH (0.1 mL). The solution was purged with Ar, 10% Pd/C (40 mg) was added and the solution purged with H_2 for 30 min, then stirred under an H_2 atmosphere for 12 h, filtered and concentrated. The two batches were combined, dissolved in an aqueous solution of KOH (0.25M, 1.0 mL) and stirred for 12 h, neutralized with 2% AcOH and lyophilized. Size exclusion chromatography on Sephadex LH-20 (MeOH) afforded **1b** (6.1 mg, 8.6 μmol, 54%). ^{1}H NMR (D_2O, 600 MHz) δ 5.33 (d, *J* = 1.4 Hz, 1H, anomeric), 5.17 (d, *J* = 1.3 Hz, 1H, anomeric), 5.05 (d, *J* = 1.5 Hz, 1H, anomeric), 4.47 (d, *J* = 7.9 Hz, 1H, anomeric), 4.16-4.13 (m, 1H), 4.10-4.05 (m, 3H), 4.01-3.62 (m, 21H), 3.56 (dd, *J* = 9.9, 7.9 Hz, 1H), 3.30-3.21 (m, 2H); ^{13}C NMR (150 MHz, D_2O) δ 103.8 (anomeric), 102.9 (anomeric), 101.2 (anomeric), 98.7 (anomeric), 79.3, 78.9, 77.4, 76.0, 73.9, 73.2, 72.3, 71.6, 71.0, 70.6, 69.5, 69.2, 67.8, 67.5, 61.9, 61.7, 60.9, 39.8; HRMS (MALDI-TOF): Calcd for $C_{26}H_{47}NO_{21}Na^+$ $[M+Na]^+$ 732.2533, found 732.2598.

_N_-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl 3,4,6-tri-_O_-benzyl-β-D-galactopyranosyl (1→4)-3,6-di-_O_-benzyl-α-D-mannopyranoside (24).



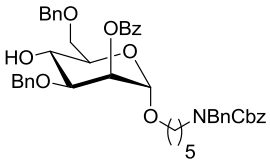
According to general procedure (F), disaccharide **12** (35 mg, 30 μmol) was subjected to microwave assisted de-esterification to give partially deprotected disaccharide **24** (32 mg, 29 μmol, 98%). $[\alpha]_D^{20}$ = + 24.2 º (c = 2.4, $CHCl_3$), IR ν_{max} (film) 3451, 3030, 2923, 2866, 1697, 1497, 1454, 1365, 1229, 1213, 1100, 1060 cm^{-1} cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.50-7.07 (m, 35H), 5.18 (d, *J* = 10.8 Hz, 2H), 4.90 (d, *J* = 11.5 Hz, 1H), 4.84-4.77 (m, 2H), 4.73-4.47 (m, 9H), 4.33 (d, *J* = 11.7 Hz, 1H), 4.25 (d, *J* = 11.7 Hz, 1H), 4.18 (t, *J* = 9.4 Hz, 1H), 4.02-3.69 (m, 7H), 3.65-3.52 (m, 2H), 3.46-3.17 (m, 6H), 1.58-1.42 (m, 4H), 1.32-1.21 (m, 2H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 139.0, 138.5, 138.0, 128.7, 128.6, 128.5, 128.4, 128.3, 128.1 (2C), 128.0 (2C), 127.9, 127.8, 127.7, 127.5, 104.2 (anomeric), 99.2 (anomeric), 81.9, 79.3, 77.5, 77.4, 77.2, 76.8, 74.7, 74.6, 73.7 (2C), 73.6, 72.5 (2C), 72.4, 70.5, 69.2, 68.9, 68.6, 67.4, 67.3, 29.2, 23.5; HRMS (MALDI-TOF): Calcd for $C_{67}H_{75}NO_{13}Na^+$ $[M+Na]^+$ 1124.5131, found 1124.5099.

5-Amino-pentanyl β-D-galactopyranosyl-(1→2)-α-D-mannopyranoside (3)



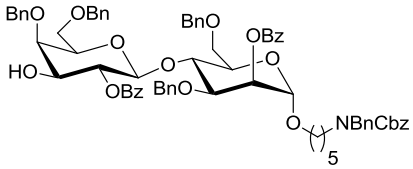
According to general procedure (G), disaccharide **24** (24 mg, 22 μmol) was subjected to Pd-catalyzed hydrogenation. Reversed phase solid phase extraction (Waters Sep-Pak®, C18) afforded **2** (7.4 mg, 17 μmol, 80%). ^{1}H NMR (D_2O, 400 MHz) δ 4.88 (d, *J* = 1.6 Hz, 1H, anomeric), 4.46 (d, *J* = 7.8 Hz, 1H, anomeric), 3.78 (m, 14H), 3.04-2.97 (m, 2H), 1.77-1.61 (m, 4H), 1.55-1.39 (m, 2H). ^{13}C NMR (100 MHz, D_2O) δ 100.8 (anomeric), 97.2 (anomeric), 74.4, 73.2, 70.3, 69.1, 68.7, 67.3, 67.3, 66.4, 65.4, 58.9, 58.1, 37.1, 25.8, 24.3, 20.2; HRMS (MALDI-TOF): Calcd for $C_{17}H_{33}NO_{11}Na^+$ $[M+Na]^+$ 450.1946, found 450.1959.

***N*-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl 2-*O*-benzoyl-3,6-di-*O*-benzyl-α-D-mannopyranoside (20)**



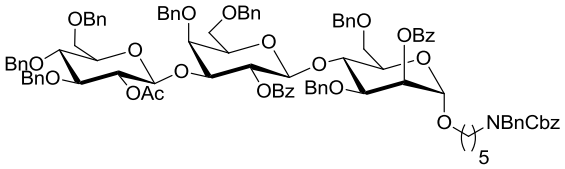
According to general procedure (A), mannosyl-imidate **5** (930 mg, 1.53 mmol) and *N*-(benzyl)benzyloxycarbonyl-5-aminopentanol (652 mg, 1.99 mmol) were reacted to give the corresponding linker-functionalized mannoside. Selective 4,6-*O*-benzylidene opening proceeded according to general procedure (B) to give **20** (929 mg, 1.20 mmol, 78% over two steps). $[\alpha]_D^{20}$ = – 15.9 º (c = 1.2, $CHCl_3$), IR ν_{max} (film) 3444, 3032, 2934, 1720, 1698, 1453, 1423, 1270, 1099 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.10-8.01 (m, 2H), 7.59-7.13 (m, 23H), 5.56 (s, 1H), 5.19 (d, *J* = 15.5 Hz, 2H), 4.94 (s, 1H, anomeric), 4.79 (d, *J* = 11.2 Hz, 1H), 4.69 (d, *J* = 12.0 Hz, 1H), 4.60 (d, *J* = 12.0 Hz, 1H), 4.55-4.46 (m, 3H), 4.13 (td, *J* = 9.3, 2.2 Hz, 1H), 3.93-3.78 (m, 4H), 3.74-3.62 (m, 1H), 3.46-3.35 (m, 1H), 3.31-3.18 (m, 2H), 2.65-2.47 (m, 1H), 1.65-1.47 (m, 4H), 1.40-1.22 (m, 2H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 165.9, 138.0, 133.3, 130.1, 129.9, 128.7, 128.6, 128. (2C), 128.2, 128.0, 127.6 (2C), 98.1 (anomeric), 77.9, 73.7, 71.6, 70.0, 67.9, 67.5, 67.3, 50.7, 50.4, 47.3, 46.3, 29.2, 28.1, 27.6, 23.6; HRMS (MALDI-TOF): Calcd for $C_{47}H_{51}NO_9Na^+$ $[M+Na]^+$ 796.3456, found 796.3447.

_N_-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl 4,6-di-_O_-benzyl-2-_O_-benzoyl-β-D-galactopyranosyl-(1→4)-2-_O_-benzoyl-3,6-di-_O_-benzyl-α-D-mannopyranoside (21)



According to general procedure (C), galactosyl-phosphate **7** (66 mg, 75 μmol) and **20** (48 mg, 63 μmol) were reacted at −40 °C to −20 °C. The reaction was quenched by the addition of NEt_3 (20% in DCM, 10 mL), stirred at room temperature for 1 h and concentrated. The crude product was purified by column chromatography (hexanes/EtOAc) to afford disaccharide **21** (68 mg, 56 μmol, 89%). $[\alpha]_D^{20}$ = + 12.6º (c = 2.9, $CHCl_3$), IR ν_{max} (film) 3454, 3031, 2926, 2866, 1723, 1698, 1602, 1453, 1422, 1364, 1267, 1098, 1069 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 8.08-7.98 (m, 4H), 7.58-7.50 (m, 2H), 7.43-7.12 (m, 34H), 5.53 (s, 1H), 5.24 (dd, *J* = 10.1, 8.0 Hz, 1H), 5.22-5.14 (m, 2H), 4.90-4.76 (m, 3H), 4.68 (q, *J*= 11.7 Hz, 3H), 4.54-4.42 (m, 3H), 4.30 (q, *J* = 11.7 Hz, 2H), 4.25-4.17 (m, 2H), 4.09-4.03 (m, 1H), 3.86 (d, *J* = 3.2 Hz, 1H), 3.80-3.71 (m, 1H), 3.69-3.15 (m, 10H), 1.59-1.42 (m, 4H), 1.33-1.14 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.6, 165.8, 138.8, 138.7, 138.2, 138.1, 137.8, 133.3, 133.2, 130.1, 130.0, 129.9 (2C), 128.7, 128.5 (2C), 128.3, 128.2, 128.0 (3C), 127.9, 127.4, 127.3 (2C), 127.2, 101.0 (anomeric), 97.6 (anomeric), 77.7, 76.6, 75.6, 75.1 (2C), 73.5, 73.3, 73.2 (2C), 71.6, 70.9, 69.4, 69.0, 68.0, 67.3, 50.7, 47.2, 46.3, 29.2, 28.0, 23.4; HRMS (MALDI-TOF): Calcd for $C_{74}H_{77}NO_{15}Na^+$ $[M+Na]^+$ 1242.5185, found 1242.5206.

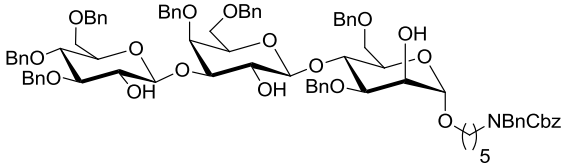
_N_-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl 2-_O_-acetyl-3,4,6-tri-_O_-benzyl-β-d-glucopyranosyl-(1→3)-4,6-di-_O_-benzyl-2-_O_-benzoyl-β-D-galactopyranosyl-(1→4)-2-_O_-benzoyl-3,6-di-_O_-benzyl-α-D-mannopyranoside (22)



Glucosyl-imidate **9** (74 mg, 111 μmol) and disaccharide **20** (68 mg, 56 μmol) were coevaporated with toluene three times and dried *in vacuo*. The mixture was dissolved in DCM (1 mL) and cooled to -78 °C. TMSOTf (2 μL, 11 μmol) was added and the mixture was warmed to -50 °C over 2 hours. The reaction was quenched by the addition of NEt_3 and concentrated. The crude product was purified by column chromatography (hexanes/EtOAc) to afford trisaccharide **22** (50 mg, 29 μmol, 53%). $[\alpha]_D^{20}$ = + 0.1 º (c = 3.3, $CHCl_3$), IR ν_{max}

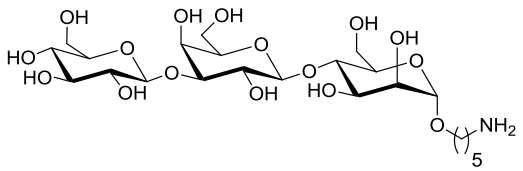
(film) 3031, 2923, 2865, 1729, 1698, 1602, 1497, 1453, 1365, 1267, 1228, 1097, 1067 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 8.00-7.92 (m, 4H), 7.40-7.08 (m, 51H), 5.58 (dd, *J* = 10.2, 8.0 Hz, 1H), 5.49 (s, 1H), 5.26-5.13 (m, 2H), 5.00 (d, *J* = 11.4 Hz, 1H), 4.96 (dd, *J* = 9.7, 8.0 Hz, 1H), 4.85-4.77 (m, 2H), 4.75-4.65 (m, 3H), 4.60-4.47 (m, 8H), 4.46-4.39 (m, 2H), 4.32-4.15 (m, 4H), 4.03 (dd, *J* = 9.0, 3.1 Hz, 1H), 3.97 (d, *J* = 2.8 Hz, 1H), 3.85 (dd, *J* = 10.2, 2.9 Hz, 1H), 3.77-3.40 (m, 11H), 3.34-3.14 (m, 4H), 1.75 (s, 3H), 1.57-1.40 (m, 4H), 1.33-1.10 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.7, 165.9, 164.8, 138.9, 138.8 (2C), 138.1 (3C), 137.8, 133.2, 133.1, 130.2, 130.1, 130.0, 129.7, 128.7 (2C), 128.6 (2C), 128.5 (2C), 128.4, 128.3 (2C), 128.2, 128.1 (2C), 128.0, 127.8 (2C), 127.7, 127.5, 127.4, 127.2, 101.6 (anomeric), 101.0 (anomeric), 97.6 (anomeric), 82.9, 79.4, 77.9, 77.4, 76.6, 75.9, 75.3, 75.2, 75.0, 74.9, 74.6, 73.8, 73.6 (2C), 73.3, 73.2, 72.7, 71.8, 71.0, 69.5, 68.8, 68.7, 67.3, 23.4, 20.5; HRMS (MALDI-TOF): Calcd for $C_{103}H_{107}NO_{21}Na^+$ $[M+Na]^+$ 1716.7228, found 1716.7208.

***N*-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl 3,4,6-tri-*O*-benzyl-β-D-glucopyranosyl-(1→3)-4,6-di-*O*-benzyl-β-D-galactopyranosyl-(1→4)-3,6-di-*O*-benzyl-α-D-mannopyranoside (25)**



According to general procedure (E), trisaccharide **22** (36 mg, 21 μmol) was subjected to saponification of the ester groups. Size exclusion chromatography on Sephadex LH-20 ($CHCl_3$/MeOH 1:1) afforded tirsaccharide **25** (28 mg, 19 μmol, 91%). $[\alpha]_D^{20}$ = + 22.3 º (c = 2.6, $CHCl_3$), IR ν_{max} (film) 3443, 3031, 2920, 2866, 1698, 1454, 1363, 1210, 1096, 1060 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.46-7.14 (m, 45H), 5.25-5.14 (m, 2H), 5.01 (dd, *J* = 19.7, 11.5 Hz, 2H), 4.93-4.71 (m, 5H), 4.66-4.48 (m, 8H), 4.45-4.40 (m, 1H), 4.28 (d, *J* = 11.7 Hz, 1H) 4.23-4.16 (m, 2H), 4.05-3.70 (m, 9H), 3.66-3.14 (m, 13H), 1.62-1.42 (m, 4H), 1.37-1.17 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 139.1, 139.0, 138.3, 138.0, 128.7, 128.6, 128.5 (3C), 128.4 (2C), 128.2, 128.1 (2C), 128.0, 127.9, 127.8, 127.7, 127.6, 127.4, 106.1 (anomeric), 104.0 (anomeric), 99.2 (anomeric), 84.7, 79.6, 77.4, 77.3, 76.1, 75.5, 75.2, 75.0 (2C), 74.6, 74.4, 74.0, 73.8, 73.6, 73.5, 72.3, 72.2, 70.3, 69.5, 69.3, 68.7, 67.4, 50.3, 47.2, 29.2, 27.5, 23.5; HRMS (MALDI-TOF): Calcd for $C_{87}H_{97}NO_{18}Na^+$ $[M+Na]^+$ 1466.6598 found, 1466.6556.

5-Amino-pentanyl β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-α-D-mannopyranoside (3)



According to general procedure (G), trisaccharide **25** (28 mg, 19 μmol) was subjected to Pd-catalyzed hydrogenation. Reversed phase solid phase extraction (Waters Sep-Pak®, C18) followed size exclusion chromatography on Sephadex LH-20 (MeOH) afforded trisaccharide **3** (8.0 mg, 14 μmol, 70%). ^{1}H NMR (D_2O, 600 MHz) δ 4.90 (d, *J* = 1.6 Hz, 1H, anomeric), 4.71 (d, *J* = 7.9 Hz, 1H, anomeric), 4.53 (d, *J* = 7.9 Hz, 1H, anomeric), 4.22 (d, *J* = 3.2 Hz, 1H), 4.03 (dd, *J* = 3.1, 1.8 Hz, 1H), 3.98 (dd, *J* = 12.1, 2.1 Hz, 1H), 3.96-3.70 (m, 12H), 3.63-3.36 (m, 5H), 3.06-3.00 (m, 2H), 1.76-1.63 (m, 4H), 1.56-1.41 (m, 2H); ^{13}C NMR (150 MHz, D_2O) δ 104.4 (anomeric), 103.3 (anomeric), 100.1 (anomeric), 82.7, 77.1, 76.4, 76.2, 75.7, 73.9, 72.0, 70.8, 70.2, 70.1, 70.0, 69.0, 68.2, 61.7, 61.1, 61.0, 40.0, 28.6, 27.2, 23.1; HRMS (MALDI-TOF): Calcd for $C_{23}H_{43}NO_{16}Na^{+}$ $[M+Na]^{+}$ 612.2474 found, 612.2465.

6.2.3 Glycan Microarray Preparation and Screening

Preparation of glycan microarrays

Anti-glycan antibody response was analysed by antigen array made of oligosaccharides antigens, **1**, **2**, **3** and **26** was prepared to screen the dog sera**.** Antigens bearing an amine linker, were immobilized on CodeLink *N*-hydroxyl succinimide (NHS) ester activated glass slides (SurModics Inc., Eden Prairie, MN, USA) with a piezoelectric spotting device (S3; Scienion, Berlin, Germany). 64 replicate array grids were printed on each slide. Microarray slides were incubated in a humid chamber to complete reaction for 24 h, quenched with 50 mM aminoethanol solution, pH 9 for 1 h at 50°C, washed three times with deionized water, and stored desiccated until use.

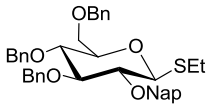
Antibody binding assays using synthetic carbohydrate antigen-based micro arrays.

A FlexWell 64 (Grace Bio-Labs, Bend, OR, USA) grid was applied to the slides. The resulting 64 wells were used for 64 individual experiments. The slide was blocked with 30 µL blocking buffer (2.5% (w/v) BSA) for 1 h at room temperature and washed 2X with 40 µL wash buffer (0.05% (v/v) Tween 20 in PBS). Blocked slides were incubated with serum dilutions in blocking buffer (20 µL) for 1 h at room temperature. Slides were washed 3X with 40 µL washing buffer and incubated with 20 µL of secondary antibodies solution in blocking buffer [anti-dog IgG DyLight® 488 (Fuller Labs, USA)]. Slides were washed with 4X with 40 µL washing buffer and centrifuged to dryness before scanning with a GenePix 4300A scanner (Bucher Biotec, Basel, Switzerland) and evaluated using the GenePix Pro 7 software (Bucher Biotec).

6.3 Synthesis of the *C. difficile* PS-I Pentasaccharide Repeating Unit

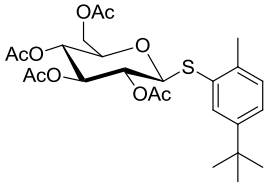
6.3.1 First Synthesis of the *C. difficile* PS-I Pentasaccharide

Ethyl 3,4,6-tri-*O*-benzyl-2-*O*-(2-naphthalenylmethyl)-1-thio-D-glucopyranoside (36)



To a solution of **37**[138] (284 mg, 0.57 mmol) in anhydrous DMF (1 mL), NaH (20.7 mg, 0.86 mmol) was added followed by NAP-Br (228 mg, 1.03 mmol) at 0 °C. The mixture was warmed to room temperature over 1 h, cooled to 0 °C and quenched by the addition of MeOH (0.1 mL). Et_2O was added and the organic layer washed with 10 mM HCl solution and with saturated aqueous $NaHCO_3$ solution. The phases were separated and the organic layer was dried over $MgSO_4$ and concentrated. Column chromatography (cyclohexane/ethyl acetate) afforded **36** (335 mg, 0.53 mmol, 92%) as mixture of α/β-anomers as a white solid. Analytical data is reported for the β-anomer. $[\alpha]_D^{20}$ = +26.1 ° (c = 5.3, $CHCl_3$), IR ν_{max} (film) 3061, 3030, 2864, 1949, 1808, 1603, 1497, 1453, 1360, 1065 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.82-7.69 (m, 4H, Ar-H), 7.52-7.09 (m, 18H, Ar-H), 5.08-5.02 (m, 1H, -CH_2-Ar), 4.93-4.77 (m, 4H, -CH_2-Ar), 4.60-4.50 (m, 3H, -CH_2-Ar), 4.47 (d, 1H, *J* = 9.7 Hz, 1-H), 3.80-3.54 (m, 4H), 3.52-3.41 (m, 2H), 2.84-2.66 (m, 2H, S-CH_2-), 1.31 (t, 3H, *J* = 7.3 Hz, CH_3); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 138.7, 138.4, 138.2, 135.6, 133.4, 133.2, 128.5 (2C), 128.5, 128.2, 128.1, 127.9 (2C), 127.8 (3C), 127.7, 127.2, 126.5, 126.1, 126.0, 86.8, 85.2 (C-1), 82.0, 79.3, 78.2, 75.9, 75.7, 75.2, 73.6, 69.3, 25.2, 15.3; HRMS (ESI): Calcd for $C_{40}H_{42}O_5S$ $[M+Na]^+$ 657.2651, found 657.2651.

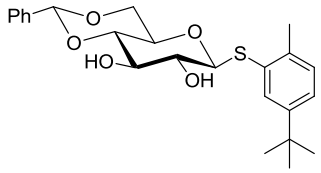
(2-Methyl-5-*tert*-butylphenyl) 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranoside (39)



Commercially purchased 1,2,3,4,6-Penta-*O*-acetyl-β-D-glucopyranose **38** (30 g, 77 mmol) was dissolved in anhydrous DCM (34 mL). 2-Methyl-5-*tert*-butyl thiophenol (17 mL, 92 mmol) was added with constant stirring. $BF_3 \cdot OEt_2$ (13.6 mL, 108 mmol) was added dropwise

and the resulting yellow solution was stirred overnight. After completion, the solution was diluted with DCM and extracted with saturated aqueous $NaHCO_3$ and H_2O, and the organic layer was dried over $MgSO_4$. The solvent was evaporated and the residue was dried *in vacuo*. The resulting yellow solid was purified by column chromatography on silica gel (cyclohexane/ethyl acetate) to afford **39** (33.4 g, 65.4 mmol, 85%). $[\alpha]_D^{20}$ = -8.0 º (c = 1.0, $CHCl_3$); IR ν_{max} (film) 2961, 1747, 1366, 1211, 1034, 912 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.52 (d, 1H, *J* = 2.0 Hz, Ar-H), 7.25-7.10 (m, 2H, Ar-H), 5.19 (dd, 1H, *J* = 9.4 Hz, 1-H), 5.10-4.98 (m, 2H, 4-H, 2-H), 4.64 (d, 1H, *J* = 10.6 Hz, 1-H), 4.23 (dd, 1H, *J* = 12.2, 5.0 Hz, 6-Ha), 4.10 (dd, 1H, *J* = 12.2, 1.9 Hz, 6-Hb), 3.71-3.63 (m, 1H, 5-H), 2.34 (s, 3H, CH_3), 2.07-2.03 (m, 6H, OAc), 2.00-1.96 (m, 6H, OAc), 1.29 (s, 9H, *t*Bu); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 170.8, 170.3, 169.5, 169.4 (C=O OAc), 149.8, 137.5 (2C), 130.53, 130.2, 125.8, 87.0 (C-1), 75.9 (C-5), 74.2 (C-3), 70.3 (C-3), 68.3(C-4), 62.4 (C-6), 31.4 (*t*Bu), 20.9 (2C), 20.7 (2C) (OAc), 20.5 (CH_3); HRMS (ESI): Calcd for $C_{25}H_{34}O_9S$ $[M+Na]^+$ 533.1816, found 533.1832.

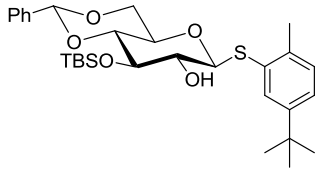
(2-Methyl-5-*tert*-butylphenyl) 4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside (41)



Thioglycoside **39** (1.5 g, 2.94 mmol) was dissolved in methanol (12 mL). Sodium methoxide (58 mg, 1.07 mmol) was added and the reaction was stirred overnight. After completion, the solution was neutralized with Amberlite IR 120 (H^+) ion exchange resin, filtered and concentrated. The remainder was dried *in vacuo* to give (2-Methyl-5-*tert*-butylphenyl) 1-thio-β-D-glucopyranoside **40** (1.0 g), which was used for the next reaction step without further purification. **40** (1.0 g) was dissolved in anhydrous acetonitrile (11.3 mL) at room temperature under argon atmosphere and benzaldehyde dimethylacetal (880 µL, 5.84 mmol) and camphorsulfonic acid (7 mg, 0.029 mmol) were added. After 2.5 h, the reaction was quenched with triethylamine, and the solvents were evaporated to give 1.5 g of colorless oil. The crude product was purified by column chromatography on silica gel (cyclohexane/ethyl acetate) to afford **41** (1.09 g, 2.53 mmol, 87%). $[\alpha]_D^{20}$ = -49.4 º (c = 1.0, CH_2Cl_2); IR ν_{max} (film) 3410, 2963, 2870, 1384, 1264, 1082, 1072, 1029, 1003, 972 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.61 (d, 1H, *J* = 2.0 Hz, Ar-H), 7.51-7.46 (m, 2H, Ar-H), 7.39-7.35 (m, 3H, Ar-H), 7.29-7.23 (m, 2H, Ar-H), 7.16 (d, 1H, *J* = 8.0 Hz, Ar-H), 5.54 (1H, s, benzylidene-H), 4.64 (d, 1H, *J* = 10.0 Hz, 1-H), 4.36 (dd, 1H, *J* = 10.3, 4.5 Hz, 6-Ha), 3.90-3.73 (m, 2H, 3-H, 6-Hb), 3.59-3.47

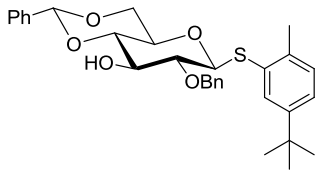
(m, 3H, 2-H, 4-H, 5-H), 2.86 (d, 1H, *J* = 2.2 Hz, OH), 2.69 (d, 1H, *J* = 2.4 Hz, OH), 2.42 (s, 3H, CH_3), 1.32 (s, 9H, *t*Bu); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 149.7, 137.1, 137.0, 131.0, 130.3, 130.2, 129.4, 128.5, 126.4, 125.5 (C-aromatic), 102.0 (C-benzylidene), 88.8 (C-1), 80.4 (C-2), 74.8 (C-3), 73.0 (C-4), 70.5 (C-5), 68.7 (C-6), 31.4 (*t*Bu), 20.6 (CH_3); HRMS (ESI): Calcd for $C_{24}H_{30}O_5S$ $[M+Na]^+$ 453.1706, found 453.1714.

(2-Methyl-5-*tert*-butylphenyl) 4,6-*O*-benzylidene-3-*O*-*tert*-butyldimethylsilyl-1-thio-β-D-glucopyranoside (42)



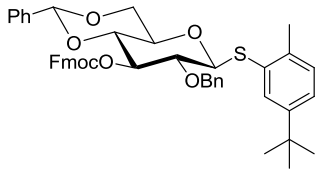
Compound **41** (658 mg, 1.53 mmol) and imidazole (208 mg, 3.06 mmol) were dissolved in anhydrous DMF (880 µL). TBSCl (346 mg, 2.29 mmol) was gradually added while stirring. After 4 h, the solvent was evaporated and the resulting oil was dissolved in DCM. The solution was extracted with 1 M HCl and saturated aqueous $NaHCO_3$ solution, the organic layer was dried over $MgSO_4$ and the solvent was evaporated. The colorless solid was dried *in vacuo* and the crude product (820 mg) was purified using flash column chromatography (cyclohexane/ethyl acetate) to afford **42** (573 mg, 1.05 mmol, 69 %). $[\alpha]_D^{20}$ = -49.1 ° (c = 1.0, CH_2Cl_2); IR ν_{max} (film) 3559, 2957, 2928, 2858, 1631, 1383, 1259, 1110, 1086, 1067, 1009 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.61 (d, 1H, *J* = 2.1 Hz, Ar-H), 7.51-7.46 (m, 2H, Ar-H), 7.39-7.33 (m, 3H, Ar-H), 7.26-7.22 (m, 1H, Ar-H), 7.15 (d, 1H, *J* = 8.0 Hz, Ar-*H*), 5.52 (s, 1H, benzylidene-H), 4.65 (d, 1H, *J* = 9.8 Hz, 1-H), 4.34 (dd, 1H, *J* = 10.4, 4.4 Hz, 6-Ha), 3.84-3.74 (m, 2H, 6-Hb, 3-H), 3.54-3.45 (m, 3H, 4-H, 5-H, 2-H), 2.42 (s, 3H, CH_3), 1.31 (s, 9H, *t*Bu), 0.88 (s, 9H, *t*Bu), 0.11 (s, 3H, CH_3), 0.04 (s, 3H, CH_3); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 149.7, 137.3, 137.0, 131.4, 130.1, 130.1, 129.1, 128.3, 126.3, 125.3 (C-aromatic), 101.8 (C-benzylidene), 89.0 (C-1), 81.2 (C-4), 76.2 (C-3), 74.0 (C-2), 70.8 (C-5), 68.8 (C-6), 31.4 (*t*Bu), 26.0 (*t*Bu), 20.6 (CH_3), -4.2 (CH_3), -4.6 (CH_3); HRMS (ESI): Calcd for $C_{30}H_{44}O_5SSi$ $[M+Na]^+$ 567.2571, found 567.2584.

(2-Methyl-5-*tert*-butylphenyl) 4,6-*O*-benzylidene-2-*O*-benzyl-1-thio-β-D-glucopyranoside (43)



To a solution of **42** (2.00 g, 3.67 mmol) in anhydrous DMF (20 mL), NaH (0.21 g, 8.81 mmol) and BnBr (1.31 mL, 11.01 mmol) were added at 0 °C. The mixture was warmed to room temperature and stirred over night. Then cooled to 0 °C, quenched with MeOH and diluted with Et_2O. The organic layers were washed with H_2O and brine, dried over $MgSO_4$ and concentrated. Column chromatography on silica gel (cyclohexane/ethyl acetate) afforded impure benzylated product (2.4 g). The benzylated product was dissolved in THF (30 mL), cooled to 0 °C and treated with a solution of TBAF (1 M in THF, 7.24 mL, 7.24 mmol). The mixture was warmed to room temperature over night and concentrated. Column chromatography on silica gel (cyclohexane/ethyl acetate) afforded **43** (1.77 g, 3.40 mmol, 93%). $[\alpha]_D^{20}$ = -11.4 ° (c = 3.7, $CHCl_3$), IR ν_{max} (film) 3463, 3033, 2962, 1810, 1670, 1602, 1488, 1455, 1384, 1264, 1215, 1088 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.64-7.61 (m, 1H, Ar-H), 7.51-7.20 (m, 11H, Ar-H), 7.17-7.12 (m, 1H, Ar-H), 5.55 (s, 1H, benzylidene-H), 4.99 (d, 1H, *J* = 10.9 Hz, -CH_2-Bn) , 4.84 (d, 1H, *J* = 10.9 Hz, -CH_2-Bn), 4.75 (d, 1H, *J* = 9.8 Hz, 1-H), 4.34 (dd, 1H, *J* = 10.5, 5.0 Hz, 6-Ha), 3.97-3.89 (m, 1H, 3-H), 3.81 (dd, 1H, *J* = 10.3 Hz, 6-Hb), 3.60 (dd, 1H, *J* = 9.4 Hz, 4-H), 3.55-3.42 (m, 2H, 2-H, 5-H), 2.52 (d, 1H, *J* = 2.4 Hz, 3-OH), 2.42 (s, 3H, CH_3), 1.31 (s, 9H, *t*Bu); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 149.7, 138.1, 137.1, 136.3, 132.8, 130.1, 129.4, 129.1, 128.7, 128.5, 128.4, 128.2, 126.4, 125.0, 101.9, 88.2 (C-1), 81.1 (C-2), 80.5 (C-4), 75.7, 75.6 (C-3), 70.1 (C-5), 68.8 (C-6), 34.6, 31.5, 20.5; HRMS (ESI): Calcd for $C_{31}H_{36}O_5S$ $[M+Na]^+$ 543.2181, found 543.2181.

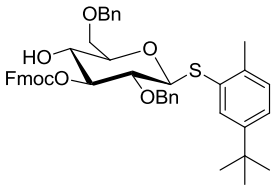
(2-Methyl-5-tert-butylphenyl) 4,6-*O*-benzylidene-2-*O*-benzyl-3-*O*-fluorenylmethoxy-carbonyl-1-thio-β-D-glucopyranoside (44)



To a solution of **43** (415 mg, 0.80 mmol) and pyridine (129 µl) in DCM (5 mL), Fmoc-Cl (309 mg, 1.20 mmol) was added and the mixture was stirred over night, diluted with DCM and the organic layers were washed with a 10 mM HCl solution and saturated aqueous

$NaHCO_3$ solution. The organic layer was dried over $MgSO_4$ and concentrated. Column chromatography on silica gel (cyclohexane/ethyl acetate) afforded **44** (561 mg, 0.76 mmol, 95%) as a white solid. $[\alpha]_D^{20}$ = -0.3 º (c = 5.9, $CHCl_3$), IR ν_{max} (film) 3033, 2961, 1955, 1754, 1605, 1451, 1385, 1251, 1077 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.79-7.73 (m, 2H, Fmoc-H), 7.65-7.13 (m, 19H, Ar-H), 5.55 (s, 1H, benzylidene-H), 5.29-5.22 (m, 1H), 4.98 (d, 1H, *J* = 10.7 Hz, -CH_2-Bn), 4.82 (d, 1H, *J* = 9.8 Hz, H-1), 4.72 (d, 1H, *J* = 10.7 Hz, -CH_2-Bn), 4.49-4.42 (m, 1H), 4.40-4.28 (m, 2H), 4.24-4.18 (m, 1H), 3.88-3.67 (m, 3H), 3.60-3.52 (m, 1H), 2.42 (s, 3H, CH_3), 1.31 (s, 9H, *t*Bu); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 154.6, 149.8, 143.5, 143.3, 141.4, 137.5, 136.9, 136.6, 130.2, 129.6, 129.2, 128.4, 128.3, 128.2, 128. (2C), 127.30, 127.27, 126.3, 126.2, 125.2, 120.1, 101.6, 88.7 (C-1), 79.5, 79.3, 78.5, 75.7, 70.3 (2C), 68.8, 46.8, 34.6, 31.4, 20.5; HRMS (ESI): Calcd for $C_{46}H_{46}O_7S$ $[M+Na]^+$ 765.2862, found 765.2886.

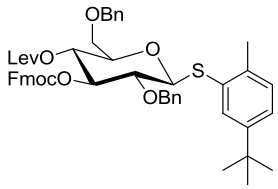
(2-Methyl-5-*tert*-butylphenyl) 2,6-di-*O*-benzyl-3-*O*-fluorenylmethoxycarbonyl-1-thio-β-D-glucopyranoside (45)



To a solution of **44** (100 mg, 0.14 mmol) in anhydrous DCM (3 mL) freshly activated molecular sieves (4 Å) were added. The mixture was cooled to -78 °C, TES (64 µl, 0.40 mmol) and TfOH (41 µl, 0.46 mmol) were added. After stirring for 3 hours at -78 °C, the reaction was quenched by the addition of pyridine, diluted with DCM and washed with a saturated aqueous $NaHCO_3$ solution. The organic phase was then dried over $MgSO_4$, filtered and concentrated. Column chromatography on silica gel (cyclohexane/ethyl acetate) afforded **45** (73 mg, 0.10 mmol, 73%). $[\alpha]_D^{20}$ = +10.5 º (c = 4.9, $CHCl_3$), IR ν_{max} (film) 3486, 3031, 2959, 1951, 1750, 1604, 1451, 1387, 1254, 1054 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.80-7.74 (m, 2H, Fmoc-H), 7.66-7.56 (m, 3H, Ar-H), 7.44-7.09 (m, 16H, Ar-H), 4.95 (dd, 1H, *J* = 9.2 Hz, 3-H), 4.92 (d, 1H, *J* = 10.7 Hz, -CH_2-Bn), 4.69 (d, 1H, *J* = 9.8 Hz, 1-H), 4.68 (d, 1H, *J* = 10.8 Hz, -CH_2-Bn), 4.61 (d, 1H, *J* = 12.0 Hz, -CH_2-Bn), 4.55 (d, 1H, *J* = 12.0 Hz, -CH_2-Bn), 4.50-4.43 (m, 1H, Fmoc-CH_2), 4.40-4.31 (m, 1H, Fmoc-CH_2), 4.26-4.20 (m, 1H, Fmoc-CH), 3.84 (ddd, 1H, *J* = 9.5, 3.6 Hz, 4-H), 3.81-3.74 (m, 2H, 6-H), 3.61 (dd, 1H, *J* = 9.5 Hz, 2-H), 3.56-3.49 (m, 1H, 5-H), 2.97 (d, 1H, *J* = 3.6 Hz, 4-OH), 2.40 (s, 1H, CH_3), 1.26 (s, 9H, *t*Bu); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 155.7, 149.8, 143.5, 143.4, 141.4, 137.7, 137.6, 136.5,

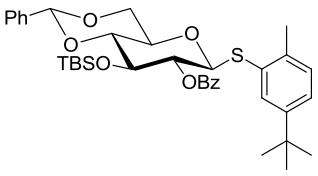
132.8, 130.1, 129.5, 128.6, 128.4, 128.2 128.0 (2C), 127.9, 127.3, 125.3, 125.2, 125.0, 120.2, 88.1 (C-1), 83.2 (C-3), 78.5 (C-2), 77.8 (C-5), 75.4, 73.9, 71.0 (C-4), 70.4, 70.3 (C-6), 46.9, 34.6, 31.4, 20.5; HRMS (ESI): Calcd for $C_{46}H_{48}O_7S$ $[M+Na]^+$ 767.3018, found 767.3038.

(2-Methyl-5-*tert*-butylphenyl) 2,6-di-*O*-benzyl-3-*O*-fluorenylmethoxycarbonyl-4-*O*-levulinoyl-1-thio-β-D-glucopyranoside (35)



To a solution of **45** (480 mg, 0.64 mmol) in DCM (8 mL) and pyridine (0.3 mL) Lev_2O (55 mg, 0.26 mmol) was added and stirred for three days. The mixture was diluted with DCM and washed with a 1 M HCl solution and with saturated aqueous $NaHCO_3$ solution. The organic layers were dried over $MgSO_4$ and concentrated. Column chromatography on silica gel (cyclohexane/ethyl acetate) afforded **35** (428 mg, 0.51 mmol, 79%). $[\alpha]_D^{20}$ = +19.2 º (c = 1.0, $CHCl_3$), IR ν_{max} (film) 3065, 2955, 1754, 1719, 1604, 1488, 1452, 1363, 1259, 1152, 1070, 1039 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.80-7.74 (m, 2H, Ar-H), 7.68-7.58 (m, 3H, Ar-H), 7.44-7.17 (m, 15H, Ar-H), 7.15-7.11 (m, 1H, Ar-H), 5.20 (dd, 1H, *J* = 9.7 Hz, 4-H), 5.15-5.07 (m, 1H, 3-H), 4.95 (d, 1H, *J* = 10.8 Hz, -CH_2-Bn), 4.71 (d, 1H, *J* = 9.8 Hz, 1-H), 4.69 (d, 1H, *J* = 10.4 Hz, -CH_2-Bn), 4.56-4.41 (m, 3H), 4.29-4.20 (m, 2H), 3.74-3.55 (m, 4H, 2-H, 4-H, 6-H), 2.60-2.52 (m, 2H, Lev-CH_2), 2.42 (s, 3H, Lev-CH_3), 2.41-2.32 (m, 2H, Lev-CH_2), 2.02 (s, 3H, CH_3), 1.26 (s, 9H, *t*Bu); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 206.0, 171.6, 154.8, 149.9, 143.7, 143. 5, 141.4, 141.3, 138.0, 137.6, 136.6, 132.7, 130.1, 129.5, 128.4, 128.2, 128.1, 128.0, 127.9, 127.7, 127.4, 127.3, 125.5, 125.4, 125.0, 120.1, 88.2, 80.5, 78.9, 77.3, 75.6, 73.7, 70.6, 69.4, 69.1, 46.7, 37.8, 34.6, 31.4, 29.7, 28.0, 20.5; HRMS (ESI): Calcd for $C_{51}H_{54}O_9S$ $[M+Na]^+$ 865.3386 found 865.3412.

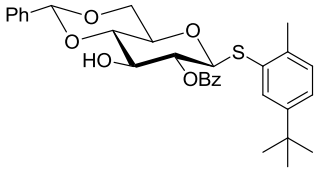
(2-Methyl-5-*tert*-butylphenyl) 2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-*tert*-butyldimethylsilyl-1-thio-β-D-glucopyranoside (46)



Thioglycoside **42** (1.00 g, 1.84 mmol) was dissolved under argon in anhydrous pyridine (4 mL). DMAP (67 mg, 0.55 mmol) was added and the solution was cooled to 0 °C. BzCl (639

µL, 5.51 mmol) was added dropwise and the solution was heated to 70 °C and stirred for 12 h. After completion, the reaction was quenched with methanol. The suspension was diluted with DCM and extracted with 1 M HCl and H_2O. Column chromatography on silica gel (cyclohexane/ethyl acetate) afforded **46** (1.05 g, 1.62 mmol, 88%). $[\alpha]_D^{20}$ = +22.9 ° (c = 1.0, CH_2Cl_2); IR ν_{max} (film) 2959, 2929, 2858, 1732, 1384, 1266, 1096, 1069 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.08 (dd, 2H, *J* = 8.3 Hz, Ar-H), 7.56 (d, 1H, *J* = 1.8 Hz, Ar-H), 7.52-7.43 (m, 5H, Ar-H), 7.37 (dd, 3H, *J* = 5.2, 2.0 Hz, Ar-H), 7.20 (dd, 1H, *J* = 8.0, 2.1 Hz, Ar-H), 7.07 (d, 1H, *J* = 8.0 Hz, Ar-*H*), 5.58 (s, 1H, benzylidene-H), 5.35 (dd, 1H, *J* = 10.3, 8.6 Hz, 2-H), 4.84 (d, 1H, *J* = 10.3 Hz, 1-H), 4.38 (dd, 1H, *J* = 10.5, 5.0 Hz, 6-Ha), 4.06 (dd, 1H, *J* = 8.9 Hz, 3-H), 3.88 (dd, 1H, *J* = 10.3, 5.0 Hz, 6-Hb), 3.69 (dd, 1H, *J* = 9.1 Hz, 4-H), 3.60-3.52 (m, 1H, 5-H), 2.18 (s, 3H, CH_3), 1.28 (s, 9H, *t*Bu), 0.70 (s, 9H, *t*Bu), -0.05 (s, 3H, CH_3), -0.14 (s, 3H, CH_3); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 133.1, 129.9, 129.8, 129.4, 129.1, 128.3, 128.1, 126.2, 125.1 (C-Ar), 101.9 (C-benzylidene), 88.1 (C-1), 81.3 (C-4), 74.3 (C-3), 73.6 (C-2), 70.6 (C-5), 68.7 (C-6), 31.3 (*t*Bu), 25.5 (*t*Bu), 20.2 (CH_3), -4.2 (CH_3), -5.0 (CH_3); HRMS (ESI): Calcd for $C_{37}H_{48}O_6SSi$ $[M+Na]^+$ 671.2833, found 671.2852.

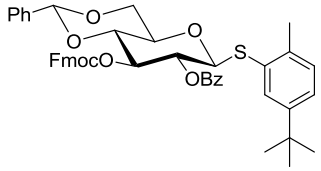
(2-Methyl-5-*tert*-butylphenyl) 2-*O*-benzoyl-4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside (47)



To a solution of **46** (200 mg, 0.31 mmol) in DMF (1 mL) a solution of TBAF·3H_2O (683 mg, 1.85 mmol) and glacial acetic acid (124 µL, 2.16 mmol) in DMF (1 mL) were added. The mixture was warmed to 35 °C for 9 h, diluted with Et_2O and washed with a 10 mM HCl solution and saturated aqueous $NaHCO_3$ solution. The organic layer was dried over $MgSO_4$ and concentrated. Column chromatography on silica gel (cyclohexane/ethyl acetate) afforded **47** (150 mg, 0.28 mmol, 91%). $[\alpha]_D^{20}$ = -5.5 ° (c = 0.8, $CHCl_3$); IR ($CHCl_3$): 3455, 2963, 2870, 1729, 1268, 1100, 1071 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.11 (d, 2H, *J* = 7.4 Hz, Ar-H), 7.64-7.33 (m, 9H, Ar-H), 7.27-7.20 (m, 1H, Ar-H), 7.10 (d, 1H, *J* = 8.0 Hz, Ar-H), 5.59 (s, 1H, benzylidene-H), 5.25 (dd, 1H, *J* = 10.1, 8.7 Hz, 2-H), 4.88 (d,1H, *J* = 10.1 Hz, 1-H), 4.40 (dd, 1H, *J* = 10.5, 5.0 Hz, 6-Ha), 4.09 (dd, 1H, *J* = 9.0, 8.7 Hz, 3-H), 3.87 (dd, 1H, *J* = 10.4, 5.0 Hz, 6-Hb), 3.71 (dd, 1H, *J* = 9.0, 9.7 Hz, 4-H), 3.57 (td, 1H, *J* = 9.7, 5.0 Hz, 5-H), 2.83 (br, 1H, 3-OH), 2.23 (s, 3H, CH_3), 1.29 (s, 9H, *t*Bu); ^{13}C-NMR (100 MHz, $CDCl_3$) δ

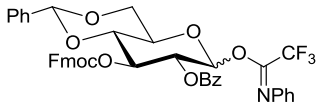
166.1 (C=O benzoyl), 149.7, 137.32, 136.9, 133.6, 131.9, 130.4, 130.2, 129.5, 128.6, 128.5, 126.4, 125.6 (aromatics), 102.1 (C-benzylidene), 87.5 (C-1), 80.9 (C-4), 74.0 (C-3), 73.6 (C-2), 70.5 (C-5), 68.7 (C-6), 34.6, 31.4 (*t*Bu), 20.4 (CH_3); HRMS (ESI): Calcd for $C_{31}H_{34}O_6S$ $[M+Na]^+$ 557.1968, found 557.1975.

(2-Methyl-5-*tert*-butylphenyl) 2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-fluorenylmethoxycarbonyl-1-thio-β-D-glucopyranoside (32)



To a solution of **47** (277 mg, 0.52 mmol) and pyridine (130 µl) in DCM (4 mL), Fmoc-Cl (268 mg, 1.04 mmol) was added and the mixture stirred overnight, diluted with DCM and the organic layers were washed with a 10 mM HCl solution and saturated aqueous $NaHCO_3$ solution. The organic layer was dried over $MgSO_4$ and concentrated. Column chromatography on silica gel (cyclohexane/ethyl acetate) afforded **32** (378 mg, 0.50 mmol, 96%). $[\alpha]_D^{20}$ = +50.2 ° (c = 4.5, $CHCl_3$), IR ν_{max} (film) 3066, 2961, 1752, 1732, 1602, 1488, 1450, 1385, 1316, 1268, 1250, 1093 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.06-7.99 (m, 2H, Ar-H), 7.73-7.67 (m, 2H, Ar-H), 7.61-7.07 (m, 19H, Ar-H), 5.60 (s, 1H, benzylidene-H), 5.51-5.36 (m, 2H, 2-H, 3-H), 4.95 (d, 1H, *J* = 9.9 Hz, 1-H), 4.46-4.39 (m, 1H, 6-*H*), 4.27-4.16 (m, 2H, Fmoc-CH_2), 4.06-4.00 (m, 1H, Fmoc-CH), 3.98-3.88 (m, 2H, 4-H, 6-H), 3.72-3.63 (m, 1H, 5-H) 2.23 (s, 1H, CH_3), 1.29 (s, 9H, *t*Bu); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 165.3, 154.6, 149.8, 143.4, 143.2, 141.3, 141.2, 137.4, 136.8, 133.6, 131.7, 130.5, 130.2, 130.1, 129.3, 129.2, 128.6, 128.4, 127.9, 127.3 (2C), 126.3, 125.8, 125.3, 125.2, 120.0 (2C), 101.8, 88.0 (C-1), 78.3 (4-H), 77.3 (C-3), 71.4 (C-2), 70.8 (C-5), 70.5, 68.7 (C-6), 46.6, 34.6, 31.7, 31.4, 20.4, 14.3; HRMS (ESI): Calcd for $C_{46}H_{44}O_8S$ $[M+Na]^+$ 779.2655, found 779.2649.

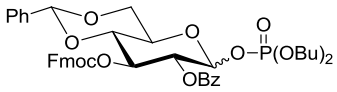
2-*O*-Benzoyl-4,6-*O*-benzylidene-3-*O*-fluorenylmethoxycarbonyl-D-glucopyranoside *N*-phenyltrifluoroacetimidate (33)



To a solution of **32** (110 mg, 0.15 mmol), TTBP (144 mg, 0.58 mmol) and NIS (98 mg, 0.44 mmol) in acetonitrile (5 mL) and H_2O (0.1 mL), AgOTf (112 mg, 0.44 mmol) was added and stirred for 4 h. The reaction was quenched with saturated aqueous $Na_2S_2O_3$ solution, and

extracted with DCM. The organic layer was dried over $MgSO_4$ and concentrated. Column chromatography on silica gel (cyclohexane/ethyl acetate) afforded the lactol which was taken directly to the next step. A solution of the lactol in DCM (8 mL) was cooled to 0 °C, $CF_3C(NPh)Cl$ (41 mg, 0.20 mmol) and Cs_2CO_3 (65 mg, 0.20 mmol) were added and the resulting solution was stirred for 2 h at room temperature, diluted with DCM, filtered through a plug of celite and concentrated. Column chromatography on silica gel (cyclohexane/ethyl acetate) afforded **33** (75 mg, 0.10 mmol, 67%) as an inseparable mixture of α/β anomers. $[\alpha]_D^{20}$ = +46.3 º (c = 3.1, $CHCl_3$), IR ν_{max} (film) 3069, 2925, 1755, 1734, 1600, 1451, 1379, 1322, 1270, 1212, 1165, 1096 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.07-8.00 (m, 2H, Ar-H), 7.75-7.68 (m, 2H, Ar-H), 7.58-7.28 (m, 14H, Ar-H), 7.20-7.09 (m, 2H, Ar-H), 6.83-6.70 (m, 2H, Ar-H), 6.13 (s, 1H, 1-H), 5.70-5.55 (m, 2H, benzylidene-H), 5.45-5.35 (m, 1H), 4.55-3.83 (m, 8H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 154.5, 143.3, 143.1, 141.3, 136.6, 133.8, 130.1, 129.4, 128.9, 128.8, 128.7, 128.4, 127.9, 127.3, 126.4, 125.3, 125.2, 120.0, 119.3, 102.0, 78.0, 77.4, 75.8, 71.8, 70.6, 68.5, 67.3, 46.6; HRMS (MALDI-TOF): Calcd for $C_{43}H_{34}F_3NO_9$ $[M+Na]^+$ 788.2083, found 788.1952.

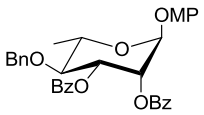
Dibutyl 2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-fluorenylmethoxycarbonyl-D-gluco-pyranoside phosphate (34)



Thioglucoside **32** (690 mg, 0.91 mmol) was coevaporated with toluene three times and dried *in vacuo*, then dissolved in anhydrous DCM (10 mL). Freshly activated molecular sieves (4 Å) and dibutyl hydrogen phosphate (542 µl, 2.73 mmol) were added and the solution cooled to 0 °C. NIS (246 mg, 1.09 mmol), followed by TfOH (10 µl, 0.11 mmol) was added and stirred at 0 °C for one hour. The reaction was quenched by the addition of pyridine, diluted with DCM and washed with aqueous $Na_2S_2O_3$ and saturated aqueous $NaHCO_3$ solutions. The organic phase was dried over $MgSO_4$, filtered and concentrated. The crude product was purified by column chromatography on silica gel (cyclohexane/ethyl acetate) to afford **34** (583 mg, 0.74 mmol, 81%) in a mixture of α/β-anomers (α/β=1:4). NMR data are reported for the β-anomer. $[\alpha]_D^{20}$ = +8.9 º (c = 3.1, $CHCl_3$), IR ν_{max} (film) 3067, 2961, 1755, 1733, 1602, 1451, 1268, 1096, 1026 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.06-7.99 (m, 2H, Ar-H), 7.72-7.66 (m, 2H, Ar-H), 7.55-7.29 (m, 12H, Ar-H), 7.18-7.11 (m, 2H, Ar-H), 5.60-5.54 (m, 2H, benzylidene-H, 1-H), 5.50 (dd,1H, *J* = 9.4 Hz, 2-H), 5.36 (dd, 1H, *J* = 9.4 Hz, 3-H), 4.49-4,41 (m, 1H, 6-H), 4.30-4.18 (m, 2H, Fmoc-CH_2), 4.10-4.01 (m, 3H, Fmoc-H, phosphate-

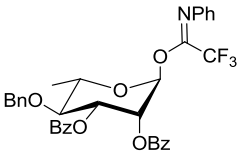
CH_2), 4.00-3.94 (m, 1H, 4-H), 3.90-3.86 (m, 1H, 6-H), 3.82-3.67 (m, 3H, phosphate-CH_2, 5-*H*), 1.67-1.60 (m, 2H, phosphate-CH_2), 1.42-1.25 (m, 4H, phosphate-CH_2), 1.10-1.01 (m, 2H, phosphate-CH_2), 0.92 (t, 3H, *J* = 7.4 Hz, phosphate-CH_3), 0.70 (3H, t, *J* = 7.4 Hz, phosphate-CH_3); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 165.1, 154.5, 143.3, 143.1, 141.3, 136.6, 133.8, 130.1, 129.4, 128.9, 128.6, 128.4, 127.9, 127.3, 127.2, 126.4, 126.3, 125.3, 125.2, 120.0, 101.9, 96.9 (2C), 78.0, 75.8, 72.6, 70.6, 68.4, 68.1, 67.1, 46.6, 32.2, 32.1, 32.0, 31.9, 18.7, 18.4, 13.7, 13.5; ^{31}P-NMR (162 MHz, $CDCl_3$) δ -2.95; HRMS (ESI): Calcd for $C_{43}H_{47}O_{12}P$ $[M+Na]^+$ 809.2703, found 809.2690.

4-Methoxyphenyl 2,3-di-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranoside (48)



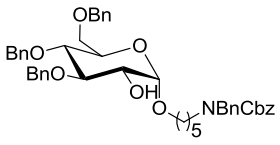
Rhamnoside **47**[140] (500 mg, 1.39 mmol) was dissolved in a solution of DCM (1 mL) and pyridine (1 mL). DMAP (68 mg, 0.56 mmol) was added and the mixture cooled to 0 °C, then BzCl (780 mg, 5.56 mmol) was added and the reaction warmed to room temperature over night. The reaction was quenched with MeOH, diluted with DCM and the organic layer was washed with a 10 mM HCl solution and saturated aqueous $NaHCO_3$ solution. The organic layer was dried over $MgSO_4$ and concentrated. Column chromatography on silica gel (cyclohexane/ethyl acetate) afforded **48** (768 mg, 1.35 mmol, 97%). $[\alpha]_D^{20}$ = +17.6 º (c = 3.1, $CHCl_3$), IR ν_{max} (film) 3064, 2934, 1725, 1602, 1506, 1452, 1363, 1273, 1213, 1094, 1027 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.11-8.05 (m, 2H, Ar-H), 7.98-7.93 (m, 2H, Ar-H), 7.67-7.61 (m, 1H, Ar-H), 7.56-7.49 (m, 3H, Ar-H), 7.40-7.35 (m, 2H, Ar-H), 7.25-7.16 (m, 5H, Ar-H), 7.08-7.03 (m, 2H, Ar-H), 6.87-6.82 (m, 2H, Ar-H), 5.94 (dd, 1H, *J* = 9.6, 3.4 Hz, 3-H), 5.79 (dd, 1H, *J* = 3.4, 1.9 Hz, 2-H), 5.54 (d, 1H, *J* = 1.8 Hz, 1-H), 4.75 (d, 1H, *J* = 10.9 Hz, -CH_2-Bn), 4.68 (d, 1H, *J* = 10.9 Hz, -CH_2-Bn), 4.20-4.11 (m, 1H, 5-H), 3.88 (dd, 1H, J_1 J_2 9.6, 4-H), 3.78 (s, 3H, -CH_3), 1.41 (d, 3H, *J* = 6.2 Hz, 6-H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 165.6 (2C), 155.2, 150.2, 137.7, 133.6, 133.3, 130.0, 129.9, 129.8, 129.7 (2C), 128.5(2C), 128.2, 128.0, 117.9, 114.7, 96.6 (C-1), 79.1 (C-4), 75.3, 72.3 (C-3), 71.2 (C-2), 68.5 (C-5), 55.8, 18.3 (C-6); HRMS (ESI): Calcd for $C_{34}H_{32}O_8$ $[M+Na]^+$ 591.1995, found 591.1985.

2,3-Di-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranoside *N*-phenyltrifluoroacetimidate (31)



CAN (2.17 g, 3.96 mmol) was added to a mixture of **48** (750 mg, 1.32 mmol) in MeCN (12 mL) and H_2O (12 mL) and stirred vigorously for 2 h. H_2O and EtOAc were added, the layers separated, the organic layer washed with H_2O and brine, dried over $MgSO_4$ and concentrated. Column chromatography on silica gel (cyclohexane/ethyl acetate) afforded the lactol as an orange solid (548 mg). A solution of the lactol (548 mg) in DCM (10 mL) was cooled to 0 °C, $CF_3C(NPh)Cl$ (438 mg, 2.11 mmol) and Cs_2CO_3 (688 mg, 2.11 mmol) were added and the resulting solution was stirred overnight at room temperature, diluted with DCM, filtered through a plug of celite and concentrated. Column chromatography on silica gel (cyclohexane/ethyl acetate) afforded **31** (619 mg, 0.98 mmol, 74%). $[\alpha]_D^{20}$ = +41.2 º (c = 4.8, $CHCl_3$), IR ν_{max} (film) 3065, 2981, 1727, 1600, 1490, 1452, 1270, 1208, 1164, 1091 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.07-8.02 (m, 2H, Ar-H), 7.96-7.89 (m, 2H, Ar-H), 7.66-7.60 (m, 1H, Ar-H), 7.57-7.46 (m, 3H, Ar-H), 7.40-7.19 (m, 9H, Ar-H), 7.14-7.07 (m, 1H, Ar-H), 6.91-6.82 (m, 2H, Ar-H), 6.35 (s, 1H, 1-H), 5.84 (s, 1H, 2-H), 5.77 (dd, 1H, *J* = 9.4, 3.3 Hz, 3-H), 4.76 (d, 1H, *J* = 10.9 Hz, -CH_2-Bn), 4.68 (d, 1H, *J* = 10.9 Hz, -CH_2-Bn), 4.21-4.08 (m, 1H, 5-H), 3.87 (dd, 1H, *J* = 9.5 Hz, 4-H), 1.48 (d, 3H, *J* = 6.1 Hz, 6-H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 165.5, 165.3, 143.4, 137.4, 133.7, 133.4, 130.0, 129.8, 129.7, 129.4, 128.9, 128.7, 128.6 (2C), 128.3, 128.2, 124.6, 119.6, 94.1 (C-1), 78.5 (C-4), 75.5, 72.0 (C-3), 70.7 (C-3), 69.6 (C-2), 18.4 (C-6); HRMS (ESI): Calcd for $C_{35}H_{30}F_3NO_7$ $[M+Na]^+$ 656.1872, found 656.1852.

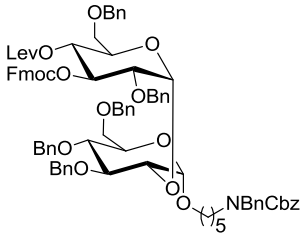
***N*-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl 3,4,6-tri-*O*-benzyl-α-D-glucopyranoside (49)**



Thioglucoside **36** (335 mg, 0.53 mmol) and $HO(CH_2)_5NBnCbz$ (518 mg, 1.58 mmol) were coevaporated with toluene three times, dried *in vacuo*, then the compounds were dissolved in a solution of anhydrous toluene:dioxane=2:1 (4.5 mL). The solution was cooled to -40 °C, treated with NIS (131 mg, 0.58 mmol) and TfOH (4.7 µl, 53 µmol) and warmed to -20 °C over 1.5 h. The reaction was quenched with pyridine, diluted with DCM and washed with

saturated aqueous $Na_2S_2O_3$ solution. The organic layer was dried over $MgSO_4$ and concentrated. Column chromatography on silica gel (cyclohexane/ethyl acetate) gave a mixture of anomers which was dissolved in DCM (10 mL) and water (1 mL) and treated with DDQ (202 mg, 0.89 mmol) at 0 °C for 2 h. The mixture was diluted with DCM and the organic layer washed with saturated aqueous $NaHCO_3$ solution, dried over $MgSO_4$ and concentrated. Column chromatography on silica gel (cyclohexane/ethyl acetate) afforded **49** (140 mg, 0.184 mmol, 35%) as a colorless oil. $[\alpha]_D^{20}$ = +53.3 º (c = 5.5, $CHCl_3$), IR ν_{max} (film) 3458, 3031, 2927, 1952, 1876, 1808, 1454, 1421, 1360, 1229, 1129, 1067 cm^{-1}; ^{1}H-NMR (400 MHz, acetone-d6) δ 7.48-7.10 (m, 25H, Ar-H), 5.15 (s, 2H), 4.99 (d, 1H, *J* = 11.4 Hz, -CH_2-Bn), 4.84 (d, 1H, *J* = 11.1 Hz, -CH_2-Bn). 4.79 (d, 1H, *J* = 11.4 Hz, -CH_2-Bn), 4.75 (s, 1H, 1-H), 4.62-4.49 (m, 5H, -CH_2-Bn), 3.84-3.66 (m, 6H), 3.62-3.47 (m, 2H), 3.40 (m, 1H), 3.31-3.18 (m, 2H, linker-CH_2-), 1.67-1.50 (m, 4H, linker-CH_2-), 1.43-1.29 (m, 2H, linker-CH_2-); ^{13}C-NMR (100 MHz, acetone-d6) δ 140.5, 139.8, 139.7, 139.5, 129.3, 129.1, 129.0, 128.9, 128.6 (2C), 128.4 (2C), 128.2, 128.0, 99.9 (C-1), 84.3, 78.7, 75.5, 75.4, 74.2, 73.7, 71.5, 70.2, 68.5, 67.4, 24.1; HRMS (ESI): Calcd for $C_{47}H_{53}NO_8$ $[M+Na]^+$ 782.3669, found 782.3633.

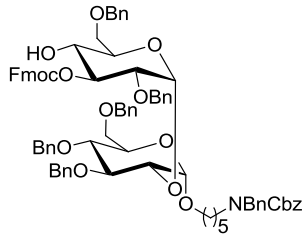
_N_-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl 2,6-di-_O_-benzyl-3-_O_-fluorenylmethoxycarbonyl-4-_O_-levulinoyl-α-D-glucopyranosyl-(1→2)-3,4,6-tri-_O_-benzyl-α-D-glucopyranoside (30)



Glucosides **35** (326 mg, 0.34 mmol) and **49** (262 mg, 0.35 mmol) were coevaporated with toluene three times and dried *in vacuo*. The mixture was dissolved in anhydrous Et_2O (3 mL), NIS (93 mg, 0.41 mmol) was added and cooled to -35°C. TfOH (3.7 µl, 41 µmol) was added and the mixture was stirred and warmed up to -10 °C in one hour. The reaction was quenched by the addition of pyridine, diluted with DCM and washed with aqueous $Na_2S_2O_3$ and saturated aqueous $NaHCO_3$ solutions. The phases were separated and the aqueous phase was extracted with DCM. The combined organic phases were dried over $MgSO_4$, filtered and concentrated. The crude product was purified by column chromatography on silica gel (cyclohexane/ethyl acetate) to afford **30** (343 mg, 0.24 mmol, 70%). $[\alpha]_D^{20}$ = +64.4 º (c = 5.9,

$CHCl_3$), IR ν_{max} (film) 3032, 2932, 1755, 1700, 1605, 1497, 1452, 1362, 1259 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.00-6.90 (m, 43H, Ar-H), 5.41 (dd, 1H, *J* = 9.7 Hz), 5.26 (dd, 1H, *J* = 9.8 Hz), 5.18-5.10 (m, 2H), 5.06 (s, 1H, anomeric-H), 5.03-4.96 (m, 2H, anomeric-H), 4.88 (d, 1H, *J* = 11.0 Hz), 4.82 (d, 1H, *J* = 10.8 Hz), 4.68-4.58 (m, 3H), 4.52-4.41 (m, 5H), 4.39-4.30 (m, 2H), 4.26 (dd, 1H, *J* = 7.5 Hz), 4.14-4.08 (m, 1H), 4.07-4.01 (m, 1H), 3.82 (dd, 1H, *J* = 9.9, 3.4 Hz), 3.80-3.56 (m, 6H), 3.34-3.31 (m, 2H), 3.28-3.06 (m, 4H), 2.54-2.42 (m, 2H), 2.32-2.17 (m, 2H), 2.00 (s, 3H, Lev-CH_3), 1.65-1.50 (m, 4H, linker-CH_2-), 1.30-1.23 (m, 4H, linker-CH_2-); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 206.0, 171.5, 154.9, 143.7, 143.6, 141.4 (2C), 138.0, 137.8, 128.6, 128.5 (2C), 128.4 (2C), 128.2, 128.1, 128.0, 127.9 (2C), 127.7 (2C), 127.5, 127.3, 126.3, 125.5, 120.1, 120.0, 95.6 (C-anomeric), 94.0 (C-anomeric), 80.7, 78.2, 77.4, 77.0, 76.8, 76.2, 75.9, 75.4, 73.7, 73.5, 72.4, 70.5, 70.3, 68.8, 68.6, 68.4, 67.3, 46.8, 37.8, 31.4, 29.8, 27.9, 23.7; HRMS (ESI): Calcd for $C_{87}H_{91}NO_{17}$ $[M+Na]^+$ 1444.6179, found 1444.6128.

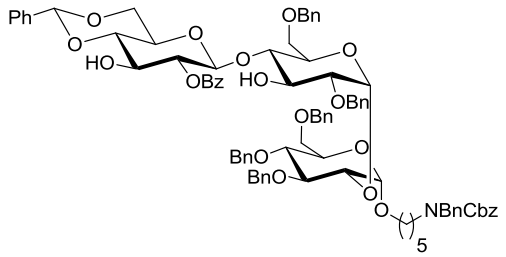
_N_-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl 2,6-di-_O_-benzyl-3-_O_-fluorenylmethoxycarbonyl-α-D-glucopyranosyl-(1→2)-3,4,6-tri-_O_-benzyl-α-D-glucopyranoside (50)



To a solution of **30** (224 mg, 0.16 mmol) in DCM (4.5 mL) hydrazine hydrate (31 µl, 0.63 mmol) dissolved in AcOH (0.4 mL) and pyridine (0.6 mL) was added and the solution stirred for 1 h. The reaction was then quenched by the addition of acetone and concentrated. Column chromatography on silica gel (cyclohexane/ethyl acetate) afforded **50** (196 mg, 0.15 mmol, 94%). $[\alpha]_D^{20}$ = +57.7 º (c = 1.7, $CHCl_3$), IR ν_{max} (film) 3423, 3031, 2926, 1753, 1697, 1605, 1586, 1497, 1452, 1422, 1362, 1255, 1068 cm^{-1}; ^{1}H-NMR (400 MHz, acetone-d6) δ 7.92-7.84 (m, 2H, Ar-H), 7.78-7.64 (m, 2H, Ar-H), 7.56-7.14 (m, 35H, Ar-H), 5.44-5.37 (m, 2H), 5.20-5.10 (m, 3H), 5.07 (d, 1H, *J* = 10.7 Hz), 4.89-4.77 (m, 3H), 4.66-4.47 (m, 8H), 4.46-4.39 (m, 2H), 4.27 (dd, 1H, *J* = 6.9 Hz), 4-20-4.14 (m, 1H), 3.99 (dd, 1H, *J* = 9.3 Hz), 3.89-3.80 (m, 2H), 3.78-3.59 (m, 7H), 3.59-3.52 (m, 1H), 3.49-3.42 (m, 1H), 3.25-3.15 (m, 2H), 2.82-2.79 (m, 1H), 1.60-1.44 (m, 4H, linker-CH_2-), 1.33-1.25 (m, 2H, linker-CH_2-); ^{13}C-NMR (100 MHz, acetone-d6) δ 155.9, 144.7, 144.6, 142.2, 142.1, 139.9, 139.8, 139.7 (2C), 139.5, 139.4,

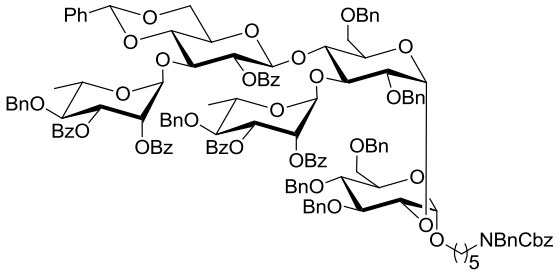
129.3 (2C), 129.1, 129.0 (2C), 128.9, 128.7, 128.6 (2C), 128.5, 128.4, 128.2 (2C), 128.1 (2C), 128.0, 127.9, 126.1, 126.0, 120.9 (2C), 96.3, 94.2, 81.8, 80.0, 79.2, 78.0, 76.5, 75.5, 73.8, 73.5, 72.1, 71.9, 71.5, 70.2, 70.08, 70.06, 69.5, 68.6, 67.4, 47.6, 27.5, 24.2; HRMS (ESI): Calcd for $C_{82}H_{85}NO_{15}$ $[M+Na]^+$ 1346.5817, found 1346.5784.

_N_-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl 2-_O_-benzoyl-4,6-_O_-benzylidene-β-D-glucopyranosyl-(1→4)-2,6-di-_O_-benzyl-α-D-glucopyranosyl-(1→2)-3,4,6-tri-_O_-benzyl-α-D-glucopyranoside (29)



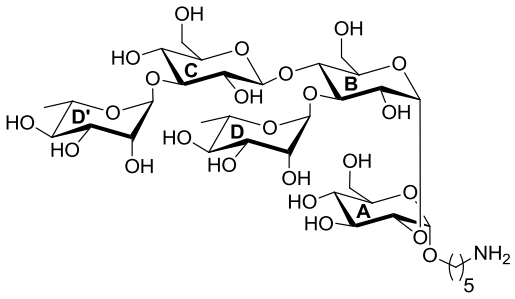
Glycosyl phosphate **34** (74 mg, 94 μmol) and **50** (48 mg, 36 μmol) were coevaporated with toluene three times, dried *in vacuo* and then dissolved in anhydrous DCM (1.0 mL). Freshly activated molecular sieves (4 Å) were added and the mixture cooled to –30 °C. TMSOTf (18 μl, 98 μmol) was added and then warmed to –7 °C over 1.5 h. The reaction was quenched with pyridine and concentrated. Column chromatography on silica gel (toluene/acetone) afforded the impure Fmoc protected trisaccharide. 20% NEt_3 in DCM (1 mL) was added to the Fmoc protected trisaccharide and stirred for 4 h, the mixture was concentrated. Column chromatography on silica gel (toluene/acetone) afforded **29** (20 mg, 14 μmol, 38 %). $[\alpha]_D^{20}$ = +8.1 º (c = 1.6, $CHCl_3$), IR ν_{max} (film) 3462, 3032, 2924, 1732, 1699, 1603, 1497, 1453, 1364, 1268, 1093 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.05-7.92 (m, 2H, Ar-H), 7.63-7.06 (m, 43H, Ar-H), 5.56 (s, 1H, benzylidene-H), 5.24 (dd, 1H, *J* = 8.5 Hz), 5.20-5.11 (m, 2H), 5.09-4.98 (m, 2H, anomeric-H), 4.88 (d, 1H, *J* = 10.7 Hz), 4.79-4.66 (m, 4H, anomeric-H), 4.62-4.54 (m, 1H), 4.49-4.36 (m, 5H), 4.19-4.05 (m, 2H), 4.03-3.91 (m, 2H), 3.89-3.44 (m, 14H), 3.39-3.04 (m, 4H), 1.57-1.36 (m, 4H, linker-CH_2-), 1.32-1.14 (m, 2H, linker-CH_2-); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 165.6, 138.5 (2C), 138.4, 138.1, 136.8, 133.7, 130.1, 129.6, 129.4, 128.7, 128.6, 128.5 (4C), 128.4, 127.98, 127.9, 127.8 (2C), 127.7, 127.4, 126.4, 102.1, 101.7 (C-anomeric), 95.8 (C-anomeric), 94.5 (C-anomeric), 81.1, 80.8, 80.6, 78.2, 77.9, 77.4, 76.1, 75.2, 74.7, 73.6, 73.4, 72.8, 72.1, 71.6, 70.4, 69.5, 68.7, 68.4, 68.2, 67.8, 67.3, 66.4, 29.8, 29.4, 23.6; HRMS (ESI): Calcd for $C_{87}H_{93}NO_{19}$ $[M+Na]^+$ 1478.6239, found 1478.6136.

***N*-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl 2,3-di-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl-(1→3)-2-*O*-benzoyl-4,6-*O*-benzylidene-3-β-D-glucopyranosyl-(1→4)-[2,3-di-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl-(1→3)]-2,6-di-*O*-benzyl-α-D-glucopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-α-D-glucopyranoside (28)**



Compounds **31** (26 mg, 41 μmol) and **29** (10 mg, 6.9 μmol) were coevaporated with toluene three times, dried *in vacuo* and dissolved in anhydrous DCM (1.0 mL). Freshly activated molecular sieves (4 Å) were added and the mixture cooled to –30 °C. TMSOTf (10 μl of a solution of 7.4 μl TMSOTf in 93 μl DCM, 4.1 μmol) was added and the reaction was stirred at –30 °C for 1.5 h. The reaction was quenched with pyridine and concentrated *in vacuo*. Column chromatography on silica gel (toluene/acetone) afforded **28** (14 mg, 5.5 μmol, 81%). $[\alpha]_D^{20}$ = + 5.2° (c =0.7, $CHCl_3$), IR ν_{max} (film) 3032, 2933, 1728, 1602, 1585, 1496, 1452, 1363, 1263, 1094, 1069 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.20-6.90 (m, 75H, Ar-H), 5.79-5.67 (m, 3H), 5.46 (s, 1H, benylidene-H), 5.33-5.29 (m, 1H), 5.28-5.21 (m, 1H), 5.17-5.08 (m, 3H, anomeric-H), 5.02 (s, 1H, anomeric-H), 4.92-4.78 (m, 4H, anomeric-H), 4.74-4.60 (m, 4H), 4.59-4.49 (m, 4H, anomeric-H), 4.48-4.44 (m, 1H), 4.43-4.31 (m, 4H), 4.29-4.13 (m, 4H, anomeric-H), 4.03-3.88 (m, 3H), 3.83-3.45 (m, 13H), 3.40-3.02 (m, 7H), 1.65 (d, 3H, *J* = 6.2 Hz, Rha-CH_3), 1.53-1.32 (m, 4H, linker-CH_2-), 1.24-1.10 (m, 2H, linker-CH_2-), 0.90 (d, 3H, *J* = 6.1 Hz, Rha-CH_3); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 165.6, 165. (2C), 164.5, 164.2, 138.3, 137.8, 137.6, 133.1, 130.1, 130.0, 129.9 (2C), 129.7, 129.4, 129.1, 129.0, 128.9, 128.8 (2C), 128.7, 128.6, 128.5 (3C), 128.4 (2C), 128.3 (2C), 128.2 (2C), 128.0 (2C), 127.9 (2C), 127.8, 127.7, 126.5, 126.4, 100.6 (C-anomeric), 100.5 (C-anomeric), 97.9 (C-anomeric), 97.5, 95.8 (C-anomeric), 93.5 (C-anomeric), 80.2, 79.2, 78.1, 77.5, 77.4, 77.2, 76.8, 76.2, 76.1, 76.0, 74.2, 74.0, 73.6, 72.9, 72.1, 71.6, 71.2, 70.9, 68.7, 67.4, 67.2, 50.6, 47.2, 46.2, 29.9, 23.6, 18.4, 17.5; HRMS (ESI): Calcd for $C_{141}H_{141}NO_{31}$ $[M+Na]^+$ 2366.9385, found 2366.9440.

5-Amino-pentanyl α-L-rhamnopyranosyl-(1→3)-β-D-glucopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→3)]-α-D-glucopyranosyl-(1→2)-α-D-glucopyranoside (27)



Fully protected pentasaccharide **28** (10 mg, 4.3 μmol) was dissolved in a solution of NaOMe in THF/MeOH (1:1, 0.5 M, 1 mL) and heated to 50 °C for 12 h. The mixture was neutralized with Amberlite IR 120 (H^+) ion exchange resin, filtered and concentrated. Size exclusion chromatography on Sephadex LH-20 ($CHCl_3$/MeOH=1:1) afforded the de-benzoylated pentasaccharide (5.6 mg), which was dissolved in a mixture of MeOH (0.9 mL), H_2O (0.1 mL) and AcOH (25 μl). The solution was purged with Ar, 10% Pd/C (10 mg) was added and the solution purged with H_2 for 30 min, then stirred under an H_2 atmosphere for 12 h, filtered and concentrated. Size exclusion chromatography on Sephadex LH-20 (MeOH) afforded **27** (2.3 mg, 2.6 μmol, 61%). NMR data are reported in Table 2, comparison with the data from native PS-I[129] is reported in Table 3. HRMS (MALDI-TOF): Calcd for $C_{35}H_{63}NO_{24}$ $[M+Na]^+$ 904.3632, found 904.3606.

Table 2: ^{1}H NMR δ (600 MHz, D_2O) and ^{13}C NMR δ (150 MHz, D_2O) of pentasaccharide **27**.[a]

	α-Glc **(A)**	*α-Glc* **(B)**	*β-Glc* **(C)**	*α-Rha* **(D)**	*α-Rha* **(D')**	*Linker*
H-1	5.18	5.09	4.53	5.24	5.14	
C-1	96.1	96.8	102.4	101.8	102.0	
H-2	3.70	3.73	3.38	4.06	4.06	
C-2	72.7	73.4	75.3	71.4	71.2	
H-3	3.70	4.03	3.61	3.88	3.81	
C-3	76.1	77.0	83.2	71.1	71.2	
H-4	3.48	3.86	3.46	3.47	3.47	
C-4	70.5	73.8	69.1	73.0	73.0	
H-5	3.82	4.05	3.45	4.43	4.03	
C-5	72.5	72.3	77.2	69.5	69.8	
H-6 a/b	3.88/3.78	3.92	3.80/3.96	1.27	1.27	
C-6	61.6	60.3	62.2	17.5	17.5	
H-1' a/b						3.79/3.59
C-1'						68.7
H-2'						1.70
C-2'						29.0
H-3'						1.49
C-3'						23.5
H-4'						1.70
C-4'						27.7
H-5'						3.01
C-5'						40.4

[a] ^{1}H and ^{13}C NMR resonances were assigned based on HSQC, HMBC, COSY and TOCSY experiments.

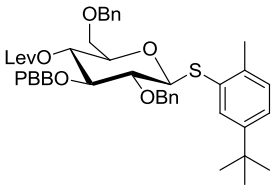
Table 3: Comparison of ^{1}H and ^{13}C NMR δ between **27** and the native PS-I repeating unit.[a]

	α-Glc	*α-Glc*	*β-Glc*	*α-Rha*	*α-Rha*
	(A)	**(B)**	**(C)**	**(D)**	**(D')**
H-1	5.18	5.09	4.53	5.24	5.14
	5.75	*5.13*	*4.53*	*5.23*	*5.17*
C-1	96.1	96.8	102.4	101.8	102.0
	93.5	*98.0*	*102.4*	*101.9*	*101.4*
H-2	3.70	3.73	3.38	4.06	4.06
	3.68	*3.70*	*3.38*	*4.07*	*4.09*
C-2	72.7	73.4	75.3	71.4	71.2
	77.3	*73.6*	*75.2*	*71.1*	*71.2*
H-3	3.70	4.03	3.61	3.88	3.81
	3.89	*4.01*	*3.62*	*3.85*	*3.97*
C-3	76.1	77.0	83.2	71.1	71.2
	72.1	*77.5*	*83.0*	*71.0*	*70.9*
H-4	3.48	3.86	3.46	3.47	3.47
	3.53	*3.86*	*3.46*	*3.46*	*4.07*
C-4	70.5	73.8	69.1	73.0	73.0
	70.1	*73.6*	*69.1*	*73.0*	*78.9*
H-5	3.82	4.05	3.45	4.43	4.03
	3.91	*4.06*	*3.45*	*4.44*	*4.12*
C-5	72.5	72.3	77.2	69.5	69.8
	73.8	*72.4*	*77.1*	*69.4*	*68.6*
H-6 a/b	3.88/3.78	3.92	3.80/3.96	1.27	1.27
	n.d.	*n.d.*	*3.80/3.95*	*1.27*	*1.33*
C-6	61.6	60.3	62.2	17.5	17.5
	n.d.	*n.d.*	*62.2*	*17.5*	*17.8*

[a] data of native PS-I reported in italic taken from: J. Ganeshapillai *et al.*, *Carbohydr. Res.*, 2008, **343**, 703.

6.3.2 Revised Synthesis of the PS-I Repeating Unit

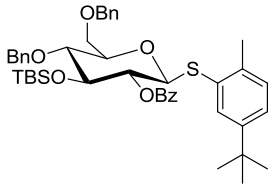
(2-Methyl-5-*tert*-butylphenyl) 2,6-di-*O*-benzyl-3-*O*-(4-bromo)benzyl-4-*O*-levulinoyl-1-thio-β-D-glucopyranoside (54)



To a solution of **43** (1.25 g, 2.40 mmol) in anhydrous DMF (12 mL), NaH (0.14 g, 5.76 mmol) was added followed by *para*-bromobenzyl (PBB) bromide (1.80 mg, 7.20 mmol) at 0 °C. The mixture was warmed to room temperature over 2 h, cooled to 0 °C and quenched by the addition of MeOH. Et_2O was added and the organic layer washed with 0.1 M HCl solution followed by saturated aqueous $NaHCO_3$ solution. The phases were separated and the organic layer was dried over $MgSO_4$ and concentrated. Column chromatography (cyclohexane/ethyl acetate) afforded the PBB protected glucoside (1.55 g) along with aromatic impurities and was taken to the next step without further purification. To a solution of the impure PBB protected glucoside in anhydrous DCM (45 mL) freshly activated molecular sieves (4 Å) were added. The mixture was cooled to -78 °C, triethylsilane (1.08 mL, 6.74 mmol) and TfOH (0.68 mL, 7.64 mmol) were added. After stirring for 4 h at –78 °C, the reaction was quenched by the addition of saturated aqueous $NaHCO_3$ solution, diluted with DCM and washed with a saturated aqueous $NaHCO_3$ solution. The organic phase was then dried over $MgSO_4$, filtered and concentrated. The crude alcohol was taken to the next step. To a solution of the crude alcohol in DCM (20 mL) at 0 °C, DMAP (274 mg, 2.24 mmol), LevOH (1.14 mL, 11.20 mmol) and DCC (2.31 g, 11.20 mmol) were added. The solution was warmed to room temperature and stirred for 16 h. The reaction was diluted with DCM and the organic layers were washed with a 0.1 M HCl solution, followed by saturated aqueous $NaHCO_3$ solution. The organic layer was dried over $MgSO_4$ and concentrated. Column chromatography on silica gel (hexanes/ethyl acetate) afforded **54** (1.54 g, 1.95 mmol, 81% over three steps). $[\alpha]_D^{20}$ = + 6.4° (c = 3.4, $CHCl_3$), IR ν_{max} (film) 2963, 1744, 1718, 1488, 1361, 1261, 1068, 1038, 1012 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.70-7.05 (m, 17H, Ar-H), 5.11-5.04 (m, 1H, 4-H), 4.97 (d, 1H, *J* = 10.4 Hz, benzyl-H), 4.77-4.60 (m, 4H, benzyl-H and 1-H), 4.48 (s, 1H), 3.70-3.54 (m, 5H), 2.64-2.55 (m, 2H, Lev-CH_2), 2.40 (s, 3H, S-CH_3), 2.35-2.29 (m, 2H, Lev-CH_2), 2.12 (s, 3H, Lev-CH_3), 1.25 (s, 9H, *t*Bu); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 206.2 (Lev-carbonyl), 171.7, 149.8, 138.1, 138.0, 137.5, 136.2, 133.2, 131.6, 130.0, 129.6, 128.9, 128.5, 128.4,

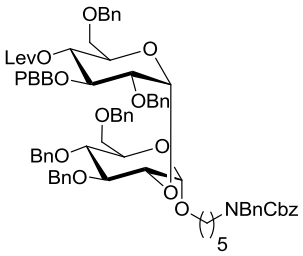
128.3, 128.1, 128.0, 127.7 (2C), 121.6, 88.3 (C-1), 84.1, 81.1, 77.4, 75.8, 74.5, 73.7, 71.3, 69.7, 37.8, 34.6, 31.4, 29.9, 28.0, 20.5; HRMS (MALDI-TOF): Calcd for $C_{43}H_{49}BrO_7SNa^+$ $[M+Na]^+$ 811.2275, found 811.2026.

(2-Methyl-5-*tert*-butylphenyl) 2-*O*-benzoyl-4,6-di-*O*-benzyl-3-*O*-*tert*-butyldimethylsilyl-1-thio-β-D-glucopyranoside (53)



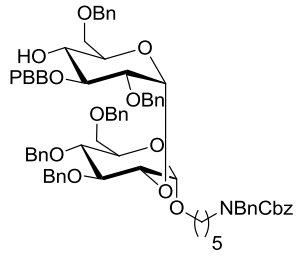
To a solution of **46** (800 mg, 1.23 mmol) in anhydrous DCM (12 mL) BH_3·THF (1 M in THF, 7.4 mL, 7.4 mmol) and TMSOTf (0.11 mL, 0.62 mmol) were added drop wise at 0°C. The reaction was warmed to room temperature over 2 h, cooled to 0°C again and quenched by drop wise addition of saturated aqueous $NaHCO_3$ solution. The Emulsion was diluted with DCM and washed with a saturated aqueous $NaHCO_3$ solution. The organic phase was then dried over $MgSO_4$, filtered and concentrated. The alcohol was taken crude to the next step. To a solution of the crude alcohol in THF/DMF (9:1, 10 mL) at 0°C, BnBr (0.18 mL, 1.50 mmol) and NaH (36 mg, 1.50 mmol) were added. The solution was warmed to room temperature over 2 h, then cooled back to 0°C and additional BnBr (0.18 mL, 1.50 mmol) was added. The reaction was warmed to room temperature over 30 min, cooled to 0°C and quenched by the addition of water. After dilution with Et_2O the phases were separated and the aqueous layer extracted with Et_2O. The organic phase was then dried over MgSO4, filtered and concentrated. Column chromatography on silica gel (hexanes/ethyl acetate) afforded **53** (797 mg, 1.08 mmol, 88% over 2 steps). $[\alpha]_D^{20}$ = + 33.3° (c = 2.1, $CHCl_3$), IR ν_{max} (film) 2956, 2858, 1732, 1453, 1263, 1092 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.14-7.00 (m, 18H, Ar-H), 5.31 (dd, 1H, *J* = 10.1 Hz and 8.9 Hz, 2-H), 4.83 (d, 1H, *J* = 11.3 Hz, benzyl-H_a), 4.72 (d, 1H, *J* = 10.2 Hz, 1-H), 4.63 (d, 1H, *J* = 11.0 Hz, benzyl-H_b), 4.58 (d, 2H, *J* = 3.1 Hz, benzyl-H), 3.95 (t, 1H, *J* = 8.7 Hz, 3-H), 3.78-3.51 (m, 4H), 2.15 (s, 3H, S-CH_3), 1.25 (s, 9H, S-*t*Bu), 0.79 (s, 9H, TBS-*t*Bu), 0.00 (s, 3H, TBS-CH_3), -0.16 (s, 3H, TBS-CH_3); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 165.6, 149.7, 138.2, 136.5, 133.2, 130.5, 130.1, 129.8, 129.2, 128.5, 128.4, 128.0, 127.7(2C), 127.6, 124.7, 88.0 (C-1), 79.5 (C-5), 78.9 (C-4), 77.0 (C-3), 75.1, 73.6 (C-2), 73.5, 69.0 (C-6), 31.4, 25.8, 20.3, -3.9, -4.1; HRMS (ESI): Calcd for $C_{44}H_{56}O_6SSiNa^+$ $[M+Na]^+$ 763.3459, found: 763.3500

***N*-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl 2,6-di-*O*-benzyl-3-*O*-(4-bromo)benzyl-α-D-glucopyranosyl-4-*O*-levulinoyl-(1→2)-3,4,6-Tri-*O*-benzyl-α-D-gluco-pyranoside (55)**



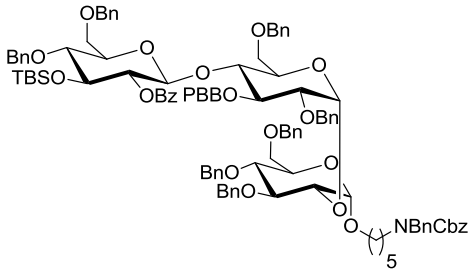
Thioglucoside **54** (323 mg, 0.41 mmol) and glucoside **49** (222 mg, 0.29 mmol) were coevaporated with toluene three times and dried *in vacuo*. The mixture was dissolved in ether (4 mL), freshly activated and acid washed molecular sieves (4 Å) and NIS (105 mg, 0.47 mmol) were added and cooled to –20 °C. TfOH (4.2 μL, 0.05 mmol) was added and the mixture was stirred and warmed up to –10 °C in one h. The reaction was quenched by the addition of pyridine, diluted with DCM and washed with aqueous $Na_2S_2O_3$ and saturated aqueous $NaHCO_3$ solutions. The phases were separated and the aqueous phase was extracted with DCM. The combined organic phases were dried over $MgSO_4$, filtered and concentrated. The crude product was purified by column chromatography on silica gel (toluene/acetone) to afford **55** (276 mg, 0.20 mmol, 69%). $[\alpha]_D^{20}$ = + 54.1° (c = 4.8, $CHCl_3$), IR ν_{max} (film) 3031, 2923, 2864, 1744, 1698, 1497, 1454, 1420, 1360, 1209 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.60-7.02 (m, 39H, Ar-H), 5.24-5.10 (m, 3-H), 5.09-4.93 (m, 3H, 2 × anomeric-H), 4.89-4.37 (m, 13H), 4.13-4.00 (m, 2H), 3.99-3.56 (m, 8H), 3.50-3.08 (m, 5H), 2.63-2.47 (m, 2H), 2.25-2.18 (m, 2H), 2.13 (s, 3H, Lev-CH_3), 1.71-1.38 (m, 4H, linker-H), 1.36-1.14 (m, 2H, linker-H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 206.3 (Lev-carbonyl), 171.4, 138.7, 138.3, 138.1, 138.1, 137.8, 131.4, 129.6, 128.7, 128.5, 128.4, 128.3, 128.1, 128.03, 127.98, 127.93, 127.88, 127.8, 127.6 (2C), 127.4, 121.4, 95.5 (C-anomeric), 93.5 (C-anomeric), 80.9, 79.3, 78.8, 78.1, 75.7, 75.3, 74.1, 73.7, 73.5, 72.3, 70.5, 70.2, 68.6, 68.3, 68.1, 67.3, 37.8, 30.0, 29.5, 27.9, 23.7; HRMS (MALDI-TOF): Calcd for $C_{79}H_{86}BrNO_{15}Na^+$ $[M+Na]^+$ 1390.5073, found 1390.5105.

***N*-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl 2,6-di-*O*-benzyl-3-*O*-(4-bromo)benzyl-α-D-glucopyranosyl-(1→2)-3,4,6-Tri-*O*-benzyl-α-D-gluco-pyranoside (56)**



To a solution of **55** (300 mg, 0.22 mmol) in DCM (5.0 mL) hydrazine hydrate (32 µL, 0.66 mmol) dissolved in AcOH (0.4 mL) and pyridine (0.6 mL) was added and the solution stirred for 1 h. The reaction was then quenched by the addition of acetone and concentrated. Column chromatography on silica gel (hexanes/ethyl acetate) afforded **56** (266 mg, 0.21 mmol, 96%). $[\alpha]_D^{20}$ = + 56.5º (c = 2.7, $CHCl_3$), IR ν_{max} (film) 3453, 2963, 1695, 1454, 1420, 1360, 1259, 1013 cm^{-1}; ^{1}H-NMR (600 MHz, $CDCl_3$) δ 7.90-7.00 (m, 39H, Ar-H), 5.25-5.13 (m, 2H), 5.10 (s, 1H, anomeric-H), 5.05 (bs, 1H, anomeric-H), 4.98-4.43 (m, 14H), 4.10-3.53 (m, 13H), 3.45-3.10 (m, 3H), 1.65-1.40 (m, 4H, linker-H), 1.34-1.15 (m, 2H, linker-H); ^{13}C-NMR (150 MHz, $CDCl_3$) δ 138.7, 138.2, 138.1, 131.6, 129.7, 128.6, 128.49, 128.45, 128.1, 128.0 (2C), 127.9 (2C), 127.7 (2C), 127.3, 121.6, 95.6 (C-anomeric), 93.9 (C-anomeric), 81.4, 81.0, 78.9, 78.1, 77.4, 77.2, 77.0, 75.8, 75.2, 74.4, 73.6, 73.6, 72.1, 71.1, 70.5, 69.3, 68.6, 68.3, 67.3, 50.3, 47.2, 46.2, 43.3, 29.5, 27.7, 23.6; HRMS (MALDI-TOF): Calcd for $C_{74}H_{80}BrNO_{13}Na^+$ $[M+Na]^+$ 1292.4705, found 1292.4701.

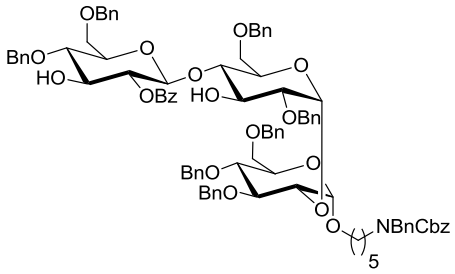
***N*-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl 2-*O*-benzoyl-4,6-di-*O*-benzyl-3-*O*-*tert*-butyldimethylsilyl-β-D-glucopyranosyl-(1→4)-2,6-di-*O*-benzyl-3-*O*-(4-bromo)benzyl-α-D-glucopyranosyl-(1→2)-3,4,6-tri-*O*-benzyl-α-D-glucopyranoside (52)**



Thioglucoside **53** (233 mg, 0.31 mmol) and disaccharide **56** (266 mg, 0.21 mmol) were coevaporated with toluene three times and dried *in vacuo*. The mixture was dissolved in DCM (7 mL), freshly activated and acid washed molecular sieves (4 Å) and NIS (80 mg, 0.36 mmol) were added and cooled to –30°C. TfOH (3.2 µL, 0.04 mmol) was added and the mixture was stirred and warmed up to –17 °C in 1 h. The reaction was quenched by the addition of pyridine, diluted with DCM and washed with aqueous $Na_2S_2O_3$ and saturated aqueous $NaHCO_3$ solutions. The phases were separated and the aqueous phase was extracted with DCM. The combined organic phases were dried over $MgSO_4$, filtered and concentrated. The crude product was purified by column chromatography on silica gel (toluene/acetone) to afford **52** (354 mg, 0.19 mmol, 92%). $[\alpha]_D^{20}$ = + 52.5º (c = 2.6, $CHCl_3$), IR ν_{max} (film) 3031, 2928, 2859, 1733, 1699, 1603, 1497, 1454, 1421, 1362, 1314, 1265, 1070 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.91-7.05 (m, 54H, Ar-H), 5.21-5.11 (m, 3H), 5.04 (s, 1H, anomeric-H),

5.01-4.95 (m, 2H), 4.81 (d, 1H, *J* = 11.3 Hz), 4.74-4.35 (m, 17H), 4.23 (d, 1H, *J* = 12.3 Hz), 3.98 (t, 1H, *J* = 9.4 Hz), 3.93-3,87 (m, 1H), 3.82 (t, 1H, *J* = 9.3 Hz), 3.74-3.66 (m, 4H), 3.64-3.45 (m, 10H), 3.42-3.36 (m, 1H), 3.34-3.06 (m, 4H), 1.56-1.35 (m, 4H), 1.25-1.09 (m, 2H), 0.79 (s, 9H, *t*Bu), 0.02 (s, 3H), -0.19 (s, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 164.8, 138.7, 138.7, 138.4, 138.4, 138.4, 138.1, 133.1, 131.1, 130.1, 130.0, 129.6, 128.7, 128.6, 128.5 (2C), 128.4 (5C), 128.2, 128.0, 127.9 (2C), 127.8, 127.7, 127.6 (2C), 127.5, 127.4, 120.8, 100.3 (C-anomeric), 96.1 (C-anomeric), 59.0 (C-anomeric) 80.5, 80.0, 79.1, 78.6, 77.7, 76.1, 75.5, 75.4, 75.3, 75.2, 74.7, 74.4, 73.8, 73.6, 73.5, 72.3, 70.6, 70.3, 69.1, 68.7, 67.6, 67.2, 50.6, 47.2, 46.3, 29.4, 28.1, 25.8, 23.6, 17.9, -3.86, -3.89; HRMS (MALDI-TOF): Calcd for $C_{107}H_{120}BrNO_{19}SiNa^+$ $[M+Na]^+$ 1852.7299 found 1852.7375.

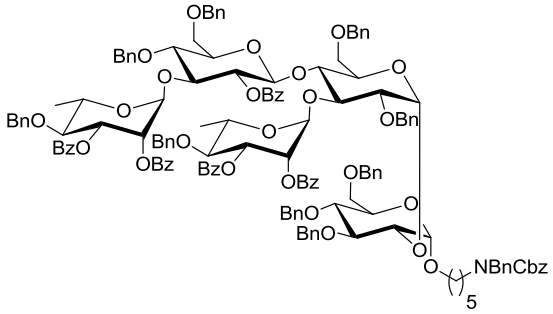
_N_-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl 2-_O_-benzoyl-4,6-di-_O_-benzyl-β-D-glucopyranosyl-(1→4)-2,6-di-_O_-benzyl-α-D-glucopyranosyl-(1→2)-3,4,6-tri-_O_-benzyl-α-D-glucopyranoside (57)



A solution of **52** (100 mg, 0.06 mmol), (3,4-dimethoxyphenyl)boronic acid (20 mg, 0.11 mmol), *tert*-butyl ammonium bromide (1.8 mg, 5.5 µmol), K_3PO_4 (35 mg, 0.16 mmol) in EtOH (4 mL) was subjected to three freeze-pump-saw cycles. To this solution $Pd(OAc)_2$ (1.2 mg, 5.5 µmol) was added and stirred for 2 h. The mixture was diluted with EtOAc and washed with saturated aqueous $NaHCO_3$ solution. The aqueous phase was back extracted with EtOAc. The combined organic phases were dried over $MgSO_4$, filtered and concentrated. The crude product was purified by column chromatography on silica gel (toluene/acetone) to afford the Suzuki coupling product (95 mg) which was dissolved in DCM/H_2O/saturated aqueous $NaHCO_3$ (100:9:1, 11 mL). To this emulsion DDQ (34 mg, 0.15 mmol) was added, stirred vigorously for 16 h, diluted with DCM and washed with saturated aqueous $NaHCO_3$ solutions. The combined organic phases were dried over $MgSO_4$, filtered and concentrated. The crude intermediate was dissolved in DMF (2.5 mL), and treated with a solution of TBAF·$3H_2O$ (137 mg, 0.43 mmol) and AcOH (29 µl, 0.51 mmol) in DMF (2.5 mL) at 50°C for three days. After dilution with Et_2O the phases were separated and the organic phase washed with a 0.1 M HCl solution, saturated aqueous $NaHCO_3$ solution and brine. The

organic phase was then dried over $MgSO_4$, filtered and concentrated. The crude product was purified by column chromatography on silica gel (toluene/acetone) to afford **57** (52 mg, 0.03 mmol, 62%). $[\alpha]_D^{20}$ = + 38.9º (c = 1.5, $CHCl_3$), IR ν_{max} (film) 3462, 3031, 2924, 2867, 1729, 1699, 1497, 1454, 1422, 1362, 1315, 1268, 1095, 1069 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.07-7.00 (m, 50H), 5.25-5.05 (m, 3H), 5.03-4.94 (m, 2H, 2 × anomeric-H), 4.90 (d, *J* = 10.6, 1H), 4.82-4.35 (m, 16H), 4.27 (d, *J* = 12.1, 1H), 4.16 (dd, *J* = 9.2 Hz and 8.8 Hz, 1H), 4.06 (d, *J* = 12.2 Hz, 1H), 3.99 (t, *J* = 9.3 Hz, 1H), 3.93-3.42 (m, 15H), 3.28 (s, 4H), 1.73-1.36 (m, 4H, linker-H), 1.34-1.08 (m, 2H, linker-H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 166.2, 139.0, 138.5, 138.4, 137.9, 137.7, 133.6, 130.1, 129.4, 128.7 (2C), 128.6 (3C), 128.5 (3C), 128.4 (2C), 128.3, 128.2 (2C), 128.0 (2C), 127.9 (2C), 127.8, 127.6 (2C), 127.4, 101.2 (C-anomeric), 95.9 (C-anomeric), 94.8 (C-anomeric), 81.6, 78.4, 78.2, 78.0, 77.5, 77.4, 77.2, 76.8, 76.6, 76.3, 75.0, 74.7, 73.9, 73.6, 73.2, 72.7, 72.2, 70.4, 69.3, 69.1, 68.7, 67.3, 50.4, 47.3, 29.5, 28.1, 23.6; HRMS (MALDI-TOF): Calcd for $C_{94}H_{101}NO_{19}Na^+$ $[M+Na]^+$ 1570.6860, found 1570.6362.

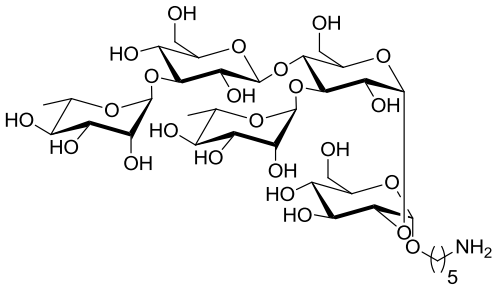
_N_-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl 2,3-di-_O_-benzoyl-4-_O_-benzyl-α-L-rhamnopyranosyl-(1→3)-2-_O_-benzoyl-4,6-_O_-benzyl-β-D-glucopyranosyl-(1→4)-[2,3-di-_O_-benzoyl-4-_O_-benzyl-α-L-rhamnopyranosyl-(1→3)]-2,6-di-_O_-benzyl-α-D-glucopyranosyl-(1→2)-3,4,6-tri-_O_-benzyl-α-D-glucopyranoside (51)



Rhamnosyl-imidate **31** (86 mg, 136 μmol) and trisaccharide **57** (42 mg, 27 μmol) were coevaporated with toluene three times, dried *in vacuo* and dissolved in anhydrous DCM (3.0 mL). Freshly activated molecular sieves (4 Å) were added and the mixture cooled to –40 °C. TMSOTf (2.5 μL, 14 μmol) was added and the reaction was warmed to –20 °C over 1.5 h. The reaction was quenched with triethylamine and concentrated. Size exclusion chromatography on Sephadex LH-20 ($CHCl_3$/MeOH 1:1) afforded **51** (58 mg, 24 μmol, 88 %). $[\alpha]_D^{20}$ = + 49.7º (c = 2.2, $CHCl_3$), IR ν_{max} (film) 3031, 2927, 2863, 1729, 1700, 1602, 1497, 1453, 1273, 1264, 1095, 1069 cm^{-1}; ^{1}H-NMR (600 MHz, $CDCl_3$) δ 8.08- 6.98 (m, 80, Ar-H), 5.89 (dd, *J* = 9.4Hz and 3.5Hz, 1H), 5.85 (dd, *J* = 3.5 Hz and 1.7 Hz, 1H), 5.66 (dd, *J*

= 9.4 Hz, 3.5, 1H), 5.49-5.42 (m, 1H), 5.39 (dd, *J* = 3.5 Hz and 1.8 Hz, 1H), 5.32- 5.25 (m, 1H), 5.16- 5.12 (m, 2H), 5.08-5.05 (m, 1H), 5.04-4.99 (m, 1H), 4.97 (d, *J*= 1.6 Hz, 1H), 4.95-4.25 (m, 21H), 4.24-3.43 (m, 20H), 3.39- 3.00 (m, 5H), 1.67 (d, *J* = 6.2 Hz, 3H), 1.60-1.32 (m, 4H), 1.32-1.06 (m, 2H), 0.94 (d, *J* = 6.1 Hz, 3H); ^{13}C-NMR (150 MHz, $CDCl_3$) δ 165.4, 165.2, 164.5, 164.1, 139.2, 138.5, 138.4, 138.2, 137.8, 137.7, 133.1, 133.0 (2C), 132.7 (2C), 130.4, 130.1 (2C), 130.0, 129.9 (2C), 129.8 (2C), 129.7, 129.5, 129.0, 128.7 (3C), 128.6 (2C), 128.5 (2C), 128.4 (3C), 128.3, 128.2 (3C), 128.0 (2C), 127.9 (2C), 127.8, 127.7, 127.4, 127.2, 99.5, 98.0, 97.7, 95.8, 93.7, 80.8, 80.4, 80.3, 79.3, 78.4, 78.3, 77.5, 76.6, 76.2, 75.9, 75.7, 75.2, 74.7, 74.2, 74.0, 73.7, 73.6, 73.3, 72.9, 72.2, 71.9, 71.2 (2C), 70.6, 70.3, 68.7, 68.1, 68.0, 67.3, 67.2, 50.6, 47.2, 46.3, 29.4, 23.6, 18.6, 17.75; HRMS (MALDI-TOF): Calcd for $C_{148}H_{149}NO_{31}Na^+$ $[M+Na]^+$ 2459.0006, found 2459.0636.

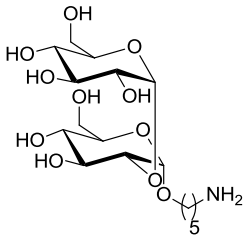
5-Amino-pentanyl α-L-rhamnopyranosyl-(1→3)-β-D-glucopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→3)]-α-D-glucopyranosyl-(1→2)-α-D-glucopyranoside (27)



To a solution of fully protected pentasaccharide **51** (23.0 mg, 9.4 μmol) in THF (1.5 mL) NaOMe (0.5 M, in MeOH, 1 mL) was added and stirred for 12 h. The mixture was neutralized with Amberlite IR 120 (H^+) ion exchange resin, filtered and concentrated. Column chromatography on silica gel (DCM/acetone/MeOH) afforded the de-benzoylated pentasaccharide (16 mg), which was dissolved in a mixture of THF (1 mL) MeOH (1 mL), H_2O (0.7 mL) and AcOH (0.1 mL). The solution was purged with Ar, 10% Pd/C (30 mg) was added and the solution purged with H_2 for 30 min, then stirred under an H_2 atmosphere for 12 h, filtered and concentrated. Size exclusion chromatography on Sephadex LH-20 (MeOH) afforded **27** (5.0 mg, 5.7 μmol, 60%). All analytical data is consistent with previously reported.

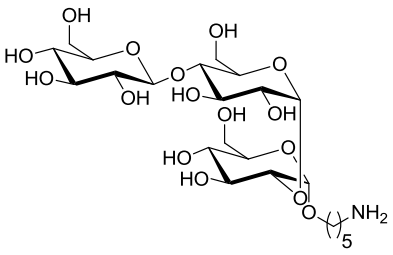
6.3.3 Synthesis of PS-I Substructures

5-Amino-pentanyl D-glucopyranosyl-(1→2)-α-D-glucopyranoside (58)



A solution of disaccharide **56** (40.0 mg, 31 µmol) in a mixture of MeOH (5.0 mL),THF (2.5 mL) H_2O (2.0 mL) and AcOH (0.5 mL) was purged with Ar. After that 10% Pd/C (70 mg) was added and the solution purged with H_2 for 30 min, then stirred under an H_2 atmosphere for 12 h, filtered and concentrated. The crude product was purified by reversed phase solid phase extraction (RP SPE) (Waters Sep-Pak®, C18) to afford **58** (13.3 mg, 31 µmol, 99%). ^{1}H-NMR (600 MHz, D_2O) δ 5.23 (d, *J* = 3.4 Hz, 1H, H-anomeric), 5.16 (d, *J*=3.6, 1H, H-anomeric), 4.02-3.80 (m, 8H), 3.75 (dd, *J* = 9.9 Hz, 3.5, 2H) 3.68-3.61 (m, 2H), 3.53 (td, J=9.6 Hz and 4.7 Hz, 2H), 3.09 (t, *J* = 7.5 Hz, 2H), 1.81-1.71 (m, 4H, linker), 1.59-1.49 (m, 2H, linker); ^{13}C-NMR (150 MHz, D_2O) δ 98.6 (anomeric), 97.9 (anomeric), 77.7, 75.4, 74.5, 74.4, 74.2, 74.0, 72.3, 72.1, 70.4, 63.3, 63.1, 42.1, 30.6, 29.2, 25.1; HRMS (MALDI-TOF): Calcd for $C_{17}H_{33}NO_{11}H^+$ $[M+H]^+$ 428.2126, found 428.2147.

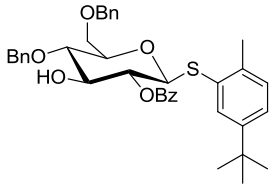
5-Amino-pentanyl β-D-glucopyranosyl-(1→4)-α-D-glucopyranosyl-(1→2)-α-D-glucopyranoside (59)



To a solution of protected trisaccharide **57** (60 mg, 39 µmol) in THF (2 mL) NaOMe (0.5 M in MeOH, 0.5 mL) was added and stirred for 4 h. The mixture was neutralized with Amberlite IR 120 (H^+) ion exchange resin, filtered and concentrated. The crude product was dissolved in a mixture of THF (5.0 mL) MeOH (2.5 mL), H_2O (2.0 mL) and AcOH (0.5 mL). The solution was purged with Ar, then 10% Pd/C (30 mg) was added and the solution purged with H_2 for 30 min, then stirred under an H_2 atmosphere for 12 h, filtered and concentrated. Purification by RP SPE (Waters Sep-Pak®, C18) afforded **59** (11.2 mg, 19 µmol, 49 %). ^{1}H-NMR (600

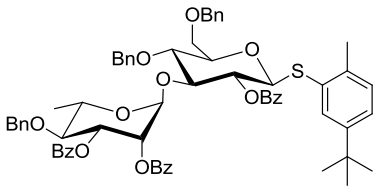
MHz, D_2O) δ 5.22 (d, *J* = 3.3 Hz, 1H, anomeric α-Glc), 5.15 (d, *J* = 3.6 Hz, 1H, anomeric α-Glc), 4.60 (d, *J*= 7.9 Hz, 1H, anomeric β-Glc), 4.14-4.08 (m, 1H), 4.03-3.91 (m, 5H), 3.90-3.79 (m, 4H), 3.78-3.72 (m, 3H), 3.71-3.62 (m, 2H), 3.62-3.47 (m, 4H), 3.40 (t, *J* = 8.7 Hz, 1H), 3.09 (t, *J* = 7.5 Hz, 2H), 1.83-1.72 (m, 4H, linker), 1.59-1.49 (m, 2H, linker). ^{13}C-NMR (150 MHz, D_2O) δ 100.7 (anomeric β-Glc), 94.0 (anomeric α-Glc), 93.4 (anomeric α-Glc), 76.8, 74.2, 73.7, 73.5, 71.3, 69.8, 69.6, 69.5, 69.2, 68.7, 67.7, 67.6, 65.9, 58.8, 57.9, 37.5, 26.1, 24.6, 20.6; HRMS (MALDI-TOF): Calcd for $C_{23}H_{43}NO_{16}Na^+$ $[M+Na]^+$ 612.2474, found 612.2424.

(2-Methyl-5-*tert*-butylphenyl) 2-*O*-benzoyl-4,6-di-*O*-benzyl-1-thio-β-D-glucopyranoside (62)



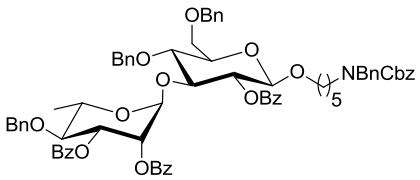
A solution of TBAF·$3H_2O$ (1.10 g, 3.48 mmol) and acetic acid (266 µl, 4.64 mmol) in DMF (4 mL) was added to a solution of **53** (430 mg, 0.58 mmol) in DMF (4 mL). The mixture was stirred for 3 days at 35°C. After dilution with Et_2O the phases were separated and the organic phase washed with a 0.1 M HCl solution, saturated aqueous $NaHCO_3$ solution and brine. The organic phase was then dried over $MgSO_4$, filtered and concentrated. Column chromatography on silica gel (hexanes/ethyl acetate) afforded **62** (335 mg, 0.53 mmol, 92 %). $[\alpha]_D^{20}$ = – 9.2° (c = 3.2, $CHCl_3$), IR ν_{max} (film) 3463, 2961, 2867, 1727, 1602, 1452, 1264, 1092 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.12-7.05 (m, 18H, Ar-H), 5.31 (dd, 1H, *J* = 9.6 Hz, 2-H), 4.83 (d, 1H, *J* = 11.2 Hz, benzyl-H_a), 4.78 (d, 1H, *J* = 10.1 Hz, 1-H), 4.65 (d, 2H, *J* = 12.0 Hz, benzyl-H_b), 4.58 (d, 1H, *J* = 12.2 Hz), 3.94 (td, 1H, *J* = 8.9 Hz and 4.1 Hz, 3-H), 3.82-3.73 (m, 2H), 3.71-3.65 (m, 1H), 3.59-3.54 (m, 1H), 2.72 (d, *J* = 4.0 Hz, 3-OH), 2.24 (s, 3H, S-CH_3), 1.25 (s, 9H, S-*t*Bu). ^{13}C-NMR (100 MHz, $CDCl_3$) δ 166.5, 149.7, 138.2, 138.1, 137.0, 133.5, 132.6, 130.1, 130.0, 129.8, 129.7, 128.6, 128.5 (2C), 128.2, 128.1, 127.8, 125.1, 86.7, 79.2, 78.1, 77.6, 75.0, 73.7 (2C), 69.0, 34.5, 31.4, 20.4; HRMS (MALDI-TOF): Calcd for $C_{38}H_{42}O_6SNa^+$ $[M+Na]^+$ 649.2594, found 649.2621.

(2-Methyl-5-*tert*-butylphenyl) 2,3-di-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl-(1→3)2-*O*-benzoyl-4,6-di-*O*-benzyl-1-thio-β-D-glucopyranoside (63)



Rhamnosyl-imidate **31** (373 mg, 0.59 mmol) and glucoside **62** (335 mg, 0.53 mmol) were coevaporated with toluene three times, dried *in vacuo* and dissolved in anhydrous DCM (3.0 mL). Freshly activated molecular sieves (4 Å) were added and the mixture cooled to –40 °C. TMSOTf (10 µL, 53 µmol) was added and the reaction was warmed to –20 °C over 1.5 h. The reaction was quenched with triethylamine and concentrated. Column chromatography on silica gel (hexanes/ethyl acetate) afforded **63** (490 mg, 0.46 mmol, 86 %). $[\alpha]_D^{20}$ = + 70.7º (c = 1.9, $CHCl_3$), IR ν_{max} (film) 2963, 1728, 1602, 1451, 1259, 1090, 1067, 1025 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.02-7.03 (m, 33H), 5.72 (dd, *J* = 9.4 Hz, 3.5, 1H), 5.53-5.42 (m, 2H), 5.22 (d, *J* = 1.9 Hz, 1H), 4.88 (d, *J* = 10.6 Hz, 1H), 4.77-4.47 (m, 6H), 4.24-4.13 (m, 2H), 3.92-3.80 (m, 3H), 3.68-3.59 (m, 2H), 2.18 (s, 3H), 1.25 (s, 9H), 1.08 (d, *J* = 6.2 Hz, 3H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ 149.8, 138.1, 138.0, 133.1, 130.3, 130.0, 129.9, 129.8, 129.7 (2C), 128.6 (2C), 128.5 (2C), 128.4 (3C), 128.3 (2C), 128.2 (2C), 128.1, 128.0 (2C), 127.9, 127.8 (3C), 125.7, 124.4, 97.6, 86.6, 79.3, 77.5, 77.2, 76.8, 75.7, 75.6, 75.0, 74.4, 73.8, 72.0, 71.3, 68.3, 67.9, 31.5, 19.5, 18.0; HRMS (MALDI-TOF): Calcd for $C_{65}H_{66}O_{12}SNa^+$ $[M+Na]^+$ 1093.4167, found 1093.4159.

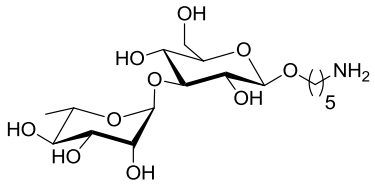
***N*-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl 2,3-di-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl-(1→3)2-*O*-benzoyl-4,6-di-*O*-benzyl-1-thio-β-D-glucopyranoside (64)**



Disaccharide **63** (50 mg, 47 µmol) and *N*-(Benzyl)benzyloxycarbonyl-5-amino-pentanol (31 mg, 93 µmol) were coevaporated with toluene three times and dried *in vacuo*. The mixture was dissolved in DCM (3 mL) and NIS (13 mg, 56 µmol) was added and cooled to –20 °C. TfOH (0.5 µL, 6 µmol) was added and the mixture was stirred and warmed up to 0 °C in 2 h. The reaction was quenched by the addition of aqueous $Na_2S_2O_3$ and saturated aqueous $NaHCO_3$. The phases were separated and the aqueous phase was extracted with DCM. The combined organic phases were dried over $MgSO_4$, filtered and concentrated. The crude

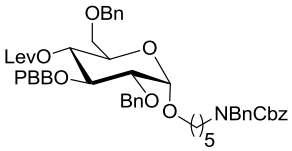
product was purified by column chromatography on silica gel (hexanes/ethyl acetates) to afford **64** (52 mg, 43 μmol, 91%). $[\alpha]_D^{20}$ = + 50.3º (c = 2.6, $CHCl_3$), IR ν_{max} (film) 3032, 2936, 1730, 1698, 1452, 1265, 1069 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.23-6.80 (m, 40H, aromatic), 5.73 (dd, *J* = 9.4 Hz and 3.5 Hz, 1H), 5.46 (dd, *J* = 3.4 Hz and 1.9 Hz, 1H), 5.35 (dd, *J* = 9.2 Hz and 7.9 Hz, 1H), 5.24 (d, *J* = 1.9 Hz, 1H, anomeric Rha), 5.14 (s, 2H), 4.89 (d, *J* = 10.6 Hz, 1H), 4.72-4.59 (m, 4H), 4.56-4.35 (m, 4H, anomeric Glc), 4.22-4.12 (m, 2H), 3.91-3.76 (m, 4H), 3.68-3.58 (m, 2H), 3.42-3.33 (m, 1H), 3.05-2.88 (m, 2H), 1.50-1.29 (m, 4H, linker), 1.24-0.98 (m, 5H, linker and Rha CH_3). ^{13}C-NMR (100 MHz, $CDCl_3$) δ 165.7, 164.8, 138.2, 138.0, 137.6, 133.1, 132.8, 130.0, 129.9 (2C), 129.8, 129.7, 128.6, 128.5, 128.4 (3C), 128.3 (2C), 128.2, 128.0, 127.9 (2C), 127.8, 127.7, 101.1 (anomeric Glc), 97.6 (anomeric Rha), 79.3, 77.8, 76.9, 75.6 (2C), 74.9, 74.6, 73.8, 71.9, 71.3, 68.9, 68.3, 67.2, 29.2, 23.2, 18.0; HRMS (MALDI-TOF): Calcd for $C_{74}H_{75}NO_{15}Na^+$ $[M+Na]^+$ 1240.5029, found 1240.4792.

5-Amino-pentanyl α-L-rhamnopyranosyl-(1→3)-β-D-glucopyranoside (60)



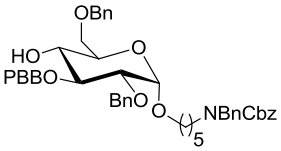
To a solution of protected disaccharide **64** (50.0 mg, 41 μmol) in THF (2 mL) NaOMe (0.5 M in MeOH, 0.5 mL) was added and stirred for 4 h. The mixture was neutralized with Amberlite IR 120 (H^+) ion exchange resin, filtered and concentrated. The crude product was dissolved in a mixture of THF (5.0 mL) MeOH (2.5 mL), H_2O (2.0 mL) and AcOH (0.5 mL). The solution was purged with Ar, then 10% Pd/C (100 mg) was added and the solution purged with H_2 for 30 min, then stirred under an H_2 atmosphere for 12 h, filtered and concentrated. Purification by RP SPE (Waters Sep-Pak®, C18) afforded **60** (12.9 mg, 30 μmol, 76%). ^{1}H-NMR (600 MHz, D_2O) δ 5.20 (s, 1H, anomeric Rha), 4.53 (d, *J* = 8.1 Hz, 1H, anomeric Glc), 4.15-4.04 (m, 2H), 4.02-3.96 (m, 2H), 3.85 (dd, *J* = 9.7 Hz and 3.3 Hz, 1H), 3.81-3.73 (m, 2H), 3.66 (t, *J* = 8.7 Hz, 1H), 3.56-3.49 (m, 3H), 3.44 (t, *J* = 8.7 Hz, 1H), 3.08 (t, *J* = 7.5 Hz, 2H), 1.75 (tt, *J* = 14.6 Hz and 7.2 Hz, 4H, linker), 1.57-1.49 (m, 2H, linker), 1.32 (d, *J* = 6.3 Hz, 3H, Rha CH_3); ^{13}C-NMR (150 MHz, D_2O) δ 100.0 (anomeric Glc), 99.1 (anomeric Rha), 80.3, 73.9, 71.8, 70.0, 68.4, 68.2, 68.1, 66.9, 66.2, 58.8, 37.4, 26.2, 24.4, 20.1, 14.5 (Rha CH_3); HRMS (MALDI-TOF): Calcd for $C_{17}H_{33}NO_{10}Na^+$ $[M+Na]^+$ 434.1997, found 434.1975.

_N_-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl 2,6-di-_O_-benzyl-3-_O_-(4-bromo)benzyl-4-_O_-levulinoyl-α-D-glucopyranoside (65)



Thioglucoside **54** (300 mg, 0.38 mmol) and *N*-(benzyl)benzyloxycarbonyl-5-amino-pentanol (200 mg, 0.61 mmol) were coevaporated with toluene three times and dried *in vacuo*. The mixture was dissolved in ether (4 mL) and dioxane (4 mL), NIS (103 mg, 0.46 mmol) was added and cooled to –10 °C. TfOH (4 µL, 46 µmol) was added and the mixture was warmed up to 0 °C in 3 h. The reaction was quenched by the addition of aqueous $Na_2S_2O_3$ and saturated aqueous $NaHCO_3$. The phases were separated and the aqueous phase was extracted with DCM. The combined organic phases were dried over $MgSO_4$, filtered and concentrated. The crude product was purified by column chromatography on silica gel (hexanes/ethyl acetates) to afford **65** (140 mg, 0.15 mmol, 39%). $[\alpha]_D^{20}$ = + 22.0º (c = 3.4, $CHCl_3$), IR ν_{max} (film) 2920, 1743, 1697, 1454, 1420, 1360, 1208, 1153, 1069, 1038 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.69-6.92 (m, 24H), 5.22-5.15 (m, 2H), 5.09- 5.03 (m, 1H), 4.81 (d, *J* = 11.9 Hz, 1H), 4.76-4.68 (m, 2H, anomeric), 4.63-4.56 (m, 2H), 4.54-4.46 (m, 4H), 3.89 (t, *J* = 9.4 Hz, 1H), 3.84-3.78 (m, 1H), 3.62-3.45 (m, 4H), 3.38-3.18 (m, 3H), 2.66-2.53 (m, 2H), 2.43-2.29 (m, 2H), 2.13 (s, 3H, Lev CH_3), 1.66-1.48 (m, 4H, linker), 1.38-1.27 (m, 2H, linker); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 206.3 (Lev carbonyl), 171.6, 138.2, 138.1, 138.0, 131.4, 129.6, 129.4, 128.7, 128.5, 128.3, 128.1, 128.0 (2C), 127.9, 127.6, 127.4, 121.3, 96.9 (anomeric), 79.8, 79.6, 74.3, 73.7, 73.2, 70.9, 69.0, 68.9, 68.3, 67.3, 37.8, 29.9 (Lev CH_3), 29.2, 28.0, 23.6; HRMS (MALDI-TOF): Calcd for $C_{52}H_{58}BrNO_{10}Na^+$ $[M+Na]^+$ 958.3134, found 958.3112.

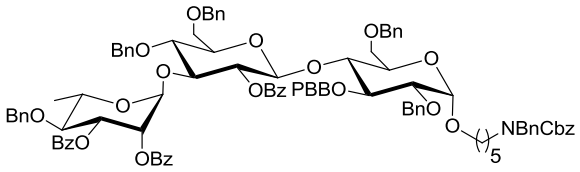
_N_-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl 2,6-di-_O_-benzyl-3-_O_-(4-bromo)benzyl-α-D-glucopyranoside (66)



To a solution of **65** (140 mg, 0.15 mmol) in DCM (5.0 mL) hydrazine hydrate (26 µL, 0.54 mmol) dissolved in AcOH (0.4 mL) and pyridine (0.6 mL) was added and the solution stirred for 1 h. The reaction was then quenched by the addition of acetone and concentrated. Column chromatography on silica gel (hexanes/ethyl acetate) afforded **66** (102 mg, 0.12

mmol, 81%). $[\alpha]_D^{20}$ = + 24.3º (c = 4.2, $CHCl_3$), IR ν_{max} (film) 3454, 3031, 2920, 1696, 1454, 1422, 1229, 1055 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.48-7.04 (m, 24H, Ar), 5.16-5.09 (m, 2H), 4.86 (d, *J* = 11.7 Hz, 1H), 4.70-4.43 (m, 8H), 3.75-3.55 (m, 6H), 3.45 (dd, *J* = 9.5 Hz and 3.6 Hz, 1H), 3.32-3.14 (m, 3H), 1.59-1.44 (m, 4H, linker), 1.33-1.23 (m, 2H, linker); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 138.3, 138.1, 138.0, 131.6, 129.5, 128.6, 128.5 (2C), 128.0 (2C), 127.9, 127.8, 127.7, 127.4, 121.6, 96.9 (anomeric), 81.7, 79.8, 74.6, 73.7, 72.9, 71.4, 70.1, 69.8, 68.1, 67.3, 50.4, 47.3, 29.2, 27.7, 23.7; HRMS (MALDI-TOF): Calcd for $C_{47}H_{52}BrNO_8Na^+$ $[M+Na]^+$ 860.2769, found 860.2508.

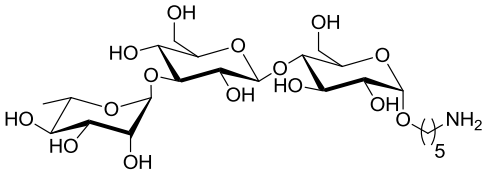
_N_-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl 2,3-di-_O_-benzoyl-4-_O_-benzyl-α-L-rhamnopyranosyl-(1→3)-2-_O_-benzoyl-4,6-_O_-benzyl-β-D-glucopyranosyl-(1→4)-2,6-di-_O_-benzyl-3-_O_-(4-bromo)benzyl-α-D-glucopyranoside (67)



Disaccharide **63** (144 mg, 0.13 mmol) and glucoside **66** (102 mg, 0.12 mmol) were coevaporated with toluene three times and dried *in vacuo*. The mixture was dissolved in DCM (4 mL) and NIS (36 mg, 0.16 mmol) was added and cooled to –20 °C. TfOH (1.4 μL, 16 μmol) was added and the mixture was warmed up to 0 °C in 2 h. The reaction was then quenched by the addition of aqueous $Na_2S_2O_3$ and saturated aqueous $NaHCO_3$. The phases were separated and the aqueous phase was extracted with DCM. The combined organic phases were dried over $MgSO_4$, filtered and concentrated. The crude product was purified by column chromatography on silica gel (hexanes/ethyl acetates) to afford **67** (200 mg, 0.12 mmol, 95%). $[\alpha]_D^{20}$ = + 36.9º (c = 5.2, $CHCl_3$), IR ν_{max} (film) 3031, 2866, 1730, 1698, 1602, 1452, 1262, 1092 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.31-6.72 (m, 54H, Ar), 5.73 (dd, *J* = 9.4 Hz and 3.4 Hz, 1H), 5.44 (dd, *J* = 3.4 Hz and 1.9 Hz, 1H), 5.37 (dd, *J* = 9.3 Hz and 8.1 Hz, 1H), 5.21 (s, 2H), 5.17-5.09 (m, 2H), 4.88 (d, *J* = 10.9 Hz, 1H), 4.76-4.39 (m, 13H), 4.31 (d, *J* = 12.2 Hz, 1H), 4.19 (dd, *J* = 9.5 Hz and 6.1 Hz, 1H), 4.03-3.63 (m, 9H), 3.49-3.42 (m, 3H), 3.37-3.15 (m, 4H), 1.59-1.40 (m, 4H), 1.28-1.11 (m, 5H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 165.2, 164.6, 164.5, 138.9, 138.5, 138.2, 138.0, 137.9 (2C), 137.6, 133.1, 133.0, 132.9, 131.1, 129.9, 129.8, 129.7, 129.6 (2C), 129.4, 129.2, 128.7, 128.6, 128.5, 128.4 (2C), 128.3, 128.2 (2C), 128.1 (2C), 128.0, 127.9 (2C), 127.7 (2C), 127.6 (2C), 127.3, 120.7, 100.3 (anomeric), 97.7 (anomeric), 96.9 (anomeric), 80.3, 79.1, 78.0, 77.4, 76.7, 75.6, 75.2, 74.9,

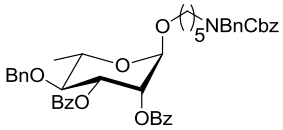
74.8, 74.5, 73.6, 73.5, 73.1, 71.9, 71.1, 69.7, 68.8, 68.3, 68.0, 67.7, 67.2, 29.0, 23.3, 17.9 (Rha CH_3); HRMS (MALDI-TOF): Calcd for $C_{101}H_{102}BrNO_{20}Na^+$ $[M+Na]^+$ 1750.6071, found 1750.5921.

5-Amino-pentanyl α-L-rhamnopyranosyl-(1→3)-β-D-glucopyranosyl-(1→4)-α-D-glucopyranoside (61)



To a solution of protected trisaccharide **67** (61 mg, 35 µmol) in THF (2 mL) NaOMe (0.5 M in MeOH, 0.5 mL) was added and stirred for 4 h. The mixture was neutralized with Amberlite IR 120 (H^+) ion exchange resin, filtered and concentrated. The crude product was dissolved in a mixture of THF (5.0 mL) MeOH (2.5 mL), H_2O (2.0 mL) and AcOH (0.5 mL). The solution was purged with Ar, then 10% Pd/C (100 mg) was added and the solution purged with H_2 for 30 min, then stirred under an H_2 atmosphere for 12 h, filtered and concentrated. Purification by RP SPE (Waters Sep-Pak®, C18) afforded **61** (15.7 mg, 27 µmol, 77%). ^{1}H-NMR (600 MHz, D_2O) δ 5.21 (s, 1H, anomeric Rha), 4.99 (d, *J* = 2.9 Hz, 1H, anomeric α-Glc), 4.61 (d, *J* = 8.0 Hz, 1H, anomeric β-Glc), 4.15-4.05 (m, 2H), 4.02-3.97 (m, 2H), 3.93-3.79 (m, 6H), 3.73-3.66 (m, 3H), 3.64-3.49 (m, 5H), 3.09 (t, *J* = 7.1 Hz, 2H), 1.81-1.71 (m, 4H, linker), 1.59-1.50 (m, 2H, linker), 1.33 (d, *J* = 6.0 Hz, 3H, Rha CH_3); ^{13}C-NMR (150 MHz, D_2O) δ 102.9 (anomeric Rha), 101.7 (anomeric β-Glc), 98.4 (anomeric α-Glc), 82.7, 79.7, 76.5, 74.5, 72.6, 72.4, 71.6, 71.1, 71.0, 70.8, 69.4, 68.6, 68.5, 61.2, 60.6, 40.0, 28.6, 27.1, 23.0, 17.1. (Rha CH_3); HRMS (MALDI-TOF): Calcd for $C_{23}H_{43}BrNO_{15}Na^+$ $[M+Na]^+$ 596.2525, found 596.2540.

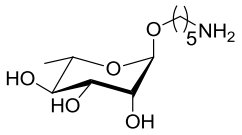
***N*-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl 2,3-di-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranoside (68)**



Rhamnosyl-imidate **31** (127 mg, 0.20 mmol) and *N*-(benzyl)benzyloxycarbonyl-5-amino-pentanol (160 mg, 0.49 mmol) were coevaporated with toluene three times, dried *in vacuo* and dissolved in anhydrous DCM (3 mL). Freshly activated molecular sieves (4 Å) were added and the mixture cooled to –30 °C. TMSOTf (3.6 µL, 20 µmol) was added and the reaction was

warmed to –20 °C over 1 h. The reaction was quenched with triethylamine and concentrated. Column chromatography on silica gel (hexanes/ethyl acetate) afforded **68** (145 mg, 0.19 mmol, 94 %). $[\alpha]_D^{20}$ = + 54.1º (c = 2.6, $CHCl_3$), IR ν_{max} (film) 2963, 1727, 1260, 1018 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.28-7.00 (m, 25H, Ar), 5.73 (app. dd, *J*=9.6, 3.4, 1H), 5.59 (s, 1H), 5.19 (d, *J* = 11.3 Hz, 2H), 4.87 (s, 1H, anomeric), 4.72 (d, *J* = 10.9 Hz, 1H), 4.65 (d, *J* = 10.9 Hz, 1H), 4.53 (s, 2H), 3.96 (s, 1H), 3.79 (t, *J* = 9.5 Hz, 1H), 3.75-3.61 (m, 1H), 3.48-3.21 (m, 3H), 1.65-1.51 (m, 4H), 1.45-1.27 (m, 5H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 165.6, 165.5, 138.1, 137.8, 133.4, 133.2, 130.0, 129.9, 129.7, 128.7, 128.6, 128.6 (2C), 128.2, 128.0 (2C), 127.4, 97.5 (anomeric), 79.3, 75.3, 72.6, 71.5, 68.0, 67.8, 67.3, 29.3, 23.6, 18.3; HRMS (MALDI-TOF): Calcd for $C_{47}H_{49}NO_9Na^+$ $[M+Na]^+$ 794.3300, found 794.3264.

5-Amino-pentanyl α-L-rhamnopyranoside (69)



To a solution of protected rhamnoside **68** (145 mg, 0.19 mmol) in THF (4 mL), NaOMe (0.5 M in MeOH, 0.5 mL) was added and stirred for 4 h. The mixture was neutralized with Amberlite IR 120 (H^+) ion exchange resin, filtered and concentrated. The crude product was dissolved in a mixture of THF (10 mL), MeOH (5 mL), H_2O (4 mL) and AcOH (1 mL). The solution was purged with Ar, then 10% Pd/C (300 mg) was added and the solution purged with H_2 for 30 min, then stirred under an H_2 atmosphere for 12 h, filtered and concentrated. Purification by RP SPE (Waters Sep-Pak®, C18) afforded **69** (44 mg, 0.18 mmol, 94%). ^{1}H-NMR (600 MHz, D_2O) δ 4.85 (s, 1H, anomeric Rha), 4.01-3.96 (m, 1H), 3.81-3.70 (m, 3H), 3.62-3.57 (m, 1H), 3.50 (t, *J* = 9.6 Hz, 1H), 3.11-3.03 (m, 2H), 1.78-1.67 (m, 4H, linker), 1.56-1.46 (m, 2H), 1.34 (d, *J* = 6.3 Hz, 3H, Rha CH_3). ^{13}C-NMR (150 MHz, D_2O) δ 98.3 (anomeric), 70.6, 70.0, 68.8, 67.1, 66.1, 38.0, 26.6, 25.1, 21.0, 15.2 (Rha CH_3); HRMS (MALDI-TOF): Calcd for $C_{11}H_{23}NO_5Na^+$ $[M+Na]^+$ 272.1468, found 272.1433.

6.4 Synthesis of PS-III Oligomers

6.4.1 Geneneral Synthetic Procedures for the Synthesis of PS-III Oligomers

General procedure (A) for removal of anomeric TBS.

Anomeric TBS-protected starting material (1.0 equiv) was dissolved in THF (reaction concentration at 0.15 M) and cooled to 0 °C. A solution of TBAF (1 M in THF, 1.2 equiv) and AcOH (1.4 equiv) was added and stirred for 1 h at 0 °C. The reaction mixture was diluted with EtOAc, washed with 0.1 N HCl, sat. aq. $NaHCO_3$ and brine. The organic layers were dried over $MgSO_4$ and concentrated. Column chromatography on silica gel (hexanes/EtOAc) afforded the corresponding lactol as a mixture of α and β anomers.

General procedure (B) for glycosyl-trichloroacetimidate synthesis.

To a solution of the lactol (1.0 equiv) in DCM (reaction concentration at 0.5 M) trichloroacetonitrile (10 equiv) and K_2CO_3 (1.7 equiv) were added and stirred for 3 h at room temperature. The crude product was concentrated and purified by column chromatography on silica gel (hexanes/EtOAc) to afford pure product as a mixture of α and β anomers.

General procedure (C) for TMSOTf-mediated glycosylation of glycosyl-imidates.

The acceptor (1.0 to 2.0 equiv) and glycosyl-trichloroacetimidate (1.0 to 1.3 equiv) were coevaporated with toluene three times and dried *in vacuo*. The residue was dissolved in DCM/Et_2O (1:1, reaction concentration at 80 to 120 mM) and freshly activated molecular sieves (4 Å) were added. The mixture was cooled to −20 °C and TMSOTf (0.1 equiv) was added. The reaction was brought to −10 °C over 1 h, then quenched by the addition of NEt_3 and concentrated under reduced pressure. Column chromatography (hexanes/EtOAc) afforded the pure product.

General procedure (D) for phosphoramidite synthesis.

The starting material (1.0 equiv) was dissolved in DCM/MeCN (1:1, reaction concentration at 25 mM) together with diisopropylammonium tetrazolide (1.0 equiv). Freshly activated molecular sieves (3 Å) were added and the mixture cooled to 0 °C. Then *N,N,N',N'*-tetraisopropylphosphordiamidite (1.2 equiv) was added and the reaction brought to room temperature over 3 h. The mixture was concentrated under reduced pressure on Isolute®

(Biotage) and purified by column chromatography on silica gel to give the phosphoramidite as a mixture of two stereoisomers.

General procedure (E) for phosphoramidite coupling, oxidation and vinyl-ether deprotection.

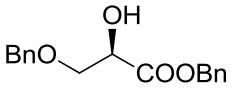
Starting alcohol (1.0 equiv) was dissolved in 5-(ethylthio)tetrazole (0.25 M in MeCN, 1.5 mL). Freshly activated molecular sieves (3 Å) and phosphoramidite (1.3 to 3.0 equiv, in 1.0 mL MeCN) were added. The reaction mixture was stirred for 1 h, then H_2O (0.5 mL) was added followed by I_2 (in 2.0 mL THF, 20 equiv) and stirred for further 3 h. The mixture was diluted with EtOAc and washed with sat. aq. $Na_2S_2O_3$, sat. aq. $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4 and concentrated. The residue was purified by SEC ($CHCl_3$/MeOH 1:1) to give the phosphotriester as a mixture of diastereomers.

General procedure (F) for final deprotection.

The phosphotriester (1.0 equiv) was dissolved in NEt_3 (1 mL), stirred for 16 h and concentrated. The residue was purified by SEC ($CHCl_3$/MeOH 1:1) to give the corresponding phosphodiester. The phosphodiester was dissolved in EtOH/H_2O/AcOH (50:50:1, reaction concentration 1 mM) and purged with Ar. After that Pd/C (10% Pd, w/w 2:1 with respect to phosphodiester) was added and the mixture purged with H_2, then transferred to a autoclave and treated with 4 bar H_2 for 20 h. The mixture was filtered, concentrated, the residue dissolved in H_2O/AcOH (100:1, reaction concentration 1 mM) and purged with Ar. After that Pd/C (10% Pd, w/w 2:1 with respect to residue) was added and the mixture purged with H_2, then transferred to a autoclave and treated with 4 bar H_2 for further 20 h. The mixture was filtered and concentrated. The crude product was dissolved in H_2O, subjected to reversed phase solid phase extraction (RP SPE) (Waters Sep-Pak®, C18) and lyophilized to give the target compound as a triethylammonium salt.

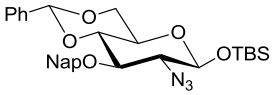
6.4.2 Synthesis of PS-III Oligomers

Benzyl (2*R*)-2-hydroxy-3-benzyloxypropanoate (77)[146,147]



To a solution of *O*-Benzyl-D-serine **80** (1.0 g, 5.1 mmol) in 2 N H_2SO_4 (12 mL) at 0 °C a solution of $NaNO_2$ (2.0 g, 29.0 mmol) was added over 2 h. After complete addition the mixture was warmed to room temperature and stirred for additional 3 h. The reaction mixture was then diluted with brine, extracted with EtOAc and the organic layer dried over Na_2SO_4 and concentrated to give the corresponding hydroxyacid. To a solution of crude hydroxyacid in DMF (20 mL) at 0 °C was added potassium carbonate (1.0 g, 7.6 mmol). Benzyl bromide (0.8 mL, 6.6 mmol) was added drop wise over 30 min, after complete addition the mixture was warmed to room temperature and let stir for 24 h. The reaction was then diluted with Et_2O and washed with sat. aq. $NaHCO_3$. The aqueous layer was extracted with Et_2O and the combined organic layers were dried over $MgSO_4$ and concentrated. Column chromatography on silica gel (hexanes/EtOAc) afforded **77** (0.92 g, 3.2 mmol, 63%). $[\alpha]_D^{20}$ = + 20.0 º (c = 1.0, $CHCl_3$). NMR data is consistent with previously reported for the (2*S*)-enantiomer.[147] HRMS (ESI): Calcd for $C_{17}H_{18}O_4Na^+$ $[M+Na]^+$ 309.1097, found 309.1114.

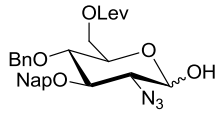
***tert*-Butyldimethylsilyl 2-azido-4,6-*O*-benzylidene-2-deoxy-3-*O*-(2-naphthalenylmethyl)-β-D-glucopyranoside (82)**



To a solution of **81**[148] (1.04 g, 2.6 mmol) in DMF (10 mL) at 0 °C, NaH (0.073 g, 3.1 mmol) was added. Naphthyl bromide (0.85 g, 3.8 mmol) was added and the reaction mixture warmed to room temperature. After stirring for 1.5 h the reaction mixture was cooled to 0 °C, quenched by the slow addition of H_2O and extracted with Et_2O. The organic layer was dried over $MgSO_4$ and concentrated. Column chromatography on silica gel (hexanes/EtOAc) afforded **82** (1.20 g, 2.2 mmol, 87%). $[\alpha]_D^{20}$ = – 83.1 º (c = 1.7, $CHCl_3$); IR ν_{max} (film) 3059, 2930, 2959, 2110, 1463, 1372, 1256, 1177, 1096 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.86 – 7.73 (m, 4H), 7.55 – 7.37 (m, 8H), 5.59 (s, 1H), 5.06 (d, *J* = 11.6 Hz, 1H), 4.97 (d, *J* = 11.6 Hz, 1H), 4.59 (d, *J* = 7.7 Hz, 1H), 4.30 (dd, *J* = 10.5, 5.0 Hz, 1H), 3.81 (t, *J* = 10.3 Hz, 1H), 3.75 (t, *J* = 9.3 Hz, 1H), 3.57 (t, *J* = 9.3 Hz, 1H), 3.44 – 3.35 (m, 2H), 0.95 (s, 9H), 0.17 (s, 3H), 0.16 (s, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 137.3, 135.5, 133.4, 133.2, 129.2, 128.4, 128.2, 128.1, 127.8, 127.1, 126.3, 126.2, 126.1, 126.0, 101.6, 97.6, 81.8, 78.7, 74.9, 68.9,

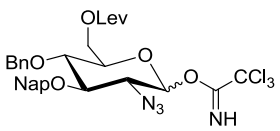
68.8, 66.5, 25.7, 18.1, -4.2, -5.1; HRMS (MALDI-TOF): Calcd for $C_{30}H_{37}N_3O_5SiNa^+$ $[M+Na]^+$ 570.2395, found 570.2343.

2-Azido-4-*O*-benzyl-2-deoxy-6-*O*-levulinoyl-3-*O*-(2-naphthalenylmethyl)-D-glucopyranoside (83)



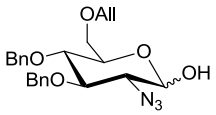
A solution of **82** (1.20 g, 2.2 mmol) in DCM (18 mL) was cooled to 0 °C and borane tetrahydrofuran complex solution (1 M, 13.2 mL, 13.2 mmol) was added slowly. After complete addition TMSOTf (0.2 mL, 1.1 mmol) was added and the reaction mixture warmed to 10 °C over 16 h. After cooling to 0 °C, the reaction was quenched by the slow addition of sat. aq. $NaHCO_3$ and extracted with DCM. The organic layer was dried over $MgSO_4$ and concentrated to give the 6-OH intermediate. The crude intermediate was dissolved in DCM (10 mL) together with DMAP (0.0027 g, 0.22 mmol) and levulinic acid (0.45 mL, 4.42 mmol). EDC (0.54 mL, 3.08 mmol) was added, the reaction mixture stirred for 1 h, concentrated and dissolved in EtOAc. The organic layer was washed with sat. aq. NH_4Cl, sat. aq. $NaHCO_3$ and brine then dried over $MgSO_4$ and concentrated. The residue was reacted according to general procedure (A) to give lactol **83** (0.983 g, 1.84 mmol, 84%) as a mixture of α and β anomers. IR ν_{max} (film) 3418, 3060, 2919, 2109, 1737, 1717, 1358, 1259, 1209, 1159, 1075 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.87 – 7.76 (m, 3.8H), 7.60 – 7.40 (m, 2.8H), 7.35 – 7.23 (m, 5.5H), 5.33 (d, *J* = 3.2 Hz, 0.6H), 5.13 – 4.94 (m, 1.5H), 4.89 (dd, *J* = 11.0, 5.7 Hz, 1.0H), 4.66 – 4.59 (m, 1.4H), 4.43 – 4.34 (m, 1.0H), 4.27 – 4.07 (m, 2.2H), 3.58 – 3.40 (m, 3.1H), 2.80 – 2.71 (m, 1.9H), 2.60 – 2.54 (m, 1.9H), 2.18 (s, 2.7H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 207.4, 207.2, 172.6 (2C), 137.7, 137.6, 135.3 (2C), 133.4, 133.2, 128.7, 128.4, 128.2, 128.1 (2C), 128.0, 127.8 (2C), 127.1, 126.3, 126.2 (2C), 126.1, 96.3, 92.1, 83.1, 80.4, 78.5, 77.6, 77.4, 75.8, 75.3, 75.2, 73.4, 69.3, 67.6, 64.2, 63.2, 63.0, 38.2, 38.0, 30.1, 30.0, 28.1, 28.0; HRMS (MALDI-TOF): Calcd for $C_{29}H_{31}N_3O_7Na^+$ $[M+Na]^+$ 556.2054, found 556.2011.

2-Azido-4-*O*-benzyl-2-deoxy-6-*O*-levulinoyl-3-*O*-(2-naphthalenylmethyl)-D-glucopyranosyl trichloroacetimidate (78)



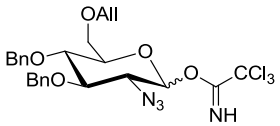
According to general procedure (B), lactol **83** (0.983 g, 1.84 mmol) was reacted with trichloroacetonitrile (1.85 mL, 18.4 mmol) and K_2CO_3 (0.43 g, 3.13 mmol) in DCM (5 mL) to afford **78** (1.25 g, 1.84 mmol, 100%) as a mixture of α and β anomers. IR ν_{max} (film) 3337, 3059, 2921, 2112, 1739, 1719, 1677, 1358, 1284, 1156, 1064 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.75 (s, 1.0H), 7.92 – 7.75 (m, 3.2H), 7.54 – 7.45 (m, 2.8H), 7.35 – 7.24 (m, 5.0H), 6.42 (d, *J* = 3.5 Hz, 0.3H), 5.64 (d, *J* = 8.3 Hz, 0.7H), 5.13 – 4.99 (m, 2.2H), 4.95 – 4.86 (m, 1.1H), 4.70 – 4.61 (m, 1.1H), 4.37 – 4.27 (m, 2.0H), 4.18 – 4.02 (m, 1.0H), 3.78 – 3.59 (m, 3.6H), 2.79 – 2.69 (m, 1.9H), 2.64 – 2.54 (m, 1.9H), 2.17 (s, 2.7H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 206.5, 172.4, 161.1, 160.8, 137.5, 137.4, 135.2, 135.1, 133.4, 133.2, 128.7 (2C), 128.5, 128.4, 128.3, 128.2, 128.1, 127.9, 127.1 (2C), 126.3 (2C), 126.2, 126.1, 96.8, 94.7, 83.1, 80.2, 77.6, 77.0, 75.9, 75.8, 75.6, 75.3, 74.1, 72.0, 65.9, 63.2, 62.6, 62.5, 38.0, 30.0 (2C), 28.0, 27.9; HRMS (MALDI-TOF): Calcd for $C_{31}H_{31}Cl_3N_4O_7Na^+$ $[M+Na]^+$ 699.1151, found 699.1136.

6-*O*-Allyl-2-azido-3,4-di-*O*-benzyl-2-deoxy-D-glucopyranoside (85)



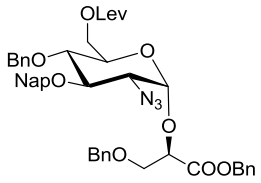
To a solution of **84**[148] (2.00 g, 3.69 mmol) in MeOH (20 mL), 0.5 M NaOMe in MeOH (1.48 mL, 0.74 mmol) was added and stirred for 1.5 h. The mixture was neutralized with Amberlite® IR 120 (H^+) ion exchange resin, filtered and concentrated. The residue was dissolved in THF (10 mL) together with TBAI (0.136 g, 0.37 mmol) and cooled to 0 °C. NaH (0.177 g, 7.38 mmol) and allyl bromide (0.64 mL, 7.38 mmol) were added and the reaction mixture warmed to room temperature. After stirring for 16 h the reaction was cooled to 0 °C and quenched by the slow addition of H_2O and extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$ and concentrated. The residue was reacted according to general procedure (A) to give lactol **85** (1.18 g, 2.77 mmol, 75%) as a mixture of α and β anomers. IR ν_{max} (film) 3416, 2918, 2872, 2103, 1454, 1359, 1269, 1131, 1086, 1045 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.42 – 7.23 (m, 9.4H), 5.90 (ddt, *J* = 17.1, 10.4, 5.8 Hz, 0.9H), 5.36 – 5.15 (m, 2.5H), 4.97 – 4.78 (m, 3.0H), 4.68 – 4.55 (m, 1.4H), 4.18 – 3.92 (m, 3.6H), 3.69 – 3.35 (m, 5.3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 138.1, 137.9 (2C), 134.4, 134.3, 128.6 (3C), 128.3, 128.2, 128.1 (2C), 128.0, 127.9, 118.0 (2C), 96.3, 92.2, 83.2, 80.2, 78.7, 77.9, 75.7 (2C), 75.2 (2C), 74.9, 72.6 (2C), 70.6, 68.8, 68.7, 67.6, 64.1; HRMS (MALDI-TOF): Calcd for $C_{23}H_{27}N_3O_5Na^+$ $[M+Na]^+$ 448.1843, found 448.1825.

6-*O*-Allyl-2-azido-3,4-di-*O*-benzyl-2-deoxy-D-glucopyranosyl trichloroacetimidate (79)



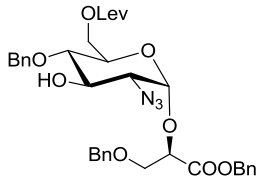
According to general procedure (B), lactol **85** (0.68 mg, 1.60 mmol) was reacted with trichloroacetonitrile (1.60 mL, 15.98 mmol) and K_2CO_3 (0.38 g, 2.72 mmol) in DCM (3 mL) to afford **79** (0.75 g, 1.31 mmol, 82%) as a mixture of α and β anomers. IR ν_{max} (film) 3342, 2870, 2111, 1675, 1455, 1357, 1285, 1058 cm^{-1}; NMR data reported for the β anomer: ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.73 (s, 1H), 7.40 – 7.28 (m, 10H), 5.89 (ddt, *J* = 17.2, 10.4, 5.6 Hz, 1H), 5.61 (d, *J* = 8.4 Hz, 1H), 5.27 (ddd, *J* = 17.2, 3.3, 1.6 Hz, 1H), 5.17 (ddd, *J* = 10.4, 3.0, 1.3 Hz, 1H), 4.95 – 4.84 (m, 3H), 4.71 – 4.67 (m, 1H), 4.09 – 3.96 (m, 2H), 3.81 – 3.66 (m, 4H), 3.60 – 3.52 (m, 2H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 161.2, 138.0, 137.9, 134.7, 128.6 (2C), 128.2, 128.1 (2C), 128.0, 117.4, 97.0, 83.2, 77.3, 76.2, 75.8, 75.2, 72.6, 68.1, 66.0; HRMS (MALDI-TOF): Calcd for $C_{25}H_{27}Cl_3N_4O_5Na^+$ $[M+Na]^+$ 591.0939, found 591.0918.

2-Azido-4-*O*-benzyl-2-deoxy-6-*O*-levulinoyl-3-*O*-(2-naphthalenylmethyl)-α-D-glucopyranosyl-(1→2)-benzyl (2*R*)-3-benzyloxypropanoate (86)



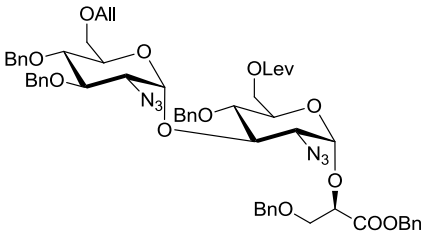
According to general procedure (C), glycosyl-imidate **78** (0.50 g, 0.74 mmol) and **77** (0.42 g, 1.48 mmol) were dissolved in DCM/Et_2O (1:1, 6 mL) and reacted to give **86** (0.48 g, 0.60 mmol, 81%). $[\alpha]_D^{20}$ = + 57.2 º (c = 3.2, $CHCl_3$); IR ν_{max} (film) 3032, 2920, 2106, 1737, 1719, 1455, 1361, 1261, 1208, 1155, 1100, 1028 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.86 – 7.75 (m, 4H), 7.54 – 7.44 (m, 3H), 7.38 – 7.23 (m, 15H), 5.26 (d, *J* = 12.1 Hz, 1H), 5.19 (d, *J* = 12.1 Hz, 1H), 5.14 – 5.06 (m, 2H), 5.02 (d, *J* = 10.8 Hz, 1H), 4.89 (d, *J* = 10.9 Hz, 1H), 4.63 – 4.49 (m, 4H), 4.23 – 4.09 (m, 4H), 3.89 – 3.78 (m, 2H), 3.64 – 3.55 (m, 1H), 3.45 (dd, *J* = 10.3, 3.6 Hz, 1H), 2.79 – 2.64 (m, 2H), 2.58 – 2.47 (m, 2H), 2.15 (s, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 206.4, 172.5, 169.2, 137.9, 137.6, 135.4, 135.2, 133.4, 133.2, 128.7, 128.6 (3C), 128.5, 128.4, 128.1, 128.0 (2C), 127.9 (2C), 127.8, 127.0, 126.2, 126.1 (2C), 96.7, 80.3, 78.0, 75.7, 75.2, 74.4, 73.5, 70.1, 69.7, 67.4, 63.3, 62.7, 37.9, 29.9, 27.9; HRMS (MALDI-TOF): Calcd for $C_{46}H_{47}N_3O_{10}Na^+$ $[M+Na]^+$ 824.3154, found 824.3115.

2-Azido-4-*O*-benzyl-2-deoxy-6-*O*-levulinoyl-α-D-glucopyranosyl-(1→2)-benzyl (2*R*)-3-benzyloxypropanoate (87)



To a mixture of **86** (0.48 g, 0.60 mmol) in DCM (20 mL) and phosphate-buffer (7 mM, pH 7.4, 2 mL) at 0 °C DDQ (0.41 g, 1.80 mmol) was added portion wise over 1 h. The reaction mixture was warmed to room temperature and stirred for further 30 min. The mixture was diluted with sat. aq. $NaHCO_3$ solution, extracted with DCM and the organic layer dried over $MgSO_4$ and concentrated. Column chromatography on silica gel (hexanes/EtOAc) gave **87** (0.32 g, 0.48 mmol, 80%). $[\alpha]_D^{20}$ = + 2.0 º (c = 3.7, $CHCl_3$); IR ν_{max} (film) 3473, 2918, 2108, 1737, 1718, 1455, 1362, 1264, 1208, 1143, 1101, 1027 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.41 – 7.29 (m, 10H), 7.28 – 7.23 (m, 5H), 5.24 (d, *J* = 12.2 Hz, 1H), 5.18 (d, *J* = 12.2 Hz, 1H), 5.10 (d, *J* = 3.6 Hz, 1H), 4.79 (d, *J* = 11.3 Hz, 1H), 4.67 (d, *J* = 11.3 Hz, 1H), 4.56 – 4.51 (m, 2H), 4.48 (d, *J* = 11.7 Hz, 1H), 4.21 – 4.07 (m, 4H), 3.86 – 3.75 (m, 2H), 3.45 (dd, *J* = 10.0, 8.7 Hz, 1H), 3.25 (dd, *J* = 10.4, 3.6 Hz, 1H), 2.80 – 2.68 (m, 2H), 2.58 – 2.50 (m, 2H), 2.16 (s, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 206.5, 172.5, 169.1, 138.0, 137.6, 135.2, 128.7, 128.6, 128.5 (2C), 128.2 (2C), 127.9, 127.8, 96.6, 78.0, 75.0, 74.4, 73.5, 72.0, 70.2, 69.3, 67.3, 62.9, 62.8, 37.9, 29.9, 27.9; HRMS (MALDI-TOF): Calcd for $C_{35}H_{39}N_3O_{10}Na^+$ $[M+Na]^+$ 684.2528, found 684.2564.

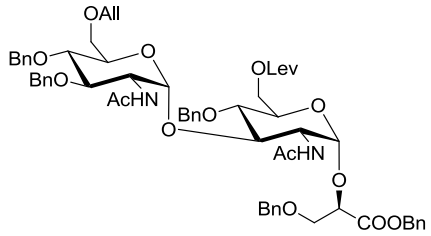
6-*O*-Allyl-2-azido-3,4-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl-(1→3)-2-azido-4-*O*-benzyl-2-deoxy-6-*O*-levulinoyl-α-D-glucopyranosyl-(1→2)-benzyl (2*R*)-3-benzyloxypropanoate (88)



According to general procedure (C), glycosyl-imidate **79** (0.36 g, 0.64 mmol) and **87** (0.32 g, 0.48 mmol) were dissolved in DCM/Et_2O (1:1, 6 mL) and reacted to give **88** (0.35 g, 0.33 mmol, 69%). $[\alpha]_D^{20}$ = + 92.4 º (c = 2.5, $CHCl_3$); IR ν_{max} (film) 3033, 2920, 2108, 1740, 1720, 1498, 1455, 1361, 1263, 1210, 1136, 1045 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.39 – 7.21 (m, 25H), 5.90 (ddt, *J* = 17.2, 10.4, 5.8 Hz, 1H), 5.57 (d, *J* = 3.8 Hz, 1H), 5.30 – 5.13 (m, 5H),

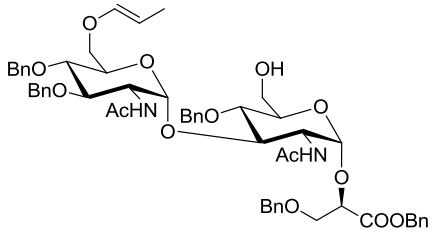
5.03 (d, *J* = 10.7 Hz, 1H), 4.95 (d, *J* = 10.7 Hz, 1H), 4.91 – 4.85 (m, 2H), 4.71 (d, *J* = 11.0 Hz, 1H), 4.59 – 4.50 (m, 4H), 4.28 – 4.12 (m, 4H), 4.09 – 4.01 (m, 3H), 3.99 – 3.90 (m, 1H), 3.90 – 3.85 (m, 2H), 3.84 – 3.72 (m, 3H), 3.66 (dd, *J* = 10.0, 8.8 Hz, 1H), 3.43 (dd, *J* = 10.3, 3.8 Hz, 1H), 3.15 (dd, *J* = 10.5, 3.7 Hz, 1H), 2.83 – 2.69 (m, 2H), 2.63 – 2.52 (m, 2H), 2.17 (s, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 206.3, 172.5, 169.0, 138.4, 138.0, 137.7, 137.6, 135.2, 134.5, 128.8, 128.7, 128.6 (3C), 128.5, 128.1, 127.9 (3C), 127.8 (3C), 127.4, 117.6, 98.6, 97.0, 80.2, 79.2, 78.2, 75.6, 75.2, 75.1 (2C), 74.3 (2C), 73.6, 72.6, 71.7, 70.2, 69.2, 68.2, 67.4, 63.5, 62.5, 61.5, 38.0, 29.9, 27.9; HRMS (MALDI-TOF): Calcd for $C_{58}H_{64}N_6O_{14}Na^+$ $[M+Na]^+$ 1091.4373, found 1091.4378.

2-*N*-Acetyl-6-*O*-allyl-3,4-di-*O*-benzyl-α-D-glucosaminopyranosyl-(1→3)-2-*N*-acetyl-4-*O*-benzyl-6-*O*-levulinoyl-α-D-glucosaminopyranosyl-(1→2)-benzyl (2*R*)-3-benzyloxypropanoate (89)



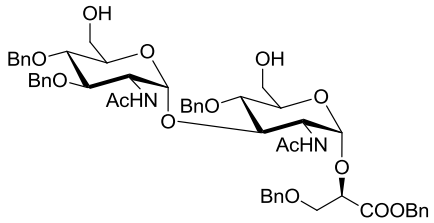
To a solution of **88** (100 mg, 94 µmol) in pyridine (2 mL) thioacetic acid (0.8 mL) was added and stirred for 24 h. The reaction mixture was concentrated and purified by column chromatography on silica gel (hexanes/acetone) to afford **89** (69 mg, 63 µmol, 67%). $[\alpha]_D^{20}$ = + 70.0 º (c = 1.9, $CHCl_3$); IR ν_{max} (film) 3311, 3032, 2918, 2870, 1739, 1719, 1668, 1530, 1455, 1365, 1209, 1123, 1059 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.29 (dd, *J* = 16.9, 8.7 Hz, 25H), 6.75 (d, *J* = 9.5 Hz, 1H), 6.12 (d, *J* = 9.9 Hz, 1H), 5.86 (ddd, *J* = 22.8, 10.8, 5.6 Hz, 1H), 5.34 (d, *J* = 3.8 Hz, 1H), 5.27 – 5.19 (m, 3H), 5.13 – 5.07 (m, 1H), 4.88 – 4.80 (m, 3H), 4.69 – 4.59 (m, 3H), 4.53 – 4.38 (m, 5H), 4.28 (td, *J* = 10.1, 3.5 Hz, 1H), 4.16 – 3.96 (m, 7H), 3.80 – 3.52 (m, 7H), 2.86 – 2.71 (m, 2H), 2.65 – 2.51 (m, 2H), 2.19 (s, 3H), 2.02 (s, 3H), 1.78 (s, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 206.5, 172.4, 171.2, 169.9, 169.8, 138.7, 138.5, 137.4, 136.8, 135.1, 134.6, 128.8 (2C), 128.6, 128.5, 128.4 (2C), 128.3, 128.2, 128.0, 127.9, 127.8, 127.7, 127.6, 117.4, 98.1, 98.0, 81.2, 79.1, 78.3, 75.3, 75.2, 74.9, 74.8, 74.6, 73.4, 72.6, 71.5, 70.2, 70.1, 69.1, 67.4, 62.3, 52.5, 51.9, 38.0, 30.0, 28.0, 23.4 (2C); HRMS (MALDI-TOF): Calcd for $C_{62}H_{72}N_2O_{16}Na^+$ $[M+Na]^+$ 1123.4774, found 1123.4747.

2-*N*-Acetyl-3,4-di-*O*-benzyl-6-*O*-prop-1-en-α-D-glucosaminopyranosyl-(1→3)-2-*N*-acetyl-4-*O*-benzyl-α-D-glucosaminopyranosyl-(1→2)-benzyl (2*R*)-3-benzyloxypropanoate (74)



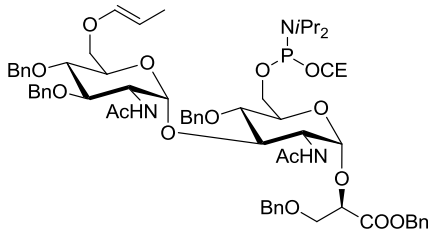
A solution of (1,5-Cyclooctadiene)bis(methyldiphenylphosphine)iridium(I) hexafluorophosphate (5.3 mg, 6.3 µmol) in degassed THF (0.5 mL) was purged with H_2 for 15 min and added to a solution of **89** (69 mg, 63 µmol) in THF (2 mL). After stirring for 30 min, sat. aq. $NaHCO_3$ solution was added and the mixture extracted with EtOAc. The organic layer was dried over $MgSO_4$ and concentrated to give the corresponding vinyl ether. To a solution of crude vinyl in DCM (2 mL), hydrazine hydrate (6 µL, 123 µmol) dissolved in AcOH (100 µL) and pyridine (150 µL) was added and the solution stirred for 25 min. Sat. aq. $NaHCO_3$ solution was then added and the mixture extracted with DCM. The organic layer was dried over $MgSO_4$ and concentrated. Column chromatography on silica gel (DCM/MeOH/acetone) afforded **74** (49 mg, 49 µmol, 78%). $[\alpha]_D^{20}$ = + 94.1 ° (c = 1.3, $CHCl_3$); IR ν_{max} (film) 3329, 3064, 3032, 2925, 1744, 1667, 1525, 1455, 1366, 1312, 1124, 1063 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.39 – 7.14 (m, 25H), 6.76 (d, *J* = 9.9 Hz, 1H), 6.69 (d, *J* = 9.1 Hz, 1H), 6.17 (dd, *J* = 12.6, 1.5 Hz, 1H), 5.32 (d, *J* = 3.7 Hz, 1H), 5.24 (d, *J* = 12.2 Hz, 1H), 5.16 (d, *J* = 12.2 Hz, 1H), 4.85 – 4.78 (m, 3H), 4.76 – 4.61 (m, 4H), 4.57 – 4.47 (m, 4H), 4.34 (td, *J* = 10.2, 3.7 Hz, 1H), 4.30 – 4.21 (m, 1H), 4.18 – 4.10 (m, 1H), 3.95 (dd, *J* = 10.0, 4.3 Hz, 1H), 3.90 – 3.65 (m, 8H), 3.60 – 3.46 (m, 2H), 1.98 (s, 3H), 1.61 (s, 3H), 1.47 (dd, *J* = 6.7, 1.5 Hz, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 171.4, 170.4, 169.8, 146.7, 138.8, 138.5, 137.5, 137.0, 135.1, 128.9, 128.8, 128.6, 128.5, 128.4, 128.3, 128.2, 128.0 (2C), 127.9, 127.8, 127.7, 127.6, 99.0, 98.4, 98.0, 81.4, 78.8, 78.0, 77.4, 75.6, 75.4, 74.8, 74.5, 73.5, 72.8, 70.8, 70.3, 67.4, 60.5, 52.5 (2C), 23.5, 23.3, 12.6; HRMS (MALDI-TOF): Calcd for $C_{57}H_{66}N_2O_{14}Na^+$ $[M+Na]^+$ 1025.4406, found 1025.4404.

2-*N*-Acetyl-3,4-di-*O*-benzyl-α-D-glucosaminopyranosyl-(1→3)-2-*N*-acetyl-4-*O*-benzyl-α-D-glucosaminopyranosyl-(1→2)-benzyl (2*R*)-3-benzyloxypropanoate (90)



To a solution of **74** (42 mg, 42 µmol) in THF (4 mL) and H_2O (1 mL), I_2 (106 mg, 0.42 mmol) was added and stirred for 3 h. The mixture was diluted with EtOAc and washed with sat. aq. $Na_2S_2O_3$, sat. aq. $NaHCO_3$ and brine. The organic layer was dried over $MgSO_4$ and concentrated. The residue was purified by column chromatography on silica gel (DCM/MeOH/acetone) to afford **90** (36 mg, 37 µmol, 89%). $[\alpha]_D^{20}$ = + 75.7 ° (c = 1.2, $CHCl_3$); IR ν_{max} (film) 3324, 3031, 2925, 1741, 1666, 1534, 1454, 1366, 1311, 1123, 1062, 1028 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.36 – 7.15 (m, 25H), 6.80 (dd, *J* = 14.8, 9.6 Hz, 2H), 5.27 – 5.11 (m, 3H), 4.85 – 4.74 (m, 4H), 4.67 (d, *J* = 10.6 Hz, 1H), 4.61 – 4.53 (m, 2H), 4.52 – 4.44 (m, 3H), 4.29 (ddd, *J* = 13.7, 9.6, 3.6 Hz, 2H), 4.07 – 3.98 (m, 1H), 3.91 – 3.67 (m, 10H), 3.48 – 3.40 (m, 1H), 1.97 (s, 3H), 1.57 (s, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 171.8, 170.4, 170.0, 138.8, 138.3, 137.5, 136.9, 135.0, 128.9, 128.8 (2C), 128.7, 128.6, 128.5, 128.4, 128.3, 128.0, 127.9 (2C), 127.8 (3C), 127.6 (2C), 98.5, 98.2, 81.3, 78.3, 75.7, 75.3, 74.9, 74.8, 73.4, 72.8, 70.3, 67.6, 62.5, 60.6, 53.0, 52.7, 23.5, 23.1; HRMS (MALDI-TOF): Calcd for $C_{54}H_{62}N_2O_{14}Na^+$ $[M+Na]^+$ 985.4093, found 985.4077.

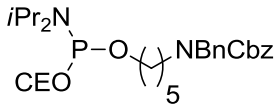
2-*N*-Acetyl-3,4-di-*O*-benzyl-6-*O*-prop-1-en-α-D-glucosaminopyranosyl-(1→3)-2-*N*-acetyl-4-*O*-benzyl-6-((*N*,*N*,-diisopropylamino)-2-cyanoethylphosphite)-α-D-glucosaminopyranosyl-(1→2)-benzyl (2*R*)-3-benzyloxypropanoate (75)



According to general procedure (D), **74** (50 mg, 50 µmol) was reacted with *N,N,N',N'*-tetraisopropylphosphordiamidite (18 mg, 60 µmol) in DCM/MeCN (2 mL). Column chromatography on silica gel (hexanes/acetone + 1% NEt_3) gave **75** (49 mg, 41 µmol, 82%) as a mixture of two diastereomers. ^{1}H-NMR (400 MHz, acetone-d_6) δ 7.33 (s, 25H), 6.74 (dd, *J* = 9.1, 3.3 Hz, 1H), 6.27 (dd, *J* = 12.6, 1.6 Hz, 1H), 5.40 (dd, *J* = 9.2, 3.8 Hz, 1H), 5.24 (qd,

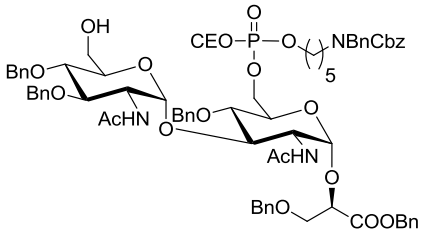
J = 12.5, 1.3 Hz, 2H), 4.90 – 4.73 (m, 7H), 4.68 – 4.51 (m, 4H), 4.32 (ddt, *J* = 14.3, 9.8, 4.2 Hz, 1H), 4.24 – 4.07 (m, 3H), 3.56 – 3.47 (m, 14H), 3.56 – 3.47 (m, 1H), 2.78 – 2.72 (m, 1H), 2.70 (t, *J* = 6.0 Hz, 1H), 1.94 – 1.86 (m, 6H), 1.46 (ddd, *J* = 6.7, 1.5, 0.8 Hz, 3H), 1.24 – 1.12 (m, 12H); ^{13}C-NMR (100 MHz, acetone-d_6) δ 170.6 (2C), 170.2 (2C), 170.0, 169.9, 147.8, 140.1, 139.7, 139.1 (2C), 139.0 (2C), 136.9, 129.4, 129.2 (2C), 129.1 (2C), 129.0 (2C), 128.9, 128.8, 128.6, 128.4 (3C), 128.3, 128.1, 99.2, 98.5, 82.0, 80.2, 79.3, 75.8 (2C), 75.5, 75.2, 74.6, 73.7, 71.2, 69.4, 67.4, 62.9, 59.7 (2C), 59.6, 59.5, 53.6 (2C), 52.7, 52.6, 44.0 (2C), 43.9, 43.8, 25.1 (2C), 25.0, 24.9 (3C), 24.8, 23.3, 23.2 (2C), 20.9, 20.8 (2C), 12.7; ^{31}P-NMR (162 MHz, acetone-d_6) δ 148.5, 148.3 (1P); HRMS (MALDI-TOF): Calcd for $C_{66}H_{83}N_4O_{15}PNa^+$ $[M+Na]^+$ 1225.5485, found 1225.5475.

_N_-Benzyl benzyl(5-((_N,N,_-diisopropylamino)-2-cyanoethylphosphite)pentyl)carbamate (76)



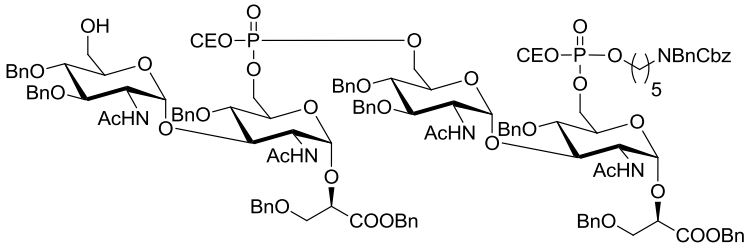
According to general procedure (D), *N*-benzyl benzyl(5-hydroxypentyl)carbamate (30 mg, 92 µmol) was reacted with *N,N,N',N'*-tetraisopropylphosphordiamidite (33 mg, 110 µmol) in DCM/MeCN (2 mL). Column chromatography on silica gel (hexanes/EtOAc + 1% NEt_3) gave **76** (42 mg, 80 µmol, 87%). IR ν_{max} (film) 2966, 2933, 2869, 1697, 1455, 1421, 1364, 1225, 1184, 1127, 1072, 1027, 975 cm^{-1}; ^{1}H-NMR (400 MHz, acetone-d_6) δ 7.56 – 7.15 (m, 10H), 5.17 (s, 2H), 4.54 (s, 2H), 3.89 – 3.75 (m, 2H), 3.63 (ddq, *J* = 13.4, 9.9, 6.7 Hz, 4H), 3.34 – 3.20 (m, 2H), 2.72 (t, *J* = 6.1 Hz, 2H), 1.67 – 1.50 (m, 4H), 1.44 – 1.29 (m, 2H), 1.18 (t, *J* = 6.5 Hz, 12H); ^{13}C-NMR (100 MHz, acetone-d_6) δ 139.5, 129.3, 129.2, 128.6 (2C), 128.0, 67.4, 64.1, 64.0, 59.5, 59.3, 43.8, 43.7, 31.7, 31.6, 25.0, 24.9 (3C), 23.9, 20.8 (2C); ^{31}P-NMR (162 MHz, acetone-d_6) δ 147.1 (1P); HRMS (ESI): Calcd for $C_{29}H_{42}N_3O_4PNa^+$ $[M+Na]^+$ 550.2805, found 550.2817.

Protected monomer: 2-*N*-Acetyl-3,4-di-*O*-benzyl-α-D-glucosaminopyranosyl-(1→3)-2-*N*-acetyl-4-*O*-benzyl-6-(5-(benzyl((benzyloxy)carbonyl)amino)pentyl (2-cyanoethyl) phosphoryl)-α-D-glucosaminopyranosyl-(1→2)-benzyl (2*R*)-3-benzyloxypropanoate (72)



According to general procedure (E), **74** (19 mg, 19 µmol) was reacted with **76** (30 mg, 57 µmol) in the presence of 5-(ethylthio)tetrazole (0.25 M in MeCN, 1.5 mL, 0.38 mmol). Oxidation with I_2 (in 2.0 mL THF, 96 mg, 0.38 mmol) and purification by SEC afforded **72** (26 mg, 18 µmol, 98%) as a mixture of diastereomers. IR ν_{max} (film) 3324, 2929, 1695, 1671, 1531, 1455, 1422, 1367, 1260, 1125, 1068, 1029 cm^{-1}; ^{1}H-NMR (400 MHz, acetone-d_6) δ 7.45 – 7.19 (m, 35H), 6.98 (d, *J* = 9.4 Hz, 1H), 5.34 (d, *J* = 3.7 Hz, 1H), 5.24 (d, *J* = 12.4 Hz, 1H), 5.21 – 5.12 (m, 3H), 4.94 (d, *J* = 3.5 Hz, 1H), 4.87 – 4.80 (m, 2H), 4.79 – 4.62 (m, 5H), 4.59 (d, *J* = 11.9 Hz, 1H), 4.52 (d, *J* = 9.0 Hz, 3H), 4.34 – 4.16 (m, 6H), 4.14 – 4.00 (m, 4H), 3.90 – 3.63 (m, 6H), 3.52 (t, *J* = 9.4 Hz, 1H), 3.31 – 3.17 (m, 2H), 2.91 – 2.80 (m, 4H), 1.90 (s, 3H), 1.84 (s, 3H), 1.71 – 1.49 (m, 4H), 1.42 – 1.24 (m, 3H); ^{13}C-NMR (100 MHz, acetone-d_6) δ 170.6, 170.4, 170.1, 170.0, 140.2, 139.9, 139.4, 139.1, 138.7, 138.6, 138.3, 136.8, 129.4, 129.3, 129.2, 129.1 (3C), 129.0 (2C), 128.9, 128.8, 128.6, 128.5, 128.4 (3C), 128.2, 128.1, 128.0, 118.2, 98.9, 98.4, 82.0, 79.5, 79.4, 76.0, 75.8 (2C), 75.4, 75.1, 74.9, 73.7, 73.4 (2C), 71.2, 71.1, 68.7, 67.5, 66.6, 63.3, 63.2 (2C), 63.1, 62.3, 53.7, 52.7, 50.8, 46.8, 30.6 (2C), 28.0, 23.4, 23.3, 23.2, 20.1, 20.0; ^{31}P-NMR (162 MHz, acetone-d_6) δ –1.4; HRMS (MALDI-TOF): Calcd for $C_{77}H_{89}N_4O_{19}PNa^+$ $[M+Na]^+$ 1427.5751, found 1427.5717.

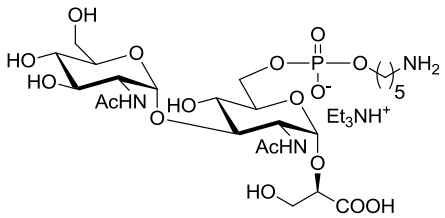
Protected dimer (73)



According to general procedure (E), **72** (26 mg, 18 µmol) was reacted with **75** (30 mg, 25 µmol) in the presence of 5-(ethylthio)tetrazole (0.25 M in MeCN, 1.5 mL, 0.38 mmol). Oxidation with I_2 (in 2.0 mL THF, 96 mg, 0.38 mmol) and purification by SEC afforded **73** (36 mg, 14 µmol, 78%) as a mixture of diastereomers. IR ν_{max} (film) 3328, 3033, 2923, 1745,

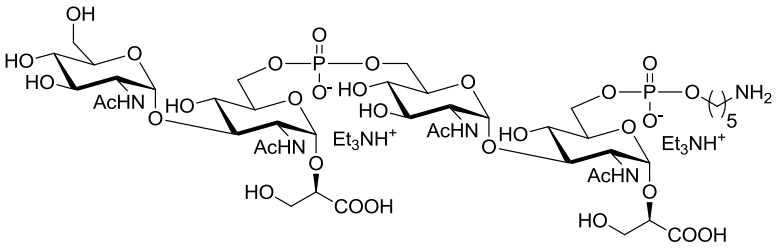
1672, 1525, 1455, 1367, 1280, 1126, 1028 cm^{-1}; ^{1}H-NMR (400 MHz, acetone-d_6) δ 7.43 – 7.17 (m, 60H), 6.93 (t, *J* = 9.9 Hz, 1H), 5.46 – 5.37 (m, 1H), 5.32 (d, *J* = 3.6 Hz, 1H), 5.26 – 5.10 (m, 6H), 5.00 – 4.92 (m, 1H), 4.90 – 4.61 (m, 14H), 4.60 – 4.43 (m, 8H), 4.31 – 4.20 (m, 9H), 4.16 – 3.96 (m, 10H), 3.92 – 3.72 (m, 10H), 3.71 – 3.61 (m, 2H), 3.55 – 3.48 (m, 1H), 3.29 – 3.17 (m, 2H), 2.87 – 2.80 (m, 6H), 2.77 – 2.67 (m, 2H), 1.94 – 1.75 (m, 12H), 1.69 – 1.46 (m, 4H), 1.39 – 1.22 (m, 2H); ^{13}C-NMR (100 MHz, acetone-d_6) δ 170.5, 170.1, 140.2 (2C), 140.1, 140.0 (3C), 139.7 (3C), 139.5 (2C), 139.1, 139.0 (2C), 138.6 (3C), 138.3, 136.9 (2C), 136.8 (2C), 129.4 (2C), 129.3 (3C), 129.2 (2C), 129.1 (4C), 129.0 (3C), 128.9 (4C), 128.6 (3C), 128.5 (3C), 128.4, 128.3, 128.2, 128.0, 118.3, 118.2, 118.1, 110.9, 98.8 (2C), 98.7, 98.5, 82.0, 81.4 (2C), 79.8 (2C), 79.6 (2C), 79.4, 78.5, 78.4, 76.2, 76.1, 75.9, 75.7, 75.6, 75.5, 75.3, 75.2, 75.1, 75.0, 74.7, 73.7 (2C), 73.4 (2C), 71.4, 71.2 (2C), 71.1, 71.0, 70.9, 67.5 (4C), 67.4, 63.5 (2C), 63.3, 63.2, 63.1, 62.3, 62.2, 53.9, 53.7 (3C), 52.7 (2C), 52.5, 50.8, 47.6, 30.7, 30.6, 30.3, 30.1, 29.9, 29.7, 28.2, 23.5, 23.4 (2C), 23.2 (2C), 20.1, 20.0 (2C), 19.9; ^{31}P-NMR (162 MHz, acetone-d_6) δ –1.5 (2P), –1.6 (2P); HRMS (MALDI-TOF): Calcd for $C_{134}H_{153}N_7O_{35}P_2Na^+$ $[M+Na]^+$ 2504.9775, found 2504.9773.

Deprotected monomer: 2-*N*-Acetyl-α-D-glucosaminopyranosyl-(1→3)-2-*N*-acetyl-6-(5-aminopentyl phosphoryl)-α-D-glucosaminopyranosyl-(1→2)-(2*R*)-3-hydroxypropanoate (70)



According to general procedure (F), protected monomer **72** (6 mg, 4.3 µmol) was first treated with NEt_3, purified by SEC and then subjected to Pd/C-catalyzed hydrogenolysis. Purification by RP SPE gave **70** (2.1 mg, 2.7 µmol, 63%) as a triethylammonium salt. ^{1}H-NMR (600 MHz, D_2O) δ 5.39 (d, *J* = 3.7 Hz, 1H), 4.94 (d, *J* = 3.6 Hz, 1H), 4.31 (dd, *J* = 4.6, 3.7 Hz, 1H), 4.13 – 4.07 (m, 3H), 4.03 – 3.98 (m, 1H), 3.96 – 3.89 (m, 6H), 3.87 – 3.80 (m, 3H), 3.69 – 3.62 (m, 2H), 3.58 – 3.51 (m, 1H), 3.03 (t, *J* = 7.5 Hz, 2H), 2.08 (s, 3H), 2.07 (s, 3H), 1.76 – 1.67 (m, 4H), 1.49 (dt, *J* = 15.3, 7.7 Hz, 2H); ^{13}C-NMR (150 MHz, D_2O) δ 177.0, 176.9, 176.4, 100.3, 99.1, 80.3, 78.8, 74.9, 74.0, 73.4, 73.1, 72.0, 68.5, 66.4, 65.4, 62.7, 56.2, 54.6, 42.0, 31.7, 28.9, 24.8, 24.6 (2C); ^{31}P-NMR (243 MHz, D_2O) δ -2.0; HRMS (MALDI-TOF): Calcd for $C_{24}H_{44}N_3O_{17}PNa^+$ $[M+Na]^+$ 700.2301, found 700.2300.

Deprotected dimer (71)



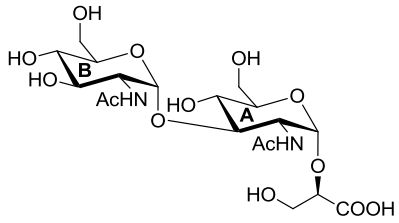
According to general procedure (F), protected dimer **73** (19 mg, 7.7 µmol) was first treated with NEt_3, purified by SEC and then subjected to Pd/C-catalyzed hydrogenolysis. Purification by RP SPE gave **71** (8.0 mg, 5.5 µmol, 72%) as a triethylammonium salt. ^{1}H-NMR (600 MHz, D_2O) δ 5.38 – 5.33 (m, 2H), 5.03 – 4.99 (m, 2H), 4.46 (dd, *J* = 6.5, 3.1 Hz, 2H), 4.22 – 4.08 (m, 8H), 4.02 – 3.83 (m, 15H), 3.82 – 3.77 (m, 1H), 3.75 – 3.71 (m, 1H), 3.70 – 3.61 (m, 4H), 3.56 – 3.52 (m, 1H), 3.03 (t, *J* = 7.5 Hz, 2H), 2.12 – 2.04 (m, 12H), 1.75 – 1.66 (m, 4H), 1.53 – 1.45 (m, 2H); ^{13}C-NMR (150 MHz, D_2O) δ 177.0 (2C), 176.9 (2C), 176.3 (2C), 100.6, 100.5, 99.2, 98.9, 79.3, 79.0, 78.3, 78.2, 74.9, 74.1 (2C), 74.0 (2C), 73.9, 73.4, 73.2, 73.0, 72.8, 72.0, 71.7, 68.6, 68.5, 66.7, 66.5, 65.1, 62.8, 56.3, 56.2, 54.6, 42.0, 31.8, 31.7, 28.9, 24.9, 24.8, 24.7, 24.6; ^{31}P-NMR (243 MHz, D_2O) δ -2.0; HRMS (MALDI-TOF): Calcd for $C_{43}H_{75}N_5O_{33}P_2Na^+$ $[M+Na]^+$ 1274.3712, found 1274.3764.

Table 4: ^{1}H NMR δ (600 MHz, D_2O) and ^{13}C NMR δ (150 MHz, D_2O) of dimer **71**.[a]

	α-GlcNAc **(A)**	*α-GlcNAc* **(B)**	*GroA*
H-1	5.01	5.36	
	4.98	*5.33*	
C-1	98.9, 99.1	100.5, 100.6	
	97.6	*99.3*	
H-2	4.10	3.93	4.46
	4.10	*3.94*	*4.41*
C-2	54.6	56.2, 56.3	78.3, 78.2
	53.1	*54.7*	*77.2*
H-3	3.97	3.65	3.92
	3.95	*3.66*	*3.92/3.96*
C-3	79.0, 79.3	74.9	68.5, 68.6
	78.0	*71.7*	*63.7*
H-4	3.72	3.54	
	3.83	*3.63*	
C-4	73.9, 74.0	72.0	
	71.3	*70.1*	
H-5	3.80	3.67	
	3.90	*3.71*	
C-5	73.0, 73.2	73.2	
	72.5	*72.5*	
H-6 a/b	4.10/4.15	3.96/3.98	
	4.12/4.17	*4.09/4.20*	
C-6	65.1	66.5	
	64.9	*65.2*	

[a] data of native LTA polymer[130] reported in italic.

2-*N*-Acetyl-α-D-glucosaminopyranosyl-(1→3)-2-*N*-acetyl-α-D-glucosaminopyranosyl-(1→2)-(2*R*)-3-hydroxypropanoate (91)



The protected repeating unit **90** (12 mg, 12 μmol) was dissolved in a mixture of EtOH (2 mL), H_2O (2 mL) and AcOH (15 μL). The mixture was purged with Ar. Pd/C (10% Pd, 20 mg) was added and the mixture purged with H_2 and stirred for 16 h under H_2 atmosphere. The mixture was filtered and concentrated. The crude product was dissolved in H_2O and subjected to reversed phase solid phase extraction (RP SPE). Further purification by SEC (MeOH) gave **91** (5.0 mg, 9.8 μmol, 78%). HRMS (MALDI-TOF): Calcd for $C_{19}H_{32}N_2O_{14}Na^+$ $[M+Na]^+$ 535.1746, found 535.1774.

Table 5: ^1H NMR δ (600 MHz, D_2O) and ^{13}C NMR δ (150 MHz, D_2O) of repeating unit **91**.[a]

	α-GlcNAc **(A)**	*α-GlcNAc* **(B)**	*GroA*
H-1	5.01	5.37	
C-1	99.0	100.3	176.4
H-2	4.07	3.92	4.46
C-2	54.6	56.3	78.1
H-3	3.97	3.66	3.95/4.01
C-3	79.0	74.8	65.1
H-4	3.73	3.53	
C-4	73.4	72.0	
H-5	3.79	3.68	
C-5	75.1	73.3	
H-6 a/b	3.79/3.86	3.79/3.86	
C-6	62.8	62.8	
NAc	2.07/2.06; 24.8/24.6; 177 ppm		

[a] ^1H and ^{13}C NMR resonances were assigned based on HSQC and COSY experiments.

6.4.3

6.4.4 Glycan Microarray Preparation and Screening

Preparation of microarrays

Oligosaccharides bearing an amine linker or proteins in coupling buffer (100 mM sodium phosphate, pH 8.5) were immobilized on CodeLink *N*-hydroxyl succinimide (NHS) ester activated glass slides (SurModics Inc., Eden Prairie, MN, USA) with a piezoelectric spotting device (S3; Scienion, Berlin, Germany). Microarray slides were incubated for 24 h in a humid chamber to complete coupling reactions, quenched with 50 mM aminoethanol solution, pH 9 for 1 h at 50°C, washed three times with deionized water, and stored desiccated until use.

Microarray binding assay

Slides were blocked with 1% bovine serum albumin (BSA) in phosphate-buffered saline (PBS) (w/v) for 1 h, washed three times with PBS and dried by centrifugation (300x g, 5 min.). A FlexWell 64 (Grace Bio-Labs, Bend, OR, USA) grid was applied to microarray slides. Resulting 64 wells were used for individual experiments. Slides were incubated with serum of CDI patients, which was diluted 1:100 in PBS, or sera of immunized mice, in a humid chamber for 1 h, washed three times with 0.1% Tween-20 in PBS (v/v) and dried by centrifugation (300x g, 5 min.). Slides were incubated with fluorescence-labeled secondary antibody, goat anti-human IgG Alexa Fluor 647 (Life Technologies, Cat.# A-21445) diluted 1:400 in 1% BSA in PBS (w/v), or goat anti-mouse IgG Alexa Fluor 647 (Life Technologies, Cat.# A-31574) diluted 1:400, in a humid chamber for 1 h, washed three times with 0.1% Tween-20 in PBS (v/v), rinsed once with deionized water and dried by centrifugation (300x g, 5 min.) prior to scanning with a GenePix 4300A microarray scanner (Molecular Devices, Sunnyvale, CA, USA). Image analysis was carried out with the GenePix Pro 7 software (Molecular Devices). The entire procedure was performed at room temperature.

6.5 Synthesis of *S. pneumoniae* Serotype 5 CPS Repeating Unit Substructures

6.5.1 Geneneral Synthetic Procedures for the Synthesis of *S. pneumoniae* Serotype 5 CPS Repeating Unit Substructures

General procedure (A) for removal of anomeric TDS.

Anomeric TDS-protected starting material (1.0 equiv) was dissolved in THF (reaction concentration at 0.15 M) and cooled to 0 °C. A solution of TBAF (1 M in THF, 10 equiv) and AcOH (12 equiv) was added, warmed to room temperature and stirred for 3 h. The reaction mixture was diluted with EtOAc, washed with 0.1 N HCl, sat. aq. $NaHCO_3$ and brine. The organic phases were dried over $MgSO_4$ and concentrated. Column chromatography on silica gel (hexanes/EtOAc) afforded the corresponding lactol as a mixture of α and β anomers.

General procedure (B) for NIS/TfOH-mediated glycosylation of thio- and selenoglycosides.

The acceptor (1.0 to 2.0 equiv) and thio- or selenoglycoside (1.0 to 1.3 equiv) were coevaporated with toluene three times and dried *in vacuo*. The residue was dissolved in DCM or MeCN (reaction concentration at 80 to 120 mM) together with freshly activated molecular sieves (4 Å) and NIS (1.2 equiv with regard to the thio- or selenoglycoside). The mixture was cooled and TfOH (0.1 equiv with regard to the thio- or selenoglycoside) was added. The reaction was stirred for 1 h, then quenched by the addition of NEt_3 and diluted with DCM. The organic layer was washed with sat. aq. $Na_2S_2O_3$ followed by sat. aq. $NaHCO_3$, dried over $MgSO_4$ and concentrated. Column chromatography (hexanes/EtOAc) afforded the pure product.

General procedure (C) for glycosyl-trichloroacetimidate synthesis with K_2CO_3.

To a solution of the lactol (1.0 equiv) in DCM (reaction concentration at 0.5 M) trichloroacetonitrile (10 equiv) and K_2CO_3 (1.7 equiv) were added and stirred for 3 h at room temperature. The crude product was concentrated and purified by column chromatography on silica gel (hexanes/EtOAc) to afford pure product.

General procedure (D) for glycosyl-trichloroacetimidate synthesis with DBU.

To a solution of the lactol (1.0 equiv) in DCM (reaction concentration at 0.5 M) at 0 °C trichloroacetonitrile (10 equiv) and DBU (1 drop) were added and stirred for 1 h at 0 °C. The crude product was concentrated and purified by column chromatography on silica gel (hexanes/EtOAc) to afford pure product.

General procedure (E) for TMSOTf-mediated glycosylation of glycosyl-imidates.

The acceptor (1.0 to 2.0 equiv) and glycosyl-trichloroacetimidate (1.0 to 1.3 equiv) were coevaporated with toluene three times and dried *in vacuo*. The residue was dissolved in DCM (reaction concentration at 80 to 120 mM) and freshly activated molecular sieves (4 Å) were added. The mixture was cooled to −30 °C and TMSOTf (0.1 equiv) was added. The reaction was brought to −20 °C, then quenched by the addition of NEt_3 and concentrated under reduced pressure. Column chromatography (hexanes/EtOAc) afforded the pure product.

General procedure (F) for thioacetic acid based azide reduction.

To a solution of azide starting material in pyridine (1 mL) thioacetic acid (0.5 mL) was added and stirred for 12 to 48 h. The reaction mixture was concentrated and purified by column chromatography on silica gel (hexanes/acetone) to afford the acetamide product.

General procedure (G) for removal of the Nap protecting group.

To a mixture of naphthylated starting material (1 equiv) in a mixture of DCM/phosphate-buffer (10:1 (v:v), 7 mM, pH 7.2, reaction concentration at 4 mM or DCM/H_2O (10:1) at 0 °C DDQ (2–3 equiv) was added portionwise over 1.5 h. The reaction mixture was warmed to room temperature and stirred for further 1.5 to 5 h. The mixture was diluted with sat. aq. $NaHCO_3$ solution, extracted with DCM and the organic layer dried over $MgSO_4$ and concentrated. Column chromatography on silica gel (hexanes/EtOAc) afforded the pure product.

General procedure (H) for TEMPO-mediated oxidation.

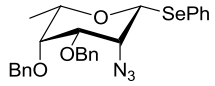
To a mixture of the primary alcohol (1.0 equiv) in DCM/H_2O (2.5:1 (v:v), reaction concentration at 7 mM) at 0 °C was added TEMPO (0.2 equiv) and BAIB (5.0 equiv). The reaction mixture was warmed to room temperature, stirred for 2 h and then diluted with H_2O and extracted with DCM. The organic phases were dried over Na_2SO_4 and concentrated. Size exclusion chromatography on Sephadex LH-20 ($CHCl_3$/MeOH = 1:1) afforded the product.

General procedure (I) for hydrogenolysis.

A solution of starting material (reaction concentration at 4 to 8 mM) in a mixture of EtOH/EtOAc/H_2O/AcOH was purged with Ar. After that 10% Pd/C was added and the solution purged with H_2 for 10 min, then stirred under an H_2 atmosphere for 12 h, filtered and concentrated. The crude product was dissolved in H_2O, subjected to reversed phase solid phase extraction (RP SPE) (Waters Sep-Pak®, C18) and lyophilized. When necessary, size exclusion chromatography on Sephadex LH-20 (MeOH) was performed.

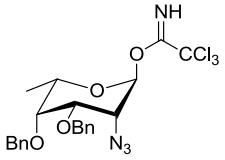
6.5.2 Synthesis of SP5 CPS Oligosaccharide Substructures

Phenyl 2-azido-3,4-di-*O*-benzyl-2-deoxy-1-seleno-α-L-pneumopyranoside (106)



To a impure mixture containing approx. 25% of selenoglycoside **105** (192 mg) in DMF (5 mL) at 0 °C, BnBr (0.21 mL, 1.8 mmol) and NaH (31 mg, 1.3 mmol) were added. After 3 h the reaction was quenched with MeOH at 0 °C and diluted with Et_2O. The organic layer was washed with 0.1 M aq. HCl solution, sat. aq. $NaHCO_3$ and brine. The organic layers were dried over $MgSO_4$ and concentrated. Column chromatography on silica gel (hexanes/ethyl acetate) afforded **106** (66 mg, 0.13 mmol, 22%). $[\alpha]_D^{20}$ = – 72.2 ° (c = 1.6, $CHCl_3$); IR ν_{max} (film) 3062, 3031, 2933, 2869, 2106, 1578, 1496, 1477, 1454, 1438, 1361, 1279, 1202, 1162, 1105, 1067 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.58 – 7.23 (m, 15H), 5.80 (d, *J*=1.5, 1H), 5.02 (*J*=11.8, 1H), 4.81 – 4.64 (m, 3H), 4.24 – 4.10 (m, 2H), 3.99 – 3.90 (m, 1H), 3.71 – 3.66 (m, 1H), 1.24 (d, *J*=6.5, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 138.4, 137.4, 133.9, 129.4, 129.1, 128.8, 128.6, 128.3, 128.2, 128.0, 127.8, 127.7, 83.9, 77.5, 75.4, 75.0, 71.4, 70.6, 60.0, 16.7; HRMS (ESI): Calcd for $C_{26}H_{27}N_3O_3SeNa^+$ $[M+Na]^+$ 532.1115, found 532.1112.

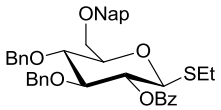
2-Azido-3,4-di-*O*-benzyl-2-deoxy-α-L-pneumopyranosyl trichloroacetimidate (101)



To a solution of **106** (30 mg, 59 µmol) in THF (1.25 mL) and H_2O (0.75 mL) was added NIS (27 mg, 180 µmol) and stirred for 2 h. The reaction was diluted with DCM and the organic layer washed with sat. aq. $Na_2S_2O_3$ followed by sat. aq. $NaHCO_3$, dried over $MgSO_4$ and

concentrated. Column chromatography (hexanes/EtOAc) afforded the free lactol (20 mg, 54 µmol, 92%) as a mixture of α and β anomers. IR ν_{max} (film) 3413, 3031, 2872, 2110, 1496, 1454, 1360, 1306, 1176, 1109, 1053, 1027 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.59 – 7.20 (m, 10H), 5.20 (d, *J* = 1.6 Hz, 0.5H), 4.99 (d, *J* = 11.7 Hz, 1H), 4.81 – 4.64 (m, 3H), 4.56 (d, *J* = 1.8 Hz, 0.5H), 4.02 (t, *J* = 3.4 Hz, 1H), 3.67 (t, *J* = 3.4 Hz, 0.5H), 3.65 – 3.63 (m, 0.5H), 3.60 – 3.55 (m, 0.5H), 3.44 (qd, *J* = 6.3, 1.1 Hz, 0.5H), 1.26 (d, *J* = 6.4 Hz, 1.5H), 1.23 (d, *J* = 6.6 Hz, 1.5H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 138.4, 138.1, 137.8, 137.3, 129.0, 128.8 (2C), 128.6, 128.4, 128.3, 128.2, 127.9, 127.7, 127.5, 127.4, 93.6, 93.2, 79.7, 76.7, 75.4, 74.9, 73.8, 71.8, 71.4, 71.2, 67.3, 60.4, 58.3, 17.0, 16.9; HRMS (ESI): Calcd for $C_{20}H_{23}N_3O_4Na^+$ $[M+Na]^+$ 392.1586, found 392.1583. According to general procedure (C), the lactol (75 mg, 0.20 mmol) was reacted with trichloroacetonitrile (0.20 mL, 2.0 mmol) and K_2CO_3 (48 mg, 0.35 mmol) in DCM (2 mL) to afford **101** (65 mg, 0.13 mmol, 62%). $[\alpha]_D^{20}$ = – 32.3 ° (c = 1.5, $CHCl_3$); IR ν_{max} (film) 3336, 2910, 2113, 1672, 1454, 1356, 1273, 1157, 1063 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.65 – 8.49 (m, 1H), 7.43 – 7.22 (m, 10H), 6.19 (d, *J* = 1.5 Hz, 1H), 5.03 (d, *J* = 11.7 Hz, 1H), 4.78 (d, *J* = 11.8 Hz, 1H), 4.70 (d, *J* = 3.7 Hz, 2H), 4.09 – 3.98 (m, 3H), 3.69 (dd, *J* = 1.6, 1.2 Hz, 1H), 1.23 (d, *J* = 6.5 Hz, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 159.9, 138.2, 137.2, 128.9, 128.8 (2C), 128.7, 128.4, 128.3, 128.2, 128.0, 127.9 (2C), 97.0, 91.0, 76.0, 75.1, 74.5, 71.2, 70.3, 56.2, 16.9; HRMS (MALDI-TOF): Calcd for $C_{22}H_{23}Cl_3N_4O_4Na^+$ $[M+Na]^+$ 535.0677, found 535.0660.

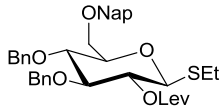
Ethyl 2-*O*-benzoyl-3,4-di-*O*-benzyl-6-*O*-(2-naphthalenylmethyl)-1-thio-β-D-glucopyranoside (108)



To a solution of **107** (584 mg, 1.15 mmol) in DCM (10 mL) BH_3·THF (1 M in THF, 6.9 mL, 6.9 mmol) and TMSOTf (0.1 mL, 0.6 mmol) were added drop wise at 0°C. The reaction was warmed to room temperature over 2 h, cooled to 0°C again and quenched by drop wise addition of sat. aq. $NaHCO_3$ solution. The emulsion was diluted with DCM and washed with a sat. aq. $NaHCO_3$ solution. The organic phase was then dried over $MgSO_4$, filtered and concentrated to give the crude alcohol. To a solution of crude alcohol in THF/DMF (9:1, 10 mL) at 0 °C, naphtyl bromide (509 mg, 2.30 mmol) and NaH (33 mg, 1.38 mmol) were added. The reaction was warmed to room temperature and stirred for 30 min, cooled to 0 °C and quenched by the addition of water. After dilution with Et_2O the organic phase was washed with 0.1 M HCl and sat. aq. $NaHCO_3$ solutions. The organic phase was then dried

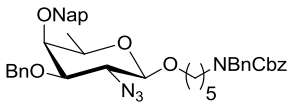
over $MgSO_4$, filtered and concentrated. Column chromatography on silica gel (hexanes/ethyl acetate) afforded **108** (626 mg, 0.97 mmol, 84%). $[\alpha]_D^{20}$ = + 29.5 º (c = 3.1, $CHCl_3$), IR ν_{max} (film) 3061, 3030, 2867, 1722, 1602, 1496, 1452, 1359, 1315, 1264, 1087, 1067, 1026 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.06 – 8.01 (m, 2H), 7.86 – 7.79 (m, 4H), 7.60 – 7.42 (m, 6H), 7.26 – 7.04 (m, 10H), 5.34 (dd, *J* = 10.0, 8.9 Hz, 1H), 4.84 – 4.54 (m, 7H), 3.88 – 3.74 (m, 4H), 3.60 (ddd, *J* = 9.5, 4.5, 2.0 Hz, 1H), 2.81 – 2.68 (m, 2H), 1.26 (t, *J* = 7.5 Hz, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 165.4, 138.0, 137.9, 135.8, 133.4, 133.3, 133.2, 130.1, 130.0, 128.5 (2C), 128.4, 128.3, 128.1 (3C), 127.9, 127.8 (2C), 126.6, 126.2, 126.0, 125.9, 84.5, 83.4, 79.7, 78.1, 75.4, 75.2, 73.7, 72.6, 69.1, 24.1, 15.1; HRMS (MALDI-TOF): Calcd for $C_{40}H_{40}O_6SNa^+$ $[M+Na]^+$ 671.2438, found 671.2478.

Ethyl 3,4-di-*O*-benzyl-2-*O*-levulinoyl-6-*O*-(2-naphthalenylmethyl)-1-thio-β-D-glucopyranoside (100)



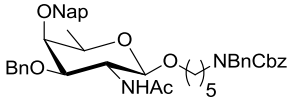
To a solution of **108** (367 mg, 0.57 mmol) in DCM (5.0 mL) was added 0.5 M NaOMe in MeOH (5.0 mL) and stirred for 16 h. The mixture was neutralized with Amberlite® IR 120 (H^+) ion exchange resin, filtered and concentrated. The residue was dissolved in DCM (5.0 mL) together with DMAP (7 mg, 0.06 mmol), levulinic acid (81 µL, 0.79 mmol) and EDC (120 µL, 0.68 mmol). The reaction mixture was stirred for 4 h, concentrated and redissolved in EtOAc. The organic layer was washed with sat. aq. NH_4Cl, sat. aq. $NaHCO_3$ and brine, then dried over $MgSO_4$ and concentrated. Column chromatography on silica gel (hexanes/ethyl acetate) afforded **100** (271 mg, 0.42 mmol, 75%). $[\alpha]_D^{20}$ = – 1.0 º (c = 1.5, $CHCl_3$), IR ν_{max} (film) 3061, 3031, 2926, 2869, 1746, 1718, 1497, 1454, 1404, 1361, 1203, 1158, 1081, 1062 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.89 – 7.73 (m, 4H), 7.55 – 7.42 (m, 3H), 7.41 – 6.97 (m, 10H), 5.11 – 5.02 (m, 1H), 4.82 – 4.69 (m, 5H), 4.56 (d, *J* = 10.8 Hz, 1H), 4.39 (d, *J* = 10.0 Hz, 1H), 3.82 – 3.68 (m, 4H), 3.53 (ddd, *J* = 9.2, 4.6, 2.1 Hz, 1H), 2.80 – 2.64 (m, 4H), 2.60 – 2.46 (m, 2H), 2.16 (s, 3H), 1.28 (t, *J* = 7.5 Hz, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 206.2, 171.6, 138.3, 137.9, 135.7, 133.3, 133.1, 128.4 (2C), 128.3, 128.2, 128.0 (2C), 127.9 (3C), 127.8 (2C), 126.5, 126.2, 125.9, 125.8, 84.4, 83.5, 79.5, 77.9, 75.2, 75.1, 73.6, 72.3, 68.9, 38.0, 29.9, 28.2, 24.0, 15.0; HRMS (MALDI-TOF): Calcd for $C_{38}H_{42}O_7SNa^+$ $[M+Na]^+$ 665.2543, found 665.2558.

_N_-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl 2-azido-3-_O_-benzyl-2-deoxy-4-_O_-(2-naphthalenylmethyl)-β-D-fucopyranoside (112)



To a solution of **96** (50 mg, 78 µmol) in DMF (4 ml) at 0 °C, BnBr (28 µL, 240 µmol), TBAI (3 mg, 8 µmol) and NaH (3 mg, 125 mmol) were added. After 3 h the reaction was quenched with MeOH at 0 °C and diluted with Et_2O. The organic layer was washed with 0.1 M aq. HCl solution, sat. aq. $NaHCO_3$ and brine. The organic layers were dried over $MgSO_4$ and concentrated. Column chromatography on silica gel (hexanes/ethyl acetate) afforded **112** (32 mg, 44 µmol, 56%). $[\alpha]_D^{20}$ = – 24.2 ° (c = 1.6, $CHCl_3$); IR ν_{max} (film) 3031, 2935, 2110, 1697, 1496, 1454, 1421, 1362, 1230, 1172, 1111, 1068 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.42 (s, 22H), 5.19 (d, *J* = 12.8 Hz, 2H), 5.09 (d, *J* = 11.9 Hz, 1H), 4.86 (d, *J* = 11.9 Hz, 1H), 4.79 – 4.68 (m, 2H), 4.51 (d, *J* = 8.8 Hz, 2H), 4.22 – 4.11 (m, 1H), 3.96 – 3.80 (m, 2H), 3.58 (d, *J* = 2.5 Hz, 1H), 3.51 – 3.36 (m, 2H), 3.34 – 3.17 (m, 3H), 1.67 – 1.47 (m, 4H), 1.46 – 1.28 (m, 2H), 1.21 (d, *J* = 6.4 Hz, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 138.0, 137.9, 135.8, 133.2, 133.1, 128.6 (2C), 128.6, 128.1 (2C), 128.0, 127.9, 127.8, 127.3, 126.8, 126.1, 126.0, 102.4, 81.1, 74.8, 74.7, 72.9, 70.6, 69.8, 67.2, 63.3, 50.6, 50.3, 47.2, 46.3, 29.3, 28.0, 27.6, 23.3, 17.1; HRMS (MALDI-TOF): Calcd for $C_{44}H_{48}N_4O_6Na^+$ $[M+Na]^+$ 751.3466, found 751.3417.

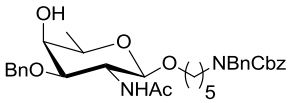
_N_-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl 2-_N_-acetyl-3-_O_-benzyl-2-deoxy-4-_O_-(2-naphthalenylmethyl)-β-D-fucosaminopyranoside (113)



According to general procedure (F), azido-monosaccharide **112** (32 mg, 44 µmol) was reacted with thioacetic acid for 24 h to give **113** (26 mg, 35 µmol, 80%). $[\alpha]_D^{20}$ = + 8.8 ° (c = 1.4, $CHCl_3$); IR ν_{max} (film) 3288, 3062, 2933, 1698, 1654, 1555, 1496, 1454, 1422, 1366, 1308, 1231, 1173, 1113, 1068 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.93 – 7.08 (m, 22H), 5.17 (d, *J* = 15.1 Hz, 2H), 5.06 (d, *J* = 11.9 Hz, 1H), 4.95 (d, *J* = 8.3 Hz, 1H), 4.84 (d, *J* = 12.0 Hz, 1H), 4.72 – 4.63 (m, 1H), 4.60 – 4.32 (m, 4H), 3.92 – 3.72 (m, 1H), 3.65 (d, *J* = 2.2 Hz, 1H), 3.57 (q, *J* = 6.3 Hz, 1H), 3.53 – 3.32 (m, 2H), 3.32 – 3.09 (m, 2H), 1.98 – 1.79 (m, 3H), 1.63 – 1.41 (m, 4H), 1.33 – 1.16 (m, 5H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 171.1, 138.0, 133.2, 133.0, 128.6, 128.1, 128.0 (2C), 127.9, 127.8, 127.3, 127.0, 126.7, 126.1, 125.9, 99.4, 78.2, 75.5,

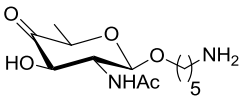
74.7, 72.6, 70.4, 69.5, 67.2, 55.5, 50.3, 47.3, 29.1, 27.5, 23.8, 23.5, 17.3; HRMS (MALDI-TOF): Calcd for $C_{46}H_{52}N_2O_7Na^+$ $[M+Na]^+$ 767.3667, found 767.3575.

_N_-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl 2-_N_-acetyl-3-_O_-benzyl-2-deoxy-β-D-fucosaminopyranoside (125)



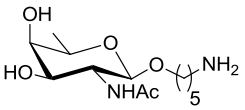
According to general procedure (G), monosaccharide **113** (20 mg, 27 μmol) was reacted with DDQ (12 mg, 53 μmol) in DCM/H_2O to give alcohol **125** (12 mg, 20 μmol, 74%). $[\alpha]_D^{20}$ = + 13.9 º (c = 1.2, $CHCl_3$); IR ν_{max} (film) 3298, 3032, 2934, 1697, 1657, 1554, 1497, 1454, 1423, 1369, 1303, 1229, 1173, 1069 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.53 – 7.07 (m, 15H), 5.16 (d, *J* = 18.4 Hz, 2H), 4.85 (d, *J* = 8.4 Hz, 1H), 4.67 (d, *J* = 11.7 Hz, 1H), 4.59 – 4.42 (m, 3H), 4.31 – 4.15 (m, 1H), 3.92 – 3.72 (m, 2H), 3.61 (q, *J* = 6.4 Hz, 1H), 3.45 – 3.10 (m, 4H), 2.34 (s, 1H), 1.99 – 1.80 (m, 3H), 1.64 – 1.43 (m, 4H), 1.36 – 1.20 (m, 5H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 170.9, 138.0, 128.7, 128.6, 128.1 (2C), 127.9, 127.4, 127.3, 99.4, 77.4, 76.7, 71.9, 70.0, 69.5, 68.7, 67.3, 54.6, 50.5, 50.3, 47.4, 29.3, 29.0, 27.5, 23.8, 23.6, 16.7; HRMS (MALDI-TOF): Calcd for $C_{35}H_{77}N_2O_7Na^+$ $[M+Na]^+$ 627.3041, found 627.2969.

5-Amino-pentanyl 2-acetamido-2,5-dideoxy-β-D-*xylo*-hexos-4-uloside (92)



To a solution of **125** (12 mg, 20 μmol) in DCM (2 mL) Dess Martin periodinane (17 mg, 40 μmol) was added and stirred for 12 h. The reaction mixture was loaded onto a silica gel column and purified by column chromatography (hexanes/acetone) to give the crude protected ketone. According to general procedure (I), the crude protected ketone was subjected to hydrogenolysis to give **92** as a minor component in a mixture of compounds. HRMS (ESI): Calcd for $C_{13}H_{26}N_2O_6Na^+$ $[M+H_2O+Na]^+$ 329.1689, found 329.1479.

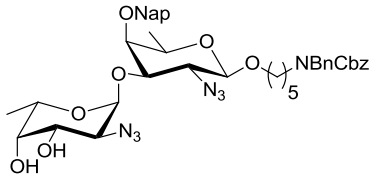
5-Amino-pentanyl 2-_N_-acetyl-2-deoxy-β-D-fucosaminopyranoside (93)



According to general procedure (I), D-fucosaminoside **113** (7 mg, 9 μmol) was subjected to hydrogenolysis to give **93** (2.5 mg, 8.6 μmol, 92%). ^{1}H-NMR (600 MHz, D_2O) δ 4.43 (d, *J* =

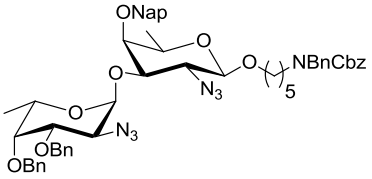
8.5 Hz, 1H), 3.94 – 3.81 (m, 2H), 3.80 – 3.68 (m, 3H), 3.64 – 3.56 (m, 1H), 3.06 – 2.96 (m, 2H), 2.05 (s, 3H), 1.69 (dt, *J* = 15.2, 7.6 Hz, 2H), 1.63 – 1.57 (m, 2H), 1.45 – 1.37 (m, 2H), 1.28 (d, *J* = 6.3 Hz, 3H); ^{13}C-NMR (150 MHz, D_2O) δ 177.2, 104.1, 73.8, 73.5, 73.1, 72.6, 54.8, 41.9, 30.8, 29.0, 24.8, 24.7, 18.1; HRMS (ESI): Calcd for $C_{13}H_{27}N_2O_5^+$ $[M+H]^+$ 291.1920, found 291.1925.

_N_-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl 2-azido-2-deoxy-α-L-fucopyranosyl-(1→3)-2-azido-2-deoxy-4-_O_-(2-naphthalenylmethyl)-β-D-fucopyranoside (109)



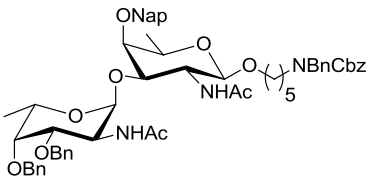
According to general procedure (E), fucosyl-imidate **97** (140 mg, 335 μmol) and **96** (100 mg, 157 μmol) were reacted in DCM (2 mL) at –20 °C to –15 °C over 30 min to give the crude disaccharide. To a solution of the crude disaccharide in in DCM/MeOH (1:1, 2mL) was added 0.5 M NaOMe in MeOH (0.1 mL) and stirred for 16 h. The mixture was neutralized with Amberlite® IR 120 (H^+) ion exchange resin, filtered and concentrated. Column chromatography (DCM/MeOH/acetone) afforded **109** (110 mg, 136 μmol, 87%). $[\alpha]_D^{20}$ = – 41.6 º (c = 1.5, $CHCl_3$); IR ν_{max} (film) 3328, 2936, 2119, 1697, 1422, 1255, 1095, 1070, 1028 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.94 – 7.72 (m, 4H), 7.59 – 7.44 (m, 3H), 7.41 – 7.03 (m, 10H), 5.24 (d, *J* = 3.6 Hz, 1H), 5.17 (d, *J* = 12.9 Hz, 2H), 4.99 (d, *J* = 12.2 Hz, 1H), 4.80 (d, *J* = 12.2 Hz, 1H), 4.49 (d, *J* = 6.9 Hz, 2H), 4.21 (t, *J* = 9.3 Hz, 1H), 3.99 – 3.84 (m, 2H), 3.59 – 3.39 (m, 6H), 3.31 – 3.12 (m, 4H), 2.30 (s, 2H), 1.69 – 1.47 (m, 4H), 1.43 – 1.27 (m, 5H), 1.10 (d, *J* = 6.6 Hz, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 136.0, 133.2, 133.1, 128.6, 128.3, 128.0, 127.9, 127.8, 127.4, 126.8, 126.6, 126.5, 126.3, 102.8, 99.9, 79.1, 78.2, 75.6, 71.4, 71.0, 69.9, 67.9, 67.3, 66.3, 63.8, 59.7, 50.6, 50.3, 47.2, 46.3, 29.3, 23.3, 17.3, 16.2; HRMS (MALDI-TOF): Calcd for $C_{43}H_{51}N_7O_9Na^+$ $[M+Na]^+$ 832.3640, found 832.3676.

***N*-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl 3,4-di-*O*-benzyl-2-azido-2-deoxy-α-L-fucopyranosyl-(1→3)-2-azido-2-deoxy-4-*O*-(2-naphthalenylmethyl)-β-D-fucopyranoside (110)**



To a solution of diol **109** (87 mg, 107 µmol) in DMF (5 mL) at 0 °C, BnBr (77 µL, 645 µmol), TBAI (4 mg, 11 µmol) and NaH (8 mg, 333 mmol) were added. After 3 h the reaction was quenched with MeOH at 0 °C and diluted with Et_2O. The organic layer was washed with 0.1 M aq. HCl solution, sat. aq. $NaHCO_3$ and brine. The organic layers were dried over $MgSO_4$ and concentrated. Column chromatography on silica gel (hexanes/ethyl acetate) afforded **110** (88 mg, 89 µmol, 83%). $[\alpha]_D^{20}$ = – 50.2 ° (c = 1.4, $CHCl_3$); IR ν_{max} (film) 2937, 2114, 1697, 1496, 1454, 1421, 1360, 1233, 1171, 1103, 1068, 1038 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.92 – 7.72 (m, 4H), 7.63 – 7.07 (m, 23H), 5.22 (d, *J* = 3.7 Hz, 1H), 5.18 (d, *J* = 13.1 Hz, 2H), 4.95 (d, *J* = 12.1 Hz, 1H), 4.83 (dd, *J* = 12.5 Hz, 2H), 4.57 – 4.36 (m, 5H), 4.21 (dd, *J* = 11.6, 8.1 Hz, 1H), 4.02 – 3.84 (m, 2H), 3.75 (dd, *J* = 10.8, 3.5 Hz, 1H), 3.64 – 3.40 (m, 6H), 3.33 – 3.15 (m, 3H), 1.70 – 1.49 (m, 4H), 1.43 – 1.26 (m, 5H), 1.00 (d, *J* = 6.5 Hz, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 138.1, 137.6, 136.0, 133.2, 133.0, 128.6, 128.5, 128.4, 128.3, 128.0, 127.9 (2C), 127.8, 127.3, 126.5, 126.4, 126.2, 102.7, 100.4, 79.4, 78.9, 75.8, 75.7, 74.9, 72.2, 70.9, 70.0, 67.2, 63.9, 59.4, 50.6, 50.3, 47.2, 46.3, 29.3, 28.0, 27.5, 23.3, 17.3, 16.8; HRMS (MALDI-TOF): Calcd for $C_{57}H_{63}N_7O_9Na^+$ $[M+Na]^+$ 1012.4579, found 1012.4502.

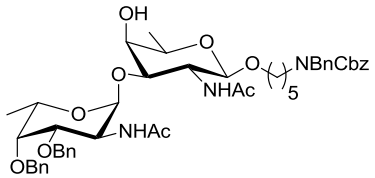
***N*-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl 2-*N*-acetyl-3,4-di-*O*-benzyl-α-L-fucosaminopyranosyl-(1→3)-2-*N*-acetyl-4-*O*-(2-naphthalenylmethyl)-β-D-fucosaminopyranoside (111)**



According to general procedure (F), azido-disaccharide **110** (88 mg, 89 µmol) was reacted with thioacetic acid for 24 h to give **111** (82 mg, 80 µmol, 90%). $[\alpha]_D^{20}$ = – 21.0 ° (c = 1.2, $CHCl_3$); IR ν_{max} (film) 3314, 2933, 1682, 1497, 1454, 1366, 1304, 1233, 1172, 1104, 1063, 1029 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.91 – 7.71 (m, 4H), 7.55 – 7.04 (m, 23H), 5.21 –

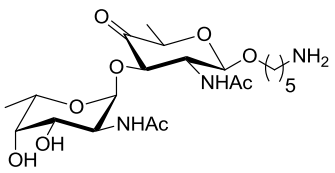
5.08 (m, 2H), 5.00 – 4.62 (m, 5H), 4.60 – 4.40 (m, 4H), 4.35 – 4.00 (m, 3H), 3.90 – 3.66 (m, 3H), 3.57 – 3.09 (m, 7H), 2.07 – 1.84 (m, 6H), 1.65 – 1.40 (m, 4H), 1.36 – 1.15 (m, 5H), 1.05 (d, *J* = 6.5 Hz, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 171.4, 170.8, 133.2, 132.9, 128.6, 128.5, 128.4 (2C), 128.3, 128.2, 128.0, 127.9, 127.8, 127.6, 127.5, 127.4, 127.3, 126.4, 126.1, 126.0, 125.5, 101.4, 100.5, 78.5, 75.5, 74.3, 72.2, 70.7, 68.5, 67.6, 67.2, 52.0, 50.5, 48.1, 47.6, 29.2, 28.7, 23.6, 23.5, 17.4, 17.0; HRMS (MALDI-TOF): Calcd for $C_{61}H_{71}N_3O_{11}Na^+$ $[M+Na]^+$ 1044.4981, found 1044.4917.

_N_-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl 2-_N_-acetyl-3,4-di-_O_-benzyl-α-L-fucosaminopyranosyl-(1→3)-2-_N_-acetyl-β-D-fucosaminopyranoside (127)



According to general procedure (G), disaccharide **111** (46 mg, 45 μmol) was reacted with DDQ (30 mg, 132 μmol) in DCM/H_2O to give alcohol **127** (30 mg, 34 μmol, 76%). $[\alpha]_D^{20}$ = – 35.7 º (c =1.0, $CHCl_3$); IR ν_{max} (film) 3290, 3088, 2932, 1702, 1646, 1548, 1497, 1454, 1422, 1372, 1304, 1227, 1101, 1063, 1024 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.41 – 7.14 (m, 20H), 5.22 – 5.07 (m, 2H), 5.01 – 4.85 (m, 2H), 4.84 – 4.31 (m, 6H), 4.29 – 4.18 (m, 1H), 4.11 – 3.89 (m, 2H), 3.87 – 3.38 (m, 6H), 3.36 – 3.04 (m, 3H), 2.00 (s, 3H), 1.91 (s, 3H), 1.63 – 1.38 (m, 4H), 1.27 (d, *J* = 6.4 Hz, 5H), 1.17 (d, *J* = 6.5 Hz, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 170.8, 128.7, 128.6, 128.5, 128.4 (2C), 128.0, 127.6, 127.5, 127.4, 101.5, 101.1, 80.4, 78.4, 75.8, 74.8, 72.0, 71.5, 70.1, 68.8, 67.4, 67.3, 51.3, 50.6, 48.8, 47.6, 28.7, 27.0, 23.6, 23.5, 17.2, 16.5; HRMS (MALDI-TOF): Calcd for $C_{50}H_{63}N_3O_{11}Na^+$ $[M+Na]^+$ 904.4355, found 904.4344.

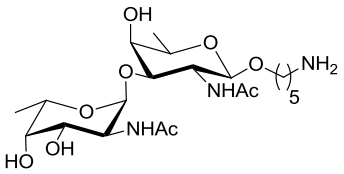
5-Amino-pentanyl 2-_N_-acetyl-α-L-fucosaminopyranosyl-(1→3)-2-acetamido-2,5-dideoxy-β-D-_xylo_-hexos-4-uloside (94)



To a solution of **127** (9 mg, 10 μmol) in DCM (2 mL) Dess Martin periodinane (9 mg, 9 μmol) was added and stirred for 12 h. The reaction mixture was loaded onto a silica gel column and purified by column chromatography (hexanes/acetone) to give the crude protected

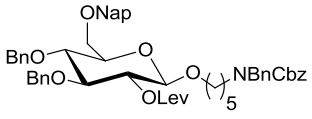
ketone. According to general procedure (I), the crude protected ketone was subjected to hydrogenolysis to give **94** as a mixture of compounds (approx. 2 mg, approx. 4 μmol, approx. 39%). ^{1}H-NMR (600 MHz, D_2O) δ 5.02 (d, *J* = 4.0 Hz, 1H), 4.49 (q, *J* = 6.5 Hz, 1H), 4.45 (d, *J* = 8.8 Hz, 1H), 4.15 (dd, *J* = 11.2, 4.1 Hz, 1H), 3.99 (dd, *J* = 11.2, 3.2 Hz, 1H), 3.92 – 3.83 (m, 3H), 3.66 – 3.54 (m, 3H), 3.01 – 2.97 (m, 2H), 2.06 (s, 3H), 1.98 (s, 3H), 1.70 – 1.64 (m, 2H), 1.61 – 1.55 (m, 2H), 1.42 – 1.36 (m, 2H), 1.29 (d, *J* = 6.4 Hz, 3H), 1.20 (d, *J* = 6.6 Hz, 3H); ^{13}C-NMR (150 MHz, D_2O) δ 177.0, 176.4, 104.0, 100.9, 95.4, 81.4, 75.9, 73.7, 72.8, 70.2, 69.9, 52.1, 42.0, 30.8, 29.1, 24.9, 24.8, 24.7, 17.9, 13.8; HRMS (ESI): Calcd for $C_{21}H_{40}N_3O_{10}^+$ $[M+H_2O+H]^+$ 494.2714, found 494.2740.

5-Amino-pentanyl 2-*N*-acetyl-α-L-fucosaminopyranosyl-(1→3)-2-*N*-acetyl-β-D-fucosaminopyranoside (95)



According to general procedure (I), disaccharide **111** (7 mg, 7 μmol) was subjected to hydrogenolysis to give **95** (2.73 mg, 5.7 μmol, 83%). ^{1}H-NMR (600 MHz, D_2O) δ 5.00 (d, *J* = 4.0 Hz, 1H), 4.41 (d, *J* = 8.5 Hz, 1H), 4.15 – 4.09 (m, 2H), 4.01 – 3.94 (m, 2H), 3.89 (dt, *J* = 10.2, 6.0 Hz, 1H), 3.85 (d, *J* = 3.0 Hz, 1H), 3.82 – 3.74 (m, 3H), 3.58 (dt, *J* = 10.1, 6.4 Hz, 1H), 3.04 – 2.96 (m, 2H), 2.05 (s, 3H), 2.00 (s, 3H), 1.72 – 1.64 (m, 2H), 1.63 – 1.55 (m, 2H), 1.44 – 1.36 (m, 2H), 1.28 (d, *J* = 6.5 Hz, 3H), 1.24 (d, *J* = 6.6 Hz, 3H); ^{13}C-NMR (150 MHz, D_2O) δ 176.8, 176.7, 104.2, 101.6, 79.4, 73.6, 73.3, 73.1, 72.7, 70.1, 69.7, 54.0, 52.1, 41.9, 30.8, 29.1, 24.8 (2C), 24.7, 18.0 (2C); HRMS (ESI): Calcd for $C_{21}H_{40}N_3O_9^+$ $[M+H]^+$ 478.2765, found 478.2775.

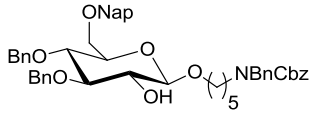
***N*-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl 3,4-di-*O*-benzyl-2-*O*-levulinoyl-6-*O*-(2-naphthalenylmethyl)-β-D-glucopyranoside (116)**



According to general procedure (B), thioglucoside **100** (30 mg, 47 μmol) was reacted with *N*-(Benzyl)benzyloxycarbonyl-5-amino-pentanol (31 mg, 93 μmol), NIS (13 mg, 56 μmol) and TfOH (0.8 μL, 9 μmol) in DCM (1 mL) at –30 °C to –20 °C over 1 h. After workup column chromatography (hexanes/EtOAc) afforded **116** (26 mg, 29 μmol, 61%). $[\alpha]_D^{20}$ = + 3.7 ° (c =

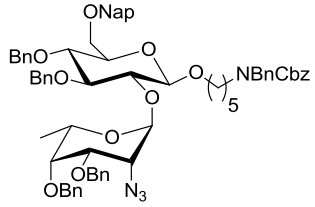
0.9, $CHCl_3$); IR ν_{max} (film) 2924, 1747, 1698, 1454, 1421, 1362, 1210, 1153, 1071 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.84 – 7.76 (m, 4H), 7.26 (s, 22H), 7.09 – 7.05 (m, 2H), 5.21 – 5.13 (m, 3H), 4.98 (t, J = 8.6 Hz, 1H), 4.77 (d, J = 10.9 Hz, 3H), 4.70 (d, J = 11.6 Hz, 2H), 4.53 – 4.44 (m, 4H), 4.37 – 4.27 (m, 1H), 3.85 – 3.59 (m, 5H), 3.53 – 3.11 (m, 3H), 2.82 – 2.39 (m, 4H), 2.17 – 2.03 (m, 2H), 1.62 – 1.43 (m, 8H), 1.37 – 1.17 (m, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 171.5, 138.4, 133.4, 133.1, 128.7, 128.5, 128.3, 128.1 (2C), 128.0, 127.9, 127.8 (2C), 126.7, 126.2, 126.0, 101.1, 83.0, 78.1, 75.2 (2C), 75.1, 73.7 (2C), 69.6, 68.8, 67.3, 50.3, 47.2, 37.9, 30.0, 29.3, 28.1, 23.3; HRMS (MALDI-TOF): Calcd for $C_{56}H_{61}NO_{10}Na^+$ $[M+Na]^+$ 930.4188, found 930.4190.

_N_-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl 3,4-di-_O_-benzyl-6-_O_-(2-naphthalenylmethyl)-β-D-glucopyranoside (117)



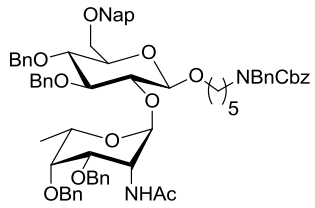
To a solution of **116** (13 mg, 14 μmol) in DCM (1 mL) hydrazine hydrate (2.8 μL, 57 μmol) dissolved in AcOH (40 μL) and pyridine (60 μL) was added and the solution stirred for 1 h. The reaction was then quenched by the addition of acetone and concentrated. Column chromatography on silica gel (hexanes/ethyl acetate) afforded **117** (10 mg, 12 μmol, 86%). $[\alpha]_D^{20}$ = + 4.9 ° (c = 1.0, $CHCl_3$); IR ν_{max} (film) 2926, 1698, 1454, 1230, 1063 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.84 – 7.01 (m, 27H), 5.16 (d, *J* = 9.8 Hz, 2H), 4.93 (t, *J* = 11.1 Hz, 1H), 4.81 (dd, *J* = 11.0, 6.1 Hz, 2H), 4.75 (d, *J* = 12.4 Hz, 1H), 4.67 (d, *J* = 12.4 Hz, 1H), 4.51 – 4.43 (m, 3H), 4.26 – 4.17 (m, 1H), 4.02 – 3.79 (m, 2H), 3.78 – 3.67 (m, 2H), 3.60 – 3.44 (m, 4H), 3.30 – 3.12 (m, 2H), 1.66 – 1.43 (m, 4H), 1.38 – 1.19 (m, 2H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 138.0, 135.7, 133.4, 133.1, 128.7, 128.6, 128.5, 128.3, 128.1, 128.0 (2C), 127.8, 127.3, 126.8, 126.2, 126.0, 103.1, 84.7, 77.7, 75.3, 75.2, 74.8, 73.7, 70.1, 69.9, 69.0, 67.3, 50.3, 47.2, 46.2, 32.4, 29.3, 27.5, 23.5; HRMS (MALDI-TOF): Calcd for $C_{51}H_{55}NO_8Na^+$ $[M+Na]^+$ 832.3820, found 832.3870.

***N*-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl 2-azido-3,4-di-*O*-benzyl-2-deoxy-α-L-pneumopyranosyl-(1→2)-3,4-di-*O*-benzyl-6-*O*-(2-naphthalenylmethyl)-β-D-glucopyranoside (118)**



According to general procedure (E), pneumosyl-imidate **101** (12 mg, 23 μmol) and **117** (10 mg, 12 μmol) were reacted in DCM (1 mL) at –30 °C to –20 °C over 30 min to give **118** (13 mg, 11 μmol, 91%). $[\alpha]_D^{20}$ = – 0.7 ° (c = 0.7, $CHCl_3$); IR ν_{max} (film) 2929, 2868, 2112, 1697, 1496, 1454, 1421, 1361, 1229, 1126, 1057 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.86 – 7.72 (m, 4H), 7.50 – 7.43 (m, 3H), 7.41 – 7.02 (m, 30H), 5.24 – 5.08 (m, 3H), 4.93 (dd, *J* = 30.9, 11.5 Hz, 2H), 4.77 – 4.44 (m, 10H), 4.26 – 4.08 (m, 2H), 3.85 – 3.67 (m, 4H), 3.66 – 3.51 (m, 5H), 3.47 – 3.29 (m, 2H), 3.25 – 3.09 (m, 2H), 1.61 – 1.38 (m, 4H), 1.32 – 1.03 (m, 5H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 137.9, 133.4, 133.1, 128.8, 128.7 (2C), 128.6 (2C), 128.4, 128.2, 128.1, 128.0, 127.9, 127.8, 127.2, 126.8, 126.3, 126.0 (2C), 101.8, 99.2, 78.7, 77.4, 75.7, 75.1 (2C), 73.8 (2C), 70.9, 68.9, 67.3, 66.9, 29.5, 23.4, 17.0; HRMS (MALDI-TOF): Calcd for $C_{71}H_{76}N_4O_{11}Na^+$ $[M+Na]^+$ 1183.5403, found 1183.5391.

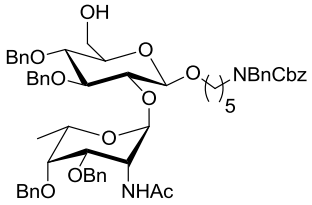
***N*-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl 2-*N*-acetyl-3,4-di-*O*-benzyl-α-L-pneumosaminopyranosyl-(1→2)-3,4-di-*O*-benzyl-6-*O*-(2-naphthalenylmethyl)-β-D-glucopyranoside (119)**



According to general procedure (F), azido-disaccharide **118** (13 mg, 11 μmol) was reacted with thioacetic acid for 24 h to give **119** (10 mg, 8 μmol, 76%). $[\alpha]_D^{20}$ = – 31.1 ° (c = 1.0, $CHCl_3$); IR ν_{max} (film) 2930, 1698, 1497, 1454, 1363, 1229, 1056 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.85 – 7.73 (m, 4H), 7.49 – 7.43 (m, 3H), 7.35 – 7.12 (m, 28H), 7.08 – 7.02 (m, 2H), 7.01 – 6.94 (m, 1H), 5.28 (s, 1H), 5.21 – 5.13 (m, 2H), 4.90 – 4.81 (m, 3H), 4.77 – 4.67 (m, 5H), 4.60 – 4.54 (m, 1H), 4.51 – 4.30 (m, 5H), 4.28 – 4.20 (m, 1H), 3.90 – 3.78 (m, 2H), 3.76 – 3.57 (m, 6H), 3.49 – 3.43 (m, 1H), 3.38 (s, 1H), 3.28 – 3.14 (m, 2H), 1.65 (s, 3H), 1.58 – 1.45 (m, 4H), 1.30 – 1.16 (m, 5H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 169.8, 138.5, 138.3,

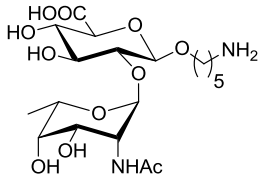
137.9, 135.7, 133.4, 133.2, 128.7 (2C), 128.5 (3C), 128.3 (2C), 128.2, 128.1, 128.0 (3C), 127.9, 127.7, 127.6, 126.8, 126.3, 126.0, 102.0, 100.3, 86.0, 79.0, 78.5, 77.4, 75.6 (2C), 75.1, 75.0, 73.8, 73.3, 70.0, 69.0, 67.3, 66.6, 50.7, 50.4, 47.6, 29.6, 27.7, 23.5, 23.3, 16.8; HRMS (MALDI-TOF): Calcd for $C_{73}H_{80}N_2O_{12}Na^+$ $[M+Na]^+$ 1199.5603, found 1199.5599.

_N_-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl 2-_N_-acetyl-3,4-di-_O_-benzyl-α-L-pneumosaminopyranosyl-(1→2)-3,4-di-_O_-benzyl-β-D-glucopyranoside (124)



According to general procedure (G), disaccharide **119** (10 mg, 8 μmol) was reacted with DDQ (6 mg, 25 μmol) for 4 h to give primary alcohol **124** (7 mg, 7 μmol, 79%). $[\alpha]_D^{20}$ = – 34.5 º (c = 0.7, $CHCl_3$); IR ν_{max} (film) 3404, 2925, 1697, 1497, 1454, 1421, 1362 1260, 1027 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.39 – 7.16 (m, 30H), 7.04 – 6.93 (m, 1H), 5.28 (s, 1H), 5.23 – 5.14 (m, 2H), 4.92 – 4.83 (m, 3H), 4.80 (d, *J* = 11.0 Hz, 1H), 4.72 (d, *J* = 12.2 Hz, 2H), 4.63 – 4.55 (m, 2H), 4.53 – 4.47 (m, 2H), 4.45 – 4.37 (m, 1H), 4.35 – 4.23 (m, 2H), 3.90 – 3.72 (m, 3H), 3.72 – 3.53 (m, 5H), 3.47 – 3.30 (m, 2H), 3.29 – 3.14 (m, 2H), 1.66 (s, 3H), 1.58 – 1.45 (m, 4H), 1.30 – 1.16 (m, 5H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 169.8, 138.5, 138.2, 138.0, 128.7 (3C), 128.5 (2C), 128.3, 128.2 (2C), 128.1, 128.0, 127.7, 127.6, 102.0, 100.4, 85.7, 78.9, 78.1, 77.4, 75.8, 75.6, 75.1, 73.2, 70.0, 67.4, 66.7, 62.1, 50.5, 47.6, 47.2, 29.4, 28.1, 27.4, 23.3, 16.7; HRMS (MALDI-TOF): Calcd for $C_{62}H_{72}N_2O_{12}Na^+$ $[M+Na]^+$ 1059.4977, found 1059.4938.

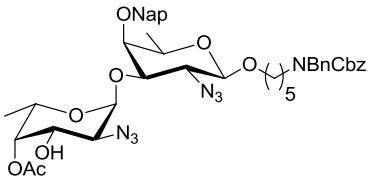
5-Amino-pentanyl 2-_N_-acetyl-α-L-pneumosaminopyranosyl-(1→2)-β-D-glucopyranosyluronate (99)



According to general procedure (H), alcohol **124** (6 mg, 6 μmol) was reacted with TEMPO (0.2 mg, 2 μmol) and BAIB (9 mg, 30 μmol) to give the protected uronate disaccharide. According to general procedure (I), the uronate disaccharide was subjected to hydrogenolysis to give **99** (1.6 mg, 3.4 μmol, 59%). ^{1}H-NMR (600 MHz, D_2O) δ 5.15 (s, 1H), 4.55 (d, *J* = 7.9

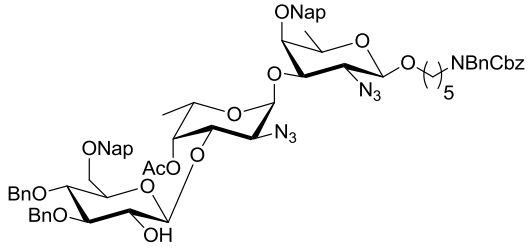
Hz, 1H), 4.33 (q, *J* = 6.7 Hz, 1H), 4.23 (d, *J* = 4.8 Hz, 1H), 4.09 (dd, *J* = 4.8, 3.2 Hz, 1H), 3.92 (dt, *J* = 10.0, 6.9 Hz, 1H), 3.85 – 3.81 (m, 1H), 3.74 – 3.65 (m, 3H), 3.55 – 3.50 (m, 1H), 3.43 (dd, *J* = 9.0, 8.0 Hz, 1H), 3.00 (t, *J* = 7.6 Hz, 2H), 2.06 (s, 3H), 1.73 – 1.66 (m, 4H), 1.49 – 1.42 (m, 2H), 1.26 (d, *J* = 6.6 Hz, 3H); ^{13}C-NMR (150 MHz, D_2O) δ 178.4, 176.74, 103.6, 102.6, 80.8, 78.8, 78.7, 74.3, 73.6, 72.7, 69.9, 66.5, 53.8, 42.0, 30.9, 28.9, 25.0, 24.7, 18.1; HRMS (ESI): Calcd for $C_{19}H_{34}N_2O_{11}Na^+$ $[M+Na]^+$ 489.2060, found 489.2060.

_N_-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl 4-_O_-acetyl-2-azido-2-deoxy-α-L-fucopyranosyl-(1→3)-2-azido-2-deoxy-4-_O_-(2-naphthalenylmethyl)-β-D-fucopyranoside (120)



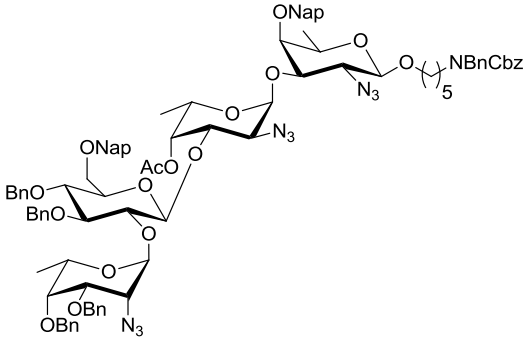
To a solution of the disaccharide **109** (110 mg, 136 μmol) in DMF (1 mL) was added trimethyl orthoacetate (104 μL, 815 μmol) and p-TSA (4 mg, 21 μmol) and the reaction mixture stirred for 30 min. Triethylamine (2 drops) was added and the solvent removed under vacuum using toluene as an azeotrope. The crude residue was taken up in 80% acetic acid (3 mL) and the reaction mixture stirred for 1 h. The solvent was removed under vacuum and azeotroped with toluene. Column chromatography on silica gel (hexanes/EtOAc) afforded **120** (92 mg, 108 μmol, 80%). $[\alpha]_D^{20}$ = – 50.9 º (c = 1.3, $CHCl_3$); IR ν_{max} (film) 3436, 2937, 2114, 1743, 1696, 1423, 1363, 1232, 1165, 1125, 1093, 1070, 1036 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.95 – 7.74 (m, 4H), 7.59 – 7.47 (m, 3H), 7.41 – 7.10 (m, 10H), 5.24 (d, *J* = 3.6 Hz, 1H), 5.17 (d, *J* = 13.3 Hz, 2H), 5.05 (d, *J* = 12.4 Hz, 1H), 4.74 (d, *J* = 12.4 Hz, 1H), 4.49 (d, *J* = 7.6 Hz, 2H), 4.41 (d, *J* = 2.2 Hz, 1H), 4.21 (dd, *J* = 11.2, 8.3 Hz, 1H), 4.01 – 3.84 (m, 2H), 3.60 – 3.37 (m, 6H), 3.31 – 3.16 (m, 3H), 2.09 (s, 3H), 1.67 – 1.48 (m, 4H), 1.44 – 1.27 (m, 5H), 0.94 (d, *J* = 6.6 Hz, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 171.3, 138.0, 136.0, 133.1 (2C), 128.6, 128.4, 127.9, 127.8, 127.3, 126.9, 126.8, 126.7, 126.5, 102.7, 99.8, 79.2, 78.1, 75.8, 72.7, 71.0, 70.0, 67.2, 66.5, 65.4, 63.9, 59.6, 50.6, 50.3, 47.2, 46.3, 29.3, 28.0, 27.5, 23.3, 20.8, 17.3, 16.1; HRMS (MALDI-TOF): Calcd for $C_{45}H_{53}N_7O_{10}Na^+$ $[M+Na]^+$ 874.3746, found 874.3737.

***N*-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl 3,4-di-*O*-benzyl-6-*O*-(2-naphthalenylmethyl)-β-D-glucopyranosyl-(1→3)-4-*O*-acetyl-2-azido-2-deoxy-α-L-fucopyranosyl-(1→3)-2-azido-2-deoxy-4-*O*-(2-naphthalenylmethyl)-β-D-fucopyranoside (121)**



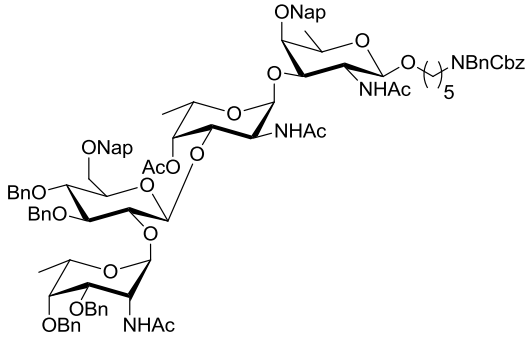
According to general procedure (B), thioglucoside **100** (75 mg, 117 μmol) was reacted with disaccharide **120** (50 mg, 59 μmol), NIS (29 mg, 129 μmol) and TfOH (1 μL, 12 μmol) in DCM (2 mL) at –20 °C to –10 °C over 1 h to give the crude trisaccharide. To a solution of the crude trisaccharide in DCM (3 mL) hydrazine hydrate (11 μL, μmol) dissolved in AcOH (40 μL) and pyridine (60 μL) was added and the solution stirred for 1 h. The reaction was then quenched by the addition of acetone and concentrated. Column chromatography on silica gel (hexanes/ethyl acetate) afforded **121** (72 mg, 54 μmol, 91%). $[\alpha]_D^{20}$ = – 19.2 ° (c = 1.0, $CHCl_3$); IR ν_{max} (film) 2936, 2115, 1697, 1454, 1360, 1235, 1069 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.87 – 7.72 (m, 8H), 7.50 – 7.17 (m, 24H), 7.10 – 7.03 (m, 2H), 5.33 (d, *J* = 3.6 Hz, 1H), 5.19 (d, *J* = 12.8 Hz, 2H), 5.05 (d, *J* = 11.3 Hz, 1H), 4.98 (d, *J* = 12.3 Hz, 1H), 4.88 – 4.70 (m, 6H), 4.60 – 4.46 (m, 4H), 4.32 – 4.19 (m, 1H), 4.09 (d, *J* = 7.0 Hz, 1H), 4.01 – 3.86 (m, 3H), 3.74 – 3.66 (m, 3H), 3.63 – 3.42 (m, 7H), 3.40 (d, *J* = 2.1 Hz, 1H), 3.33 – 3.18 (m, 3H), 2.13 (s, 3H), 1.71 – 1.50 (m, 4H), 1.47 – 1.21 (m, 5H), 0.90 (d, *J* = 6.5 Hz, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 174.1, 138.4, 138.2, 138.0, 137.9, 135.6, 133.2, 133.0, 132.9, 128.5, 128.4, 128.3, 128.1 (2C), 128.0, 127.9, 127.8, 127.7(2C), 127.6 (2C), 127.5, 127.3, 127.2, 126.7, 126.5, 126.4, 126.3, 126.1, 126.0, 125.7, 102.7, 100.3, 100.2, 85.1, 82.0, 77.9, 77.4, 77.0, 76.7, 75.5, 75.5, 75.0, 74.9, 74.5, 74.2, 73.4, 70.8, 69.8, 68.5, 67.1, 63.8, 58.1, 29.2, 27.9, 23.2, 17.1, 16.6; HRMS (MALDI-TOF): Calcd for $C_{76}H_{83}N_7O_{15}Na^+$ $[M+Na]^+$ 1356.5839, found 1356.5896.

***N*-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl 2-azido-3,4-di-*O*-benzyl-2-deoxy-α-L-pneumopyranosyl-(1→2)-3,4-di-*O*-benzyl-6-*O*-(2-naphthalenylmethyl)-β-D-glucopyranosyl-(1→3)-4-*O*-acetyl-2-azido-2-deoxy-α-L-fucopyranosyl-(1→3)-2-azido-2-deoxy-4-*O*-(2-naphthalenylmethyl)-β-D-fucopyranoside (122)**



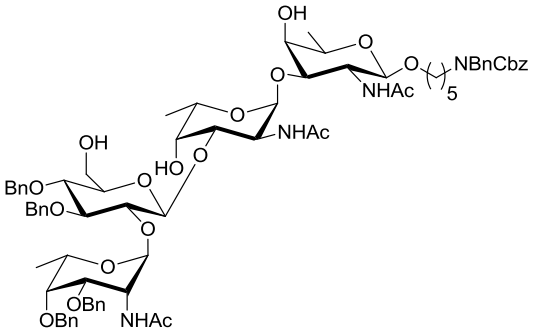
According to general procedure (E), pneumosyl-imidate **101** (100 mg, 196 μmol) and trisaccharide **121** (58 mg, 43 μmol) were reacted in DCM (2 mL) at –30 °C to –20 °C over 30 min to give **122** (40 mg, 24 μmol, 55%). $[\alpha]_D^{20}$ = – 38.3 ° (c = 1.2, $CHCl_3$); IR ν_{max} (film) 2936, 2114, 1744, 1697, 1496, 1454, 1361, 1231, 1065 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.85 – 6.94 (m, 44H), 5.34 (d, *J* = 3.7 Hz, 1H), 5.23 (s, 1H), 5.21 – 5.10 (m, 3H), 4.96 (dd, *J* = 11.8, 3.2 Hz, 2H), 4.88 (d, *J* = 11.3 Hz, 1H), 4.77 – 4.36 (m, 12H), 4.27 – 4.14 (m, 2H), 4.08 – 3.99 (m, 1H), 3.95 – 3.85 (m, 2H), 3.81 – 3.76 (m, 1H), 3.74 – 3.60 (m, 5H), 3.58 – 3.37 (m, 8H), 3.33 – 3.16 (m, 3H), 1.90 (s, 3H), 1.64 – 1.46 (m, 4H), 1.42 – 1.22 (m, 5H), 1.19 (d, *J* = 6.5 Hz, 3H), 0.83 (d, *J* = 6.4 Hz, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 170.3, 138.4, 138.0, 137.9, 137.7, 135.6, 133.2, 133.1, 133.0, 128.9, 128.7, 128.6 (2C), 128.5, 128.4, 128.3, 128.2, 128.1, 128.0 (2C), 127.9, 127.8, 127.7, 127.4, 127.2, 126.8, 126.5, 126.4, 126.1, 125.9, 125.8, 102.8, 100.1, 98.6, 96.7, 84.8, 78.7, 78.5, 77.4, 76.0, 75.3, 75.1, 75.0, 74.7, 73.3, 71.0, 70.9, 69.9, 68.9, 68.7, 67.3, 65.7, 63.9, 57.4, 57.0, 29.3, 27.6, 23.3, 20.9, 17.6, 16.8, 16.3; HRMS (MALDI-TOF): Calcd for $C_{96}H_{104}N_{10}O_{18}Na^+$ $[M+Na]^+$ 1707.7422, found 1707.7445.

***N*-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl 2-*N*-acetyl-3,4-di-*O*-benzyl-α-L-pneumosaminopyranosyl-(1→2)-3,4-di-*O*-benzyl-6-*O*-(2-naphthalenylmethyl)-β-D-glucuronatepyranosyl-(1→3)-4-*O*-acetyl-2-*N*-acetyl-α-L-fucosaminopyranosyl-(1→3)-2-*N*-acetyl-4-*O*-(2-naphthalenylmethyl)-β-D-fucosaminopyranoside (123)**



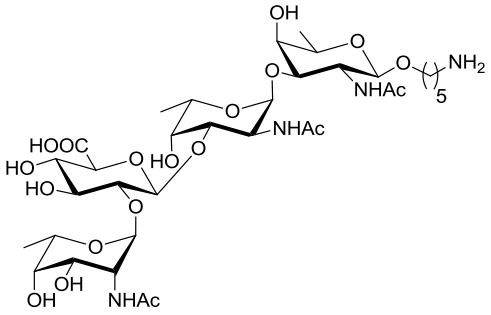
According to general procedure (F), azido-tetrasaccharide **122** (40 mg, 24 μmol) was reacted with thioacetic acid for 48 h to give **123** (31 mg, 18 μmol, 75%). $[\alpha]_D^{20}$ = – 58.3 º (c = 1.2, $CHCl_3$); IR ν_{max} (film) 2963, 1740, 1674, 1519, 1454, 1365, 1260, 1234, 1025 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.89 – 6.61 (m, 44H), 5.40 – 5.04 (m, 6H), 4.95 – 4.80 (m, 2H), 4.79 – 4.11 (m, 16H), 4.10 – 3.92 (m, 2H), 3.91 – 3.73 (m, 4H), 3.74 – 3.54 (m, 4H), 3.54 – 3.31 (m, 4H), 3.30 – 3.02 (m, 4H), 2.00 (s, 3H), 1.90 – 1.85 (m, 3H), 1.68 (s, 3H), 1.58 – 1.38 (m, 4H), 1.38 – 1.23 (m, 5H), 1.20 (d, *J* = 6.4 Hz, 3H), 1.10 – 1.00 (m, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 171.2, 170.7 (2C), 170.0, 138.5, 138.1, 137.9, 137.7, 136.2, 133.4, 133.1 (2C), 133.0, 128.8, 128.7, 128.5 (2C), 128.4, 128.2, 128.0, 127.9, 127.7 (2C), 127.6, 127.5, 127.4, 127.3, 126.7, 126.3, 125.9, 125.5, 100.7, 98.7, 98.6, 97.1, 84.1, 79.1, 78.4, 77.4, 75.6, 75.0, 74.5, 74.0, 73.8, 73.5, 73.3, 73.1, 70.7, 70.1, 69.7, 69.3, 68.1, 67.3, 66.9, 66.0, 50.4, 48.6, 47.6, 47.4, 29.0, 27.2, 23.8, 23.4, 23.1, 21.0, 17.3, 16.9, 16.7; HRMS (MALDI-TOF): Calcd for $C_{102}H_{116}N_4O_{21}Na^+$ $[M+Na]^+$ 1755.8024, found 1755.8089.

***N*-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl 2-*N*-acetyl-3,4-di-*O*-benzyl-α-L-pneumosaminopyranosyl-(1→2)-3,4-di-*O*-benzyl-β-D-glucopyranosyl-(1→3)-2-*N*-acetyl-α-L-fucosaminopyranosyl-(1→3)-2-*N*-acetyl-β-D-fucosaminopyranoside (129)**



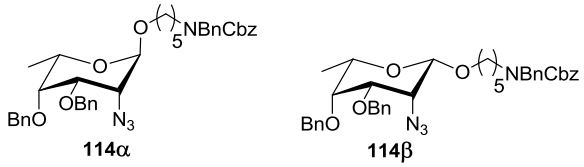
To a solution of **123** (19 mg, 11 μmol) in DCM/MeOH (1:1, 1 mL) was added 0.5 M NaOMe in MeOH (0.5 mL) and stirred for 16 h. The mixture was neutralized with Amberlite® IR 120 (H^+) ion exchange resin, filtered and concentrated. According to general procedure (G), the crude material was reacted with DDQ (9 mg, 40 μmol) in DCM/H_2O to give triol **129** (10 mg, 7 μmol, 64%). $[\alpha]_D^{20}$ = – 69.7 ° (c = 1.0, $CHCl_3$); IR ν_{max} (film) 3400, 3030, 2933, 1656, 1524, 1497, 1454, 1421, 1366, 1305, 1232, 1055 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.53 – 6.98 (m, 30H), 5.25 – 5.01 (m, 4H), 4.94 – 4.70 (m, 4H), 4.67 – 4.34 (m, 7H), 4.28 – 4.10 (m, 4H), 4.00 – 3.10 (m, 19H), 2.06 (s, 3H), 1.99 – 1.94 (m, 3H), 1.70 (s, 3H), 1.63 – 1.37 (m, 4H), 1.37 – 1.07 (m, 11H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 171.6, 170.9, 170.1, 138.3, 128.7, 128.6 (2C), 128.5, 128.4, 128.3, 128.2, 128.1, 127.7, 127.5, 127.3, 127.2, 126.8, 101.7, 101.4, 101.2, 100.6, 99.5, 84.2, 80.8, 78.5, 77.5, 77.4, 77.2, 76.8, 75.7, 75.5, 75.2, 72.7, 72.5, 71.8, 71.3, 70.2, 69.7, 68.7, 68.6, 68.2, 67.3, 66.9, 61.9, 51.4, 50.6, 49.5, 48.5, 47.9, 23.9, 23.8, 23.5, 21.2, 16.6; HRMS (MALDI-TOF): Calcd for $C_{78}H_{98}N_4O_{20}Na^+$ $[M+Na]^+$ 1433.6666, found 1433.6640.

5-Amino-pentanyl 2-*N*-acetyl-α-L-pneumosaminopyranosyl-(1→2)-β-D-glucopyranosyluronate-(1→3)-2-*N*-acetyl-α-L-fucosaminopyranosyl-(1→3)-2-*N*-acetyl-β-D-fucosaminopyranoside (102)



According to general procedure (H), triol **129** (10 mg, 7 μmol) was reacted with TEMPO (0.3 mg, 2 μmol) and BAIB (6 mg, 19 μmol) to give the protected uronate tetrasaccharide. According to general procedure (I), the uronate tetrasaccharide was subjected to hydrogenolysis to give **102** (4.9 mg, 5.8 μmol, 83%). ^{1}H-NMR (600 MHz, D_2O) δ 5.18 (s, 1H), 5.07 (d, *J* = 3.9 Hz, 1H), 4.69 (d, *J* = 7.8 Hz, 1H), 4.65 (q, *J* = 6.7 Hz, 1H), 4.41 (d, *J* = 8.6 Hz, 1H), 4.37 (dd, *J* = 11.6, 3.0 Hz, 1H), 4.18 (d, *J* = 4.8 Hz, 1H), 4.12 (dd, *J* = 4.7, 3.2 Hz, 1H), 4.10 – 4.06 (m, 2H), 4.03 (dd, *J* = 11.6, 3.9 Hz, 1H), 3.94 (t, *J* = 9.6 Hz, 1H), 3.88 (dt, *J* = 10.1, 6.0 Hz, 1H), 3.82 – 3.74 (m, 4H), 3.73 – 3.68 (m, 2H), 3.61 – 3.52 (m, 2H), 3.51 (dd, *J* = 9.2, 7.9 Hz, 1H), 3.02 – 2.98 (m, 2H), 2.05 (s, 3H), 2.04 (s, 3H), 2.00 (s, 3H), 1.71 – 1.65 (m, 2H), 1.61 – 1.56 (m, 2H), 1.44 – 1.37 (m, 2H), 1.28 (d, *J* = 6.5 Hz, 3H), 1.24 (d, *J* = 6.6 Hz, 3H), 1.15 (d, *J* = 6.6 Hz, 3H); ^{13}C-NMR (150 MHz, D_2O) δ 178.4, 176.8, 176.5, 176.3, 104.2, 102.1, 101.0, 98.2, 79.1, 78.8, 78.1, 74.3, 73.7, 73.5, 73.2, 73.1, 72.6, 69.8, 69.6, 68.9, 66.4, 54.0, 53.7, 51.0, 41.9, 30.8, 29.0, 25.0, 24.9, 24.8, 24.7, 18.1, 18.0, 17.9; HRMS (ESI): Calcd for $C_{35}H_{60}N_4O_{19}Na^+$ $[M+Na]^+$ 863.3744, found 864.3774.

***N*-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl 2-azido-3,4-di-*O*-benzyl-2-deoxy-L-pneumopyranosides (114α and 114β)**

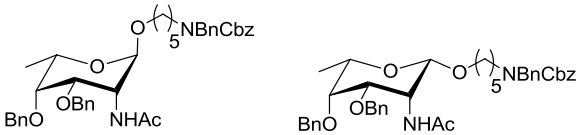


114α **114β**

According to general procedure (E), pneumosyl-imidate **101** (30 mg, 58 μmol) and *N*-(Benzyl)benzyloxycarbonyl-5-amino-pentanol (38 mg, 117 μmol) were reacted in DCM (1 mL) at –30 °C to –20 °C over 30 min to give α and β anomers **114α** (28 mg, 41 μmol, 71%) and **114β** (10 mg, 15 μmol, 25%). **114α**: $[\alpha]_D^{20}$ = – 18.0 ° (c = 1.3, $CHCl_3$); IR ν_{max} (film) 2934, 2111, 1697, 1496, 1454, 1422, 1360, 1229, 1061 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ

7.60 – 7.06 (m, 20H), 5.18 (d, *J* = 13.0 Hz, 2H), 4.99 (d, *J* = 11.7 Hz, 1H), 4.79 – 4.69 (m, 3H), 4.64 (d, *J* = 11.8 Hz, 1H), 4.50 (d, *J* = 6.9 Hz, 2H), 3.98 – 3.85 (m, 2H), 3.83 – 3.73 (m, 1H), 3.62 (s, 1H), 3.58 – 3.45 (m, 1H), 3.37 – 3.15 (m, 3H), 1.58 – 1.41 (m, 4H), 1.31 – 1.16 (m, 5H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 138.4, 137.8, 128.8, 128.7, 128.6, 128.3, 128.1, 127.9 (2C), 127.7, 127.4, 98.6, 77.2, 75.2, 74.9, 71.2, 67.7, 67.3, 66.9, 58.1, 50.6, 50.3, 47.2, 46.2, 29.1, 23.5, 16.9. **114β**: $[\alpha]_D^{20}$ = + 59.2 º (c = 0.9, $CHCl_3$); IR ν_{max} (film) 2928, 2110, 1697, 1496, 1454, 1421, 1359, 1229, 1119, 1070 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.55 – 7.09 (m, 20H), 5.17 (d, *J* = 12.4 Hz, 2H), 5.02 (d, *J* = 11.9 Hz, 1H), 4.77 – 4.70 (m, 2H), 4.62 (d, *J* = 12.2 Hz, 1H), 4.53 – 4.44 (m, 2H), 4.31 (d, *J* = 14.7 Hz, 1H), 3.96 – 3.78 (m, 2H), 3.52 (s, 2H), 3.44 – 3.14 (m, 4H), 1.62 – 1.45 (m, 4H), 1.38 – 1.20 (m, 5H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 138.6, 138.1, 137.6, 128.7, 128.6, 128.2, 128.1, 128.0, 127.9, 127.6 (2C), 127.4, 100.1, 78.8, 77.5, 77.2, 76.8, 74.8, 74.1, 71.8, 70.8, 69.3, 67.2, 58.8, 29.3, 23.4, 16.9; HRMS (MALDI-TOF): Calcd for $C_{40}H_{46}N_4O_6Na^+$ $[M+Na]^+$ 701.3310, found 701.3337.

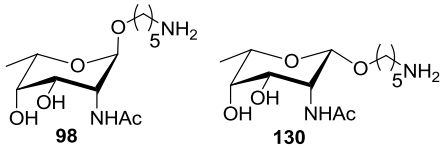
_N_-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl 2-_N_-acetyl-3,4-di-_O_-benzyl-L-pneumosaminopyranosides (115α and 115β)



According to general procedure (F), azido-pneumosides **114α** (28 mg, 41 μmol) and **114β** (10 mg, 15 μmol) were individually reacted with thioacetic acid for 12 h to give **115α** (19 mg, 27 μmol, 66%) and **115β** (7 mg, 10 μmol, 68%). **115α**: $[\alpha]_D^{20}$ = – 47.4 º (c = 1.9, $CHCl_3$); IR ν_{max} (film) 3404, 2935, 1698, 1675, 1515, 1497, 1454, 1421, 1361, 1305, 1228, 1120, 1055, 1040 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.43 – 7.12 (m, 20H), 5.18 (d, *J* = 14.2 Hz, 2H), 4.90 (d, *J* = 10.3 Hz, 1H), 4.75 – 4.68 (m, 2H), 4.60 (d, *J* = 10.3 Hz, 1H), 4.56 – 4.40 (m, 4H), 3.92 – 3.79 (m, 2H), 3.67 – 3.60 (m, 1H), 3.59 – 3.15 (m, 5H), 1.74 (s, 3H), 1.61 – 1.40 (m, 4H), 1.35 – 1.15 (m, 5H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 170.4, 138.3, 138.2, 138.0, 128.7, 128.6, 128.5 (2C), 128.2, 128.1, 127.9, 127.7 (2C), 127.4, 127.3, 100.1, 78.7, 75.7, 72.8, 69.9, 67.7, 67.3, 66.3, 50.6, 50.3, 48.0, 47.2, 46.2, 29.3, 28.1, 27.7, 23.5, 16.8. **115β**: $[\alpha]_D^{20}$ = + 18.3 º (c = 0.7, $CHCl_3$); IR ν_{max} (film) 3420, 2929, 2865, 1698, 1677, 1521, 1454, 1421, 1367, 1312, 1230, 1113, 1058 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.42 – 7.13 (m, 20H), 6.79 (d, *J* = 9.8 Hz, 1H), 5.16 (d, *J* = 11.6 Hz, 2H), 4.91 (d, *J* = 10.2 Hz, 1H), 4.84 – 4.73 (m, 2H), 4.58 (d, *J* = 10.2 Hz, 1H), 4.52 – 4.42 (m, 3H), 4.28 (d, *J* = 13.1 Hz, 1H), 3.80 – 3.65 (m, 1H), 3.59 – 3.51 (m, 2H), 3.50 – 3.35 (m, 2H), 3.27 – 3.10 (m, 2H), 1.76 (s, 3H), 1.59 – 1.43 (m, 4H),

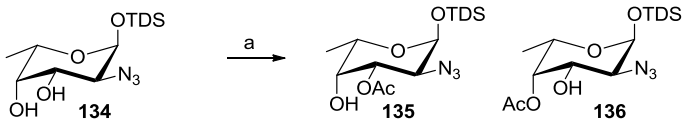
1.34 – 1.17 (m, 5H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 170.7, 138.2, 138.1, 128.8, 128.6 (2C), 128.5, 128.1, 128.0, 127.9 (2C), 127.8, 127.3, 100.5, 77.7, 76.2, 75.7, 71.4, 69.8, 67.2, 47.9, 29.3, 23.6, 23.3, 16.8; HRMS (MALDI-TOF): Calcd for $C_{42}H_{50}N_2O_7Na^+$ $[M+Na]^+$ 717.3510, found 717.3543.

5-Amino-pentanyl 2-*N*-acetyl-L-pneumosaminopyranosides (98 and 130)



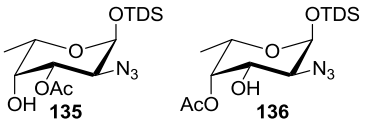
According to general procedure (I), pneumosaminosides **115α** (19 mg, 27 μmol) and **115β** (7 mg, 10 μmol) were individually submitted to hydrogenolysis to give **98** (7 mg, 24 μmol, 88%) and **130** (2 mg, 7 μmol, 68%), respectively. **98**: ^{1}H-NMR (400 MHz, D_2O) δ 4.75 (s, 1H), 4.13 – 3.93 (m, 3H), 3.78 (s, 1H), 3.71 – 3.61 (m, 1H), 3.55 – 3.44 (m, 1H), 3.03 – 2.91 (m, 2H), 2.01 (s, 3H), 1.74 – 1.57 (m, 4H), 1.49 – 1.35 (m, 2H), 1.22 (d, *J* = 6.6 Hz, 3H); ^{13}C-NMR (150 MHz, D_2O) δ 176.8, 101.6, 73.5, 70.2, 69.5, 66.6, 53.9, 42.0, 30.5, 29.2, 25.0, 18.1. **130**: ^{1}H-NMR (400 MHz, D_2O) δ 4.61 (d, *J* = 1.7 Hz, 1H), 4.29 (d, *J* = 5.0 Hz, 1H), 3.88 (dd, *J* = 4.5, 3.4 Hz, 1H), 3.81 (dt, *J* = 9.9, 6.3 Hz, 1H), 3.71 – 3.58 (m, 3H), 3.00 – 2.93 (m, 2H), 2.02 (s, 3H), 1.68 – 1.54 (m, 4H), 1.42 – 1.33 (m, 2H), 1.25 (d, *J* = 6.5 Hz, 3H); ^{13}C-NMR (150 MHz, D_2O) δ 177.2, 102.4, 74.2, 72.9, 72.0, 70.1, 54.5, 42.0, 30.8, 29.3, 25.9, 25.2, 24.8, 18.0; HRMS (ESI): Calcd for $C_{13}H_{26}N_2O_5Na^+$ $[M+Na]^+$ 313.1739, found 313.1750.

6.5.3 Synthesis of Oligosaccharides 132–133

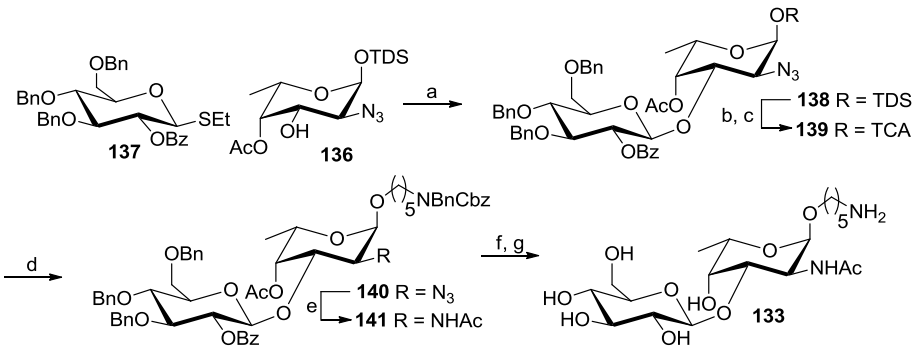


Scheme 51: Synthesis of **135** and **136**. Reagents and conditions: a) AcCl, DCM/pyridine, 0 °C, **135**: 72%, **136**: 10%.

Thexyldimethylsilyl 3-*O*-acetyl-2-azido-2-deoxy-α-L-fucopyranoside (135) and Thexyldimethylsilyl 4-*O*-acetyl-2-azido-2-deoxy-α-L-fucopyranoside (136)

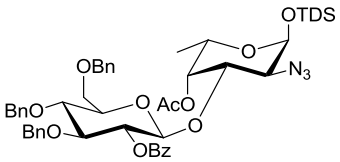


To a solution of diol **134** (200 mg, 0.60 mmol) in DCM/pyridine (4:1 (v/v), 10 mL), a solution of AcCl (51 µL, 0.72 mmol) in DCM (1 mL) was added drop-wise at 0 °C and stirred for 6 h at the same temperature. The mixture was diluted with DCM and washed with sat. aq. $NaHCO_3$. The combined organic layers were dried over $MgSO_4$, filtered and concentrated. Column chromatography on silica gel (DCM/MeOH/acetone) afforded **135** (163 mg, 0.44 mmol, 72%). $[\alpha]_D^{20}$ = + 4.4 º (c = 1.0, $CHCl_3$); IR ν_{max} (film) 3483, 2958, 2868, 2111, 1747, 1726, 1465, 1370, 1251, 1171, 1145, 1069, 1039 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 4.66 (dd, *J* = 10.8, 3.1 Hz, 1H), 4.50 (d, *J* = 7.6 Hz, 1H), 3.77 (d, *J* = 2.6 Hz, 1H), 3.61 (qd, *J* = 6.4, 0.8 Hz, 1H), 3.55 (dd, *J* = 10.8, 7.6 Hz, 1H), 2.16 (s, 3H), 1.66 (dt, *J* = 13.7, 6.9 Hz, 1H), 1.28 (d, *J* = 6.5 Hz, 3H), 0.89 (d, *J* = 1.0 Hz, 3H), 0.88 (s, 9H), 0.19 (s, 6H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 170.3, 97.3, 74.0, 70.4, 69.5, 63.5, 34.0, 25.0, 21.2, 20.1, 20.0, 18.6, 18.5, 16.4, -1.9, -3.2; HRMS (MALDI-TOF): Calcd for $C_{16}H_{31}N_3O_5SiNa^+$ $[M+Na]^+$ 396.1925, found 396.1911. **Thexyldimethylsilyl 4-*O*-acetyl-2-azido-2-deoxy-α-L-fucopyranoside (136)** (22 mg, 0.06 mmol, 10%) was obtained as a byproduct. $[\alpha]_D^{20}$ = + 5.3 º (c = 1.3, $CHCl_3$); IR ν_{max} (film) 3462, 2959, 2869, 2112, 1744, 1465, 1443, 1380, 1252, 1185, 1114, 1073 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 5.09 (dd, *J* = 3.5, 1.1 Hz, 1H), 4.48 (d, *J* = 7.6 Hz, 1H), 3.65 (qd, *J* = 6.4, 1.2 Hz, 1H), 3.57 (dd, *J* = 10.4, 3.6 Hz, 1H), 3.40 (dd, *J* = 10.4, 7.6 Hz, 1H), 2.19 (s, 3H), 1.68 (dt, *J* = 13.7, 6.9 Hz, 1H), 1.17 (d, *J* = 6.5 Hz, 3H), 0.93 – 0.86 (m, 12H), 0.19 (d, *J* = 1.6 Hz, 6H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 171.7, 97.2, 71.9, 71.1, 69.4, 66.5, 34.0, 25.0, 21.0, 20.1, 20.0, 18.6, 18.3, 16.5, -1.9, -3.0; HRMS (MALDI-TOF): Calcd for $C_{16}H_{31}N_3O_5SiNa^+$ $[M+Na]^+$ 396.1925, found 396.1954.



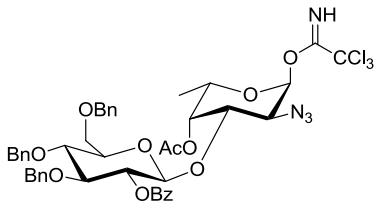
Scheme 52: Synthesis of **133**. Reagents and conditions: a) NIS/TfOH, DCM, 4 Å MS, –25 °C to –20 °C, 92%; b) TBAF/AcOH, THF, 0° C to rt; c) trichloroacetonitrile, DBU, DCM, 0 °C, 81% over two steps; d) $HO(CH_2)_5NBnCbz$, TMSOTf, DCM, 4Å MS, –30°C to –20 °C, 87%, α/β = 1:6; e) AcSH, pyridine, 79%; f) NaOMe, MeOH; g) H_2, Pd/C, EtOH/EtOAc/H_2O/AcOH, 89% over two steps.

Thexyldimethylsilyl 2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-glucopyranosyl-(1→3)-4-*O*-acetyl-2-azido-2-deoxy-α-L-fucopyranoside (138)



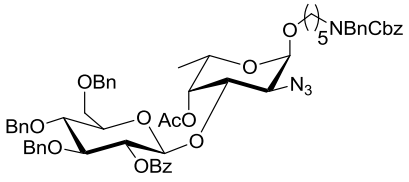
According to general procedure (B), thioglucoside **137** (109 mg, 0.18 mmol) was reacted with fucoside **136** (62 mg, 0.17 mmol), NIS (45 mg, 0.20 mmol) and TfOH (1.8 µL, 20 µmol) in DCM (1 mL) at –25 °C to –20 °C over 1 h. After workup column chromatography (hexanes/EtOAc) afforded **138** (139 mg, 0.15 mmol, 92%). $[\alpha]_D^{20}$ = – 13.9 ° (c = 1.9, $CHCl_3$); IR ν_{max} (film) 2958, 2867, 2113, 1744, 1496, 1453, 1364, 1267, 1234, 1179, 1095, 1071 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.10 – 8.03 (m, 2H), 7.60 – 7.54 (m, 1H), 7.50 – 7.42 (m, 2H), 7.41 – 7.21 (m, 10H), 7.16 – 7.02 (m, 5H), 5.32 (dd, *J* = 9.1, 7.7 Hz, 1H), 5.12 (d, *J* = 2.8 Hz, 1H), 4.82 (d, *J* = 11.0 Hz, 1H), 4.76 – 4.66 (m, 4H), 4.63 (dd, *J* = 11.0, 6.5 Hz, 2H), 4.36 (d, *J* = 7.7 Hz, 1H), 3.89 (dd, *J* = 10.4, 3.5 Hz, 1H), 3.86 – 3.80 (m, 2H), 3.79 – 3.72 (m, 2H), 3.62 (ddd, *J* = 9.4, 5.3, 1.9 Hz, 1H), 3.50 – 3.41 (m, 2H), 1.73 – 1.62 (m, 1H), 1.49 (s, 3H), 1.07 (d, *J* = 6.4 Hz, 3H), 0.92 – 0.87 (m, 12H), 0.17 (d, *J* = 4.6 Hz, 6H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 170.6, 164.9, 138.6, 138.0, 137.8, 133.3, 130.2, 129.8, 128.6, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.7, 127.6 (2C), 97.1, 96.8, 83.0, 78.1, 75.7, 75.3, 75.1, 74.6, 73.7, 73.5, 69.0, 68.9, 67.8, 63.9, 34.0, 25.0, 20.2, 20.1 (2C), 18.6 (2C), 16.5, -1.8, -3.0; HRMS (MALDI-TOF): Calcd for $C_{50}H_{63}N_3O_{11}SiNa^+$ $[M+Na]^+$ 932.4124, found 932.4125.

2-*O*-Benzoyl-3,4,6-tri-*O*-benzyl-β-D-glucopyranosyl-(1→3)-4-*O*-acetyl-2-azido-2-deoxy-α-L-fucopyranosyl trichloroacetimidate (139)



According to general procedure (A), disaccharide **138** (55 mg, 60 µmol) was reacted with TBAF (1 M in THF, 600 µL, 600 µmol) and AcOH (42 µL, 725 µmol) in THF (2 mL) to give the crude lactol. According to general procedure (D), the crude lactol was reacted with trichloroacetonitrile (60 µL, 600 µmol) and DBU in DCM (2 mL) at 0 °C to afford **139** (44 mg, 48 mmol, 81%). $[\alpha]_D^{20}$ = – 64.8 ° (c = 1.8, $CHCl_3$)IR ν_{max} (film) 2871, 2115, 1743, 1673, 1496, 1453, 1361, 1267, 1229, 1093, 1070, 1027 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.67 (s, 1H), 8.11 – 8.03 (m, 2H), 7.60 – 7.56 (m, 1H), 7.50 – 7.43 (m, 2H), 7.42 – 7.26 (m, 8H), 7.24 – 7.18 (m, 2H), 7.16 – 7.03 (m, 5H), 6.35 (d, *J* = 3.6 Hz, 1H), 5.38 – 5.30 (m, 2H), 4.84 – 4.78 (m, 2H), 4.73 (d, *J* = 11.0 Hz, 1H), 4.67 – 4.54 (m, 5H), 4.21 (q, *J* = 6.4 Hz, 1H), 3.88 – 3.71 (m, 5H), 3.61 (ddd, *J* = 9.5, 4.7, 1.8 Hz, 1H), 1.44 (s, 3H), 1.08 (d, *J* = 6.5 Hz, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 170.3, 165.0, 160.9, 138.3, 137.9, 137.8, 133.4, 130.3, 129.7, 128.6, 128.5 (2C), 128.4, 128.2, 128.1, 128.0, 127.8, 127.7, 96.8, 95.4, 91.1, 82.9, 78.1, 75.8, 75.4, 75.2, 73.9, 73.7, 70.8, 69.0, 68.2, 68.0, 57.2, 19.9, 16.3; HRMS (ESI): Calcd for $C_{44}H_{45}Cl_3N_4O_{11}Na^+$ $[M+Na]^+$ 933.2048, found 933.2051.

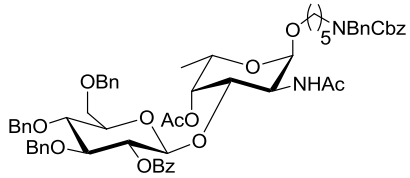
***N*-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl 2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-glucopyranosyl-(1→3)-4-*O*-acetyl-2-azido-2-deoxy-L-fucopyranoside (140)**



According to general procedure (E), disaccharide-imidate **139** (44 mg, 48 µmol) and *N*-(benzyl)benzyloxycarbonyl-5-amino-pentanol (32 mg, 96 µmol) were reacted in DCM (2 mL) at –30 °C to –20 °C over 30 min to give the α and β anomers of **140** (45 mg, 42 µmol, 87%) in a ratio α/β=1:6. Analytical data is given for the α anomer. $[\alpha]_D^{20}$ = – 68.5 ° (c = 0.5, $CHCl_3$); IR ν_{max} (film) 2927, 2111, 1743, 1698, 1454, 1267, 1091 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.06 (d, *J* = 7.9 Hz, 2H), 7.57 (t, *J* = 7.4 Hz, 1H), 7.44 (d, *J* = 7.6 Hz, 2H), 7.39 – 7.27 (m, 15H), 7.23 – 7.01 (m, 10H), 5.31 (t, *J* = 8.4 Hz, 1H), 5.25 (s, 1H), 5.16 (d, *J* = 7.8 Hz, 2H),

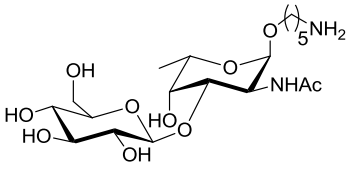
4.85 – 4.78 (m, 2H), 4.74 – 4.69 (m, 2H), 4.63 – 4.52 (m, 4H), 4.45 (s, 3H), 4.01 – 3.90 (m, 1H), 3.85 – 3.69 (m, 4H), 3.59 (dd, *J* = 8.6, 4.1 Hz, 2H), 3.41 (dd, *J* = 10.8, 3.6 Hz, 2H), 3.18 (d, *J* = 20.7 Hz, 2H), 1.56 – 1.43 (m, 4H), 1.42 (s, 3H), 1.35 – 1.18 (m, 3H), 1.02 (d, *J* = 6.4 Hz, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 170.5, 165.0, 138.4, 138.0, 137.8, 133.3, 130.3, 129.8, 128.7, 128.6, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.7 (2C), 98.6, 97.4, 83.1, 78.1, 77.4, 75.7, 75.4, 75.2, 73.7, 73.6, 70.9, 69.1, 68.9, 68.5, 67.3, 65.0, 57.8, 50.6, 47.2, 46.2, 29.2, 23.4, 20.0, 16.3; HRMS (ESI): Calcd for $C_{62}H_{68}N_4O_{13}Na^+$ $[M+Na]^+$ 1099.4681, found 1099.4679.

_N_-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl 2-_O_-benzoyl-3,4,6-tri-_O_-benzyl-β-D-glucopyranosyl-(1→3)-2-_N_-acetyl-4-_O_-acetyl-α-L-fucosaminopyranoside (141)

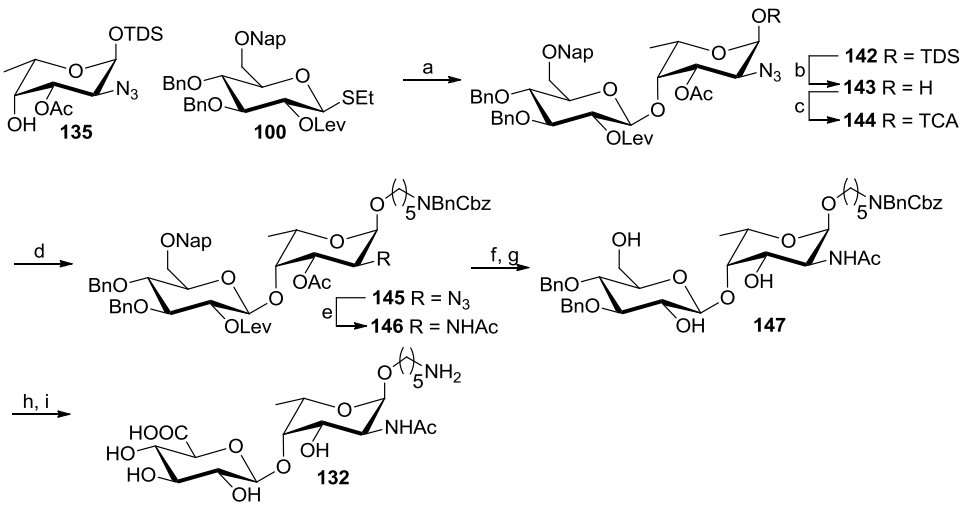


According to general procedure (F), azido-disaccharide **140** (10 mg, 9 μmol) was reacted with thioacetic acid for 48 h to give **141** (8 mg, 7 μmol, 79%). $[\alpha]_D^{20}$ = – 5.5 º (c = 1.0, $CHCl_3$); IR ν_{max} (film) 2928, 1743, 1697, 1454, 1365, 1266, 1232, 1091 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.06 – 8.01 (m, 2H), 7.61 – 7.55 (m, 1H), 7.47 – 7.42 (m, 2H), 7.38 – 7.26 (m, 15H), 7.26 – 7.20 (m, 2H), 7.18 – 7.06 (m, 8H), 6.50 – 6.27 (m, 1H), 5.22 – 5.12 (m, 5H), 4.80 (d, *J* = 10.9 Hz, 1H), 4.69 (d, *J* = 10.9 Hz, 1H), 4.66 – 4.60 (m, 2H), 4.50 (dd, *J* = 22.3, 11.3 Hz, 5H), 4.08 (s, 2H), 3.98 – 3.86 (m, 1H), 3.82 – 3.49 (m, 6H), 3.43 – 3.28 (m, 1H), 3.28 – 3.06 (m, 2H), 1.83 (s, 3H), 1.64 (s, 3H), 1.55 – 1.42 (m, 4H), 1.29 – 1.16 (m, 2H), 1.01 (d, *J* = 6.5 Hz, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 170.7, 165.1, 138.0, 137.8, 137.6, 133.3, 130.1, 129.8, 128.7 (2C), 128.6, 128.4 (2C), 128. (2C), 127.9, 127.8, 98.9, 97.1, 82.9, 77.8, 77.4, 75.2 (2C), 74.5, 73.5, 73.3, 72.6, 69.9, 68.9, 68.3, 67.3, 64.8, 49.7, 29.2, 23.4, 23.1, 20.2, 16.3; HRMS (MALDI-TOF): Calcd for $C_{64}H_{72}N_2O_{14}Na^+$ $[M+Na]^+$ 1115.4876, found 1115.4890.

5-Amino-pentanyl β-D-glucopyranosyl-(1→3)-2-_N_-acetyl-α-L-fucosaminopyranoside (133)

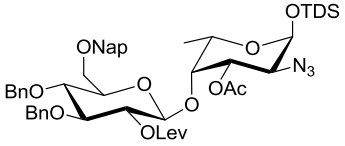


To a solution of **141** (8 mg, 7 μmol) in MeOH (1 mL) was added 0.5 M NaOMe in MeOH (0.2 mL) and stirred for 16 h. The mixture was neutralized with Amberlite® IR 120 (H^+) ion exchange resin and the resulting diol was filtered and concentrated. According to general procedure (I), the crude diol was submitted to hydrogenolysis to give **133** (3.4 mg, 7.5 μmol, 89%). ^{1}H-NMR (600 MHz, D_2O) δ 4.95 (d, *J* = 3.7 Hz, 1H), 4.55 (d, *J* = 7.9 Hz, 1H), 4.19 (dd, *J* = 11.3, 3.7 Hz, 1H), 4.13 (dd, *J* = 11.3, 2.9 Hz, 1H), 4.09 (q, *J* = 6.6 Hz, 1H), 4.03 (d, *J* = 2.5 Hz, 1H), 3.98 (dd, *J* = 12.2, 2.1 Hz, 1H), 3.75 – 3.68 (m, 2H), 3.54 – 3.45 (m, 3H), 3.38 (t, *J* = 9.5 Hz, 1H), 3.31 (dd, *J* = 9.4, 8.0 Hz, 1H), 3.03 – 2.98 (m, 2H), 2.05 (s, 3H), 1.73 – 1.62 (m, 4H), 1.49 – 1.41 (m, 2H), 1.27 (d, *J* = 6.6 Hz, 3H); ^{13}C-NMR (150 MHz, D_2O) δ 177.1, 103.0, 99.3, 78.7, 78.1, 77.8, 75.4, 72.3, 71.3, 70.4, 69.0, 63.6, 50.9, 42.0, 30.5, 29.0, 24.8, 24.6, 18.1; HRMS (ESI): Calcd for $C_{19}H_{36}N_2O_{10}Na^+$ $[M+Na]^+$ 475.2268, found 475.2273.



Scheme 53: Synthesis of **132**. Reagents and conditions: a) NIS/TfOH, DCM, 4 Å MS, –30 °C to –20 °C, 89%; b) TBAF/AcOH, THF, 0° C to rt, 95%; c) trichloroacetonitrile, DBU, DCM, 0 °C, 86%; d) $HO(CH_2)_5NBnCbz$, TMSOTf, DCM, 4Å MS, –30°C to –20 °C, 90%, α/β = 1:3; e) AcSH, pyridine, 70%; f) DDQ, DCM, phosphate buffer, pH 7.2, 0 °C to rt; g) NaOMe, MeOH, 60% over two steps; h) TEMPO, BAIB, DCM, H_2O, 0 °C to rt; i) H_2, Pd/C, EtOH/H_2O/AcOH, 57% over two steps.

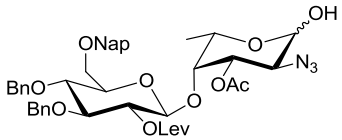
Thexyldimethylsilyl 3,4-di-*O*-benzyl-2-*O*-levulinoyl-6-*O*-(2-naphthalenylmethyl)-β-D-glucopyranosyl-(1→4)-3-*O*-acetyl-2-azido-2-deoxy-α-L-fucopyranoside (142)



According to general procedure (B), thioglucoside **100** (140 mg, 218 μmol) was reacted with fucoside **135** (50 mg, 134 μmol), NIS (54 mg, 240 μmol) and TfOH (2.4 μL, 27 μmol) in

DCM (1 mL) at –30 °C to –20 °C over 1 h. After workup column chromatography (hexanes/EtOAc) afforded **142** (114 mg, 119 μmol, 89%). $[\alpha]_D^{20}$ = – 13.0 º (c = 1.8, $CHCl_3$); IR ν_{max} (film) 2958, 2867, 2112, 1748, 1721, 1603, 1509, 1455, 1364, 1240, 1147, 1072 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.87 – 7.76 (m, 4H), 7.52 – 7.43 (m, 3H), 7.35 – 7.25 (m, 5H), 7.23 – 7.12 (m, 3H), 7.04 – 6.98 (m, 2H), 5.16 (dd, *J* = 9.6, 8.1 Hz, 1H), 4.80 – 4.75 (m, 3H), 4.70 (d, *J* = 12.1 Hz, 1H), 4.60 (d, *J* = 12.2 Hz, 1H), 4.49 – 4.39 (m, 3H), 4.30 (d, *J* = 8.1 Hz, 1H), 3.94 (d, *J* = 3.4 Hz, 1H), 3.85 – 3.79 (m, 1H), 3.76 – 3.65 (m, 3H), 3.58 (dd, *J* = 11.0, 7.6 Hz, 1H), 3.52 (q, *J* = 6.5 Hz, 1H), 3.39 (ddd, *J* = 9.8, 3.5, 2.0 Hz, 1H), 2.93 – 2.81 (m, 1H), 2.68 – 2.63 (m, 1H), 2.62 – 2.58 (m, 1H), 2.54 – 2.45 (m, 1H), 2.16 (s, 3H), 2.11 (s, 3H), 1.67 (dt, *J* = 13.7, 6.9 Hz, 1H), 1.25 (d, *J* = 6.5 Hz, 3H), 0.92 (s, 3H), 0.90 (s, 3H), 0.89 (s, 6H), 0.18 (s, 3H), 0.15 (s, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 206.4, 171.4, 171.2, 138.3, 137.9, 135.1, 133.3, 133.1, 128.4 (3C), 128.1, 128.0, 127.9, 127.8 (3C), 126.6, 126.3, 126.1, 125.8, 101.9, 97.2, 82.9, 77.5, 75.1, 75.0 (2C), 74.3, 73.8, 73.4, 72.8, 70.0, 68.7, 62.8, 38.1, 34.0, 29.9, 28.0, 24.9, 21.0, 20.0 (2C), 18.6, 18.5, 16.4, -2.0, -3.1; HRMS (MALDI-TOF): Calcd for $C_{52}H_{67}N_3O_{12}SiNa^+$ $[M+Na]^+$ 976.4386, found 976.4333.

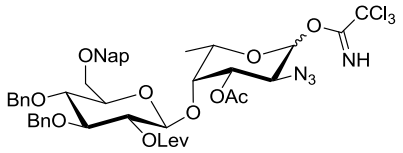
3,4-Di-*O*-benzyl-2-*O*-levulinoyl-6-*O*-(2-naphthalenylmethyl)-β-D-glucopyranosyl-(1→4)-3-*O*-acetyl-2-azido-2-deoxy-L-fucopyranoside (143)



According to general procedure (A), disaccharide **142** (105 mg, 0.11 mmol) was reacted with TBAF (1.0 mL, 1.00 mmol) and AcOH (70 μL, 1.22 mmol) in THF (2 mL) to give lactol **143** (81 mg, 0.10 mmol, 95%) as a mixture of α and β anomers. IR ν_{max} (film) 3424, 2869, 2111, 1742, 1717, 1454, 1363, 1237, 1151, 1059 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.87 – 7.75 (m, 4H), 7.53 – 7.41 (m, 3H), 7.34 – 7.25 (m, 5H), 7.22 – 7.10 (m, 3H), 7.03 – 6.95 (m, 2H), 5.37 – 5.28 (m, 0.5H) 5.25 (d, *J* = 3.5 Hz, 0.5H), 5.16 – 5.02 (m, 1.5H), 4.80 – 4.65 (m, 4H), 4.60 (d, *J* = 12.1 Hz, 1H), 4.50 – 4.43 (m, 2H), 4.35 (d, *J* = 8.0 Hz, 0.5H), 4.31 (d, *J* = 8.1 Hz, 0.5H), 4.19 (q, *J* = 6.6 Hz, 0.5H), 4.07 (dd, *J* = 11.1, 3.1 Hz, 1H), 3.88 – 3.56 (m, 6H), 3.41 – 3.35 (m, 1H), 2.92 – 2.62 (m, 2H), 2.55 – 2.44 (m, 1H), 2.24 (s, 1.5H), 2.15 (s, 1.5H), 2.12 (s, 1.5H), 2.09 (s, 1.5H), 1.29 (d, *J* = 6.5 Hz, 1.5H), 1.19 (d, *J* = 6.6 Hz, 1.5H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 208.9, 206.4, 171.9, 171.7, 171.2, 171.1, 138.5, 138.4, 137.9, 135.1 (2C), 133.3, 133.1, 128.5 (2C), 128.4 (2C), 128.1, 127.9 (4C), 127.8 (3C), 127.7, 126.8, 126.6, 126.4, 126.1, 125.9, 125.8, 101.7, 100.9, 96.8, 92.2, 83.0, 82.9, 77.8, 77.6, 75.4, 75.2, 75.1,

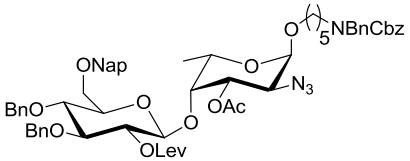
75.0 (2C), 73.9 (2C), 73.6, 73.3, 72.8, 70.7, 70.4, 68.8, 68.6, 65.8, 62.8, 57.9, 38.1, 37.6, 30.5, 30.0, 28.1 (2C), 21.1, 21.0, 17.0, 16.3; HRMS (MALDI-TOF): Calcd for $C_{44}H_{49}N_3O_{12}Na^+$ $[M+Na]^+$ 834.3208, found 834.3222.

3,4-Di-*O*-benzyl-2-*O*-levulinoyl-6-*O*-(2-naphthalenylmethyl)-β-D-glucopyranosyl-(1→4)-3-*O*-acetyl-2-azido-2-deoxy-L-fucopyranosyl trichloroacetimidate (144)



According to general procedure (D), lactol **143** (81 mg, 100 µmol) was reacted with trichloroacetonitrile (100 µL, 998 µmol) and DBU in DCM (2 mL) at 0 °C to afford **144** (82 mg, 86 mmol, 86%) as a mixture of α and β anomers. Analytical data is given for the α anomer. $[\alpha]_D^{20} = -88.7$ º (c = 1.5, $CHCl_3$); IR ν_{max} (film) 3336, 3060, 3030, 2906, 2868, 2113, 1746, 1718, 1673, 1363, 1273, 1243, 1144, 1063, 1028 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.67 (s, 1H), 7.90 – 7.75 (m, 4H), 7.52 – 7.42 (m, 3H), 7.35 – 7.25 (m, 5H), 7.23 – 7.10 (m, 3H), 7.05 – 6.96 (m, 2H), 6.36 (d, *J* = 3.6 Hz, 1H), 5.14 (dd, *J* = 9.6, 8.1 Hz, 1H), 5.08 (dd, *J* = 11.1, 3.1 Hz, 1H), 4.80 – 4.69 (m, 4H), 4.60 (d, *J* = 12.1 Hz, 1H), 4.47 (d, *J* = 10.8 Hz, 1H), 4.34 (d, *J* = 8.0 Hz, 1H), 4.22 – 4.13 (m, 2H), 4.10 (dd, *J* = 11.1, 3.6 Hz, 1H), 3.87 – 3.72 (m, 3H), 3.71 – 3.64 (m, 1H), 3.40 (ddd, *J* = 9.8, 3.5, 2.0 Hz, 1H), 2.84 – 2.64 (m, 2H), 2.61 – 2.46 (m, 2H), 2.17 (s, 3H), 2.13 (s, 3H), 1.24 (d, *J* = 6.6 Hz, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 206.3, 171.7, 171.0, 161.1, 138.3, 137.9, 135.0, 133.3, 133.2, 128.5, 128.4, 128.1, 128.0, 127.9, 127.8 (2C), 126.7, 126.4, 126.1, 125.9, 101.7, 95.2, 91.1, 82.9, 77.5, 75.1, 74.5, 73.9, 73.5, 70.7, 68.7, 68.5, 56.6, 38.1, 30.0, 28.1, 21.0, 16.3; HRMS (ESI): Calcd for $C_{46}H_{49}Cl_3N_4O_{12}Na^+$ $[M+Na]^+$ 977.2310, found 977.2312.

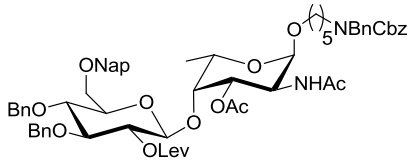
***N*-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl 3,4-Di-*O*-benzyl-2-*O*-levulinoyl-6-*O*-(2-naphthalenylmethyl)-β-D-glucopyranosyl-(1→4)-3-*O*-acetyl-2-azido-2-deoxy-L-fucopyranoside (145)**



According to general procedure (E), disaccharide-imidate **144** (17 mg, 18 µmol) and *N*-(benzyl)benzyloxycarbonyl-5-amino-pentanol (12 mg, 36 µmol) were reacted in DCM (1 mL) at –30 °C to –20 °C over 30 min to give the α and β anomers of **145** (18 mg, 16 µmol, 90%) in

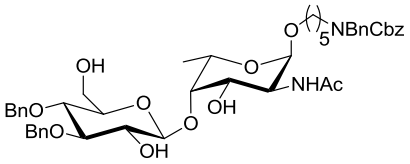
a ratio α/β=1:3. Analytical data is given for the α anomer. $[\alpha]_D^{20}$ = – 70.3 º (c = 0.3, $CHCl_3$); IR ν_{max} (film) 2936, 2110, 1746, 1699, 1497, 1454, 1421, 1362, 1244, 1131, 1054 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.84 – 7.73 (m, 4H), 7.49 – 7.40 (m, 3H), 7.34 – 7.21 (m, 14H), 7.19 – 7.09 (m, 4H), 7.00 – 6.95 (m, 2H), 5.18 – 5.07 (m, 3H), 5.00 (dd, *J* = 11.1, 2.6 Hz, 1H), 4.78 – 4.65 (m, 5H), 4.57 (d, *J* = 12.0 Hz, 1H), 4.51 – 4.42 (m, 3H), 4.28 (d, *J* = 7.9 Hz, 1H), 4.04 (s, 1H), 3.92 – 3.86 (m, 1H), 3.77 (d, *J* = 9.4 Hz, 1H), 3.72 – 3.57 (m, 4H), 3.36 (d, *J* = 9.6 Hz, 2H), 3.26 – 3.15 (m, 2H), 2.82 – 2.57 (m, 3H), 2.50 (t, *J* = 7.0 Hz, 2H), 2.13 (s, 3H), 2.08 (s, 3H), 1.54 – 1.46 (m, 4H), 1.33 – 1.25 (m, 2H), 1.15 (d, *J* = 6.4 Hz, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 206.4, 171.7, 171.2, 138.4, 137.9, 135.1, 133.4, 133.2, 128.7, 128.5 (2C), 128.1, 128.0, 127.9 (3C), 127.8, 127.4, 126.7, 126.4, 126.1, 125.9, 101.7, 98.0, 83.0, 77.6, 77.4, 75.4, 75.1 (2C), 75.0, 73.9, 73.5, 70.3, 68.7, 68.3, 67.3, 65.6, 57.1, 38.2, 30.0, 29.3, 28.1, 23.5, 21.1, 16.3; HRMS (MALDI-TOF): Calcd for $C_{64}H_{72}N_4O_{14}Na^+$ $[M+Na]^+$ 1143.4937, found 1143.4974.

_N_-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl 3,4-Di-_O_-benzyl-2-_O_-levulinoyl-6-_O_-(2-naphthalenylmethyl)-β-D-glucopyranosyl-(1→4)-2-_N_-acetyl-3-_O_-acetyl-α-L-fucosaminopyranoside (146)



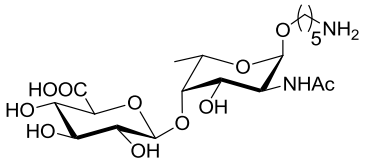
According to general procedure (F), azido-disaccharide **145** (28 mg, 25 μmol) was reacted with thioacetic acid for 24 h to give **146** (20 mg, 18 μmol, 70%). $[\alpha]_D^{20}$ = – 53.6 º (c = 1.0, $CHCl_3$); IR ν_{max} (film) 2926, 1694, 1454, 1364, 1247, 1053 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.92 – 7.72 (m, 4H), 7.60 – 7.08 (m, 21H), 7.06 – 6.90 (m, 2H), 5.90 – 5.30 (m, 1H), 5.27 – 5.02 (m, 3H), 4.84 (d, *J* = 11.4 Hz, 1H), 4.80 – 4.67 (m, 5H), 4.64 – 4.57 (m, 1H), 4.56 – 4.39 (m, 4H), 4.34 (d, *J* = 8.0 Hz, 1H), 3.99 – 3.46 (m, 7H), 3.39 (d, *J* = 9.7 Hz, 1H), 3.36 – 3.10 (m, 3H), 2.89 – 2.62 (m, 2H), 2.62 – 2.44 (m, 2H), 2.15 (s, 3H), 2.04 (s, 3H), 1.92 (s, 3H), 1.66 – 1.41 (m, 4H), 1.39 – 1.22 (m, 2H), 1.19 (d, *J* = 5.6 Hz, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 206.8, 171.9, 171.3, 171.1, 138.4, 137.9, 135.3, 133.4, 133.1, 128.7, 128.6, 128.5 (2C), 128.4, 128.3, 128.1, 128.0 (2C), 127.8 (2C), 127.7, 126.5, 126.3, 126.0, 125.9, 101.8, 97.5, 83.2, 77.7, 77.4, 75.5, 75.1, 74.9, 73.9, 73.5, 70.3, 69.0, 67.3, 65.6, 50.3, 47.5, 47.2, 38.2, 38.0, 30.0, 29.3, 28.1, 27.5, 23.9, 23.5, 21.2, 16.4; HRMS (MALDI-TOF): Calcd for $C_{66}H_{76}N_2O_{15}Na^+$ $[M+Na]^+$ 1159.5138, found 1159.5148.

_N_-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl 3,4-Di-_O_-benzyl-β-D-glucopyranosyl-(1→4)-2-_N_-acetyl-α-L-fucosaminopyranoside (147)



According to general procedure (G), disaccharide **146** (20 mg, 18 μmol) was reacted with DDQ (12 mg, 53 μmol) for 1.5 h to give the crude primary alcohol. To a solution of crude primary alcohol in MeOH (1 mL) was added 0.5 M NaOMe in MeOH (0.3 mL) and stirred for 16 h. The mixture was neutralized with Amberlite® IR 120 (H^+) ion exchange resin, filtered and concentrated. Column chromatography (DCM/MeOH/acetone) afforded **147** (9 mg, 11 μmol, 60%). $[\alpha]_D^{20}$ = – 45.2 º (c = 0.7, $CHCl_3$); IR ν_{max} (film) 3354, 2925, 1695, 1542, 1497, 1454, 1422, 1361, 1231, 1049 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.68 – 6.84 (m, 20H), 6.07 – 5.61 (m, 1H), 5.18 (d, *J* = 11.9 Hz, 2H), 5.01 – 4.76 (m, 3H), 4.76 – 4.58 (m, 2H), 4.57 – 4.33 (m, 4H), 4.02 – 3.80 (m, 2H), 3.80 – 3.42 (m, 8H), 3.39 – 3.14 (m, 3H), 2.53 (s, 1H), 2.01 (s, 3H), 1.91 – 1.63 (m, 2H), 1.63 – 1.45 (m, 4H), 1.36 – 1.26 (m, 5H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 172.0, 138.6, 137.9, 128.7, 128.6 (2C), 128.3, 128.1, 128.0, 127.9 (2C), 103.4, 98.1, 84.3, 83.3, 77.7, 77.4, 76.3, 75.4, 75.2, 74.9, 70.5, 67.4, 67.2, 61.9, 50.3, 47.0, 46.2, 29.3, 27.5, 23.7, 16.8; HRMS (MALDI-TOF): Calcd for $C_{48}H_{60}N_2O_{12}Na^+$ $[M+Na]^+$ 879.4038, found 879.4004.

5-Amino-pentanyl β-D-glucopyranosyluronate-(1→4)-2-_N_-acetyl-α-L-fucosaminopyranoside (132)



According to general procedure (H), triol **147** (8 mg, 9 μmol) was reacted with TEMPO (0.3 mg, 2 μmol) and BAIB (15 mg, 47 μmol) to give the protected uronate disaccharide. According to general procedure (I), the uronate disaccharide was subjected to hydrogenolysis to give **132** (2.6 mg, 5.3 μmol, 57%). ^{1}H-NMR (600 MHz, D_2O) δ 4.89 (d, *J* = 3.7 Hz, 1H), 4.53 (d, *J* = 7.3 Hz, 1H), 4.20 – 4.12 (m, 2H), 4.05 (d, *J* = 2.9 Hz, 1H), 3.93 (dd, *J* = 11.2, 3.0 Hz, 1H), 3.77 – 3.72 (m, 1H), 3.70 (dt, *J* = 8.1, 5.6 Hz, 1H), 3.59 – 3.45 (m, 4H), 3.06 – 2.97 (m, 2H), 2.05 (s, 3H), 1.73 – 1.63 (m, 4H), 1.49 – 1.42 (m, 2H), 1.34 (d, *J* = 6.6 Hz, 3H); ^{13}C-NMR (150 MHz, D_2O) δ 178.1, 177.1, 105.5, 99.6, 82.5, 78.8, 77.9, 75.8, 74.4, 70.5, 69.6,

69.5, 53.2, 42.0, 30.6, 29.1, 24.9, 24.5, 17.9; HRMS (ESI): Calcd for $C_{19}H_{34}N_2O_{11}Na^+$ $[M+Na]^+$ 489.2060, found 489.2063.

6.5.4 Glycan Microarray Preparation and Screening

64 well glycan arrays were printed using a Scienion S3 microarray printer on CodeLink™ NHS activated glass slides (Surmodics). All compounds (proteins and glycans containing an aminolinker) were dissolved in printing buffer (50 mM sodium phosphate, pH 8.5). The printed slides were stored overnight in a humidity saturated chamber for complete reaction, dried by centrifugation at 1200 g and stored at 4 °C afterwards. Prior to use, slides were washed with water, quenched with quenching solution (50 mM sodium phosphate, 100 mM ethanolamine, pH 7.4) at room temperature for 2 h, washed with water and blocked for 1 h at room temperature with 1% BSA in PBS. After washing with PBS, the slides were dried by centrifugation. A 64 well gasket was applied to the slides and primary antibody incubation (serum dilutions in 1% BSA-PBS) was carried out at room temperature for 1 h. The wells were washed three times for 10 min with PBS containing 0.1% Tween-20. Secondary antibody dilutions were pipetted into the wells. After 1h incubation at room temperature in the dark, the slides were washed three times for 10 min with PBS containing 0.1% Tween-20 and once shortly with water. The gasket was removed and the slides were dried by centrifugation. Fluorescence was read out using a GenePix 4300A microarray scanner (Bucher Biotec, Basel, Switzerland) with a 488 nm laser for FITC excitation and a 594 nm laser for AlexaFluor 594. GenePix Pro 7 (Bucher Biotec) was used for analysis.

7 Appendix

7.1 Synthesis of inner core oligosaccharides of the lipopolysaccharide of Gram-negative bacteria

During the work on this dissertation I contributed to several further scientific projects that are only mentioned briefly here. These projects focus on diversity-oriented syntheses of inner core oligosaccharides of the lipopolysaccharide (LPS) of *Neisseria meningitidis* and other pathogenic Gram-negative bacteria.

The work was carried out together with Dr. You Yang and resulted in the following publications:

Yang, Y.; Oishi, S.; **Martin, C. E.**; Seeberger, P. H.: Diversity-oriented synthesis of inner core oligosaccharides of the lipopolysaccharide of pathogenic Gram-negative bacteria. *J. Am. Chem. Soc.*, **2013**, *135*, 6262.

Yang, Y.; **Martin, C. E.**; Seeberger, P. H.: Total synthesis of the core tetrasaccharide of *Neisseria meningitidis* lipopolysaccharide, a potential vaccine candidate for meningococcal diseases. *Chem. Sci.*, **2012**, *3*, 896.

7.2 Abbreviations

Å	Ångström, 10^{-10} m
Ac	acetyl
All	allyl
aq.	aqueous
Ar	aromatic
Bn	benzyl
Bu	butyl
Bz	benzoyl
c	concentration
CAN	ceric (IV) ammonium nitrate
cat.	catalytic
Cbz	benzyloxycarbonyl
CDI	*Clostridium difficile* infection
CE	2-cyanoethyl
CL	cutaneous leishmaniasis
CSA	camphorsulfonic acid
CPS	capsular polysaccharide
δ	chemical shift
d	doublet
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCC	*N*,*N´*-dicyclohexylcarbodiimide
DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
DIPC	*N*,*N´*-diisopropylcarbodiimide
DIPEA	diisopropylethylamine
DMAP	4-(*N*,*N*-dimethylamino)pyridine
DMF	*N*,*N*-dimethylformamide
DMTST	dimethylsulfonium triflate
DTBMP	2,6-di-*tert*-butyl-4-methylpyridine
DMSO	dimethylsulfoxide
EDC	*N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide
equiv	equivalent
ESI	electrospray ionization

Et	ethyl
ETT	5-(ethylthio)tetrazole
Fmoc	9-fluorenylmethyloxycarbonyl
	N-acetyl fucosamine
Gal	galactose
Glc	glucose
GlcA	glucuronic acid
GlcNAc	*N*-acetyl glucosamine
GPI	glycosylphosphatidylinositol
GroA	glyceric acid
h	hour(s)
Hib	*Haemophilus influenza* type B
HRMS	high resolution mass spectroscopy
Hz	herz
Ig	immunoglobulin
IR	infrared spectroscopy
J	coupling constant
KDO	3-deoxy-D-manno-2-octulosonic acid
Lev	levulinoyl
LevOH	levulinic acid
LPG	lipophosphoglycan
LTA	lipoteichoic acid
m	multiplet
M	molar
MALDI	matrix assisted laser desorption/ionization
Man	mannose
Me	methyl
min	minute(s)
MS	molecular sieves
NAP	2-naphthylmethyl
NBS	*N*-bromosuccinimide
NHS	*N*-hydroxysuccinimide
NIS	*N*-iodosuccinimide
nm	nanometer

NMR	nuclear magentic resonance
PBB	*para*-bromobenzyl
PG	protecting group
Ph	phenyl
Piv	pivaloyl
PMB	*para*-methoxybenzyl
PneNAc	*N*-acetyl pneumosamine
ppm	parts per million
Pr	propyl
quant.	quantitative
Rha	rhamnose
rt	room temperature
s	singulet
Sug	2-acetamido-2,5-dideoxy-D-*xylo*-hexos-4-ulose
t	triplet
TBAF	tetrabutylammonium fluoride
TBAI	tetrabutylammonium iodide
TBS	*tert*-butyldimethylsilyl
TCA	trichloroacetimidate
TDS	thexyldimethylsilyl
TEMPO	(2,2,6,6-tetramethylpiperidin-1-yl)oxidanyl
Tf	trifluoromethanesulfonyl
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography
TMS	trimethylsilyl
TOF	time of flight
Tol	toluene
Ts	Tosyl
TTBP	2,4,6-tri-*tert*-butylpyrimidine
PS	polysaccharide
p-TsOH	*para*-toluenesulfonic acid
VL	visceral leishmaniasis

7.3 *Curriculum vitae* Christopher E. Martin

Due to data privacy the CV is not available in the online version.

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