MODERN APPROACHES TO THE SYNTHESIS OF DIVERSE CLASSES OF OLIGOSACCHARIDES

Moderne synthetische benaderingen van diverse oligosacchariden

(met een samenvatting in het Nederlands)

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List of Abbreviations

Alloc	Allyloxycarbonyl
Ac	Acetyl
AAL	*Aleuria Aurantia* Lectin
Bn	Benzyl
Bu	Butyl
BSA	Bovine serum albumin
Boc	tert-Butyloxycarbonyl
BSP	1-Benzenesulfinyl piperidine
Bsmoc	1,1-Dioxobenzo[*b*]thiophene-2-ylmethyloxycarbonyl
CAN	Ceric ammonium nitrate
CMP	Cytidine monophosphate
CIAP	Calf intestinal alkaline phosphatase
CID	Collision-induced Dissociation
CE	Capillary Electrophoresis
Cbz	Carboxybenzyl
DDQ	2,3-Dichloro-5,6-dicyano-1,4-benzoquinone
DPS	Diphenyl sulfoxide
DTBMP	2,6-Di-tert-butyl-4-methylpyridine
DMTST	Dimethyl(methylthio)sulfonium trifluoromethanesulfonate
DMAP	4-Dimethylaminopyridine
DMF	Dimethylformamide
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DTT	Dithiothreitol
DMSO	Dimethylsulfoxide
DC-SIGN	Dendritic Cell-Specific Intercellular adhesion molecule-3-Grabbing Non-integrin
ESI-TOF	Electrospray Ionization – Time of Flight
Et	Ethyl
EDCI	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
ECL	*Erythrina cristagalli* Lectin
Fmoc	Fluorenylmethyloxycarbonyl
FUT	Fucosyltransferase
Gal	Galactose
GlcNAc	*N*-acetyl-D-glucosamine
GalT1	*β*-(1,4)-Galactosyltransferase
*β*3GnT2	*β*-(1,3)-*N*-acetyl-D-glucosamine transferase
GDP-Fuc	6-Deoxy-*β*-L-galactopyranosylguanosine 5′-diphosphate
HPLC	High Performance Liquid Chromatography
HILIC	Hydrophilic Interaction Liquid Chromatography

Hpα1,3FT	*Helicobacter pylori* α(1,3) Fucosyltransferase
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HRMS	High Resolution Mass Spectrometry
IAV HA	Influenza A virus hemagglutinin
ICAM-1	Intercellular Adhesion Molecule 1
LacNAc	*N*-Acetyllactosamine
Lev	Levulinoyl
LC-MS	Liquid Chrmoatography – Mass Spectrometry
Le^x	Lewis X
Man	D-Mannose
MS	Mass Spectrometry
MALDI-TOF	Matrix-Assisted Laser Desorption/Ionization – Time of Flight
MAL-II	*Maackia amurensis* Lectin II
ManNAc	*N*-Acetyl-D-mannosamine
MRSA	Methicillin-Resistant *Staphylococcus aureus*
Nap	2-Napthyl
Neu5Ac	*N*-Acetylneuraminic acid
NIS	*N*-Iodosuccinimide
NPhth	*N*-Phthaloyl
NHS	*N*-Hydroxysuccinimide
PmST1	*Pasteurella multocida* α-(2,3)-sialyltransferase
PMB	*p*-Methoxybenzyl
Piv	Pivaloyl
ST3GalIV	Mammalian α-(2,3)-sialyltransferase
SLe^x	Sialyl Lewis x
SNA	*Sambucus nigra* lectin
SSTI	Soft Tissue Infections
TBS	*t*-Butyl-dimethylsilyl
TMSOTf	Trimethylsilyl trifluoromethanesulfonate
TLC	Thin Layer Chromatography
Tf	Trifluoromethanesulfonyl
TTBP	2,4,6-Tri-*t*-butyl-pyrimidine
TMEDA	Tetramethylethylenediamine
THF	Tetrahydrofuran
Troc	2,2,2-Trichloroethoxycarbonyl
TFA	Trifluoroacetyl
TDS	(Dimethyl)thexylsilyl
TBAF	Tetrabutylammonium fluoride
UDP	Uridine diphosphate
VIM-2	Verona integron-encoded metallo-β-lactamase 2
WGA	Wheat germ agglutinin

Chapter 1

General Introduction

The last century has witnessed impressive developments in carbohydrate chemistry through advances in the art and science of organic synthesis.[1] Stereoselective assembly of complex oligosaccharides has become a "scale" to measure the capabilities of accumulated methodologies to their limits.[2] These endeavors in the synthesis of oligosaccharides provide many benefits for understanding selectivity principles of the most important reaction in carbohydrate science, namely glycosylation.

Two general approaches are used to form a glycosidic bond: chemical and enzymatic methods. Chemical glycosylation entails a reaction between a glycosyl donor and glycosyl acceptor in the presence of a suitable promoter (Figure 1). As a prerequisite, hydroxyls of the glycosylation partners must be modified with blocking/protecting groups.[3] This is done to ensure that only desired hydroxyl groups of a carbohydrate participate in a glycosylation reaction. Upon activation, the promoter-assisted departure of the leaving group in **1** results in the formation of a glycosyl cation **3**, which then gets stabilized via an oxocarbenium ion intermediate.[4] The incoming nucleophile **4**, glycosyl acceptor, can then attack either from the top or bottom of the ring thus forming a glycosidic bond. This gives rise to either 1,2-*trans* (**5**) or 1,2-*cis* (**6**) glycosides with respect to the C-2 moiety. Mixtures of anomers will be produced if glycosylations are uncontrolled.[5] Interestingly, many novel anomeric leaving groups have been described,[6] however, only two or three of those are widely employed and recycled throughout synthetic projects of even utmost difficulty.[7] Hence, in case of a complex target,[8] which is build up by consecutive glycosylations, the choice of protecting groups and anomeric leaving groups must be balanced in a way to consistently obtain high yielding coupling reactions. This is a highly laborious process, and only specialized laboratories are capable of synthesizing glycans in a reproducible manner. This problem has stimulated the development of enzymatic methods.

The *in vitro* enzymatic synthesis of oligosaccharides employs glycosyltransferases,[9] glycosidases[10] and phosphorylases.[11] Obviously, there are no requirements of installing protecting groups, because highly specific glycosyltransferases stereo- and regioselectively catalyze formation of a glycosidic bond, where appropriate sugar nucleotides such as **7** are used as donors. Thus, what takes more steps to chemically prepare a disaccharide, can now be

accomplished in one step only, greatly reducing waste and purification efforts. The drawback of enzyme mediated oligosaccharide synthesis is that not all enzymes can readily be expressed on a scale required for synthetic purposes. This is particularly the case for many bacterial oligosaccharides.[12]

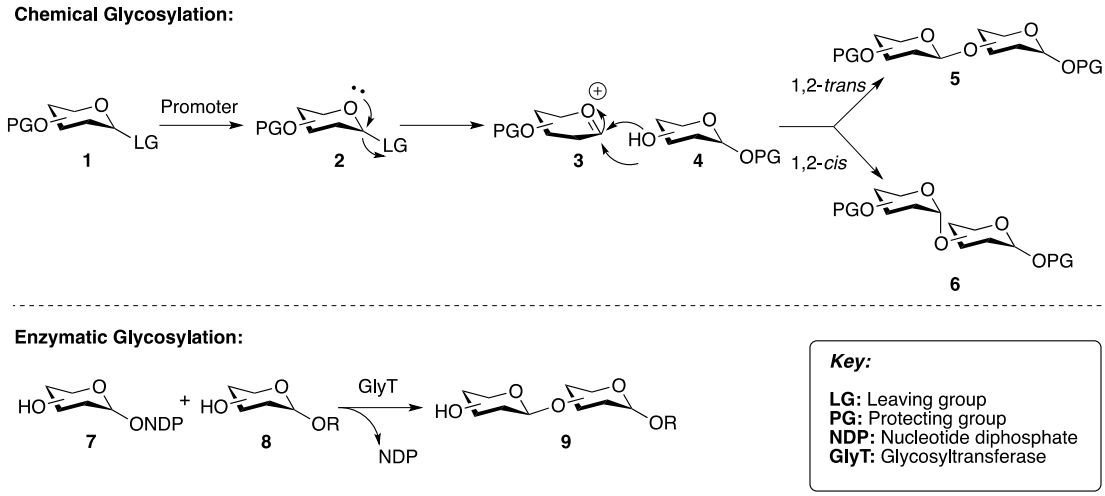

Figure 1. General principles of chemical and enzymatic glycosylations

Two examples are mentioned below to demonstrate the scope of each glycosylation type (Figure 2). A highly branched mycobacterial arabinogalactan **10**, which contains 92 sugar units, was created from three monosaccharide building blocks.[13] The synthesis of various sub-structures required to eventually reach this complex target is overwhelming, totaling well over 100 chemical manipulations. Therefore, it is obvious how much effort, patience, and manpower were involved. On the other hand, cancer-associated antigens globopentaose **11** (Gb5), fucosyl-Gb5 **12** (GloboH), and sialyl-Gb5 **13** (SSEA4) were only constructed in 3 or 4 steps by using overexpressed glycosyltransferases.[14] Effective *in situ* regeneration of required sugar nucleotides enabled multigram synthesis of the above penta- and hexasaccharides with only two or three purification steps. Contrary to enzymatic synthesis, a related chemoenzymatic approach is useful for the synthesis of oligosaccharides having unusual modifications and linkages. These include, for example, *O*-sulfated SLe^x, C9/5-modified glycosides of sialic acid, protecting group-modified *O*- and *N*-glycans, and a few examples of echinoderm-type gangliosides with interesting neurological properties. Unnaturally modified oligosaccharides are useful, first of all, for chemical biology research because synthetic modifications (e.g. reporting tags) make it possible to track metabolic processes. Secondly, artificial modifications are frequently introduced to either prevent or stimulate a glycosyltransferase to selectively act upon one desired monosaccharide, thus ignoring the rest of the oligosaccharide chain. These synthetic moieties are often introduced chemically at the pre-enzymatic stages of the synthesis, and it is very important to make sure they are stable during all manipulations until no longer needed. Not all modifications are tolerated by glycosyltransferases, making it difficult to pre-estimate which group to install for higher chances of success. Alternatively, some glycosyltransferases are capable of utilizing sugar-nucleotides containing pre-installed modifications, and this shorter route is useful for direct buildup of artificial oligosaccharides. Unfortunately, expert synthetic and purification knowledge are required to synthesize these modified sugar nucleotides.

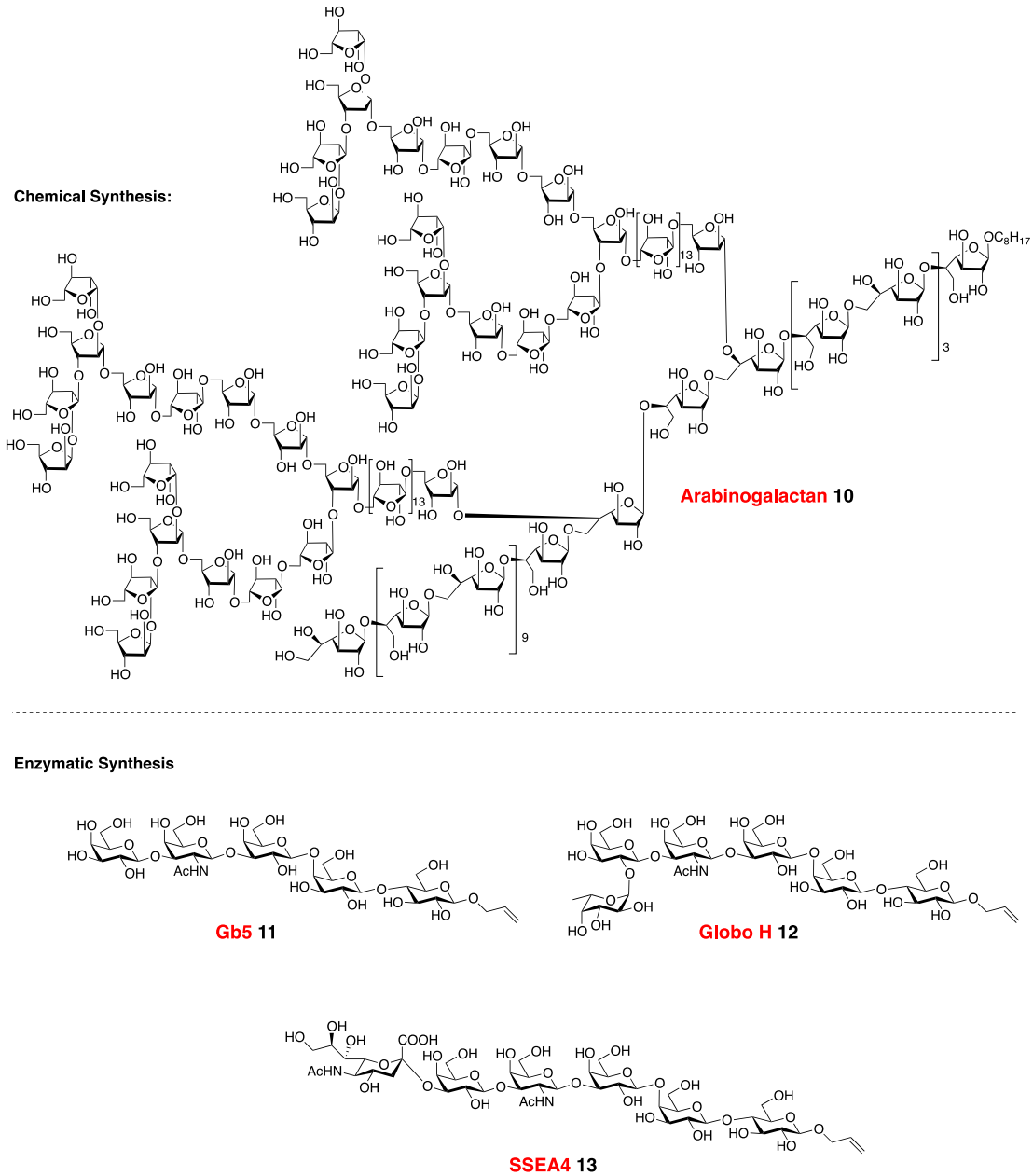

Figure 2. Examples of chemically and enzymatically prepared oligosaccharides.

Current synthetic strategies and methodologies are inefficient to acquire compounds such as **10**, where more than 100 steps are involved. To streamline oligosaccharide synthesis in the 21st century, one has to analyze why our traditional synthetic and enzymatic methodologies are limiting.[15] Either approach when used in isolation is not capable of delivering large glycans with highly complex architectures. For example, a major difficulty in the synthesis of asymmetric *N*-glycans is the availability of core oligosaccharides. Enzymes responsible for assembly of these substructures are difficult to isolate on a preparative scale, because many are transmembrane and sometimes unstable to manipulate. Chemoenzymatic approach is a more suitable alternative, because chemical preparation of core oligosaccharides is already well-established. These precursors can additionally be modified with temporary protecting groups, which enables specific glycosyltransferases to selectively extend a branch of interest. As a result, high degree of glycan asymmetry can be accomplished, and this combined approach lightens the burden of a large number of synthetic steps.

Excess of synthetic steps remains the most obvious obstacle, which precludes delivery of any oligosaccharide within a short timeframe. It is for this reason that reduction of step count is always the main objective of those involved in the construction of complex molecules.[16] The importance of "step-economical" approach to oligosaccharide synthesis definitely receives the highest priority bringing carbohydrate chemists to Hendrickson's formulation[17] of "ideal synthesis:"

"The ideal synthesis creates a complex molecule…in a sequence of only construction reactions involving no intermediary refunctionalizations, and leading directly to the target, not only its skeleton but also its correctly placed functionality."

Given the reliance of carbohydrate chemistry on use of protecting groups, it is not possible yet to define clear guidelines on how to reduce the number of steps and reach the Hendrickson's "ideal synthesis". Obviously, rejecting the use of protecting groups all together is impossible at this stage. Moreover, finding reliable, standardized procedures for these routine transformations is often a time-consuming and tedious process if one has to decide which reaction conditions to try first in order to have the best chances of success. Above all, any of such selected procedures should be scalable. In this respect, finding the right glycosylation conditions is especially difficult, because current literature is overpopulated with many methods to choose from. Glycosylation is especially difficult, because current literature is overpopulated with many methods to choose from. A more desirable technology could perhaps combine enzymatic catalysis, chemical synthesis, and solid-phase automation.[18]

Through diverse oligosaccharide examples of what today constitutes a complex target, this thesis details four total synthetic oligosaccharide projects. Each project emphasizes the importance of scalable delivery of required monosaccharide building blocks, selection of most optimal synthetic routes, and rational, experience-based choice of protecting groups so that even the most fragile functionalities can be preserved. Special attention is also devoted to the so-called "hybrid" synthesis, whereby chemical and enzymatic approaches are combined together to step-economically deliver the products. Finally, it is shown which types of complex oligosaccharides have reached the state of enzyme-catalyzed assembly, and the ones that are not.

Thus, the following **Chapter 2** describes a general chemoenzymatic approach to access bi-, tri-, and tetra-antennary asymmetric complex *N*-linked glycans directly starting from one single precursor. This starting oligosaccharide was obtained on a large scale (~350 mg) through chemical synthesis. Highly specific mammalian glycosyltransferases further enabled efficient elaboration of the synthetic template giving an asymmetric tetra-antennary *N*-glycan composed of 21 monosaccharides. Asymmetry was achieved through chemical installation of artificial sugars on two branches of the precursor, which could be cleaved by specific glycosidases to be activated towards recognition by a glycosyltransferase of choice. Unfortunately, the long-standing problem of selective fucosylation of oligo-lactosamine could not be addressed at that moment, and this methodology was developed separately and subsequently described in **Chapter 3**. It was discovered that replacing the native *N*-acetyl moieties of tri-lactosamine with either free NH_2 or a removable NHBoc groups blocks C-3 glycosylation by a fucosyltransferase. Thus a wide range of tri-LacNAc precursors was synthesized having different arrangements of NH_2/NHBoc moieties, and this approach enabled synthesis of all possible variants of mono- and di-fucosylated tri-lactosamines.

The second half of this thesis is dedicated to chemical assembly of capsular oligosaccharides found in bacteria. Therefore, **Chapter 4** focuses on the chemical synthesis of an orthogonally protected trisaccharide derived from the polysaccharide of *Staphylococcus aureus* Type 5, which is an attractive candidate for the development of immunotherapies. The challenging α-fucosylation and β-mannosylation are addressed through the careful choice of protecting groups. Lactamization of a β-D-Man*p*NAc moiety during deprotection was avoided by a late stage oxidation approach. Versatility of the trisaccharide was demonstrated by its transformation into a spacer-containing repeating unit suitable for immunological investigations.

Finally, synthesis of *Streptococcus pneumoniae* 35B capsular polysaccharide (CPS) oligomers is described in **Chapter 5**. Protein-CPS conjugate vaccines have been highly successful in reducing the incidence of infections caused by pneumococci. Increased use of two licensed CPS vaccines PCV7 and PCV13 resulted in emergence of a serotype 35B, antigens of which are not included in any current formulations. This invasive serotype has recently become the most common clinical isolate, and accounts for 90% of all infections. Alerted by the urgent need to include serotype 35B antigens in current vaccines, we have developed a scalable approach which successfully delivers well-defined synthetic polysaccharide fragments ready for conjugation and immediate clinical use. This discovery is based on a modular synthetic route producing oligosaccharides up to a pentadecamer (15 sugar units). Contrary to previous beliefs, we show that CPS *O*-acetylation is essential for immunogenicity. In this respect, our binding studies with ficolin-2, a plasma protein known to activate certain components of the complement pathway, demonstrate that de-*O*-acetylated CPS fragments are no longer being recognised.

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Chapter 2

A Chemoenzymatic Approach for the Preparation of Asymmetric Bi-, Tri- and Tetra-Antennary N-Glycans from a Common Precursor

Introduction

Of all post-translational modifications of proteins, complex *N*-linked glycans are the most prominent in terms of complexity and diversity.[1] All eukaryotic *N*-linked glycans have a common pentasaccharide core that can be modified by various *N*-acetyllactosamine (*β*-Gal(1,4)GlcNAc, LacNAc) moieties, which in turn can be elaborated by glycosyltransferases to give highly complex branched structures.[2] The structural diversity of *N*-glycans arises from the degrees and patterns of branching, various numbers of LacNAc repeating units, and further elaborations such as sialylation and fucosylation.[3] Structural studies have shown that the *N*-glycans are usually asymmetrically substituted having a unique appendage at each branching point. Variations in *N*-glycan structures occur during embryogenesis, cell activation, morphogenesis, cell cycle entry, and during oncogenesis.[4] These differences are largely based on cell-specific expressions of collection of glycosyltransferases. Furthermore, the degree of branching of *N*-glycans appears to be sensitive to the metabolic flux,[5] and it has been proposed that changes in branching cooperate with the regulation of cell proliferation and differentiation.[6]

Diverse libraries of well-defined complex glycans are urgently needed for the fabrication of the next generation of microarrays,[7] as standards for the development of analytical protocols to determine exact structures and quantities of isolated glycans, and for the elucidation of pathways of glycoconjugate biosynthesis. Often well-defined oligosaccharides can only be obtained by chemical or enzymatic approaches.[8a, b, 7, 8c, d] Despite that most complex *N*-glycans have asymmetrical architectures, synthetic efforts have almost exclusively focused on the preparation of incomplete or *symmetrically* branched structures.[9]

Recently, we reported a chemoenzymatic approach that for the first time can provide libraries of complex *asymmetrically* branched *N*-glycans.[10] It exploits chemically synthesized multi-antennary *N*-glycans that at each antenna can selectively be extended by a panel of glycosyltransferases. The latter was possible because specific antennae were temporarily rendered inactive for enzymatic extension by acetylation of a GlcNAc and LacNAc appendage. At an appropriate stage of synthesis, the esters can be removed to give substrates that can selectively be extended by appropriate glycosyltransferases. The methodology made it possible to prepare a tri-antennary *N*-glycan having different complex oligosaccharide extensions at each antenna. Other laboratories are adopting this methodology for the preparation of panels of complex *N*-glycans.[11]

Although the chemoenzymatic approach gave entry into *N*-glycans of unprecedented complexity, it requires a unique precursor for each type of branched oligosaccharide. Furthermore, we have observed that acetyl esters are prone to hydrolysis during multiple enzymatic extensions, and could not serve as an appropriate protecting group when complex tetra-antennary *N*-glycans were targeted.[12] To address these difficulties, we describe here a more versatile chemoenzymatic methodology that makes it possible to prepare any bi-, tri-, and tetra-antennary asymmetric *N*-glycans from a single precursor (**1**) through enzymatic transformations (Figure 1). The universal precursor could be obtained by sequential removal of the orthogonal protecting groups of core pentasaccharide **2** and glycosylation with glycosyl donors **3**-**6**. The resulting tetra-antennary glycan has a terminal GlcNAc, LacNAc, and unnatural Gal-α(1,4)-GlcNAc and Manβ(1,4)-GlcNAc moiety. It was anticipated that relevant glycosyltransferases would only modify LacNAc and not the other terminal structures allowing selective extension of this arm. At an appropriate stage of the synthesis, the β-GlcNAc terminating antenna can be converted into LacNAc by galactosylation using Gal-T1, which can then enzymatically be extended into a complex structure. Next, the unnatural α-Gal and β-Man terminating antennae can sequentially be de-caged by an appropriate glycosidase[13] to liberate a terminal β-GlcNAc moiety, which can then be converted into LacNAc and elaborated by our panel of glycosyltransferases. It was also envisaged that asymmetric bi- and tri-antennary glycans could be obtained by removal of a terminal β-GlcNAc moiety by treatment with β-*N*-acetylglucosaminidase. To demonstrate the power of the methodology, we describe here the synthesis of one of the glycoforms of a tetra-antennary *N*-glycan that was observed in human ductal invasive breast carcinoma tissue and has the potential to serve as a biomarker.[14] It represents the most complex *N*-glycan ever synthesized by chemoenzymatic means. It is the expectation that the availability of such synthetic standard will allow quantitation of the biomarker in biological samples by mass spectrometry. To further illustrate the flexibility of the methodology, the common precursor **1** was employed to synthesize tri-antennary positional isomers **8** and **9** and a bi-antennary glycan **10**. Analysis of the new compounds by multistage mass spectrometry, including MS^3, showed unique fragmentation patterns of the isomeric glycans. This data will facilitate the assignment of positional isomers in complex biological samples.

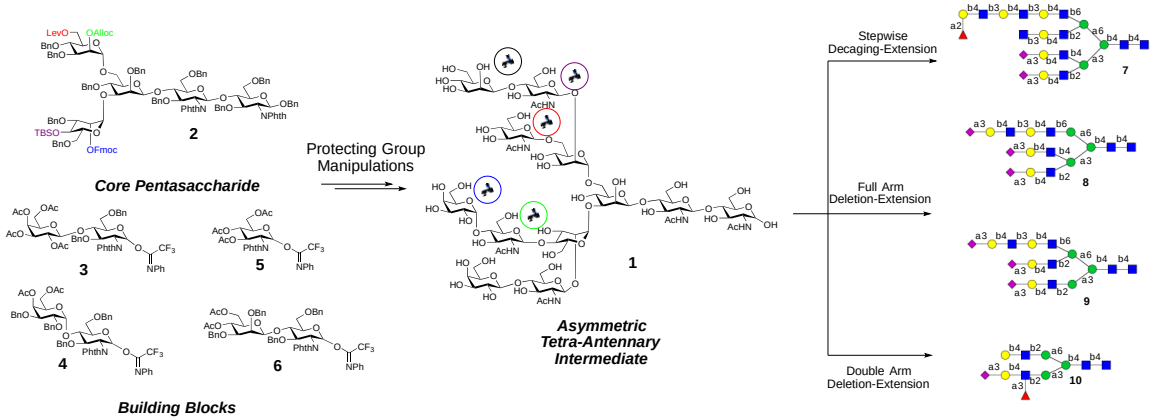
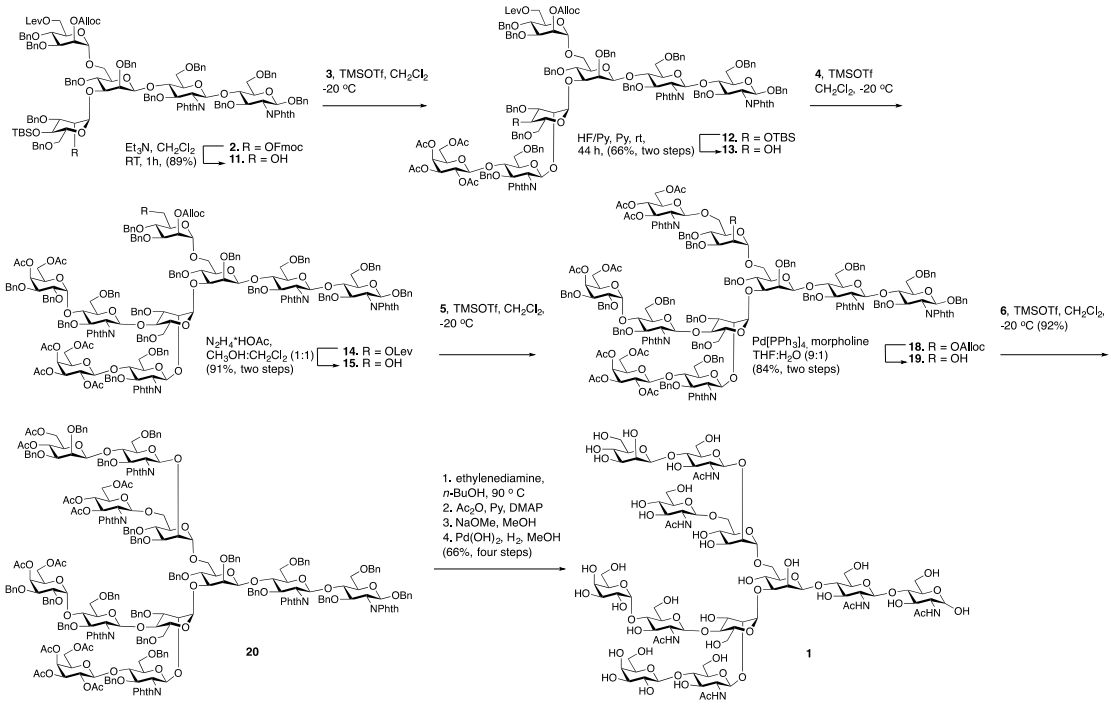


Figure 1. Asymmetric bi-, tri, and tetra-antennary *N*-glycans from common intermediate **1**



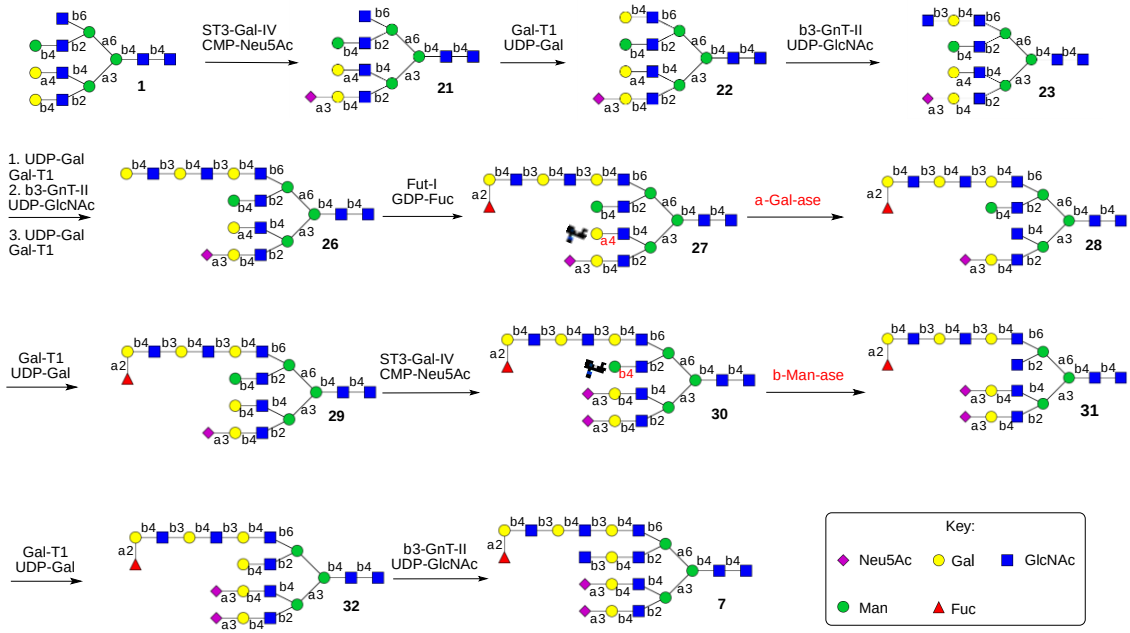
Scheme 1. Chemical assembly of common intermediate **1**

Results and Discussion

Chemical synthesis of the common intermediate. Core pentasaccharide **2**, which is modified by the orthogonal protecting groups levulinoyl (Lev), fluorenylmethyloxycarbonyl (Fmoc), allyloxycarbonyl (Alloc), and *t*-butyl-dimethylsilyl (TBS) at positions where branching points may occur, was prepared from appropriate monosaccharide building blocks (see Scheme S1 for synthesis of **2**). Furthermore, *N*-phenyl trifluoroacetimidate-based[15] glycosyl donors **3**–**6** were synthesized for the stepwise extension of **2** to give target compound **1** (see Scheme S4 for synthesis of **3**-**6**). Treatment of **2** with the non-nucleophilic base triethylamine resulted in the selective removal of the Fmoc protecting group without affecting the other base sensitive functionalities to give glycosyl acceptor **11** in an excellent yield of 89% (Scheme 1). Glycosylation of **11** with *N*-phenyl trifluoroacetimidate **3** in the presence of trimethylsilyl trifluoromethanesulfonate (TMSOTf) at –20 ºC gave heptasaccharide **12**, which was immediately treated with HF/pyridine to remove TBS ether to give acceptor **13** in a 66% overall yield. A TMSOTf promoted glycosylation of **13** with donor **4** proceeded efficiently to give, after purification by Biogel SX-1 (toluene:acetone, 1:1, v:v), the nonasaccharide **14** in a yield of 91 %. The latter glycosylation required a high concentration of donor and acceptor (>100 mM) otherwise a significant quantity of the glycosyl acceptor was recovered resulting in a modest yield of product. It was also found that the order of protecting group removal and glycosylation was critical for the successful preparation of **14**. First removal of the TBS ether of **2** followed by glycosylation with **4** gave the corresponding heptasaccharide in a low yield of 14% (see Scheme S2). Probably, the electron withdrawing Fmoc protecting group at C-2 significantly reduces the glycosyl acceptor reactivity of the C-4 hydroxyl of **16**. The observations indicate that the C-4 hydroxyl has a much high reactivity when the C-2 hydroxyl is glycosylated.

The Lev ester of **14** was selectively removed by treatment with hydrazine acetate to give alcohol **15** which was coupled with glycosyl donor **5** using TMSOTf as a promoter to provide the desired decasaccharide **18**. Next, the Alloc protecting group of **18** was removed by treatment with $Pd[PPh_3]_4$ and the resulting acceptor **19** was coupled with donor **6** using standard conditions to give dodecasaccharide **20** in high yield. Global deprotection of **20** was accomplished in four steps by first treatment with ethylenediamine in *n*-butanol at 90 ºC to remove the phthaloyl-protecting groups, which was followed by re-acetylation of the resulting free amines and hydroxyls by acetic anhydride in pyridine. Finally, the esters were selectively cleaved by catalytic sodium methoxide and hydrogenation over $Pd(OH)_2$ afforded the required tetra-antennary glycan **1**. The orthogonal protecting groups described here are more convenient than the previously employed set of protecting groups,[10] and in particular the replacement of a 2-naphthylmethyl (Nap) by TBS ether was advantageous because cleavage of the former by DDQ required great care and could result in side reactions such as cleavage of primary benzyl ethers. Also the use of phthaloyl instead of Troc for protection of amino functions gave a more robust precursor.

Enzymatic diversification of common intermediate. The stage was now set for regioselective extension of each antenna of **1** to give entry into highly complex asymmetric tetra-antennary *N*-linked glycans (Scheme 2). Compounds of such complexity have not been synthesized before and would demonstrate the power of the new methodology. Towards this end, glycan **7** was selected as a target which is a putative compound observed in a glycoproteomic study of human ductal invasive breast carcinoma tissue samples and may be attractive for biomarker development for early disease diagnosis.[14]

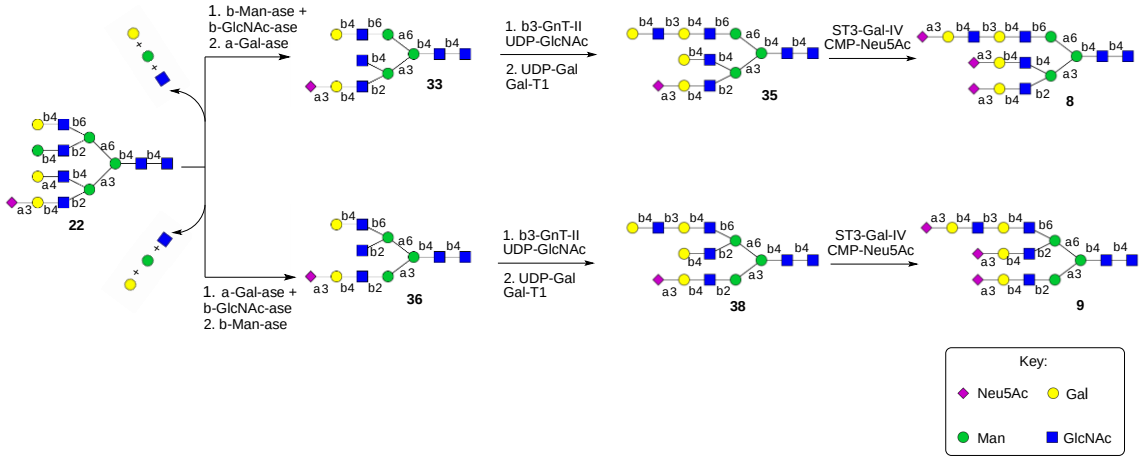


Scheme 2. Synthesis of asymmetrical tetra-antennary glycan **7** from precursor **1**

The use of a bacterial *α*(2,3)-sialyltransferase (PmST1),[16] cytidine-5'-monophospho-*N*-acetylneuraminic acid (CMP-Neu5Ac), and calf intestine alkaline phosphatase (CIAP) resulted in sialylation of the LacNAc moiety and terminal *α*-Gal moiety of the C-2 and C-3 arms. Fortunately, this lack of selectivity could be addressed by using the mammalian *α*(2,3)-sialyltransferase ST3Gal-IV[17] and this enzyme exhibited absolute selectivity for the LacNAc antenna, and could selectively convert compound **1** into mono-sialylated derivative **21**. A downfield shift of the *β*-Gal H-1 supported the site of transfer of the Neu5Ac residue. The GlcNAc moiety of the *β*(1,6)-antenna of **21** was converted into LacNAc by using recombinant mammalian *β*(1,4)-galactosyltransferase (Gal-T1), uridine-5'-diphosphogalactose (UDP-Gal) and CIAP, and complete conversion to **22** was observed after an incubation time of 10 h. Surprisingly, the use of bovine Gal-T1 led only to partial galactosylation of **21**. Treatment of **22** with *β*(1,3)-*N*-acetylglucosaminyltransferase (*β*3GnT-II),[18] uridine-5'-diphospho-*N*-acetylglucosamine (UDP-GlcNAc) and CIAP resulted in the introduction of a GlcNAc residue at the *β*(1,6)-branch to give **23**. Full assignment of ^{1}H NMR spectra (900 MHz) combined with a band-selective 2D TOCSY experiments confirmed that only the terminal *β*-Gal of the *β*(1,6)-arm was modified by GlcNAc. In this respect, only one new doublet at 4.69 ppm corresponding to H-1 of the anomeric *β*-GlcNAc moiety was observed. In addition, an upfield shift of the H-1 and the downfield shift of H-4 protons of the *β*-Gal residue (4.31 and 4.0 ppm, respectively) supported that only the LacNAc moiety of the *β*(1,6)-arm was extended and that the *α*-Gal residue at the *β*(1,4)-arm was unaffected by the enzymatic transformation. Permethylation of the product followed by mass spectrometric (MS) analysis confirmed the attachment of a single GlcNAc moiety (*m/z* 3574.7880). Controlled extension of the *β*(1,6)-arm to give a tri-LacNAc residue was accomplished by a repetition of galactosylation by Gal-T1 and *N*-acetylglucosaminylation with *β*3GnT-II to give the octadecasaccharide **26**. After each step, the product was purified by size-exclusion chromatography, and the progress of the reactions was monitored by MS of permethylated derivatives. If any starting material was observed, the reaction was prolonged until a homogenous product was obtained.

Next, the terminal LacNAc moiety of the β(1,6)-arm was converted into an H-type antigen by treatment with mammalian α(1,2)-fucosyltransferase (Fut-I)[19] in the presence of GDP-Fuc to furnish **27**. This intermediate was purified by semi-preparative HPLC using an amide HILIC column (10 x 250 mm, Waters Inc.) under isocratic conditions (55% CH_3CN:100 mM ammonium formate, pH 3.4) with the UV (210 nm) detection to give the homogeneous nonadecasaccharide **27** in a sufficient quantity for further enzymatic modification of the β(1,2)- and β(1,4)-antennae.

At this point in the synthesis, the β(1,4)- and the β(1,2)-antenna were sequentially de-caged by treatment with either α-galactosidase or β-mannosidase, respectively followed by elaboration of the resulting terminal GlcNAc moieties into complex structures. Thus, the α-Gal moiety was removed by treatment with the α-galactosidase from green coffee beans, which proceeded smoothly to give glycan **28**. The resulting terminal GlcNAc residue of **28** could easily be converted into a sialyl LacNAc moiety by subsequent treatment with Gal-T1 and ST3Gal-IV to give the di-sialylated glycan **30**. Finally, the β(1,2)-arm was elaborated by first removal of the capping β-mannoside using *Helix pomatia* β-mannosidase. Mass spectrometric analysis of the product revealed that in addition to the β-mannoside, also the α-fucoside had been cleaved, which probably was due to contamination with a fucosidase. Fortunately, the use of the inhibitor 1-deoxyfuconojirimycin abolished the loss of the fucosyl residue to give reliable entry into glycan **31**. Finally, the targeted asymmetric 21-mer **7** was obtained by galactosylation of **31** with Gal-T1 to give **32**, which was further extended by β(1,3)GlcNAc unit by employing β3GnT-II. Homogeneity of glycan **7** was confirmed by LC-MS using an amide HILIC column (55% CH_3CN:100 mM ammonium formate, pH ± 3.4).

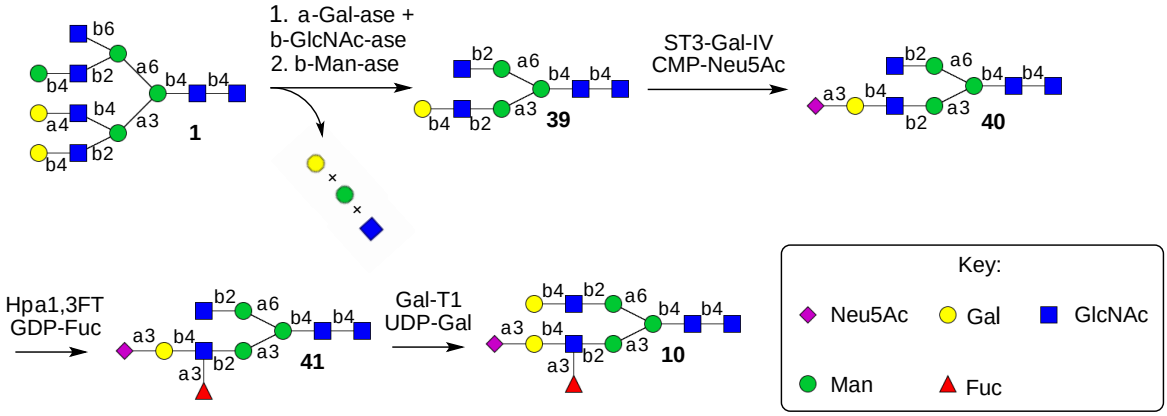


Scheme 3. Preparation of isomeric tri-antennary glycans **8** and **9** from common precursor **22**

Next, attention was focused on the synthesis of tri-antennary glycans from the universal precursor. It was expected that selective removal of the terminal β-Man or α-Gal of the β(1,2)- or β(1,4)-antenna of **22** followed by treatment with β-*N*-acetylglucosaminidase to remove the revealed terminal GlcNAc moiety would provide tri-antennary structures **33** and **36**, respectively (Scheme 3). The latter compounds can then be elaborated to the tri-antennary glycans **8** and **9**, which are positional isomers. Thus, treatment of compound **22** with a mixture of α-galactosidase and β-*N*-acetylglucosaminidase from Jack bean resulted in deletion of the β(1,4)-arm as shown by MS. Next, the reaction mixture was heat deactivated and subsequent addition of β-mannosidase and incubation for additional 2 h afforded homogenous tri-antennary glycan **36**. Alternatively, exposure of **22** to β-mannosidase and β-*N*-acetylglucosaminidase resulted in the removal of the β(1,2) appendage, and after the

addition of the α-galactosidase and further incubation for 2 h, glycan **33** was obtained. Detailed inspection of NMR data as well as the permethylation followed by MALDI-TOF MS analysis confirmed that the trimming process had proceeded with the expected regio-selectivity. For both compounds, the anomeric proton for α-Gal (5.44 ppm) had disappeared while a similar signal for the β-Gal (4.47 ppm) was still present, which confirmed that the β(1,6)-arm had remained intact. The one-pot multiple-enzyme procedure[16b] made the transformation very efficient.

Treatment of **33** and **36** with β3GnT-II and UDP-GlcNAc followed by bis-galactosylation with Gal-T1 and UDP-Gal afforded tri-antennary glycans **35** and **38**, respectively containing a terminal β(1,6)-di-LacNAc moiety. Sialylation of **35** and **38** with ST3Gal-IV in the presence of CMP-Neu5Ac gave the positional isomers **8** and **9**, respectively.



Scheme 4. One-pot multiple-enzyme approach for the preparation of bi-antennary glycans from common precursor **1**

The final goal was to synthesize a bi-antennary glycan from common tetra-antennary precursor **1** by a one-pot multiple-enzyme approach (Scheme 4). Sequential treatment of **1** with the α-galactosidase and β-*N*-acetylglucosaminidase followed by heat-deactivation and further processing with β-mannosidase lead to clean formation of bi-antennary glycan **39** (LC-MS on a HILIC column, 65% CH_3CN:100 mM ammonium formate, pH±3.4). Sialylation of the LacNAc moiety of the β(1,2)-arm with ST3Gal-IV resulted in the formation of glycan **40**. Antenna-selective installation of a sialyl-Le^x moiety was easily accomplished by exposing the newly formed α(2,3)-sialyl LacNAc to GDP-Fuc and *Helicobacter pylori* α(1,3)-fucosyltransferase (Hpα1,3FT)[20] providing **41**. The final step involved galactosylation of the β (1,2)-arm of **41** by employing Gal-T1 and UDP-Gal to furnish bi-antennary glycan **10**.

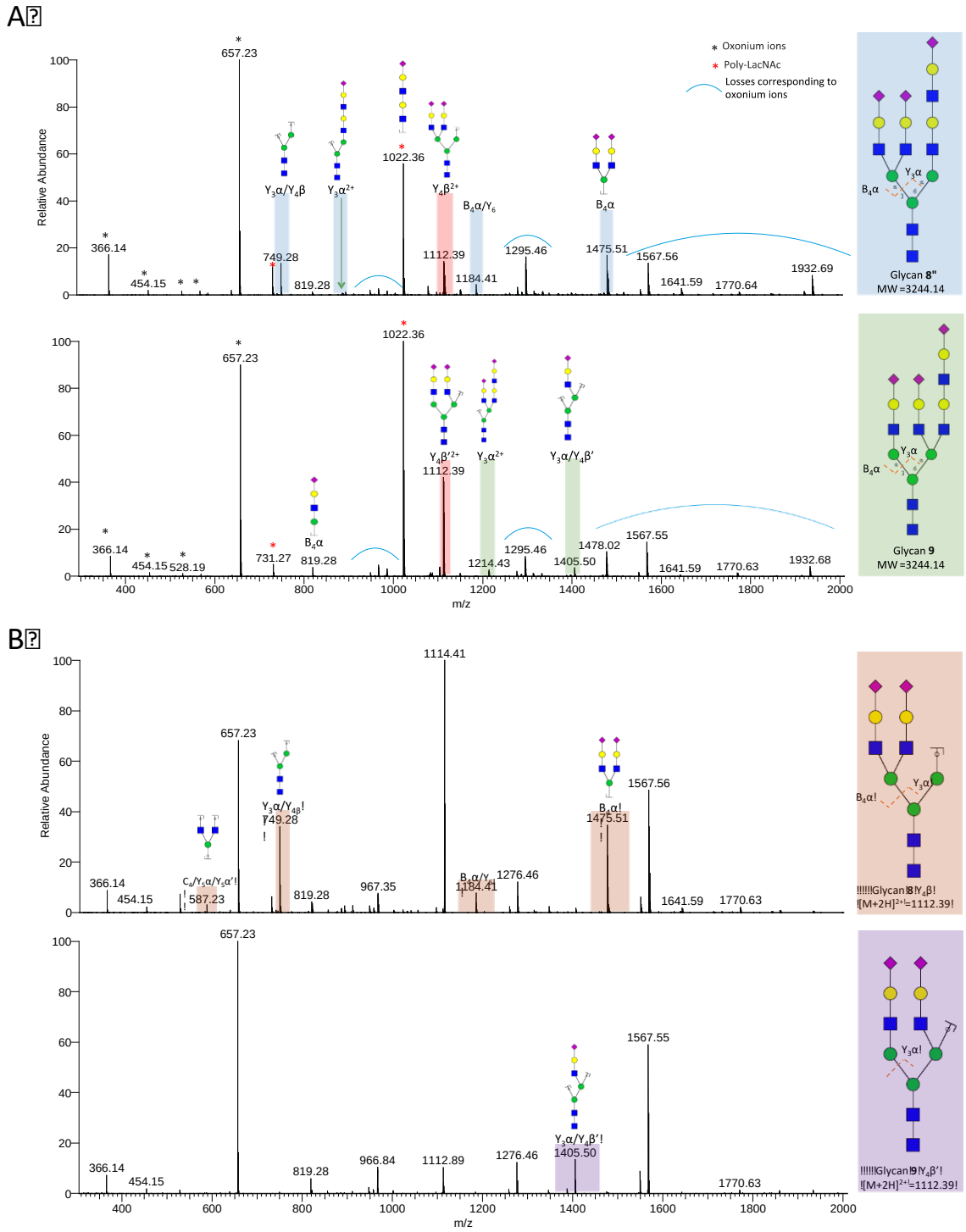


Figure 2. A. Multistage mass spectrometry can distinguish the isomer glycans **8** and **9**. CID MS^2 of the triply charged precursor ion at m/z 1082.39. Fragment ions indicative for distinguishing the two isomers are color-coded. **B**. CID-MS^3. The MS^2 fragment ion at m/z 1112.39 was subjected to MS^3 to reveal the structural differences between these two isobaric fragment ions. Fragment ions that can distinguish the two isomers are color-coded.

Multistage mass spectrometry of isomeric glycans. The use of MS in glycomics is driven by the need to obtain quantitative and structural information of glycans of glycoproteins present in biological samples to understand metabolic or disease processes and to discover and validate putative new biomarkers.[21] In general, glycans are enzymatically or chemically released from glycoproteins, separated by chromatography and analyzed by MALDI-TOF or ESI-TOF MS. Although powerful, such studies often do not provide exact structures of glycans because of their isobaric nature (*i.e.* different structures with identical molecular weights). It is anticipated that the use of well-defined glycan standards will make it possible to examine chromatographic behavior and fragmentation patterns in multistage mass spectrometry, which may reveal unique features of glycan isomers that may facilitate exact structure identification.[22]

$[M+3H]^{3+}$ precursor ions corresponding to native/non-permethylated glycan **8** and glycan **9** were isolated and subjected to CID to obtain fragments that may distinguish these two isomers. Oxonium ions were observed in the low mass range as well as the corresponding fragments resulting from the losses of oxonium ions from precursor ions in the higher mass range. A peak at *m/z* 1022 is indicative of di-*N*-acetyllactosamine containing sialic acid (Neu5Ac-di-LacNAc), which can further lose Neu5Ac as observed by the presence of peak at *m/z* 731. To confirm the branching structures of glycans **8** and **9**, we searched for unique and structurally informative peaks in each spectrum, mainly the branching specific $B_4\alpha$ and $Y_3\alpha$ fragment pairs. For glycan **8**, MS^2 CID branching specific fragments (Figure 2, top panel, shaded in blue) were observed as $B_4\alpha$ at *m/z* 1475 and its' Y counterpart fragment $Y_3\alpha^{2+}$ at *m/z* 885 confirmed the presence of two Neu5Ac-LacNAc units on *α*(1,3)-branch and Neu5Ac-di-LacNAc on *α*(1,6)-branch, respectively. The presence of two Neu5Ac-LacNAc units on the *α*(1,3)-branch is further supported by the $B_4\alpha/Y_6$ *m/z* 1184 peak (loss of one Neu5Ac from $B_4\alpha$). Doubly protonated fragment $Y_4\beta^{2+}$ at *m/z* 1112 (shaded in red) resulting from the loss of Neu5Ac-di-LacNAc from precursor ion. $Y_4\beta^{2+}$ can further lose the entire *α*(1,3)branch and give rise to a $Y_3\alpha$/ $Y_4\beta$ fragment at *m/z* 749. Although $Y_3\alpha^{2+}$ is barely visible in the spectra, it can still be confidently assigned with signal to noise ratio (S/N) of 45. The low abundance thereof is due to the lability of Neu5Ac-di-LacNAc unit in the gas phase resulting once again in $Y_3\alpha$/ $Y_4\beta$ fragment.

The CID MS^2 spectrum of glycan **9** provided two unique fragment peaks (Figure 2A, bottom panel, shaded in green). A unique peak is observed for the $Y_3\alpha^{2+}$ *m/z* 1214 fragment ion, resulting from the loss of Neu5Ac-LacNAc-Man from *α*(1,3)-branch, which can be found as $B_4\alpha$ fragment ion at *m/z* 819. Loss of Neu5Ac-LacNAc-Man from $Y_4\beta'^{2+}$ *m/z* 1112 results in second unique peak $Y_3\alpha/Y_4\beta'$ found at *m/z* 1405. These two peaks corroborate glycan **9** branching pattern consisting of one Neu5Ac-LacNAc unit on the *α*(1,3)-branch, and Neu5Ac-LacNAc Neu5Ac-di-LacNAc on the α6-branch.

In order to validate the annotation of the *m/z* 1112, which appears in both MS^2 spectra, MS^3 CID analyses were performed for each glycan (Figure 2B). Fragmentation of the $Y_4\beta^{2+}$ *m/z* 1112 fragment, belonging to glycan **8** MS^2 CID (Figure 2B, top panel shaded in orange) produced $B_4\alpha$ fragment at *m/z* 1475 as well as the $Y_3\alpha/Y_4\beta$ *m/z* 749 ion. These informative fragments can be formed if both Neu5Ac-LacNAc blocks are present on the *α*(1,3) branch. In contrast, the MS^3 CID fragmentation of the $Y_4\beta'^{2+}$ *m/z* 1112 ion isolated from glycan **9** MS^2 CID (Figure 2B, bottom panel shaded in purple) resulted in a distinct $Y_3\alpha/Y_4\beta'$ *m/z* 1405 peak which can only be formed if the two Neu5Ac-LacNAc units are found on different branches within **9**. These results clearly demonstrate the importance of MS^3 to distinguish

isobaric fragments of glycans that cannot be distinguished based on mass measurements alone.

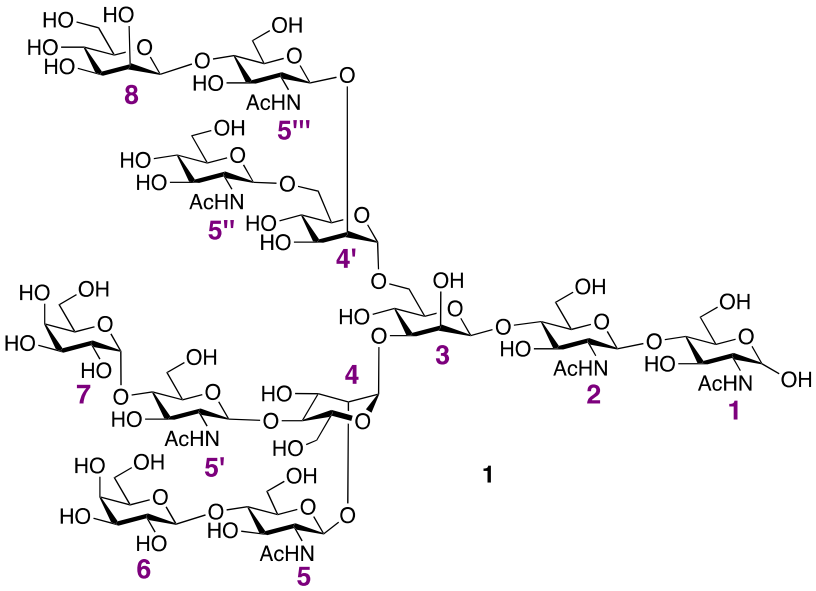
Although it is possible to achieve richer fragmentation spectra of glycans by permethylation or measuring them as adducts of alkali metals, the resulting spectra require expert knowledge of glycan fragmentation for unambiguous annotation. We have measured glycans **8** and **9** as potassium and sodium adducts in positive mode as well as chlorinated glycans in negative mode, and although the resulting fragmentation spectra are rich in information, we have found that CID of protonated glycans provides sufficient details to distinguish isobaric glycans **8** and **9** and result in a simple fragmentation pathway consisting mainly of glycosidic bond cleavages and thus simplifying spectral annotation.

Conclusion

Although naturally occurring complex *N*-linked oligosaccharides are usually asymmetrically substituted having a unique saccharide appendage at each branching point, previous synthetic efforts have almost exclusively been directed to the preparation of simpler symmetrical structures.[9a, 9d-j, l, m, o] This stems from the difficulties of controlling diversification at the various sites of branching, especially when several different complex terminal structures need to be appended. To address this challenge, we describe here a chemoenzymatic methodology that makes it possible to prepare any bi-, tri-, and tetra-antennary asymmetric *N*-glycan from a common precursor (**1**). This latter precursor is a tetra-antennary glycan that at positions where branching may occur is modified by LacNAc, GlcNAc and unnatural α-Gal(1,4)GlcNAc and β-Man(1,4)GlcNAc. We have found that relevant mammalian glycosyltransferases will only recognize the LacNAc containing antenna of the common precursor as a substrate, therefore allowing unique extension of this arm. At an appropriate stage of synthesis, the antenna containing a terminal GlcNAc residue can be "armed" by conversion into LacNAc by a galactosyltransferase, which can then be further extended by our panel of glycosyltransferases into a complex structure. The antenna that are modified by the unnatural hexoses α-Gal and β-Man can be de-caged by an appropriate glycosidase to reveal a terminal β-GlcNAc moiety, and thus the process of conversion into LacNAc and extension by glycosyltransferases can be repeated to give entry into asymmetrical tetra-antennary glycan. The use of unnatural distal hexoses to temporarily block an antenna from enzymatic modification is much more robust than our previously reported approach[10] based on acetylation of terminal GlcNAc or LacNAc residues. The new strategy made it possible, for the first time, to prepare a tetra-antennary *N*-glycan that at each branching point has a different appendage. We also describe that the common precursor can easily be converted into bi- and tri-antennary glycans by deletion of an antenna by the action β-*N*-acetylglucosaminidase, which selectively cleaves a terminal GlcNAc residue. This process can be performed by an efficient one-pot multiple-enzyme procedure. The latter strategy made it possible to prepare two isomeric tri-antennary *N*-glycans. As proof of principle to demonstrate the usefulness of the methodology, the isomers were subjected to multistage mass spectrometry to discover diagnostic ions that can determine the branching pattern of *N*-glycans in complex biological samples.

Experimental

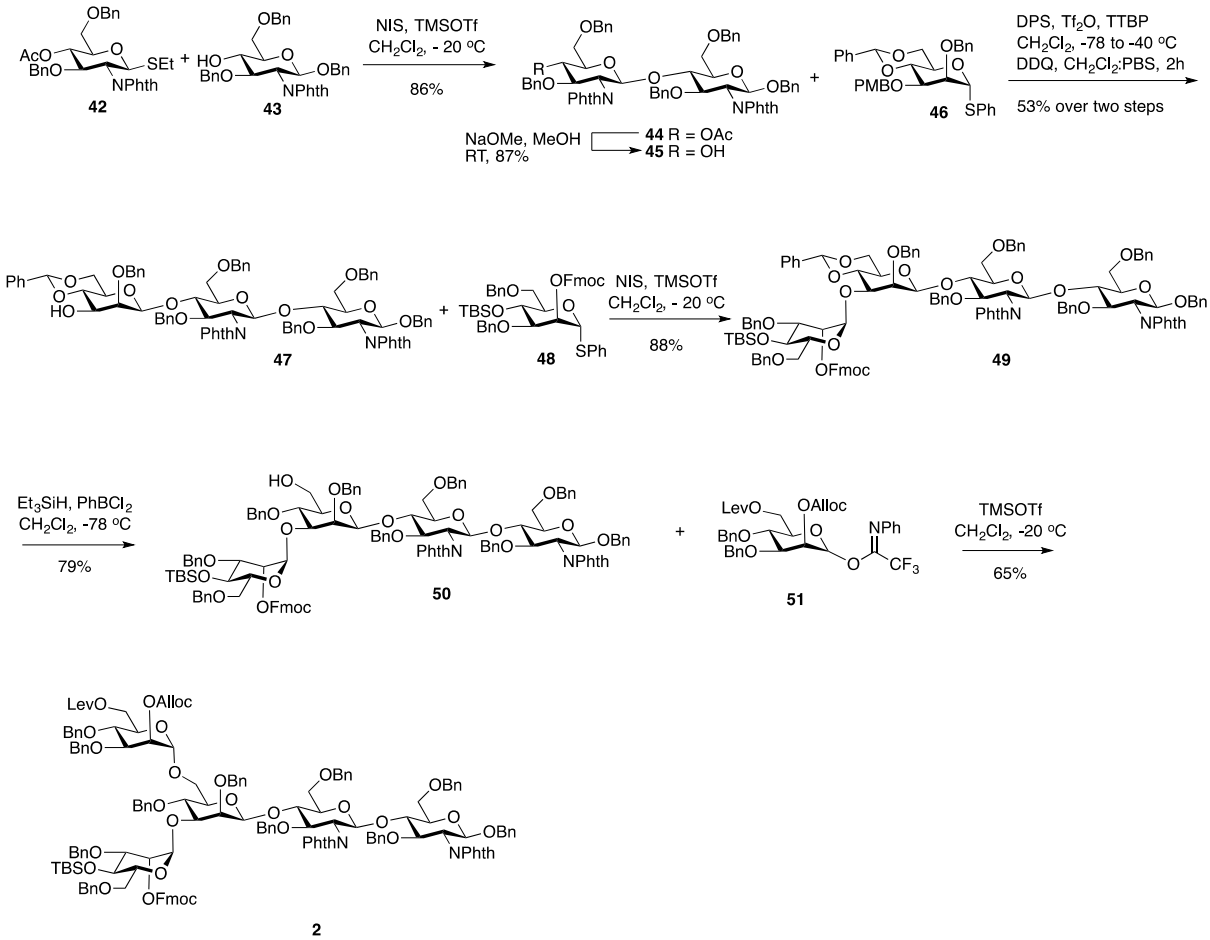
General Methods. All reagents, unless otherwise stated, were purchased from Sigma-Aldrich. ^{1}H and ^{13}C NMR spectra were recorded on Varian Mercury 300, 400, 500, or 600 MHz. Chemical shifts are reported in parts per million (ppm) relative to $CDCl_3$ as the internal standard. NMR data are presented as follows: Chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, dd = doublet of doublet, m = multiplet and/or multiple resonances); coupling constant are reported in Hertz (Hz). All NMR signals were assigned on the basis of ^{1}H NMR, COSY and HSQC experiments. Mass spectra were recorded on either on an Applied Biosystems SCIEX MALDI TOF/TOF 5800 mass spectrometer, a Shimadzu Biotech Axima-CFR MALDI-TOF, or a high resolution Shimadzu LCMS-IT-TOF mass spectrometer. Column chromatography was performed on silica gel G60 (Silicycle, 60-200 µm, 60 Å). TLC analysis was conducted on Silicagel 60 F254 (EMD Chemicals Inc.) with detection by UV light (254 nm) where applicable, and by charring with 10% sulfuric acid in ethanol or a solution of $(NH_4)_6Mo_7O_2*4H_2O$ (25 g/L) in 10% sulfuric acid in ethanol. All reactions were carried out under argon atmosphere unless specified otherwise. Unless otherwise stated, all reactions were carried out at room temperature (RT) in glassware with magnetic stirring. Solutions in organic solvents were dried with $MgSO_4$ and concentrated at 40 ^{o}C/2 kPa. Molecular sieves were flame-dried under vacuum immediately prior to use.



NMR Nomenclature

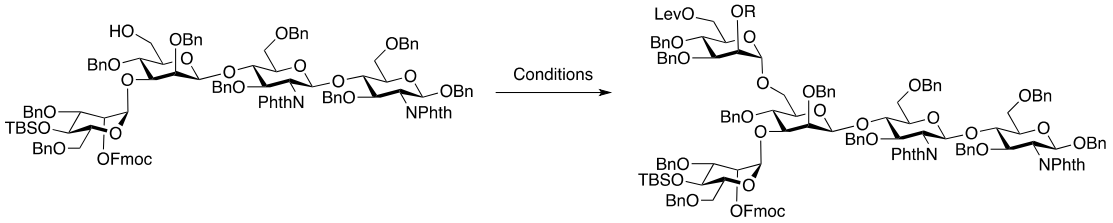
Individual monosaccharides within glycans have been labeled as shown in **Figure S1**. Starting from the reducing end of the chitobiose core, these were labeled as GlcNAc-1 (GlcN-1) and GlcNAc-2 (GlcN-2) respectively; the *β*-mannoside of the core pentasaccharide is labeled as Man-3, the *β*-mannoside of the unnatural Man-*β*-(1→4)-GlcNAc terminus was labeled as Man-8; the *α*-3 mannoside as Man-4, and the *α*-6 mannoside as Man-4', followed by the *N*-acetylglucosamine residues as GlcNAc-5, 5', 5'', 5'''. The *α*-4 galactoside was labeled as Gal-7.

Figure S1. Monosaccharide labeling system for compounds within the chemical synthesis section.



Scheme S1. Synthesis of a core-pentasaccharide with 4 orthogonal protecting groups.

Pentasaccharide **2** was prepared from building blocks **42, 43, 46, 48,** and **51**. Thus, the known acceptor **43**[23] was glycosylated with the thioglycoside donor **42**[9m] to give a chitobiose core **44** in 86% yield, which was further treated with NaOMe in methanol to give acceptor **45**. Pre-activation[24] of mannosyl donor **46** with diphenyl sulfoxide (DPS) and Tf_2O in presence of tri-*t*-butylpyrimidine (TTBP), followed by adding the alcohol **45** resulted in the formation of a *β*-mannoside, which was isolated in 53% yield over two steps after the DDQ-mediated oxidative cleavage of the PMB group.[9l] Next, trisaccharide **47** was coupled with the mannosyl donor **48** under the NIS-TMSOTf conditions furnishing tetrasaccharide **49** in high yield as only the *α*-anomer. The benzylidene acetal of **49** was then regioselectively opened[10] using triethylsilane and $PhBCl_2$ to give the glycosyl acceptor **50** in 79% yield. Constructing the final mannose-*α*-(1→6)-mannose glycosidic linkage proved to be the most difficult step within the entire assembly sequence. For this purpose, multiple types of glycosyl donors were examined by varying promoters and reaction temperatures (Table S1). Satisfactory, it was found that pentasaccharide **2** could be obtained in 65% yield by coupling the acceptor **50** with the donor **51.**



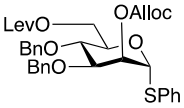
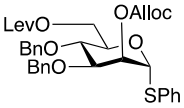
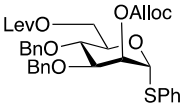
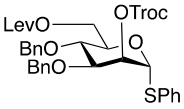
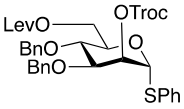
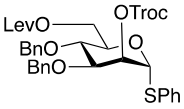
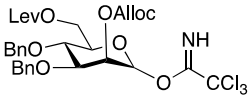
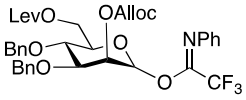
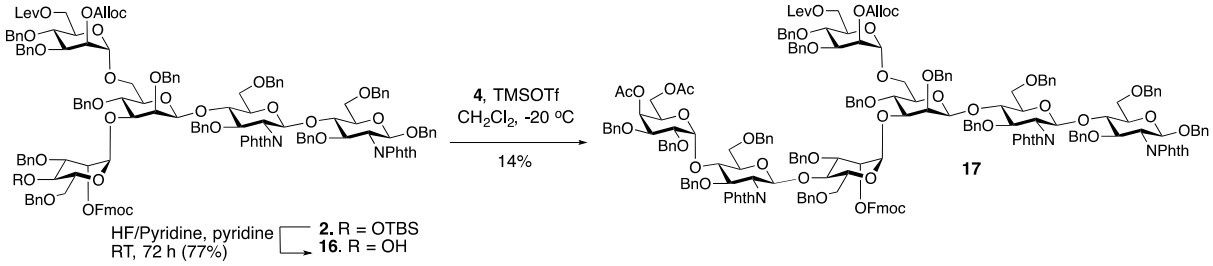
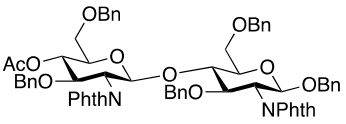
Entry	Donor	Conditions	Results/Yield
1		MeOTf (8 eq), CH_2Cl_2, DTBMP RT, 18 h	Acceptor recovered
2		DMTST (4 eq), CH_2Cl_2, RT	Acceptor recovered
3		DPS, Tf_2O, TTBP, CH_2Cl_2 -78 ^oC	Complex mixture
4		NIS, TMSOTf, CH_2Cl_2, -20 ^oC	Complex mixture
5		NIS, AgOTf, CH_2Cl_2, -20 ^oC	Complex mixture
6		DPS, Tf_2O, TTBP, CH_2Cl_2 -78 ^oC	Complex mixture
7		TMSOTf, CH_2Cl_2, -20 to -10 ^oC	67%
8		TMSOTf, CH_2Cl_2, -20 to -10 ^oC	65%

Table S1. Coupling optimization of the acceptor **50** with a panel of glycosyl donors



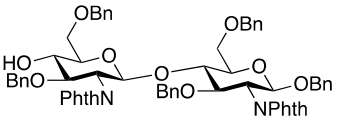
Scheme S2. Low-yielding coupling of acceptor **16** with donor **4**.

Benzyl 4-*O*-acetyl-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-*β*-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-*β*-D-glucopyranoside (44). A mixture of donor **42** (23.4 g, 40.65 mmol), acceptor **43** (20.0 g, 34.0 mmol), and 4Å flame-dried molecular sieves, was stirred in CH_2Cl_2 (300 mL) for 5 min. NIS (9.3 g, 40.65 mmol) was added and the reaction mixture was cooled to – 20 ºC. TMSOTf (1.2 mL, 6.8 mmol) was added and the mixture was stirred at – 20 ºC for 10 min, after which it was warmed to 0 ºC and stirring was continued for another 30 min. The reaction was quenched with Et_3N (5 mL), diluted with CH_2Cl_2 (200 mL), and washed with 10% $Na_2S_2O_3$ (200 mL). The organic phase was dried ($MgSO_4$), filtered, and the filtrate was concentrated *in vacuo* to give the crude product as an orange syrup. Purification by silica gel chromatography using EtOAc:Hexane (1:4 to 3:7) as eluent gave the title disaccharide as a white foam. (32.05 g, 86%). R_f = 0.3 (EtOAc:Hexane, 3:7). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.91 (3H, s, OAc), 3.30 (1H, br d), 3.37 – 3.60 (6H, m, H-3, GlcN-2; H-6a, GlcN-1; H-6b, GlcN-1; H-6a, GlcN-2; H-6b, GlcN-2; H-3, GlcN-1), 4.08 – 4.22 (2H, m, H-2, GlcN-2; H-5, GlcN-1), 4.22 – 4.39 (3H, m, CH*H*Ph; CH*H*Ph ; H-2, GlcN-1), 4.39 – 4.63 (7H, m, CH_2Ph X 3; H-5, GlcN-2), 4.67 (1H, d, *J* = 12.2 Hz, CH*H*Ph), 4.81 (1H, d, *J* = 12.2 Hz, CH*H*Ph), 4.94 (1H, br d, H-1, GlcN-2), 5.14 (1H, t, *J* = 9.2 Hz, H-4, GlcN-2), 5.33 (1H, d, *J* = 8.3 Hz, H-1, GlcN-1), 6.77 – 7.89 (33H, m, Ar-H). ^{13}C NMR (75 MHz, $CDCl_3$): 169.8, 167.7, 138.7, 138.6, 138.1, 137.7, 137.0, 134.3, 134.1, 133.5, 131.7, 128.4 x 4, 128.3, 128.1 x 2, 127.8, 127.7, 127.5, 127.4 x 2, 127.3, 126.7, 123.1, 97.1(C-1, GlcN-2), 97.0 (C-1, GlcN-1), 76.8 (C-5, GlcN-2), 76.3 x 2 (C-5, GlcN-1), 74.5 x 2 ($\underline{C}H_2$Ph), 72.05 – 73.08 (C-4, GlcN-2; 4 x *C*H_2Ph), 69.4, 68.1 (C-6, GlcN-1+GlcN-2), 56.2 (C-2, GlcN-2), 55.8 (C-2, GlcN-2), 20.8 (*C*H_3CO). MALDI-TOF-MS (*m/z*): [M + Na]$^+$ calcd for $C_{65}H_{60}N_2NaO_{14}$ 1115.3942; found 1115.3958. The analytical data are in accordance with literature data.[25]



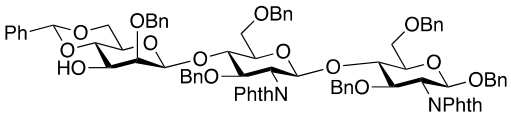
44

Benzyl 3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-*β*-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-*β*-D-glucopyranoside (45). Disaccharide **44** (28.0 g, 25.6 mmol) was dissolved in methanol, followed by the addition of guanidinium hydrochloride (5.0 g). To the resulting solution was added 1 M NaOMe until pH~9.0, and the reaction mixture was stirred for 2 h. Acetic acid was then added, and the product mixture was concentrated *in vacuo*, applied to a column of silica gel, and purified using EtOAc:Hexane (3:7) as eluent to give the disaccharide acceptor as a white foam. 23.54 g (87%). R_f = 0.3 (EtOAc:Hexane, 3:7). ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.75 (1H, s), 3.29 (1H, br d), 3.34 – 3.60 (5H, m), 3.70 (1H, dd, *J* = 10.3, 4.3 Hz), 3.81 (1H, t, *J* = 8.80 Hz), 4.07 – 4.30 (7H, m), 4.31 – 4.57 (8H, m), 4.69 (1H, d, *J* = 12.7 Hz), 4.73 – 4.83 (2H, m), 4.95 (1H, d, *J* = 8.2 Hz), 5.31 (1H, d, *J* = 8.6 Hz), 6.79 – 7.89 (33H, m, Ar-H). ^{13}C NMR (75 MHz, $CDCl_3$): 168.3, 167.8, 138.6, 138.5, 138.3, 137.7, 134.0, 133.8, 133.5, 131.9, 131.4, 128.5, 128.4, 128.1, 127.9 x 2, 127.7, 127.6, 127.5, 127.4, 127.3, 126.6, 123.6, 123.1, 97.2, 96.9, 78.3, 76.5, 75.5, 74.5, 74.3, 74.1, 73.7, 72.7 x 2, 71.0, 70.5, 68.1, 65.4, 56.0. MALDI-TOF-MS (*m/z*): [M + Na]$^+$ calcd for $C_{63}H_{58}N_2NaO_{13}$ 1073.3837; found 1073.3840. The analytical data are in accordance with literature data.[9l]



45

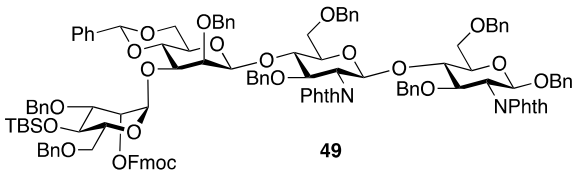
Benzyl 2-*O*-benzyl-4,6-benzylidene-*β*-D-mannopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-*β*-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-*β*-D-glucopyranoside (47).



47

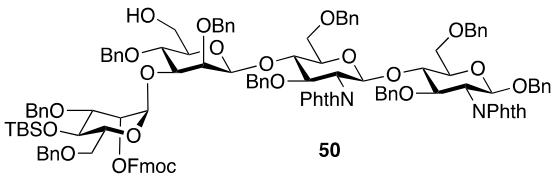
A mixture of mannosyl donor **46**[9l, m] (16.07 g, 28.16 mmol), diphenyl sulfoxide (5.7 g, 28.16 mmol), TTBP (7.5 g, 28.16 mmol) and 4Å flame-dried molecular sieves was stirred in CH_2Cl_2 (200 mL) for 5 min. The mixture was cooled to -78 ^oC, and Tf_2O (4.7 mL, 28.16 mmol) was added along the wall of the flask. After stirring for 10 min at – 78 ^oC, a solution of disaccharide **45** (20.0 g, 18.78 mmol) in CH_2Cl_2 (100 mL+20mL x 2 washings) was added dropwise over a period of 30 min with the aid of a dropping funnel. The reaction mixture was stirred for additional 30 min at – 78 ^oC, after which it was gradually warmed to – 40 ^oC, and then quenched with Et_3N (10 mL). The mixture was diluted with CH_2Cl_2 (200 mL) and washed with sat. $NaHCO_3$ (300 mL). The organic phase was then dried ($MgSO_4$), filtered, and the filtrate was concentrated *in vacuo* to give a thick syrup, which was subjected to a short silica gel column using EtOAc:Hexane (3:7) as eluent, affording inseparable *α*/*β*-trisaccharide anomers as a white foam (29.5 g). This material was dissolved in CH_2Cl_2 (100 mL), cooled to 0 ^oC, and phosphate buffer (pH 7.0, 10 mL, 0.5 M) was added, and the mixture was further treated with DDQ (5.6 g, 25 mmol). Stirring was continued for 2 h, after which the reaction was carefully quenched with sat. $NaHCO_3$ (100 mL), diluted with CH_2Cl_2 (200 mL), and the organic phase was washed with an additional portion of sat. $NaHCO_3$ (200 mL). The organic phase was further dried ($MgSO_4$), filtered, and the filtrate was concentrated *in vacuo*. Purification of the residue by silica gel chromatography using EtOAc:Toluene (1:9 to 15:85) as eluent gave the desired trisaccharide acceptor as a white foam. (13.71 g, 53%, over two steps). R_f = 0.3 (EtOAc:Toluene, 1:4). ^1H NMR (500 MHz, $CDCl_3$): δ 2.31 (1H, d, *J* = 9.1 Hz, OH), 3.12 (1H, ddd, *J* = 5.5, 9.8 Hz, H-5, Man-3), 3.20 (1H, d, *J* = 10.2 Hz, H-5, GlcN-2), 3.32 (1H, dd, *J* = 2.9, 9.8 Hz, H-3, GlcN-2), 3.42 – 3.53 (4H, m, H-6a, GlcN-1; H-5, GlcN-1, H-6a, GlcN-2), 3.54 – 3.60 (3H, m, H-6b, GlcN-2; H-6b, GlcN-1; H-2, Man-3;), 3.63 – 3.73 (3H, m, H-3, Man-3; H-6a, Man-3; H-4, GlcN-2;), 4.07 – 4.28 (8H,m, H-6b, Man-3; H-3, GlcN-1; H-4, GlcN-1; H-2, GlcN-2; H-2, GlcN-1; H-4, Man-3), 4.39 (2H, m, C*H*HPh; CH*H*Ph), 4.46 (1H, d, *J* = 12.5 Hz, C*H*HPh), 4.49 – 4.55 (3H, m, CH*H*Ph; CH*H*Ph; C*H*HPh), 4.60 (1H, d, *J* = 11.6 Hz, C*H*HPh), 4.65 – 4.72 (3H,m, CH*H*Ph; H-1, Man-3; C*H*HPh), 4.85 (1H, d, *J* = 12.5 Hz, CH*H*Ph), 4.90 (1H, d, *J* = 12.5 Hz, CH*H*Ph), 4.96 (1H, d, *J* = 8.8 Hz, H-1, GlcN-2), 5.01 (1H, d, 12.3 Hz, C*H*HPh), 5.29 (1H, d, *J* = 8.16 Hz, H-1, GlcN-1), 5.43 (1H, s, benzylidine C*H*Ph), 6.73 – 6.90 (43 H, m, Ar-H). MALDI-TOF-MS (*m/z*): $[M + Na]^+$ calcd for $C_{83}H_{78}N_2NaO_{18}$ 1413.5147; found 1413.5153. The remaining analytical data are in accordance with that of previously reported.[9l]

Benzyl 3,6-di-*O*-benzyl-4-*O*-*t*-butyldimethylsilyl-2-*O*-fluorenylmethoxycarbonyl-*α*-D-mannopyranosyl-(1→3)-2-*O*-benzyl-4,6-benzylidene-*β*-D-mannopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-*β*-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-*β*-D-glucopyranoside (49). To a mixture of the donor **48** (16.7 g, 21.16 mmol), acceptor **47** (14.72 g, 10.57 mmol), and 4Å flame-dried molecular sieves in dry CH_2Cl_2 (200 mL) was added NIS (4.8 g, 21.14 mmol). The resulting solution was cooled to – 20 ºC upon which TMSOTf (383 µL, 2.11 mmol) was added, and stirring was continued at – 20 ºC for 10 min. The reaction was then quenched with pyridine (10 mL), diluted with CH_2Cl_2 (100 mL), washed with 10% $Na_2S_2O_3$ (100 mL), and dried ($MgSO_4$), filtered, and the filtrate was concentrated *in vacuo*. The obtained residue was purified using EtOAc:Toluene (1:9 to 1:4) as eluent to give the desired tetrasaccharide as a white solid. (20.0 g, 88%). R_f = 0.4 (EtOAc:Toluene 1:9). ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.02 (6H, d, TBS-Si-CH_3 x 2), 0.83 (9H, s, TBS-C-CH_3 X3), 3.06 (1H, m, H-5, Man-3), 3.22 (1H, d, *J* = 9.7 Hz, H-5, GlcN-2), 3.32 (1H, d, *J* = 9.7 Hz, H-3, GlcN-2), 3.37 (1H, d, *J* = 11.3 Hz, H-6a, GlcN-2), 3.47 (2H, m, H-6a, Man-4; H-6a,Man-3), 3.59 (2H, m, H-6b, Man-3; H-6b, GlcN-2), 3.69 – 3.87 (6H, m, H-6a, GlcN-1; H-6b, Man-4; H-3, Man-4; H-6b, GlcN-1; H-2, Man-3), 3.92 (3H, m, H-3, GlcN-1; H-3, Man-3), 4.01 (3H, m), 4.05 – 4.74 (41H, m; H-2, GlcN-2; H-2, GlcN-1; H-4, GlcN-2; CH_2Ph protons, H-1; Man-3), 4.80 – 4.99 (7H, m, CH_2Ph protons; Fmoc CH_2; H-1, GlcN-2), 5.29 (1H, d, *J* = 7.8 Hz, H-1, GlcN-1), 5.36 (1H, s, H-1, Man-4), 5.41 (1H, s, H-2, Man-4), 5.47 (1H, s, benzylidine C*H*Ph), 6.71 – 7.92 (61H, m, Ar-H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 167.6, 143.6, 143.3, 141.2, 138.6, 138.5, 138.5, 138.3, 137.8, 137.7, 137.3, 137.2, 133.4, 131.7, 128.6, 128.4, 128.3, 128.2, 128.0, 127.9, 127.8, 127.8, 127.7, 127.6, 127.5, 127.4, 127.3, 127.2, 126.9, 125.9, 125.3, 123.1, 119.9, 101.1, 97.1, 78.5, 76.2, 74.5, 73.4, 73.2, 72.7, 71.5, 70.5, 70.2, 55.7, 46.5, 25.9, 18.2, -3.9, -5.0. MALDI-TOF-MS (*m/z*): [M + Na]$^+$ calcd for $C_{124}H_{124}N_2O_{25}Si$, 2091.8160; found 2091.3216.



49

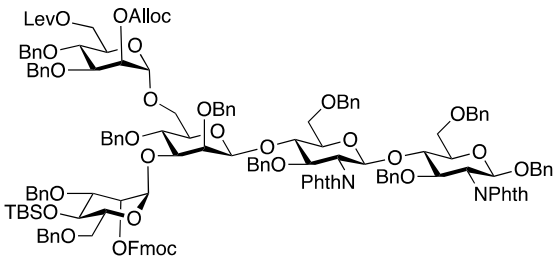
Benzyl 3,6-di-*O*-benzyl-4-*O*-*t*-butyldimethylsilyl-2-*O*-fluorenylmethoxycarbonyl-*α*-D-mannopyranosyl-(1→3)-2,4-di-*O*-benzyl-*β*-D-mannopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-*β*-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-*β*-D-glucopyranoside (50). Tetrasaccharide **49** (21.7 g, 10.48 mmol) was dissolved in CH_2Cl_2 (500 mL), followed by adding 4Å flame-dried molecular sieves. The resulting mixture was cooled to – 78 ºC, and Et_3SiH (3.3 mL) was added. $PhBCl_2$ (1.8 mL) was introduced and the resulting solution was warmed to –60 ºC, and stirring was continued at –60 ºC for 30 min. A second portion of Et_3SiH (3.3 mL) and $PhBCl_2$ (1.8 mL) was added, and stirring was continued at –60 ºC for another 20 min. The reaction mixture was quenched with pyridine (20 mL), further diluted with CH_2Cl_2 (100 mL), and then washed with sat. $NaHCO_3$ (100 mL). The organic phase was dried ($MgSO_4$), filtered, and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel chromatography using (Acetone:Hexane, 1:9 to 3:7) as eluent gave the desired acceptor tetrasaccharide as a white solid. (17.5 g, 79%). R_f = 0.3 (EtOAc:Toluene, 1:4). ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.00 (6H, d,TBS-Si-CH_3 X2), 0.79 (9H, s,TBS-C-CH_3 X3), 3.07 (1H, m, H-5, Man-3), 3.23 (1H, d, *J* = 9.2 Hz, H-5, GlcN-2), 3.32 (1H, dd, *J* = 3.2, 9.8 Hz, H-3, GlcN-2), 3.35 – 3.47 (3H, m, H-6a, GlcN-2; H-6a, Man-3; H-6a, Man-4), 3.56 (1H, d, *J* = 11.2 Hz, H-6b, Man-3), 3.59 – 3.69 (5H, m, H-6b, Man-4; H-6b, GlcN-2; H-3, GlcN-1), 3.72 – 3.82 (4H, m, H-6b, GlcN-1;



50

H-2, Man-3; H-3, Man-4; H-6a, GlcN-1), 3.91 – 4.01 (4H, m), 4.06 (1H, t, *J* = 10.0 Hz), 4.11 – 4.33 (10H, m; H-2, GlcN-2; H-2, GlcN-1; H-4, GlcN-2), 4.34 – 4.46 (5H, m, CH_2Ph protons), 4.47 – 4.59 (8H, m, CH_2Ph protons), 4.59 – 4.66 (4H, m, H-1, Man-3; CH_2Ph protons), 4.70 (2H, m ,CH_2Ph protons), 4.86 (2H, m, CH_2Ph protons), 4.97 (2H, m, H-1, GlcN-2, C*H*HPh), 5.06 (1H, d, *J* = 11.2 Hz, CH*H*Ph), 5.29 (3H, m, H-1, Man-4; H-1, GlcN-1; H-2, Man-4), 6.58 – 7.91 (61H, m, Ar-H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 167.6, 154.5, 143.5, 143.3, 141.2, 141.2, 138.9, 138.7, 138.6, 138.4, 138.1, 137.8, 137.8, 137.6, 137.2, 131.7, 128.5, 128.4, 128.3, 128.3, 128.2, 128.1, 128.0, 128.0, 127.9, 127.8, 127.7, 127.7, 127.6, 127.5, 127.5, 127.5, 127.4, 127.3, 127.3, 127.2, 127.2, 127.0, 126.9, 126.8, 125.3, 123.1, 120.0, 100.8, 99.6, 97.1, 97.1, 81.1, 78.4, 78.2, 78.0, 76.0, 75.4, 75.0, 74.8, 74.8, 74.7, 74.5, 74.5, 74.3, 73.8, 73.4, 73.1, 72.7, 72.1, 71.2, 70.4, 70.3, 69.8, 68.1, 67.9, 67.4, 62.0, 56.5, 55.7, 46.5, 25.9, 18.1, -0.0, -3.9, -5.0. MALDI-TOF-MS (*m/z*): $[M + Na]^+$ calcd for $C_{124}H_{126}N_2NaO_{25}Si$, 2093.8317; found 2093.4475.

Benzyl 2-*O*-allyloxycarbonyl-3,4-di-*O*-benzyl-6-*O*-levulenoyl-*α*-D-mannopyranosyl-(1→6)-3,6-di-*O*-benzyl-4-*O*-*t*-butyldimethylsilyl-2-*O*-fluorenylmethoxycarbonyl-*α*-D-mannopyranosyl-(1→3)-2,4-di-*O*-benzyl-*β*-D-mannopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-*β*-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-*β*-D-glucopyranoside (2).

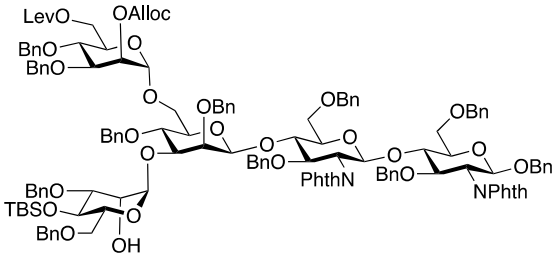


2

To a mixture of the donor **51** (13.1 g, 6.3 mmol), acceptor **50** (9.05 g, 12.6 mmol) and 4Å flame-dried molecular sieves in CH_2Cl_2 (100 mL) at –20 ºC was added TMSOTf (500 µL, 2.52 mmol). The reaction mixture was gradually warmed up to –10 ºC over a period of 30 min. The solution was cooled to –20 ºC and a second portion of donor (9.00 g, 12.6 mmol)[1] in CH_2Cl_2 (20 mL) was then slowly added along the wall of the flask. A further portion of TMSOTf (500 µL, 2.52 mmol) was added, and the reaction was stirred for another 20 min at – 20 ºC, after which it was warmed to -10 ºC, quenched with pyridine (5 mL), diluted with CH_2Cl_2, and washed with sat. $NaHCO_3$. The organic phase was dried ($MgSO_4$), filtered, and the filtrate was concentrated *in vacuo* to give the crude product. Silica gel chromatography with acetone:hexane (3:7) – EtOAc:Toluene (1:9) as eluent furnished the desired pentasaccharide as a white solid. (11.1 g, 65%). R_f = 0.3 (EtOAc:Hexane, 1:1). ^{1}H NMR (600 MHz, $CDCl_3$): δ 0.00 (6H, d, TBS-Si-CH_3 x 2), 0.79 (9H, s, TBS-C-CH_3 x 3), 2.08 (3H, s, Lev CH_2COCH_3), 2.43 (2H, t, *J* = 6.6 Hz, Lev $COOCH_2CH_2$), 2.58 (2H, t, *J* = 6.6 Hz, Lev $COOCH_2CH_2$), 3.12 (2H, m, H-5, Man-3; H-5, GlcN-2), 3.24 (1H, dd, *J* = 9.6, 3.2 Hz, H-3, GlcN-2), 3.35 (1H, dd, *J* = 11.2, 4.2 Hz, H-6a Man-3), 3.41 (1H, dd, *J* = 11.2, 2.2 Hz, H-6a, GlcN-1), 3.47 (1H, d, *J* = 10.4 Hz, H-6a, GlcN-2), 3.56 – 3.64 (3H, m, H-3, GlcN-1; H-2, Man-3; H-6b, Man-3), 3.69 (2H, m, H-6b, GlcN-1; H-6b GlcN-2), 3.75 – 3.84 (3H, m, H-6a, Man-4; H-6b, Man-4), 3.90 (1H, t, *J* = 9.2 Hz), 3.97 (1H, t, *J* = 9.2 Hz), 4.01 – 4.69 (24 H, m, H-6a, Man-4'; H-6b Man-4'; CH_2Ph protons; H-1, Man-3), 4.79 (2H, m), 4.83 – 4.93 (3H, m, H-1, GlcN-2; H-1, Man-4'), 5.12 (2H, m, H-2, Man-4'; alloc HC=CH*H*), 5.15 – 5.24 (3H, m, alloc HC=C*H*H; H-1, GlcN-1; H-1, Man-4), 5.32 (1H, s, H-2, Man-4), 5.71 (1H, m, alloc *H*C=CH_2), 6.62 – 7.80 (m, Ar-H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 223.9, 220.7, 211.9, 206.4, 202.4, 201.1, 197.5, 191.1, 183.2, 181.9, 181.7, 172.3, 169.8, 168.1, 167.5, 167.3, 166.2, 162.0, 157.0, 154.4, 154.0, 151.3, 150.5, 143.5, 143.2, 141.2, 141.2, 140.1, 139.1, 138.8, 138.6, 138.4, 138.2, 138.1, 137.9, 137.9, 137.7, 137.1, 137.0, 133.7, 133.4, 131.8, 131.6, 131.5, 128.5, 128.5, 128.4, 128.3, 128.3, 128.2, 128.2, 128.2, 128.1, 128.1, 128.0, 128.0, 128.0, 127.9, 127.8, 127.7, 127.7, 127.6, 127.5, 127.5, 127.5, 127.5, 127.4, 127.4, 127.3, 127.3, 127.2, 127.2, 126.8, 126.7, 126.4, 125.3, 123.5, 123.0, 122.1, 120.0, 118.7, 113.6, 110.0, 101.9, 99.7, 97.8, 97.0, 81.7, 79.7, 78.2, 78.1, 77.3, 77.2, 77.1, 77.0, 76.8, 76.6, 76.5, 75.9, 75.1, 74.9, 74.7, 74.6, 74.5, 74.5, 74.4, 74.4, 74.3, 73.9, 73.6, 73.4, 73.0, 72.7, 72.6, 72.0, 71.7, 71.1, 71.1, 70.4, 70.3, 69.9, 69.8, 68.4, 68.1, 68.0, 67.5, 66.5, 63.2, 56.5, 55.7, 53.3, 46.5, 37.8, 29.8, 29.7, 27.9, 27.5, 25.9, 25.7, 22.7, 18.1, 13.7, 4.9, 1.0, -3.9, -5.0. MALDI-TOF-MS (*m/z*): $[M + Na]^+$ calcd for $C_{153}H_{158}N_2NaO_{34}Si$, 2618.0363; found 2618.0166.

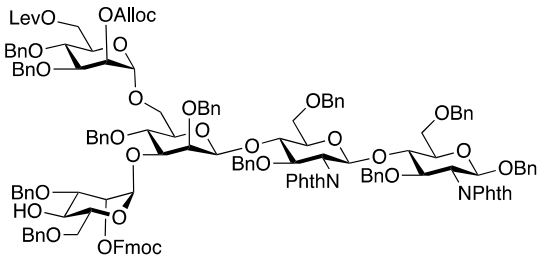
[1] This glycosylation does not go to completion with 2 eq. of the donor, hence a second addition was required. The target pentasaccharide is slightly more polar than the acceptor as evident by TLC (EtOAc:Hexane, 1:1).

Benzyl [2-*O*-allyloxycarbonyl-3,4-di-*O*-benzyl-6-*O*-levulenoyl-*α*-D-mannopyranosyl-(1→6)]-[3,6-di-*O*-benzyl-4-*O*-*t*-butyldimethylsilyl-*α*-D-mannopyranosyl-(1→3)]-2,4-di-*O*-benzyl-*β*-D-mannopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-*β*-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-*β*-D-glucopyranoside (11). To a solution of **2** (5.2 g, 2 mmol) in CH_2Cl_2 (20 mL) was added Et_3N (1.2 mL, 8 mmol), and the sreaction was left stirring for 1 h, after which TLC (EtOAc:CH_2Cl_2:Hexane, 3:2:5) showed no more starting material present. The reaction mixture was concentrated *in vacuo*, absorbed onto silica gel and purified using (EtOAc:CH_2Cl_2:Hexane, 3:2:5) as eluent to give the intermediate acceptor as a white solid. (4.2 g, 89%). R_f = 0.3 (EtOAc:Hexane, 1:1). ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.00 (6H, d, TBS-Si-CH_3 x 2), 0.84 (9H, s,TBS-C-CH_3 x 3), 2.11 (3H, s, Lev CH_2COCH_3), 2.46 (2H, t, *J* = 6.6 Hz, Lev $COOCH_2CH_2$), 2.61 (2H, t, *J* = 6.6 Hz, Lev $COOCH_2CH_2$), 3.14 (2H, m, H-5, Man-3; H-5, GlcN-2), 3.28 (1H, dd, *J* = 8.6, 2.5 Hz, H-3, GlcN-2), 3.38 (1H, *J* = 10.7, 3.5 Hz, H-6a, Man-3), 3.44 (1H, d, *J* = 10.0 Hz, H-6a, GlcN-1), 3.6 (1H, d, *J* = 10.5 Hz, H-6a, GlcN-2), 3.53 – 3.91 (16H, m, H-6b, Man-4; H-6a, Man-4; H-3, GlcN-1; H-6b, Man-3; H-6b GlcN-1; H-6b, GlcN-2; H-2, Man-3; H-4, Man-4' ; H-3, Man-4' ; H-3, Man-4), 3.97 – 4.30 (12H, m, H-6a, Man-4' ; H-6b, Man-4' ; H-2, GlcN-1; H-2, GlcN-2), 4.30 – 4.70 (19H, m, CH_2Ph protons; H-1; Man-3), 4.80 – 4.97 (5H, m, CH_2Ph protons; H-1, GlcN-2; H-1, Man-4'), 5.10 – 5.18 (3H, m; alloc HC=CH*H*; H-1, GlcN-1; H-2, Man-4'), 5.20 – 5.28 (2H, m, alloc HC=C*H*H; H-1, Man-4), 5.75 (1H, m, alloc *H*C=CH_2), 6.67 – 7.8 (Ar-H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 206.4, 172.2, 167.5, 154.4, 154.0, 143.5, 143.2, 141.2, 141.2, 139.1, 138.6, 138.4, 138.2, 138.1, 137.9, 137.9, 137.7, 137.1, 131.5, 128.5, 128.5, 128.3, 128.3, 128.2, 128.2, 128.2, 128.1, 128.1, 128.0, 128.0, 127.9, 127.8, 127.7, 127.6, 127.5, 127.5, 127.5, 127.4, 127.3, 127.3, 127.2, 127.2, 126.8, 126.7, 125.3, 123.0, 120.0, 118.7, 99.7, 97.8, 97.0, 81.7, 79.7, 78.2, 78.1, 77.3, 77.2, 77.0, 76.8, 76.6, 76.5, 75.9, 75.1, 74.9, 74.7, 74.6, 74.5, 74.5, 74.4, 74.4, 73.9, 73.6, 73.4, 73.0, 72.6, 72.0, 71.7, 71.1, 71.1, 70.4, 70.3, 69.9, 69.8, 68.4, 68.1, 68.0, 63.2, 56.5, 55.7, 46.5, 37.8, 29.8, 27.9, 25.9, 18.1, 1.0, -3.9, -5.0. MALDI-TOF-MS (*m/z*): $[M + Na]^+$ calcd for $C_{138}H_{148}N_2NaO_{32}Si$, 2395.9682; found 2395.3738.



11

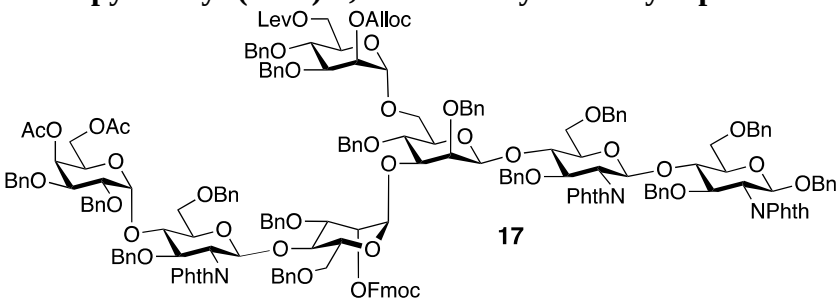
Benzyl [2-*O*-allyloxycarbonyl-3,4-di-*O*-benzyl-6-*O*-levulenoyl-*α*-D-mannopyranosyl-(1→6)]-[3,6-di-*O*-benzyl-2-*O*-fluorenylmethoxycarbonyl-*α*-D-mannopyranosyl]-(1→3)-2,4-di-*O*-benzyl-*β*-D-mannopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-*β*-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-*β*-D-glucopyranoside (16). To a solution of **2** (147 mg, 0.056 mmol) in pyridine (0.5 mL) was added HF-pyridine (pre-diluted 1 mL of 70% HF-pyridine complex in 1 mL pyridine) at RT, and the reaction mixture was left stirring for 72 h, after which TLC (EtOAc:Hexane, 1:1) showed the cleavage to be complete furnishing a slightly more polar product. The reaction mixture was then diluted with CH_2Cl_2 (10 mL) and washed successively with H_2O (10 mL) and sat. $NaHCO_3$ (10 mL). The organic phase was then dried ($MgSO_4$), filtered and the filtrate was concentrated *in vacuo* to give a residue, which was chromatographed with (EtOAc:CH_2Cl_2:Hexane, 3:2:5) as eluent affording the acceptor as a



16

white solid. (108 mg, 77%). R_f = 0.3 (EtOAc:Hexane, 1:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.12 (3H, s, Lev CH_2COCH_3), 2.48 (2H, t, *J* = 6.6 Hz, Lev $COOCH_2CH_2$), 2.63 (2H, t, *J* = 6.6 Hz, Lev $COOCH_2CH_2$), 3.18 (2H, d, *J* = 9.7 Hz, H-5, Man-3; H-5, GlcN-2), 3.30 (1H, dd, *J* = 9.0, 3.0 Hz, H-3, GlcN-2), 3.40 (1H, dd, *J* = 11.2, 3.8 Hz, H-6a, Man-3), 3.45 (1H, m, H-6a GlcN-1), 3.51 (2H, m, H-6a, GlcN-2; H-3, GlcN-1), 3.58 – 3.71 (4H, m, H-2, Man-3; H-6b, GlcN-1; H-4, Man-4), 3.73 (1H, d, H-6b, GlcN-2), 3.82 (1H, *J* = 11.6, 3.2 Hz, H-6b, Man-3), 3.87 (1H, dd, *J* = 8.3, 2.8 Hz, H-3, Man-4'), 3.91 – 4.04 (4H,m, H-6a, Man-4; H-6b, Man-4), 4.04 – 4.74 (34H, m, H-6a, Man-4' ; H-6b, Man-4' ; CH_2Ph protons; H-1, Man-3), 4.81 - 4.91 (4H, m; H-1, GlcN-2; CH_2Ph protons), 4.96 (1H, d, *J* = 7.8 Hz; H-1, Man-4'), 5.08 – 5.37 (7H, m; alloc HC=CH*H*, H-2; Man-4'; alloc HC=C*H*H; H1, GlcN-1; H-1, Man-4; H-2, Man-4), 5.75 (1H, m, alloc HC=CH_2), 6.68 – 7.80 (Ar-H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 206.9, 206.4, 172.3, 172.2, 169.7, 168.1, 167.6, 167.4, 157.5, 154.5, 154.3, 154.1, 143.5, 143.4, 143.3, 143.2, 141.2, 141.1, 139.1, 139.0, 138.8, 138.6, 138.4, 138.3, 138.2, 137.9, 137.8, 137.7, 137.2, 133.7, 133.4, 131.8, 131.7, 131.5, 131.5, 128.6, 128.5, 128.4, 128.3, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.7, 127.6, 127.5, 127.4, 127.3, 127.2, 127.1, 126.9, 126.8, 125.6, 125.3, 125.2, 123.5, 123.1, 120.0, 119.9, 118.7, 101.9, 99.6, 97.9, 97.0, 81.9, 79.7, 78.1, 77.5, 77.2, 77.0, 76.7, 76.6, 76.0, 75.1, 74.9, 74.7, 74.6, 74.5, 74.4, 74.0, 73.7, 73.5, 73.1, 72.7, 72.2, 71.8, 71.6, 71.2, 70.9, 70.5, 70.4, 70.0, 69.6, 68.5, 68.1, 67.6, 66.6, 63.2, 56.6, 55.7, 46.6, 46.5, 39.5, 37.9, 30.9, 29.8, 27.9, 25.9, 20.9, 18.2. MALDI-TOF-MS (*m/z*): $[M + Na]^+$ calcd for $C_{147}H_{144}N_2NaO_{34}$, 2505.7388; found 2505.4054.

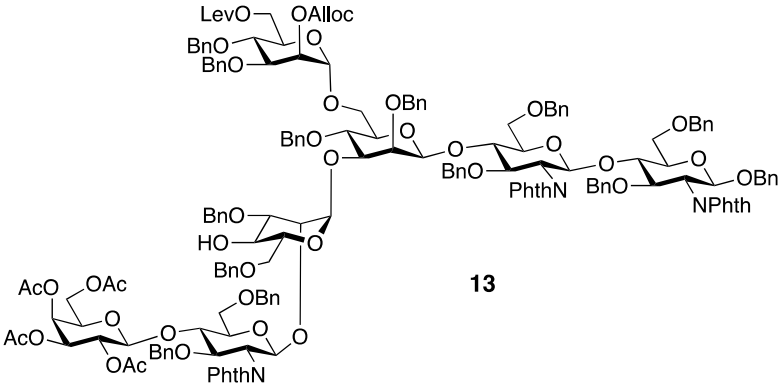
Benzyl [2-*O*-allyloxycarbonyl-3,4-di-*O*-benzyl-6-*O*-levulenoyl-*α*-D-mannopyranosyl-(1→6)]-[2,3-di-*O*-benzyl-4,6-di-*O*-acetyl-*α*-D-galactopyranosyl-(1→4)-2-deoxy-3,6-di-*O*-benzyl-2-pthalimido-*β*-D-glucopyranosyl-(1→4)3,6-di-*O*-benzyl-2-*O*-fluorenylmethoxycarbonyl-*α*-D-mannopyranosyl]-(1→3)-2,4-di-*O*-benzyl-*β*-D-mannopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-*β*-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-*β*-D-glucopyranoside (17).



To a mixture of the donor **4** (45 mg, 0.041 mmol), acceptor **16** (51 mg, 0.020 mmol) and 4Å flame-dried molecular sieves in CH_2Cl_2 (3 mL) at -20 °C was added TMSOTf (2 µL). Stirring was continued at -20 °C for 20 min, after which the reaction mixture was quenched with pyridine (100 µL), filtered, the filtrate was concentrated *in vacuo*, and passed through Biogel SX-1 (toluene:acetone, 1:1) as mobile phase to give the desired heptasaccharide as a white solid. (10 mg, 14%). R_f = 0.3 (EtOAc:Toluene, 1:4). ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.92 (3H, s, Acetyl CH_3), 2.06 (3H, s, Acetyl CH_3), 2.07 (3H, s, Lev CH_2COCH_3), 2.41 (2H, t, *J* = 6.7 Hz, Lev $COOCH_2CH_2$), 2.56 (2H, t, *J* = 6.7 Hz, Lev $COOCH_2CH_2$), 2.99 (1H, d, *J* = 10.0 Hz), 3.05 (1H, d, *J* = 9.5 Hz, H-5, GlcN-5'), 3.19 – 3.27 (3H, m, H-5, Man-3; H-5, GLcN-2), 3.29 – 3.51 (9H, m, H-3, GLcN-2; H-6a, Man-3; H-6a, GlcN-2; H-6a, Man-4; H-4, GlcN-5'), 3.53 – 3.64 (4H, m, H-6a, GlcN-1; H-6b, Man-3; H-6b, Glc-N-2; H-6b, Man-4), 3.67 – 3.76 (5H, m, H-2, Gal-7; H-6b, GlcN-1), 3.78 – 3.86 (5H, m, H-6a, Man-4'; H-6a, Gal-7; H-6b, Gal-7; H-6b, Man-4'), 3.92 – 4.73 (42H, m, H-2, GlcN-1; H-2, GlcN-2; H-2, GlcN-5'; H-1, Man-3, CH_2Ph protons), 4.74 – 5.34 (17H, m; H-1, GlcN-2; H-1, Man-4'; H-1, Man-4; H-4, Gal-7; alloc HC=C*H*H; H-1, GlcN-1; H-2, Man-4'; alloc HC=CH*H*), 5.46 (3H, m, H-2, Man-4; H-1, Gal-7; H-1, GlcN-5'), 5.69 (1H, m; alloc *H*C=CH_2). ^{13}C NMR (151 MHz, $CDCl_3$) δ 133.5, 133.4, 129.8,

129.6, 128.0, 127.9, 127.8, 127.6, 126.1, 126.0, 125.6, 125.4, 124.3, 123.2, 123.0, 119.9, 118.2, 99.3, 98.1, 97.8, 97.1, 97.0, 81.4, 80.4, 79.5, 77.9, 77.5, 76.5, 76.4, 75.2, 75.0, 74.7, 74.5, 74.4, 74.2, 74.1, 74.0, 73.5, 73.3, 73.0, 72.9, 72.7, 72.6, 71.7, 71.3, 71.2, 70.6, 70.5, 70.4, 70.3, 70.1, 68.5, 68.4, 68.3, 68.2, 67.8, 67.4, 67.0, 66.8, 66.3, 66.2, 63.1, 62.1, 62.0, 56.5, 55.8, 37.7, 29.6, 27.9, 20.9, 20.7. MALDI-TOF-MS (*m/z*): $[M + Na]^+$ calcd for $C_{199}H_{195}N_3NaO_{47}$, 3401.2859 found 3402.0988.

Benzyl [2-*O*-allyloxycarbonyl-3,4-di-*O*-benzyl-6-*O*-levulenoyl-*α*-D-mannopyranosyl-(1→6)]-[2,3,4,6-tetra-*O*-acetyl-*β*-D-galactopyranosyl-(1→4)-2-deoxy-3,6-di-*O*-benzyl-2-phthalimido-*β*-D-glucopyranosyl-(1→2)-3,6-di-*O*-benzyl-2-*O*-*α*-D-mannopyranosyl-(1→3)]-2,4-di-*O*-benzyl-*β*-D-mannopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-*β*-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-*β*-D-glucopyranoside (13). To a mixture of the donor **3** (2.3 g, 2.36 mmol), acceptor **11** (2.8 g, 1.18 mmol), and 4Å flame-dried molecular sieves in CH_2Cl_2 (20 mL) at – 20 ºC was added TMSOTf (85 µL, 0.472 mmol). The reaction mixture was stirred at – 20 ºC for 15 min, after which TLC (EtOAc:CH_2Cl_2:Hexane, 4:2:4) showed the acceptor was fully consumed into a more polar product. The reaction mixture was quenched with Et_3N (1 mL), filtered, and the solution was washed with sat. $NaHCO_3$ (10 mL). The organic phase was concentrated *in vacuo* to give a residue, which was chromatographed using EtOAc:CH_2Cl_2:Hexane (2:2:5→2:2:4→2:2:3) as eluent to provide the crude **12** containing some residual hydrolyzed donor (5.0 g in total). This heptasaccharide was dissolved in pyridine (10 mL) and transferred into a Teflon flask. To this solution was added HF-pyridine (pre-diluted 10 mL of 70% HF-pyridine complex in 10 mL of pyridine), and stirring was continued for 44 h. The reaction mixture was then diluted with CH_2Cl_2 (50 mL) and washed successively with H_2O (30 mL) and sat. $NaHCO_3$ (30 mL). The organic phase was then dried ($MgSO_4$), filtered, and the filtrate was concentrated *in vacuo* to give a residue, which was chromatographed with (EtOAc:CH_2Cl_2:Hexane, 4:2:4) as eluent affording the target acceptor as a white solid. (2.38 g, 66%, two steps). R_f = 0.3 (EtOAc:CH_2Cl_2:Hexane, 2:2:2). ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.00 (3H, s, Acetyl CH_3), 2.04 (3H, s, Acetyl CH_3), 2.06 (3H, s, Acetyl CH_3), 2.08 (3H, s, Acetyl CH_3), 2.13 (3H, s, Lev CH_2COCH_3) , 2.45 (2H, t, *J* = 7.1 Hz, Lev $COOCH_2CH_2$), 2.62 (2H, t, *J* = 7.1 Hz, Lev $COOCH_2CH_2$), 2.76 (1H, dd, *J* = 7.3, 10.8 Hz), 2.84 (1H, m), 3.03 (1H, d, *J* = 9.7 Hz, H-5, GlcN-5), 3.21 – 3.31 (4H, m, H-5, Man-3; H-5, Glc-N-2; H-3, GlcN-2), 3.33 – 3.79 (33H, m, H-3, GlcN-5; H-4, GlcN-5; H-6a, Man-3; H-6a, GlcN-1; H-6a, GlcN-2; H-6b, GlcN-2; H-6b, Man-3; H-6b, GlcN-1; H-6a, Man-4; H-6b, Man-4), 3.83 (1H, dd, *J* = 8.5, 3.0 Hz), 3.88 – 4.29 (43H, m, H-6a, Man-4' ; H-6b, Man-4' ; H-6a, Gal-6; H-6b, Gal-6; H-2, GlcN-5; H-2, GlcN-2; H-2, GlcN-1; CH_2Ph protons), 4.32 – 4.76 (42H, m, H-1, Gal-6; H-1, Man-3; CH_2Ph protons), 4.77 – 4.88 (12H, m), 4.88 – 4.99 (8H, m, H-2, Man-4' ; H-1, GlcN-2; H-1, Man-4' ; H-1, GlcN-1; H-1, Man-4; alloc HC=C*H*H; alloc HC=CH*H*), 5.06 (1H, s, H-3, Gal-6), 5.13 – 5.29 (9H, m, H-2, Gal-6; H-1, GlcN-5; H-4, Gal-6), 5.75 (1H, m; alloc *H*C=CH_2), 6.65 – 7.81 (Ar-H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 206.4, 190.5, 186.8, 178.2, 172.3, 170.3, 170.2, 170.0, 169.0, 168.0, 167.6, 167.4, 154.0, 138.9, 138.8, 138.7, 138.6, 138.4, 138.4, 138.3, 138.0, 137.9, 137.9, 137.8, 137.8,



137.2, 133.5, 131.8, 131.7, 131.5, 128.8, 128.7, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 128.0, 127.9, 127.8, 127.7, 127.6, 127.5, 127.4, 127.3, 127.1, 126.8, 126.0, 123.0, 118.7, 101.2, 100.3, 98.9, 97.8, 97.0, 95.6, 80.9, 79.1, 78.4, 78.0, 77.7, 77.4, 77.2, 77.0, 76.7, 76.6, 76.4, 76.1, 75.2, 74.5, 74.4, 74.2, 74.0, 73.7, 73.5, 73.3, 72.9, 72.6, 72.3, 71.6, 71.3, 71.2, 71.1, 70.4, 70.4, 70.3, 69.9, 69.5, 68.5, 68.1, 67.6, 66.9, 63.1, 60.7, 56.5, 55.7, 55.5, 37.9, 29.8, 29.7, 27.9, 25.9, 21.0, 20.9, 20.7, 20.6, 14.1.. MALDI-TOF-MS (*m/z*): $[M + Na]^+$ calcd for $C_{174}H_{177}N_3NaO_{47}$, 3083.1450 found 3082.6494.

General Procedures

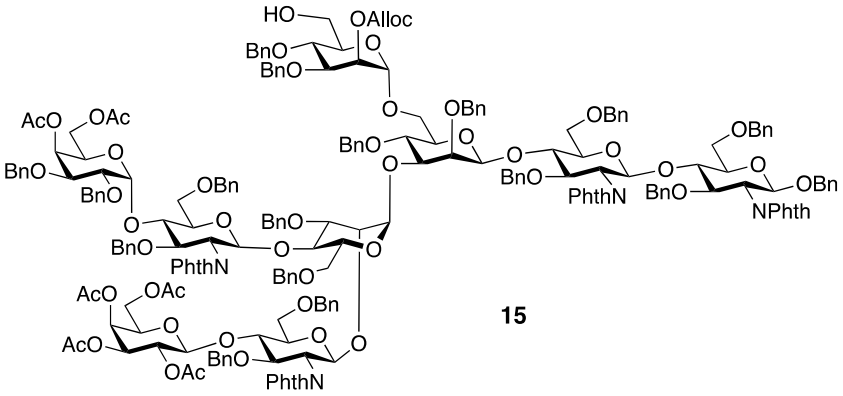
a) General glycosylation procedure for the synthesis of glycans **14** – **20**: A mixture of the acceptor (0.22 mmol, 100 mM)[2], *N*-phenyl imidate donor (0.44 mmol, 200 mM), and 4Å flame-dried molecular sieves in CH_2Cl_2 (2.5 mL) was cooled to – 20 ºC, followed by adding TMSOTf (0.088 mmol, 0.2 eq). The reaction was stirred at to – 20 ºC for 15 min, after which another portion of the donor (0.22 mmol) was added and stirring was continued for another 15 min, before the reaction was quenched with Et_3N (0.5 mL). The resulting mixture was then filtered, the filtrate was concentrated to dryness *in vacuo*, and passed through the BioGel SX-1 column (toluene:acetone, 1:1) as mobile phase to provide the pure glycan as a clear syrup. Freeze-drying from benzene affords the product as a white solid.

b) General procedure for the cleavage of the Lev ester: To a solution of a glycan (0.22 mmol) in CH_2Cl_2:CH_3OH (1:1) was added solid hydrazine acetate (2.2 mmol), and the reaction was stirred at for 2 h, after which it was diluted with CH_2Cl_2 (10 mL) and washed with sat. $NaHCO_3$ (10 mL). The organic phase was dried ($MgSO_4$), filtered, and the filtrate was concentrated *in vacuo*. The product was passed through the BioGel SX-1 column (toluene:acetone, 1:1) as mobile phase to provide pure glycan as a clear syrup. Freeze-drying from benzene affords the product as a white solid.

c) General procedure for the cleavage of the Alloc carbonate: To a solution of a glycan (0.22 mmol) in THF_2:H_2O (9:1) containing morpholine (0.88 mmol, 4 eq) was added tetrakis(triphenylphosphine) palladium (0.022 mmol, 0.1 eq), and the reaction was stirred for 2 h, after which it was concentrated *in vacuo*, the product was loaded onto a silica gel column and eluted with tolunene:EtOAc (6:4) as eluent to give the crude product as a clear syrup. This crude material was passed through BioGel SX-1 column (toluene:acetone, 1:1) as mobile phase to provide the pure glycan as a clear syrup. Freeze-drying from benzene affords the product as a white solid.

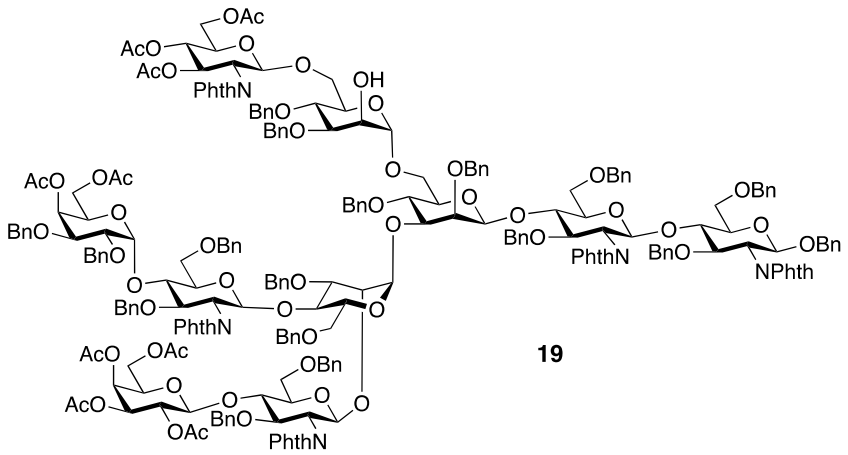
[2] For the synthesis of compounds **14**– **20** high concentration (100 mM) during glycosylations is crucial for reproducibility. Reported yields were ensured to be consistent by repeating the synthesis of compound three times on 0.15 – 0.22 mmol scale. The mixture of a donor and acceptor were freeze-dried from benzene before each glycosylation step.

Benzyl [2-*O*-allyloxycarbonyl-3,4-di-*O*-benzyl-*α*-D-mannopyranosyl-(1→6)]-[2,3,4,6-tetra-*O*-acetyl-*β*-D-galactopyranosyl-(1→4)-2-deoxy-3,6-di-*O*-benzyl-2-pthalimido-*β*-D-glucopyranosyl-(1→2)-2,3-di-*O*-benzyl-4,6-di-*O*-acetyl-*α*-D-galactopyranosyl-(1→4)-2-deoxy-3,6-di-*O*-benzyl-2-pthalimido-*β*-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-*O*-*α*-D-mannopyranosyl-(1→3)]-2,4-di-*O*-benzyl-*β*-D-mannopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-*β*-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-*β*-D-glucopyranoside (15).



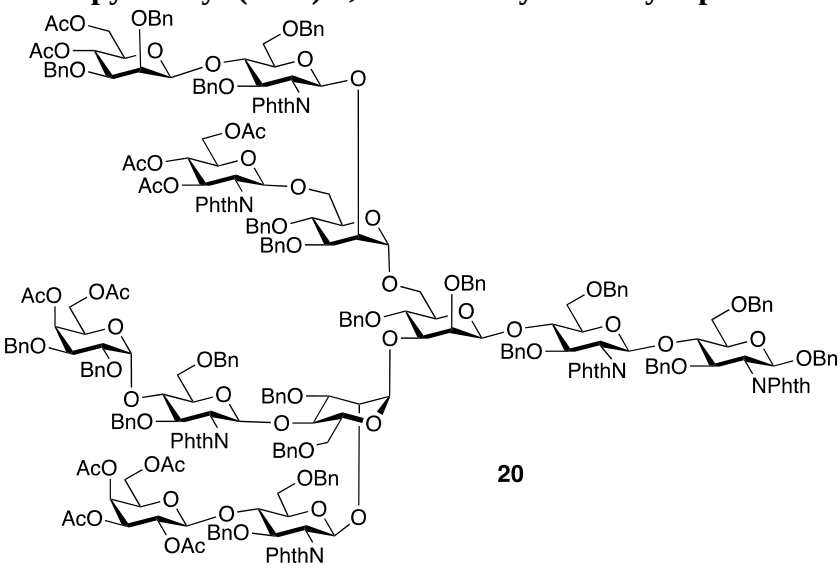
White solid. (730 mg, 91%). Glycosylation of **13** and with **4** over two steps with the subsequent removal of Lev ester according to the General Procedure **a)** and **b)**. R_f = 0.4 (EtOAc:toluene, 4:6). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.97 (3H, s, Acetyl CH_3), 2.0 (3H, s, Acetyl CH_3), 2.01 (3H, s, Acetyl CH_3), 2.02 (3H, s, Acetyl CH_3), 2.04 (3H, s, Acetyl CH_3), 2.09 (3H, s, Acetyl CH_3), 2.62 (1H, broad m), 2.86 (1H, d, *J* = 9.7 Hz, H-3, GlcN-5'), 3.04 (1H, d, *J* = 10.2 Hz,), 3.13 – 3.30 (3H, m, H-6a, GlcN-5'; H-6b, GlcN-5'), 3.31 – 4.60 (43H, m, H-5, Man-3; H-5, GlcN-2; H-3, GlcN-2; H-3, GlcN-5; H-4, GlcN-5; H-6a, Man-3; H-6a, GlcN-1; H-6a, GlcN-2; H-6b, GlcN-1; H-6b, GlcN-2; H-6b, Man-3; H-6a, Man-4; H-6b, Man-4; H-6a, Gal-6; H-6b, Gal-6; H-6a, Man-4'; H-6b, Man-4' ; H-6a, Gal-7; H-6b, Gal-7; H-2, Gal-7; H-2, GlcN-5' ; H-2, GlcN-2; H-2, GlcN-1; H-3, Gal-7; H-2, GlcN-5; H-1, Gal-6; H-1, Man-3; CH_2Ph protons), 4.59 – 4.88 (8H, m, H-2, Man-4' ; H-1, GlcN-2; H-1, Man-4' ; H-1, GlcN-1 ; H-4, Gal-7; H-2, Gal-6), 4.92 (1H, d, *J* = 8.6 Hz), 5.03 (1H, s), 5.08 – 5.29 (5H, m, H-1, Man-4 ; H-1, GlcN-5; H-1, GlcN-5' ; Alloc HC=C*H*H; Alloc HC=CH*H*), 5.47 (2H, m, H-1, Gal-7 ; H-4, Gal-6), 5.71 (1H, m, Alloc *H*C=CH_2), 6.62 – 7.85 (Ar-H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.4, 170.3, 170.2, 170.0, 169.0, 168.1, 167.6, 167.4, 154.4, 154.0, 138.9, 138.8, 138.7, 138.6, 138.6, 138.5, 138.4, 138.2, 138.1, 138.1, 138.0, 137.9, 137.8, 137.2, 133.4, 133.2, 131.7, 131.5, 128.7, 128.5, 128.4, 128.3, 128.3, 128.2, 128.2, 128.1, 128.0, 128.0, 127.9, 127.8, 127.7, 127.6, 127.5, 127.4, 127.3, 127.2, 127.1, 127.0, 126.9, 126.8, 126.7, 126.2, 123.5, 123.0, 118.7, 104.7, 100.8, 100.2, 98.4, 97.8, 97.0, 80.2, 78.6, 78.1, 77.8, 77.4, 77.2, 77.0, 76.7, 76.6, 76.1, 75.5, 75.2, 74.7, 74.4, 74.1, 73.8, 73.5, 73.2, 72.9, 72.7, 72.4, 72.1, 71.9, 71.3, 71.2, 70.5, 70.4, 69.6, 69.5, 68.4, 68.1, 67.5, 67.0, 62.0, 61.9, 60.7, 56.4, 55.8, 55.4, 29.7, 21.7, 20.9, 20.8, 20.7, 20.7, 20.6. MALDI-TOF-MS (*m/z*): $[M + Na]^+$ calcd for $C_{221}H_{222}N_4NaO_{58}$, 3882.4443 found 3882.8464.

Benzyl [3,4,6-tri-*O*-acetyl-2-deoxy-2-pthalimido-*β*-D-glucopyranosyl-(1→6)-3,4-di-*O*-benzyl-*α*-D-mannopyranosyl-(1→6)]-[2,3,4,6-tetra-*O*-acetyl-*β*-D-galactopyranosyl-(1→4)-2-deoxy-3,6-di-*O*-benzyl-2-pthalimido-*β*-D-glucopyranosyl-(1→2)-2,3-di-*O*-benzyl-4,6-di-*O*-acetyl-*α*-D-galactopyranosyl-(1→4)-2-deoxy-3,6-di-*O*-benzyl-2-pthalimido-*β*-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-*O*-*α*-D-mannopyranosyl-(1→3)]-2,4-di-*O*-benzyl-*β*-D-mannopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-*β*-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-*β*-D-glucopyranoside (19).

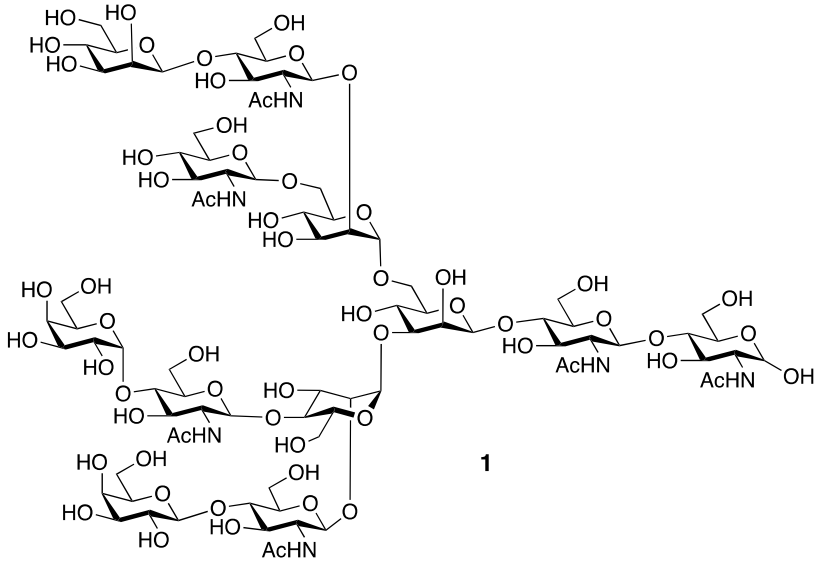


White solid. (792 mg, 84%). Glycosylation of **15** with **5** over two steps with the subsequent removal of Alloc carbonate according to the General Procedure **a)** and **c)**. R_f = 0.3 (EtOAc:toluene, 3:7). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.99 (3H, s, Acetyl CH_3), 2.00 (3H, s, Acetyl CH_3), 2.02 (3H, s, Acetyl CH_3), 2.03 (3H, s, Acetyl CH_3), 2.03 (3H, s, Acetyl CH_3), 2.04 (3H, s, Acetyl CH_3), 2.06 (3H, s, Acetyl CH_3), 2.11 (3H, s, Acetyl CH_3), 2.60 (1H, d, *J* = 10.0 Hz), 2.70 (1H, d, *J* = 10.5 Hz), 3.04 (3H, m, H-6a, Man-3; H-6a, GlcN-1; H-6a, GlcN-2), 3.14 – 3.79 (m, H-5, Man-3; H-6a, GlcN-5' ; H-6b, Man-3; H-6b, GlcN-5' ; H-6b, GlcN-1 ; H-6b, GlcN-2; H-3,GlcN-5; H-4, GlcN-5; H-6a, Man-4' ; H-6b, Man-4' ; H-6a, Gal-6; H-6b, Gal-6; H-6a, Man-4; H-6b, Man-4; H-6a, Gal-7; H-6b, Gal-7; H-6a, GlcN-5" ; H-6b, GlcN-5"), 3.79 – 3.96 (m, H-2, GlcN-2; H-2, GlcN-5' ; H-2, GlcN-1; H-3, Gal-7; H-2, GlcN-5), 3.96 – 4.59 (m; H-2, GlcN-5" ; H-1, Gal-6; H-1, GlcN-5" ; H-1, Man-3; CH_2Ph protons), 4.60 – 4.89 (m, H-1, GlcN-2; H-1, Man-4' ; H-1, GlcN-1; CH_2Ph protons), 4.96 (1H, d, *J* = 8.9 Hz,), 5.08 – 5.21 (5H, m, H-2, Gal-6; H-3, Gal-6; H-1, Man-4), 5.27 (3H, m; H-4, Gal-7; H-1, GlcN-5, H-1, GlcN-5'), 5.47 (2H, m, H-1, Gal-7; H-4, Gal-6), 5.79 (1H, dd, J = 11.0, 9.2 Hz, H-3, GlcN-5"), 6.67 – 7.86 (Ar-H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.7, 170.4, 170.3, 170.2, 170.1, 170.0, 169.5, 169.0, 168.2, 167.6, 167.5, 167.1, 139.0, 138.9, 138.7, 138.6, 138.4, 138.3, 138.1, 138.0, 137.9, 137.7, 137.6, 137.1, 134.2, 133.9, 133.6, 133.4, 133.3, 131.8, 131.7, 131.5, 131.2, 129.3, 129.0, 128.9, 128.9, 128.7, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.7, 127.6, 127.5, 127.3, 127.2, 127.1, 127.0, 126.9, 126.8, 126.7, 126.2, 123.5, 123.1, 100.7, 100.2, 99.9, 98.5, 98.3, 97.8, 97.3, 97.0, 96.0, 80.2, 79.1, 78.3, 77.8, 77.5, 77.2, 77.0, 76.8, 76.6, 76.3, 76.1, 75.5, 75.2, 74.7, 74.5, 74.4, 74.3, 74.1, 73.8, 73.7, 73.5, 73.2, 73.1, 72.7, 72.4, 71.9, 71.7, 71.2, 70.7, 70.6, 70.5, 70.4, 69.7, 69.5, 69.0, 68.4, 68.0, 67.5, 67.1, 67.0, 66.3, 61.9, 60.7, 56.5, 56.4, 55.8, 55.5, 54.5, 29.7, 20.9, 20.8, 20.7, 20.6, 20.5. MALDI-TOF-MS (*m/z*): $[M + Na]^+$ calcd for $C_{237}H_{237}N_5NaO_{65}$, 4215.5291 found 4215.8687.

Benzyl [2,3-di-*O*-benzyl-4,6-di-*O*-acetyl-*β*-D-mannopyranosyl-(1→4)-2-deoxy-3,6-di-*O*-benzyl-2-pthalimido-*β*-D-glucopyranosyl-(1→2)-3,4,6-tri-*O*-acetyl-2-deoxy-2-pthalimido-*β*-D-glucopyranosyl-(1→6)-3,4-di-*O*-benzyl-*α*-D-mannopyranosyl-(1→6)]-[2,3,4,6-tetra-*O*-acetyl-*β*-D-galactopyranosyl-(1→4)-2-deoxy-3,6-di-*O*-benzyl-2-pthalimido-*β*-D-glucopyranosyl-(1→2)-2,3-di-*O*-benzyl-4,6-di-*O*-acetyl-*α*-D-galactopyranosyl-(1→4)-2-deoxy-3,6-di-*O*-benzyl-2-pthalimido-*β*-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-*O*-*α*-D-mannopyranosyl-(1→3)]-2,4-di-*O*-benzyl-*β*-D-mannopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-*β*-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-*β*-D-glucopyranoside (20). White solid; 843 mg (89%). Glycosylation of **19** and **6** according to the General Procedure **a)**. R_f = 0.5 (EtOAc:toluene, 4:6). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.91 – 2.10 (33H, m, Acetyl CH_3 X 11), 2.25 (1H, m), 2.46 (1H, d, *J* = 9.7 Hz), 2.52 – 2.67 (3H, m), 2.91 (1H, d, J = 9.2 Hz, H-5, GlcN-2), 2.96 – 3.37 (16H, m, H-6a, GlcN-5'; H-6b, GlcN-5' ; H-5, GlcN-5''' ; H-5, Man-8; H-6a, Man-3; H-6a, GlcN-1; H-6b, GlcN-2; H-6a, Man-8; H-6b, Man-3; H-6b, GlcN-1; H-6b, GlcN-2; H-3, GlcN-5; H-4, GlcN-5), 3.38 – 3.63 (11H, m, H-6b, Man-8; H-6a, GlcN-5'''; H-6b, GlcN-5''' ; H-6a, GlcN-5'' ; H-6b, GlcN-5'' ; H-6a, Man-4' ; H-6b, Man-4' ; H-6a, Gal-6; H-6b, Gal-6), 3.64 – 4.22 (34H, m, H-6a, Man-4; H-6b, Man-4 ; H-6a, Gal-7; H-6b, Gal-7; H-3, GlcN-5''', CH_2Ph protons; H-1, Gal-6; H-2, GlcN-5''' ; H-2, GlcN-5'' ; H-1, Man-8), 4.24 -4.94 (37H, m, CH_2Ph protons; H-1, GlcN-5'' ; H-1, Man-3; H-1, GlcN-2; H-2, GlcN-5' GlcN-2; H-1, Man-4' ; H-1, GlcN-1; H-3, GlcN-5''; H-1, Man-4), 5.07 – 5.15 (3H, m, H-1, GlcN-5; H-1, GlcN-5' ; H-2, Gal-6), 5.18 – 5.27 (3H, m, H-3, Gal-6; H-4, Gal-7; H-1, Gal-7), 5.40 – 5.49 (3H, m, H-1,GlcN-5''' ; H-4, GlcN-5'' ; H-4, Gal-6), 6.59 – 7.80 (97H, m). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.9, 170.6, 170.4, 170.3, 170.2, 170.0, 169.7, 169.5, 169.0, 168.1, 168.0, 167.6, 167.4, 167.0, 139.0, 138.9, 138.8, 138.7, 138.6, 138.5, 138.4, 138.1, 138.0, 137.9, 137.8, 137.8, 137.1, 133.4, 131.8, 131.7, 131.4, 130.9, 128.9, 128.8, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.7, 127.6, 127.5, 127.5, 127.4, 127.3, 127.2, 127.1, 127.0, 126.9, 126.8, 126.1, 123.4, 123.1, 101.4, 100.1, 98.5, 97.8, 97.0, 95.9, 80.2, 79.7, 79.1, 77.5, 77.3, 77.0, 76.9, 76.6, 76.1, 75.5, 75.2, 74.5, 74.3, 74.2, 74.0, 73.8, 73.4, 73.1, 72.7, 72.4, 71.9, 71.4, 71.2, 70.5, 70.4, 69.5, 68.8, 68.1, 67.5, 67.0, 62.9, 62.0, 60.7, 56.4, 55.8, 55.5, 54.3, 38.7, 30.4, 29.7, 23.7, 23.0, 20.9, 20.9, 20.8, 20.7, 20.7, 20.6, 20.4. MALDI-TOF-MS (*m/z*): $[M + Na]^+$ calcd for $C_{289}H_{288}N_6NaO_{78}$, 5112.8652; found 5115.8843.

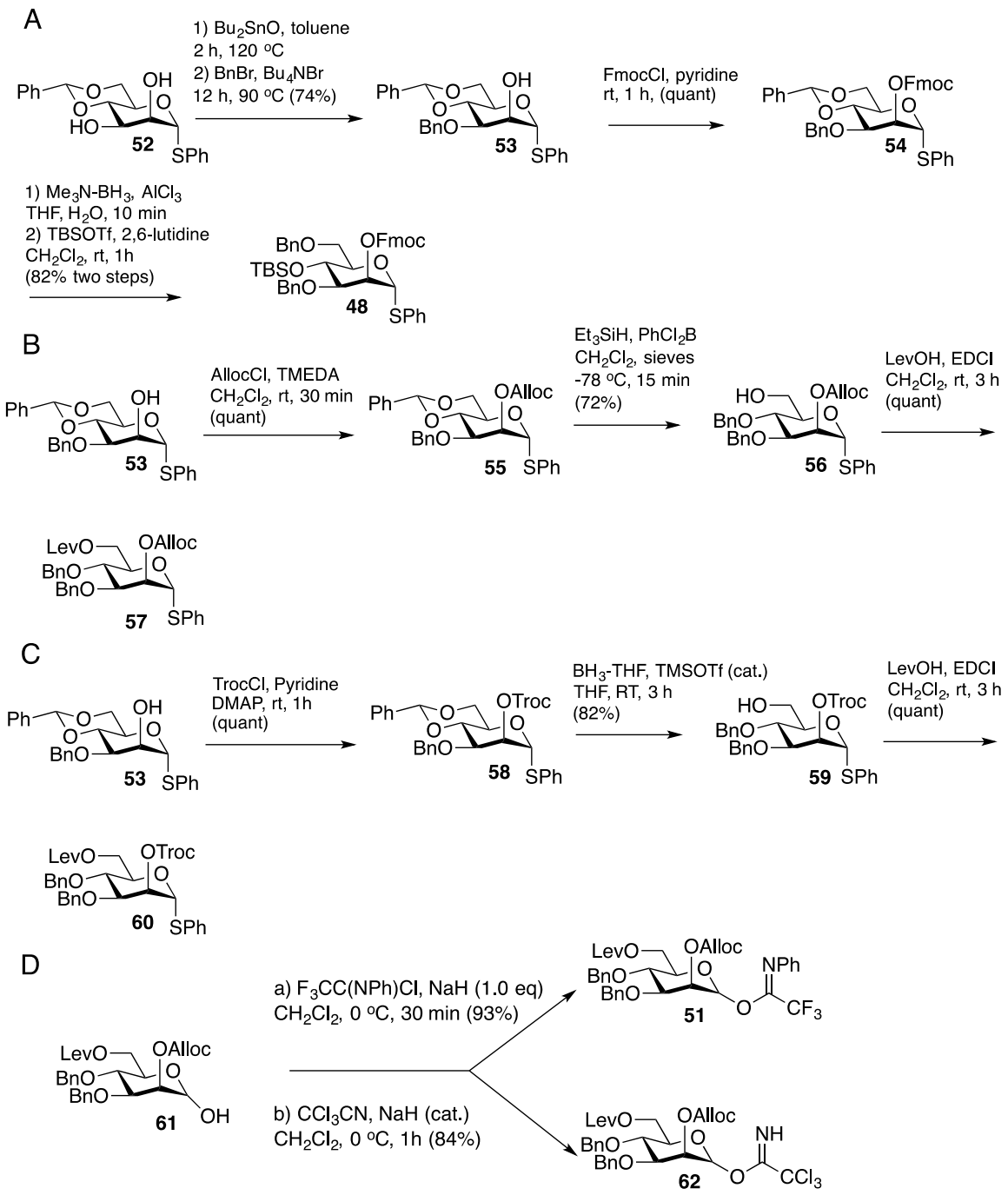


[[[*β*-D-Galactopyranosyl-(1→4)-2-acetamido-2-deoxy-*β*-D-glucopyranosyl-(1→2)]-[*α*-D-galactopyranosyl-(1→4)-acetamido-2-deoxy-*β*-D-glucopyranosyl-(1→4)]-*α*-D-mannopyranosyl-(1→3)]-[[*β*-D-mannopyranosyl-(1→4)-2-acetamido-2-deoxy-*β*-D-glucopyranosyl-(1→2)]-[2-acetamido-2-deoxy-*β*-D-glucopyranosyl-(1→6)-*α*-D-mannopyranosyl]]]-*β*-D-mannopyranosyl-(1→4)-2-acetamido-2-deoxy-*β*-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy- *α/β*-D-glucopyranose (1).



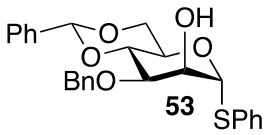
To a suspension of **20** (329.0 mg) in *n*BuOH (5 mL) was added ethylenediamine (5 mL) and the resulting clear solution was heated at 90 ºC for 16 h. The reaction mixture was concentrated *in vacuo*, co-evaporated with toluene, and the resulting residue was dissolved in pyridine:Ac_2O (10 mL, 1:1), after which DMAP (cat) was added. The reaction was left stirring for 2 h, after which TLC (acetone:toluene, 3:7) showed the presence of one major product with R_f = 0.6. The reaction mixture was concentrated *in vacuo*, and the resulting crude product was briefly chromatographed with (acetone:toluene, 3:7) as eluent to give product which was additionally passed through BioGel SX-1 column (toluene:acetone, 1:1) as mobile phase to provide the acetylated intermediate as a clear syrup. This material was then dissolved in MeOH, after which 1 M NaOMe (250 µL) was added and the deacetylation was left proceeding at RT for 2 h. The reaction mixture was neutralized with the Amberlite 120 H^+ resin, filtered and concentrated *in vacuo*. This syrup was dissolved in MeOH:H_2O (10 mL, 1:1), followed by adding $Pd(OH)_2$ (150 mg, 20%, Degussa type) and the reaction mixture was left stirring under the atmosphere of hydrogen for 24 h. After that time it was filtered, the filtrate was concentrated *in vacuo*, and passed through the BioGel P-2 column to give the desired glycan as a white solid. 94 mg (66%, over four steps). Additional purification (30 mg) by HPLC with a semi-preparative amide HILIC column (10 x 250 mm, Waters Inc.) under isocratic conditions (65% CH_3CN:100 mM Ammonium formate, pH±3.4) with the UV (210 nm) detection affords analytically pure glycan. ^{1}H NMR (500 MHz, D_2O): δ 1.99 – 2.12 (18H, m, 6 x NHAc), 3.38 – 3.49 (4H, m), 3.49 – 4.01 (64H, m), 4.02 – 4.11 (3H, m), 5.08 – 4.25 (3H, m), 4.46 (1H, d, *J* = 8.0 Hz, Gal-6, H-1), 4.51 – 4.63 (5H, m, GlcNAc-2, -5, -5’, -5’’, -5’’’, H-1 x 5), 4.70 (1H, d, *J* = 7.8 Hz, GlcNAc-1, H-1*β*), 4.73 (2H, s, Man-3, Man-8, H-1), 4.87 (1H, s, Man-4’, H-1), 5.13 (1H, s, Man-4, H-1), 5.19 (1H, s, GlcNAc-1, H-1*α*), 5.44 (1H, d, *J* = 2.7 Hz, Gal-7, H-1). ^{13}C NMR (101 MHz, D_2O) δ 102.9, 101.5, 101.3, 100.0, 99.7, 99.5, 99.0, 90.4, 90.3, 80.3, 78.7, 76.5, 76.0, 75.9, 75.2, 74.4, 73.8, 72.5, 71.8, 71.7, 70.9, 70.5, 70.1, 70.0, 69.9, 69.1, 68.9, 68.5, 68.0, 67.5, 66.5, 65.3, 65.1, 62.7, 61.2, 61.0, 60.6, 55.1, 54.8, 53.6, 29.1, 28.1, 22.3, 21.0.

MALDI-TOF-MS (*m/z*): $[M + Na]^+$ calcd for $C_{84}H_{140}N_6O_{61}$, 2231.7930; found 2231.2700.

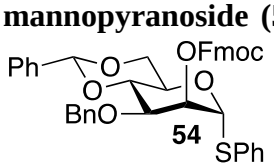


Scheme S3. Preparation of mannose building blocks **48**, **51**, **57**, **60, 62**

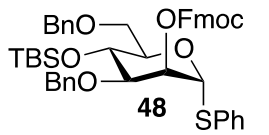
Phenyl 3-*O*-benzyl-4,6-*O*-benzylidene-1-thio-*α*-D-mannopyranoside (53). To a suspension of phenyl 4,6-*O*-benzylidene-1-thio-*α*-D-mannopyranoside (7.22 g, 20.03 mmol)[25] in toluene (50 mL) was added Bu_2SnO (7.5 g, 30.0 mmol) and the mixture was heated at 120 °C for 2 h. To the resulting clear solution was added Bu_4NBr (500 mg, cat) and BnBr (3.6 mL, 30.00 mmol) and the reaction mixture was left stirring at 90 °C for 12 h. The mixture was diluted with CH_2Cl_2 (100 mL) and washed with sat. $NaHCO_3$ (100 mL). The organic phase was then vacuum-filtered through a filter paper on a Buchner funnel to collect the precipitated tin residues. The organic phase was dried ($MgSO_4$), filtered, and the filtrate was concentrated to give the crude product. Chromatography with EtOAc:Hexane (3:7) as eluent affords the title compound as a clear oil. (6.67 g, 74%). R_f = 0.4 (EtOAc:Hexane, 3:7). The analytical data are in accordance with the literature data.[26]



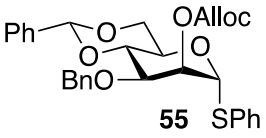
Phenyl 3-*O*-benzyl-4,6-*O*-benzylidene-2-*O*-fluorenylmethoxycarbonyl-1-thio-*α*-D-mannopyranoside (54). To a solution of **53** (9.0 g, 20.03 mmol) in pyridine (50 mL) was added DMAP (500 mg, cat), followed by Fmoc-Cl (7.8 g, 30.1 mmol) and the mixture was stirred for 30 min. After that time, second portion of Fmoc-Cl (2.6 g, 10 mmol) was added and stirring was continued for another 30 min, upon which TLC (EtOAc:Hexane, 3:7) showed the reaction to be complete. The solvent was removed *in vacuo*, and the residue was re-dissolved in CH_2Cl_2 (100 mL) and the organic layer was washed with 1 M HCl (50 mL). The organic phase was dried ($MgSO_4$), filtered and the filtrate was concentrated to give the title compound as a yellow oil. Chromatography with EtOAc:Hexane (1:9→1:4) as eluent gave the desired product as a white foam. (12.9 g, almost quant). R_f = 0.7 (EtOAc:Hexane, 3:7). ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.95 (t, *J* = 10.2 Hz, 1H, H-6a), 4.08 (dd, *J* = 9.8, 3.4 Hz, 1H, H-3), 4.21 – 4.32 (m, 3H, H-6b, H-4, Fmoc C*H*), 4.32 – 4.52 (m, 3H, C*H*HPh, H-5, CH*H*Ph), 4.80 (s, 2H, Fmoc CH_2), 5.48 (dd, *J* = 3.4, 1.5 Hz, 1H, H-2), 5.60 (d, *J* = 1.5 Hz, 1H, H-1), 5.71 (s, 1H, benzylidine C*H*Ph), 7.15 – 7.82 (m, 22H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 196.3, 179.6, 171.1, 165.4, 154.6, 143.4, 143.1, 141.3, 141.2, 137.8, 137.4, 132.9, 132.2, 129.2, 129.0, 128.5, 128.4, 128.2, 128.1, 127.9, 127.7, 127.2, 126.1, 125.3, 125.2, 120.1, 101.7, 86.9, 78.5, 75.6, 74.1, 72.6, 70.4, 68.4, 65.3, 46.7.



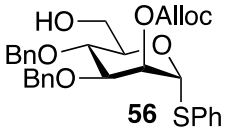
Phenyl 3,6-di-*O*-benzyl-4-*O*-*t*-butyldimethylsilyl-2-*O*-fluorenylmethoxycarbonyl-1-thio-*α*-D-mannopyranoside (48). To a solution of **54** (12.5 g, 18.57 mmol) in THF 100 mL was added BH_3-NMe_3 (7.0 g, 92.9 mmol), followed by adding $AlCl_3$ (12.1 g, 91.0 mmol) in four portions. Upon dissolution of $AlCl_3$, H_2O (670 μL, 37.14 mmol) was added and stirring was continued for 1h, after which TLC (EtOAc:Hexane, 3:7) showed the reaction to be complete. The reaction mixture was quenched with H_2O (100 mL), diluted with CH_2Cl_2 (100 mL), and successively washed with 1 M HCl (100 mL) and sat. $NaHCO_3$ (150 mL). The organic phase was dried ($MgSO_4$), filtered, and the filtrate was concentrated *in vacuo* to give the crude product as an oil. This alcohol was dissolved in anhydrous CH_2Cl_2 (20 mL), followed by adding 2,6-lutidine (22 mL, 188.7 mmol), and the solution was cooled to 0 °C. TBSOTf (6.5 mL, 30 mmol) was added, and stirring was continued for 1 h, TLC (EtOAc:Hexane, 3:7) showed the reaction to be complete. The solvents were then removed, and the residue was re-dissolved in CH_2Cl_2 and washed with 1 M HCl. The organic phase was then dried ($MgSO_4$), filtered and filtrate was concentrated to afford the crude product as a clear oil. Chromatography with EtOAc:Hexane (5:95→1:9) as eluent gave the desired product as a clear oil. (12.0 g, 82%). R_f = 0.7 (EtOAc:Hexane, 3:7). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.07 (d, *J* = 12.3 Hz, 6H, TBS-Si-CH_3 X2), 0.87 (s, 9H, TBS-C-CH_3 X3), 3.75 (dd,



J = 8.9, 3.1 Hz, 1H, H-3), 3.80 – 3.91 (m, 2H, H-6a, H-6b), 4.10 (t, *J* = 8.8 Hz, 1H, H-4), 4.20 – 4.43 (m, 4H, C*H*HPh, Fmoc CH, CH*H*Ph, H-5), 4.55 (d, *J* = 11.3 Hz, 1H, C*H*HPh), 4.62 (s, 2H, Fmoc CH_2), 4.73 (d, *J* = 11.3 Hz, 1H, CH*H*Ph), 5.42 – 5.50 (m, 1H, H-2), 5.62 (d, *J* = 1.4 Hz, 1H, H-1), 7.09 – 7.83 (m, 23H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 143.4, 143.2, 141.2, 137.5, 132.3, 129.0, 128.2, 128.1, 127.8, 127.6, 127.5, 127.4, 127.2, 125.3, 120.0, 86.0, 78.6, 74.0, 73.7, 73.2, 71.3, 70.3, 69.5, 68.1, 46.6, 26.0, 18.2, -3.8, -4.9. MALDI-TOF-MS (*m/z*): $[M + Na]^+$ calcd for $C_{47}H_{52}NaO_7SSi$, 811.3101; found 811.3420.



Phenyl 2-*O*-allyloxycarbonyl-3-*O*-benzyl-4,6-*O*-benzylidene-1-thio-*α*-D-mannopyranoside (55). To a solution of alcohol **53** (2.5 g, 5.57 mmol) in CH_2Cl_2 (20 mL) was added TMEDA (1.5 mL, 11.14 mmol), followed by Alloc-Cl (1.3 mL, 11.14 mmol), and the reaction mixture was left stirring for 30 min, after which a second portion of TMEDA (1.5 mL, 11.14 mmol) and Alloc-Cl (1.3 mL, 11.14 mmol) were added, and stirring was continued for another 15 min. The mixture was diluted with CH_2Cl_2 (20 mL) and washed with 1 M HCl. The organic phase was dried ($MgSO_4$), filtered, and the filtrate was concentrated to give the crude product. The crude product was purified on a short silica gel column with EtOAc:Hexane (1:95→3:7) as eluent to give the desired product as a clear oil. (2.48 g, almost quant). R_f = 0.7 (EtOAc:Hexane, 3:7). This product was then used in the next step without additional purification.



Phenyl 2-*O*-allyloxycarbonyl-3,4-di-*O*-benzyl-1-thio-*α*-D-mannopyranoside (56). A mixture of **55** (2.48 g, 4.64 mmol), Et_3SiH (1.9 mL, 11.6 mmol) and 4Å flame-dried molecular sieves was stirred in CH_2Cl_2 (20 mL). The solution was cooled to -78 ºC, after which $PhCl_2B$ (782 µL, 6.03 mmol) was added, and the reaction was stirred at -78 ºC for 15 min. The reaction mixture was then warmed to – 60 ºC and quenched with Et_3N (5 mL). The mixture was then diluted with CH_2Cl_2 (20 mL) and washed with 1 M HCl (20 mL). The organic phase was dried ($MgSO_4$), filtered, and the filtrate was concentrated *in vacuo* to give the crude product. Chromatography on silica gel with EtOAc:Hexane (3:7) as eluent gave the desired product as a clear oil. (1.79 g, 72%). R_f = 0.4 (EtOAc:Hexane, 3:7). ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.81 (dd, *J* = 6.8, 4.2 Hz, 2H; H-6a; H-6b), 3.87 – 4.02 (m, 2H, H-4; H-3), 4.19 (dt, *J* = 7.2, 3.6 Hz, 1H; H-5), 4.59 – 4.69 (m, 4H, CH*H*Ph; CH*H*Ph; alloc CH_2), 4.79 (d, *J* = 11.4 Hz, 1H, C*H*HPh), 4.93 (d, *J* = 10.9 Hz, 1H, C*H*HPh), 5.27 (dd, *J* = 10.4, 1.2 Hz, 1H, alloc HC=CH*H*), 5.37 (dq, *J* = 17.2, 1.4 Hz, 1H, alloc HC=C*H*H), 5.42 (dd, *J* = 2.8, 1.7 Hz, 1H, H-2), 5.55 (d, *J* = 1.3 Hz, 1H, H-1), 5.85 – 6.01 (m, 1H, alloc *H*C=CH_2), 7.21 – 7.54 (m, 15H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 154.4, 138.1, 137.6, 133.0, 132.3, 131.3, 129.2, 128.4, 128.4, 128.1, 128.0, 128.0, 127.9, 119.2, 86.0, 78.3, 75.4, 74.3, 74.2, 73.1, 72.0, 68.9, 62.0. MALDI-TOF-MS (*m/z*): $[M + Na]^+$ calcd for $C_{30}H_{32}NaO_7S$, 559.1766; found 559.3104.

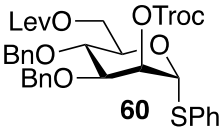
Phenyl 2-*O*-allyloxycarbonyl-3,4-di-*O*-benzyl-6-*O*-levulenoyl-1-thio-*α*-D-mannopyranoside (57).[27] To a solution of **56** (1.79 g, 3.33 mmol) in CH_2Cl_2 (30 mL) was added LevOH (679 µL, 6.67 mmol), DMAP (100 mg, cat) and EDCI (1.3 g, 6.67 mmol). The reaction mixture was left stirring for 3 h, after which it was diluted with CH_2Cl_2 (20 mL), and washed with sat. $NaHCO_3$ (20 mL). The organic phase was dried ($MgSO_4$), filtered, and the filtrate was concentrated *in vacuo*. Chromatography on silica gel with EtOAc:Hexane (3:7) as eluent gave the desired product as a clear oil. (1.79 g, almost quant). R_f = 0.25 (EtOAc:Hexane, 3:7). ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.16 (s, 3H, Lev CH_2COCH_3), 2.50 – 2.62 (m, 2H; Lev $COOCH_2$), 2.71 (t, *J* = 6.4 Hz, 2H, Lev CH_2COCH_3), 3.83 (t, *J* = 9.1 Hz, 1H, H-4), 3.96 (dd, *J* = 9.2, 3.1 Hz, 1H, H-3), 4.23 – 4.43 (m, 3H, H-6a, H-5, H-6b), 4.53 – 4.68 (m, 4H, C*H*HPh, alloc CH_2, C*H*HPh), 4.77 (d, *J* = 11.3 Hz, 1H, CH*H*Ph), 4.92 (d, *J* = 10.8 Hz, 1H, CH*H*Ph), 5.22 – 5.36 (m, 1H, alloc HC=C*H*H), 5.40 (dd, *J* = 2.9, 1.6 Hz, 2H; alloc HC=CH*H*; H-2), 5.57 (d, *J* = 1.6 Hz, 1H, H-1), 5.82 – 6.02 (m, 1H, alloc *H*C=CH_2), 7.21 – 7.52 (m, 15H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 206.4, 172.4, 154.4, 137.9, 137.4, 133.1, 132.0, 131.3, 129.1, 128.4, 128.1, 128.1, 127.9, 127.9, 127.9, 119.2, 85.8, 78.3, 75.4, 74.2, 74.0, 72.0, 70.8, 68.9, 63.3, 37.9, 29.8, 27.9. MALDI-TOF-MS (*m/z*): $[M + Na]^+$ calcd for $C_{35}H_{38}NaO_9S$, 657.2134; found 657.2197.

Phenyl 3-*O*-benzyl-4,6-*O*-benzylidene-2-*O*-2,2,2-trichloroethoxycarbonyl-1-thio-*α*-D-mannopyranoside (58). To a solution of alcohol **53** (5.33 g, 11.82 mmol) in pyridine (20 mL) was added DMAP (cat) and Troc-Cl (2.5 mL, 17.7 mmol). The reaction mixture was left stirring for 1 h, after which TLC (EtOAc:Hexane, 3:7) showed that it went to completion. The solution was concentrated, and the residue was diluted with CH_2Cl_2 (30 mL), and washed with 1 M HCl (30 mL). The organic phase was dried ($MgSO_4$), filtered, and the filtrate was concentrated *in vacuo* to give the crude product as a clear oil. Chromatography on silica gel with EtOAc:Hexane (1:9→1:4) as eluent gave the desired product as a clear oil. (7.3 g, almost quant). R_f = 0.25 (EtOAc:Hexane, 3:7). ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.91 (t, *J* = 10.2 Hz, 1H, H-6a), 4.02 – 4.16 (m, 1H, H-3), 4.17 – 4.30 (m, 2H, H-6b, H-4), 4.38 (td, *J* = 9.8, 4.7 Hz, 1H, H-5), 4.78 (dd, *J* = 3.7, 2.6 Hz, 4H, C*H*HPh, troc CH_2, CH*H*Ph), 5.50 (dd, *J* = 3.3, 1.3 Hz, 1H, H-2), 5.59 (d, *J* = 1.1 Hz, 1H, H-1), 5.67 (s, 1H, benzylidine C*H*Ph), 7.27 – 7.57 (m, 15H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 153.6, 137.7, 137.3, 132.7, 132.2, 129.3, 129.0, 128.4, 128.2, 127.8, 127.7, 126.1, 101.6, 94.2, 86.7, 78.3, 77.5, 76.5, 76.3, 74.0, 72.7, 68.3, 65.3. MALDI-TOF-MS (*m/z*): $[M + Na]^+$ calcd for $C_{29}H_{27}Cl_3NaO_7S$, 647.0441; found 647.1434.

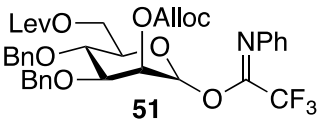
Phenyl 3,4-di-*O*-benzyl-2-*O*-2,2,2-trichloroethoxycarbonyl-1-thio-*α*-D-mannopyranoside (59). To a solution of the starting material **58** (7.5 g, 12 mmol) in 1 M BH_3-THF (60 mL) was added TMSOTf (500 µL, 2.4 mmol), and the reaction mixture was left stirring for 3 h, after which it was diluted with CH_2Cl_2 (100 mL) and washed with sat. $NaHCO_3$ (100 mL). The organic phase was dried ($MgSO_4$), filtered, and the filtrate was concentrated *in vacuo* to give the crude product, which was chromatographed with EtOAc:Hexane (1:9→1:4) as eluent giving the desired product as a clear oil. (6.1 g, 82%). R_f = 0.55 (EtOAc:Hexane, 3:7). ^{1}H NMR (300 MHz, $CDCl_3$): δ ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.77 – 3.88 (m, 2H, H-6a, H-6b), 3.89 – 4.03 (m, 2H, H-4; H-3), 4.16 – 4.26 (m, 1H, H-5), 4.66 (dd, *J* = 11.1, 7.6 Hz, 2H, C*H*HPh; C*H*HPh), 4.73 – 4.81 (m, 3H, CH*H*Ph, troc CH_2), 4.93 (d, *J* = 10.9 Hz, 1H, CH*H*Ph), 5.47 (dd, *J* = 2.8, 1.7 Hz, 1H, H-2), 5.57 (d, *J* = 1.3 Hz, 1H, H-1), 7.26 – 7.52 (m, 15H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 153.6, 138.0, 137.5, 132.8, 132.3, 129.3, 128.5, 128.4,

128.2, 128.1, 128.0, 127.9, 127.9, 94.2, 85.8, 78.3, 77.4, 77.0, 77.0, 76.6, 75.4, 75.3, 74.0, 73.2, 72.2, 61.9. ^{13}C NMR (75 MHz, $CDCl_3$) δ 153.6, 138.0, 137.5, 132.8, 132.3, 129.3, 128.5, 128.4, 128.2, 128.1, 128.0, 127.9, 127.9, 85.8, 78.3, 75.4, 75.3, 74.0, 73.2, 72.2, 61.9. MALDI-TOF-MS (*m/z*): $[M + Na]^+$ calcd for $C_{29}H_{27}Cl_3NaO_7S$, 647.0441; found 647.1434.

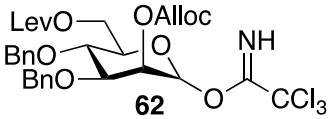
Phenyl 3,4-di-*O*-benzyl-6-*O*-levulenoyl-2-*O*-2,2,2-trichloroethoxycarbonyl-1-thio-*α*-D-mannopyranoside (60). To a solution of **59** (9.77 g, 15.55 mmol) in CH_2Cl_2 (30 mL) was added LevOH (3.2 mL, 31.1 mmol), DMAP (500 mg, cat) and EDCI (6.0 g, 31.1 mmol). The reaction mixture was left stirring for 3 h, after which it was diluted with CH_2Cl_2 (100 mL), and washed with sat. $NaHCO_3$ (100 mL). The organic phase was dried ($MgSO_4$), filtered, and the filtrate was concentrated *in vacuo*. Chromatography on silica gel with EtOAc:Hexane (3:7) as eluent gave the desired product as a clear oil. (10.8 g, almost quant). R_f = 0.3 (EtOAc:Hexane, 3:7). ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.18 (s, 3H, Lev CH_2COCH_3), 2.57 (t, *J* = 6.6 Hz, 2H, Lev $COOCH_2$), 2.71 (t, *J* = 6.7 Hz, 2H, Lev CH_2COCH_3), 3.85 (t, *J* = 8.9 Hz, 1H, H-4), 3.92 – 4.03 (m, 1H, H-3), 4.26 – 4.43 (m, 3H, H-5, H-6a, H-6b), 4.63 (m, 2H, C*H*HPh, C*H*HPh), 4.71 – 4.83 (m, 3H, troc CH_2; CH*H*Ph), 4.92 (d, *J* = 10.8 Hz, 1H, CH*H*Ph), 5.44 (s, 1H, H-2), 5.57 (s, 1H, H-1), 7.22 – 7.52 (m, 13H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 206.4, 172.3, 153.6, 137.8, 137.3, 132.9, 132.0, 129.2, 128.5, 128.2, 128.1, 128.0, 127.9, 94.2, 85.6, 78.3, 75.4, 75.1, 74.1, 74.0, 72.1, 70.9, 63.1, 37.8, 37.7, 29.9, 27.9. MALDI-TOF-MS (*m/z*): $[M + Na]^+$ calcd for $C_{34}H_{35}Cl_3NaO_9S$, 747.0965; found 747.0848.



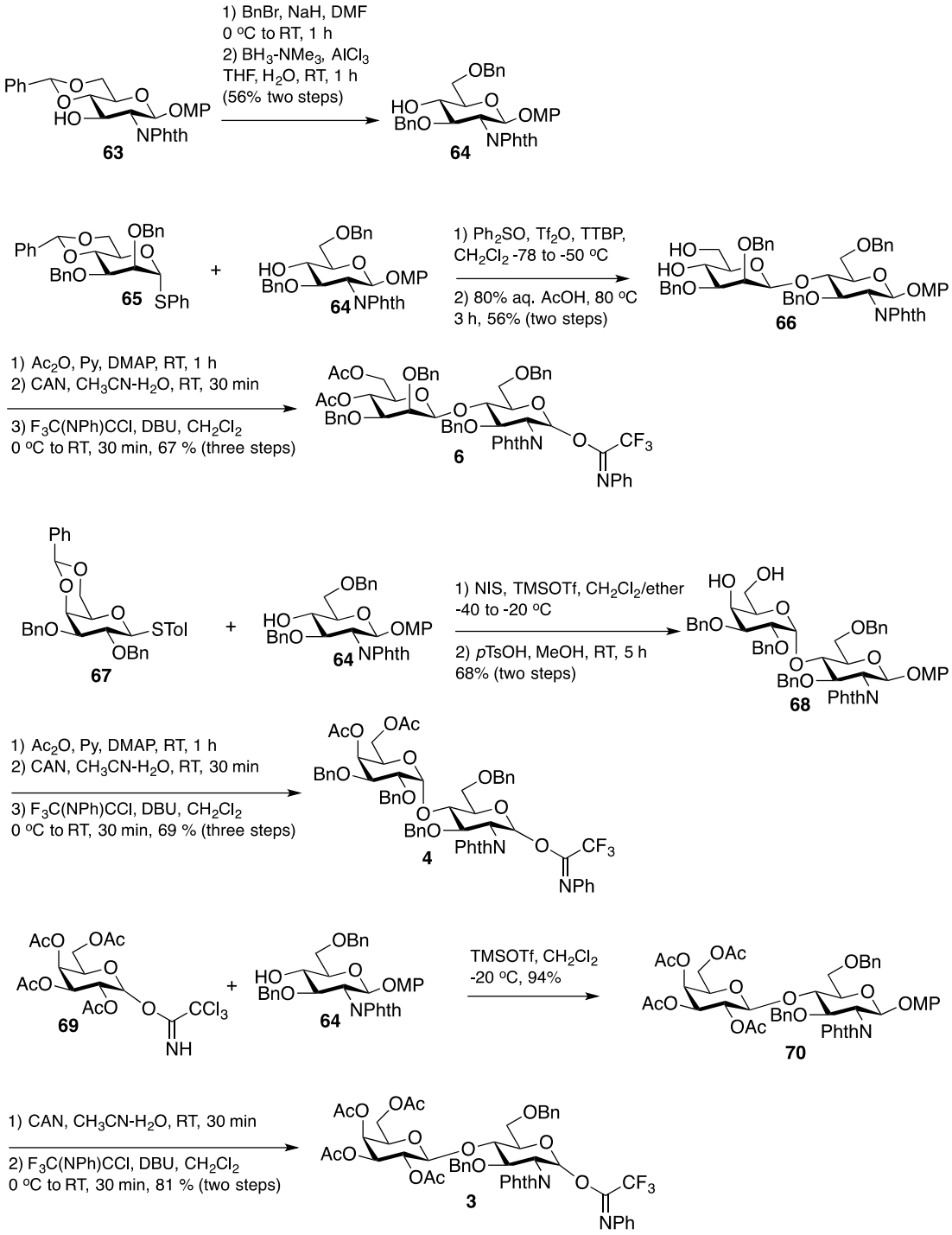
(*N*-Phenyl)-2,2,2-trifluoroacetimidate-3,4-di-*O*-benzyl-6-*O*-levulenoyl-D-mannopyranoside (51). Hemiacetal[10] **61** (8.1 g, 19.92 mmol) was dissolved in CH_2Cl_2 (30 mL) and $CF_3(NPh)CCl$ (3.6 mL, 22.3 mmol) was added. The mixture was cooled to 0 ºC, and NaH (600 mg, 14.92 mmol) was added in one portion. Stirring was continued at 0 ºC for 30 min, after which the reaction mixture was concentrated *in vacuo* and chromatographed with EtOAc:Hexane (1:9→3:7) as eluent to give the title imidate as a yellow foam. (9.42 g, 93%). R_f = 0.7 (EtOAc:Hexane, 1:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.17 (s, 3H, Lev CH_2COCH_3), 2.61 (t, *J* = 6.6 Hz, 2H, Lev $COOCH_2$), 2.68 – 2.81 (m, 2H, Lev CH_2COCH_3), 3.66 – 3.91 (m, 1H, H-4), 3.96 (m, 1H, H-5), 4.05 (dd, *J* = 9.1, 3.2 Hz, 1H, H-3), 4.25 – 4.45 (m, 2H, H-6a, H-6b), 4.64 (ddd, *J* = 21.2, 10.9, 4.2 Hz, 4H; C*H*HPh; alloc CH_2; C*H*HPh), 4.79 (dd, *J* = 11.3, 5.3 Hz, 1H, CH*H*Ph), 4.92 (dd, *J* = 10.7, 3.0 Hz, 1H, CH*H*Ph), 5.22 – 5.45 (m, 2H; alloc HC=C*H*H ; alloc HC=CH*H*), 5.62 (s, 1H, H-2), 5.71 – 6.04 (m, 1H, alloc *H*C=CH_2), 6.26 (br s, 1H, H-1), 6.84 (dd, *J* = 11.1, 8.0 Hz, 2H, aromatic protons), 7.12 - 7.33 (m, 15H, aromatic protons). ^{13}C NMR (75 MHz, $CDCl_3$) δ 206.5, 172.3, 154.9, 137.7, 137.3, 137.1, 131.3, 131.2, 128.8, 128.5, 128.2, 128.1, 128.0, 124.5, 119.4, 119.3, 119.0, 94.0, 79.3, 75.5, 75.3, 74.4, 73.4, 73.2, 72.3, 71.8, 71.0, 70.2, 69.1, 69.0, 62.8, 37.9, 29.8, 28.0, 27.9.



2,2,2-Trichloroacetimidate-3,4-di-*O*-benzyl-6-*O*-levulenoyl-D-mannopyranoside (62).



Hemiacetal **61** (581 mg, 1.07 mmol) was dissolved in dry CH_2Cl_2 (5 mL) followed by the addition of CCl_3CN (536 μL). The solution was cooled to 0 ^oC followed by adding NaH (20.0 mg, cat). It was left stirring for further 1 h, after which it was concentrated *in vacuo* and chromatographed with EtOAc:Hexane (1:9→1:1) as eluent to give the title imidate as a yellow foam. (624 mg, 84%). R_f = 0.7 (EtOAc:Hexane, 1:1). ^1H NMR (300 MHz, $CDCl_3$): δ 2.17 (s, 3H, Lev CH_2COCH_3), 2.58 (t, *J* = 6.4 Hz, 2H, Lev $COOCH_2$), 2.73 (t, *J* = 6.4 Hz, 2H, Lev CH_2COCH_3), 3.88 (t, *J* = 9.6 Hz, 1H, H-4), 4.03 (m, 2H, H-5, H-3), 4.33 (d, *J* = 3.4 Hz, 2H, H-6a, H-6b), 4.53 – 4.72 (m, 4H, C*H*HPh, C*H*HPh, alloc CH_2), 4.78 (d, *J* = 11.4 Hz, 1H, CH*H*Ph), 4.92 (d, *J* = 10.7 Hz, 1H, CH*H*Ph), 5.29 (d, *J* = 11.5 Hz, 2H, H-2, alloc HC=C*H*H), 5.34 – 5.46 (m, 1H, alloc HC=CH*H*), 5.95 (m, 1H, alloc *H*C=CH_2), 6.31 (d, 1H, H-1), 7.21 – 7.42 (m, 10H), 8.72 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 206.4, 172.3, 159.8, 154.3, 137.7, 137.3, 131.2, 128.5, 128.4, 128.3, 128.2, 128.0, 119.4, 94.8, 77.2, 75.5, 73.3, 72.5, 72.1, 71.0, 69.1, 62.8, 37.9, 29.8, 27.9.



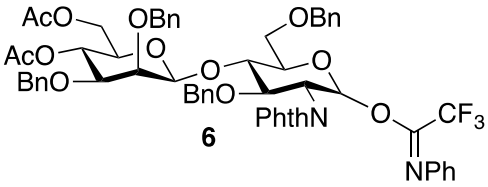
Scheme S4. Preparation of building blocks **3**, **4**, and **6**.

4-Methoxyphenyl 3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-*β*-D-glucopyranoside (64). 4-Methoxylphenyl 4,6-*O*-benzylidene-2-deoxy-2-phthalimido-*β*-D-glucopyranoside[28] (25.17 g, 49.9 mmol) was dissolved in anhydrous DMF (100 mL), after which BnBr (9 mL, 75 mmol) was added. The solution was cooled to 0 ºC, and NaH (3.0 g, 75 mmol) was added in one portion. The reaction mixture was stirred for 1 h, after which TLC (EtOAc:Hexane, 3:7) showed it was complete. The reaction mixture was neutralized with AcOH:MeOH (10 mL, 1:1), and then with sat. $NaHCO_3$ (10 mL). The mixture was concentrated *in vacuo*, diluted with CH_2Cl_2 (200 mL), and washed with H_2O (100 mL). The organic phase was dried ($MgSO_4$), filtered, and the filtrate was concentrated to give the crude product, which was used in the next step without further purification. The above material was dissolved in THF (500 mL) and BH_3-NMe_3 (15.0 g, 199.0 mmol) was added and the solution was cooled to 0 ºC. $AlCl_3$ (27.0 g, 199 mmol) was slowly added in multiple portions. Upon dissolution of $AlCl_3$, H_2O (1.8 mL) was carefully added and the reaction mixture was stirred for 1 h. It was then neutralized with H_2O (100 mL), concentrated *in vacuo*, diluted with CH_2Cl_2 (200 mL), and successively washed with H_2O (100 mL) and sat. $NaHCO_3$ (100 mL). The organic phase was dried ($MgSO_4$), filtered, and the filtrate was concentrated *in vacuo* to give the crude product, which was loaded on silica gel and chromatographed with EtOAc:Hexane (1:9→1:4→3:7) as eluent to give the title acceptor as a light-yellow foam. (16.8 g, 56%, two steps). R_f = 0.25 (EtOAc:Hexane, 3:7). ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.70 (s, 3H, OC*H*$_3$), 3.72 – 3.77 (m, 1H, H-5), 3.83 (m, 2H, H-6a, H-6b), 3.90 (t, *J* = 8.9 Hz, 1H, H-3), 4.30 (dd, *J* = 10.7, 8.2 Hz, 1H, H-2), 4.41 (dd, *J* = 10.7, 8.3 Hz, 1H, H-4), 4.52 – 4.70 (m, 3H, C*H*HPh; C*H*HPh; CH*H*Ph), 4.77 (d, *J* = 12.1 Hz, 1H, CH*H*Ph), 5.66 (d, *J* = 8.3 Hz, 1H, H-1), 6.60 – 7.92 (m, 18H). The remaining analytical data are in accordance with that of previously reported.[29]

4-Methoxylphenyl 2,3-di-*O*-benzyl-*β*-D-mannopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-*β*-D-glucopyranoside (66). A mixture of phenyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-1-thio-*α*-D-mannopyranoside[25] (6.8 g, 12.6 mmol), diphenyl sulfoxide (2.6 g, 12.6 mmol) TTBP (3.3 g, 12.6 mmol) and 4Å flame-dried molecular sieves was stirred in CH_2Cl_2 (100 mL) for 5 min. The reaction mixture was then cooled to -78 ºC, and Tf_2O (2.1 mL, 12.6 mmol) was added along the wall of the flask. After 10 min at – 78 ºC, a solution of **64** (5.0 g, 18.78 mmol) in dry CH_2Cl_2 (10 mL + (2 mL x 2) washings) was slowly added along the wall of the flask. The reaction was stirred for additional 30 min at – 78 ºC, after which it was gradually warmed to – 50 ºC, and then quenched with Et_3N (5 mL). The mixture was then diluted with CH_2Cl_2 (50 mL) and washed with sat. $NaHCO_3$ (50 mL). The organic phase was dried ($MgSO_4$), filtered, and the filtrate was concentrated *in vacuo* to give the crude product, which was directly dissolved in 80% aq. AcOH (100 mL) and the resulting solution was heated at 80 ºC for 3 h. The reaction mixture was concentrated *in vacuo*, absorbed on silica gel, and purified using EtOAc:toluene (0:1→1:4→3:7) as eluent giving the pure diol as a white foam. (4.98 g, 56%, two steps). R_f = 0.3 (EtOAc:Hexane, 1:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.18 (m, 2H, H-5, GlcN; H-5, Man), 3.49 (m, 5.4 Hz, 1H, H-6a, Man), 3.64 – 3.89 (m, 9H, H-6a, GlcN ; H-4, GlcN ; H-6b, Man ; H-6b, GlcN ; H-3, Man; H-2, Man; OCH_3), 4.10 (t, *J* = 8.4, 7.8, 1H, H-3, GlcN), 4.27 – 4.61 (m, 7H, C*H*HPh; H-2, GlcN ; H-4, Man ; CH*H*Ph ; C*H*HPh; C*H*HPh ; H-1, Man), 4.65 – 5.00 (m, 4H; CH*H*Ph ; CH*H*Ph; CH*H*Ph ; C*H*HPh), 5.66 (d, *J* = 8.2 Hz, 1H, H-1, GlcN), 6.67 – 7.92 (m, 28H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 155.4, 150.8, 138.5, 138.3, 137.8, 137.7, 133.8, 131.5, 128.6, 128.5, 128.2, 128.0, 127.9, 127.6, 127.6, 127.5, 127.1, 123.4, 118.7, 114.4, 101.1, 97.7, 82.0, 78.7, 75.9, 75.0,

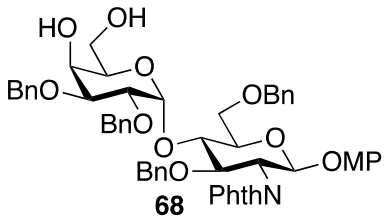
74.7, 74.4, 74.2, 73.7, 71.3, 68.6, 67.2, 62.7, 55.6. MALDI-TOF-MS (*m/z*): $[M + H]^+$ calcd for $C_{55}H_{56}NO_{13}$, 938.3752; found 938.2343.

(*N*-Phenyl)-2,2,2-trifluoroacetimidate 4,6-di-*O*-acetyl-2,3-di-*O*-benzyl-*β*-D-mannopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-D-glucopyranoside (6).



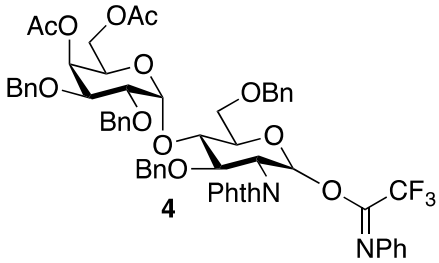
Disaccharide **66** (4.08 g, 4.27 mmol) was dissolved in pyridine (10 mL) followed by adding DMAP (cat) and Ac_2O (1.7 mL), and the reaction mixture was stirred for 1h. It was then quenched with methanol and concentrated. The resulting syrup was then dissolved in CH_2Cl_2 (20 mL) and washed with 1 M HCl (20 mL). The organic phase was dried ($MgSO_4$), filtered, and the filtrate was concentrated *in vacuo* to give an intermediate, which was dissolved in CH_3CN (20 mL), and then H_2O (2 mL) was added. Ceric ammonium nitrate (4.7 g, 8.54 mmol) was added at 0 ºC, and the reaction mixture was for 30 min. The reaction mixture was diluted with EtOAc (50 mL) and successively washed with H_2O (30 mL) and sat. $NaHCO_3$ (30 mL). The organic phase was dried ($MgSO_4$), filtered, and the filtrate was concentrated *in vacuo* to afford a residue, which was chromatographed with EtOAc:Hexane (3:7→1:1) as eluent to give the lactol as an orange foam. To a solution of this hemiacetal in dry CH_2Cl_2 (10 mL) was added $CF_3C(NPh)Cl$ (1.1 mL, 6.4 mmol) and DBU (700 µL, 4.7 mmol) at 0 ºC. The reaction mixture was stirred at 0 ºC for 30 min, after which it was concentrated and chromatographed with EtOAc:Hexane (3:7) as eluent to give the imidate as a light-yellow foam. (3.1 g, 67%, over three steps). R_f = 0.6 (EtOAc:Hexane, 1:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.96 (s, 3H, Acetyl CH_3), 2.03 (s, 3H, Acetyl CH_3), 3.26 (dd, *J* = 9.8, 2.8 Hz, 1H, H-3, Man), 3.31 – 3.44 (m, 1H, H-5, Man), 3.76 (d, *J* = 2.7 Hz, 1H, H-2, Man), 3.95 – 4.20 (m, 5H, H-6a, Man; H-6b, Man), 4.22 – 4.57 (m, 8H, CH_2Ph protons; H-1, Man), 4.68 (d, *J* = 12.1 Hz, 1H, C*H*HPh), 4.75 – 4.94 (m, 3H, CH_2Ph protons), 5.29 (t, *J* = 9.8 Hz, 1H, H-4, Man), 6.53 – 7.81 (m, 29H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.9, 169.6, 138.6, 138.4, 137.9, 137.7, 133.8, 131.4, 128.6, 128.4, 128.1, 128.0, 127.9, 127.8, 127.7, 127.5, 127.3, 126.9, 124.4, 123.4, 119.3, 101.3, 79.4, 78.6, 76.7, 75.4, 74.6, 74.3, 74.0, 73.5, 72.5, 71.5, 68.0, 62.9, 54.7, 20.9, 20.7.

4-Methoxylphenyl 2,3-di-*O*-benzyl-*α*-D-galactopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-*β*-D-glucopyranoside (68). A mixture of the donor **67**[30] (12.2 g, 22.0 mmol), acceptor **64** (8.74 g, 14.67 mmol) and 4Å flame-dried molecular sieves was stirred in CH_2Cl_2:Et_2O (150 mL, 1:2) for 5 min. NIS (5.0 g, 22.0 mmol) was added and the reaction mixture was cooled to – 40 ºC and TMSOTf (530 µL, 2.9 mmol) was added. After 10 min the reaction mixture was warmed to – 20 ºC, after which it was quenched with Et_3N (5 mL). The solution was then filtered, diluted with CH_2Cl_2 (100 mL) and washed with 10 % $Na_2S_2O_3$ (50 mL). The organic phase was dried ($MgSO_4$), filtered, and the filtrate was concentrated *in vacuo* to provide the crude product, which was briefly chromatographed using EtOAc:Toluene (1:4) as eluent. The isolated material was directly dissolved in MeOH (100 mL) and *p*-TsOH (1.0 g) was added, after which the reaction mixture was stirred for 5 h. It was then neutralized with Et_3N (10 mL), concentrated *in vacuo*, and purified using EtOAc:Toluene (1:4) as eluent to give the disaccharide diol as a white foam. (9.08 g, 68%, two steps, *α* only). R_f = 0.3 (EtOAc:Toluene, 1:4). ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.62 – 3.96 (m, 15H, OCH_3 ; H-6a, GlcN; H-6b, GlcN ; H-2, Gal ; H-5, GlcN ; H-4, GlcN), 4.06 (s, 1H, H-3, Gal), 4.10 – 4.20 (m, 1H, H-3, GlcN), 4.31 (d, *J* = 12.2 Hz, 1H, H-6a, Gal), 4.42 – 4.80 (m, 11H, H-6b, Gal; H-2, GlcN ; H-5, Gal ; H-4, Gal, CH_2Ph



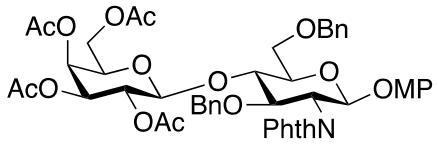
X4), 5.54 (d, J = 3.4 Hz, 1H, H-1, Gal), 5.59 (d, J = 8.2 Hz, 1H, H-1, GlcN), 6.62 – 7.76 (m, 28H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 155.4, 150.8, 138.0, 138.0, 137.8, 133.7, 131.5, 128.5, 128.3, 128.0, 128.0, 127.9, 127.8, 127.7, 127.4, 127.0, 123.3, 118.8, 114.4, 97.8, 97.5, 80.2, 77.3, 75.7, 75.3, 74.0, 73.6, 72.4, 70.2, 68.9, 68.7, 63.1, 55.6, 55.5. MALDI-TOF-MS (*m/z*): $[M + Na]^+$ calcd for $C_{55}H_{55}NNaO_{13}$, 960.3571; found 960.7290.

(*N*-Phenyl)-2,2,2-trifluoroacetimidate 4,6-di-*O*-acetyl-2,3-di-*O*-benzyl-*α*-D-galactopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-*β*-D-glucopyranoside (4). Disaccharide **68** (1.55 g, 1.52 mmol) was dissolved in pyridine (5 mL) followed by adding DMAP (cat) and Ac_2O (575 μL), and the reaction mixture was stirred for 1 h. It was quenched with methanol and concentrated. The resulting syrup was then dissolved in CH_2Cl_2 (20 mL) and washed with 1 M HCl (10 mL). The organic phase was dried ($MgSO_4$), filtered, and the filtrate was concentrated to give an intermediate, which was dissolved in CH_3CN (10 mL), and H_2O (1 mL) was added. Ceric ammonium nitrate (1.3 g, 2.28 mmol) was added at 0 ºC, and the reaction mixture was stirred for 30 min. It was diluted with EtOAc (20 mL) and successively washed with H_2O (20 mL) and sat. $NaHCO_3$ (20 mL). The organic phase was dried ($MgSO_4$), filtered, and the filtrate was concentrated to afford a residue, which was chromatographed with EtOAc:Hexane (3:7→1:1) eluent to give the lactol as an orange foam. To a solution of this hemiacetal in dry CH_2Cl_2 (10 mL) was added $CF_3C(NPh)Cl$ (260 μL, 1.6 mmol) and DBU (227 μL, 1.52 mmol) at 0 ºC. The reaction mixture was stirred at 0 ºC for 30 min, after which it was concentrated *in vacuo* and chromatographed with EtOAc:Hexane (3:7) as eluent to give the imidate as a light-yellow foam. (1.04 g, 69%, over three steps). R_f = 0.6 (EtOAc:Hexane, 1:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.05 (s, 3H, Acetyl CH_3), 2.13 (s, 3H, Acetyl CH_3), 3.67 – 3.85 (m, 2H, H-6a, GlcN ; H-2, Gal), 3.85 – 4.05 (m, 3H, H-3, Gal; H-6b, GlcN; H-6a, Gal), 4.05 – 4.27 (m, 4H, H-6b, Gal; H-5, Gal; H-3, GlcN, C*H*HPh), 4.48 (m, 2H, H-4, GlcN; H-2, GlcN), 4.55 – 4.82 (m, 7H, CH*H*Ph , CH_2Ph X3), 5.48 – 5.54 (m, 2H, H-1, Gal; H-4, Gal), 6.43 – 7.82 (m, 32H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.4, 170.3, 167.5, 142.9, 138.0, 137.9, 137.8, 133.8, 131.4, 128.8, 128.6, 128.5, 128.4, 128.3, 128.3, 128.1, 128.1, 128.0, 127.9, 127.8, 127.8, 127.7, 127.7, 127.5, 127.0, 124.4, 123.4, 119.3, 98.3, 93.4, 79.7, 77.5, 76.4, 75.7, 75.5, 75.2, 74.6, 74.2, 73.3, 71.9, 68.3, 67.5, 67.5, 62.4, 54.7, 20.9, 20.8, 14.2.



4

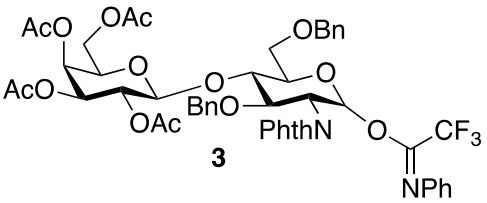
4-Methoxylphenyl 2,3,4,6-tetra-*O*-acetyl-*β*-D-galactopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-*β*-D-glucopyranoside (70). A mixture of the donor **69**[23] (8.1g, 17.62 mmol), acceptor **64** (7.0 g, 11.75 mmol) and 4Å flame-dried molecular sieves was stirred in CH_2Cl_2 (50 mL) for 5 min. The reaction mixture was then cooled to – 20 ºC, followed by adding TMSOTf (426 μL, 2.35 mmol). It was then stirred at – 20 ºC for 15 min, neutralized with Et_3N (5 mL), filtered, diluted with CH_2Cl_2 (50 mL), and the organic solution was washed with 10 % $Na_2S_2O_3$ (100 mL). The organic phase was dried ($MgSO_4$), filtered, and the filtrate was concentrated *in vacuo* to provide the crude product, which was chromatographed using EtOAc:Hexane (3:7) as eluent to give the disaccharide as a white foam. (10.18 g, 94%). R_f = 0.4 (EtOAc:Hexane, 1:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.91 – 2.12 (4 X s, 12H, Acetyl CH_3), 3.57 – 3.79 (m, 6H, H-4, GlcN; H-5, GlcN; H-6a, GlcN; OCH_3, H-6b, GlcN), 3.91 – 4.04 (m, 2H, H-6a, Gal; H-6b, Gal), 4.07 – 4.18 (m, 1H, H-3, GlcN), 4.26 – 4.55 (m, 4H, H-5, Gal; H-2, GlcN; C*H*HPh; C*H*HPh), 4.61 (d, J = 8.0 Hz, 1H, H-1, Gal), 4.74 – 4.92 (m, 3H, CH*H*Ph,; CH*H*Ph; H-3, Gal), 5.16 (dd, J = 10.4, 8.0 Hz,



70

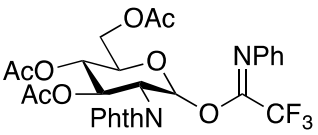
1H, H-2, Gal), 5.28 (d, J = 3.0 Hz, 1H, H-4, Gal), 5.60 (d, J = 8.1 Hz, 1H, H-1, GlcN), 6.63 – 7.86 (m, 18H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.3, 170.3, 170.2, 170.0, 169.1, 155.4, 150.8, 138.4, 137.8, 133.7, 131.5, 128.7, 128.6, 128.3, 128.0, 128.0, 127.9, 127.9, 127.8, 127.2, 127.1, 123.3, 118.7, 117.4, 114.5, 114.3, 100.4, 97.6, 77.9, 75.0, 74.5, 73.6, 71.0, 70.5, 69.5, 67.5, 66.9, 60.7, 55.5, 20.8, 20.7, 20.6, 20.5, 20.5.MALDI-TOF-MS (*m/z*): [M + Na]$^+$ calcd for $C_{49}H_{51}NNaO_{17}$, 948.3055; found 948.4044.

(*N*-Phenyl)-2,2,2-trifluoroacetimidate 2,3,4,6-tetra-*O*-acetyl-*β*-D-galactopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-*β*-D-glucopyranoside (3). Disaccharide **70** (3.0 g, 3.23mmol) dissolved in CH_3CN (10 mL), and then H_2O (1 mL) was added. Ceric ammonium nitrate (3.5 g, 6.46 mmol) was added at 0 ºC, and the reaction mixture was then stirred for 30 min. It was diluted with EtOAc (50 mL) and successively washed with H_2O (20 mL) and sat. $NaHCO_3$ (30 mL). The organic phase was dried ($MgSO_4$), filtered, and the filtrate was concentrated *in vacuo* to afford a residue, which was chromatographed with EtOAc:Hexane (3:7→1:1) as eluent to give the lactol as an orange foam. To a solution of this hemiacetal in dry CH_2Cl_2 (10 mL) was added $CF_3C(NPh)Cl$ (780 µL, 1.6 mmol) and DBU (485 µL, 1.52 mmol) at 0 ºC. The reaction mixture was stirred at 0 ºC for 30 min, after which it was concentrated and chromatographed with EtOAc:Hexane (3:7) to give the imidate as a light-yellow foam. (3.1 g, 81%, over two steps). R_f = 0.6 (EtOAc:Hexane, 1:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.92 – 2.10 (4 x s, 12H, Acetyl CH_3), 3.63 (t, J = 6.7 Hz, 1H, H-3, GlcN), 3.73 (s, 1H, H-6a, GlcN), 3.88 – 4.05 (m, 3H, H-6b, GlcN; H-6a, Gal; H-6b, Gal), 4.11 (q, J = 7.1 Hz, 2H; H-5, GlcN; H-4, GlcN), 4.21 – 4.61 (m, 5H, H-5, Gal; H-2, GlcN; C*H*HPh; CH*H*Ph; H-1, Gal), 4.75 – 4.88 (m, 3H, H-3, Gal; C*H*HPh; CH*H*Ph), 5.07 – 5.18 (m, 1H, H-2, Gal), 5.26 (d, J = 3.2 Hz, 1H, H-4, Gal), 6.48 – 7.85 (m, 15H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.3, 170.2, 170.0, 169.1, 138.3, 137.5, 133.9, 131.4, 128.6, 128.6, 128.2, 127.9, 127.2, 124.3, 123.4, 100.2, 77.2, 76.2, 75.5, 74.5, 73.6, 70.9, 70.5, 69.4, 66.9, 60.7, 54.6, 20.7, 20.7, 20.6, 20.5.



3

(*N*-Phenyl)-2,2,2-trifluoroacetimidate 3,4,6-tetra-*O*-acetyl-2-deoxy-2-phthalimido-D-glucopyranoside (5). To a solution of 3,4,6-tetra-*O*-acetyl-2-deoxy-2-phthalimido-D-glucopyranose[31] (3.0 g, 6.9 mmol) in dry CH_2Cl_2 (20 mL) was added $CF_3C(NPh)Cl$ (1.7 mL, 10.35 mmol) and DBU (1.0 mL, 6.9 mmol) at 0 ºC. The reaction mixture was stirred at 0 ºC for 1 h, after which it was concentrated *in vacuo* and chromatographed with EtOAc:Hexane (3:7) as eluent to give the imidate as a light-yellow foam. (3.3 g, 82%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.85 (s, 3H, Acetyl CH_3), 2.01 (s, 3H, Acetyl CH_3), 2.08 (s, 3H, Acetyl CH_3), 4.13 (t, J = 11.7 Hz, 1H, H-6a), 4.31 (d, J = 11.7 Hz, 1H, H-6b), 4.55 (broad m, 1H, H-2), 5.22 (t, J = 9.6 Hz, 1H, H-3), 5.86 (broad s, 1H, H-4), 6.71 (s, 1H), 7.57 (m, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.6, 170.0, 169.3, 167.3, 142.7, 134.5, 131.2, 128.7, 128.3, 124.6, 123.8, 120.4, 119.2, 92.6, 77.3, 72.7, 70.4, 68.2, 61.4, 53.6, 20.7, 20.5, 20.4.



5

Enzymatic Synthesis

General methods. All enzymatic reactions were performed in aqueous buffers with an appropriate pH for each enzyme. β3-Gnt-II (*β*-(1→3)-glucosaminyltransferase), ST3-Gal-IV (*α*-(2→3)-sialyltransferase) and Fut-I (*α*-(1→2)-fucosyltransferase) were provided by Dr K. W. Moremen (Complex Carbohydrate Research Center, Athens, GA, USA). Mammalian *β*-(1→4)-galactosyltransferase was purchased from Roche Diagnostic Corporation (Indianapolis, IN). Alkaline phosphatase from calf intestine (CIAP) was purchased from Sigma-Aldrich. Uridine 5'-diphospho-*N*-acetylglucosamine (UDP-GlcNAc) was purchased from Sigma-Aldrich; uridine 5'-diphosphogalactose (UDP-Gal) and cytidine 5'monophospho-*N*-acetylneuraminic acid (CMP-Neu5Ac) were purchased from Roche Diagnostic Corporation (Indianapolis, IN); guanosine 5'-diphospho-L-fucose (GDP-Fuc) was purchased form Carbosynth Limited (UK). Water was purified by NANOpure Diamond™ water system (Barnstead D3750 Hollow Fibre Filter). All enzymatic reaction, unless otherwise stated, were monitored by mass spectrometry preceded by permethylation, and either recorded on an Applied Biosystems SCIEX MALDI TOF/TOF 5800 mass spectrometer or a Shimadzu Biotech Axima-CFR MALDI-TOF using dihydroxybenzoiuc acid or 4-hydroxycinnamic acid as matrices respectively. Gel filtration chromatography was performed using a column (30 cm x 1.5 cm) packed with Sephadex™ G-25 Superfine (GE Healthcare), eluted with deionized water. All enzymatic reactions were forced to go to full completion by adding excess of glycosyltransferases until all starting material had disappeared. This approach enabled efficient product isolation and purification. All nuclear magnetic resonance (NMR) spectra were acquired on 400, 600, 800, or 900 MHz Varian/Agilent Direct Drive, alternatively on 750 or 900 MHz Bruker spectrometers operating at 25 ^{o}C unless otherwise stated. Data were collected using standard pulse programs from the spectrometer library. Samples were dissolved in 99.96% D_2O. Chemical shifts were referenced to the residual HDO signal at 4.79 ppm. For integration of 1D proton spectra, data were acquired with recycling delays of 10 seconds and a small tip angle. The residual HDO signal was suppressed by a low-power presaturation pulse. Typically, 2D homonuclear spectra were collected as a 1750 X 512 complex point data set with a spectral width of 7.8 ppm. The "zTOCSY" sequence was run with a 80 msec dipsi-2 mixing sequence; the NOESY mixing time was 300 ms. The "HSQCAD" sequence was used for the carbon-proton correlated spectra. Data were generally processed with Mnova (Mestrelab Inc.)

Enzyme	Source
ST3-GalIV, *α*-(2→3)-sialyltransferase	Gift of Dr. K. W. Moremen (CCRC)
PmST-1, *α*-(2→3)-sialyltransferase	Chemily LLC
***β*3-Gnt-II**, *β*-(1→3)-glucosaminyltransferase	Gift of Dr. K. W. Moremen (CCRC)
Fut-I, *α*-(1→2)-fucosyltransferase	Gift of Dr. K. W. Moremen (CCRC)
Gal-T1[3], *β*-(1→4)-galactosyltransferase	Roche Diagnostic Corporation (Indianapolis, IN)
Alkaline phosphatase from calf intestine (**CIAP**)	Sigma-Aldrich
***N*-Acetyl-*β*-glucosaminidase** (from Jack bean)	Sigma-Aldrich
***β*-Mannosidase** (crude extract from *Helix pomatia*)	Sigma-Aldrich
***α*-Galactosidase** (from green coffee beans)	Sigma-Aldrich
Hpα1,3FT, *α*-(1→3)-fucosyltransferase	Chemily LLC

[3] In all our syntheses, this enzyme has been proved to be more stable and active compared to the related bovine Gal-T1 (available from Sigma-Aldrich). Unlike the bovine Gal-T1, this enzyme does not lose its activity upon prolonged storage at -20 ^{o}C.

General Experimental Procedures

General Procedure for *α*(2→3) Sialylation. Glycan **1** (28.0 mg), **29** (5.8 mg), **35** (5.0 mg), **38** (6.0 mg) or **39** (6.0 mg) and CMP-Neu5Ac (1.2 eq per sialic acid) were dissolved in sodium cacodylate buffer (50 mM, pH~7.6) containing BSA (0.1 %). CIAP (10 mU) and ST3Gal-IV (3.3 mU/μmol substrate for mono-sialylation or 6.6 mU/μmol substrate for bis-sialylation) were added to achieve a final concentration of glycan ranging from 4 – 7 mM. The resulting reaction mixture was incubated at 37 ^{o}C for 18 h. In case MALDI (after permethylation) showed the remaining starting material additional CMP-Neu5Ac (1 or 2 eq), CIAP (10 mU) and ST3Gal-IV (3.3 mU/μmol substrate for mono-sialylation or 6.6 mU/μmol substrate for bis-sialylation) were added and incubated at 37 ^{o}C until no more starting material could be detected. The reaction mixture was quenched by adding an equal volume of ethanol, after which the mixture was centrifuged, and the supernatant subjected to gel filtration over Sephadex G-25. Fractions containing product, which were detected using TLC (dipping into the anisaldehyde stain followed by charring at 150 ^{o}C), were combined and lyophilized to give the respective products **21** (27.0 mg, 86%), **30** (5.9 mg, 94%), **8** (5.4 mg, 88%), **9** (6.3 mg, 86%) and **40** (5.9 mg, 82%) as white fluffy solids.

General Procedure for *α*(1→3) Fucosylation. Glycan **40** (5.9 mg) and GDP-Fucose (2 eq per fucose) were dissolved in Tris buffer (50 mM, pH~7.5) containing $MnCl_2$ (10 mM). CIAP (10 mU) and HP*α*(1→3)FucT (6.6 mU/μmol of substrate) were added to achieve a final concentration of 4 mM. The resulting mixture was incubated at 37 ^{o}C for 18 h. In case MALDI-TOF-MS (after permethylation) showed the remaining starting material additional GDP-Fucose (1 or 2 eq), CIAP (10 mU) and HP*α*(1→3)FucT (3.3 mU/μmol substrate) were added and incubation at 37 ^{o}C was continued until no more starting material could be detected. The reaction mixture was quenched by adding an equal volume of ethanol, after which it was centrifuged, and the supernatant subjected to gel filtration over Sephadex G-25. Fractions containing product were identified using TLC (dipping into the anisaldehyde stain followed by charring at 150 ^{o}C), combined and lyophilized to give the product **41** as a white fluffy solid. (5.9 mg, 92%).

General Procedure for *α*(1→2) Fucosylation. Glycan **26** (22.0 mg) and GDP-Fucose (2 eq per fucose) were dissolved in Tris buffer (50 mM, pH~7.5) containing $MnCl_2$ (10 mM). CIAP (10 mU) and Fut-I (150 μL, 1 mg/mL) were added to achieve a final concentration of 4 mM. The resulting mixture was incubated at 37 ^{o}C for 18 h. In case MALDI-TOF-MS (after permethylation) showed the remaining starting material additional GDP-Fucose (1 or 2 eq), CIAP (10 mU) and Fut-I were added and incubated at 37 ^{o}C until no more starting material could be detected. The reaction mixture was quenched by adding an equal volume of ethanol, after which it was centrifuged, and the supernatant subjected to gel filtration over Sephadex G-25. Fractions containing product were detected using TLC (dipping into the anisaldehyde stain followed by charring at 150 ^{o}C), were combined and lyophilized to give the product **27** as a white fluffy solid. Purification by HILIC (55% MeCN-100 mM Ammonium formate, pH 3.4) affords pure product. (11.5 mg, 51% over 7 steps)

General Procedure for *β*(1→4) Galactosylation. Glycans **21** (24.0 mg), **23** (24.0 mg), **25** (22.0 mg), **28** (5.8 mg), **31** (5.0 mg), **34** (5.0 mg), **37** (6.0 mg), **41** (5.9 mg) and UDP-Gal (2 eq per galactoside) were dissolved in Tris buffer (50 mM, pH±7.5) containing BSA (0.1%) and $MnCl_2$ (20 mM). CIAP (10 mU) and Gal-T1 (3.4 mU/μmol) were added to achieve a final concentration of 4 mM. The reaction mixture was then incubated at 37 ^{o}C for 10 h. The reaction mixture was quenched by adding an equal volume of ethanol, after which it was

centrifuged, and the supernatant subjected to gel filtration over Sephadex G-25. Fractions containing product were identified using TLC (dipping into the anisaldehyde stain followed by charring at 150 ^{o}C), combined and lyophilized to give the respective products **22** (24.0 mg, 94%), **24** (24.6 mg, 97%), **26** (22.0 mg, 95%), **29** (5.8 mg, 95%), **32** (5.0 mg, 96%), **35** (5.0 mg, 87%), **38** (6.0 mg, 88%), and **10** (5.9 mg, 92%) as white fluffy solids.

General Procedure for installation of *β*(1→3) *N*-acetylglucosamine moieties. Glycan **22** (24.0 mg), **24** (23.0 mg), **32** (5.0 mg), **33** (5.1 mg), and **36** (6.0 mg) and UDP-GlcNAc (1.5 eq) were dissolved in HEPES buffer (50 mM, pH±7.3) containing KCl (25 mM), $MgCl_2$ (2 mM), and DTT (1 mM). To this, CIAP (10 mU) and β3Gnt-II (6.0 mU/μmol) were added to achieve a final concentration of glycan at 4 mM. The resulting mixture was then incubated at 37 ^{o}C for 12 h. The reaction mixture was quenched by adding an equal volume of ethanol, after which it was centrifuged, and the supernatant subjected to gel filtration over Sephadex G-25. Fractions containing product were identified using TLC (dipping into the anisaldehyde stain followed by charring at 150 ^{o}C), combined and lyophilized to give the respective products **23** (24.0 mg, 93%), **25** (23.0 mg, 94%), **7** (4.9 mg, 93%), **34** (5.0 mg, 89%), and **37** (6.0 mg, 91%) as white fluffy solids.

Sequential One-Pot Three-Enzyme Conversion of 22 to 36. Glycan **22** (10.0 mg) was dissolved in 50 mM NaOAc buffer (pH~5.5) (500 μL) followed by adding 10 mM Zanamivir and the appropriate amounts of *α*-galactosidase from green coffee beans and the reaction mixture was then incubated at 37 ^{o}C for 2 h. In case MALDI-TOF-MS (after permethylation) showed the remaining starting material additional portion of the enzyme was added and incubation at 37 ^{o}C was continued until no more starting material could be detected (2 h). After this time 10 mM deoxygalactonojirimycin hydrochloride was added followed by adding appropriate amounts of the *Canavalia ensiformis β-N*-acetylglucosaminidase (5 U) and the reaction mixture was then incubated for 2 days, after this time additional amounts of the *Canavalia ensiformis β-N*-acetylglucosaminidase (5 U) was added and incubation was continued for another 2 days. After this time the reaction mixture was heated at 100 ^{o}C for 10 min, after which appropriate amounts of the *Helix pomatia β*-mannosidase were added and incubation was continued for another 3 h. In case MALDI-TOF-MS (after permethylation) showed the remaining starting material, additional portion of the *Helix pomatia β*-mannosidase was added and incubation was continued at 37 ^{o}C until no more starting material could be detected (2 h). After this time the reaction mixture was heated at 100 ^{o}C for 10 min, centrifuged, the supernatant was lyophilized, and the resulting product was desalted using the BioGel P-2 column. Additional purification by HPLC with a semi-preparative amide HILIC column (10 x 250 mm, Waters Inc.) under isocratic conditions (65% CH_3CN:100 mM Ammonium formate, pH~3.4) with the UV (210 nm) detection affords analytically pure **36** (6.0 mg, 74%) as a white solid.

Sequential One-Pot Three-Enzyme Conversion of 22 to 33. Glycan **22** (10.0 mg) was dissolved in 50 mM NaOAc buffer (pH~5.5) (500 μL) followed by adding 10 mM Zanamivir and the appropriate amounts of the *Helix pomatia β*-mannosidase and the *Canavalia ensiformis β-N*-acetylglucosaminidase (5 U). The reaction mixture was then incubated at 37 ^{o}C for 6 h. In case MALDI-TOF-MS (after permethylation) showed the remaining starting material, additional portion of the above enzymes was added and incubation was continued at 37 ^{o}C until no more starting material could be detected (2 h). After this time the reaction mixture was heated at 100 ^{o}C for 10 min, after which appropriate amounts of *α*-galactosidase from green coffee beans was added and incubation was continued for another 2 h. In case MALDI-TOF-MS (after permethylation) showed the remaining starting material additional

portion of the *α*-galactosidase from green coffee beans was added and incubation was continued at 37 ºC until no more starting material could be detected (2 h). After this time the reaction mixture was heated at 100 ºC for 10 min, centrifuged, the supernatant was lyophilized, and the resulting product was desalted using the BioGel P-2 column. Additional purification by HPLC with a semi-preparative amide HILIC column (10 x 250 mm, Waters Inc.) under isocratic conditions (60% CH_3CN:100 mM Ammonium formate, pH±3.4) with the UV (210 nm) detection affords analytically pure **33** (5.1 mg, 63%) as a white solid.

Sequential One-Pot Three-Enzyme Conversion of 1 to 39. Glycan **1** (10.0 mg) was dissolved in 50 mM NaOAc buffer (pH~5.5) (500 µL) and the appropriate amounts of *α*-galactosidase from green coffee beans was added and the reaction mixture was incubated at 37 ºC for 3h, after which 10 mM deoxygalactonojirimycin hydrochloride was added together with the *Canavalia ensiformis β-N*-acetylglucosaminidase (5 U). The reaction mixture was then incubated at 37 ºC for 6 h. In case MALDI-TOF-MS (after permethylation) showed the remaining starting material, additional portion of the above enzymes was added and incubation was continued at 37 ºC until no more starting material could be detected (2 h). After this time the reaction mixture was heated at 100 ºC for 10 min, after which appropriate amounts of the *Helix pomatia β*-mannosidase were added and incubation was continued for another 2 h. In case MALDI-TOF-MS (after permethylation) showed the remaining starting material additional portion of the the *Helix pomatia β*-mannosidase was added and incubated at 37 ºC until no more starting material could be detected (2 h). After this time the reaction mixture was heated at 100 ºC for 10 min, centrifuged, the supernatant was lyophilized, and the resulting product was desalted using the BioGel P-2 column. Additional purification by HPLC with a semi-preparative amide HILIC column (10 x 250 mm, Waters Inc.) under isocratic conditions (60% CH_3CN:100 mM ammonium formate, pH~3.4) with the UV (210 nm) detection affords analytically pure **39** (6.0 mg, 89%) as a white solid.

General Procedure for *α*-Galactose Removal with Subsequent Installation of a *β*-Galactose Moiety. Glycan **27** (5.8 mg) was dissolved in 50 mM NaOAc buffer (pH~5.5) (500 µL) followed by adding the appropriate amounts of *α*-galactosidase from green coffee beans and the reaction mixture was then incubated at 37 ºC for 2 h. In case MALDI-TOF-MS (after permethylation) showed the remaining starting material, additional portion of the enzyme was added and incubated at 37 ºC until no more starting material could be detected (2 h). After this time the reaction mixture was heated at 100 ºC for 10 min, centrifuged, the supernatant was lyophilized, and the resulting product was desalted using the BioGel P-2 column. Fractions containing the product were pooled and lyophilized. This glycan and UDP-Gal (1.5 - 2 eq) were dissolved in Tris buffer (50 mM, pH~7.5) containing BSA (0.1%) and $MnCl_2$ (20 mM). CIAP (10 mU) and Gal-T1 (3.4 mU/µmol) were added to achieve a final concentration of 4 mM. The reaction mixture was then incubated at 37 ºC for 10 h. The reaction mixture was quenched by adding an equal volume of ethanol, after which it was centrifuged, and the supernatant subjected to gel filtration over Sephadex G-25. Fractions containing product were identified using TLC (dipping into the anisaldehyde stain followed by charring at 150 ºC), combined and lyophilized to give **29** (5.8 mg, almost quant.) as a white fluffy solid.

General Procedure for *β*-Mannose Removal with Subsequent Installation of a *β*-Galactose Moiety. Glycan **30** (6.1 mg) was dissolved in 50 mM NaOAc buffer (pH~5.5) (500 µL) followed by adding 10 mM deoxyfuconojirimycin hydrochloride and the appropriate amounts of the *Helix pomatia β*-mannosidase the reaction mixture was then incubated at 37 ºC for 2 h. In case MALDI-TOF-MS (after permethylation) showed the

remaining starting material, additional portion of the enzyme was added and incubated at 37 ^{o}C until no more starting material could be detected (2 h). After this time the reaction mixture was heated at 100 ^{o}C for 10 min, centrifuged, the supernatant was lyophilized, and the resulting product was desalted using the BioGel P-2 column. Fractions containing the product were pooled and lyophilized. This glycan and UDP-Gal (1.5 - 2 eq) were dissolved in Tris buffer (50 mM, pH~7.5) containing BSA (0.1%) and $MnCl_2$ (20 mM). CIAP (10 mU) and Gal-T1 (3.4 mU/μmol) were added to achieve a final concentration of 4 mM. The reaction mixture was then incubated at 37 ^{o}C for 10 h. The reaction mixture was quenched by adding an equal volume of ethanol, after which it was centrifuged, and the supernatant subjected to gel filtration over Sephadex G-25. Fractions containing product were identified using TLC (dipping into the anisaldehyde stain followed by charring at 150 ^{o}C), were combined and lyophilized to give **32** (5.0 mg, 82%) as a white fluffy solid.

Permethylation Analysis

Permethylation of glycans and their analysis was achieved using the procedure described below.

Preparation of Base. DMSO (1.5 mL) was added to 50% aq. NaOH (100 μL) and MeOH (200 μL) in a pyrex tube. The tube was vortexed and then centrifuged to bring the gel to the bottom of the tube. The top layer solution was removed and the gel was washed with DMSO (x5). To the clean gel, DMSO (1 mL) was added and the gel was broken by vortexing.

Permethylation. Iodomethane (125 μL) and the broken gel (350 μL) were added to the glycan (~8 μg) dissolved in DMSO (200 μL). The tube was purged with N_2 and vortexed continuously for 10 min, after which water (1.5 mL) was added. The excess iodomethane was removed by a flow of N_2, and the permethylated glycans was extracted with CH_2Cl_2. The organic layer was extracted further with water (x5). The clean CH_2Cl_2 extract was transferred to another pyrex tube and was dried using a flow of N_2. MeOH (20 μL) was added to the tube, vortexed, and used for attaining the mass spectra.

NMR Nomenclature

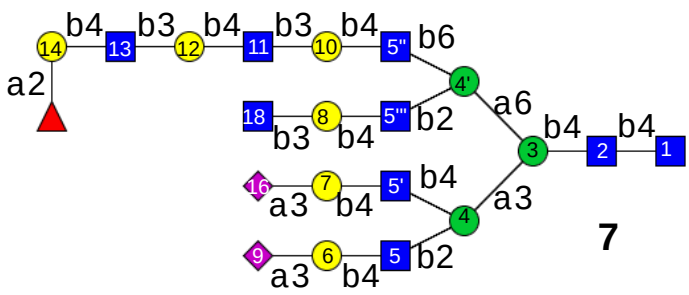


Figure S2. Monosaccharide labeling used for compounds within the enzymatic synthesis section.

The monosaccharide residues in the following glycans have been labelled as depicted in **Figure S2**. Starting from the reducing end of the chitobiose core, these were labeled as GlcNAc-1 (GlcN-1) and GlcNAc-2 (GlcN-2) respectively; the *β*-mannoside of the core pentasaccharide is labeled as Man-3, the *β*-mannoside of the unnatural Man-*β*-(1→4)-GlcNAc terminus was labeled as Man-8 however, when replaced with a *β*-galactoside after the trimming process, the newly formed galactoside receives the same number.; the *α*-3 mannoside as Man-4, and the *α*-6 mannoside as Man-4', followed by the *N*-acetylglucosamine residues as GlcNAc-5, 5', 5'', 5'''. The *α*-4 galactoside was labeled as Gal-7, however, when replaced with a *β*-galactoside after the trimming process, the newly formed galactoside receives the same number. Sialic acids are labelled as Neu5Ac-9 and Neu5Ac-16; for tri-antennary glycans, where additional sialic acid is present, it was named as Neu5Ac-19. Galactose residues within the tri-lactosamine component were labelled as Gal-10, Gal-12, and Gal-14 beginning from the reducing end, whereas the *N*-acetylglucosamine residues within this chain were labelled as GlcNAc-11 and GlcNAc-13. Since only one fucoside is present in any given compound, it was abbreviated as Fuc.

NMR Analysis

Structural Analysis of Precursor 1. The enzymatic transformations could be established by either analysing appearance or disappearance of certain anomeric signals corresponding to either added or deleted monosaccharide units. Gal-7 is the only *α*-linked galactoside in the precursor **1** and could be identified as a doublet with $J_{1,2}$ = 2.7 Hz, appearing in the most de-shielded region of the spectrum (**Figure S3**). Reducing end GlcNAc-1-*α*/*β* could be identified in the ^{1}H spectra as doublets with $J_{1,2}$ = 2.5 Hz and with $J_{1,2}$ = 7.7 Hz respectively. Their identity was also supported by the HSQC spectrum, having the most shielded resonances in their ^{13}C spectrum, which is in agreement with previous work. *β*-Linked Man-3 and Man-8 appeared in a more shielded region (4.64 ppm) as compared to the branching Man-4 and Man-4' (5.13 and 4.87 ppm respectively). The anomeric signals of branching GlcNAc residues (2, 5, 5', 5'', and 5''') overlapped, and hence were identified as a multiplet. The only *β*-linked galactoside appeared at 4.46 ppm with $J_{1,2}$ = 8.0 Hz, being consistent with the *β*-linkage.

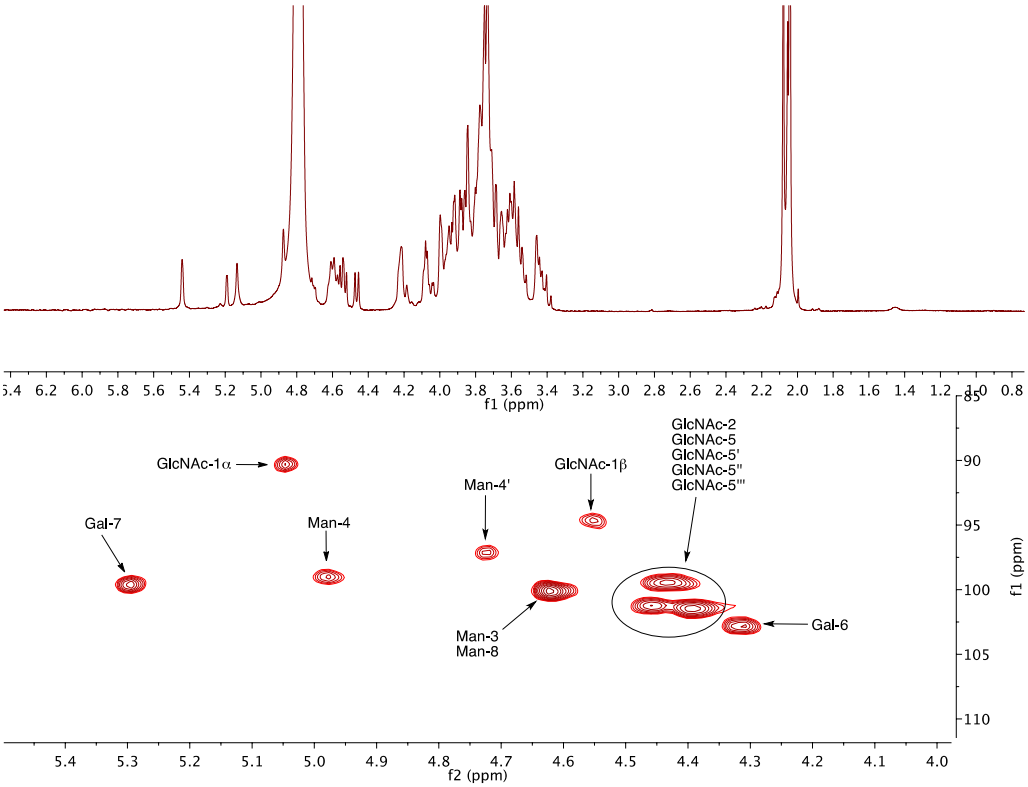


Figure S3. ^{1}H and HSQC spectra of the common oligosaccharide precursor **1**

NMR Analysis of Arm-Specific Addition of Neu5Ac and GlcNAc Moieties. Exclusive addition of sialic acid catalyzed by a specific mammalian ST3Gal-IV enzyme to the bottom β(1,2)-arm was confirmed by the downfield shift of the H-1 of Gal-6 into the region where it overlaps with the branching GlcNAc moieties (**Figure S4**). Addition of a single β(1,3)-GlcNAc moiety to the β(1,6)-arm of **22** to give **23** could be determined through comparative TOCSY experiments (**Figure S5**). A significant shift of H-4 (Gal-10) from 3.95 ppm to 4.16 ppm are consistent with the β(1,3)-linkage and internalization of Gal-10.[10] Finally, the anomeric signal of GlcNAc-11 appears as a doublet at 4.69 ppm ($J_{1,2}$ = 8.1 Hz) downfield of the original branching GlcNAcs. Additionally, H-1 (Gal-10) shifted upfield, which also confirms that it was the Gal-10 that was modified by β3-GnT-II enzyme. No changes were observed in resonances belonging to Gal-7 (α-Gal), confirming that unnatural α-Gal is not a substrate for β3-GnT-II.

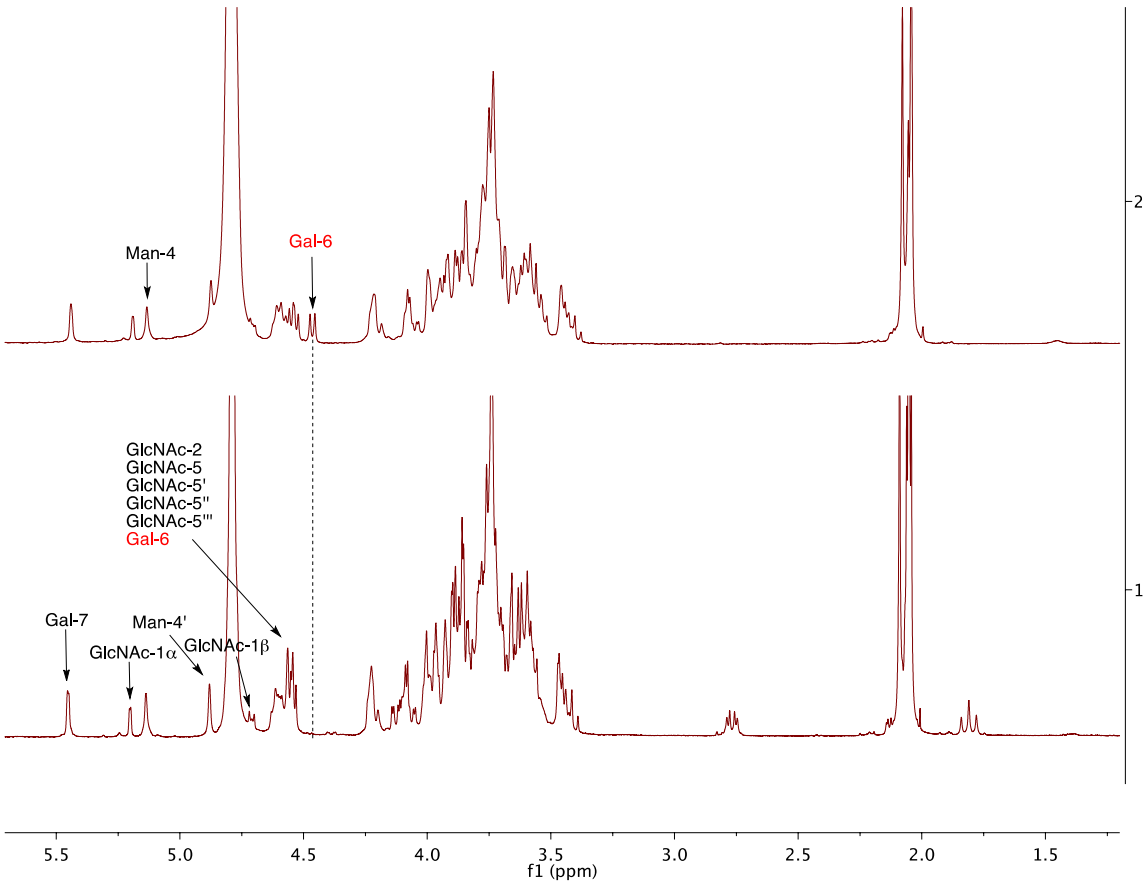


Figure S4. Monitoring arm-specific addition of Neu5Ac. Top: compound **1**; bottom: product **21**.

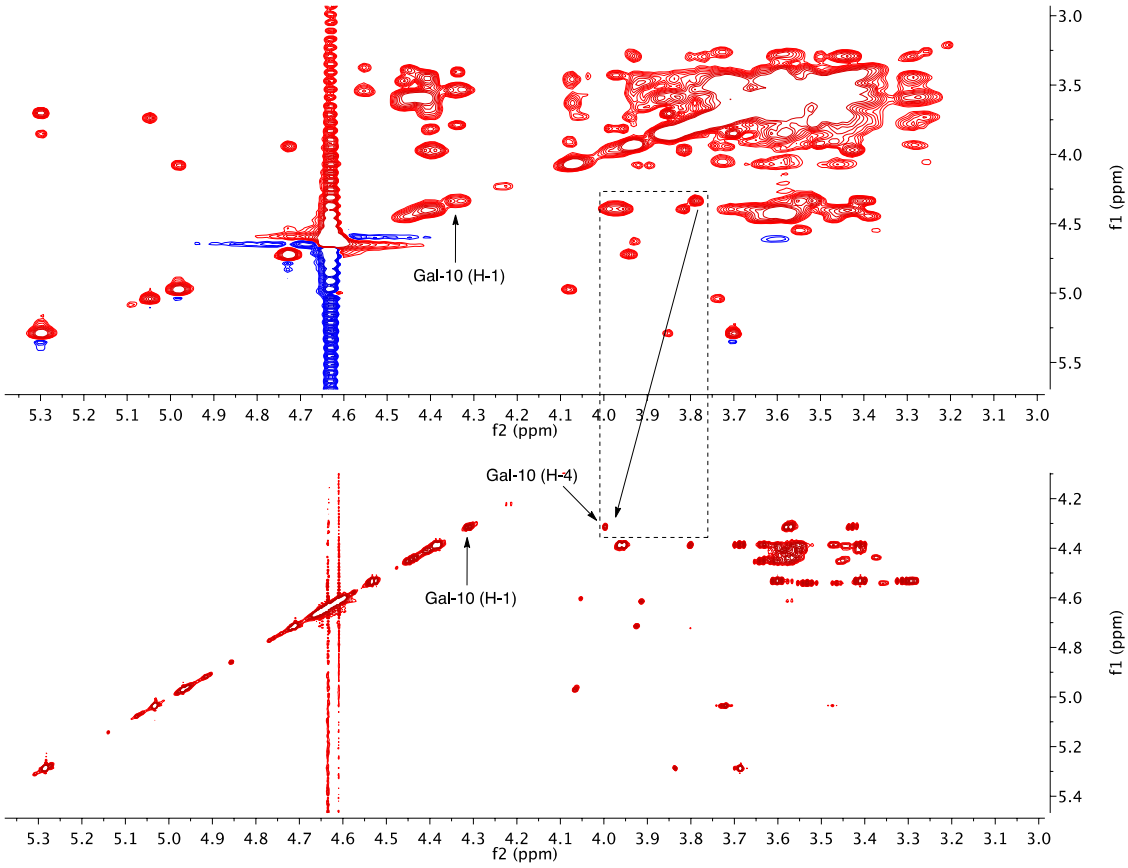


Figure S5. TOCSY spectra of compounds **22** (top panel) and **23** (bottom panel)

NMR Analysis of Selective Trimming of Caging *α*-Galactoside. Removal of *α*-Gal using *α*-galactosidase proceeded quantitatively, which is manifested by the disappearance of the anomeric H-1 at 5.40 ppm (**Figure S6**). This event in itself serves as an important checkpoint providing a strong evidence that the bottom *β*-(1,2) and top *β*-(1,6) arms were previously modified regioselectivity. Loss of enzymatic control at any previous step would result in internalization of the *α*-Gal, which would disable it from being cleaved by an exo-*α*-galactosidase when converting **27** to **28.** This logic also allows to exclude possibilities of obtaining mixtures of glycans in case enzymatic transfers are only partially specific. If this took place, trimming of the *α*-Gal would not be complete and its anomeric signal could still be detected within the ^{1}H NMR spectra as a less intense resonance belonging to contaminating by-products. Addition of the *β*-linked GlcNAc to give **29**, was first confirmed by MALDI-TOF-MS, and the anomeric signal of the newly installed Gal-7*β* overlapped with those of Gal-10 and Gal-12 appearing as a broad doublet at 4.47 ppm.

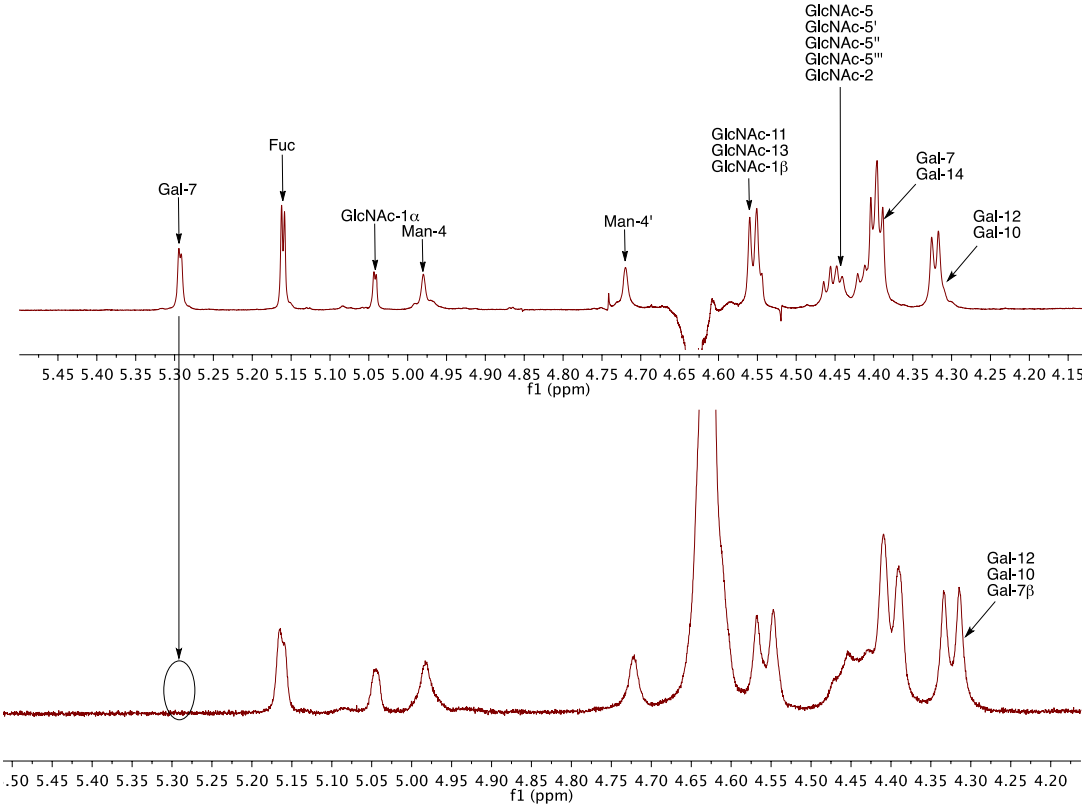


Figure S6. ^{1}H spectra of **27** (top panel) and **29** (bottom panel).

NMR Analysis of Asymmetrical Tetra-Antennary Glycan 7

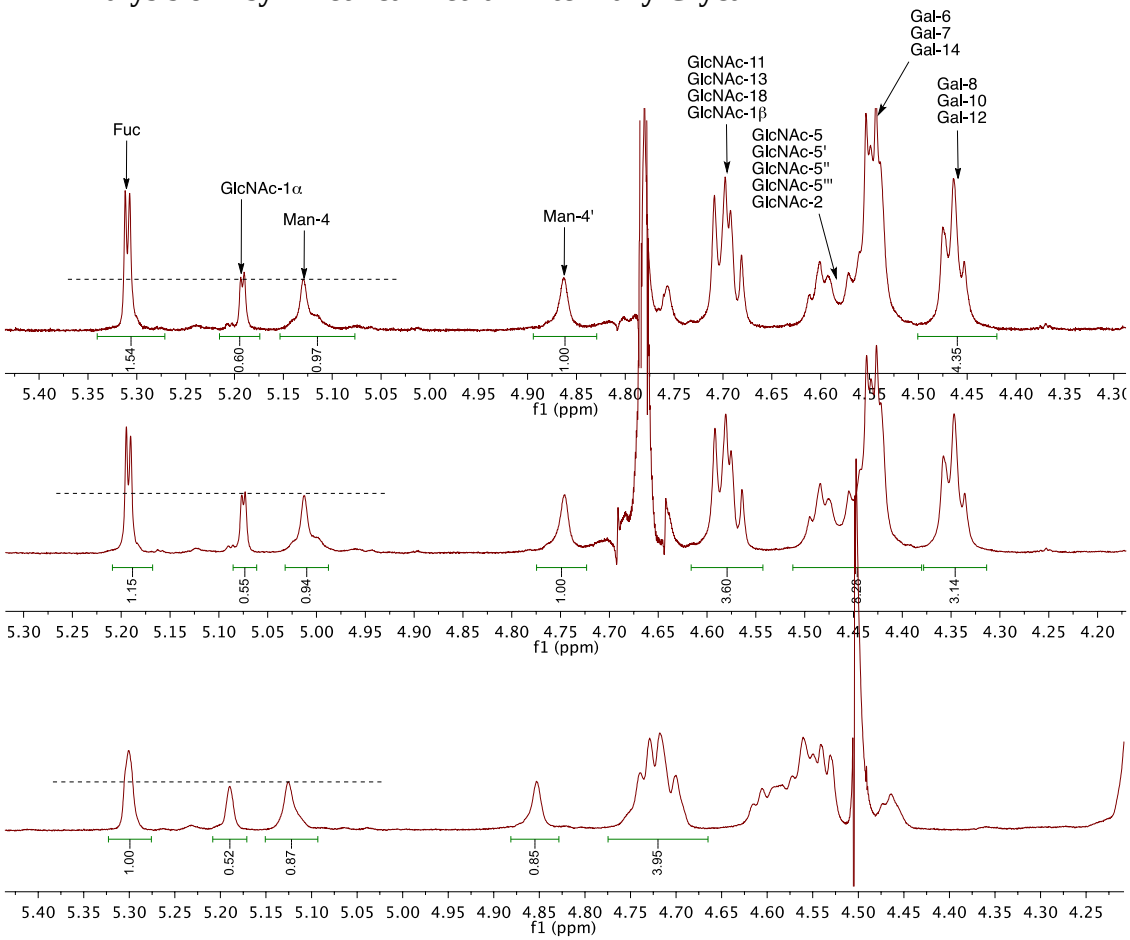


Figure S7. ^{1}H NMR (750 MHz) spectra (anomeric region) of glycan **7**; top panel: T = 298 K, no recycle delay; middle panel: T = 298 K, recycle delay = 10 s; bottom panel: T = 328 K, recycle delay = 10 s.

After installation of the second Neu5Ac moiety, the *β*(1,2)-arm had to be liberated to install another LacNAc unit. Trimming of the terminal *β*(1,4)-Man was straightforward, however, it was not possible to follow this particular step by NMR, since the Man-8 signals cannot be distinguished from Man-3 due to the overlap. MALDI permethylation analysis (see page S53), however, clearly showed the removal of one hexose upon treatment with *Helix pomatia* *β*-mannosidase. Again, this reaction was quantitative, supporting that the bottom *β*-(1,2) and *β*-(1,4) arms along with the top *β*-(1,6) arms were previously modified selectively and under expected regioselectivity. Loss of enzymatic control at any previous stages of this 13-step synthesis would result in internalization of the *β*(1,4)-Man, which would disable it from being cleaved by an exo-*β*-mannosidase when converting **30** to **31**. Therefore, *α*-Gal and *β*-Man not only serve as caging moieties in synthetic *N*-glycans, but are also there to verify whether enzymatic reactions are arm-specific, and whether the desired asymmetry had been achieved. Finally, introduction of Gal-8 was straightforward, however, its anomeric signal again overlapped with those of Gal-10 and Gal-12. On the other hand, transfer of a new *β*(1,3)-GlcNAc residue (GlcNAc-18) was manifested by the appearance of the new anomeric signal at 4.68 ppm, being more shielded than the remaining *β*(1,3)-GlcNAc moieties belonging to the tri-lactosamine unit, which is consistent with a terminal *β*(1,3)-linkage. This was also

accompanied by a slight upfield shift of the anomeric Gal-8 signal to 4.47 ppm, which is also in accord with modification of that particular galactose by a β(1,3)-GlcNAc.

Finally, for this asymmetric 21-mer, it has been observed that the branching Man-4 and Man-4' anomeric NMR signals exhibit unusually low intensity as compared to the Fuc, Gal and GlcNAc moieties of the tri-lactosamine platform. This effect was observed when performing the experiment at standard temperature and with a short relaxation delay (**Figure S7**, top panel). To prove that this is indeed an artefact, the relaxation delay was set to 10 s, which resulted in increase of intensities of the anomeric Man-4 and Man-4' signals and restoration of more accurate integration signals (1.15 for Fuc and 1.0 for Man-4'). The same effect could be achieved by increasing the experiment temperature from 298 to 328 K (**Figure S7**, bottom panel). To show that it is only the Man-4 and Man-4' that are being affected by these experimental NMR changes, the overall integral ratio of Gal-8+Gal-10+Gal-12 relative to Fuc remains 2.7 regardless of changes in the temperature or relaxation delay. The above data suggest that the branching Man-4 and Man-4' could be conformationally more constrained than the sugar moieties belonging to the distal sites of large *N*-glycans. Interestingly, the overall rigidity of the *N*-glycan chitobiose has already been demonstrated within the conserved core pentasaccharide alone.[32] Our NMR data collected within a more complex system indicate that the rigidity could also be extended towards the branching mannoses.

Full NMR Assignments of Final *N*-Glycans

Table S2. ^{1}H NMR of compound **10**.

10	**H1**	**H2**	**H3**	**H4**	**H5**	**H6**	**Fuc-CH$_3$**
GlcNAc-1α	5.19	3.87	3.63	NA	NA	NA	—
Man-4	5.11	4.19	3.89	3.61	3.74	NA	—
Man-4'	4.93	4.11	3.89	3.61	NA	NA	—
Man-3	4.76	4.25	3.78	3.62	NA	NA	—
GlcNAc-1β	4.69	3.69	3.62	3.52	NA	NA	—
GlcNAc-2	4.61	3.80	3.75	3.61	NA	NA	—
GlcNAc-5	4.56	3.73	3.69	3.57	NA	NA	—
GlcNAc-5''	4.59	3.75	3.74	3.57	NA	NA	—
Gal-6	4.51	3.52	4.08	3.93	NA	NA	—
Gal-10	4.46	3.54	3.67	3.93	NA	NA	—
Neu5Ac-9	—	—	2.76; 1.79	3.68	3.85	3.66	—
Fuc	5.12	3.68	3.90	3.77	4.81	—	1.16

Table S3. ^{1}H NMR of compound **8**.

8	**H1**	**H2**	**H3**	**H4**	**H5**	**H6**	**Fuc-CH$_3$**
GlcNAc-1α	5.19	3.88	3.66	NA	NA	NA	—
Man-4	5.11	4.19	3.89	3.61	3.74	NA	—
Man-4'	4.93	4.14	3.89	3.61	NA	NA	—
Man-3	4.76	4.25	3.78	3.62	NA	NA	—
GlcNAc-1β	4.69	3.69	3.62	3.52	NA	NA	—
GlcNAc-2	4.61	3.80	3.75	3.61	NA	NA	—
GlcNAc-5	4.56	3.73	3.69	3.57	NA	NA	—
GlcNAc-5'	4.55	3.76	3.84-3.89	3.71-3.76	3.54-3.58	NA	NA
GlcNAc-5''	4.59	3.75	3.74	3.57	NA	NA	—
Gal-6	4.51	3.55	4.04	3.88	3.49-3.66	3.49-3.66	—
Gal-10	4.39	3.50	3.66	4.09	NA	NA	—
Gal-12	4.51	3.55	4.04	3.88	3.49-3.66	3.49-3.66	—
Gal-15	4.51	3.55	4.04	3.88	3.49-3.66	3.49-3.66	—
Neu5Ac-9	—	—	2.76; 1.80	3.68	3.71	NA	—
Neu5Ac-16	—	—	2.76; 1.80	3.68	3.71	NA	—
Neu5Ac-19	—	—	2.76; 1.80	3.68	3.71	NA	—

Table S4. ^{1}H NMR of compound **9**.

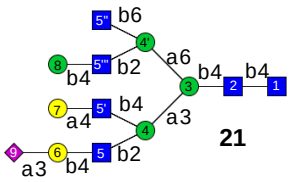
9	**H1**	**H2**	**H3**	**H4**	**H5**	**H6**	**Fuc-CH$_3$**
GlcNAc-1α	5.19	3.88	3.66	NA	NA	NA	–
Man-4	5.11	4.19	3.89	3.61	3.74	NA	–
Man-4'	4.93	4.14	3.89	3.61	NA	NA	–
Man-3	4.76	4.25	3.78	3.62	NA	NA	–
GlcNAc-1β	4.69	3.69	3.62	3.52	NA	NA	–
GlcNAc-2	4.61	3.80	3.75	3.61	NA	NA	–
GlcNAc-5	4.55	3.73	3.69	3.57	NA	NA	–
GlcNAc-5'''	4.58	3.75	3.63	3.61	NA	NA	–
GlcNAc-5''	4.54	3.75	3.74	3.57	NA	NA	–
Gal-6	4.55	3.60	4.11	3.96	3.49-3.66	3.49-3.66	–
Gal-10	4.46	3.50	3.94	4.09	NA	NA	–
Gal-12	4.51	3.60	4.11	4.16	3.49-3.66	3.49-3.66	–
Gal-15	4.51	3.60	4.11	3.96	3.49-3.66	3.49-3.66	–
Neu5Ac-9	–	–	2.76; 1.79	3.68	3.71	NA	–
Neu5Ac-16	–	–	2.76; 1.79	3.68	3.71	NA	–
Neu5Ac-19	–	–	2.76; 1.79	3.68	3.71	NA	–

Table S5. ^{1}H NMR of compound **7**.

7	**H1**	**H2**	**H3**	**H4**	**H5**	**H6**	**Fuc-CH$_3$**
GlcNAc-1α	5.19	3.87	3.63	NA	NA	NA	—
Man-4	5.11	4.19	3.89	3.61	3.74	NA	—
Man-4'	4.93	4.11	3.89	3.61	NA	NA	—
Man-3	4.76	4.25	3.78	3.62	NA	NA	—
GlcNAc-1β	4.69	3.69	3.62	3.52	NA	NA	—
GlcNAc-2	4.61	3.80	3.75	3.61	NA	NA	—
GlcNAc-5	4.56	3.83	3.69	3.57	NA	NA	—
GlcNAc-5'	4.54	3.87	3.74	3.57	NA	NA	—
GlcNAc-5'''	4.59	3.75	3.63	3.61	NA	NA	—
GlcNAc-5''	4.56	3.74-3.78	3.72-3.75	3.72-3.75	NA	NA	—
GlcNAc-11	4.71	3.81	3.72-3.75	3.72-3.75	3.60	NA	—
GlcNAc-13	4.71	3.81	3.72-3.75	3.72-3.75	NA	NA	—
GlcNAc-18	4.68	3.72-3.75	3.72-3.75	3.72-3.75	NA	NA	—
Gal-6	4.54-4.58	3.52	4.14	3.91	NA	NA	—
Gal-7	4.54-4.58	3.52	4.14	3.91	NA	NA	—
Gal-8	4.47	3.57	3.47-3.69	4.16-4.17	NA	NA	—
Gal-10	4.48	3.47-3.69	3.47-3.69	4.16-4.17	NA	NA	—
Gal-12	4.48	3.47-3.69	3.47-3.69	4.16-4.17	NA	NA	—
Gal-14	4.54-4.58	3.47-3.69	3.47-3.69	NA	NA	NA	—
Neu5Ac-9	—	—	2.68; 1.75	3.67	3.843	3.65	—
Neu5Ac-16	—	—	2.68; 1.75	3.67	3.843	3.65	—
Fuc	5.23	3.60-3.75	3.60-3.75	3.60-3.75	4.16	—	1.23

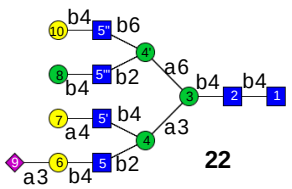
NMR Data of Enzymatic Intermediates

Glycan **21**



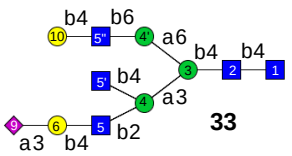
^{1}H NMR (600 MHz, D_2O): δ 1.83 (t, *J* = 12.1, 1H, H-3$_{ax}$, Neu5Ac-9), 1.97 – 2.15 (m, 21H), 2.70 – 2.81 (dd, J = 12.1, 4.0 Hz, 1H, H-3$_{eq}$, Neu5Ac-9), 3.36 – 3.49 (m, 4H), 3.61 (m, 48H), 4.03 – 4.10 (m, 3H), 4.13 (ad, *J* = 10.1 Hz, 1H, H-3, Gal-6), 4.21 (m, 3H), 4.49 – 4.63 (m, 6H, H-1 x 6, Gal-6, GlcNAc-5 – 5’’’, GlcNAc-2), 4.69 (d, *J* = 7.6 Hz, 1H), 4.74 – 4.76 (s x 2, 1H x 2, Man-3, Man-8 overlap with D_2O), 4.87 (s, 1H, Man-4’), 5.12 (s, 1H, Man-4), 5.19 (d, *J* = 1.8 Hz, 1H, GlcNAc-1), 5.44 (d, *J* = 2.1 Hz, 1H, Gal-7). Per-OMe MALDI-TOF-MS (*m/z*): [M + Na]$^+$ calcd for $C_{138}H_{243}N_7NaO_{69}$, 3125.5619; found 3126.0703.

Glycan **22**



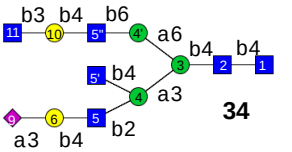
^{1}H NMR (400 MHz, D_2O) δ 1.81 (t, *J* = 12.1 Hz, 1H, H-3$_{ax}$, Neu5Ac-9), 1.98 – 2.20 (m, 21H), 2.76 (dd, *J* = 12.3, 4.5 Hz, 1H, H-3$_{eq}$, Neu5Ac-9), 3.37 – 3.48 (m, 3H), 3.49 – 4.03 (m), 4.03 – 4.17 (m, 5H), 4.21 (m, 3H), 4.49 (d, *J* = 7.6 Hz, 1H, H-1, Gal-10), 4.52 – 4.64 (m, 6H, H-1 x 6, Gal-6, GlcNAc-5 – 5’’’, GlcNAc-2), 4.70 (d, *J* = 7.9 Hz, 1H, H-1, GlcNAc-1), 4.73 – 4.77 (s x 2, 1H x 2, Man-3, Man-8 overlap with D_2O), 4.88 (s, 1H, H-1, Man-4’), 5.13 (s, 1H, H-1, Man-4), 5.19 (s, 1H, H-1, GlcNAc-1), 5.49 (s, 1H, H-1, Gal-7). Per-OMe MALDI-TOF-MS (*m/z*): [M + Na]$^+$ calcd for $C_{147}H_{259}N_7NaO_{74}$, 3329.6617; found 3329.0444.

Glycan **33**



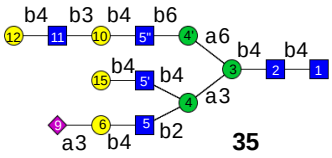
^{1}H NMR (600 MHz, D_2O) δ 1.80 (t, *J* = 12.4 Hz, 1H, H-3$_{ax}$, Neu5Ac-9), 2.01 – 2.14 (m, 18H), 2.76 (dd, *J* = 12.3, 4.5 Hz, 1H, H-3$_{eq}$, Neu5Ac-9), 3.44 – 4.07 (m), 4.11 (ad, 1H), 4.17 (ad, 1H), 4.22 (s, 1H), 4.48 (d, *J* = 7.6 Hz, 1H, H-1, Gal-10), 4.51 – 4.64 (m, 5H, H-1 x 5, Gal-6, GlcNAc-5, 5’, 5’’, GlcNAc-2), 4.70 (d, *J* = 7.9 Hz, 1H, H-1, GlcNAc-1), 4.77 (s, 1H, H-1, Man-3, overlap with D_2O), 4.88 (s, 1H, H-1, Man-4’), 5.12 (s, 1H, H-1, Man-4), 5.19 (s, 1H, H-1, GlcNAc-1). Per-OMe MALDI-TOF-MS (*m/z*): [M + Na]$^+$ calcd for $C_{118}H_{208}N_6NaO_{59}$, 2676.3358; found 2675.799.

Glycan **34**



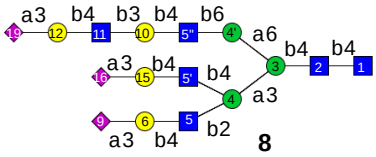
^{1}H NMR (400 MHz, D_2O) δ 1.80 (t, *J* = 12.4 Hz, 1H, H-3$_{ax}$, Neu5Ac-9), 2.06 – 2.19 (m, 21H), 2.75 (dd, *J* = 12.3, 4.5 Hz, 1H, H-3$_{eq}$, Neu5Ac-9), 3.42 – 4.07 (m), 4.11 (dd, *J* = 9.8, 2.8 Hz, 1H, H-3, Gal-6), 4.14 - 4.18 (m, 2H), 4.21 (s, 2H), 4.46 (d, *J* = 7.6 Hz, 1H, H-1, Gal-10), 4.50 – 4.63 (m, 5H, H-1 x 5, Gal-6, GlcNAc-5, 5’, 5’’, GlcNAc-2), 4.75 (d, *J* = 8.8 Hz, 2H, H-1 x 2, GlcNAc-1, GlcNAc-11), 4.82 (s, 1H, H-1, Man-3, overlap with D_2O), 4.87 (s, 1H, H-1, Man-4’), 5.11 (s, 1H, H-1, Man-4), 5.19 (s, 1H, H-1, GlcNAc-1). Per-OMe MALDI-TOF-MS (*m/z*): [M + Na]$^+$ calcd for $C_{118}H_{208}N_6NaO_{59}$, 2921.4621; found 2921.394.

Glycan **35**



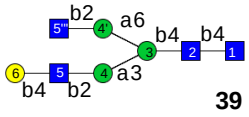
^{1}H NMR (400 MHz, D_2O) δ 1.80 (t, *J* = 12.4 Hz, 1H, H-3_{ax}, Neu5Ac-9), 2.06 – 2.19 (m, 21H), 2.75 (dd, *J* = 12.3, 4.5 Hz, 1H, H-3_{eq}, Neu5Ac-9), 3.42 – 4.07 (m), 4.11 (dd, *J* = 9.8, 2.8 Hz, 1H, H-3, Gal-6), 4.14 - 4.18 (m, 3H), 4.21 (s, 2H), 4.47 (m, 3H, H-1 x 3, Gal-10,-12,-15), 4.50 – 4.63 (m, 5H, H-1 x 5, Gal-6, GlcNAc-5, 5', 5'', GlcNAc-2), 4.71 (d, *J* = 8.8 Hz, 2H, H-1 x 2, GlcNAc-1, GlcNAc-11), 4.82 (s, 1H, H-1, Man-3, overlap with D_2O), 4.87 (s, 1H, H-1, Man-4'), 5.11 (s, 1H, H-1, Man-4), 5.19 (s, 1H, H-1, GlcNAc-1). Per-OMe MALDI-TOF-MS (*m/z*): $[M + Na]^+$ calcd for $C_{147}H_{259}N_7NaO_{74}$, 3329.6617; found 3329.973.

Glycan **8**



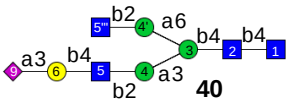
^{1}H NMR (750 MHz, D_2O) δ 1.80 (t, *J* = 12.4 Hz, 3H, H-3_{ax} x 3, Neu5Ac-9,-16,-19), 1.99 – 2.12 (m, 27H), 2.68 (dd, *J* = 12.3, 4.5 Hz, 1H, H-3_{eq} x 3, Neu5Ac-9,-16,-19), 3.54 – 3.92 (m), 3.94 – 4.05 (m, 10H), 4.11 (dd, *J* = 9.8, 2.9 Hz, 3H, H-3, Gal-6,-15,-12), 4.16 (m, 2H), 4.21 (m, 2H), 4.46 (d, *J* = 7.9 Hz, 1H, H-1, Gal-10), 4.43 – 4.56 (m, 7H, H-1 x 7, Gal-6,-15,-12, GlcNAc-5, 5', 5'', GlcNAc-2), 4.69 (d, *J* = 8.5 Hz, 2H, H-1 x 2, GlcNAc-1, GlcNAc-11), 4.75 (s, 1H, H-1, Man-3, overlap with D_2O), 4.88 (s, 1H, H-1, Man-4'), 5.11 (s, 1H, H-1, Man-4), 5.19 (d, *J* = 2.6 Hz, 1H, H-1, GlcNAc-1). Per-OMe MALDI-TOF-MS (*m/z*): $[M + Na]^+$ calcd for $C_{179}H_{313}N_9NaO_{90}$, 4052.0090; found 4052.655.

Glycan **39**



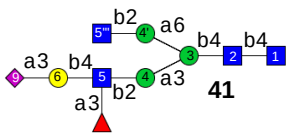
^{1}H NMR (600 MHz, D_2O) δ 1.98 – 2.14 (m, 12H), 3.41 – 4.02 (m), 4.11 (s, 1H, H-2, Man-4'), 4.21 (s, 1H, H-2, Man-4), 4.26 (d, *J* = 2.7 Hz, 1H, H-2, Man-3), 4.47 (d, *J* = 7.8 Hz, 1H, Gal-6), 4.51 – 4.65 (m, 3H, GlcNAc-2,-5,-5'''), 4.70 (d, *J* = 7.5 Hz, 1H, GlcNAc-1), 4.79 (s, 1H, H-1, Man-3, overlap with D_2O), 4.92 (s, 1H, H-1, Man-4'), 5.12 (s, 1H, H-1, Man-4), 5.19 (s, 1H, GlcNAc-1). MALDI-TOF-MS (*m/z*): $[M + Na]^+$ calcd for $C_{56}H_{94}N_4NaO_{41}$1501.5291; found 1501.2272.

Glycan **40**



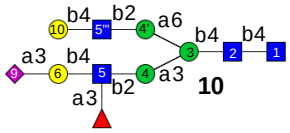
^{1}H NMR (600 MHz, D_2O) δ 1.80 (t, *J* = 12.1, 1H, H-3_{ax}, Neu5Ac-9), 1.95 – 2.22 (m, 15H), 2.76 (dd, *J* = 12.4, 4.6 Hz, 1H, H-3_{eq}, Neu5Ac-9), 4.08 – 4.16 (m, 2H, H-3 [Neu5Ac-9], H-2 [Man-4]), 4.21 (s, 1H, H-2, Man-4), 4.25 (s, 1H, H-2, Man-3), 4.44 – 4.65 (m, 4H, GlcNAc-2,-5,-5'''', Gal-6), 4.70 (d, *J* = 7.5 Hz, 1H, H-1, GlcNAc-1), 4.79 (s, 1H, H-1, Man-3, overlap with D_2O), 4.92 (s, 1H, H-1, Man-4'), 5.12 (s, 1H, H-1, Man-4), 5.19 (s, 1H, GlcNAc-1). Per-OMe MALDI-TOF-MS (*m/z*): $[M + Na]^+$ calcd for $C_{98}H_{173}N_5NaO_{49}$, 2227.1097; found 2226.6748.

Glycan **41**



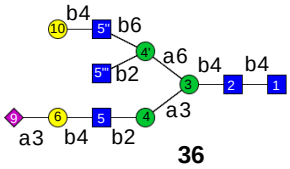
^{1}H NMR (600 MHz, D_2O) δ 1.17 (d, *J* = 6.8 Hz, 3H, H-6, Fuc), 1.80 (t, *J* = 12.1, 1H, H-3$_{ax}$, Neu5Ac-9), 1.95 – 2.22 (m, 15H), 2.76 (dd, *J* = 12.4, 4.6 Hz, 1H, H-3$_{eq}$, Neu5Ac-9), 4.08 – 4.16 (m, 2H, H-3 [Neu5Ac-9], H-2 [Man-4]), 4.21 (s, 1H, H-2, Man-4), 4.25 (s, 1H, H-2, Man-3), 4.52 (d, *J* = 7.9 Hz, 1H, H-1, Gal-6) 4.54 – 4.65 (m, 3H, GlcNAc-2,-5,-5'''), 4.70 (d, *J* = 7.5 Hz, 1H, H-1, GlcNAc-1), 4.79 (s, 1H, H-1, Man-3, overlap with D_2O), 4.92 (s, 1H, H-1, Man-4'), 4.12 (m, 2H, H-1 [Man-4], H-1 [Fuc]), 5.19 (s, 1H, GlcNAc-1). Per-OMe MALDI-TOF-MS (*m/z*): [M + Na]$^+$ calcd for $C_{106}H_{187}N_5NaO_{53}$, 2401.1989; found 2401.0806.

Glycan **10**



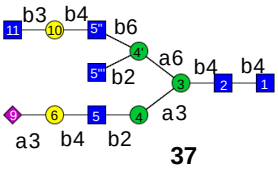
^{1}H NMR (600 MHz, D_2O) δ 1.16 (d, *J* = 6.8 Hz, 3H, H-6, Fuc), 1.79 (t, *J* = 12.1, 1H, H-3$_{ax}$, Neu5Ac-9), 1.90 – 2.06 (m, 15H), 2.76 (dd, *J* = 12.4, 4.6 Hz, 1H, H-3$_{eq}$, Neu5Ac-9), 3.92 – 4.19 (m, 2H, H-3 [Neu5Ac-9], H-2 [Man-4]), 4.11 (s, 1H, H-2, Man-4'), 4.25 (s, 1H, H-2, Man-3), 4.46 (d, *J* = 7.8 Hz, 1H, H-1, Gal-10), 4.51 (d, *J* = 7.9 Hz, 1H, H-1, Gal-6) 4.59 – 4.61 (m, 3H, GlcNAc-2,-5,-5'''), 4.69 (d, *J* = 7.5 Hz, 1H, H-1, GlcNAc-1), 4.76 (s, 1H, H-1, Man-3, overlap with D_2O), 4.93 (s, 1H, H-1, Man-4'), 5.11 (m, 2H, H-1 [Man-4], H-1 [Fuc]), 5.19 (s, 1H, GlcNAc-1). Per-OMe MALDI-TOF-MS (*m/z*): [M + Na]$^+$ calcd for $C_{115}H_{203}N_5NaO_{58}$, 2605.2987; found 2605.3123.

Glycan **36**



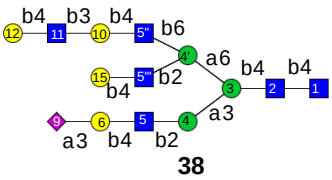
^{1}H NMR (400 MHz, D_2O) δ 1.79 (t, *J* = 12.4 Hz, 1H, H-3$_{ax}$, Neu5Ac-9), 1.98 – 2.10 (m, 18H), 2.75 (dd, *J* = 12.3, 4.5 Hz, 1H, H-3$_{eq}$, Neu5Ac-9), 3.36 – 4.03 (m), 4.06 – 4.13 (m, 2H, H-3 – Gal-6, H-2 – Man-4'), 4.19 (m, 2H, including H-2 – Man-4), 4.25 (s, 1H), 4.47 (d, *J* = 7.6 Hz, 1H, H-1, Gal-10), 4.51 – 4.63 (m, 5H, H-1 x 5, Gal-6, GlcNAc-5, 5''', 5'', GlcNAc-2), 4.69 (d, *J* = 7.9 Hz, 1H, H-1, GlcNAc-1), 4.82 (s, 1H, H-1, Man-3, overlap with D_2O), 4.86 (s, 1H, H-1, Man-4'), 5.12 (s, 1H, H-1, Man-4), 5.19 (s, 1H, H-1, GlcNAc-1). Per-OMe MALDI-TOF-MS (*m/z*): [M + Na]$^+$ calcd for $C_{118}H_{208}N_6NaO_{59}$, 2676.3358; found 2675.799.

Glycan **37**



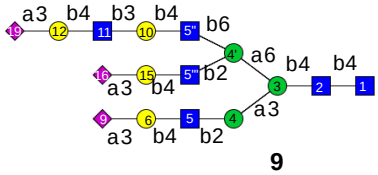
^{1}H NMR (400 MHz, D_2O) δ 1.79 (t, *J* = 12.4 Hz, 1H, H-3$_{ax}$, Neu5Ac-9), 1.98 – 2.10 (m, 18H), 2.75 (dd, *J* = 12.3, 4.5 Hz, 1H, H-3$_{eq}$, Neu5Ac-9), 3.36 – 4.03 (m), 4.08 (m, 1H, H-2, Man-4'), 4.12 (1H, dd, *J* = 2.9, 9.2 Hz, H-3, Gal-6), 4.15 (d, 1H, H-4, Gal-10) 4.17 – 4.23 (m, 2H, including H-2 – Man-4), 4.24 (s, 1H), 4.46 (d, *J* = 7.6 Hz, 1H, H-1, Gal-10), 4.51 – 4.63 (m, 5H, H-1 x 5, Gal-6, GlcNAc-5, 5''', 5'', GlcNAc-2), 4.69 (d, *J* = 8.5 Hz, 1H, H-1, GlcNAc-11, including GlcNAc-1), 4.82 (s, 1H, H-1, Man-3, overlap with D_2O), 4.86 (s, 1H, H-1, Man-4'), 5.12(s, 1H, H-1, Man-4), 5.19 (s, 1H, H-1, GlcNAc-1). Per-OMe MALDI-TOF-MS (*m/z*): [M + Na]$^+$ calcd for $C_{118}H_{208}N_6NaO_{59}$, 2921.4621; found 2921.394.

Glycan **38**



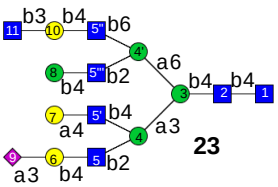
^{1}H NMR (400 MHz, D_2O) δ 1.79 (t, *J* = 12.4 Hz, 1H, H-3$_{ax}$, Neu5Ac-9), 1.98 – 2.10 (m, 18H), 2.75 (dd, *J* = 12.3, 4.5 Hz, 1H, H-3$_{eq}$, Neu5Ac-9), 3.36 – 4.03 (m), 4.08 (m, 1H, H-2, Man-4'), 4.12 (1H, dd, *J* = 2.9, 9.2 Hz, H-3, Gal-6), 4.15 (d, 1H, H-4, Gal-10) 4.17 – 4.23 (m, 2H, including H-2 – Man-4), 4.24 (s, 1H), 4.42 - 4.50 (m, 3H, H-1 x 3, Gal-10,-12,-17), 4.51 – 4.63 (m, 5H, H-1 x 5, Gal-6, GlcNAc-5, 5''', 5'', GlcNAc-2), 4.69 (d, *J* = 8.5 Hz, 1H, H-1, GlcNAc-11, including GlcNAc-1), 4.82 (s, 1H, H-1, Man-3, overlap with D_2O), 4.86 (s, 1H, H-1, Man-4'), 5.12 (s, 1H, H-1, Man-4), 5.19 (s, 1H, H-1, GlcNAc-1). Per-OMe MALDI-TOF-MS (*m/z*): [M + Na]$^+$ calcd for $C_{147}H_{259}N_7NaO_{74}$, 3329.6617; found 3329.526.

Glycan **9**



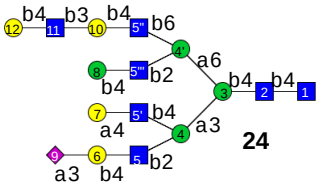
^{1}H NMR (750 MHz, D_2O) δ 1.79 (t, *J* = 12.4 Hz, 1H, H-3$_{ax}$, Neu5Ac-9), 1.98 – 2.10 (m, 27H), 2.75 (dd, *J* = 12.3, 4.5 Hz, 1H, H-3$_{eq}$, Neu5Ac-9), 3.46 – 4.01 (m), 4.08 (s, 1H, H-2, Man-4'), 4.10 (3H, dd, *J* = 2.9, 9.2 Hz, H-3, Gal-6,-12,-17), 4.14 (s, 1H, H-4, Gal-10) 4.17 – 4.23 (m, 2H, including H-2 – Man-4), 4.24 (s, 1H), 4.45 (s, 1H, H-1, Gal-10), 4.51 – 4.61 (m, 7H, H-1 x 5, Gal-6,-12,-17, GlcNAc-5, 5''', 5'', GlcNAc-2), 4.69 (d, *J* = 8.5 Hz, 1H, H-1, GlcNAc-11, including GlcNAc-1), 4.75 (s, 1H, H-1, Man-3, overlap with D_2O), 4.86 (s, 1H, H-1, Man-4'), 5.12 (s, 1H, H-1, Man-4), 5.19 (s, 1H, H-1, GlcNAc-1). Per-OMe MALDI-TOF-MS (*m/z*): [M + Na]$^+$ calcd for $C_{179}H_{313}N_9NaO_{90}$, 4052.0090; found 4052.655. ESI-MS (*m/z*): [M+3H]$^{3-}$1080.3678.

Glycan **23**



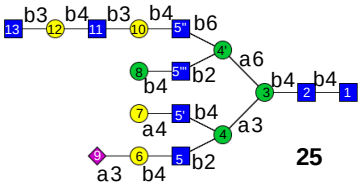
^{1}H NMR (900 MHz, D_2O) δ 1.81 (t, *J* = 12.2, 1H, H-3$_{ax}$, Neu5Ac-9), 1.99 – 2.12 (m, 24H), 2.76 (dd, *J* = 12.4, 4.5 Hz, 1H, H-3$_{eq}$, Neu5Ac-9), 4.03 – 4.06 (m, 2H), 4.07 – 4.10 (m, 2H, including H-2 [Man-4']), 4.12 (dd, *J* = 9.9, 2.8 Hz, 1H, H-3, Gal-6), 4.16 (d, *J* = 3.1 Hz, 1H, H-4, Gal-10), 4.21 (m, 2H, H-2 x 2, Man-4, Man-3), 4.47 (d, *J* = 7.9 Hz, 1H, Gal-10), 4.58 (m, 6H, H-1 x 6, Gal-6, GlcNAc-5 – 5''', -2), 4.69 (d, *J* = 8.1 Hz, 2H, H-1, GlcNAc-1, GlcNAc-11), 4.79 (s, 2H, H-1 x 2, Man-8, Man-3, overlap with D_2O), 4.87 (s, 1H, H-1, Man-4'), 5.12 (s, 1H, H-1, Man-4), 5.19 (d, *J* = 2.7 Hz, 1H, H-1, GlcNAc-1), 5.45 (d, *J* = 2.9 Hz, 1H, H-1, Gal-7). Per-OMe MALDI-TOF-MS (*m/z*): [M + Na]$^+$ calcd for $C_{158}H_{278}N_8NaO_{79}$, 3574.7880; found 3574.7703.

Glycan **24**



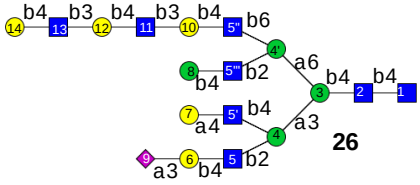
^{1}H NMR (600 MHz, D_2O) δ 1.80 (t, *J* = 12.2, 1H, H-3$_{ax}$, Neu5Ac-9), 1.99 – 2.12 (m, 24H), 2.76 (dd, *J* = 12.4, 4.5 Hz, 1H, H-3$_{eq}$, Neu5Ac-9), 4.03 – 4.06 (m, 2H), 4.07 – 4.10 (m, 2H, including H-2 [Man-4']), 4.12 (dd, *J* = 9.9, 2.8 Hz, 1H, H-3, Gal-6), 4.16 (d, *J* = 3.1 Hz, 1H, H-4, Gal-10), 4.21 (m, 2H, H-2 x 2, Man-4, Man-3), 4.45 – 4.49 (m, 2H, Gal-10, Gal-12), 4.58 (m, 6H, H-1 x 6, Gal-6, GlcNAc-5 – 5''', -2), 4.71 (d, *J* = 8.1 Hz, 2H, H-1, GlcNAc-1, GlcNAc-11), 4.79 (s, 2H, H-1 x 2, Man-8, Man-3, overlap with D_2O), 4.87 (s, 1H, H-1, Man-4'), 5.12 (s, 1H, H-1, Man-4), 5.19 (d, *J* = 2.7 Hz, 1H, H-1, GlcNAc-1), 5.45 (d, *J* = 2.9 Hz, 1H, H-1, Gal-7). Per-OMe MALDI-TOF-MS (*m/z*): [M + Na]$^+$ calcd for $C_{167}H_{294}N_8NaO_{84}$, 3778.8877; found 3778.1443.

Glycan **25**



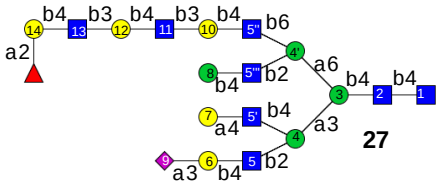
^{1}H NMR (600 MHz, D_2O) δ 1.66 (t, *J* = 12.2, 1H, H-3$_{ax}$, Neu5Ac-9), 1.83 – 1.96 (m, 27H), 2.62 (dd, *J* = 12.4, 4.5 Hz, 1H, H-3$_{eq}$, Neu5Ac-9), 3.87 – 3.90 (m, 2H), 3.91 – 3.94 (m, 2H, including H-2 [Man-4']), 3.98 (d, *J* = 9.9Hz, 1H, H-3, Gal-6), 4.01 (broad s, 2H, H-4 x 2, Gal-10, Gal-12), 4.05 (m, 2H, H-2 x 2, Man-4, Man-3), 4.33 (d, 2H, H-1 x 2, Gal-10, Gal-12), 4.42 (m, 6H, H-1 x 6, Gal-6, GlcNAc-5 – 5''', -2), 4.53 – 4.58 (m, 3H, H-1 x 3, GlcNAc-1, GlcNAc-11,-13), 4.63 (s, 2H, H-1 x 2, Man-8, Man-3, overlap with D_2O), 4.73 (s, 1H, H-1, Man-4'), 4.99 (s, 1H, H-1, Man-4), 5.05 (d, *J* = 2.7 Hz, 1H, H-1, GlcNAc-1), 5.30 (d, *J* = 2.9 Hz, 1H, H-1, Gal-7). Per-OMe MALDI-TOF-MS (*m/z*): [M + Na]$^+$ calcd for $C_{178}H_{313}N_9NaO_{89}$, 4024.0141; found 4025.9487.

Glycan **26**



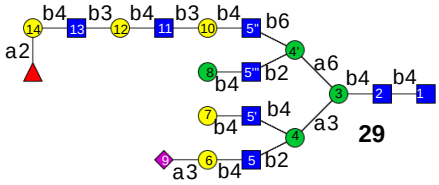
^{1}H NMR (600 MHz, D_2O) δ 1.80 (t, *J* = 12.2, 1H, H-3$_{ax}$, Neu5Ac-9), 1.96 – 2.12 (m, 27H), 2.76 (dd, *J* = 12.4, 4.5 Hz, 1H, H-3$_{eq}$, Neu5Ac-9), 3.50 – 4.02 (m), 4.03 – 4.06 (m, 2H), 4.07 – 4.10 (m, 2H, including H-2 [Man-4']), 4.12 (d, *J* = 9.9Hz, 1H, H-3, Gal-6), 4.17 (broad s, 2H, H-4 x 2, Gal-10, Gal-12), 4.21 (m, 2H, H-2 x 2, Man-4, Man-3), 4.42 - 4.51 (m, 3H, H-1 x 3, Gal-10, Gal-12, Gal-14), 4.58 (m, 6H, H-1 x 6, Gal-6, GlcNAc-5, – 5''', -2), 4.69 – 4.74 (m, 3H, H-1 x 3, GlcNAc-1, GlcNAc-11,-13), 4.79 (s, 2H, H-1 x 2, Man-8, Man-3, overlap with D_2O), 4.87 (s, 1H, H-1, Man-4'), 5.12 (s, 1H, H-1, Man-4), 5.19 (d, *J* = 2.7 Hz, 1H, H-1, GlcNAc-1), 5.44 (d, *J* = 2.9 Hz, 1H, H-1, Gal-7). Per-OMe MALDI-TOF-MS (*m/z*): [M + Na]$^+$ calcd for $C_{187}H_{329}N_9NaO_{94}$, 4228.1138; found 4228.9512.

Glycan **27**



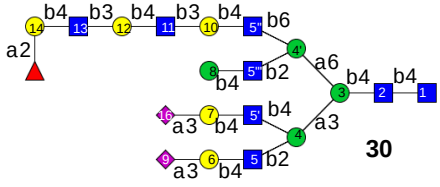
^{1}H NMR (900 MHz, D_2O) δ 1.23 (d, *J* = 6.7 Hz, 3H, H-6, Fuc), 1.89 (t, *J* = 12.2, 1H, H-3$_{ax}$, Neu5Ac-9), 2.10 – 2.18 (m, 27H), 2.85 (dd, *J* = 12.4, 4.5 Hz, 1H, H-3$_{eq}$, Neu5Ac-9), 3.47 – 3.50 (m, 3H), 3.59 – 4.11 (m), 4.03 – 4.04 (m, 2H), 4.07– 4.10 (m, 2H, including H-2 [Man-4']), 4.12 (d, *J* = 9.9Hz, 1H, H-3, Gal-6), 4.17 (broad s, 2H, H-4 x 2, Gal-10, Gal-12), 4.21 (m, 2H, H-2 x 2, Man-4, Man-3), 4.43 - 4.51 (m, 3H, H-1 x 3, Gal-10, Gal-12, Gal-14), 4.58 (m, 6H, H-1 x 6, Gal-6, GlcNAc-5 – 5''', -2), 4.69 – 4.74 (m, 3H, H-1 x 3, GlcNAc-1, GlcNAc-11,-13), 4.79 (s, 2H, H-1 x 2, Man-8, Man-3, overlap with D_2O), 4.87 (s, 1H, H-1, Man-4'), 5.12 (s, 1H, H-1, Man-4), 5.19 (d, *J* = 2.7 Hz, 1H, H-1, GlcNAc-1), 5.40 (d, *J* = 3.2 Hz, 1H, H-1, Fuc), 5.44 (d, *J* = 2.9 Hz, 1H, H-1, Gal-7). Per-OMe MALDI-TOF-MS (*m/z*): $[M + Na]^+$ calcd for $C_{195}H_{343}N_9NaO_{98}$, 4402.2031; found 4400.805.

Glycan **29**



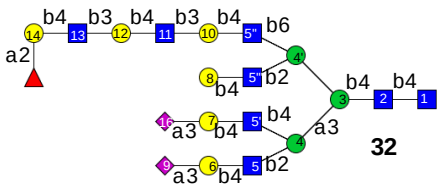
^{1}H NMR (400 MHz, D_2O) δ 1.23 (d, *J* = 6.7 Hz, 3H, H-6, Fuc), 1.81 (t, *J* = 12.2, 1H, H-3$_{ax}$, Neu5Ac-9), 1.97 – 2.17 (m, 27H), 2.76 (dd, *J* = 12.4, 4.5 Hz, 1H, H-3$_{eq}$, Neu5Ac-9), 3.36 – 4.06 (m), 4.02 – 4.06 (m, 2H), 4.06– 4.09 (m, 2H, including H-2 [Man-4']), 4.10 (d, *J* = 9.9Hz, 1H, H-3, Gal-6), 4.16 (broad s, 2H, H-4 x 2, Gal-10, Gal-12), 4.23 (broad d, 2H, H-2 x 2, Man-4, Man-3), 4.43 - 4.51 (m, 3H, H-1 x 3, Gal-10, Gal-12, Gal-15), 4.52 – 4.66 (m, 7H, H-1 x 6, Fuc, Gal-6, GlcNAc-5 – 5''', -2), 4.72 (d, 3H, H-1 x 3, GlcNAc-1, GlcNAc-11,-13), 4.78 (s, 2H, H-1 x 2, Man-8, Man-3, overlap with D_2O), 4.87 (s, 1H, H-1, Man-4'), 5.13 (s, 1H, H-1, Man-4), 5.19 (s, 1H, H-1, GlcNAc-1), 5.32 (s, 1H, H-1, Fuc). Per-OMe MALDI-TOF-MS (*m/z*): $[M + Na]^+$ calcd for $C_{195}H_{343}N_9NaO_{98}$, 4402.2031; found 4402.476.

Glycan **30**



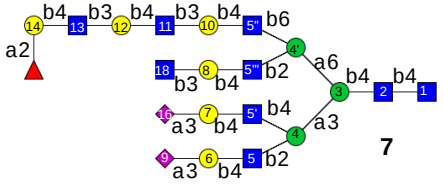
^{1}H NMR (400 MHz, D_2O) δ 1.23 (d, *J* = 6.7 Hz, 3H, H-6, Fuc), 1.81 (t, *J* = 12.2, 2H, H-3$_{ax}$ x 2, Neu5Ac-9, -16), 2.01 – 2.12 (m, 30H), 2.76 (dd, *J* = 12.4, 4.5 Hz, 2H, H-3$_{eq}$ x 2, Neu5Ac-9, -16), 3.36 – 4.06 (m), 4.02 – 4.05 (m, 2H), 4.06 – 4.09 (m, 2H, including H-2 [Man-4']), 4.10 (d, *J* = 9.9Hz, 2H, H-3 x 2, Gal-6, -15), 4.16 (broad s, 2H, H-4 x 2, Gal-10, Gal-12), 4.22 (broad d, 2H, H-2 x 2, Man-4, Man-3), 4.47 (d, 2H, H-1 x 2, Gal-10, Gal-12), 4.52 – 4.65 (m, 7H, H-1 x 6, Fuc, Gal-6, -15, GlcNAc-5 – 5''', -2), 4.71 (d, 3H, H-1 x 3, GlcNAc-1, GlcNAc-11,-13), 4.78 (s, 2H, H-1 x 2, Man-8, Man-3, overlap with D_2O), 4.87 (s, 1H, H-1, Man-4'), 5.13 (s, 1H, H-1, Man-4), 5.19 (s, 1H, H-1, GlcNAc-1), 5.31 (s, 1H, H-1, Fuc). Per-OMe MALDI-TOF-MS (*m/z*): $[M + Na]^+$ calcd for $C_{211}H_{371}N_{10}NaO_{106}$, 4764.3845; found 4763.771.

Glycan **32**



^{1}H NMR (750 MHz, D_2O) δ 1.23 (d, *J* = 6.7 Hz, 3H, H-6, Fuc), 1.81 (t, *J* = 12.2, 2H, H-3_{ax} x 2, Neu5Ac-9, -16), 2.01– 2.11 (m, 30H), 2.76 (dd, *J* = 12.4, 4.5 Hz, 2H, H-3_{eq} x 2, Neu5Ac-9, -16), 3.30 – 3.99 (m), 3.96 – 4.06 (m, 2H), 4.00 – 4.03 (m, 2H, including H-2 [Man-4']), 4.12 (d, *J* = 9.9 Hz, 2H, H-3 x 2, Gal-6, -15), 4.14 – 4.18 (broad m, 2H, H-4 x 2, Gal-10, Gal-12), 4.19 – 4.25 (broad d, 3H, H-2 x 2, Man-4, Man-3), 4.47 (d, 3H, H-1 x 3, Gal-10, Gal-12, Gal-17), 4.52 – 4.62 (m, 7H, H-1 x 6, Fuc, Gal-6, -15, GlcNAc-5 – 5''', -2), 4.70 (d, 3H, H-1 x 3, GlcNAc-1, GlcNAc-11,-13), 4.76 (s, 1H, H-1, Man-3, overlap with D_2O), 4.87 (s, 1H, H-1, Man-4'), 5.13 (s, 1H, H-1, Man-4), 5.19 (s, 1H, H-1, GlcNAc-1), 5.31 (s, 1H, H-1, Fuc). 4559.680 (Glycan without *β*-Man). Per-OMe MALDI-TOF-MS (*m/z*): $[M + Na]^+$ calcd for $C_{211}H_{371}N_{10}NaO_{106}$, 4764.3845; found 4765.438 (Glycan with *β*-Gal).

Glycan **7**



^{1}H NMR (750 MHz, D_2O) δ 1.23 (d, *J* = 6.7 Hz, 3H, H-6, Fuc), 1.75 (t, *J* = 12.2, 2H, H-3_{ax} x 2, Neu5Ac-9, -16), 1.93 – 2.03 (m, 30H), 2.68 (dd, *J* = 12.4, 4.5 Hz, 2H, H-3_{eq} x 2, Neu5Ac-9, -16), 3.30 – 3.99 (m), 4.11 (s, 1H, H-2 [Man-4']), 4.14 (d, *J* = 9.9 Hz, 2H, H-3 x 2, Gal-6, -14), 4.16 – 4.17 (broad m, 3H, H-4 x 3, Gal-10, Gal-12, Gal-17), 4.19 – 4.25 (broad d, 3H, including H-2 x 2, Man-4, Man-3), 4.48 (m, 3H, H-1 x 3, Gal-10, Gal-12, Gal-17), 4.54 – 4.64 (m, 7H, H-1 x 6, Gal-6, -15, GlcNAc-5 – 5''', -2), 4.71 – 4.75 (m, 4H, H-1 x 3, GlcNAc-1β, GlcNAc-11,-13,-18), 4.76 (s, 1H, H-1, Man-3, overlap with D_2O), 4.93 (s, 1H, H-1, Man-4'), 5.11 (s, 1H, H-1, Man-4), 5.19 (d, *J* = 2.5 Hz, 1H, H-1, GlcNAc-1), 5.23 (s, 1H, H-1, Fuc). ESI-MS (*m/z*): $[M+2H]^{2-}$ calcd for $C_{154}H_{253}N_{11}O_{111}$ 2015.2173; found 2015.2086; $[M+3H]^{3-}$ 1343.4000.

HPLC-HRMS and CE-HRMS Data of the Final *N*-Glycans

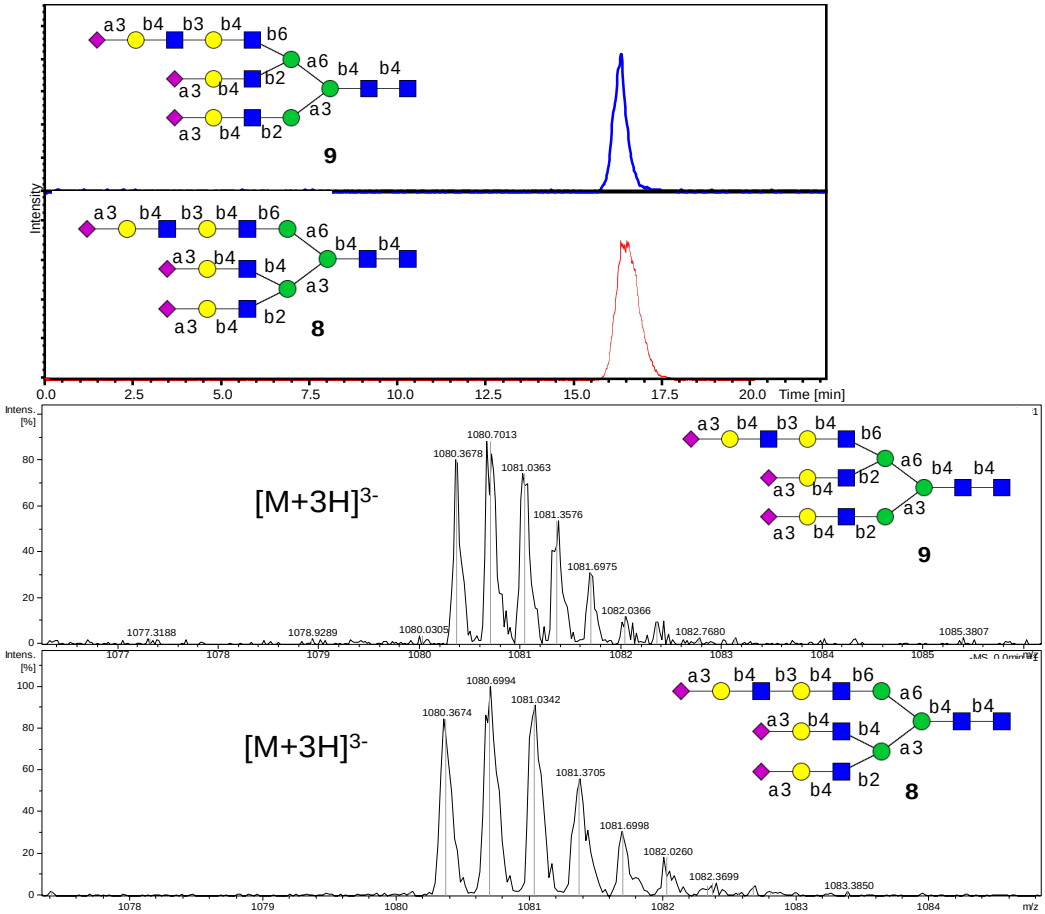


Figure S8. High resolution CE-MS chromatogram of **8** and **9** with sheath liquid (CH_3OH:10 mM NH_4OH); negative mode, capillary length = 90 cm, 80 mM NH_4OAc, pH±9.3, 30 kV.

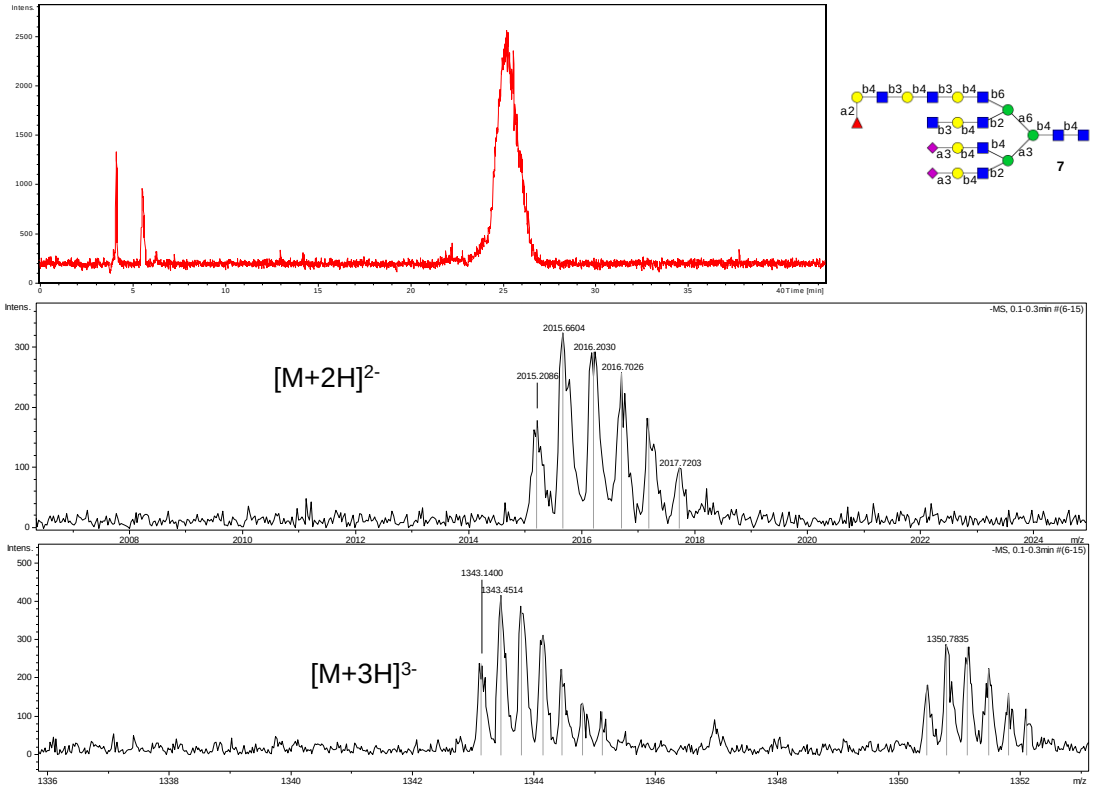


Figure S9. High resolution HILIC-MS chromatogram of **7** (isocratic [55:45 - CH_3CN:100 mM NH_4COOH pH±3.4]); mass spectrometry data were acquired in the negative mode.

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Chapter 3

Protecting Group-Controlled Enzymatic Glycosylation of Oligo-N-Acetyllactosamine Derivatives

Introduction

Fucosylated glycans mediate a wide range of physiological and disease processes such as leukocyte adhesion during inflammation,[1] fertilization,[2] tissue development[3] and tumor metastasis.[4] These structures are also employed by pathogens for infection, and for example, *Helicobacter pylori* adheres to the human gastric mucosa by binding to sialyl-di-Lewis^x (SLe^{x}Le^x) containing glycolipids.[5] The latter glycoconjugates are also expressed during intestinal inflammation, which may contribute to virulence and the chronic nature of these infections. Pathogens including *Helicobacter pylori*,[6] *Haemophilus influenzae* and parasitic worms such as *Schistosoma mansoni*[7] also express fucosylated glycans such as Lewis^x, which can modulate immune responses by preventing the induction of antibodies directed to the epitopes shared by the microorganism, or by interacting with host glycan binding proteins such as DC-SIGN to induce immune-modulatory responses.[8]

In humans, the structural diversity of fucosylated glycans arises from thirteen fucosyltransferases that possess unique tissue distributions and acceptor preferences. Six 1,3/4-fucosyltransferases (FUT3-7 and 9) catalyze the fucosylation of *N*-acetylglucosamine (GlcNAc) moieties of *N*-acetyllactosamine (LacNAc) residues, and in concert with 1,2-fucosyltransferases (FUT1 and FUT2) and various sialyltransferases create the ABO blood groups and Lewis antigens.[3b] The 1,3/4-fucosyltransferases exhibit different acceptor selectivities. For example, FUT5 preferentially transfers fucosyl residues to internal GlcNAc moieties of polylactosamine chains to create antigens such as VIM-2,[9] whereas FUT6 preferentially modifies distal GlcNAc residues of neutral and sialylated polylactosamine structures resulting in the formation of Lewis^x (Le^x) and sialyl-Le^x epitopes (SLe^x).[10] FUT7[11] modifies only sialylated acceptors whereas FUT9[12] is selective for neutral glycans but will also modify sialylated acceptors.

Due to their involvement in plethora of biological and disease processes, the chemical and enzymatic synthesis of Lewis and blood group antigens has received considerable attention,[13] and the availability of well-defined compounds has made it possible to examine at a molecular level how these antigens are recognized by lectins, antibodies and glycan binding proteins. It has also made it possible to design inhibitors,[14] which for example target E-selectin (ICAM-1) by mimicking its natural SLe^x ligand, proving attractive compounds for the treatment of sickle cell anemia and cancer. The preparation of complex fucosylated poly-*N*-acetyllactosamine derivatives has, however, received little attention,[15] and in particular there are no general solutions to selective incorporation of fucosides and other functionalities into these backbones. Such epitopes have, however, been implicated in important biological processes. For example, VIM-2 is a sialylated glycosphingolipid that occurs on human neutrophils and is believed to be a functional receptor for E-selectin.[16] Studies with isolated glycolipids indicate that the pattern of fucosylation of the poly-LacNAc backbone of VIM-2 is critical for binding. Furthermore, the epitope SLe^xLe^x has been found on cancer cells and may serve as a cancer biomarker.[17] It is also upregulated during inflammation and can serve as a ligand for *Helicobacter pylori*. Recently, SLe^xLe^x was found on the extracellular matrix of human oocytes (zona pellucida)[2] and may serve as a ligand for human sperm binding.[18]

Here, we report a chemoenzymatic "stop and go" strategy[19] that can give easy access to a library of differentially fucosylated and sialylated oligo-LacNAc derivatives from a single precursor. It is based on the finding that lactosamine derivatives, having a free amine or the amino function modified by a tert-butyloxycarbonyl (Boc) protecting group, are resistant to fucosylation by recombinant FUT5 and Hp39-FT.[20] These lactosamine derivatives can, however, easily be converted into their natural counterpart (Figure 1A). To exploit this observation, we developed an efficient synthetic route for hexasaccharide **1** in which the amines of the LacNAc repeating units are uniquely masked as a free amine, trifluoracetyl (TFA) and Boc (Figure 1B). It has been shown that through simple manipulations, this common precursor can be converted into hexasaccharides **2-7** in which the GlcN moieties are differentially modified as free amines or Boc groups thereby temporarily blocking specific lactosamine moieties from enzymatic modification (Figure 1C). As anticipated, mono- and bis-fucosylated derivatives **8a-13a** were readily obtained by treatment of compounds **2-7** with either FUT5 or Hp39-FT in the presence GDP-fucose followed by removal of Boc and acetylation of the free amines (Figure 1D). These compounds could be sialylated by ST3Gal4[21] to give sialoglycans **8b-13b**, and thus from a single precursor 12 different derivatives were obtained. The compounds were printed as glycan microarrays and examined for binding to a number of lectins, glycan binding proteins including E-selectin and DC-SIGN and several IAV HAs. The data indicate that the pattern of fucosylation can modulate the interaction with glycan binding proteins.

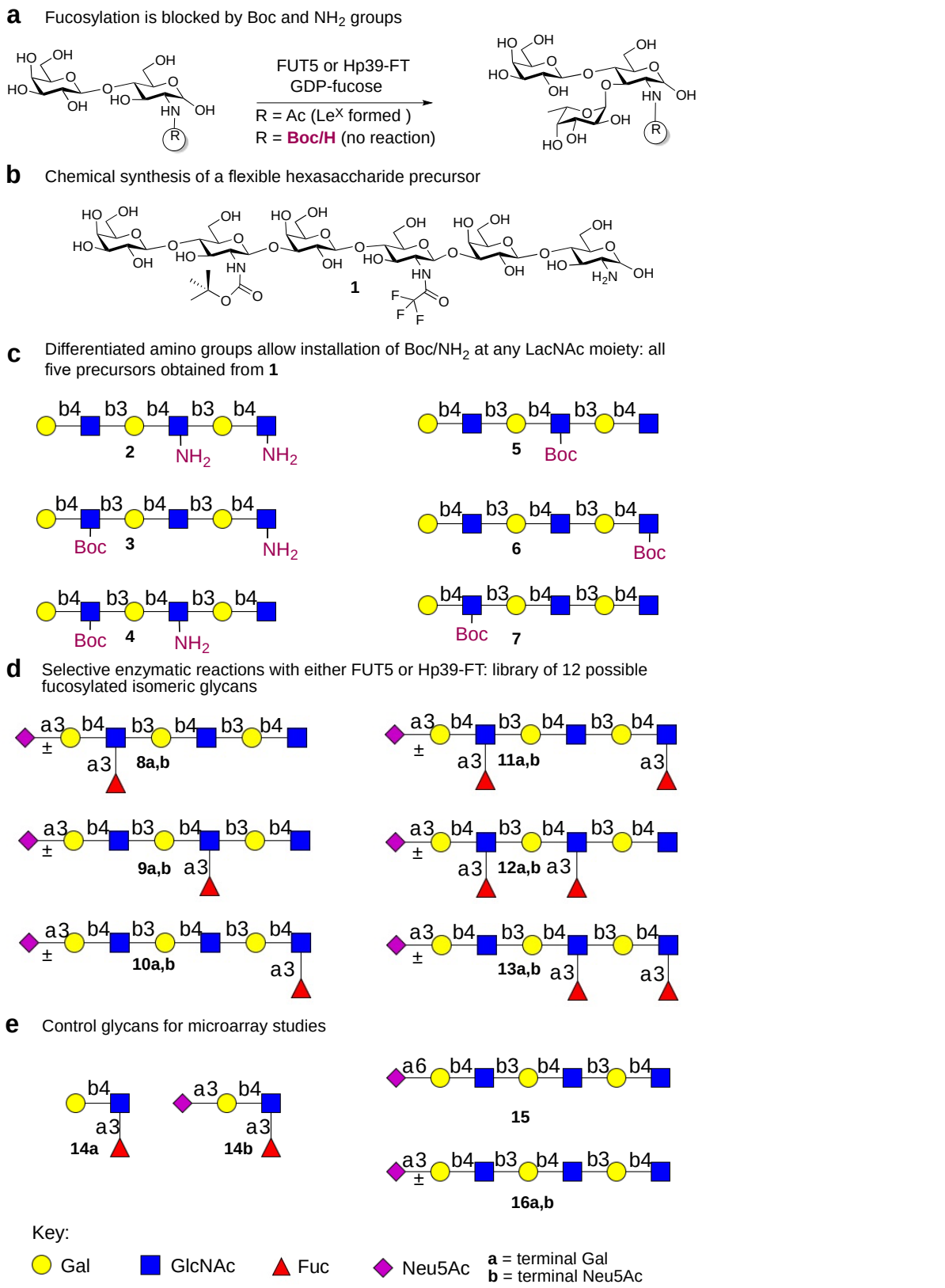


Figure 1. Synthetic hexasaccharide **1** was used to create a library of 12 differentially fucosylated and sialylated derivatives.

Results and Discussion

Modulation fucosyltransferase activity. To implement a "stop and go" strategy for the selective enzymatic modification of oligo-LacNAc derivatives, we explored a range of chemical entities to temporary block fucosylation. For this purpose, a range of lactosamine derivatives were prepared in which the amine was modified as either NHAc (**17**), NHTFA (**18**), NHCbz (**19**), NHBsmoc (**20**),[22] or NHBoc (**21**) (Figure 2). The model substrates were exposed to the microbial fucosyl transferase Hp39-FT in the presence of GDP-fucose, and the conversion to the corresponding Le^x containing oligosaccharides **23** – **27** was monitored by capillary electrophoresis – mass spectrometry (CE-MS).[23] Ammonium borate was used as an additive to assist separation of the neutral molecules. It was found that LacNAc **17** and LacNHTFA **18** were readily converted into the Le^x containing products **23** and **24**. On the other hand, the presence of the Cbz- and Bsmoc- protecting groups of **19** and **20,** respectively, considerably slowed down the fucosylation and only partial product formation was observed. Strikingly, no conversion to Le^x was observed for LacNHBoc **21** and $LacNH_2$ **22**. Similar results were obtained when FUT5 was employed. These findings indicate that selective incorporation of either Boc or NH_2 groups into poly-LacNAc chains should result in site-specific fucosylation.

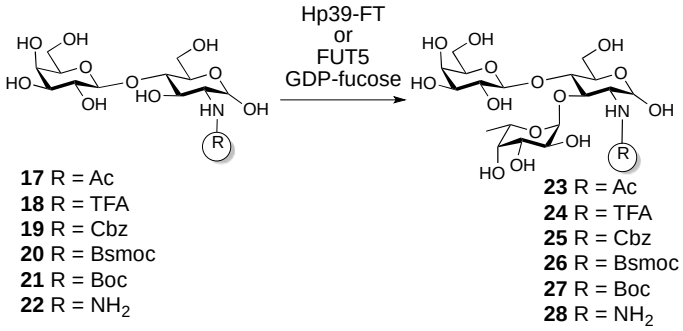


Figure 2. Set of model disaccharides used to identify protecting groups that can temporarily blocking fucosylation by Hp39-FT or FUT5.

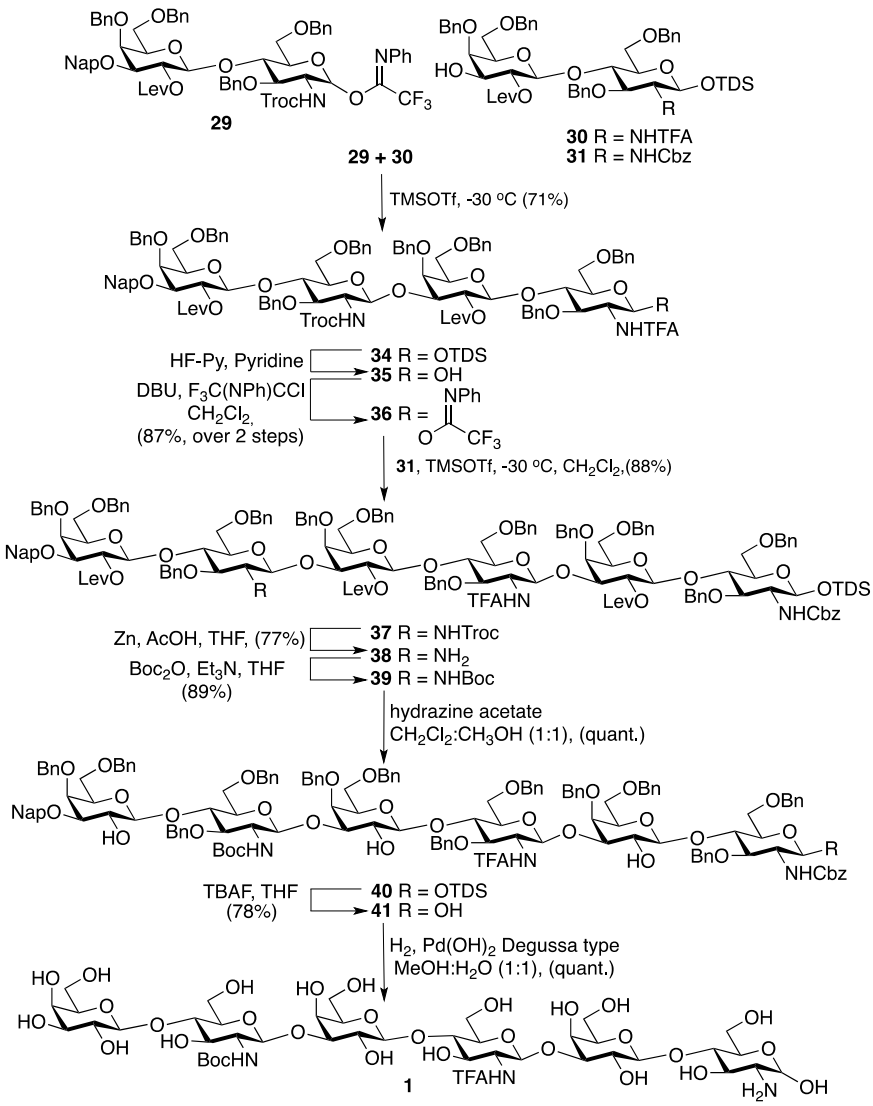
Chemical synthesis of a universal precursor. The next challenge was the preparation of a universal oligo-LacNAc derivative that can give easy access to compounds having different patterns of NHBoc or NH_2 to block enzymatic fucosylation of specific LacNAc units. It was envisaged that hexasaccharide **1** would serve this purpose. In this respect, the $LacNH_2$ unit can either be acetylated ("on" state) or modified as a benzyloxycarbonyl moiety which temporary blocks this site but at a late stage in the synthesis can be converted to a free amine to block fucosylation ("off" state). The LacNTFA containing residue can either be converted to a free amine by base treatment ("off"), which when desired can be acetylated to give a LacNAc residue ("on"). The Boc group can either be maintained ("off") or removed and then acetylated resulting in the formation of a LacNAc residue ("on").

Target hexasacharide **1** was assembled from building blocks **29**-**31** to provide hexasaccharide **37** in which the amino functions of the LacNAc moieties are protected as trifluoroacetamido (TFA), 2,2,2-trichloroethoxycarbonyl (Troc) and benzyloxycarbonyl (Cbz) (Scheme 1). The Troc protecting group of this compound can selectively be removed by treatment with Zn[24] to give amine **38** that can then be modified as Boc to provide hexasaccharide **39**, which after hydrogenation would yield key intermediate **1**. Installation of Boc at a late stage of the synthesis was important because glycosyl donors having this function at C-2 are prone to oxazolidinone formation resulting in low yields of product.

Building blocks **29**-**31** were prepared from of a common disaccharide in which the amine was masked as an azide, while the anomeric center and the C-3' hydroxyl were temporary protected as TDS[25] and Nap ether, respectively (see ESI, Scheme S1) Trimethylsilyl

trifluoromethanesulfonate (TMSOTf)-catalyzed glycosylation of imidate **29** with glycosyl acceptor **30** furnished, after purification by silica gel column chromatography, tetrasaccharide **34** in 71% yield. Subsequent HF/pyridine-mediated cleavage of the anomeric TDS group gave lactol **35**, which was treated with 2,2,2-trifluoro-*N*-phenylacetimidoyl chloride in the presence of DBU to give donor **36** in 87% yield over two steps. Glycosylation of **36** with a slight excess of acceptor **31** in the presence of TMSOTf provided the hexasaccharide **37** in a yield of 88%. The robustness of the synthetic approach was demonstrated by synthesizing the hexasaccharide in gram quantities.

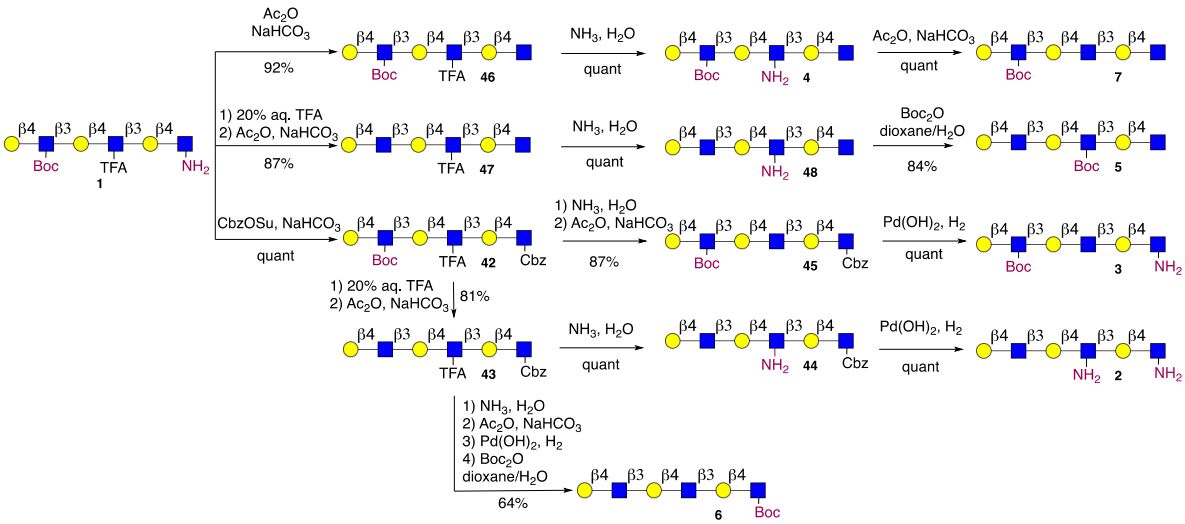
Intermediate **37** was treated with Zn dust in the presence of acetic acid in THF to afford free amine **38** (77%), which was reacted with Boc_2O to yield the key hexasaccharide **39** (Scheme 1). With this material in hand, an orchestrated sequence of deprotection steps was performed. Thus, the Lev esters of **39** were hydrolyzed by treatment with excess of hydrazine acetate in a mixture of CH_2Cl_2 and CH_3OH resulting in the formation of triol **40**, which was treated with TBAF to cleave the anomeric TDS group to provide intermediate **41** in a yield of 78%. Finally, the benzyl ethers, the Nap ether, and Cbz carbamate were simultaneously removed by catalytic hydrogenation to give the target compound **1** in quantitative yield. This efficient one-step global deprotection was achieved by using a Degussa-type $Pd(OH)_2$ catalyst, and under the mild reaction conditions the TFA and Boc groups remained intact.



Scheme 1. Chemical synthesis of required building blocks.

Further diversification of common precursor 1. Having prepared a sufficient quantity of **1**, the stage was set to synthesize compounds **2-7** (Scheme 2) having different arrangements of Boc/NH_2/NHAc groups. It was expected that lactosamine moieties modified by Boc or NH_2 would be resistant, whereas those containing a natural acetamido function would readily be modified by an α(1,3)-fucosyltransferase (Scheme 2). Each of the three amino moieties of **1** can be uniquely manipulated because the TFA group can be cleaved under mild basic conditions (dilute aqueous ammonia) while the Boc group is removable with dilute aqueous trifluoroacetic acid (20%). The Cbz group is stable to both basic and acidic conditions but can readily be cleaved by catalytic hydrogenation over Pd/C without affecting the TFA and Boc protecting groups. Thus, compound **1** was expected to be an appropriate precursor for the preparation of compounds **2-7**.

In a first sequence of reactions, the free amine of key intermediate **1** was acetylated with acetic anhydride in the presence of $NaHCO_3$ to give **46**, which was treated with dilute aqueous ammonia to remove the TFA protecting group providing target compound **4** having a free amine and Boc group at the central and distal lactosamine moieties, respectively. Acetylation of the free amine of **4** gave the second target compound **7**. Alternatively, exposure of **1** to aqueous TFA to remove the Boc group followed acetylation of the resulting amine gave **47**, which was subsequently treated with aqueous ammonia (→**48**) and Boc anhydride to provide **5**. In another sequence of reactions, the amine of compound **1** was protected as a Cbz carbamate by reaction with CbzOSu to give **42**, which was subsequently treated with aqueous ammonia and acetic anhydride to convert the trifluoracetamido into an acetamido moiety providing compound **45**, which was subjected to catalytic hydrogenation over $Pd(OH)_2$ to remove the Cbz protecting group resulting in the formation of compound **3**. Derivative **2**, having free amines at the proximal and distal LacNAc moieties, was also prepared starting from **42** by treatment with 20% trifluoracetic acid followed by acetylation of the resulting amine with acetic anhydride to convert Boc into acetamido (→**43**), which was followed by hydrolysis of the TFA protecting group with ammonia and cleavage of the Cbz moiety by catalytic hydrogenation. Finally, hexasaccharide **6**, having a blocking Boc moiety at the proximal LacNAc, was obtained by conversion of TFA into acetamido, while transforming Cbz into Boc by standard manipulations in four steps in 64% overall yield from intermediate **43**.

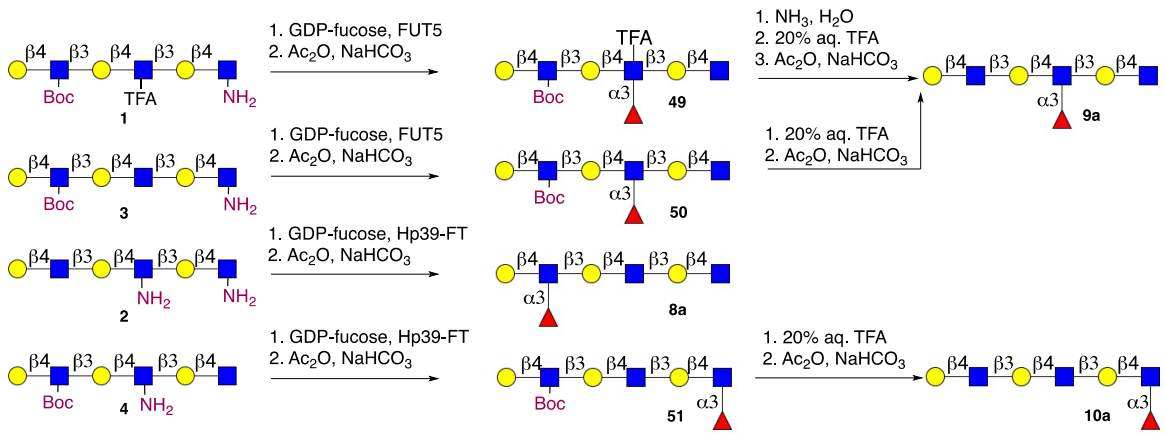


Scheme 2. Diversification of common intermediate **1** into substrates **2-7**.

Next, attention was focused on the selective fucosylation of compounds **2-7** to give mono-fucosylated derivatives **8a-10a** (Scheme 3), as well as the di-fucosylated products **11a – 13a** respectively (Scheme 4). For this purpose, we selected the mammalian fucosyl transferase FUT5, which preferentially forms internal Le^x moieties and Hp39-FT that favors fucosylation

of terminal LacNAc acceptors. Gratifyingly, treatment of **1** with FUT5 in presence of GDP-fucose followed by *N*-acetylation of the free amine gave the expected mono-fucosylated heptasaccharide **49** in a yield of 88% after purification by HPLC using a semi-preparative HILIC column (Scheme 3). Full assignment of ^{1}H NMR spectra (750 MHz) combined with ^{1}H-^{1}H COSY, ^{1}H-^{1}H TOCSY and ^{1}H-^{13}C HSCQC experiments confirmed the position of the fucoside of **49**. Having both the TFA and the Boc group in **49** assists in identification of otherwise overlapping GlcNAc H-2 signals, which proved to be useful in compound characterization. In this instance, a downfield shift of H-2 of GlcNHTFA from 3.90 to 4.09 ppm (t, J = 9.7 Hz), along with the appearance of one anomeric fucoside at 5.04 ppm (d, J = 4.1 Hz) confirmed the presence of the fucoside at the central lactosamine moiety. In this respect, it is known that α(1,3)-fucosylation of LacNAc is accompanied by a downfield shift of H-2 of GlcNAc along with an upfield shift of Gal H-4.[15, 26] The H-2 signal of GlcNHBoc and GlcNHAc of **49** were unchanged (3.48 ppm, dd, J = 10.3, 8.3 Hz) providing further support of the site of fucosylation. A small amount (10%) of a regioisomer was formed in which the reducing end was fucosylated (see SI for full compound characterization). After *N*-acetylation, this by-product could readily by removed by HPLC. The required compound **9a** was obtained by cleavage of the TFA and Boc blocking groups of **49** using standard procedures followed by acetylation of the resulting free amines. A similar regioselectivity was observed when **3** was subjected to FUT5 to give, after *N*-acetylation, compound **50** which by a simple two-step procedure could also be transformed into **9a**.

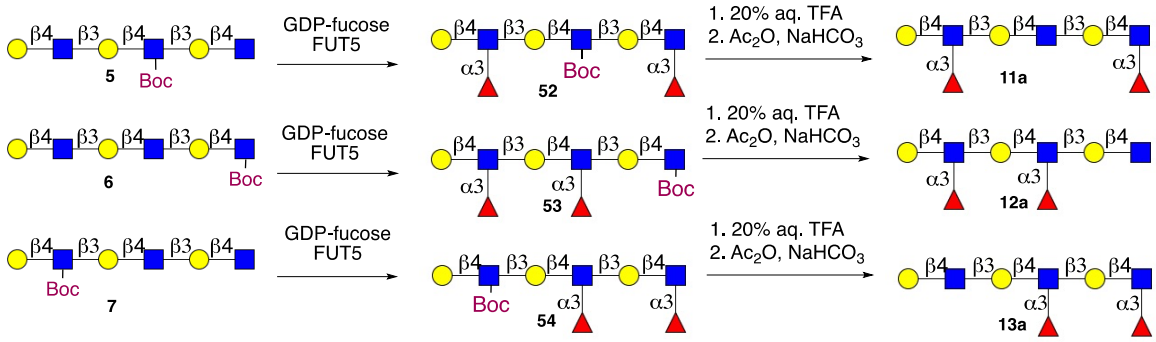
Treatment of hexsaccharide **2** with Hp39-FT and GDP-fucose followed by acetylation of the two free amines resulted in the exclusive formation of **8a**, which was isolated in yield of 73% after purification by HPLC using a HILIC column. NMR analysis established that the fucoside was located at the distal LacNAc moiety (see SI). Similarly, fucosylation of **4** with Hp39-FT followed by *N*-acetylation resulted in the clean formation of mono-fucosylated glycan **51**, which was isolated in 84% yield. The latter derivative was converted into **10a** by removal of Boc with 20% aqueous trifluoracetic acid followed by acetylation of the amines with acetic anhydride in the presence of $NaHCO_3$. Importantly, these conditions were sufficiently mild that the sensitive fucosides were unaffected.



Scheme 3. Selective enzymatic mono-fucosylation.

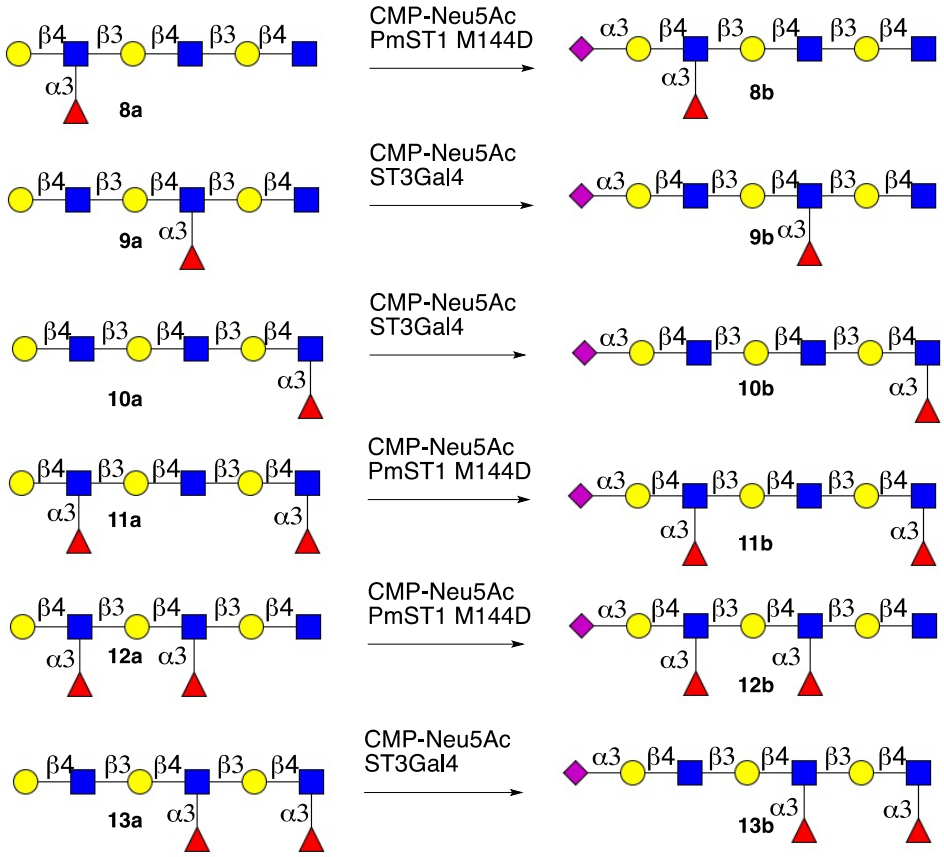
Having successfully prepared all possible mono-fucosylated regio-isomers, attention was focused on selective di-fucosylation. Thus, exposure of **5** to FUT5 and GDP-fucose resulted in the formation of di-Le^{x}-containing derivative **52** as the only product, which was isolated in a yield of 90% (Scheme 4). NMR analysis indicated that H-2 of GlcNHBoc in **52** had remained unchanged (3.48 ppm, t, J = 9.6 Hz), whereas the H-2 signals of the GlcNAc moieties had significantly shifted downfield. In addition, an upfield shift of the H-1α of GlcNAc as well as the appearance of another doublet at 5.13 ppm (J = 4.2 Hz) corresponding to non-reducing end fucoside confirmed the structure of the compound. The di-fucosylated

positional isomers **53** and **54** were obtained by similar procedures starting from glycans **6** and **7**, respectively (see SI for compound characterization). Compounds **52**, **53**, and **54** were converted into **11a**, **12a**, and **13a** by removal of the Boc group followed by acetylation of the amines using standard procedures (Scheme 4).



Scheme 4. Selective enzymatic di-fucosylation followed by removal of Boc groups.

Sialylation of compounds **9a**, **10a**, and **13a** which have a terminal LacNAc moiety was accomplished by the mammalian sialyltransferase ST3Gal4 in the presence of CMP-Neu5Ac to give compounds **9b**, **10b**, and **13b**, respectively (Scheme 5). On the other hand, sialylation of glycans **8a**, **11a**, and **12a** bearing a terminal Le^x moiety was achieved by using the mutant bacterial sialyl transferase PmST1 M144D.[27] We also discovered that these glycans could be sialylated with ST3Gal4, although prolonged treatment (3 days) and large amounts of enzyme were required.



Scheme 5. Sialylation of compounds **8a-13a** by either ST3Gal4 or PmST1 M144D to give the sialosides **9b-13b**, respectively.

Glycan microarray studies. Compounds **8a**,**b**-**13a**,**b** (Fig. 1d) and control derivatives **14a**,**b**, **15**, and **16a**,**b** (Fig. 1e) were printed as glycan micro-arrays to examine whether the pattern of fucosylation of oligo-LacNAc derivatives can modulate binding of lectins. For this purpose, the compounds were modified with a 2-[(methylamino)oxy]ethanamine linker,[28] and the resulting derivatives printed on *N*-hydroxysuccinimide (NHS)-activated glass slides in replicates of 6 (100 µM in sodium phosphate (250 mM, pH 8.5) buffer). After overnight incubation in a saturated NaCl chamber, unreacted esters were quenched by the addition of ethanolamine (50 mM in Tris (100 mM, pH 9.0) buffer).

Sub-arrays were exposed to a range of plant lectins including *Aleuria aurantia* (AAL), *Erythrina cristagalli* (ECL), *Maackia amurensis* Ⅱ (MAL-II), wheat germ agglutinin (WGA) and *Sambucus nigra* agglutinin (SNA), the mammalian glycan binding proteins E-selectin and DC-SIGN and several recombinant HAs of IAVs. Detection of binding was accomplished by using AlexaFluor635 (lectins, E-selectin, DC-SIGN) or 647 (HAs). To analyze the data, the compounds were organized according to increasing numbers of fucosides.

The plant lectin AAL is known to bind α1,2-, α1,3-, and α1-6-fucosides. Our array screen (Fig. 3a) indicate, however, that the position of an α1,3-fucoside within an oligo-LacNAc chain is important for recognition, and only terminal Lewis^x (Le^x) and sialyl Lewis^x (SLe^x) containing compounds showed good responsiveness (**8a**,**b**, **11a**,**b**, **12a**,**b**, **14a**,**b**). The location of a fucoside is also important for the recognition of ECL, which is a lectin that recognizes terminal galactose/*N*-acetylgalactosamine residues. As expected, only glycans having a terminal Gal moiety (**9a**, **10a**, **13a**, **16a**) where bound by ECL (Fig. 3b). Fucosylation of Gal at the non-reducing terminus that creates the terminal Le^x (**8a**) antigen, however, abolished binding whereas the presence of a fucoside at the central and the non-reducing LacNAc moiety were tolerated. MAL-II is known to bind 2,3-sialylated glycans and in this case a fucoside at the central and reducing LacNAc moiety (**9b**, **10b**, **13b**) are tolerated, whereas this is not the case for terminal fucosylation that creates the SLe^x epitope (Fig. 3c). WGA, which is a lectin that binds GlcNAc moieties, demonstrated a remarkable recognition profile, as the major recognition site was present at the reducing end (Fig. 3d). This was apparent as only compounds that have an unmodified GlcNAc moiety at the reducing end (**8a**,**b**, **9a**,**b**, **12a**,**b**, **15**, **16a**,**b**) exhibited responsiveness. Fucosylation at the central and terminal LacNAc moiety had no impact on WGA binding. As expected, SNA only showed responsiveness to glycan **15** which is modified by a 2,6-sialoside (Fig. S2).

Next, we examined binding properties of E-selectin, which is an inflammatory protein that recognized fucosylated glycans on leukocytes, thereby initiating rolling and tethering of these cells to sites of inflammation. There is conflicting data regarding the ligand requirements of E-selectin.[29] On the one hand, SLe^x is a well-recognized ligand for E-selectin. On the other hand, it has been proposed that VIM-2, which is a sialylated glycosphingolipid expressed on human neutrophils containing an internal fucoside is the functional E-selectin receptor.[16b, 29] Studies with isolated compounds have indicated that positional isomers of VIM-2 are poorer receptors including SLe^x containing glycans. In our microarray screen, only compounds containing a SLe^X moiety (**8b, 11b**, **12b**) were recognized by E-selectin (Fig. 3e). Internally fucosylated motifs such as **9b** (related to VIM-2), **10b**, and **13b** showed no detectable binding. Thus, the E-selectin appears to bind only to SLe^x containing compounds, yet it cannot be excluded that additional functionalities and molecular environment could enhance binding affinities of compounds such as VIM-2, and for example the presence of a ceramide may be important receptor determinant.

DC-SIGN, which also belongs to the family of C-type lectins, is a protein expressed by dendritic cells that plays an important role in pathogen detection and innate immunity.[30] It has been proposed that this protein has a preference for Le^x moieties of *N*-and *O*-linked glycans. Our results demonstrate (Fig. 3f) that glycans bearing terminal Le^x (**8a, 11a, 12a,**) show better

responsiveness compared to their SLe^x containing counterparts (**8b**, **11b**, **12b**). Interestingly, compounds **9a** and **13a** which have Le^x at the central LacNAc moiety, are also recognized by DC-SIGN. No binding of compounds **10a** and **10b** was observed, which have Le^x at the reducing LacNAc residue. The latter finding is in agreement with the observation that the chitobiose core of *N*-linked glycans modified by an 1,6- or 1,3-fucoside is not recognized by DC-SIGN.[31]

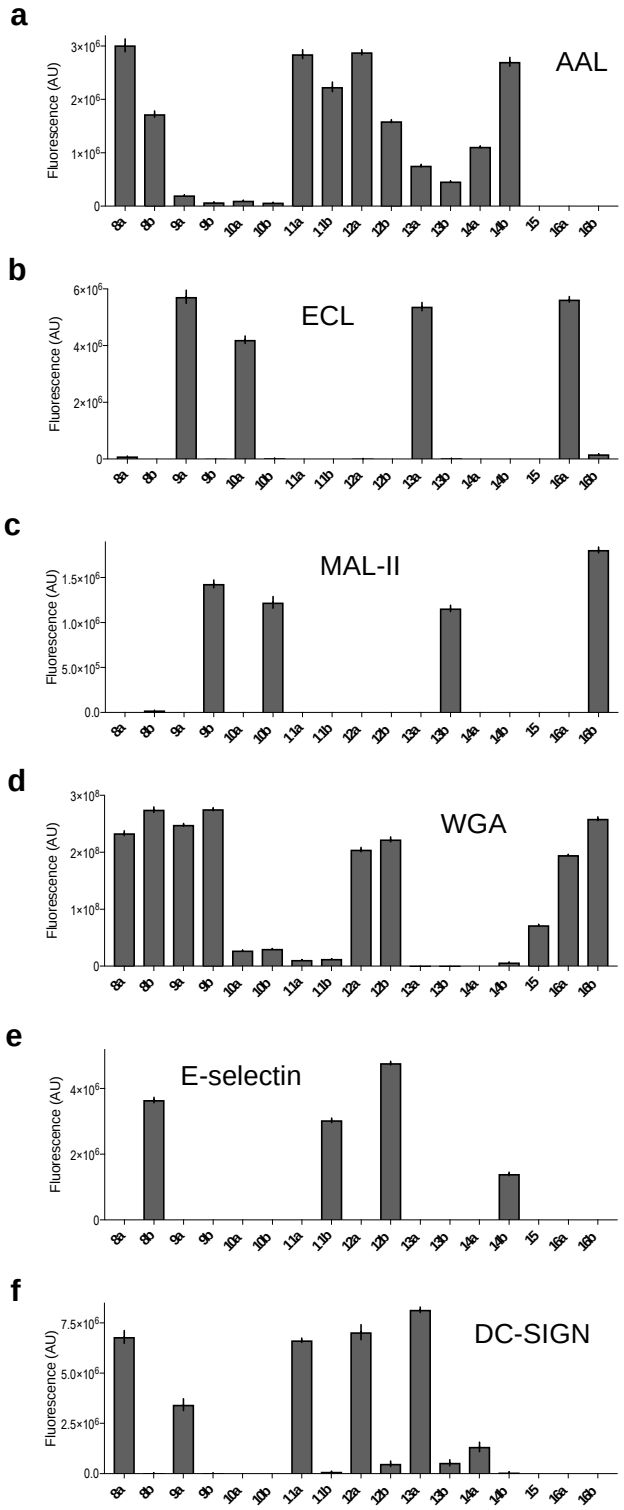


Figure 3. Microarray results of synthetic glycan library at 100 µM with (a) AAL (1 µg/mL); (b) ECL (10 µg/mL; (c) MAL-II (10 µg/mL); (d) WGA (10 µg/mL); (e) E-selectin (2 µg/mL); and (f) DC-SIGN (10 µg/mL). The lowest concentration required for good responsiveness in the optimum dynamic range was selected for all proteins examined. Bars represent the mean ± SD.

Finally, we have examined the receptor usage of HAs of several IAVs. It is generally accepted that avian and human IAV bind α(2,3)- and α(2,6)-sialosides, respectively. Evidence is emerging that this binary differentiation is an oversimplification and other features, such as branching and the presence of extended LacNAc moieties, can modulate HA binding.[32] Furthermore, the presence of a fucosyl residue at a terminal LacNAc moiety to create SLe^x may be a species barrier between chicken and duck.[33] To gain further insight in IAV transmission between the latter two species, the new glycan microarray was used to examine the receptor requirements of influenza viruses isolated from a chicken and a zoonotic human infection (Fig. 4). A chicken influenza virus, A/chicken/Ibaraki/1/2005 (H5N2), showed a exclusive specificity for SLe^x containing glycans (**8b**, **11b**, **12b**, **14b**). Computational analysis has indicated that this glycan-binding specificity is determined by amino acid residues at positions 222 and 227. Therefore, we also examined the R222K R227S mutant, and as expected this HA recognized non-fucosylated 2,3-sialoside **16b**. Interestingly, a fucosyl moiety at the non-reducing LacNAc moiety (**8b**, **11b**, **12b**) almost abolished binding whereas such a residue at the central and reducing LacNAc moiety (**9b**, **10b**, **13b**) reduced responsivenss indicating this HA preferentially binds to extended and unmodified sialyl LacNAc epitopes. As a control, we analyzed A/Vietnam/1203/05 (H5N1) that contains K222 and S227. This HA recognized 2,3-sialyl-LacNAc containing compounds (**9b**, **10b**, **13b**, **16b**) and fucosylation of this residue greatly reduced binding (**8b**, **11b**, **12b**) as previously observed.[34] Fucosylation of the central and reducing LacNAc moiety had only minor influence on binding. Therefore, terminal SLe^x is indeed a species barrier in H5Nx viruses.

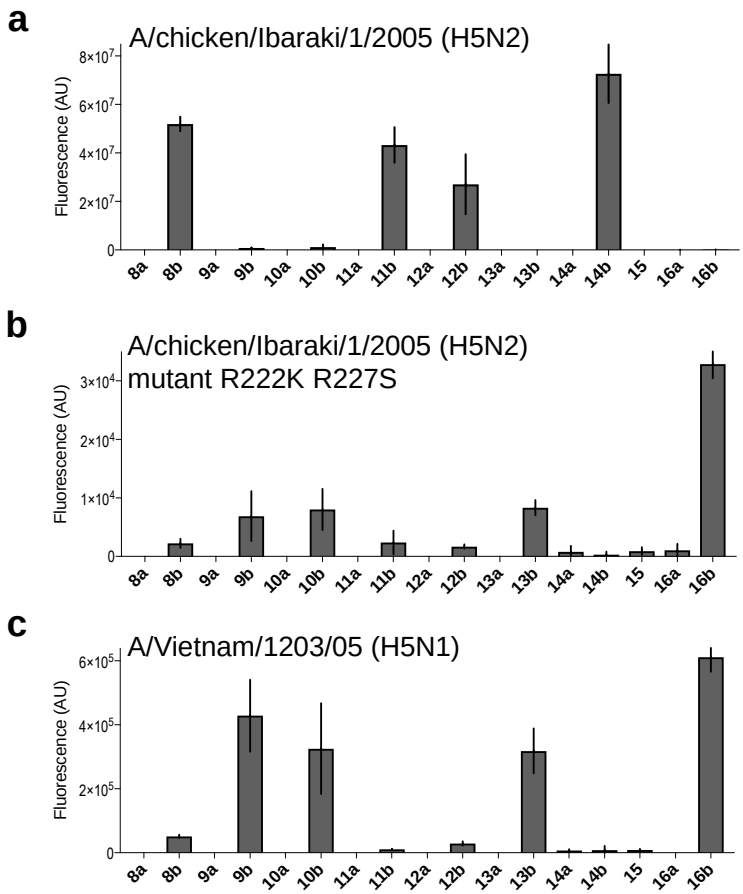


Figure 4. Microarray results of synthetic glycan library at 100 µM with HAs of (a) A/chicken/Ibaraki/1/2005 (H5N2) (50 µg/mL); (b) A/chicken/Ibaraki/1/2005 (H5N2) mutant R222K R227S (50 µg/mL; and (c) A/Vietnam/1203/05 (H5N1) (50 µg/mL). Bars represent the mean ± SD.

Conclusion

Poly-*N*-acetyllactosamine chains are important constituents of *N*- and *O*-linked glycans and glycolipids.[35] The termini of these chains can be modified by various forms of fucosylation and sialylation to create Lewis antigens and ABO blood groups. The internal LacNAc moieties can also be modified by fucosylation to create more complex epitopes. In addition, sulfation of C-6 position of GlcNAc and Gal and branching at Gal to create so-called I-antigens can occur.[35] The preparation of complex modified poly-LacNAc chains has received little attention and to address this deficiency, we describe here a chemoenzymatic strategy that can provide selectively fucosylated compounds starting from a single precursor (**1**). It has also the potential to site-specifically install other functionalities. The new method is based on the finding that $LacNH_2$ and LacNHBoc derivatives are resistant to fucosylation by FUT5 and Hp39-FT. Thus, treatment of hexasaccharides **2-7**, which have different patterns of $LacNH_2$ and LacNHBoc units followed by conversion of NH_2 and NHBoc into NHAc, gave a panel of selectively mono- and bis-fucosylated glycans (**8a-13a**). We also found that the compounds **8a-13a** could be further diversified by exposure to a 2,3-sialyltransferase to give 3'-sialylLacNAc and SLe^x containing compounds (**8b-13b**). It is expected that other types of terminal modifications can be installed and for example exposure of glycans **8a-13a** to FUT2 should create an H-type antigen or Le^y epitopes. Furthermore, we anticipate that the activity of other enzymes that modify poly-*N*-acetyllactosamine chains, such specific sulfotransferases I-branching GlcNAc transferases, will also be impacted by chemical modifications on LacNAc, thereby further expanding the scope of the methodology.

We set ourselves the challenge to prepare compounds **2-7** from a single precursor (**1**), which required the identification of a set of protecting groups that allowed each amino function of an oligo-LacNAc chain to be uniquely modified. We addressed this challenge by synthesizing compound **41** that has Boc, TFA and CBz as amino-protecting groups, and partial deprotection of this compounds gave derivative **1**, having NHBoc, NHTFA, and NH_2 and through simple manipulations compounds **2-7** could easily be obtained.

Glycan microarray binding studies with the new compounds revealed that the pattern of fucosylation of poly-*N*-acetyllactosamine chains influences lectin binding. For example, a number of lectins, such as AAL, ECL and E-selectin, recognize terminal epitopes and fucosylation of the central or reducing LacNAc moiety did not greatly impact binding. On the other hand, MAL-II binds to reducing LacNAc and does not tolerate a fucoside at this position. DC-SIGN showed yet another binding pattern and recognized terminal and central Le^x moieties but not such an epitope at the reducing end. HA of a chicken influenza virus, (A/chicken/Ibaraki/1/2005) required an SLe^x moiety as fucosylation of the central and non-reducing LacNAc were not bound. On the other hand, HA of A/Vietnam/1203/05 H5N1 does not tolerate fucosylation at the non-reducing terminus, but tolerated this modification at the central and non-reducing LacNAc moiety. These results highlight the new collection of compounds can uncover novel aspects of glycan-binding specificities of a wide range of biologically interesting lectins. Plant lectins are widely used to profile glycan structures expressed by cells and a detailed knowledge of the binding profiles will facilitate more precise glycomic profiling. Knowledge of the ligand requirement of glycan binding proteins such as E-selectin, DC-SIGN, and viral HAs will provide insight in the expression of glycans and specific disease processes.

Experimental

General Methods. All reagents, unless otherwise stated, were purchased from Sigma-Aldrich. 2-Deoxy-2-trifluoroacetamido-D-glucose was purchased from Carbosynth Limited (UK). 1,1-dioxobenzo[b]thiophen-2-ylmethyl N-succimidyl carbonate (BsmocOSu) was purchased from Alfa Aesar. ^{1}H and ^{13}C NMR spectra were recorded on Varian Mercury 400 MHz instrument. Chemical shifts are reported in parts per million (ppm) relative to $CDCl_3$ as the internal standard. NMR data are presented as follows: Chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, dd = doublet of doublets, m = multiplet and/or multiple resonances, app = apparent); coupling constants are reported in Hertz (Hz). All NMR signals were assigned on the basis of ^{1}H NMR, COSY and HSQC experiments. Mass spectra were recorded on either on an Applied Biosystems SCIEX MALDI TOF/TOF 5800 mass spectrometer, a Shimadzu Biotech Axima-CFR MALDI-TOF, or a high resolution Shimadzu LCMS-IT-TOF mass spectrometer. Column chromatography was performed on silica gel G60 (Silicycle, 60-200 µm, 60 Å). TLC analysis was conducted on Silicagel 60 F254 (EMD Chemicals Inc.) with detection by UV light (254 nm) where applicable, and by charring with 10% sulfuric acid in ethanol or a solution of $(NH_4)_6Mo_7O_2*4H_2O$ (25 g/L) in 10% sulfuric acid in ethanol. All reactions were carried out under argon atmosphere unless specified otherwise. Unless otherwise stated, all reactions were carried out at room temperature (RT) in glassware with magnetic stirring. Solutions in organic solvents were dried with Na_2SO_4 and concentrated at 40 ^{o}C/2 kPa. Molecular sieves were flame-dried under vacuum immediately prior to use.

Oligosaccharide Nomenclature for NMR Spectroscopy

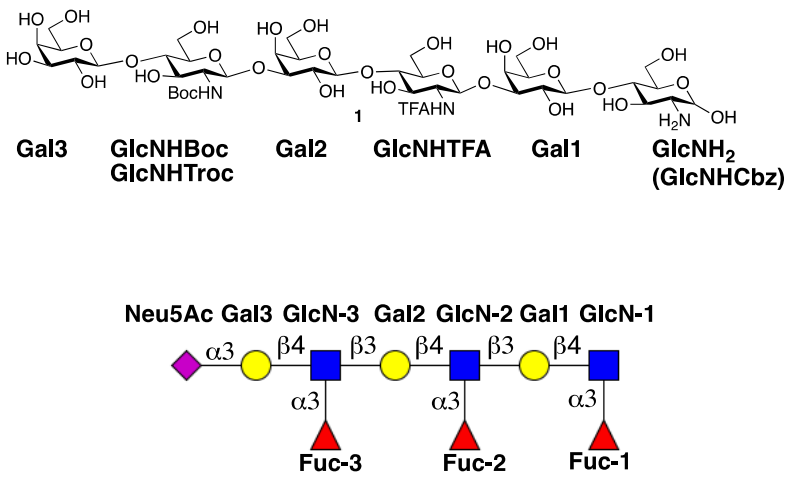
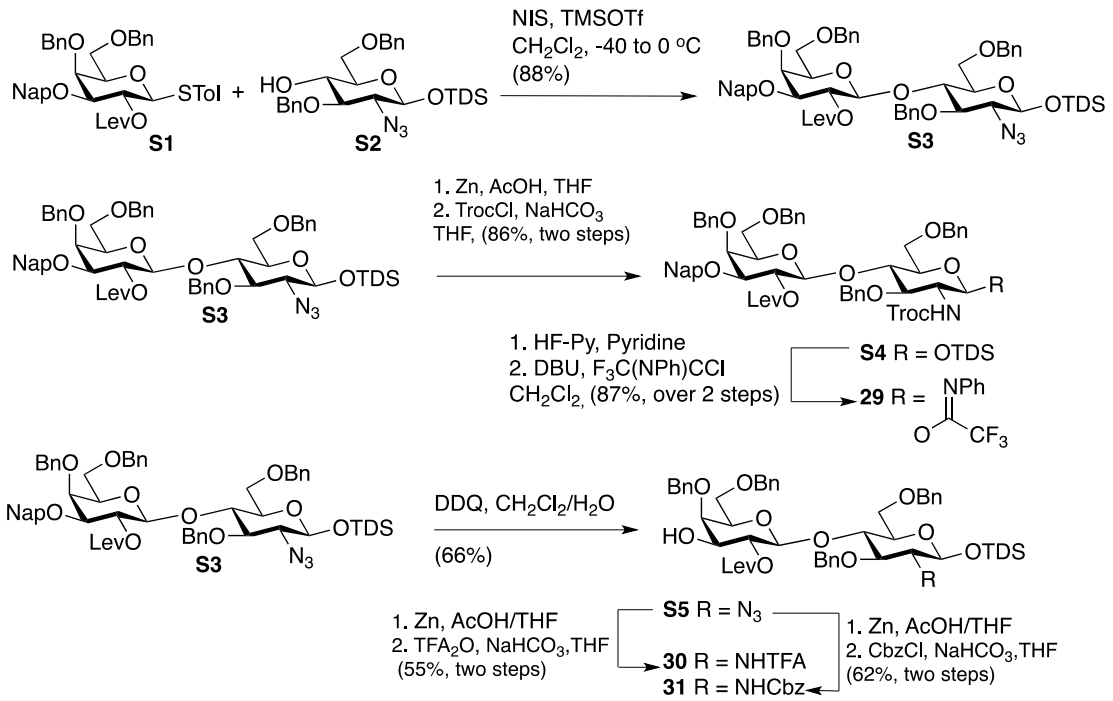


Figure S1. Monosaccharide labeling system for compounds within the chemical and enzymatic synthesis sections

Monosaccharide label units were individually specified as depicted in **Figure S1**. During the chemical synthesis the reducing end glucosamine unit is named either as GlcNHCbz or $GlcNH_2$, while the following glucosamines are either specified as GlcNHTFA or GlcNHBoc (GlcNHTroc where appropriate). In some cases where signals of amino sugars overlap, no distinction is being made, and those glucosamines are labeled as GlcN. Galactose residues received their names either as Gal1, Gal2 or Gal3. During the enzymatic synthesis stage the reducing end glucosamine receives the name of GlcN-1, while the following glucosamines are either specified as GlcN-2 or GlcN-3. Galactose residues are likewise labeled either as Gal1, Gal2 or Gal3. Reducing end fucose receives an acronym of Fuc-1, while the remaining fucoses are given the names of Fuc-2 and Fuc-3. Sialic acid is labeled as Neu5Ac as per **Figure S1**.

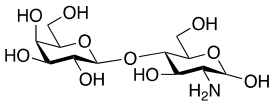
Preparation of Disaccharide Building Blocks



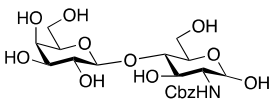
Scheme S1. Synthesis of the key disaccharide **S3**, from which building blocks **29 – 31** were further synthesized.

2-Deoxy-2-trifluoroacetamido-*α,β*-D-glucopyranosyl-(1→4)-*β*-D-galactopyranoside (18). 2-Trifluoroacetamido-2-deoxy-*α,β*-D-glucopyranose (225 mg, 0.82 mmol) was dissolved in 50 mM Tris-HCl buffer (pH=7.5) containing 200 mM $MnCl_2$. UDP-Gal (333 mg, 0.54 mmol) was added, followed by NmLgtB (crude cell lysate), and the reaction mixture was incubated at 37 °C for 2 h, if TLC (EtOAc:MeOH:H_2O, 7:2:1) showed some remaining starting material, extra portion of the buffer and NmLgtB enzyme were added, and incubation was continued as above. After that time, ethanol was added and the resulting solution was centrifuged. Supernatant was further concentrated *in vacuo*, the crude product was applied to a Biogel P-2 column and the product was eluted with water. Fractions containing the desired disaccharide were combined, concentrated and freeze-dried to give the product as a white solid (345 mg, 92%). R_f = 0.7 (EtOAc:MeOH:H_2O, 7:2:1).^{1}H NMR (400 MHz, D_2O): δ 3.53 – 3.59 (1H, m, H-2^{Gal}), 3.64 (1H, m, H-5^{Gal}), 3.66 – 3.72 (1H, m, H-3^{Gal}), 3.72 – 3.80 (5H, m, H-6$_{(a+b)}$^{Gal} incl.), 3.82 (2H, m, H-2$^{\beta GlcN}$), 3.91 (1H, m, H-6a^{GlcN}), 3.94 (1H, d, *J* = 3.0 Hz, H-4^{Gal}), 3.99 (1H, m, H-6b^{GlcN}), 4.01 – 4.05 (3H, m, H-2$^{\alpha GlcN}$), 4.50 (1H, d, *J* = 7.7 Hz, H-1^{Gal}), 4.84 (1H, ad, H-1$^{\beta GlcN}$), 5.30 (1H, d, *J* = 2.3 Hz, H-1$^{\alpha GlcN}$). ^{13}C NMR (101 MHz, D_2O) δ 103.0 (C-1), 94.2, 90.1, 78.6, 75.5, 75.0, 72.6, 71.9, 71.0, 70.4, 68.8, 68.6, 61.1, 60.0, 59.6, 56.8. ESI-MS: $C_{14}H_{22}F_3NNaO_{11}$ calcd. 460.1043, found 460.1048.

2-Amino-2-deoxy-*α,β*-D-glucopyranosyl-(1→4)-*β*-D-galactopyranoside (22). To a solution of LacNHTFA (345 mg) in water (10 mL) was added 30% NH_3 solution (5 mL) and the reaction mixture was kept at room temperature for 4 h. If TLC (EtOAc:MeOH:H_2O, 7:2:1) shows incomplete hydrolysis, incubation may continue at 37 ºC for 2 h. The solution was freeze-dried to afford the product as a white solid, which was used in the next step without further purification. 340 mg (almost quantitative). R_f = 0.0 (EtOAc:MeOH:H_2O, 7:2:1). ^{1}H NMR (400 MHz, D_2O): δ 3.02 (1H, dd, *J* = 8.61, 10.4 Hz, H-2$^{\beta GlcN}$), 3.33 (1H, dd, *J* = 3.51, 10.6 Hz, H-2$^{\alpha GlcN}$), 3.52 – 3.60 (1H, m, H-2^{Gal}), 3.66 (1H, m, H-5^{Gal}), 3.69 (1H, dd, *J* = 3.6, 10.0 Hz, H-3^{Gal}), 3.71 – 3.83 (4H, m, H-6 incl.), 3.84 (1H, m, H-3$^{\beta GlcN}$), 3.90 (2H, m, H-6), 3.94 (1H, d, *J* = 3.4 Hz, H-4^{Gal}), 4.01 (1H, m, H-3$^{\alpha GlcN}$), 4.04 (1H, m), 4.48 (1H, d, *J* = 7.8 Hz, H-1^{Gal}), 4.95 (1H, d, J = 8.0 Hz, H-1$^{\beta GlcN}$), 5.45 (1H, d, J = 3.5 Hz, H-1$^{\alpha GlcN}$). ^{13}C NMR (101 MHz, D_2O) δ 103.1, 92.9, 89.0, 78.2, 75.5, 75.2, 72.6, 71.0, 70.4, 68.6 (x2), 61.2, 59.9 (x2), 59.8, 56.6, 54.2 (x2). ESI-MS: $C_{12}H_{23}NNaO_{10}$ calcd. 364.1220, found 364.1224.

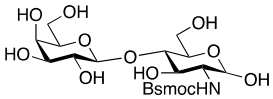


2-(*t*-Butyloxycarbonylamino)-2-deoxy-*α,β*-D-glucopyranosyl-(1→4)-*β*-D-galactopyranoside (21). Lactosamine (116 mg, 0.26 mmol) was dissolved in MeOH:H_2O (1:1, 500 µL), followed by adding solid $NaHCO_3$ (45 mg, 0.52 mmol) and Boc_2O (87 mg, 0.39 mmol). The reaction mixture was kept at RT, after which it was directly loaded on Biogel P-2 and the product was eluted with water. Fractions containing the product were pooled, concentrated and lyophilized to give the product as a white solid. 133 mg (89%). R_f = 0.7 (EtOAc:MeOH:H_2O, 7:2:1). ^{1}H NMR (400 MHz, D_2O): δ 1.46 (9H, s, Boc), 3.37 (1H, t, J = 9.2 Hz, H-2$^{\beta GlcN}$), 3.57 (1H, m, H-2^{Gal}), 3.60 (1H, m, H-5^{Gal}), 3.64 (1H, m, H-2$^{\alpha GlcN}$), 3.69 (2H, m, H-3$^{\beta GlcN}$, H-3^{Gal}), 3.70 – 3.86 (5H, m, H-6 incl.), 3.90 (2H, m, H-6), 3.95 (1H, brs, H-4^{Gal}), 3.97 (1H, m), 4.50 (1H, d, H-1^{Gal}), 4.72 (1H, d, H-1$^{\beta GlcN}$), 5.22 (1H, s, H-1$^{\alpha GlcN}$).^{13}C NMR (101 MHz, D_2O) δ 103.1, 95.3, 91.2, 78.8, 75.6, 72.7 (x2), 71.1, 70.4, 69.8, 68.7, 61.2, 60.2 (x2), 57.7, 54.9. 27.7 (x3). ESI-MS: $C_{17}H_{31}NNaO_{12}$ calcd. 464.1744, found 464.1742.

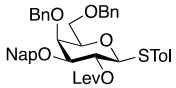


2-(Benzyloxycarbonylamino)-2-deoxy-*α,β*-D-glucopyranosyl-(1→4)-*β*-D-galactopyranoside (19). Lactosamine (120 mg, 0.34 mmol) was dissolved in MeOH:H_2O (1:1), followed by adding solid $NaHCO_3$ (45 mg, 0.52 mmol) and CbzOSu (172 mg, 0.69 mmol). The reaction mixture was kept at RT, after which it was directly loaded on Biogel P-2 and the product was eluted with water. Fractions containing the product were pooled, concentrated and lyophilized to give the product as a white solid. 138 mg (83%). R_f = 0.7 (EtOAc:MeOH:H_2O, 7:2:1). ^{1}H NMR (400 MHz, D_2O): δ 3.44 (1H, m, H-2$^{\beta GlcN}$), 3.56 (1H, m, H-2^{Gal}), 3.61 (1H, m, H-5^{Gal}), 3.65 (2H, m, H-6), 3.67 (2H, m, H-3$^{\beta GlcN}$, H-3^{Gal}), 3.70 (1H, m, H-2$^{\alpha GlcN}$), 3.72 (1H, m), 3.74 (1H, m), 3.86 (1H, m), 3.89 – 3.96 (4H, m, H-4^{Gal}, H-6 incl.), 4.48 (1H, d, *J* = 7.5 Hz, H-1^{Gal}), 4.73 (1H, d, *J* = 8.5 Hz, H-1$^{\beta GlcN}$), 5.11 (2H, m, $\underline{CH_2}$OBn), 5.22 (1H, d, J = 3.71 Hz, H-1$^{\alpha GlcN}$), 7.41 – 7.48 (5H, m, Ar-H).^{13}C NMR (101 MHz, D_2O) δ 128.7, 127.8, 126.6, 103.0, 95.1, 91.1, 78.6, 75.5, 75.0, 72.6, 71.1, 70.4, 69.8, 68.7, 67.2, 66.9, 62.7 (x2), 60.2, 60.1, 58.1, 55.3. ESI-MS: $C_{20}H_{29}NNaO_{12}$ calcd. 498.1587, found 498.1592.

2-(1,1-Dioxobenzo[b]thiophen-2-ylmethylcarbonylamino)-2-deoxy-*α,β*-D-glucopyranosyl-(1→4)-*β*-D-galactopyranoside (20). Lactosamine (50 mg, 0.15 mmol) was dissolved in MeOH:H_2O (1:1), followed by adding solid $NaHCO_3$ (45 mg, 0.52 mmol) and BsmocOSu (102 mg, 0.30 mmol). The reaction mixture was kept at RT, after which it was directly loaded on Biogel P-2 and the product was eluted with water. Fractions containing the product were pooled, concentrated and lyophilized to give the product as a white solid. 70 mg (83%). R_f = 0.7 (EtOAc:MeOH:H_2O, 7:2:1). ^{1}H NMR (400 MHz, D_2O): δ 3.45 (1H, br t, H-2$^{\beta GlcN}$), 3.57 (1H, m, H-2^{Gal}), 3.62 (1H, m, H-5^{Gal}), 3.64 – 3.73 (2H, m, H-3$^{\beta GlcN}$, H-3^{Gal}), 3.69 (1H, m), 3.73 (1H, m, H-2$^{\alpha GlcN}$), 3.74 (1H, m), 3.75 (1H, m), 3.77 (2H, m, H-6), 3.90 (2H, m, H-6), 3.94 (1H, br s, H-4^{Gal}), 3.98 (1H, m), 4.49 (1H, d, *J* = 8.0 Hz, H-1^{Gal}), 4.76 (1H, d, *J* = 9.2 Hz, H-1$^{\beta GlcN}$), 5.11 – 5.26 (2H, m, CH_2 Bsmoc), 5.25 (1H, d, *J* = 3.6 Hz, H-1$^{\alpha GlcN}$), 7.52 (1H, s, Ar-H), 7.59 (1H, m, Ar-H), 7.67 (1H, m, Ar-H), 7.73 (1H, m, Ar-H), 7.84 (1H, m, Ar-H). ^{13}C NMR (101 MHz, D_2O) δ 135.0, 132.8, 131.2, 126.4, 121.7, 102.9, 95.0, 91.0, 78.6, 75.5, 75.0, 72.6, 71.1, 70.4, 69.8, 68.7, 61.2, 60.1, 58.3, 57.4, 55.5. ESI-MS: $C_{22}H_{29}NNaO_{14}S$ calcd. 586.1206, found 586.1212.



4-Methyphenyl 4,6-dibenzyl-2-*O*-levulinoyl-3-(2-naphthyl)-1-thio-*β*-D-galactopyranoside (S1). 4-Methyl-4,6-*O*-benzylidene-1-thio-*β*-D-galactopyranoside[36] (50.0 g, 133 mmol) was suspended in toluene (300 mL), after which Bu_2SnO (40.0 g, 159.6 mmol) was added and the suspension was boiled at 110 ºC for 2 h. The resulting clear solution was concentrated, re-dissolved in DMF (200 mL), followed by adding CsF (30.0 g, 199.5 mmol) and NapBr (44.0 g, 199.5 mmol) and the mixture was stirred at RT for 3 h. DMF was then removed *in vacuo*, and the crude mixture was dissolved in CH_2Cl_2 (500 mL) and washed successively with H_2O (500 mL) and $NaHCO_3$ (500 mL). The organic phase containing paste-like tin residues was vacuum-filtered through a Büchner funnel, after which it was dried and concentrated to give 4-methyphenyl 4,6-*O*-benzylidene-3-(2-naphthyl)-1-thio-*β*-D-galactopyranoside as a white solid, which was used directly in the next step. To a solution of the above material in CH_2Cl_2 (200 mL) was added Et_3N (57 mL, 399 mmol) and DMAP (17.0 g, 140 mmol). The reaction mixture was cooled to 0 ºC and PivCl (25 mL, 199.5 mmol) was slowly added. Stirring was continued at RT for 1 h, after which TLC (EtOAc:Hexane, 3:7) showed the starting material was fully consumed. The mixture was diluted with CH_2Cl_2 (200 mL) and washed with 1 M HCl (300 mL). The organic layer was dried and concentrated *in vacuo*, and the obtained solid was used in the next step without further purification. This material was dissolved in CH_2Cl_2/MeOH (1:9, 300 mL), after which catalytic amount of *p*-TsOH was added and the reaction mixture was stirred overnight. This was followed by neutralization with Et_3N and concentration *in vacuo*. The resulting solid was crystallized from EtOAc/Hexane to give the desired product as a white powder. 50.9 g (72%, over three steps). R_f = 0.5 (EtOAc:Hexane, 1:1). This product was then dissolved in DMF, followed by adding NaH (6.1 g, 153 mmol) and BnBr (18 mL, 153 mmol) at 0 ºC, and the mixture was left stirring at that temperature for 30 min, and then for additional 1 h at RT. Neutralization with MeOH at 0 ºC, followed by concentration *in vacuo* afforded the crude product as a solid which was directly dissolved in CH_2Cl_2/MeOH (1:9), followed by adding LiOH (50.0 g), and the reaction mixture was heated at 60 ºC for 48 h, after which the Piv group was completely removed. The solution was then concentrated *in vacuo*, diluted with CH_2Cl_2 (400 mL) and washed with water (300 mL). The organic layer was dried and concentrated *in vacuo* providing a solid which was directly dissolved in CH_2Cl_2 (300 mL) followed by adding LevOH (15 mL, 147 mmol), EDCI (28 g, 147 mmol) and DMAP (1.0 g, catalytic) and the reaction was stirred at RT for 1 h, after which TLC (EtOAc:Hexane, 3:7) showed it was complete. The reaction mixture was diluted with CH_2Cl_2 (200 mL) and washed with sat. $NaHCO_3$ (200 mL), and the organic phase was dried and concentrated *in vacuo*. Crystallization from EtOAc/Hexane gave the desired product as a white powder. 50.4 g (72%, over three steps). $[\alpha]_D^{24}$ +9.5 (c=1, $CHCl_3$). R_f = 0.3 (EtOAc:Hexane, 3:7). ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.09 (3H, s, $\underline{CH_3}$ Lev), 2.26 (3H, s, $\underline{CH_3}$



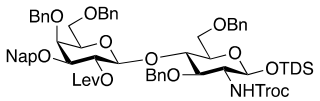
STol), 2.54 – 2.57 (2H, m, $\underline{CH_2}$ Lev), 2.65 – 2.68 (2H, m, $\underline{CH_2}$ Lev), 3.57 – 3.64 (4H, m, H-3, H-5, H-6), 3.99 (1H, d, *J* = 2.4 Hz, H-4), 4.40 (2H, app q, $\underline{CH_2}$ OAr), 4.53 (1H, d, *J* = 10.0 Hz, H-1), 4.59 (1H, d, *J* = 11.1 Hz, C<u>H</u>HOAr), 4.69 (1H, d, *J* = 12.1 Hz, C<u>H</u>HOAr), 4.81 (1H, d, *J* = 12.1 Hz, CH<u>H</u>OAr), 4.95 (1H, d, *J* = 11.1 Hz, CH<u>H</u>OAr), 5.41 (1H, app t, *J* = 9.9 Hz, H-2), 7.00 (2H, d, *J* = 7.9 Hz, Ar-H), 7.20 -7.34 (9H, m, Ar-H), 7.37 (2H, d, *J* = 7.9Hz, Ar-H), 7.74 – 7.84 (8H, m, Ar-H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 206.3, 171.5, 138.5, 137.9, 137.6, 135.4, 133.2, 133.0, 132.6, 129.7, 129.5, 128.4, 128.2 (x2), 128.0, 127.9, 127.8, 127.7, 127.5, 126.4, 126.2, 126.0, 125.7, 87.1, 81.3, 77.6, 74.4, 73.6, 72.9 72.2, 70.2, 68.8, 37.9, 29.8, 28.2, 21.1. ESI-MS: $(C_{43}H_{44}O_7+NH_4)^+$ calcd. 722.3151, found 722.3149.

Dimethylthexylsilyl 4,6-dibenzyl-2-*O*-levulinoyl-3-(2-naphthyl)-*β*-D-galactopyranosyl-(1→4)-2-azido-2-deoxy-3,6-dibenzyl-*β*-D-glucopyranoside (S3). To a mixture of dimethylthexylsilyl 2-azido-3,6-dibenzyl-2-deoxy-*β*-D-glucopyranose[18] (22.0 g, 41.6 mmol) and glycosyl donor **55** (44.0 g, 62.4 mmol) in CH_2Cl_2 (300 mL) was added NIS (14.0 g, 62.4 mmol) and the solution was cooled to -40 °C followed by adding TMSOTf (1mL). The glycosylation mixture was stirred at that temperature for 30 min, and then warmed to 0 °C, and further stirred at that temperature for 15 min, followed by quenching with Et_3N. The mixture was diluted with CH_2Cl_2 (200 mL) and washed with 10% $Na_2S_2O_3$ (200 mL), after which the organic phase was dried and concentrated *in vacuo*, affording a syrup which was chromatographed using EtOAc:Hexane (1:4) as eluent to give the target product as oil. 40.5 g (88%). $[\alpha]_D^{24}$ -5.2 (c=1, $CHCl_3$). R_f = 0.3 (EtOAc:Hexane, 1:4). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.19 (6H, 2 x s, Si($\underline{CH_3}$)$_2$), 0.90 (12H, m, C($\underline{CH_3}$)$_2$, CH($\underline{CH_3}$)$_2$), 1.67 (1H, m, $\underline{CH}$(CH_3)$_2$), 2.08 (3H, s, $\underline{CH_3}$ Lev), 2.44 (2H, m, C$\underline{H_2}$ Lev), 2.59 (1H, m, C<u>H</u>H Lev), 2.65 (1H, m, CH<u>H</u> Lev), 3.26 (1H, m, H-2^{GlcN}), 3.30 (1H, m, H-3^{GlcN}), 3.33 (1H, m, H-6$_a$), 3.36 – 3.40 (2H, m, H-5^{Gal}, H-5^{GlcN}), 3.45 (1H, dd, *J* = 10.0, 2.8 Hz, H-3^{Gal}), 3.52 (1H, t, *J* = 8.5Hz, H-6$_b^{GlcN}$), 3.65 (1H, dd, *J* = 11.1, 1.7 Hz, H-6$_a^{Gal}$), 3.77 (1H, dd, *J* = 11.6, 3.6 Hz, H-6$_b^{Gal}$), 3.92 (1H, dd, *J* = 9.6, 8.8 Hz, H-4^{GlcN}), 4.0 (1H, d, *J* = 2.8 Hz, H-4^{Gal}), 4.23 (1H, d, *J* = 11.7 Hz, C<u>H</u>HOAr), 4.33 (1H, d, *J* = 11.7 Hz, CH<u>H</u>OAr), 4.45 (1H, d, *J* = 8.0 Hz, H-1^{GlcN}), 4.46 (1H, d, *J* = 11.6 Hz, CH<u>H</u>OAr), 4.51 (1H, d, *J* = 8.0 Hz, H-1^{Gal}), 4.57 (1H, d, *J* = 11.6 Hz, C<u>H</u>HOAr), 4.66 (3H, m C$\underline{H_2}$OAr, CH<u>H</u>OAr), 4.83 (1H, d, *J* = 12.5 Hz, C<u>H</u>HOAr), 5.00 (1H, d, *J* = 10.8 Hz, C<u>H</u>HOAr), 5.37 (1H, dd, *J* = 10.1, 8.1 Hz, H-2^{Gal}), 7.06 – 7.92 (27H, m, Ar-H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 206.2, 171.4, 138.7, 138.6, 138.2, 137.9, 135.5, 133.2, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.7, 127.6, 127.5, 127.4, 127.2, 126.2, 126.1, 126.0, 125.5, 100.4, 96.8, 81.0, 80.3, 76.0, 75.2, 75.0, 74.7, 73.5, 73.4, 73.3, 72.8, 72.4, 71.8, 68.5, 68.1, 67.9, 37.7, 33.9, 29.8, 27.9, 24.8, 20.0, 19.9, 18.5, 18.4, -2.1, -3.2. ESI-MS: $(C_{64}H_{77}N_3O_{12}Si+NH_4)^+$ calcd. 1125.5620, found 1125.5616.

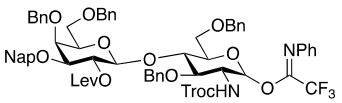
Dimethylthexylsilyl 4,6-dibenzyl-2-*O*-levulinoyl-*β*-D-galactopyranosyl-(1→4)-2-azido-2-deoxy-3,6-dibenzyl-*β*-D-glucopyranoside (S5). To a solution of disaccharide **S3** (9.9 g, 8.9 mmol) in CH_2Cl_2 (200 mL) was added water (20 mL) and DDQ (3.1 g, 13.39 mmol), and the reaction mixture was left stirring at RT for 1.5 h. The mixture was quenched with sat. $NaHCO_3$, filtered, and washed with sat. $NaHCO_3$ once more. The organic phase was dried and concentrated *in vacuo* to give a crude product, which was chromatographed using EtOAc:Hexane (3:7) to give the pure product as a clear oil. 7.19 g (66%). $[\alpha]_D^{24}$ -18.0 (c=1, $CHCl_3$). R_f = 0.4 (EtOAc:Hexane, 3:7). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.19 (6H, 2 x s, Si($\underline{CH_3}$)$_2$), 0.90 (12H, m, C($\underline{CH_3}$)$_2$, CH($\underline{CH_3}$)$_2$), 1.67 (1H, m, $\underline{CH}$(CH_3)$_2$), 2.13 (3H, s, $\underline{CH_3}$ Lev), 2.34 (1H, d, *J* = 9.3 Hz, 3-OH^{Gal}), 2.48 (2H, app t, *J* = 7.26 Hz, C$\underline{H_2}$ Lev), 2.60 – 2.79 (2H, m, C$\underline{H_2}$ Lev), 3.24 (1H, m, H-2^{GlcN}), 3.26 (1H, m, H-3^{GlcN}), 3.34 (1H, m, H-5^{GlcN}), 3.36 (1H, m, H-5^{Gal}), 3.39 – 3.52 (3H, m, H-3^{Gal}, H-6^{GlcN}), 3.64 (1H, dd, *J* = 11.1, 1.7 Hz, H-6$_a^{Gal}$), 3.79 (1H, dd, *J* = 11.6, 3.6 Hz, H-6$_b^{Gal}$), 3.82 (1H, d, *J* = 3.5 Hz, H-4^{Gal}), 3.91 (1H, dd, *J* = 9.4, 8.6 Hz, H-4^{GlcN}), 4.22 (1H, d, *J* = 11.5 Hz, CH<u>H</u>OAr), 4.33 (1H, d, *J* = 11.5 Hz, C<u>H</u>HOAr), 4.42 (1H, d, *J* = 7.03 Hz, H-1^{GlcN}), 4.45 (1H, d, *J* = 8.62 Hz, CH<u>H</u>OAr), 4.50 (1H,

d, J = 12.5 Hz, CH<u>H</u>OAr), 4.51 (1H, d, J = 8.0 Hz, H-1^{Gal}), 4.60 – 4.71 (3H, m, <u>CH$_2$</u>OAr, C<u>H</u>HOAr), 4.95 (1H, dd, J = 10.0, 8.2 Hz, H-2^{Gal}), 4.96 (1H, d, J = 10.6 Hz, C<u>H</u>HOAr), 7.14 – 7.39 (20H, m, Ar-H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 206.6, 172.6, 138.7, 138.3, 137.8, 128.5, 128.4 (x2), 128.0, 127.8 (x2), 127.3, 100.1, 96.9, 81.0, 76.4, 76.3, 75.3, 75.1, 75.0, 74.3, 73.6, 73.4, 73.3, 73.0, 68.5, 68.0, 67.7, 38.0, 34.0, 29.8, 28.0, 24.8, 20.0, 19.9, 18.5, 18.4, -2.1, -3.2. ESI-MS: $(C_{53}H_{69}N_3O_{12}Si+NH_4)^+$ calcd. 985.4994, found 985.4998.

Dimethylthexylsilyl 4,6-dibenzyl-2-*O*-levulinoyl-3-(2-naphthyl)-*β*-D-galactopyranosyl-(1→4)-3,6-dibenzyl-2-deoxy-2-(2,2,2-trichloroethylcarbonylamino)-*β*-D-glucopyranoside (S4). To a solution of disaccharide **S3** (11.9 g, 10.7 mmol) in THF (50mL) was added AcOH (1 mL) and freshly activated Zn powder (13.0 g), and the reaction mixture was left stirring at RT for 1 h, after which time TLC (EtOAc:Hexane, 3:7) showed it was complete. The solution was filtered, concentrated to dryness, and the syrup was re-dissolved in THF (50 mL), followed by adding solid $NaHCO_3$ (5.0 g) and TrocCl (2.3 mL, 16.1 mmol), after which the solution was left stirring at ambient temperature for 30 min. The reaction mixture was filtered, concentrated in *vacuo*, and chromatographed using EtOAc:Hexane (1:4) as eluent to give the desired product as oil. 11.5 g (86%). $[\alpha]_D^{24}$ +7.1 (c=1, $CHCl_3$). R_f = 0.7 (EtOAc:Hexane, 3:7).^{1}H NMR (400 MHz, $CDCl_3$): δ 0.13 (6H, 2 x s, Si(<u>CH$_3$</u>)$_2$), 0.83 (12H, m, C(<u>CH$_3$</u>)$_2$, CH(<u>CH$_3$</u>)$_2$), 1.60 (1H, m, <u>CH</u>(CH$_3$)$_2$), 2.09 (3H, s, <u>CH$_3$</u> Lev), 2.46 (2H, app t, J = 7.26 Hz, C<u>H$_2$</u> Lev), 2.58 – 2.79 (2H, m, C<u>H$_2$</u> Lev), 3.30 (1H, m, H-2^{GlcN}), 3.38 (1H, m H-6$_a^{GlcN}$), 3.41 (1H, m, H-5^{Gal}), 3.44 (1H, m, H-5^{GlcN}), 3.45 (1H, m, H-3^{Gal}), 3.51 (1H, t, J = 6.9 Hz, H-6$_b^{GlcN}$), 3.68 (1H, dd, J = 11.1, 1.7 Hz, H-6$_a^{Gal}$), 3.74 (1H, m, H-3^{GlcN}), 3.76 (1H, dd, J = 11.6, 3.6 Hz, H-6$_b^{Gal}$), 3.95 (1H, t, J = 9.2 Hz, H-4^{GlcN}), 3.98 (1H, d, J = 2.9 Hz, H-4^{Gal}), 4.26 (1H, d, J = 11.5 Hz, CH<u>H</u>OAr), 4.34 (1H, d, J = 11.5 Hz, C<u>H</u>HOAr), 4.46 (1H, d, J = 12.2 Hz, C<u>H</u>HOAr), 4.50 (1H, d, J = 8.5 Hz, H-1^{Gal}), 4.53 – 4.60 (2H, m, 2xC<u>H</u>HOAr), 4.62 – 4.69 (3H, m, C<u>H$_2$</u> Troc, C<u>H</u>HOAr), 4.80 (1H, br m, H-1^{GlcN}), 4.82 (1H, d, J = 12.3 Hz, C<u>H</u>HOAr), 4.93 – 4.99 (2H, m, <u>CH$_2$</u>OAr), 5.02 (1H, br m, N<u>H</u>), 5.37 (1H, dd, J = 10.1, 8.01 Hz, H-2^{Gal}), 7.08 – 7.92 (27H, m Ar-H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 206.2, 171.4, 138.8, 138.7, 138.2, 137.9, 135.5, 133.2, 132.9, 128.4, 128.3, 128.1 (x2), 128.0, 127.8 (x2), 127.7 (x2), 127.6, 127.5, 127.4, 127.1, 126.2, 126.1, 125.5, 100.3, 95.4, 80.2, 78.4, 76.4, 74.9, 74.6, 74.3, 73.5, 73.4, 73.3, 72.8, 72.3, 71.8, 68.4, 68.0, 59.3, 37.7, 34.0, 29.8, 27.9, 24.8, 20.0 (x2), 18.5 (x2), -1.9, -3.4. ESI-MS: $(C_{67}H_{80}Cl_3NO_{14}Si+NH_4)^+$ calcd. 1273.4757, found 1273.4755.



(*N*-Phenyl)-2,2,2-trifluoroacetimidate-4,6-dibenzyl-2-*O*-levulinoyl-3-(2-naphthyl)-*β*-D-galactopyranosyl-(1→4)-3,6-dibenzyl-2-deoxy-2-(2,2,2-trichloroethylcarbonylamino)-D-glucopyranoside (29). To a solution of disaccharide **S4** (8.5 g, 6.7 mmol) in pyridine (30 mL) was added HF/Py solution at 0 ^oC, and the reaction mixture was then further stirred at RT for 4 h. The mixture was diluted with CH_2Cl_2 (100 mL) and was successively washed with H_2O and sat. $NaHCO_3$. The organic phase was dried and concentrated *in vacuo* to give an oil, which was directly dissolved in CH_2Cl_2 (100 mL) and treated with CF_3(NPh)CCl (1.8 mL, 11.25 mmol) and DBU (1 mL, 6.7 mmol), and the reaction mixture was stirred at that temperature for 30 min. The solution was then concentrated *in vacuo*, and the resulting product was chromatographed using EtOAc:Hexane (1:4) to give the imidate as a clear oil. 7.5 g (78%, over two steps). ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.07 (3H, s, <u>CH$_3$</u> Lev), 2.37 – 2.83 (4H, m, C<u>H$_2$</u>x2 Lev), 3.27 – 3.59 (m, incl. H-2^{GlcN}, H-6^{GlcN,} H-5^{GlcN}, H-5^{Gal,}), 3.60 – 4.09 (m), 4.21 – 4.46 (m), 4.51 – 4.71 (m), 4.74 – 4.85 (m), 4.97 – 5.05 (m), 5.39 (2H, m, H-1^{Gal}, H-1^{GlcN}), 6.76 (d, Ar-H), 6.98 (m, Ar-H), 7.69 – 7.88 (m, Ar-H). ^{13}C NMR (101 MHz, d_6-DMSO) δ 206.9, 206.8, 171.7, 139.2, 138.6, 136.7, 136.5, 136.4, 133.2, 132.9, 129.4, 129.4, 129.3, 128.7 (x2), 128.6 (x2), 128.5 (x2), 128.3, 128.2 (x2), 128.1 (x3), 128.0 (x4), 127.9 (x3), 127.8 (x2), 126.6, 126.5, 126.4 (x2), 126.1,

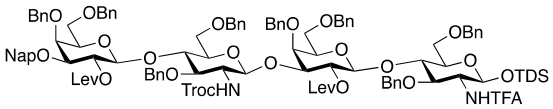


121.5 119.6, 96.7, 79.6, 77.1, 74.9, 74.6 (x2), 74.0, 73.9, 73.4, 72.9, 72.8, 72.1, 71.3, 69.8, 68.6, 60.2, 56.1, 54.9, 37.6, 30.0, 28.0, 21.2.

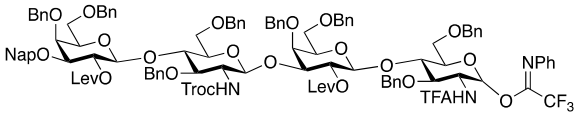
Dimethylthexylsilyl 4,6-dibenzyl-2-*O*-levulinoyl-*β*-D-galactopyranosyl-(1→4)- 3,6-dibenzyl-2-deoxy-2-trifluoroacetamido-*β*-D-glucopyranoside (30). To a solution of disaccharide **S5** (7.26 g, 7.5 mmol) in THF (50 mL) was added AcOH (1 mL) and freshly activated Zn powder (13.0 g), and the reaction mixture was left stirring at RT for 1 h, after which time TLC (EtOAc:Hexane, 3:7) showed it was complete. The solution was filtered, concentrated to dryness, and the syrup was re-dissolved in THF, followed by adding solid $NaHCO_3$ (5.0 g) and trifluoroacetic anhydride (2 mL, 14.9 mmol). The reaction mixture was stirred at RT for 30 min, after which it was filtered, concentrated *in vacuo*, applied to a column of silica gel and the product was eluted with EtOAc:Hexane (3:7) to give the title product as a clear foam. 4.2 g (55%, over two steps). $[\alpha]_D^{24}$ -9.0 (c=1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.13 (6H, 2 x s, Si($\underline{CH_3}$)$_2$), 0.79 – 0.86 (12H, m, C($\underline{CH_3}$)$_2$, CH($\underline{CH_3}$)$_2$), 1.59 (1H, m, $\underline{CH}$(CH_3)$_2$), 2.18 (3H, s, $\underline{CH_3}$ Lev), 2.54 (2H, app t, *J* = 7.26 Hz, C$\underline{H_2}$ Lev), 2.68 – 2.82 (2H, m, C$\underline{H_2}$ Lev), 3.44 (1H, m, H-6$_a^{GlcN}$), 3.52 (3H, m, H-3^{Gal}, H-5^{Gal}, H-5^{GlcN}), 3.54 (1H, m, H-6$_b^{GlcN}$), 3.57 (1H, m, H-2^{GlcN}), 3.72 (1H, dd, *J* = 11.1, 2.75 Hz, H-6$_a^{Gal}$), 3.82 (1H, m, H-6$_b^{Gal}$), 3.83 (1H, m, H-3^{GlcN}), 3.86 (1H, d, *J* = 3.7 Hz, H-4^{Gal}), 4.00 (1H, t, *J* = 8.4 Hz, H-4^{GlcN}), 4.33 (1H, d, *J* = 12.0 Hz, C$\underline{H}$HOAr), 4.41 (1H, d, *J* = 12.0 Hz, C$\underline{H}$HOAr), 4.52 (1H, d, *J* = 8.4 Hz, H-1^{Gal}), 4.54 (2H, m, 2 x C$\underline{H}$HOAr), 4.63 – 4.72 (3H, m, $\underline{CH_2}$OAr, C$\underline{H}$HOAr), 4.90 (2H, m, C$\underline{H}$HOAr, H-1^{GlcN}), 4.98 (1H, dd, *J* = 9.7, 7.9 Hz, H-2^{Gal}), 6.32 (1H, d, *J* = 8.49 Hz, NH), 7.16 – 7.41 (20H, m, Ar-H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 127.9, 127.8, 99.9, 94.5, 77.3, 76.3, 76.2, 75.2, 74.9, 74.2 (x2), 73.5, 73.4, 73.2, 68.2, 67.7, 58.3, 38.0, 34.0, 29.9, 28.0 (x2), 19.9, 18.4. ESI-MS: $(C_{55}H_{70}F_3NO_{13}Si+NH_4)^+$ calcd. 1055.4912, found 1055.4907.

Dimethylthexylsilyl 4,6-dibenzyl-2-*O*-levulinoyl-*β*-D-galactopyranosyl-(1→4)-2-(benzyloxycarbonylamino)-3,6-dibenzyl-2-deoxy-*β*-D-glucopyranoside (31). To a solution of disaccharide **S5** (4.0 g, 4.13 mmol) in THF (50 mL) was added AcOH (1 mL) and freshly activated Zn powder (5.0 g), and the reaction mixture was left stirring at RT for 1 h, after which time TLC (EtOAc:Hexane, 3:7) showed it was complete. The solution was filtered, concentrated to dryness, and the syrup was re-dissolved in THF, followed by adding solid $NaHCO_3$ (5.0 g) and CbzCl (884 µL, 6.2 mmol). The reaction mixture was stirred at RT for 30 min, after which it was filtered, concentrated *in vacuo*, applied to a column of silica gel and the product was eluted with EtOAc:Hexane (3:7 to 4:6) to give the title product as a clear oil. 2.75 g (62%, over two steps). $[\alpha]_D^{24}$ +4.3 (c=1, $CHCl_3$). R_f = 0.4 (EtOAc:Hexane, 4:6). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.10 (6H, 2 x s, Si($\underline{CH_3}$)$_2$), 0.78 – 0.84 (12H, m, C($\underline{CH_3}$)$_2$, CH($\underline{CH_3}$)$_2$), 1.59 (1H, m, $\underline{CH}$(CH_3)$_2$), 2.15 (3H, s, $\underline{CH_3}$ Lev), 2.37 (1H, d, *J* = 8.6 Hz, 3-OH^{Gal}), 2.45 – 2.59 (2H, m, C$\underline{H_2}$ Lev), 2.73 (2H, m, C$\underline{H_2}$ Lev), 3.21 (1H, br m, H-2^{GlcN}), 3.38 (1H, m, H-6$_a^{GlcN}$), 3.43 (1H, m, H-5^{GlcN}), 3.49 (3H, m, H-3^{Gal}, H-3^{GlcN}, H-6$_b^{GlcN}$), 3.68 (1H, br d, H-6$_a^{Gal}$), 3.79 (1H, dd, *J* = 11.7, 3.9 Hz, H-6$_b^{Gal}$), 3.84 (1H, d, *J* = 3.3 Hz, H-4^{Gal}), 3.93 (1H, t, *J* = 8.9 Hz, H-4^{GlcN}), 4.26 (1H, d, *J* = 12.0 Hz, C$\underline{H}$HOAr), 4.36 (1H, d, *J* = 12.0 Hz, C$\underline{H}$HOAr), 4.48 – 4.54 (3H, m, H-1^{Gal}, 2 x C$\underline{H}$HOAr), 4.61 – 4.71 (3H, m, C$\underline{H}$HOAr, $\underline{CH_2}$OAr), 4.85 (1H, br s, H-1^{GlcN}), 4.90 (1H, d, *J* = 10.5 Hz, C$\underline{H}$HOAr), 4.97 (1H, dd, *J* = 9.5, 8.0 Hz, H-2^{Gal}), 5.03 (2H, s, $\underline{CH_2}$ Cbz). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.5, 155.6, 139.0, 137.8, 136.5, 128.4 (x2), 128.3, 128.1, 128.0 (x2), 127.9, 127.8, 127.7 (x3), 127.6 (x2), 127.1, 100.0, 95.4, 78.6, 76.8, 76.3, 75.2, 74.8, 74.3, 74.2, 73.5, 73.4, 73.2, 73.0, 68.3, 67.8, 66.5, 59.5, 38.0, 34.0, 30.9, 29.8, 28.0, 24.8, 20.0 (x2), 18.5 (x2), -2.0, -3.5. ESI-MS: $(C_{61}H_{77}NO_{14}Si+NH_4)^+$ calcd. 1093.5457, found 1093.5458.

Dimethylthexylsilyl 4,6-dibenzyl-2-*O*-levulinoyl-3-(2-naphthyl)-*β*-D-galactopyranosyl-(1→4)-3,6-dibenzyl-2-deoxy-2-(2,2,2-trichloroethylcarbonylamino)-*β*-D-glucopyranosyl-(1→3)- 4,6-dibenzyl-2-*O*-levulinoyl-*β*-D-galactopyranosyl-(1→4)- 3,6-dibenzyl-2-deoxy-2-trifluoroacetamido-*β*-D-glucopyranoside (32). A mixture of donor **29** (7.7 g, 6.02 mmol), acceptor **30** (4.81 g, 4.63 mmol) and flame-dried 4Å molecular sieves was stirred in anhydrous CH_2Cl_2 (25 mL) at RT for 10 min. The solution was then cooled to – 40 ºC, followed by adding TMSOTf (170 µL, 0.92 mmol), and the reaction was stirred at that temperature for 10 min, after which it was warmed to -20 ºC and quenched with Et_3N. The solution was then concentrated, absorbed on silica gel and purified EtOAc:Hexane (3:7) to give the target tetrasaccharide as a white solid. 7.2 g (71%). R_f = 0.3 (EtOAc:Hexane, 3:7). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.19 (6H, 2 x s, Si($\underline{CH_3}$)$_2$), 0.87 – 0.92 (12H, m, C($\underline{CH_3}$)$_2$, CH($\underline{CH_3}$)$_2$), 1.67 (1H, m, $\underline{CH}$(CH_3)$_2$), 2.07 (3H, s, $\underline{CH_3}$ Lev), 2.20 (3H, s, $\underline{CH_3}$ Lev), 2.31 (1H, m, C$\underline{H}$H Lev), 2.40 – 2.76 (6H, m, CH_2 Lev), 2.91 (1H, m, CH$\underline{H}$ Lev), 3.21 (1H, m, H-2^{GlcNHTFA}), 3.24 (1H, m, H-6$_a^{GlcN}$), 3.25 (1H, m), 3.27 (1H, m), 3.30 (1H, m, H-6$_a^{GlcN}$), 3.34 (1H, m), 3.39 (1H, m, H-6$_b^{GlcN}$), 3.42 (1H, m, H-3^{Gal3}), 3.44 (1H, m,), 3.46 (1H, m, H-6$_b^{GlcN}$), 3.51 (1H, m, H-3^{GlcN}), 3.65 (2H, m, H-6$_a^{Gal3}$, H-6$_a^{Gal2}$), 3.70 (1H, m, H-2^{GlcNHTroc}), 3.74 (1H, m), 3.76 (2H, m, H-6$_b^{Gal3}$, H-6$_b^{Gal2}$), 3.82 (2H, m, incl. H-3^{Gal2}), 3.91 (2H, m, 2 x H-4^{GlcN}), 3.95 (1H, d, *J* = 2.4 Hz, H-4^{Gal}), 4.15 (2H, m, 2 x C$\underline{H}$HOAr), 4.26 (2H, m, 2 x C$\underline{H}$HOAr), 4.40 (2H, m, C$\underline{H}$HOAr, H-1^{GlcNHTFA}), 4.45 – 4.71 (12H, m, $\underline{CH_2}$OAr, $\underline{CH_2}$ Troc), 4.45 (1H, m, H-1^{Gal2}), 4.50 (1H, m, H-1^{Gal3}), 4.62 (1H, m, H-1^{GlcNHTroc}), 4.82 (2H, m, 2 x C$\underline{H}$HOAr), 4.93 – 5.01 (3H, m, 3 x C$\underline{H}$HOAr), 5.08 (1H, d, *J* = 12.5 Hz, C$\underline{H}$HOAr), 5.23 (1H, dd, *J* = 9.6, 7.8 Hz, H-2^{Gal2}), 5.37 (1H, dd, *J* = 10.2, 7.8 Hz, H-2^{Gal3}), 7.04 – 7.94 (47H, m, Ar-H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 201.1, 171.5, 170.9, 154.5, 139.2 (x2), 138.7, 138.5, 138.1 (x2), 138.0, 137.9, 135.5, 133.2, 133.0, 128.4, 128.3 (x3), 128.2 (x2), 128.1 (x2), 128.0 (x2), 127.8 (x3), 127.7 (x3), 127.6 (x2), 127.5, 127.4, 127.2, 127.1, 126.8, 126.2 (x2), 126.0, 125.5, 101.3, 100.6, 100.4, 96.8, 81.0, 80.2, 80.0, 78.0, 77.2, 76.0, 75.9, 75.3 (x2), 75.0, 74.9, 74.7, 74.6, 74.3, 73.7, 73.4 (x3), 73.1, 72.6, 72.5, 71.7, 68.5, 68.3, 68.1, 67.9, 57.6, 37.7, 37.6, 33.9, 30.1, 29.8, 27.9, 27.7, 24.8, 20.0, 19.9, 18.5, 18.4, -2.1, -3.3. ESI-MS: $(C_{114}H_{130}Cl_3F_3N_2O_{26}Si+NH_4)^+$ calcd. 2150.8043, found 2150.8057.



(*N*-Phenyl)-2,2,2-trifluoroacetimidate-4,6-dibenzyl-2-*O*-levulinoyl-3-(2-naphthyl)-*β*-D-galactopyranosyl-(1→4)-3,6-dibenzyl-2-deoxy-2-(2,2,2-trichloroethylcarbonylamino)-*β*-D-glucopyranosyl-(1→3)- 4,6-dibenzyl-2-*O*-levulinoyl-*β*-D-galactopyranosyl-(1→4)- 3,6-dibenzyl-2-deoxy-2-trifluoroacetamido-D-glucopyranoside (34). Tetrasaccharide **32** (7.2 g, 3.37 mmol) was dissolved in pyridine (20 mL), followed by adding a pre-diluted solution of HF-pyridine (15 mL of HF-py in 20 mL of pyridine) and the reaction was left stirring at RT overnight. The mixture was then diluted with CH_2Cl_2 (100 mL) and H_2O (100 mL). The organic phase was then washed with sat. $NaHCO_3$ (100 mL), dried and concentrated to give a crude lactol **35** which was used without further purification. This material was then dissolved in anhydrous CH_2Cl_2 (50 mL), after which the solution was cooled to 0 ºC, followed by adding $F_3CC(NPh)Cl$ (812 µL) and DBU (563 µL). The solution was stirred for 10 min, after which it was concentrated and chromatographed using EtOAc:Hexane (0:1 to 3:7) to provide the imidate as a white solid. 6.3 g (87%, over two steps). ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.07 (3H, s, $\underline{CH_3}$ Lev), 2.18 – 2.21 (3H, s x 2, $\underline{CH_3}$ Lev *α* and *β* isomers), 2.40 – 2.76 (6H, m, $\underline{CH_2}$ Lev), 2.79 – 3.00 (2H, m, C$\underline{H}$H Lev), 3.26 (1H, m, H-6$_a^{GlcN}$), 3.35 – 3.52 (m, incl. H-6$^{GlcNHTroc+GlcNHTFA}$), 3.43 (1H, m H-3^{Gal2}), 3.64 – 4.05 (m), 3.71 (1H, m, H-2^{GlcNHTroc}), 3.75 (1H, m, H-3^{Gal2}), 3.79 (1H, m, H-3^{Gal2}), 4.17 – 4.74 (m), 4.21 (1H, m, H-2^{GlcNHTFA}), 4.28 (1H, m, H-2^{GlcNHTFA}), 4.35 (1H, m, H-1^{Gal2}), 4.36 (1H, m, H-1^{Gal2}), 4.52 (1H, m, H-1^{Gal3}), 4.63 (1H, m, H-1^{GlcNHTroc}), 4.82 (2H, m, 2 x C$\underline{H}$HOAr), 4.91 – 5.08 (m, C$\underline{H}$HOAr), 5.20 (1H, br t, H-2^{Gal2}), 5.27 (1H, dd, *J* = 9.6, 7.8 Hz,



H-2^{Gal2}), 5.37 (1H, m, H-2^{Gal3}), 6.22 (1H, d, J = 7.7 Hz, H-1^{GlcNHTFA}), 6.75 (2H, d, Ar-H), 7.07 – 7.88 (m, Ar-H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 206.2, 171.5, 139.1, 138.7, 137.9, 137.7, 135.5, 133.2, 133.0, 128.7, 128.5, 128.4 (x3), 128.3 (x2), 128.2, 128.1 (x2), 128.0 (x2), 127.9, 127.8 (x3), 127.7 (x2), 127.6 (x5), 127.4, 127.3, 127.1, 126.9, 126.8, 126.2 (x3), 126.0 (x2), 125.5, 119.2, 103.2, 101.9, 100.6, 100.2, 95.9, 80.2, 77.2, 75.5, 75.0, 74.6, 73.9, 73.6, 73.5, 73.4 (x3), 73.1, 72.6, 72.4, 71.9, 71.7, 71.0, 69.0, 68.4, 68.2, 67.9, 67.0, 65.5, 57.6, 37.7 (x2), 37.5, 30.1, 29.8, 27.9.

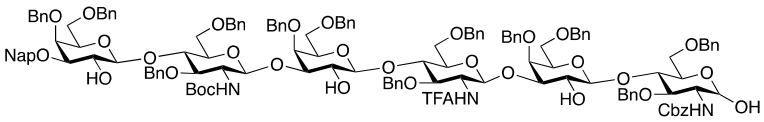
Dimethylthexylsilyl 4,6-dibenzyl-2-*O*-levulinoyl-3-(2-naphthyl)-*β*-D-galactopyranosyl-(1→4)-3,6-dibenzyl-2-deoxy-2-(2,2,2-trichloroethylcarbonylamino)-*β*-D-glucopyranosyl-(1→3)-4,6-dibenzyl-2-*O*-levulinoyl-*β*-D-galactopyranosyl-(1→4)-3,6-dibenzyl-2-deoxy-2-trifluoroacetamido-D-glucopyranoside-(1→3)-4,6-dibenzyl-2-*O*-levulinoyl-*β*-D-galactopyranosyl-(1→4)-2-(benzyloxycarbonylamino)-3,6-dibenzyl-2-deoxy-*β*-D-glucopyranoside (35). A mixture of tetrasaccharide imidate **34** (3.5 g, 1.61 mmol) and acceptor **31** (2.1 g, 1.94 mmol) was stirred in anhydrous CH_2Cl_2 (20 mL) in the presence of flame-dried 4Å molecular sieves at RT for 10 min. The solution was then cooled to – 30 ºC upon which TMSOTf (60 µL, 0.322 mmol) was added and the mixture was stirred at that temperature for 15 min, after which the reaction was quenched with Et_3N, concentrated, absorbed on silica gel and purified using EtOAc:Toluene (1:4 to 3:7) to give the desired hexasaccharide as a white solid. 4.32 g (88%). R_f = 0.6 (EtOAc:Hexane, 1:1). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.10 (6H, 2 x s, Si($\underline{CH_3}$)$_2$), 0.78 – 0.88 (12H, m, C($\underline{CH_3}$)$_2$, CH($\underline{CH_3}$)$_2$), 1.59 (1H, m, $\underline{CH}$(CH_3)$_2$), 2.06 (3H, s, $\underline{CH_3}$ Lev), 2.12 (3H, s, $\underline{CH_3}$ Lev), 2.16 (3H, s, $\underline{CH_3}$ Lev), 2.36 – 2.74 (10H, m, CH_2 Lev), 2.83 (1H, m, C$\underline{H}$HLev), 2.99 (1H, m, C$\underline{H}$HLev), 3.21 (1H, m H-2^{GlcNHCbz}), 3.24 (2H, m, H-6^{GlcN}), 3.32 – 3.57 (m, incl. H-6^{GlcN}), 3.43 (1H, m, H-3^{Gal3}), 3.54 (1H, m, H-3^{GlcN}), 3.64 – 3.84 (m, incl. H-6^{Gal1,2,3}), 3.71 (1H, m, H-2^{GlcNHTroc}), 3.80 (1H, m, H-3$^{Gal1or\,2}$), 3.85 – 3.99 (m), 3.93 (1H, m, H-4^{GlcN}), 3.95 (1H, m, H-3^{Gal}), 3.97 (1H, br d, H-4^{Gal3}), 4.08 (2H, m, 2 x C$\underline{H}$HOAr), 4.14 (1H, m, H-2^{GlcNHTFA}), 4.16 – 4.33 (m, C$\underline{H_2}$OAr), 4.42 (2H, m, H-1^{Gal1,2}), 4.39 – 4.58 (m, $\underline{CH_2}$OAr), 4.51 (1H, m, H-1^{Gal3}), 4.59 – 4.70 (m, $\underline{CH_2}$OAr), 4.62 (1H, m, H-1^{GlcNHTroc}), 4.74 (1H, d, J = 8.4 Hz, H-1^{GlcNHTFA}), 4.78 – 4.92 (m, $\underline{CH_2}$OAr), 4.85 (1H, m, H-1^{GlcNHCbz}), 4.93 – 5.10 (m, $\underline{CH_2}$OAr, $\underline{CH_2}$ Cbz), 5.25 (1H, dd, J = 10.8, 9.2 Hz, H-2^{Gal1or2}), 5.30 (1H, dd, J = 10.1, 8.3 Hz, H-2^{Gal1or2}), 5.37 (1H, dd, J = 9.8, 7.7 Hz, H-2^{Gal3}), 6.19 (1H, d, J = 8.7 Hz, NH^{Troc}), 6.99 – 7.56 (m, Ar-H), 7.62 (1H, d, J = 9.3 Hz, NH^{TFA}), 7.76 – 7.87 (m, Ar-H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 210.8, 209.2, 206.2, 171.6, 171.1, 170.6, 157.7, 157.4, 155.6, 154.5, 139.2 (x3), 138.9, 138.8, 138.7, 138.3, 138.1, 138.0 (x2), 137.9, 137.8, 136.5, 135.5, 133.2, 133.0, 128.5 (x2), 128.4 (x2), 128.3 (x2), 128.2, 128.1, 128.0 (x2), 127.9 (x4), 127.8 (x3), 127.7 (x2), 127.6 (x2), 127.5 (x2), 127.4, 127.2 (x2), 127.1, 127.0, 126.9, 126.3, 126.2, 126.0, 125.5, 101.4, 100.7, 100.3, 99.9, 80.2, 80.0, 79.7, 77.2, 76.5, 76.4, 75.9, 75.7, 75.6, 75.2, 75.1, 75.0, 74.9, 74.8, 74.6, 74.4, 73.8, 73.7, 73.5, 73.5, 73.4, 73.3, 73.2, 72.6, 72.5, 71.8, 68.6, 68.3, 67.9, 66.6, 59.2, 57.6, 55.2, 37.8, 37.7, 34.0, 30.1, 29.8 (x2), 27.9, 27.7, 24.8, 20.0 (x2), 18.5 (x2), -2.0, -3.5. MALDI-MS ($C_{167}H_{187}Cl_3F_3N_3NaO_{39}Si$) calcd. 3071.1426, found 3071.1384.

Dimethylthexylsilyl 4,6-dibenzyl-2-*O*-levulinoyl-3-(2-naphthyl)-*β*-D-galactopyranosyl-(1→4)-2-(*t*-butyloxycarbonylamino)-3,6-dibenzyl-2-deoxy-*β*-D-glucopyranosyl-(1→3)-4,6-dibenzyl-2-*O*-levulinoyl-*β*-D-galactopyranosyl-(1→4)-3,6-dibenzyl-2-deoxy-2-trifluoroacetamido-D-glucopyranoside-(1→3)-4,6-dibenzyl-2-*O*-levulinoyl-*β*-D-galactopyranosyl-(1→4)-2-(benzyloxycarbonylamino)-3,6-dibenzyl-2-deoxy-*β*-D-glucopyranoside (37). To a solution of hexasaccharide **35** (3.76 g, 1.23 mmol) in THF (50 mL) was added acetic acid (2 mL) and Zn dust (15.0 g) and the resulting reaction mixture was stirred at RT for 1 h, after which it was filtered, diluted with CH_2Cl_2 (100 mL) and washed with sat. $NaHCO_3$. The organic layer was then dried and concentrated to give the intermediate amine as a white solid. This material was then dissolved in THF (50 mL), followed by adding Et_3N (520 µL, 3.69 mmol) and Boc_2O (2.7 g, 12.3 mmol). The reaction was left stirring at RT for 2 h, after which it was concentrated, absorbed on silica gel and purified using EtOAc:Toluene (3:7) to give the desired product as white solid. 2.9 g (77%, over two steps). R_f = 0.5 (EtOAc:Toluene, 3:7). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.10 (6H, 2 x s, Si($\underline{CH_3}$)$_2$), 0.78 – 0.88 (12H, m, C($\underline{CH_3}$)$_2$, CH($\underline{CH_3}$)$_2$), 1.40 (9H, s, 3x CH_3 Boc), 1.59 (1H, m, $\underline{CH}$(CH_3)$_2$), 2.06 (3H, s, $\underline{CH_3}$ Lev), 2.12 (3H, s, $\underline{CH_3}$ Lev), 2.16 (3H, s, $\underline{CH_3}$ Lev), 2.36 – 2.74 (10H, m, CH_2 Lev), 2.83 (1H, m, C$\underline{H}$HLev), 2.99 (1H, m, C$\underline{H}$HLev), 3.20 (1H, m, H-2^{GlcNHCbz}), 3.21 (2H, H-6^{GlcN}). 3.25 – 3.58 (m, incl. H-6^{GlcN}), 3.43 (1H, m, H-3^{Gal3}), 3.52 (2H, m, H-2^{GlcNHBoc}, H-3^{GlcN}), 3.63 – 3.85 (m, incl H-6^{Gal1,2,3}), 3.71 (1H, m, H-3^{Gal1or2}), 3.86 – 3.97 (m), 3.95 (3H, m, H-3^{Gal1or2}, 2 x H-4^{GlcN}), 3.97 (1H, s, H-4^{Gal3}), 4.06 (1H, d, CH$\underline{H}$OAr), 4.14 (1H, m, H-2^{GlcNHTFA}), 4.20 – 4.71 (m, incl. $\underline{CH_2}$OAr), 4.43 (2H, m, H-1^{Gal1,2}), 4.50 (1H, m, H-1^{Gal3}), 4.65 (1H, m, H-1^{GlcNHBoc}), 4.75 (1H, d, *J* = 7.8 Hz, H-1^{GlcNHTFA}), 4.80 – 4.89 (m, incl. $\underline{CH_2}$OAr), 4.85 (1H, m, H-1^{GlcNHCbz}), 4.94 – 5.11 (m, $\underline{CH_2}$OAr, $\underline{CH_2}$ Cbz), 5.26 (1H, m, H-2^{Gal1or2}), 5.30 (1H, H-2^{Gal1or2}), 5.37 (1H, dd, *J* = 9.6, 7.6 Hz, H-2^{Gal3}), 5.53 (1H, br d, NH^{Boc}), 7.04 – 7.50 (m, Ar-H), 7.62 (1H, d, *J* = 9.6 Hz, NH^{TFA}), 7.76 – 7.87 (m, Ar-H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 128.2, 127.9, 127.8, 126.2, 126.1, 125.5, 100.6, 100.4, 99.9, 80.2, 77.1, 76.5, 76.2, 75.6, 75.1, 74.9, 74.8, 74.7 (x4), 74.4, 73.6, 73.5 (x4), 73.4 (x3), 73.3 (x2), 73.2, 72.7, 72.4, 71.8 (x3), 68.6, 68.3 (x3), 68.2, 68.1, 66.5, 59.1, 57.0, 55.2, 37.69, 29.9, 29.7 (x2), 28.3, 27.8, 20.0, 18.5, -2.1, -3.6. ESI-MS: $(C_{169}H_{194}F_3N_3O_{39}Si+2NH_4)^{+2}$ calcd. 1505.1849, found 1505.1830.

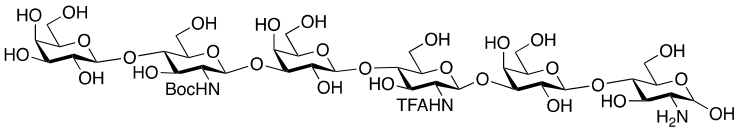
Dimethylthexylsilyl 4,6-dibenzyl-3-(2-naphthyl)-*β*-D-galactopyranosyl-(1→4)-2-(*t*-butyloxycarbonylamino)-3,6-dibenzyl-2-deoxy-*β*-D-glucopyranosyl-(1→3)-4,6-dibenzyl-*β*-D-galactopyranosyl-(1→4)-3,6-dibenzyl-2-deoxy-2-trifluoroacetamido-D-glucopyranoside-(1→3)-4,6-dibenzyl-*β*-D-galactopyranosyl-(1→4)-2-(benzyloxycarbonylamino)-3,6-dibenzyl-2-deoxy-*β*-D-glucopyranoside (38). To a solution of hexasaccharide **37** (2.9 g, 0.97 mmol) in CH_2Cl_2:MeOH (1:1, 40 mL) was added hydrazine acetate (2.7 g, 29.2 mmol), and the mixture was stirred at RT for 6 h, after which it was concentrated, diluted with CH_2Cl_2 (100 mL) and washed with water (100 mL). The organic phase was dried and concentrated to give the crude product, which was purified on silica gel using acetone:hexane (1:1) to provide the desired hexasaccharide as a white solid. 2.53 g (95%). $[\alpha]_D^{24}$ -0.7 (c=1, $CHCl_3$). R_f = 0.3 (EtOAc:Toluene, 3:7). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.10 (6H, 2 x s, Si($\underline{CH_3}$)$_2$), 0.78 – 0.88 (12H, m, C($\underline{CH_3}$)$_2$, CH($\underline{CH_3}$)$_2$), 1.40 (9H, s, 3x CH_3 Boc), 1.59 (1H, m, $\underline{CH}$(CH_3)$_2$), 3.24 (1H, m, H-2^{GlcNHCbz}), 3.26 – 3.59 (m, incl. H-6^{GlcN}), 3.39 (1H, m, H-2^{GlcNHBoc}), 3.60 – 4.08 (m, incl. H-6^{Gal1,2,3}), 3.76 (1H, m, H-2^{Gal}), 3.84 (1H, m, H-2^{Gal}), 3.96 (1H, m, H-2^{Gal}), 3.87 (1H, m, H-2^{GlcNHTFA}), 4.16 – 4.36 (m, $\underline{CH_2}$OAr), 4.43 – 4.73 (m, incl. $\underline{CH_2}$OAr), 4.48 (1H, m, H-1^{Gal}), 4.52 (1H, m, H-1^{Gal}), 4.56 (1H, m, H-1^{Gal}), 4.71 – 4.98 (m, incl. CH_2OAr), 4.76 (1H, m, H-1^{GlcNHTFA}), 4.82 (1H, m, H-1^{GlcNHBoc}), 4.85 (1H, m, H-1^{GlcNHCbz}), 5.03 (2H, m, $\underline{CH_2}$ Cbz), 7.06

– 7.88 (m, Ar-H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 139.1, 138.8, 138.8, 138.7, 138.4, 138.0, 138.0, 137.9, 137.9, 137.8, 137.7, 137.4, 135.6, 133.3, 133.0, 129.0, 129.0, 128.4, 128.4, 128.4, 128.4, 128.3, 128.3, 128.3, 128.2, 128.1, 128.1, 128.1, 128.0, 128.0, 127.9, 127.9, 127.8, 127.8, 127.7, 127.7, 127.7, 127.6, 127.6, 127.6, 127.5, 127.4, 127.3, 127.3, 127.2, 127.2, 126.3, 126.2, 125.9, 125.5, 125.3, 105.0, 103.7, 103.4, 102.7, 101.5, 95.6, 82.9, 82.0, 81.7, 80.2, 80.0, 77.2, 76.9, 76.4, 75.2, 75.2, 74.7, 74.7, 74.7, 74.7, 74.6, 74.5, 74.4, 74.3, 74.2, 73.9, 73.6, 73.6, 73.5, 73.5, 73.4, 73.4, 73.1, 72.9, 72.3, 72.0, 68.7, 68.4, 68.3, 68.2, 66.7, 60.3, 56.3, 34.0, 28.3, 24.8, 20.0, 19.9, 18.5, 18.5, -2.0, -3.5. ESI-MS: $(C_{154}H_{176}F_3N_3O_{33}Si+2NH_4)^{+2}$ calcd. 1358.1297, found 1358.1345.

4,6-Dibenzyl-3-(2-naphthyl)-*β*-D-galactopyranosyl-(1→4)-2-(*t*-butyloxycarbonylamino)-3,6-dibenzyl-2-deoxy-*β*-D-glucopyranosyl-(1→3)-4,6-dibenzyl-*β*-D-galactopyranosyl-(1→4)-3,6-dibenzyl-2-deoxy-2-trifluoroacetamido-D-glucopyranoside-(1→3)-4,6-dibenzyl-*β*-D-galactopyranosyl-(1→4)-2-(benzyloxycarbonylamino)-3,6-dibenzyl-2-deoxy-*β*-D-glucopyranoside (39). Hexasaccharide **38** (2.5 g, 0.9 mmol) was dissolved in THF (50 mL) followed by adding TBAF (1.5 g, 4.76 mmol) and the mixture was left stirring at RT overnight. The mixture was diluted with CH_2Cl_2 (100 mL) and washed with water (100 mL), and the organic phase was dried and concentrated to give the crude product, which was purified on silica gel using EtOAc:Toluene (1:1) to give the title product as a white solid. 1.89 g (78%). R_f = 0.3 (EtOAc:Toluene, 4:6). $[\alpha]_D^{24}$ +10.9 (c=1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.40 (9H, s, 3x CH_3 Boc), 3.24 – 3.58 (m, incl. H-6^{GlcN}), 3.38 (1H, m, H-2^{GlcN}), 3.60 – 4.07 (m, incl. H-6^{Gal}, H-4^{Gal}, H-4^{GlcN}), 4.13 – 4.33 (m, C<u>H</u>HOAr), 4.42 – 4.66 (m, C<u>H</u>HOAr), 4.44 (2H, m, H-1^{Gal1,2}), 4.50 (1H, m, H-1^{Gal3}), 4.74 – 4.98 (m, C<u>H</u>HOAr), 4.79 (1H, m, H-1^{GlcN}), 5.04 (2H, m, <u>CH_2</u> Cbz), 5.11 (1H, m, H-1^{GlcN}), 7.11 – 7.33 (m, Ar-H), 7.43 – 7.49 (m, Ar-H), 7.75 – 7.85 (m, Ar-H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 156.1, 139.0, 138.7 (x2), 138.0, 137.9, 137.8, 137.7, 135.6, 133.2, 133.0, 128.4 (x3), 128.3 (x2), 128.2 (x2), 128.1 (x3), 128.0, 127.9 (x3), 127.8, 127.7 (x4), 127.6 (x2), 127.5 (x2), 127.4, 127.3, 127.2, 126.3, 126.1, 125.9, 125.5, 103.4, 102.6, 101.4, 92.2, 81.7, 79.1, 77.2, 76.9, 75.4, 74.7, 74.6, 74.4, 74.3, 73.6, 73.5, 73.4 (x3), 73.3, 72.8, 72.3, 72.2, 71.8, 70.3, 68.6, 68.4, 68.1, 66.9, 57.8, 28.3. ESI-MS: $(C_{146}H_{158}F_3N_3O_{33}+2NH_4)^{+2}$ calcd. 1287.0708, found 1287.0717.



β-D-Galactopyranosyl-(1→4)-2-(*t*-butyloxycarbonylamino)-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-2-deoxy-2-trifluoroacetamido-D-glucopyranoside-(1→3)-β-D-galactopyranosyl-(1→4)-2-amino-2-deoxy-α,β-D-glucopyranoside (1). The starting hexasaccharide **39** (1.7 g, 0.66 mmol) was dissolved in methanol (30 mL), after which H_2O (3 mL) and AcOH (0.5 mL) were added. $Pd(OH)_2$ (20% on carbon, Degussa type) (500 mg) was then, and the reaction mixture was left stirring under the atmosphere of hydrogen at 1 atm for 16 h. The mixture was then filtered through Celite, concentrated, and passed through Biogel P-2 column. Fractions containing the desired product were pooled and lyophilized to afford the desired hexasaccharide as a white solid (675 mg, 85%). R_f = 0.2 (EtOAc:MeOH:H_2O, 3:2:1).



^{1}H (400 MHz, D_2O): δ (ppm)

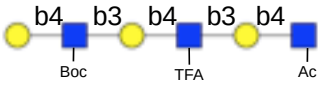
	H-1	H-2	H-3	H-4	H-5	H-6
GlcN-1α	5.42 d, *J* = 2.5 Hz	3.27 dd, *J* = 10.3, 2.5 Hz	3.99	3.74	3.88	n/a
GlcN-1β	4.87	2.94 t, *J* = 9.63 Hz	3.71	3.64	n/a	n/a
Gal-1	4.45	3.60	3.74	4.19	n/a	n/a
GlcN-2	4.84	3.90	3.62	n/a	n/a	n/a
Gal-2	4.51	3.64	3.78	4.15	n/a	n/a
GlcN-3	4.74	3.47	3.71	3.57	n/a	n/a
Gal-3	4.49	3.59	n/a	3.93	n/a	n/a

^{13}C (101 MHz, D_2O): δ (ppm)

	C-1	C-2	C-4
GlcN-1α	89.3	54.3	n/a
GlcN-1β	93.6	56.7	n/a
Gal-1	103.1	n/a	68.1
GlcN-2	102.0	55.8	n/a
Gal-2	103.1	n/a	68.1
GlcN-3	102.5	56.5	n/a
Gal-3	103.1	n/a	68.5

MALDI TOF-MS *m/z* $C_{143}H_{72}F_3N_3O_{31}Na$ calcd 1206.4000, found 1206.9751

β-D-Galactopyranosyl-(1→4)-2-deoxy-2-(*t*-butyloxycarbonylamino)-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-2-deoxy-2-trifluoroacetamido-D-glucopyranoside-(1→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-α,β-D-glucopyranoside (44). Hexasaccharide **1** (40 mg, 0.034 mmol) was dissolved in H_2O (200 μL) followed by adding solid $NaHCO_3$ (29 mg, 0.34 mmol) and Ac_2O (34 μL). The reaction mixture was sonicated for 2 min, after which it was further incubated for 1 h at 37 ºC. The resulting solution was loaded on Biogel P-2 column and further eluted with H_2O. Fractions containing the desired product were pooled and lyophilized to give the hexasaccharide as a white solid (38 mg, 92%).



^{1}H (400 MHz, D_2O): δ (ppm)

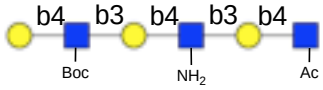
	H-1	H-2	H-3	H-4	H-5	H-6
GlcN-1α	5.22 d *J* = 2.0 Hz	3.91	3.75	n/a	n/a	n/a
GlcN-1β	4.73	3.71	n/a	n/a	n/a	n/a
Gal-1	4.45	3.60	3.74	4.19 d *J* = 3.1 Hz	n/a	n/a
GlcN-2	4.84	3.89	3.64	n/a	n/a	n/a
Gal-2	4.51	3.64	3.78	4.15 d *J* = 3.1 Hz	n/a	n/a
GlcN-3	4.75	3.47 dd *J* = 9.3 Hz	3.72	3.59	n/a	n/a
Gal-3	4.49	3.59	n/a	3.93	n/a	n/a

^{13}C (101 MHz, D_2O): δ (ppm)

	C-1	C-2	C-4
GlcN-1α	90.7	53.8	n/a
GlcN-1β	94.8	56.5	n/a
Gal-1	102.9	n/a	68.3
GlcN-2	102.3	55.9	n/a
Gal-2	102.9	n/a	68.3
GlcN-3	102.6	n/a	n/a
Gal-3	102.9	56.7	68.8

MALDI TOF-MS *m/z* calcd $C_{45}H_{74}F_3N_3NaO_{32}$ $(M+Na)^+$ 1248.4105, found 1248.5070.

***β*-D-Galactopyranosyl-(1→4)-2-deoxy-2-(*t*-butyloxycarbonylamino)-*β*-D-glucopyranosyl-(1→3)-*β*-D-galactopyranosyl-(1→4)-2-amino-2-deoxy-D-glucopyranoside-(1→3)-*β*-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-*α*,*β*-D-glucopyranoside (4).** Hexasaccharide **44** (38 mg, 0.034 mmol) was dissolved in H_2O (1 mL) followed by adding 30% NH_3 solution (200 µL). The reaction mixture was sonicated for 2 min, after which it was left standing at RT for 3 h. The resulting solution was lyophilized to give the hexasaccharide as a white solid. 35 mg (quant). Standard acetylation with $Ac_2O/NaHCO_3$ affords compound **7**, which is further desalted on Biogel P2, followed lyophilization to give a white solid.



^{1}H (600 MHz, D_2O) for **4**: δ (ppm)

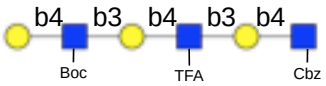
	H-1	H-2	H-3	H-4	H-5	H-6
GlcN-1*α*	5.21 d, *J* = 2.5 Hz	3.91	3.77	3.99	n/a	n/a
GlcN-1*β*	4.72	3.72	3.73	3.98	n/a	n/a
Gal-1	4.53	3.70	3.86	4.20	n/a	n/a
GlcN-2	4.75	2.90 br d	3.66		n/a	n/a
Gal-2	4.47	3.65	3.78	4.14	3.66	n/a
GlcN-3	4.74	3.46	3.69	3.98	3.59	n/a
Gal-3	4.48	3.55	3.67	3.93 d, *J* = 3.55	3.56	n/a

^{13}C (150 MHz, D_2O): δ (ppm)

	C-1	C-2	C-4
GlcN-1*α*	90.6	53.7	n/a
GlcN-1*β*	94.8	56.3	n/a
Gal-1	102.6	n/a	68.3
GlcN-2	102.6	56.2	n/a
Gal-2	103.0	n/a	68.3
GlcN-3	103.0	56.5	n/a
Gal-3	103.0	n/a	68.5

MALDI TOF-MS *m/z* calcd $C_{43}H_{75}N_3NaO_{31}$ $(M+Na)^+$ 1152.4282, found 1152.4485.

β-D-Galactopyranosyl-(1→4)-2-deoxy-2-(*t*-butyloxycarbonylamino)-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-2-deoxy-2-trifluoroacetamido-D-glucopyranoside-(1→3)-β-D-galactopyranosyl-(1→4)-2-(benzyloxycarbonylamino)-2-deoxy-α,β-D-glucopyranoside (40). Hexasaccharide **1** (70 mg, 0.059 mmol) was dissolved in H_2O (500 μL) followed by adding solid $NaHCO_3$ (25 mg, 0.29 mmol) and CbzOSu (147 mg, 0.59 mmol). The reaction mixture was sonicated for 2 min, after which it was further incubated for 1 h at 37 °C. The resulting solution was loaded on Biogel P-2 column and further eluted with H_2O. Fractions containing the desired product were pooled and lyophilized to give the hexasaccharide as a white solid (77 mg, quant).



^{1}H (400 MHz, D_2O): δ (ppm)

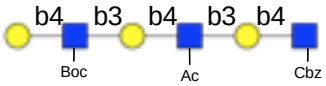
	H-1	H-2	H-3	H-4	H-5	H-6
GlcN-1α	5.22 br s	3.69	3.72	3.99	n/a	n/a
GlcN-1β	4.72	3.44	n/a	n/a	n/a	n/a
Gal-1	4.45	3.60	3.74	4.19	n/a	n/a
GlcN-2	4.84	3.89	n/a	n/a	n/a	n/a
Gal-2	4.51	3.64	3.78	4.15	n/a	n/a
GlcN-3	4.75	3.47	n/a	n/a	n/a	n/a
Gal-3	4.49	3.59	3.67	3.94	3.56	n/a

^{13}C (101 MHz, D_2O): δ (ppm)

	C-1	C-2	C-4
GlcN-1α	91.0	55.3	n/a
GlcN-1β	94.9	57.0	n/a
Gal-1	102.8	n/a	68.2
GlcN-2	101.9	55.8	n/a
Gal-2	102.8	n/a	68.2
GlcN-3	102.4	56.7	n/a
Gal-3	102.8	n/a	68.6

MALDI TOF-MS *m/z* calcd $C_{51}H_{78}F_3N_3NaO_{33}$ $(M+Na)^+$ 1340.4367, found 1340.5149.

***β*-D-Galactopyranosyl-(1→4)-2-deoxy-2-(*t*-butyloxycarbonylamino)-*β*-D-glucopyranosyl-(1→3)-*β*-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-D-glucopyranoside-(1→3)-*β*-D-galactopyranosyl-(1→4)-2-(benzyloxycarbonylamino)-2-deoxy-*α*,*β*-D-glucopyranoside (43).** Hexasaccharide **40** (30 mg, 0.022 mmol) was dissolved in H_2O (500 µL) followed by adding 30% NH_3 solution (200 µL). The reaction mixture was left standing at RT for 3 h, after which it was directly lyophilized. The resulting white solid was then re-dissolved in H_2O (200 µL) followed by adding solid $NaHCO_3$ (18 mg, 0.22 mol) and Ac_2O (22 µL, 0.22 mmol). The reaction mixture was sonicated for 2 min, after which it was further incubated for 1 h at 37 ºC. The resulting solution was loaded on Biogel P-2 column and further eluted with H_2O. Fractions containing the desired product were pooled and lyophilized to give the hexasaccharide as a white solid (25 mg, 87%).



^{1}H (600 MHz, D_2O): δ (ppm)

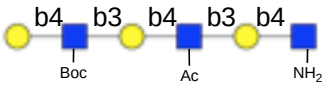
	H-1	H-2	H-3	H-4	H-5	H-6
GlcN-1*α*	5.21 d, *J* = 3.8 Hz	3.68	3.72	3.97	n/a	n/a
GlcN-1*β*	4.71	3.44 br s	n/a	n/a	n/a	n/a
Gal-1	4.45	3.60	3.74	4.19 d, *J* = 3.8 Hz	n/a	n/a
GlcN-2	4.71	3.81	3.60	3.98	n/a	n/a
Gal-2	4.51	3.64	3.78	4.15 d, *J* = 3.5 Hz	n/a	n/a
GlcN-3	4.74 d, *J* = 8.6 Hz	3.47 dd, *J* = 10.2, 8.7 Hz	3.70	3.98	3.59	n/a
Gal-3	4.49	3.59	3.67	3.94 d, *J* = 3.5 Hz	3.56	n/a

^{13}C (150 MHz, D_2O): δ (ppm)

	C-1	C-2	C-4
GlcN-1*α*	91.0	55.2	n/a
GlcN-1*β*	95.1	58.0	n/a
Gal-1	102.9	n/a	68.4
GlcN-2	102.7	55.2	n/a
Gal-2	102.9	n/a	68.4
GlcN-3	102.5	56.6	n/a
Gal-3	102.9	n/a	68.6

MALDI TOF-MS *m/z* calcd $C_{51}H_{81}N_3NaO_{33}$ $(M+Na)^+$ 1286.4650, found 1287.1291.

***β*-D-Galactopyranosyl-(1→4)-2-deoxy-2-(*t*-butyloxycarbonylamino)-*β*-D-glucopyranosyl-(1→3)-*β*-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-D-glucopyranoside-(1→3)-*β*-D-galactopyranosyl-(1→4)-2-amino-2-deoxy-*α*,*β*-D-glucopyranoside (3).** Hexasaccharide **43** (25 mg, 0.019 mmol) was dissolved in H_2O (300 μL) after which $Pd(OH)_2$ (20% on carbon, Degussa type) (10 mg) was introduced and the reaction was stirred under the atmosphere of hydrogen at 1 atm for 2 h. The mixture was then filtered through Celite and lyophilized to provide pure product as a white solid (23 mg, quant).



^{1}H (600 MHz, D_2O): δ (ppm)

	H-1	H-2	H-3	H-4	H-5	H-6
GlcN-1*α*	5.42 d, *J* = 3.6 Hz	3.28 dd, *J* = 10.0, 2.7 Hz	3.99	3.74	3.88	n/a
GlcN-1*β*	4.90 br d	2.97 br t	3.71	n/a	n/a	n/a
Gal-1	4.45	3.60	3.74	4.19 d, *J* = 3.2 Hz	n/a	n/a
GlcN-2	4.71	3.81	3.60	3.98	n/a	n/a
Gal-2	4.51	3.64	3.78	4.15 d, *J* = 2.8 Hz	n/a	n/a
GlcN-3	4.74 d, *J* = 8.1 Hz	3.47 dd, *J* = 10.1, 8.8 Hz	3.70	3.98	3.59	n/a
Gal-3	4.49	3.59	3.67	3.94 d, *J* = 3.3 Hz	3.56	n/a

^{13}C (150 MHz, D_2O): δ (ppm)

	C-1	C-2	C-4
GlcN-1*α*	89.1	54.0	n/a
GlcN-1*β*	93.2	56.7	n/a
Gal-1	102.9	n/a	68.3
GlcN-2	102.7	55.2	n/a
Gal-2	102.9	n/a	68.3
GlcN-3	102.4	56.6	n/a
Gal-3	102.9	n/a	68.6

MALDI TOF-MS *m/z* calcd $C_{43}H_{75}N_3NaO_{31}$ $(M+Na)^+$ 1152.4282, found 1152.9528.

***β*-D-Galactopyranosyl-(1→4)-2-acetamido-2-deoxy-*β*-D-glucopyranosyl-(1→3)-*β*-D-galactopyranosyl-(1→4)-2-deoxy-2-trifluoroacetamido-D-glucopyranoside-(1→3)-*β*-D-galactopyranosyl-(1→4)-2-(benzyloxycarbonylamino)-2-deoxy-*α*,*β*-D-glucopyranoside (41).** Hexasaccharide **40** (32 mg, 0.025 mmol) was dissolved in 20% aq TFA (300 µL) and the resulting solution was then left standing at RT for 3h, after which it was lyophilized to provide the free amine as a white solid. This material was then dissolved in H_2O (200 µL) followed by adding solid $NaHCO_3$ (21 mg, 0.25 mmol) and Ac_2O (25 µL, 0.25 mmol). The reaction mixture was sonicated for 2 min, after which it was further incubated for 1 h at 37 ºC. The resulting solution was loaded on Biogel P-2 column and further eluted with H_2O. Fractions containing the desired product were pooled and lyophilized to give the hexasaccharide as a white solid (24 mg, 81%).

^{1}H (400 MHz, D_2O): δ (ppm)

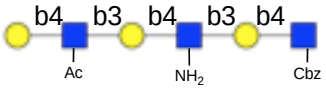
	H-1	H-2	H-3	H-4	H-5	H-6
GlcN-1*α*	5.22 d, *J* = 3.2 Hz	3.69	3.72	3.97	n/a	n/a
GlcN-1*β*	4.72	3.44	n/a	n/a	n/a	n/a
Gal-1	4.45	3.60	3.74	4.18	n/a	n/a
GlcN-2	4.84 d, *J* = 7.8 Hz	3.89	n/a	n/a	n/a	n/a
Gal-2	4.50	3.64	3.78	4.18	n/a	n/a
GlcN-3	4.72 d, *J* = 8.1 Hz	3.83	3.60	3.98	n/a	n/a
Gal-3	4.48	3.59	3.67	3.94	3.56	n/a

^{13}C (101 MHz, D_2O): δ (ppm)

	C-1	C-2	C-4
GlcN-1*α*	90.9	55.3	n/a
GlcN-1*β*	95.0	58.0	n/a
Gal-1	102.9	n/a	68.2
GlcN-2	102.2	55.8	n/a
Gal-2	102.9	n/a	68.2
GlcN-3	102.8	55.2	n/a
Gal-3	102.9	n/a	68.6

MALDI TOF-MS *m/z* calcd $C_{48}H_{72}F_3N_3NaO_{32}$ $(M+Na)^+$ 1282.3949, found 1282.3980.

***β*-D-Galactopyranosyl-(1→4)-2-acetamido-2-deoxy-*β*-D-glucopyranosyl-(1→3)-*β*-D-galactopyranosyl-(1→4)-2-amino-2-deoxy-D-glucopyranoside-(1→3)-*β*-D-galactopyranosyl-(1→4)-2-(benzyloxycarbonylamino)-2-deoxy-*α*,*β*-D-glucopyranoside**



(42). Hexasaccharide **41** (24 mg, 0.019 mmol) was dissolved in H_2O (500 µL) followed by adding 30% NH_3 solution (200 µL). The reaction mixture was left standing at RT for 3 h, after which it was directly lyophilized to provide the desired hexasaccharide as a white solid (23 mg, quant).

^{1}H (600 MHz, D_2O): δ (ppm)

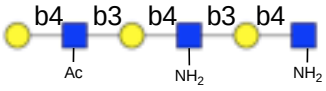
	H-1	H-2	H-3	H-4	H-5	H-6
GlcN-1*α*	5.21	3.69	3.72	3.97	n/a	n/a
GlcN-1*β*	4.73 br s	3.44	n/a	n/a	n/a	n/a
Gal-1	4.53	3.70	3.86	4.20	3.72	n/a
GlcN-2	4.69	2.83	3.66	n/a	n/a	n/a
Gal-2	4.46	3.60	3.74	4.18	3.66	n/a
GlcN-3	4.71	3.81	3.60	3.98	3.59	n/a
Gal-3	4.50	3.55 dd, *J* = 9.5, 7.8 Hz	3.67	3.94	3.56	n/a

^{13}C (150 MHz, D_2O): δ (ppm)

	C-1	C-2	C-4
GlcN-1*α*	90.9	55.3	n/a
GlcN-1*β*	95.1	58.0	n/a
Gal-1	102.6	n/a	68.4
GlcN-2	103.7	56.4	n/a
Gal-2	103.0	n/a	68.4
GlcN-3	102.7	55.1	n/a
Gal-3	102.8	70.9	68.5

MALDI TOF-MS *m/z* calcd $C_{49}H_{79}N_3NaO_{32}$ $(M+Na)^+$ 1244.4544, found 1244.1862.

***β*-D-Galactopyranosyl-(1→4)-2-acetamido-2-deoxy-*β*-D-glucopyranosyl-(1→3)-*β*-D-galactopyranosyl-(1→4)-2-amino-2-deoxy-D-glucopyranoside-(1→3)-*β*-D-galactopyranosyl-(1→4)-2-amino-2-deoxy-*α*,*β*-D-glucopyranoside (2).** Hexasaccharide **42** (23 mg, 0.019 mmol) was dissolved in H_2O (300 μL) after which $Pd(OH)_2$ (20% on carbon, Degussa type) (10 mg) was introduced and the reaction was stirred under the atmosphere of hydrogen at 1 atm for 2 h. The mixture was then filtered through Celite and lyophilized to provide pure product as a white solid (22 mg, quant).



^{1}H (400 MHz, D_2O): δ (ppm)

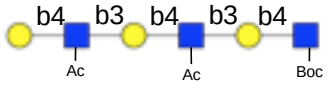
	H-1	H-2	H-3	H-4	H-5	H-6
GlcN-1*α*	5.43 d, *J* = 3.8 Hz	3.25 d, *J* = 10.5, 3.6 Hz	3.97	3.78	n/a	n/a
GlcN-1*β*	4.85	2.90	3.71	3.64	n/a	n/a
Gal-1	4.55 d, *J* = 8.0 Hz	3.74	3.88	4.23 d, *J* = 3.0 Hz	n/a	n/a
GlcN-2	4.84	2.96	3.74	n/a	n/a	n/a
Gal-2	4.48 d, *J* = 8.1 Hz	3.64	3.75	4.19 d, *J* = 3.0 Hz	n/a	n/a
GlcN-3	4.74 d, *J* = 7.9 Hz	3.84	3.62	3.99	n/a	n/a
Gal-3	4.52 d, *J* = 7.9 Hz	3.57	3.71	3.97	3.56	n/a

^{13}C (101 MHz, D_2O): δ (ppm)

	C-1	C-2	C-4
GlcN-1*α*	89.9	54.3	n/a
GlcN-1*β*	94.1	56.6	n/a
Gal-1	102.7	n/a	68.5
GlcN-2	102.4	56.3	n/a
Gal-2	103.1	n/a	68.5
GlcN-3	102.9	55.3	n/a
Gal-3	102.8	71.0	68.6

MALDI TOF-MS *m/z* calcd $C_{38}H_{67}N_3NaO_{29}$ $(M+Na)^+$ 1052.3758, found 1052.3980.

β-D-Galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-D-glucopyranoside-(1→3)-β-D-galactopyranosyl-(1→4)-2-(*t*-butyloxycarbonylamino)-2-deoxy-α,β-D-glucopyranoside (6). Hexasaccharide **41** (40 mg, 0.032 mmol) was dissolved in H_2O (500 µL) and 30% NH_3 solution (200 µL) was added, and the reaction mixture was left standing at RT for 3 h, after which it was directly lyophilized. The resulting solid was dissolved in H_2O (300 µL) and solid $NaHCO_3$ (27 mg, 0.32 mmol) followed by Ac_2O (31 µL, 0.32 mmol). The reaction mixture was sonicated for 2 min, after which it was further incubated for 1 h at 37 °C. The resulting solution was loaded on Biogel P-2 column and further eluted with H_2O. Fractions containing the desired product were pooled and lyophilized to give the intermediate hexasaccharide, which was dissolved in H_2O (300 µL). $Pd(OH)_2$ (20% on carbon, Degussa type) (10 mg) was introduced and the reaction was stirred under the atmosphere of hydrogen at 1 atm for 2 h. The mixture was then filtered through Celite, upon which Boc_2O (70 mg, 0.32 mmol) in 1,4-dioxane (300 µL) was added, and the solution was further incubated at 60 °C for 2h. The reaction mixture was directly lyophilized, re-dissolved in H_2O (200 µL) and loaded on Biogel P-2 column and further eluted with H_2O. Fractions containing the desired product were pooled and lyophilized to give the hexasaccharide as a white solid (24 mg, 64 %).



^{1}H (600 MHz, D_2O): δ (ppm)

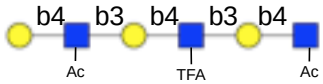
	H-1	H-2	H-3	H-4	H-5	H-6
GlcN-1α	5.20 br d	3.61	n/a	3.96	n/a	n/a
GlcN-1β	4.70	3.36 dd, *J* = 9.4 Hz	3.66	n/a	n/a	n/a
Gal-1	4.47	3.59	3.74	4.16	n/a	n/a
GlcN-2	4.71 d, *J* = 8.8 Hz	3.81	3.60	3.97	n/a	n/a
Gal-2	4.47	3.59	3.74	4.16	n/a	n/a
GlcN-3	4.71 d, *J* = 8.8 Hz	3.81	3.60	3.97	n/a	n/a
Gal-3	4.49	3.55 dd, *J* = 9.7, 7.9 Hz	3.68	3.94	n/a	n/a

^{13}C (150 MHz, D_2O): δ (ppm)

	C-1	C-2	C-4
GlcN-1α	91.0	54.7	n/a
GlcN-1β	95.1	57.6	n/a
Gal-1	102.8	n/a	68.2
GlcN-2	102.7	55.1	n/a
Gal-2	102.8	n/a	68.2
GlcN-3	102.7	55.1	n/a
Gal-3	102.8	70.9	68.4

MALDI TOF-MS *m/z* calcd $C_{45}H_{77}N_3NaO_{32}$ $(M+Na)^+$ 1194.4388, found 1194.9600.

β-D-Galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-2-deoxy-2-trifluoroacetamido-D-glucopyranoside-(1→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-α,β-D-glucopyranoside (45).



Hexasaccharide **1** (40 mg, 0.033 mmol) was dissolved in 20% aq TFA (1 mL) and the reaction mixture was kept at RT for 3 h, after which it was lyophilized. The obtained solid was then dissolved in H_2O (200 µL) followed by adding solid $NaHCO_3$ (27 mg, 0.33 mmol) and Ac_2O (33 µL, 0.33 mmol). The reaction mixture was sonicated for 2 min, after which it was further incubated for 1 h at 37 ºC. The resulting solution was loaded on Biogel P-2 column and further eluted with H_2O. Fractions containing the desired product were pooled and lyophilized to give the target hexasaccharide as a white solid (34 mg, 87%).

^{1}H (400 MHz, D_2O): δ (ppm)

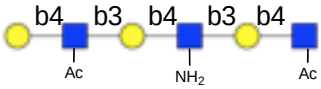
	H-1	H-2	H-3	H-4	H-5	H-6
GlcN-1α	5.24 br s	3.93	3.79	4.00	n/a	n/a
GlcN-1β	4.76	3.74	3.99	n/a	n/a	n/a
Gal-1	4.49	3.62	3.77	4.19 d, *J* = 3.2 Hz	n/a	n/a
GlcN-2	4.86 d, *J* = 7.7 Hz	3.93	3.64	n/a	n/a	n/a
Gal-2	4.49	3.62	3.77	4.21 d, *J* = 3.1 Hz	n/a	n/a
GlcN-3	4.74 d, *J* = 8.0 Hz	3.85	3.60	3.98	3.59	n/a
Gal-3	4.51 d, *J* = 8.1 Hz	3.58	3.67	3.96	3.56	n/a

^{13}C (101 MHz, D_2O): δ (ppm)

	C-1	C-2	C-4
GlcN-1α	90.6	53.8	n/a
GlcN-1β	94.9	56.4	n/a
Gal-1	102.7	n/a	68.1
GlcN-2	102.0	55.7	n/a
Gal-2	102.7	n/a	68.1
GlcN-3	102.7	55.2	n/a
Gal-3	102.7	70.9	68.6

MALDI TOF-MS *m/z* calcd $C_{42}H_{68}F_3N_3NaO_{31}$ $(M+Na)^+$ 1190.3687, found 1190.8792.

β-D-Galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-2-amino-2-deoxy-D-glucopyranoside-(1→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-α,β-D-glucopyranoside (46).



Hexasaccharide **45** (24 mg, 0.019 mmol) was dissolved in H_2O (500 μL) followed by adding 30% NH_3 solution (200 μL). The reaction mixture was left standing at RT for 3 h, after which it was directly lyophilized to provide the desired hexasaccharide as a white solid (23 mg, quant).

^{1}H (600 MHz, D_2O): δ (ppm)

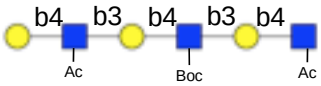
	H-1	H-2	H-3	H-4	H-5	H-6
GlcN-1α	5.21 d, *J* = 2.6 Hz	3.91	3.90	3.99	n/a	n/a
GlcN-1β	4.72	3.71	3.73	3.98	n/a	n/a
Gal-1	4.53 d, *J* = 8.3 Hz	3.70	3.86	4.20 d, *J* = 3.3 Hz	3.72	n/a
GlcN-2	4.74	2.87	3.63	n/a	n/a	n/a
Gal-2	4.45 d, *J* = 8.6 Hz	3.65	3.78	4.14 d, *J* = 3.5 Hz	3.66	n/a
GlcN-3	4.71 d, *J* = 8.7 Hz	3.80	3.60	3.98	3.59	n/a
Gal-3	4.48 d, *J* = 8.1 Hz	3.55 dd, *J* = 8.2, 9.8 Hz	3.67	3.93 d, *J* = 3.6 Hz	3.56	n/a

^{13}C (150 MHz, D_2O): δ (ppm)

	C-1	C-2	C-4
GlcN-1α	90.6	53.8	n/a
GlcN-1β	94.9	56.3	n/a
Gal-1	102.7	n/a	68.3
GlcN-2	103.2	56.2	n/a
Gal-2	103.1	n/a	68.3
GlcN-3	102.8	55.2	n/a
Gal-3	102.9	n/a	68.6

MALDI TOF-MS *m/z* calcd $C_{42}H_{68}F_3N_3NaO_{31}$ $(M+Na)^+$ 1190.3687, found 1190.8792.

***β*-D-Galactopyranosyl-(1→4)-2-acetamido-2-deoxy-*β*-D-glucopyranosyl-(1→3)-*β*-D-galactopyranosyl-(1→4)-2-(*t*-butyloxycarbonylamino)-2-deoxy-D-glucopyranoside-(1→3)-*β*-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-*α*,*β*-D-glucopyranoside (5).**



Hexasaccharide **46** (23 mg, 0.019 mmol) was dissolved in H_2O (200 µL) upon which Boc_2O (41 mg, 0.19 mmol) in 1,4-dioxane (100 µL) was added, and the solution was further incubated at 60 ºC for 2 h. The reaction mixture was directly lyophilized, re-dissolved in H_2O (200 µL) and loaded on Biogel P-2 column and further eluted with H_2O. Fractions containing the desired product were pooled and lyophilized to give the hexasaccharide as a white solid (21 mg, 84%).

^{1}H (600 MHz, D_2O): δ (ppm)

	H-1	H-2	H-3	H-4	H-5	H-6
GlcN-1*α*	5.22 d, *J* = 2.5 Hz	3.91	3.77	3.98	n/a	n/a
GlcN-1*β*	4.73	3.72	n/a	3.99	n/a	n/a
Gal-1	4.48	3.62	3.73	4.14	n/a	n/a
GlcN-2	4.74	3.46 t, *J* = 9.9 Hz	3.70	3.97	n/a	n/a
Gal-2	4.48	3.62	3.80	4.16	n/a	n/a
GlcN-3	4.71 d, *J* = 8.4 Hz	3.81	3.57	3.95	3.56	n/a
Gal-3	4.48	3.55 dd, *J* = 8.0, 9.8 Hz	3.68	3.93	3.55	n/a

^{13}C (150 MHz, D_2O): δ (ppm)

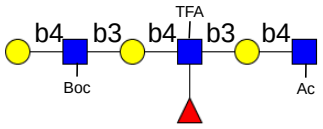
	C-1	C-2	C-4
GlcN-1*α*	90.5	53.5	n/a
GlcN-1*β*	94.7	56.1	n/a
Gal-1	102.8	n/a	68.3
GlcN-2	102.5	56.4	n/a
Gal-2	102.8	n/a	68.3
GlcN-3	102.7	55.3	n/a
Gal-3	102.8	71.0	68.5

MALDI TOF-MS *m/z* calcd $C_{45}H_{77}N_3NaO_{32}$ $(M+Na)^+$ 1194.4388, found 1194.9600.

Enzymatic Synthesis

General Methods. All enzymatic reactions were performed in aqueous buffers with an appropriate pH for each enzyme. Guanosine 5'-diphospho-L-fucose (GDP-Fuc) was purchased form Carbosynth Limited (UK). Hp39-FT was purchased from Chemily LLC (Atlanta, USA). FUT5 was provided by Dr. Kelley W. Moremen (CCRC, USA), which was expressed according to the published protocol.[3] Water was purified by NANOpure Diamond™ water system (Barnstead D3750 Hollow Fibre Filter). All enzymatic reactions, unless otherwise stated, were monitored by mass spectrometry on a Shimadzu Biotech Axima-CFR MALDI-TOF using 4-hydroxycinnamic acid as a matrix. All enzymatic reactions were forced to go to full completion by adding excess of glycosyltransferases until all starting material had disappeared. This approach enabled efficient product isolation and purification. All nuclear magnetic resonance (NMR) spectra were acquired on either 400, 600, or 750 MHz Bruker spectrometers operating at 25 ^{o}C unless otherwise stated. Data were collected using standard pulse programs from the spectrometer library. Samples were dissolved in 99.96% D_2O. Chemical shifts were referenced to the residual HDO signal at 4.79 ppm. For integration of 1D proton spectra, data were acquired with recycling delays of 10 seconds and a small tip angle. Data were generally processed with Mnova (Mestrelab Inc.).

β-D-Galactopyranosyl-(1→4)-2-deoxy-2-(*t*-butyloxycarbonylamino)-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-[α-L-fucopyranosyl-(1→3)-2-deoxy-2-trifluoroacetamido-D-glucopyranoside]-(1→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-α,β-D-glucopyranoside (47).



Hexasaccharide **1** (20.0 mg, 0.017 mmol) and GDP-fucose (12 mg, 0.020 mmol) were dissolved in water 500 µL, after which 200 mM $MnCl_2$ (200 µL) and 1 M Tris-HCl (pH=7.5) (200 µL) were added. Total volume of the reaction mixture was then adjusted to 1.5 mL, upon which the FUT5 (1 mg/mL) enzyme solution (300 µL) was added, and the mixture was incubated at 37 ºC overnight. Further portion of 1 M Tris-HCl (pH=7.5) (200 µL) was added, followed by adding one additional portion of the FUT5 (1mg/mL) enzyme solution (300 µL) to bring the reaction to completion. Ice-cold ethanol (1 mL) was then added to precipitate the enzyme and the mixture was centrifuged. The resulting supernatant was then concentrated and loaded on Biogel P-2 column and further eluted with H_2O. Fractions containing the desired product were pooled and lyophilized to give the crude product mixture as a white solid. This material was then dissolved in H_2O (200 µL) followed by adding solid $NaHCO_3$ (7 mg, 0.085 mmol) and Ac_2O (10 µL, 0.085 mmol). The reaction mixture was sonicated for 2 min, after which it was further incubated for 1 h at 37 ºC. The resulting solution was loaded on Biogel P-2 column and further eluted with H_2O. Fractions containing the desired product were pooled and lyophilized to give the crude product. Additional purification by HPLC with a semi-preparative amide HILIC column (10 x 250 mm, Waters Inc.) under isocratic conditions (75% CH_3CN:10 mM Ammonium formate) with the UV (210 nm) detection affords analytically pure **47** (17 mg, 88%). RT = 38 min (75% CH_3CN:10 mM ammonium formate).

^{1}H (750 MHz, D_2O): δ (ppm)

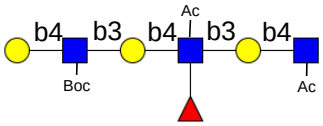
	H-1	H-2	H-3	H-4	H-5	H-6
GlcN-1α	5.21 d, *J* = 2.7 Hz	3.90	3.73	3.98	n/a	n/a
GlcN-1β	4.72 d, *J* = 8.0 Hz	3.71	3.73	3.98	n/a	n/a
Gal-1	4.45 d, *J* = 7.8 Hz	3.58	3.74	4.18	3.59	n/a
GlcN-2	4.80	4.09 dd, *J* = 9.7 Hz	3.96	3.99	3.64	n/a
Gal-2	4.48 d, *J* = 7.9 Hz	3.60	3.76	4.12	3.62	n/a
GlcN-3	4.69 d, *J* = 8.6 Hz	3.48 dd, *J* = 10.3, 8.3 Hz	3.67	3.97	n/a	n/a
Gal-3	4.48 d, *J* = 7.8 Hz	3.55	3.68	3.94 d, *J* = 3.6 Hz	3.56	n/a
Fuc-2	5.04 d, *J* = 4.1 Hz	3.69	3.88	3.77	4.86	1.16 d, *J* = 6.8 Hz

^{13}C (187 MHz, D_2O): δ (ppm)

	C-1	C-2	C-3	C-4	C-5	C-6
GlcN-1α	90.6	53.5	n/a	n/a	n/a	n/a
GlcN-1β	94.9	56.1	n/a	n/a	n/a	n/a
Gal-1	102.9	n/a	n/a	68.3	n/a	n/a
GlcN-2	101.8	56.4	n/a	n/a	n/a	n/a
Gal-2	101.9	n/a	n/a	68.3	n/a	n/a
GlcN-3	102.6	55.3	n/a	n/a	n/a	n/a
Gal-3	102.9	71.0	n/a	68.5	n/a	n/a
Fuc-2	99.0	67.5	69.3	71.8	66.8	16.1

MALDI TOF-MS *m/z* calcd $C_{51}H_{84}F_3N_3NaO_{36}$ $(M+Na)^+$ 1394.4684, found 1394.9209.

***β*-D-Galactopyranosyl-(1→4)-2-deoxy-2-(*t*-butyloxycarbonylamino)-*β*-D-glucopyranosyl-(1→3)-*β*-D-galactopyranosyl-(1→4)-[*α*-L-fucopyranosyl-(1→3)-2-acetamido-2-deoxy-D-glucopyranoside]-(1→3)-*β*-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-*α*,*β*-D-glucopyranoside (48).** Hexasaccharide **3** (10.0 mg, 8 μmol) and GDP-fucose (6 mg, 10 μmol) were dissolved in water 200 μL, after which 100 mM $MnCl_2$ (200 μL) and 1 M Tris-HCl (pH=7.5) (100 μL) were added. Total volume of the reaction mixture was then adjusted to 0.75 mL, upon which the FUT5 (1mg/mL) enzyme solution (150 μL) was added, and the mixture was incubated at 37 ºC overnight. Further portion of 1 M Tris-HCl (pH=7.5) (200 μL) was added, followed by adding one additional portion of the FUT5 (1mg/mL) enzyme solution (150 μL) to bring the reaction to completion. Ice-cold ethanol (1 mL) was then added to precipitate the enzyme and the mixture was centrifuged. The resulting supernatant was then concentrated and loaded on Biogel P-2 column and further eluted with H_2O. Fractions containing the desired product were pooled and lyophilized to give the crude product mixture as a white solid. This material was then dissolved in H_2O (200 μL) followed by adding solid $NaHCO_3$ (7 mg, 0.085 mmol) and Ac_2O (10 μL, 0.085 mmol). The reaction mixture was sonicated for 2 min, after which it was further incubated for 1 h at 37 ºC. The resulting solution was loaded on Biogel P-2 column and further eluted with H_2O. Fractions containing the desired product were pooled and lyophilized to give the crude product. Additional purification by HPLC with a semi-preparative amide HILIC column (10 x 250 mm, Waters Inc.) under isocratic conditions (70% CH_3CN:10 mM ammonium formate) with the UV (210 nm) detection affords analytically pure **48** (8.5 mg, 84%). RT = 22 min (70% CH_3CN:10 mM ammonium formate).



^{1}H (750 MHz, D_2O): δ (ppm)

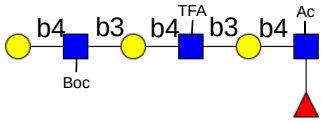
	H-1	H-2	H-3	H-4	H-5	H-6
GlcN-1*α*	5.21 d, *J* = 2.8 Hz	3.90	3.73	3.98	n/a	n/a
GlcN-1*β*	4.72	3.71	3.73	3.98	n/a	n/a
Gal-1	4.45	3.58	3.74	4.18 d, *J* = 3.4 Hz	3.59	n/a
GlcN-2	4.73	3.97	3.85	3.98	3.60	n/a
Gal-2	4.48	3.60	3.76	4.12 d, *J* = 3.4 Hz	3.62	n/a
GlcN-3	4.69 d, *J* = 8.8 Hz	3.48 dd, *J* = 10.3, 8.6 Hz	3.67	3.97	n/a	n/a
Gal-3	4.48	3.55	3.68	3.94	3.56	n/a
Fuc-2	5.13 d, *J* = 4.3 Hz	3.69	3.91	3.78	4.84	1.16 d, *J* = 6.8 Hz

^{13}C (187 MHz, D_2O): δ (ppm)

	C-1	C-2	C-3	C-4	C-5	C-6
GlcN-1*α*	90.5	53.7	n/a	n/a	n/a	n/a
GlcN-1*β*	94.9	56.2	n/a	n/a	n/a	n/a
Gal-1	102.9	n/a	n/a	68.3	n/a	n/a
GlcN-2	102.5	56.2	n/a	n/a	n/a	n/a
Gal-2	101.9	n/a	n/a	68.3	n/a	n/a
GlcN-3	102.7	56.6	n/a	n/a	n/a	n/a
Gal-3	102.9	70.9	n/a	68.6	n/a	n/a
Fuc-2	98.7	67.7	69.3	71.8	66.7	16.1

MALDI TOF-MS *m/z* calcd $C_{51}H_{87}N_3NaO_{36}$ $(M+Na)^+$ 1340.4697, found 1340.9797.

β-D-Galactopyranosyl-(1→4)-2-deoxy-2-(*t*-butyloxycarbonylamino)-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-2-deoxy-2-trifluoroacetamido-D-glucopyranoside]-(1→3)-β-D-galactopyranosyl-(1→4)-[α-L-fucopyranosyl-(1→3)-2-acetamido-2-deoxy-α,β-D-glucopyranoside] (S6). Obtained as a by-product during the synthesis of heptasaccharide **47**. RT = 31 min (75% CH_3CN:10 mM ammonium formate).



^{1}H (750 MHz, D_2O): δ (ppm)

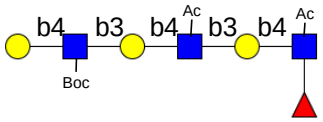
	H-1	H-2	H-3	H-4	H-5	H-6
GlcN-1α	5.10	4.16	4.01	3.92	n/a	n/a
GlcN-1β	4.75	3.88	n/a	n/a	n/a	n/a
Gal-1	4.44	3.56	3.76	4.15	3.61	n/a
GlcN-2	4.84	3.90	3.64	n/a	n/a	n/a
Gal-2	4.51 d, *J* = 8.2 Hz	3.62	3.80	4.17	n/a	n/a
GlcN-3	4.73	3.46 dd, *J* = 8.7 Hz	3.75	3.94	3.56	n/a
Gal-3	4.48 d, *J* = 7.9 Hz	3.58	3.60	3.96	3.56	n/a
Fuc-1	5.10 d, *J* = 3.6 Hz	3.70	3.92	3.78	4.85	1.14

^{13}C (187 MHz, D_2O): δ (ppm)

	C-1	C-2	C-3	C-4	C-5	C-6
GlcN-1α	91.2	54.1	n/a	n/a	n/a	n/a
GlcN-1β	94.8	56.9	n/a	n/a	n/a	n/a
Gal-1	101.8	n/a	n/a	68.2	n/a	n/a
GlcN-2	101.9	55.8	n/a	n/a	n/a	n/a
Gal-2	102.8	n/a	n/a	68.2	n/a	n/a
GlcN-3	102.6	56.6	n/a	n/a	n/a	n/a
Gal-3	102.8	n/a	n/a	68.6	n/a	n/a
Fuc-1	98.7	67.7	69.3	71.8	66.7	16.1

MALDI TOF-MS *m/z* calcd $C_{51}H_{84}F_3N_3NaO_{36}$ $(M+Na)^+$ 1394.4684, found 1394.9209.

β-D-Galactopyranosyl-(1→4)-2-deoxy-2-(*t*-butyloxycarbonylamino)-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-D-glucopyranoside]-(1→3)-β-D-galactopyranosyl-(1→4)-[α-L-fucopyranosyl-(1→3)-2-acetamido-2-deoxy-α,β-D-glucopyranoside] (49).



Hexasaccharide **4** (23.0 mg, 0.020 mmol) and GDP-fucose (14 mg, 0.020 mmol) were dissolved in water 500 µL, after which 200 mM $MnCl_2$ (200 µL) and 1 M Tris-HCl (pH=7.5) (200 µL) were added. Total volume of the reaction mixture was then adjusted to 1.5 mL, upon which the Hp-α(1,3)-FT (1U/mL) enzyme solution (300 µL) was added, and the mixture was incubated at 37 °C overnight. Further portion of 1 M Tris-HCl (pH=7.5) (200 µL) was added, followed by adding one additional portion of the Hp-α(1,3)-FT (1U/mL) enzyme solution (300 µL) to bring the reaction to completion. Ice-cold ethanol (1 mL) was then added to precipitate the enzyme and the mixture was centrifuged. The resulting supernatant was then concentrated and loaded on Biogel P-2 column and further eluted with H_2O. Fractions containing the desired product were pooled and lyophilized to give the crude product mixture as a white solid. This material was then dissolved in H_2O (200 µL) followed by adding solid $NaHCO_3$ (7 mg, 0.085 mmol) and Ac_2O (10 µL, 0.085 mmol). The reaction mixture was sonicated for 2 min, after which it was further incubated for 1 h at 37 °C. The resulting solution was loaded on Biogel P-2 column and further eluted with H_2O. Fractions containing the desired product were pooled and lyophilized to give the crude product. Additional purification by HPLC with a semi-preparative amide HILIC column (10 x 250 mm, Waters Inc.) under isocratic conditions (75% CH_3CN:10 mM Ammonium formate) with the UV (210 nm) detection affords analytically pure **49** (19.3 mg, 84%). RT = 45 min (75% CH_3CN:10 mM ammonium formate).

^{1}H (600 MHz, D_2O): δ (ppm)

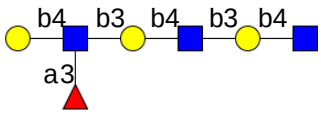
	H-1	H-2	H-3	H-4	H-5	H-6
GlcN-1*α*	5.10	4.16 dd, *J* = 10.4, 4.17 Hz	4.01	3.92	n/a	n/a
GlcN-1*β*	4.73	3.87	n/a	n/a	n/a	n/a
Gal-1	4.44	3.56	3.76	4.18	n/a	n/a
GlcN-2	4.71	3.80	3.60	3.98	n/a	n/a
Gal-2	4.50 d, *J* = 8.5 Hz	3.65	3.78	4.14	n/a	n/a
GlcN-3	4.73	3.46 dd, *J* = 8.8, 10 Hz	3.75	3.94	3.59	n/a
Gal-3	4.49 d, *J* = 7.6 Hz	3.54	3.67	3.94	3.56	n/a
Fuc-1	5.10 d, *J* = 3.6 Hz	3.70	3.92	3.78	4.85	1.14

^{13}C (150 MHz, D_2O): δ (ppm)

	C-1	C-2	C-3	C-4	C-5	C-6
GlcN-1*α*	91.2	54.1	n/a	n/a	n/a	n/a
GlcN-1*β*	94.8	56.9	n/a	n/a	n/a	n/a
Gal-1	101.8	n/a	n/a	68.2	n/a	n/a
GlcN-2	102.8	55.2	n/a	n/a	n/a	n/a
Gal-2	102.8	n/a	n/a	68.2	n/a	n/a
GlcN-3	102.6	56.6	n/a	n/a	n/a	n/a
Gal-3	102.8	n/a	n/a	68.6	n/a	n/a
Fuc-1	98.8	67.7	69.3	71.8	66.7	16.1

MALDI TOF-MS *m/z* calcd $C_{51}H_{87}N_3NaO_{36}$ $(M+Na)^+$ 1340.4697, found 1341.1925.

β-D-Galactopyranosyl-(1→4)-[α-L-fucopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranosyl]-(1→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-D-glucopyranoside]-(1→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-α,β-D-glucopyranoside (8a). Hexasaccharide **2** (7.5 mg, 7.3 μmol) and GDP-fucose (5.5 mg, 8.74 μmol) were dissolved in water 200 μL, after which 100 mM $MnCl_2$ (200 μL) and 1 M Tris-HCl (pH=7.5) (100 μL) were added. Total volume of the reaction mixture was then adjusted to 0.75 mL, upon which the Hp-α(1,3)-FT (1U/mL) enzyme solution (150 μL) was added, and the mixture was incubated at 37 ºC overnight. Further portion of 1 M Tris-HCl (pH=7.5) (200 μL) was added, followed by adding one additional portion of the Hp-α(1,3)-FT (1U/mL) enzyme solution (150 μL) to bring the reaction to completion. Ice-cold ethanol (1 mL) was then added to precipitate the enzyme and the mixture was centrifuged. The resulting supernatant was then concentrated and loaded on Biogel P-2 column and further eluted with H_2O. Fractions containing the desired product were pooled and lyophilized to give the crude product mixture as a white solid. This material was then dissolved in H_2O (200 μL) followed by adding solid $NaHCO_3$ (7 mg, 0.085 mmol) and Ac_2O (10 μL, 0.085 mmol). The reaction mixture was sonicated for 2 min, after which it was further incubated for 1 h at 37 ºC. The resulting solution was loaded on Biogel P-2 column and further eluted with H_2O. Fractions containing the desired product were pooled and lyophilized to give the crude product. Additional purification by HPLC with a semi-preparative amide HILIC column (10 x 250 mm, Waters Inc.) under isocratic conditions (70% CH_3CN:10 mM Ammonium formate) with the UV (210 nm) detection affords pure **8a** (5.4 mg, 73%). RT = 46 min (70% CH_3CN:10 mM ammonium formate).



^{1}H (600 MHz, D_2O): δ (ppm)

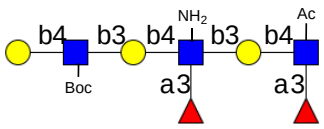
	H-1	H-2	H-3	H-4	H-5	H-6
GlcN-1α	5.21 d, *J* = 2.5 Hz	3.91	3.73	3.98	n/a	n/a
GlcN-1β	4.71	3.72	3.73	3.98	n/a	n/a
Gal-1	4.47	3.59	3.74	4.17	3.59	n/a
GlcN-2	4.70	3.80	n/a	n/a	n/a	n/a
Gal-2	4.47	3.65	3.74	4.17	3.62	n/a
GlcN-3	4.70	3.98	3.85	3.89	3.57	n/a
Gal-3	4.47	3.51 dd, *J* = 9.7, 7.8 Hz	3.66 dd, *J* = 9.9, 3.4 Hz	3.94 d, *J* = 3.4 Hz	3.56	n/a
Fuc-3	5.14 d, *J* = 4.1 Hz	3.70	3.91	3.81	4.85	1.14

^{13}C (150 MHz, D_2O): δ (ppm)

	C-1	C-2	C-3	C-4	C-5	C-6
GlcN-1α	90.5	53.8	n/a	n/a	n/a	n/a
GlcN-1β	94.8	56.2	n/a	n/a	n/a	n/a
Gal-1	102.3	n/a	n/a	68.3	n/a	n/a
GlcN-2	102.7	55.2	n/a	n/a	n/a	n/a
Gal-2	102.3	n/a	n/a	68.3	n/a	n/a
GlcN-3	102.7	56.0	n/a	n/a	n/a	n/a
Gal-3	101.8	71.0	72.4	68.3	n/a	n/a
Fuc-3	98.6	67.7	69.3	71.8	66.7	16.1

MALDI TOF-MS *m/z* calcd $C_{48}H_{81}N_3NaO_{35}$ $(M+Na)^+$ 1282.4548, found 1282.0057.

β-D-Galactopyranosyl-(1→4)-2-deoxy-2-(*t*-butyloxycarbonylamino)-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-[α-L-fucopyranosyl-2-acetamido-2-deoxy-D-glucopyranoside]-(1→3)-β-D-galactopyranosyl-(1→4)-[α-L-fucopyranosyl-(1→3)-2-acetamido-2-deoxy-α,β-D-glucopyranoside] (S7). Hexasaccharide **4** (10.0 mg, 8 μmol) and GDP-fucose (12 mg, 20 μmol) were dissolved in water 200 μL, after which 100 mM $MnCl_2$ (200 μL) and 1 M Tris-HCl (pH=7.5) (100 μL) were added. Total volume of the reaction mixture was then adjusted to 0.5 mL, upon which the FUT5 (1 mg/mL) enzyme solution (200 μL) was added, and the mixture was incubated at 37 °C overnight. Further portion of 1 M Tris-HCl (pH=7.5) (100 μL) was added, followed by adding one additional portion of the FUT5 (1mg/mL) enzyme solution (200 μL) to bring the reaction to completion. Ice-cold ethanol (1mL) was then added to precipitate the enzyme and the mixture was centrifuged. The resulting supernatant was then concentrated and loaded on Biogel P-2 column and further eluted with H_2O. Fractions containing the desired product were pooled and lyophilized to give the crude product mixture as a white solid. Additional purification by HPLC with a semi-preparative amide HILIC column (10 x 250 mm, Waters Inc.) under isocratic conditions (70% CH_3CN:10 mM ammonium formate) with the UV (210 nm) detection affords analytically pure **S7**. RT = 65 min (70% CH_3CN:10 mM ammonium formate). Acetylation of **S7** under $Ac_2O/NaHCO_3$ further gives compound **52**, which is used in deprotection steps without further characterization. Alternatively, glycan **52** can be also prepared directly from **7** by following the above fucosylation procedure (8.9 mg, 90%).



^{1}H (750 MHz, D_2O) for **S7**: δ (ppm)

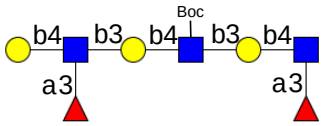
	H-1	H-2	H-3	H-4	H-5	H-6
GlcN-1α	5.12	4.17 dd, *J* = 10.2, 3.8 Hz	4.01	3.92	n/a	n/a
GlcN-1β	4.75	3.88	n/a	n/a	n/a	n/a
Gal-1	4.50 d, *J* = 8.3 Hz	3.70	3.86	4.20 d, *J* = 3.0 Hz	n/a	n/a
GlcN-2	4.97	3.30	3.95 – 4.05	3.95 – 4.05	n/a	n/a
Gal-2	4.50 d, *J* = 8.5 Hz	3.65	3.77	4.13 d, *J* = 2.5 Hz	3.66	n/a
GlcN-3	4.71 d, *J* = 8.2 Hz	3.48 dd, *J* = 9.3 Hz	3.69	3.98	3.59	n/a
Gal-3	4.48 d, *J* = 8.0 Hz	3.55 dd, *J* = 9.5, 7.8 Hz	3.67	3.94 d, *J* = 3.4 Hz	3.56	n/a
Fuc-1	5.10	3.69	3.91	3.80	4.84	1.17
Fuc-2	5.13	n/a	n/a	n/a	4.74	1.20

^{13}C (187 MHz, D_2O): δ (ppm)

	C-1	C-2	C-3	C-4	C-5	C-6
GlcN-1α	91.2	53.8	n/a	n/a	n/a	n/a
GlcN-1β	94.8	56.2	n/a	n/a	n/a	n/a
Gal-1	101.7	n/a	n/a	68.3	n/a	n/a
GlcN-2	100.4	55.2	n/a	n/a	n/a	n/a
Gal-2	101.7	n/a	n/a	68.3	n/a	n/a
GlcN-3	102.7	56.0	n/a	n/a	n/a	n/a
Gal-3	102.9	71.0	72.4	68.3	n/a	n/a
Fuc-1	100.7	67.7	69.3	71.8	66.7	16.1
Fuc-2	98.7	n/a	n/a	n/a	67.9	n/a

MALDI TOF-MS *m/z* calcd $C_{55}H_{95}N_3NaO_{39}$ $(M+Na)^+$ 1444.5440, found 1444.4213.

β-D-Galactopyranosyl-(1→4)-[α-L-fucopyranosyl-2-acetamido-2-deoxy]-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-2-(*t*-butyloxycarbonylamino)-2-deoxy-D-glucopyranoside-(1→3)-β-D-galactopyranosyl-(1→4)-[α-L-fucopyranosyl-2-acetamido-2-deoxy-α,β-D-glucopyranoside] (50). Hexasaccharide **5** (10.0 mg, 8 μmol) and GDP-fucose (12 mg, 20 μmol) were dissolved in water 200 μL, after which 100 mM $MnCl_2$ (200 μL) and 1 M Tris-HCl (pH=7.5) (100 μL) were added. Total volume of the reaction mixture was then adjusted to 0.5 mL, upon which the FUT5 (1 mg/mL) enzyme solution (200 μL) was added, and the mixture was incubated at 37 ºC overnight. Further portion of 1 M Tris-HCl (pH=7.5) (100 μL) was added, followed by adding one additional portion of the FUT5 (1mg/mL) enzyme solution (200 μL) to bring the reaction to completion. Ice-cold ethanol (1mL) was then added to precipitate the enzyme and the mixture was centrifuged. The resulting supernatant was then concentrated and loaded on Biogel P-2 column and further eluted with H_2O. Fractions containing the desired product were pooled and lyophilized to give the crude product mixture as a white solid. Additional purification by HPLC with a semi-preparative amide HILIC column (10 x 250 mm, Waters Inc.) under isocratic conditions (65% CH_3CN:10 mM ammonium formate) with the UV (210 nm) detection affords analytically pure **50** (9.0 mg, 90%). RT = 14 min (65% CH_3CN:10 mM ammonium formate).



^{1}H (600 MHz, D_2O): δ (ppm)

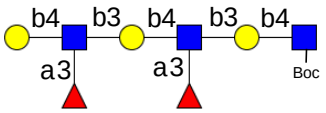
	H-1	H-2	H-3	H-4	H-5	H-6
GlcN-1α	5.10	4.16	4.01	3.92	n/a	n/a
GlcN-1β	4.73	3.86	n/a	n/a	n/a	n/a
Gal-1	4.47 d, *J* = 8.1 Hz	3.59	3.75	4.11 d, *J* = 3.1 Hz	3.61	n/a
GlcN-2	4.69 d, *J* = 9.3 Hz	3.48 d, *J* = 9.6 Hz	3.66	3.98	n/a	n/a
Gal-2	4.47 d, *J* = 8.1 Hz	3.59	3.73	4.16 d, *J* = 3.7 Hz	3.59	n/a
GlcN-3	4.70	3.97	3.85	3.89	3.57	n/a
Gal-3	4.47 d, *J* = 8.1 Hz	3.51	3.66 dd, *J* = 9.6, 3.5 Hz	3.92 d, *J* = 3.1 Hz	3.56	n/a
Fuc-1	5.10 d, *J* = 3.84 Hz	3.69	3.91	3.78	4.84	1.16
Fuc-3	5.13 d, *J* = 4.2 Hz	3.70	3.93	3.82	4.84	1.18 *J* = 6.9 Hz

^{13}C (150 MHz, D_2O): δ (ppm)

	C-1	C-2	C-3	C-4	C-5	C-6
GlcN-1α	91.1	54.2	n/a	n/a	n/a	n/a
GlcN-1β	94.8	56.9	n/a	n/a	n/a	n/a
Gal-1	101.9	n/a	n/a	68.3	n/a	n/a
GlcN-2	102.6	56.4	n/a	n/a	n/a	n/a
Gal-2	102.9	n/a	n/a	68.3	n/a	n/a
GlcN-3	102.6	56.0	n/a	n/a	n/a	n/a
Gal-3	101.9	71.1	72.4	68.3	n/a	n/a
Fuc-1	98.7	67.7	69.3	71.8	66.7	16.1
Fuc-3	98.6	67.7	69.3	71.9	66.7	16.1

MALDI TOF-MS *m/z* calcd $C_{57}H_{97}N_3NaO_{40}$ $(M+Na)^+$ 1486.5546, found 1487.0371.

β-D-Galactopyranosyl-(1→4)-[α-L-fucopyranosyl-2-acetamido-2-deoxy-β-D-glucopyranosyl]-(1→3)-β-D-galactopyranosyl-(1→4)-[α-L-fucopyranosyl-2-acetamido-2-deoxy-D-glucopyranoside]-(1→3)-β-D-galactopyranosyl-(1→4)-2-(*t*-butyloxycarbonylamino)-2-deoxy-α,β-D-glucopyranoside (51). Hexasaccharide **6** (8.0 mg, 6.83 μmol) and GDP-fucose (13 mg, 20.5 μmol) were dissolved in water 200 μL, after which 100 mM $MnCl_2$ (200 μL) and 1 M Tris-HCl (pH=7.5) (100 μL) were added. Total volume of the reaction mixture was then adjusted to 0.5 mL, upon which the FUT5 (1 mg/mL) enzyme solution (200 μL) was added, and the mixture was incubated at 37 °C overnight. Further portion of 1 M Tris-HCl (pH=7.5) (100 μL) was added, followed by adding one additional portion of the FUT5 (1 mg/mL) enzyme solution (200 μL) to bring the reaction to completion. Ice-cold ethanol (1 mL) was then added to precipitate the enzyme and the mixture was centrifuged. The resulting supernatant was then concentrated and loaded on Biogel P-2 column and further eluted with H_2O. Fractions containing the desired product were pooled and lyophilized to give the crude product mixture as a white solid. Additional purification by HPLC with a semi-preparative amide HILIC column (10 x 250 mm, Waters Inc.) under isocratic conditions (65% CH_3CN:10 mM ammonium formate) with the UV (210 nm) detection affords analytically pure **51** (7.1 mg, 91%). RT = 13 min (65% CH_3CN:10 mM ammonium formate).



^{1}H (600 MHz, D_2O): δ (ppm)

	H-1	H-2	H-3	H-4	H-5	H-6
GlcN-1α	5.20 br d	3.61	n/a	3.96	n/a	n/a
GlcN-1β	4.70	3.36 dd, *J* = 9.4 Hz	3.66	n/a	n/a	n/a
Gal-1	4.46	3.50	3.74	4.19 d, *J* = 3.0 Hz	n/a	n/a
GlcN-2	4.72	3.98	3.85	3.89	3.57	n/a
Gal-2	4.45	3.50	3.74	4.12 d, *J* = 3.0 Hz	3.59	n/a
GlcN-3	4.72	3.98	3.85	3.89	3.57	n/a
Gal-3	4.47	3.59	3.66	3.92	3.56	n/a
Fuc-2	5.12 d, *J* = 4.2 Hz	3.70	3.92	3.78	4.84	1.16 d, *J* = 7.2 Hz
Fuc-3	5.14	3.70	3.93	3.80	4.84	1.18 *J* = 7.3 Hz

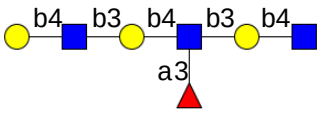
^{13}C (150 MHz, D_2O): δ (ppm)

	C-1	C-2	C-3	C-4	C-5	C-6
GlcN-1α	91.1	54.9	n/a	n/a	n/a	n/a
GlcN-1β	95.2	57.6	n/a	n/a	n/a	n/a
Gal-1	102.9	n/a	n/a	68.5	n/a	n/a
GlcN-2	102.6	56.0	n/a	n/a	n/a	n/a
Gal-2	101.8	n/a	n/a	68.3	n/a	n/a
GlcN-3	102.6	56.0	n/a	n/a	n/a	n/a
Gal-3	101.8	n/a	n/a	68.5	n/a	n/a
Fuc-2	98.8	67.7	69.3	71.8	66.7	16.1
Fuc-3	98.6	67.7	69.3	71.9	66.7	16.1

MALDI TOF-MS *m/z* calcd $C_{57}H_{97}N_3NaO_{40}$ $(M+Na)^+$ 1486.5546, found 1487.1865.

General Procedure for the Removal of C-2 Boc Group with Subsequent Installation of Acetamido (Ac) Functionalities in Glycans 9a – 13a. Heptasaccharides (**48 - 49**) or octasaccharides (**50 - 52**) were dissolved in 20% aq. TFA (200 µL), and the resulting reaction mixture was kept at RT for 3 h, after which it was directly freeze-dried. The obtained white solid was dissolved in water (200 µL) followed by adding solid $NaHCO_3$ (10 eq) and Ac_2O (5 eq). The reaction mixture was sonicated for 2 min, after which it was further incubated for 1 h at 37 °C. The resulting solution was loaded on Biogel P-2 column and further eluted with H_2O. Fractions containing the desired product were pooled and lyophilized to give the product as a white solid.

β-D-Galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-[α-L-fucopyranosyl-(1→3)-2-acetamido-2-deoxy-D-glucopyranoside]-(1→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-α,β-D-glucopyranoside (9a)



Obtained 7.9 mg (quant., starting with 8.0 mg of starting material).

^{1}H (750 MHz, D_2O): δ (ppm)

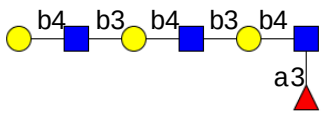
	H-1	H-2	H-3	H-4	H-5	H-6
GlcN-1α	5.21 br s	3.90	3.77	3.97	n/a	n/a
GlcN-1β	4.74	3.73	3.74	3.98	n/a	n/a
Gal-1	4.47	3.59	3.72	4.16	n/a	n/a
GlcN-2	4.71	3.96	3.83	3.92	n/a	n/a
Gal-2	4.45	3.52	3.72	4.11	n/a	n/a
GlcN-3	4.71 d, *J* = 8.8 Hz	3.81	3.60	3.98	3.59	n/a
Gal-3	4.49 d, *J* = 8.1 Hz	3.54	3.67	3.93 d, *J* = 3.4 Hz	n/a	n/a
Fuc-2	5.12 d, *J* = 4.3 Hz	3.69	3.91	3.78	4.84	1.16 d, *J* = 6.7 Hz

^{13}C (187 MHz, D_2O): δ (ppm)

	C-1	C-2	C-3	C-4	C-5	C-6
GlcN-1α	90.6	53.8	n/a	n/a	n/a	n/a
GlcN-1β	94.9	56.1	n/a	n/a	n/a	n/a
Gal-1	103.0	n/a	n/a	68.4	n/a	n/a
GlcN-2	102.7	56.1	n/a	n/a	n/a	n/a
Gal-2	101.8	n/a	n/a	68.4	n/a	n/a
GlcN-3	102.7	55.2	n/a	n/a	n/a	n/a
Gal-3	103.0	n/a	n/a	68.6	n/a	n/a
Fuc-2	98.8	67.7	69.3	71.8	66.7	16.1

MALDI TOF-MS *m/z* calcd $C_{48}H_{81}N_3NaO_{35}$ $(M+Na)^+$ 1282.4548, found 1282.0078.

β-D-Galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-D-glucopyranoside]-(1→3)-β-D-galactopyranosyl-(1→4)-[α-L-fucopyranosyl-(1→3)-2-acetamido-2-deoxy-α,β-D-glucopyranoside] (10a).



Obtained 11.5 mg (quant., starting with 12.0 mg of starting material).

^{1}H (600 MHz, D_2O): δ (ppm)

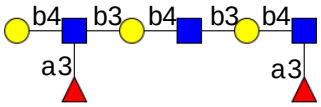
	H-1	H-2	H-3	H-4	H-5	H-6
GlcN-1α	5.10	4.16	3.99	3.92	n/a	n/a
GlcN-1β	4.74	3.87	n/a	n/a	n/a	n/a
Gal-1	4.45	3.53	3.73	4.11	n/a	n/a
GlcN-2	4.71 d, *J* = 9.0 Hz	3.81	3.60	3.98	3.59	n/a
Gal-2	4.47	3.58	3.74	4.17	n/a	n/a
GlcN-3	4.71 d, *J* = 9.0 Hz	3.81	3.60	3.98	3.59	n/a
Gal-3	4.49	3.54	3.68	3.94	n/a	n/a
Fuc-1	5.10	3.69	3.91	3.78	4.84	1.16 d, *J* = 6.7 Hz

^{13}C (150 MHz, D_2O): δ (ppm)

	C-1	C-2	C-3	C-4	C-5	C-6
GlcN-1α	91.0	54.0	n/a	n/a	n/a	n/a
GlcN-1β	94.7	56.7	n/a	n/a	n/a	n/a
Gal-1	101.7	n/a	n/a	68.2	n/a	n/a
GlcN-2	102.7	55.1	n/a	n/a	n/a	n/a
Gal-2	102.8	n/a	n/a	68.2	n/a	n/a
GlcN-3	102.7	55.1	n/a	n/a	n/a	n/a
Gal-3	102.8	n/a	n/a	68.5	n/a	n/a
Fuc-2	98.7	67.7	69.3	71.8	66.7	16.1

MALDI TOF-MS *m/z* calcd $C_{48}H_{81}N_3NaO_{35}$ $(M+Na)^+$ 1282.4548, found 1282.0054.

β-D-Galactopyranosyl-(1→4)-[α-L-fucopyranosyl-2-acetamido-2-deoxy]-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-D-glucopyranoside-(1→3)-β-D-galactopyranosyl-(1→4)-[α-L-fucopyranosyl-2-acetamido-2-deoxy-α,β-D-glucopyranoside] (11a).



Obtained 8.7 mg (quant., starting with 9.0 mg of starting material)

^{1}H (600 MHz, D_2O): δ (ppm)

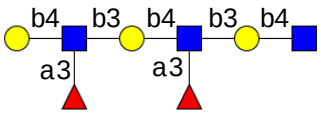
	H-1	H-2	H-3	H-4	H-5	H-6
GlcN-1α	5.08	4.15	3.99	3.92	n/a	n/a
GlcN-1β	4.71	3.86	n/a	n/a	n/a	n/a
Gal-1	4.45	3.53	3.73	4.11 d, *J* = 3.4 Hz	n/a	n/a
GlcN-2	4.69	3.79	3.60	3.98	3.59	n/a
Gal-2	4.47	3.58	3.74	4.17 d, *J* = 3.4 Hz	n/a	n/a
GlcN-3	4.69	3.96	3.85	3.89	3.57	n/a
Gal-3	4.47	3.51	3.64 dd, *J* = 9.8, 3.6 Hz	3.89	n/a	n/a
Fuc-1	5.08 d, *J* = 3.5 Hz	3.69	3.91	3.78	4.84	1.16
Fuc-3	5.12 d, *J* = 4.0 Hz	3.69	3.91	3.78	4.84	1.16

^{13}C (150 MHz, D_2O): δ (ppm)

	C-1	C-2	C-3	C-4	C-5	C-6
GlcN-1α	91.1	54.0	n/a	n/a	n/a	n/a
GlcN-1β	94.6	56.8	n/a	n/a	n/a	n/a
Gal-1	101.7	n/a	n/a	68.2	n/a	n/a
GlcN-2	102.5	55.0	n/a	n/a	n/a	n/a
Gal-2	102.7	n/a	n/a	68.2	n/a	n/a
GlcN-3	102.5	55.9	n/a	n/a	n/a	n/a
Gal-3	101.7	n/a	n/a	68.2	n/a	n/a
Fuc-1	98.6	67.7	69.3	71.8	66.7	16.1
Fuc-3	98.5	67.7	69.3	71.8	66.7	16.1

MALDI TOF-MS *m/z* calcd $C_{54}H_{91}N_3NaO_{39}$ $(M+Na)^+$ 1428.5127, found 1428.4937.

β-D-Galactopyranosyl-(1→4)-[α-L-fucopyranosyl-2-acetamido-2-deoxy-β-D-glucopyranosyl]-(1→3)-β-D-galactopyranosyl-(1→4)-[α-L-fucopyranosyl-2-acetamido-2-deoxy-D-glucopyranoside]-(1→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-α,β-D-glucopyranoside (12a).



Obtained 6.8 mg (quant., starting with 7.1 mg of starting material)

^{1}H (600 MHz, D_2O): δ (ppm)

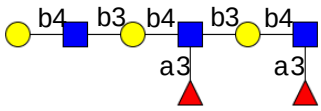
	H-1	H-2	H-3	H-4	H-5	H-6
GlcN-1α	5.21 d, *J* = 2.8 Hz	3.90	3.90	3.98	3.73	n/a
GlcN-1β	4.72	3.72	n/a	n/a	n/a	n/a
Gal-1	4.48	3.55	3.72	4.16 d, *J* = 3.2 Hz	n/a	n/a
GlcN-2	4.71	3.97	3.88	3.88	3.60	n/a
Gal-2	4.46	3.50	3.71	4.10 d, *J* = 3.6 Hz	n/a	n/a
GlcN-3	4.71	3.97	3.88	3.88	3.60	n/a
Gal-3	4.48	3.52	3.66 dd, *J* = 9.8, 3.5 Hz	3.91	n/a	n/a
Fuc-2	5.12 d, *J* = 4.2 Hz	3.70	3.91	3.78	4.84	1.16 d, *J* = 6.8 Hz
Fuc-3	5.14 d, *J* = 4.2 Hz	3.70	3.91	3.78	4.84	1.16 d, *J* = 6.6 Hz

^{13}C (150 MHz, D_2O): δ (ppm)

	C-1	C-2	C-3	C-4	C-5	C-6
GlcN-1α	90.6	53.7	n/a	n/a	n/a	n/a
GlcN-1β	94.9	56.2	n/a	n/a	n/a	n/a
Gal-1	103.0	n/a	n/a	68.3	n/a	n/a
GlcN-2	102.6	56.0	n/a	n/a	n/a	n/a
Gal-2	101.8	n/a	n/a	68.3	n/a	n/a
GlcN-3	102.6	56.0	n/a	n/a	n/a	n/a
Gal-3	101.8	n/a	n/a	68.4	n/a	n/a
Fuc-2	98.6	67.7	69.3	71.8	66.7	16.1
Fuc-3	98.6	67.7	69.3	71.8	66.7	16.1

MALDI TOF-MS *m/z* calcd $C_{54}H_{91}N_3NaO_{39}$ $(M+Na)^+$ 1428.5127, found 1428.0002.

β-D-Galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-[α-L-fucopyranosyl-2-acetamido-2-deoxy-D-glucopyranoside]-(1→3)-β-D-galactopyranosyl-(1→4)-[α-L-fucopyranosyl-(1→3)-2-acetamido-2-deoxy-α,β-D-glucopyranoside] (13a).



Obtained 7.8 mg (quant., starting with 8.0 mg of starting material)

^{1}H (600 MHz, D_2O): δ (ppm)

	H-1	H-2	H-3	H-4	H-5	H-6
GlcN-1α	5.10	4.16 dd, *J* = 3.3, 10.1 Hz	3.99	3.89	n/a	n/a
GlcN-1β	4.73 d, *J* = 7.7 Hz	3.88	3.96	3.87	n/a	n/a
Gal-1	4.45	3.52	3.71	4.10	n/a	n/a
GlcN-2	4.71	3.97	3.96	3.87	3.59	n/a
Gal-2	4.45	3.52	3.71	4.10	n/a	n/a
GlcN-3	4.71	3.81	3.96	3.87	3.59	n/a
Gal-3	4.49 d, *J* = 7.7 Hz	3.55	3.67	3.93 d, *J* = 3.6 Hz	n/a	n/a
Fuc-1	5.10 d, *J* = 3.4 Hz	3.69	3.91	3.78	4.84	1.16
Fuc-2	5.13 d, *J* = 4.2 Hz	3.69	3.91	3.78	4.84	1.16

^{13}C (150 MHz, D_2O): δ (ppm)

	C-1	C-2	C-3	C-4	C-5	C-6
GlcN-1α	91.1	54.0	n/a	n/a	n/a	n/a
GlcN-1β	94.7	56.8	n/a	n/a	n/a	n/a
Gal-1	101.6	n/a	n/a	68.2	n/a	n/a
GlcN-2	102.6	55.9	n/a	n/a	n/a	n/a
Gal-2	101.6	n/a	n/a	68.2	n/a	n/a
GlcN-3	102.6	55.0	n/a	n/a	n/a	n/a
Gal-3	102.8	n/a	n/a	68.4	n/a	n/a
Fuc-2	98.7	67.7	69.3	71.8	66.7	16.1
Fuc-3	98.7	67.7	69.3	71.8	66.7	16.1

MALDI TOF-MS *m/z* calcd $C_{54}H_{91}N_3NaO_{39}$ $(M+Na)^+$ 1428.5127, found 1428.0019.

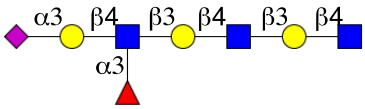
General Procedure for the Enzymatic *α*-(2→3) Sialylation using ST3Gal-IV.

Glycan **9a**, **10a**, **13a,** and CMP-Neu5Ac (1.2 eq) were dissolved in sodium cacodylate buffer (50 mM, pH~7.6) containing BSA (0.1 %). CIAP (10 mU) and ST3Gal-IV (3.3 mU/µmol) were added to achieve a final concentration of glycan ranging from 5 – 10 mM. The resulting reaction mixture was incubated at 37 ºC for 18 h. In case MALDI (after permethylation) or ESI showed the remaining starting material additional CMP-Neu5Ac (1 eq), CIAP (10 mU) and ST3Gal-IV (3.3 mU/µmol) were added and incubated at 37 ºC until no more starting material could be detected. The reaction mixture was quenched by adding an equal volume of ethanol, after which the mixture was centrifuged, and the supernatant subjected to gel filtration over Biogel P-2. Fractions containing product, which were detected using TLC (dipping into the anisaldehyde stain followed by charring at 150 ºC), were combined and lyophilized to give the respective products **9b**, **10b, 13b** as white fluffy solids.

General Procedure for the Enzymatic *α*-(2→3) Sialylation of Glycans Containing Terminal Lewis^{X} Moieties using PmST1 M144D Mutant Enzyme.

Glycan **8a**, **11a**, **12a** and CMP-Neu5Ac (1.2 eq) were dissolved in Tris-HCl buffer (50 mM, pH~8.0). CIAP (10 mU) and appropriate amounts of PmST1 stock solution (1 U/mL) were added to achieve a final concentration of glycan ranging from 5 – 10 mM. The resulting reaction mixture was incubated at 37 ºC for 6 h. In case MALDI (after permethylation) or ESI showed the remaining starting material additional CMP-Neu5Ac (1 eq), CIAP (10 mU) and PmST1 M144D (3.3 mU/µmol) were added and incubated at 37 ºC until no more starting material could be detected. The reaction mixture was quenched by adding an equal volume of ethanol, after which the mixture was centrifuged, and the supernatant subjected to gel filtration over Biogel P-2. Fractions containing product, which were detected using TLC (dipping into the anisaldehyde stain followed by charring at 150 ºC), were combined and lyophilized to give the respective products **8b**, **11b**, **12b** as white fluffy solids.

α-2,4-Bis(5-acetamido-3,5-dideoxy-D-glycero-β-D-galacto-non-2-ulopyranosylate)-(2→3)-β-D-galactopyranosyl-(1→4)-[α-L-fucopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranosyl]-(1→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-D-glucopyranoside]-(1→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-α,β-D-glucopyranoside (8b).



Obtained 2.8 mg (85%, starting with 3.1 mg of starting material)

^{1}H (600 MHz, D_2O): δ (ppm)

	H-1	H-2	H-3	H-4	H-5	H-6
GlcN-1α	5.20 br s	3.88	3.72	3.97	n/a	n/a
GlcN-1β	4.71	3.72	3.73	3.98	n/a	n/a
Gal-1	4.46 d, *J* = 8.0 Hz	3.57	3.71	4.15	3.57	n/a
GlcN-2	4.69	3.79	3.72	3.96	3.58	n/a
Gal-2	4.46 d, *J* = 8.0 Hz	3.57	3.71	4.15	3.57	n/a
GlcN-3	4.69	3.96	3.72	3.96	3.58	n/a
Gal-3	4.52 d, *J* = 8.0 Hz	3.52 d, *J* = 9.5, 8.1 Hz	4.08 dd, *J* = 10.0, 3.2 Hz	3.91	n/a	n/a
Fuc-3	5.11 d, *J* = 4.1 Hz	3.66	3.90	3.80	4.85	1.16 d, *J* = 6.9 Hz

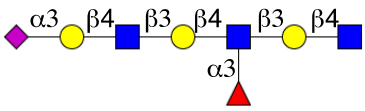
	H-3	H-4	H-5	H-6	H-7	H-8	H-9
Neu5Ac	2.76 dd, *J* = 12.9, 4.8 Hz 1.79 dd, *J* = 12.8 Hz	3.66	3.84	3.65	3.59	3.89	3.64 3.87

^{13}C (150 MHz, D_2O): δ (ppm)

	C-1	C-2	C-3	C-4	C-5	C-6
GlcN-1α	90.5	51.8	n/a	n/a	n/a	n/a
GlcN-1β	94.9	56.2	n/a	n/a	n/a	n/a
Gal-1	103.0	n/a	n/a	68.2	n/a	n/a
GlcN-2	102.7	55.2	n/a	n/a	n/a	n/a
Gal-2	103.0	n/a	n/a	68.2	n/a	n/a
GlcN-3	102.7	56.1	n/a	n/a	n/a	n/a
Gal-3	101.6	69.3	n/a	67.4	n/a	n/a
Fuc-3	98.6	67.7	69.3	71.8	66.7	16.1
Fuc-3	98.7	67.7	69.3	71.8	66.7	16.1

MALDI TOF-MS *m/z* calcd $C_{59}H_{97}N_4O_{43}$ $(M - H)^-$ 1549.5527, found 1549.1666.

α-2,4-Bis(5-acetamido-3,5-dideoxy-D-glycero-β-D-galacto-non-2-ulopyranosylate)-(2→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-[α-L-fucopyranosyl-(1→3)-2-acetamido-2-deoxy-D-glucopyranoside]-(1→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-α,β-D-glucopyranoside (9b).



Obtained 3.2 mg (quant., starting with 3.0 mg of starting material)

^{1}H (600 MHz, D_2O): δ (ppm)

	H-1	H-2	H-3	H-4	H-5	H-6
GlcN-1α	5.20 br s	3.89	3.72	3.96	n/a	n/a
GlcN-1β	4.71	3.71	3.73	3.98	n/a	n/a
Gal-1	4.45	3.59	3.71	4.15 d, *J* = 3.3 Hz	3.59	n/a
GlcN-2	4.70	3.96	3.87	3.95	3.56	n/a
Gal-2	4.44	3.51	3.71	4.09 d, *J* = 3.3 Hz	3.59	n/a
GlcN-3	4.68 d, *J* = 8.8 Hz	3.78	3.73	3.82	3.57	n/a
Gal-3	4.55 d, *J* = 7.6 Hz	3.56	4.11 dd, *J* = 9.8, 2.8 Hz	3.95	n/a	n/a
Fuc-2	5.11 d, *J* = 4.3 Hz	3.68	3.91	3.78	4.84	1.16

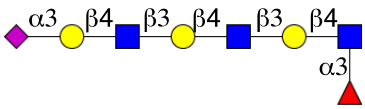
	H-3	H-4	H-5	H-6	H-7	H-8	H-9
Neu5Ac	2.76 dd, *J* = 12.9, 4.8 Hz 1.79 dd, *J* = 12.8 Hz	3.66	3.84	3.65	3.59	3.89	3.64 3.87

^{13}C (150 MHz, D_2O): δ (ppm)

	C-1	C-2	C-3	C-4	C-5	C-6
GlcN-1α	90.4	53.7	n/a	n/a	n/a	n/a
GlcN-1β	94.7	56.5	n/a	n/a	n/a	n/a
Gal-1	102.4	n/a	n/a	68.2	n/a	n/a
GlcN-2	102.6	56.0	n/a	n/a	n/a	n/a
Gal-2	101.8	n/a	n/a	68.2	n/a	n/a
GlcN-3	102.6	55.2	n/a	n/a	n/a	n/a
Gal-3	102.4	69.3	n/a	67.3	n/a	n/a
Fuc-2	98.6	67.7	69.3	71.8	66.7	16.1

MALDI TOF-MS *m/z* calcd $C_{59}H_{97}N_4O_{43}$ $(M - H)^-$ 1549.5527, found 1549.1458.

α-2,4-Bis(5-acetamido-3,5-dideoxy-D-glycero-β-D-galacto-non-2-ulopyranosylate)-(2→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-D-glucopyranoside]-(1→3)-β-D-galactopyranosyl-(1→4)-[α-L-fucopyranosyl-(1→3)-2-acetamido-2-deoxy-α,β-D-glucopyranoside] (10b).



Obtained 2.5 mg (quant., starting with 2.4 mg of starting material)

^{1}H (600 MHz, D_2O): δ (ppm)

	H-1	H-2	H-3	H-4	H-5	H-6
GlcN-1α	5.09	4.15	3.99	3.94	n/a	n/a
GlcN-1β	4.72 d, J = 8.0 Hz	3.86	3.96	3.87	3.59	n/a
Gal-1	4.44	3.51	3.71	4.09	n/a	n/a
GlcN-2	4.69 d, J = 8.0 Hz	3.79	3.73	3.82	3.57	n/a
Gal-2	4.46 d, J = 7.9 Hz	3.58	3.72	4.15	3.58	n/a
GlcN-3	4.69 d, J = 8.0 Hz	3.79	3.73	3.82	3.57	n/a
Gal-3	4.55 d, J = 8.1 Hz	3.56	4.11 dd, J = 10.5, 2.7 Hz	3.95	n/a	n/a
Fuc-1	5.09 d, J = 3.6 Hz	3.68	3.91	3.78	4.84	1.16

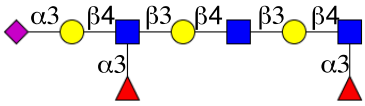
	H-3	H-4	H-5	H-6	H-7	H-8	H-9
Neu5Ac	2.76 dd, J = 12.9, 4.8 Hz 1.79 dd, J = 12.8 Hz	3.66	3.84	3.65	3.59	3.89	3.64 3.87

^{13}C (150 MHz, D_2O): δ (ppm)

	C-1	C-2	C-3	C-4	C-5	C-6
GlcN-1α	91.1	54.1	n/a	n/a	n/a	n/a
GlcN-1β	94.8	56.9	n/a	n/a	n/a	n/a
Gal-1	101.8	n/a	n/a	68.2	n/a	n/a
GlcN-2	102.8	55.0	n/a	n/a	n/a	n/a
Gal-2	102.8	n/a	n/a	68.2	n/a	n/a
GlcN-3	102.8	55.0	n/a	n/a	n/a	n/a
Gal-3	102.5	69.3	75.5	67.4	n/a	n/a
Fuc-1	98.6	67.7	69.3	71.8	66.7	16.1

MALDI TOF-MS *m/z* calcd $C_{59}H_{97}N_4O_{43}$ $(M - H)^-$ 1549.5527, found 1549.1516.

α-2,4-Bis(5-acetamido-3,5-dideoxy-D-glycero-β-D-galacto-non-2-ulopyranosylate)-(2→3)-β-D-galactopyranosyl-(1→4)-[α-L-fucopyranosyl-2-acetamido-2-deoxy]-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-D-glucopyranoside-(1→3)-β-D-galactopyranosyl-(1→4)-[α-L-fucopyranosyl-2-acetamido-2-deoxy-α,β-D-glucopyranoside] (11b).



Obtained 4.0 mg (88%, starting with 4.6 mg of starting material)

^{1}H (600 MHz, D_2O): δ (ppm)

	H-1	H-2	H-3	H-4	H-5	H-6
GlcN-1α	5.09	4.15	3.96	3.94	n/a	n/a
GlcN-1β	4.73	3.86	3.95	3.87	3.59	n/a
Gal-1	4.44	3.52	3.71	4.09	n/a	n/a
GlcN-2	4.69	3.79	3.73	3.82	3.57	n/a
Gal-2	4.46	3.57	3.72	4.15	3.58	n/a
GlcN-3	4.70	3.96	3.72	3.96	3.57	n/a
Gal-3	4.52 d, *J* = 7.4 Hz	3.52	4.08 dd, *J* = 10.5, 2.8 Hz	3.93	n/a	n/a
Fuc-1	5.09 d, *J* = 3.6 Hz	3.68	3.91	3.78	4.84	1.16
Fuc-3	5.12 d, *J* = 4.5 Hz	3.68	3.91	3.78	4.84	1.16

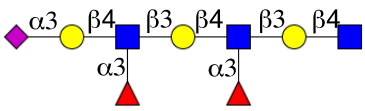
	H-3	H-4	H-5	H-6	H-7	H-8	H-9
Neu5Ac	2.76 dd, *J* = 12.9, 4.8 Hz 1.79 dd, *J* = 12.8 Hz	3.66	3.84	3.65	3.59	3.89	3.64 3.87

^{13}C (150 MHz, D_2O): δ (ppm)

	C-1	C-2	C-3	C-4	C-5	C-6
GlcN-1α	91.1	54.1	n/a	n/a	n/a	n/a
GlcN-1β	94.6	55.9	n/a	n/a	n/a	n/a
Gal-1	101.5	n/a	n/a	68.2	n/a	n/a
GlcN-2	102.5	54.9	n/a	n/a	n/a	n/a
Gal-2	102.4	n/a	n/a	68.2	n/a	n/a
GlcN-3	102.5	55.8	n/a	n/a	n/a	n/a
Gal-3	101.5	69.3	75.6	67.2	n/a	n/a
Fuc-1	98.7	67.7	69.3	71.8	66.7	16.1
Fuc-3	98.7	67.7	69.3	71.8	66.7	16.1

MALDI TOF-MS *m/z* calcd $C_{65}H_{107}N_4O_{47}$ $(M - H)^-$ 1695.6106, found 1695.1848.

α-2,4-Bis(5-acetamido-3,5-dideoxy-D-glycero-β-D-galacto-non-2-ulopyranosylate)-(2→3)-β-D-galactopyranosyl-(1→4)-[α-L-fucopyranosyl-2-acetamido-2-deoxy-β-D-glucopyranosyl]-(1→3)-β-D-galactopyranosyl-(1→4)-[α-L-fucopyranosyl-2-acetamido-2-deoxy-D-glucopyranoside]-(1→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-α,β-D-glucopyranoside (12b).



Obtained 3.2 mg (85%, starting with 3.8 mg of starting material)

^{1}H (600 MHz, D_2O): δ (ppm)

	H-1	H-2	H-3	H-4	H-5	H-6
GlcN-1α	5.20	3.88	3.72	3.96	n/a	n/a
GlcN-1β	4.71	3.71	3.73	3.98	n/a	n/a
Gal-1	4.45	3.59	3.71	4.15 d, *J* = 2.7 Hz	3.59	n/a
GlcN-2	4.69	3.96	3.87	3.95	3.56	n/a
Gal-2	4.43	3.49	3.70	4.09	3.59	n/a
GlcN-3	4.69	3.96	3.87	3.95	3.57	n/a
Gal-3	4.52	3.53	4.07 dd, *J* = 10.5, 3.2 Hz	3.92	n/a	n/a
Fuc-2	5.10 d, *J* = 4.0 Hz	3.66	3.90	3.80	4.85	1.16
Fuc-3	5.12 d, *J* = 4.3 Hz	3.66	3.90	3.80	4.85	1.16

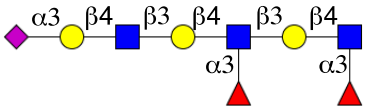
	H-3	H-4	H-5	H-6	H-7	H-8	H-9
Neu5Ac	2.76 dd, *J* = 12.9, 4.8 Hz 1.79 dd, *J* = 12.8 Hz	3.66	3.84	3.65	3.59	3.89	3.64 3.87

^{13}C (150 MHz, D_2O): δ (ppm)

	C-1	C-2	C-3	C-4	C-5	C-6
GlcN-1α	90.4	53.6	n/a	n/a	n/a	n/a
GlcN-1β	94.8	55.9	n/a	n/a	n/a	n/a
Gal-1	102.7	n/a	n/a	68.2	n/a	n/a
GlcN-2	102.4	55.9	n/a	n/a	n/a	n/a
Gal-2	101.7	n/a	n/a	68.2	n/a	n/a
GlcN-3	102.4	55.9	n/a	n/a	n/a	n/a
Gal-3	101.4	69.3	75.6	67.2	n/a	n/a
Fuc-1	98.6	67.7	69.3	71.8	66.7	16.1
Fuc-3	98.6	67.7	69.3	71.8	66.7	16.1

MALDI TOF-MS *m/z* calcd $C_{65}H_{107}N_4O_{47}$ $(M - H)^-$ 1695.6106, found 1695.1777.

***A*-2,4-Bis(5-acetamido-3,5-dideoxy-D-glycero-*β*-D-galacto-non-2-ulopyranosylate)-(2→3)-*β*-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-*β*-D-glucopyranosyl-(1→3)-*β*-D-galactopyranosyl-(1→4)-[*α*-L-fucopyranosyl-2-acetamido-2-deoxy-D-glucopyranoside]-(1→3)-*β*-D-galactopyranosyl-(1→4)-[*α*-L-fucopyranosyl-(1→3)-2-acetamido-2-deoxy-*α*,*β*-D-glucopyranoside] (13b).**



Obtained 3.1 mg (quant., starting with 3.0 mg of starting material)

^{1}H (600 MHz, D_2O): δ (ppm)

	H-1	H-2	H-3	H-4	H-5	H-6
GlcN-1*α*	5.09	4.15 dd, *J* = 9.6, 2.8 Hz	3.99	3.94	n/a	n/a
GlcN-1*β*	4.72	3.86	3.96	3.87	3.59	n/a
Gal-1	4.44	3.50	3.70	4.09	3.58	n/a
GlcN-2	4.69	3.96	3.88	3.88	3.60	n/a
Gal-2	4.44	3.50	3.70	4.09	3.58	n/a
GlcN-3	4.69	3.79	3.73	3.82	3.57	n/a
Gal-3	4.55 d, *J* = 8.0 Hz	3.56	4.11 dd, *J* = 10.5, 2.7 Hz	3.95	n/a	n/a
Fuc-1	5.09 d, *J* = 3.6 Hz	3.68	3.91	3.78	4.84	1.16
Fuc-2	5.12 d, *J* = 3.7 Hz	3.68	3.91	3.78	4.84	1.16

	H-3	H-4	H-5	H-6	H-7	H-8	H-9
Neu5Ac	2.76 dd, *J* = 12.9, 4.8 Hz 1.79 dd, *J* = 12.8 Hz	3.66	3.84	3.65	3.59	3.89	3.64 3.87

^{13}C (150 MHz, D_2O): δ (ppm)

	C-1	C-2	C-3	C-4	C-5	C-6
GlcN-1*α*	90.9	53.9	n/a	n/a	n/a	n/a
GlcN-1*β*	94.6	56.5	n/a	n/a	n/a	n/a
Gal-1	101.7	n/a	n/a	68.2	n/a	n/a
GlcN-2	102.5	55.9	n/a	n/a	n/a	n/a
Gal-2	101.7	n/a	n/a	68.2	n/a	n/a
GlcN-3	102.5	55.1	n/a	n/a	n/a	n/a
Gal-3	102.5	69.3	75.4	67.3	n/a	n/a
Fuc-1	98.6	67.7	69.3	71.8	66.7	16.1
Fuc-2	98.6	67.7	69.3	71.8	66.7	16.1

MALDI TOF-MS *m/z* calcd $C_{65}H_{107}N_4O_{47}$ $(M - H)^-$ 1695.6106, found 1695.1848.

General Procedure for the Installation of a Bi-Functional Spacer.

Glycans **8b – 13b** (4 – 20 mM) and linker 2-[(methylamino)oxy]ethanamine (100 eq) were dissolved in aqueous NaOAc buffer (0.1 M, 50 – 100 μL), and the final pH was adjusted to 5.0. The reaction mixture was incubated at 37 ^{o}C for 6 days. The reactions were monitored by TLC (EtOAc:AcOH:MeOH:H_2O; 5:3:3:0.5) and ESI-MS. To remove excess of the linker and salt, the obtained glycans were quickly passed through a VertiPakTM FL-PR/Carbograph cartridge. Glycan-linker conjugates were obtained as white solids after lyophilization.

HPLC and ESI-MS Spectra of Final Compounds

Conditions.

Column: Waters Xbridge Amide Column (4.6 x 250 mm, 5 µm)

Mobile phase: A: 10 mM ammnonium formate in H_2O (pH=3.5); B: CH_3CN

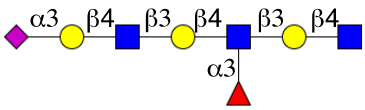
62% B for 50 min

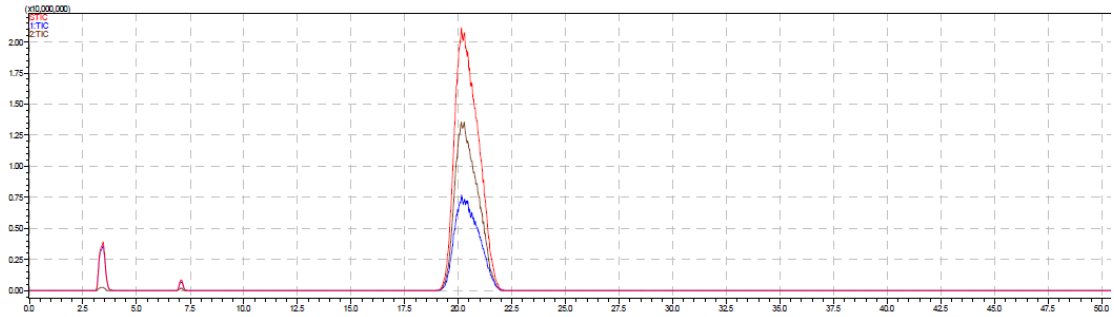
Flow Rate: 0.8mL/min

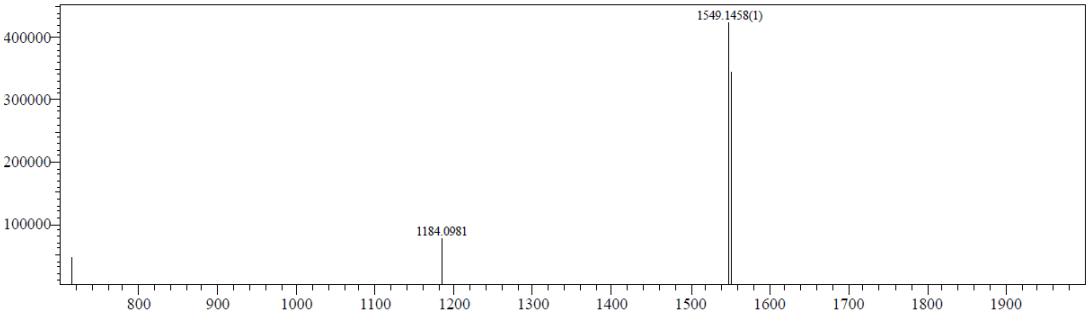
Detection: ESI-MS (negative mode).

Glycan 9b

Retention time = 20.32 min

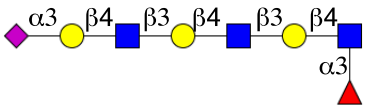


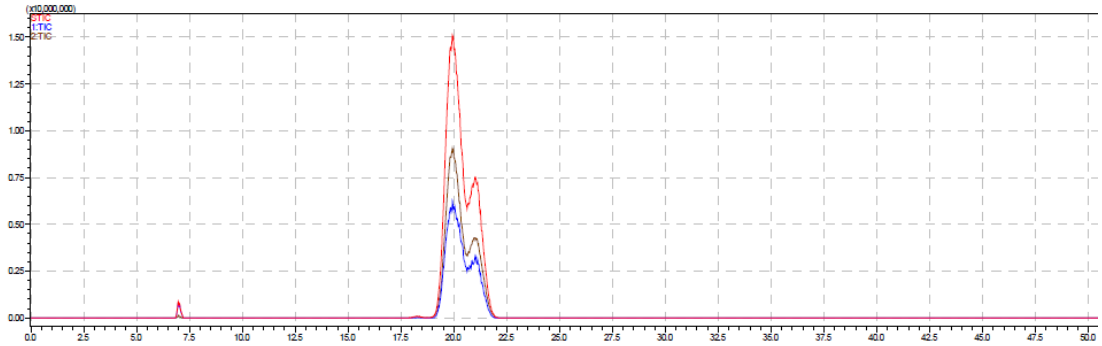


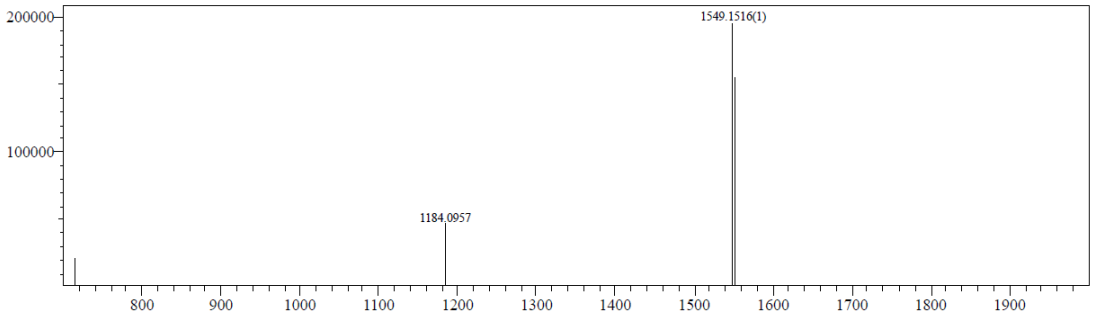


Glycan 10b

Retention time = 20.03 min

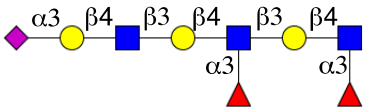


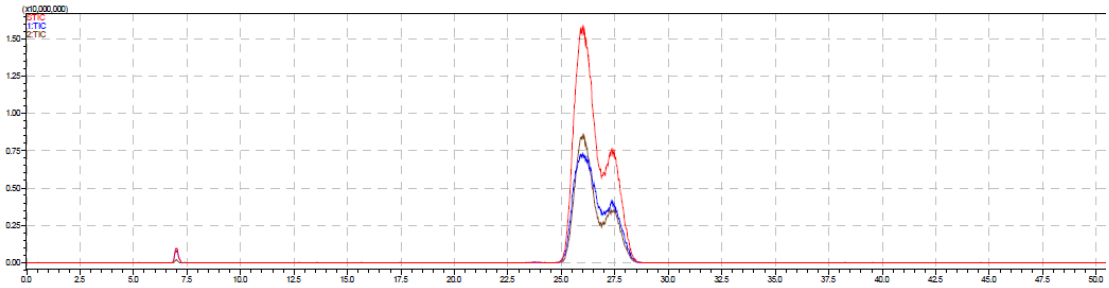


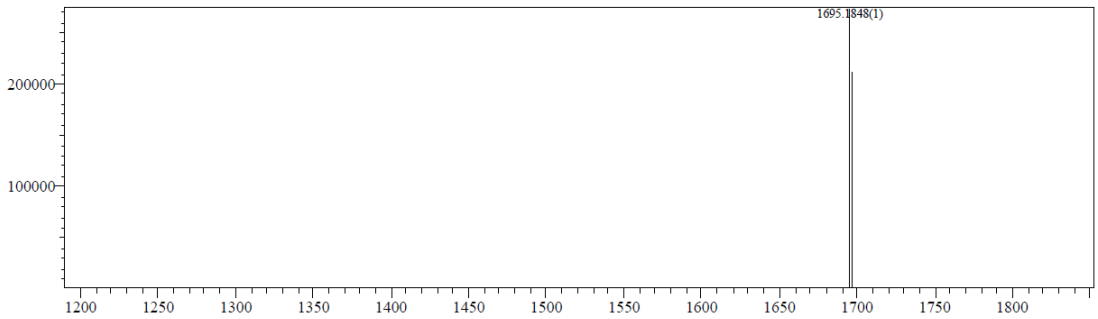


Glycan 13b

Retention time = 26.06 min

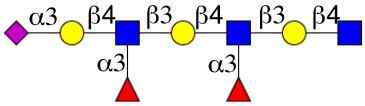


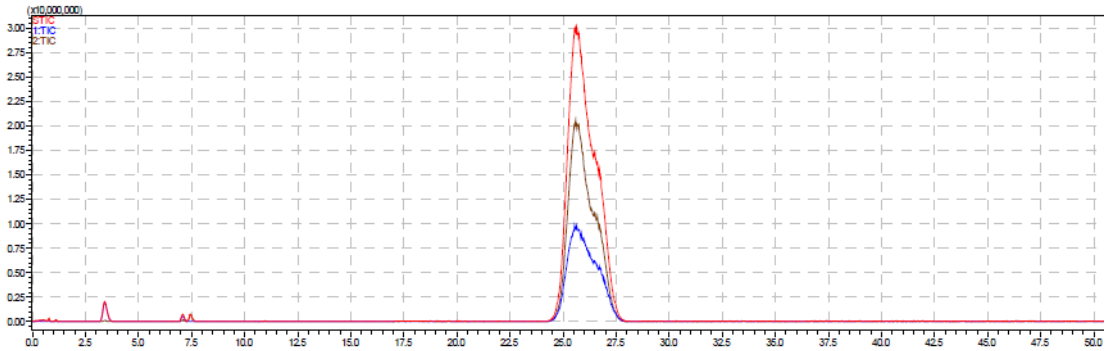


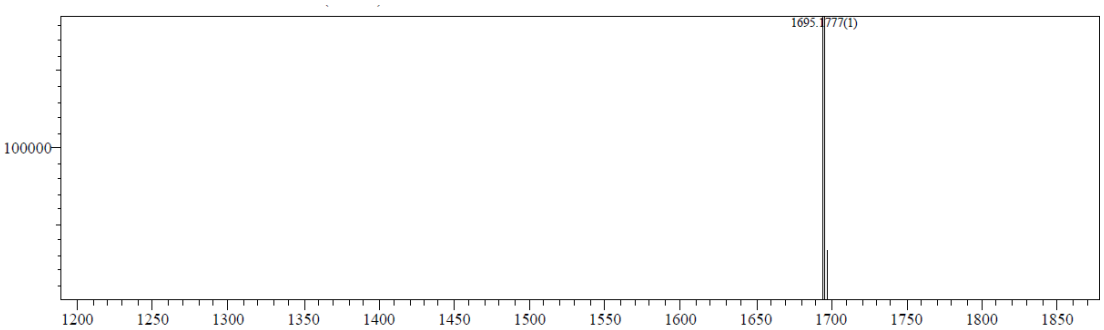


Glycan 12b

Retention time = 25.71 min

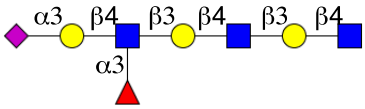


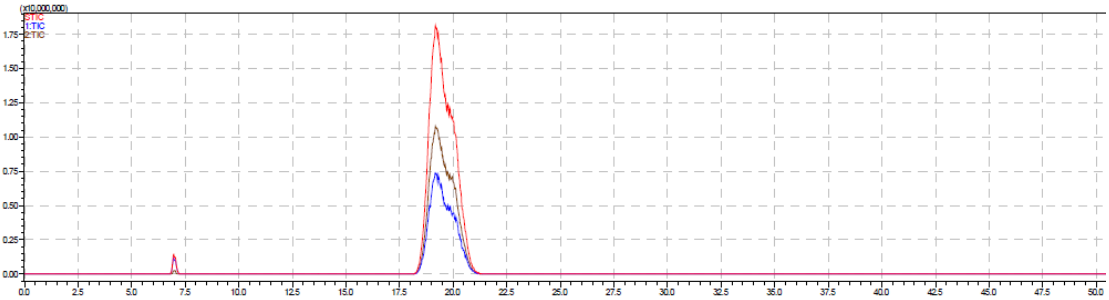


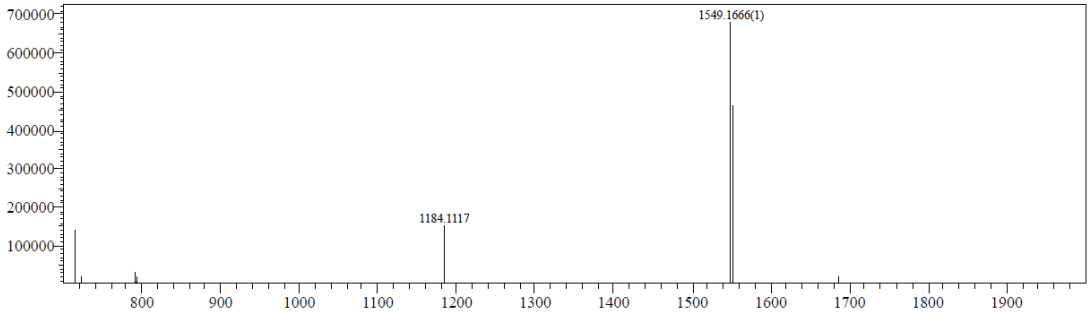


Glycan 8b

Retention time = 19.24 min

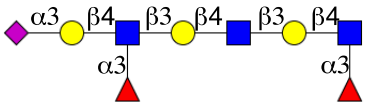


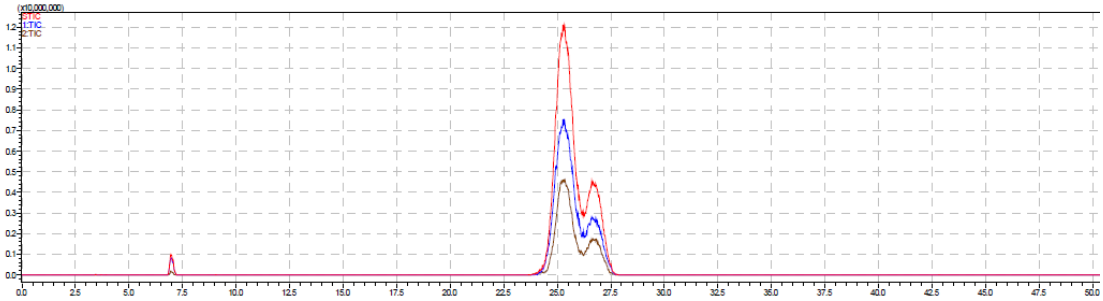


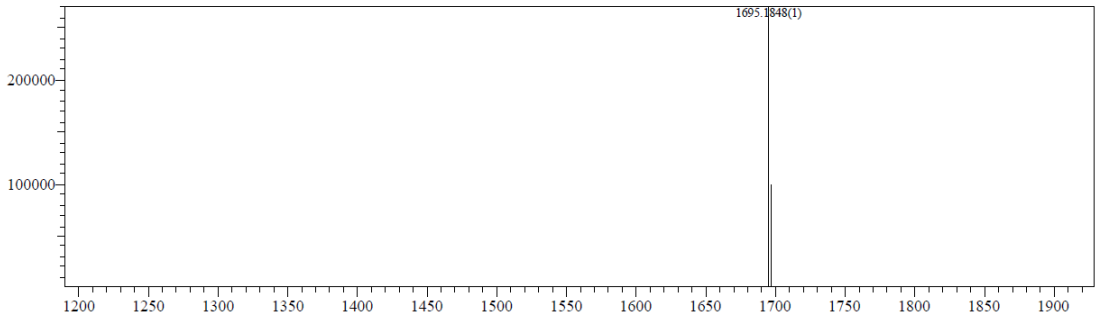


Glycan 11b

Retention time = 25.36 min







Microarray Studies

Recombinant Has

The HAs A/chicken/Ibaraki/1/2005 9h5N20, its mutant R222K R227S, and A/Vietnam/1203/04/H5N1 were cloned into the pCD5 expression vector as described previously.[3] The pCD5 expression vector was adapted so that the HA-encoding cDNAs are cloned in frame with DNA sequences coding for a signal sequence, a GCN4 trimerization motif (RMKQIEDKIEEIESKQKKIENEIARIKK), a super folder GFP,[4] and the Strep-tag II (WSHPQFEKGGGSGGGSWSHPQFEK); IBA, Germany). The HA proteins were expressed in HEK293S GnTI(-) cells and purified from the cell culture supernatants as described previously.[3]

Glycan array printing, screening and analyses

Microarrays were constructed by piezoelectric non-contact printing (sciFLEXARRAYER S3, Scienion Inc) of the synthetic glycans on activated glass slides (Nexterion Slide H, Schott Inc). Compounds (100 µM) were printed (drop volume ~400 pL, 1 drop per spot) at 50 % relative humidity as replicates of 6 in sodium phosphate (250 mM), pH 8.5 buffer with on each slide 24 subarrays (3x8). After overnight incubation in a saturated NaCl chamber (providing a 75% relative humidity environment), the remaining activated esters were quenched with ethanolamine (50 mM) in TRIS (100 mM), pH 9.0. Next, slides were rinsed with DI water, dried by centrifugation, and stored in a desiccator at RT.

Sub-arrays (26x21 spots) were incubated with 50 µL biotinylated lectins (AAL, ECL, MAL-II, WGA, and SNA; all obtained from Vector Labs) at the indicated concentrations premixed with Streptavidin-AlexaFluor635 (5 µg/mL; ThermoFisher Scientific, S32364) in TSM binding buffer (20 mM Tris Cl, pH 7.4, 150 mM NaCl, 2 mM $CaCl_2$, 2 mM $MgCl_2$, 0.05% Tween, 1% BSA) for 1 h followed by washing. Wash steps involved 4 successive washes of the whole slides with TSM wash buffer (20 mM Tris Cl, pH 7.4, 150 mM NaCl, 2 mM $CaCl_2$, 2 mM $MgCl_2$, 0.05% Tween-20) - TSM buffer (20 mM Tris Cl, pH 7.4, 150 mM NaCl, 2 mM $CaCl_2$, 2 mM $MgCl_2$) - 2x deionized H_2O with 5 min soak times.

Similarly as described for the plant lectins, recombinant human E-selectin--Fc Chimera (R&D systems, 724-ES) and recombinant human DC-SIGN--Fc Chimera (R&D systems, 161-DC) were assayed at the indicated concentrations premixed with anti-IgG Fc-biotin (5 µg/mL; ThermoFisher Scientific, A18833) and Streptavidin-AlexaFluor635 (5 µg/mL).

Recombinant HAs were precomplexed with mouse anti-streptag-Alexa647 and goat-anti mouse-Alexa647 antibodies in a 4:2:1 molar ratio in 50 µL PBS-T. After incubation on ice for 15 min, the mixtures were added to the sub-arrays for 90 min. Slides were subsequently washed by successive rinses with PBS-T, PBS, and deionized H_2O.

All incubation and wash steps were performed at RT. Washed arrays were dried by centrifugation and immediately scanned for fluorescence on a GenePix 4000 B microarray scanner (Molecular Devices). The detection gain was adjusted to avoid saturation of the signal. The data were processed with GenePix Pro 7 software and further analyzed using our home written Microsoft Excel macro. After removal of the lowest and highest value of the six replicates, the mean fluorescent intensities (corrected for mean background) and standard deviations (SD) were calculated (n=4). Data were fitted using Prism software (GraphPad Software, Inc). Bar graphs represent the mean ± SD for each compound. The lowest possible protein concentration was employed at which good responsiveness was observed to achieve an appropriate dynamic range.

Results and discussion plant lectins. The plant lectin AAL is known to bind α1,2-, α1,3-, and α1-6-fucosides. Our array screen indicates (Fig. S2a), however, that the position of an α1,3-fucoside within an oligo-LacNAc chain is important for recognition, and only terminal Lewis^x (Le^x) and sialyl Lewis^x (SLe^x) containing compounds showed good responsiveness (**8a**,**b**, **11a**,**b**, **12a**,**b**, **14a**,**b**). The location of a fucoside is also important for the recognition of ECL, which is a lectin that recognizes terminal galactose/*N*-acetylgalactosamine residues. As expected, only glycans having a terminal Gal moiety (**9a**, **10a**, **13a**, **16a**) where bound by ECL (Fig. S2b). Fucosylation of Gal at the non-reducing terminus that creates the terminal Le^x (**8a**) antigen, however, abolished binding whereas the presence of a fucoside at the central and the non-reducing LacNAc moiety were tolerated. MAL-II is known to bind 2,3-sialylated glycans and in this case a fucoside at the central and reducing LacNAc moiety (**9b**, **10b**, **13b**) are tolerated, whereas this is not the case for terminal fucosylation that creates the SLe^x epitope (Fig. S2c). WGA, which is a lectin that binds GlcNAc moieties, demonstrated a remarkable recognition profile, as the major recognition site was present at the reducing end (Fig. S2d). This was apparent as only compounds that have an unmodified GlcNAc moiety at the reducing end (**8a**,**b**, **9a**,**b**, **12a**,**b**, **15**, **16a**,**b**) exhibited responsiveness. Fucosylation at the central and terminal LacNAc moiety had no impact on WGA binding. As expected, SNA only showed responsiveness to glycan **15** which is modified by a 2,6-sialoside (Fig. S2e).

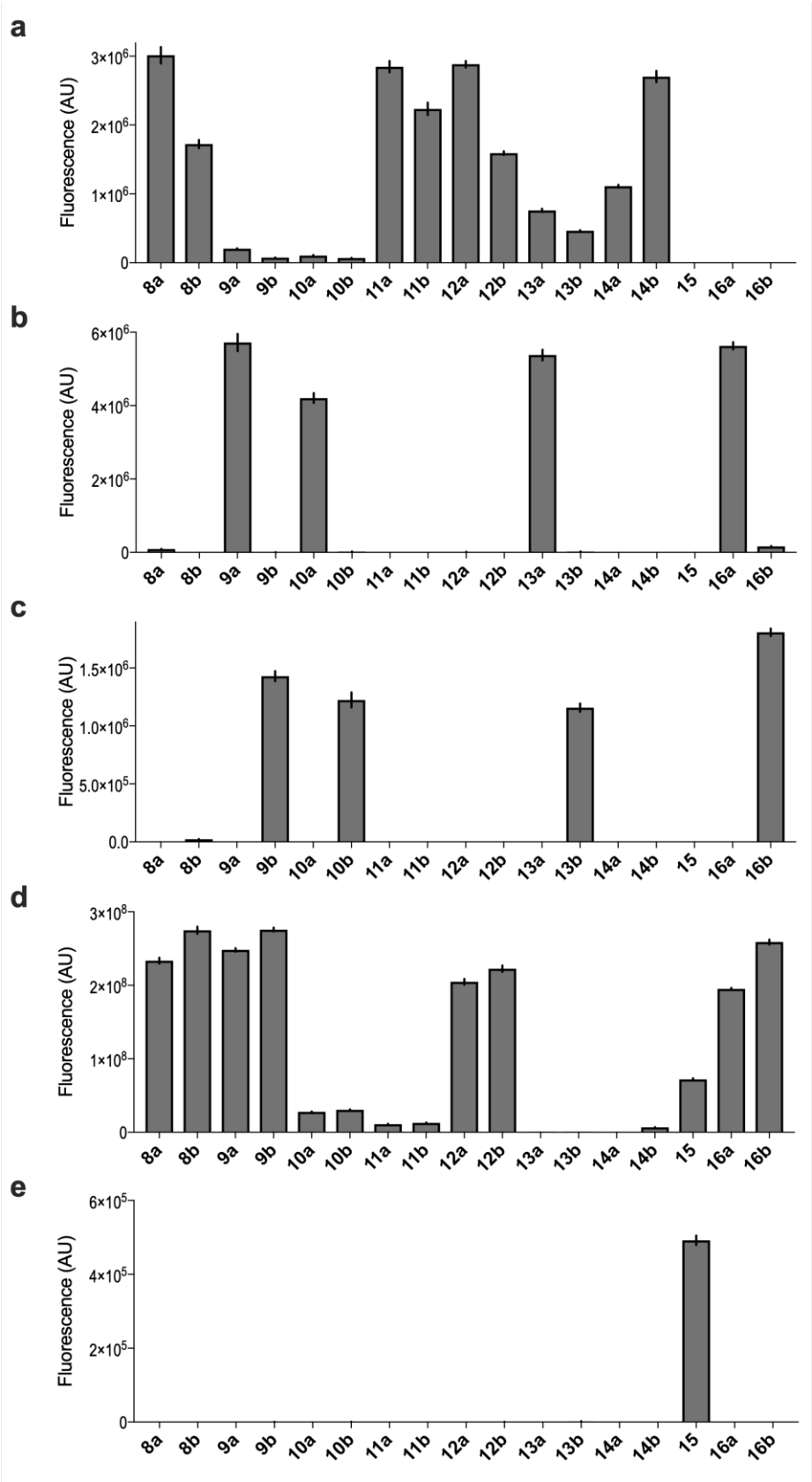


Figure S2. Microarray results of synthetic glycan library at 100 µM with (a) AAL (1 µg/mL); (b) ECL (10 µg/mL; (c) MAL-II (10 µg/mL); (d) WGA (10 µg/mL); and (e) SNA (10 µg/mL). The lowest concentration required for good responsiveness in the optimum dynamic range was selected for all lectins examined. Bars represent the mean ± SD.

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Chapter 4

Synthesis of Staphylococcus aureus Type 5 Trisaccharide Repeating Unit: Solving the Problem of Lactamization

Introduction

Staphylococcus aureus is a commensal of the human skin that can cause skin and soft tissue infections (SSTIs). As many as 20% of individuals receiving surgical and antibiotic therapy suffer from recurrent SSTIs, which can lead to invasive disease with bacteremia, sepsis of endocarditis.[1] Of particular concern are infections caused by antibiotic (methicillin)-resistant strains (MRSA) for which there are limited treatment options.[2] Addressing SSTIs through the development of new antibiotics alone is not sustainable due to the continued increase in antibiotic-resistance of MRSA isolates.[3] Passive or active immunization of patients may offer an attractive alternative for the treatment of infections caused by MRSAs, and particularly immunotherapies based on the capsular polysaccharide of *S. aureus* are offering exciting avenues.[1]

There are twelve known serotypes of the *S. aureus* capsular polysaccharides. However, type 5 and 8 comprise the majority of reported isolates.[4] The structures of the type 5 and 8 polysaccharides have been deduced by NMR as being →4)-*β*-D-Man*p*NAcA-(1→4)-*α*-L-Fuc*p*NAc(3-OAc)-(1→3)-*β*-D-Fuc*p*NAc-(1→, and →3)-*β*-D-Man*p*NAcA(4-OAc)-(1→3)-*α*-L-Fuc*p*NAc-(1→3)-*β*-D-Fuc*p*NAc-(1→, respectively.[5] Thus, the two polysaccharides share common monosaccharides and only differ in the location of the acetyl esters and position of an anomeric linkage. A bivalent vaccine composed of *S. aureus* capsular polysaccharide types 5 and 8 conjugated to *Pseudomonas aeruginosa* exotoxin A (rEPA) gave 60% protection. However, it failed to confer long term protection for end stage renal disease patients, who are often affected by these infections.[6] It is thought that well-defined oligosaccharides obtained by chemical synthesis could facilitate the development of more efficacious vaccines and therapeutic antibodies. In this respect, chemical synthesis makes it possible to install a reactive linker for controlled conjugation to carrier proteins, which is often required for immunological studies. This feature is particularly relevant for the *S. aureus* polysaccharides, which are highly functionalized, and contain sensitive functional groups such as acetyl esters

that complicate conjugation chemistry. Furthermore, chemical synthesis can provide substructures and structural analogs required for establishing structure-activity relationships, or be used to determine minimal epitope requirements to elicit protective immune responses.[7]

Previous efforts to prepare a repeating unit of *S. aureus* type 5 polysaccharide that can be conjugated to a carrier protein in a controlled manner, failed due to difficulties of differentiating amino groups in the saccharide moiety and the amino propyl linker that was needed for conjugation purposes.[8] Specifically, reduction of azido moieties of the sugar moiety led to lactam formation of the mannosaminuronic moiety, whereas hydrogenation of the same compound resulted in the reduction of the azides and removal of the benzyloxycarbonyl-protecting group of the amino propyl linker. Birch reduction had been reported for the deprotection of a similar oligosaccharide.[9] However this method could not be applied to the target compound due to the presence of a base-sensitive acetyl ester.

In addition to the difficulties associated with deprotection, the preparation of the repeating unit of *S. aureas* type 5 polysaccharide (Figure 1) has a number of other synthetic challenges. Specifically, it requires efficient syntheses of rare monosaccharides such as D-and L-fucosamines, and the installation of glycosidic linkages having a 1,2-*cis*-configuration including a *N*-acetyl-*β*-D-mannosaminuronic acid (*β*-D-Man*p*NAcA) and *N*-acetyl-*α*-L-fucosamine (*α*-L-Fuc*p*NAc) glycosides. Furthermore, the target compound contains acetyl esters and, therefore, base sensitive protecting groups need to be avoided. Finally, orthogonal protecting groups need to be identified that allow the preparation of a glycosyl donor and acceptor for further oligosaccharide assembly.

Results and Discussion

We envisaged that monosaccharide building blocks **1**, **2a-b** and **3** would make it possible to assemble trisaccharide **6**, which could then be converted into the spacer containing repeating unit of *S. aureus* type 5 polysaccharide (**4**, Figure 1). Selective removal of the anomeric dimethylthexylsilyl (TDS)[10] ether of **6** would provide a route to a glycosyl donor whereas hydrolysis of the benzylidene acetal followed by regio-selective protection of the C-6 hydroxyl of the resulting diol would give a potential acceptor for oligosaccharide assembly. Furthermore, it was anticipated that the *β*-D-Man*p*N_3 glycoside of **6** could be installed by employing glycosyl donor **1** by *in-situ* conversion into an *α*-anomeric triflate and subsequent nucleophilic displacement with a sugar alcohol.[11] Lactam formation of the *β*-D-Man*p*NAcA moiety would be avoided by a late stage oxidation of the C-6 hydroxyl of the *β*-D-Man*p*NAc residue after reduction of the C-2 azido group, followed by acetylation of the resulting amine. Finally, the azido moiety at C-2 of the glycosyl donors **2a-b** would allow the installation of a *α*-2-amino-fucoside, whereas the 2,2,2-trichloroethoxycarbamate (Troc) moiety function at C-2 of **3** would make it possible to form a *β*-2-amino-fucoside.

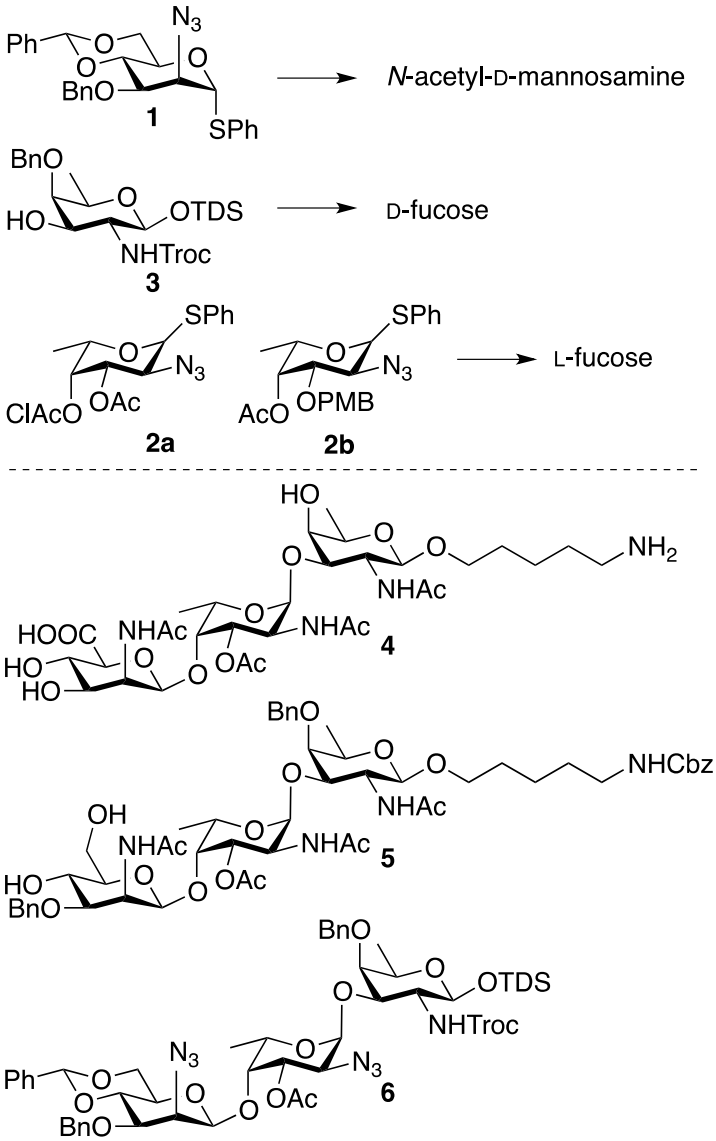
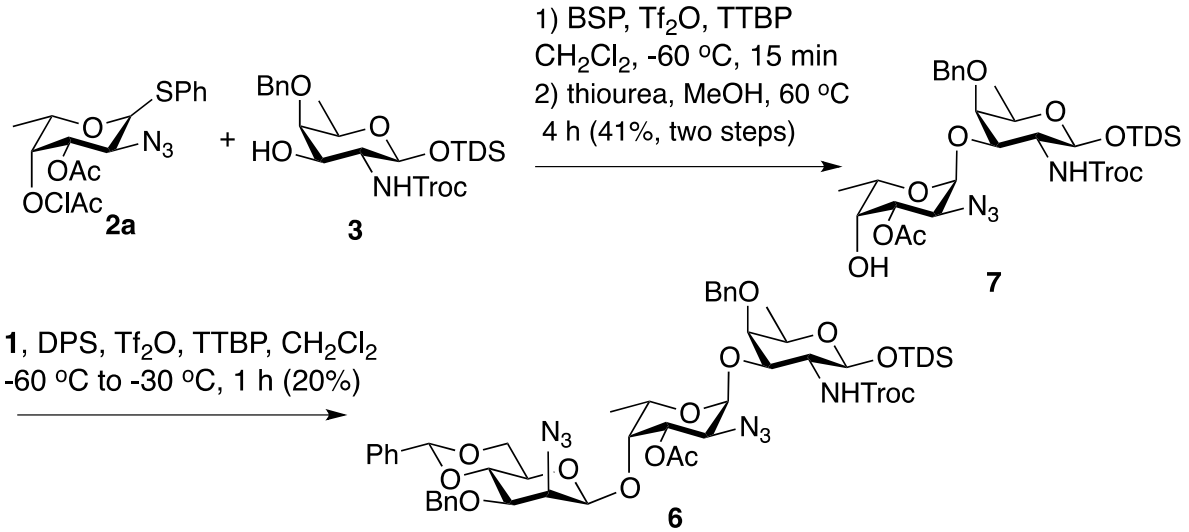


Figure 1. Retrosynthetic analysis

Monosaccharide building block **1** was synthesized by a modification of a reported procedure[11a] starting from inexpensive *N*-acetyl-D-mannosamine (see ESI). Building blocks **2a-b** and **3** were prepared by efficient approaches starting from commercially available L-and D-fucose, respectively (see ESI).

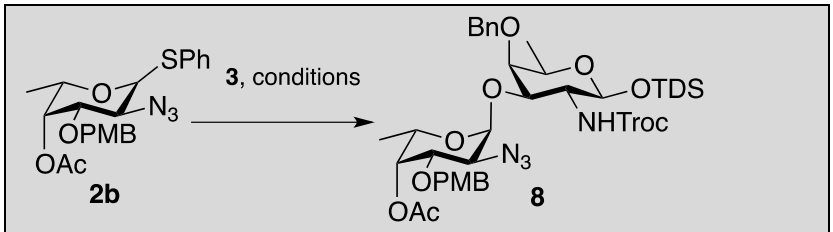
Previous studies employing 2-azido-L-fucose as a glycosyl donor[12] indicated that methyl triflate (MeOTf) is an appropriate promoter to obtain the corresponding glycosides in good yield. However, MeOTf-promoted coupling of **2a** with **3** proceeded sluggishly resulting in the degradation of acceptor **3**, probably due to the acid sensitivity of the anomeric TDS group (Scheme 1). The use of 2,6-di-*tert*-butyl-4-methylpyridine (DTBMP) as an acid scavenger did prevent acceptor decomposition.[13] However, the glycosylation still proceeded very slowly, and after a reaction time of 18 h, **7** was isolated in only a trace amount (<5%) after deprotection of C-4. An *N*-iodosuccinimide (NIS)/trimethylsilyl trifluoromethanesulfonate (TMSOTf) promoted glycosylation of **2a** with **3** gave disaccharide **7** in a low yield of 32% along with unidentified byproducts possibly resulting from acid-mediated degradation of the acceptor. The target fucoside was obtained in an improved yield of 41% when the glycosylation was performed under neutral conditions employing 1-benzenesulfinyl

piperidine (BSP)/trifluoromethanesulfonic anhydride (Tf_2O) as the activator system[14] at –60 °C. Use of the more powerful diphenylsulfoxide (DPS) activator did not improve the yield of the glycosylation. However, in each case, only *α*-fucoside formation was observed and although the yields of the glycosylations were modest, a sufficient quantity of **7** could be prepared to examine installation of the *β*-mannosamine residue.



Scheme 1. Attempted assembly of trisaccharide **6**

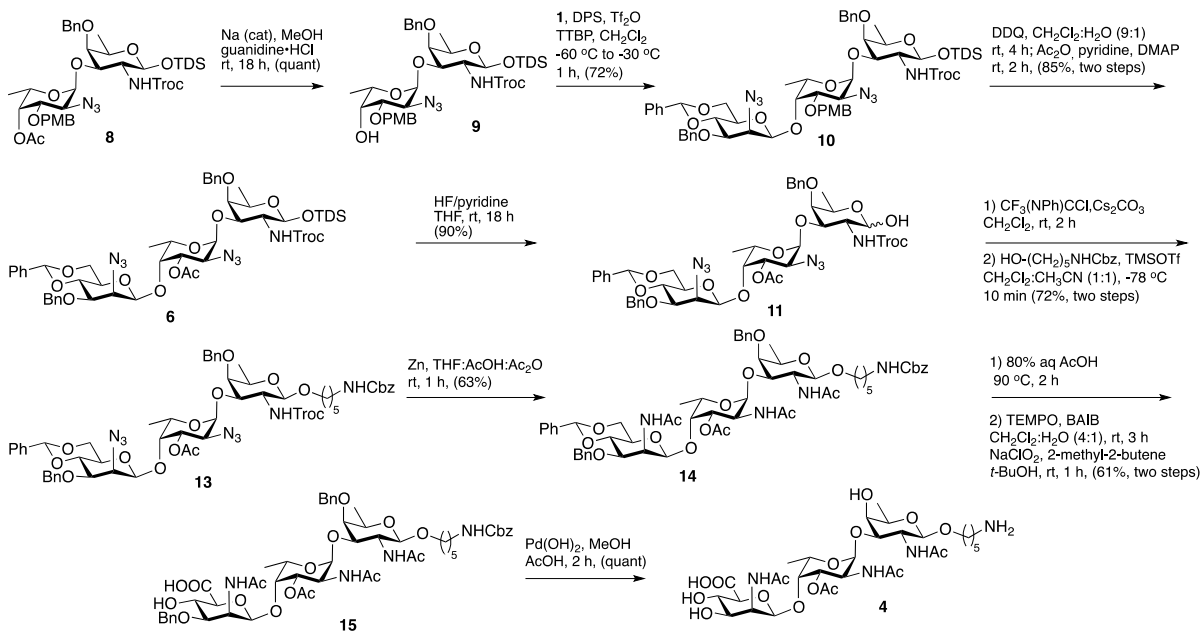
β-Mannosides are difficult to install due to the axial C-2 substituent, which hinders the attack of an incoming nucleophile from the *β*-face and the Δ-anomeric effect that stabilizes the *α*-anomer.[15] Recent studies indicate that preactivation of phenyl 2-azido-2-deoxy-3-*O*-benzyl-4,6-benzylidene-1-thio-*α*-D-mannopyranoside **1** with the powerful DPS/Tf_2O promoter system[11b] offers an attractive approach for selective *β*-glycosylations. Thus, activation of donor **1** was achieved at low temperature (–60 °C) with DPS/Tf_2O in the presence of 2,4,6-tri-*tert*-butylpyrimidine (TTBP) in CH_2Cl_2. Subsequent dropwise addition of **7** followed by slow warming to –30 °C afforded the desired *β*-mannoside **6** in a disappointing yield of 20%.



Promoter/conditions	yield	*β*/*α*
BSP, Tf_2O, TTBP, CH_2Cl_2, -60 °C	30%	*α* only
DPS, Tf_2O, TTBP, CH_2Cl_2, -60 °C	30%	*α* only
NIS, TMSOTf, CH_2Cl_2, -60 °C	73%	1/4
NIS, AgOTf, CH_2Cl_2, -60 °C	70%	1/4
NIS, TMSOTf, CH_2Cl_2/Et_2O (4:1), -60 °C	72%	1/4

Table 1. Coupling optimization of **2b** with **3**

It is known that the axial C-4 hydroxyl of galactosides and fucosides are of low reactivity,[16] which in the case of glycosyl acceptor **7** is attenuated by the neighboring electron-withdrawing acetyl ester at C-3. To improve the glycosyl accepting properties of **7**, the acetyl ester at C-3 was replaced by an electron-donating *p*-methoxybenzyl (PMB) group.[17] It was expected that the newly required glycosyl donor **2b** would be more reactive than **2a**, thus providing an opportunity to improve the challenging *α*-fucosylation. For the purpose of the latter glycosylation, multiple reaction conditions were examined by varying the solvent, temperature and the use of additives (Table 1). It was found that a coupling of **2b** with **3** in the presence of NIS/TMSOTf in CH_2Cl_2 at -60 ºC gave disaccharide **8** in a much improved yield of 73% as mainly the *α*-anomer (*α*:*β*, 4:1). Surprisingly, the nature of the promoter influenced the outcome of the glycosylation and the use of BSP/Tf_2O in CH_2Cl_2 at -60 ºC gave only the *α*-anomer ($J_{1,2}$ = 3.6 Hz) albeit in a significantly lower yield. Next, removal of the acetyl ester of **8** using sodium methoxide in methanol (Scheme 2) gave acceptor **9**, which was condensed with **1** using DPS/Tf_2O as the promoter to give the expected *β*-mannoside **10** in an excellent yield of 72%. The *β*-configuration of the newly formed glycosidic linkage was unambiguously confirmed by ^{13}C-NMR spectroscopy ($^1J_{CH,\beta}$ = 159.8 Hz)[18] and the chemical shift of H-5 (~ 3.1 ppm). The minor *α*-anomer was isolated by silica gel chromatography in a yield of 5-8% (see ESI).



Scheme 2. Improved assembly of target tetrasaccharide **4**

Encouraged by these results, the flexibility of synthon **10** was demonstrated by performing a glycosylation-deprotection-oxidation-deprotection sequence to prepare spacer-containing repeating unit **4**. First, the PMB ether of **10** was removed by oxidation with 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (DDQ),[19] which was followed by acetylation of the resulting hydroxyl to give **6** in a 85% yield over two steps. Subsequent cleavage of anomeric TDS group using hydrogen fluoride-pyridine complex gave hemiacetal **11**, which was transformed into the corresponding *N*-phenyl trifluoroacetimidate **12** by reaction with 2,2,2-trifluoro-*N*-phenylacetimidoyl chloride in the presence of Cs_2CO_3. A TMSOTf-catalyzed glycosylation of **12** with 5-(benzyloxycarbonyl)aminopentanol in CH_2Cl_2:CH_3CN (1:1, v/v) gave **13** as only the *β*-anomer (72%). Trisaccharide **13** was then treated with Zn in a solution of THF/AcOH/Ac_2O[10] to simultaneously convert the azido and Troc groups into acetamido

moieties, affording compound **14** in high yield. Hydrolysis of the benzylidene acetal followed by selective oxidation of the primary hydroxyl of the resulting diol **5** by a modification of Huang's one pot TEMPO/NaOCl – $NaClO_2$ procedure,[20] gave carboxylate **15** in a yield of 61% over two steps. Finally, the remaining benzyl ethers and benzyloxycarbamate were removed by hydrogenation to furnish the target trisaccharide **4**.

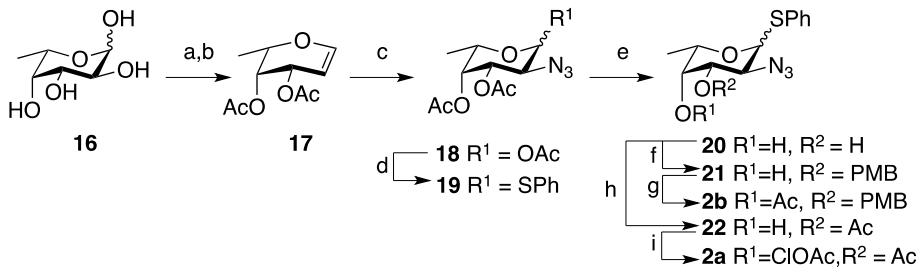
Conclusion

In conclusion, a flexible orthogonally protected trisaccharide *β*-D-Man*p*N_3-(1→4)-*α*-L-Fuc*p*N_3-(1→3)-*β*-D-Fuc*p*NHTroc has been prepared in high yield with excellent anomeric selectivity for the challenging *α*-fucosylation and *β*-mannosylation. Systematic modification of the protecting groups of these unusual glycosyl donors was critical for achieving high anomeric selectivities. Late-stage regioselective oxidation of a highly functionalized repeating unit containing *β*-D-Man*p*NAcA was key to avoid the commonly observed lactam formation. The strategic principles described in this communication will be relevant to the preparation of other biologically important polysaccharides containing *β*-D-Man*p*NAcA moieties.[21] Furthermore, our findings will guide the synthesis of larger *S. aureus* oligosaccharide fragments required for the development of a fully synthetic vaccine.

Experimental

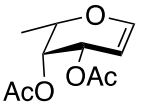
General Procedures: ^{1}H and ^{13}C NMR spectra (from HSQC) were recorded on Varian Mercury 300 MHz or Varian INOVA 500 MHz spectrometer. Chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane (TMS) as the internal standard. NMR data are presented as follows: Chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, dd = doublet of doublet, m = multiplet and/or multiple resonances), coupling constant in Hertz (Hz), integration. For oligosaccharide assignments the following abbreviations were used: δ^{D} – D-fucose; δ^{L} – L-fucose; δ^{M} – 2-azido-D-mannose (*N*-acetyl-D-mannosaminuronic acid). All NMR signals were assigned on the basis of ^{1}H NMR, COSY, ^{13}C and HSQC experiments. For compounds **24**, **26**, and **6 - 15** only HSQC data are provided (blue resonances indicate the CH_2 groups, red resonances indicate the CH/CH_3 groups). Mass spectra were recorded on a high resolution Shimadzu LCMS-IT-TOF mass spectrometer. Column chromatography was performed on silica gel G60 (Silicycle, 60-200 μm, 60 Å). TLC analysis was conducted on Silicagel 60 F254 (EMD Chemicals inc.) with detection by UV-absorption (254nm) were applicable, and by spraying with 20% sulfuric acid in ethanol followed by charring at ~150 °C or by spraying with a solution of $(NH_4)_6Mo_7O_2*4H_2O$ (25 g/L) in 10% sulfuric acid in ethanol followed by charring at ~150 °C. All reactions were carried out under an argon atmosphere unless specified otherwise.

Scheme S1. Synthesis of the glycosyl donors **2a-b**.



Reagents and conditions: a) Ac_2O, $HClO_4$ (cat.), 0 °C, 10 min; b) HBr/AcOH, CH_2Cl_2, 4 h; c) Zn, NaH_2PO_4, EtOAc:H_2O, 30 min; c) NaN_3, CAN, CH_3CN, 15 °C, 3h, then NaOAc, AcOH, Ac_2O (cat.), 100 °C, 1h, (**18**: 63%, from L-fucose); d) thiophenol, BF_3-OEt_2, CH_2Cl_2, rt, 4h, (**19:** 82%); e) NaOMe, MeOH, rt, 30 min; f) Bu_2SnO, toluene, 110 °C, 2h, then *p*-methoxybenzyl chloride, Bu_4NBr, 90 °C, 16h, (**21:** 92%); g) Ac_2O, pyridine, DMAP, rt 2h, (**2b:** quant.); h) AcCl, pyridine, rt, 2h, (**22**: 73%); i) $(ClOAc)_2$, pyridine, rt, 1h, (**2a**: 83%).

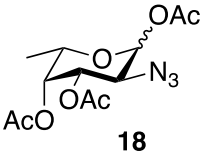
3,4-Di-*O*-acetyl-L-fucal (17). To a cooled (0 °C) suspension of L-fucose (10.0 g, 61.0 mmol) in acetic anhydride (50 mL) was added perchloric acid (1 drop) and the reaction mixture was stirred at this temperature for 30 min. after which the resulting clear solution was concentrated under reduced pressure affording a syrup, which was re-dissolved in CH_2Cl_2 (30 mL). HBr/AcOH (20 mL) was added to the resulting solution and stirring was continued at room temperature (RT) for 4 h. After this time, TLC (EtOAc:Hexane, 1:1, v/v) indicated the completion of the reaction. The mixture was then diluted with CH_2Cl_2 (100 mL), washed with water, and then with sat. $NaHCO_3$ (300 mL). The aqueous layer was then extracted with CH_2Cl_2 (2 x 100 mL), and the combined organic phases were dried ($MgSO_4$) and concentrated under reduced pressure to afford the crude bromide as a yellow syrup, which was used in the next step without further purification. R_f = 0.7 (EtOAc:Hexane, 1:1). To a solution of the crude bromide (11.8 g, 33.5 mmol) in EtOAc (200 mL) was subsequently added sat. aq. NaH_2PO_4 (100 mL), and zinc dust (20.0 g) gradually over a period of 30 min. The reaction mixture was stirred at RT for a further 15 min, after which TLC (EtOAc:Hexane, 1:1, v/v) indicated the reaction had gone to



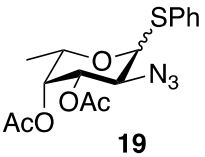
17

completion. The organic layer was separated and washed with sat. $NaHCO_3$ (300 mL), dried ($MgSO_4$) and concentrated under reduced pressure to afford the target glycal as colorless syrup, which was used in the next step without further purification. A small amount was purified by silica gel column chromatography using EtOAc:Hexane (3:7, v/v) as the eluent for characterization purposes. R_f = 0.7 (EtOAc:Hexane, 1:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.25 (3H, d, H-6, *J* = 7.1 Hz), 2.00 (3H, s, OAc), 2.21 (3H, s, OAc), 4.23 (1H, q, H-5, *J* = 7.1 Hz), 4.64 (1H, ddd, H-3 *J* = 1.8, 6.3, 9.8 Hz), 5.3 (1H, m H-2) 5.61 (1H, m H-4), 6.46 (1H, dd, H-1, *J* = 1.8, 6.3 Hz). ^{13}C NMR ($CDCl_3$): δ 17.9, 22.0, 22.2, 66.2, 67.5, 72.3, 100.0, 147.2, 171.4, 171.7. The spectral data are in accordance with that of previously reported.[22]

2-Azido-2-deoxy-1,3,4-tri-*O*-acetyl-L-fucopyranose (18). To as solution of the crude glycal (1.46 g) in CH_3CN (30 mL) was added NaN_3 (700 mg, 10.2 mmol) and the resulting mixture was the cooled to -20 ºC after which CAN (12.0 g, 20.4 mmol) was added. The reaction mixture was stirred at -20 ºC for 1 h, after which it was allowed to gradually attain 0 ºC within 2 h. At this point, TLC (EtOAc:Hexane, 1:1) showed the absence of starting material. The reaction mixture was diluted with Et_2O (100 mL) and washed with water (300 mL). The aqueous layer was extracted with Et_2O (100 mL), and the combined organic layers were dried ($MgSO_4$) and concentrated *in vacuo* to afford a syrup that was dissolved in AcOH (20 mL). NaOAc (3.0 g) and Ac_2O (cat. 1 mL) was added to the solution and the resulting reaction mixture was heated at 100 ºC for 1 h. The reaction mixture was then concentrated *in vacuo*, re-dissolved in CH_2Cl_2 (50 mL) and successively washed with water (100 mL) and sat. $NaHCO_3$ (100 mL). The organic layer was dried ($MgSO_4$) and concentrated to syrup, which was purified by silica gel column chromatography using EtOAc:Hexane (1:9, v/v) as the eluent to give the title compound as a white solid. 1.12 g (63% starting from L-fucose). R_f = 0.4 (EtOAc:Hexane, 3:7) as an α/β-mixture (1:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.14 (3H, d, H-6, *J* = 6.6 Hz), 1.20 (3H, d, *J* = 6.8 Hz), 2.05 – 2.18 (18 H, s x 6, OAc), 3.80 (1H, dd, H-2$^\beta$, *J* = 8.9, 1.9 Hz), 3.89 (2H, m, H-2$^\alpha$, H-5$^\beta$), 4.19 (1H, q, H-5$^\alpha$, *J* = 7.3 Hz), 4.87 (1H, dd, H-3$^\beta$, *J* = 4.0, 10.4 Hz), 5.22 (1H, d, H-4$^\beta$, *J* = 4.0), 5.31 (2H, m, H3$^\alpha$, H-4$^\alpha$), 5.52 (1H, d, H-1$^\beta$, 8.9 Hz), 6.28 (1H, d, H-1$^\alpha$, *J* = 5.0 Hz). ^{13}C NMR$^\alpha$ ($CDCl_3$): δ 17.2, 21.9 x 2, 22.3, 57.9, 68.4, 70.3, 71.2, 92.0, 170.0, 170.8 171.6. ^{13}C NMR$^\beta$ ($CDCl_3$): δ = 17.2, 21.9 x 2, 22.2, 60.8, 70.4, 71.4, 72.8, 94.0, 169.8, 170.7, 171.3. The spectral data are in accordance with previously reported data.[22]



Phenyl 2-azido-3,4-di-*O*-acetyl-1-thio-L-fucopyranoside (19). To a solution of **18** (4.7 g, 14.9 mmol) in CH_2Cl_2 (20 mL) was added thiophenol (2.5 mL, 22.35 mmol) and BF_3-OEt_2 (3.7 mL, 29.8 mmol) and the reaction mixture was left stirring at RT overnight. It was diluted with CH_2Cl_2 (100 mL) and washed with sat. $NaHCO_3$ (100 mL). The organic layer was dried ($MgSO_4$) and concentrated *in vacuo*. Purification of the residue on silica gel using EtOAc:Hexane (1:9, v/v) as the eluent afforded the product as a clear oil. 5.1 g (92 %). R_f = 0.55 (EtOAc:Hexane, 3:7, v/v). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.13 (3H, d, H-6, *J* = 6.85 Hz), 1.23 (3H, d, H-6, *J* = 6.23), 2.04 (3H, s, OAc), 2.06 (3H, s, OAc), 2.11 (3H, s, OAc), 2.18 (3H, s, OAc), 3.63 (1H, t, H-2$^\beta$, *J* = 9.0 Hz), 3.78 (1H, q, H-5$^\beta$, *J* = 7.0 Hz), 4.28 (1H, dd, H-2$^\alpha$, *J* = 6.0, 10.9 Hz), 4.5 (1H, d, H-1$^\beta$, *J* = 9.9 Hz), 4.61 (1H, q, H-5$^\alpha$, *J* = 5.9 Hz), 4.85 (1H, dd, H-3$^\beta$, *J* = 4.0, 10.1 Hz), 5.33 (1H, d, H-4$^\alpha$, *J* = 4.0 Hz), 5.64 (1H, d, H-1$^\alpha$, *J* = 6.0 Hz), 7.26 – 7.38 (6H, m, Ar-H), 7.45 – 7.52 (2H, m, Ar-H), 7.62 (1H, m, Ar-H). ^{13}C NMR ($CDCl_3$): δ 15.6, 16.5, 20.55 x 4, 58.0, 59.1, 65.8, 70.2, 70.3, 72.9, 73.4, 86.4, 87.1, 127.5 – 133.2 (Ar-C). ESI HRMS (*m/z*): $[M + Na]^+$ calcd for $C_{16}H_{19}N_3O_4S$, 338.0964; found 338.0964.



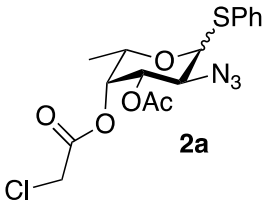
Phenyl 2-azido-3-methoxybenzyl-1-thio-L-fucopyranoside (21). Compound **19** (6.7 g, 18.3 mmol) was dissolved in methanol (50 mL), followed by the addition of a catalytic amount of Na. The reaction mixture was stirred at RT for 20 min, after which it was neutralized with AcOH and the resulting solution was concentrated to dryness to afford crude diol **20**. This intermediate was dissolved in toluene (100 mL) and Bu_2SnO (6.0 g, 248.94 mmol) was added to the resulting solution. The suspension was heated at 110 ºC for 1 h, after which the solution became clear. 4-Methoxybenzyl chloride (3.7 mL, 27.45 mmol) and Bu_4NBr (8.8 g, 27.45 mmol) were then added and heating was continued for further 4 h at 100 ºC, after which TLC (EtOAc:Hexane, 1:1, v/v) showed full conversion of the starting material into a single product. The reaction mixture was concentrated under reduced pressure and the residue purified by silica gel column chromatography using EtOAc:Hexane (1:9 to 1:4, v/v) as the eluent to afford the target product as a yellow oil. 6.8 g (92 %). R_f = 0.3 (EtOAc:Hexane, 3:7, v/v). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.29 (3H, d, H-6$^\alpha$, *J* = 6.91 Hz), 1.36 (3H, d, H-6$^\beta$, *J* = 6.14), 3.39 (1H, dd, H-3$^\beta$, *J* = 5.0, 10.0 Hz), 3.54 (1H, m, H-5$^\beta$), 3.73 (1H, dd, H-3$^\alpha$, *J* = 4.0, 10.2 Hz), 3.80 (3H, s, *p*-OMe), 3.81 (2H, m, H-2$^\beta$, H-4$^\beta$), 3.82 (3H, s, *p*-OMe), 3.86 (1H, s, H-4$^\alpha$), 4.20 (1H, dd, H-2$^\alpha$, *J* = 6.39, 11.0 Hz), 4.33 (1H, d, H-1$^\beta$, *J* = 10.0 Hz), 4.40 (1H, q, H-5$^\alpha$, *J* = 6.4 Hz), 4.59 – 4.72 (2H, m, C$\underline{H_2}$OPMB), 5.57 (1H, d, H-1$^\alpha$, *J* = 5.0 Hz), 6.87 – 6.95 (2H, m, Ar-H), 7.24 – 7.36 (4H, m, Ar-H), 7.48 (2H, d, Ar-H), 7.59 (1H, m, Ar-H). ^{13}C NMR ($CDCl_3$): δ 16.0, 16.6, 55.1, 59.3, 60.8, 66.9, 68.0, 68.6, 71.7, 72.0, 74.45, 77.8, 80.9, 85.9, 87.3, 114.0, 127.47 – 133.29 (Ar-C). ESI HRMS (*m/z*): [M + Na]$^+$ calcd for $C_{20}H_{23}N_3O_4S$, 424.1307; found 424.1305.

Phenyl 2-azido-4-*O*-acetyl-3-methoxybenzyl-1-thio-L-fucopyranoside (2b). To a solution of **21** (6.8 g, 16.9 mmol) in pyridine (30 mL) was added a catalytic amount of DMAP (500 mg) and Ac_2O (10 mL). The reaction mixture was stirred at RT for 2 h, after which TLC (EtOAc:Hexane, 3:7, v/v) showed full consumption of the starting material. Methanol (20 mL) was added and the resulting mixture was concentrated *in vacuo*. The residue was purified by silica gel column chromatography using EtOAc:Hexane (1:4, v/v) as the eluent to give the title compound as a yellow oil in quantitative yield. R_f = 0.7 (EtOAc:Hexane, 3:7, v/v). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.15 (3H, d, H-6$^\alpha$, *J* = 5.8 Hz), 1.24 (3H, d, H-6$^\beta$, *J* = 6.9), 2.07 (3H, s, OAc$^\beta$), 2.16 (3H, s, OAc$^\alpha$), 3.34 (1H, dd, H-3$^\beta$, *J* = 3.1, 9.8 Hz), 3.54 (1H, t, H-2$^\beta$, *J* = 9.6 Hz), 3.65 (1H, q, H-5$^\beta$, *J* = 6.2 Hz), 3.79 (3H, s, *p*-OMe), 3.81 (4H, m, H-3$^\alpha$, *p*-OMe), 4.15 (1H, dd, H-2$^\alpha$, *J* = 5.61, 10.34 Hz), 4.35 – 4.49 (2H, m, C$\underline{H_2}$OPMB), 4.53 (1H, q, H-5$^\alpha$, *J* = 7.0 Hz), 4.61 – 4.71 (2H, m, C$\underline{H_2}$OPMB), 5.31 (1H, d, H-4$^\beta$, *J* = 3.0 Hz), 5.45 (1H, d, H-4$^\alpha$, *J* = 2.6 Hz), 5.58 (1H, d, H-1$^\alpha$, *J* = 6.0 Hz), 6.87 – 6.95 (2H, m, Ar-H), 7.24 – 7.36 (4H, m, Ar-H), 7.48 (2H, d, Ar-H), 7.59 (1H, m, Ar-H). ^{13}C NMR ($CDCl_3$): δ 16.0, 16.8, 23.0, 23.0, 55.2, 55.2, 59.6, 60.5, 66.0, 68.2, 69.17, 71.2, 73.2, 75.8, 79.2, 86.0, 87.4, 114.0, 127.47 – 133.29 (Ar-C). ESI HRMS (*m/z*): [M + Na]$^+$ calcd for $C_{22}H_{25}N_3O_5S$, 466.1413; found 466.1405.

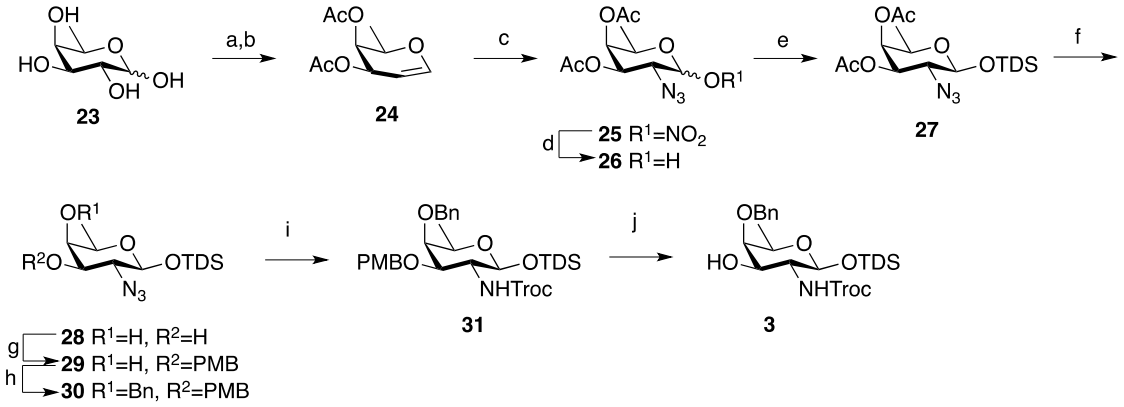
Phenyl 2-azido-2-deoxy-3-*O*-acetyl-1-thio-L-fucopyranoside (22). To a solution of **19** (350 mg, 0.96 mmol) in methanol (5 mL) was added a catalytic amount of Na. The reaction mixture was stirred at RT for 15 min, after which it was neutralized with Amberlite IR-120 H$^+$ resin. The solution was filtered and concentrated *in vacuo* to afford **20** as a white solid, which was used in the next step without further purification. To a solution of the above intermediate in pyridine (5 mL) was added acetyl chloride (82 μL, 1.15

mmol) at 0 ºC and stirring was continued for 1 h at RT, after which a further portion of acetyl chloride (41 µL, 0.57 mmol) was added and stirring was continued for another 30 min. After this time TLC (EtOAc:Hexane, 1:1, v/v) showed full consumption of the starting material, and the reaction was quenched with methanol (5 mL). The resulting solution was concentrated under reduced pressure, and the residue purified by silica gel column chromatography using EtOAc:Hexane, (1:4 to 3:7, v/v) as the eluent to afford the target product as a white foam. 220 mg (73%). R_f = 0.6 (EtOAc:Hexane, 1:1, v/v). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.25 (3H, d, H-6$^\alpha$, *J* = 5.8 Hz), 1.32 (3H, d, H-6$^\beta$ *J* = 6.8 Hz), 2.15 (3H, s, OAc$^\beta$), 2.18 (3H, s, OAc$^\alpha$), 3.65 (2H, m, H-2$^\beta$, H-5$^\beta$), 3.83 (1H, d, H-4$^\beta$, *J* = 4.0 Hz), 3.99 (1H, d, H-4$^\alpha$, *J* = 2.5 Hz), 4.35 (1H, dd, H-2$^\alpha$, *J* = 6.0, 10.9 Hz), 4.45 (1H, d, H-1$^\beta$, *J* = 10.4 Hz), 4.53 (1H, q, H-5$^\alpha$, *J* = 6.6 Hz), 4.78 (1H, dd, H-3$^\beta$, *J* = 3.0, 10.5 Hz), 5.09 (1H, dd, H-3$^\alpha$, *J* = 3.0, 10.9 Hz), 5.61 (1H, d, H-1$^\alpha$, *J* = 5.2 Hz), 7.24 – 7.35 (6H, m, Ar-H), 7.45 – 7.51 (2H, m, Ar-H), 7.6 (1H, m Ar-H). ^{13}C NMR ($CDCl_3$): δ 15.9, 16.5, 20.9 x 2, 57.9, 59.3, 61.1, 66.8, 69.3, 69.6, 69.7, 73.1, 74.7, 75.8, 86.4, 87.2, 127.0 – 133.5 (Ar-C). ESI HRMS (*m/z*): [M + Na]$^+$ calcd for $C_{14}H_{17}N_3O_4S$, 346.0837; found 346.0850.

Phenyl 2-azido-2-deoxy-3-*O*-acetyl-4-*O*-chloroacetyl-1-thio-α-L-fucopyranoside (2a). To a solution of **22** (210 mg, 0.65 mmol) in pyridine (5 mL) at 0 ºC was added chloroacetic anhydride (223 mg, 1.3 mmol) and the reaction mixture was stirred at this temperature for 1 h. Next, methanol (1 mL) was added and the mixture was concentrated under reduced pressure to give a residue that was purified by silica gel column chromatography using EtOAc:Hexane (1:9, v/v) as the eluent to give the title compound as a clear oil. 160 mg (64%, isolated α). R_f = 0.3 (EtOAc:Hexane, 1:9, v/v). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.15 (3H, d, H-6, *J* = 6.78 Hz), 2.07 (3H, s, OAc), 4.16 (2H, s, Cl$\underline{CH_2}$CO), 4.27 (1H, H-2, *J* = 6.17, 11.3 Hz), 4.66 (1H, q, *J* = 7.34 Hz), 5.20 (1H, H-3, *J* = 2.8, 10.8 Hz), 5.38 (1H, H-4, *J* = 2.8 Hz), 5.65 (1H, H-1, *J* = 5.6 Hz), 7.27 – 7.37 (2H, m, Ar-H), 7.43 – 7.51 (2H, m, Ar-H). ^{13}C NMR ($CDCl_3$): δ 15.9, 20.8, 40.5, 58.0, 65.5, 70.4, 72.5, 87.0, 127.8 – 137.1 (Ar-C). ESI HRMS (*m/z*): unstable to ESI conditions.



Scheme S2. Synthesis of the acceptor **3**.



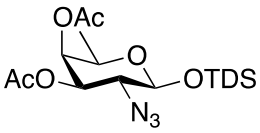
Reagents and conditions: a) Ac_2O, $HClO_4$ (cat.), 0 °C, 10 min; b) HBr/AcOH, CH_2Cl_2, 4 h; c) Zn, NaH_2PO_4, EtOAc:H_2O, 30 min; c) NaN_3, CAN, CH_3CN, 15 °C, 3h; d) Thiocresol, DIPEA, CH_3CN (**26**: 63%, from D-fucose); e) TDSCl, imidazole, CH_2Cl_2, rt, overnight, (**27:** 92%); f) NaOMe, MeOH, rt, 30 min, (**28**: quant.); g) Bu_2SnO, toluene, 110 °C, 2h, then *p*-methoxybenzyl chloride, Bu_4NBr, 90 °C, 16h, (**29:** 90%); h) BnBr, NaH, rt 2h, (**30:** 86%.); i) Zn, THF, AcOH, rt, then TrocCl, $NaHCO_3$,THF, rt, 15 min, (**31**: 82%); j) DDQ, CH_2Cl_2:H_2O (9:1), rt, 1h. (**3**: 72%)

3,4-Di-*O*-acetyl-D-fucal (24). To a cooled (0 °C) suspension of D-fucose (10.0 g, 61 mmol) in acetic anhydride (50 mL) was added perchloric acid (1 drop). The reaction mixture was stirred at that temperature for 30 min, after which the resulting clear solution was concentrated *in vacuo* to afford a syrup, which was re-dissolved in CH_2Cl_2 (30 mL). HBr/AcOH (20 mL) was added to the resulting solution and the reaction mixture was stirred at RT for 4 h. After this time, TLC (EtOAc:Hexane, 1:1, v/v) showed completion of the reaction. The mixture was then diluted with CH_2Cl_2 (100 mL), washed with water, and then with sat. $NaHCO_3$ (300 mL). The aqueous layer was extracted with CH_2Cl_2 (2 x 100 mL), and the combined organic layers were dried ($MgSO_4$) and concentrated *in vacuo* to afford the crude bromide as a yellow syrup, which was used in the next step without further purification. To a solution of the bromide in EtOAc (200 mL) was added sat. acq. NaH_2PO_4 (100 mL). Zinc dust (60.0 g) was then gradually added over a period of 30 min, and the resulting mixture was stirred for another 15 min at RT, after which full consumption of starting material was observed by TLC. The organic layer was then separated, washed with sat. $NaHCO_3$ (200 mL), dried ($MgSO_4$) and concentrated affording crude product which was purified on silica gel using EtOAc:Hexane (3:7) affording the target glycal as a clear oil. 11.2 g (86%, three steps). R_f = 0.7 (EtOAc:Hexane, 1:1, v/v). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.27 (3H, d, H-6, *J* = 6.3 Hz), 2.01 (3H, s, OAc), 2.15 (3H, s, OAc), 4.21 (1H, q, H-5, *J* = 6.78 Hz), 4.63 (1H, ddd, H-3, *J* = 1.8, 6.3, 9.8 Hz), 5.28 (1H, d, H-2, *J* = 5.1 Hz), 5.57 (1H, broad s, H-4), 6.46 (1H, dd, H-1, *J* = 1.6, 6.2 Hz). ^{13}C NMR ($CDCl_3$): δ 16.6, 20.9 x 2, 64.8, 66.4, 71.6, 98.3, 146.0. ESI HRMS (*m/z*): $[M + Na]^+$ calcd for $C_{10}H_{14}O_5$, 237.0739; found 237.0704. The spectral data are in accordance with that of previously reported.[23]

2-Azido-2-deoxy-3,4-di-*O*-acetyl-α,β-D-fucopyranose (26). To as solution of **24** (11.2 g, 52.3 mmol) in CH_3CN (250 mL) was added NaN_3 (5.1 g, 78.45 mmol) and the resulting mixture was then cooled to -20 °C after which CAN (87.0 g, 156.9 mmol) was added. The reaction mixture was stirred at -20 °C for 1h, and then allowing to gradually attain 0 °C within 2 h, after which TLC (EtOAc:Hexane, 1:1, v/v) showed the absence of starting material. The reaction mixture was diluted with Et_2O (200 mL) and washed with water (300

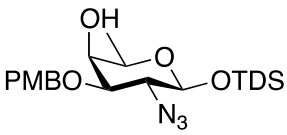
mL). The aqueous layer was extracted with Et_2O (200 mL), and the combined organic layers were dried ($MgSO_4$) and concentrated *in vacuo* to syrup**.** The crude nitrate **25** was dissolved in CH_3CN (100 mL) followed by the addition of thiocresol (9.7 g, 78.45 mmol) and *N,N*-diisopropylethylamine (18 mL, 104.6 mmol). The reaction mixture was stirred at RT for 10 min, after which it was diluted with CH_2Cl_2 (200 mL) and washed with 1 M HCl (200 mL). The organic layer was separated, dried ($MgSO_4$) and concentrated *in vacuo*. Silica gel column chromatography using EtOAc:Hexane, (1:9 to 2:3, v/v) as the eluent afforded the target hemiacetal as a yellow syrup. 9.0 g (63%, from D-fucose). R_f = 0.5 (EtOAc:Hexane, 1:1, v/v). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.13 (3H, d, H-6$^\beta$, *J* = 6.8 Hz), 1.20 (3H, d, H-6$^\alpha$, *J* = 6.5 Hz), 2.04 (6H, s, OAc x 2), 2.16 (3H, s, OAc), 2.17 (3H, s, OAc), 3.38 (1H, broad s, 1-OH), 3.62 (1H, dd, H-2$^\beta$, *J* = 8.0, 10.9 Hz), 3.71 (1H, dd, H-2$^\alpha$, *J* = 3.5, 11.0 Hz), 3.80 (1H, q, H-5$^\beta$, *J* = 6.5 Hz), 4.04 (1H, broad s, 1-OH), 4.39 (1H, q, H-5$^\alpha$, *J* = 7.0 Hz), 4.65 (1H, dd, H-1$^\beta$, *J* = 5.7, 8.3 Hz), 4.80 (1H, dd, H-3$^\beta$, *J* = 3.5, 10.5 Hz), 5.18 (1H, d, H-4$^\beta$, *J* = 3.0 Hz), 5.29 (1H, d, H-4$^\alpha$, *J* = 3.2 Hz), 5.34 – 5.42 (2H, m, H-1$^\alpha$, H-3$^\alpha$).^{13}C NMR ($CDCl_3$): δ 15.8, 16.0, 20.5, 20.5, 58.1, 62.0, 64.7, 68.9, 69.4, 69.5, 70.7, 71.4, 92.2, 96.0. ESI HRMS (*m/z*): $[M + Na]^+$ calcd for $C_{10}H_{15}N_3O_6$, 296.0859; found 296.0881. The analytical data are in accordance with that of previously reported.[24]

Dimethylthexylsilyl 2-azido-2-deoxy-3,4-di-*O*-acetyl-β-D-fucopyranoside (27). Compound **26** (9.0 g, 33 mmol) was dissolved in CH_2Cl_2 (50 mL), and imidazole (6.8 g, 99 mmol) was added. The solution was stirred at RT for 5 min. TDSCl (9.8 mL) was then added and the reaction mixture was stirred at RT overnight. The reaction mixture was diluted with CH_2Cl_2 (100 mL) and washed with 1 M HCl (100 mL). The organic layer was then dried ($MgSO_4$) and concentrated *in vacuo*. The crude product was then chromatographed on silica gel using EtOAc:Hexane (1:9, v/v) as the eluent to give the target compound as a clear oil. 11.7 g (92%). R_f = 0.8 (EtOAc:Hexane, 1:4, v/v). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.19 – 0.2 (6H, 2s, Si($\underline{CH_3}$)$_2$), 0.9 (12H, m, 12H, C(C$\underline{H_3}$)$_2$, CH(C$\underline{H_3}$)$_2$), 1.17 (3H, d, H-6, *J* = 6.3 Hz), 1.68 (1H, m, C$\underline{H}$(CH_3)$_2$), 2.04 (3H, s, OAc), 2.17 (3H, s, OAc), 3.54 (1H, dd, H-2, *J* = 8.0, 11.2 Hz), 4.52 (1H, d, H-1, *J* = 8.0 Hz), 4.75 (1H, dd, H-3, *J* = 4.0, 11.2 Hz), 5.15 (1H, d, H-4, *J* = 3.0 Hz). ^{13}C NMR ($CDCl_3$): δ -2.7, 16.1, 19.8, 20.4 x 2, 33.8, 63.1, 68.9, 69.8, 71.2, 96.9. ESI HRMS (*m/z*): $[M + Na]^+$ calcd for $C_{18}H_{33}N_3O_6Si$, 438.2036; found 438.2071.



27

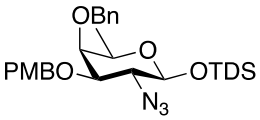
Dimethylthexylsilyl 2-Azido-2-deoxy-3-*O*-*p*-methoxybenzyl-β-D-fucopyranoside (29). Diacetate **27** (1.4 g, 3.3 mmol) was dissolved in anhydrous CH_3OH (10 mL) followed by the addition of a catalytic amount of Na. The reaction mixture was stirred at ambient temperature for 1 h. It was then neutralized with AcOH, concentrated *in vacuo*, and the resulting product was used in the next step without further purification. To a solution of crude **28** in toluene (20 mL) was added Bu_2SnO (982 mg, 4 mmol) and the reaction mixture was heated at 100 °C for 1 h. The solvent was then evaporated and the product was re-dissolved in toluene (20 mL), after which *p*-methoxybenzyl chloride (680 µL, 4.95 mmol) and Bu_4NBr (1.6 g, 4.95 mmol) were added. Stirring was continued at 90 °C for 2 h, after which TLC (EtOAc:Hexane, 1:1) indicated consumption of the starting material. The reaction mixture was concentrated *in vacuo*, and the residue was then re-dissolved in CH_2Cl_2 (50 mL). The organic layer was then washed with H_2O (50 mL), after which it was dried ($MgSO_4$) and concentrated *in vacuo*. Column chromatography over silica gel using EtOAc:Hexane, (5:95 to 1:9, v/v) as the eluent afforded the product as a clear oil. 1.2 g (90%). R_f = 0.5 (EtOAc:Hexane, 3:7). ^{1}H NMR (300 MHz,



29

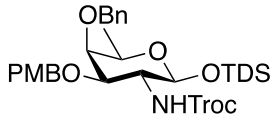
$CDCl_3$): δ 0.16 – 0.17 (6H, 2s, Si($\underline{CH_3}$)$_2$), 0.88 (12H, m, 12H, C(C$\underline{H_3}$)$_2$, CH(C$\underline{H_3}$)$_2$), 1.31 (3H, d, H-6, *J* = 6.5 Hz), 1.66 (1H, m, C$\underline{H}$(CH_3)$_2$), 2.29 (1H, d, 4-OH), 3.23 (1H, dd, H-3, *J* = 3.2, 10.2 Hz), 3.46 (2H, m, H-4, H-5), 3.66 (1H, broad t, H-2), 3.81 (3H, s, *p*-O$\underline{CH_3}$), 4.38 (1H, d, H-1, *J* = 7.8 Hz), 4.63 (2H, s, C$\underline{H_2}$OPMB), 6.90 (2H, d, Ar-H), 7.30 (2H, d, Ar-H). ^{13}C NMR ($CDCl_3$): δ 16.2, 18.4, 20.0, 33.8, 55.2, 65.0, 68.2, 70.1, 71.6, 79.0, 96.8, 114.0, 114.0, 129.6, 129.6. ESI HRMS (*m/z*): [M + Na]$^+$ calcd for $C_{22}H_{37}N_3O_5Si$, 474.2400; found 474.2377.

Dimethylthexylsilyl-2-Azido-2-deoxy-4-*O*-benzyl-3-*O*-*p*-methoxybenzyl-β-D-fucopyranoside (30). Compound **29** (1.5 g, 3.3 mmol) was dissolved in DMF (20 mL), and the solution was cooled to 0 ^{o}C. NaH (265 mg, 6.6 mmol, 60% oil dispersion) and benzyl bromide (784 µL, 6.6 mmol) were subsequently added allowing release of the formed hydrogen gas. The reaction mixture was stirred at RT for 1 h, after which the starting material has been fully consumed. The reaction mixture was neutralized with AcOH (5 mL), concentrated *in vacuo*, diluted with CH_2Cl_2 (50 mL) and washed with sat. $NaHCO_3$ (100 mL). The organic layer was dried ($MgSO_4$) and concentrated *in vacuo* to afforded a residue that was purified over silica gel using EtOAc:Hexane (0:100 to 5:95, v/v) as the eluent to afford the target product as a clear oil. 1.5 g (86%). R_f = 0.7 (EtOAc:Hexane, 3:7, v/v). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.17 – 0.18 (6H, 2s, Si($\underline{CH_3}$)$_2$), 0.90 (12H, m, 12H, C(C$\underline{H_3}$)$_2$, CH(C$\underline{H_3}$)$_2$), 1.16 (3H, d, H-6, *J* = 6.5 Hz), 1.68 (1H, m, C$\underline{H}$(CH_3)$_2$), 3.25 (1H, dd, H-3, *J* = 3.2, 10.4 Hz), 3.38 (1H, q, H-5, *J* = 6.2 Hz), 3.48 (1H, d, H-4, *J* = 2.6 Hz), 3.68 (1H, dd, H-2, *J* = 7.6, 10.5 Hz), 3.82 (3H, s, *p*-O$\underline{CH_3}$), 4.38 (1H, d, H-1, *J* = 7.5 Hz), 4.52 (1H, d, CH$\underline{H}$OBn, *J* = 12.0 Hz), 4.66 (2H, m, C$\underline{H_2}$OPMB), 4.94 (1H, d, C$\underline{H}$HOBn, *J* = 12.0 Hz), 6.91 (2H, d, Ar-H), 7.33 (7H, m, Ar-H). ^{13}C NMR ($CDCl_3$): δ 17.0, 18.5, 20.0, 33.9, 55.3, 65.6, 70.5, 71.8, 72.4, 74.5, 75.1, 80.6, 97.2, 113.8, 129.4. ESI HRMS (*m/z*): [M + Na]$^+$ calcd for $C_{29}H_{43}N_3O_5Si$, 564.2870; found 564.2852.



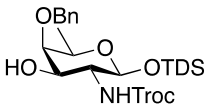
30

Dimethylthexylsilyl-2-(2,2,2-trichloroethoxy)-carbonylamino-2-deoxy-4-*O*-benzyl-3-*O*-*p*-methoxybenzyl-β-D-fucopyranoside (31). Compound **30** (830 mg, 1.53 mmol) was dissolved in THF (5 mL) followed by the addition of AcOH (500 µL) and Zn dust (1.0 g). The reaction mixture was stirred at RT for 1 h, after which it was filtered and the filtrate concentrated *in vacuo* to dryness. The resulting syrup was re-dissolved in THF (10 mL), followed by the addition of solid $NaHCO_3$ (1.0 g) and 2,2,2-trichloroethoxycarbonyl chloride (455 µL, 2.94 mmol). The reaction mixture was stirred at room temperatire for 15 min, after which TLC (EtOAc:Hexane, 3:7) showed it was complete. The reaction mixture was then filtered, concentrated to dryness and the residue purified on silica gel using EtOAc:Hexane (1:9 to 1:4, v/v) as the eluent to give the title compound as a colorless oil. 867 mg (82 %, two steps). R_f = 0.65 (EtOAc:Hexane, 3:7, v/v). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.09 – 0.13 (6H, 2s, Si($\underline{CH_3}$)$_2$), 0.84 (12H, m, 12H, C(C$\underline{H_3}$)$_2$, CH(C$\underline{H_3}$)$_2$), 1.18 (3H, d, H-6, *J* = 6.4 Hz), 1.61 (1H, m, C$\underline{H}$(CH_3)$_2$), 3.47 (1H, q, H-5, *J* = 6.4 Hz), 3.55 (1H, broad m, H-2), 3.59 (1H, d, H-4, *J* = 2.2 Hz), 3.81 (3H, s, *p*-O$\underline{CH_3}$), 4.44 (1H, d, CH$\underline{H}$OBn, *J* = 12.0 Hz), 4.65 (4H, m, C$\underline{H_2}$OPMB, $Cl_3CC\underline{H_2}O$), 4.82 (1H, broad d, H-1), 4.94 (1H, d, C$\underline{H}$HOBn, *J* = 12.0 Hz), 5.01 (1H, broad s, NH), 6.87 (2H, d, Ar-H), 7.31 (7H, m, Ar-H). ^{13}C NMR ($CDCl_3$): δ -2.7, 17.1, 18.5, 20.0, 34.0, 55.3, 56.7, 70.4, 71.6, 74.5, 74.9, 77.7, 95.2, 113.8, 128.3, 129.5. ESI HRMS (*m/z*): [M + Na]$^+$ calcd for $C_{32}H_{46}Cl_3NO_7Si$, 712.2007; found 712.2021.



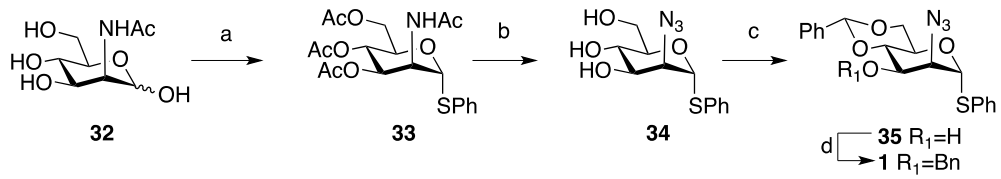
31

Dimethylthexylsilyl-2-(2,2,2-trichloroethoxy)-carbonylamino-2-deoxy-4-*O*-benzyl-β-D-fucopyranoside (3). Compound **31** (1.1 g, 1.6 mmol) was dissolved in a mixture of CH_2Cl_2:H_2O (10 mL, 9:1, v/v). DDQ (544 mg, 2.44 mmol) was added and the resulting reaction mixture was stirred at RT for 2 h. It was then diluted with CH_2Cl_2 (20 mL), washed with sat. $NaHCO_3$ (50 mL). The organic layer dried ($MgSO_4$) and concentration under reduced pressure and the residue was purified over silica gel using EtOAc:Hexane (1:9 to 1:4, v/v) as the eluent to afford the target compound as a white foam. 650 mg, (72%). R_f = 0.4 (EtOAc:Hexane, 3:7, v/v). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.12 – 0.16 (6H, 2s, Si($\underline{CH_3}$)$_2$), 0.85 (12H, m, 12H, C(C$\underline{H_3}$)$_2$, CH(C$\underline{H_3}$)$_2$), 1.26 (3H, d, H-6, *J* = 6.4 Hz), 1.61 (1H, m, C$\underline{H}$(CH$_3$)$_2$), 3.53 (3H, m, H-2, H-4, H-5), 3.80 (1H, broad d, H-3), 4.60 (1H, d, H-1, *J* = 8.6 Hz), 4.66 – 4.85 (4H, m, C$\underline{H_2}$OBn, Cl_3CC$\underline{H_2}$O), 5.04 (1H, broad d, NH), 7.27 – 7.42 (5H, m, Ar-H). ^{13}C NMR ($CDCl_3$): δ -2.7, 16.9, 18.4, 19.9, 33.4, 58.2, 70.7, 72.6, 74.8, 75.9, 79.0, 95.8, 128.16. ESI HRMS (*m/z*): [M + Na]$^+$ calcd for $C_{24}H_{38}Cl_3NO_6Si$, 592.1432; found 592.1410.



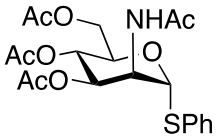
3

Scheme 3S. Optimized synthesis of donor **1**.



Reagents and conditions: a) Ac_2O, pyridine, rt, 2h, then thiophenol, BF_3-OEt_2, DCE, 60 °C, overnight, (**33**: 78%, two steps); b) Na (cat.), MeOH, rt, 30 min, then $Ba(OH)_2$, H_2O, 90 °C, overnight, then Im-SO_2N_3, K_2CO_3, $CuSO_4$, CH_3OH, rt 2h (**34**: 55%, 3 steps); c) Benzaldehyde dimethyl acetal, CSA, CH_3CN, rt, 4h, (**35**: 75%); d) BnBr, NaH, DMF, rt, 3h, (**1:** 91%)

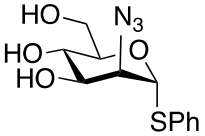
Phenyl 2-acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-1-thio-α-D-mannopyranoside (33). *N*-Acetyl-D-mannosamine **32** (10.0 g, 45.2 mmol) was suspended in pyridine (50 mL), followed by the addition of Ac_2O (50 mL). The reaction mixture was stirred at RT for 3 h, after which it was concentrated *in vacuo*, diluted with CH_2Cl_2 (200 mL) and washed with 1 M HCl (200 mL). The organic layer dried ($MgSO_4$), concentrated *in vacuo* to afford an intermediate peracetate as a white foam, which was used directly in the next step without further purification. The above product was dissolved in 1,2-dichloroethane (200 mL), and thiophenol (7.5 mL, 67.8 mmol) and BF_3-OEt_2 (11.1 mL, 90 mmol) were added. The reaction mixture was heated at 55 °C overnight, after which time the solution had turned deep purple and TLC (EtOAc) showed the presence of a single product. The reaction mixture was diluted with CH_2Cl_2 (50 mL) and washed with sat. $NaHCO_3$. The organic layer was dried ($MgSO_4$), concentrated *in vacuo* to afford a residue, which was subjected to chromatography over silica gel using EtOAc:Hexane (1:4 to 1:1 to 7:3, v/v) as the eluent to give the title compound. 15.5 g (78%, two steps). R_f = 0.6 (EtOAc). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.99 – 2.06 (12H, 4s, OAc x 4), 4.03 (1H, dd, H-6$_a$, *J* =3.2, 12.4 Hz), 4.28 (1H, dd, H-6$_b$, *J* = 6.2, 12.4 Hz), 4.60 (1H, m, H-5), 4.84 (1H, ddd, H-2, *J* = 1.2, 4.2, 9.2 Hz), 5.12 (1H, t, H-4, *J* = 10.1 Hz), 5.28 (1H, dd, H-3, *J* = 4.2, 10.1 Hz), 5.4 (1H, H-1, *J* = 1.1 Hz), 6.08 (1H, d, NH, *J* = 9.4 Hz), 7.24 – 7.34 (3H, m, Ar-H), 7.42 – 7.50 (2H, m, Ar-H). ^{13}C NMR ($CDCl_3$): δ 20.7 x



33

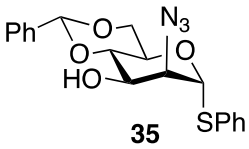
3, 23.6, 51.3, 62.4, 66.5, 68.9, 69.7, 86.9, 128.8, 131.9. ESI HRMS (*m/z*): $[M + Na]^+$ calcd for $C_{20}H_{25}NO_8S$, 462.1199; found 462.1192.

Phenyl 2-azido-2-deoxy-1-thio-α-D-mannopyranoside (34). To a solution of **22** (15.5 g, 35.2 mmol) in methanol (100 mL) was added a small piece of Na. The reaction mixture was stirred at RT for 1 h, after which it was neutralized with AcOH, concentrated *in vacuo*, redissolved in H_2O (100 mL) followed by the addition of $Ba(OH)_2*8H_2O$ (22.2 g, 70.5 mmol). The reaction mixture was heated at 90 °C overnight, after which TLC showed the presence of the free amine [R_f = 0.4 (EtOAc:CH_3OH:H_2O, 7:2:1)]. The reaction mixture was then neutralized with H_2SO_4 until pH~6.0 and the resulting suspension was centrifuged to remove $BaSO_4$. The solution was then concentrated to ~30 mL of H_2O, after which CH_3OH (100 mL) was added. To the resulting solution was added $CuSO_4$ (100 mg), K_2CO_3 (9.7 g, 70.4 mmol) and $ImSO_2HSO_4$[25] (12.5 g, 45.76 mmol) and the mixture was stirred at RT for 3 h. The solution was then concentrated *in vacuo*, redissolved in CH_2Cl_2 (100 mL) and washed with 1 M HCl (50 mL). The organic layer was separated and dried ($MgSO_4$). Concentration of the resulting solution *in vacuo* afforded a residue that was purified on silica gel using CH_3OH:CH_2Cl_2 (5:95 to 1:9, v/v) as the eluent to afford the target compound as a yellow syrup. 6.3 g (55%, two steps). R_f = 0.56 (CH_3OH:CH_2Cl_2, 1:9). ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.79 – 3.96 (2H, m, H-6_{a+b}), 3.94 – 3.15 (3H, m, H-3, H-4, H-5), 4.2 (1H, d, H-2, *J* = 3.6 Hz), 5.51 (1H, s, H-1), 7.27 – 7. 49 (5H, m, Ar-H). ^{13}C NMR ($CDCl_3$): 61.7, 65.1, 67.9, 71.7, 73.1, 86.4, 129.0, 132.0. ESI HRMS (*m/z*): $[M - H]^-$ calcd for $C_{12}H_{15}N_3O_4S$, 296.0783; found 296.0707.



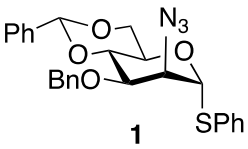
34

Phenyl 2-azido-2-deoxy-4,6-*O*-benzylidene-1-thio-α-D-mannopyranoside (35). Triol **34** (9.22 g, 31.0 mmol) was dissolved in CH_3CN (100 mL), and benzaldehyde dimethyl acetal (7 mL, 46.5 mmol) and camphorsulfonic acid (1.4 g, 6.2 mmol) were added. The reaction mixture was stirred at RT for 3 h, after which TLC (EtOAc:Hexane, 1:1, v/v) showed the reaction had gone to completion. The mixture was neutralized with Et_3N (5mL) and concentrated *in vacuo*. Purification of the resulting syrup over silica gel using EtOAc:Hexane (1:9 to 1:4, v/v) as the eluent afforded the target compound as a white foam. 8.9 g (75%). R_f = 0.6 (EtOAc:Hexane, 3:7). ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.69 (1H, d, 3-OH, *J* = 3.91 Hz), 3.82 (1H, t, H-6_a, *J* = 10.4 Hz), 3.97 (1H, t, H-4, *J* = 10.1 Hz), 4.18 – 4.38 (4H, m, H-6_b, H-5, H-3), 5.49 (1H, s, H-1), 5.60 (1H, C<u>H</u>Ph), 7.28 – 7.55 (10H, m, Ar-H). ^{13}C NMR ($CDCl_3$): δ 64.6, 65.0, 68.3, 69.3, 79.0, 86.9, 102.4, 126.0 – 132.1 (Ar-C). ESI HRMS (*m/z*): $[M + Na]^+$ calcd for $C_{19}H_{19}N_3O_4S$, 408.0994; found 408.0964.

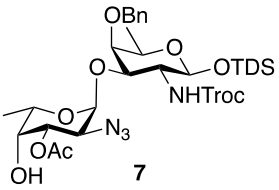


35

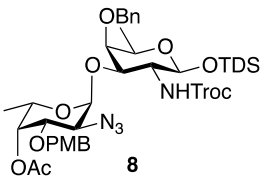
Phenyl 2-azido-2-deoxy-3-*O*-benzyl-4,6-*O*-benzylidene-1-thio-α-D-mannopyranoside (1). Alcohol **35** (6.68 g, 17.33 mmol) was dissolved in DMF (30 mL). The resulting solution was then cooled (0 °C), and NaH (2.0 g, 51.9 mmol, 60% dispersion in oil) was added. To the resulting suspension was added BnBr (3.1 mL, 26 mmol) and the reaction mixture was stirred at RT for 3 h. It was then carefully quenched by the addition of CH_3OH (10 mL) and the resulting clear solution was concentrated *in vacuo*. Column chromatography of the residue over silica gel using EtOAc:Hexane (0:100 to 5:95, v/v) as the eluent afforded the target compound as a white syrup. 7.8 g (91 %). R_f = 0.8 (EtOAc:Hexane, 3:7, v/v). ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.84 (1H, t, H-6$_a$, *J* = 10.4 Hz), 4.10 – 4.25 (4H, m, H-6$_b$, H-4, H-3, H-2), 4.32 (1H, m, H-5), 4.76 (1H, d, C<u>H</u>HOBn, *J* = 11.7 Hz), 4.94 (1H, d, CH<u>H</u>OBn, *J* = 11.7 Hz), 5.43 (1H, s, H-1), 5.64 (1H, s, C<u>H</u>Ph), 7.28 – 7.58 (15H, m, Ar-H). ^{13}C NMR ($CDCl_3$): δ 64.3, 65.2, 68.5, 73.5, 75.7, 79.1, 87.3, 101.5, 126.0 – 132.0 (Ar-C). ESI HRMS (*m/z*): $[M + Na]^+$ calcd for $C_{26}H_{25}N_3O_4S$, 498.1463; found 498.1441.



Dimethylthexylsilyl-2-azido-2-deoxy-3-*O*-acetyl-α-L-fucopyranosyl-(1→3)-2-(2,2,2-trichloroethoxy)-carbonylamino-2-deoxy-4-*O*-benzyl-β-D-fucopyranoside (7). To a solution of the donor **2a** (309 mg, 0.77 mmol) in CH_2Cl_2 (5 mL) at RT was added BSP (200 mg, 0.92 mmol), TTBP (383 mg, 1.54 mmol), and 3 Å flame-dried molecular sieves. The resulting suspension was cooled (-60 °C), followed by the addition of Tf_2O (155 µL, 0.92 mmol). The resulting mixture was stirred at this temperature for 10 min., after which acceptor **3** (527 mg, 0.924 mmol) in CH_2Cl_2 (2 mL) was slowly added along the wall of the flask. The reaction mixture was kept at -60 °C for 15 min, after which it was warmed to -50 °C and stirred for additional 15 min. The reaction mixture was quenched with Et_3N, warmed to RT, diluted with CH_2Cl_2 (20 mL) and washed with 10% $Na_2S_2O_3$ (30 mL). The organic layer was dried ($MgSO_4$) and concentrated *in vacuo* affording a residue that was dissolved in methanol (20 mL) followed by the addition of thiourea (690 mg, 9 mmol). The resulting mixture was heated at 60 °C for 4 h, after which TLC showed the reaction had gone to completion. The reaction mixture was concentrated *in vacuo*, and the residue purified by silica gel column chromatography using EtOAc:Hexane (3:7 to 1:1, v/v) as the eluent to afford the target compound as a white foam. 250 mg (41%, two steps). R_f = 0.3 (EtOAc:Hexane, 3:7, v/v). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.11 – 0.15 (6H, 2s, Si(<u>CH$_3$</u>)$_2$), 0.78 – 0.92 (12H, m, 12H, C(C<u>H$_3$</u>)$_2$, CH(C<u>H$_3$</u>)$_2$), 1.12 (3H, d, H-6^L, *J* = 6.6 Hz), 1.30 (3H, d, H-6^D, *J* = 6.5 Hz), 1.62 (1H, m, C<u>H</u>(CH$_3$)$_2$), 1.93 (1H, s, 4-OH^L), 2.16 (3H, s, OAc), 3.47 – 3.68 (4H, m, H-2^L, H-2^D, H-4^D, H-5^D), 3.71 (1H, s, H-4^L), 3.86 (1H, q, *J* = 6.4 Hz, H-5^L), 4.25 (1H, broad d, H-3^D, *J* = 10.5 Hz), 4.56 (1H, d, C<u>H</u>HOBn, *J* = 12.8 Hz), 4.73 – 4.89 (3H, m, CH<u>H</u>OBn, Cl_3C<u>H_2</u>OCO), 4.98 (1H, d, H-1^D, *J* = 8.5 Hz), 5.06 (1H, d, H-1^L, *J* = 3.6 Hz), 5.20 (1H, dd, H-3^L, *J* = 3.0 Hz, 11.3 Hz), 5.41 (1H, d, NH, *J* = 7.3 Hz), 7.22 – 7.45 (5H, m, Ar-H). ^{13}C NMR ($CDCl_3$): δ -3.43, -1.72, 15.9, 17.5, 19.9, 20.8, 34.0, 56.8, 57.4, 66.0, 69.9, 71.0, 74.5, 74.7, 78.3, 79.6, 94.8, 99.7, 124.4 – 131.0 (Ar-C).

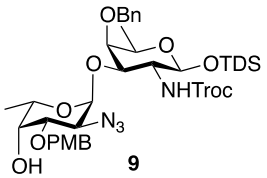


Dimethylthexylsilyl-2-azido-2-deoxy-4-*O*-acetyl-3-*O*-*p*-methoxybenzyl-α-L-fucopyranosyl-(1→3)-2-(2,2,2-trichloroethoxy)-carbonylamino-2-deoxy-4-*O*-benzyl-β-D-fucopyranoside (8). A mixture of donor **2b** (4.79 g, 10.8 mmol), acceptor **3** (4.7 g, 8.3 mmol) and 3Å flame-dried molecular sieves was stirred in CH_2Cl_2 (200 mL) at RT for 15 min. NIS (2.6 g, 11.62 mmol) was then added and the mixture was cooled to -60 °C, after which TMSOTf (300 µL, 1.66 mmol) was added. The reaction mixture was

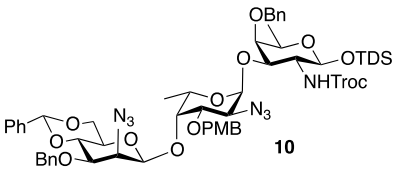


then stirred at to -60 ºC for 15 min after which it warmed to -50 ºC over a period of 15 min. It was quenched with Et_3N (5 mL), and allowed to warm to RT. The mixture was diluted with CH_2Cl_2 (100 mL) and washed with 10% $Na_2S_2O_3$. The organic layer was dried ($MgSO_4$) and concentrated *in vacuo* to afford a residue that was purified by silica gel column chromatography using EtOAc:Hexane (1:9 to 15:85, v/v) as the eluent to give the target disaccharide as a white foam. 4.5 g (60%, isolated α). R_f = 0.65 (EtOAc:Hexane, 3:7, v/v). ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.15 – 0.18 (6H, 2s, Si($\underline{CH_3}$)$_2$), 0.84 – 0.92 (12H, m, 12H, C(C$\underline{H_3}$)$_2$, CH(C$\underline{H_3}$)$_2$), 1.06 (3H, d, H-6^L, *J* = 6.5 Hz), 1.32 (3H, d, H-6^D, *J* = 6.5 Hz), 1.65 (1H, m, C$\underline{H}$(CH$_3$)$_2$), 2.13 (3H, s, OAc), 3.51 – 3.61 (3H, m, H-2^L, H-2^D, H-4^D), 3.66 (1H, q, H-5^D, *J* = 6.70 Hz), 3.75 (1H, dd, H-3^L, *J* = 3.0, 10.3 Hz), 3.79 (3H, s, *p*-OC$\underline{H_3}$), 3.88 (1H, q, H-5^L, *J* = 6.70 Hz), 4.26 (1H, broad d, H-3^D, *J* = 10.5 Hz), 4.34 (1H, d, C$\underline{H}$HOBn, *J* = 10.5 Hz), 4.55 – 4.61 (2H, m, CH$\underline{H}$OBn, Cl$_3$CC$\underline{H}$HCO), 4.67 (1H, d, C$\underline{H}$HOPMB), 4.80 (1H, d, Cl$_3$CCH$\underline{H}$CO), 4.89 (1H, d, CH$\underline{H}$OPMB, *J* = 12.5 Hz), 5.02 (1H, d, H-1^L, *J* = 3.7 Hz), 5.03 (1H, d, H-1^D, *J* = 8.1 Hz), 5.15 (1H, H-4^L, *J* = 2.56 Hz), 5.45 (1H, d, NH, *J* = 7.0 Hz), 6.87 (2H, d, Ar-H), 7.26 – 7.41 (8H, m, Ar-H). ^{13}C NMR ($CDCl_3$): δ -3.48, -1.77, 16.1, 17.1, 18.6, 20.0, 20.6, 33.9, 55.1, 56.9, 59.3, 65.4, 69.2, 70.5, 71.2, 73.8, 74.6, 75.5, 78.2, 80.7, 94.7, 99.9, 113.9, 126.02 – 131.4 (Ar-C). ESI HRMS (*m/z*): [M + Na]$^+$ calcd for $C_{40}H_{57}Cl_3N_4O_{11}Si$, 925.2756; found 925.2735.

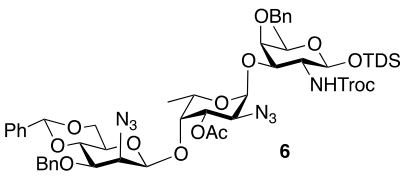
Dimethylthexylsilyl-2-azido-2-deoxy-3-*O*-*p*-methoxybenzyl-α-L-fucopyranosyl-(1→3)-2-(2,2,2-trichloroethoxy)-carbonylamino-2-deoxy-4-*O*-benzyl-β-D-fucopyranoside (9). To a solution of **8** (4.5 g, 4.9 mmol) in CH_3OH (50 mL) was added guanidine hydrochloride (5.0 g) and to the resulting solution was added a small piece of Na. The reaction mixture was then stirred at RT for 16 h, after which TLC (EtOAc:Hexane, 3:7, v/v) showed the deprotection had gone to completion. The reaction mixture was neutralized with AcOH (1 mL), concentrated *in vacuo* and chromatographed using EtOAc:Hexane (3:7 to 1:1, v/v) as the eluent to provide the title compound as a white solid. 3.8 g (quant). R_f = 0.3 (EtOAc:Hexane, 3:7, v/v). ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.14 – 0.17 (6H, 2s, Si($\underline{CH_3}$)$_2$), 0.84 – 0.91 (12H, m, 12H, C(C$\underline{H_3}$)$_2$, CH(C$\underline{H_3}$)$_2$), 1.20 (3H, d, H-6^L, *J* = 5.8 Hz), 1.30 (3H, d, H-6^D, *J* = 6.6 Hz), 1.65 (1H, m, C$\underline{H}$(CH$_3$)$_2$), 2.26 (1H, s, 4-OH), 3.50 – 3.67 (5H, m, H-2^L, H-2^D, H-4^D, H-5^D, H-4^L), 3.70 (1H, dd, H-3^L, *J* = 2.7, 10.3 Hz), 3.80 (3H, s, *p*-OC$\underline{H_3}$), 3.88 (1H, m, H-5^L), 4.20 (1H, broad d, H-3^D, *J* = 10.5 Hz), 4.51 (1H, d, C$\underline{H}$HOBn, *J* = 10.5 Hz), 4.58 – 4.63 (2H, m, CH$\underline{H}$OBn, Cl$_3$CC$\underline{H}$HCO), 4.71 (1H, d, C$\underline{H}$HOPMB), 4.79 (1H, d, Cl$_3$CCH$\underline{H}$CO), 4.87 (1H, d, CH$\underline{H}$OPMB, *J* = 12.5 Hz), 5.00 (2H, d, H-1^D, H-1^L, *J* = 3.7 Hz), 5.42 (1H, d, NH, *J* = 7.3 Hz), 6.88 (2H, d, Ar-H), 7.25 – 7.41 (8H, m, Ar-H). ^{13}C NMR ($CDCl_3$): δ -3.41, -1.8, 16.0, 17.2, 18.5, 20.0, 33.9, 55.2, 56.9, 59.2, 66.1, 68.6, 70.5, 71.6, 74.7, 75.3, 76.0, 78.3, 80.5, 94.9, 100.0, 114.0, 126.9 – 129.8 (Ar-C). ESI HRMS (*m/z*): [M + Na]$^+$ calcd for $C_{38}H_{55}Cl_3N_4O_{10}Si$, 883.2651; found 883.2631.



Dimethylthexylsilyl-2-azido-2-deoxy-3-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranosyl-(1→4)-2-azido-2-deoxy-3-*O*-*p*-methoxybenzyl-α-L-fucopyranosyl-(1→3)-2-(2,2,2-trichloroethoxy)-carbonylamino-2-deoxy-4-*O*-benzyl-β-D-fucopyranoside (10). A mixture of the donor **1** (717 mg, 1.5 mmol), diphenyl sulfoxide (330 mg, 1.6 mmol), TTBP (585 mg, 2.32 mmol) and 3Å flame-dried molecular sieves was stirred in CH_2Cl_2 (20 mL) at RT for 10 min. The resulting solution was cooled (-60 °C), after which Tf_2O (270 µL, 1.62 mmol) was added. The reaction mixture was stirred at -60 °C for 10 min, after which a solution of acceptor **9** (1.0 g, 1.16 mmol) in CH_2Cl_2 (2 mL) was added slowly along the wall of the flask. The reaction mixture was stirred at -60 °C for 15 min, after which it was allowed to warm to -30 °C within 1 h. TLC (EtOAc:Hexane, 3:7, v/v) showed the acceptor has been converted into a less polar product. The reaction was then quenched with sat. $NaHCO_3$ (1 mL) and the resulting mixture was allowed to warm to RT, after which it was diluted with CH_2Cl_2 (20 mL) and washed with sat. $NaHCO_3$ (20 mL). The organic layer was dried ($MgSO_4$), concentrated *in vacuo* and the residue purified by silica gel column chromatography using EtOAc:Hexane (1:9 to 1:4, v/v) as the eluent. First eluted was the α-anomer (R_f =0.6, EtOAc:Hexane, 3:7). The second elution afforded the target trisaccharide as a white solid. 990 mg (72%). R_f = 0.45 (EtOAc:Hexane, 3:7). ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.13 – 0.17 (6H, 2s, Si($\underline{CH_3}$)$_2$), 0.83 – 0.91 (12H, m, 12H, C(C$\underline{H_3}$)$_2$, CH(C$\underline{H_3}$)$_2$), 1.14 (3H, d, H-6^L, *J* = 6.4 Hz), 1.28 (3H, d, H-6^D, *J* = 6.4 Hz), 1.64 (1H, m, C$\underline{H}$(CH$_3$)$_2$), 3.12 (1H, ddd, H-5^M, *J* = 5.0, 9.5 Hz), 3.53 (1H, d, H-4^D, *J* = 3.1 Hz), 3.55 – 3.69 (4H, m, H-2^D, H-5^D, H-3^M, H-6$_a^M$), 3.72 (1H, dd, H-3^L, *J* = 3.1, 11.3 Hz), 3.77 (3H, s, *p*-OC$\underline{H_3}$), 3.79 – 3.88 (3H, m, H-2^M, H-5^L, H-2^L), 3.99 (1H, t, H-4^M, *J* = 9.1 Hz), 4.03 (1H, d, H-4^L, *J* = 4.0 Hz), 4.11 (1H, dd, H-6$_b^M$, *J* = 4.6, 10.6 Hz), 4.18 (1H, broad d, H-3^D, *J* = 11.3 Hz), 4.46 (1H, d, C$\underline{H}$HOBn, *J* = 11.9 Hz), 4.55 – 4.60 (2H, m, benzylic H), 4.62 (1H, s, H-1^M), 4.69 – 4.83 (4H, m, benzylic H, Cl$_3$CC$\underline{H_2}$OCO), 4.88 (1H, d, CH$\underline{H}$OBn, *J* = 12.7 Hz), 4.98 (2H, m, H-1^L, H-1^D), 5.46 (1H, d, NH, *J* = 7.8 Hz), 5.52 (1H, s, C$\underline{H}$Ph), 6.87 (2H, d, Ar-H), 7.28 – 7.42 (16H, m, Ar-H), 7.46 (2H, d, Ar-H). ^{13}C NMR ($CDCl_3$): δ -3.4, -1.7, 16.9, 17.3, 17.8 – 20.8, 33.9, 55.2, 56.7, 59.16, 63.2, 66.6, 67.4, 68.0, 70.5, 70.8, 72.9, 74.7, 74.9, 75.1, 75.9, 76.0, 78.1, 78.5, 80.3, 95.1, 99.9, 101.1 ($J_{C,H}$ = 159.9 Hz), 101.5, 113.8, 125.9 – 130.0 (Ar-C). ESI HRMS (*m/z*): [M + Na]$^+$ calcd for $C_{58}H_{74}Cl_3N_7O_{14}Si$, 1248.4026; found 1248.4026.

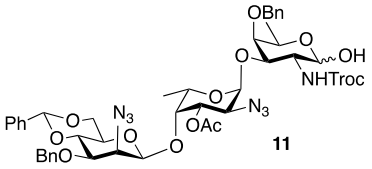


Dimethylthexylsilyl-2-azido-2-deoxy-3-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranosyl-(1→4)-2-azido-2-deoxy-3-*O*-acetyl-α-L-fucopyranosyl-(1→3)-2-(2,2,2-trichloroethoxy)-carbonylamino-2-deoxy-4-*O*-benzyl-β-D-fucopyranoside (6). To as solution of trisaccharide **10** (3.0 g, 2.4 mmol) in CH_2Cl_2 (30 mL) was added H_2O (3 mL), followed by the addition of DDQ (817 mg, 3.6 mmol). The reaction mixture was vigorously stirred at RT for 2 h, after which a further portion of DDQ (410 mg, 1.6 mmol) was added and stirring was continued for another 1 h, after which TLC (EtOAc:Hexane, 3:7, v/v) showed the reaction had gone to completion. R_f = 0.4 (EtOAc:Hexane, 3:7, v/v). The mixture was diluted with CH_2Cl_2 (50 mL) and washed with sat. $NaHCO_3$ (100 mL). The organic layer was dried ($MgSO_4$), filtered and the filtrate was concentrated *in vacuo* to afford the intermediate alcohol, which was dissolved in pyridine:Ac_2O (2:1, 15 mL). To the resulting solution was added DMAP (20 mg, cat.) and stirring was continued at RT for 2 h, after which the starting material had been fully consumed. The reaction mixture was concentrated *in vacuo* and the residue purified by silica gel column chromatography using EtOAc:Hexane (1:9 to 1:4, v/v) as the eluent to afford the

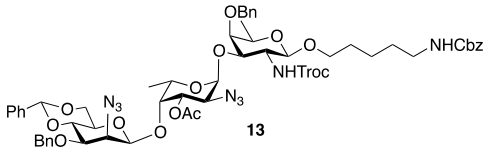


title compound as a white foam. 2.3 g (85%, two steps). R_f = 0.5 (EtOAc:Hexane, 3:7, v/v). ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.06 – 0.09 (6H, 2s, Si($\underline{CH_3}$)$_2$), 0.74 – 0.83 (12H, m, 12H, C($C\underline{H_3}$)$_2$, CH($C\underline{H_3}$)$_2$), 1.01 (3H, d, H-6^L, *J* = 5.4 Hz), 1.21 (3H, d, H-6^D, *J* = 6.6 Hz), 1.56 (1H, m, C$\underline{H}$(CH_3)$_2$), 2.06 (3H, s, OAc), 3.17 (1H, ddd, H-5^M, *J* = 4.3, 9.5 Hz), 3.58 (1H, d, H-4^D, *J* = 3.2 Hz), 3.59 – 3.65 (2H, m, H-5^D, H-2^D), 3.67 (1H, dd, H-3^M, *J* = 3.2, 9.7 Hz), 3.82 (1H, t, H-6$_a^M$, *J* = 9.7 Hz), 3.91 – 4.03 (3H, m, H-5^L, H-4^M, H-2^L), 4.09 (2H, s, H-2^D, H-2^M), 4.18 (1H, broad d, H-3^D, *J* = 12.4 Hz), 4.29 (1H, dd, H-6$_b^M$, *J* = 5.3, 10.5 Hz), 4.50 (1H, s, H-1^M), 4.60 (1H, d, CH$\underline{H}$OBn, *J* = 12.2 Hz), 4.76 (1H, CH$\underline{H}$OBn, *J* = 13.0 Hz), 4.80 – 4.85 (3H, m, C$\underline{H}$HOBn, $Cl_3CC\underline{H_2}OCO$), 4.89 (1H, d, C$\underline{H}$HOBn, *J* = 13.0 Hz), 4.97 (1H, d, H-1^D, *J* = 8.3 Hz), 5.03 (1H, dd, H-3^L, *J* = 2.9 Hz, 11.3 Hz), 5.05 (1H, d, H-1^L, *J* = 3.6 Hz), 5.45 (1H, d, NH, *J* = 7.8 Hz), 5.60 (1H, s, C$\underline{H}$Ph), 7.29 – 7.45 (12H, m, Ar-H), 7.46 – 7.51 (3H, m, Ar-H). ^{13}C NMR ($CDCl_3$): δ -3.4, -1.7, 16.6, 17.4, 18.6, 20.2, 21.4, 34.0, 56.7, 57.6, 62.7, 66.0, 67.6, 68.3, 70.4, 70.5, 72.9, 74.7, 75.8, 76.3, 78.3, 78.8, 79.0, 95.1, 99.3, 101.3, 101.8, 126.1 – 129.5 (Ar-C). ESI HRMS (*m/z*): [M + Na]$^+$ calcd for $C_{52}H_{68}Cl_3N_7O_{14}Si$, 1170.3557; found 1170.3569.

2-Azido-2-deoxy-3-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranosyl-(1→4)-2-azido-2-deoxy-3-*O*-acetyl-α-L-fucopyranosyl-(1→3)-2-(2,2,2-trichloroethoxy)-carbonylamino-2-deoxy-4-*O*-benzyl-α/β-D-fucopyranose (11). To a solution of trisaccharide **6** (2.3 g, 1.87 mmol) in THF (20 mL) (HDPE flask was used) was added HF/pyridine solution (3 mL) at 0 ºC and the reaction mixture was stirred at RT overnight. After this time, it was diluted with CH_2Cl_2 (50 mL) and washed with H_2O (50 mL) and then with sat. $NaHCO_3$ (30 mL). The organic layer was dried ($MgSO_4$), filtered and the filtrate concentrated *in vacuo* to provide a residue that was purified by silica gel column chromatography using EtOAc:Hexane (3:7 to 1:1, v/v) as the eluent to give a mixture of anomers. 1.7 g (90%) α/β = 1:5. R_f = 0.5 (α), 0.3 (β) (EtOAc:Hexane, 1:1). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.09 (3H, d, H-6^L, *J* = 6.0 Hz), 1.22 (3H, d, H-6^D, *J* = 6.0 Hz), 2.15 (3H, s, OAc), 3.11 (1H, s, 1-OH), 3.26 (1H, ddd, H-5^M, 3.61 – 3.70 (2H, m, H-4^D, H-3^M), 3.82 (1H, t, H-6$_a^M$, *J* = 9.7 Hz), 4.0 (2H, m, H-4^M, H-2^D), 4.04 – 4.21 (5H, m, H-5^L, H-5^D, H-4^L, H-2^L, H-2^M), 4.29 (1H, dd, H-6$_b^M$, *J* = 4.6, 10.3 Hz), 4.36 (1H, broad m, H-3^D), 4.52 (1H, s, H-1^M), 4.67 – 4.93 (m, C$\underline{H_2}$OBn x 2, $Cl_3CC\underline{H_2}OCO$, H-1$^{\beta D}$), 5.01 (1H, m, H-3^L), 5.06 (1H, d, H-1^L, *J* = 3.21 Hz), 5.46 (1H, s, H-1$^{\alpha D}$), 5.60 (1H, s, C$\underline{H}$Ph), 5.91 (1H, d, NH, *J* = 8.4 Hz), 7.30 – 7.52 (15H, m, Ar-H). ^{13}C NMR ($CDCl_3$): δ 16.5, 16.7, 21.5, 51.4, 58.2, 62.8, 66.7, 66.9, 67.5, 68.3, 71.0, 72.9, 74.8 x 2, 75.8, 76.2, 78.3, 78.4, 91.9, 99.2, 101.4, 101.8, 126.0 – 129.3 (Ar-C). ESI HRMS (*m/z*): [M + Na]$^+$ calcd for $C_{44}H_{50}Cl_3N_7O_{14}Si$, 1028.2379; found 1028.2367.

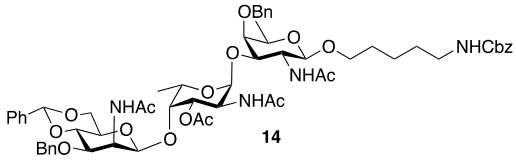


5-(Benzyloxycarbonyl)aminopentyl 2-azido-2-deoxy-3-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranosyl-(1→4)-2-azido-2-deoxy-3-*O*-acetyl-α-L-fucopyranosyl-(1→3)-2-(2,2,2-trichloroethoxy)-carbonylamino-2-deoxy-4-*O*-benzyl-β-D-fucopyranoside (13). To a solution of hemiacetal **11** (842 mg, 0.84 mmol) in CH_2Cl_2 (10 mL) was added Cs_2CO_3 (1.4 g, 4.2 mmol), followed by the addition of $CF_3(NPh)Cl$ (410 µL, 2.52 mmol), The reaction was left stirring at RT for 2 h after which TLC (EtOAc:Hexane, 3:7, v/v) showed the starting material had been fully consumed. R_f = 0.3 (β), 0.5 (α) (EtOAc:Hexane, 3:7, v/v). The reaction mixture was filtered through a pad of Celite® and the filtrate concentrated *in vacuo*. The resulting imidate **12** was used in the next step without further purification.[1] The mixture of crude **12** and benzyl *N*-(5-hydroxypentyl)carbamate[26] was dissolved in a mixture of CH_3CN:CH_2Cl_2 (1:1, v/v 10 mL) followed by adding 3Å flame-dried molecular sieves. The resulting solution was cooled (-60 ºC), after which TMSOTf (50 µL) was added. The reaction mixture was stirred at that temperature for 15 min. It was then warmed to -40 ºC and quenched with Et_3N. The resulting solution was filtered, concentrated *in vacuo*, and the residue purified by silica gel column chromatography using EtOAc:Hexane (3:7 to 1:1, v/v) as the eluent to give the title compound as a white solid. 630 mg (72%, two steps). R_f = 0.5 (EtOAc:Hexane, 1:1). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.1 (3H, d, H-6^L, *J* = 6.2 Hz), 1.32 (3H, d, H-6^D, 6.0 Hz), 1.34 – 1.64 (6H, m, C$\underline{H_2}$ x 3 linker), 2.15 (3H, s, OAc), 3.20 (2H, m, C$\underline{H_2}$NHCbz), 3.26 (1H, ddd, H-5^M, *J* = 5.1, 9.5 Hz), 3.45 (1H, m, OC$\underline{H}$H$(CH_2)_4$NHCbz), 3.57 (1H, d, H-4^D, *J* = 3.0 Hz), 3.61 (1H, m, H-5^D), 3.64 (1H, m, H-2^D), 3.67 (1H, dd, H-3^M, *J* = 4.3, 10.6 Hz), 3.82 (1H, t, H-6$_a^M$, *J* = 10.0 Hz), 3.92 – 3.98 (3H, m, H-2^L, H-5^L, OCH$\underline{H}$$(CH_2)_4$NHCbz), 4.00 (1H, t, H-4^M, *J* = 10.0 Hz), 4.08 (1H, d, H-2^M, *J* = 4.0 Hz), 4.10 (1H, d, H-4^L, 3.0 Hz), 4.17 (1H, m, H-3^D), 4.29 (1H, dd, H-6$_b^M$, *J* = 4.6, 10.4 Hz), 4.5 (1H, d, H-1^M), 4.65 – 4.91 (7H, m, C$\underline{H_2}$OBn x 2, Cl_3CC$\underline{H_2}$OCO, H-1^D), 5.01 (1H, H-3^L, *J* = 3.4, 11.3 Hz), 5.05 (1H, d, H-1^L, *J* = 3.7 Hz), 5.12 (2H, s, C$\underline{H_2}$ NHCbz), 5.6 (1H, s, C$\underline{H}$Ph), 5.73 (1H, broad s, NH), 7.29 – 7.51 (20H, m, Ar-H). ^{13}C NMR ($CDCl_3$): δ 16.6, 17.2, 21.2, 22.8, 28.8, 29.8, 41.1, 54.8, 57.4, 62.8, 66.0, 66.7, 67.4, 68.3, 69.3, 70.5, 70.5, 72.8, 74.4, 75.0, 75.7, 76.3, 78.3, 78.9, 78.9, 99.3, 100.0, 101.4, 101.6, 126.0 – 129.6 (Ar-C). ESI HRMS (*m/z*): [M + Na]$^+$ calcd for $C_{57}H_{67}Cl_3N_8O_{16}$, 1247.3638; found 1247.3637.

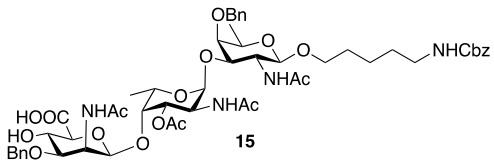


[1] Purification of this glycosyl imidate results in its partial hydrolysis on silica gel, and is not necessary provided the lactol is pure before commencing this step

5-(Benzyloxycarbonyl)aminopentyl 2-acetamido-2-deoxy-3-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranosyl-(1→4)-2-acetamido-2-deoxy-3-*O*-acetyl-α-L-fucopyranosyl-(1→3)-2-acetamido-2-deoxy-4-*O*-benzyl-β-D-fucopyranoside (14). To a solution of **13** (152 mg, 0.12 mmol) in THF (5 mL) was added AcOH (1 mL) and Ac_2O (1 mL). To the resulting mixture was added Zn dust (500 mg) and the reaction mixture was stirred at RT for 1 h, after which TLC (CH_3OH:CH_2Cl_2, 5:95, v/v) showed the transformation had gone to completion. The solution was filtered, and the filtrate concentrated *in vacuo*. The residue was purified on silica gel using CH_3OH:CH_2Cl_2 (1:99 to 3:97 to 5:95, v/v) affording the title compound as a glassy solid. 88 mg (63%). R_f = 0.2 (CH_3OH:CH_2Cl_2, 5:95). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.17 (3H, d, H-6^L, *J* = 6.0 Hz), 1.26 – 1.64 (9H, m, CH_2 x 3 linker, H-6^D), 1.96 (3H, s, NHAc), 2.05 (3H, s, OAc), 2.09 (3H, s, NHAc), 2.24 (3H, s, NHAc), 3.17 (2H, broad t, C$\underline{H_2}$NHCbz), 3.33 (1H, ddd, H-5^M, *J* = 4.9, 9.8 Hz), 3.46 (2H, m, H-4^D, OC$\underline{H}$H$(CH_2)_4$NHCbz), 3.64 (1H, m, H-5^D), 3.74 (1H, m, H-3^M), 3.78 – 4.03 (5H, m, H-4^L, H-6$_a^M$, H-5^L, H-4^M, OC$\underline{H}$H$(CH_2)_4$NHCbz), 4.25 (1H, dd, H-6$_b^M$, *J* = 6.25, 10.6 Hz), 4.41 (2H, m, H-2^D, H-1^D), 4.54 (2H, s, H-1^M, H-2^L), 4.69 (1H, d, CH$\underline{H}$OBn, *J* = 12.2 Hz), 4.80 (1H, m, H-3^L), 4.80-04.89 (3H, m, C$\underline{H_2}$OBn, C$\underline{H}$HOBn), 4.96 – 5.06 (2H, m, H-1^L, H-2^M), 5.10 (2H, m, C$\underline{H_2}$ NHCbz), 5.62 (1H, s, C$\underline{H}$Ph), 6.21 (1H, broad s, NH), 6.31 (1H, broad s, NH) , 6.80 (1H, broad s, NH), 7.24 – 7.54 (20H, m, Ar-H). ^{13}C NMR ($CDCl_3$): δ 16.6, 17.2, 21.3, 22.8, 23.4, 23.5, 23.5, 29.5, 41.1, 46.5, 50.5, 52.1, 55.2, 66.7, 66.7, 67.4, 68.3, 68.3, 70.6, 70.8, 71.4, 74.8, 75.6, 77.0, 78.0, 78.7, 99.4, 100.9, 100.9, 101.6, 125.6 – 129.5 (Ar-C). ESI HRMS (*m/z*): $[M + Na]^+$ calcd for $C_{60}H_{76}N_4O_{17}$, 1147.5103; found 1147.5093.



5-(Benzyloxycarbonyl)aminopentyl 2-acetamido-2-deoxy-3-*O*-benzyl-β-D-mannopyranosyluronate-(1→4)-2-acetamido-2-deoxy-3-*O*-acetyl-α-L-fucopyranosyl-(1→3)-2-acetamido-2-deoxy-4-*O*-benzyl-β-D-fucopyranoside (15). Trisaccharide **14** (50 mg, 0.054 mmol) was dissolved in 80% aq. AcOH (5 mL) and the resulting solution was heated at 90 °C for 2 h, after which TLC showed the reaction had gone to completion. The mixture was concentrated *in vacuo* and the resulting diol **5** was dissolved in CH_2Cl_2 (5 mL) followed by adding H_2O (2 mL), TEMPO (3.0 mg, cat.) and BAIB (35 mg, 0.108 mmol). The reaction mixture was stirred at room temperature for 2 h, after which *t*-BuOH (5 mL), 2-methyl-2-butene (1 mL) and a solution of $NaClO_2$ (50.0 mg, 0.44 mM) and NaH_2PO_4 (40 mg, 0.34 mM) in H_2O (0.2 mL) were added. The mixture was then stirred for additional 1 h, after which TLC showed no starting material remaining. The reaction mixture was diluted with CH_2Cl_2 (10 mL) and washed with sat. $NaHCO_3$ (20 mL). The organic layer was dried ($MgSO_4$) and concentrated *in vacuo* to afford a residue that was purified by silica gel column chromatography using CH_3OH:CH_2Cl_2 (1:4, v/v) as the eluent to give the title compound as a white solid. 30 mg (61%, two steps). R_f = 0.2 (CH_3OH:CH_2Cl_2, 1:4, v/v). ^{1}H NMR (500 MHz, d_6-DMSO): δ 1.15 – 1.47 (12H, m, CH_2 x 3 linker, H-6^L, H-6^D), 1.73 (3H, s, NHAc), 1.81 (3H, s, NHAc), 1.93 (3H, s, OAc), 1.94 (3H, s, NHAc), 2.96 (2H, m, C$\underline{H_2}$NHCbz), 3.31 (1H, m, OC$\underline{H}$H$(CH_2)_4$NHCbz), 3.36 (1H, m, H-4^D), 3.43 (1H, m, H-3^M), 3.52 (1H, s, H-5^M), 3.59 (1H, m, H-5^D), 3.64 (1H, m, OCH$\underline{H}$$(CH_2)_4$NHCbz), 3.70 (1H, t, H-4^M, *J* = 9.5 Hz), 3.75 (1H, m, H-3^D), 3.90 (1H, broad m, H-2^D), 4.01 (1H, s, H-4^L), 4.05 (1H, m, H-5^L), 4.18 (2H, m, H-2^L, H-1^D), 4.38 (1H, d, C$\underline{H}$HOBn, *J* = 12.2 Hz), 4.60 – 4.73 (3H, H-1^M, C$\underline{H_2}$OBn), 4.80 (1H,



dd, H-2^{M}, J = 5.0, 10.2 Hz), 4.90 (2H, m, H-3^{L}, H-1^{L}), 4.96 – 5.03 (3H, m, CH<u>H</u>OBn, C<u>H$_2$</u> NHCbz), 7.11 – 7.46 (15H, m, Ar-H). ^{13}C NMR (d_6-DMSO): δ = 16.6, 17.2, 19.6, 21.2, 23.0, 23.2, 23.2, 23.2, 29.2, 29.3, 29.5, 40.6, 46.8, 48.6, 51.4, 65.3, 66.7, 67.3, 68.5, 68.7, 70.3, 70.3, 74.7, 77.1, 77.7, 77.9, 79.3, 79.8, 98.8, 101.5, 101.6, 126.8 – 129.6. ESI HRMS (*m/z*): [M + Na]$^{+}$ calcd for $C_{53}H_{70}N_4O_{18}$, 1073.4583; found 1073.4547.

Aminopentyl 2-acetamido-2-deoxy-β-D-mannopyranosyluronate-(1→4)-2-acetamido-2-deoxy-3-*O*-acetyl-α-L-fucopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-fucopyranoside (4). To a solution of **15** (30 mg, 0.029 mmol) in methanol was added AcOH (100 μL) followed by the addition of Pd(OH)$_2$ (20% w/w on carbon, Degussa type) (50 mg). The reaction mixture was stirred under an atmosphere of hydrogen (1 atmosphere) for 1 h, after which it was filtered, and then another portion of the catalyst was added (50 mg) and stirring was continued for 2 h. After this time TLC (EtOAc:CH$_3$OH:H$_2$O, 5:2:1, v/v) showed the presence of a single product (R_f = 0.15). The mixture was filtered and lyophilization of the filtrate afforded **4** in quantitative yield as a white foam. ^{1}H NMR (500 MHz, D$_2$O): δ 1.12 (3H, d, H-6^{L}, J = 6.0 Hz), 1.15 (3H, d, H-6^{D}), 1.27 (2H, m, CH$_2$ linker), 1.46 (2H, m, CH$_2$ linker), 1.55 (2H, m, CH$_2$ linker), 1.86 (3H, s, NHAc), 1.89 (3H, s, NHAc), 1.96 (3H, s, OAc), 2.01 (3H, s, NHAc), 2.87 (2H, t, C<u>H$_2$</u>NHCbz, J = 8.9 Hz), 3.42 – 3.49 (2H, m, H-4^{D}, OCH<u>H</u>(CH$_2$)$_4$NHCbz), 3.52 (1H, t, H-4^{M}, J = 8.9 Hz), 3.66 (4H, m, H-3^{D}, H-3^{M}, H-5^{D}, H-5^{M}), 3.76 (1H, m, OC<u>H</u>H(CH$_2$)$_4$NHCbz), 3.86 (1H, t, H-2^{D}, J = 10.2 Hz), 4.07 (1H, q, H-5^{L}), 4.09 (1H, d, H-4^{L}, J = 3.1 Hz), 4.25 (1H, dd, H-2^{L}, J = 4.5, 12.1 Hz), 4.47 (1H, d, H-2^{M}, J = 4.5 Hz), 4.62 (1H, s, H-1^{M}), 4.88 (1H, d, H-1^{L}, J = 3.7 Hz), 4.90 (1H, dd, H-3^{L}, J = 3.1, 11.5 Hz). ^{13}C NMR (D$_2$O): 15.2, 20.2, 22.0, 22.0, 22.0, 22.4, 26.3, 28.0, 39.2, 47.1, 51.2, 52.8, 66.7, 69.4, 69.9, 69.9, 69.9, 70.5, 75.9, 77.1, 78.5, 99.0, 99.7, 101.4. ESI HRMS (*m/z*): [M - H]$^{-}$ calcd. for $C_{31}H_{51}N_4O_{16}$, 735.3300 found 735.3284.

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Chapter 5

Synthesis of Oligosaccharides Derived from Streptococcus pneumoniae Serotype 35B for Glycoconjugate Vaccine Development

Introduction

Streptococcus pneumoniae is a gram-positive bacterium, which is a common cause of pneumonia worldwide. Pneumococci frequently cause other illnesses including severe ear infections, meningitis, and bacteraemia.[1] These infections can result in long-lasting problems such as hearing loss, brain damage, and even death. The most effective way to combat pneumococcal disease remains preventative vaccination. A currently employed vaccine, PCV13, is composed of capsular polysaccharides (CPS) conjugated to carrier proteins and it protects against 13 serotypes that commonly cause pneumococcal infections. This is insufficient because the 83 remaining *S. pneumoniae* serotypes, each having a unique CPS structure, are not part of this formulation.[2] Because of the wide use of PCV13, and earlier PCV7 vaccines, the prevalence of other serotypes is increasing, reducing the protection by immunizations.[3] This so-called antigenic shift is favouring the spread of invasive nonvaccine serotype 35B, which is known for its resistance to penicillin treatments.[4] As a result, a number of government agencies have designated serotype 35B as the next likely vaccine candidate, because it is currently the most lethal among all serotypes affecting adults and children in the United States and Europe.[5]

The successful use of PCV13 and PCV7 is due to chemical conjugation of isolated CPS to carrier proteins such as CRM197, which enhances antigenicity of the native polysaccharide. The use of native CPS does, however, complicate glycoconjugate vaccine development. Studies have shown that oligosaccharides of intermediate length conjugated to a carrier protein elicit more specific antibodies than conjugates containing larger polysaccharides.[6] Furthermore, isolation of well-defined CPS fragments from bacterial cultures is difficult. Serotype 35B is an especially difficult case because its CPS is modified with a base-labile

acetyl ester. If traditional protein conjugation methods are used, there is a possibility that *O*-acetyl esters are hydrolysed, which may result in loss of antigenicity. It is anticipated that well-defined oligosaccharides obtained by chemical synthesis could facilitate the development of more efficient and better-characterised glycoconjugate vaccines. In particular, chemical synthesis enables installation of a reactive linker for controlled conjugation to a carrier protein.[7] Noteworthy, only three CPS structures (7F, 9V and 18C) amongst the 13 serotypes within the PCV13 vaccine are *O*-acetylated. Polysaccharide *O*-acetylation is known to play an important role in functional immunity to some vaccine oligosaccharides such as meningococcal serogroup A[8] and *Salmonella typhi* V[9], but considered less important for *S. pneumoniae* strains.[10]

Herein, we report an efficient synthetic route that can provide well-defined oligomers derived from CPS 35B. In addition, by removing the acetyl esters, oligomers derived from serotype 35D were easily obtained. Binding studies showed that *O*-acetylation is essential for recognition by ficolin-2, which is a serum protein believed to be important in controlling respiratory infections. Ficolin-2 can activate the lectin pathway of the complement system, which may contribute to long-lasting immunity. Collectively, our data provides a rationale why the closely related serotype 35D might escape immune detection and thus be more invasive.

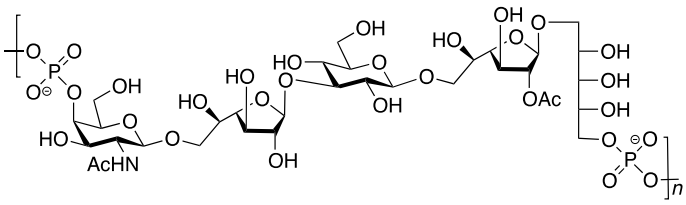


Figure 1. Structure of *S. pneumoniae* 35B CPS.

Results and Discussion

Isolated CPS antigen of 35B was determined to be a high molecular weight polymer composed of D-galactofuranose, D-glucose, *N*-acetyl-D-galactosamine, and ribitol.[11] These monosaccharides are all *trans*-linked through glycosidic bonds to give a pentasaccharide repeating unit, which is further polymerized through phosphodiester linkages (Figure 1). One of the galactofuranose residues is modified at C2 with an acetyl ester. This important feature distinguishes 35B from closely related and also invasive serotype 35D, lacking the acetyl moiety.

Chemical synthesis of *S. pneumoniae* 35B pentasaccharide repeating unit and its oligomers has not been described. The biomedical importance of this serotype gives an impetus to develop a scalable synthetic route that can give access to immunogenic conjugates without the risk of contamination from bacterial cultures. To provide synthetic polysaccharides of defined length as tools to address current vaccination needs, a scalable solution phase strategy was implemented. The synthetic route combines protecting groups used by both DNA and peptide chemistry,[12] and employs a flexible Fmoc-functionalized pentasaccharide building block that can be elongated by means of *H*-phosphonate chemistry.[13]

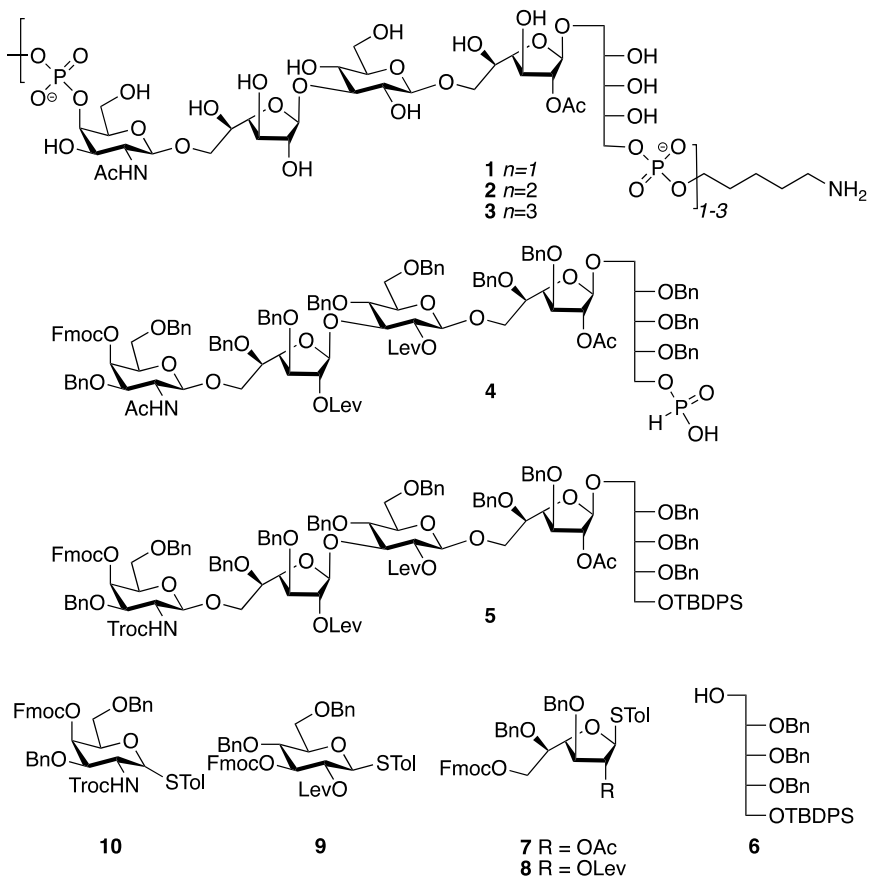


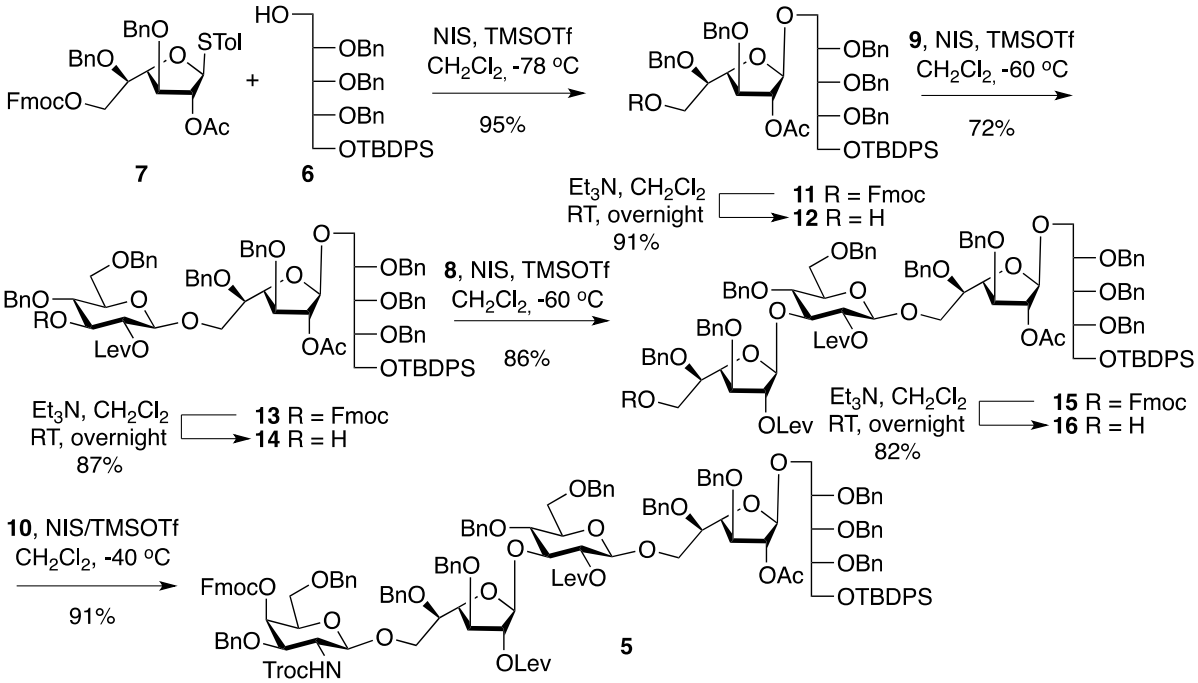
Figure 2. Structure of *S. pneumoniae* 35B capsular polysaccharide and building blocks required for the assembly.

Thus, a modular synthetic approach was developed to assemble vaccine candidates **1**–**3**, which are composed of one, two and three repeating units. Similar to oligonucleotide chemistry, phosphodiester linkages between pentasaccharide units were repeatedly formed using a bifunctional *H*-phosphonate building block **4**, which was first conjugated to an aminopentyl spacer. To avoid potential cleavage of acid-labile Gal*f* units during the following coupling cycles, we were in search of protecting groups to replace the dimethoxytrityl (DMTr) moiety traditionally used in oligonucleotide synthesis.[12] This was achieved by implementing the Fmoc strategy because of its proven performance in peptide synthesis[14] and orthogonality to employed levulinoyl (Lev) and acetyl esters.

We envisaged that the key phosphonate **4** could be accessed on a large scale through flexible pentasaccharide **5**, which is equipped with four orthogonal protecting groups. First, Lev esters were employed to ensure that glucose and non-acetylated Gal*f* units could be glycosylated with high *β*-anomeric selectivity. The use of the Lev esters was important to preserve the acetyl ester during the deprotection procedure. Next, TBDPS and Fmoc moieties were installed at the sites where phosphodiesters occur, and both can be independently cleaved using HF-pyridine and triethylamine, respectively.[15] Finally, galactosamine unit was derivatized with a C2 NHTroc group to enhance the yield of a desired *β*-glycoside, and this group can also be selectively converted to a native acetamido moiety without affecting other part of the saccharide.

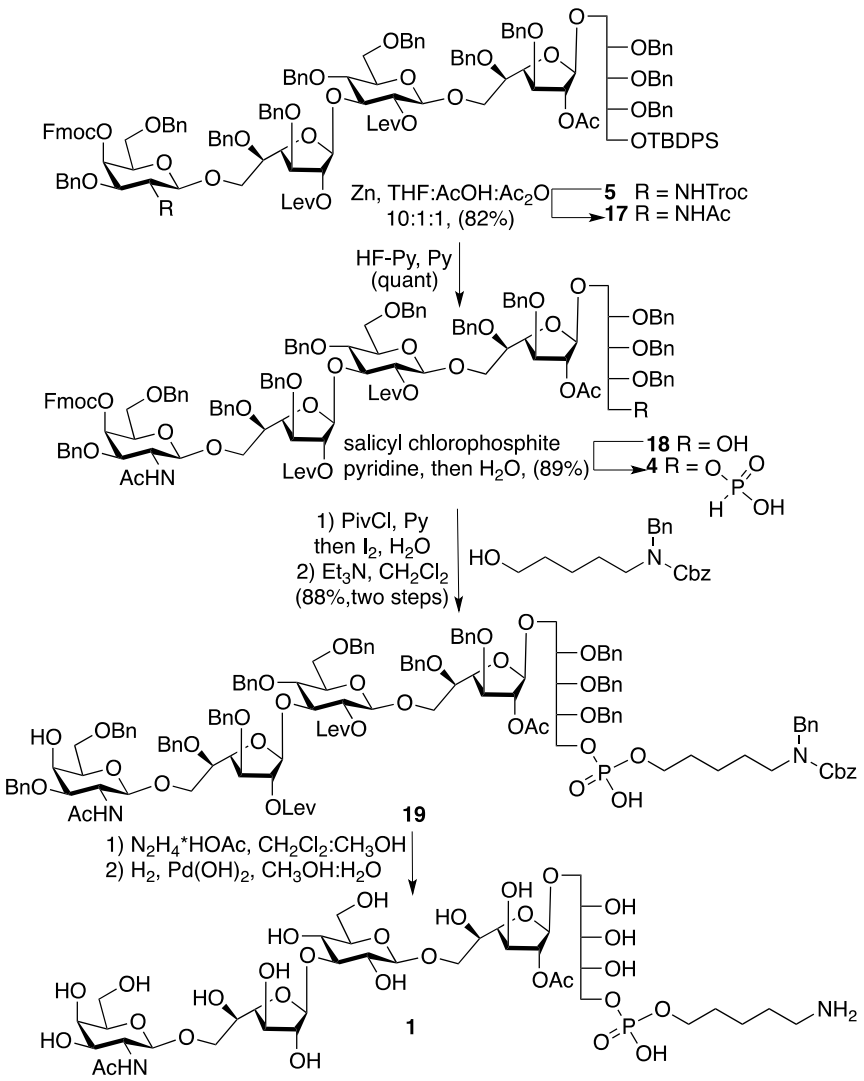
Common precursor **5** was assembled from the five monosaccharide building blocks **6** – **10**. First, ribitol derivative **6**, was prepared in large quantities (~45.0 g) in four steps from

crystalline ribose dithioacetal.[16] Previous syntheses of ribitol acceptors are lengthy[13] and not scalable. Traditional C-5 allyl protection was replaced by a more convenient TBDPS moiety, which no longer requires any toxic palladium catalysts for cleavage and is suitable for NIS/TMSOTf glycosylation conditions. Furthermore, building blocks **7** – **10** share two common structural features to keep glycosylation events operationally simple. An STol[17] leaving group is placed to ensure shelf stability and high glycosylation efficiencies of **7** – **10**. Second, all following glycosylation sites in **7** – **10** were protected with an Fmoc group, which excludes potential difficulties when deprotecting intermediate oligosaccharides.



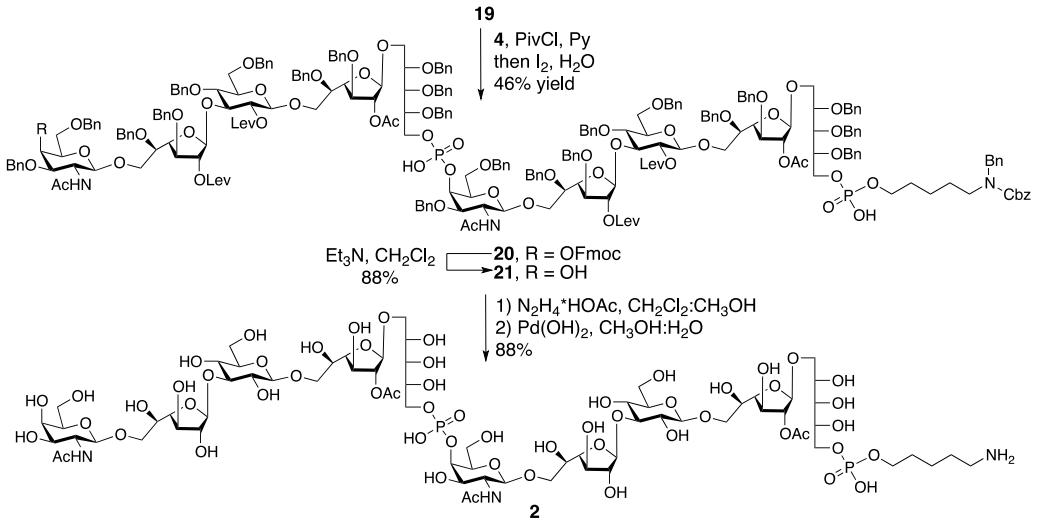
Scheme 1. Synthesis of the core pentasaccharide **5**.

With the building blocks **6**–**10** in hand, a sequence of glycosylation reactions was performed to assemble **5** (Scheme 1). An NIS/TMSOTf-catalyzed[18] glycosylation of **6** with **7** furnished disaccharide **11** in 95% yield. Et_3N-mediated cleavage of the Fmoc protecting group liberated a hydroxyl to give acceptor **12** in high yield, which was coupled with the glucosyl donor **9** providing trisaccharide **13** in 72% yield. Next, the Fmoc protecting group of **13** was removed using standard conditions to afford acceptor **14**, which was coupled with the Gal*f* donor **8** using NIS/TMSOTf as the promotor to provide tetrasaccharide **15** in 86% yield. The Fmoc protecting group of **15** was cleaved to give the acceptor **16**, which was further reacted with the thioglycoside donor **10** providing **5** in a high yield of 91%. Interestingly, **3** was obtained in a much lower yield when a trichloroacetimidate[19] donor was used (data not shown). The developed synthetic route made it easy to provide **5** in a large quantity (15.7 g).



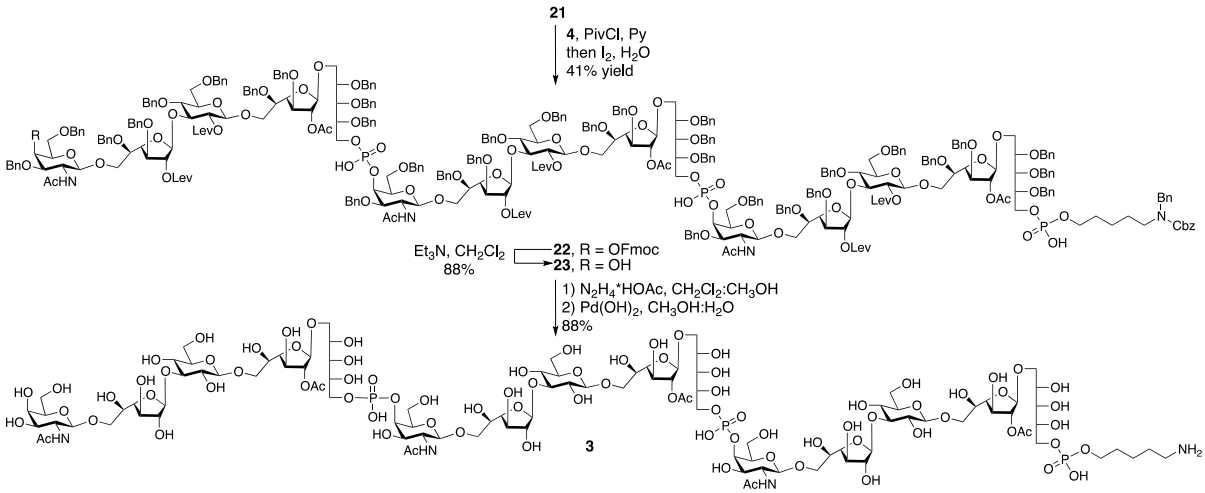
Scheme 2. Synthesis of key phosphonate **4** and its further functionalization with a C5 linker to give **1**.

The flexibility of synthon **5** was demonstrated by performing a phosphitylation – linker attachment – deprotection sequence (Scheme 2). First, the Troc carbamate of **5** was converted into an acetamido moiety by treatment with Zn dust in a solution of THF/AcOH/Ac_2O[20] affording **17** in high yield. Next, the TBDPS group was cleaved using a hydrogen fluoride – pyridine complex to provide alcohol **18** in quantitative yield. Subsequent installation of the crucial *H*-phosphonate group was accomplished by using salicyl chlorophosphite[21] (van Boom's reagent) to give **4** in an excellent yield of 89%. Coupling *N*-(benzyl)benzyloxycarbonyl-protected aminopentanol[22] and **4** using pivaloyl chloride (PivCl) as activator, followed by *in situ* oxidation with iodine in pyridine/water and subsequent removal of the Fmoc group generated fragment **19** in 88% yield over two steps.[13] Finally, Lev esters were selectively deprotected using hydrazine acetate in CH_2Cl_2/CH_3OH mixture,[23] and the remaining benzyl ethers were removed by hydrogenation[23] at ambient pressure to furnish the first desired target **1**.



Scheme 3. Synthesis of decasaccharide **2**

The versatility of phosphonate **4** was showcased by synthesizing deca- and pentadecasaccharide fragments **2** and **3**, respectively (Schemes 3 and 4). The 5 + 5 block coupling involving of **19** and **4** using PivCl as activator afforded **20** in 46% yield after purification by silica gel chromatography. Traces of starting material **19** could only be removed from the desired **20** by incorporation of acetonitrile into eluent system, which is unusual for standard silica gel columns (see SI). Subsequent removal of the Fmoc group gave alcohol **21** in an excellent 88% yield. This material was further treated with hydrazine acetate to remove the Lev esters, followed by catalytic hydrogenation over $Pd(OH)_2$ cleaving all 24 benzyl groups to provide decasaccharide **2** in 88% yield. Pentadecasaccharide **3** was synthesized according to the above methodology, albeit the coupling yield between **21** and **4** was modest (41%), which was attributed to poor nucleophilicity of GalNAc 4OH group (Scheme 4).



Scheme 4. Synthesis of pentadecasaccharide **3**

Next, attention was focused on printing compounds **1 – 3** as microarrays to investigate binding with ficolin-L and M (Figure 2). The compounds were dissolved in a printing buffer (pH 8.5) and printed on *N*-hydroxysuccinimide (NHS)-activated glass slides at a concentration of 100 μM and printed in replicates of 10. A second set of slides was incubated with 100 mM NaOH (pH 11.0) at 40 ºC for 2 h to effect de-*O*-acetylation.

The array results uncovered that the length of CPS fragments together with preserved *O*-acetylation significantly influence the recognition and binding to ficolin-2 (Figure 3). Ficolin-2 (ficolin-L) is a protein widely present in liver and blood plasma. Ficolin-2 was shown to activate the lectin pathway after binding to various capsulated bacteria, which provides a strong theoretical basis to believe ficolin-2 may be important in respiratory infections. Critically, it has been established that ficolin-A-deficient knockout mice exhibited reduced survival rates following the infection with *S. penumoniae* compared to the wild-type mice.[24] The specificity of ficolin-2 recognition remains still uncertain despite extensive studies with *O*-acetylated and neutral oligosaccharide ligands.[25] Additionally, role of *N*-acetylation is also obscure. For example, it has been shown that ficolin-2 binding could be inhibited by various *N*-acetylated compounds,[26] while *N*-acetylated CPS of *S. pneumoniae* were an exception as the binding was still observed.[27]

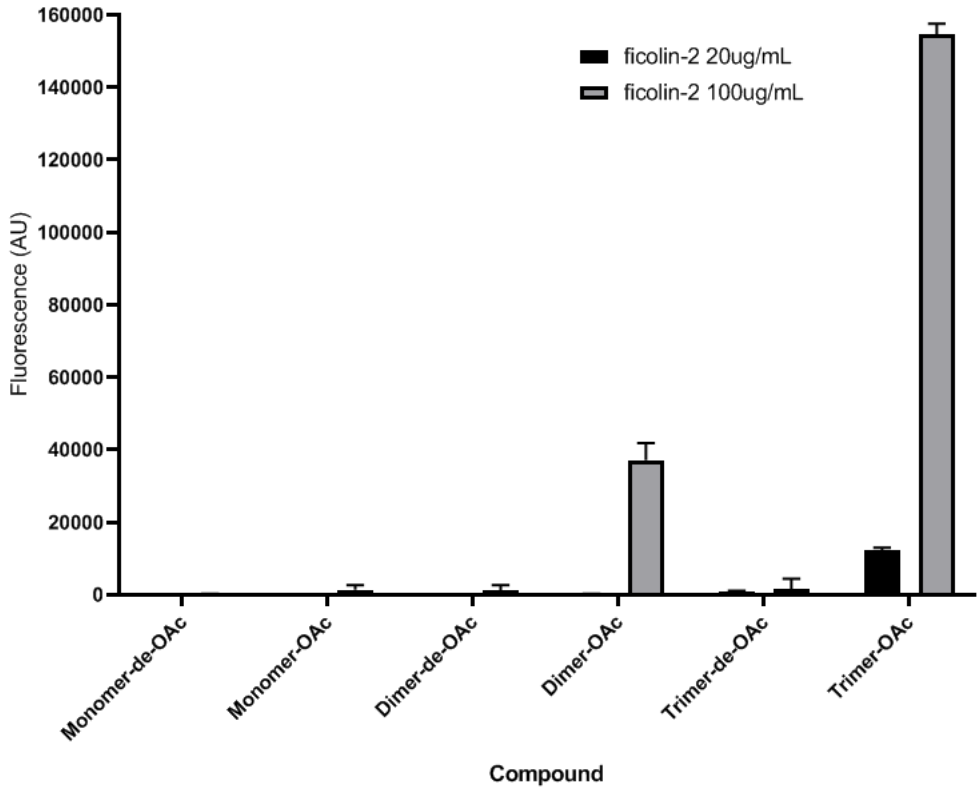


Figure 3. Microarray results of synthetic CPS fragments at 100 μM with ficolin-2 (20 or 100 μg/mL).

Our data show strong binding of ficolin-2 to compound **3** (*O*-acetylated trimer), whereas no binding was observed for **1** (*O*-acetylated monomer) and weak binding for **2** (*O*-acetylated dimer). Remocal of the O-acetyl esters abolished binding of **1 – 3**. Furthermore, compounds **1 – 3** exhibited no binding to ficolin-1 (ficolin-M). Collectively, our data indicate that ficolin-2 has complex binding requirements, which is not only *O*-acetylation dependent, but also the size of ligands. It cannot be excluded that the actual conformation of *O*-acetylated oligosaccharides plays an important role as well.

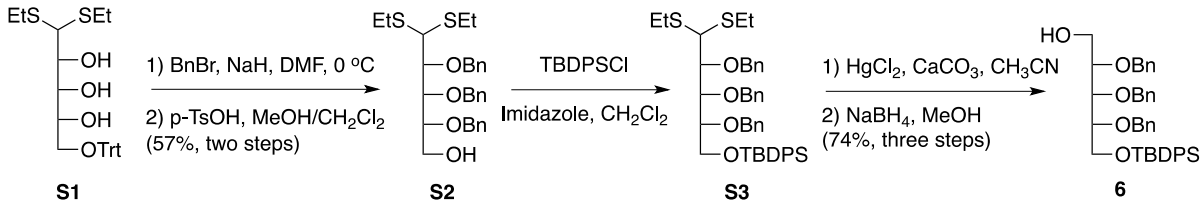
We propose that lack of *O*-acetylation of CPS can be a mechanism of immune evasion, which has recently been reflected as a microevolution of serotype 35B into a genetically similar serotype 35D[28]. The serotype 35D capsule is identical to that of serotype 35B except for the absence of an *O*-acetyl group at one of the galactofuranose residues. Therefore, hydroxide-induced de-*O*-acetylation of **1 – 3** gave well-defined CPS fragments of immunologically related 35D, which were not recognised by ficolin-2. *S. pneumoniae* has already demonstrated a remarkable ability to adapt during the use of conjugate vaccines. Therefore, it is not surprising that an increase in recently reported global distribution of 35D, among young children in the post-PCV13 era is posing a new problem. Our data underline the invasive potential conferred by the loss of *O*-acetylation in the pneumococcal capsule, which was established at a molecular level.

Conclusion

In summary, a scalable synthetic route yielded three promising oligosaccharide candidates that are useful for vaccine development of *S. pneumoniae* 35B, a serotype for urgent consideration in next-generation pneumococcal vaccines. Our modular approach could deliver compounds of up to 15 monosaccharides in length. Only one common pentasaccharide building block equipped with four orthogonal protecting groups was employed to cover the wide range of synthetic intermediates. Careful selection of protecting groups was key to preserve the biologically-important *O*-acetyl esters. Our findings and strategic principles will be relevant to the preparation of other emerging serotypes of *S. pneumoniae*, because many share common structural motifs and phosphodiester bonds through which repeating units are linked. Finally, the synthetic antigens have been coupled to CRM197, and the resulting conjugates will be used in immunization studies in rabbits.

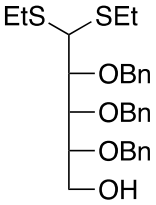
Experimental

General Methods. All reagents, unless otherwise stated, were purchased from Sigma-Aldrich. ^{1}H and ^{13}C NMR spectra were recorded either on Varian Mercury 400 MHz, Bruker 600 or 750 MHz instrument. Chemical shifts are reported in parts per million (ppm) relative to D_2O or $CDCl_3$ as the internal standards. NMR data are presented as follows: Chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, dd = doublet of doublets, m = multiplet and/or multiple resonances, app = apparent); coupling constants are reported in Hertz (Hz). All NMR signals were assigned on the basis of ^{1}H NMR, COSY and HSQC experiments. For oligosaccharide assignments the following abbreviations were used: δ^{Rib} – D-ribose; δ^{Gal1} – D-galactofuranose (acetylated); δ^{Glc} – D-glucose; δ^{Gal2} – galactofuranose (non-acetylated); δ^{GalN} –*N*-acetyl-D-galactosamine. Mass spectra were recorded on either on an Applied Biosystems SCIEX MALDI TOF/TOF 5800 mass spectrometer, a Shimadzu Biotech Axima-CFR MALDI-TOF, or a high resolution Shimadzu LCMS-IT-TOF mass spectrometer. Column chromatography was performed on silica gel G60 (Silicycle, 60-200 μm, 60 Å). TLC analysis was conducted on Silicagel 60 F254 (EMD Chemicals Inc.) with detection by UV light (254 nm) where applicable, and by charring with 10% sulfuric acid in ethanol or a solution of $(NH_4)_6Mo_7O_2*4H_2O$ (25 g/L) in 10% sulfuric acid in ethanol. All reactions were carried out under argon atmosphere unless specified otherwise. Unless otherwise stated, all reactions were carried out at room temperature (RT) in glassware with magnetic stirring. Solutions in organic solvents were dried with Na_2SO_4 and concentrated at 40 °C/2 kPa. Molecular sieves were flame-dried under vacuum immediately prior to use.



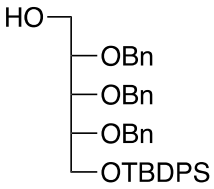
Scheme S1. Synthesis of acceptor **6**

2,3,4-Tri-*O*-benzyl-D-ribose diethyl dithioacetal (S2)

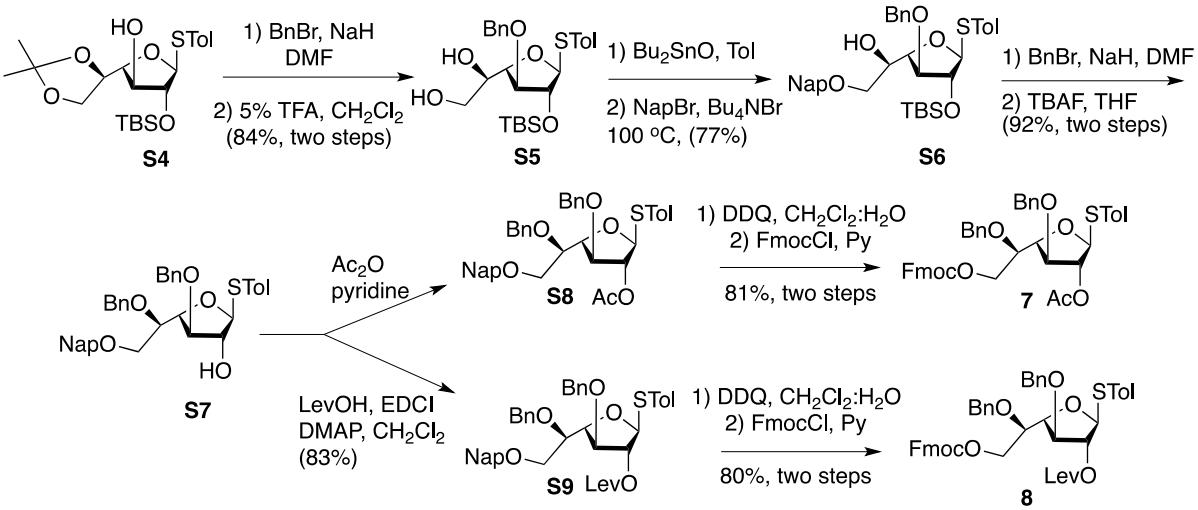


Compound **S1**[29] (4.3 g, 8.58 mmol) was dissolved in anhydrous DMF (20 mL), after which NaH (1.7 g, 42.9 mmol) and BnBr (4.6 mL, 38.61 mmol) were added at 0 °C, and the reaction mixture was allowed to stir at that temperature for 2 h. The mixture was then quenched with MeOH/AcOH (10 mL, 1:1) and concentrated to dryness. The obtained syrup was dissolved in CH_2Cl_2 (100 mL), and the solution was washed with water, and the organic phase was dried and concentrated. The resulting syrup was dissolved in methanol, after which a catalytic amount of *p*-TsOH was added, and the reaction mixture was stirred at rt for 1 h. The mixture was concentrated, absorbed on silica gel, and purified using EtOAc:Hexane (5:95 to 1:9) to afford the title product as a clear syrup (2.7 g, 57% over 2 steps). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.19 (6H, m, $SCH_2\underline{CH_3}$ x 2), 2.22 (1H, dd, *J* = 7.1, 5.9 Hz, 6-OH), 2.52 – 2.68 (4H, m, $S\underline{CH_2}CH_3$ x 2), 3.68 – 3.79 (2H, m, H-6 x 2), 3.84 – 3.91 (2H, m, H-4, H-5), 4.19 (2H, m, H-2, H-1), 4.55 – 4.73 (4H, m, $OC\underline{H_2}Ph$ x 2), 4.83 (1H, d, *J* = 11.0 Hz, OCH<u>H</u>Ph), 4.93 (1H, d, *J* = 11.0 Hz, OC<u>H</u>HPh), 7.24 – 7.36 (15H, m, Ar-H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 138.2, 138.1, 137.9, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9 x 2, 127.8, 127.8 x 3, 127.7 x 2, 127.6, 126.9, 82.3, 79.7, 79.6, 74.9, 73.8, 72.0, 61.7, 53.7, 26.0, 25.2, 14.4 x 2. MALDI-MS: $[M + Na]^+$ $C_{30}H_{38}NaO_4S_2$ calcd. 549.2109, found 549.2123.

_t_-Butyldiphenylsilyl 2,3,4-tri-_O_-benzyl-D-ribose (6)

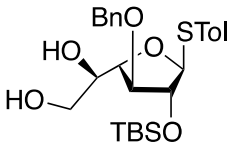


Compound **S2** (40.4 g, 76.69 mmol) was dissolved in CH_2Cl_2 (100 mL) followed by adding imidazole (16.0 g, 230.07 mmol) and TBDPSCl (26 mL, 92.03 mmol), after which the reaction mixture was left stirring at rt for 1 h. The mixture was washed with 1M HCl (100 mL), and the organic phase was dried and concentrated to give crude **S3** as a syrup, which was directly dissolved in CH_3CN (100 mL) followed by adding $CaCO_3$ (15.5 g, 153.38 mmol) and $HgCl_2$ (42.0 g, 153.38 mmol) at 0 °C. The reaction mixture was stirred at that temperature for 1 h, and then at rt for 1 h. The mixture was then filtered and concentrated *in vacuo* to dryness. This material was dissolved in CH_3OH (200 mL), after which $NaBH_4$ (10.0 g) was added at 0 °C, and stirring was continued at that temperature for 30 min. The mixture was neutralized with AcOH, and concentrated *in vacuo* to dryness. The residue was loaded on silica gel and purified using EtOAc:Hexane (5:95 to 1:9) as a mobile phase to give the target product as a clear oil (42.0 g, 82% over three steps). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.09 (9H, s, $(CH_3)_3$ of TBDPS), 2.34 (1H, t, J = 5.98 Hz, 1-OH), 3.77 (4H, m, incl. H-1, H-4), 3.92 (2H, d, J = 4.5 Hz, H-5), 3.99 (1H, t, J = 4.8 Hz), 4.54 – 4.74 (6H, m, $\underline{CH_2}$Ph), 7.21 – 7.71 (25H, m, Ar-H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 138.3, 138.2, 138.1, 135.7, 135.6, 133.4, 133.3, 129.7, 129.7, 128.4, 128.4, 128.3, 128.0, 127.8, 127.7, 127.7, 127.7, 127.7, 127.5, 79.7, 79.0, 79.0, 73.9, 72.6, 71.8, 63.5, 61.5, 26.9, 19.2. MALDI-MS: $[M + Na]^+$ $C_{42}H_{48}NaO_5Si$ calcd. 683.3169, found 683.3154.



Scheme S2. Synthesis of **7** and **8**

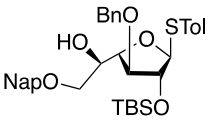
4-Methyphenyl 2-_O_-_t_-butyldimethylsilyl-3-_O_-benzyl-1-thio-_β_-D-galactofuranoside (S5)



Compound **S4**[30] (26.0 g, 59.0 mmol) was dissolved in DMF (100 mL), and BnBr (10.5 mL, 88.5 mmol) and NaH (3.6 g, 88.5 mmol) were added at 0 °C in succession. The reaction mixture was then stirred at 0 °C for 15 min, and then at rt for 1 h. The reaction mixture was quenched by the addition of MeOH:AcOH (1:1), concentrated under reduced pressure to give the product as an oil. This material was dissolved in CH_2Cl_2 (100 mL), after which TFA (10 mL) and water (2 mL) were added, and the reaction mixture was left stirring at rt for 3 h, after which it was diluted with CH_2Cl_2 (100 mL), washed with water and sat. $NaHCO_3$ in succession. The organic phase was concentrated under reduced pressure, and the product was absorbed on silica gel and purified using EtOAc:Hexane (3:7) as a phase to give the target product as a clear oil (24.5 g, 84% over two steps). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.12 (6H, s, CH_3 x 2 of TBS), 0.88 (9H, s, tBu of

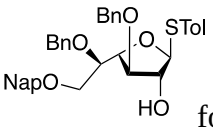
TBS), 2.17 (1H, dd, J = 8.7, 4.6 Hz, 6-OH), 2.32 (3H, s, CH_3 of STol), 2.62 (1H, d, J = 6.8 Hz, 5-OH), 3.61 – 3.72 (2H, m, H-6), 3.77 (1H, m, H-5), 3.99 (1H, dd, J = 6.0, 2.7 Hz, H-3), 4.30 (1H, dd, J = 3.7, 6.0 Hz, H-4), 4.34 (1H, t, J = 2.5 Hz, H-2), 4.57 (1H, d, J = 11.8 Hz, CHHPh), 4.70 (1H, d, J = 11.8 Hz, CHHPh), 5.24 (1H, d, J = 2.17 Hz, H-1), 7.09 – 7.39 (9H, m, Ar-H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 137.8, 137.4, 132.5, 130.8, 129.8, 128.5, 128.0, 127.7, 94.4, 85.7, 85.7, 82.9, 81.6, 72.5, 70.8, 64.6, 25.7, 25.7, 25.6, 21.1, 17.9, -4.4, -5.0. MALDI-MS: $[M + Na]^+$ $C_{26}H_{38}NaO_5SSi$ calcd. 513.2107, found 513.2112.

4-Methyphenyl 2-*O*-*t*-butyldimethylsilyl-3-*O*-benzyl-6-*O*-(2-naphthyl)-1-thio-*β*-D-galactofuranoside (S6)



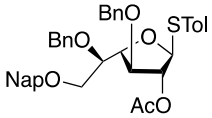
Compound **S5** (3.51 g, 7.15 mmol) was dissolved in toluene (20 mL), followed by adding Bu_2SnO (2.1 g, 8.58 mmol), and the reaction mixture was stirred at 100 °C for 2 h. After complete dissolution of Bu_2SnO, NapBr (2.3 g, 10.72 mmol) and Bu_4NBr (2.3 g, 7.15 mmol) were added, and stirring was further continued for 6 h. The reaction mixture was then concentrated, diluted with CH_2Cl_2, and washed with water and sat. $NaHCO_3$ in succession. The organic phase was then filtered, dried and concentrated. The crude product was purified on silica gel using EtOAc:Hexane (5:95 to 1:9) as a mobile phase, giving the target product as clear oil (3.53 g, 77%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.12 (6H, s, CH_3 x 2 of TBS), 0.88 (9H, s, *t*Bu of TBS), 2.32 (3H, s, CH_3 of STol), 2.57 (1H, d, J = 6.8 Hz, 5-OH), 3.60 (2H, m, H-6), 3.95 – 4.02 (2H, m, H-3, H-5), 4.36 (1H, t, J = 2.39 Hz, H-2), 4.40 (1H, dd, J = 5.40, 3.04 Hz, H-4), 5.27 (1H, d, J = 2.1 Hz, H-1), 7.02 – 7.84 (16H, m, Ar-H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 137.6, 137.4, 135.6, 133.2, 133.0, 132.3, 131.2, 129.7, 128.5, 128.2, 127.9, 127.9, 127.7, 127.7, 126.5, 126.1, 125.8, 125.7, 94.3, 85.6, 82.2, 81.6, 73.5, 72.4, 71.5, 69.9, 25.7, 21.1, 17.9, -4.4, -5.0. MALDI-MS: $[M + Na]^+$ $C_{37}H_{46}NaO_5SSi$ calcd. 653.2733, found 653.2737.

4-Methyphenyl 3,5-di-*O*-benzyl-6-*O*-(2-naphthyl)-1-thio-*β*-D-galactofuranoside (S7)



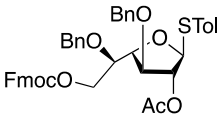
Compound **S6** (3.39 g, 5.37 mmol) was dissolved in DMF (20 mL), after which BnBr (963 μL, 8.05 mmol) and NaH (322 mg, 8.05 mmol) were added at 0 °C, and the reaction mixture was left stirring at that temperature for 1 h, after which it was quenched with CH_3OH, followed by adding AcOH. The reaction mixture was then concentrated, dissolved in THF (20 mL) followed by adding TBAF (3.4 g, 10.74 mmol, hydrate form), and stirring was continued at rt for 3 h. The solution was then concentrated, diluted with CH_2Cl_2 and washed with water. The organic phase was then dried and concentrated to give a residue, which was purified on silica gel using EtOAc:Hexane (3:7) as a mobile phase to give the product as a clear oil (3.03 g, 92%, over two steps). ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.29 (3H, s, CH_3 of STol), 3.66 (1H, d, J = 10.7 Hz, 2-OH), 3.72 – 3.79 (2H, m, H-5, H-6a), 3.79 – 3.85 (2H, m, H-6b, H-3), 4.27 (1H, d, J = 10.8 Hz, H-2), 4.44 (1H, d, J = 11.8 Hz, CHHAr), 4.47 – 4.50 (2H, m, H-4, CHHAr), 4.64 (1H, d, J = 11.9 Hz, CHHAr), 4.70 (2H, s, CH_2Ar), 4.76 (1H, d, J = 11.2 Hz, CHHAr), 5.40 (1H, s, H-1), 7.02 – 7.85 (21H, m, Ar-H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 137.5, 136.9, 136.9, 135.4, 133.2, 133.0, 131.9, 131.6, 129.6, 128.6, 128.5, 128.4, 128.3, 128.2, 127.9, 127.8, 127.8, 127.7, 126.5, 126.1, 125.9, 125.6, 95.1, 85.3, 83.8, 79.0, 76.9, 73.7, 73.4, 72.0, 70.4, 21.0. MALDI-MS: $[M + Na]^+$ $C_{38}H_{38}NaO_5S$ calcd. 629.2338, found 629.2345.

4-Methyphenyl 2-*O*-acetyl-3,5-di-*O*-benzyl-6-*O*-(2-naphthyl)-1-thio-*β*-D-galactofuranoside (S8)



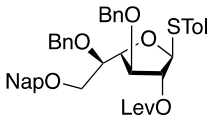
Compound **S7** (3.03 g, 4.99 mmol) was dissolved in CH_2Cl_2 (20 mL), followed by adding pyridine (1.6 mL, 19.96 mmol) and Ac_2O (943 µL, 9.98 mmol). The reaction mixture was left stirring at rt for 2 h, after which it was diluted with CH_2Cl_2 (50 mL), washed with 1 M HCl, concentrated, and purified on silica gel using EtOAc:Hexane (1:4) as a mobile phase, to give the product as a clear oil (3.18 g, quant.). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.90 (3H, s, OAc), 2.29 (3H, s, CH_3 of STol), 3.67 (1H, dd, *J* = 9.9, 5.1 Hz, H-6a), 3.71 – 3.81 (2H, m, H-6b, H-5), 3.97 (1H, d, *J* = 6.0 Hz, H-3), 4.34 (1H, d, *J* = 11.5 Hz, CH*H*Ar), 4.41 (1H, d, *J* = 11.5 Hz, CH*H*Ar), 4.49 (1H, dd, *J* = 6.2, 2.9 Hz, H-4), 4.61 – 4.71 (4H, m, CH_2Ar), 5.19 (1H, t, *J* = 1.5 Hz, H-2), 5.49 (1H, s, H-1), 6.97 – 7.86 (21H, m, Ar-H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.9, 138.2, 137.6, 137.4, 135.6, 133.2, 133.0, 132.2, 130.3, 129.6, 128.4, 128.2, 128.2, 128.2, 128.1, 127.9, 127.8, 127.7, 127.7, 126.3, 126.1, 125.8, 125.6, 91.0, 82.6, 82.3, 82.0, 76.4, 73.6, 73.5, 72.1, 70.6, 21.1, 20.8. MALDI-MS: [M + Na]$^+$ $C_{40}H_{40}NaO_6S$ calcd. 671.2443, found 671.2450.

4-Methyphenyl 2-*O*-acetyl-3,5-di-*O*-benzyl-6-*O*-fluorenylmethoxycarbonyl-1-thio-*β*-D-galactofuranoside (7)



To a solution of compound **S8** (13.8 g, 21.2 mmol) in CH_2Cl_2 (100 mL) were added H_2O (10 mL) and DDQ (7.1 g, 31.9 mmol), and the reaction mixture was left stirring at rt for 1 h. The reaction mixture was quenched with sat. $NaHCO_3$, filtered, and washed with sat. $NaHCO_3$. The organic phase was dried, concentrated, and the product purified on silica gel to give the alcohol as a clear oil. This material was then dissolved in pyridine (50 mL), after which Fmoc-Cl (7.6 g, 29.3 mmol) was added at 0 ^{o}C and the reaction mixture was left stirring at rt for 1 h. After that time pyridine was removed *in vacuo*, the crude mixture was dissolved in CH_2Cl_2 (100 mL), washed with 1 M HCl, the organic phase was then dried and concentrated to give the product, which was purified on silica gel using EtOAc:Hexane (1:4) as a mobile phase, providing the target donor as a white foam (12.6 g, 81%, over two steps). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.96 (3H, s, OAc), 2.29 (3H, s, STol), 3.85 (1H, m, H-5), 3.97 (1H, d, *J* = 5.7 Hz, H-3), 4.23 (1H, t, *J* = 7.4 Hz, CH of Fmoc), 4.32 – 4.40 (5H, m, H-6, CH<u>H</u>Ph, CH_2 of Fmoc), 4.45 (1H, d, *J* = 12.4 Hz, CH<u>H</u>Ph), 4.50 (1H, dd, *J* = 5.7, 3.4 Hz, H-4), 4.64 – 4.70 (2H, m, C<u>H</u>HPh x 2), 5.22 (1H, t, *J* = 1.4 Hz, H-2), 5.50 (1H, s, H-1), 7.05 – 7.77 (22H, m, Ar-H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.8, 155.0, 143.3, 141.3, 137.7, 137.4, 132.4, 130.1, 129.7, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.9, 127.9, 127.2, 125.1, 125.1, 120.0, 91.3, 82.6, 81.8, 81.7, 75.0, 73.5, 72.2, 70.0, 67.4, 46.7, 21.1, 21.0, 20.8. MALDI-MS: [M + Na]$^+$ $C_{44}H_{42}NaO_8S$ calcd. 753.2498, found 753.2494.

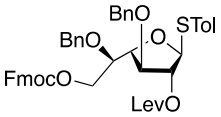
4-Methyphenyl 2-*O*-levulinolyl-3,5-di-*O*-benzyl-6-*O*-(2-naphthyl)-1-thio-*β*-D-galactofuranoside (S9)



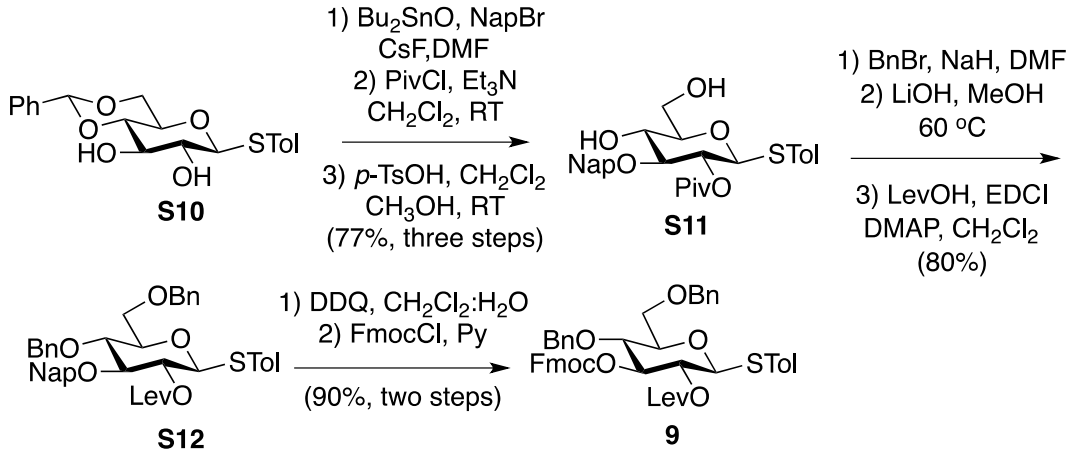
Compound **S7** (12.54 g, 20.6 mmol) was dissolved in CH_2Cl_2 (100 mL), followed by adding LevOH (3.6 mL, 31 mmol), EDCI (6.0 g, 31 mmol) and DMAP (catalytic amount). The reaction mixture was left stirring at rt for 2 h., after which it was diluted with CH_2Cl_2 (100 mL), washed with sat. $NaHCO_3$, the organic phase was dried (Na_2SO_4), concentrated and purified on silica gel using EtOAc:Hexane (3:7 to 2:3) as a mobile system to give the target product as a clear oil (12.0 g, 83%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.12 (3H, s, CH_3 of Lev), 2.27 (3H, s, CH_3 of STol), 2.41 (2H, m, CH_2 of Lev), 2.61 (2H, m, CH_2 of Lev), 3.66 (1H, m, H-6a), 3.73 (1H, m, H-6b), 3.79 (1H, m, H-5), 4.00 (1H, d, *J* = 6.1 Hz, H-3), 4.38 (2H, m, CH_2Ar),

4.49 (1H, dd, *J* = 5.7, 3.3 Hz, H-4), 4.66 (4H, m, CH_2Ar x 2), 5.22 (1H, t, *J* = 1.6 Hz, H-2), 5.48 (1H, s, H-1), 6.99 – 7.83 (21H, m, Ar-H), ^{13}C NMR (101 MHz, $CDCl_3$) δ 206.1, 171.7, 138.2, 137.6, 137.4, 135.6, 133.2, 132.9, 132.3, 130.3, 129.6, 128.4, 128.4, 128.2, 128.2, 128.2, 127.9, 127.9, 127.7, 126.3, 126.1, 125.8, 125.6, 90.9, 82.4, 82.1, 82.1, 76.4, 73.6, 73.5, 72.1, 70.5, 37.7, 29.7, 27.8, 21.1. MALDI-MS: $[M + Na]^+$ $C_{43}H_{44}NaO_7S$ calcd. 727.2705, found 727.2712.

4-Methyphenyl 2-*O*-levulinoyl-3,5-di-*O*-benzyl-6-*O*-fluorenylmethoxycarbonyl-1-thio-*β*-D-galactofuranoside (8)

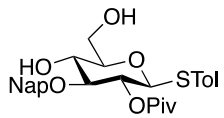


Compound **S9** (15.0 g, 21.3 mmol) was dissolved in CH_2Cl_2 (100 mL), H_2O (10 mL) and DDQ (6.3 g, 31.9 mmol) were then added, and the reaction mixture was left stirring at rt for 1 h. It was quenched with sat. $NaHCO_3$, filtered, and washed with sat. $NaHCO_3$. The organic phase was dried, concentrated, and the product purified on silica gel to give the alcohol as a clear oil. This material was then dissolved in pyridine (50 mL), after which Fmoc-Cl (8.3 g, 31.9 mmol) was added at 0 ºC and the reaction mixture was left stirring at rt for 1 h. After that time pyridine was removed *in vacuo*, the crude mixture was dissolved in CH_2Cl_2 (100 mL), washed with 1 M HCl, the organic phase was then dried and concentrated to give the product, which was purified on silica gel using EtOAc:Hexane (3:7) system as a mobile phase, providing the target donor as a white foam (13.3 g, 80%, over two steps). ^1H NMR (400 MHz, $CDCl_3$): δ 2.14 (3H, s, CH_3 of Lev), 2.28 (3H, s, CH_3 of STol), 2.48 (2H, m, CH_2 of Lev), 2.68 (2H, m, CH_2 of Lev), 3.84 (1H, m, H-5), 4.22 (1H, t, *J* = 7.4 Hz, CH of Fmoc), 4.29 – 4.45 (6H, m, H-6, CH_2 of Fmoc, C<u>H</u>HAr x 2), 4.49 (1H, dd, *J* = 3.5, 5.8 Hz, H-4), 4.63 (1H, d, *J* = 11.3 Hz, C<u>H</u>HAr), 4.68 (1H, d, *J* = 11.8 Hz, C<u>H</u>HAr), 5.24 (1H, t, *J* = 1.68 Hz, H-2), 5.47 (1H, s, H-1), 7.04 – 7.78 (22H, m, Ar-H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 206.1, 171.7, 154.9, 143.3, 143.3, 141.3, 141.2, 137.7, 137.7, 137.4, 132.5, 130.0, 129.6, 128.4, 128.3, 128.2, 128.2, 127.9, 127.9, 127.9, 127.9, 127.2, 125.1, 125.1, 120.1, 120.0, 91.1, 82.5, 81.9, 81.6, 75.0, 73.5, 72.2, 69.9, 69.9, 67.4, 46.8, 46.7, 37.7, 29.7, 27.8, 21.1. MALDI-MS: $[M + Na]^+$ $C_{47}H_{46}NaO_9S$ calcd. 809.2760, found 809.2778.



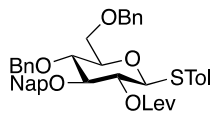
Scheme S3. Synthesis of **9**

4-Methyphenyl 2-*O*-pivaloyl-3-*O*-(2-naphthyl)-1-thio-*β*-D-glucopyranoside (S11)



Diol **S10**[31] (33.75 g, 90.13 mmol) was suspended in toluene (200 mL), followed by adding Bu_2SnO (27.0 g, 108.15 mmol), and the mixture was heated at 110 °C until clear solution was obtained. The reaction mixture was concentrated, dissolved in DMF (100 mL) after which CsF (21.0 g, 135.19 mmol) and NapBr (26.0 g, 117.169 mmol) were added, and the reaction mixture was left stirring at rt until the starting material was fully consumed. The reaction mixture was concentrated, the crude intermediate was dissolved in CH_2Cl_2 and washed with water and sat. $NaHCO_3$. The organic phase was then filtered, dried and concentrated to give the intermediate as a white solid. To a solution of this material in CH_2Cl_2 (200 mL) was added Et_3N (38 mL, 270.4 mmol) and DMAP (11.0 g, 90.13 mmol), after which the solution was cooled to 0 °C, and PivCl (17 mL, 135.2 mmol) was added dropwise, and the reaction mixture was left stirring at rt for 1 h, after which it was diluted with CH_2Cl_2, washed with 1 M HCl, and the organic phase was dried and concentrated to provide a crude intermediate as a syrup, which was dissolved in CH_2Cl_2:CH_3OH (1:1, 200 mL), and CSA (5.0 g) was then added. The reaction mixture was left stirring at rt overnight, after which it was concentrated, diluted with CH_2Cl_2, washed with sat. $NaHCO_3$, the organic phase was dried (Na_2SO_4) and concentrated to give a crude product, which was crystallized from ethanol to provide a pure title compound as an off-yellow powder (35.0 g, 77% over three steps). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.25 (9H, s, CH_3 x 3 Piv), 2.32 (3H, s, CH_3 of STol), 2.69 (1H, br m, 4-OH), 3.37 (1H, m, H-5), 3.61 (1H, t, *J* = 8.6 Hz, H-3), 3.68 (1H, t, *J* = 9.0 Hz, H-4), 3.76 (1H, dd, *J* = 11.7, 4.59 Hz, H-6a), 3.87 (1H, dd, *J* = 12.5, 3.4 Hz, H-6b), 4.63 (1H, d, *J* = 9.7 Hz, H-1), 4.78 (1H, d, *J* = 11.5 Hz, C*H*HAr), 4.88 (1H, d, *J* = 11.5 Hz, CH*H*Ar), 5.05 (1H, dd, *J* = 9.7, 9.0 Hz, H-2), 7.08 – 7.8 (11H, m, Ar-H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 176.8, 138.3, 138.2, 135.4, 135.4, 133.2, 133.0, 132.7, 132.7, 129.7, 129.2, 129.1, 128.4, 128.4, 127.9, 127.7, 126.3, 126.3, 126.2, 126.0, 126.0, 125.8, 125.3, 87.0, 84.3, 79.3, 79.3, 74.7, 71.4, 70.2, 62.5, 27.2, 21.1. MALDI-MS: $[M + Na]^+$ $C_{29}H_{34}NaO_6S$ calcd. 533.1974, found 533.1983.

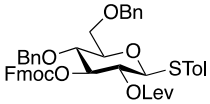
4-Methyphenyl 4,6-di-*O*-benzyl-2-*O*-levulinoyl-3-*O*-(2-naphthyl)-1-thio-*β*-D-glucopyranoside (S12)



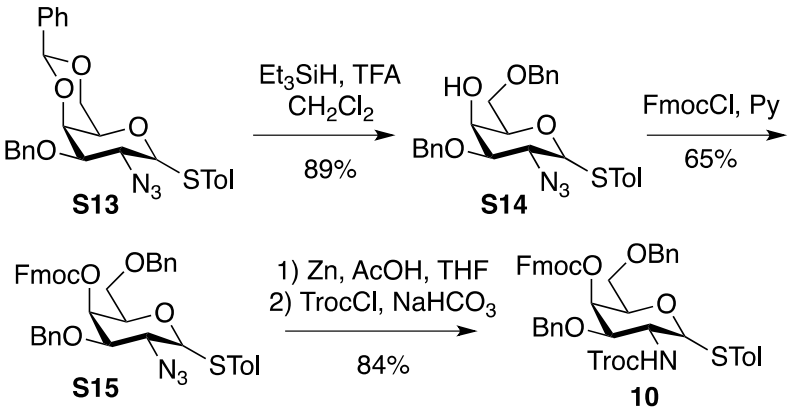
Diol **S11** (34.0 g, 66.5 mmol) was dissolved in anhydrous DMF (200 mL) followed by adding NaH (7.8 g, 199.5 mmol) and BnBr (24 mL, 199.5 mmol) at 0 °C and the reaction mixture was stirred at that temperature for 30 min. It was then neutralized with CH_3OH, concentrated, and the resulting intermediate was dissolved in CH_3OH/CH_2Cl_2 (200 mL, 3:1), LiOH (5.0 g, 199.5

mmol) was then added and the reaction was heated at 60 ºC for 12 h. The mixture was then concentrated, dissolved in CH_2Cl_2 (300 mL), and washed with H_2O (200 mL), the organic phase was dried and concentrated to afford an intermediate which was directly dissolved in CH_2Cl_2 (200 mL), followed by adding LevOH (12 mL, 99.75 mmol), EDCI (19.2 g, 99.75) and DMAP (1.6 g, 13.3 mmol). The reaction mixture was stirred at RT for 2 h, after which it was washed with sat. $NaHCO_3$ (200 mL), dried and concentrated to give the crude product as an oil, which was chromatographed using EtOAc:Hexane (3:7), (37.0 g, 80% over three steps). R_f = 0.4 (EtOAc:Hexane, 2:3). ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.08 (3H, s, CH_3 of Lev), 2.28 (3H, s, CH_3 of STol), 2.37 – 2.62 (4H, m, CH_2 x 2 of Lev), 3.52 (1H, m, H-5), 3.67 (1H, dd, *J* = 8.9 Hz, H-4), 3.72 (1H, m, H-3), 3.71 – 3.80 (3H, m, H-6 x 2, H-3), 4.50 – 4.60 (3H, C*H*HAr x 2, H-1), 4.79 (1H, d, *J* = 10.9 Hz, C*H*HAr), 4.85 (1H, d, *J* = 11.7 Hz, C*H*HAr), 4.93 (1H, d, *J* = 11.7 Hz, C*H*HAr), 5.00 (1H, dd, *J* = 9.0, 9.9 Hz, H-2), 7.00 – 7.84 (21H, m, Ar-H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 206.1, 171.4, 138.2, 138.1, 137.9, 135.7, 133.2, 133.2, 132.9, 129.6, 128.7, 128.4, 128.3, 128.1, 127.9, 127.8, 127.7, 127.6, 127.5, 126.6, 126.0, 126.0, 125.9, 86.2, 84.4, 79.4, 77.8, 75.3, 75.1, 73.5, 72.2, 68.9, 37.7, 29.7, 28.1, 21.1. MALDI-MS: $[M + Na]^+$ $C_{43}H_{44}NaO_7S$ calcd. 727.2705, found 727.2717.

4-Methyphenyl 4,6-di-*O*-benzyl-2-*O*-levulinoyl-3-*O*-fluorenylmethyl-1-thio-*β*-D-glucopyranoside (9)

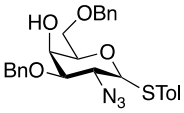


Compound **S12** (5.0 g, 7.09 mmol) was dissolved in CH_2Cl_2 (20 mL), after which H_2O (2 mL) was added followed by DDQ (2.4 g, 10.64 mmol). The reaction mixture was left stirring at RT for 1 h, after which it was neutralized with sat. $NaHCO_3$, diluted with CH_2Cl_2 (50 mL), and the organic layer was then dried and concentrated to give an oil, which was purified on silica gel using EtOAc:Hexane (3:7) as a mobile phase. This intermediate was then dissolved in pyridine (20 mL), after which Fmoc-Cl (2.7 g, 10.6 mmol) was added and the mixture was left stirring at RT for 1 h, after which the reaction mixture was concentrated, re-dissolved in CH_2Cl_2 (50 mL) and washed with 1M HCl. The organic layer was then dried and concentrated to give the product which was chromatographed using EtOAc:Hexane (1:10 to 3:7), (6.74 g, 90% over two steps). R_f = 0.6 (EtOAc:Hexane, 3:7). ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.04 (3H, s, CH_3 of Lev), 2.29 (3H, s, CH_3 of STol), 2.54 – 2.75 (4H, m, CH_2 x 2 of Lev), 3.54 (1H, m, H-5), 3.70 – 3.79 (3H, m, H-4, H-6 x 2), 4.22 (2H, m, CH of Fmoc, CH*H*Fmoc), 4.48 (1H, m, C*H*HFmoc), 4.50 – 4.59 (4H, m, CH_2Ar), 4.61 (1H, d, *J* = 9.7 Hz, H-1), 7.01 – 7.73 (18H, m, Ar-H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 205.8, 171.3, 154.6, 143.6, 143.2, 141.2, 141.1, 138.4, 138.1, 137.5, 133.7, 129.6, 128.3, 128.3, 127.9, 127.8, 127.8, 127.8, 127.6, 127.6, 127.1, 125.3, 125.2, 119.9, 85.6, 80.7, 79.1, 75.6, 74.9, 73.5, 70.4, 70.4, 68.6, 46.6, 37.7, 29.6, 28.0, 21.1. MALDI-MS: $[M + Na]^+$ $C_{47}H_{46}NaO_9S$ calcd. 809.2760, found 809.2748.



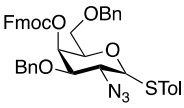
Scheme S4. Synthesis of donor **10**

4-Methyphenyl 2-azido-2-deoxy-3,6-di-*O*-benzyl-1-thio-*α,β*-D-galactopyranoside (S14)



To a solution of **S13**[32] (21.67 g, 44.26 mmol) in CH_2Cl_2 (160 mL) containing Et_3SiH (36 mL, 221.3 mmol) was added TFA (17 mL, 221.3 mmol) at 0 °C. The reaction mixture was stirred at that temperature for 30 min, and further at RT for 2 h. The reaction mixture was diluted with CH_2Cl_2 (50 mL) and washed with sat. $NaHCO_3$ (100 mL). The organic phase was then dried and concentrated to give the crude product as an oil, which was chromatographed using EtOAc:Hexane (1:4 to 3:7) to give the pure product as a clear oil, (21.0 g, 89%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.29 (3H, s, CH_3 of STol), 2.30 (3H, s, CH_3 of STol), 2.42 (1H, m, 4-OH), 2.62 (1H, m, 4-OH), 3.37 (1H, dd, *J* = 9.0, 3.7 Hz, H-3), 3.53 (1H, m, *J* = 5.61 Hz, H-5), 3.58 (1H, dd, *J* = 9.76 Hz, H-2), 3.67 – 3.81 (3H, m, H-6 x 2, H-3), 4.04 (1H, m, H-4), 4.14 (1H, m, H-4), 4.27 (1H, dd, *J* = 10.1, 5.2 Hz), 4.30 (1H, d, *J* = 10.1 Hz, H-1), 4.51 (1H, m, H-5), 4.52 – 4.76 (4H, m, CH_2Ar), 5.52 (1H, d, *J* = 5.5 Hz, H-1), 6.99 – 7.50 (14H, m, Ar-H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 138.5, 138.0, 137.9, 137.8, 137.0, 137.0, 133.8, 133.0, 129.8, 129.7, 129.2, 128.7, 128.6, 128.6, 128.4, 128.4, 128.3, 128.2, 128.0, 128.0, 128.0, 127.8, 127.8, 127.8, 127.7, 127.6, 127.4, 87.7, 86.3, 81.1, 77.8, 77.0, 73.7, 73.6, 72.0, 71.9, 69.7, 69.5, 69.3, 66.7, 65.6, 60.8, 59.7, 21.1, 21.1. MALDI-MS: $[M + Na]^+$ $C_{27}H_{29}N_3NaO_4S$ calcd. 514.1776, found 514.1790.

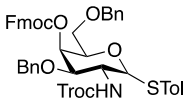
4-Methyphenyl 2-azido-2-deoxy-3,6-di-*O*-benzyl-4-*O*-fluorenylmethyl-1-thio-*α,β*-D-galactopyranoside (S15)



To a solution of compound **S14** (21.0 g, 42.71mmol) in pyridine (100 mL) was added DMAP (cat.) and the solution was cooled to 0 °C, followed by adding Fmoc-Cl (16.7 g, 64.06 mmol). The reaction mixture was left stirring at that temperature for 3 h. It was concentrated, dissolved in CH_2Cl_2 (150 mL) and washed with 1M HCl (100 mL). The organic phase was then dried and concentrated to give the crude product as an oil, which was chromatographed using EtOAc:Toluene (0:1 to 5:95) as a solvent system, (19.5 g, 65%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.27 (3H, s, CH_3 of STol), 2.30 (3H, s, CH_3 of STol), 3.48 (1H, dd, *J* = 9.7, 3.1 Hz, H-3), 3.55 – 3.74 (4H, H-6 x 2, H-5, H-2), 3.84 (1H, dd, *J* = 10.5, 3.18 Hz, H-3), 4.14 (1H, t, *J* = 7.0 Hz, CH of Fmoc), 4.22 – 4.30 (3H, m, H-2, C*H*HAr x 2), 4.34 – 4.54 (4H, m, H-1, C*H*HAr, CH_2 of Fmoc), 4.58 (1H, d, *J* = 10.8 Hz, C*H*HAr), 4.71 (1H, t, *J* = 6.7 Hz, H-5), 4.77 (1H, d, *J* = 11.0 Hz, C*H*HAr), 4.81 (1H, d, *J* = 10.8 Hz, C*H*HAr), 5.41 (1H, d, *J* = 3.15 Hz, H-4), 5.51 (1H, d, *J* = 2.0 Hz, H-4), 5.55 (1H, d, *J* = 5.5 Hz, H-1), 6.99 – 7.80 (22H, m, Ar-H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.8, 154.8, 143.5, 143.5, 143.1, 143.0, 141.3, 141.2, 141.2, 138.5, 138.1, 137.6, 137.5, 136.8, 133.6, 133.1, 129.8, 129.7, 128.9, 128.4, 128.4, 128.3, 128.3, 128.2,

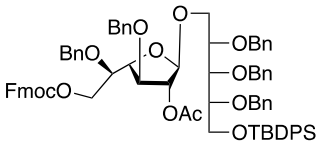
127.9, 127.9, 127.9, 127.9, 127.9, 127.8, 127.8, 127.7, 127.6, 127.2, 127.1, 127.1, 125.3, 125.0, 125.0, 120.0, 120.0, 119.9, 87.6, 86.5, 79.4, 77.2, 76.2, 75.9, 73.7, 73.6, 71.9, 71.8, 70.9, 70.1, 70.1, 69.6, 68.7, 68.2, 67.9, 61.0, 59.9, 46.5, 46.5, 21.2, 21.1. MALDI-MS: [M + Na]$^+$ $C_{42}H_{39}N_3NaO_6S$ calcd. 736.2457, found 736.2478.

4-Methyphenyl 2-(2,2,2-trichloroethylcarbonylamino)-2-deoxy-3,6-di-*O*-benzyl-4-*O*-fluorenylmethyl-1-thio-*α,β*-D-galactopyranoside (10)



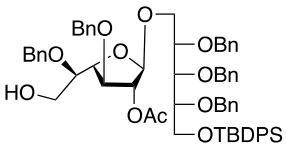
Azide **S15** (19.2 g, 26.89 mmol) was dissolved in THF (50 mL), followed by adding AcOH (5 mL). Zn dust (20.0 g) was then added, and the reaction mixture was stirred at RT for 1 h, after which it was filtered and concentrated to dryness. The resulting crude amine was dissolved in THF (20 mL), after which solid $NaHCO_3$ (10.0 g) and Troc-Cl (5.5 mL, 40.34 mmol) were added, and the reaction mixture was stirred at RT for further 10 mins. The reaction mixture was diluted with CH_2Cl_2 (100 mL) and washed with sat. $NaHCO_3$. The organic phase was dried and concentrated affording a solid, which was crystallized from EtOAc to give product as a white powder, (19.5 g, 84%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.27 (3H, s, CH_3 of STol), 2.30 (3H, s, CH_3 of STol), 3.56 – 3.72 (4H, m, H-3, H-2, H-6 x 2), 3.81 (1H, br t, H-5), 3.98 (1H, br d, H-3), 4.20 (1H, m, H of Fmoc), 4.29 (2H, m, CHHAr), 4.38 – 4.53 (6H, m, CH_2 of Fmoc, CHHAr, CH_2 of Troc), 4.56 (1H, m, H-2), 4.64 – 4.87 (4H, m, H-5, CHHAr x 3), 5.06 (1H, br d, H-1), 5.49 (1H, d, *J* = 2.8 Hz, H-4), 5.54 (1H, br d, H-4), 5.69 (1H, d, *J* = 5.1 Hz, H-1), 7.02 – 7.78 (22H, m, Ar-H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.9, 154.9, 154.0, 153.7, 143.6, 143.5, 143.1, 143.1, 141.3, 141.3, 141.2, 141.2, 138.1, 138.1, 137.7, 137.6, 137.2, 137.2, 132.8, 132.7, 129.9, 129.9, 129.7, 129.3, 128.9, 128.5, 128.4, 128.4, 128.4, 128.3, 128.1, 128.0, 127.9, 127.9, 127.9, 127.8, 127.8, 127.8, 127.8, 127.2, 127.2, 125.5, 125.4, 125.2, 125.1, 120.0, 120.0, 119.9, 105.0, 95.4, 89.5, 86.3, 78.5, 77.2, 75.9, 75.7, 75.6, 74.7, 74.4, 74.0, 73.7, 73.6, 71.5, 71.1, 70.4, 70.2, 70.2, 70.1, 69.1, 68.2, 68.1, 52.8, 51.3, 46.6, 46.5, 21.1, 21.1. MALDI-MS: [M + Na]$^+$ $C_{45}H_{42}Cl_3NNaO_8S$ calcd. 884.1594, found 884.1612.

2-*O*-Acetyl-3,5-di-*O*-benzyl-6-*O*-fluorenylmethoxycarbonyl-*β*-D-galactofuranosyl-(1→1)-*t*-butyldiphenylsilyl 2,3,4-tri-*O*-benzyl-D-ribose (11)



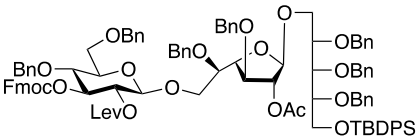
To a mixture of the donor **7** (9.0 g, 12.3 mmol), acceptor **6** (9.7 g, 14.7 mmol), NIS (4.1 g, 18.5 mmol), and activated molecular sieves in dry CH_2Cl_2 (50 mL) was added TMSOTf (222 μL, 1.23 mmol) at -78 ºC. The reaction mixture was stirred at that temperature for 10 min, and then gradually warmed to -40 ºC at which point it was quenched with pyridine. The reaction mixture was diluted with CH_2Cl_2 (50 mL) and washed with 10% $Na_2S_2O_3$. The organic layer was dried and concentrated to give a syrup which was chromatographed using EtOAc:Hexane (1:4) to give the pure product as a clear foam, (15.8 g, 95%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.03 (9H, s, CH_3 x 3 of TBDPS), 1.93 (3H, s, OAc), 3.65 (1H, dd, *J* = 9.9, 2.0 Hz, H-5$_a^{Rib}$), 3.71 – 3.81 (2H, m, H-5^{Gal1},H^{Rib}), 3.84 (1H, d, *J* = 5.3 Hz, H-3), 3.87 – 3.94 (5H, m, H-5$_b^{Rib}$, H-1^{Rib} x 2, H^{Rib} x 2), 4.16 (1H, dd, *J* = 5.5, 3.6 Hz, H-4^{Gal1}), 4.19 – 4.24 (2H, m, H-6$_a^{Gal}$, CH of Fmoc), 4.27 – 4.40 (5H, m, H-6$_b^{Gal1}$, CH_2 of Fmoc, CHHAr x 2), 4.51 – 4.72 (8H, m, CHHAr), 4.97 (1H, s, H-1^{Gal1}), 5.07 (1H, s, H-2^{Gal1}), 7.1 – 7.77 (43H, m, ArH). ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.8, 154.9, 143.3, 141.2, 138.7, 138.7, 138.5, 137.8, 137.7, 135.7, 135.7, 135.6, 135.6, 133.6, 133.4, 129.5, 129.5, 128.4, 128.3, 128.3, 128.3, 128.2, 128.2, 128.2, 128.2, 128.0, 128.0, 127.9, 127.8, 127.8, 127.8, 127.7, 127.7, 127.6, 127.6, 127.6, 127.6, 127.3, 127.2, 127.2, 127.1, 127.1, 125.1, 120.0, 105.7, 83.0, 82.2, 81.0, 79.9, 78.6, 77.9, 77.2, 75.3, 73.6, 73.5, 72.5, 72.4, 72.1, 69.9, 68.1, 67.2, 63.7, 46.7, 26.9, 20.8, 19.2, 0.0. MALDI-MS: [M + Na]$^+$ $C_{79}H_{82}NaO_{13}Si$ calcd. 1289.5422, found 1289.5435.

2-*O*-Acetyl-3,5-di-*O*-benzyl-*β*-D-galactofuranosyl-(1→1)-*t*-butyldiphenylsilyl 2,3,4-tri-*O*-benzyl-D-ribose (12)



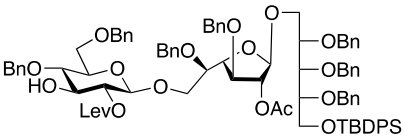
Disaccharide **11** (15.0 g, 11.8 mmol) was dissolved in CH_2Cl_2 (50 mL), after which Et_3N (16 mL, 118.3 mmol) was added and the reaction mixture was stirred at RT overnight. It was then concentrated, loaded on silica gel and chromatographed using EtOAc:Hexane (3:7), (11.3 g, 91%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.03 (9H, s, CH_3 x 3 of TBDPS), 1.98 (3H, s, OAc), 3.52 (1H, m, H-5^{Gal1}), 3.60 (2H, m, H-6^{Gal1}), 3.64 (1H, m, H-5$_a^{Rib}$), 3.76 (1H, m, H^{Rib}), 3.86 (1H, d, *J* = 5.6 Hz, H-3^{Gal1}), 3.87 – 3.96 (5H, m, H-1^{Rib}, H-5$_b^{Rib}$, H^{Rib} x 2), 4.20 (1H, dd, *J* = 4.1, 5.6 Hz, H-4^{Gal}), 4.39 (1H, d, *J* = 6.2 Hz, C*H*HAr), 4.42 (1H, d, *J* = 6.4 Hz, C*H*HAr), 4.50 – 4.60 (5H, m, C*H*HAr), 4.64 – 4.70 (4H, m, C*H*HAr), 4.94 (1H, s, H-1^{Gal1}), 5.08 (1H, d, *J* = 1.0 Hz, H-2^{Gal1}), 7.11 – 7.43 (30H, m, Ar-H), 7.63 (5H, m, Ar-H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.8, 138.7, 138.6, 138.5, 138.1, 137.6, 135.7, 135.6, 133.5, 133.3, 129.5, 129.5, 128.3, 128.3, 128.2, 128.2, 128.1, 128.0, 127.8, 127.8, 127.8, 127.7, 127.7, 127.6, 127.6, 127.6, 127.4, 127.3, 127.2, 105.6, 83.2, 82.9, 81.0, 79.8, 78.6, 78.0, 77.6, 77.2, 73.6, 72.8, 72.5, 72.4, 72.2, 67.2, 63.6, 62.1, 26.9, 20.8, 19.2, 0.0. MALDI-MS: [M + Na]$^+$ $C_{79}H_{82}NaO_{13}Si$ calcd. 1067.4742 found 1067.4756.

4,6-Di-*O*-benzyl-2-*O*-levulinoyl-3-*O*-fluorenylmethyl-*β*-D-glucopyranosyl-(1→6)-2-*O*-acetyl-3,5-di-*O*-benzyl-*β*-D-galactofuranosyl-(1→1)-*t*-butyldiphenylsilyl 2,3,4-tri-*O*-benzyl-D-ribose (13)



To a mixture of the donor **9** (2.5 g, 3.19 mmol), acceptor **12** (2.57 g, 2.45 mmol), NIS (715 mg, 3.19 mmol), and activated molecular sieves in dry CH_2Cl_2 (20 mL) was added TMSOTf (88 µL, 0.49 mmol) at -78 ºC. The reaction mixture was stirred at that temperature for 10 min, and then gradually warmed to -40 ºC at which point it was quenched with pyridine. The reaction mixture was diluted with CH_2Cl_2 (50 mL) and washed with 10% $Na_2S_2O_3$. The organic layer was dried and concentrated to give a syrup which was chromatographed using EtOAc:Hexane (1:4) to give the pure product as a clear foam, (3.0 g, 72%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.04 (9H, s, CH_3 x 3 of TBDPS), 1.87 (3H, s, OAc), 1.88 (3H, s, OLev), 2.23 – 2.54 (4H, m, CH_2 of Lev), 3.43 (1H, m, H-5^{Glc}), 3.63 (1H, m, H-5$_a^{Rib}$), 3.69 (2H, m, H-6^{Gal1}), 3.74 (2H, m, H-5^{Gal1}, H-6$_a^{Glc}$), 3.76 (1H, m, H-3^{Gal1}), 3.78 (1H, m, H^{Rib}), 3.86 (1H, m, H-4^{Glc}), 3.87 (1H, m, H-5$_b^{Rib}$), 3.89 (4H, m, H-1^{Rib}, H^{Rib} x 2), 3.99 (1H, m, H-6$_b^{Glc}$), 4.00 (1H, m, H-4^{Gal1}), 4.19 (1H, d, *J* = 11.4 Hz, C*H*HAr), 4.23 – 4.25 (2H, m, CH of Fmoc, C*H*HAr), 4.30 (1H, d, *J* = 11.3 Hz, C*H*HAr), 4.45 (1H, d, H-1^{Glc}), 4.42 – 4.69 (14H, m, CH_2Ar, CH_2 of Fmoc, H-1^{Glc}), 4.95 (1H, s, H-1^{Gal1}), 5.02 (1H, s, H-2^{Gal1}), 5.06 (1H, m, H-3^{Glc}), 5.08 (1H, m, H-2^{Glc}), 7.00 – 7.76 (53H, m, Ar-H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 205.6, 171.4, 171.1, 169.8, 154.6, 143.6, 143.3, 141.2, 141.1, 138.7, 138.7, 138.5, 138.4, 137.8, 137.7, 137.5, 135.7, 135.6, 133.5, 133.4, 129.5, 129.5, 128.4, 128.3, 128.3, 128.3, 128.2, 128.2, 128.2, 128.2, 128.1, 128.0, 127.8, 127.8, 127.8, 127.8, 127.8, 127.8, 127.7, 127.7, 127.7, 127.6, 127.6, 127.6, 127.6, 127.4, 127.3, 127.2, 127.1, 125.3, 125.2, 119.9, 105.8, 100.8, 83.1, 83.0, 81.1, 79.9, 79.5, 78.6, 78.0, 77.2, 76.4, 75.7, 74.9, 74.6, 74.0, 73.6, 73.5, 72.5, 72.4, 72.1, 72.0, 71.8, 70.4, 68.0, 67.3, 63.7, 46.6, 37.6, 29.4, 27.8, 26.9, 21.0, 20.7, 19.2, 14.2, 0.0. MALDI-MS: $[M + Na]^+$ $C_{104}H_{110}NaO_{20}Si$ calcd. 1729.7257 found 1729.7269.

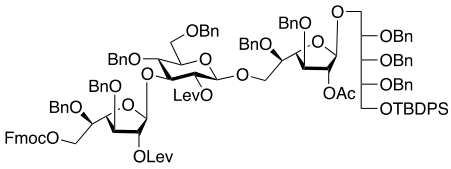
4,6-Di-*O*-benzyl-2-*O*-levulinoyl-3-*β*-D-glucopyranosyl-(1→6)-2-*O*-acetyl-3,5-di-*O*-benzyl-*β*-D-galactofuranosyl-(1→1)-*t*-butyldiphenylsilyl 2,3,4-tri-*O*-benzyl-D-ribose (14)



Trisaccharide **13** (2.8 g, 1.64 mmol) was dissolved in CH_2Cl_2 (20 mL), after which Et_3N (1.14 mL, 8.19 mmol) was added and the reaction mixture was stirred at RT overnight. It was then concentrated, loaded on silica gel and chromatographed using EtOAc:Toluene (1:4), (2.1 g, 87%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.04 (9H, s, CH_3 x 3 of TBDPS), 1.85 (3H, s, OAc), 2.07 (3H, s, OLev) 2.20 (1H, m, C*H*H of OLev), 2.44 (1H, m, C*H*H of OLev), 2.52 (1H, m, C*H*H of OLev), 2.70 (1H, m, C*H*H of OLev), 3.07 (1H, d, *J* = 3.17 Hz, 3-OH^{Glc}), 3.39 (1H, m, H-5^{Glc}), 3.62 (1H, m, H-5$_a^{Rib}$), 3.63 (1H, m, H-4^{Glc}), 3.67 (2H, m, H-6^{Gal1}), 3.71 (2H, m, H-5^{Gal1}, H-6$_a^{Glc}$), 3.75 (1H, m, H-3^{Gal1}), 3.79 (1H, m, H-3^{Glc}), 3.84 (1H, m, H-5$_b^{Rib}$), 3.88 (4H, m, H-1^{Rib}, H^{Rib} x 2), 3.99 (1H, m, H-6$_b^{Glc}$), 3.99 (1H, m, H-4^{Gal1}), 4.17 (1H, d, *J* = 11.6 Hz, C*H*HAr), 4.30 (1H, d, *J* = 11.7 Hz, C*H*HAr), 4.39 (1H, d, *J* = 7.8 Hz, H-1^{Glc}), 4.42 – 4.69 (11H, m, CH_2Ar), 4.87 (1H, d, *J* = 11.0 Hz), 4.91 (1H, m, H-2^{Glc}), 4.95 (1H, s, H-1^{Gal1}), 5.00 (1H, s, H-2^{Gal1}), 7.09 – 7.69 (45H, m, Ar-H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 207.8, 172.2, 169.8, 138.7, 138.6, 138.5, 138.5, 138.2, 137.9, 137.7, 135.7, 135.6, 133.5, 133.4, 129.5, 129.5, 129.0, 128.4, 128.4, 128.3, 128.3, 128.2, 128.2, 128.2, 128.1, 128.0, 127.8, 127.8, 127.8, 127.7, 127.7, 127.6, 127.6, 127.6, 127.6, 127.5, 127.5, 127.4, 127.3, 127.2, 127.2, 125.3, 105.8, 100.6, 83.0, 82.9, 81.1, 79.9, 78.6, 77.9, 77.2, 76.4, 76.1, 74.8, 74.8, 74.5, 74.0, 73.6, 73.5, 72.5, 72.4, 72.0, 68.5,

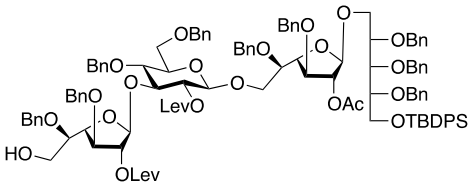
67.2, 63.7, 38.4, 29.6, 28.1, 26.9, 21.4, 20.7, 19.2, 0.0. MALDI-MS: [M + Na]$^+$ $C_{89}H_{100}NaO_{18}Si$ calcd. 1507.6577 found 1507.6598.

2-*O*-Levulinoyl-3,5-di-*O*-benzyl-6-*O*-fluorenylmethyl-*β*-D-galactofuranosyl-(1→3)-4,6-di-*O*-benzyl-2-*O*-levulinoyl-*β*-D-glucopyranosyl-(1→6)-2-*O*-acetyl-3,5-di-*O*-benzyl-*β*-D-galactofuranosyl-(1→1)-*t*-butyldiphenylsilyl 2,3,4-tri-*O*-benzyl-D-ribose (15)



To a mixture of the donor **8** (1.6 g, 2.12 mmol), acceptor **14** (2.1 g, 1.41 mmol), NIS (480 mg, 2.12 mmol), and activated molecular sieves in dry CH_2Cl_2 (20 mL) was added TMSOTf (51 µL, 0.282 mmol) at -78 ºC. The reaction mixture was stirred at that temperature for 10 min, and then gradually warmed to -40 ºC at which point it was quenched with pyridine. The reaction mixture was diluted with CH_2Cl_2 (50 mL) and washed with 10% $Na_2S_2O_3$. The organic layer was dried and concentrated to give a syrup which was chromatographed using EtOAc:Toluene (5:95 to 1:9) to give the pure product as a clear foam, (2.6 g, 86%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.04 (9H, s, CH_3 x 3 of TBDPS), 1.86 (3H, s, OAc), 1.94 (3H, s, OLev), 2.09 (3H, s, OLev), 2.44 (4H, m, C*H*H of OLev), 2.62 (4H, m, C*H*H of OLev), 3.40 (1H, m, H-5^{Glc}), 3.59 (2H, m, incl. H-5^{Gal2}), 3.60 – 3.68 (m, H-5$_a^{Rib}$, H-6^{Gal1}), 3.70 (2H, m, H-6$_a^{Glc}$), 3.72 (1H, m, H-5^{Gal1}), 3.75 (1H, m, H-3^{Gal1}), 3.78 (m, H^{Rib}), 3.86 (1H, m, H-5$_b^{Rib}$), 3.88 (m, H-1^{Rib}, H^{Rib}, H-3^{Gal2}), 3.93 – 3.94 (m, H-3^{Glc}, H-4^{Glc}), 3.96 (m, H-6$_b^{Glc}$), 3.98 (1H, m, H-6$_a^{Gal2}$), 4.00 (1H, m, H-4^{Gal1}), 4.13 – 4.20 (m, H-6$_b^{Gal2}$, C*H*HAr), 4.20 (1H, m, CH of Fmoc), 4.22 (1H, m, H-4^{Gal2}), 4.29 (1H, d, C*H*HAr), 4.31 (1H, m, H-1^{Glc}), 4.32 – 4.60 (m, CH*H*Ar), 4.62 – 4.70 (4H, m, C*H*HAr), 4.88 (1H, d, *J* = 11.4 Hz, C*H*HAr), 4.95 (1H, s, H-1^{Gal1}), 5.0 (1H, s, H-2^{Gal1}), 5.08 (2H, m, H-2^{Gal2}, H-2^{Glc}), 5.31 (1H, s, H-1^{Gal2}), 7.04 – 7.80 (63H, Ar-H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 206.2, 205.9, 171.6, 171.2, 169.8, 154.9, 143.3, 141.3, 138.7, 138.6, 138.6, 138.5, 138.4, 138.0, 137.8, 137.7, 137.7, 135.7, 135.6, 133.6, 133.4, 129.5, 129.5, 128.3, 128.3, 128.3, 128.3, 128.2, 128.2, 128.2, 128.1, 128.0, 128.0, 128.0, 127.9, 127.8, 127.8, 127.8, 127.7, 127.7, 127.7, 127.7, 127.7, 127.6, 127.6, 127.6, 127.6, 127.6, 127.6, 127.5, 127.4, 127.3, 127.3, 127.2, 127.2, 127.1, 127.1, 125.1, 120.0, 105.8, 105.7, 100.9, 83.1, 83.1, 82.7, 82.6, 81.1, 80.7, 79.9, 78.6, 77.9, 77.2, 76.1, 75.4, 75.2, 74.4, 74.0, 73.8, 73.6, 73.4, 73.2, 72.5, 72.4, 72.1, 72.0, 71.8, 69.8, 68.6, 68.4, 67.2, 63.7, 46.7, 37.7, 37.5, 29.7, 29.5, 27.9, 27.8, 26.9, 20.7, 19.2, 0.0. MALDI-MS: [M + Na]$^+$ $C_{129}H_{138}NaO_{27}Si$ calcd. 2169.9092 found 2169.9105

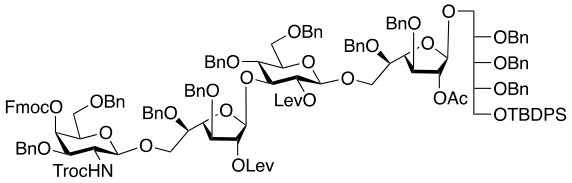
2-*O*-Levulinoyl-3,5-di-*O*-benzyl-*β*-D-galactofuranosyl-(1→3)-4,6-di-*O*-benzyl-2-*O*-levulinoyl-*β*-D-glucopyranosyl-(1→6)-2-*O*-acetyl-3,5-di-*O*-benzyl-*β*-D-galactofuranosyl-(1→1)-*t*-butyldiphenylsilyl 2,3,4-tri-*O*-benzyl-D-ribose (16)



Tetrasaccharide **14** (2.6 g, 1.21 mmol) was dissolved in CH_2Cl_2 (20 mL), after which Et_3N (1.7 mL, 12.1 mmol) was added and the reaction mixture was stirred at RT overnight. It was then concentrated, loaded on silica gel and chromatographed using EtOAc:Toluene (1:4), (1.9 g, 82%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.03 (9H, s, CH_3 x 3 of TBDPS), 1.86 (3H, s, OAc), 1.94 (3H, s, OLev), 2.09 (3H, s, OLev), 2.44 (4H, m, C*H*H of OLev), 2.64 (4H, m, C*H*H of OLev), 3.37 (1H, m, H-5^{Gal2}), 3.38 (1H, m, H-6$_a^{Gal2}$), 3.40 (1H, m, H-5^{Glc}), 3.47 (1H, m, H-6$_b^{Gal2}$), 3.58 – 3.67 (4H, m, H-6^{Gal1}, H-5$_a^{Rib}$), 3.70 (1H, m, H-6^{Glc}), 3.71 (1H, m, H-5^{Gal1}), 3.74 (1H, m, H-3^{Gal1}), 3.77 (1H, H^{Rib}), 3.86 (1H, m, H-5$_b^{Rib}$), 3.88 (m, H-1^{Rib}, H-4^{Glc}), 3.90 (1H, m, H-3^{Gal2}), 3.94 (1H, m, H-3^{Glc}), 3.96 (1H, m, H-6$_b^{Glc}$), 3.99 (1H, dd, *J* = 5.8, 2.2 Hz, H-4^{Gal1}), 4.16 – 4.23 (2H, m, C*H*HAr x

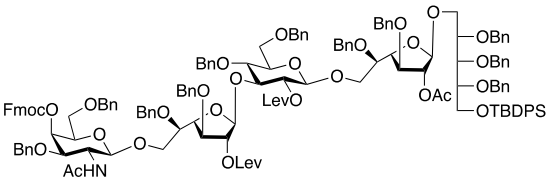
2), 4.26 (1H, dd, J = 3.9, 4.9 Hz, H-4^{Gal2}), 4.27 – 4.59 (11H, m, C*H*HAr), 4.31 (1H, m, H-1^{Glc}), 4.62 – 4.72 (4H, m, C*H*HAr), 4.89 (1H, d, J = 11.4 Hz, C*H*HAr), 4.95 (1H, s, H-1^{Gal1}), 5.01 (1H, s, H-2^{Gal1}), 5.08 (1H, dd, J = 9.3, 8.2 Hz, H-2^{Glc}), 5.09 (1H, s, H-2^{Gal2}), 5.31 (1H, s, H-1^{Gal2}). ^{13}C NMR (101 MHz, $CDCl_3$) δ 206.3, 206.0, 171.6, 171.2, 169.8, 138.7, 138.6, 138.6, 138.5, 138.3, 138.1, 137.8, 137.7, 137.6, 135.7, 135.6, 133.6, 133.4, 129.5, 129.5, 128.3, 128.3, 128.3, 128.3, 128.2, 128.2, 128.2, 128.2, 128.2, 128.1, 128.1, 128.0, 128.0, 127.9, 127.9, 127.9, 127.8, 127.8, 127.8, 127.7, 127.7, 127.7, 127.7, 127.6, 127.6, 127.6, 127.6, 127.5, 127.4, 127.3, 127.3, 127.2, 105.8, 105.6, 100.9, 83.2, 83.1, 83.0, 82.8, 81.1, 80.8, 79.9, 78.6, 78.0, 77.2, 76.5, 76.1, 75.2, 74.5, 74.0, 73.8, 73.6, 73.4, 72.5, 72.4, 72.2, 72.0, 71.8, 68.4, 67.2, 63.7, 61.8, 37.8, 37.5, 29.7, 29.5, 27.8, 26.9, 20.7, 19.2, 0.0. MALDI-MS: [M + Na]$^+$ $C_{114}H_{128}NaO_{25}Si$ calcd. 1947.8412 found 1947.8427.

2-(2,2,2-Trichloroethylcarbonylamino)-2-deoxy-3,6-di-*O*-benzyl-4-*O*-fluorenylmethyl-*β*-D-galactopyranosyl-(1→6)-2-*O*-levulinoyl-3,5-di-*O*-benzyl-*β*-D-galactofuranosyl-(1→3)-4,6-di-*O*-benzyl-2-*O*-levulinoyl-*β*-D-glucopyranosyl-(1→6)-2-*O*-acetyl-3,5-di-*O*-benzyl-*β*-D-galactofuranosyl-(1→1)-*t*-butyldiphenylsilyl 2,3,4-tri-*O*-benzyl-D-ribose (5)



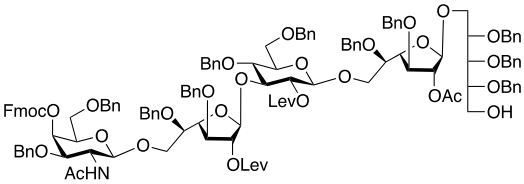
To a mixture of the donor **10** (8.4 g, 9.7 mmol), acceptor **16** (12.5 g, 6.49 mmol), NIS (2.1 g, 9.7 mmol), and activated molecular sieves in dry CH_2Cl_2 (100 mL) was added TMSOTf (235 µL, 1.298 mmol) at -50 ºC. The reaction mixture was stirred at that temperature for 10 min, and then gradually warmed to -40 ºC at which point it was quenched with pyridine. The reaction mixture was diluted with CH_2Cl_2 (50 mL) and washed with 10% $Na_2S_2O_3$. The organic layer was dried and concentrated to a syrup which was chromatographed using EtOAc:Toluene (1:4 to 3:7) to give the pure product as a white solid, (15.7 g, 91%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.03 (9H, s, CH_3 x 3 of TBDPS), 1.86 (3H, s, OAc), 1.94 (3H, s, OLev), 2.09 (3H, s, OLev), 2.44 (4H, m, C*H*H of OLev), 2.64 (4H, m, C*H*H of OLev), 3.37 (1H, m, H-5^{Glc}), 3.56 – 3.65 (8H, m, H-2^{GalN}, H-6^{Gal1}, H-6^{Gal2}, H-6$_a^{GalN}$), 3.70 (2H, m, H-6$_b^{GalN}$, H-5^{Gal1}), 3.73 – 3.81 (5H, m, H-3^{GalN}, H-6$_a^{Glc}$, H-3^{Gal1}, H-3^{Gal2}, H^{Rib}), 3.84 (1H, m, H-5^{Rib}), 3.88 (m, inc. H-1^{Rib}, H-3^{Glc}, H-4^{Glc}, H^{Rib}), 3.95 (1H, m, H-6$_b^{Glc}$), 3.99 (1H, dd, J = 5.4, 1.9 Hz, H-4^{Gal1}), 4.10 (1H, at, H-4^{Gal2}), 4.12 – 4.22 (3H, m, C*H*HAr, CH of Fmoc), 4.23 – 4.35 (m, C*H*HAr), 4.30 (1H, H-1^{Glc}), 4.37 – 4.60 (m, CHHAr), 4.49 (1H, H-1^{GalN}), 4.61 – 4.69 (3H, m, CHHAr), 4.74 (1H, d, J = 10.8 Hz, CHHAr), 4.88 (1H, d, J = 11.0 Hz, CHHAr), 4.95 (1H, s, H-2^{Gal1}), 5.01 (1H, s, H-2^{Gal2}), 5.06 (1H, dd, J = 8.2, 9.5 Hz, H-2^{Glc}), 5.30 (1H, s, H-1^{Gal2}), 5.46 (1H, d, J = 2.7 Hz, H-4^{GalN}), 7.04 – 7.79 (73H, m, Ar-H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 206.4, 206.0, 171.7, 171.2, 169.8, 155.0, 153.9, 143.6, 143.1, 141.3, 141.2, 138.7, 138.6, 138.6, 138.6, 138.5, 138.4, 137.9, 137.7, 137.7, 137.5, 137.4, 135.7, 135.6, 133.5, 133.4, 129.5, 129.5, 128.5, 128.3, 128.3, 128.3, 128.3, 128.3, 128.2, 128.2, 128.2, 128.2, 128.1, 128.1, 128.0, 128.0, 127.9, 127.9, 127.9, 127.8, 127.8, 127.7, 127.7, 127.7, 127.6, 127.6, 127.6, 127.5, 127.4, 127.4, 127.3, 127.2, 127.2, 127.2, 125.5, 125.2, 119.9, 105.9, 100.8, 83.4, 83.1, 82.9, 81.1, 80.9, 79.9, 78.6, 77.9, 77.2, 77.1, 76.6, 76.2, 75.1, 74.5, 74.0, 73.9, 73.7, 73.6, 73.4, 72.5, 72.4, 72.0, 71.8, 71.7, 71.4, 70.1, 69.7, 67.6, 67.2, 63.7, 54.4, 46.6, 37.7, 37.5, 29.7, 29.6, 27.9, 27.7, 26.9, 20.7, 19.2, 0.0. MALDI-MS: [M + Na]$^+$ $C_{152}H_{162}Cl_3NNaO_{33}Si$ calcd. 2684.9762 found 2684.9778.

2-Acetamido-2-deoxy-3,6-di-*O*-benzyl-4-*O*-fluorenylmethyl-*β*-D-galactopyranosyl-(1→6)-2-*O*-levulinoyl-3,5-di-*O*-benzyl-*β*-D-galactofuranosyl-(1→3)-4,6-di-*O*-benzyl-2-*O*-levulinoyl-*β*-D-glucopyranosyl-(1→6)-2-*O*-acetyl-3,5-di-*O*-benzyl-*β*-D-galactofuranosyl-(1→1)-*t*-butyldiphenylsilyl 2,3,4-tri-*O*-benzyl-D-ribose (17)



To a solution of pentasaccharide **5** (1.0 g, 0.37 mmol) in THF (5 mL) was added Zn dust (1.0 g), Ac_2O (0.5 mL) and AcOH (0.5 mL), and the reaction mixture was left stirring at RT for 1 h, after which it was filtered, concentrated, absorbed on silica gel and purified using acetone:toluene (1:9 to 1:4) to give the product as a white solid (787 mg, 82%).^{1}H NMR (400 MHz, $CDCl_3$): δ 1.04 (9H, s, CH_3 x 3 of TBDPS), 1.63 (3H, s, NHAc), 1.86 (3H, s, OAc), 1.94 (3H, s, OLev), 2.09 (3H, s, OLev), 2.44 (4H, m, C*H*H of OLev), 2.64 (4H, m, C*H*H of OLev), 3.36 – 3.44 (2H, m, H-5^{Glc}, H-2^{GalN}), 3.53 – 3.67 (7H, m, H-6^{Gal1}, H-6^{Gal2}, H-5^{Gal2}, H-5$_a^{Rib}$, H-6$_a^{GalN}$), 3.70 (1H, m, H-6$_a^{Glc}$), 3.71 (1H, m, H-5^{Gal1}), 3.74 (1H, m, H-3^{Gal1}), 3.77 (3H, m, H-5^{GalN}, H-3^{Gal2}, H^{Rib}), 3.81 (1H, m, H-6$_b^{GalN}$), 3.84 (1H, m, H-5$_b^{Rib}$), 3.87 – 3.93 (4H, m, H-3^{Glc}, H-4^{Glc}, H-1^{Rib}), 3.96 (1H, m, H-6$_b^{Glc}$), 3.99 (1H, dd, *J* = 2.3, 5.6 Hz, H-4^{Gal1}), 4.11 (1H, m, H-4^{Gal2}), 4.15 – 4.22 (3H, m, H-3^{GalN}, CH of Fmoc, C*H*HAr), 4.27 – 4.33 (3H, m, H-1^{Glc}, CH*H*Ar x 2), 4.33 – 4.70 (m, C*H*HAr), 4.74 (1H, d, *J* = 11.6 Hz, C*H*HAr), 4.81 (1H, d, *J* = 8.3 Hz, H-1^{GalN}), 4.92 (1H, d, *J* = 11.6 Hz, C*H*HAr), 4.96 (1H, s, H-1^{Gal1}), 5.01 (1H, s, H-2^{Gal1}), 5.04 (1H, s, H-2^{Gal2}), 5.06 (1H, dd, *J* = 8.4, 9.4 Hz, H-2^{Glc}), 5.19 (1H, d, *J* = 7.8 Hz, NH), 5.31 (1H, s, H-1^{Gal2}), 5.46 (1H, d, *J* = 2.5 Hz, H-4^{GalN}), 7.06 – 7.77 (73H, m, Ar-H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 206.4, 206.0, 171.7, 171.3, 170.4, 169.8, 155.1, 143.6, 143.2, 141.3, 141.2, 138.7, 138.6, 138.6, 138.6, 138.5, 138.5, 137.9, 137.8, 137.7, 137.7, 137.6, 135.7, 135.6, 135.6, 133.6, 133.4, 129.5, 129.5, 128.4, 128.4, 128.3, 128.3, 128.2, 128.2, 128.2, 128.2, 128.2, 128.1, 128.0, 128.0, 128.0, 127.9, 127.9, 127.8, 127.8, 127.8, 127.8, 127.7, 127.7, 127.7, 127.7, 127.6, 127.6, 127.6, 127.6, 127.5, 127.4, 127.3, 127.3, 127.2, 127.2, 127.1, 125.4, 125.2, 120.0, 119.9, 105.9, 105.8, 105.0, 100.8, 100.3, 83.3, 83.1, 83.0, 81.1, 79.9, 78.6, 77.9, 77.2, 76.6, 76.4, 75.1, 74.7, 74.5, 74.0, 73.9, 73.7, 73.6, 73.4, 73.1, 72.5, 72.4, 72.0, 71.7, 71.6, 70.8, 70.2, 70.1, 68.4, 67.7, 67.2, 63.7, 54.6, 46.6, 37.7, 37.5, 29.7, 29.6, 27.9, 27.7, 26.9, 23.4, 20.7, 19.2, 0.0. MALDI-MS: $[M + Na]^+$ $C_{151}H_{163}NNaO_{32}Si$ calcd. 2553.0825 found 2553.0843.

2-Acetamido-2-deoxy-3,6-di-*O*-benzyl-4-*O*-fluorenylmethyl-*β*-D-galactopyranosyl-(1→6)-2-*O*-levulinoyl-3,5-di-*O*-benzyl-*β*-D-galactofuranosyl-(1→3)-4,6-di-*O*-benzyl-2-*O*-levulinoyl-*β*-D-glucopyranosyl-(1→6)-2-*O*-acetyl-3,5-di-*O*-benzyl-*β*-D-galactofuranosyl-(1→1)-2,3,4-tri-*O*-benzyl-D-ribose (18)

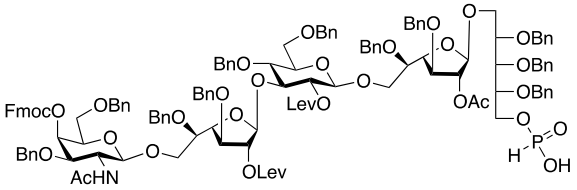


Pentasaccharide **17** (787 mg, 0.31 mmol) was dissolved in pyridine (6 mL) and cooled to 0 ºC, followed by adding HF-Py (4 mL). The reaction mixture was left stirring at RT for 30 min, after which it was diluted with CH_2Cl_2 (20 mL), washed with water (20 mL), and then with sat. $NaHCO_3$ (20 mL). The organic phase was dried and concentrated *in vacuo* to give a crude residue, which was chromatographed using acetone:toluene (1:4) to give the desired product as a white solid (680 mg, 86%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 1.63 (3H, s, NHAc), 1.86 (3H, s, OAc), 1.94 (3H, s, OLev), 2.09 (3H, s, OLev), 2.24 (1H, m, 1-OH^{Rib}), 2.44 (4H, m, C*H*H of OLev), 2.64 (4H, m, C*H*H of OLev), 3.36 – 3.44 (2H, m, H-5^{Glc}, H-2^{GalN}), 3.53 – 3.67 (7H, m, H-6^{Gal1}, H-6^{Gal2}, H-5^{Gal2},

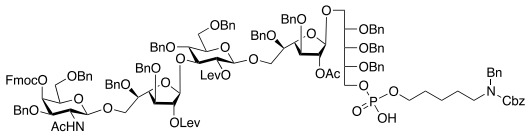
H-5$_a^{Rib}$, H-6$_a^{GalN}$), 3.70 (1H, m, H-6$_a^{Glc}$), 3.71 (1H, m, H-5^{Gal1}), 3.74 (1H, m, H-3^{Gal1}), 3.77 (3H, m, H-5^{GalN}, H-3^{Gal2}, H^{Rib}), 3.81 (1H, m, H-6$_b^{GalN}$), 3.84 (1H, m, H-5$_b^{Rib}$), 3.87 – 3.93 (4H, m, H-3^{Glc}, H-4^{Glc}, H-1^{Rib}), 3.96 (1H, m, H-6$_b^{Glc}$), 3.99 (1H, m, H-4^{Gal1}), 4.11 (1H, m, H-4^{Gal2}), 4.15 – 4.22 (3H, m, H-3^{GalN}, CH of Fmoc, C*H*HAr), 4.27 – 4.33 (3H, m, H-1^{Glc}, CH*H*Ar x 2), 4.33 – 4.70 (m, C*H*HAr), 4.74 (1H, d, *J* = 11.6 Hz, C*H*HAr), 4.81 (1H, d, *J* = 8.3 Hz, H-1^{GalN}), 4.92 (1H, d, *J* = 11.6 Hz, C*H*HAr), 4.96 (1H, s, H-1^{Gal1}), 5.01 (1H, s, H-2^{Gal1}), 5.04 (1H, s, H-2^{Gal2}), 5.06 (1H, dd, *J* = 8.4, 9.4 Hz, H-2^{Glc}), 5.19 (1H, d, *J* = 7.8 Hz, NH), 5.31 (1H, s, H-1^{Gal2}), 5.46 (1H, s, H-4^{GalN}), 7.03 – 7.79 (63H, m, Ar-H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 120.0, 125.5, 125.2, 128.0, 127.0, 70.3, 105.7, 81.2, 105.9, 74.6, 100.3, 71.8, 72.5, 73.9, 72.2, 72.0, 72.3, 72.2, 73.6, 74.5, 70.1, 73.7, 71.9, 100.9, 72.2, 70.1, 70.1, 72.2, 73.9, 73.4, 46.7, 74.9, 73.2, 83.4, 83.2, 71.6, 78.9, 66.7, 77.7, 70.6, 83.0, 71.7, 76.5, 78.9, 61.5, 71.6, 66.7, 68.4, 68.1, 70.9, 76.7, 75.3, 54.7, 37.8, 29.9, 29.8, 20.9, 23.6. MALDI-MS: $[M + Na]^+$ $C_{135}H_{145}NNaO_{32}$ calcd. 2314.9647 found 2316.4943.

2-Acetamido-2-deoxy-3,6-di-*O*-benzyl-4-*O*-fluorenylmethyl-*β*-D-galactopyranosyl-(1→6)-2-*O*-levulinoyl-3,5-di-*O*-benzyl-*β*-D-galactofuranosyl-(1→3)-4,6-di-*O*-benzyl-2-*O*-levulinoyl-*β*-D-glucopyranosyl-(1→6)-2-*O*-acetyl-3,5-di-*O*-benzyl-*β*-D-galactofuranosyl-(1→1)-5-[5-benzyl(benzyloxycarbonyl)aminopentyl]-phosphoryl-2,3,4-tri-*O*-benzyl-D-ribose (4)



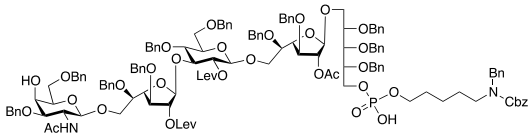
To a solution of pentasaccharide **18** (2.0 g, 0.87 mmol) in pyridine (10 mL), was added dioxane (5 mL), and after that he reaction was cooled to 0 ºC, followed by adding salicyl chlorophosphite (883 mg, 4.35 mmol). Stirring was continued at RT for 2 h. A second portion of salicyl chlorophospite (883 mg, 4.35 mmol) was added to bring the reaction to completion. Water was then added, and the solution was then concentrated *in vacuo*, loaded on silica gel and chromatographed using CH_3CN, then CH_3CN:CH_2Cl_2 (1:1), and CH_3OH:CH_2Cl_2:CH_3CN (1:4:5) to give the desired phosphonate as a white solid. (2.04 g, 89%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.65 (3H, s, NHAc), 1.84 (3H, s, OAc), 1.93 (3H, s, OLev), 2.09 (3H, s, OLev), 2.29 – 2.60 (8H, m, C*H*H of OLev), 3.36 – 3.44 (2H, m, H-5^{Glc}, H-2^{GalN}), 3.53 – 3.67 (7H, m, H-6^{Gal1}, H-6^{Gal2}, H-5^{Gal2}, H-5$_a^{Rib}$, H-6$_a^{GalN}$), 3.70 (1H, m, H-6$_a^{Glc}$), 3.71 (1H, m, H-5^{Gal1}), 3.74 (1H, m, H-3^{Gal1}), 3.77 (3H, m, H-5^{GalN}, H-3^{Gal2}, H^{Rib}), 3.81 (1H, m, H-6$_b^{GalN}$), 3.84 (1H, m, H-5$_b^{Rib}$), 3.87 – 3.93 (4H, m, H-3^{Glc}, H-4^{Glc}, H-1^{Rib}), 3.96 (1H, m, H-6$_b^{Glc}$), 3.99 (1H, m, H-4^{Gal1}), 4.11 (1H, m, H-4^{Gal2}), 4.15 – 4.22 (3H, m, H-3^{GalN}, CH of Fmoc, C*H*HAr), 4.27 – 4.33 (3H, m, H-1^{Glc}, CH*H*Ar x 2), 4.33 – 4.70 (m, C*H*HAr), 4.74 (1H, d, *J* = 11.6 Hz, C*H*HAr), 4.81 (1H, d, *J* = 8.3 Hz, H-1^{GalN}), 4.92 (1H, d, *J* = 11.6 Hz, C*H*HAr), 4.96 (1H, s, H-1^{Gal1}), 5.01 (1H, s, H-2^{Gal1}), 5.04 (1H, s, H-2^{Gal2}), 5.06 (1H, dd, *J* = 8.4, 9.4 Hz, H-2^{Glc}), 5.19 (1H, d, *J* = 7.8 Hz, NH), 5.31 (1H, s, H-1^{Gal2}), 5.46 (1H, s, H-4^{GalN}), 5.90 (1H, br s, H-phosphonate), 7.03 – 7.79 (63H, m, Ar-H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 206.4, 205.9, 183.0, 171.7, 171.2, 170.4, 169.9, 155.1, 143.6, 143.2, 141.3, 141.2, 138.5, 138.5, 138.2, 138.1, 138.1, 137.9, 137.8, 137.7, 137.6, 128.4, 128.4, 128.4, 128.3, 128.3, 128.3, 128.2, 128.2, 128.2, 128.2, 128.1, 128.0, 128.0, 128.0, 127.9, 127.9, 127.8, 127.7, 127.7, 127.7, 127.6, 127.6, 127.5, 127.5, 127.3, 127.2, 127.1, 125.4, 125.2, 120.0, 106.0, 105.8, 100.8, 100.3, 83.1, 83.0, 81.2, 78.9, 78.8, 77.6, 77.2, 75.1, 74.7, 74.5, 74.0, 73.9, 73.8, 73.7, 73.4, 73.1, 72.5, 72.1, 72.0, 71.9, 71.7, 71.6, 70.2, 70.0, 68.5, 67.7, 66.6, 61.4, 54.6, 46.6, 37.7, 37.5, 29.7, 29.6, 27.9, 27.7, 23.4, 20.7. MALDI-MS: $[M + Na]^+$ $C_{135}H_{146}NNaO_{34}P$ calcd. 2378.9362 found 2378.9464.

2-Acetamido-2-deoxy-3,6-di-*O*-benzyl-4-*O*-fluorenylmethyl-*β*-D-galactopyranosyl-(1→6)-2-*O*-levulinoyl-3,5-di-*O*-benzyl-*β*-D-galactofuranosyl-(1→3)-4,6-di-*O*-benzyl-2-*O*-levulinoyl-3-*O*-*β*-D-glucopyranosyl-(1→6)-2-*O*-acetyl-3,5-di-*O*-benzyl-*β*-D-galactofuranosyl-(1→1)-5-[5-benzyl(benzyloxycarbonyl)aminopentyl]-phosphoryl-2,3,4-tri-*O*-benzyl-D-ribose (18a)



To a solution of the phosphonate **4** (500 mg, 0.212 mmol) and linker (347 mg, 1.06 mmol) in pyridine (10 mL) was added PivCl (104 µL, 0.848 mmol), and the reaction mixture was left stirring at that temperature for 2 h. I_2 (538 mg, 2.1 mmol) and H_2O (0.5 mL) were added, and the reaction mixture was additionally stirred at RT for 2 h. The reaction mixture was neutralized with sat. $Na_2S_2O_3$, concentrated *in vacuo,* re-dissolved in CH_2Cl_2, and washed with water. The organic phase was dried and concentrated *in vacuo* to give the residue which was further chromatographed to give the desired product as a white solid (500 mg, 88%). ^{1}H NMR (750 MHz, $CDCl_3$): δ 1.15 – 1.39 (4H, br m, CH_2 x 2 linker), 1.65 (3H, s, NHAc), 1.84 (3H, s, OAc), 1.93 (3H, s, OLev), 2.09 (3H, s, OLev), 2.29 – 2.60 (8H, m, C*H*H of OLev), 2.87 – 3.04 (4H, m, CH_2 x 2 linker), 3.36 – 3.44 (2H, m, H-5^{Glc}, H-2^{GalN}), 3.53 – 3.67 (7H, m, H-6^{Gal1}, H-6^{Gal2}, H-5^{Gal2}, H-5$_a^{Rib}$, H-6$_a^{GalN}$), 3.70 (1H, m, H-6$_a^{Glc}$), 3.71 (1H, m, H-5^{Gal1}), 3.74 (1H, m, H-3^{Gal1}), 3.77 (3H, m, H-5^{GalN}, H-3^{Gal2}, H^{Rib}), 3.81 (1H, m, H-6$_b^{GalN}$), 3.84 (1H, m, H-5$_b^{Rib}$), 3.87 – 3.93 (4H, m, H-3^{Glc}, H-4^{Glc}, H-1^{Rib}), 3.96 (1H, m, H-6$_b^{Glc}$), 3.99 (1H, m, H-4^{Gal1}), 4.11 (1H, m, H-4^{Gal2}), 4.15 – 4.22 (3H, m, H-3^{GalN}, CH of Fmoc, C*H*HAr), 4.27 – 4.33 (3H, m, H-1^{Glc}, CH*H*Ar x 2), 4.33 – 4.70 (m, C*H*HAr), 4.74 (1H, d, *J* = 11.6 Hz, C*H*HAr), 4.81 (1H, d, *J* = 8.3 Hz, H-1^{GalN}), 4.92 (1H, d, *J* = 11.6 Hz, C*H*HAr), 4.96 (1H, s, H-1^{Gal1}), 5.01 (1H, s, H-2^{Gal1}), 5.04 (1H, s, H-2^{Gal2}), 5.06 (1H, dd, *J* = 8.4, 9.4 Hz, H-2^{Glc}), 5.19 (1H, d, *J* = 7.8 Hz, NH), 5.31 (1H, s, H-1^{Gal2}), 5.46 (1H, s, H-4^{GalN}), 5.90 (1H, br s, H-phosphonate), 7.03 – 7.79 (83H, m, Ar-H). ^{13}C NMR (189 MHz, $CDCl_3$) δ 108.7, 108.6, 103.6, 103.0, 86.1, 85.8, 85.7, 83.8, 83.7, 80.8, 79.6, 79.5, 79.2, 79.1, 77.7, 77.4, 77.2, 76.7, 76.5, 76.3, 76.1, 75.8, 75.7, 74.7, 74.4, 74.3, 73.5, 72.9, 72.7, 71.1, 70.4, 69.9, 69.8, 67.9, 57.2, 53.0, 50.0, 49.3, 48.8, 40.4, 40.3, 40.2. ESI-MS: $[M + H]^-$ $C_{155}H_{169}N_2O_{37}P$ calcd. 2681.1142 found 2681.1072.

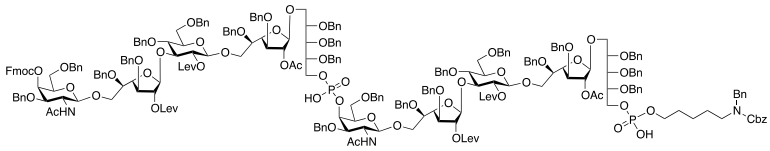
2-Acetamido-2-deoxy-3,6-di-*O*-benzyl-*β*-D-galactopyranosyl-(1→6)-2-*O*-levulinoyl-3,5-di-*O*-benzyl-*β*-D-galactofuranosyl-(1→3)-4,6-di-*O*-benzyl-2-*O*-levulinoyl-*β*-D-glucopyranosyl-(1→6)-2-*O*-acetyl-3,5-di-*O*-benzyl-*β*-D-galactofuranosyl-(1→1)-5-[5-benzyl(benzyloxycarbonyl)aminopentyl]-phosphoryl-2,3,4-tri-*O*-benzyl-D-ribose (19)



To a solution of pentasaccharide **18a** (500 mg, 0.18 mmol) in CH_2Cl_2 (10 mL) was added Et_3N (0.5 mL) and the reaction mixture was left stirring at RT overnight. The mixture was concentrated, absorbed on silica gel, and chromatographed using CH_3OH:CH_2Cl_2 (1:9) to give the desired product as a white solid in quantitative yield. (440 mg, quant). ^{1}H NMR (750 MHz, $CDCl_3$): δ 1.15 – 1.39 (4H, br m, CH_2 x 2 linker), 1.65 (3H, s, NHAc), 1.84 (3H, s, OAc), 1.93 (3H, s, OLev), 2.09 (3H, s, OLev), 2.29 – 2.60 (8H, m, C*H*H of OLev), 2.87 – 3.04 (4H, m, CH_2 x 2 linker), 3.36 – 3.44 (2H, m, H-5^{Glc}, H-2^{GalN}), 3.53 – 3.67 (7H, m, H-6^{Gal1}, H-6^{Gal2}, H-5^{Gal2}, H-5$_a^{Rib}$, H-6$_a^{GalN}$), 3.70 (1H, m, H-6$_a^{Glc}$), 3.71 (1H, m, H-5^{Gal1}), 3.74 (1H, m, H-3^{Gal1}), 3.77 (3H, m, H-5^{GalN}, H-3^{Gal2}, H^{Rib}), 3.81 (1H, m, H-6$_b^{GalN}$), 3.84 (1H, m, H-5$_b^{Rib}$), 3.87 – 3.93 (4H, m, H-3^{Glc}, H-4^{Glc}, H-1^{Rib}), 3.96 (1H, m, H-

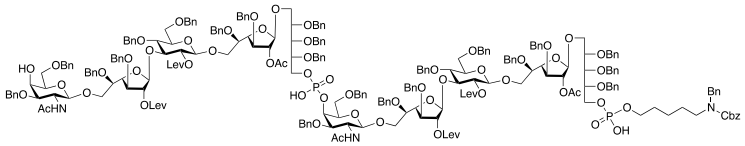
6_b^{Glc}), 3.99 (1H, m, H-4^{Gal1}), 4.11 (1H, m, H-4^{Gal2}), 4.15 – 4.22 (2H, m, H-3^{GalN}, C*H*HAr), 4.27 – 4.33 (5H, m, H-1^{Glc}, CH_2NBnCbz, CH*H*Ar x 2), 4.33 – 4.70 (m, C*H*HAr), 4.74 (1H, d, *J* = 11.6 Hz, C*H*HAr), 4.83 (1H, br d, H-1^{GalN}), 4.92 (1H, d, *J* = 11.6 Hz, C*H*HAr), 4.96 (1H, s, H-1^{Gal1}), 5.01 (1H, s, H-2^{Gal1}), 5.03 (2H, m, CH_2 of Cbz), 5.04 (1H, s, H-2^{Gal2}), 5.06 (1H, dd, *J* = 8.4, 9.4 Hz, H-2^{Glc}), 5.25 (1H, br d, NH), 5.31 (1H, s, H-1^{Gal2}), 7.03 – 7.79 (75H, m, Ar-H). ^{13}C NMR (189 MHz, $CDCl_3$) δ 108.7, 108.5, 103.5, 102.9, 85.9, 85.8, 85.7, 83.8, 80.9, 79.7, 79.5, 79.1, 77.7, 77.2, 76.7, 76.5, 76.4, 76.3, 76.1, 76.0, 75.9, 75.7, 75.5, 74.8, 74.7, 74.3, 74.2, 72.8, 71.6, 71.5, 71.4, 71.1, 69.8, 68.2, 67.7, 56.3, 52.8, 49.9, 48.8, 40.4, 40.3, 40.2. ESI-MS: $[M + H]^-$ $C_{140}H_{159}N_2O_{35}P$ calcd. 2549.0461 found 2459.0378.

Compound 20



To a solution of the phosphonate **4** (330 mg, 0.14 mmol) and alcohol **19** (295 mg, 0.12 mmol) in pyridine (5 mL) was added PivCl (88 µL, 0.72 mmol), and the reaction mixture was left stirring at that temperature for 2 h. I_2 (538 mg, 2.1 mmol) and H_2O (0.5 mL) were added, and the reaction mixture was additionally stirred at RT for 2 h. The reaction mixture was neutralized with sat. $Na_2S_2O_3$, concentrated *in vacuo*, re-dissolved in CH_2Cl_2, and washed with water. The organic phase was dried and concentrated *in vacuo* to give the residue which was further chromatographed to give the desired product as a white solid (271 mg, 46%). Purification conditions: 1) 100% CH_3CN; 2) CH_2Cl_2:CH_3CN (1:1); 3) 3:47:50 CH_3OH:CH_2Cl_2:CH_3CN; 4) CH_3OH:CH_2Cl_2 (1:9). ^{1}H NMR (750 MHz, $CDCl_3$): δ 1.15 – 1.39 (4H, br m, CH_2 x 2 linker), 1.65 (6H, s, NHAc x 2), 1.84 (6H, s, OAc x 2), 1.93 (6H, s, OLev x 2), 2.09 (6H, s, OLev x 2), 2.29 – 2.60 (16H, m, C*H*H of OLev), 2.87 – 3.04 (4H, m, CH_2 x 2 linker), 3.36 – 3.44 (4H, m, 2 x H-5^{Glc}, 2 x H-2^{GalN}), 3.53 – 3.67 (14H, m, 2 x H-6^{Gal1}, 2 x H-6^{Gal2}, 2 x H-5^{Gal2}, 2 x H-5_a^{Rib}, H-6_a^{GalN}), 3.70 (2H, m, 2 x H-6_a^{Glc}), 3.71 (2H, m, 2 x H-5^{Gal1}), 3.74 (2H, m, 2 x H-3^{Gal1}), 3.77 (6H, m, 2 x H-5^{GalN}, 2 x H-3^{Gal2}, 2 x H^{Rib}), 3.81 (2H, m, 2 x H-6_b^{GalN}), 3.84 (2H, m, 2 x H-5_b^{Rib}), 3.87 – 3.93 (8H, m, 2 x H-3^{Glc}, 2 x H-4^{Glc}, H-1^{Rib}), 3.96 (2H, m, 2 x H-6_b^{Glc}), 3.99 (2H, m, 2 x H-4^{Gal1}), 4.11 (2H, m, 2 x H-4^{Gal2}), 4.15 – 4.22 (4H, m, 2 x H-3^{GalN}, CH of Fmoc, 1 x C*H*HAr), 4.27 – 4.33 (10H, m, 2 x H-1^{Glc}, CH_2NBnCbz, CH*H*Ar x 2), 4.33 – 4.70 (m, C*H*HAr), 4.74 (1H, d, *J* = 11.6 Hz, C*H*HAr), 4.83 (2H, br d, 2 x H-1^{GalN}), 4.92 (1H, d, *J* = 11.6 Hz, C*H*HAr), 4.96 (2H, s, 2 x H-1^{Gal1}), 5.01 (2H, s, 2 x H-2^{Gal1}), 5.03 (2H, m, CH_2 of Cbz), 5.04 (2H, s, 2 x H-2^{Gal2}), 5.06 (2H, dd, 2 x H-2^{Glc}), 5.22 (2H, br d, 2 x NH), 5.31 (2H, s, 2 x H-1^{Gal2}), 5.46 (2H, s, 2 x H-4^{GalN}), 7.03 – 7.79 (128H, m, Ar-H). ^{13}C NMR (189 MHz, $CDCl_3$) δ 108.7, 108.5, 103.0, 86.1, 85.7, 83.7, 79.9, 79.5, 79.3, 79.1, 77.7, 77.5, 77.1, 76.7, 76.5, 76.3, 76.2, 76.1, 75.8, 75.7, 74.7, 74.5, 74.4, 74.3, 73.5, 72.9, 72.7, 71.1, 70.4, 69.6, 68.2, 57.2, 52.9, 52.7, 49.3, 40.3, 40.2. ESI-MS: $[M + H]^-$ $C_{275}H_{303}N_3O_{69}P_2$ calcd. 2406.4884 found 2406.4820.

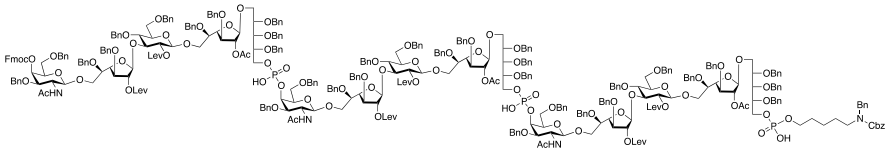
Compound 21



To a solution of decasaccharide **20** (271 mg, 0.056 mmol) in CH_2Cl_2 (5 mL) was added Et_3N (0.5 mL) and the reaction mixture was left stirring at RT overnight. The mixture was concentrated and passed through a Biogel SX1 column with a toluene:acetone (1:1) as a mobile phase giving the desired product as a white solid. (250 mg, 96%). ^{1}H NMR (750 MHz, $CDCl_3$): δ 1.15 – 1.39 (4H, br m, CH_2 x 2

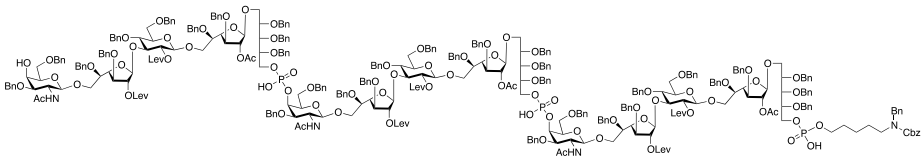
linker), 1.65 (6H, s, NHAc x 2), 1.84 (6H, s, OAc x 2), 1.93 (6H, s, OLev x 2), 2.09 (6H, s, OLev x 2), 2.29 – 2.60 (16H, m, C*H*H of OLev), 2.87 – 3.04 (4H, m, CH_2 x 2 linker), 3.36 – 3.44 (4H, m, 2 x H-5^{Glc}, 2 x H-2^{GalN}), 3.53 – 3.67 (14H, m, 2 x H-6^{Gal1}, 2 x H-6^{Gal2}, 2 x H-5^{Gal2}, 2 x H-5$_a^{Rib}$, H-6$_a^{GalN}$), 3.70 (2H, m, 2 x H-6$_a^{Glc}$), 3.71 (2H, m, 2 x H-5^{Gal1}), 3.74 (2H, m, 2 x H-3^{Gal1}), 3.77 (6H, m, 2 x H-5^{GalN}, 2 x H-3^{Gal2}, 2 x H^{Rib}), 3.81 (2H, m, 2 x H-6$_b^{GalN}$), 3.84 (2H, m, 2 x H-5$_b^{Rib}$), 3.87 – 3.93 (8H, m, 2 x H-3^{Glc}, 2 x H-4^{Glc}, H-1^{Rib}), 3.96 (2H, m, 2 x H-6$_b^{Glc}$), 3.99 (2H, m, 2 x H-4^{Gal1}), 4.11 (2H, m, 2 x H-4^{Gal2}), 4.15 – 4.22 (3H, m, 2 x H-3^{GalN}, 1 x C*H*HAr), 4.27 – 4.33 (10H, m, 2 x H-1^{Glc}, CH_2NBnCbz, CH*H*Ar x 2), 4.33 – 4.70 (m, C*H*HAr), 4.74 (1H, d, *J* = 11.6 Hz, C*H*HAr), 4.83 (2H, br d, 2 x H-1^{GalN}), 4.92 (1H, d, *J* = 11.6 Hz, C*H*HAr), 4.96 (2H, s, 2 x H-1^{Gal1}), 5.01 (2H, s, 2 x H-2^{Gal1}), 5.03 (2H, m, CH_2 of Cbz), 5.04 (2H, s, 2 x H-2^{Gal2}), 5.06 (2H, dd, 2 x H-2^{Glc}), 5.22 (2H, br d, 2 x NH), 5.31 (2H, s, 2 x H-1^{Gal2}), 5.46 (2H, s, 2 x H-4^{GalN}), 7.03 – 7.79 (120H, m, Ar-H). ^{13}C NMR (189 MHz, $CDCl_3$) δ 108.6, 108.4, 103.6, 102.9, 85.9, 85.8, 85.7, 83.9, 83.8, 81.3, 80.7, 79.6, 79.4, 79.3, 79.1, 77.7, 77.2, 76.7, 76.5, 76.4, 76.3, 76.1, 76.0, 75.7, 75.5, 75.1, 74.8, 74.7, 74.4, 74.2, 73.2, 72.8, 71.6, 71.1, 69.9, 69.8, 68.2, 68.1, 67.7, 62.2, 56.4, 55.2, 53.0, 52.8, 49.7, 48.8, 47.8, 40.4, 40.3, 40.2. ESI-MS: [M + H]$^-$ $C_{260}H_{293}N_3NaO_{67}P_2$ calcd. 2295.4544 found 2295.4433.

Compound 22



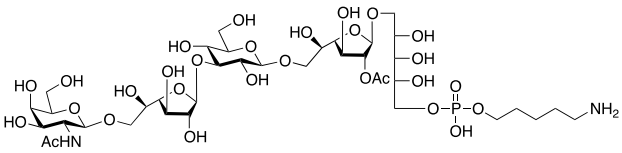
To a solution of the phosphonate **4** (110 mg, 0.047 mmol) and decasaccharide **21** (144 mg, 0.03 mmol) in pyridine (5 mL) was added PivCl (40 µL, 0.72 mmol), and the reaction mixture was left stirring at that temperature for 4 h. I_2 (80 mg, 0.313 mmol) and H_2O (0.5 mL) were added, and the reaction mixture was additionally stirred at RT for 2 h. The reaction mixture was neutralized with sat. $Na_2S_2O_3$, concentrated *in vacuo*, re-dissolved in CH_2Cl_2, and washed with water. The organic phase was dried and concentrated *in vacuo* to give the residue which was further chromatographed to give the desired product as a white solid (89 mg, 41%). Purification conditions: 1) 100% CH_3CN; 2) CH_2Cl_2:CH_3CN (1:1); 3) 2:48:50 CH_3OH:CH_2Cl_2:CH_3CN; 4) 1:4:5 CH_3OH:CH_2Cl_2:CH_3CN. ^{1}H NMR (750 MHz, $CDCl_3$): δ 1.15 – 1.39 (4H, br m, CH_2 x 2 linker), 1.65 (9H, s, NHAc x 3), 1.84 (9H, s, OAc x 3), 1.93 (9H, s, OLev x 3), 2.09 (9H, s, OLev x 3), 2.29 – 2.60 (24H, m, C*H*H of OLev), 2.87 – 3.04 (4H, m, CH_2 x 2 linker), 3.36 – 3.44 (6H, m, 3 x H-5^{Glc}, 3 x H-2^{GalN}), 3.53 – 3.67 (13H, m, 3 x H-6^{Gal1}, 3 x H-6^{Gal2}, 3 x H-5^{Gal2}, 3 x H-5$_a^{Rib}$, H-6$_a^{GalN}$), 3.70 (3H, m, 3 x H-6$_a^{Glc}$), 3.71 (3H, m, 3 x H-5^{Gal1}), 3.74 (3H, m, 3 x H-3^{Gal1}), 3.77 (9H, m, 3 x H-5^{GalN}, 3 x H-3^{Gal2}, 3 x H^{Rib}), 3.81 (3H, m, 3 x H-6$_b^{GalN}$), 3.84 (3H, m, 3 x H-5$_b^{Rib}$), 3.87 – 3.93 (7H, m, 3 x H-3^{Glc}, 3 x H-4^{Glc}, H-1^{Rib}), 3.96 (3H, m, 3 x H-6$_b^{Glc}$), 3.99 (3H, m, 3 x H-4^{Gal1}), 4.11 (3H, m, 3 x H-4^{Gal2}), 4.15 – 4.22 (5H, m, 3 x H-3^{GalN}, CH of Fmoc, 1 x C*H*HAr), 4.27 – 4.33 (16H, m, 3 x H-1^{Glc}, CH_2NBnCbz, CH*H*Ar x 2), 4.33 – 4.70 (m, C*H*HAr), 4.74 (1H, d, C*H*HAr), 4.83 (3H, br d, 3 x H-1^{GalN}), 4.92 (1H, d, C*H*HAr), 4.96 (3H, s, 3 x H-1^{Gal1}), 5.01 (3H, s, 3 x H-2^{Gal1}), 5.03 (2H, m, CH_2 of Cbz), 5.04 (3H, s, 3 x H-2^{Gal2}), 5.06 (3H, dd, 3 x H-2^{Glc}), 5.22 (3H, br d, 3 x NH), 5.31 (3H, s, 3 x H-1^{Gal2}), 5.46 (3H, s, 3 x H-4^{GalN}), 7.03 – 7.79 (183H, m, Ar-H). ^{13}C NMR (189 MHz, $CDCl_3$) δ 108.7, 108.5, 103.0, 86.1, 85.7, 83.7, 79.9, 79.5, 79.3, 79.1, 77.7, 77.5, 77.1, 76.7, 76.5, 76.3, 76.2, 76.1, 75.8, 75.7, 74.7, 74.5, 74.4, 74.3, 73.5, 72.9, 72.7, 71.1, 70.4, 69.6, 68.2, 57.2, 52.9, 52.7, 49.3, 40.3, 40.2. MALDI-MS: [M + Na]$^+$ $C_{395}H_{437}N_4NaO_{101}P_3$ calcd. 6967.8293 found 6967.1736.

Compound 23



To a solution of pentadecasaccharide **22** (89 mg, 0.013 mmol) in CH_2Cl_2 (5 mL) was added Et_3N (0.5 mL) and the reaction mixture was left stirring at RT overnight. The mixture was concentrated and passed through a Biogel SX1 column with a toluene:acetone (1:1) as a mobile phase giving the desired product as a white solid. (80 mg, 92%). ^{1}H NMR (750 MHz, $CDCl_3$): δ 1.15 – 1.39 (4H, br m, CH_2 x 2 linker), 1.65 (9H, s, NHAc x 3), 1.84 (9H, s, OAc x 3), 1.93 (9H, s, OLev x 3), 2.09 (9H, s, OLev x 3), 2.29 – 2.60 (24H, m, C*H*H of OLev), 2.87 – 3.04 (4H, m, CH_2 x 2 linker), 3.36 – 3.44 (6H, m, 3 x H-5^{Glc}, 3 x H-2^{GalN}), 3.53 – 3.67 (13H, m, 3 x H-6^{Gal1}, 3 x H-6^{Gal2}, 3 x H-5^{Gal2}, 3 x H-5$_a^{Rib}$, H-6$_a^{GalN}$), 3.70 (3H, m, 3 x H-6$_a^{Glc}$), 3.71 (3H, m, 3 x H-5^{Gal1}), 3.74 (3H, m, 3 x H-3^{Gal1}), 3.77 (9H, m, 3 x H-5^{GalN}, 3 x H-3^{Gal2}, 3 x H^{Rib}), 3.81 (3H, m, 3 x H-6$_b^{GalN}$), 3.84 (3H, m, 3 x H-5$_b^{Rib}$), 3.87 – 3.93 (7H, m, 3 x H-3^{Glc}, 3 x H-4^{Glc}, H-1^{Rib}), 3.96 (3H, m, 3 x H-6$_b^{Glc}$), 3.99 (3H, m, 3 x H-4^{Gal1}), 4.11 (3H, m, 3 x H-4^{Gal2}), 4.15 – 4.22 (4H, m, 3 x H-3^{GalN}, 1 x C*H*HAr), 4.27 – 4.33 (16H, m, 3 x H-1^{Glc}, CH_2NBnCbz, CH*H*Ar x 2), 4.33 – 4.70 (m, C*H*HAr), 4.74 (1H, d, C*H*HAr), 4.83 (3H, br d, 3 x H-1^{GalN}), 4.92 (1H, d, C*H*HAr), 4.96 (3H, s, 3 x H-1^{Gal1}), 5.01 (3H, s, 3 x H-2^{Gal1}), 5.03 (2H, m, CH_2 of Cbz), 5.04 (3H, s, 3 x H-2^{Gal2}), 5.06 (3H, dd, 3 x H-2^{Glc}), 5.22 (3H, br d, 3 x NH), 5.31 (3H, s, 3 x H-1^{Gal2}), 5.46 (3H, s, 3 x H-4^{GalN}), 7.03 – 7.79 (175H, m, Ar-H). ^{13}C NMR (189 MHz, $CDCl_3$) δ 108.6, 108.5, 103.6, 102.9, 85.9, 85.8, 85.8, 85.7, 83.8, 79.8, 79.7, 79.6, 79.5, 79.5, 79.1, 77.7, 77.2, 77.2, 76.7, 76.5, 76.5, 76.3, 76.1, 76.1, 75.7, 75.5, 74.7, 74.7, 74.3, 74.2, 74.2, 72.8, 72.8, 71.6, 71.5, 69.6, 68.2, 56.4, 52.7, 40.4, 40.3, 40. ESI-MS: $[M + H]^-$ $C_{380}H_{427}N_4O_{99}P_3$ calcd. 2240.9328 found 2240.8775.

Compound 1



Pentasaccharide **19** (120 mg, 0.048 mmol) was dissolved in CH_2Cl_2:CH_3OH (1:1, 10 mL), after which solid N_2H_4*HOAc (120 mg) and the reaction mixture was left stirring at RT overnight. The mixture was concentrated *in vacuo*, and passed through Biogel SX1. This material was dissolved in CH_3OH:H_2O (4:1, 10 mL), after which AcOH (100 µL) was added, followed by the addition of the Degussa type $Pd(OH)_2$ (20% w/w) (50 mg). The reaction mixture was then hydrogenated overnight, filtered through Celite, and concentrated to afford the target pentasaccharide as a white solid. (40 mg, 78% over two steps). ESI-MS: $[M + H]^-$ $C_{38}H_{69}N_2O_{29}P$ calcd. 1048.3724 found 1048.3716.

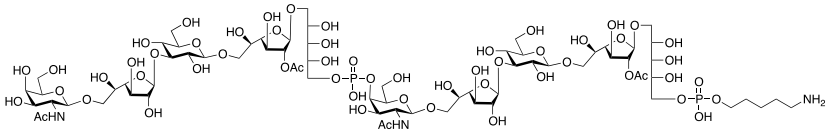
^{1}H (750 MHz, D_2O): δ (ppm)

	H-1	H-2	H-3	H-4	H-5	H-6
Rib	3.84/3.74	4.03	3.78	3.94	4.14	n/a
Gal1	5.19	4.97, d, *J* = 2.05Hz	4.26 dd, *J* = 5.6, 1.9 Hz	4.10	3.80	n/a
Glc	4.56 d, *J* = 8.4 Hz	3.47	3.66	3.49	3.50	n/a
Gal2	5.29 d, *J* = 1.9 Hz	4.18	4.08	3.95	3.72	n/a
GalN	4.50 d, *J* = 8.8 Hz	3.92	3.75	n/a	n/a	n/a

^{13}C (187 MHz, D_2O): δ (ppm)

	C-1	C-2	C-3	C-4	C-5	C-6
Rib	68.3	70.2	71.5	69.5	67.2	n/a
Gal1	105.3	83.5	75.5	83.4	71.3	n/a
Glc	105.8	73.6	82.6	68.5	76.0	n/a
Gal2	108.6	81.4	76.4	84.0	71.3	n/a
GalN	105.5	55.8	n/a	n/a	n/a	n/a

Compound 2



Decasaccharide **21** (100 mg, 0.021 mmol) was dissolved in CH_2Cl_2:CH_3OH (1:1, 5 mL), after which solid N_2H_4*HOAc (100 mg) and the reaction mixture was left stirring at RT overnight. The mixture was concentrated *in vacuo*, and passed through Biogel SX1. This material was dissolved in CH_3OH:H_2O (4:1, 10 mL), after which AcOH (100 µL) was added, followed by the addition of the Degussa type $Pd(OH)_2$ (20% w/w) (50 mg). The reaction mixture was then hydrogenated overnight, filtered through Celite, and concentrated to afford the target Pentasaccharide as a white solid. (34 mg, 82% over two steps). ESI-MS: $[M + H]^-$ $C_{71}H_{125}N_3O_{57}P_2$ calcd. 996.8225 found 996.8202.

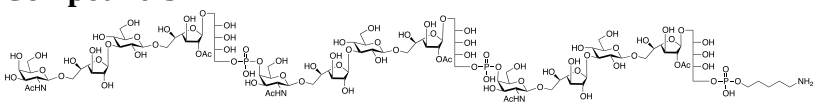
^{1}H (750 MHz, D_2O): δ (ppm)

	H-1	H-2	H-3	H-4	H-5	H-6
Rib	3.84/3.74	4.03	3.78	3.94	4.14	n/a
Gal1	5.19	4.97	4.25	4.10	3.80	n/a
Glc	4.56 d, *J* = 7.9 Hz	3.47	3.66	3.49	3.50	n/a
Gal2	5.29	4.18	4.08	3.95	3.72	n/a
GalN-1	4.56	3.97	3.85	4.52 dd, *J* = 9.9, 3.8 Hz	n/a	n/a
GalN-2	4.50 d, *J* = 8.4 Hz	3.92	3.75	n/a	n/a	n/a

^{13}C (187 MHz, D_2O): δ (ppm)

	C-1	C-2	C-3	C-4	C-5	C-6
Rib	68.3	70.2	71.5	69.5	67.2	n/a
Gal1	105.3	83.5	75.5	83.4	71.3	n/a
Glc	105.8	73.6	82.6	68.5	76.0	n/a
Gal2	108.6	81.4	76.4	84.0	71.3	n/a
GalN	105.5	55.8	n/a	n/a	n/a	n/a
GalN-2	105.7	55.8	n/a	76.2	n/a	n/a

Compound 3



Pentadecasaccharide **23** (131 mg, 0.019 mmol) was dissolved in CH_2Cl_2:CH_3OH (1:1, 5 mL), after which solid N_2H_4*HOAc (100 mg) and the reaction mixture was left stirring at RT overnight. The mixture was concentrated *in vacuo*, and passed through Biogel SX1. This material was dissolved in CH_3OH:H_2O (4:1, 10 mL), after which AcOH (100 µL) was added, followed by the addition of the Degussa type $Pd(OH)_2$ (20% w/w) (50 mg). The reaction mixture was then hydrogenated overnight, filtered through Celite, and concentrated to afford the target pentasaccharide as a white solid. (34 mg, 82% over two steps). ESI-MS: $[M + H]^-$ $C_{104}H_{181}N_4O_{85}P_3$ calcd. 979.6392 found 979.6513.

^{1}H (750 MHz, D_2O): δ (ppm)

	H-1	H-2	H-3	H-4	H-5	H-6
Rib	3.84/3.74	4.03	3.78	3.94	4.14	n/a
Gal1	5.19	4.97	4.25	4.10	3.80	n/a
Glc	4.56 d, *J* = 7.9 Hz	3.47	3.66	3.49	3.50	n/a
Gal2	5.29	4.18	4.08	3.95	3.72	n/a
GalN-1	4.56	3.97	3.85	4.52 dd, *J* = 9.9, 3.8 Hz	n/a	n/a
GalN-2	4.56	3.97	3.85	4.52 dd, *J* = 9.9, 3.8 Hz	n/a	n/a
GalN-3	4.50 *J* = 8.4 Hz	3.92	3.75	n/a	n/a	n/a

^{13}C (187 MHz, D_2O): δ (ppm)

	C-1	C-2	C-3	C-4	C-5	C-6
Rib	68.3	70.2	71.5	69.5	67.2	n/a
Gal1	105.3	83.5	75.5	83.4	71.3	n/a
Glc	105.8	73.6	82.6	68.5	76.0	n/a
Gal2	108.6	81.4	76.4	84.0	71.3	n/a
GalN-1	105.7	55.8	n/a	76.2	n/a	n/a
GalN-2	105.7	55.8	n/a	76.2	n/a	n/a
GalN-3	105.5	55.8	n/a	n/a	n/a	n/a

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Chapter 6

Summary and Concluding Remarks

Chapter 1 introduces the reader to the synthesis of complex carbohydrates, which is one of the most vigorous and challenging disciplines for organic chemists to excel at. Delivery of these highly sophisticated molecules enabled by new advances in stereoselective construction of glycosidic bonds is central to chemical biology, drug discovery, and vaccinology endeavours. Among the most exciting aspects of oligosaccharide synthesis in the last few decades has been the interplay between traditional chemical synthesis methodologies, use of enzymes, and solid phase automation technologies. It is hoped, that through this work, the reader would gain a better understanding of how these subdisciplines advance and complement each other in new directions providing access to molecules otherwise inaccessible previously.

Despite the field's great achievements, however, full control of stereochemistry of the glycosylation reaction is difficult. Poor glycosylation efficiency and routine use of protecting groups are obstacles to step-efficient delivery of complex glycans, which can fortunately be addressed by use of glycosyltransferases. Even then, not each and every oligosaccharide can be synthesized at will. Experience shows that only mammalian glycans can be chemoenzymatically prepared on a scale useful for biological applications. It is further shown that implementation of temporary protecting groups in oligosaccharides can modulate the activity of glycosyltransferases so that they extend only specific parts of a complex molecule. In contrast, availability of synthetically useful enzymes to make bacterial oligosaccharides is still scarce, and for this reason components of capsular polysaccharides are most commonly prepared by chemical means, which is very tedious. Presence of directly inaccessible rare sugars complicates the overall assembly route far from making it as efficient as its peptide and phosphate forming bond counterparts. Before we can enjoy the rapid and widespread

construction of any desired oligosaccharide, new solutions should be implemented to fill current gaps.

In **Chapter 2** a chemoenzymatic methodology is described that makes it possible, for the first time, to prepare any bi-, tri-, and tetra-antennary asymmetric *N*-glycan from a single precursor. It is based on the chemical synthesis of a tetra-antennary glycan that has *N*-acetylglucosamine (GlcNAc), *N*-acetyllactosamine (LacNAc), and unnatural Galα(1,4)-GlcNAc and Manβ(1,4)-GlcNAc appendages. Mammalian glycosyltransferases recognize only the terminal LacNAc moiety as a substrate and thus this structure can be uniquely extended. Next, the β-GlcNAc terminating antenna can be converted into LacNAc by galactosylation and can then be enzymatically modified into a complex structure. The unnatural α-Gal and β-Man terminating antennae can sequentially be de-caged by an appropriate glycosidase to liberate a terminal β-GlcNAc moiety, which can be converted into LacNAc and then elaborated by a panel of glycosylransferases. Asymmetric bi- and tri-antennary glycans could be obtained by removal of a terminal β-GlcNAc moiety by treatment with β-*N*-acetylglucosaminidase and selective extension of the other arms. The power of the methodology is demonstrated by the preparation of an asymmetric tetra-antennary *N*-glycan found in human breast carcinoma tissue, which represents the most complex *N*-glycan ever synthesized. Multistage mass spectrometry of the two isomeric tri-antennary glycans uncovered unique fragment ions that will facilitate identification of exact structures of glycans in biological samples.

Strategies employed to chemoenzymatically synthesize asymmetric *N*-glycans were further elaborated in **Chapter 3** to manipulate various fucosyltransferases. Fucosylated glycans play key roles in many biological processes such as immune regulation, selectin-mediated leukocyte recruitment, host-microbe interactions, and blood transfusion reactions. Although chemical and enzymatic procedures have been developed to prepare fucosylated epitopes, such as Lewis and blood group antigens, the synthesis of complex fucosylated oligo-*N*-acetyllactosamine has received little attention. In particular, there are no general solutions to selectively incorporate fucosides and other functionalities into these oligosaccharide backbones. The lack of such compounds has hampered elucidation by which the pattern of fucosylation influences binding of glycan binding proteins. Here, we describe a chemoenzymatic strategy that can give a library of differentially fucosylated and sialylated oligosaccharides starting from a single chemically synthesized tri-*N*-acetyllactosamine derivative. It is based on the finding that lactosamine derivatives that have a free amine or a tert-butyloxycarbonyl (Boc) protecting group at the amino function, are resistant to fucosylation by recombinant fucosyl transferases. To exploit this observation, we prepared from a single hexasaccharide precursor six different hexasaccharides in which the glucosamine moieties of LacNAc are either acetylated (GlcNAc) or modified as a free amine ($GlcNH_2$) or Boc (GlcNHBoc). Fucosylation of the resulting compounds by recombinant fucosyl transferases FUT5 and Hp39-FT resulted in only modification of the natural GlcNAc moieties providing access to six selectively mono- and bis-fucosylated oligosaccharides. Next, the $GlcNH_2$ or GlcNHBoc moieties were converted into the natural GlcNAc counterparts. The resulting compounds could be further diversified by sialylation by ST3Gal4 or PmST1 M144D to give twelve different compounds. The library of differentially fucosylated and sialylated oligosaccharides were printed to give a glycan microarray which was probed by a panel of plant lectins and a set of mammalian glycan binding and viral proteins including E-selectin, DC-SIGN, and influenza A virus (IAV) hemagglutinins (HAs). These studies demonstrated that the pattern of fucosylation can modulate protein binding and uncovered that the antigen VIM-2 is not recognized by E-selectin.

Remainder of this thesis is dedicated to chemical assembly of bacterial oligosaccharides. Therefore, **Chapter 4** focuses on the chemical synthesis of an orthogonally protected trisaccharide derived from the polysaccharide of *Staphylococcus aureus* Type 5, which is an attractive candidate for the development of immunotherapies. The challenging α-fucosylation and β-mannosylation are addressed through the careful choice of protecting groups. Lactamization of a β-D-Man*p*NAc moiety during deprotection was avoided by a late stage oxidation approach. Versatility of the trisaccharide was demonstrated by its transformation into a spacer-containing repeating unit suitable for immunological investigations.

Finally, synthesis of *Streptococcus pneumoniae* 35B capsular polysaccharide (CPS) oligomers is described in **Chapter 5**. Protein-CPS conjugate vaccines have been highly successful in reducing the incidence of pneumococcal infections caused by *S. pneumoniae.* Increased use of two licensed CPS vaccines PCV7 and PCV13 resulted in emergence of a serotype 35B, antigens of which are not included in any current formulations. This invasive serotype has recently become the most common clinical isolate, and accounts for 90% of all infections. Alerted by the urgent need to include serotype 35B antigens in current vaccines, we have developed a scalable approach which successfully delivers well-defined synthetic polysaccharide fragments ready for conjugation and immediate clinical use. This discovery is based on a “hybrid” DNA/peptide synthetic route producing oligosaccharides up to a pentadecamer (15 sugar units). Conjugates of these antigens are currently employed in immunization studies in rabbits and results will be reported in the due course.

Nederlandse samenvatting

Hoofdstuk 1 laat de lezer kennismaken met de synthese van complexe koolhydraten, een van de krachtigste en meest uitdagende disciplines voor organische chemici om inuit te blinken. Aflevering van deze zeer geavanceerde moleculen die mogelijk worden gemaakt door nieuwe ontwikkelingen in de stereoselectieve constructie van glycosidebindingen staat centraal in chemische biologie, geneesmiddelenontdekking en vaccinologie-inspanningen. Een van de meest opwindende aspecten van oligosacharidesynthese in de afgelopen decennia is de wisselwerking tussen traditionele chemische synthesemethoden, het gebruik van enzymen en automatiseringstechnologieën in vaste fase. Het is te hopen dat de lezer door dit werk een beter begrip zou krijgen van hoe deze subdisciplines elkaar voortbewegen en aanvullen in nieuwe richtingen, die toegang bieden tot moleculen, die voorheen ontoegankelijk waren.

In **hoofdstuk 2** wordt een chemoenzymatische methode beschreven, die het voor het eerst mogelijk maakt om elke bi-, tri- en tetra-antennaire asymmetrische *N*-glycanen te bereiden uit een enkele voorloper. Het is gebaseerd op de chemische synthese van een tetra-antennaire glycan met *N*-acetylglucosamine (GlcNAc), *N*-acetyllactosamine (LacNAc) en onnatuurlijke Gal-α(1,4)-GlcNAc en Man-β-(1,4)-GlcNAc aanhangsels. Glycosyltransferasen van zoogdieren herkennen alleen het terminale LacNAc-deel als een substraat en dus kan deze structuur op unieke wijze worden uitgebreid. Vervolgens kan de β-GlcNAc-eindantenne worden omgezet in LacNAc door het galactosyleren en vervolgens enzymatisch gemodificeerd worden in een complexe structuur. De onnatuurlijke α-Gal en β-Man eindigende antennes kunnen achtereenvolgens worden verwijderd door een geschikte glycosidase om een terminale β-GlcNAc-eenheid vrij te maken, die kan worden omgezet in LacNAc en vervolgens uitgewerkt door een panel van glycosyltransferasen. Asymmetrische bi- en tri-antennaire glycanen kunnen worden verkregen door verwijdering van een terminale β-GlcNAc-eenheid door behandeling met β-*N*-acetylglucosaminidase en selectieve verlenging van de andere armen. De kracht van de methodologie wordt aangetoond door de bereiding van een asymmetrische tetra-antennaire *N*-glycan, die wordt gevonden in menselijk borstcarcinoomweefsel, dat de meest complexe *N*-glycan vertegenwoordigt, die ooit is gesynthetiseerd. Meertraps massaspectrometrie van de twee isomere tri-antennaire glycanen onthulde unieke fragmentionen die identificatie van exacte structuren van glycanen in biologische monsters zullen vergemakkelijken.

Strategieën gebruikt om asymmetrische *N*-glycanen chemo-enzymatisch te syntheseren werden in **Hoofdstuk 3** verder uitgewerkt om verschillende fucosyltransferasen te manipuleren. Gefucosyleerde glycanen spelen een sleutelrol in veel biologische processen, zoals immuunregulatie, selectine-gemedieerde leukocytenwerving, gastheer-microbe-interacties en bloedtransfusiereacties. Hoewel chemische en enzymatische procedures zijn ontwikkeld om gefucosyleerde epitopen te bereiden, zoals Lewis en bloedgroepantigenen, heeft de synthese van complexe gefucosyleerde oligo-*N*-acetyllactosamine weinig aandacht gekregen. In het bijzonder zijn er geen algemene oplossingen om selectief fucosiden en andere functionaliteiten in deze botten van oligosacharide op te nemen. Het ontbreken van dergelijke verbindingen heeft de opheldering bemoeilijkt waardoor het patroon van het fucosyleren de binding van glycan-bindende eiwitten beïnvloedt. Hier beschrijven we een chemo-enzymatische strategie die een bibliotheek van differentieel gefucosyleerde en gesialyleerde oligosacchariden kan opleveren, uitgaande van een enkel chemisch gesynthetiseerd tri-N-acetyllactosamine-derivaat. Het is gebaseerd op de bevinding dat lactosaminederivaten die een vrije amine of een tert-butyloxycarbonyl (Boc) beschermende groep bij de aminofunctie

hebben, resistent zijn tegen fucosylering door recombinante fucosyltransferasen. Om deze waarneming te benutten, hebben we uit een enkele hexasacharidevoorloper zes verschillende hexasachariden bereid waarin de glucosaminedelen van LacNAc ofwel geacetyleerd (GlcNAc) of gemodificeerd als een vrije amine (GlcNH2) of Boc (GlcNHBoc) zijn. Fucosylering van de resulterende verbindingen door recombinante fucosyltransferasen FUT5 en Hp39-FT resulteerde in alleen modificatie van de natuurlijke GlcNAc-eenheden die toegang verschaffen tot zes selectief mono- en bis-gefucosyleerde oligosacchariden. Vervolgens werden de GlcNH2- of GlcNHBoc-eenheden omgezet in de natuurlijke GlcNAc-tegenhangers. De resulterende verbindingen zouden verder kunnen worden gediversifieerd door sialylering door ST3Gal4 of PmST1 M144D om twaalf verschillende verbindingen te geven. De bibliotheek van differentieel gefucosyleerde en gesialyleerde oligosachariden werd geprint om een glycan-microarray te geven die werd gesondeerd door een panel van plantlectines en een set zoogdierlijke glycanbinding en virale eiwitten waaronder E-selectine, DC-SIGN en influenza A-virus (IAV) hemagglutinines (HA's). Deze studies toonden aan dat het patroon van fucosylering eiwitbinding kan moduleren en ontdekte dat het antigeen VIM-2 niet wordt herkend door E-selectine.

De rest van dit proefschrift is gewijd aan de chemische assemblage van bacteriële oligosachariden. Daarom richt **hoofdstuk 4** zich op de chemische synthese van een orthogonaal beschermd trisaccharide afgeleid van de polysaccharide van *Staphylococcus aureus* Type 5, dat een aantrekkelijke kandidaat is voor de ontwikkeling van immunotherapieën. De uitdagende α-fucosylatie en β-mannosylatie worden aangepakt door de zorgvuldige keuze van beschermende groepen. Lactamisatie van een β-D-ManpNAc-deel tijdens ontscherming werd vermeden door een oxidatiebenadering in de laatste stap. Veelzijdigheid van het trisaccharide werd aangetoond door zijn transformatie in een spacer bevattende herhalende eenheid die geschikt is voor immunologisch onderzoek.

Tenslotte wordt de synthese van *Streptococcus pneumoniae* 35B capsulaire polysacharide (CPS) oligomeren beschreven in **Hoofdstuk 5**. Eiwit-CPS-geconjugeerde vaccins zijn zeer succesvol geweest bij het verminderen van de incidentie van pneumokokkeninfecties veroorzaakt door *S. pneumoniae*. Toenemend gebruik van twee gelicentieerde CPS-vaccins PCV7 en PCV13 resulteerde in de opkomst van een serotype 35B, waarvan antigenen niet zijn opgenomen in huidige formuleringen. Dit invasieve serotype is onlangs het meest voorkomende klinische isolaat geworden en is verantwoordelijk voor 90% van alle infecties. Gealarmeerd door de dringende noodzaak om serotype 35B-antigenen in huidige vaccins op te nemen, hebben we een schaalbare benadering ontwikkeld die met succes goed gedefinieerde synthetische polysaccharidefragmenten levert klaar voor conjugatie en onmiddellijk klinisch gebruik. Deze ontdekking is gebaseerd op een "hybride" synthetische DNA / peptide-route die oligosachariden produceert tot een pentadecamer (15 suikereenheden). Conjugaten van deze antigenen worden momenteel gebruikt in immunisatiestudies bij konijnen en de resultaten zullen te zijner tijd worden gerapporteerd.

List of Publications

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10. Gagarinov, I.A., A.D. Srivastava, G.J. Boons, and Z. Wang. 2017. A multigram synthesis of phenyl 2-azido-3-*O*-benzyl-2-deoxy-4,6-*O*-benzylidene-1-thio-*α*-D-mannopyranoside (Chapter 21). In: *Carbohydrate Chemistry: Proven Synthetic Methods*, Volume 4 (C. Vogel and P. Murphy, eds). CRC Press, Taylor & Francis Group, Boca Raton, FL, Chapter 21.

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