Chemo-enzymatic Synthesis of *N*-glycans of *Schistosoma mansoni* and their Molecular Interactions with Glycan Binding Proteins

Chemo-enzymatische Synthese van *N*-glycanen van *Schistosoma mansoni* en hun Moleculaire Interacties met Glycaan Bindende Eiwitten
(met een samenvatting in het Nederlands)

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List of Abbreviations

AAL	*Aleuria Aurantia* Lectin
Ac	Acetyl
ACN	Acetonitrile
Alloc	Allyloxycarbonyl
Asn	Asparagine
Bn	Benzyl
Bu	Butyl
CAN	Ceric ammonium nitrate
CIAP	Calf intestinal alkaline phosphatase
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DCM	Dichloromethane
DC-SIGN	Dendritic Cell-Specific Intercellular adhesion molecule-3-Grabbing Non-integrin
DDQ	2,3-Dichloro-5,6-dicyano-1,4-benzoquinone
DIPEA	*N,N*-Diisopropylethylamine
DMAP	4-Dimethylaminopyridine
DMF	Dimethylformamide
DPS	Diphenyl sulfoxide
DTBMP	2,6-Di-*tert*-butyl-4-methylpyridine
ECL	*Erythrina cristagalli* Lectin
EDCI	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
ESI-TOF	Electrospray Ionizaiton – Time of Flight
Et	Ethyl
Fmoc	Fluorenylmethyloxycarbonyl
Fut	Fucosyltransferase
Gal	Galactose
Gal-T	Galactosyltransferase
GalNAc	*N*-acetyl-D-galactosamine
GBP	Glycan Binding Protein
GDP-Fuc	6-Deoxy-β-L-galactopyranosylguanosine-5'-diphosphate
Glc	Glucose
GlcNAc	*N*-acetyl-D-glucosamine
Gn-T	*N*-acetyl-D-glucosaminyltransferase
GPI	Glycosylphosphatidylinisotol
HEPES	2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid
HILIC	Hydrophilic Interaction Liquid Chromatography
HPLC	High Performance Liquid Chromatography

HRMS	High Resolution Mass Spectrometry
ICAM-1	Intercellular Adhesion Molecule-1
Lev	Levulinoyl
LLO	Lipid Linked Oligosaccharide
MALDI-TOF	Matrix - Assisted Laser Desorption Ionization – Time of Flight
Me	Methyl
Ms	Methane sulfonyl
Nap	2-methylnaphthyl
NIS	*N*-iodosuccinimide
NPhth	*N*-Phthaloyl
NHS	*N*-Hydroxysuccinimide
OMP	4-methoxyphenyl
PMB	4-methoxybenzyl
Py	Pyridine
RT	Room Temperature
Ser	Serine
TBAB	Tetrabutylammonium bromide
TBAF	Tetrabutylammonium fluoride
TBS	(*t*-Butyl)dimethylsilyl
TDS	(Dimethyl)thexylsilyl
TfOH	Trifluoromethanesulfonic acid
TFA	Trifluroacetic acid
THF	Tetrahydrofuran
Thr	Threonine
TLC	Thin Layer Chromatography
TMSOTf	Trimethylsilyl trifluoromethanesulfonate
Tol	Toluene
Tos	4-Toluenesulfonyl
Troc	2,2,2-Trichloroethoxycarbonyl chloride
TTBP	2,4,6-Tri-*t*-butyl-pyrimidine
UDP	Uridine diphosphate
WGA	Wheat germ agglutinin
Xyl-T	D-Xylose transferase

CHAPTER 1

General Introduction

Cell glycans are recognized by a multitude of glycan binding proteins (GBPs or lectins) thereby mediating many biological functions.[1] Almost all cell surface and secreted proteins are glycosylated. The structures of glycans are not hardwired in the genome, instead, their occurrence is dependent on the action of enzymes, their localization and the availability of sugar nucleotide donors. To examine the glycome is challenging and depends on the isolation of glycans from natural sources, which often yields small quantities of heterogenous mixtures that are difficult to analyze and use in biophysical studies. There is a huge opportunity to exploit the field of synthesis of glycans to provide analytical standards and to study these biomolecules at a molecular level via glycan-lectin interactions, micro-array binding studies, STD-NMR, molecular modeling and other biophysical studies

Glyco-conjugates have been classified according to the type of linkage between a glycan and protein or lipid (Figure 1).[2] Broadly, the major types of glycosylations found across various species are glycoproteins including: *N*-glycans (covalently linked to the side chain amide of an asparagine residue) and *O*-glycans (linked to the hydroxyl group of serine, threonine or tyrosine residue), proteoglycans (glycosaminoglycan or GAG chains linked to serine residue), glycosphingolipids (linked to the hydroxyl group of a ceramide lipid moiety), and glycosylphosphatidylinisotol (GPI) anchored proteins (linked to phosphodiester). Glycans are attached to proteins through co-translational or post-translational mechanisms by the action of nucleotide sugar donors and glycosyltransferases or glycosidases. Protein glycosylation is important in all aspects of the biological functioning of a living organism, and any defects in glycosylations can cause mis-functioning of these biological processes or even death.

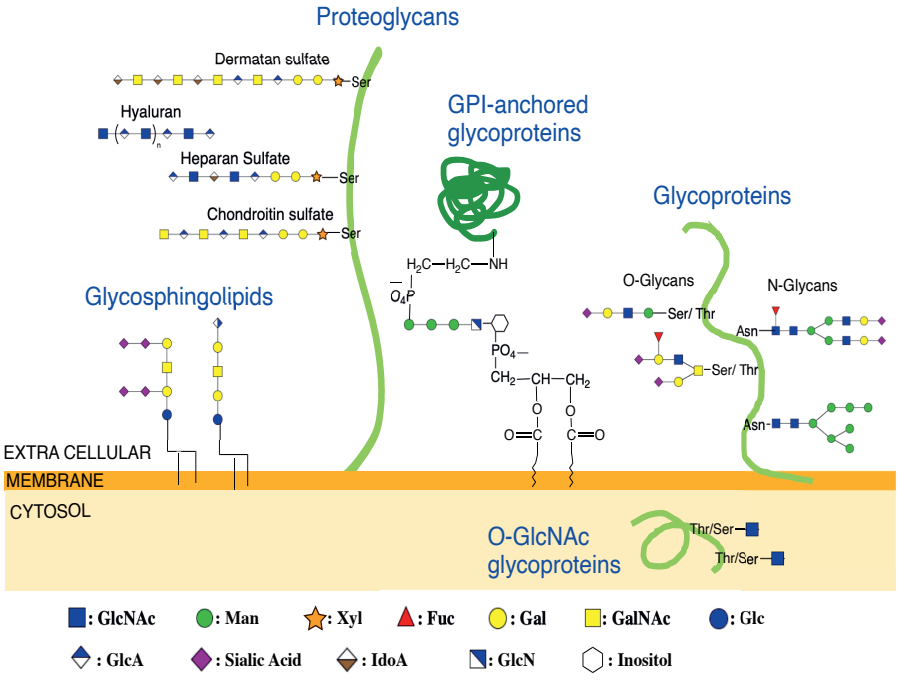


Figure 1. Types of Glycans present on cell surface of mammals.

Glycoproteins. *N*-linked and *O*-linked glycosylations are one of the most abundant type of post-translational modifications found in nature (Figure 2). Glycoproteins play important roles in various biological functions such as cell-cell communication, fertilization and proper protein folding[3] as described further along in this thesis. Apart from *N*- and *O*- glycans, glycoproteins also display a rare *C*-linked glycosylation in which the anomeric carbon of a mannosyl (Man) moiety is attached to the aromatic C-2 on the tryptophan (Trp) side chain. *C*-glycans are found in proteins like thrombospodin, RNAse2, interleukin-12, etc.[4]

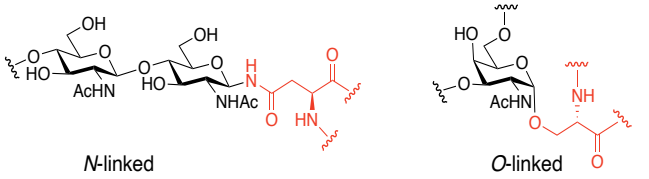


Figure 2. Structures of *N*-linked and *O*-linked glycoproteins.

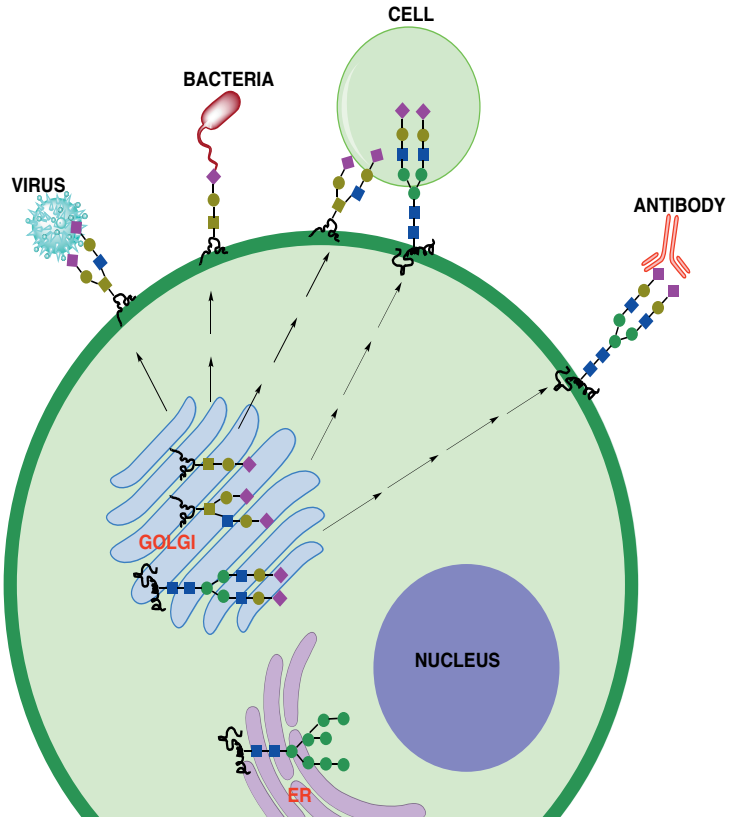


Figure 3. Interactions between cell surface glycans and receptors.

Biological significance of Glycans. The cells of living organisms comprise of a thick layer of oligosaccharides attached to underlying proteins or lipids, coating the surface of cells. This layer of glycans is called the "glycocalyx" and the first indication of its existence dates back to early 1940s when evidence of an "extraneous coating of organic material on the plasma membrane of invertebrate eggs" was reported.[5] Further studies indicated that glycans were responsible for interactions between the cell and its immediate environment including a number of glycan binding proteins[6] (Figure 3). Further, glycans are important for communicating with many proteins or lectins comprising of enzymes or antibodies and also with bacterial and viral antigens.[7] Glycan - proteins interactions have been implicated in cell-cell communication, fertilization, development, differentiation, apoptosis, immune-modulation, cell-signaling, protein folding and expulsion of mis-folded proteins, among others.[8] Abnormalities in glycosylation can lead to tumorigenesis, metastasis, neurological disorders, inflammation, congenital and auto-immune diseases.[9] Apart from glycan-lectin interactions, the study of glycan-glycan interactions are now also deemed

highly important to open new avenues for studying biological mechanisms. Individual glycan interactions are often too weak for any consequential biological relevance, however, their arrangement into glycoclusters and superstructures leads to higher avidity of binding, thus increasing the affinity mani-fold.[10] Examples include the aggregation of cells, signal transduction in melanoma and cell adhesion.[11] Further, there is an abundance of evidence suggesting the importance of glycans in pathogen recognition and development of innate immune responses. Improper or altered *N*-glycosylation is implicated in tumor growth, cancer and auto-immune diseases. Altered protein glycosylation also serves as a biomarker for diseases such as inflammation and cancer and the study of the glycan architectural assembly becomes imperative to understand such phenomena. For eg., increased sialylation and fucosylation to form Lewis^x, sialyl Lewis^x and poly-sialylated epitopes have been observed in colorectal cancer and lung cancer.[12] Hyper-expression of the *N*-acetylglucosamine (GlcNAc) moiety at β-1,6 branch, brought about by *N*-acetylglucosaminyltransferase V (GnT-V) are indicators of gastric carcinogenesis.[13]

Biosynthesis of *N*-glycans. *N*-glycans are the oligosaccharides covalently linked to the side chain of asparagine that are part of an asparagine-X-serine/threonine sequon, where X is any amino acid except for Proline. *N*-glycosylation has a very well-defined consensus sequence and *N*-glycans are probably the most complex and ubiquitous form of glycoprotein found across all domains of life.[14] The biosynthesis of *N*-glycans starts on the cytoplasmic face of Endoplasmic Reticulum (ER) and is facilitated by several glycosyltransferases and endoglycosidases enzymes. The first step in the biosynthesis is the transfer of phosphorylated *N*-acetylglucosamine (GlcNAc) to the dolichol phosphate (P-Dol) moiety.[15] The product of this reaction, called the dolichol pyrophosphate *N*-acetylglucosamine (GlcNAc-P-P-Dol), is then elongated by an additional GlcNAc, and five mannose (Man) moieties to generate the $Man_5GlcNAc_2$-P-P-Dol oligosaccharide moiety. The enzyme "flippase" translocates this $Man_5GlcNAc_2$-P-P-Dol across the lumen side of the ER where it is further elongated by four mannose and three glucose (Glc) residues. This fourteen sugar oligosaccharide $Glc_3Man_9GlcNAc_2$-P-P-Dol is then transferred *en bloc* from the dolichol precursor to the asparagine of an Asn-X-Ser/Thr peptide sequon by the enzyme oligosaccharyl transferase (OST).[16] The oligosaccharide is then further diversified by trimming and maturation in the Endoplasmic Reticulum (ER) by action of various enzymes to generate characteristic *N*-glycans. This entire process is important for the proper folding of glycoproteins (Figure 4). The proper folding of glycoproteins is mediated by calnexin and calreticulin. Mis-folded proteins are transferred to proteasome for degradation and expulsion from the ER.[17]

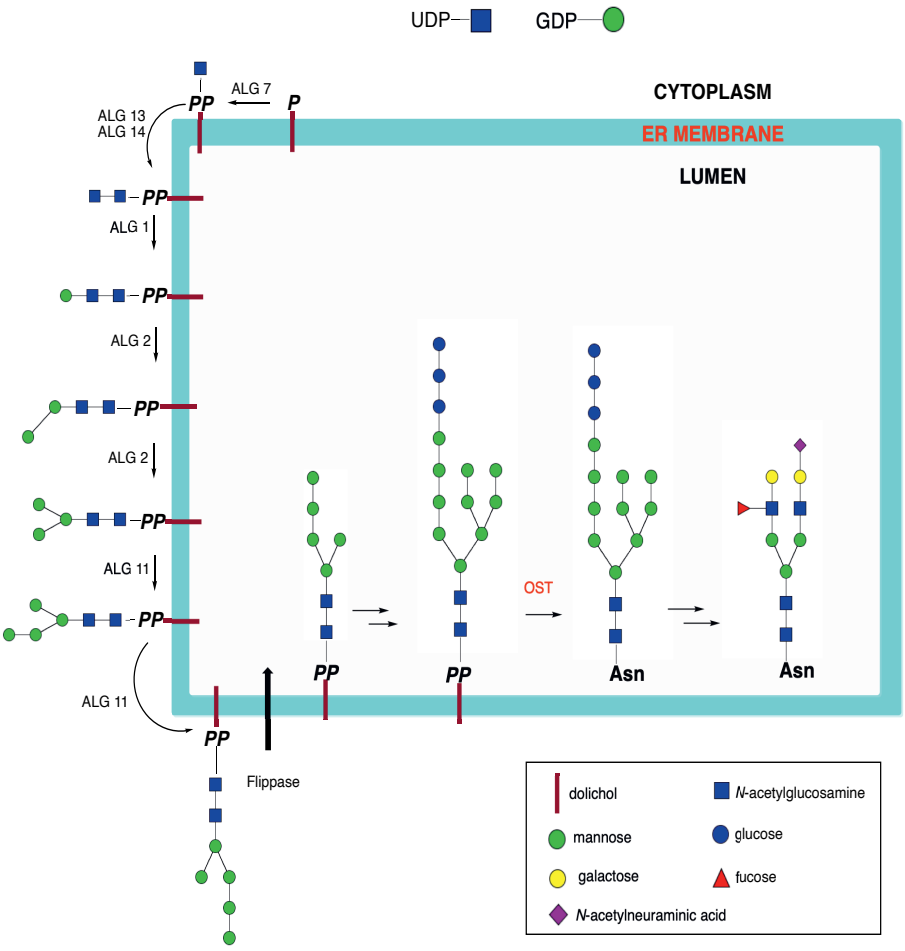


Figure 4. Biosynthesis of *N*-glycans in eukaryotes.

Properly folded glycoproteins are transferred to the *cis*-Golgi, where they undergo further trimming and maturation by a panel of glycosidases and glycosyltransferases to generate three types of *N*-glycans, which share a common $Man_3GlcNAc_2$ core. The first and also the simplest form of *N*-glycan is the High Mannose type, in which the $Man_3GlcNAc_2$ core is extended by six α-mannoside moieties. The second is the complex type of *N*-glycan in which the α3 and α6 mannosides of the core are extended by two to four GlcNAc residues, to generate bi-antennary, tri-antennary or tetra-antennary glycans. These GlcNAc residues are generally extended by

galactose (Gal) or *N*-acetylgalactosamine (GalNAc) moieties, which are further modified with motifs like *N*-acetylneuraminic acid (NeuAc) and fucose (Fuc). In mammals, the complex type *N*-glycan core is usually modified with the reducing end GlcNAc moiety containing an α6 linked fucoside. Another variation of the complex type *N*-glycan is when an extra GlcNAc moiety caps the central β-mannoside of the core, in effect "bisecting" the glycan in the middle, hence aptly named the *Bisecting Complex* type *N*-glycan. The third type of *N*-glycan can be described as a combination of the previous two types and is referred to as the hybrid *N*-glycan. In this case, the α3 mannose of the core can be extended by one or two complex type antennae, whereas the α6 mannose is extended by several mannosides (Figure 5).

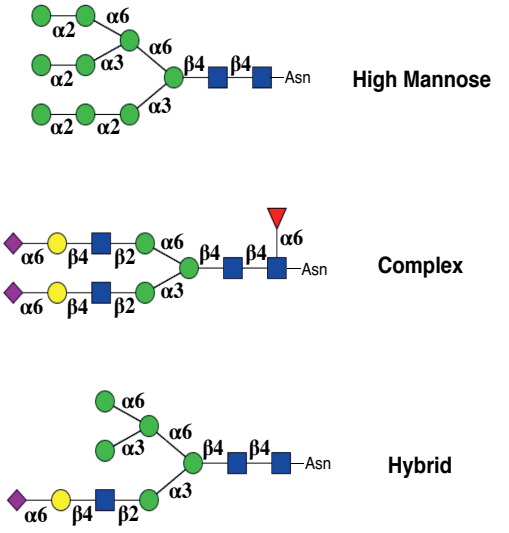
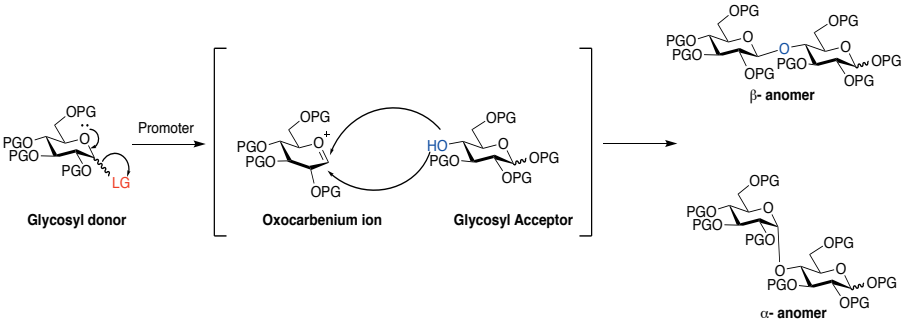


Figure 5. Types of *N*-Glycans.

General Strategies employed for synthesis of glycans. Synthetic chemistry is an important tool to study properties of glycans. Tremendous progress has been made in method development for the synthesis of complex oligosaccharides over the past 40 years. Streamlining the synthesis of complex oligosaccharides requires tuning several parameters as described in the following sections. *N*-glycans show highly diverse and asymmetrical branching patterns,[18] and isolating sufficient amounts from biological sources is not efficient for their applicability as standards, as components of comprehensive libraries, or to study interactions and mechanisms at a molecular level. Synthetic procedures for the construction of well-defined glycans gives access to isomerically pure and well-characterized standards as a component of the glycome database,

paving way for in depth investigation of glycan-protein interactions, for example for host-microbe interactions and immunological responses.[19]

1) Stereoselectivity. The synthesis of complex oligosaccharides revolves around the stereoselective formation of glycosidic bonds. The two possible outcomes of a glycosylation reaction are an α or β linkage. A typical glycosylation reaction occurs between a glycosyl donor and acceptor in which the donor contains a leaving group at the anomeric center and acts as an electrophile. The leaving group of the donor departs in the presence of a promoter (or activator), thus generating an oxocarbenium ion intermediate. This is followed by an attack by the hydroxyl group of an acceptor from either the α or β face resulting in the respective glycosides (Scheme 1).

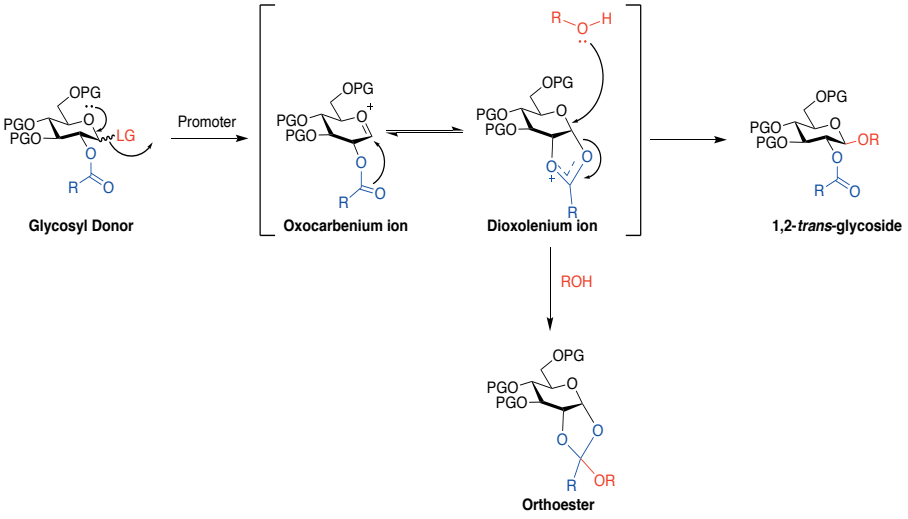


Scheme 1. General glycosylation pathway (LG = Leaving Group; PG = Protecting Group).

The stereoselective formation of glycosidic bonds is affected by a number of factors such as neighboring group participation, solvent participation, long range couplings, additives, and temperature. A number of important factors affecting the outcome of glycosylations are discussed as below.

a) Neighboring Group Participation. 1,2-*trans* anomeric glycosidic linkages can be introduced by using an ester protecting group at the C-2 position of a donor. The leaving group at the anomeric center of the glycosyl donor departs in the presence of a promoter resulting in the formation of an oxocarbenium ion. The 2-*O*-acyl group performs neighboring group participation to stabilize the oxocarbenium ion resulting in the formation of a five-membered dioxolenium ion.[20] As a result, the α– face is blocked and nucleophilic attack by the hydroxyl of acceptor occurs from the β–face, resulting in the formation of β–glycoside. However, a nucleophilic attack at the electron deficient dioxolenium ion can result in the formation of ortho-ester, which is an oft-observed side product when acetyl esters are used at C-2. Use of bulkier esters like benzoyls and pivaloyls, which stabilize the diaxolenium ion better, avoid ortho-ester formation (Scheme 2).

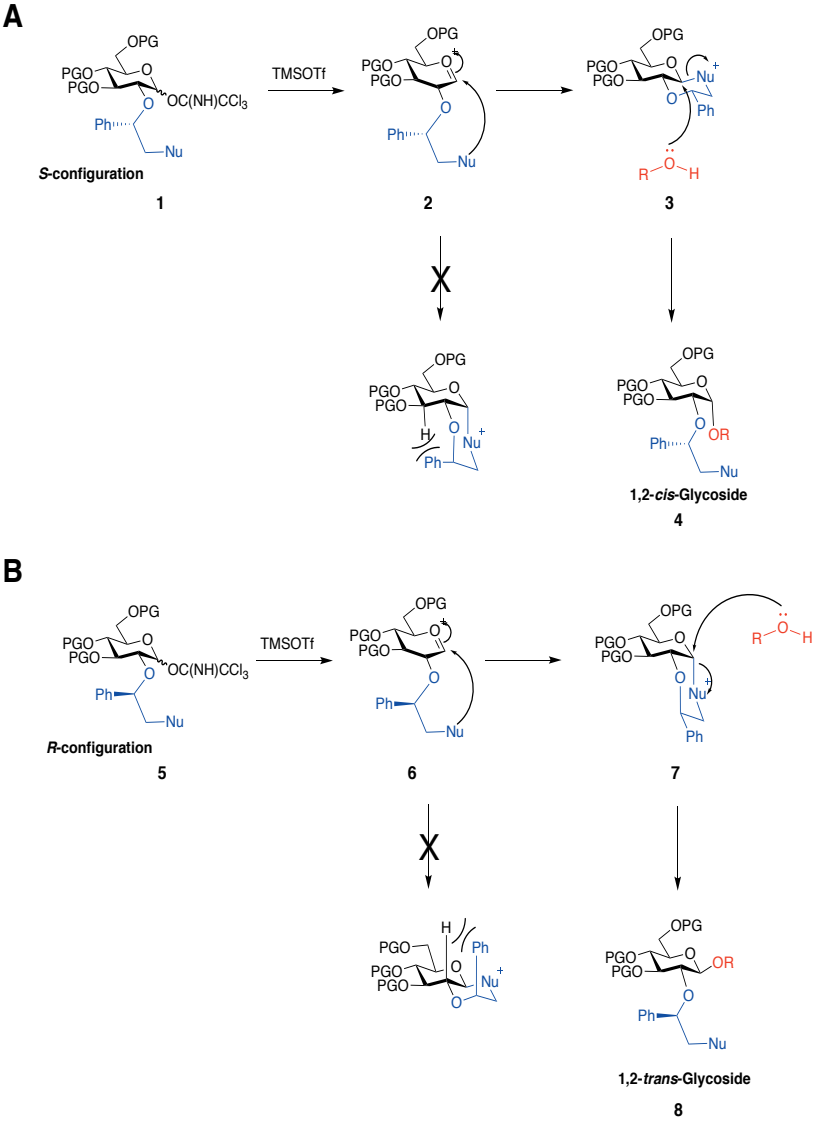
For 2-deoxy-2-amino glycosides, protecting groups such as phthaloyl (Phth), tricholroethoxycarbonyl (Troc), trichloroacetyl (TCA), trifluoroacetyl (TFA), alloxycarbonyl (alloc), etc. perform similar neighboring group participation.



Scheme 2. Depiction of Neighboring Group Participation during Glycosylation.

b) C-2 auxiliaries. Conventional synthesis of 1,2-*cis*-glycosides is dependent on a non-participating group at C-2 and controlling reaction conditions such as solvent, temperature, and the promoter. Further, tuning the protecting groups of the acceptor and donor is important for achieving acceptable anomeric selectivities. However, even the best optimization reactions often yield mixtures of α and β anomers. In 2005, Boons and co-workers introduced the first generation of chiral auxiliaries at the C-2 position of glycosyl donors that can undergo neighboring group participation for stereoselective 1,2-cis glycosylations (Scheme 3). The auxiliary comprises of a substituted ethyl moiety with a nucleophilic group. Treatment with a promoter leads to the formation of an oxonium ion which is attacked by the nucleophilic group on the auxiliary. This forms a six-membered ring, resulting in either a *cis*- or a *trans*- decalin ion intermediate.[21] The *(S)*-auxiliary **1** leads to the formation of sterically favorable *trans*-decalin intermediate **3** ultimately resulting in the 1,2-*cis*-glycoside **4**. On the other hand, the *(R)*-auxiliary forms a *cis*- decalin intermediate **7**, thus generating the 1,2-*trans*- glycoside **8** upon reaction with an acceptor. The first generation of chiral auxiliaries employed *(S)*-ethoxycarbonylbenzyl as the nucleophilic group to generate 1,2-*cis*- glycosides, with α:β ratios ranging from 20:1 to 4:1. This selectivity was substantially improved by applying the more nucleophilic thiophenyl ether group. In this case, only the α product was observed, and the methodology was employed to synthesize a branched polysaccharide derived from the fungus *Pseudallescheria boydii*, which causes fungal

phagocytosis and activates innate immune responses.[22] These were also used as an alternative methodology for the synthesis of β-mannosides via a *trans*- decalin sulfonium ion intermediate.[23] In this case, it was essential to use $^{1}C_{4}$ locked conformation of mannuronic acid, so that the thio-ether C-2 auxiliary could accomplish neighboring group participation.



Scheme 3. Use of Chiral Auxilliaries to generate *cis* and *trans* glycosides.

The second generation of chiral auxiliaries employing the more nucleophilic thiophenyl ether have proved to be a more superior way of installing 1,2-*cis*-linkages and β-mannosides.[24] Successful formation of α-linkages in 2-deoxyglycosides, was performed by attaching the chiral auxiliary to its C-6 position.[25]

Further improvement on the chiral auxiliaries were performed by Turnbull and co-workers (Figure 6).[26] To afford high α-stereoselectivity, it was necessary to install electron withdrawing groups like esters at the C-3 position of Turnbull's donors. Several studies have been undertaken for obtaining ideal reaction conditions for glycosylation involving chiral auxiliaries, as they are cost-efficient, chemically inert and easy to install and remove, and hence are popular alternatives to install 1,2-cis glycosidic linkages.[22, 27]

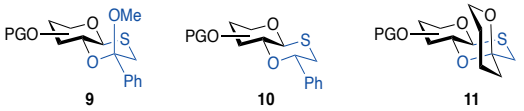
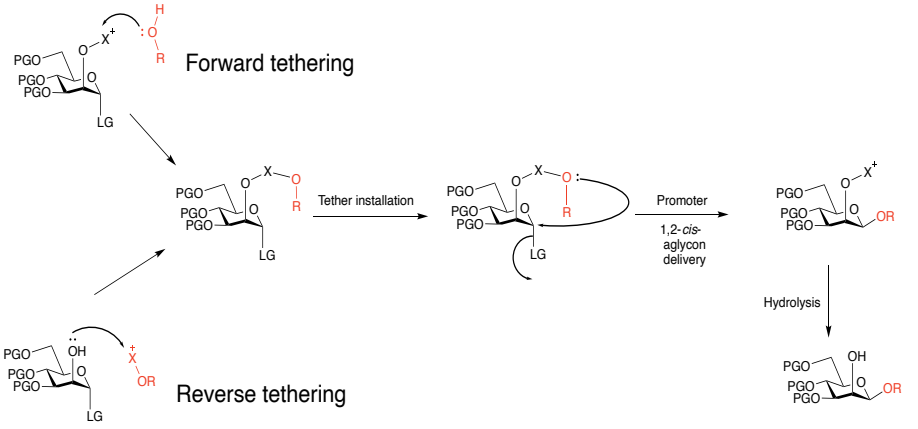


Figure 6. Turnbull's chiral auxiliary glycosyl donors.

c) Intramolecular Aglycone Delivery (IAD). Initially developed by Barresi and Hindsgaul,[28] this methodology utilizes the stereochemistry at C-2 of a glycosyl donor to generate 1,2-*cis*-glycosides by a "tethering-glycosylation" method (Scheme 4). The tether prearranges the donor and acceptor by linking the *O*-2 of the glycosyl donor and the hydroxyl group of acceptor. The acceptor alcohol is connected to the tether, resulting in a five-membered transition state yielding 1,2-*cis*-glycoside. This method is widely used for synthesizing β-mannosides.[28-29]



Scheme 4. IAD to generate 1,2-*cis*-glycosides.

Subsequently, long range IAD using silicon tethers were employed to generate 1,2-*trans*-linkages as well.[30] The most commonly used tethering groups are listed in Figure 7.[31]

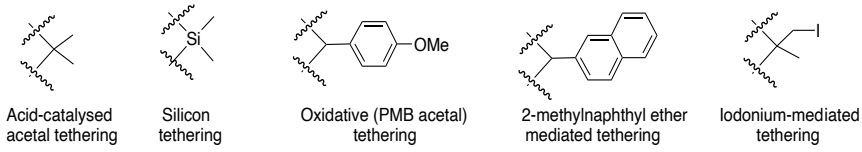
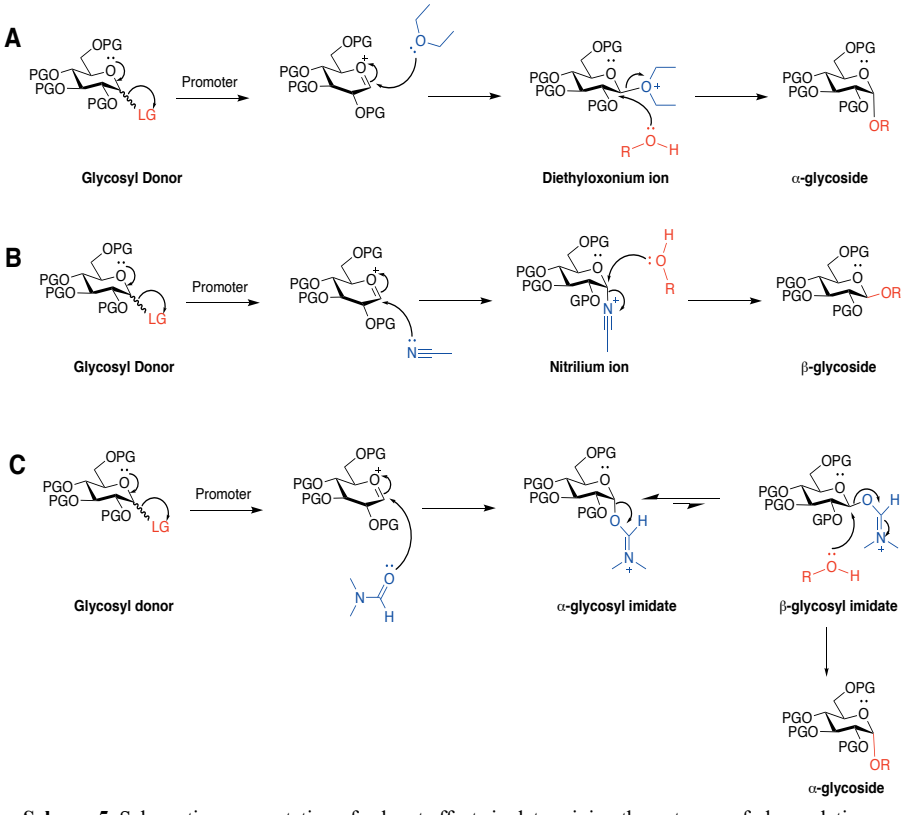


Figure 7. Types of protecting groups used for C-2 tethering.

d) Solvent Effect. Several glycosylations have been aided in achieving the desired stereo outcome by using solvents such as diethyl ether (Et_2O), acetonitrile (ACN) and dimethylformamide (DMF). The role of solvents becomes important in glycosylations marked by an absence of C-2 participating groups in the glycosyl donors (Scheme 5).



Scheme 5. Schematic representation of solvent effects in determining the outcome of glycosylations.

Solvents described herein are widely used as additives directly participating in the glycosylation reaction proceeding via an S_N2 mechanism. For example, diethyl ether participates in the glycosylation by forming a diethyloxonium ion, which attains the more stable β or equatorial configuration, thus directing an axial acceptor attack affording the α product. On the other hand, in presence of acetonitrile, the nitrilium ion generated attains an axial configuration, thus blocking the α-face and generating a β-product. The comparatively newly discovered DMF-mediated glycosylation has been used to drive the result towards an α-glycosylation. Mechanistic studies undertaken on the reacting glycosyl donor (imidate or triflate) have pointed towards the formation of α– imidate while the corresponding β–imidate was not detected, thus signifying that the β-intermediate is more reactive leading to attack from alpha side.[32]

e) Remote group participation. Another way for the construction of 1,2-cis-glycosides is through remote neighboring group participation, also known as long range participation. It is most useful for the construction of 1,2-*cis*-glycosides acting in conjunction along with C-2 non-participating group. Donors having 3-*O*-, 4-*O*- and 6-*O*-esters have been widely studied to improve the stereochemical outcomes.[33] The mechanism of remote group participation or long range participation was recently demonstrated by comprehensive studies including DFT computations and infra-red ion spectroscopy of the intermediate dioxolenium ions using model glycosylation reactions of glucosyl, mannosyl and galactosyl donors.[34] The detailed studies performed indicate the strength of remote group participation in the order: 3-Ac-Man > > 4-Ac-Gal > 3-Ac-Glc∼ 3-Ac-Gal > 4-Ac-Glc> 4-Ac-Man ∼ 6-Ac-Glc/Gal/Man, concluding that the most prominent effect is observed for C-3 acetyl of mannosides.

Cryogenic infra-red spectroscopy has proven to be a useful to detect transition state cations like oxocarbenium or acyloxonium. ions[20b, 35] Pagel and co-workers had already investigated that cryogenic IR spectroscopy is an important approach to detect glycosyl cations. This technique provided evidence that an acetyl at C-2 can form a covalent bond with the anomeric carbon generating an acyloxonium-type intermediate.[36] This distortion of ring then results in the desired stereochemical results. Extending this principle to galactosides in which the C-4 and C-6 positions were protected by acetyl esters and benzyl ethers, it was found that 4-Ac and 2,6-di-Ac followed by 6-Bn groups are most suitable for alpha product formations through long range participation.

In the recent years, Demchenko and co-workers have extensively studied the stereochemical outcomes of remote picoloyl and picolinyl substituted donors. Using these groups at C-3, C-4 and C-6 positions ensured a highly β-selective glycosylation of galactosides, mannosides and rhamnosides.[37] The reaction mechanism was proposed to proceed via “hydrogen-bond mediated aglycone delivery” (HAD). Further, the stereoselectivity could be switched to α-selective by manipulating the remote 6-*O*-picolinyl and picolyl protecting groups, by temporarily blocking their respective *N*-atoms by coordination with a metal such as palladium.[38] Other studies include

the use of an excess of triflic acid with remote 3-*O*-picoloyl group to yield β-glucosides, even in the absence of a C-2 participating group,[39] and the synthesis of a novel 8-*O*-picoloylated sialyl donor, yielding several α(2,6)-linked disaccharides.[40]

2) Glycosidic Bond Formation by Activation of Glycosyl donors.

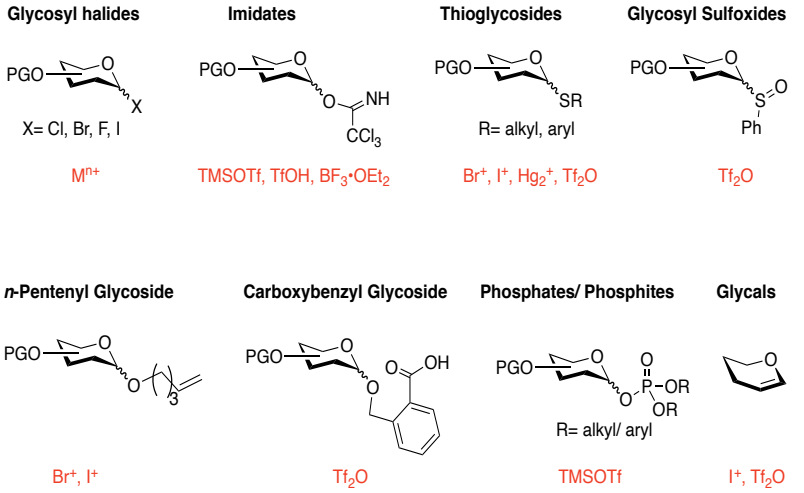


Figure 8. Some common glycosyl donors and their respective promoters used for *O*-glycosylation.

For a long time, the Koenigs and Knorr[41] donors comprising of glycosyl chlorides and bromides were the only methods used for the synthesis of glycosides. These donors were activated by heavy metal salts such as silver oxide and silver carbonate. Later, mercury salts like mercuric bromide and mercuric cyanide were used as promoters.[42] For the synthesis of 1,2-*cis*-glycosides, *in situ* anomerization using tetra alkyl ammonium halides was employed. To overcome the problem of the instability of glycosyl bromides and chlorides, the more stable glycosyl fluorides were developed, and perhaps, their most important use was in the synthesis of the challenging 1,2-*cis*-linked (β-linked) mannosides.[43] Glycosyl fluorides can be activated under milder reaction conditions with Lewis acids, thus dispensing with toxic, heavy metal salt promoters used previously. Glycosyl iodides in general are highly reactive, and hence have been commonly used in IAD to generate β-glycosides[44], especially in formation of anomeric linker-linked products.[45]

Glycosyl trichloroacetimitates developed by Richard Schmidt and coworkers in 1980,[46] are still the most widely used glycosyl donors today for oligosaccharide synthesis. These can be activated by catalytic amounts of Lewis acids like $BF_3.Et_2O$, TMSOTf, TfOH, AgOTf, etc. The presence of a C-2 participating group results in the formation of 1,2-trans-glycosides whereas a non-

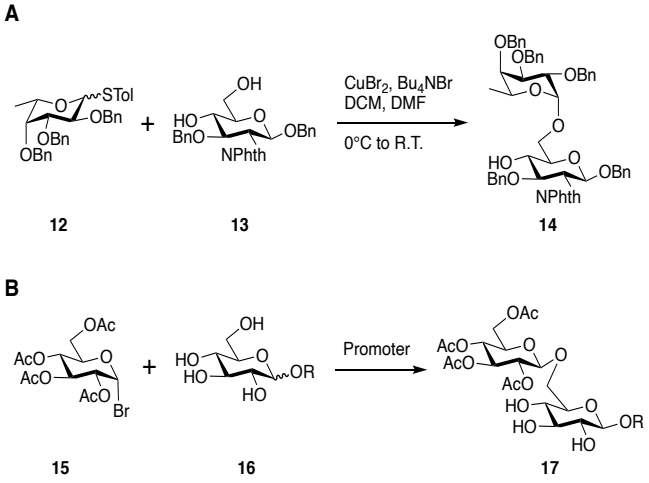
participating group at C-2 can give anomeric mixtures. Yu and coworkers then developed the more superior *N*-phenyltrifluroacetimidate[47] which prevents the formation of rearranged donors.

Thioglycosides, perhaps the most versatile and efficient glycosyl donors, were first reported by Ferrier and coworkers in 1973.[48] They are a very popular choice of glycosyl donors owing to their high stability in both acidic and basic conditions. These can be activated under relatively mild conditions in the presence of a thiophile such as *N*-iodosuccinimide (NIS), activated by Lewis acids like trimethylsilyl trifluoromethanesulfonate (TMSOTf) or trifluoromethanesulfonic acid (TfOH). Hence, thioglycosides are often used as orthogonal activation alternative in presence of other glycosyl donors. Other activation reagents for thioglycosides are diphenyl sulfoxide or 1-benzenesulfinylpiperidine in presence of trifluoromethanesulfonic anhydride (Tf_2O) or dimethyl(methylthio)sulfonium triflate (DMTST).[49] The oxidation of thioglycosides results in the formation of glycosyl sulfoxides, that can be activated by Tf_2O. An advantage of using glycosyl sulfoxides as donors, is that they can be activated orthogonally to thioglycosides, and this aspect can be employed in selective glycosylations.

The use of glycosyl phosphates as donors in glycosylations was reported in 2001 by Seeberger and coworkers for the installation of β-glucosidic, β-galactosidic, α-fucosidic, α-mannosidic, β-glucuronic acid, and β-glucosamine linkages. These donors afforded good results, even with hindered and electron-deficient substrates, as well as for *S*-glycosides and *C*-glycosides.[50] Glycosyl phosphites have also been used as donors using activators like TMSOTf and *tert*-butyldimethylsilyl trifluoromethanesulfonate (TBSOTf) or the triflate salts of tertiary amines.[51]

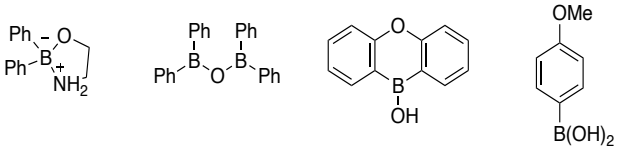
Glycals can be activated by NIS or Iodine Dicollidine Perchlorate (IDCP) and are especially useful for the formation of 2-deoxy-glycosides. The promoter facilitates the formation of cyclic iodonium intermediate of the glycal, which acts as a donor for attack by an acceptor from the less sterically hindered α side. Glycals can also be used to generate 2,3-unsaturated glycosides, in a reaction known as the Ferrier glycosylation.[52] The successful conversion to unsaturated glycosides is dependent on addition of an appropriate catalyst.[53] Further, glycals are also used to generate 2-amino-2-deoxyglycosides by azido-nitration reaction using sodium azide and ceric ammonium nitrate (CAN). This results in the formation of 2-azido-2-deoxy-glycosyl nitrates.[54] The anomeric nitrate group can then be hydrolysed and converted into the desired glycosyl donor. Danishefsky and coworkers have contributed to the use of glycals in glycosylations by “aza-glycosylation” and epoxide formation.[55] Glycals have also been used as donors in the synthesis of *C*-glycosides.[56] *n*-Pentenyl glycosides,[57] carboxybenzyl glycosides [58] and the ortho-alkynylbenzoate are some other examples of glycosyl donors used for difficult glycosylations.[59]

3) Regioselectivity. Carbohydrates contain several hydroxyls and sometimes also amino- and carboxylic acid moieties. Generally, during a chemical glycosylation, only one hydroxyl group is left exposed to reaction, whereas the others are protected, thus eliminating the chances of side reactions. Regioselective glycosylations are an alternative that can be used to glycosylate at the desired hydroxyl in the presence of other free hydroxyls, without causing undesirable side reactions. Another advantage is that the glycosyl acceptor requires fewer steps to synthesize. There are several parameters that need to be considered for regioselective glycosylations. For example, primary hydroxyls are more reactive than secondary hydroxyls. Reactivity differences between axial and equatorial hydroxyls have also often exploited, in accordance with following the general reactivity order of primary hydroxyl > secondary equatorial hydroxyl > secondary axial hydroxyl.



Scheme 6. Regioselective glycosylation of primary hydroxyls.

This reactivity difference was employed by Lemieux and coworkers in 1975, to synthesize α-linked disaccharides in an *in situ* anomerization protocol using copper bromide and tetrabutylammonium bromide.[60] Since then, this method has been used to introduce a core α-1,6-linked fucose in synthesis of *N*-glycans selectively on the C-6 primary hydroxyl in presence of other secondary unprotected hydroxyls (compound **14**, Scheme 6A).[61] Glycosyl halides as donors can be activated using mild promoters such a silver carbonate (Ag_2CO_3)[62] or silver triflate (AgOTf) in the presence of 2,4-lutidine and glycosylated with unprotected or partially protected acceptors, to yield the 1-6 linked glycosides as the major product.[63] Diphenylborinic acid derivatives have been used by Taylor and coworkers as catalysts to control regioselectivity in glycosylations with multiple free hydroxyl groups.[64] This methodology was applied in the impressive synthesis of a branched pentasaccharide derived from a Saponin natural product, *Spergularia ramose*.[65]

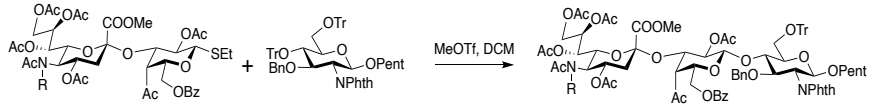


18 **19** **20** **21**

Figure 9. Organo-Boron catalysts used in Regioselective Glycosylations.

Bis(tributyltin)oxide was used by Ogawa and coworkers in 1978 for the regioselective glycosylation on unprotected methyl α-D-mannopyranoside by forming a bis-dibutyltin intermediate at the 2,3 and 4,6 positions. This caused an increased nucleophilicity of hydroxyls at C-3, and C-6 resulting in glycosyl bond formation at these positions when subjected to reaction with glycosyl halide.[66]

Protecting group manipulations play an important role to achieve successful and high yielding regioselective glycosylations. For example, capping a primary and secondary hydroxyl with a trityl group, resulted in a high regioselective and stereoselective glycosylation at the secondary trityl position.[67] This strategy was employed in the synthesis of a trisaccharide derived from the capsular polysaccharide of *Streptococcus* group B type III. The secondary trityl group enhances the nucleophilicity of C-4 hydroxyl, thus resulting in high yield (70% to 95%) of the desired product (Scheme **7**).[68]



22 **23** **24**

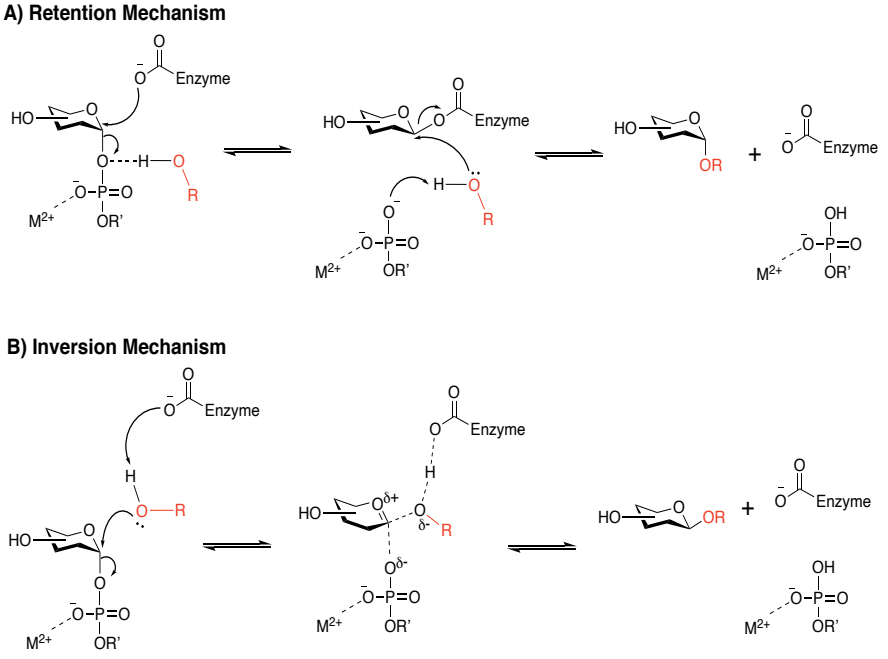
R= H/ Me/ Ac

Scheme 7. Use of trityl group for regioselective glycosylation of secondary hydroxyls.

4) Enzymatic Reactions. Enzymes, broadly divided into two categories: glycosyltransferases and glycosidases, are catalysts for the synthesis of complex oligosaccharides in nature. Enzymatic reactions proceed with excellent regio- and stereoselectivity, and hence eliminate the use of temporary protecting group strategies employed in chemical synthesis. Glycosyltransferases are the enzymes that catalyze the transfer of a monosaccharide residue from an activated glycosyl donor onto an acceptor. The sugar donors are present in the form of nucleoside diphosphate (UDP-Gal, UDP-GlcNAc, GDP-Man, GDP-Fuc) as well as nucleoside monophosphate (CMP-NeuAc). Acceptors encompass a wide range of substrates, including mono and oligosaccharides, proteins,

lipids and other small molecules.[69] The two most important mechanisms in enzymatic reactions brought about glycosyltransferases, are the inversion and the retention type (Scheme 8).[70] The retention type mechanism is much debated and is said to proceed through an acyl-glycoside intermediate, while the inversion type mechanism proceeds through an oxocarbenium ion intermediate via S_N2 type reaction.[71]

Glycosidases on the other hand hydrolyze glycosidic linkages. *Exo*-glycosidases cleave monosaccharide residues from the non-reducing end, while *endo*-glycosidases cleave internal glycosidic linkages. Some glycosidases degrade the extracellular carbohydrates, and others, like α-glucosidases and α-mannosidases bring about the trimming and maturation of *N*-linked glycans, resulting in proper protein folding. Glycosidases also follow inversion and retention type mechanisms (Scheme 8).[72]



Scheme 8. Mechanism of enzymatic reactions.

Application of chemical and enzymatic techniques for the synthesis of *N*-glycans. The focus on the need to synthesize *N*-glycans to understand their biological roles can be attributed to the groups of Ogawa and Hans Paulsen, who made significant contributions in this field and accomplished many milestones.[73] The small, impure quantities being isolated from biological sources were not sufficient to study and analyze all the important biological roles *N*-glycans. The

first synthesis of an *N*-glycan was reported in 1986 by Ogawa and co-workers, who accomplished the synthesis of a bi-antennary complex type *N*-glycan containing a terminal sialic acid.[74] Further, the synthesis of the "bisected" type complex *N*-glycan, in which the central mannose had an additional GlcNAc at the hydroxyl of C4 position, was also accomplished.[75] These impressive achievements have since then, lead to the generation of more diverse and complex *N*-glycans. The synthesis of such complex oligosaccharides was quite labor intensive and therefore, combination of chemical synthesis with enzymatic transformations proved to be advantageous, as it gave regioselective and stereoselective products without the need of cumbersome protection-deprotection cycles of temporary protecting groups. An elegant strategy to combine the two methods was reported by Unverzagt and co-workers in 1996, when the synthesis of a bi-antennary undecasaccharide *N*-glycan- asparagine (Asp) conjugate, a partial structure of human transferrin and ribonuclease, was accomplished. The galactosides and sialic acids at the non-reducing end were installed enzymatically (Figure **9**).[76]

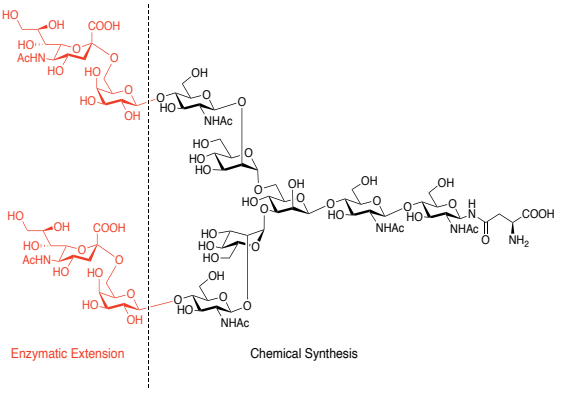
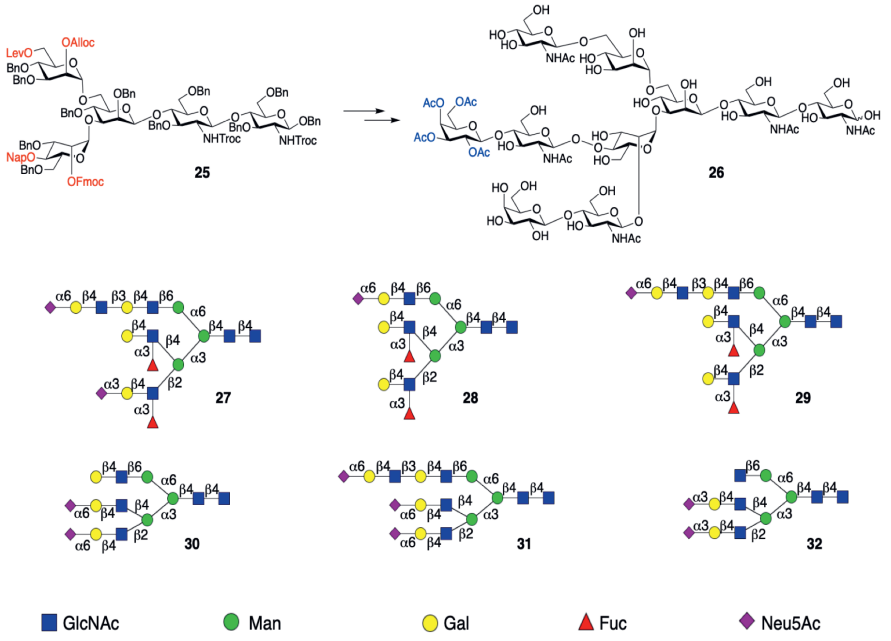


Figure 9. Unverzagt's chemo-enzymatic synthesis of *N*-glycans.

Towards the end of the 20th century, some major advancements had been made in the synthesis of *N*-glycans, and the understanding of their biological functions. By now, it was also understood that a majority of these glycans showed diverse and asymmetrical branching patterns.[18] Realizing the biological relevance of *N*-glycans, many groups made foray into the synthesis of pure and well-characterized compounds. Unverzagt and coworkers continued to develop the synthetic strategies for their synthesis, in the process synthesizing a number of complex type *N*-glycans.[77] Huang and coworkers designed a pre-activation based strategy for the synthesis of a core-fucosylated bi-antennary *N*-glycan.[78] Other prominent contributions towards the advancement of their synthesis were made by groups of Profs. Ito,[61a, 79] Danishefsky,[80] and Chi-Huey Wong.[81]

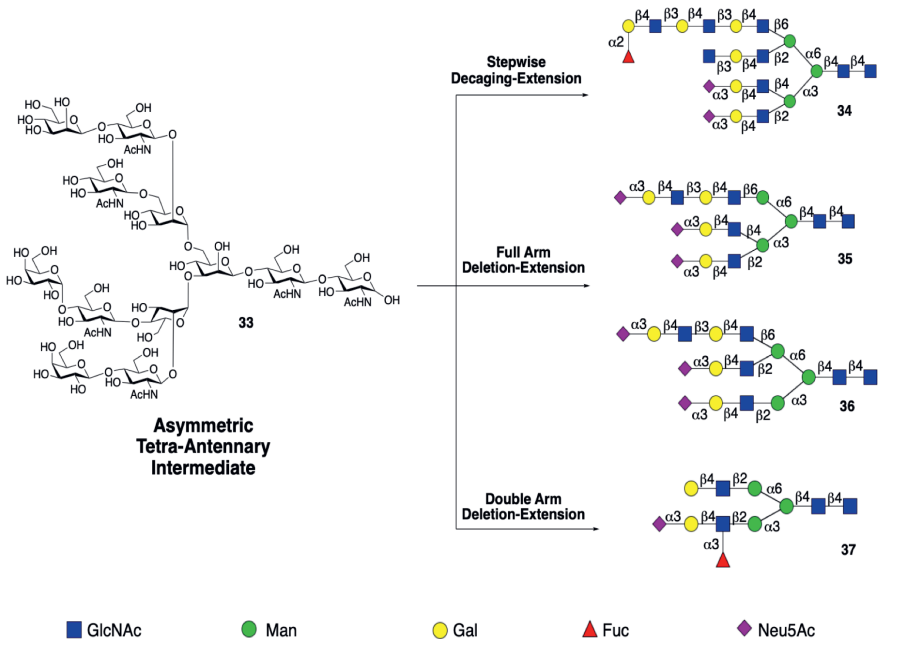
A major step forwards for the synthesis of asymmetrical *N*-glycans was accomplished by Boons and co-workers with the chemical synthesis of pentasaccharide **19**, a common precursor to all

eukaryotic *N*-glycans, which comprised of four orthogonal protecting groups at the typical branching positions.[82] Each of these branches could be extended independently by chemical glycosylation with different donors. The LacNAc arm of **26** was extended first, while the other two arms capped with an acetylated galactose and an unmodified GlcNAc remained inactive to the enzymes. Next, the acetyl esters were cleaved exposing the second LacNAc, which was subjected to enzymatic modifications. Finally, the exposed GlcNAc was converted into LacNAc using a galactosyl transferase, thus resulting in the synthesis of a library of asymmetrical *N*-glycans. (Scheme 9).



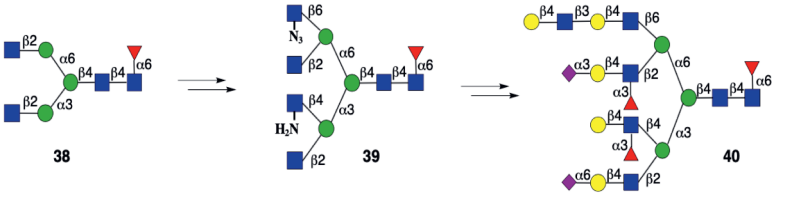
Scheme 9. Synthesis of asymmetric tri-antennary *N*-glycan.

Although the complexity of *N*-glycans synthesized by this method was unprecedented, major disadvantages were that the synthesis was restricted to a tri-antennary compounds, and that the acetyl esters were unstable and hydrolyzed during prolonged enzymatic reactions. To overcome these issues, another strategy was then utilized by Boons and co-workers for the synthesis of a highly complex, asymmetric tetra-antennary *N*-glycan. Taking inspiration from previous work by Ito and coworkers,[79d, 79e] this strategy employed installing unnatural sugars to cap enzymatic sites. At a desired stage, each unnatural sugar moiety could be independently cleaved by its respective glycosidase, in essence exposing only one arm at a time for modification. Thus, using one common precursor coupled with a clever use of enzymatic manipulations, gave way to tetra, tri and bi-antennary (Scheme 10).[83]



Scheme 10. Synthesis of asymmetric tetra, tri and bi-antennary *N*-glycans from a common precursor.

Total Enzymatic Synthesis. The synthesis of such complex oligosaccharides involves more than hundred steps for synthesis, requiring a high level of expertise in synthesis. To streamline the synthesis of *N*-glycans, and to democratize such a complicated synthesis, Boons and co-workers introduced a fully enzymatic extension, on a naturally derived *N*-glycan obtained from egg yolk. This bi-antennary *N*-glycan was converted into a tetra-antennary structure by treatment with enzymes *N*-acetylglucosaminyltransferases MGAT4 and MGAT5.



Scheme 11. Fully enzymatically synthesized tetra-antennary *N*-glycan.

Further, the *N*-protecting groups were differentially capped, and at an appropriate stage, uncapped and asymmetrically extended. Using this methodology, the previously required more than hundred steps were reduced to a mere ten steps (Scheme 11).

An automated fully enzymatic '*catch and release*' methodology was recently introduced which could yield a number of oligosaccharides, including an asymmetrical bi-antennary *N*-glycan.[84] For this, an *N*-glycan derived from egg yolk was modified by a sulfonated tag at the reducing end which facilitated solid phase extraction. The glycan release was followed by glycosyltransferase catalyzed enzymatic reactions in solution. The total number of steps was considerably reduced and the overall yield substantially superior.

Scope of the thesis. Although advances in the synthesis of glycans have reached an unparalleled complexity, the synthesis of glycans of lower species like disease causing parasites and bacteria, have still not been fully explored, owing to a poor understanding of their enzyme expression. As such, there is a tremendous lag in the amount of information available with respect to their interaction and invasion of humans. Research suggests that seemingly unimportant changes in glycosylation patterns in such pathogens can have unpredictable and immense effects on biological functions. Until we gain extensive advancement in technologies like gene expression and recombinant protein expression, chemical approach aided with enzymatic extensions seems to be the only way to get access to comprehensive libraries of desired glycans.

S. mansoni is a helminthic parasite that utilizes glycan expression on its cell surface to invade its host and manipulate their immune system. Thus, the following **Chapter 2** of this thesis gives a general overview of the epidemiology and immunogenicity in human hosts upon infection. *S. mansoni* modulates the dendritic cell-specific intercellular adhesion molecule-3-grabbing nonintegrin (DC-SIGN), a C-type lectin, to generate a modified immune response affecting the adaptive immunity which reduces inflammation to cause chronic infections in hosts.

S. mansoni express a number of glycan motifs which contain highly unusual fucoside linkages, which are recognized as immunogenic epitopes. The chemical synthesis of two characteristic terminal epitopes GalNAcβ1,4(Fucα1,3)GlcNAc (LDN-F) and GalNAcβ1,4(Fucα1,2Fucα1,3)GlcNAc (LDN-DF) expressed in the glyco-conjugates of these parasites in a stage-dependent manner, is discussed in **Chapter 3**. The synthesis was accomplished by careful selection of protecting groups to ensure high stereo-selectivity of glycosylations. The binding of these epitopes with DC-SIGN was studied by STD-NMR, molecular modeling and micro-array. The studies established the importance of multivalency to increase avidity and selectivity of binding from a biological perspective.

Building on the conclusions derived by studying the minimal epitopes discussed above, it was decided to expand our understanding of immune-modulation by synthesizing three characteristic

N-glycans of *S. mansoni*, via a chemo-enzymatic methodology, which is discussed in **Chapter 4**. The *N*-glycans were attributed with characteristic features such as a core xyloside, core fucoside and terminal epitopes like Lewis^x, LDN-F and di-Lewis^x. These glycans were studied by STD-NMR, computation, and electron microscopy and it was concluded that multi-antennary glycans bind with higher affinity to DC-SIGN compared to mono-valent minimal epitopes. It was presented that the multi-antennary glycans could cross-link DC-SIGN tetramers into a dense network which could contribute to clustering of DC-SIGN, thus enhancing antigen recognition.

The versatility of the chemo-enzymatic strategy was elucidated in **Chapter 5** with the synthesis of a library of *N*-glycans varying in terminal epitopes and branching patterns. Careful manipulation of orthogonal protecting groups along with a panel of glycosyltransferases were used to synthesize bi-antennary, tri-antennary and positional isomers from a common precursor. Access to such well-defined glycans will enable future studies to identify the ideal candidate that can generate an appropriate antibody response in infections, thus activating the immune system towards death and expulsion of the parasite.

Chapter 6 summarizes the research described herein and concludes with the description of attempts at the synthesis of an unnatural lipid-linked *N*-glycan core, which could inhibit the enzyme Oligosaccharyl Transferase (OST). OST catalyzes *en bloc* transfer of the pre-assembled oligosaccharide to the carboxamide side chain of asparagine residue to yield *N*-linked glycoproteins. The structural information on OST is sparse, and the synthesis of an inhibitory analog that could trap OST in an intermediate state, could be an appropriate starting point towards the full structural elucidation of OST.

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CHAPTER 2

Schistosomiasis and Immunogenicity in Humans

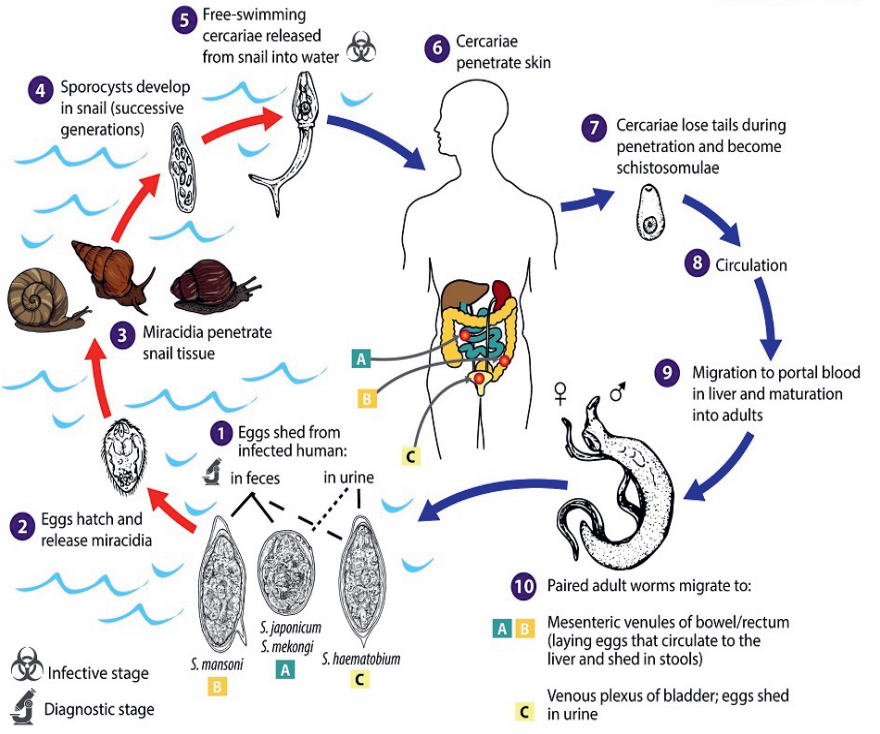
Introduction. Schistosomiasis, also known as Bilharziasis is a disease caused by long living, multi-cellular parasitic helminths of the trematode genus *Schistosoma.*[1] These parasites are one of the most prevalent pathogenic agents in the world, causing chronic and debilitating diseases in humans and animals alike, infecting snails as intermediate host. The three main species of this genus infecting humans are *S. mansoni, S. japonicum* and *S. haematobium*. The extent of schistosomiasis permeation in human populations is evidenced by the fact that it affects an estimated 290 million people worldwide, causing around 200,000 deaths annually. Schistosomes have developed remarkable strategies to remain undetected and evade the host immune responses. Hence, schistosomiasis has an exceptionally high chronicity and morbidity rate, with some cases in which these parasites survive in the human gut for up to forty years.[2] Schistosomiasis has been classified as a Neglected Tropical Disease (NTD) and infects the populations living in some of the poorest parts of the world.

The symptoms of schistosomiasis manifest owing to the host's immune response against the eggs, and not against the worms *per se*. The symptoms of acute schistosomiasis include fever, muscle aches, abdominal pain and cough. Chronic schistosomiasis infections can remain undetected for years,[3] leading to organ damage, and fibroid formation, ultimately resulting in bloody urine and diarrhea, seizures, anemia resulting in poor quality of life.

Life cycle of schistosomes. Schistosomes have a very complex life cycle employing snails (genus *Oncomelania*) as the intermediate host, eventually ending up in human hosts. Schistosomes start their life cycle when eggs are excreted through urine or feces of infected individuals. These eggs hatch when they come in contact with water, thus releasing larva known as miracidia. The free-living, multi-ciliated miracidia, searches for, and penetrates a suitable snail host. Here, the miracidium converts into the primary (mother) sporocyte and starts reproducing asexually in the snail host. After around two weeks, secondary (daughter) sporocytes escape, and produce thousands of cercariae. Cercariae is the second and ultimate free-living, multi-cellular larval stage, which is excreted from the snail body, in search of the next host. Humans are infected when they come in contact with water contaminated with this free-swimming cercariae. The acetabular glands of cercariae help it in breaching skin surface and gaining entry into the human hosts.

To adapt to the physiological conditions inside the human body, the cercariae loses its tail and convert into schistosomula, at which point it enters the blood stream. The blood circulation takes schistosomules to various organs like the heart, lung and liver, where they attain sexual maturity to become adult male and female worms. Surprisingly, the male worms can attain sexual maturity independently, however, female worms require pairing with male worms to undergo physical and reproductive maturation. After the coupling process, the female worms start producing eggs continuously, laying approximately 300 eggs per day. The eggs pass through intestinal and urogenital tracts, to be expelled from the human body through excreta, and continue their life cycle. Most of these eggs are unable to be expelled from the body, and schistosomiasis occurs as a result of egg deposition and entrapment in between tissues and organs (Figure 1).

Epidemiology and pathogenicity. The World Health Organization characterizes schistosomiasis as endemic to the poorest tropical regions, particularly in farming and fishing communities.[4] About 90% of patients requiring treatment inhabit the sub-Saharan regions of Africa. *S. mansoni*, the species most prevalent in affecting humans, occurs in Africa, the Middle Eastern region, Brazil, Venezuela and Suriname. Schistosomiasis is a debilitating and chronic disease, often occurring with other helminthic infections. Therefore, it is difficult to estimate the extent of its impact on the overall health of vulnerable populations.[5] Still, it is likely that around 800 million people are at a severe risk of getting infected.[6] Contamination of water with the cercaria passed through the fecal matter of infected individuals, and further consumption of this contaminated water transmits and propagates the infection by keeping the life cycle of these parasites active. The most susceptible age groups to infection are children and young adults. The cause for this could be the death of the worm (average life span of the worm being around five years) and decrease in water contact as the age of the host increases or the development of immunological resistance against re-infection over time.[7]



The stages of the schistosome life cycle. (1) elimination from the host as eggs in feces or urine (diagnostic stage), (2) hatching of miracidia, (3) infection of species-specific aqueous snail intermediate hosts, (4) proliferation of sporocysts within snails, (5) release of cercariae into water (infective stage), (6) infection of host by skin penetration, (7) development into schistosomulae, (8) circulation, (9) maturation within portal vasculature, and (10) migration of paired adult worms to target organs. Elimination of schistosome eggs in either feces or urine depends on whether the adults reside in the mesenteric venules of the bowel/rectum.

Figure 1. provided by A. J. da Silva and M. Moser for copyright-free dissemination through the Public Health Image Library of the Centers for Disease Control and Prevention.

Immune response against schistosomiasis. In the human immune system, dendritic cells (DCs) are the first line of defense that an antigen encounters and function as a link between the antigen non-specific innate immune response and the antigen specific adaptive immune response.[8] Dendritic cells are the most important Antigen presenting cells (APCs) that express several pattern recognition receptors (PRRs), such as toll-like receptors (TLRs), RIG-I-like receptors (RLRs), NOD-like receptors (NLRs), and C-type lectin receptors (CLRs). Pathogens express pathogen-associated molecular patterns (PAMPs), which are recognized by PRRs.[9] Upon encountering a PAMP, DCs use these receptors for pathogen binding, internalization, antigen presentation, and immune activation.[10] Of all the species of schistosomes, *S. mansoni* has been most widely studied and is also the most implicated in human schistosomiasis. The different stages in the life cycle of *S. mansoni* produce a complex mixture of glycoproteins, glycolipids, and other glycoconjugates that are recognized by the PRRs of dendritic cells.

C-type lectins and the role of DC-SIGN. C-type lectins are considered to be a superfamily of more than a thousand proteins subdivided into 17 groups based on their domain architecture.[11] C-type lectins express one or more carbohydrate recognition domains (CRDs) that can recognize carbohydrates (or glycan) of pathogens in a Ca^{2+} dependent manner, capture them and present them to lymphocytes. C-type lectins also comprise of many domains that lack the components for a Ca^{2+} dependent binding, thus recognizing a much broader set of ligands like proteins and lipids.[12] C-type lectins like macrophage galactose-type C-type lectin (MGL), dendritic cell-specific intercellular adhesion molecule-3-grabbing nonintegrin (DC-SIGN), the mannose receptor (MR), and Dectin-1 contain one or more CRDs that are responsible for driving the Th2 response upon infection with parasites like *S. mansoni.*[13]

DC-SIGN is a calcium dependent carbohydrate binding protein that binds to epitopes displaying mannose and fucose moieties.[14] DC-SIGN forms a link between adaptive and innate immune response and is responsible for maintaining Th1/Th2 balance. The extracellular domain of DC-SIGN is composed of a neck region made up of seven and a half tandem repeats of 23 amino acids, followed by a CRD.[15] The neck region presents itself in the form of an α-helix which is expressed in the form of tetramers and directs the CRD away from the cell surface.[15a, 15c] The presentation of DC-SIGN in a tetrameric form increases the specificity and avidity of ligands that would be too weak to bind individually with a monomeric form.[16]

Fucose signaling in DC-SIGN. DC-SIGN recognizes a remarkably broad set of fucosylated glycans through its carbohydrate recognition domain (CRD). Fucose is also found abundantly expressed in the glycoprotein repertoire of *S. mansoni*. DC-SIGN uses fucose-specific pathogen-associated molecular patterns (PAMP) recognition to induce a Th2 immune response.[17] *S. mansoni* manipulate the Th2 response and produce an anti-inflammatory environment thus suppressing allergies and inflammations and ensuring their undetected survival in the host.[18] The characteristic *N*-glycans of *S. mansoni* are dominated by terminal GalNAc-GlcNAc residues (Lac-di-NAc).

These modifications found very rarely in mammalian glycoconjugates, are omnipresent in *S. mansoni*, particularly when they are decorated with fucoses in unique linkages. Analytical studies show a glycan profile dominated by mono and bis-fucosylated Lac-di-NAc, namely GalNAcβ1-4(Fuca1-3)GlcNAc (LDN-F) and GalNAcβ1-4(Fucα1-2Fucα1-3)GlcNAc (LDN-DF).[19] Several studies have shown that binding of DC-SIGN is fucose mediated, and that the epitope presentation is essential for good binding efficiencies.[20] Thus acquiring well-characterized glycan standards is an important prerequisite to studying glycan-lectin interactions.

Human Immuno-modulation/ regulation in schistosomiasis. Amongst all species of schistosomes, the most widely studied is *S. mansoni* because it is also responsible for majority of infections caused in humans. A large body of literature is dedicated to understanding the exemplary immuno-modulatory effects exhibited by *S. mansoni*, affecting both adaptive and acquired immunity. The average life span of an adult schistosome worm in human body is five years during which it lays thousands of eggs. These parasites ensure their survival in the hosts by developing a plethora of mechanisms and tactics to evade or manipulate the immune system. This includes immuno-modulation affecting the Th2 response in hosts, living intracellularly in host tissues, developing glycocalyx that make the immune defense system helpless, and changing the glycan (antigen) expressions during their developmental stages to resemble that of the host.[21]

Schistosomes like *S. mansoni* have developed an immune-modulation strategy that not only helps them overcome host immunity, but also regulate it in such a way so as to make the host conditions apt for their survival and thriving, to ensure higher chronicity in infections, sometimes even for decades.[3] Upon infection, *S. mansoni* initially produce a Th1 response, which then switches to a "modified" Th2 response in the host, in which an anti-inflammatory environment is created which is most favorable for their survival and tends to suppress allergies or inflammations in the host. In a modified Th2 response, an induction of Th2 immune response in the hosts is accompanied by a suppression of Th1 pro-inflammatory cytokines (like IFN-γ).[18] There is T-cell hyporesponsiveness, T-cell anergy, and upregulation of Treg cells, which suppresses the inflammatory Th1/Th17 response.[21a] This results in a controlled cellular inflammation and tissue damage, and hence a subdued immune response It is of note that in normal infections, a typical Th2 response would produce an uncontrolled inflammation, thus resulting in expulsion of the pathogen from the body. Thus, *S. mansoni* drives a DC-SIGN response towards Th2 which causes persistence of infection in the host.[22]

Prevention and Cure. Currently, the only treatment employed for schistosomiasis is the drug Praziquantel which is being employed for mass treatments of entire communities endemic to this infection. The mechanism of praziquantel action has been well investigated. It works through the influx of Ca^{2+} into the parasite which results in paralysis of the worm and tegument damage, exposing the worm surface antigen to host immune action. A continuous immune action against the worm is required to inhibit tissue recovery and ultimately worm death.[23] The downside of

praziquantel treatment is that it is not effective in all stages of the life cycle of *S. mansoni*. It is effective in killing adult schistosome worms but has no effect on the eggs or the sexually immature stages involved in the infection stage, and therefore schistosomiasis continues to re-emerge.[24] Further, treatment with praziquantel is often accompanied with severe side-effects including seizures, bloody diarrhea, irregular heartbeat and fatigue. Lastly, overuse of the drug can bring about drug resistance, and thus the only weapon against schistosomiasis will be rendered useless.

To curb such high infection rates, it is logical to concentrate on the advancement of vaccine development and there have been several attempts to synthesize vaccine candidates for schistosomiasis.[25] Despite showing promise in early stages, none could reach the desired efficiency for all species of schistosomes, and hence the vaccination research program is currently stalled. The primary reasons for the failure of vaccination programs is that the correct antigen has not been identified yet, and that the vaccine formulation was not successful in order to induce protective immunity.[25c] Normon Stoll, in his pioneering article published in 1947, "This wormy world", attempted to calculate the extent of human helminthiasis in the world. He predicted that schistosomiasis would be controlled and cured by the end of the 20th century.[26] It is a sobering thought that little has changed since then, despite accumulating decades of research data. To curb schistosomiasis, the best strategy would be to apply an integrated approach which includes mass drug administration, vaccine development, health education and sanitation awareness.[25d]

Biosynthesis of *N*-glycans of *S. mansoni*. The genome of *S. mansoni* was reported in 2009, in which at least 11,000 genes were annotated encoding over 13,000 transcripts. By 2012, the genome sequence of other species of schistosomes including *S. japonicum* and *S. heamtobium* were also deciphered. These developments can in future lead way to understanding the complex glycan biosynthesis of these parasites.The biosynthesis seemingly proceeds through the conventional *N*-glycan biosynthetic pathway with the sequential addition of fourteen sugars onto the dolichol phosphate in the cytoplasm of ER by enzymes homologous to the mammalian the *ALG* gene enzymes.[27] Similar to mammalian *N*-glycans, there in an *en bloc* transfer of the fourteen membered glycan structure to Asn-X-Ser/Thr sequon of a protein, by a set of homologs of the oligosaccharyl transferase.[28] A review by Cummings and co-workers summarised succinctly the enzymes involved in the biosynthesis of *N*-glycans of schistosomes.[27] Some examples of the enzymes and their mammalian counterparts are listed below:

Enzymes	**Mammalian enzymes**	***S. mansoni* analogs**
GlcNAcT	ALG 7, 13, 14	Smp 0454303, Smp 082710
ManT	ALG 1, 2, 3, 9, 11, 12	Smp 177080, Smp161590, Smp103930
GlcT	ALG 6, 8, 10	Smp096910, Smp 15120
Glucosidases	Glucosidase I, II	Smp 024580, Smp 018760
Mannosidases	MAN1A1, A2	Smp018750, Smp143430

Table 1. Mammalian enzymes and their *S. mansoni* counterparts.

Synthesis of *N*-glycans of Schistosomes for parasitological diagnosis. As described in chapter 1, there are many glycan structures associated with glyco-conjugates (lipids, proteins), on cell surface of both, the parasite and the host, acting as the contact site for cellular signaling, recognition and communication. *N*-glycans are expressed in all stages in the life cycle of *S. mansoni* and the epitopes expressed on these *N*-glycans also change during its maturation to worm stage.[29] *S. mansoni* rely on "glycan mimicry", i.e., they evolved with their hosts and express glycan structures fairly similar to their hosts and it is suggested that glycan mimicry is an important factor in immune-evasion by *S. mansoni*.[21a] However, recent studies hint that seemingly slight and unremarkable modifications on the *N*-glycans are recognized as antigenic motifs.[30]

A considerable amount of effort has been put into understanding the immunological manipulations caused during schistosomiasis and synthesizing the glycans to test their antigenic properties. Hada and co-workers synthesized three neutral glycosphingolipids and six oligosaccharide derivatives found in *S. mansoni* to explore their potential as a diagnostic tool for the detection of schistosomiasis from patients' sera.[31] Hokke and co-workers synthesized spacer linked highly fucosylated linear oligosachharides typical to *S. mansoni*. [32] These epitopes were then used to screen an anti-schistosome monoclonal antibodies library by SPR. This is an important tool for the diagnosis of schistosomiasis. Ito *et al.* synthesized complex type *N*-glycans characteristic of schistosome eggs.[33] Taking this work forward, Reichardt and co-workers synthesized a library of *N*-glycans of *S. mansoni* and studied their binding with the human lectin DC-SIGN and with serum antibodies of patients suffering from schistosomiasis.[34] Recently, they also synthesized *O*-glycan cores observed in the infectious stages of schistosomiasis, the mucin core 2 and the *S. mansoni* core and studied their binding with C-type lectins like DC-SIGN, DC-SIGNR and MGL.[35]

The access to pure glycans in sufficient quantities is paramount in investigating their immunological implications. However, despite tremendous progress in the field of synthesis of complex oligosaccharides including enzymatic reactions and automated synthesis, the synthesis of glycans of lower organisms like parasites and bacteria is still lagging owing to poor understanding of their genome and enzyme expression. Such glycans are therefore exclusively obtained by chemical synthesis. Chemical synthesis is often tedious, owing to continuous protection and deprotection strategies, difficulty in exclusive stereoselectivity during glycosylation and overall low step efficiency.

However, the research conducted until now points to an optimistic future and have laid a sound foundation for future experiment, with an aim to design highly complex and comprehensive sets of libraries of *N*-glycans to study their interaction of DC-SIGN. The chemo-enzymatic synthetic strategies described in this thesis aim to democratize the synthesis of such complex oligosaccharides, which can be used for other relevant research. In case of *S. mansoni*, the study of their characteristic epitopes and *N*-glycans at a molecular level through STD-NMR, molecular

modeling and micro-array can point to the perfect glycan structure(s) that can then be screened as antagonists to generate anti-DC-SIGN antibodies to manipulate the Th1/Th2 balance to result in immune activation, inflammation, and expulsion of the parasite from the host.

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CHAPTER 3

Mono- and Di-Fucosylated Glycans of the Parasitic Worm *S. mansoni* are Recognized Differently by the Innate Immune Receptor DC-SIGN

Abstract. The parasitic worm, *Schistosoma mansoni* expresses unusual fucosylated glycans such as LDN-F and LDN-DF in a stage-dependent manner that have been implicated in shaping host immune responses. We have developed a synthetic approach for mono- and di-fucosylated LacdiNAc (LDN-F and LDN-DF, respectively), which are epitopes expressed on glycolipids and glycoproteins of *S. mansoni*. The synthetic strategy is based on the use of monosaccharide building blocks consisting of carefully selected amino- protecting groups, facilitating high yielding and stereoselective glycosylations. The molecular interaction between the synthetic glycans and DC-SIGN was studied by NMR and molecular modeling, which demonstrated that the α-1,3-fucoside of LDN-F can coordinate with the Ca^{2+} ion of the canonical binding site of DC-SIGN allowing for additional interactions with the underlying LDN backbone. The 1,2-fucoside of LDN-DF can be complexed in a similar manner, however, in this binding mode GlcNAc and GalNAc of the LDN backbone are place away from the protein surface resulting in a substantially lower binding affinity. Glycan microarray binding studies showed that the avidity and selectivity of binding is greatly enhanced when the glycans are presented multivalently, and in this format Le^x and LDN-F gave strong responsiveness whereas no binding was detected for LDN-DF. The data indicates that *S. mansoni* has developed a strategy to avoid detection by DC-SIGN in a stage -dependent manner by the addition of an extra fucoside to its ligands.

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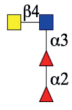
Introduction. Schistosomes are parasitic helminths that infect over 250 million people worldwide and are responsible for 280,000 deaths annually. They manipulate the host's immune system to establish chronic infections.[1] They can manipulate the host's immune system to establish chronic infections. Although the molecular basis of these immune-moldulatory mechanisms remain poorly understood, it has been established that glycans of schistosomes can induce specific innate immune responses in the infected host, which in turn affects adaptive immunity. This occurs through an interplay between Toll like receptors (TLRs) and C-Type lectin receptors (CLRs) of dendritic cells (DCs) thereby tuning immune responses towards an immune activation or tolerant state.[2] Schistosomes can biosynthesize a vast array of glycoconjugates, many of which are expressed at specific stages of their complex life cycle. The glycoproteins and glycolipids of schistosomes lack sialic acid and contain a variety of terminal glycan epitopes, including fucosylated antigens such as, Lewis-x (Le^x), pseudo-Lewis-y (pseudo Le^y), GalNAcβ1,4(Fucα1,3)GlcNAc (LDN-F), Fucα1,3GalNAcβ1,4(Fucα1,3)GlcNAc (F-LDN-F), GalNAcβ1,4(Fucα1,2Fucα1,3)GlcNAc (LDN-DF) and Fucα1,2Fucα1,3GalNAcβ1,4(Fucα1,2Fucα1,3)GlcNAc (DF-LDN-DF) (Table 1).[3] These glycan motifs are rarely found in mammalian glycoconjugates but are typical signatures of schistosomes.[2a, 4]

Dendritic cell-specific ICAM-3 grabbing nonintegrin (DC-SIGN), which is a C-type lectin expressed by immature dendritic cells, has been implicated in schistosomiasis.[5] DC-SIGN binds in a Ca^{2+}-dependent manner glycan motifs shared by many microorganisms, thereby facilitating pathogen internalization, targeting to lysosomes for antigen processing and presentation. It is also a signaling receptor and binding of glycans can trigger signal transduction pathways leading to the expression of specific cytokine profiles that in turn control T-cell polarization.[6] The carbohydrate binding domain of DC-SIGN is flexible and can recognize high-mannose structures as well as a variety of fucosylated glycans such as Le^x, Le^y and even histo blood group A and B antigens.[7]

Glycan Structure	Abbreviation	Symbol
GalNAcβ1-4GlcNAc-	LDN	
Galβ1-4(Fucα1-3)GlcNAc-	Le^x	
GalNAcβ1-4(Fucα1-3)GlcNAc-	LDN-F	
GalNAcβ1-4(Fucα1-2Fucα1-3)GlcNAc-	LDN-DF	

Key: ■ : N-Acetyl-D-glucosamine ▲ : L-Fucose
□ : N-Acetyl-D-galactosamine ○ : D-Galactose

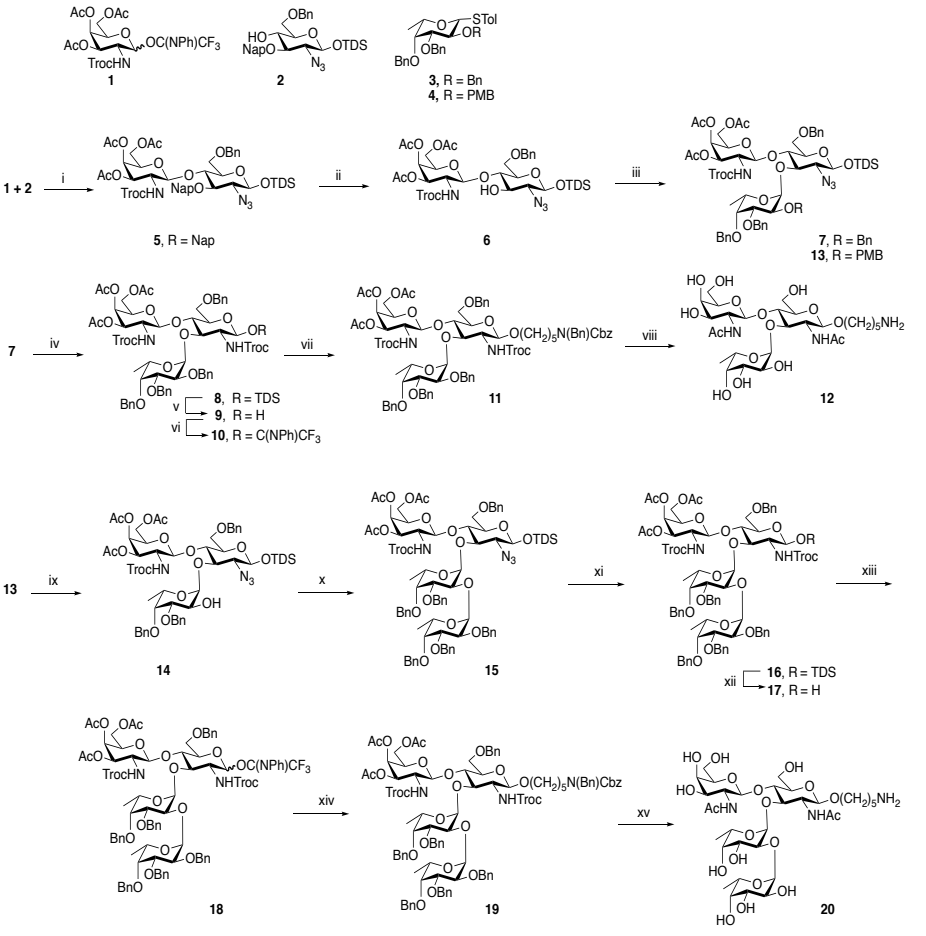
Table 1. Major glycan structures expressed by *Schistosoma mansoni*.

The binding of LDN-F by DC-SIGN has only been demonstrated indirectly,[8] however, due to the structural similarity to Le^x, it is to be expected that it is an appropriate ligand for this immune receptor. The use of blocking antibodies has indicated that DC-SIGN cannot detect difucosylated LDN-DF.[7] This glycan is expressed in a stage dependent manner by the pathogen,[9] and it appears that the action of only one fucosyl transferase that converts LDN-F into LDN-DF abolishes immune detection. DC-SIGN has broad ligand specificity and can recognize a plethora of fucosylated glycans, and thus an important question to address is how LDN-DF can avoid detection by DC-SIGN.

In this chapter, we report the first chemical synthesis of LDN-DF and LDN-F by a convergent block synthetic approach. The availability of these compound made it possible to examine their molecular recognition by DC-SIGN using STD-NMR, chemical shift perturbation, trNOESY and molecular modeling studies. It was found that the binding of LDN-F is in fact very similar to that of structurally related Le^x.[7b] A surprising finding was that DC-SIGN can also interact with the terminal fucoside of LDN-DF, however, in this case no further interactions are possible with the underlying glycan moiety resulting in a lower affinity binding. The interaction of DC-SIGN with LDN-DF, LDN-F, Le^x, di-Le^x and SLe^x-Le^x was also examined in a glycan microarray format, which showed strong responsiveness of Le^x and LDN-F but no binding to LDN-DF was detected, indicating that through multivalent interactions avidity and selectivity of binding is substantially enhanced.

Chemical Synthesis. The preparation of *Schistosoma*-derived glycans has received relatively little attention,[10] and the chemical synthesis of LDN-DF has not been reported. The latter compound represents a challenging synthetic target because it requires careful selection of amino protecting groups to construct an LDN derivative that can be glycosylated with a fucosyl donor having a temporary protecting group at C-2 to allow installation of the subsequent 1,2-linked fucoside. The amino protecting groups need to be selected in such a way that they promote the introduction of β-glycosides, but do not sterically block the introduction of the 1,3-fucoside. Furthermore, the target compounds need to be modified by an aminopentyl linker, which is required for glycan microarray printing.

We found that the monosaccharide building blocks **1**-**4** are appropriate for the assembly of aminopentyl modified LDN-F (**12**) and LDN-DF (**20**) (Scheme **1**). In this respect, the NHTroc protecting group of **1** assures that a β-glycoside is formed when glycosylated with acceptor **2**, which has its C-2 amino group masked as azide. The presence of the latter functional group is important because after oxidative removal of the Nap ether, an acceptor is formed (**6**) that sterically is sufficiently unencumbered for glycosylation with fucosyl donor **3**. The latter compound also has a removable PMB ether allowing the installation of an 1,2-fucoside using donor **4**. After assembly of a tetrasaccharide, the azide can be converted into Troc and a glycosylation with properly protected aminopentanol will give linker modified compound LDN-DF (**19**) as only the β- anomer.



Reagents and conditions. i) TMSOTf, DCM, -30 °C, 91%; ii) DDQ, DCM, DCM/H_2O, 84%; iii) DPS/Tf_2O, DCM, -60 °C to -40 °C, 68% for **7** and 73% for **13**; iv) (a) PPh_3, THF/H_2O (b) trocCl, NEt_3, DCM, 63% (over two steps); v) HF/py in pyridine; vi) 2,2,2-Trifluoro-*N*-phenylacetimidoyl Chloride, DBU, DCM; vii) $HO(CH_2)_5N(Bn)Cbz$, TMSOTf, DCM, -30 °C, 66% (over 3 steps); viii) (a) Zn dust, AcOH, Ac_2O, THF (b) NaOMe, MeOH (c) Pd/C, H_2, H_2O/ MeOH, 45% (over three steps); ix) DDQ, DCM/H_2O, 74%; x) TMSOTf, DCM/ DMF, -30 °C to +5 °C, 83%; xi) (a) PPh_3, THF/H_2O (b) TrocCl, NEt_3, DCM; xii) HF/py in pyridine; xiii) 2,2,2-Trifluoro-*N*-phenylacetimidoyl Chloride, DBU, DCM; xiv) $HO(CH_2)_5N(Bn)Cbz$, TMSOTf, DCM, -30 °C, 31% (over five steps); xv) (a) Zn dust, AcOH, Ac_2O, THF (b) NaOMe, MeOH (c) Pd/C, H_2, H_2O/MeOH, 37% (over three steps).

Scheme 1. Synthesis of LDN-F and LDN-DF epitope.

A TMSOTf-mediated glycosylation of *N*-phenyl trifluoracetimidate donor **1** with acceptor **2** afforded disaccharide **5** in a yield of 91%. The Nap ether of **5** was removed using DDQ in a mixture of DCM and H_2O to give acceptor **6** in a yield of 84%. Next, fucosyl donors **3** and **4**, having Bn and PMB ether at C-2, respectively, were preactivated with DPS/ Tf_2O in the presence of TTBP at -60 °C,[11] which was followed by the addition of acceptor **6**. The resulting reaction mixture was allowed to slowly warm to -40ºC resulting in formation of trisaccharides **7** and **13**, which were isolated as mainly the a-anomers in good yield (**7**, 68%; $J_{1,2}$ = 3.5 Hz; $^1J_{C,H}$ = 174.9 Hz) and (**13**, 73%; $J_{1,2}$ = 3.8 Hz; $^1J_{C,H}$ = 175.4 Hz). Alternative glycosylation conditions result in poor anomeric selectivity (Table S1). Trisaccharide **7** was further modified with an anomeric aminopentyl linker and to attain β-anomeric selectivity, the azide was converted into NHTroc (→**8**) by a two-step procedure entailing reduction using triphenyl phosphine followed by reaction of the resulting amine with TrocCl. Next, glycosyl donor **10** was prepared by removal of the anomeric TDS ether of **8** with HF/pyridine followed by reaction with *N*-phenyl trifluoroacetimidate in the presence of DBU. As anticipated, a TMSOTf mediated glycosylation of **10** with *N*-benzyloxycarbonyl-*N*-benzyl-5 aminopentanol gave **11** as only the β-anomer in 87% yield. The latter trisaccharide was globally deprotected by a three-step procedure in which the NHTroc was converted to NHAc using zinc dust, followed by global deacetylation and hydrogenation to provide spacer modified LDN-F **12**. A number of alternative strategies were explored to prepare LND-F, and it was found that judicious selection of amino protecting groups was critical, and the use of bulky protecting groups such as *N*-phthalimido resulted in a donor-acceptor reactivity mismatch, resulting in almost immediate hydrolysis or degradation of donor (Table S1). In addition to donor **4** having a temporary PMB ether, several alternatives were examined but these gave disappointing results (Table S1).

Next, attention was focused on the preparation of spacer modified LDN-DF **20**. Thus, treatment of trisaccharide **13** with DDQ in DCM/H_2O afforded acceptor **14**. Several reaction conditions were explored (Table S2) to install a fucoside, and tetrasaccharide **15** was obtained with high a-anomeric selectivity in good yield (83%) when the glycosylation was performed in DCM in the presence of DMF[12] and promoted by 1 equivalent of TMSOTf at -20 °C followed by slow warming to +5 °C ($J_{1,2}$ = 3.9 Hz; $^1J_{C,H}$ = 173.3 Hz). The anomeric TDS group of **15** was removed and the resulting lactol (**17**) converted into a *N*-phenyl trifluoroacetimidate **18**, which was coupled with *N*-benzyloxycarbonyl-*N*-benzyl-5-aminopentanol to give **19**. Global deprotection of the latter compounds gave target tetrasaccharide **20** in an overall good yield (37% over three steps).

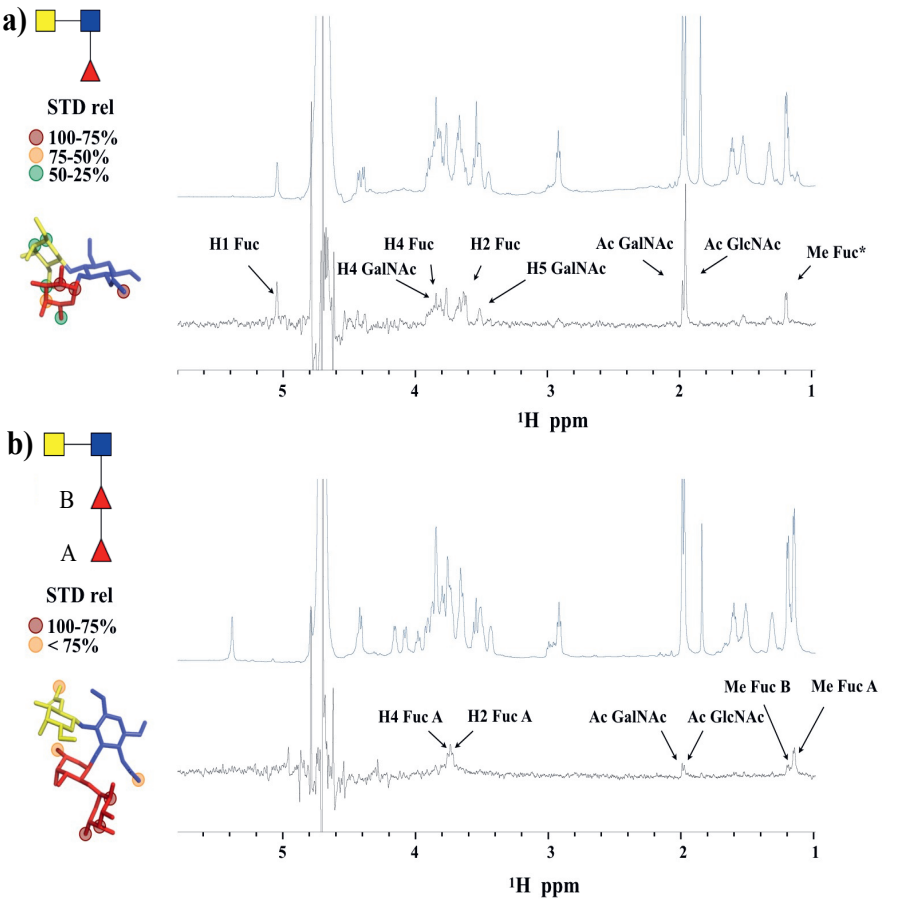
Molecular Basis of Binding of LDN-F and LDN-DF to DC-SIGN. The molecular basis of the interactions of DC-SIGN with LDN-F (**12**) and LDN-DF (**20**) in solution was examined by combining Saturation Transference Difference (STD-NMR), chemical shift perturbation analysis of the protein backbone, Nuclear Overhauser Effect (NOE) evaluation, and molecular modeling. The NMR-based strategy combines molecular recognition analysis from the perspective of the protein and ligand thereby providing a detailed atomic view of the interactions. ^{1}H-STD-NMR

experiments were performed to establish which parts of LDN-F and LDN-DF make direct contacts with the lectin. ^{1}H-^{15}N HSQC experiments on the ^{15}N-labeled carbohydrate recognition domain (CRD) of DC-SIGN were conducted to identify protein residues involved in ligand binding and to establish whether differences exist between the two epitopes. Furthermore, ^{1}H-^{15}N HSQC titration experiments provided binding affinity estimations. Finally, trNOESY experiments characterized the ligand conformation in the bound state and additionally provided short intermolecular distances between the ligand and the protein, thus defining the precise binding pose.

^{1}H Saturation Transfer Difference (STD) NMR. ^{1}H-STD NMR spectra resulting from the interaction of LDN-F and LDN-DF with DC-SIGN extracellular domain (ECD) which tetramerizes in solution, along with the corresponding reference spectra are shown in Figures 1a and 1b, respectively. It demonstrates that DC-SIGN can bind both glycans but with substantial differences in binding mode. Both ligands bind through the terminal fucoside. The STD data for LDN-F are similar to those previously reported for the structurally related Le^{x} trisaccharide,[7b] indicating a similar mode of binding. As for Le^{x}, LDN-F is recognized by DC-SIGN through the fucoside and additional contacts are made with the GalNAc pyranosyl ring and the *N*-acetyl group of the GlcNAc moiety. The *N*-acetyl moiety of GalNAc exhibited only a weak STD effect, indicating that it does not contribute substantially to binding (Figure 1a).

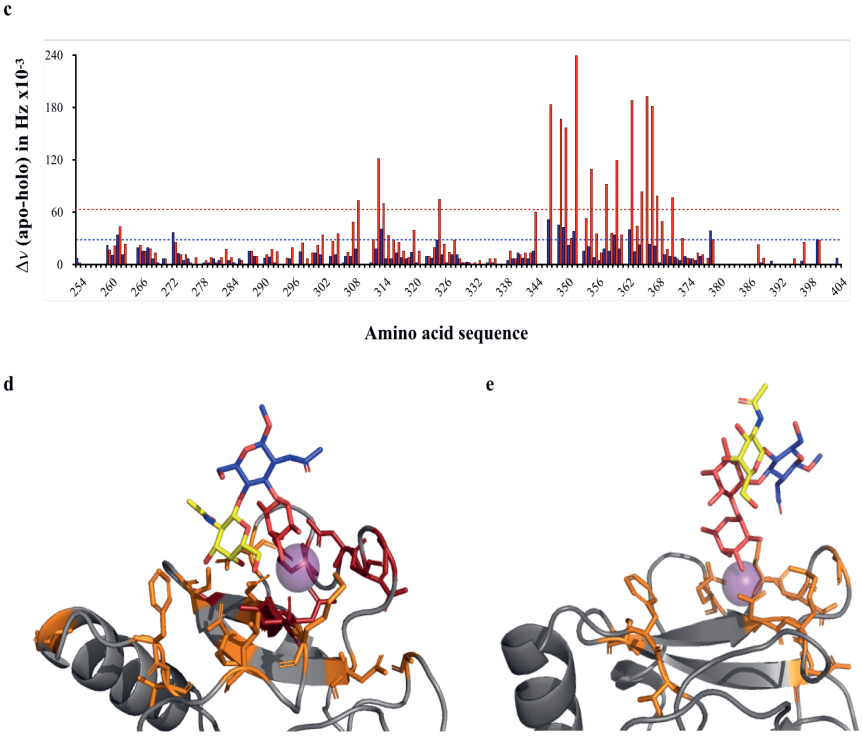
Detectable STD effects of the interaction of DC-SIGN with LDN-DF were restricted to H2, H4 and Me of the terminal 1,2-fucoside with a remarkable absence of STD signals from the internal 1,3-fucoside and the GalNAc and GlcNAc moieties (Figure 1b). The much lower intensities of the STD signals of the LDN-DF compared to those acquired for the LDN-F suggests weaker binding of the former. The blank ^{1}H-STD NMR experiments of LDN-F and LDN-DF ligands alone are shown in SI (Figures S1 and S2).

Chemical shift perturbation analysis. The primary carbohydrate binding site of DC-SIGN is composed of an extended loop (from W343 to D355) and residues in β-strand-4 (from N363 to D367), which surround the principal Ca^{+2} ion.[13] Additionally, residues in β-strands-3 and -2 (from E356 to G361 and F313, respectively) shape a secondary binding region. Chemical shift perturbation analysis using the ^{15}N-labeled protein was performed to examine which residues of the CRD of DC-SIGN interact with the glycans. Thus, the ^{15}N-labeled CRD DC-SIGN was titrated with LDN-F and LND-DF and ^{1}H-^{15}N-HSQC spectra were acquired at every titration point (Figure S3). Both ligands provided similar chemical shift perturbation (CSP) profiles involving amino acids of the primary and secondary binding site. However, upon addition of the same number of equivalents, the CSP of the lectin backbone NH resonances were much larger for LDN-F than for LDN-DF (Figure 1c), indicating weaker binding of the latter compound. Indeed, fitting of the CSP to the corresponding binding isotherms for LDN-F yielded a K_D of 1.5 ± 0.4 mM (Figure S3). Protein saturation was not possible with LND-DF providing an imprecise K_D estimation, but substantially larger than 6 mM.



[1]H STD-NMR spectra for the interaction of full-length DC-SIGN with (a) LDN-F and (b) LDN-DF. Relative STD and epitope mapping are shown on the left side for both ligands and refer to double difference between the STD spectrum in the presence of the protein and that in the absence of it. STD spectra were acquired in the same conditions, however, drastic difference in absolute STD intensities between the two ligands exist. Specifically, the maximum STD signal detected for LDN-DF ligand corresponds to just the 25% of the strongest STD.

Figure 1. STD-NMR spectra.

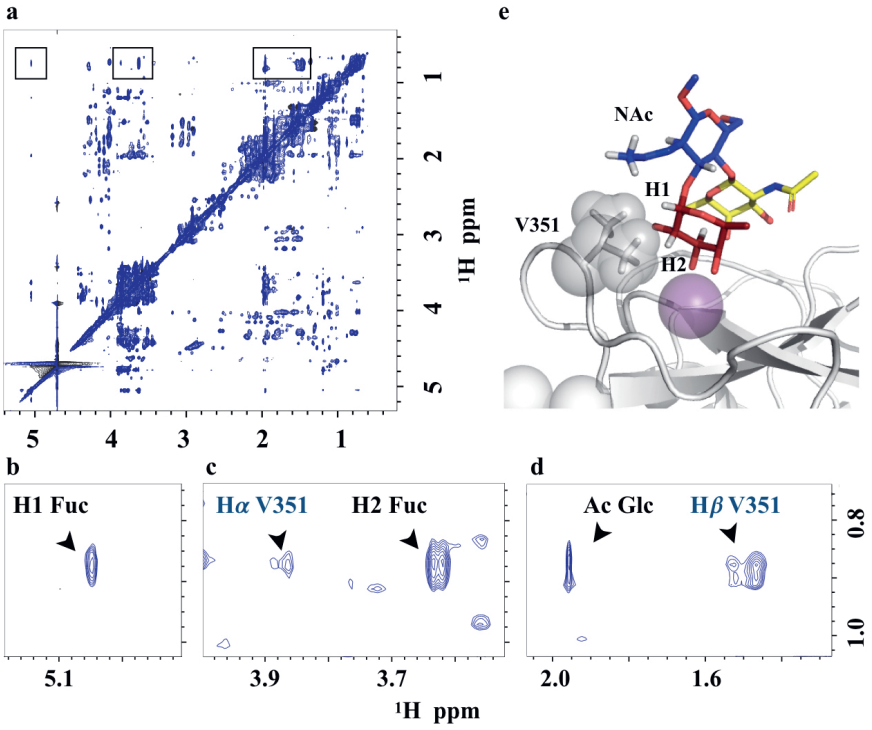


Interaction of full-length DC-SIGN (c) Average chemical shift perturbation upon the addition of LDN-F (red) and LDN-DF (blue). Structural models for the complexes of CRD DC-SIGN with the LDN-F ligand (d) and the LDN-DF ligand (e) obtained from MD simulations.

Figure 2. Molecular Modeling.

Transferred NOESY. Recently,[7d] the molecular complexes of DC-SIGN with A and B histo blood group antigens have been studied by trNOESY experiments. Key intermolecular NOEs between the Hγ protons of V351 with H1 and H2 of the fucoside moiety were observed. The importance of the van der Waals stabilizing contacts involving the Me groups of V351 for the recognition of branched fucosylated epitopes has also been shown by X-ray crystallography,[14] and is in agreement with mutagenesis studies, demonstrating that substitution of this residue has important implications for the binding of Lewis type epitopes.[15] Therefore, a trNOESY experiment was carried out for LDN-F in the presence of 0.2 equivalents of the DC-SIGN CRD. After addition of the protein to the NMR tube, strong negative NOE were observed for the ligand protons, which contain information on the bound ligand conformation. Inter-molecular NOE correlations between

the methyl groups of V351 and H1-Fuc, H2-Fuc, and the methyl group of GlcNAc were observed (Figure 2). Consistent with the STD analysis, Fuc H1 and H2 are in close contact with the lectin. Moreover, the strong STD described above for the methyl moiety of the GlcNAc residue is supported by the tr-NOE correlation between this group and V351. As control, the NOESY spectrum of the free ligand was measured which displayed very weak negative NOE effects. Unfortunately, a good tr-NOESY spectrum could not be recorded for the LDN-DF complex, probably due to its rather low binding affinity.



(a) NOESY spectrum of the complex of DC-SIGN with LDN-F; (b, c and d) The quest of protein/ligand intermolecular NOEs; (e) Three-dimensional model from the MD simulation showing the key intermolecular NOEs between the ligand and the V351 residue of the lectin.

Figure 3. NOESY spectrum.

Molecular Modeling. The NMR data were employed to derive three-dimensional models for the complexes of DC-SIGN with LDN-F and LDN-DF. For LDN-F, the experimental STD and intermolecular NOE data show close contacts of H1-Fuc, H2-Fuc and Ac-GlcNAc with the methyl group of V351 of the protein. For the histo blood A and B antigens,[16] it has been demonstrated that DC-SIGN binds the fucosyl ring exclusively through coordination of the C-3 and C-4 hydroxyls with the Ca^{2+} ion. The crystallographic structure of DC-SIGN complexed with Le^x (pdb code 1SL5) also fulfills this requirement.[14] Therefore, the pyranosyl ring of LDN-F was superimposed onto the corresponding monosacc haride in the deposited 1SL5 structure. The resulting binding pose was minimization by MD simulation that resulted in a structure that is in excellent agreement with the experimental data, including HSQC chemical shift perturbation and epitope mapping through STD. For LDN-DF, a similar approach was used by superimposing the terminal 1,2-linked fucoside in the primary Ca^{2+} binding site. This starting binding pose placed H2 of the terminal fucoside in close proximity to the protein backbone, which is in agreement with the STD data. The alternative binding pose through the inner fucoside was discarded due to steric clashes. Analysis of the MD trajectory showed that for the complex of LDN-F with DC-SIGN, the ligand conformation remained fairly well defined as revealed by the low dispersion of the φ and ψ angles (Figure S4). This arrangement favors hydrophobic contacts between V351 and the Fuc and GlcNAc moieties, which were maintained throughout the entire MD run (Figure 1d). Additionally, the bound geometries were validated by simulating the STD spectrum with CORCEMA-ST.[17] The match between the expected and the experimental STD intensities for LDN-F was excellent, further supporting the proposed binding model (Figure S5).

The derived bound structure for LDN-DF was very different (Figure 2e). In this case, the LND backbone was far from the protein with only the terminal fucoside making interactions. In this case, the contacts between the Ac of GlcNAc and Hγ protons of V351 were only transient and there was not a preferential spatial arrangement of both groups to make substantial van der Waals contacts. Although the LDN-F moiety of LDN-DF preserved conformational rigidity, the α1,2-fucoside linkage was rather flexible (Figure S1) providing a loosely defined epitope presentation around the primary binding site. In this case, the fitting between the CORCEMA-ST simulations with the experimental STD was less accurate, which is probably due to the weak STD signals and the inability of the MD simulation to reproduce the flexibility of the complex.

Glycan Microarray Binding Studies. The CRDs of DC-SIGN are clustered in tetramers, and such an arrangement can greatly amplify the avidity and specificity when interacting with glycans epitopes that are present in a multivalent arrangement.[18] To examine the importance of multivalency, LDN-F (12) and LDN-DF (20) and a number of control glycans including Le^x (21), Le^x-Le^x (22), SLe^x (23) and SLe^x-Le^x (24), all of which are equipped with an anomeric aminopentyl moiety, were immobilized on *N*-hydroxysuccinimide (NHS)-activated glass slides in replicates of 6 by piezoelectric printing. After incubation overnight in a saturated NaCl chamber,

unreacted esters were quenched with ethanolamine. First, the glycan microarray was probed with biotinylated *Aleuria aurantia* lectin, which recognizes α1,2- α1,3- and α1,6-fucosides, and Streptavidin-AlexaFluor635. As anticipated all compounds showed strong responsiveness (Figure S3) confirming proper spot morphology and printing. Next, sub-arrays were incubated with various concentrations of recombinant human DC-SIGN-Fc chimera premixed with anti-IgG Fc-biotin and Streptavidin-AlexaFluor635 in TSM binding buffer containing Ca^{2+}. After incubation for 1 h, the slide was washed, dried by centrifugation, and scanned for fluorescence intensity. LDN-F (**12**), Le^x (**21**) and Le^x-Le^x (**22**) exhibited strong responsiveness whereas no binding was detected for LDN-DF (**20**) (Figure 3). This observation indicates that the avidity and selectivity of binding is greatly enhanced when the binding is probed on a multivalent surface. At a higher concentration of DC-SIGN, SLe^x-Le^x (**24**) also exhibited responsiveness whereas this was not the case for SLe^x, indicating that the internal Le^x moiety can be recognized by DC-SIGN. When the microarray binding studies were performed in the absence of Ca^{2+}, no binding was observed confirming specificity of binding.

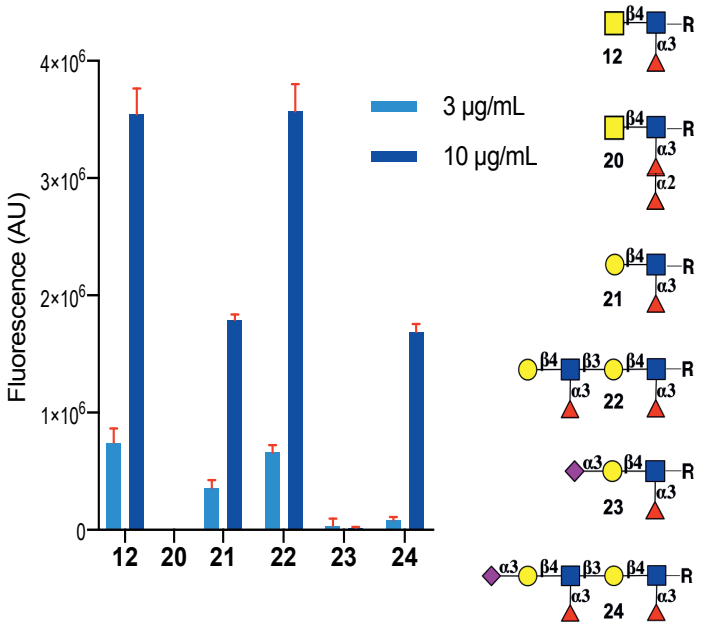


Figure 4. Microarray results of the glycan library printed at 100 µM for binding to DC-SIGN (3 and 10 µg/mL). Bars represent the mean ± SD.

Conclusion.

A number of pathogens can hijack or evade the host's immune system by phase-variable glycosylation.[19] *S. mansoni* is such a pathogen that can cause life-long infections, alternating latent with active phases and re-activation of the host's immune system. During the life cycle of *S. mansoni*, certain fucosylated glycans such as LDN, Le^x, LDN-F and LDN-DF are expressed in a stage-dependent manner.[4, 20] There are indications that the pattern-recognition receptor DC-SIGN can recognize some but not all of these glycans,[2a] suggesting they play a role in shaping host immune responses. Additionally, the molecular structure of DC-SIGN may change upon binding of different classes of glycans resulting in tailored immune responses.[21] Pathogens, such as *S. mansoni*, may exploit their phase variable glycosylation to modulate lectin recognition and subsequent host immune response. We have investigated, at a molecular level, in which way the terminal 1,2-fucoside of LDN-DF influences recognition by DC-SIGN. Such a study required well-defined glycans, which were obtained by a chemical approach in which amino protecting groups were carefully selected to facilitate high yielding and stereoselective chemical glycosylations. The molecular recognition LDN-F and LDN-DF by DC-SIGN was studied by NMR assisted by molecular modeling, which revealed that in solution it can recognize both glycans but with substantial differences in affinity and binding mode. The HSQC titration experiments provided a dissociation constant for LDN-F of 1.5 ± 0.4 mM at the monovalent level, whereas the k_D for LDN-DF could only be estimated but is substantially larger than 6 mM. In the case of the LDN-F, the α1,3-fucoside coordinates with the Ca^{2+}-ion of the CRD of DC-SIGN, placing the GlcNAc residue in close proximity to the protein surface thereby allowing for additional interactions. The affinity and structural model for LDN-F and Le^x [7b] are very similar, indicating that the presence of a β4GalNAc *vs.* a β4Gal moiety does not substantially alter binding.

The terminal α1,2-linked fucoside of LDN-DF can also bind into the canonical binding site of DC-SIGN but in this case, the GlcNAc and GalNAc residues are placed away from the protein surface preventing additional contacts, and as a result the binding affinity is approximately an order of magnitude lower.

Previous studies[2a, 22] have shown that antibodies directed against Le^x and LDN-F can block the binding of DC-SIGN to soluble egg antigen of *S. mansoni* whereas an antibody against LDN-DF had no effect on binding. These observations lead to the conclusion that DC-SIGN can recognize Le^x and LDN-F but not LDN-DF. Our studies have shown that LDN-DF can interact with DC-SIGN albeit with a substantial lower affinity than for Le^x and LDN-F. The glycan microarray studies showed that the avidity and selectivity of binding is greatly enhanced when the glycans are presented in a multivalent manner, and in this format Le^x and LDN-F gave strong responsiveness whereas no binding was detected for LDN-DF. The extracellular domain of DC-SIGN occurs as a tetramer. Furthermore, the glycans of *S. mansoni* are presented on its cell surface as glycoproteins and glycolipids, and thus can make multivalent interactions with DC-SIGN. Such interactions are

biologically highly relevant and can result in a substantial enhancement in avidity[18a] and magnify selectivites.[23]

The phase variable expression of glycans such Le^x, LDN-F and LDN-DF that interact differently with DC-SIGN, may offer *S. mansoni* a strategy to skew host immune responses in a stage dependent manner.[24] This may be important because some developmental stages, such as the development of schistosomes into sexually mature, egg-producing adults, require host-derived innate immune signals. Other stages, such as egg deposition require T_H2 responses. Additionally, to avoid fatal immunopathology, schistosome should also be able to dampen host immunity by stimulating regulatory immune cells, such as Bregs and Tregs. It appears that *S. mansoni* has developed an efficient strategy for phase switching and by the simple addition a fucoside, it can avoid detection by DC-SIGN. Further studies are required to determine the importance of the density of specific glycans during the different life stages of Schistosomes and their influence on DC-SIGN detection and subsequent skewing of host immune responses. Furthermore, structures such as LDN-DF and LDN-F are part of complex oligosaccharides in which multiple of these epitopes can be presented, and potentially such structures can make multi-valent interactions leading to high avidity of binding. The synthesis of such structures will make it possible to investigate such interactions at the molecular level. In addition to DC-SIGN, other C-type lectins have been implicated in sensing helminth glycans by human DCs. Among those, the macrophage galactose-type lectin MGL exhibits high specificity for *S. mansoni* glycans terminating in GalNAc.[25] It is conceivable that these lectins act in concert to detect patterns of glycans thereby shaping immune responses. Finally, the novel synthesis of LDN-F and LDN-DF may boost for further biological studies to address the role of unique fucosylated glycans in *S. mansoni* infectivity.

Experimental Procedures.

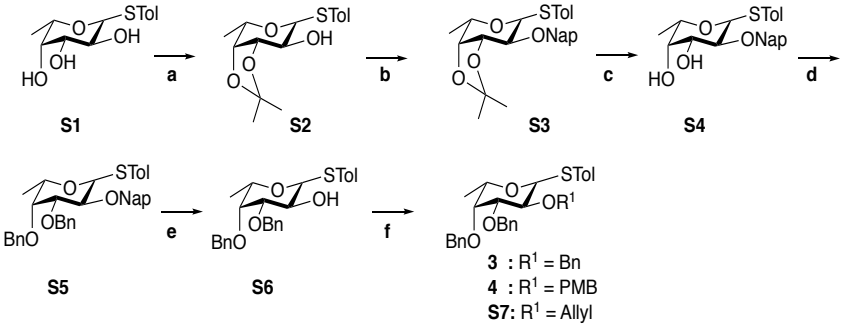
1. Chemical Synthesis of Building Blocks for Optimization Reactions.

1.1 General Methods.

Reactions were performed using flame-dried glassware with anhydrous solvents under an atmosphere of argon unless otherwise noted. Proton nuclear magnetic resonance (^{1}H -NMR) spectra were recorded with Varian 400 (at 400 MHz) or Bruker 600 (at 600 MHz) spectrometers. Multiplicities are assigned as singlet (s), broad singlet (br s), doublet (d), doublet of doublets (dd), triplet of doublets (td), triplet (t), quartet (q) or multiplet (m). Carbon nuclear magnetic resonance (^{13}C) spectra were recorded with Varian 400 (at 101 MHz) or Bruker 600 (at 151 MHz) spectrometers. Spectra were assigned using gCOSY and multiplicity-edited gHSQC experiments. Tetramethylsilane (TMS) was used as an internal standard in all ^{1}H and ^{13}C spectra (δ = 0 ppm)

when applicable. Mass spectra was recorded using high resolution Shimadzu LCMS-IT-TOF or Kratos Analytical Maxima-CFR MALDI-TOF system. All reactions were stirred under Argon, until otherwise stated. Column chromatography was performed on silica gel G60 (Silicycle, 60-200 μm, 60 Å). Thin layer chromatography (TLC) analysis was conducted on Silicagel 60 F254 (EMD Chemicals Inc.) coated aluminum sheets. Plates were visualized by UV light (254 nm) and by charring with 10% sulfuric acid in ethanol and/or Hanessian's stain. Size exclusion chromatography was carried out on bio-beads S-X1 (40-80 μm) or bio-gel P2 (45-90 μm). Acid washed molecular sieves (4 Å) were flame activated under vacuum prior to reactions. The final compounds were purified by HPLC using HILIC column (XBridge® Amide 5 μm, 10 mm x 250 mm, Waters) using UV detection (210 nm) and lyophilized by dissolving the compound in water and freezing using liquid nitrogen.

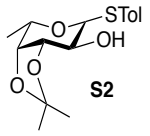
1.2 Synthetic Schemes.



Reagents and Conditions **: a)** 2,2-dimethoxy propane, p-tolunesulfonic acid, DMF, R.T., 12h, 96% ; **b)** NapBr, NaH, DMF, R.T., 15 mins, 83% ; **c)** *p*-TSA, MeOH, DCM, R.T. 24h, 89% ; **d)** BnBr, NaH, DMF, R.T, 1h, 92% ; **e)** DDQ, DCM, H_2O, R.T, 1h, 86% **f) NaH,** PMB-Cl or Bn-Br or Allyl-Cl, DMF, 0°C, 1h, 85 to 90%

Scheme S1. Synthesis of fucose donors.

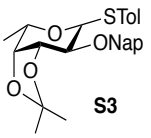
4-methylphenyl 3,4-*O*-isopropylidine-1-thio-β-L-fucopyranoside (S2):



S2

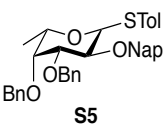
Triol **S1** (8.0 g, 29.5 mmol) was dissolved in DMF (80 mL) under argon atmosphere, followed by the addition of 2,2-dimethoxy propane (7.3 mL, 59.0 mmol) and *p*-Toluenesulfonic acid monohydrate (1.1 g, 5.9 mmol). The reaction mixture was stirred overnight, after which the TLC (petroleum ether (PE): EtOAc, 7: 3, v: v, R_f = 0.56) showed the completion of reaction. The reaction was quenched with Et_3N (10 mL) and concentrated *in vacuo*. The residue was purified by silica gel column chromatography using PE: EtOAc (9: 1, v: v to 8:2, v: v) which gave the desired product as a transparent sticky syrup, as majority beta product. (8.8 g, 96%). R_f = 0.62 (PE: EtOAc, 7: 3, v: v). For NMR studies and subsequent reactions, the beta product was isolated and used. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.43 to 7.09 (4H, m, H-Ar), 4.34 (1H, d, H-1, *J* = 10.2 Hz), 4.01 (2H, m, H-3, H-4), 3.82 (1H, m, H-5), 3.49 (1H, dd, H-2, *J* = 10.2 Hz, 6.3 Hz), 2.31 (3H, s, C<u>H</u>$_3$ of STol), 1.40 to 1.32 (9H, m, 2x C<u>H</u>$_3$ of iso-propylidene, C<u>H</u>$_3$ of Fuc); ^{13}C NMR (101 MHz, $CDCl_3$): δ 138.23 to 128.25 (C-Ar), 88.14 (C-1), 79.05 (C-3), 77.32, 77.20, 77.00, 76.68 (C-4), 72.76 (C-5), 71.28 (C-2), 28.08 and 26.32 (<u>C</u>H$_3$ of iso-propylidene), 21.10 (<u>C</u>H$_3$ of STol), 16.90 (<u>C</u>H$_3$ of Fuc). ESI (*m/z*): $[M + NH_4]^+$ calculated for $C_{16}H_{22}O_4S$, 328.1583; found 328.1587.

4-methylphenyl 3,4-*O*-isopropylidine-2-*O*-(2-methylnaphthyl)-1-thio-β-L-fucopyranoside (S3): Compound **S2** (8.0 g, 25.8 mmol) was dissolved in DMF (50 mL) followed by the addition of NaH (2.0 g, 51.6 mmol, 60% dispersion in oil) and NapBr (8.5 g, 38.7 mmol). The reaction mixture was stirred for 30 min, after which it was quenched with AcOH (10 mL) and solvent was evaporated *in vacuo*. The residue was diluted with DCM (100 mL) and washed successively with water (100 mL) and saturated $NaHCO_3$ (100 mL). The organic phase was dried over $MgSO_4$, filtered, and the filtrate was concentrated *in vacuo*. Silica gel column chromatography using PE: EtOAc (9: 1, v: v to 8: 2, v: v) afforded the product as a transparent oil. (9.8 g, 84%). R_f = 0.59 (PE: EtOAc, 9: 1, v: v). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.81 to 7.04 (11H, m, H-Ar), 4.97 (1H, CH<u>H</u> of Nap, *J* = 11.5 Hz), 4.83 (1H, d, C<u>H</u>H of Nap, *J* = 11.5 Hz), 4.54 (1H, d, H-1, *J* = 9.8 Hz), 4.24 (1H, t, H-3, *J* = 6.1 Hz), 4.03 (1H, dd, H-4, *J* = 5.6 Hz, 2.1 Hz), 3.79 (1H, m, H-5), 3.51 (1H, dd, H-2, *J* = 9.5 Hz, 6.5 Hz), 2.30 (3H, s, C<u>H</u>$_3$ of STol), 1.38 (3H, d, *J* = 6.7 Hz, C<u>H</u>$_3$ of Fuc), 1.37 to 1.35 (6H, 2s, 2x C<u>H</u>$_3$ of iso-propylidene); ^{13}C NMR (101 MHz, $CDCl_3$): δ 137.51 to 125.76 (C-Ar), 86.47 (C-1), 79.90 (C-4), 78.02 (C-2), 77.31, 76.99, 76.67, 76.44 (C-3), 73.43 (<u>C</u>H$_2$ of Nap), 72.37 (C-5), 27.84 and 26.38 (<u>C</u>H$_3$ of iso-propylidene), 21.09 (<u>C</u>H$_3$ of STol), 16.87 (<u>C</u>H$_3$ of Fuc). ESI (*m/z*): $[M + NH_4]^+$ calculated for $C_{27}H_{30}O_4S$, 468.2209; found 468.2218.



S3

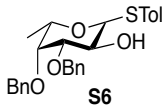
4-methylphenyl 3,4-di-*O*-benzyl-2-*O*-(2-methylnaphthyl)-1-thio-β-L-fucopyranoside (S5):



S5

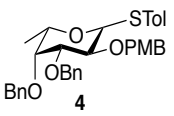
Compound **S3** (9.5 g, 21.1 mmol) was dissolved in the solvent system DCM (50 mL) and MeOH (50 mL), followed by the addition of *p*-Toluenesulfonic acid monohydrate (665 mg, 3.5 mmol) and stirred for 24 h, after which the iso-propylidine ring fell off to give the desired diol. The reaction mixture was quenched with Et_3N (5 mL), following which the solvent was evaporated *in vacuo* giving the crude mixture as a white foamy powder. This crude residue was used in further reaction without any purification. R_f = 0.42 (DCM: MeOH, 9: 1, v: v). The crude diol **S4** (6.2 g, 15.1 mmol) was dissolved in anhydrous DMF (100 mL), followed by the addition of NaH (2.4 g, 59.9 mmol, 60% dispersion in oil) and BnBr (5.3 mL, 45.0 mmol). The reaction mixture was stirred for one h, after which it was quenched with AcOH (10 mL) and solvent was evaporated *in vacuo*. The residue was diluted with DCM and washed successively with water and saturated $NaHCO_3$ solution. The organic phase was dried over $MgSO_4$, filtered and the filtrate was concentrated *in vacuo*. Silica gel column chromatography using PE: EtOAc (9: 1, v: v to 7: 3, v: v) afforded the product as a white amorphous powder. (7.9 g, 90%). R_f = 0.51 (PE: EtOAc, 9: 1, v: v). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.95 to 6.89 (21H, m, H-Ar), 4.96 (3H, m, PhC<u>H</u>H, PhCH<u>H</u>, CH<u>H</u> of Nap), 4.76 (2H, s, PhC<u>H</u>H, PhCH<u>H</u>), 4.68 (1H, d, C<u>H</u>H of Nap, *J* = 11.7 Hz), 4.59 (1H, d, H-1, *J* = 9.6 Hz), 3.96 (1H, t, H-2, *J* = 9.4 Hz), 3.63 (2H, m, H-3, H-4), 3.52 (1H, m, H-5), 2.30 (3H, s, C<u>H</u>$_3$ of STol), 1.28 (3H, d, C<u>H</u>$_3$ of Fuc, *J* = 6.3 Hz); ^{13}C NMR (101 MHz, $CDCl_3$): δ 138.75 to 125.72 (C-Ar), 87.89 (C-1), 84.60 (C-4), 77.31 (C-2), 77.17, 76.99, 76.67, 76.64 (C-3), 75.53 (Ph<u>C</u>H$_2$), 74.58 (C-5), 74.56 (<u>C</u>H$_2$ of Nap), 72.81 (Ph<u>C</u>H$_2$), 21.08 (<u>C</u>H$_3$ of STol), 17.30 (<u>C</u>H$_3$ of Fuc). ESI (*m/z*): $[M + NH_4]^+$ calculated for $C_{38}H_{38}O_4S$, 608.2835; found 608.2832.

4-methylphenyl 3,4-di-*O*-benzyl-1-thio-β-L-fucopyranoside (S6): Compound **S5** (7.0 g, 11.8 mmol) was dissolved in DCM: H_2O (100 mL, 9: 1, v: v) followed by the addition of DDQ (5.8 g, 23.7 mmol). The reaction mixture was stirred in dark for 2 h, following which the TLC showed full conversion of starting material to product. R_f = 0.44 (PE: EtOAc, 7: 3, v: v). The mixture was diluted by DCM (100 mL) and washed successively with saturated $NaHCO_3$ (70 mL) and water (100 mL). The organic phase was dried over $MgSO_4$, filtered and the filtrate was concentrated *in vacuo*. Silica gel column chromatography with PE: EtOAc (8: 2, v: v to 6: 4, v: v) afforded the product as a yellowish amorphous powder. (4.7 g, 88.6%). R_f = 0.44 (PE: EtOAc, 7: 3, v: v). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.64 to 6.93 (14H, m, H-Ar), 4.92 (1H, d, PhCH<u>H</u>), 4.72 (2H, s, PhC<u>H</u>H, PhCH<u>H</u>), 4.62 (1H, d, PhC<u>H</u>H, *J* = 11.4 Hz), 4.42 (1H, d, H-1, *J* = 9.7 Hz), 3.93 (1H, t, H-2, *J* = 9.3 Hz), 3.61 (1H, d, H-4, *J* = 2.6 Hz), 3.54 (1H, m, H-5), 3.45 (1H, dd, H-3, *J* = 9.4 Hz, 2.7 Hz), 2.29 (3H, s, C<u>H</u>$_3$ of STol), 1.26 (3H, d, C<u>H</u>$_3$ of Fuc, *J* = 6.6 Hz); ^{13}C NMR (101 MHz, $CDCl_3$) δ 138.64 to 127.36 (C-Ar), 88.73 (C-1), 83.54 (C-3), 77.29, 77.17, 76.97 (C-4), 76.65, 76.37, 74.95 (C-5), 74.52 (Ph<u>C</u>H$_2$), 72.55 (Ph<u>C</u>H$_2$), 68.89 (C-2), 21.09 (<u>C</u>H$_3$ of STol), 17.27 (<u>C</u>H$_3$ of Fuc). ESI (*m/z*): $[M + NH_4]^+$ calculated for $C_{27}H_{30}O_4S$, 468.2209; found 468.2212.



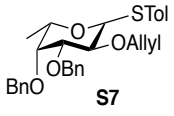
S6

4-methylphenyl 3,4-di-*O*-benzyl-2-*O*-*p*-methoxybenzyl-1-thio-β-L-fucopyranoside (4): Compound **S6** (2.0 g, 4.4 mmol) was dissolved in DMF (20 mL), followed by the addition of NaH (280 mg, 7.0 mmol, 60% dispersion in oil) and 4-methoxybenzyl chloride (710 μL, 5.2 mmol). The reaction mixture was stirred for 1 h, after which it was quenched with AcOH (2 mL). The solvent was evaporated *in vacuo* and the residue was diluted with DCM (50 mL) and washed successively with water (20 mL) and saturated $NaHCO_3$ solution (20 mL). The organic layers were collected, dried over $MgSO_4$ and concentrated *in vacuo*. Silica gel column chromatography using PE: EtOAc (9: 1, v: v) afforded the required product as a colorless syrup. (2.2 g, 88%). R_f = 0.53 (PE: EtOAc, 9: 1, v: v). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.60 to 6.71 (18H, m, H-Ar), 4.99 (1H, d, PhCH<u>H</u>, *J* = 11.7 Hz), 4.68 (5H, m, PhC<u>H</u>H, 2x Ph-C<u>H</u>$_2$), 4.52 (1H, d, H-1, *J* = 9.7 Hz), 3.86 (1H, t, H-2, *J* = 9.3 Hz), 3.78 (3H, s, OC<u>H</u>$_3$ of PMB), 3.61 (1H, d, H-4, *J* = 2.4 Hz), 3.55 (1H, dd, H-3, *J* = 9.2 Hz, 2.7 Hz), 3.48 (1H, m, H-5), 2.28 (3H, s, C<u>H</u>$_3$ of STol), 1.26 (3H, d, C<u>H</u>$_3$ of Fuc, *J* = 6.4 Hz); ^{13}C NMR (101 MHz, $CDCl_3$): δ 159.22 to 113.71 (C-Ar), 87.89 (C-1), 84.56 (C-3), 77.31, 76.99, 76.86 (C-2), 76.67 (C-4), 76.62, 75.15 (Ph<u>C</u>H$_2$), 74.51 (C-5), 72.84 (Ph<u>C</u>H$_2$), 55.27 (O<u>C</u>H$_3$ of PMB), 21.09 (<u>C</u>H$_3$ of STol), 17.28 (<u>C</u>H$_3$ of Fuc). ESI (*m/z*): $[M + NH_4]^+$ calculated for $C_{35}H_{38}O_5S$, 588.2784; found 588.2781.

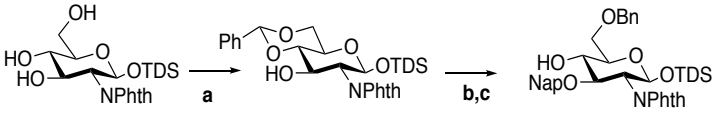


4

4-methylphenyl 2-*O*-allyl-3,4-di-*O*-benzyl-1-thio-β-L-fucopyranoside (S7): Compound **S6** (2.0 g, 4.4 mmol) was dissolved in DMF (20 mL), followed by the addition of NaH (280 mg, 7.0 mmol, 60% dispersion in oil) and allyl bromide (571 μL, 6.6 mmol). The mixture was stirred for 1 h, after which it was quenched with AcOH (2 mL). The solvent was evaporated *in vacuo*, the residue was diluted with DCM (50 mL) and washed successively with water (50 mL) and saturated $NaHCO_3$ (50 mL) solution. The organic phase was dried over $MgSO_4$, filtered and the filtrate was concentrated *in vacuo*. Silica gel column chromatography using PE: EtOAc (9: 1, v: v) afforded the required product as a colorless syrup. (1.6 g, 74%), R_f = 0.49 (PE: EtOAc, 9: 1, v: v). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.55 to 6.89 (14H, m, H-Ar), 5.97 (1H, m, OCH$_2$C<u>H</u>=CH$_2$ of Allyl), 5.18 (2H, m, OCH$_2$CH=C<u>H</u>$_2$ of Allyl), 4.96 (1H, d, PhCH<u>H</u>, *J* = 11.8 Hz), 4.71 (2H, m, PhCH<u>H</u>, PhC<u>H</u>H), 4.64 (1H, d, PhC<u>H</u>H, *J* = 11.8 Hz), 4.46 (1H, d, H-1, *J* = 9.4 Hz), 4.26 (2H, m, OC<u>H</u>$_2$CH=CH$_2$ of Allyl), 3.74 (1H, t, H-2, *J* = 9.3 Hz), 3.59 (1H, d, H-4, *J* = 2.8 Hz), 3.49 (2H, m, H-5, H-3), 2.29 (3H, s, C<u>H</u>$_3$ of STol), 1.23 (3H, d, C<u>H</u>$_3$ of Fuc, *J* = 6.4 Hz); ^{13}C NMR (101 MHz, $CDCl_3$): δ 138.76 to 136.99 (C-Ar), 135.07 (OCH$_2$<u>C</u>H=CH$_2$ of Allyl), 132.10 to 127.37 (C-Ar), 116.89 (OCH$_2$CH=<u>C</u>H$_2$ of Allyl), 87.85 (C-1), 84.40 (C-3), 77.30, 77.04, 76.98, 76.70 (C-2), 76.67 (C-4), 74.55 (Ph<u>C</u>H$_2$), 74.48 (H-5), 74.33 (O<u>C</u>H$_2$CH=CH$_2$ of Allyl), 72.90 (Ph<u>C</u>H$_2$), 21.06 (<u>C</u>H$_3$ of STol), 17.23 (<u>C</u>H$_3$ of Fuc). ESI (*m/z*): $[M + NH_4]^+$ calculated for $C_{30}H_{34}O_4S$, 508.2522; found 508.2513.



S7

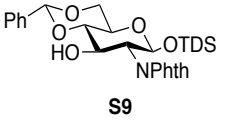


S8 **S9** **S10**

Reagents and Conditions **: a)** Benzaldehyde dimethyl acetal, p-tolunesulfonic acid, ACN, R.T., 2h, 85% ; **b)** NapBr, NaH, DMF, 0ºC, 2h **c)** TfOH, Et_3SiH, DCM, mol. sieves, -60ºC, 30 mins, 76% over two steps

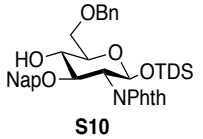
Scheme S2. Synthesis of acceptor S10.

Dimethylthexylsilyl 4,6-*O*-benzylidine-2-deoxy-2-phthalimido-β-D-glucopyranoside (S9): To a suspension of triol **S8** (10.0 g, 22.2 mmol) in dry acetonitrile (100 mL) was added benzaldehyde dimethyl acetal (4.0 mL, 26.6 mmol) and *p*-Toluenesulfonic acid monohydrate (764 mg, 4.4 mmol). The reaction mixture was stirred at room temperature for 6 h, after which it was quenched with Et_3N (5 mL). The solvent was concentrated *in vacuo* and the residue was purified by silica gel column chromatography using PE: EtOAc (9: 1, v: v to 7: 3, v: v), which afforded the target compound as a white amorphous solid, (10.1 g, 85%). R_f = 0.47 (PE: EtOAc, 8: 2, v: v). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.87 to 7.32 (9H, m, H-Ar), 5.55 (1H, s, C<u>H</u>Ph of benzylidene), 5.47 (1H, d, H-1, *J* = 8.4 Hz), 4.62 (1H, dd, H-3, *J* = 10.5 Hz, 8.6 Hz), 4.31 (1H, m, H-6b), 4.19 (1H, dd, H-2, *J* = 10.5 Hz, 8.2 Hz), 3.81 (1H, m, H-6a), 3.61 (2H, m, H-4, H-5), 1.38 [1H, m, C<u>H</u>$(CH_3)_2$ of TDS], 0.62 [12H, m, C($C\underline{H}_3)_2$, CH($C\underline{H}_3)_2$ of TDS], 0.09 to -0.04 (6H, 2s, 2x $C\underline{H}_3$-Si of TDS); ^{13}C NMR (101 MHz, $CDCl_3$): δ 137.03 to 123.27 (C-Ar), 101.95 (<u>C</u>HPh of benzylidene), 93.88 (C-1), 82.40 (C-4), 77.31, 77.00, 76.68, 68.74 (C-6), 68.44 (C-3), 66.20 (C-5), 58.64 (C-2), 33.79 (<u>C</u>H$(CH_3)_2$ of TDS), 19.79 to 18.16 (4x <u>C</u>H_3 of TDS), -0.02 to -1.86 (2x <u>C</u>H_3-Si of TDS). ESI (*m/z*): $[M+ Na]^+$ calculated for $C_{29}H_{37}NO_7Si$, 562.2237; found 562.2233.



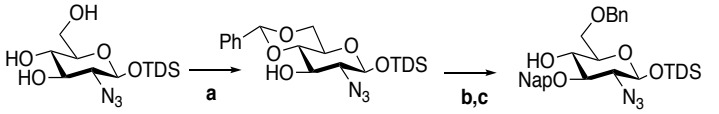
S9

Dimethylthexylsilyl 6-*O*-benzyl-3-*O*-(2-methylnaphthyl)-2-deoxy-2-phthalimido-β-D-glucopyranoside (S10): A solution of **S9** (10.0 g, 18.5 mmol) in DMF (100 mL) was cooled down to 0 ºC, followed by the sequential addition of NapBr (5.3 g, 24.1 mmol) and NaH (1.1 g, 27.8 mmol, 60% dispersion in oil). The reaction mixture was stirred at this temperature for 2 h, after which it was quenched with AcOH (5 mL). The solvent was evaporated *in vacuo* and the residue was diluted with DCM, washed with saturated $NaHCO_3$ (100 mL) and water (100 mL) and dried over $MgSO_4$. The organic phase was filtered, and the filtrate was concentrated *in vacuo* and was used in the next step without purification. The residue was stirred with pre-activated molecular sieves (20 g) in 100 mL DCM for 30 min. The mixture was cooled down to -



S10

60 ºC, followed by the sequential addition of triethylsilane (4.2 mL, 26.5 mmol) and trifluoromethanesulfonic acid (1.8 mL, 19.9 mmol). The reaction mixture was stirred at this temperature for 30 min, after which it was quenched with a mixture of Et_3N: MeOH (5 mL, 1:1, v: v). The mixture was warmed to room temperature, the molecular sieves were filtered off and the filtrate was diluted by DCM and washed with saturated $NaHCO_3$ (50 mL) and dried over $MgSO_4$. The organic phase was filtered, and the filtrate was concentrated *in vacuo*. Silica gel column chromatography using PE: EtOAc (9: 1, v: v to 7: 3, v: v) yielded the product as a pale-yellow powder. (6.9 g, 76% over two steps). R_f = 0.53 (PE: EtOAc, 8: 2, v: v). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.70 to 7.09 (16H, m, H-Ar), 5.32 (1H, d, H-1, *J* = 8.2 Hz), 4.93 (1H, d, CHH of Nap, *J* = 12.6 Hz), 4.64 (3H, m, CHH of Nap, PhCHH, PhCHH), 4.32 (1H, dd, H-3, *J* = 10.6 Hz, 8.0 Hz), 4.08 (1H, dd, H-2, *J* = 10.0 Hz, 8.0 Hz), 3.81 (3H, m, H-6a, H-4, H-6b), 3.65 (1H, m, H-5), 2.98 (1H, d, OH, *J* = 2.4 Hz), 1.31 [1H, m, CH(CH$_3$)$_2$ of TDS], 0.54 [12H, m, C(CH$_3$)$_2$, CH(CH$_3$)$_2$ of TDS], 0.06 to -0.11 (6H, 2s, 2x CH$_3$-Si of TDS); ^{13}C NMR (101 MHz, $CDCl_3$): δ 137.67 to 125.49 (C-Ar), 93.36 (C-1), 78.93 (C-3), 77.29, 77.18, 76.98, 76.66, 74.79 (CH$_2$ of Nap), 74.61 (C-4), 73.79 (PhCH$_2$), 73.46 (C-5), 71.05 (C-6), 57.38 (C-2), 33.79 (CH(CH$_3$)$_2$ of TDS), 24.42, 19.80 to 18.10 (4x CH$_3$ of TDS), -1.85 to -3.92 (2x CH$_3$-Si of TDS). ESI (*m/z*): [M+ NH_4]$^+$ calculated for $C_{40}H_{47}NO_7Si$, 699.3466; found 699.3460.

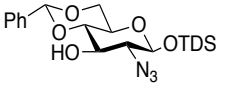


S11 **S12** **2**

Reagents and Conditions **: a)** Benzaldehyde dimethyl acetal, p-tolunesulfonic acid, ACN, R.T., 6h, 77% ; **b)** NapBr, NaH, DMF, 0ºC, 2h **c)** TfOH, Et_3SiH, DCM, mol. sieves, -60ºC, 30 mins, 73% over two steps

Scheme S3. Synthesis of acceptor **2**

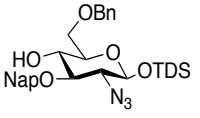
Dimethylthexylsilyl 2-deoxy-2-azido-4,6-*O*-benzylidine-β-D-glucopyranoside (S12): To a suspension of triol **S11**, (10.6 g, 30.5 mmol) in dry acetonitrile (100 mL) was added benzaldehyde dimethyl acetal (5.5 mL, 36.6 mmol) and *p*-Toluenesulfonic acid monohydrate (1.2 g, 6.1 mmol) and the reaction mixture was stirred at room temperature for 6 h after which the TLC (PE: EtOAc, 7: 3, v: v) showed the reaction had gone to completion. The reaction mixture was quenched with Et_3N (5 mL) and concentrated *in vacuo*. Silica gel column chromatography using PE: EtOAc (8: 2, v: v) afforded the target compound as a transparent syrup, (10.3 g, 78%). R_f = 0.59 (PE: EtOAc, 8: 2, v: v). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.96 to 7.20 (5H, m, Ar-H), 5.51 (1H, s, CHPh



S12

of benzylidene), 4.60 (1H, d, H-1, J = 7.5 Hz), 4.26 (1H, dd, H-6b, J = 10.5 Hz, 5.1 Hz), 3.75 (1H, t, H-6a, J = 10.1 Hz), 3.57 (2H, m, H-3, H-4), 3.37 (1H, m, H-5), 3.29 (1H, dd, H-2, J = 9.4 Hz, 7.7 Hz), 1.65 [1H, m, C<u>H</u>$(CH_3)_2$ of TDS], 0.89 [12H, m, C(C<u>H</u>$_3)_2$, CH(C<u>H</u>$_3)_2$ of TDS], 0.19 (6H, d, 2x C<u>H</u>$_3$-Si of TDS, J = 8.3 Hz); ^{13}C NMR (101 MHz, $CDCl_3$): δ 136.86 to 126.22 (C-Ar), 101.96 (<u>C</u>HPh of benzylidene), 97.36 (C-1), 80.74 (C-3), 77.32 , 77.21, 77.00, 76.69, 71.88 (C-4), 69.11 (C-2), 68.54 (C-6), 66.23 (C-5), 34.14 (<u>C</u>H$(CH_3)_2$ of TDS), 19.91 to 18.36 (4x <u>C</u>H_3 of TDS), -0.03 to -3.23 (2x <u>C</u>H_3-Si of TDS). ESI (m/z): [M+ NH_4]$^+$ calculated for $C_{21}H_{33}N_3O_5Si$, 453.2533; found 453.2531.

Dimethylthexylsilyl 2-deoxy-2-azido-6-*O*-benzyl-3-*O*-(2-methylnaphthyl)-β-D-glucopyranoside (2): A solution of **S12** (10.1 g, 23.2 mmol) in DMF (100 mL) was cooled down to 0 ºC, followed by the sequential addition of NapBr (6.1 g, 27.6 mmol) and NaH (1.4 g, 34.5 mmol, 60% dispersion in oil). The reaction mixture was stirred at this temperature for 2 h, after which it was quenched with AcOH (5 mL). The solvent was evaporated *in vacuo*, the residue was diluted with DCM, and washed with $NaHCO_3$ (100 mL) and water (150 mL). The organic phase was dried over $MgSO_4$, filtered and the filtrate was concentrated *in vacuo*. The residue was used in further step without purification. The residue was stirred with pre-activated molecular sieves (20 g) in DCM (100 mL) for 30 min after which it was cooled down to -60 ºC, followed by the sequential addition of triethylsilane (8.2 mL, 51.0 mmol) and trifluoromethanesulfonic acid (2.2 mL, 25.5 mmol). The reaction mixture was stirred at this temperature for 30 min, after which it was quenched with a mixture of Et_3N: MeOH (5 mL, 1: 1, v: v). The molecular sieves were filtered off, the filtrate was diluted by DCM and washed with saturated $NaHCO_3$ (100 mL) and dried over $MgSO_4$. The organic phase was filtered, and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography using PE: EtOAc (8: 2, v: v to 6: 4 v: v), which yielded the product as a transparent sticky syrup, (7.5 g, 73% over two steps). R_f = 0.54 (PE: EtOAc, 8.5: 1.5, v: v). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.87 to 7.23 (12H, m, H-Ar), 5.06 (1H, d, CH<u>H</u> of Nap, J = 11.5 Hz), 4.93 (1H, d, C<u>H</u>H of Nap, J = 11.5 Hz), 4.56 (2H, m, PhC<u>H</u>H, PhCH<u>H</u>), 4.51 (1H, d, H-1, J = 7.7 Hz), 3.69 (3H, m, H-4, H-6a, H-6b), 3.34 (3H, m, H-2, H-5, H-3), 1.67 [1H, m, C<u>H</u>$(CH_3)_2$ of TDS], 0.89 [12H, m, C(C<u>H</u>$_3)_2$, CH(C<u>H</u>$_3)_2$ of TDS], 0.19 (6H, d, 2x C<u>H</u>$_3$-Si of TDS, J = 6.0 Hz); ^{13}C NMR (101 MHz, $CDCl_3$): δ 137.74 to 125.89 (C-Ar), 97.03 (C-1), 82.34 (C-3), 77.31, 77.00, 76.68, 74.99 (<u>C</u>H_2 of Nap), 73.88 (PhCH_2), 73.70 (C-5), 72.16 (C-4), 70.43 (Ph<u>C</u>H_2), 68.27 (C-2), 33.89 (<u>C</u>H$(CH_3)_2$ of TDS), 19.96 to 18.38 (4x <u>C</u>H_3 of TDS), -0.02 to -3.28 (2x <u>C</u>H_3-Si of TDS). ESI (m/z): [M+ NH_4]$^+$ calculated for $C_{32}H_{43}N_3O_5Si$, 595.3316; found 595.3313.



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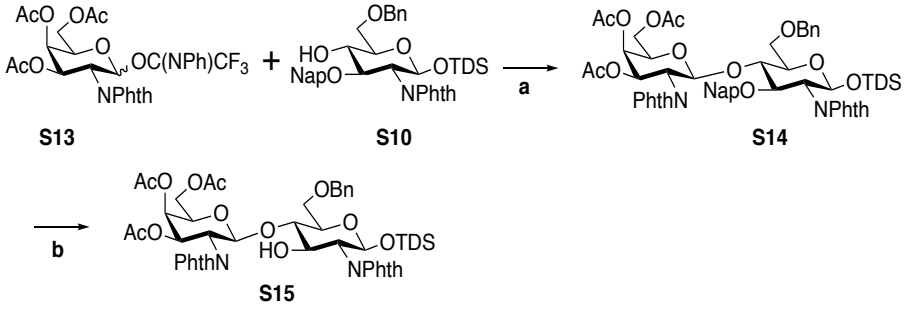
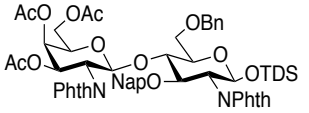


Reagents and Conditions **: a)** TMSOTf, DCM, -30 °C, 92% ; **b)** DDQ, DCM/H_2O = 9:1, 88%

Scheme S4. Synthesis of LDN acceptor S15.

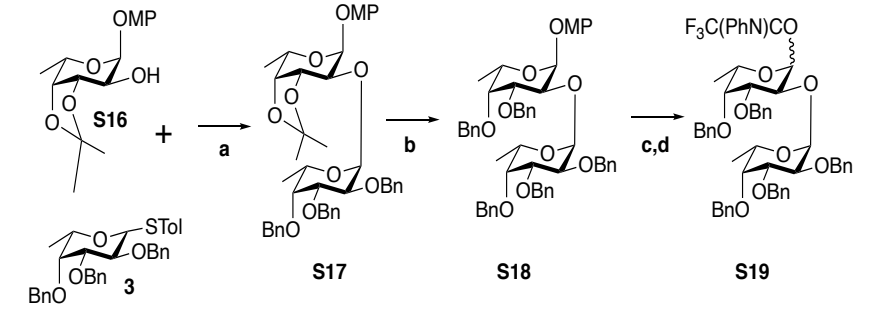
Dimethylthexylsilyl [3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-galactopyranosyl]-(1→4)-6-*O*-benzyl-3-*O*-(2-methylnaphthyl)-2-deoxy-2-phthalimido-β-D-glucopyranoside (S14): *N*-phenyltrifluoroimidate donor **S13** (5.2 g, 8.6 mmol), with acceptor **S10** (4.5 g, 6.6 mmol) was dissolved in DCM (50 mL) and stirred with pre-activated molecular sieves (10 g) for 30 min.



S14

The mixture was then cooled down to -30 ºC, followed by the addition of TMSOTf (240 µL, 1.3 mmol). The reaction was quenched after 20 min with Et_3N (500 µL). The mixture was warmed up to room temperature and then concentrated *in vacuo*. Silica gel column chromatography with PE: EtOAc (9: 1, v: v to 7: 3, v: v) yielded the product as white amorphous powder (6.7 g, 92%). R_f = 0.58 (PE: EtOAc, 6: 4 v: v). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.01 to 6.98 (20H, m, H-Ar), 5.83 (1H, dd, H-3 GalN, *J* = 11.4 Hz, 3.5 Hz), 5.56 (1H, d, H-1 GalN, *J* = 8.4 Hz), 5.40 (1H, d, H-4 GalN, *J* = 3.4 Hz), 5.14 (1H, d, H-1 GlcN, *J* = 8.4 Hz), 4.98 (1H, d, CH<u>H</u> of Nap, *J* = 12.6 Hz), 4.65 (1H, d, C<u>H</u>H of Nap, *J* = 12.6 Hz), 4.51 (3H, m, PhC<u>H</u>H, PhCH<u>H</u>, H-2 GalN), 4.32 (1H, dd, H-3 GlcN, *J* = 11.0 Hz, 8.7 Hz), 4.11 (4H, m, H-2 GlcN, H-6a GalN, H-6b GalN, H-5 GalN), 3.87 (1H, t, H-4 GlcN, *J* = 6.7 Hz), 3.41 (3H, m, H-5 GlcN, H-6a GlcN, H-6b GlcN), 2.05 to 1.82 (9H, 3s, 3x C<u>H</u>$_3$ of Ac), 1.26 [1H, m, C<u>H</u>$(CH_3)_2$ of TDS] , 0.49 [12H, m, C$(C\underline{H}_3)_2$, CH$(C\underline{H}_3)_2$ of TDS], -0.02 to -0.22 (6H, 2s, 2x C<u>H</u>$_3$-Si of TDS); ^{13}C NMR (101 MHz, $CDCl_3$): δ 170.35 to 168.29 (3x <u>C</u>OCH$_3$ of Ac), 138.30 to 123.48 (C-Ar), 97.58 (C-1 GalN), 93.17 (C-1 GlcN), 77.32, 77.20, 77.18, 77.00, 76.79 (C-3 GlcN), 76.68 (C-5 GalN), 74.43 (C-5 GlcN), 74.40 (<u>C</u>H$_2$ of Nap), 72.74 (Ph<u>C</u>H$_2$), 70.65 (C-4 GlcN), 67.90 (C-6 GlcN), 67.83 (C-3 GalN), 66.64 (C-4 GalN), 61.09 (C-6 GalN), 57.70 (C-2 GlcN), 52.10 (C-2 GalN), 33.77 (<u>C</u>H$(CH_3)_2$ of TDS), 20.67 to 20.49 (3x <u>C</u>H$_3$ of Ac), 19.76 to 18.07 (4x <u>C</u>H$_3$ of TDS), -0.02 to -3.99 (2x <u>C</u>H$_3$-Si of TDS). ESI (*m/z*): $[M+ NH_4]^+$ calculated for $C_{60}H_{66}N_2O_{16}Si$, 1116.4525; found 1116.4527.

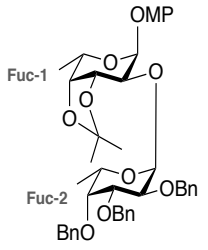
Dimethylthexylsilyl [3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-galactopyranosyl]-(1→4)-6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (S15): Compound **S14** (6.7 g, 6.1 mmol) was dissolved in the solvent system DCM: H_2O (100 mL, 9: 1, v: v), followed by the addition of DDQ (2.8 g, 12.2 mmol), and allowed to stir in the dark for 3 h after which it was diluted by DCM (100 mL) and washed with saturated $NaHCO_3$ (150 mL) and water (100 mL). The organic fractions were then dried over $MgSO_4$ and filtered, and the filtrate was concentrated *in vacuo*. Silica gel column chromatography using PE: EtOAc (8: 2, v: v to 6: 4, v: v) gave the product as a white amorphous powder. (5.2 g, 88%). R_f = 0.48 (PE: EtOAc, 6: 4, v: v). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.95 to 6.98 (13H, m, H-Ar), 5.83 (1H, dd, H-3 GalN, *J* = 11.4 Hz, 3.5 Hz), 5.44 (2H, m, H-4 GalN, H-1 GalN), 5.32 (1H, d, H-1 GlcN, *J* = 8.1 Hz), 4.55 (1H, dd, H-2 GalN, *J* = 11.4 Hz, 8.5 Hz), 4.42 (1H, m, H-3 GlcN), 4.17 (1H, d, H-5 GalN, *J* = 1.8 Hz), 4.09 (5H, m, H-2 GlcN, H-6a GalN, H-6b GalN, PhC<u>H</u>H, PhCH<u>H</u>), 3.68 (1H, t, H-4 GlcN, *J* = 9.0 Hz), 3.49 (1H, m, H-5 GlcN), 3.21 (2H, m, H-6a GlcN, H-6b GlcN), 2.16 to 1.82 (9H, 3s, 3x C<u>H</u>$_3$ of Ac), 1.36 [1H, m, C<u>H</u>(CH$_3$)$_2$ of TDS], 0.59 [12H, m, C(C<u>H</u>$_3$)$_2$, CH(C<u>H</u>$_3$)$_2$ of TDS], 0.02 to -0.10 (6H, 2s, 2x C<u>H</u>$_3$-Si of TDS); ^{13}C NMR (101 MHz, $CDCl_3$): δ 170.44 to 167.37 (3x <u>C</u>OCH$_3$ of Ac), 138.04 to 123.62 (C-Ar), 99.33 (C-1 GalN), 93.20 (C-1 GlcN), 82.60 (C-4 GlcN), 77.32 , 77.20, 77.00, 76.68, 73.86 (C-5 GlcN), 72.81 (Ph<u>C</u>H$_2$), 71.20 (C-5 GalN), 69.71 (C-3 GlcN), 68.00 (C-6 GlcN), 67.61 (C-3 GalN), 66.40 (C-4 GalN), 61.85 (C-6 GalN), 57.97 (C-2 GlcN), 51.32 (C-2 GalN), 33.91 (<u>C</u>H(CH$_3$)$_2$ of TDS), 20.64 to 20.23 (3x <u>C</u>H$_3$ of Ac), 19.86 to 18.15 (4x <u>C</u>H$_3$ of TDS), -0.03 to -3.90 (2x <u>C</u>H$_3$-Si of TDS). ESI (*m/z*): $[M+ Na]^+$ calculated for $C_{49}H_{58}N_2O_{16}Si$, 981.3453; found 981.3456.



Reagents and Conditions **: a)** NS/TMSOTf, DCM, -20 to -5°C, DCM:DMF=1:1, 86% ; **b)** i) pTSA, MeOH, DCM ; ii) BnBr, NaH, DMF, 84% (over two steps); **c)** CAN, ACN:H_2O= 4:1 **d)** N-phenylimidate, DBU, DCM, 71% (over two steps)

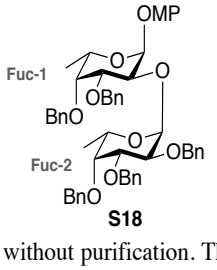
Scheme S5. Synthesis of fucose disaccharide donor.

***p*-methoxyphenyl [2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl]-(1→2)-3,4-*O*-isopropylidine-α-L-fucopyranoside (S17):** Acceptor **S16** (5.0 g, 16.1 mmol) and donor **3** (9.6 g, 17.8 mmol), were dissolved in the solvent system DCM: DMF (100 mL, 1: 1, v: v) and stirred with pre-activated molecular sieves (15.0 g) for 30 min, after which the temperature was brought down to -20 ºC. This was followed by the sequential addition of NIS (4.3 g, 19.35 mmol) and TMSOTf (2.3 mL, 12.9 mmol). The reaction mixture was warmed up to -5 ºC over a period of 1 h, after which the TLC showed complete consumption of acceptor. The reaction mixture was quenched with Et_3N (10 mL) and warmed up to room temperature. The sieves were filtered off, and the filtrate was concentrated *in vacuo*. The residue was diluted by DCM (150 mL) and washed with saturated $NaHCO_3$ (200 mL) solution and 5% sodium thiosulphate solution respectively (100 mL). The organic phase was dried over $MgSO_4$ and filtered, and the filtrate was concentrated *in vacuo*. Silica gel column chromatography using (PE: EtOAc (9: 1, v: v to 7: 3, v: v) yielded the product as transparent syrup. (10.1 g, 86%). R_f = 0.51 (PE: EtOAc, 8: 2, v: v). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.47 to 6.69 (19H, m, H-Ar), 5.46 (1H, d, H-1 Fuc-2, *J* = 3.4 Hz), 4.97 (2H, m, H-1 Fuc-1, PhCHH), 4.72 (5H, m, PhCHH, 2x PhCHH, 2x PhCHH), 4.49 (1H, dd, H-3 Fuc-2, *J* = 8.0 Hz, 5.4 Hz), 4.23 (2H, m, H-5 Fuc-2, H-5 Fuc-1), 4.12 (2H, m H-2 Fuc-1, H-4 Fuc-2), 4.02 (1H, dd, H-3 Fuc-1, *J* = 10.1 Hz, 3.6 Hz), 3.94 (1H, dd, H-2 Fuc-2, *J* = 8.1 Hz, 3.4 Hz), 3.74 (4H, m, H-4 Fuc-1, OCH_3 of OMP), 1.53 to 1.37 (6H, 2s, 2x CH_3 of iso-propylidine), 1.32 (3H, d, CH_3 of Fuc-1, *J* = 6.7 Hz), 1.12 (3H, d, CH_3 of Fuc-2, *J* = 6.6 Hz); ^{13}C NMR (101 MHz, $CDCl_3$): δ 154.97 to 114.60 (C-Ar), 94.84 (C-1 Fuc-2), 94.76 (C-1 Fuc-1), 79.29 (C-2 Fuc-1), 77.60 (C-4 Fuc-1), 77.34, 77.02, 76.71, 76.04 (C-4 Fuc-2), 75.79 (C-3 Fuc-1), 74.86 ($PhCH_2$), 74.71 (C-3 Fuc-2), 73.03 ($PhCH_2$), 72.90 ($PhCH_2$), 72.58 (C-2 Fuc-2), 66.27 (C-5 Fuc-1), 63.69 (C-5 Fuc-2), 55.64 (OCH_3 of OMP), 28.54 to 26.46 (2x CH_3 of iso-propylidine), 16.50 (CH_3 Fuc-1), 16.33 (CH_3 Fuc-2). ESI (*m/z*): $[M + NH_4]^+$ calculated for $C_{43}H_{50}O_{10}$, 744.3748; found 744.3709.



S17

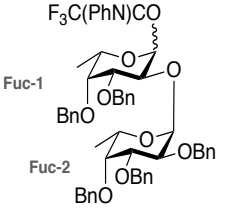
***p*-methoxyphenyl [2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl]-(1→2)-3,4-di-*O*-benzyl-α-L-fucopyranoside (S18):** The disaccharide **S17** (10.1 g, 13.9 mmol) was dissolved in the solvent system DCM (70 mL) and MeOH (50 mL), followed by the addition of *p*-Toluenesulfonic acid monohydrate (665 mg, 3.5 mmol) and stirred overnight, after which the iso-propylidine ring fell off to give the desired diol. The solvent was removed *in vacuo*, the residue was diluted by DCM and washed with saturated $NaHCO_3$. The organic fractions were dried over $MgSO_4$ and filtered. The filtrate was concentrated, and the residue was dried on high vacuum for 2 h. This was further used for benzylation without purification. The residue was dissolved in dry DMF (100 mL) and cooled down to 0 ºC. This was followed by the addition of NaH (1.6 g, 40.8 mmol, 60% dispersion in oil) and BnBr (4.8 mL, 40.8 mmol), and the reaction mixture was stirred at this temperature for 30 min after which it



S18

was quenched with AcOH (5 mL) and the solvent was removed *in vacuo*. Silica gel column chromatography using at first pure PE and then increasing the polarity to PE: EtOAc (9: 1, v: v), gave the product as a white amorphous powder. (9.1 g, 76% over two steps). R_f = 0.54 (PE: EtOAc, 9: 1, v: v). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.45 to 6.66 (29H, m, H-Ar), 5.42 (1H, d, H-1 Fuc-2, *J* = 3.7 Hz), 4.97 (2H, m, H-1 Fuc-1, PhCHH), 4.70 (5H, m, PhCHH, 2x PhCHH, 2x PhCHH), 4.11 (4H, m, H-2 Fuc-1, H-5 Fuc-1, H-5 Fuc-2, H-2 Fuc-2), 3.95 (1H, dd, H-3 Fuc-2, *J* = 10.1 Hz, 2.8 Hz), 3.85 (2H, m, H-3 Fuc-1, H-4 Fuc-2), 3.70 (4H, m, H-4 Fuc-1, OCH_3-OMP), 1.28 (3H, d, CH_3 of Fuc-1, *J* = 6.6 Hz), 1.14 (3H, d, CH_3 of Fuc-2, *J* = 6.3 Hz); ^{13}C NMR (101 MHz, $CDCl_3$): δ 155.11 to 114.47 (C-Ar), 98.47 (C-1 Fuc-1), 98.12 (C-1 Fuc-2), 78.70 (C-3 Fuc-2), 78.23 (C-3 Fuc-1), 77.46, 77.30 (C-4 Fuc-1), 77.19, 76.99, 76.67 (C-2 Fuc-1), 75.87 ($PhCH_2$), 74.91 ($PhCH_2$), 73.16 to 73.10 (3x $PhCH_2$), 71.38 (C-4 Fuc-2), 68.93 (C-2 Fuc-1), 67.79 (C-2 Fuc-2), 66.31 (C-5 Fuc-1), 55.63 (C-5 Fuc-2), 16.58 (CH_3 of Fuc-2), 16.10 (CH_3 of Fuc-2). ESI (*m/z*): $[M + NH_4]^+$ calculated for $C_{54}H_{58}O_{10}$, 884.4374; found 844.4381.

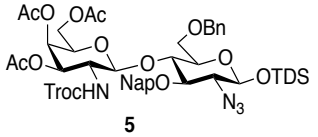
(*N*-Phenyl)-2,2,2-trifluoroacetimidate [2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl]-(1→2)-3,4-di-*O*-benzyl-α/β-L-fucopyranoside (S19): Compound **S18** (4.0 g, 4.6 mmol) was dissolved in acetonitrile (40 mL) and water (10 mL) was added. This was followed by the addition of ceric ammonium nitrate (5.1 g, 9.2 mmol). The reaction mixture was stirred under argon, in the dark, for 2 h, after which TLC (PE: EtOAc = 6: 4, v: v) showed complete consumption of starting material. The solvent was evaporated *in vacuo*, and the residue was dissolved in DCM (50 mL), and washed with water (50 mL) and saturated $NaHCO_3$ (100 mL) and the organic phase was dried over $MgSO_4$ and filtered. The filtrate was concentrated *in vacuo* and dried over high vacuum over a period of 2 h. This residue was then used further without purification. The residue was dissolved in DCM followed by the addition of DBU (344 μL, 2.3 mmol) and 2,2,2-trifluoro-*N*-phenylacetimidoyl chloride (1.4 mL, 6.9 mmol). The reaction mixture was stirred for 30 min. After this time, the solvent was evaporated, and the residue was quickly purified using PE: EtOAc (9: 1, v: v to 6: 4, v: v). The product was dried over high vacuum for 30 min. This afforded the disaccharide as a colorless syrup. (3.1 g, 71 % over two steps). R_f = 0.61 (PE: EtOAc, 7: 3, v: v). The imidate product showed hydrolysis when dissolved in $CDCl_3$, hence NMR and mass were not recorded as the product was unstable and used for next reaction immediately.



S19

2. Final Synthesis of LDN-F and LDN-DF Epitopes

Dimethylthexylsilyl [3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,2,2,-trichloroethoxy)carbonylamino-β-D-galactopyranosyl]-(1→4)-2-deoxy-2-azido-6-*O*-benzyl-3-*O*-(2-methylnaphthyl)-β-D-glucopyranoside (5): *N*-phenyltrifluoroimidate donor **1** (9.4 g, 14.5 mmol) with acceptor **2** (7.0 g, 12.1 mmol) were dissolved in DCM (50 mL), and allowed to stir with pre-activated molecular sieves for 30 min. The mixture was then cooled down to -30 ºC, followed by the addition of TMSOTf (657 μL, 3.6 mmol). The reaction was quenched after 10 min with Et_3N (2 mL). Concentration of mixture *in vacuo* and silica gel column chromatography with PE: EtOAc (9: 1, v: v to 6: 4, v: v) yielded the product as white solid. (10.9 g, 93%). R_f = 0.48 (PE: EtOAc, 7: 3, v: v). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.90 to 7.27 (12H, m, H-Ar), 5.20 (1H, d, H-4 GalN, *J* = 3.2 Hz), 5.12 (1H, d, CHH of Nap, *J* = 10.3 Hz), 4.83 (2H, m, CHH of Nap, PhCHH), 4.68 (3H, m, CH_2 of Troc, H-3 GalN), 4.40 (3H, m, PhCHH, H-1 GlcN, H-1 GalN), 3.98 (2H, m, H-5 GalN, H-6b GalN), 3.78 (3H, m, H-2 GalN, H-6a GlcN, H-6a GalN), 3.58 (2H, m, H-4 GlcN, H-6b GlcN), 3.31 (3H, m, H-5 GlcN, H-3 GlcN, H-2 GlcN), 2.01 to 1.94 (9H, 3s, 3x CH_3 of Ac), 1.65 [1H, m, $CH(CH_3)_2$ of TDS] , 0.88 [12H, m, $C(CH_3)_2$, $CH(CH_3)_2$ of TDS], 0.17 (6H, d, 2x CH_3-Si of TDS); ^{13}C NMR (101 MHz, $CDCl_3$): δ 170.13 to 170.10 (3x $COCH_3$ of Ac), 154.06 ($COOCH_2$ of Troc), 137.40 to 125.67 (C-Ar), 100.69 (C-1 GalN), 97.05 (C-1 GlcN), 80.72 (C-3 GlcN), 77.30, 76.98, 76.66 (C-5 GalN), 74.93 (CH_2 of Nap), 74.42 (CH_2 of Troc), 74.18 (C-5 GlcN), 73.81 ($PhCH_2$), 70.36 (C-3 GalN), 70.21 (C-4 GlcN), 68.42 (C-2 GlcN), 67.74 (C-6 GlcN), 66.08 (C-4 GalN), 60.79 (C-6 GalN), 52.76 (C-2 GalN), 33.90 ($CH(CH_3)_2$ of TDS), 20.83 to 20.55 (3x CH_3 of Ac), 19.97 to 18.37 (4x CH_3 of TDS), -0.03 to -3.27 (2x CH_3-Si of TDS). ESI (*m/z*): $[M+ NH_4]^+$ calculated for $C_{47}H_{61}Cl_3N_4O_{14}Si$, 1056.3363; found 1056.3351.



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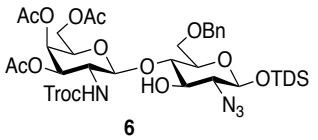
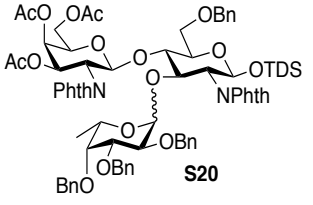
Dimethylthexylsilyl [3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxy)carbonylamino-β-D-galactopyranosyl]-(1→4)-2-deoxy-2-azido-6-*O*-benzyl-β-D-glucopyranoside (6): Compound **5** (10.9 g, 11.3 mmol) was dissolved in DCM: H_2O (100 mL, 9: 1, v: v), followed by the addition of DDQ (2.8 g, 12.2 mmol), and stirred in the dark for 3 h, after which it was diluted by DCM (100 mL) and washed with saturated $NaHCO_3$ (250 mL) and water (100 mL). The organic fractions were dried over $MgSO_4$, filtered, and the filtrate was then concentrated. Silica gel column chromatography using PE: EtOAc (9: 1, v: v to 7: 3, v: v) gave the product as a pale-yellow amorphous powder. (9.0 g, 88%). R_f = 0.52 (PE: EtOAc, 7.5: 2.5, v: v). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.55 to 7.16 (5H, Ar-H), 5.27 (1H, d, H-4 GalN, *J* = 3.2 Hz), 4.81 (1H, d, PhCHH, *J* = 11.8 Hz), 4.66 (3H, m, H-3 GalN, CH_2 of Troc), 4.44 (1H, d, H-1 GlcN, *J* = 7.9 Hz), 4.37 (1H, d, PhCHH, *J* = 11.8 Hz), 4.12 (4H, m, H-1 GalN, H-6a GalN, H-6b GalN, NH of Troc), 3.87 (2H,



6

m, H-5 GalN, H-2 GalN), 3.68 (2H, m, H-3 GlcN, H-6b GlcN), 3.50 (2H, m, H-4 GlcN, H-6a GlcN), 3.33 (1H, m, H-5 GlcN), 3.22 (1H, dd, H-2 GlcN, J = 9.7 Hz, 7.6 Hz), 2.12 to 1.95 (9H, 3s, 3x C$\underline{H}_3$ of Ac), 1.66 [1H, m, C$\underline{H}$(CH$_3$)$_2$ of TDS], 0.88 [12H, m, C(C$\underline{H}_3$)$_2$, CH(C$\underline{H}_3$)$_2$ of TDS], 0.17 (6H, d, 2x C$\underline{H}_3$-Si of TDS); ^{13}C NMR (101 MHz, CDCl$_3$): δ 170.59 to 170.05 (3x $\underline{C}$OCH$_3$ of Ac), 154.12 ($\underline{C}$OOCH$_2$ of Troc), 137.98 to 127.33 (C-Ar), 102.06 (C-1 GalN), 96.65 (C-1 GlcN), 81.17 (C-3 GlcN), 77.35, 77.24, 77.04, 76.72, 74.57 ($\underline{C}$H$_2$ of Troc), 73.56 (Ph$\underline{C}$H$_2$), 73.49 (C-5 GlcN), 73.25 (C-4 GlcN), 71.17 (C-5 GalN), 70.08 (C-3 GalN), 68.05 (C-2 GlcN), 67.45 (C-6 GlcN), 66.24 (C-4 GalN), 61.63 (C-6 GalN), 52.03 (C-2 GalN), 33.88 ($\underline{C}$H(CH$_3$)$_2$ of TDS), 20.57 to 20.48 (3x $\underline{C}$H$_3$ of Ac), 19.97 to 18.39 (4x $\underline{C}$H$_3$ of TDS), -0.03 to -3.25 (2x $\underline{C}$H$_3$-Si of TDS).ESI (*m/z*): [M+ NH$_4$]$^+$ calculated for $C_{36}H_{53}Cl_3N_4O_{14}Si$; 916.2737; found 916.2726.

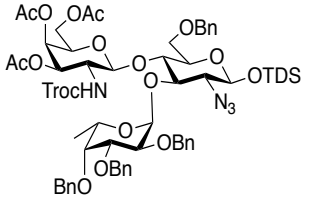
Dimethylthexylsilyl [2,3,4-tri-*O*-benzyl-α/β-L-fucopyranosyl]-(1→3)-[3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-galactopyranosyl]-(1→4)-6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (S20) : Donor **3** (1.0 g, 1.8 mmol), DPS (748 mg, 3.7 mmol) and TTBP (1.1 g, 4.6 mmol) were dissolved in DCM (10 mL) and allowed to stir with pre-activated molecular sieves (2 g) for 30 min. The temperature was brought down to -70 ºC, followed by the addition of Tf_2O (620 µL, 3.7 mmol). After 10 min, a solution of acceptor **S15** (886 mg, 0.925 mmol) in anhydrous DCM (5 mL) was added dropwise along the wall of the flask, and the reaction mixture was stirred for 2 h, during which the temperature was slowly raised to -40º C. The TLC (PE: EtOAc, 7: 3, v: v) showed complete consumption of acceptor, at which point, the reaction was quenched using Et_3N (1 mL). The molecular sieves were filtered off, and the solvent was removed *in vacuo*. Silica gel column chromatography using PE: EtOAc (9: 1, v: v to 6: 4, v: v) gave the product as a white amorphous powder in an inseparable α: β ratio of 8: 2. (1.1 g, 88%). R_f = 0.52 (PE: EtOAc, 7: 3, v: v). For the NMR analysis, the proton signals for alpha and beta product are assigned with superscript ($^{\alpha}$ or $^{\beta}$). ^{1}H NMR (400 MHz, CDCl$_3$): δ 8.01 to 6.83 (32H, m, H-Ar), 5.83 (H-3 GalN$^{\beta}$), 5.75 (1H, dd, H-3 GalN$^{\alpha}$, J = 11.6 Hz, 3.5 Hz), 5.58 (1H, d, H-1 GalN$^{\alpha}$, J = 8.5 Hz), 5.43 (H-4 GalN$^{\beta}$, H-1 GalN$^{\beta}$), 5.34 (1H, d, H-4 GalN$^{\alpha}$, J = 3.3 Hz), 5.21 (1H, d, H-1 GlcN$^{\alpha}$, J = 8.1 Hz), 5.16 (H-1 GlcN$^{\beta}$), 4.87 (1H, d, H-1 Fuc$^{\alpha}$, J = 3.3 Hz), 4.80 (1H, d, PhC$\underline{H}$H, J = 11.8 Hz), 4.58 (5H, m, PhCH$\underline{H}$, 2x PhC$\underline{H}$H, 2x PhCH$\underline{H}$, H-5 GalN$^{\alpha}$), 4.47 (1H, m, H-5 Fuc$^{\alpha}$), 4.39 (2H, m, PhC$\underline{H}$H, H-2 GalN$^{\alpha}$), 4.22 (3H, m, H-6b GalN$^{\alpha}$, H-4 Fuc$^{\alpha}$, H-2 GlcN$^{\alpha}$), 4.11 (1H, d, PhCH$\underline{H}$, J = 12.6 Hz), 4.01 (H-6a Gal$^{\beta}$), 3.88 (2H, m, H-3 GlcN$^{\alpha}$, H-6a GalN$^{\alpha}$), 3.76 (2H, m, H-2 Fuc$^{\alpha}$, H-4 GlcN$^{\alpha}$), 3.65 (1H, s, H-3 Fuc$^{\alpha}$), 3.55 (2H, m, H-6a GlcN$^{\alpha}$, H-6b GlcN$^{\alpha}$), 3.37 (1H, m, H-5 GlcN$^{\alpha}$), 3.28 (H-5 GlcN$^{\beta}$), 1.92 to 1.77 (9H, 3s, 3x C$\underline{H}_3$ of Ac), 1.31 [4H, m, C$\underline{H}$(CH$_3$)$_2$ of TDS, C$\underline{H}_3$ of Fuc], 0.55 [12H, m, C(C$\underline{H}_3$)$_2$, CH(C$\underline{H}_3$)$_2$ of TDS], 0.01 to -0.18 (6H, 2s, 2x C$\underline{H}_3$-Si of TDS); ^{13}C NMR (101 MHz, CDCl$_3$): δ 170.14 to 167.25 (3x $\underline{C}$OCH$_3$ of Ac), 138.97 to 123.09 (C-Ar), 97.99 (C-1 Fuc$^{\alpha}$), 96.90 (C-1 GalN$^{\alpha}$), 92.99 (C-1 GlcN$^{\alpha}$), 79.51 (C-3 GlcN$^{\alpha}$),



S20

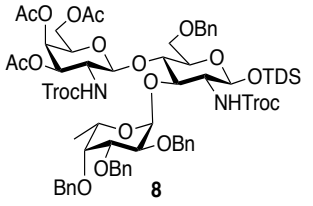
77.76 (C-3 Fuc$^{\alpha}$), 77.30, 76.99, 76.67, 75.02 (C-2 Fuc$^{\alpha}$), 74.95 (C-4 Fuc$^{\alpha}$), 74.70 (C-5 GlcN$^{\alpha}$), 74.44 (Ph<u>C</u>H$_2$), 74.38 (C-5 GalN$^{\alpha}$), 72.71 (Ph<u>C</u>H$_2$), 72.61 (Ph<u>C</u>H$_2$), 72.46, 70.67, 68.13 (C-6 GlcN$^{\alpha}$), 67.81 (C-3 GalN$^{\alpha}$), 66.90 (C-5 Fuc$^{\alpha}$), 66.56 (C-4 GalN$^{\alpha}$), 60.67 (C-6 GalN$^{\alpha}$), 58.39 (C-2 GlcN$^{\alpha}$), 51.86 (C-2 GalN$^{\alpha}$), 33.82 (<u>C</u>H(CH$_3$)$_2$ of TDS), 20.60 to 20.42 (3x <u>C</u>H$_3$ of Ac), 19.83 to 18.13 (4x <u>C</u>H$_3$ of TDS), 16.84 (<u>C</u>H$_3$ of Fuc$^{\alpha}$), -1.97 to -3.80 (2x <u>C</u>H$_3$-Si of TDS). ESI (*m/z*): [M+ Na]$^+$ calculated for $C_{76}H_{86}N_2O_{20}Si$; 1397.5441; found 1397.5450.

Dimethylthexylsilyl [2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl]-(1→3)-[3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxy)carbonylamino-β-D-galactopyranosyl]-(1→4)-2-deoxy-2-azido-6-*O*-benzyl-β-D-glucopyranoside (7): Donor **3** (2.1 g, 4.4 mmol), DPS (900 mg, 4.4 mmol) and TTBP (912 mg, 4.4 mmol) were dissolved in DCM (20 mL) and stirred with pre-activated molecular sieves (2 g) for 30 min. After this time, the temperature was brought down to -60 ºC, followed by the addition of Tf_2O (750 µL, 4.4 mmol). After 10 min at this temperature, a solution of acceptor **6** (1.0 g, 1.1 mmol) in anhydrous DCM (5 mL), was added dropwise along the wall of the flask and the reaction mixture was stirred for 1 h, during which the temperature was slowly raised to -40 ºC. The TLC (PE: EtOAc, 7: 3, v: v) showed complete consumption of acceptor, at which point, the reaction was quenched with Et_3N (2 mL). The molecular sieves were filtered off, and DCM was removed *in vacuo*. Silica gel column chromatography using PE: EtOAc (9: 1, v: v to 7: 3, v: v) gave the product as a white amorphous powder. (1.0 g, 68%); R_f = 0.55 (PE: EtOAc, 7: 3, v: v). ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.31 to 7.03 (20H, m, H-Ar), 5.34 (1H, d, H-1 Fuc, *J* = 3.5 Hz), 5.03 (1H, d, H-4 GalN, *J* = 3.4 Hz), 4.80 to 4.54 (7H, m, 3x PhCH<u>H</u>, 3x PhC<u>H</u>H, PhCH<u>H</u>), 4.49 (3H, m, H-5 Fuc, C<u>H</u>$_2$ of Troc), 4.36 (1H, m, H-3 GalN), 4.33 (1H, d, H-1 GlcN, *J* = 7.8 Hz), 4.22 (2H, m, PhC<u>H</u>H, H-1 GalN), 3.93 (2H, m, H-2 Fuc, H-6b GalN), 3.75 (3H, m, H- 6a GalN, H-3 Fuc, H-5 GalN), 3.54 (2H, m, H-6b GlcN, H-3 GlcN), 3.43 (2H, m, H-2 GalN, H-4 GlcN), 3.36 (2H, m, H-6a GlcN, H-4 Fuc), 3.27 (1H, dd, H-2 GlcN, *J* = 9.9 Hz, 7.7 Hz), 3.08 (1H, m, H-5 GlcN), 1.83 to 1.55 (9H, 3s, 3x C<u>H</u>$_3$ of Ac), 1.49 [1H, m, C<u>H</u>(CH$_3$)$_2$ of TDS], 1.06 (3H, C<u>H</u>$_3$ of Fuc), 0.70 [12H, m, C(C<u>H</u>$_3$)$_2$, CH(C<u>H</u>$_3$)$_2$ of TDS], 0.00 (6H, d, 2x C<u>H</u>$_3$-Si of TDS); ^{13}C NMR (151 MHz, $CDCl_3$): δ 170.22 to 169.80 (3x <u>C</u>OCH$_3$ of Ac), 154.04 (<u>C</u>OOCH$_2$ of Troc), 139.15 to 126.97 (C-Ar), 100.43 (C-1 GalN), 97.40 (C-1 GlcN), 97.21 (C-1 Fuc), 79.80 (C-3 Fuc), 77.34, 77.22, 77.02, 76.98 (C-3 GlcN), 75.60 (C-2 Fuc), 74.64 (C-5 GalN), 74.57 (C-4 Fuc), 74.27 (C-5 GlcN), 74.04 to 72.61 (4x Ph<u>C</u>H$_2$), 70.56 (C-3 GalN), 70.24 (C-4 GlcN), 68.71 (C-2 GlcN), 68.22 (C-6 GlcN), 66.08 (C-5 Fuc), 65.83 (C-4 GalN), 60.15 (C-6 GalN), 52.20 (C-2 GalN), 33.95 (<u>C</u>H(CH$_3$)$_2$ of TDS), 20.64 to 20.35 (3x <u>C</u>H$_3$ of Ac), 20.01, 19.98 to 18.36 (4x <u>C</u>H$_3$ of TDS), 16.82 (<u>C</u>H$_3$ of Fuc), -2.09 to -3.02 (2x <u>C</u>H$_3$-Si of TDS). ESI (*m/z*): [M+ Na]$^+$ calculated for $C_{63}H_{81}Cl_3N_4O_{18}Si$, 1337.4278; found 1337.4285.



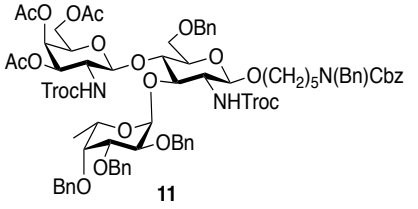
7

Dimethylthexylsilyl [2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl]-(1→3)-[3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxy)carbonylamino-β-D-galactopyranosyl]-(1→4)-[6-*O*-benzyl-2-deoxy-2-(2,2,2-trichloroethoxy)]carbonylamino-β-D-glucopyranoside (8): Compound **7** (1.0 g, 0.76 mmol) was dissolved in THF (20 mL) and water (4 mL) was added. This was followed by the addition of trimethylphosphine (392 µL, 3.8 mmol). The reaction mixture was stirred under argon for 2 h, following which the solvent was evaporated *in vacuo* and co-evaporated with toluene twice. The residue (800 mg, 0.6 mmol) was dissolved in DCM (20 mL), followed by the addition of 2,2,2-trichloroethyl chloroformate (170 µL, 1.24 mmol) and triethylamine (173 µL, 1.24 mmol). The reaction mixture was stirred for 1 h after which it was diluted by DCM (100 mL) and washed with water (150 mL). The organic layer was dried over $MgSO_4$ and filtered, and the filtrate was concentrated *in vacuo*. Silica gel column chromatography using PE: EtOAc (9: 1, v: v to 7: 3, v: v) afforded the product as a yellowish amorphous solid. (700.0 mg, 77%); R_f = 0.58 (PE: EtOAc, 7: 3 v: v). ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.48 to 7.07 (20H, m, H-Ar), 5.22 (1H, d, NH of Troc, *J* = 7.1 Hz), 5.19 (1H, d, H-4 GalN, *J* = 3.1 Hz), 5.09 (1H, d, H-1 Fuc, *J* = 2.8 Hz), 5.01 (1H, d, H-1 GlcN, *J* = 7.4 Hz), 4.76 (11H, m, 2x CH_2 of Troc, 3x PhCHH, 3x PhCHH), 4.53 (2H, m, H-5 Fuc, PhCHH), 4.46 (1H, d, H-1 GalN, *J* = 9.0 Hz), 4.37 (1H, d, PhCHH, *J* = 11.7 Hz), 4.07 (3H, m, H-6b GalN, H-2 Fuc, H-3 GlcN), 3.85 (3H, m, H-3 Fuc, H-6a GalN, H-5 GalN), 3.67 (2H, m, H-6b GlcN, H-4 Fuc), 3.58 (2H, m, H-2 GalN, H-4 GlcN), 3.50 (1H, d, H-6a GlcN, *J* = 10.1 Hz), 3.32 (1H, m, H-5 GlcN), 2.94 (1H, m, H-2 GlcN), 1.97 to 1.76 (9H, 3s, 3x CH_3 of Ac), 1.51 [1H, m, $CH(CH_3)_2$ of TDS], 1.17 (3H, CH_3 of Fuc), 0.77 [12H, m, $C(CH_3)_2$, $CH(CH_3)_2$ of TDS], 0.03 (6H, d, 2x CH_3-Si of TDS); ^{13}C NMR (151 MHz, $CDCl_3$): δ 172.08 to 171.87 (3x $COCH_3$ of Ac), 155.92 to 155.33 (2x $COOCH_2$ of Troc), 140.84 to 128.99 (C-Ar), 101.87 (C-1 GalN), 99.52 (C-1 Fuc), 96.15 (C-1 GlcN), 81.81 (C-3 Fuc), 79.17, 79.04 (C-3 GlcN), 78.96 (C-4 Fuc), 78.85, 78.75, 77.06 (C-5 GalN), 76.63 (C-2 Fuc), 76.37 to 76.15 (2x $PhCH_2$), 75.95 (C-5 GlcN), 75.88 to 74.53 (2x $PhCH_2$), 72.13 (C-4 GlcN), 70.39 (C-6 GlcN), 68.14 (C-5 Fuc), 67.99 (C-4 GalN), 63.44 (C-2 GlcN), 62.31 (C-6 GalN), 54.29 (C-2 GalN), 35.97 ($CH(CH_3)_2$ of TDS), 22.59 to 22.06 (3x CH_3 of Ac), 21.89 to 19.22 (4x CH_3 of TDS), 18.84 (CH_3 of Fuc), -0.01 to -1.58 (2x CH_3-Si of TDS). ESI (*m/z*): $[M+NH_4]^+$ calculated for $C_{66}H_{84}Cl_6N_2O_{20}Si$, 1480.3862; found 1480.3878.



8

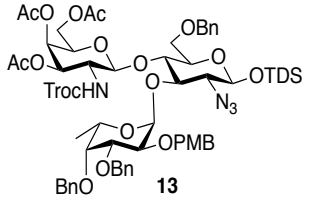
5-(*N*-Benzyloxycarbonyl,*N*-Benzyl)aminopentyl [2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl]-(1→3)-[3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxy)carbonylamino-β-D-galactopyranosyl]-(1→4)-[6-*O*-benzyl-2-deoxy-2-(2,2,2-trichloroethoxy)]carbonylamino-β-D-glucopyranoside (11): Compound **8** (700 mg, 0.47 mmol) was dissolved in the solvent system DCM: pyridine (30 mL, 1: 2, v: v), followed by dropwise addition of HF in pyridine (70% HF, 30% pyridine; 5 mL). The reaction mixture was stirred at room temperature for 12 h, after which it was quenched with solid $NaHCO_3$ (10 g), till all CO_2 bubbling stopped. The salts were filtered off, the solvent was evaporated *in vacuo*, and the residue was re-dissolved in DCM (50 mL), followed by washing with water (100 mL) and saturated $NaHCO_3$ (100 mL). The organic layer was dried over $MgSO_4$, filtered and the filtrate was concentrated and put for the next stage without further purification. The residue (554 mg, 0.42 mmol) was dissolved in DCM (20 mL), followed by the addition of 2,2,2-trifluoro-*N*-phenylacetimidoyl chloride (130 μL, 0.63 mmol) and DBU (62 μL, 0.42 mmol). The reaction was stirred for 30 min, after which the solvent was evaporated and product was purified using silica gel column chromatography (PE to PE: EtOAc, 7: 3, v: v). The product was dried over high vacuum for 30 min, and immediately put for glycosylation reaction. The imidate donor (570 mg, 0.38 mmol) and 5-(*N*-benzyloxycarbonyl,*N*-benzyl)aminopentyl linker (626 mg, 1.91 mmol) were dissolved in DCM (10 mL), and stirred with pre-activated molecular sieves (1.5 g), under argon for 30 min. The reaction mixture was cooled down to -50 °C, followed by the addition of TfOH (6.8 μL, 0.076 mmol). The temperature was slowly warmed up to -30 °C, after which the TLC showed complete consumption of donor and a new product spot formed. The reaction mixture was quenched with Et_3N (10 μL), the sieves were filtered off, and the solvent was evaporated. Silica gel column chromatography using PE: EtOAc (9: 1, v: v to 7: 3, v: v) afforded the product as a transparent syrup (509 mg, 66% over 3 steps). R_f = 0.52 (PE: EtOAc, 7: 3, v: v). ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.65 to 6.96 (30H, m, H-Ar), 5.24 (1H, d, H-4 GalN, J = 3.1 Hz), 5.15 (3H, m, C<u>H</u>$_2$ of Cbz of N(Bn)Cbz Linker, H-1 Fuc), 4.99 to 4.66 (9H, m, 3x PhC<u>H</u>H, 3xPhCH<u>H</u>, H-1 GlcN, CH_2 of Troc), 4.60 (3H, m, H-5 Fuc, H-3 GalN, PhCH<u>H</u>), 4.43 (4H, m, C<u>H</u>$_2$ of Bn of N(Bn)Cbz Linker, PhC<u>H</u>H, H-1 GalN), 4.16 (3H, m, H-6a GalN, H-3 GlcN, H-2 Fuc), 3.92 (3H, m, H-3 Fuc, H-5 GalN, H-6b GalN), 3.75 (3H, m, H-6b GlcN, OCH<u>H</u>$(CH_2)_4$N(Bn)Cbz, H-4 Fuc), 3.60 (3H, m, H-2 GalN, H-6a GlcN, H-4 GlcN), 3.37 (2H, m, OC<u>H</u>H$(CH_2)_4$N(Bn)Cbz, H-5 GlcN), 3.17 (3H, m, O$(CH_2)_4$C<u>H</u>$_2$N(Bn)Cbz Linker, H-2 GlcN), 2.01 to 1.81 (9H, 3s, 3x C<u>H</u>$_3$ of Ac), 1.48 (4H, m, 2x C<u>H</u>$_2$ of linker), 1.22 (5H, m, C<u>H</u>$_3$ of Fuc, C<u>H</u>$_2$ of linker); ^{13}C NMR (151 MHz, $CDCl_3$): δ 171.38 to 170.01 (3x <u>C</u>OCH$_3$ of Ac), 156.81 to 153.68 (2x <u>C</u>OOCH$_2$ of Troc), 138.97 to 128.28 (C-Ar), 100.23 (C-1 GalN), 99.98 (C-1 GlcN), 97.61 (C-1 Fuc), 79.94 (C-3 Fuc), 77.51, 77.29 (C-4 Fuc), 77.08, 76.60 (C-2 Fuc), 74.89 (C-5 GalN), 74.49 (C-3 GlcN), 74.38 (Ph<u>C</u>H$_2$), 74.25 (C-5 GlcN), 73.83 to 72.64 (2x Ph<u>C</u>H$_2$), 70.19 (C-3 GalN), 69.68 (C-4 GlcN), 68.25 (C-6 GlcN), 67.24 (<u>C</u>H$_2$ of Cbz of N(Bn)Cbz Linker), 66.43 (C-5 Fuc), 66.02 (C-4 GalN), 64.42, 60.47, 60.42 (C-6 GalN),



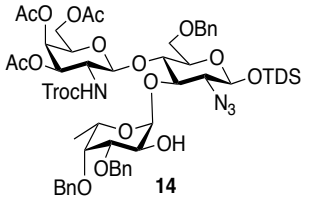
11

58.74 (C-2 GlcN), 54.19, 52.35 (C-2 GalN), 50.54, 50.32 ($\underline{C}H_2$ of Bn of N(Bn)Cbz), 47.24, 46.18 ($O(CH_2)_4\underline{C}H_2N$(Bn)Cbz Linker), 40.98, 33.86, 31.96, 30.66, 30.39, 29.73, 29.69, 29.15, 29.03, 28.50, 27.90 to 27.33 (2x CH_2) of linker, 24.02, 23.14, 22.74, 21.11, 21.07, 20.99, 20.69 to 20.61 (3x $\underline{C}H_3$ of Ac), 19.17, 17.62, 17.37, 16.96 ($\underline{C}H_3$ of Fuc). ESI (*m/z*): $[M+ Na]^+$ calculated for $C_{78}H_{89}Cl_6N_3O_{22}$, 1652.3967; found 1652.3955.

Dimethylthexylsilyl [3,4-di-*O*-benzyl-2-*O*-p-methoxybenzyl-α-L-fucopyranosyl]-(1→3)-[3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxy)carbonylamino-β-D-galactopyranosyl]-(1→4)-2-deoxy-2-azido-6-*O*-benzyl-β-D-glucopyranoside (13): Donor **4** (4.0 g, 7.0 mmol), DPS (1.4 g, 7.0 mmol) and TTBP (1.74 g, 7.0 mmol) were dissolved in DCM (25 mL) and stirred with pre-activated molecular sieves (6.0 g) for 30 min, after which the temperature was brought down to -60 ºC, followed by the addition of Tf_2O (1.2 mL, 7.0 mmol). After 10 min, a solution of acceptor **6** (1.6 g, 1.7 mmol) in anhydrous DCM (6 mL) was added dropwise along the wall of the flask, and the reaction mixture was stirred for 1 h, during which the temperature was slowly raised to -40 ºC. After TLC (Tol: EtOAc, 8.5: 1.5, v: v) showed complete consumption of acceptor, the reaction was quenched using Et_3N (5 mL). The molecular sieves were filtered off, and the solvent was removed *in vacuo*. Silica gel column chromatography using Tol: EtOAc (9: 1, v: v to 7: 3, v: v) gave the product as a white amorphous powder. (1.8 g, 73%). R_f = 0.49 (Tol: EtOAc, 8.5: 1.5, v: v). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.55 to 6.67 (19H, m, H-Ar), 5.48 (1H, d, H-1 Fuc, *J* = 3.8 Hz), 5.21 (1H, d, H-4 GalN, *J* = 3.2 Hz), 4.92 (2H, m, PhC$\underline{H}$H, PhC$\underline{H}$H), 4.81 (3H, m, CH_2 of Troc, PhCH$\underline{H}$), 4.67 (6H, m, H-3 GalN, H-5 Fuc, 3x PhCH$\underline{H}$, PhC$\underline{H}$H), 4.50 (1H, d, H-1 GlcN, *J* = 7.6 Hz), 4.40 (2H, m, PhC$\underline{H}$H, H-1 GalN), 4.11 (2H, m, H-2 Fuc, H-6b GalN), 3.92 (3H, m, H- 6a GalN, H-3 Fuc, H-5 GalN), 3.76 (5H, m, H-6b GlcN, H-3 GlcN, *O*CH_3 of PMB), 3.58 (4H, m, H-6a GlcN, H-4 Fuc, H-2 GalN, H-4 GlcN), 3.46 (1H, m, H-2 GlcN), 3.26 (1H, m, H-5 GlcN), 2.00 to 1.74 (9H, 3s, 3x C$\underline{H}_3$ of Ac), 1.67 [1H, m, C$\underline{H}(CH_3)_2$ of TDS], 1.22 (3H, d, C$\underline{H}_3$ of Fuc, *J* = 7.1 Hz), 0.88 [12H, m, C(C$\underline{H}_3)_2$, CH(C$\underline{H}_3)_2$ of TDS], 0.18 (6H, d, 2x C$\underline{H}_3$-Si of TDS); ^{13}C NMR (101 MHz, $CDCl_3$): δ 170.04 to 169.75 (3x $\underline{C}OCH_3$ of Ac), 153.89 ($\underline{C}OOCH_2$ of Troc), 139.08 to 113.39 (C-Ar), 100.38 (C-1 GalN), 97.36 (C-1 GlcN), 97.30 (C-1 Fuc), 79.88 (C-3 Fuc), 77.31, 76.99 (C-3 GlcN), 76.67, 75.03, 74.54 (C-2 Fuc), 74.40 (C-5 GalN), 74.02 (C-4 Fuc), 73.92 (C-5 GlcN), 72.80 to 72.71 (Ph$\underline{C}H_2$), 70.10 (C-4 GlcN), 68.75 (C-2 GlcN), 68.12 (C-6 GlcN), 66.02 (C-5 Fuc), 65.79 (C-4 GalN), 60.11 (C-6 GalN), 55.21 (O$\underline{C}H_3$ of PMB), 52.17 (C-2 GalN), 33.85 ($\underline{C}H(CH_3)_2$ of TDS), 20.63 to 20.46 (3x $\underline{C}H_3$ of Ac), 19.97 to 18.35 (4x $\underline{C}H_3$ of TDS), 16.82 ($\underline{C}H_3$ of Fuc), -0.03 to -3.03 (2x $\underline{C}H_3$-Si of TDS). ESI (*m/z*): $[M+ Na]^+$ calculated for $C_{64}H_{83}Cl_3N_4O_{19}Si$; 1367.4384; found 1367.4367.

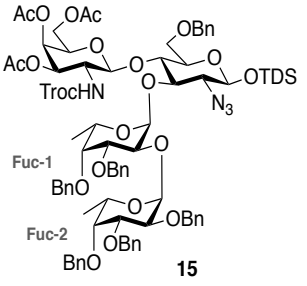


Dimethylthexylsilyl [3,4-di-*O*-benzyl-α-L-fucopyranosyl]-(1→3)-[3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxy)carbonylamino-β-D-galactopyranosyl]-(1→4)-6-*O*-benzyl-2-deoxy-2-azido-β-D-glucopyranoside (14): Trisaccharide **13** (1.5 g, 1.11 mmol) was dissolved in DCM: H_2O (20 mL, 9: 1, v: v) followed by the addition of DDQ (506 mg, 2.2 mmol), and was stirred for 2 h, after which the TLC showed full conversion of starting material to product. The reaction mixture was then diluted by DCM (50 mL) and washed with water (20 mL) and saturated $NaHCO_3$ (100 mL). The organic layer was dried over $MgSO_4$ and filtered, and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography using Tol: EtOAc (8: 2, v: v) which gave the product as a white amorphous solid. (1.0 g, 74%). R_f = 0.47 (Tol: EtOAc, 8.5: 1.5, v: v). ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.65 to 7.06 (15H, m, H-Ar), 5.46 (1H, d, H-1 Fuc, *J* = 4.2 Hz), 5.22 (1H, d, H-4 GalN, *J* = 3.5 Hz), 4.95 to 4.61 (6H, m, CH_2 of Troc, 2x PhCH<u>H</u>, 2x PhC<u>H</u>H), 4.54 (2H, m, H-5 Fuc, H-3 GalN), 4.47 (1H, d, H-1 GlcN, *J* = 7.8 Hz), 4.41 (2H, m, H-1 GalN, PhC<u>H</u>H), 4.21 (1H, m, H-2 Fuc), 4.09 (1H, m, H-6b GalN), 3.90 (2H, m, H-6a GalN, H-3 Fuc), 3.72 (2H, m, H-6b GlcN, H-5 GalN), 3.63 (3H ,m, H-2 GalN, H-4 GlcN, H-3 GlcN), 3.54 (2H, m, H-6a GlcN, H-4 Fuc), 3.30 (2H, m, H-5 GlcN, H-2 GlcN), 2.03 to 1.76 (9H, 3s, 3x C<u>H</u>$_3$ of Ac), 1.67 [1H, m, C<u>H</u>$(CH_3)_2$ of TDS] , 1.22 (3H, d, C<u>H</u>$_3$ of Fuc, *J* = 6.2 Hz), 0.88 [12H, m, C(C<u>H</u>$_3)_2$, CH(C<u>H</u>$_3)_2$ of TDS], 0.17 (6H, d, 2x C<u>H</u>$_3$-Si of TDS); ^{13}C NMR (151 MHz, $CDCl_3$): δ 170.04 to 169.78 (3x <u>C</u>OCH$_3$ of Ac), 153.91 (<u>C</u>OOCH$_2$ of Troc), 138.68 to 127.12 (C-Ar), 100.07 (C-1 GalN), 98.41(C-1 Fuc), 97.22 (C-1 GlcN), 80.63 (C-5 GalN), 77.30 , 77.18, 76.98, 76.66, 76.09 (C-3 GlcN), 75.14 (C-4 Fuc), 74.39 (C-5 GlcN), 74.37, 73.98 (C-3 Fuc), 73.90 to 70.36 (3x Ph<u>C</u>H$_2$), 70.12 <u>C</u>H$_2$ of Troc), 69.29 (C-4 GlcN), 69.13 (C-5 Fuc), 68.94 (C-2 Fuc), 68.55 (C-2 GlcN), 67.96 (C-3 GalN), 67.72 (C-6 GlcN), 65.91 (C-4 GalN), 60.44 (C-6 GalN), 52.15 (C-2 GalN), 33.84 (<u>C</u>H$(CH_3)_2$ of TDS), 20.67 to 20.46 (3x <u>C</u>H$_3$ of Ac), 19.97 to 18.34 (4x <u>C</u>H$_3$ of TDS), 16.90 (<u>C</u>H$_3$ of Fuc), -0.04 to -3.19 (2x <u>C</u>H$_3$-Si of TDS). ESI (*m/z*): $[M+ Na]^+$ calculated for $C_{56}H_{75}Cl_3N_4O_{18}Si$, 1247.3809; found 1247.3815.



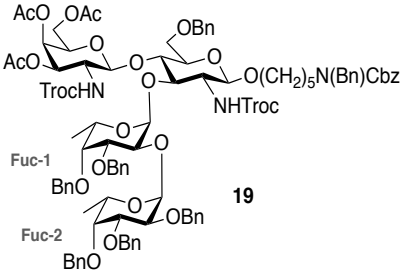
14

Dimethylthexylsilyl 2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl-(1→2)-[3,4-di-*O*-benzyl-α-L-fucopyranosyl]-(1→3)-[3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxy)carbonylamino-β-D-galactopyranosyl]-(1→4)-2-azido-2-deoxy-6-*O*-benzyl-β-D-glucopyranoside (15): Acceptor **14** (800 mg, 0.65 mmol) and donor **3** (625 mg, 1.3 mmol), were dissolved in the solvent system DCM (5 mL) and DMF (5 mL) and stirred with pre-activated molecular sieves (2 g) for 30 min, after which the temperature was brought down to -30 ºC. This was followed by the sequential addition of NIS (293 mg, 1.3 mmol) and TMSOTf (118 µL, 0.65 mmol). The reaction mixture was warmed up to +5 ºC over a period of one h, after which the TLC showed complete consumption of acceptor. The reaction mixture was quenched with Et_3N (1 mL). The solvent was removed *in vacuo*, the residue was diluted by DCM and washed with saturated $NaHCO_3$ (20 mL) and 5% sodium thio-sulphate (50 mL) respectively. The organic phase was dried over $MgSO_4$ and filtered, and the filtrate was concentrated *in vacuo*. The residue was purified using silica gel column chromatography (Tol: EtOAc, 9: 1, v: v to 7: 3, v: v) yielded the product as a pale-yellow amorphous powder. (890 mg, 83%). R_f = 0.61 (Tol: EtOAc, 8: 2, v: v). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.61 to 6.98 (30H, Ar-H), 5.69 (1H, d, H-1 Fuc-2, *J* = 3.6 Hz), 5.31 (1H, d, H-1 Fuc-1, *J* = 3.3 Hz), 5.23 (1H, d, H-4 GalN, *J* = 3.2 Hz), 4.73 to 5.02 (8H, m, CH_2 of Troc, 3x PhCHH, PhCHH), 4.64 (4H, m, H-3 GalN, H-5 Fuc-1, PhCHH, PhCHH), 4.49 (4H, m, H-1 GalN, H-1 GlcN, PhCHH, PhCHH), 4.43 (3H, m, H-2 Fuc-2, PhCHH, PhCHH), 4.20 (2H, m, H-6b GalN, H-5 Fuc-2), 4.07 (2H, m, H-6a GalN, H-2 Fuc-1), 3.96 (1H, m, H-5 GalN), 3.80 (2H, m H-4 Fuc-2, H-3 Fuc-1), 3.69 (3H, m, H-6b GlcN, H-4 GlcN, H-3 GlcN), 3.59 (2H, m, H-4 Fuc-1, H-2 GalN), 3.47 (1H, m, H-6a GlcN), 3.22 (3H, m, H-5 GlcN, H-2 GlcN, H-3 Fuc-2), 1.97 to 1.79 (9H, 3s, 3x CH_3 of Ac), 1.60 [1H, m, $CH(CH_3)_2$ of TDS], 1.24 (3H, m, CH_3 of Fuc-1), 1.01 (3H, d, CH_3 of Fuc-2, *J* = 6.6 Hz), 0.83 [12H, m, $C(CH_3)_2$, $CH(CH_3)_2$ of TDS], 0.13 (6H, d, 2x CH_3-Si of TDS); ^{13}C NMR (101 MHz, $CDCl_3$): δ 170.23 to 169.74 (3x $COCH_3$ of Ac), 153.93 ($COOCH_2$ of Troc), 139.24 to 126.13 (C-Ar), 100.50 (C-1 GalN), 97.14 (C-1 GlcN), 94.80 (C-1 Fuc-2), 93.25 (C-1 Fuc-1), 79.63 (C-4 Fuc-2), 78.27 (C-5 GalN), 77.49 (C-4 GlcN), 77.31, 77.19 (C-3 Fuc-2), 77.08, 76.99, 76.67, 76.07 (C-2 Fuc-1), 74.82 (C-3 Fuc-1), 74.70 ($PhCH_2$), 74.43 ($PhCH_2$), 74.38 (C-5 GlcN), 74.10 to 74.01 (2x ($PhCH_2$), 73.63 (C-4 Fuc-1), 72.54 to 72.46 (2x$PhCH_2$, CH_2 of Troc), 70.36 (C-3 GlcN), 70.23 (C-3 GalN), 69.17 (C-2 Fuc-2), 68.40 (C-6 GlcN), 67.69 (C-2 GlcN), 65.98 (C-4 GalN), 65.90 (C-5 Fuc-1), 65.82 (C-5 Fuc-2), 60.49 (C-6 GalN), 52.30 (C-2 GalN), 33.69 ($CH(CH_3)_2$ of TDS), 31.90, 29.67, 29.24, 20.68 to 20.51 (3x CH_3 of Ac), 19.94 to 18.31 (4x CH_3 of TDS), 16.78 (CH_3 of Fuc-1), 16.14 (CH_3 of Fuc-2), -0.03 to -3.01 (2x CH_3-Si of TDS). ESI (*m/z*): $[M+Na]^+$ calculated for $C_{83}H_{103}Cl_3N_4O_{22}Si$, 1663.5797; found 1663.5792.



15

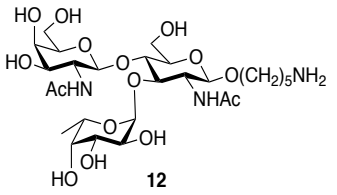
5-(*N*-Benzyloxycarbonyl,*N*-Benzyl)aminopentyl 2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl-(1→2)-[3,4-di-*O*-benzyl-α-L-fucopyranosyl]-(1→3)-[3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,2,2,-trichloroethoxy)carbonylamino-β-D-galactopyranosyl]-(1→4)-6-*O*-benzyl-2-deoxy-2-(2,2,2-trichloroethoxy)carbonylamino-β-D-glucopyranoside (19): Compound **15** (850 mg, 0.53 mmol) was dissolved in THF (9 mL) and H_2O (1 mL), followed by the addition of trimethyl phosphine (283 µL, 2.7 mmol). The reaction mixture was stirred under argon for 2 h. After this time, the solvent was evaporated, and the residue was co-evaporated with toluene thrice. The residue was used further without purification. The residue was dissolved in DCM (10 mL), followed by the addition of 2,2,2-trichloroethyl chloroformate (136 µL, 0.99 mmol) and Et_3N (138 µL, 0.99 mmol). The reaction mixture was then allowed to stir for 1 h, after which the TLC (R_f = 0.58, Tol: EtOAc, 8: 2, v: v) showed installation of Troc group at the free amine of C-2 of GlcN. The solvent was evaporated, the residue was dissolved in DCM (20 mL), and washed with water (20 mL) and 1M HCl (30 mL). The organic layer was dried over $MgSO_4$, filtered, and the filtrate was concentrated and used for next step. The Troc installed compound **16** (500 mg, 0.28 mmol) was dissolved in DCM (5 mL) and pyridine (10 mL), followed by dropwise addition of HF in pyridine (70% HF, 30% pyridine; 3 mL). The reaction mixture was stirred for 12 h, after which it was quenched with solid $NaHCO_3$ (2 g), till all CO_2 bubbling stopped. The salts were filtered off, the solvent was evaporated *in vacuo*, and the residue was re-dissolved in DCM (10 mL), followed by washing with water (10 mL) and saturated $NaHCO_3$ (15 mL). The organic layer was dried over $MgSO_4$, filtered, and the filtrate was concentrated, affording compound **17** and put for the next stage without further purification. Compound **17** (370 mg, 0.224 mmol) was dissolved in DCM (20 mL), followed by the addition of 2,2,2-trifluoro-*N*-phenylacetimidoyl chloride (70 µL, 0.337 mmol) and DBU (334 µL, 0.224 mmol). The reaction was stirred for 30 min, after which the solvent was evaporated and product was purified using silica gel column chromatography (Tol to Tol: EtOAc, 8: 2, v: v). The product **18** was dried over high vacuum for 30 min, and immediately put for glycosylation reaction. The imidate donor **18** (350 mg, 0.19 mmol) and 5-(*N*-Benzyloxycarbonyl,*N*-Benzyl)aminopentyl linker (316 mg, 0.98 mmol) were dissolved in DCM (5 mL), and stirred with pre-activated molecular sieves (500 mg), under argon for 30 min. The mixture was cooled down to -50 °C, followed by the addition of TfOH (6.0 µL, 0.069 mmol). The temperature was slowly warmed up to -30 °C, after which the TLC showed complete consumption of donor and a new product spot formed. The reaction mixture was quenched with Et_3N (50 µL), the sieves were filtered off, and the solvent was evaporated. Silica gel column chromatography using Tol: EtOAc (9: 1, v: v to 7: 3, v: v) afforded the product **19** as a transparent syrup (312.0 mg, 31% over 5 steps). R_f = 0.62 (Tol: EtOAc, 8.5: 1.5, v: v). ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.51 to 7.05 (40H, m, H-Ar), 5.26 (1H, d, H-4 GalN, *J* = 3.3 Hz), 5.14 (3H, m, C<u>H</u>$_2$ of Cbz of N(Bn)Cbz linker, H-1 Fuc-1, H-1 Fuc-



19

2), 4.96 (3H, m, H-1 GlcN, PhC<u>H</u>H, PhCH<u>H</u>), 4.86 (4H, m, 2x PhC<u>H</u>H, 2x PhCH<u>H</u>), 4.67 (9H, m, PhC<u>H</u>H, H-5 Fuc-1, H-3 GalN, 2x C<u>H</u>$_2$ of Troc, PhC<u>H</u>H, PhCH<u>H</u>), 4.51 (2H, m, PhC<u>H</u>H, PhCH<u>H</u>), 4.40 (5H, m, C<u>H</u>$_2$ of Bn of N(Bn)Cbz linker, H-2 Fuc-2, PhCH<u>H</u>, H-1 GalN), 4.25 (1H, m, H-5 Fuc-2), 4.11 (4H, m, H-6a GalN, H-6b GalN, H-2 Fuc-1, H-3 GlcN), 3.95 (2H, m, H-4 Fuc-2, H-5 GalN), 3.81 (1H, m, H-3 Fuc-1), 3.71 (3H, m, H-6b GlcN, H-4 Fuc-1, OCH<u>H</u>(CH$_2$)$_4$N(Bn)Cbz), 3.58 (3H, m, H-6a GlcN, H-2 GalN, H-4 GlcN), 3.33 (3H, m, OC<u>H</u>H(CH$_2$)$_4$N(Bn)Cbz, H-5 GlcN, H-3 Fuc-2), 3.08 (3H, m, O(CH$_2$)$_4$C<u>H</u>$_2$N(Bn)Cbz, H-2 GlcN), 2.05 to 1.84 (9H, 3s, 3x C<u>H</u>$_3$ of Ac), 1.40 (4H, m, 2x C<u>H</u>$_2$ of Linker), 1.24 (5H, m, C<u>H</u>$_3$ of Fuc-1, C<u>H</u>$_2$ of Linker), 0.81 (3H, d, C<u>H</u>$_3$ of Fuc-2); ^{13}C NMR (151 MHz, $CDCl_3$): δ 170.23 to 169.95 (3x <u>C</u>OCH$_3$ of Ac), 153.99 to 153.33 (2x <u>C</u>OOCH$_2$ of Troc) 138.80 to 127.20 (C-Ar), 100.00 (C-1 GalN), 99.73 (C-1 GlcN), 95.82 (C-1 Fuc-1), 95.69 (C-1 Fuc-2), 80.74 (C-4 Fuc-2), 78.49 (C-5 GalN), 77.37 (C-4 Fuc-1), 77.15, 76.94 (C-5 GlcN), 75.65 (C-2 Fuc-1), 74.61 (C-3 Fuc-1), 74.52 (Ph<u>C</u>H$_2$), 74.37 (C-3 GlcN), 74.14 (Ph<u>C</u>H$_2$), 74.06 (C-3 Fuc-2), 73.99 to 72.10 (4x Ph<u>C</u>H$_2$, 2x CH$_2$ of Troc), 71.23 (C-2 Fuc-2), 70.21 (C-3 GalN), 70.21 (C-4 GlcN), 69.78 (O<u>C</u>H$_2$(CH$_2$)$_4$N(Bn)Cbz), 68.09 (C-6 GlcN), 67.20 (<u>C</u>H$_2$ of Cbz of N(Bn)Cbz linker), 66.34 (C-5 Fuc-1), 66.11 (C-4 GalN), 65.95 (C-5 Fuc-2), 60.31 (C-6 GalN), 59.13 (C-2 GlcN), 52.29 (C-2 GalN), 50.51 (<u>C</u>H$_2$ of Bn of N(Bn)Cbz linker), 50.19, 47.14, 46.21, 40.93, 33.85, 31.97, 30.37, 29.74, 29.40, 29.31, 28.47, 27.96, 27.46, 23.94, 23.16, 22.74, 20.90, 20.70 to 20.59 (3x <u>C</u>H$_3$ of Ac), 17.57, 17.35, 16.84 (<u>C</u>H$_3$ of Fuc-1), 16.15 (<u>C</u>H$_3$ of Fuc-2). ESI (*m/z*): $[M+ NH_4]^+$ calculated for $C_{98}H_{111}Cl_6N_3O_{26}$, 1959.6610; found 1959.1591.

5-aminopentyl α-L-fucopyranosyl-(1→3)-[2-deoxy-2-acetamido)-β-D-galactopyranosyl]-(1→4)-2-deoxy-2-acetamido-β-D-glucopyranoside (12): Compound **11** (500 mg, 0.3 mmol) was dissolved in THF (20 mL). To this solution, was added zinc (Zn) dust (1.0 g, pre-activated with 1N HCl), Acetic anhydride (1.0 mL) and acetic acid (250 μL). The reaction mixture was stirred for 30 min, after which, TLC (Tol: acetone, 9: 1, v: v), showed complete consumption of starting material. The Zn dust was filtered off, and the residue was co-evaporated with toluene. The residue was dissolved in DCM (10 mL) and MeOH (10 mL), followed by the addition of 1M NaOMe (250 μL). After 2 h, the reaction mixture was quenched Amberlite® IR 120 hydrogen form resin (500 mg). The resin was filtered off, solvent was evaporated *in vacuo* and the residue was dried over high vacuum. This was used for the next reaction without any purification. The residue was dissolved in solvent system MeOH (5 mL) and water (5 mL), followed by the addition of palladium hydroxide (Degussa type, 100 mg), and AcOH (50 μL). The reaction mixture was stirred under hydrogen for 24 h, after which MALDI showed that the product had completely formed. The catalyst was carefully filtered off using celite, the solvent was evaporated *in vacuo*, and the residue was purified over P-2 Biogel using 50 mM ammonium bicarbonate as eluent. The compound was further purified using HILIC HPLC, using



12

a gradient solvent system ACN: 50 mM NH_4HCO_3 (95: 5, v: v to 80: 20, v: v). The resulting product was a white cotton-like solid after lyophilization (89 mg, 45 % over three steps).

^{1}H NMR (600 MHz, D_2O):

12	H1	H2	H3	H4	H5	H6	Fuc-CH_3	NHAc
GlcNAc	4.33, d, *J*= 8.3 Hz	3.77	3.82	3.71	3.39	3.45, 3.77	-	1.92
GalNAc	4.37, d, *J*= 8.4 Hz	3.85	3.79	3.76	3.46	3.62, 3.80	-	1.94
Fuc	4.99, d, *J*= 3.8 Hz	3.57	3.59	3.72	4.74	N.A.	1.14, d, *J*= 6.6 Hz	-

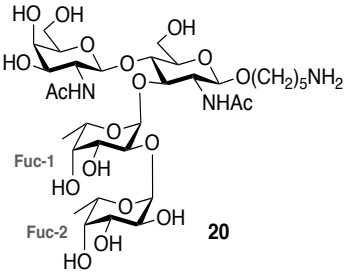
^{13}C NMR (151 MHz, D_2O):

12	C1	C2	C3	C4	C5	C6	**C**$COCH_3$ of NHAc	CO**C**H_3 of NHAc
GlcNAc	100.91	55.59	67.29	71.94	75.39	70.11	174.80	22.13
GalNAc	100.69	52.30	69.10	73.29	74.80	59.95	174.07	22.07
Fuc	98.42	67.64	70.65	74.61	66.89	15.31	-	-

Linker Peak	^{1}H Signal	^{13}C Signal
OCH_2CH_2**CH_2**$CH_2CH_2NH_2$	1.27	22.21
$OCH_2CH_2CH_2$**CH_2**CH_2NH_2	1.47	26.33
OCH_2**CH_2**$CH_2CH_2CH_2NH_2$	1.55	28.02
$OCH_2CH_2CH_2CH_2$**CH_2**NH_2	2.86	39.25
O**CH_2**$CH_2CH_2CH_2CH_2NH_2$	3.62	61.42

ESI (*m*/*z*): [M+ Na^+] calculated for $C_{27}H_{49}N_3O_{15}$, 678.3061; found 678.3076.

5-aminopentyl α-L-fucopyranosyl-(1→2)-α-L-fucopyranosyl-(1→3)-[2-deoxy-2-acetamido-β-D-galactopyranosyl]-(1→4)-2-deoxy-2-acetamido-β-D-glucopyranoside (20): Compound **19** (310 mg, 0.16 mmol) was dissolved in THF (15 mL). To this solution was added Zn dust (800 mg, pre-activated with 1N HCl), acetic anhydride (800 µL) and acetic acid (200 µL). The reaction mixture was stirred for 30 min, after which, TLC (Tol: acetone, 9: 1, v: v), showed complete consumption of starting material. The Zn dust was filtered off, and the residue was co-evaporated with toluene. The residue was dissolved in DCM (10 mL) and MeOH (10 mL), followed by the addition of 1M NaOMe (200 µL). After 2 h, the reaction mixture was quenched with Amberlite® IR 120 hydrogen form resin (600 mg). The resin was filtered off, solvent was evaporated *in vacuo* and the residue was dried over high vacuum. This was used for the next reaction without any purification. The residue was dissolved in solvent system MeOH (5 mL) and water (5 mL), followed by the addition of palladium hydroxide (Degussa type, 80 mg), and AcOH (50 µL). The reaction mixture was stirred under hydrogen for 24 h, after which MALDI showed that the product had completely formed. The catalyst was filtered off using celite, the solvent was evaporated, and the residue was purified over P-2 gel using 50 mM ammonium bicarbonate as eluent. The compound was further purified using HILIC HPLC, using a gradient solvent system ACN: 50 mM NH_4HCO_3 (95: 5, v: v to 75: 25, v: v). The resulting product was a white cotton-like solid after lyophilization. (48 mg, 38% over three steps).



20

^{1}H NMR (600 MHz, D_2O):

20	**H1**	**H2**	**H3**	**H4**	**H5**	**H6**	**Fuc-CH_3**	**NHAc**
GlcNAc	4.36	3.71	3.87	3.77	3.37	3.44, 3.79	-	1.92
GalNAc	4.36	3.84	3.80	4.03, dd, *J*= 10.4, 3.3 Hz	3.46	3.62, 3.82	-	1.94
Fuc-1	4.74	3.66	3.59	3.75	4.67	N.A.	1.15, d, *J* = 6.5 Hz	-
Fuc-2	5.34, d, *J*= 3.5 Hz	3.64	3.93	3.68	4.11	N.A.	1.10, d, *J* = 6.7 Hz	-

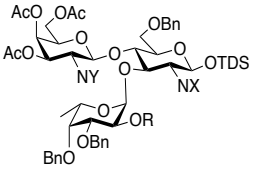
^{13}C NMR (151 MHz, D_2O):

12	**C1**	**C2**	**C3**	**C4**	**C5**	**C6**	**C**$COCH_3$ of NHAc	CO**C**H_3 of NHAc
GlcNAc	100.70	56.15	68.93	71.58	75.22	70.17	174.75	22.46
GalNAc	101.07	52.34	67.84	67.22	75.14	59.85	174.50	22.26
Fuc-1	95.12	71.65	70.49	71.50	66.81	15.23	-	-
Fuc-2	94.14	72.99	73.12	71.81	66.88	15.27	-	-

Linker Peak	**^{1}H Signal**	**^{13}C Signal**
OCH_2CH_2**CH_2**$CH_2CH_2NH_2$	1.25	22.15
$OCH_2CH_2CH_2$**CH_2**CH_2NH_2	1.46	27.77
OCH_2**CH_2**$CH_2CH_2CH_2NH_2$	1.51	28.28
$OCH_2CH_2CH_2CH_2$**CH_2**NH_2	2.75	39.25
O**CH**$HCH_2CH_2CH_2CH_2NH_2$,	3.60	61.44
OCH**H**$CH_2CH_2CH_2CH_2NH_2$	3.70	-

ESI (*m*/*z*): [M+ Na^+] calculated for $C_{33}H_{59}N_3O_{19}$, 824.3640; found 824.3663.

3. Tables of Optimization of Synthesis Protocols



NX and NY are different N-ptotecting groups

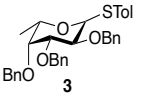
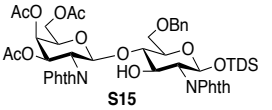
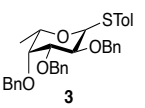
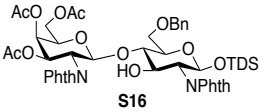
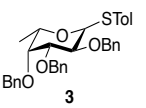
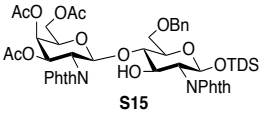
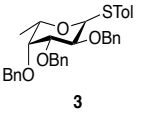
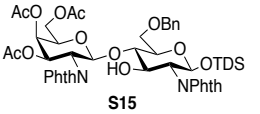
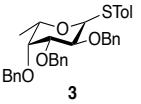
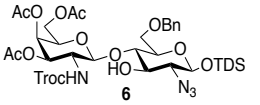
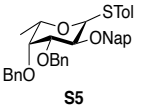
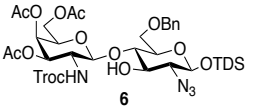
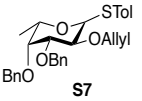
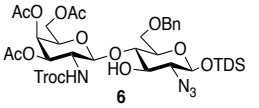
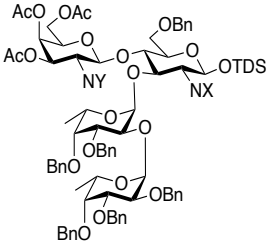
Donor	Acceptor	Promoter	Result
 **3**	 **S15**	NIS/TMSOTf;DCM; -40 to -20 °C	Donor hydrrolysis, acceptor unreacted
 **3**	 **S16**	MeOTf, DMTST, 0°C	Donor hydrrolysis, acceptor unreacted
 **3**	 **S15**	$CuBr_2$/TBAB, 0°C to R.T., 2h	Donor hydrrolysis, acceptor unreacted
 **3**	 **S15**	DPS/ Tf_2O ; DCM -70 to -40 °C	88% product; α:β = 8:2, inseparable mixture **S20**
 **3**	 **6**	DPS/ Tf_2O ; DCM -70 to -40 °C	68% only α product; **7**
 **S5**	 **6**	DPS/ Tf_2O ; DCM -70 to -40 °C	No reaction, acceptor starts degrading beyond -40 °
 **S7**	 **6**	DPS/ Tf_2O ; DCM -70 to -40 °C	Donor degradation

Table S1. Trial Reactions for Assembly of LDN-F Trisaccharide.



NX and NY are different N-ptotecting groups

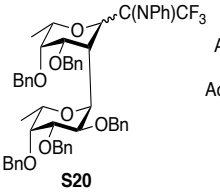
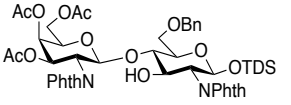
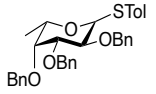
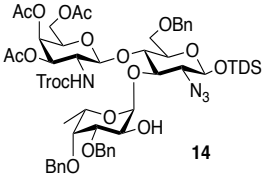
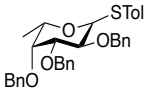
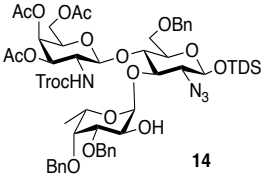
Donor	Acceptor	Promoter	Result
 **S20**	 **S15**	TMSOTf; DCM; -40 to -20 °C	Donor hydrrolysis, acceptor unreacted
 **3**	 **14**	NIS/TMSOTf; DCM -20 to -5 °C	Product is α:β mixture = 1:1; Reaction does not go to completion, acceptor recovered
 **3**	 **14**	$CuBr_2$/ TBAB, DCM: DMF= 1:1 0 °C to R.T.	Trace product; hydrolysed donor

Table S2. Trial Reactions for Assembly of LDN-DF Tetrasaccharide.

4. NMR and Molecular Modeling Studies

Protein expression and purification. The extracellular domain of DC-SIGN was obtained as previously described.[16] The carbohydrate recognition domain of DC-SIGN in its ^{15}N labeled form was obtained as previously described.[7d]

^{1}H Saturation transfer difference (STD) NMR. The samples for saturation-transfer difference (STD) NMR experiments were prepared using the extracellular domain of DC-SIGN at 10 μM concentration in 25 mM Tris-d11, 150 mM NaCl, 4 mM $CaCl_2$ in D_2O (pD 8) using lectin/ligand ratios of 1:60. The temperature was set to 298 K. STD experiments were performed at 600 MHz Bruker spectrometer, using standard Bruker pulse sequences without water suppression nor protein spin-lock filter. Protein saturation was achieved with a Gaussian-shaped pulse of 49 ms. The on-resonance frequency was set at aliphatic regions (0.76 ppm) and the off-resonance frequency at 100 ppm. Blank STD experiments of the ligands alone were acquired in the same conditions. The results of blank ^{1}H-STD NMR experiments for ligands **12** and **20** are shown in Figures **S1** and **S2** respectively.

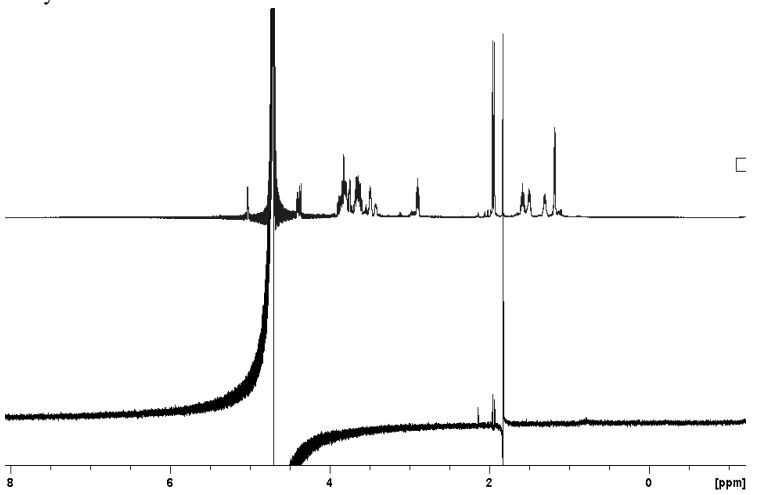


Figure S1. Blank ^{1}H-STD experiment of the free LDN-F. Ligand concentration is 780 μM in D_2O.

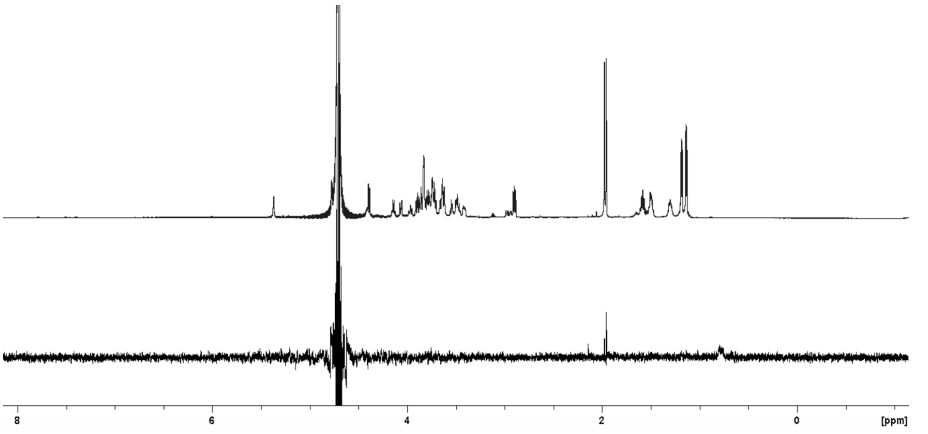


Figure S2. Blank ^{1}H-STD experiment of the free LDN-DF. Ligand concentration is 780 μM in D_2O.

Chemical shift perturbation analysis. ^{1}H-^{15}N-HSQC-based experiments were performed using ^{15}N-labeled CRD DC-SIGN at 50 μM, with 2 mM DTT-d_{10}, at 800 MHz Bruker spectrometer equipped with a cryoprobe, at 310 K. Eight and ten titration points were acquired for ligands **12** and **20** respectively, with ligand concentrations varying from 0.0 to 0.5 mM for the former and from 0.0 to 1.5 mM for latter. Averaged chemical shift perturbation (CSP) and dissociation constants (k_D) were calculated using the CcpNmr Analysis 2.4.2.[26] The chemical shift perturbation analysis was performed based on the protein backbone assignment deposited in the BMRB database with the code 27854. The results from this analysis are shown in Figure **S3**.

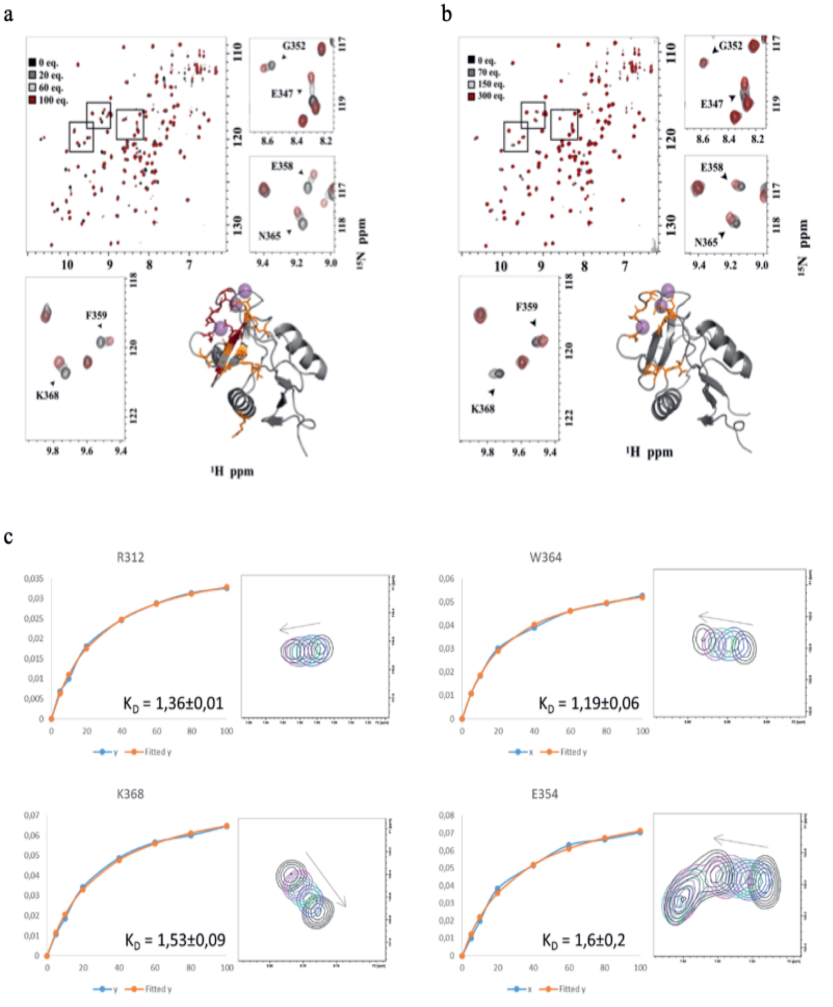


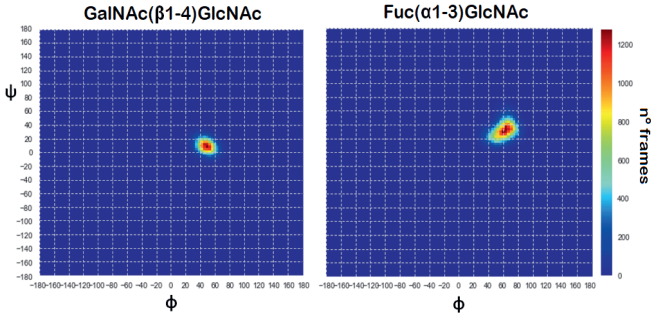
Figure S3. Superimposition of ^{1}H-^{15}N HSQC spectra (black, apo DC-SIGN; from dark gray to red, in the presence of increasing equiv. of LDN-F (a) and LDN-DF (b), respectively. Some regions of the spectra are enlarged in boxes. Perturbed residues are mapped onto the protein (cartoon representation). Most affected residues are in red, while less ones are in orange. c) Average chemical shift perturbation *versus* protein/ligand ratio for selected aminoacids for the determination of the k_D for the binding of LDN-F ligand.

Transferred NOESY spectrum for glycan **12**, was acquired at 800 MHz Bruker spectrometer equipped with a cryoprobe in the presence of 0.2 equivalents of DC-SIGN (180 μM of protein), with a mixing time of 400 ms, at 298 K.

Molecular Modeling. Initial geometries of ligands **12** and **20** were built in the Glycam web (http://glycam.org). Proton-proton distances derived from NOESY spectra (using the isolated spin-pair approximation) were used to check the goodness of the minimized structures. The initial pdb coordinates for CRD of DC-SIGN were derived from the crystal structure Protein Database (PDB) 1SL5. The magnesium ion was replaced by calcium, and the fucose pyranose ring of glycans **12** and **20** was superimposed onto the corresponding sugar in the deposited 1SL5 structure. The resulting binding poses were used as starting points for molecular dynamics (MD) simulations. The MD simulations were performed using the Amber16 program[27] with the ff99SB force field parameters for protein and GLYCAM_06h for the saccharides. Thereafter, the starting 3D geometries were placed into a 12 Å octahedral box of explicit TIP3P waters, and counterions were added to maintain electroneutrality. Two consecutive minimization stages were performed involving (1) only the water molecules and ions and (2) the whole system with a higher number of cycles, using the steepest descent algorithm. The system was subjected to two rapid molecular dynamic simulations (heating and equilibration) before starting the real dynamic simulation. The equilibrated structures were the starting points for the final MD simulations at constant temperature (300 K) and pressure (1 atm). 500 ns Molecular dynamics simulations without constraints were recorded, using an NPT ensemble with periodic boundary conditions, a cutoff of 10 Å, and the particle mesh Ewald method. A total of 250 000 000 molecular dynamics steps were run with a time step of 1 fs per step. Coordinates and energy values were recorded every 10000 steps (10 ps) for a total of 25 000 MD models. A detailed analysis of the glycosydic linkages for glycans **12** and **20** was performed along the MD trajectory using the cpptraj module included in Amber-Tools 16 package and are represented in Figure **S4.**

MD 500 ns

LDN-F



LDN-DF:

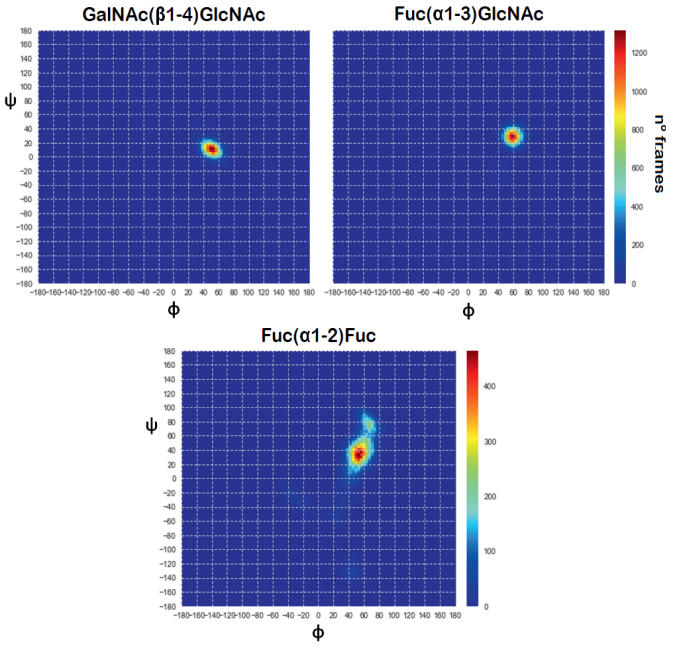


Figure S4. Glycans conformation analysis. Phi and Psi dihedral angles are monitored for the glycosidic linkages of **12** (top) and **20** (bottom) along 500 ns of MD simulation in complex with DC-SIGN.

Corcema-ST Calculation. Corcema-ST matlab scripts were applied to the modeled structures of the complexes obtained by molecular dynamics calculations. Average structures from MD simulations for both ligands were selected and were analyzed by Corcema-ST.[17] The input parameters used in the calculations were: 2 s saturation time; amino acid in a radius of 10 Å around the ligand; direct irradiation on methyl groups of Ile, Leu, Val, Thr and Ala (as an approximation to 0.76 ppm experimental irradiation frequency); experimental k_D dissociation constants, 1.0 mM for **12** and 6.0 mM for **20** and experimental ligands/protein concentrations. An optimal k_{on} of 10^4 L mol^{-1} s^{-1} was determined by fitting the theoretical absolute STDs with the experimental one. Correlation times of 0.5 and 67ns for the ligands in the free and bound forms, respectively, were estimated following an empirical approximation and considering a 160.855 kDa tetramer form for the DC-SIGN. The comparison between the experimental STD NMR intensities and the CORCEMA-ST calculated profiles are represented in Figure **S5.**

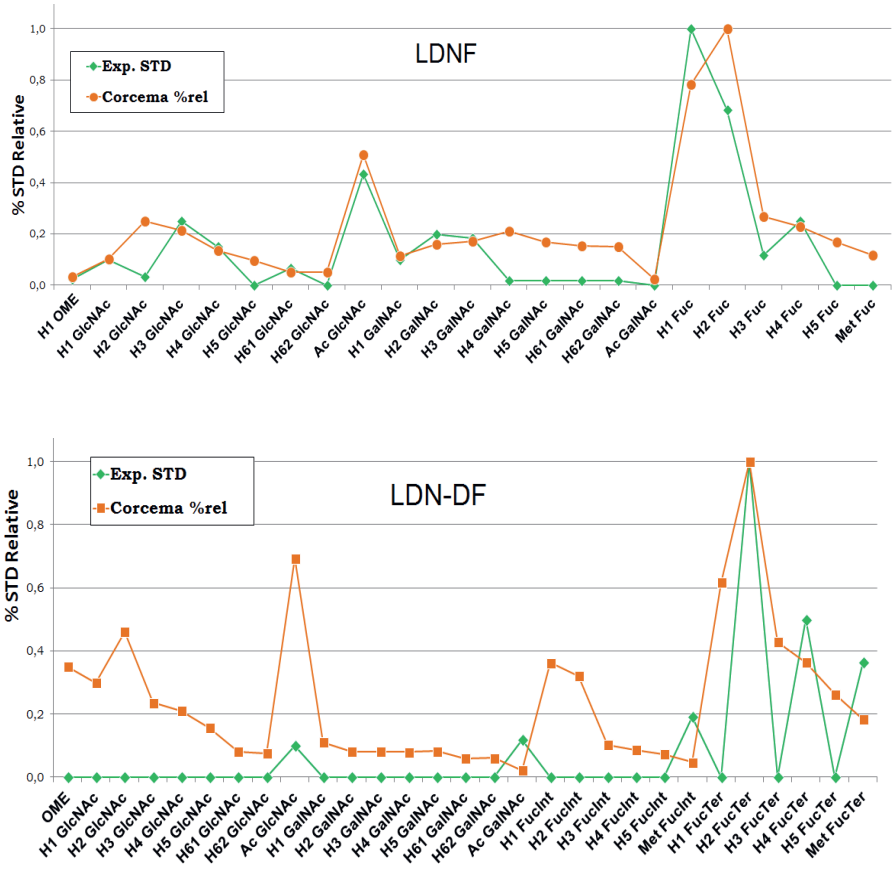


Figure S5. Comparison between the experimental STD intensities and the CORCEMA-ST profiles obtained from the MD simulations. The CORCEMA values were estimated using 50 frames randomly selected and the following parameters were set: 60 eq of ligand, $k_{on} = 10^4\,M^{-1}\,s^{-1}$, $t_{sat} = 2$ sec, Δ_{sat} = Aliphatic (I, L, V, A, T), $K_a = 10^3\,M^{-1}$, $B_0 = 600$ MHz, $\tau_{free\ lig} = 0{,}5$ ns, $\tau_{bound\ lig} = 67$ ns, $\tau_{internal} = 0{,}01$ ns, rholeak = 0.1.

5. Microarray Studies

Glycan array printing: The synthetic compounds (100 μM in sodium phosphate (250 mM), pH 8.5 buffer) were printed on activated glass slides (Nexterion Slide H, Schott Inc) by piezoelectric non-contact printing (sciFLEXARRAYER S3, Scienion Inc) with a drop volume of ~400 pL and 1 drop per spot at 50% relative humidity. Compounds were printed as replicates of 6, 32x25 spots per subarray, and 24 subarrays (3x8) per slide. After overnight incubation in a saturated NaCl chamber (75% relative humidity), 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.

Screening: To validate printing sub-arrays were incubated at RT with 50 μL of a mixture of biotinylated *Aleuria aurantia* lectin (AAL; Vector Labs, B-1395) and 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 4 successive washes of the whole slide 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 each 5 min soak time.

Recombinant human DC-SIGN--Fc Chimera (R&D systems, 161-DC) was assayed at the indicated concentrations premixed with anti-IgG Fc-biotin (5 μg/mL; ThermoFisher Scientific, A18833) and Streptavidin-AlexaFluor635 (5 μg/mL) in TSM binding buffer and washed were performed as described above.

Analyses: 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 6 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.

Printing validation: *Aleuria aurantia* lectin (AAL) binds to fucose linked (α-1,2, α-1,3, and α-1,6) structures. As expected all glycans show binding to AAL (Fig. **S6**).

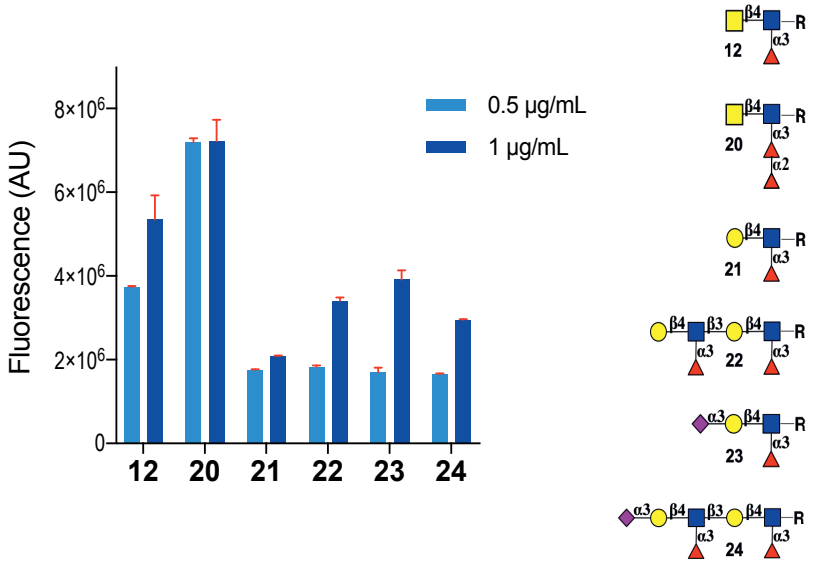


Figure S6. Microarray results of the glycan library at 100 μM for binding to AAL (0.5 and 1 μg/mL). Bars represent the mean ± SD.

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CHAPTER 4

Chemoenzymatic Synthesis of Complex *N*-glycans of the parasite *S. mansoni* to examine the importance of epitope presentation on DC-SIGN recognition

Abstract. The importance of multivalency for *N*-glycan-protein interactions has mainly been studied by attachment of minimal epitopes to artificial multivalent scaffold and not in the context of multi-antennary glycans. *N*-glycans can be modified by bisecting GlcNAc, core xylosides and fucosides, and extended *N*-acetyl lactosamine moieties. The impact of such modifications on glycan recognition are also not well understood. We describe here a chemoenzymatic methodology that can provide *N*-glycans expressed by the parasitic worm *S. mansoni* having unique epitopes at each antenna and contain core xyloside. NMR, computational and electron microscopy were employed to investigate recognition of the glycans by the human lectin DC-SIGN. It revealed that core xyloside does not influence terminal epitope recognition. The multi-antennary glycans bound with higher affinity to DC-SIGN compared to mono-valent counterparts, which was attributed to proximity-induced effective concentration. The multi-antennary glycans cross-linked DC-SIGN into a dense network, which likely is relevant for antigen uptake and intracellular routing.

Adapted from: **A.D. Srivastava,** L. Unione, M. Bunyatov, I.A. Gagarinov, N.G.A. Abrescia, S. Delgado, A. Ardá and G.J. Boons. 2021, *Angew. Chem. Int. Ed.*, 60, 19287-19296.

Introduction. Glycan binding proteins play key roles in the battle between host and pathogens. Pathogens often express glyco-epitopes that can be detected by glycan binding proteins of the host such as the mannose-binding lectin, macrophage mannose receptor, or C-type lectins, resulting in a diverse range of immune responses. The host also expresses glycan binding proteins such as the Siglec's and complement factor H that can detect self-glycan signatures to maintain immune-homeostasis.[1] A number of microbes have developed ways to achieve molecular mimicry of host glycans to avoid immune detection and establish infections.[2]

Binding and structural studies have indicated that glycan binding proteins recognize relatively small oligosaccharide motifs often found at termini of complex glycans.[3] There are however, indications that the complex architecture of glycans can modulate recognition of minimal epitopes.[4] For example, *N*-glycans which have branched structures potentially can present multiple minimal epitopes that can engage with multiple glycan binding proteins resulting in increased binding avidities. It may also facilitate glycoconjugate clustering, which in turn may influence multiple downstream processes. Multivalency has primarily been studied by attachment of minimal epitopes to artificial multivalent scaffold,[5] and not in the context of natural multi-antennary glycans. In addition, *N*-glycans can be modified by bisecting *N*-acetyl glucosamine (GlcNAc), core xylosides and fucosides, and extended *N*-acetyl lactosamine (LacNAc) moieties. The impact of such modification on glycan recognition are also not well understood. These deficiencies are mainly due to inaccessibility of structurally defined complex glycans.

Schistosomes are parasitic helminths that cause chronic infections in humans associated with high morbidity.[6] *N*-glycans of *S. mansoni* exhibit structural heterogeneity due variations in core modifications, the number of antennae, and their extensions into various epitopes. The expression is regulated in stage-specific manner,[7] and for example during the egg and cercaria stage, Schistosomes abundantly decorate the core of *N*-glycans by xylose.[8]

The termini of glycans of Schistosomes are often fucosylated to present epiropes such as Lewis X (Le^x), GalNAc β1,4(Fuc α1,3)GlcNAc (LDN-F) and di-Lewis X (di-Le^x).[7] These epitopes can be recognized by DC-SIGN on dendritic cells.[9] DC-SIGN is a C-type lectin that interacts with conserved molecular patterns that are shared by a large group of microorganisms. It facilitates internalization of pathogens for processing and antigen presentation.[10] Pathogens can also exploit DC-SIGN for infection and dissemination making it an important therapeutic target.[11]

DC-SIGN is a homo-tetrameric Ca^{2+} dependent lectin in which each monomer presents a carbohydrate recognition domain (CRD).[12] Potentially, it can engage in multivalent interactions resulting to high avidity of binding.[13] *In vivo*, multivalent glycans on the pathogen surface promote DC-SIGN clustering, thus facilitating antigen uptake. There are indications that the density of the surface exposed glycans influence the cellular signaling, which in turn lead to enhancement or suppression of proinflammatory response.[14] The molecular details by which these complexes are

formed are poorly understood. Moreover, little is known of the preferences of DC-SIGN for *S. mansoni* derived glycans and if there are restrictions related to glycan valency or size. The latter is due to the difficulties of synthesizing highly complex glycans that are modified by core xylose and have unusual epitopes such as LacdiNAc (LDN) and LDN-F.

Here, we report a synthetic methodology that can provide *N*-glycans expressed by the parasitic worm *S. mansoni*. It includes compounds that have asymmetrical architectures and contain core xylose and terminal epitope such as LacdiNAc and mono- and di-Lewis X (Le^x). NMR, computational and electron microscopy were employed to investigate the importance of glycan complexity for recognition by the human lectin Dendritic cell-specific ICAM-3 grabbing nonintegrin (DC-SIGN). It revealed that core xyloside does not influence terminal epitope presentation and recognition. Furthermore, it was found that the multi-antennary glycans bind with higher affinity to DC-SIGN compared to mono-valent minimal epitopes, which was attributed to proximity-induced effective concentration. Finally, the studies revealed that the multi-antennary glycan can cross-link DC-SIGN into a dense network, which likely is relevant for antigen uptake and intracellular routing.

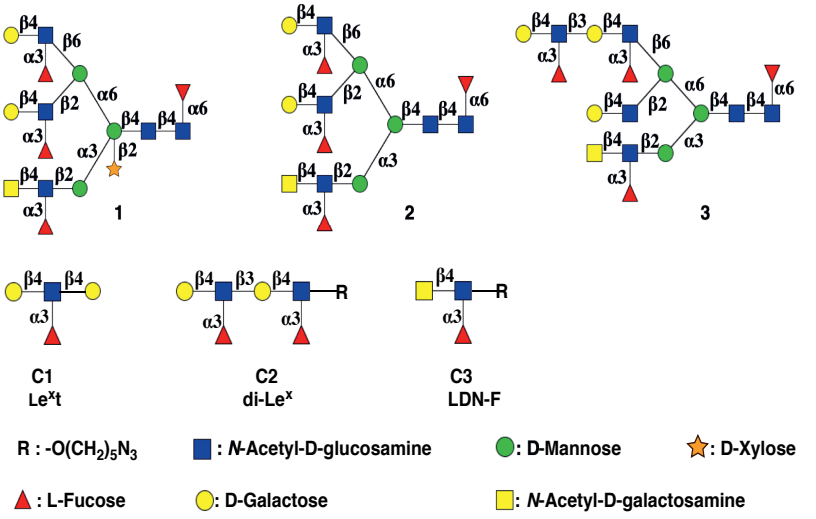


Figure 1. Schematic representation of the different glycans (**1-3**) and their fragments (**C1-C3**) studied herein.

Results and Discussion.

Chemoenzymatic synthesis. We set out to develop a chemoenzymatic methodology that can provide asymmetric complex glycans expressed by *S. mansoni* such as compounds **1**, **2** and **3** (Figure 1). The compounds are modified by core xyloside, a Lac-di-NAc moiety and various patterns of fucosylation. In parallel, reference compounds Le^Xt (**C1**, Galβ1,4-(Fucα1,3)GlcNAcβ1,3Galα-*O*-aminopropyl; Le^X tetraose, Elicityl), di-Le^X (**C2**, Galβ1,4-(Fucα1,3)GlcNAcβ1,3Galβ1,4-(Fucα1,3)GlcNAcβ-*O*-azidopentyl) and LDN-F (**C3**, GalNAcβ1,4-(Fucα1,3)GlcNAcβ-*O*-azidopentyl) were prepared or purchased for NMR studies described below. Challenging aspects of the preparation of compounds such as **1** are the installation of a core xyloside and the decoration of each antennae by unique appendages. The β-1,2-xylosyltransferase from *Arabidopsis thaliana* (XYLT), which potentially can install a core xyloside, operates early in the biosynthesis of *N*-glycans and has narrow substrate specify. In particular, it cannot transfer xylose when an antenna is modified by a galactoside, which greatly complicates the construction of asymmetrical glycans.[15] Thus, we opted for a synthetic strategy in which the xyloside was introduced by chemical glycosylation to give precursors that can be elaborated by glycosyl transferases into asymmetrical multi-antennary glycans.

Thus, heptasaccharide **4a** was prepared which is modified by a core xyloside, and at potential branching points is modified by the orthogonal protecting groups levulinoyl (Lev), fluorenylmethyloxycarbonyl (Fmoc), allyloxycarbonyl (Alloc), and t-butyl-dimethylsilyl (TBS).[16] Sequential removal of these protecting groups will give acceptors that can be extended by glycosyl donors such as **5**, **6** and **7** to provide, after deprotecting, a compound such as **8** and **9** (Figure 2). It was anticipated that the Lac-di-NAc moiety of **8** and **9** can selectively be modified by glycosyl transferases. In the next stage of synthesis, the terminal Gal can be converted into LacNAc, and then enzymatically extended into a complex structure. It was expected that compound **4b** would give entry into complex glycans lacking core xylose. The synthesis of the donors **5**, **6** and **7** is presented in the Experimental Section. (Scheme S4).

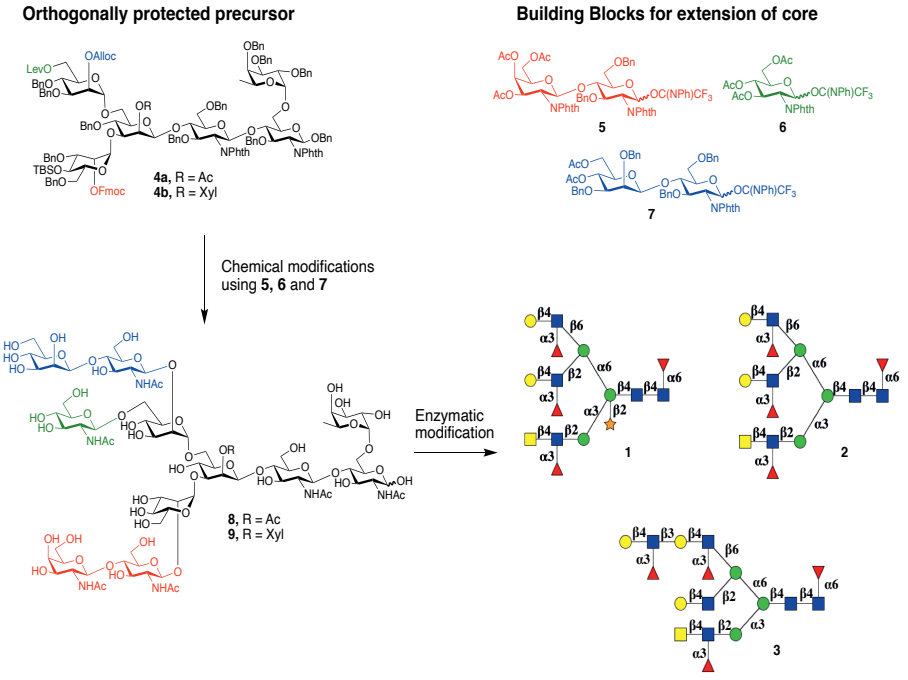


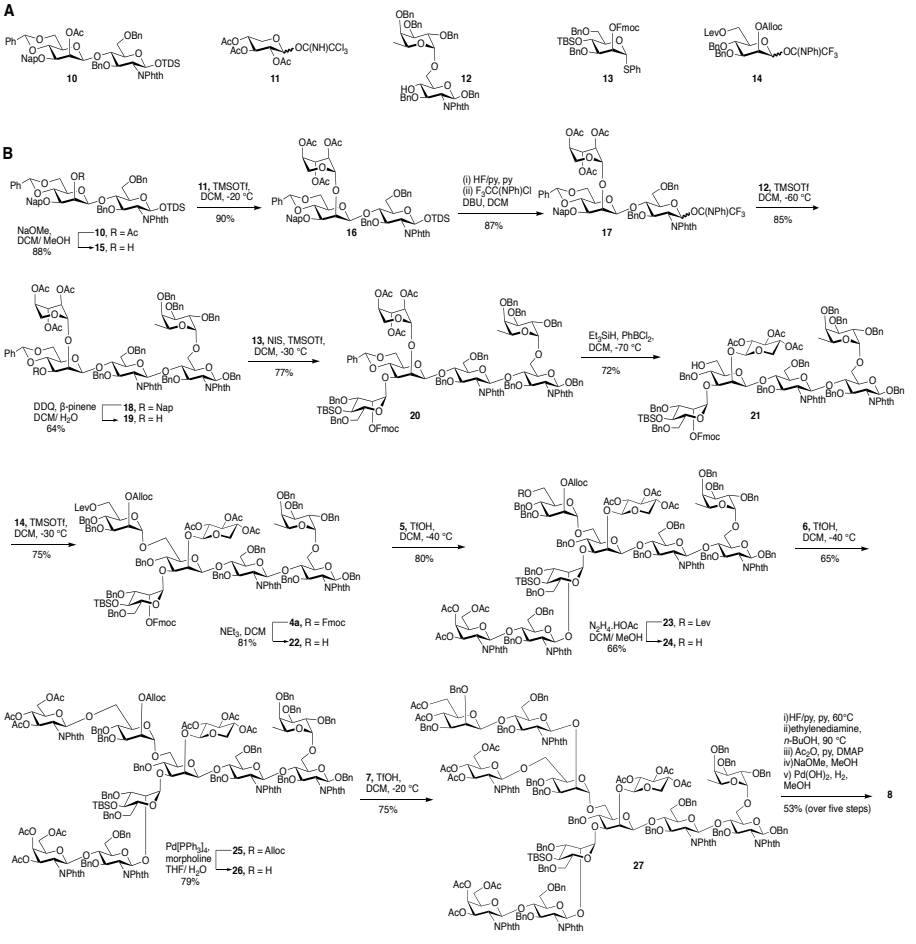
Figure 2. Overview of the synthetic strategy for the preparation of asymmetrical glycans with and without core xyloside. The coupling of the common precursors, **4a** and **4b**, which display orthogonal protecting groups at key branching points, with glycosyl donors **5-7** followed by global deprotection afforded asymmetric tri-antennary glycans, **8** and **9**, respectively. The latter intermediates were used as substrates for enzymatic extensions of each antenna to yield the asymmetrical glycans **1**, **2** and **3**, which differ for in presence or absence of core-xylose, fucosylation of the arms and for the presence of a di-Le^X moiety. Antenna selective arm extension was possible because the unnatural β-mannoside at the C-2' arm temporarily blocks it from enzymatic modification. It can, however, be unmasked by a β-mannosidase and after enzymatic galactosylation, a precursor is obtained that can be elaborated by many glycosyl transferases into a complex structure. The strategy also exploits that many glycosyl transferases can modify LacNAc but not GlcNAc. The latter can, however, be converted into LacNAc by galactosylation with GalT1.

It was envisaged that compound **10** (Scheme 1) would be an appropriate starting material for the synthesis of core xylosylated *N*-glycan **4a**. Its mannosyl moiety is protected at C-2 by an acetyl ester, which can selectively be cleaved to generate an acceptor that can be xylosylated with donor **11** to provide glycan **16**. The anomeric TDS can be cleaved by HF pyridine to give a lactol that can be converted into donor **17** for glycosylation with acceptor **12** to give pentasaccharide **18**. The naphthylmethyl (NAP) ether and the benzylidene acetal of the resulting compound can selectively be cleaved or reductively opened, respectively to give acceptors that can be glycosylated with thio-manosyl donor **13** and *N*-phenyl trifluoroacetimidate donor **14** to provide fully protected

heptasaccharide **4a**, which is an appropriate precursor for antennae selective extension. Alternatively, the acetyl ester of compound **10** can be left intact to give access to non-xylosylated *N*-glycan precursors **4b** (see Experimental, Scheme S2 and S3). Thus, disaccharide **10** was treated with sodium methoxide in methanol to give acceptor **15**, which was coupled with trichloroacetimidate donor **11** to provide xyloside bearing trisaccharide **16** as only the β-anomer. The xylosyl moiety adopted a $^1C_4$ conformation as evident from the coupling constant between H-1 and H-2 ($^3J_{1,2}$ = 3.7 Hz) indicating a di-equatorial orientation. Others have observed that xylosides protected by acetyl esters in apolar solvent can adopt a $^1C_4$ conformation.[17] It has been suggested that the resulting anomeric effect overcomes unfavorable steric interactions of axial substituents. It appears that protecting groups at neighboring glycosyl moieties can influence the conformation of the xyloside, indicating that steric hindrance of these entities exert a conformational control. Compound **16** was treated with HF in pyridine to remove the anomeric dimethylthexylsilyl group, and the resulting anomeric lactol was reacted with 2,2,2-trifluoro-*N*-phenylacetimidoyl chloride in presence of DBU to form *N*-phenyl trifluroacetimidate[18] donor **17** in a yield of 87% over two steps. The latter compound was glycosylated with disaccharide acceptor **12**[19] in presence of TMSOTf as the catalyst to furnish **18** in a yield of 85%. Next, the Nap ether of **18** was oxidatively removed by DDQ in the presence of β-pinene as an acid scavenger[20] to afford acceptor **19** (64%). A NIS/TMSOTf mediated glycosylation of thio-manosyl donor **13**, having the orthogonal protecting groups Fmoc and TBS at C-2 at C-4, respectively (Scheme S5) with acceptor **19** gave hexasaccharide **20** in a yield of 77% as only the α-anomer. The benzylidene acetal of **20** was regioselectively opened by treatment with triethylsilane (Et_3SiH) and dichlorophenylborane ($PhBCl_2$) in DCM at -78°C to furnish acceptor **21** having a hydroxyl at the C-6 position of the central mannoside. As previously observed,[17a, 21] upon removing the bulky benzylidene group, the xyloside moiety adopted the $^4C_1$ conformation, which is reflected by an increase in the $^3J_{1,2}$ coupling constant from the diequatorial (3.7 Hz) to the diaxial orientation (7 Hz). Finally, a TMSOTf-mediated glycosylation of mannosyl trichoroacetimidate **14**, modified by the orthogonal protecting groups Alloc and Lev (Scheme S5), with acceptor **21** gave heptasaccharide **4a** in a yield of 75%. The corresponding non-xylosylated hexasaccharide core **4b** was synthesized following a similar glycosylation strategy (Scheme **S2**).

Next, attention was focused on the installation of the various branching points to give tri-antennary precursor glycan **8** by subsequent removal of the orthogonal protecting groups, glycosylation with donors **5**, **6** and **7** followed by deprotection. Treatment of heptasaccharide **4a** with Et_3N resulted in the selective removal of the Fmoc protecting group without affecting other functionalities to give glycosyl acceptor **22** in a good yield of 81%. The latter acceptor was glycosylated with *N*-phenyl trifluoroacetimidate donor **5** in the presence of trifluoromethanesulfonic acid (TfOH) at -40 °C to afford **23** (80%). The Lev ester of **23** was selectively removed by treatment with hydrazine acetate to provide acceptor **24** (66%), which was coupled with *N*-phenyl trifluoroacetimidate glycosyl donor **6** using TfOH as promoter at -40 °C to afford **25** in a yield of 65%. Next, the Alloc protecting group was removed by treatment with tetrakis(triphenylphosphine)-palladium-0

($Pd[PPh_3]_4$) and morpholine to afford acceptor **26**, which was then glycosylated with donor **7** using the standard procedure to afford tri-antennary glycan **27** in 75% yield.

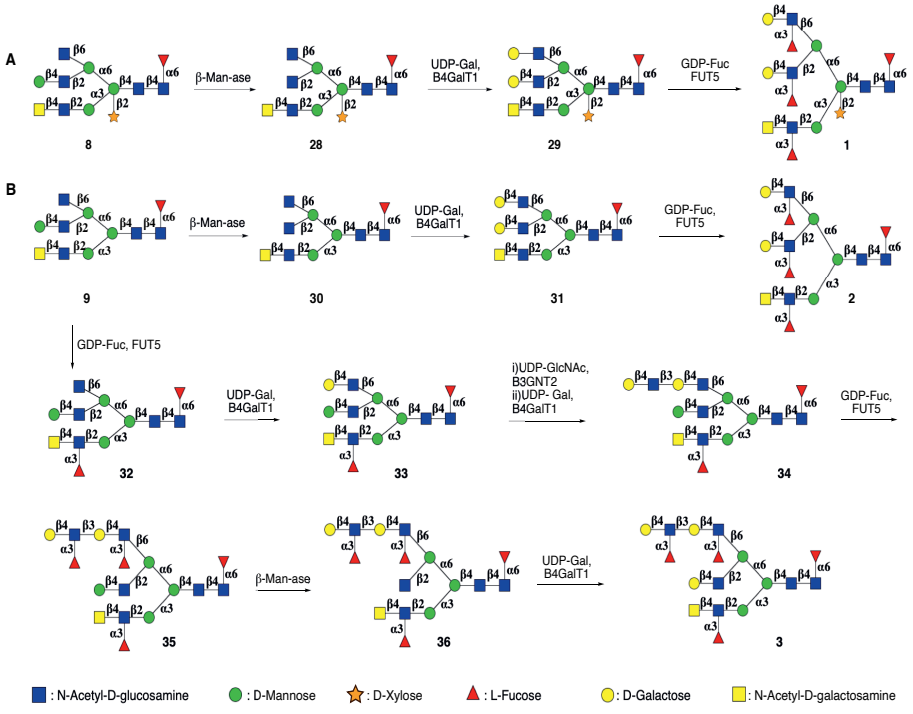


Scheme 1. Synthetic scheme for synthesis of *N*-glycan **8** from key building blocks **10-14**.

The latter compound was subjected to global deprotection over five steps entailing treatment with HF/pyridine to remove TBS ether, followed by heating under reflux with ethylenediamine in *n*-butanol to cleave the phthalimide-protecting groups. The exposed free amines and hydroxyls were acetylated by acetic anhydride in pyridine followed by cleavage of the esters by sodium methoxide. Finally, the benzyl ethers were removed by catalytic hydrogenation in the presence of palladium hydroxide ($Pd[OH]_2$) which afforded the required tri-antennary glycans **8** containing core xylose.

The non-xylosylated hexasaccharide core **4b** was extended by a similar glycosylation sequence to generate glycan **9** (Scheme S3).

Finally, compounds **8** and **9** were treated with *Helix pomatia β-mannosidase* and the inhibitor 1-deoxyfuconojirimycin,[22] to avoid the cleavage of core α-fucoside, to yield **28** and **30**, respectively (Scheme 2). The two GlcNAc termini of the α6-arm were simultaneously galactosylated by B4GalT1 and UDP-galactose to give derivatives **29** and **31** having terminal LacNAc moieties. The latter glycans were fucosylated at all three arms by using FUT5 which modified the LacNAc and LacdiNAc substrates, affording compounds **1** and **2**. The synthesis of asymmetric glycan **3** started by treatment of **9** with FUT5 to transform the LDN moiety into LDN-F epitope providing **32**. A key point in our chemoenzymatic strategy was the capping of the α6β2-arm by a mannosyl moiety, and thus exposure of **32** to B4GalT1 resulted in the selective galactosylation of the α6β6-arm. Exposure of the resulting compounds to B3GNT2 and then B4GalT1 resulted in the installation of a di-LacNAc moiety to give compound **34**. The di-LacNAc moiety was transformed into Le^xLe^x epitope by treatment with FUT5 to give **35**. The GlcNAc residue of the α6β2-arm of **35** was unmasked by treatment with β-mannosidase to give **36** which was galactosylated by B4GalT1 provide target compound **3**.



Scheme 2. Enzymatic Extension from precursors **8** and **9**.

NMR studies. The interaction of DC-SIGN with the xylosylated (**1**) and non-xylosylated (**2**) glycans was examined by NMR. Saturation Transference Difference (^{1}H-STD-NMR) experiments were performed using recombinant extracellular domain (ECD) of DC-SIGN as receptor, which is organized as a tetramer in solution. The resulting ^{1}H-STD NMR profiles for **1** and **2** were very similar (Figure 3a and b). No signals originating from the xyloside were observed in the ^{1}H-STD spectrum of compound **1**, and thus it appears it does not directly participate in lectin binding. Comparing the ^{1}H-STD profiles of the complex glycans **1** and **2** with those of Le^x (**C1**) showed that no contacts between the lectin and the underlying glycan take place (Figure 3c).

Due to severe ^{1}H NMR signal overlap, untangling a possible preference of DC-SIGN for the two Le^x moieties and the LDN-F epitope (Figure 1) at the different branches of the glycans was challenging. However, for compound **1**, the NMR signals of the acetyl moieties at the GlcNAc residues of the three arms appear at different NMR chemical shifts, allowing unequivocal assignment by NOE spectroscopy (Figure 3a quest). The results from the ^{1}H-STD NMR experiment showed that all GlcNAc residues contribute equally to the binding. A model of the two glycans in solution further supported that the three branches are equally accessible for lectin binding (Figure 3d, 3e).

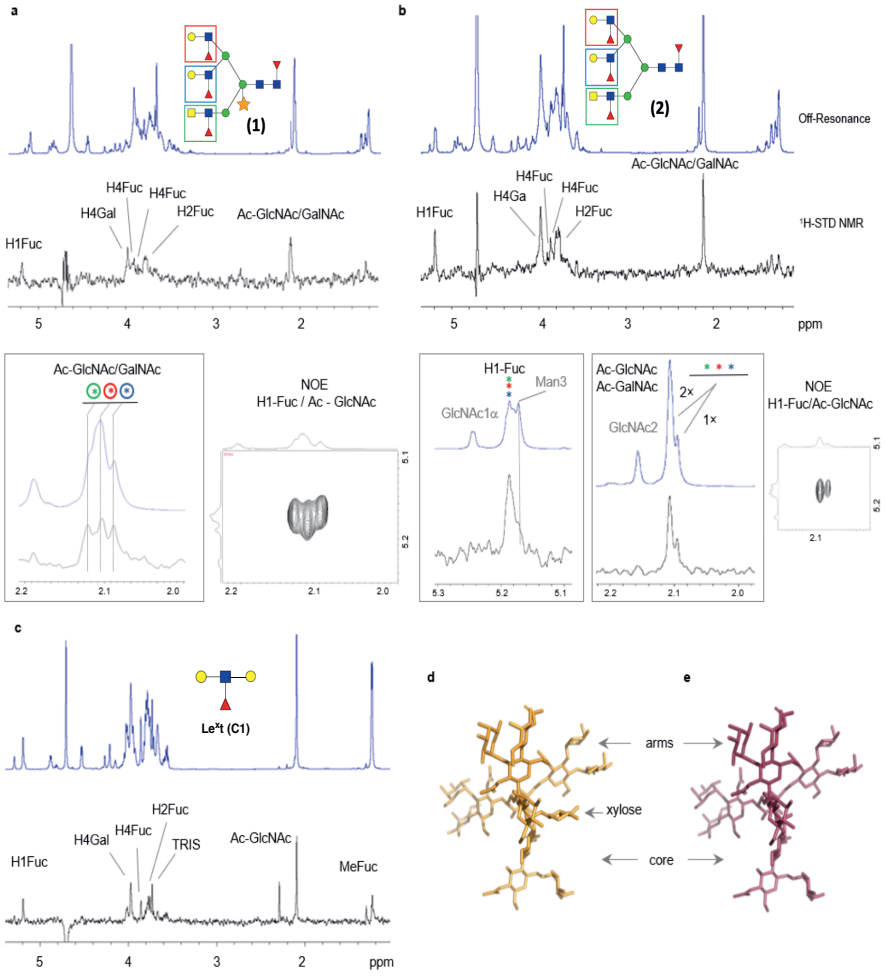


Figure 3. ^{1}H STD-NMR spectra obtained for the complexes of DC-SIGN (ECD) with (a) glycan (**1**), (b) glycan (**2**) and (c) the minimal epitope: the Le^{x} trisaccharide (**C1**). The specific and unique ^{1}H-STD NMR signals are highlighted, along with the NOE cross peaks employed for their assignment. In particular, the 2D sections correspond to the NOE correlations between the anomeric protons of the fucose residues and the methyl protons of the corresponding acetamide moieties for each of the three arms. The ^{1}H-STD NMR profiles are derived from the double difference between the STD spectrum in the presence and in absence of the protein. Irradiation frequency was set at 0.4 ppm. Protein saturation was achieved with a Gaussian-shaped pulse of 49 ms (Gauss 1.1000, with a power of 1e^{-05} W. Water suppression was applied by using the excitation sculpting. (d) and (e). Representative 3D models for glycan (**1**) and (**2**) respectively, obtained from MD simulations analysis. The models support the accessibility of the three arms for providing interactions with the lectin. Alternative views are provided in the experimental section (Figure S8).

S. mansoni often presents extended *N*-acetyllactosamine (Galβ1,4-GlcNAcβ1,3-Galβ1,4-GlcNAc; poly-LN) chains which can be modified by fucosylation to form poly-Lewis^x (poly-Le^x).[23] The asymmetric glycan **3** presents an extended arm with two repeating Le^x motifs, an LDN-F epitope and a non-fucosylated LacNAc epitope (Figure 1). The ^{1}H-STD NMR analysis of **3** with DC-SIGN (ECD) (Figure 4a) showed a similar profile as for glycans **1** and **2** indicating that the terminal Le^x and LDN-F epitope are equally well recognized. In contrast, the STD signals from the internal Le^x motif were barely visible, indicating that this moiety is less available for lectin recognition. This notion was further assessed by analyzing the interaction of the hexasaccharide **C2** (di-Le^x, Figure 1) to DC-SIGN (Figure 4b). The simpler NMR spectra for this compound allowed a clear differentiation of the contributions of the internal and terminal Le^x moieties. In particular, the ^{1}H-STD intensities from the internal Le^x moiety only accounted for ~15% of those from terminal Le^x.

Next, the interactions of DC-SIGN with the complex glycans **1**, **2** and **3** were examined from the protein perspective using receptor-based NMR methods. In particular, the chemical shift perturbation (CSP) analysis of the ^{15}N labeled DC-SIGN carbohydrate recognition domain (CRD) was performed (SI section 5.5). The tri-antennary glycans **1**, **2** and **3** showed similar CSPs, which did not extend beyond the canonical carbohydrate binding site, supporting that despite the complex structures, there are no additional contacts between the protein and glycans. However, the induced CSPs were significantly stronger than those observed for LDN-F (**C3**)[24] and Le^x (**C1**)[25] for the same number of equivalents.

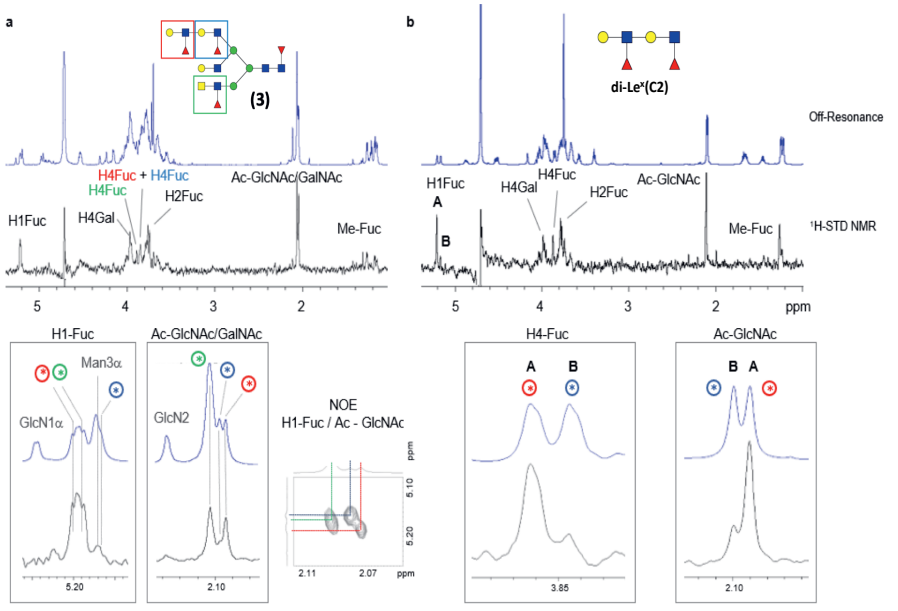


Figure 4. ^{1}H STD-NMR spectra recorded for the interaction of DC-SIGN (ECD) with (a) glycan (**3**) and (b) diLe^{x} hexasaccharide (**C2**). The specific and unique ^{1}H-STD NMR signals are highlighted, along with the NOE cross peaks employed for their assignments. Irradiation frequency was set at 0.4 ppm. Protein saturation was achieved with a Gaussian-shaped pulse of 49 ms (Gauss 1.1000, with a power of 1e^{-05} W. Water suppression was applied by using the excitation sculpting.

To confirm that the multi-antennary glycans bind stronger than their monovalent counterparts, we used 2-fluoro-fucose (2-F-Fuc) as probe to determine relative binding affinities. The addition of the lectin to the fluorinated probe causes a dramatic decrease in ^{19}F transverse relaxation time (T2) with respect to the free form, which is indicative of 2-F-Fuc binding. Next 0.5 equivalent with respect to the probe of compounds **1**, **2** and **3** were added. The addition of a competitor molecule causes a recovery in T2 for the fluorinated probe, which is proportional to the relative affinities of the tested compounds (Figure 5). Although subtle differences were observed, the results indicate that the three glycans bind DC-SIGN with a similar affinity.

Next, we compared the relative affinity of the synthetized multi-antennary N-glycans with respect to the monovalent counterpart. Thus, in a new sample tube containing 2-F-Fuc and DC-SIGN (ECD), we added 0.5 equivalents of LDN-F (**C3**), again with respect to the fluorinated probe. In this case, the competition with the monovalent compound caused only a marginal recovery in transverse relaxation time (T2), which indicates a much lower affinity of **C3** for the lectin. Because the multivalent molecules contain three putative epitopes for lectin binding, we further added the

compound **C3** to reach 1.5 equivalents with respect to the fluorinated probe, equalizing in this way the concentration of binder epitopes with respect to the multivalent N-glycans. Nevertheless, the recovery in T2 transverse relaxation time of the fluorinated probe is lower than that observed using the multivalent ligands, which demonstrate that a multivalent presentation enhances glycan-lectin binding.

Multivalency is often attributed to a favorable spatial organization of a multivalent ligand that that can make multiple interactions with a multivalent protein. DC-SIGN is a tetramer with their CRD binding sites separated by ~40 Å.[26] The spatial distance between two terminal epitopes at the same glycan was estimated as approx. 20 Å (Figure 2d and 2e). Thus, the possibility that one glycan can simultaneously bind to two different CRDs within the same tetramer through a chelating effect is unlikely. It has been proposed that "rebinding" can also contribute to cooperativity,[27] and in this model, as soon as a single ligand-receptor complex dissociates, the presence of another ligand will increase the probability of another binding event. It is likely that the trimeric glycans exhibit higher affinities due to rebinding.

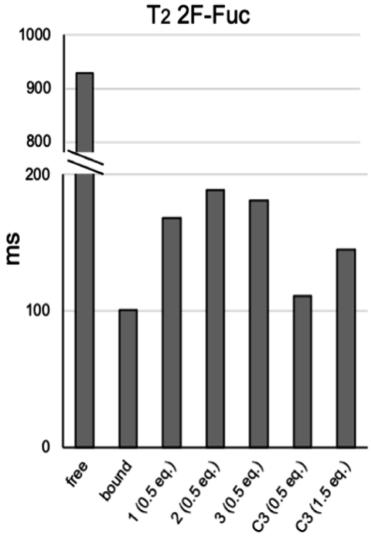


Figure 5. ^{19}F-NMR relaxation filter experiments performed for the fluorine-containing monosaccharide 2-fluoro-fucose (2F-Fuc) in absence and in presence of the tetrameric lectin DC-SIGN. The addition of competitor molecules, **1-3** and **C3** causes a recovery in the transverse relaxation time (T2) for the fluorinated probe, which is proportional to the relative affinities of the tested compounds.

Multivalent presentation and lattice formation. It is possible that the antenna of compounds **1**-**3** engage with more than one DC-SIGN tetramer to form a higher ordered complexes.[28] To examine such a mode of binding, transmission electron microscopy (TEM) was employed to provide support for the existence of glycan-lectin complexes. The apo DC-SIGN (ECD) was used as control. In this format, lectin alone gave a soluble monodisperse organization (Figure 6a-c). Conversely, incubation of the multivalent glycans **1** and **2** with DC-SIGN (ECD) generated a tangled network (Figure 6d-e). It is likely, the aggregates arise from the cross-linking of two or more DC-SIGN CRDs at different ECD tetramers by the multivalent glycans. In fact, we observed protein aggregation in the sample containing glycans **1**, **2** and **3** but not in those containing the monovalent ligands Le^X (**C1**) and LDN-F (**C3**), (Figure S14). These observations further support our hypothesis that multi-antennary glycans may be able to cross-link DC-SIGN (EDC) tetramers. Molecular modelling studies support that the glycans have appropriate geometries to engage with two DC-SIGN tetramers (Figure 6g-h). The 3D model of DC-SIGN ECD assembled into tetramers was generated as previously described[13] (see experimental procedures, section 6.3).

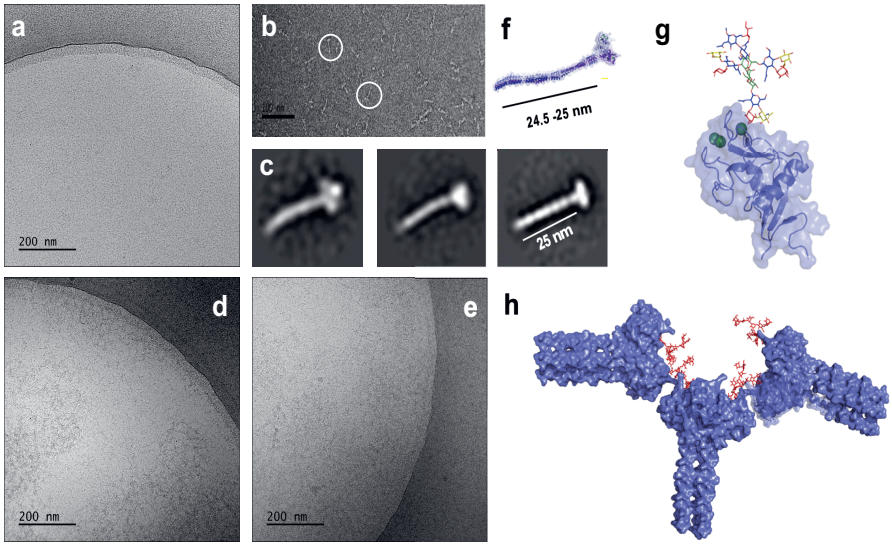


Figure 6. Electron microscopy of DC-SIGN interacting with multivalent glycans. Cryo-TEM images at 50,000x magnification of apo DC-SIGN (a) and in the presence of compound **1** (d) and **2** (e). (b) apo DC-SIGN visualised by negative-stain; the white circles mark the individual molecules. Scale bar is 100 nm. (c) three class-averages resulting from 2D classification of extracted particles from negative-stain 2D images and indicating the tetrameric organization of DC-SIGN (see Experimemtal Section). (f) 3D model of DC-SIGN ECD assembled into tetramers. (g) 3D model of DC-SIGN CRD showing the interaction with compound **1** as a single molecule. (h) 3D models of DC-SIGN ECD (assembled into tetramers) showing the putative interaction with compound **1** and forming an inter-connected network.

Conclusion.

S. Mansoni expresses fucosylated glycans that can be recognized by DC-SIGN on the cell surface of DCs. The structures of these antigenic glycans and their density on pathogen surface probably represents a first barrier for host detection. The molecular basis of complex formation between DC-SIGN and glycans is not well understood. Moreover, little is known about a possible preference of DC-SIGN for *S. mansoni* derived glycans, and it is also unclear whether glycan complexity can modulate binding. The latter is due to difficulties of synthesizing highly complex glycans expressed by *S. Mansoni* required for structural and functional studies. Current approaches are limited to relatively simple epitopes or symmetrical structures.[23-24, 28] To address these limitations, we have developed a chemoenzymatic approach that can provide *N*-glycans derived from *S. mansoni* including compounds having core xylose and antenna with unusual structures such as Lac-di-NAc and di-Le^x. The compounds made it possible to examine the influence of glycan complexity on DC-SIGN recognition. It revealed that the core xyloside does not influence terminal epitope presentation and lectin binding. Recently, the interaction of DC-SIGN with symmetric bi-antennary glycans with and without core-xylose was examined by microarray technology.[29] It showed the core xylose abolished lectin binding for the inner-core mannosyl residues. Together with the NMR data presented here, it supports a model in which the xyloside masks or distort the conformation of the glycan-core but does not impact the presentation of the Le^x epitopes. Xylosylated glycans are highly expressed from the egg to cercaria stage of development, but substantially decreases during the schistosomula and adult worm stages.[8] The stage-specific expression of core xylosylated glycans implies a role in snail–schistosome interactions but may be less relevant during intra-mammalian stages of development. The NMR studies also uncovered that the multi-antennary glycans bind with higher affinity to DC-SIGN compared to mono- valent minimal epitopes. The STD experiments indicated that only the terminal epitopes of compounds **1-3** engage with DC-SIGN and no further interactions were observed between protein and glycan. Modeling studies showed that the distance between the terminal epitopes is too short to engage with two different CRDs within the same tetramer. Thus, it is unlikely that avidity enhancement occurs through a classical chelate effect.[30] Although enhancement of affinity through proximity-induced effective concentrations has received relatively little attention, it is likely that the higher affinities of **1-3** can be accounted to such an effect.

Multiple biological properties of DC-SIGN, such as antigen uptake and processing, can be attributed to receptor clustering. Recently, it was shown that DC-SIGN mediated uptake of glycoconjugates by immature moDCs does not directly correlate with their affinity,[14] and instead it appeared that the size of the cluster is critical for antigen uptake and routing.[31] Based on this study, we propose that multi-antennary glycans may be able to cross-link DC-SIGN (EDC) tetramers into a dense network, where, the nodes are tetramers of DC-SIGN connected through the multivalent glycans. Physiologically, DC-SIGN is embedded in the cellular membrane and, recently, the relevance of proper protein presentation was demonstrated.[28] Although our in-

solution studies do not represent *N*-glycan presentation on the pathogen surface, it is conceivable that lectins and glycans presented at a surface can form similar latices and that the branched nature of *N*-glycans contribute to cluster size, which in turn will impact update and intracellular transport. Future studies will focus on investigating DC-SIGN clustering and internalization using nano- or micro-structures, decorated with the types of complex glycans presented herein. The complex glycans will also be important to unravel structural elements required for triggering different signaling pathways during *S. mansoni* infection, as well as for the further developing of glycan-based vaccines.

Experimental Procedure.

1. Chemical Synthesis.

1.1 General Methods: Reactions were performed using flame-dried glassware with anhydrous solvents under an atmosphere of argon unless otherwise noted. Proton nuclear magnetic resonance (^{1}H -NMR) spectra were recorded with Varian 400 (at 400 MHz) or Bruker 600 (at 600 MHz) spectrometers. Multiplicities are assigned as singlet (s), broad singlet (br s), doublet (d), doublet of doublets (dd), triplet of doublets (td), triplet (t), quartet (q) or multiplet (m). Carbon nuclear magnetic resonance (^{13}C) spectra were recorded with Varian 400 (at 101 MHz) or Bruker 600 (at 151 MHz) spectrometers. Spectra were assigned using gCOSY and multiplicity-edited gHSQC experiments. Tetramethylsilane (TMS) was used as an internal standard in all ^{1}H and ^{13}C spectra (δ = 0 ppm) when applicable. Mass spectra was recorded using high resolution Shimadzu LCMS-IT-TOF or Kratos Analytical Maxima-CFR MALDI-TOF system. Column chromatography was performed on silica gel G60 (Silicycle, 60-200 μm, 60 Å). Thin layer chromatography (TLC) analysis was conducted on Silicagel 60 F254 (EMD Chemicals Inc.) coated aluminum sheets. Plates were visualized by UV light (254 nm) and by charring with 10% sulfuric acid in ethanol and/or Hanessian's stain. Size exclusion chromatography was carried out on bio-beads S-X1 (40-80 μm) or bio-gel P2 (45-90 μm). Acid washed molecular sieves (4 Å) were flame activated under vacuum prior to reactions.

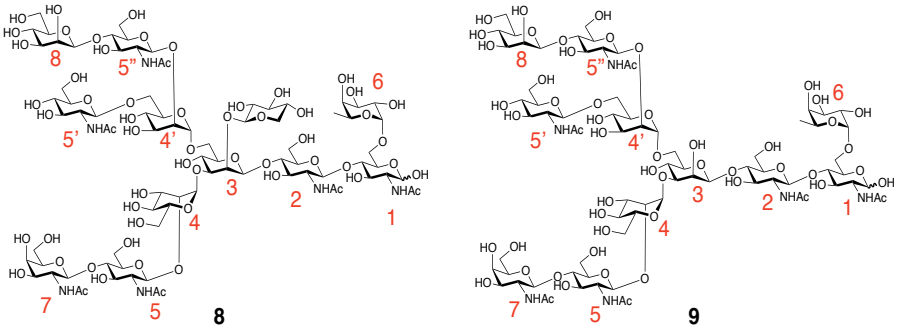
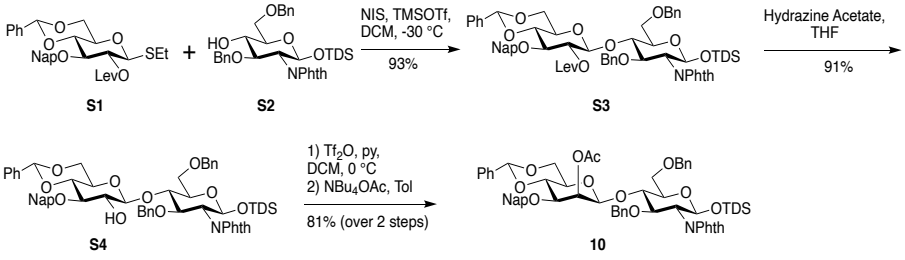


Figure S1. Monosaccharide nomenclature system for NMR assignments.

For the simpler building blocks, the monosaccharides were denoted with their known abbreviations, for eg., Glucose (Glc), *N*-acetyl glucosamine (GlcN), Mannose (Man), *N*-acetyl galactosamine (GalN) and Fucose (Fuc). For the oligosaccharide glycans, the individual monosaccharides have been labeled from the reducing end of the glycans as shown in Figure S1. For eg., the *N*-acetyl glucosamine residues from the reducing end of the glycans were labeled as GlcN-1 and GlcN-2 respectively; the β-mannoside of the core pentasaccharide is labeled as Man-3, the α-3 and α-6 mannosides were labeled as Man-4 and Man-4' respectively. The mannose moeity of the unnatural Man-β-(1→4)-GlcNAc terminus was labeled as Man-8;The *N*-acetylglucosamine residues as GlcNAc-5, 5', 5". The *N*-acetyl galactosamine of the GalN-β-(1→4)-GlcN terminus was denoted as GalN-7.



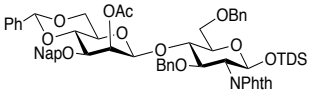
Scheme S1: Synthesis of Building Block **10.**

Dimethylthexylsilyl [4,6-*O*-benzylidene-2-*O*-levulenoyl-3-*O*-(2-methylnaphthyl)-β-D-glucopyranosyl]-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (S3): Donor **S1** (24.6 g, 44.8 mmol) and acceptor **S2** (23.5 g, 37.3 mmol), were dissolved in DCM (300 ml) and stirred with pre-activated molecular sieves (50 g) for 20 minutes. The mixture was then cooled down to -30 ºC, followed by the addition of NIS (10.5 g, 46.7 mmol) and TfOH (692 µL, 7.5 mmol). The reaction mixture was warmed up to -10 ºC over a period of 30 minutes, after which it was quenched with Et_3N (5 mL). The mixture was diluted by DCM (200 mL) and washed with 10% $Na_2S_2O_3$ (200 mL). The organic phase was dried over $MgSO_4$ and the filtrate was concentrated *in vacuo* to give the crude product as a brown syrup. Silica gel column chromatography with Tol: EtOAc (9.5: 0.5, v: v to 8: 2, v: v) yielded the product as white amorphous powder (38.8 g, 93%). R_f = 0.59 (Tol: EtOAc, 9: 1 v: v). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.93 to 6.82 (26H, m, H-Ar), 5.48 (1H, s, PhC<u>H</u> of benzylidene), 5.30 (1H, d, H-1 GlcN, *J* = 7.9 Hz), 5.01 (2H, m, H-2 Glc, PhC<u>H</u>H), 4.82 (1H, d, PhCH<u>H</u>, *J* = 12.2 Hz), 4.74 (2H, m, C<u>H</u>$_2$ of Nap), 4.63 (1H, d, *J* =8.1 Hz), 4.45 (2H, m, PhC<u>H</u>$_2$), 4.24 (2H, m, H-6b Glc, H-3 GlcN), 4.06 (2H, m, H-2 GlcN, H-3 Glc), 3.82 (1H, m, H-6b GlcN), 3.66 (3H, m, H-6a GlcN, H-5 GlcN, H-4 Glc), 3.50 (2H, m, H-6a Glc, H-4 GlcN), 3.26 (1H, m, H-5 Glc), 2.62 (2H, m, C<u>H</u>$_2$COCH$_3$ of Lev), 2.42 (2H, m, COOC<u>H</u>$_2$ of Lev), 2.10 (3H, s, CH$_2$COC<u>H</u>$_3$ of Lev), 1.35 [1H, m, C<u>H</u>(CH$_3$)$_2$ of TDS], 0.56 [12H, m, C(C<u>H</u>$_3$)$_2$, CH(C<u>H</u>$_3$)$_2$ of TDS], 0.09 to -0.08 (6H, 2s, 2x C<u>H</u>$_3$-Si of TDS); ^{13}C NMR (101 MHz, $CDCl_3$): δ 206.53, 206.10, 172.60, 171.21, 138.69, 138.10, 137.84, 137.24, 135.79, 133.22, 133.15, 133.08, 132.93, 132.09, 129.01, 128.93, 128.65, 128.51, 128.48, 128.45, 128.43, 128.36, 128.31, 128.29, 128.26, 128.20, 128.14, 128.00, 127.96, 127.89, 127.87, 127.84, 127.81, 127.73, 127.68, 127.66, 127.38, 127.29, 126.98, 126.52, 126.43, 126.28, 126.24, 126.06, 125.98, 125.92, 125.85, 125.80, 125.28, 101.22, 101.04, 100.65, 93.38, 93.28, 81.71, 78.54, 78.46, 78.03, 77.33, 77.02, 76.70, 76.37, 74.87, 74.48, 74.25, 74.10, 73.76, 73.71, 73.62, 73.56, 68.63, 67.94, 66.62, 65.91, 57.74, 57.38, 37.92, 37.76, 37.67, 33.87, 33.83, 29.87, 29.82, 28.02, 27.80, 24.47, 21.45, 19.89, 19.85, 19.71, 19.66, 18.28, 18.16, -1.82, -3.81, -3.88. MALDI-TOF-MS (*m/z*): [M+ Na]$^+$ calculated for $C_{65}H_{73}NO_{14}SiNa$, 1142.4698; found 1142.4762.

Dimethylthexylsilyl [4,6-*O*-benzylidene-3-*O*-(2-methylnaphthyl)-β-D-glucopyranosyl]-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (S4): Compound **S3** (38.5 g, 34.4 mmol) was dissolved in THF (200 mL), followed by the addition of hydrazine acetate (4.8 g, 51.6 mmol), and stirred for 3 hours after which the solvent was evaporated *in vacuo* and the residue was diluted by DCM (300 mL) and washed with water and brine. The organic fractions were then dried over $MgSO_4$ and filtered, and the filtrate was concentrated *in vacuo*. Silica gel column chromatography using Pet. Ether: EtOAc

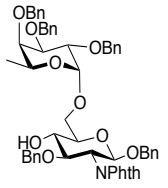
(8: 2, v: v to 6: 4, v: v) gave the product as a white amorphous powder. (32.2 g, 91%). R_f = 0.56 (Pet. Ether: EtOAc, 7: 3, v: v). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.98 to 6.74 (26H, m, H-Ar), 5.48 (1H, s, PhC<u>H</u> of benzylidene), 5.30 (1H, d, C<u>H</u>H of Nap, *J* = 10.7 Hz), 4.94 (1H, d, CH<u>H</u> of Nap, *J* = 10.7 Hz), 4.72 (3H, m, H-1 Glc, PhCH<u>H</u>, PhCH<u>H</u>), 4.59 (1H, d, PhC<u>H</u>H, *J* = 12.3 Hz), 4.38 (2H, m, H-3 GlcN, PhC<u>H</u>H), 4.12 (3H, m, H-2 GlcN, H-6b Glc, H-3 Glc), 4.02 (1H, m, H-6a Glc), 3.74 (1H, d, H-6b GlcN, *J* = 11.1 Hz), 3.58 (5H, m, H-6a GlcN, H-4 GlcN, H-4 Glc, H-5 GlcN, H-2 Glc), 3.23 (2H, m, H-5 Glc, O<u>H</u>), 1.35 [1H, m, C<u>H</u>$(CH_3)_2$ of TDS], 0.57 [12H, m, C($C\underline{H}_3$)$_2$, CH($C\underline{H}_3$)$_2$ of TDS], 0.08 to -0.10 (6H, 2s, 2x $C\underline{H}_3$-Si of TDS); ^{13}C NMR (101 MHz, $CDCl_3$): δ 138.51, 138.30, 137.74, 137.31, 135.90, 133.35, 133.27, 132.99, 132.91, 132.32, 128.95, 128.88, 128.71, 128.47, 128.44, 128.40, 128.31, 128.26, 128.22, 128.13, 127.96, 127.93, 127.89, 127.85, 127.80, 127.77, 127.68, 127.64, 127.39, 127.34, 127.28, 127.08, 126.70, 126.60, 126.47, 126.16, 126.07, 126.06, 126.04, 125.99, 125.96, 125.87, 125.81, 125.40, 125.13, 103.62, 101.24, 93.47, 93.38, 81.26, 80.30, 78.92, 77.95, 77.32, 77.00, 76.68, 75.43, 75.15, 74.67, 74.55, 74.35, 74.06, 73.76, 73.60, 68.65, 68.41, 66.28, 65.46, 60.37, 57.89, 57.40, 33.86, 33.83, 24.48, 21.03, 19.86, 19.67, 18.28, 18.16, 14.18, -1.81, -1.85, -3.80, -3.88. MALDI-TOF-MS (*m/z*): $[M+Na]^+$ calculated for $C_{60}H_{67}NO_{12}SiNa$, 1044.4330; found 1044.4125.

Dimethylthexylsilyl [2-*O*-acetyl-4,6-*O*-benzylidene-3-*O*-(2-methylnaphthyl)-β-D-mannopyranosyl]-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (10): Compound **S4** (32.0 g, 31.3 mmol) was dissolved in anhydrous DCM (200 mL) and the mixture was cooled down to 0 °C. This was followed by the addition of anhydrous pyridine (7.6 mL, 93.9 mmol) and Tf_2O (10.5 mL, 62.2 mmol) and the mixture was stirred at this temperature for 1 hour after which it was diluted by DCM (300 mL) and washed with sat. $NaHCO_3$ (150 mL). The organic fractions were then dried over $MgSO_4$ and filtered, and the filtrate was concentrated *in vacuo*, resulting in crude product as a yellow syrup. The crude product was then dissolved in dry Toluene (300 mL) followed by the addition of tetrabutylammonium acetate (47.1 g, 156.5 mmol). The suspension was sonicated under Ar atmosphere for 3 hours, after which the solvent was evaporated *in vacuo*, and the residue was purified by silica gel column chromatography using Pet. Ether: EtOAc (9: 1, v: v to 6: 4, v: v) which provided the product as a white amorphous powder. (27.1 g, 81% over two steps). R_f = 0.53 (Pet. Ether: EtOAc, 7: 3, v: v). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.95 to 6.82 (26H, m, H-Ar), 5.55 (1H, s, PhC<u>H</u> of benzylidene), 5.53 (1H, d, H-2 Man, *J* = 3.3 Hz), 5.31 (1H, d, H-1 GlcN, *J* = 8.1 Hz), 4.83 (2H, m, PhC<u>H</u>H, C<u>H</u>H of Nap), 4.71 (3H, m, H-1 Man, CH<u>H</u> of Nap, PhCH<u>H</u>), 4.45 (2H, dd, PhC<u>H</u>$_2$, *J* = 12.1 Hz, 3.6 Hz), 4.28 (1H, dd, H-3 GlcN, *J* = 10.8 Hz, 8.5 Hz), 4.13 (3H, m, H-2 GlcN, H-6b Man, H-4 GlcN), 3.92 (1H, t, H-4 Man, *J* = 9.7 Hz), 3.81 (1H, m, H-6a Man), 3.61 (4H, m, H-6a GlcN, H-3 Man, H-5 GlcN, H-6b GlcN), 3.18 (1H, m, H-5 Man), 2.19 (3H, s, C<u>H</u>$_3$ of Ac), 1.36 [1H, m, C<u>H</u>$(CH_3)_2$ of TDS], 0.60 [12H, m, C($C\underline{H}_3$)$_2$, CH($C\underline{H}_3$)$_2$ of TDS], 0.10 to -0.06 (6H, 2s, 2x $C\underline{H}_3$-Si of TDS); ^{13}C NMR (101 MHz, $CDCl_3$): δ 170.32, 138.66, 137.90, 137.46,

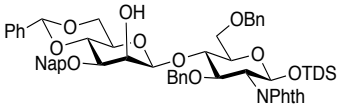


135.27, 133.66, 133.29, 132.97, 131.67, 128.96, 128.45, 128.21, 128.10, 127.95, 127.93, 127.89, 127.80, 127.74, 127.73, 127.61, 127.13, 126.19, 126.17, 126.01, 125.83, 125.39, 123.04, 101.57, 99.51, 93.42, 79.23, 77.88, 77.34, 77.23, 77.03, 76.75, 76.71, 75.75, 74.40, 74.32, 73.47, 71.49, 69.19, 68.62, 68.47, 66.98, 57.77, 33.87, 24.49, 21.07, 20.14, 19.88, 19.71, 18.58, 18.28, 18.17, -0.01, -1.46, -1.84, -3.80. MALDI-TOF-MS (*m/z*): $[M+ Na]^+$ calculated for $C_{62}H_{69}NO_{13}SiNa$, 1086.4436; found 1086.4643.

Benzyl 2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl-(1→6)-3-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (12): Compound was synthesized according to reported literature[19] (20.1 g, 22.2 mmol). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.10 to 6.69 (29H, m, H-Ar), 5.12 (1H, m, H-1 GlcN), 4.99 (1H, d, PhC<u>H</u>H, *J* = 12.1 Hz), 4.80 (8H, m, PhC<u>H</u>H, 3x Ph<u>CH</u>$_2$, H-1 Fuc), 4.46 (2H, m, PhCH<u>H</u>, PhCH<u>H</u>), 4.18 (2H, m, H-2 GlcN, H-3 GlcN), 4.11 (1H, m, H-2 Fuc), 4.03 (2H, m, H-5 Fuc, H-4 GlcN), 3.90 (3H, m, H-6a GlcN, H-6b GlcN, H-3 Fuc), 3.79 (1H, O<u>H</u>), 3.70 (1H, s, H-4 Fuc), 3.57 (1H, m, H-5 GlcN), 1.14 (3H, d, C<u>H</u>$_3$ of Fuc, *J* = 6.5 Hz); ^{13}C NMR (101 MHz, $CDCl_3$): δ 167.77, 138.55, 138.51, 138.45, 138.36, 138.08, 137.65, 137.11, 133.61, 131.67, 128.48, 128.43, 128.41, 128.38, 128.36, 128.33, 128.30, 128.26, 128.23, 128.21, 128.19, 128.11, 128.10, 128.03, 128.00, 127.94, 127.91, 127.88, 127.83, 127.78, 127.73, 127.68, 127.65, 127.64, 127.60, 127.56, 127.53, 127.52, 127.47, 127.26, 127.21, 123.17, 100.14, 98.61, 97.37, 97.31, 91.80, 84.34, 83.71, 81.28, 79.30, 79.11, 78.01, 77.63, 77.50, 77.39, 77.28, 77.08, 76.76, 76.51, 76.40, 75.10, 74.94, 74.76, 74.62, 74.22, 74.19, 73.72, 73.44, 73.15, 72.99, 72.92, 72.43, 70.80, 70.70, 68.50, 66.87, 66.61, 55.62, 55.39, 16.79, 16.64, 15.59, 0.03. MALDI-TOF-MS (*m/z*): $[M+ Na]^+$ calculated for $C_{55}H_{55}NO_{11}Na$, 928.3673; found 928.3342.

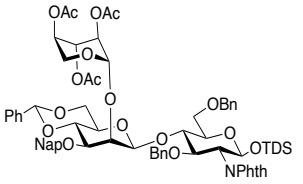


Dimethylthexylsilyl [4,6-*O*-benzylidine-3-*O*-(2-methylnaphthyl)-β-D-mannopyranosyl]-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (15): Compound **10** (17.0 g, 15.9 mmol), was dissolved in DCM (200 mL), followed by the addition of 1 N NaOMe (5 mL) in MeOH (20 mL), and stirred for 3 hours after which the reaction was quenched by AcOH (5 mL). The solvent was evaporated *in vacuo* and the residue was purified by silica gel column chromatography using Pet. Ether: EtOAc (8: 2, v: v to 6: 4, v: v) gave the product as a white amorphous powder. (14.4 g, 88%). R_f = 0.54 (Pet. Ether: EtOAc, 7: 3, v: v). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.91 to 6.74 (26H, m, H-Ar), 5.52 (1H, s, PhC<u>H</u> of benzylidene), 5.31 (1H, d, H-1 GlcN, *J* = 8.0 Hz), 4.85 (3H, m, PhCH<u>H</u>, C<u>H</u>$_2$ of Nap), 4.66 (2H, m, PhC<u>H</u>H, H-1 Man), 4.45 (2H, m, PhC<u>H</u>$_2$), 4.38 (1H, dd, H-3 GlcN, *J* = 10.7 Hz, 8.6 Hz), 4.09 (5H, m, H-2 GlcN, H-2 Man, H-3 Man, H-4 Man, H-6b Man), 3.79 (1H, dd, H-6a Man, *J* = 11.4 Hz, 3.1 Hz), 3.63 (3H, m, H-5 GlcN, H-6a GlcN, H-6b GlcN), 3.53 (1H, dd, H-

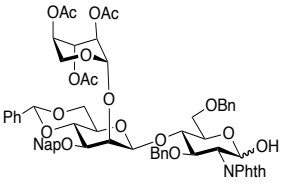


4 GlcN, *J* = 9.6 Hz, 3.1 Hz), 3.19 (1H, m, H-5 Man), 2.63(1H, OH), 1.35 [1H, m, CH(CH_3)$_2$ of TDS], 0.58 [12H, m,C(CH_3)$_2$, CH(CH_3)$_2$ of TDS], -0.08 to 0.09 (6H, 2s, 2x CH_3-Si of TDS); ^{13}C NMR (101 MHz, $CDCl_3$): δ 171.12, 138.48, 137.85, 137.49, 135.41, 133.68, 133.22, 133.02, 131.66, 129.11, 128.92, 128.44, 128.38, 128.28, 128.24, 128.21, 128.20, 127.93, 127.91, 127.80, 127.70, 127.67, 127.10, 127.00, 126.56, 126.23, 126.12, 126.11, 126.05, 125.94, 125.74, 125.65, 123.05, 101.53, 100.80, 93.43, 78.93, 78.72, 78.21, 77.39, 77.33, 77.21, 77.01, 76.95, 76.80, 76.69, 74.53, 74.35, 73.61, 73.38, 72.35, 69.61, 68.85, 68.68, 68.53, 66.83, 60.37, 57.84, 53.41, 33.87, 24.48, 21.03, 20.22, 19.87, 19.69, 18.28, 18.16, 14.18, -0.02, -1.85, -3.79. MALDI-TOF-MS (*m/z*): [M+ Na]$^+$ calculated for $C_{60}H_{67}NO_{12}SiNa$, 1044.4330 ; found 1044.4541.

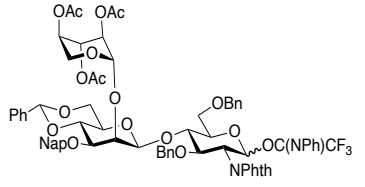
Dimethylthexylsilyl [2,3,4-tri-*O*-acetyl-β-D-xylopyranosyl-(1→2)-4,6-*O*-benzylidine-3-*O*-(2-methylnaphthyl)-β-D-mannopyranosyl]-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (16): Imidate donor **11** (9.4 g, 21.1 mmol) and acceptor **15** (14.4 g, 14.1 mmol), were dissolved in DCM (250 mL) and stirred with pre-activated molecular sieves (30 g) for 20 minutes. The mixture was then cooled down to -20 ºC, followed by the addition of TMSOTf (1.15 mL, 6.3 mmol). The reaction mixture was warmed up to -10 ºC over a period of 30 minutes, after which it was quenched with Et_3N (5 mL). The sieves were filtered off and the mixture was concentrated *in vacuo* to give the crude product as a brown syrup. Silica gel column chromatography with Tol: EtOAc (9.5: 0.5, v: v to 8: 2, v: v) yielded the product as white amorphous powder. (16.3 g, 90%). R_f = 0.50 (Tol: EtOAc, 8.5: 1.5, v: v). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.87 to 6.79 (26H, m, H-Ar), 5.48 (1H, s, PhCH of benzylidene), 5.35 (1H, d, H-1 GlcN, *J* = 8.1 Hz), 5.15 (1H, d, H1-Xyl, *J* = 3.7 Hz), 5.10 (1H, t, H-3 Xyl, *J* = 5.6 Hz), 5.04 (1H, m, H-4 Xyl), 4.85 (4H, m, H-2 Xyl, CH_2 of Nap, PhCHH), 4.58 (3H, m, H-5b Xyl, PhCHH, H-1 Man), 4.35 (3H, m, H-3 GlcN, PhCH_2), 4.11 (3H, m, H-2 GlcN, H-6b GlcN, H-2 Man), 3.95 (2H, m, H-4 Man, H-3 Man), 3.61 (3H, m, H-4 GlcN, H-6a Man, H-6b Man), 3.47 (3H, m, H-5a Xyl, H-6a GlcN, H-5 GlcN), 3.15 (1H, m, H-5 Man), 2.09 to 2.00 (9H, 3s, 3x CH_3 of Ac), 1.36 [1H, m, CH(CH_3)$_2$ of TDS], 0.60 [12H, m, C(CH_3)$_2$, CH(CH_3)$_2$ of TDS], 0.09 to -0.06 (6H, 2s, 2xCH_3-Si of TDS); ^{13}C NMR (101 MHz, $CDCl_3$): δ 206.90, 169.91, 169.37, 169.00, 138.81, 137.75, 137.47, 135.68, 133.62, 133.25, 132.94, 131.67, 129.00, 128.92, 128.43, 128.24, 128.19, 128.06, 127.86, 127.83, 127.79, 127.67, 127.56, 127.43, 126.88, 126.13, 126.11, 126.07, 125.85, 125.44, 125.27, 123.03, 102.30, 101.53, 99.17, 93.39, 80.92, 78.26, 77.34, 77.23, 77.03, 76.96, 76.76, 76.71, 74.72, 74.58, 74.32, 73.53, 71.95, 68.98, 68.68, 68.56, 68.35, 67.34, 60.26, 57.77, 33.88, 30.89, 24.49, 21.43, 20.98, 20.92, 20.80, 19.88, 19.70, 18.28, 18.17, -0.02, -1.84, -3.46, -3.74. MALDI-TOF-MS (*m/z*): [M+ Na]$^+$ calculated for $C_{71}H_{81}NO_{19}SiNa$, 1302.5070; found 1302.5166.



2,3,4-tri-*O*-acetyl-β-D-xylopyranosyl-(1→2)-4,6-*O*-benzylidine-3-*O*-(2-methylnaphthyl)-β-D-mannopyranosyl]-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-α/β-D-glucopyranoside (S5): Compound **16** (16.2 g, 12.7 mmol) was dissolved in pyridine (150 mL) followed by dropwise addition of HF in pyridine (70% HF, 30% pyridine; 25 mL). The mixture was stirred for 15 hours after which it was quenched by solid $NaHCO_3$ (50 g), till all CO_2 bubbling stopped. The salts were filtered off, the solvent was evaporated *in vacuo*, and the residue was re-dissolved in DCM (200 mL), followed by washing with water (100 mL) and saturated $NaHCO_3$ (100 mL). The organic phase was dried over $MgSO_4$, filtered, and the filtrate was concentrated, and the residue was purified using silica gel column chromatography (Pet. Ether: EtOAc, 8: 2, v: v to Pet: EtOAc, 4: 6, v: v), which provided the product as a white foamy solid. (12.2 g, 84 %). R_f = 0.34 (Pet. Ether: EtOAc, 1: 1, v: v). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.96 to 6.71 (26H, m, H-Ar), 5.45 (1H, s, PhC<u>H</u> of benzylidene), 5.34 (1H, d, H-1 GlcN, *J* = 8.9 Hz), 5.03 (3H, m, H-4 Xyl, H-3 Xyl, H-1 Xyl), 4.82 (4H, m, H-2 Xyl, C<u>H</u>$_2$ of Nap, PhCH<u>H</u>), 4.60 (2H, m, H-5b Xyl, PhC<u>H</u>H), 4.44 (2H, m, H-3 GlcN, H-1 Man), 4.30 (2H, m, PhC<u>H</u>$_2$), 4.07 (3H, m, H-2 GlcN, H-6b GlcN, H-2 Man), 3.89 (2H, m, H-4 Man, H-3 Man), 3.66 (3H, m, H-4 GlcN, H-6a Man, H-6b Man), 3.40 (3H, m, H-5a Xyl, H-6a GlcN, H-5 GlcN), 3.09 (1H, m, H-5 Man), 2.16 to 1.92 (9H, 3s, 3x C<u>H</u>$_3$ of Ac); ^{13}C NMR (101 MHz, $CDCl_3$): δ 169.94, 169.27, 168.97, 168.10, 155.37, 138.74, 137.42, 137.37, 135.64, 133.76, 133.24, 132.93, 131.55, 128.96, 128.49, 128.20, 128.09, 128.00, 127.83, 127.66, 127.31, 126.98, 126.91, 126.13, 126.03, 125.88, 125.34, 123.28, 101.94, 101.54, 99.05, 92.99, 80.42, 78.23, 77.33, 77.21, 77.01, 76.88, 76.69, 75.04, 74.59, 74.51, 73.56, 71.90, 68.57, 68.29, 68.09, 67.20, 59.95, 57.54, 55.75, 20.98, 20.93, 20.79, 14.17, -0.02. MALDI-TOF-MS (*m/z*): $[M+ Na]^+$ calculated for $C_{63}H_{63}NO_{19}Na$, 1160.3892; found 1160.3767.

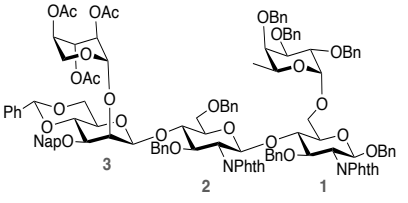


(*N*-Phenyl)-2,2,2-trifluoroacetimidate 2,3,4-tri-*O*-acetyl-β-D-xylopyranosyl-(1→2)-4,6-*O*-benzylidine-3-*O*-(2-methylnaphthyl)-β-D-mannopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-α/β–D-glucopyranoside (17): Compound **S5** (12.2 g, 10.7 mmol) was dissolved in DCM (150 mL), followed by the addition of 2,2,2-Trifluoro-*N*-phenylacetimidoyl chloride (2.7 mL, 12.8 mmol) and DBU (1.6 mL, 10.7 mmol). The reaction mixture was stirred for 30 minutes, after which the solvent was evaporated and the residue was purified using silica gel column chromatography (Pet. Ether: EtOAc, 9: 1, v: v to Pet. Ether: EtOAc, 1: 1, v: v), which provided the product as an off-white foamy powder. (12.5 g, 87%). R_f = 0.61 (Pet. Ether: EtOAc, 1: 1, v: v). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.05 to 6.55 (31H, m, H-Ar), 5.45 (1H, s, PhC<u>H</u> of benzylidene), 5.34 (1H, d, H-1 GlcN□, *J* = 8.5 Hz), 5.29 (1H, d, H-1 GlcN□, *J* = 3.3 Hz), 5.17 to 4.90 (4H, m, H-3 Xyl, H-4 Xyl, H-1 Xyl, PhC<u>H</u>H), 4.89



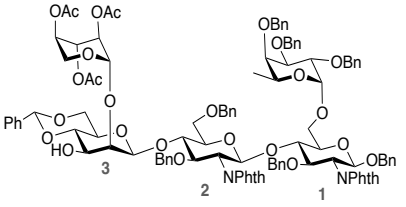
to 4.70 (4H, m, H-2 Xyl, C<u>H</u>$_2$ of Nap, PhCH<u>H</u>), 4.62 (2H, m, H-5b Xyl, PhC<u>H</u>H), 4.54 to 4.33 (3H, m, H-2 GlcN□ □ H-3 GlcN, H-1 Man), 4.33 to 4.20 (2H, m, PhC<u>H</u>H, PhCH<u>H</u>), 4.16 to 3.93 (4H, m, H-2 GlcN□, H-6b GlcN, H-2 Man, H-3 Man), 3.86 (1H, m, H-4 Man), 3.63 (3H, m, H-4 GlcN, H-6a Man, H-6b Man), 3.48 (1H, m, H-5a Xyl), 3.37 (2H, m, H-6a GlcN, H-5 GlcN), 3.08 (1H, m, H-5 Man), 2.18 to 1.92 (9H, 3s, 3x C<u>H</u>$_3$ of Ac); ^{13}C NMR (101 MHz, $CDCl_3$): δ170.00, 169.97, 169.30, 169.18, 169.01, 168.11, 138.96, 138.69, 137.39, 137.30, 135.58, 135.07, 134.18, 133.79, 133.24, 132.94, 131.54, 129.35, 129.00, 128.51, 128.21, 128.12, 128.09, 128.06, 128.05, 127.86, 127.85, 127.83, 127.77, 127.67, 127.32, 126.99, 126.94, 126.89, 126.33, 126.14, 126.13, 126.05, 125.99, 125.90, 125.34, 125.30, 123.59, 123.30, 120.42, 104.98, 101.93, 101.56, 101.28, 99.16, 99.04, 93.01, 92.90, 80.37, 78.23, 77.31, 76.99, 76.84, 76.67, 76.00, 75.07, 74.61, 74.49, 74.15, 73.58, 71.92, 71.79, 69.99, 68.55, 68.27, 68.07, 67.98, 67.70, 67.21, 66.97, 59.94, 59.48, 57.53, 55.72, 21.07, 20.98, 20.95, 20.93, 20.79, -0.03. Compound was unstable under MALDI-TOF-MS conditions.

Benzyl 2,3,4-tri-*O*-acetyl-β-D-xylopyranosyl-(1→2)-4,6-*O*-benzylidine-3-*O*-(2-methylnaphthyl)-β-D-mannopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl-(1→6)-3-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (18): Donor **17** (12.5 g, 9.5 mmol) and acceptor **12** (10.3 g, 11.4 mmol), were dissolved in DCM (100 mL) and stirred with pre-activated molecular sieves (25 g) for 20 minutes. The mixture was then cooled down to -60 ºC, followed by the addition of TMSOTf (619 µL, 3.4 mmol). The reaction mixture was warmed up to -40 ºC over a period of 45 minutes, after which it was quenched with Et_3N (5 mL). The sieves were filtered off and the mixture was concentrated *in vacuo* to give the crude product as a brown syrup. Silica gel column chromatography with DCM: Acetone (9.9: 0.1, v: v to 9: 1, v: v) yielded the product as white amorphous powder. (16.5 g, 85 %). R_f = 0.58 (DCM: Acetone, 9.5: 0.5, v: v). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.94 to 6.61 (60H, m, H-Ar), 5.48 (1H, d, H-1 GlcN-2, *J* = 8.4 Hz), 5.44 (1H, s, PhC<u>H</u> of benzylidene), 5.02 (1H, d, H-1 Xyl, *J* = 3.4 Hz), 4.95 (4H, m, H-4 Xyl, H-3 Xyl, H-1 GlcN-1, C<u>H</u>H of Nap), 4.80 (9H, m, H-2 Xyl, H-1 Fuc, 3x PhC<u>H</u>$_2$, CH<u>H</u> of Nap), 4.55 (4H, m, PhCH<u>H</u>, PhC<u>H</u>H, PhCH<u>H</u>, H-1 Man-3), 4.38 (5H, m, H-5b Xyl, PhC<u>H</u>H, PhC<u>H</u>$_2$, H-3 GlcN-1), 4.22 (4H, m, H-2 GlcN-2, PhC<u>H</u>H, PhCH<u>H</u>, H-3 GlcN-2), 4.09 (5H, m, H-2 GlcN-1, H-6b GlcN-1, H-2 Man-3, H-4 Fuc, H-2 Fuc), 3.92 (4H, m, H-5 Fuc, H-3 Fuc, H-4 Man-3, H-3 Man-3), 3.67 (4H, m, H-6b GlcN-2, H-6b Man-3, H-5 GlcN-2, H-4 GlcN-2), 3.41 (4H, m, H-6a GlcN-2, H-6a GlcN-1, H-6a Man-3, H-5 GlcN-1), 3.23 (2H, m, H-5a Xyl, H-4 GlcN-1), 3.03 (1H, m, H-5 Man-3), 2.09 to 1.73 (9H, 3s, 3x C<u>H</u>$_3$ of Ac), 0.99 (1H, d, C<u>H</u>$_3$ of Fuc, *J* = 6.5 Hz); ^{13}C NMR (101 MHz, $CDCl_3$): δ 169.85, 169.25, 169.02, 167.63, 138.97, 138.84, 138.73, 138.62, 137.53,



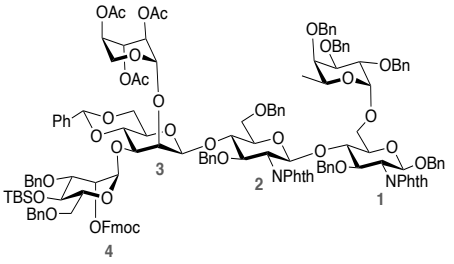
137.50, 137.04, 135.67, 133.92, 133.81, 133.48, 133.27, 132.94, 131.62, 131.39, 128.91, 128.52, 128.47, 128.43, 128.40, 128.37, 128.35, 128.28, 128.17, 128.16, 128.12, 128.05, 128.03, 127.91, 127.86, 127.84, 127.74, 127.66, 127.60, 127.56, 127.54, 127.46, 127.44, 127.42, 127.33, 127.32, 126.94, 126.82, 126.16, 126.15, 126.10, 125.85, 125.39, 123.58, 123.07, 101.79, 101.54, 98.87, 97.03, 96.84, 96.63, 80.00, 79.44, 78.19, 77.59, 77.44, 77.12, 76.88, 76.80, 76.73, 76.42, 75.56, 75.13, 74.73, 74.46, 74.33, 74.08, 73.86, 73.81, 73.16, 73.07, 72.59, 71.63, 69.94, 69.41, 69.02, 68.55, 68.46, 68.14, 67.44, 66.00, 64.34, 60.27, 56.59, 55.78, 29.65, 20.91, 20.75, 20.52, 16.61, 16.43, 14.19. MALDI-TOF-MS (*m/z*): $[M+ Na]^+$ calculated for $C_{118}H_{116}N_2O_{29}Na$, 2047.7561; found 2048.7192.

Benzyl 2,3,4-tri-*O*-acetyl-β-D-xylopyranosyl-(1→2)-4,6-*O*-benzylidine-β-D-mannopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl-(1→6)-3-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (19): Compound **18** (16.1 g, 7.9 mmol) was dissolved in the solvent system DCM (200 mL) and H_2O (20 mL), followed by the addition of DDQ (3.6 g, 15.8 mmol) and β-pinene (3.7 mL, 23.7 mmol). The mixture was stirred in dark for 48 hours after which reaction did not proceed any further. The solvent was evaporated *in vacuo* and the residue was diluted by DCM (300 mL) and washed with water (200 mL) and sat. $NaHCO_3$ (300 mL). The organic phase was dried over $MgSO_4$ and filtered, and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography and using Tol: EtOAc (9: 1, v: v to 7: 3, v: v). The recovered starting material was re-subjected to the above reaction conditions, and purification by silica gel column chromatography gave the combined product as yellow amorphous solid. (9.7 g, 64%). R_f = 0.47 (Tol: EtOAc, 7: 3, v: v). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.98 to 6.67 (48H, m, H-Ar), 5.59 (1H, d, H-1 GlcN-2, *J* = 8.2 Hz), 5.42 (1H, s, PhC<u>H</u> of benzylidene), 4.93 (9H, m, H-1 Fuc, H-1 GlcN-1, H-2 Xyl, H-4 Xyl, H-3 Xyl, 2x PhC<u>H</u>$_2$), 4.75 (4H, m, PhC<u>H</u>H, PhC<u>H</u>$_2$, H-1 Xyl), 4.61 (5H, m, PhCH<u>H</u>, H-1 Man-3, PhC<u>H</u>$_2$, PhCH<u>H</u>), 4.48 (3H, m, PhC<u>H</u>$_2$, H-3 GlcN-1), 4.33 (3H, m, H-2 GlcN-2, PhC<u>H</u>H, H-3 GlcN-2), 4.20 (3H, m, H-2 GlcN-1, H-3 Man-3, H-2 Fuc), 4.02 (6H, m, H-5b Xyl, H-5 Fuc, H-6b GlcN-2, H-2 Man-3, H-3 Fuc, H-4 Fuc), 3.88 (2H, m, H-6b Man-3, H-4 Man-3), 3.75 (1H, d, H-6b GlcN-1, *J* = 11.2 Hz), 3.60 (4H, m, H-5 GlcN-1, H-5 GlcN-2, H-6a Man-3, H-4 GlcN-2), 3.37 (3H, m, H-6a GlcN-1, H-6a GlcN-2, H-4 GlcN-1), 3.13 (1H, m, H-5 Man-3), 3.03 (1H, bs, O<u>H</u>), 2.86 (1H, dd, H-5a Xyl, *J* = 11.8 Hz, 8.9 Hz), 2.07 to 1.79 (9H, 3s, 3x C<u>H</u>$_3$ of Ac), 1.02 (1H, d, C<u>H</u>$_3$ of Fuc, *J* = 6.5 Hz); ^{13}C NMR (101 MHz, $CDCl_3$) : δ 171.10, 169.72, 169.68, 169.55, 169.45, 169.29, 168.44, 167.68, 138.86, 138.80, 138.73, 138.57, 138.43, 137.58, 137.25, 137.02, 133.95, 133.61, 133.30, 131.64, 129.06, 128.68, 128.64, 128.57, 128.47, 128.41, 128.38, 128.32, 128.26, 128.22, 128.20, 128.15, 128.10,



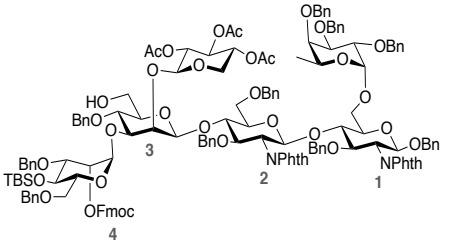
128.07, 128.01, 127.99, 127.92, 127.91, 127.85, 127.83, 127.80, 127.67, 127.63, 127.57, 127.54, 127.51, 127.47, 127.39, 127.37, 127.29, 127.21, 127.19, 126.96, 126.37, 126.34, 126.20, 123.68, 123.18, 102.04, 101.52, 99.92, 97.00, 96.71, 96.64, 79.87, 79.48, 79.07, 77.59, 77.55, 77.43, 77.23, 77.05, 76.92, 76.15, 75.21, 74.89, 74.77, 74.38, 74.34, 73.83, 73.77, 73.45, 73.08, 72.58, 70.95, 70.92, 70.66, 70.12, 69.98, 68.44, 68.38, 67.95, 67.43, 66.08, 64.13, 61.58, 60.38, 56.36, 55.83, 21.05, 21.05, 20.77, 20.72, 20.68, 20.66, 16.43, 14.23. MALDI-TOF-MS (*m/z*): $[M+ Na]^+$ calculated for $C_{107}H_{108}N_2O_{29}Na$, 1884.7038; found 1885.6401.

Benzyl [3,6-di-*O*-benzyl-4-*O*-*t*-butyldimethylsilyl-2-*O*-fluorenylmethoxycarbonyl-α-D-mannopyranosyl]-(1→3)-2,3,4-tri-*O*-acetyl-β-D-xylopyranosyl-(1→2)-4,6-*O*-benzylidine-β-D-mannopyranosyl]-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl-(1→6)-3-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (20): A mixture of acceptor **19** (9.3 g, 4.9 mmol) and donor **13** (7.8 g, 9.9 mmol), was stirred in DCM (100 mL) with pre-activated molecular sieves (20 g) for 20 minutes. The mixture was then cooled down to -30 ºC, followed by the sequential addition of NIS (2.22 g, 9.9 mmol) and TMSOTf (717 µL, 3.96 mmol). The reaction mixture was warmed up to -20 ºC over a period of 30 minutes, after which it was quenched with pyridine (10 mL). The sieves were filtered off, the mixture was diluted by DCM (200 mL) and washed with 5% $Na_2S_2O_4$ (100 mL) and sat. $NaHCO_3$ (150 mL). The mixture was concentrated *in vacuo* to give the crude product as a brown syrup. Silica gel column chromatography with Tol: EtOAc (9: 1, v: v to 7: 3, v: v) yielded the product as an off-white amorphous powder. (9.7 g, 77 %). R_f = 0.51 (Tol: EtOAc, 8: 2, v: v). ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.85 to 6.65 (77H, m, H-Ar), 5.46 (1H, d, H-1 GlcN-2, *J* = 8.6 Hz), 5.40 (2H, m, PhC<u>H</u> of benzylidene, H-2 Man-4), 5.27 (1H, s, H-1 Man-4), 4.96 (7H, m, H-1 Xyl, H-2 Xyl, H-1 GlcN-1, H-4 Xyl, H-3 Xyl), 4.82 (5H, m, H-1 Fuc, 2x PhC<u>H</u>$_2$), 4.72 (2H, m, PhC<u>H</u>$_2$), 4.64 to 4.39(10H, m, C<u>H</u>$_2$ of Fmoc, PhC<u>H</u>$_2$ protons, H-1 Man-3), 4.31 (5H, m, H-3 GlcN-1, PhC<u>H</u>$_2$ protons), 4.16 (8H, m, C<u>H</u> of Fmoc, H-2 GlcN-1, H-2 GlcN-2, H-3 GlcN-2, H-2 Man-3, PhC<u>H</u>$_2$), 4.01 (4H, m, H-4 Man-3, H-3 Man-3, H-2 Fuc, H-4 Fuc), 3.91 (2H, m, H-5 Fuc, H-3 Fuc), 3.77 (6H, m, H-6b GlcN-1, H-6a Man-4, H-6b Man-4, H-3 Man-4, H-5 GlcN-1, H-4 GlcN-2), 3.63 (4H, m, H-4 Man-4, H-5 Man-4, H-5 GlcN-2, H-6b GlcN-2), 3.50 (1H, dd, H-6b Man-3, *J* = 11.2 Hz, 2.9 Hz), 3.29 (4H, m, H-5a Xyl, H-6a GlcN-2, H-6a GlcN-1, H-6a Man-3), 2.96 (1H, m, H-5 Man-3), 2.08 to 1.78 (9H, 3s, 3x C<u>H</u>$_3$ of Ac), 0.99 (3H, d, C<u>H</u>$_3$ of Fuc, *J* = 6.5 Hz), 0.80 (9H, s, 3x C-C<u>H</u>3 of TBS), 0.03 to -0.02 (6H, d, 2x C<u>H</u>$_3$-Si of TBS); ^{13}C NMR (151 MHz, $CDCl_3$) : δ 169.58, 169.36, 167.71, 154.51, 143.65, 143.39, 143.34, 141.27, 141.24, 138.97, 138.95, 138.83, 138.69, 138.63,



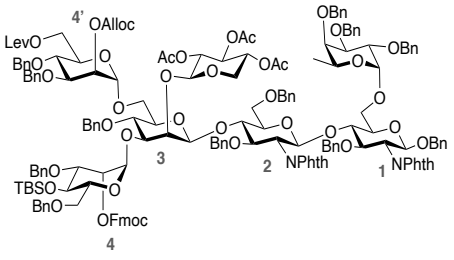
138.41, 137.91, 137.87, 137.78, 137.24, 137.11, 134.00, 133.54, 131.71, 129.07, 128.72, 128.65, 128.59, 128.51, 128.46, 128.44, 128.42, 128.35, 128.27, 128.18, 128.11, 128.08, 127.98, 127.91, 127.87, 127.84, 127.76, 127.73, 127.66, 127.61, 127.56, 127.53, 127.51, 127.49, 127.44, 127.40, 127.31, 127.22, 127.13, 126.92, 125.98, 125.38, 125.34, 123.15, 120.01, 101.71, 101.20, 99.59, 98.84, 97.24, 97.13, 96.70, 79.48, 79.33, 78.06, 77.76, 77.46, 77.34, 77.14, 76.82, 76.61, 76.03, 75.69, 75.29, 74.79, 74.61, 74.45, 74.02, 73.82, 73.53, 73.35, 73.26, 73.13, 72.62, 71.58, 70.96, 70.65, 70.41, 70.25, 69.99, 69.66, 68.64, 68.34, 68.08, 67.99, 67.27, 66.07, 64.49, 61.17, 56.46, 55.83, 46.57, 30.93, 26.05, 26.01, 21.50, 20.84, 20.81, 20.72, 18.20, 16.49, -3.87, -4.95, -5.25. MALDI-TOF-MS (*m/z*): $[M+ Na]^+$ calculated for $C_{148}H_{154}N_2O_{36}SiNa$, 2585.9948; found 2586.1830.

Benzyl [3,6-di-*O*-benzyl-4-*O*-*t*-butyldimethylsilyl-2-*O*-fluorenylmethoxycarbonyl-α-D-mannopyranosyl-(1→3)-2,3,4-tri-*O*-acetyl-β-D-xylopyranosyl-(1→2)-4-*O*-benzyl-β-D-mannopyranosyl]-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl-(1→6)-3-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (21): Compound **20** (9.7 g, 3.8 mmol) was dissolved in DCM (150 mL) and stirred with pre-activated molecular sieves (30 g) for 30 minutes, after which the temperature was brought down to -70 °C. The mixture was stirred at this temperature for another 30 minutes after which Et_3SiH (1.2 mL, 7.6 mmol) and $PhBCl_2$ (1.5 mL, 11.4 mmol). The mixture was stirred at this temperature for 20 minutes after which it was quenched with pyridine (20 mL). The mixture was further diluted by DCM (200 mL), the sieves were filtered off, and the mixture was washed with sat. $NaHCO_3$ (100 mL). The organic phase was dried over $MgSO_4$ and filtered, and the filtrate was concentrated *in vacuo*. Silica gel column chromatography using Tol: EtOAc (9: 1, v: v to 7: 3, v: v) gave the product as yellow amorphous powder. (7.1 g, 72%). R_f= 0.27 (Tol: EtOAc, 8: 2, v: v). ^1H NMR (600 MHz, $CDCl_3$): δ 7.89 to 6.59 (80H, m, H-Ar), 5.37 (1H, d, H-1 GlcN-2, *J* = 8.3 Hz), 5.25 (1H, s, H-2 Man-4), 5.16 (1H, s, H-1 Man-4), 5.03 to 4.80 (9H, m, H-4 Xyl, H-3 Xyl, H-2 Xyl, H-1 Fuc, H-1 GlcN-1, H-1 Xyl, PhC$\underline{H}_2$ protons), 4.70 (5H, m, H-1 Man-3, PhC$\underline{H}_2$, C$\underline{H}_2$ of Fmoc), 4.53 (6H, m, PhC$\underline{H}_2$ protons), 4.39 (4H, m, H-3 GlcN-1, PhC$\underline{H}_2$ protons), 4.18 (10H, m, C$\underline{H}$ of Fmoc, H-2 GlcN-2, H-2 GlcN-1, H-5b Xyl, H-4 Man-4, H-3 GlcN-2, H-2 Man-3, PhC$\underline{H}_2$ protons), 3.92 (4H, m, H-5 Fuc, H-3 Man-3, H-2 Fuc, H-4 Fuc), 3.70 (6H, m, H-6b GlcN-2, H-6b Man-3, H-6a Man-4, H-6b Man-4, H-4 GlcN-2, H-3 Fuc), 3.50 (5H, m, H-6b GlcN-1, H-6a Man-3, H-4 GlcN-1, H-5 GlcN-2, H-5 GlcN-1), 3.28 (4H, m, H-5a Xyl, H-6a GlcN-2, H-6a GlcN-1, H-4 Man-3), 2.92 (1H, m, H-5 Man-3), 2.09 to 1.72 (9H, 3s, 3x C$\underline{H}_3$ of Ac), 0.94 (3H, d, C$\underline{H}_3$ of Fuc, *J* = 6.5 Hz), 0.79 (9H, s, 3x C-C$\underline{H}$3 of TBS), 0.01 to -0.02



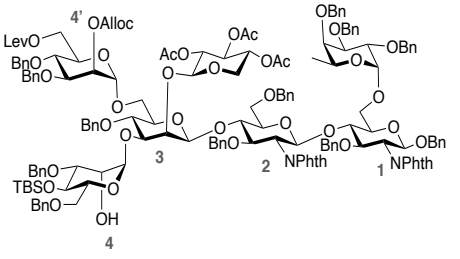
(6H, d, 2x C<u>H</u>$_3$-Si of TBS; ^{13}C NMR (151 MHz, $CDCl_3$) : δ 169.68, 169.62, 169.42, 167.68, 154.55, 143.58, 143.34, 141.29, 141.27, 138.97, 138.83, 138.70, 138.58, 138.42, 137.91, 137.79, 137.73, 137.11, 134.15, 133.84, 133.48, 131.69, 130.35, 129.08, 128.58, 128.55, 128.47, 128.44, 128.42, 128.27, 128.23, 128.21, 128.19, 128.17, 128.10, 128.04, 127.95, 127.89, 127.86, 127.81, 127.71, 127.67, 127.58, 127.56, 127.54, 127.51, 127.43, 127.39, 127.31, 127.23, 126.89, 126.86, 125.45, 125.35, 123.15, 120.03, 100.99, 99.89, 98.89, 97.23, 97.18, 96.67, 79.77, 79.58, 79.25, 77.75, 77.43, 77.30, 77.09, 76.88, 76.74, 76.22, 75.71, 75.67, 75.34, 75.20, 74.78, 74.74, 74.48, 74.21, 74.03, 73.88, 73.46, 73.27, 73.17, 73.14, 72.56, 72.29, 71.34, 70.32, 70.23, 70.12, 69.98, 69.66, 68.31, 67.99, 67.90, 66.04, 64.49, 61.83, 60.72, 56.52, 55.81, 46.58, 30.96, 26.04, 21.50, 20.86, 20.79, 20.72, 18.17, 16.48, 0.05, -3.83, -4.94. MALDI-TOF-MS (*m/z*): $[M+Na]^+$ calculated for $C_{148}H_{156}N_2O_{36}SiNa$, 2588.0105; found 2589.5942.

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)-2,3,4-tri-*O*-acetyl-β-D-xylopyranosyl-(1→2)-4-*O*-benzyl-β-D-mannopyranosyl]-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl-(1→6)-3-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (4a): A mixture of acceptor **21** (7.1 g, 2.8 mmol) and donor **14** (7.9 g, 11.1 mmol), was stirred in DCM (100 mL) with pre-activated molecular sieves (20 g) for 20 minutes. The mixture was then cooled down to -30 ºC, followed by the addition of TMSOTf (603 µL, 3.33 mmol). The reaction mixture was warmed up to -15 ºC over a period of 30 minutes, after which it was quenched with pyridine (10 mL). The sieves were filtered off and the mixture was concentrated *in vacuo.* The residue was purified by silica gel column chromatography using DCM to DCM: Acetone (95: 5, v: v) which yielded the product as a white amorphous powder. (6.6 g, 75 %). R_f = 0.64 (DCM: Acetone, 9.5: 0.5, v: v). ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.87 to 6.59 (85H, m, H-Ar), 5.78 (1H, m, C<u>H</u>=CH$_2$ of alloc), 5.33 (2H, m, H-2 Man-4, H-1 GlcN-2), 5.19 (4H, m, H-2 Man-4', H-1 Man-4, CH=C<u>H</u>$_2$ of alloc), 5.07 (2H, m, H-4 Xyl, H-3 Xyl), 4.88 (2H, m, H-1 GlcN-1, PhCH<u>H</u>), 4.97 (1H, m, H-2 Xyl), 4.84 to 4.71 (10H, m, H-1 Fuc, H-1 Man-4', H-1 Xyl, PhC<u>H</u>$_2$ protons), 4.64 (4H, m, H-1 Man-3, PhC<u>H</u>H, PhC<u>H</u>$_2$ protons), 4.58 to 4.37 (14H, PhC<u>H</u>$_2$ protons, C<u>H</u>$_2$ of Fmoc, OC<u>H</u>$_2$ of alloc), 4.34 (2H, m, H-3 GlcN-1, PhCH<u>H</u>), 4.30 to 4.13 (8H, m, C<u>H</u> of Fmoc, H-2 GlcN-1, H-2 GlcN-2, H-5b Xyl, H-3 GlcN-2, PhC<u>H</u>$_2$ protons), 4.12 to 3.95 (8H, m, H-6a Man-4', H-6b Man-4', H-2 Man-3, H-4 Man-4, H-2 Fuc, H-3 Man-3), 3.93 to 3.76 (7H, m, H-6b Man-3, H-5 Fuc, H-5 Man-4, H-5 Man-4' , H-3 Man-4', H-4 Fuc), 3.67 (3H, m, H-6a Man-4, H-6b Man-4, H-3 Fuc), 3.59 (6H, m, H-6b GlcN-2, H-6b GlcN-



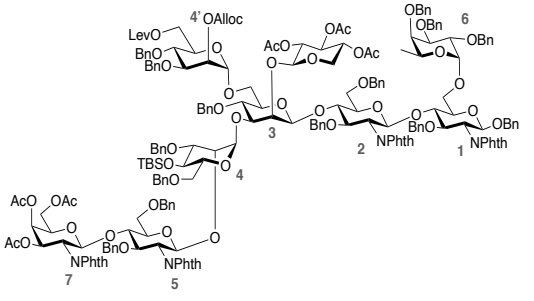
1, H-6a Man-3, H-4 GlcN-2, H-5 GlcN-1, H-3 Man-4), 3.49 (3H, m, H-6a GlcN-2, H-4 GlcN-1, H-5 GlcN-2), 3.26 (2H, m, H-6a GlcN-1, H-4 Man-3), 3.04 (2H, m, H-5a Xyl, H-5 Man-3), 2.56 (2H, t, $COOCH_2CH_2$ of Lev, J = 6.6 Hz), 2.40 (2H, t, $COOCH_2CH_2$ of Lev, J = 6.6 Hz), 2.07 (3H, s, CH_2COCH_3 of Lev), 2.04 to 1.69 (9H, 3s, 3x CH_3 of Ac), 0.91 (3H, d, CH_3 of Fuc, J = 6.5 Hz), 0.76 (9H, s, 3x C-CH_3 of TBS), 0.03 to -0.07 (6H, d, 2x -CH_3-Si of TBS); ^{13}C NMR (151 MHz, $CDCl_3$) δ 207.01, 206.46, 172.31, 169.81, 169.49, 169.41, 167.65, 154.49, 154.34, 143.62, 143.31, 141.26, 141.23, 138.96, 138.92, 138.83, 138.60, 138.44, 138.37, 138.31, 138.13, 137.91, 137.84, 137.08, 133.49, 131.59, 128.59, 128.51, 128.47, 128.42, 128.39, 128.28, 128.26, 128.24, 128.19, 128.15, 128.07, 127.99, 127.97, 127.91, 127.85, 127.79, 127.67, 127.61, 127.54, 127.52, 127.50, 127.47, 127.39, 127.37, 127.32, 127.29, 127.26, 127.20, 127.11, 126.85, 125.38, 125.35, 123.15, 119.99, 118.87, 101.97, 100.28, 99.44, 97.38, 97.27, 97.08, 96.59, 80.58, 79.37, 78.74, 77.79, 77.72, 77.49, 77.27, 77.06, 76.96, 76.85, 76.61, 76.14, 75.56, 75.06, 75.02, 74.87, 74.72, 74.63, 74.56, 74.51, 74.07, 73.66, 73.30, 73.06, 72.99, 72.57, 72.20, 71.92, 71.88, 71.36, 71.31, 71.25, 70.29, 69.92, 69.88, 69.71, 69.27, 68.61, 68.00, 66.12, 65.97, 64.53, 63.15, 62.02, 56.39, 55.77, 53.46, 46.54, 37.87, 30.96, 29.81, 27.88, 26.03, 20.74, 20.51, 18.19, 16.44, 0.03, -3.91, -5.02. MALTI-TOF-MS (*m/z*): [M+ Na]$^+$ calculated for $C_{177}H_{188}N_2O_{45}SiNa$, 3112.2151; found 3113.0973.

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)-2,3,4-tri-*O*-acetyl-β-D-xylopyranosyl-(1→2)-4-*O*-benzyl-β-D-mannopyranosyl]-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl-(1→6)-3-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (22): Compound **4a** (6.6 g, 2.1 mmol) was dissolved in DCM (80 mL) followed by the addition of Et_3N (10 mL). The mixture was stirred for 3 hours after which it was concentrated *in vacuo*. The residue was purified by silica gel column chromatography using solvent gradient DCM to DCM: Acetone (9.5: 0.5, v: v) which afforded the product as an off-white amorphous powder. (4.9 g, 81%). R_f = 0.51 (DCM: Acetone, 9.5: 0.5, v: v). ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.87 to 6.55 (65H, m, H-Ar), 5.80 (1H, m, CH=CH_2 of alloc), 5.35 (1H, d, H-1 GlcN-2, J = 8.5 Hz), 5.31 to 4.94 (10H, m, H-2 Xyl, H-4 Xyl, H-3 Xyl, H-2 Man-4', H-1 Man-4, CH=CH_2 of alloc), 4.94 to 4.71 (11H, m, H-1 Fuc, H-1 Man-4', H-1 Xyl, H-1 GlcN-1, $PhCH_2$ protons), 4.67 (3H, m, H-1 Man-3, $PhCH_2$), 4.66 to 4.36 (16H, m, $PhCH_2$ protons, OCH_2 of alloc), 4.28 (3H, m, $PhCH_2$, H-3 GlcN-1), 4.20 to 4.02 (8H, m, H-2 GlcN-2, H-2 GlcN-1, H-5b Xyl, H-6a Man-4, H-6b Man-4', H-3 Man-4', H-3 Man-3, H-3 GlcN-2), 3.99 to 3.74 (9H, m, H-



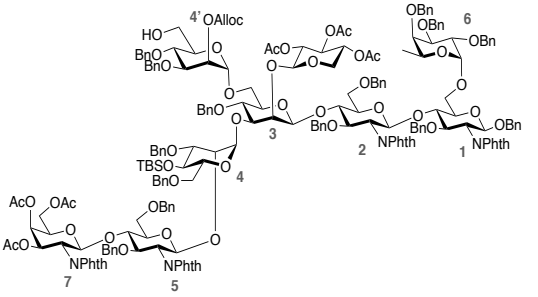
6b Man-3, H-5 Fuc, H-4 Man-4, H-3 Man-4, H-5 Man-4, H-5 Man-4', H-2 Fuc, H-2 Man-3, H-3 Man-4'), 3.72 to 3.42 (12H, m, H-6a GlcN-2, H-6b GlcN-2, H-6b GlcN-1, H-6a Man-3, H-4 GlcN-1, H-6a Man-4, H-6b Man-4, H-3 Fuc, H-4 GlcN-2, H-5 GlcN-1, H-5 GlcN-2, H-2 Man-4), 3.27 (2H, m, H-6a GlcN-1, H-4 Man-3), 3.06 (2H, m, H-5a Xyl, H-5 Man-3), 2.58 (2H, t, $COOCH_2CH_2$ of Lev, J = 6.6 Hz), 2.42 (2H, t, $COOCH_2CH_2$ of Lev, J = 6.6 Hz), 2.09 (3H, s, CH_2COCH_3 of Lev), 2.04 to 1.68 (9H, 3s, 3x CH_3 of Ac), 0.92 (3H, d, CH_3 of Fuc, J = 6.5 Hz), 0.79 (9H, s, 3x C-CH_3 of TBS), 0.00 to -0.05 (6H, d, 2x CH_3-Si of TBS); ^{13}C NMR (151 MHz, $CDCl_3$): δ 206.38, 172.25, 169.70, 169.50, 169.35, 167.59, 154.29, 138.90, 138.86, 138.79, 138.56, 138.42, 138.27, 138.25, 138.18, 137.86, 137.84, 137.03, 133.41, 131.63, 131.53, 128.52, 128.49, 128.45, 128.41, 128.38, 128.36, 128.33, 128.20, 128.17, 128.13, 128.11, 128.09, 128.04, 128.01, 127.99, 127.96, 127.92, 127.89, 127.82, 127.73, 127.60, 127.53, 127.50, 127.47, 127.46, 127.44, 127.42, 127.39, 127.38, 127.31, 127.27, 127.03, 126.78, 123.06, 118.81, 102.05, 101.37, 99.99, 97.34, 97.22, 97.09, 96.56, 80.62, 79.97, 79.29, 77.84, 77.72, 77.34, 77.23, 77.03, 76.88, 76.71, 76.18, 75.52, 75.11, 75.03, 74.77, 74.68, 74.54, 74.40, 74.05, 73.71, 73.25, 73.02, 72.94, 72.50, 71.90, 71.78, 71.69, 71.44, 71.16, 69.87, 69.80, 69.66, 69.26, 68.56, 68.05, 67.53, 66.23, 65.93, 64.54, 63.09, 61.90, 59.51, 56.36, 55.72, 53.42, 50.77, 38.13, 37.82, 37.08, 32.73, 31.91, 31.22, 30.01, 29.76, 29.71, 29.68, 29.64, 29.34, 27.85, 27.07, 25.98, 25.84, 22.68, 20.71, 20.69, 20.50, 18.09, 16.39, 14.11, 13.42, 0.40, -0.01, -3.88, -5.03. MALDI-TOF-MS (*m/z*): $[M+Na]^+$ calculated for $C_{162}H_{178}N_2O_{43}SiNa$, 2890.1470; found 2890.5249.

Benzyl [2-*O*-allyloxycarbonyl-3,4-di-*O*-benzyl-6-*O*-levulenoyl-α-D-mannopyranosyl-(1→6)]-[3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-galactopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside-(1→2)-3,6-di-*O*-benzyl-4-*O*-*t*-butyldimethylsilyl-α-D-mannopyranosyl-(1→3)]-2,3,4-tri-*O*-acetyl-β-D-xylopyranosyl-(1→2)-4-*O*-benzyl-β-D-mannopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl-(1→6)-3-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (23): A mixture of acceptor **22** (4.8 g, 1.7 mmol) and donor **5** (5.4 g, 5.1 mmol), was stirred in DCM (50 mL) with pre-activated molecular sieves (10 g) for 20 minutes, after which the mixture was cooled down to -40 ºC, followed by the addition of TfOH (137 µL, 1.53 mmol). The mixture was warmed up to -20 ºC over a period of 30 minutes, after which it was quenched with Et_3N (1 mL). The sieves were filtered off and the mixture was concentrated *in vacuo.* The residue was purified by silica gel column chromatography using Tol: EtOAc (9: 1, v: v to 1:1, v: v) which afforded the product as an off-



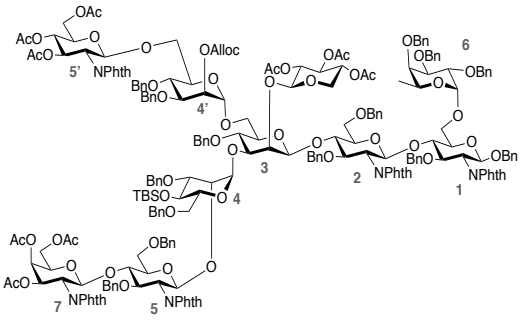
white amorphous powder. (5.1 g, 80%). R_f = 0.46 (Tol: EtOAc, 7: 3, v: v). ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.32 to 6.61 (86H, m, H-Ar), 6.03 (1H, m, C<u>H</u>=CH_2 of alloc), 5.94 (1H, d, H-3 GalN-7, *J* = 11.1 Hz), 5.57 (1H, d, H-1 GlcN-2, *J* = 8.3 Hz), 5.49 (3H, m, H-2 Man-4', H-1 GlcN-5, CH=C<u>H</u>H of alloc), 5.35 (2H, m, H-4 GalN-7, CH=CH<u>H</u> of alloc), 5.25 (1H, t, H-4 Xyl, *J* = 8.8 Hz), 5.20 to 5.00 (9H, m, H-2 Xyl, H-3 Xyl, H-1 Man-4, 3x PhC<u>H_2</u> protons), 4.99 to 4.89 (5H, m, H-1 Xyl, H-1 GlcN-1, PhC<u>H_2</u> protons), 4.85 (3H, m, H-1 GalN-7, H-1 Man-4', PhC<u>H</u>H), 4.81 to 4.62 (11H, m, H-2 GalN-7, H-2 GlcN-5, H-1 Fuc-6, PhC<u>H_2</u> protons, OC<u>H_2</u> of alloc), 4.61 to 4.43 (9H, m, H-1 Man-3, H-3 GlcN-1, PhC<u>H_2</u> protons), 4.41 to 4.24 (9H, m, H-2 GlcN-2, H-2 GlcN-1, H-3 GlcN-2, H-3 GlcN-5, PhC<u>H_2</u> protons), 4.24 to 4.01 (12H, m, H-6a GalN-7, H-6b GalN-7, H-6a Man-4', H-6b Man-4', H-5a Xyl, H-4 GlcN-1, H-4 GlcN-2, H-4 GlcN-5, H-3 Fuc-6, H-2 Fuc-6, H-4 Man-3, H-3 Man-4), 3.93 (4H, m, H-5 Fuc-6, H-2 Man-4, H-5 GalN-7, PhCH<u>H</u>), 3.79 (5H, m, H-6a GlcN-2, H-6a Man-4, H-2 Man-3, H-5 Man-4', H-5 Man-4), 3.68 (3H, m, H-6a Man-3, H-4 Man-4', H-3 Man-4'), 3.57 (5H, m, H-4 Man-4, H-6b Man-4, H-6a GlcN-5, H-3 Man-3, H-4 Fuc-6), 3.45 (4H, m, H-6b GlcN-2, H-6b Man-3, H-5 GlcN-2, H-5 GlcN-1), 3.16 (2H, m, H-5b Xyl, H-6a GlcN-1), 3.08 (1H, m, H-6b GlcN-1), 2.78 (3H, m, COOC<u>H_2</u>CH_2 of Lev, H-5 Man-3), 2.54 (3H, m, COOCH_2C<u>H_2</u> of Lev, H-6b GlcN-5), 2.33 (3H, s, CH_2COC<u>H</u>3 of Lev), 2.23 to 1.86 (19H, m, 6x C<u>H_3</u> of Ac, H-5 GlcN-5), 1.13 (3H, d, C<u>H_3</u> of Fuc-6, *J* = 6.3 Hz), 0.82 (9H, s, 3x C-C<u>H</u>3 of TBS), 0.11 to -0.06 (6H, d, 2x C<u>H_3</u>-Si of TBS); ^{13}C NMR (151 MHz, $CDCl_3$): δ 206.42, 172.30, 172.22, 170.30, 170.26, 169.77, 169.69, 169.41, 169.33, 168.09, 167.57, 167.40, 154.34, 138.96, 138.90, 138.82, 138.53, 138.46, 138.43, 138.34, 138.26, 138.17, 138.09, 137.94, 137.82, 137.11, 133.51, 131.96, 131.57, 131.54, 129.12, 129.06, 128.91, 128.78, 128.64, 128.53, 128.48, 128.44, 128.42, 128.39, 128.37, 128.32, 128.31, 128.26, 128.22, 128.19, 128.15, 128.08, 128.00, 127.94, 127.93, 127.90, 127.87, 127.83, 127.78, 127.75, 127.71, 127.66, 127.62, 127.60, 127.54, 127.51, 127.43, 127.37, 127.35, 127.29, 127.25, 127.13, 127.02, 126.87, 125.60, 125.34, 123.87, 123.50, 123.11, 118.91, 101.13, 100.27, 97.33, 97.25, 96.66, 79.27, 77.70, 77.57, 77.45, 77.25, 76.93, 76.49, 75.73, 75.20, 75.00, 74.72, 74.23, 74.02, 73.63, 73.25, 72.92, 72.81, 72.70, 72.56, 71.99, 71.80, 71.61, 71.27, 70.48, 69.95, 69.70, 68.60, 68.07, 67.90, 66.67, 65.97, 60.87, 56.30, 55.78, 55.62, 52.07, 37.85, 31.94, 31.48, 30.24, 29.83, 29.82, 29.70, 29.38, 27.85, 26.05, 26.02, 25.95, 22.72, 21.49, 20.76, 20.71, 20.66, 20.62, 20.53, 20.51, 18.14, 18.06, 17.83, 16.45, 14.19, 0.08, -3.81, -5.26, -5.38. MALDI-TOF-MS (*m/z*): $[M+ Na]^+$ calculated for $C_{210}H_{222}N_4O_{58}SiNa$, 3778.4212; found 3778.5018.

Benzyl [2-*O*-allyloxycarbonyl-3,4-di-*O*-benzyl-α-D-mannopyranosyl-(1→6)]-[3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-galactopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside-(1→2)-3,6-di-*O*-benzyl-4-*O*-*t*-butyldimethylsilyl-α-D-mannopyranosyl-(1→3)]-2,3,4-tri-*O*-acetyl-β-D-xylopyranosyl-(1→2)-4-*O*-benzyl-β-D-mannopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl-(1→6)-3-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (24): Compound **23** (2.5 g, 0.66 mmol) was dissolved in DCM (20 mL) and MeOH (10 mL), followed by the addition of solid hydrazine acetate (304 mg, 3.3 mmol). The mixture was stirred for 1 hour after which it was concentrated *in vacuo* and the residue was purified by silica gel column chromatography using Pet. Ether: EtOAc (8: 2, v: v to 1:1, v: v) which afforded the product as a white foamy solid. (1.6 g, 66%). R_f = 0.49 (Tol: EtOAc, 7: 3, v: v). ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.17 to 6.35 (86H, m, H-Ar), 5.83 (1H, m, C<u>H</u>=CH_2 of alloc), 5.74 (1H, dd, H-3 GalN-7, *J* = 11.8 Hz, 3.6 Hz), 5.38 (1H, d, H-1 GlcN-2, *J* = 8.1 Hz), 5.33 (1H, d, H-2 Man-4', *J* = 3.6 Hz), 5.28 (2H, m, H-1 GlcN-5, CH=C<u>H</u>H of alloc), 5.18 (1H, m, CH=CH<u>H</u> of alloc), 5.12 (1H, m, H-4 GalN-7), 5.04 (1H, t, H-4 Xyl, *J* = 8.9 Hz), 4.98 to 4.71 (14H, m, H-2 Xyl, H-3 Xyl, H-1 Man-4, H-1 Xyl, H-1 GlcN-1, PhC<u>H</u>$_2$ protons), 4.65 (4H, m, H-1 GalN-7, H-1 Man-4', PhC<u>H</u>$_2$), 4.60 to 4.41 (11H, m, H-2 GalN-7, H-1 Fuc-6, PhC<u>H</u>$_2$ protons, OC<u>H</u>$_2$ of alloc), 4.41 to 4.24 (7H, m, H-1 Man-3, H-3 GlcN-1, PhC<u>H</u>$_2$ protons), 4.25 to 4.08 (7H, m, H-2 GlcN-2, H-2 GlcN-1, H-2 GlcN-5, H-3 GlcN-2, H-3 GlcN-5, PhC<u>H</u>H protons), 4.07 to 3.81 (9H, m, H-6a GalN-7, H-6b GalN-7, H-5a Xyl, H-4 GlcN-1, H-4 GlcN-2, H-4 GlcN-5, H-3 Fuc-6, H-2 Fuc-6, H-4 Man-3), 3.77 (4H, m, H-5 Fuc-6, H-2 Man-4, H-5 GalN-7, PhCH<u>H</u>), 3.69 to 3.49 (5H, m, H-6a GlcN-2, H-6a Man-4, H-2 Man-3, H-5 Man-4', H-5 Man-4), 3.49 to 3.31 (8H, m, H-6a Man-4', H-6b Man-4', H-6a Man-3, H-4 Man-4, H-6b Man-4, H-6a GlcN-5, H-3 Man-3, H-3 Man-4', H-4 Man-4'), 3.26 (5H, m, H-6b GlcN-2, H-6b Man-3, H-4 Fuc-6, H-5 GlcN-2, H-5 GlcN-1), 2.97 (3H, m, H-5b Xyl, H-6a GlcN-1, H-6b GlcN-1), 2.38 (2H, m, H-5 Man-3, H-6b GlcN-5), 2.11 to 1.67 (19H, m, 6x C<u>H</u>$_3$ of Ac, H-5 GlcN-5), 0.93 (3H, d, C<u>H</u>$_3$ of Fuc-6, *J* = 6.5 Hz), 0.63 (9H, s, 3x C-C<u>H</u>3 of TBS), -0.07 to -0.20 (6H, d, 2x C<u>H</u>$_3$-Si of TBS); ^{13}C NMR (151 MHz, $CDCl_3$, signals from edited HSQCAD experiment): δ 123.82, 123.64, 134.68, 123.19, 133.57, 123.35, 127.96, 133.56, 128.33, 126.01, 128.19, 76.97, 127.86, 127.95, 127.93, 127.74, 125.64, 127.67, 125.67, 127.87, 127.47, 127.84, 127.84, 126.97, 131.69, 67.86, 96.81, 96.71, 66.63, 97.27, 118.95, 71.95, 71.83, 71.16, 96.64, 69.29, 74.68, 72.64, 74.95, 97.46, 72.94, 100.17, 97.30, 71.42, 73.02, 94.85, 69.98, 74.87, 68.65, 73.66, 52.04, 71.43, 74.85, 74.73, 101.18, 70.52, 72.94, 76.62, 69.96, 72.86, 75.47, 55.72, 76.96, 76.65, 60.94, 60.89, 75.21, 75.60, 79.46, 77.88, 72.84, 71.09, 66.00, 73.41, 73.97, 70.50, 68.39, 64.59, 76.07, 77.80, 61.87, 65.97, 74.20,



67.61, 70.74, 71.89, 74.13, 64.63, 78.27, 61.84, 68.02, 74.07, 70.81, 20.75, 20.69, 25.06, 20.73, 20.54, 17.30, 73.34, 18.01, 20.55, 16.47, 26.03, 24.50, 26.02, 0.02, -5.23, -3.80, -5.35. MALDI-TOF-MS (*m/z*): $[M+Na]^+$ calculated for $C_{205}H_{216}N_4O_{56}SiNa$, 3680.3844; found 3680.4160.

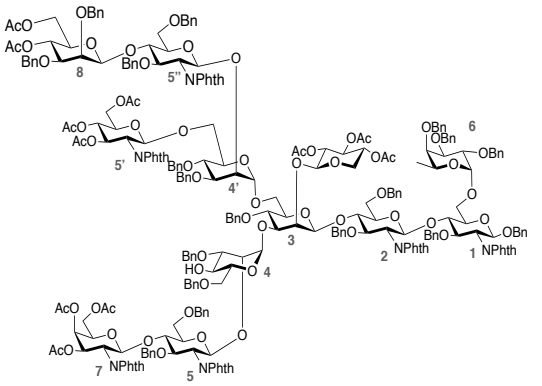
Benzyl [3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-galactopyranosyl-(1→6)-2-*O*-allyloxycarbonyl-3,4-di-*O*-benzyl-α-D-mannopyranosyl-(1→6)]-[3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-galactopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside-(1→2)-3,6-di-*O*-benzyl-4-*O*-*t*-butyldimethylsilyl-α-D-mannopyranosyl-(1→3)]-2,3,4-tri-*O*-acetyl-β-D-xylopyranosyl-(1→2)-4-*O*-benzyl-β-D-mannopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl-(1→6)-3-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (25): A mixture of acceptor **24** (1.6 g, 0.44 mmol) and donor **6** (1.07 g, 1.76 mmol), was stirred in DCM (15 mL) with pre-activated molecular sieves (3 g) for 10 minutes, after which the mixture was cooled down to -40 ºC, followed by the addition of TfOH (16 µL, 0.18 mmol). The mixture was warmed up to -10 ºC over a period of 30 minutes, after which it was quenched with Et_3N (150 µL). The sieves were filtered off and the mixture was concentrated *in vacuo*.



The residue was purified by silica gel column chromatography using Tol: EtOAc (9: 1, v: v to 6: 4, v: v) and then passed through BioGel SX-1 column using Tol: Acetone (1: 1, v: v) as mobile phase, which afforded the product as an off-white amorphous powder. (1.19 g, 65%). R_f = 0.57 (Tol: EtOAc, 7: 3, v: v). ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.12 to 6.41 (90H, m, H-Ar), 5.85 (1H, m, C<u>H</u>=CH_2 of alloc), 5.74 (2H, m, H-3 GalN-7, H-3 GlcN-5'), 5.39 (1H, d, H-1 GlcN-2, *J* = 8.7 Hz), 5.34 (1H, d, H-2 Man-4', *J* = 3.1 Hz), 5.29 (2H, m, H-1 GlcN-5, CH=C<u>H</u>H of alloc), 5.21 (2H, m, H-1 GlcN-5', CH=CH<u>H</u> of alloc), 5.07 (2H, m, H-4 GlcN-5', H-4 GalN-7), 5.00 (1H, m, H-4 Xyl), 4.96 to 4.82 (7H, m, H-2 Xyl, H-3 Xyl, H-1 Man-4, PhC<u>H</u>$_2$ protons), 4.82 to 4.71 (4H, m, H-1 Xyl, H-1 GlcN-1, PhC<u>H</u>$_2$ protons), 4.71 to 4.57 (5H, m, H-1 GalN-7, PhC<u>H</u>$_2$ protons), 4.56 to 4.41 (10H, m, H-2 GalN-7, H-1 Fuc-6, PhC<u>H</u>$_2$ protons, OC<u>H</u>$_2$ of alloc), 4.41 to 4.30 (7H, m, H-1 Man-4', H-1 Man-3, PhC<u>H</u>$_2$ protons), 4.26 (5H, m, H-2 GlcN-5', H-6a GlcN-5', PhC<u>H</u>$_2$, H-3 GlcN-1), 4.20 to 4.05 (10H, m, H-2 GlcN-1, H-2 GlcN-2, H-2 GlcN-5, H-6b GlcN-5', H-3 GlcN-2, H-3 GlcN-5, PhC<u>H</u>$_2$ protons), 4.04 to 3.87 (8H, m, H-6a GalN-7, H-6b GalN-7, H-5a Xyl, H-4 GlcN-1, H-4 GlcN-2, H-4 GlcN-5, H-3 Fuc-6, H-2 Fuc-6), 3.84 to 3.69 (7H, m, H-5 Fuc-6, H-6a GlcN-1, H-2 Man-4, PhC<u>H</u>H, H-5 GalN-7, H-3 Man-4, H-5 Man-4'), 3.66 to 3.48 (6H, m, H-6a Man-4', H-6a Man-4, H-2 Man-3, H-5 GlcN-5', H-5 Man-4, H-4 Man-4'), 3.44 to 3.19 (11H, m,

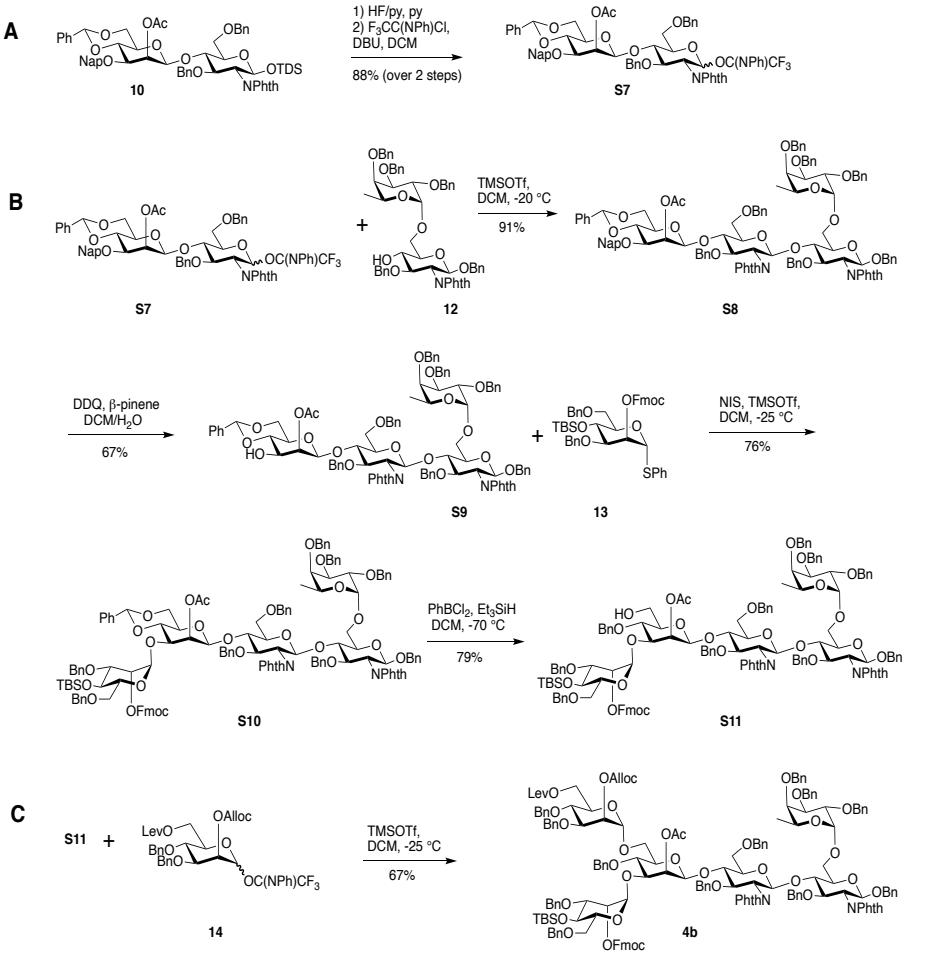
H-6b Man-4', H-6a GlcN-2, H-4 Man-4, H-6b Man-4, H-6b GlcN-1, H-6a GlcN-5, H-3 Man-3, H-3 Man-4', H-5 GlcN-2, H-4 Fuc-6, H-5 GlcN-1), 2.99 (2H, m, H-5b Xyl, H-6a Man-3), 2.91 (1H, d, H-6b Man-3, *J* = 10.6 Hz), 2.79 (1H, d, H-6b GlcN-2, *J* = 10.7 Hz), 2.43 (1H, m, H-5 Man-3), 2.29 (1H, m, H-6b GlcN-5), 2.09 to 1.65 (28H, m, 9x C$\underline{H}_3$ of Ac, H-5 GlcN-5), 0.93 (3H, d, C$\underline{H}_3$ of Fuc-6, *J* = 6.5 Hz), 0.62 (9H, s, 3x C-C$\underline{H}$3 of TBS), -0.09 to -0.28 (6H, d, 2x C$\underline{H}_3$-Si of TBS); ^{13}C NMR (151 MHz, $CDCl_3$, signals from edited HSQCAD experiment): δ 123.91, 123.63, 134.85, 123.22, 133.61, 123.47, 128.02, 123.33, 133.54, 128.18, 126.09, 128.21, 128.13, 76.96, 127.99, 125.80, 128.06, 127.76, 127.72, 124.08, 125.61, 127.79, 127.96, 128.05, 127.50, 127.81, 127.83, 126.79, 131.70, 67.87, 70.70, 96.77, 66.65, 118.82, 97.29, 118.82, 98.55, 118.81, 68.98, 71.93, 71.47, 96.62, 71.16, 69.23, 74.69, 72.57, 97.41, 97.80, 72.88, 100.07, 73.18, 70.78, 94.80, 70.02, 74.72, 74.03, 52.07, 68.54, 74.75, 70.89, 72.90, 97.45, 70.58, 101.34, 72.98, 72.96, 69.98, 69.97, 76.59, 54.42, 62.03, 72.89, 75.63, 72.91, 55.82, 77.05, 62.00, 76.71, 60.92, 75.91, 60.86, 69.71, 79.29, 75.27, 79.34, 80.03, 72.66, 66.01, 73.76, 71.10, 69.12, 73.43, 77.19, 70.52, 71.69, 68.33, 64.62, 76.13, 77.83, 74.02, 68.46, 70.85, 69.12, 67.77, 74.02, 66.36, 74.14, 64.60, 78.46, 70.34, 68.02, 61.92, 68.00, 66.38, 73.86, 70.81, 22.89, 20.73, 19.18, 20.69, 20.73, 20.54, 19.00, 73.33, 20.53, 29.68, 16.47, 10.21, 24.52, 26.02, 0.04, -5.20, -3.80, -6.87, -5.35. MALDI-TOF-MS (*m/z*): $[M+Na]^+$ calculated for $C_{225}H_{235}N_5O_{65}SiNa$, 4097.4904; found 4097.6391.

Benzyl [3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-galactopyranosyl-(1→6)-4,6-di-*O*-acetyl-2,3-di-*O*-benzyl-β-D-mannopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→2)-3,4-di-*O*-benzyl-α-D-mannopyranosyl]-(1→6)-[3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-galactopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside-(1→2)-3,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→3)]-2,3,4-tri-*O*-acetyl-β-D-xylopyranosyl-(1→2)-4-*O*-benzyl-β-D-mannopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl-(1→6)- 3-*O*-benzyl-2-deoxy- 2-phthalimido-β-D-glucopyranoside (S6): Compound **25** (1.1 g, 0.27 mmol) was dissolved in THF (30 mL) and H_2O (3 mL), followed by the addition of tetrakis(triphenylphosphine)palladium (62 mg, 0.054 mmol) and morpholine (95 µL, 1.08 mmol). The mixture was stirred for 2 hours after which it was concentrated *in vacuo* and the residue was purified by silica gel column chromatography using Pet. Ether: EtOAc (8: 2,



v: v to 3:7, v: v) which afforded the product as a white amorphous solid. (850 mg, 79%). R_f = 0.41 (Tol: EtOAc, 7: 3, v: v). A mixture of acceptor **24** (850 mg, 0.21 mmol) and donor **7** (926 mg, 0.85 mmol), was stirred in DCM (10 mL) with pre-activated molecular sieves (2 g) for 10 minutes, after which the mixture was cooled down to -40 ºC, followed by the addition of TfOH (7.6 µL, 0.085 mmol). The mixture was warmed up to -20 ºC over a period of 30 minutes, after which it was quenched with Et_3N (100 µL). The sieves were filtered off and the mixture was concentrated *in vacuo.* The residue was purified by silica gel column chromatography using Tol: EtOAc (8: 2, v: v to 1: 1, v: v) and then passed through BioGel SX-1 column using Tol: Acetone (1: 1, v: v) as mobile phase, which afforded the product **25** as an off-white amorphous powder (774mg, 76%). To a suspension of **25** (774 mg, 0.161 mmol) in pyridine (10 mL) was added HF in pyridine (70% HF, 30% pyridine; 1.0 mL) dropwise. The mixture was stirred for 6 hours at 60 °C, after which it was quenched by solid $NaHCO_3$, till all CO_2 bubbling stopped. The salts were filtered off, the solvent was evaporated *in vacuo*, and the residue was re-dissolved in DCM, followed by successive washing with water and saturated $NaHCO_3$. The residue was passed through BioGel SX-1, providing intermediate compound **S6** (602 mg, 78%). R_f = 0.39 (Tol: EtOAc, 6: 4, v: v). ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.11 to 6.41 (104H, m, H-Ar), 5.78 (1H, dd, H-3 GalN, *J* = 11.5 Hz, 3.3 Hz), 5.42 (1H, m, H-3 GlcN-5'), 5.35 (3H, m, H-1 GlcN-2, H-1 GlcN-5, H-4 Man-8), 5.25 (1H, m, H-4 GlcN-5'), 5.05 to 4.71 (15H, m, H-4 GalN-7, H-4 Xyl, H-1 GlcN-5', H-1 GlcN-5", H-2 Xyl, H-3 Xyl, H-1 Man-4, H-1 Man-8, PhC$\underline{H}_2$ protons), 4.70 to 4.38 (19H, m, H-2 GalN-7, H-1 Xyl, H-1 GlcN-1, H-1 Fuc-6, H-1 GalN-7, PhC$\underline{H}_2$ protons), 4.38 to 4.20 (8H, m, H-3 GlcN-1, H-1 Man-4', H-1 Man-3, PhC$\underline{H}_2$ protons), 4.20 to 3.98 (16H, m, H-2 GlcN-5', H-2 GlcN-5, H-2 GlcN-2, H-2 GlcN-1, H-5 Man-8, H-3 GlcN-2, H-3 GlcN-5", H-3 GlcN-5, H-6a GalN-7, H-6a Man-8, H-6b Man-8, H-6a GlcN-5', H-3 Man-8, PhC$\underline{H}_2$ protons), 3.98 to 3.76 (13H, m, H-2 GlcN-5", H-6b GalN-7, H6b GlcN-5', H-5a Xyl, H-5 Fuc-6, H-5 GlcN-5', H-4 GlcN-1, H-4 GlcN-2, H-4 GlcN-5, H-4 GlcN-5", PhC$\underline{H}_2$ protons), 3.75 to 3.48 (8H, m, H-6a Man-4', H-6a GlcN-1, H-5 GalN-7, H-4 Man-4, H-3 Fuc-6, H-3 Man-3, H-3 Man-4', H-2 Fuc-6), 3.47 to 3.15 (15H, m, H-6b Man-4', H-2 Man-4', H-6a GlcN-2, H-6b GlcN-1, H-6a GlcN-5", H-6b GlcN-5", H-6a Man-4, H-6a GlcN-5, H-4 Fuc-6, H-2 Man-3, H-2 Man-4, H-5 Man-4', H-5 Man-4, H-4 Man-3, H-4 Man-4'), 3.11 (1H, m, H-5 GlcN-2), 2.98 (4H, m, H-6a Man-3, H-6b Man-3, H-5 GlcN-5", H-5 GlcN-1), 2.82 (1H, m, H-5b Xyl), 2.69 (2H, m, H-6b GlcN-2, H-6b Man-4), 2.58 (1H, m, H-6b GlcN-5), 2.47 (1H, m, H-5 Man-3), 2.09 to 1.65 (34H, m, 11x C$\underline{H}_3$ of Ac, H-5 GlcN-5), 0.94 (3H, d, C$\underline{H}_3$ of Fuc-6, *J* = 6.3 Hz); ^{13}C NMR (151 MHz, $CDCl_3$, signals from edited HSQCAD experiment): δ 123.94, 123.69, 135.04, 123.32, 133.66, 128.05, 123.25,

127.88, 128.32, 125.80, 128.08, 76.95, 127.97, 127.99, 127.84, 127.94, 127.63, 127.80, 127.84, 126.90, 67.87, 67.82, 70.64, 96.59, 66.67, 68.08, 71.86, 96.62, 68.74, 74.12, 97.64, 72.89, 73.18, 70.77, 98.39, 99.88, 95.02, 70.03, 74.75, 52.06, 101.50, 73.58, 72.91, 71.34, 101.63, 97.07, 73.21, 75.03, 55.74, 76.96, 76.84, 74.83, 62.85, 71.74, 61.02, 73.10, 62.88, 61.06, 79.40, 75.16, 73.16, 54.40, 71.98, 61.76, 65.98, 80.55, 74.80, 71.31, 76.15, 76.97, 70.57, 64.52, 64.44, 77.76, 66.71, 71.05, 68.70, 72.50, 74.07, 64.51, 79.58, 66.18, 68.01, 70.36, 61.73, 66.22, 30.95, 29.42, 20.76, 20.74, 20.86, 20.75, 20.66, 20.54, 73.39, 20.61, 30.33, 59.68, 30.28, 22.68, 31.50, 28.12, 29.70, 31.98, 31.27, 16.46, 14.17, 25.98, 0.03. MALDI-TOF-MS (*m/z*): $[M+ Na]^+$ calculated for $C_{267}H_{268}N_6O_{76}Na$, 4796.7188; found 4797.0277.



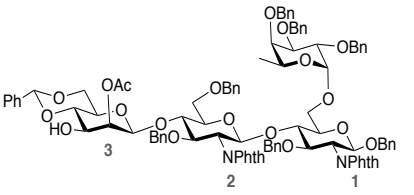
Scheme S2: Synthesis of core fucosylated hexasaccharide with orthogonal protecting groups.

***N*-phenyltrifluoracetimidate [2-*O*-acetyl-4,6-*O*-benzylidene-3-*O*-(2-methylnaphthyl)-β-D-mannopyranosyl]-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (S7):** Compound **10** (10.0 g, 9.4 mmol) was dissolved in pyridine (100 mL) followed by dropwise addition of HF in pyridine (70% HF, 30% pyridine; 15 mL). The mixture was stirred for 15 hours after which it was quenched by solid $NaHCO_3$ (50 g), till all CO_2 bubbling stopped. The salts were filtered off, the solvent was evaporated *in vacuo*, and the residue was re-dissolved in DCM (150 mL), followed by washing with water (100 mL) and saturated $NaHCO_3$ (150 mL). The organic phase was dried over $MgSO_4$, filtered, and the filtrate was concentrated, affording compound with silyl group removed as a white foamy solid, which was used for the next reaction without further purification. This crude compound (7.7 g, 8.4 mmol) was dissolved in DCM (100 mL), followed by the addition of 2,2,2-Trifluoro-*N*-phenylacetimidoyl chloride (2.1 mL, 10.1 mmol) and DBU (1.2 mL, 8.4 mmol). The reaction mixture was stirred for 30 minutes, after which the solvent was evaporated and the residue was purified using silica gel column chromatography (Tol to Tol: EtOAc, 8: 2, v: v), which provided the product as an off-white foamy powder. (9.1 g, 88.3% over two steps). R_f= 0.63 (Tol: EtOAc, 9: 1, v: v). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.98 to 6.48 (31H, m, H-Ar), 5.54 (1H, s, PhC<u>H</u> of benzylidene), 5.46 (1H, s, H-2 Man), 4.83 (2H, m, PhC<u>H</u>H, C<u>H</u>H of Nap), 4.67 (3H, m, H-1 Man, CH<u>H</u> of Nap, PhCH<u>H</u>), 4.40 (3H, m, PhC<u>H</u>$_2$, H-2 GlcN), 4.18 (3H, m, H-3 GlcN, H-4 GlcN, H-6b Man), 3.90 (1H, t, H-4 Man, *J* = 9.6 Hz), 3.73 (2H, bd, H-6a GlcN, H-6b GlcN), 3.58 (1H, m, H-6a Man), 3.48 (1H, m, H-3 Man), 3.13 (1H, m, H-5 Man), 2.18 (3H, s, C<u>H</u>$_3$ of Ac); ^{13}C NMR (101 MHz, $CDCl_3$): δ170.30, 167.60, 143.00, 138.35, 137.54, 137.51, 135.32, 134.02, 133.37, 133.05, 131.44, 129.09, 128.66, 128.61, 128.31, 128.21, 128.16, 128.13, 128.07, 128.02, 127.83, 127.70, 127.60, 127.37, 126.28, 126.19, 126.13, 125.95, 125.40, 124.51, 123.51, 119.37, 101.68, 99.30, 93.43, 78.18, 77.87, 77.34, 77.12, 76.91, 76.56, 75.67, 75.15, 74.71, 73.51, 71.48, 69.10, 68.48, 67.67, 67.02, 60.43, 54.80, 53.50, 21.11. Compound was unstable under MALDI-TOF-MS conditions.

Benzyl [2-*O*-acetyl-4,6-*O*-benzylidene-3-*O*-(2-methylnaphthyl)-β-D-mannopyranosyl]-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl-(1→6)-3-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (S8): Donor **S7** (7.7 g, 7.1 mmol) and Acceptor **12** (5.6 g, 6.1 mmol), were dissolved in DCM (50 mL) and stirred with pre-activated molecular sieves (12 g) for 20 minutes. The mixture was then cooled down to -40 ºC, followed by the addition of TMSOTf (386 µL, 2.1 mmol). The reaction mixture was warmed up to -20 ºC over a period of 45 minutes, after which it was quenched with Et_3N (1 mL). The

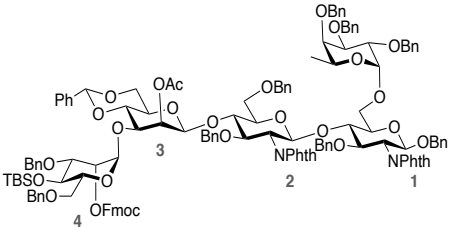
mixture was concentrated *in vacuo* to give the crude product as a brown syrup. Silica gel column chromatography with Tol: EtOAc (9.5: 0.5, v: v to 8: 2, v: v) yielded the product as white amorphous powder. (10.2 g, 91 %). R_f = 0.48 (Tol: EtOAc, 9: 1, v: v). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.01 to 6.63 (55H, m, H-Ar), 5.51 (3H, m, H-1 GlcN-2, PhC<u>H</u> of benzylidene, H-2 Man-3), 4.99 to 4.73 (9H, m, H-1 GlcN-1, H-1 Fuc, 3x PhC<u>H</u>$_2$ protons, PhC<u>H</u>H of Nap), 4.64 (4H, m, PhC<u>H</u>$_2$, PhCH<u>H</u> of Nap, H-1 Man-3), 4.51 (2H, m, PhC<u>H</u>$_2$), 4.40 (2H, m, PhC<u>H</u>$_2$), 4.30 (3H, m, H-3 GlcN-1, PhC<u>H</u>$_2$), 4.25 to 4.05 (6H, m, H-3 GlcN-2, H-2 GlcN-2, H-3 Fuc, H-6a Man-3, H-2 GlcN-1, H-4 GlcN-1), 4.00 (3H, m, H-5 Fuc, H-4 GlcN-2, H-2 Fuc), 3.84 (1H, t, H-4 Man, *J* = 9.5 Hz), 3.74 (3H, m, H-6a GlcN-2, H-6a GlcN-1, H-4 Fuc), 3.64 (2H, m, H-6b GlcN-1, H-5 GlcN-1), 3.50 (2H, m, H-6b Man-3, H-3 Man-3), 3.36 (1H, m, H-6b GlcN-2), 3.23 (1H, m, H-5 GlcN-2), 3.09 (1H, m, H-5 Man-3), 1.84 (3H, s, C<u>H</u>$_3$ of Ac), 0.97 (3H, d, C<u>H</u>$_3$ of Fuc, *J* = 6.5 Hz); ^{13}C NMR (101 MHz, $CDCl_3$): δ 169.99, 168.23, 167.65, 139.23, 138.96, 138.78, 138.72, 138.64, 137.76, 137.47, 137.04, 135.31, 133.48, 133.30, 132.96, 131.82, 131.66, 131.44, 129.03, 128.95, 128.49, 128.45, 128.42, 128.37, 128.21, 128.16, 128.12, 128.10, 128.07, 128.03, 127.96, 127.92, 127.77, 127.71, 127.66, 127.64, 127.61, 127.55, 127.48, 127.46, 127.34, 127.15, 126.84, 126.19, 126.11, 126.00, 125.82, 125.36, 125.29, 123.57, 123.15, 101.52, 99.35, 96.96, 96.75, 96.59, 79.69, 79.40, 77.78, 77.61, 77.34, 77.03, 76.91, 76.71, 76.09, 75.76, 75.22, 75.10, 74.71, 74.60, 74.35, 73.98, 73.62, 73.34, 73.16, 72.78, 71.33, 69.81, 68.87, 68.42, 68.01, 66.83, 65.96, 63.60, 56.46, 55.80, 20.45, 16.39, -0.00. MALDI-TOF-MS (*m/z*): $[M+ Na]^+$ calculated for $C_{109}H_{104}N_2O_{23}Na$, 1831.6928; found 1831.6942.

Benzyl [2-*O*-acetyl-4,6-*O*-benzylidene-β-D-mannopyranosyl]-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl-(1→6)-3-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (S9): Compound **S8** (10.1 g, 5.6 mmol) was dissolved in the solvent system DCM (200 mL) and H_2O (20 mL), followed by the addition of DDQ (2.6 g, 11.1 mmol) and β-pinene (3.5 mL, 22.1 mmol). The mixture was stirred in dark for 36 hours after which the solvent was evaporated *in vacuo* and the residue was diluted by DCM (300 mL) and washed with water (100 mL) and sat. $NaHCO_3$ (150 mL). The organic phase was dried over $MgSO_4$ and filtered, and the filtrate was concentrated *in vacuo*. Silica gel column chromatography using Tol: EtOAc (9: 1, v: v to 7: 3, v: v) gave the product as yellow amorphous solid. (6.2 g, 67%). R_f = 0.32 (Tol: EtOAc, 9: 1, v: v). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.05 to 6.54 (48H, m, H-Ar), 5.51(1H, d, H-1 GlcN-2, *J* = 8.2 Hz), 5.43 (1H, s, PhC<u>H</u> of benzylidene), 5.28 (1H, s, H-2 Man-3), 4.92 (2H, m, PhCH<u>H</u>, H-1 GlcN-1), 4.84 (6H, m, PhC<u>H</u>H, H-1 Fuc, 2x PhC<u>H</u>$_2$), 4.74 (2H, m, PhC<u>H</u>H, H-1 Man-3), 4.57 (4H, m, PhCH<u>H</u>, PhC<u>H</u>$_2$, PhCH<u>H</u>), 4.41 (2H, m, PhC<u>H</u>$_2$), 4.30 (3H, m, PhC<u>H</u>H, H-3 GlcN-2, H-3 GlcN-1), 4.22 to 4.05 (5H, m, H-2 GlcN-2, H-2



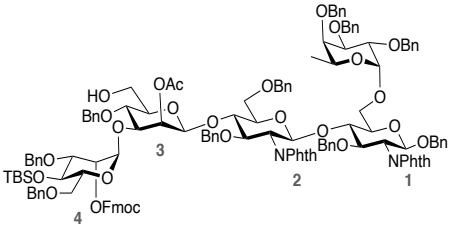
GlcN-1, H-6a Man-3, H-4 GlcN-1, H-3 Fuc), 3.98 (3H, m, H-5 Fuc, H-4 GlcN-2, H-2 Fuc), 3.81 to 3.60 (7H, m, H-6a GlcN-2, H-6a GlcN-1, H-6b GlcN-1, H-3 Man-3, H-4 Fuc, H-5 GlcN-1, H-4 Man-3), 3.49 (1H, t, H-6b Man-3, J = 10.1 Hz), 3.35 (1H, m, H-6b GlcN-2), 3.22 (1H, m, H-5 GlcN-2), 3.12 (1H, m, H-5 Man-3), 1.81 (3H, s, C$\underline{H}_3$ of Ac), 0.96 (3H, d, C$\underline{H}_3$ of Fuc, J = 6.5 Hz); ^{13}C NMR (101 MHz, $CDCl_3$): δ 170.29, 167.62, 139.20, 138.88, 138.76, 138.68, 138.64, 137.86, 137.03, 133.49, 131.66, 129.20, 129.01, 128.52, 128.47, 128.43, 128.41, 128.38, 128.28, 128.20, 128.09, 128.05, 127.91, 127.86, 127.77, 127.70, 127.67, 127.64, 127.53, 127.46, 127.43, 127.40, 127.36, 127.32, 127.14, 126.83, 126.20, 125.27, 123.57, 123.14, 101.99, 99.36, 96.90, 96.70, 96.59, 79.66, 78.49, 77.63, 77.33, 77.22, 77.01, 76.90, 76.70, 76.12, 75.19, 75.16, 74.69, 74.58, 74.35, 73.99, 73.62, 73.40, 73.27, 72.71, 71.13, 69.88, 69.80, 68.38, 67.95, 66.56, 65.95, 63.58, 56.50, 55.80, 20.37, 16.38. MALDI-TOF-MS (*m/z*): $[M+ Na]^+$ calculated for $C_{98}H_{96}N_2O_{23}Na$, 1691.6302; found 1691.6331.

Benzyl [3,6-di-*O*-benzyl-4-*O*-*t*-butyldimethylsilyl-2-*O*-fluorenylmethoxycarbonyl-α-D-mannopyranosyl]-(1→3)-[2-*O*-acetyl-4,6-*O*-benzylidene-β-D-mannopyranosyl]-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl-(1→6)-3-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (S10): A mixture of acceptor **S9** (4.9 g, 2.9 mmol) and donor **13** (2.8 g, 3.5 mmol), was stirred in DCM (30 mL) with pre-activated molecular sieves (8 g) for 20 minutes. The mixture was then cooled down to -25 ºC, followed by the sequential addition of NIS (983 mg, 4.3 mmol) and TMSOTf (160 µL, 0.88 mmol). The reaction mixture was warmed up to -5 ºC over a period of 25 minutes, after which it was quenched with pyridine (5 mL). The sieves were filtered off, the mixture was diluted by DCM (100 mL) and washed with 5% $Na_2S_2O_4$ (100 mL) and sat. $NaHCO_3$ (50 mL). The mixture was concentrated *in vacuo* to give the crude product as a brown syrup. Silica gel column chromatography with Tol: EtOAc (9: 1, v: v to 7: 3, v: v) yielded the product as an off-white amorphous powder. (5.2 g, 76 %). R_f = 0.63 (Tol: EtOAc, 8: 2, v: v). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.95 to 6.62 (66H, m, H-Ar), 5.43 (3H, m, H-1 GlcN-2, PhC$\underline{H}$ of benzylidene, H-2 Man-4), 5.30 (1H, d, H-1 Man-4, J = 1.3 Hz), 5.24 (1H, s, H-2 Man-3), 4.97 to 4.76 (10H, m, H-1 GlcN-1, H-1 Fuc, 4x PhC$\underline{H}_2$ protons), 4.71 (1H, m, PhC$\underline{H}$H), 4.76 to 4.46 (H-1 Man-3, PhC$\underline{H}_2$ protons, C$\underline{H}_2$ of Fmoc), 4.37 (4H, m, PhC$\underline{H}_2$ protons, H-3 GlcN-1), 4.27 (3H, m, H-3 GlcN-2, PhCH$\underline{H}$), 4.18 (5H, m, C$\underline{H}$ of Fmoc, H-2 GlcN-1, H-2 GlcN-2, H-4 GlcN-1, H-3 Fuc), 4.13 to 3.86 (8H, m, H-5 Fuc, H-6a Man-3, H-4 Man-4, H-4 GlcN-2, H-2 Fuc, H-5 Man-4, H-3 Man-3), 3.77 (5H, m, H-6a GlcN-2, H-6a GlcN-1, H-6a Man-4, H-6b Man-4, H-3 Man-4), 3.59 (4H, m, H-6b GlcN-1, H-4 Fuc, H-4 Man-3, H-5 GlcN-1), 3.44 (1H, m, H-6b Man-3), 3.31 (1H, m, H-6b GlcN-2), 3.22 (1H, m, H-5 GlcN-2), 3.01



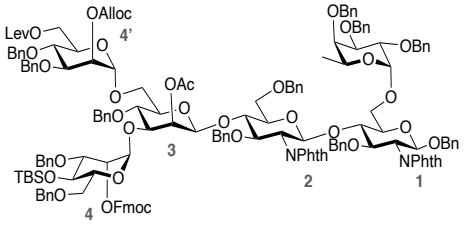
(1H, m, H-5 Man-3), 1.82 (3H, s, C<u>H</u>$_3$ of Ac), 0.95 (3H, d, C<u>H</u>$_3$ of Fuc, J = 6.5 Hz), 0.84 (9H, s, 3x C-C<u>H</u>$_3$ of TBS), 0.04 to -0.03 (6H, d, 2x C<u>H</u>$_3$-Si of TBS); ^{13}C NMR (101 MHz, $CDCl_3$): δ 168.49, 166.94, 153.81, 142.86, 142.55, 140.51, 140.48, 138.48, 138.21, 138.10, 138.04, 137.97, 137.78, 137.18, 137.15, 136.39, 136.36, 133.13, 132.78, 130.98, 130.74, 128.06, 127.85, 127.79, 127.75, 127.70, 127.63, 127.52, 127.42, 127.36, 127.29, 127.25, 127.12, 127.07, 127.03, 126.89, 126.85, 126.77, 126.74, 126.71, 126.63, 126.60, 126.55, 126.49, 126.44, 126.13, 125.22, 124.63, 124.59, 122.89, 122.45, 119.25, 100.50, 98.47, 97.44, 96.21, 95.91, 78.72, 78.16, 78.06, 77.07, 77.02, 76.66, 76.54, 76.34, 76.20, 76.02, 75.58, 74.83, 74.68, 74.02, 73.79, 73.38, 73.01, 72.93, 72.64, 72.56, 72.37, 72.20, 72.04, 71.03, 70.37, 70.18, 69.82, 69.51, 69.11, 68.67, 67.64, 67.05, 66.86, 65.59, 65.26, 62.98, 55.84, 55.10, 45.79, 25.29, 25.19, 19.66, 17.51, 15.70, -4.56, -5.59. MALDI-TOF-MS (*m/z*): [M+ Na]$^+$ calculated for $C_{139}H_{142}N_2O_{30}SiNa$, 2369.9314; found 2369.9405.

Benzyl [3,6-di-*O*-benzyl-4-*O*-*t*-butyldimethylsilyl-2-*O*-fluorenylmethoxycarbonyl-α-D-mannopyranosyl]-(1→3)-[2-*O*-acetyl-4-*O*-benzyl-β-D-mannopyranosyl]-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl-(1→6)-3-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (S11): Compound **S10** (4.5 g, 1.9 mmol) was dissolved in DCM (100 mL) and stirred with pre-activated molecular sieves (20 g) for 30 minutes, after which the temperature was brought down to -70 °C. The mixture was stirred at this temperature for another 30 minutes after which Et_3SiH (611 µL, 3.8 mmol) and $PhBCl_2$ (743 µL, 5.7 mmol). The mixture was stirred at this temperature for 20 minutes after which it was quenched with pyridine (10 mL). The mixture was further diluted by DCM (200 mL), the sieves were filtered off, and the mixture was washed with sat. $NaHCO_3$ (150 mL). The organic phase was dried over $MgSO_4$ and filtered, and the filtrate was concentrated *in vacuo*. Silica gel column chromatography using Tol: EtOAc (9: 1, v: v to 6: 4, v: v) gave the product as yellow amorphous powder. (3.6 g, 79%). R_f = 0.35 (Tol: EtOAc, 8: 2, v: v). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.88 to 6.63 (66H, m, H-Ar), 5.42 (2H, m H-2 Man-4, H-1 GlcN-2), 5.25 (1H, s, H-1 Man-4), 5.21 (1H, s, H-2 Man-3), 4.99 to 4.78 (8H, m, H-1 GlcN-1, H-1 Fuc, $PhCH_2$ protons), 4.70 (3H, m, PhC<u>H</u>H, PhC<u>H</u>$_2$), 4.65 to 4.36 (12H, m, PhC<u>H</u>H, PhC<u>H</u>$_2$ protons, C<u>H</u>$_2$ of Fmoc), 4.29 (5H, m, H-3 GlcN-1, $PhCH_2$ protons), 4.18 (4H, m, C<u>H</u> of Fmoc, H-2 GlcN-2, H-2 GlcN-1, H-3 GlcN-2), 4.09 (4H, m, H-4 Man-4, H-4 GlcN-1, H-3 Fuc, H-4 GlcN-2), 3.97 (3H, m, H-5 Fuc, H-5 Man-4, H-2 Fuc), 3.76 (3H, m, H-6a Man-4, H-6b Man-4, H-3 Man-4), 3.72 to 3.56 (7H, m, H-6a GlcN-2, H-6a GlcN-1, H-6a Man-3, H-4 Fuc, H-4 Man-3, H-5 GlcN-1), 3.51 (2H, m, H-6b Man-3, H-3 Man-3), 3.39 (1H, m, H-6b GlcN-2), 3.27 (2H, m, H-6b GlcN-1, H-5 GlcN-2), 2.97 (1H, m, H-5 Man-3),

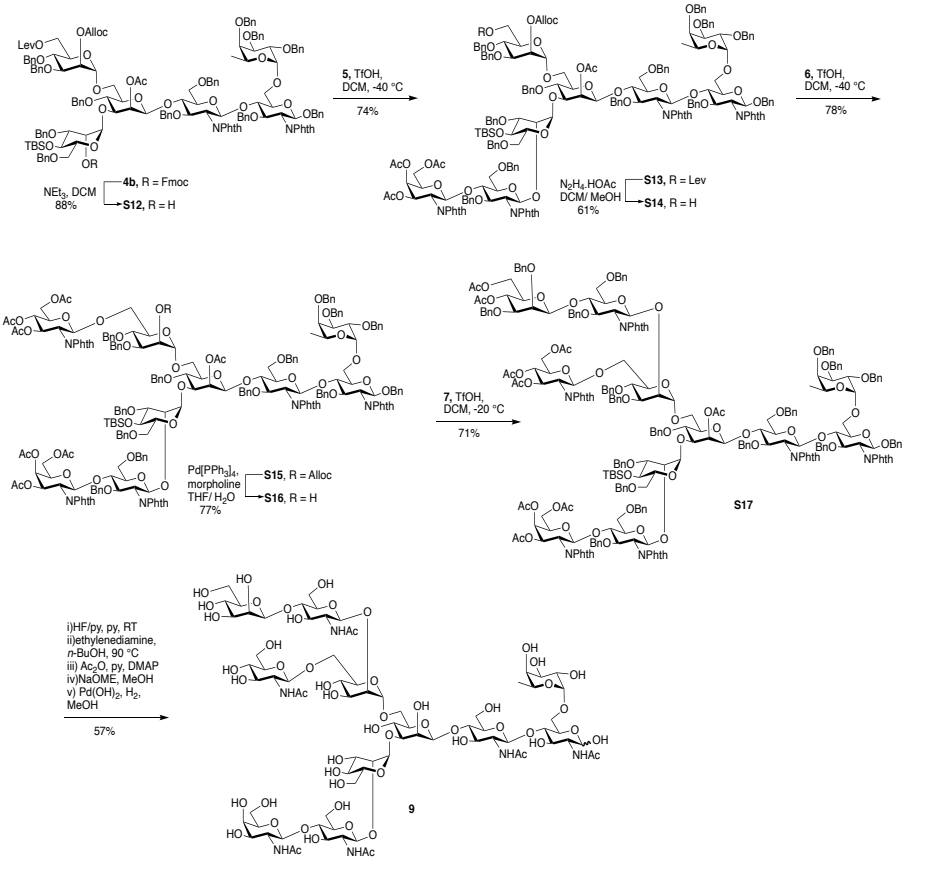


1.88 (3H, s, C<u>H</u>$_3$ of Ac), 0.96 (3H, d, C<u>H</u>$_3$ of Fuc, J = 6.3 Hz), 0.86 (9H, s, 3x C-C<u>H</u>$_3$ of TBS), 0.03 to -0.01 (6H, d, 2x C<u>H</u>$_3$-Si of TBS); ^{13}C NMR (101 MHz, $CDCl_3$): δ 169.36, 167.92, 154.82, 143.76, 143.55, 141.47, 139.44, 139.21, 139.08, 138.95, 138.80, 138.08, 137.94, 137.33, 134.28, 133.70, 131.96, 129.83, 128.79, 128.70, 128.64, 128.61, 128.59, 128.45, 128.38, 128.32, 128.26, 128.09, 128.00, 127.97, 127.80, 127.72, 127.65, 127.59, 127.54, 127.50, 127.46, 127.05, 125.58, 123.37, 120.22, 115.61, 99.37, 98.45, 97.32, 97.17, 96.89, 79.60, 78.36, 78.07, 77.93, 77.62, 77.50, 77.30, 76.98, 76.77, 76.08, 75.88, 75.43, 75.33, 75.27, 75.00, 74.89, 74.60, 74.37, 74.03, 73.60, 73.52, 73.36, 73.03, 72.39, 71.38, 71.04, 70.54, 70.10, 69.49, 68.00, 67.68, 66.24, 64.00, 61.81, 56.78, 56.08, 46.78, 26.22, 20.80, 18.44, 16.67, -3.58, -4.70. MALDI-TOF-MS (*m/z*): [M+ Na]$^+$ calculated for $C_{139}H_{144}N_2O_{30}SiNa$, 2371.9471; found 2371.9453.

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-*O*-acetyl-4-*O*-benzyl-β-D-mannopyranosyl]-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl-(1→6)-3-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (4b): A mixture of acceptor **S11** (3.6 g, 1.5 mmol) and donor **14** (4.3 g, 6.0 mmol), was stirred in DCM (50 mL) with pre-activated molecular sieves (10 g) for 20 minutes. The mixture was then cooled down to -45 ºC, followed by the addition of TMSOTf (108 µL, 0.60 mmol). The reaction mixture was warmed up to -25 ºC over a period of 30 minutes, after which it was quenched with pyridine (2 mL). The sieves were filtered off and the mixture was concentrated *in vacuo.* The residue was purified by silica gel column chromatography using DCM to DCM: Acetone (95: 5, v: v) which yielded the product as a white amorphous powder. (2.9 g, 67 %). R_f = 0.62 (DCM: Acetone, 9.7: 0.3, v: v). ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.85 to 6.59 (76H, m, H-Ar), 5.66 (1H, m, 5.78, C<u>H</u>=CH$_2$ of alloc), 5.44 (1H, d, H-2 Man-4', J = 2.4 Hz), 5.33 (1H, d, H-1 GlcN-2, J = 8.3 Hz), 5.27 (1H, m, H-2 Man-4), 5.23 (1H, d, H-1 Man-4', J = 1.4 Hz), 5.13 (3H, m, H-2 Man-3, CH=C<u>H</u>$_2$ of alloc), 5.04 (1H, d, H-1 Man-4, J = 1.6 Hz), 4.91 (3H, m, H-1 GlcN-1, PhC<u>H</u>$_2$), 4.83 (7H, m, H-1 Fuc, PhC<u>H</u>$_2$ protons, C<u>H</u>$_2$ of Fmoc), 4.72 (2H, m, PhC<u>H</u>$_2$ protons), 4.65 (4H, m, H-1 Man-3, PhC<u>H</u>$_2$ protons) 4.62 to 4.51 (6H, m, PhC<u>H</u>$_2$ protons, OC<u>H</u>$_2$ of alloc), 4.50 to 4.24 (11H, m, PhC<u>H</u>$_2$ protons), 4.20 (2H, m, C<u>H</u> of Fmoc, PhCH<u>H</u>), 4.17 to 4.04 (10H, m, H-2 GlcN-1, H-2 GlcN-2, H-6a Man-4', H-6b Man-4', H-4 Man-4, PhCH<u>H</u>, H-3 GlcN-1, H-3 GlcN-2, H-3 Fuc, H-3 Man-4'), 3.98 (2H, m, H-4 GlcN-1, H-4 GlcN-2), 3.91 (2H, m, H-5 Fuc, H-2 Fuc), 3.85 (1H, dd, H-4 Man-3, J = 8.9 Hz, 3.1 Hz), 3.81 to 3.69 (8H, m, H-6a Man-4, H-6b Man-4, H-6a Man-3, H-6b Man-3, H-5 Man-4, H-3 Man-4, H-6a GlcN-1, H-5 Man-4'), 3.67 to 3.58 (3H, m, H-6a GlcN-2, H-3 Man-3, H-4 Man-4'), 3.55 (2H, m, H-6b GlcN-1, H-5 GlcN-1), 3.42 (1H, m, H-4 Fuc), 3.20 (2H, m, H-6b GlcN-2,

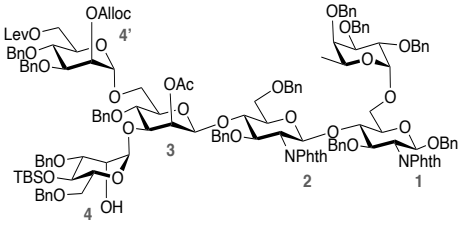


H-5 GlcN-2), 3.03 (1H, m, H-5 Man-3), 2.62 (2H, t, $COOCH_2CH_2$ of Lev, J = 6.6 Hz), 2.46 ((2H, t, $COOCH_2CH_2$ of Lev, J = 6.6 Hz), 2.09 (3H, s, CH_2COCH_3 of Lev), 1.96 (3H, s, CH_3 of Ac), 0.93 (3H, d, CH_3 of Fuc, J = 6.5 Hz), 0.86 (9H, s, 3x C-CH_3 of TBS), 0.04 to -0.01 (6H, d, 2x CH_3-Si of TBS); ^{13}C NMR (151 MHz, $CDCl_3$): δ 207.01, 206.54, 172.34, 169.46, 167.75, 167.55, 154.61, 154.20, 143.56, 143.31, 141.24, 139.17, 139.03, 138.86, 138.71, 138.55, 138.08, 137.96, 137.89, 137.69, 137.09, 133.72, 133.49, 131.40, 129.06, 128.54, 128.48, 128.46, 128.40, 128.35, 128.30, 128.25, 128.19, 128.14, 128.10, 128.08, 128.01, 128.00, 127.96, 127.87, 127.76, 127.73, 127.71, 127.65, 127.61, 127.56, 127.47, 127.42, 127.38, 127.33, 127.28, 127.24, 126.78, 125.38, 123.44, 123.21, 119.99, 118.74, 99.27, 99.22, 97.75, 96.92, 96.82, 96.57, 79.45, 79.24, 78.14, 78.00, 77.85, 77.50, 77.26, 77.05, 76.84, 76.62, 75.77, 75.71, 75.24, 74.95, 74.74, 74.63, 74.13, 73.90, 73.86, 73.81, 73.41, 73.31, 72.93, 72.66, 72.04, 71.90, 71.44, 71.09, 71.03, 70.33, 69.97, 69.80, 69.24, 68.48, 67.85, 67.40, 65.92, 65.50, 63.74, 63.16, 56.60, 55.78, 53.45, 46.53, 37.91, 30.96, 29.83, 27.94, 25.99, 20.59, 18.22, 16.41, 0.02, -3.83, -4.97. MALDI-TOF-MS (*m/z*): $[M+Na]^+$ calculated for $C_{168}H_{176}N_2O_{39}SiNa$, 2896.1517; found 2896.1744.

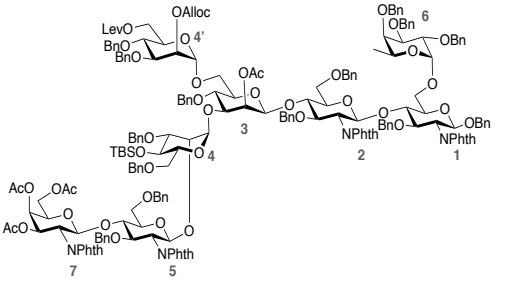


Scheme S3: Synthetic Scheme for extension of core hexasaccharide **4b.**

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-*O*-acetyl-4-*O*-benzyl-β-D-mannopyranosyl]-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl-(1→6)-3-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (S12): Compound **4b** (2.9 g, 1.1 mmol) was dissolved in DCM (100 mL) followed by the addition of Et_3N (5 mL). The mixture was stirred for 3 hours after which it was concentrated *in vacuo*. The residue was purified by silica gel column chromatography using solvent gradient DCM to DCM: Acetone (9.5: 0.5, v: v) which afforded the product as an off-white amorphous powder. (2.6 g, 88%). R_f = 0.54 (DCM: Acetone, 9.7: 0.3, v: v). ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.91 to 6.50 (68H, m, H-Ar), 5.70 (1H, m, C<u>H</u>=CH_2 of alloc), 5.43 (1H, d, H-2 Man-4', *J* = 3.1 Hz), 5.36 (1H, d, H-1 GlcN-2, *J* = 8.2 Hz), 5.15 (4H, m, CH=C<u>H</u>$_2$ of alloc, H-2 Man-3, H-1 Man-4'), 5.05 (1H, d, H-1 Man-4, *J* = 1.3 Hz), 4.94 (3H, m, H-1 GlcN-1, PhC<u>H</u>$_2$ protons), 4.89 to 4.69 (9H, H-1 Fuc, PhC<u>H</u>$_2$ protons), 4.66 (1H, H-1 Man-3), 4.63 to 4.25 (20H, m, PhC<u>H</u>$_2$ protons, OC<u>H</u>$_2$ of alloc)), 4.20 to 4.06 (8H, m, H-2 GlcN-2, H-2 GlcN-1, H-6a Man-4', H-6b Man-4', H-3 GlcN-1, H-3 GlcN-2, H-3 Man-4', H-3 Fuc), 4.0 (2H, m, H-4 Man-4, H-4 GlcN-1), 3.96 to 3.81 (5H, m, H-5 Fuc, H-2 Man-4, H-4 GlcN-2, H-2 Fuc, H-4 Man-3), 3.79 to 3.62 (9H, m, H-6a Man-3, H-6b Man-3, H-6a GlcN-1, H-6a Man-4, H-6b Man-4, H-5 Man-4', H-5 Man-4, H-4 Man-4', H-3 Man-4), 3.57 (4H, m, H-6a GlcN-2, H-6b GlcN-1, H-3 Man-3, H-5 GlcN-1), 3.47 (1H, d, H-4 Fuc), 3.23 (2H, m, H-6b GlcN-2, H-5 GlcN-2), 3.07 (1H, m, H-5 Man-3), 2.64 (2H, t, COOC<u>H</u>$_2$CH$_2$ of Lev, *J* = 6.6 Hz), 2.49 (2H, t, COOCH$_2$C<u>H</u>$_2$ of Lev, *J* = 6.6 Hz), 2.12 (3H, s, CH2COC<u>H</u>3 of Lev), 1.94 (3H, s, C<u>H</u>$_3$ of Ac), 0.96 (3H, d, C<u>H</u>$_3$ of Fuc, *J* = 6.6 Hz), 0.89 (9H, s, 3x C-C<u>H</u>$_3$ of TBS), 0.05 to 0.03 (6H, d, 2x C<u>H</u>$_3$-Si of TBS); ^{13}C NMR (151 MHz, $CDCl_3$): δ 206.47, 172.31, 169.35, 167.76, 167.64, 167.52, 154.22, 139.17, 139.02, 138.97, 138.86, 138.74, 138.71, 138.60, 138.54, 138.47, 138.09, 137.98, 137.94, 137.79, 137.73, 137.10, 133.68, 133.45, 131.81, 131.69, 131.51, 131.41, 131.39, 129.04, 128.69, 128.52, 128.45, 128.43, 128.38, 128.35, 128.33, 128.29, 128.28, 128.26, 128.23, 128.17, 128.15, 128.12, 128.09, 128.05, 127.98, 127.92, 127.83, 127.79, 127.77, 127.74, 127.71, 127.68, 127.65, 127.63, 127.57, 127.54, 127.48, 127.46, 127.44, 127.41, 127.39, 127.34, 127.32, 127.18, 127.13, 126.77, 125.31, 123.41, 123.12, 118.76, 118.33, 101.53, 99.45, 97.70, 96.90, 96.87, 96.59, 80.36, 79.67, 79.26, 78.98, 77.88, 77.70, 77.38, 77.26, 77.06, 76.74, 76.60, 75.90, 75.73, 75.60, 75.25, 74.95, 74.72, 74.69, 74.57, 74.20, 73.97, 73.86, 73.82, 73.40, 73.30, 73.30, 73.16, 72.94, 72.66, 71.91, 71.44, 71.23, 69.97, 69.79, 69.10, 68.74, 68.50, 67.86, 66.87, 65.92, 65.65, 63.81, 63.13, 56.60, 55.78, 53.81, 53.45, 37.89, 30.92, 29.80, 29.29, 27.93, 25.98, 25.96, 21.46, 20.55, 18.16, 16.39, 0.01, -3.80. MALDI-TOF-MS (*m/z*): $[M+ Na]^+$ calculated for $C_{153}H_{166}N_2O_{37}SiNa$, 2674.0836; found 2674.1021.

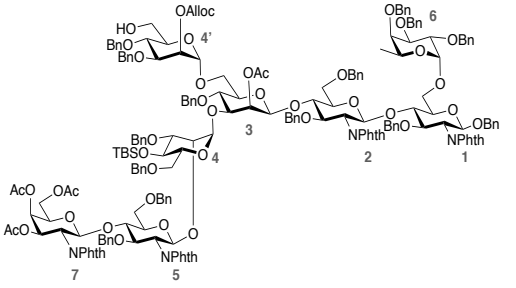


Benzyl [2-*O*-allyloxycarbonyl-3,4-di-*O*-benzyl-6-*O*-levulenoyl-α-D-mannopyranosyl]-(1→6)-[3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-galactopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl]-(1→2)-[3,6-di-*O*-benzyl-4-*O*-*t*-butyldimethylsilyl-α-D-mannopyranosyl]-(1→3)-[2-*O*-acetyl-4-*O*-benzyl-β-D-mannopyranosyl]-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl-(1→6)-3-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (S13): A mixture of acceptor **S12** (2.5 g, 0.94 mmol) and imidate donor **5** (3.5 g, 3.3 mmol), was stirred in DCM (20 mL) with pre-activated molecular sieves (5 g) for 20 minutes, after which the mixture was cooled down to -40 ºC, followed by the addition of TfOH (30 µL, 0.33 mmol). The mixture was warmed up to -20 ºC over a period of 30 minutes, after which it was quenched with Et_3N (200 µL). The sieves were filtered off and the mixture was concentrated *in vacuo.* The residue was purified by silica gel column chromatography using Tol: EtOAc (9: 1, v: v to 1:1, v: v) which afforded the product as an off-white amorphous powder. (2.5 g, 74%). R_f = 0.39 (Tol: EtOAc, 7: 3, v: v). ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.16 to 6.49 (86H, m, H-Ar), 5.73 (1H, d, H-3 GalN-7, *J* = 11.3 Hz), 5.66 (1H, m, C<u>H</u>=CH$_2$ of alloc), 5.37 (1H, d, H-1 GlcN-2, *J* = 8.2 Hz), 5.31 (2H, m, H-4 GalN-7, H-1 Man-4'), 5.22 (1H, d, H-1 Man-4', *J* = 7.1 Hz), 5.13 (3H, m, H-2 Man-4', CH=C<u>H</u>$_2$ of alloc), 5.02 (1H, s, H-2 Man-3), 4.92 to 4.76 (11H, m, H-1 Man-4, H-1 GlcN-5, H-1 GalN-7, H-1 GlcN-1, PhC<u>H</u>$_2$ protons), 4.70 (3H, m, H-1 Fuc-6, PhC<u>H</u>$_2$ protons), 4.65 to 4.54 (5H, m, PhC<u>H</u>$_2$ protons), 4.51 (5H, m, H-2 GalN-7, PhC<u>H</u>$_2$ protons), 4.46 to 4.19 (13H, m, H-1 Man-3, PhC<u>H</u>$_2$ protons, OC<u>H</u>$_2$ of alloc), 4.17 to 3.98 (14H, m, H-2 GlcN-2, H-2 GlcN-1, H-2 GlcN-5, H-6a GalN-7, H-6a Man-4', H-6b Man-4', H-3 GlcN-1, H-3 GlcN-2, H-3 GlcN-5, PhC<u>H</u>$_2$ protons), 3.98 to 3.87 (6H, m, H-6b GalN-7, H-4 GlcN-1, H-4 GlcN-2, H-3 Fuc, H-2 Fuc, PhC<u>H</u>$_2$ protons), 3.81 (3H, m, H-5 Fuc-6, H-2 Man-4, H-4 Man-3), 3.73 to 3.49 (9H, m, H-6a GlcN-2, H-6a Man-3, H-6b Man-3, H-5 Man-4, H-4 Man-4', H-5 GalN-7, H-4 GlcN-5, H-3 Man-4', H-3 Man-4), 3.45 (3H, m, H-4 Man-4, H-6a Man-4, H-3 Man-3), 3.36 (2H, m, H-6a GlcN-5, H-5 GlcN-1), 3.31 (1H, m, H-6b Man-4), 3.18 (3H, m, H-6b GlcN-2, H-4 Fuc-6, H-5 GlcN-2), 3.04 (2H, m, H-6a GlcN-1, H-6b GlcN-1), 2.63 (2H, t, COOC<u>H</u>$_2$CH$_2$ of Lev, *J* = 6.6 Hz), 2.58 (2H, m, H-6b GlcN-5, H-5 Man-3), 2.46 (2H, t, COOCH$_2$C<u>H</u>$_2$ of Lev, *J* = 6.6 Hz), 2.13 (3H, s, CH2COC<u>H</u>3 of Lev), 2.08 to 1.81 (13H, m, 4x C<u>H</u>$_3$ of Ac, H-5 GlcN-5), 0.91 (3H, d, C<u>H</u>$_3$ of Fuc, *J* = 6.4 Hz), 0.75 (9H, s, 3x C-C<u>H</u>$_3$ of TBS), -0.11 to -0.19 (6H, d, 2x C<u>H</u>$_3$-Si of TBS); ^{13}C NMR (151 MHz, $CDCl_3$, signals from edited HSQCAD experiment): δ 123.87, 123.63, 134.73, 123.35, 123.23, 133.55, 123.34, 133.58, 133.53, 128.02, 123.19, 128.04, 128.24, 126.08, 77.00, 125.86, 125.71, 128.02, 128.02, 124.12, 125.66, 127.50, 125.91, 125.57, 127.95, 125.83, 127.72, 128.08, 127.84, 67.89, 131.47, 96.85, 96.75, 66.70, 53.72, 97.28, 118.80, 118.78, 70.99, 118.77, 71.74, 74.79, 74.81, 97.28, 74.33, 73.07, 96.79, 75.27, 72.70, 73.38, 95.23, 73.94, 73.94, 74.78, 74.82,



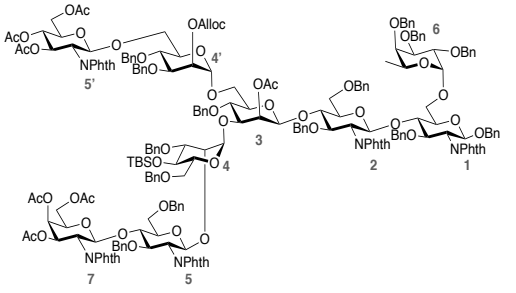
69.87, 73.70, 73.72, 52.10, 75.28, 71.42, 74.23, 74.54, 72.77, 98.70, 69.89, 69.92, 55.60, 75.55, 63.10, 72.79, 55.89, 77.10, 68.47, 56.62, 76.55, 72.90, 60.94, 60.90, 75.82, 72.78, 79.08, 65.93, 70.98, 73.75, 77.88, 73.36, 70.50, 73.80, 69.98, 63.79, 65.86, 77.97, 68.10, 67.36, 74.59, 70.61, 76.78, 68.06, 73.86, 63.77, 68.27, 68.26, 28.01, 37.99, 74.03, 70.61, 38.09, 28.03, 29.92, 28.40, 19.17, 20.72, 73.54, 20.54, 19.02, 28.62, 40.99, 30.42, 31.51, 29.74, 31.98, 23.94, 20.90, 20.66, 14.47, 16.48, 14.25, 0.08, -3.64, -6.64, -5.12. MALDI-TOF-MS (*m/z*): $[M+ Na]^+$ calculated for $C_{201}H_{210}N_4O_{52}SiNa$, 3562.3578; found 3562.3604.

Benzyl [2-*O*-allyloxycarbonyl-3,4-di-*O*-benzyl-α-D-mannopyranosyl]-(1→6)-[3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-galactopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl]-(1→2)-[3,6-di-*O*-benzyl-4-*O*-*t*-butyldimethylsilyl-α-D-mannopyranosyl]-(1→3)-[2-*O*-acetyl-4-*O*-benzyl-β-D-mannopyranosyl]-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl-(1→6)-3-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (S14): Compound **S13** (2.3 g, 0.65 mmol) was dissolved in DCM (20 mL) and MeOH (10 mL), followed by the addition of solid hydrazine acetate (299 mg, 3.25 mmol). The mixture was stirred for 1 hour after which it was concentrated *in vacuo* and the residue was purified by silica gel column chromatography using Pet. Ether: EtOAc (8: 2, v: v to 1:1, v: v) which afforded the product as a white foamy solid. (1.4 g, 61%). R_f = 0.34 (Pet. Ether: EtOAc, 1:1, v: v). ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.25 to 6.72 (86H, m, H-Ar), 5.93 (1H, d, H-3 GalN-7, *J* = 11.2 Hz), 5.77 (1H, m, C<u>H</u>=CH$_2$ of alloc), 5.56 (1H, d, H-1 GlcN-2, *J* = 8.3 Hz), 5.52 (1H, s, H-4 GalN-7), 5.40 (1H, d, Man-4', *J* = 6.8 Hz), 5.27 (5H, m, H-2 Man-4', H-2 Man-3, H-1 Man-4, CH=C<u>H</u>$_2$ of alloc), 5.14 to 4.96 (10H, m, H-1 GalN-7, H-1 GlcN-5, H-1 GlcN-1, PhC<u>H</u>$_2$ protons), 4.87 (4H, m, H-1 Fuc-6, PhC<u>H</u>$_2$ protons), 4.79 to 4.37 (18H, m, H-2 GalN-7, H-1 Man-3, PhC<u>H</u>$_2$ protons, OC<u>H</u>$_2$ of alloc), 4.36 to 4.07 (19H, m, H-2 GlcN-1, H-2 GlcN-2, H-2 GlcN-5, H-6a GalN-7, H-6b GalN-7, H-3 GlcN-1, H-3 GlcN-2, H-3 GlcN-5, H-4 GlcN-2, H-4 GlcN-1, H-3 Fuc-6, H-2 Fuc-6), 4.00 (3H, m, H-5 Fuc-6, H-2 Man-4, H-4 Man-3), 3.93 to 3.76 (6H, m, H-6a Man-4', H-6b Man-4', H-6a Man-3, H-5 GalN-7, H-5 Man-4, H-4 Man-4'), 3.75 to 3.58 (7H, m, H-6a GlcN-2, H-6b Man-3, H-4 Man-4, H-6a Man-4, H-3 Man-3, H-5 Man-4', H-3 Man-4), 3.54 (2H, m, H-6a GlcN-5, H-5 GlcN-1), 3.44 (1H, m, H-6b Man-4), 3.38 (1H, m, H-4 Fuc), 3.30 (2H, m, H-6b GlcN-2, H-5 GlcN-2), 3.24 (2H, m, H-6a GlcN-1, H-6b GlcN-1), 2.74 (2H, m, H-6b GlcN-5, H-5 Man-3), 2.27 to 1.98 (13H, m, 4x C<u>H</u>$_3$ of Ac, H-5 GlcN-5), 1.11 (3H, d, C<u>H</u>$_3$ of Fuc, *J* = 6.4 Hz), 0.93 (9H, s, 3x C-C<u>H</u>$_3$ of TBS), 0.01 to -0.01 (6H, d, 2x C<u>H</u>$_3$-Si of TBS); ^{13}C NMR



(151 MHz, $CDCl_3$, signals from edited HSQCAD experiment): δ 123.89, 123.65, 134.71, 123.50, 123.19, 123.25, 123.37, 133.59, 133.55, 133.59, 127.93, 126.33, 123.20, 127.92, 126.30, 128.29, 128.38, 128.24, 126.12, 76.96, 127.93, 125.77, 125.65, 127.90, 124.15, 127.49, 125.92, 128.04, 125.53, 127.85, 125.83, 127.45, 128.06, 127.90, 67.94, 131.36, 96.79, 66.65, 53.74, 97.24, 70.94, 118.79, 118.77, 118.76, 72.08, 97.30, 74.81, 96.60, 97.88, 73.34, 96.67, 75.34, 73.07, 95.18, 73.90, 70.05, 74.84, 75.21, 69.84, 71.58, 73.71, 52.10, 73.69, 72.83, 98.63, 69.93, 98.33, 55.56, 60.41, 75.58, 72.80, 55.79, 77.10, 76.64, 56.52, 68.44, 60.88, 72.97, 60.88, 75.93, 78.31, 72.80, 79.01, 65.94, 71.01, 74.16, 77.84, 77.78, 73.86, 61.90, 70.54, 64.68, 61.91, 63.65, 77.93, 72.27, 74.30, 68.04, 64.74, 67.29, 74.46, 70.64, 76.71, 74.33, 67.98, 73.88, 73.62, 63.62, 68.24, 70.62, 74.55, 20.79, 19.21, 33.91, 19.26, 73.51, 20.50, 18.96, 28.53, 40.99, 30.34, 29.00, 17.36, 30.33, 22.77, 31.39, 29.69, 28.12, 31.98, 14.24, 23.92, 20.88, 16.43, 17.46, 14.22, 7.98, 19.78, 24.43, 25.96, -3.67, -6.69, -5.16. MALDI-TOF-MS (*m/z*): $[M+Na]^+$ calculated for $C_{196}H_{204}N_4O_{50}SiNa$, 3464.3210; found 3464.3224.

Benzyl [3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→6)-2-*O*-allyloxycarbonyl-3,4-di-*O*-benzyl-α-D-mannopyranosyl]-(1→6)-[3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-galactopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→2)-3,6-di-*O*-benzyl-4-*O*-*t*-butyldimethylsilyl-α-D-mannopyranosyl]-(1→3)-[2-*O*-acetyl-4-*O*-benzyl-β-D-mannopyranosyl]-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl-(1→6)-3-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (S15): A mixture of acceptor **S14** (1.4 g, 0.41 mmol) and donor **6** (986 mg, 1.6 mmol), was stirred in DCM (10 mL) with pre-activated molecular sieves (2 g) for 10 minutes, after which the mixture was cooled down to -40 ºC, followed by the addition of TfOH (15 µL, 0.16 mmol). The mixture was warmed up to -20 ºC over a period of 30 minutes, after which it was quenched with Et_3N (150 µL). The sieves were filtered off and the mixture was concentrated *in vacuo.* The residue was purified by silica gel column chromatography using Tol: EtOAc (9: 1, v: v to 6: 4, v: v) and then passed through BioGel SX-1 column using Tol: Acetone (1: 1, v: v) as mobile phase, which afforded the product as an off-white amorphous powder. (1.25 g, 78%). R_f = 0.37 (Tol: EtOAc, 7: 3, v: v). ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.09 to 6.53 (90H, m, H-Ar), 5.81 (1H, t, H-3 GlcN-5', *J* = 9.7 Hz), 5.74 (1H, d, H-3 GalN-7, *J* = 11.3 Hz), 5.62 (1H, m, C<u>H</u>=CH_2 of alloc), 5.38 (1H, d, H-1 GlcN-2, *J* = 8.1 Hz), 5.31 (2H, m, H-4 GalN-7, H-1 Man-4'), 5.19 (1H, d, H-1 GlcN-5, *J* = 7.7 Hz), 5.13 (4H, m, H-2 Man-4', H-4 GlcN-5', CH=C<u>H</u>$_2$ of alloc), 4.98 to 4.78 (9H, m, H-2 Man-3,

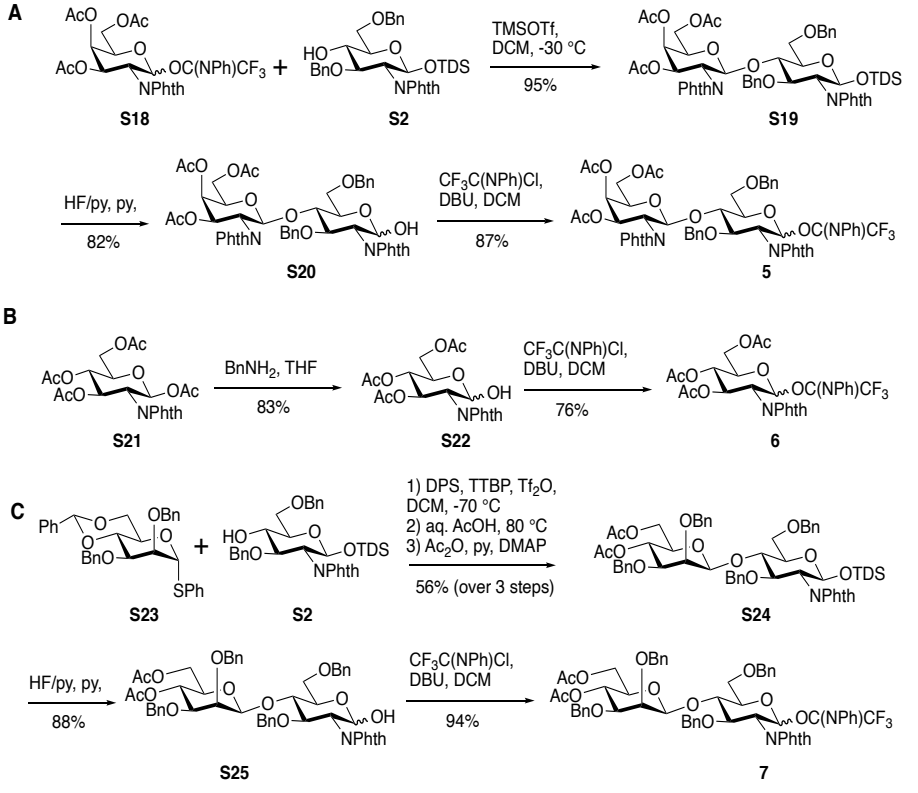


H-1 Man-4, H-1 GalN-7, H-1 GlcN-5', PhC$\underline{H}_2$ protons), 4.69 (3H, m, H-1 GlcN-1, PhC$\underline{H}_2$ protons), 4.56 (7H, m, H-2 GalN-7, H-1 Fuc, PhC$\underline{H}_2$ protons), 4.48 to 4.20 (16H, m, H-2 GlcN-5', H-1 Man-3, H-6a GlcN-5', PhC$\underline{H}_2$ protons, OC$\underline{H}_2$ of alloc), 4.19 to 3.98 (13H, m, H-2 GlcN-1, H-2 GlcN-2, H-2 GlcN-5, H-6b GlcN-5', H-6a GalN-7, H-3 GlcN-1, H-3 GlcN-2, H-3 GlcN-5, PhC$\underline{H}_2$ protons), 3.98 to 3.76 (11H, m, H-6b GalN-7, H-5 Fuc-6, H-6a Man-3, H-2 Man-4, H-4 Man-4', H-4 GlcN-1, H-4 GlcN-2, H-4 GlcN-5, H-3 Fuc-6, H-2 Fuc-6, PhC$\underline{H}_2$ protons), 3.64 (3H, m, H-5 GalN-7, H-5 Man-4, H-5 GlcN-5'), 3.53 (2H, m, H-6a Man-4', H-3 Man-4), 3.45 (4H, m, H-4 Man-4, H-6b Man-3, H-6a Man-4, H-3 Man-3), 3.39 to 3.26 (6H, m, H-6a GlcN-2, H-6b Man-4, H-6a GlcN-5, H-5 Man-4', H-3 Man-4', H-5 GlcN-1), 3.20 (1H, m, H-4 Fuc-6), 3.15 to 2.98 (5H, m, H-6b GlcN-2, H-6b Man-4', H-6a GlcN-1, H-6b GlcN-1, H-5 GlcN-2), 2.54 (1H, m, H-6b GlcN-5), 2.46 (1H, m, H-5 Man-3), 2.10 to 1.75 (22H, m, 7x C$\underline{H}_3$ of Ac, H-5 GlcN-5), 0.92 (3H, d, C$\underline{H}_3$ of Fuc, J = 6.4 Hz), 0.74 (9H, s, 3x C-C$\underline{H}_3$ of TBS), -0.12 to -0.19 (6H, d, 2x C$\underline{H}_3$-Si of TBS); ^{13}C NMR (151 MHz, $CDCl_3$, signals from edited HSQCAD experiment): δ 123.91, 123.62, 128.01, 134.75, 123.42, 123.26, 133.59, 123.37, 126.32, 133.69, 127.98, 123.19, 128.05, 128.52, 128.08, 76.97, 128.26, 125.85, 127.44, 128.09, 125.66, 127.49, 127.89, 127.45, 128.53, 127.83, 70.65, 67.97, 131.49, 65.60, 96.77, 66.64, 98.37, 97.43, 118.54, 70.93, 69.07, 118.48, 74.83, 96.58, 71.44, 97.88, 73.02, 96.70, 73.06, 95.12, 74.83, 69.88, 73.80, 52.07, 97.98, 71.21, 74.87, 74.92, 72.84, 62.06, 98.39, 54.45, 69.91, 74.97, 68.28, 75.57, 61.99, 72.81, 55.77, 77.37, 76.69, 56.45, 74.62, 73.09, 60.85, 60.86, 75.80, 69.14, 72.86, 78.84, 65.89, 68.34, 71.03, 71.80, 73.29, 70.58, 77.53, 77.49, 63.68, 77.91, 68.81, 70.87, 67.31, 70.68, 76.80, 65.93, 68.06, 74.33, 73.93, 73.57, 63.67, 68.22, 65.89, 74.09, 30.97, 29.89, 20.74, 20.75, 19.22, 20.50, 29.86, 16.43, 24.40, 25.93, 0.02, -3.67, -5.19. MALDI-TOF-MS (*m/z*): $[M+ Na]^+$ calculated for $C_{216}H_{223}N_5O_{59}SiNa$, 3881.4270; found 3881.4741.

Benzyl [3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→6)-4,6-di-*O*-acetyl-2,3-di-*O*-benzyl-β-D-mannopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→2)-3,4-di-*O*-benzyl-α-D-mannopyranosyl]-(1→6)-[3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-galactopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→2)-3,6-di-*O*-benzyl-4-*O*-*t*-butyldimethylsilyl-α-D-mannopyranosyl]-(1→3)-[2-*O*-acetyl-4-*O*-benzyl-β-D-mannopyranosyl]-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl-(1→6)-3-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (S17):

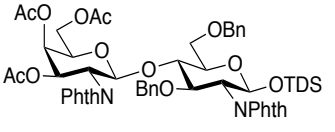
Compound **S15** (1.2 g, 0.31 mmol) was dissolved in THF (30 mL) and H_2O (3 mL), followed by the addition of tetrakis(triphenylphosphine)palladium (72 mg, 0.062 mmol) and morpholine (108 µL, 1.24 mmol). The mixture was stirred for 2 hours after which it was concentrated *in vacuo* and the residue was purified by silica gel column chromatography using Pet. Ether: EtOAc (8: 2, v: v to 3:7, v: v) which afforded the product as a white amorphous solid. (918 mg, 77%). R_f = 0.32 (Tol: EtOAc, 7:3, v: v). A mixture of acceptor **S16** (900 mg, 0.24 mmol) and donor **7** (1.0 g, 0.95 mmol), was stirred in DCM (10 mL) with pre-activated molecular sieves (2 g) for 10 minutes, after which the mixture was cooled down to -40 ºC, followed by the addition of TfOH (8.5 µL, 0.095 mmol). The mixture was warmed up to -20 ºC over a period of 30 minutes, after which it was quenched with Et_3N (100 µL). The sieves were filtered off and the mixture was concentrated *in vacuo.* The residue was purified by silica gel column chromatography using Tol: EtOAc (8: 2, v: v to 1: 1, v: v) and then passed through BioGel SX-1 column using Tol: Acetone (1: 1, v: v) as mobile phase, which afforded the product as an off-white amorphous powder. (823 mg, 71%). R_f = 0.35 (Tol: EtOAc, 6: 4, v: v). ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.14 to 6.44 (1140H, m, H-Ar), 5.76 (1H, dd, H-3 GalN-7, *J* = 11.5 Hz, 3.4 Hz), 5.35 (3H, m, H-4 GalN-7, H-3 GlcN-5', H-1 GlcN-2), 5.26 (2H, m, H-4 Man-8, H-1 Man-4'), 5.13 (1H, d, H-4 GlcN-5', *J* = 3.3 Hz), 4.95 to 4.82 (9H, m, H-2 Man-3, H-1 Man-4, H-1 GlcN-5, PhC$\underline{H}_2$ protons), 4.81 to 4.71 (6H, m, H-1 GalN-7, H-1 GlcN-5", H-1 GlcN-1, H-1 GlcN-5', PhC$\underline{H}_2$), 4.70 to 4.50 (10H, m, H-2 GalN-7, H-1 Fuc-6, PhC$\underline{H}_2$ protons), 4.49 to 4.22 (13H, m, H-1 Man-8, H-1 Man-3, PhC$\underline{H}_2$ protons), 4.21 to 4.03 (13H, m, H-2 GlcN-1, H-2 GlcN-2, H-2 GlcN-5, H-2 GlcN-5', H-6a GalN-7, H-6a Man-8, H-6a GlcN-5', H-3 GlcN-1, H-3 GlcN-2, H-3 GlcN-5, H-3 GlcN-5", PhC$\underline{H}_2$ protons), 4.03 to 3.90 (7H, m, H-6b GalN-7, H-6b GlcN-5', H-4 GlcN-1, H-4 GlcN-2, H-4 GlcN-5, H-4 GlcN-5", PhC$\underline{H}$H), 3.90 to 3.75 (8H, m, H-2 GlcN-5", H-6b Man-8, H-5 Fuc-6, H-4 Man-4', H-2 Man-4, H-3 Fuc-6, H-2 Fuc-6, PhCH$\underline{H}$), 3.70 (2H, m, H-5 Man-4, H-2 Man-4'), 3.64 to 3.51 (5H, m, H-6a GlcN-5", H-6a GlcN-5, H-5 GalN-7, H-5 Man-8, H-5 GlcN-5'), 3.50 to 3.28 (11H, m, H-4 Man-4, H-6b GlcN-5, H-6a Man-4, H-6a GlcN-1, H-6a Man-3, H-2 Man-8, H-5 Man-4', H-3 Man-

3, H-3 Man-8, H-3 Man-4'), 3.21 (5H, m, H-6b GlcN-5", H-6a Man-4', H-6b Man-4, H-4 Man-3, H-3 Man-4), 3.13 (1H, m, H-5 GlcN-1), 3.04 (1H, m, H-5 GlcN-5"), 2.97 (1H, m, H-6a GlcN-2), 2.86 (2H, m, H-6b GlcN-2, H-6b Man-3), 2.68 (1H, m, H-6b Man-4'), 2.54 (4H, m, H-6b GlcN-1, H-5 Man-3, H-4 Fuc-6, H-5 GlcN-2), 2.10 to 1.81 (30H, 9x CH_3 of Ac, H-5 GlcN-5), 0.92 (3H, d, CH_3 of Fuc-6, J = 6.3 Hz), 0.74 (9H, s, 3x C-C$\underline{H}_3$ of TBS), -0.13 to -0.20 (6H, d, 2x C$\underline{H}_3$-Si of TBS); ^{13}C NMR (151 MHz, $CDCl_3$) δ 123.98, 123.62, 135.11, 123.49, 123.36, 133.66, 133.70, 133.58, 128.18, 126.55, 123.15, 127.90, 126.30, 128.22, 128.19, 76.95, 127.78, 125.80, 127.74, 128.08, 125.34, 127.95, 126.27, 127.92, 125.90, 128.02, 124.31, 125.88, 127.53, 125.99, 127.91, 127.85, 125.33, 127.11, 67.82, 70.68, 96.57, 66.65, 96.90, 68.10, 71.00, 96.60, 68.65, 74.31, 97.86, 97.05, 72.99, 94.98, 98.62, 73.38, 69.88, 74.22, 69.70, 52.02, 74.14, 73.78, 101.44, 71.46, 72.83, 72.78, 99.28, 69.90, 97.30, 69.29, 73.23, 55.83, 76.36, 62.93, 60.98, 72.83, 76.94, 73.10, 62.95, 60.88, 75.57, 72.78, 54.39, 79.16, 65.98, 61.67, 71.21, 75.07, 73.33, 70.54, 76.50, 64.06, 67.87, 77.96, 67.21, 70.90, 73.82, 67.93, 77.42, 68.91, 76.73, 72.51, 66.05, 68.89, 64.04, 79.64, 73.91, 74.33, 70.53, 68.14, 68.09, 70.94, 66.04, 70.96, 73.72, 70.42, 73.15, 19.98, 21.52, 30.98, 29.43, 20.80, 20.77, 19.24, 20.90, 20.76, 20.55, 19.02, 20.33, 73.43, 29.81, 14.88, 16.42, 24.42, 25.96, 0.03, -3.71, -5.16. MALDI-TOF-MS (*m/z*): $[M+ Na]^+$ calculated for $C_{264}H_{270}N_6O_{70}SiNa$, 4694.7419; found 4694.7738.



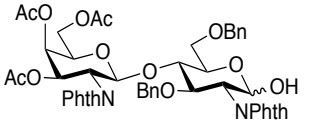
Scheme S4: Synthesis of Epitopes for extension at orthogonal sites.

Dimethylthexylsilyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-galactopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (S19): A mixture of *N*-phenyltrifluoroimidate donor **S18** (6.2 g, 10.3 mmol) and acceptor **S2** (5.0 g, 7.9 mmol), was stirred with pre-activated molecular sieves (50 g) in DCM (50 mL) for 20 minutes. The mixture was then cooled down to -30 ºC, followed by the addition of TMSOTf (373 µL, 2.1 mmol). The reaction mixture was warmed up to -10 ºC over a period of 30 minutes, after which it was quenched with Et_3N (5 mL). The sieves were filtered off, and the mixture was concentrated *in vacuo* to give the crude product as a yellow syrup. Silica gel column chromatography with Tol: EtOAc (9.5: 0.5, v: v to 7: 3, v: v) yielded the product as white amorphous powder (7.9 g, 95%). R_f = 0.44 (Tol: EtOAc, 8.5: 1.5, v: v). ^{1}H NMR (600 MHz,

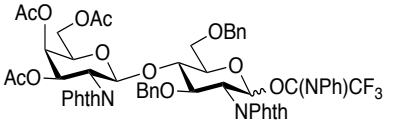


$CDCl_3$): δ 8.00 to 6.85 (18H, m, H-Ar), 5.80 (1H, dd, H-3 GalN, *J* = 11.8 Hz, 2.6 Hz), 5.51 (1H, d, H-1 GalN, *J* = 8.5 Hz), 5.38 (1H, s, H-4 GalN), 5.18 (1H, d, H-1 GlcN, *J* = 8.4 Hz), 4.85 (1H, d, PhCHH, *J* = 12.6 Hz), 4.51 (3H, m, PhCHH, PhCHH, H-2 GalN), 4.44 (1H, d, PhCHH, *J* = 11.2 Hz), 4.25 (1H, t, H-3 GlcN, *J* = 8.8 Hz), 4.16 (1H, m, H-5 GalN), 4.12 to 3.94 (3H, m, H-2 GlcN, H-6a GalN, H-6b GalN), 3.80 (1H, t, H-4 GlcN, *J* = 6.7 Hz), 3.49 to 3.30 (3H, m, H-5 GlcN, H-6a GlcN, H-6b GlcN), 2.11 to 1.78 (9H, 3s, 3x CH_3 of Ac), 1.31 [1H, m, $CH(CH_3)_2$ of TDS] , 0.54 [12H, m, $C(CH_3)_2$, $CH(CH_3)_2$ of TDS], 0.01 to -0.21 (6H, 2s, 2x CH_3-Si of TDS); ^{13}C NMR (151 MHz, $CDCl_3$): δ 172.21, 172.14, 171.64, 170.21, 169.42, 140.66, 140.25, 136.32, 136.26, 135.57, 133.61, 133.34, 133.31, 130.94, 130.18, 130.13, 129.84, 129.60, 129.29, 129.21, 128.92, 125.71, 125.39, 125.05, 99.34, 95.16, 79.16, 79.02, 78.95, 78.80, 78.74, 78.58, 78.33, 76.38, 75.71, 74.67, 72.52, 69.85, 69.75, 68.54, 62.90, 59.65, 53.98, 35.76, 26.36, 22.60, 22.56, 22.42, 21.75, 21.58, 20.18, 20.06, 1.92, 0.00, -1.99. MALDI-TOF-MS (*m/z*): $[M+ Na]^+$ calculated for $C_{56}H_{64}N_2O_{16}SiNa$, 1071.3923; found 1071.3985.

3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-galactopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (S20): Compound **S19** (7.5 g, 7.1 mmol) was dissolved in pyridine (100 mL) followed by dropwise addition of HF in pyridine (70% HF, 30% pyridine; 10 mL). The mixture was stirred for 15 hours after which it was quenched by solid $NaHCO_3$ (50 g), till all CO_2 bubbling stopped. The salts were filtered off, the solvent was evaporated *in vacuo*, and the residue was re-dissolved in DCM, followed by washing with water (100 mL) and saturated $NaHCO_3$ (150 mL). The organic phase was dried over $MgSO_4$, filtered, and the filtrate was concentrated. The residue was purified by silica gel column chromatography with Pet. Ether: EtOAc (8: 2, v: v to 1:1, v: v) as eluent to give the affording the product as a white foamy solid. (5.3 g, 82%). ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.00 to 6.85 (18H, m, H-Ar), 5.76 (1H, dd, H-3 GalN, *J* = 11.5 Hz, 3.3 Hz), 5.44 (1H, d, H-1 GalN, *J* = 8.5 Hz), 5.36 (1H, s, H-4 GalN), 5.18 (1H, d, H-1 GlcN, *J* = 8.6 Hz), 4.87 (1H, d, PhCHH, *J* = 12.6 Hz), 4.47 (4H, m, PhCHH, PhCHH, H-2 GalN, PhCHH), 4.27 (1H, t, H-3 GlcN, *J* = 10.0 Hz), 4.19 (1H, m, H-5 GalN), 4.07 to 3.95 (3H, m, H-2 GlcN, H-6a GalN, H-6b GalN), 3.69 (1H, t, H-4 GlcN, *J* = 6.8 Hz), 3.57 to 3.37 (3H, m, H-5 GlcN, H-6a GlcN, H-6b GlcN), 2.09 to 1.79 (9H, 3s, 3x CH_3 of Ac); ^{13}C NMR (151 MHz, $CDCl_3$): δ 170.34, 170.30, 169.81, 168.45, 168.39, 168.10, 167.52, 138.75, 138.54, 138.20, 138.08, 134.55, 134.50, 134.27, 133.84, 131.54, 131.39, 131.36, 131.30, 129.05, 128.40, 128.37, 128.25, 127.96, 127.95, 127.76, 127.62, 127.59, 127.50, 127.18, 127.13, 123.90, 123.61, 123.57, 123.31, 97.13, 96.97, 92.79, 92.55, 77.36, 77.15, 76.94, 76.44, 75.89, 75.86, 74.38, 74.26, 74.07, 73.60, 72.89, 72.83, 70.60, 70.57, 69.77, 68.24, 68.10, 67.79, 67.76, 66.60, 66.57, 60.85, 60.46, 57.37, 55.77, 52.02, 31.93, 29.70, 28.95, 22.71, 21.07, 20.69, 20.64, 20.62, 20.51. MALDI-TOF-MS (*m/z*): $[M+ Na]^+$ calculated for $C_{48}H_{46}N_2O_{16}Na$, 929.2745; found 929.2819.

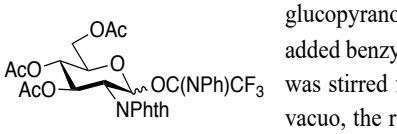


(*N*-Phenyl)-2,2,2-trifluoroacetimidate 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-galactopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (5): Compound **S20** (5.3 g, 5.8 mmol) was dissolved in DCM (50 mL), followed by the addition of



2,2,2- Trifluoro-*N*-phenylacetimidoyl chloride (1.5 mL, 7.5 mmol) and DBU (867 µL, 5.8 mmol). The reaction mixture was stirred for 30 minutes, after which the solvent was evaporated *in vacuo* and the residue was purified using silica gel column chromatography (Pet. Ether: EtOAc, 9: 1, v: v to 7: 3, v: v), which provided the product as an off-white foamy powder. (5.5 g, 87%). R_f = 0.63 (Pet. Ether: EtOAc, 7: 3, v: v). ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.98 to 6.81 (22H, m, H-Ar), 6.53 (1H, br s, H-1 GlcN), 5.79 (1H, dd, H-3 GalN, *J* = 11.2 Hz, 2.9 Hz), 5.48 (1H, d, H-1 GalN, *J* = 8.5 Hz), 5.36 (1H, d, H-4 GalN, *J* = 2.6 Hz), 4.89 (1H, d, PhCHH, *J* = 10.6 Hz), 4.47 (4H, m, PhCHH, PhCHH, H-2 GalN, PhCHH), 4.34 (2H, m, H-3 GlcN, H-2 GlcN) 4.11 to 3.98 (2H, m, H-6a GalN, H-6b GalN), 3.70 (1H, t, H-4 GlcN, *J* = 6.9 Hz), 3.63 to 3.35 (3H, m, H-5 GlcN, H-6a GlcN, H-6b GlcN), 2.09 to 1.79 (9H, 3s, 3x CH_3 of Ac); ^{13}C NMR (151 MHz, $CDCl_3$): δ 171.18, 170.31, 170.24, 169.77, 168.39, 167.51, 142.97, 138.31, 138.06, 134.56, 134.51, 134.01, 131.42, 131.41, 131.35, 128.52, 128.40, 128.03, 127.88, 127.65, 127.54, 127.26, 124.25, 123.89, 123.54, 123.50, 119.20, 97.13, 77.33, 77.12, 76.91, 76.36, 75.34, 75.22, 74.26, 72.73, 70.66, 67.77, 66.58, 60.91, 60.41, 54.58, 51.99, 49.78, 23.42, 21.07, 20.71, 20.65, 20.53, 14.22. Compound was unstable under MALDI-TOF-MS conditions.

(*N*-Phenyl)-2,2,2-trifluoroacetimidate 3,4,6-tetra-*O*-acetyl-2-deoxy-2-phthalimido-α/β-D-glucopyranoside (6): To a solution of 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-phthalimido-β-D-



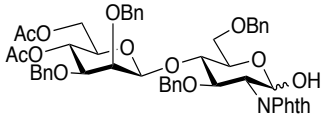
glucopyranose **S21** (28.7 g, 60.2 mmol) in anhydrous THF was added benzylamine (4.7 mL, 43.1 mmol), and the reaction mixture was stirred for 10 hours, after which the solvent was removed in vacuo, the residue was dissolved in DCM (300 mL), and washed with 1N HCl (200 mL). The organic phase was dried over $MgSO_4$, filtered, and the filtrate was concentrated *in vacuo* to afford compound **S22** as a yellow oil. This residue was purified by silica gel column chromatography with Pet. Ether: EtOAc (8: 2, v: v to 4: 6, v: v). To a solution of 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranose **S22** in DCM (150 mL) was added 2,2,2-Trifluoro-*N*-phenylacetimidoyl chloride (15.0 mL, 72.2 mmol) and DBU (9.5 mL, 45.9 mmol) at 0 °C. The reaction mixture was stirred at this temperature for 1 hour, after which it was concentrated *in vacuo* and purified by silica gel column chromatography with Pet. Ether: EtOAc

(9: 1, v: v to 1: 1, v: v) as eluent to give the imidate as a light-yellow foam. (25.2 g, 76% over two steps). R*f* = 0.62 (Pet. Ether: EtOAc, 6: 4, v: v). NMR data corresponds to reported literature.[16c]

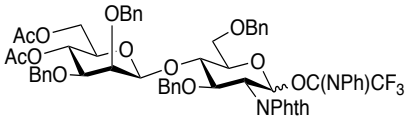
Dimethylthexylsilyl 4,6-di-*O*-acetyl-2,3-di-*O*-benzyl-β-D-mannopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (S24): A mixture of phenyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-1-thio-α-D-mannopyranoside donor **S23** (8.5 g, 15.8 mmol), diphenyl sulfoxide (3.2 g, 15.8 mmol), TTBP (3.9 g, 15.8 mmol) and pre-activated molecular sieves was stirred in DCM (40 mL) for 20 minutes. The mixture was then cooled to -70 ºC, followed by the addition of Tf_2O (2.6 mL, 15.8 mmol) along the wall of the flask. After 10 minutes at -70 ºC, a solution of **S2** (5.0 g, 7.9 mmol) in dry DCM (15 mL) was slowly added along the wall of the flask. The reaction was stirred for additional 30 minutes at -70 ºC, after which it was gradually warmed to -50 ºC, and then quenched with NEt_3 (15 mL). The sieves were filtered off, the filtrate was diluted with DCM (100 mL) and washed with sat. $NaHCO_3$ (50 mL). The organic phase was dried over $MgSO_4$, filtered, and the filtrate was concentrated *in vacuo* to give the crude product, which was directly dissolved in 80% aq. AcOH (150 mL) and the resulting solution was heated at 80 °C for 3 hours. The mixture was concentrated *in vacuo*, and the residue was re-dissolved in DCM and washed with sat. $NaHCO_3$ (100 mL). The organic phase was dried over $MgSO_4$, filtered, the filtrate was concentrated and co-evaporated with toluene thrice. The residue was further dissolved in pyridine (100 mL), followed by the addition of DMAP (500 mg, cat.) and Ac_2O (50 mL). The mixture was stirred for 2 hours, after which 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 (50 mL) after which the solvent was evaporated, and the residue was purified by silica gel column chromatography using Pet. Ether: EtOAc (8: 2, v: v to 1: 1, v: v) as eluent giving the pure compound as a white foamy powder. (4.7 g, 56% over three steps). R*f* = 0.64 (Pet. Ether: EtOAc, 6: 4, v: v). ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.71 to 6.64 (24H, m, H-Ar), 5.22 (2H, m, H-4 Man, H-1 GlcN), 4.76 (3H, m, PhC$\underline{H}_2$, PhC$\underline{H}$H), 4.57 (1H, d, PhCH$\underline{H}$, *J* = 11.8 Hz), 4.50 (1H, s, H-1 Man), 4.42 to 4.32 (3H, m, PhCH$\underline{H}$, PhCH_2), 4.22 (1H, d, PhC$\underline{H}$H, *J* = 11.8 Hz), 4.16 (1H, dd, H-3 GlcN, *J* = 10.7 Hz, 8.8 Hz), 4.02 (2H, m, H-2 GlcN, H-6a Man), 3.91 (2H, m, H-6b Man, H-4 GlcN), 3.71 (1H, d, H-2 Man, *J* = 2.5 Hz), 3.55 (2H, m, H-6a GlcN, H-6b GlcN), 3.45 (1H, m, H-5 GlcN), 3.31 (1H, m, H-5 Man), 3.21 (1H, dd, H-3 Man, *J* = 9.8 Hz, 2.8 Hz), 1.94 to 1.81 (6H, 2s, 2x C$\underline{H}_3$ of Ac), 1.27 [1H, m, C$\underline{H}$(CH_3)$_2$ of TDS] , 0.50 [12H, m, C(C$\underline{H}_3$)$_2$, CH(C$\underline{H}_3$)$_2$ of TDS], 0.03 to -0.20 (6H, 2s, 2x C$\underline{H}_3$-Si of TDS); ^{13}C NMR (151 MHz, $CDCl_3$): δ 172.76, 171.54, 170.04, 169.46, 140.72, 140.41, 139.90, 139.73, 135.54, 135.40, 133.57, 133.42, 130.34, 130.23, 130.19, 130.16, 129.98, 129.92, 129.86, 129.74, 129.63, 129.58, 129.56, 129.53, 129.47, 129.25, 129.16, 129.13, 128.68, 124.89, 124.77, 103.37, 95.24, 81.38, 81.26, 79.18, 78.97, 78.76, 78.66, 76.45, 76.17, 76.07, 75.83, 75.36, 74.43, 74.30, 73.21, 70.64, 69.89, 64.78, 59.62, 35.70, 33.74, 31.50, 26.31, 22.74, 22.68, 22.55, 22.18, 21.71,

21.54, 20.45, 20.12, 20.01, 16.03, 0.24, 0.00, -1.99, -2.09. MALDI-TOF-MS (*m/z*): $[M+ Na]^+$ calculated for $C_{60}H_{71}NO_{14}SiNa$, 1080.4542; found 1080.4505.

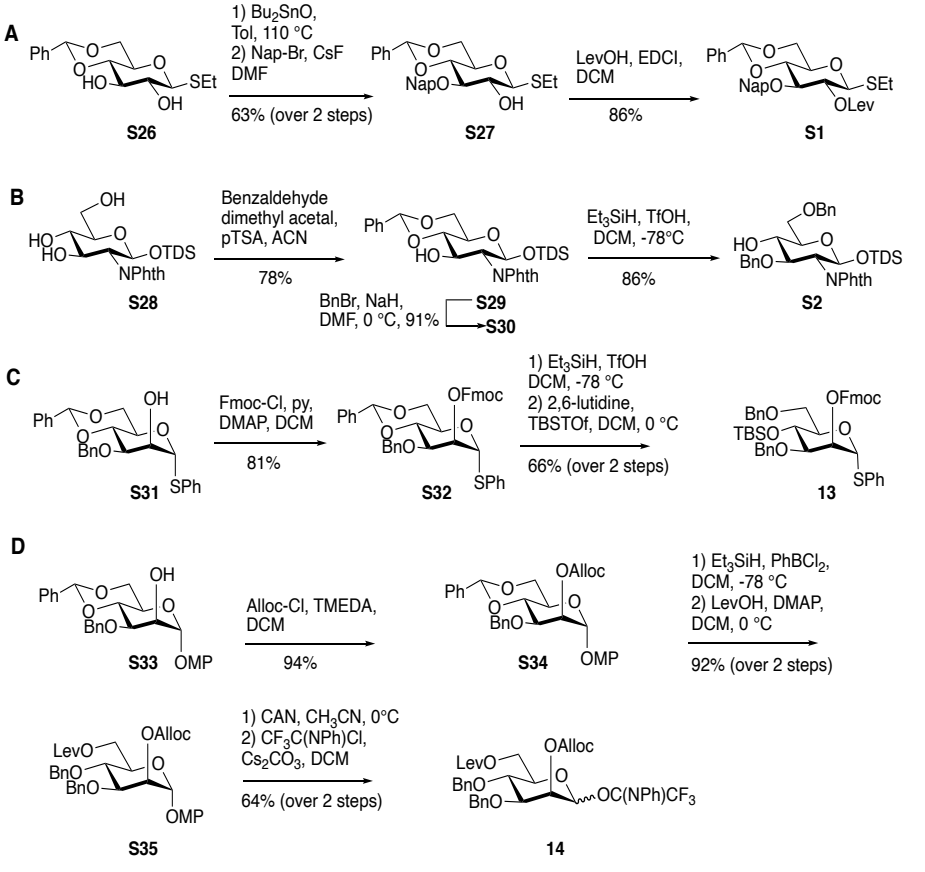
4,6-di-*O*-acetyl-2,3-di-*O*-benzyl-β-D-mannopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (S25): Compound **S24** (4.5 g, 4.2 mmol) was dissolved in pyridine (70 mL) followed by dropwise addition of HF in pyridine (70% HF, 30% pyridine; 6 mL). The mixture was stirred for 12 hours after which it was quenched by solid $NaHCO_3$ (50 g), till all CO_2 bubbling stopped. The salts were filtered off, the solvent was evaporated *in vacuo*, and the residue was re-dissolved in DCM (100 mL), followed by successive washing with water and saturated $NaHCO_3$ (150 mL). The organic phase was dried over $MgSO_4$, filtered, and the filtrate was concentrated. The residue was purified by silica gel column chromatography with Pet. Ether: EtOAc (8: 2, v: v to 1:1, v: v) as eluent to give the affording the product as a white foamy powder. (3.7 g, 88%). ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.82 to 6.67 (24H, m, H-Ar), 5.30 (2H, m, H-4 Man, H-1 GlcN), 4.93 to 4.79 (3H, m, PhC<u>H</u>$_2$, PhC<u>H</u>H), 4.66 (1H, d, PhCH<u>H</u>, *J* = 11.9 Hz), 4.52 to 4.38 (4H, m, H-1 Man, PhCH<u>H</u>, PhC<u>H</u>$_2$), 4.31 (2H, m, PhC<u>H</u>H, H-3 GlcN), 4.15 to 3.98 (4H, m, H-2 GlcN, H-6a Man, H-6b Man, H-4 GlcN), 3.73 (1H, d, H-2 Man, *J* = 2.8 Hz), 3.68 (1H, m, H-6a GlcN), 3.59 (2H, m, H-6b GlcN, H-5 GlcN), 3.36 (1H, m, H-5 Man), 3.23 (1H, dd, H-3 Man, *J* = 9.7 Hz, 2.6 Hz), 2.02 to 1.92 (6H, 2s, 2x C<u>H</u>$_3$ of Ac); ^{13}C NMR (151 MHz, $CDCl_3$): δ 170.95, 169.70, 168.11, 138.82, 138.53, 137.93, 137.76, 133.78, 131.61, 128.61, 128.45, 128.18, 128.00, 127.97, 127.86, 127.79, 127.78, 127.50, 127.29, 126.91, 123.29, 101.41, 93.01, 79.43, 79.23, 77.26, 77.05, 76.84, 74.66, 74.54, 74.37, 74.08, 73.61, 72.43, 71.42, 68.56, 68.02, 62.91, 60.45, 57.57, 20.95, 20.74, 0.03. MALDI-TOF-MS (*m/z*): $[M+ Na]^+$ calculated for $C_{52}H_{53}NO_{14}Na$, 938.3364; found 938.3771.



(*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-β-D-glucopyranoside (7): Compound **S25** (3.7 g, 4.0 mmol) was dissolved in DCM (50 mL), followed by the addition of 2,2,2- Trifluoro-*N*-phenylacetimidoyl chloride (1.1 mL, 5.3 mmol) and DBU (598 µL, 4.0 mmol). The reaction mixture was stirred for 30 minutes, after which the solvent was evaporated *in vacuo* and the residue was purified using silica gel column chromatography (Pet. Ether: EtOAc, 9: 1, v: v to 6: 4, v: v), which provided the product as an off-white amorphous powder. (4.1 g, 94%). R_f = 0.58 (Pet. Ether: EtOAc, 6: 4, v: v). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.92 to 6.72 (28H, m, H-Ar), 6.63 (1H, br s, H-1 GlcN), 5.29 (1H, t, H-4 Man, *J* = 9.8 Hz), 4.84 (3H, m, PhC<u>H</u>$_2$,



PhCHH), 4.68 (1H, d, PhCHH, J = 12.1 Hz), 4.52 (1H, s, H-1 Man), 4.48 to 4.27 (5H, m, 2x $PhCH_2$, PhCHH), 4.17 to 3.95 (3H, m, H-6a Man, H-3 GlcN, H-6b Man), 3.74 (1H, d, H-2, Man, J = 2.7 Hz), 3.36 (1H, m, H-5 Man), 3.25 (1H, dd, H-3, Man, J = 9.8 Hz, 2.8 Hz), 2.12 to 1.84 (3H, 2s, CH_3 of Ac); ^{13}C NMR (101 MHz, $CDCl_3$): δ 170.86, 169.64, 142.98, 138.56, 138.45, 137.88, 137.69, 133.85, 131.43, 128.57, 128.40, 128.14, 127.97, 127.91, 127.85, 127.83, 127.77, 127.74, 127.47, 127.26, 126.94, 124.36, 123.38, 119.30, 101.25, 79.44, 78.58, 77.45, 77.03, 76.66, 76.60, 75.37, 74.59, 74.32, 74.03, 73.50, 72.45, 71.46, 68.02, 62.90, 54.71, 30.90, 20.90, 20.70. Compound was unstable under MALDI-TOF-MS conditions.



Scheme S5: Synthesis of Building Blocks.

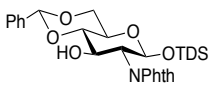
Ethane 4,6-*O*-benzylidene-3-*O*-(2-methylnaphthyl)-1-thio-β-D-glucopyranoside (S27):

To a suspension of diol compound **S26** (34.0 g, 109 mmol) in anhydrous toluene (200 mL) was added Bu_2SnO (40.7 g, 163.5 mmol) and the mixture was refluxed at 110 °C for 2 hours, till the solution became clear. The mixture was then cooled to room temperature and solvent was evaporated *in vacuo.* The residue was dissolved in DMF (200 mL), followed by the addition of 2-(bromomethyl)naphthalene (28.9 g, 130.7 mmol) and CsF (33g, 217.9 mmol). The mixture was stirred overnight after which it was concentrated *in vacuo*. The residue was dissolved in DCM (400 mL) and washed with sat. $NaHCO_3$ (500 mL). The organic phase was then filtered over Buchner funnel to remove the precipitated salts, and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography using Tol: EtOAc (9: 1, v: v to 6:4, v: v) which afforded the product as a white foamy solid. (31.2 g, 63%). R_f = 0.53 (Tol: EtOAc, 8:2, v: v). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.93 to 7.21 (12H, m, H-Ar), 5.58 (1H, s, PhC<u>H</u> of benzylidene), 5.12 (1H, d, CH<u>H</u> of Nap, *J* = 11.9 Hz), 4.99 (1H, d, C<u>H</u>H of Nap, *J* = 11.9 Hz), 4.45 (1H, d, H-1, *J* = 9.7 Hz), 4.35 (1H, q, H-6a), 3.74 (3H, m, H-6b, H-3, H-4), 3.61 (1H, m, H-2), 3.48 (1H, m, H-5), 2.73 (2H, m, SC<u>H</u>$_2$CH$_3$), 1.31 (3H, t, SCH$_2$C<u>H</u>$_3$, *J* = 7.4 Hz); ^{13}C NMR (101 MHz, $CDCl_3$): δ 197.50, 137.22, 136.95, 135.71, 133.25, 133.02, 129.02, 128.33, 128.27, 128.21, 127.93, 127.69, 127.66, 126.82, 126.26, 126.06, 126.03, 125.97, 125.87, 101.89, 101.34, 86.63, 81.39, 81.17, 80.35, 77.34, 77.23, 77.03, 76.71, 74.66, 73.21, 73.11, 71.04, 70.75, 68.64, 53.42, 24.58, 15.24, -0.00. MALDI-TOF-MS (*m/z*): $[M + Na]^+$: calculated for $C_{26}H_{28}O_5SNa$, 475.1555; found 475.1607.

Ethane 4,6-*O*-benzylidene-2-*O*-levulenoyl-3-*O*-(2-methylnaphthyl)-1-thio-β-D-glucopyranoside (S1): Compound **S27** (30.0 g, 66.3 mmol) was dissolved in DCM (200 mL) and the mixture was cooled down to 0 °C. This was followed by the addition of LevOH (10.2 mL, 99.5 mmol) and EDCI (15.4 g, 99.5 mmol). The mixture was stirred for 6 hours, during which the temperature was warmed to room temperature. The mixture was then diluted with DCM (200 mL) and washed with sat. $NaHCO_3$ (200 mL). The organic phase was dried over $MgSO_4$ and filtered, and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography using Tol: EtOAc (9: 1, v: v to 7: 3, v: v) which afforded the product as a white foamy solid. (31.4 g, 86%). R_f = 0.47 (Tol: EtOAc, 8:2, v: v). ^{1}H NMR (400MHz, $CDCl_3$): δ 7.90 to 7.21 (12H, m, H-Ar), 5.59 (1H, s, PhC<u>H</u> of benzylidene), 5.08 (1H, m, H-2), 5.02 (1H, d, CH<u>H</u> of Nap, *J* = 12.0 Hz), 4.89 (1H, d, C<u>H</u>H of Nap, *J* = 12.0 Hz), 4.44 (1H, d, H-1, *J* = 10.1 Hz), 4.37 (1H, q, H-6a), 3.78 (3H, m, H-6b, H-3, H-4), 3.47 (1H, m, H-5), 2.66 (4H, m, SC<u>H</u>$_2$CH$_3$, C<u>H</u>$_2$COCH$_3$), 2.51 (2H, m, COOC<u>H</u>$_2$), 2.10 (3H, s, Lev CH$_2$COC<u>H</u>$_3$), 1.24 (3H, t, SCH$_2$C<u>H</u>$_3$, *J* = 7.4 Hz); ^{13}C NMR (101 MHz, $CDCl_3$): δ 206.11, 171.41, 137.18, 135.66, 133.20, 133.01, 132.98,

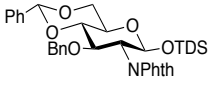
129.06, 128.33, 128.30, 128.10, 128.02, 127.92, 127.67, 126.82, 126.79, 126.14, 126.11, 126.06, 126.04, 125.88, 101.29, 84.17, 81.45, 79.46, 77.44, 77.31, 77.12, 76.80, 74.33, 73.47, 71.67, 70.64, 68.57, 53.48, 37.75, 37.67, 29.76, 28.00, 27.77, 24.02, 14.87. MALDI-TOF-MS (*m/z*): $[M + Na]^+$: calculated for $C_{31}H_{34}O_7SNa$, 573.1923; found 573.1960.

Dimethylthexylsilyl 4,6-*O*-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranoside (S29): To a suspension of triol **S28** (42.0 g, 93.1 mmol) in anhydrous acetonitrile (200 mL) was added



benzaldehyde dimethyl acetal (18.2 mL, 121.0 mmol) and *p*-Toluenesulfonic acid monohydrate (3.5 g, 18.6 mmol). The mixture was stirred for 6 hours, after which it was quenched with Et_3N (20 mL). The solvent was concentrated *in vacuo* and the residue was purified by silica gel column chromatography using Pet. Ether: EtOAc (9: 1, v: v to 7: 3, v: v), which afforded the target compound as a white amorphous solid, (39.2 g, 78%). R_f = 0.47 (Pet. Ether: EtOAc, 8: 2 v: v). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.87 to 7.32 (9H, m, H-Ar), 5.55 (1H, s, PhC<u>H</u> of benzylidene), 5.47 (1H, d, H-1, *J* = 8.4 Hz), 4.62 (1H, dd, H-3, *J* = 10.5 Hz, 8.6 Hz), 4.31 (1H, q, H-6b), 4.19 (1H, dd, H-2, *J* = 10.5 Hz, 8.2 Hz), 3.81(1H, m, H-6a), 3.61(2H, m, H-4, H-5), 1.38 [1H, m, C<u>H</u>$(CH_3)_2$ of TDS], 0.62 [12H, m, C$(C\underline{H}_3)_2$, CH$(C\underline{H}_3)_2$ of TDS], 0.09 to -0.04 (6H, 2s, 2x C<u>H</u>$_3$-Si of TDS); ^{13}C NMR (101 MHz, $CDCl_3$): δ 137.03, 134.05, 131.69, 129.31, 128.35, 126.32, 123.27, 101.95, 93.88, 82.40, 77.31, 77.00, 76.68, 68.74, 68.44, 66.20, 58.64, 33.79, 24.47, 19.79, 19.60, 18.29, 18.16, -0.02, -1.86, -3.82. MALDI-TOF-MS (*m/z*): $[M + Na]^+$: calculated for $C_{29}H_{37}NO_7SiNa$, 562.2237; found 562.2313.

Dimethylthexylsilyl 3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranoside (S30): A solution of **S29** (39.0 g, 72.3 mmol) in DMF (200 mL) was cooled



down to 0 ºC, followed by the sequential addition of BnBr (12.9 mL, 108.4 mmol) and NaH (5.8 g, 144.6 mmol, 60% dispersion in oil). The mixture was stirred at this temperature for two hours, after which it was quenched with AcOH. The solvent was evaporated *in vacuo* and the residue was diluted with DCM (200 mL), washed with saturated $NaHCO_3$ (200 mL) and water (100 mL) and dried over $MgSO_4$. The organic phase was filtered, and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography using Pet. Ether: EtOAc (9: 1, v: v to 7: 3, v: v), which afforded the target compound as a transparent sticky syrup. (41.6 g, 91%). R_f = 0.66 (Pet. Ether: EtOAc, 8.5: 1.5, v: v). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.11 to 6.75 (14H, m, H-Ar), 5.60 (1H, s, PhC<u>H</u> of benzylidene), 5.41 (1H, d, H-1, *J* = 8.4 Hz), 4.77 (1H, d, PhC<u>H</u>H, *J* = 12.2 Hz), 4.50 (1H, d, PhCH<u>H</u>, *J* = 12.2 Hz), 4.42 (1H, dd, H-3, *J* = 10.6 Hz, 9.1 Hz), 4.33 (1H, q, H-6b), 4.16 (1H, dd, H-2, *J* = 10.6 Hz, 8.2 Hz), 3.63 (1H, m, H-5), 3.83 (2H, m, H-6a, H-4), 1.35 [1H, m, C<u>H</u>$(CH_3)_2$ of TDS], 0.60 [12H, m, C$(C\underline{H}_3)_2$, CH$(C\underline{H}_3)_2$ of TDS], 0.06 to -0.09 (6H, 2s, 2x C<u>H</u>$_3$-Si of TDS);

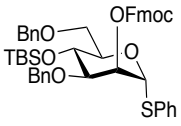
^{13}C NMR (101 MHz, $CDCl_3$): δ 38.05, 137.42, 133.76, 131.67, 128.97, 128.55, 128.25, 128.04, 128.00, 127.31, 126.08, 123.13, 101.33, 93.92, 83.23, 77.31, 76.99, 76.67, 74.36, 73.92, 68.85, 66.22, 57.91, 33.77, 24.47, 19.77, 19.61, 18.26, 18.15, -0.03, -1.88, -3.87. MALDI-TOF-MS (*m/z*): $[M + Na]^+$: calculated for $C_{29}H_{37}NO_7SiNa$, 562.2237; found 562.2313

Dimethylthexylsilyl 3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (S2): A solution of compound **S30** (41.5 g, 65.9 mmol) was stirred with pre-activated molecular sieves (100 g) in DCM (300 mL) for 30 minutes. The mixture was cooled down to -78 ºC, followed by the sequential addition of triethylsilane (21.1 mL, 131.8 mmol) and trifluoromethanesulfonic acid (9.9 mL, 112.1 mmol). The mixture was stirred at this temperature for half an hour, after which it was quenched with a mixture of Et_3N: MeOH (40 mL, 1:1, v: v). The mixture was warmed to room temperature, the molecular sieves were filtered off and the filtrate was diluted by DCM (200 mL) and washed with sat. $NaHCO_3$ (150 mL) and dried over $MgSO_4$. The organic phase was filtered, and the filtrate was concentrated *in vacuo*. Silica gel column chromatography using Pet. Ether: EtOAc (9: 1, v: v to 7: 3, v: v) yielded the product as an off-white semi-solid. (35.6 g, 86%). R_f = 0.28 (Pet. Ether: EtOAc, 8.5: 1.5, v: v). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.88 to 6.88 (14H, m, H-Ar), 5.35 (1H, d, H-1, *J* = 8.0 Hz), 4.71 (1H, d, PhC<u>H</u>H, *J* = 12.2 Hz), 4.60 (2H, m, PhC<u>H</u>$_2$), 4.53 (1H, d, PhCH<u>H</u>, *J* = 12.2 Hz), 4.42 (1H, dd, H-3, *J* = 10.9 Hz, 8.4 Hz), 4.10 (1H, dd, H-2, *J* = 10.9 Hz, 8.1 Hz), 3.78 (3H, m, H-6a, H-6b H-4), 3.63 (1H, m, H-5), 2.84 (1H, d, O<u>H</u>, *J* = 2.5 Hz), 1.34 [1H, m, C<u>H</u>$(CH_3)_2$ of TDS], 0.57 [12H, m, C$(C\underline{H}_3)_2$, CH$(C\underline{H}_3)_2$ of TDS], 0.08 to -0.07 (6H, 2s, 2x C<u>H</u>$_3$-Si of TDS); ^{13}C NMR (101 MHz, $CDCl_3$): δ 138.30, 137.71, 133.73, 128.47, 128.14, 127.86, 127.81, 127.68, 127.38, 93.38, 78.47, 77.31, 77.19, 76.99, 76.67, 74.47, 74.05, 73.77, 73.55, 70.97, 57.38, 33.82, 24.45, 19.83, 19.64, 18.26, 18.14, -0.03, -1.82, -3.89. MALDI-TOF-MS (*m/z*): $[M + Na]^+$: calculated for $C_{36}H_{45}NO_7SiNa$, 654.2863; found 654.2988

Phenyl 3-*O*-benzyl-4,6-*O*-benzylidene-2-*O*-fluorenylmethoxycarbonyl-1-thio-α-D-mannopyranoside (S32): To a solution of **S31** (30.0 g, 66.6 mmol) in DCM (200 mL) was added DMAP (500 mg, cat), pyridine (60 mL) and Fmoc-Cl (34.5 g, 133.2 mmol) and the mixture was stirred for 2 hours. After that time, a second portion of Fmoc-Cl (8.6 g, 33.3 mmol) was added and stirring was continued for another 30 min, upon which TLC (Pet. Ether: EtOAc, 7: 3) showed the full consumption of starting material. The mixture was then diluted by DCM (200 mL) and washed with 1 N HCl (100 mL). The organic phase was dried over $MgSO_4$, filtered and the filtrate was concentrated. The residue was purified by silica gel column chromatography with Pet. Ether: EtOAc (9:1, v: v to 6:4, v: v) as eluent which gave the desired product as a white foam. (36.3 g, 81%). R*f* = 0.69 (Pet. Ether: EtOAc, 7: 3, v: v). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.82 to 7.15 (22H,

m, H-Ar), 5.71 (s, 1H, PhC<u>H</u> of benzylidene), 5.60 (1H, d, H-1, *J* = 1.5 Hz), 5.48 (1H, dd, H-2, *J* = 3.4 Hz, 1.5 Hz), 4.80 (2H, s, C<u>H</u>$_2$ of Fmoc), 4.52 to 4.30 (3H, m, PhC<u>H</u>H, H-5, PhCH<u>H</u>), 4.32 to 4.21 (3H, m, H-6a, H-4, C<u>H</u> of Fmoc), 4.08 (1H, dd, H-3, *J* = 9.8 Hz, 3.4 Hz), 3.95 (1H, t, H-6b, *J* = 10.2 Hz); ^{13}C NMR (101 MHz, $CDCl_3$): δ 154.62, 143.41, 143.10, 141.29, 141.24, 137.79, 137.38, 132.87, 132.21, 129.25, 129.00, 128.37, 128.23, 128.12, 127.92, 127.89, 127.74, 127.68, 127.22, 127.19, 126.13, 125.29, 125.16, 120.05, 101.65, 86.92, 78.51, 77.46, 77.03, 76.61, 75.56, 74.06, 72.56, 70.45, 68.40, 65.26, 46.66. MALDI-TOF-MS (*m/z*): $[M + Na]^+$: calculated for $C_{41}H_{36}O_7SNa$, 695.2079; found 695.2103

Phenyl 3,6-di-*O*-benzyl-4-*O*-*t*-butyldimethylsilyl-2-*O*-fluorenylmethoxycarbonyl-1-thio-α-D-mannopyranoside (13): A solution of compound **S32** (36.3 g, 54.0 mmol) was stirred with pre-activated molecular sieves (70 g) in DCM (300 mL) for 30 minutes. The mixture was cooled down to -78 ºC, followed by the sequential addition of Et_3SiH (17.3 mL, 108.0 mmol) and trifluoromethanesulfonic acid (8.1 mL, 91.8 mmol). The mixture was stirred at this temperature for half an hour, after which it was quenched with a mixture of Et_3N: MeOH (40 mL, 1:1, v: v). The mixture was warmed to room temperature, the molecular sieves were filtered off and the filtrate was diluted by DCM (200 mL) and washed with sat. $NaHCO_3$ (200 mL) and dried over $MgSO_4$. The organic phase was filtered, and the filtrate was concentrated *in vacuo* to give the crude product as an oil. This crude product was dissolved in anhydrous CH_2Cl_2 (200 mL), and cooled down to 0 °C, followed by the addition of 2,6-lutidine (9.1 mL, 77.9 mmol), and TBSOTf (14.3 mL, 62.3 mmol), and stirring was continued for 1 h, after which the mixture was diluted by DCM and washed with sat. $NaHCO_3$ (200 mL) and 1 N HCl (200 mL). The organic phase was then dried over MgSO4, filtered and filtrate was concentrated to afford the crude product as a clear oil. Silica gel column chromatography with Pet. Ether: EtOAc (9: 1, v: v to 6: 4, v: v) afforded the product a transparent oil. (28.4 g, 66% over two steps). R_f = 0.33 (Pet. Ether: EtOAc, 8: 2, v: v). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.83 to 7.09 (23H, m, H-Ar), 5.61 (1H, d, H-1, *J* = 1.4 Hz), 5.45 (1H, m, H-2), 4.72 (1H, d, PhCH<u>H</u>, *J* = 11.3 Hz), 4.60 (2H, s, C<u>H</u>$_2$ of Fmoc), 4.53 (1H, d, PhC<u>H</u>H, *J* = 11.3 Hz), 4.43 to 4.20 (4H, m, PhC<u>H</u>H, C<u>H</u> of Fmoc, PhCH<u>H</u>, H-5), 4.08 (1H, t, H-4, *J* = 8.8 Hz), 3.91 to 3.80 (2H, m, H-6a, H-6b), 3.75 (1H, dd, H-3, *J* = 8.9 Hz, 3.1 Hz), 0.87 (9H, s, 3x C-C<u>H</u>$_3$ of TBS), 0.07 (6H, d, 2x C<u>H</u>$_3$-Si of TBS *J* = 12.3 Hz); ^{13}C NMR (101 MHz, $CDCl_3$): δ 157.66, 154.57, 143.41, 143.36, 143.24, 141.33, 141.24, 141.23, 138.45, 137.52, 136.48, 133.50, 132.32, 131.88, 131.85, 129.05, 128.99, 128.36, 128.26, 128.22, 128.13, 128.01, 127.91, 127.87, 127.85, 127.77, 127.72, 127.61, 127.49, 127.47, 127.39, 127.35, 127.24, 127.19, 127.13, 125.28, 125.18, 120.11, 120.09, 120.00, 86.02, 85.33, 78.62, 77.34, 77.22, 77.02, 76.70, 74.03, 73.79, 73.67, 73.20, 72.20, 71.28, 70.31, 69.91, 69.53, 68.14, 46.78, 46.55, 25.95, 25.77, 25.65, 24.51, 18.19, 17.97, 0.00, -3.57, -3.82, -4.26, -4.81, -4.94. MALDI-TOF-MS (*m/z*): $[M + Na]^+$: calculated for $C_{47}H_{52}O_7SSiNa$, 811.3101; found 811.3420.

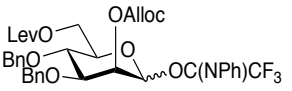


4-Methoxyphenyl 2-*O*-allyloxycarbonyl-3,4-di-*O*-benzyl-1-thio-α-D-mannopyranoside (S34): To a solution of compound **S33** (16 g, 34.5 mmol) in DCM (100 mL) was added TMEDA (7.7 mL, 51.7 mmol), followed by Alloc-Cl (4.4 mL, 41.4 mmol), and the reaction mixture was left stirring for 30 minutes, after 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 30 minutes. The mixture was diluted with DCM (200 mL) and washed with 1 N HCl (100 mL). The organic phase was dried over $MgSO_4$, filtered, and the filtrate was concentrated to give the crude product. The crude product was purified by silica gel column chromatography with Pet. Ether: EtOAc (9:1, v: v to 1:1, v: v) as eluent to give the desired product as a transparent oil. (17.8 g, 94%). R*f* = 0.65 (Pet. Ether: EtOAc, 7: 3, v: v). ^{1}H NMR (400 MHz, CDCl3): δ 7.54 to 6.75 (14H, m, H-Ar), 5.92 (1H, m, C<u>H</u>=CH$_2$ of alloc), 5.63 (1H, s, PhC<u>H</u> of benzylidene), 5.48 (1H, d, H-1, *J* = 1.5 Hz), 5.38 (2H, m, H-2, CH=CH<u>H</u> of alloc), 5.26 (1H, dd, CH=C<u>H</u>H of alloc, *J* = 10.5 Hz, 1.2 Hz), 4.78 (2H, s, PhC<u>H</u>$_2$), 4.65 (2H, m, OC<u>H</u>$_2$ of alloc), 4.18 (3H, m, H-6a, H-4, H-3), 4.00 (1H, m, H-5), 3.83 (1H, t, H-6b, *J* = 10.2 Hz), 3.76 (3H, s, OC<u>H</u>$_3$); ^{13}C NMR (101 MHz, CDCl$_3$): δ 126.08, 127.91, 128.29, 128.25, 128.17, 117.90, 117.88, 114.69, 101.59, 97.56, 119.28, 73.54, 119.28, 119.27, 119.28, 70.75, 72.47, 73.58, 71.36, 69.02, 68.50, 73.71, 78.20, 78.21, 64.43, 68.51, 53.92, 55.62, 54.51, 56.73. MALDI-TOF-MS (*m/z*): [M + Na]$^+$: calculated for $C_{31}H_{32}O_9Na$, 571.1944; found 571.2296

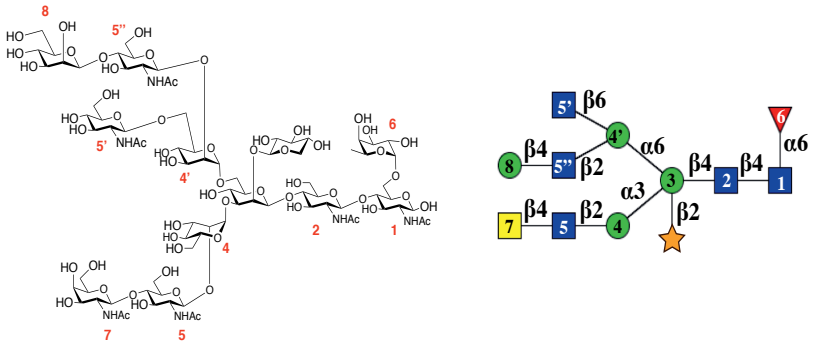
4-Methoxyphenyl 2-*O*-allyloxycarbonyl-3,4-di-*O*-benzyl-6-*O*-levulenoyl-1-thio-α-D-mannopyranoside (S35): A mixture of **S34** (17.8 g, 32.5 mmol), Et_3SiH (7.8 mL, 48.7 mmol) and pre-activated molecular sieves was stirred in DCM (150 mL) for 30 minutes. The solution was cooled to -78 ºC, after which $PhBCl_2$ (5.5 mL, 42.3 mmol) was added, and the reaction was stirred at -78 ºC for 15 min after which it was quenched with Et_3N (20 mL). The mixture was then diluted with DCM (200 mL) and washed with sat. $NaHCO_3$ (100 mL). The organic phase was dried over $MgSO_4$, filtered, and the filtrate was concentrated *in vacuo* to give the crude product as a transparent oil. This was used in the next step without further purification. A solution of the crude product in DCM (200 mL) was cooled to 0 °C, followed by the addition of LevOH (5.6 mL, 48.7 mmol), DMAP (500 mg, cat) and EDCI (10.1 g, 65.0 mmol). The reaction mixture was left stirring for 3 h, after which it was diluted with DCM (200 mL) and washed with sat. $NaHCO_3$ (200 mL). The organic phase was dried over $MgSO_4$, filtered, and the filtrate was concentrated *in vacuo*. Chromatography on silica gel with Pet. Ether: EtOAc (8: 2, v: v to 1: 1, v: v) as eluent gave the desired product as a transparent oil. (19.4 g, 92%). R*f* = 0.54 (Pet. Ether: EtOAc, 7: 3, v: v). ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.47 to 6.69 (14H, m, H-Ar), 5.93 (1H, m, C<u>H</u>=CH$_2$ of alloc), 5.48

(1H, d, H-1, J = 1.8 Hz), 5.40 to 5.32 (2H, m, H-2, CH=CHH of alloc), 5.27 (1H, dd, CH=CHH of alloc, J = 10.5 Hz, 1.2 Hz), 4.93 (1H, d, PhCHH, J = 10.9 Hz), 4.82 ((1H, d, PhCHH, J = 11.4 Hz), 4.66 (3H, m, OCH_2 of alloc, PhCHH), 4.59 (1H, d, PhCHH, J = 10.9 Hz), 4.30 (2H, m, H-6a, H-6b), 4.19 (1H, dd, H-3, J = 9.2 Hz, 3.2 Hz), 3.97 (1H, m, H-5), 3.83 (1H, t, H-4, J = 9.6 Hz), 3.76 (3H, s, OCH_3), 2.69 (2H, m, $COOCH_2CH_2$ of Lev), 2.55 (2H, m, $COOCH_2CH_2$ of Lev), 2.15 (3H, s, CH_2COCH_3 of Lev); ^{13}C NMR (151 MHz, $CDCl_3$): δ 206.49, 172.38, 155.30, 154.60, 149.74, 137.98, 137.89, 137.78, 131.34, 129.06, 128.47, 128.44, 128.25, 128.14, 128.01, 127.88, 127.85, 125.32, 119.28, 117.88, 114.62, 96.59, 77.95, 77.27, 77.06, 76.85, 75.40, 73.90, 72.23, 72.04, 70.37, 69.00, 63.15, 55.65, 37.90, 29.83, 27.95, 21.49, 0.03. MALDI-TOF-MS (*m/z*): $[M + Na]^+$: calculated for $C_{31}H_{32}O_9Na$, 571.1944; found 571.2296.

(*N*-Phenyl)-2,2,2-trifluoroacetimidate-3,4-di-*O*-benzyl-6-*O*-levulenoyl-α/β-D-mannopyranoside (14): To a solution of compound **S35** (19.4 g, 29.9 mmol) in acetonitrile (200 mL) was added ceric ammonium nitrate (49.1 g, 89.7 mmol) at 0 °C, and the reaction mixture was stirred for 2 hours, after which the solvent was evaporated *in vacuo*. The residue was diluted with EtOAc (300 mL) and successively washed with H_2O (200 mL) and sat. $NaHCO_3$ (200 mL). The organic phase was dried over $MgSO_4$, filtered, and the filtrate was concentrated *in vacuo* to afford a residue, which was chromatographed with Pet. Ether: EtOAc (7: 3, v: v to 1: 1, v: v) as eluent to give the lactol as an orangish foamy solid. To a solution of this hemiacetal in anhydrous DCM (150 mL), was added $CF_3C(NPh)Cl$ (7.4 mL, 35.9 mmol) and Cs_2CO_3 (29.2 g, 89.7 mmol). The reaction mixture was stirred for 3 hours, after which it was diluted by DCM (200 mL) and the salts were filtered off. The mixture was concentrated, and the residue was purified by silica gel column chromatography with Pet. Ether: EtOAc (8: 2, v: v to 1: 1, v: v) as eluent to give the imidate product as an off-white foamy powder. (13.6 g, 64%, over two steps). R*f* = 0.7 (Pet. Ether: EtOAc, 7: 3, v: v). ^1H NMR (400 MHz, $CDCl_3$): δ 7.45 to 6.72 (15H, m, H-Ar), 6.26 (1H, br s, H-1), 5.95 (1H, m, $CH=CH_2$ of alloc), 5.62 (1H, s, H-2), 5.38 (2H, m, CH=CHH of alloc), 5.27 (2H, m, CH=CHH of alloc), 4.92 (1H, m, PhCHH), 4.77 (1H, m, PhCHH), 4.72 to 4.54 (4H, m, PhCHH, OCH_2 of alloc, PhCHH), 4.35 (2H, m, H-6a, H-6b), 4.04 (1H, dd, H-3, J = 9.1 Hz, 3.2 Hz), 3.81 (2H, m, H-4, H-5), 2.74 (2H, m, $COOCH_2CH_2$ of Lev), 2.61 (1H, t, $COOCH_2CH_2$ of Lev, J = 6.6 Hz, 2H, Lev), 2.17 (3H, s, CH_2COCH_3 of Lev); ^{13}C NMR (101 MHz, $CDCl_3$): δ 206.47, 172.30, 154.85, 137.66, 137.33, 137.12, 131.27, 131.16, 128.80, 128.49, 128.46, 128.23, 128.16, 128.13, 127.99, 127.96, 124.53, 119.36, 119.30, 119.05, 93.96, 79.28, 77.45, 77.36, 77.02, 76.60, 75.54, 75.30, 74.36, 73.38, 73.24, 72.30, 71.80, 71.04, 70.18, 69.12, 69.03, 62.82, 37.88, 29.78, 27.99, 27.94. Compound was unstable under MALDI-TOF-MS conditions.



2. Deprotection conditions to yield glycan 8.



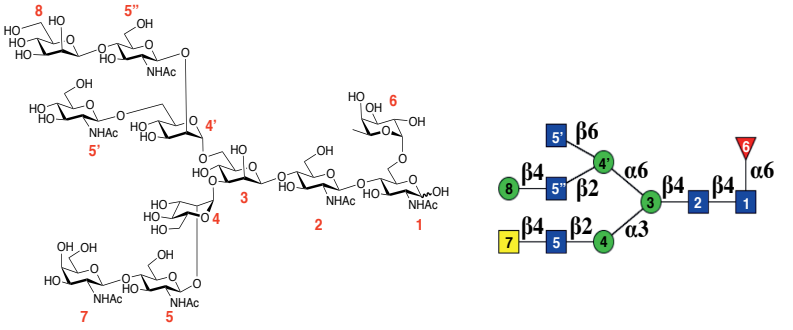
To a suspension of **27** (600 mg, 0.125 mmol) in *n*BuOH (10 mL) was added ethylenediamine (10 mL) and the resulting clear solution was heated at 90 °C for 16 h. The mixture was concentrated *in vacuo*, co-evaporated with toluene 5 times, and the resulting residue was dissolved in pyridine: Ac_2O (20 mL, 1: 1, v: v), after which DMAP (50 mg, cat) was added. The reaction was left stirring for 2 h, after which TLC (Tol: Acetone, 7: 3, v: v) showed the presence of one major product. The mixture was concentrated *in vacuo*, and the resulting crude product was briefly chromatographed with (Tol: Acetone, 7: 3, v: v) as eluent to give product which was additionally passed through BioGel SX-1 column (Tol: Acetone, 1: 1, v: v) as mobile phase to provide the acetylated intermediate as a clear syrup. This material was then dissolved in MeOH, after which 1 M NaOMe (500 µl) was added, and the deacetylation was left proceeding at for 2 hours. The reaction was neutralized with the Amberlite 120 H^+ resin, filtered and the filtrate was 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 48 hours, after which MALDI showed the product peak. The mixture was then filtered to remove catalyst, and the filtrate was concentrated *in vacuo*, passed through the BioGel P-4 column and lyophilized to give the desired glycan as a white cotton-like solid. (144 mg, 53% over four steps). The glycan was additionally purified (in batches of 15 to 20 mg) by HPLC with a semi-preparative amide HILIC column (10 x 250 mm, Waters Inc.) by the gradient solvent system CH_3CN: H_2O (9: 1, v: v to 1: 1, v: v) with the UV (210 nm) detector to afford analytically pure glycan used for further enzymatic reactions.

^{1}H NMR (600 MHz, D_2O):

8	H1	H2	H3	H4	H5	H6	Fuc-CH_3
GlcNAc-1α	5.07 (d, *J* = 2.6 Hz)	3.80	3.89	3.66	NA	NA	–
Man-4	5.03	4.05	3.81	3.42	NA	3.80, 3.51	–
Man-3	4.75	4.17	3.79	3.71	3.75	3.85, 3.60	–
Man-4'	4.74	3.99	3.75	3.31	3.68	4.09, 3.46	–
Man-8	4.65	3.97	3.55	NA	NA	NA	–
GlcNAc-1β	4.58	3.63	3.65	NA	NA	NA	–
GlcNAc-2	4.55	3.69	3.67	3.54	NA	NA	–
GlcNAc-5"	4.46 (d, *J* = 7.8 Hz)	3.64	3.53	3.63	3.39	NA	–
GlcNAc-5'	4.42	3.66	3.55	3.62	3.39	NA	–
GlcNAc-5	4.40	3.66	3.46	3.64	3.40	NA	–
GalNAc-7	4.40	3.84	3.63	3.85	NA	NA	–
Xyl (core)	4.32 (d, *J* = 7.6 Hz)	3.28	3.35	NA	3.14, 3.89	–	–
Fuc-6 (core) α,β	4.78	3.69	3.79	NA	3.99, 4.02	–	1.10

MALDI-TOF-MS (*m/z*): $[M+ Na]^+$ calculated for $C_{78}H_{130}N_6O_{55}Na$, 2053.7458; found 2053.8128.

3. Deprotection conditions to yield glycan 9.



To a suspension of **S17** (815 mg, 0.17 mmol) in pyridine (10 mL) was added HF in pyridine (70% HF, 30% pyridine; 1.0 mL) dropwise. The mixture was stirred for 6 hours at 60 °C, after which it was quenched by solid $NaHCO_3$, till all CO_2 bubbling stopped. The salts were filtered off, the solvent was evaporated *in vacuo*, and the residue was re-dissolved in DCM, followed by successive washing with water and saturated $NaHCO_3$. The organic phase was dried over $MgSO_4$, filtered, and the filtrate was concentrated. To a suspension of the residue in *n*BuOH (10 mL) was added ethylenediamine (10 mL) and the resulting clear solution was heated at 90 °C for 16 h. The mixture was concentrated *in vacuo*, co-evaporated with toluene 5 times, and the resulting residue was dissolved in pyridine: Ac_2O (20 mL, 1: 1, v: v), after which DMAP (50 mg, cat) was added. The reaction was left stirring for 2 h, after which TLC (Tol: Acetone, 7: 3, v: v) showed the presence of one major product. The mixture was concentrated *in vacuo*, and the resulting crude product was briefly chromatographed with (Tol: Acetone, 7: 3, v: v) as eluent to give product which was additionally passed through BioGel SX-1 column (Tol: Acetone, 1: 1, v: v) as mobile phase to provide the acetylated intermediate as a clear syrup. This material was then dissolved in MeOH, after which 1 M NaOMe (500 µl) was added and the deacetylation was left proceeding at for 2 hours. The reaction was neutralized with the Amberlite 120 H^+ resin, filtered and the filtrate was 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 48 hours, after which MALDI showed the product peak. The mixture was then filtered to remove catalyst, and the filtrate was concentrated *in vacuo*, passed through the BioGel P-4 column and lyophilized to give the desired glycan as a white cotton-like solid. (203 mg, 57% over five steps). The glycan was additionally purified (in batches of 15 to 20 mg) by HPLC with a semi-preparative amide HILIC column (10 x 250 mm, Waters Inc.) by the gradient solvent system CH_3CN: H_2O (9: 1, v: v to 1: 1, v: v) with the UV (210 nm) detector to afford analytically pure glycan used for further enzymatic reactions.

^{1}H NMR (600 MHz, D_2O)

9	H1	H2	H3	H4	H5	H6	Fuc-CH_3
GlcNAc-1α	5.07 (d, *J* = 3.0 Hz)	3.78	3.89	3.69	NA	3.73, 3.60	–
Man-4	5.00 (s)	4.07	3.80	3.38	3.47	3.82, 3.50	–
Man-4'	4.75	3.97	3.76	3.29	3.71	4.09, 3.47	–
Man-3	4.65	4.13	3.67	3.71	3.46	3.81, 3.63	–
Man-8	4.65	3.96	3.55	3.62	3.34	NA	–
GlcNAc-1β	4.58 (d, *J* = 8.2 Hz)	3.59	3.63	NA	NA	NA	–
GlcNAc-2	4.55	3.67	3.65	3.50	NA	NA	–
GlcNAc-5"	4.48 (d, *J* = 7.3 Hz)	3.62	3.38	3.61	3.38	NA	–
GlcNAc-5	4.44	3.62	3.47	3.61	3.39	NA	–
GlcNAc-5'	4.42	3.61	3.48	3.62	3.39	NA	
GalNAc-7	4.40	3.82	3.60	3.82	NA	NA	–
Fuc-6 (core) (α, β)	4.78	3.68	3.81	3.68	3.98, 4.02	–	1.10

MALDI-TOF-MS (*m/z*): $[M+ Na]^+$ calculated for $C_{78}H_{130}N_6O_{55}Na$, 2053.7458; found 2053.8128.

4. Enzymatic Reactions.

4.1 General methods. All enzymatic reactions were performed in aqueous buffers with an appropriate pH for each enzyme. B3GNT2 [β-(1→3)-glucosaminyltransferase], FUT5 [α-(1→3)-fucosyltransferase] and B4GalT1 [β-(1→4)-galactosyltransferase] were provided by Dr. K. W. Moremen (Complex Carbohydrate Research Center, Athens, GA, USA). *Helix pomatia* β-mannosidase and 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) were purchased from Roche Diagnostic Corporation (Indianapolis, IN); guanosine 5'-diphospho-L-fucose (GDP-Fuc) was synthesized chemically using reported procedures.[32] All enzymatic reactions, unless otherwise stated, were monitored by mass spectrometry, and recorded on a Shimadzu Biotech Axima-CFR MALDI-TOF using dihydroxybenzoic acid or 4- hydroxycinnamic acid as matrices. Gel filtration

chromatography was performed using a column (30 cm x 1.5 cm) packed with BioGel P-4 or P-6 (GE Healthcare), eluted with deionized water. Glycans were purified by HPLC using HILIC column (XBridge® Amide 5 µm, 10 mm x 250 mm, Waters) HPLC grade Acetonitrile and de-ionised water as eluents under UV detection (210 nm). Glycans were lyophilized by dissolving the compound in water and freezing using liquid nitrogen. All enzymatic reactions were driven to full completion by adding excess of glycosyltransferases until all starting material was consumed, as detected by MALDI-TOF-MS. This approach enabled efficient product isolation and purification. All nuclear magnetic resonance (NMR) spectra were acquired on 400, 500 or 600, MHz Varian/Agilent Direct Drive, operating at 25 °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 HOD signal at 4.79 ppm.

4.2 Expression and purification of Human Glycosyl Transferses.

The catalytic domains of all human glycosyl transferases were expressed as a soluble secreted fusion protein for production in mammalian (HEK293) suspension cultures.[33] The coding regions were amplified from Mammalian Gene Collection clones using primers that appended a tobacco etch virus (TEV) protease cleavage site[34] to the NH_2-terminal end of the coding region and attL1 and attL2 Gateway adaptor sites were extended on the 5′ and 3′ terminal ends of the coding region during transfer to pDONR221 vector backbone.[33b] The pDONR221 clones were then recombined via LR clonase reaction into a custom Gateway adapted version of the pGEn2 mammalian expression vector[33b] to assemble a recombinant coding region comprised of a 25 amino acid NH_2-terminal signal sequence from the *T. cruzi* lysosomal α-mannosidase[35] followed by an 8xHis tag, 17 amino acid AviTag,[36] "superfolder" GFP,[37] the nine amino acid sequence encoded by attB1 recombination site, followed by the TEV protease cleavage site and the respective glycosyltransferase catalytic domain coding region.

Suspension culture HEK293 cells (Freestyle 293-F cells, Life Technologies, Grand Island, NY) were transfected as previously described[33] and the culture supernatant was subjected to Ni^{2+}-NTA superflow chromatography (Qiagen, Valencia, CA). Enzyme preparations were eluted with 300 mM imidazole, concentrated by ultrafiltration, and subjected to gel filtration on a Superdex 75 column (GE Healthcare) preconditioned with a buffer containing 20 mM HEPES, pH 7.0, 100 mM NaCl, 10% glycerol, 0.05% Na azide. Peak fractions were pooled and concentrated to ~1 mg/mL using an ultrafiltration pressure cell membrane (Millipore, Billerica, MA) with a 10 kDa molecular weight cutoff.

Enzyme	Amino Acid residues	Uniprot ID
B3GNT2	35-397	Q9NY97
B4GalT1	63-398	P15291
FUT5	40-374	Q11128

Table S1. Enzyme expression details.

4.3 General Procedures for enzymatic reactions

General procedure for α(1→3) Fucosylation: Glycan and GDP-Fucose (2 eq per fucose) were dissolved in Tris buffer (50 mM, pH~7.3) containing $MnCl_2$ (10 mM), CIAP (10 mU). Recombinant α(1,3)-Fuctosyltransferases FUT5 (6.6 mU/μmol of substrate) was added to achieve a final concentration of 4 mmol. The resulting mixture was incubated at 37 ºC for 12 h. In case MALDI-TOF-MS showed the remaining starting material additional GDP-Fucose (1 or 2 eq), CIAP (10 mU) and enzyme FUT5 was added and incubation at 37 ºC was continued until no more starting material could be detected. The reaction mixture was quenched by adding methanol (10 μL), after which it was passed through Biogel P-4 or P-6 column. Fractions containing product were identified using TLC (dipping into the anisaldehyde stain followed by charring at 150 ºC), combined and lyophilized to give the product.

General Procedure for β(1→4) Galactosylation. Glycans 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 B4GalT1 (3.4 mU/μmol) were added to achieve a final concentration of 4 mmol. The reaction mixture was then incubated at 37 ºC for 10 h. The reaction mixture was quenched by adding methanol (10 μL) and passed through Biogel P-4 or P-6 column. Fractions containing product were identified using TLC (dipping into the anisaldehyde stain followed by charring at 150 ºC), combined and lyophilized to give the respective products as white fluffy solids.

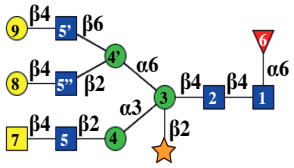
General Procedure for installation of β(1→3) *N*-acetylglucosamine moieties. Glycans and UDP-GlcNAc (2 eq) were dissolved in HEPES buffer (50 mM, pH~7.3) containing KCl (25 mM), $MgCl_2$ (2 mM), and DTT (1 mM). To this, CIAP (10 mU) and B3GNT2 (6.0 mU/μmol) were added to achieve a final concentration of glycan at 4 mM. The resulting mixture was then incubated at 37 ºC for 12 h. The reaction mixture was quenched by adding methanol (10 □L) and passed through Biogel P-4 or P-6 column. Fractions containing product were identified using TLC (dipping into the anisaldehyde stain followed by charring at 150 ºC), combined and lyophilized to give the respective products as white fluffy solids.

General Procedure for β-Mannose Removal with subsequent Installation of a β-Galactose Moiety. Glycan was dissolved in 100 mM NaOAc buffer (500 μL, pH~5.0) 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 6 h. In case MALDI-TOF-MS 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. 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 combined and lyophilized. This glycan and UDP-Gal (1.5 - 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 B4GalT1 (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 methanol (10 μL), after which it was passed through Biogel P-4 or P-6 column. Fractions containing product were identified using TLC (dipping into the anisaldehyde stain followed by charring at 150 ºC), were combined and lyophilized to give products as a white cotton-like solid.

4.4 Enzymatic Reactions on Glycans

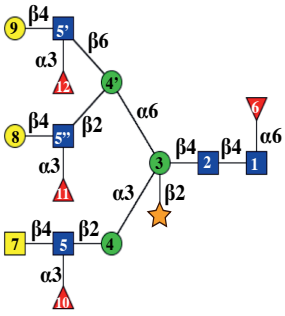
Glycan 29: Glycan **8** (5.0 mg) was dissolved in 100 mM NaOAc buffer (500 μL, pH~5.0) followed by adding 10 mM deoxyfuconojirimycin hydrochloride (20 μL) and *Helix pomatia* β-mannosidase (10 μL) the reaction mixture was then incubated at 37 ºC for 12 h. After this time the reaction mixture was heated 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 combined and lyophilized. This glycan **28** and UDP-Gal (4 eq) were dissolved in Tris buffer (50 mM, pH~7.5) containing BSA (0.1%) and $MnCl_2$ (20 mM). CIAP (10 mU) and B4GalT1 (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 methanol (10 μL), after which it was passed through Biogel P-4 column. Fractions containing product were combined and lyophilized to give the product **29** as a white fluffy solid (5.4 mg). MALDI-TOF-MS (*m/z*): $[M+ Na]^+$ calculated for $C_{89}H_{148}N_6O_{64}Na$, 2347.8409; found 2348.1302.



29	H1	H2	H3	H4	H5	H6	Fuc-CH_3
GlcNAc-1α	5.06 (d, *J* = 3.1 Hz)	3.78	3.88	3.66	NA	NA	–
Man-4	5.03	4.02	3.78	3.39	NA	3.79, 3.49	–
Man-3	4.75	4.14	3.76	3.69	3.76	3.83, 3.58	–
Man-4'	4.74	3.97	3.74	3.30	3.68	4.10, 3.46	–
GlcNAc-1β	4.57 (d, *J* = 8.2 Hz)	3.59	3.61	NA	NA	NA	–
GlcNAc-2	4.55	3.65	3.64	3.51	NA	NA	–
GlcNAc-5"	4.46	3.62	3.52	3.61	3.37	NA	–
GlcNAc-5'	4.43 (d, *J* = 8.3 Hz)	3.68	3.52	3.62	3.37	NA	–
GlcNAc-5	4.40	3.66	3.45	3.62	3.38	NA	–

GalNAc-7	4.40	3.82	3.62	3.83	NA	NA	–
Gal-8	4.37	3.42	3.56	3.81	NA	NA	–
Gal-9	4.35	3.42	3.55	3.80	NA	NA	–
Xyl (core)	4.32 (d, *J* = 7.4 Hz)	3.27	3.33	NA	3.13, 3.89	–	–
Fuc-6 (core) α,β	4.78	3.67	3.78	NA	3.98, 4.02	–	1.10

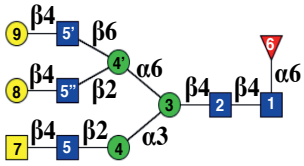
Glycan 1: Glycan **29** (5.4 mg) and GDP-Fucose (6 eq) were dissolved in Tris buffer (50 mM, pH~7.3) containing $MnCl_2$ (10 mM), CIAP (10 mU). Recombinant α(1,3)-Fuctosyltransferase FUT5 (6.6 mU/μmol of substrate) was added to achieve a final concentration of 4 mmol. The resulting mixture was incubated at 37 ºC for 12 h. After this time, additional GDP-Fucose (2 eq), CIAP (10 mU) and enzymes were added and incubation at 37 ºC was continued until no more starting material could be detected by MALDI-TOF. The reaction mixture was quenched by adding methanol (10 μL), after which it was passed through Biogel P-6 column. Fractions containing the product were combined and lyophilized to give the product as a white fluffy solid. This product was put for HPLC purification using HILIC column, using solvent gradient ACN: H_2O (90: 10, v: v to 50: 50, v: v). The fractions containing the product were concentrated and lyophilized yielding the purified glycan **1** as a white cotton like fluffy solid (3.0 mg). MALDI-TOF-MS (*m/z*): $[M+ Na]^+$ calculated for $C_{107}H_{178}N_6O_{76}Na$, 2786.0146; found 2786.7738.



1	**H1**	**H2**	**H3**	**H4**	**H5**	**H6**	**Fuc-CH₃**
GlcNAc-1α	5.10 (d, *J* = 3.1 Hz)	3.81	3.90	3.67	NA	NA	–
Man-4	5.06	4.05	3.81	3.42	3.47	3.84, 3.51	–
Man-3	4.79	4.18	3.80	3.69	3.78	3.83, 3.60	–
Man-4'	4.76	4.00	3.77	3.32	3.69	4.12, 3.48	–
GlcNAc-1β	4.61 (d, *J* = 8.3 Hz)	3.63	3.66	NA	NA	NA	–
GlcNAc-2	4.59	3.68	3.67	3.53	NA	NA	–

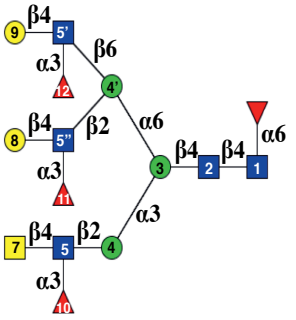
GlcNAc-5”	4.52	3.65	3.56	3.62	3.43	NA	–
GlcNAc-5’	4.48 (d, *J* = 7.1 Hz)	3.72	3.56	3.63	3.42	NA	–
GlcNAc-5	4.43 (d, *J* = 8.3 Hz)	3.69	3.48	3.63	3.43	NA	–
GalNAc-7	4.38	3.85	3.64	3.85	NA	NA	–
Gal-8	4.37	3.44	3.57	3.82	NA	NA	–
Gal-9	4.37	3.43	3.56	3.80	NA	NA	–
Xyl (core)	4.36	3.32	3.32	NA	3.17, 3.93	–	–
Fuc-6 (core) α,β	4.82	3.71	3.82	NA	4.02, 4.06	–	1.14
Fuc-10	5.04	3.88	3.86	3.67	4.77	–	1.10
Fuc-11	5.04	3.84	3.82	3.65	4.80	–	1.18
Fuc-12	5.03	3.85	3.79	3.64	4.80	–	1.17

Glycan 31: Glycan **9** (5.0 mg) was dissolved in 100 mM NaOAc buffer (500 µL, pH~5.0) followed by adding 10 mM deoxyfuconojirimycin hydrochloride (20 µL) and *Helix pomatia* β-mannosidase (10 µL) the reaction mixture was then incubated at 37 ºC for 12 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 combined and lyophilized. This glycan **30** and UDP-Gal (4 eq) were dissolved in Tris buffer (50 mM, pH~7.5) containing BSA (0.1%) and $MnCl_2$ (20 mM). CIAP (10 mU) and B4GalT1 (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 methanol (10 µL), after which it was passed through Biogel P-4 column. Fractions containing product were combined and lyophilized to give the product as a white fluffy solid (5.5 mg). MALDI-TOF-MS (*m/z*): $[M+Na]^+$ calculated for $C_{84}H_{140}N_6O_{60}Na$, 2215.7458; found 2215.9522.



31	H1	H2	H3	H4	H5	H6	Fuc-CH_3
GlcNAc-1α	5.07 (d, *J*= 3.1 Hz)	3.78	3.88	3.69	NA	3.72, 3.60	–
Man-4	5.00	4.07	3.80	3.38	3.44	3.81, 3.49	–
Man-4'	4.76	3.97	3.75	3.29	3.70	4.09, 3.46	–
Man-3	4.65	4.14	3.67	3.71	3.43	3.81,3.63	–
GlcNAc-1β	4.58 (d, *J*= 8.0 Hz)	3.59	3.63	NA	NA	NA	–
GlcNAc-2	4.54	3.67	3.65	3.50	NA	NA	–
GlcNAc-5"	4.48 (d, *J*= 7.5 Hz)	3.66	3.38	3.61	3.38	NA	–
GlcNAc-5	4.44	3.64	3.47	3.61	3.39	NA	–
GlcNAc-5'	4.43	3.61	3.48	3.62	3.39	NA	
GalNAc-7	4.40 (d, *J*= 8.5 Hz)	3.82	3.60	3.82	NA	NA	
Gal-8	4.37	3.44	3.55	3.83	3.53	NA	–
Gal-9	4.35	3.42	3.54	3.83	3.52	NA	
Fuc-6 (core) α, β	4.78	3.69	3.81	3.68	3.98, 4.02	–	1.10

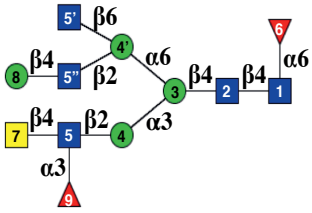
Glycan 2: Glycan **31** (5.5 mg) and GDP-Fucose (6 eq) were dissolved in Tris buffer (50 mM, pH~7.3) containing $MnCl_2$ (10 mM), CIAP (10 mU). Recombinant α(1,3)-Fuctosyltransferase FUT5 (6.6 mU/μmol of substrate) were added to achieve a final concentration of 4 mmol. The resulting mixture was incubated at 37 ºC for 12 h. After this time, additional GDP-Fucose (2 eq), CIAP (10 mU) and enzymes were added and incubation at 37 ºC was continued until no more starting material could be detected by MALDI-TOF. The reaction mixture was quenched by adding methanol (10 μL), after which it was passed through Biogel P-6 column. Fractions containing the product were combined and lyophilized to give the product as a white fluffy solid. This product was put for HPLC purification using HILIC column, using solvent gradient ACN: H_2O (90: 10, v: v to 50: 50, v: v). The fractions containing the product were concentrated and lyophilized yielding the purified glycan **2** as a white cotton like



fluffy solid (3.0 mg). MALDI-TOF-MS (*m/z*): $[M+ Na]^+$ calculated for $C_{102}H_{170}N_6O_{72}Na$, 2653.9723; found 2654.01917.

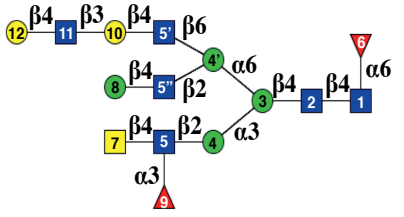
2	H1	H2	H3	H4	H5	H6	Fuc-CH_3
GlcNAc-1α	5.06 (d, *J* = 2.9 Hz)	3.77	3.86	3.69	NA	3.72, 3.61	–
Man-4	5.00	4.05	3.78	3.39	3.44	3.81, 3.48	–
Man-4'	4.74	3.96	3.74	3.30	3.69	4.09, 3.46	–
Man-3	4.65	4.14	3.67	3.71	3.46	3.81, 3.65	–
GlcNAc-1β	4.48 (d, *J* = 8.1 Hz)	3.58	3.62	NA	NA	NA	–
GlcNAc-2	4.54	3.66	3.65	3.51	NA	NA	–
GlcNAc-5"	4.48	3.71	3.41	3.65	NA	NA	–
GlcNAc-5	4.45	3.72	3.43	3.66	NA	NA	–
GlcNAc-5'	4.43	3.76	3.46	3.63	3.38	NA	–
GalNAc-7	4.35	3.85	3.63	3.82	NA	NA	–
Gal-8	4.33	3.53	3.55	3.85	3.52	NA	–
Gal-9	4.33	3.56	3.59	3.89	3.55	NA	–
Fuc-6 (core) α, β	4.77	3.68	3.81	3.68	3.98, 4.01	–	1.10
Fuc-10	5.01	3.83	3.82	3.64	4.76	–	1.15
Fuc-11	5.00	3.80	3.78	3.62	4.72	–	1.06
Fuc-12	4.98	3.82	3.75	3.61	4.73	–	1.06

Glycan 32: Glycan **9** (7.0 mg) and GDP-Fucose (2 eq) were dissolved in Tris buffer (50 mM, pH~7.3) containing $MnCl_2$ (10 mM), CIAP (10 mU). Recombinant α(1,3)-Fuctosyltransferases FUT5 (6.6 mU/μmol of substrate) were added to achieve a final concentration of 4 mmol. The resulting mixture was incubated at 37 ºC for 12 h. The reaction mixture was quenched by adding methanol (10 μL), after which it was passed through Biogel P-4 column. Fractions containing the product were combined and lyophilized to give the product as a white fluffy solid (7.0 mg). MALDI-TOF-MS (*m/z*): $[M+ Na]^+$ calculated for $C_{84}H_{140}N_6O_{59}Na$, 2199.8037; found 2200.0307.



32	H1	H2	H3	H4	H5	H6	Fuc-CH_3
GlcNAc-1α	5.07 (d, *J* = 2.2 Hz)	3.78	3.88	3.69	NA	3.72, 3.60	–
Man-4	4.99	4.06	3.80	3.38	3.43	3.81, 3.49	–
Man-4'	4.75	3.98	3.76	3.28	3.70	4.10, 3.47	–
Man-3	4.65	4.14	3.67	3.72	3.41	3.80, 3.63	–
Man-8	4.65	3.96	3.55	NA	NA	NA	–
GlcNAc-1β	4.58 (d, *J* = 8.2 Hz)	3.59	3.63	NA	NA	NA	–
GlcNAc-2	4.55	3.66	3.65	3.50	NA	NA	–
GlcNAc-5"	4.48 (d, *J* = 6.5 Hz)	3.61	3.38	3.61	3.38	NA	–
GlcNAc-5	4.44	3.63	3.46	3.60	3.39	NA	–
GlcNAc-5'	4.41	3.60	3.47	3.61	3.39	NA	–
GalNAc-7	4.33 (d, *J* = 8.4 Hz)	3.86	3.62	3.82	NA	NA	–
Fuc-6 (core) (α, β)	4.78	3.68	3.81	3.68	3.98, 4.02	–	1.10
Fuc-9	5.02 (d, *J* = 3.4 Hz)	3.84	3.82	3.66	4.75	–	1.15

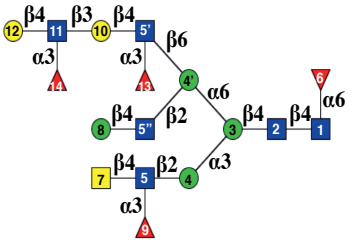
Glycan 34: Glycan **32** (7.0 mg) and UDP-Gal (2 eq) were dissolved in Tris buffer (50 mM, pH~7.5) containing BSA (0.1%) and $MnCl_2$ (20 mM). CIAP (10 mU) and B4GalT1 (3.4 mU/μmol) were added to achieve a final concentration of 4 mM. The reaction mixture was then incubated at 37 ºC for 10 h. The reaction mixture was quenched by adding methanol (10 μL), after which it was passed through Biogel P-4 column. Fractions containing product were combined and lyophilized to give the glycan **33** (7.5 mg). This glycan and UDP-GlcNAc (2 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 B3GNT2 (6.0 mU/μmol) were added to achieve a final concentration of glycan at 4 mM. The resulting mixture was then incubated at 37 ºC for 12 h. The reaction mixture was quenched by adding methanol (10 μL) and passed through Biogel P-6 column. The fractions containing product were combined and lyophilized to give the intermediate



glycan, which was again put for galactosylation reaction using the conditions described above, yielding glycan **34** (8.0 mg). This product was put for HPLC purification using HILIC column, using solvent gradient ACN: H_2O (90: 10, v: v to 50: 50, v: v). The fractions containing the product were concentrated and lyophilized yielding the purified glycan as a white cotton like fluffy solid (5.0 mg). MALDI-TOF-MS (*m/z*): $[M+ Na]^+$ calculated for $C_{104}H_{173}N_7O_{74}Na$, 2726.9887; found 2727.1029.

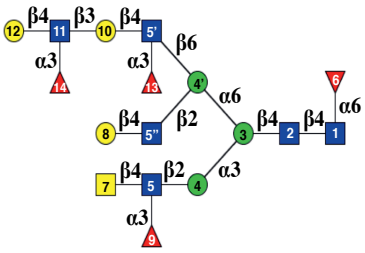
34	**H1**	**H2**	**H3**	**H4**	**H5**	**H6**	**Fuc-CH_3**
GlcNAc-1α	5.10 (d, *J* = 3.0 Hz)	3.80	3.93	3.72	NA	3.73, 3.59	–
Man-4	5.02	4.09	3.82	3.41	3.45	3.83, 3.52	–
Man-4'	4.79	3.99	3.79	3.32	3.73	4.12, 3.50	–
Man-3	4.67	4.17	3.68	3.75	3.43	3.83, 3.66	–
Man-8	4.68	3.98	3.56	NA	NA	NA	–
GlcNAc-11	4.62	3.71	3.57	3.70	NA	NA	–
GlcNAc-1β	4.60	3.62	3.65	NA	NA	NA	–
GlcNAc-2	4.58	3.69	3.67	3.53	NA	NA	–
GlcNAc-5"	4.51 (d, *J* = 7.2 Hz)	3.64	3.40	3.65	3.42	NA	–
GlcNAc-5	4.46	3.65	3.48	3.63	3.41	NA	–
GlcNAc-5'	4.46	3.67	3.54	3.66	3.42	NA	–
GalNAc-7	4.39	3.89	3.64	3.85	NA	NA	–
Gal-10	4.38	3.50	3.59	3.88	3.57	NA	–
Gal-12	4.36	3.46	3.58	3.88	3.55	NA	–
Fuc-6 (core) (α, β)	4.81	3.72	3.84	3.72	4.01, 4.05	–	1.13
Fuc-9	5.05 (d, *J* = 3.8 Hz)	3.86	3.85	3.68	4.79	–	1.18

Glycan 35: Glycan **34** (5.0 mg) and GDP-Fucose (4 eq) were dissolved in Tris buffer (50 mM, pH~7.3) containing $MnCl_2$ (10 mM), CIAP (10 mU). Recombinant α(1,3)-Fuctosyltransferase FUT5 (6.6 mU/μmol of substrate) was added to achieve a final concentration of 4 mmol. The resulting mixture was incubated at 37 ºC for 12 h. After this time, additional GDP-Fucose (2 eq), CIAP (10 mU) and FUT5 enzyme was added and incubation at 37 ºC was continued until no more starting material could be detected by MALDI-TOF. The reaction mixture was quenched by adding methanol (10 μL), after which it was passed through Biogel P-6 column. Fractions containing the product were combined and lyophilized to give the product as a white fluffy solid (5.6 mg). MALDI-TOF-MS (*m/z*): $[M+ Na]^+$ calculated for $C_{116}H_{193}N_7O_{82}Na$, 3019.1045; found 3019.4936.



35	H1	H2	H3	H4	H5	H6	Fuc-CH_3
GlcNAc-1α	5.07 (d, *J* = 3.1 Hz)	3.78	3.90	3.69	NA	3.72, 3.58	–
Man-4	4.99	4.05	3.79	3.38	3.43	3.81, 3.49	–
Man-4'	4.78	3.96	3.75	3.29	3.69	4.09, 3.47	–
Man-3	4.66	4.14	3.66	3.73	3.42	3.81, 3.65	–
Man-8	4.65	3.95	3.54	NA	NA	NA	–
GlcNAc-11	4.59	3.74	3.59	3.72	NA	NA	–
GlcNAc-1β	4.58	3.60	3.63	NA	NA	NA	–
GlcNAc-2	4.55	3.65	3.64	3.51	NA	NA	–
GlcNAc-5"	4.48 (d, *J* = 7.4 Hz)	3.60	3.38	3.63	NA	NA	–
GlcNAc-5	4.44	3.71	3.43	3.59	3.38	NA	–
GlcNAc-5'	4.43	3.76	3.52	3.63	3.39	NA	–
GalNAc-7	4.34	3.86	3.61	3.83	NA	NA	–
Gal-10	4.34	3.54	3.56	3.85	3.53	NA	–
Gal-12	4.33	3.55	3.60	3.91	3.56	NA	–
Fuc-6 (core) (α, β)	4.75	3.69	3.81	3.68	3.97, 4.02	–	1.09
Fuc-9	5.02	3.82	3.80	3.64	4.75	–	1.15
Fuc-13	5.02	3.80	3.78	3.61	4.72	–	1.06
Fuc-14	4.99	3.77	3.76	3.60	4.71	–	1.04

Glycan 3: Glycan **35** (5.6 mg) was dissolved in 100 mM NaOAc buffer (500 µL, pH~5.0) followed by adding 10 mM deoxyfuconojirimycin hydrochloride (20 µL) and *Helix pomatia* β-mannosidase (10 µL) the reaction mixture was then incubated at 37 ºC for 12 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 combined and lyophilized. This glycan **36** and UDP-Gal (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 B4GalT1 (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 methanol (10 µL), after which it was passed through Biogel P-4 column. Fractions containing product were combined and lyophilized to give the product as a white fluffy solid (5.0 mg). This product was put for HPLC purification using HILIC column, using solvent gradient ACN: H_2O (90: 10, v: v to 50: 50, v: v). The fractions containing the product were concentrated and lyophilized yielding the purified glycan **3** as a white cotton like fluffy solid (2.5 mg). MALDI-TOF-MS (*m/z*): $[M+ Na]^+$ calculated for $C_{116}H_{193}N_7O_{82}Na$, 3019.1045; found 3019.3130.



3	H1	H2	H3	H4	H5	H6	Fuc-CH_3
GlcNAc-1α	5.06 (d, *J* = 2.8 Hz)	3.78	3.90	3.69	NA	3.71, 3.58	–
Man-4	4.99	4.05	3.79	3.38	3.43	3.81, 3.48	–
Man-4'	4.78	3.97	3.74	3.29	3.69	4.09, 3.47	–
Man-3	4.65	4.14	3.66	3.73	3.41	3.81, 3.65	–
GlcNAc-11	4.59	3.75	3.58	3.72	NA	NA	–
GlcNAc-1β	4.58	3.59	3.63	NA	NA	NA	–
GlcNAc-2	4.55	3.66	3.63	3.50	NA	NA	–
GlcNAc-5"	4.48 (d, *J* = 6.9 Hz)	3.60	3.38	3.63	NA	NA	–
GlcNAc-5	4.45	3.71	3.44	3.59	3.39	NA	–
GlcNAc-5'	4.43	3.76	3.52	3.63	3.38	NA	–
Gal-8	4.36	3.44	3.54	3.81	NA	NA	–
GalNAc-7	4.34	3.87	3.62	3.83	NA	NA	–
Gal-10	4.33	3.54	3.55	3.85	3.51	NA	–
Gal-12	4.33	3.55	3.60	3.91	3.55	NA	–
Fuc-6 (core) (α, β)	4.75	3.69	3.81	3.68	3.99, 4.02	–	1.10

Fuc-9	5.02	3.83	3.81	3.64	4.76	–	1.15
Fuc-13	5.02	3.80	3.78	3.61	4.72	–	1.06
Fuc-14	4.98	3.77	3.76	3.60	4.71	–	1.04

5. Molecular interaction studies by NMR

5.1 Protein expression and purification. The extracellular domain of DC-SIGN was obtained as previously described.[38] The carbohydrate recognition domain of DC-SIGN in its ^{15}N labelled form was obtained as previously described.[39]

5.2 ^{1}H Saturation transfer difference (STD) NMR. The samples for saturation-transfer difference (STD) NMR experiments were prepared using the extracellular domain of DC-SIGN at 10 μM concentration in 25 mM Tris-d11, 150 mM NaCl, 4 mM $CaCl_2$ in D_2O (pD 8) using lectin/ligand ratios of 1:50. The temperature was set to 298 K. STD experiments were performed at 600 MHz Bruker spectrometer, using standard Bruker pulse sequences with water suppression by using the excitation sculpting and without protein spin-lock filter. Protein saturation was achieved with a Gaussian-shaped pulse of 49 ms (Gauss 1.1000, with a power of $1e^{-05}$ W). Different irradiation frequencies for only the ligand and in presence of the protein (Fig. S2 a and b, respectively) were tested. We found that the irradiation at 0.4 ppm was the best compromise between optimal protein saturation and minimal ligand direct irradiation. Thus, the on- resonance frequency was set at aliphatic regions (0.4 ppm) and the off-resonance frequency at 100 ppm. Blank STD experiments of the ligands alone were acquired in the same conditions. The results of blank ^{1}H-STD NMR experiments for ligands **1-3** are shown in Figs S3, S4 and S5 respectively.

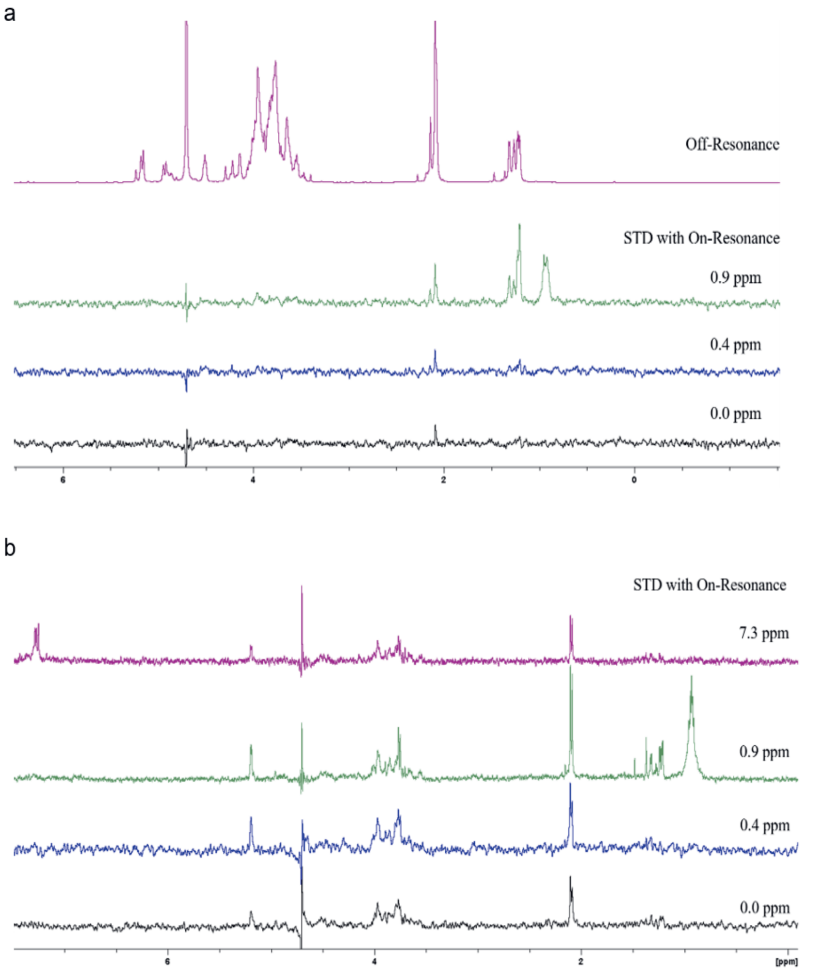


Figure S2. Different irradiation frequency tested for ^{1}H-STD NMR experiments. (a) Only the ligand. (b) In the presence of the protein.

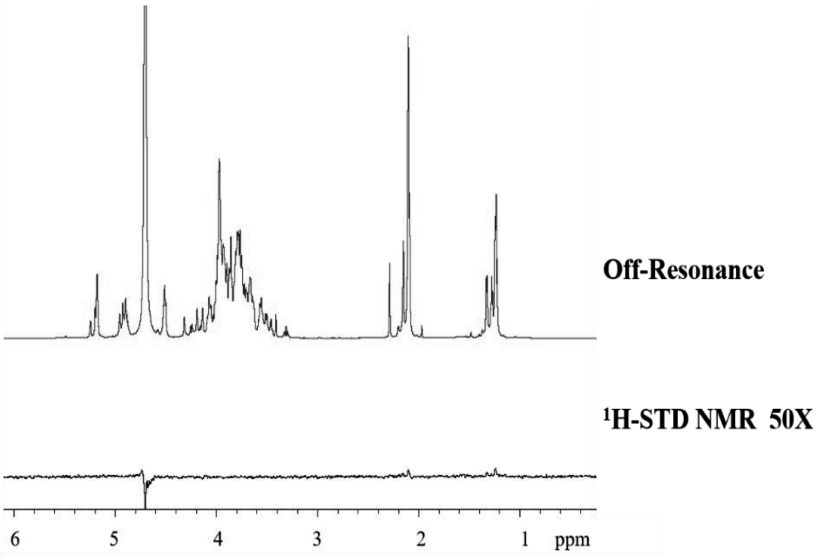


Figure S3. Blank ^{1}H-STD experiment of the free ligand **1**. Ligand concentration is 500 µM in D_2O.

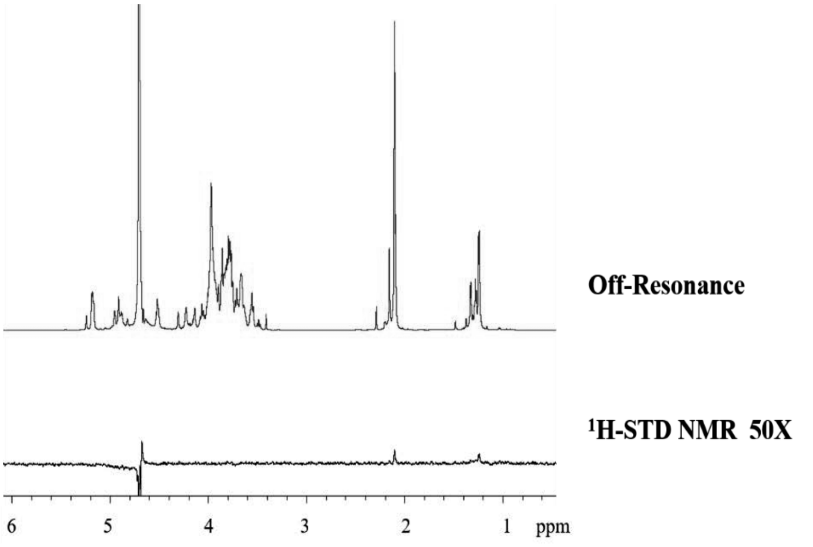


Figure S4. Blank ^{1}H-STD experiment of the free ligand **2**. Ligand concentration is 500 µM in D_2O.

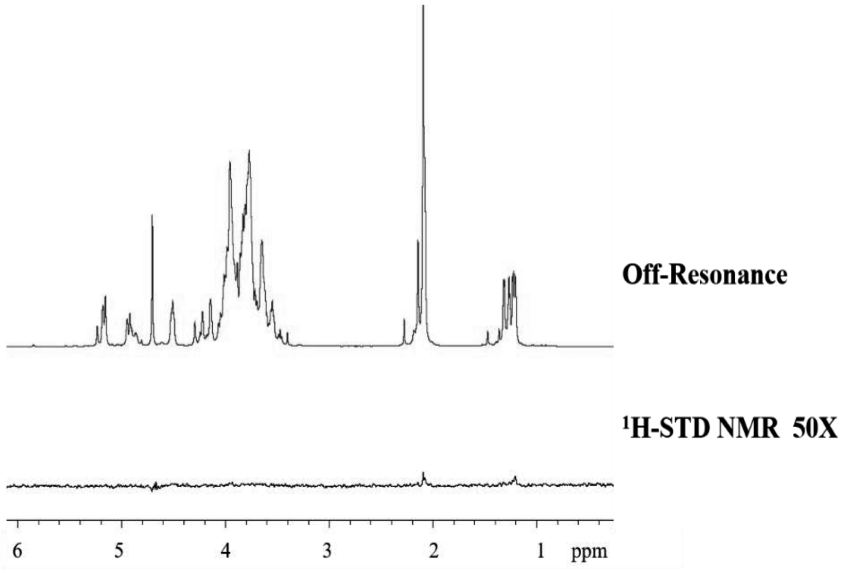


Figure S5. Blank ^{1}H-STD experiment of the free ligand **3**. Ligand concentration is 500 µM in D_2O.

5.3 ^{19}F CPMG NMR. ^{19}F CPMG spectra were acquired in a 600 MHz spectrometer equipped with a Bruker selective ^{19}F–^{1}H decoupling (SEF) probe at 298 K, on samples containing 8 mM of DC-SIGN ECD, the concentration is calculated with respect to the tetramer, and 0.8 mM of the fluorinated probes (2-fluoro-Fucose). Competitors glycans **1-3** were successively added to the sample at a final concentration of 0.4 mM. All samples were measured in buffer 25 mM Tris-d11, 150 mM NaCl, 4 mM $CaCl_2$ in H_2O or D_2O (pH/D 8). The standard CPMG Bruker pulse sequence was modified as described.[40] Twenty four (24) points were acquired with total echo times from 8 to 5200 ms, with τ = 2 ms. Data were analyzed with the T1T2 relaxation module of Topspin3.5.

5.4 Chemical shift perturbation analysis. ^{1}H-^{15}N-HSQC-based experiments were performed using ^{15}N-labeled CRD DC-SIGN at 50 µM, with 2 mM DTT-d10, at 800 MHz Bruker spectrometer equipped with a cryoprobe, at 310 K. Five titration points were acquired for ligands **1-3**, with ligand concentrations varying from 0.0 to 1.0 mM. Averaged chemical shift perturbation (CSP) was calculated using the CcpNmr Analysis 2.4.2.3. The chemical shift perturbation analysis was performed based on the protein backbone assignment deposited in the BMRB database with the code 27854.

5.5 Receptor-based NMR experiments. The CSPs induced on the ^{15}N labeled DC-SIGN (CRD) cross peaks by titration with compound (1-3) were compared (Figure S6). The observed perturbations took place at the primary carbohydrate binding site, which is composed by residues in an extended loop and in β-strand-4 (from W343 to D355 and from N363 to D367, respectively) and at the secondary binding region (from E356 to G361).

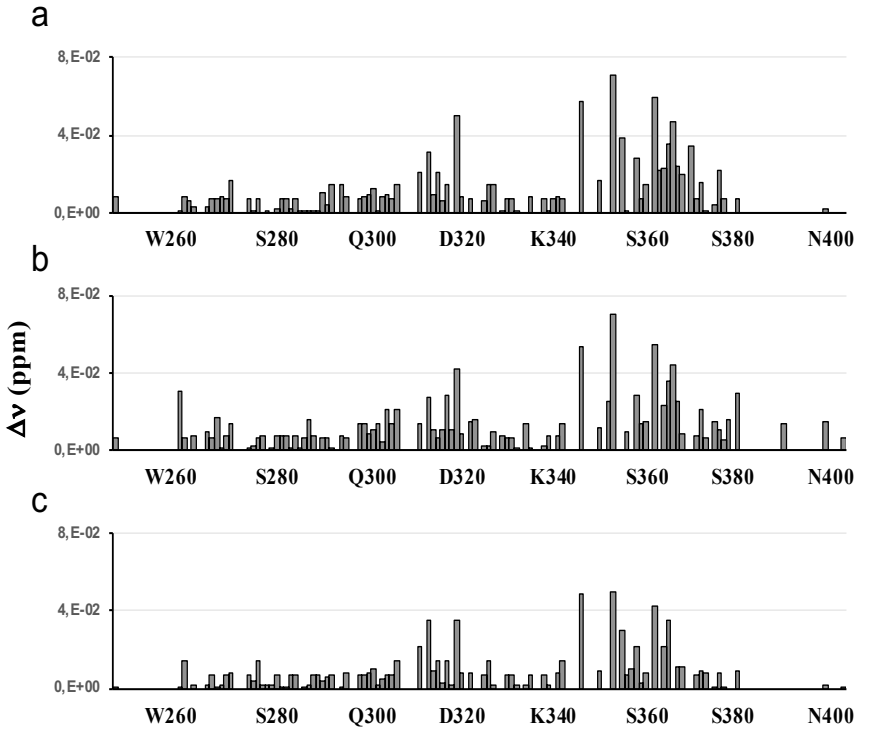


Figure S6. Average chemical shift perturbation (CSPs) produced on the ^{1}H-^{15}N resonances of the DC-SIGN (CRD) upon the addition of 20 eq. of (a) glycan (**1**), (b) glycan (**2**) and (c) glycan (**3**).

6. Molecular Modeling

6.1 Molecular Dynamic Simulations. Initial geometries of ligands 1-3 were built in the Glycam web (http://glycam.org). The MD simulations were performed using the Amber16 program4 with the GLYCAM_06h force field parameters. Thereafter, the starting 3D geometries were placed into a 10 Å octahedral box of explicit TIP3P waters, and counterions were added to maintain electroneutrality. Two consecutive minimization stages were performed involving (1) only the water molecules and ions and (2) the whole system with a higher number of cycles, using the steepest descent algorithm. The system was subjected to two rapid molecular dynamic simulations (heating and equilibration) before starting the real dynamic simulation. The equilibrated structures were the starting points for the final MD simulations at constant temperature (300 K) and pressure (1 atm). 100 ns Molecular dynamics simulations without constraints were recorded, using an NPT ensemble with periodic boundary conditions, a cut-off of 10 Å, and the particle mesh Ewald method. A total of 50 000 000 molecular dynamics steps were run with a time step of 1 fs per step. Coordinates and energy values were recorded every 50000 steps (50 ps) for a total of 1 000 MD models. The detailed analysis of the glycosydic linkages for glycans 1-3 was performed along the MD trajectory using the cpptraj module included in Amber-Tools 16 package.

6.2 Conformational analysis. We performed molecular modelling studies to determine the main conformational distribution of the synthetized glycans **1**, **2** and **3**. Molecular dynamic (MD) simulation were performed for each compound during 100 ns. The short MD trajectory satisfactory explored the expected conformational distribution of triantennary glycans, with the main flexibility at the O5C5C6O6 w dihedral angles. All the f dihedral angles respected the *exo*-anomeric effect, while the y dihedral angles showed the expected wider distribution (data not shown). The distribution of the w dihedral angles along the MD trajectory of compound **1** is shown in figure S6. The presence of the xylose ring does not hamper the expected flexibility of branched glycans. Both dihedral angles mainly populate the gt and gg conformations, giving rise to four main conformers. The four main conformers (gg;gt), (gt;gt), (gg;gg) and (gt;gg) are represented for compounds **1** and **3** in figure S7.

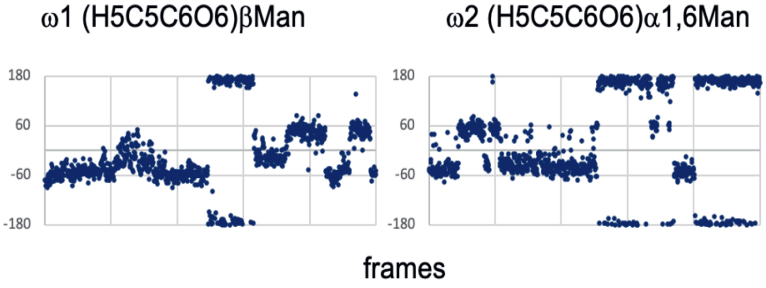


Figure S7. Map of the w dihedral angles for glycan 1 as generated by analysis along the 100 ns MD simulation.

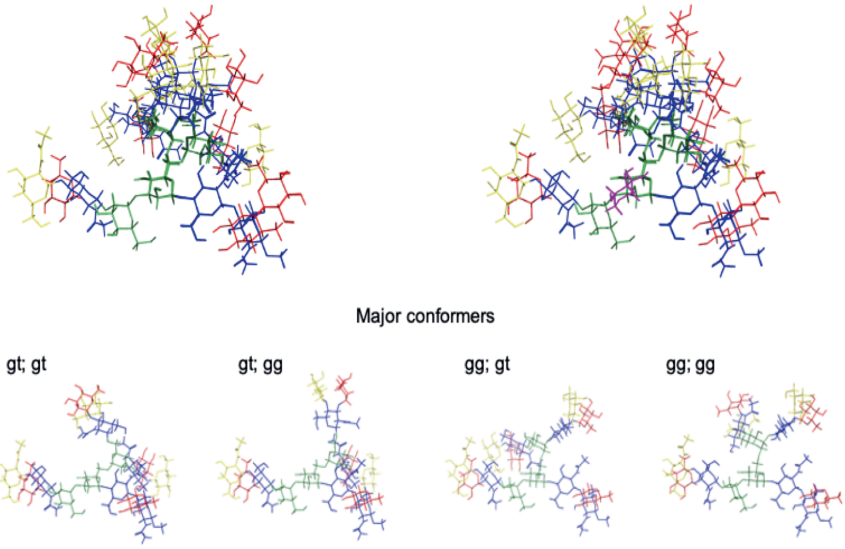


Figure S8. Representation of the average conformational distribution for glycan **1** (right) and **2** (left). The main conformers are defined as gt;gt (w1 -60, w2 -60), gt;gg (w1 -60, w2 ±180), gg;gt (w1 ±180, w2 -60) and gg;gg (w1 ±180, w2 ±180). The structures are derived from MD simulations study.

6.3 Modelling of the bound states. The initial pdb coordinates for CRD of DC-SIGN were derived from the crystal structure Protein Database (PDB) 1SL5, while the model of DC-SIGN ECD was generated as previously described.[41] Briefly, to build the model of the protein's head, the DC-SIGN CRD (PDB code 1sl5) was superimposed on the mannose-binding protein CRD (PDB code 1hup), which represents the prototype of the C-type family of lectin and for which an oligomeric structure is available. Four copies of DC-SIGN CRD in this orientation were generated. To model the neck, 8 repeating units of 23 residues (PDB code 3JQH) were linearly assembled and four of those were arranged as the four a-helices observed in the structure of the tetramer of DC-SIGNR CRDs (PDB code 1xar). We underline that our model for the DC-SIGN ECD tetramer is an approximation. However, the generated model satisfactorily resemble the one previously described, which is derived from SAXS data and nicely fits the apo DC-SIGN structure as visualized by negative-stain technique. To generate protein/ ligand complexes, the fucose pyranose rings of the Le^x and LDN-F motives of glycans **1-3** were manually superimposed onto the corresponding sugar in the deposited 1sl5 structure. Glycans structures used to model the binding poses were derived from the conformational study described above. All alternative binding poses were generated for each ligand and are represented in figure S8.

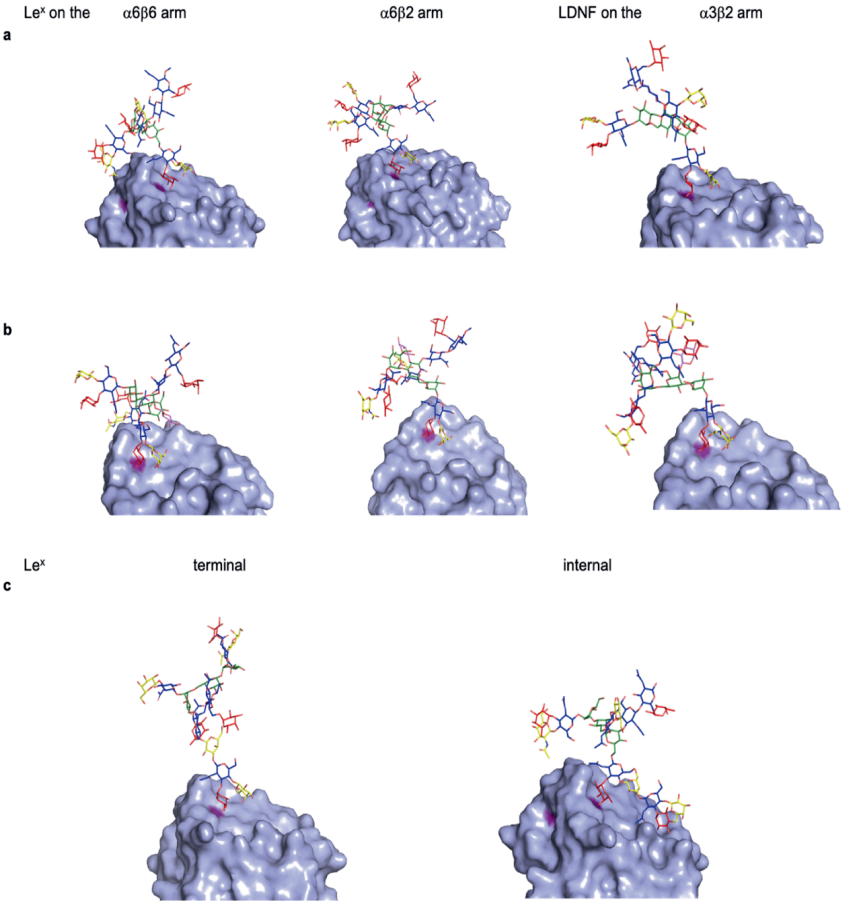


Figure S9. Models of the binding mode for the complex between CRD DC-SIGN and the glycans (a) **2**, (b) **1** and (c) **3**. All possible binding models are represented for compounds **1** and **3**, each corresponding to a different arm of the triantennary glycans. Two alternative binding modes are presented for the glycan **3**, one in which the terminal Le^x motif is recognized and the second in which the internal Le^x is into the binding pocket.

7. EM sample preparation and TEM data collection

Sample and cryo-grid optimization were performed using the in-house EM facilities. The apo DC-SIGN sample (4ul at 1.5mg/ml) and the samples of DC-SIGN in presence of the ligands **1** and **2**, at 1 to 50 molar ratio (4ul at 1.5mg/ml protein and 1.4mg/ml ligand) were pipetted onto 200-mesh Quantifoil R 2/2 Cu 200. The grids were vitrified using the automated vitrification robot Vitrobot (Mark III-FEI). Different blot pad position and blotting times were tested with offset number 0 and 3 s blotting giving satisfactory results in terms of ice thickness relative to particle's size. All the samples were prepared in triplicate and the grids were inspected on a JEM-2200FS/CR (JEOL, Ltd.) electron microscope operating at 200kV field emission gun (FEG) and equipped with an omega in-column energy filter. About 50 cryo-images were recorded on an UltraScan 4000 SP (4008x4008 pixels) cooled slow-scan CCD camera (GATAN) at defocus of -2.5/ -3.0 micron with a total dose of 20 e–/Å^2 at a nominal magnification of ×50,000, producing a pixel size at the specimen of 2.0 Å.

For negative-stain EM the sample was applied to glow-discharged carbon-coated copper grids and stained with 2% (w/v) uranyl acetate. Micrographs were taken under low dose condition on a JEOL JEM-1230 LaB6 transmission electron microscope operated at 100 kV and equipped with an Orius SC1000 CCD camera. More than 100 micrographs with a nominal magnification of 40,000 (1.78 Å per pixel) were recorded. A set of 1,086, particles of the sample were manually selected and this set of particles was subjected to an iterative reference-free two-dimensional alignment and classification procedure using the Relion algorithm in the Scipion package.

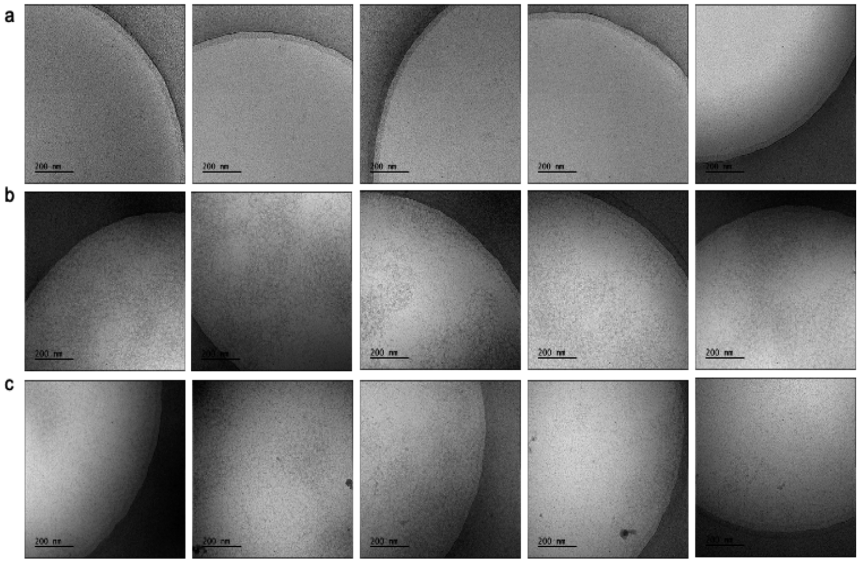


Figure S10. Cryo-EM images acquired for (a) apo DC-SIGN, (b) DC-SIGN in presence of ligand **1** and (c) ligand **2**. Nominal magnification is ×50,000. Bar, 200 nm.

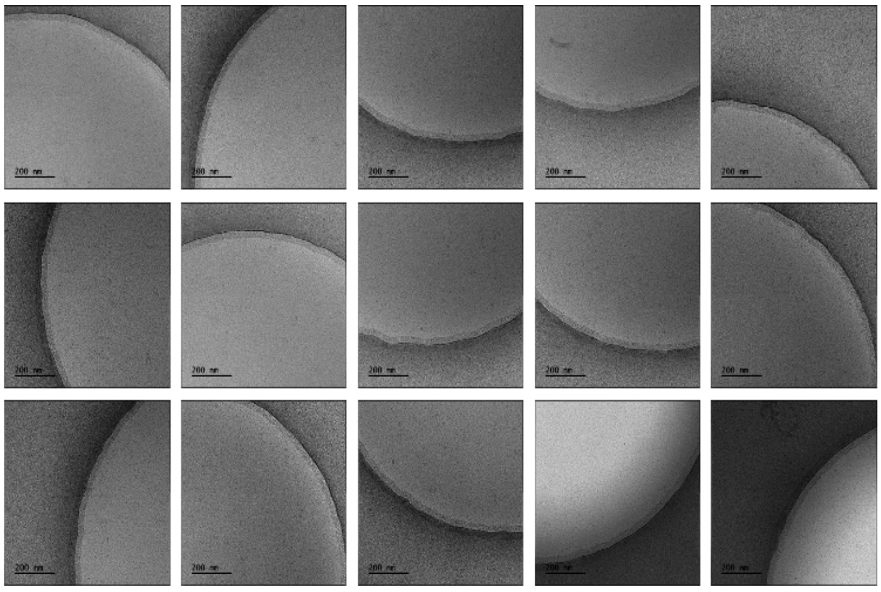


Figure S11. Cryo-EM images acquired for *apo* DC-SIGN. Nominal magnification is ×50,000. Bar, 200 nm.

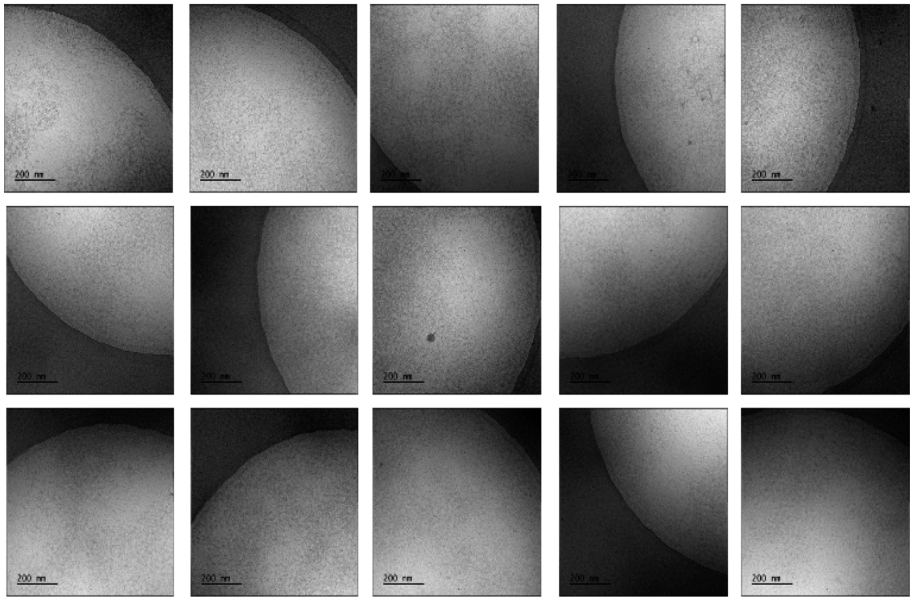


Figure S12. Cryo-EM images acquired for DC-SIGN in complex with ligand **1**. Nominal magnification is ×50,000. Bar, 200 nm.

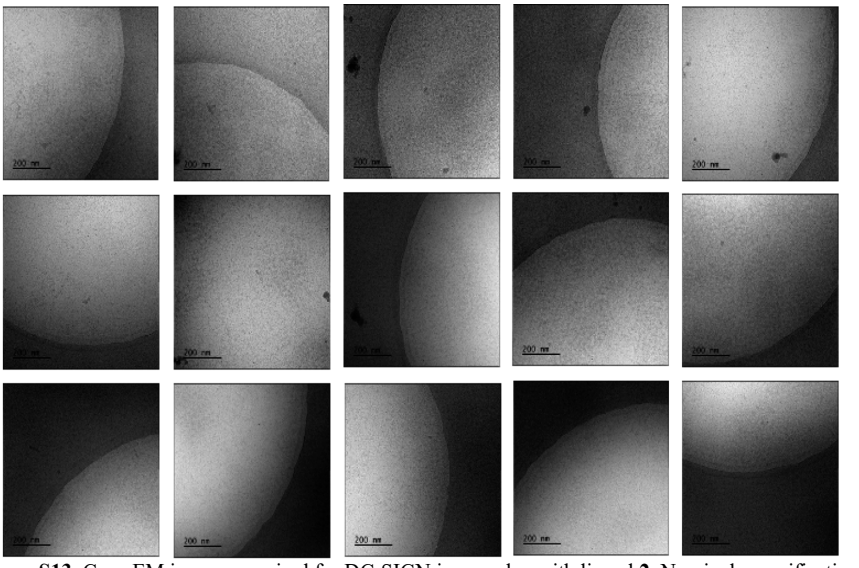


Figure S13. Cryo-EM images acquired for DC-SIGN in complex with ligand **2**. Nominal magnification is ×50,000. Bar, 200 nm.

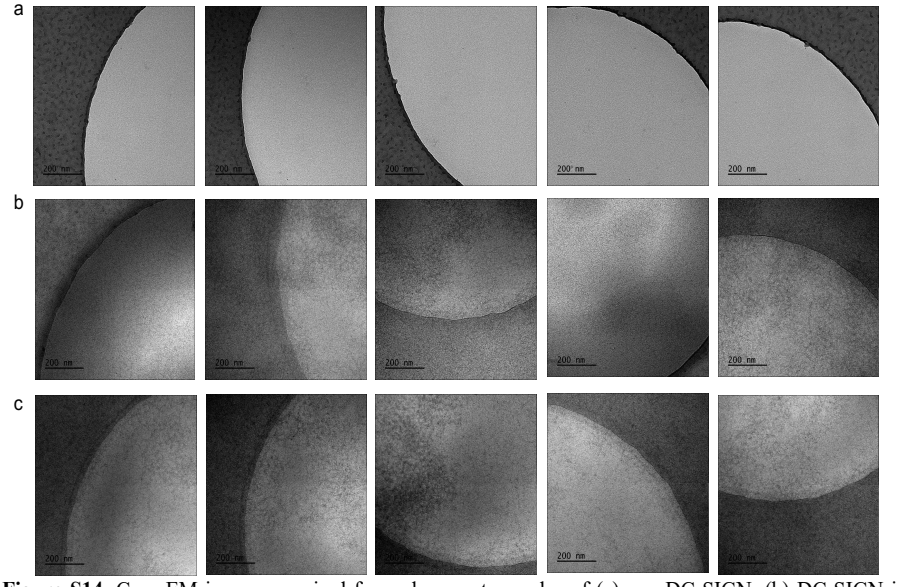


Figure S14. Cryo-EM images acquired for carbon coat samples of (a) apo DC-SIGN, (b) DC-SIGN in presence of ligand **1** and (c) ligand **2**. Nominal magnification is ×50,000. Bar, 200 nm.

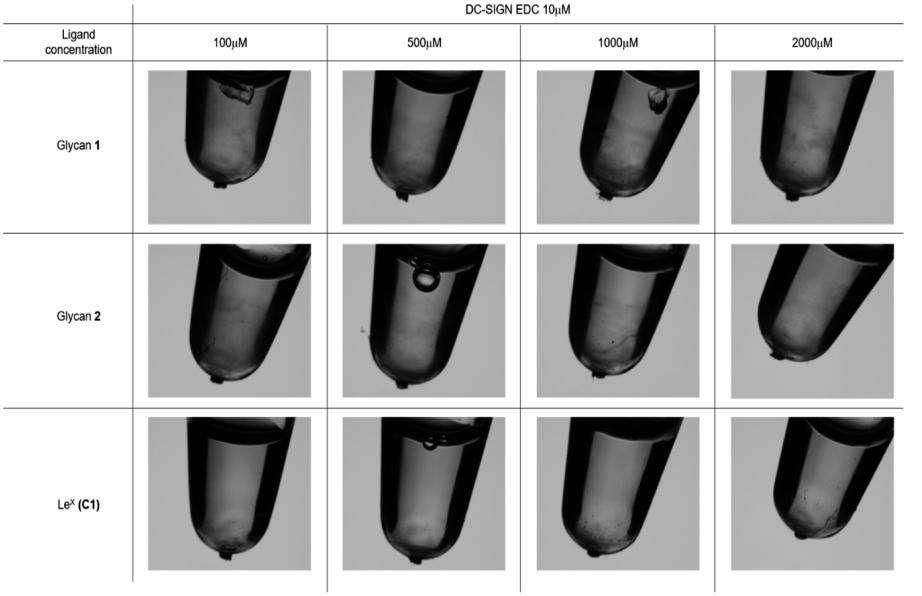


Figure S15. Pictures of the samples containing DC-SIGN (ECD) in the presence of different concentrations of ligands **1**, **2** and **C1**. The presence of aggregates are visible for ligands **1** and **2**, but not for ligand **C1**.

DC-SIGN EDC 10 μM	Absorbance at 600 nM			
Ligand Concentration	100 μM	500 μM	1000 μM	2000 μM
Glycan **1**	0.27	0.25	0.17	0.18
Glycan **2**	0.27	0.20	0.18	0.16
Le^X (**C1**)	0.04	0.00	0.04	0.06

Table S2. Absorbance at 600 nm.

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CHAPTER 5

Synthesis and micro-array studies of a library of characteristic *N*-glycans of *S. mansoni*

Abstract. *N*-glycans of *S. mansoni* are highly complex carbohydrates that cover the pathogen cell surface and mediate host-pathogen interaction. The dense coat of carbohydrates is made of uniquely branched glycans. Key epitopes for host infection are presented in multiple copy and variable presentation, which contribute to shaping host immune response. The chemoenzymatic synthesis of a library of asymmetrically branched multi-antennary glycans which are representative of the characteristic *S. mansoni N*-glycans is described here. The synthesized glycans account for multiple structural presentations of immunogenic epitopes. The access to a well-defined library of such characteristic glycans can be used as prospective candidates for testing antibody response against sera of infected patients and to use these as biological markers to identify the best ligands to activate the immune system towards a more desirable pro-inflammatory Th1 response.

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Introduction. The synthesis of three characteristic *N*-glycans of *S. mansoni* and their application as probes to study interactions with DC-SIGN is Chapter 4. It was demonstrated that epitope presentation is an important factor recognition by DC-SIGN. The importance of multivalency in *N*-glycans and protein interaction was established. These in-depth studies were made possible due to a carefully planned and strategically designed chemical route aided with enzymatic modifications which afforded previously un-attainable, asymmetrical, highly complex and pure *N*-glycans. To further establish the scope of the chemo-enzymatic strategy, a wider range of other characteristic *N*-glycans which differed in terminal epitopes and branching patterns was prepared.

Ito and co-workers[1] reported the first synthesis of *N*-glycans characteristic of eggs of *S. mansoni*, to gain access to well-defined carbohydrates to investigate their roles as adjuvants[2] towards a T_H2 immune response driven by *S. mansoni* upon infection.[3] Subsequently, in a convergent chemo-enzymatic approach, Reichardt and co-workers synthesized a library of *N*-glycans of *S. mansoni* and studied their binding with plant lectins, DC-SIGN and with serum antibodies of patients suffering from schistosomiasis.[4] Through the sera studies, they observed an age-dependent immune response towards a range of glycan motifs and immunologically relevant differences between children and adult groups infected with this parasite. Recently, they also observed differences in binding affinities of positional isomers of these glycans upon interaction with a range of C-type lectins.[5] Although these studies made it possible to study the antigenic properties of *N*-glycans, it did not allow to explore the importance of glycan architecture for immune responses because all the compounds were either symmetrically branched or truncated *N*-glycans.

Analytical studies have revealed that a majority of *N*-glycans are asymmetrically branched, and the *N*-glycans of *S. mansoni* express highly unusual glycosidic linkages and branching patterns.[6] The expression of many glycan elements appears to be developmentally regulated by the differential expression of glycosyltransferases during the different lifecycle stages. For example, the pentasaccharide core of *N*-glycans of *S. mansoni* is modified in a stage-dependent manner by motifs such as a core xyloside and fucoside.[6c, 7] Core xylosides are abundantly expressed in the *N*-glycan core during the egg and cercaria stage, but absent in the adult worm stage.[6c] However, till date the reason behind this is poorly understood. Further structural complexity arises due to varying extensions and branching patterns of the core pentasaccharide resulting in the so called "appendages" or "arms". The most common terminal epitopes consist of GalNAc β1,4GlcNAc (Lac-di-NAc) and Gal β1,4GlcNAc (LacNAc), often presented as repeating units which may be further elaborated with fucosides to display epitopes such as GalNAcβ1,4(Fucα1,3) GlcNAc (LDN-F) and Lewis-x (Le^x). The expression of these epitopes is also stage-dependent, with Le^x expression in cercariae shifting towards Lac-di-NAc based termini in *N*-glycans of adult worms.[8] This means that among the complex-glycans in adult worms, the Lac-di-NAc and LDN-F antennae are dominant. The *N*-glycans of *S. mansoni* are also marked by a characteristic lack of sialic acids which is a terminal epitope expressed extensively in eukaryotic *N*-glycans. Although the *N*-glycans of *S. mansoni* are marked with slight structural differences than their eukaryotic counterparts, they

use these minor differences to exhibit immune-evasion in hosts and evade detection for years.[9] We envisaged that the construction of a well-defined library of asymmetric *N*-glycans displaying epitopes expressed most abundantly over various stages in the life cycle of *S. mansoni*, would open avenues to identifying promising candidates that could generate antibodies and activate the immune response in hosts.

Results and Discussions. The construction of the library of glycans was conceived considering the stage-dependent expression of core xyloside and the diversity in expression of terminal epitopes such as LacNAc, poly-Le^x and LDN-F. We envisaged that core structures **1** and **2**, modified by the presence and absence of core xyloside respectively, would be appropriate starting material for the preparation of the desired library of asymmetrically branched *N*-glycans (Fig. 1). Thus, compounds **1** and **2**, were protected with orthogonal protecting groups levulinoyl (Lev), fluorenylmethyloxycarbonate (Fmoc), allyloxycarbonate (Alloc), and *t*-butyldimethylsilyl (TBS) at the typical branching points. Sequential removal of Fmoc, Lev and Alloc followed by glycosylation with the predetermined glycosyl donors **3**, **4** and **5**[10] and global deprotection were expected to result in glycan structures **7** and **8**. On the other hand, sequential removal of Fmoc, TBS and Lev, followed by glycosylation at the typical branching points with donors **3**, **4** and **6** would afford the tri-antennary positional isomer **9**. The glycan precursors **7**, **8** and **9** were furnished with the Lac-di-NAc moiety at the β(1,2) branch of α3 mannoside which could easily be converted to the LDN-F epitope upon treatment with fucosyl transferases. Further, the glycans consisted of a β(1,6) GlcNAc moiety at the α6 mannoside, which is unreactive to most glycosyl transferases except galactosyl transferases. At an appropriate stage, GlcNAc be converted to LacNAc and elaborated with glycosyl transfereases into a complex structure. To impart asymmetry, the other branching positions of the glycans were extended with either unnatural Galα(1,4)GlcNAc or Manβ(1,4)GlcNAc moiety. The unnatural galactoside or mannoside sugar moieties block modification by common mammalian glycosyl transferases, however, they can individually be unmasked by an appropriate glycosidase to expose the inner β-GlcNAc moiety, which can be converted into a desired terminal epitope such as LacNAc, Le^x or di-Le^x. The synthesis of precursors **7** and **8** by chemical extension of compounds **1** and **2**, respectively, was described in chapter 4. The versatility of our synthetic strategy was demonstrated by utilizing precursor **1** for the construction of the core xylosylated positional isomer **9.**

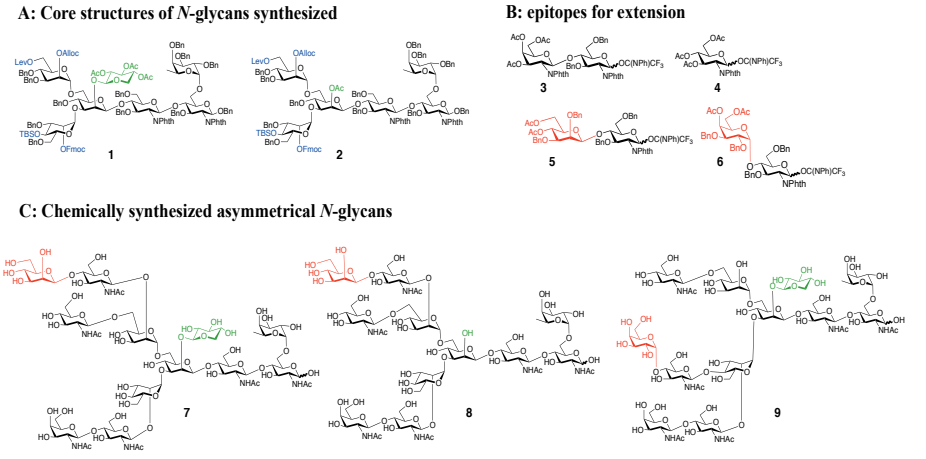
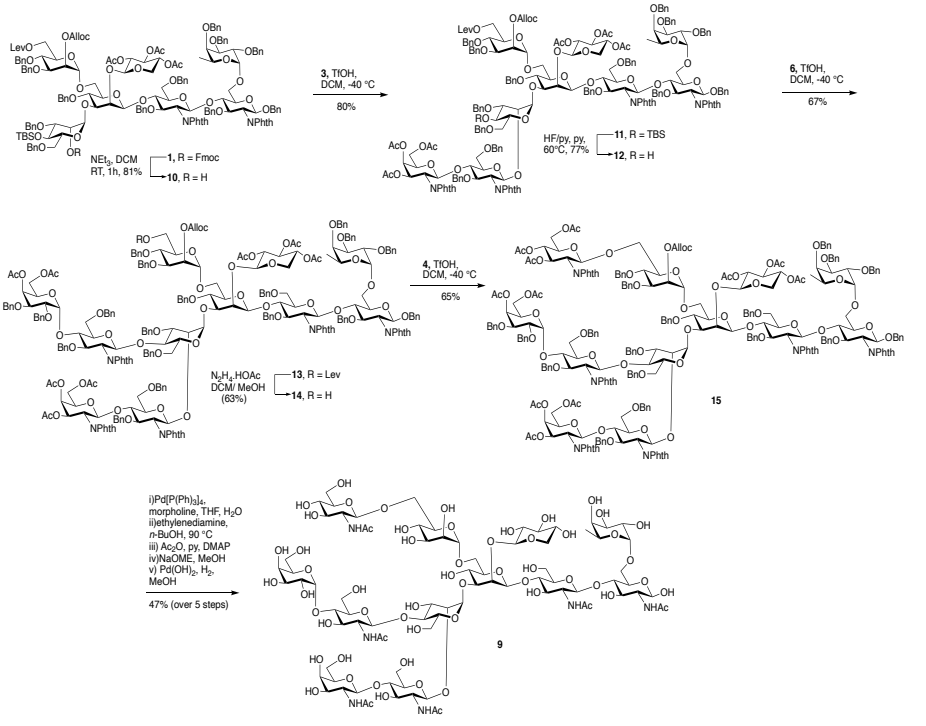


Figure 1. Overview. A) Compounds **1** and **2** are the core structures with orthogonal protecting groups (highlighted in blue); B) The epitopes for glycosylation at typical branching points. The unnatural glycosidic linkages β–Man and α–Gal (highlighted in red) can be cleaved at a convenient point by their respective glycosidases. C) Precursors for enzymatic reactions.

Thus, compound **1** was treated with non-nucleophilic base triethyl amine (Et_3N) to cleave the Fmoc protecting group, and as expected it did not affect other protecting such groups such as Lev, Alloc and TBS, to yield glycosyl acceptor **10** in 81% yield. A TfOH-mediated glycosylation with trifluoroacetimidate donor **3** with acceptor **10** in DCM at -78 °C afforded compound **11** (80%), which was subjected to HF/pyridine to selectively cleave the TBS ether to generate glycosyl acceptor **12** (77%). The latter compound was glycosylated with the donor **6** in the presence of TfOH as catalyst to yield **13** in 67% yield. Finally, the Lev ester was cleaved using hydrazine acetate to provide glycosyl acceptor **14** (63%) which was glycosylated with donor **4** in presence of TfOH to yield the triantennary glycan **15**. This compound was deprotected starting with the removal of Alloc group by treatment with ($Pd[PPh_3]_4$), followed by cleavage of the phthalimide-protecting groups by heating under reflux with ethylenediamine in *n*-butanol. The resulting free amines and hydroxyls were acetylated by acetic anhydride in pyridine followed by cleavage of the esters by sodium methoxide. Finally, the benzyl ethers were removed by catalytic hydrogenation over palladium hydroxide ($Pd[OH]_2$) affording the desired tri-antennary glycan **9** (47% over five steps), which is a positional isomer of **7**.



Scheme 1. Synthesis of positional isomer xylosylated *N*-glycans **9**.

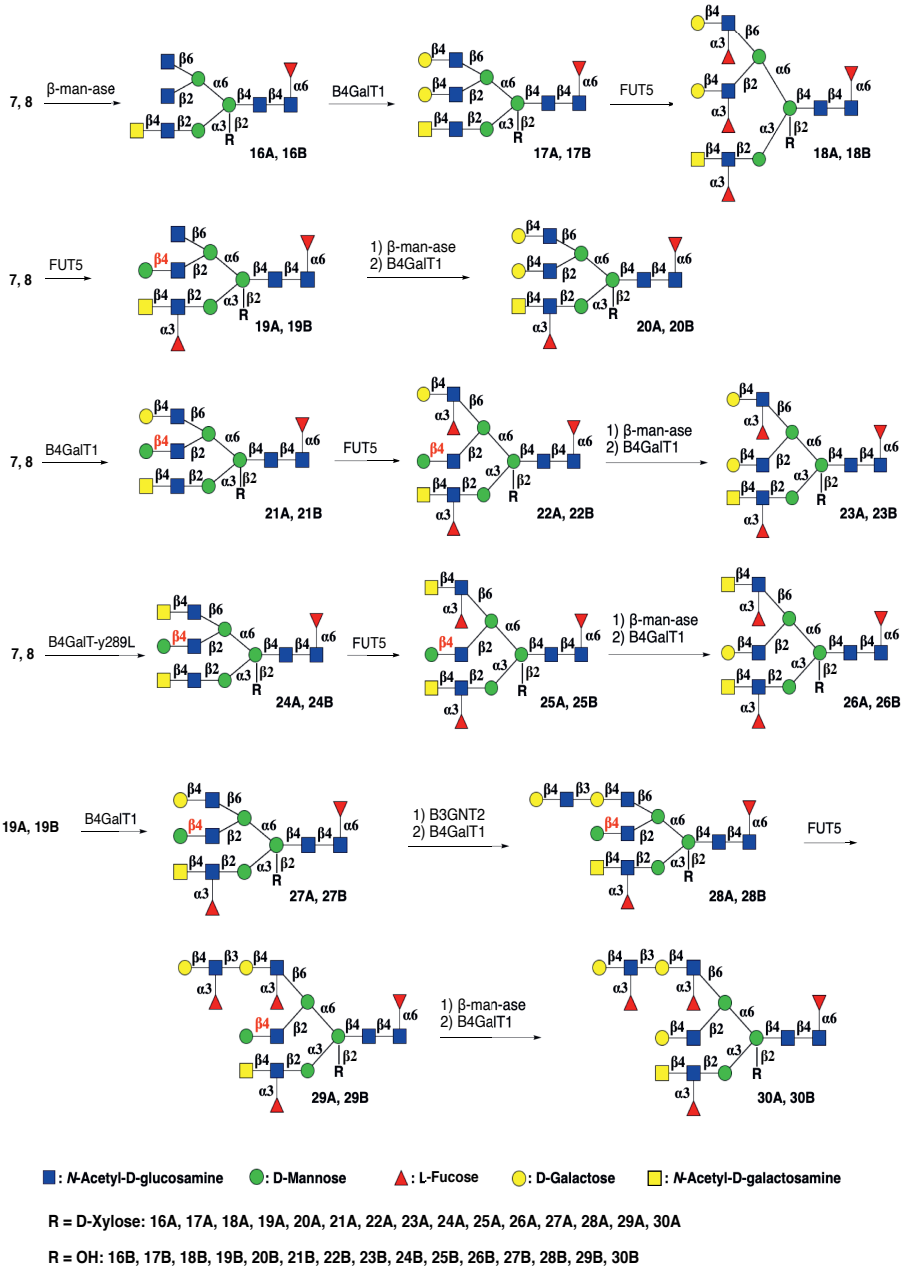
Previously, we demonstrated the versatility of our enzymatic protocols by utilizing glycans **7** and **8**, to synthesize three distinct, highly complex asymmetrical glycans (Chapter 4). Next, we further broadened the scope of our chemo-enzymatic strategy by employing precursors **7, 8** and **9** to synthesize a library of asymmetrical, characteristic *N*-glycans of *S. mansoni* differing in terminal epitope presentation. The enzymatic elaborations commenced by treatment of glycan **7** and **8** with *Helix pomatia β-mannosidase* and the fucoside inhibitor 1-deoxyfuconojirimycin[11] to avoid the cleavage of the core fucoside, to remove the unnatural β-mannoside providing glycans **16A** and **16B** (Scheme 3). The resulting glycans possess two terminal GlcNAc moieties that were converted into LacNAc by treatment with recombinant mammalian β(1,4)-galactoysltransferase (B4GalT1) in the presence of uridine-5'-diphosphogalactose (UDP-Gal), and Calf Intestinal Alkaline Phosphatase (CIAP) at 37 °C for 10 h to afford **17A** and **17B**, respectively. The latter glycans were fucosylated by mammalian α(1,3)-fucosyltransferase (FUT5) in the presence of guanosine-5'-diphosphofucose (GDP-Fuc), and CIAP, which resulted in the fucosylation of the two LacNAc and the LacdiNAc moieties resulting in the formation of glycans **18A** and **18B**.

Changing the sequence of enzymatic transformations gave access to glycans **20A** and **20B**. Thus, **7** and **8** were treated with FUT5 in the presence of GDP-Fuc and CIAP which selectively fucosylated the Lac-di-NAc moiety leaving the unnatural Manβ(1,4)-GlcNAc and terminal GlcNAc moieties unmodified, resulting in the formation of glycans **19A** and **19B**. These glycans were subjected to β-mannosidase in presence of 1-deoxyfuconojirimycin followed by bis-galactosylation with B4GalT1 and UDP-Gal to afford the mono-fucosylated tri-antennary glycans **20A** and **20B**.

In yet other sequences of reactions, glycans **7** and **8** were converted into and **23A**, **23B**, **26A** and **26B**. Thus, treatment of **7** and **8** with with B4GalT1 and UDP-Gal yielded **21A** and **21B**, respectively. These compounds were bis-fucosylation with GDP-Fuc and FUT5 to generate glycans **22A** and **22B** which contain a Le^x and LDN-F moiety. Next, the latter compounds were subjected to β-mannosidase to remove the unnatural mannoside at the α6β2 arm and the resulting terminal GlcNAc moiety was galactosylated with B4GalT1 and UDP-Gal resulting in the formation of **23A** and **23B**. Similarly, treatment of **7** and **8** with mutant β(1,4)-*N*-acetylgalactosaminyltransferase (B4GalT-y289L)[12] which exhibits a very high GalNAc-T activity, in the presence of uridine-5'-diphospho-*N*-acetylgalactosamine (UDP-GalNAc) yielded **24A** and **24B**. These glycans were bis-fucosylation with GDP-Fuc and FUT5 to provide the **25A** and **25B.** The latter compounds were subjected to β-mannosidase treatment followed by modification with B4GalT1 and UDP-Gal resulting in the formation of **26A** and **26B**.

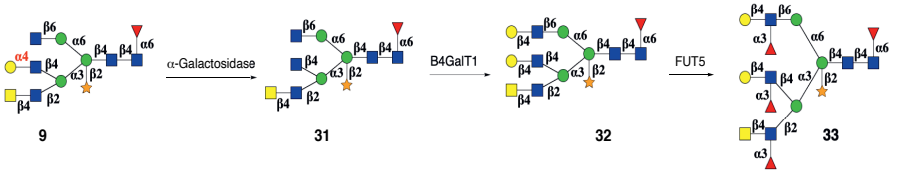
Finally, we also exposed glycans **19A** and **19B** to B4GalT1 which resulted in the selective galactosylation of the α6β6-arm. Sequential treatment of **19A** and **19B** with β(1,3)-glucosaminyltransferase B3GNT2 and B4GalT1 resulted in the installation of a di-LacNAc moiety to give compounds **28A** and **28B**. The di-LacNAc moiety was transformed into a di-Le^x epitope by treatment with FUT5 to afford glycans **29A** and **29B**. The GlcNAc residue of the α6β2-arm of **40** was unmasked by treatment with β-mannosidase which was galactosylated by B4GalT1 to provide target compounds **30A** and **30B**.

The progress of enzymatic reactions was followed by mass spectrometric analysis, and if starting material was detected, the reactions were prolonged till a homogeneous product was obtained. After each step, the product was purified by either size-exclusion chromatography or semipreparative HPLC using an amide HILIC column (10 × 250 mm, Waters Inc.) under gradient conditions (9: 1 CH_3CN: Milli-Q H_2O to 1: 1 CH_3CN: Milli-Q H_2O, v: v) with the UV (210 nm) detection.



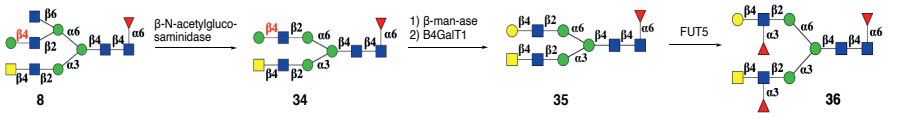
Scheme 3. Enzymatic extensions to generate a library of *N*-glycans

To demonstrate further versatility of the chemoenzymatic approach, the xylosylated tri-antennary positional isomeric glycan **9** was subjected to α-galactosidase from green coffee beans to afford the intermediate glycan **31** (Scheme 4). The exposed GlcNAc moieties were converted into LacNAc by treatment with B4GalT1 and UDP-Gal resulting in the formation of **32**. As a final step, **32** was subjected to fucosylation using standard conditions to afford the xylosylated, **33** which is a positional isomer of 18A.



Scheme 4. Synthesis of a tri-antennary positional isomer using glycan precursor **9**.

The versaility of our enzymatic manipulation strategy was also demonstrated by the synthesis of a bi-antennary glycan from precursor **8** (Scheme 5). Thus, **8** was treated with β-*N*-acetylglucosaminidase from *Canavalia ensiformis* (Jack Bean) at 37°C, followed by heat-deactivation of the enzyme at 60°C, which cleaved the β6-GlcNAc arm, thus affording bi-antennary glycan intermediate **34**. In a one-pot multiple enzymes approach, the mixture was sequentially treated with β-mannosidase followed by heat deactivation, and then subjected to galactosylation of the exposed GlcNAc moiety at β(1,2)arm by B4GalT1 and UDP-Gal to afford **35**. Subsequently, fucosylation with FUT5 and GDP-Fucose resulted in bi-antennary glycan **36**.



Scheme 5. Synthesis of a bi-antennary glycan using tri-antennary glycan precursor **8**.

Glycan micro-array development. Glycan-binding proteins (GBPs), such as C-type lectins, galectins or siglecs recognize glycans to mediate various biological functions.[13] The immune system of the hosts uses these GBPs to detect pathogens to generate an appropriate antibody response, and to discriminate against self/ non-self-antigens. For example, upon infection with *S. mansoni*, majority of the antibody response is directed towards antigenic glycan motifs, particularly the Lac-di-NAc epitope and its fucosylated variants.[6d, 14]

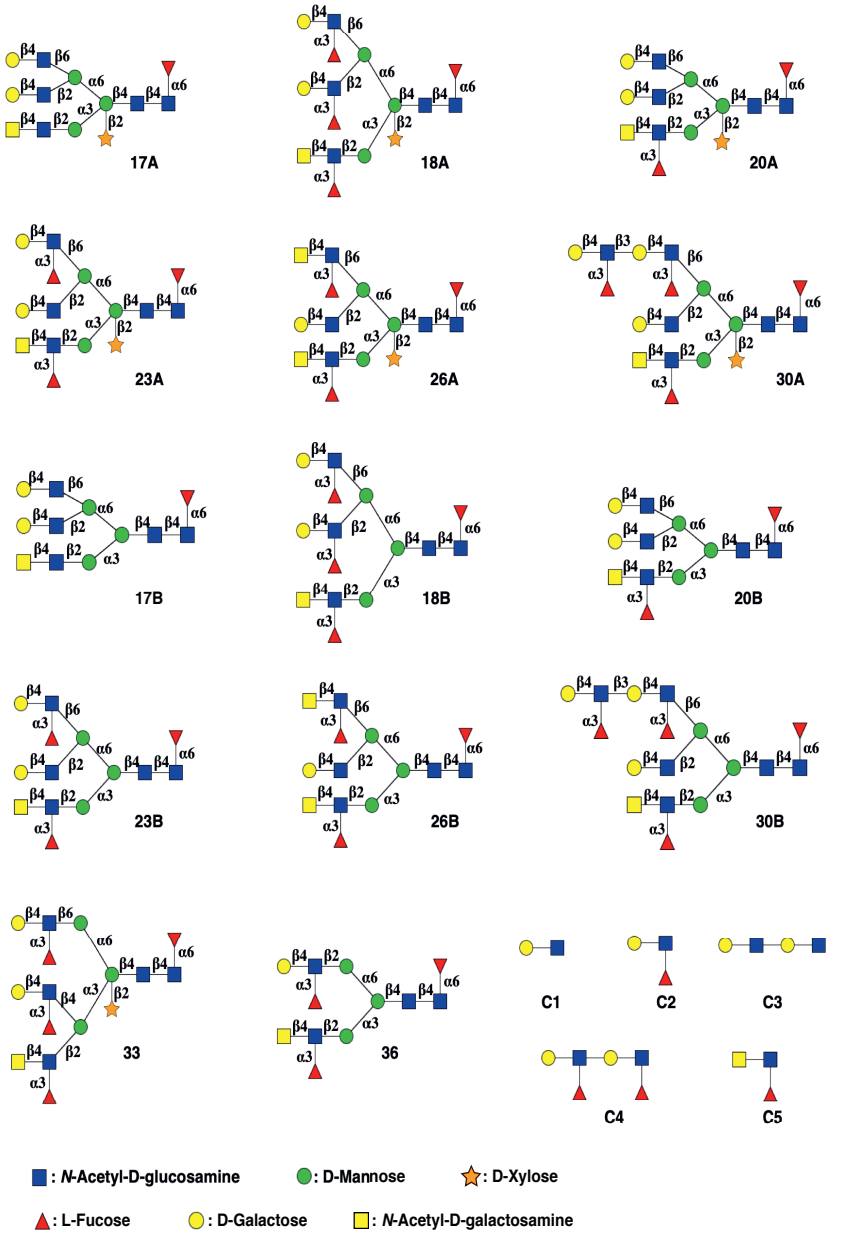


Figure 4. Library of *N*-glycans synthesized.

Glycan arrays utilize the cluster-like properties of glycans to display multi-valent binding to lectins[15] and have emerged as an important tool in the study of carbohydrate-protein interactions owing to their highly advantageous property of utilizing only miniscule amounts of precious glycans as well as the ability to simultaneously screen diverse set of glycan libraries and study their binding interactions with multiple proteins.[15b, 16] The typically weak protein binding with a single glycan ligand can increase by manifolds when presented in the context of multiple carbohydrate recognition domains on a protein or the multivalent presentation of glycans as clusters, dendrimers or on arrays.[17]

To provide immobilization on *N*-hydroxysuccinimide (NHS)-activated glass slides, the reference compounds **C1** to **C5** were all equipped with an anomeric aminopentyl moiety, while the library of *N*-glycans (Figure 4) were modified by an anomeric bi-functional amino-containing linker by treatment with 2-[(methylamino)oxy]ethanamine[18] (See Experimental Procedures, Section 1.3). Compounds were printed in replicates of 6 by piezoelectric printing. After incubation overnight in a saturated NaCl chamber, unreacted esters were quenched with ethanolamine. The glycan sub-arrays were first probed with biotinylated lectins to confirm proper spot morphology and printing. Hence, the lectins *Aleuria aurantia* (AAL) specific for α(1,2)-, α(1,3)- and α(1,6)fucosides, *Erythrina cristagalli* (ECL) specific for terminal galactosides or terminal *N*-acetylgalactosamine moiety, soybean agglutinin (SBA) specific for terminal galactosides, and wheat germ agglutinin (WGA) specific for GlcNAc residues were preincubated with Streptavidin-AlexaFluor635. Next binding of the immobilized oligosaccharides was established by measuring fluorescence intensity using a microarray scanner.

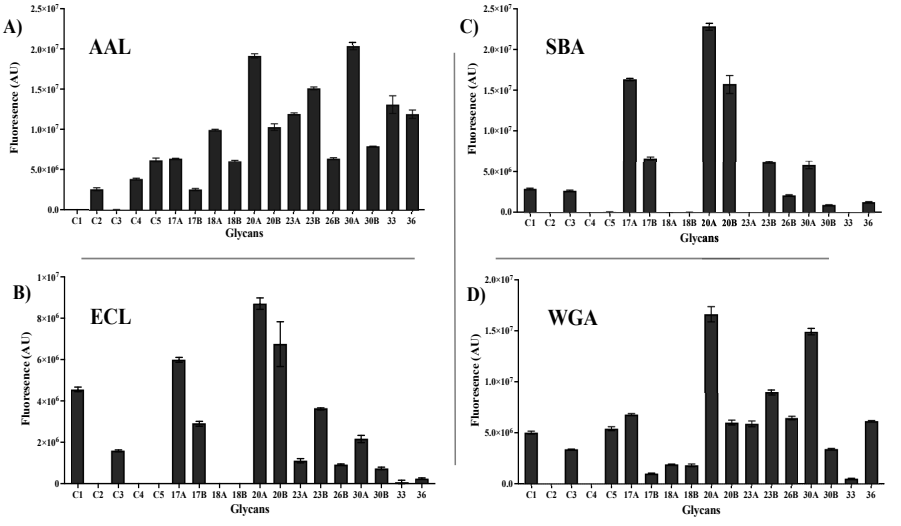


Figure 5. Micro-array studies with lectins A) AAL; B) ECL; C) SBA; D) WGA

The reference compounds **C2**, **C4** and **C5** comprising of α(1,3)fucosides showed binding response towards AAL. All tri-antennary glycans containing a core α(1,6)fucoside, and glycans **18A**,**B**; **20A**,**B**; **23A**,**B**; **30A**,**B**, **33** and **36** also containing one or more α(1,3)fucosides presented as Lewis-x or LDN-F showed responsiveness to AAL (Figure 5A). The lectin ECL recognizes terminal LacNAc moiety, but the affinity is diminished if these moieties are elaborated with a fucoside, as indicated by a positive response with the fucoside-lacking reference compounds **C1** and **C3**. Thus, glycans **17A**,**B and 20A**,**B** comprising of multiple LacNAc moieties, showed a strong binding (Figure 5B). Similarly, the galactoside binding of SBA showed prominent binding towards compounds **17A,B** and **20A,B** which constitute multiple terminal and unsubstituted galactosides (Figure 5C). The GlcNAc binding lectin WGA showed a responsiveness to all glycans with diminished binding to tris-fucosylated glycans **18A,B** and **33** (Figure 5D). An anomaly was observed with compound **26A**, which did not show proper spot morphology and no obvious binding towards the described four lectins, hence this compound was excluded from binding studies.

Conclusion and Future Prospectives. *S. mansoni* express minimal epitopes on their *N*-glycans, in a stage dependent manner in their life cycle[6c] and it is important to access well-characterized glycans representative of each stage to understand their immunological implications. To this end, a versatile chemo-enzymatic strategy that could generate a well-defined library of previously inaccessible and incredibly complex *N*-glycans of *S. mansoni* was developed. From a limited range of chemically synthesized precursors and a judicious application of highly specific mammalian glycosyltransferases and glycosidases, it was possible to synthesize both bi-antennary and tri-antennary glycans, with distinct terminal epitope presentation and branching patterns. Asymmetry was achieved through chemical installation of unnatural glycosidic linkages on the precursors **7**, **8** and **9** which could be independently cleaved by specific glycosidases releasing the inner natural sugar moieties that were activated for enzymatic extension by appropriate glycosyltransferases.

The glycans were subjected to initial micro-array studies with plant lectins. The binding profiles of common plant lectins are very well-studied and are useful tools for identifying and characterizing glycan epitopes.[19] Up till now, a reliable assessment of binding relationship between complex glycans and lectins was lacking due to unavailability of such diverse structures. Future experiments can thus unravel the perfect glycan(s) that generate anti-glycan antibodies to activate the immune system towards inflammation and expulsion of the parasite. For this purpose, our chemo-enzymatic methodology can be used to prepare any *N*-glycan with any glycosidic architecture of choice. These will give an opportunity for a more “natural” presentation of antigenic carbohydrate motifs for the study of glycan-immunogenicity and possibly the development of anti-glycan vaccines.

1. Experimental Procedures.

1.1 General Methods.

The chemical synthesis of precursors **1**, **2** and **3** as well as the core compounds, epitopes for extension and other intermediates is described in Chapter 4 of this Thesis. The precursors were further elaborated using a panel of enzymes to generate a library of fourteen *N*-glycans. All enzymatic reactions were performed in aqueous buffers at an appropriate pH for each enzyme. β(1→4)-galactosyltransferase (B4GalT1), β(1→3)-glucosaminyltransferase (B3GNT2), α(1→3)-fucosyltransferase (FUT5) and mutant β(1→4)-galactosyltransferase (B4GalT-y289l, transfer of galactosaminyl residue) were provided by Dr K. W. Moremen (Complex Carbohydrate Research Center, Athens, GA, USA). Alkaline phosphatase from calf intestine (CIAP), β-mannosidase (crude extract from *Helix pomatia*), α-Galactosidase (from Green Coffee Bean) and *Canavalia ensiformis* β-*N*-acetylglucosaminidase (from Jack bean) were purchased from Sigma-Aldrich. Uridine 5'-diphospho-*N*-acetylglucosamine (UDP-GlcNAc) was purchased from Sigma-Aldrich; uridine 5'-diphosphogalactose (UDP-Gal) were purchased from Roche Diagnostic Corporation (Indianapolis, IN); guanosine 5'-diphospho-L-fucose (GDP-Fuc) was synthesized chemically using reported procedures.[20] All enzymatic reactions, unless otherwise stated, were monitored by mass spectrometry, and recorded on a Shimadzu Biotech Axima-CFR MALDI-TOF using dihydroxybenzoic acid or 4- hydroxycinnamic acid as matrices respectively. Size exclusion gel chromatography was performed using columns (40 cm x 2 cm or 45 cm x 0.5 cm) packed with BioGel P-4 or P-6 (GE Healthcare) and eluted with deionized water. All enzymatic reactions were brought to full completion by adding excess of glycosyltransferases and sugar donors until all starting material was consumed. All nuclear magnetic resonance (NMR) spectra were acquired on 400 MHz Varian/Agilent Direct Drive or 600 MHz Bruker spectrometers, operating at 25 °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 HOD signal at 4.70 ppm. The glycans were purified by HPLC using HILIC column (XBridge® Amide 5 μm, 10 mm x 250 mm, Waters) using UV detection (210 nm) and lyophilized by dissolving the compound in water and freezing using liquid nitrogen.

1.2 Expression and purification of Human Glycosyl Transferases.

The catalytic domains of all human glycosyl transferases were expressed as a soluble secreted fusion protein for production in mammalian (HEK293) suspension cultures.[21] The coding regions were amplified from Mammalian Gene Collection clones using primers that appended a tobacco etch virus (TEV) protease cleavage site[22] to the NH_2-terminal end of the coding region and attL1 and attL2 Gateway adaptor sites were extended on the 5′ and 3′ terminal ends of the coding region

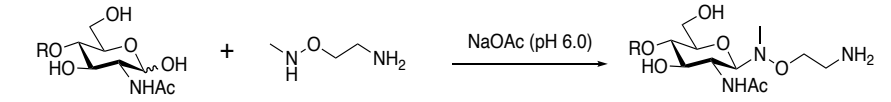
during transfer to pDONR221 vector backbone.[21b] The pDONR221 clones were then recombined via LR clonase reaction into a custom Gateway adapted version of the pGEn2 mammalian expression vector[21b] to assemble a recombinant coding region comprised of a 25 amino acid NH_2-terminal signal sequence from the *T. cruzi* lysosomal α-mannosidase[23] followed by an 8xHis tag, 17 amino acid AviTag,[24] "superfolder" GFP,[25] the nine amino acid sequence encoded by attB1 recombination site, followed by the TEV protease cleavage site and the respective glycosyltransferase catalytic domain coding region.

Suspension culture HEK293 cells (Freestyle 293-F cells, Life Technologies, Grand Island, NY) were transfected as previously described[21] and the culture supernatant was subjected to Ni^{2+}-NTA superflow chromatography (Qiagen, Valencia, CA). Enzyme preparations were eluted with 300 mM imidazole, concentrated by ultrafiltration, and subjected to gel filtration on a Superdex 75 column (GE Healthcare) preconditioned with a buffer containing 20 mM HEPES, pH 7.0, 100 mM NaCl, 10% glycerol, 0.05% Na azide. Peak fractions were pooled and concentrated to ~1 mg/mL using an ultrafiltration pressure cell membrane (Millipore, Billerica, MA) with a 10 kDa molecular weight cutoff.

Enzyme	Amino Acid residues	Uniprot ID
B3GNT2	35-397	Q9NY97
B4GalT1	63-398	P15291
FUT5	40-374	Q11128
B4GalT-y289L	130-402	-

Table S1. Enzyme expression details.

1.3 General Procedure for linkering of glycans and their purification.



R = extended glycan structures | Bi-functional Linker | Linker modified glycans

Scheme S1. General procedure for linkering of all complex glycans for printing on NHS-activated glass slides for micro-array studies.

Free reducing glycan (0.1 to 0.3 mg) and bi-functional spacer (20 equiv.) were dissolved in 0.1 M acetate aqueous buffer (pH 6.0), to attain a glycan concentration of 2 mM. The mixture was incubated at 37 °C for 72 h and monitored by MALDI-TOF MS. The reaction mixture was loaded over Biogel P-2 size exclusion filtration column (50 cm x 1 cm) with 0.1 M NH_4HCO_3 (aq) as

eluent, to remove excess linker and salt. Fractions containing the glycans were lyophilized, and the final products were characterized by MALDI-TOF MS and ^{1}H NMR. It is worth mentioning that the linkering reactions may not reach full completion. However, the material without the bi-functional linker could not be printed on the NHS-activated glass slides.

1.4 Glycan array printing.

The glycans (100 µM in sodium phosphate (250 mM), pH 8.5 buffer) were printed on activated glass slides (Nexterion Slide H, Schott Inc) by piezoelectric non-contact printing (sciFLEXARRAYER S3, Scienion Inc) with a drop volume of ~400 pL and 1 drop per spot at 50% relative humidity. Compounds were printed as replicates of 6, 32x25 spots per subarray, and 24 subarrays (3x8) per slide. After overnight incubation in a saturated NaCl chamber (75% relative humidity), 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.

Screening. To validate printing sub-arrays were incubated at RT with 50 µL of a mixture of biotinylated lectins (AAL, ECL, SBA and WGA; all obtained from Vector Labs) and 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 4 successive washes of the whole slide 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 each 5 min soak time.
Recombinant human DC-SIGN-Fc Chimera (R&D systems, 161-DC) was assayed at the indicated concentration premixed with anti-IgG Fc-biotin (5 µg/mL; ThermoFisher Scientific, A18833) and Streptavidin-AlexaFluor635 (5 µg/mL) in TSM binding buffer and washes were performed as described above.

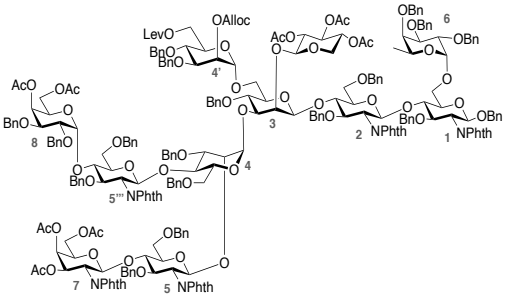
Analyses. Washed arrays were dried by centrifugation and immediately scanned for fluorescence on a GenePix 4000 B microarray scanner (Molecular Devices). The detection gain was adjusted to avoid saturation of the signal. The data were processed with GenePix Pro 7 software and further analyzed using our home written Microsoft Excel macro. After removal of the lowest and highest value of the 6 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.

1.5 Monosaccharide nomenclature system for NMR assignments.

For the simpler building blocks, the monosaccharides were denoted with their known abbreviations, for *e.g.*, Glucose (Glc), *N*-acetyl glucosamine (GlcN), Mannose (Man), *N*-acetyl galactosamine (GalN) and Fucose (Fuc). For the oligosaccharide glycans, the individual monosaccharides have been labeled from the reducing end of the glycans as shown in Figure S1. For eg., the *N*-acetyl glucosamine residues from the reducing end of the glycans were labeled as GlcN-1 and GlcN-2 respectively; the β-mannoside of the core pentasaccharide is labeled as Man-3, the α-3 and α-6 mannosides were labeled as Man-4 and Man-4' respectively. The mannose moeity of the unnatural Man-β-(1→4)-GlcNAc terminus was labeled as Man-8; the *N*-acetylglucosamine residues as GlcNAc-5, 5', 5". The *N*-acetyl galactosamine of the GalN-β-(1→4)-GlcN terminus was denoted as GalN-7.

1.6 NMR assignments.

Benzyl [2-*O*-allyloxycarbonyl-3,4-di-*O*-benzyl-6-*O*-levulenoyl-α-D-mannopyranosyl-(1→6)]-[3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-galactopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside-(1→2)-2,3-di-*O*-benzyl-4,6-di-*O*-acetyl-α-D-galactopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-pthalimido-β-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→3)]-2,3,4-tri-*O*-acetyl-β-D-xylopyranosyl-(1→2)-4-*O*-benzyl-β-D-mannopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl-(1→6)-3-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (13): Compound **11** (150 mg, 0.039 mmol, synthesis described in chapter 4) was dissolved in pyridine (5 mL) followed by dropwise addition of HF in pyridine (70% HF, 30% pyridine; 1 mL). The mixture was stirred for 10 hours at 60 °C, after which it was quenched by solid $NaHCO_3$ (1 g), till all CO_2 bubbling stopped. The salts were filtered off, the solvent was evaporated *in vacuo*, and the residue was re-dissolved in DCM, followed by washing with water and saturated $NaHCO_3$ (10 mL). The organic phase was dried over $MgSO_4$, filtered, and the filtrate was concentrated, and the residue was purified using silica gel column chromatography (Pet. Ether: EtOAc, 8: 2, v: v to Pet: EtOAc, 2: 8, v: v), which provided the product **12** as a white foamy solid. (110 mg, 77 %). R_f = 0.61 (Pet. Ether: EtOAc, 4: 6, v: v). A mixture this acceptor (110 mg, 0.03 mmol) and imidate donor **6** (130 mg, 0.12 mmol), was stirred

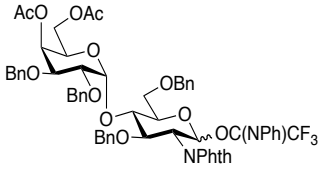


in DCM (1.5 mL) with pre-activated molecular sieves (300 mg) for 10 minutes, after which the mixture was cooled down to -40 ºC, followed by the addition of TfOH (1.1 µL, 0.012 mmol). The mixture was warmed up to -20 ºC over a period of 30 minutes, after which it was quenched with Et_3N (15 µL). The sieves were filtered off and the mixture was concentrated *in vacuo.* The residue was purified by silica gel column chromatography using Pet. Ether: EtOAc (9: 1, v: v to 6: 4, v: v) and then passed through BioGel SX-1 column using Tol: Acetone (1: 1, v: v) as mobile phase, which afforded the product **13** as an off-white amorphous powder. (92 mg, 67%). R_f = 0.33 (Pet. Ether: EtOAc, 1: 1, v: v). ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.07 to 6.42 (110H, m, H-Ar), 5.82 (1H, m, C<u>H</u>=CH_2 of alloc), 5.74 (1H, dd, H-3 GalN-7, *J* = 11.4 Hz, 3.4 Hz), 5.51 (1H, d, H-1 Gal-8, *J* = 3.4 Hz), 5.46 (1H, d, H-2 Man-4', *J* = 1.9 Hz), 5.33 (3H, m, H-4 Gal-8, H-1 GlcN-2, CH=C<u>H</u>H of alloc), 5.25 (1H, m, CH=CH<u>H</u> of alloc), 5.14 (4H, m, H-4 GalN-7, H-4 Xyl, H-1 GlcN-5, H-1 Man-4), 5.07 to 4.82 (9H, m, H-2 Xyl, H-3 Xyl, H-1 Xyl, H-1 GlcN-1, PhC<u>H</u>$_2$ protons), 4.77 (5H, m, PhC<u>H</u>$_2$ protons), 4.72 to 4.52 (14H, m, H-1 GlcN-5"', H-1 GalN-7, H-1 Man-4', H-1 Fuc-6, OC<u>H</u>$_2$ of alloc, PhC<u>H</u>$_2$ protons), 4.51 to 4.33 (13H, m, H-2 GalN-7, H-3 GlcN-5"', PhC<u>H</u>$_2$ protons, PhCH<u>H</u>), 4.28 (4H, m, H-2 Gal-8, PhC<u>H</u>$_2$ protons), 4.23 to 4.04 (14H, m, H-2 GlcN-1, H-2 GlcN-2, H-2 GlcN-5, H-6a Man-4', H-6b Man-4', H-3 GlcN-1, H-3 GlcN-2, H-3 GlcN-5, H-1 Man-3, PhC<u>H</u>$_2$ protons), 4.03 to 3.83 (12H, m, H-2 GlcN-5"', H-6a GalN-7, H-6b GalN-7, H-6a Gal-8, H-6b Gal-8, H-5a Xyl, H-4 GlcN-1, H-5 GalN-7, H-4 GlcN-2, H-4 GlcN-5, H-4 GlcN-5"', H-3 Fuc-6), 3.83 to 3.67 (8H, m, H-5 Fuc-6, H-6a Man-4, H-6b Man-4, H-2 Man-4, PhC<u>H</u>H, H-2 Fuc-6, H-4 Man-3, H-5 Gal-8), 3.66 to 3.41 (11H, m, H-6a GlcN-2, H-6a Man-3, H-2 Man-3, H-4 Man-4, H-3 Man-4', H-4 Man-4', H-5 Man-4, H-3 Gal-8, H-3 Man-4, PhC<u>H</u>$_2$), 3.37 to 3.23 (4H, m, H-6b GlcN-2, H-4 Fuc-6, H-3 Man-3, H-5 GlcN-5"'), 3.17 (1H, d, H-6b Man-3, *J* = 9.9 Hz), 3.07 (2H, m, H-5b Xyl, H-6a GlcN-5"'), 2.96 (3H, m, H-6a GlcN-1, H-6a GlcN-5, H-5 GlcN-1), 2.81 (2H, m, H-6b GlcN-1, H-5 GlcN-2), 2.57 (3H, m, COOC<u>H</u>$_2$CH$_2$ of Lev, H-6b GlcN-5), 2.45 (1H, m, H-5 Man-3), 2.35 (2H, m, COOCH$_2$C<u>H</u>$_2$ of Lev), 2.20 to 1.65 (27H, m, 8x C<u>H</u>$_3$ of Ac, CH$_2$COC<u>H</u>$_3$ of Lev), 1.50 (1H, d, H-5 GlcN-5, *J* = 9.8 Hz), 0.98 (3H, d, C<u>H</u>$_3$ of Fuc-6, *J* = 6.3 Hz); ^{13}C NMR (151 MHz, $CDCl_3$, signals from edited HSQCAD experiment): δ 123.74, 123.66, 134.84, 123.35, 133.56, 123.18, 127.66, 123.08, 133.36, 126.28, 133.26, 128.10, 128.15, 133.36, 76.95, 128.14, 128.01, 127.80, 125.65, 128.03, 127.95, 127.68, 127.71, 127.61, 127.53, 127.71, 127.97, 127.05, 126.88, 131.58, 67.96, 97.55, 67.66, 96.63, 66.67, 118.97, 118.97, 118.95, 98.84, 71.74, 97.41, 71.78, 69.12, 74.87, 96.69, 96.64, 71.33, 72.92, 97.36, 75.16, 74.66, 72.86, 73.52, 98.04, 72.32, 74.74, 99.83, 97.39, 73.72, 70.08, 95.38, 75.19, 52.04, 71.46, 68.60, 73.29, 80.39, 72.88, 74.76, 70.07, 67.01, 73.13, 72.93, 100.96, 55.83, 76.76, 62.03, 76.94, 75.15, 56.23, 60.88, 75.81, 62.62, 79.29, 76.94, 75.61, 78.10, 65.99, 68.83, 75.26, 79.37, 71.67, 72.98, 73.69, 75.00, 77.80, 64.76, 70.49, 74.63, 73.74, 72.21, 66.42, 69.65, 74.19, 64.76, 66.49, 61.88, 78.80, 68.12, 69.80, 73.85, 37.93, 73.57, 27.87, 30.97, 20.97, 29.86, 20.92, 20.77, 20.72, 20.54, 20.50, 20.53, 29.70, 16.57, 16.47. MALTI-TOF-MS (*m/z*): $[M+ Na]^+$ calculated for $C_{256}H_{259}N_5O_{71}Na$, 4561.6708; found 4561.7013.

Benzyl [3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→6)-2-*O*-allyloxycarbonyl-3,4-di-*O*-benzyl-α-D-mannopyranosyl-(1→6)]-[3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-galactopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside-(1→2)-2,3-di-*O*-benzyl-4,6-di-*O*-acetyl-α-D-galactopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-pthalimido-β-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→3)]-2,3,4-tri-*O*-acetyl-β-D-xylopyranosyl-(1→2)-4-*O*-benzyl-β-D-mannopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl-(1→6)-3-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (15): Compound **13** (90 mg, 0.0198 mmol) was dissolved in DCM (2 mL) and MeOH (1 mL), followed by the addition of solid hydrazine acetate (9.1 mg, 0.099 mmol). The mixture was stirred for 1 hour after which it was concentrated *in vacuo* and the residue was purified by silica gel column chromatography using Pet. Ether: EtOAc (8: 2, v: v to 1:1, v: v) which afforded the product **14** as a white foamy solid. (55 mg, 63%). This acceptor (55 mg, 0.012 mmol), and imidate donor **4** (30 mg, 0.049 mmol), was stirred in DCM (1 mL) with pre-activated molecular sieves (100 mg) for 20 minutes, after which the mixture was cooled down to -40 ºC, followed by the addition of TfOH (0.5 µL, 0.0049 mmol). The mixture was warmed up to -20 ºC over a period of 30 minutes, after which it was quenched with Et_3N (10 µL). The sieves were filtered off and the mixture was concentrated *in vacuo.* The residue was purified by silica gel column chromatography using Tol: EtOAc (9: 1, v: v to 6: 4, v: v) and then passed through BioGel SX-1 column using Tol: Acetone (1: 1, v: v) as mobile phase, which afforded the product as an off-white amorphous powder. (38.1 mg, 65%). R_f = 0.60 (Tol: EtOAc, 7: 3, v: v). ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.07 to 6.41 (110H, m, H-Ar), 5.84 (1H, m, C<u>H</u>=CH_2 of alloc), 5.74 (2H, m, H-3 GalN-7, H-3 GlcN-5'), 5.50 (1H, d, H-1 Gal-8, *J* = 3.1 Hz), 5.45 (1H, s, H-2 Man-4'), 5.33 (3H, m, H-4 Gal-8, H-1 GlcN-2, CH=C<u>H</u>H of alloc), 5.20 (2H, m, H-1 GlcN-5, CH=CH<u>H</u> of alloc), 5.10 (4H, m, H-4 GlcN-5', H-4 GalN-7, H-1 Man-4, H-1 GlcN-5'), 4.96 (4H, m, H-2 Xyl, H-4 Xyl, H-1 Xyl, PhC<u>H</u>H), 4.87 (3H, m, H-3 Xyl, H-1 GlcN-1, PhCH<u>H</u>), 4.78 (5H, m, Ph<u>C</u>H_2 protons), 4.71 to 4.54 (13H, m, H-3 Gal-8, H-1 GalN-7, H-1 GlcN-5"', H-1 Fuc-6, Ph<u>C</u>H_2 protons, O<u>C</u>H_2 of alloc), 4.52 to 4.34 (12H, m, H-2 GalN-7, H-3 GlcN-5"', H-1 Man-4', Ph<u>C</u>H_2 protons), 4.34 to 4.20 (7H, m, H-2 GlcN-5', H-6a Man-4', H-5 GlcN-5', Ph<u>C</u>H_2 protons), 4.19 to 3.97 (19H, m, H-2 GlcN-1, H-2 GlcN-2, H-2 GlcN-5, H-2 GlcN-5"', H-6b Man-4', H-6a GlcN-5', H-6b GlcN-5', H-6a GalN-7, H-6a Gal-8, H-6b Gal-8, H-2 Gal-8, H-3 GlcN-1, H-3 GlcN-2, H-3 GlcN-5, H-4 GlcN-1, Ph<u>C</u>H_2 protons), 3.96 to 3.76 (8H, m, H-6b GalN-7, H-5a Xyl, H-5 Fuc-6, H-5 GalN-7, H-4 GlcN-2, H-4 GlcN-5, H-4 GlcN-5"', H-3 Fuc-6), 3.76 to 3.67 (7H, m, H-6a Man-4, H-2 Man-4, PhC<u>H</u>H, H-4 Man-4, H-3 Man-4', H-2 Fuc-6, H-5 Gal-8), 3.65 to 3.43 (11H, m, H-6a GlcN-2, H-2 Man-3, H-4 Man-4', H-5 Man-4, H-5 Man-4', H-3 Man-4, H-

4 Man-3, PhC<u>H</u>$_2$ protons), 3.38 to 3.19 (6H, m, H-6b GlcN-2, H-6a Man-3, H-6b Man-4, H-4 Fuc-6, H-3 Man-3, H-5 GlcN-5"'), 3.12 to 2.87 (6H, m, H-5b Xyl, H-6a GlcN-5"', H-6b GlcN-5"', H-6a GlcN-5, H-5 GlcN-1, H-6a GlcN-1), 2.82 (2H, m, H-6b GlcN-1, H-5 GlcN-2), 2.74 (1H, d, H-6b Man-3, J = 11.1 Hz), 2.52 (1H, m, H-6b GlcN-5), 2.35 (1H, m, H-5 Man-3), 2.16 to 1.80 (33H, m, 11x C<u>H</u>$_3$ of Ac), 1.66 (1H, m, H-5 GlcN-5), 0.98 (3H, d, C<u>H</u>$_3$ of Fuc-6, J = 6.3 Hz); ^{13}C NMR (151 MHz, $CDCl_3$, signals from edited HSQCAD experiment): δ 123.80, 134.91, 133.54, 123.44, 129.18, 132.21, 127.66, 123.06, 133.35, 133.22, 128.07, 126.12, 128.15, 133.35, 76.99, 78.48, 128.14, 128.10, 125.33, 127.85, 125.68, 127.90, 127.56, 127.87, 125.62, 127.47, 127.00, 126.78, 131.68, 67.89, 70.67, 97.53, 67.66, 96.55, 66.66, 98.55, 118.83, 98.81, 71.77, 71.69, 97.38, 68.99, 69.08, 74.92, 96.69, 71.49, 72.86, 97.38, 72.83, 73.60, 98.16, 72.13, 74.69, 99.78, 70.15, 73.63, 88.56, 95.38, 74.79, 52.07, 73.33, 68.54, 80.34, 72.86, 97.39, 70.11, 66.97, 62.03, 54.37, 75.63, 73.13, 55.81, 101.00, 76.95, 62.00, 69.62, 56.25, 60.88, 75.79, 61.05, 79.34, 79.24, 61.98, 76.95, 75.68, 66.01, 71.67, 75.29, 72.97, 77.57, 68.72, 79.46, 74.86, 71.39, 77.94, 70.59, 64.89, 74.48, 72.20, 68.95, 74.11, 74.15, 64.95, 70.21, 68.02, 61.86, 78.90, 69.85, 73.81, 66.34, 21.50, 20.94, 20.90, 20.72, 19.15, 20.78, 19.25, 20.70, 20.53, 27.32, 20.51, 13.68, 26.88, 30.36, 29.66, 29.73, 14.23, 16.57, 12.18, 13.67, 26.80, 0.09. MALTI-TOF-MS (*m/z*): [M+ Na]$^+$ calculated for $C_{271}H_{272}N_6O_{78}Na$, 4880.7400; found 4880.7862.

(*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-α/β-D-glucopyranoside (6): Imidate donor **6** was prepared according to reported literature.[26] ^{1}H NMR (400 MHz, $CDCl_3$): δ



7.82 to 6.43 (32 H, m, H-Ar), 5.54 to 5.48 (2H, m, H-1 Gal, H-4 Gal), 4.82 to 4.55 (7H, m, PhCH<u>H</u>, 3x PhC<u>H</u>$_2$), 4.48 (2H, m, H-4 GlcN, H-2 GlcN), 4.27 to 4.05 (4H, m, H-6b Gal, H-5 Gal, H-3 GlcN, PhC<u>H</u>H), 4.05 to 3.84 (3H, m, H-3 Gal, H-6b GlcN, H-6a Gal), 3.85 to 3.67 (2H, m, H-6a GlcN, H-2 Gal), 2.13 (3H, s, C<u>H</u>$_3$ of Ac), 2.05 (3H, s, C<u>H</u>$_3$ of Ac). ^{13}C NMR (101 MHz, $CDCl_3$): δ 170.4, 170.3, 167.2, 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. Compound was unstable under MALDI-TOF-MS conditions.

1.7 General Enzymatic Procedure

General procedure for α(1→3) Fucosylation: Glycan and GDP-Fucose (2 eq per fucoside) were dissolved in Tris buffer (50 mM, pH~7.3) containing $MnCl_2$ (10 mM), CIAP (10 mU). Recombinant α(1,3)-Fuctosyltransferases FUT5 (6.6 mU/μmol of substrate) were added to achieve a final concentration of 4 mmol. The resulting mixture was incubated at 37 °C for 12 h. In case MALDI-TOF-MS showed the remaining starting material additional GDP-Fucose (1 or 2 eq), CIAP (10 mU) and enzyme FUT5 was added and incubation at 37 °C was continued until no more starting material could be detected. The reaction mixture was quenched by adding methanol (10 μL), after which it was passed through Biogel P-4 or P-6 column. Fractions containing product were identified using TLC (dipping into the anisaldehyde stain followed by charring at 150 °C), combined and lyophilized to give the product.

General Procedure for β(1→4) Galactosylation. Glycans 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 B4GalT1 (3.4 mU/μmol) were added to achieve a final concentration of 4 mmol. The reaction mixture was then incubated at 37 °C for 10 h. The reaction mixture was quenched by adding methanol (10 μL) and passed through Biogel P-4 or P-6 column. Fractions containing product were identified using TLC (dipping into the anisaldehyde stain followed by charring at 150 °C), combined and lyophilized to give the respective products as white fluffy solids.

General Procedure for installation of β(1→3) *N*-acetylglucosamine moieties. Glycans and UDP-GlcNAc (2 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 B3GNT2 (6.0 mU/μmol) were added to achieve a final concentration of glycan at 4 mM. The resulting mixture was then incubated at 37 °C for 12 h. The reaction mixture was quenched by adding methanol (10 μL) and passed through Biogel P-4 or P-6 column. Fractions containing product were identified using TLC (dipping into the anisaldehyde stain followed by charring at 150 °C), combined and lyophilized to give the respective products as white fluffy solids.

General Procedure for installation of β(1→4) *N*-acetylgalactosamine moieties. Glycans and UDP-GalNAc (2 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 mutant B4GalT-y289l (6.6 mU/μmol) were added to achieve a final concentration of glycan at 4 mM. The resulting mixture was then incubated at 37 °C for 12 h. The reaction mixture was quenched by adding methanol (10 μL) and passed through Biogel P-4 or P-6 column. Fractions containing product were identified using TLC (dipping into the anisaldehyde stain followed by charring at 150 °C), combined and lyophilized to give the respective products as white fluffy solids.

General Procedure for unnatural β-Mannose removal with Subsequent Installation of a β-Galactose Moiety. Glycan was dissolved in 100 mM NaOAc buffer (500 μL, pH~5.0) followed

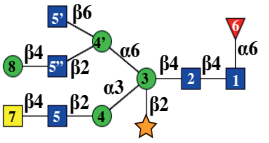
by addition of 10 mM deoxyfuconojirimycin hydrochloride and the appropriate amounts of the *Helix pomatia* β-mannosidase the reaction mixture was then incubated at 37 °C for 6 h. In case MALDI-TOF-MS showed the remaining starting material, additional portion of the enzyme was added and incubated at 37 °C until no more starting material could be detected. After this time the reaction mixture was heated at 100 °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 combined and lyophilized. This glycan and UDP-Gal (1.5 - 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 B4GalT1 (3.4 mU/μmol) were added to achieve a final concentration of 4 mM. The reaction mixture was then incubated at 37 °C for 10 h. The reaction mixture was quenched by adding methanol (10 μL), after which it was passed through Biogel P-4 or P-6 column. Fractions containing product were identified using TLC (dipping into the anisaldehyde stain followed by charring at 150 °C), were combined and lyophilized to give products as a white cotton-like solid.

General Procedure for unnatural α-galactose removal with Subsequential installation of a β-galactosyl moiety. Glycan was dissolved in 100 mM NaOAc buffer (500 μL, pH~5.0) followed by addition of the appropriate amounts of the α-galactosidase (from Green Coffee Bean) and the reaction mixture was incubated at 37 °C for 12 h. In case MALDI-TOF-MS showed the remaining starting material, additional portion of the enzyme was added and incubated at 37 °C until no more starting material could be detected. After this time the reaction mixture was heated at 100 °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 combined and lyophilized. This glycan and UDP-Gal (1.5 - 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 B4GalT1 (3.4 mU/μmol) were added to achieve a final concentration of 4 mM. The reaction mixture was then incubated at 37 °C for 10 h. The reaction mixture was quenched by adding methanol (10 μL), after which it was passed through Biogel P-4 or P-6 column. Fractions containing product were identified using TLC (dipping into the anisaldehyde stain followed by charring at 150 °C), were combined and lyophilized to give products as a white cotton-like solid.

General Procedure for the removal of β-*N*-acetylglucosamine. Glycan was dissolved in 100 mM NaOAc buffer (500 μL, pH~5.0) followed by addition of the appropriate amounts of *Canavalia ensiformis* β-*N*-acetylglucosaminidase (5 U). The reaction mixture was then incubated for 2 h. After this time the reaction mixture was heated at 100 °C for 10 min, centrifuged, the supernatant was lyophilized, and the resulting product was desalted using the BioGel P-2 column. Fractions containing product were identified using TLC (dipping into the anisaldehyde stain followed by charring at 150 °C), were combined and lyophilized to give products as a white cotton-like solid.

1.8 Enzymatic elaborations and NMR Assignments of Glycans

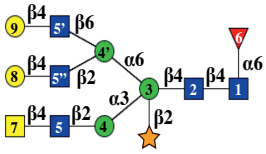
Glycan 7: Detailed synthesis described in chapter 4.



7	H1	H2	H3	H4	H5	H6	Fuc-CH_3
GlcNAc-1α	5.07 (d, *J* = 2.6 Hz)	3.80	3.89	3.66	NA	NA	–
Man-4	5.03	4.05	3.81	3.42	NA	3.80, 3.51	–
Man-3	4.75	4.17	3.79	3.71	3.75	3.85, 3.60	–
Man-4'	4.74	3.99	3.75	3.31	3.68	4.09, 3.46	–
Man-8	4.65	3.97	3.55	NA	NA	NA	–
GlcNAc-1β	4.58	3.63	3.65	NA	NA	NA	–
GlcNAc-2	4.55	3.69	3.67	3.54	NA	NA	–
GlcNAc-5"	4.46 (d, *J* = 7.8 Hz)	3.64	3.53	3.63	3.39	NA	–
GlcNAc-5'	4.42	3.66	3.55	3.62	3.39	NA	–
GlcNAc-5	4.40	3.66	3.46	3.64	3.40	NA	–
GalNAc-7	4.40	3.84	3.63	3.85	NA	NA	–
Xyl (core)	4.32 (d, *J* = 7.6 Hz)	3.28	3.35	NA	3.14, 3.89	–	–
Fuc-6 (core) α,β	4.78	3.69	3.79	3.72	3.99, 4.02	–	1.10

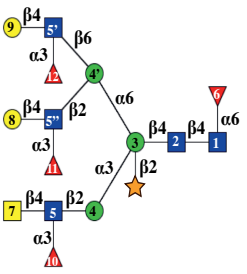
MALDI-TOF-MS (*m/z*): $[M+Na]^+$ calculated for $C_{83}H_{138}N_6O_{59}Na$, 2185.7880; found 2185.8172.

Glycan **17A:** Glycan **7** (5.0 mg) was dissolved in 100 mM NaOAc buffer (500 µL, pH~5.0) followed by adding 10 mM deoxyfuconojirimycin hydrochloride (20 µL) and *Helix pomatia* β-mannosidase (10 µL) the reaction mixture was then incubated at 37 °C for 12 h. After this time the reaction mixture was heated at 100 °C for 10 min, centrifuged, the supernatant was lyophilized, and the resulting product **16A** was desalted using the BioGel P-2 column. Fractions containing the product were combined and lyophilized. This glycan and UDP-Gal (4 eq) were dissolved in Tris buffer (50 mM, pH~7.5) and B4GalT1 was added to achieve a final concentration of 4 mM. The reaction mixture was then incubated at 37 °C for 10 h. The reaction mixture was quenched by adding methanol (10 µL), after which it was passed through Biogel P-4 column. Fractions containing product were combined and lyophilized to give the product as a white fluffy solid (5.5 mg). MALDI-TOF-MS (*m/z*): $[M+Na]^+$ calculated for $C_{89}H_{148}N_6O_{64}Na$, 2347.8409; found 2347.9092.



17A	H1	H2	H3	H4	H5	H6	Fuc-CH_3
GlcNAc-1α	5.06 (d, *J* = 3.1 Hz)	3.78	3.88	3.66	NA	NA	–
Man-4	5.03	4.02	3.78	3.39	NA	3.79, 3.49	–
Man-3	4.75	4.14	3.76	3.69	3.76	3.83, 3.58	–
Man-4'	4.74	3.97	3.74	3.30	3.68	4.10, 3.46	–
GlcNAc-1β	4.57 (d, *J* = 8.2 Hz)	3.59	3.61	NA	NA	NA	–
GlcNAc-2	4.55	3.65	3.64	3.51	NA	NA	–
GlcNAc-5"	4.46	3.62	3.52	3.61	3.37	NA	–
GlcNAc-5'	4.43 (d, *J* = 8.3 Hz)	3.74	3.52	3.62	3.37	NA	–
GlcNAc-5	4.40	3.66	3.45	3.62	3.38	NA	–
GalNAc-7	4.40	3.82	3.62	3.83	NA	NA	–
Gal-8	4.37	3.42	3.56	3.81	NA	NA	–
Gal-9	4.35	3.42	3.55	3.80	NA	NA	–
Xyl (core)	4.32 (d, *J* = 7.4 Hz)	3.27	3.33	NA	3.13, 3.89	–	–
Fuc-6 (core) α,β	4.78	3.67	3.78	3.73	3.98, 4.02	–	1.10

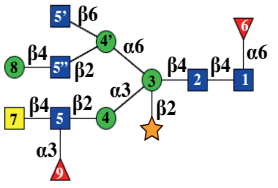
Glycan **18A:** Glycan **17A** (3.5 mg) and GDP-Fucose (6 eq) were dissolved in Tris buffer (50 mM, pH~7.3) containing $MnCl_2$ (10 mM), CIAP (10 mU). Recombinant α(1,3)-Fuctosyltransferase FUT5 (6.6 mU/μmol of substrate) was added to achieve a final concentration of 4 mmol. The resulting mixture was incubated at 37 °C for 12 h. After this time, additional GDP-Fucose (2 eq), CIAP (10 mU) and enzyme was added and incubation at 37 °C was continued until no more starting material could be detected by MALDI-TOF. The reaction mixture was quenched by adding methanol (10 μL), after which it was passed through Biogel P-6 column. Fractions containing the product were combined and lyophilized to give the product as a white fluffy solid. This product was put for HPLC purification using HILIC column, using solvent gradient ACN: H_2O (90: 10, v: v to 50: 50, v: v). The fractions containing the product were concentrated and lyophilized yielding the purified glycan **18A** as a white cotton like fluffy solid (2.5 mg). MALDI-TOF-MS (*m/z*): $[M+Na]^+$ calculated for $C_{107}H_{178}N_6O_{76}Na$, 2786.0146; found 2786.2809.



18A	H1	H2	H3	H4	H5	H6	Fuc-CH_3
GlcNAc-1α	5.10 (d, *J* = 3.1 Hz)	3.81	3.90	3.67	NA	NA	–
Man-4	5.06	4.05	3.81	3.42	3.47	3.84, 3.51	–
Man-3	4.79	4.18	3.80	3.69	3.78	3.83, 3.60	–
Man-4'	4.76	4.00	3.77	3.32	3.69	4.12, 3.48,	–
GlcNAc-1β	4.61 (d, *J* = 8.3 Hz)	3.63	3.66	NA	NA	NA	–
GlcNAc-2	4.59	3.68	3.67	3.53	NA	NA	–
GlcNAc-5"	4.52	3.65	3.56	3.62	3.43	NA	–
GlcNAc-5'	4.48 (d, *J* = 7.1 Hz)	3.80	3.56	3.63	3.42	NA	–
GlcNAc-5	4.43 (d, *J* = 8.3 Hz)	3.69	3.48	3.63	3.43	NA	–
GalNAc-7	4.38	3.87	3.64	3.85	NA	NA	–
Gal-8	4.37	3.44	3.57	3.82	NA	NA	–
Gal-9	4.37	3.43	3.56	3.80	NA	NA	–
Xyl (core)	4.36	3.32	3.32	NA	3.17, 3.93	–	–
Fuc-6 (core) α,β	4.82	3.71	3.82	3.72	4.02, 4.06	–	1.14
Fuc-10	5.04	3.88	3.86	3.67	4.77	–	1.10

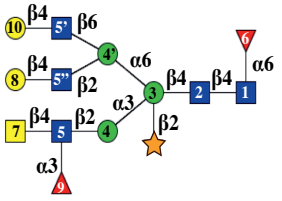
Fuc-11	5.04	3.84	3.82	3.65	4.80	–	1.18
Fuc-12	5.03	3.85	3.79	3.64	4.80	–	1.17

Glycan 19A: Glycan **7** (7.0 mg) and GDP-Fucose (2 eq) were dissolved in Tris buffer (50 mM, pH~7.3) containing $MnCl_2$ (10 mM), CIAP (10 mU). Recombinant α(1,3)-Fuctosyltransferase FUT5 (6.6 mU/μmol of substrate) was added to achieve a final concentration of 4 mmol. The resulting mixture was incubated at 37 °C for 12 h until no more starting material could be detected by MALDI-TOF. The reaction mixture was quenched by adding methanol (10 μL), after which it was passed through Biogel P-6 column. Fractions containing the product were combined and lyophilized to give the product as a white fluffy solid (7.0 mg). MALDI-TOF-MS (*m/z*): $[M+ Na]^+$ calculated for $C_{89}H_{148}N_6O_{63}Na$, 2331.8459; found 2332.0061.



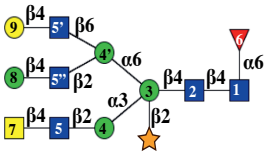
19A	H1	H2	H3	H4	H5	H6	Fuc-CH_3
GlcNAc-1α	5.11 (d, *J* = 3.2 Hz)	3.83	3.92	3.68	NA	NA	–
Man-4	5.07	4.07	3.83	3.44	NA	3.82, 3.53,	–
Man-3	4.83	4.20	3.81	3.73	3.78	3.86, 3.62	–
Man-4'	4.80	4.02	3.77	3.33	3.70	4.15, 3.50,	–
Man-8	4.70	4.00	3.59	NA	NA	NA	–
GlcNAc-1β	4.63	3.66	3.68	NA	NA	NA	–
GlcNAc-2	4.60	3.73	3.71	3.57	NA	NA	–
GlcNAc-5"	4.51 (d, *J* = 7.9 Hz)	3.68	3.57	3.66	3.43	NA	–
GlcNAc-5'	4.47	3.69	3.58	3.65	3.43	NA	–
GlcNAc-5	4.44	3.69	3.49	3.68	3.44	NA	–
GalNAc-7	4.38	3.85	3.65	3.86	NA	NA	–
Xyl (core)	4.37	3.32	3.38	NA	3.18, 3.95	–	–
Fuc-6 (core) α,β	4.79	3.71	3.82	3.72	4.04, 4.07	–	1.15
Fuc-9	5.06	3.88	3.86	3.69	4.81	–	1.20

Glycan **20A:** Glycan **19A** (2.0 mg) was dissolved in 100 mM NaOAc buffer (500 µL, pH~5.0) followed by adding 10 mM deoxyfuconojirimycin hydrochloride (20 µL) and *Helix pomatia* β-mannosidase (10 µL) the reaction mixture was then incubated at 37 °C for 12 h. After this time the reaction mixture was heated at 100 °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 combined and lyophilized. This glycan and UDP-Gal (4 eq) were dissolved in Tris buffer (50 mM, pH~7.5) containing BSA (0.1%) and $MnCl_2$ (20 mM). CIAP (10 mU) and B4GalT1 (3.4 mU/µmol) were added to achieve a final concentration of 4 mM. The reaction mixture was then incubated at 37 °C for 10 h. The reaction mixture was quenched by adding methanol (10 µL), after which it was passed through Biogel P-4 column. This product was put for HPLC purification using HILIC column, using solvent gradient ACN: H_2O (90: 10, v: v to 50: 50, v: v). The fractions containing the product were concentrated and lyophilized yielding the purified glycan **20A** as a white cotton like fluffy solid (1.0 mg). MALDI-TOF-MS (*m/z*): $[M+ Na]^+$ calculated for $C_{95}H_{158}N_6O_{68}Na$, 2493.8988; found 2494.1189.



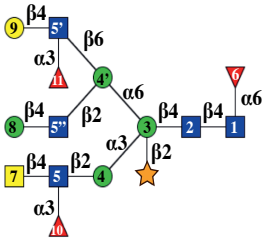
20A	H1	H2	H3	H4	H5	H6	Fuc-CH_3
GlcNAc-1α	5.11 (d, *J* = 3.2 Hz)	3.78	3.92	3.69	NA	NA	–
Man-4	5.02	4.02	3.80	3.44	NA	3.57, 3.83	–
Man-3	4.76	4.14	3.81	3.75	3.79	3.60, 3.73	–
Man-4'	4.74	3.97	3.74	3.38	3.72	3.45, 4.08	–
GlcNAc-1β	4.57	3.65	3.67	NA	NA	NA	–
GlcNAc-2	4.55	3.77	3.73	3.58	NA	NA	–
GlcNAc-5"	4.46	3.60	3.49	3.61	3.40	NA	–
GlcNAc-5'	4.43 (d, *J* = 8.1 Hz)	3.67	3.56	3.63	3.46	NA	–
GlcNAc-5	4.39	3.78	3.45	3.69	3.51	NA	–
Gal-8	4.36	3.43	3.59	3.80	NA	NA	–
Gal-10	4.36	3.48	3.58	3.81	NA	NA	–
GalNAc-7	4.34	3.85	3.67	3.91	NA	NA	–
Xyl (core)	4.32	3.27	3.34	NA	3.12, 3.89	–	–
Fuc-6 (core) α,β	4.78	3.73	3.85	3.71	3.98, 4.02	–	1.10
Fuc-9	5.01	3.83	3.78	3.64	4.74	–	1.15

Glycan **21A:** Glycan **7** (3.0 mg) and UDP-Gal (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 B4GalT1 (3.4 mU/μmol) were added to achieve a final concentration of 4 mM. The reaction mixture was then incubated at 37 °C for 10 h. The reaction mixture was quenched by adding methanol (10 μL), after which it was passed through Biogel P-4 column. Fractions containing product were combined and lyophilized to give the product as a white fluffy solid (3.0 mg). MALDI-TOF-MS (*m/z*): $[M+ Na]^+$ calculated for $C_{89}H_{148}N_6O_{64}Na$, 2347.8409; found 2348.1163.



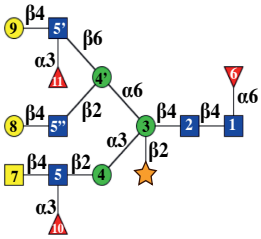
21A	H1	H2	H3	H4	H5	H6	Fuc-CH_3
GlcNAc-1α	5.07 (d, *J* = 3.0 Hz)	3.78	3.88	3.65	NA	NA	–
Man-4	5.03	4.03	3.79	3.41	NA	3.51, 3.81	–
Man-3	4.76	4.14	3.77	3.69	3.75	3.60, 3.85	–
Man-4'	4.74	3.96	3.73	3.30	3.67	3.45, 4.10	–
Man-8	4.65	3.95	3.54	NA	NA	NA	–
GlcNAc-1β	4.58	3.61	3.64	NA	NA	NA	–
GlcNAc-2	4.55	3.69	3.66	3.53	NA	NA	–
GlcNAc-5"	4.46 (d, *J* = 7.8 Hz)	3.64	3.53	3.64	3.38	NA	–
GlcNAc-5'	4.43 (d, *J* = 8.1 Hz)	3.66	3.58	3.62	3.40	NA	–
GlcNAc-5	4.40	3.63	3.43	3.64	3.41	NA	–
GalNAc-7	4.40	3.82	3.61	3.85	NA	NA	–
Gal-9	4.36 (d, *J* = 7.9 Hz)	3.48	3.57	3.82	NA	NA	–
Xyl (core)	4.32 (d, *J* = 7.7 Hz)	3.27	3.34	NA	3.14, 3.89	–	–
Fuc-6 (core) α,β	4.78	3.70	3.72	3.72	4.02, 3.98	–	1.10

Glycan **22A:** Glycan **21A** (3.0 mg) and GDP-Fucose (4 eq) were dissolved in Tris buffer (50 mM, pH~7.3) containing $MnCl_2$ (10 mM), CIAP (10 mU). Recombinant α(1,3)-Fuctosyltransferase FUT5 (6.6 mU/μmol of substrate) was added to achieve a final concentration of 4 mmol. The resulting mixture was incubated at 37 °C for 12 h. After this time, additional GDP-Fucose (2 eq), CIAP (10 mU) and enzymes were added and incubation at 37 °C was continued until no more starting material could be detected by MALDI-TOF. The reaction mixture was quenched by adding methanol (10 μL), after which it was passed through Biogel P-6 column. Fractions containing the product were combined and lyophilized to give the product as a white fluffy solid (3.6 mg). MALDI-TOF-MS (*m/z*): $[M+ Na]^+$ calculated for $C_{101}H_{168}N_6O_{72}Na$, 2639.9567; found 2640.1707.



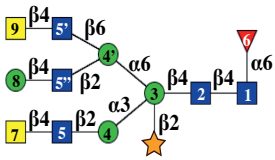
22A	H1	H2	H3	H4	H5	H6	Fuc-CH_3
GlcNAc-1α	5.06 (d, *J* = 3.2 Hz)	3.78	3.88	3.65	NA	NA	–
Man-4	5.02	4.01	3.77	3.40	NA	3.49, 3.81	–
Man-3	4.74	4.14	3.75	3.68	3.75	3.60, 3.85	–
Man-4'	4.73	3.96	3.72	3.29	3.67	3.45, 4.09	–
Man-8	4.64	3.95	3.53	NA	NA	NA	–
GlcNAc-1β	4.57 (d, *J* = 8.1 Hz)	3.62	3.64	NA	NA	NA	–
GlcNAc-2	4.55	3.68	3.67	3.53	NA	NA	–
GlcNAc-5"	4.46	3.69	3.53	3.64	3.37	NA	–
GlcNAc-5'	4.44	3.77	3.59	3.62	3.42	NA	–
GlcNAc-5	4.40 (d, *J* = 8.2 Hz)	3.80	3.47	3.68	3.45	NA	–
GalNAc-7	4.34	3.84	3.64	3.86	NA	NA	–
Gal-9	4.34	3.46	3.56	3.84	NA	NA	–
Xyl (core)	4.31	3.28	3.36	NA	3.13, 3.90	–	–
Fuc-6 (core) α,β	4.78	3.69	3.74	3.73	3.98, 4.03	–	1.10
Fuc-10	5.00	3.79	3.78	3.64	4.75	–	1.15
Fuc-11	5.00	3.83	3.80	3.66	4.73	–	1.06

Glycan **23A**: Glycan **22A** (3.6 mg) was dissolved in 100 mM NaOAc buffer (500 µL, pH~5.0) followed by adding 10 mM deoxyfuconojirimycin hydrochloride (20 µL) and *Helix pomatia* β-mannosidase (10 µL) the reaction mixture was then incubated at 37 °C for 12 h. After this time the reaction mixture was heated at 100 °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 combined and lyophilized. This glycan, UDP-Gal (2 eq) were dissolved in Tris buffer (50 mM, pH~7.5). CIAP (10 mU) and B4GalT1 were added to achieve a final concentration of 4 mM. The reaction mixture was then incubated at 37 °C for 10 h. The reaction mixture was quenched by adding methanol (10 µL), after which it was passed through Biogel P-4 column. Fractions containing product were combined and lyophilized. This product was put for HPLC purification using HILIC column, using solvent gradient ACN: H_2O (90: 10, v: v to 50: 50, v: v). The fractions containing the product were concentrated and lyophilized yielding the purified glycan **23A** as a white cotton like solid (2.0 mg). MALDI-TOF-MS (*m/z*): $[M+ Na]^+$ calculated for $C_{101}H_{168}N_6O_{72}Na$, 2639.9567; found 2640.1910.



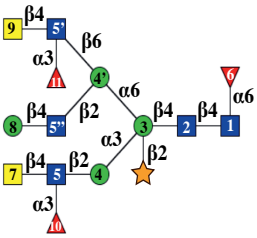
23A	H1	H2	H3	H4	H5	H6	Fuc-CH_3
GlcNAc-1α	5.10 (d, *J* = 2.8 Hz)	3.81	3.91	3.68	NA	NA	–
Man-4	5.06	4.04	3.80	3.44	NA	3.50, 3.83	–
Man-3	4.78	4.18	3.79	3.69	3.78	3.62, 3.85	–
Man-4'	4.77	4.00	3.75	3.33	3.69	3.49, 4.12	–
GlcNAc-1β	4.61	3.64	3.68	NA	NA	NA	–
GlcNAc-2	4.58	3.67	3.70	3.56	NA	NA	–
GlcNAc-5"	4.49	3.71	3.56	3.67	3.41	NA	–
GlcNAc-5'	4.48	3.83	3.61	3.64	3.45	NA	–
GlcNAc-5	4.43	3.80	3.50	3.71	3.48	NA	–
GalNAc-7	4.39	3.89	3.67	3.89	NA	NA	–
Gal-8	4.38	3.49	3.59	3.88	NA	NA	–
Gal-9	4.37	3.51	3.61	3.86	NA	NA	–
Xyl (core)	4.35	3.31	3.40	NA	3.17, 3.93	–	–
Fuc-6 (core) α,β	4.81	3.73	3.78	3.73	4.06, 4.01	–	1.13
Fuc-10	5.04	3.82	3.84	3.68	4.77	–	1.18
Fuc-11	5.04	3.85	3.83	3.69	4.76	–	1.10

Glycan **24A:** Glycan **7** (3.0 mg) and UDP-GalNAc (2 eq) were dissolved in HEPES buffer (50 mM, pH~7.5) containing BSA (0.1%) and $MnCl_2$ (20 mM). CIAP (10 mU) and B4GalT-y289l (3.4 mU/μmol) were added to achieve a final concentration of 4 mM. The reaction mixture was then incubated at 37 °C for 10 h. The reaction mixture was quenched by adding methanol (10 μL), after which it was passed through Biogel P-4 column. Fractions containing product were combined and lyophilized to give the product as a white fluffy solid (3.0 mg). MALDI-TOF-MS (*m/z*): $[M+ Na]^+$ calculated for $C_{91}H_{151}N_7O_{64}Na$, 2388.8674; found 2388.8099.



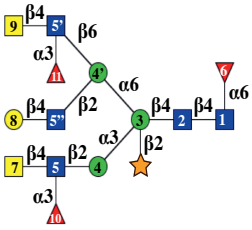
24A	H1	H2	H3	H4	H5	H6	Fuc-CH_3
GlcNAc-1α	5.12 (d, *J* = 3.0 Hz)	3.83	3.93	3.71	NA	NA	–
Man-4	5.09	4.08	3.84	3.46	NA	3.56, 3.85	–
Man-3	4.81	4.20	3.83	3.74	3.80	3.66, 3.87	–
Man-4'	4.79	4.02	3.78	3.36	3.72	3.49, 4.17	–
Man-8	4.71	4.01	3.59	NA	NA	NA	–
GlcNAc-1β	4.63 (d, *J* = 8.2 Hz)	3.65	3.68	NA	NA	NA	–
GlcNAc-2	4.61	3.73	3.71	3.59	NA	NA	–
GlcNAc-5"	4.52 (d, *J* = 8.1 Hz)	3.69	3.58	3.70	3.44	NA	–
GlcNAc-5'	4.47	3.70	3.62	3.66	3.45	NA	–
GlcNAc-5	4.46	3.68	3.48	3.68	3.47	NA	–
GalNAc-7	4.46	3.88	3.67	3.90	NA	NA	–
GalNac-9	4.45	3.85	3.65	3.89	NA	NA	–
Xyl (core)	4.38 (d, *J* = 7.7 Hz)	3.32	3.39	NA	3.21, 3.95	–	–
Fuc-6 (core) α,β	4.84	3.75	3.78	3.71	4.04, 4.08	–	1.15

Glycan **25A:** Glycan **24A** (3.0 mg) and GDP-Fucose (4 eq) were dissolved in Tris buffer (50 mM, pH~7.3) containing $MnCl_2$ (10 mM), CIAP (10 mU). Recombinant α(1,3)-Fuctosyltransferase FUT5 (6.6 mU/μmol of substrate) were added to achieve a final concentration of 4 mmol. The resulting mixture was incubated at 37 °C for 12 h. After this time, additional GDP-Fucose (2 eq), CIAP (10 mU) and enzymes were added and incubation at 37 °C was continued until no more starting material could be detected by MALDI-TOF. The reaction mixture was quenched by adding methanol (10 μL), after which it was passed through Biogel P-6 column. Fractions containing the product were combined and lyophilized to give the product as a white fluffy solid (3.3 mg). MALDI-TOF-MS (*m/z*): $[M+ Na]^+$ calculated for $C_{103}H_{171}N_7O_{72}Na$, 2680.9832; found 2681.3575.



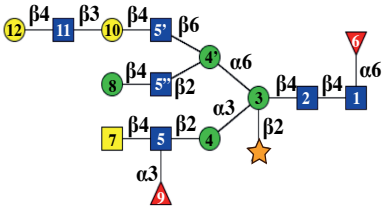
25A	H1	H2	H3	H4	H5	H6	Fuc-CH_3
GlcNAc-1α	5.06 (d, *J* = 3.1 Hz)	3.77	3.88	3.64	NA	NA	–
Man-4	5.02	4.01	3.78	3.41	NA	3.49, 3.81	–
Man-3	4.75	4.14	3.75	3.68	3.76	3.60, 3.85	–
Man-4'	4.73	3.96	3.72	3.30	3.67	3.45, 4.09	–
Man-8	4.64	3.95	3.53	NA	NA	NA	–
GlcNAc-1β	4.57 (d, *J* = 8.1 Hz)	3.58	3.62	3.65	NA	NA	–
GlcNAc-2	4.55	3.61	3.67	3.52	NA	NA	–
GlcNAc-5"	4.46 (d, *J* = 8.6 Hz)	3.67	3.53	3.65	3.38	NA	–
GlcNAc-5'	4.40	3.77	3.60	3.62	3.43	NA	–
GlcNAc-5	4.39	3.81	3.48	3.68	3.47	NA	–
GalNAc-7	4.34	3.86	3.66	3.87	NA	NA	–
GalNac-9	4.34	3.85	3.64	3.85	NA	NA	–
Xyl (core)	4.31	3.29	3.37	NA	3.98, 4.02	–	–
Fuc-6 (core) α,β	4.78	3.69	3.74	3.71	3.98, 4.02	–	1.10
Fuc-10	5.01	3.81	3.80	3.65	4.76	–	1.15
Fuc-11	5.01	3.83	3.81	3.67	4.75	–	1.15

Glycan **26A**: Glycan **25A** (3.3 mg) was dissolved in 100 mM NaOAc buffer (500 µL, pH~5.0) followed by adding 10 mM deoxyfuconojirimycin hydrochloride (20 µL) and *Helix pomatia* β-mannosidase (10 µL) the reaction mixture was then incubated at 37 °C for 12 h. After this time the reaction mixture was heated at 100 °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 combined and lyophilized. This glycan and UDP-Gal (2 eq) were dissolved in Tris buffer (50 mM, pH~7.5). CIAP and B4GalT1 were added, and the reaction mixture was then incubated at 37 °C for 10 h. The reaction mixture was quenched by adding methanol (10 µL), after which it was passed through Biogel P-4 column. This product was put for HPLC purification using HILIC column, using solvent gradient ACN: H_2O (90: 10, v: v to 50: 50, v: v). The fractions containing the product were concentrated and lyophilized yielding the purified glycan **26A** as a white cotton like fluffy solid (1.8 mg). MALDI-TOF-MS (*m/z*): $[M+ Na]^+$ calculated for $C_{103}H_{171}N_7O_{72}Na$, 2680.9832; found 2681.3196.



26A	H1	H2	H3	H4	H5	H6	Fuc-CH_3
GlcNAc-1α	5.10 (d, *J* = 3.0 Hz)	3.82	3.91	3.68	NA	NA	–
Man-4	5.06	4.05	3.81	3.44	NA	3.50, 3.83	–
Man-3	4.79	4.18	3.79	3.70	3.76	3.62, 3.85	–
Man-4'	4.77	4.00	3.75	3.33	3.69	3.49, 4.12	–
GlcNAc-1β	4.62	3.64	3.68	NA	NA	NA	–
GlcNAc-2	4.59	3.70	3.72	3.56	NA	NA	–
GlcNAc-5"	4.50 (d, *J* = 7.8 Hz)	3.72	3.57	3.69	3.43	NA	–
GlcNAc-5'	4.44	3.83	3.61	3.63	3.47	NA	–
GlcNAc-5	4.43	3.82	3.52	3.71	3.48	NA	–
GalNAc-7	4.39	3.90	3.66	3.89	NA	NA	–
Gal-8	4.39	3.50	3.59	3.90	NA	NA	–
GalNac-9	4.37	3.88	3.68	3.92	NA	NA	–
Xyl (core)	4.36	3.32	3.41	NA	3.17, 3.94	–	–
Fuc-6 (core) α,β	4.81	3.73	3.78	3.72	4.06, 4.02	–	1.14
Fuc-10	5.04	3.86	3.84	3.68	4.79	–	1.19
Fuc-11	5.04	3.87	3.83	3.69	4.78	–	1.19

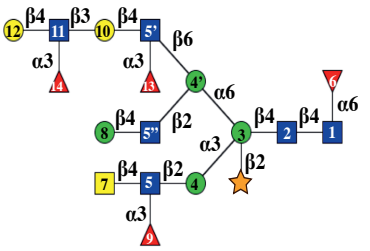
Glycan **28A:** Glycan **19A** (5.0 mg) and UDP-Gal (2 eq) were dissolved in Tris buffer (50 mM, pH~7.5) and CIAP and B4GalT1 were added to achieve a final concentration of 4 mM. The reaction mixture was then incubated at 37 °C for 10 h. The reaction mixture was quenched by adding methanol (10 µL), after which it was passed through Biogel P-4 column. Fractions containing product were combined and lyophilized to give the galactosylated glycan **27A** (5.2 mg). This glycan and UDP-GlcNAc (2 eq) were dissolved in HEPES buffer. To this, CIAP and B3GNT2 and the resulting mixture was then incubated at 37 °C for 12 h and then quenched with methanol (10 µL) and purified over P-4 column. This intermediate glycan was again put for galactosylation reaction using the conditions described above, yielding glycan **28A** (6.0 mg). This product was put for HPLC purification using HILIC column, using solvent gradient ACN: H_2O (90: 10, v: v to 50: 50, v: v). The fractions containing the product were concentrated and lyophilized yielding the purified glycan **39** as a white cotton like fluffy solid (4.0 mg). MALDI-TOF-MS (*m/z*): $[M+ Na]^+$ calculated for $C_{109}H_{181}N_7O_{78}Na$, 2859.0310; found 2859.2184.



28A	H1	H2	H3	H4	H5	H6	Fuc-CH_3
GlcNAc-1α	5.06 (d, *J* = 3.2 Hz)	3.77	3.89	3.69	NA	3.71, 3.57	–
Man-4	5.02	4.02	3.77	3.39	3.45	3.82, 3.49	–
Man-3	4.75	4.14	3.65	3.73	3.42	3.81, 3.65	–
Man-4'	4.74	3.96	3.73	3.32	3.71	4.13, 3.43	–
Man-8	4.65	3.95	3.53	NA	NA	NA	–
GlcNAc-11	4.60	3.70	3.56	3.69	NA	NA	–
GlcNAc-1β	4.59	3.62	3.68	NA	NA	NA	–
GlcNAc-2	4.56	3.78	3.66	3.52	NA	NA	–
GlcNAc-5"	4.46 (d, *J* = 8.0 Hz)	3.64	3.58	3.64	3.40	NA	–
GlcNAc-5'	4.43 (d, *J* = 7.8 Hz)	3.68	3.51	3.62	3.42	NA	–
GlcNAc-5	4.39	3.79	3.49	3.60	3.41	NA	–
GalNAc-7	4.37	3.87	3.62	3.81	NA	NA	–
Gal-10	4.35	3.53	3.56	3.86	3.56	NA	–
Gal-12	4.34	3.56	3.58	3.88	3.54	NA	–
Xyl (core)	4.32	3.33	3.37	NA	3.13, 3.90	–	–

Fuc-6 (core) α,β	4.78	3.69	3.82	3.71	3.98, 4.03	–	1.10
Fuc-9	5.00	3.83	3.81	3.69	4.75	–	1.15

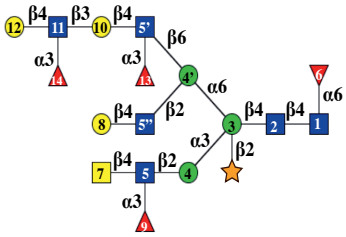
Glycan **29A:** Glycan **28A** (4.0 mg) and GDP-Fucose (4 eq) were dissolved in Tris buffer (50 mM, pH~7.3) containing $MnCl_2$ (10 mM), CIAP (10 mU). Recombinant α(1,3)-Fuctosyltransferase FUT5 (6.6 mU/μmol of substrate) was added to achieve a final concentration of 4 mmol. The resulting mixture was incubated at 37 °C for 12 h. After this time, additional GDP-Fucose (2 eq), CIAP (10 mU) and FUT5 enzyme was added and incubation at 37 °C was continued until no more starting material could be detected by MALDI-TOF. The reaction mixture was quenched by adding methanol (10 μL), after which it was passed through Biogel P-6 column. Fractions containing the product were combined and lyophilized to give the product as a white fluffy solid (4.3 mg). MALDI-TOF-MS (*m/z*): $[M+ Na]^+$ calculated for $C_{121}H_{201}N_7O_{86}Na$, 3151.1468; found 3151.4019.



29A	**H1**	**H2**	**H3**	**H4**	**H5**	**H6**	**Fuc-CH_3**
GlcNAc-1α	5.07 (d, *J* = 3.1 Hz)	3.78	3.89	3.70	NA	3.73, 3.59	–
Man-4	5.03	4.01	3.78	3.39	3.46	3.82, 3.49	–
Man-3	4.76	4.15	3.66	3.73	3.43	3.82, 3.60	–
Man-4'	4.74	3.97	3.73	3.31	3.71	4.10, 3.46	–
Man-8	4.66	3.95	3.54	NA	NA	NA	–
GlcNAc-11	4.60 (d, *J* = 8.3 Hz)	3.70	3.57	3.69	NA	NA	–
GlcNAc-1β	4.59	3.62	3.63	NA	NA	NA	–
GlcNAc-2	4.56	3.67	3.65	3.52	NA	NA	–
GlcNAc-5"	4.46	3.78	3.59	3.64	3.41	NA	–
GlcNAc-5'	4.45	3.83	3.52	3.62	3.43	NA	–
GlcNAc-5	4.40	3.79	3.48	3.60	3.42	NA	–
GalNAc-7	4.35	3.88	3.61	3.82	NA	NA	–
Gal-10	4.34	3.53	3.56	3.85	NA	NA	–

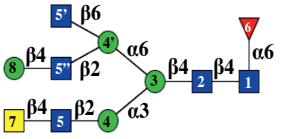
Gal-12	4.34	3.56	3.58	3.87	NA	NA	–
Xyl (core)	4.32	3.34	3.37	NA	3.14, 3.91	–	–
Fuc-6 (core) α,β	4.79	3.70	3.82	3.69	4.03, 3.99	–	1.10
Fuc-9	5.01 (d, *J* = 4.0 Hz)	3.82	3.81	3.64	4.76	–	1.16
Fuc-13	5.03	3.81	3.79	3.62	4.73	–	1.06
Fuc-14	4.99 (d, *J* = 3.8 Hz)	3.79	3.78	3.64	4.71	–	1.04

Glycan **30A:** Glycan **29A** (4.3 mg) was dissolved in 100 mM NaOAc buffer (500 µL, pH~5.0) followed by adding 10 mM deoxyfuconojirimycin hydrochloride (20 µL) and *Helix pomatia* β-mannosidase (10 µL) the reaction mixture was then incubated at 37 °C for 12 h. After this time the reaction mixture was heated at 100 °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 combined and lyophilized. This glycan and UDP-Gal (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 B4GalT1 (3.4 mU/µmol) were added to achieve a final concentration of 4 mM. The reaction mixture was then incubated at 37 °C for 10 h. The reaction mixture was quenched by adding methanol (10 µL), after which it was passed through Biogel P-4 column. Fractions containing product were combined and lyophilized to give the product as a white fluffy solid (4.5 mg). This product was put for HPLC purification using HILIC column, using solvent gradient ACN: H_2O (90: 10, v: v to 50: 50, v: v). The fractions containing the product were concentrated and lyophilized yielding the purified glycan **30A** as a white cotton like fluffy solid (2.5 mg). MALDI-TOF-MS (*m/z*): $[M+ Na]^+$ calculated for $C_{121}H_{201}N_7O_{86}Na$, 3151.1468; found 3151.3990.



30A	H1	H2	H3	H4	H5	H6	Fuc-CH_3
GlcNAc-1α	5.07 (d, *J* = 3.0 Hz)	3.81	3.92	3.71	NA	3.77, 3.65	–
Man-4	5.06	4.05	3.81	3.41	3.49	3.83, 3.53	–
Man-3	4.79	4.18	3.68	3.75	3.46	3.86, 3.62	–
Man-4'	4.77	4.00	3.76	3.35	3.74	4.13, 3.47	–
GlcNAc-11	4.64	3.70	3.61	3.72	NA	NA	–
GlcNAc-1β	4.61	3.63	3.65	NA	NA	NA	–
GlcNAc-2	4.59	3.69	3.68	3.54	NA	NA	–
GlcNAc-5"	4.50	3.78	3.63	3.65	3.44	NA	–
GlcNAc-5'	4.48	3.82	3.55	3.68	3.45	NA	–
GlcNAc-5	4.43 (d, *J* = 8.1 Hz)	3.84	3.51	3.63	3.47	NA	–
GalNAc-7	4.39	3.87	3.66	3.85	NA	NA	–
Gal-8	4.37	3.56	3.54	3.87	NA	NA	–
Gal-10	4.37	3.58	3.60	3.89	NA	NA	–
Gal-12	4.36	3.59	3.57	3.91	NA	NA	–
Xyl (core)	4.35	3.36	3.39	NA	3.18, 3.93	–	–
Fuc-6 (core) α,β	4.82	3.73	3.86	3.72	4.06, 4.02	–	1.14
Fuc-9	5.04 (d, *J* = 3.8 Hz)	3.86	3.84	3.68	4.79	–	1.19
Fuc-13	5.06	3.84	3.82	3.66	4.76	–	1.10
Fuc-14	5.03 (d, *J* = 3.6 Hz)	3.83	3.80	3.65	4.74	–	1.07

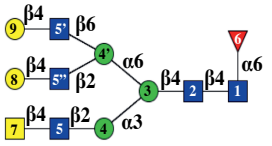
Glycan **8:** Synthesis described in chapter 4.



8	H1	H2	H3	H4	H5	H6	Fuc-CH_3
GlcNAc-1α	5.07 (d, *J* = 3.0 Hz)	3.78	3.89	3.69	NA	3.73, 3.60	–
Man-4	5.00 (s)	4.07	3.80	3.38	3.47	3.82, 3.50	–
Man-4'	4.75	3.97	3.76	3.29	3.71	4.09, 3.47	–
Man-3	4.65	4.13	3.67	3.71	3.46	3.81, 3.63	–
Man-8	4.65	3.96	3.55	3.62	3.34	NA	–
GlcNAc-1β	4.58 (d, *J* = 8.2 Hz)	3.59	3.63	NA	NA	NA	–
GlcNAc-2	4.55	3.67	3.65	3.50	NA	NA	–
GlcNAc-5"	4.48 (d, *J* = 7.3 Hz)	3.62	3.38	3.61	3.38	NA	–
GlcNAc-5	4.44	3.62	3.47	3.61	3.39	NA	–
GlcNAc-5'	4.42	3.61	3.48	3.62	3.39	NA	
GalNAc-7	4.40	3.82	3.60	3.82	NA	NA	–
Fuc-6 (core) (α, β)	4.78	3.68	3.81	3.68	3.98, 4.02	–	1.10

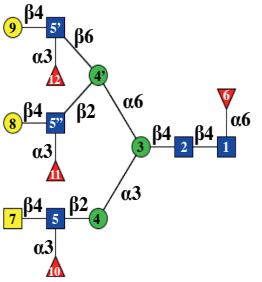
MALDI-TOF-MS (*m/z*): $[M+ Na]^+$ calculated for $C_{78}H_{130}N_6O_{55}Na$, 2053.7458; found 2053.8128.

Glycan **17B**: Glycan **8** (5.0 mg) was dissolved in 100 mM NaOAc buffer (500 µL, pH~5.0) followed by adding 10 mM deoxyfuconojirimycin hydrochloride (20 µL) and *Helix pomatia* β-mannosidase (10 µL) the reaction mixture was then incubated at 37 °C for 12 h. After this time the reaction mixture was heated at 100 °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 combined and lyophilized. This glycan and UDP-Gal (4 eq) were dissolved in Tris buffer (50 mM, pH~7.5) containing BSA (0.1%) and $MnCl_2$ (20 mM). CIAP (10 mU) and B4GalT1 (3.4 mU/µmol) were added to achieve a final concentration of 4 mM. The reaction mixture was then incubated at 37 °C for 10 h. The reaction mixture was quenched by adding methanol (10 µL), after which it was passed through Biogel P-4 column. Fractions containing product were combined and lyophilized to give the product as a white fluffy solid (5.0 mg). MALDI-TOF-MS (*m/z*): $[M+Na]^+$ calculated for $C_{84}H_{140}N_6O_{60}Na$, 2215.7986; found 2215.6672.



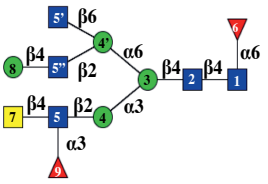
17B	H1	H2	H3	H4	H5	H6	Fuc-CH_3
GlcNAc-1α	5.07 (d, *J* = 3.1 Hz)	3.78	3.88	3.69	NA	3.72, 3.60	–
Man-4	5.00	4.07	3.80	3.38	3.44	3.81, 3.49	–
Man-4'	4.76	3.97	3.75	3.29	3.70	4.09, 3.46	–
Man-3	4.65	4.14	3.67	3.71	3.43	3.81, 3.63	–
GlcNAc-1β	4.58 (d, *J* = 8.0 Hz)	3.59	3.63	NA	NA	NA	–
GlcNAc-2	4.54	3.67	3.65	3.50	NA	NA	–
GlcNAc-5"	4.48 (d, *J* = 7.5 Hz)	3.66	3.38	3.61	3.38	NA	–
GlcNAc-5	4.44	3.64	3.47	3.61	3.39	NA	–
GlcNAc-5'	4.43	3.61	3.48	3.62	3.39	NA	
GalNAc-7	4.40 (d, *J* = 8.5 Hz)	3.82	3.60	3.82	NA	NA	
Gal-8	4.37	3.44	3.55	3.83	3.53	NA	–
Gal-9	4.35	3.42	3.54	3.83	3.52	NA	
Fuc-6 (core) (α, β)	4.78	3.69	3.81	3.68	3.98, 4.02	–	1.10

Glycan **18B:** Glycan **17B** (2.5 mg) and GDP-Fucose (6 eq) were dissolved in Tris buffer (50 mM, pH~7.3) containing $MnCl_2$ (10 mM), CIAP (10 mU). Recombinant α(1,3)-Fuctosyltransferase FUT5 (6.6 mU/μmol of substrate) were added to achieve a final concentration of 4 mmol. The resulting mixture was incubated at 37 °C for 12 h. After this time, additional GDP-Fucose (2 eq), CIAP (10 mU) and enzymes were added and incubation at 37 °C was continued until no more starting material could be detected by MALDI-TOF. The reaction mixture was quenched by adding methanol (10 μL), after which it was passed through Biogel P-6 column. Fractions containing the product were combined and lyophilized to give the product as a white fluffy solid. This product was put for HPLC purification using HILIC column, using solvent gradient ACN: H_2O (90: 10, v: v to 50: 50, v: v). The fractions containing the product were concentrated and lyophilized yielding the purified glycan **18B** as a white cotton like fluffy solid (1.7 mg). MALDI-TOF-MS (*m/z*): $[M+Na]^+$ calculated for $C_{102}H_{170}N_6O_{72}Na$, 2653.9723; found 2654.2361.



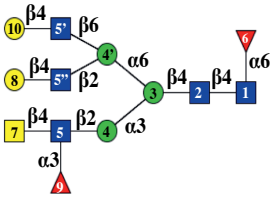
18B	H1	H2	H3	H4	H5	H6	Fuc-CH_3
GlcNAc-1α	5.06 (d, *J* = 2.9 Hz)	3.77	3.86	3.69	NA	3.72, 3.61	–
Man-4	5.00	4.05	3.78	3.39	3.44	3.81, 3.48	–
Man-4'	4.74	3.96	3.74	3.30	3.69	4.09, 3.46	–
Man-3	4.65	4.14	3.67	3.71	3.46	3.81, 3.65	–
GlcNAc-1β	4.48 (d, *J* = 8.1 Hz)	3.58	3.62	NA	NA	NA	–
GlcNAc-2	4.54	3.66	3.65	3.51	NA	NA	–
GlcNAc-5"	4.48	3.71	3.41	3.65	NA	NA	–
GlcNAc-5	4.45	3.72	3.43	3.66	NA	NA	–
GlcNAc-5'	4.43	3.76	3.46	3.63	3.38	NA	–
GalNAc-7	4.35	3.85	3.63	3.82	NA	NA	–
Gal-8	4.33	3.53	3.55	3.85	3.52	NA	–
Gal-9	4.33	3.56	3.59	3.89	3.55	NA	–
Fuc-6 (core) α, β	4.77	3.68	3.81	3.68	3.98, 4.01	–	1.10
Fuc-10	5.01	3.83	3.82	3.64	4.76	–	1.15
Fuc-11	5.00	3.80	3.78	3.62	4.72	–	1.06
Fuc-12	4.98	3.82	3.75	3.61	4.73	–	1.06

Glycan 19B: Glycan **8** (10.0 mg) and GDP-Fucose (2 eq) were dissolved in Tris buffer (50 mM, pH~7.3) containing $MnCl_2$ (10 mM), CIAP (10 mU). Recombinant α(1,3)-Fuctosyltransferase FUT5 (6.6 mU/μmol of substrate) were added to achieve a final concentration of 4 mmol. The resulting mixture was incubated at 37 °C for 12 h. After this time, additional GDP-Fucose (2 eq), CIAP (10 mU) and enzyme were added and incubation at 37 °C was continued until no more starting material could be detected by MALDI-TOF. The reaction mixture was quenched by adding methanol (10 μL), after which it was passed through Biogel P-6 column. Fractions containing the product were combined and lyophilized to give the product as a white fluffy solid (10.0 mg). MALDI-TOF-MS (*m/z*): $[M+ Na]^+$ calculated for $C_{84}H_{140}N_6O_{59}Na$, 2199.8037; found 2199.9138.



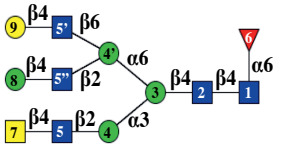
19B	H1	H2	H3	H4	H5	H6	Fuc-CH_3
GlcNAc-1α	5.07 (d, *J* = 2.2 Hz)	3.78	3.88	3.69	NA	3.72, 3.60	–
Man-4	4.99	4.06	3.80	3.38	3.43	3.81, 3.49	–
Man-4'	4.75	3.98	3.76	3.28	3.70	4.10, 3.47	–
Man-3	4.65	4.14	3.67	3.72	3.41	3.80, 3.63	–
Man-8	4.65	3.96	3.55	NA	NA	NA	–
GlcNAc-1β	4.58 (d, *J* = 8.2 Hz)	3.59	3.63	NA	NA	NA	–
GlcNAc-2	4.55	3.66	3.65	3.50	NA	NA	–
GlcNAc-5"	4.48 (d, *J* = 6.5 Hz)	3.61	3.38	3.61	3.38	NA	–
GlcNAc-5	4.44	3.63	3.46	3.60	3.39	NA	–
GlcNAc-5'	4.41	3.60	3.47	3.61	3.39	NA	–
GalNAc-7	4.33 (d, *J* = 8.4 Hz)	3.86	3.62	3.82	NA	NA	–
Fuc-6 (core) (α, β)	4.78	3.68	3.81	3.68	3.98, 4.02	–	1.10
Fuc-9	5.02 (d, *J* = 3.4 Hz)	3.84	3.82	3.66	4.75	–	1.15

Glycan **20B:** Glycan **19B** (3.0 mg) was dissolved in 100 mM NaOAc buffer (500 µL, pH~5.0) followed by adding 10 mM deoxyfuconojirimycin hydrochloride (20 µL) and *Helix pomatia* β-mannosidase (10 µL) the reaction mixture was then incubated at 37 °C for 12 h. After this time the reaction mixture was heated at 100 °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 combined and lyophilized. This glycan and UDP-Gal (4 eq) were dissolved in Tris buffer (50 mM, pH~7.5) containing BSA (0.1%) and $MnCl_2$ (20 mM). CIAP (10 mU) and B4GalT1 (3.4 mU/µmol) were added to achieve a final concentration of 4 mM. The reaction mixture was then incubated at 37 °C for 10 h. The reaction mixture was quenched by adding methanol (10 µL), after which it was passed through Biogel P-4 column. This product was put for HPLC purification using HILIC column, using solvent gradient ACN: H_2O (90: 10, v: v to 50: 50, v: v). The fractions containing the product were concentrated and lyophilized yielding the purified glycan as a white cotton like fluffy solid (1.5 mg). MALDI-TOF-MS (*m/z*): $[M+ Na]^+$ calculated for $C_{90}H_{150}N_6O_{64}Na$, 2361.8565; found 2361.6023.



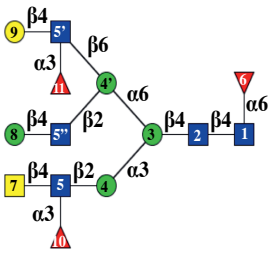
20B	H1	H2	H3	H4	H5	H6	Fuc-CH_3
GlcNAc-1α	5.10 (d, *J* = 2.8 Hz)	3.81	3.90	3.71	NA	3.75, 3.63	–
Man-4	5.03	4.09	3.83	3.40	3.46	3.85, 3.52	–
Man-4'	4.79	4.00	3.77	3.32	3.77	4.13, 3.50	–
Man-3	4.67	4.17	3.68	3.74	NA	3.83, 3.66	–
GlcNAc-1β	4.61	3.62	3.65	NA	NA	NA	–
GlcNAc-2	4.58	3.70	3.68	NA	NA	NA	–
GlcNAc-5"	4.50	3.64	3.42	3.41	NA	NA	–
GlcNAc-5	4.47	3.66	3.48	3.42	NA	NA	–
GlcNAc-5'	4.46	3.63	3.49	3.45	NA	NA	–
Gal-8	4.40	3.57	3.59	3.83	3.44	NA	–
Gal-10	4.39	3.60	3.61	3.85	3.46	NA	–
GalNAc-7	4.35	3.88	3.64	3.88	3.43	NA	–
Fuc-6 (core) (α, β)	4.81	3.72	3.83	NA	4.02, 4.05	–	1.14
Fuc-9	5.05 (d, *J* = 3.7 Hz)	3.86	3.84	3.69	4.78	–	1.19

Glycan 21B: Glycan **8** (3.0 mg) and UDP-Gal (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 B4GalT1 (3.4 mU/μmol) were added to achieve a final concentration of 4 mM. The reaction mixture was then incubated at 37 °C for 10 h. The reaction mixture was quenched by adding methanol (10 μL), after which it was passed through Biogel P-4 column. Fractions containing product were combined and lyophilized to give the product as a white fluffy solid (3.0 mg). MALDI-TOF-MS (*m/z*): $[M+ Na]^+$ calculated for $C_{84}H_{140}N_6O_{60}Na$, 2215.7986; found 2216.0514.



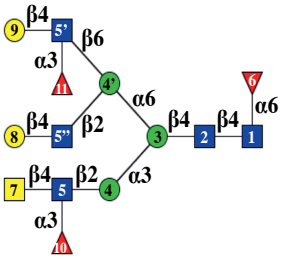
21B	H1	H2	H3	H4	H5	H6	Fuc-CH_3
GlcNAc-1α	5.10, (d, *J* = 2.9 Hz)	3.81	3.93	3.72	NA	3.75, 3.63	–
Man-4	5.04	4.10	3.84	3.42	3.47	3.83, 3.52	–
Man-4'	4.79	4.00	3.79	3.33	3.72	4.12, 3.50	–
Man-3	4.69	4.17	3.71	3.74	3.47	3.84, 3.67	–
Man-8	4.68	3.99	3.57	NA	NA	NA	–
GlcNAc-1β	4.61, (d, *J* = 8.1 Hz)	3.63	3.65	NA	NA	NA	–
GlcNAc-2	4.58	3.70	3.68	3.54	NA	NA	–
GlcNAc-5"	4.51	3.68	3.42	3.64	3.43	NA	–
GlcNAc-5	4.48	3.67	3.50	3.65	3.42	NA	–
GlcNAc-5'	4.47	3.64	3.51	3.64	3.42	NA	–
GalNAc-7	4.44 (d, *J* = 8.3 Hz)	3.85	3.63	3.84	NA	NA	–
Gal-9	4.40 (d, *J* = 7.4 Hz)	3.45	3.57	3.85	3.55	NA	–
Fuc-6 (core) (α, β)	4.82	3.72	3.83	3.72	4.01, 4.05	–	1.10

Glycan **22B:** Glycan **21B** (3.0 mg) and GDP-Fucose (4 eq) were dissolved in Tris buffer (50 mM, pH~7.3) containing $MnCl_2$ (10 mM), CIAP (10 mU). Recombinant α(1,3)-Fuctosyltransferase FUT5 (6.6 mU/μmol of substrate) were added to achieve a final concentration of 4 mmol and incubation at 37 °C was continued until no more starting material could be detected by MALDI-TOF. The reaction mixture was quenched by adding methanol (10 μL), after which it was passed through Biogel P-6 column. Fractions containing the product were combined and lyophilized to give the product as a white fluffy solid (3.5 mg). MALDI-TOF-MS (*m/z*): $[M+ Na]^+$ calculated for $C_{96}H_{160}N_6O_{68}Na$, 2507.9144; found 2508.1067.



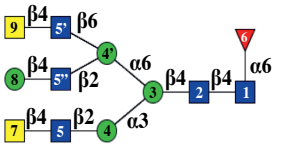
22B	H1	H2	H3	H4	H5	H6	Fuc-CH_3
GlcNAc-1α	5.10 (d, *J* = 2.9 Hz)	3.81	3.93	3.71	NA	3.75, 3.64	–
Man-4	5.05	4.09	3.84	3.41	3.47	3.83, 3.51	–
Man-4'	4.80	4.01	3.78	3.33	3.72	4.12, 3.52	–
Man-3	4.69	4.17	3.71	3.74	3.48	3.84, 3.68	–
Man-8	4.69	3.99	3.57	NA	NA	NA	–
GlcNAc-1β	4.62 (d, *J* = 8.4 Hz)	3.63	3.65	NA	NA	NA	–
GlcNAc-2	4.58	3.71	3.69	3.54	NA	NA	–
GlcNAc-5"	4.52 (d, *J* = 8.0 Hz)	3.68	3.43	3.65	3.44	NA	–
GlcNAc-5	4.49	3.74	3.51	3.65	3.42	NA	–
GlcNAc-5'	4.47	3.69	3.52	3.66	3.45	NA	–
Gal-9	4.39	3.53	3.59	3.86	NA	NA	–
GalNAc-7	4.37	3.84	3.64	3.83	NA	NA	–
Fuc-6 (core) (α, β)	4.82	3.72	3.83	3.74	4.02, 4.06	–	1.14
Fuc-10	5.04	3.84	3.83	3.64	4.79	–	1.19
Fuc-11	5.02	3.82	3.81	3.63	4.76	–	1.10

Glycan **23B**: Glycan **22B** (3.5 mg) was dissolved in 100 mM NaOAc buffer (500 µL, pH~5.0) followed by adding 10 mM deoxyfuconojirimycin hydrochloride (20 µL) and *Helix pomatia* β-mannosidase (10 µL) the reaction mixture was then incubated at 37 °C for 12 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 combined and lyophilized. This glycan and UDP-Gal (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 B4GalT1 (3.4 mU/µmol) were added to achieve a final concentration of 4 mM. The reaction mixture was then incubated at 37 °C for 10 h. The reaction mixture was quenched by adding methanol (10 µL), after which it was passed through Biogel P-4 column. Fractions containing product were combined and lyophilized to give the product as a white fluffy solid. This product was put for HPLC purification using HILIC column, using solvent gradient ACN: H_2O (90: 10, v: v to 50: 50, v: v). The fractions containing the product were concentrated and lyophilized yielding the purified glycan **23B** as a white cotton like solid (2.0 mg). MALDI-TOF-MS (*m/z*): $[M+ Na]^+$ calculated for $C_{96}H_{160}N_6O_{68}Na$, 2507.9144; found 2508.1620.



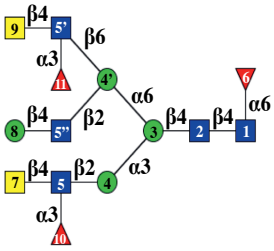
23B	H1	H2	H3	H4	H5	H6	Fuc-CH_3
GlcNAc-1α	5.10 (d, *J* = 2.9 Hz)	3.81	3.93	3.72	NA	3.75, 3.64	–
Man-4	5.05	4.09	3.84	3.40	3.48	3.82, 3.53	–
Man-4'	4.79	4.00	3.78	3.33	3.70	4.12, 3.52	–
Man-3	4.68	4.18	3.72	3.74	3.48	3.84, 3.69	–
GlcNAc-1β	4.61	3.63	3.65	NA	NA	NA	–
GlcNAc-2	4.58	3.71	3.69	3.55	NA	NA	–
GlcNAc-5"	4.52	3.69	3.43	3.65	3.44	NA	–
GlcNAc-5	4.48	3.74	3.53	3.66	3.42	NA	–
GlcNAc-5'	4.47	3.69	3.52	3.66	3.43	NA	–
Gal-8	4.39	3.55	3.62	3.86	NA	NA	–
Gal-9	4.39	3.53	3.60	3.86	NA	NA	–
GalNAc-7	4.37	3.84	3.64	3.84	NA	NA	–
Fuc-6 (core) (α, β)	4.82	3.72	3.83	3.75	4.01, 4.05	–	1.13
Fuc-10	5.04	3.85	3.84	3.64	4.79	–	1.18
Fuc-11	5.02	3.82	3.81	3.64	4.77	–	1.09

Glycan **24B:** Glycan **8** (3.0 mg) and UDP-GalNAc (2 eq) were dissolved in HEPES buffer (50 mM, pH~7.5) containing BSA (0.1%) and $MnCl_2$ (20 mM). CIAP (10 mU) and B4GalT-y289l (3.4 mU/μmol) were added to achieve a final concentration of 4 mM. The reaction mixture was then incubated at 37 °C for 10 h. The reaction mixture was quenched by adding methanol (10 μL), after which it was passed through Biogel P-4 column. Fractions containing product were combined and lyophilized to give the product as a white fluffy solid (3.0 mg). MALDI-TOF-MS (*m/z*): $[M+ Na]^+$ calculated for $C_{86}H_{143}N_7O_{60}Na$, 2256.8251; found 2257.1463.



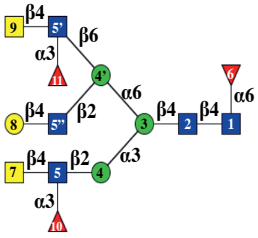
24B	H1	H2	H3	H4	H5	H6	Fuc-CH_3
GlcNAc-1α	5.10 (d, *J* = 2.8 Hz)	3.80	3.92	3.72	NA	3.76, 3.63	–
Man-4	5.03	4.10	3.84	3.42	3.47	3.83, 3.52	–
Man-4'	4.78	3.99	3.81	3.33	3.72	4.13, 3.51	–
Man-3	4.68	4.16	3.70	3.74	3.45	3.85, 3.68	–
Man-8	4.68	3.98	3.56	NA	NA	NA	–
GlcNAc-1β	4.61	3.62	3.64	NA	NA	NA	–
GlcNAc-2	4.58	3.71	3.67	3.54	NA	NA	–
GlcNAc-5"	4.51	3.68	3.42	3.65	3.43	NA	–
GlcNAc-5	4.48	3.67	3.50	3.65	3.42	NA	–
GlcNAc-5'	4.45	3.63	3.52	3.64	3.43	NA	–
GalNAc-7	4.44	3.85	3.63	3.85	NA	NA	–
GalNac-9	4.44	3.87	3.64	3.87	NA	NA	–
Fuc-6 (core) (α, β)	4.82	3.72	3.83	3.71	4.01, 4.05	–	1.13

Glycan **25B:** Glycan **24B** (3.0 mg) and GDP-Fucose (4 eq) were dissolved in Tris buffer (50 mM, pH~7.3) containing $MnCl_2$ (10 mM), CIAP (10 mU). Recombinant α(1,3)-Fuctosyltransferase FUT5 (6.6 mU/μmol of substrate) were added to achieve a final concentration of 4 mmol. The resulting mixture was incubated at 37 °C was continued until no more starting material could be detected by MALDI-TOF. The reaction mixture was quenched by adding methanol (10 μL), after which it was passed through Biogel P-6 column. Fractions containing the product were combined and lyophilized to give the product as a white fluffy solid (3.5 mg). MALDI-TOF-MS (*m/z*): $[M+Na]^+$ calculated for $C_{98}H_{163}N_7O_{68}Na$, 2548.9410; found 2549.4137.



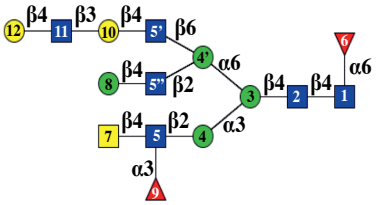
25B	H1	H2	H3	H4	H5	H6	Fuc-CH_3
GlcNAc-1α	5.10 (d, *J* = 2.3 Hz)	3.81	3.93	3.72	NA	3.75, 3.63	–
Man-4	5.04	4.10	3.84	3.41	3.48	3.83, 3.52	–
Man-4'	4.79	4.00	3.78	3.34	3.73	4.12, 3.54	–
Man-3	4.68	4.17	3.71	3.74	3.48	3.85, 3.69	–
Man-8	4.67	3.99	3.57	NA	NA	NA	–
GlcNAc-1β	4.61	3.63	3.65	NA	NA	NA	–
GlcNAc-2	4.58	3.71	3.69	3.55	NA	NA	–
GlcNAc-5"	4.51 (d, *J* = 7.6 Hz)	3.69	3.43	3.66	3.45	NA	–
GlcNAc-5	4.47	3.70	3.51	3.65	3.42	NA	–
GlcNAc-5'	4.45	3.69	3.52	3.66	3.46	NA	–
GalNAc-7	4.39	3.84	3.64	3.85	NA	NA	–
GalNac-9	4.37	3.87	3.63	3.87	NA	NA	–
Fuc-6 (core) (α, β)	4.81	3.72	3.83	3.75	4.01, 4.05	–	1.14
Fuc-10	5.05	3.87	3.85	3.65	4.81	–	1.19
Fuc-11	5.02	3.86	3.83	3.64	4.79	–	1.19

Glycan **26B**: Glycan **25B** (3.6 mg) was dissolved in 100 mM NaOAc buffer (500 µL, pH~5.0) followed by adding 10 mM deoxyfuconojirimycin hydrochloride (20 µL) and *Helix pomatia* β-mannosidase (10 µL) the reaction mixture was then incubated at 37 °C for 12 h. After this time the reaction mixture was heated at 100 °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 combined and lyophilized. This glycan and UDP-Gal (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 B4GalT1 (3.4 mU/µmol) were added to achieve a final concentration of 4 mM. The reaction mixture was then incubated at 37 °C for 10 h. The reaction mixture was quenched by adding methanol (10 µL), after which it was passed through Biogel P-4 column. Fractions containing product were combined and lyophilized to give the product as a white fluffy solid. This product was put for HPLC purification using HILIC column, using solvent gradient ACN: H_2O (90: 10, v: v to 50: 50, v: v). The fractions containing the product were concentrated and lyophilized yielding the purified glycan **26B** as a white cotton like solid (2.0 mg). MALDI-TOF-MS (*m/z*): $[M+ Na]^+$ calculated for $C_{96}H_{160}N_6O_{68}Na$, 2507.9144; found 2508.1620.



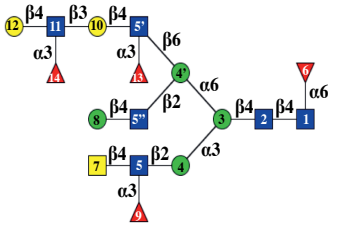
26B	H1	H2	H3	H4	H5	H6	Fuc-CH_3
GlcNAc-1α	5.10 (d, *J* = 3.1 Hz)	3.81	3.93	3.72	NA	3.75, 3.63	–
Man-4	5.05	4.09	3.84	3.41	3.48	3.82, 3.52	–
Man-4'	4.79	4.00	3.78	3.34	3.70	4.12, 3.51	–
Man-3	4.68	4.17	3.72	3.75	3.48	3.84, 3.70	–
GlcNAc-1β	4.61	3.63	3.65	NA	NA	NA	–
GlcNAc-2	4.58	3.71	3.69	3.54	NA	NA	–
GlcNAc-5"	4.51	3.69	3.44	3.65	3.45	NA	–
GlcNAc-5	4.47 (d, *J* = 8.2 Hz)	3.72	3.55	3.67	3.43	NA	–
GlcNAc-5'	4.44 (d, *J* = 8.4 Hz)	3.68	3.54	3.66	3.43	NA	–
Gal-8	4.39	3.55	3.62	3.86	NA	NA	–
GalNAc-7	4.38	3.85	3.66	3.85	NA	NA	–
GalNac-9	4.38	3.89	3.67	3.86	NA	NA	–
Fuc-6 (core) (α, β)	4.82	3.72	3.84	3.75	4.00, 4.04	–	1.14
Fuc-10	5.05	3.86	3.84	3.65	4.79	–	1.19
Fuc-11	5.02	3.85	3.82	3.64	4.78	–	1.19

Glycan **28B:** Glycan **19B** (7.0 mg) and UDP-Gal (2 eq) were dissolved in Tris buffer (50 mM, pH~7.5) and CIAP and B4GalT1 were added to achieve a final concentration of 4 mM. The reaction mixture was then incubated at 37 °C for 10 h after which it was quenched by adding methanol (10 µL) and passed through Biogel P-4 column. Fractions containing product were combined and lyophilized to give the galactosylated glycan (5.2 mg) to yield **27B**. This glycan and UDP-GlcNAc (2 eq) were dissolved in HEPES buffer (50 mM, pH~7.3), CIAP and B3GNT2 were added and incubated at 37 °C for 12 h. The intermediate glycan was again put for galactosylation reaction using the conditions described above, yielding glycan **28B** (6.5 mg). This product was put for HPLC purification using HILIC column, using solvent gradient ACN: H_2O (90: 10, v: v to 50: 50, v: v). The fractions containing the product were concentrated and lyophilized yielding the purified glycan as a white cotton like fluffy solid (5.0 mg). MALDI-TOF-MS (*m/z*): $[M+ Na]^+$ calculated for $C_{104}H_{173}N_7O_{74}Na$, 2726.9887; found 2727.1029.



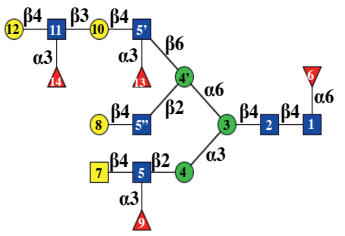
28B	H1	H2	H3	H4	H5	H6	Fuc-CH_3
GlcNAc-1α	5.10 (d, *J* = 3.0 Hz)	3.80	3.93	3.72	NA	3.73, 3.59	–
Man-4	5.02	4.09	3.82	3.41	3.45	3.83, 3.52	–
Man-4'	4.79	3.99	3.79	3.32	3.73	4.12, 3.50	–
Man-3	4.67	4.17	3.68	3.75	3.43	3.83, 3.66	–
Man-8	4.68	3.98	3.56	NA	NA	NA	–
GlcNAc-11	4.62	3.71	3.57	3.70	NA	NA	–
GlcNAc-1β	4.60	3.62	3.65	NA	NA	NA	–
GlcNAc-2	4.58	3.69	3.67	3.53	NA	NA	–
GlcNAc-5"	4.51 (d, *J* = 7.2 Hz)	3.64	3.40	3.65	3.42	NA	–
GlcNAc-5	4.46	3.65	3.48	3.63	3.41	NA	–
GlcNAc-5'	4.46	3.67	3.54	3.66	3.42	NA	–
GalNAc-7	4.39	3.89	3.64	3.85	NA	NA	–
Gal-10	4.38	3.50	3.59	3.88	3.57	NA	–
Gal-12	4.36	3.46	3.58	3.88	3.55	NA	–
Fuc-6 (core) (α, β)	4.81	3.72	3.84	3.72	4.01, 4.05	–	1.13
Fuc-9	5.05 (d, *J* = 3.8 Hz)	3.86	3.85	3.68	4.79	–	1.18

Glycan **29B:** Glycan **28B** (5.0 mg) and GDP-Fucose (4 eq) were dissolved in Tris buffer (50 mM, pH~7.3) containing $MnCl_2$ (10 mM), CIAP (10 mU). Recombinant α(1,3)-Fuctosyltransferase FUT5 (6.6 mU/μmol of substrate) was added to achieve a final concentration of 4 mmol. The resulting mixture was incubated at 37 °C for 12 h. After this time, additional GDP-Fucose (2 eq), CIAP (10 mU) and FUT5 enzyme was added and incubation at 37 °C was continued until no more starting material could be detected by MALDI-TOF. The reaction mixture was quenched by adding methanol (10 μL), after which it was passed through Biogel P-6 column. Fractions containing the product were combined and lyophilized to give the product as a white fluffy solid (5.6 mg). MALDI-TOF-MS (*m/z*): $[M+ Na]^+$ calculated for $C_{116}H_{193}N_7O_{82}Na$, 3019.1045; found 3019.4936.



29B	H1	H2	H3	H4	H5	H6	Fuc-CH_3
GlcNAc-1α	5.07 (d, *J* = 3.1 Hz)	3.78	3.90	3.69	NA	3.72, 3.58	–
Man-4	4.99	4.05	3.79	3.38	3.43	3.81, 3.49	–
Man-4'	4.78	3.96	3.75	3.29	3.69	4.09, 3.47	–
Man-3	4.66	4.14	3.66	3.73	3.42	3.81, 3.65	–
Man-8	4.65	3.95	3.54	NA	NA	NA	–
GlcNAc-11	4.59	3.74	3.59	3.72	NA	NA	–
GlcNAc-1β	4.58	3.60	3.63	NA	NA	NA	–
GlcNAc-2	4.55	3.65	3.64	3.51	NA	NA	–
GlcNAc-5"	4.48 (d, *J* = 7.4 Hz)	3.60	3.38	3.63	NA	NA	–
GlcNAc-5	4.44	3.71	3.43	3.59	3.38	NA	–
GlcNAc-5'	4.43	3.76	3.52	3.63	3.39	NA	–
GalNAc-7	4.34	3.86	3.61	3.83	NA	NA	–
Gal-10	4.34	3.54	3.56	3.85	3.53	NA	–
Gal-12	4.33	3.55	3.60	3.91	3.56	NA	–
Fuc-6 (core) (α, β)	4.75	3.69	3.81	3.68	3.97, 4.02	–	1.09
Fuc-9	5.02	3.82	3.80	3.64	4.75	–	1.15
Fuc-13	5.02	3.80	3.78	3.61	4.72	–	1.06
Fuc-14	4.99	3.77	3.76	3.60	4.71	–	1.04

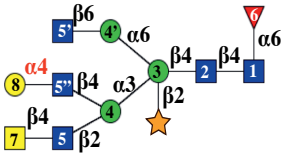
Glycan **30B:** Glycan **29B** (5.6 mg) was dissolved in 100 mM NaOAc buffer (500 µL, pH~5.0) followed by adding 10 mM deoxyfuconojirimycin hydrochloride (20 µL) and *Helix pomatia* β-mannosidase (10 µL) the reaction mixture was then incubated at 37 °C for 12 h. After this time the reaction mixture was heated at 100 °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 combined and lyophilized. This glycan and UDP-Gal (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 B4GalT1 (3.4 mU/µmol) were added to achieve a final concentration of 4 mM. The reaction mixture was then incubated at 37 °C for 10 h. The reaction mixture was quenched by adding methanol (10 µL), after which it was passed through Biogel P-6 column. Fractions containing product were combined and lyophilized to give the product as a white fluffy solid (4.6 mg). This product was put for HPLC purification using HILIC column, using solvent gradient ACN: H_2O (90: 10, v: v to 50: 50, v: v). The fractions containing the product were concentrated and lyophilized yielding the purified glycan **53** as a white cotton like fluffy solid (2.5 mg). MALDI-TOF-MS (*m/z*): $[M+ Na]^+$ calculated for $C_{116}H_{193}N_7O_{82}Na$, 3019.1045; found 3019.3130.



30B	H1	H2	H3	H4	H5	H6	Fuc-CH_3
GlcNAc-1α	5.06 (d, *J* = 2.8 Hz)	3.78	3.90	3.69	NA	3.71, 3.58	–
Man-4	4.99	4.05	3.79	3.38	3.43	3.81, 3.48	–
Man-4'	4.78	3.97	3.74	3.29	3.69	4.09, 3.47	–
Man-3	4.65	4.14	3.66	3.73	3.41	3.81, 3.65	–
GlcNAc-11	4.59	3.75	3.58	3.72	NA	NA	–
GlcNAc-1β	4.58	3.59	3.63	NA	NA	NA	–
GlcNAc-2	4.55	3.66	3.63	3.50	NA	NA	–
GlcNAc-5"	4.48 (d, *J* = 6.9 Hz)	3.60	3.38	3.63	NA	NA	–
GlcNAc-5	4.45	3.71	3.44	3.59	3.39	NA	–
GlcNAc-5'	4.43	3.76	3.52	3.63	3.38	NA	–
Gal-8	4.36	3.44	3.54	3.81	NA	NA	–
GalNAc-7	4.34	3.87	3.62	3.83	NA	NA	–
Gal-10	4.33	3.54	3.55	3.85	3.51	NA	–
Gal-12	4.33	3.55	3.60	3.91	3.55	NA	–
Fuc-6 (core)	4.75	3.69	3.81	3.68	3.99, 4.02	–	1.10

(α, β)							
Fuc-9	5.02	3.83	3.81	3.64	4.76	–	1.15
Fuc-13	5.02	3.80	3.78	3.61	4.72	–	1.06
Fuc-14	4.98	3.77	3.76	3.60	4.71	–	1.04

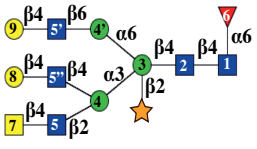
Glycan **9:** Compound **15** (38 mg, 0.017 mmol) was dissolved in THF (30 mL) and H_2O (3 mL), followed by the addition of tetrakis(triphenylphosphine)palladium (62 mg, 0.054 mmol) and morpholine (95 µL, 1.08 mmol). The mixture was stirred for 2 hours after which it was concentrated *in vacuo* and the residue was purified by silica gel column chromatography using Pet. Ether: EtOAc (8: 2, v: v to 3:7, v: v) which afforded the product as a white amorphous solid. To a suspension of this product (38 mg, 0.0078 mmol) in *n*BuOH (3 mL) was added ethylenediamine (1 mL) and the resulting clear solution was heated at 90 °C for 16 h. The mixture was concentrated *in vacuo*, co-evaporated with toluene 5 times, and the resulting residue was dissolved in pyridine: Ac_2O (20 mL, 1: 1, v: v), after which DMAP (10 mg, cat) was added. The reaction was left stirring for 2 h, after which TLC (Tol: Acetone, 7: 3, v: v) showed the presence of one major product. The mixture was concentrated *in vacuo*, and the resulting crude product was briefly chromatographed with (Tol: Acetone, 7: 3, v: v) as eluent to give product which was additionally passed through BioGel SX-1 column (Tol: Acetone, 1: 1, v: v) as mobile phase to provide the acetylated intermediate as a clear syrup. This material was then dissolved in MeOH, after which 1 M NaOMe (100 µl) was added and the deacetylation was left proceeding at for 2 hours. The reaction was neutralized with the Amberlite 120 H^+ resin, filtered and the filtrate was concentrated *in vacuo*. This syrup was dissolved in MeOH: H_2O (2 mL, 1: 1), followed by adding $Pd(OH)_2$ (20 mg, 20%, Degussa type) and the reaction mixture was left stirring under the atmosphere of hydrogen for 48 hours, after which MALDI showed the product peak. The mixture was then filtered to remove catalyst, and the filtrate was concentrated *in vacuo*, passed through the BioGel P-4 column and lyophilized to give the desired glycan **9** as a white cotton-like solid. (8 mg, 47% over four steps). MALDI-TOF-MS (*m/z*): $[M+ Na]^+$ calculated for $C_{83}H_{138}N_6O_{59}Na$, 2185.7880; found 2185.9024.



^{1}H NMR (600 MHz, D_2O)

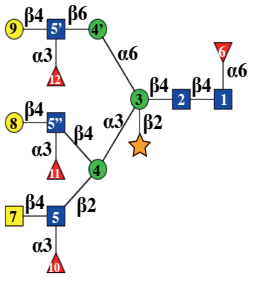
9	H1	H2	H3	H4	H5	H6	Fuc-CH_3
GlcNAc-1α	5.10 (d, *J* = 3.0 Hz)	3.82	3.91	3.68	NA	NA	–
Man-4	5.02	4.10	3.83	3.45	NA	NA	–
Man-3	4.80	4.17	3.79	3.71	3.76	3.61, 3.83	–
Man-4'	4.77	4.12	3.75	3.32	3.70	3.48, 4.09	–
GlcNAc-1β	4.62 (d, *J* = 8.0 Hz)	3.66	3.68	NA	NA	NA	–
GlcNAc-2	4.59	3.71	3.69	3.55	NA	NA	–
GlcNAc-5"	4.47	3.82	3.53	3.65	3.40	NA	–
GlcNAc-5'	4.46	3.75	3.58	3.63	3.39	NA	–
GlcNAc-5	4.44	3.74	3.50	3.67	3.42	NA	–
GalNAc-7	4.44	3.85	3.63	3.84	NA	NA	–
Xyl (core)	4.31 (d, *J* = 7.7 Hz)	3.30	3.36	3.13, 3.93	NA	–	–
Fuc-6 (core) α,β	4.82	3.73	3.81	3.73	3.99, 4.02	–	1.13
Gal-8	5.36 (d, *J* = 2.2 Hz)	3.76	3.60	3.85	NA	NA	–

Glycan **32**: Glycan **9** (4.0 mg) was dissolved in 100 mM NaOAc buffer (500 µL, pH~5.0) followed by addition of α-Galactosidase (from Jack Bean; 15 µL) the reaction mixture was then incubated at 37 °C for 12 h. After this time the reaction mixture was heated at 100 °C for 10 min, centrifuged, the supernatant was lyophilized, and the resulting product **31** was desalted using the BioGel P-2 column. Fractions containing the product were combined and lyophilized. This glycan and UDP-Gal (4 eq) were dissolved in Tris buffer (50 mM, pH~7.5) containing BSA (0.1%) and $MnCl_2$ (20 mM). CIAP (10 mU) and B4GalT1 (3.4 mU/µmol) were added to achieve a final concentration of 4 mM. The reaction mixture was then incubated at 37 °C for 10 h. The reaction mixture was quenched by adding methanol (10 µL), after which it was passed through Biogel P-4 column. Fractions containing product **32** were combined and lyophilized to give the product as a white fluffy solid (4.1 mg). MALDI-TOF-MS (*m/z*): $[M+ Na]^+$ calculated for $C_{89}H_{148}N_6O_{64}Na$, 2347.8409; found 2348.0634.



32	H1	H2	H3	H4	H5	H6	Fuc-CH_3
GlcNAc-1α	5.10 (d, *J* = 3.1 Hz)	3.81	3.89	3.68	NA	NA	–
Man-4	5.02	4.10	3.78	3.41	NA	3.51, 3.75	–
Man-3	4.80	3.89	3.76	3.72	3.69	3.59, 3.82	–
Man-4'	4.77	4.12	3.74	3.32	3.68	NA	–
GlcNAc-1β	4.61 (d, *J* = 8.3 Hz)	3.64	3.67	NA	NA	NA	–
GlcNAc-2	4.59	3.70	3.68	3.51	NA	NA	–
GlcNAc-5"	4.49 (d, *J* = 8.4 Hz)	3.76	3.58	3.63	3.37	NA	–
GlcNAc-5'	4.46	3.81	3.56	3.66	3.40	NA	–
GlcNAc-5	4.44	3.72	3.50	3.63	3.41	NA	–
GalNAc-7	4.44	3.85	3.65	3.86	NA	NA	–
Gal-8	4.40	3.44	3.58	3.84	NA	NA	–
Gal-9	4.40	3.46	3.57	3.86	NA	NA	–
Xyl (core)	4.31 (d, *J* = 7.7 Hz)	3.29	3.35	NA	3.14, 3.92	–	–
Fuc-6 (core) α,β	4.82	3.73	3.80	NA	4.02, 4.06	–	1.13

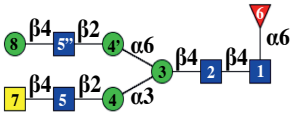
Glycan **33:** Glycan **32** (2.5 mg) and GDP-Fucose (6 eq) were dissolved in Tris buffer (50 mM, pH~7.3) and CIAP and FUT5 was added to achieve a final concentration of 4 mmol. The resulting mixture was incubated at 37 °C for 12 h. After this time, additional GDP-Fucose (2 eq), and enzymes were added and incubation at 37 °C was continued until no more starting material could be detected by MALDI-TOF. The reaction mixture was quenched by adding methanol (10 µL), after which it was passed through Biogel P-6 column. Fractions containing the product were combined and lyophilized to give the product, which was put for HPLC purification using HILIC column, using solvent gradient ACN: H_2O (90: 10, v: v to 50: 50, v: v). The fractions containing the product were concentrated and lyophilized yielding the purified glycan **33** as a white cotton



like fluffy solid (1.5 mg). MALDI-TOF-MS (*m/z*): $[M+ Na]^+$ calculated for $C_{107}H_{178}N_6O_{76}Na$, 2786.0146; found 2786.3007.

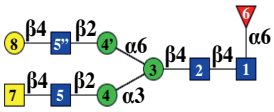
33	H1	H2	H3	H4	H5	H6	Fuc-CH_3
GlcNAc-1α	5.10 (d, *J* = 3.1 Hz)	3.80	3.91	3.67	NA	NA	–
Man-4	5.01	4.09	3.85	3.42	3.47	3.51, 3.77	–
Man-3	4.79	4.11	3.83	3.69	3.78	3.60, 3.87	–
Man-4'	4.77	4.09	3.78	3.37	3.72	3.62, 4.10	–
GlcNAc-1β	4.61 (d, *J* = 8.3 Hz)	3.69	3.70	NA	NA	NA	–
GlcNAc-2	4.59	3.72	3.71	3.55	NA	NA	–
GlcNAc-5"	4.49 (d, *J* = 7.9 Hz)	3.86	3.60	3.66	3.48	NA	–
GlcNAc-5'	4.46	3.82	3.59	3.68	3.47	NA	–
GlcNAc-5	4.43	3.87	3.63	3.70	3.53	NA	–
GalNAc-7	4.37	3.89	3.69	3.88	NA	NA	–
Gal-8	4.36	3.47	3.59	3.84	NA	NA	–
Gal-9	4.36	3.48	3.58	3.86	NA	NA	–
Xyl (core)	4.31 (d, *J* = 7.5 Hz)	3.30	3.34	NA	3.15, 3.93	–	–
Fuc-6 (core) α,β	4.82	3.73	3.84	3.75	4.02, 4.06	–	1.14
Fuc-10	5.04	3.85	3.89	3.69	4.77	–	1.10
Fuc-11	5.04	3.83	3.86	3.68	4.76	–	1.10
Fuc-12	5.02	3.81	3.83	3.64	4.80	–	1.18

Glycan **34:** Glycan **8** (5.0 mg) was dissolved in 100 mM NaOAc buffer (500 µL, pH~5.0) followed by addition of *Canavalia ensiformis* β-*N*-acetylglucosaminidase (15 µL). The reaction mixture was then incubated for 2 h. If the reaction was prolonged beyond 2 h, the removal of GalNAc moiety was observed. Therefore, the reaction was quenched after 2 h, by heating at 100 °C for 10 min. The mixture was centrifuged, the supernatant was lyophilized, and the resulting product was desalted using the BioGel P-2 column. Fractions were combined and lyophilized to give product as a white cotton-like solid. This product was put for HPLC purification using HILIC column, using solvent gradient ACN: H_2O (90: 10, v: v to 50: 50, v: v). This removed the unwanted fragments and impurities. The fractions containing the product were concentrated and lyophilized yielding the purified glycan **34** as a white cotton like solid (2.0 mg). MALDI-TOF-MS (*m/z*): $[M+ Na]^+$ calculated for $C_{70}H_{117}N_5O_{50}Na$, 1850.6664; found 1850.7916.



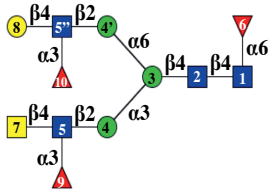
34	H1	H2	H3	H4	H5	H6	Fuc-CH_3
GlcNAc-1α	5.10 (d, *J* = 3.1 Hz)	3.82	3.93	3.73	NA	3.75, 3.63	–
Man-4	5.03	4.10	3.84	3.43	3.47	3.83, 3.52	–
Man-4'	4.85	4.02	3.81	3.35	3.72	4.14, 3.52	–
Man-3	4.68	4.17	3.71	3.75	3.47	3.84, 3.69	–
Man-8	4.68	3.99	3.57	NA	NA	NA	–
GlcNAc-1β	4.61	3.64	3.65	NA	NA	NA	–
GlcNAc-2	4.59	3.67	3.69	3.54	NA	NA	–
GlcNAc-5"	4.50	3.69	3.42	3.64	3.43	NA	–
GlcNAc-5	4.48	3.72	3.53	3.68	3.45	NA	–
GalNAc-7	4.44 (d, *J* = 8.6 Hz)	3.85	3.66	3.84	NA	NA	–
Fuc-6 (core) (α, β)	4.81	3.72	3.83	3.74	4.01, 4.05	–	1.13

Glycan **35**: Glycan **34** (2.0 mg) was dissolved in 100 mM NaOAc buffer (500 µL, pH~5.0) followed by adding 10 mM deoxyfuconojirimycin hydrochloride (20 µL) and *Helix pomatia* β-mannosidase (10 µL) the reaction mixture was then incubated at 37 °C for 12 h. After this time the reaction mixture was heated at 100 °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 combined and lyophilized. This glycan and UDP-Gal (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 B4GalT1(3.4 mU/µmol) were added to achieve a final concentration of 4 mM. The reaction mixture was then incubated at 37 °C for 10 h. The reaction mixture was quenched by adding methanol (10 µL), after which it was passed through Biogel P-4 column. Fractions containing product were combined and lyophilized to give the product as a white fluffy solid (1.5 mg). MALDI-TOF-MS (*m/z*): $[M+ Na]^+$ calculated for $C_{70}H_{117}N_5O_{50}Na$, 1850.6664; found 1850.8051.



35	H1	H2	H3	H4	H5	H6	Fuc-CH_3
GlcNAc-1α	5.10 (d, *J* = 3.1 Hz)	3.81	3.92	3.73	NA	3.75, 3.64	–
Man-4	5.03	4.10	3.84	3.43	3.49	3.85, 3.52	–
Man-4'	4.85	4.03	3.82	3.37	3.72	4.13, 3.53	–
Man-3	4.68	4.17	3.71	3.76	3.47	3.84, 3.70	–
GlcNAc-1β	4.62 (d, *J* = 8.2 Hz)	3.62	3.66	NA	NA	NA	–
GlcNAc-2	4.59	3.66	3.69	3.55	NA	NA	–
GlcNAc-5"	4.50 (d, *J* = 8.3 Hz)	3.69	3.42	3.63	3.43	NA	–
GlcNAc-5	4.48	3.71	3.51	3.68	3.46	NA	–
GalNAc-7	4.43 (d, *J* = 8.6 Hz)	3.85	3.66	3.84	NA	NA	–
Gal-8	4.39 (d, *J* = 7.9 Hz)	3.54	3.63	3.85	NA	NA	–
Fuc-6 (core) (α, β)	4.82	3.73	3.83	3.76	4.02, 4.05	–	1.14

Glycan **36**: Glycan **35** (1.5 mg) and GDP-Fucose (4 eq) were dissolved in Tris buffer (50 mM, pH~7.3) containing $MnCl_2$ (10 mM), CIAP (10 mU). Recombinant α(1,3)-Fuctosyltransferase FUT5 (6.6 mU/μmol of substrate) was added to achieve a final concentration of 4 mmol. The resulting mixture was incubated at 37 °C for 12 h. After this time, additional GDP-Fucose (2 eq), CIAP (10 mU) and enzymes were added and incubation at 37 °C was continued until no more starting material could be detected by MALDI-TOF. The reaction mixture was quenched by adding methanol (10 μL), after which it was passed through Biogel P-6 column. Fractions containing the product were combined and lyophilized to give the product as a white fluffy solid (1.6 mg). MALDI-TOF-MS (*m/z*): $[M+Na]^+$ calculated for $C_{82}H_{137}N_5O_{58}Na$, 2142.7822; found 2142.9947.



35	H1	H2	H3	H4	H5	H6	Fuc-CH_3
GlcNAc-1α	5.07	3.84	3.93	3.72	NA	3.74, 3.63	–
Man-4	5.02	4.06	3.84	3.42	3.49	3.82, 3.53	–
Man-4'	4.80	4.00	3.78	3.34	3.72	4.12, 3.52	–
Man-3	4.67	4.17	3.71	3.74	3.49	3.83, 3.71	–
GlcNAc-1β	4.64	3.60	3.62	NA	NA	NA	–
GlcNAc-2	4.57	3.76	3.73	3.55	NA	NA	–
GlcNAc-5"	4.48	3.68	3.46	3.65	3.44	NA	–
GlcNAc-5	4.44	3.70	3.52	3.65	3.42	NA	–
GalNAc-7	4.35	3.87	3.68	3.89	NA	NA	–
Gal-8	4.34	3.56	3.64	3.87	NA	NA	–
Fuc-6 (core) (α, β)	4.81	3.72	3.84	3.77	4.00, 4.04	–	1.12
Fuc-9	5.02	3.85	3.83	3.64	4.76	–	1.14
Fuc-10	4.99	3.81	3.79	3.67	4.75	–	1.14

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CHAPTER 6

Summary and Future Perspectives

The glycome comprises of glyco-conjugates like glycoproteins and glyco-lipids that are generated in a non-templated fashion, post-translationally through the action of more than 200 glycosyltransferases. *N*-linked glycans are the most diverse and abundant types of glycoproteins expressed across all domains of life.[1] They bind to glycan binding proteins called lectins for recognition and interactions performing numerous biological processes and play key roles in host – pathogen interactions. **Chapter 1** provides an overview of the biological relevance of *N*-glycans in cell-cell communication, receptor recognition, immune-modulation, etc. and the strategies employed by chemists for the synthesis of such complex oligosaccharides. In nature, glycans are present as heterogeneous mixtures of many forms of glyco-conjugates and are difficult to isolate as pure standards. Since the synthesis of the first bi-antennary *N*-glycan in 1980s, the strategies of chemical synthesis aided with enzymatic reactions have evolved tremendously to yield asymmetrical *N*-glycans of unprecedented complexity. This was possible due to innumerable inventions and improvements in reaction strategies over the years, which have been discussed in brief. The incorporation of enzyme-aided reactions in the synthesis route revolutionized this field by affording absolute regio- and stereoselectivity, facilitating the synthesis of oligosaccharides of unprecedented complexity. However, although eukaryotic *N*-glycans have been accessed quite efficiently, the glycans of lower disease-causative pathogens like bacterial and parasitic glycans are still difficult to achieve owing to unusual carbohydrate epitopes, glycosylation patterns and inaccessibility of synthetically useful enzymes. Chemical synthesis still dominates the pathway to obtain such unusual glycans. The following chapters elucidate the strategies employed to achieve desired glycans that trigger host-pathogen interactions and studied by multi-disciplinary approaches.

A number of pathogens can hijack or evade the host's immune system by phase-variable glycosylation. *S. mansoni* is a pathogen that can cause life-long infections, alternating latent with active phases and re-activation of the host's immune system. **Chapter 2** is the introduction of schistosomiasis and a discussion of the strategies employed by *S. mansoni* to mute the host immunological response. *S. mansoni* are called the "master manipulators" of the immune system and ensure their survival by reducing inflammation and inducing a modified immunological response. The pattern-recognition receptor Dendritic cell-specific ICAM-3 grabbing nonintegrin (DC-SIGN), is a C-type lectin that maintains the balance between Th1 and Th2 immune responses, has a broad substrate specificity and can recognize a plethora of fucosylated glycans. *S. mansoni* display highly fucosylated glycan structures and are known to target DC-SIGN upon infection. A brief explanation of their mechanism of invasion and the long-term immunological implication in hosts is provided in this chapter.

During its life cycle, *S. Mansoni* expresses various fucosylated glycans including Lac-di-NAc, Le^x, LDN-F and LDN-DF in a stage-dependent manner. There are indications that DC-SIGN can recognize some of these fucosylated glycans selectively, indicating that phase variable expression of glycans may shape host immune responses. Thus, an important question addressed in **Chapter 3**, is how certain fucosylated structures can avoid detection by DC-SIGN. In this chapter, we report the first chemical synthesis of the fucosylated glycans LDN-F and LDN-DF. NMR and molecular modeling studies demonstrated that the α1,3-fucoside of LDN-F can coordinate with the Ca^{2+}-ion of the canonical binding site of DC-SIGN placing the GlcNAc residue in close proximity to the protein surface allowing for additional interactions. The α1,2- linked fucoside of LDN-DF can also be placed into this binding site but in this case, the GlcNAc and GalNAc residues cannot make interactions with the protein surface resulting in a substantially lower binding affinity. Glycan microarray binding studies showed that the avidity and selectivity of binding is greatly enhanced when the glycans are presented multivalently, and in this format Le^x and LDN-F gave strong responsiveness whereas no binding was detected for LDN-DF. Based on these observations, it was proposed that phase variable expression of glycans such Le^x, LDN-F and LDN-DF that interact differently with DC-SIGN offers *S. mansoni* a strategy to skew the host immune response in a manner appropriate for specific developmental stages.

Binding and structural studies have indicated that glycan binding proteins recognize relatively small oligosaccharide motifs often found at termini of complex glycans. However, the complex architecture of *N*-glycans displaying diverse branching patterns can potentially present multiple minimal epitopes that can engage with multiple glycan binding proteins resulting in increased binding avidities. In **Chapter 4**, a chemoenzymatic methodology to provide three characteristic *N*-glycans expressed by the parasitic worm *S. mansoni* having unique epitopes at each antenna and marked by the presence or absence of core xyloside, is described. A careful glycosylation strategy aided with the action of a panel of highly specific mammalian glycosyltransferases and glycosidases were used to achieve asymmetry. STD-NMR, computational and electron microscopy were employed to investigate recognition of the glycans by the human lectin DC-

SIGN. It was revealed that core xyloside does not influence terminal epitope recognition. Furthermore, it was found that the multi-antennary glycans bind with higher affinity to DC-SIGN compared to mono-valent minimal epitopes, which was attributed to proximity-induced effective concentration. Finally, the studies uncovered that the multi- antennary glycan can cross-link DC-SIGN into a dense network, which is likely relevant for antigen uptake and intracellular routing. These studies demonstrate that unique insight can be obtained in molecular aspects of protein-glycan interactions through a multidisciplinary approach.

The versatility and superiority of our newly developed chemo-enzymatic approach was used to synthesize a library of biologically important glycans of *S. mansoni* that were previously not accessible. These glycans were asymmetrically modified by core xylosides and fucosides, extended LacNAc and multiple Lac-di-NAc moieties as explained in **Chapter 5.** The strategy employed unnatural glycosidic linkages like α-galactoside and β-mannoside as capping motifs at the termini, that could be sequentially removed by appropriate glycosidase at a desired stage, to liberate the inner β-GlcNAc moiety that could be independently extended by a panel of mammalian glycosyltransferases. Further, a one-pot enzymatic strategy to yield bi-antennary glycans by the cleavage of an entire terminal antenna was developed. The library was used for preliminary micro-array studies with plant lectins. The accessibility of structurally defined complex glycans could be the ideal foundation for future studies to generate the perfect glycan motifs that can generate anti-bodies and activate the host immune system towards an appropriate response against schistosomiasis.

Future Perspectives.

Synthesis of an unnatural Lipid-linked *N*-Glycan core as an Inhibitory analog for Oligosaccharyltransferase. The importance of *N*-glycosylation in numerous biological functions and their underlying interactions with glycan binding proteins have been well described in this thesis. The biosynthesis of native oligosaccharides involves a cascade of membrane embedded glycosyltransferases extending the lipid-linked oligosaccharide (LLO). An LLO is the assembly of an oligosaccharide with phospho-lipid tails, typically dolichol pyrophosphate in eukaryotes and archaea, and undecaprenyl pyrophosphate (UndPP) in eubacteria.[2] The biosynthesis starts in the cytoplasmic side of the ER membrane and then flipped into the lumen, where it undergoes further extension. Here, the enzyme Oligosaccharyl transferase (OST) catalyzes *en bloc* transfer of the pre-assembled oligosaccharide from LLO donor (containing pyrophosphate or phosphate as leaving group), to the carboxamide side chain of asparagine residue (glycosyl acceptor), in a nucleophilic substitution mechanism to yield *N*-linked glycoproteins (Figure 1).[3]

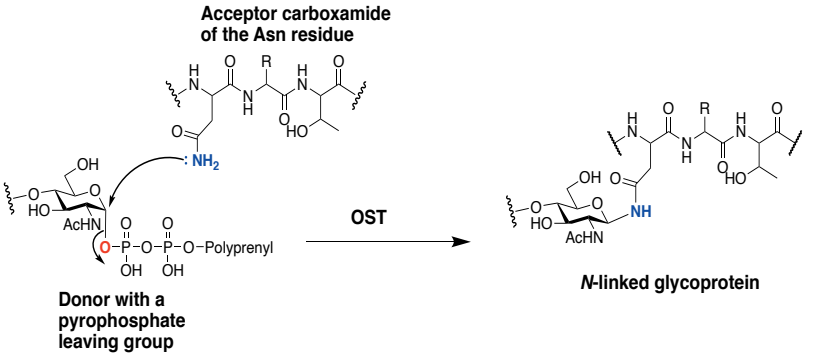


Figure 1. OST catalyzed *N*-linked glycoprotein synthesis.

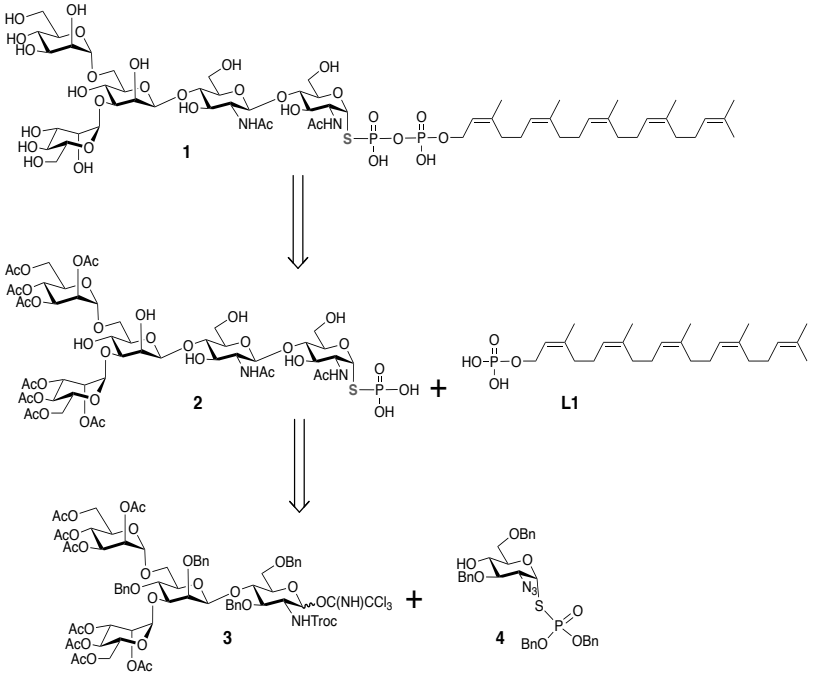
The structure of OST differs across the different domains of life, i.e., eukaryotic, bacterial and archaea. For example, while the bacterial and archaeal OST is a monomeric enzyme,[4] the eukaryotic OST is a complex octamer.[5] The eukaryotic OST protein complex comprises of STT3 (staurosporine and temperature sensitivity 3), the largest and most conserved catalytic subunit of OST.[6] The archaeal and bacterial OSTs are single subunit enzymes homologous to the STT3 subunit of the eukaryotic OST,[7] which are referred to as "AglB" (archaeal glycosylation B) and "PglB" (protein glycosylation B), respectively. All OST catalytic enzymes share a common topology signature of an *N*-terminal transmembrane (TM) part and a soluble *C*-terminal domain.[2] EL5 is the external loop present in all STT3 proteins and has a dual function in binding peptide (*C*-EL5) and LLO (*N*-EL5). In the absence of the nascent protein, the full-length EL5 is flexible, disordered and disengaged from the OST core. EL5 becomes ordered upon peptide binding, which affixes the peptide and creates an active site. This is followed by a mechanism in which binding with LLO is initiated, resulting in a nucleophilic attack by the carboxamide group of the Asn acceptor, leading to formation of *N*-linked glycoproteins (*N*-glycans).[4a, 8] Although it was established that *C*-EL5 and *N*-EL5 behave independently, their exact mechanistic pathways, the structural information on OST and its integration into the ER machinery is scarce and inconclusive.

Recently, Locher and coworkers described the crystal structure of PglB from the gram-negative bacteria *Campylobacter jejuni*[9] by trapping PglB in an intermediate state bound to an acceptor peptide and an unnatural synthetic LLO (monosaccharide GlcNAc-phosphonate) before the glycan transfer could proceed. Further, they discovered that in the presence of LLO the disordered *N*-EL5 adopts a defined three-dimensional conformation, shielding the active site. To access the active site, the glycan moiety of LLO must thread under EL5. They also speculated as to the mechanism of the OST (PglB), concluding that two possible binding mechanisms could be operating, i.e., if peptide binds first, *C*-EL5 becomes ordered whereas if LLO binds first, *N*-EL5 becomes ordered.

They speculated that binding of LLO before the binding of peptide could be a more efficient mechanistic route for OST, because when both *N*-EL5 and *C*-EL5 are disengaged, the large glycan moiety could more easily access the active site, decrease the EL5 mobility and inhibit the catalytic rate of OST.

To test this theory, we envisaged that a pentasaccharide thio-phosphate analog, such as **1** (Scheme 1) would be an appropriate non-hydrolysable analogue to the natural *O*-linked substrate bringing about the desired inhibition of OST. It was envisaged that further extension of the pentasaccharide could be performed by the enzyme ALG11 that adds additional mannosides, to afford the desired heptasaccharide LLO which would be flipped into the lumen by the flippase Rft1p where it would continue to grow, ultimately reaching a stage appropriate for trapping OST in an intermediate state.

From a retrosynthetic perspective, the desired LLO **1** could be accessed by a carbonyldiimidazole (CDI)-mediated coupling of α-thiophosphate pentasaccharide **2** with nerylphosphate lipid **L1**. Previous studies indicate that the length of lipid chain was important for OST inhibiton and that the C-20 lipid **L1** was a good candidate for proper inhibition.[10] It was envisaged that compound **2** could in turn be prepared by the glycosylation of tetrasaccharide donor **3** and α-thioglycopyranosyl acceptor **4** (Scheme 1).

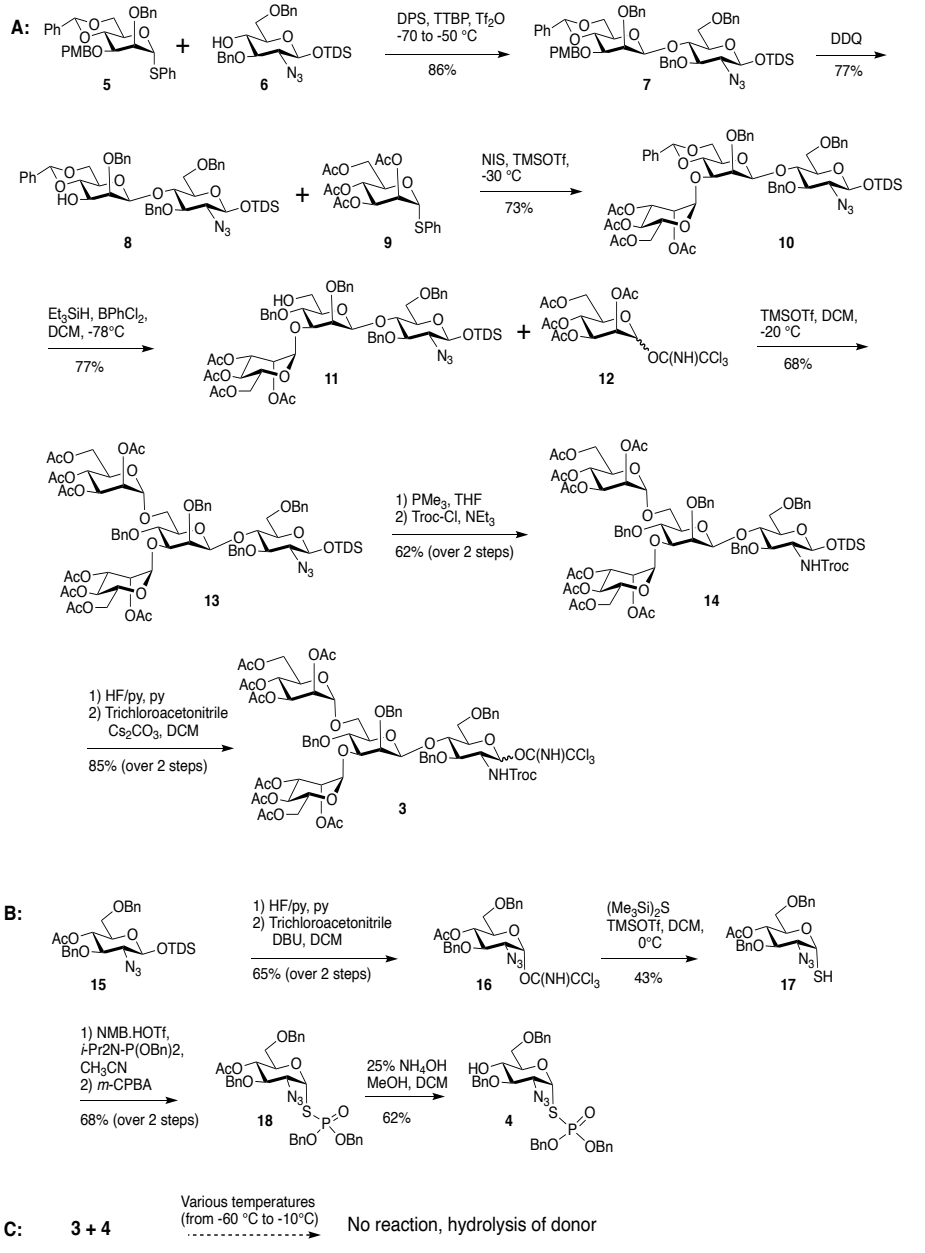


Scheme 1. Proposed retrosynthesis route to obtain desired lipid-linked *N*-glycan.

To this end, thio-manosyl donor **5** (Scheme 2A) was deemed to be an appropriate starting material for the synthesis of the desired acetimidate donor **3**. Thus, donor **5** was pre-activated with DPS/ Tf_2O in the presence of TTBP at -70 °C,[11] which was followed by the addition of acceptor **6**, which afforded disaccharide **7** in 86% yield. Next, the PMB ether of **7** was oxidatively removed by DDQ in the to afford acceptor **8** (77%). A NIS/TMSOTf mediated glycosylation of thio-manosyl donor **9**, with acceptor **8** gave trisaccharide **10** in a yield of 73% as only the α-anomer. The benzylidene acetal of **10** was regioselectively opened by treatment with triethylsilane (Et_3SiH) and dichlorophenylborane ($PhBCl_2$) in DCM at -78°C to furnish acceptor **11** having a hydroxyl at the C-6 position of the central mannoside. Finally, a TMSOTf-mediated glycosylation of mannosyl trichoroacetimidate **12**, with acceptor **11** gave tetrasaccharide **13** in a yield of 68%. To attain a β-anomeric selectivity in the next glycosylation reaction, tetrasaccharide **13** was further modified, by converting the azide into NHTroc (→**14**) by a two-step procedure entailing reduction using triphenyl phosphine followed by reaction of the resulting amine with TrocCl. Next, glycosyl donor **3** was prepared by removal of the anomeric TDS ether of **14** with HF/pyridine followed by reaction with trichloroacetonitrile in the presence of cesium carbonate (Cs_2CO_3).

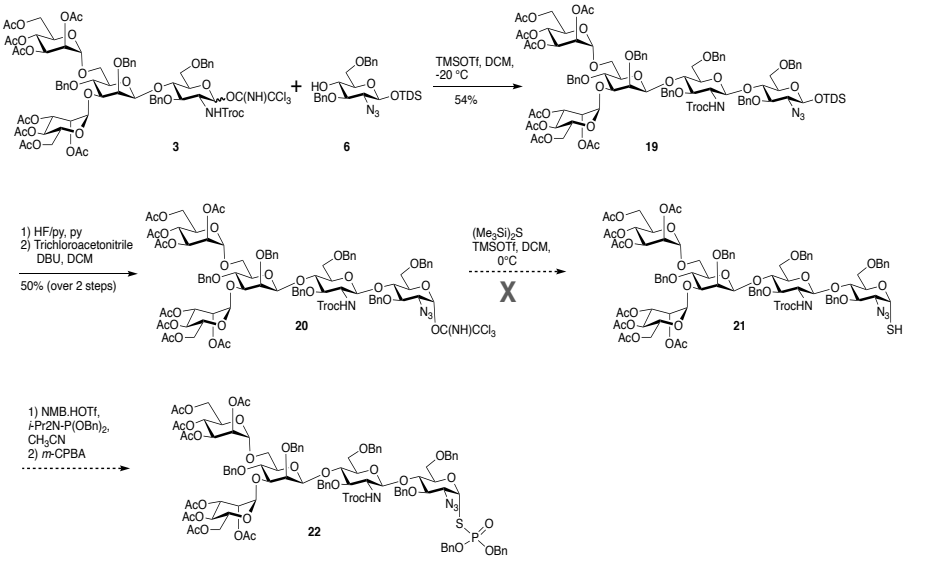
Next, attention was focused on the synthesis of anomeric thiophosphate modified acceptor **4**. Construction of an α-glycosylthiol linkage is challenging on protected sugar substrates, owing to side reactions and decomposition with traditional methods utilizing H_2S[12] or Lawesson's reagent.[13] To this end, the protocol introduced by Schmidt and coworkers[14] in which *O*-glycosyl trichloroacetimidate donors reacted with the sterically hindered bis(trimethylsilyl)sulfide (BTMSS) as *S*-nucleophile, in the presence of catalytic TMSOTf, resulted in the generation of α-glycopyranosylthiols, was applied. In our hands, this protocol proved to be a superior method in the generation of α-*N*-acetylglucosaminylthiol exclusively. Thus, the anomeric TDS ether of compound **15** was removed by a treatment with HF/pyridine and the resulting lactol was converted to α-acetimidate donor **16** in the presence of trichloroacetonitrile and DBU, in a decent yield of 65% over two steps. Using Schmidt's protocol described above, compound **16** was converted to α-glycosylmercaptan **17** by a TMSOTf-catalyzed reaction with BTMSS (43%). **17** was then converted to thiophosphate compound **18** in a two-step procedure entailing an *N*-methylbenzimidazolium triflate (NMB.TfOH)[15] catalyzed coupling with dibenzyl(*N,N*-diisopropyl)phosphoramidite followed by oxidation with 3-chloroperbenzoic acid (*m*CPBA) in a decent yield of 68%.[16] Finally, the acetyl ester was cleaved off using 25% ammonium hydroxide yielding desired acceptor **4**.

Unfortunately, glycosylation attempts with donor **3** and acceptor **4** proved to be unsuccessful. Despite several trials with catalytic TMSOTf or TfOH, over a range of temperatures (from -60 °C to -10 °C), we ended up with hydrolysed donor and an unreactive acceptor (Scheme 2C).



Scheme 2. Proposed chemical synthesis of core pentasaccharide.

Next, we attempted an alternative approach for the synthesis of α-glycopyranosylthiol **21** (Scheme 3). With acetimidate donor **3** and acceptor **6** in hand, a TMSOTf- mediated glycosylation afforded pentasaccharide **19** in a modest yield of 54%. The anomeric TDS ether of compound **19** was removed by a treatment with HF/pyridine and the resulting lactol was converted to α-acetimidate donor **20** in the presence of trichloroacetonitrile and DBU (50%). Unfortunately, the installation of an α-thiol moiety by a TMSOTf-catalyzed reaction with BTMSS at the reducing end (→**21**) resulted in immediate hydrolysis of **20**.



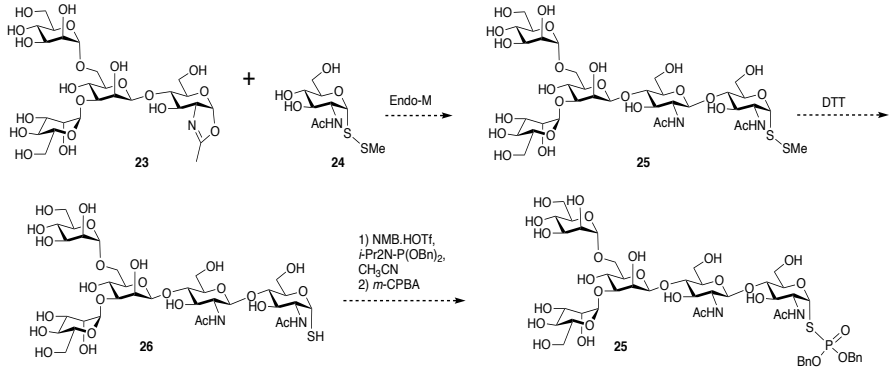
Scheme 3. Synthesis attempt with an alternative strategy.

Future experiments: Utilizing transglycosylation methodology by ENGases. Through our attempts with chemical modifications, it was observed that the installation of an anomeric thio-phosphate on oligosaccharides resulted in either side reactions at other sites capped with protecting groups or decomposition of the substrate.

Glycosyltransferases and endoglycosidases are attractive strategies to elaborate sugar chains for glycopeptide/glycoprotein synthesis. In a highly convergent approach utilizing the transglycosylation property of endo-*N*acetylglucosaminidase (ENGase) enzymes (Endo-A from *Arthrobacter Protophormiae* and Endo-M from *Mucor hiemalis*),[17] intact *N*-glycans could be synthesized in a protecting group free manner, with absolute regiospecificity and stereoselectivity.[18] However, natural donor substrates such as *N*-glycans or *N*-glycopeptides suffered from low transglycosylation yields and hydrolysis of product. To circumvent these

problems, synthetic sugar oxazolines were introduced as donor substrates for glycopeptide synthesis.[19] The activated oxazolines of compounds such as the $Man_3GlcNAc$ and $Man_9GlcNAc$ act as donors in transglycosylation reaction with GlcNAc acceptor in the presence of Endo-A and Endo-M mutants[17g, 20] to afford corresponding glycopeptides or glycoproteins with truncated or modified *N*-glycans.

It is therefore proposed that for future studies, pursuing a transglycosylation reaction between oxazoline tetrasaccharide **23** and acceptor **24** (Scheme 4) could be an attractive strategy for preparing desired LLO **1**. The synthesis of α-disulfide linked GlcNAc acceptor has been reported previously.[21] Such di-sulfide protecting groups are stable, prevent anomerization and can be reduced at a convenient stage to yield an anomeric α-thiol, ready for conjugation with a phosphate group. Previous studies investigating the acceptor specificity of Endo-M mutants, allude that the enzyme requires an equatorial hydroxyl group at C-4 of the acceptor GlcNAc, and that the stereochemistry around C-1 is not important.[22] We speculate that the tranglycosylation methodology could be an important tool for a protecting-group free transformation to yield the anomeric disulfide **25.** The disulfide group can be reduced using dithiothreitol (DTT),[23] followed by a benzimidazole triflate coupling with phosphate moiety as described previously. Further, a successful transglycosylation reaction using substrate such as **24** could open new avenues to develop other substrates for re-engineering and re-constructing glycoproteins and glycopeptides for advancement in therapeutics and synthesis of bacterial and eukaryotic peptidoglycans as novel vaccine candidates.[9]



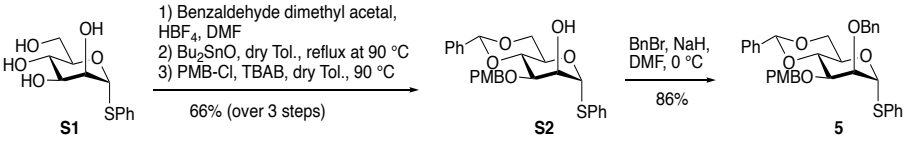
Scheme 4. Future plans to utilize transglycosylation reactions for the synthesis of LLO **1**.

Experimental Procedures.

1.1 General Methods.

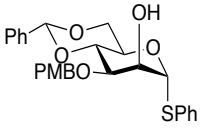
Reactions were performed using flame-dried glassware with anhydrous solvents under an atmosphere of argon unless otherwise noted. Proton nuclear magnetic resonance (^{1}H -NMR) spectra were recorded with Varian 400 (at 400 MHz) or Bruker 600 (at 600 MHz) spectrometers. Multiplicities are assigned as singlet (s), broad singlet (br s), doublet (d), doublet of doublets (dd), triplet of doublets (td), triplet (t), quartet (q) or multiplet (m). Carbon nuclear magnetic resonance (^{13}C) spectra were recorded with Varian 400 (at 101 MHz) or Bruker 600 (at 151 MHz) spectrometers. Phosphorus nuclear magnetic resonance (^{31}P) were recorded with Varian 400 (at 162 MHz). Spectra were assigned using gCOSY and multiplicity-edited gHSQC experiments. Tetramethylsilane (TMS) was used as an internal standard in all ^{1}H and ^{13}C spectra (δ = 0 ppm) when applicable. Mass spectra was recorded using high resolution Shimadzu LCMS-IT-TOF or Kratos Analytical Maxima-CFR MALDI-TOF system. Column chromatography was performed on silica gel G60 (Silicycle, 60-200 mm, 60 Å). Thin layer chromatography (TLC) analysis was conducted on Silicagel 60 F254 (EMD Chemicals Inc.) coated aluminum sheets. Plates were visualized by UV light (254 nm) and by charring with 10% sulfuric acid in ethanol and/or Hanessian's stain. Size exclusion chromatography was carried out on bio-beads S-X1 (40-80 mm) or bio-gel P2 (45-90 mm). Acid washed molecular sieves (4 Å) were flame activated under vacuum prior to reactions.

1.2 NMR Assignments.



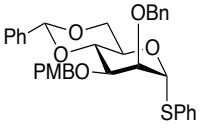
Scheme S1: Synthesis of mannopyranoside donor

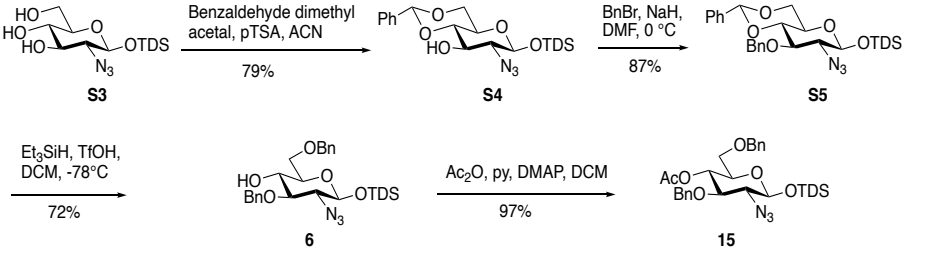
Phenyl 4,6-*O*-benzylidene-3-*O*-(4-methoxybenzyl)-1-thio-α-D-mannopyranoside (S2): Compound **S1 (**9 g, 33.1 mmol) was suspended in dry DMF, followed by the addition of benzaldehyde dimethyl acetal (5.4 mL, 36.4 mmol) and tetrafluoroboric acid diethyl ether complex solution (6.4 mL, 39.7 mmol). The mixture was stirred at R.T. for 12 h, after which it was quenched with Et_3N (15 mL). This solution was then poured over ice crystals, causing the desired



product to crash out. The product was then filtered and left to dry under high vacuum. This intermediate diol compound was then used for next reaction without further purification. The diol compound (7 g, 19.4 mmol) was suspended in dry toluene (100 mL), followed by the addition of dibutyl tin oxide (9.6 g, 38.8 mmol) and heating at 90 °C for 2 h, after which a clear solution was obtained. To this solution was added PMB-Cl (3.4 mL, 25.2 mmol) and TBAB (3.8 g, 25.2 mmol) and the heating was continued for another 4 h. After this time, the solution was cooled and the solvent was evaporated. The residue was dissolved in DCM (200 mL), followed by washing with sat. $NaHCO_3$ solution twice (200 mL). The organic layers were collected, dried over $MgSO_4$ and concentrated *in vacuo*. Silica gel column chromatography using Toluene: EtOAc (9: 1, v: v to 7:3, v: v) afforded the required product as a yellow syrup. (6.1 g, 66%). R_f = 0.53 (Tol: EtOAc, 8: 2, v: v). ^{1}H NMR (400MHz, $CDCl_3$): δ 7.67 to 6.81 (14H, m, Ar-H), 5.65 (1H, s, PhCH of benzylidene), 5.50 (1H, d, H-1, *J* = 1.3 Hz), 4.73 (1H, d, PhC<u>H</u>H, *J* = 11.6 Hz), 4.60 (1H, d, PhCH<u>H</u>, *J* = 11.6 Hz), 4.29 (2H, m, H-3, H-4), 4.21 (1H, m, H-6b), 3.97 (2H, m, H-2, H-5), 3.88 (1H, m, H-6a), 3.81 (3H, s, OC<u>H</u>$_3$). ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.22, 137.77, 137.62, 133.79, 131.63, 130.43, 129.34, 129.12, 128.86, 128.42, 128.18, 128.09, 127.84, 127.62, 126.11, 126.09, 113.77, 101.50, 87.14, 79.02, 78.08, 77.35, 77.03, 76.71, 75.83, 73.03, 72.75, 68.52, 65.50, 55.28. MALDI-TOF-MS (*m/z*): $[M + Na]^+$: calculated for $C_{27}H_{28}O_6SNa$, 503.1504; found 503.2010.

Phenyl 2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-(4-methoxybenzyl)-1-thio-α-D-mannopyranoside (5): Compound **S2** (6.1 g, 12.7 mmol) was dissolved in DMF cooled to 0 °C. This was followed by the addition of BnBr (2.3 mL, 19 mmol) and NaH (1g, 25 mmol, 60% dispersion in oil). The mixture was stirred at this temperature for 2 h, after which it was quenched with AcOH (50 mL). The solvent was evaporated *in vacuo* and the residue was diluted with DCM (100 mL), washed with saturated $NaHCO_3$ (100 mL) and water (100 mL) and dried over $MgSO_4$. The organic phase was filtered, and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography using Pet. Ether: EtOAc (8: 2, v: v to 6: 4, v: v), which afforded the target compound as a transparent syrup. (6.3 g, 86%). R_f = 0.46 (Pet. Ether: EtOAc, 8.5: 1.5, v: v). ^{1}H NMR (400MHz, $CDCl_3$): δ 7.69 to 6.58 (19H, m, Ar-H), 5.65 (1H, s, PhCH of benzylidene), 5.50 (1H, d, H-1, *J* = 1.3 Hz), 4.73 (3H, m, PhC<u>H</u>$_2$, PhC<u>H</u>H), 4.60 (1H, d, PhCH<u>H</u>, *J* = 11.3 Hz), 4.29 (2H, m, H-3, H-4), 4.22 (1H, m, H-6b), 3.97 (2H, m, H-2, H-5), 3.86 (1H, m, H-6a), 3.81 (3H, s, OC<u>H</u>$_3$). ^{13}C NMR (101 MHz, $CDCl_3$): δ 159.22, 137.77, 137.62, 133.79, 131.63, 130.43, 129.34, 129.12, 128.86, 128.42, 128.18, 128.09, 127.84, 127.62, 126.11, 126.09, 113.77, 101.50, 87.14, 79.02, 78.08, 77.35, 77.03, 76.71, 75.83, 73.03, 72.75, 68.52, 65.50, 55.28. MALDI-TOF-MS (*m/z*): $[M + Na]^+$: calculated for $C_{34}H_{34}O_6SNa$, 593.1974; found 593.2081.





Scheme S2. Synthesis of Building blocks **5** and **15**.

Dimethylthexylsilyl 2-deoxy-2-azido-4,6-*O*-benzylidine-β-D-glucopyranoside (S4): To a suspension of triol **S3**, (21.4 g, 61.6 mmol) in dry acetonitrile (200 mL) was added benzaldehyde dimethyl acetal (12 mL, 80.1 mmol) and *p*-Toluenesulfonic acid monohydrate (2.4 g, 12.2 mmol) and the reaction mixture was stirred at room temperature for 6 h after which the TLC (PE: EtOAc, 7: 3, v: v) showed the reaction had gone to completion. The reaction mixture was quenched with NEt_3 (10 mL) and concentrated *in vacuo*. Silica gel column chromatography using PE: EtOAc (8: 2, v: v) afforded the target compound as a transparent syrup, (21.1 g, 79%). R_f = 0.59 (PE: EtOAc, 8: 2, v: v). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.96 to 7.20 (5H, m, Ar-H), 5.51 (1H, s, C<u>H</u>Ph of benzylidene), 4.60 (1H, d, H-1, *J* = 7.5 Hz), 4.26 (1H, dd, H-6b, *J* = 10.5 Hz, 5.1 Hz), 3.75 (1H, t, H-6a, *J* = 10.1 Hz), 3.57 (2H, m, H-3, H-4), 3.37 (1H, m, H-5), 3.29 (1H, dd, H-2, *J* = 9.4 Hz, 7.7 Hz), 1.65 [1H, m, C<u>H</u>$(CH_3)_2$ of TDS], 0.89 [12H, m, C($C\underline{H}_3)_2$, CH($C\underline{H}_3)_2$ of TDS], 0.19 (6H, d, 2x $C\underline{H}_3$-Si of TDS, *J* = 8.3 Hz); ^{13}C NMR (101 MHz, $CDCl_3$): δ 136.86 to 126.22 (C-Ar), 101.96, 97.36, 80.74, 77.32 , 77.21, 77.00, 76.69, 71.88, 69.11, 68.54, 66.23, 34.14, 19.91, 19.72, 18.56 18.36, -0.03 to -3.23. MALDI-TOF-MS (*m/z*): $[M + Na]^+$: calculated for $C_{21}H_{33}N_3O_5SiNa$, 458.2087; found 458.2369.

Dimethylthexylsilyl 2-deoxy-2-azido-3-*O*-benzyl-4,6-*O*-benzylidine-β-D-glucopyranoside (S5): Compound **S4** (21 g, 48.2 mmol), was suspended in DMF (200 mL) and the mixture was cooled down to down to 0 ºC, followed by the sequential addition of BnBr (9.7 mL, 81.9 mmol) and NaH (3.8 g, 96.4 mmol, 60% dispersion in oil). The mixture was stirred at this temperature for two hours, after which it was quenched with AcOH. The solvent was evaporated *in vacuo* and the residue was diluted with DCM (200 mL), washed with saturated $NaHCO_3$ and water and dried over $MgSO_4$. The organic phase was filtered, and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography using Pet. Ether: EtOAc (9: 1, v: v to 7: 3, v: v), which afforded the target compound as a transparent sticky syrup. (22.2 g, 87%). R_f = 0.62 (Pet. Ether:

EtOAc, 9: 1, v: v). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.55 to 7.16 (10H, m, H-Ar), 5.56 (1H, s, PhC<u>H</u> of benzylidene), 4.91 (1H, d, PhC<u>H</u>H, *J* = 11.2 Hz), 4.79 (1H, d, PhCH<u>H</u>, *J* = 11.2 Hz), 4.57 (1H, d, H-1, *J* = 7.8 Hz), 4.28 (1H, dd, H-6b, *J* = 10.6 Hz, 5.1 Hz), 3.73 (2H, m, H-6a, H-3), 3.52 (1H, t, H-4, *J* = 9.4 Hz), 3.36 (2H, m, H-2, H-5), 1.67 [1H, m, C<u>H</u>$(CH_3)_2$ of TDS], 0.90 [12H, m, C(C<u>H</u>$_3)_2$, CH(C<u>H</u>$_3)_2$ of TDS], 0.19 (6H, 2s, 2x C<u>H</u>$_3$-Si of TDS); ^{13}C NMR (101 MHz, $CDCl_3$): δ 137.99, 137.22, 129.02, 128.56, 128.40, 128.34, 128.26, 128.14, 128.09, 127.78, 127.77, 127.62, 126.76, 126.01, 125.99, 101.30, 97.33, 81.66, 78.96, 77.33, 77.01, 76.69, 74.80, 72.11, 68.86, 68.64, 66.26, 34.19, 33.91, 24.81, 20.37, 19.97, 19.84, 18.55, 18.48, 18.39, -2.10, -3.25, -3.32. MALDI-TOF-MS (*m/z*): $[M + Na]^+$: calculated for $C_{28}H_{39}N_3O_5SiNa$, 548.2557; found 548.2824.

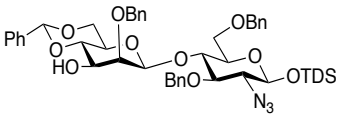
Dimethylthexylsilyl 2-deoxy-2-azido-3,6-di-*O*-benzyl-β-D-glucopyranoside (6): A solution of compound **S5** (22.15 g, 42.1 mmol) was stirred with pre-activated molecular sieves (50 g) in DCM (150 mL) for 30 minutes. The mixture was cooled down to -78 ºC, followed by the sequential addition of triethylsilane (10.1 mL, 63.2 mmol) and trifluoromethanesulfonic acid (7.4 mL, 84.2 mmol). The mixture was stirred at this temperature for half an hour, after which it was quenched with a mixture of NEt_3: MeOH (40 mL, 1:1, v: v). The mixture was warmed to room temperature, the molecular sieves were filtered off and the filtrate was diluted by DCM (200 mL) and washed with sat. $NaHCO_3$ and dried over $MgSO_4$. The organic phase was filtered, and the filtrate was concentrated *in vacuo*. Silica gel column chromatography using Pet. Ether: EtOAc (9: 1, v: v to 7: 3, v: v) yielded the product as a transparent syrup. (16 g, 72%). R_f = 0.32 (Pet. Ether: EtOAc, 8.5: 1.5, v: v). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.47 to 7.21 (10H, m, H-Ar), 4.92 (1H, d, PhC<u>H</u>H, *J* = 11.3 Hz), 4.78 (1H, d, PhCH<u>H</u>, *J* = 11.3 Hz), 4.58 (2H, m, PhC<u>H</u>$_2$), 4.52 (1H, d, H-1, *J* = 7.8 Hz), 3.72 (2H, m, H-6a, H-6b), 3.65 (1H, t, H-4), 3.41 (1H, m, H-5), 3.27 (2H, m, H-2, H-3), 2.71 (1H, d, O<u>H</u>, *J* = 2.5 Hz), 1.70 [1H, m, C<u>H</u>$(CH_3)_2$ of TDS], 0.93 [12H, m, C(C<u>H</u>$_3)_2$, CH(C<u>H</u>$_3)_2$ of TDS], 0.22 (6H, 2s, 2x C<u>H</u>$_3$-Si of TDS); ^{13}C NMR (101 MHz, $CDCl_3$): δ 138.26, 137.84, 128.59, 128.45, 128.05, 127.97, 127.77, 127.62, 97.06, 82.50, 77.38, 77.06, 76.75, 74.97, 74.05, 73.71, 71.94, 70.37, 68.28, 33.93, 24.84, 20.00, 19.89, 18.53, 18.42, -2.02, -3.25. MALDI-TOF-MS (*m/z*): $[M + Na]^+$: calculated for $C_{28}H_{41}N_3O_5SiNa$, 550.2713; found 550.2951.

Dimethylthexylsilyl 2-deoxy-2-azido-4-*O*-acetyl-3,6-di-*O*-benzyl-β-D-glucopyranoside (15): Compound **6** (10 g, 18.9 mmol) was dissolved in DCM (200 mL), followed by the addition of dry pyridine (20 mL), acetic anhydride (15 mL) and DMAP (200 mg). The reaction mixture was stirred for 30 minutes, after which it was quenched by MeOH (1 mL). The solvent was evaporated *in vacuo*, the residue was diluted with DCM (200 mL) and washed with sat. $NaHCO_3$

and 1 N HCl. The organic phase was dried over $MgSO_4$, filtered, and the filtrate was evaporated in vacuo to afford the product as a yellowish syrup. The product was then used in the next reaction without further purification. (10.1 g, quantitative yield). R_f = 0.47 (Pet. Ether: EtOAc, 8: 2, v: v). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.44 to 7.08 (10H, m, H-Ar), 4.97 (1H, m, H-4), 4.81 (1H, d, PhCHH, *J* = 11.5 Hz), 4.61 (1H, d, PhCHH, *J* = 11.5 Hz), 4.51 (3H, m, $PhCH_2$, H-1), 3.51 (3H, m, H-6a, H-6b, H-5), 3.37 (2H, m, H-2, H-3), 1.84 (3H, s, CH_3 of OAc), 1.67 [1H, m, $CH(CH_3)_2$ of TDS], 0.90 [12H, m, $C(CH_3)_2$, $CH(CH_3)_2$ of TDS], 0.21 (6H, 2s, 2x CH_3-Si of TDS); ^{13}C NMR (101 MHz, $CDCl_3$): δ 169.69, 137.91, 137.88, 129.02, 128.42, 128.31, 128.22, 127.86, 127.80, 127.66, 127.62, 125.29, 123.82, 96.96, 80.23, 77.34, 77.02, 76.71, 74.71, 73.56, 73.43, 70.99, 69.82, 68.47, 33.91, 24.82, 21.45, 20.80, 19.96, 19.86, 18.49, 18.39, -2.06, -3.29. MALDI-TOF-MS (*m/z*): $[M + Na]^+$: calculated for $C_{30}H_{43}N_3O_6SiNa$, 592.2819; found 592.2772.

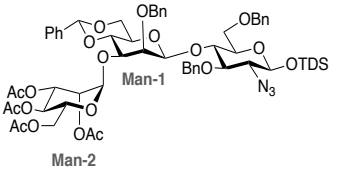
Dimethylthexylsilyl [4,6-*O*-benzylidene-2-*O*-benzyl-3-*O*-(4-methoxybenzyl)-β-D-mannopyranosyl](1→4)-3,6-di-*O*-benzyl-2-deoxy-2-azido-β-D-glucopyranoside (7): Glycosyl donor **5** (6.3 g, 11 mmol), DPS (3.6 g, 17.9 mmol) and TTBP (4.4 g, 17.9 mmol) were dissolved in DCM (80 mL) and stirred with pre-activated molecular sieves (10 g) for 30 min. After this time, the temperature was brought down to -60 ºC, followed by the addition of Tf_2O (3 mL, 17.9 mmol). After 10 min at this temperature, a solution of acceptor **6** (5.8 g, 11 mmol) in anhydrous DCM (5 mL), was added dropwise along the wall of the flask and the reaction mixture was stirred for 1 h, during which the temperature was slowly raised to -40 ºC. The TLC (PE: EtOAc, 8: 2, v: v) showed complete consumption of acceptor, at which point, the reaction was quenched with Et_3N (10 mL). The molecular sieves were filtered off, and DCM was removed *in vacuo*. Silica gel column chromatography using PE: EtOAc (9: 1, v: v to 7: 3, v: v) gave the product as a white amorphous powder. (6.1 g, 86%). R_f = 0.66 (Pet. Ether: EtOAc, 8: 2, v: v). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.34 to 6.56 (24H, m, H-Ar), 5.33 (1H, s, PhCH of benzylidene), 4.86 (1H, d, PhCHH, *J* = 10.2 Hz), 4.66 to 4.38 (5H, m, 2x $PhCH_2$, PhCHH), 4.36 (2H, m, H-1 GlcN, PhCHH), 4.24 (3H, m, $PhCH_2$, H-1 Man), 3.84 (2H, m, H-6a GlcN, H-3 GlcN), 3.72 (1H, m, H-2 GlcN), 3.59 (4H, m, OCH_3, H-2 Man), 3.34 (3H, m, H-6a Man, H-6b GlcN, H-6b Man), 3.25 (1H, dd, H-4 GlcN, *J* = 9.6 Hz, 3.1 Hz), 3.10 (3H, m, H-3 Man, H-4 Man, H-5 GlcN), 2.93 (1H, m, H-5 Man), 1.48 [1H, m, $CH(CH_3)_2$ of TDS], 0.69 [12H, m, $C(CH_3)_2$, $CH(CH_3)_2$ of TDS], 0.01 (6H, 2s, 2x CH_3-Si of TDS); ^{13}C NMR (101 MHz, $CDCl_3$): δ 26.16, 128.28, 128.31, 128.17, 128.26, 127.82, 76.97, 129.16, 127.68, 113.78, 101.37, 75.09, 75.10, 75.11, 75.11, 72.41, 72.42, 75.11, 73.66, 101.69, 72.45, 72.46, 97.01, 73.63, 78.74, 68.52, 77.52, 55.30, 77.20, 69.01, 68.69, 68.65, 78.08, 68.47, 81.33, 74.86, 67.47, 34.04, 17.03, 18.49, 34.02, 20.00, -2.05, -3.05, -2.12. MALDI-TOF-MS (*m/z*): $[M + Na]^+$: calculated for $C_{56}H_{69}N_3O_{11}SiNa$, 1010.4599; found 1010.4616.

Dimethylthexylsilyl [4,6-*O*-benzylidene-2-*O*-benzyl-β-D-mannopyranosyl](1→4)-3,6-di-*O*-benzyl-2-deoxy-2-azido-β-D-glucopyranoside (8): Compound **7** (6.1g, 7 mmol) was dissolved in



the solvent system DCM (200 mL) and H_2O (20 mL), followed by the addition of DDQ (3.6 g, 15.8 mmol). The mixture was stirred in dark for 3 h after which the reaction was worked up by washing with water (200 mL) and sat. $NaHCO_3$ (300 mL). The organic phase was dried over $MgSO_4$ and filtered, and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography and using Pet. Ether: EtOAc (9: 1, v: v to 7: 3, v: v) afforded the product as yellow amorphous solid. (4.7 g, 77%). R_f = 0.49 (Pet. Ether: EtOAc, 9: 1, v: v). ^{1}H NMR (400 MHz, $CDCl_3$): 7.30 to 7.03 (20H, m, H-Ar), 5.25 (1H, s, PhCH of benzylidene), 4.83 (1H, d, PhCHH, *J* = 10.4 Hz), 4.76 (1H, d, PhCHH, *J* = 10.2 Hz), 4.46 (4H, m, PhCHH, PhCHH, H-1 GlcN, PhCHH), 4.28 (2H, m, PhCHH, H-1 Man), 3.88 (1H, m, H-6a GlcN), 3.79 (1H, t, H-3 GlcN *J* = 9.2 Hz), 3.53 (1H, m, H-3 Man,), 3.41 (3H, m, H-2 GlcN, H-6b GlcN, H-6a Man), 3.27 (1H, t, H-6b Man, *J* = 10.4 Hz), 3.12 (3H m, H-2 Man, H-4 GlcN, H-5 GlcN), 2.90 (1H, m, H-5 Man), 2.15 (1H, OH), 1.48 [1H, m, CH$(CH_3)_2$ of TDS], 0.71 [12H, m, C$(CH_3)_2$, CH$(CH_3)_2$ of TDS], -0.02 (6H, 2s, 2x CH_3-Si of TDS); ^{13}C NMR (101 MHz, $CDCl_3$): δ 140.67, 140.14, 139.72, 139.27, 131.17, 130.63, 130.54, 130.31, 130.21, 130.10, 130.05, 130.00, 129.88, 129.87, 129.58, 128.33, 104.03, 103.82, 99.06, 83.35, 81.20, 80.98, 79.76, 79.29, 79.08, 78.87, 77.87, 77.12, 76.89, 75.81, 73.02, 70.60, 70.50, 70.42, 69.05, 62.45, 36.02, 26.92, 25.88, 23.11, 22.08, 21.96, 20.57, 20.46, 16.26, 2.05, -0.00, -1.13. MALDI-TOF-MS (*m/z*): $[M + Na]^+$: calculated for $C_{48}H_{61}N_3O_{10}SiNa$, 890.4024; found 890.4063.

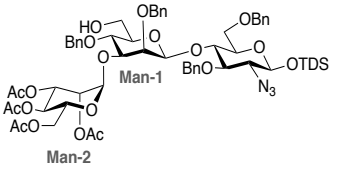
Dimethylthexylsilyl [2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl]-(1→3)-[2-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranosyl]-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-azido-β-D-glucopyranoside (10): A mixture of acceptor **8** (4.9 g, 5.6 mmol) and thio-manosyl donor **9** (4.9



g, 11.2 mmol), was stirred in DCM (60 mL) with pre-activated molecular sieves (10 g) for 20 min. The mixture was then cooled down to -25 ºC, followed by the sequential addition of NIS (2.5 g, 11.2 mmol) and TfOH (200 µL, 2.2 mmol). The reaction mixture was warmed up to -5 ºC over a period of 25 min, after which it was quenched with Et_3N (5 mL). The sieves were filtered off, the mixture was diluted by DCM (200 mL) and washed with 5% $Na_2S_2O_3$ (200 mL) and sat. $NaHCO_3$ (150 mL). The mixture was concentrated *in vacuo* to give the crude product as a yellow syrup. Silica gel column chromatography with Pet. Ether: EtOAc (9: 1, v: v to 7: 3, v: v) yielded the product as a transparent syrup. (5.1 g, 73 %). R_f = 0.57 (Pet. Ether: EtOAc, 7: 3, v: v). ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.32 to 7.01 (20H, m, H-Ar), 5.26 (1H, s, PhCH of benzylidene), 5.21 (2H, m, H-3 Man-2, H-4 Man-2), 5.03 (2H, m, H-1 Man-2, H-2 Man-2), 4.86 (1H, d, PhCHH, *J* = 10.2 Hz), 4.65 (2H, s, PhCH_2), 4.46 (3H, m, H-1 GlcN, PhCH_2), 4.28 (2H, m, PhCHH, H-1 Man-1),

3.99 (1H, dd, H-6a Man-2, J = 12.2 Hz, 4.3 Hz), 3.84 (3H, m, H-6a GlcN, H-3 GlcN, H-5 Man-2), 3.78 (1H, m H-6b Man-2), 3.59 (2H, m, H-2 GlcN, H-2 Man-1), 3.48 (3H, m, H-3 Man-1, H-6b GlcN, H-6a Man-1), 3.15 (3H, m, H-6b Man-1, H-4 GlcN, H-4 Man-1, H-5 GlcN), 2.86 (1H, m, H-5 Man-1), 1.82 (12H, m, 4x C$\underline{H}_3$ of Ac), 1.45 [1H, m, C$\underline{H}$(CH$_3$)$_2$ of TDS], 0.69 [12H, m, C(C$\underline{H}_3$)$_2$, CH(C$\underline{H}_3$)$_2$ of TDS], 0.01 (6H, 2s, 2x C$\underline{H}_3$-Si of TDS); ^{13}C NMR (151 MHz, CDCl$_3$): δ173.18, 172.63, 172.59, 171.96, 171.87, 171.80, 171.65, 171.61, 171.58, 140.67, 140.13, 140.08, 139.86, 139.70, 139.20, 134.66, 134.10, 131.25, 130.85, 130.69, 130.60, 130.50, 130.48, 130.44, 130.38, 130.20, 130.17, 130.12, 130.06, 129.95, 129.87, 129.83, 129.79, 129.76, 129.71, 129.59, 129.53, 128.91, 128.05, 128.02, 103.21, 103.16, 101.80, 100.74, 99.05, 87.73, 85.39, 85.13, 83.35, 80.40, 79.85, 79.34, 79.13, 78.91, 78.81, 78.48, 77.44, 77.12, 77.09, 76.12, 75.82, 75.64, 75.19, 74.90, 74.20, 72.94, 71.56, 71.43, 71.25, 70.85, 70.75, 70.49, 70.43, 70.28, 69.99, 69.10, 68.41, 68.34, 67.36, 64.58, 64.49, 63.49, 62.43, 55.50, 36.04, 35.97, 26.91, 23.10, 22.93, 22.87, 22.79, 22.77, 22.74, 22.72, 22.70, 22.62, 22.10, 22.03, 21.97, 21.90, 20.58, 20.47, 16.26, 2.06, 0.00, -0.93, -1.11, -1.18. MALDI-TOF-MS (*m/z*): [M + Na]$^+$: calculated for $C_{62}H_{79}N_3O_{19}SiNa$, 1220.4975; found 1220.5110.

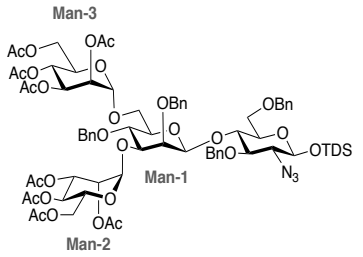
Dimethylthexylsilyl [2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl]-(1→3)-[2,4-di-*O*-benzyl-β-D-mannopyranosyl]-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-azido-β-D-glucopyranoside (11):



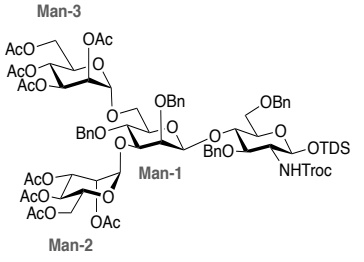
Compound **10** (5.1 g, 4.2 mmol) was dissolved in DCM (100 mL) and stirred with pre-activated molecular sieves (12 g) for 30 min, after which the temperature was brought down to -70 °C. The mixture was stirred at this temperature for another 30 min after which Et$_3$SiH (1.0 mL, 6.4 mmol) and PhBCl$_2$ (1.1 mL, 8.4 mmol). The mixture was stirred at this temperature for 20 min after which it was quenched with Et$_3$N (10 mL). The mixture was further diluted by DCM (200 mL), the sieves were filtered off, and the mixture was washed with sat. NaHCO$_3$ (150 mL). The organic phase was dried over MgSO$_4$ and filtered, and the filtrate was concentrated *in vacuo*. Silica gel column chromatography using Pet. Ether: EtOAc (9: 1, v: v to 4: 6, v: v) afforded the product as yellow amorphous powder. (3.9 g, 77%). R_f = 0.41 (Pet: EtOAc, 6: 4, v: v). ^{1}H NMR (600 MHz, CDCl$_3$): δ 7.35 to 6.94 (20H, m, H-Ar), 5.16 (2H, m, H-3 Man-2, H-4 Man-2), 5.03 (1H, t, H-2 Man-2, J = 9.6 Hz), 4.91 (1H, s, H-1 Man-2), 4.81 (1H, m, PhCH$\underline{H}$), 4.59 to 4.41 (4H, m, 2x PhC$\underline{H}_2$), 4.35 (2H, m, H-1 GlcN, PhCH$\underline{H}$), 4.29 (2H, m, PhC$\underline{H}$H, H-1 Man-1), 3.90 (1H, dd, H-6a Man-2, J = 12.2 Hz, 4.3 Hz), 3.74 (2H, m, H-6b Man-2, H-3 GlcN), 3.64 (2H, H-5 Man-2, H-2 GlcN), 3.58 (1H, d, H-2 Man-1, J = 2.9 Hz), 3.47 (2H, m, H-6a GlcN, H-6b GlcN), 3.36 (2H, m, H-6a Man-1, H-3 Man-1), 3.14 (3H, m, H-4 GlcN, H-4 Man-1, H-5 GlcN), 3.05 (1H, m, H-6b Man-1), 2.86 (1H, m, H-5 Man-1), 1.81 (12H, m, 4x C$\underline{H}_3$ of Ac), 1.46 [1H, m, C$\underline{H}$(CH$_3$)$_2$ of TDS], 0.69 [12H, m, C(C$\underline{H}_3$)$_2$, CH(C$\underline{H}_3$)$_2$ of TDS], -0.01 (6H, 2s, 2x C$\underline{H}_3$-Si of TDS); ^{13}C NMR (151 MHz, CDCl$_3$): δ 171.48, 171.21, 155.45, 150.42, 135.71, 135.59, 132.99, 132.94, 129.88, 127.80, 127.75, 118.20,

114.68, 97.75, 77.26, 77.04, 76.89, 76.83, 73.22, 72.04, 70.64, 63.77, 60.82, 60.43, 55.67, 26.86, 26.73, 21.07, 21.04, 20.80, 19.28, 14.22. MALDI-TOF-MS (*m/z*): $[M + Na]^+$: calculated for $C_{62}H_{81}N_3O_{19}SiNa$, 1222.5131; found 1222.5327.

Dimethylthexylsilyl [2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl]-(1→6)-[2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl]-(1→3)-[2,4-di-*O*-benzyl-β-D-mannopyranosyl]-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-azido-β-D-glucopyranoside (13): A mixture of acceptor **11** (3.9 g, 3.2 mmol) and imidate donor **12** (6.4 g, 13.0 mmol), was stirred in DCM (70 mL) with pre-activated molecular sieves (10 g) for 20 min. The mixture was then cooled down to -45 ºC, followed by the addition of TMSOTf (232 µL, 1.28 mmol). The reaction mixture was warmed up to -15 ºC over a period of 30 min, after which it was quenched with Et_3N (2 mL). The sieves were filtered off and the mixture was concentrated *in vacuo.* The residue was purified by silica gel column chromatography using Pet. Ether: EtOAc 8: 2, v: v to 1: 1, v: v) which yielded the product as a pale-yellow amorphous powder. (3.2 g, 68 %). R_f = 0.37 (Pet: EtOAc, 6: 4, v: v). ^1H NMR (600 MHz, $CDCl_3$): δ 7.28 to 6.95 (20H, m, H-Ar), 5.17 (2H, m, H-3 Man-2, H-4 Man-2), 5.05 (3H, m, H-2 Man-2, H-2 Man-3, H-4 Man-3), 4.98 (H-3 Man-3), 4.91 (1H, s, H-1 Man-2), 4.80 (2H, m, $PhCH_2$), 4.64 (1H, d, PhCHH, *J* = 11.5 Hz), 4.60 (1H, d, H-1 Man-3, *J* = 1.4 Hz), 4.52 (3H, m, $PhCH_2$, PhCHH), 4.39 (2H, m, H-1 GlcN, PhCHH), 4.31 (2H, m, PhCHH, H-1 Man-1), 3.96 to 3.78 (3H, m, H-6a Man-2, H-6a Man-3, H-5 Man-3), 3.71 (1H, m, H-6b Man-2), 3.62(3H m, H-3 GlcN, H-5 Man-2, H-2 GlcN), 3.54 (1H, m, H-2 Man-1), 3.49 (2H, m, H-6a GlcN, H-6b GlcN), 3.44 to 3.33 (3H, m, H-6a Man-1, H-3 Man-1, H-6b Man-3), 3.25 to 3.08 (5H, m, H-4 GlcN, H-4 Man-1, H-5 GlcN, H-6b Man-1, H-5 Man-1), 1.96 to 1.71 (24H, m, 8x CH_3 of Ac), 1.48 [1H, m, $CH(CH_3)_2$ of TDS], 0.71 [12H, m, $C(CH_3)_2$, $CH(CH_3)_2$ of TDS], 0.00 (6H, 2s, 2x CH_3-Si of TDS); ^{13}C NMR (151 MHz, $CDCl_3$): δ 127.57, 128.27, 127.85, 100.42, 99.64, 97.48, 96.88, 75.14, 75.12, 74.97, 74.56, 74.29, 73.74, 69.48, 69.17, 69.14, 69.12, 67.47, 65.79 65.58, 62.48, 61.98, 20.86, 20.76, 20.81, 20.76, 20.62, 34.00, 18.51, 19.95, -3.10. MALDI-TOF-MS (*m/z*): $[M + Na]^+$: calculated for $C_{76}H_{99}N_3O_{28}SiNa$, 1552.6082; found 1552.6198.

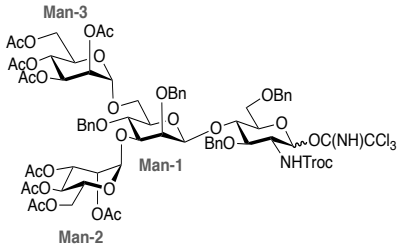


Dimethylthexylsilyl [2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl]-(1→6)-[2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl]-(1→3)-[2,4-di-*O*-benzyl-β-D-mannopyranosyl]-(1→4)-3,6-di-*O*-benzyl-2-deoxy-(2,2,2,-trichloroethoxy)carbonylamino-β-D-glucopyranoside (14):



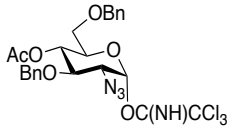
Compound **13** (2.3 g, 1.5 mmol) was dissolved in THF (30 mL) and water (5 mL) was added. This was followed by the addition of trimethylphosphine (775 µL, 7.5 mmol). The reaction mixture was stirred under argon for 2 h, following which the solvent was evaporated *in vacuo* and co-evaporated with toluene twice. The residue (1.9 g, 1.3 mmol) was dissolved in DCM (20 mL), followed by the addition of 2,2,2-trichloroethyl chloroformate (360 µL, 2.6 mmol) and triethylamine (900 µL, 6.5 mmol). The reaction mixture was stirred for 1 h after which it was diluted by DCM (100 mL) and washed with water. The organic layer was dried over $MgSO_4$ and filtered, and the filtrate was concentrated *in vacuo*. Silica gel column chromatography using PE: EtOAc (8: 2, v: v to 1: 1, v: v) afforded the product as a yellowish amorphous solid. (1.6, 62% over two steps); R_f = 0.40 (PE: EtOAc, 6: 4 v: v). ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.29 to 6.99 (20H, m, H-Ar), 5.22 (1H, d, NH of Troc, *J* = 8.3 Hz), 5.25 to 5.00 (6H, m, H-3 Man-2, H-4 Man-2, H-2 Man-2, H-2 Man-3, H-4 Man-3, H-3 Man-3), 4.93 (1H, s, H-1 Man-2), 4.80 (2H, m, PhCH_2), 4.68 (2H, m, PhCHH, H-1 GlcN), 4.63(1H, s, H-1 Man-3), 4.59 to 4.35 (8H, m, 2x PhCH_2, PhCHH, H-1 Man-1, CH_2 of Troc), 3.96 to 3.84 (3H, m, H-6a Man-2, H-6a Man-3, H-5 Man-3), 3.71 (2H, m, H-6b Man-2, H-3 GlcN), 3.62 (3H m, H-5 Man-2, H-2 Man-1, H-4 GlcN), 3.56 (2H, m, H-6a GlcN, H-6b GlcN), 3.51 to 3.32 (7H, m, H-6a Man-1, H-3 Man-1, H-6b Man-3, H-4 Man-1, H-5 GlcN, H-6b Man-1, 3.25 (1H, m, H-2 GlcN), 3.17 (1H, m, H-5 Man-1), 1.99 to 1.75 (24H, m, 8x CH_3 of Ac), 1.44 [1H, m, CH$(CH_3)_2$ of TDS], 0.68 [12H, m, C$(CH_3)_2$, CH$(CH_3)_2$ of TDS], -0.03 (6H, 2s, 2x CH_3-Si of TDS); ^{13}C NMR (151 MHz, $CDCl_3$), extracted form HSQC : δ 127.52, 128.28, 127.92, 128.12, 127.99, 69.24, 65.65, 69.26, 65.73, 65.92, 69.58, 99.65, 74.24, 73.50, 75.06, 95.60, 97.18, 74.20, 73.59, 99.95, 74.53, 75.03, 73.62, 60.37, 62.29, 76.73, 62.46, 68.38, 77.85, 68.92, 74.90, 68.78, 62.15, 67.23, 81.85, 75.03, 59.25, 74.68, 20.81, 20.81, 20.74, 20.69, 20.73, 33.99, 14.21, 17.01, 18.56, 20.00, -1.82, -3.54. MALDI-TOF-MS (*m/z*): $[M + Na]^+$: calculated for $C_{79}H_{102}Cl_3NO_{30}SiNa$, 1700.5219; found 1700.6135.

2,2,2-Trichloroacetimidate [2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl]-(1→6)-[2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl]-(1→3)-[2,4-di-*O*-benzyl-β-D-mannopyranosyl]-(1→4)-3,6-di-*O*-benzyl-2-deoxy-(2,2,2,-trichloroethoxy)carbonylamino-α/β-D-glucopyranoside (3):



Compound **14** (1.6 g, 0.95 mmol) was dissolved in the solvent system DCM: pyridine (30 mL, 1: 2, v: v), followed by dropwise addition of HF in pyridine (70% HF, 30% pyridine; 5 mL). The reaction mixture was stirred at room temperature for 12 h, after which it was quenched with solid $NaHCO_3$ (10 g), till all CO_2 bubbling stopped. The salts were filtered off, the solvent was evaporated *in vacuo*, and the residue was re-dissolved in DCM (20 mL), followed by washing with water (50 mL) and saturated $NaHCO_3$ (100 mL). The organic layer was dried over $MgSO_4$, filtered and the filtrate was concentrated and put for the next stage without further purification. The residue (1.4 g, 0.91 mmol) was dissolved in DCM (20 mL), followed by the addition of trichloroacetonitrile (460 µL, 4.5 mmol) and DBU (210 µL, 1.4 mmol). The reaction was stirred for 30 min, after which the solvent was evaporated and product was purified using silica gel column chromatography PE: EtOAc (8: 2, v: v to 1: 1, v: v) afforded the product as a white amorphous solid. (1.35, 85% over two steps); R_f = 0.58 (PE: EtOAc, 6: 4 v: v). Product showed hydrolysis under NMR and MALDI-TOF-MS conditions, hence was stored at -20 °C till further use in reactions.

2,2,2-Trichloroacetimidate 2-deoxy-2-azido-4-*O*-acetyl-3,6-di-*O*-benzyl-α-D-glucopyranoside (16): Compound **15** (5 g, 8.8 mmol) was dissolved in the solvent system DCM: pyridine (80 mL, 1: 2, v: v), followed by dropwise addition of HF in pyridine (70% HF, 30% pyridine; 15 mL). The reaction mixture was stirred at room temperature for 12 h, after which it was quenched with solid $NaHCO_3$ (30 g), till all CO_2 bubbling stopped. The salts were filtered off, the solvent was evaporated *in vacuo*, and the residue was re-dissolved in DCM (100 mL), followed by washing with water (150 mL) and saturated $NaHCO_3$ (150 mL). The organic layer was dried over $MgSO_4$, filtered and the filtrate was concentrated and put for the next stage without further purification. The residue (3.5 g, 8.2 mmol) was dissolved in DCM (50 mL), followed by the addition of trichloroacetonitrile (4.1 mL, 41 mmol) and DBU (1.8 mL, 12.3 mmol). The reaction was stirred for 10 min, which resulted in the formation of alpha product, after which the solvent was evaporated and product was purified using silica gel column chromatography PE: EtOAc (9: 1, v: v to 6: 4, v: v), which afforded the product as a white amorphous solid. (3.2 g, 65% over two steps); R_f = 0.63 (PE: EtOAc, 7: 3 v: v). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.66 (1H, s, NH), 7.44 to 7.08 (10H, m, H-Ar), 6.39 (1H, s, H-1),



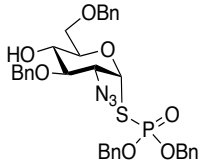
5.27 (1H, m, H-4), 4.59 (2H, m, 2x PhC<u>H</u>$_2$), 4.05 (2H, m, H-2, H-3), 3.82 (1H, m, H-5), 3.51 (2H, m, H-6a, H-6b), 1.90 (3H, s, C<u>H</u>$_3$ of OAc); ^{13}C NMR (101 MHz, $CDCl_3$): δ 128.70, 128.48, 128.42, 128.40, 128.33, 128.25, 128.23, 127.97, 127.91, 127.89, 94.51, 70.35, 68.60, 66.83, 60.38, 53.83, 52.56, 21.04. Compound was unstable under MALDI-TOF-MS conditions.

2-deoxy-2-azido-4-*O*-acetyl-3,6-di-*O*-benzyl-1-thio-α-D-glucopyranoside (17): To a solution of **16** (900 mg, 1.58 mmol) and bis(trimethylsilyl) sulfide (500 µL, 2.37 mmol) in DCM (15 mL) was added TMSOTf (43 µL, 0.237 mmol) at 0 °C. The mixture was stirred at this temperature for 1 h, then poured into saturated $NaHCO_3$ (10 mL) solution and extracted with DCM (50 mL). The organic layer was washed successively with water and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography using PE: EtOAc (9: 1, v: v to 7: 3, v: v), which afforded the product as a white amorphous solid. (300 mg, 43%); R_f= 0.59 (PE: EtOAc, 7: 3 v: v). ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.31 to 7.21 (10H, m, H-Ar), 5.70 (1H, t, H-1, *J* = 5.1 Hz), 5.10 (1H, dd, H-4, *J* = 9.5 Hz, 1.1 Hz), 4.82 (1H, d, PhC<u>H</u>H, *J* = 10.9 Hz), 4.63 ((1H, d, PhCH<u>H</u>, *J* = 10.9 Hz), 4.50 (2H, dd, PhC<u>H</u>$_2$, *J* = 17.3 Hz, 11.3 Hz), 4.27 (1H, m, H-5), 3.88 (1H, m, H-2), 3.79 (1H, t, H-3, *J* = 9.4 Hz), 3.49 (2H, m, H-6a, H-6b), 1.84 (3H, s, C<u>H</u>$_3$ of OAc), ; ^{13}C NMR (151 MHz, $CDCl_3$): δ 129.30, 129.18, 128.16, 128.03, 127.82, 127.55, 126.52, 126.41, 78.34, 75.25, 75.28, 73.65, 70.42, 70.25, 78.72, 63.60, 78.80, 68.68, 21.01. MALDI-TOF-MS (*m/z*): $[M + Na]^+$: calculated for $C_{22}H_{25}N_3O_5SNa$, 466.1413; found 466.1501.

Dibenzylthiophosphinate 2-deoxy-2-azido-4-*O*-acetyl-3,6-di-*O*-benzyl-1-thio-α-D-glucopyranoside (18): A solution of compound **17** (100 mg, 0.22 mmol) in DCM (1 mL), was added dropwise to a solution of dibenzyl *N,N*-diisopropylphosphoramidite (152 mL, 0.44 mmol), benzimidazolium triflate (240 mL, 0.9 mmol) and activated molecular sieves (100 mg) in ACN (1 mL) and DCM (1 mL), held at -50 °C during addition under Ar. The reaction mixture was warmed to -25 °C over a period of 2 h, after which it was cooled back to -50 °C and *m*CPBA (280 mg, 1.13 mmol) was added. The reaction was stirred for 2 h, during which it was warmed to room temperature. The mixture was filtered through Celite, diluted with DCM, washed sequentially with 20% aq. Na_2SO_3 (5 mL), 1 N aq. HCl (5 mL), and water (5 mL), dried over $MgSO_4$, and then concentrated. The residue was purified by silica gel column chromatography using PE: EtOAc (9: 1, v: v to 6: 4, v: v), which afforded the product as a white amorphous solid. (105 mg, 68%); R_f= 0.50 (PE: EtOAc, 7: 3 v: v). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.43 to 7.03 (20H, m, H-Ar), 5.96 (1H, dd, H-1, *J* = 10.1 Hz, 5.3 Hz), 5.14 (1H, dd, H-4, *J* = 9.5 Hz, 1.1 Hz), 5.08 (2H, m, PhC<u>H</u>$_2$), 5.02 (2H, m, PhC<u>H</u>$_2$), 4.79 (1H, d, PhC<u>H</u>H, *J* = 10.9

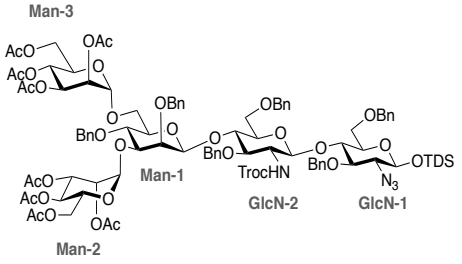
Hz), 4.60 (1H, d, PhCHH, *J* = 10.9 Hz), 4.50 (2H, m, PhCH_2), 4.07 (1H, m, H-5), 3.85 (1H, m, H-2), 3.69 (1H, t, H-3, *J* = 9.7 Hz), 3.60 (1H, m, H-6a), 3.53 (1H, m, H-6b), 1.86 (3H, s, CH_3 of OAc), ; ^{13}C NMR (101 MHz, $CDCl_3$): δ 133.26, 128.29, 128.06, 126.62, 126.46, 123.20, 123.05, 90.93, 89.43, 79.29, 79.21, 79.07, 75.22, 75.20, 73.68, 71.37, 70.55, 68.51, 67.18, 67.16, 69.00, 68.68, 63.62, 22.26; ^{31}P NMR (162 MHz, $CDCl_3$): δ 23.92. MALDI-TOF-MS (*m/z*): $[M + Na]^+$: calculated for $C_{36}H_{38}N_3O_8PSNa$, 726.2015; found 726.5637.

Dibenzylthiophosphinate 2-deoxy-2-azido-3,6-di-*O*-benzyl-1-thio-α-D-glucopyranoside (4):



Compound **18 (**92 mg, 0.13 mmol) was dissolved in DCM (1 mL) and MeOH (1 mL), followed by the addition of a solution of 25% NH_4OH (500 µL). The reaction was stirred at room temperature for 12 h, after which the solvent was evaporated, and the residue was purified by silioca gel column chromatography using PE: EtOAc (9: 1, v: v to 4: 6, v: v), which afforded the product as a white amorphous solid. (60 mg, 68%); R_f = 0.53 (PE: EtOAc, 6: 4 v: v). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.40 to 7.00 (20H, m, H-Ar), 5.96 (1H, dd, H-1, *J* = 10.5 Hz, 5.1 Hz), 5.08 (2H, m, PhCH_2), 5.02 (2H, m, PhCH_2), 4.77 (1H, d, PhCHH, *J* = 10.9 Hz), 4.60 (1H, d, PhCHH, *J* = 10.9 Hz), 4.50 (2H, m, PhCH_2), 4.07 (1H, m, H-5), 3.85 (1H, m, H-2), 3.73 (2H, m, H-3), (3.55 (2H, m, H-6a, H-6b), 1.85 (3H, s, CH_3 of OAc) ; ^{31}P NMR (162 MHz, $CDCl_3$): δ 24.06. MALDI-TOF-MS (*m/z*): $[M + Na]^+$: calculated for $C_{34}H_{36}N_3O_7PSNa$, 684.1901; found 684.2084.

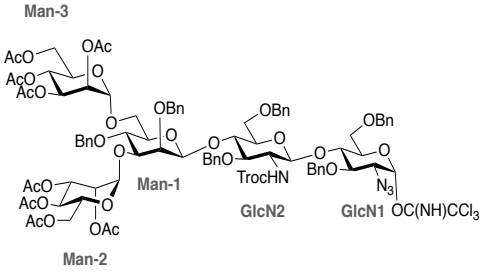
Dimethylthexylsilyl [2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl]-(1→6)-[2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl]-(1→3)-[2,4-di-*O*-benzyl-β-D-mannopyranosyl]-(1→4)-3,6-di-*O*-benzyl-2-deoxy-(2,2,2,-trichloroethoxy)carbonylamino-β-D-glucopyranosyl-(1→4)-2-deoxy-2-azido-3,6-di-*O*-benzyl-β-D-glucopyranoside (19): A mixture of acceptor **6** (100 mg, 0.19 mmol) and imidate donor **3** (290 mg, 0.17 mmol), was stirred in DCM (1 mL) with pre-activated molecular sieves (500 mg) for 20 min. The mixture was then cooled down to -20 ºC, followed by the addition of TMSOTf (6 µL, 0.034 mmol). The reaction mixture was warmed up to -5 ºC over a period of 30 min, after which it was quenched with Et_3N (50 µL).



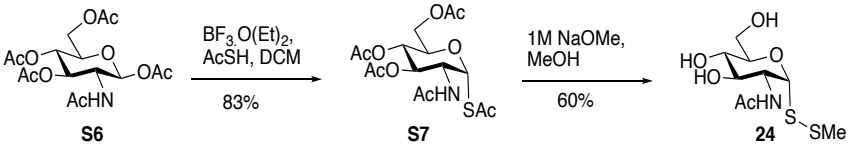
The sieves were filtered off and the mixture was concentrated *in vacuo.* The residue was purified by silica gel column chromatography using Pet. Ether: EtOAc 8: 2, v: v to 1: 1, v: v) which yielded the product as a white amorphous powder. (210 mg, 54 %). R_f = 0.57 (Pet: EtOAc, 6: 4, v: v). ^{1}H

NMR (600 MHz, $CDCl_3$): δ 7.43 to 6.97 (30H, m, H-Ar), 5.31 (1H, d, NH of Troc, J = 8.8 Hz), 5.21 to 4.98 (6H, m, H-3 Man-2, H-4 Man-2, H-2 Man-2, H-2 Man-3, H-4 Man-3, H-3 Man-3), 4.94 (2H, m, H-1 Man-2, PhCHH), 4.83 (3H, m, $PhCH_2$, PhCHH), 4.68 (2H, m, PhCHH, H-1 GlcN-2), 4.57 (2H, m, $PhCH_2$) 4.65 (2H, m, H-1 Man-3, H-1 GlcN-1), 4.59 to 4.35 (8H, m, 2x $PhCH_2$, PhCHH, H-1 Man-1, CH_2 of Troc), 3.96 to 3.84 (3H, m, H-6a Man-2, H-6a Man-3, H-5 Man-3), 3.74 (4H, m, H-6b Man-2, H-3 GlcN-2, H-6a GlcN-1, H-6b GlcN-2), 3.67 to 3.59 (4H m, H-5 Man-2, H-2 Man-1, H-4 GlcN-2, H-4 GlcN-1), 3.55 (2H, m, H-6a GlcN-1, H-6b GlcN-1), 3.49 (1H, m, H-5 GlcN-1), 3.40 to 3.32 (7H, m, H-6a Man-1, H-3 Man-1, H-6b Man-3, H-4 Man-1, H-5 GlcN, H-6b Man-1), 3.45 to 3.19 (3H, m, H-2 GlcN-2, H-2 GlcN-1, H-3 GlcN-1), 3.14 (1H, m, H-5 Man-1), 1.93 to 1.71 (24H, m, 8x CH_3 of Ac), 1.70 [1H, m, $CH(CH_3)_2$ of TDS], 0.88 [12H, m, $C(CH_3)_2$, $CH(CH_3)_2$ of TDS], 0.23 (6H, 2s, 2x CH_3-Si of TDS); ^{13}C NMR (151 MHz, $CDCl_3$), extracted form HSQC : δ 138.57, 137.80, 128.59, 128.31, 128.45, 128.15, 128.01, 127.94, 127.52, 127.92, , 127.77, 127.49, 81.75, 77.95, 75.06, 75.03, 75.01, 74.94, 74.58, 74.34, 74.27, 73.62, 73.51, 73.50, 69.61, 69.26, 69.22, 68.68, 68.38, 67.28, 65.92, 65.73, 65.65, 62.46, 62.29, 62.13, 60.37, 59.17, 33.93, 20.81, 20.81, 20.74, 20.73, 20.69, 20.10, 18.56, 17.08, 14.21, , -1.82, -3.54. MALDI-TOF-MS (*m/z*): $[M + Na]^+$: calculated for $C_{99}H_{123}Cl_3N_4O_{34}SiNa$, 2067.6751; found 2067.6884.

2,2,2-Trichloroacetimidate 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl]-(1→6)-[2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl]-(1→3)-[2,4-di-*O*-benzyl-β-D-mannopyranosyl]-(1→4)-3,6-di-*O*-benzyl-2-deoxy-(2,2,2,-trichloroethoxy)carbonylamino-β-D-glucopyranosyl-(1→4)-2-deoxy-2-azido-3,6-di-*O*-benzyl-α-D-glucopyranoside (20): Compound **19** (200 mg, 0.097 mmol) was dissolved in the solvent system DCM: pyridine (8 mL, 1: 2, v: v), followed by dropwise addition of HF in pyridine (70% HF, 30% pyridine; 3 mL). The reaction mixture was stirred at room temperature for 12 h, after which it was quenched with solid $NaHCO_3$ (2 g), till all CO_2 bubbling stopped. The salts were filtered off, the solvent was evaporated *in vacuo*, and the residue was re-dissolved in DCM (10 mL), followed by washing with water (15 mL) and saturated $NaHCO_3$ (15 mL). The organic layer was dried over $MgSO_4$, filtered and the filtrate was concentrated and put for the next stage without further purification. The residue (152 mg, 0.08 mmol) was dissolved in DCM (5 mL), followed by the addition of trichloroacetonitrile (40 µL, 0.4 mmol) and DBU (18 µL, 0.12 mmol). The reaction was stirred for 10 min, which resulted in the formation of alpha product, after which the solvent was evaporated and product was purified using silica gel column chromatography PE: EtOAc (9: 1, v: v to 1: 1, v: v), which afforded

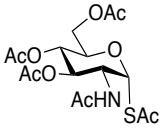


the product as a yellow amorphous solid. (102 mg, 50% over two steps); R_f = 0.65 (PE: EtOAc, 1: 1 v: v). ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.81 (1H, s, NH), 7.44 to 6.99 (30H, m, H-Ar), 6.49 (1H, s, H-1), 5.38 (1H, d, NH of Troc, *J* = 8.1 Hz), 5.29 to 4.90 (6H, m, H-3 Man-2, H-4 Man-2, H-2 Man-2, H-2 Man-3, H-4 Man-3, H-3 Man-3), 4.94 (5H, m, H-1 Man-2, PhCHH, PhCH_2, PhCHH), 4.72 (2H, m, PhCHH, H-1 GlcN-2), 4.61 to 4.55 (4H, m, PhCH_2, H-1 Man-3, H-1 GlcN-1), 4.51 to 4.30 (8H, m, 2x PhCH_2, PhCHH, H-1 Man-1, CH_2 of Troc), 3.95 to 3.75 (7H, m, H-6a Man-2, H-6a Man-3, H-5 Man-3, H-6b Man-2, H-3 GlcN-2, H-6a GlcN-1, H-6b GlcN-2), 3.65 to 3.55 (4H m, H-5 Man-2, H-2 Man-1, H-4 GlcN-2, H-4 GlcN-1), 3.51 (3H, m, H-6a GlcN-1, H-6b GlcN-1, H-5 GlcN-1), 3.49 (7H, m, H-6a Man-1, H-3 Man-1, H-6b Man-3, H-4 Man-1, H-5 GlcN, H-6b Man-1), 3.39 to 3.23 (3H, m, H-2 GlcN-2, H-2 GlcN-1, H-3 GlcN-1), 3.12 (1H, m, H-5 Man-1), 1.89 to 1.67 (24H, m, 8x CH_3 of Ac). Compound was unstable under MALDI-TOF-MS conditions.



Scheme S3. Synthesis of anomeric di-sulfide compound **24**.

Acetyl 2-acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-1-thio-α-D-glucopyranoside (S7): To a solution of compound **S6** (700 mg, 1.8 mmol) in anhydrous DCM (10 mL) was added boron trifluoride diethyl etherate (440 µL, 3.6 mmol) and thioacetic acid (260 µL, 3.6 mmol). The reaction was stirred for 12 h, after which it was quenched by adding water (20 mL). The organic layer was collected and dried over $MgSO_4$. The solvent was evaporated, and the residue was purified by silica gel column chromatography Tol: EtOAc (9: 1, v: v to 1: 1, v: v), which afforded the product as a white amorphous solid. (610 mg, 83%). R_f = 0.52 (Tol: EtOAc, 8: 2, v: v). ^{1}H NMR (600 MHz, $CDCl_3$): ^{1}H NMR (400 MHz, $CDCl_3$): 6.13 (1H, d, H-1, *J* = 3.6 Hz), 5.19 (2H, m, H-4, H-3), 4.44 (1H, m, H-2), 4.21 (1H, dd, H-6a), 4.03 (1H, dd, H-6b), 3.96 (1H, m, H-5), 2.17 to 1.90 (12H, 4s, 3x CH_3 of Ac and CH_3 of NHAc); ^{13}C NMR (101 MHz, $CDCl_3$): δ 90.60, 70.64, 69.68, 67.44, 61.51, 61.50, 51.04, 23.09, 20.66, 20.63, 20.63. MALDI-TOF-MS (*m/z*): [M + Na]$^+$: calculated for $C_{16}H_{23}NO_9SNa$, 428.0991; found 428.2102.



Methyl 2-acetamido-2-deoxy-1-dithio-α-D-glucopyranoside (24): To a solution of compound **S7** (600 mg, 1.48 mmol) in anhydrous MeOH, was added 1M NaOMe solution in MeOH (1 mL). The reaction mixture was stirred for 1 h, after which it was neutralized by adding Amberlite-H form resin. The resin was filtered, and the solvent was removed *in vacuo*. The residue was purified by silica gel column chromatography using DCM: MeOH (9: 1, v: v) which afforded the desired product as an amorphous solid (255 mg, 60%).[1]H NMR (400 MHz, $CDCl_3$): 5.08 (1H, d, H-1, J = 3.4 Hz), 5.19 (2H, m, H-4, H-3), 3.81 (1H, m, H-2), 3.76 (2H, m, H-6a, H-3), 3.66 (2H, m, H-6b, H-4), 3.32 (1H, m, H-5), 2.70 (3H, s, C$\underline{H}_3$ of S-Me), to 1.97 (3H, s, C$\underline{H}_3$ of NHAc); ^{13}C NMR (101 MHz, $CDCl_3$): δ 91.28, 71.43, 71.30, 70.75, 61.44, 54.31, 38.23, 21.56. MALDI-TOF-MS (*m/z*): $[M + Na]^+$: calculated for $C_9H_{17}NO_5S_2Na$, 306.0446; found 306.0605.

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Nederlandse Samenvatting

Het glycoom bestaat uit glycoconjugaten zoals glycoproteïnen en glycolipiden die post-translationeel worden gegenereerd door de werking van meer dan 200 glycosyltransferasen. *N*-glycanen zijn de meest diverse en overvloedige soorten glycoproteïnen die tot expressie worden gebracht in alle domeinen van het leven. Ze binden aan glycaanbindende eiwitten, lectines genaamd, voor herkenning en interacties die talrijke biologische processen uitvoeren en spelen een sleutelrol in interacties tussen gastheer en ziekteverwekker. **Hoofdstuk 1** geeft een overzicht van de biologische relevantie van *N*-glycanen in cel-cel communicatie, receptorherkenning, immuunmodulatie, en de strategieën die door chemici worden gebruikt voor de synthese van dergelijke complexe oligosachariden. In de natuur zijn glycanen aanwezig als heterogene mengsels van vele vormen van glycoconjugaten en zijn ze als zuivere standaarden moeilijk te isoleren. Sinds de synthese van het eerste bi-antennaire *N*-glycaan in de jaren tachtig, zijn de strategieën van chemische synthese, geholpen door enzymatische reacties, enorm geëvolueerd om asymmetrische *N*-glycanen van ongekende complexiteit op te leveren. Dit was mogelijk dankzij talloze uitvindingen en verbeteringen in reactiestrategieën door de jaren heen, die in het kort zijn besproken. De integratie van door enzymen ondersteunde reacties in de syntheseroute zorgde voor een revolutie op dit gebied door absolute regio- en stereoselectiviteit te bieden, waardoor de synthese van oligosachariden van ongekende complexiteit werd vergemakkelijkt. Hoewel eukaryote *N*-glycanen vrij efficiënt zijn verkregen, zijn de glycanen van lagere ziekteveroorzakende pathogenen zoals bacteriële en parasitaire glycanen nog steeds moeilijk te bereiken vanwege ongebruikelijke koolhydraatepitopen, glycosyleringspatronen en ontoegankelijkheid van synthetisch bruikbare enzymen. Chemische synthese domineert nog steeds de weg om dergelijke ongebruikelijke glycanen te verkrijgen. De volgende hoofdstukken lichten de strategieën toe die worden gebruikt om de gewenste glycanen te verkrijgen die gastheer-pathogeen-interacties veroorzaken en worden bestudeerd door multidisciplinaire benaderingen.

Een aantal pathogenen kan het immuunsysteem van de gastheer kapen of omzeilen door fasevariabele glycosylering. *S. mansoni* is een ziekteverwekker die levenslange infecties kan veroorzaken, latente afwisseling met actieve fasen en reactivering van het immuunsysteem van de gastheer. **Hoofdstuk 2** is de introductie van schistosomiasis en een bespreking van de strategieën die door *S. mansoni* worden gebruikt om de immunologische respons van de gastheer te dempen. *S. mansoni* worden de meester-manipulators van het immuunsysteem genoemd en zorgen voor hun overleving door ontstekingen te verminderen en een gewijzigde immunologische reactie op te wekken. De patroonherkenningsreceptor Dendritische cel-specifieke ICAM-3 grijpende non-

integrine (DC-SIGN), is een C-type lectine dat de balans tussen Th1 en Th2 immuunresponsen handhaaft, een brede substraatspecificiteit heeft en een overvloed aan gefucosyleerde glycanen kan herkennen. *S. mansoni* vertoont sterk gefucosyleerde glycaanstructuren en het is bekend dat ze zich bij infectie op DC-SIGN richten. In dit hoofdstuk wordt een korte uitleg gegeven van hun invasiemechanisme en de immunologische implicaties op lange termijn in gastheren.

Tijdens zijn levenscyclus brengt *S. Mansoni* verschillende gefucosyleerde glycanen tot expressie, waaronder Lac-di-NAc, Le^x, LDN-F en LDN-DF op een fase-afhankelijke manier. Er zijn aanwijzingen dat DC-SIGN sommige van deze gefucosyleerde glycanen selectief kan herkennen, wat aangeeft dat fasevariabele expressie van glycanen de immuunresponsen van de gastheer kan vormen. Een belangrijke vraag die in **Hoofdstuk 3** aan de orde komt, is dus hoe bepaalde gefucosyleerde structuren detectie door DC-SIGN kunnen vermijden. In dit hoofdstuk rapporteren we de eerste chemische synthese van de gefucosyleerde glycanen LDN-F en LDN-DF. NMR en moleculaire modelleringsstudies toonden aan dat het α1,3-fucoside van LDN-F kan coördineren met het Ca^{2+}-ion van de canonieke bindingsplaats van DC-SIGN, waardoor het GlcNAc residu dicht bij het eiwitoppervlak wordt geplaatst, waardoor extra interacties mogelijk zijn. Het α1,2-gebonden fucoside van LDN-DF kan ook in deze bindingsplaats worden geplaatst, maar in dit geval kunnen de GlcNAc en GalNAc residuen geen interacties aangaan met het eiwitoppervlak, wat resulteert in een aanzienlijk lagere bindingsaffiniteit. Glycan microarray bindingsonderzoeken toonden aan dat de aviditeit en selectiviteit van binding aanzienlijk wordt verbeterd wanneer de glycanen multivalent worden gepresenteerd, en in dit formaat gaven Le^x en LDN-F een sterke respons terwijl er geen binding werd gedetecteerd voor LDN-DF. Op basis van deze waarnemingen werd voorgesteld dat fasevariabele expressie van glycanen zoals Le^x, LDN-F en LDN-DF die verschillend interageren met DC-SIGN, *S. mansoni* een strategie biedt om de immuunrespons van de gastheer te scheeftrekken op een manier die geschikt is voor specifieke ontwikkelingsstoornissenstadia.

Bindings en structuurstudies hebben aangetoond dat glycaanbindende eiwitten relatief kleine oligosacharidemotieven herkennen die vaak worden gevonden aan de uiteinden van complexe glycanen. De complexe architectuur van *N*-glycanen die verschillende vertakkingspatronen vertonen, kan echter mogelijk meerdere minimale epitopen presenteren die kunnen interageren met meerdere glycan bindende eiwitten, wat resulteert in verhoogde bindingslusten. In **Hoofdstuk 4** wordt een chemo-enzymatische methodologie beschreven om drie karakteristieke *N*-glycanen te verschaffen die tot expressie worden gebracht door de parasitaire worm *S. mansoni* met unieke epitopen bij elke antenne en gekenmerkt door de aan of afwezigheid van kern xyloside. Een zorgvuldige glycosyleringsstrategie, geholpen door de werking van een panel van zeer specifieke zoogdierglycosyltransferasen en glycosidasen, werd gebruikt om asymmetrie te bereiken. STD-NMR, computationele en elektronenmicroscopie werden gebruikt om de herkenning van de glycanen door het menselijke lectine DC-SIGN te onderzoeken. Er werd onthuld dat kern xyloside de herkenning van terminale epitoop niet beïnvloedt. Verder werd gevonden dat de multi-

antennaire glycanen met hogere affiniteit binden aan DC-SIGN in vergelijking met monovalente minimale epitopen, wat werd toegeschreven aan nabijheid-geïnduceerde effectieve concentratie. Ten slotte hebben de onderzoeken aangetoond dat het multi-antennaire glycaan DC-SIGN kan verknopen tot een dicht netwerk, wat waarschijnlijk relevant is voor de opname van antigeen en intracellulaire routering. Deze studies tonen aan dat uniek inzicht kan worden verkregen in moleculaire aspecten van eiwit-glycaan interacties door middel van een multidisciplinaire benadering.

De veelzijdigheid en superioriteit van onze nieuw ontwikkelde chemo-enzymatische benadering werd gebruikt om een bibliotheek van biologisch belangrijke glycanen van *S. mansoni* te synthetiseren die voorheen niet toegankelijk waren. Deze glycanen werden asymmetrisch gemodificeerd door kern xylosiden en fucosiden, verlengde LacNAc en meerdere Lac-di-NAc resten, zoals uitgelegd in **Hoofdstuk 5**. De strategie gebruikte onnatuurlijke glycosidische bindingen zoals α-galactoside en β-mannoside als overkappingsmotieven aan de termini, die achtereenvolgens verwijderd konden worden door een geschikte glycosidase in een gewenst stadium, om terminale β-GlcNAc rest vrij te maken die onafhankelijk zou kunnen worden verlengd door een panel van zoogdierglycosyltransferasen. Verder werd een één-pot enzymatische strategie ontwikkeld om bi-antennaire glycanen te verkrijgen door de splitsing van een volledige terminale antenne. De bibliotheek werd gebruikt voor voorlopige microarray-onderzoeken met plantaardige lectines. De toegankelijkheid van structureel gedefinieerde complexe glycanen zou de ideale basis kunnen zijn voor toekomstige studies om de perfecte glycan-motieven te genereren die antilichamen kunnen genereren en het immuunsysteem van de gastheer kunnen activeren voor een passende reactie tegen schistosomiasis

List of Publications

A.D. Srivastava, L. Unione, M. Bunyatov, I.A. Gagarinov, N.G.A. Abrescia, S. Delgado, A. Ardá and G.J. Boons. Chemoenzymatic synthesis of complex *N*-glycans of the parasite *S. mansoni* to examine the importance of epitope presentation on DC-SIGN recognition. **2021**, *Angew. Chem. Int. Ed.*, 60, 19287-19296.

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I.A. Gagarinov, T. Fang, L. Liu, **A.D. Srivastava** and G.J. Boons. Synthesis of Staphylococcus Aureus Type 5 Trisachharide Repeating Unit: Solving the problem of Lactamization. **2015,** *Org. Lett.,* 17(4), 928-931.

Curriculum Vitae

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