Chemical, Enzymatic and Chemo-enzymatic Synthesis of Complex Oligosaccharides as Probes of Glycan Functions

by

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Abstract

Carbohydrates, an essential class of biomolecules, mediate a variety of biological processes. Recent advances in chemical, enzymatic and chemo-enzymatic synthesis have facilitated access to an increasing number of structurally well-defined oligosaccharides. These molecules are serving as invaluable probes for deciphering the biological functions of these glycans and understanding the molecular mechanism underlying their functions, as well as exploring their potential for medicinal use. This thesis is focused on the synthesis of oligosaccharides related to three projects using different synthetic strategies, purely chemical, purely enzymatic and chemo-enzymatic.

In the first example, lacto-*N*-fucopentaose III (LNFPIII), a previously reported immunomodulatory glycan was synthesized via a concise chemical approach, which features two highly regio- and stereoselective glycosylations for the construction of the pentasaccharide, a Birch reduction for deprotection of benzyl ethers, and a thiol–ene coupling for modification of the aglycon. Preliminary biological studies confirmed the potential of LNFPIII to be used as a tolerance-inducing agent in ABO-incompatible (ABOi) heart transplants.

In a second example, as part of ABOi heart transplant program, we are interested in creating an ABO-glycan microarray for accurate detection of anti-donor antibody responses in patients undergoing an ABOi transplant. To better mimic natural glycan structures and reveal the effect of structural complexity on antibody–antigen recognition, a panel of poly-LacNAc extended biantennary *N*- and *O*-linked glycans capped with blood group A or B antigens were synthesized via an enzymatic approach. These glycans will be used in a glycan microarray to screen serum samples from ABOi heart transplant patients.

In the final example, a chemo-enzymatic approach was developed to prepare a library of structurally defined group B Streptococcus type III (GBSIII) oligosaccharides differing in size from 10 up to 50 residues. The route developed involves chemical synthesis of the oligosaccharide backbone and introduction of the side chain by glycosyltransferases. These compounds will be used as probes to gain deeper insight into the role of carbohydrates in activation of the immune system.

Preface

This thesis is an original work by Junfeng Zhang. The synthetic work described in Chapter 2 of this thesis has been published as: Zhang, J.; Zou, L.; Lowary, T. L. Synthesis of the tolerance inducing oligosaccharide lacto-N-fucopentaose III bearing an activated linker. *ChemistryOpen*, **2013**, *2*, 156–163. I was responsible for the synthesis, characterization as well as manuscript composition. Lu Zou assisted with conjugate synthesis. Todd L. Lowary was the supervisory author. The biological study was carried out through collaboration with Professor Jordi Ochando at Icahn School of Medicine at Mount Sinai. The result was published as: Conde, P.; Rodriguez, M.; van der Touw, W.; Jimenez, A.; Burns, M.; Miller, J.; Brahmachary, M.; Chen, H.-M.; Boros, P.; Rausell-Palamos, F.; Yun, T. J.; Riquelme, P.; Rastrojo, A.; Aguado, B.; Stein-Streilein, J.; Tanaka, M.; Zhou, L.; Zhang, J.; Lowary, T. L.; Ginhoux, F.; Park, C. G.; Cheong, C.; Brody, J.; Turley, S. J.; Lira, S. A.; Bronte, V.; Gordon, S.; Heeger, P. S.; Merad, M.; Hutchinson, J.; Chen, S.-H.; Ochando, J. DC-SIGN^{+} macrophages control the induction of transplantation tolerance. *Immunity* **2015**, *42*, 1143–1158. I provided the compound to Professor Jordi Ochando; his group and their collaborators conducted all the biological studies.

The work described in Chapter 3 is a collaboration with Professor James C. Paulson at the Scripps Research Institute, I conducted all the synthetic work in his lab, which has not been published.

The work described in Chapter 4 was done solely by me and has not been published.

Dedicated to All My Friends and Family!

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Next, I would like to offer my special thanks to Prof. James C. Paulson for giving me the opportunity to learn enzymatic synthesis in his laboratory at the Scripps Research Institute in La Jolla California, Dr. Wenjie Peng for his guidance and help in the Paulson laboratory, Prof. Warren W. Wakarchuk (Ryerson University) for providing us the clones of enzymes required for my research project and Mr. Ruixiang Blake Zheng for enzyme expression.

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Table of Contents

List of Figures

List of Schemes

List of Abbreviations

$[\alpha]_D$	specific rotation (sodium D line)
1-P	1-phosphate
12-mer	dodecasaccharide
18-mer	octadecasaccharide
24-mer	tetracosasaccharide
30-mer	triacontasaccharide
Å	Angstrom
ABOi	ABO-incompatible
Ac	acetyl
ADP	adenosine diphosphate
AgOTf	silver trifluoromethanesulfonate
APC	antigen presenting cell
app	apparent
Ar	aromatic
ATP	adenosine triphosphate
BCR	B cell receptor
BDA	butane-2,3-diacetal
Bn	benzyl
BnAb	broadly neutralizing antibody
br s	broad singlet (NMR spectra)
BSA	bovine serum albumin
BSP	1-benzenesulfinyl piperidine

Bz	benzoyl
C_5-epi	C_5-epimerase
CD4	cluster of differentiation 4
CIAP	calf intestinal alkaline phosphatase
CMP-Neu5Ac	cytidine-5′-monophospho-*N*-acetylneuraminic acid
COSY	correlation spectroscopy
CPS	capsular polysaccharide
CSA	camphorsulfonic acid
d	doublet (NMR spectra)
DBU	diazabicyclo[5.4.0]undec-7-ene
DC-SIGN	dendritic cell-specific intercellular adhesionmolecule-3-grabbing non-integrin
DCC	*N*,*N*′-dicyclohexylcarbodiimide
DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
DIBAL-H	diisobutylaluminium hydride
DMAP	4-dimethylaminopyridine
DMDO	dimethyldioxirane
DMF	*N,N*-dimethylformamide
DMTST	dimethyl(methylthio)sulfonium trifluoromethanesulfonate
DTBMP	2,6-di-*tert*-butyl-4-methylpyridine
DTBS	4,6-*O*-di-*t*-butylsilyl
EGCase	endoglycoceramidase II
ENGases	endo-β-*N*-acetylglucosaminidases
ESI	electrospray ionization

Fmoc	9-fluorenylmethoxycarbonate
Fuc	fucose
FucT	fucosyltransferase
FucT II	α-(1→2)-Fucosyltransferase
Gal	galactose
GalNAc	*N*-acetylgalactosamine
GalNAcE	UDP-GalNAc 4'-epimerase
GalNAcT	*N*-acetylgalactosaminyltransferase
GalT	galactosyltransferase
GalT-1	bovine β-(1→4)-galactosyltransferase
GalT-5	human β-(1→3)-galactosyltransferase
GBP	glycan-binding protein
GBSIII	group B Streptococcus type III
GDP	guanosine diphosphate
GDP-Fuc	guanosine diphosphate fucose
Glc	glucose
GlcNAc	*N*-acetylglucosamine
GlcNAcT	*N*-acetylglucosaminyltransferase
GTA	α-(1→3)-*N*-acetylgalactosaminyltransferase
GTB	α-(1→3)-galactosyltransferase
GTP	guanosine triphosphate
HA	hemagglutinin
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
Hib	*Haemophilus influenz*ae type b

HIV-1	human immunodeficiency virus type 1
HMBC	Heteronuclear Multiple Bond Correlation
HMQC	Heteronuclear Multiple Quantum Coherence
HSA	human serum albumin
HSQC	Heteronuclear Single Quantum Coherence
IgG	immunoglobulin G
IgM	immunoglobulin M
KfiA	*E. coli* K5 *N*-acetyl glucosaminyltransferase
L	leaving group
LacNAc	*N*-acetyllactosamine
Lev	levulinoyl
LNFPIII	lacto-*N*-fucopentaose III
m	multiplet (NMR spetra)
MALDI-TOF	Matrix-Assisted Laser Desorption/Ionization Time-of-Flight
Man	mannose
MeOTf	methyl trifluoromethanesulfonate
MHCII	major histocompatibility complex class II
NAP	2-naphthylmethyl
Naph	2-naphthyl
NBS	*N*-bromosuccinimide
NIS	*N*-iodosuccinimide
NmCSS	*N. meningitides* CMP-Neu5Ac synthetase
NMR	nuclear magnetic resonance

NST	*N*-sulfotransferase
OST	*O*-sulfotransferase
P	protecting group
p-TsOH	*p*-toluenesulfonic acid
PAPS	3′-phosphoadenosine-5′-phosphosulfate
Pd2,6ST	*Photobacterium damselea* α-(2→6) sialytransferase
PEP	phosphoenolpyruvate
Ph	phenyl
$Ph_3PAuNTf_2$	[bis(trifluoromethanesulfonyl)imidate]-(triphenylphosphine)gold(I)
Pi	phosphate
Piv	pivaloyl
PK	pyruvate kinase
pmHS2	heparosan synthase-2
PmST	*Paseurella multocida* α-(2→3)-sialyltransferase
PNP	*p*-nitrophenol
PPA	pyrophosphate
PPh_3	triphenylphosphine
PPi	pyrophosphate
ppm	parts per million
q	quartet (NMR spectra)
R_f	retention factor
s	singlet (NMR spectra)
SiaT	sialyltransferase

t	triplet (NMR spectra)
TACA	tumor-associated carbohydrate antigen
TBAF	tetrabutylammonium fluoride
TBAI	tetrabutylammonium iodide
TBDPS	*tert*-butyldiphenylsilyl
TBDPSCl	*tert*-butyl(chloro)diphenylsilane
TCR	T cell receptor
Tf_2O	trifluoromethanesulfonic anhydride
TFA	trifluoroacetic acid
TfOH	trifluoromethanesulfonic acid
THF	tetrahydrofuran
TLC	thin layer chromatography
TMSOTf	trimethylsilyl trifluoromethanesulfonate
Tol	*p*-tolyl
Tris	tris(hydroxymethyl)aminomethane
Troc	2,2,2-trichloroethoxycarbonyl
TT	tetanus toxin
TTBP	2,4,6-tri-*tert*-butylpyrimidine
UDP	uridine diphosphate
UDP-Gal	uridine diphosphate galactose
UDP-GalNAc	uridine diphosphate *N*-acetylgalactosamine
UDP-Glc	uridine diphosphate glucose
UDP-GlcNAc	uridine diphosphate *N*-acetylglucosamine
ULMW	ultralow molecular weight

UTP	uridine triphosphate
V1V2	variable 1 and 2
ZPS	zwitterionic polysaccharide
β3GlcNAcT	β-(1→3)-*N*-acetylglucosaminyltransferase
β4GalT/GalE	β-(1→4)-GalT/UDP-Gal 4'-epimerase

Chapter 1: Introduction

1.1 Biological importance of carbohydrates

Carbohydrates, also referred to as glycans, are a class of complex and structurally diverse biomolecules found in all organisms. They are mainly located on the cell surface and in extracellular matrices in the form of oligosaccharides, polysaccharides and glycoconjugates (*e.g.,* glycoproteins, glycolipids, peptidoglycans). It is now well recognized that these molecules mediate a myriad of biological processes including organismal development, cell adhesion and signaling, viral and bacterial infection, tumorigenesis, inflammatory and immune responses.[1,2] An in-depth understanding of their biological functions will shed light on development of carbohydrate-based diagnostics, vaccines and therapeutics.[3] However, progress towards the understanding of structure–function relationships of glycans lags far behind that of proteins and nucleic acids, due to the lack of diverse libraries of structurally well-defined oligosaccharides.[4]

Access to these glycans is a prerequisite for deciphering their biological functions and their underlying molecular mechanisms.[5] Given the small quantity and heterogeneity of glycans obtained from natural sources, a synthetic approach is often more practical to provide many of these compounds.[6] However, in contrast to proteins and nucleic acids, oligosaccharides are much more challenging to synthesize because of their branched structures (rather than linear for both proteins and nucleic acids) and the requirement for stereocontrol of the installation of glycosidic linkages. Therefore, extensive efforts have been devoted to push forward the synthesis of structurally well-defined complex glycans. Despite ongoing challenges, tremendous progress has been achieved, which mainly relies on two general synthetic strategies: 1) chemical synthesis and 2) enzymatic (including chemo-enzymatic) synthesis.[5-7]

1.2 Chemical synthesis of oligosaccharides

1.2.1 Glycosylations and anomeric stereocontrol

An essential transformation involved in the chemical synthesis of oligosaccharides is glycosylation. This is a reaction in which the hydroxyl group of glycosyl acceptor attacks the anomeric position of glycosyl donor after the leaving group of the donor is activated, resulting in the formation of a glycosidic bond.[8] In general, glycosyl donors and glycosyl acceptors are equipped with protecting groups to mask and differentiate multiple hydroxyl groups, which ensures the regioselectivity of glycosylation. After each glycosylation, selective deprotection is performed to unmask a specific hydroxyl group for the next round of glycosylation. Or, all of the protecting groups are removed in one or more steps. In the former case, the glycosylation–deprotection cycle is repeated until the desired compound is constructed. To date, a variety of glycosyl donors have been developed (Figure 1.1), making the synthesis of complex oligosaccharides more predictable.[9,10]

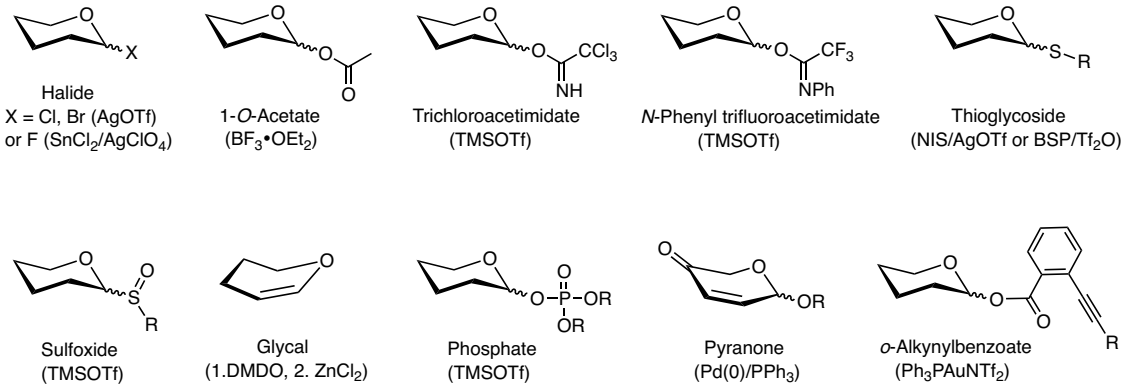
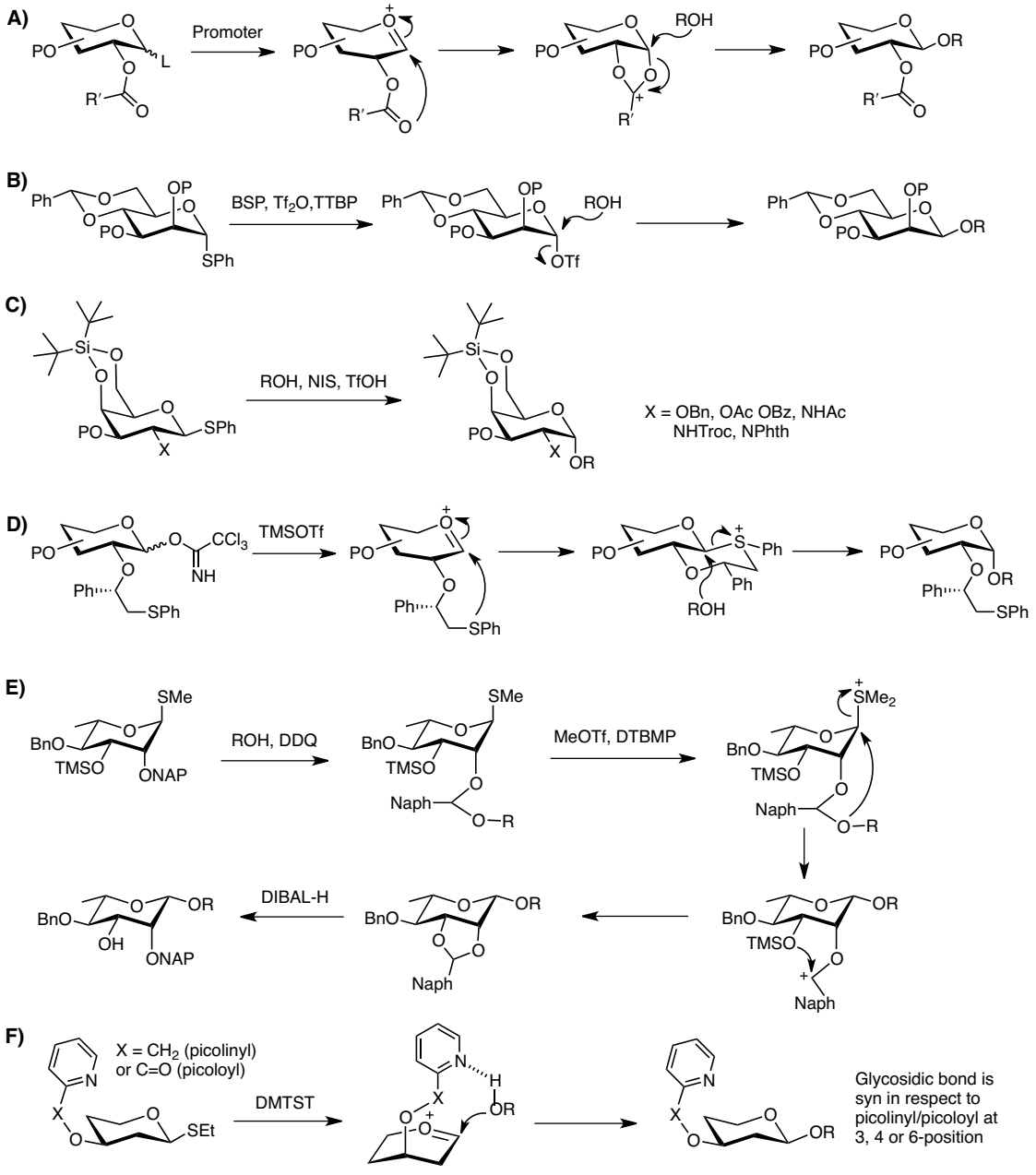


Figure 1.1 Commonly used glycosyl donors and their corresponding promoters in parentheses. AgOTf, silver trifluoromethanesulfonate; BSP, 1-benzenesulfinyl piperidine; DMDO, dimethyldioxirane; NIS, N-iodosuccinimide; $Ph_3PAuNTf_2$, [bis(trifluoromethanesulfonyl)imidate](triphenylphosphine)gold(I); PPh_3, triphenylphosphine; TMSOTf, trimethylsilyl trifluoromethanesulfonate; Tf_2O, trifluoromethanesulfonic anhydride.

The seeming simplicity of the synthetic strategy masks its complexity in terms of anomeric control. Because the glycosidic bond can be formed with either the α or β stereochemistry, particular care has to be taken with regard to the reaction. Glycosylation stereoselectivity can be tuned by the steric and electronic properties of protecting groups, the conformation of the donor and the acceptor, the lability of the anomeric leaving group, as well as the solvent, temperature, and activator.[11-13]

The stereoselective construction of 1,2-*trans*-glycosidic linkages can be achieved by a reliable strategy (Scheme 1.1A), in which an acyloxy group is introduced at the C-2 position of the glycosyl donor to direct the attack of the nucleophile from only one face of the oxacarbenium ion via neighboring group participation.[14] In contrast, the construction of 1,2-*cis*-glycosidic linkages such as α-glucosides, α-galactosides, β-mannosides and β-rhamnosides, still poses synthetic challenges to chemists. Although α-glucosylation and α-galactosylation can benefit from the anomeric effect when nonparticipating groups such as benzyloxy ethers or azides are present at the C-2 position, mixtures of anomers are often obtained despite extensive optimization efforts. The situation is worse for β-mannosides and β-rhamnosides, because glycosidic bond formation not only suffers from the inability to use neighboring group participation at C-2 but also needs to overcome the anomeric effect. To address the problems encountered in the 1,2-*cis*-glycosidic bond construction, several innovative approaches have been devised.[12,13] Some representative examples are described in Scheme 1.1B–F, including 4,6-*O*-benzylidene acetal-directed β-mannoside formation,[15] 4,6-*O*-di-*t*-butylsilyl (DTBS)-controlled α-galactosylation,[16,17] chiral-auxiliary controlled glycosylation,[18] 2-naphthylmethyl ether (NAP)-mediated intramolecular aglycon delivery[19,20] as well as hydrogen bond mediated aglycon delivery[21,22].



Scheme 1.1 A) The stereoselective formation of 1,2-*trans*-glycosidic linkages by C-2 neighboring group participation; B–F) Representative approaches for the stereoselective formation of 1,2-*cis*-glycosidic linkages: B) 4,6-*O*-benzylidene acetal-directed β-mannosylation; C) DTBS-controlled α-galactosylation; D) Chiral-auxiliary controlled glycosylation; E) NAP-mediated intramolecular aglycon delivery; F) Hydrogen bond mediated aglycon delivery. L, leaving group; P, protecting group; Naph, 2-naphthyl; DIBAL-H, diisobutylaluminium hydride; DMTST, dimethyl(methylthio)sulfonium trifluoromethanesulfonate; DTBMP, 2,6-di-*tert*-butyl-4-methylpyridine; DDQ, 2,3-dichloro-5,6-dicyano-1,4-benzoquinone; MeOTf, methyl trifluoromethanesulfonate; TTBP, 2,4,6-tri-*tert*-butylpyrimidine; TfOH, trifluoromethanesulfonic acid.

1.2.2 One-pot synthesis of oligosaccharides

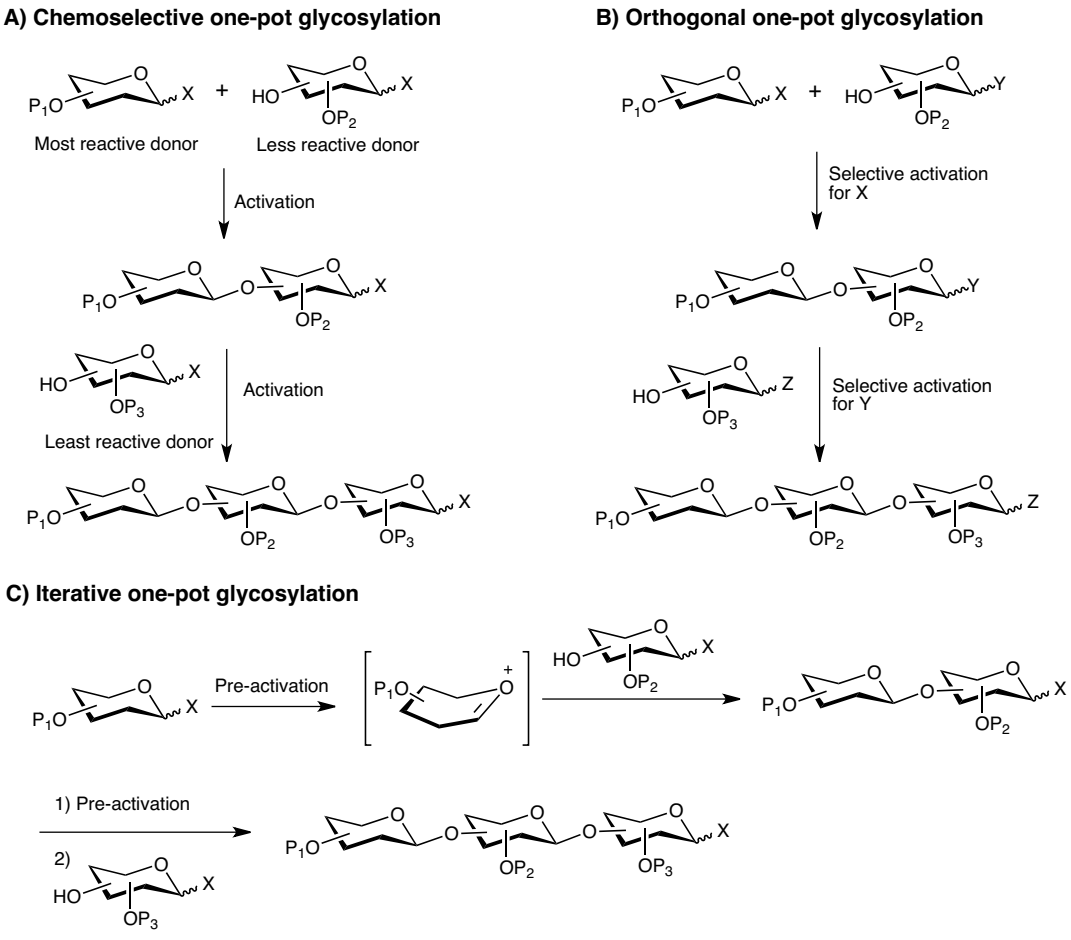
Although the advent of new and efficient glycosylation methodologies enables chemists to access many complex oligosaccharides, conventional stepwise solution phase synthesis is a tedious and time-consuming process requiring multiple protection–deprotection steps and intermediate isolation/purification. To address this major drawback, tremendous efforts have been paid to streamline oligosaccharide assembly. One of the approaches that has been developed is one-pot glycosylation, in which multiple glycosidic bonds are created via sequential glycosylation in a single reaction vessel without the need for multistep workups and purification of intermediates. One-pot glycosylation methods can be classified into three types: 1) chemoselective glycosylation, 2) orthogonal glycosylation and 3) iterative (pre-activation) glycosylation.[23,24]

1.2.2.1 Chemoselective one-pot glycosylation

The concept underlying chemoselective glycosylation is that glycosyl donors can be either armed (reactive) or disarmed (less reactive).[25] The relative reactivity of glycosyl donors carrying the same anomeric leaving group can be modulated by manipulation of the number of electron-donating or electron-withdrawing protecting groups, allowing for the selective activation of an armed glycosyl donor in the presence of a disarmed glycosyl donor. This minimizes the need to worry about activation and self-coupling of the disarmed glycosyl donor. In chemoselective one pot glycosylation (Scheme 1.2A), the most reactive glycosyl donor is selectively activated and and is allowed to couple with a less reactive glycosyl donor, which bears a hydroxyl group. This generates an intermediate that is subsequently activated and coupled with the least reactive donor, resulting in the assembly

of an oligosaccharide from the non-reducing end to the reducing end.

The Wong group has done systematic work to quantity the relative reactivity of thioglycosides and created a database to evaluate the suitability of specific glycosyl donors for the synthesis of a target oligosaccharide.[26,27] The efficiency of this method has been demonstrated by the synthesis of oligosaccharides such as the Globo-H hexasaccharide and heparin-like oligosaccharides.[27] However, an inherent limitation of this method is the requirement of a large number of glycosyl donors differing in relative reactivity, especially for the assembly of complex oligosaccharides containing multiple identical linkages.



Scheme 1.2 One-pot multistep glycosylation strategies.

1.2.2.2 Orthogonal one-pot glycosylation

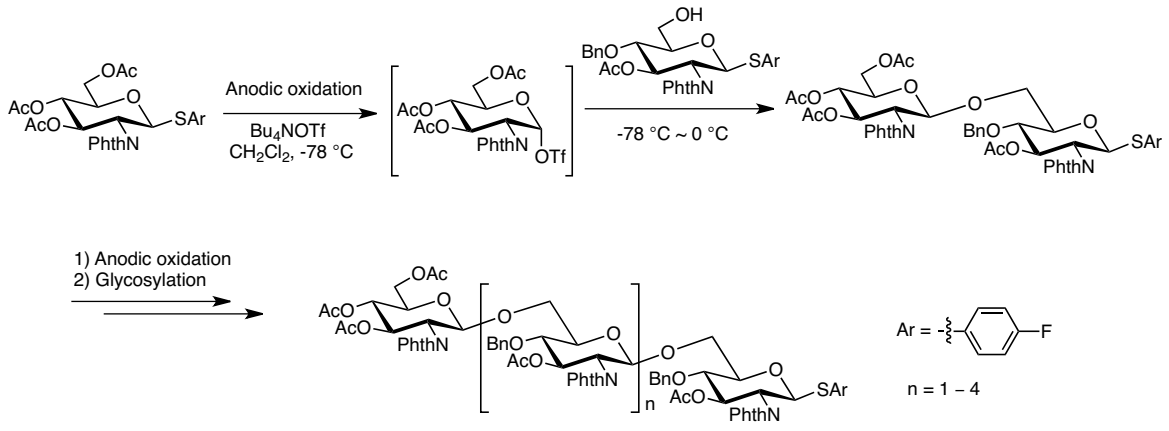
The orthogonal one-pot strategy uses glycosyl donors equipped with different anomeric leaving groups (*e.g.,* glycosyl fluorides and thioglycosides), in which one donor can be selectively activated in the presence of the other by using selective promoters (Scheme 1.2B). The building blocks are assembled sequentially by a series of selective activations and glycosylations.[28,29] In comparison with the chemoselective one-pot strategy, sequential glycosylation depends on the promoters instead of the relative reactivity of the building blocks.

1.2.2.3 Iterative one-pot glycosylation

The iterative glycosylation strategy exploits the assembly of glycosylating agents bearing the same anomeric leaving group (Scheme 1.2C). It starts with pre-activation of a glycosyl donor in the absence of glycosyl acceptor to generate a reactive intermediate, followed by the addition of an acceptor to yield a product that can be pre-activated and treated with another acceptor for further elongation of the oligosaccharide.[30] Compared with chemoselective and orthogonal one-pot strategies, the pre-activation strategy is the most convenient and straightforward approach to rapidly construct complex oligosaccharides. It does not require extensive protecting group manipulations to tune the relative reactivity of the building blocks in an armed–disarmed fashion nor does it require the installation of different anomeric leaving groups. This dramatically simplifies the design and synthesis of building blocks.

To date, the iterative one-pot strategy has mainly used BSP/Tf_2O as the promoters to activate thioglycoside donors to form glycosyl triflate intermediates. These species in

turn react with thioglycoside-based acceptors added subsequently to the reaction mixture leading to a new glycosidic bond.[24] Recently, Nokami and coworkers developed a new iterative one-pot method based on electrochemical oxidation (Scheme 1.3).[31,32] The formation of a glycosyl triflate intermediate results from anodic oxidation of thioglycosides in the presence of tetra-*n*-butylammonium triflate (*n*-Bu_4NOTf) rather than activation by the electrophilic chemical promoters mentioned above. An automated electrochemical synthesizer has also been developed and used to facilitate the synthesis of oligosaccharides via this electrochemical method.



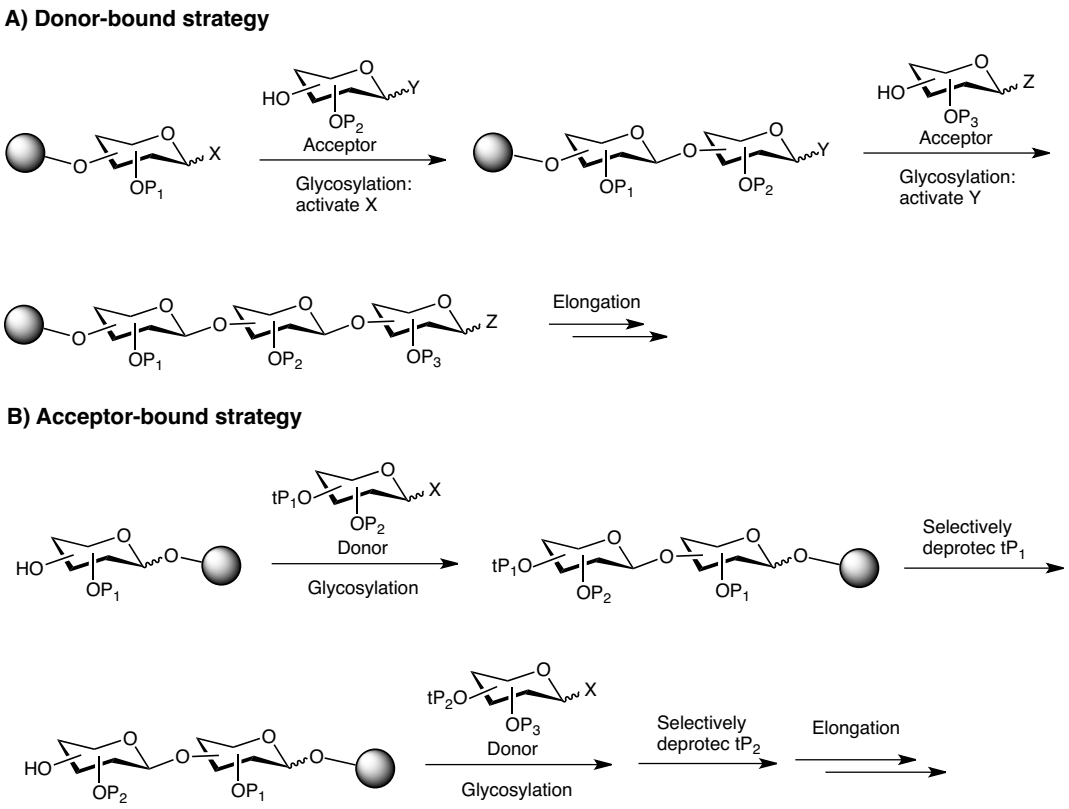
Scheme 1.3 Iterative electrochemical assembly of thioglycosides by Nokami and coworkers.[31]

1.2.3 Solid phase synthesis of oligosaccharide

Besides one-pot strategies, the past few decades have witnessed the development of solid phase synthesis to automate the assembly of oligosaccharides. Inspired by the success of solid-phase peptide synthesis, the concept of solid-phase oligosaccharide synthesis was initially proposed by Fréchet and Schuerch in the 1970s.[33] However, the lack of efficient glycosylation methods on solid support impeded progress in this area for 20 years.

Recently, advances in synthetic carbohydrate chemistry rejuvenated chemists' enthusiasm for exploring the feasibility of solid-phase oligosaccharide synthesis. This work has included fundamental investigations regarding solid support resins, linkers, glycosylating agents and synthetic strategies, as well as the construction of an automated synthesizer. [34-37]

In principle, either the glycosyl donor or the glycosyl acceptor can be attached to the solid support to initiate the synthesis.[38] In the donor-bound strategy, an anchored glycosyl donor is exposed to a solution-phase acceptor bearing an orthogonal anomeric leaving group, which facilitates chain elongation (Scheme 1.4A). The major drawback of this approach is that side reactions of the donor (hydrolysis or aglycon transfer) result in the termination of chain elongation and reduction in overall yield. This makes this strategy less attractive. In the acceptor-bound strategy, the anchored nucleophilic acceptor is treated with an excess of glycosyl donor together with promoter in solution to create a new glycosidic linkage. After washing away unreacted donor, side product and reagent, the anchored intermediate undergoes selective deprotection to unveil a hydroxyl group for a subsequent glycosylation cycle (Scheme 1.4B). In contrast to the donor-bound strategy, the acceptor-bound strategy is more advantageous, because each glycosylation reaction can be driven to completion by using an excess of the glycosyl donor, which is in solution as are its degradation by-products. Therefore, most solid phase oligosaccharide syntheses are based on the acceptor-bound strategy.



Scheme 1.4 Solid-phase oligosaccharide synthesis strategies.

A significant breakthrough in solid-phase oligosaccharide synthesis was made in 2001 by Seeberger, who introduced the first automated oligosaccharide synthesizer and demonstrated the construction of several oligosaccharides.[39] Over the past decade, the efficiency and versatility of the synthesizer has been significantly improved, resulting in the first commercial, fully automated oligosaccharide synthesizer.[35] The synthesizer mainly uses three types of glycosylating agents, thioglycosides, glycosyl phosphate and glycosyl acetimidates. Similar to peptide synthesis, 9-fluorenylmethoxycarbonate (Fmoc) was chosen as a temporary protecting group to mask the hydroxyl group that serves as nucleophile for subsequent glycosylation cycle. Levulinoyl (Lev) esters, silyl ethers and NAP groups can be used as orthogonal protecting groups to allow the assembly of branched

oligosaccharides. To date, the effectiveness of the synthesizer is evidenced by an impressive number of diverse and complex oligosaccharides that have been constructed.[37] Some representative examples are shown in Figure 1.2, and include a β-(1→4)-linked mannuronic acid oligomer (**1.1**),[40] a dodecasaccharide of β-(1→6)-linked *N*-acetylglucosamine (GlcNAc) residues (**1.2**),[35] fragments of plant arabinoxylan (**1.3**)[41] and arabinogalactan (**1.4**),[42] α-(1→6)-linked mannan chains up to a 30-mer (**1.5**)[43] as well as protected chondroitin sulfate glycosaminoglycans (**1.6**)[44]. These examples illustrate that not only *trans*-glycosidic linkages can be introduced on solid phase, but also some challenging *cis*-glycosidic linkages can be installed with excellent stereoselectivity.

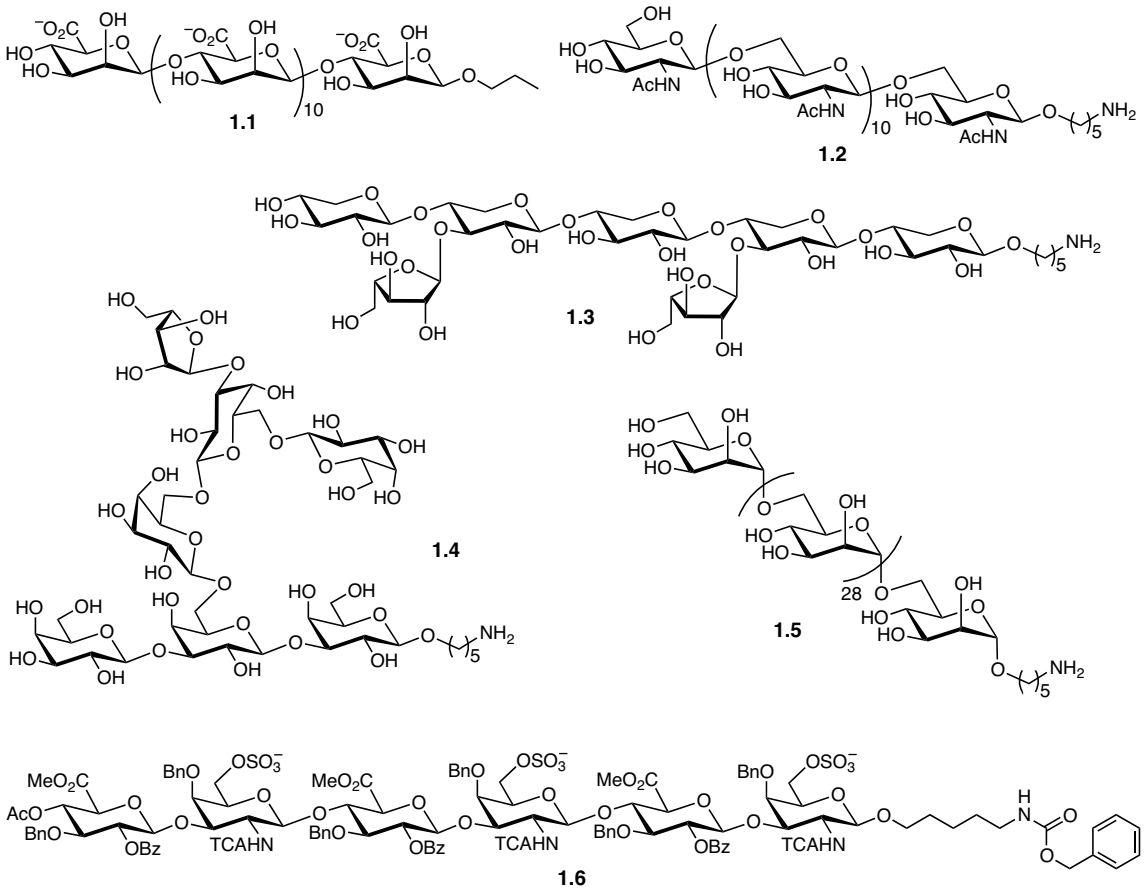


Figure 1.2 Examples of oligosaccharide synthesized by automated solid-phase synthesizer.

Compared with solution phase synthesis, solid-phase synthesis offers a considerably faster approach to access to complex oligosaccharides. The bottleneck of solid-phase oligosaccharide synthesis is access to large quantities of diverse building blocks that can be assembled on the synthesizer. Furthermore, highly stereoselective solid-phase glycosylation methods for the construction of challenging glycosidic linkages need to be developed. Therefore, there is still a long way to go before this methodology can achieve the level of generality and accessibilty associated with solid-phase peptide synthesis.

1.3 Enzymatic (chemo-enzymatic) synthesis of oligosaccharides

In chemical synthesis, protecting groups play an essential role in directing the regioselectivity and stereoselectivity of glycosylation reactions. Despite ongoing progress, absolute stereocontrol of glycosidic bond formation remains the key challenge in oligosaccharide synthesis. In contrast, nature addresses the challenge by using enzymes to catalyze the assembly of glycans with exquisite regio- and stereoselectivity under mild conditions without protecting groups. Inspired by nature, enzymatic and chemo-enzymatic approaches have been developed, and are gaining popularity recently due to the availability of a large number of well-identified and characterized carbohydrate-processing enzymes, including glycosyltransferases and glycosidases.

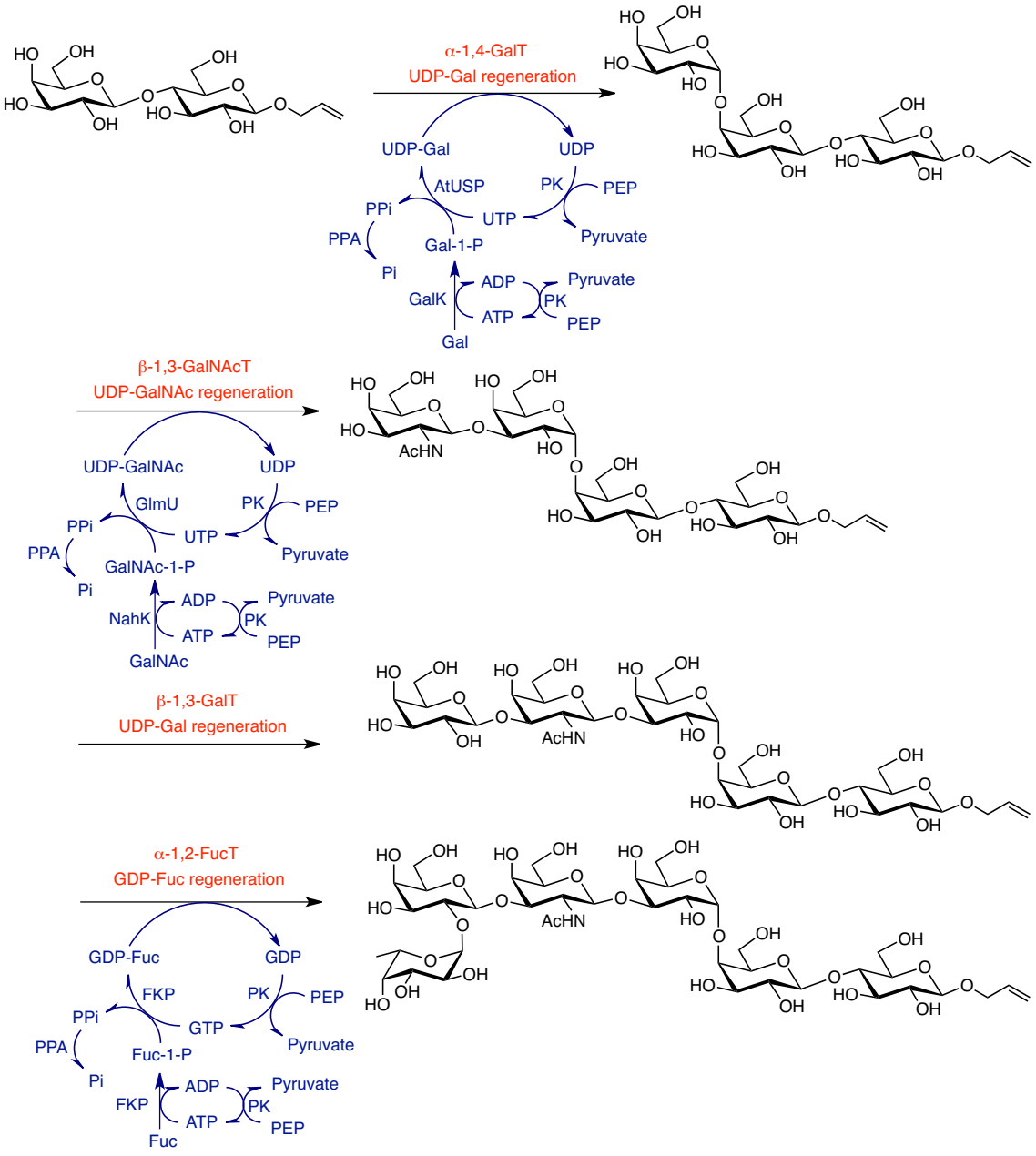
1.3.1 Synthesis of oligosaccharides using glycosyltransferases

Glycosyltransferases, an essential class of enzymes involved in glycan biosynthesis, catalyze the transfer of a sugar residue from the corresponding sugar nucleotide or sugar lipid phosphate onto an acceptor molecule.[45] Bacterial glycosyltransferases have been

proven to be suitable candidates for synthetic use, as they are generally more promiscuous and can be obtained more readily by cloning and overexpression in *Escherichia coli* than their mammalian counterparts.[46] In the past decade, the concerns associated with enzymatic synthesis–limited enzyme availability, high cost of sugar nucleotides and feedback inhibition caused by accumulation of nucleoside phosphate byproduct during the course of reaction–have been relieved to some extent.[27] As a result, glycosyltransferases have drawn unprecedented attention in the synthesis of complex oligosaccharides.[47]

1.3.1.1 One-pot multienzyme approach to synthesis oligosaccharides

To date, a growing number of bacterial glycosyltransferases with broad substrate specificity have become available from either commercial sources or academic labs.[48] More importantly, one-pot multienzyme approaches, which combine glycosyltransferases with enzymes that allow in situ sugar nucleotide regeneration from inexpensive precursors, have been established for the effective construction of glycosidic linkages. These approaches not only alleviate concerns regarding the high cost of sugar nucleotides, but also overcome feedback inhibition.[27] The efficiency of this approach is illustrated by a recent study regarding large-scale enzymatic synthesis of the tumor-associated antigen Globo H carried out by Wong and coworkers (Scheme 1.5).[49]



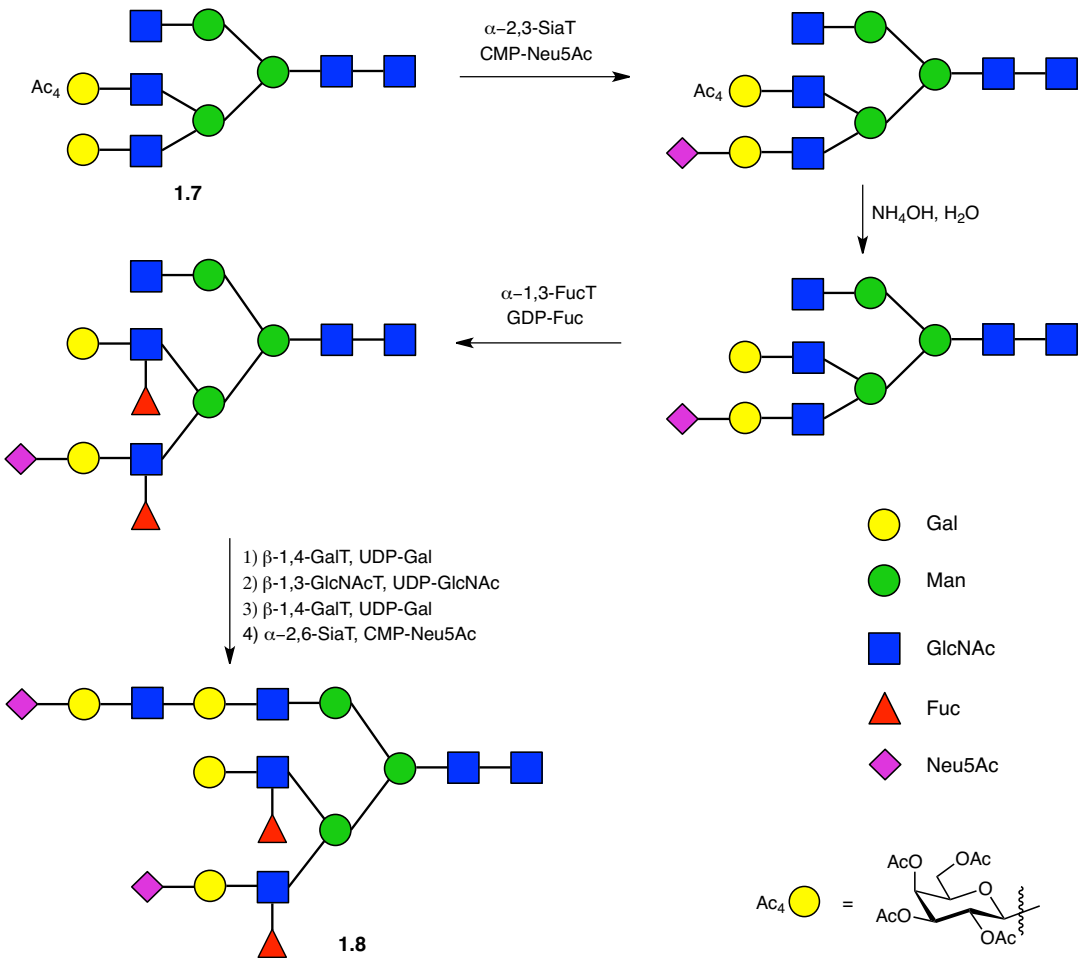
Scheme 1.5 Large-scale enzymatic synthesis of Globo H by Wong and coworkers.[49] Gal, galactose; GalT, galactosyltransferase; Fuc, fucose; FucT, fucosyltransferase; GalNAc, *N*-acetylgalactosamine; GalNAcT, *N*-acetylgalactosaminyltransferase; GalK, galactokinase; AtUSP, UDP-Gal pyrophosphorylase; NahK, GalNAc kinase; FKP, bifunctional enzyme with L-fucose-1-phosphate guanyltransferase and L-fucose kinases activities; GlmU, UDP-GalNAc pyrophosphorylase; UDP, uridine diphosphate; GDP, guanosine diphosphate; PK, pyruvate kinase; PEP, phosphoenolpyruvate; UTP, uridine triphosphate; GTP, guanosine triphosphate; ATP, adenosine triphosphate; ADP, adenosine diphosphate; Pi, phosphate; PPi, pyrophosphate; PPA, pyrophosphatase; 1-P, 1-phosphate.

The synthesis relied on a sequential transfer of four sugar units (Gal, GalNAc, Gal and Fuc) to the lactose substrate by using four glycosyltransferases (α-(1→4)-GalT, β-(1→3)-GalNAcT, β-(1→3)-GalT and α-(1→2)-FucT, respectively), in combination with effective UDP-Gal, UDP-GalNAc and GDP-Fuc regeneration from corresponding monosaccharide catalyzed by kinases and sugar nucleotide pyrophosphorylases. The reactions proceeded in aqueous buffer solutions in high yields with complete regio- and stereoselectivity, circumventing the need for numerous protecting group manipulations. The enzymatic reaction cycles also featured the regeneration of UTP/GTP and ATP from byproducts UDP/GDP and ADP, respectively, through reaction with phosphoenolpyruvate catalyzed by pyruvate kinase. Notably, Globo H can be obtained in gram quantities with relatively low cost in just four steps from a lactose substrate.

1.3.1.2 Chemo-enzymatic synthesis of complex oligosaccharides

Chemo-enzymatic approaches integrate the flexibility of chemical synthesis with the specificity of enzymatic transformations to provide a powerful alternative to access to various complex oligosaccharides. An elegant recent example was reported by Boons, Paulson and coworkers, who devised a chemo-enzymatic approach to achieve a library of asymmetrically-substituted tri-antennary *N*-linked glycans.[50] The strategy consists of the chemical synthesis of tri-antennary decasaccharide precursor **1.7** and selective extension of each branch with a series of glycosyltransferases to form asymmetric structures (*e.g.,* **1.8** in Scheme 1.6). In this process, chemical modification (acetylation) plays an important role in differentiating the three branches, making selective enzymatic extension possible. A broader library of asymmetric glycans can be readily generated using this approach, while it

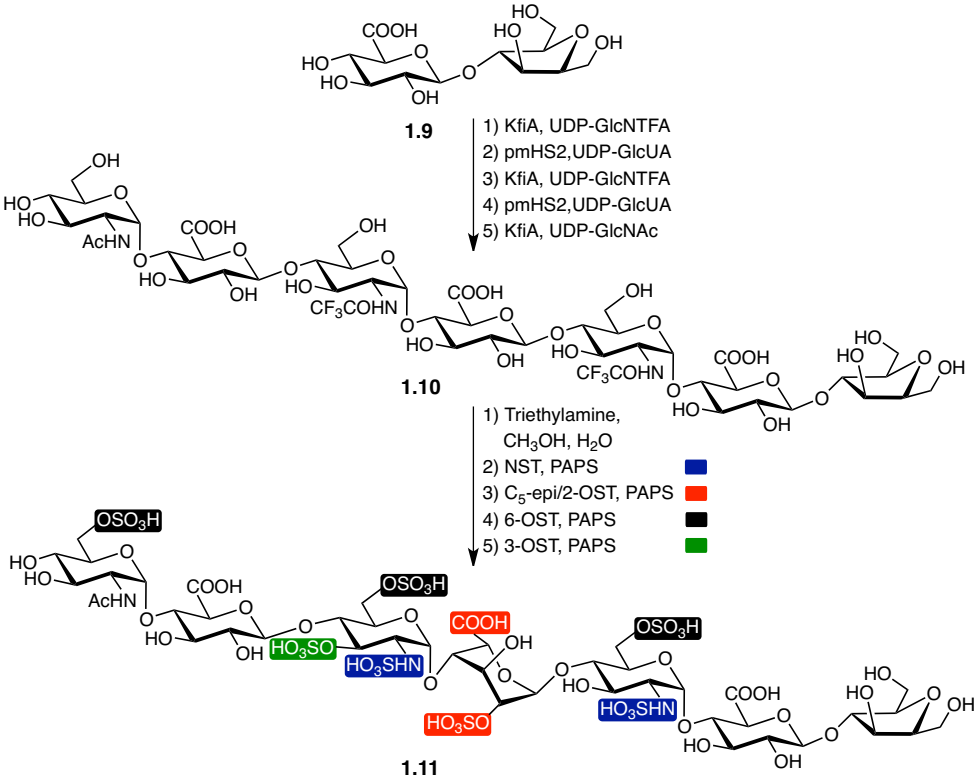
is a formidable task for traditional chemical synthesis.



Scheme 1.6 Chemo-enzymatic route to asymmetrically substituted tri-antennary glycans by Boons and coworkers.[50] SiaT, sialyltransferase; CMP-Neu5Ac, cytidine monophosphate-*N*-acetylneuraminic acid; GlcNAcT, *N*-acetylglucosaminyltransferase; UDP-GlcNAc, uridine diphosphate *N*-acetylglucosamine; Man, mannose.

The power of chemo-enzymatic strategies is also highlighted by the synthesis of homogeneous heparin oligosaccharides developed by Liu and coworkers.[51] Scheme 1.7 illustrates the process of backbone elongation and subsequent modification to synthesize a ultralow molecular weight (ULMW) heparin construct.[52] Elongation of the disaccharide starting material **1.9** was performed by alternate use of two bacterial glycosyltransferases,

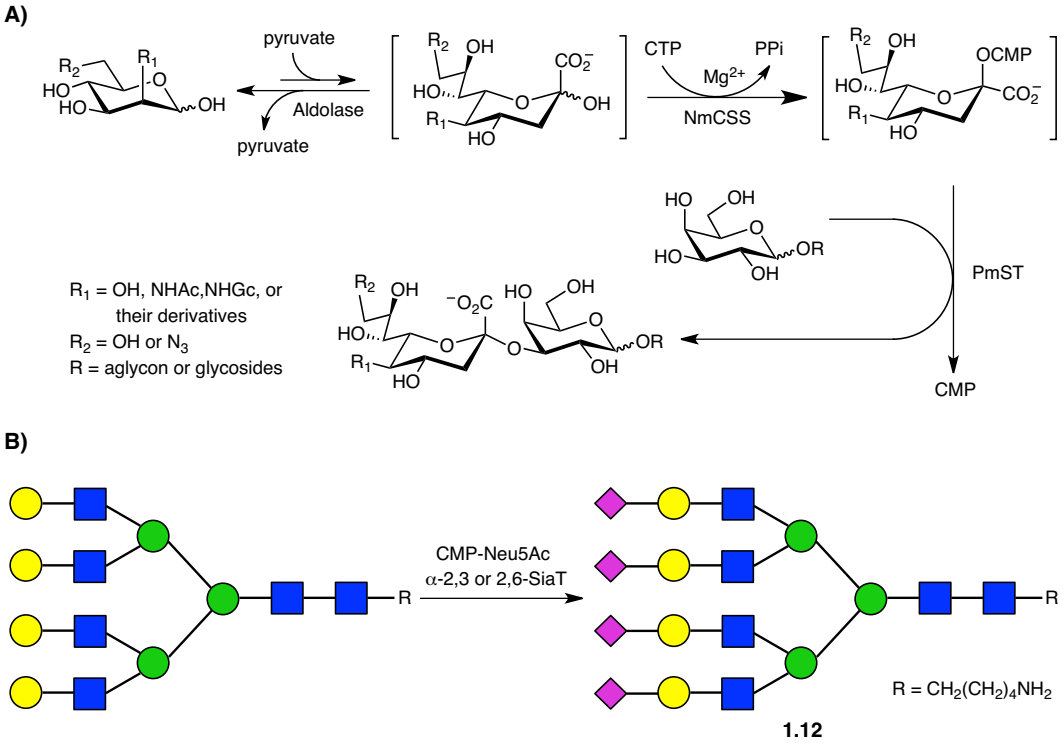
E. coli K5 GlcNAcT (KfiA) and heparosan synthase-2 (pmHS2) from *Pasteurella multocida*, resulting in the formation of heptasaccharide **1.10** containing repeating disaccharide units of *N*-acetyl or *N*-trifluoroacetyl glucosamine and glucuronic acid. This compound was converted to the desired product **1.11** after a series of chemo-enzymatic modifications, including *N*-sulfation, followed by epimerization/2-*O*-sulfation, 6-*O*-sulfation and 3-*O*-sulfation. Careful design of target structures and optimized sequences of sulfo group installation was key to implementing this approach. Taking advantage of a chemo-enzymatic approach, low molecular weight heparins up to dodecasaccharides have been successfully synthesized.[53]



Scheme 1.7 Chemo-enzymatic synthesis of ULMW heparin construct **1.11** by Liu and coworkers.[52] NST, N-sulfotransferase; PAPS, 3′-phosphoadenosine-5′-phosphosulfate; C_5-epi, C_5-epimerase; OST, O-sulfotransferase.

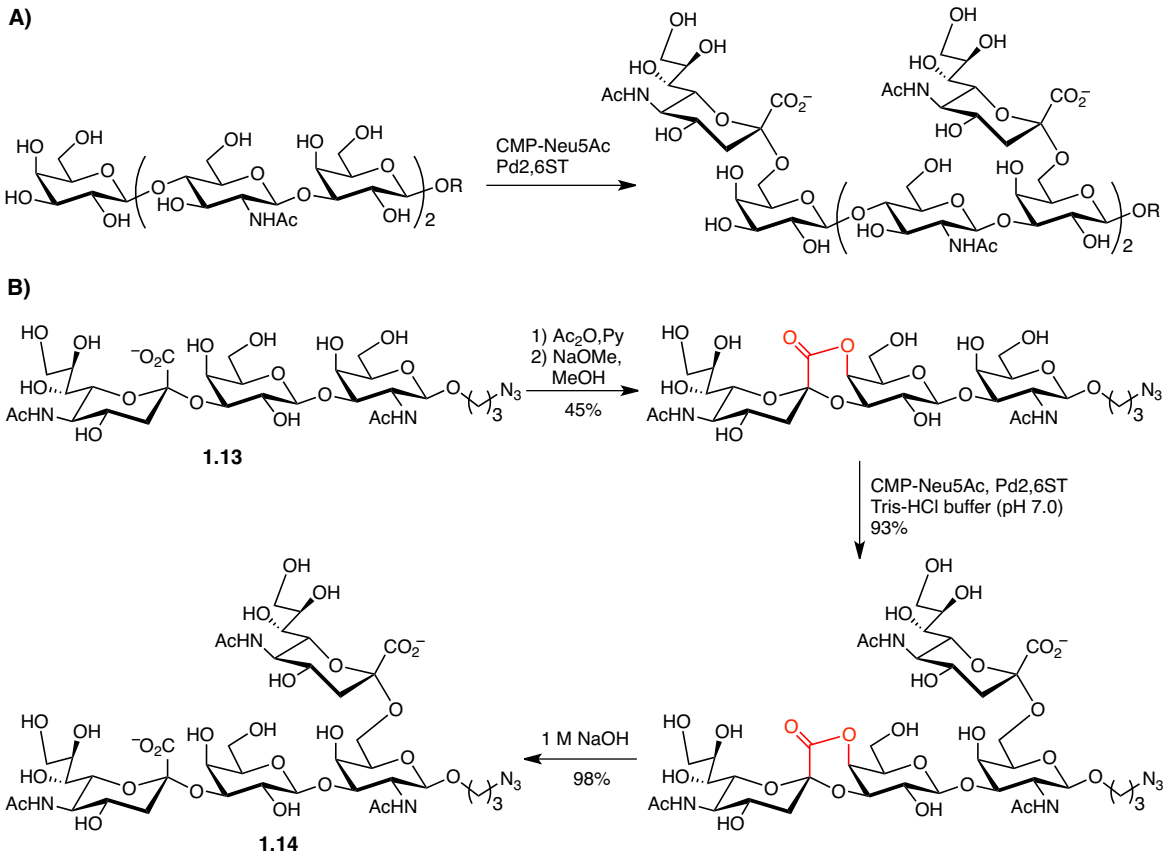
1.3.1.3 Enzymatic and chemo-enzymatic synthesis of sialosides

Among all the glycosyltransferases, sialyltransferases (SiaTs) have drawn special attention, because chemical α-sialylation still poses a formidable challenge owing to the hindered anomeric center and the lack of participating group at C-3 to direct the stereoselectivity of glycosylation. SiaTs have been used as reliable tools to construct sialosides containing α-(2→3), α-(2→6) and α-(2→8) linkages. In addition, the promiscuity of SiaTs allows for versatile structural modification at the C-3, C-5, C-7, C-8, and/or C-9 position of sialic acid.[54] In a pioneering study, Chen and coworkers devised a one-pot three-enzyme system for the efficient synthesis of naturally occurring or structurally modified sialosides.[55,56] In this system, natural or modified CMP-sialic acid was generated in *situ* from the corresponding *N*-acetyl mannosamine or mannose derivatives together with pyruvate using a recombinant *E. coli* K12 sialic acid aldolase and a recombinant *N. meningitides* CMP-Neu5Ac synthetase (NmCSS). Once formed, the modified sialic acid derivative was then transferred to a suitable galactose acceptor by a multifuntional *Paseurella multocida* α-(2→3)-SiaT (PmST) (Scheme 1.8A). Later, the wild type PmST was engineered to yield a mutant (PmST M144D),[57] which can catalyze the sialylation more efficiently especially when a poor acceptor is used. More recently, Wong and coworkers reported the rapid production of sialylated (α-(2→3) or α-(2→6)) bi-, tri- and tetra-antennary *N*-glycans through chemical synthesis of complex *N*-glycans, followed by SiaT-mediated sialylation (*e.g.,* **1.12** in Scheme 1.8B).[58] In another study, Seeberger and coworkers combined solid phase synthesis with enzymatic synthesis to produce α-(2→3)-sialylated glycans.[59]



Scheme 1.8 A) One-pot three-enzyme system for the synthesis of sialosides by Chen and coworkers;[55] B) Enzymatic sialylation of synthetic tetra-antennary N-glycans by Wong and coworkers.[58]

In addition to terminally sialylated glycans, SiaT-mediated sialylation can also provide access to internally sialylated glycans. A recent investigation carried out by Paulson and coworkers uncovered that a recombinant *Photobacterium damselea* α-(2→6)-SiaT (Pd2,6ST) can sialylate both terminal and internal galactose residues in poly-LacNAc extended glycans to form multiple Neu5Ac-α-(2→6)-Gal epitopes (Scheme 1.9A). Taking advantage of this unique regioselectivity, a library of *N*- and *O*-linked glycans with multiple α-(2→6)-sialylated poly-LacNAc motifs was generated and incorporated into a glycan microarray to probe their biological functions.[60]



Scheme 1.9 A) Pd2,6ST-catalyzed multisialylation of glycans with poly-LacNAc extension by Paulson and coworkers;[60] B) Cao and coworker's chemo-enzymatic synthesis of a ganglioside disialyl tetrasaccharide epitope via a regioselective sialylation.[61] A key feature is the formation of a lactone moiety, which prevents sialylation of the Gal residue. Tris, tris(hydroxymethyl)aminomethane.

Shortly after Paulson's discovery, Cao and coworkers described a chemo-enzymatic approach to access ganglioside disialyl tetrasaccharide epitopes (Scheme 1.9B).[61] The synthesis started with α-(2→3)-SiaT-catalyzed sialyation of a β-Gal-(1→3)-GalNAc substrate to form trisaccharide **1.13**. Subsequent chemical modification and Pd2,6ST-catalyzed regioselective sialylation of the internal GalNAc residue to generated the desired tetrasaccharide **1.14**. Key to implementing this approach was the formation of a lactone in the trisaccharide that prevented α-(2→6)-sialylation of the Gal residue, resulting in

regioselective sialylation of GalNAc residue.

1.3.2 Synthesis of oligosaccharides using glycosidases and glycosynthases

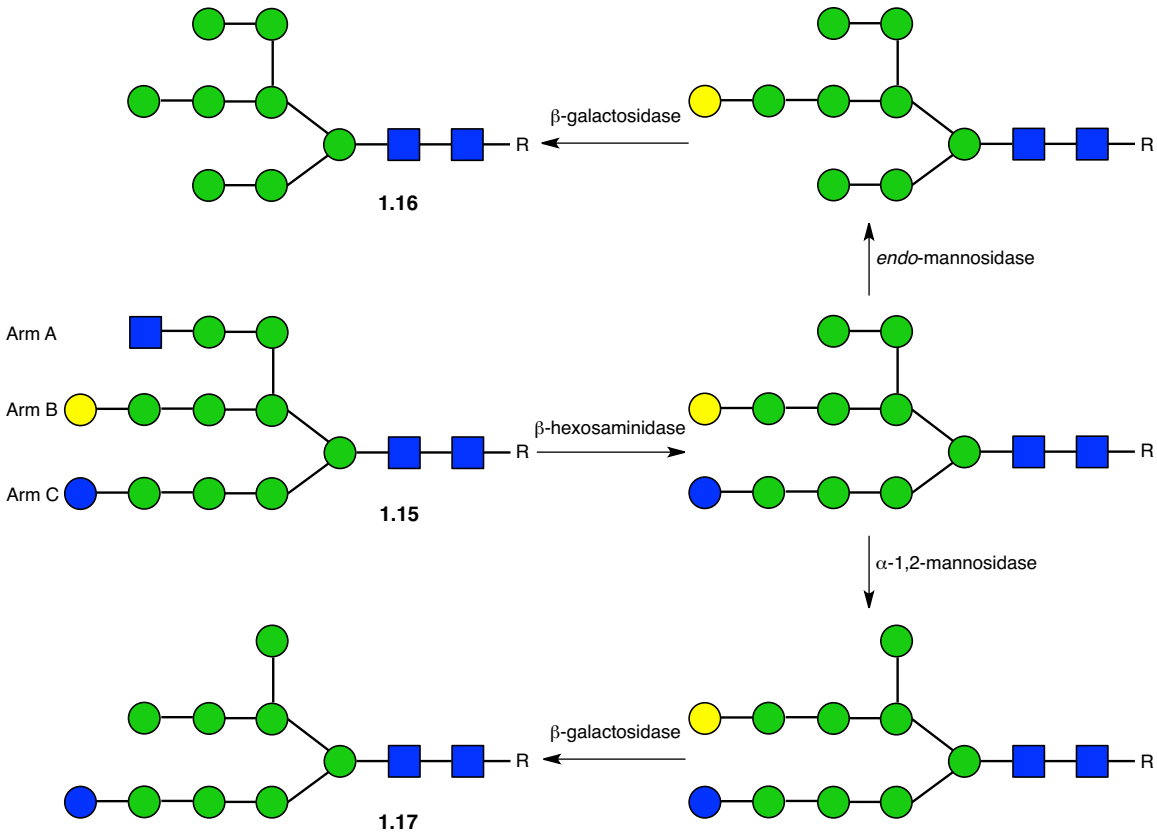
Glycosidases are a class of enzymes that catalyze the hydrolytic cleavage of glycosidic bonds. They can be classified as exo-glycosidases or endo-glycosidases, depending upon whether they cleave the nonreducing terminal monosaccharide or hydrolyze internal glycosidic bonds to cleave more than one monosaccharide, respectively. In general, glycosidases are wildly available, highly stable and soluble, and more promiscuous than glycosyltransferases. Therefore, they are considered as attractive biocatalysts in complex oligosaccharide synthesis.[48]

1.3.2.1 Top-down chemo-enzymatic approach

In vivo, glycosidases are responsible for glycan cleavage (trimming) and remodeling. Drawing inspiration from this, Ito and coworkers devised a top-down chemo-enzymatic approach to access a library of high-mannose-type glycans (Scheme 1.10).[62] The strategy relied upon the chemical synthesis of non-natural tetradecasaccharide **1.15**, in which three branches, A, B and C were capped by different sugar residues, β-GlcNAc, β-Gal, and α-glucose (α-Glc), respectively. Subsequent digestions with various defined sets of glycosidases in an orthogonal manner enabled the formation of a range of high-mannose-type *N*-linked glycans. For example, precursor **1.15** underwent a sequential treatment with β-*N*-acetylhexosaminidase, endo-*α*-mannosidase and β-galactosidase to selectively cleave the GlcNAc in Arm A, the Glc-Man disaccharide in Arm C and the Gal in Arm B, respectively, to afford decasaccharide **1.16**. In this process, if an α-(1→2)-mannosidase was

used to replace the endo-α-mannosidase, decasaccharide **1.17** was achieved instead.

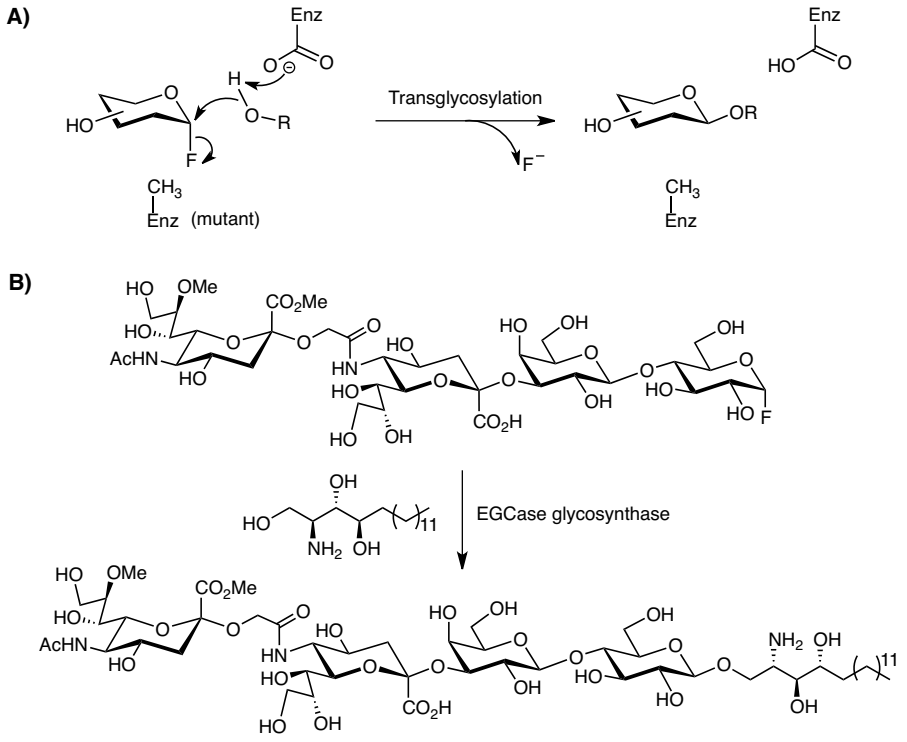
A problem encountered in this approach is that that terminal Gal residue in Arm B turned out to be quite robust against enzymatic hydrolysis and a large amount of β-galactosidase was required. To tackle this issue, a renewed approach was developed.[63] Instead of using Gal, an isopropylidene group was installed on the terminal Man residue to mask Arm B, which can be readily removed by treatment with 10% aqueous acetic acid solution without cleaving glycosidic linkages. The power of this top-down chemo-enzymatic approach was illustrated by the generation of thirty-seven high-mannose-type glycans for elucidating the functions of glycans involved in glycoprotein quality control.



Scheme 1.10 Top down chemo-enzymatic synthesis of high mannose-type glycans by Ito and coworkers.[62]

1.3.2.2 Glycosynthase-catalyzed transglycosylation

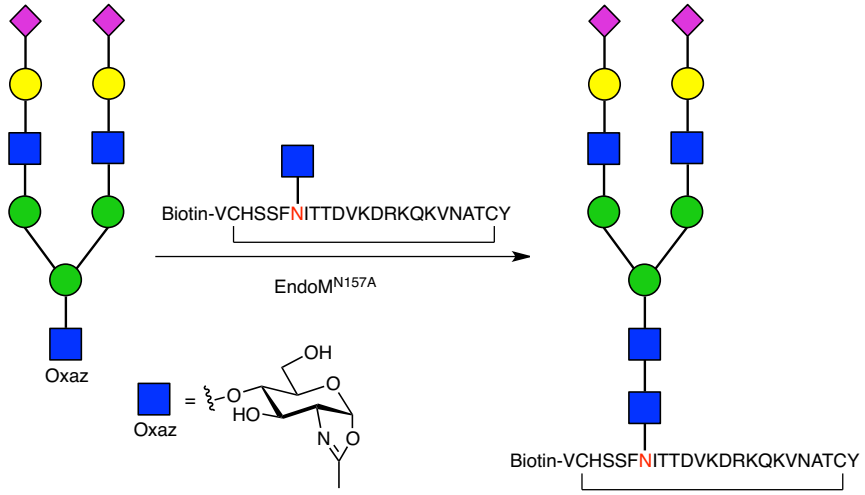
Glycosidase-catalyzed hydrolytic cleavage of glycosidic bonds is a reversible process. In principle, it is possible to use the reverse hydrolytic activity of glycosidases for glycosidic bond formation. Although the use of activated glycosyl donors, such as *p*-nitrophenyl glycosides and glycosyl fluorides, help to drive the equilibrium toward the glycosylation direction, glycosidase-catalyzed transglycosylations generally feature low yields and suffer from product hydrolysis. To overcome these problems, Withers and coworkers introduced 'glycosynthases', engineered glycosidases where nucleophilic catalytic site aspartate or glutamate residues are replaced by small hydrophobic residues, such as alanine or glycine, to knock out the hydrolysis activity (Scheme 1.11A).[64] Glycosynthases can catalyze transglycosylations using activated glycosyl donors (*e.g.*, glycosyl fluorides) and proceed in high yield without significant hydrolysis of the product.[65] To date, the utility of glycosynthases has been exemplified by the efficient construction of a number of oligosaccharides, for example, β-mannosides,[66] cyclic β-glucans,[67] isoprimeverosides[68] and a chitin tetrasaccharide.[69] In a recent investigation, Withers and coworkers reported the glycosynthase-mediated synthesis of the neurogenic starfish ganglioside LLG-3.[70] An endoglycoceramidase II (EGCase) mutant E351S/D314Y discovered by directed evolution was used to catalyze the coupling of a tetrasaccharide fluoride donor with a lipid (Scheme 1.11B), giving the product.



Scheme 1.11 A) The mechanism of a glycosynthase-catalyzed transglycosylation; B) Key step in the synthesis of neurogenic starfish ganglioside LLG-3 by Withers and coworkers.[70]

Another significant achievement in the field is the invention of glycosynthases derived from endo-β-*N*-acetylglucosaminidases (ENGases) for *N*-linked glycan remodeling and synthesis. ENGases, an essential class of endo-glycosidases, catalyze not only the cleavage of the β-GlcNAc-(1→4)-β-GlcNAc linkage in *N*-linked glycans, but also transglycosylation to reconstruct this linkage. In 2008, Yamamoto, Wang and coworkers reported the first glycosynthase (N175A mutant) generated by site-directed mutagenesis of Endo-M, which was devoid of hydrolytic activity but which was able to take synthetic sugar oxazolines as glycosyl donors for transglycosylation.[71] Notably, this glycosynthase has been used as a reliable tool to synthesize homogenous glycopeptides and glycoproteins,[72] including HIV-1 glycopeptide antigens reported recently by Wang and

coworkers.[73] The key step in synthesis of the glycopeptides employed the aforementioned N175A mutant glycosynthase to transfer a synthetic decasaccharide in the form of activated glycan oxazaline to a β-GlcNAc moiety in a cyclic peptide (Scheme 1.12).



Scheme 1.12 Chemo-enzymatic synthesis of glycopeptides carrying a defined N-glycan at the Asn[160] site using the N175A mutant glycosynthase by Wang and coworkers.[73] Ligation site are shown in red, amino acids are represented by their single-leter codes.

1.4 Applications of synthetic oligosaccharides

The recent developments in carbohydrate synthesis including new glycosylation methods, one-pot strategies, solid phase synthesis, and enzymatic and chemo-enzymatic approaches have facilitated access to an increasing number of structurally well-defined oligosaccharides. Such molecules are invaluable probes to gain insight into many glycan-mediated biological and pathological processes. Furthermore, better understanding the biological functions of glycans leads to development, for example, of carbohydrate-based diagnostics and vaccines.[74] In this section, a few applications of synthetic glycans are discussed.

1.4.1 Synthetic glycans to investigate the binding specificity of glycan-binding protein

Glycans mediate a range of biological events through their interaction with glycan-binding proteins (GBPs). Studying the binding specificity of GBPs is crucial to unravel their functions. A technical breakthrough that has accelerated the screening of GBPs was the invention of printed glycan microarrays (Figure 1.3A). In this technology, glycans are immobilized onto activated solid surfaces in a multivalent fashion and then treated with the sample of interest such as an isolated or recombinant GBP. Assessment of the binding specificity is achieved using fluorescence or other detection methods.[75] Glycan microarrays represent an attractive platform because they require a small amount of glycans and offer the benefit of simultaneous comparison of many glycan–protein interactions. Synthetic oligosaccharides are a major source of glycans for glycan microarrays and have proven to be useful for identifying ligand specificities of lectins, antibodies and other GBPs.[76,77]

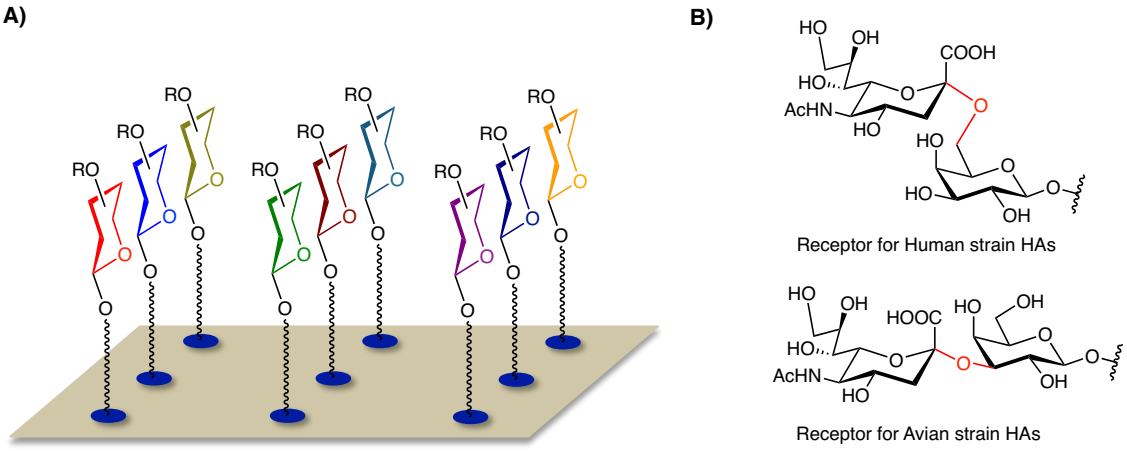


Figure 1.3 A) Example of a microarray with various glycans immobilized on the surface; B) Sialoside receptors for human- and avian-adapted influenza virus hemagglutinins.

One of the targets that has been explored is influenza virus hemagglutinins (HA), a major GBP on the surface of influenza viruses. HA initiates virus infection through

interactions with sialoside receptors on host cells. The study of glycan binding preferences of HAs from different strains using glycan microarrays confirmed the hypothesis that human-adapted strains preferably bind to α-Neu5Ac-(2→6)-Gal moieties and avian-adapted strains prefer α-Neu5Ac-(2→3)-Gal moieties (Figure 1.3B).[78] Further investigation using glycan microarrays composed of a library of synthetic linear or bi-antennary sialosides differing in length revealed the influence of poly-LacNAc chains on HA specificity.[79,80] It was also found that a set of synthetic sialosides could be used to differentiate influenza virus subtypes by comparing HA binding profiles.[79] In another recent study, Sasisekharan and coworkers employed glycan microarrays to determine the mutations of HA required for avian influenza H5N1 to switch receptor specificity to humans.[81] Therefore, it was envisioned that microarrays of synthetic glycans could provide a reliable tool to identify influenza virus strains in the early stage of epidemics and also to monitor changes in receptor binding profiles of virus mutants.

1.4.2 Synthetic carbohydrate-based diagnostics

Anti-carbohydrate antibodies are considered to be biomarkers to diagnose various infections. Due to their well-defined structures, synthetic glycans represent promising candidates for detection of glycan specific antibodies present in patient serum.[82-84] The advantage of synthetic glycans over natural glycans in disease diagnosis is highlighted by a recent investigation that explored the diagnosis of brucellosis. This bacterial disease can be transmitted from animals to humans and poses a serious threat to both animal and human health.[85] The *Brucella* cell-wall polysaccharide is a potent antigen, which produces a robust antibody response. This glycan contains two antigenic motifs, the A-antigen and M-antigen

(Figure 1.4A). Current brucellosis diagnostic tests employing isolated natural antigens to detect antibodies often give rise to false-positive results, because the A-antigen is also found in other bacteria. To address this problem, Bundle, McGiven and coworkers synthesized six *Brucella* oligosaccharide antigens and attached them to proteins to form the corresponding glycoconjugates.[86] These chemically defined glycoconjugate antigens allow anti-A and anti-M antibodies to be detected independently. Surprisingly, a simple disaccharide M-antigen epitope glycoconjugate (Figure 1.4B) was able to detect unique anti-M antibodies in the sera of animals and humans infected with brucellosis, providing an unambiguous diagnostic tool to distinguish brucellosis from infections caused by other bacteria carrying the same A-antigen epitope.

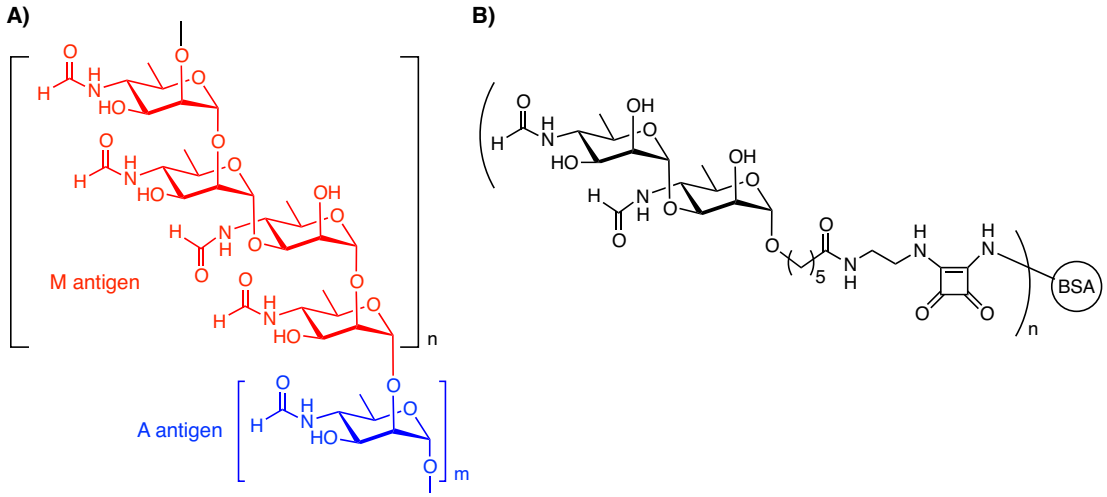


Figure 1.4 A) Structure of *Brucella* cell-wall polysaccharide with the two antigenic epitopes highlighted; B) Structure of glycoconjugate containing a synthetic disaccharide M-antigen epitope for brucellosis diagnosis.[86] BSA, bovine serum albumin.

1.4.3 Synthetic carbohydrate-based vaccines

The unique polysaccharides and glycoconjugates on the surfaces of invasive pathogens have served as attractive targets for vaccine development to prevent life-threatening infectious diseases. Given the fact that most of carbohydrates themselves are poorly immunogenic,[87] carbohydrate-based vaccines usually require covalent attachment to carrier proteins to form glycoconjugates, which can effectively elicit the production of high affinity immunoglobulin G (IgG) antibodies, resulting in long-lived immune responses.[88] Although several vaccines derived from natural sources have achieved success, increasing attention is being paid to chemically defined glycoconjugates vaccines.[89,90] These synthetic constructs open up the possibility of enhancing consistency and efficacy of glycoconjugate vaccines, and facilitating investigations of immunological mechanisms.

In the design of synthetic glycoconjugate vaccines, the length of the oligosaccharide chains, the immunogenicity of the linker, the conjugation method as well as the conjugation site are key considerations that must be taken into account.[89] The most successful example of a glycoconjugate vaccine is a against the bacterium *Haemophilus influenzae* type b (Hib), which was developed in Cuba. This is the first clinically approved synthetic oligosaccharide-based vaccine, which is generated by coupling a synthetic capsular polysaccharide antigen from Hib to tetanus toxin (TT) (Figure 1.5A).[91] Another representative example is the anti-candidiasis glycoconjugate vaccine reported by Hu and coworkers (Figure 1.5B).[92] The strategy relied upon a tyrosine-selective alkynylation of the carrier protein CRM_{197}, followed by click chemistry mediated conjugation to couple a synthetic β-(1→3)-glucan pentasaccharide to CRM_{197}. This is the first example of a glycoconjugate vaccine carrying an oligosaccharide at tyrosine. Despite low carbohydrate

loading and a short glycan length, the vaccine was able to elicit a desirable level of IgG antibodies in immunized mice.

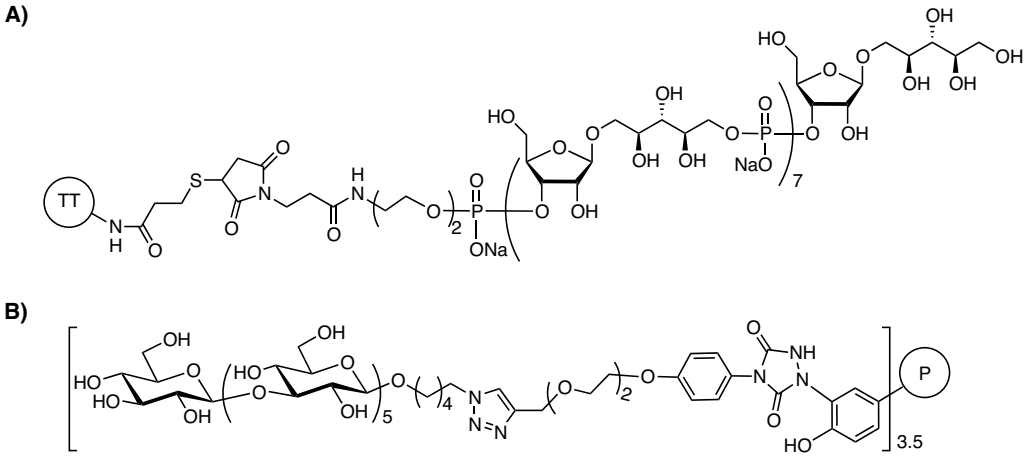


Figure 1.5 A) Synthetic carbohydrate-based vaccine for *Haemophilus influenzae* type b (Hib);[91] B) Anti-candidiasis glycoconjugate vaccine based on tyrosine-selective conjugation.[92]

The concept of synthetic carbohydrate-based vaccines has also been applied to human immunodeficiency virus type 1 (HIV-1).[93] Inspired by the discovery that the broadly neutralizing antibody (BnAb) 2G12 isolated from an HIV-infected patient binds exclusively to the high-mannose epitope on HIV envelope protein gp120,[94,95] a variety of glycoconjugates with multivalent display of synthetic oligomannoses such as $Man_9GlcNAc_2$ and Man_4 have been constructed for the development of HIV vaccines. Unfortunately, the antibodies induced by these immunogens in animal models failed to bind the native gp120 glycans or neutralize HIV.[93] Recently, a newly isolated BnAb, PG9, which exhibits 10-fold higher HIV-neutralizing activity than 2G12, was found to recognize a glycopeptide epitope located in the variable 1 and 2 (V1V2) region of gp120. This has prompted new interest in HIV vaccine design.[96] In a pioneering study, Danishefsky and coworkers reported several synthetic gp120 V1V2 glycopeptides derived from a HIV-1

strain to mimic the binding epitope of PG9.[97] The glycopeptide bearing two closely spaced $Man_5GlcNAc_2$ or $Man_3GlcNAc_2$ glycans at Asn^{156} and Asn^{160} (Figure 1.6) exhibited high affinity to PG9. Further study revealed that the dimers of these glycopeptides linked by a disulfide-bond were capable of binding both to mature BnAbs and receptors on naïve B cells, suggesting the potential of generating BnAbs that recognize these glycans.[98]

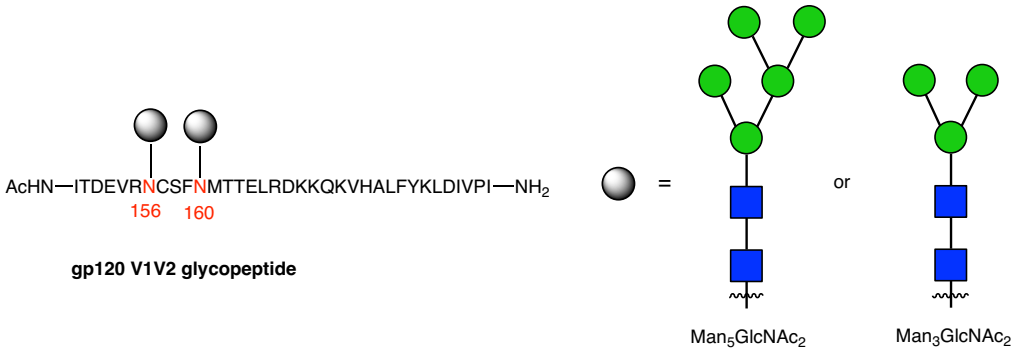


Figure 1.6 Structure of a synthetic gp120 V1V2 glycopeptide bearing two N-linked glycans synthesized by Danishefsky and coworkers.[97] Ligation sites are shown in red, amino acids are represented by their single-letter codes.

In addition, tumor-associated carbohydrate antigens (TACAs) on cancer cells are emerging as a predominant target for cancer vaccine design.[99,100] An elegant example is the synthesis of a glycoconjugate vaccine by conjugating a synthetic peptide backbone containing five TACAs, Globo-H, GM2, STn, TF and Tn, to the carrier protein KLH (Figure 1.7). This vaccine is currently in clinical trials.[101] Other representative achievements include an optimized Globo-H vaccine (CRM197 conjugate),[102] a three-component vaccine consisting of a TLR2 agonist, a T-helper peptide epitope and a tumor-associated glycopeptide,[103] as well as a four-component vaccine composed of a mucin glycopeptide and three different T-helper peptide epitopes.[104]

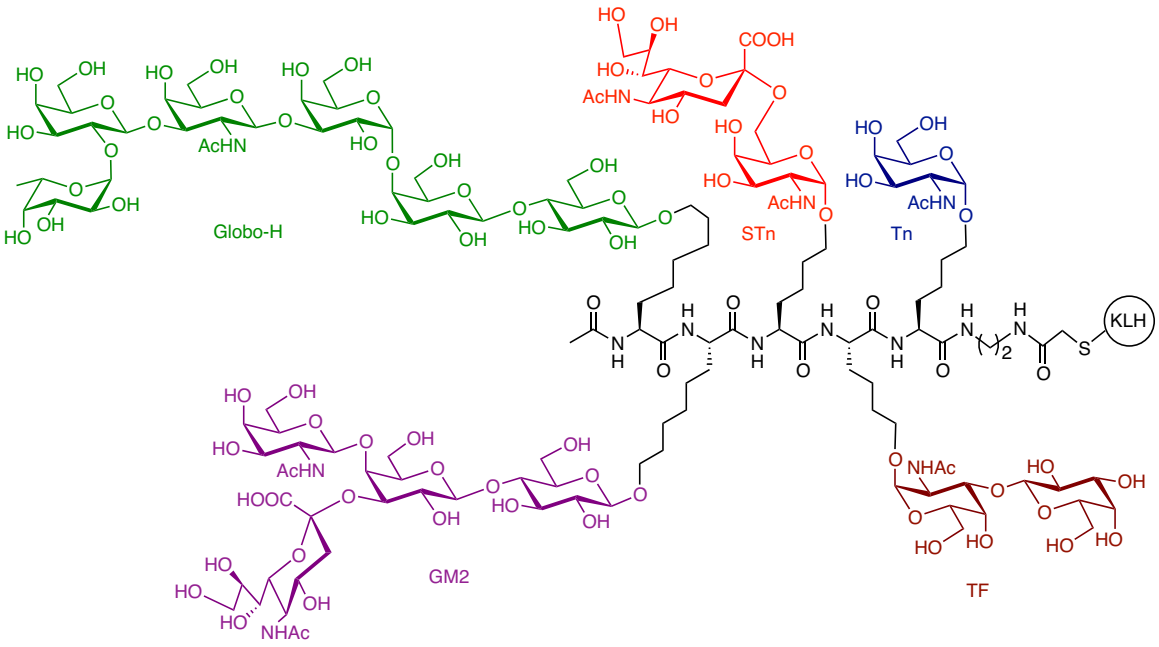


Figure 1.7 Structure of a synthetic glycoconjugate vaccine containing five different tumor-associated antigens on a single peptide backbone.[101]

1.5 Overview of thesis research

As discussed previously, recent developments in chemical, enzymatic and chemo-enzymatic synthesis has enabled a growing number of structurally defined oligosaccharides to become accessible. These molecules are serving as invaluable probes for deciphering the biological functions of these glycans and understanding the molecular mechanism underlying their functions as well as exploring the potential for medicinal use. In this thesis, I will describe three projects in the field of synthetic chemistry, which are targeted to preparing glycans for different biological investigations.

In the past few years, we have been interested in accessing devices (nanoparticles or stents)[105] carrying synthetic ABO blood group antigens and tolerance-inducing glycans for use in inducing specific B-cell tolerance during immune development, with the aim to

extend the window for ABO-incompatible (ABOi) heart transplants. This is a collaboration between our group and clinicians in the Faculty of Medicine and Dentistry, as well as investigators in the National Institute for Nanotechnology. As part of this program, we wanted to determine if lacto-*N*-fucopentaose III (LNFPIII), a previously reported immunomodulatory glycan,[106] could serve as a tolerance-inducing agent to promote immune tolerance to ABO blood group antigens in neonates. Therefore, I developed a concise synthetic route to LNFPIII and its corresponding human serum albumin (HSA) conjugate. The details of the chemical synthesis and preliminary biological results obtained in animal model will be described in Chapter 2.

As part of this larger program, another issue we are aiming to address is the accurate detection of anti-donor antibody responses in patients undergoing an ABOi transplant. The current clinical standard assay, erythrocyte agglutination, cannot differentiate subtype-specific ABO antibodies, and hence provides imprecise information for donor-specific antibodies, owing to different expression of subtype antigens between erythrocytes and hearts.[107] Recently, we have created an ABO-glycan microarray containing synthetic type I−VI ABH antigens to characterize anti-ABO subtype antibodies. We demonstrated the precise assessment of donor-specific antibodies responses in heart transplant patients.[108] Despite the success we have achieved, the AB antigens used in the microarray are just terminal tetrasaccharide (A,B) or trisaccharide (H) fragments of the natural antigen-containing glycans, which are typically expressed on the ends of long poly-*N*-acetyllactosamine (poly-LacNAc) chains. To better mimic natural glycan structures and reveal the effect of structural complexity on antibody–antigen recognition, a panel of poly-LacNAc extended biantennary *N*- and *O*-linked glycans capped with blood group A or B

antigens were synthesized via an enzymatic approach. This work is described in Chapter 3. These glycans will be used in a glycan microarray to screen serum samples from heart transplant patients. Moreover, the microarray containing both small and complex AB antigens will provide additional insight into the binding specificities of monoclonal subtype specific antibodies.

Chapter 4 is focused on a different project. As mentioned in section 1.4.3, glycoconjugate vaccines are superior to pure carbohydrate antigens in inducing immune responses, and have offered enormous health benefits. However, the mechanism of the immune system activation by glycoconjugate vaccine remains unclear. A recently proposed mechanistic model suggested that the carbohydrate epitope can be presented by major histocompatibility complex class II (MHCII) in the presence of another molecule (for example, a peptide) and then be recognized by T cells.[109] Deeper insights into this new mechanism and understanding the interaction between the glycopeptide, MHCII and T cell receptors require a library of structurally well-defined complex oligosaccharides. Therefore, we developed a chemo-enzymatic strategy to prepare a library of defined group B Streptococcus type III (GBSIII) oligosaccharides, involving the chemical synthesis of the oligosaccharide backbone and introduction of the side chain by glycosyltransferases.

Chapter 2: Synthesis and evaluation of the tolerance inducing activity of lacto-*N*-fucopentaose III

2.1 Introduction

Lacto-*N*-fucopentaose III (LNFPIII), a pentasaccharide containing the Lewis^X trisaccharide antigen, is an immunomodulatory glycan that is present on schistosome eggs.[110] The expression of LNFPIII on schistosome eggs has been shown to suppress host immune responses, which enables the parasite to escape detection of the mammalian host immune system thus facilitating survival.[110] LNFPIII is also found in breast milk and the urine of pregnant women, as well as the fetal brain, and has been speculated to have a similar protective immunomodulatory effect in the fetus.[110,111] Recently, Burlingham and coworkers demonstrated that a LNFPIII conjugate can prolong organ survival in neonatal heart transplantation models.[106] Although the biological role that LNFPIII plays in graft prolongation remains to be further investigated, a preliminary mechanistic study suggested that the LNFPIII conjugate significantly upregulated the expression of programmed death ligand 1,[106] which negatively regulates immune responses through binding with its receptor, programmed cell death protein 1, expressed on the surface of activated T cells, B cells and macrophages.[112] This observation suggests that LNFPIII is a potential tolerance inducing oligosaccharide.

Drawing inspiration from the knowledge that ABOi heart transplants during infancy can result in spontaneous development of immunological tolerance to donor A/B antigens, we are interested in accessing devices (nanoparticles or stents) carrying synthetic ABO blood group antigens and tolerance-inducing glycans for use in inducing specific B-cell tolerance during immune development, with the aim to extend the window for ABOi heart transplants.[113] As part of this program, we wanted to determine if LNFPIII, presented together with the ABO blood group structures, could promote immune tolerance to these

antigens in neonates, and decipher the mechanism underlying the tolerance inducing activity of LNFPIII. Carrying out these studies required access to milligram quantities of LNPFIII functionalized with a linker that would allow its attachment surfaces, *e.g.*, proteins as well as amine-coated nanoparticles or stents.[105] Although previous synthesis of LNPFIII derivatives have been reported by Sinaÿ[114] and Zhang,[115] none of these compounds was suitably functionalized for our purposes. I describe here the synthesis of LNPFIII bearing an activated ester moiety in the aglycon (**2.1**, Figure 2.1) and its corresponding HSA conjugate, as well as the preliminary evaluation of the tolerance inducing activity of the LNFPIII-HSA conjugate.

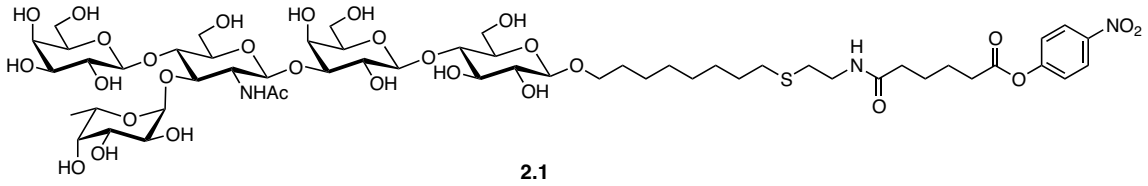


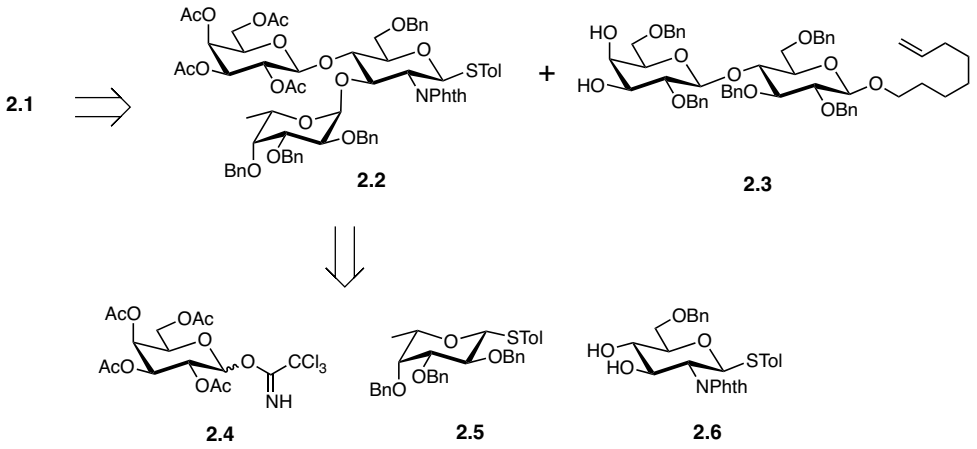
Figure 2.1 Structure of LNFPIII **2.1** with activated flexible linker.

2.2 Results and discussion

2.2.1 Synthesis of LNFPIII 2.1 and its HSA conjugate

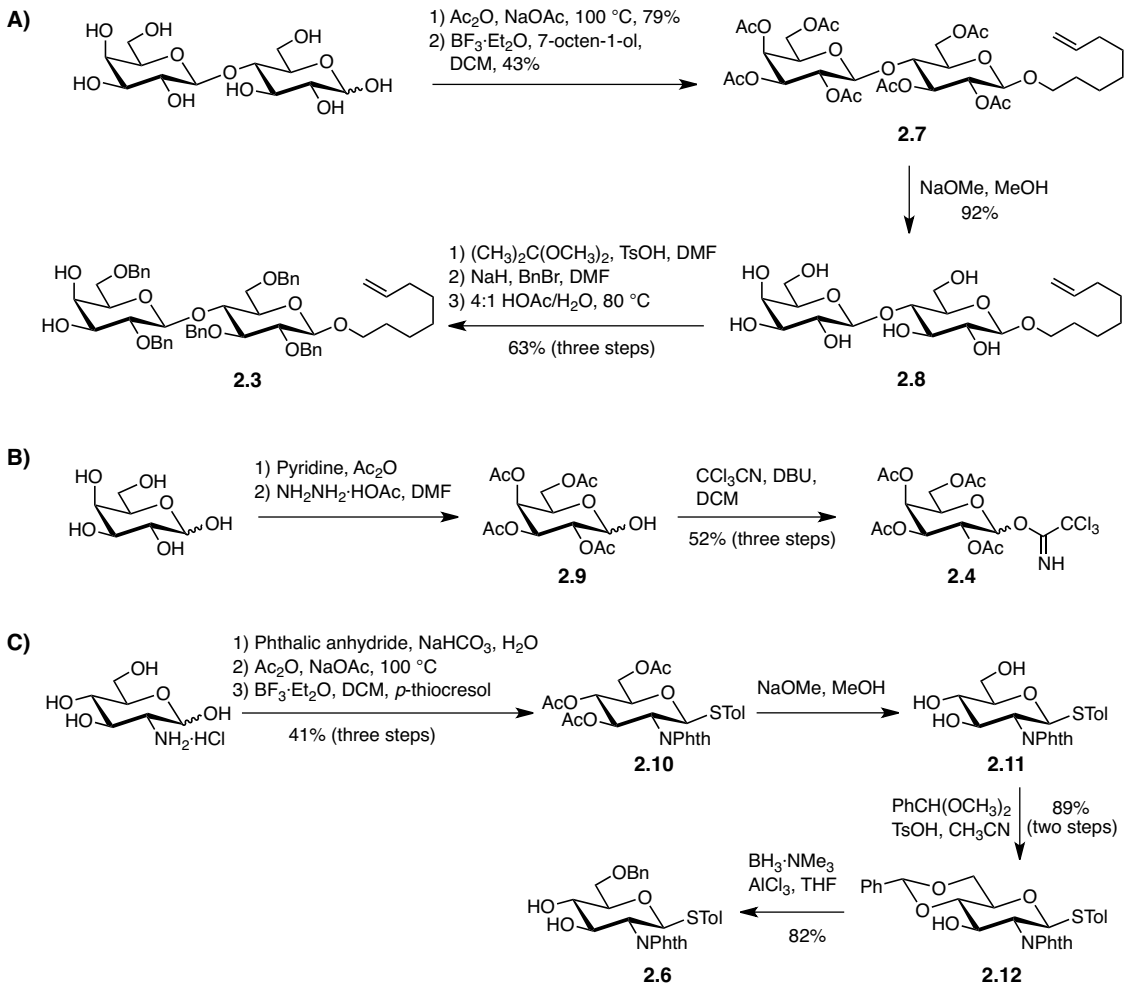
I envisioned (Scheme 2.1) constructing pentasaccharide **2.1** from four readily available carbohydrates: D-galactose, L-fucose, D-glucosamine and lactose, via trisaccharide thioglycoside **2.2** and disaccharide diol **2.3**. An important feature was a regio- and stereoselective 3+2 glycosylation, a key strategy in earlier routes to LNPFIII derivatives,[114,115] followed by global deprotection and introduction of the activated ester. Thioglycoside **2.2** can be assembled through regioselective condensation of

trichloroacetimidate **2.4** with diol **2.6** followed by treatment of the product with thioglycoside **2.5**.



Scheme 2.1 Retrosynthetic analysis of **2.1**.

As illustrated in Scheme 2.2A, diol **2.3** was prepared from lactose. First, acetylation was performed in acetic anhydride at 100 °C in the presence of sodium acetate to form preferentially the β-anomer of lactose octaacetate (α/β = 1:5). This compound was then coupled with 7-octen-1-ol using $BF_3 \cdot OEt_2$ as the promoter to generate octenyl glycoside **2.7** in 43% overall yield. The ^{1}H NMR spectrum of **2.7** showed the anomeric proton H-1 at 4.45 ppm as a doublet with a coupling constant between H-1 and H-2 of 8.0 Hz, indicating the newly formed glycosidic linkage was β. Then, deacetylation of **2.7** using a catalytic amount of sodium methoxide afforded **2.8** in 92% yield. Finally, installation of an isopropylidene ketal at the 3′ and 4′ positions of **2.8**, benzylation of the remaining five hydroxyl groups followed by acid hydrolysis of the isopropylidene ketal enabled the conversion of **2.8** to diol **2.3** in 63% yield over three steps.[116]



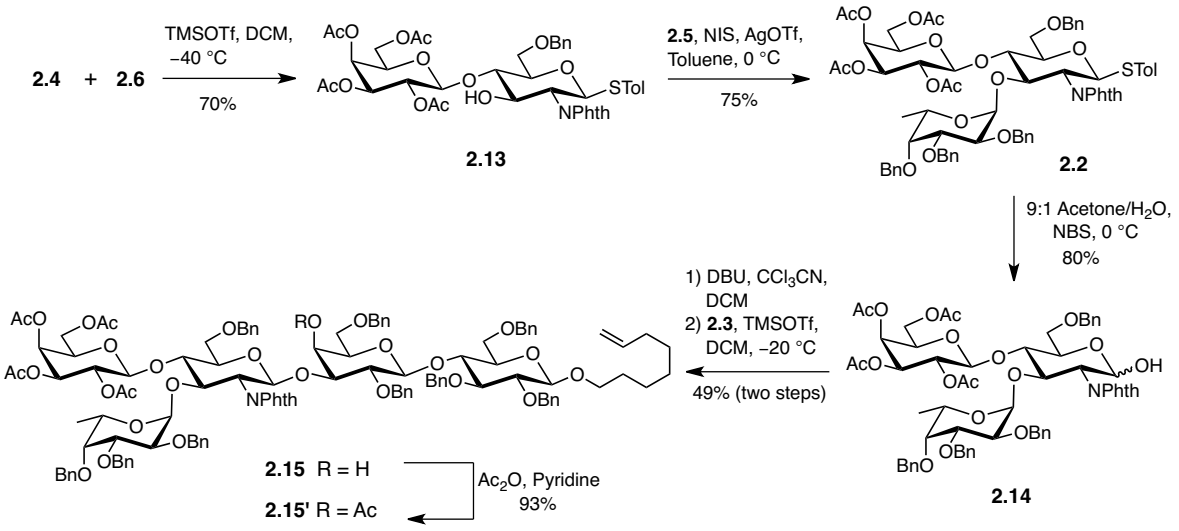
Scheme 2.2 Synthesis of building blocks **2.3** **2.4** and **2.6**.

Trichloroacetimidate **2.4**[117] was readily achieved from D-galactose (Scheme 2.2B). Peracetylation and subsequent selective anomeric deacetylation led to hemiacetal **2.9**, which was treated with trichloroacetonitrile in the presence of a catalytic amount of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) to provide **2.4** in 52% yield over three steps. Building block **2.6** was derived from D-glucosamine hydrochloride in six steps (Scheme 2.2C). The phthalimide group was used to protect the C-2 amino group and the remaining hydroxyl groups were acetylated to form an intermediate that was coupled with *p*-thiocresol

in the presence of $BF_3 \cdot OEt_2$ to yield thioglycoside **2.10**. Subsequently, deacetylation of **2.10** followed by installation of a benzylidene acetal across O-4 and O-6 resulted in **2.12**,[26] which underwent regioselective reductive opening of the benzylidene ring using $BH_3 \cdot NMe_3$ and $AlCl_3$[118] to afford the desired compound in 82% yield. Crude building block **2.5**,[26] synthesized by a previous group member, was purified and used directly.

With **2.3**–**2.6** in hand, the construction of the pentasaccharide was carried out (Scheme 2.3). First, trichloroacetimidate **2.4** was coupled with diol **2.6** in the presence of TMSOTf to generate regioselectively the β-(1→4)-linked disaccharide **2.13** in 70% yield. The β-stereochemistry was confirmed by the H-1, H-2 coupling constant of the galactopyranosyl residue ($^3J_{\text{H-1,H-2}}$ = 8.0 Hz). The regioselectivity of the glycosylation was determined by a two-dimensional NMR experiment. In the HMBC spectrum of **2.13** (shown in the experimental section), the expected correlations between H-1_{Gal} and C-4_{GlcNAc}, H-4_{GlcNAc} and C-1_{Gal} are both observed, while correlations between H-1_{Gal} and C-3_{GlcNAc}, H-3_{GlcNAc} and C-1_{Gal} are not, indicating that the newly formed glycosidic linkage is the desired β-(1→4)-linked disaccharide instead of the β-(1→3)-linked isomer. The unusual regioselectivity of this glycosylation has been reported previously and can be rationalized by matched–mismatched glycosylation.[119,120] In this case, disarmed donor **2.4** reacts preferentially with the less reactive C-4 hydroxyl group in diol **2.6**. In addition, the steric effect induced by the *N*-phthalimido group at the C-2 position also likely contributes to the regioselectivity.[119] Subsequently, the fucosylation of disaccharide **2.13** with thioglycoside **2.5** in toluene using NIS and AgOTf as the promotor provided trisaccharide **2.2** in 75% yield with excellent stereoselectivity. The anomeric proton of the fucopyranoside residue in **2.2** appeared around 4.84 ppm in the ^{1}H NMR spectrum,

overlapping with a methylene proton from the benzyl groups. Therefore, the one-bond heteronuclear coupling constant at the anomeric centre of the fucose residue ($^1J_{C\text{-}1,H\text{-}1}$)[121] was used to determine the stereochemistry at the newly formed linkage; the value is 170.3 Hz, which unambiguously confirms the α-stereochemistry of this residue. The armed nature of **2.5** relative to **2.13**[26] allows this reaction to proceed efficiently without competing activation of the disaccharide acceptor or trisaccharide product.



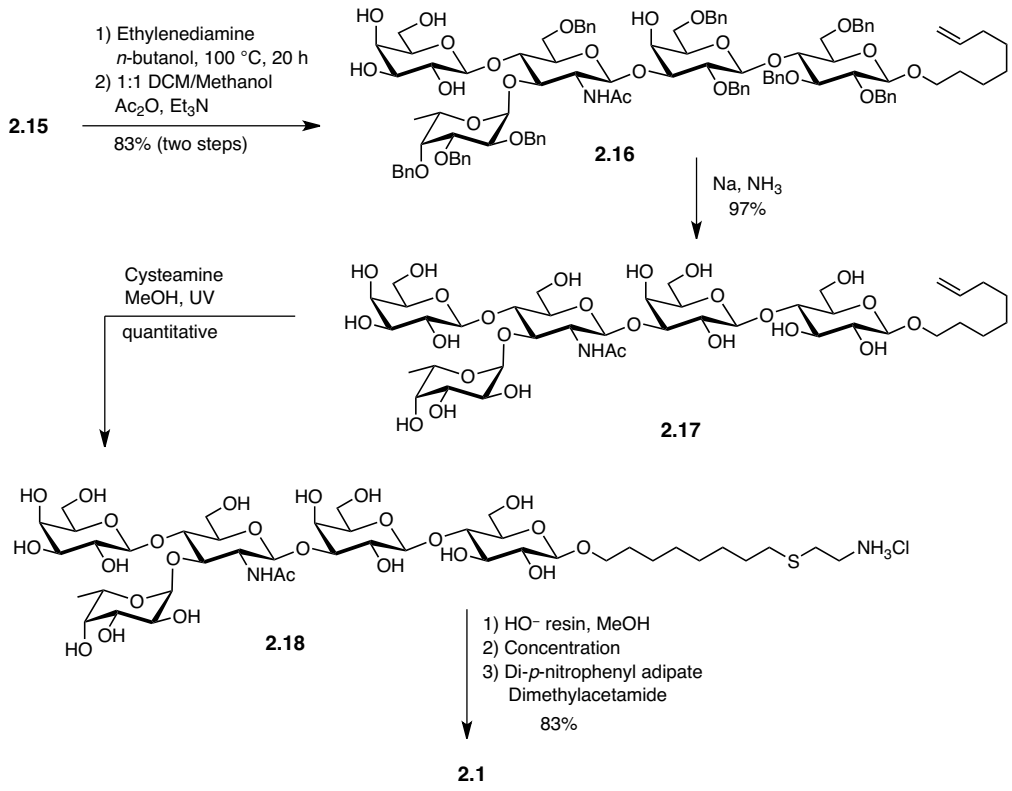
Scheme 2.3 Synthesis of protected pentasaccharide **2.15**.

The final planned glycosylation reaction, coupling of trisaccharide **2.2** and diol **2.3**, cannot be carried out using NIS and AgOTf activation because of the incompatibility of these conditions with the alkene functionality in the aglycone. To circumvent this problem, an alternative promotor system using diphenyl sulfoxide in combination with Tf_2O[122] was explored, but the desired product was obtained in low yield. Although other thioglycoside activation conditions, for example using DMTST could have been explored, I chose instead to convert **2.2** into an alternate glycosyl donor. Therefore, thioglycoside **2.2** was treated

with *N*-bromosuccinimide (NBS) in acetone–water (9:1) to afford hemiacetal **2.14** in 80% yield. This compound was subsequently converted to corresponding trichloroacetimidate by treatment with trichloroacetonitrile (CCl_3CN) in the presence of catalytic amount of DBU. The freshly made donor was then used in a reaction with diol acceptor **2.3** to give the pentasaccharide **2.15** in 49% yield over two steps. The β-stereochemistry of the newly formed linkage was established based on the $^{3}J_{\text{H-1, H-2}}$ (8.5 Hz) and $^{1}J_{\text{C-1,H-1}}$ (165.5 Hz) values. To confirm the regioselectivity, a small amount of pentasaccharide **2.15** was acetylated to generate **2.15′**. Upon comparison of ^{1}H NMR spectra of these two compounds, a broad signal at 4.05 ppm in **2.15** shifted to 5.46 ppm in **2.15′** and appeared as a doublet of doublets (J = 3.6 and 0.6 Hz). The values of these coupling constants indicated that the acetyl group was introduced onto O-4′, confirming that the newly introduced glycosidic linkage was β-(1→3) not β-(1→4).

With the pentasaccharide assembled, treatment of **2.15** with ethylenediamine in *n*-butanol at 100 °C for 20 h (Scheme 2.4), followed by selective *N*-acetylation, led to formation of **2.16**. Birch reduction was conducted to remove the benzyl groups, while keeping intact the alkene functionality for further modification. The fully unprotected pentasaccharide **2.17** was obtained in 97% yield. A UV-promoted thiol–ene reaction[123] was then performed to further functionalize the octenyl linker with a cysteamine residue, leading to the corresponding amine salt **2.18** in quantitative yield. Finally, the amine salt was converted to the free amine by exchange with HO^- resin, followed by coupling with di-*p*-nitrophenyl adipate[124] in dimethylacetamide to yield the desired highly reactive *p*-nitrophenol (PNP) ester **2.1** in 83% overall yield. Conversion of **2.1** into the corresponding HSA conjugate was then done by treatment of HSA with **2.1** in phosphate buffer (pH =

7.5), resulting in the incorporation of up to 21 pentasaccharide moieties into the glycoconjugate by varying the amount of **2.1** used for conjugation.



Scheme 2.4 Synthesis of **2.1**.

2.2.2 Evaluation of the tolerance inducing activity of LNFPIII-HSA conjugate

Recently, Ochando and coworkers demonstrated that blockade of an essential signaling pathway (the cluster of differentiation 4 (CD4)–CD4 ligand costimulatory pathway) involved in immune system activation, promotes the accumulation of suppressive macrophages in transplanted hearts. These suppressive macrophages can suppress immune responses by inhibiting killer T cell proliferation and stimulating suppressor T cell expansion, resulting in the induction of tolerance and long-term organ survival.[125] A further mechanistic study revealed that the function of suppressive macrophages is controlled by

dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN), a C-type lectin expressed on the surface of macrophages. DC-SIGN recognizes and binds to mannose or fucose-containing carbohydrates, such as $Lewis^X$. They found that suppressive macrophages accumulated in donor hearts with impaired $Lewis^X$ expression did not exhibit immune regulatory function, and the transplant recipients suffered from acute rejection, indicating that $Lewis^X$-mediated DC-SIGN signaling pathway is crucial for the function of suppressive macrophages and for the induction of transplantation tolerance.[126]

Given the presence of a $Lewis^X$ fragment in LNFPIII and the immunosuppressive activity of LNFPIII reported previously, it was of interest to investigate whether LNFPIII functions as a ligand for DC-SIGN to induce transplantation tolerance to mismatched organ. In collaboration with Ochando, transplant recipients containing $Lewis^X$-deficient donor hearts were treated with the LNFPIII-HSA conjugate, which led to transplantation tolerance. This indicates that LNFPIII/DC-SIGN signaling is able to activate the immune regulatory function of suppressive macrophages. The result provides new insight into the mechanisms that accounts for the tolerance-inducing activity of LNFPIII, in particular, that is does so through binding to DC-SIGN.

2.3 Summary

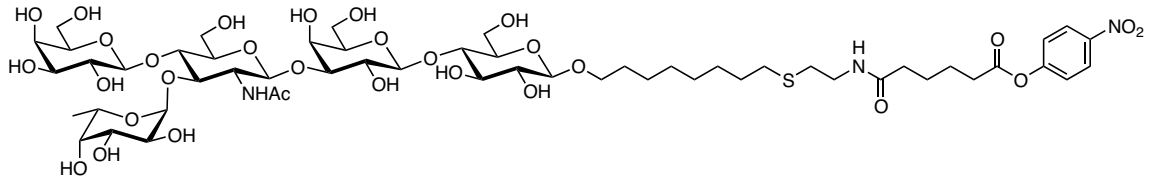
In conclusion, I have achieved the synthesis of LNPFIII functionalized with reactive PNP ester and the corresponding HSA conjugate. The synthetic strategy features two highly regio- and stereoselective glycosylations for the construction of disaccharide **2.13** and pentasaccharide **2.15** based on the reactivity difference between two hydroxyl groups in acceptor **2.6** and **2.3**, respectively. Birch reduction enabled the deprotection of benzyl

ethers while leaving the octenyl linker intact, which allowed further functionalization to form the PNP ester and, in turn, the HSA conjugate. Moreover, it was found that LNFPIII is able to bind to DC-SIGN for induction of transplantation tolerance. The observation also sheds light on the mechanism underlying the tolerance inducing activity of LNFPIII.

2.4 Experimental section

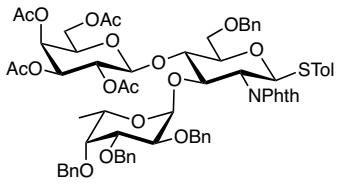
General methods for chemical synthesis: All reagents were purchased from commercial sources and were used without further purification unless noted. Dry solvents used in reactions were purified by successive passage through columns of alumina and copper under an argon atmosphere. All reactions were carried out under a positive pressure of argon unless otherwise stated, monitored by TLC on Silica Gel 60-F_{254} (0.25 mm), and the spots were visualized under UV light (254 nm) and/or stained by charring with acidified anisaldehyde solution in ethanol. Column chromatography was performed on Silica Gel 60 (40–60 μm) or C_{18} silica gel (35–70 μm, Toronto Research Chemicals). ^{1}H NMR spectra were recorded at 500 or 600 MHz, and chemical shifts were referenced to $CHCl_3$ (7.26 ppm, $CDCl_3$), CD_2HOD (3.31 ppm CD_3OD), or HOD (4.79 ppm, D_2O). ^{13}C NMR spectra were recorded at 126 MHz and chemical shifts were referenced to $CDCl_3$ (77.06 ppm, $CDCl_3$), CD_3OD (49.0 ppm, CD_3OD) or external acetone (31.07 ppm, D_2O). Assignments of NMR spectra were made on the basis of two-dimensional experiments (^{1}H–^{1}H COSY, HSQC and HMBC) and the stereochemistry of the newly formed glycosidic linkages was confirmed by measuring $^{1}J_{C\text{-}1,H\text{-}1}$ values via an ^{1}H-coupled HSQC experiment. In the data provided below the resonances on particular residues are indicated by an increasing number of primes (′) moving from the reducing to non-reducing end. For example, in **2.15** H-1 is

H-1 of the Glc residue, H-1′′ is H-1 of the GlcNAc residue, and H-1′′′′ is H-1 of the Fuc residue. Electrospray-ionization mass spectra were recorded on an Agilent Technologies 6220 TOF spectrometer on samples dissolved in CH_2Cl_2 or CH_3OH. The matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrum was obtained in the linear positive mode of ionization on a Bruker Daltonics (Bremen, GmbH) UltrafleXtreme MALDI TOF/TOF mass spectrometer using sinapinic acid as the matrix. Optical rotations were measured on Perkin Elmer 241 polarimeter at 22 ± 2 °C in units of degree mL/(g·dm).



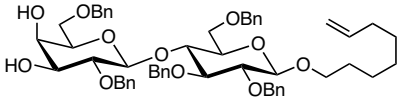
6-[[2-[[8-(β-D-Galactopyranosyl-(1→4)-[α-L-fucopyranosyl-(1→3)]-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl-oxy)octyl]thio]ethyl]amino]-6-oxo-hexanoic acid *p*-nitrophenyl ester (2.1): Compound **2.18** (10 mg, 9.3 μmol) was dissolved in CH_3OH (5 mL) and treated with Amberlite IR400 HO^- ion-exchange resin to convert the hydrochloride salt to the free amine. The solution was filtered, concentrated and dried overnight under high vacuum. Then the residue was dissolved in dimethylacetamide (0.5 mL) and treated with di-*p*-nitrophenyl adipate (18 mg, 46.4 μmol). After stirring at room temperature for 4 h, the solution was concentrated under high vacuum to dryness. The residue was subjected to C_{18} chromatography using gradient elution (0.5% aqueous AcOH → 60:40 CH_3OH–0.5% aqueous AcOH to give **2.1** (10 mg, 83%) as a white foam after lyophilization. ^1H NMR (600 MHz, CD_3OD): δ 8.29 (d, J = 9.2

Hz, 2H, Ar), 7.37 (d, *J* = 9.2 Hz, 2H, Ar), 5.05 (d, *J* = 4.0 Hz, 1H, H-1′′′′), 4.83–4.82 (m, 1H, H-5′′′′), 4.69 (d, *J* = 8.1 Hz, 1H, H-1′′), 4.43 (d, *J* = 7.6 Hz, 1H, one of H-1, H-1′, H-1′′′), 4.36 (d, *J* = 7.7 Hz, 1H, one of H-1, H-1′, H-1′′′), 4.27 (d, *J* = 7.8 Hz, 1H, one of H-1, H-1′, H-1′′′), 4.04 (d, *J* = 3.0 Hz, 1H), 3.95 (dd, *J* = 6.3, 3.3 Hz, 1H), 3.92–3.81 (m, 8H), 3.79–3.75 (m, 3H), 3.71–3.49 (m, 12H), 3.46–3.41 (m, 3H), 3.39–3.36 (m, 1H), 3.35 (t, *J* = 7.0 Hz, 2H, SCH_2CH_2N), 3.22 (dd, *J* = 9.1, 7.9 Hz, 1H), 2.66 (t, *J* = 7.1 Hz, 2H, $OC(O)CH_2$), 2.62 (t, *J* = 7.0 Hz, 2H, SCH_2CH_2N), 2.53 (t, *J* = 7.3 Hz, 2H, $SCH_2(CH_2)_6CH_2O$), 2.25 (t, *J* = 7.0 Hz, 2H, $NHC(O)CH_2$), 1.97 (s, 3H, NHAc), 1.77–1.73 (m, 4H, $NC(O)CH_2(CH_2)_2CH_2C(O)O$), 1.61–1.55 (m, 4H, $OCH_2CH_2(CH_2)_4CH_2CH_2S$), 1.39–1.30 (m, 8H, $OCH_2CH_2(CH_2)_4CH_2CH_2S$), 1.17 (d, *J* = 6.6 Hz, 3H, H-6′′′′); ^{13}C NMR (126 MHz, CD_3OD): δ 174.3 (C=O), 173.1 (C=O), 171.2 (C=O), 155.7 (Ar), 145.4 (Ar), 124.7 (2C, Ar), 122.5 (2C, Ar), 103.6, 102.8, 102.5 (C-1, C-1′, C-1′′′), 102.4 (C-1′′), 98.8 (C-1′′′′), 82.4, 79.1, 75.8, 75.3, 75.2, 75.1, 75.02, 74.99, 73.5 (2C), 73.3, 72.3, 71.4, 70.1, 69.8, 69.5 ($OCH_2(CH_2)_6CH_2S$), 68.6 (2C), 68.4, 66.3 (C-5′′′′), 61.4, 61.0, 60.5, 59.8 (C-6, C-6′, C-6′′, C-6′′′), 56.3 (C-2′′), 38.7 (SCH_2CH_2N), 35.2 ($NHC(O)CH_2$), 33.2 ($OC(O)CH_2$), 31.2 (SCH_2CH_2N), 30.8 ($SCH_2(CH_2)_6CH_2O$), 29.3 (2C), 29.0, 28.8, 28.4 (5 × $SCH_2(CH_2)_6CH_2O$), 25.6, 24.8, 23.8 ($SCH_2(CH_2)_6CH_2O$, 2 × $NC(O)CH_2(CH_2)_2CH_2C(O)O$), 21.8 (NHAc), 15.2 (C-6′′′′); HRMS-ESI *m/z* $[M + Na]^+$ calcd for $C_{54}H_{87}N_3NaO_{30}S$: 1312.4987, found: 1312.4986.



***p*-Tolyl 2,3,4-6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-[2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl-(1→3)]-6-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (2.2):** A mixture of thioglycoside **2.5** (2.74 g, 5.07 mmol), acceptor **2.13** (1.70 g, 2.03 mmol) and powdered 4 Å molecular sieves was suspended in toluene (30 mL) and stirred at room temperature for 1 h. The solution was then cooled to 0 °C, and then *N*-iodosuccinimide (1.10 g, 4.88 mmol) and silver trifluoromethanesulfonate (125 mg, 0.49 mmol) were added. After stirring at 0 °C for 2 h, Et_3N (1 mL) was added and the mixture was filtered through Celite. The filtrate was concentrated and the resulting residue was subjected to flash chromatography (hexane–EtOAc 2:1) to afford trisaccharide **2.2** (1.90 g, 75%) as a white foam. R_f = 0.24 (hexane–EtOAc 2:1); $[\alpha]_D$ = +7.1 (c = 0.9, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.75–7.70 (m, 4H, Ar), 7.45–7.01 (m, 24H, Ar), 5.44 (d, J = 10.5 Hz, 1H, H-1), 5.26 (dd, J = 3.5, 1.0 Hz, 1H, H-4′), 5.04 (dd, J = 10.4, 8.2 Hz, 1H, H-2′), 4.84–4.79 (m, 4H, H-1′′, H-3′, 2 × OCH_2Ph), 4.77–4.72 (m, 2H, H-1′, H-3), 4.65–4.60 (m, 3H, H-5′′, 2 × OCH_2Ph), 4.58 (A of ABq, J = 11.7 Hz, 1H, OCH_2Ph), 4.53–4.48 (m, 2H, H-2, OCH_2Ph), 4.46, 4.28 (ABq, J = 12.3 Hz, 2H, 2 × OCH_2Ph), 4.18–4.13 (m, 2H, H-4, H-6a′), 3.98 (dd, J = 10.7, 5.7 Hz, 1H, H-6b′), 3.92–3.88 (m, 2H, H-3′′, H-6a), 3.85–3.80 (m, 2H, H-2′′, H-6b) 3.64 (d, J = 1.3 Hz, 1H, H-4′′), 3.57–3.55 (m, 2H, H-5, H-5′), 2.29 (s, 3H, ArMe), 2.03 (s, 3H, OAc), 2.02 (s, 3H, OAc), 1.97 (s, 3H, OAc), 1.86 (s, 3H, OAc), 1.21 (d, J = 6.5 Hz, 3H, H-6′′); ^{13}C NMR (126 MHz, $CDCl_3$): δ 170.0 (C=O), 169.8 (C=O), 168.7 (C=O), 138.9 (Ar), 138.7 (Ar), 138.2 (Ar), 138.1 (Ar), 137.9 (Ar), 134.2 (Ar), 133.3

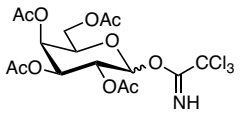
(Ar), 131.8 (Ar), 129.6 (Ar), 128.6 (Ar), 128.3 (Ar), 128.24 (Ar), 128.17 (Ar), 128.1 (Ar), 128.02 (Ar), 127.97 (Ar), 127.86 (Ar), 127.4 (Ar), 127.2 (Ar), 127.1 (Ar), 127.0 (Ar), 123.7 (Ar), 99.5 (C-1′), 97.6 (C-1″), 84.4 (C-1), 79.8 (C-3″), 79.6 (C-5), 77.2 (C-4″), 75.0 (C-4), 74.5 (C-2″) 74.2 (O*C*H_2Ph), 73.64 (C-3), 73.58, 73.0, 72.3 (3 × O*C*H_2Ph), 71.0 (C-3′), 70.4 (C-5′), 69.0 (C-2′), 67.85 (C-6), 66.76 (C-4′), 66.5 (C-5″), 60.2 (C-6′), 55.6 (C-2), 21.1, 20.7, 20.62, 20.55, 20.54 (4 × OAc, ArMe); 16.7 (C-6″); HRMS-ESI *m/z* $[M + Na]^+$ calcd for $C_{69}H_{73}NNaO_{19}S$: 1274.4390, found: 1274.4385.



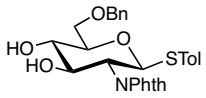
7-Octen-1-yl 2,6-di-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (2.3): To a solution of **2.7** (2.89 g, 3.87 mmol) in 1:3 CH_2Cl_2–CH_3OH (40 mL) was added 1.0 M $NaOCH_3$ (2.7 mL). After stirring at room temperature overnight, the solution was neutralized with Amberlite IR120 H^+ ion-exchange resin. The solution was filtered and the filtrate was concentrated to afford **2.8** (1.61 g, 92%) as a white solid. A portion of **2.8** (1.65 g, 3.65 mmol) and *p*-toluenesulfonic acid (*p*-TsOH) (41 mg) were suspended in a mixture of dry DMF (8 mL) and 2,2-dimethoxypropane (24 mL). After stirring at 85 °C for 1.5 h, the solution was cooled to room temperature, neutralized with Et_3N (1 mL), concentrated and dried. The resulting residue and BnBr (2.9 mL, 24.3 mmol) were dissolved in dry DMF (40 mL), to which NaH (60%, 1.22 g, 30.5 mmol) was added in portions at 0 °C, followed by vigorous stirring. After stirring overnight at room temperature, CH_3OH (4 mL) was added. The solution was concentrated, dissolved in EtOAc (200 mL) and washed with water and brine. The organic layer was dried over

Na_2SO_4, filtered, concentrated to dryness. Subsequently, the obtained crude residue was treated with 80% aqueous AcOH (160 mL) at 80 °C for 3 h. The solution was concentrated, dissolved in EtOAc (200 mL) and washed with saturated aqueous $NaHCO_3$, water and brine. The organic layer was dried over Na_2SO_4, filtered, concentrated and the residue was subjected to flash chromatography (hexane–EtOAc 5:2) to afford diol **2.3** (2.07 g, 63% over three steps) as a white solid. R_f = 0.40 (hexane–EtOAc 2:1); $[\alpha]_D$ = +16.4 (*c* = 0.9, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.39–7.22 (m, 25H, Ar), 5.81 (ddt, *J* = 17.0, 10.3, 6.7 Hz, 1H, C*H*=CH_2), 5.02–4.92 (m, 3H, CH=CH_2, OCH_2Ph), 4.91, 4.72 (ABq, *J* = 11.0 Hz, 2H, 2 × OCH_2Ph), 4.82, 4.68 (ABq, *J* = 11.5 Hz, 2H, 2 × OCH_2Ph), 4.79 (A of ABq, *J* = 11.0 Hz, 1H, OCH_2Ph), 4.61 (A of ABq, *J* = 12.5 Hz, 1H, OCH_2Ph), 4.47–4.44 (m, 3H, H-1′, 2 × OCH_2Ph), 4.41–4.38 (m, 2H, H-1, OCH_2Ph), 3.99 (app t, *J* = 9.5 Hz, 1H, H-4), 3.97–3.92 (m, 2H, H-4′, octenyl OCH_2), 3.82 (dd, *J* = 11.0, 4.0 Hz, 1H, H-6a), 3.77 (dd, *J* = 11.0, 2.0 Hz, 1H, H-6b), 3.64–3.58 (m, 2H, H-3, H-6a′), 3.54–3.49 (m, 2H, H-6b′, octenyl OCH_2), 3.45–3.39 (m, 4H, H-2, H-2′, H-3′, H-5), 3.37–3.35 (m, 1H, H-5′), 2.48 (d, *J* = 3.5 Hz, 1H, 4′-OH), 2.41 (d, *J* = 4.0 Hz, 1H, 3′-OH), 2.06–2.02 (m, 2H, CH_2CH=CH_2), 1.69–1.62 (m, 2H, $OCH_2CH_2(CH_2)_3CH_2CH=CH_2$), 1.45–1.31 (m, 6H, $OCH_2CH_2(CH_2)_3CH_2CH=CH_2$); ^{13}C NMR (126 MHz, $CDCl_3$): δ 139.2 (Ar), 139.1 (*C*H=CH_2), 138.7 (Ar), 138.4 (Ar), 138.3 (Ar), 138.0 (Ar), 128.5 (Ar), 128.4 (Ar), 128.32 (Ar), 128.30 (Ar), 128.08 (Ar), 128.06 (Ar), 128.0 (Ar), 127.93 (Ar), 127.86 (Ar), 127.7 (Ar), 127.6 (Ar), 127.57 (Ar), 127.54 (Ar), 127.3 (Ar), 114.3 (CH=*C*H_2), 103.7 (C-1), 102.6 (C-1′), 82.9 (C-3), 81.8, 80.1 (C-2, C-2′), 76.7 (C-4), 75.2 (O*C*H_2Ph), 75.1 (C-5), 74.93, 74.90 (2 × O*C*H_2Ph), 73.6 (C-3′), 73.5, 73.2 (2 × O*C*H_2Ph), 72.9 (C-5′), 70.0 (octenyl OCH_2), 68.8 (C-4′), 68.7 (C-6′), 68.4 (C-6), 33.8, 29.7, 29.0, 28.9, 26.1 (5 × octenyl CH_2); HRMS-ESI *m/z* $[M + Na]^+$ calcd for

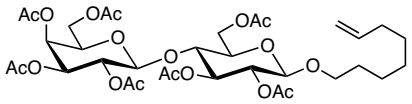
$C_{55}H_{66}NaO_{11}$: 925.4497, found: 925.4489.



2,3,4,6-Tetra-*O*-acetyl-D-galactopyranosyl trichloroacetimidate (2.4): D-Galactose (5 g, 28 mmol) was dissolved in pyridine (70 mL) and cooled to 0 °C before Ac_2O (15 mL, 160 mmol) was added. After stirring overnight at room temperature, the reaction mixture was concentrated, diluted with EtOAc (300 mL) and washed with water, saturated aqueous $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4, filtered, concentrated to dryness. The resulting residue was dissolved in DMF (100 mL), to which hydrazine acetate (2.90 g, 31.5 mmol) was added. After stirring at room temperature for 4 h, the solution was diluted with EtOAc (400 mL) and washed with water and brine. The organic layer was dried over Na_2SO_4, filtered and concentrated to dryness to afford crude **2.9**. Subsequent, all the crude **2.9** and CCl_3CN (17.54 mL, 175 mmol) were dissolved in dry CH_2Cl_2 (100 mL) and cooled to 0 °C before DBU (0.04 mL, 0.25 mmol) and added. After stirring at room temperature for 4 h, the solution was concentrated and the residue was subjected to flash chromatography (hexanes–EtOAc 3:1) to yield **2.4** (7.10 g, 52% over three steps) as a white solid. ^{1}H NMR (500 MHz; $CDCl_3$) of α anomer: δ 8.66 (s, 1H, NH), 6.60 (d, J = 3.5 Hz, 1H, H-1), 5.56 (dd, J = 3.1, 1.0 Hz, 1H, H-4), 5.42 (dd, J = 10.9, 3.2 Hz, 1H, H-3), 5.36 (dd, J = 10.9, 3.5 Hz, 1H, H-2), 4.45–4.42 (m, J = 6.7 Hz, 1H, H-5), 4.16 (dd, J = 11.3, 6.6 Hz, 1H, H-6a), 4.08 (dd, J = 11.3, 6.6 Hz, 1H, H-6b), 2.16 (s, 3H, OAc), 2.02 (s, 3H, OAc), 2.014 (s, 3H, OAc), 2.009 (s, 3H, OAc). The ^{1}H NMR spectral data were consistent with that reported.[117]



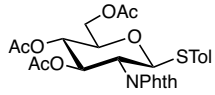
***p*-Tolyl 6-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (2.6):** Benzylidene acetal **2.12** (1.82 g, 3.62 mmol) and $BH_3 \cdot NMe_3$ (1.06 g, 14.50 mmol) were dissolved in THF (26 mL) and cooled to 0 °C, then an ice-cold solution of $AlCl_3$ (2.90 g, 21.74 mmol) in THF (10 mL) was added. After stirring at room temperature for 3 h, the solution was concentrated, dissolved in EtOAc (200 mL) and washed with saturated aqueous $NaHCO_3$, water and brine. The organic layer was dried over Na_2SO_4, filtered, concentrated and the residue was subjected to flash chromatography (hexane–EtOAc 3:4) to afford diol **2.6** (1.50 g, 82%) as a white solid. R_f = 0.29 (hexane–EtOAc 3:4); $[\alpha]_D$ = +16.3 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (500 MHz; $CDCl_3$): δ 7.87–7.85 (m, 2H, Ar), 7.75–7.74 (m, 2H, Ar), 7.40–7.29 (m, 7H, Ar), 7.04–7.02 (m, 2H, Ar), 5.56 (d, *J* = 10.3 Hz, 1H, H-1), 4.63, 4.58 (ABq, *J* = 11.4 Hz, 2H, 2 × OC*H*$_2$Ph), 4.35 (dd, *J* = 10.3, 8.3 Hz, 1H, H-3), 4.21 (app t, *J* = 10.3 Hz, 1H, H-2), 3.85 (dd, *J* = 10.5, 4.5 Hz, 1H, H-6a), 3.81 (dd, *J* = 10.5, 4.5 Hz, 1H, H-6b), 3.68–3.60 (m, 2H, H-4, H-5), 2.30 (s, 3H, ArMe); ^{13}C NMR (126 MHz; $CDCl_3$): δ 138.2 (Ar), 137.7 (Ar), 134.2 (Ar), 133.3 (Ar), 131.7 (Ar), 129.6 (Ar), 128.5 (Ar), 128.2 (Ar), 127.9 (Ar), 127.8 (Ar), 83.8 (C-1), 77.8 (C-5), 73.8 (OC*H*$_2$Ph), 73.6 (C-4), 72.8 (C-3), 70.5 (C-6), 55.4 (C-2), 21.1 (ArMe); HRMS-ESI *m/z* $[M + Na]^+$ calcd for $C_{28}H_{27}NNaO_6S$: 528.1451, found: 528.1451.



7-Octen-1-yl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (2.7): D-Lactose (10.00 g, 29.2 mmol) and NaOAc (2.5 g, 30.5 mmol)

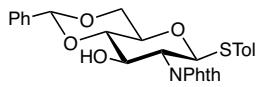
were heated in acetic anhydride (80 mL) at 100 °C for 5 h. Then the solution was allowed to cool to room temperature and poured into ice-water (1 L). The precipitate was filtered and recrystallized from EtOAc–hexane to yield peracetylated lactose (15.60 g, 79%) as colorless crystals. A portion of this compound (6.78 g, 10 mmol), 7-octen-1-ol (2.4 mL, 16 mmol) and 4 Å molecular sieves was dissolved in CH_2Cl_2 (80 mL) and stirred at at room temperature for 0.5 h. Then the solution was cooled to 0 °C and $BF_3{\cdot}Et_2O$ (3.8 mL, 30 mmol) was added. After stirring at room temperature for 24 h, Et_3N (5 mL) was added and the reaction mixture was filtered through Celite. The filtrate was concentrated and the residue was subjected to flash chromatography (hexane–EtOAc 4:3) to afford **2.7** (3.2 g, 43%) as white foam. R_f = 0.39 (hexane–EtOAc 1:1); $[\alpha]_D$ = −11.6 (*c* = 1.1, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.79 (ddt, *J* = 17.0, 10.3, 6.7 Hz, 1H, C*H*=CH_2), 5.34 (dd, *J* = 3.4, 1.1 Hz, 1H, H-4′), 5.19 (app t, *J* = 9.5 Hz, 1H, H-3), 5.10 (dd, *J* = 10.4, 7.9 Hz, 1H, H-2′), 5.01–4.91 (m, 3H, CH=CH_2, H-3′), 4.88 (dd, *J* = 9.5, 8.0 Hz, 1H, H-2), 4.49–4.46 (m, 2H, H-1′, H-6a), 4.45 (d, *J* = 8.0 Hz, 1H, H-1), 4.15–4.06 (m, 3H, H-6b, H-6a′, H-6b′), 3.88–3.85 (m, 1H, H-5′), 3.82 (dt, *J* = 9.7, 6.8 Hz, 1H, octenyl OCH_2), 3.79 (app t, *J* = 9.5 Hz, 1H, H-4), 3.59 (ddd, *J* = 9.9, 5.1, 2.1 Hz, 1H, H-5), 3.44 (dt, *J* = 9.7, 6.8 Hz, 1H, octenyl OCH_2), 2.15 (s, 3H, OAc), 2.11 (s, 3H, OAc), 2.06–2.03 (m, 14H, 4 × OAc, $OCH_2CH_2(CH_2)_3CH_2CH{=}CH_2$), 1.96 (s, 3H, OAc), 1.57–1.51 (m, 2H, $OCH_2CH_2(CH_2)_3CH_2CH{=}CH_2$), 1.39–1.25 (m, 6H, $OCH_2CH_2(CH_2)_3CH_2CH{=}CH_2$); ^{13}C NMR (126 MHz, $CDCl_3$): δ 170.4 (C=O), 170.3 (C=O), 170.1 (C=O), 170.0 (C=O), 169.8 (C=O), 169.6 (C=O), 169.1 (C=O), 139.0 (*C*H=CH_2), 114.3 (CH=*C*H_2), 101.1 (C-1′), 100.6 (C-1), 76.4 (C-4), 72.9 (C-3), 72.6 (C-5), 71.8 (C-2), 71.0 (C-3′), 70.7 (C-5′), 70.2 (octenyl OCH_2), 69.1 (C-2′), 66.6 (C-4′), 62.1 (C-6), 60.8 (C-6′), 33.7, 29.4, 28.84, 28.77, 25.7 (5 ×

octenyl CH_2); 20.9, 20.8, 20.71, 20.66, 20.65 (2C), 20.5 (7 × OAc); HRMS-ESI *m/z* [M + Na]$^+$ calcd for $C_{34}H_{50}NaO_{18}$: 769.2889, found: 769.2880.



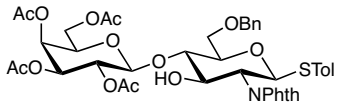
***p*-Tolyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (2.10)**: To a solution of D-glucosamine hydrochloride (10 g, 46.4 mmol) and NaOH (2.40 g, 60 mmol) in distilled water (50 mL) was added phthalic anhydride (7.56 g, 51 mmol). After stirring overnight at room temperature, the formed precipitate was filtered, washed with cold water and ether, and dried overnight under high vacuum to yield a desired intermediate (6.88 g, 48%). A portion of the intermediate (6.09 g, 19.7 mmol) and NaOAc (0.48 g, 5.9 mmol) were suspended in acetic anhydride (50 mL). After stirring at 100 °C for 5 h, the solution was cooled, diluted with EtOAc (300 mL) and washed with water, saturated aqueous $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4, filtered, concentrated to dryness to afford the acetylated intermediate in quantitative yield (9.4 g). Subsequently, a portion of the acetylated intermediate (8.32 g, 17.43 mmol) and *p*-thiocresol (2.60 g, 20.92 mmol) were dissolved in dry CH_2Cl_2 (120 mL). Then the solution was cooled to 0 °C and $BF_3{\cdot}Et_2O$ (4.5 mL) was added slowly. After stirring overnight, while warming to room temperature, the solution was neutralized with Et_3N, concentrated and the residue was subjected to flash chromatography (hexane–EtOAc 5:3) to afford **2.10** (8.15 g, 86%) as a white foam. R_f = 0.39 (hexane–EtOAc 1:1). $[\alpha]_D$ = +38.2 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.88–7.85 (m, 2H, Ar), 7.77–7.74 (m, 2H, Ar), 7.31–7.28 (m, 2H, Ar), 7.09–7.05 (m, 2H, Ar), 5.77 (dd, *J* = 10.2, 9.2 Hz, 1H, H-3), 5.65 (d, *J* = 10.5 Hz, 1H, H-1), 5.12 (dd, *J* = 10.2, 9.2 Hz, 1H, H-4), 4.32 (t, *J* = 10.4 Hz, 1H, H-2),

4.28 (dd, J = 12.2, 5.0 Hz, 1H, H-6a), 4.20 (dd, J = 12.2, 2.4 Hz, 1H, H-6b), 3.88 (ddd, J = 10.2, 5.0, 2.4 Hz, 1H, H-5), 2.32 (s, 3H, ArMe), 2.10 (s, 3H, OAc), 2.01 (s, 3H, OAc), 1.83 (s, 3H, OAc); ^{13}C NMR (126 MHz, $CDCl_3$): δ 170.6 (C=O), 170.1 (C=O, 169.5 (C=O), 138.8 (Ar), 134.0 (Ar), 129.7 (Ar), 127.0 (Ar), 123.7 (Ar), 83.2 (C-1), 75.9 (C-5), 71.7 (C-3), 68.8 (C-4), 62.2 (C-6), 53.7 (C-2), 21.2 (ArMe), 20.8, 20.7, 20.4 (3C, 3 × OAc); HRMS-ESI m/z $[M + Na]^+$ calcd for $C_{27}H_{27}NNaO_9S$: 564.1299, found: 564.1298.



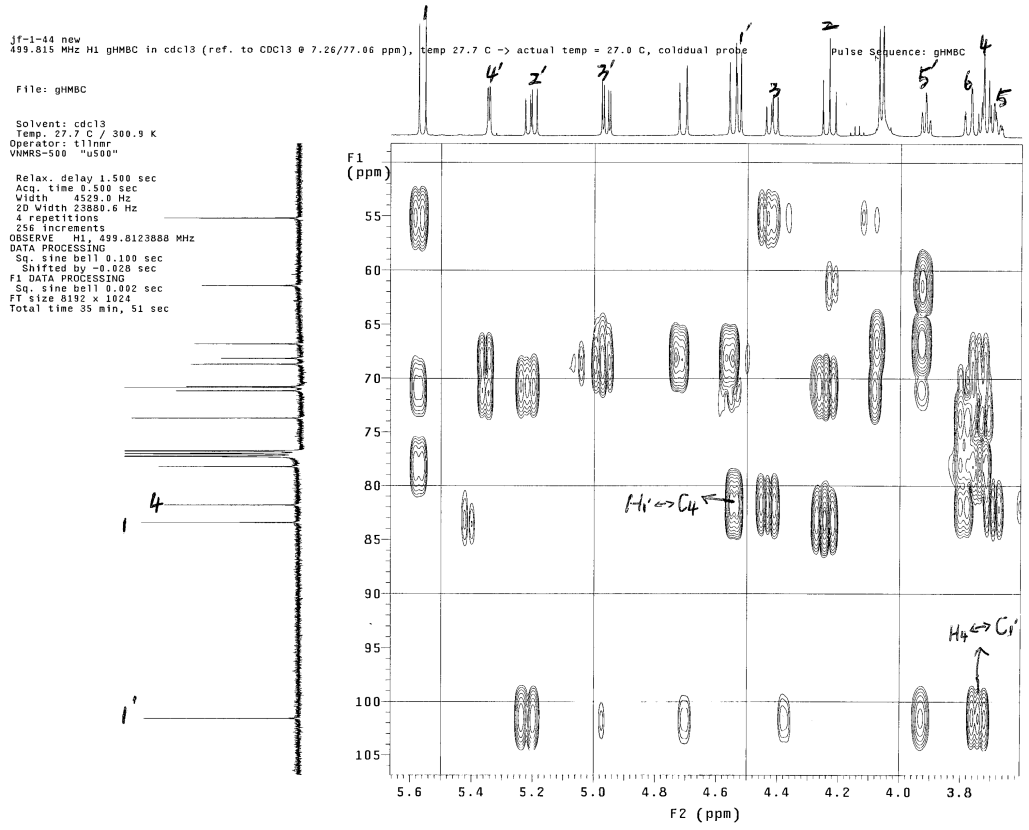
***p*-Tolyl 4,6-*O*-benzylidene-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (2.12)**: To a solution of **2.10** (5.42 g, 10.00 mmol) in 1:3 CH_2Cl_2–CH_3OH (80 mL) was added 1.0 M $NaOCH_3$ (1.5 mL). After stirring at room temperature for 30 minutes, the solution was neutralized with Amberlite IR120 H^+ ion-exchange resin. The resulting mixture was filtered and the filtrate was concentrated and dried to afford crude **2.11**. Without further purification, all the **2.11** and *p*-TsOH (0.19 g) were suspended in a mixture of dry MeCN (70 mL) and benzaldehyde dimethyl acetal (6 mL). After stirring overnight at room temperature, the solution was neutralized with Et_3N (1 mL), concentrated and the residue was subjected to flash chromatography (hexane–EtOAc 2:1) to afford **2.12** (4.46 g, 89% over two steps) as a white foam. R_f = 0.30 (hexane–EtOAc 2:1); $[\alpha]_D$ = +33.6 (c = 0.9, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.94–7.81 (m, 2H, Ar), 7.78–7.72 (m, 2H, Ar), 7.50–7.45 (m, 2H, Ar), 7.40–7.35 (m, 3H, Ar), 7.30–7.27 (m, 2H, Ar), 7.09–7.05 (m, 2H, Ar), 5.63 (d, J = 10.5 Hz, 1H, H-1), 5.56 (s, 1H, PhCH(O)$_2$), 4.63 (t, J = 9.5 Hz, 1H, H-3), 4.40 (dd, J = 10.5, 4.9 Hz, 1H, H-6a), 4.31 (t, J = 10.3 Hz, 1H, H-2), 3.82 (t, J = 10.2 Hz, 1H, H-6b), 3.69 (td, J = 9.7, 4.9 Hz, 1H, H-5), 3.59 (t, J = 9.2 Hz, 1H, H-4), 2.40 (s, 1H, br

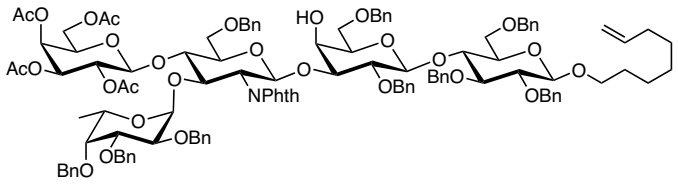
s, 3-OH), 2.31 (s, 3H, ArMe); ^{13}C NMR (126 MHz, $CDCl_3$): δ 168.3 (C=O), 167.6 (C=O), 138.5 (Ar), 136.9 (Ar), 134.2 (Ar), 133.4 (Ar), 131.74 (Ar), 131.66 (Ar), 129.7 (Ar), 129.4 (Ar), 128.4 (Ar), 127.8 (Ar), 126.3 (Ar), 123.9 (Ar), 123.4 (Ar), 102.0 (Ph*C*H(O)$_2$), 84.5 (C-1), 82.0 (C-4), 70.3 (C-5), 69.8 (C-3), 68.6 (C-6), 55.7 (C-2), 21.2 (ArMe); HRMS-ESI *m/z* $[M + Na]^+$ calcd for $C_{28}H_{25}NNaO_6S$: 526.1295, found: 526.1295.



***p*-Tolyl 2,3,4-6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-6-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (2.13):** A mixture of trichloroacetimidate **2.4** (1.80 g, 3.65 mmol), diol **2.6** (1.76 g 3.48 mmol) and powdered 4 Å molecular sieves was suspended in CH_2Cl_2 (40 mL) and stirred at room temperature for 1 h. The solution was then cooled to –40 °C, and then TMSOTf (63 μL) was added drop wise. After stirring at –40 °C for 2 h, the mixture was allowed to warm to room temperature. Then, Et_3N (1 mL) was added and the mixture was filtered through Celite. The filtrate was concentrated and the residue was subjected to flash chromatography (hexane–EtOAc 4:3) to afford **2.13** (2.21 g, 70%) as a white foam. R_f = 0.39 (hexane–EtOAc 1:1); $[\alpha]_D$ = +18.5 (*c* = 0.9, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.88–7.82 (m, 2H, Ar), 7.75–7.71 (m, 2H, Ar), 7.41–7.37 (m, 2H, Ar), 7.36–7.30 (m, 5H, Ar), 7.02–6.99 (m, 2H, Ar), 5.53 (d, *J* = 10.5 Hz, 1H, H-1), 5.31 (dd, *J* = 3.5, 1.0 Hz, 1H, H-4′), 5.18 (dd, *J* = 10.5, 8.0 Hz, 1H, H-2′), 4.93 (dd, *J* = 10.5, 3.5 Hz, 1H, H-3′), 4.68, 4.52 (ABq, *J* = 12.0 Hz, 2H, 2 × OC*H*$_2$Ph), 4.50 (d, *J* = 8.0 Hz, 1H, H-1′), 4.39 (dd, *J* = 10.5, 8.0 Hz, 1H, H-3), 4.20 (app t, *J* = 10.5 Hz, 1H, H-2), 4.05–4.00 (m, 3H, H-6a′, H-6b′, 3-OH), 3.89 (dt, *J* = 6.5, 1.0 Hz, 1H, H-5′), 3.76–3.64 (m,

4H, H-4, H-5, H-6a, H-6b), 2.27 (s, 3H, ArMe), 2.11 (s, 3H, OAc), 2.00 (s, 3H, OAc), 1.97 (s, 3H, OAc), 1.89 (s, 3H, OAc); ^{13}C NMR (126 MHz, $CDCl_3$): δ 170.4 (C=O), 170.1 (C=O), 169.9 (C=O), 169.2 (C=O), 168.2 (C=O), 167.5 (C=O), 138.3 (Ar), 138.2 (Ar), 134.1 (Ar), 133.7 (Ar), 131.9 (Ar), 131.8 (Ar), 129.6 (Ar), 128.5 (Ar), 127.84 (Ar), 127.81 (Ar), 123.6 (Ar), 123.3 (Ar), 101.6 (C-1′), 83.4 (C-1), 81.8 (C-4), 78.2 (C-5), 73.7 (O*C*H_2Ph), 71.2 (C-5′), 70.87 (C-3), 70.78 (C-3′), 68.7 (C-2′), 68.2 (C-6), 66.8 (C-4′), 61.4 (C-6′), 55.2 (C-2), 21.1, 20.7, 20.6, 20.5, 20.3 (5C, 4 × OAc, ArMe); HRMS-ESI *m/z* [M + Na]$^+$ calcd for $C_{42}H_{45}NNaO_{15}S$: 858.2402, found: 858.2395. The regioselectivity of this glycosylation was confirmed from the following HMBC spectrum.

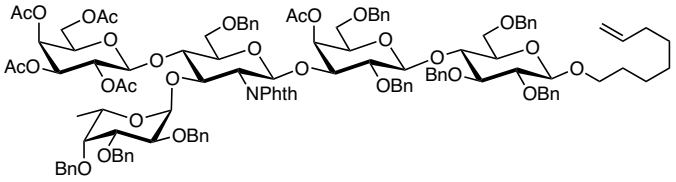




7-Octen-1-yl 2,3,4-6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-[2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl-(1→3)]-6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→3)-2,6-di-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (2.15): To a solution of trisaccharide **2.2** (809 mg, 0.65 mmol) in 9:1 acetone–water (7 mL) was added NBS (287 mg, 1.62 mmol) at 0 °C. After stirring at 0 °C for 0.5 h, saturated aqueous $NaHCO_3$ (2 mL) was added. The solution was concentrated, and then the residue was dissolved in EtOAc (80 mL) and washed with water and brine. The organic layer was dried over Na_2SO_4, filtered, concentrated and the residue was subjected to flash chromatography (hexane–EtOAc 4:5) to afford **2.14** (590 mg, 80%) as a white solid. A solution of **2.14** (455 mg, 0.39 mmol) in CH_2Cl_2 (4 mL) was treated with trichloroacetonitrile (0.28 mL, 2.75 mmol) and catalytic amount of DBU, and stirred at room temperature for 4 h. Concentration and flash chromatography (hexanes–EtOAc 1:1) afforded trichloroacetimidate that was immediately used in the next step. The trichloroacetimidate achieved in previous step, diol **2.3** (354 mg, 0.39 mmol) and powdered 4 Å molecular sieves were suspended in CH_2Cl_2 (4 mL) and stirred at room temperature for 1 h. The solution was then cooled to −30 °C, to which TMSOTf (14 μL) was added. The mixture was allowed to warm to room temperature after stirring at −30 °C for 2 h, Et_3N (1 mL) was added and the mixture was filtered through Celite. The filtrate was concentrated and the resulting residue was subjected to flash chromatography (hexane–EtOAc 5:2) to afford **2.15** (390 mg, 49% over two steps) as a white foam. R_f = 0.46 (hexane–EtOAc 5:3);

$[\alpha]_D$ = +5.5 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.42–7.07 (m, 45H, Ar), 7.01 (d, *J* = 7.2 Hz, 2H, Ar), 6.79 (d, *J* = 7.2 Hz, 2H, Ar), 5.78 (ddt, *J* = 17.0, 10.3, 6.7 Hz, 1H, C*H*=CH_2), 5.32 (d, *J* = 8.5 Hz, 1H, H-1′′), 5.25 (dd, *J* = 3.5, 0.8 Hz, 1H, H-4′′′), 5.03 (dd, *J* = 10.4, 8.2 Hz, 1H, H-2′′′), 4.97 (dq, *J* = 17.1, 1.8 Hz, 1H, CH=CH_2), 4.93–4.89 (m, 2H, CH=CH_2, OCH_2Ph), 4.84–4.78 (m, 4H, H-1′′′′, H-3′′′, 2 × OCH_2Ph), 4.74–4.65 (m, 5H, H-1′′′, H-3′′, 3 × OCH_2Ph), 4.60–4.53 (m, 4H, H-5′′′′, 3 × OCH_2Ph), 4.48–4.42 (m, 4H, H-2′′, 3 × OCH_2Ph), 4.33 (A of ABq, *J* = 12.2 Hz, 2H, 2 × OCH_2Ph), 4.30–4.25 (m, 2H, H-1′, OCH_2Ph), 4.21–4.17 (m, 3H, H-1, 2 × OCH_2Ph), 4.16–4.12 (m, 3H, H-4′′, H-6a′′′, OCH_2Ph), 4.05 (br s, 1H, H-4′), 3.95 (dd, *J* = 10.9, 5.9 Hz, 1H, H-6b′′′), 3.85–3.81 (m, 4H, H-3′′′′, H-4, H-6a′′, octenyl OCH_2), 3.75 (dd, *J* = 12.2 Hz, 1H, H-2′′′′), 3.71–3.68 (m, 2H, H-6b′′, H-6a′), 3.61–3.58 (m, 3H, H-4′′′′, H-5′′, H-5′′′), 3.49 (dd, *J* = 9.6, 5.6 Hz, 1H, H-6b′), 3.42 (dd, *J* = 10.8 Hz, 4.2 Hz, 1H, H-6a), 3.42–3.35 (m, 5H, H-2′, H-3′, H-3, H-5′, octenyl OCH_2), 3.32–3.27 (m, 2H, H-2, H-6b), 2.96 (ddd, *J* = 9.6, 4.2, 1.8 Hz, 1H, H-5), 2.71 (br s, 1H, 4′-OH), 2.03–1.99 (m, 8H, 2 × OAc, $OCH_2CH_2(CH_2)_3CH_2CH=CH_2$), 1.96 (s, 3H, OAc), 1.85 (s, 3H, OAc), 1.62–1.55 (m, 2H, $OCH_2CH_2(CH_2)_3CH_2CH=CH_2$), 1.37–1.26 (m, 6H, $OCH_2CH_2(CH_2)_3CH_2CH=CH_2$), 1.19 (d, *J* = 6.6 Hz, 3H, H-6′′′′); ^{13}C NMR (126 MHz, $CDCl_3$): δ 170.1 (C=O), 170.0 (C=O), 169.9 (C=O), 168.7 (C=O), 139.10 (Ar), 139.06 (*C*H=CH_2), 138.8 (Ar), 138.75 (Ar), 138.65 (Ar), 138.57 (Ar), 138.56 (Ar), 138.4 (Ar), 138.2 (Ar), 137.6 (Ar), 133.9 (Ar), 131.3 (Ar), 128.7 (Ar), 128.4 (Ar), 128.27 (Ar), 128.25 (2C, Ar), 128.16 (Ar), 128.13 (Ar), 128.11 (Ar), 128.02 (Ar), 128.0 (Ar), 127.85 (Ar), 127.83 (Ar), 127.75 (Ar), 127.5 (Ar), 127.4 (Ar), 127.2 (2C, Ar), 127.1 (Ar), 127.0 (Ar), 126.6 (Ar), 126.3 (Ar), 123.3 (Ar), 114.2 (CH=*C*H_2), 103.5 (C-1), 102.0 (C-1′), 99.6

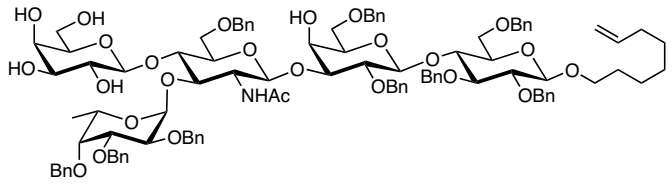
(C-1‴), 99.0 (C-1″), 97.6 (C-1⁗), 83.5 (C-3), 82.9 (C-2′), 81.8 (C-2), 79.7 (C-3⁗), 78.1 (C-3′), 77.2 (C-4⁗), 75.9 (C-4), 75.4 (O*C*H$_2$Ph), 75.3 (C-4″), 75.1 (C-5″), 74.9 (O*C*H$_2$Ph), 74.75, 74.72 (C-2⁗, C-5), 74.21, 74.16, 73.8, 73.4, 73.0, 72.9 (6 × O*C*H$_2$Ph), 72.64, 72.60 (C-3″, C-5′), 72.4 (O*C*H$_2$Ph), 71.0 (C-3‴), 70.5 (C-5‴), 69.9 (octenyl OCH$_2$), 69.1 (C-2‴), 68.5, 68.0, 67.9 (C-6, C-6′, C-6″), 67.5 (C-4′), 66.8 (C-4‴), 66.6 (C-5⁗), 60.3 (C-6‴), 56.2 (C-2″), 33.7, 29.7, 28.9, 28.8, 26.0 (5 × octenyl CH$_2$), 20.7, 20.62, 20.56, 20.5 (4 × OAc), 16.8 (C-6⁗); HRMS-ESI *m/z* $[M + Na]^+$ calcd for $C_{117}H_{131}NNaO_{30}$: 2052.8648, found: 2052.8612.



7-Octen-1-yl 2,3,4-6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-[2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl-(1→3)]-6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→3)-4-*O*-acetyl-2,6-di-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (2.15′): A solution of **2.15** (10 mg) in pyridine (1 mL) and acetic anhydride (0.5 mL) was stirred overnight, and then concentrated and the residue was subjected to flash chromatography (hexane–EtOAc 5:2) to afford **2.15′** (9.5 mg, 93%) as a white foam. R_f = 0.45 (hexane–EtOAc 5:3); $[\alpha]_D$ = +4.2 (*c* = 0.8, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.45–7.09 (m, 45H, Ar), 7.02–7.01 (m, 2H, Ar), 6.87–6.85 (m, 2H, Ar), 5.80 (ddt, *J* = 17.0, 10.3, 6.7 Hz, 1H, C*H*=CH$_2$), 5.46 (dd, *J* = 3.6, 0.6 Hz, 1H, H-4′), 5.28 (dd, *J* = 3.6, 1.0 Hz, 1H, H-4‴), 5.26 (d, *J* = 8.2 Hz, 1H, H-1″), 5.04 (dd, *J* = 10.4, 8.2 Hz, 1H, H-2‴), 5.00–4.97 (m, 1H, CH=C*H*$_2$), 4.95–4.92 (m, 2H, CH=C*H*$_2$, OC*H*$_2$Ph), 4.90 (A

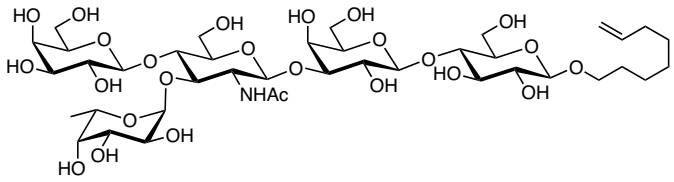
of ABq, J = 10.5 Hz, 1H, OCH_2Ph), 4.86–4.83 (m, 3H, H-1′′′, H-3′′′, OCH_2Ph), 4.81, 4.57 (ABq, J = 11.8 Hz, 2H, 2 × OCH_2Ph), 4.78 (d, J = 3.6 Hz, 1H, H-1′′′′), 4.75 (dd, J = 10.2, 9.0 Hz, 1H, H-3′′), 4.69–4.63 (m, 5H, H-5′′′′, 4 × OCH_2Ph), 4.55 (A of ABq, J = 11.7 Hz, 1H, OCH_2Ph), 4.47 (A of ABq, J = 12.2 Hz, 1H, OCH_2Ph), 4.43–4.40 (m, 3H, H-2′′, 2 × OCH_2Ph), 4.30–4.26 (m, 3H, H-1′, 2 × OCH_2Ph), 4.23–4.15 (m, 5H, H-1, H-4′′, H-6a′′′, 2 × OCH_2Ph), 4.00 (A of ABq, J = 11.8 Hz, 1H, OCH_2Ph), 3.97 (dd, J = 10.8, 5.7 Hz, 1H, H-6b′′′), 3.93 (dd, J = 5.4, 3.0 Hz, 1H, H-6a′′), 3.89–3.83 (m, 4H, H-3′′′′, H-4, H-6b′′, octenyl OCH_2), 3.77 (dd, J = 10.2, 3.7 Hz, 1H, H-2′′′′), 3.63–3.60 (m, 2H, H-4′′′′, H-5′′′), 3.56–3.52 (m, 2H, H-3′, H-5′′), 3.47–3.41 (m, 3H, H-5′, H-6a, octenyl OCH_2), 3.38 (m, 2H, H-3, H-6a′), 3.33–3.29 (m, 4H, H-2, H-2′, H-6b, H-6b′), 2.98–2.96 (ddd, J = 9.6, 3.6, 1.8 Hz, 1H, H-5), 2.09 (s, 3H, OAc), 2.05 (s, 3H, OAc), 2.03–2.02 (m, 5H, OAc, $OCH_2CH_2(CH_2)_3CH_2CH{=}CH_2$), 1.97 (s, 3H, OAc), 1.85 (s, 3H, OAc), 1.63–1.59 (m, 2H, $OCH_2CH_2(CH_2)_3CH_2CH{=}CH_2$), 1.39–1.29 (m, 6H, $OCH_2CH_2(CH_2)_3CH_2CH{=}CH_2$), 1.21 (d, J = 6.0 Hz, 3H, C-6′′′′); ^{13}C NMR (126 MHz; $CDCl_3$): δ170.02 (C=O), 170.01 (C=O), 169.96 (C=O), 169.86 (C=O), 168.8 (C=O), 139.12 (Ar), 139.05 ($CH{=}CH_2$), 138.89 (Ar), 138.69 (Ar), 138.68 (Ar), 138.4 (Ar), 138.28 (Ar), 138.27 (Ar), 138.23 (Ar), 138.19 (Ar), 133.8 (Ar), 131.3 (Ar), 128.5 (Ar), 128.31 (Ar), 128.26 (Ar), 128.15 (Ar), 128.13 (Ar), 128.09 (Ar), 128.01 (Ar), 127.94 (Ar), 127.92 (Ar), 127.90 (Ar), 127.83 (Ar), 127.79 (2C, Ar), 127.78 (Ar), 127.53 (Ar), 127.48 (Ar), 127.41 (Ar), 127.3 (Ar), 127.13 (Ar), 127.11 (Ar), 127.00 (Ar), 126.9 (Ar), 126.4 (Ar), 123.3 (Ar), 114.2 ($CH{=}CH_2$), 103.6 (C-1), 102.0 (C-1′), 99.5 (C-1′′′), 99.1 (C-1′′), 97.2 (C-1′′′′), 82.6 (C-3), 81.6, 78.84, 78.79 (C-2, C-2′, C-3′), 79.7 (C-3′′′′), 77.2 (C-4′′′′), 75.7 (C-4), 75.4 (C-5′′), 75.2 (OCH_2Ph), 74.96 (C-4′′),

74.92 (O*C*H$_2$Ph), 74.7, 74.5 (C-5, C-2′′′′), 74.20, 74.15, 73.6, 73.50, 73.0, 72.7 (6 × O*C*H$_2$Ph), 72.6 (C-5′), 72.4 (O*C*H$_2$Ph), 72.0 (C-3′′), 71.0 (C-3′′′), 70.4 (C-5′′′), 69.94 (C-4′), 69.90 (octenyl OCH$_2$), 69.0 (C-2′′′), 68.3, 67.7, 67.6 (C-6, C-6′, C-6′′), 66.8 (C-4′′′), 66.4 (C-5′′′′), 60.2 (C-6′′′), 56.6 (C-2′′), 33.7, 29.7, 28.9, 28.8, 26.0 (5 × octenyl CH$_2$), 20.83, 20.75, 20.64, 20.56, 20.54 (5 × OAc), 16.7 (C-6′′′′); HRMS-ESI *m/z* [M + Na]$^+$ calcd for $C_{119}H_{133}NNaO_{31}$: 2094.8754, found: 2094.8751.



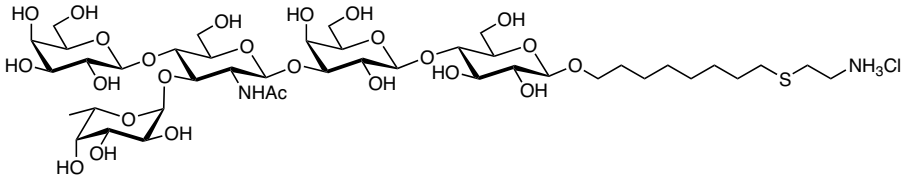
7-Octen-1-yl β-D-galactopyranosyl-(1→4)-[2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl-(1→3)]-6-*O*-benzyl-2-deoxy-2-acetamido-β-D-glucopyranosyl-(1→3)-2,6-di-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (2.16): A solution of pentasaccharide **2.15** (352 mg, 0.17 mmol) in 1-butanol (15 mL) was treated with ethylenediamine (3 mL), followed by stirring at 100 °C for 20 h. The solution was concentrated to dryness. The crude residue was dissolved in 1:2 CH_2Cl_2–CH_3OH (6 mL), to which acetic anhydride (1 mL) and Et_3N (1 mL) were added. After stirring at room temperature for 5 h, the solution was concentrated, and the residue was subjected to flash chromatography (toluene–acetone 3:2). Further purification by C_{18} chromatography (1:1 CH_3OH–water → 100% CH_3OH) afforded **2.16** (255 mg, 83%) as a white foam. R_f = 0.51 (toluene–acetone 4:5); $[\alpha]_D$ = −19.6 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.42–7.19 (m, 45H), 5.80 (ddt, *J* = 17.0, 10.3, 6.7 Hz, 1H, C*H*=CH$_2$), 5.67 (d, *J* = 7.2 H, 1H, NH), 5.16 (d, *J* = 7.5 Hz, 1H, H-1′′), 5.05 (d, *J* = 3.6 Hz, 1H, H-1′′′′), 5.00–4.97 (m, 2H,

CH=CH_2, OCH_2Ph), 4.94–4.87 (m, 4H, CH=CH_2, 3 × OCH_2Ph), 4.74–4.70 (m, 4H, 4 × OCH_2Ph), 4.66–4.63 (m, 2H, 2 × OCH_2Ph), 4.60–4.55 (m, 3H, 3 × OCH_2Ph), 4.53, 4.47 (ABq, J = 12.2 Hz, 2H, 2 × OCH_2Ph), 4.44–4.38 (m, 4H, H-1′, H-1‴, 2 × OCH_2Ph), 4.35–4.32 (m, 2H, H-1, H-3″), 4.29 (A of ABq, J = 12.0 Hz, 1H, OCH_2Ph), 4.12–4.08 (m, 2H, H-5⁗, OH), 4.05–4.01 (m, 3H, H-2⁗), 3.94–3.86 (m, 5H, H-3⁗, H-6a‴, octenyl OCH_2), 3.78–3.76 (m, 1H, H-4″), 3.74–3.65 (m, 6H, H-6b‴), 3.62–3.58 (m, 2H, H-4⁗), 3.54–3.46 (m, 7H, H-2′, H-2‴, H-3, H-3′, octenyl OCH_2), 3.44–3.33 (m, 4H, H-2, H-2″), 3.30–3.27 (m, 1H), 2.99–2.97 (m, 2H, OH), 2.72 (br s, 1H, OH), 2.05–2.01 (m, 2H, $OCH_2CH_2(CH_2)_3CH_2CH{=}CH_2$), 1.64–1.62 (m, 2H, $OCH_2CH_2(CH_2)_3CH_2CH{=}CH_2$), 1.40–1.32 (m, 9H, NHAc, $OCH_2CH_2(CH_2)_3CH_2CH{=}CH_2$), 1.11 (d, J = 6.0 Hz, 3H, H-6⁗); ^{13}C NMR (126 MHz, $CDCl_3$): δ 170.9 (C=O), 139.2 (Ar), 139.1 ($CH{=}CH_2$), 139.0 (Ar), 138.7 (Ar), 138.44 (Ar), 138.43 (Ar), 138.38 (Ar), 138.2 (Ar), 137.5 (Ar), 128.6 (Ar), 128.51 (Ar), 128.47 (Ar), 128.31 (Ar), 128.27 (Ar), 128.25 (Ar), 128.20 (Ar), 128.1 (Ar), 128.04 (2C, Ar), 128.01 (Ar), 127.91 (Ar), 127.86 (Ar), 127.71 (Ar), 127.67 (Ar), 127.66 (Ar), 127.63 (Ar), 127.53 (Ar), 127.51 (Ar), 127.46 (Ar), 127.39 (Ar), 127.37 (Ar), 127.2 (Ar), 114.2 (CH=CH_2), 103.6 (C-1), 102.2 (C-1′), 100.1 (C-1‴), 99.7 (C-1″), 98.0 (C-1⁗), 82.9 (C-3), 82.3 (C-2′), 81.9 (C-2), 79.4, 79.0 (C-3′, C-3⁗), 77.2 (C-4⁗), 76.6, 76.5, 76.4, 76.0, 75.3 (OCH$_2$Ph), 75.14, 75.08, 74.95 (OCH$_2$Ph), 74.93 (OCH$_2$Ph), 74.8, 74.7 (OCH$_2$Ph), 74.1 (OCH$_2$Ph), 73.7, 73.6, 73.4, 73.2 (3 × OCH$_2$Ph), 72.9, 72.4 (OCH$_2$Ph), 71.7, 70.0, 69.9, 68.9, 68.4 (C-6, C-6′, C-6″, octenyl OCH_2), 69.3, 67.9, 67.5 (C-5⁗), 63.0 (C-6‴), 57.5 (C-2″), 33.7, 29.7, 28.95, 28.86, 26.0 (5 × octenyl CH_2), 22.9 (NHAc), 16.7 (C-6⁗); HRMS-ESI m/z $[M + Na]^+$ calcd for $C_{103}H_{123}NNaO_{25}$: 1796.8276, found: 1796.8254.



7-Octen-1-yl β-D-galactopyranosyl-(1→4)-[α-L-fucopyranosyl-(1→3)]-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (2.17): Sodium was added to freshly collected liquid ammonia (~ 8 mL) at −78 °C until the blue color of the solution persisted. Then, a solution of **2.16** (144 mg, 0.73 mmol) in THF (4 mL) and CH_3OH (30 μL) was added dropwise at −78 °C. After 2 h, CH_3OH (5 mL) was added and the solution was concentrated to dryness. The residue was dissolved in CH_3OH (30 mL), neutralized with Amberlite IR120 H^+ ion-exchange resin, filtered and concentrated. The crude residue was purified by C_{18} chromatography using gradient elution (100% water → 3:7 CH_3OH–water) to give **2.17** (76 mg, 97%) as a white solid. $[\alpha]_D$ = −45.6 (*c* = 0.9, CH_3OH); ^{1}H NMR (600 MHz, D_2O): δ 5.91 (ddt, *J* = 17.2, 10.4, 6.7 Hz, 1H, C*H*=CH_2), 5.11 (d, *J* = 4.0 Hz, 1H, H-1′′′′), 5.06–5.02 (m, 1H, CH=CH_2), 4.97–4.95 (m, 1H, CH=CH_2), 4.82 (q, *J* = 6.7 Hz, 1H, H-5′′′′), 4.70 (d, *J* = 8.3 Hz, 1H, H-1′′), 4.46 (d, *J* = 7.8 Hz, 1H, H-1′′′), 4.45 (d, *J* = 7.8 Hz, 1H, H-1′), 4.42 (d, *J* = 7.8 Hz, 1H, H-1), 4.14 (d, *J* = 3.5 Hz, 1H), 3.96–3.84 (m, 9H), 3.79–3.55 (m, 17H), 3.48 (dd, *J* = 9.8, 7.8 Hz, 1H, H-2′), 3.28 (dd, *J* = 9.6, 7.8 Hz, 1H, H-2′′′), 2.07–2.03 (m, 2H, $OCH_2CH_2(CH_2)_3CH_2CH=CH_2$), 2.01 (s, 3H, NHAc), 1.63–1.59 (m, 2H, $OCH_2CH_2(CH_2)_3CH_2CH=CH_2$), 1.41–1.30 (m, 6H, $OCH_2CH_2(CH_2)_3CH_2CH=CH_2$), 1.16 (d, *J* = 6.6 Hz, 3H, H-6′′′′); ^{13}C NMR (126 MHz, D_2O): δ 175.7 (C=O), 141.3 (*C*H=CH_2), 115.0 (CH=*C*H_2), 103.9 (C-1), 103.5, 103.0, 102.8 (C-1′, C-1′′, C-1′′′), 99.6 (C-1′′′′), 83.1, 79.4, 76.1, 75.9, 75.8, 75.7 (2C), 75.4, 74.1, 73.8,

73.5, 72.9, 72.0, 71.7, 70.9, 70.2, 69.33, 69.28, 68.7, 67.7 (C-5′′′′), 62.5, 61.9, 61.1, 60.6 (C-6, C-6′, C-6′′, C-6′′′), 57.0 (C-2′′), 34.0 (octenyl CH_2), 29.6 (octenyl CH_2), 29.0 (2C, octenyl CH_2), 25.8 (octenyl CH_2), 23.2 (NHAc), 16.3 (C-6′′′′); HRMS-ESI *m/z* $[M + Na]^+$ calcd for $C_{40}H_{69}NNaO_{25}$: 986.4051, found: 986.4047.



8-[(2-Aminoethyl)thio]-1-octyl β-D-galactopyranosyl-(1→4)-[α-L-fucopyranosyl-(1→3)]-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (2.18): Compound **2.17** (38 mg, 0.039 mmol) and cysteamine hydrochloride (44 mg, 0.39 mmol) were dissolved in dry CH_3OH (0.5 mL) in a quartz tube. The solution was degassed and the tube was filled with argon. After irradiation with UV light for 2.5 h, the solution was concentrated and the resulting residue was subjected to C_{18} chromatography using gradient elution (0.5% aqueous AcOH → 3:7 CH_3OH –0.5% aqueous AcOH) to afford the corresponding amine salt **2.18** (42 mg) in quantitative yield. $[\alpha]_D$ = –41.1 (*c* = 0.9, CH_3OH); ^{1}H NMR (600 MHz, D_2O): δ 5.11 (d, *J* = 3.9 Hz, 1H, H-1′′′′), 4.83–4.80 (m, 1H, H-5′′′′), 4.70 (d, *J* = 8.4 Hz, 1H, H-1′′), 4.46 (d, *J* = 7.8 Hz, 1H, H-1′′′), 4.45 (d, *J* = 7.8 Hz, 1H, H-1′), 4.42 (d, *J* = 7.8 Hz, 1H, H-1), 4.14 (d, *J* = 2.9 Hz, 1H), 3.96–3.84 (m, 9H), 3.79–3.55 (m, 17H), 3.48 (dd, *J* = 9.6, 7.8 Hz, 1H, H-2′), 3.29–3.26 (m, 1H, H-2′′′), 3.20 (t, *J* = 6.7 Hz, 2H, SCH_2CH_2N), 2.83 (t, *J* = 6.7 Hz, 2H, SCH_2CH_2N), 2.58 (t, *J* = 7.3 Hz, 2H, $SCH_2(CH_2)_6CH_2O$), 2.01 (s, 3H, NHAc), 1.63–1.56 (m, 4H, $OCH_2CH_2(CH_2)_4CH_2CH_2S$), 1.38–1.31 (m, 8H, $OCH_2CH_2(CH_2)_4CH_2CH_2S$), 1.16

(d, J = 6.6 Hz, 3H, H-6′′′′); ^{13}C NMR (126 MHz, D_2O): δ 175.7 (C=O), 103.9 (C-1), 103.5, 103.0, 102.8 (C-1′, C-1′′, C-1′′′), 99.6 (C-1′′′′), 83.1, 79.4, 76.1, 75.90, 75.86, 75.7 (2C), 75.5, 74.1, 73.8, 73.5, 72.9, 72.0, 71.7, 70.9, 70.2, 69.34, 69.27, 68.7, 67.7 (C-5′′′′), 62.5, 61.9, 61.1, 60.6 (C-6, C-6′, C-6′′, C-6′′′), 57.0 (C-2′′), 39.4 (SCH_2CH_2N), 31.7 ($SCH_2(CH_2)_6CH_2O$), 29.7 ($SCH_2(CH_2)_6CH_2O$), 29.5 (SCH_2CH_2N), 29.3, 29.1(2C), 28.8, 25.9 (5 × $SCH_2(CH_2)_6CH_2O$), 23.2 (NHAc), 16.3 (C-6′′′′); HRMS-ESI m/z $[M + H]^+$ calcd for $C_{42}H_{77}N_2O_{25}S$: 1041.4531, found: 1041.4517.

Preparation of HSA conjugate:

Compound **2.1** (1.5 mg) was dissolved in DMF (15 μL) and injected into a solution of HSA (1.5 mg) in phosphate buffer (0.3 mL, pH = 7.5). The reaction was left at room temperature for one day, and then the mixture was dialyzed against deionized water (5 × 4 L). A white solid was obtained after lyophilization. The degree of incorporation of the pentasaccharide into the glycoconjugate was determined to be 21 by MALDI-TOF mass spectrometry.

Chapter 3: Enzymatic synthesis of a panel of biantennary *N*- and *O*-linked glycans capped with A and B blood group antigens

3.1 Introduction

The discovery of the ABO histo-blood group system dates back to 1900,[127] but it was not until 1957 that the structures of the carbohydrate epitopes responsible for the classification of ABO blood types were elucidated by Morgan and Watkins.[128] The ABO(H) antigens are terminal oligosaccharides found not only on red blood cells but also in tissues and secretions in the form of glycoproteins, glycolipids or free oligosaccharides. These epitopes are synthesized by a series of specific glycosyltransferases (Figure 3.1A).[129,130] The H antigen is a disaccharide synthesized by the FucT-catalyzed transfer of a Fuc residue from GDP-Fuc to a β-Gal residue in α-(1→2) linkage.[129] The A and B antigens are trisaccharides built upon the H antigen core by the action of an α-(1→3)-GalNAcT (GTA) and an α-(1→3)-GalT (GTB), respectively. GTA is responsible for transferring GalNAc from UDP-GalNAc to the H antigen to generate the A antigen,[131] whereas GTB transfers Gal from UDP-Gal to the H antigen to form the B antigen.[132]

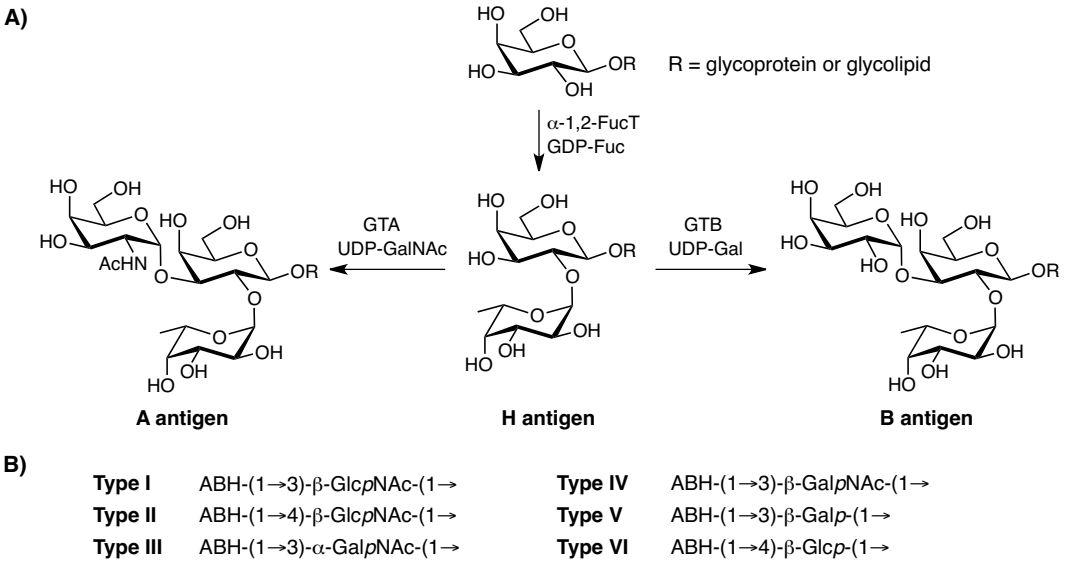


Figure 3.1 A) Biosynthesis of ABH blood group antigens; B) Definition of type I−type VI blood group antigen subtypes.

Based on the adjacent monosaccharide residue and position to which the antigen β-Gal moiety is linked, ABH antigens can be further classified into six subtypes, type I−type VI (Figure 3.1B).[129] To date, limited attention has been paid to unveiling the role these subtypes play in biology. Although a few reports have suggested that these subtypes are expressed differentially on erythrocytes and tissues,[133] the detailed expression profiles and biological importance of ABH subtypes remain to be further investigated.

The ABH antigens are the most important clinical consideration in organ transplantation. ABO blood group incompatibility between the donor and recipient will initiate rejection of the organ, a process that is caused by antibodies the patient has against foreign antigens on the organ. This rejection process results in a loss of the organ and/or patient death.[134] Despite these risks, worldwide shortages of organs for transplantation have stimulated many attempts to perform ABOi transplants. Improved immunosuppressive therapies and antibody removal strategies have led to some success in ABOi liver and kidney transplants.[135] In addition, it has been discovered that ABOi heart transplants during infancy can result in spontaneous development of immunological tolerance to donor A/B antigens, due to delayed production of anti-ABO antibodies during normal infancy.[136] Following an ABOi organ transplant, it is essential to monitor the presence of donor-specific antibodies (those that recognize the transplanted organ). The current clinical standard assay for measuring anti-ABO antibodies is erythrocyte agglutination, using reagent erythrocytes to screen the serum of patients for the presence of donor-specific antibodies. Data analysis of this assay assumes that ABH subtype antigens expressed by erythrocytes are identical to those in the organs, which neglects the possibility that subtypes are expressed differentially on erythrocytes and organs.

Recently, using synthetic type I−VI ABH antigens[137-139] (Figure 3.2) to generate subtype specific monoclonal antibodies, we obtained detailed expression profiles of subtype antigens and confirmed that they are expressed differentially between erythrocytes and organs.[107] Cardiac endothelium only expresses subtype II ABH antigens; thus, only antibodies against subtype II A or B antigens can be regarded as donor-specific (and therefore deleterious) in the context of ABOi heart transplants. Although subtype II antigens are present on erythrocytes of all blood groups, erythrocytes of A_1 and A_2 blood groups additionally express subtype III/IV antigens.[107] The erythrocyte agglutination assay cannot differentiate subtype-specific anti-ABO antibodies, and hence provides imprecise information about the presence of potentially deleterious antibodies in the serum of patients.

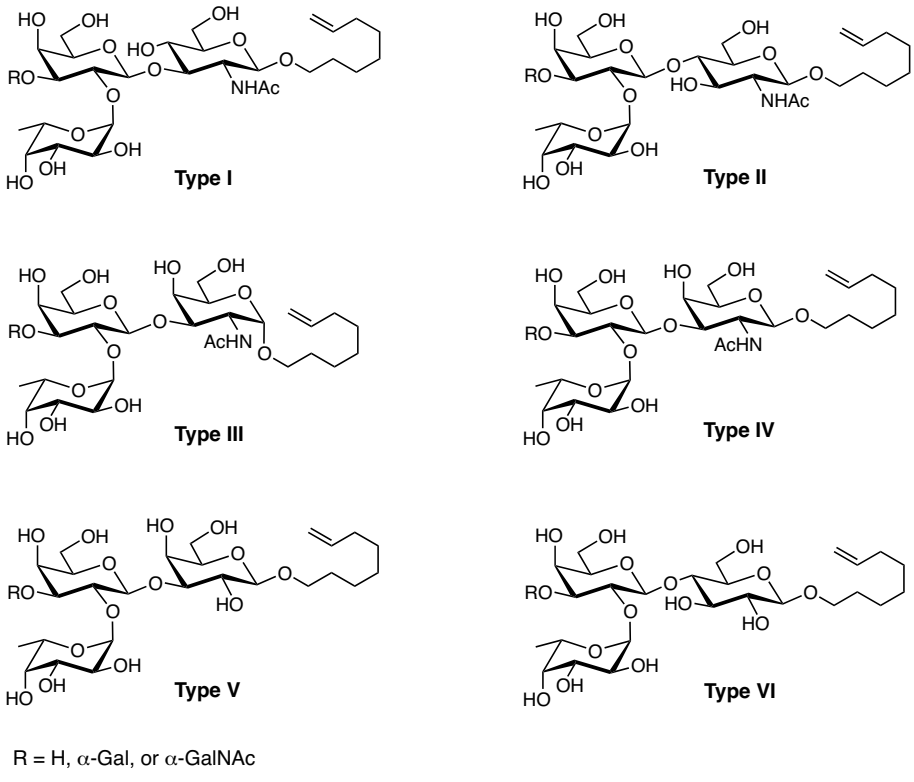


Figure 3.2 Structures of synthetic type I−VI ABH antigens synthesized by Meloncelli and Lowary.[137-139]

To address this important clinical issue, we created an ABO-glycan microarray containing synthetic type I−VI ABH antigens for the quantitative characterization of anti-ABO subtype-specific antibodies, and demonstrated the precise assessment of donor-specific antibodies responses in heart transplant patients. In particular, the results showed that patients that had undergone an ABOi heart transplant (*e.g.*, an O individual receiving an A heart) lacked antibodies against the subtype II antigens (*e.g.*, A-type II) but had normal levels against the subtype I and III–VI antigens. This suggests that the patient has developed tolerance specifically to the antigens found on the transplanted organ.[108]

Despite the success we have achieved, the ABH antigens used in the microarray are just the terminal tetrasaccharide (A, B) or trisaccharide (H) fragments of the natural antigen containing glycans, which are typically expressed at the ends of long poly-*N*-acetyl-lactosamine (poly-LacNAc) chains. The most common poly-LacNAc extension is composed of β-Gal-(1→4)-β-GlcNAc-(1→3) tandem repeats that extend *N*- and *O*-linked glycans. Increasing evidence shows that internal poly-LacNAc epitopes contribute to the biology mediated by glycan-binding proteins.[140-142]

Motivated by their biological importance, and the need to prepare structurally defined antigens containing poly-LacNAc chains, an enzymatic approach employing a β-(1→3)-*N*-acetylglucosaminyltransferase (β3GlcNAcT) from *Helicobacter pylori* (H. *pylori*)[143] in combination with recombinant bacterial or mammalian GalT has been developed for the efficient construction of poly-LacNAc extensions on various complex glycans.[80,144-146] Taking advantage of these advances, a panel of poly-LacNAc extended biantennary *N*- and *O*-linked glycans capped with blood group A or B antigens (Figure 3.3) were designed and their synthesis is described here. Given the size and complexity of the

molecules, I will use the Consortium for Functional Glycomics nomenclature (defined in Figure 3.3) in this chapter. As these are all enzymatic transformations, no protecting groups were involved. The goal was to prepare compounds that better mimic natural glycan structures, which could reveal the effect of structural complexity on antibody–antigen recognition. Because all of the enzymes and chemicals required for the synthesis are available in the Paulson laboratory at the Scripps Research Institute in La Jolla California, I conducted all the synthesis of these compounds in his lab as part of a collaboration. The obtained compounds will be incorporated into a glycan microarray to screen the serum samples from ABOi organ transplant patients. Moreover, these complex A/B antigens will provide additional insight into the binding specificities of monoclonal subtype specific antibodies, which have been developed by us and others.[107,147]

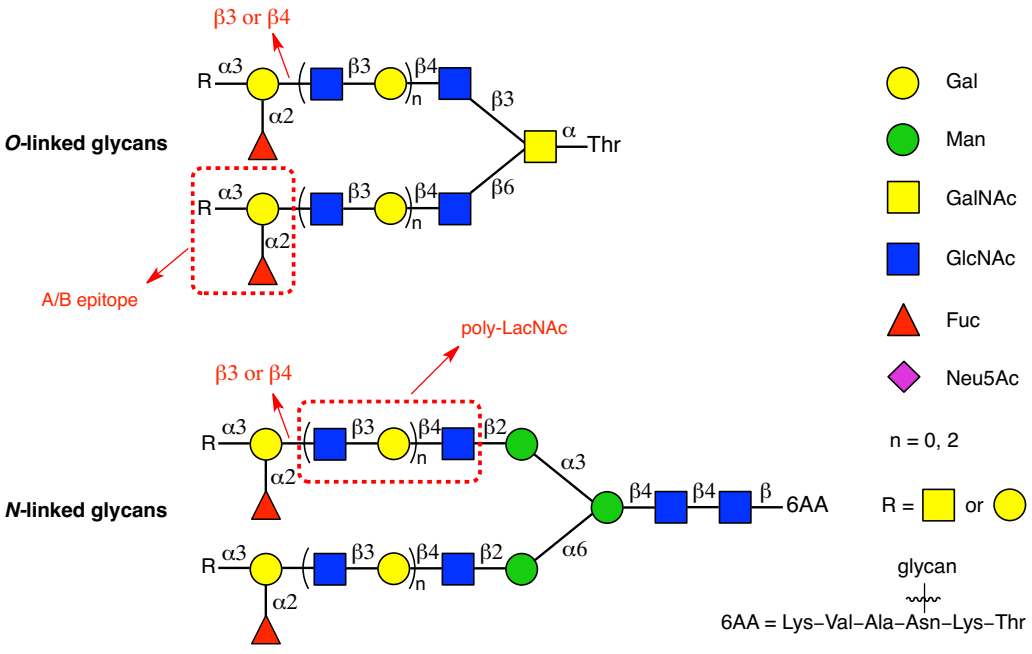


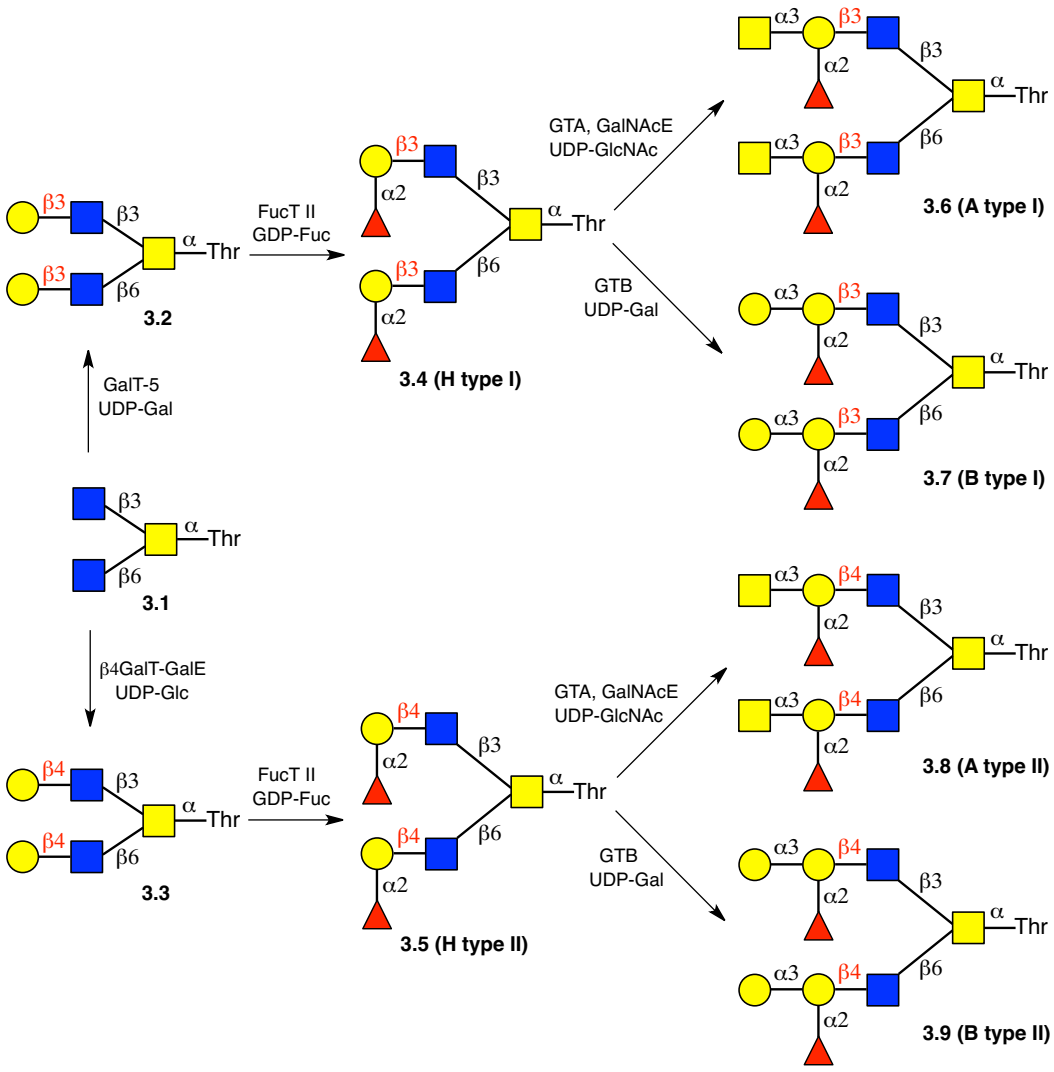
Figure 3.3 Structures of biantennary *N*- and *O*-linked glycans capped with A or B blood group antigens.

3.2 Results and discussion

3.2.1 Enzymatic synthesis of *O*-linked glycans capped with A/B antigen

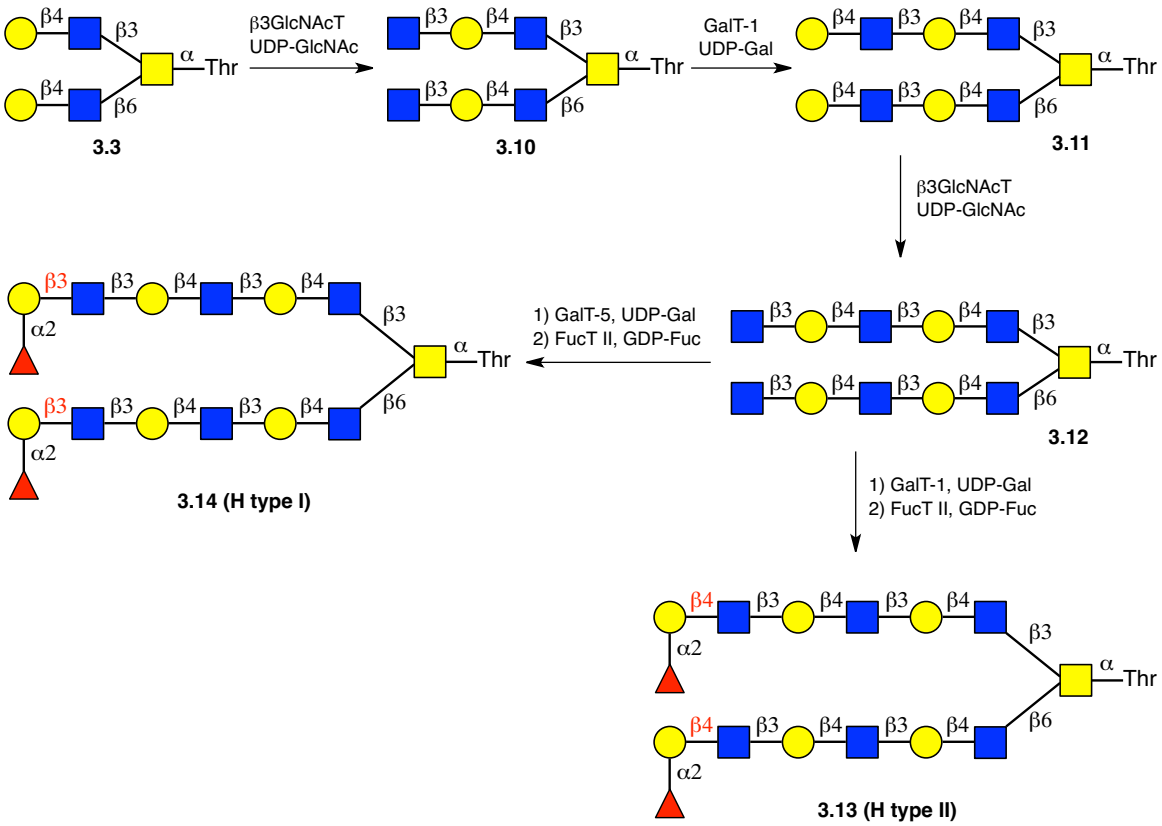
The commercially available threonine (Thr)-conjugated *O*-glycan core 4 trisaccharide **3.1**, β-GlcNAc-(1→3)-[β-GlcNAc-(1→6)]-α-GalNAc-Thr, served as the starting material for the enzymatic construction of the *O*-linked glycans. Scheme 3.1 describes the synthesis of four A/B antigens with short chains. First, incubation of **3.1** with recombinant human β-(1→3)-GalT (GalT-5)[148] and UDP-Gal afforded the type I precursor **3.2** with two terminal β-(1→3) linked Gal residues. Alternatively, the conversion of **3.1** to the type II precursor **3.3**, with two terminal Gal residues in β-(1→4) linkages, was catalyzed by a recombinant β-(1→4)-GalT/UDP-Gal 4'-epimerase (β4GalT/GalE) fusion protein,[149] which can generate UDP-Gal in situ from comparatively cheaper uridine diphosphate glucose (UDP-Glc), and then transfer the Gal from UDP-Gal to the terminal GlcNAc. Subsequently, fucosylation of **3.2** and **3.3** with an α-(1→2)-FucT (FucT II) and GDP-Fuc resulted in the formation of the H type I antigen **3.4** and H type II antigen **3.5**, respectively. In the last step, given the high price of UDP-GalNAc, a one-pot reaction combining UDP-GalNAc 4'-epimerase (GalNAcE)-catalyzed[150] conversion of UDP-GlcNAc to UDP-GalNAc and GTA[151] resulted in the transfer of GalNAc to H antigens. This was performed to yield the desired A type I antigen **3.6** and A type II antigen **3.8**. Similarly, treatment of the H antigens **3.4** and **3.5** with GTB[152] and UDP-Gal allowed the efficient construction of the desired B type I antigen **3.7** and B type II antigen **3.9**. It is worth noting that the GTA and GTB enzymes used here are bacterial homologues of human blood group glycosyltransferases, derived from *E. coli* O86 and *Helicobacter mustelae*, respectively. These bacterial transferases possess high activity and flexible substrate

specificity, and can be easily expressed in *E. coli*, making them attractive biocatalysts for synthesizing human blood group antigens.



Scheme 3.1 Enzymatic synthesis of A type I antigen **3.6**, B type I antigen **3.7**, A type II antigen **3.8** and B type II antigen **3.9** on an *O*-linked glycan scaffold.

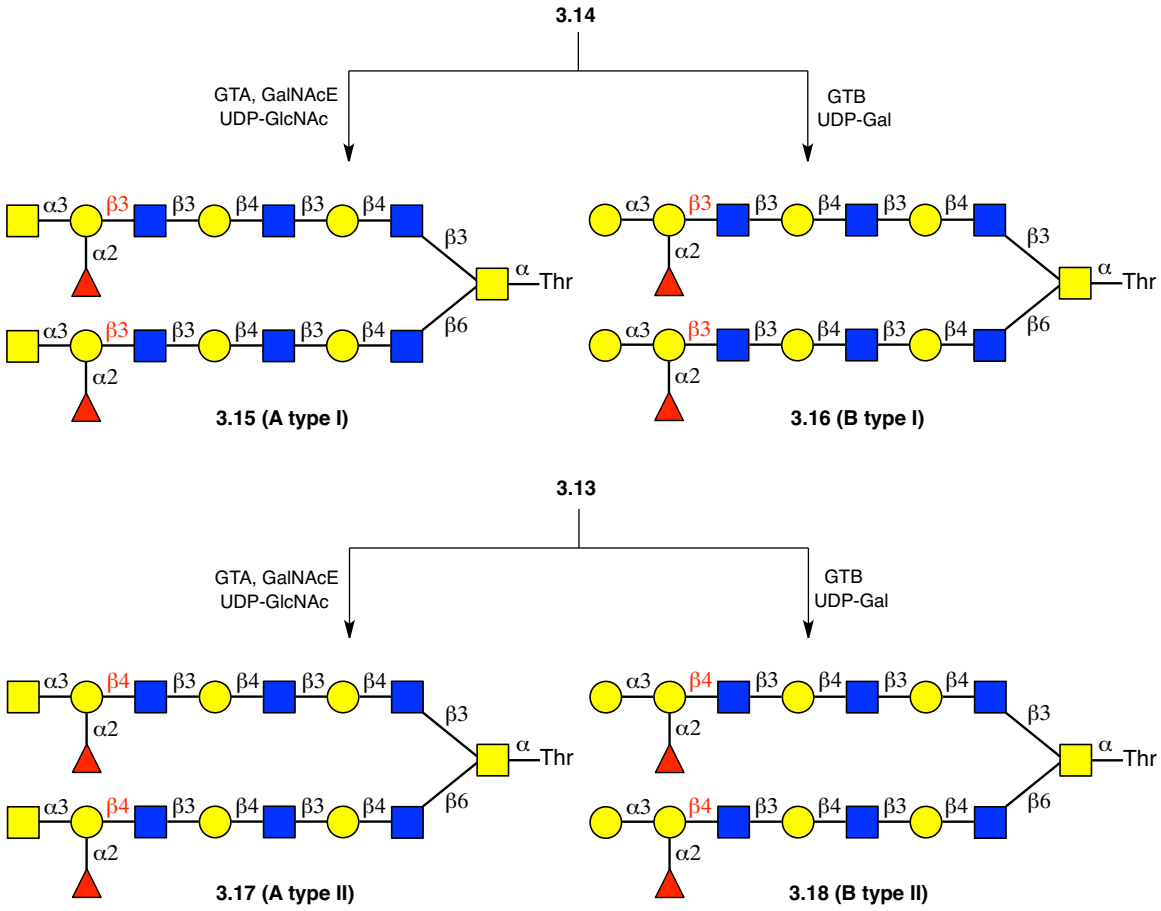
To synthesize A/B antigens that better mimic the natural glycans, LacNAc extensions were introduced by alternating reactions using β3GlcNAcT and bovine β-(1→4)-GalT (GalT-1) with the appropriate sugar nucleotides as illustrated in Scheme 3.2. GalT-1 has the same function as the β4GalT/GalE used previously in the synthesis of **3.3**. However, we chose to use GalT-1 in this case because it exhibits higher efficiency in the galactosylation of branched glycans such those shown in Scheme 3.2.[145]



Scheme 3.2 Enzymatic synthesis of H type II antigen **3.13** and H type I antigen **3.14** on an *O*-linked glycan scaffold. Key differences in linkages are indicated in red.

To prepare the target molecules, intermediate **3.3** was incubated with β3GlcNAcT and UDP-GlcNAc to form **3.10**, which underwent galactosylation catalyzed by GalT-1 to

give **3.11** with an extra LacNAc unit at each branch. Another round of LacNAc extension, followed by fucosylation provided access to the H type II antigen **3.13** Alternatively, extension of **3.11** by the sequential use of β3GlcNAcT, GalT-5 and FucT II resulted in the formation of the H type I antigen **3.14**. These two H antigens were then converted to the desired A type I antigen **3.15**, B type I antigen **3.16,** A type II antigen **3.17**, and B type II antigen **3.18** by using GTA or GTB (Scheme 3.3) and the appropriate sugar nucleotides.



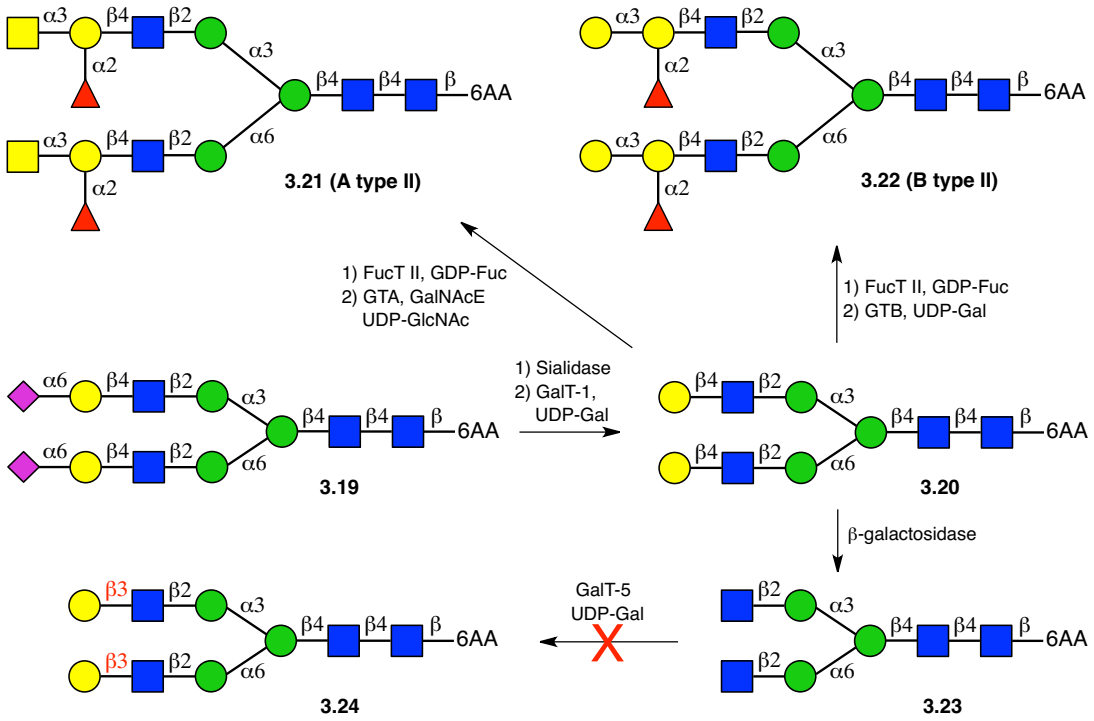
Scheme 3.3 Enzymatic synthesis of A type I antigen **3.15**, B type I antigen **3.16**, A type II antigen **3.17** and B type II antigen **3.18** on an *O*-linked glycan scaffold. Key differences in linkages are indicated in red.

In all the glycosyltransferase-catalyzed reactions, calf intestinal alkaline phosphatase (CIAP) was added to degrade the byproduct nucleoside phosphate (UDP or GDP) to prevent feedback inhibition of the enzymes.[27] Generally, four equivalents of the donor were used to push the reaction to completion, resulting in near quantitative conversion of the substrate to the product. The pH of reaction solution was kept in the optimal range for the glycosyltransferases being used, and 2 N NaOH solution was added to adjust the pH, if necessary. The reaction was monitored by TLC and MALDI-TOF mass spectrometry. If starting material or an intermediate was observed in the MS spectra, the reaction was allowed to go on and, if necessary, more donor and enzyme were added until the reaction reached completion. After the reaction, the mixture was centrifuged and the product, which was present in the supernatant, was purified by size exclusion chromatography. A similar strategy was applied to the synthesis of the *N*-linked glycans, which is described in the next section.

3.2.2 Enzymatic synthesis of *N*-linked glycans capped with A/B antigen

The enzymatic synthesis of the *N*-linked glycan targets started with **3.19** (Scheme 3.4), a commercially available natural glycopeptide isolated from hen egg yolk.[153] This glycopeptide contains the core structure of all *N*-linked glycans, α-Man-(1→3)-[α-Man-(1→6)]-β-Man-(1→4)-β-GlcNAc-(1→4)-β-GlcNAc-Asn-X-Ser/Thr. This compound, however, has two terminal sialic acid residues that must be removed before the enzymatic reactions can proceed. Removal of these sialic acid residues in **3.19**, followed by a series of enzymatic transformations described in the synthesis of *O*-linked glycans resulted in a panel of *N*-linked glycans containing A/B antigens at the non-reducing terminus.

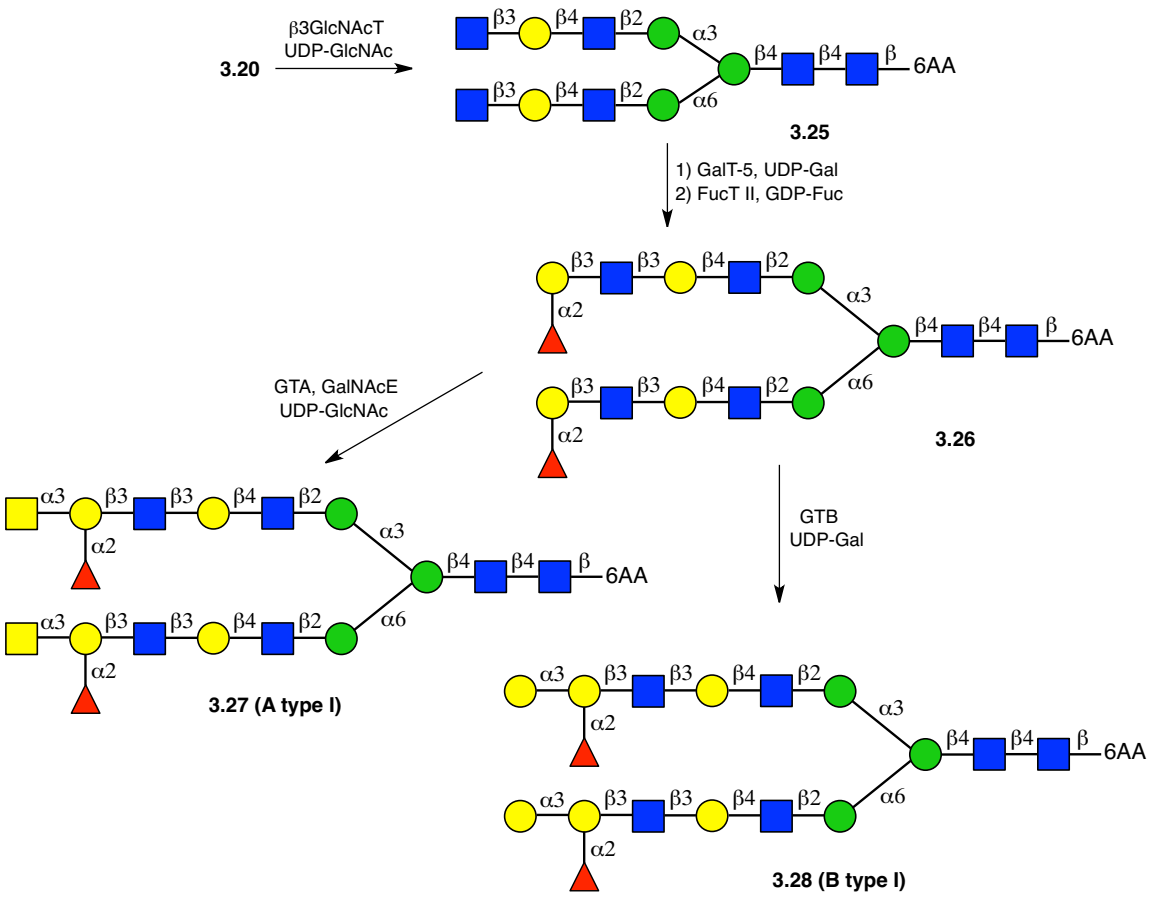
As depicted in Scheme 3.4, **3.19** was subjected to sialidase-catalyzed cleavage of the terminal sialic acid residues to afford the type II precursor **3.20**. A problem encountered in this reaction is that product, **3.20**, underwent slow hydrolysis in the reaction mixture to lose a Gal residue. This led to the formation of a small amount of mono-Gal terminated intermediate in the product. This product presumably forms as a result of the presence of a trace amount of galactosidase in the commercial sialidase used. To address this problem, the product obtained after the sialidase treatment was incubated with GalT-1 and UDP-Gal before further use to ensure both branches are terminated by a Gal residue. Then, **3.20** was fucosylated using GDP-Fuc and FucT II. Subsequent treatment of this product with either GTA, GalNAcE and UDP-GlcNAc, or GTB and UDP-Gal, gave the A type II antigen **3.21** and the B type II antigen **3.22**, respectively.



Scheme 3.4 Enzymatic synthesis of A type II antigen **3.21** and B type II antigen **3.22** as well as an effort toward the synthesis of the type I precursor **3.24** on an *N*-linked glycan scaffold.

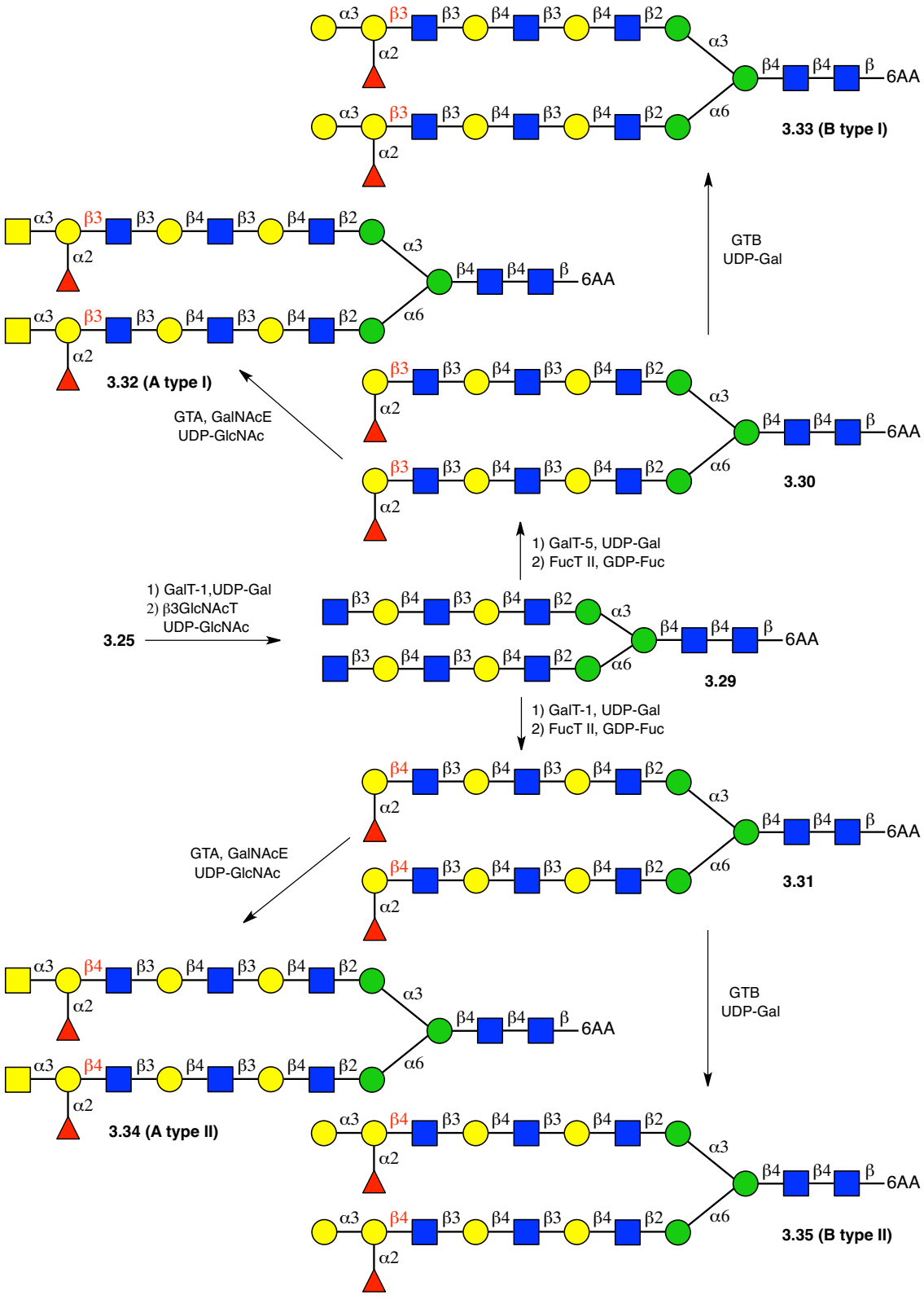
To synthesize the type I antigens, the terminal β-(1→4) linked Gal residues needed to be substituted by a β-(1→3)-linked Gal. It was anticipated that a β-galactosidase-mediated hydrolysis of terminal Gal residues followed by β-(1→3)-galactosylation would provide the type I precursor **3.24**. The hydrolysis of **3.20** went smoothly to yield **3.23**. Unfortunately, β-(1→3)-galactosylation of **3.23** catalyzed by GalT-5 did not proceed efficiently. A mixture of staring material and mono-galactosylated intermediate was obtained, together with a trace amount of the desired **3.24**. Several efforts were made to push this reaction to completion by adding more enzyme, more UDP-Gal and leaving the reaction longer time. However, no improved results were seen. This observation indicated that GalT-5 does not recognize β-(1→2)-GlcNAc terminated branched glycans, which is in contrast to β-(1→3)-GlcNAc terminated branched glycans.

To achieve a type I precursor, the glycan needs to be terminated by GlcNAc in β-(1→3) linkage. Therefore, **3.20** was incubated with β3GlcNAcT and UDP-GlcNAc to add a β-(1→3)-GlcNAc at each branch (Scheme 3.5). The resulting compound, **3.25**, was then treated with GalT-5 and UDP-Gal. This β-(1→3) galactosylation proceeded efficiently. Subsequently, the galactosylated intermediate underwent Fuc II catalyzed α-(1→2)-fucosylation to afford the type I H antigen **3.26**, from which the desired A type I antigen **3.27** and B type I antigen **3.28** were readily obtained using GTA and GTB, respectively. Thus, the type I products were obtained, although because of the difficulties with GalT-5, they were one LacNAc residue longer than the Type II products.



Scheme 3.5 Enzymatic synthesis of A type I antigen **3.27** and B type I antigen **3.28** on an *N*-linked glycan scaffold.

Scheme 3.6 describes the synthesis of A/B antigens with an additional LacNAc extension. Sequential addition of β-(1→4)-linked Gal, β-(1→3)-linked GlcNAc, β-(1→3) linked Gal and finally α-(1→2)-linked Fuc to the terminus of **3.25** resulted in the formation of the H type I antigen **3.30**. Similarly, the H type II antigen **3.31** was readily achieved from **3.25** via β3GlcNAcT, GalT-1 and FucT II catalyzed transformations. Further elongation of **3.30** and **3.31** catalyzed by GTA or GTB led to A type I antigen **3.32**, B type I antigen **3.33**, A type II antigen **3.34** and B type II antigen **3.35**.



Scheme 3.6 Enzymatic synthesis of A type I antigen **3.32**, B type I antigen **3.33**, A type II antigen **3.34** and B type II antigen **3.35** on an *N*-linked glycan scaffold. Key differences in linkages are indicated in red.

3.3 Summary

A panel of poly-LacNAc extended biantennary *N*- and *O*-linked glycans capped with the A or B blood group antigens was successfully synthesized by an enzymatic approach. The goal was to provide compounds that better mimic natural glycan structures. The glycosyltransferase-catalyzed reactions proceed with exquisite regio- and stereoselectivity under mild conditions without protecting groups. LacNAc extensions were introduced by alternating reactions using β3GlcNAcT and GalT-1. The bacterial homologues of GTA and GTB were employed to catalyze the conversion of the H antigen to the corresponding A and B antigens. The obtained compounds have been incorporated into a glycan microarray with the aim of revealing the effect of structural complexity on antibody–antigen recognition. The screening of the serum samples from ABOi transplant patients and monoclonal subtype specific antibodies using the glycan microarray are in progress.

3.4 Experimental section

General methods for enzymatic synthesis: All the enzymatic reactions were performed in aqueous buffer solutions at the optimal pH for each enzyme at 37 °C. A unit of enzyme (U) is defined as the amount of enzyme that catalyzes the conversion of 1 μmol substrate per minute. Water was purified by a NanoPure Infinity Ultrapure water system (Barnstead). The reactions were monitored by TLC on Silica Gel 60-F_{254} (0.25 mm), and the spots were visualized by charring with 10% sulfuric acid in ethanol. Gel filtration chromatography was performed on a column (113 cm x 0.7 cm) or (47 cm x 1 cm) packed with Sephadex^{TM} G-25 Superfine (GE Healthcare) using 0.1 M NH_4HCO_3 aqueous solution as the eluent. Mass

spectra were recorded with an Applied Biosystems DE MALDI-TOF using dihydroxybenzoic acid as the matrix. NMR spectra were recorded on Bruker DRX-500 or 600 MHz, or Agilent 700 MHz instruments at 25 °C, and chemical shifts were referenced to HOD (4.79 ppm, D_2O).

Materials: Recombinant β3GlcNAcT,[145] Gal-T5,[148] β4GalT/GalE[149] and GalNAcE[150] were expressed in the Paulson lab as previously reported. GTA and GTB were kindly provided by Prof. George Peng Wang (Georgia State University). FucT II was obtained from Shawn DeFrees (Neose Technologies, Inc.). GalT-1 was purchased from Sigma-Aldrich. Neuraminidase (Sialidase from *Clostridium perfringens*; 1 U/100 μL) was purchased from Roche Applied Science (Indianapolis, IN, USA). Alkaline Phosphatase from calf intestine (10,000 U/mL) was purchased from New England Biolabs (Ipswich, MA, USA). The *O*-linked glycan core 4 trisaccharide **3.1** was purchased from TCI (Tokyo Chemical Industry Co., Ltd.). The *N*-linked glycan precursor **3.19** was isolated from hen egg yolk as previously reported.[153] UDP-Gal was purchased from EMD Chemicals Inc. (USA). UDP-GlcNAc was a gift from Tokyo Research Laboratories, Kyowa. Other chemicals were obtained from commercial suppliers and used as received unless otherwise noted.

General procedure A for GalT-1 catalyzed reaction: The GlcNAc terminated substrate (0.5–6 mg, 5 mM) and UDP-Gal (4 equiv.) were dissolved in Tris–HCl buffer (50 mM, pH 8.0) containing BSA (0.1 mg/mL) and $MnCl_2$ (20 mM). After adding GalT-1 (10 mU/μmol substrate) and CIAP (100 mU/mL), the resulting mixture was incubated at 37 °C for 5 h. The reaction was monitored by TLC using EtOH–NH_4OH–H_2O (5:2:1) or *i*-PrOH–

$NH_4OH–H_2O$ (4:3:1 or 3:3:1) as the eluant. The reaction mixture was centrifuged and the supernatant was subjected to purification on a Sephadex™ G-25 Superfine gel filtration column (eluent 0.1 M NH_4HCO_3). Fractions containing the product were combined and lyophilized to give the desired products as an amorphous white solid.

General procedure B for GalT-5 catalyzed reaction: The GlcNAc terminated substrate (0.5–5 mg, 5–10 mM) and UDP-Gal (4 equiv.) were dissolved in Tris–HCl buffer (50 mM, pH 8.0) containing $MnCl_2$ (15 mM). After adding GalT-5 (6 mU/μmol substrate) and CIAP (100 mU/mL), the resulting mixture was incubated overnight at 37 °C. The reaction was monitored by TLC with EtOH–NH_4OH–H_2O (5:2:1) or *i*-PrOH–NH_4OH–H_2O (4:3:1 or 3:3:1) as the eluant. The reaction mixture was centrifuged and the supernatant was subjected to purification on a Sephadex™ G-25 Superfine gel filtration column (eluent 0.1 M NH_4HCO_3). Fractions containing the desired product were combined and lyophilized to give the product as an amorphous white solid.

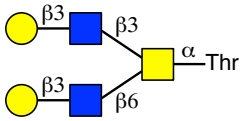
General procedure C for β3GlcNAcT catalyzed reaction: The galactose terminated substrate (0.5–8 mg, 5 mM) and UDP-GlcNAc (4 equiv.) were dissolved in 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES)–NaOH buffer (50 mM, pH 7.5) containing KCl (25 mM), $MgCl_2$ (2 mM) and dithiothreitol (1 mM). After adding β3GlcNAcT (7 mU/μmol substrate) and CIAP (100 mU/ml), the resulting mixture was incubated at 37 °C for 6 h. The reaction was monitored by TLC with EtOH–NH_4OH–H_2O (5:2:1) or *i*-PrOH–NH_4OH–H_2O (4:3:1 or 3:3:1) as the eluant. The reaction mixture was centrifuged and the supernatant was subjected to purification on a Sephadex™ G-25

Superfine gel filtration column (eluent 0.1 M NH_4HCO_3). Fractions containing the desired product were combined and lyophilized to give the product as an amorphous white solid.

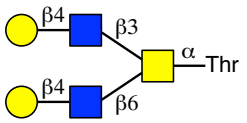
General procedure D for FucT II catalyzed reaction: The galactose terminated substrate (0.5–3 mg, 10 mM) and GDP-Fuc (4 equiv.) were dissolved in crude FucT II (0.08 U/mL) Tris–HCl buffer solution (0.08 U/mL, 50 mM, pH 8.0) containing $MnCl_2$ (20 mM). After adding CIAP (100 mU/mL), the resulting mixture was incubated overnight at 37 °C. During the course of the reaction, the pH was re-adjusted to 8.0 with 2 N NaOH as necessary. The reaction was monitored by TLC with EtOH–NH_4OH–H_2O (5:2:1) or *i*-PrOH–NH_4OH–H_2O (4:3:1 or 3:3:1) as the eluant. The reaction mixture was centrifuged and the supernatant was subjected to purification on a Sephadex™ G-25 Superfine gel filtration column (eluent 0.1 M NH_4HCO_3). Fractions containing the desired product were combined and lyophilized to give the product as an amorphous white solid.

General procedure E for GTA catalyzed reaction: The H antigen substrate (0.5–1.5 mg, 5 mM) and UDP-GlcNAc (4 equiv.) were dissolved in Tris–HCl buffer (50 mM, pH 7.5) containing $MnCl_2$ (25 mM). After adding GTA (120 mU/μmol substrate), GalNAcE (12 mU/μmol substrate) and CIAP (100 mU/mL), the resulting mixture was incubated at 37 °C for 3 h. The reaction was monitored by TLC with EtOH–NH_4OH–H_2O (5:2:1) or *i*-PrOH–NH_4OH–H_2O (4:3:1 or 3:3:1) as the eluant. The reaction mixture was centrifuged and the supernatant was subjected to purification on a Sephadex™ G-25 Superfine gel filtration column (eluent 0.1 M NH_4HCO_3). Fractions containing the desired product were combined and lyophilized to give the product as an amorphous white solid.

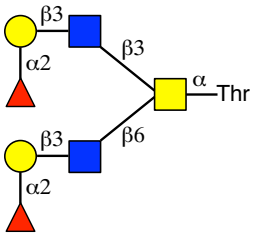
General procedure F for GTB catalyzed reaction: The H antigen substrate (0.5–1.5 mg, 5 mM) and UDP-Gal (4 equiv.) were dissolved in Tris–HCl buffer (50 mM, pH 7.5) containing $MnCl_2$ (25 mM). After adding GTB (200 mU/μmol substrate) and CIAP (100 mU/mL), the resulting mixture was incubated at 37 °C for 3 h. The reaction was monitored by TLC with EtOH–NH_4OH–H_2O (5:2:1) or *i*-PrOH–NH_4OH–H_2O (4:3:1 or 3:3:1) as the eluant. The reaction mixture was centrifuged and the supernatant was subjected to purification on a Sephadex™ G-25 Superfine gel filtration column (eluent 0.1 M NH_4HCO_3). Fractions containing the desired product were combined and lyophilized to give the product as an amorphous white solid.



β-D-Galactopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-[β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→6)]-2-acetamido-2-deoxy-α-D-galactopyranosyl-L-threonine (3.2): The GalT-5 catalyzed conversion of **3.1** (5 mg, 6.86 μmol) to **3.2** (5.7 mg, 78%) was performed following general procedure B described above. ^1H NMR (500 MHz, D_2O) δ 4.92 (br s, 1H), 4.65–4.54 (m, 2H), 4.53–4.45 (m, 2H), 4.45–4.37 (m, 1H), 4.28–4.08 (m, 4H), 4.07–3.90 (m, 5H), 3.89–3.83 (m, 2H), 3.83–3.61 (m, 17H), 3.61–3.50 (m, 3H), 2.08 (s, 3H), 2.07 (s, 3H), 2.04 (s, 3H), 1.39 (d, J = 6.5 Hz, 3H); MALDI-TOF MS *m/z* $[M + H]^+$ calcd for $C_{40}H_{69}N_4O_{28}$: 1053, found: 1053.

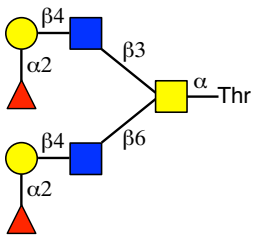


β-D-Galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-[β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→6)]-2-acetamido-2-deoxy-α-D-galactopyranosyl-L-threonine (3.3): To a solution of **3.1** (10.0 mg, 13.72 μmol) and UDP-Glc (33.5 mg, 54.87 μmol) in 1.24 mL Tris–HCl buffer (50 mM, pH 8.0, 20 mM $MnCl_2$) was added 76 μL β4GalT/GalE (1 U/mL) and 2 μL CIAP. The resulting mixture was incubated overnight at 37 °C. The reaction was monitored by TLC with EtOH–NH_4OH–H_2O (5:2:1) as the eluant. Purification on a Sephadex™ G-25 Superfine gel filtration column (eluent 0.1 M NH_4HCO_3) provided **3.3** (11.5 mg, 80%) as a white amorphous solid. ^{1}H NMR (600 MHz, D_2O) δ 4.84 (d, *J* = 3.8 Hz, 1H), 4.55 (d, *J* = 8.0 Hz, 1H), 4.52 (d, *J* = 8.4 Hz, 1H), 4.43 (d, *J* = 7.8 Hz, 1H), 4.42 (d, *J* = 7.8 Hz, 1H), 4.20–4.10 (m, 4H), 4.05 (dd, *J* = 10.9, 2.9 Hz, 1H), 3.96–3.87 (m, 6H), 3.81–3.44 (m, 21H), 2.02 (s, 3H), 1.99 (s, 3H), 1.96 (s, 3H), 1.27 (d, *J* = 6.6 Hz, 3H); MALDI-TOF MS *m/z* $[M + H]^+$ calcd for $C_{40}H_{69}N_4O_{28}$: 1053, found: 1053.



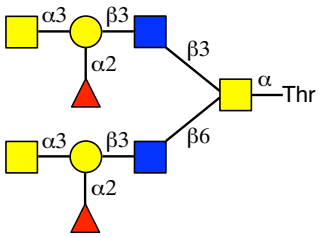
α-L-Fucopyranosyl-(1→2)-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-[α-L-fucopyranosyl-(1→2)-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→6)]-2-acetamido-2-deoxy-α-D-

galactopyranosyl-L-threonine (3.4): The FucT II catalyzed conversion of **3.2** (2.2 mg, 2.09 μmol**)** to **3.4** (2.4 mg, 86%) was performed following general procedure D described above. ^{1}H NMR (600 MHz, D_2O) δ 5.17 (d, *J* = 3.9 Hz, 1H), 5.16 (d, *J* = 3.9 Hz, 1H), 4.87 (d, *J* = 3.7 Hz, 1H), 4.65 (d, *J* = 7.7 Hz, 1H), 4.62 (d, *J* = 7.7 Hz, 1H), 4.50 (d, *J* = 8.4 Hz, 1H), 4.44 (d, *J* = 8.5 Hz, 1H), 4.41–4.36 (m, 1H), 4.31 (q, *J* = 6.7 Hz, 1H), 4.23 (q, *J* = 6.6 Hz, 1H), 4.20–4.13 (m, 3H), 4.08 (dd, *J* = 10.9, 2.7 Hz, 1H), 4.01–3.95 (m, 3H), 3.94–3.85 (m, 4H), 3.84–3.61 (m, 20H), 3.59–3.54 (m, 2H), 3.53–3.47 (m, 3H), 3.47–3.42 (m, 1H), 2.07 (s, 3H), 2.05 (s, 3H), 2.01 (s, 3H), 1.35 (d, *J* = 6.7 Hz, 3H), 1.21 (d, *J* = 6.6 Hz, 3H), 1.20 (d, *J* = 6.6 Hz, 3H); MALDI-TOF MS *m/z* $[M + Na]^+$ calcd for $C_{52}H_{88}N_4NaO_{36}$: 1368, found: 1368.

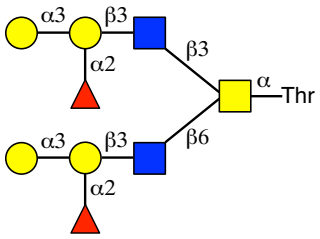


α-L-Fucopyranosyl-(1→2)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-[α-L-fucopyranosyl-(1→2)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→6)]-2-acetamido-2-deoxy-α-D-galactopyranosyl-L-threonine (3.5): The FucT II catalyzed conversion of **3.3** (2.4 mg, 2.28 μmol**)** to **3.5** (2.2 mg, 73%) was performed following general procedure D described above. ^{1}H NMR (600 MHz, D_2O) δ 5.30 (d, *J* = 3.3 Hz, 1H), 5.29 (d, *J* = 2.5 Hz, 1H), 4.89 (d, *J* = 3.6 Hz, 1H), 4.57 (d, *J* = 8.1 Hz, 1H), 4.54–4.51 (m, 3H), 4.41–4.35 (m, 1H), 4.23–4.17 (m, 4H), 4.14 (dd, *J* = 8.4, 2.3 Hz, 1H), 4.09 (dd, *J* = 10.7, 2.6 Hz, 1H), 3.98 (d, *J* = 10.6 Hz, 1H), 3.94 (d, *J* = 10.8 Hz, 1H), 3.91 (dd, *J* = 11.2, 2.8 Hz, 1H), 3.89–3.84 (m, 4H),

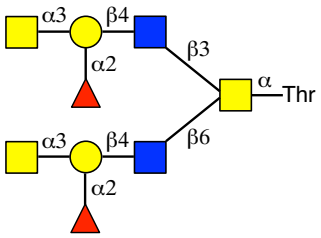
3.82–3.59 (m, 25H), 3.49–3.45 (m, 1H), 3.44–3.39 (m, 1H), 2.05 (s, 3H), 2.04 (s, 3H), 2.00 (s, 3H), 1.34 (d, J = 6.7 Hz, 3H), 1.22 (d, J = 6.0 Hz, 3H), 1.21 (d, J = 6.0 Hz, 3H); MALDI-TOF MS m/z $[M + Na]^+$ calcd for $C_{52}H_{88}N_4NaO_{36}$: 1368, found: 1368.



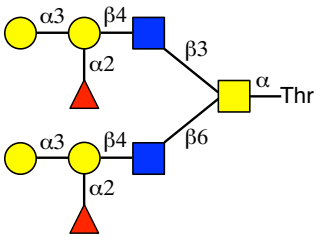
2-Acetamido-2-deoxy-α-D-galactopyranosyl-(1→3)-[α-L-fucopyranosyl-(1→2)]-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-[2-acetamido-2-deoxy-α-D-galactopyranosyl-(1→3)-[α-L-fucopyranosyl-(1→2)]-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→6)]-2-acetamido-2-deoxy-α-D-galactopyranosyl-L-threonine (3.6): The GTA catalyzed conversion of **3.4** (1.4 mg, 1.04 μmol) to **3.6** (1.5 mg, 82%) was performed following general procedure E described above. ^{1}H NMR (700 MHz, D_2O) δ 5.20 (d, J = 4.4 Hz, 1H), 5.19 (d, J = 4.4 Hz, 1H), 5.13 (d, J = 3.9 Hz, 2H), 4.85 (d, J = 3.9 Hz, 1H), 4.67 (d, J = 7.6 Hz, 1H), 4.64 (d, J = 7.6 Hz, 1H), 4.47 (d, J = 8.4 Hz, 1H), 4.41 (d, J = 8.5 Hz, 1H), 4.40–4.36 (m, 1H), 4.33 (q, J = 6.6 Hz, 1H), 4.27–4.22 (m, 3H), 4.19–4.16 (m, 4H), 4.14 (dd, J = 11.1, 3.7 Hz, 1H), 4.12 (d, J = 3.5 Hz, 1H), 4.11 (dd, J = 8.1, 3.5 Hz, 1H), 4.05 (dd, J = 11.0, 3.2 Hz, 1H), 4.00–3.95 (m, 2H), 3.94–3.84 (m, 9H), 3.82–3.66 (m, 19H), 3.66–3.59 (m, 4H), 3.57 (dd, J = 10.4, 3.3 Hz, 1H), 3.50–3.44 (m, 3H), 3.44–3.40 (m, 1H), 2.03 (s, 3H), 2.02 (s, 3H), 1.991 (s, 3H), 1.989 (s, 3H), 1.98 (s, 3H), 1.33 (d, J = 6.7 Hz, 3H), 1.19 (d, J = 6.6 Hz, 3H), 1.18 (d, J = 6.6 Hz, 3H); MALDI-TOF MS m/z $[M + Na]^+$ calcd for $C_{68}H_{114}N_6NaO_{46}$: 1774, found: 1774.



α-D-Galactopyranosyl-(1→3)-[α-L-fucopyranosyl-(1→2)]-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-[α-D-galactopyranosyl-(1→3)-[α-L-fucopyranosyl-(1→2)]-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→6)]-2-acetamido-2-deoxy-α-D-galactopyranosyl-L-threonine (3.7): The GTB catalyzed conversion of **3.4** (0.7 mg, 0.52 μmol**)** to **3.7** (0.86 mg, 98%) was performed following general procedure F described above. ^{1}H NMR (700 MHz, D_2O) δ 5.19 (d, *J* = 3.8 Hz, 2H), 5.18 (d, *J* = 4.0 Hz, 1H), 5.17 (d, *J* = 4.0 Hz, 1H), 4.84 (d, *J* = 3.7 Hz, 1H), 4.68 (d, *J* = 7.6 Hz, 1H), 4.66 (d, *J* = 7.6 Hz, 1H), 4.47 (d, *J* = 8.4 Hz, 1H), 4.41 (d, *J* = 8.5 Hz, 1H), 4.39–4.35 (m, 1H), 4.33 (q, *J* = 6.6 Hz, 1H), 4.26 (q, *J* = 6.7 Hz, 1H), 4.23 (d, *J* = 2.3 Hz, 2H), 4.20 (t, *J* = 6.2 Hz, 2H), 4.14 (dd, *J* = 11.2, 3.7 Hz, 1H), 4.12–4.10 (m, 2H), 4.04 (dd, *J* = 10.9, 3.1 Hz, 1H), 4.00–3.95 (m, 2H), 3.94–3.81 (m, 10H), 3.81–3.58 (m, 23H), 3.56 (dd, *J* = 10.4, 3.2 Hz, 1H), 3.53–3.43 (m, 4H), 3.43–3.40 (m, 1H), 2.04 (s, 3H), 2.03 (s, 3H), 1.98 (s, 3H), 1.32 (d, *J* = 6.7 Hz, 3H), 1.18 (d, *J* = 6.6 Hz, 3H), 1.17 (d, *J* = 6.6 Hz, 3H); MALDI-TOF MS *m/z* $[M + Na]^+$ calcd for $C_{64}H_{108}N_4NaO_{46}$: 1692, found: 1692.

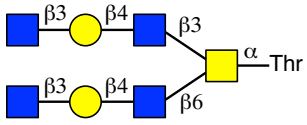


2-Acetamido-2-deoxy-α-D-galactopyranosyl-(1→3)-[α-L-fucopyranosyl-(1→2)]-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-[2-acetamido-2-deoxy-α-D-galactopyranosyl-(1→3)-[α-L-fucopyranosyl-(1→2)]-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→6)]-2-acetamido-2-deoxy-α-D-galactopyranosyl-L-threonine (3.8): The GTA catalyzed conversion of **3.5** (0.6 mg, 0.44 μmol) to **3.8** (0.56 mg, 72%) was performed following general procedure E described above. ^{1}H NMR (700 MHz, D_2O) δ 5.30 (d, *J* = 2.5 Hz, 2H), 5.13 (d, *J* = 3.6 Hz, 2H), 4.86 (d, *J* = 3.7 Hz, 1H), 4.57– 4.53 (m, 3H), 4.50 (d, *J* = 8.5 Hz, 1H), 4.40–4.34 (m, 1H), 4.29–4.24 (m, 2H), 4.21–4.14 (m, 7H), 4.10 (dd, *J* = 8.2, 2.4 Hz, 1H), 4.05 (dd, *J* = 10.5, 2.4 Hz, 1H), 3.98–3.89 (m, 6H), 3.89–3.83 (m, 5H), 3.81–3.55 (m, 27H), 3.45–3.40 (m, 1H), 3.39–3.34 (m, 1H), 2.01 (s, 3H), 2.00 (s, 3H), 1.99 (s, 6H), 1.97 (s, 3H), 1.32 (d, *J* = 6.7 Hz, 3H), 1.21 (d, *J* = 6.4 Hz, 3H), 1.20 (d, *J* = 6.4 Hz, 3H); MALDI-TOF MS *m/z* $[M + Na]^+$ calcd for $C_{68}H_{114}N_6NaO_{46}$: 1774, found: 1774.



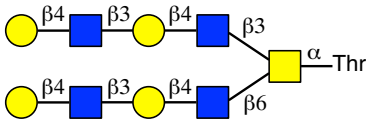
α-D-Galactopyranosyl-(1→3)-[α-L-fucopyranosyl-(1→2)]-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-[α-D-galactopyranosyl-(1→3)-

[α-L-fucopyranosyl-(1→2)]-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→6)]-2-acetamido-2-deoxy-α-D-galactopyranosyl-L-threonine (3.9): The GTB catalyzed conversion of **3.5** (1.1 mg, 0.82 μmol) to **3.9** (1.15 mg, 85%) was performed following general procedure F described above. ^{1}H NMR (700 MHz, D_2O) δ 5.28 (d, *J* = 4.1 Hz, 2H), 5.20 (d, *J* = 1.9 Hz, 2H), 4.87 (d, *J* = 3.8 Hz, 1H), 4.57 (d, *J* = 7.7 Hz, 2H), 4.54 (d, *J* = 8.1 Hz, 1H), 4.50 (d, *J* = 8.5 Hz, 1H), 4.41–4.36 (m, 1H), 4.29–4.23 (m, 4H), 4.19–4.13 (m, 4H), 4.10 (dd, *J* = 8.4, 2.8 Hz, 1H), 4.06 (dd, *J* = 10.8, 2.6 Hz, 1H), 3.98–3.61 (m, 38H), 3.60–3.56 (m, 1H), 3.45–3.40 (m, 1H), 3.39–3.34 (m, 1H), 2.01 (s, 3H), 2.00 (s, 3H), 1.97 (s, 3H), 1.32 (d, *J* = 6.7 Hz, 3H), 1.20 (d, *J* = 6.4 Hz, 3H), 1.19 (d, *J* = 6.4 Hz, 3H); MALDI-TOF MS *m/z* $[M + Na]^+$ calcd for $C_{64}H_{108}N_4NaO_{46}$: 1692, found: 1692.

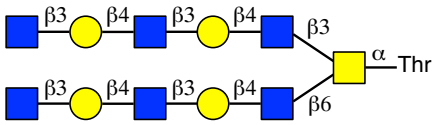


2-Acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-[2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→6)]-2-acetamido-2-deoxy-α-D-galactopyranosyl-L-threonine (3.10): The β3GlcNAcT catalyzed conversion of **3.3** (6 mg, 5.69 μmol) to **3.10** (7.3 mg, 88%) was performed following general procedure C described above. ^{1}H NMR (600 MHz, D_2O) δ 4.89 (d, *J* = 3.8 Hz, 1H), 4.67 (d, *J* = 8.4 Hz, 1H), 4.66 (d, *J* = 8.4 Hz, 1H), 4.58 (d, *J* = 7.7 Hz, 1H), 4.55 (d, *J* = 8.4 Hz, 1H), 4.45 (d, *J* = 7.8 Hz, 1H), 4.44 (d, *J* = 7.8 Hz, 1H), 4.41–4.35 (m, 1H), 4.20 (dd, *J* = 11.1, 3.8 Hz, 1H), 4.18 (d, *J* = 2.9 Hz, 1H), 4.16–4.12 (m, 3H),

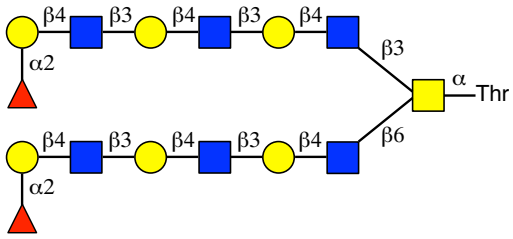
4.09 (dd, *J* = 10.8, 2.4 Hz, 1H), 3.99 (d, *J* = 10.4 Hz, 1H), 3.93 (d, *J* = 11.4 Hz, 2H), 3.88 (d, *J* = 12.6 Hz, 2H), 3.85–3.79 (m, 2H), 3.79–3.62 (m, 20H), 3.62–3.51 (m, 6H), 3.46–3.40 (m, 4H), 2.05 (s, 3H), 2.03 (s, 3H), 2.02 (s, 6H), 2.00 (s, 3H), 1.34 (d, *J* = 6.7 Hz, 3H); MALDI-TOF MS *m/z* $[M + Na]^+$ calcd for $C_{56}H_{94}N_6NaO_{38}$: 1481, found: 1481.



β-D-Galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-[β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→6)]-2-acetamido-2-deoxy-α-D-galactopyranosyl-L-threonine (3.11): The GalT-1 catalyzed conversion of **3.10** (7.3 mg, 5.00 μmol**)** to **3.11** (8.1 mg, 91%) was performed following general procedure A described above. ^{1}H NMR (600 MHz, D_2O) δ 4.85 (d, *J* = 3.8 Hz, 1H), 4.66 (d, *J* = 8.4 Hz, 1H), 4.65 (d, *J* = 8.4Hz, 1H), 4.54 (d, *J* = 7.7 Hz, 1H), 4.52 (d, *J* = 8.5 Hz, 1H), 4.43 (d, *J* = 7.8 Hz, 2H), 4.42 (d, *J* = 8.4 Hz, 1H), 4.41 (d, *J* = 7.8 Hz, 1H), 4.36–4.30 (m, 1H), 4.16 (dd, *J* = 10.8, 3.6 Hz, 1H), 4.14 (d, *J* = 3.0 Hz, 1H), 4.13–4.09 (m, 3H), 4.06 (dd, *J* = 11.4, 3.0 Hz, 1H), 3.96 (d, *J* = 10.8 Hz, 1H), 3.94–3.86 (m, 6H), 3.83–3.45 (m, 42H), 2.01 (s, 3H), 2.00 (s, 3H), 1.99 (s, 6H), 1.96 (s, 3H), 1.30 (d, *J* = 6.7 Hz, 3H); MALDI-TOF MS *m/z* $[M + H]^+$ calcd for $C_{68}H_{115}N_6O_{48}$: 1783, found: 1783.

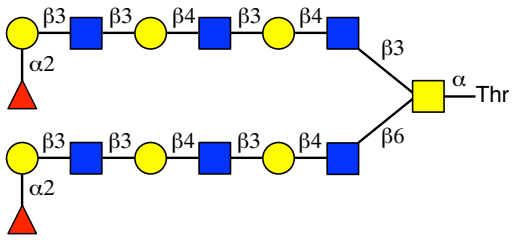


2-Acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-[2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→6)]-2-acetamido-2-deoxy-α-D-galactopyranosyl-L-threonine (3.12): The β3GlcNAcT catalyzed conversion of **3.11** (8 mg, 4.48 μmol) to **3.12** (9.24 mg, 94%) was performed following general procedure C described above. ^{1}H NMR (600 MHz, D_2O) δ 4.88 (d, *J* = 3.8 Hz, 1H), 4.69 (d, *J* = 3.7 Hz, 1H), 4.68–4.65 (m, 3H), 4.58 (d, *J* = 7.5 Hz, 1H), 4.55 (d, *J* = 8.4 Hz, 1H), 4.47–4.41 (m, 4H), 4.36 (qd, *J* = 6.3, 1.6 Hz, 1H), 4.20 (dd, *J* = 11.1, 3.7 Hz, 1H), 4.17 (d, *J* = 2.4 Hz, 1H), 4.16–4.11 (m, 5H), 4.11–4.05 (m, 1H), 3.99 (d, *J* = 10.8 Hz, 1H), 3.96–3.91 (m, 4H), 3.90–3.86 (m, 2H), 3.85–3.64 (m, 38H), 3.62–3.50 (m, 10H), 3.49–3.40 (m, 4H), 2.05 (s, 3H), 2.03 (s, 3H), 2.023 (s, 6H), 2.019 (s, 6H), 1.99 (s, 3H), 1.34 (d, *J* = 6.6 Hz, 3H); MALDI-TOF MS *m/z* $[M + Na]^+$ calcd for $C_{84}H_{140}N_8NaO_{58}$: 2213, found: 2213.



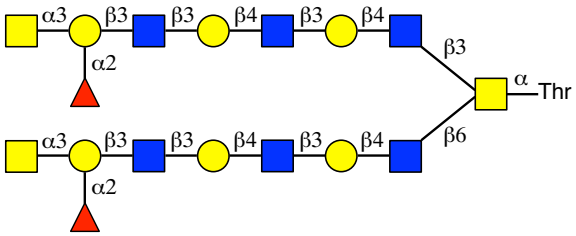
α-L-Fucopyranosyl-(1→2)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-

glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-[α-L-fucopyranosyl-(1→2)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→6)]-2-acetamido-2-deoxy-α-D-galactopyranosyl-L-threonine (3.13): 3.12 (3.26 mg, 1.49 μmol) underwent GalT-1 catalyzed galactosylation described in general procedure A, followed by FucT II catalyzed fucosylation described in general procedure D to give **3.13** (2.8 mg, 67%) as a white amorphous solid. ^{1}H NMR (600 MHz, D_2O) δ 5.29 (d, *J* = 3.0 Hz, 2H), 4.89 (d, *J* = 3.5 Hz, 1H), 4.68 (d, *J* = 8.4 Hz, 4H), 4.57 (d, *J* = 7.4 Hz, 1H), 4.56–4.51 (m, 3H), 4.48–4.42 (m, 4H), 4.40–4.34 (m, 1H), 4.23–4.16 (m, 4H), 4.16–4.11 (m, 5H), 4.09 (d, *J* = 9.3 Hz, 1H), 4.01–3.90 (m, 7H), 3.89–3.63 (m, 60H), 3.61–3.50 (m, 8H), 3.47–3.42 (m, 2H), 2.05 (s, 3H), 2.03 (s, 3H), 2.025 (s, 6H), 2.018 (s, 6H), 1.99 (s, 3H), 1.34 (d, *J* = 6.6 Hz, 3H), 1.21 (d, *J* = 6.6 Hz, 6H); MALDI-TOF MS *m/z* $[M + Na]^+$ calcd for $C_{108}H_{180}N_8NaO_{76}$: 2829, found: 2829.



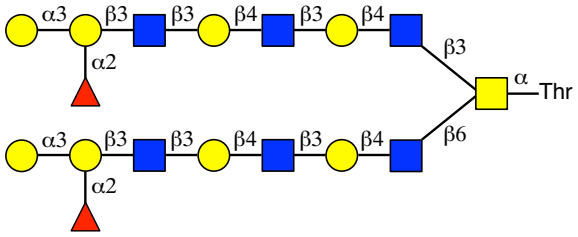
α-L-Fucopyranosyl-(1→2)-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-[α-L-fucopyranosyl-(1→2)-β-D-galactopyranosyl-(1→3)-2-

acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→6)]-2-acetamido-2-deoxy-α-D-galactopyranosyl-L-threonine (3.14): 3.12 (3 mg, 1.37 μmol) underwent GalT-5 catalyzed galactosylation described in general procedure B, followed by FucT II catalyzed fucosylation described in general procedure D to give **3.14** (2.91 mg, 76%) as a white amorphous solid. ^{1}H NMR (600 MHz, D_2O) δ 5.18 (d, *J* = 4.1 Hz, 2H), 4.89 (d, *J* = 3.7 Hz, 1H), 4.69 (d, *J* = 8.3 Hz, 1H), 4.68 (d, *J* = 8.3 Hz, 1H), 4.63 (d, *J* = 7.6 Hz, 2H), 4.60 (d, *J* = 8.4 Hz, 2H), 4.57 (d, *J* = 7.2 Hz, 1H), 4.55 (d, *J* = 8.4 Hz, 1H), 4.46–4.41 (m, 4H), 4.40–4.35 (m, 1H), 4.28 (q, *J* = 6.5 Hz, 2H), 4.20 (dd, *J* = 11.2, 3.5 Hz, 1H), 4.17 (d, *J* = 2.4 Hz, 1H), 4.16–4.11 (m, 5H), 4.09 (d, *J* = 8.7 Hz, 1H), 4.01–3.85 (m, 11H), 3.85–3.62 (m, 52H), 3.62–3.44 (m, 14H), 2.05 (s, 3H), 2.04 (s, 6H), 2.03 (s, 3H), 2.02 (s, 6H), 1.99 (s, 3H), 1.34 (d, *J* = 6.6 Hz, 3H), 1.22 (d, *J* = 6.6 Hz, 6H); MALDI-TOF MS *m/z* $[M + Na]^+$ calcd for $C_{108}H_{180}N_8NaO_{76}$: 2829, found: 2829.



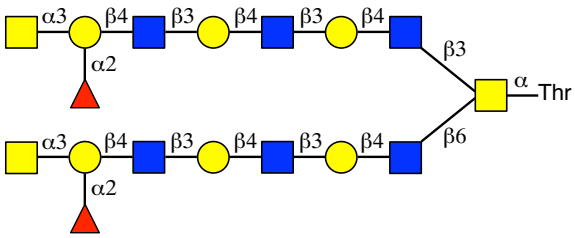
2-Acetamido-2-deoxy-α-D-galactopyranosyl-(1→3)-[α-L-fucopyranosyl-(1→2)]-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-[2-acetamido-2-deoxy-α-D-galactopyranosyl-(1→3)-[α-L-fucopyranosyl-(1→2)]-β-D-

galactopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→6)]-2-acetamido-2-deoxy-α-D-galactopyranosyl-L-threonine (3.15): The GTA catalyzed conversion of **3.14** (1.47 mg, 0.52 μmol) to **3.15** (1.43 mg, 87%) was performed following general procedure E described above. ^{1}H NMR (700 MHz, D_2O) δ 5.21 (d, *J* = 4.2 Hz, 2H), 5.14 (d, *J* = 3.9 Hz, 2H), 4.86 (d, *J* = 3.8 Hz, 1H), 4.68–4.63 (m, 4H), 4.58 (d, *J* = 8.4 Hz, 2H), 4.55 (d, *J* = 7.2 Hz, 1H), 4.52 (d, *J* = 8.4 Hz, 1H), 4.44–4.39 (m, 4H), 4.38–4.33 (m, 1H), 4.30 (q, *J* = 6.6 Hz, 2H), 4.28–4.24 (m, 2H), 4.21–4.16 (m, 5H), 4.14 (d, *J* = 2.6 Hz, 1H), 4.13–4.08 (m, 5H), 4.06 (d, *J* = 8.6 Hz, 1H), 4.00–3.88 (m, 13H), 3.86 (d, *J* = 10.7 Hz, 2H), 3.82–3.60 (m, 54H), 3.60–3.43 (m, 14H), 2.02 (s, 9H), 2.001 (s, 3H), 1.996 (s, 6H), 1.99 (s, 6H), 1.97 (s, 3H), 1.31 (d, *J* = 6.7 Hz, 3H), 1.20 (d, *J* = 6.6 Hz, 6H); MALDI-TOF MS *m/z* $[M + Na]^+$ calcd for $C_{124}H_{206}N_{10}NaO_{86}$: 3235, found: 3235.



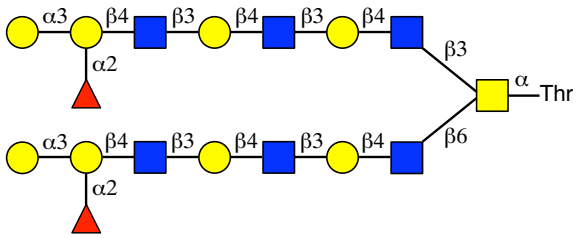
α-D-Galactopyranosyl-(1→3)-[α-L-fucopyranosyl-(1→2)]-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-[α-D-galactopyranosyl-(1→3)-[α-L-fucopyranosyl-(1→2)]-β-D-galactopyranosyl-(1→3)-2-acetamido-2-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

glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→6)]-2-acetamido-2-deoxy-α-D-galactopyranosyl-L-threonine (3.16): The GTB catalyzed conversion of **3.14** (1.44 mg, 0.51 μmol) to **3.16** (1.46 mg, 91%) was performed following general procedure F described above. ^{1}H NMR (700 MHz, D_2O) δ 5.21–5.17 (m, 4H), 4.86 (d, *J* = 3.5 Hz, 1H), 4.69–4.64 (m, 4H), 4.58 (d, *J* = 8.3 Hz, 2H), 4.55 (d, *J* = 6.8 Hz, 1H), 4.52 (d, *J* = 8.4 Hz, 1H), 4.44–4.39 (m, 4H), 4.38–4.33 (m, 1H), 4.30 (q, *J* = 6.6 Hz, 2H), 4.25–4.20 (m, 4H), 4.17 (dd, *J* = 11.1, 3.5 Hz, 1H), 4.15 (br s, 1H), 4.13–4.08 (m, 5H), 4.06 (d, *J* = 8.7 Hz, 1H), 3.99–3.94 (m, 3H), 3.93–3.82 (m, 14H), 3.81–3.63 (m, 53H), 3.59–3.48 (m, 13H), 3.47–3.43 (m, 2H), 2.02 (s, 9H), 2.00 (s, 3H), 1.99 (s, 6H), 1.97 (s, 3H), 1.32 (d, *J* = 6.7 Hz, 3H), 1.20 (d, *J* = 6.6 Hz, 6H); MALDI-TOF MS *m/z* $[M + Na]^+$ calcd for $C_{120}H_{200}N_8NaO_{86}$: 3153, found: 3153.



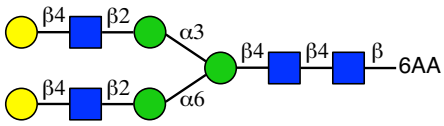
2-Acetamido-2-deoxy-α-D-galactopyranosyl-(1→3)-[α-L-fucopyranosyl-(1→2)]-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-[2-acetamido-2-deoxy-α-D-galactopyranosyl-(1→3)-[α-L-fucopyranosyl-(1→2)]-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-

galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→6)]-2-acetamido-2-deoxy-α-D-galactopyranosyl-L-threonine (3.17): The GTA catalyzed conversion of **3.13** (1.3 mg, 0.46 μmol) to **3.17** (1.24 mg, 83%) was performed following general procedure E described above. ^{1}H NMR (700 MHz, D_2O) δ 5.31 (d, *J* = 4.1 Hz, 2H), 5.14 (d, *J* = 4.0 Hz, 2H), 4.86 (d, *J* = 3.8 Hz, 1H), 4.68–4.63 (m, 4H), 4.59–4.54 (m, 3H), 4.52 (d, *J* = 8.4 Hz, 1H), 4.45–4.39 (m, 4H), 4.38–4.32 (m, 1H), 4.27 (q, *J* = 6.7 Hz, 2H), 4.22–4.13 (m, 8H), 4.13–4.08 (m, 5H), 4.06 (d, *J* = 8.4 Hz, 1H), 3.97–3.88 (m, 11H), 3.88–3.84 (m, 4H), 3.82–3.61 (m, 58H), 3.59–3.49 (m, 8H), 3.43–3.38 (m, 2H), 2.02 (s, 3H), 2.002 (s, 6H), 1.999 (s, 9H), 1.99 (s, 6H), 1.97 (s, 3H), 1.31 (d, *J* = 6.7 Hz, 3H), 1.21 (d, *J* = 6.6 Hz, 6H); MALDI-TOF MS *m/z* $[M + Na]^+$ calcd for $C_{124}H_{206}N_{10}NaO_{86}$: 3235, found: 3235.



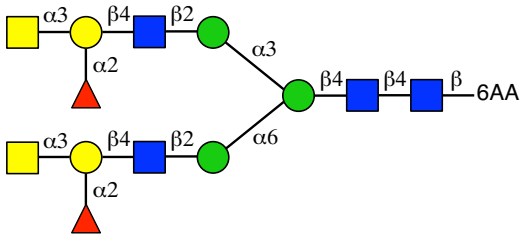
α-D-Galactopyranosyl-(1→3)-[α-L-fucopyranosyl-(1→2)]-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-[α-D-galactopyranosyl-(1→3)-[α-L-fucopyranosyl-(1→2)]-β-D-galactopyranosyl-(1→4)-2-acetamido-2-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

glucopyranosyl-(1→6)]-2-acetamido-2-deoxy-α-D-galactopyranosyl-L-threonine (3.18): The GTB catalyzed conversion of **3.13** (1.5 mg, 0.53 μmol) to **3.18** (1.45 mg, 87%) was performed following general procedure F described above. ^{1}H NMR (700 MHz, D_2O) δ 5.29 (d, J = 4.1 Hz, 2H), 5.20 (d, J = 2.7 Hz, 2H), 4.86 (d, J = 3.6 Hz, 1H), 4.68–4.64 (m, 4H), 4.58 (d, J = 7.7 Hz, 2H), 4.55 (d, J = 7.2 Hz, 1H), 4.52 (d, J = 8.4 Hz, 1H), 4.45–4.39 (m, 4H), 4.38–4.32 (m, 1H), 4.28–4.22 (m, 4H), 4.19–4.13 (m, 4H), 4.13–4.08 (m, 5H), 4.06 (d, J = 8.3 Hz, 1H), 3.98–3.83 (m, 17H), 3.82–3.61 (m, 58H), 3.59–3.48 (m, 8H), 3.42–3.38 (m, 2H), 2.02 (s, 3H), 2.002 (s, 3H), 1.999 (s, 6H), 1.99 (s, 6H), 1.97 (s, 3H), 1.32 (d, J = 6.7 Hz, 3H), 1.19 (d, J = 6.6 Hz, 6H); MALDI-TOF MS *m/z* $[M + Na]^+$ calcd for $C_{120}H_{200}N_8NaO_{86}$: 3153, found: 3153.



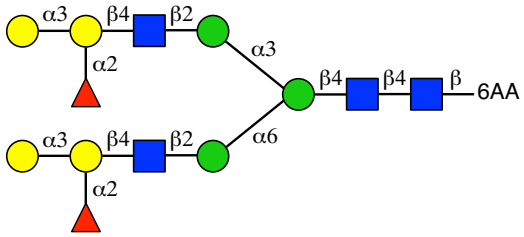
β-D-Galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-α-D-mannopyranosyl-(1→3)-[β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-α-D-mannopyranosyl-(1→6)]-β-D-mannopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(KVA)NKT (3.20): To a aqueous solution (160 μL) containing **3.19** (16 mg, 5.58 μmol) and sodium acetate (50 mM) was added 3.2 μL neuraminidase (1U/100 μL). The resulting mixture was incubated overnight at 37 °C. The reaction was monitored by TLC with *i*-PrOH–NH_4OH–H_2O (4:3:1) as the eluant. Purification on a Sephadex™ G-25 Superfine gel filtration column (eluent 0.1 M NH_4HCO_3) provided **3.20** (11.4 mg, 89%) as a white amorphous solid. ^{1}H NMR (500 MHz, D_2O) δ 5.12 (s, 1H), 5.05 (d, *J* = 9.9 Hz,

1H), 4.93 (s, 1H), 4.80 (s, 1H), 4.68 (t, *J* = 6.7 Hz, 1H), 4.62 (d, *J* = 7.9 Hz, 1H), 4.59 (d, *J* = 7.9 Hz, 2H), 4.50–4.45 (m, 2H), 4.40 (t, *J* = 7.2 Hz, 1H), 4.35–4.28 (m, 1H), 4.27–4.17 (m, 3H), 4.14 (d, *J* = 4.1 Hz, 1H), 4.13–4.09 (m, 2H), 4.03–3.42 (m, 52H), 3.02–2.95 (m, 4H), 2.87 (dd, *J* = 16.0, 5.8 Hz, 1H), 2.75 (dd, *J* = 16.5, 7.6 Hz, 1H), 2.08 (s, 3H), 2.054 (s, 3H), 2.050 (s, 3H), 2.01 (s, 3H), 1.94–1.83 (m, 2H), 1.81–1.73 (m, 1H), 1.73–1.56 (m, 6H), 1.50–1.39 (m, 4H), 1.38 (d, *J* = 7.1 Hz, 3H), 1.17 (d, *J* = 6.4 Hz, 3H), 0.97 (d, *J* = 6.7 Hz, 6H); MALDI-TOF MS *m/z* $[M + H]^+$ calcd for $C_{90}H_{156}N_{13}O_{54}$: 2283, found: 2284.



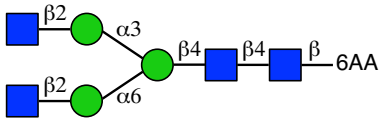
2-Acetamido-2-deoxy-α-D-galactopyranosyl-(1→3)-[α-L-fucopyranosyl-(1→2)]-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-α-D-mannopyranosyl-(1→3)-[2-acetamido-2-deoxy-α-D-galactopyranosyl-(1→3)-[α-L-fucopyranosyl-(1→2)]-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-α-D-mannopyranosyl-(1→6)]-β-D-mannopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(KVA)NKT (3.21): 3.20 (2.2 mg, 0.96 μmol) underwent FucT II catalyzed fucosylation described in general procedure D to afford corresponding H antigen (2.0 mg, 81%). A portion of the H antigen (0.72 mg, 0.28 μmol) was converted to **3.21** (0.68 mg, 82%) as described in general procedure E. ^{1}H NMR (700 MHz, D_2O) δ 5.37 (s, 2H), 5.20 (s, 2H), 5.13 (s, 1H), 5.06 (d, *J* = 9.5 Hz, 1H), 4.93 (s, 1H), 4.80 (s, 1H), 4.69 (t, *J*

= 6.5 Hz, 1H), 4.65–4.59 (m, 3H), 4.59–4.53 (m, 2H), 4.43 (t, *J* = 6.9 Hz, 1H), 4.37–4.20 (m, 12H), 4.20–4.15 (m, 2H), 4.10 (s, 2H), 4.05–3.56 (m, 59H), 3.55–3.49 (m, 2H), 3.48–3.41 (m, 2H), 3.06–2.97 (m, 4H), 2.88 (dd, *J* = 15.4, 5.4 Hz, 1H), 2.76 (dd, *J* = 17.5, 8.4 Hz, 1H), 2.10 (s, 3H), 2.07 (s, 6H), 2.06 (s, 6H), 2.02 (s, 3H), 1.97–1.85 (m, 3H), 1.83–1.65 (m, 5H), 1.53–1.41 (m, 5H), 1.40 (d, *J* = 7.0 Hz, 3H), 1.27 (d, *J* = 6.4 Hz, 3H), 1.25 (d, *J* = 6.4 Hz, 3H) 1.20 (d, *J* = 6.1 Hz, 3H), 0.99 (d, *J* = 6.6 Hz, 6H); MALDI-TOF MS *m/z* $[M + H]^+$ calcd for $C_{118}H_{202}N_{15}O_{72}$: 2982, found: 2982.

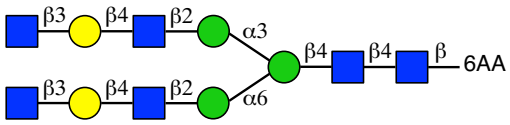


α-D-Galactopyranosyl-(1→3)-[α-L-fucopyranosyl-(1→2)]-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-α-D-mannopyranosyl-(1→3)-[α-D-galactopyranosyl-(1→3)-[α-L-fucopyranosyl-(1→2)]-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-α-D-mannopyranosyl-(1→6)]-β-D-mannopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(KVA)NKT (3.22): The H antigen (1 mg, 0.39 μmol) used for synthesis of **3.21** was converted to **3.22** (0.88 mg, 78%) as described in general procedure F. ^{1}H NMR (700 MHz, D_2O) δ 5.35 (s, 2H), 5.26 (s, 2H), 5.13 (s, 1H), 5.06 (d, *J* = 9.7 Hz, 1H), 4.93 (s, 1H), 4.77 (s, 1H), 4.69 (t, *J* = 6.6 Hz, 1H), 4.66–4.60 (m, 3H), 4.57 (d, *J* = 8.5 Hz, 1H), 4.55 (d, *J* = 8.4 Hz, 1H), 4.42 (t, *J* = 7.1 Hz, 1H), 4.36–4.25 (m, 8H), 4.21 (t, *J* = 5.5 Hz, 2H), 4.19–4.15 (m, 2H), 4.12–4.07 (m, 2H), 4.04–3.55 (m, 61H), 3.55–3.49 (m, 2H), 3.48–3.40 (m, 2H), 3.05–2.99 (m, 4H), 2.88 (dd, *J* = 16.3, 5.8 Hz,

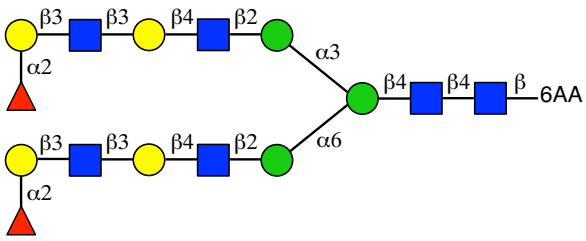
1H), 2.76 (dd, *J* = 16.2, 7.2 Hz, 1H), 2.10 (s, 3H), 2.07 (s, 6H), 2.02 (s, 3H), 1.97–1.86 (m, 3H), 1.83–1.66 (m, 5H), 1.53–1.41 (m, 5H), 1.40 (d, *J* = 7.1 Hz, 3H), 1.26 (d, *J* = 6.6 Hz, 3H), 1.24 (d, *J* = 6.6 Hz, 3H), 1.20 (d, *J* = 6.2 Hz, 3H), 0.99 (d, *J* = 6.7 Hz, 6H); MALDI-TOF MS *m/z* $[M + H]^+$ calcd for $C_{114}H_{196}N_{13}O_{72}$: 2900, found: 2900.



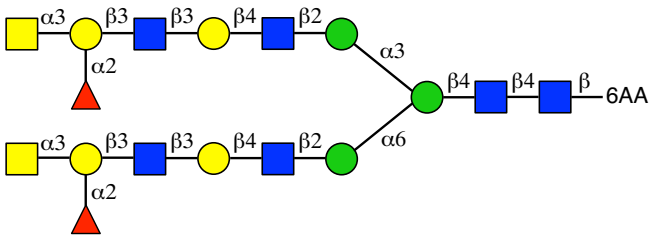
2-Acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-α-D-mannopyranosyl-(1→3)-[2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-α-D-mannopyranosyl-(1→6)]-β-D-mannopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy- β -D-glucopyranosyl-(KVA)NKT (3.23): A sodium acetate buffer (132 µL, 50 mM, pH 5.0) containing **3.20** (3 mg, 1.31 µmol), GlcNAc (50 mM), protease inhibitor (103 µL) and galactosidase (12 µL) was incubated at 37 °C for 3 h. The reaction was monitored by TLC with *i*-PrOH–NH_4OH–H_2O (4:3:1) as the eluant. Purification on a Sephadex™ G-25 Superfine gel filtration column (eluent 0.1 M NH_4HCO_3) provided **3.23** (2.3 mg, 89%) as a white amorphous solid. ^{1}H NMR (500 MHz, D_2O) δ 5.12 (s, 1H), 5.05 (d, *J* = 9.5 Hz, 1H), 4.92 (s, 1H), 4.80 (s, 1H), 4.68 (t, *J* = 6.5 Hz, 1H), 4.61 (d, *J* = 7.4 Hz, 1H), 4.56 (d, *J* = 8.3 Hz, 2H), 4.41 (t, *J* = 7.0 Hz, 1H), 4.33–4.29 (m, 1H), 4.27–4.17 (m, 3H), 4.17–4.08 (m, 3H), 4.02–3.38 (m, 40H), 3.04–2.95 (m, 4H), 2.87 (dd, *J* = 16.0, 5.9 Hz, 1H), 2.75 (dd, *J* = 16.5, 7.7 Hz, 1H), 2.08 (s, 3H), 2.06 (s, 6H), 2.01 (s, 3H), 1.94–1.84 (m, 2H), 1.82–1.64 (m, 7H), 1.52–1.40 (m, 4H), 1.38 (d, *J* = 7.0 Hz, 3H), 1.17 (d, *J* = 6.5 Hz, 3H), 0.97 (d, *J* = 6.7 Hz, 6H); MALDI-TOF MS *m/z* $[M + H]^+$ calcd for $C_{78}H_{136}N_{13}O_{44}$: 1959, found: 1959.



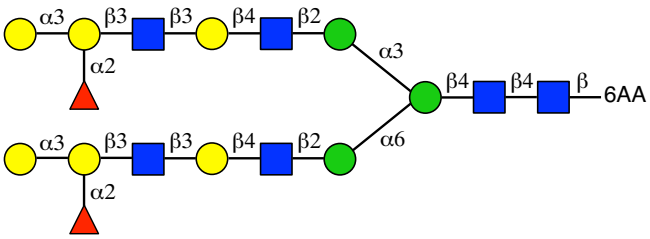
2-Acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-α-D-mannopyranosyl-(1→3)-[2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-α-D-mannopyranosyl-(1→6)]-β-D-mannopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(KVA)NKT (3.25): The β3GlcNAcT catalyzed conversion of **3.20** (6 mg, 2.63 μmol**)** to **3.25** (6.5 mg, 92%) was performed following general procedure C described above. ^{1}H NMR (600 MHz, D_2O) δ 4.98 (s, 1H), 4.91 (d, *J* = 9.6 Hz, 1H), 4.80 (s, 1H), 4.63 (s, 1H), 4.60–4.51 (m, 3H), 4.48 (d, *J* = 7.6 Hz, 1H), 4.44 (d, *J* = 7.9 Hz, 2H), 4.32 (d, *J* = 7.8 Hz, 1H), 4.31 (d, *J* = 7.8 Hz, 1H), 4.28–4.24 (m, 1H), 4.16 (q, *J* = 7.2 Hz, 1H), 4.12 (s, 1H), 4.10–4.07 (m, 1H), 4.06 (s, 1H), 4.04–4.01 (s, 2H), 4.00 (d, *J* = 4.2 Hz, 1H), 3.99–3.95 (m, 2H), 3.88–3.25 (m, 62H), 2.91–2.78 (m, 4H), 2.73 (dd, *J* = 16.0, 5.0 Hz, 1H), 2.61 (dd, *J* = 16.0, 7.6 Hz, 1H), 2.00–1.94 (m, 1H), 1.94 (s, 3H), 1.92 (s, 3H), 1.91 (s, 3H), 1.906 (s, 3H), 1.902 (s, 3H), 1.87 (s, 3H), 1.81–1.71 (m, 1H), 1.68–1.59 (m, 1H), 1.59–1.41 (m, 6H), 1.40–1.19 (m, 4H), 1.24 (d, *J* = 7.1 Hz, 3H), 1.04 (d, *J* = 6.4 Hz, 3H), 0.83 (d, *J* = 6.8 Hz, 6H); MALDI-TOF MS *m/z* $[M + H]^+$ calcd for $C_{106}H_{182}N_{15}O_{64}$: 2689, found: 2689.



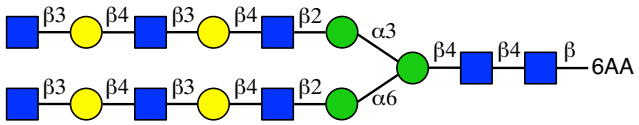
α-L-Fucopyranosyl-(1→2)-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-α-D-mannopyranosyl-(1→3)-[α-L-fucopyranosyl-(1→2)-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-α-D-mannopyranosyl-(1→6)]-β-D-mannopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(KVA)NKT (3.26): **3.25** (3 mg, 1.12 μmol) underwent GalT-5 catalyzed galactosylation described in general procedure B, followed by FucT II catalyzed fucosylation described in general procedure D to give **3.26** (2.5 mg, 68%) as a white amorphous solid. ^{1}H NMR (600 MHz, D_2O) δ 5.18 (d, *J* = 4.0 Hz, 2H), 5.10 (s, 1H), 5.03 (d, *J* = 9.6 Hz, 1H), 4.91 (s, 1H), 4.75 (s, 1H), 4.68–4.65 (m, 1H), 4.63 (d, *J* = 7.6 Hz, 2H), 4.62–4.58 (m, 3H), 4.57 (d, *J* = 8.0 Hz, 2H), 4.43 (d, *J* = 7.7 Hz, 1H), 4.42 (d, *J* = 7.7 Hz, 1H), 4.39 (t, *J* = 7.2 Hz, 1H), 4.31–4.25 (m, 3H), 4.24 (s, 1H), 4.22–4.19 (m, 1H), 4.17 (d, *J* = 2.6 Hz, 1H), 4.15–4.07 (m, 5H), 4.01–3.42 (m, 80H), 3.01–2.96 (m, 4H), 2.85 (dd, *J* = 16.2, 5.6 Hz, 1H), 2.73 (dd, *J* = 16.2, 7.7 Hz, 1H), 2.12–2.07 (m, 1H) 2.06 (s, 3H), 2.05 (s, 3H), 2.04 (s, 3H), 2.03 (s, 3H), 2.03 (s, 3H), 1.99 (s, 3H), 1.92–1.84 (m, 1H), 1.78–1.63 (m, 7H), 1.47–1.38 (m, 4H), 1.37 (d, *J* = 7.1 Hz, 3H), 1.22 (d, *J* = 6.5 Hz, 6H), 1.16 (d, *J* = 6.4 Hz, 3H), 0.95 (d, *J* = 6.7 Hz, 6H); MALDI-TOF MS *m/z* $[M + H]^+$ calcd for $C_{130}H_{222}N_{15}O_{82}$: 3306, found: 3306.



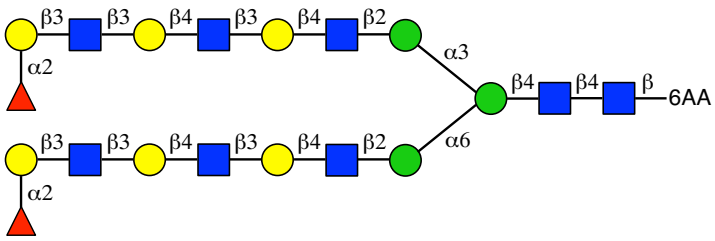
2-Acetamido-2-deoxy-α-D-galactopyranosyl-(1→3)-[α-L-fucopyranosyl-(1→2)]-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-α-D-mannopyranosyl-(1→3)-[2-acetamido-2-deoxy-α-D-galactopyranosyl-(1→3)-[α-L-fucopyranosyl-(1→2)]-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-α-D-mannopyranosyl-(1→6)]-β-D-mannopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(KVA)NKT (3.27): The GTA catalyzed conversion of **3.26** (1.4 mg, 0.42 μmol**)** to **3.27** (1.2 mg, 76%) was performed following general procedure E described above. ^{1}H NMR (700 MHz, D_2O) δ 5.27 (d, *J* = 4.1 Hz, 2H), 5.20 (d, *J* = 3.8 Hz, 2H), 5.14 (s, 1H), 5.07 (d, *J* = 9.7 Hz, 1H), 4.95 (s, 1H), 4.78 (s, 1H), 4.75–4.68 (m, 3H), 4.66–4.62 (m, 3H), 4.60 (d, *J* = 7.8 Hz, 2H), 4.47 (d, *J* = 7.8 Hz, 1H), 4.46 (d, *J* = 7.8 Hz, 1H), 4.45–4.40 (m, 1H), 4.40–4.35 (m, 3H), 4.35–4.29 (m, 3H), 4.29–4.22 (m, 6H), 4.21 (d, *J* = 2.8 Hz, 1H), 4.19–4.10 (m, 4H), 4.06–3.71 (m, 64H), 3.70–3.48 (m, 22H), 3.07–2.99 (m, 4H), 2.92–2.70 (m, 2H), 2.16–2.12 (m, 1H), 2.10 (s, 3H), 2.09 (s, 3H), 2.08 (s, 3H), 2.072 (s, 3H), 2.067 (s, 3H), 2.062 (s, 6H), 2.03 (s, 3H), 1.95–1.85 (m, 2H), 1.84–1.63 (m, 6H), 1.55–1.36 (m, 7H), 1.27 (d, *J* = 6.4 Hz, 6H), 1.19 (d, *J* = 6.4 Hz, 3H), 0.99 (d, *J* = 6.9 Hz, 6H); MALDI-TOF MS *m/z* $[M + Na]^+$ calcd for $C_{146}H_{247}N_{17}NaO_{92}$: 3734, found: 3735.



α-D-Galactopyranosyl-(1→3)-[α-L-fucopyranosyl-(1→2)]-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-α-D-mannopyranosyl-(1→3)-[α-D-galactopyranosyl-(1→3)-[α-L-fucopyranosyl-(1→2)]-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-α-D-mannopyranosyl-(1→6)]-β-D-mannopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(KVA)NKT (3.28): The GTB catalyzed conversion of **3.26** (1.6 mg, 0.48 μmol) to **3.28** (1.28 mg, 73%) was performed following general procedure F described above. ^{1}H NMR (700 MHz, D_2O) δ 5.27–5.22 (m, 4H), 5.13 (s, 1H), 5.06 (d, *J* = 9.6 Hz, 1H), 4.94 (s, 1H), 4.77 (s, 1H), 4.75–4.66 (m, 1H), 4.65–4.61 (m, 3H), 4.59 (d, *J* = 7.9 Hz, 2H), 4.46 (d, *J* = 7.6 Hz, 1H), 4.45 (d, *J* = 7.6 Hz, 1H), 4.42 (t, *J* = 7.2 Hz, 1H), 4.40–4.31 (m, 4H), 4.31–4.24 (m, 6H), 4.23–4.19 (m, 2H), 4.18–4.14 (m, 3H), 4.13–4.07 (m, 2H), 4.05–3.48 (m, 86H), 3.05–2.99 (m, 4H), 2.88 (dd, *J* = 16.2, 5.4 Hz, 1H), 2.80–2.72 (m, 1H), 2.09 (s, 3H), 2.08 (s, 3H), 2.076 (s, 3H), 2.062 (s, 3H), 2.058 (s, 3H), 2.02 (s, 3H), 1.96–1.86 (m, 3H), 1.82–1.66 (m, 5H), 1.53–1.36 (m, 8H), 1.25 (d, *J* = 6.6 Hz, 6H), 1.19 (d, *J* = 6.4 Hz, 3H), 0.98 (d, *J* = 6.8 Hz, 6H); MALDI-TOF MS *m/z* [M + Na]$^{+}$ calcd for $C_{142}H_{241}N_{15}NaO_{92}$: 3652, found: 3653.

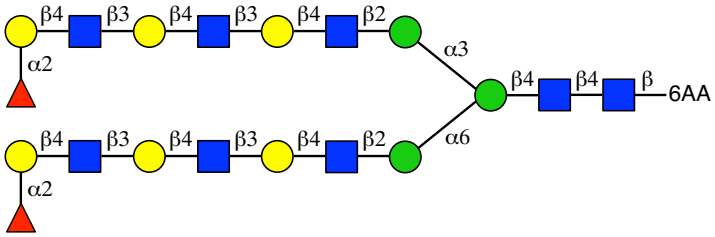


2-Acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-α-D-mannopyranosyl-(1→3)-[2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-α-D-mannopyranosyl-(1→6)]-β-D-mannopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(KVA)NKT (3.29): 3.25 (6 mg, 2.23 μmol) underwent GalT-1 catalyzed galactosylation described in general procedure A, followed by installment of GlcNAc residues described in general procedure C to give **3.29** (5.9 mg, 77%) as a white amorphous solid. ^{1}H NMR (600 MHz, D_2O): δ = 5.09 (s, 1H), 5.02 (d, J = 9.7 Hz, 1H), 4.91 (s, 1H), 4.74 (s, 1H), 4.70–4.63 (m, 5H), 4.59 (d, J = 8.0 Hz, 1H), 4.56 (d, J = 7.7 Hz, 2H), 4.47–4.40 (m, 4H), 4.38 (dd, J = 8.1, 6.2 Hz, 1H), 4.28 (q, J = 7.1 Hz, 1H), 4.23 (s, 1H), 4.20 (dd, J = 6.4, 4.2 Hz, 1H), 4.17 (d, J = 2.8 Hz, 1H), 4.15–4.12 (m, 4H), 4.11 (d, J = 4.2 Hz, 1H), 4.10–4.07 (m, 2H), 3.99–3.51 (m, 77H), 3.50–3.40 (m, 7H), 2.97–2.86 (m, 4H), 2.85 (dd, J = 16.2, 5.4 Hz, 1H), 2.73 (dd, J = 16.2, 7.7 Hz, 1H), 2.06 (s, 3H), 2.05 (m, 1H), 2.03 (s, 3H), 2.02 (s, 3H), 2.02 (s, 9H), 2.01 (s, 3H), 1.98 (s, 3H), 1.92–1.82 (m, 1H), 1.78–1.70 (m, 1H), 1.69–1.52 (m, 6H), 1.50–1.20 (m, 4H), 1.36 (d, J = 7.1 Hz, 3H), 1.15 (d, J = 6.4 Hz, 3H), 0.94 (d, J = 6.7 Hz, 6H); MALDI-TOF MS *m/z* $[M + H]^+$ calcd for $C_{134}H_{228}N_{17}O_{84}$: 3419, found: 3419.



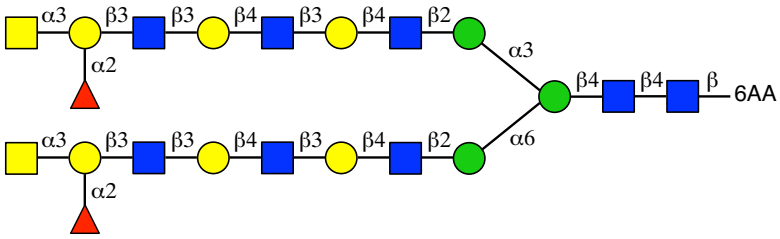
α-L-Fucopyranosyl-(1→2)-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-α-D-mannopyranosyl-(1→3)-[α-L-fucopyranosyl-(1→2)-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-α-D-mannopyranosyl-(1→6)]-β-D-mannopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(KVA)NKT (3.30): **3.29** (3 mg, 0.88 μmol) underwent GalT-5 catalyzed galactosylation described in general procedure B, followed by FucT II catalyzed fucosylation described in general procedure D to give **3.30** (2.4 mg, 68%) as a white amorphous solid. ^{1}H NMR (600 MHz, D_2O) δ 5.18 (d, *J* = 4.0 Hz, 2H), 5.10 (s, 1H), 5.03 (d, *J* = 9.6 Hz, 1H), 4.91 (s, 1H), 4.74 (s, 1H), 4.70–4.65 (m, 3H), 4.63 (d, *J* = 7.6 Hz, 2H), 4.62–4.58 (m, 3H), 4.56 (d, *J* = 7.6 Hz, 2H), 4.46–4.42 (m, 4H), 4.39 (t, *J* = 7.2 Hz, 1H), 4.32–4.25 (m, 3H), 4.24 (s, 1H), 4.22–4.19 (m, 1H), 4.17 (d, *J* = 2.8 Hz, 1H), 4.16–4.11 (m, 6H), 4.11–4.08 (m, 2H), 4.00–3.43 (m, 101H), 3.01–2.95 (m, 4H), 2.85 (dd, *J* = 16.2, 5.5 Hz, 1H), 2.73 (dd, *J* = 16.0, 7.6 Hz, 1H), 2.12–2.08 (m, 1H), 2.06 (s, 3H), 2.04 (s, 6H), 2.034 (s, 3H), 2.029 (s, 3H), 2.020 (s, 3H), 2.016 (s, 3H), 1.99 (s, 3H), 1.90–1.84 (m, 1H), 1.79–1.56 (m, 7H), 1.47–1.37 (m, 4H), 1.36 (d, *J* = 7.2 Hz,

3H), 1.22 (d, J = 6.6 Hz, 6H), 1.15 (d, J = 6.4 Hz, 3H), 0.95 (d, J = 6.7 Hz, 6H); MALDI-TOF MS m/z $[M + H]^+$ calcd for $C_{158}H_{268}N_{17}O_{102}$: 4037, found: 4037.



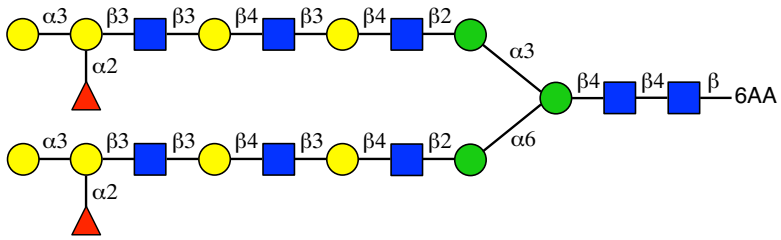
α-L-Fucopyranosyl-(1→2)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-α-D-mannopyranosyl-(1→3)-[α-L-fucopyranosyl-(1→2)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-α-D-mannopyranosyl-(1→6)]-β-D-mannopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(KVA)NKT (3.31): **3.29** (2.77 mg, 0.81 μmol) underwent GalT-1 catalyzed galactosylation described in general procedure A, followed by FucT II catalyzed fucosylation described in general procedure D to give **3.31** (2.05 mg, 63%) as a white amorphous solid. ^{1}H NMR (600 MHz, D_2O) δ 5.29 (d, J = 3.0 Hz, 2H), 5.10 (s, 1H), 5.03 (d, J = 9.7 Hz, 1H), 4.91 (s, 1H), 4.75 (s, 1H), 4.70–4.65 (m, 5H), 4.60 (d, J = 8.2 Hz, 1H), 4.56 (d, J = 7.8 Hz, 2H), 4.53 (d, J = 7.7 Hz, 2H), 4.48–4.41 (m, 4H), 4.39 (t, J = 7.2 Hz, 1H), 4.31–4.27 (m, 1H), 4.25–4.16 (m, 5H), 4.16–4.05 (m, 8H), 3.99–3.42 (m, 101H), 3.01–2.96 (m, 4H), 2.85 (dd, J = 15.7, 5.0 Hz, 1H),

2.73 (dd, J = 16.4, 7.8 Hz, 1H), 2.14–2.07 (m, 1H), 2.06 (s, 3H), 2.03 (s, 3H), 2.03 (s, 3H), 2.02 (s, 6H), 2.02 (s, 3H), 2.02 (s, 3H), 1.99 (s, 3H), 1.92–1.83 (m, 1H), 1.79–1.57 (m, 7H), 1.50–1.38 (m, 4H), 1.36 (d, J = 7.2 Hz, 3H), 1.21 (d, J = 6.6 Hz, 6H), 1.15 (d, J = 6.4 Hz, 3H), 0.95 (d, J = 6.7 Hz, 6H); MALDI-TOF MS m/z $[M + H]^+$ calcd for $C_{158}H_{268}N_{17}O_{102}$: 4037, found: 4037.



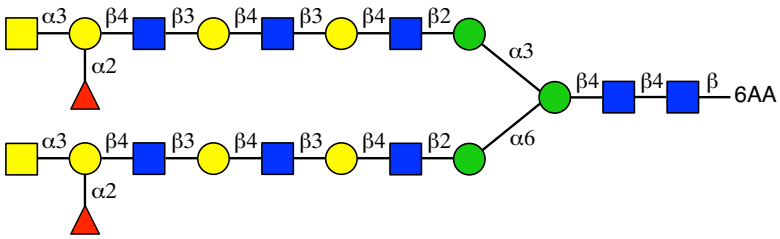
2-Acetamido-2-deoxy-α-D-galactopyranosyl-(1→3)-[α-L-fucopyranosyl-(1→2)]-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-α-D-mannopyranosyl-(1→3)-[2-acetamido-2-deoxy-α-D-galactopyranosyl-(1→3)-[α-L-fucopyranosyl-(1→2)]-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-α-D-mannopyranosyl-(1→6)]-β-D-mannopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(KVA)NKT (3.32): The GTA catalyzed conversion of **3.30** (1.1 mg, 0.27 μmol**)** to **3.32** was performed following general procedure E described above. Unfortunately, most of the product was lost during purification and so a yield was not

calculated. MALDI-TOF MS *m/z* $[M + Na]^+$ calcd for $C_{174}H_{293}N_{19}NaO_{112}$: 4465, found: 4465.



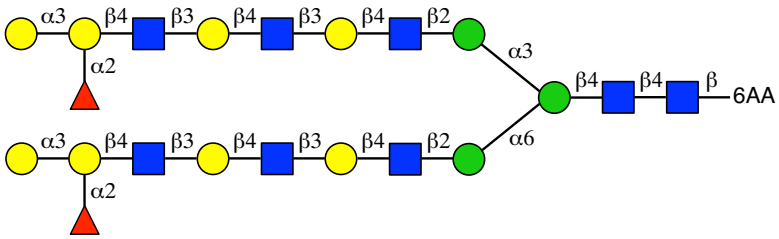
α-D-Galactopyranosyl-(1→3)-[α-L-fucopyranosyl-(1→2)]-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-α-D-mannopyranosyl-(1→3)-[α-D-galactopyranosyl-(1→3)-[α-L-fucopyranosyl-(1→2)]-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-α-D-mannopyranosyl-(1→6)]-β-D-mannopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(KVA)NKT (3.33): The GTB catalyzed conversion of **3.30** (1.1 mg, 0.27 μmol) to **3.33** (1 mg, 84%) was performed following general procedure F described above. ^{1}H NMR (700 MHz, D_2O) δ 5.28–5.22 (m, 4H), 5.13 (s, 1H), 5.06 (d, *J* = 9.7 Hz, 1H), 4.94 (s, 1H), 4.77 (s, 1H), 4.75–4.67 (m, 5H), 4.66–4.61 (m, 3H), 4.59 (d, *J* = 7.7 Hz, 2H), 4.49–4.45 (m, 4H), 4.43 (t, *J* = 7.0 Hz, 1H), 4.39–4.25 (m, 10H), 4.20 (d, *J* = 2.0 Hz, 1H), 4.19–4.14 (m, 5H), 4.12 (br s, 1H), 4.10 (t, *J* = 6.7 Hz, 1H), 4.07–3.46 (m, 109H), 3.05–2.99 (m, 4H), 2.88 (dd, *J* = 16.2, 5.6 Hz, 1H), 2.76 (dd, *J* =

16.5, 7.4 Hz, 1H), 2.09 (s, 3H), 2.08 (s, 6H), 2.064 (s, 3H), 2.060 (s, 3H), 2.051 (s, 3H), 2.047 (s, 3H), 2.02 (s, 3H), 1.96–1.87 (m, 3H), 1.82–1.68 (m, 6H), 1.52–1.41 (m, 4H), 1.40 (d, J = 7.1 Hz, 3H), 1.25 (d, J = 6.6 Hz, 6H), 1.21 (d, J = 5.8 Hz, 3H), 0.99 (d, J = 6.7 Hz, 6H); MALDI-TOF MS m/z $[M + Na]^+$ calcd for $C_{170}H_{287}N_{17}NaO_{112}$: 4383, found: 4383.



2-Acetamido-2-deoxy-α-D-galactopyranosyl-(1→3)-[α-L-fucopyranosyl-(1→2)]-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-α-D-mannopyranosyl-(1→3)-[2-acetamido-2-deoxy-α-D-galactopyranosyl-(1→3)-[α-L-fucopyranosyl-(1→2)]-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-α-D-mannopyranosyl-(1→6)]-β-D-mannopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(KVA)NKT (3.34): The GTA catalyzed conversion of **3.31** (1 mg, 0.25 μmol) to **3.34** (0.94 mg, 85%) was performed following general procedure E described above. ^{1}H NMR (700 MHz, D_2O) δ 5.37 (d, J = 3.8 Hz, 2H), 5.20 (d, J = 3.5 Hz, 2H), 5.14 (s, 1H), 5.07 (d, J = 8.5 Hz, 1H), 4.95 (s, 1H), 4.78 (s, 1H), 4.75–4.67 (m, 5H), 4.63 (d, J =

7.5 Hz, 3H), 4.60 (d, *J* = 7.6 Hz, 2H), 4.52–4.45 (m, 4H), 4.45–4.40 (m, 1H), 4.36–4.30 (m, 3H), 4.28–4.20 (m, 9H), 4.20–4.11 (m, 7H), 4.04–3.44 (m, 108H), 3.08–2.97 (m, 4H), 2.93–2.84 (m, 1H), 2.82–2.70 (m, 1H), 2.10 (s, 3H), 2.08–2.04 (m, 24H), 2.03 (s, 3H), 1.95–1.82 (m, 3H), 1.82–1.66 (m, 6H), 1.52–1.36 (m, 7H), 1.27 (d, *J* = 6.4 Hz, 6H), 1.19 (d, *J* = 5.9 Hz, 3H), 0.99 (d, *J* = 6.7 Hz, 6H); MALDI-TOF MS *m/z* $[M + Na]^+$ calcd for $C_{174}H_{293}N_{19}NaO_{112}$: 4465, found: 4465.



α-D-Galactopyranosyl-(1→3)-[α-L-fucopyranosyl-(1→2)]-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-α-D-mannopyranosyl-(1→3)-[α-D-galactopyranosyl-(1→3)-[α-L-fucopyranosyl-(1→2)]-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-α-D-mannopyranosyl-(1→6)]-β-D-mannopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(KVA)NKT (3.35): The GTB catalyzed conversion of **3.31** (1 mg, 0.25 μmol) to **3.35** (0.96 mg, 89%) was performed following general procedure F described above. ^{1}H NMR (700 MHz, D_2O) δ 5.35 (d, *J* = 4.1 Hz, 2H),

5.26 (br s, 2H), 5.14 (s, 1H), 5.06 (d, J = 9.6 Hz, 1H), 4.94 (s, 1H), 4.78 (s, 1H), 4.75–4.67 (m, 5H), 4.67–4.62 (m, 3H), 4.60 (d, J = 8.1 Hz, 2H), 4.52–4.45 (m, 4H), 4.42 (t, J = 7.0 Hz, 1H), 4.35–4.29 (m, 5H), 4.28–4.19 (m, 5H), 4.19–4.15 (m, 5H), 4.15–4.10 (m, 2H), 4.04–3.44 (m, 110H), 3.05–2.98 (m, 4H), 2.88 (dd, J = 16.3, 5.1 Hz, 1H), 2.77 (dd, J = 16.1, 7.4 Hz, 1H), 2.10 (s, 3H), 2.07 (s, 3H), 2.066–2.058 (m, 9H), 2.056 (s, 3H), 2.052 (s, 3H), 2.02 (s, 3H), 1.94–1.87 (m, 1H), 1.82–1.59 (m, 8H), 1.52–1.41 (m, 4H), 1.40 (d, J = 7.1 Hz, 3H), 1.26 (d, J = 6.5 Hz, 6H), 1.19 (d, J = 6.3 Hz, 3H), 0.98 (d, J = 6.6 Hz, 6H); MALDI-TOF MS *m/z* $[M + Na]^+$ calcd for $C_{170}H_{287}N_{17}NaO_{112}$: 4383, found: 4383.

Chapter 4: Chemo-enzymatic synthesis of group B *Streptococcus* type III (GBSIII) glycoconjugates for understanding the mechanism of glycoconjugate vaccine activation of the immune system

4.1 Introduction

Capsular polysaccharides (CPSs), a class of high-molecular-weight biologically important molecules expressed on the surface of bacteria, have been identified as promising vaccine candidates to prevent life-threatening infectious diseases.[154] Effective generation of protective antibodies of the IgG class requires the involvement of both T cells and B cells of the immune system. T cells are involved in activating B cells, which lead to IgG antibodies. However, CPSs, like all carbohydrates, are T cell-independent antigens. When they are used in pure form for immunization, they can only induce the production of low-affinity immunoglobulin M (IgM) antibodies, not high-affinity IgG antibodies. This is because of the lack of T cell involvement in B cell activation.[155] To circumvent this problem, glycoconjugate vaccines, in which the CPS is covalently attached to a carrier protein have been developed. Glycoconjugate vaccines effectively elicit T cell help for B cell activation, resulting in the production of polysaccharide-specific IgG antibodies and long-lived immune responses.[156]

To date, glycoconjugate vaccines derived from either natural CPSs or synthetic oligosaccharide fragments of them have offered enormous health benefits. Representative examples include Menactra vaccine against *Neisseria meningitides*, the Prevnar vaccine against *Streptococcus pneumonia* and a vaccine against *Haemophilus influenzae* type b (Hib).[3] However, the mechanisms of glycoconjugate vaccine activation of the adaptive immune system, especially the glycoconjugate processing and presentation pathways, remain to be fully dissected.[156,157] Figure 4.1 illustrates the traditional hypothesis that accounts for the production of protective IgG antibodies from glycoconjugate vaccines.[158] After being recognized by B-cell receptors (BCRs) present on polysaccharide-specific pre-

B cells and taken into B-cells, the glycoconjugate is processed by proteases in endosomes to generate peptide fragments. These peptide fragments bind to MHCII molecules, and then are presented on the B-cell surface to $CD4^+$ T cells in the context of MHCII. Subsequently, interaction between the peptide/MHCII complex and the T cell receptor (TCR) of $CD4^+$ T cells, along with costimulatory pathways, activates T cells to release cytokines. These cytokines in turn stimulate B cells maturation to memory B cells and induce polysaccharide-specific IgM to IgG switching. The logic behind this model is built on the assumption that peptides are the only antigens capable of being presented by MHCII to T cells, because carbohydrates fail to induce T cell responses.

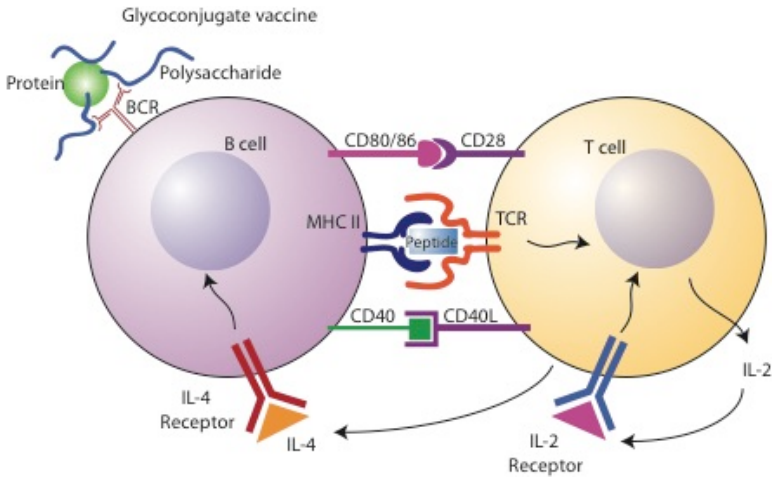


Figure 4.1 Traditional mechanistic model for T cell activation by glycoconjugate vaccines. (Taken from: Avci, F. Y.; Li, X.; Tsuji, M.; Kasper, D. L. Nature Medicine **2011**, 17, 1602–1609.)

Since proposed, tremendous efforts have been made to test this 'peptide-only' presentation hypothesis and explore other possible mechanisms. Recently, emerging evidence has led to an understanding that peptides are not the only antigens capable of being presented to MHCII for T cell recognition.[156] A representative example is

zwitterionic polysaccharides (ZPSs), which can be processed by antigen presenting cells (APCs) and presented by MHCII to $CD4^+$ T cells, leading antibody production.[159] This discovery not only provided the first mechanistic insight into how carbohydrates themselves can be presented to activate T cells, but also opened up the possibility that the carbohydrate portion of glycoconjugate vaccines, not just the peptide portion, can be somehow presented for T cell recognition.

In 2011, Kasper and coworkers proposed a new model illustrating the presentation of carbohydrate epitopes of glycoconjugates vaccines for T cell recognition (Figure 4.2).[109] For this study they employed a glycoconjugate vaccine derived from group B *Streptococcus* type III (GBSIII) CPS. This CPS has a pentasaccharide repeating unit (Figure 4.2A). They found that glycopeptides generated from endolysosomal processing of GBSIII glycoconjugate binds to MHCII via the peptide domain to form a MHCII–glycopeptide complex, which allows the presentation of the glycan to $CD4^+$ T cells (Figure 4.2B). The successful isolation of glycan-specific $CD4^+$ T cell clones indicated glycan presentation on the APC surface. The binding of glycan-specific TCRs to the glycan domain of the MHCII–glycopeptide complex contributes to immune system activation. In this model, the peptide domain serves as a bridge to connect MHCII with the glycan domain, resulting in the presentation of the carbohydrate by MHCII. Moreover, the presented glycopeptide is estimated to contain approximately eight GBSIII pentasaccharide-repeating units, in another words, a 40-residue polysaccharide linked to a peptide. This study greatly expands our understanding of the role of carbohydrates in activation of the adaptive immune system.

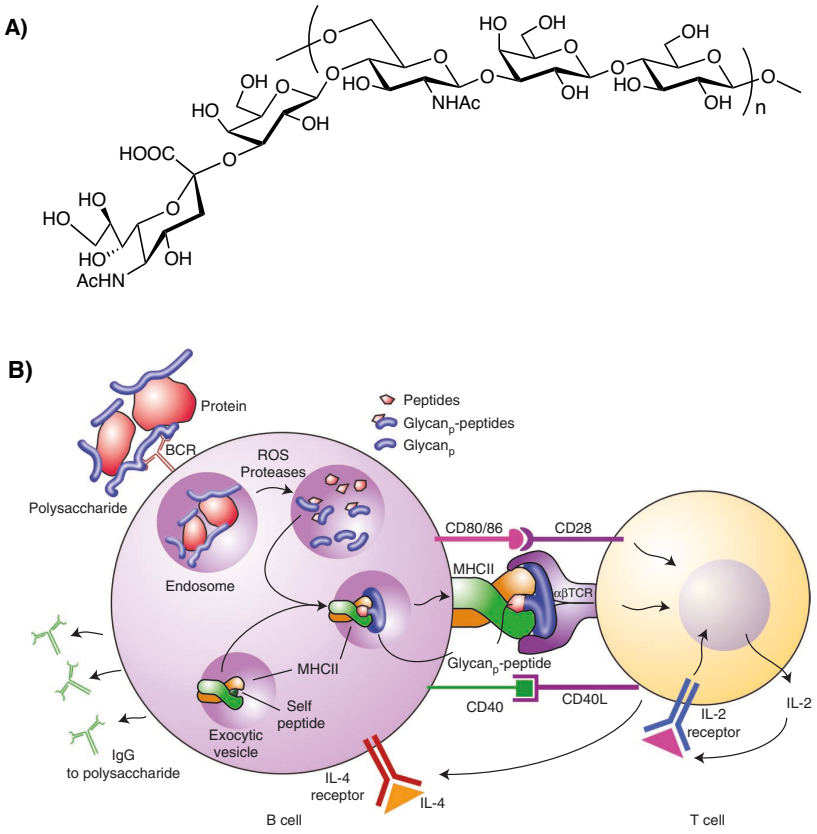


Figure 4.2 A) Structure of GBSIII CPS; B) New proposed mechanistic model for T cell activation by glycoconjugate vaccines. (Taken from: Avci, F. Y.; Li, X.; Tsuji, M.; Kasper, D. L. Nature Medicine **2011**, 17, 1602–1609.)

Despite the logic of this new mechanism, the size of glycan domain (40-mer) presented for T cell recognition in Kasper's study is surprising. In particular, this suggests that other glycoconjugate vaccines produced from synthetic fragments of CPSs, which typically contain fewer than 10 sugar residues,[90] may not be optimal for protection. Unambiguous confirmation of the mechanism outlined in Figure 4.2B still requires more conclusive evidence such as the crystal structure of a MHCII–glycopeptide complex or a MHCII–glycopeptide–TCR complex. Given the heterogeneity of GBSIII polysaccharide

derived from natural sources, a better understanding of the interaction between MHCII, glycopeptides and the TCR requires access to structurally well-defined oligosaccharides up to as large as a 40-mer. Although attempts to synthesize GBSIII oligosaccharides have been made before,[160,161] oligosaccharides containing more than two GBSIII pentasaccharide-repeating units have not been synthesized, mainly due to the synthetic challenges posed by structural complexity. Therefore, I started this synthetic project to access a library of well-defined GBSIII oligosaccharides differing in size (**4.1**–**4.5**, Figure 4.3). The goal was to prepare a homologous series of molecules containing two, four, six, eight and ten repeating units. The preparation of the smaller molecules would allow the chemistry to be optimized on a more tractable system, which would allow more straightforward access to the more complicated species. All of the compounds were synthesized with an aminoethyl group, which would allow conjugation to a peptide. The glycoconjugates generated from these compounds will be used to further explore the mechanism proposed in Figure 4.2B and will provide additional structural insight into the interaction between MHCII, glycopeptides and the TCR.

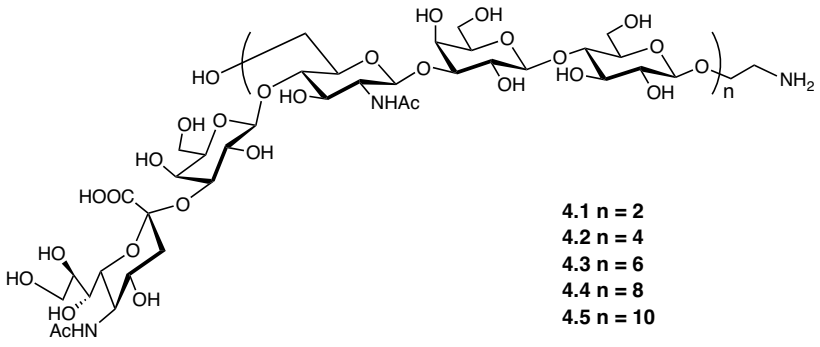


Figure 4.3 Structures of well-defined GBSIII oligosaccharides **4.1**–**4.5**.

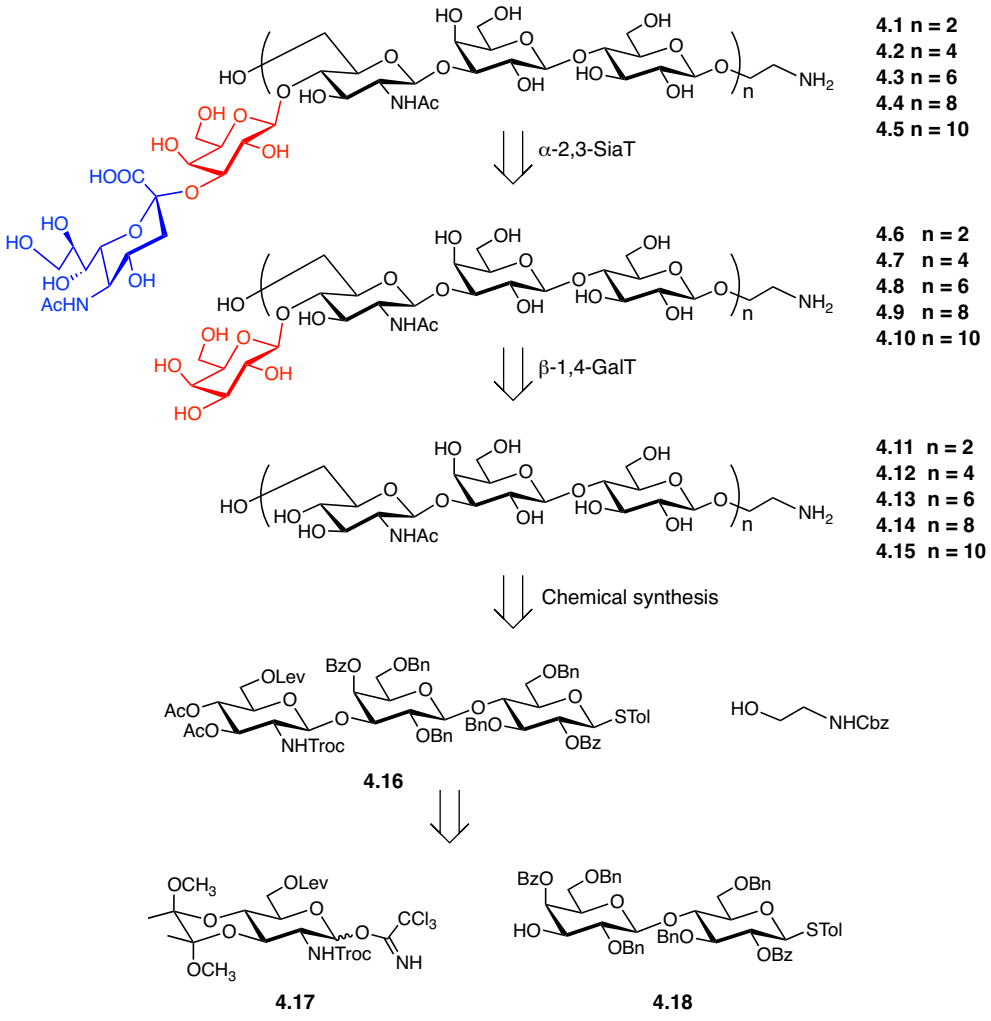
4.2 Results and discussion

4.2.1 Retrosynthetic analysis of GBSIII oligosaccharides

Five GBSIII oligosaccharides ranging in size from two to ten pentasaccharide-repeating units were targeted for synthesis. Given the presence of sialic acid residues in the repeating unit, the branched structure, and the size of the targets, we anticipated it would be a formidable challenge to synthesize these compounds via a solely chemical approach. As discussed in section 1.3 of Chapter 1, glycosyltransferases, an essential class of enzymes involved in glycan biosynthesis, are drawing increasing attention in complex oligosaccharide synthesis because they can catalyze the construction of glycosidic linkages with exquisite regio- and stereoselectivity under mild conditions without protecting groups.[47] In the case of these targets, we thought a chemo-enzymatic approach, which would integrate the flexibility of chemical synthesis with the specificity of enzymatic transformations, would be the most efficient way to access these complex oligosaccharides.[50]

Based on structural analysis of GBSIII oligosaccharides, we proposed that the side chain disaccharide epitope (α-Neu5NAc-(2→3)-β-Gal-(1→4)-) attached to the GlcNAc residue in each repeating unit could be constructed by glycosyltransferases. The enzymes required for these glycosylations, a β-(1→4)-GalT[162] and a α-(2→3)-SiaT,[54] are available. Therefore, the chemo-enzymatic approach we developed to synthesize **4.1**–**4.5**, involved the chemical synthesis of backbone oligosaccharides **4.11**–**4.15** followed by glycosyltransferase-catalyzed attachment of the α-Neu5NAc-(2→3)-β-Gal-(1→4) sequence to each GlcNAc residue in **4.11**–**4.15** (Scheme 4.1). The β-(1→4)-GalT-catalyzed multi-galactosylation of **4.11**–**4.15** will afford **4.6**–**4.10**, which are substrates for α-(2→3)-SiaT-

catalyzed multi-sialylation to yield the desired GBSIII oligosaccharides **4.1**–**4.5**. The protected backbone oligosaccharides, precursors for **4.11**–**4.15**, can be constructed from trisaccharide **4.16** via sequential chain extension. Trisaccharide **4.16** can be assembled from the glucosamine-based trichloroacetimidate **4.17** and the lactose-based thioglycoside **4.18**. The rationale behind the choice of protecting groups will be discussed in the following section. The ethylamine aglycon can be derived from commercially available *N*-Z-ethanolamine.

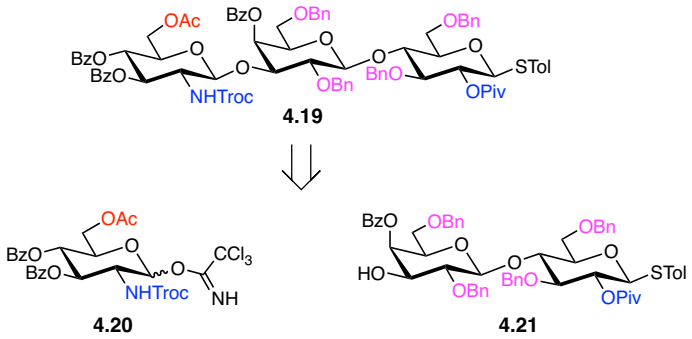


Scheme 4.1 Retrosynthetic analysis for the chemo-enzymatic synthesis of of GBSIII oligosaccharides **4.1**–**4.5**. The residues introduced by glycosyltransferases are shown in red and blue.

4.2.2 Synthesis of trisaccharide building block 4.16 and its dimer

4.2.2.1 Rationale for the initial design of the trisaccharide building block

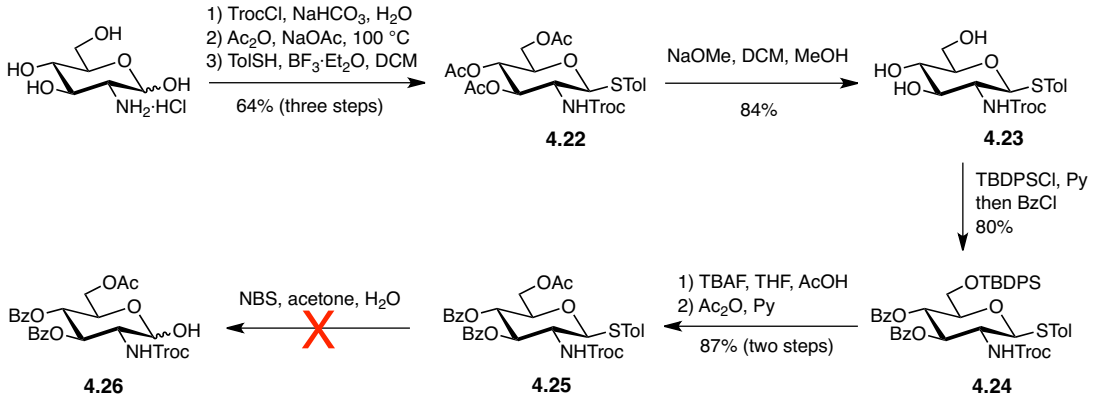
Access to large amounts of a trisaccharide building block is a prerequisite for implementing this chemo-enzymatic approach to the targets. Initially, the trisaccharide I designed was compound **4.19** (Scheme 4.2) instead of **4.16**, which I expected could be assembled from building blocks **4.20** and **4.21**. The GBSIII oligosaccharide backbone is composed of alternating GlcNAc and lactose residues connected via 1,2-*trans*-β-linkages. Therefore, neighboring participating groups 2,2,2-trichloroethoxycarbonyl (Troc) and pivaloyl (Piv) were chosen to mask the amino group of glucosamine and O-2 of lactose, respectively. The acetyl group installed on O-6 of the glucosamine residue could be selectively removed in the presence of benzoyl groups at a later stage to unmask the hydroxyl group for subsequent glycosylation. Considering that the chain extension employing **4.19**, or dimer of **4.19,** as the donor might suffer from the low reactivity, four benzyl groups, instead of benzoyl groups, were introduced to the lactose residue to increase its reactivity. Although removing a large number of benzyl groups from complex oligosaccharides by hydrogenation at the end of the synthesis can be difficult, I anticipated that this problem could be addressed by Birch reduction.



Scheme 4.2 Initial design of trisaccharide **4.19** and its two building blocks **4.20** and **4.21**.

4.2.2.2 Synthesis of D-glucosamine building block 4.17

With this original analysis in mind, I started my attempt to synthesize building block **4.20** from D-glucosamine hydrochloride (Scheme 4.3). First, the C-2 amino group was protected as a Troc carbamate and the remaining hydroxyl groups were acetylated in acetic anhydride at 100 °C in the presence of sodium acetate. The resulting intermediate was coupled with *p*-thiocresol in the presence of $BF_3 \cdot OEt_2$ to yield thioglycoside **4.22** in 64% yield over three steps. The choice of Troc as the *N*-protecting group was made based on the consideration of facile introduction and deprotection, β-selective glycosylation, as well as stability under a range of reaction conditions.[163] Compound **4.22** was then deacetylated using a catalytic amount of sodium methoxide in methanol affording **4.23** in 84% yield. Thioglycoside **4.23** then underwent a one-pot regioselective silylation with *tert*-butyl(chloro)diphenylsilane (TBDPSCl) and benzoylation to generate **4.24** in 80% yield. Subsequently, cleavage of the TBDPS ether in **4.24** followed by acetylation led to the formation of **4.25** in 87% yield over two steps.



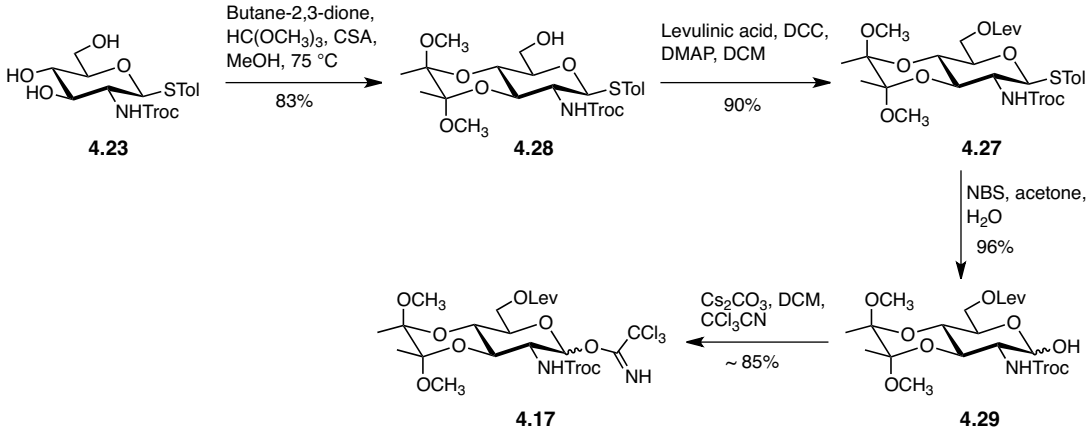
Scheme 4.3 Synthesis of thioglycoside **4.25** (precursor for **4.20**).

The conversion of thioglycoside **4.25** to trichloroacetimidate **4.20** required the formation of hemiacetal **4.26**. Unfortunately, **4.25** remained intact under the commonly used hydrolysis conditions (NBS in acetone–water), and none of the desired **4.26** was obtained. An attempt to hydrolyze **4.25** using NIS/AgOTf or NIS/TfOH in dichloromethane with a suitable amount of water also failed, indicating **4.25** is super-disarmed. A reasonable explanation is that the electron-withdrawing protecting groups installed at O-3, O-4 and O-6 positions significantly reduced the reactivity of thioglycoside. This did not bode well for future glycosylations involving compounds with this protecting group pattern.

To address this issue, I turned to tuning the reactivity of thioglycoside by introducing electron-donating groups at O-3 and O-4 positions. A survey of protecting groups led me to the butane-2,3-diacetal (BDA) group, which provides a convenient way to protect vicinal diequatorial diols.[164] On the other hand, the Lev ester drew my attention for the orthogonal protection of the C-6 hydroxyl group; its deprotection by hydrazine acetate makes it superior to acetyl groups as a temporary protecting group. As a result, thioglycoside **4.27** (Scheme 4.4) with a BDA across O-3 and O-4 and a Lev ester located at O-6 was proposed.

The synthesis of **4.27** is illustrated in Scheme 4.4. Thioglycoside **4.23** was treated with butane-2,3-dione, trimethylorthoformate and a catalytic amount of camphorsulfonic acid (CSA) in methanol at reflux to selectively protect the C-3 and C-4 hydroxyl groups with a BDA group;[164] the desired compound **4.28** was obtained in 83% yield. Then, the C-6 hydroxyl group was protected as a Lev ester by treatment with levulinic acid, *N*,*N*′-dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP), resulting in the formation of **4.27** in 90% yield. Subsequently, the conversion of **4.27** to

trichloroacetimidate **4.17** was studied. To my delight, NBS-promoted hydrolysis of **4.28** proceeded smoothly to afford hemiacetal **4.29** in 96% yield, which was treated with trichloroacetonitrile and cesium carbonate (Cs_2CO_3) to provide trichloroacetimidate **4.17**. After the reaction reached completion, the insoluble Cs_2CO_3 was removed by filtration through Celite. Crude **4.17** was dried under high vacuum and then used immediately for glycosylation reactions.

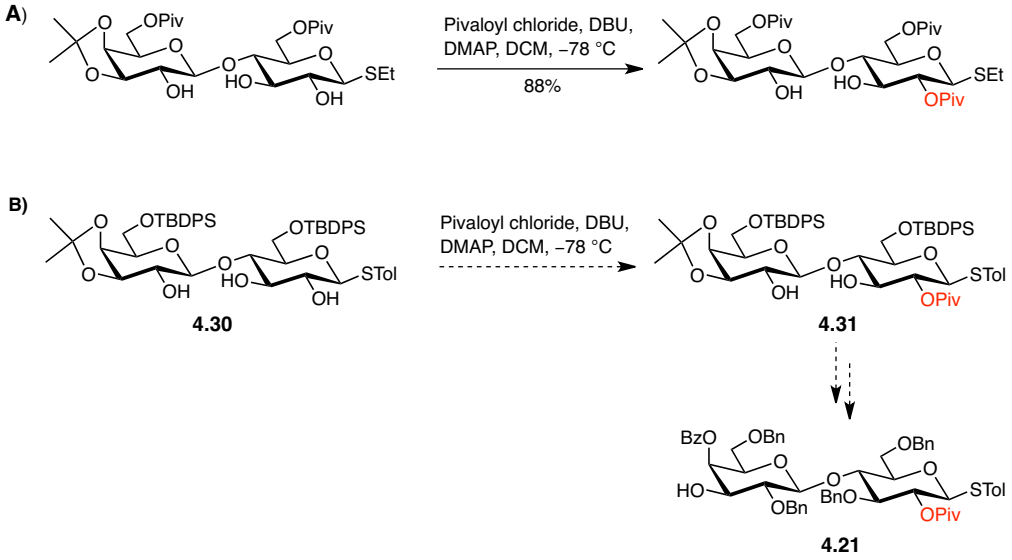


Scheme 4.4 Synthesis of trichloroacetimidate **4.17**.

4.2.2.3 Synthesis of D-lactose-based building block 4.18

My initial design of the lactose-based building block **4.21**, with a Piv ester installed at O-2 instead of other neighboring participating groups (*e.g.*, benzoyl), was inspired by a study regarding regioselective mono-pivaloylation carried out by Goto and coworkers.[165] They found that a bulky Piv group can be regioselectively installed at O-2 of a 6,6′-di-*O*-pivaloyl-thiolactoside derivative by treatment with pivaloyl chloride (1 equiv.) at −78 °C in the presence of DBU (1 equiv.) and DMAP (0.2 equiv.) (Scheme 4.5A). To take advantage of this methodology, compound **4.30** (Scheme 4.5B) equipped with TBDPS ether at O-6

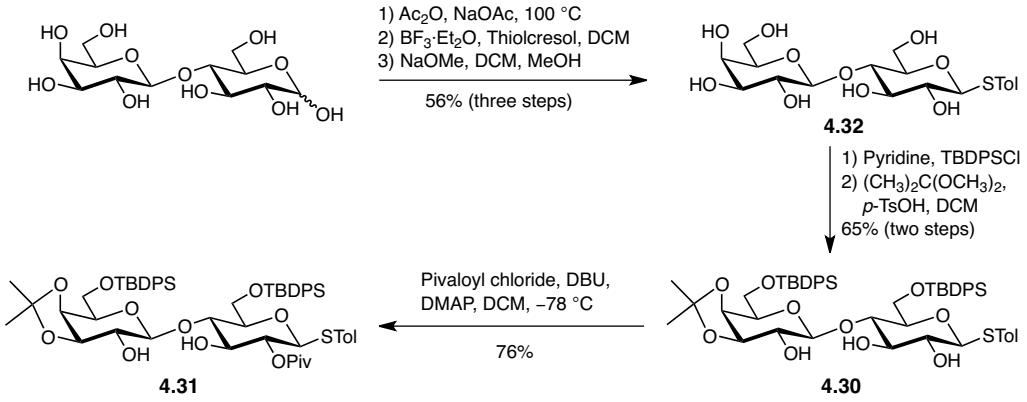
and O-6′ positions was designed to mimic the substrate in Goto's study. It was envisioned that regioselective mono-pivaloylation of **4.30** would result in **4.31**, which could be further converted to building block **4.21** via protecting group manipulations.



Scheme 4.5 A) Regioselective mono-pivaloylation developed by Goto and coworkers;[165] B) Synthetic plan for building block **4.21**.

To test this idea, **4.30** was synthesized from D-lactose in five steps (Scheme 4.6). Acetylation of lactose in acetic anhydride at 100 °C in the presence of sodium acetate formed preferentially the β-anomer of lactose octaacetate, which underwent coupling with *p*-thiocresol using $BF_3 \cdot OEt_2$ as the promoter. The product of this reaction was deacetylated using a catalytic amount of sodium methoxide to afford the deprotected thioglycoside **4.32** in 56% yield over three steps. Then, selective silylation of the 6-OH and 6′-OH groups using TBDPSCl, and installation of an isopropylidene ketal at the O-3′- and O-4′ positions enabled the conversion of **4.32** to triol **4.30** in 65% yield over two steps. With **4.30** in hand,

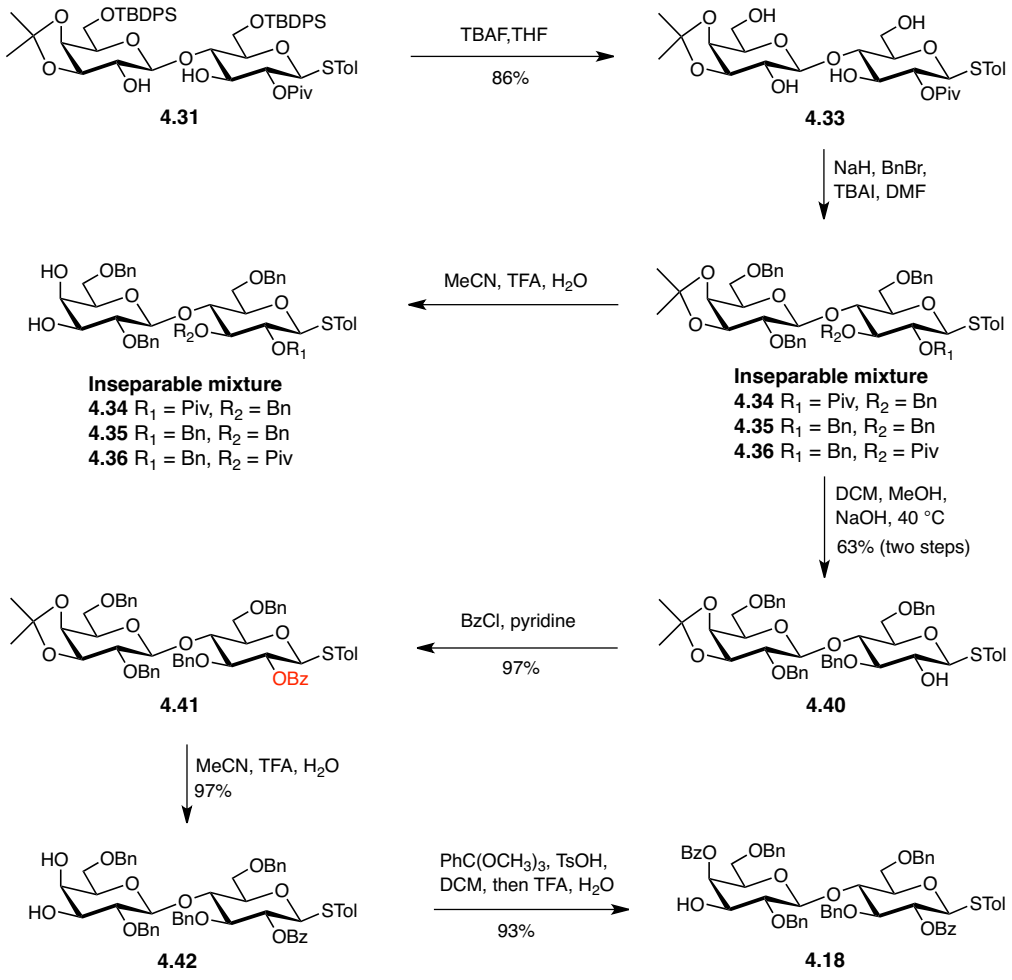
regioselective mono-pivaloylation of **4.30** at the O-2 position was performed using the method mentioned above. Although three pivaloylated compounds were observed, the desired compound, **4.31**, was isolated in 76% yield. The two side products were derived from mono-pivaloylation at the O-3 position and di-pivaloylation at O-2 and O-2′.



Scheme 4.6 Synthesis of intermediate **4.31**.

With this success, I explored the conversion of **4.31** to the initially designed building block **4.21**. As described in Scheme 4.7, desilylation of **4.31** using tetrabutylammonium fluoride (TBAF) afforded **4.33** in 86% yield, which was subjected to benzylation using benzyl bromide and sodium hydride to furnish **4.34**. A problem encountered under these basic reaction conditions was the partial hydrolysis and migration of the Piv ester, which resulted in two side products **4.35** and **4.36**. Although optimization of the reaction by adding a catalytic amount of tetrabutylammonium iodide (TBAI) and lowering the reaction temperature to 0 °C reduced the amount of **4.35** and **4.36**, the separation of **4.34** was still a challenge because these three compounds overlapped completely on TLC. To circumvent this problem, benzylation of **4.33** employing freshly prepared silver oxide and benzyl bromide was attempted. It was anticipated that the

hydrolysis and migration of Piv would be prevented under these less basic reaction conditions. Unfortunately, the reaction was very slow and a number of spots were observed on TLC after a long reaction time. Therefore, this approach was abandoned. Instead, the inseparable mixture of **4.34**, **4.35** and **4.36** obtained by benzylation of **4.33** using benzyl bromide, sodium hydride and TBAI was carried forward to the next step, which was the removal of isopropylidene using 10% trifluoroacetic acid (TFA) in acetonitrile. After cleavage of the acetal the resulting diols **4.37**, **4.38** and **4.39** were still inseparable.



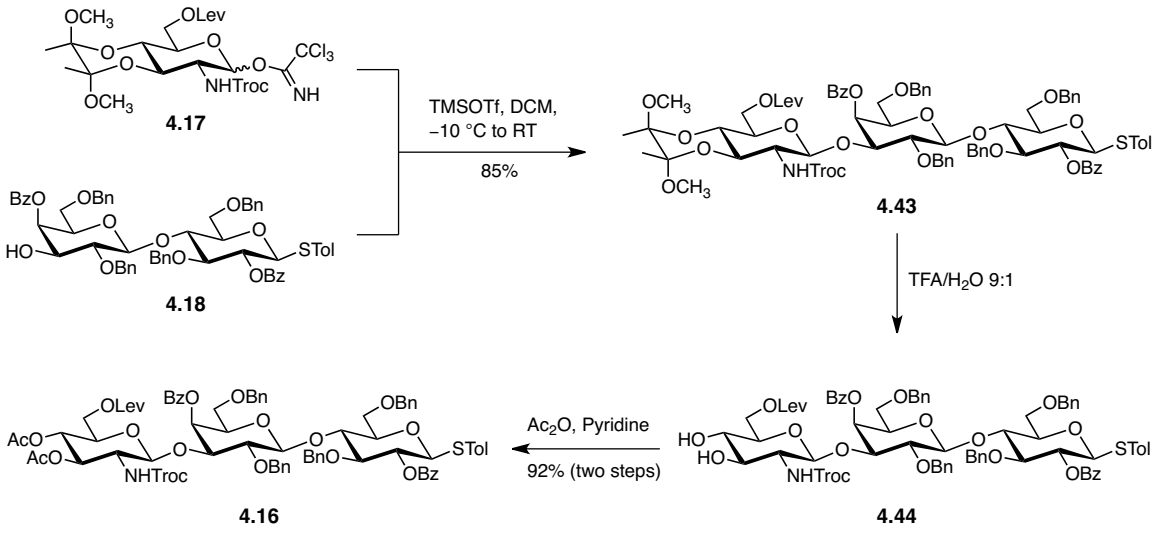
Scheme 4.7 Synthesis of building block **4.18**.

The separation problem motivated me to explore an indirect approach: removal of the Piv ester temporarily for separation purposes. TLC analysis revealed that treatment of the inseparable mixture of **4.34**, **4.35** and **4.36** with sodium methoxide at room temperature led to the formation of two new polar spots, suggesting that the desired alcohol **4.40** could be separated. However, I found that the Piv ester was quite robust against methanolysis. The reaction did not reach completion despite the addition of a large amount of sodium methoxide and using long reaction times. Finally, the reaction was pushed to completion by a using large amount of sodium hydroxide and keeping the reaction at 40 °C overnight. Under these conditions, **4.40** was successfully isolated in 63% yield over two steps.

To synthesize initial target **4.21**, **4.40** needed to be converted back to **4.34** by pivaloylation. However, given the robustness of the Piv ester to hydrolysis, I decided to change the synthetic plan by masking the 2-OH group of **4.40** with a benzoyl ester to simplify the late-stage deprotection. Benzoylation of **4.40** produced **4.41**, which was subjected to 10% TFA in acetonitrile to remove the isopropylidene acetal, furnishing diol **4.42** in 94% yield over two steps. Subsequently, **4.42** was treated with trimethylorthobenzoate and a catalytic amount of *p*-TsOH to install an orthoester across the O-3′ and O-4′ positions. This intermediate underwent TFA and water initiated regioselective ring opening to form a benzoyl ester at the O-4′ position,[166] leading to lactose-based acceptor **4.18** in 93% yield. The only difference between **4.18** and the initial target **4.21** is the identity of the neighboring participating group at the O-2 position (Bz in place of Piv).

4.2.2.4 Synthesis of trisaccharide building block 4.16

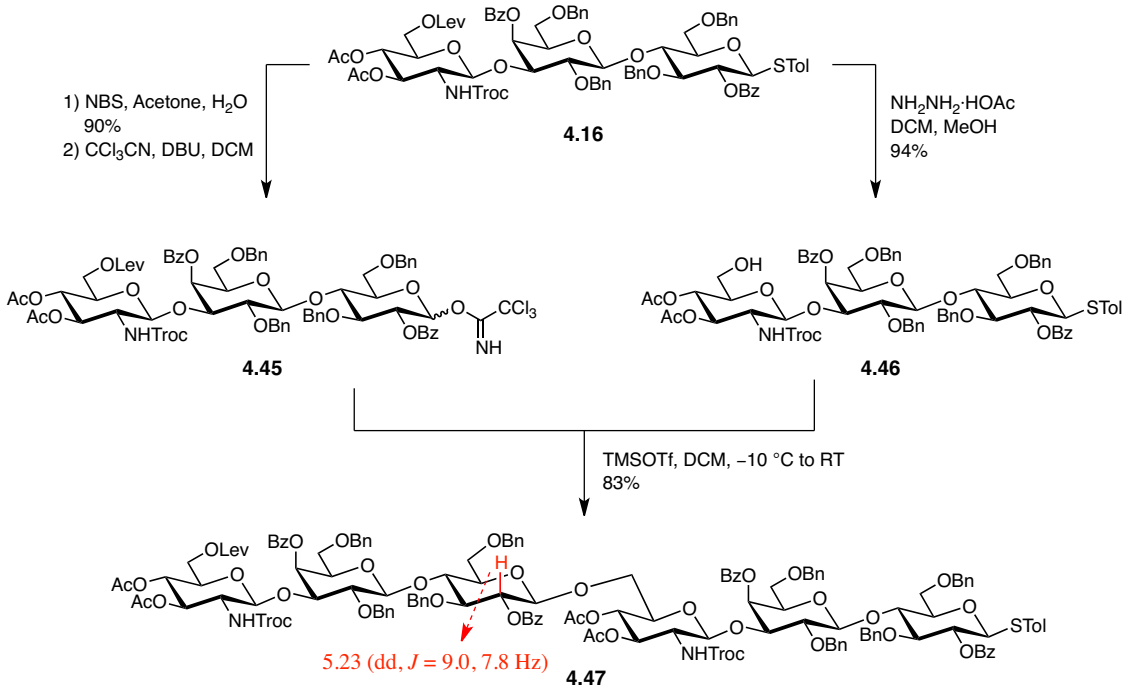
As discussed above, the problems encountered in the course of synthesis of initially designed building blocks (**4.20** and **4.21**) led me to prepare two more suitable derivatives **4.17** and **4.18**. With these two compounds in hand, the assembly of trisaccharide was carried out (Scheme 4.8). Freshly prepared **4.17** was coupled with acceptor **4.18** using TMSOTf as the promoter to furnish trisaccharide **4.43** in 85% yield. The stereoselectivity of the glycosylation was directed by neighboring group participation of the Troc carbamate at the glucosamine C-2 position. In theory, **4.43** could be used directly as the key building block to construct the GBSIII backbone oligosaccharides. However, given the concern that glycosidic bonds are susceptible to hydrolysis under BDA deprotection conditions (90% aqueous TFA),[167] I thought it would be better to replace the BDA with another protecting group (*e.g.*, acetate esters) at this stage. This would also allow a simpler global deprotection at the end of the synthesis. Thus, **4.43** was subjected to 90% aqueous TFA to cleave the BDA. It turned out that conversion of **4.43** to diol **4.44** was finished in five minutes; longer reaction times might result in trisaccharide decomposition. The diol was then acetylated to provide **4.16** in 92% yield over two steps. The β-stereochemistry of the glucosamine residue in trisaccharide was established based on the $^{3}J_{\text{H-1}'',\text{H-2}''}$ value (8.4 Hz) obtained from the ^{1}H NMR spectrum of **4.16**.



Scheme 4.8 Synthesis of trisaccharide building block **4.16**.

4.2.2.5 Synthesis of hexasaccharide thioglycoside building block (dimer of 4.16)

Using trisaccharide **4.16** as the donor for oligosaccharide assembly would allow the chain to be extended only by one repeating unit after each glycosylation. To facilitate the chain extension, a hexasaccharide thioglycoside, a dimer of **4.16**, was synthesized. As depicted in Scheme 4.9, treatment of thioglycoside **4.16** with NBS in acetone–water (9:1) afforded a hemiacetal intermediate in 90% yield, which was converted to trichloroacetimidate **4.45** by treatment with trichloroacetonitrile and a catalytic amount of DBU. The acceptor **4.46** was readily achieved from **4.16** by selective removal, in 94% yield, of the Lev group using hydrazine acetate. Subsequently, the freshly prepared donor **4.45** was subjected to glycosylation with acceptor **4.46** in the presence of TMSOTf, resulting in the desired hexasaccharide **4.47** in 83% yield.



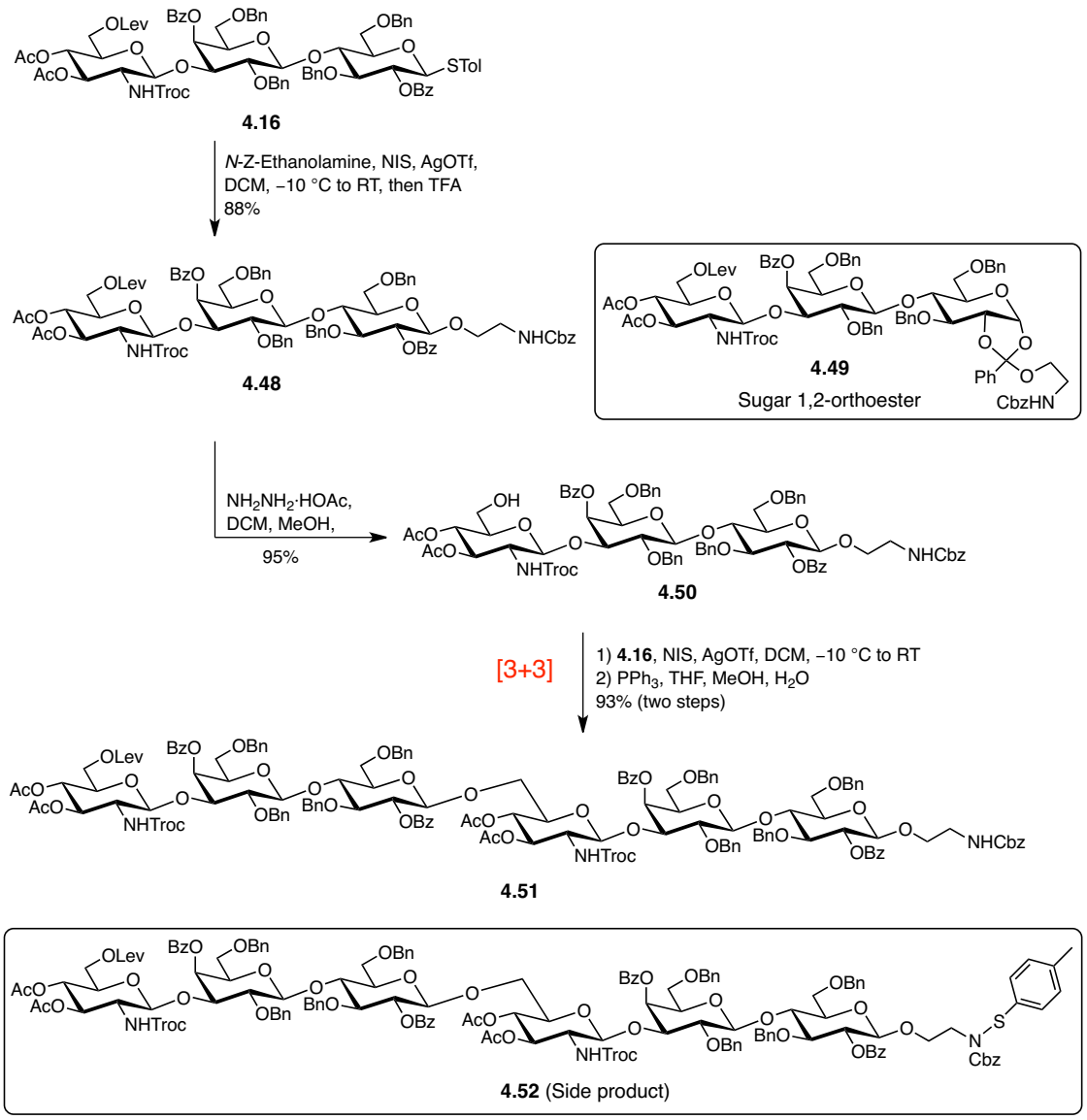
Scheme 4.9 Synthesis of hexasaccharide building block **4.47**.

The stereoselectivity of this glycosylation was directed by neighboring group participation of the benzoyl group at the O-2 position of the glucose residue in **4.45**. Analysis of the 1D and 2D NMR spectra of **4.47** enabled the identification of the anomeric proton of the glucose residue closer to the nonreducing end, which overlapped with a few other protons to form a multiplet around 4.64 ppm. Through careful analysis of the ^{1}H–^{1}H COSY spectrum, it was also found that H-2 of this glucose residue appeared around 5.23 ppm as a doublet of doublets (J = 9.0 and 7.8 Hz). The smaller of these J values was attributed to the H-1/H-2 coupling, confirming that the newly introduced glycosidic linkage was β.

4.2.3 Efforts toward synthesis of backbone oligosaccharides 4.11–4.15

4.2.3.1 Assembly of protected backbone oligosaccharides with different lengths

With the key building blocks **4.16** and **4.47** in hand, the assembly of backbone oligosaccharides with different lengths was investigated. As illustrated in Scheme 4.10, thioglycoside **4.16** and commercially-available *N*-Z-ethanolamine were coupled through NIS/AgOTf promoted glycosylation. Although TLC indicated complete conversion of **4.16**, the desired trisaccharide **4.48** was only isolated in 65%–70% yield, mainly due to the formation of the 1,2-orthoester **4.49** (~25%). To improve the yield, the crude product obtained after the glycosylation was subjected to TFA (5% in dichloromethane). Under these acidic conditions, **4.49** rearranged to **4.48**, leading to isolation of trisaccharide **4.48** in 88% yield. The stereochemistry of the newly formed glycosidic linkage was confirmed by measuring $^{3}J_{\text{H-1,H-2}}$, which had a value of 8.1 Hz, clearly indicating the β-stereochemistry.

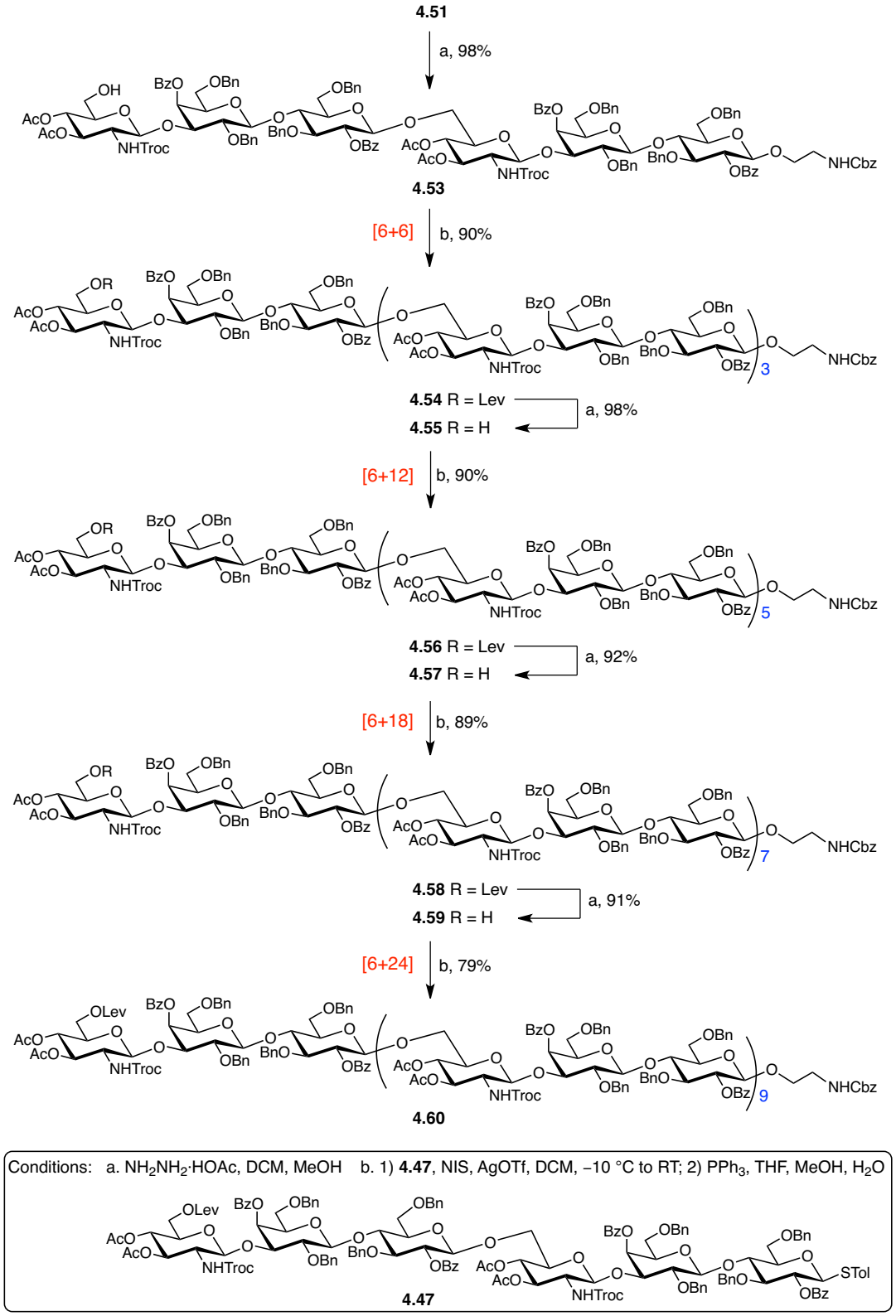


Scheme 4.10 Synthesis of hexasaccharide **4.51**.

After introducing the linker at the reducing end, attention was moved to chain extension at O-6 of the glucosamine residue at the nonreducing end. Treatment of **4.48** with hydrazine acetate resulted in selective removal of the Lev group at O-6″ in the presence of the benzoyl groups affording **4.50** in 95% yield (Scheme 4.10). Subsequently, an NIS/AgOTf-mediated glycosylation was performed to couple **4.50** and thioglycoside **4.16**.

In addition to the desired hexasaccharide **4.51**, a minor byproduct with a very similar retention factor on TLC was isolated. Structure elucidation of the byproduct by NMR spectroscopy and mass spectrometry led to its identification as **4.52**. Presumably this compound is formed from the reaction of **4.51** with the *p*-tolylsulfenyl iodide generated by activation of donor **4.16** with iodonium ion derived from NIS. Interestingly, **4.52** can be converted back to **4.51** by treatment with PPh_3. To simply the isolation of desired product and improve the yield, the crude residue obtained after the glycosylation was treated with PPh_3, before purification. Using this approach, hexasaccharide (6-mer) **4.51** was isolated in 93% yield.

With this improved glycosylation protocol involving a post-treatment with PPh_3 developed, **4.51** was further elongated to afford a library of oligosaccharides with different lengths by sequential addition of a hexasaccharide unit to the growing chain. Each round of chain extension featured a selective Lev deprotection followed by a NIS/AgOTf-promoted glycosylation using hexasaccharide **4.47** as the donor. As outlined in Scheme 4.11, selective removal of the Lev groups of **4.51** was achieved using hydrazine acetate in 98% yield. The resulting alcohol, **4.53**, was then glycosylated with **4.47** to generate a 90% yield of dodecasaccharide (12-mer) **4.54**. Subsequently, another round of chain extension enabled the conversion of **4.54** to octadecasaccharide (18-mer) **4.56** (88% yield over two steps). Next, tetracosasaccharide (24-mer) **4.58** was obtained in 82% yield over two steps. from **4.56**. Finally, **4.56** was further elongated using the same sequence of reaction to produce the desired triacontasaccharide (30-mer) **4.60** in 72% yield over two steps. In the synthesis of these large oligosaccharides, 1.2 equivalent of donor **4.47** was used, and all the glycosylation reactions proceeded efficiently, giving excellent yields of the products.



Scheme 4.11 Synthesis of 12-mer **4.54**, 18-mer **4.56**, 24-mer **4.58** and 30-mer **4.60**.

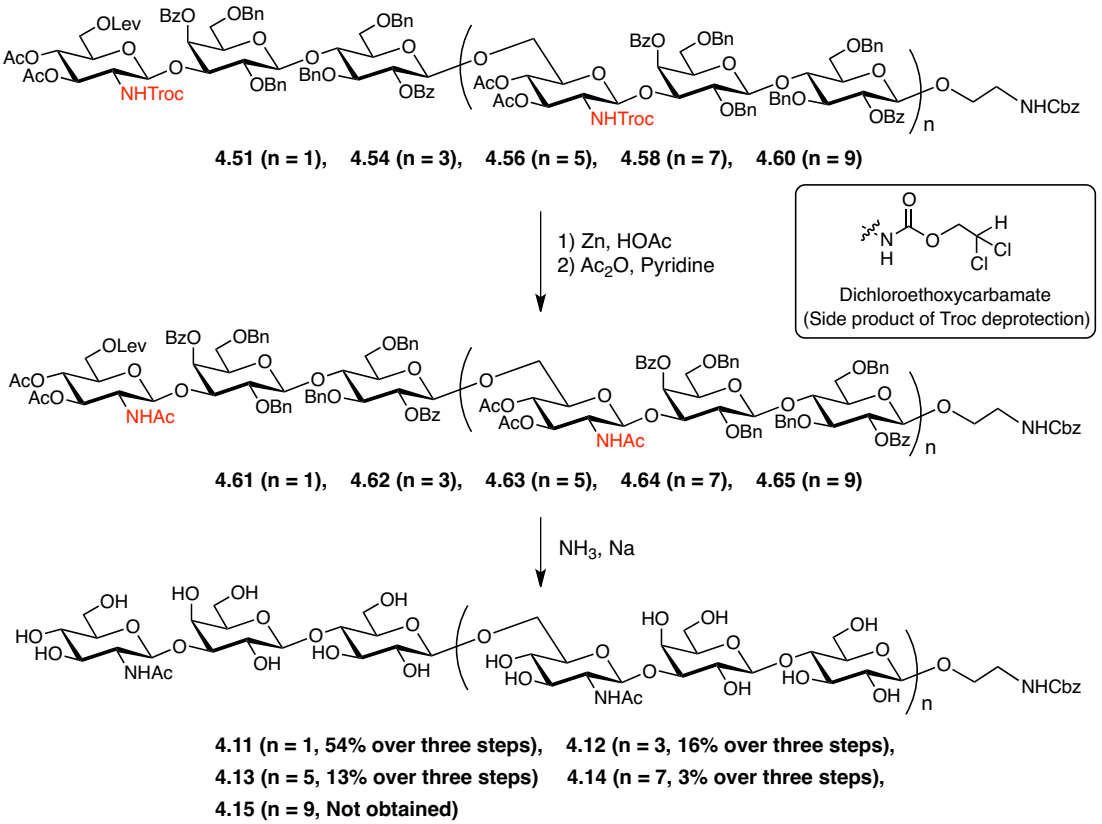
Because of the size of these molecules, and the fact that they were composed of a single repeating unit, there were, as expected, significant overlap in the ^{1}H and ^{13}C NMR spectra. Although the donor **4.47** had a neighboring participating group at C-2, which we expected would provide only the β-glycosides, it was necessary to establish this. In ^{13}C NMR spectra, the anomeric carbons of the all of the glucose residues were between 100 and 102 ppm, indicating β-stereoselectivity of the glycosylations in the course of chain extension. If the α-glycoside has been formed, resonances at higher field (95–100 ppm) would be expected.

4.2.3.2 Deprotection of oligosaccharides

After successful construction of 6-mer **4.51**, 12-mer **4.54**, 18-mer **4.56**, 24-mer **4.58** and 30-mer **4.60**, deprotection of these oligosaccharides was studied. It was anticipated that removal of the Troc group, followed by *N*-acetylation and a global deprotection by Birch reduction would furnish the desired oligosaccharides **4.11**–**4.15**, which could be used in the enzymatic reactions.

The Troc group is generally removed via a reductive elimination process, which commonly employs zinc in acetic acid. Moreover, it is essential to use freshly activated zinc to achieve the best reaction yield.[168] As described in Scheme 4.12, the fully protected oligosaccharides were treated with activated zinc in acetic acid. A common problem was encountered in these reactions: the formation of dichloroethoxycarbamate side products resulting from substitution of a chlorine atom by hydrogen.[169] In the case of **4.51**, **4.54** and **4.56**, a small amount of side products having a single dichloroethoxycarbonyl group were observed; the desired products **4.61**, **4.62** and **4.63** were obtained after *N*-acetylation of the

crude reaction mixture. However, in the case of **4.58** and **4.60**, a significant amount of side products having one or two dichloroethoxycarbonyl groups were observed. After *N*-acetylation, crude **4.64** and **4.65** were obtained, which could not be purified from the species containing the dichlorocarbamate groups. Attempts to convert the side products to the desired products by treatment with more zinc in acetic acid failed, indicating that the dichloroethoxycarbonyl group is inert under these reductive conditions. The use of freshly made zinc–copper couple[170] in acetic acid also failed to prevent the formation of dichloroethoxycarbamate side products. In addition, removal of multiple Troc groups under basic conditions (LiOH, NaOH, or KOH) was proven to be ineffective. Therefore, I decided to carry the obtained pure **4.61**–**4.63** and the impure **4.64** and **4.65** onto the next step.



Scheme 4.12 Synthesis of 6-mer **4.11**, 12-mer **4.12**, 18-mer **4.13** and 24-mer **4.14**.

Given the numbers of benzyl groups in **4.61**–**4.65** (up to as many as 40), Birch reduction was employed for debenzylation. Under the basic reaction conditions, the O-acyl groups can also be removed. Deprotection of **4.61** proceeded smoothly, providing **4.11** in 54% yield (three-step yield from **4.51**). For the longer oligosaccharides, the yield decreased significantly. Decasaccharide **4.12** and octadecasacchride **4.13** were obtained in 16% and 13%, respectively, over three steps. The 24-mer **4.14** derived from **4.64** was only isolated in 3% yield over three steps. Unfortunately, no desired deprotected 30-mer **4.15** was observed from the deprotection of **4.65**. Instead, a series of mass peaks smaller than the desired one were observed in the MALDI spectrum, which match the intermediates generated from **4.15** by sequential loss of a terminal sugar residue, suggesting that the product decomposed under Birch reduction condition.

4.2.4 Enzymatic modifications of synthetic oligosaccharides

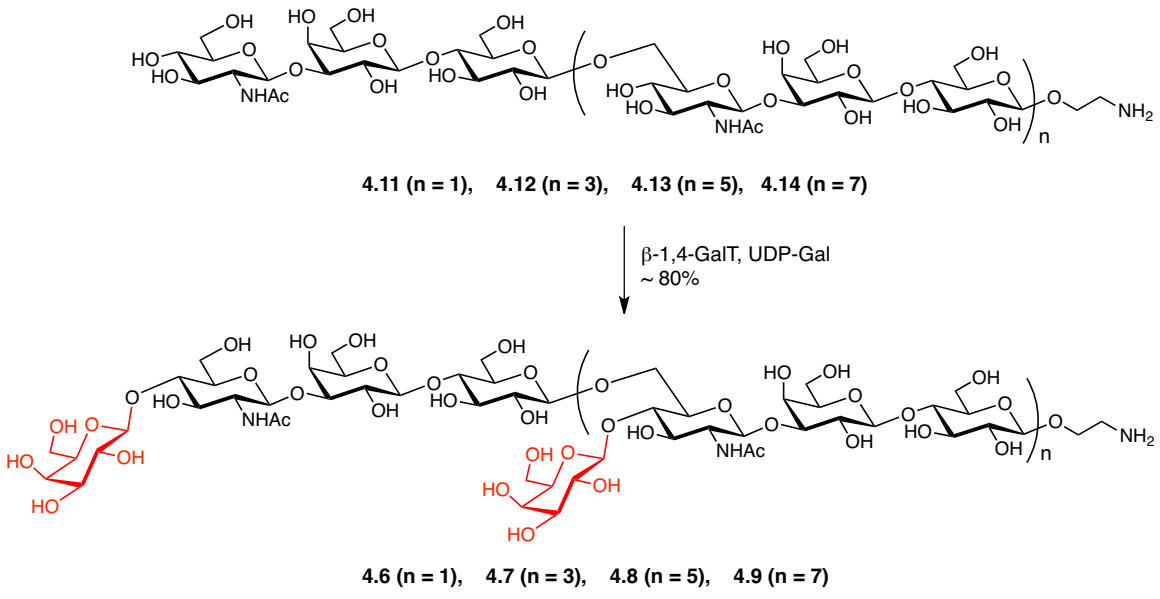
Although the deprotection of the backbone oligosaccharides remains to be further investigated and optimized, 80 mg of the 6-mer, 25 mg of the 12-mer, 20 mg of the 18-mer and 4 mg of the 24-mer were obtained. I then turned my attention to the glycosyltransferase-catalyzed attachment of the α-Neu5NAc-(2→3)-β-Gal-(1→4) sequence to the GlcNAc residue in each repeating unit of the oligosaccharides.

4.2.4.1 β-(1→4)-GalT catalyzed multi-galactosylation

A recombinant *Helicobacter pylori* β-(1→4)-GalT was employed for multi-galactosylation of the oligosaccharides (Scheme 4.13). This enzyme was expressed by Mr. Blake Zheng in our lab according to a previous report.[162] It is well documented that this β-

(1→4)-GalT is capable of transferring a galactose residue from UDP-Gal to GlcNAc acceptors in a β-(1→4) linkage, and exhibits broad substrate specificity. However, in my synthetic oligosaccharides, the O-6 position of many of the GlcNAc residues is connected to a glucose residue. Whether the enzyme could tolerate substitution at O-6 of GlcNAc was unknown.

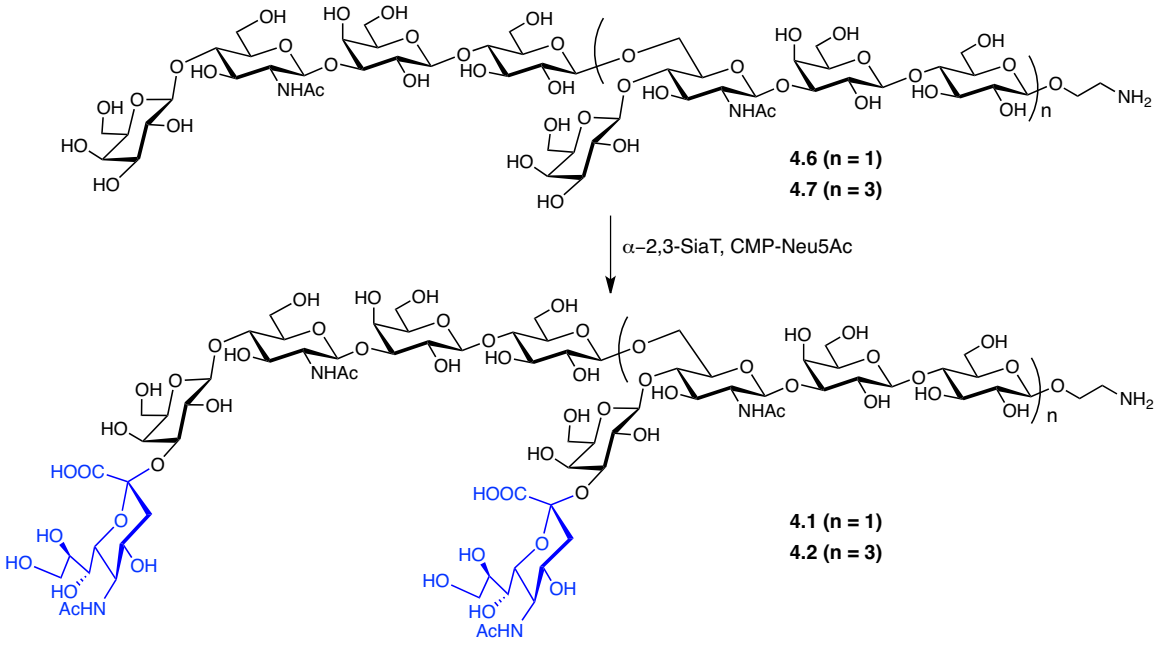
To answer this question, enzymatic reaction was performed on each substrate. A HEPES buffer (pH 7.4) solution containing the substrate (2 mM), UDP-Gal (2 equiv./GlcNAc residue), $MnCl_2$ (5 mM), alkaline phosphatase and β-(1→4)-GalT was incubated overnight at 37 °C. To my delight, all the reactions proceeded without difficulty, and each GlcNAc residue in the oligosaccharides was galactosylated. After purification by size exclusion chromatography, the desired products **4.6–4.9** were obtained in about 80% yield.



Scheme 4.13 Enzymatic synthesis of **4.6–4.9** by β-(1→4)-GalT catalyzed multi-galactosylation.

4.2.4.2 α-(2→3)-SiaT catalyzed multi-sialylation

After successfully introducing a galactose residue to each GlcNAc in the oligosaccharides, the last step, α-(2→3)-SiaT catalyzed multi-sialylation, was studied. A recombinant α-(2→3)-SiaT from *Campylobacter jejuni*, which was expressed by Mr. Blake Zheng in our lab according to a previous report,[171] was employed for the multi-sialylation. This α-(2→3)-SiaT is capable of transferring a sialic acid residue from CMP-Neu5Ac to galactose acceptors in a α-(2→3) linkage. Because the O-3 of the galactose residue in the backbone is connected to a GlcNAc residue, only the O-3 of the galactose residue in the side chain can be sialylated. As described in Scheme 4.14, the α-(2→3)-SiaT catalyzed multi-sialylation was first carried out on hexasaccharide **4.6**. A HEPES buffer (pH 7.5) solution containing **4.6** (1 mM), CMP-Neu5Ac (4 equiv.), $MnCl_2$ (10 mM), $MgCl_2$ (10 mM), alkaline phosphatase (100 mU/mL) and α-(2→3)-SiaT was incubated at 37 °C.



Scheme 4.14 Attempted enzymatic synthesis of **4.1** and **4.2** by α-(2→3)-SiaT catalyzed multi-sialylation.

Generally, MALDI-TOF MS is used as a reliable tool to characterize large oligosaccharides. However, the labile nature of sialic acid poses a challenge for characterization of sialylated oligosaccharides. When characterized by MALDI, sialylated oligosaccharides usually decompose in-source by loss of sialic acid.[172] To overcome this problem, we collaborated with the Klassen lab to characterize these compounds by ESI MS. The ESI spectrum indicated the conversion of **4.6** to **4.1** was not complete no matter how much enzyme and donor were added, and how long the reaction was left for incubation. A mixture of mono-sialylated and di-sialylated (**4.1**) compounds was always obtained. When the reaction was performed on **4.7**, a mixture of mono-sialylated, di-sialylated, tri-sialylated and tetra-sialylated (**4.2**) compounds were obtained. We postulated that the incomplete conversion was due to sialidase activity of the SiaT. It has been recognized that many bacterial SiaTs also exhibit sialidase activities,[55,173] which catalyze the cleavage of sialic acid from the product and prevent the reaction from reaching completion.

To push the muti-sialylation reaction to completion, the sialidase activity of SiaT has to be inhibited. One solution is incubation the substrate in HEPES buffer at a pH above 8.5. Under these conditions, SiaTs normally only exhibit transferase activity without significant sialidase activity.[55] The other solution is to use mammalian SiaTs, such as the rat α-(2→3)-SiaT, which do not have sialidase activities.[54] These two solutions will be explored in the future.

4.3 Summary

In conclusion, a chemo-enzymatic approach was developed to synthesize a library of structurally well-defined GBSIII oligosaccharides (**4.1**–**4.5)**, ranging in size from two to

ten pentasaccharide-repeating units. The approach started with chemical synthesis of the backbone oligosaccharides (**4.11**–**4.15**) from a glucosamine-based building block **4.17** and a lactose-based building block **4.18**. In the synthesis of **4.17**, a BDA group was used to protect 3,4-*trans* diol and increase the reactivity of anomeric leaving group. The synthesis of **4.18** featured a regioselective mono-pivaloylation at O-2. From **4.17** and **4.18**, a trisaccharide thioglycoside **4.16** and its dimer **4.47** were readily obtained via efficient glycosylations. Using **4.16** and **4.47** as key building blocks, a library of protected backbone oligosaccharides were successfully assembled by sequential addition of a hexasaccharide unit to the growing chain. In this process, an NIS/AgOTf promoted glycosylation protocol involving a post-treatment with PPh_3 was developed to increase the yield of glycosylation reactions and simplify the purification of the products. Although the deprotection of backbone oligosaccharides suffered from low yields and remains to be further investigated, four of the desired deprotected backbone oligosaccharides, containing up to 24-monosaccharide residues (**4.11**–**4.14**) were achieved. The 30-mer was not obtained, and its synthesis will require optimization of the deprotection protocol.

Subsequently, glycosyltransferase-catalyzed attachment of α-Neu5NAc-(2→3)-β-Gal-(1→4) side chain to **4.11**–**4.14** was investigated. A β-(1→4)-GalT from *H. pylori* catalyzed multi-galactosylation of **4.11**–**4.14** proceeded smoothly affording **4.6**–**4.9** in about 80% yield for each compound. In the α-(2→3)-SiaT catalyzed multi-sialylation, it was found that the sialidase activity of the SiaT resulted in incomplete conversion of substrate to the desired muti-sialylated product. To achieve all the desired GBSIII oligosaccharides (**4.1**–**4.5**), optimization of the deprotection of the backbone oligosaccharides is required, and the problems encountered in SiaT catalyzed multi-

sialylation needs to be addressed in the future.

4.4 Experimental section

General methods for chemical synthesis: All reagents were purchased from commercial sources and were used without further purification unless noted. Dry solvents used in reactions were purified by successive passage through columns of alumina and copper under an argon atmosphere. All reactions were carried out under a positive pressure of argon unless otherwise stated, monitored by TLC on Silica Gel 60-F_{254} (0.25 mm), and the spots were visualized under UV light (254 nm) and/or stained by charring with acidified anisaldehyde solution in ethanol. Column chromatography was performed on Silica Gel 60 (40–60 μm) or C_{18} silica gel (35–70 μm, Toronto Research Chemicals).

General methods for enzymatic synthesis: The genes for β-(1→4)-GalT and (2→3)-SiaT were kindly provided by Prof. Warren W. Wakarchuk (Ryerson University). Recombinant β-(1→4)-GalT[162] and (2→3)-SiaT[171] were expressed by Mr. Blake Zheng in our lab according to previous reports. CMP-Neu5Ac and UDP-Gal were purchased from Roche Diagnostics. Shrimp alkaline phosphatase (1,000 U/mL) was purchased from New England Biolabs (Ipswich, MA, USA). HEPES buffer (50 mM, pH 7.5) was made in house. The enzymatic reactions were performed in aqueous buffer solutions at the optimal pH for each enzyme at 37 °C. A unit of enzyme (U) is defined as the amount of enzyme that catalyzes the conversion of 1 μmol substrate per min. The reactions were monitored by TLC on Silica Gel 60-F_{254} (0.25 mm) using *i*-PrOH–NH_4OH–H_2O (3:3:1 or 2:3:1) as the eluent, and the spots were visualized by charring with 10% sulfuric acid in ethanol. Gel filtration

chromatography was performed on a column (47 cm x 1 cm) packed with Sephadex G-15 (GE Healthcare) using 0.1 M NH_4HCO_3 aqueous solution as the eluent.

Compound characterization: ^{1}H NMR spectra were recorded at 500, 600 or 700 MHz, and chemical shifts were referenced to $CHCl_3$ (7.26 ppm, $CDCl_3$), CD_2HOD (3.31 ppm CD_3OD), or HOD (4.79 ppm, D_2O). ^{13}C NMR spectra were recorded at 126 MHz and chemical shifts were referenced to $CDCl_3$ (77.06 ppm, $CDCl_3$), CD_3OD (49.0 ppm, CD_3OD) or external acetone (31.07 ppm, D_2O). Assignments of NMR spectra were made on the basis of two-dimensional experiments (^{1}H–^{1}H COSY, HSQC and HMBC) and the stereochemistry of the newly formed glycosidic linkages was confirmed by values of $^3J_{H\text{-}1,H\text{-}2}$ and anomeric carbon resonances. In the data provided below the resonances on particular residues are indicated by an increasing number of primes (′) moving from the reducing to non-reducing end. For example, in **4.16** H-1 is H-1 of the Glc residue, H-1′ is H-1 of the Gal residue, and H-1′′ is H-1 of the GlcNTroc residue. Electrospray-ionization mass spectra were recorded on an Agilent Technologies 6220 TOF spectrometer on samples dissolved in CH_2Cl_2, CH_3OH or H_2O. MALDI mass spectrum was obtained in the linear positive mode of ionization on a Bruker Daltonics (Bremen, GmbH) UltrafleXtreme MALDI TOF/TOF mass spectrometer using sinapinic acid as the matrix. Optical rotations were measured on Perkin Elmer 241 polarimeter at 22 ± 2 °C in units of degree mL/(g·dm).

Prodecure for activation of zinc dust: Commercial zinc dust (20 g) was activated by stirring with 10% HCl aqueous solution (500 mL) for 5 min, then the solution was decanted and the zinc duct was washed several times with distilled water, ethanol and ether before

drying under high vacuum. The activated zinc dust was used immediately.

General procedure A for NIS/AgOTf promoted glycosylation involving a post-treatment with PPh_3: A mixture of acceptor (0.40–1.25 g), thioglycoside donor (1.1–1.2 equiv.) and powdered 4 Å molecular sieves was suspended in dry CH_2Cl_2 (10–20 mL) and stirred at room temperature for 1 h. The solution was cooled to −10 °C before NIS (2.5 equiv.) and AgOTf (0.25 equiv.) were added. After stirring for 1 h while warming to room temperature, Et_3N (1 mL) was added and the mixture was filtered through Celite. The filtrate was concentrated and the resulting residue was dissolved in 1:1 THF–CH_3OH (15 mL), to which PPh_3 (1.3 equiv.) and a few drops of water were added. After stirring for 10 min at room temperature, the mixture was concentrated and the resulting residue was subjected to flash chromatography to afford the desired product as a white foam.

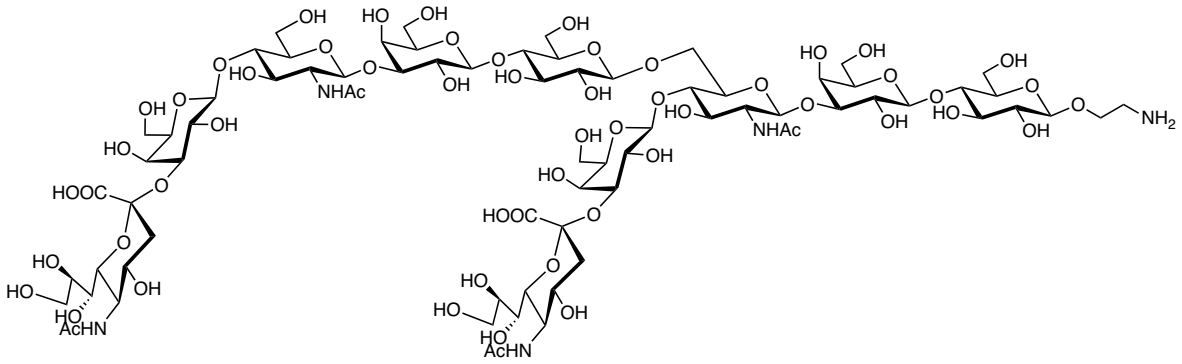
General procedure B for deprotection of 4.51, 4.54, 4.56, 4.58, 4.60: To a solution of substrate (300 mg) in AcOH (50 mL) was added freshly activated zinc dust (7.5 g). After stirring overnight at room temperature, the mixture was filtered through Celite. The filtrate was concentrated, dissolved in CH_2Cl_2 (200 mL) and washed with water, saturated aqueous $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4, filtered and concentrated to dryness. The resulting residue was dissolved in pyridine (15 mL), to which acetic anhydride (2 mL) was added. After stirring overnight at room temperature, the solution was concentrated, dissolved in CH_2Cl_2 (100 mL) followed by washing with saturated aqueous $NaHCO_3$, water and brine. The organic layer was dried over Na_2SO_4, filtered and concentrated. The resulting residue was subjected to flash chromatography (CH_2Cl_2–MeOH

40:1) to afford the corresponding *N*-acetylated intermediate (**4.61**–**4.65**). Subsequently, Birch reduction was conducted to fully deprotect **4.61**–**4.65**. Sodium was added to freshly collected liquid ammonia (~ 20 mL) at −78 °C until the blue color of the solution persisted. Then, a solution of *N*-acetylated intermediate (100 mg) in THF (1.5 mL) and CH_3OH (20 μL) was added dropwise at −78 °C. After 2 h, CH_3OH (3 mL) was added and the solution was concentrated to dryness. The resulting residue was dissolved in H_2O (10 mL), neutralized with Amberlite IR120 H^+ ion-exchange resin, filtered and concentrated. The resulting crude residue was purified by Iatrobeads chromatography (*i*-PrOH–NH_4OH–H_2O 3:3:1 or 2:3:1) followed by C_{18} chromatography (100% water → 1:4 CH_3OH–water) to give fully deprotected backbone oligosaccharide **4.11**–**4.14**. (**4.15** was not achieved due to product decomposition under Birch reduction conditions).

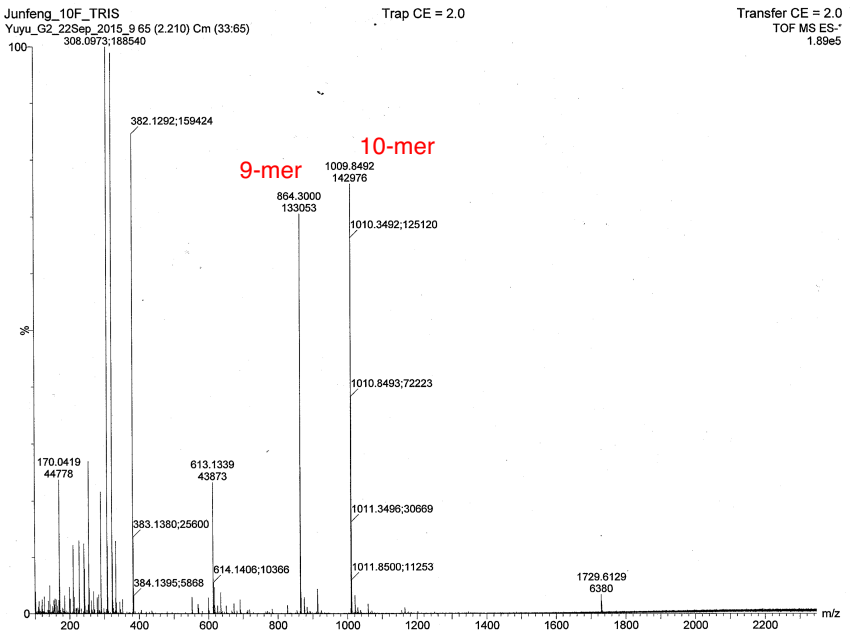
General procedure C for β-(1→4)-GalT catalyzed multi-galactosylation: The substrate **4.11**–**4.14** (3–5 mg, 2 mM) and UDP-Gal (2 equiv./GlcNAc residue) were dissolved in HEPES buffer (50 mM, pH 7.5) containing $MnCl_2$ (5 mM). After adding β-(1→4)-GalT (100 μL/mg substrate) and alkaline phosphatase (1 μL), the resulting mixture was incubated overnight at 37 °C. The reaction was monitored by TLC using *i*-PrOH–NH_4OH–H_2O (3:3:1 or 2:3:1) as the eluant. The reaction mixture was centrifuged and the supernatant was subjected to purification on a Sephadex G-15 gel filtration column (eluent 0.1 M NH_4HCO_3). Fractions containing the product were combined and lyophilized to give the desired product **4.6**–**4.9** as an amorphous white solid.

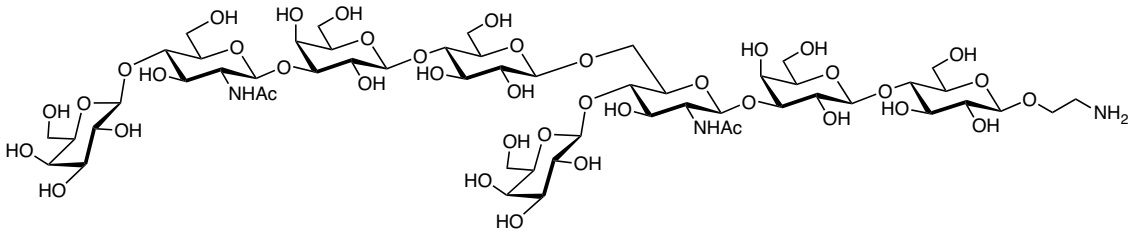
General procedure D for α-(2→3)-SiaT catalyzed multi-sialylation: The substrate (1

mg, 1 mM) and CMP-Neu5Ac (2 equiv./repeating unit) were dissolved in HEPES buffer (50 mM, pH 7.5) containing $MnCl_2$ (10 mM) and $MgCl_2$ (10 mM). After adding α-(2→3)-SiaT (100 μL/mg substrate) and alkaline phosphatase (1 μL), the resulting mixture was incubated at 37 °C. The reaction was monitored by TLC using *i*-PrOH–NH_4OH–H_2O (5:2:1) as the eluant.



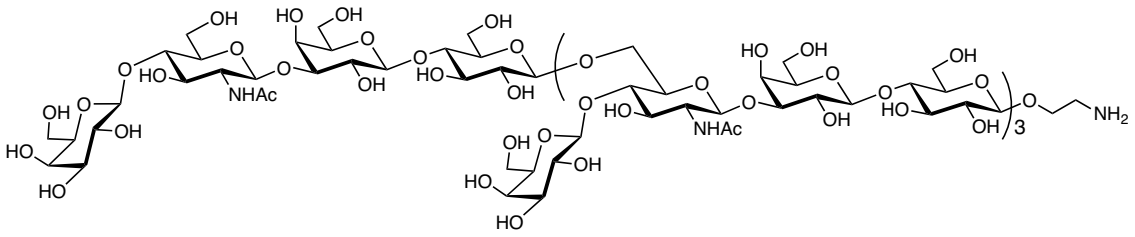
2-Aminoethyl 5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyrano-sylonate-(2→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-[5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyrano-sylonate-(2→3)-β-D-galactopyranosyl-(1→4)]-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (4.1): The α-(2→3)-SiaT catalyzed catalyzed conversion of **4.6** (1.0 mg**)** to **4.1** was performed following general procedure D described above. The ESI spectrum (shown below) indicated that the reaction was not completed despite many optimization efforts. A mixture of monosialylated compound (9-mer) and the desired disialylated (10-mer) were obtained.





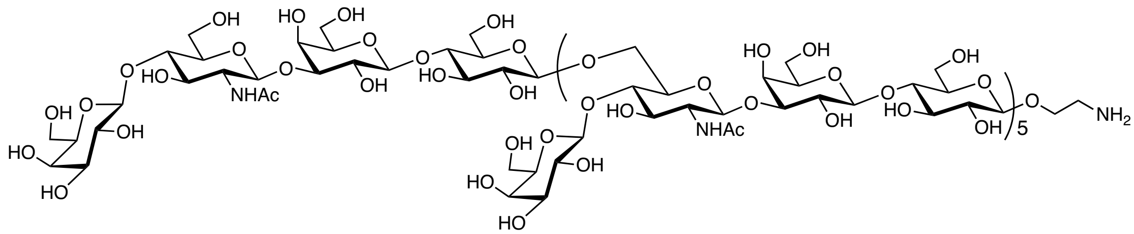
2-Aminoethyl β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-[β-D-galactopyranosyl-(1→4)]-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (4.6): The β-(1→4)-GalT catalyzed conversion of **4.11** (5.0 mg, 4.48 μmol) to **4.6** (5.5 mg, 85%) was performed following general procedure C described above. ^{1}H NMR (700 MHz, D_2O) δ: 4.74–4.68 (m, 2H), 4.58–4.51 (m, 3H), 4.48 (d, J = 7.8 Hz, 1H), 4.46–4.40 (m, 2H), 4.28 (d, J = 10.4 Hz, 1H), 4.19–4.07 (m, 3H), 4.02–3.90 (m, 7H), 3.89–3.69 (m, 24H), 3.69–3.56 (m, 11H), 3.56–3.51 (m, 2H), 3.41–3.33 (m, 2H), 3.32–3.19 (m, 2H), 2.04 (s, 6H, 2 × NHAc); ^{13}C NMR (126

MHz, D_2O): δ 175.9, 103.93, 103.92, 103.8, 103.7, 103.6, 102.9, 83.1, 83.0, 79.4, 79.24, 79.19, 78.7, 76.3, 76.2, 75.90, 75.87, 75.8, 75.7, 75.5, 75.3, 75.2, 74.3, 73.7, 73.6, 73.50, 73.48, 73.2, 73.0, 72.0, 71.9, 71.0, 69.6, 69.5, 69.3, 69.2, 68.6, 67.1, 62.1, 62.01, 61.98, 61.9, 61.1, 60.94, 60.87, 56.2, 40.4, 23.2; MALDI-TOF *m/z* $[M + Na]^+$ calcd for $C_{54}H_{93}N_3NaO_{41}$: 1462.5, found: 1462.7.



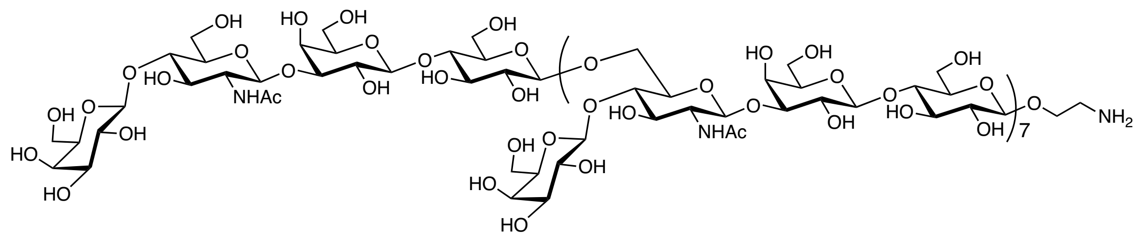
2-Aminoethyl β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-[β-D-galactopyranosyl-(1→4)]-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-[β-D-galactopyranosyl-(1→4)]-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-[β-D-galactopyranosyl-(1→4)]-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (4.7): The β-(1→4)-GalT catalyzed conversion of **4.12** (3.2 mg, 1.47 μmol) to **4.7** (3.4 mg, 82%) was performed following general procedure C described above. ^{1}H NMR (700 MHz, D_2O): δ 4.74–4.68 (m, 4H), 4.58–4.51 (m, 7H), 4.48 (d, *J* = 7.8 Hz, 1H), 4.46–4.41 (m, 4H), 4.31–4.23 (m, 3H), 4.19–4.08 (m, 5H), 4.01–3.90 (m, 13H), 3.89–3.69 (m, 48H), 3.69–3.57 (m, 21H), 3.56–3.52 (m, 4H), 3.40–3.33 (m, 4H), 3.29–3.21 (m, 2H), 2.04 (s, 12H, 4 × NHAc); ^{13}C NMR (126 MHz, D_2O): δ 175.9, 103.9, 103.8, 103.7, 103.6, 102.9, 83.1, 83.0, 79.4, 79.24, 79.18, 78.7, 78.6, 76.3, 76.2, 75.9, 75.8, 75.7, 75.5, 75.3, 75.2, 74.3, 73.7, 73.6,

73.50, 73.48, 73.2, 73.0, 72.0, 71.9, 71.0, 69.6, 69.5, 69.3, 69.2, 68.5, 67.0, 62.1, 62.01, 61.97, 61.9, 61.1, 60.94, 60.87, 56.2, 40.4, 23.2; MALDI-TOF *m/z* $[M + Na]^+$ calcd for $C_{106}H_{179}N_5NaO_{81}$: 2841.0, found: 2841.1.

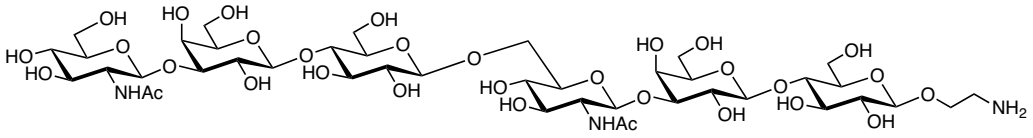


2-Aminoethyl β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-[β-D-galactopyranosyl-(1→4)]-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-[β-D-galactopyranosyl-(1→4)]-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-[β-D-galactopyranosyl-(1→4)]-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-[β-D-galactopyranosyl-(1→4)]-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-[β-D-galactopyranosyl-(1→4)]-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (4.8): The β-(1→4)-GalT catalyzed conversion of **4.13** (3.0 mg, 0.93 μmol) to **4.8** (3.1 mg, 79%) was performed following general procedure C described above. ^{1}H NMR (700 MHz, D_2O) δ: 4.74–4.65 (m, 6H), 4.64–4.51 (m, 11H), 4.48 (d, J = 7.8 Hz, 1H), 4.47–4.39 (m, 6H), 4.34–4.24 (m, 5H), 4.21–4.09 (m, 7H), 4.05–3.49 (m, 128H), 3.40–3.32 (m, 6H), 3.28–3.22 (m, 2H), 2.04 (s, 18H, 6 × NHAc); MALDI-TOF *m/z* [M +

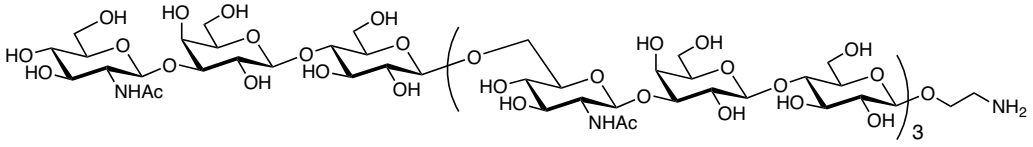
Na]$^{+}$ calcd for $C_{158}H_{265}N_7NaO_{121}$: 4219.5, found: 4219.8.



2-Aminoethyl β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-[β-D-galactopyranosyl-(1→4)]-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-[β-D-galactopyranosyl-(1→4)]-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-[β-D-galactopyranosyl-(1→4)]-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-[β-D-galactopyranosyl-(1→4)]-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-[β-D-galactopyranosyl-(1→4)]-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-[β-D-galactopyranosyl-(1→4)]-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-[β-D-galactopyranosyl-(1→4)]-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (4.9): The β-(1→4)-GalT catalyzed conversion of **4.14** (4 mg, 0.93 μmol) to **4.9** was performed following general procedure C described above. Exact mass of $C_{210}H_{351}N_9O_{161}$: 5575.0, ESI-MS *m/z* [M – 3H]$^{3-}$ found: 1858.0232 (M = 5577.1). Unfortunately, **4.9** was lost during purification.

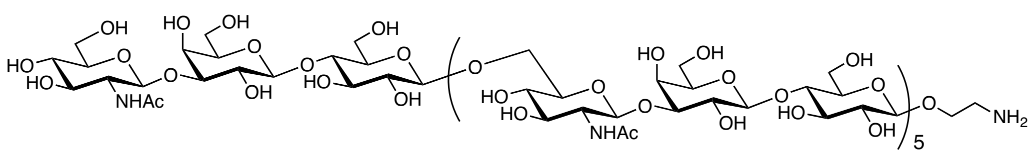


2-Aminoethyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (4.11): The conversion of **4.51** (300 mg, 102.72 μmol) to **4.11** (62 mg, 54%) was performed following general procedure B described above. ^{1}H NMR (700 MHz, D_2O): δ 4.693 (d, *J* = 8.4 Hz, 1H), 4.687 (d, *J* = 8.4 Hz, 1H), 4.544 (d, *J* = 7.8 Hz, 1H), 4.537 (d, *J* = 7.8 Hz, 1H), 4.44 (d, *J* = 7.9 Hz, 1H), 4.437 (d, *J* = 7.9 Hz, 1H), 4.21 (d, *J* = 10.3 Hz, 1H), 4.17–4.10 (m, 3H), 4.01–3.92 (m, 3H), 3.92–3.86 (m, 2H), 3.83–3.69 (m, 13H), 3.69–3.52 (m, 12H), 3.49–3.42 (m, 2H), 3.40–3.33 (m, 2H), 3.30–3.24 (m, 2H), 2.04 (s, 6H, 2 × NHAc); ^{13}C NMR (126 MHz, D_2O): δ 175.99, 103.9, 103.81, 103.78, 102.9, 82.9, 79.3, 79.2, 76.6, 75.91, 75.88, 75.8, 75.7, 75.3, 75.2, 74.6, 74.4, 73.8, 73.7, 71.0, 70.7, 70.6, 69.7, 69.32, 69.29, 66.8, 62.0, 61.9, 61.5, 61.1, 60.9, 56.7, 56.6, 40.4, 23.2; MALDI-TOF *m/z* $[M + Na]^+$ calcd for $C_{42}H_{73}N_3NaO_{31}$: 1138.4, found: 1138.5.



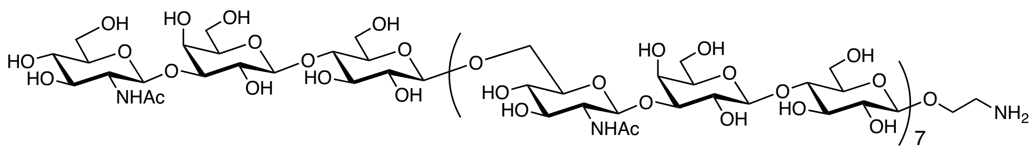
2-Aminoethyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-2-

acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (4.12): The conversion of **4.54** (300 mg, 54.08 μmol) to **4.12** (19 mg, 16%) was performed following general procedure B described above. ^{1}H NMR (700 MHz, D_2O): δ 4.72–4.66 (m, 4H), 4.57–4.50 (m, 4H), 4.46–4.40 (m, 4H), 4.23–4.18 (m, 3H), 4.18–4.07 (m, 5H), 4.03–3.92 (m, 5H), 3.92–3.85 (m, 4H), 3.84–3.69 (m, 25H), 3.69–3.51 (m, 26H), 3.50–3.42 (m, 2H), 3.39–3.31 (m, 4H), 3.30–3.21 (m, 2H), 2.04 (s, 12H, 4 × NHAc); ^{13}C NMR (126 MHz, D_2O): δ 175.9, 103.9, 103.81, 103.77, 102.9, 82.9, 79.3, 79.2, 76.65, 75.89, 75.8, 75.7, 75.6, 75.3, 75.2, 74.6, 74.4, 73.8, 73.7, 71.01, 70.99, 70.7, 70.6, 69.6, 69.3, 67.0, 62.0, 61.5, 61.1, 60.9, 56.7, 56.6, 40.4, 23.2; MALDI-TOF *m/z* [M + Na]$^+$ calcd for $C_{82}H_{139}N_5NaO_{61}$: 2192.8, found: 2192.8.



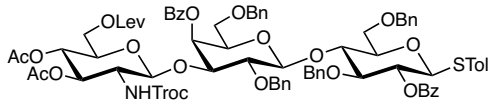
2-Aminoethyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (4.13): The conversion of **4.56** (300 mg, 36.70 μmol) to **4.13** (15.5 mg, 13%) was performed following general procedure B described above. ^{1}H NMR (700 MHz, D_2O) δ: 4.73–4.65 (m, 6H),

4.57–4.51 (m, 6H), 4.47–4.41 (m, 6H), 4.24–4.18 (m, 5H), 4.18–4.10 (m, 7H), 4.02–3.92 (m, 7H), 3.92–3.85 (m, 6H), 3.84–3.69 (m, 37H), 3.69–3.51 (m, 39H), 3.50–3.42 (m, 3H), 3.40–3.32 (m, 6H), 3.30–3.24 (m, 2H), 2.04 (s, 18H, 6 × NHAc); ^{13}C NMR (126 MHz, D_2O): δ 175.9, 103.9, 103.83, 103.82, 103.77, 102.9, 82.98, 82.95, 79.3, 79.2, 76.65, 75.90, 75.78, 75.68, 75.64, 75.33, 75.21, 74.56, 74.46, 73.75, 73.68, 71.02, 71.00, 70.69, 70.56, 69.6, 69.3, 66.8, 62.0, 61.5, 61.1, 61.0, 56.7, 56.6, 40.4, 23.2; MALDI-TOF *m/z* [M + Na]$^+$ calcd for $C_{122}H_{205}N_7NaO_{91}$: 3247.2, found: 3247.2.



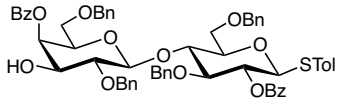
2-Aminoethyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (4.14): The conversion of **4.58** (300 mg, 27.77 µmol) to **4.14** (4 mg, 3%) was performed following general procedure B described

above. Exact mass of $C_{162}H_{271}N_9O_{121}$: 4278.5, ESI-MS *m/z* $[M - 3H]^{3-}$ found: 1425.1517 (M = 4278.5).



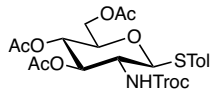
***p*-Tolyl 3,4-di-*O*-acetyl-2-deoxy-6-*O*-levulinyl-2-(2,2,2-trichlorethoxycarbonylamino)-β-D-glucopyranosyl-(1→3)-4-*O*-benzoyl-2,6-di-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2-*O*-benzoyl-3,6-di-*O*-benzyl-1-thio-β-D-glucopyranoside (4.16):** A solution of **4.43** (4.31 g, 2.75 mmol) in 9:1 TFA–H_2O (20 mL) was stirred at room temperature for 5 min, and was then was diluted with EtOAc (300 mL) and washed with saturated aqueous $NaHCO_3$, water and brine. The organic layer was dried over Na_2SO_4, filtered, concentrated to dryness to yield crude **4.44**. Without further purification, crude **4.44** was treated with acetic anhydride (5 mL) in pyridine (20 mL) overnight at room temperature. Then the solution was concentrated, dissolved in EtOAc (300 mL) and washed with saturated aqueous $NaHCO_3$, water and brine. The organic layer was dried over Na_2SO_4, filtered, concentrated and the resulting residue was subjected to flash chromatography (hexane–EtOAc 1:1) to afford **4.16** (3.90 g, 92% over two steps) as a white solid. R_f = 0.34 (hexane–EtOAc 1:1); $[\alpha]_D$ = +19.9 (*c* = 0.9, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.04–7.98 (m, 4H, Ar), 7.60–7.55 (m, 1H, Ar), 7.51 (t, *J* = 7.2 Hz, 1H, Ar), 7.46–7.39 (m, 4H, Ar), 7.38–7.29 (m, 12H, Ar), 7.25–7.17 (m, 5H, Ar), 7.06 (d, *J* = 7.3 Hz, 2H, Ar), 6.99 (d, *J* = 7.8 Hz, 2H, Ar), 6.97 (d, *J* = 7.4 Hz, 1H, Ar), 6.87–6.81 (m, 2H, Ar), 5.66 (br s, 1H, H-4′), 5.22 (app t, *J* = 9.6 Hz, 1H, H-2), 4.96–4.88 (m, 3H, H-4″, OC*H*$_2$Ph, OC*H*$_2$CCl$_3$), 4.86 (app t, *J* = 9.7 Hz, 1H, H-3″), 4.76 (d, *J* = 8.4 Hz, 1H, H-1″), 4.72–4.65 (m, 3H, H-1, OC*H*$_2$Ph, OC*H*$_2$CCl$_3$), 4.64–4.58 (m, 2H, H-1′, OC*H*$_2$Ph), 4.46 (A of ABq, *J* = 11.9 Hz, 1H,

OC*H*$_2$Ph), 4.35 (d, *J* = 8.3 Hz, 1H, N*H*Troc), 4.30 (A of ABq, *J* = 11.7 Hz, 1H, OC*H*$_2$Ph), 4.28–4.22 (m, 2H, H-6a″, OC*H*$_2$Ph), 4.21– 4.15 (m, 3H, H-6b″, 2 × OC*H*$_2$Ph), 4.13 (app t, *J* = 9.3 Hz, 1H, H-4), 3.91 (dd, *J* = 9.6, 3.5 Hz, 1H, H-3′), 3.88–3.83 (m, 1H, H-6a), 3.82–3.76 (m, 2H, H-3, H-6b), 3.74–3.67 (m, 2H, H-5′, H-2′), 3.62–3.57 (m, 1H, H-5″), 3.55–3.47 (m, 2H, H-2″, H-5), 3.41–3.35 (m, 1H, H-6a′), 3.31 (dd, *J* = 9.9, 6.0 Hz, 1H, H-6b′), 2.72 (ddd, *J* = 18.2, 7.3, 6.3 Hz, 1H, OC(O)CH$_2$C*H*$_2$C(O)), 2.67–2.61 (m, 1H, OC(O)CH$_2$C*H*$_2$C(O)), 2.61–2.46 (m, 2H, OC(O)C*H*$_2$CH$_2$C(O)), 2.26 (s, 3H, ArMe), 2.13 (s, 3H, C(O)C*H*$_3$), 2.00 (s, 3H, OAc), 1.91 (s, 3H, OAc); ^{13}C NMR (126 MHz, $CDCl_3$): δ 206.6 (C=O), 172.5 (C=O), 170.4 (C=O), 169.4 (C=O), 165.1 (2C, 2 × C=O), 153.6 (C=O), 138.4 (Ar), 138.3 (Ar), 138.2 (Ar), 138.1 (Ar), 138.0 (Ar), 133.4 (Ar), 133.1 (Ar), 132.92 (Ar), 132.90 (Ar), 130.2 (Ar), 130.1 (Ar), 129.9 (Ar), 129.8 (Ar), 129.6 (Ar), 128.8 (Ar), 128.7 (Ar), 128.43 (Ar), 128.35 (Ar), 128.1 (Ar), 128.0 (Ar), 127.89 (Ar), 127.88 (Ar), 127.8 (Ar), 127.7 (Ar), 127.6 (Ar), 127.23 (Ar), 127.18 (Ar), 102.5 (C-1′), 100.8 (C-1″), 95.5 (CCl_3), 86.4 (C-1), 81.7 (C-3), 80.8 (C-2′), 79.8 (C-5), 77.6 (C-3′), 76.2 (C-4), 75.1 (O*C*H$_2$CCl$_3$), 74.3, 74.1, 73.7, 73.6 (4C, 4 × O*C*H$_2$Ph), 73.0 (C-5′), 71.84 (2C), 71.76 (3C, C-2, C-3″, C-5″), 70.2 (C-4′), 68.6 (C-4″), 68.3 (C-6′), 68.2 (C-6), 62.1 (C-6″), 56.2 (C-2″), 37.9, 27.9 (2C, *C*H$_2$*C*H$_2$), 29.8 (CH$_2$C(O)*C*H$_3$), 21.1 (ArMe), 20.7, 20.6 (2C, 2 × OAc); HRMS-ESI *m/z* $[M + Na]^+$ calcd for $C_{79}H_{82}Cl_3NNaO_{22}S$: 1556.4007, found: 1556.4033.



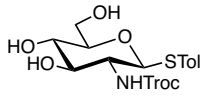
***p*-Tolyl 4-*O*-benzoyl-2,6-di-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2-*O*-benzoyl-3,6-di-*O*-benzyl-1-thio-β-D-glucopyranoside (4.18):** To a solution of **4.42** (6.88 g, 7.54 mmol) and *p*-TsOH (143 mg, 0.75 mmol) in CH_2Cl_2 (60 mL) was added trimethylorthobenzoate (3.89 mL, 22.61 mmol). After stirring at room temperature for 2 h, the solution was neutralized with Et_3N (1 mL) and concentrated to dryness. The resulting residue was then dissolved in CH_3CN (40 mL), to which TFA (2 mL) and H_2O (1 mL) were added. After stirring at room temperature for 10 min, the solution was neutralized with Et_3N (3 mL), concentrated and the resulting residue was subjected to flash chromatography (hexane–EtOAc 5:2) to afford **4.18** (7.13 g, 93%) as a white foam. R_f = 0.38 (hexane–EtOAc 2:1); $[\alpha]_D$ = −5.5 (*c* = 0.9, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.05–8.02 (m, 2H, Ar), 8.00–7.97 (m, 2H, Ar), 7.61–7.57 (m, 1H, Ar), 7.52 (tt, *J* = 7.6, 1.3 Hz, 1H, Ar), 7.48–7.43 (m, 2H, Ar), 7.41–7.38 (m, 2H, Ar), 7.36–7.27 (m, 12H, Ar), 7.21–7.11 (m, 7H, Ar), 7.05–7.00 (m, 3H, Ar), 6.94–6.90 (m, 2H, Ar), 5.60 (dd, *J* = 3.4, 0.8 Hz, 1H, H-4′), 5.24 (dd, *J* = 9.9, 9.2 Hz, 1H, H-2), 4.95 (A of ABq, *J* = 11.1 Hz, 1H, OC*H*$_2$Ph), 4.85 (A of ABq, *J* = 11.2 Hz, 1H, OC*H*$_2$Ph), 4.75–4.69 (m, 3H, H-1, 2 × OC*H*$_2$Ph), 4.61–4.57 (m, 2H, H-1′, OC*H*$_2$Ph), 4.48 (A of ABq, *J* = 11.9 Hz, 1H, OC*H*$_2$Ph), 4.34 (A of ABq, *J* = 11.9 Hz, 1H, OC*H*$_2$Ph), 4.19 (B of ABq, *J* = 11.9 Hz, 1H, OC*H*$_2$Ph), 4.13 (dd, *J* = 9.6, 9.2 Hz, 1H, H-4), 3.89 (dd, *J* = 11.1, 4.2 Hz, 1H, H-6a), 3.86–3.78 (m, 3H, H-6b, H-3, H-3′), 3.70–3.65 (m, 1H, H-5′), 3.58–3.53 (m, 2H, H-2′, H-5), 3.40–3.34 (m, 2H, H-6a, H-6b), 2.37 (d, *J* = 2.2 Hz, 1H, 3′-OH), 2.29 (s, 3H, ArMe); ^{13}C NMR (126 MHz, $CDCl_3$): δ 166.4 (C=O), 165.1 (C=O), 138.4 (Ar), 138.3 (Ar), 138.15 (Ar), 138.14 (Ar), 137.7 (Ar), 133.5 (Ar), 133.2

(Ar), 133.1 (Ar), 130.2 (Ar), 129.9 (Ar), 129.8 (Ar), 129.7 (Ar), 129.6 (Ar), 128.7 (Ar), 128.6 (Ar), 128.42 (Ar), 128.40 (Ar), 128.38 (Ar), 128.3 (Ar), 127.98 (Ar), 127.97 (Ar), 127.9 (Ar), 127.72 (Ar), 127.68 (Ar), 127.64 (Ar), 127.59 (Ar), 127.3 (Ar), 102.7 (C-1′), 86.4 (C-1), 81.8 (C-3), 80.3, 79.8 (2C, C-2′, C-5), 76.5 (C-4), 75.3, 74.3, 73.5, 73.4 (4C, 4 × O*C*H_2Ph) 72.8 (C-3′), 72.4 (C-2), 71.9 (C-5′), 70.3 (C-4′), 68.2 (C-6′), 67.6 (C-6), 21.2 (ArMe); HRMS-ESI *m/z* $[M + Na]^+$ calcd for $C_{61}H_{60}NaO_{12}S$: 1039.3698, found: 1039.3691.



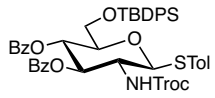
***p*-Tolyl 3,4,6-tri-*O*-acetyl-2-deoxy-1-thio-2-(2,2,2-trichlorethoxycarbonylamino)-β-D-glucopyranoside (4.22):** To a solution of D-glucosamine hydrochloride (10 g, 46.40 mmol) and $NaHCO_3$ (9.74 g, 116 mmol) in distilled water (150 mL) was added 2,2,2-trichlorethoxycarbonyl chloride (9.4 mL, 69 mmol). After stirring at room temperature, the formed precipitate was filtered, washed with cold water and ether, and dried overnight under high vacuum. The obtained intermediate (13.05 g) and NaOAc (0.90 g, 10.97 mmol) were suspended in acetic anhydride (60 mL). After stirring at 100 °C for 4 h, the solution was cooled, diluted with EtOAc (400 mL) and washed with water, saturated aqueous $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4, filtered, concentrated to dryness to afford the acetylated intermediate (19.23 g). Subsequently, the acetylated intermediate and *p*-thiocresol (5.49 g, 44.16 mmol) were dissolved in dry CH_2Cl_2 (150 mL). Then the solution was cooled to 0 °C and $BF_3{\cdot}Et_2O$ (11.3 mL, 92 mmol) was added slowly. After stirring overnight, while warming to room temperature, the solution was neutralized with Et_3N, concentrated and the resulting residue was subjected to flash chromatography (hexane–EtOAc 3:1) to afford thioglycoside **4.22** (17.4 g, 64% over three

steps) as a white foam. R_f = 0.63 (hexane–EtOAc 4:3); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.41 (d, *J* = 8.1 Hz, 2H, Ar), 7.12 (d, *J* = 7.9 Hz, 2H, Ar), 5.27 (t, *J* = 9.8 Hz, 1H, H-3), 5.18 (d, *J* = 8.9 Hz, 1H, N*H*Troc), 5.01 (t, *J* = 9.7 Hz, 1H, H-4), 4.80 (m, 2H, H-1, C*H*$_2$CCl$_3$), 4.72 (d, *J* = 12.0 Hz, 1H, C*H*$_2$CCl$_3$), 4.23 (dd, *J* = 12.2, 5.2 Hz, 1H, H-6a), 4.17 (dd, *J* = 12.2, 2.3 Hz, 1H, H-6b), 3.70 (ddd, *J* = 9.8, 5.0, 2.1 Hz, 1H, H-5), 3.64 (q, *J* = 9.8 Hz, 1H, H-2), 2.35 (s, 3H, ArMe), 2.08 (s, 3H, OAc), 2.01 (s, 3H, OAc), 2.00 (s, 3H, OAc); ^{13}C NMR (126 MHz, $CDCl_3$): δ 170.62 (C=O), 170.58 (C=O), 169.5 (C=O), 153.9 (C=O), 138.8 (Ar), 133.7 (2C, Ar), 129.8 (2C, Ar), 127.9 (Ar), 95.4 (CCl_3), 86.7 (C-1), 75.9 (C-5), 74.6 (O*C*H$_2$CCl$_3$), 73.2 (C-3), 68.5 (C-4), 62.3 (C-6), 55.1 (C-2), 21.2 (ArMe), 20.8, 20.7, 20.6 (3C, 3 × OAc).



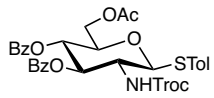
***p*-Tolyl 2-deoxy-1-thio-2-(2,2,2-trichlorethoxycarbonylamino)-β-D-glucopyranoside (4.23):** To a solution of **4.22** (11.24 g, 19.15 mmol) in 1:1 CH_2Cl_2–CH_3OH (80 mL) was added 4.4 M $NaOCH_3$ (0.87 mL). After stirring at room temperature for 3 h, the solution was neutralized with Amberlite IR120 H^+ ion-exchange resin. Then the solution was filtered and the filtrate was concentrated and the resulting residue was subjected to flash chromatography (CH_2Cl_2–MeOH 14:1) to afford **4.22** (7.41 g, 84%) as a white foam. R_f = 0.31 (CH_2Cl_2–MeOH 10:1); ^{1}H NMR (600 MHz, CD_3OD): δ 7.39 (d, *J* = 8.1 Hz, 2H, Ar), 7.10 (d, *J* = 8.0 Hz, 2H, Ar), 4.87 (d, *J* = 12.1 Hz, 1H, C*H*$_2$CCl$_3$), 4.71 (d, *J* = 12.1, 1H, C*H*$_2$CCl$_3$), 4.70 (d, *J* = 9.6, 1H, H-1) 3.85 (dd, *J* = 12.1, 2.2 Hz, 1H, H-6a), 3.67 (dd, *J* = 12.1, 5.7 Hz, 1H, H-6b), 3.48–3.39 (m, 2H, H-2, H-3), 3.32 (t, *J* = 9.6 Hz, 1H, H-4), 3.27 (ddd, *J* = 9.6, 5.7, 2.2 Hz, 1H, H-5), 2.30 (s, 3H, ArMe); ^{13}C NMR (126 MHz, CD_3OD): δ

156.8 (C=O), 138.7 (Ar), 133.2 (2C, Ar), 131.8 (Ar), 130.5 (2C, Ar), 97.2 (CCl_3), 89.0 (C-1), 82.0 (C-5), 77.3 (C-3), 75.6 (O*C*H_2CCl_3), 71.9 (C-4), 62.9 (C-6), 58.3 (C-2), 21.1 (ArMe).

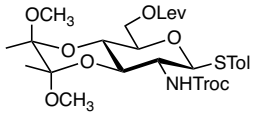


***p*-Tolyl 3,4-di-*O*-benzoyl-6-*O*-*tert*-butyldiphenylsilyl-2-deoxy-1-thio-2-(2,2,2-trichlorethoxycarbonylamino)-β-D-glucopyranoside (4.24):** To a solution of **4.23** (92 mg, 0.20 mmol) in pyridine (2 mL) was added TBDPSCl (57 μL, 0.22 mmol). After stirring overnight at room temperature, benzoyl chloride (75 μL, 0.60 mmol) was added to the solution. Stirring was continued for another 10 h before MeOH (0.5 mL) was added. Then the solution was concentrated and the resulting residue was subjected to flash chromatography (hexane–EtOAc 8:1) to afford **4.24** (145 mg, 80%) as a white foam. R_f = 0.37 (hexane–EtOAc 6:1); $[\alpha]_D$ = −25.8 (*c* = 1.1, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.93–7.89 (m, 2H, Ar), 7.82 (d, *J* = 7.3 Hz, 2H, Ar), 7.72–7.69 (m, 2H, Ar), 7.58–7.55 (m, 2H, Ar), 7.52–7.46 (m, 4H, Ar), 7.38–7.27 (m, 8H, Ar), 7.16 (t, *J* = 7.6 Hz, 2H, Ar), 7.07 (d, *J* = 7.9 Hz, 2H, Ar), 5.67 (t, *J* = 9.9 Hz, 1H, H-3), 5.59 (t, *J* = 9.6 Hz, 1H, H-4), 5.26 (d, *J* = 9.2 Hz, 1H, N*H*Cbz), 4.94 (d, *J* = 10.2 Hz, 1H, H-1), 4.73 (d, *J* = 12.0 Hz, 1H, OC*H*$_2$CCl$_3$), 4.62 (d, *J* = 12.0 Hz, 1H, OC*H*$_2$CCl$_3$), 3.97–3.90 (q, *J* = 9.9 Hz, 1H, H-2), 3.87–3.77 (m, 3H, H-5, H-6a, H-6b), 2.33 (s, 3H, ArMe), 1.04 (s, 9H, 3 × CH_3); ^{13}C NMR (126 MHz, $CDCl_3$): δ 166.5 (C=O), 165.0 (C=O), 154.0 (C=O), 138.4 (Ar), 135.7 (Ar), 135.6 (Ar), 133.5 (Ar), 133.4 (Ar), 133.3 (Ar), 132.9 (Ar), 130.0 (Ar), 129.9 (Ar), 129.75 (Ar), 129.68 (Ar), 129.6 (Ar), 129.2 (Ar), 128.9 (Ar), 128.5 (Ar), 128.4 (Ar), 127.7 (Ar), 127.6 (Ar), 95.3 (CCl_3), 87.1 (C-1), 79.4 (C-5), 74.5 (O*C*H_2CCl_3), 74.2 (C-3), 68.8 (C-4),

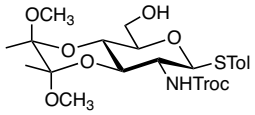
62.8 (C-6), 55.6 (C-2), 26.7 (3C, 3 × CH_3), 21.2 (ArMe), 19.2 (Si*C*$(CH_3)_3$); HRMS-ESI *m/z* $[M + Na]^+$ calcd for $C_{46}H_{46}Cl_3NNaO_8SSi$: 928.1671, found: 928.1669.



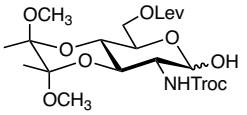
***p*-Tolyl 6-*O*-acetyl-3,4-di-*O*-benzoyl-2-deoxy-1-thio-2-(2,2,2-trichlorethoxycarbonylamino)-β-D-glucopyranoside (4.25):** To a solution of **4.24** (132 mg, 0.15 mmol) in 9:1 THF–AcOH (3 mL) was added TBAF (0.30 mL of a 1M solution in THF, 0.30 mmol). After stirring overnight at 40 °C, the solution was concentrated and the resulting residue was subjected to flash chromatography (hexane–EtOAc 3:1). The resulting compound (91 mg) was treated with acetic anhydride (0.5 mL) in pyridine (2.5 mL) overnight at room temperature. The solution was concentrated and the resulting residue was subjected to flash chromatography (hexane–EtOAc 4:1) to afford **4.25** (90 mg, 87% over two steps) as a white foam. R_f = 0.57 (hexane–EtOAc 2:1); $[\alpha]_D$ = –35.2 (*c* = 0.9, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.90–7.85 (m, 4H, Ar), 7.51–7.44 (m, 4H, Ar), 7.36–7.31 (m, 4H, Ar), 7.14 (d, *J* = 8.0 Hz, 2H, Ar), 5.72 (t, *J* = 9.9 Hz, 1H, H-3), 5.48 (t, *J* = 9.7 Hz, 1H, H-4), 5.34 (d, *J* = 9.2 Hz, 1H, N*H*Cbz), 4.95 (d, *J* = 10.3 Hz, 1H, H-1), 4.72 (d, *J* = 12.0 Hz, 1H, OCH_2CCl$_3$), 4.61 (d, *J* = 12.0 Hz, 1H, OCH_2CCl$_3$), 4.30–4.24 (m, 2H, H-6b, H-6a), 3.98–3.88 (m, 2H, H-2, H-5), 2.36 (s, 3H, ArMe), 2.03 (s, 3H, OAc); ^{13}C NMR (126 MHz, $CDCl_3$): δ 170.6 (C=O), 166.3 (C=O), 165.2 (C=O), 154.0 (C=O), 138.8 (Ar), 133.7 (Ar), 133.6 (Ar), 130.0 (Ar), 129.82 (Ar), 129.79 (Ar), 128.8 (Ar), 128.7 (Ar), 128.5 (Ar), 128.4 (Ar), 128.0 (Ar), 95.3 (CCl_3), 87.0 (C-1), 76.1 (C-5), 74.5 (O*C*H_2CCl$_3$), 73.7 (C-3), 69.3 (C-4), 62.8 (C-6), 55.5 (C-2), 21.3 (ArMe), 20.7 (OAc); HRMS-ESI *m/z* $[M + Na]^+$ calcd for $C_{32}H_{30}Cl_3NNaO_9S$: 732.0599, found: 732.0597.



(2′*S*,3′*S*) *p*-Tolyl 2-deoxy-3,4-*O*-(2′,3′-dimethoxybutane-2′,3′-diyl)-6-*O*-levulinyl-1-thio-2-(2,2,2-trichlorethoxycarbonylamino)-β-D-glucopyranoside (4.27): 4.28 (9.0 g, 15.65 mmol), levulinic acid (2.18 g, 18.78 mmol) and DMAP (95 mg, 0.78 mmol) were dissolved in CH_2Cl_2 (80 mL). The solution was cooled to 0 °C before DCC (4.20 g, 20.35 mmol) was added. After stirring at room temperature for 2 h, hexane (80 mL) was added and the solution was cooled to 0 °C. Then the cold solution was filtered to remove the precipitate and the filtrate was concentrated and the resulting residue was subjected to flash chromatography (hexane–EtOAc 5:2) to afford **4.27** (9.50 g, 90%) as a white foam. R_f = 0.3 (hexane–EtOAc 2:1); $[\alpha]_D$ = +91.7 (*c* = 0.9, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.41 (d, *J* = 7.8 Hz, 2H, Ar), 7.10 (d, *J* = 7.8 Hz, 2H, Ar), 5.05 (d, *J* = 6.6 Hz, 1H, N*H*Troc), 4.99 (d, *J* = 9.6 Hz, 1H, H-1), 4.76, 4.73 (ABq, *J* = 11.7 Hz, 2H, OC*H*$_2$CCl$_3$), 4.44 (dd, *J* = 11.9, 2.0 Hz, 1H, H-6a), 4.17 (dd, *J* = 11.9, 5.5 Hz, 1H, H-6b), 4.09 (app t, *J* = 9.6 Hz, 1H, H-3), 3.74–3.68 (m, 1H, H-5), 3.58 (app t, *J* = 9.6 Hz, 1H, H-4), 3.37–3.28 (m, 1H, H-2), 3.22 (s, 3H, OCH_3), 3.20 (s, 3H, OCH_3), 2.79–2.71 (m, 2H, OC(O)CH$_2$C*H*$_2$C(O) 2.60 (t, *J* = 6.6 Hz, 2H, OC(O)C*H*$_2$CH$_2$C(O)), 2.34 (s, 1H, ArMe), 2.19 (s, 1H, C(O)C*H*$_3$), 1.26 (s, 6H, 2 × CH_3); ^{13}C NMR (126 MHz, $CDCl_3$): δ 206.4 (C=O), 172.5 (C=O), 153.6 (C=O), 138.6 (Ar), 133.8 (2C, Ar), 129.8 (2C, Ar), 128.0 (Ar), 100.1, 99.8 (2C, 2 × O*C*(OCH$_3$)), 95.6 (CCl_3), 85.8 (C-1), 75.6 (C-5), 74.5 (O*C*H$_2$CCl$_3$), 69.9 (C-3), 67.0 (C-4), 62.7 (C-6), 54.2 (C-2), 48.1, 48.0 (2C, 2 × OCH_3), 37.9, 27.9 (2C, CH_2CH_2) 29.9 (CH$_2$C(O)*C*H$_3$), 21.2 (ArMe), 17.7, 17.6 (2C, 2 × CH_3); HRMS-ESI *m/z* [M + Na]$^+$ calcd for $C_{27}H_{36}Cl_3NNaO_{10}S$: 694.1018, found: 694.1010.

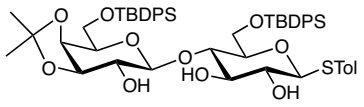


(2′*S*,3′*S*) *p*-Tolyl 2-deoxy-3,4-*O*-(2′,3′-dimethoxybutane-2′,3′-diyl)-1-thio-2-(2,2,2-trichlorethoxycarbonylamino)-β-D-glucopyranoside (4.28): 4.23 (7.44 g, 16.15 mmol) was dissolved in MeOH (100 mL), to which butane-2,3-dione (1.7 mL, 19.38 mmol), trimethylorthoformate (7.07 ml, 64.59 mmol) and CSA (0.45 g, 1.94 mmol) was added. After heating at reflux overnight, the solution was cooled, neutralized with Et_3N (1 mL) and concentrated. The resulting residue was subjected to flash chromatography (hexane–EtOAc 5:2) to afford **4.28** (7.70 g, 83%) as a white foam. R_f = 0.45 (hexane–EtOAc 3:2); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.40–7.37 (m, 2H, Ar), 7.14–7.10 (m, 2H, Ar), 5.08 (d, *J* = 6.7 Hz, 1H, N*H*Troc), 5.03 (d, *J* = 9.7 Hz, 1H, H-1), 4.75 (ABq, *J* = 12.5 Hz, 2H, OCH_2CCl$_3$), 4.11–4.05 (m, 1H, H-3), 3.88 (dd, *J* = 12.0, 2.8 Hz, 1H, H-6a), 3.73 (dd, *J* = 12.0, 4.6 Hz, 1H, H-6b), 3.63 (app t, *J* = 9.7 Hz, 1H, H-4), 3.59–3.54 (m, 1H, H-5), 3.37–3.30 (m, 1H, H-2), 3.23 (s, 3H, OCH_3), 3.21 (s, 3H, OCH_3), 2.34 (s, 3H, ArMe), 1.273 (s, 3H, CH_3), 1.269 (s, 3H, CH_3); ^{13}C NMR (126 MHz, $CDCl_3$): δ 153.6 (C=O), 138.7 (Ar), 133.6 (2C, Ar), 129.9 (2C, Ar), 128.0 (Ar), 100.1, 99.6 (2C, 2 × O*C*OCH_3), 95.6 (CCl_3), 85.9 (C-1), 77.9 (C-5), 74.5 (O*C*H_2CCl_3), 69.9 (C-3), 66.8 (C-4), 61.5 (C-6), 54.4 (C-2), 48.02, 47.96 (2C, 2 × OCH_3), 21.2 (ArMe), 17.7, 17.6 (2C, 2 × CH_3); HRMS-ESI *m/z* $[M + Na]^+$ calcd for $C_{22}H_{30}Cl_3NNaO_8S$: 596.0650, found: 596.0642.



(2′*S*,3′*S*) 2-deoxy-3,4-*O*-(2′,3′-dimethoxybutane-2′,3′-diyl)-6-*O*-levulinyl-2-(2,2,2-trichlorethoxycarbonylamino)-β-D-glucopyranose (4.29): To a solution of **4.27** (1.05 g,

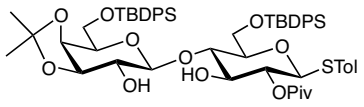
1.55 mmol) in 9:1 acetone–water (10 mL) was added NBS (691 mg, 3.88 mmol) at 0 °C. After stirring at 0 °C for 1 h, saturated aqueous $NaHCO_3$ (2 mL) was added. The solution was concentrated, and then the resulting residue was dissolved in EtOAc (100 mL) and washed with water and brine. The organic layer was dried over Na_2SO_4, filtered, concentrated and the resulting residue was subjected to flash chromatography (hexane–EtOAc 1:1) to afford **4.29** (855 mg, 96%) as a white solid. R_f = 0.26 (hexane–EtOAc 1:1); ^{1}H NMR (600 MHz, $CDCl_3$): δ 5.31 (t, *J* = 2.5 Hz, 1H, H-1), 5.19 (d, *J* = 8.6 Hz, 1H, N*H*Cbz), 4.82 (d, *J* = 12.1 Hz, 1H, OC*H*$_2$CCl$_3$), 4.66 (d, *J* = 12.1 Hz, 1H, OC*H*$_2$CCl$_3$), 4.47–4.41 (m, 1H, H-6a), 4.20–4.13 (m, 2H, H-5, H-6b), 3.98–3.92 (m, 2H, H-2, H-3), 3.72–3.66 (m, 1H, H-4), 3.59 (d, *J* = 2.7 Hz, 1H, 1-OH), 3.25 (s, 3H, OCH_3), 3.23 (s, 3H, OCH_3), 2.82–2.68 (m, 2H, OC(O)CH$_2$C*H*$_2$C(O)), 2.62–2.56 (m, 2H, OC(O)C*H*$_2$CH$_2$C(O)), 2.19 (s, 3H, CH$_2$C(O)C*H*$_3$), 1.28 (s, 3H, CH_3), 1.27 (s, 3H, CH_3); ^{13}C NMR (126 MHz, $CDCl_3$): δ 207.2 (C=O), 172.5 (C=O), 154.3 (C=O), 100.0, 99.8 (2C, 2 × O*C*OCH$_3$), 95.7 (CCl_3), 92.2 (C-1), 74.6 (O*C*H$_2$CCl$_3$), 67.85, 67.77 (2C, C-3, C-5), 67.0 (C-4), 62.6 (C-6), 53.0 (C-2), 48.0 (2C, 2 × OCH_3), 38.1, 28.0 (2C, *C*H$_2$*C*H$_2$), 29.9 (CH$_2$C(O)*C*H$_3$), 17.8, 17.7 (2C, 2 × CH_3).



***p*-Tolyl 6-*O*-*tert*-butyldiphenylsilyl-3,4-*O*-isopropylidene-β-D-galactopyranosyl-(1→4)-6-*O*-*tert*-butyldiphenylsilyl-1-thio-β-D-glucopyranoside (4.30):** To an ice-cold solution of **4.32** (9.19 g, 20.49 mmol) in pyridine (100 mL) was added TBDPSCl (11.73 mL, 45.08 mmol). After stirring overnight, while warming to room temperature, MeOH (10 mL) was

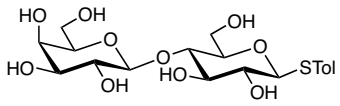
added and the solution was concentrated, dissolved in EtOAc (400 mL) and washed with water and brine. The organic layer was dried over Na_2SO_4, filtered, concentrated and the resulting residue was subjected to flash chromatography (CH_2Cl_2–MeOH 17:1). The resulting residue and *p*-TsOH (350 mg, 1.85 mmol) were suspended in 2,2-dimethoxypropane (120 mL). After stirring at room temperature for 3 h, Et_3N (10 mL) was added and the solution was concentrated and the resulting residue was subjected to flash chromatography (hexane–EtOAc 3:2). to afford **4.30** (12.69 g, 64% over two steps) as a white solid. R_f = 0.41 (hexane–EtOAc 1:1); $[\alpha]_D$ = –6.7 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.80–7.77 (m, 2H, Ar), 7.75–7.72 (m, 2H, Ar), 7.68–7.63 (m, 4H, Ar), 7.51–7.47 (m, 2H, Ar), 7.45–7.34 (m, 12H, Ar), 7.04 (d, *J* = 7.9 Hz, 2H, Ar), 4.49 (d, *J* = 9.7 Hz, 1H, H-1), 4.34 (d, *J* = 1.4 Hz, 1H, 3-OH), 4.33 (d, *J* = 8.3 Hz, 1H, H-1′), 4.15 (dd, *J* = 5.4, 1.9 Hz, 1H, H-4′), 4.00–3.95 (m, 3H, H-3′, H-6a, H-6b), 3.93 (dd, *J* = 8.7, 6.4 Hz, 1H, H-6a′), 3.91–3.88 (m, 1H, H-5′), 3.86 (dd, *J* = 8.7, 5.3 Hz, 1H, H-6b′), 3.68 (t, *J* = 9.2 Hz, 1H, H-4), 3.60 (dt, *J* = 8.8, 1.3 Hz, 1H, H-3), 3.51–3.47 (dt, *J* = 7.8, 3.3 Hz 1H, H-2′), 3.45–3.41 (m, 1H, H-5), 3.38 (dt, *J* = 8.8, 1.3 Hz, 1H, H-2), 2.57 (d, *J* = 1.6 Hz, 1H, 2-OH), 2.31 (s, 3H, ArMe), 2.11 (d, *J* = 3.3 Hz, 1H, 2′-OH), 1.50 (s, 3H, CH_3), 1.31 (s, 3H, CH_3), 1.06 (s, 9H, 3 × CH_3), 1.04 (s, 9H, 3 × CH_3); ^{13}C NMR (126 MHz, $CDCl_3$): δ 138.2 (Ar), 135.9 (2C, Ar), 135.7 (4C, Ar), 135.6 (2C, Ar), 133.6 (2C, Ar), 133.4(Ar), 133.1 (Ar), 133.0 (Ar), 132.8 (Ar), 129.87 (2C, Ar), 129.86 (2C, Ar), 129.7 (2C, Ar), 128.2 (Ar), 127.9 (2C, Ar), 127.82 (2C, Ar), 127.77 (2C, Ar), 127.7 (2C, Ar), 110.3 ((CH_3)$_2$*C*(O)$_2$), 103.0 (C-1′), 87.6 (C-1), 80.0 (C-4), 79.1 (C-5), 78.9 (C-3′), 76.2 (C-3), 74.1 (C-5′), 73.6 (C-2′), 73.1 (C-4′), 71.9 (C-2), 62.9, 62.5 (2C, C-6, C-6′), 28.2 ((CH$_3$)$_2$C(O)$_2$), 26.9 (3C, SiC(CH$_3$)$_3$), 26.8 (3C, SiC(CH$_3$)$_3$), 26.3 ((CH$_3$)$_2$C(O)$_2$), 21.2 (ArMe), 19.3, 19.2 (2C, 2 × SiC(CH$_3$)$_3$);

HRMS-ESI *m/z* $[M + Na]^+$ calcd for $C_{54}H_{68}NaO_{10}SSi_2$: 987.3964, found: 987.3962.



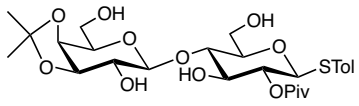
***p*-Tolyl 6-*O*-*tert*-butyldiphenylsilyl-3,4-*O*-isopropylidene-β-D-galactopyranosyl-(1→4)-6-*O*-*tert*-butyldiphenylsilyl-2-*O*-pivaloyl-1-thio-β-D-glucopyranoside (4.31):** A stirred solution of **4.30** (10.69 g, 11.07 mmol) and DMAP (268 mg, 2.20 mmol) in dry CH_2Cl_2 (150 mL) was cooled to −78 °C, to which DBU (1.9 mL, 12.74 mmol) was added. After stirring for 30 min at −78 °C, pivaloyl chloride (1.57 mL, 12.74 mmol) was added dropwise to the solution, and stirring was continued for 3.5 h at −78 °C. MeOH (2 mL) was added before the solution was warmed to room temperature. Then the solution was concentrated and the resulting residue was subjected to flash chromatography (hexane–EtOAc 4:1) to afford **4.31** (8.87 g, 76%) as a white foam. R_f = 0.50 (hexane–EtOAc 3:1); $[\alpha]_D$ = −3.2 (*c* = 0.9, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.79–7.72 (m, 4H, Ar), 7.70–7.64 (m, 4H, Ar), 7.46–7.33 (m, 14H, Ar), 7.03 (d, *J* = 7.9 Hz, 2H, Ar), 4.91 (dd, *J* = 10.1, 8.8 Hz, 1H, H-2), 4.58 (d, *J* = 10.2 Hz, 1H, H-1), 4.32 (d, *J* = 8.3 Hz, 1H, H-1′), 4.14 (dd, *J* = 5.4, 2.0 Hz, 1H, H-4′), 4.07 (br s, 1H, OH), 4.00–3.91 (m, 4H, H-6a, H-6b, H-3′, H-6a′), 3.88 (dt, *J* = 6.0, 2.0 Hz 1H, H-5′), 3.83 (dd, *J* = 9.2, 5.7 Hz, 1H, H-6b′), 3.77–3.68 (m, 2H, H-3, H-4)), 3.49–3.39 (m, 2H, H-2′, H-5), 2.30 (s, 3H, ArMe), 2.11 (br s, 1H, OH), 1.48 (s, 3H, CH_3), 1.30 (s, 3H, CH_3), 1.23 (s, 9H, 3 × CH_3), 1.08 (s, 9H, 3 × CH_3), 1.04 (s, 9H, 3 × CH_3); ^{13}C NMR (126 MHz, $CDCl_3$): δ 176.69 (C=O), 137.82 (Ar), 135.95 (Ar), 135.72 (Ar), 135.68 (Ar), 135.64 (Ar), 133.43 (Ar), 133.12 (Ar), 133.10 (Ar), 132.79 (Ar), 132.76 (Ar), 130.12 (Ar), 129.83 (Ar), 129.79 (Ar), 129.65 (Ar), 127.86 (Ar), 127.81 (Ar), 127.77 (Ar), 127.71

(Ar), 110.22 ($(CH_3)_2C(O)_2$), 103.08 (C-1′), 87.13 (C-1), 80.80 (C-4), 79.01 (C-5), 78.82 (C-3′), 74.69 (C-3), 74.09 (C-5′), 73.69 (C-2′), 73.12 (C-4′), 71.49 (C-2), 63.06 (C-6), 62.51 (C-6′), 38.74($C(O)C(CH_3)_3$), 28.21 ($(CH_3)_2C(O)_2$), 27.18 (3C, 3 × $C(CH_3)_3$), 26.88 (3C, $C(CH_3)_3$), 26.78 (3C, 3 × $C(CH_3)_3$), 26.29 ($(CH_3)_2C(O)_2$), 21.18 (ArMe), 19.36, 19.17 (2C, 2 × $SiC(CH_3)_3$); HRMS-ESI *m/z* $[M + Na]^+$ calcd for $C_{59}H_{76}NaO_{11}SSi_2$: 1071.4539, found: 1071.4521.



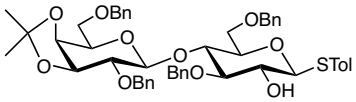
***p*-Tolyl β-D-galactopyranosyl-(1→4)-1-thio-β-D-glucopyranoside (4.32):** D-Lactose (10 g, 29.2 mmol) and NaOAc (2.4 g, 30 mmol) were heated in acetic anhydride (60 mL) at 100 °C for 5 h. The solution was cooled, diluted with EtOAc (400 mL) and washed with water, saturated aqueous $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4, filtered, concentrated to dryness. The resulting peracetylated lactose and *p*-thiocresol (4.35 g, 35.04 mmol) were dissolved in dry CH_2Cl_2 (150 mL). The solution was cooled to 0 °C before $BF_3{\cdot}Et_2O$ (8.74 mL) was added dropwise. After stirring overnight, while warming to room temperature, the solution was neutralized with Et_3N, concentrated and the resulting residue was subjected to flash chromatography (hexane–EtOAc 3:2) to afford a thioglycoside intermediate (15.23 g). Subsequently, this compound was treated with 3.0 M $NaOCH_3$ (4.78 mL) in 1:3 CH_2Cl_2–CH_3OH (80 mL) overnight at room temperature. The solution was neutralized with Amberlite IR120 H^+ ion-exchange resin, filtered and the filtrate was concentrated to afford **4.32** (9.19 g, 70% over three steps) as a white solid. ^1H NMR (500 MHz, CD_3OD): δ 7.45 (d, J = 8.0 Hz, 2H), 7.12 (d, J = 8.0 Hz, 2H), 4.52 (d, J =

9.8 Hz, 1H), 4.34 (d, *J* = 7.6 Hz, 1H), 3.88 (dd, *J* = 12.2, 2.5 Hz, 1H), 3.84–3.78 (m, 2H), 3.76 (dd, *J* = 11.4, 7.5 Hz, 1H), 3.68 (dd, *J* = 11.4, 4.6 Hz, 1H), 3.58–3.49 (m, 4H), 3.46 (dd, *J* = 9.7, 3.3 Hz, 1H), 3.40 (ddd, *J* = 9.2, 4.2, 2.4 Hz, 1H), 3.26–3.20 (m, 1H), 2.30 (s, 3H).



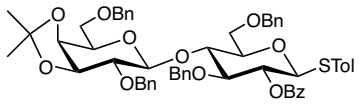
***p*-Tolyl 3,4-*O*-isopropylidene-β-D-galactopyranosyl-(1→4)-2-*O*-pivaloyl-1-thio-β-D-glucopyranoside (4.33):** To a solution of **4.31** (8.85 g, 8.43 mmol) in THF (50 mL) was added TBAF (33.73 mL of 1M solution in THF, 33.73 mmol). After stirring at room temperature for 3 h, the solution was neutralized with AcOH and concentrated. The resulting residue was dissolved in EtOAc (400 mL) and washed with saturated aqueous $NaHCO_3$, water and brine. The organic layer was dried over Na_2SO_4, filtered, concentrated and the resulting residue was subjected to flash chromatography (CH_2Cl_2–MeOH 19:1) to afford **4.33** (4.17 g, 86%) as a white foam. R_f = 0.30 (CH_2Cl_2–MeOH 15:1); $[\alpha]_D$ = +3.6 (*c* = 0.9, MeOH); ^{1}H NMR (600 MHz, CD_3OD): δ 7.37 (d, *J* = 7.8 Hz, 2H, Ar), 7.12 (d, *J* = 7.8 Hz, 2H, Ar), 4.77 (dd, *J* = 10.2, 8.4 Hz, 1H, H-2), 4.69 (d, *J* = 10.2 Hz, 1H, H-1), 4.36 (d, *J* = 8.4 Hz, 1H, H-1′), 4.16 (dd, *J* = 5.4, 2.4 Hz, 1H, H-4′), 4.03 (dd, *J* = 7.2, 5.4, Hz, 1H, H-3′), 3.92–3.86 (m, 2H, H-5′, H-6a), 3.82 (dd, *J* = 12.0, 4.2 Hz 1H, H-6b), 3.76 (dd, *J* = 11.7, 8.1 Hz, 1H, H-6a′) 3.73–3.68 (m, 2H, H-3, H-6b′), 3.66 (app t, *J* = 9.3 Hz, 1H, H-4), 3.46–3.40 (m, 2H, H-2′, H-5), 2.30 (s, 3H, ArMe), 1.44 (s, 3H, CH_3), 1.30 (s, 3H, CH_3), 1.24 (s, 9H, 3 × C(CH_3)$_3$); ^{13}C NMR (126 MHz, CD_3OD): δ 178.6 (C=O), 139.1 (Ar), 133.6 (2C, Ar), 131.0 (Ar), 130.6 (2C, Ar), 111.1 ((CH_3)$_2$*C*(O)$_2$), 104.0 (C-1′), 87.8 (C-1), 80.9 (C-3′), 80.7 (C-5), 80.0 (C-4), 75.8 (C-3), 75.5 (C-5′), 75.1 (C-4′), 74.5 (C-2′), 73.1

(C-2), 62.5 (C-6′), 61.8 (C-6), 39.8 ($C(CH_3)_3$), 28.4, 26.5 (2C, 2 × $(CH_3)_2C(O)_2$), 27.6 (3C, 3 × $C(CH_3)_3$), 21.1 (ArMe); HRMS-ESI *m/z* $[M + Na]^+$ calcd for $C_{27}H_{40}NaO_{11}S$: 595.2184, found: 595.2173.



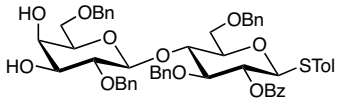
***p*-Tolyl 2,6-di-*O*-benzyl-3,4-*O*-isopropylidene-β-D-galactopyranosyl-(1→4)-3,6-di-*O*-benzyl-1-thio-β-D-glucopyranoside (4.40):** Compound **4.33** (3.71 g, 6.48 mmol), BnBr (9.23 mL, 77.74 mmol) and TBAI (2.39 g, 6.48 mmol) were suspended in dry DMF (50 mL), to which NaH (60%, 1.30 g, 32.39 mmol) was added in portions at 0 °C, followed by vigorous stirring at 0 °C for 1 h. The mixture was warmed to room temperature and stirred for 1 h before CH_3OH (4 mL) was added. The solution was concentrated, dissolved in EtOAc (300 mL) and washed with water and brine. The organic layer was dried over Na_2SO_4, filtered, concentrated and the resulting residue was subjected to flash chromatography (hexane–EtOAc 4:1). The resulting benzylated intermediate was dissolved in 1:1 CH_2Cl_2–CH_3OH (60 mL), to which NaOH (6.00 g) was added. After stirring overnight at 40 °C, the solution was concentrated, dissolved in EtOAc (200 mL) and washed with water and brine. The organic layer was dried over Na_2SO_4, filtered, concentrated and the resulting residue was subjected to flash chromatography (hexane–EtOAc 4:1) to afford **4.40** (3.46 g, 63% over two steps) as a white foam. R_f = 0.37 (hexane–EtOAc 3:1); $[\alpha]_D$ = −8.5 (*c* = 0.8, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.47–7.43 (m, 2H, Ar), 7.41–7.37 (m, 2H, Ar), 7.36–7.21 (m, 18H, Ar), 7.03 (d, *J* = 8.0 Hz, 2H, Ar), 4.98, 4.71 (ABq, *J* = 11.0 Hz, 2H, 2 × OCH_2Ph), 4.80, 4.68 (ABq, *J* = 11.8 Hz, 2H, 2 ×

OCH_2Ph), 4.56 (A of ABq, J = 12.0 Hz, 1H, OCH_2Ph), 4.51, 4.36 (ABq, J = 12.0 Hz, 2H, 2 × OCH_2Ph), 4.47–4.42 (m, 3H, H-1, H-1′, OCH_2Ph), 4.13 (dd, J = 5.6, 1.5 Hz, 1H, H-4′), 4.07 (dd, J = 6.8, 5.6 Hz, 1H, H-3′), 3.92 (app t, J = 9.4 Hz, 1H, H-4), 3.85 (dd, J = 11.2, 4.1 Hz, 1H, H-6a), 3.77 (dd, J = 11.2, 1.6 Hz, 1H, H-6b), 3.74–3.67 (m, 2H, H-5′, H-6a′), 3.61–3.54 (m, 1H, H-6b′), 3.52–3.45 (m, 2H, H-3, H-5), 3.39 (app t, J = 9.2 Hz, 1H, H-2), 3.36 (dd, J = 8.0, 6.8 Hz, 1H, H-2′), 2.44 (br s, 1H, 2-OH), 2.30 (s, 3H, ArMe), 1.40 (s, 3H, CH_3), 1.35 (s, 3H, CH_3); ^{13}C NMR (126 MHz, $CDCl_3$): δ 138.8 (Ar), 138.45 (Ar), 138.44 (Ar), 138.40 (Ar), 138.3 (Ar), 133.7 (2C, Ar), 129.7 (2C, Ar), 128.4 (2C, Ar), 128.34 (2C, Ar), 128.32 (2C, Ar), 128.31 (2C, Ar), 128.2 (2C, Ar), 127.9 (2C, Ar), 127.8 (Ar), 127.65 (2C, Ar), 127.63 (2C, Ar), 127.58 (2C, Ar), 127.56 (2C, Ar), 127.53 (2C, Ar), 127.51 (2C, Ar), 109.8 ((CH_3)$_2$*C*(O)$_2$), 102.0 (C-1′), 87.7 (C-1), 84.2 (C-3), 80.7 (C-2′), 79.8 (C-3′), 79.4 (C-5), 75.9 (C-4), 75.0 (O*C*H$_2$Ph), 73.7 (C-4′), 73.5, 73.4, 73.2 (3C, 3 × O*C*H$_2$Ph), 72.0 (C-5′), 71.6 (C-2), 69.0 (C-6′), 68.3 (C-6), 28.0 ((CH_3)$_2$*C*(O)$_2$), 26.4 ((CH_3)$_2$*C*(O)$_2$), 21.2 (ArMe); HRMS-ESI *m/z* [M + Na]$^+$ calcd for $C_{50}H_{56}NaO_{10}S$: 871.3486, found: 871.3472.



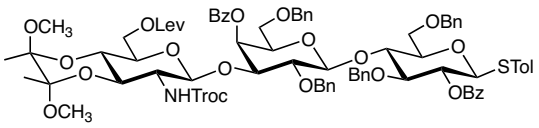
***p*-Tolyl 2,6-di-*O*-benzyl-3,4-*O*-isopropylidene-β-D-galactopyranosyl-(1→4)-2-*O*-benzoyl-3,6-di-*O*-benzyl-1-thio-β-D-glucopyranoside (4.41):** To a ice-cold solution of **4.40** (3.30 g, 3.89 mmol) in pyridine (25 mL) was added benzoyl chloride (1.36 mL, 11.67 mmol). After stirring overnight, while warming to room temperature, MeOH (2 mL) was added, and the solution was concentrated, dissolved in EtOAc (300 mL) followed by washing with water and brine. The organic layer was dried over Na_2SO_4, filtered and

concentrated. The resulting residue was subjected to flash chromatography (hexane–EtOAc 5:1) to afford **4.41** (3.62 g, 97%) as a white foam. R_f = 0.40 (hexane–EtOAc 4:1); $[\alpha]_D$ = +28.9 (*c* = 0.9, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ: 8.04–8.01 (m, 2H, Ar), 7.60–7.56 (m, 1H, Ar), 7.47–7.43 (m, 2H, Ar), 7.39–7.37 (m, 2H, Ar), 7.36–7.24 (m, 15H, Ar), 7.14–7.11 (m, 2H, Ar), 7.09– 6.98 (m, 5H, Ar), 5.22 (dd, *J* = 10.0, 9.1 Hz, 1H, H-2), 4.84 (A of ABq, *J* = 11.0 Hz, 1H, OC*H*$_2$Ph), 4.81 (A of ABq, *J* = 11.8 Hz, 1H, OC*H*$_2$Ph), 4.72 (d, *J* = 10.0 Hz, 1H, H-1), 4.70 (B of ABq, *J* = 11.6 Hz, 1H, OC*H*$_2$Ph), 4.59 (B of ABq, *J* = 11.0 Hz, 1H, OC*H*$_2$Ph), 4.54 (A of ABq, *J* = 11.9 Hz, 1H, OC*H*$_2$Ph), 4.48–4.42 (m, 3H, H-1′, 2 × OC*H*$_2$Ph), 4.31 (A of ABq, *J* = 12.0 Hz, 1H, OC*H*$_2$Ph), 4.12 (dd, *J* = 5.6, 2.1 Hz, 1H, H-4′), 4.07 (dd, *J* = 6.7, 5.6 Hz, 1H, H-3′), 4.04 (dd, *J* = 9.6, 9.0 Hz, 1H, H-4), 3.86 (dd, *J* = 11.1, 4.5 Hz, 1H, H-6a,), 3.81 (dd, *J* = 11.1, 1.8 Hz, 1H, H-6b), 3.77 (t, *J* = 8.9 Hz, 1H, H-3), 3.71 (td, *J* = 6.4, 2.0 Hz, 1H, H-5′), 3.65 (dd, *J* = 9.8, 6.5 Hz, 1H, H-6a′), 3.55 (ddd, *J* = 9.8, 4.4, 1.9 Hz, 1H, H-5), 3.51 (dd, *J* = 9.8, 6.2 Hz, 1H, H-6b′), 3.37 (dd, *J* = 8.0, 6.7 Hz, 1H, H-2′), 2.28 (s, 3H, ArMe), 1.40 (s, 3H, CH_3), 1.35 (s, 3H, CH_3); ^{13}C NMR (126 MHz, $CDCl_3$): δ 165.1 (C=O), 138.45 (Ar), 138.40 (Ar), 138.39 (Ar), 138.35 (Ar), 138.0 (Ar), 133.3 (Ar), 133.0 (Ar), 130.2 (Ar), 129.9 (Ar), 129.6 (Ar), 129.0 (Ar), 128.4 (Ar), 128.35 (Ar), 128.30 (Ar), 128.26 (Ar), 128.0 (Ar), 127.9 (Ar), 127.7 (Ar), 127.6 (Ar), 127.54 (Ar), 127.50 (Ar), 127.3 (Ar), 109.8 ($(CH_3)_2C(O)_2$), 102.2 (C-1′), 86.4 (C-1), 82.1 (C-3), 80.7 (C-2′), 79.8, 79.4 (2C, C-3′, C-5), 76.5 (C-4), 74.6 (O*C*H$_2$Ph), 73.7 (C-4′), 73.5, 73.4, 73.3 (3C, 3 × O*C*H$_2$Ph), 72.1 (2C, C-2, C-5′), 69.0 (C-6′), 68.3 (C-6), 28.0 ($(CH_3)_2C(O)_2$), 26.4 ($(CH_3)_2C(O)_2$), 21.2 (ArMe); HRMS-ESI *m/z* $[M + Na]^+$ calcd for $C_{57}H_{60}NaO_{11}S$: 975.3749, found: 975.3734.



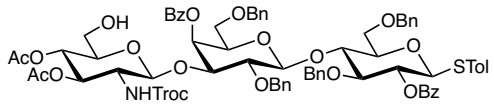
***p*-Tolyl 2,6-di-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2-*O*-benzoyl-3,6-di-*O*-benzyl-1-thio-β-D-glucopyranoside (4.42):** Compound **4.41** (4.04 g, 4.24 mmol) was dissolved in MeCN (27 mL), to which TFA (3 mL) and H_2O (1 mL) were added. After stirring at room temperature for 30 min, the solution was neutralized with Et_3N, then concentrated and the resulting residue was subjected to flash chromatography (hexane–EtOAc 1:1) to afford diol **4.42** (3.76 g, 97%) as a white foam. R_f = 0.33 (hexane–EtOAc 1:1); $[\alpha]_D$ = +19.7 (*c* = 0.7, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.04–7.99 (m, 2H, Ar), 7.61–7.55 (m, 1H, Ar), 7.47–7.42 (m, 2H, Ar), 7.41–7.22 (m, 17H, Ar), 7.16–7.12 (m, 2H, Ar), 7.09–6.98 (m, 5H, Ar), 5.23 (dd, *J* = 9.8, 9.2 Hz, 1H, H-2), 4.89, 4.64 (ABq, *J* = 11.4 Hz, 2H, 2 × OC*H*$_2$Ph), 4.85, 4.70 (ABq, *J* = 11.5 Hz, 2H, 2 × OC*H*$_2$Ph), 4.72 (d, *J* = 10.0 Hz, 1H, H-1), 4.57 (A of ABq, *J* = 11.9 Hz, 1H, OC*H*$_2$Ph), 4.49–4.44 (m, 2H, H-1′, OC*H*$_2$Ph), 4.41, 4.37 (ABq, *J* = 12.0 Hz, 2H, 2 × OC*H*$_2$Ph), 4.09 (t, *J* = 8.9 Hz, 1H, H-4), 3.98–3.94 (m, 1H, H-4′), 3.87 (dd, *J* = 11.1, 4.2 Hz, 1H, H-6a), 3.83 (dd, *J* = 11.1, 1.8 Hz, 1H, H-6b), 3.79 (t, *J* = 8.9 Hz, 1H, H-3), 3.60 (dd, *J* = 10.0, 6.2 Hz, 1H, H-6a′), 3.54 (ddd, *J* = 9.8, 4.1, 1.9 Hz, 1H, H-5), 3.52–3.44 (m, 3H, H-2′, H-3′, H-6b′), 3.40–3.35 (m, 1H, H-5′), 2.58 (d, *J* = 3.2 Hz, 1H, OH), 2.40 (d, *J* = 4.4 Hz, 1H, OH), 2.28 (s, 3H, ArMe); ^{13}C NMR (126 MHz, $CDCl_3$): δ 165.1 (C=O), 138.5 (Ar), 138.3 (2C, Ar), 138.1 (Ar), 137.9 (Ar), 133.4 (Ar), 133.0 (Ar), 130.2 (Ar), 129.9 (Ar), 129.6 (Ar), 128.8 (Ar), 128.6 (Ar), 128.5 (Ar), 128.4 (Ar), 128.3 (Ar), 128.1 (Ar), 128.0 (Ar), 127.8 (Ar), 127.65 (Ar), 127.63 (Ar), 127.6 (Ar), 127.2 (Ar), 102.9 (C-1′), 86.3 (C-1), 81.9 (C-3), 80.1 (C-2′), 79.8 (C-5), 76.6 (C-4), 75.0, 74.5, 73.58 (3C, 3 × OCH_2Ph), 73.56 (C-3′), 73.3 (OCH_2Ph), 72.9 (C-5′), 72.1 (C-2), 69.03 (C-4′),

68.96 (C-6′), 68.4 (C-6), 21.2 (ArMe); HRMS-ESI *m/z* $[M + Na]^+$ calcd for $C_{54}H_{56}NaO_{11}S$: 935.3436, found: 935.3430.



(2′*S*,3′*S*) *p*-Tolyl 2-deoxy-3,4-*O*-(2′,3′-dimethoxybutane-2′,3′-diyl)-6-*O*-levulinyl-2-(2,2,2-trichlorethoxycarbonylamino)-β-D-glucopyranosyl-(1→3)-4-*O*-benzoyl-2,6-di-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2-*O*-benzoyl-3,6-di-*O*-benzyl-1-thio-β-D-glucopyranoside (4.43): To a stirring solution of **4.29** (2.76 g, 4.87 mmol) and trichloroacetonitrile (4.87 mL, 48.66 mmol) in CH_2Cl_2 (30 mL) was added Cs_2CO_3 (15.85 g, 48.66 mmol). After stirring at room temperature for 1 h, the mixture was filtered through Celite and the filtrate was concentrated and dried under high vacuum. The resulting trichloroacetimidate **4.17**, acceptor **4.18** (3.30 g, 3.24 mmol) and powdered 4 Å molecular sieves were suspended in dry CH_2Cl_2 (40 mL) and stirred at room temperature for 1 h. The solution was then cooled to −10 °C, to which TMSOTf (88 μL, 0.49 mmol) was added. The mixture was allowed to warm to room temperature after stirring at −10 °C for 30 min, Et_3N (1 mL) was added and the mixture was filtered through Celite. The filtrate was concentrated and the resulting residue was subjected to flash chromatography (hexane–EtOAc 7:4) to afford **4.43** (4.32 g, 85%) as a white foam. R_f = 0.34 (hexane–EtOAc 3:2); $[\alpha]_D$ = +65.1 (*c* = 0.9, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.04–7.96 (m, 4H, Ar), 7.60–7.54 (m, 1H, Ar), 7.53–7.47 (m, 1H, Ar), 7.46–7.41 (m, 2H, Ar), 7.40–7.17 (m, 19H, Ar), 7.05 (d, *J* = 7.1 Hz, 2H, Ar), 7.01–6.93 (m, 3H, Ar), 6.86–6.79 (m, 2H, Ar), 5.62 (d, *J* = 2.6 Hz, 1H, H-4′), 5.22 (t, *J* = 9.6 Hz, 1H, H-2), 4.94–4.86 (m, 2H, OC*H*$_2$Ph, OC*H*$_2$$CCl_3$),

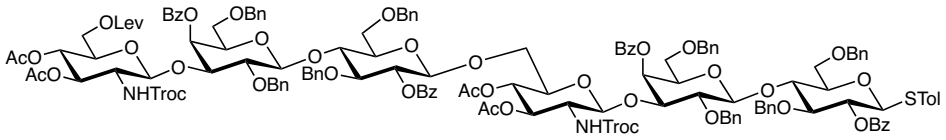
4.83–4.75 (m, 1H, H-1″), 4.73–4.66 (m, 3H, H-1, OC*H*$_2$Ph, OC*H*$_2$CCl$_3$), 4.62 (A of ABq, *J* = 12.0 Hz, 1H, OC*H*$_2$Ph), 4.59 (d, *J* = 7.8 Hz, 1H, H-1′), 4.52 (d, *J* = 11.6 Hz, 1H, H-6a″), 4.49–4.37 (m, 3H, 3 × OC*H*$_2$Ph), 4.32, 4.18 (ABq, *J* = 11.8 Hz, 2H, 2 × OC*H*$_2$Ph), 4.16–4.07 (m, 3H, H-6b″, H-4, N*H*Troc), 3.88–3.80 (m, 2H, H-3′, H-6a), 3.77 (t, *J* = 9.0 Hz, 1H, H-3), 3.75–3.70 (m, 2H, H-6b, H-5′), 3.67 (dd, *J* = 9.6, 7.8 Hz, 1H, H-2′), 3.64–3.56 (m, 3H, H-3″, H-4″, H-5″), 3.46 (dd, *J* = 10.1, 2.7 Hz, 1H, H-5), 3.42 (dd, *J* = 10.1, 6.1 Hz, 1H, H-6a′), 3.34–3.25 (m, 2H, H-2″, H-6b′), 3.20 (s, 3H, OCH$_3$), 3.06 (s, 3H, OCH$_3$), 2.71–2.65 (m, 2H, OC(O)CH$_2$C*H*$_2$C(O)), 2.59–2.51 (m, 2H, OC(O)C*H*$_2$CH$_2$C(O)), 2.26 (s, 3H, ArMe), 2.12 (s, 3H, C(O)C*H*$_3$), 1.24 (s, 3H, CH$_3$), 1.19 (s, 3H, CH$_3$); ^{13}C NMR (126 MHz, CDCl$_3$): δ 206.7 (C=O), 172.6 (C=O), 165.13 (C=O), 165.06 (C=O), 138.6 (Ar), 138.3 (Ar), 138.2 (Ar), 138.05 (Ar), 138.03 (Ar), 133.3 (Ar), 133.0 (Ar), 132.9 (Ar), 130.3 (Ar), 130.1 (Ar), 129.9 (Ar), 129.8 (Ar), 129.5 (Ar), 128.8 (Ar), 128.6 (Ar), 128.4 (Ar), 128.3 (Ar), 128.03 (Ar), 127.97 (Ar), 127.8 (Ar), 127.7 (Ar), 127.5 (Ar), 127.2 (Ar), 126.9 (Ar), 102.4 (C-1′), 101.1 (C-1″), 99.8, 99.7 (2C, 2 × O*C*(OCH$_3$)) 95.6 (CCl$_3$), 86.4 (C-1), 81.6 (C-3), 80.7 (C-2′), 79.7 (C-5), 77.6 (C-3′), 76.2 (C-4), 74.8 (O*C*H$_2$CCl$_3$), 74.2, 74.1, 73.62, 73.55 (4C, 4 × O*C*H$_2$Ph), 73.1 (C-5′), 71.81, 71.78 (3C, C-2, C-3″, C-5″), 70.7 (C-4′), 68.5 (C-6′), 68.0 (C-6), 66.6 (C-4″), 61.9 (C-6″), 55.4 (C-2″), 48.0, 47.9 (2C, 2 × OCH$_3$), 37.8, 27.9 (2C, *C*H$_2$*C*H$_2$), 29.8 (CH$_2$C(O)*C*H$_3$), 21.1 (ArMe), 17.56, 17.55 (2C, 2 × CH$_3$); HRMS-ESI *m/z* [M + Na]$^+$ calcd for $C_{81}H_{88}Cl_3NNaO_{22}S$: 1586.4476, found: 1586.4497.



***p*-Tolyl 3,4-di-*O*-acetyl-2-deoxy-2-(2,2,2-trichlorethoxycarbonylamino)-β-D-glucopyranosyl-(1→3)-4-*O*-benzoyl-2,6-di-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2-*O*-**

benzoyl-3,6-di-*O*-benzyl-1-thio-β-D-glucopyranoside (4.46): A solution of **4.16** (1.00 g, 0.65 mmol) and hydrazine acetate (120 mg, 1.30 mmol) in 4:1 CH_2Cl_2–CH_3OH (10 mL) was stirred at room temperature for 4 h. Then the solution was concentrated and the residue was subjected to flash chromatography (hexane–EtOAc 5:4) to afford **4.46** (0.88 g, 94%) as a white foam. R_f = 0.40 (hexane–EtOAc 1:1); $[\alpha]_D$ = +9.8 (*c* = 0.9, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.06–7.99 (m, 4H, Ar), 7.59–7.53 (m, 2H, Ar), 7.46–7.41 (m, 2H, Ar), 7.41–7.20 (m, 19H, Ar), 7.03–6.93 (m, 5H, Ar), 6.81 (t, *J* = 7.7 Hz, 2H, Ar), 5.80 (d, *J* = 2.1 Hz, 1H, H-4′), 5.23–5.13 (m, 2H, H-2, H-3″), 4.89 (app t, *J* = 9.1 Hz, 1H, H-4″), 4.86–4.75 (m, 5H, H-1″, N*H*Troc, OC*H*$_2$CCl$_3$, OC*H*$_2$Ph), 4.67 (d, *J* = 10.0 Hz, 1H, H-1), 4.65 (A of ABq, *J* = 11.1 Hz, 1H, OC*H*$_2$Ph), 4.59 (d, *J* = 7.5 Hz, 1H, H-1′), 4.51 (A of ABq, *J* = 11.9 Hz, 1H, OC*H*$_2$Ph), 4.42 (m, 2H, 2 × OC*H*$_2$Ph), 4.24, 4.19 (ABq, *J* = 11.4 Hz, 2H, 2 × OC*H*$_2$Ph), 4.07 (app t, *J* = 9.3 Hz, 1H, H-4), 3.92 (A of ABq, *J* = 12.2 Hz, 1H, OC*H*$_2$Ph), 3.80 (dd, *J* = 11.0, 3.7 Hz, 1H, H-6a), 3.77–3.64 (m, 6H, H-3, H-2′, H-3′, H-6b, H-6a″, 6″-OH), 3.61 (t, *J* = 6.3 Hz, 1H, H-5′), 3.56–3.44 (m, 3H, H-5″, H-2″, H-6b″), 3.42 (dd, *J* = 9.8, 2.8 Hz, 1H, H-5), 3.31–3.22 (m, 2H, H-6a′, H-6b′), 2.27 (s, 3H, ArMe), 2.01 (s, 3H, OAc), 1.93 (s, 3H, OAc); ^{13}C NMR (126 MHz, $CDCl_3$): δ 170.5 (C=O), 169.6 (C=O), 166.3 (C=O), 165.0 (C=O), 153.8 (C=O), 138.4 (Ar), 138.3 (Ar), 138.2 (Ar), 138.1 (Ar), 137.8 (Ar), 133.52 (Ar), 133.48 (Ar), 133.0 (Ar), 130.1 (Ar), 129.9 (Ar), 129.8 (Ar), 129.6 (Ar), 129.5 (Ar), 128.55 (Ar), 128.49 (Ar), 128.4 (Ar), 128.3 (Ar), 127.92 (Ar), 127.87 (Ar), 127.83 (Ar), 127.76 (Ar), 127.6 (Ar), 127.5 (Ar), 127.3 (Ar), 127.2 (Ar), 102.4 (C-1′), 101.7 (C-1″), 95.4 (CCl_3), 86.2 (C-1), 81.7, 81.6 (2C, C-3, C-3′), 79.7, 79.1 (2C, C-2′, C-5), 76.4 (C-4), 75.2 (O*C*H$_2$CCl$_3$), 74.7(C-5″), 74.3, 74.0, 73.7, 73.3 (4C, 4 × O*C*H$_2$Ph), 72.5 (C-5′), 72.0, 71.8 (2C, C-2, C-3″), 70.6 (C-4′), 68.4 (C-4″), 68.1, 68.0 (2C, C-6, C-6′), 61.3

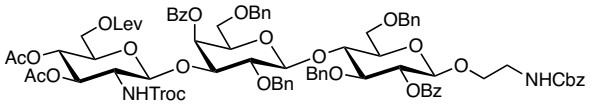
(C-6″), 56.6 (C-2″), 21.1, 20.7, 20.6 (3C, ArMe, 2 × OAc); HRMS-ESI *m/z* $[M + Na]^+$ calcd for $C_{74}H_{76}Cl_3NNaO_{20}S$: 1458.3639, found: 1458.3652.



***p*-Tolyl 3,4-di-*O*-acetyl-2-deoxy-6-*O*-levulinyl-2-(2,2,2-trichlorethoxycarbonylamino)-β-D-glucopyranosyl-(1→3)-4-*O*-benzoyl-2,6-di-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2-*O*-benzoyl-3,6-di-*O*-benzyl-1-thio-β-D-glucopyranosyl-(1→6)-3,4-di-*O*-acetyl-2-deoxy-2-(2,2,2-trichlorethoxycarbonylamino)-β-D-glucopyranosyl-(1→3)-4-*O*-benzoyl-2,6-di-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2-*O*-benzoyl-3,6-di-*O*-benzyl-1-thio-β-D-glucopyranoside (4.47):** To a solution of trisaccharide **4.16** (1.50 g, 0.98 mmol) in 9:1 acetone–water (20 mL) was added NBS (0.52 g, 2.93 mmol) at 0 °C. After stirring at 0 °C for 1 h, saturated aqueous $NaHCO_3$ (2 mL) was added. The solution was concentrated, and then the resulting residue was dissolved in EtOAc (100 mL) and washed with water and brine. The organic layer was dried over Na_2SO_4, filtered, concentrated and and the resulting residue was subjected to flash chromatography (hexane–EtOAc 1:1) to afford a hemiacetal intermediate (1.26 g, 85%). Subsequently, this compound (1.26 g, 0.88 mmol) was dissolved in CH_2Cl_2 (10 mL) and cooled to 0 °C, to which trichloroacetonitrile (0.88 mL, 8.81 mmol) and a catalytic amount of DBU (1 drop) were added. The solution was stirred at room temperature for 1 h. Concentration and flash chromatography of the resulting residue (hexanes–EtOAc 1:1) afforded the corresponding trichloroacetimidate, which was immediately used for the following glycosylation. The obtained trichloroacetimidate, acceptor **4.46** (975 mg, 0.68 mmol) and powdered 4 Å molecular

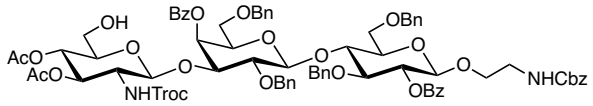
sieves were suspended in CH_2Cl_2 (15 mL) and stirred at room temperature for 1 h. The solution was then cooled to −10 °C, to which TMSOTf (19 μL) was added. The mixture was allowed to warm to room temperature after stirring at −10 °C for 30 min, Et_3N (1 mL) was added and the mixture was filtered through Celite. The filtrate was concentrated and the resulting residue was subjected to flash chromatography (hexane–acetone 2:1) to afford hexasaccharide **4.47** (1.61 g, 83%) as a white foam. R_f = 0.40 (hexane–acetone 3:2); $[\alpha]_D$ = +22.2 (*c* = 0.9, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.05–7.90 (m, 8H, Ar), 7.60–7.55 (m, 1H, Ar), 7.54–7.13 (m, 43H, Ar), 7.08–7.03 (m, 4H, Ar), 7.01–6.93 (m, 4H, Ar), 6.87–6.79 (m, 4H, Ar), 5.67 (br s, 1H, H-4_{Gal}), 5.51 (br s, 1H, H-4_{Gal}), 5.24 (t, *J* = 9.6 Hz, 1H, H-2_{Glc}), 5.21 (dd, *J* = 9.0, 7.8 Hz, 1H, H-2_{Glc}), 4.99–4.82 (m, 6H), 4.82–4.52 (m, 14H), 4.48 (d, *J* = 12.0 Hz, 1H), 4.44 (d, *J* = 11.9 Hz, 1H), 4.42 (d, *J* = 7.3 Hz, 1H), 4.37–4.29 (m, 3H), 4.29–4.10 (m, 9H), 4.03 (t, *J* = 9.3 Hz, 1H, H_{Glc}-4), 3.93–3.84 (m, 3H), 3.83–3.64 (m, 9H), 3.63–3.57 (m, 2H), 3.56–3.42 (m, 6H), 3.42–3.30 (m, 3H), 3.23 (dd, *J* = 10.0, 6.3 Hz, 1H), 2.75–2.68 (m, 1H, OC(O)$CH_2C\mathit{H}_2$C(O)), 2.67–2.60 (m, 1H, OC(O)$CH_2C\mathit{H}_2$C(O)), 2.60–2.47 (m, 2H, OC(O)$C\mathit{H}_2CH_2$C(O)) 2.27 (s, 3H, ArMe), 2.13 (s, 3H, $CH_2C(O)C\mathit{H}_3$), 2.01 (s, 3H, OAc), 1.94 (s, 3H, OAc), 1.91 (s, 3H, OAc), 1.86 (s, 3H, OAc); ^{13}C NMR (126 MHz, $CDCl_3$): δ 206.6, 172.5, 170.4, 170.3, 169.7, 169.4, 165.1, 153.6, 138.4, 138.3, 138.2, 138.1, 138.04, 138.01, 137.97, 133.2, 133.1, 132.9, 132.8, 130.3, 130.2, 130.1, 129.9, 129.79, 129.77, 129.7, 129.6, 129.0, 128.81, 128.79, 128.53, 128.49, 128.39, 128.36, 128.3, 128.2, 128.1, 128.06, 128.0, 127.90, 127.88, 127.8, 127.73, 127.69, 127.6, 127.5, 127.22, 127.17, 127.0, 102.5 (C-1_{Gal}), 102.4 (C-1_{Gal}), 100.9 (C-1_{Glc}), 100.8 (2C, 2 × C-$1_{GlcNTroc}$), 95.5 ($C\mathrm{Cl}_3$), 86.5(C-1_{Glc}), 81.8, 81.2, 80.8, 80.3, 79.7, 77.5, 77.3, 76.5, 76.1, 75.5, 75.1, 75.0, 74.5, 74.2, 74.1, 73.64, 73.61, 73.5, 73.04, 72.95, 71.9, 71.82, 71.77, 70.3, 70.0, 68.6,

68.5, 68.24, 68.16, 67.8, 62.1, 56.1, 56.0, 37.9, 29.8, 27.9, 21.1, 20.7 (2C), 20.6, 20.5; HRMS-ESI *m/z* [M + 2Na]$^{2+}$ calcd for $C_{146}H_{150}Cl_6N_2Na_2O_{42}S$: 1445.3650, found: 1445.3635.



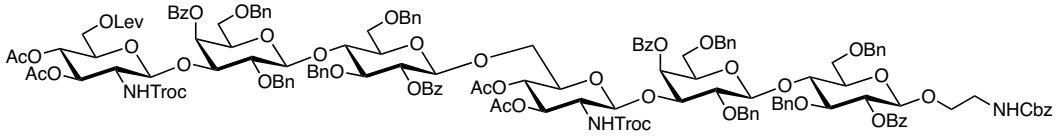
2-(Benzyloxycarbonylamino)ethyl 3,4-di-*O*-acetyl-2-deoxy-6-*O*-levulinyl-2-(2,2,2-trichlorethoxycarbonylamino)-β-D-glucopyranosyl-(1→3)-4-*O*-benzoyl-2,6-di-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2-*O*-benzoyl-3,6-di-*O*-benzyl-β-D-glucopyranoside (4.48): A mixture of trisaccharide **4.16** (1.50 g, 0.98 mmol), *N*-Z-ethanolamine (572 mg, 2.93 mmol) and powdered 4 Å molecular sieves was suspended in dry CH_2Cl_2 (15 mL) and stirred at room temperature for 1 h. The solution was cooled to −10 °C before NIS (439 mg, 1.95 mmol) and AgOTf (75 mg, 0.29 mmol) were added. After stirring for 1 h, while warming to room temperature slowly, TFA (0.80 mL) was added and the mixture was stirred at room temperature for 30 min. Then, Et_3N (2 mL) was added, the mixture was filtered through Celite and the filtrate was concentrated. The resulting residue was subjected to flash chromatography (hexane–acetone 2:1) to afford **4.48** (1.38 g, 88%) as a white foam. R_f = 0.39 (hexane–acetone 3:2); $[\alpha]_D$ = +24.2 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.00 (d, *J* = 7.7 Hz, 2H, Ar), 7.97 (d, *J* = 7.5 Hz, 2H, Ar), 7.55–7.48 (m, 2H, Ar), 7.45–7.40 (m, 2H, Ar), 7.39–7.17 (m, 22H, Ar), 7.09 (d, *J* = 7.3 Hz, 2H, Ar), 7.00 (t, *J* = 7.4 Hz, 1H, Ar), 6.90–6.84 (m, 2H, Ar), 5.66 (s, 1H, H-4′), 5.29 (app t, *J* = 5.4 Hz, 1H, N*H*Cbz), 5.23 (dd, *J* = 9.3, 8.1 Hz, 1H, H-2), 5.01 (A of ABq, *J* = 12.3 Hz, 1H, OC*H*$_2$Ph), 4.97–4.92 (m, 2H, OC*H*$_2$Ph, H-4″), 4.92–4.85 (m, 3H, H-3″,

OCH_2CCl$_3$, OCH_2Ph), 4.76 (d, J = 8.5 Hz, 1H, H-1″), 4.73 (A of ABq, J = 11.2 Hz, 1H, OCH_2Ph), 4.68 (d, J = 11.9 Hz, 1H, OCH_2CCl$_3$), 4.61 (A of ABq, J = 11.4 Hz, 1H, OCH_2Ph), 4.57 (d, J = 7.5 Hz, 1H, H-1′), 4.50 (d, J = 8.1 Hz, 1H, H-1), 4.43–4.36 (m, 2H, NHTroc, OCH_2Ph), 4.34 (A of ABq, J = 11.8 Hz, 1H, OCH_2Ph), 4.29–4.23 (m, 2H, H-6a″, OCH_2Ph), 4.23–4.14 (m, 4H, H-6b″, H-4, 2 × OCH_2Ph), 3.87 (dd, J = 9.6, 3.2 Hz, 1H, H-3′), 3.84–3.80 (m, 2H, H-6a, OCH_2CH$_2$NH), 3.78 (app t, J = 9.0 Hz, 1H, H-3), 3.74–3.67 (m, 3H, H-2′, H-5′, H-6b), 3.67–3.64 (m, 1H, OCH_2CH$_2$NH), 3.63–3.59 (m, 1H, H-5″), 3.52 (app q, J = 9.5 Hz, 1H, H-2″), 3.47 (m, 1H, H-5), 3.44–3.39 (m, 1H, H-6a′), 3.38–3.35 (m, 1H, H-6b′), 3.35–3.29 (m, 2H, CH_2NHCbz), 2.72 (ddd, J = 18.2, 7.4, 6.3 Hz, 1H, OC(O)CH$_2$CH_2C(O)), 2.67–2.61 (m, 1H, OC(O)CH$_2$CH_2C(O)), 2.61–2.46 (m, 2H, OC(O)CH_2CH$_2$C(O)), 2.13 (s, 3H, C(O)CH_3), 2.01 (s, 3H, OAc), 1.91 (s, 3H, OAc); ^{13}C NMR (126 MHz, CDCl$_3$): δ 206.6 (C=O), 172.4 (C=O), 170.4 (C=O), 169.4 (C=O), 165.2 (C=O), 165.1 (C=O), 156.4 (C=O), 153.6 (C=O), 138.4 (Ar), 138.2 (Ar), 138.0 (Ar), 137.96 (Ar), 136.6 (Ar), 133.1 (Ar), 132.9 (Ar), 130.2 (Ar), 129.85 (Ar), 129.79 (Ar), 129.76 (Ar), 128.8 (Ar), 128.5 (Ar), 128.4 (Ar), 128.3 (Ar), 128.1 (Ar), 128.0 (Ar), 127.98 (Ar), 127.9 (Ar), 127.8 (Ar), 127.6 (Ar), 127.3 (Ar), 127.1 (Ar), 102.4 (C-1′), 101.6 (C-1), 100.8 (C-1″), 95.5 (CCl$_3$), 80.7 (C-2′), 80.0 (C-3), 77.6 (C-3′), 76.1 (C-4), 75.3 (C-5), 75.0 (OCH$_2$CCl$_3$), 74.1, 74.0, 73.6, 73.5 (4C, 4 × OCH$_2$Ph), 73.1, 73.0 (2C, C-2, C-5′), 71.84, 71.76 (2C, C-3″, C-5″), 70.3 (C-4′), 69.3 (OCH$_2$CH$_2$NH), 68.6 (C-4″), 68.4 (C-6′), 67.8 (C-6), 66.5 (NHC(O)OCH$_2$Ph), 62.1 (C-6″), 56.2 (C-2″), 41.1 (CH$_2$NHCbz), 37.9, 27.9 (2C, CH$_2$$CH_2$), 29.8 (CH$_2$C(O)$CH_3$), 20.6, 20.5 (2C, 2 × OAc); HRMS-ESI *m/z* [M + Na]$^+$ calcd for C$_{82}$H$_{87}$Cl$_3$N$_2$NaO$_{25}$: 1627.4556, found: 1627.4543.



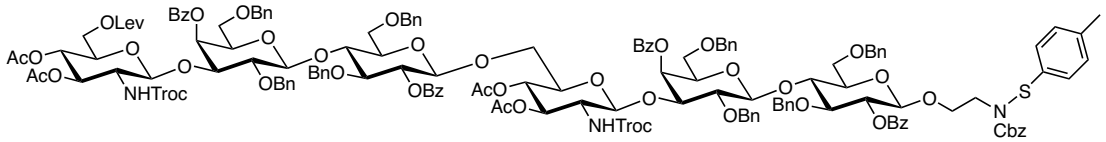
2-(Benzyloxycarbonylamino)ethyl 3,4-di-*O*-acetyl-2-deoxy-2-(2,2,2-trichlorethoxycarbonylamino)-β-D-glucopyranosyl-(1→3)-4-*O*-benzoyl-2,6-di-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2-*O*-benzoyl-3,6-di-*O*-benzyl-β-D-glucopyranoside (4.50): A solution of **4.48** (640 mg, 0.40 mmol) and hydrazine acetate (73 mg, 0.80 mmol) in 4:1 CH_2Cl_2–CH_3OH (10 mL) was stirred at room temperature for 4 h. Then, the solution was concentrated and the resulting residue was subjected to flash chromatography (hexane–EtOAc 1:1) to afford **4.50** (551 mg, 92%) as a white foam. R_f = 0.44 (hexane–EtOAc 4:5); $[\alpha]_D$ = +16.9 (*c* = 1.2, $CHCl_3$); ^{1}H NMR (700 MHz, $CDCl_3$): δ 8.07–8.03 (m, 2H, Ar), 7.96 (d, *J* = 7.6 Hz, 2H, Ar), 7.58–7.54 (m, 1H, Ar), 7.52 (t, *J* = 7.3 Hz, 1H, Ar), 7.42–7.19 (m, 24H, Ar), 7.05 (d, *J* = 7.2 Hz, 2H, Ar), 6.99 (t, *J* = 7.4 Hz, 1H, Ar), 6.85 (t, *J* = 7.6 Hz, 2H, Ar), 5.80 (s, 1H, H-4′), 5.33– 5.27 (m, 1H, N*H*Cbz), 5.24–5.13 (m, 2H, H-2, H-3″), 5.01, 4.93 (ABq, *J* = 12.3 Hz, 2H, OCH_2CCl$_3$), 4.91–4.87 (m, 2H, H-4″, N*H*Troc), 4.85, 4.69 (ABq, *J* = 11.2 Hz, 2H, 2 × OCH_2Ph), 4.83–4.79 (m, 2H, H-1″, OCH_2Ph), 4.76 (A of ABq, *J* = 11.3 Hz, 1H, OCH_2Ph), 4.55 (d, *J* = 7.2 Hz, 1H, H-1′), 4.52, 4.33 (ABq, *J* = 11.7 Hz, 2H, 2 × OCH_2Ph), 4.48 (d, *J* = 7.9 Hz, 1H, H-1), 4.42, 3.92 (ABq, *J* = 11.7 Hz, 2H, 2 × OCH_2Ph), 4.29, 4.22 (ABq, *J* = 11.5 Hz, 2H, 2 × OCH_2Ph), 4.12 (app t, *J* = 9.0 Hz, 1H, H-4), 3.83–3.75 (m, 2H, OCH_2CH$_2$NH, H-6a), 3.75–3.67 (m, 5H, H-3, H-2′, H-3′, H-6a″, 6″-OH), 3.66–3.58 (m, 3H, OCH_2CH$_2$NH, H-6b, H-5′), 3.58–3.44 (m, 3H, H-5″, H-6b″, H-2″), 3.42–3.36 (m, 1H, H-5), 3.36–3.26 (m, 4H, OCH$_2$CH_2NH, H-6a′, H-6b′), 2.02 (s, 3H, OAc), 1.93 (s, 3H, OAc); ^{13}C NMR (126 MHz, $CDCl_3$): δ 170.5 (C=O), 169.6 (C=O), 166.3 (C=O), 165.2 (C=O), 156.4 (C=O), 153.8 (C=O), 138.4 (Ar), 138.2

(Ar), 138.1 (Ar), 137.8 (Ar), 136.6 (Ar), 133.5 (Ar), 133.1 (Ar), 129.9 (Ar), 129.8 (Ar), 129.7 (Ar), 129.6 (Ar), 128.6 (Ar), 128.5 (Ar), 128.44 (2C, Ar), 128.36 (2C, Ar), 128.02 (Ar), 127.98 (Ar), 127.89 (Ar), 127.87 (Ar), 127.8 (Ar), 127.73 (Ar), 127.68 (Ar), 127.6 (Ar), 127.3 (Ar), 127.2 (Ar), 102.3 (C-1′), 101.8 (C-1″), 101.5 (C-1), 95.4 (CCl_3), 81.7, 80.0, 79.0 (3C, C-2′, C-3, C-3′), 76.3 (C-4), 75.2 (C-5), 75.1 (OCH_2CCl_3), 74.7 (C-5″), 74.0 (2C), 73.7, 73.2 (4C, 4 × OCH_2Ph), 73.0, 72.5, 72.0 (3C, C-2, C-3″, C-5′), 70.6 (C-4′), 69.3 (OCH_2CH_2NH), 68.4 (C-4″), 68.1, 67.8 (2C, C-6′, C-6), 66.5 ($NHC(O)OCH_2Ph$), 61.3 (C-6″), 56.6 (C-2″), 41.1(OCH_2CH_2NH), 20.7, 20.6 (2C, 2 × OAc); HRMS-ESI *m/z* $[M + Na]^+$ calcd for $C_{77}H_{81}Cl_3N_2NaO_{23}$: 1529.4188, found: 1529.4195.



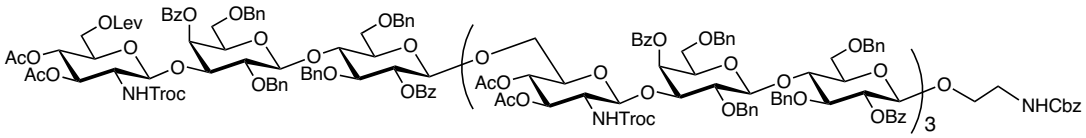
2-(Benzyloxycarbonylamino)ethyl 3,4-di-*O*-acetyl-2-deoxy-6-*O*-levulinyl-2-(2,2,2-trichlorethoxycarbonylamino)-β-D-glucopyranosyl-(1→3)-4-*O*-benzoyl-2,6-di-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2-*O*-benzoyl-3,6-di-*O*-benzyl-β-D-glucopyranosyl-(1→6)-3,4-di-*O*-acetyl-2-deoxy-2-(2,2,2-trichlorethoxycarbonylamino)-β-D-glucopyranosyl-(1→3)-4-*O*-benzoyl-2,6-di-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2-*O*-benzoyl-3,6-di-*O*-benzyl-β-D-glucopyranoside (4.51): The glycosylation was performed following general procedure A described above, with acceptor **4.50** (0.92 g, 0.61 mmol), donor **4.16** (1.03 g, 0.67 mmol), NIS (302 mg, 1.34 mmol) and AgOTf (31 mg, 0.12 mmol) in dry CH_2Cl_2 (20 mL). The resulting crude residue was purified by chromatography (hexane–acetone 3:2) to afford hexasaccharide **4.51** (1.65 g, 93%) as a white foam. R_f = 0.28 (hexane–acetone 3:2); $[\alpha]_D$ =

−13.1 (*c* = 0.8, $CHCl_3$); ^{1}H NMR (700 MHz, $CDCl_3$): δ 8.05–7.92 (m, 8H, Ar), 7.56–7.46 (m, 4H, Ar), 7.45–7.14 (m, 43H, Ar), 7.11–7.04 (m, 4H, Ar), 7.02–6.96 (m, 2H, Ar), 6.89–6.81 (m, 4H, Ar), 5.68 (s, 1H, H-4_{Gal}), 5.52 (s, 1H, H-4_{Gal}), 5.36–5.31 (m, 1H, N*H*Cbz), 5.26–5.20 (m, 2H, 2 × H-2_{Glc}), 5.02 (d, *J* = 12.3 Hz, 1H), 4.99–4.84 (m, 7H), 4.81 (t, *J* = 9.6 Hz, 1H), 4.79–4.54 (m, 11H), 4.53–4.47 (m, 2H), 4.44–4.31 (m, 5H), 4.30–4.12 (m, 9H), 4.08 (t, *J* = 8.9 Hz, 1H), 3.94–3.86 (m, 3H), 3.86–3.64 (m, 12H), 3.64–3.43 (m, 8H), 3.43–3.31 (m, 5H), 3.28 (dd, *J* = 9.9, 6.4 Hz, 1H), 2.72 (dt, *J* = 18.2, 6.8 Hz, 1H, OC(O)CH_2C*H_2*C(O)), 2.64 (dt, *J* = 18.2, 6.2 Hz, 1H, OC(O)CH_2C*H_2*C(O)), 2.62–2.48 (m, 2H, OC(O)C*H_2*CH_2C(O)), 2.14 (s, 3H, CH_2C(O)C*H_3*), 2.02 (s, 3H, OAc), 1.96 (s, 3H, OAc), 1.92 (s, 3H, OAc), 1.87 (s, 3H, OAc); ^{13}C NMR (126 MHz, $CDCl_3$): δ 206.6, 172.5, 170.4, 170.3, 169.8, 169.4, 165.3, 165.1, 156.4, 153.6, 153.5, 138.4, 138.3, 138.2, 138.05, 137.96, 137.8, 136.7, 133.1, 133.0, 132.9 (2C), 130.24, 130.22, 130.1, 129.8, 129.8, 128.81, 128.79, 128.53, 128.47, 128.4, 128.3, 128.2, 128.1, 128.02, 127.99, 127.96, 127.93, 127.91, 127.90, 127.85, 127.79, 127.74, 127.67, 127.5, 127.47, 127.3, 127.23, 127.16, 127.0, 102.4 (2 × C-1_{Gal}), 101.6 (C-1_{Glc}), 100.8 (3C, C-1_{Glc}, 2 × C-$1_{GlcNTroc}$), 95.51, 95.47, 81.1, 80.8, 80.3, 80.1, 77.5, 77.3, 76.5, 76.4, 76.1, 75.5, 75.3, 75.1, 74.9, 74.1, 73.6, 73.5, 73.05, 73.0, 72.96, 72.8, 71.9, 71.80, 71.77, 70.3, 70.2, 70.0, 69.4, 68.6, 68.5, 68.4, 68.2, 67.8, 67.7, 66.5, 62.1, 56.1, 56.0, 41.2, 37.9, 29.8, 27.9, 20.7 (2C), 20.6, 20.5; HRMS-ESI *m/z* $[M + 2Na]^{2+}$ calcd for $C_{149}H_{155}Cl_6N_3Na_2O_{45}$: 1480.8924, found: 1480.8967.



***N*-Benzyloxycarbonyl-*N*-p-tolylthio-2-aminoethyl 3,4-di-*O*-acetyl-2-deoxy-6-*O*-levulinyl-2-(2,2,2-trichlorethoxycarbonylamino)-β-D-glucopyranosyl-(1→3)-4-*O*-benzoyl-2,6-di-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2-*O*-benzoyl-3,6-di-*O*-benzyl-β-D-glucopyranosyl-(1→6)-3,4-di-*O*-acetyl-2-deoxy-2-(2,2,2-trichlorethoxycarbonylamino)-β-D-glucopyranosyl-(1→3)-4-*O*-benzoyl-2,6-di-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2-*O*-benzoyl-3,6-di-*O*-benzyl-β-D-glucopyranoside (4.52):** This is a byproduct formed in the synthesis of **4.51**. ^{1}H NMR (700 MHz, $CDCl_3$): δ 8.06–7.90 (m, 8H, Ar), 7.56–7.46 (m, 4H, Ar), 7.45–7.14 (m, 44H, Ar), 7.11–7.05 (m, 4H, Ar), 7.05–7.01 (m, 3H, Ar), 6.99 (t, *J* = 7.1 Hz, 2H, Ar), 6.88–6.82 (m, 4H, Ar), 5.68 (s, 1H, H-4$_{Gal}$), 5.53 (s, 1H, H-4$_{Gal}$), 5.26 (t, *J* = 8.7 Hz, 1H, H-2$_{Glc}$), 5.23 (t, *J* = 8.7 Hz, 1H, H-2$_{Glc}$), 5.07–4.99 (m, 2H), 4.99–4.84 (m, 6H), 4.81 (t, *J* = 9.6 Hz, 1H), 4.79–4.54 (m, 11H), 4.50 (d, *J* = 11.9 Hz, 2H), 4.44 (d, *J* = 7.4 Hz, 1H), 4.41 (d, *J* = 12.2 Hz, 1H), 4.38–4.31 (m, 3H), 4.29–4.08 (m, 10H), 3.99–3.87 (m, 4H), 3.83–3.68 (m, 11H), 3.66 (d, *J* = 10.5 Hz, 1H), 3.63–3.58 (m, 2H), 3.57–3.43 (m, 5H), 3.43–3.33 (m, 4H), 3.28 (dd, *J* = 10.0, 6.2 Hz, 1H), 2.73 (ddd, *J* = 18.1, 10.6, 6.8 Hz, 1H, OC(O)CH_2CH_2C(O)), 2.64 (dt, *J* = 18.2, 6.3 Hz, 1H, OC(O)CH_2CH_2C(O)), 2.62–2.48 (m, 2H, OC(O)C$H_2$$CH_2$C(O)), 2.30 (s, 3H, ArMe), 2.14 (s, 3H, CH_2C(O)CH_3), 2.02 (s, 3H, OAc), 1.96 (s, 3H, OAc), 1.92 (s, 3H, OAc), 1.87 (s, 3H, OAc); ^{13}C NMR (126 MHz, $CDCl_3$): δ 206.6, 172.5, 170.4, 170.3, 169.8, 169.4, 165.11, 165.09, 157.6, 153.6, 138.4, 138.3, 138.2, 138.03, 137.97, 137.4, 136.0, 134.4, 133.0, 132.9, 130.24, 130.19, 130.1, 129.9, 129.8, 128.81, 128.79, 128.53, 128.47, 128.4, 128.33, 128.28, 128.2, 128.1, 128.04, 127.97, 127.9,

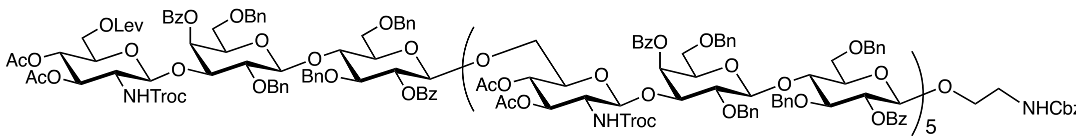
127.79, 127.76, 127.73, 127.70, 127.55, 127.47, 127.22, 127.20, 127.0, 102.37, 102.35, 101.2, 100.9, 95.52, 95.46, 81.2, 80.8, 80.3, 80.2, 77.5, 77.3, 76.6, 76.3, 76.1, 75.51, 75.45, 75.1, 74.9, 74.2, 74.1, 73.6, 73.5, 73.0, 72.9, 72.84, 72.78, 71.9, 71.81, 71.76, 70.3, 70.2, 70.0, 68.6, 68.53, 68.49, 68.3, 68.2, 67.8, 67.7, 66.7, 62.1, 56.1, 56.0, 53.0, 37.9, 29.8, 27.9, 21.1, 20.7 (2C), 20.6, 20.5; MALDI-TOF MS *m/z* [M + Na]$^+$ calcd for $C_{156}H_{161}Cl_6N_3NaO_{45}S$: 3060.8, found: 3060.6



2-(Benzyloxycarbonylamino)ethyl 3,4-di-*O*-acetyl-2-deoxy-6-*O*-levulinyl-2-(2,2,2-trichlorethoxycarbonylamino)-β-D-glucopyranosyl-(1→3)-4-*O*-benzoyl-2,6-di-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2-*O*-benzoyl-3,6-di-*O*-benzyl-β-D-glucopyranosyl-(1→6)-3,4-di-*O*-acetyl-2-deoxy-2-(2,2,2-trichlorethoxycarbonylamino)-β-D-glucopyranosyl-(1→3)-4-*O*-benzoyl-2,6-di-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2-*O*-benzoyl-3,6-di-*O*-benzyl-β-D-glucopyranosyl-(1→6)-3,4-di-*O*-acetyl-2-deoxy-2-(2,2,2-trichlorethoxycarbonylamino)-β-D-glucopyranosyl-(1→3)-4-*O*-benzoyl-2,6-di-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2-*O*-benzoyl-3,6-di-*O*-benzyl-β-D-glucopyranoside (4.54): A solution of **4.51** (944 mg, 0.32 mmol) and hydrazine acetate (60 mg, 0.65 mmol) in 4:1 CH_2Cl_2–CH_3OH (10 mL) was stirred at room temperature for 4 h.

Then the solution was concentrated and the resulting residue was subjected to flash chromatography (hexane–EtOAc 4:5) to afford **4.53** (896 mg, 98%). Subsequently, glycosylation was performed following general procedure A described above, with acceptor **4.53** (700 mg, 0.25 mmol), donor **4.47** (797 mg, 0.28 mmol), NIS (138 mg, 0.61 mmol) and AgOTf (16 mg, 0.06 mmol) in dry CH_2Cl_2 (15 mL). The resulting crude residue was purified by chromatography (hexane–acetone 3:2) to afford dodecasaccharide **4.54** (1.23 g, 90%) as a white foam. R_f = 0.38 (hexane–acetone 5:4); $[\alpha]_D$ = +20.9 (c = 1.0, $CHCl_3$); ^{1}H NMR (700 MHz, $CDCl_3$): δ 8.03–7.90 (m, 16H, Ar), 7.55–7.44 (m, 8H, Ar), 7.43–7.12 (m, 81H, Ar), 7.10–7.02 (m, 8H, Ar), 7.00–6.93 (m, 4H, Ar), 6.88–6.79 (m, 8H, Ar), 5.67 (s, 1H, H-4$_{Gal}$), 5.57–5.48 (m, 3H, 3 × H-4 $_{Gal}$), 5.35–5.30 (m, 1H, N*H*Cbz), 5.26–5.19 (m, 4H, 4 × H-2$_{Glc}$), 5.02 (d, J = 12.3 Hz, 1H), 4.98–4.84 (m, 11H), 4.84–4.78 (m, 3H), 4.78–4.41 (m, 30H), 4.38 (d, J = 6.7 Hz, 1H), 4.37–4.31 (m, 6H), 4.30–4.04 (m, 19H), 3.94–3.87 (m, 5H), 3.85–3.64 (m, 22H), 3.63–3.42 (m, 18H), 3.41–3.31 (m, 7H), 3.30–3.24 (m, 3H), 2.74–2.69 (m, 1H, OC(O)CH_2CH_2C(O)), 2.64 (dt, J = 18.2, 6.3 Hz, 1H, OC(O)CH_2CH_2C(O)), 2.61–2.48 (m, 2H, OC(O)CH_2CH_2C(O)), 2.13 (s, 3H, CH_2C(O)C*H*$_3$), 2.01 (s, 3H, OAc), 1.968 (s, 3H, OAc), 1.966 (s, 3H, OAc), 1.96 (s, 3H, OAc), 1.91 (s, 3H, OAc), 1.87 (s, 3H, OAc), 1.859 (s, 3H, OAc), 1.855 (s, 3H, OAc); ^{13}C NMR (126 MHz, $CDCl_3$): δ 206.6, 172.4, 170.4, 170.3, 169.7, 169.4, 165.2, 165.1, 156.4, 153.5, 138.3, 138.2, 138.02, 137.99, 137.9, 137.7, 136.6, 133.1, 133.0, 132.8, 130.2, 130.1, 129.7, 128.8, 128.49, 128.47, 128.45, 128.43, 128.34, 128.30, 128.29, 128.2, 128.1, 127.99, 127.96, 127.93, 127.88, 127.85, 127.8, 127.7, 127.6, 127.5, 127.4, 127.24, 127.19, 127.0, 102.4, 102.33, 102.29, 101.5, 100.8, 95.5, 81.2, 81.1, 80.8, 80.4, 80.3, 80.0, 77.5, 77.31, 77.26, 77.1, 76.8, 76.5, 76.4, 76.20, 76.17, 76.0, 75.5, 75.4, 75.3, 75.1, 74.9, 74.33, 74.30,

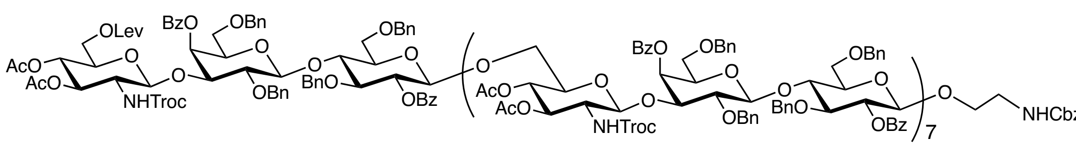
74.14, 74.10, 73.60, 73.57, 73.5, 73.05, 72.97, 72.9, 72.8, 72.7, 71.84, 71.76, 71.7, 70.3, 70.2, 70.1, 70.0, 69.4, 68.6, 68.52, 68.46, 68.3, 68.2, 67.8, 67.7, 67.6, 66.5, 62.1, 56.1, 55.9, 41.1, 37.8, 29.8, 27.9, 20.6, 20.52, 20.51; HRMS-ESI *m/z* $[M + 2Na]^{2+}$ calcd for $C_{283}H_{291}Cl_{12}N_5Na_2O_{85}$: 2794.7341, found: 2794.7331.



2-(Benzyloxycarbonylamino)ethyl 3,4-di-*O*-acetyl-2-deoxy-6-*O*-levulinyl-2-(2,2,2-trichlorethoxycarbonylamino)-β-D-glucopyranosyl-(1→3)-4-*O*-benzoyl-2,6-di-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2-*O*-benzoyl-3,6-di-*O*-benzyl-β-D-glucopyranosyl-(1→6)-3,4-di-*O*-acetyl-2-deoxy-2-(2,2,2-trichlorethoxycarbonylamino)-β-D-glucopyranosyl-(1→3)-4-*O*-benzoyl-2,6-di-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2-*O*-benzoyl-3,6-di-*O*-benzyl-β-D-glucopyranosyl-(1→6)-3,4-di-*O*-acetyl-2-deoxy-2-(2,2,2-trichlorethoxycarbonylamino)-β-D-glucopyranosyl-(1→3)-4-*O*-benzoyl-2,6-di-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2-*O*-benzoyl-3,6-di-*O*-benzyl-β-D-glucopyranosyl-(1→6)-3,4-di-*O*-acetyl-2-deoxy-2-(2,2,2-trichlorethoxycarbonylamino)-β-D-glucopyranosyl-(1→3)-4-*O*-benzoyl-2,6-di-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2-*O*-benzoyl-3,6-di-*O*-benzyl-β-D-glucopyranosyl-(1→6)-3,4-di-*O*-acetyl-2-deoxy-2-(2,2,2-trichlorethoxycarbonylamino)-β-D-glucopyranosyl-(1→3)-4-*O*-benzoyl-2,6-di-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2-*O*-benzoyl-3,6-di-*O*-benzyl-β-D-

glucopyranosyl-(1→6)-3,4-di-*O*-acetyl-2-deoxy-2-(2,2,2-trichlorethoxycarbonylamino)-β-D-glucopyranosyl-(1→3)-4-*O*-benzoyl-2,6-di-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2-*O*-benzoyl-3,6-di-*O*-benzyl-β-D-glucopyranoside (4.56): A solution of **4.54** (1.27 g, 0.23 mmol) and hydrazine acetate (42 mg, 0.46 mmol) in 4:1 CH_2Cl_2–CH_3OH (10 mL) was stirred at room temperature for 4 h. Then the solution was concentrated and the resulting residue was subjected to flash chromatography (hexane–EtOAc 4:5) to afford **4.55** (1.23 g, 98%). Subsequently, glycosylation was performed following general procedure A described above, with acceptor **4.55** (1.24 g, 0.23 mmol), donor **4.47** (744 mg, 0.26 mmol), NIS (128 mg, 0.57 mmol) and AgOTf (15 mg, 0.06 mmol) in dry CH_2Cl_2 (20 mL). The resulting crude residue was purified by chromatography (hexane–acetone 5:4) to afford octadecasaccharide **4.56** (1.67 g, 90%) as a white foam. R_f = 0.36 (hexane–acetone 1:1); $[\alpha]_D$ = +20.0 (*c* = 1.1, $CHCl_3$); ^{1}H NMR (700 MHz, $CDCl_3$): δ 8.04–7.91 (m, 24H, Ar), 7.56–7.45 (m, 12H, Ar), 7.45–7.19 (m, 113H), 7.19–7.13 (m, 6H, Ar), 7.12–7.03 (m, 12H, Ar), 7.02–6.95 (m, 6H, Ar), 6.89–6.80 (m, 12H, Ar), 5.69 (s, 1H, H-4_{Gal}), 5.63–5.49 (m, 5H, 5 × H-4_{Gal}), 5.36–5.33 (m, 1H, N*H*Cbz), 5.28–5.19 (m, 6H, 6 × H-2_{Glc}), 5.03 (d, *J* = 12.3 Hz, 1H), 5.00–4.86 (m, 14H), 4.86–4.80 (m, 5H), 4.80–4.43 (m, 47H), 4.42–4.32 (m, 9H), 4.30–4.06 (m, 29H), 3.97–3.89 (m, 7H), 3.88–3.65 (m, 32H), 3.65–3.43 (m, 28H), 3.43–3.32 (m, 9H), 3.32–3.25 (m, 5H), 2.76–2.70 (m, 1H, OC(O)CH_2CH_2C(O)), 2.68–2.62 (m, 1H, OC(O)CH_2CH_2C(O)), 2.62–2.49 (m, 2H, OC(O)CH_2CH_2C(O)), 2.15 (s, 3H, CH_2C(O)CH_3), 2.06 (s, 3H, OAc), 2.02 (s, 3H, OAc), 1.99 (s, 6H, 2 × OAc), 1.98 (s, 3H, OAc), 1.97 (s, 3H, OAc), 1.93 (s, 3H, OAc), 1.883 (s, 3H, OAc), 1.875 (s, 9H, 3 × OAc), 1.87 (s, 3H, OAc); ^{13}C NMR (126 MHz, $CDCl_3$): δ 206.5, 172.4, 170.33, 170.26, 169.7, 169.3, 165.2, 165.1, 156.4, 153.5, 138.3,

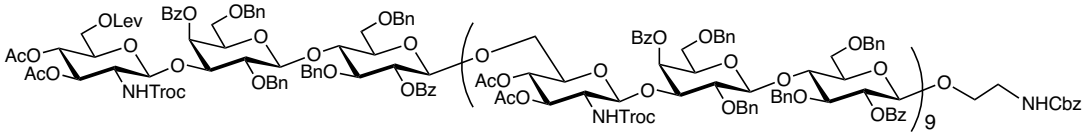
138.22, 138.21, 138.0, 137.9, 137.7, 136.6, 133.0, 132.8, 130.2, 130.0, 129.7, 128.7, 128.4, 128.4, 128.31, 128.26, 128.2, 128.1, 128.0, 127.93, 127.91, 127.86, 127.8, 127.74, 127.66, 127.6, 127.5, 127.4, 127.21, 127.16, 126.9, 102.34, 102.30, 102.27, 101.5, 100.8, 95.5, 81.2, 81.1, 80.8, 80.4, 80.3, 80.0, 77.5, 77.3, 76.5, 76.4, 76.1, 76.0, 75.5, 75.4, 75.3, 75.0, 74.9, 74.3, 74.12, 74.07, 73.6, 73.58, 73.55, 73.5, 73.0, 72.94, 72.88, 72.7, 71.7, 70.2, 70.1, 70.0, 69.4, 68.6, 68.5, 68.3, 68.2, 67.74, 67.69, 67.6, 66.5, 62.1, 56.1, 55.9, 53.4, 41.1, 37.8, 29.7, 27.9, 20.6, 20.5; ESI-MS *m/z* $[M + 3Na]^{3+}$ for $C_{417}H_{427}Cl_{18}N_7Na_3O_{125}$: found: 2748.0378 (M=8175.1). The observed isotope peak is consistent with theoretical isotope peak 8175.2.



2-(Benzyloxycarbonylamino)ethyl 3,4-di-*O*-acetyl-2-deoxy-6-*O*-levulinyl-2-(2,2,2-trichlorethoxycarbonylamino)-β-D-glucopyranosyl-(1→3)-4-*O*-benzoyl-2,6-di-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2-*O*-benzoyl-3,6-di-*O*-benzyl-β-D-glucopyranosyl-(1→6)-3,4-di-*O*-acetyl-2-deoxy-2-(2,2,2-trichlorethoxycarbonylamino)-β-D-glucopyranosyl-(1→3)-4-*O*-benzoyl-2,6-di-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2-*O*-benzoyl-3,6-di-*O*-benzyl-β-D-glucopyranosyl-(1→6)-3,4-di-*O*-acetyl-2-deoxy-2-(2,2,2-trichlorethoxycarbonylamino)-β-D-glucopyranosyl-(1→3)-4-*O*-benzoyl-2,6-di-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2-*O*-benzoyl-3,6-di-*O*-benzyl-β-D-glucopyranosyl-(1→6)-3,4-di-*O*-acetyl-2-deoxy-2-(2,2,2-trichlorethoxycarbonylamino)-β-D-glucopyranosyl-(1→3)-4-*O*-benzoyl-2,6-di-*O*-

benzyl-β-D-galactopyranosyl-(1→4)-2-*O*-benzoyl-3,6-di-*O*-benzyl-β-D-glucopyranosyl-(1→6)-3,4-di-*O*-acetyl-2-deoxy-2-(2,2,2-trichlorethoxycarbonylamino)-β-D-glucopyranosyl-(1→3)-4-*O*-benzoyl-2,6-di-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2-*O*-benzoyl-3,6-di-*O*-benzyl-β-D-glucopyranosyl-(1→6)-3,4-di-*O*-acetyl-2-deoxy-2-(2,2,2-trichlorethoxycarbonylamino)-β-D-glucopyranosyl-(1→3)-4-*O*-benzoyl-2,6-di-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2-*O*-benzoyl-3,6-di-*O*-benzyl-β-D-glucopyranosyl-(1→6)-3,4-di-*O*-acetyl-2-deoxy-2-(2,2,2-trichlorethoxycarbonylamino)-β-D-glucopyranosyl-(1→3)-4-*O*-benzoyl-2,6-di-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2-*O*-benzoyl-3,6-di-*O*-benzyl-β-D-glucopyranosyl-(1→6)-3,4-di-*O*-acetyl-2-deoxy-2-(2,2,2-trichlorethoxycarbonylamino)-β-D-glucopyranosyl-(1→3)-4-*O*-benzoyl-2,6-di-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2-*O*-benzoyl-3,6-di-*O*-benzyl-β-D-glucopyranoside (4.58): A solution of **4.56** (1.57 g, 0.19 mmol) and hydrazine acetate (36 mg, 0.39 mmol) in 4:1 CH_2Cl_2–CH_3OH (15 mL) was stirred at room temperature for 4 h. Then the solution was concentrated and the resulting residue was subjected to flash chromatography (CH_2Cl_2–MeOH 75:1) to afford **4.57** (1.42 g, 92%). Subsequently, glycosylation was performed following procedure A described above, with acceptor **4.57** (980 mg, 0.12 mmol), donor **4.47** (415 mg, 0.15 mmol), NIS (68 mg, 0.30 mmol) and AgOTf (6 mg, 0.02 mmol) in dry CH_2Cl_2 (15 mL). The resulting crude residue was purified by chromatography (hexane–acetone 1:1) to afford tetracosasaccharide **4.58** (1.17 g, 89%) as a white foam. R_f = 0.29 (hexane–acetone 1:1); $[\alpha]_D$ = +20.6 (c = 1.2, $CHCl_3$); ^{1}H NMR

(700 MHz, $CDCl_3$): δ 8.11–7.92 (m, 32H, Ar), 7.57–7.48 (m, 16H, Ar), 7.47–7.21 (m, 149H, Ar), 7.21–7.16 (m, 8H, Ar), 7.15–7.06 (m, 16H, Ar), 7.05–6.96 (m, 8H, Ar), 6.93–6.81 (m, 16H, Ar), 5.72 (s, 1H, H-4_{Gal}), 5.67–5.53 (m, 7H, 7 × H-4_{Gal}), 5.42–5.36 (m, 1H, N*H*Cbz), 5.32–5.21 (m, 8H, 8 × H-2_{Glc}), 5.05 (d, *J* = 12.3 Hz, 1H), 5.03–4.89 (m, 19H), 4.89–4.83 (m, 7H), 4.83–4.46 (m, 63H), 4.44 (d, *J* = 4.9 Hz, 2H), 4.42–4.35 (m, 9H), 4.34–4.08 (m, 37H), 4.03–3.91 (m, 10H), 3.91–3.68 (m, 42H), 3.68–3.46 (m, 38H), 3.46–3.35 (m, 11H), 3.35–3.25 (m, 7H), 2.78–2.71 (m, 1H, OC(O)CH_2C*H_2*C(O)), 2.67 (dt, *J* = 18.1, 6.2 Hz, 1H, OC(O)CH_2C*H_2*C(O)), 2.64–2.51 (m, 2H, OC(O)C*H_2*CH_2C(O)), 2.16 (s, 3H, $CH_2C(O)C$*H_3*), 2.04 (s, 3H, OAc), 2.03–2.00 (m, 18H, 6 × OAc), 2.00 (s, 3H, OAc), 1.94 (s, 3H, OAc), 1.903 (s, 3H, OAc), 1.896 (s, 15H, 5 × OAc), 1.89 (s, 3H, OAc); ^{13}C NMR (126 MHz, $CDCl_3$): δ 206.5, 172.3, 170.3, 170.2, 169.6, 169.3, 165.2, 165.0, 156.3, 153.4, 138.3, 138.18, 138.16, 137.9, 137.7, 136.6, 132.9, 132.7, 130.1, 130.0, 129.6, 128.7, 128.4, 128.2, 128.1, 128.0, 127.88, 127.86, 127.80, 127.78, 127.7, 127.60, 127.55, 127.44, 127.35, 127.2, 127.1, 126.9, 102.2, 101.5, 100.7, 95.4, 81.1, 81.0, 80.7, 80.3, 80.2, 80.0, 77.4, 77.3, 76.5, 76.3, 76.1, 76.0, 75.4, 75.2, 75.0, 74.8, 74.2, 74.0, 73.6, 73.52, 73.49, 73.4, 73.0, 72.9, 72.8, 72.7, 71.7, 70.1, 70.0, 69.9, 69.3, 68.6, 68.4, 68.3, 68.1, 67.7, 67.6, 67.5, 66.4, 62.0, 56.0, 55.8, 41.0, 37.8, 29.7, 27.8, 20.6, 20.4; MS-ESI *m/z* $[M + 4Na]^{4+}$ calcd for $C_{551}H_{563}Cl_{24}N_9Na_4O_{165}$: 2722.2022, found: 2722.1911 (isotope peak).



2-(Benzyloxycarbonylamino)ethyl 3,4-di-*O*-acetyl-2-deoxy-6-*O*-levulinyl-2-(2,2,2-trichlorethoxycarbonylamino)-β-D-glucopyranosyl-(1→3)-4-*O*-benzoyl-2,6-di-*O*-

benzyl-β-D-galactopyranosyl-(1→4)-2-*O*-benzoyl-3,6-di-*O*-benzyl-β-D-glucopyranosyl-(1→6)-3,4-di-*O*-acetyl-2-deoxy-2-(2,2,2-trichlorethoxycarbonylamino)-β-D-glucopyranosyl-(1→3)-4-*O*-benzoyl-2,6-di-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2-*O*-benzoyl-3,6-di-*O*-benzyl-β-D-glucopyranosyl-(1→6)-3,4-di-*O*-acetyl-2-deoxy-2-(2,2,2-trichlorethoxycarbonylamino)-β-D-glucopyranosyl-(1→3)-4-*O*-benzoyl-2,6-di-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2-*O*-benzoyl-3,6-di-*O*-benzyl-β-D-glucopyranosyl-(1→6)-3,4-di-*O*-acetyl-2-deoxy-2-(2,2,2-trichlorethoxycarbonylamino)-β-D-glucopyranosyl-(1→3)-4-*O*-benzoyl-2,6-di-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2-*O*-benzoyl-3,6-di-*O*-benzyl-β-D-glucopyranosyl-(1→6)-3,4-di-*O*-acetyl-2-deoxy-2-(2,2,2-trichlorethoxycarbonylamino)-β-D-glucopyranosyl-(1→3)-4-*O*-benzoyl-2,6-di-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2-*O*-benzoyl-3,6-di-*O*-benzyl-β-D-glucopyranosyl-(1→6)-3,4-di-*O*-acetyl-2-deoxy-2-(2,2,2-trichlorethoxycarbonylamino)-β-D-glucopyranosyl-(1→3)-4-*O*-benzoyl-2,6-di-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2-*O*-benzoyl-3,6-di-*O*-benzyl-β-D-glucopyranosyl-(1→6)-3,4-di-*O*-acetyl-2-deoxy-2-(2,2,2-trichlorethoxycarbonylamino)-β-D-glucopyranosyl-(1→3)-4-*O*-benzoyl-2,6-di-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2-*O*-benzoyl-3,6-di-*O*-benzyl-β-D-

glucopyranosyl-(1→6)-3,4-di-*O*-acetyl-2-deoxy-2-(2,2,2-trichlorethoxycarbonylamino)-β-D-glucopyranosyl-(1→3)-4-*O*-benzoyl-2,6-di-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2-*O*-benzoyl-3,6-di-*O*-benzyl-β-D-glucopyranosyl-(1→6)-3,4-di-*O*-acetyl-2-deoxy-2-(2,2,2-trichlorethoxycarbonylamino)-β-D-glucopyranosyl-(1→3)-4-*O*-benzoyl-2,6-di-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2-*O*-benzoyl-3,6-di-*O*-benzyl-β-D-glucopyranoside (4.60): A solution of **4.58** (1.16 g, 0.11 mmol) and hydrazine acetate (20 mg, 0.21 mmol) in 4:1 CH_2Cl_2–CH_3OH (15 mL) was stirred at room temperature for 4 h. Then the solution was concentrated and the resulting residue was subjected to flash chromatography (CH_2Cl_2–MeOH 75:1) to afford **4.59** (1.05 g, 91%). Subsequently, glycosylation was performed following general procedure A described above, with acceptor **4.59** (680 mg, 0.06 mmol), donor **4.47** (217 mg, 0.08 mmol), NIS (36 mg, 0.16 mmol) and AgOTf (3 mg, 0.01 mmol) in dry CH_2Cl_2 (10 mL). The resulting crude residue was purified by chromatography (hexane–acetone 1:1) to afford triacontasaccharide **4.60** (660 mg, 79%) as a white foam. R_f = 0.43 (hexane–acetone 4:5); $[\alpha]_D$ = +20.5 (c = 1.0, $CHCl_3$); ^{1}H NMR (700 MHz, $CDCl_3$): δ 8.12–7.91 (m, 40H, Ar), 7.59–7.48 (m, 20H, Ar), 7.48–7.21 (m, 185H, Ar), 7.21–7.16 (m, 10H, Ar), 7.15–7.06 (m, 20H, Ar), 7.05–6.97 (m, 10H, Ar), 6.92–6.81 (m, 20H, Ar), 5.72 (s, 1H, H-4_{Gal}), 5.68–5.52 (m, 9H, 9 × H-4_{Gal}), 5.42–5.37 (m, 1H, N*H*Cbz), 5.34–5.21 (m, 10H, 10 × H-2_{Glc}), 5.05 (d, J = 12.3 Hz, 1H), 5.03–4.90 (m, 22H), 4.90–4.84 (m, 9H), 4.83–4.43 (m, 81H), 4.42–4.35 (m, 11H), 4.34–4.10 (m, 46H), 4.02–3.69 (m, 63H), 3.68–3.47 (m, 49H), 3.47–3.25 (m, 24H), 2.75 (dt, J = 18.0, 6.7 Hz, 1H, OC(O)CH_2CH_2C(O)), 2.67 (dt, J = 18.2, 6.1 Hz, 1H, OC(O)CH_2CH_2C(O)), 2.64–2.51 (m, 2H, OC(O)C$H_2$$CH_2$C(O)), 2.16 (s, 3H, CH_2C(O)CH_3), 2.04 (s, 3H, OAc), 2.03–1.99 (m,

24H, 8 × OAc), 1.98 (s, 3H, OAc), 1.95 (s, 3H, OAc), 1.93–1.85 (m, 27H, 9 × OAc); ^{13}C NMR (126 MHz, $CDCl_3$): δ 206.5, 172.3, 170.23, 170.16, 169.6, 169.2, 165.1, 165.0, 156.3, 153.4, 138.2, 138.1, 137.9, 137.6, 136.5, 132.9, 132.7, 130.1, 130.0, 129.6, 128.6, 128.4, 128.2, 128.1, 128.0, 127.85, 127.82, 127.77, 127.7, 127.64, 127.56, 127.5, 127.4, 127.3, 127.13, 127.08, 126.9, 102.2, 101.4, 100.7, 95.4, 81.0, 80.6, 80.3, 79.9, 77.4, 77.3, 76.5, 76.3, 76.1, 75.9, 75.3, 75.2, 75.0, 74.8, 74.2, 74.0, 73.5, 73.4, 72.9, 72.8, 72.7, 71.7, 70.1, 69.9, 69.8, 69.3, 68.5, 68.4, 68.3, 68.1, 67.65, 67.60, 67.5, 66.4, 62.0, 56.0, 55.8, 53.4, 41.0, 37.7, 29.6, 27.8, 20.5, 20.4; ESI-MS *m/z* $[M + 5Na]^{5+}$ for $C_{685}H_{699}Cl_{30}N_{11}Na_5O_{205}$: found: 2708.8733 (M=13429.4). The observed isotope peak is consistent with theoretical isotope peak 13429.5.

Chapter 5: Summary and future work

5.1 Summary and future work

In this thesis, I have described the synthesis of oligosaccharides related to three projects using different synthetic strategies. This includes the chemical synthesis of LNFPIII and its HSA-conjugate (Chapter 2), the enzymatic synthesis of biantennary *N*- and *O*-linked glycans capped with A/B blood group antigens (Chapter 3), and the chemo-enzymatic synthesis of GBSIII oligosaccharides (Chapter 4).

5.1.1 Chemical synthesis of LNFPIII and its HSA conjugate

In Chapter 2, I developed a concise synthetic route to lacto-*N*-fucopentaose (LNFPIII) and its HSA conjugate. Key transformations include a two-step sequential glycosylation for the assembly of the protected Lewis^X trisaccharide, a highly regio- and stereoselective glycosylation for the construction of the pentasaccharide, a Birch reduction for removal of benzyl ethers, and a thiol–ene coupling for the aglycon functionalization for the following conjugation. Moreover, in collaboration with Ochando, we found that LNFPIII is able to function as a ligand for DC-SIGN to induce transplantation tolerance to mismatched organ.[126] This study not only provides insight into the mechanism underlying the immunomodulatory activity of LNFPIII, but also confirmed the potential of LNFPIII to be used as a tolerance-inducing agent in ABOi heart transplants.

The ultimate goal of this project is to extend the window for ABOi heart transplants. Future work will be focused on preparation and evaluation of devices (nanoparticles or stents) carrying synthetic ABO blood group antigens and LNFPIII, through collaboration with clinicians in the Faculty of Medicine and Dentistry and investigators in the National Institute for Nanotechnology.

5.1.2 Enzymatic synthesis of biantennary *N*- and *O*-linked glycans capped with A/B blood group antigens

In Chapter 3, a panel of poly-LacNAc extended biantennary *N*- and *O*-linked glycans capped with blood group A or B antigens were successfully synthesized from a natural *N*-glycopeptide isolated from hen egg yolk and an *O*-glycan core 4 trisaccharide, respectively, via an enzymatic approach. All the reactions were catalyzed by glycosyltransferases and proceeded in aqueous buffer solutions in high yields with exquisite regio- and stereoselectivity. Alternating use of β3GlcNAcT and GalT-1 enabled the branches of the core structures to be elongated by LacNAc units.[145] The terminal A or B antigen epitopes were installed by FucT II and bacterial homologues of GTA[151] or GTB[152].

Through collaboration with Professor James C. Paulson, the synthesized *N*- and *O*-linked glycans, equipped with at least one free amine in the reducing end, have been printed on slides modified by an *N*-hydroxy succinimide-activated carboxylic acid. Formation of amide bonds enables the glycans to be covalently immobilized on the surface to generate a glycan microarray (Figure 5.1). The screening of the serum samples from ABOi transplant patients and monoclonal subtype specific antibodies using the glycan microarray will be conducted. The result could provide additional insight into the binding specificities of monoclonal subtype specific antibodies, and reveal if the poly-LacNAc extensions contribute to A/B antigen recognition by anti-A/B antibodies.

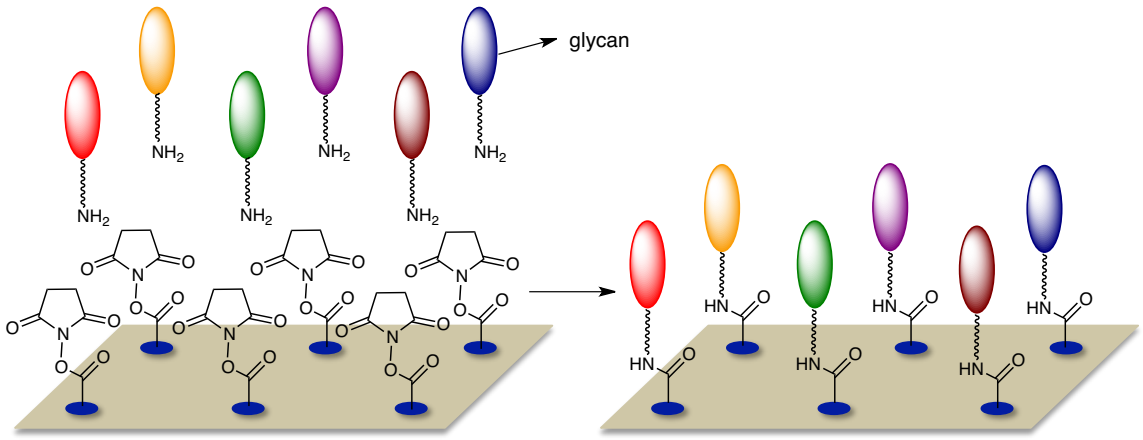


Figure 5.1 Incorporation of *N*- and *O*-linked glycans into a glycan microarray.

5.1.3 Chemo-enzymatic synthesis of GBSIII oligosaccharides

In Chapter 4, I developed a chemo-enzymatic strategy to prepare a library of defined group B *Streptococcus* type III (GBSIII) oligosaccharides, involving chemical synthesis of the oligosaccharide backbone and introduction of side chain by glycosyltransferases. Starting from a glucosamine-based building block **4.17** and a lactose-based building block **4.18**, a trisaccharide thioglycoside **4.16** and its dimer **4.47** were synthesized via efficient glycosylations. Using an improved NIS/AgOTf promoted glycosylation protocol involving a post-treatment with PPh_3, five protected backbone oligosaccharides differing in size were successfully assembled by sequential addition of a hexasaccharide unit to the growing chain. After deprotection, four of the desired deprotected backbone oligosaccharides (**4.11**–**4.14**) were achieved. The multi-galactosylation of **4.11**–**4.14** using a recombinant *H. pylori* β-(1→4)-GalT afforded **4.6**–**4.9** in about 80% yield for each compound. The α-(2→3)-SiaT catalyzed multi-sialylation suffered from incomplete conversion of substrate to the desired muti-sialylated product. At this point, the desired GBSIII oligosaccharides (**4.1**–**4.5**) were not obtained.

To achieve all the desired GBSIII oligosaccharides (**4.1**–**4.5**), optimization of the deprotection of the backbone oligosaccharides is required in the future. Given the difficulties encountered in the step of multiple Troc groups deprotection, the Troc group could be replaced by *N*,*N*-diacetyl group before the longer backbone oligosaccharides are assembled, which will facilitate the late stage deprotection. In addition, the problem encountered in the SiaT catalyzed multi-sialylation needs to be addressed. One possible solution to the sialylation problem is incubation of the substrate in HEPES buffer at a pH above 8.5 to inhibit sialidase activity of SiaTs.[55] Another solution is to use mammalian SiaTs, such as the rat α-(2→3)-SiaT, which does not have sialidase activities.[54] Once **4.1**–**4.5** are obtained, they will be attached to peptide to form glycoconjugates, which will be used as immunogens to investigate the antibody responses in animal model.

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