Synthesis of Lipopolysaccharide O-antigens from *Klebsiella pneumoniae* Serotype O2a as Molecular Probes for Biosynthetic Studies

by

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Abstract

Many Gram-negative bacteria are pathogenic organisms that can cause disease and death. As antibiotic resistance and nosocomial disease occurrences both increase, these infections are becoming more problematic. These issues can be partially attributed to lipopolysaccharide (LPS), which is a major structural and immunomodulatory molecule found in the outer membrane of Gram-negative bacteria. The O-antigenic polysaccharide (O-PS), the terminal domain of LPS, is a major virulence factor. Understanding the biosynthesis of these molecules may allow for new treatments of these pathogenic diseases.

One manner by which O-PS is biosynthesized is the ATP-binding cassette (ABC) transporter-dependent pathway. In this process, the full length O-PS is synthesized in the cytoplasm, and then the transporter flips it to the periplasmic side, prior to extension to full-length LPS. There are two prototypical systems in this pathway: 1) termination of chain extension by a modification (*E. coli* O9a) and 2) termination without a chain modification (*K. pneumoniae* O2a). While the glycosyltransferases (GTases) and chain length control mechanisms have been identified for *E. coli* O9a, many questions still remain in the *K. pneumoniae* O2a system. This includes: 1) What are the precise roles of the GTases involved in O-PS assembly?, 2) How is the chain length controlled?, and 3) What is the mechanism by which the O-PS chain is flipped across the membrane by the ABC transporter?. Investigating these questions requires access to structurally well-defined, synthetic glycans. This thesis focuses on the synthesis of glycan acceptors that will be used as molecular probes to further elucidate the *K. pneumoniae* O-PS biosynthetic pathway.

In one investigation, three fluorescein-based probes, two disaccharides and one monosaccharide, were chemically synthesized. The challenge in generating the galactofuranose

ring in these molecules was overcome using a kinetically controlled I_2-catalyzed cyclization of D-galactose dithioacetal. Completely stereoselective glycosylations were achieved by the use of the appropriate donor: 2-*O*-benzoylated galactofuranoside donors gave the desired 1,2-*trans*-β linkages, and DTBS-protected galactopyranoside donors gave the more challenging 1,2-*cis*-α linkages. The monosaccharide probe was obtained via a concise, three-step synthetic route. Preliminary investigations with these probes have clarified the activities of the three GTases involved in its O-PS assembly in *K. pneumoniae* O2a.

The second investigation centers on the synthesis of lipid-linked oligosaccharides from *K. pneumonia* O2a. Our targets are five molecules, differing in size from nine monosaccharide residues up to 41 monosaccharide residues. The highly convergent route developed gave the desired target backbones (up to the 33-mer) in substantial quantities. Conversion of these backbones to the final targets gave three of the target glycans (the 9-mer, 17-mer, and 25-mer). These synthetic glycans will be used to further elucidate the O-PS biosynthetic pathway found in *K. pneumoniae* O2a.

Preface

The work described in this thesis was done solely by me and has not been published.

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List of Abbreviations

$[\alpha]_D$	specific rotation (sodium D line)
°C	degrees Celsius
12-mer	dodecasaccharide
13-mer	tridecasaccharide
16-mer	hexadecasaccharide
17-mer	heptadecasaccharide
20-mer	icosasaccharide
24-mer	tetracosasaccharide
25-mer	pentacosasaccharide
28-mer	octacosasaccharide
32-mer	dotriacontasaccharide
33-mer	tritriacontasaccharide
41-mer	hentetracontasaccharide
8-mer	octasaccharide
9-mer	nonasaccharide
Å	Angstrom
A. aeolicis	*Aquifex aeolicis*
ABC	ATP-binding cassette
Ac	acetyl
AcOH	acetic acid
ACP	acyl carrier protein
ADP	adenosine diphosphate

AgOTf	silver trifluoromethanesulfonate
All	allyl
Ar	aromatic
ATP	adenosine triphosphate
BDA	benzaldehyde dimethyl acetal
$BF_3 \cdot OEt_2$	boron trifluoride etherate
Bn	benzyl
br s	broad singlet (NMR spectra)
Bz	benzoyl
CA	California
calcd	calculated
CAN	ceric ammonium nitrate
CBM	carbohydrate binding motif
CD14	cluster of differentiation 14 receptor
CDI	1,1'-carbonyldiimidazole
CMP	cytidine monophosphate
Cp_2ZrCl_2	zirconocene dichloride
CSA	camphorsulfonic acid
d	doublet (NMR spectra)
DBU	diazabicyclo[5.4.0]undec-7-ene
DCC	*N*,*N'*-dicyclohexylcarbodiimide
DCTB	*trans*-2-[3-(4-*tert*-butylphenyl)-2-methyl-2-propenylidene]malononitrile

DIAD	diisopropyl azodicarboxylate
DIPEA	*N*,*N*-diisopropylethylamine
DMAP	4-dimethylaminopyridine
dm	decimeter
DMF	*N*,*N'*-dimethylformamide
DMP	dimethoxypropane
DNA	deoxyribonucleic acid
DTBS	4,6-*O*-di-*tert*-butylsilylidene
$DTBS(OTf)_2$	4,6-*O*-di-*tert*-butylsilylidene bis(trifluoromethanesulfonate)
dTDP	deoxythymidine diphosphate
E. coli	*Escherichia coli*
ESI	electrospray ionization
Et	ethyl
Et_2O	diethyl ether
Et_3N	triethylamine
EtOAc	ethyl acetate
FITC	fluorescein isothiocyanate
g	grams
Gal*f*	D-galactofuranose
Gal*p*	D-galactopyranose
GDP	guanosine diphosphate
gem	geminal
Glc	D-glucose

GlcNAc	*N*-acetylglucosamine
GTase(s)	glycosyltransferase(s)
h	hour
Hep	L(or D)-*glycero*-D-*manno*-heptose
HPLC	high performance liquid chromatography
HRMS	high resolution mass spectroscopy
Hz	hertz
i-$Pr_2NP(OBn)_2$	dibenzyl *N*,*N*-diisopropylphosphoramidite
Im	imidazole
$Ir(COD)(PMePh_2)_2 \cdot PF_6$	(1,5-cyclooctadiene)bis(methyldiphenylphosphine)iridium(I) hexafluorophosphate
K. pneumoniae	*Klebsiella pneumoniae*
Kdo	3-deoxy-D-*manno*-oct-2-ulosonic acid
Lev	levulinoyl
LPS	lipopolysaccharide
Lpt	LPS transport
LRMS	low resolution mass spectrometry
m	multiplet (NMR spectra)
M	molar
m-CPBA	*meta*-chloroperoxybenzoic acid
M.S.	molecular sieves
mAb	monoclonal antibody
MALDI-TOF	Matrix-Assisted Laser Desorption Ionization/Time-of-Flight

Man*p*	D-mannopyranose
MD-2	lymphocyte antigen 96 protein
mg	milligrams
MHz	megahertz
min	minutes
mL	milliliters
mM	millimolar
mmol	millimoles
MW	molecular weight
N	normal
N/O	not observed
NBD	nucleotide-binding domain
NDP	nucleotide diphosphate
NIS	N-iodosuccinimide
NMP	nucleotide monophosphate
NMR	nuclear magnetic resonance
O-PS	O-antigenic polysaccharide
ON	Ontario
PCP	polysaccharide copolymerase
$Pd(OH)_2/C$	palladium hydroxide on carbon
Pd/C	palladium on carbon
Ph	phenyl
pH	activity of hydrogen; ($-\log[H_3O^+]$)

P_i	phosphate
PIF	pyranoside-into-furanoside
PMe_3	trimethylphosphine
PMP	*p*-methoxyphenol
PP	pyrophosphate
PPh_3	triphenylphosphine
ppm	parts per million
Pr	propyl
psi	pounds per square inch
Py	pyridinium
q	quartet (NMR spectra)
R. terrigena	*Raoultella terrigena*
RBF	round bottom flask
R_f	retardation factor
RNA	ribonucleic acid
rt	room temperature
RU	repeating units
s	singlet (NMR spectra)
SAH	*S*-adenosyl homocysteine
SAM	*S*-adenosyl methionine
t	triplet (NMR spectra)
TBAF	tetrabutylammonium fluoride
TBDPS	*tert*-butyldiphenylsilyl

TBS	*tert*-butyldimethylsilyl
TBSOTf	*tert*-butyldimethylsilyl trifluoromethanesulfonate
TFA	trifluoroacetic acid
TfOH	trifluoromethanesulfonic acid
THF	tetrahydrofuran
TLC	thin layer chromatography
TLR4	toll-like receptor 4
TMD	transmembrane domain
TMSEt	trimethylsilyl ethyl
TMSOTf	trimethylsilyl trifluoromethanesulfonate
TOF	time-of-flight
Tol	*p*-tolyl
UDP	uridine diphosphate
UDP-Gal*f*	uridine diphosphate galactofuranose
UDP-Gal*p*	uridine diphosphate galactopyranose
UDP-GlcNAc	uridine diphosphate *N*-acetylglucosamine
UDP-Man*p*	uridine diphosphate mannopyranose
UMP	uridine monophosphate
und	undecaprenol
USA	United States of America
v/v	volume by volume
µL	microliters
µmol	micromoles

Chapter 1: Introduction

1.1 The Outer Membrane of Gram-negative Bacteria

Many Gram-negative bacteria are pathogens that cause disease,[1,2] and if the infections are untreated, they can lead to death.[3,4] These organisms cause the following infections and diseases: urinary tract infections,[5] gonorrhea,[6] wound infections,[7] bloodstream infections,[8] pneumonia,[9] cancer,[10,11] and many others. Such infections have become even more problematic because they have emerged as nosocomial diseases in people with already compromised immune systems, leading to a higher chance of death.[12,13] Bacterial infections are also becoming more difficult to treat due to increasing antibiotic resistance.[14-16] In Gram-negative bacteria, both this virulence and resistance increase can be partially attributed to their outer membrane (which is found in most, but not all Gram-negative bacteria[17]) and the structural components that are part of the outer membrane.[2,18]

1.2 Lipopolysaccharide

A major component of the bacterial outer membrane and a major virulence factor found in Gram-negative bacteria is lipopolysaccharide, or LPS.[2,19] Lipopolysaccharide is an important component of the cell membrane and contributes significantly to its structural integrity.[2,19] LPS is also an important immunomodulatory molecule, as it elicits immune responses from infected hosts.[2] This molecule also affords a protective layer that helps shield the bacteria from antibiotics and other kinds of chemical and environmental attacks.[2] LPS consists of three components: lipid A, the core oligosaccharide, and the O-antigenic polysaccharide (O-PS) repeating units (Figure 1.1).[2,19]

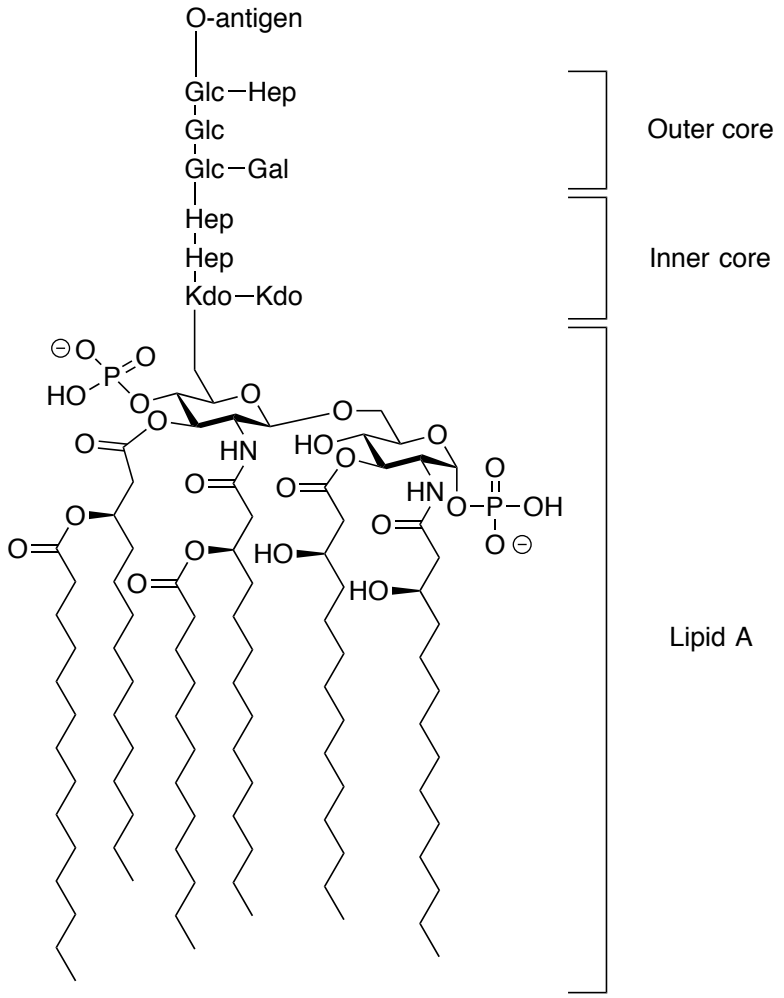


Figure 1.1 The general structure of lipopolysaccharide (LPS). Kdo, 3-deoxy-D-manno-oct-2-ulosonic acid; Hep, L-*glycero*-D-*manno*-heptose, or D-*glycero*-D-*manno*-heptose; Glc, D-glucose; Gal, D-galactose.

1.2.1 Components of Lipopolysaccharide (LPS)

Lipid A is a disaccharide that consists of two glucosamine sugar residues, six acyl chains, and two phosphates,[19] and it is strictly conserved throughout all Gram-negative bacteria.[2] The six acyl chains and two phosphates anchor LPS to the bacterial cell outer membrane,[2,19] projecting the core oligosaccharide and O-PS away from the surface. Lipid A is also responsible for the activation of TLR4, MD-2, and CD14 receptors, part of the host's innate immune responses.[20]

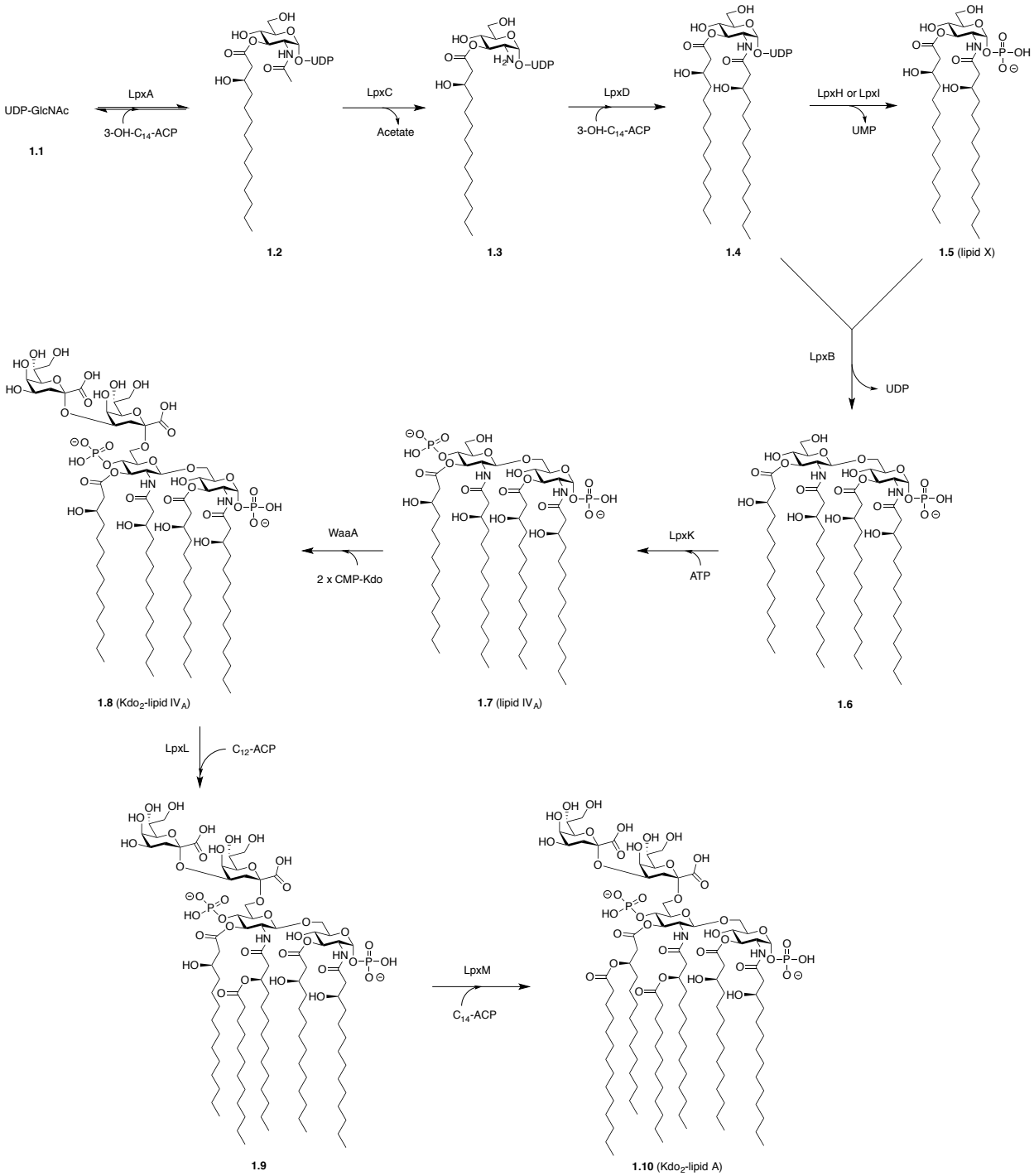
The core oligosaccharide connects lipid A to the O-PS. Like lipid A, the inner core region is also conserved in Gram-negative bacteria.[2] It consists of two 3-deoxy-D-*manno*-oct-2-ulosonic acid (Kdo) residues that are linked to the lipid A moiety. The inner core is further comprised of L-*glycero*-D-*manno*-heptose, or D-*glycero*-D-*manno*-heptose (Hep) residues.[2] In the outer core region, however, the conservation of residues dissipates between different species of bacteria.[21] Many different structures have been reported, but there is a narrow range of structures in each species of bacteria.[21] These outer core structures often contain hexose residues, including D-glucose (Glc), D-galactose (Gal), and D-mannose (Man).[2]

The O-PS, is the "tail" component of the LPS. It is projected out into the extracellular matrix away from the cell membrane. The O-PS chains are extremely variable, even within the same species.[2] They appear to have a primary role of protecting the bacteria from external hazards.[2] These molecules also have an important function in host–pathogen interplay as essential virulence determinants[22] and are accountable for the resistance to non-specific, complement-mediated serum killing.[23,24]

1.2.2 Biosynthesis of LPS

LPS biosynthesis (Figure 1.2) begins at the interface between the cytosol and inner membrane. The first step is the synthesis of the Kdo_2-lipid A tetrasaccharide intermediate in a nine-step enzymatic pathway (Scheme 1.1), frequently referred to as the Raetz pathway.[21] First, UDP-GlcNAc (**1.1**) is converted to 3-*O*-acyl-UDP-GlcNAc **1.2** by the enzyme LpxA in the presence of 3-OH-C_{14}-acyl carrier protein (ACP).[25,26] That product then has the *N*-acetate removed by the enzyme LpxC to give 3-*O*-acyl glucosamine derivative **1.3**.[27-29] That species is then acylated at the nitrogen by the enzyme LpxD, once again in the presence of 3-OH-C_{14}-ACP,

to give the UDP-GlcN-diacyl product **1.4**,[30] which is then cleaved at the pyrophosphate bond by LpxH[31] (or LpxI[32] in some species of bacteria), forming the intermediate product, lipid X (**1.5**).



Scheme 1.1 Biosynthesis of the $(Kdo)_2$-lipid A domain of LPS; the Raetz pathway.

The structure of LpxB has been recently reported.[33] This enzyme catalyzes the introduction of the characteristic β–(1→6)-glycosidic linkage of the disaccharide, condensing one molecule of UDP-diacyl-GlcN **1.4** as a donor and one molecule of lipid X **1.5** as an acceptor, forming the disaccharide-1-phosphate **1.6**.[34] This disaccharide is then phosphorylated at the 4' position by the enzyme LpxK to form the diphosphate lipid IV_A (**1.7**).[35] Lipid IV_A (**1.7**) is then glycosylated twice by the enzyme WaaA, using CMP-Kdo as the donor, forming the product Kdo_2-lipid IV_A (**1.8**).[36] The final two acyl chains are added to Kdo_2-lipid IV_A (**1.8**) by the two enzymes LpxL and LpxM, respectively, forming the final product in the Raetz pathway, the tetrasaccharide Kdo_2-lipid A (**1.10**).[37,38]

After synthesis of Kdo_2-lipid A (**1.10**), various glycosyltransferases (GTases) add residues to the Kdo_2-lipid A moiety to generate the lipid A–core oligosaccharide intermediate. This process also occurs on the surface of the inner membrane.[21] Once addition of the core residues to lipid A is complete, the lipid A–core oligosaccharide is then flipped across the inner membrane from the cytosol to the periplasm by the ATP-binding-cassette (ABC) transporter[39-43] MsbA (Figure 1.2), driven by the hydrolysis of ATP.[2,21,44-46]

The O-PS is assembled by a separate process in the cytoplasm, which is described in more detail in Section 1.3. Once the O-PS is produced and transferred to the periplasm, it is ligated to the lipid A–core oligosaccharide by the enzyme WaaL (Figure 1.2).[47-49] After ligation, the full-length LPS is then transported to the outer membrane by the LPS transport (Lpt) machinery proteins (Figure 1.2).[50-52] The Lpt system is a seven-protein complex (LptA–LptG) that bridges[53] the inner and outer membranes,[54] and is driven forward by the hydrolysis of ATP.[55,56]

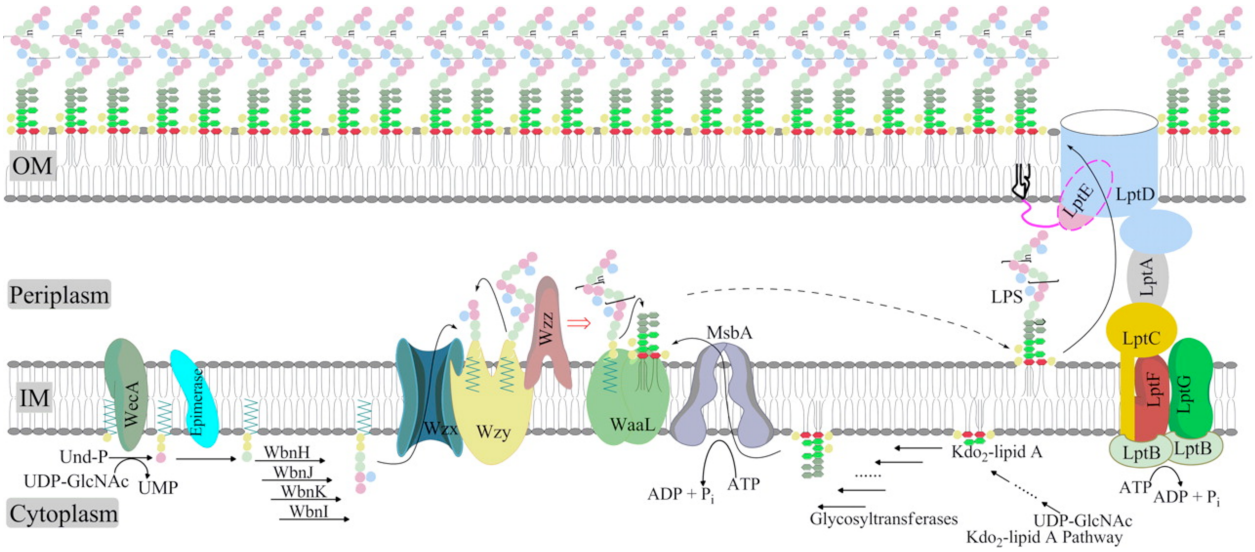


Figure 1.2 Assembly of LPS. In the cytoplasm, the Kdo2–Lipid A moiety is elaborated with additional carbohydrates to give the lipid A–core structure, which is "flipped" to the periplasm by the ABC transporter MsbA. Separately, the O-PS is assembled in the cytoplasm and transferred to the periplasm (the Wzy-dependent pathway [Section 1.3] is used as an example in this figure). In the periplasm, the two components are ligated by the enzyme WaaL. The LPS transport (Lpt) proteins then carry the full length LPS to the cell surface. (Reproduced with permission from Han, W.; Wu, B.; Li, L.; Zhao, G.; Woodward, R.; Pettit, N.; Cai, L.; Thon, V.; Wang, P. G. *J. Biol. Chem.* **2012**, *287*, 5357). OM, outer membrane; IM, inner membrane.

The Lpt machinery has a protein complex at the inner membrane, $LptB_2FGC$, and a protein complex at the outer membrane, LptDE, with the protein LptA (possibly as multiple monomers), creating a bridge[53] that spans the periplasm.[51,54] LptB was first identified as a nucleotide-binding domain (NBD) of an ABC transporter that exists as two distinct NBDs that dimerize upon binding ATP.[56] LptF and LptG were identified as transmembrane domains (TMDs) of this transporter.[57] All three are vital components of LPS transport to the outer membrane.[58] Recently, a nucleotide-free crystal structure of the $LptB_2FG$ protein complex was reported.[59] It was proposed that the complex first binds to ATP, inducing LptB dimerization, which then opens up the LptFG domains, extracting LPS from the inner membrane into its hydrophobic cavity, thus beginning its transit to the outer membrane (Figure 1.3).[52,59]

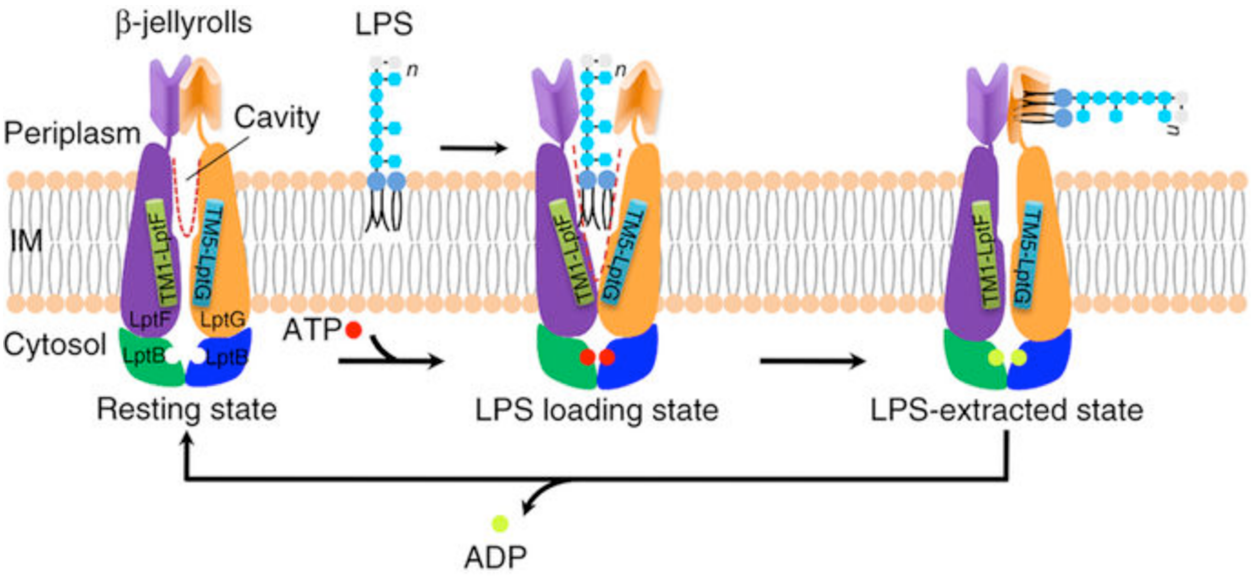


Figure 1.3 Binding of LPS to $LptB_2FG$. After ATP activation of the $LptB_2FG$ protein complex, the cavity of the TMD opens and extracts LPS from the inner membrane. After ADP is released, $LptB_2FG$ undergoes another conformational change and puts LPS into the Lpt hydrophobic channel, where it begins its transit to the outer membrane. (Reproduced with permission from Luo, Q.; Yang, X.; Yu, S.; Shi, H.; Wang, K.; Le Xiao; Zhu, G.; Sun, C.; Li, T.; Li, D.; Zhang, X.; Zhou, M.; Huang, Y. *Nat. Struct. Mol. Biol.* **2017**, *24*, 469).

LptC is part of the ABC-transporter protein complex, though not part of the ABC transporter itself. It interacts with LptF and/or LptG,[58] along with the C-terminus of LptA,[60,61] to connect the ABC transporter extraction complex to the bridge formed by LptA,[54] possibly as a quality control step for trans-envelope bridge formation.[52] The LptA bridge,[53] specifically the N terminus of LptA, interacts with the N-terminus of LptD,[60] which is part of the LptDE protein complex that inserts the LPS into the outer membrane.[62]

LptD and LptE interact strongly, and both are crucial for LPS transport.[62,63] These two proteins form a plug-and-barrel structure,[64-66] and LPS probably passes through the β-barrel lumen to the cell surface, after a conformational change in the LptDE complex upon arrival of the LPS.[64,67,68] LptE is proposed to be more than just a plug; potentially having a role in

assembling the LPS onto the cell surface,[63,69] ending the long journey of LPS to the outer membrane of Gram-negative bacteria (Figure 1.4).

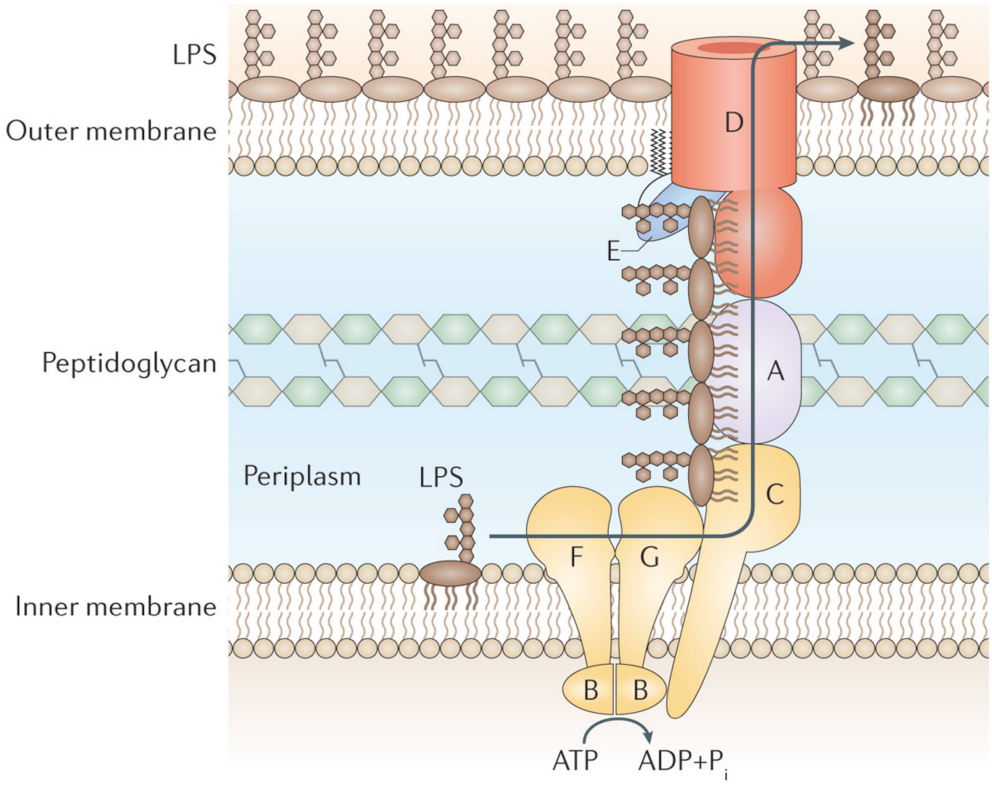


Figure 1.4 Lpt protein bridge.[53] After the Lpt proteins carry LPS across the periplasm, LPS completes its journey by being inserted into the outer membrane. (Reproduced with permission from Okuda, S.; Sherman, D. J.; Silhavy, T. J.; Ruiz, N.; Kahne, D. *Nat. Rev. Micro.* **2016**, *14*, 337).

1.3 Mechanisms of O-antigenic Polysaccharide (O-PS) Biosynthesis

O-PS biosynthesis follows one of three different pathways: 1) the ABC transporter-dependent pathway, 2) the Wzy-dependent pathway, or 3) the synthase dependent pathway (Figure 1.5). Undecaprenyl diphosphate-linked intermediates are the substrates for all three of these pathways, and a member of polyprenol-phosphate sugar-1-phosphate transferase family,

WecA (or a homolog of WecA), initiates all three.[2] These three pathways will now be discussed in further detail.

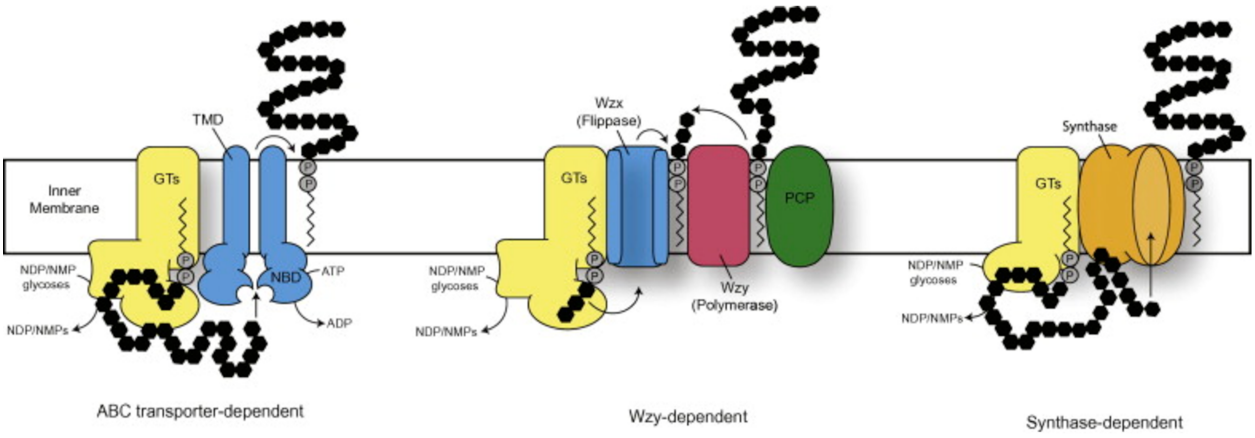


Figure 1.5 The three mechanisms of O-PS biosynthesis. (Reproduced with permission from Greenfield, L. K.; Whitfield, C. *Carbohydr. Res.* **2012**, *356*, 12).

1.3.1 ABC Transporter-dependent Pathway

In this pathway, O-PS biosynthesis is initiated by WecA, which forms und-PP-GlcNAc.[2,70] The O-PS is then polymerized to its full length by GTases at the cytoplasmic face of the inner membrane.[2,71] Once the O-PS reaches its full length, it is then exported to the periplasmic face of the inner membrane by an ABC transporter that consists of two NBDs and two TMDs,[71] with ATP hydrolysis driving the process of exportation.[2] The O-PS chain length is controlled by one of two types of mechanisms: either by termination of chain extension by a modification, or by a coupled process where the O-PS does not have a modification to determine chain length.[71] To date, three different types of terminal modification residues have been identified: *O*-methyl groups, methyl phosphates, or unique sugar residues (Figure 1.6). These two prototypical systems will be expanded upon in further detail in Section 1.4.

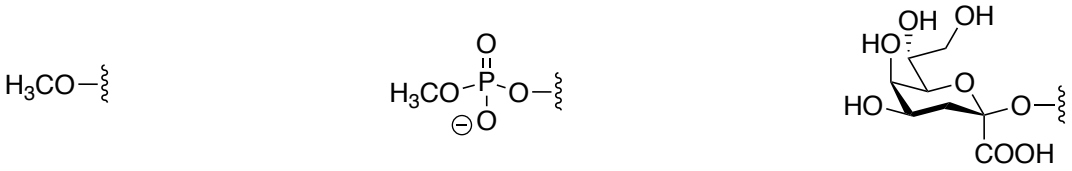


Figure 1.6 Examples of truncating groups. *O*-methyl (left), methyl phosphate (middle), and unique sugar residue Kdo (right) truncating groups will all be discussed in further detail in Section 1.4.1.

1.3.2 Wzy-dependent Pathway

In this pathway, GTases produce individual O-PS repeating units on the cytoplasmic face of the inner membrane.[2,71] A flippase, Wzx, then exports these individual repeating units to the periplasmic face.[2,71,72] On the periplasmic face, the O-PS is then polymerized by the enzyme, Wzy.[2,71,73,74] Wzy polymerizes the O-PS by adding individual repeating units in a block-wise manner, ligating the reducing end of the nascent O-PS polymer to individual repeating unit blocks, until it reaches its full length.[2,71,73,74] The enzyme Wzz, a member of the polysaccharide copolymerase (PCP) family, is believed to regulate O-PS chain length.[75]

1.3.3 Synthase-dependent Pathway

In this pathway, a polymerizing glycosyltransferase, also known as a synthase, produces the O-PS polymer in the cytoplasm. This enzyme is also believed to be accountable for exporting the O-PS to the periplasm.[2,71] The only example of this system has been observed in the bacteria *Salmonella enterica* serovar Borreze O:54.[76]

The synthesis is also initiated by WecA, forming an und-PP-GlcNAc primer.[77] This primer is then committed to O-PS synthesis by the GTase, WbbE, which adds the first residue of

the repeating unit.[76,77] The GTase, WbbF, is suggested to then be responsible for chain polymerization and O-PS export to the periplasm.[2] This is the least well characterized pathway of all three O-PS biosynthetic systems.[71]

1.4 Prototypical Systems of ABC Transporter-Dependent O-PS Biosynthesis

As mentioned above, control of O-PS chain length in ABC-transporter dependent systems arises in one of two ways: termination of chain extension by the addition of a modification, or when such a modification is not present, a coupled process between the GTases and the transporter. These systems will be discussed in more detail below. Control of chain length in the synthesis of polysaccharides is a topic of significant current study.[78-82] Unlike the synthesis of other essential biopolymers – DNA, RNA, and proteins – this process is not template driven. As such, unraveling the mechanisms by which this occurs in nature is of great interest.

1.4.1 Termination of Chain Extension by a Modification (*E. coli* O9a, *R. terrigena*)

Escherichia coli's O-PS has been implicated in TLR4 activation of myeloid cells.[83] It also assists the bacteria in resisting the host complement system, particular antibiotics, and additional natural environmental stresses.[71] For these reasons, it has been studied extensively as a prototypical system of O-PS biosynthesis. The three main serotypes that have been studied for this purpose are serotypes O8, O9, and O9a. All three have O-antigens that consist of polymannan repeating units, but with differing glycosidic linkages (Figure 1.7). The O-PS of *E. coli* O8 consists of a →3)-β-D-Man*p*-(1→2)-α-D-Man*p*-(1→2)-α-D-Man*p*-(1→ repeating unit polymer.[84] The O-PS of *E. coli* O9 consists of a →2)-α-D-Man*p*-(1→2)-α-D-Man*p*-(1→2)-α-D-

Man*p*-(1→3)-α-D-Man*p*-(1→3)-α-D-Man*p*-(1→ repeating unit polymer.[85] The O-PS of *E. coli* O9a consists of a →2)-α-D-Man*p*-(1→2)-α-D-Man*p*-(1→3)-α-D-Man*p*-(1→3)-α-D-Man*p*-(1→ repeating unit polymer.[86] In 1997, it was reported that a monoclonal antibody (mAb) discriminated between the O9 and O9a serotypes.[87] In this study, the authors observed that the O9a serotype was actually the dominant strain in *E. coli* O9.[87] Based on this conclusion, many studies of the O-PS biosynthetic system have since been focused on *E. coli* O8/O9a. For these reasons, I will also focus on these two serotypes, O8 and O9a, in this introduction.

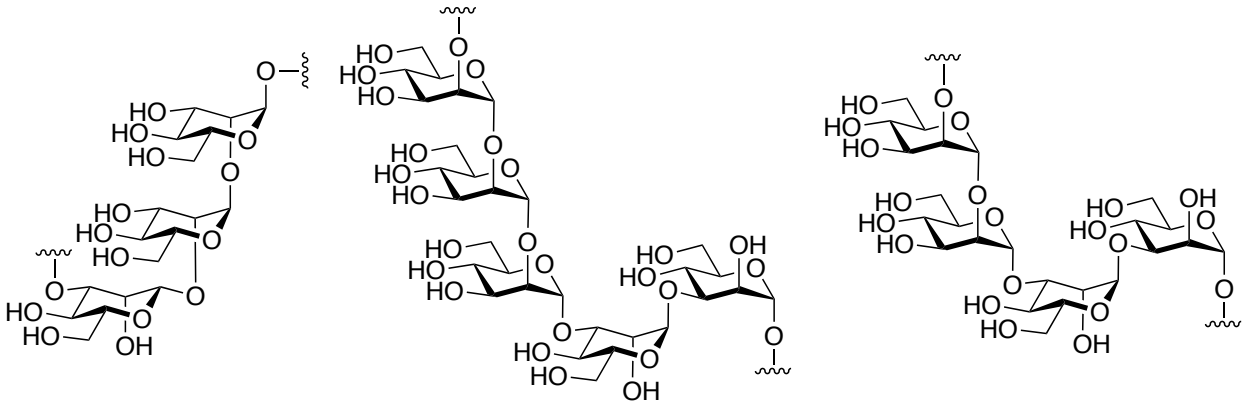
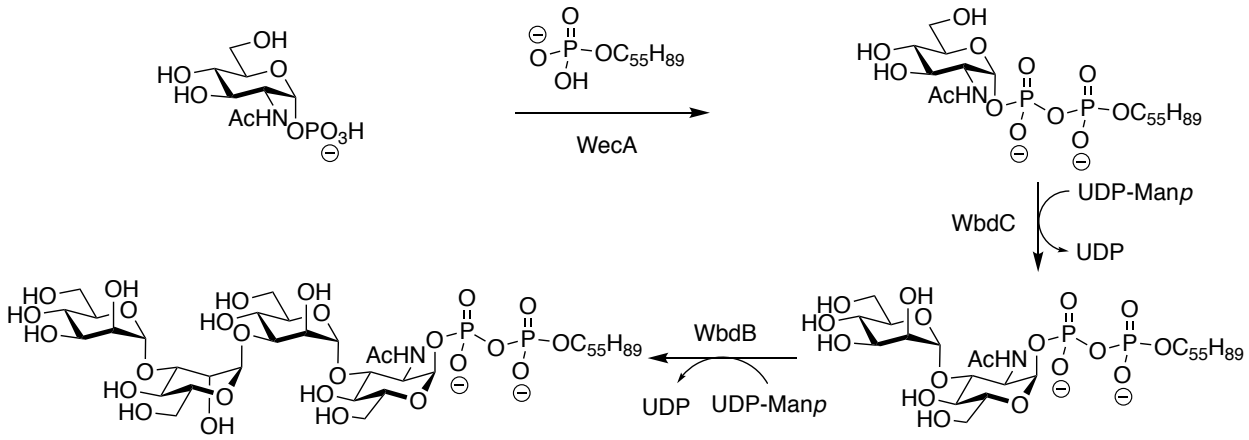


Figure 1.7 The repeating unit structures of the O-antigens from *Escherichia coli* serotypes O8 (left), O9 (middle), and O9a (right).

1.4.1.1 Initiation of O-PS Biosynthetic Pathway in *E. coli* O8/O9a (Scheme 1.2)

Biosynthesis of the O-PS begins with an undecaprenyl-pyrophosphate acceptor located on the cytoplasmic face of the inner membrane.[70] The enzyme, WecA, ligates GlcNAc-1-phosphate to undecaprenyl phosphate, forming the acceptor und-PP-GlcNAc.[88,89] The GTase, $WbdC_{O8/O9a}$, is a monofunctional α-(1→3) mannosyltransferase that next adds a single mannose residue to the und-PP-GlcNAc acceptor.[90] The GTase, $WbdB_{O8/O9a}$, is an another α-(1→3) mannosyltransferase that installs, in succession, two mannose residues to the WbdC product,

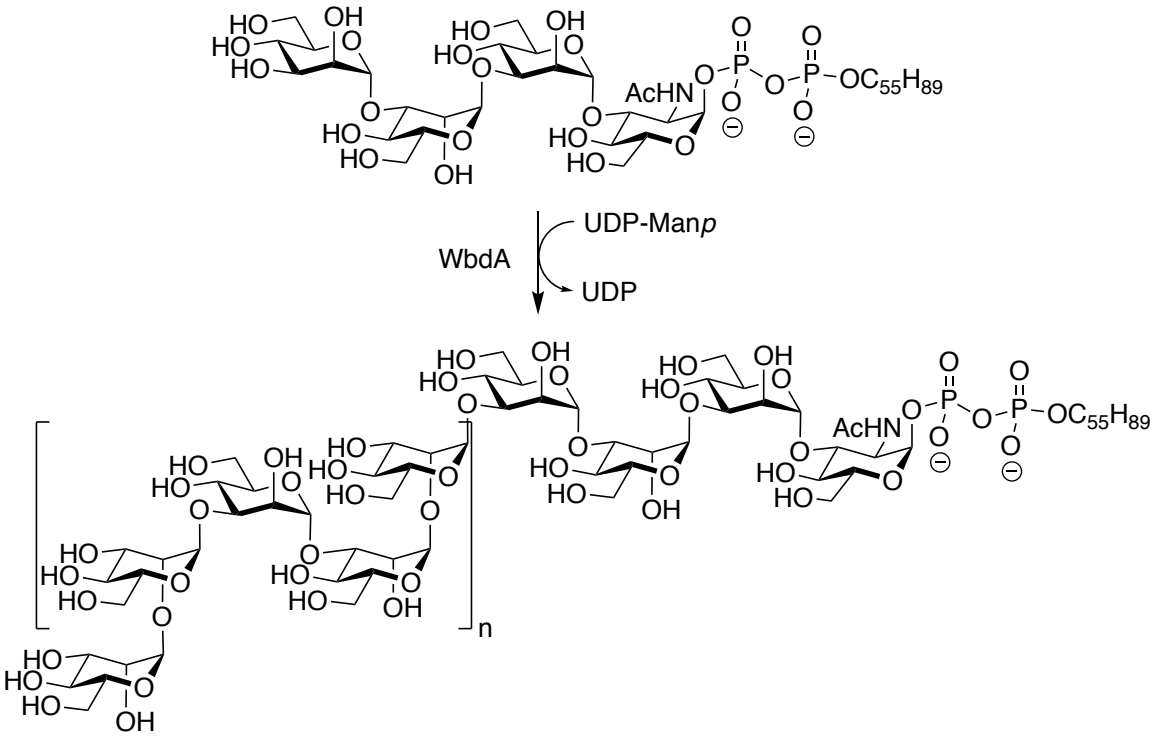
forming the und-PP-tetrasaccharide intermediate, as the final enzymatic step in the initiation of O-PS biosynthesis in *E. coli* O8/O9a.[90]



Scheme 1.2 The three enzymatic steps involved in the initiation of O-PS biosynthesis in *E. coli* O8/O9a.

1.4.1.2 Polymerization of O-PS in *E. coli* O8/O9a

The GTase, WbdA, is serotype-specific enzyme that is solely responsible for the polymerization of the O-PS in *E. coli* O8/O9a (Scheme 1.3).[90] $WbdA_{O9a}$ is a bifunctional GTase with α-(1→2) and α-(1→3) mannosyltransferase activity,[90] with both the C-terminus and N-terminus required for biosynthesis.[91] $WbdA_{O9a}$ has two distinct active domains, rather than one that makes both linkages.[92] Similarly, $WbdA_{O8}$ is a trifunctional GTase with α-(1→2), α-(1→3), and β-(1→2) mannosyltransferase activities,[90] with three distinct active domains, one for each linkage.[92]



Scheme 1.3 WbdA is the GTase responsible for O-PS chain elongation. Shown for *E. coli* O9a in this scheme.

1.4.1.3 Chain Termination, Chain Length Control, and Export of O-PS in *E. coli* O8/O9a

After the O-PS is polymerized to its full length, the enzyme WbdD is responsible for controlling chain length by truncating the O-PS with a terminal modification (Figure 1.8).[93] $WbdD_{O8}$ is a methyltransferase that adds a methyl group to the terminal end of the O-PS.[93] $WbdD_{O9a}$ is a bifunctional kinase–methyltransferase that adds a methyl phosphate[94] to stop polymerization, with its kinase activity controlling the chain length.[93] This chain termination occurs prior to, and is essential for, export.[93] WbdD coordinates chain termination with export by the ABC-transporter.[93]

Figure 1.8 The truncating groups in *E. coli* O8 and O9a. WbdD is responsible for truncating the O-PS by addition of a methyl group in *E. coli* 08 (left) or a methyl phosphate in *E. coli* O9a (right) in turn, controlling the chain length.

To control the chain length of the O-PS in *E. coli* O9a, WbdD–WbdA forms an enzyme complex, with the C-terminal domain of WbdD interacting with WbdA.[95] The crystal structure of the WbdA–WbdD complex shows that the C-terminus of WbdD is essential for trimer formation with WbdA, for its kinase activity, and for recognition of the correct polymeric O-PS substrate.[96] The stoichiometry of WbdA–WbdD enzyme complex (1:3) is also crucial in controlling the chain length.[97] The C-terminal domain of WbdD contains an extended coiled-coil region that physically seperates WbdA from the catalytic domain of WbdD.[81] The length of the coiled-coil domain in WbdD is ~200 Å (Figure 1.9), which is in close agreement with the length of 14 repeating units, the mean distribution of the O9a O-PS repeating units (~210 Å in length).[81] It is proposed that WbdA would grow the O-PS polymer until it is long enough to reach the kinase active site of WbdD.[81] In effect, the coiled-coil domain acts as a molecular ruler, [75,98] terminating the chain growth once O-PS reaches a certain length.

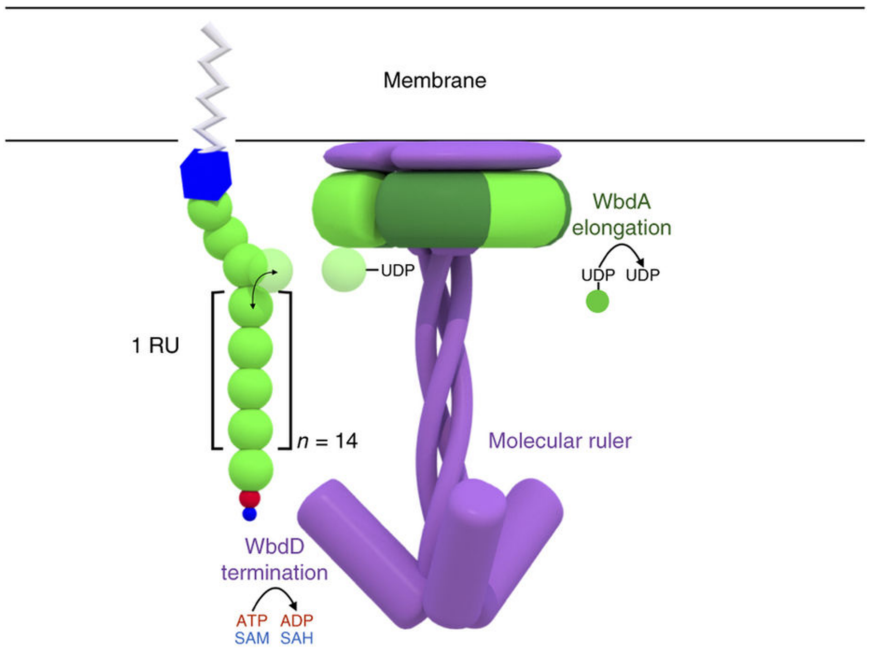


Figure 1.9 Proposed model for O-PS chain elongation and termination in *E. coli* O9a. (Reproduced with permission from Hagelueken, G.; Clarke, B. R.; Huang, H.; Tuukkanen, A.; Danciu, I.; Svergun, D. I.; Hussain, R.; Liu, H.; Whitfield, C.; Naismith, J. H. *Nat. Struct. Mol. Biol* **2015**, *22*, 50).

In *E. coli* 08 and O9a, the ABC-transporters are specific for their cognate O-PS substrates.[99] The C-terminal domain of the NBD (Wzt) has a specific role in substrate recognition; it has a structural element, a carbohydrate binding motif (CBM), that determines the substrate specificity of the ABC-transporter.[100] Both of the NBDs (named Wzt in these systems) are required for the export of the O-PS, and the C-terminus of Wzt binds its substrate by recognizing the residue at the terminal end of the O-PS.[100] After recognition of the O-PS by the CBM, O-PS is then exported to the periplasm by the ABC-transporter, where it is ligated to lipid A–core oligosaccharide by the enzyme WaaL to make full length LPS (Figure 1.10).

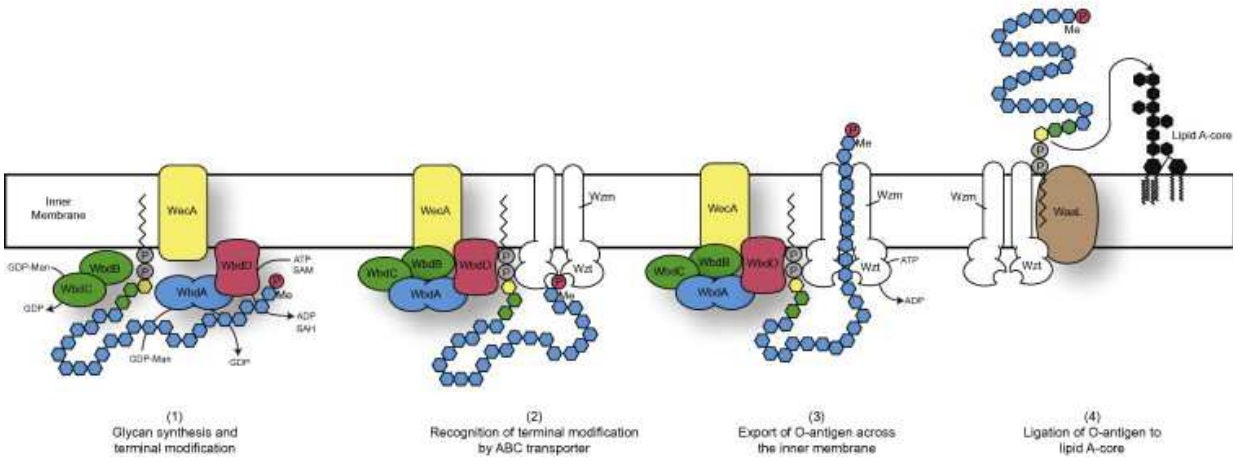


Figure 1.10 Cartoon overview of the ABC transporter-dependent O-PS biosynthetic complex from *E. coli* O8 and O9a. (Reproduced with permission from Greenfield, L. K.; Whitfield, C. *Carbohydr. Res.* **2012**, *356*, 12).

1.4.1.4 Polymerization, Chain Termination, and Export of O-PS in *R. terrigena*

Recently, a second version of the prototypical terminal chain modification system of controlling O-PS chain length was reported.[82] In *Raoultella terrigena* (and *Klebsiella pneumoniae* serotype O12), a single protein, WbbB, incorporates polymerization, termination, and chain-length control.[82] After WecA initiates the process by forming the und-PP-GlcNAc, the enzyme WbbB synthesizes the full length O-PS in *R. terrigena* (Figure 1.11).

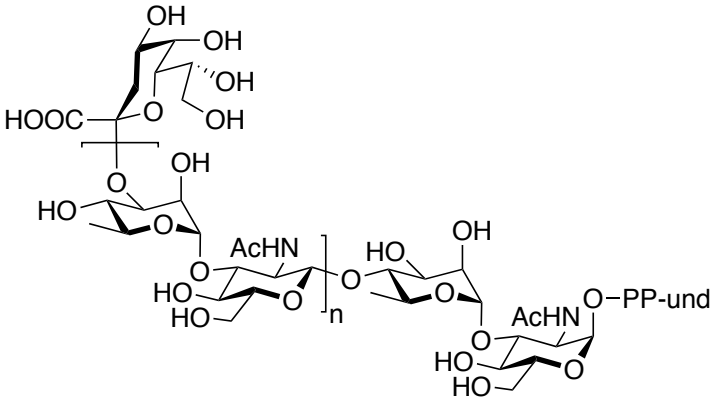


Figure 1.11 The structure of the lipid linked O-PS precursor intermediate from *Raoultella terrigena*.

WbbB is a trifunctional enzyme with α-(1→4)-rhamnosyltransferase, β-(1→3)-N-acetylglucosaminyltransferase, and β-(2→3)-Kdo glycosyltransferase activities; all from new GTase families: GT102, GT103, and GT99, respectively.[82] The two GTases from families GT102 and GT103 are responsible for polymerization of O-PS.[82] WbbB forms a membrane-bound dimer, with an extended coiled-coil domain that contains, at its terminus, the GT99 domain. This enzymatic activity caps the O-PS chain with a single Kdo residue (Figure 1.12), and is responsible for controlling the chain length.[82] Only after the Kdo residue caps the O-PS polymer, O-PS is recognized by the CBM[101] of the ABC-transporter.[102] This second example shows that this highly organized mechanism of O-PS synthesis with terminal modifications of O-PS polymers, extended coiled-coil domains acting as molecular rulers, and CBMs are conserved themes found throughout nature.[82,101]

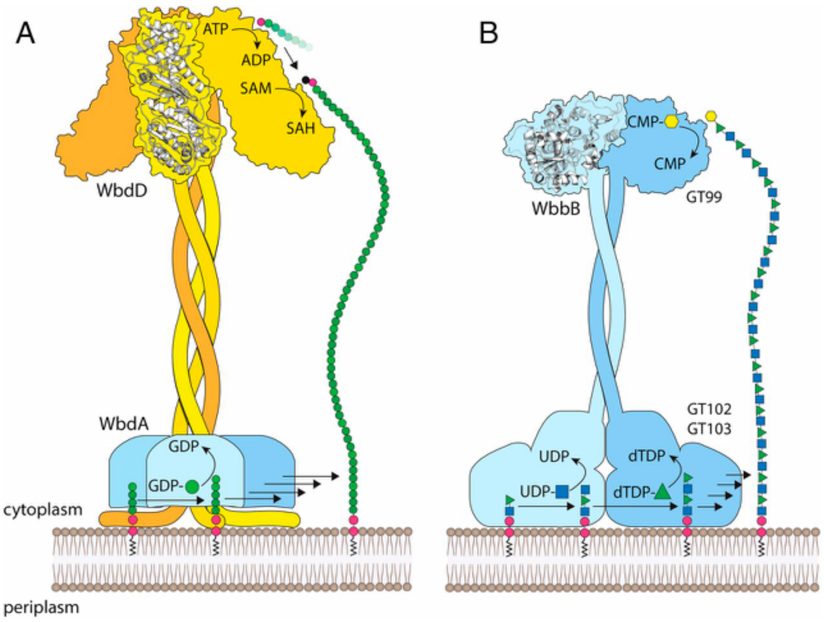


Figure 1.12 Cartoon representation of the O-PS chain elongation and termination mechanisms from *E. coli* O9a (A) and *R. terrigena* (B). (Reproduced with permission from Williams, D. M.; Ovchinnikova, O. G.; Koizumi, A.; Mainprize, I. L.; Kimber, M. S.; Lowary, T. L.; Whitfield, C. *Proc. Natl. Acad. Sci. USA* **2017**, *114* (7), E1215).

1.4.1.5 Mechanism of O-PS Export by ABC Transporters with CBMs

Early in 2018, the structure of the O-PS ABC transporter from *A. aeolicis* was reported by Zimmer and co-workers, and a mechanism for O-PS export was proposed (Figure 1.13).[103] In the model, the transporter exists in a resting state until the O-PS with the terminal modification becomes tethered to the CBM.[103] After tethering, the "gate helix" of the NBD at the cytosolic interface recognizes and binds to the PP-GlcNAc moiety, which opens the transporter channel.[103] The undecaprenyl lipid is then spontaneously "flipped" across the membrane, while the lipid is likely still anchored in the membrane.[103] After the lipid is flipped, the polymer is transported to the periplasm through the transmembrane channel.[103] This channel is large enough to facilitate ~8–10 sugar residues and contains a clustering of aromatic amino acid residues, which mediate

transport of the O-PS[103] via CH-π stacking interactions.[104] After release of the O-PS, the transporter returns to its resting state.[103]

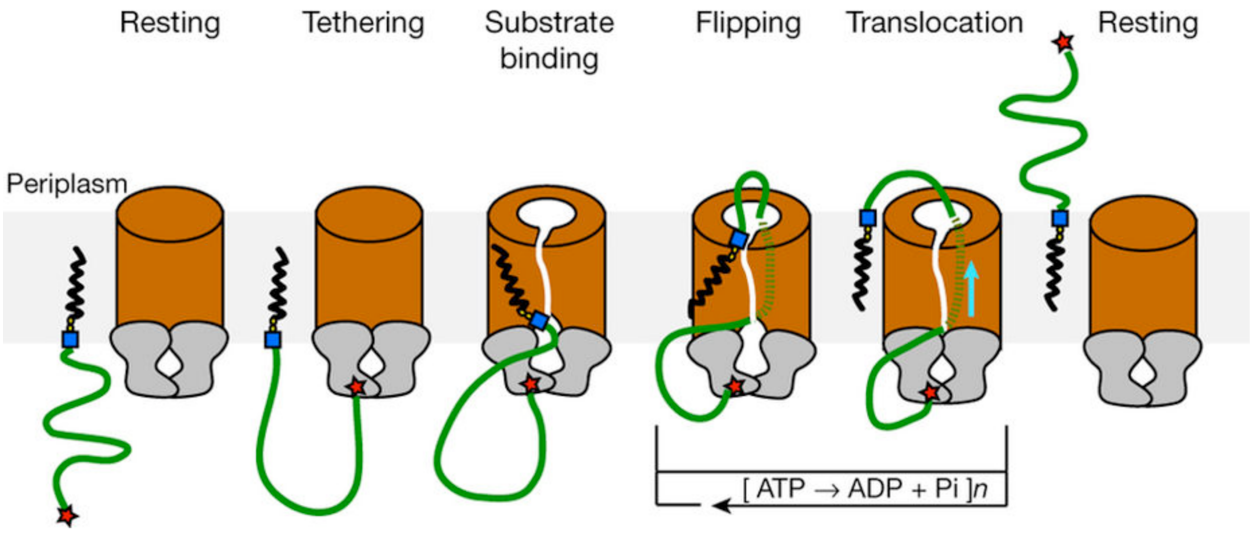


Figure 1.13 Postulated mechanism of O-PS export. First, the CBM tethers the terminal modification (star) to the transporter. Next the PP-GlcNAc moiety binds to and opens the gate to the membrane channel. After the und-lipid is flipped, the carbohydrate polymer is translocated to the periplasm by repeated cycles of ATP hydrolysis. (Reproduced with permission from Bi, Y.; Mann, E.; Whitfield, C.; Zimmer, J. *Nature* **2018**, *553*, 361).

1.4.2 Termination without a Chain Modification (*K. pneumoniae* O2a)

In 1989, a high molecular weight fraction of LPS from *Klebsiella pneumoniae* serotype O1 was shown to be an important virulence determinant by demonstrating that the molecule is responsible for the bacteria's resistance to nonspecific, complement-mediated serum killing.[23] The field then set out to determine and fully characterize this molecule to find a way to combat this virulence determinant. In the early 90's, two different research groups reported their findings at nearly the same time. Both Whitfield and co-workers,[105] as well as Kol and co-workers,[106,107] found that the O-antigens in *Klebsiella pneumoniae* serotype O1 were glycans composed of galactose-containing repeat units. Two distinct O-antigens were described for serotype O1: D-

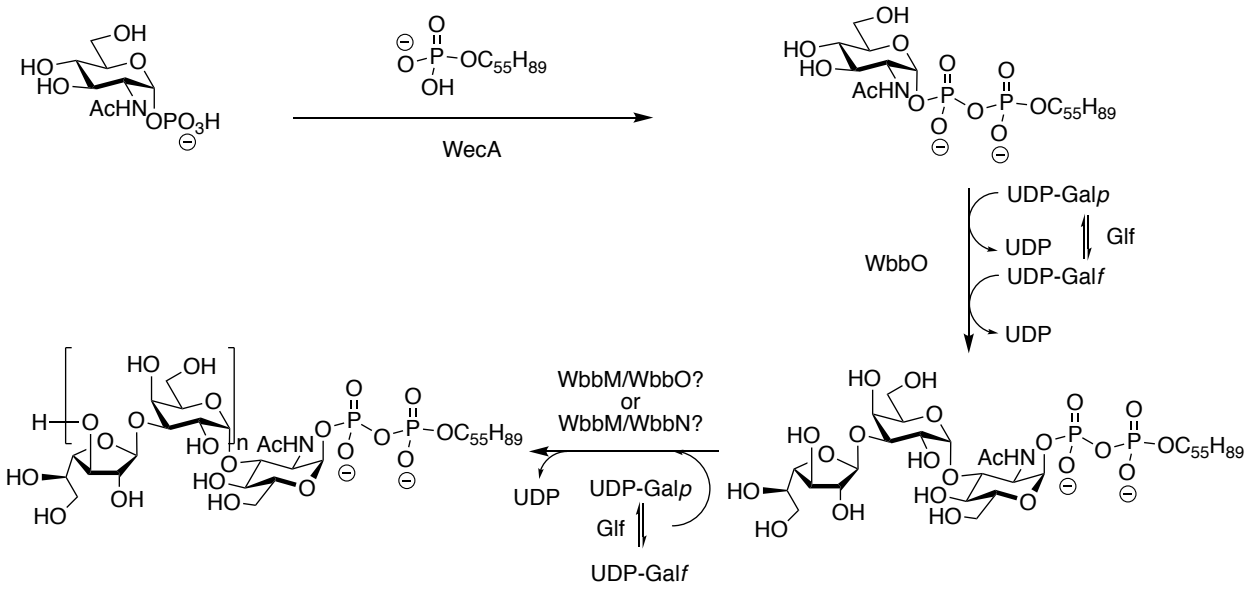
galactan I and D-galactan II (Figure 1.14). D-galactan I consists of a →3)-β-D-Gal*f*-(1→3)-α-D-Gal*p*-(1→ repeating unit polymer. D-galactan II consists of a →3)-α-D-Gal*p*-(1→3)-β-D-Gal*p*-(1→ repeating unit polymer. Serotype O1 LPS has a D-galactan I core in its O-PS, which is capped by an additional D-galactan II polymer.[105] *Klebsiella pneumoniae* serotype O2a was later shown to have D-galactan I as its only O-PS structure.[108] Other serotypes: O2c, O6, and O8, also have D-galactan I as a major component of their LPS.[108]

Figure 1.14 Repeating unit structures from the D-galactan I (left) and D-galactan II (right) O-antigens found in *Klebsiella pneumoniae* serotype O1.

After identification of the O-antigens in *Klebsiella pneumoniae*, the pathway of O-antigen biosynthesis was studied as a potential target for therapeutics.[22] In 1992, the genes responsible for D-galactan I biosynthesis were first described,[109] but the genes responsible for D-galactan II biosynthesis could not be elucidated until 2014.[110] As the majority of *K. pneumoniae* serotypes contain D-galactan I as the core O-PS,[108] biochemical and chemical studies have focused on D-galactan I, including my own studies. For this reason, I now discuss the current understanding of the D-galactan I O-PS biosynthetic pathway from *K. pneumoniae* O2a.

1.4.2.1 Initiation and Polymerization of O-PS in *K. pneumoniae* O2a

As in *E. coli* O8/O9a and *R. terrigena*, O-PS biosynthesis in *K. pneumoniae* O2a begins with the formation of und-PP-GlcNAc by WecA on the cytoplasmic face of the inner membrane (Scheme 1.4).[88,109,111] The biosynthesis of the O-PS in *K. pneumoniae* O2a requires UDP-Gal*p* and UDP-Gal*f*, which are provided by UDP-galactose-4-epimerase,[109] and UDP-galactopyranose mutase,[112] respectively. There are three predicted GTases that are responsible for polymerization of the O-PS (WbbM, WbbN, and WbbO),[113] but only two have confirmed GTase activity *in vitro* (WbbM and WbbO).[113,114] All three enzymes interact to form a membrane-associated complex.[113]



Scheme 1.4 Putative O-PS biosynthetic pathway in *K. pneumoniae* O2a.

It is believed that WbbO is a bifunctional galactosyltransferase that uses und-PP-GlcNAc as an acceptor and adds the first Gal*p* and first Gal*f* residues to the O-PS, forming und-PP-GlcNAc-Gal*p*-Gal*f* as its product.[111,114] The N-terminus of WbbO is a hydrophobic region

partially responsible for its membrane association, and its GTase activity is confined to the C-terminus.[113]

WbbM is a 73 kDa galactosyltransferase[115] that is postulated to add the third residue, a Gal*p* residue, to the growing O-PS.[114] WbbM contains two independently folded domains: a predicted GTase C-terminal domain with α-(1→3) galactopyranosyltransferase activity and a separate N-terminal domain; both domains are required for O-PS biosynthesis *in vivo*.[113]

The enzyme WbbN is predicted to be a GTase;[113] however, *in vitro* experiments have been unable to confirm this activity.[114] Mutation of a large portion of its putative GTase domain suggested that WbbN is indeed a glycosyltransferase, and that it is required for biosynthesis of D-galactan I polymer.[113] There is discrepancy between the *in vitro* studies, which suggest that polymer extension requires only WbbM and WbbO,[114] and the *in vivo* studies, which suggest WbbN is an essential glycosyltransferase required for D-galactan I synthesis.[113,114] This discrepancy warrants further investigation of these three enzymes.

In addition, the entire process described above and outlined in Scheme 1.4 has little biochemical support. To unequivocally determine the catalytic activities of the GTases, recombinant enzymes need to be studied with the appropriate synthetic substrates, which were not available at the time of earlier studies.[113]

1.4.2.3 Chain Termination and Export of O-PS in *K. pneumoniae* O2a

The O-PS of *K. pneumoniae* O2a, D-galactan I, does not have a chain-terminating residue. Therefore, O-PS is synthesized by a mechanism for O-PS biosynthesis different from *E. coli* O9a and *R. terrigena*.[80] The ABC-transporter of *K. pneumoniae* O2a can export *E. coli* O9a O-PS when expressed in *E. coli* O9a, suggesting the transporter does not have a substrate-

specific binding domain, such as a CBM.[80] In *K. pneumoniae* O2a, O-PS polymerization and export are temporally coupled, and this process determines chain length.[80] This coordination of O-PS polymerization and export is crucial in this system.[80]

In particular, the stoichiometry of the ABC transporter and O-PS biosynthetic machinery is critical in determining O-PS chain length in *K. pneumoniae* O2a.[80] Overexpression of the ABC-transporter leads to a substantial reduction in the average O-PS chain length.[80] Control of O-PS chain length is lost without a functional ABC-transporter, leading to chains that are longer than the native O-PS; moreover, these chains are not substrates for export after polymerization.[80]

It is unclear whether this effect is because there is a disconnection in the synthesis and export process, or if there is a maximum length that the transporter can export, or even both.[80] Without a distinct binding motif in the ABC-transporter, the transporter might recognize a conserved feature of the O-PS, such as the lipid or the GlcNAc residue at the reducing end.[80] Based on the putative mechanism discussed in Section 1.4.1.5, this is likely the case, as the *K. pneumoniae* O2a transporter shares key structural features to transporters with CBMs.[103] If the reducing end is recognized, then the ABC transporter might actively pull the O-PS chain away from the GTases to terminate chain elongation, or it could be possible that when the O-PS reaches the appropriate length, the GTases are physically removed from the growing chain.[80] Many questions still remain about this second mechanism (Figure 1.15) of O-PS polymerization, chain length control, and export.

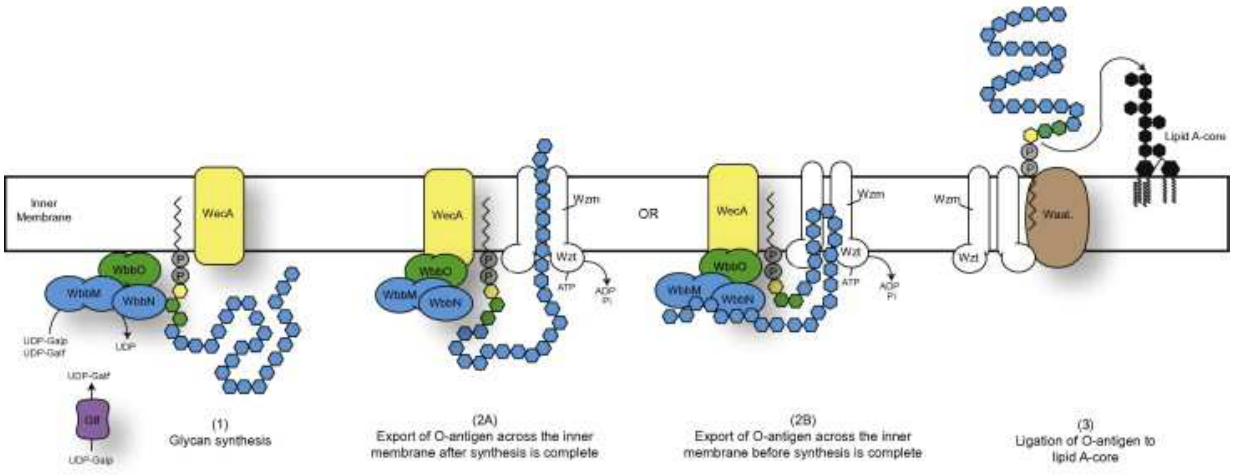


Figure 1.15 Cartoon overview of the ABC transporter-dependent O-PS biosynthetic complex from *K. pneumoniae* O2a. (Taken with permission from Greenfield, L. K.; Whitfield, C. *Carbohydr. Res.* **2012**, *356*, 12).

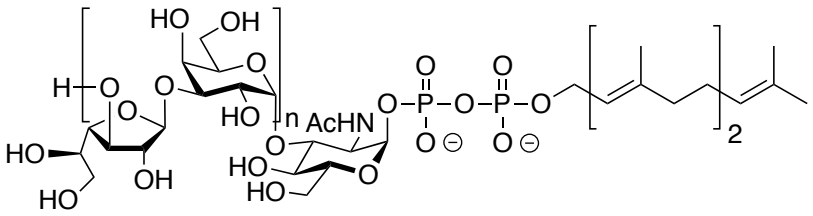
1.5 Statement of Research Purpose

Many questions remain about the details of LPS biosynthesis, particularly the O-PS. Because the O-antigens are important virulence factors of pathogenic Gram-negative bacteria, understanding their assembly could provide new treatments and antibiotics that target these pathogenic bacteria.[22] Because the *Klebsiella pneumoniae* serotype O2a O-PS biosynthetic pathway does not have a chain termination modification,[80] it is a particularly interesting system to investigate. Questions of particular interest are: What are the exact roles of each GTase? How is the chain length controlled? And what is the mechanism of transport across the inner membrane? To address these questions, we need access to chemically pure, structurally well-defined, synthetic glycans.[113]

In *Klebsiella pneumoniae* O2a, the mechanism of transport requires an ABC transporter.[115] The transporter is critical to regulating the chain elongation of the O-PS, and that process of transport across the inner membrane to the periplasm is coupled to the chain

elongation of the O-PS.[80] There are three membrane-located GTases that are responsible for the chain elongation of the O-antigen: WbbM, WbbN, and WbbO.[113] However, the substrates for each GTase have been unclear to this point, despite the various biochemical experiments that have tried to decipher the roles of the GTases in this pathway.[113,114] Without an understanding of the catalytic activities of these GTases, we cannot understand the assembly of the O-PS and transport across the inner membrane, prior to the ligation to the lipid A core to make the full length LPS.

In Chapter 2, I discuss the synthesis of low molecular weight acceptors that are being used to unequivocally assign the roles of each GTase. These compounds will provide insight into the roles of each GTase, but this still leaves questions about how the chain length is controlled and the mechanism of transport across the membrane. To answer these questions, we need access to oligosaccharides that better mimic the native substrates. In Chapter 3, I discuss the synthesis of large, lipid-linked oligosaccharides related to *K. pneumoniae* O2a O-PS. Our targets are five molecules, of varying repeating unit length (4, 8, 12, 16, and 20) that resemble the native O-PS precursor for D-galactan I (Figure 1.16).[105] These molecules, containing up to 41 monosaccharide residues, include a short isoprenyl diphosphate lipid at the reducing end. Based on earlier work,[116] we believe this shorter lipid will serve as an effective mimic of the native undecaprenol phosphate. These compounds will be used to further elucidate the biosynthetic pathway of the LPS in *Klebsiella pneumoniae*.



where n = 4, 8,12, 16, and 20

Figure 1.16 Five target molecules related to D-galactan I.

Chapter 2: Synthesis of Probes to Elucidate Glycosyltransferase Activities in *K. pneumoniae* O2a O-PS Biosynthesis

2.1 Introduction

To elucidate the O-PS biosynthetic pathway from *K. pneumoniae* O2a, we need a clear understanding of the glycosyltransferase (GTase) activities involved. There are three GTases – WbbM, WbbN, WbbO – that are responsible for the synthesis of the *K. pneumoniae* O2a O-PS, which is also known as D-galactan I.[80,113] The activities of these three GTases are coupled with an ABC-transporter that is responsible for O-PS translocation to the periplasm, where the O-PS is ligated to the core oligosaccharide to make full length LPS.[80] In 2001, the first analysis of the GTases from D-galactan I biosynthesis was reported; however, the results were inconclusive, and the precise role of each enzyme could not be determined.[114] More recent biochemical experiments have also not been able to further clarify the catalytic activities of the GTases in this system.[113]

In the most recent study, the authors stated that the only way to unequivocally determine the activities of the GTases would be to use chemically pure, synthetic acceptors as substrates, which at the time were not available.[113] Our lab has previously collaborated with these authors using synthetic acceptors to determine the roles of the GTases involved in the *E. coli* O9a LPS biosynthesis.[90,92,94,117] Therefore, we believed that we could also contribute, with my synthetic chemistry skills, to deciphering the roles of the GTases in *K. pneumoniae* O2a.

We envisioned that fluorescein-tagged probes would be good substrates for these GTases. The fluorescein moiety would ease the analysis of the biochemical experiments by allowing convenient monitoring by TLC or HPLC. We decided to synthesize two disaccharides, **2.1** and **2.2**, and a monosaccharide, **2.3** (Figure 2.1). Disaccharides **2.1** and **2.2** would mimic the repeating unit of the O-PS glycan; **2.1** would replicate an intermediate bearing a terminal Gal*p* monosaccharide, and **2.2** would replicate an intermediate bearing a terminal Gal*f*

monosaccharide. Compound **2.3** would emulate the GlcNAc-PP-und intermediate that serves as the acceptor for addition of the first carbohydrate residue. Using these synthetic small molecule acceptors, we anticipated that we could unequivocally determine the catalytic activities of WbbM, WbbN, and WbbO. In this chapter, I describe the synthesis of **2.1**–**2.3**.

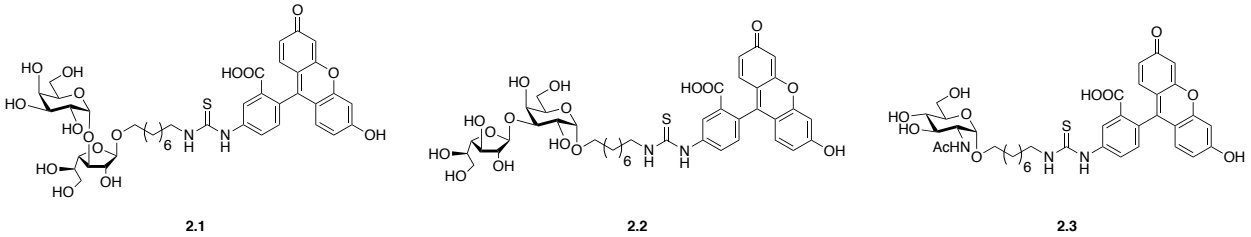


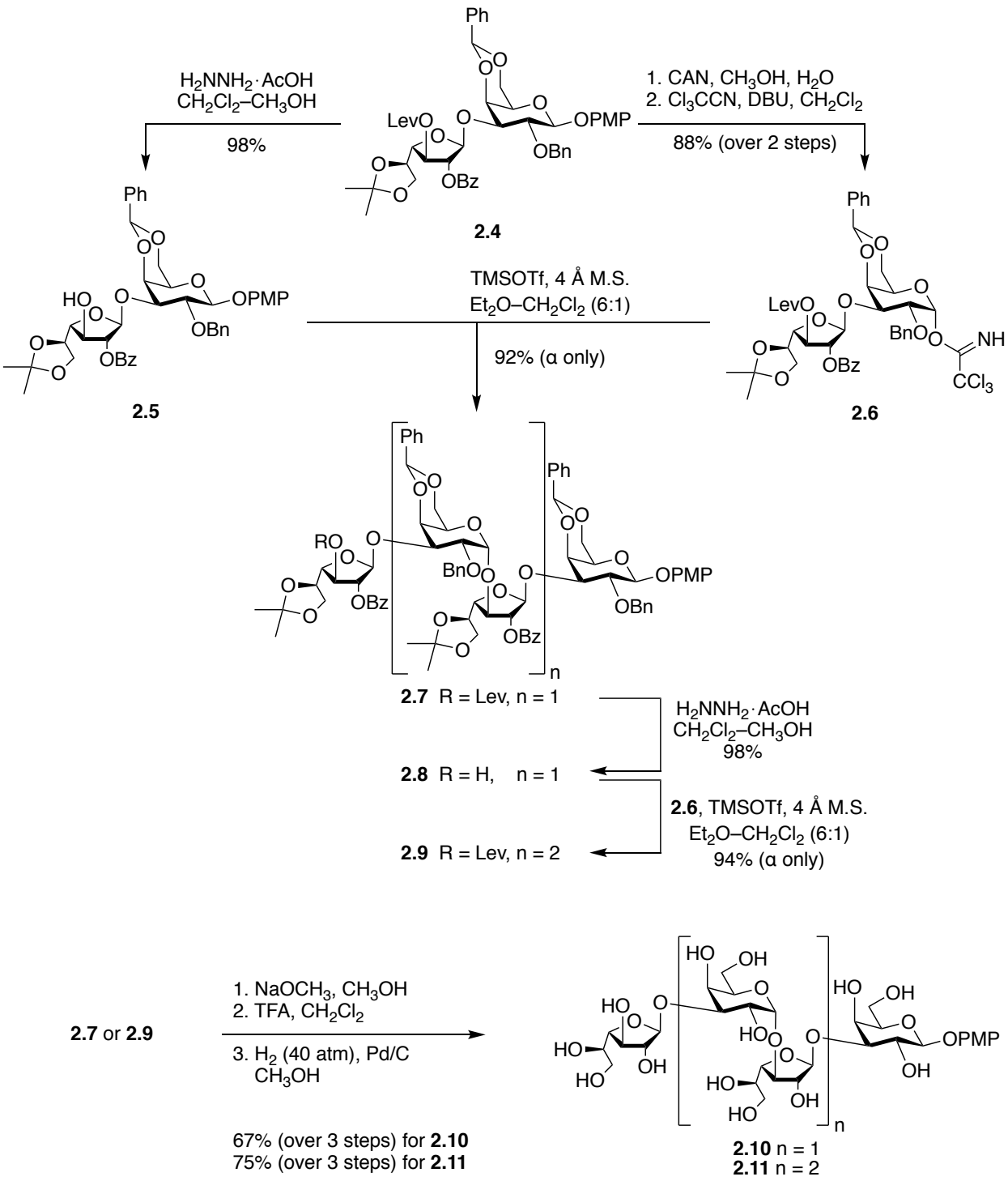
Figure 2.1 Three fluorescein-tagged biosynthetic probes **2.1**–**2.3**.

2.1.1 Previous Syntheses of D-Galactan I Fragments

There are five previous reports of the synthesis of D-galactan I fragments.[118-122] Two of these reports feature synthesis of the Gal*f*-Gal*p* disaccharide repeating unit,[118,119] one publication describes construction of the Gal*p*-Gal*f* disaccharide repeating unit,[121] and the other two papers report preparation of tetra- or hexasaccharide fragments.[120,122] Key challenges in the synthesis of these targets include: 1) generation of the galactofuranose ring system, which is the thermodynamically least stable natural cyclic form, and 2) stereoselective introduction of the α-galactopyranoside residue. These syntheses used different methods to overcome the challenges in synthesizing galactofuranosides, such as kinetically-controlled cyclization with $FeCl_3$ as a Lewis acid,[120,123] a per-*O*-silylation of furanose derivatives,[119] or a pyranoside-into-furanoside (PIF) rearrangement.[121,122] In three syntheses, the activation of the glycosyl donors at low temperatures, in ethereal solvents for two of them,[120,122] was responsible for the stereoselective

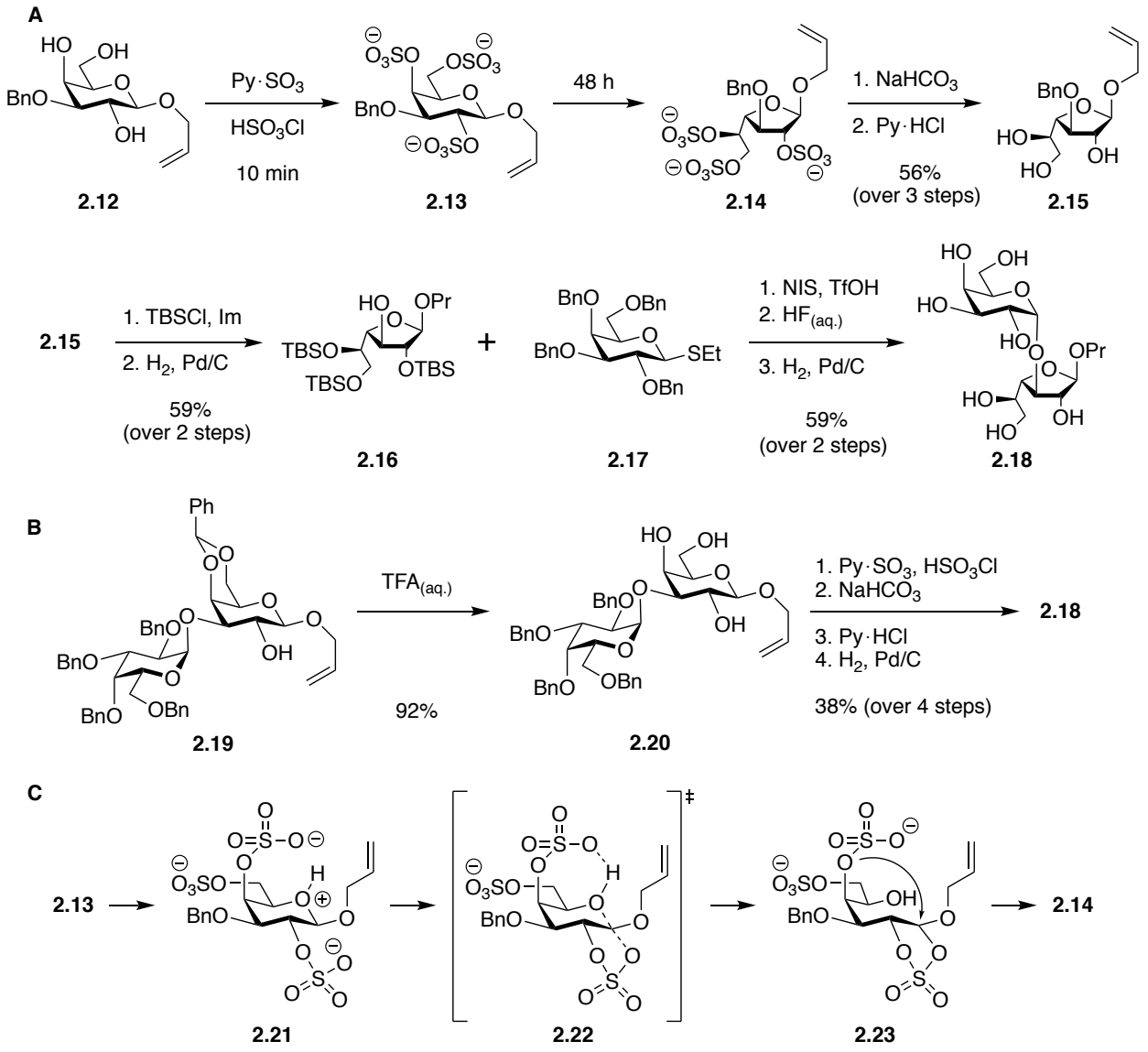
addition of the α-galactopyranoside residue.[120-122] Two reports did not install the α-galactopyranosyl linkage, as the Gal*p* residue was left as a free reducing sugar.[118,119] I will not further discuss these two papers,[118,119] as the authors did not address this challenge. I will instead focus on the reports that address both challenges.[120-122]

Zhu and Yang reported the synthesis of tetra- and hexasaccharide fragments related to the D-galactan I (Scheme 2.1).[120] The authors used $FeCl_3$ to generate the galactofuranose ring,[123] which was converted to a donor and glycosylated to give disaccharide **2.4**. This key conserved building block **2.4** first installed the 1,2-*trans*-β linkage. Disaccharide **2.4** was then converted to alcohol **2.5**, which was used as an acceptor in a 2 + 2 glycosylation. Compound **2.4** was also converted to imidate **2.6**, which would be used as a donor in their 2 + 2 and 4 + 2 glycosylations. The authors chose to elongate the fragments via the formation of the 1,2-*cis*-α glycosidic linkage, a more challenging linkage to install. Using a mixture of ether and dichloromethane as the solvent, both the 2 + 2 and the 4 + 2 glycosylation reactions gave compounds **2.7** and **2.9**, respectively, as the desired α-anomers in high yields. After deprotection, the authors obtained the tetra- and hexasaccharide fragments, **2.10** and **2.11**, respectively.



Scheme 2.1 Synthesis of the tetra- and hexasaccharide fragments related to D-galactan I reported by Zhu and Yang.[120] Their synthesis featured a key conserved disaccharide intermediate **2.4**.

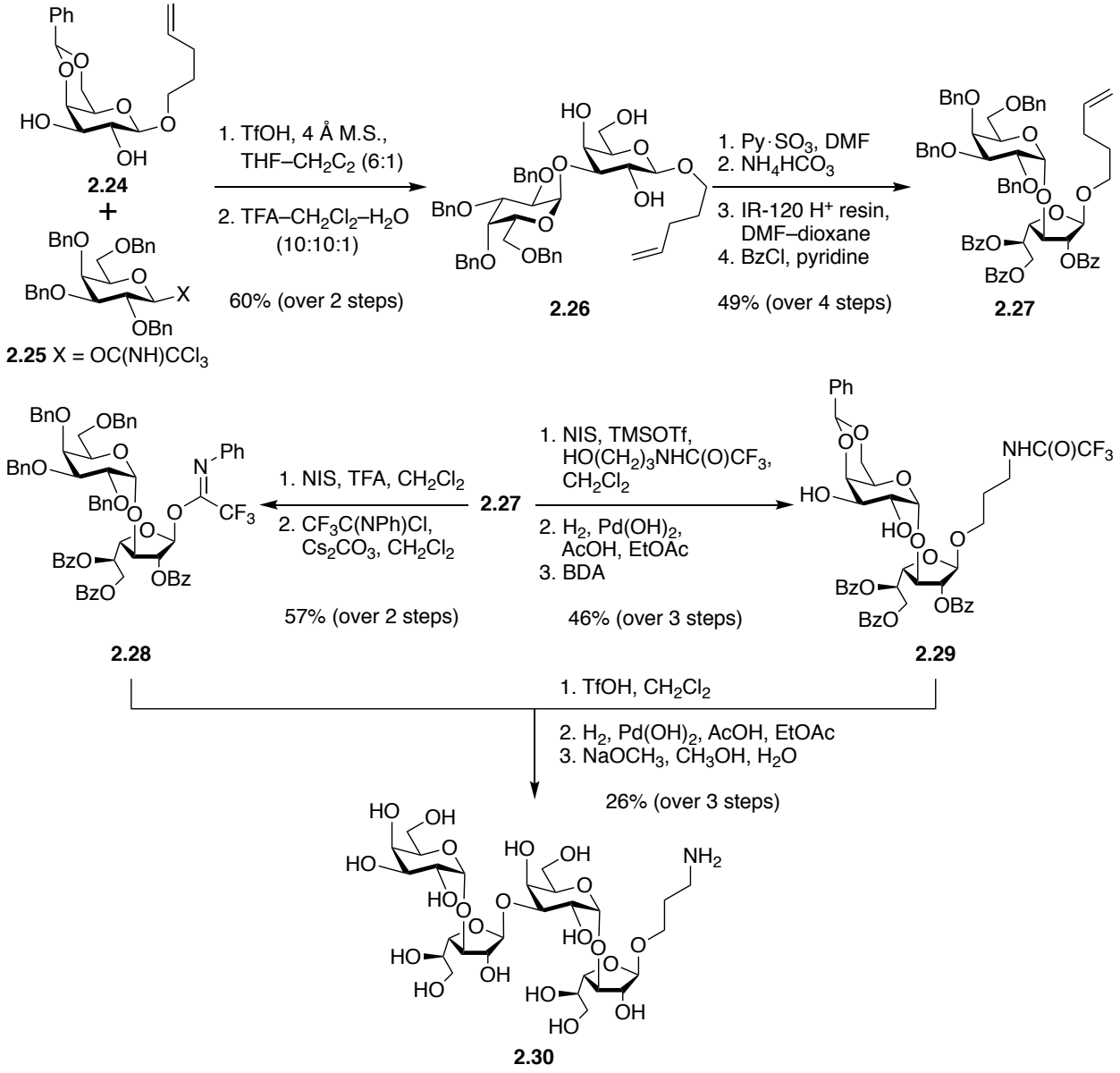
Nifantiev and co-workers published two sequential reports on the synthesis of compounds related to D-galactan I.[121,122] These reports featured a pyranoside-into-furanoside (PIF) rearrangement, which the authors used to overcome the challenge of synthesizing galactofuranosides. In the first publication,[121] the authors used the PIF rearrangement to form the galactofuranose ring **2.15** (Scheme 2.2A), converted it to acceptor **2.16**, which was then reacted with galactopyranosyl donor **2.17** at –40 °C to access the α-galactopyranosyl linkage. After deprotection, the authors obtained **2.18**. The authors also showed that the PIF rearrangement can be done on appropriately protected disaccharides (Scheme 2.2B), converting **2.19** into the same disaccharide **2.18**. Mechanistically, it is believed that both the 2-*O*-sulfate and the 4-*O*-sulfate on the galactopyranose ring are involved in the ring opening/closing of the PIF rearrangement (Scheme 2.2C).[121,124]



Scheme 2.2 A) Using a pyranoside-into-furanoside (PIF) rearrangement, Nifantiev and co-workers synthesized disaccharide **2.18** related to D-galactan I.[121] B) They also showed the PIF arrangement works on appropriate disaccharides, converting **2.20** to **2.18**. C) The authors' mechanistic proposal for the PIF rearrangement, featuring intermediates **2.21** and **2.23** and transition state **2.22**.

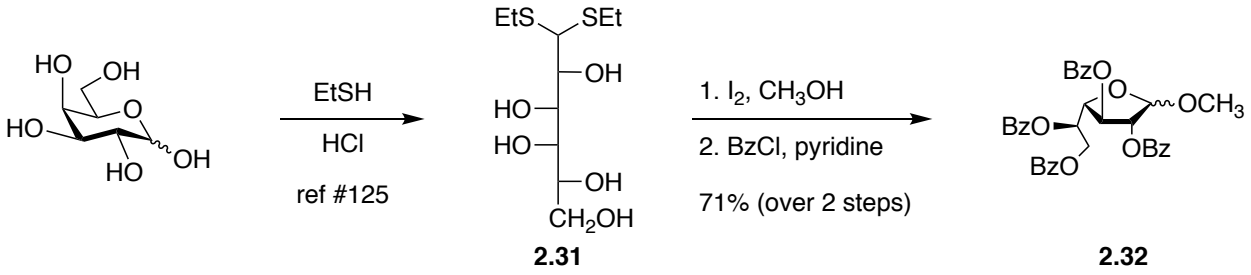
Nifantiev and co-workers later reported the synthesis of a tetrasaccharide related to D-galactan I (Scheme 2.3).[122] The authors initially formed the more challenging 1,2-*cis*-α glycosidic linkage. Activation of pyranoside donor **2.25** at –80 °C in THF–CH_2Cl_2 in the presence of **2.24**, and subsequent acid hydrolysis gave **2.26**. Disaccharide **2.26** then underwent

the PIF rearrangement, followed by benzoylation to give their conserved building block **2.27**. Disaccharide **2.27** was then either converted to donor **2.28** or to diol **2.29**. Using these disaccharides, the authors then installed the easier 1,2-*trans*-β linkage that gave the desired tetrasaccharide **2.30** after deprotection.



Scheme 2.3 Nifantiev and co-workers' synthesis of a tetrasaccharide related to D-galactan I.[122] It featured a conserved building block, disaccharide **2.27**. BDA, benzaldehyde dimethyl acetal.

A limitation of these syntheses is that most did not install a linker or tag to facilitate biochemical experiments. Adding a fluorescein tag was one of our primary objectives. Also, solutions used by both sets of authors to generate galactofuranose rings were a limitation, as I believed our lab developed a higher-yielding and more reliable method to access such species from D-galactose dithioacetal **2.31**[125] (Scheme 2.4).[126] In the work described above, low temperature activation of glycosyl donors in ethereal solvents was the primary method for installing the α-galactopyranoside residues. This approach could be applied in my synthesis, but other options exist. Overall, these reports leave room for improvement in synthesizing D-galactan I fragments.



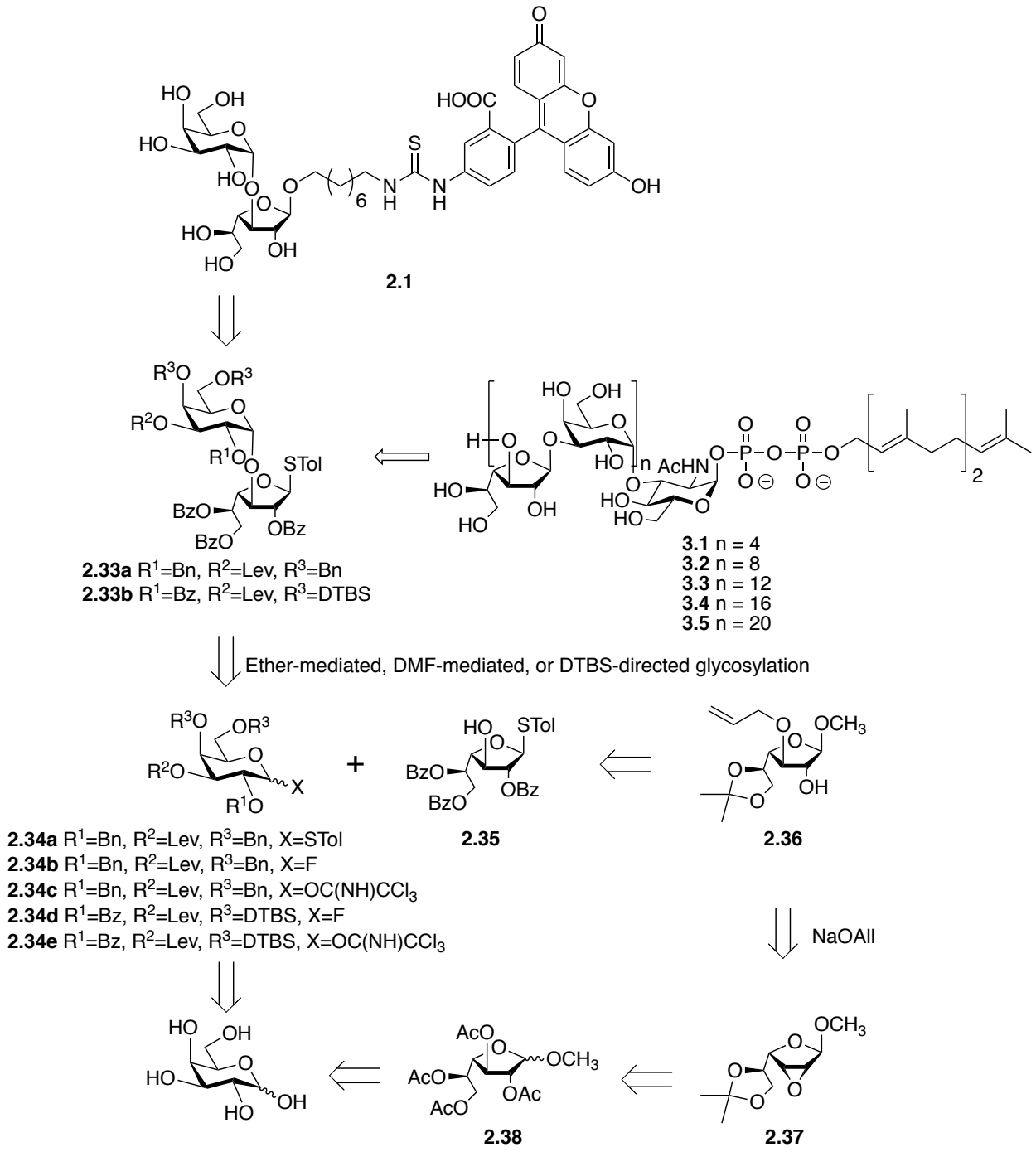
Scheme 2.4 Kinetically-controlled I_2-catalyzed cyclization of dithioacetal **2.31** to generate galactofuranosides.[126]

The use of a conserved disaccharide building block was common to the syntheses described above, and this seemed advantageous for my own synthesis. This approach would reduce the total number of steps and purifications, and allow me to focus on preparing large amounts of a single building block. I desired to install the more challenging 1,2-*cis*-α glycosidic linkage first as it would allow me to use the easier 1,2-*trans*-β glycosidic linkage in making target molecules **3.1**–**3.5**. This was the approach used by Nifantiev and co-workers in 2017.[122] If

I could obtain the "correct" disaccharide building block, then I could use it in the syntheses of the target **2.1**, and the larger probes **3.1**–**3.5**.

2.1.2 Original Synthetic Design for Small Molecule Probe 2.1 and Oligosaccharide Targets 3.1–3.5

I proposed a synthetic strategy for the synthesis of compound **2.1** that featured key disaccharide building block **2.33a/2.33b** (Scheme 2.5). I envisioned protecting the C-2 hydroxyl group on the galactofuranose residue of this disaccharide with a benzoyl group to ensure complete stereoselectivity, via neighboring group participation, in the subsequent 1,2-*trans*-β glycosylations. Therefore, stereoselective glycosylation of **2.33a** or **2.33b** with 8-azido-1-octanol, followed by global deprotection and conjugation to fluorescein isothiocyannate (FITC), would give the target compound **2.1**. I hoped that **2.33a** or **2.33b** would also be a key building block in the synthesis of the large target molecules **3.1–3.5**. To overcome the challenge of installing the α-galactopyranosyl linkage, I wanted to explore using a variety of galactopyranosyl donors (**2.34**) in various glycosylation conditions, such as DMF-mediated α-glycosylations (**2.34a**),[127] ether mediated α-glycosylations (**2.34b** and **2.34c**),[128] and di-*tert*-butyl-silylidene (DTBS) directed α-glycosylations (**2.34d** and **2.34e**),[129] using galactofuranoside acceptor **2.35** as a coupling partner. The thiogalactofuranoside acceptor **2.35** could easily be obtained from compound **2.36** via simple protecting group manipulations. Compound **2.36** can be obtained from the known 2,3-anhydro-guloside **2.37**,[130] via the selective opening of the epoxide with sodium allyloxide. Compound **2.37** would be obtained from galactofuranoside **2.38**.[131] Ultimately, all of the key building blocks can be obtained from D-galactose.

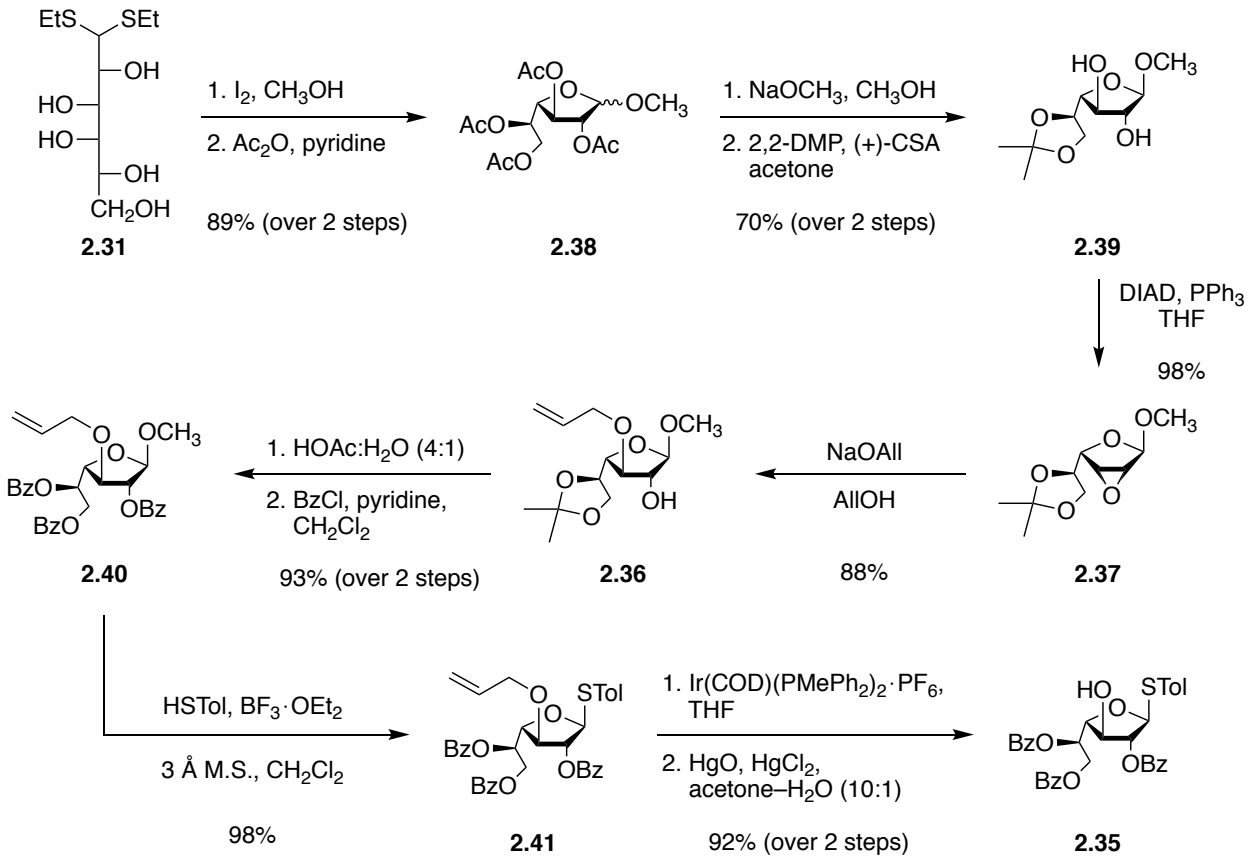


Scheme 2.5 Retrosynthetic analysis for compound **2.1**. Building block **2.33** could also be used for the synthesis of larger oligosaccharide targets **3.1**–**3.5**.

2.2 Results and Discussion

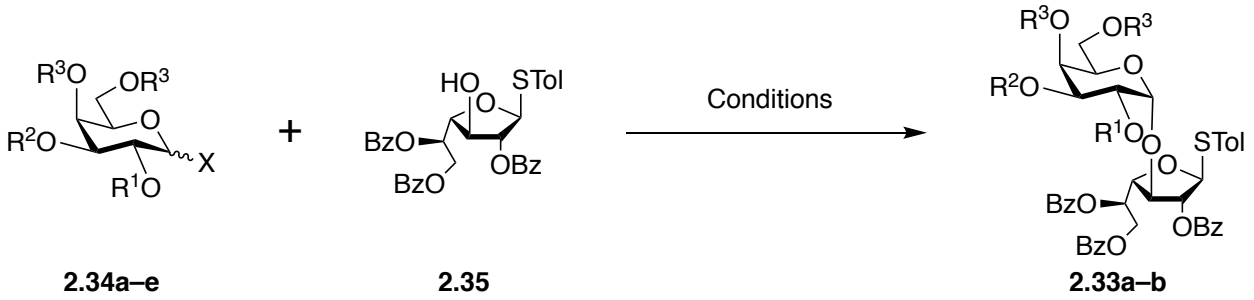
2.2.1 Synthesis of Galactofuranoside Acceptor 2.35 (Scheme 2.6)

Synthesis of compound **2.35** began with the I_2-catalyzed cyclization[126] of dithioacetal **2.31**[125] to give the known peracetylated methyl glycoside **2.38**[131] in 89% yield over two steps. Compound **2.38** was then converted to 2,3-anhydro-guloside **2.37**[130] in 69% yield over three steps. Opening of the epoxide to give galactofuranoside **2.36** was achieved in 88% yield by reaction with sodium allyloxide in allyl alcohol, which our group has used to open 2,3-anhydro-furanosides in previous studies.[132] The stereochemistry of **2.36** was confirmed by matching $^3J_{H\text{-}H}$ values for the proton signals around the galactofuranose ring with known compounds, including the singlet H-1 signal, indicative of a β-galactofuranoside.[133] The β-configuration of the guloside substrate controls the regioselectivity in the epoxide opening, since nucleophilic attack on the C-3 position of the galactofuranose ring is sterically preferred.[130] Removal of the isopropylidene group, followed by benzoylation, gave the fully protected monosaccharide **2.40** in 93% yield. Treatment of **2.40** with *p*-thiocresol and boron trifluoride gave the thioglycoside **2.41** in 98% yield. Conversion into thiogalactofuranoside acceptor **2.35** was achieved in two steps in 92 % yield. First, isomerization of the double bond was achieved using $Ir(COD)(PMePh_2)_2{\cdot}PF_6$ as the catalyst; then hydrolysis of the resulting vinyl ether was done by treatment with mercuric oxide and mercuric chloride in acetone–H_2O.



Scheme 2.6 Synthesis of galactofuranoside acceptor **2.35**.

With thioglycoside acceptor **2.35** in hand, attempts to obtain the disaccharide **2.33** were investigated. Many different conditions were tried with a variety of galactopyranosyl donors (**2.34a-e**) in the presence of thioglycoside **2.35** (Table 2.1); however, the desired disaccharide **2.33** was never obtained in appreciable yield, let alone enough for use as a conserved building block for the synthesis of both target **2.1** and targets **3.1**–**3.5**. The plan involving the disaccharide building block **2.33** was thus abandoned.



2.34a–e **2.35** **2.33a–b**

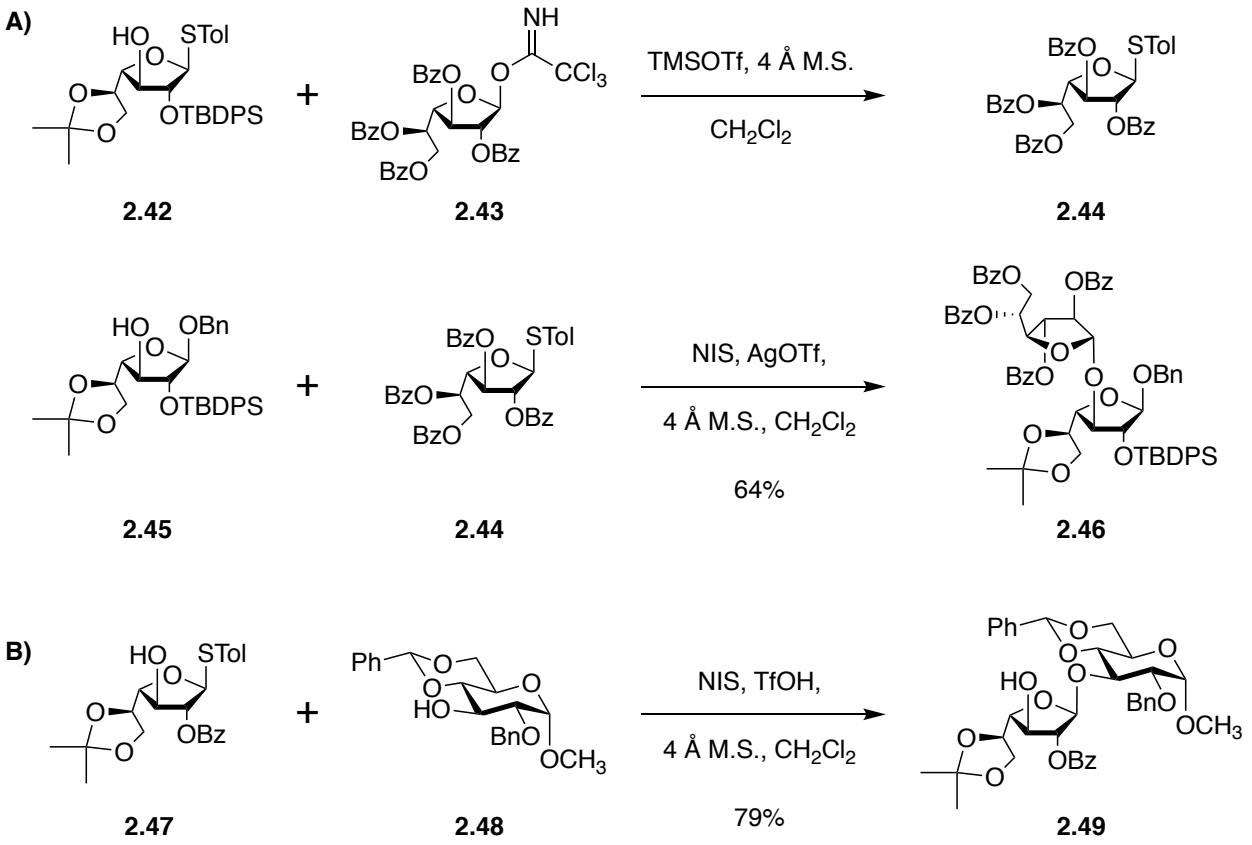
Donor	R^1	R^2	R^3	X	Conditions	Product	Yield
2.34a	Bn	Lev	Bn	STol	i. DMF, 3 Å M.S., CH_2Cl_2 ii. TMSOTf, NIS iii. **2.19**, 3 h, 0 °C	**2.33a**	17%
2.34b	Bn	Lev	Bn	F	Cp_2ZrCl_2, AgOTf, **2.19**, Et_2O, 3 Å M.S., rt, 1 h	**2.33a**	N/O
2.34c	Bn	Lev	Bn	$OC(NH)CCl_3$	TMSOTf, **2.19**, Et_2O, 3 Å M.S., –10 °C, 30 min	**2.33a**	11%
2.34d	Bz	Lev	DTBS	F	Cp_2ZrCl_2, AgOTf, **2.19**, CH_2Cl_2, 3 Å M.S., rt, 18 h	**2.33b**	N/O
2.34e	Bz	Lev	DTBS	$OC(NH)CCl_3$	TMSOTf, **2.19**, CH_2Cl_2, 3 Å M.S., –10 °C, 30 min	**2.33b**	N/O

Table 2.1 Selected examples of glycosylation results with acceptor **2.35** and donors **2.34a–e**. (N/O = Not Observed)

2.2.2 Revised Retrosynthetic Design and Synthesis of Disaccharide Probe 2.1

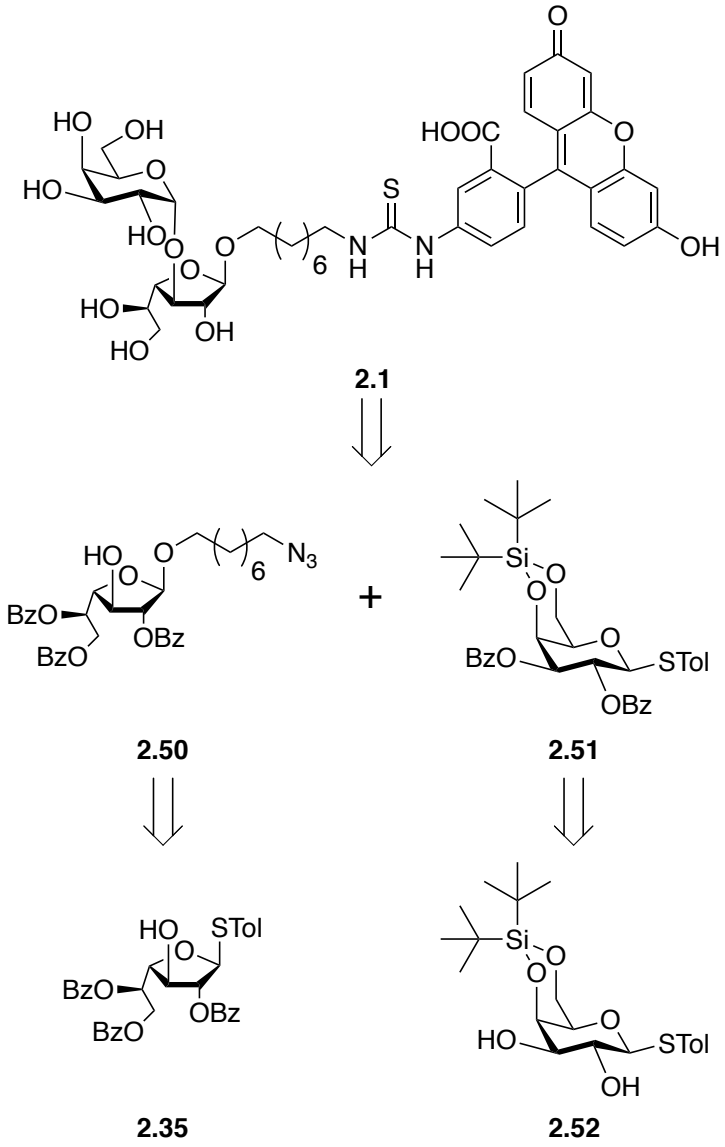
I concluded that the issue in section 2.2.1 was likely the acceptor **2.35**. At around the same time that I came to this conclusion, two different groups published reports with similar results to mine (Scheme 2.7). Wang and co-workers[134] reported that their attempts to use thiogalactofuranoside **2.42** as an acceptor in glycosylations with imidate **2.43** resulted in only the aglycone transfer product **2.44** and none of the desired disaccharide product. On the other hand, when the O-glycoside **2.45** was used as the acceptor with thioglycoside **2.44**, the authors obtained the desired disaccharide **2.46** in 64% yield. Yang and co-workers[135] reported the

selective glycosylation of the C-3 hydroxyl group of galactopyranose **2.48** in the presence of the C-3 hydroxyl of the galactofuranosyl donor **2.47**, producing disaccharide **2.49** in 79% yield. These combined results show that the C-3 hydroxyl of thiogalactofuranosides are weak nucleophiles in glycosylation reactions, possibly due to the conformation of the galactofuranose ring. Comparison of the ^{1}H NMR signals of H-1 of β-thiogalactofuranosides to H-1 of β-*O*-galactofuranosides supports this hypothesis. For β-thiogalactofuranoside **2.35**, the H-1 signal is a doublet ($^{3}J_{1,2}$ = 3.4 Hz), whereas the H-1 signal of β-*O*-galactofuranoside **2.50** is a singlet, substantiating that these rings are in different conformations.



Scheme 2.7 Previous reports of thioglycoside alcohols used in glycosylations. A) Wang and co-workers[134] reported that there was no desired disaccharide product observed when thioglycoside **2.42** was used as the acceptor in the reaction with **2.43**; however, when O-glycoside **2.45** was used as the acceptor with donor **2.44**, the desired product disaccharide **2.46** was obtained in moderate yield. B) Yang and co-workers[135] reported the selective glycosylation of the 3-OH of the galactopyranose ring **2.48** in the presence of the 3-OH of the galactofuranose ring **2.47**.

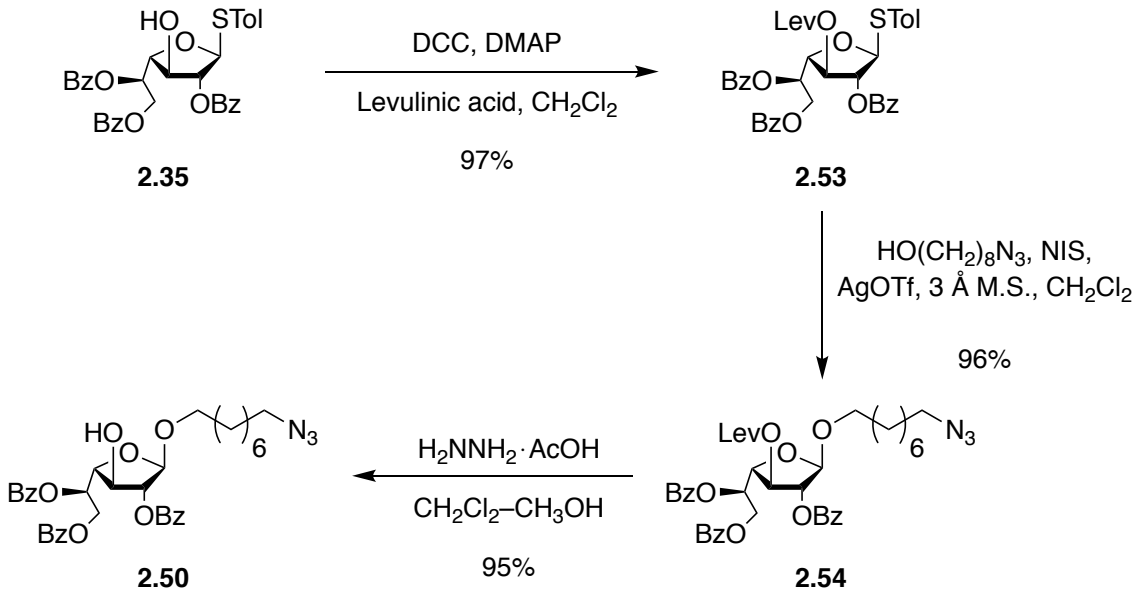
Thus, I changed my route to access **2.1** (Scheme 2.8). I hypothesized that the O-glycoside acceptor **2.50** would have improved reactivity as an acceptor in the 1,2-*cis*-α glycosylation. To ensure complete 1,2-*cis*-α stereoselectivity in the glycosylation, I envisioned using the DTBS-protected galactopyranoside **2.51** as the donor. Galactopyranoside **2.51** would come from compound **2.52**, which can be prepared from D-galactose.[136] The galactofuranose acceptor could be obtained from compound **2.35**. I envisioned that this more straightforward synthetic route would alleviate the difficulties found using compound **2.35** as an acceptor.



Scheme 2.8 Revised retrosynthesis of target **2.1**.

Synthesis of acceptor **2.50** began from the previously discussed thiogalactofuranoside **2.35** (Scheme 2.9). Protection of the C-3 hydroxyl group of compound **2.35** with a levulinoyl ester gave the galactofuranosyl donor **2.53** in 97% yield. Activation of donor **2.53** with NIS and AgOTf in the presence of 8-azido-1-octanol gave the β-O-glycoside **2.54** in 96% yield. A singlet at 5.25 ppm in the ^{1}H NMR spectrum confirmed the β-glycosidic linkage.[133] The desired

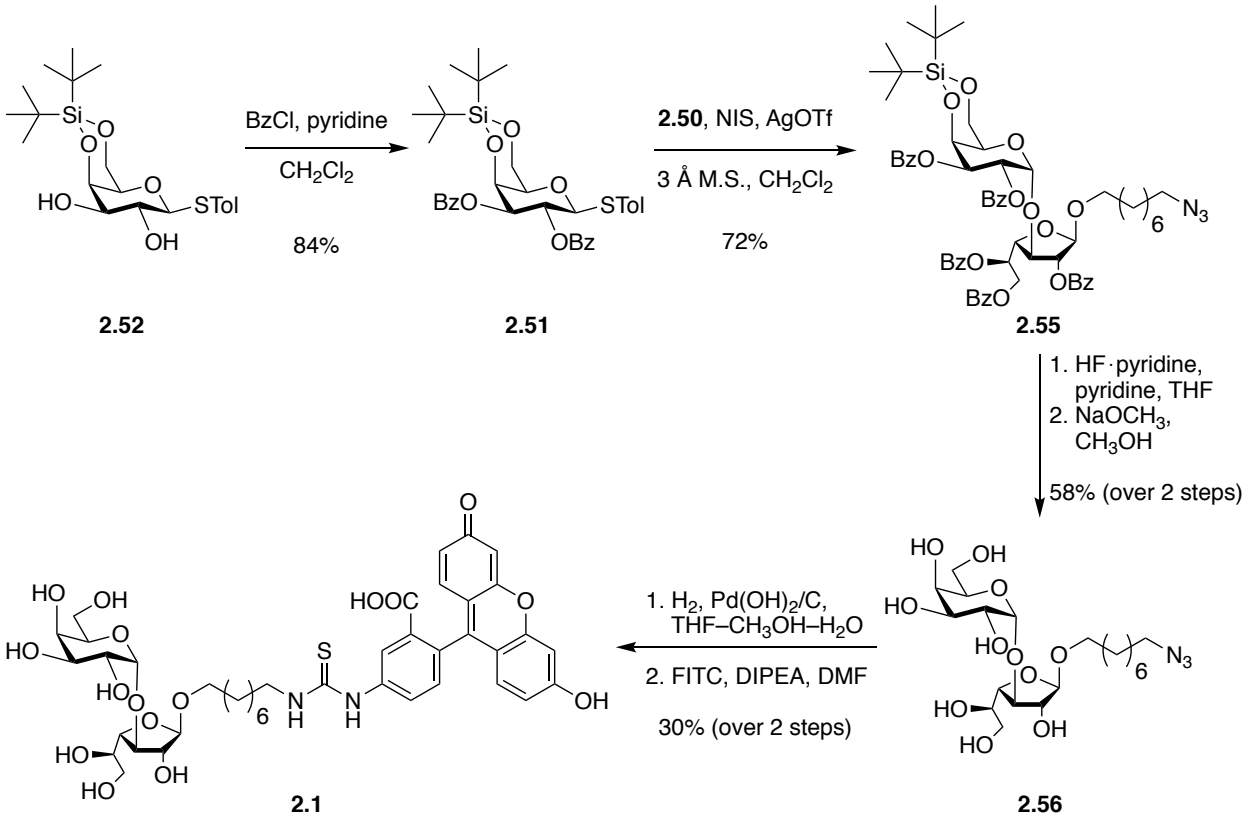
galactofuranoside acceptor **2.50** was obtained after removal of the levulinoyl ester with $H_2NNH_2 \cdot AcOH$ in 95% yield.



Scheme 2.9 Synthesis of galactofuranoside **2.50**.

Having developed an approach to azidooctyl glycoside **2.50**, the synthesis of target **2.1** (Scheme 2.10) began with the preparation of galactopyranosyl donor **2.51**, which was obtained in 84% yield by benzoylation of **2.52**.[136] Activation of **2.51** with NIS and AgOTf in the presence of galactofuranoside acceptor **2.50** gave the desired disaccharide **2.55** in 72% yield with α-only selectivity. The magnitude of the $^3J_{H1\text{-}H2}$, 3.7 Hz, for the anomeric proton of the newly formed glycosidic bond confirmed the α-stereochemistry. Removal of the DTBS group with HF·pyridine, followed by removal of the benzoyl esters with catalytic sodium methoxide, gave the deprotected disaccharide **2.56** in 58% yield over two steps. Extractions in the work-up protocols may have resulted in loss of yield in these reactions. Reduction of the azide by hydrogenolysis, then treatment of the intermediate amine with FITC and 1% DIPEA in DMF

gave the first desired disaccharide target **2.1** in 30 % yield over the two steps. This low yield may be due to the compound's poor solubility in water because the hydrophobic fluorescein moiety is hydrophobic. Hydrophobicity led to difficulties in isolation by C18 chromatography, as some fractions of **2.1** contained hydrophobic impurities.

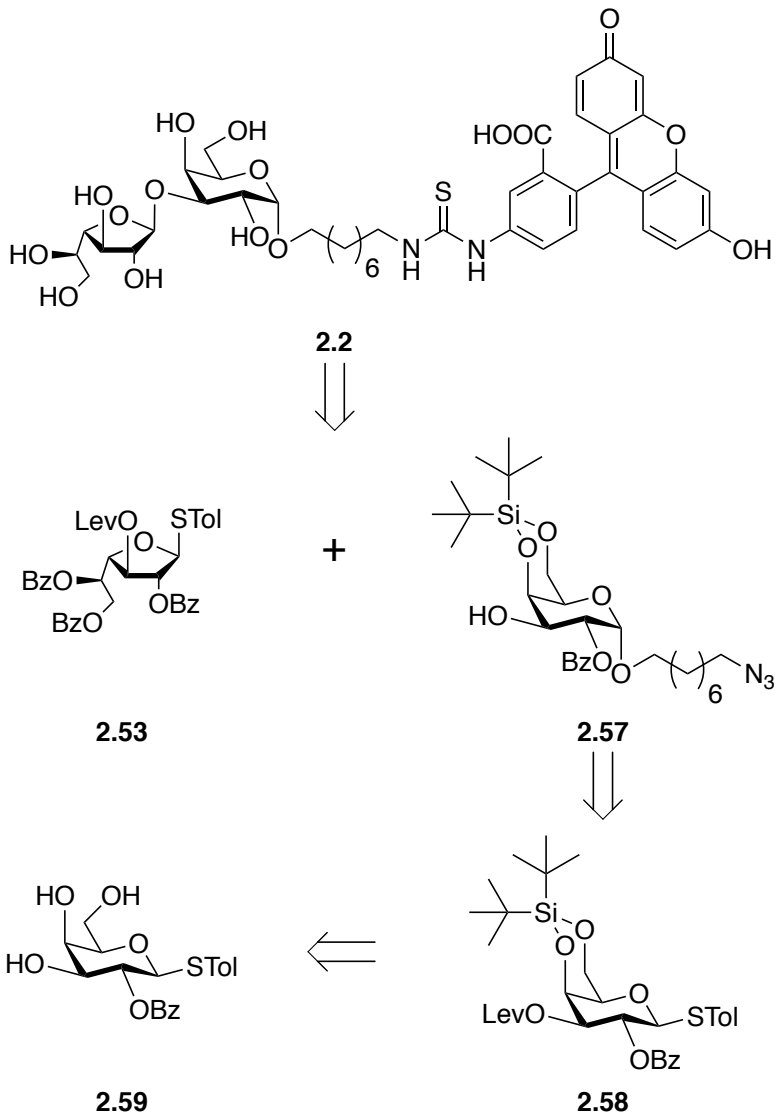


Scheme 2.10 Synthesis of target compound **2.1**.

2.2.3 Retrosynthetic Design and Synthesis of Disaccharide Probe 2.2

With the synthesis of **2.1** complete, I anticipated a similar synthetic strategy to obtain the second disaccharide target **2.2** (Scheme 2.11). Glycosylation of the previously discussed galactofuranosyl donor **2.53** with the 8-azidooctyl galactopyranoside **2.57**, followed by deprotection and conjugation to FITC, would give **2.2**. I envisioned galactopyranoside acceptor

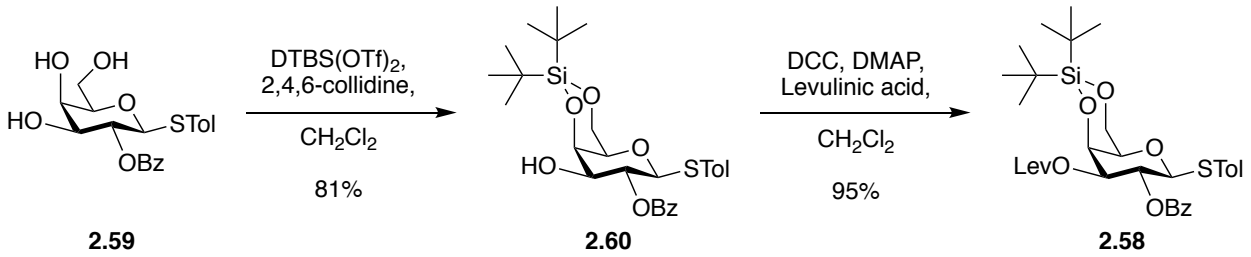
2.57 coming from **2.58** with differentially protected O-2 and O-3 positions. DTBS protection of the O-4 and O-6 position of **2.59**,[137] followed by protection of the O-3 position with a levulinoyl ester, would give **2.58**.



Scheme 2.11 Retrosynthesis of target **2.2**.

With this plan in place, synthesis of target **2.2** began with **2.59**, which was treated with $DTBS(OTf)_2$ and 2,4,6-collidine in CH_2Cl_2 to give galactopyranoside **2.60** in 81% yield (Scheme

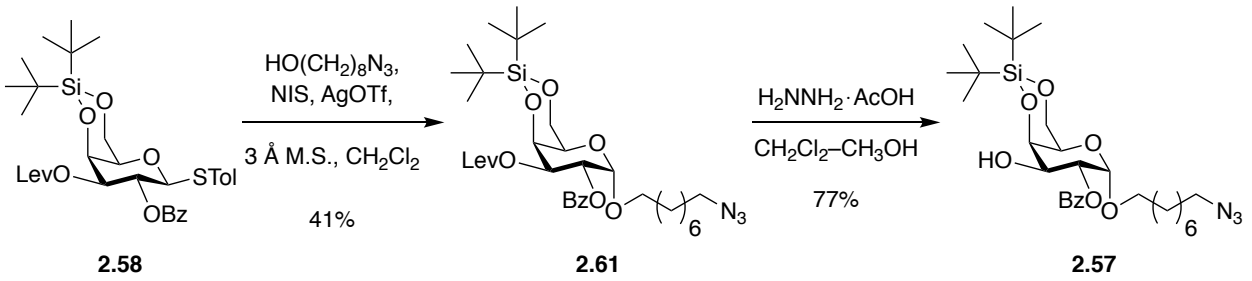
2.12). Treatment of **2.60** with DCC, DMAP, and levulinic acid in CH_2Cl_2 provided the desired differentially protected galactopyranoside **2.58** in 95% yield.



2.59 **2.60** **2.58**

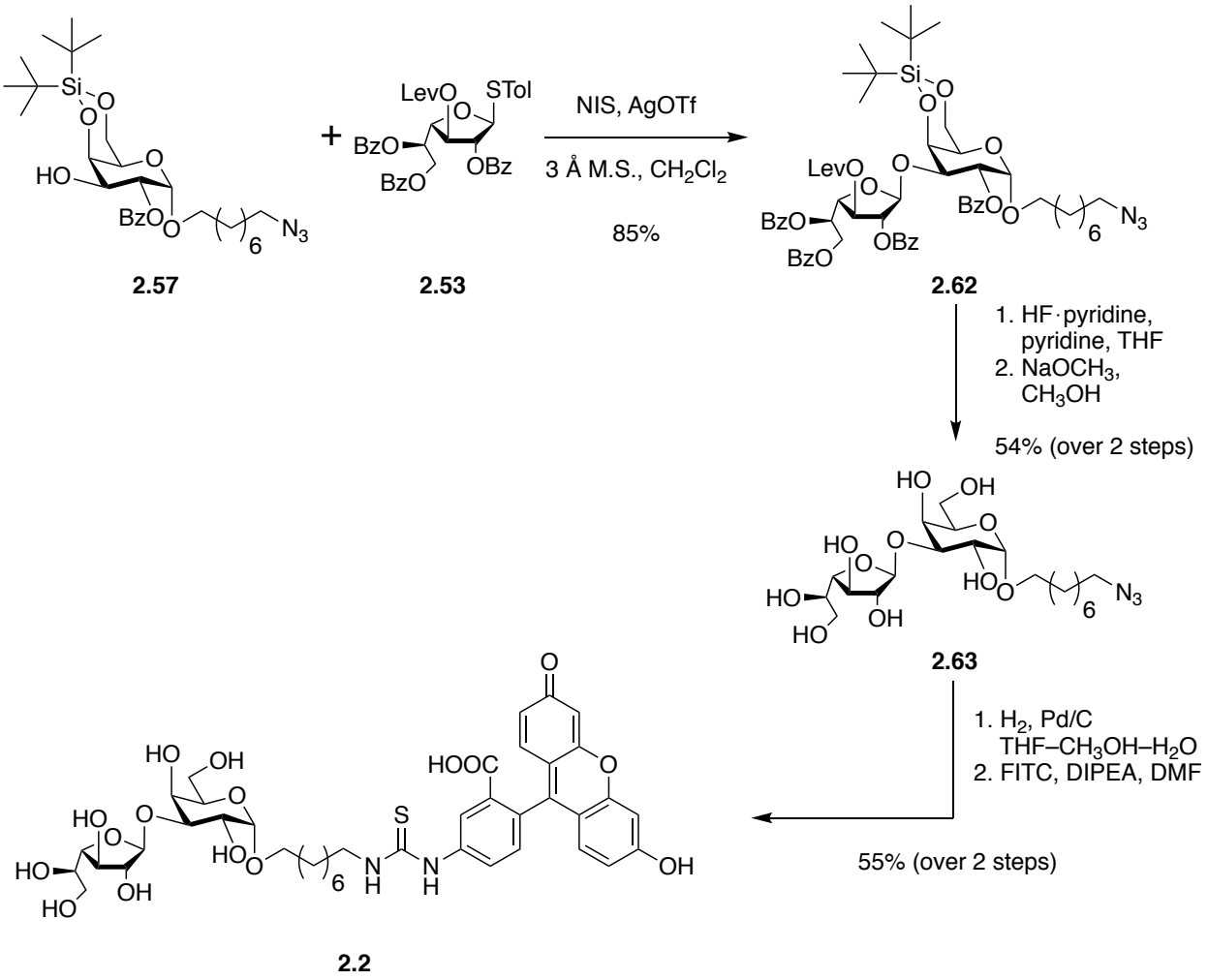
Scheme 2.12 Synthesis of galactopyranoside donor **2.58**.

Next, I synthesized galactopyanoside acceptor **2.54** (Scheme 2.13). Activation of the thioglycoside **2.58** with NIS and AgOTf in the presence of 8-azido-1-octanol gave the desired monosaccharide **2.61**, albeit in 41% yield. The magnitude of the $^3J_{H1\text{-}H2}$, 3.7 Hz, for H-1 of **2.61** confirmed the α-stereochemistry of the glycosidic linkage. Changing the acid activator to TfOH, increasing the reaction temperature, or leaving the reaction for a longer time did not improve the yield. This is likely a case where the donor–acceptor pair were not properly matched,[138,139] leading to poor reactivity, as both donor **2.58** and 8-azido-1-octanol were recovered in high percentages. Removal of the levulinoyl ester from compound **2.61** gave the desired galactopyranoside acceptor **2.57** in 77% yield.



Scheme 2.13 Synthesis of galactopyranoside **2.57**.

With acceptor **2.57** and donor **2.53** in hand, synthesis of **2.2** commenced (Scheme 2.14). Activation of the galactofuranosyl **2.53** with NIS and AgOTf in the presence of galactopyranoside acceptor **2.57** gave the desired disaccharide **2.62** in 85% yield. The new singlet at 5.60 ppm in the ^{1}H NMR spectrum confirmed the β-(1→3) glycosidic linkage.[133] Treatment with HF·pyridine gave the diol intermediate that was treated with catalytic sodium methoxide to give **2.63** in 54% yield over two steps. Once again, the low yield might have resulted from the work-up protocol of these two reactions. After hydrogenolysis and conjugation of the amine to FITC, the second target **2.2** was obtained in 55% yield over two steps. As with **2.1**, fluorescein-tagged target **2.2** was poorly soluble in water, which led to modest separation of **2.2** and the hydrophobic impurities by C18 chromatography, reducing the yield.

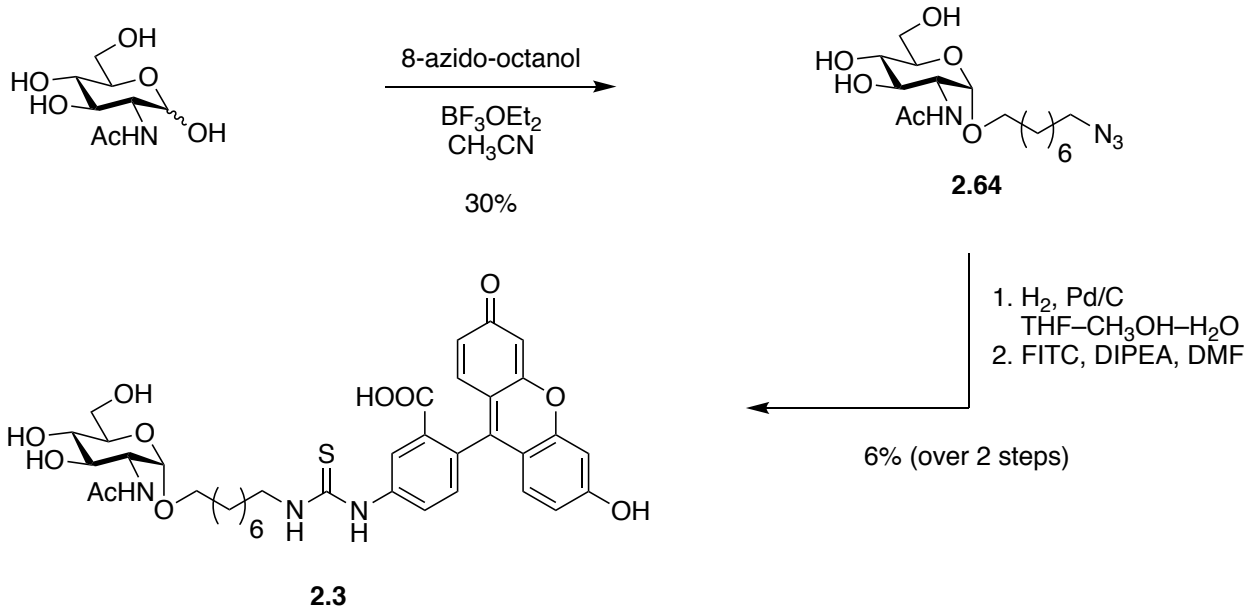


Scheme 2.14 Synthesis of target compound **2.2**.

2.2.4 Synthesis of GlcNAc Probe 2.3 (Scheme 2.14)

To obtain the third fluorescein-tagged target **2.3** effeciently, I treated *N*-acetylglucosamine with boron trifluoride in the presence of 8-azido-1-octanol in acetonitrile at reflux. These conditions gave the thermodynamically favored α-glycoside **2.64** in a single step, albeit in 30% yield. The α-stereochemistry of **2.64** was confirmed by the magnitude of the $^{3}J_{H1\text{-}H2}$, 3.6 Hz, for H-1. Hydrogenolysis and amine conjugation with FITC and 1% DIPEA in DMF gave the crude target **2.3**. Despite the success in isolating targets **2.1** and **2.2**, the purification of

target **2.3** proved to be challenging, given its insolubility in a range of solvents. Ultimately, crude compound **2.3** was purified by trituration with toluene, then by trituration with H_2O, and lastly by purification by preparative reverse phase HPLC. Afterwards, I obtained the pure desired target **2.3** in 6% yield over the two steps.



Scheme 2.15 Synthesis of target compound **2.3**.

2.3 Summary

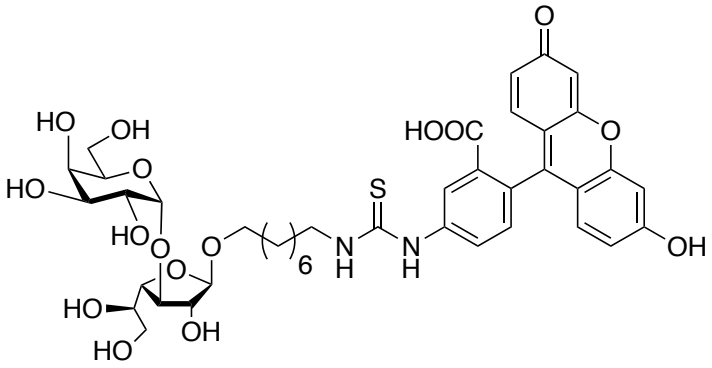
In conclusion, I synthesized the fluorescein-labeled target compounds **2.1**–**2.3**. I was unable to synthesize a conserved disaccharide building block for targets **2.1** and **3.1**–**3.5**, however; as thioglycoside **2.35** was a poor acceptor in the glycosylation reactions, possibly due to the confirmation of the galactofuranose ring. After revising my synthetic strategy, the first target **2.1** was obtained in good overall yield. The key step was an α-selective glycosylation between a DTBS-protected galactopyranosyl donor **2.51** and 8-azidooctyl galactofuranoside

2.50. My success in accessing this compound suggests that O-glycoside **2.50** is a more reactive acceptor than thioglycoside **2.35**. For synthesis of **2.2**, a β-selective glycosylation between a 2-*O*-benzoyl-galactofuranosyl donor **2.53** and acceptor **2.57** was a key step. To obtain acceptor **2.57**, differentially protected galactopyranosyl donor **2.58** was obtained as a key synthetic intermediate. Purification of target **2.3** was found to be difficult, but was achieved with purification by first trituration, then by preparative HPLC, to obtain appreciable amounts of target **2.3**, albeit in low yields. These three target compounds **2.1**–**2.3** are now being used in collaboration with the labs of Chris Whitfield and Matt Kimber at the University of Guelph to determine the precise catalytic activities of the glycosyltransferases WbbM, WbbN, and WbbO, from the *K. pneumoniae* O2a O-PS biosynthetic pathway. Preliminary results with **2.1** and **2.2** suggest that WbbN and WbbO are responsible for initiation of O-PS biosynthesis, and that WbbM is a bifunctional GTase responsible for polymerization of the O-PS in *K. pneumoniae* O2a (Clarke and Whitfield, personal communication).

2.4 Experimental Section

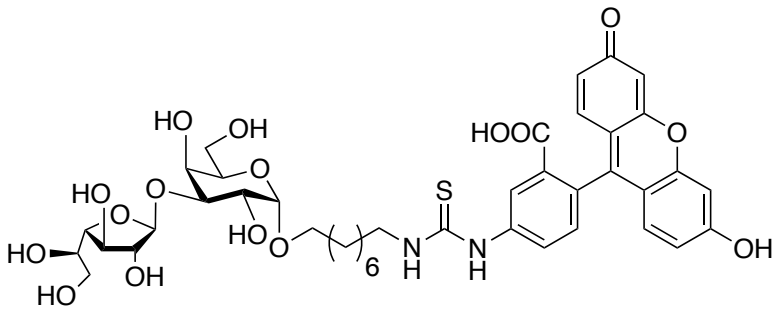
General Methods: All reagents, except 8-azido-1-octanol[140] and sodium allyloxide,[141] were purchased from commercial sources and used without further purification. Dry CH_2Cl_2, THF, and CH_3CN were taken from a solvent purification system after passage through alumina columns. Dry DMF was taken from a solvent purification system after passage through a 5 Å M.S. column. Dry CH_3OH was obtained via storage in a sealed bottle with activated 4 Å M.S. overnight at rt. Unless otherwise stated, all reactions were carried out under an argon atmosphere and were monitored by TLC on silica gel 60 F_{254} (0.25 mm, Merck). Spots were visualized by UV light and/or by charring with 10% H_2SO_4 in EtOH. In the processing of reaction mixtures, solutions of organic solvents were washed with equal amounts of aqueous solutions. Column chromatography was performed on silica gel 60 (40–60 μm) or C_{18} silica gel (35–70 μm). ^{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 (D_2O, 4.79 ppm). ^{13}C NMR spectra were recorded at 126 or 176 MHz, and chemical shifts were referenced to internal $CDCl_3$ (77.06 ppm, $CDCl_3$), CD_3OD (49.00 ppm, CD_3OD), or external acetone (31.07 ppm, D_2O). All NMR assignments were made by appropriate 2D NMR experiments, and the stereochemistry of the newly formed glycosidic bonds was confirmed by $^3J_{H1\text{-}H2}$ and anomeric ^{13}C chemical shifts. In the following data, the resonances of particular residues are indicated by an increasing number of primes (‘) moving from the reducing end to the non-reducing end. Optical rotations were measured on a Perkin Elmer 241 polarimeter at 22 ± 2 °C in units of (degree·mL)/(dm·g). Electrospray ionization spectra were recorded on an Aligent Technologies 6220 TOF spectrometer with samples dissolved in CH_3OH or H_2O. HPLC purification was carried out with a Waters (Waters Ltd., Mississauga, ON, Canada) Delta 600 pump, Waters 600 controller, with a

Waters XTerra Prep RP18 10 μm reverse-phase preparatory column (Ø 10 x 250 mm) monitored with a Waters 2996 photodiode array detector with Empower 2 software.



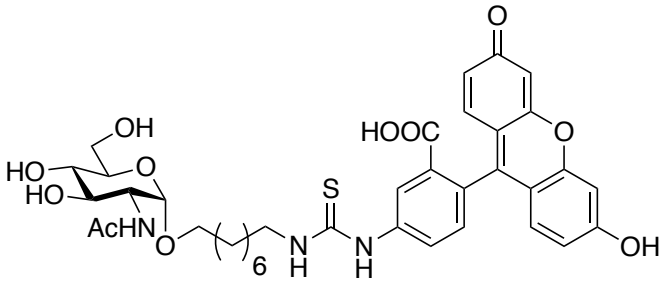
8-(Fluoresceinthiourea)-octyl α-D-galactopyranosyl-(1→3)-β-D-galactofuranoside; DIPEA salt (2.1). A suspension of **2.56** (16 mg; 0.030 mmol) and $Pd(OH)_2/C$ (3.0 mg) in THF–H_2O–CH_3OH (1.2 mL; 3:2:1 v/v/v) was placed under an atmosphere of H_2, and stirred for 4 h at rt. The mixture was filtered through Celite and concentrated to give the crude amine (14 mg). The crude amine was dissolved in dry DMF (1.0 mL) with 1% DIPEA (0.01 mL) in an RBF protected from light. FITC (15 mg, 0.040 mmol) was added to the mixture and stirred for 2 h, while being protected from light. The mixture was concentrated, and purified by C_{18} column chromatography (gradient of 1% → 100% CH_3OH–H_2O) and lyophilized to yield **2.1** (8 mg, 30% over 2 steps), as a fluffy orange solid. R_f 0.63 (7:2:1 EtOAc–CH_3OH–H_2O); $[\alpha]_D$ +10.7 (*c* 0.3, CH_3OH); ^{1}H NMR (700 MHz, CD_3OD, δ_H) 8.02 – 7.93 (m, 1 H, ArH), 7.78 – 7.69 (m, 1 H, ArH), 7.19 (d, *J* = 8.2 Hz, 1 H, ArH), 7.11 – 7.01 (m, 2 H, ArH), 6.67 (d, *J* = 2.2 Hz, 2 H, ArH), 6.62 (dd, *J* = 9.0, 2.2 Hz, 2 H, ArH), 4.95 (d, *J* = 3.9 Hz, 1 H, H-1'), 4.87 (d, *J* = 1.7 Hz, 1 H, H-1), 4.21 (dd, *J* = 3.6, 1.8 Hz, 1 H), 4.13 (dd, *J* = 6.9, 3.8 Hz, 1 H), 4.03 – 3.99 (m, 1 H), 3.94 (dd, *J* = 6.9, 3.6 Hz, 1 H), 3.91 – 3.88 (m, 1 H), 3.81 – 3.60 (m, 11 H, octyl OCH_2, DIPEA C<u>H</u>$(CH_3)_2$), 3.43 (dt, *J* = 9.6, 6.3 Hz, 1 H, octyl OCH_2), 3.18 (q, *J* = 7.4 Hz, 1.25 H, DIPEA C<u>H</u>$_2CH_3$), 1.71 – 1.64 (m, 2 H, octyl CH_2), 1.63 – 1.55 (m, 2 H, octyl CH_2), 1.44 – 1.36 (m, 8 H, octyl CH_2), 1.36 – 1.29 (m,

9 H, DIPEA CH(C<u>H</u>$_3$)$_2$, DIPEA CH$_2$C<u>H</u>$_3$); ^{13}C NMR (126 MHz, CD$_3$OD, δ_C) 182.3 (C=S), 172.5 (C=O), 157.6 (Ar), 141.6 (Ar), 132.0 (Ar), 129.4 (Ar), 127.6 (Ar), 123.5 (Ar), 118.9 (Ar), 114.4 (Ar), 109.4 (C-1), 103.8 (Ar), 102.1 (C-1'), 87.9, 82.7, 81.5, 73.1, 72.7, 71.4, 71.3, 70.2, 68.8 (octyl OCH$_2$), 64.5 (C-6), 63.0 (C-6'), 55.6, 45.8 (DIPEA <u>C</u>H(CH$_3$)$_3$), 43.7 (DIPEA <u>C</u>H$_2$CH$_3$), 30.8 (octyl CH$_2$), 30.5 (octyl CH$_2$), 30.4 (octyl CH$_2$), 30.0 (octyl CH$_2$), 28.0 (octyl CH$_2$), 27.3 (octyl CH$_2$), 18.0 (DIPEA CH(<u>C</u>H$_3$)$_3$), 13.2 (DIPEA CH$_2$<u>C</u>H$_3$); HRMS–ESI–TOF calcd for [M–H]$^-$ $C_{41}H_{49}N_2O_{16}S$: 857.2808. Found 857.2808.



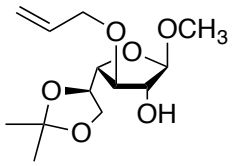
8-(Fluoresceinthiourea)-octyl β-D-galactofuranosyl-(1→3)-α-D-galactopyranoside; DIPEA salt (2.2). A suspension of **2.63** (10 mg; 0.02 mmol) and Pd(OH)$_2$/C (5.0 mg) in THF (2.0 mL), CH$_3$OH (2.0 mL), and H$_2$O (0.1 mL) was placed under an atmoshphere of H$_2$, and stirred for 5 h at rt. The mixture was filtered and concentrated to give the crude amine (10 mg). The crude amine was dissolved in dry DMF (1.0 mL) with 1% DIPEA (0.01 mL) in an RBF protected from light. FITC (10 mg, 0.03 mmol) was added to the mixture and stirred for 4 h, while being protected from light. The mixture was concentrated, and purified by C_{18} column chromatography (gradient of 1% → 100% CH$_3$OH–H$_2$O) and lyophilized to yield **2.2** (10 mg, 55% over 2 steps) as a fluffy orange solid. R_f 0.65 (7:2:1 EtOAc–CH$_3$OH–H$_2$O); $[\alpha]_D$ +13.4 (*c* 0.2, CH$_3$OH); ^{1}H NMR (700 MHz, CD$_3$OD, δ_H) 8.01 (s, 1 H, ArH), 7.78 – 7.71 (m, 1 H, ArH), 7.18 (d, *J* = 8.2 Hz, 1 H, ArH), 6.99 – 6.90 (m, 2 H, ArH), 6.68 (d, *J* = 2.2 Hz, 2 H, ArH), 6.61 (dd, *J* = 8.9, 2.2 Hz, 2

H, ArH), 5.22 – 5.16 (m, 1 H, H-1'), 4.81 (d, *J* = 3.8 Hz, 1 H, H-1), 4.09 – 4.05 (m, 3 H), 4.04 – 4.01 (m, 1 H), 3.89 (dd, *J* = 10.1, 3.8 Hz, 1 H), 3.85 – 3.80 (m, 2 H), 3.77 – 3.66 (m, 5 H, octyl OCH_2), 3.65 – 3.58 (m, 0.75 H, 2 x DIPEA $C\underline{H}(CH_3)_2$), 3.46 (dt, *J* = 9.7, 6.5 Hz, 1 H, octyl OCH_2), 3.19 (q, *J* = 7.4 Hz, 3.75 H, DIPEA $C\underline{H}_2CH_3$), 1.72 – 1.59 (m, 4 H, octyl CH_2), 1.45 – 1.38 (m, 8 H, octyl CH_2), 1.36 – 1.32 (m, 5.63 H, DIPEA $CH(C\underline{H}_3)_2$, DIPEA $CH_2C\underline{H}_3$); ^{13}C NMR (126 MHz, CD_3OD, δ_C) 172.1 (C=O), 155.1 (Ar), 141.9 (Ar), 131.5 (Ar), 111.1 (C-1'), 103.7 (Ar), 100.3 (C-1), 85.5, 82.9, 79.0, 78.6, 72.6, 72.1, 70.9, 69.2 (octyl OCH_2), 64.3 (C-6), 62.7 (C-6'), 55.7, 45.8 (DIPEA $\underline{C}H(CH_3)_3$), 43.7 (DIPEA $\underline{C}H_2CH_3$), 30.6 (octyl CH_2), 30.5 (octyl CH_2), 30.4 (octyl CH_2), 30.0 (octyl CH_2), 28.0 (octyl CH_2), 27.3 (octyl CH_2), 18.0 (DIPEA $CH(\underline{C}H_3)_3$), 13.2 (DIPEA $CH_2\underline{C}H_3$); HRMS–ESI–TOF calcd for $[M–H]^-$ $C_{41}H_{49}N_2O_{16}S$: 857.2808. Found 857.2796.



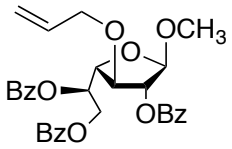
8-(Fluoresceinthiourea)-octyl 2-acetamido-2-deoxy-α-D-glucopyranoside (2.3). A suspension of **2.64** (50 mg; 0.13 mmol) and $Pd(OH)_2/C$ (12.0 mg) in THF (2.5 mL), CH_3OH (3.0 mL), and H_2O (0.1 mL) was placed under an atmosphere of H_2, and stirred for 4 h at rt. The mixture was filtered and concentrated to give the crude amine (49 mg). The crude amine was dissolved in dry DMF (3.0 mL) with 1% DIPEA (0.03 mL) in an RBF protected from light. FITC (26 mg, 0.73 mmol) was added to the mixture and stirred for 16 h, while being protected from light. The mixture was concentrated, and the crude product was triturated with toluene and with H_2O. The

residue was further purified by HPLC (eluent A 84:9:7 H_2O–CH_3CN–CH_3OH and eluent B CH_3CN; linear gradient of 100% eluent A to 60% eluent A, 40% eluent B over 30 min at 10 mL/min). Fractions containing **2.3** were combined, concentrated down, and lyophilized to yield **2.3** (3 mg, 6% over 2 steps) as an orange solid. R_f 0.74 (7:2:1 EtOAc–CH_3OH–H_2O); $[\alpha]_D$ +130.0 (*c* 0.01, $CHCl_3$); ^{1}H NMR (600 MHz, CD_3OD, δ_H) 7.96 – 7.88 (m, 2 H, ArH), 7.74 – 7.69 (m, 1 H, ArH), 7.20 (d, J = 8.3 Hz, 1 H, ArH), 6.74 – 6.54 (m, 5 H, ArH), 4.79 (d, $J_{1,2}$ = 3.7 Hz, 0.5 H, H-1 rotamer), 4.78 (d, $J_{1,2}$ = 3.7 Hz, 0.5 H, H-1 rotamer), 3.88 – 3.83 (m, 1 H, H-2), 3.83 – 3.78 (m, 1 H, H-6a), 3.74 – 3.62 (m, 3 H, H-6b, H-3, octyl OCH_2), 3.61 – 3.55 (m, 2 H, H-4, octyl CH_2N), 3.43 – 3.37 (m, 1 H, octyl OCH_2), 3.36 – 3.33 (m, 1 H, H-5), 2.91 – 2.87 (m, 1 H, octyl CH_2N), 1.98 (2 singlets, 3 H, $C(O)CH_3$ rotamers), 1.70 – 1.57 (m, 4 H, octyl CH_2), 1.45 – 1.35 (m, 8 H, octyl CH_2); ^{13}C NMR (126 MHz, CD_3OD, δ_C) 182.1 (C=S), 173.6 ($C(O)CH_3$), 163.3 (Ar), 132.4 (Ar), 130.2 (Ar), 120.9 (Ar), 103.9 (Ar), 98.4 (C-1), 73.8 (C-4), 72.8 (C-3), 72.4 (C-5), 68.9 (octyl OCH_2), 62.8 (C-6), 55.6 (C-2), 46.0 (octyl CH_2N), 30.8 (octyl CH_2), 30.53 (octyl CH_2), 30.47 (octyl CH_2), 30.0 (octyl CH_2), 28.0 (octyl CH_2), 27.3 (octyl CH_2), 22.6 ($C(O)\underline{C}H_3$); HRMS–ESI–TOF calcd for $[M–H]^-$ $C_{37}H_{42}N_3O_{11}S$: 736.2546. Found 736.2550.



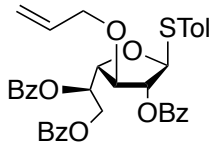
Methyl 3-*O*-allyl-5,6-di-*O*-isopropylidene-β-D-galactofuranoside (2.36). Compound **2.37**[130] (2.77 g; 12.8 mmol) was treated with 1 M NaOAll in AllOH (46.0 mL), and heated to reflux. The reaction mixture was stirred for 16 h at reflux, before being cooled to rt and diluted with EtOAc (75 mL). The organic layer was washed with H_2O three times, and then with brine. The combined aqueous layers were re-extracted with CH_2Cl_2. The combined organic layers were

dried with Na_2SO_4, filtered, and concentrated. The crude product was purified by silica gel column chromatography (3:1→2:1 hexanes–EtOAc) to yield **2.36** (3.08 g, 88%) as a clear, colorless oil. R_f 0.30 (3:2 hexanes–EtOAc); $[\alpha]_D$ –100 (*c* 1.10, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 5.92 (dddd, J_{trans} = 17.2 Hz, J_{cis} = 10.4 Hz, ^3J = 6.0 Hz, ^3J = 5.4 Hz, 1 H, H_2C=C<u>H</u>CH_2O), 5.32 (dd, J_{trans} = 17.2 Hz, J_{gem} = 1.6 Hz, 1 H, <u>H</u>$_2$C=CHCH_2O), 5.24 (dd, J_{cis} = 10.4 Hz, J_{gem} = 1.3 Hz, 1 H, <u>H</u>$_2$C=CHCH_2O), 4.92 (s, 1 H, H-1), 4.29 (ddd, $J_{5,6a}$ = 7.3 Hz, $J_{5,6b}$ = 7.1 Hz, $J_{4,5}$ = 2.5 Hz, 1 H, H-5), 4.18 (dddd, J_{gem} = 13.0 Hz, ^3J = 5.4 Hz, ^4J = 1.4 Hz, ^4J = 1.4 Hz, 1 H, H_2C=CHC<u>H</u>$_2$O), 4.14 (dd, $J_{3,4}$ = 2.7 Hz, $J_{4,5}$ = 2.5 Hz, 1 H, H-4), 4.12 – 4.02 (m, 4 H, H-2, H_2C=CHC<u>H</u>$_2$O, H-6a, H-6b), 3.84 (d, $J_{3,4}$ = 2.7 Hz, 1 H, H-3), 3.42 (s, 3 H, OCH_3), 3.37 (d, $J_{2,OH}$ = 10.8 Hz, 1 H, OH), 1.45 (s, 3 H, C(CH_3)$_2$), 1.41 (s, 3 H, C(CH_3)$_2$); ^{13}C NMR (126 MHz, $CDCl_3$, δ_C) 134.3 (H_2<u>C</u>=CHCH_2O), 118.0 (H_2C=<u>C</u>HCH_2O), 110.7 (C-1), 109.9 (<u>C</u>(CH_3)$_2$), 85.9 (C-4), 82.9 (C-3), 77.4 (C-2), 76.4 (C-5), 71.3 (H_2C=CH<u>C</u>H_2O), 65.7 (C-6), 55.4 (OCH_3), 25.8 (C(<u>C</u>H_3)$_2$), 25.7 (C(<u>C</u>H_3)$_2$); HRMS–ESI–TOF calcd for $[M+Na]^+$ $C_{13}H_{22}NaO_6$: 297.1309. Found 297.1318.



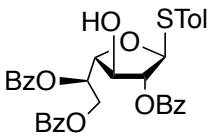
Methyl 3-*O*-allyl-2,5,6-tri-*O*-benzoyl-β-D-galactofuranoside (2.40). Compound **2.36** (3.11 g; 11.3 mmol) was treated with a solution of AcOH–H_2O (40.0 mL, 4:1 v/v), and the mixture was stirred for 25.5 h at rt before being concentrated down with the aid of toluene. The crude oil was dissolved in pyridine (36.0 mL), and cooled to 0 °C. BzCl (5.15 mL, 44.3 mmol) was added slowly dropwise to the mixture, and stirred for 24 h, while warming to rt. Excess BzCl was quenched by the addition of ice-cold water (50 mL), and the solution was diluted with EtOAc (50 mL). The organic layer was washed, in succession, with solutions of 1 M HCl, saturated

sodium bicarbonate, and brine, before being dried with Na_2SO_4, filtered, and concentrated. The crude product was purified by column chromatography (8:1→5:1 hexanes–EtOAc) to yield **2.40** (5.74 g, 93% over 2 steps) as a fluffy, white solid. R_f 0.27 (6:1 hexanes–EtOAc); $[\alpha]_D$ –13.1 (*c* 0.70, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$, δ_H) 8.10 – 8.05 (m, 2 H, ArH), 8.03 – 7.98 (m, 2 H, ArH), 7.93 – 7.89 (m, 2 H, ArH), 7.58 – 7.52 (m, 3 H, ArH), 7.44 – 7.38 (m, 2 H, ArH), 7.38 – 7.31 (m, 4 H, ArH), 5.94 – 5.84 (m, 2 H, H-5, H_2C=C<u>H</u>-CH_2O), 5.32 (d, $J_{2,3}$ = 1.0 Hz, 1 H, H-2), 5.25 (dddd, J_{trans} = 17.2 Hz, J_{gem} = 1.5 Hz, ^4J = 1.5 Hz, ^4J = 1.5 Hz, 1 H, <u>H</u>$_2$C=CH-CH_2O), 5.18 – 5.13 (m, 2 H, H-1, <u>H</u>$_2$C=CH-CH_2O), 4.75 (dd, $J_{6a,6b}$ = 11.8 Hz, $J_{5,6b}$ = 4.5 Hz, 1 H, H-6a), 4.70 (dd, $J_{6a,6b}$ = 11.8 Hz, $J_{5,6b}$ = 7.5 Hz, 1 H, H-6b), 4.49 (dd, $J_{3,4}$ = 5.8, $J_{4,5}$ = 3.6 Hz, 1 H, H-4), 4.29 (dddd, J_{gem} = 12.7 Hz, ^3J = 5.4 Hz, ^4J = 1.3 Hz, ^4J = 1.3 Hz, 1 H, H_2C=CH-C<u>H</u>$_2$O), 4.15 – 4.07 (m, 2 H, H-3, H_2C=CH-C<u>H</u>$_2$O), 3.46 (s, 3 H, OCH_3); ^{13}C NMR (126 MHz, $CDCl_3$, δ_C) 166.1 (C=O), 165.9 (C=O), 165.4 (C=O), 133.70 (H_2C=<u>C</u>HCH_2O), 133.68 (Ar), 133.4 (Ar), 133.3 (Ar), 133.1 (Ar), 130.2 (Ar), 129.9 (Ar), 129.72 (Ar), 129.69 (Ar), 129.64 (Ar), 129.57 (Ar), 129.2 (Ar), 128.49 (Ar), 128.45 (Ar), 128.42 (Ar), 128.39 (Ar), 118.6 (H_2<u>C</u>=CHCH_2O), 107.1 (C-1), 83.3 (C-3), 81.7 (C-4), 81.4 (C-2), 71.9 (H_2C=CH<u>C</u>H_2O), 70.3 (C-5), 63.6 (C-6), 54.9 (OCH_3); HRMS–ESI–TOF calcd for $[M+Na]^+$ $C_{31}H_{30}NaO_9$: 569.1788. Found 569.1770.



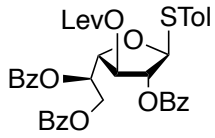
***p*-Tolyl 3-*O*-allyl-2,5,6-tri-*O*-benzoyl-1-thio-β-D-galactofuranoside (2.41).** A suspension of **2.40** (2.8 g; 5.1 mmol) and thiocresol (0.90 g; 7.2 mmol) and 3 Å M.S. in dry CH_2Cl_2 (28 mL) was stirred for 30 min at rt. The mixture was cooled to 0 °C, and $BF_3{\cdot}OEt_2$ (2.1 mL; 17 mmol) was added slowly dropwise. The reaction mixture was stirred for 22.5 h while warming to rt. A solution of saturated sodium bicarbonate (75 mL) was added to the mixture, which was diluted

with CH_2Cl_2 (75 mL) and filtered. The organic layer was washed with a solution of saturated sodium bicarbonate twice more, before being dried with Na_2SO_4, filtered, and concentrated. The crude product was purified by silica gel column chromatography (6:1 hexanes–EtOAc) to yield **2.41** (3.2 g, 98%) as a clear, colorless oil. R_f 0.33 (6:1 hexanes–EtOAc); $[\alpha]_D$ –83 (*c* 0.30, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 8.09 – 8.03 (m, 2 H, ArH), 8.03 – 7.97 (m, 2 H, ArH), 7.94 – 7.88 (m, 2 H, ArH), 7.59 – 7.52 (m, 3 H, ArH), 7.46 – 7.31 (m, 8 H, ArH), 7.09 – 7.03 (m, 2 H, ArH), 5.96 – 5.84 (m, 2 H, H-5, H_2C=C<u>H</u>CH_2O), 5.67 (s, 1 H, H-1), 5.48 (t, $J_{2,3}$ = 1.5 Hz, 1 H, H-2), 5.28 (dddd, J_{trans} = 17.2 Hz, J_{gem} = 1.5 Hz, ^4J = 1.4 Hz, ^4J = 1.2 Hz, 1 H, <u>H</u>$_2$C=CHCH_2O), 5.17 (dddd, J_{cis} = 10.5 Hz, J_{gem} = 1.5 Hz, ^4J = 1.4 Hz, ^4J = 1.2 Hz, 1 H, <u>H</u>$_2$C=CHCH_2O), 4.80 (dd, $J_{3,4}$ = 6.2 Hz, $J_{4,5}$ = 3.6 Hz, 1 H, H-4), 4.71 (dd, $J_{6a,6b}$ = 11.8 Hz, $J_{5,6a}$ = 4.6 Hz, 1 H, H-6a), 4.65 (dd, $J_{6a,6b}$ = 11.8 Hz, $J_{5,6b}$ = 7.4 Hz, 1 H, H-6b), 4.29 (dddd, J_{gem} = 12.8 Hz, ^3J = 5.4 Hz, ^4J = 1.4 Hz, ^4J = 1.4 Hz, 1 H, H_2C=CHC<u>H</u>$_2$O), 4.19 – 4.15 (m, 1 H, H-3), 4.12 (dddd, J_{gem} = 12.8 Hz, ^3J = 6.3 Hz, ^4J = 1.2 Hz, ^4J = 1.2 Hz, 1 H, H_2C=CHC<u>H</u>$_2$O), 2.32 (s, 3 H, $ArCH_3$); ^{13}C NMR (126 MHz, $CDCl_3$, δ_C) 166.1 (C=O), 165.9 (C=O), 165.3 (C=O), 138.1 (Ar), 133.6 (H_2C=<u>C</u>H-CH_2O), 133.4 (Ar), 133.3 (Ar), 133.13 (Ar), 133.07 (Ar), 129.9 (Ar), 129.73 (Ar), 129.71 (Ar), 129.66 (Ar), 129.6 (Ar), 129.5 (Ar), 129.1 (Ar), 128.5 (Ar), 128.40 (Ar), 128.39 (Ar), 118.5 (H_2<u>C</u>=CH-CH_2O), 91.5 (C-1), 83.3 (C-3), 82.2 (C-2), 81.0 (C-4), 71.8 (H_2C=CH-<u>C</u>H_2O), 70.2 (C-5), 63.4 (C-6), 21.1 ($ArCH_3$); HRMS–ESI–TOF calcd for $[M+Na]^+$ $C_{37}H_{34}NaO_8S$: 661.1872. Found 661.1861.

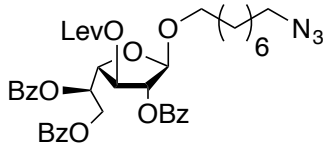


***p*-Tolyl 2,5,6-tri-*O*-benzoyl-1-thio-β-D-galactofuranoside (2.35).** To a solution of **2.41** (504 mg; 0.900 mmol) in dry THF (18.0 mL) was added $Ir(COD)(PMePh_2)_2 \cdot PF_6$ (27.0 mg; 3.50

mol%). The stirring mixture was then degassed, and placed under an inert atmosphere of argon, and was cooled to 0 °C. The solution was degassed again, and the flask was flushed with H_2 for 2 min, and then for 1 min, until the solution turned clear and colorless. The solution was then degassed once more, and placed under an inert atmosphere of argon, after which the solution turned a slight pinkish-red color. The reaction mixture was stirred for 4.75 h at rt. The solution was then concentrated, and the crude residue was dissolved in acetone–H_2O (25.0 mL; 10:1 v/v). HgO (279 mg; 1.28 mmol), followed by $HgCl_2$ (304 mg; 1.10 mmol) were added to the solution. The mixture was stirred for 15 min, before being diluted with EtOAc (25 mL), and washed, in succession, with an aqueous solution of 10% KI three times, with a solution of saturated sodium thiosulfate three times, and with H_2O twice. The organic layer was dried with Na_2SO_4, filtered, and concentrated. The crude product was purified by silica gel column chromatography (2:1 hexanes–EtOAc) to yield **2.35** (430 mg, 92%) as a fluffy, white solid. R_f 0.32 (3:1 hexanes–EtOAc); $[\alpha]_D$ –132 (*c* 0.30, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 8.09 – 7.98 (m, 6 H, ArH), 7.64 – 7.52 (m, 3 H, ArH), 7.46 – 7.36 (m, 8 H, ArH), 7.07 – 7.00 (m, 2 H, ArH), 5.84 (ddd, $J_{5,6b}$ = 7.0 Hz, $J_{5,6a}$ = 4.5 Hz, $J_{4,5}$ = 4.5 Hz, 1 H, H-5), 5.68 (d, $J_{1,2}$ = 3.4 Hz, 1 H, H-1), 5.16 (dd, $J_{2,3}$ = 3.7 Hz, $J_{1,2}$ = 3.4 Hz, 1 H, H-2), 4.75 (dd, $J_{6a,6b}$ = 11.8 Hz, $J_{5,6a}$ = 4.5 Hz, 1 H, H-6a), 4.71 – 4.63 (m, 2 H, H-6b, H-4), 4.31–4.24 (m, 1 H, H-3), 3.46 (d, $J_{3,OH}$ = 3.0 Hz, 1 H, OH), 2.32 (s, 3 H, $ArCH_3$); ^{13}C NMR (126 MHz, $CDCl_3$, δ_C) 167.2 (C=O), 166.1 (C=O), 166.0 (C=O), 138.4 (Ar), 133.8 (Ar), 133.34 (Ar), 133.30 (Ar), 133.1 (Ar), 129.91 (Ar), 129.90 (Ar), 129.8 (Ar), 129.7 (Ar), 129.6 (Ar), 129.5 (Ar), 129.0 (Ar), 128.7 (Ar), 128.5 (Ar), 128.4 (Ar), 89.3 (C-1), 86.7 (C-2), 80.6 (C-4), 77.2 (C-3), 70.5 (C-5), 63.2 (C-6), 21.2 ($ArCH_3$); HRMS–ESI–TOF calcd for $[M+Na]^+$ $C_{34}H_{30}NaO_8S$: 621.1559. Found 621.1548.

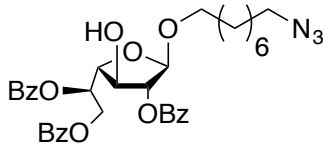


***p*-Tolyl 2,5,6-tri-*O*-benzoyl-3-*O*-levulinoyl-1-thio-β-D-galactofuranoside (2.53).** To a solution of **2.35** (1.99 g; 3.34 mmol) and DMAP (0.040 g; 0.334 mmol) in dry CH_2Cl_2 (45.0 mL), was added DCC (1.40 g; 6.70 mmol), followed by levulinic acid (0.800 g; 6.70 mmol). The reaction mixture was stirred for 18 h at rt, before being filtered and concentrated. The crude product was purified by silica gel column chromatography (3:1→2:1 hexanes–EtOAc) to yield **2.53** (2.25 g, 97%) as a white solid. R_f 0.35 (2:1 hexanes–EtOAc); $[\alpha]_D$ –67.2 (*c* 0.1, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 8.10 – 8.00 (m, 4 H, ArH), 7.91 – 7.85 (m, 2 H, ArH), 7.59 – 7.49 (m, 3 H, ArH), 7.46 – 7.39 (m, 4 H, ArH), 7.39 – 7.26 (m, 5 H, ArH), 7.10 – 7.04 (m, 2 H, ArH), 5.99 (ddd, $J_{5,6b}$ = 6.8 Hz, $J_{5,6a}$ = 5.1 Hz, $J_{4,5}$ = 3.7 Hz, 1 H, H-5), 5.71 (d, $J_{1,2}$ = 1.7 Hz, 1 H, H-1), 5.50 (dd, $J_{1,2}$ = 1.7 Hz, $J_{2,3}$ = 1.7 Hz, 1 H, H-2), 5.44 (dd, $J_{3,4}$ = 5.3 Hz, $J_{2,3}$ = 1.7 Hz, 1 H, H-3), 4.81 (dd, $J_{3,4}$ = 5.3 Hz, $J_{4,5}$ = 3.7 Hz, 1 H, H-4), 4.70 (dd, $J_{6a,6b}$ = 12.0 Hz, $J_{5,6a}$ = 5.1 Hz, 1 H, H-6a), 4.67 (dd, $J_{6a,6b}$ = 12.0 Hz, $J_{5,6b}$ = 6.8 Hz, 1 H, H-6b), 2.82 – 2.76 (m, 2 H, $CH_2C\underline{H}_2C(O)CH_3$), 2.69 – 2.62 (m, 2 H, $C\underline{H}_2CH_2C(O)CH_3$), 2.32 (s, 3 H, $ArCH_3$), 2.19 (s, 3 H, $CH_2CH_2C(O)C\underline{H}_3$); ^{13}C NMR (126 MHz, $CDCl_3$, δ_C) 206.0 (C=O), 171.8 (C=O), 166.0 (C=O), 165.6 (C=O), 165.3 (C=O), 138.3 (Ar), 133.4 (Ar), 133.3 (Ar), 133.2 (Ar), 133.1 (Ar), 130.0 (Ar), 129.84 (Ar), 129.82 (Ar), 129.79 (Ar), 129.6 (Ar), 129.5 (Ar), 129.1 (Ar), 128.8 (Ar), 128.43 (Ar), 128.40 (Ar), 91.32 (C-1), 82.2 (C-2), 80.9 (C-4), 77.6 (C-3), 70.2 (C-5), 63.2 (C-6), 38.0 ($CH_2\underline{C}H_2C(O)CH_3$), 29.7 ($CH_2CH_2C(O)\underline{C}H_3$), 27.9 ($\underline{C}H_2CH_2C(O)CH_3$), 21.2 ($ArCH_3$); HRMS–ESI–TOF calcd for $[M+Na]^+$ $C_{39}H_{36}NaO_{10}S$: 719.1927. Found 719.1915.



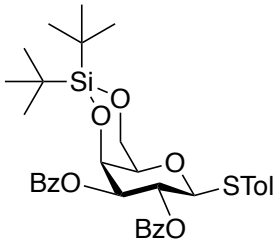
8-Azidooctyl 2,5,6-tri-*O*-benzoyl-3-*O*-levulinoyl-β-D-galactofuranoside (2.54). A suspension of **2.53** (207 mg; 0.298 mmol), 8-azido-1-octanol (56.0 mg; 0.330 mmol) and 3 Å M.S. in dry CH_2Cl_2 (4.0 mL) was stirred for 30 min at rt. The suspension was cooled to –10 °C, and NIS (76.0 mg; 0.330 mmol) was added, followed by AgOTf (16.0 mg; 0.060 mmol). The reaction mixture was stirred for 1.5 h at –10 °C, before being neutralized by the addition of Et_3N. A solution of saturated sodium thiosulfate (5.0 mL) was added, and the mixture was filtered. The organic layer was washed with brine, dried with Na_2SO_4, filtered, and concentrated. The crude product was purified by silica gel column chromatography (3:1 hexanes–EtOAc) to yield **2.54** (212 mg, 96%) as a clear, colorless oil. R_f 0.28 (3:1 hexanes–EtOAc); $[\alpha]_D$ –11.2 (*c* 0.40, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 8.12 – 8.06 (m, 2 H, ArH), 8.06 – 8.00 (m, 2 H, ArH), 7.93 – 7.87 (m, 2 H, ArH), 7.59 – 7.50 (m, 3 H, ArH), 7.46 – 7.26 (m, 6 H, ArH), 5.97 (ddd, $J_{5,6b}$ = 6.9 Hz, $J_{5,6a}$ = 5.0 Hz, $J_{4,5}$ = 3.4 Hz, 1 H, H-5), 5.41 – 5.35 (m, 1 H, H-3), 5.31 (d, $J_{2,3}$ = 1.3 Hz, 1 H, H-2), 5.25 (s, 1 H, H-1), 4.74 (dd, $J_{6a,\ 6b}$ = 12.0 Hz, $J_{5,6a}$ = 5.0 Hz, 1 H, H-6a), 4.71 (dd, $J_{6a,\ 6b}$ = 12.0 Hz, $J_{5,6b}$ = 6.9 Hz, 1 H, H-6b), 4.52 (dd, $J_{3,4}$ = 5.4 Hz, $J_{4,5}$ = 3.4 Hz, 1 H, H-4), 3.73 (dt, *J* = 9.7 Hz, 6.8 Hz, 1 H, octyl OCH_2), 3.52 (dt, *J* = 9.7 Hz, 6.5 Hz, 1 H, octyl OCH_2), 3.28 (t, *J* = 7.0 Hz, 2 H, octyl CH_2N_3), 2.84 – 2.76 (m, 1 H, $CH_2C\underline{H}_2C(O)CH_3$), 2.76 – 2.68 (m, 1 H, $CH_2C\underline{H}_2C(O)CH_3$), 2.67 – 2.60 (m, 2 H, $C\underline{H}_2CH_2C(O)CH_3$), 2.17 (s, 3 H, $CH_2CH_2C(O)C\underline{H}_3$), 1.67 – 1.57 (m, 4 H, octyl CH_2), 1.43 – 1.29 (m, 8 H, octyl CH_2); ^{13}C NMR (126 MHz, $CDCl_3$, δ_C) 206.0 (C=O), 172.0 (C=O), 166.1 (C=O), 165.7 (C=O), 165.4 (C=O), 133.33 (Ar), 133.25 (Ar), 133.1 (Ar), 130.0 (Ar), 129.80 (Ar), 129.77 (Ar), 129.7 (Ar), 129.6 (Ar), 129.0 (Ar), 128.43 (Ar), 128.40 (Ar), 105.6 (C-1), 82.1 (C-2), 80.9 (C-4), 77.3 (C-3), 70.2 (C-5), 67.7 (octyl

OCH_2), 63.4 (C-6), 51.5 (octyl CH_2N_3), 37.9 ($CH_2\underline{C}H_2C(O)CH_3$), 29.7 ($CH_2CH_2C(O)\underline{C}H_3$), 29.4 (octyl CH_2), 29.2 (octyl CH_2), 29.1 (octyl CH_2), 28.8 (octyl CH_2), 27.8 ($\underline{C}H_2CH_2C(O)CH_3$), 26.7 (octyl CH_2), 26.0 (octyl CH_2); HRMS–ESI–TOF calcd for $[M+Na]^+$ $C_{40}H_{45}N_3NaO_{11}$: 766.2952. Found 766.2952.



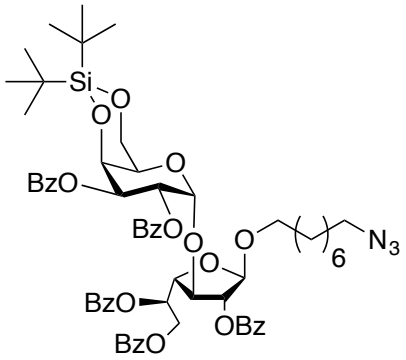
8-Azidooctyl 2,5,6-tri-*O*-benzoyl-β-D-galactofuranoside (2.50). To a solution of **2.54** (209 mg; 0.280 mmol) in dry CH_2Cl_2 (6.00 mL) and dry CH_3OH (0.50 mL), was added $H_2NNH_2{\cdot}AcOH$ (36.0 mg; 0.391 mmol). The reaction mixture was stirred for 19 h at rt, before being diluted with CH_2Cl_2 (15 mL), and washed with H_2O. The organic layer was dried with Na_2SO_4, filtered, and concentrated. The crude product was purified by silica gel column chromatography (4:1 hexanes–EtOAc) to yield **2.50** (172 mg, 95%) as a white solid. R_f 0.28 (3:1 hexanes–EtOAc); $[\alpha]_D$ –35 (*c* 0.20, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$, δ_H) 8.09 – 8.04 (m, 2 H, ArH), 8.03 – 7.97 (m, 4 H, ArH), 7.59 – 7.51 (m, 3 H, ArH), 7.44 – 7.35 (m, 6 H, ArH), 5.83 (ddd, $J_{5,6b}$ = 7.4 Hz, $J_{4,5}$ = 4.5 Hz, $J_{5,6a}$ = 4.3 Hz, 1 H, H-5), 5.30 (s, 1 H, H-1), 5.12 (dd, $J_{2,3}$ = 2.9 Hz, $J_{1,2}$ = 1.1 Hz, 1 H, H-2), 4.76 (dd, $J_{6a,6b}$ = 11.8 Hz, $J_{5,6a}$ = 4.3 Hz, 1 H, H-6a), 4.68 (dd, $J_{6a,6b}$ = 11.8 Hz, $J_{5,6b}$ = 7.4 Hz, 1 H, H-6b), 4.46 (dd, $J_{3,4}$ = 6.7 Hz, $J_{4,5}$ = 4.5 Hz, 1 H, H-4), 4.27 – 4.21 (m, 1 H, H-3), 3.75 (dt, *J* = 9.6, 6.7 Hz, 1 H, octyl OCH_2), 3.50 (dt, *J* = 9.5, 6.5 Hz, 1 H, octyl OCH_2), 3.29 – 3.22 (m, 3 H, octyl CH_2N_3, OH), 1.66 – 1.57 (m, 4 H, octyl CH_2), 1.41 – 1.29 (m, 8 H, octyl CH_2); ^{13}C NMR (126 MHz, $CDCl_3$, δ_C) 166.9 (C=O), 166.1 (C=O), 166.0 (C=O), 133.6 (Ar), 133.3 (Ar), 133.1 (Ar), 129.9 (Ar), 129.8 (Ar), 129.7 (Ar), 129.64 (Ar), 129.58 (Ar), 128.9 (Ar), 128.5 (Ar), 128.40 (Ar), 105.2 (C-1), 86.4 (C-2), 81.6 (C-4), 77.2 (C-3), 70.6 (C-5), 68.1 (Octyl OCH_2), 63.4 (C-6), 51.5 (octyl CH_2N_3), 29.5 (octyl CH_2), 29.2 (octyl CH_2), 29.0 (octyl

CH_2), 28.8 (octyl CH_2), 26.7 (octyl CH_2), 26.0 (octyl CH_2); HRMS–ESI–TOF calcd for $[M+Na]^+$ $C_{35}H_{39}N_3NaO_9$: 668.2584. Found 668.2583.



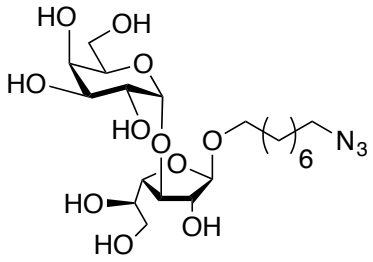
***p*-Tolyl 2,3-di-*O*-benzoyl-4,6-di-*O*-di-*tert*-butylsilylidene-1-thio-β-D-galactopyranoside (2.51).** To a solution of **2.52**[136] (103 mg, 0.240 mmol) in pyridine (2.0 mL), was added BzCl (0.8 mL, 0.7 mmol) slowly dropwise at 0 °C. The reaction mixture was stirred for 20 h while warming to rt. Excess BzCl was quenched by the addition of ice-cold H_2O (15.0 mL), and the mixture was diluted with CH_2Cl_2 (20.0 mL). The organic layer was washed, in succession, with solutions of 2 % HCl, saturated sodium bicarbonate, and brine, before being dried with Na_2SO_4, filtered, and concentrated. The crude product was purified by column chromatography (9:1 hexanes–EtOAc) to yield **2.51** (128 mg, 84%) as a fluffy, white solid. R_f 0.18 (9:1 hexanes–EtOAc); $[\alpha]_D$ +138 (*c* 0.50, $CHCl_3$); ^{1}H NMR (700 MHz, $CDCl_3$, δ_H) 8.00 – 7.96 (m, 4 H, ArH), 7.53 – 7.48 (m, 2 H, ArH), 7.40 – 7.35 (m, 6 H, ArH), 7.07 – 7.04 (m, 2 H, ArH), 5.89 (dd, $J_{1,2}$ = 10.0 Hz, $J_{2,3}$ = 9.8 Hz, 1 H, H-2), 5.17 (dd, $J_{2,3}$ = 9.8 Hz, $J_{3,4}$ = 3.2 Hz, 1 H, H-3), 4.88 – 4.83 (m, 2 H, H-1, H-4), 4.31 (dd, $J_{6a,6b}$ = 12.4 Hz, $J_{5,6a}$ = 1.6 Hz, 1 H, H-6a), 4.28 (dd, $J_{6a,6b}$ = 12.4 Hz, $J_{5,6b}$ = 2.3 Hz, 1 H, H-6b), 3.60 (br s, 1 H, H-5), 2.30 (s, 3 H, $ArCH_3$), 1.14 (s, 9 H, $SiC(CH_3)_3$), 0.94 (s, 9 H, $SiC(CH_3)_3$); ^{13}C NMR (126 MHz, $CDCl_3$, δ_C) 166.2 (C=O), 165.4 (C=O), 138.1 (Ar), 133.2 (Ar), 133.1 (Ar), 129.9 (Ar), 129.82 (Ar), 129.80 (Ar), 129.7 (Ar), 129.5 (Ar), 128.4 (Ar), 128.3 (Ar), 87.9 (C-1), 75.5 (C-3), 75.0 (C-5), 70.5 (C-4), 68.3 (C-2), 67.2 (C-6), 27.51

(SiC($\underline{C}H_3)_3$), 27.46 (SiC($\underline{C}H_3)_3$), 23.3 (Si$\underline{C}(CH_3)_3$), 21.1 ($ArCH_3$), 20.7 (Si$\underline{C}(CH_3)_3$); HRMS–ESI–TOF calcd for $[M+Na]^+$ $C_{35}H_{42}NaO_7SSi$: 657.2313. Found 657.2307.



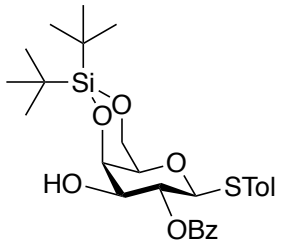
8-Azidooctyl 2,3-di-*O*-benzoyl-4,6-di-*O*-di-*tert*-butylsilylidene-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranoside (2.55). A suspension of **2.51** (172 mg; 0.280 mmol), **2.50** (115 mg; 0.200 mmol) and 3 Å M.S. in dry CH_2Cl_2 (4.00 mL) was stirred for 30 min at rt. The suspension was cooled to –10 °C, and NIS (56.0 mg; 0.250 mmol) was added, followed by AgOTf (12.0 mg; 0.040 mmol). The reaction mixture was stirred for 1 h at –10 °C, before being neutralized by the addition of Et_3N. A solution of saturated sodium thiosulfate (8.0 mL) was added, and the mixture was filtered. The organic layer was washed with brine, dried with Na_2SO_4, filtered, and concentrated. The crude product was purified by silica gel column chromatography (gradient of 6:1→3:1 hexanes–EtOAc) to give **2.55** (150 mg, 72%) as a white solid. R_f 0.43 (4:1 hexanes–EtOAc); $[\alpha]_D$ +23 (*c* 0.30, $CHCl_3$); ^1H NMR (700 MHz, $CDCl_3$, δ_H) 8.04 – 8.00 (m, 2 H, ArH), 8.00 – 7.95 (m, 4 H, ArH), 7.89 – 7.83 (m, 4 H, ArH), 7.57 – 7.49 (m, 5 H, ArH), 7.42 – 7.29 (m, 9 H, ArH), 7.28 – 7.25 (m, 1 H, ArH), 5.79 (dd, $J_{2',3'}$ = 10.6 Hz, $J_{1',2'}$ = 3.7 Hz, 1 H, H-2'), 5.65 – 5.59 (m, 2 H, H-3', H-5), 5.49 (d, $J_{1',2'}$ = 3.7 Hz, 1 H, H-1'), 5.41 (d, $J_{2,3}$ = 1.3 Hz, 1 H, H-2), 5.16 (s, 1 H, H-1), 4.93 (d, $J_{3',4'}$ = 2.9 Hz, 1 H, H-4'), 4.51 (dd, $J_{6a,6b}$ = 11.8 Hz, $J_{5,6a}$ = 7.2 Hz, 1 H, H-6a), 4.47 (dd, $J_{6a,6b}$ = 11.8 Hz, $J_{5,6b}$ = 4.2 Hz, 1 H, H-6b), 4.42 – 4.36 (m, 2 H, H-3, H-4), 4.33 – 4.29 (m, 1 H, H-6a'), 4.22 (br s, 1 H, H-5'), 4.18 – 4.13 (m, 1 H,

H-6b'), 3.68 (dt, *J* = 9.6, 6.6 Hz, 1 H, octyl OCH_2), 3.46 (dt, *J* = 9.6, 6.6 Hz, 1 H, octyl OCH_2), 3.23 (t, *J* = 7.0 Hz, 2 H, octyl CH_2N_3), 1.63 – 1.56 (m, 4 H, octyl CH_2), 1.38 – 1.25 (m, 8 H, octyl CH_2), 1.09 (s, 9 H, $SiC(CH_3)_3$), 0.96 (s, 9 H, $SiC(CH_3)_3$); ^{13}C NMR (126 MHz, $CDCl_3$, δ_C) 166.2 (C=O), 165.84 (C=O), 165.76 (C=O), 165.6 (C=O), 165.3 (C=O), 133.4 (Ar), 133.3 (Ar), 133.2 (Ar), 133.11 (Ar), 133.08 (Ar), 129.89 (Ar), 129.85 (Ar), 129.74 (Ar), 129.68 (Ar), 129.66 (Ar), 129.5 (Ar), 129.3 (Ar), 129.0 (Ar), 128.43 (Ar), 128.40 (Ar), 128.37 (Ar), 128.33 (Ar), 128.31 (Ar), 105.7 (C-1), 98.2 (C-1'), 83.1 (C-4), 82.1 (C-2), 81.4 (C-3), 71.11 (C-4'), 71.07 (C-2'), 69.6 (C-5), 68.2 (C-3'), 67.8 (C-5'), 67.7 (octyl OCH_2), 66.8 (C-6'), 63.4 (C-6), 51.5 (octyl CH_2N_3), 29.7 (octyl CH_2), 29.3 (octyl CH_2), 29.1 (octyl CH_2), 28.8 (octyl CH_2), ($SiC(\underline{C}H_3)_3$), 27.3 ($SiC(\underline{C}H_3)_3$), 26.7 (octyl CH_2), 26.0 (octyl CH_2), 23.3 ($Si\underline{C}(CH_3)_3$), 20.7 ($Si\underline{C}(CH_3)_3$); HRMS–ESI–TOF calcd for $[M+Na]^+$ $C_{63}H_{73}N_3NaO_{16}Si$: 1178.4658. Found 1178.4653.



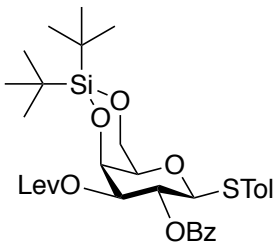
8-Azidooctyl α-D-galactopyranosyl-(1→3)-β-D-galactofuranoside (2.56). To a solution of **2.55** (148 mg; 0.130 mmol) in THF (3.00 mL) and pyridine (1.00 mL), was added HF·pyridine (0.100 mL) at 0 °C. The mixture was stirred for 23 h while warming to rt, before being neutralized by the addition of a solution of saturated sodium bicarbonate (25 mL) and diluted with EtOAc (25 mL). The organic layer was washed with brine, dried with Na_2SO_4, filtered, and concentrated. The crude product was purified by silica gel column chromatography (2:1→1:1 hexanes–EtOAc) to give the diol product (100 mg). This compound was dissolved in dry CH_3OH (30.0 mL), and a small piece of solid sodium was added. The reaction mixture was stirred for 3

d, before being neutralized by the addition of AcOH, diluted with H_2O (10 mL), and washed with CH_2Cl_2. The aqueous layer was concentrated and purified by C_{18} column chromatography (gradient of 1% → 30% CH_3OH–H_2O) and lyophilized to yield **2.56** (37 mg, 58% over 2 steps) as a white solid. R_f 0.41 (7:2:1 EtOAc–CH_3OH–H_2O); $[\alpha]_D$ +29 (*c* 0.50, H_2O); ^{1}H NMR (700 MHz, D_2O, δ_H) 5.01 (d, *J* = 3.8 Hz, 1 H, H-1'), 4.98 (s, 1 H, H-1), 4.22 (dd, *J* = 2.8, 1.6 Hz, 1 H), 4.12 (dd, *J* = 6.0, 4.0 Hz, 1 H), 4.04 – 3.98 (m, 2 H), 3.95 (d, *J* = 2.2 Hz, 1 H), 3.85 – 3.79 (m, 2 H), 3.79 – 3.73 (m, 1 H), 3.73 – 3.60 (m, 5 H), 3.54 (dt, *J* = 9.9, 6.3 Hz, 1 H, octyl OCH_2), 3.28 (t, *J* = 6.9 Hz, 2 H, octyl CH_2N_3), 1.60 – 1.53 (m, 4 H, octyl CH_2), 1.36 – 1.26 (m, 8 H, octyl CH_2); ^{13}C NMR (126 MHz, D_2O, δ_C) 108.5 (C-1), 100.8 (C-1'), 85.8, 82.9, 80.3, 72.4, 71.9, 70.22, 70.20, 69.4, 69.2, 63.8, 62.1, 52.2 (octyl CH_2N_3), 29.6 (octyl CH_2), 29.22 (octyl CH_2), 29.17 (octyl CH_2), 28.9 (octyl CH_2), 26.8 (octyl CH_2), 26.2 (octyl CH_2); HRMS–ESI–TOF calcd for $[M+Na]^+$ $C_{20}H_{37}N_3NaO_{11}$: 518.2326. Found 518.2314.



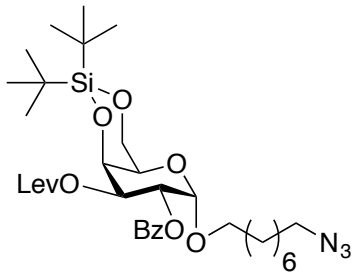
***p*-Tolyl 2-*O*-benzoyl-4,6-di-*O*-di-*tert*-butylsilylidene-1-thio-β-D-galactopyranoside (2.60).** To a solution of **2.59**[137] (23.0 g, 59.0 mmol) in CH_2Cl_2 (235 mL) and 2,4,6-collidine (19.5 mL, 147 mmol) was added DTBS(OTf)$_2$ (23.0 mL, 70.8 mmol) slowly dropwise over the course of 15 min at 0 °C. The reaction mixture was stirred at 0 °C for 1.5 h, before being diluted with H_2O (150 mL). The organic layer was washed once with a solution of saturated aqueous sodium bicarbonate and once with H_2O, before being dried with Na_2SO_4, filtered, and concentrated. The crude oil was purified by silica gel column chromatography (5:1→4:1 hexanes–EtOAc) to yield

2.60 (25.4 g, 81%) as a fluffy, white solid. R_f 0.41 (4:1 hexanes–EtOAc); $[\alpha]_D$ +27 (*c* 0.50, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$, δ_H) 8.13 – 8.07 (m, 2 H, ArH), 7.64 – 7.57 (m, 1 H, ArH), 7.52 – 7.44 (m, 2 H, ArH), 7.41 – 7.36 (m, 2 H, ArH), 7.11 – 7.05 (m, 2 H, ArH), 5.44 (dd, $J_{1,2}$ = 10.1 Hz, $J_{2,3}$ = 9.7 Hz, 1 H, H-2), 4.76 (d, $J_{1,2}$ = 10.1 Hz, 1 H, H-1), 4.52 (dd, $J_{3,4}$ = 3.4 Hz, $J_{4,5}$ = 0.9 Hz, 1 H, H-4), 4.33 (dd, $J_{6a,6b}$ = 12.5, $J_{5,6a}$ = 1.7 Hz, 1 H, H-6a), 4.30 (dd, $J_{6a,6b}$ = 12.4, $J_{5,6a}$ = 2.2 Hz, 1 H, H-6b), 3.74 (ddd, $J_{3,OH}$ = 11.5 Hz, $J_{2,3}$ = 9.7 Hz, $J_{3,4}$ = 3.4 Hz, 1 H, H-3), 3.53 (br s, 1 H, H-5), 2.77 (d, $J_{3,OH}$ = 11.5 Hz, 1 H, OH), 2.33 (s, 3 H, $ArCH_3$), 1.15 (s, 9 H, $SiC(CH_3)_3$), 1.09 (s, 9 H, $SiC(CH_3)_3$); ^{13}C NMR (126 MHz, $CDCl_3$, δ_C) 166.2 (C=O), 138.0 (Ar), 133.2 (Ar), 133.1 (Ar), 129.99 (Ar), 129.95 (Ar), 129.6 (Ar), 128.4 (Ar x 2), 87.4 (C-1), 75.2 (C-5), 73.9 (C-3), 73.0 (C-4), 71.7 (C-2), 67.1 (C-6), 27.54 ($SiC(\underline{C}H_3)_3$), 27.52 ($SiC(\underline{C}H_3)_3$), 23.4 ($Si\underline{C}(CH_3)_3$), 21.1 ($ArCH_3$), 20.8 ($Si\underline{C}(CH_3)_3$); HRMS–ESI–TOF calcd for $[M+Na]^+$ $C_{28}H_{38}NaO_6SSi$: 553.2056. Found 553.2051.



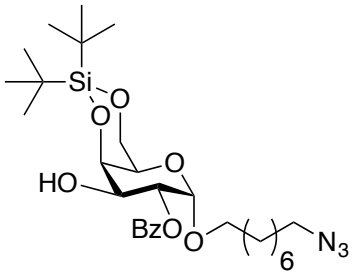
***p*-Tolyl 2-*O*-benzoyl-4,6-di-*O*-di-*tert*-butylsilylidene-3-*O*-levulinoyl-1-thio-β-D-galactopyranoside (2.58).** To a solution of **2.60** (3.35 g; 6.60 mmol) and DMAP (0.085 g; 0.66 mmol) in dry CH_2Cl_2 (39.0 mL), was added DCC (2.74 g; 13.2 mmol), followed by levulinic acid (1.55 g; 13.2 mmol). The reaction mixture was stirred for 17 h at rt, before being filtered and concentrated. The crude product was purified by silica gel column chromatography (4:1→3:1 hexanes–EtOAc) to yield **2.58** (3.78 g, 95%) as a white solid. R_f 0.30 (3:1 hexanes–EtOAc); $[\alpha]_D$ +75 (*c* 0.70, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$, δ_H) 8.11 – 8.00 (m, 2 H, ArH), 7.54 – 7.41 (m,

2 H, ArH), 7.41 – 7.30 (m, 2 H, ArH), 7.11 – 6.98 (m, 2 H, ArH), 5.74 (dd, $J_{1,2}$ = 10.1 Hz, $J_{2,3}$ = 9.9 Hz, 1 H, H-2), 4.99 (dd, $J_{2,3}$ = 9.9 Hz, $J_{3,4}$ = 3.0 Hz, 1 H, H-3), 4.80 (d, $J_{1,2}$ =10.1 Hz, 1 H, H-1), 4.73 (d, $J_{3,4}$ = 3.0 Hz, 1 H, H-4), 4.30 (dd, $J_{6a,6b}$ = 12.4 Hz, $J_{5,6b}$ = 1.5 Hz, 1 H, H-6a), 4.26 (dd, $J_{6a,6b}$ = 12.4 Hz, $J_{5,6b}$ = 2.2 Hz, 1 H, H-6b), 3.54 (br s, 1 H, H-5), 2.63 (dd, J = 7.0, 6.5 Hz, 2 H, $CH_2C\underline{H}_2C(O)CH_3$), 2.54 (ddd, J = 7.0, 6.5, 2.7 Hz, 2 H, $C\underline{H}_2CH_2C(O)CH_3$), 2.32 (s, 3 H, $ArCH_3$), 2.07 (s, 3 H, $CH_2CH_2C(O)C\underline{H}_3$), 1.15 (s, 9 H, $SiC(CH_3)_3$), 1.09 (s, 9 H, $SiC(CH_3)_3$); ^{13}C NMR (126 MHz, $CDCl_3$, δ_C) 205.8 (C=O), 172.1 (C=O), 165.4 (C=O), 138.1 (Ar), 133.3 (Ar), 133.1 (Ar), 130.0 (Ar), 129.9 (Ar), 129.6 (Ar), 128.5 (Ar x 2), 87.9 (C-1), 75.1 (C-3), 74.9 (C-5), 70.4 (C-4), 68.2 (C-2), 67.1 (C-6), 37.8 ($CH_2\underline{C}H_2C(O)CH_3$), 29.7 ($CH_2CH_2C(O)\underline{C}H_3$), 28.3 ($\underline{C}H_2CH_2C(O)CH_3$), 27.6 ($SiC(\underline{C}H_3)_3$), 27.5 ($SiC(\underline{C}H_3)_3$), 23.3 ($Si\underline{C}(CH_3)_3$), 21.1 ($ArCH_3$), 20.7 ($Si\underline{C}(CH_3)_3$); HRMS–ESI–TOF calcd for $[M+Na]^+$ $C_{33}H_{44}NaO_8SSi$: 651.2424. Found 651.2413.



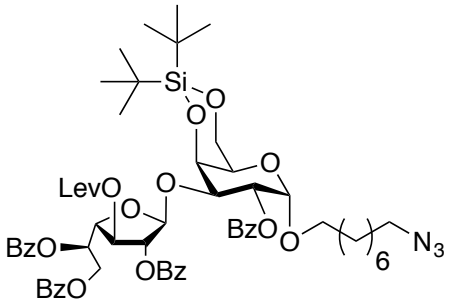
8-Azidooctyl 2-*O*-benzoyl-4,6-di-*O*-di-*tert*-butylsilylidene-3-*O*-levulinoyl-α-D-galactopyranoside (2.61). A suspension of **2.58** (37 mg; 0.06 mmol), 8-azido-1-octanol (15 mg; 0.090 mmol) and 3 Å M.S. in dry CH_2Cl_2 (2.0 mL) was stirred for 30 min at rt. The suspension was cooled to –10 °C, and NIS (16 mg; 0.07 mmol) was added, followed by AgOTf (7.0 mg; 0.02 mmol). The reaction mixture was stirred for 1.5 h at –10 °C, before being neutralized by the addition of Et_3N. A solution of saturated sodium thiosulfate (2.0 mL) was added, and the mixture was filtered. The organic layer was washed with brine, dried with Na_2SO_4, filtered, and concentrated. The crude product was purified by silica gel column chromatography (6:1

hexanes–EtOAc) to yield **2.61** (16 mg, 41%) as a clear, colorless oil. R_f 0.31 (4:1 hexanes–EtOAc); $[\alpha]_D$ +72 (*c* 0.20, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 8.10 – 8.04 (m, 2 H, ArH), 7.64 – 7.56 (m, 1 H, ArH), 7.51 – 7.44 (m, 2 H, ArH), 5.55 (dd, $J_{2,3}$ = 10.6 Hz, $J_{1,2}$ = 3.7 Hz, 1 H, H-2), 5.37 (dd, $J_{2,3}$ = 10.6 Hz, $J_{3,4}$ = 3.0 Hz, 1 H, H-3), 5.24 (d, $J_{1,2}$ = 3.7 Hz, 1 H, H-1), 4.75 (d, $J_{3,4}$ = 3.0 Hz, 1 H, H-4), 4.31 (dd, $J_{6a,6b}$ = 12.5 Hz, $J_{5,6a}$ = 2.1 Hz, 1 H, H-6a), 4.20 (dd, $J_{6a,6b}$ = 12.5 Hz, $J_{5,6b}$ = 1.6 Hz, 1 H, H-6b), 3.86 (br s, 1 H, H-5), 3.69 (dt, *J* = 9.9, 6.4 Hz, 1 H, octyl OCH_2), 3.41 (dt, *J* = 9.9, 6.4 Hz, 1 H, octyl OCH_2), 3.22 (t, *J* = 7.0 Hz, 2 H, octyl CH_2N_3), 2.72 – 2.65 (m, 2 H, $CH_2C\underline{H}_2C(O)CH_3$), 2.62 – 2.55 (m, 2 H, $C\underline{H}_2CH_2C(O)CH_3$), 2.10 (s, 3 H, $CH_2CH_2C(O)C\underline{H}_3$), 1.57 – 1.48 (m, 4H, octyl CH_2), 1.32 – 1.23 (m, 8 H, octyl CH_2), 1.13 (s, 9 H, $SiC(CH_3)_3$), 1.06 (s, 9 H, $SiC(CH_3)_3$); ^{13}C NMR (126 MHz, $CDCl_3$, δ_C) 205.9 (C=O), 172.3 (C=O), 166.1 (C=O), 133.3 (Ar), 129.9 (Ar), 129.8 (Ar), 129.7 (Ar), 129.49 (Ar), 128.45 (Ar), 96.5 (C-1), 71.1 (C-4), 70.8 (C-3), 68.7 (C-2), 68.4 (octyl OCH_2), 67.0 (C-6), 66.9 (C-5), 51.4 (octyl CH_2N_3), 37.9 ($CH_2\underline{C}H_2C(O)CH_3$), 29.7 ($CH_2CH_2C(O)\underline{C}H_3$), 29.3 (octyl CH_2), 29.2 (octyl CH_2), 29.0 (octyl CH_2), 28.8 (octyl CH_2), 28.3 ($\underline{C}H_2CH_2C(O)CH_3$), 27.6 ($SiC(\underline{C}H_3)_3$), 27.3 ($SiC(\underline{C}H_3)_3$), 26.6 (octyl CH_2), 26.0 (octyl CH_2), 23.4 ($Si\underline{C}(CH_3)_3$), 20.8 ($Si\underline{C}(CH_3)_3$); HRMS–ESI–TOF calcd for $[M+Na]^+$ $C_{34}H_{53}N_3NaO_9Si$: 698.3449. Found 698.3453.



8-Azidooctyl 2-*O*-benzoyl-4,6-di-*O*-di-*tert*-butylsilylidene-α-D-galactopyranoside (2.57). To a solution of **2.61** (41 mg; 0.060 mmol) in dry CH_2Cl_2 (4.0 mL) and dry CH_3OH (1.0 mL), was added $H_2NNH_2 \cdot AcOH$ (6.0 mg; 0.07 mmol). The reaction mixture was stirred for 23 h at rt,

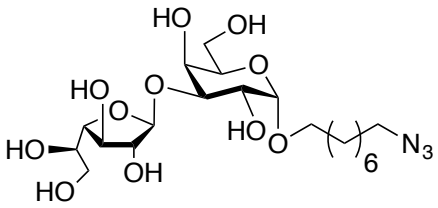
before being diluted with CH_2Cl_2 (10 mL), and washed with H_2O. The organic layer was dried with Na_2SO_4, filtered, and concentrated. The crude product was purified by silica gel column chromatography (6:1 hexanes–EtOAc) to yield **2.57** (27 mg, 77%) as a clear, colorless oil. R_f 0.46 (4:1 hexanes–EtOAc); ^{1}H NMR (600 MHz, $CDCl_3$, δ_H) 8.15 – 8.10 (m, 2 H, ArH), 7.63 – 7.55 (m, 1 H, ArH), 7.50 – 7.43 (m, 2 H, ArH), 5.35 (dd, $J_{2,3}$ = 10.1 Hz, $J_{1,2}$ = 3.7 Hz, 1 H, H-2), 5.14 (d, $J_{1,2}$ = 3.7 Hz, 1 H, H-1), 4.55 (d, $J_{3,4}$ = 3.0 Hz, 1 H, H-4), 4.35 (dd, $J_{6a,6b}$ = 12.5 Hz, $J_{5,6a}$ = 2.1 Hz, 1 H, H-6a), 4.22 (dd, $J_{6a,6b}$ = 12.5 Hz, $J_{5,6b}$ = 1.6 Hz, 1 H, H-6b), 4.13 (ddd, $J_{3,OH}$ = 11.4 Hz, $J_{2,3}$ = 10.1 Hz, $J_{3,4}$ = 3.0 Hz, 1 H, H-3), 3.86 (br s, 1 H, H-5), 3.70 (dt, J = 10.0, 6.3 Hz, 1 H, octyl OCH_2), 3.44 (dt, J = 10.0, 6.5 Hz, 1 H, octyl OCH_2), 3.23 (t, J = 7.0 Hz, 2 H, octyl CH_2N_3), 2.59 (d, $J_{3,OH}$ = 11.4 Hz, 1 H, OH), 1.61 – 1.52 (m, 4 H, octyl CH_2), 1.38 – 1.17 (m, 8 H, octyl CH_2), 1.13 (s, 9 H, $SiC(CH_3)_3$), 1.10 (s, 9 H, $SiC(CH_3)_3$); ^{13}C NMR (126 MHz, $CDCl_3$, δ_C) 166.7 (C=O), 133.1 (Ar), 130.0 (Ar), 129.9 (Ar), 129.8 (Ar), 129.5 (Ar), 128.3 (Ar), 96.9 (C-1), 73.8 (C-3), 71.8 (C-4), 68.6 (C-2), 68.3 (octyl OCH_2), 67.1 (C-6), 66.9 (C-5), 51.4 (octyl CH_2N_3), 29.4 (octyl CH_2), 29.2 (octyl CH_2), 29.1 (octyl CH_2), 28.8 (octyl CH_2), 27.6 ($SiC(\underline{C}H_3)_3$), 27.3 ($SiC(\underline{C}H_3)_3$), 26.6 (octyl CH_2), 26.0 (octyl OCH_2), 23.4 ($Si\underline{C}(CH_3)_3$), 20.8 ($Si\underline{C}(CH_3)_3$); HRMS–ESI–TOF calcd for $[M+Na]^+$ $C_{39}H_{47}N_3NaO_7Si$: 600.3081. Found 600.3067.



8-Azidooctyl 2,5,6-tri-*O*-benzoyl-3-*O*-levulinoyl-β-D-galactofuranosyl-(1→3)-2-*O*-benzoyl-4,6-di-*O*-di-*tert*-butylsilylidene-α-D-galactopyranoside (2.62). A suspension of **2.57** (27 mg; 0.050 mmol), **2.53** (37 mg; 0.054 mmol) and 3 Å M.S. in dry CH_2Cl_2 (2.0 mL) was stirred for 30

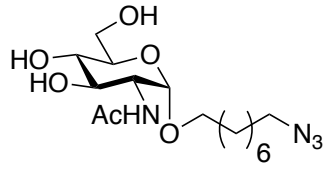
min at rt. The suspension was cooled to 0 °C, and NIS (13 mg; 0.060 mmol) was added, followed by AgOTf (3 mg; 0.01 mmol). The reaction mixture was stirred for 1 h at 0 °C, before being neutralized by the addition of Et_3N. A solution of saturated sodium thiosulfate (3.0 mL) was added, and the mixture was filtered. The organic layer was washed with brine, dried with Na_2SO_4, filtered, and concentrated. The crude product was purified by silica gel column chromatography (4:1 hexanes–acetone) to give **2.62** (46 mg, 85%) as a white solid. R_f 0.26 (4:1 hexanes–acetone); ^{1}H NMR (700 MHz, $CDCl_3$, δ_H) 8.10 – 8.04 (m, 4 H, ArH), 8.02 – 7.98 (m, 2 H, ArH), 7.71 – 7.67 (m, 2 H, ArH), 7.57 – 7.45 (m, 4 H, ArH), 7.42 – 7.38 (m, 2 H, ArH), 7.38 – 7.32 (m, 4 H, ArH), 7.27 – 7.22 (m, 2 H, ArH), 5.98 (ddd, $J_{5',6b'}$ = 7.2 Hz, $J_{5',6a'}$ = 3.9 Hz, $J_{4',5'}$ = 3.9 Hz, 1 H, H-5'), 5.60 (s, 1 H, H-1'), 5.50 (dd, $J_{2,3}$ = 10.5 Hz, $J_{1,2}$ = 3.7 Hz, 1 H, H-2), 5.44 (d, $J_{3',4'}$ = 5.0 Hz, 1 H, H-3'), 5.30 (s, 1 H, H-2'), 5.27 (d, $J_{1,2}$ = 3.7 Hz, 1 H, H-1), 4.76 (dd, $J_{6a',6b'}$ = 11.9 Hz, $J_{5',6a'}$ = 3.9 Hz, 1 H, H-6a'), 4.69 (dd, $J_{6a',6b'}$ = 11.9, $J_{5',6b'}$ = 7.2 Hz, 1 H, H-6b'), 4.65 (dd, $J_{3',4'}$ = 5.0 Hz, $J_{4',5'}$ = 3.9 Hz, 1 H, H-4'), 4.61 (d, $J_{3,4}$ = 2.9 Hz, 1 H, H-4), 4.25 (dd, $J_{2,3}$ = 10.5 Hz, $J_{3,4}$ = 2.9 Hz, 1 H, H-3), 4.23 – 4.20 (m, 1 H, H-6a), 4.18 – 4.13 (m, 1 H, H-6b), 3.74 (br s, 1 H, H-5), 3.65 (dt, J = 10.1, 6.3 Hz, 1 H, octyl OCH_2), 3.39 (dt, J = 10.1, 6.5 Hz, 1 H, octyl OCH_2), 3.19 (t, J = 7.0 Hz, 2 H, octyl CH_2N_3), 2.74 (dt, J = 18.3, 7.1 Hz, 1 H, $CH_2C\underline{H}_2C(O)CH_3$), 2.65 (dt, J = 18.3, 6.3 Hz, 1 H, $CH_2C\underline{H}_2C(O)CH_3$), 2.61 – 2.49 (m, 2 H, $C\underline{H}_2CH_2C(O)CH_3$), 2.13 (s, 3 H, $CH_2CH_2C(O)C\underline{H}_3$), 1.53 – 1.46 (m, 4 H, octyl CH_2), 1.27 – 1.20 (m, 8 H, octyl CH_2), 1.12 (s, 9 H, $SiC(CH_3)_3$), 1.06 (s, 9 H, $SiC(CH_3)_3$); ^{13}C NMR (176 MHz, $CHCl_3$, δ_C) 205.8 (C=O), 172.0 (C=O), 166.3 (C=O), 166.1 (C=O), 165.5 (C=O), 164.8 (C=O), 133.3 (Ar), 133.2 (Ar), 133.1 (Ar), 132.9 (Ar), 129.94 (Ar), 129.92 (Ar), 129.8 (Ar), 129.74 (Ar), 129.69 (Ar), 129.6 (Ar), 129.5 (Ar), 128.8 (Ar), 128.42 (Ar), 128.41 (Ar), 128.3 (Ar), 128.23 (Ar), 107.6 (C-1'), 96.5 (C-1), 81.70 (C-2'), 81.67 (C-4'), 77.1 (C-3'), 75.1 (C-3), 74.0

(C-4), 70.6 (C-2), 70.2 (C-5'), 68.2 (octyl CH_2), 67.3 (C-5), 67.0 (C-6), 63.5 (C-6'), 51.4 (octyl CH_2N_3), 37.8 ($CH_2\underline{C}H_2C(O)CH_3$), 29.7 ($CH_2CH_2C(O)\underline{C}H_3$), 29.3 (octyl CH_2), 29.1 (octyl CH_2), 29.0 (octyl CH_2), 28.8 (octyl CH_2), 27.7 ($\underline{C}H_2CH_2C(O)CH_3$), 27.6 ($SiC(\underline{C}H_3)_3$), 27.4 ($SiC(\underline{C}H_3)_3$), 26.6 (octyl CH_2), 26.0 (octyl CH_2), 23.4 ($Si\underline{C}(CH_3)_3$), 20.8 ($Si\underline{C}(CH_3)_3$); HRMS–ESI–TOF calcd for $[M+Na]^+$ $C_{61}H_{75}N_3NaO_{17}Si$: 1172.4763. Found 1172.4747.



8-Azidooctyl β-D-galactofuranosyl-(1→3)-α-D-galactopyranoside (2.63). To a solution of **2.62** (43 mg; 0.040 mmol) in THF (2.0 mL) and pyridine (1.0 mL), was added HF·pyridine (0.05 mL) at 0 °C. The mixture was stirred for 14.5 h while warming to rt, before being neutralized by the addition of a solution of saturated sodium bicarbonate (10 mL) and diluted with EtOAc (15 mL). The organic layer was washed with brine, dried with Na_2SO_4, filtered, and concentrated. The crude product was purified by column chromatography (3:1 hexanes–EtOAc) to give the diol product (32 mg). This compound was then dissolved in dry CH_3OH (11 mL), and a small piece of solid sodium was added. The reaction mixture was stirred for 19 h, before being neutralized by the addition of Amberlyst-15 H+ resin, filtered, and concentrated. The crude product was purified by C_{18} column chromatography (gradient of 1% → 40% CH_3OH–H_2O) and lyophilized to yield **2.61** (10 mg, 54% over 2 steps) as a white solid. R_f 0.62 (7:2:1 EtOAc–CH_3OH–H_2O); $[\alpha]_D$ +24 (*c* 0.50, CH_3OH); ^1H NMR (700 MHz, CD_3OD, δ_H) 5.17 (s, 1 H, H-1'), 4.79 (d, *J* = 3.8 Hz, 1 H, H-1), 4.07 – 3.99 (m, 4 H), 3.88 – 3.84 (m, 1 H), 3.83 – 3.76 (m, 2 H), 3.75 – 3.66 (m, 4 H), 3.64 – 3.56 (m, 2 H), 3.44 (dt, *J* = 9.6, 6.5 Hz, 1 H, octyl OCH_2), 3.26 (t, *J* = 6.9 Hz, 2 H, octyl CH_2N_3), 1.69 – 1.61 (m, 2 H, octyl CH_2), 1.61 – 1.54 (m, 2 H, octyl CH_2),

1.43 – 1.34 (m, 8 H, octyl CH_2); ^{13}C NMR (126 MHz, CD_3OD, δ_C) 111.1 (C-1'), 100.3 (C-1), 85.5, 82.9, 79.0, 78.6, 72.6, 72.1, 70.9, 69.4, 69.2, 64.3, 62.7, 52.5 (octyl CH_2N_3), 30.6 (octyl CH_2), 30.5 (octyl CH_2), 30.2 (octyl CH_2), 29.9 (octyl CH_2), 27.8 (octyl CH_2), 27.3 (octyl CH_2); HRMS–ESI–TOF calcd for $[M+Na]^+$ $C_{20}H_{37}N_3NaO_{11}$: 518.2326. Found 518.2312.



8-Azidooctyl 2-acetamido-2-deoxy-α-D-glucopyranoside (2.64). To a suspension of *N*-acetylglucosamine (3.02 g; 13.6 mmol) and 8-azido-1-octanol (7.00 g; 40.7 mmol) in dry CH_3CN (110 mL), was added $BF_3{\cdot}OEt_2$ (0.85 mL; 6.9 mmol) slowly dropwise. The reaction mixture was heated to reflux and stirred for 15 h. After cooling to rt, the solution was concentrated to dryness. The crude residue was purified by column chromatography (100% EtOAc → 90% EtOAc–CH_3OH) to yield **2.64** (1.51 g; 30%) as a white solid. R_f 0.28 (9:1 EtOAc–CH_3OH); $[\alpha]_D$ +122 (*c* 1.00, CH_3OH); ^1H NMR (700 MHz, CD_3OD, δ_H) 4.79 (d, *J* = 3.6 Hz, 1 H, H-1), 3.87 (dd, *J* = 10.7, 3.6 Hz, 1 H, H-2), 3.81 (dd, *J* = 11.9, 2.3 Hz, 1 H, H-6a), 3.74 – 3.63 (m, 3 H, H-6b, octyl OCH_2, H-3), 3.58 (ddd, *J* = 9.8, 5.6, 2.3 Hz, 1 H, H-5), 3.40 (dt, *J* = 9.9, 6.3 Hz, 1 H, octyl OCH_2), 3.37 – 3.33 (m, 1 H, H-4), 3.28 (t, *J* = 6.9 Hz, 2 H, octyl CH_2N_3), 1.98 (s, 3 H, $C(O)CH_3$), 1.65 – 1.55 (m, 4 H, octyl CH_2), 1.44 – 1.31 (m, 8 H, octyl CH_2); ^{13}C NMR (126 MHz, CD_3OD, δ_C) 173.6 (C=O), 98.4 (C-1), 73.8 (C-5), 72.8 (C-3), 72.4 (C-4), 68.9 (octyl OCH_2), 62.8 (C-6), 55.6 (C-2), 52.5 (octyl CH_2N_3), 30.5 (octyl CH_2), 30.4 (octyl CH_2), 30.3 (octyl CH_2), 29.9 (octyl CH_2), 27.8 (octyl CH_2), 27.2 (octyl CH_2), 22.6 ($C(O)\underline{C}H_3$); HRMS–ESI–TOF calcd for $[M+Na]^+$ $C_{16}H_{30}N_4NaO_6$: 397.2058. Found 397.2057.

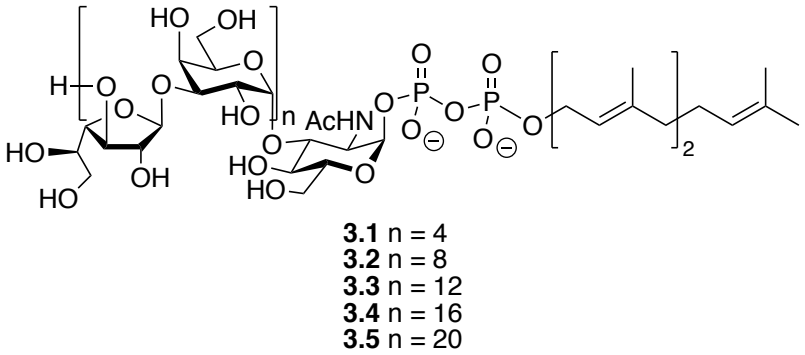
Chapter 3: Synthesis of Oligosaccharide Probes to Establish the Chain-Length Control and Export Mechanism in *K. pneumoniae* O2a O-PS Biosynthesis

3.1 Introduction

As described in Chapter 1, three GTases and an ABC-transporter are responsible for the synthesis and export of the O-PS in *K. pneumoniae* O2a, and these processes are obligatorily coupled to one another.[80,111] Chain length is controlled by a different mechanism from the one found in *E. coli* O9a and *R. terrigena*.[80] In *E. coli* O9a and *R. terrigena*, the O-PS is modified at the terminal end to terminate polymerization of the glycan, and the carbohydrate binding motifs (CBMs) for the ABC transporters recognize this modified moiety, prior to export of the O-PS.[81,82] In *K. pneumoniae* O2a, the lack of a CBM in the ABC transporter,[80] as well as unclear roles of the GTases in chain length control,[113,114] make this system interesting for further study.

Despite the targets from Chapter 2 resolving the roles of the GTases from *K. pneumoniae* O2a, many questions still remain with this complex pathway.[80,113] For example, how is the O-PS chain length is controlled without a terminal modification?[71,80] And how does the ABC transporter recognize the O-PS and exports it to the periplasm?[80] To unravel these processes, synthetic oligosaccharides that better resemble the native glycans must be used to study this system.

In this chapter, I discuss the synthesis of lipid-linked oligosaccharides related to D-galactan I from *K. pneumoniae* O2a.[105] We targeted the synthesis of five molecules **3.1**–**3.5** (Figure 3.1) of variable repeating unit length (4, 8, 12, 16, and 20), which contain up to 41 monosaccharide residues and a farnesyl diphosphate lipid. Based on previous studies,[116] we believe these compounds will be good surrogates of the native glycans and we will be able to further clarify the intricate O-PS biosynthetic pathway from *K. pneumoniae* O2a.

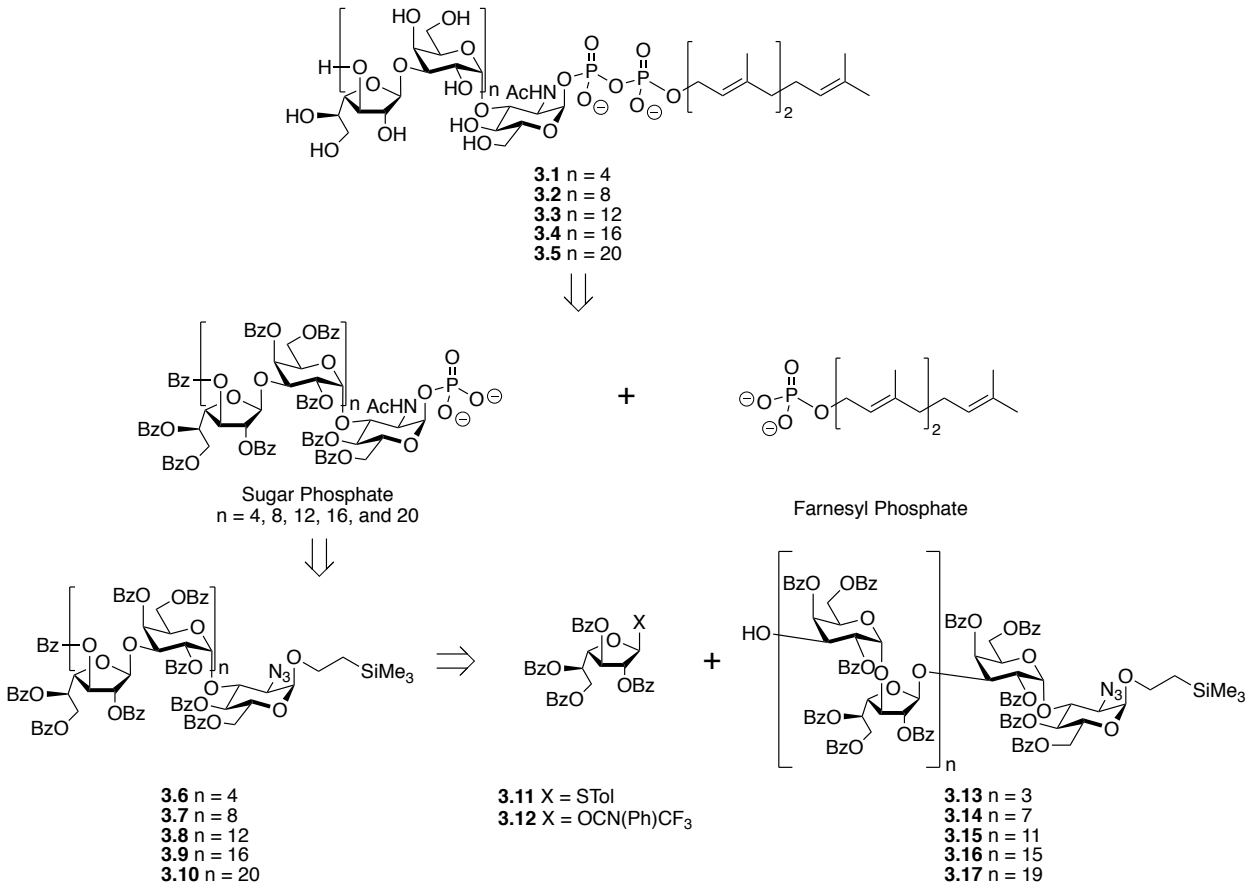


3.1 n = 4
3.2 n = 8
3.3 n = 12
3.4 n = 16
3.5 n = 20

Figure 3.1 Target molecules (**3.1**–**3.5**) related to D-galactan I from *K. pneumoniae*.

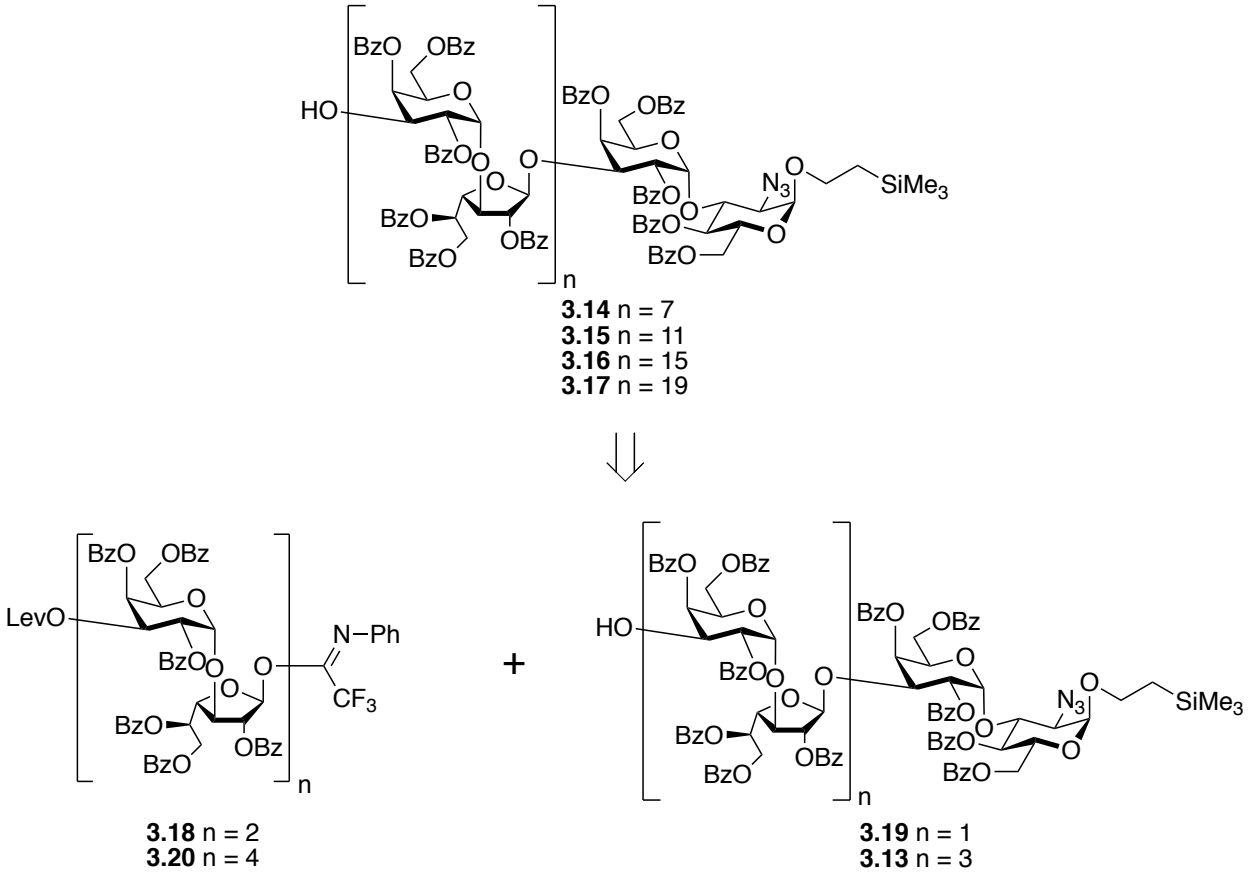
3.1.1 Retrosynthetic Analysis for Target Molecules 3.1–3.5

We believed that we could attain the five targets (**3.1**–**3.5**) solely via chemical synthesis (Scheme 3.1). Given the charged and acid-sensitive nature of the diphosphate moiety, we envisioned installing it late in our synthesis. After installation of the diphosphate, we needed to be able to cleave the chosen protecting groups in the presence of the diphosphate. We believed that the removal of acyl groups could be achieved in a mild manner that would retain the diphosphate. Benzoyl groups were chosen, as they would give good stereoselectivity in the preceding glycosylations, yet could still be removed under mild conditions. To achieve the phosphate coupling, we needed to obtain large sugar-1-phosphates (where n = 4, 8, 12, 16, and 20) that could be coupled to farnesyl phosphate.[116] Using this approach, undecaprenyl phosphate could also be used as a coupling partner in the future. These sugar-1-phosphates would come from protecting group modifications at the reducing end of compounds **3.6**–**3.10**, which can come from n + 1 glycosylations with galactofuranosyl donors **3.11** or **3.12** and oligosaccharide acceptors **3.13**–**3.17**.



Scheme 3.1 Retrosynthesis of target compounds **3.1**–**3.5**.

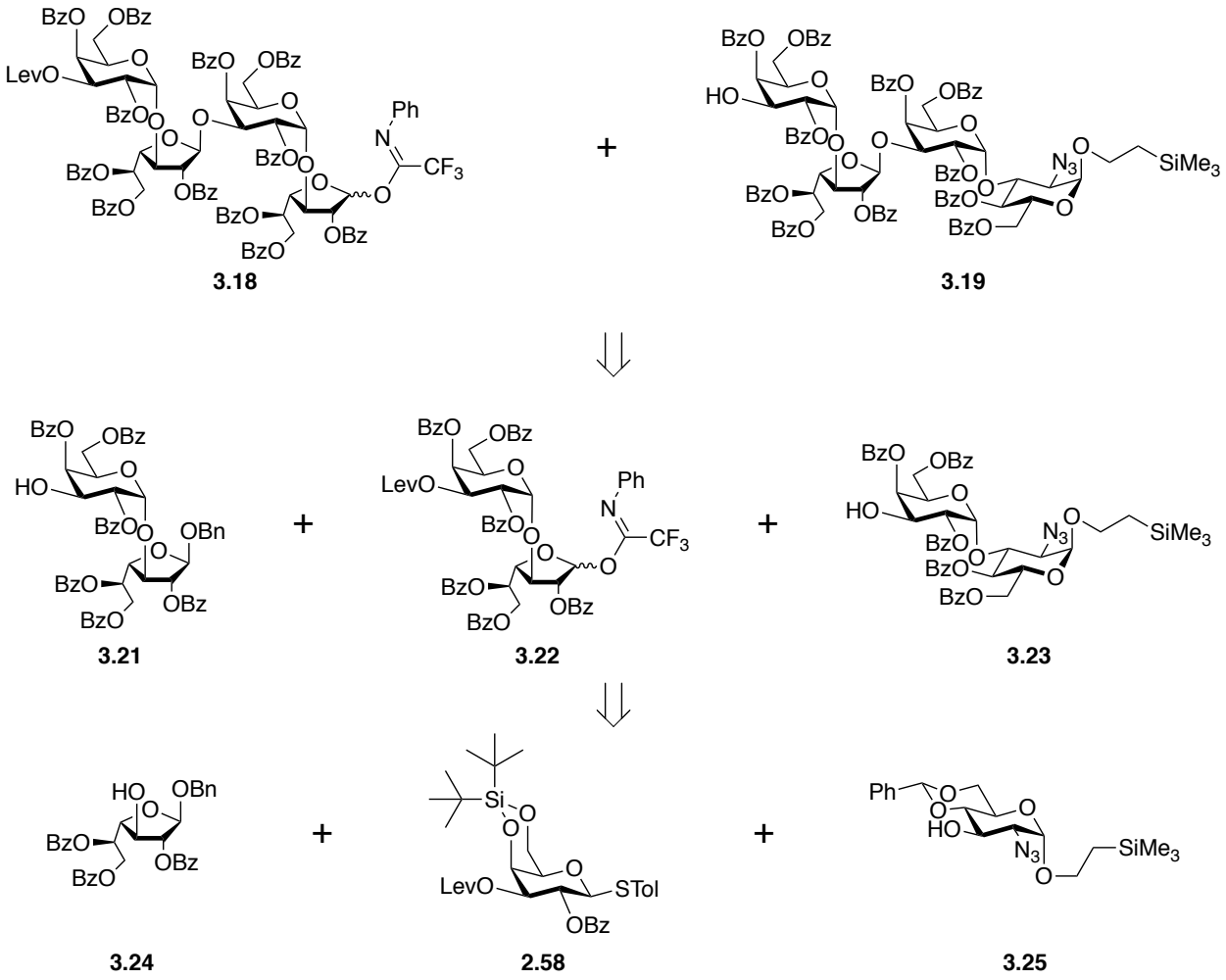
To quickly assemble the oligosaccharide acceptors, we initially envisioned doing the coupling of large building blocks (≥ 8 monosaccharide residues) together (Scheme 3.2), as this would minimize the number of steps and expedite the synthesis of the glycan. To do this, we initially desired to try 8 + 8 glycosylations with octasaccharide (8-mer) donor **3.20** and 8-mer acceptor **3.13**. If these larger coupling strategies failed, however, we could fall back on an n + 4 glycosylation strategy with tetrasaccharide **3.18**, which would give the desired oligosaccharide acceptors, albeit after a greater number of steps. The first oligosaccharide acceptor, 8-mer **3.13**, would be achieved from this n + 4 glycosylation strategy with tetrasaccharides **3.18** and **3.19**. As such, these two approaches seemed reasonable.



Scheme 3.2 Retrosynthetic rationale to elongate the synthetic glycan.

Compounds **3.18** and **3.19** would come from acceptor disaccharides, **3.21** and **3.23**, and conserved disaccharide donor **3.22** (Scheme 3.3). The 2-*O*-benzoyl protecting groups of all of these previously discussed donors (**3.11**, **3.12**, **3.18**, and **3.22**) would ensure β-selectivity in the glycosylations as the synthetic glycan grows. The three disaccharide building blocks would come from monosaccharides, **3.24** and **3.25**, and conserved galactopyranoside donor **2.58**, whose synthesis was described in Section 2.2.3. The DTBS group of donor **2.58** ensures α-selectivity[129] in the 1 + 1 glycosylations, installing the more difficult 1,2-*cis*-α glycosidic linkage first. I chose **3.24** as my galactofuranoside acceptor, as Chapter 2 showed the importance of using *O*-galactofuranosides, instead of thioglycosides, as acceptors. Acceptor **3.24** can be synthesized

from D-galactose, and acceptor **3.25** can be obtained from the hydrochloride salt of D-glucosamine.

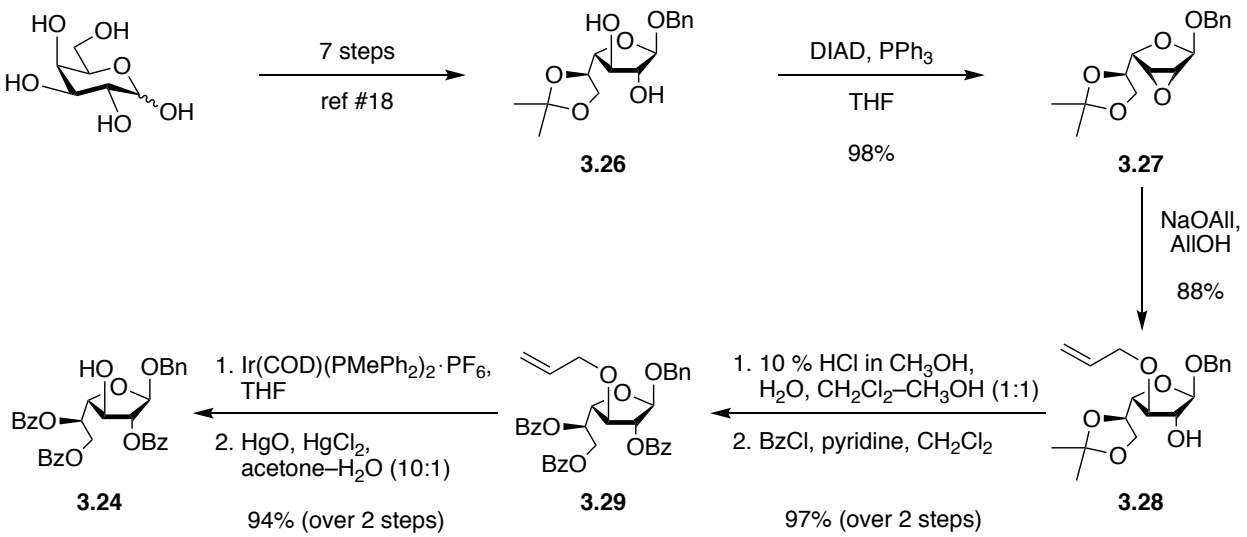


Scheme 3.3 Retrosynthesis of tetrasaccharides **3.18** and **3.19**.

3.2 Results and Discussion

3.2.1 Synthesis of Galactofuranoside Acceptor 3.24

Based on Chapter 2, I targeted *O*-galactofuranoside **3.24** as my first important building block. The synthesis of **3.24** (Scheme 3.4) began with D-galactose, which in seven steps was converted to known galactofuranoside **3.26** in 26% yield over seven steps.[134] Compound **3.26** was treated with PPh_3 and DIAD, which gave 2,3-anhydro-guloside **3.27** in 98% yield. As these conditions are a known protocol for the synthesis of 2,3-anhydro-gulosides, the stereochemistry of **3.27** was confirmed by matching the $^3J_{H\text{-}H}$ values with similar known compounds.[130] Compound **3.27** was heated to reflux in a 1 M solution of NaOAll in AllOH to give the selectively protected 3-*O*-allyl galactofuranoside **3.28** in 88% yield. Comparison of the $^3J_{H\text{-}H}$ values (including the H-1 singlet)[133] to known compounds[130] and **2.20**, confirmed the stereochemistry of **3.28**. Hydrolysis of the isopropylidene acetal, followed by benzoylation, gave the fully protected galactofuranoside **3.29** in 97% yield. The desired acceptor **3.24** was obtained in 94% yield after removal of the allyl group. This was achieved with the same method as in Chapter 2: isomerization of the double bond to the vinyl ether with $Ir(COD)(PMePh_2)_2{\cdot}PF_6$, which was then hydrolyzed with $HgCl_2$ and HgO in acetone–H_2O.



Scheme 3.4 Synthesis of galactofuranoside acceptor **3.24**.

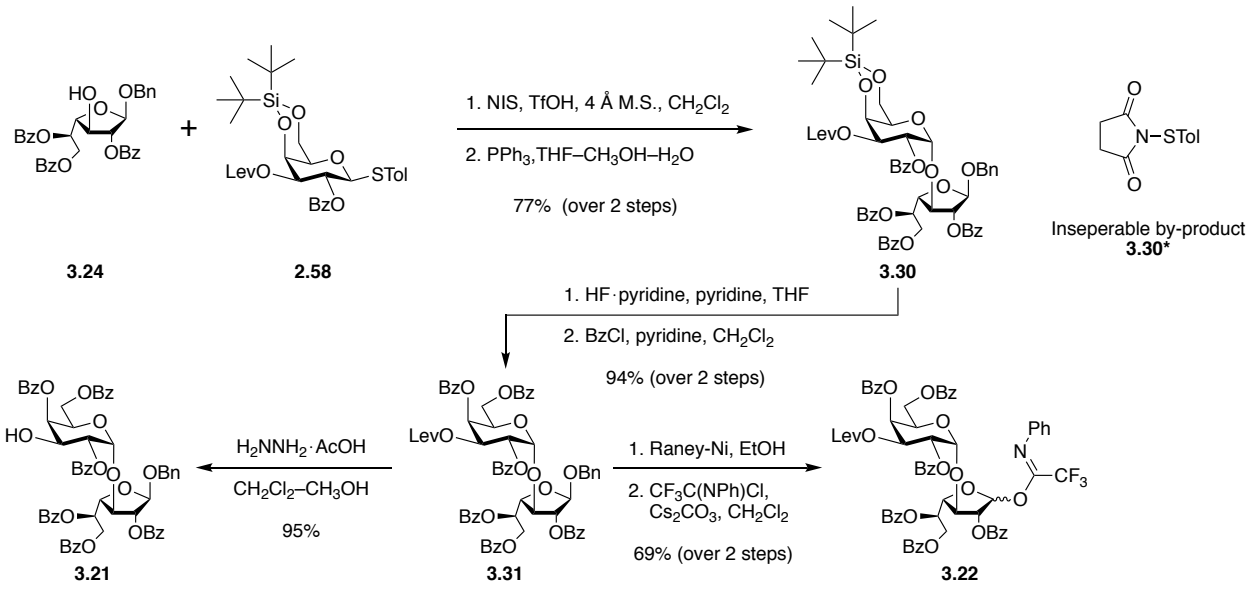
3.2.2 Synthesis of Disaccharide Acceptor 3.21 and Disaccharide Donor 3.22 (Scheme 3.5)

With the synthesis of **3.24** complete and donor **2.58** in hand, the disaccharide building blocks **3.21** and **3.22** were my next targets. To start, galactopyranoside **2.58** was activated with NIS and TfOH in the presence of **3.24**. Treatment of the crude reaction mixture with PPh_3 was necessary to remove an inseparable by-product, **3.30***. Other colleagues in the lab had also observed this compound in the glycosylation reaction mixtures, and they found the N–S bond of this by-product could be cleaved with PPh_3. This additional step eased the purification of the glycosylation mixture and gave the pure α-disaccharide **3.30** in 77% yield. The magnitude of the $^3J_{H1\text{-}H2}$, 3.7 Hz, for H-1 of the galactopyranose ring confirmed the α-selectivity.

I removed the DTBS group from compound **3.30** and replaced it with benzoyl groups. Installing the benzoyl groups ensured that there would be no later complications from removing multiple DTBS groups with harsh fluoride-based reagents, such as TBAF or HF·pyridine.

Therefore, **3.30** was treated with HF·pyridine, which gave the diol intermediate. The crude diol was benzoylated to give **3.31** in 94% yield over the two steps.

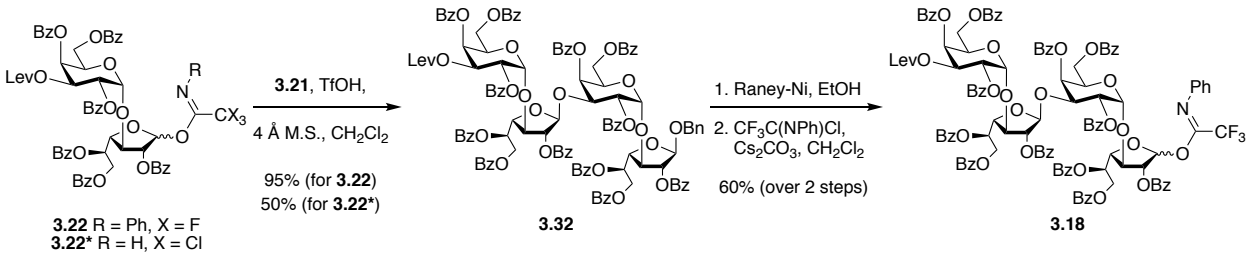
Conserved building block **3.31** could then be either converted to acceptor **3.21** or donor **3.22**. Removal of the levulinic group transpired without issue, as treatment of **3.30** with H_2NNH_2·AcOH gave acceptor **3.21** in 95% yield. Surprisingly, removal of the anomeric benzyl group proved to be a challenge. Standard hydrogenation did not show conversion of **3.31**, and transfer hydrogenation conditions did not yield the desired lactol. High pressure hydrogenations (>2000 psi) did yield the lactol, but in low yield after stirring for multiple days. Oxidative conditions with $NaBrO_3$ and $Na_2S_2O_4$[142] also gave the lactol, but in modest yields. Eventually, a report by Marino and co-workers[143] led me to try heating **3.31** in EtOH at reflux in the presence of Raney-Ni. These conditions gave the lactol in good yield, but the concentration of Raney-Ni and time of the reaction must be carefully monitored, as reduction of the ketone in the levulinic ester can also occur. Treatment of the lactol product with $CF_3C(NPh)Cl$ and Cs_2CO_3 gave imidate **3.22** in 69% yield over the two steps.



Scheme 3.5 Synthesis of disaccharide acceptor **3.21** and disaccharide donor **3.22**.

3.2.3 Synthesis of Tetrasaccharide Donor 3.18 (Scheme 3.6)

Synthesis of tetrasaccharide donor **3.18** began with the disaccharides **3.21** and **3.22**. Activation of imidate **3.22** with TfOH in the presence of **3.21** gave the tetrasaccharide **3.32** in 95% yield with complete β-selectivity. Use of the *N*-phenyl-trifluoroacetimidate **3.22** was instrumental for the excellent yield of this glycosylation. When trichloroacetimidate **3.22*** was used, the tetrasaccharide **3.32** was obtained in a modest 50% yield. As the H-1 peak from the newly formed glycosidic linkage overlapped in a complex multiplet with six other protons, the ^{1}H NMR spectrum could not be used to confirm the stereochemistry of the glycosidic linkage. However, a new peak at 107.1 ppm in the ^{13}C NMR spectrum confirmed the newly formed β-galactofuranosyl linkage.[133,144] Once again, the removal of the anomeric benzyl group was achieved by treatment of **3.32** with Raney-Ni in EtOH at reflux. The intermediate lactol was treated with $CF_3C(NPh)Cl$ and Cs_2CO_3 to give the key imidate donor **3.18** in 60% yield over two steps.

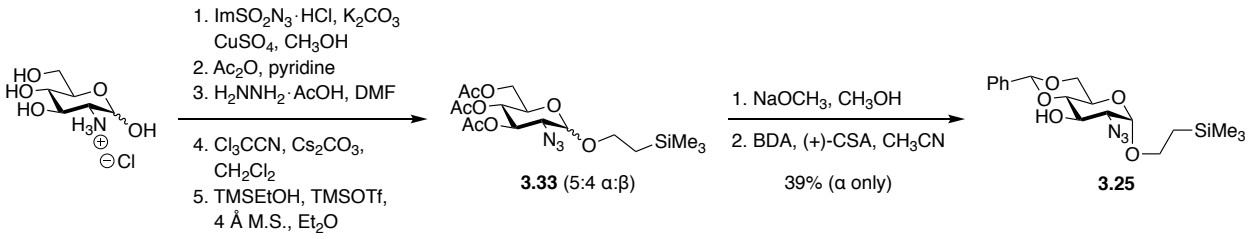


Scheme 3.6 Synthesis of tetrasaccharide donor **3.18**.

3.2.4 Synthesis of Glucosamine-Based Acceptor 3.25

To begin the synthesis of acceptor **3.25** (Scheme 3.7), the amine of D-glucosamine·HCl was converted to an azide using the azide-transfer reagent, imidazole-1-sulfonyl azide

hydrochloride ($ImSO_2N_3 \cdot HCl$).[145] I thought that this modification would eliminate potential complications from side reactions that other nitrogen protecting groups are known to undergo, such as the opening of *N*-phthalamido groups in basic conditions, difficulties in reduction of *N*-benzyl groups, or challenges in removing *N*-trichloroacetyl groups via reduction or Zemplin conditions.[146,147] In my own work, I observed the *N*-benzoylation of a Troc-protected amine in a different substrate, which further pushed me to use the azide. This azide-intermediate was then converted to the 2-(trimetheylsilyl)ethyl (TMSEt) glycoside **3.33**[148] in four steps. The TMSEt group was chosen because a former colleague had success removing this group in a closely related project.[149] The acetyl groups were removed by treatment of **3.33** with catalytic sodium methoxide, and the triol was converted to **3.25** by protection of the 4-OH and 6-OH groups with a benzylidene acetal upon treatment with benzaldehyde dimethyl acetal (BDA) and (+)-camphorsulfonic acid (CSA). As **3.33** was a 5:4 mixture of α/β anomers, the yield of the pure α-glycoside **3.25** suffered because the β-isomer of **3.25** was also obtained in modest yield.

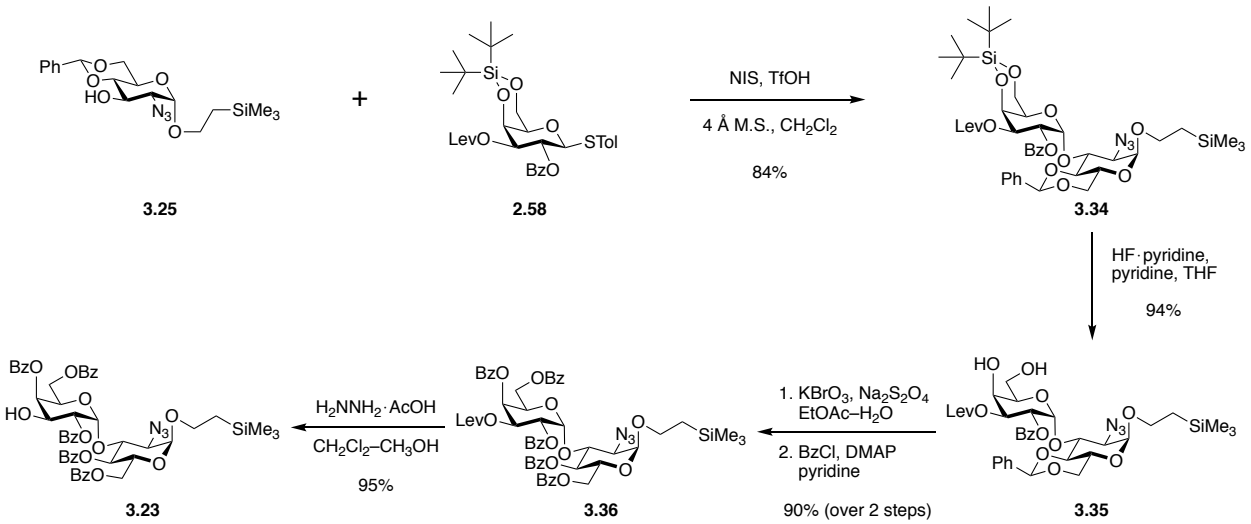


Scheme 3.7 Synthesis of 2-azido-2-deoxy-glucopyranoside acceptor **3.25**.

3.2.5 Synthesis of Tetrasaccharide Acceptor 3.19

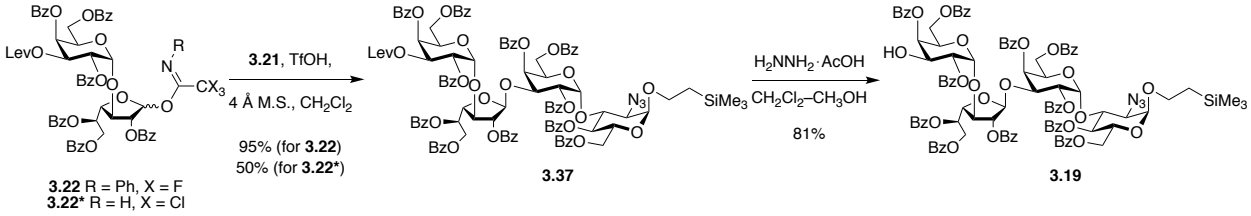
With the synthesis of **3.25** completed, assembly of tetrasaccharide **3.19** commenced. It began with the activation of donor **2.58** with NIS and TfOH in the presence of **3.25** (Scheme

3.8). This gave disaccharide **3.34** in 84% yield with α-only selectivity. The stereoselectivity of the glycosylation was confirmed by the magnitude of $^{3}J_{H1\text{-}H2}$, 3.0 Hz, from H-2 of the galactopyranose ring in the ^{1}H NMR spectrum. As was done earlier in Section 3.2.2, early removal of the DTBS group with HF·pyridine gave the diol **3.35** in 94% yield. Removal of the benzylidene in the presence of the TMSEt group with acidic conditions only gave the desired product in modest yields. Oxidative conditions with $KBrO_3$ and $Na_2S_2O_4$ cleanly removed the benzylidene acetal, but gave a mixture of the 4-*O* and 6-*O*-benzoyl regioisomers in a combined 93% yield. This was not an issue because the mixture can be converted to fully protected disaccharide **3.36** by treatment of the regioisomers with BzCl and DMAP in pyridine heated at reflux. Heating the reaction at reflux was necessary because the 4-OH of the glucosamine residue was slow to benzoylate and needed to be pushed to go to completion. These conditions gave **3.36** in 97% yield. Removal of the levulinoyl ester by treatment of **3.36** with H_2NNH_2·AcOH gave alcohol **3.23** in 95% yield.



Scheme 3.8 Synthesis of the reducing end disaccharide acceptor **3.23**.

Activation of imidate **3.22** with TfOH in the presence of alcohol **3.23** gave tetrasaccharide **3.37** in 95% yield with complete β-selectivity (Scheme 3.9). As in the case for the formation of **3.32**, the excellent yield of **3.37** was due to use of the *N*-phenyl-trifluoroacetimidate **3.22**. When the trichloroacetimidate **3.22*** was used in this glycosylation reaction, compound **3.37** was only isolated in a modest 50% yield. A new singlet at 5.33 ppm in the ^{1}H NMR spectrum confirmed the newly formed β-(1→3)-glycosidic linkage.[133] Removal of the levulinoyl ester with H_2NNH_2·AcOH gave the tetrasaccharide acceptor **3.19** in 81% yield.

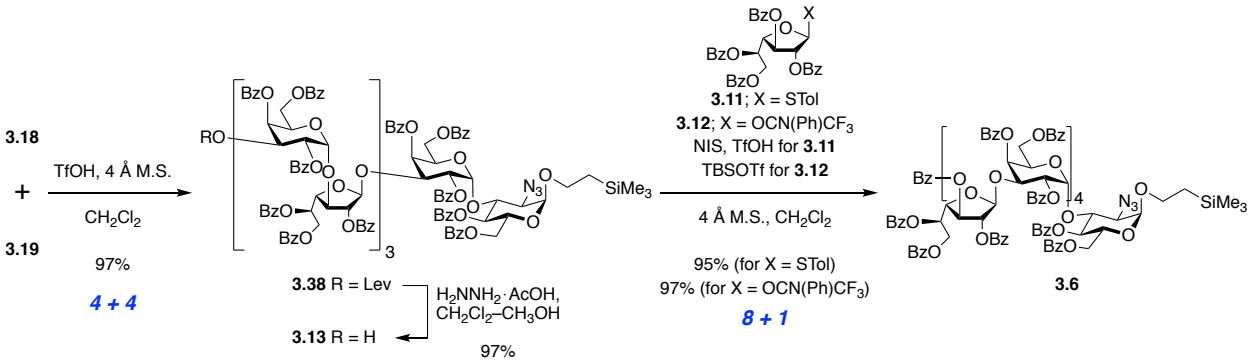


Scheme 3.9 Synthesis of the tetrasaccharide acceptor **3.19**.

3.2.6 Synthesis of Nonasaccharide (9-mer) Target 3.1

Having developed an approach to both tetrasaccharides **3.19** and **3.18**, synthesis of the first target **3.1** began. First, imidate donor **3.18** was activated with TfOH in the presence of the tetrasaccharide **3.19**, which gave the 8-mer **3.38** in 97% yield (Scheme 3.10). As **3.38** was an 8-mer comprised of three repeating units, the ^{1}H spectrum contained significant overlap. Peaks at 108.4, 108.2, and 107.6 ppm in the ^{13}C NMR spectrum, however, confirmed that there were three β-galactofuranosyl linkages. Treatment of **3.38** with H_2NNH_2·AcOH gave the 8-mer acceptor **3.13** in 97% yield. The target backbone **3.6** was obtained in 97% yield via the activation of

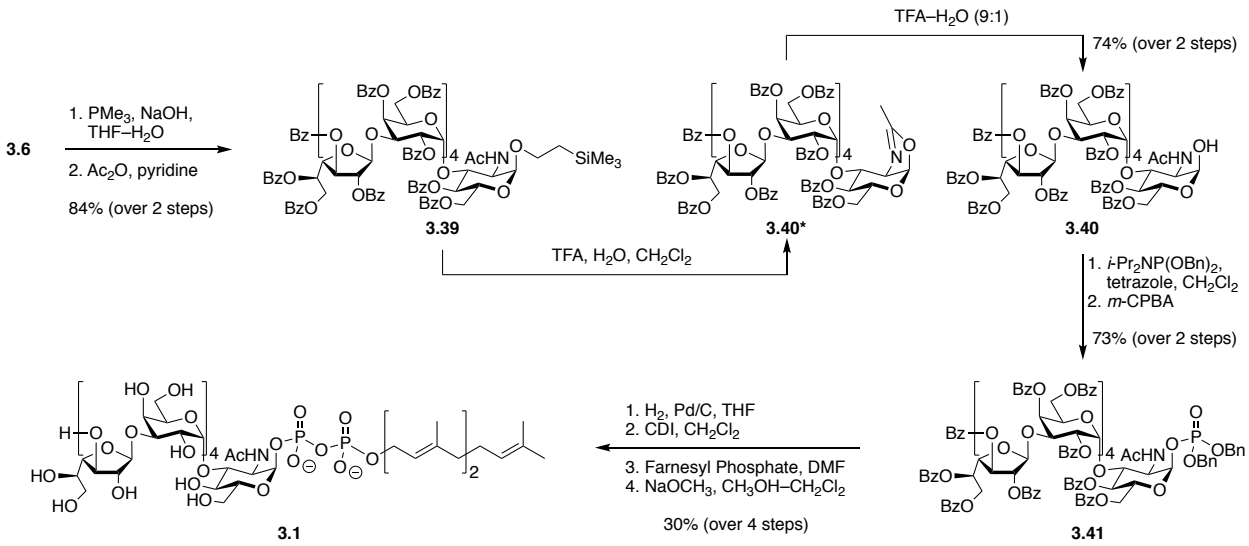
monosaccharide imidate **3.12** with TBSOTf in the presence of **3.13**. Imidate donor **3.12** was more reactive than thioglycoside donor **3.11**, as **3.11** required more equivalents and a longer reaction time (~24 h) to give comparable yields to **3.12** in glycosylations with **3.13**. A new peak at 108.3 ppm in the ^{13}C NMR spectrum confirmed the new β-(1→3) linkage.[133]



Scheme 3.10 Synthesis of the 9-mer target backbone **3.6**.

With target backbone **3.6** in hand, conversion to target **3.1** began (Scheme 3.11). First, the azide needed to be reduced, but standard hydrogenation conditions did not show any conversion of the starting material. Use of PMe_3 and NaOH in THF–H_2O did, however, give the desired amine product. Once reduced, the intermediate amine was acetylated to give **3.39** in 84% over two steps. Acidic hydrolysis of the TMSEt group was challenging. Treatment of **3.39** with TFA and H_2O in CH_2Cl_2 initially gave an oxazoline by-product **3.40***. However, this crude oxazoline was treated with TFA–H_2O (9:1) to give the desired lactol **3.40** in 74% yield. Lactol **3.40** was converted to dibenzyl phosphate **3.41** in 73% over two steps. This was achieved by treatment of **3.40** with *i*-$Pr_2NP(OBn)_2$ and tetrazole and oxidation of the intermediate phosphite with *m*-CPBA. Conversion to **3.1** started with hydrogenation of **3.41**, after which the phosphate was activated with CDI. The CDI-activated sugar phosphate was combined with farnesyl

phosphate and stirred for six days in DMF, before benzoyl group removal with sodium methoxide. The 9-mer target **3.1** was acquired in 30% yield over four steps. Hydrogenation and CDI-activation proceeded in near quantitative yields, so the loss in yield occurred over the last two steps. Phosphate–phosphate coupling reactions are typically poor-yielding reactions and take a very long time to complete.[116,150] During this lengthy reaction time, side reactions occur. One such side reaction that I observed was the formation of oxazoline **3.40***, which could not be converted to the desired product. Formation of the triphosphate sugar-lipid or self-coupling of the sugar phosphate to itself are also possible by-products and are further reasons for the low yields commonly found with this chemistry. The deprotection also needed to be done carefully; if the desired diphosphate was left for too long in a highly concentrated solution of sodium methoxide, cleavage of the diphosphate could be an issue. To circumvent this issue, the protected diphosphate intermediate was treated with a 5 mM sodium methoxide in CH_2Cl_2–CH_3OH for three days. Using these mild conditions, a lengthy reaction time was required to remove all benzoyl groups. Isolation of pure target **3.1** was confirmed by ^{1}H NMR and HR-MS analysis, as the ^{1}H NMR spectrum showed the correct number of H's with the expected shifts and HR-MS confirmed the correct molecular formula.



Scheme 3.11 Synthesis of the 9-mer target **3.1**.

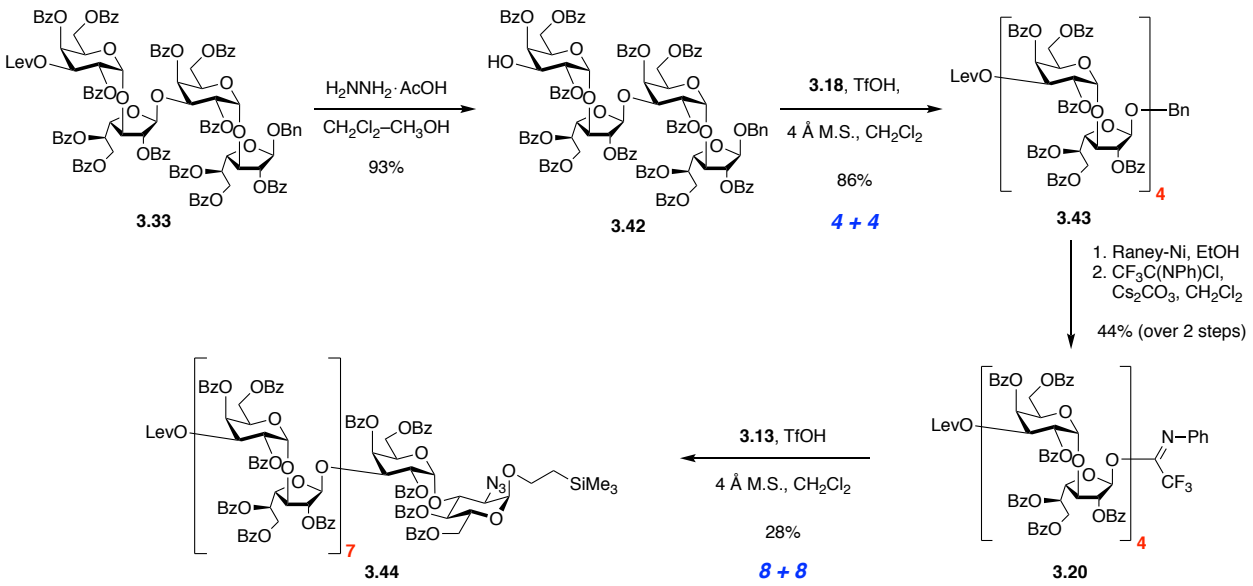
3.2.8 Efforts Using "Large" Glycosyl Donors to Obtain Oligosaccharide Acceptors

Having synthesized the 9-mer target **3.1**, I focused my attention on the synthesis of the larger targets. As described in Section 3.1.1, I desired to do the coupling of large (≥ 8 monosaccharide residues) building blocks. I hence planned to use an 8 + 8 + 1 glycosylation strategy to obtain the heptadecasaccharide (17-mer) target, **3.2**.

As I already synthesized 8-mer acceptor **3.13**, I needed to obtain 8-mer donor **3.20** to attempt the 8 + 8 glycosylation to elongate the glycan (Scheme 3.12). To begin, I removed the levulinoyl ester from tetrasaccharide **3.33** to obtain alcohol **3.42** in 93% yield. Imidate donor **3.18** was activated with TfOH in the presence of **3.42** to obtain 8-mer **3.43** in 86% yield. This 8-mer **3.43** was converted to a donor via the same method as in Section 3.2.2. First, reduction of the benzyl group with Raney-Ni, and then imidate formation by treating the lactol intermediate with $CF_3C(NPh)Cl$ and Cs_2CO_3. The desired imidate **3.20** was only obtained in 44% yield over the two steps due to Raney-Ni reduction of the benzyl group being troublesome. The two issues

in this reduction were 1) substrate solubility and 2) chemoselectivity of the reduction. The larger 8-mer substrate was less soluble in EtOH than the tetrasaccharide substrates, but this was overcome by adding EtOAc as a co-solvent to the reaction mixture. The chemoselectivity problem was more difficult to overcome. As stated in 3.2.2, the time of the reaction and the concentration of the Raney-Ni has to be carefully monitored to avoid reduction of the ketone. However, the rate of reduction of the ketone relative to the rate of reduction of the benzyl group increased with the larger substrates. By TLC and other analyses, the reduction of the ketone after the formation of the lactol was faster with this 8-mer **3.43**. The formation of the undesired di-reduced product was observed before the starting material was consumed. This over-reduction of **3.43** was the cause of the loss of yield.

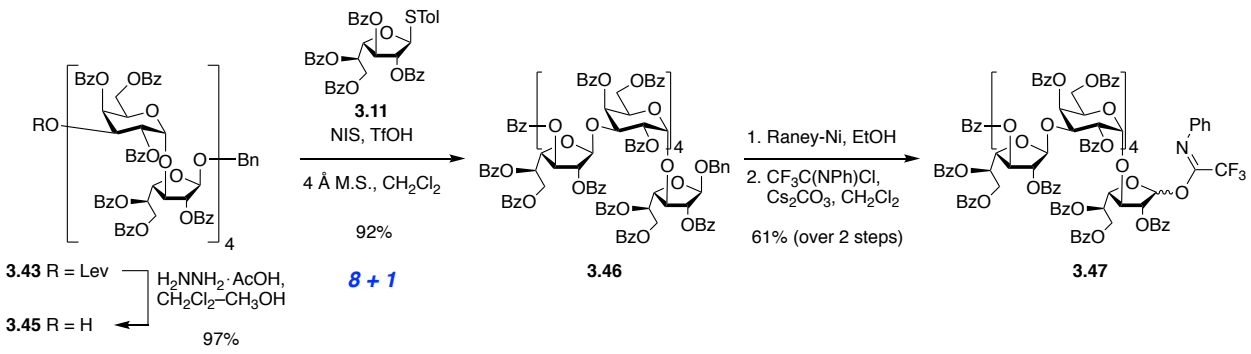
Despite these difficulties, I did obtain enough 8-mer imidate donor **3.20** to attempt the 8 + 8 glycosylation. I knew with this sterically demanding donor and acceptor combination, as well the inductive effects of the many benzoyl groups in these molecules, that this glycosylation was going to be challenging. I used inverse glycosylation conditions, as I hoped the higher concentration of the acceptor relative to the donor would bring these two compounds together in good yield. Inverse glycosylation conditions are as follows: the acceptor is first dissolved in the reaction solvent. The activator is added. Lastly the donor is slowly added to the reaction mixture. I dissolved acceptor **3.13** in CH_2Cl_2, added TfOH, and then added a solution of **3.20** in CH_2Cl_2 dropwise to the reaction mixture. I obtained the desired hexadecasaccharide (16-mer) **3.44** in 28% yield.



Scheme 3.12 The use of an 8 + 8 glycosylation strategy to achieve 16-mer **3.44**.

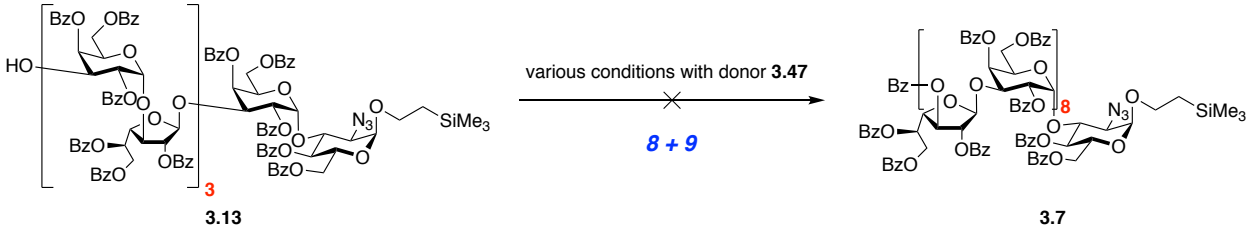
As both the glycosylation and the reduction were low yielding, I abandoned this route. I did not think the chemoselectivity problem in the reduction of **3.43** would allow material attempt optimization of the 8 + 8 glycosylation. However, if I removed the levulinic ester in **3.43**, added an additional monosaccharide residue, and then reduced the benzyl group, I would obtain a large glycosyl donor without the chemoselectivity issues. I would then have enough material to optimize the glycosylations.

To do this, I first removed the levulinic group by treating **3.43** with $H_2NNH_2 \cdot AcOH$, which gave alcohol **3.45** in 97% yield (Scheme 3.13). Activation of **3.11** with NIS/TfOH in the presence of **3.45** gave the desired 9-mer **3.46** in 92% yield. Reduction of the benzyl group, followed by imidate formation, gave the 9-mer imidate donor **3.47** in 61% yield over two steps, an improvement possible because the chemoselectivity of the reduction was no longer an issue.



Scheme 3.13 Synthesis of the 9-mer imidate donor **3.47**.

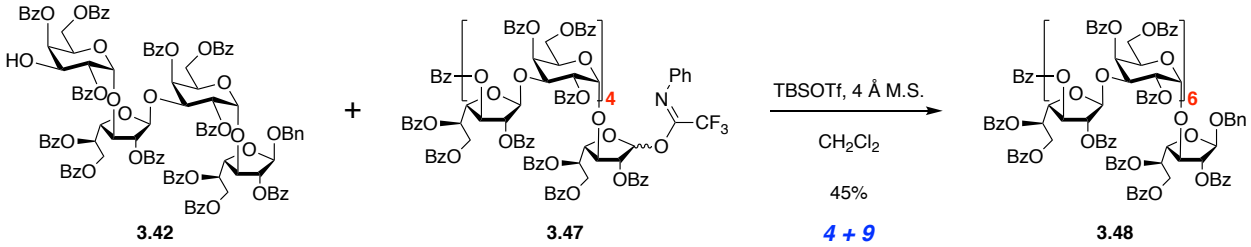
As I now had 9-mer donor **3.47**, I then attempted to do an 8 + 9 glycosylation to directly obtain the heptadecasaccharide (17-mer) target **3.7**. Despite the many conditions tried, the desired 17-mer product **3.7** could not be isolated. With this lack of success, I decided to use smaller building blocks in the glycosylation reactions to determine what the problems were.



Acceptor Equivalents (3.13)	Donor Equivalents (3.47)	Activator	Outcome
1.0	1.3	TfOH	trace by NMR
1.0	1.3	TfOH	trace by NMR
1.0	1.3	TfOH	trace by NMR
1.0	1.3	TBSOTf	N/O
1.4	1.0	TBSOTf	N/O

Table 3.1 Conditions tried to obtain target backbone **3.7**. (N/O = Not Observed).

Possibly the electron withdrawing inductive effects of the many benzoyl groups on the 8-mer acceptor **3.13** was reducing the yield. I believed the donor was not the issue, and an acceptor with fewer benzoyl groups would be a better nucleophile in the glycosylation. To test this hypothesis, the 9-mer imidate **3.47** was activated with TBSOTf in the presence of tetrasaccharide alcohol **3.42**. The desired tridecasaccharide (13-mer) **3.48** was obtained, but only in a modest 45% yield. This was around a 40% loss in yield when compared to the 4 + 4 with the same acceptor **3.42** and tetrasaccharide donor **3.18**. Contrary to my hypothesis, the use of these large donors appeared to be the reason for the low yields, and that, for this system, there might be a limit to the size of the glycosyl donor that can be used to give good yields of the products.

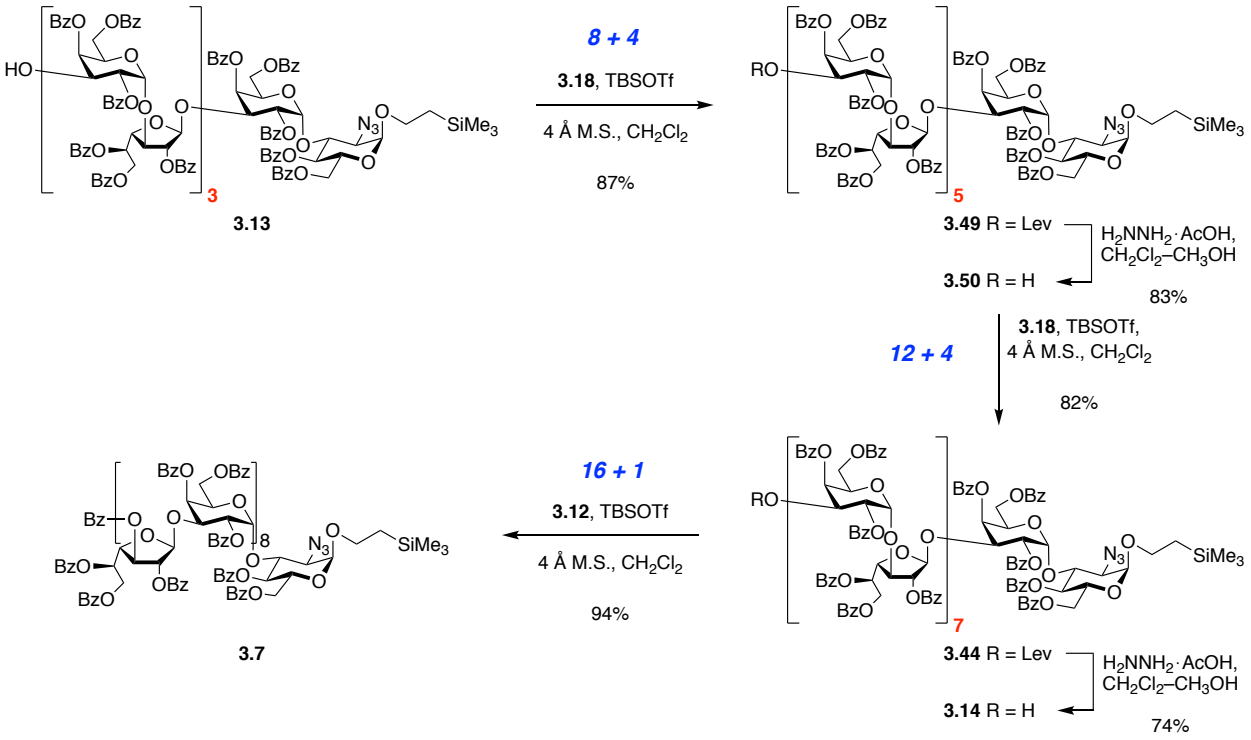


Scheme 3.14 Synthesis of the 13-mer **3.48**.

3.2.9 Synthesis of 17-mer Target 3.2

With little success in using large glycosyl donors to obtain the oligosaccharide acceptors **3.14**–**3.17**, the n + 4 glycosylation strategy was investigated to obtain the 17-mer target **3.2**. To do this, an 8 + 4 + 4 + 1 glycosylation strategy was used (Scheme 3.15). Previously synthesized 8-mer **3.13** was combined with tetrasaccharide imidate **3.18**, which was activated with TBSOTf to give the desired dodecasaccharide (12-mer) **3.49** in 87% yield. This result supports the notion of a limit to glycosyl donor size, as the 8 + 4 glycosylation was high yielding. The levulinic

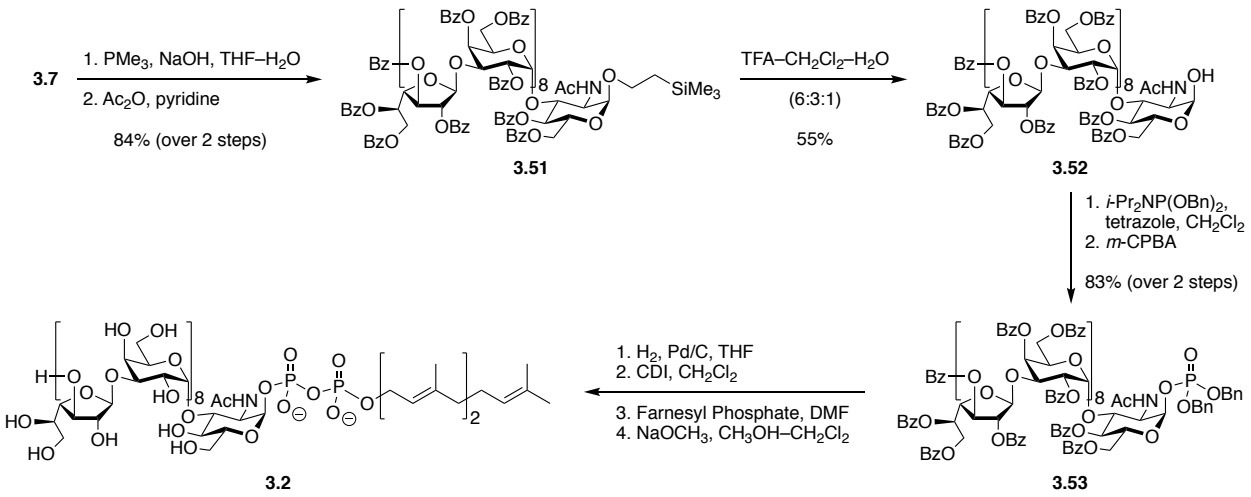
group was then removed with H_2NNH_2·AcOH to give **3.50** in 83% yield. Activation of tetrasaccharide **3.18** with TBSOTf in the presence of **3.50** gave the 16-mer **3.44** in 82% yield. The levulinoyl ester was removed with H_2NNH_2·AcOH to give acceptor **3.14** in 74% yield. The 17-mer target backbone **3.7** was obtained by activation of galactofuranosyl imidate **3.12** with TBSOTf in the presence of **3.14**. This 16 + 1 glycosylation gave the desired product **3.7** in 94% yield.



Scheme 3.15 Synthesis of the 17-mer target backbone **3.7**.

To acquire the 17-mer target **3.2**, the same approach used to obtain **3.1** was followed (Scheme 3.16). Reduction of the azide of **3.7** with PMe_3 and acetylation to convert the amine to an *N*-acetyl group gave compound **3.51** in 84% over two steps. Once again, acidic hydrolysis was challenging, but treatment of **3.51** with a solution of TFA–CH_2Cl_2–H_2O (6:3:1) gave the

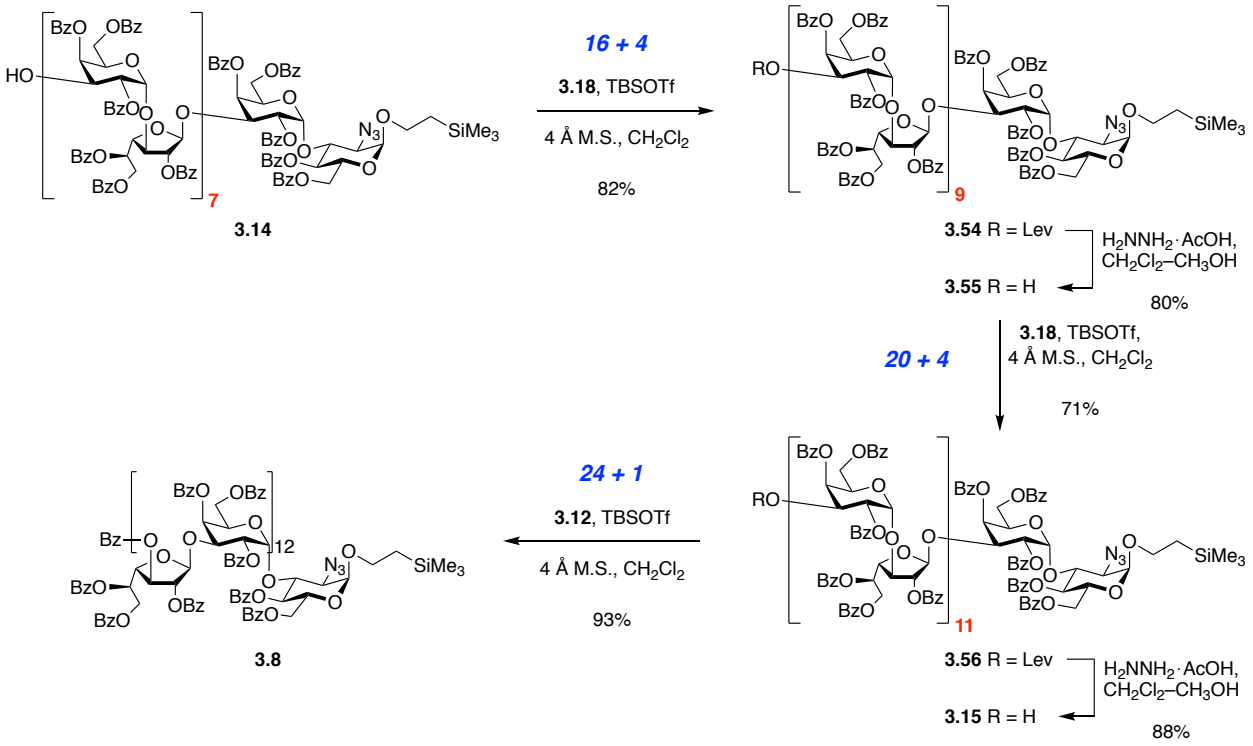
desired lactol **3.52**, albeit in a modest 55% yield. Dibenzyl phosphate **3.53** was obtained in 83% yield over two steps. This was achieved by first treating **3.52** with tetrazole and *i*-$Pr_2N(OBn)_2$ for 2 h, followed by addition of *m*-CPBA to oxidize to the phosphate in a single pot. Using the same method from Section 3.2.6, hydrogenation of **3.53**, followed by treatment of the intermediate with CDI gave the CDI-activated sugar phosphate, which was then mixed with farnesyl phosphate in DMF and stirred for 13 days. This protected phosphate was treated with sodium methoxide to remove the benzoyl groups. When treated with 5 mM $NaOCH_3$ to completely remove all of the benzoyl groups, the phosphate linkage began to break down, as this reaction took more than 5 days. To circumvent this issue, I shortened the reaction time to three hours by treating the protected diphosphate with 50 mM $NaOCH_3$ in CH_3OH–CH_2Cl_2, as this was the procedure that a former colleague used on a closely related project.[149] Even with this increase in $NaOCH_3$ concentration, there were still mono- and di-benzoylated by-products from incomplete deprotection. Although I found no evidence of the breaking of the diphosphate bond (aside from the low yield), I did not let the reaction go any longer because I feared that the diphosphate would cleave if. At this time, target **3.2** has not been purified fully, as <1 mg of a mixture was obtained. However, the HRMS–ESI–TOF spectrum (calcd for $[M–2H]^{2–}$ $C_{119}H_{201}NO_{92}P_2$: 1588.0205. Found 1588.0155) showed the presence of **3.2** in the mixture.



Scheme 3.16 Synthesis of the 17-mer target **3.2**.

3.2.10 Synthesis of Pentacosasaccharide (25-mer) Target 3.3

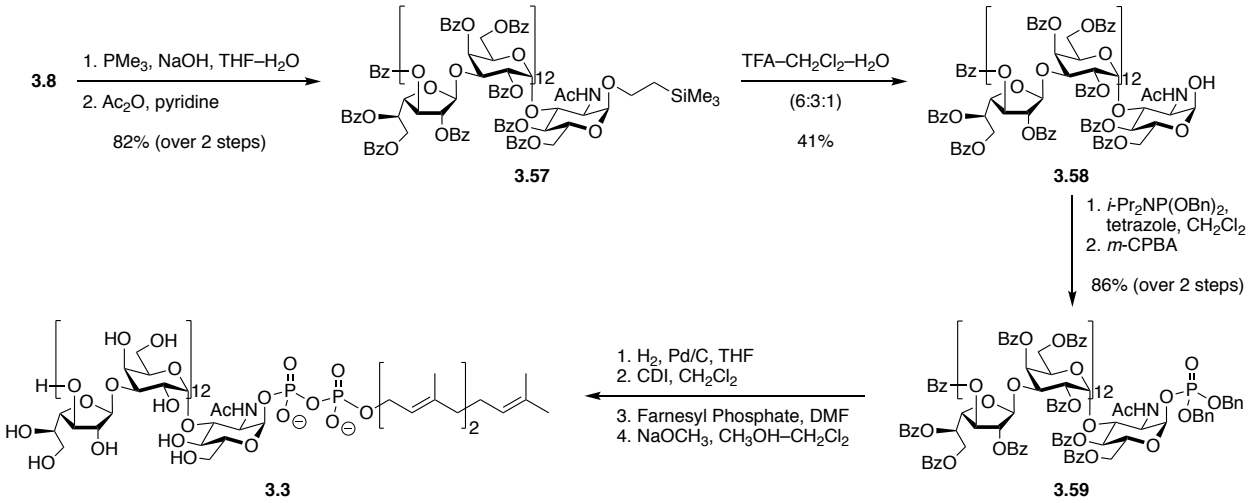
To access the 25-mer target **3.3**, I employed a 16 + 4 + 4 + 1 glycosylation strategy that began from the previously discussed 16-mer acceptor **3.14** (Scheme 3.17). Similar to what was done previously for the smaller targets, tetrasaccharide imidate **3.18** was activated with TBSOTf in the presence of **3.14**, which gave the icosasaccharide (20-mer) **3.54** in 82% yield. Treatment of **3.54** with H_2NNH_2·AcOH gave **3.55** in 80% yield. Alcohol **3.55** was combined with **3.18**, which was activated with TBSOTf. This 20 + 4 glycosylation yielded tetracosasaccharide (24-mer) **3.56** in 71% yield, which was treated with H_2NNH_2·AcOH to give the 24-mer acceptor **3.15** in 88% yield. Activation of galactofuranosyl imidate **3.12** with TBSOTf in the presence of **3.15** gave the 25-mer target backbone **3.8** in 93% yield.



Scheme 3.17 Synthesis of the 25-mer target backbone **3.8**.

Using the same approach as that for the synthesis of **3.1** and **3.2**, target backbone **3.8** was converted to **3.3** (Scheme 3.18). Compound **3.8** was treated first with PMe_3 and then with Ac_2O to give **3.57** in 82% yield over two steps. Removal of the TMSEt group was again an issue. Treatment of **3.57** with a solution of TFA–CH_2Cl_2–H_2O (6:3:1) did give lactol **3.58**, albeit in a very modest 41% yield. As the reaction was stirred for a lengthy amount of time (~5 days) to fully consume the starting material, the loss of yield was likely a result of the unselective hydrolysis of other glycosidic linkages or the benzoyl groups. The TLC showed spots on the baseline that looked to be sugars when the TLC was analyzed. Lactol **3.58** gave dibenzyl phosphate **3.59** in 86% yield over two steps, via phosphite formation with *i*-$Pr_2N(OBn)_2$ and tetrazole, followed by *m*-CPBA oxidation to the phosphate. Hydrogenation of **3.59**, followed by coupling of the CDI-activated sugar to farnesyl phosphate in DMF over 18 days, then global

deprotection with sodium methoxide gave the desired 25-mer target **3.3**. Isolation of **3.3** was difficult. A large portion of the substrate was converted to an oxazoline during the phosphate coupling step, and during the deprotection step, a portion of the phosphate was cleaved during the lengthy reaction time (>5 days). Due to these complications, I isolated <1 mg of a mixture that contained **3.3**, confirmed by HRMS–ESI–TOF analysis (calcd for $[M–3H]^{3}$ $C_{167}H_{281}NO_{132}P_2$: 1490.4854. Found 1490.4850).

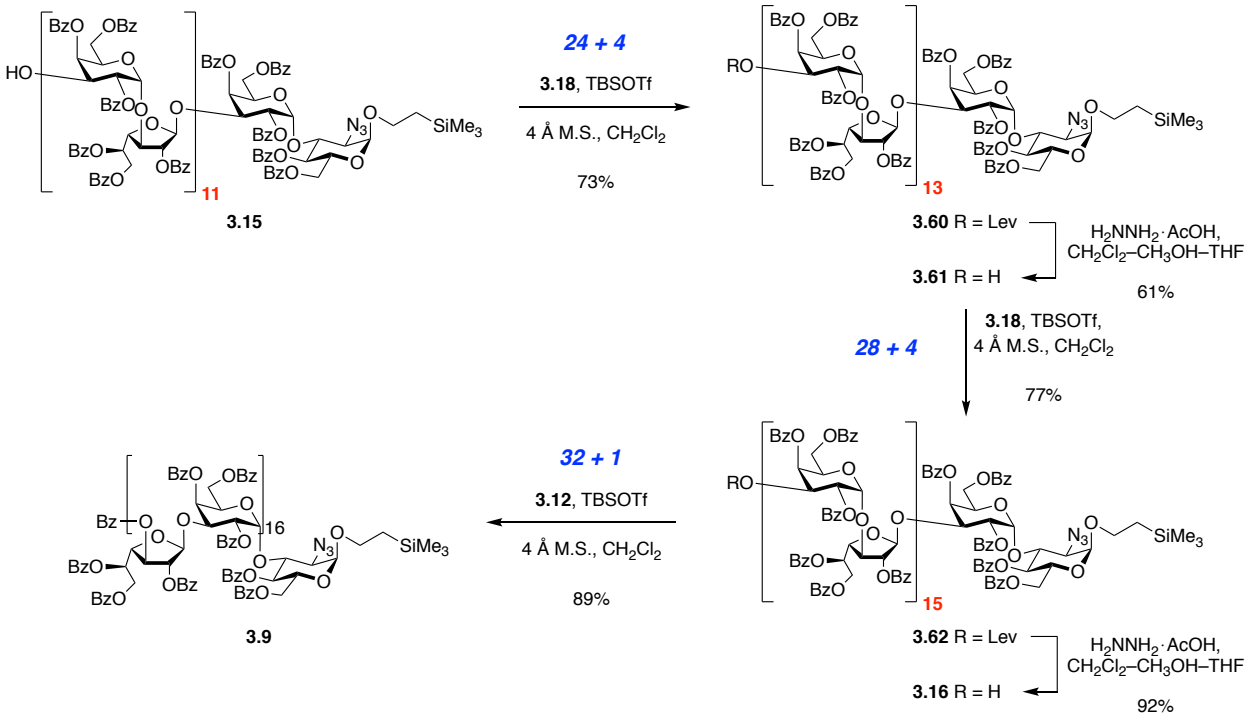


Scheme 3.18 Synthesis of the 25-mer target **3.3**.

3.2.11 Efforts Towards the Synthesis of the Tritriacontasaccharide (33-mer) Target 3.4

Synthesis of target backbone **3.9** was achieved via a 24 + 4 + 4 + 1 glycosylation strategy (Scheme 3.19). It began with 24-mer acceptor **3.15** being glycosylated with **3.18** using TBSOTf as an activator to give octacosasaccharide (28-mer) **3.60** in 73% yield. At this point in the synthesis, purification became an issue, as the large MW compounds had very subtle changes in polarity between various intermediates. Also, the removal of the levulinic esters was slow, as the reaction would take >6 h to go to completion, which is far longer than the reaction times I

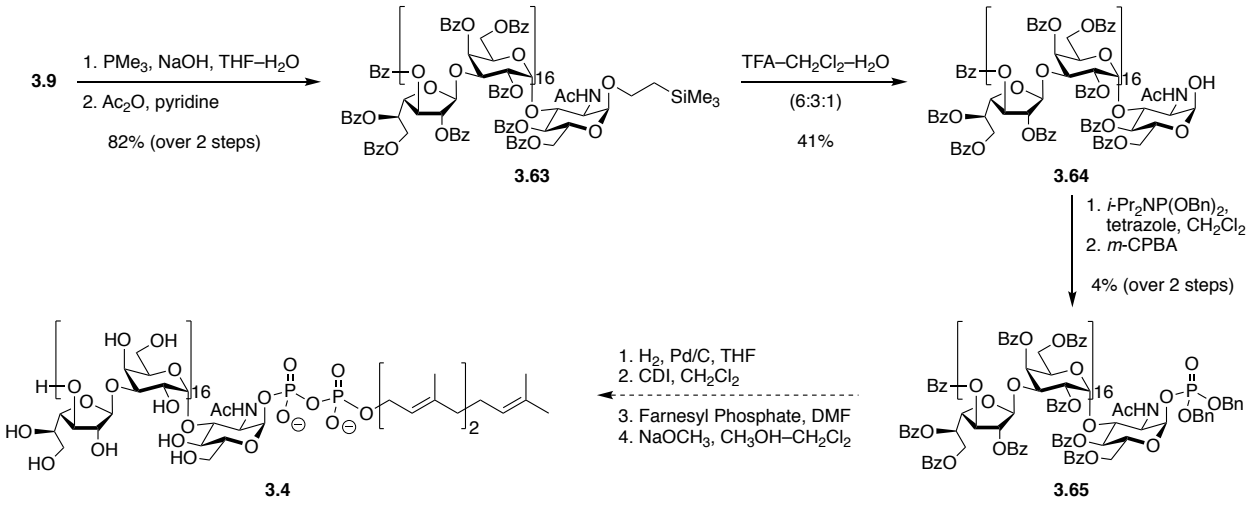
observed for the smaller compounds (≤ 1 h). For these reasons, removal of the levulinic group on **3.60** needed to be pushed to completion to isolate pure **3.61**. To achieve this goal, the conditions were changed from stirring **3.60** in CH_2Cl_2–CH_3OH at rt with 2.4 equivalents of H_2NNH_2·AcOH to stirring **3.60** in CH_2Cl_2–CH_3OH–THF at 60 °C with 10 equivalents of H_2NNH_2·AcOH. Using these modified conditions, alcohol **3.61** was isolated in 61% yield. The 28 + 4 glycosylation between **3.61** and **3.18** gave dotriacontasaccharide (32-mer) **3.62** in 77% yield. Addition of 3.8 equivalents of **3.18** to the reaction was needed to consume the 28-mer acceptor **3.61**. In the previous glycosylation studies, no more than 2.0 equivalents of donor **3.18** were used. Using the more forceful conditions to remove the levulinoyl ester from **3.62**, the 32-mer acceptor **3.16** was isolated in 92% yield. As only ~100 mg of **3.16** was isolated, I did not pursue the synthesis of the hentetracontasaccharide (41-mer) **3.5**. Instead, all of compound **3.16** was coupled with imidate **3.12** to complete the synthesis of 33-mer **3.4**. Addition of TBSOTf to a mixture of **3.16** and **3.12** activated the latter, and the 33-mer **3.9** was obtained in 89% yield.



Scheme 3.19 Synthesis of the 33-mer target backbone **3.9**.

With the synthesis of **3.9** complete, I turned my attention to the 33-mer target **3.4** (Scheme 3.20). As the previously used strategy worked for targets **3.1**–**3.3**, I started with PMe_3 reduction of the azide on **3.9**, followed by acetylation of the amine. These two steps gave the desired product **3.63** in a combined 82% yield. Acidic hydrolysis of **3.63** with TFA–CH_2Cl_2–H_2O (6:3:1) gave the 33-mer lactol **3.64** in 41% yield. The loss of yield may have been due to the unselective cleavage of other glycosidic bonds. Next, I converted **3.64** to the dibenzyl phosphate **3.65**. Again, this was achieved in one pot by first addition of tetrazole and *i*-$Pr_2NP(OBn)_2$, followed by the addition of *m*-CPBA. However, I was only able isolate **3.65** in 4% yield, as the formation and removal of by-products were a challenge. As I used a large excess of reagents to push this reaction to completion in a short time, it is possible that the high concentration of reagents may have caused the formation of a substantial amount of by-products. However, some of the desired product **3.65** did form in the reaction mixture, but its isolation was challenging, as

3.65 and by-products of the reaction had very subtle differences in polarity. Use silica gel column chromatography and size exclusion chromatography always gave inseparable mixtures. Multiple runs of preparative TLC allowed the isolation of **3.65**. Only obtained 1 mg of **3.65** was obtained after the multiple purification attempts. Based on this, I abandoned the synthesis of target **3.4**.



Scheme 3.20 Attempted synthesis of the 33-mer target **3.4**.

3.3 Summary

In conclusion, I synthesized three lipid-linked oligosaccharides **3.1**–**3.3** ranging from 9 to 25 monosaccharide units, and a fully protected 33-mer **3.65**. The isolation of pure **3.2** and **3.3**, however, has been unsuccessful so far. I was also unable to convert **3.65** to the 33-mer target **3.4**, due to the lack of sufficient material. I also abandoned the attempted synthesis of 41-mer target **3.5**, again due to a lack of material (the 32-mer **3.16**). My synthetic route featured the installation of the more challenging 1,2-*cis*-α glycosidic linkages first, using acceptors **3.24** and **3.25,** and donor **2.58**; this allowed me to elongate the synthetic glycan via the 1,2-*trans*-β glycosidic

linkage. It also featured a conserved disaccharide donor **3.22** that contained a 2-*O*-benzoyl group, which ensured the formation of the 1,2-*trans*-β linkage when glycosylated with acceptors **3.21** and **3.23**. These glycosylations led to key tetrasaccharide building blocks **3.18** and **3.19**.

Although the coupling of larger building blocks (8 + 8 and 8 + 9 glycosylations) ultimately failed (as there might be a limit to the size of glycosyl donors in this system), the n + 4 glycosylations with 2-*O*-benzoylated tetrasaccharide donor **3.18**, followed by removal of the levulinic group, led to the oligosaccharide acceptors **3.11**–**3.14** in good yields with complete β-selectivity in all cases. The n + 1 glycosylations all gave ~90% yield with complete β-selectivity for the target backbones **3.6**–**3.9**. This synthetic route should also enable the synthesis of the 41-mer target backbone **3.10**; however, I did not have enough material of the 32-mer **3.16** to complete its synthesis.

To obtain the lipid-linked oligosaccharide targets **3.1**–**3.5**, conversion of the target backbones **3.6**–**3.10** to targets **3.1**–**3.5** warrants further study. For **3.6**–**3.9**, reduction of the azide and acetylation of the amine gave the desired products in >80% yield in all cases. However, conversion of the TMSEt glycoside to the lipid diphosphate needs further optimization to achieve the synthesis of **3.4** and **3.5**, and if more material of **3.1**–**3.3** is required for future experiments. Removal of the TMSEt group was problematic, as the conditions used gave the lactols (**3.52**, **3.58**, and **3.64**) in <55% yield. The modest yields might have resulted from the α-stereochemistry of the TMSEt glycoside or the inductive effects of the many (27 to 99) benzoyl esters. Formation of the dibenzyl phosphates proceeded smoothly in most cases, except in the case of the 33-mer **3.65** where the formation of by-products with similar polarities proved to be very challenging and needs to be worked out. Lastly, the phosphate coupling and global deprotection needs to be improved in all cases, as the phosphate coupling is a typically poor

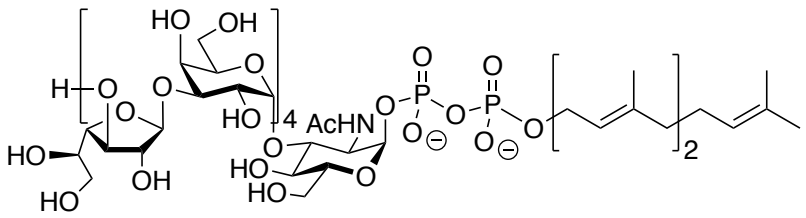
reaction.[116,150] Based on a former colleague's results,[149] these late stage steps might be improved if the benzoyl groups are replaced with acetyl groups prior to the hydrolysis of the TMSEt glycoside.

Compounds **3.1**, and possibly **3.2** and **3.3**, will be sent to the laboratory of our collaborator, Chris Whitfield, at the University of Guelph. These oligosaccharides will then be used to further elucidate the mechanism of chain length control and export of the O-PS in *K. pneumoniae* O2a.

3.4 Experimental Section

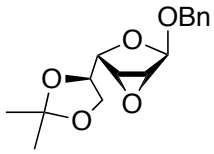
General Methods: All reagents, except sodium allyloxide[141] and farnesyl phosphate,[116] were purchased from commercial sources and used without further purification. Dry CH_2Cl_2, THF, and CH_3CN were taken from a solvent purification system after successive passage through alumina columns. Dry CH_3OH was obtained via storage in a sealed bottle with activated 4 Å M.S. overnight at rt. Unless otherwise stated, all reactions were carried out under an argon atmosphere and were monitored by TLC on silica gel 60 F_{254} (0.25 mm, Merck). Spots were visualized by UV light and/or by charring with 10% H_2SO_4 in EtOH. In the processing of reaction mixtures, solutions of organic solvents were washed with equal amounts of aqueous solutions. Column chromatography was performed on silica gel 60 (40–60 μm), C_{18} silica gel (35–70 μm), or Sephradex LH-20 gel (27–163 μm). ^{1}H NMR spectra were recorded at 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 (D_2O, 4.79 ppm). ^{13}C NMR spectra were recorded at 125 MHz, and chemical shifts were referenced to internal $CDCl_3$ (77.06 ppm, $CDCl_3$), CD_3OD (49.00 ppm, CD_3OD), or external acetone (31.07 ppm, D_2O). All NMR assignments were made by appropriate 2D NMR experiments, and the stereochemistry of the newly formed glycosidic bonds was confirmed by $^3J_{H1\text{-}H2}$ values and anomeric ^{13}C chemical shifts. In the following data, the resonances of particular residues are indicated by an increasing number of primes (‘) moving from the reducing end to the non-reducing end. Optical rotations were measured on a Perkin Elmer 241 polarimeter at 22 ± 2 °C in units of (degree·mL)/(dm·g). Electrospray ionization spectra were recorded on an Aligent Technologies 6220 TOF spectrometer with samples dissolved in CH_3OH or H_2O. MALDI mass spectra were obtained in the linear or reflectron positive mode of ionization on a Voyager Elite (AB Sciex, Foster City, CA, USA) MALDI–TOF

mass spectrometer using *trans*-2-[3-(4-*tert*-butylphenyl)-2-methyl-2-propenylidene]malononitrile (DCTB) as the matrix with NaCl added to the samples.



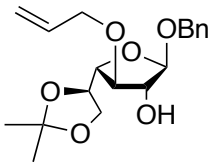
Farnesyl-diphosphate β-D-galactofuranosyl-(1→3)-α-D-galactopyranosyl-(1→3)-β-D-galactofuranosyl-(1→3)-α-D-galactopyranosyl-(1→3)-β-D-galactofuranosyl-(1→3)-α-D-galactopyranosyl-(1→3)-β-D-galactofuranosyl-(1→3)-α-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-α-D-glucopyranoside; Triethylamine Salt (3.1). Compound **3.41** (41 mg, 0.010 mmol) and Pd/C (23 mg, 10% w/w) were suspended in THF (5.0 mL). The flask was placed under an atmoshphere of H_2, and the reaction mixture was stirred for 15 h at rt. The reaction mixture was diluted with Et_3N (0.10 mL) and filtered through Celite to give the crude phosphate (40 mg). The crude phosphate was dissolved in dry CH_2Cl_2 (3.0 mL), and CDI (22 mg, 0.14 mmol) was added to the solution. The reaction mixture was stirred for 3 h at rt, before excess CDI was quenched by the addition of 5% CH_3OH in CH_2Cl_2 (0.15 mL). The mixture was stirred for an additional 30 min at rt, before being concentrated down. The residue was dissolved in DMF (1.0 mL) and farnesyl phosphate[116] (43 mg, 0.14 mmol) was added. The reaction mixture was stirred for 6 d at rt and concentrated. The crude residue was purified by Sephradex LH-20 size exclusion chromatography (1:1 CH_2Cl_2–CH_3OH) to give the protected sugar-lipid diphosphate (38 mg). This compound was dissolved in dry CH_2Cl_2 (2.0 mL) and dry CH_3OH (3.5 mL). A 10 mM solution of $NaOCH_3$ in CH_3OH (2.5 mL) was added to the mixture, and the reaction mixture was stirred for 3 d, before being neutralized with Amberlyst-15 NH_4^+ resin,

filtered, and concentrated. The crude residue was purified by C_{18} silica gel chromatography (gradient of 1%→50% CH_3OH–H_2O) and lyophilized to yield **3.1** (5.0 mg, 30% over four steps) as a fluffy, white solid. $[\alpha]_D$ +32 (*c* 0.10, H_2O); ^1H NMR (700 MHz, D_2O, δ_H) 5.55 – 5.50 (m, 1 H, H-1_{Glc}), 5.48 – 5.42 (m, 1 H, $OCH_2C\underline{H}$=C), 5.25 – 5.17 (m, 6 H, 4 x H-$1_{Gal\mathit{f}}$, 2 x $CH_2C\underline{H}$=C), 5.10 (d, *J* = 2.5 Hz, 4 H, 4 x H-$1_{Gal\mathit{p}}$), 4.54 – 4.48 (m, 2 H, $OC\underline{H}_2CH$=C), 4.45 – 4.38 (m, 4 H), 4.30 – 4.24 (m, 4 H), 4.21 – 4.18 (m, 1 H), 4.18 – 4.01 (m, 13 H), 3.99 – 3.86 (m, 12 H), 3.86 – 3.78 (m, 5 H), 3.78 – 3.65 (m, 15 H), 3.21 (q, *J* = 7.1 Hz, 6 H, $N(C\underline{H}_2CH_3)_3$), 2.21 – 2.15 (m, 2 H, 2 x $C\underline{H}_2CH$=C), 2.15 – 2.09 (m, 7 H, $C(O)CH_3$, 4 x $C\underline{H}_2CH$=C), 2.07 – 2.02 (m, 2 H, 2 x $C\underline{H}_2CH$=C), 1.73 (s, 3 H), 1.70 (s, 3 H), 1.65 – 1.61 (m, 6 H), 1.29 (t, *J* = 7.1 Hz, 9 H, $N(CH_2C\underline{H}_3)_3$); ^{13}C NMR (126 MHz, D_2O, δ_C) 175.4 (C=O), 130.6 (CH=<u>C</u>), 130.4 (CH=<u>C</u>), 129.5 (CH=<u>C</u>), 125.4 (<u>C</u>H=C), 125.2 (<u>C</u>H=C), 120.4 (d, *J* = 8.6 Hz, <u>C</u>H=C), 110.3 (3 x C-$1_{Gal\mathit{f}}$), 110.0 (C-$1_{Gal\mathit{f}}$), 100.4 (C-$1_{Gal\mathit{p}}$), 100.0 (C-$1_{Gal\mathit{p}}$), 99.6 (C-$1_{Gal\mathit{p}}$), 97.7 (C-$1_{Gal\mathit{p}}$), 95.6 (d, *J* = 6.9 Hz, C-1_{Glc}), 85.4, 83.7, 82.8, 82.4, 80.6, 78.0, 77.7, 72.2, 71.7, 71.5, 70.5, 70.2, 70.0, 68.1, 64.1 (d, *J* = 4.0 Hz), 63.6, 62.1, 61.3, 61.1, 53.1 (d, *J* = 11.8 Hz, C-2_{Glc}), 47.7 ($N(\underline{C}H_2CH_3)_3$), 39.8 (2 x Allyl CH_2), 26.8 (Allyl CH_2), 26.5 (Allyl CH_2), 25.9 (Allyl CH_3), 23.3 ($C(O)\underline{C}H_3$), 18.0 (Allyl CH_3), 16.6 (Allyl CH_3), 16.3 (Allyl CH_3), 9.20 ($N(CH_2\underline{C}H_3)_3$); ^{31}P NMR (162 MHz, D_2O, δ_P) -10.8 (d, *J* = 38.0 Hz), -13.2 (d, *J* = 57.3 Hz); HRMS–ESI–TOF calcd for $[M\text{-}2H]^{2-}$ $C_{71}H_{119}NO_{52}P_2$: 939.8092. Found 939.8092.



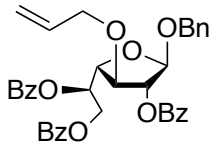
Benzyl 2,3-anhydro-5,6-di-*O*-isopropylidene-β-D-gulofuranoside (3.27). To a solution of **3.26**[134] (13.4 g, 43.2 mmol) in dry THF (400 mL) cooled to 0 °C, was added PPh_3 (14.6 g, 55.8

mmol). Subsequently at 0 °C, DIAD (11.0 mL, 55.8 mmol) was added slowly dropwise over the course of 20 min. The reaction mixture was stirred for 1 h, while slowly warming to rt. The solution was concentrated, and the residue was purified by silica gel column chromatography (6:1 hexanes–EtOAc) to yield **3.27** (12.4 g, 98%) as a clear, colorless oil. R_f 0.63 (2:1 hexanes–EtOAc); $[\alpha]_D$ –44 (*c* 0.50, $CHCl_3$); ^{1}H NMR (700 MHz, $CDCl_3$, δ_H) 7.37 – 7.26 (m, 5 H, ArH), 5.20 (s, 1 H, H-1), 4.82 (d, J_{gem} = 11.7 Hz, 1 H, OCH_2Ph), 4.55 (d, J_{gem} = 11.7 Hz, 1 H, OCH_2Ph), 4.30 (ddd, $J_{4,5}$ = 6.9 Hz, $J_{5,6b}$ = 6.6 Hz, $J_{5,6a}$ = 6.5 Hz, 1 H, H-5), 4.12 (dd, $J_{6a,6b}$ = 8.4 Hz, $J_{5,6a}$ = 6.5 Hz, 1 H, H-6a), 4.09 (d, $J_{4,5}$ = 6.9 Hz, 1 H, H-4), 3.93 (dd, $J_{6a,6b}$ = 8.4 Hz, $J_{5,6b}$ = 6.6 Hz, 1 H, H-6b), 3.68 (d, $J_{2,3}$ = 2.9 Hz, 1 H, H-2), 3.62 (d, $J_{2,3}$ = 2.9 Hz, 1 H, H-3), 1.47 (s, 3 H, $C(C\underline{H}_3)_2$), 1.38 (s, 3 H, $C(C\underline{H}_3)_2$); ^{13}C NMR (126 MHz, $CHCl_3$, δ_C) 137.3 (Ar), 128.5 (Ar), 128.01 (Ar), 128.98 (Ar), 110.0 ($\underline{C}(CH_3)_2$), 100.7 (C-1), 77.4 (C-4), 75.2 (C-5), 70.1 ($O\underline{C}H_2Ph$), 65.7 (C-6), 55.8 (C-2), 53.5 (C-3), 26.7 ($C(\underline{C}H_3)_2$), 25.3 ($C(\underline{C}H_3)_2$); HRMS–ESI–TOF calcd for $[M+Na]^+$ $C_{16}H_{24}NaO_5$: 315.1203. Found 315.1204.



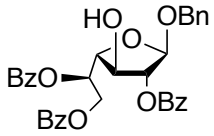
Benzyl 3-*O*-allyl-5,6-di-*O*-isopropylidene-β-D-galactofuranoside (3.28). Compound **3.27** (12.4 g, 42.4 mmol) was treated with a 1.0 M solution of NaOAll in AllOH (200 mL) and heated to reflux. The reaction mixture was stirred for 15 h at reflux, before being cooled to rt, and diluted with EtOAc (150 mL) and H_2O (150 mL). The aqueous layer was then re-extracted with EtOAc (150 mL), and the combined organic layers were washed with brine, dried with Na_2SO_4, filtered, and concentrated. The resulting residue was purified by silica gel column chromatography (3:1→2:1 Hexanes–EtOAc) to yield **3.28** (13.0 g, 88%) as a clear, slightly yellow oil. R_f 0.43

(2:1 Hexanes–EtOAc); $[\alpha]_D$ –108 (*c* 1.10, $CHCl_3$); ^1H NMR (700 MHz, $CDCl_3$, δ_H) 7.36 – 7.29 (m, 4 H, ArH), 7.28 – 7.23 (m, 1 H, ArH), 5.95 – 5.86 (m, 1 H, H_2C=C<u>H</u>CH_2O), 5.34 – 5.28 (m, 1 H, <u>H</u>$_2$C=CHCH_2O), 5.23 – 5.18 (m, 1 H, <u>H</u>$_2$C=CHCH_2O), 5.07 (s, 1 H, H-1), 4.79 (d, J_{gem} = 12.4 Hz, 1 H, OCH_2Ph), 4.54 (d, J_{gem} = 12.4 Hz, 1 H, OCH_2Ph), 4.27 (ddd, *J* = 7.5, 7.5, 2.4 Hz, 1 H, H-5), 4.18 – 4.11 (m, 3 H, H-2, H-4, H_2C=CHC<u>H</u>$_2$O), 4.07 – 4.01 (m, 2 H, H-6a, H_2C=CHC<u>H</u>$_2$O), 3.99 (dd, *J* = 7.5, 7.5 Hz, 1 H, H-6b), 3.84 (d, *J* = 2.9 Hz, 1 H, H-3), 3.29 (d, *J* = 10.6 Hz, 1 H, OH), 1.41 (s, 3 H, C(C<u>H</u>$_3$)$_2$)), 1.38 (s, 3 H, C(C<u>H</u>$_3$)$_2$)); ^{13}C NMR (126 MHz, $CHCl_3$, δ_C) 138.0 (Ar), 134.3 (H_2C=<u>C</u>HCH_2O), 128.3 (Ar), 127.6 (Ar), 127.5 (Ar), 117.6 (H_2<u>C</u>=CHCH_2O), 109.9 (<u>C</u>(CH$_3$)$_2$), 108.3 (C-1), 86.0 (C-3), 82.8 (C-4), 77.5 (C-2), 76.4 (C-5), 71.1 (H_2C=CH<u>C</u>H$_2$O), 68.8 (O<u>C</u>H$_2$Ph), 65.7 (C-6), 25.8 (C(CH$_3$)$_2$), 25.7 (C(CH$_3$)$_2$); HRMS–ESI–TOF calcd for $[M+Na]^+$ $C_{19}H_{26}NaO_6$: 373.1622. Found 373.1617.



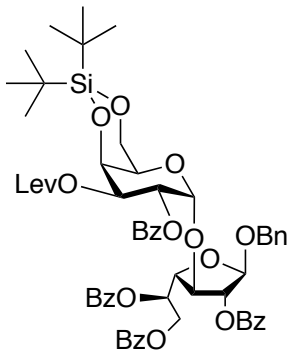
Benzyl 2,5,6-tri-*O*-benzoyl-3-*O*-allyl-β-D-galactofuranoside (3.29). Compound **3.28** (13.4 g, 38.3 mmol) was dissolved in CH_2Cl_2–CH_3OH (440 mL, 1:1 v/v) and H_2O (5.00 mL) was added. A solution of 10% HCl in CH_3OH (9.00 mL) was added dropwise to the mixture and stirred for 6 h at rt, before being neutralized by the addition of Et_3N and concentrated to dryness. The crude residue (13.7 g) was dissolved in dry CH_2Cl_2 (353 mL) and pyridine (91.0 mL), and cooled to 0 °C. BzCl (27.5 mL, 237 mmol) was added slowly dropwise to the mixture and stirred for 45 h, while warming to rt. The mixture was cooled to 0 °C, and excess BzCl was quenched by the dropwise addition of CH_3OH (50.0 mL), before being concentrated to dryness, with the aid of toluene. The residue was dissolved in CH_2Cl_2 (400 mL), washed with 1 N HCl three times, a solution of saturated sodium bicarbonate once, and brine once. The organic layer was dried with

Na_2SO_4, filtered, and concentrated. The crude residue was purified by silica gel column chromatography (6:1→5:1 hexanes–EtOAc) to yield **3.29** (23.1 g, 97%) as a fluffy, white solid. R_f 0.42 (4:1 hexanes–EtOAc); $[\alpha]_D$ –40 (*c* 0.70, $CHCl_3$); ^{1}H NMR (700 MHz, $CDCl_3$, δ_H) 8.06 – 8.02 (m, 2 H, ArH), 7.99 – 7.95 (m, 2 H, ArH), 7.88 – 7.84 (m, 2 H, ArH), 7.54 – 7.49 (m, 3 H, ArH), 7.39 – 7.25 (m, 11 H, ArH), 5.90 – 5.81 (m, 2 H, H-5, H_2C=C<u>H</u>CH_2O), 5.37 (d, $J_{2,3}$ = 1.1 Hz, 1 H, H-2), 5.27 (s, 1 H, H-1), 5.24 – 5.18 (m, 1 H, <u>H</u>$_2$C=CHCH_2O), 5.14 – 5.09 (m, 1 H, <u>H</u>$_2$C=CHCH_2O), 4.77 (d, J_{gem} = 12.2 Hz, 1 H, OCH_2Ph), 4.66 – 4.63 (m, 2 H, H-6a, H-6b), 4.61 (d, J_{gem} = 12.2 Hz, 1 H, OCH_2Ph), 4.48 (dd, $J_{3,4}$ = 6.0, $J_{4,5}$ = 3.4 Hz, 1 H, H-4), 4.29 – 4.24 (m, 1 H, H_2C=CHC<u>H</u>$_2$O), 4.11 – 4.04 (m, 2 H, H_2C=CHC<u>H</u>$_2$O, H-3); ^{13}C NMR (126 MHz, $CHCl_3$, δ_C) 166.1 (C=O), 165.9 (C=O), 165.3 (C=O), 137.3 (Ar), 133.8 (H_2C=<u>C</u>HCH_2O), 133.31 (Ar), 133.26 (Ar), 133.1 (Ar), 129.9 (Ar), 129.73 (Ar), 129.68 (Ar), 129.65 (Ar), 129.56 (Ar), 129.2 (Ar), 128.44 (Ar), 128.41 (Ar), 128.40 (Ar), 128.0 (Ar), 127.8 (Ar), 118.4 (H_2<u>C</u>=CHCH_2O), 105.1 (C-1), 83.4 (C-3), 81.9 (C-2), 81.4 (C-4), 71.8 (C-5), 70.2 (H_2C=CH<u>C</u>H_2O), 68.9 (O<u>C</u>H_2Ph), 63.5 (C-6); HRMS–ESI–TOF calcd for $[M+Na]^+$ $C_{37}H_{34}NaO_9$: 645.2101. Found 645.2095.



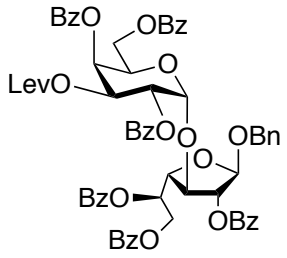
Benzyl 2,5,6-tri-*O*-benzoyl-β-D-galactofuranoside (3.24). To a solution of **3.29** (21.9 g, 35.2 mmol) in dry THF (500 mL) was added $Ir(COD)(PMePh_2)_2 \cdot PF_6$ (100 mg, 0.300 mol%). The stirring mixture was degassed, and placed under an inert atmosphere of argon, and cooled to 0 °C. The mixture was degassed again, and the flask was flushed with H_2 for 2 min, and then for 1 min, until the solution turned clear and colorless. The solution was degassed once more, and placed under an inert atmosphere of argon, after which the solution turned a slight pinkish-red

color. The reaction mixture was stirred for 20.5 h at rt. The solution was concentrated, and the crude residue was dissolved in acetone–H_2O (550 mL; 10:1 v/v). HgO (9.85 g; 45.6 mmol), followed by $HgCl_2$ (14.2 g; 52.5 mmol) were added to the solution. The mixture was stirred for 2.5 h, before being concentrated and diluted with EtOAc (500 mL). It was washed, in succession, with an aqueous solution of 10% KI three times, with a solution of saturated sodium thiosulfate three times, and with H_2O twice. The organic layer was dried with Na_2SO_4, filtered, and concentrated. The crude product was purified by silica gel column chromatography (4:1→2:1 hexanes–EtOAc) to yield **3.24** (19.3 g, 94%) as a fluffy, white solid. R_f 0.29 (3:1 hexanes–EtOAc); $[\alpha]_D$ –53 (*c* 0.50, $CHCl_3$); ^{1}H NMR (700 MHz, $CDCl_3$, δ_H) 8.05 – 8.01 (m, 2 H, ArH), 7.98 (s, 2 H, ArH), 7.96 – 7.91 (m, 2 H, ArH), 7.57 – 7.48 (m, 3 H, ArH), 7.39 – 7.31 (m, 9 H, ArH), 7.30 – 7.27 (m, 1 H, ArH), 5.81 (ddd, $J_{5,6b}$ = 7.1 Hz, $J_{5,6a}$ = 4.5 Hz, $J_{4,5}$ = 4.5 Hz, 1 H, H-5), 5.38 (s, 1 H, H-1), 5.18 (d, $J_{2,3}$ = 2.9 Hz, 1 H, H-2), 4.78 (d, J_{gem} = 11.9 Hz, 1 H, OCH_2Ph), 4.70 (dd, $J_{6a,6b}$ = 11.8 Hz, $J_{5,6a}$ = 4.5 Hz, 1 H, H-6a), 4.65 (dd, $J_{6a,6b}$ = 11.8 Hz, $J_{5,6b}$ = 7.1 Hz, 1 H, H-6b), 4.60 (d, J_{gem} = 11.9 Hz, 1 H, OCH_2Ph), 4.48 (dd, $J_{3,4}$ = 7.0 Hz, $J_{4,5}$ = 4.5 Hz, 1 H, H-4), 4.24 (ddd, $J_{3,4}$ = 7.0 Hz, $J_{3,OH}$ = 4.5 Hz, $J_{2,3}$ = 2.9 Hz, 1 H, H-3), 3.24 (d, $J_{3,OH}$ = 4.5 Hz, 1 H, OH); ^{13}C NMR (126 MHz, $CHCl_3$, δ_C) 166.8 (C=O), 166.1 (C=O), 166.0 (C=O), 137.1 (Ar), 133.6 (Ar), 133.3 (Ar), 133.1 (Ar), 129.9 (Ar), 129.8 (Ar), 129.7 (Ar), 129.6 (Ar), 129.5 (Ar), 128.9 (Ar), 128.51 (Ar), 128.49 (Ar), 128.40 (Ar), 128.0 (Ar), 127.9 (Ar), 104.5 (C-1), 86.4 (C-2), 81.8 (C-4), 77.3 (C-3), 70.5 (C-5), 69.4 ($O\underline{C}H_2Ph$), 63.3 (C-6); HRMS–ESI–TOF calcd for $[M+Na]^+$ $C_{34}H_{30}NaO_9$: 605.1782. Found 605.1779.



Benzyl 2-*O*-benzoyl-4,6-di-*O*-di-*tert*-butylsilylidene-3-*O*-levulinoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranoside (3.30). Compounds **2.58** (3.84 g; 6.11 mmol) and **3.24** (5.01 g; 8.61 mmol) were dissolved in CH_2Cl_2–toluene and were concentrated to dryness on high vacuum overnight. Dry CH_2Cl_2 (90.0 mL) and 4 Å M.S. were then added to the flask, and the mixture was stirred for 30 min at rt. The suspension was cooled to 0 °C, and NIS (3.02 g; 13.3 mmol) was added, followed by TfOH (120 μL; 1.33 mmol). The reaction mixture was stirred for 1 h, while warming to rt, before being neutralized by the addition of Et_3N. A solution of saturated sodium thiosulfate (50.0 mL) was added, and the mixture was filtered. The organic layer was separated, dried with Na_2SO_4, filtered, and concentrated. The residue was dissolved in THF–CH_3OH (90.0 mL, 1:1 v/v), and H_2O (1.0 mL, 56 mmol) was added, followed by PPh_3 (3.50 g, 13.3 mmol), and the mixture was stirred for 30 min at rt, before being concentrated. The residue was dissolved in CH_2Cl_2 (100 mL) and washed with H_2O. The organic layer was separated, dried with Na_2SO_4, filtered, and concentrated. The crude residue was purified by silica gel column chromatography (24:1→19:1 toluene–EtOAc) to give **3.30** (5.08 g, 77%) as a white, fluffy solid. R_f 0.32 (19:1 toluene–EtOAc); $[\alpha]_D$ +49 (*c* 2.0, $CHCl_3$); ^{1}H NMR (700 MHz, $CDCl_3$, δ_H) 8.01 – 7.93 (m, 4 H, ArH), 7.85 – 7.80 (m, 4 H, ArH), 7.53 – 7.46 (m, 3 H, ArH), 7.43 – 7.37 (m, 1 H, ArH), 7.37 – 7.26 (m, 12 H, ArH), 5.60 – 5.54 (m, 2 H, H-2', H-5), 5.45 (d, $J_{2,3}$ = 1.4 Hz, 1 H, H-2), 5.42 (d, $J_{1',2'}$ = 3.8 Hz, 1 H, H-1'), 5.37 (dd, $J_{2',3'}$ = 10.7 Hz,

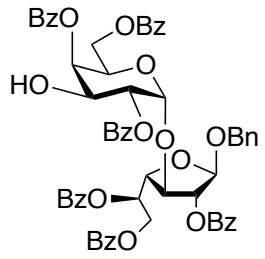
$J_{3',4'}$ = 2.9 Hz, 1 H, H-3'), 5.23 (s, 1 H, H-1), 4.71 (d, J_{gem} = 11.9 Hz, 1 H, OCH_2Ph), 4.69 (d, $J_{3',4'}$ = 2.9 Hz, 1 H, H-4'), 4.53 (d, J_{gem} = 11.9 Hz, 1 H, OCH_2Ph), 4.45 (dd, $J_{6a,6b}$ = 11.8 Hz, $J_{5,6a}$ = 7.4 Hz, 1 H, H-6a), 4.41 – 4.36 (m, 2 H, H-6b, H-4), 4.35 – 4.31 (m, 1 H, H-3), 4.10 (dd, $J_{6a',6b'}$ = 12.5, $J_{5',6a'}$ = 1.8 Hz, 1 H, H-6a'), 4.07 – 4.01 (m, 2 H, H-6b', H-5'), 2.65 (t, J = 6.7 Hz, 2 H, $CH_2C\underline{H}_2C(O)CH_3$), 2.56 (t, J = 6.7 Hz, 2 H, $C\underline{H}_2CH_2C(O)CH_3$), 2.07 (s, 3 H, $CH_2CH_2C(O)C\underline{H}_3$), 1.04 (s, 9 H, $SiC(CH_3)_3$), 1.01 (s, 9 H, $SiC(CH_3)_3$); ^{13}C NMR (126 MHz, $CDCl_3$, δ_C) 205.9 (C=O), 172.1 (C=O), 165.8 (C=O), 165.7 (C=O), 165.6 (C=O), 165.3 (C=O), 137.2 (Ar), 133.3 (Ar), 133.2 (Ar), 133.1 (Ar), 129.9 (Ar), 129.72 (Ar), 129.65 (Ar), 129.5 (Ar), 129.4 (Ar), 129.3 (Ar), 128.9 (Ar), 104.9 (C-1), 98.1 C-1'), 83.2 (C-3), 81.81 (C-2), 81.80 (C-4), 70.9 (C-4'), 70.7 (C-3'), 69.7 (C-5), 68.9 ($O\underline{C}H_2Ph$), 68.1 (C-2'), 67.7 (C-5'), 66.6 (C-6'), 63.3 (C-6), 37.9 ($CH_2\underline{C}H_2C(O)CH_3$), 29.7 ($CH_2CH_2C(O)\underline{C}H_3$), 28.3 ($\underline{C}H_2CH_2C(O)CH_3$), 27.6 ($SiC(\underline{C}H_3)_3$), 27.3 ($SiC(\underline{C}H_3)_3$), 23.3 ($Si\underline{C}(CH_3)_3$), 20.7 ($Si\underline{C}(CH_3)_3$); HRMS–ESI–TOF calcd for $[M+Na]^+$ $C_{60}H_{66}NaO_{17}Si$: 1086.4069. Found 1086.3961.



Benzyl 2,4,6-tri-*O*-benzoyl-3-*O*-levulinoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranoside (3.31). To a solution of **3.30** (5.18 g; 4.77 mmol) in THF (100 mL) and pyridine (10.8 mL), was added HF·pyridine (0.50 mL) at 0 °C. The mixture was stirred for 21 h, while warming to rt, before being neutralized by the addition of a solution of saturated sodium bicarbonate (60 mL) and diluted with EtOAc (60 mL). The aqueous layer was re-extracted twice more with EtOAc (60 mL, 60 mL). The combined organic layers were washed with brine, dried

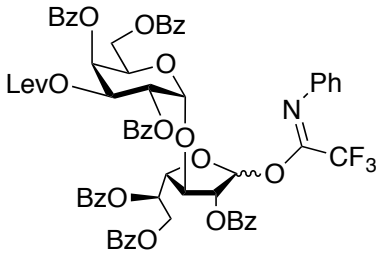
with Na_2SO_4, filtered, and concentrated. The crude residue was dissolved in CH_2Cl_2 (225 mL) and pyridine (32.0 mL), and cooled to 0 °C. BzCl (21.0 mL, 181 mmol) was added slowly dropwise at 0 °C. The reaction mixture was stirred for 23 h, while warming to rt, before excess BzCl was quenched by the addition of ice-cold H_2O (150 mL), and the mixture diluted with CH_2Cl_2 (25.0 mL). The organic layer was separated, and washed, in succession, with a solution of 1 N HCl three times, a solution of saturated aqueous $NaHCO_3$, and brine, before being dried with Na_2SO_4, filtered, and concentrated. The crude residue was purified by silica gel column chromatography (2:1→3:2 hexanes–EtOAc) to yield **3.31** (5.17 g, 94%) as a fluffy, white solid. R_f 0.28 (2:1 hexanes–EtOAc); $[\alpha]_D$ +41 (*c* 0.60, $CHCl_3$); ^{1}H NMR (700 MHz, $CDCl_3$, δ_H) 8.11 – 8.07 (m, 3 H, ArH), 8.00 – 7.95 (m, 2 H, ArH), 7.92 – 7.88 (m, 4 H, ArH), 7.86 – 7.79 (m, 4 H, ArH), 7.63 – 7.56 (m, 2 H, ArH), 7.54 – 7.33 (m, 10 H, ArH), 7.32 – 7.19 (m, 10 H, ArH), 5.82 (dd, $J_{3',4'}$ = 3.4 Hz, $J_{4',5'}$ = 1.1 Hz, 1 H, H-4'), 5.75 (dd, $J_{2',3'}$ = 10.8 Hz, $J_{3',4'}$ = 3.4 Hz, 1 H, H-3'), 5.73 (d, $J_{1',2'}$ = 3.7 Hz, 1 H, H-1'), 5.63 (d, $J_{2,3}$ = 0.8 Hz, 1 H, H-2), 5.59 – 5.52 (m, 2 H, H-5, H-2'), 5.19 (s, 1 H, H-1), 4.79 – 4.74 (m, 1 H, H-5), 4.66 (d, J_{gem} = 11.7 Hz, 1 H, OCH_2Ph), 4.48 (d, J_{gem} = 11.7 Hz, 1 H, OCH_2Ph), 4.46 – 4.40 (m, 4 H, H-6a, H-6b, H-4, H-6a'), 4.33 – 4.28 (m, 2 H, H-3, H-6b'), 2.66 (ddd, *J* = 18.2, 7.1, 6.7 Hz, 1 H, $CH_2C\underline{H}_2C(O)CH_3$), 2.53 (ddd, *J* = 18.2, 7.0, 6.5 Hz, 1 H, $CH_2C\underline{H}_2C(O)CH_3$), 2.47 (ddd, *J* = 17.1, 7.1, 7.0 Hz, 1 H, $C\underline{H}_2CH_2C(O)CH_3$), 2.38 (ddd, *J* = 17.1, 6.7, 6.5 Hz, 1 H, $C\underline{H}_2CH_2C(O)CH_3$), 1.98 (s, 3 H, $CH_2CH_2C(O)C\underline{H}_3$); ^{13}C NMR (126 MHz, $CDCl_3$, δ_C) 205.9 (C=O), 171.7 (C=O), 165.82 (C=O), 165.76 (C=O), 165.72 (C=O), 165.70 (C=O), 165.68 (C=O), 165.3 (C=O), 137.1 (Ar), 133.7 (Ar), 133.6 (Ar), 133.4 (Ar), 133.30 (Ar), 133.14 (Ar), 133.08 (Ar), 132.9 (Ar), 130.2 (Ar), 130.0 (Ar), 129.81 (Ar), 129.80 (Ar), 129.77 (Ar), 129.65 (Ar), 129.61 (Ar), 129.5 (Ar), 129.2 (Ar), 129.1 (Ar), 129.0 (Ar), 128.8 (Ar), 128.6 (Ar), 128.52 (Ar), 128.49 (Ar), 128.45 (Ar), 128.36 (Ar), 128.34 (Ar),

128.26 (Ar), 128.2 (Ar), 128.0 (Ar), 127.9 (Ar), 104.6 (C-1), 97.9 (C-1'), 84.4 (C-3), 82.2 (C-4), 81.1 (C-2), 70.2 (C-5), 68.92 (C-4'), 68.88 (O<u>C</u>H_2Ph), 68.7 (C-2'), 68.1 (C-3'), 67.6 (C-5'), 63.3 (C-6'), 62.1 (C-6), 37.8 (CH_2<u>C</u>H_2C(O)CH_3), 29.5 (CH_2CH_2C(O)<u>C</u>H_3), 28.0 (<u>C</u>H_2CH_2C(O)CH_3); HRMS–ESI–TOF calcd for $[M+Na]^+$ $C_{66}H_{58}NaO_{19}$: 1177.3465. Found 1177.3463.



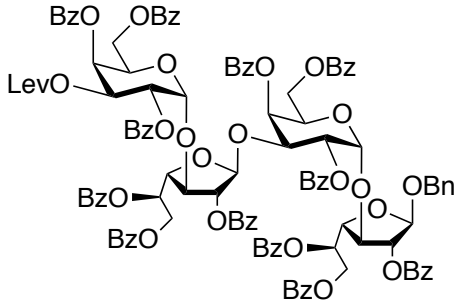
Benzyl 2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranoside (3.21). To a solution of **3.31** (4.07 g; 3.53 mmol) in CH_2Cl_2–CH_3OH (105 mL, 10:1 v/v) was added H_2NNH_2·AcOH (806 mg, 8.75 mmol). The reaction mixture was stirred for 1 h at rt, before being diluted with CH_2Cl_2 (20.0 mL) and washed with H_2O. The organic layer was dried with Na_2SO_4, filtered, and concentrated. The crude residue was purified by silica gel column chromatography (3:1→2:1 hexanes–EtOAc) to yield **3.21** (3.55 g, 95%) as a fluffy, white solid. R_f 0.37 (2:1 hexanes–EtOAc); $[\alpha]_D$ +25 (*c* 0.30, $CHCl_3$); ^{1}H NMR (700 MHz, $CDCl_3$, δ_H) 8.12 – 8.08 (m, 2 H, ArH), 8.05 – 8.01 (m, 2 H, ArH), 7.95 – 7.86 (m, 6 H, ArH), 7.83 – 7.79 (m, 2 H, ArH), 7.60 – 7.55 (m, 1 H, ArH), 7.54 – 7.38 (m, 8 H, ArH), 7.38 – 7.32 (m, 4 H, ArH), 7.32 – 7.25 (m, 6 H, ArH), 7.24 – 7.16 (m, 4 H, ArH), 5.75 (dd, $J_{3',4'}$ = 3.4 Hz, $J_{4',5'}$ = 1.0 Hz, 1H, H-4'), 5.71 (ddd, *J* = 8.0, 4.2, 4.2 Hz, 1H, H-5), 5.66 – 5.62 (m, 2 H, H-1', H-2), 5.44 (dd, $J_{2',3'}$ = 10.4 Hz, $J_{1',2'}$ = 3.7 Hz, 1 H, H-2'), 5.20 (s, 1 H, H-1), 4.70 – 4.64 (m, 2 H, H-5', OCH_2Ph), 4.55 – 4.47 (m, 5 H, OCH_2Ph, H-3', H-4, H-6a, H-6b), 4.44 (dd, $J_{6a',6b'}$ = 11.4 Hz, $J_{5',6a'}$ = 7.0 Hz, 1 H, H-6a'), 4.39 (dd, $J_{6a',6b'}$ = 11.4, $J_{5',6b'}$ = 5.9 Hz, 1 H, H-6b'), 4.35 (d, $J_{3,4}$ = 4.7 Hz, 1 H, H-3); ^{13}C NMR (126 MHz, $CDCl_3$, δ_C) 166.9 (C=O), 166.3 (C=O), 165.9 (C=O),

165.8 (C=O), 165.3 (C=O), 137.1 (Ar), 133.53 (Ar), 133.49 (Ar), 133.3 (Ar), 133.20 (Ar), 133.16 (Ar), 132.9 (Ar), 130.1 (Ar), 129.83 (Ar), 129.80 (Ar), 129.77 (Ar), 129.74 (Ar), 129.65 (Ar), 129.63 (Ar), 129.5 (Ar), 129.19 (Ar), 129.16 (Ar), 128.8 (Ar), 128.6 (Ar), 128.51 (Ar), 128.47 (Ar), 128.40 (Ar), 128.36 (Ar), 128.27 (Ar), 128.2 (Ar), 128.1 (Ar), 128.0 (Ar), 104.7 (C-1), 97.9 (C-1'), 84.0 (C-3), 82.3 (C-4), 81.1 (C-2), 71.9 (C-2'), 71.5 (C-4'), 70.1 (C-5), 68.9 (OCH_2Ph), 68.1 (C-5'), 67.7 (C-3'), 63.4 (C-6), 62.5 (C-6'); HRMS–ESI–TOF calcd for $[M+Na]^+$ $C_{61}H_{52}NaO_{17}$: 1079.3097. Found 1079.3106.



2,4,6-tri-*O*-Benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranoside *N*-phenyl trifluoroacetimidate (3.22). To a suspension of **3.31** (545 mg, 0.480 mmol) in absolute EtOH (45.0 mL), was added Raney-Ni (1.0 mL as a settled slurry in H_2O). The mixture was heated to reflux and stirred vigorously at reflux for 1 h, before cooling to rt. The suspension was filtered through Celite, concentrated, and purified by silica gel column chromatography (6:1 toluene–EtOAc) to yield the intermediate lactol (402 mg, 80%). This compound (1.39 g, 1.32 mmol) was dissolved in dry CH_2Cl_2 (40.0 mL). $CF_3CN(Ph)Cl$ (0.31 mL, 2.0 mmol), followed by Cs_2CO_3 (1.29 g, 3.95 mmol), were added to the solution, and the reaction mixture was stirred for 16 h at rt, before being filtered and concentrated. The crude residue was purified by silica gel column chromatography (2:1 hexanes–EtOAc) to yield **3.22** (1.39 g, 86%, >19:1 β:α) as a fluffy, white solid. R_f 0.29 (2:1 hexanes–EtOAc); $[\alpha]_D$ +34 (*c* 1.2, $CHCl_3$); ^1H NMR data of the β isomer (700 MHz, $CDCl_3$, δ_H) 8.14 – 8.09 (m, 2 H, ArH), 8.02 – 7.97 (m, 2 H, ArH), 7.94 – 7.88

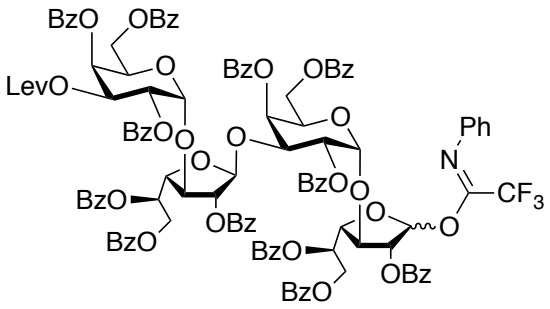
(m, 4 H, ArH), 7.86 – 7.82 (m, 2 H, ArH), 7.80 (d, *J* = 7.6 Hz, 2 H, ArH), 7.63 – 7.58 (m, 1 H, ArH), 7.54 – 7.38 (m, 7 H, ArH), 7.38 – 7.31 (m, 4 H, ArH), 7.27 – 7.21 (m, 7 H, ArH), 7.10 – 7.04 (m, 1 H, ArH), 6.76 (d, *J* = 7.5 Hz, 2 H, H-1, ArH), 5.96 – 5.93 (m, 1 H, H-4'), 5.81 – 5.75 (m, 3 H, H-1'), 5.61 – 5.54 (m, 2 H), 4.85 – 4.82 (m, 1 H), 4.66 (br s, 1 H), 4.58 – 4.47 (m, 3 H), 4.46 – 4.38 (m, 2 H), 2.66 (dt, *J* = 18.2, 7.1 Hz, 1 H, $CH_2C\underline{H}_2C(O)CH_3$), 2.54 (dt, *J* = 18.2, 6.5 Hz, 1 H, $CH_2C\underline{H}_2C(O)CH_3$), 2.47 (dt, *J* = 17.2, 7.1 Hz, 1 H, $CH_2C\underline{H}_2C(O)CH_3$), 2.38 (dt, *J* = 17.2, 6.5 Hz, 1 H, $CH_2C\underline{H}_2C(O)CH_3$), 1.98 (s, 3 H, $CH_2CH_2C(O)C\underline{H}_3$); ^{13}C NMR data of the β isomer (126 MHz, $CDCl_3$, δ_C) 205.9 (C=O), 171.7 (C=O), 165.9 (C=O), 165.74 (C=O), 165.72 (C=O), 165.66 (C=O), 165.55 (C=O), 165.1 (C=O), 143.2, 133.63, 133.56, 133.52, 133.3, 133.1, 133.0, 130.0, 129.90, 129.84, 129.82, 129.78, 129.70, 129.64, 129.57, 129.47, 129.3, 129.1, 129.0, 128.9, 128.74, 128.66, 128.5, 128.42, 128.38, 128.31, 128.28, 124.5, 119.4 (C-1), 98.0 (C-1'), 84.5, 83.4, 80.8, 69.9, 68.8, 68.7, 67.9, 67.7, 63.0, 62.1, 37.8 ($CH_2\underline{C}H_2C(O)CH_3$), 29.5 ($CH_2CH_2C(O)\underline{C}H_3$), 27.9 ($\underline{C}H_2CH_2C(O)CH_3$); HRMS–ESI–TOF calcd for $[M+Na]^+$ $C_{67}H_{56}F_3NNaO_{19}$: 1258.3291. Found 1258.3274.



Benzyl 2,4,6-tri-*O*-benzoyl-3-*O*-levulinoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranoside (3.32). Compounds **3.21** (999 mg; 0.950 mmol) and **3.22** (1.39 g; 1.13 mmol) were dissolved in CH_2Cl_2–toluene and were concentrated to dryness on high

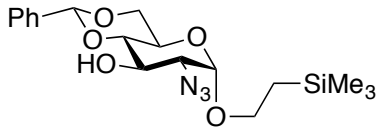
vacuum overnight. Dry CH_2Cl_2 (37.0 mL) and 4 Å M.S. were added to the flask, and the mixture was stirred for 30 min at rt. The suspension was cooled to 0 °C, and TfOH (0.008 mL; 0.1 mmol) was added. The reaction mixture was stirred for 1 h at 0 °C, before being neutralized by the addition of Et_3N, filtered, and concentrated. The crude residue was purified by silica gel column chromatography (gradient of 3:2→11:9 hexanes–EtOAc) to give **3.32** (1.90 g, 95%) as a white, fluffy solid. R_f 0.26 (3:2 hexanes–EtOAc); $[\alpha]_D$ +43 (*c* 0.40, $CHCl_3$); ^{1}H NMR (700 MHz, $CDCl_3$, δ_H) 8.22 – 8.18 (m, 2 H, ArH), 8.09 – 8.05 (m, 2 H, ArH), 8.03 – 7.97 (m, 4 H, ArH), 7.94 – 7.75 (m, 14 H, ArH), 7.59 – 7.09 (m, 41 H, ArH), 6.98 – 6.93 (m, 2 H, ArH), 5.95 (d, *J* = 3.5 Hz, 1 H), 5.75 (d, *J* = 3.8 Hz, 1 H, H-1$_{Gal p}$), 5.59 – 5.48 (m, 8 H, H-1$_{Gal f}$), 5.46 (d, *J* = 3.6 Hz, 1 H, H-1$_{Gal p}$), 5.31 (d, *J* = 2.7 Hz, 1 H), 5.18 (s, 1 H, H-1$_{Gal f}$), 4.87 (dd, *J* = 7.4, 2.7 Hz, 1 H), 4.74 – 4.67 (m, 2 H), 4.61 – 4.54 (m, 3 H, OCH_2Ph), 4.50 – 4.42 (m, 2 H, OCH_2Ph), 4.39 – 4.35 (m, 3 H), 4.33 – 4.27 (m, 2 H), 4.22 – 4.13 (m, 2 H), 4.05 (dd, *J* = 11.2, 5.8 Hz, 1 H), 3.87 (dd, *J* = 11.2, 7.2 Hz, 1 H), 2.61 (dt, *J* = 18.3, 7.0 Hz, 1 H, $CH_2C\underline{H}_2C(O)CH_3$), 2.53 (dt, *J* = 18.3, 6.7 Hz, 1 H, $CH_2C\underline{H}_2C(O)CH_3$), 2.46 – 2.33 (m, 2 H, $C\underline{H}_2CH_2C(O)CH_3$), 1.96 (s, 3 H, $CH_2CH_2C(O)C\underline{H}_3$); ^{13}C NMR (126 MHz, $CDCl_3$, δ_C) 206.0 (C=O), 171.2 (C=O), 165.85 (C=O), 165.84 (C=O), 165.80 (C=O), 165.78 (C=O), 165.75 (C=O), 165.70 (C=O), 165.61 (C=O), 165.59 (C=O), 165.51 (C=O), 165.2 (C=O), 164.5 (C=O), 137.1 (Ar), 133.7 (Ar), 133.43 (Ar), 133.41 (Ar), 133.30 (Ar), 133.28 (Ar), 133.17 (Ar), 133.12 (Ar), 133.09 (Ar), 133.05 (Ar), 132.9 (Ar), 132.80 (Ar), 132.75 (Ar), 130.1 (Ar), 129.94 (Ar), 129.91 (Ar), 129.87 (Ar), 129.85 (Ar), 129.80 (Ar), 129.64 (Ar), 129.61 (Ar), 129.59 (Ar), 129.48 (Ar), 129.44 (Ar), 129.37 (Ar), 129.34 (Ar), 129.31 (Ar), 129.26 (Ar), 129.22 (Ar), 129.15 (Ar), 129.13 (Ar), 128.9 (Ar), 128.8 (Ar), 128.6 (Ar), 128.48 (Ar), 128.45 (Ar), 128.40 (Ar), 128.36 (Ar), 128.33 (Ar), 128.32 (Ar), 128.28 (Ar), 128.20 (Ar), 128.15 (Ar), 128.09 (Ar), 127.98 (Ar), 127.87 (Ar), 108.1 (C-1$_{Gal f}$),

104.4 (C-1$_{Galf}$), 99.1 (C-1$_{Galp}$), 97.6 (C-1$_{Galp}$), 85.5, 83.7, 82.1, 81.5, 80.5, 72.8, 71.2, 70.9, 70.2, 69.9, 68.63, 68.60, 68.4, 68.0, 67.7, 67.3, 63.7, 63.3, 62.7, 61.7, 37.9 ($CH_2\underline{C}H_2C(O)CH_3$), 29.6 ($CH_2CH_2C(O)\underline{C}H_3$), 27.9 ($\underline{C}H_2CH_2C(O)CH_3$); HRMS–ESI–TOF calcd for $[M+Na]^+$ $C_{120}H_{102}NaO_{35}$: 2125.6094. Found 2125.6125.



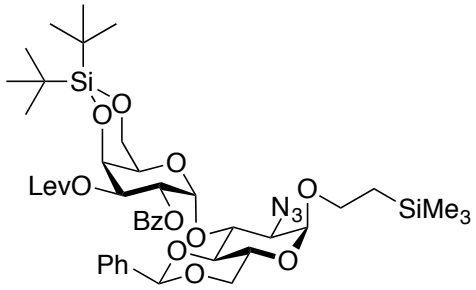
2,4,6-tri-*O*-Benzoyl-3-*O*-levulinoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranoside *N*-phenyl trifluoroacetimidate (3.18). To a suspension of **3.32** (5.08 g, 2.42 mmol) in absolute EtOH (225 mL), was added Raney-Ni (5.0 mL as a settled slurry in H_2O). The mixture was heated to reflux and stirred vigorously at reflux for 1 h, before cooling to rt. The suspension was filtered through Celite, concentrated, and purified by silica gel column chromatography (6:1→4:1 toluene–EtOAc) to yield the intermediate lactol (3.20 g, 66%). This compound (3.17 g, 1.58 mmol) was dissolved in dry CH_2Cl_2 (79.0 mL). $CF_3CN(Ph)Cl$ (0.40 mL, 2.5 mmol), followed by Cs_2CO_3 (1.56 g, 4.79 mmol), were added to the solution, and the reaction mixture was stirred for 18.5 h h at rt, before being filtered and concentrated. The crude residue purified by silica gel column chromatography (3:2 hexanes–EtOAc) to yield **3.18** (3.11 g, 90%, 4:1 β:α) as a fluffy, white solid. R_f 0.18 (3:2 hexanes–EtOAc); $[\alpha]_D$ –47 (*c* 0.30, $CHCl_3$); ^{1}H NMR data of the β isomer (600 MHz, $CDCl_3$, δ_H) 8.27 – 8.23 (m, 2 H, ArH), 8.11 – 8.06 (m, 2 H, ArH), 8.06 – 7.99 (m, 5 H, ArH), 7.97 – 7.86 (m, 8 H, ArH), 7.86 – 7.78 (m, 6 H, ArH),

7.64 – 7.28 (m, 27 H, ArH), 7.28 – 7.22 (m, 6 H, ArH), 7.22 – 7.13 (m, 5 H, ArH), 7.11 – 7.00 (m, 3 H, ArH), 6.78 – 6.73 (m, 2 H, ArH, H-$1_{Gal\mathit{f}}$), 6.05 – 6.02 (m, 1 H), 5.84 (d, J = 3.7 Hz, 1 H, H-$1_{Gal\mathit{p}}$), 5.78 – 5.75 (m, 1 H), 5.64 – 5.52 (m, 7 H, H-$1_{Gal\mathit{f}}$), 5.49 (d, J = 3.7 Hz, 1 H, H-$1_{Gal\mathit{p}}$), 5.35 (d, J = 2.6 Hz, 1 H), 4.91 (dd, J = 7.4, 2.9 Hz, 1 H), 4.82 – 4.76 (m, 1 H), 4.73 – 4.59 (m, 3 H), 4.59 – 4.52 (m, 2 H), 4.52 – 4.46 (m, 2 H), 4.43 – 4.33 (m, 2 H), 4.25 – 4.16 (m, 2 H), 4.08 (dd, J = 11.2, 5.8 Hz, 1 H), 3.89 (dd, J = 11.2, 7.2 Hz, 1 H), 2.69 – 2.52 (m, 2 H, $CH_2C\underline{H}_2C(O)CH_3$), 2.49 – 2.37 (m, 2 H, $C\underline{H}_2CH_2C(O)CH_3$), 1.99 (s, 3 H, $CH_2CH_2C(O)C\underline{H}_3$); ^{13}C NMR data of the β isomer (126 MHz, $CDCl_3$, δ_C) 205.9 (C=O), 171.2 (C=O), 165.81 (C=O), 165.79 (C=O), 165.74 (C=O), 165.68 (C=O), 165.65 (C=O), 165.60 (C=O), 165.56 (C=O), 165.51 (C=O), 165.47 (C=O), 165.0 (C=O), 164.5 (C=O), 143.2, 133.7, 133.5, 133.4, 133.3, 133.24, 133.18, 133.11, 133.09, 133.01, 132.97, 132.95, 132.90, 132.80, 132.75, 130.1, 130.0, 129.89, 129.86, 129.83, 129.80, 129.76, 129.64, 129.60, 129.50, 129.49, 129.45, 129.35, 129.33, 129.27, 129.19, 129.16, 129.0, 128.90, 128.86, 128.7, 128.6, 128.5, 128.43, 128.41, 128.32, 128.29, 128.24, 128.16, 128.12, 128.07, 119.4 (C-$1_{Gal\mathit{f}}$), 108.0 (C-$1_{Gal\mathit{f}}$), 99.1 (C-$1_{Gal\mathit{p}}$), 97.9 (C-$1_{Gal\mathit{p}}$), 85.6, 84.6, 83.0, 82.2, 80.8, 80.6, 72.4, 71.2, 70.9, 70.0, 69.9, 68.7, 68.4, 68.2, 67.8, 67.3, 63.4, 62.9, 62.7, 61.7, 37.9 ($CH_2\underline{C}H_2C(O)CH_3$), 29.5 ($CH_2CH_2C(O)\underline{C}H_3$), 28.0 ($\underline{C}H_2CH_2C(O)CH_3$); HRMS–ESI–TOF calcd for $[M+2Na]^{2+}$ $C_{121}H_{100}F_3NNa_2O_{35}$: 1114.7906. Found 1114.7910.



2-(Trimethylsilyl)ethyl 2-azido-2-deoxy-4,6-di-*O*-benzylidene-α-D-glucopyranoside (3.25). To a solution of **3.33**[148] (1.45 g, 3.48 mmol) in CH_2Cl_2–CH_3OH (20.0 mL, 1:1 v/v) was added a small piece of solid sodium. The mixture was stirred at rt for 96 h, before being neutralized with

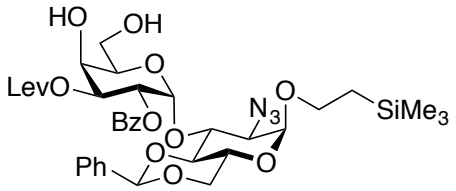
Amberlyst-15 H^+ resin, filtered, and concentrated. The residue was dissolved in CH_3CN (10.0 mL). BDA (1.05 mL, 6.96 mmol), followed by (+)-CSA (165 mg, 0.696 mmol) were added to the solution, and the solution was heated to 50 °C and stirred for 16 h, before being neutralized by the addition of Et_3N and concentrated. The resulting residue was purified by silica gel column chromatography (8:1:1 hexanes–CH_2Cl_2–EtOAc) to yield **3.25** (510 mg, 39%, α only) as a clear, colorless oil. R_f 0.23 (8:1:1 hexanes–CH_2Cl_2–EtOAc); $[\alpha]_D$ +121 (*c* 0.70, $CHCl_3$); ^{1}H NMR (700 MHz, $CDCl_3$, δ_H) 7.51 – 7.46 (m, 2 H, ArH), 7.41 – 7.34 (m, 3 H, ArH), 5.54 (s, 1 H, C<u>H</u>Ph), 4.92 (d, $J_{1,2}$ = 3.7 Hz, 1 H, H-1), 4.28 (dd, $J_{6a,6b}$ = 10.4 Hz, $J_{5,6}$ = 5.0 Hz, 1 H, H-6a), 4.23 (ddd, $J_{2,3}$ = 10.0 Hz, $J_{3,4}$ = 9.3 Hz, $J_{3,OH}$ = 2.6 Hz, 1 H, H-3), 3.89 (ddd, $J_{5,6b}$ = 10.4 Hz, $J_{4,5}$ = 10.0 Hz, $J_{5,6a}$ = 5.0 Hz, 1 H, H-5), 3.82 (ddd, *J* = 11.3, 10.0, 5.8 Hz, 1 H, OC<u>H</u>$_2$CH$_2$Si), 3.73 (dd, $J_{6a,6b}$ = 10.4 Hz, $J_{5,6b}$ =10.4 Hz, 1 H, H-6b), 3.57 (ddd, *J* = 11.3, 10.0, 5.6 Hz, 1 H, OC<u>H</u>$_2$CH$_2$Si), 3.51 (dd, $J_{4,5}$ = 10.0 Hz, $J_{3,4}$ = 9.3 Hz, 1 H, H-4), 3.27 (dd, $J_{2,3}$ = 10.0 Hz, $J_{1,2}$ =3.7 Hz, 1 H, H-2), 2.58 (d, $J_{3,OH}$ = 2.6 Hz, 1 H, OH), 1.06 (ddd, *J* = 13.8, 11.3, 5.6 Hz, 1 H, OCH$_2$C<u>H</u>$_2$Si), 1.00 (ddd, *J* = 13.8, 11.3, 5.8 Hz, 1 H, OCH$_2$C<u>H</u>$_2$Si), 0.04 (s, 9 H, Si(C<u>H</u>$_3$)$_3$); ^{13}C NMR (176 MHz, $CHCl_3$, δ_C) 136.9 (Ar), 129.4 (Ar), 128.4 (Ar), 126.3 (Ar), 102.1 (<u>C</u>HPh), 98.0 (C-1), 82.0 (C-4), 69.0 (C-3), 68.9 (C-6), 66.3 (O<u>C</u>H$_2$CH$_2$Si), 63.1 (C-2), 62.4 (C-5), 18.1 (OCH$_2$<u>C</u>H$_2$Si), -1.4 (Si(<u>C</u>H$_3$)$_3$); HRMS–ESI–TOF calcd for $[M+Na]^+$ $C_{18}H_{27}N_3NaO_5Si$: 416.1612. Found 416.1608.



2-(Trimethylsilyl)ethyl 2-*O*-benzoyl-4,6-di-*O*-di-*tert*-butylsilylidene-3-*O*-levulinoyl-α-D-galactopyranosyl-(1→3)-2-azido-2-deoxy-4,6-di-*O*-benzylidene-α-D-glucopyranoside (3.34).

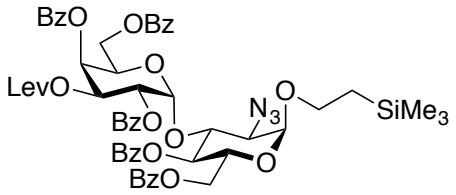
Compounds **2.58** (3.21 g; 5.11 mmol) and **3.25** (2.20 g; 5.60 mmol) were dissolved in CH_2Cl_2–toluene and were concentrated to dryness on high vacuum overnight. Dry CH_2Cl_2 (45.0 mL) and 4 Å M.S. were added to the flask, and the mixture was stirred for 30 min at rt. The suspension was cooled to 0 °C, and NIS (1.73 g; 7.69 mmol) was added, followed by TfOH (100 μL; 1.13 mmol). The reaction mixture was stirred for 1 h, while warming to rt, before being neutralized by the addition of Et_3N. A solution of saturated sodium thiosulfate (50.0 mL) was added, and the mixture was filtered. The organic layer was washed with brine, dried with Na_2SO_4, filtered, and concentrated. The crude residue was purified by silica gel column chromatography (4:1 hexanes–EtOAc) to give **3.34** (3.85 g, 84%) as a white, fluffy solid. R_f 0.24 (4:1 hexanes–EtOAc); $[\alpha]_D$ +135 (*c* 0.70, $CHCl_3$); ^{1}H NMR (700 MHz, $CDCl_3$, δ_H) 7.78 – 7.74 (m, 2 H, ArH), 7.54 – 7.48 (m, 1 H, ArH), 7.36 – 7.27 (m, 3 H, ArH), 7.27 – 7.22 (m, 3 H, ArH), 7.04 – 7.00 (m, 2 H, ArH), 5.67 – 5.61 (m, 2 H, C<u>H</u>Ph, H-3'), 5.37 (dd, $J_{2',3'}$ = 10.1 Hz, $J_{1',2'}$ = 3.0 Hz, 1 H, H-2'), 4.95 (d, $J_{1,2}$ = 3.7 Hz, 1 H, H-1), 4.81 (s, 1 H, H-4'), 4.76 – 4.72 (m, 1 H, H-1'), 4.35 (dd, $J_{2,3}$ = 10.0 Hz, $J_{3,4}$ = 9.0 Hz, 1 H, H-3), 4.30 (d, $J_{6a,6b}$ = 1.8 Hz, 2 H, H-6a', H-6b'), 4.20 – 4.17 (m, 1 H, H-5'), 4.13 (dd, $J_{6a,6b}$ = 10.2 Hz, $J_{5,6a}$ = 4.9 Hz, 1 H, H-6a), 3.82 (ddd, $J_{5,6b}$ = 10.0 Hz, $J_{4,5}$ = 9.3 Hz, $J_{5,6a}$ = 4.9 Hz, 1 H, H-5), 3.77 (ddd, *J* = 11.8, 9.9, 5.6 Hz, 1 H, OC<u>H</u>$_2$CH$_2$Si), 3.58 – 3.51 (m, 2 H, OC<u>H</u>$_2$CH$_2$Si, H-6b), 3.46 (dd, $J_{4,5}$ = 9.3 Hz, $J_{3,4}$ = 9.0 Hz, 1 H, H-4), 3.10 (dd, $J_{2,3}$ = 10.0 Hz, $J_{1,2}$ = 3.7 Hz, 1 H, H-2), 2.64 – 2.59 (m, 2 H, CH$_2$C<u>H</u>$_2$C(O)CH$_3$), 2.55 – 2.50 (m, 2 H, C<u>H</u>$_2$CH$_2$C(O)CH$_3$), 2.02 (s, 3 H, CH$_2$CH$_2$C(O)C<u>H</u>$_3$), 1.12 – 0.92 (m, 20 H, 2 x SiC(CH$_3$)$_3$, OCH$_2$C<u>H</u>$_2$Si), 0.01 (s, 9 H, Si(CH$_3$)$_3$); ^{13}C NMR (126 MHz, $CDCl_3$, δ_C) 205.9 (C=O), 172.2 (C=O), 165.1 (C=O), 136.3 (Ar), 133.0 (Ar), 129.7 (Ar), 129.5 (Ar), 128.9 (Ar), 128.2 (Ar), 128.0 (Ar), 125.8 (Ar), 101.3 (C-1'), 98.3 (C-1), 97.1 (<u>C</u>HPh), 82.8 (C-4), 72.5 (C-3), 71.1 (C-4'), 70.3 (C-2'), 68.7 (C-6), 67.8 (C-5'), 67.4 (C-3'), 67.2 (C-6'), 66.3 (O<u>C</u>H$_2$CH$_2$Si), 62.3 (C-5),

61.4 (C-2), 37.9 ($CH_2\underline{C}H_2C(O)CH_3$), 29.7 ($CH_2CH_2C(O)\underline{C}H_3$), 28.3 ($\underline{C}H_2CH_2C(O)CH_3$), 27.6 ($SiC(\underline{C}H_3)_3$), 27.3 ($SiC(\underline{C}H_3)_3$), 23.3 ($Si\underline{C}(CH_3)_3$), 20.7 ($Si\underline{C}(CH_3)_3$), 18.2 ($OCH_2\underline{C}H_2Si$), -1.5 ($Si(\underline{C}H_3)_3$); HRMS–ESI–TOF calcd for $[M+Na]^+$ $C_{44}H_{63}N_3NaO_{13}Si_2$: 920.3792. Found 920.3795.



2-(Trimethylsilyl)ethyl 2-*O*-benzoyl-3-*O*-levulinoyl-α-D-galactopyranosyl-(1→3)-2-azido-2-deoxy-4,6-di-*O*-benzylidene-α-D-glucopyranoside (3.35). To a solution of **3.34** (3.05 g; 4.77 mmol) in THF (200 mL) and pyridine (50.0 mL), was added HF·pyridine (0.30 mL) at 0 °C. The mixture was stirred for 18 h, while warming to rt, before being neutralized by the addition of a solution of saturated sodium bicarbonate (175 mL) and diluted with EtOAc (100 mL). The aqueous layer was re-extracted twice more with EtOAc (100 mL, 100 mL). The combined organic layers were washed with brine, dried with Na_2SO_4, filtered, and concentrated. The crude residue was purified by silica gel column chromatography (gradient of 66%→100% EtOAc–hexanes) to yield **3.35** (3.04 g, 94%) as fluffly, white solid. R_f 0.40 (1:2 hexanes–EtOAc); $[\alpha]_D$ +120 (*c* 0.20, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$, δ_H) 7.81 – 7.76 (m, 2 H, ArH), 7.58 – 7.52 (m, 1 H, ArH), 7.40 – 7.26 (m, 5 H, ArH), 7.10 – 7.05 (m, 2 H, ArH), 5.75 (d, $J_{1',2'}$ = 3.9 Hz, 1 H, H-1'), 5.67 (dd, $J_{2',3'}$ = 10.8 Hz, $J_{1',2'}$ = 3.9 Hz, 1 H, H-2'), 5.45 (dd, $J_{2',3'}$ = 10.8 Hz, $J_{3',4'}$ = 3.0 Hz, 1 H, H-3'), 5.00 (d, $J_{1,2}$ = 3.7 Hz, 1 H, H-1), 4.79 (s, 1 H, C$\underline{H}$Ph), 4.45 (m, 1 H, H-4'), 4.35 (dd, $J_{2,3}$ = 10.0 Hz, $J_{3,4}$ = 9.0 Hz, 1 H, H-3), 4.30 (m, 1 H, H-5'), 4.16 (dd, $J_{6a,6b}$ = 10.2 Hz, $J_{5,6a}$ = 4.9 Hz, 1 H, H-6a), 4.09 – 3.99 (m, 2 H, H-6a', H-6b'), 3.90 – 3.77 (m, 2 H, OC$\underline{H}_2$CH$_2$Si, H-5), 3.62 – 3.52 (m, 2 H, OC$\underline{H}_2$CH$_2$Si, H-6b), 3.48 (dd, $J_{3,4}$ = 9.0 Hz, $J_{4,5}$ = 9.0 Hz, 1 H, H-4), 3.45 – 3.43 (m, 1 H, OH), 3.21 (dd, $J_{2,3}$ = 10.0 Hz, $J_{1,2}$ = 3.7 Hz, 1 H, H-2), 2.78 – 2.65 (m, 2 H,

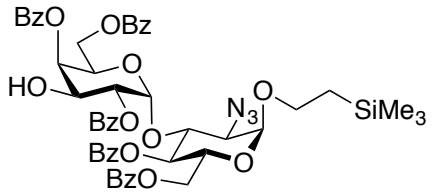
$CH_2C\underline{H}_2C(O)CH_3$), 2.58 – 2.50 (m, 2 H, $C\underline{H}_2CH_2C(O)CH_3$, OH), 2.46 (ddd, *J* = 16.7, 6.7, 5.5 Hz, 1 H, $C\underline{H}_2CH_2C(O)CH_3$), 2.12 (s, 3 H, $CH_2CH_2C(O)C\underline{H}_3$), 1.09 (ddd, *J* = 13.9, 11.5, 5.6 Hz, 1 H, $OCH_2C\underline{H}_2Si$), 1.02 (ddd, *J* = 13.8, 11.5, 5.8 Hz, 1 H, $OCH_2C\underline{H}_2Si$), 0.05 (s, 9 H, $Si(CH_3)_3$); ^{13}C NMR (126 MHz, $CDCl_3$, δ_C) 207.8 (C=O), 172.0 (C=O), 165.0 (C=O), 136.3 (Ar), 133.1 (Ar), 129.7 (Ar), 129.5 (Ar), 129.0 (Ar), 128.3 (Ar), 128.0 (Ar), 125.9 (Ar), 101.3 ($\underline{C}$HPh), 98.2 (C-1), 97.1 (C-1'), 82.8 (C-4), 72.6 (C-3), 70.5 (C-3'), 69.8 (C-4'), 69.2 (C-5'), 68.7 ($O\underline{C}H_2CH_2Si$), 67.5 (C-2'), 66.3 (C-6), 63.8 (C-6'), 62.2 (C-5), 61.5 (C-2), 38.4 ($CH_2\underline{C}H_2C(O)CH_3$), 29.7 ($CH_2CH_2C(O)\underline{C}H_3$), 28.4 ($\underline{C}H_2CH_2C(O)CH_3$), 18.2 ($OCH_2\underline{C}H_2Si$), -1.5 ($Si(\underline{C}H_3)_3$); HRMS–ESI–TOF calcd for $[M+Na]^+$ $C_{36}H_{47}N_3NaO_{13}Si$: 780.2770. Found 780.2772.



2-(Trimethylsilyl)ethyl 2,4,6-tri-*O*-benzoyl-3-*O*-levulinoyl-α-D-galactopyranosyl-(1→3)-2-azido-2-deoxy-4,6-di-*O*-benzoyl-α-D-glucopyranoside (3.36). To a solution of **3.35** (809 mg, 1.07 mmol) in EtOAc (27.0 mL), was added a solution of $KBrO_3$ (1.07 g, 6.41 mmol) in H_2O (16.0 mL). A solution of $Na_2S_2O_4$ (1.12 g, 6.41 mmol) in H_2O (16.0 mL) was added dropwise to the biphasic mixture, and the reaction mixture was stirred for 30 min at rt, before the organic layer was separated and washed with a solution of saturated aqueous $Na_2S_2O_3$. The aqueous layer was re-extracted with EtOAc (50 mL), and the combined organic layers were dried with Na_2SO_4, filtered, and concentrated. The crude residue was purified by silica gel column chromatography (gradient of 75%→100% EtOAc–hexanes) to yield the di-*O*-benzoylated intermediate (764 mg, 93%) as a 4:1 mixture of the 4-*O*-benzoyl and 6-*O*-benzoyl regioisomers. The combined regioisomers (764 mg, 0.988 mmol) were dissolved in pyridine (120 mL). DMAP (754 mg, 6.17

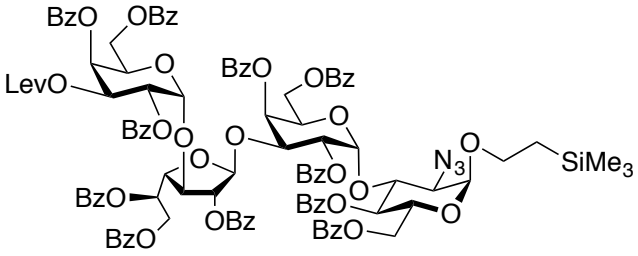
mmol) was added, and the solution was cooled to 0 °C, before BzCl (0.72 mL, 6.2 mmol) was added slowly dropwise to the solution. After the addition of BzCl, the solution was slowly heated to reflux, and the reaction mixture was stirred at reflux for 15 h, before being cooled to rt, diluted with toluene (250 mL), and concentrated. The residue was dissolved in CH_2Cl_2 (100 mL), diluted with H_2O (100 mL), and the organic layer was seperated. The organic layer was washed, in succession, with solutions of 1 N HCl three times, saturated aqueous $NaHCO_3$, and brine, before being dried with Na_2SO_4, filtered, and concentrated. The crude residue was purified by silica gel column chromatography (2:1→1:1 hexanes–EtOAc) to yield **3.36** (1.04 g, 97%) as a fluffly, white solid. R_f 0.71 (4:1 toluene–EtOAc); $[\alpha]_D$ +95 (*c* 0.30, $CHCl_3$); ^{1}H NMR (700 MHz, $CDCl_3$, δ_H) 8.08 – 8.04 (m, 2 H, ArH), 8.03 – 7.98 (m, 2 H, ArH), 7.92 – 7.88 (m, 2 H, ArH), 7.72 – 7.68 (m, 2 H, ArH), 7.58 – 7.47 (m, 6 H, ArH), 7.46 – 7.42 (m, 2 H, ArH), 7.41 – 7.38 (m, 2 H, ArH), 7.38 – 7.34 (m, 2 H, ArH), 7.34 – 7.30 (m, 2 H, ArH), 7.28 – 7.23 (m, 3 H, ArH), 5.90 (dd, $J_{3',4'}$ = 3.3 Hz, $J_{4',5'}$ = 1.6 Hz, 1 H, H-4'), 5.68 (dd, $J_{2',3'}$ = 10.9 Hz, $J_{3',4'}$ = 3.3 Hz, 1 H, H-3'), 5.57 (d, $J_{1',2'}$ = 3.8 Hz, 1 H, H-1'), 5.54 (dd, $J_{2',3'}$ = 10.9 Hz, $J_{1',2'}$ = 3.8 Hz, 1 H, H-2'), 5.31 (dd, $J_{4,5}$ = 10.1 Hz, $J_{3,4}$ = 9.0 Hz, 1 H, H-4), 5.11 (d, $J_{1,2}$ = 3.7 Hz, 1 H, H-1), 5.07 – 5.02 (m, 1 H, H-5'), 4.67 (dd, $J_{6a',6b'}$ = 11.1 Hz, $J_{5',6a'}$ = 5.5 Hz, 1 H, H-6a'), 4.47 (dd, $J_{2,3}$ = 10.3 Hz, $J_{3,4}$ = 9.0 Hz, 1 H, H-3), 4.36 – 4.31 (m, 2 H, H-6a, H-6b'), 4.27 (dd, $J_{6a,6b}$ = 12.2 Hz, $J_{5,6b}$ = 6.5 Hz, 1 H, H-6b), 4.18 (ddd, $J_{4,5}$ = 10.1 Hz, $J_{5,6b}$ = 6.5 Hz, $J_{5,6a}$ = 3.2 Hz, 1 H, H-5), 3.80 (ddd, *J* = 11.8, 10.1, 5.9 Hz, 1 H, OC$\underline{H}_2$CH_2Si), 3.59 (ddd, *J* = 11.9, 10.1, 5.3 Hz, 1 H, OC$\underline{H}_2$CH_2Si), 3.41 (dd, $J_{2,3}$ = 10.3 Hz, $J_{1,2}$ = 3.7 Hz, 1 H, H-2), 2.45 (dt, *J* = 18.3, 7.1 Hz, 1 H, CH_2C$\underline{H}_2$C(O)CH_3), 2.36 (dt, *J* = 18.3, 6.7 Hz, 1 H, CH_2C$\underline{H}_2$C(O)CH_3), 2.30 – 2.19 (m, 2 H, C$\underline{H}_2$$CH_2$C(O)$CH_3$), 1.85 (s, 3 H, CH_2CH_2C(O)C$\underline{H}_3$), 1.04 (ddd, *J* = 13.7, 11.9, 5.3 Hz, 1 H, OCH_2C$\underline{H}_2$Si), 0.98 (ddd, *J* = 13.7, 11.8, 5.8 Hz, 1 H, OCH_2C$\underline{H}_2$Si), -0.03 (s, 9 H, Si$(CH_3)_3$); ^{13}C NMR (126 MHz, $CDCl_3$, δ_C) 205.7

(C=O), 171.7 (C=O), 166.01 (C=O), 165.97 (C=O), 165.7 (C=O), 165.4 (C=O), 164.7 (C=O), 133.5 (Ar), 133.39 (Ar), 133.35 (Ar), 133.1 (Ar), 133.0 (Ar), 130.1 (Ar), 129.90 (Ar), 129.85 (Ar), 129.70 (Ar), 129.66 (Ar), 129.6 (Ar), 129.5 (Ar), 129.2 (Ar), 128.6 (Ar), 128.5 (Ar), 128.4 (Ar), 128.33 (Ar), 128.28 (Ar), 128.1 (Ar), 98.4 (C-1'), 97.0 (C-1), 75.2 (C-3), 72.5 (C-4), 68.9 (C-4'), 68.0 (C-5), 67.8 (C-2'), 67.7 (C-3'), 67.5 (C-5'), 66.3 ($O\underline{C}H_2CH_2Si$), 63.5 (C-6), 62.0 (C-6'), 61.3 (C-2), 37.7 ($CH_2\underline{C}H_2C(O)CH_3$), 29.4 ($CH_2CH_2C(O)\underline{C}H_3$), 27.9 ($\underline{C}H_2CH_2C(O)CH_3$), 18.1 ($OCH_2\underline{C}H_2Si$), -1.6 ($Si(CH_3)_3$); HRMS–ESI–TOF calcd for $[M+Na]^+$ $C_{57}H_{59}N_3NaO_{17}Si$: 1108.3506. Found 1108.3529.



2-(Trimethylsilyl)ethyl 2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2-azido-2-deoxy-4,6-di-*O*-benzoyl-α-D-glucopyranoside (3.23). To a solution of **3.36** (1.04 g; 0.960 mmol) in CH_2Cl_2–CH_3OH (100 mL, 1:1 v/v) was added H_2NNH_2·AcOH (188 mg, 2.04 mmol). The reaction mixture was stirred for 4 h at rt, before being diluted with CH_2Cl_2 (40.0 mL) and washed with H_2O. The organic layer was then dried with Na_2SO_4, filtered, and concentrated. The crude residue was purified by silica gel column chromatography (7:3 hexanes–EtOAc) to yield **3.36** (890 mg, 94%) as a fluffy, white solid. R_f 0.26 (2:1 hexanes–EtOAc); $[\alpha]_D$ +104 (*c* 0.70, $CHCl_3$); ^{1}H NMR (700 MHz, $CDCl_3$, δ_H) 8.09 – 8.05 (m, 2 H, ArH), 8.05 – 8.01 (m, 2 H, ArH), 7.93 – 7.89 (m, 2 H, ArH), 7.81 – 7.77 (m, 2 H, ArH), 7.58 – 7.45 (m, 7 H, ArH), 7.45 – 7.40 (m, 2 H, ArH), 7.40 – 7.35 (m, 3 H, ArH), 7.35 – 7.30 (m, 2 H, ArH), 7.27 – 7.22 (m, 3 H, ArH), 5.87 (dd, $J_{3',4'}$ = 3.2 Hz, $J_{4',5'}$ = 1.2 Hz, 1 H, H-4'), 5.55 (d, $J_{1',2'}$ = 3.8 Hz, 1 H, H-1'), 5.40 (dd, $J_{2',3'}$ = 10.6 Hz, $J_{1',2'}$ = 3.8 Hz, 1 H, H-2'), 5.35 (dd, $J_{4,5}$ = 10.0 Hz, $J_{3,4}$ = 9.3 Hz, 1 H, H-4), 5.09

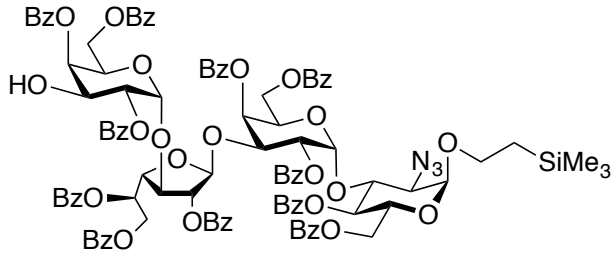
(d, $J_{1,2}$ = 3.7 Hz, 1 H, H-1), 4.97 – 4.92 (m, 1 H, H-5'), 4.67 (dd, $J_{6a',6b'}$ = 11.1 Hz, $J_{5',6a'}$ = 5.6 Hz, 1 H, H-6a'), 4.50 – 4.45 (m, 2 H, H-3, H-3'), 4.39 – 4.34 (m, 2 H, H-6b', H-6a), 4.31 (dd, $J_{6a,6b}$ = 12.1 Hz, $J_{5,6b}$ = 6.6 Hz, 1 H, H-6b), 4.20 (ddd, $J_{4,5}$ = 10.0 Hz, $J_{5,6b}$ = 6.6 Hz, $J_{5,6a}$ = 3.3 Hz, 1 H, H-5), 3.78 (ddd, J = 11.8, 10.1, 5.7 Hz, 1 H, OC$\underline{H}_2$CH$_2$Si), 3.57 (ddd, J = 11.9, 10.1, 5.3 Hz, 1 H, OC$\underline{H}_2$CH$_2$Si), 3.43 (dd, $J_{2,3}$ = 10.3 Hz, $J_{1,2}$ = 3.7 Hz, 1 H, H-2), 2.20 (d, $J_{3',OH}$ = 6.2 Hz, 1 H, OH), 1.02 (ddd, J = 13.6, 11.9, 5.3 Hz, 1 H, OCH$_2$C$\underline{H}_2$Si), 0.96 (ddd, J = 13.7, 12.0, 5.7 Hz, 1 H, OCH$_2$C$\underline{H}_2$Si), -0.04 (s, 9 H, Si(CH$_3$)$_3$); ^{13}C NMR (126 MHz, CDCl$_3$, δ_C) 166.34 (C=O), 166.33 (C=O), 166.1 (C=O), 166.0 (C=O), 164.8 (C=O), 133.5 (Ar), 133.4 (Ar), 133.3 (Ar), 133.04 (Ar), 133.00 (Ar), 130.1 (Ar), 130.0 (Ar), 129.9 (Ar), 129.69 (Ar), 129.68 (Ar), 129.6 (Ar), 129.2 (Ar), 128.8 (Ar), 128.6 (Ar), 128.4 (Ar), 128.32 (Ar), 128.29 (Ar), 128.0 (Ar), 98.2 (C-1'), 96.9 (C-1), 74.7 (C-3), 72.7 (C-4), 71.5 (C-4'), 70.8 (C-2'), 68.1 (C-5), 67.9 (C-5'), 67.0 (C-3'), 66.3 (O$\underline{C}$H$_2$CH$_2$Si), 63.6 (C-6), 62.4 (C-6'), 61.7 (C-2), 18.0 (OCH$_2\underline{C}$H$_2$Si), -1.5 (Si(CH$_3$)$_3$); HRMS–ESI–TOF calcd for [M+Na]$^+$ $C_{52}H_{53}N_3NaO_{15}Si$: 1010.3138. Found 1010.3137.



2-(Trimethylsilyl)ethyl 2,4,6-tri-*O*-benzoyl-3-*O*-levulinoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2-azido-2-deoxy-4,6-di-*O*-benzoyl-α-D-glucopyranoside (3.37). Compounds **3.23** (508 mg; 0.515 mmol) and **3.22** (838 mg; 0.678 mmol) were dissolved in CH_2Cl_2–toluene and were concentrated to dryness on high vacuum overnight. Dry CH_2Cl_2 (15.0 mL) and 4 Å M.S. were

added to the flask, and the mixture was stirred for 60 min at rt. The suspension was cooled to 0 °C, and TfOH (4.8 μL; 0.054 mmol) was added. The reaction mixture was stirred for 1 h at 0 °C, before being neutralized by the addition of Et_3N, filtered, and concentrated. The crude residue was purified by silica gel column chromatography (gradient of 3:2→11:9 hexanes–EtOAc) to give **3.37** (993 mg, 95%) as a white, fluffy solid. R_f 0.26 (3:2 hexanes–EtOAc); $[\alpha]_D$ +63 (*c* 0.20, $CHCl_3$); ^{1}H NMR (700 MHz, $CDCl_3$, δ_H) 8.21 – 8.16 (m, 2 H, ArH), 8.07 – 8.03 (m, 3 H, ArH), 7.98 – 7.92 (m, 4 H, ArH), 7.89 – 7.85 (m, 2 H, ArH), 7.83 – 7.79 (m, 2 H, ArH), 7.74 – 7.70 (m, 2 H, ArH), 7.62 – 7.58 (m, 2 H, ArH), 7.56 – 7.22 (m, 30 H, ArH), 7.22 – 7.17 (m, 4 H, ArH), 7.15 – 7.10 (m, 2 H, ArH), 7.00 – 6.95 (m, 2 H, ArH), 6.00 (dd, $J_{3',4'}$ = 3.4 Hz, $J_{4',5'}$ = 1.6 Hz, 1 H, H-4'), 5.60 – 5.55 (m, 2 H, H-1', H-2'), 5.54 – 5.46 (m, 4 H, H-5'', H-2''', H-3''', H-4'''), 5.37 (d, $J_{1''',2'''}$ = 3.6 Hz, 1 H, H-1'''), 5.33 (s, 1 H, H-1''), 5.25 (dd, $J_{4,5}$ = 10.1 Hz, $J_{3,4}$ = 9.0 Hz, 1 H, H-4), 5.12 (d, $J_{2'',3''}$ = 2.8 Hz, 1 H, H-2''), 5.08 (d, $J_{1,2}$ = 3.7 Hz, 1 H, H-1), 4.99 – 4.94 (m, 1 H, H-5'), 4.78 (dd, $J_{3'',4''}$ = 7.4 Hz, $J_{2'',3''}$ = 2.8 Hz, 1 H, H-3''), 4.68 – 4.60 (m, 2 H, H-6a'', H-6a'), 4.58 – 4.53 (m, 1 H, H-3'), 4.47 – 4.40 (m, 2 H, H-3, H-6b''), 4.35 – 4.24 (m, 3 H, H-6a, H-6b, H-6b'), 4.19 – 4.09 (m, 2 H, H-5, H-5'''), 4.01 (dd, $J_{3'',4''}$ = 7.4 Hz, $J_{4'',5''}$ = 2.7 Hz, 1 H, H-4''), 3.98 (dd, $J_{6a''',6b'''}$ = 11.1 Hz, $J_{5''',6a'''}$ = 5.5 Hz, 1 H, H-6a'''), 3.81 (dd, $J_{6a''',6b'''}$ = 11.1 Hz, $J_{5''',6b'''}$ = 7.5 Hz, 1 H, H-6b'''), 3.78 (ddd, *J* = 12.0, 10.2, 5.7 Hz, 1 H, $OC\underline{H}_2CH_2Si$), 3.57 (ddd, *J* = 12.0, 10.2, 5.3 Hz, 1 H, $OC\underline{H}_2CH_2Si$), 3.36 (dd, $J_{2,3}$ = 10.2 Hz, $J_{1,2}$ = 3.7 Hz, 1 H, H-2), 2.58 (dt, *J* = 18.3, 7.1 Hz, 1 H, $CH_2C\underline{H}_2C(O)CH_3$), 2.50 (dt, *J* = 18.3, 6.7 Hz, 1 H, $CH_2C\underline{H}_2C(O)CH_3$), 2.43 – 2.31 (m, 2 H, $C\underline{H}_2CH_2C(O)CH_3$), 1.94 (s, 3 H, $CH_2CH_2C(O)C\underline{H}_3$), 1.02 (ddd, *J* = 13.6, 12.0, 5.3 Hz, 1 H, $OCH_2C\underline{H}_2Si$), 0.96 (ddd, *J* = 13.7, 12.0, 5.7 Hz, 1 H, $OCH_2C\underline{H}_2Si$), -0.04 (s, 9 H, $Si(CH_3)_3$); ^{13}C NMR (126 MHz, $CDCl_3$, δ_C) 205.9 (C=O), 171.1 (C=O), 166.1 (C=O), 165.97 (C=O), 165.93 (C=O), 165.74 (C=O), 165.67 (C=O), 165.62 (C=O), 165.52 (C=O), 165.50

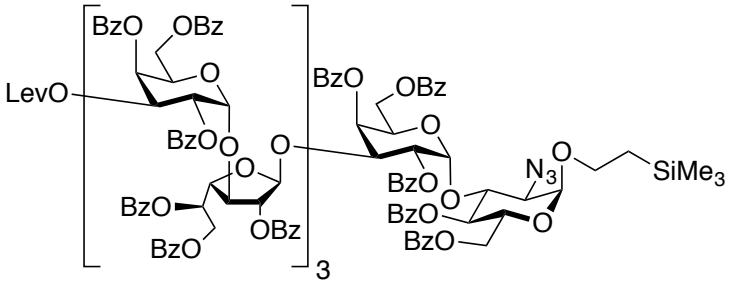
(C=O), 165.0 (C=O), 164.8 (C=O), 164.1 (C=O), 133.6 (Ar), 133.4 (Ar), 133.3 (Ar), 133.2 (Ar), 132.97 (Ar), 132.94 (Ar), 132.87 (Ar), 132.7 (Ar), 132.6 (Ar), 130.0 (Ar), 129.9 (Ar), 129.82 (Ar), 129.76 (Ar), 129.68 (Ar), 129.65 (Ar), 129.61 (Ar), 129.56 (Ar), 129.51 (Ar), 129.47 (Ar), 129.3 (Ar), 129.2 (Ar), 128.9 (Ar), 128.8 (Ar), 128.73 (Ar), 128.67 (Ar), 128.5 (Ar), 128.4 (Ar), 128.33 (Ar), 128.28 (Ar), 128.26 (Ar), 128.24 (Ar), 128.1 (Ar), 127.9 (Ar), 127.7 (Ar), 107.7 (C-1''), 99.0 (C-1'''), 98.3 (C-1'), 96.9 (C-1), 85.6 (C-4''), 81.8 (C-2''), 80.6 (C-3''), 75.0 (C-3), 72.5 (C-4), 71.9 (C-3'), 71.0 (C-4'), 70.1 (C-2'), 69.8 (C-5''), 68.7 (C-3'''), 68.3 (C-4'''), 68.02 (C-5), 68.01 (C-5'), 67.8 (C-2'''), 67.1 (C-5'''), 66.3 (OCH_2CH_2Si), 63.61 (C-6''), 63.56 (C-6), 62.6 (C-6'), 61.6 (C-6'''), 61.5 (C-2), 37.8 ($CH_2\underline{C}H_2C(O)CH_3$), 29.5 ($CH_2CH_2C(O)\underline{C}H_3$), 28.0 ($\underline{C}H_2CH_2C(O)CH_3$), 18.0 ($OCH_2\underline{C}H_2Si$), -1.6 ($Si(CH_3)_3$); HRMS–ESI–TOF calcd for $[M+Na]^+$ $C_{111}H_{103}N_3NaO_{33}Si$: 2056.6135. Found 2056.6182.



2-(Trimethylsilyl)ethyl 2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2-azido-2-deoxy-4,6-di-*O*-benzoyl-α-D-glucopyranoside (3.19). To a solution of **3.37** (995 mg; 0.489 mmol) in CH_2Cl_2–CH_3OH (39 mL, 1:1 v/v) was added H_2NNH_2·AcOH (81 mg, 0.88 mmol). The reaction mixture was stirred for 4.5 h at rt, before being diluted with CH_2Cl_2 (25.0 mL) and washed with H_2O. The organic layer was dried with Na_2SO_4, filtered, and concentrated. The crude residue was purified by silica gel column chromatography (2:1→3:2 hexanes–EtOAc) to yield **3.19** (770 mg, 81%) as a fluffy, white solid. R_f 0.29 (2:1 hexanes–EtOAc); $[\alpha]_D$ +65 (*c*

0.40, $CHCl_3$); ^{1}H NMR (700 MHz, $CDCl_3$, δ_H) 8.17 – 8.12 (m, 2 H, ArH), 8.11 – 8.05 (m, 4 H, ArH), 7.99 – 7.95 (m, 2 H, ArH), 7.91 – 7.85 (m, 6 H, ArH), 7.78 – 7.73 (m, 2 H, ArH), 7.67 – 7.63 (m, 2 H, ArH), 7.63 – 7.58 (m, 1 H, ArH), 7.53 – 7.45 (m, 6 H, ArH), 7.45 – 7.32 (m, 15 H, ArH), 7.32 – 7.27 (m, 4 H, ArH), 7.22 – 7.12 (m, 7 H, ArH), 7.12 – 7.07 (m, 2 H, ArH), 7.07 – 7.02 (m, 2 H, ArH), 6.06 (dd, $J_{3',4'}$ = 3.4 Hz, $J_{4',5'}$ = 1.4 Hz, 1 H, H-4'), 5.67 (dd, $J_{2',3'}$ = 10.7 Hz, $J_{1',2'}$ = 3.8 Hz, 1 H, H-2'), 5.60 (d, $J_{1',2'}$ = 3.8 Hz, 1 H, H-1'), 5.54 (ddd, $J_{5'',6a''}$ = 8.2 Hz, $J_{5'',6b''}$ = 3.1 Hz, $J_{4'',5''}$ = 2.8 Hz, 1 H, H-5''), 5.40 (s, 1 H, H-1''), 5.31 – 5.24 (m, 3 H, H-1''', H-2''', H-4), 5.17 – 5.13 (m, 1 H, H-4'''), 5.11 (d, $J_{1,2}$ = 3.7 Hz, 1 H, H-1), 5.05 (d, $J_{2'',3''}$ = 1.3 Hz, 1 H, H-2''), 5.03 – 4.98 (m, 1 H, H-5'), 4.73 (dd, $J_{3'',4''}$ = 5.9 Hz, $J_{4'',5''}$ = 2.8 Hz, 1 H, H-4''), 4.68 (dd, $J_{6a'',6b''}$ = 11.9 Hz, $J_{5'',6a''}$ = 8.2 Hz, 1 H, H-6a''), 4.61 (dd, $J_{6a',6b'}$ = 11.3 Hz, $J_{5',6a'}$ = 5.5 Hz, 1 H, H-6a'), 4.57 (dd, $J_{2',3'}$ = 10.7 Hz, $J_{3',4'}$ = 3.4 Hz, 1 H, H-3'), 4.50 – 4.44 (m, 2 H, H-6b'', H-3), 4.35 (dd, $J_{6a',6b'}$ = 11.3 Hz, $J_{5',6b'}$ = 7.1 Hz, 1 H, H-6b'), 4.33 – 4.28 (m, 2 H, H-6a, H-6b), 4.18 (ddd, $J_{4,5}$ = 10.1 Hz, $J_{5,6a}$ = 5.8 Hz, $J_{5,6b}$ = 4.2 Hz, 1 H, H-5), 4.13 (dd, $J_{6a''',6b'''}$ = 10.9 Hz, $J_{5''',6a'''}$ = 5.6 Hz, 1 H, H-6a'''), 4.10 – 4.06 (m, 1 H, H-5'''), 4.01 (m, 1 H, H-3''), 3.96 (dd, $J_{6a''',6b'''}$ = 10.9 Hz, $J_{5''',6b'''}$ = 7.1 Hz, 1 H, H-6b'''), 3.82 – 3.75 (m, 2 H, $OC\underline{H}_2CH_2Si$, H-3'''), 3.59 (ddd, J = 12.0, 10.1, 5.2 Hz, 1 H, $OC\underline{H}_2CH_2Si$), 3.39 (dd, $J_{2,3}$ = 10.2 Hz, $J_{1,2}$ = 3.7 Hz, 1 H, H-2), 1.88 (d, $J_{3''',OH}$ = 5.5 Hz, 1 H, OH), 1.03 (ddd, J = 13.7, 12.0, 5.3 Hz, 1 H, $OCH_2C\underline{H}_2Si$), 0.97 (ddd, J = 13.7, 12.0, 5.7 Hz, 1 H, $OCH_2C\underline{H}_2Si$), -0.04 (s, 9 H, $Si(CH_3)_3$); ^{13}C NMR (126 MHz, $CDCl_3$, δ_C) 166.5 (C=O), 166.2 (C=O), 166.1 (C=O), 166.0 (C=O), 165.9 (C=O), 165.78 (C=O), 165.76 (C=O), 165.6 (C=O), 165.2 (C=O), 164.8 (C=O), 164.0 (C=O), 133.8 (Ar), 133.37 (Ar), 133.36 (Ar), 133.3 (Ar), 133.1 (Ar), 133.03 (Ar), 132.96 (Ar), 132.93 (Ar), 132.85 (Ar), 132.5 (Ar), 108.0 (C-1''), 99.2 (C-1'''), 98.5 (C-1'), 96.9 (C-1), 85.5 (C-3''), 82.2 (C-4''), 81.1 (C-2''), 75.1 (C-3), 72.7 (C-3'), 72.5 (C-4), 71.23 (C-2'''), 71.19 (C-4'), 71.0 (C-4'''), 69.9

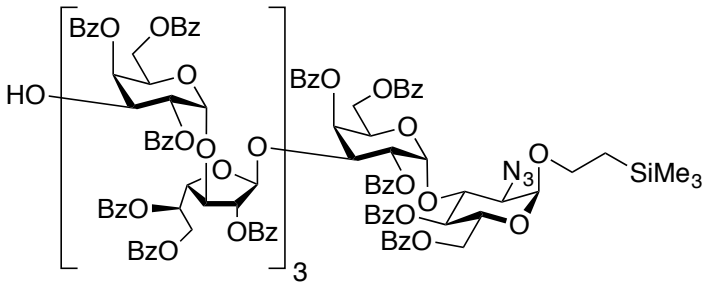
(C-5''), 69.7 (C-2'), 68.10 (C-5'), 68.07 (C-5), 67.4 (C-3'''), 67.0 (C-5'''), 66.3 (OCH_2CH_2Si), 63.8 (C-6''), 63.6 (C-6), 62.5 (C-6'), 61.9 (C-6'''), 61.5 (C-2), 18.0 ($OCH_2C\underline{H_2}Si$), -1.6 ($Si(CH_3)_3$); HRMS–ESI–TOF calcd for $[M+NH_4]^+$ $C_{106}H_{101}N_4O_{31}Si$: 1953.6214. Found 1953.6257.



2-(Trimethylsilyl)ethyl 2,4,6-tri-*O*-benzoyl-3-*O*-levulinoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2-azido-2-deoxy-4,6-di-*O*-benzoyl-α-D-glucopyranoside (3.38). Compounds **3.19** (136 mg; 70.0 μmol) and **3.18** (198 mg; 91.0 μmol) were dissolved in CH_2Cl_2–toluene and were concentrated to dryness on high vacuum overnight. Dry CH_2Cl_2 (4.0 mL) and 4 Å M.S. were added to the flask, and the mixture was stirred for 30 min at rt. The suspension was cooled to 0 °C, and TfOH (1.00 μL; 0.011 mmol) was added. The reaction mixture was stirred for 1 h at 0 °C, before being neutralized by the addition of Et_3N, filtered, and concentrated. The crude residue was purified by silica gel column chromatography (gradient of 1:1→2:3 hexanes–EtOAc) to give **3.38** (266 mg, 97%) as a white, fluffy solid. R_f 0.27 (1:1 hexanes–EtOAc); $[\alpha]_D$ +38 (*c* 0.20, $CHCl_3$); ^1H NMR (700 MHz, $CDCl_3$, δ_H) 8.11 – 8.04 (m, 4 H, ArH), 8.04 – 7.91 (m, 16 H, ArH), 7.91 – 7.84 (m, 4 H, ArH), 7.80 – 7.76 (m, 4 H,

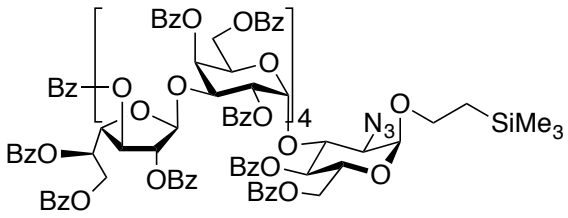
ArH), 7.76 – 7.66 (m, 8 H, ArH), 7.66 – 7.62 (m, 2 H, ArH), 7.56 – 7.49 (m, 4 H, ArH), 7.49 – 7.13 (m, 57 H, ArH), 7.10 – 6.95 (m, 14 H, ArH), 6.94 – 6.90 (m, 2 H, ArH), 5.98 (dd, *J* = 3.2, 1.5 Hz, 1 H), 5.81 – 5.79 (m, 1 H), 5.78 – 5.74 (m, 1 H), 5.65 – 5.56 (m, 4 H, H-$1_{Gal\textit{p}}$), 5.55 – 5.40 (m, 8 H, H-$1_{Gal\textit{p}}$), 5.37 (m, 2 H, 2 x H-$1_{Gal\textit{p}}$), 5.35 (s, 1 H, H-$1_{Gal\textit{f}}$), 5.32 (s, 1 H, H-$1_{Gal\textit{f}}$), 5.29 – 5.26 (m, 1 H), 5.26 – 5.22 (m, 2 H), 5.19 (s, 1 H, H-$1_{Gal\textit{f}}$), 5.12 (d, *J* = 2.8 Hz, 1 H), 5.06 (d, *J* = 3.7 Hz, 1 H, H-1_{Glc}), 4.96 – 4.92 (m, 1 H), 4.75 (dd, *J* = 7.5, 2.2 Hz, 1 H), 4.68 – 4.61 (m, 3 H), 4.61 – 4.53 (m, 3 H), 4.50 (dd, *J* = 12.0, 3.2 Hz, 1 H), 4.45 – 4.37 (m, 2 H), 4.36 – 4.11 (m, 10 H), 4.10 – 3.99 (m, 5 H), 3.97 – 3.90 (m, 2 H), 3.87 (dd, *J* = 11.1, 7.1 Hz, 1 H), 3.75 (ddd, *J* = 12.0, 10.2, 5.7 Hz, 1 H, OC$\underline{H}_2CH_2Si$), 3.71 (dd, *J* = 11.1, 6.6 Hz, 1 H), 3.55 (ddd, *J* = 12.0, 10.2, 5.2 Hz, 1 H, OC$\underline{H}_2CH_2Si$), 3.34 (dd, *J* = 10.2, 3.7 Hz, 1 H, H-2_{Glc}), 2.57 (dt, *J* = 18.2, 7.1 Hz, 1 H, $CH_2C\underline{H}_2C(O)CH_3$), 2.49 (dt, *J* = 18.3, 6.8 Hz, 1 H, $CH_2C\underline{H}_2C(O)CH_3$), 2.42 – 2.29 (m, 2 H, $C\underline{H}_2CH_2C(O)CH_3$), 1.93 (s, 3 H, $CH_2CH_2C(O)C\underline{H}_3$), 0.99 (ddd, *J* = 13.7, 12.0, 5.2 Hz, 1 H, $OCH_2C\underline{H}_2Si$), 0.93 (ddd, *J* = 13.7, 12.1, 5.7 Hz, 1 H, $OCH_2C\underline{H}_2Si$), -0.05 (s, 9 H, $Si(CH_3)_3$); ^{13}C NMR (126 MHz, $CDCl_3$, δ_C) 205.9 (C=O), 171.0 (C=O), 166.2 (C=O), 166.03 (C=O), 165.96 (C=O), 165.91 (C=O), 165.86 (C=O), 165.80 (C=O), 165.71 (C=O), 165.69 (C=O), 165.67 (C=O), 165.62 (C=O), 165.55 (C=O), 165.52 (C=O), 165.51 (C=O), 165.49 (C=O), 165.45 (C=O), 165.3 (C=O), 165.2 (C=O), 164.8 (C=O), 164.4 (C=O), 164.0 (C=O), 133.6 (Ar), 133.41 (Ar), 133.37 (Ar), 133.35 (Ar), 133.31 (Ar), 133.24 (Ar), 133.20 (Ar), 133.1 (Ar), 133.01 (Ar), 132.97 (Ar), 132.94 (Ar), 132.92 (Ar), 132.87 (Ar), 132.83 (Ar), 132.79 (Ar), 132.73 (Ar), 132.70 (Ar), 132.5 (Ar), 132.4 (Ar), 108.4 (C-$1_{Gal\textit{f}}$), 108.2 (C-$1_{Gal\textit{f}}$), 107.6 (C-$1_{Gal\textit{f}}$), 99.5 (C-$1_{Gal\textit{p}}$), 99.4 (C-$1_{Gal\textit{p}}$), 99.1 (C-$1_{Gal\textit{p}}$), 98.1 (C-$1_{Gal\textit{p}}$), 96.8 (C-1_{Glc}), 85.3, 84.9, 82.5, 82.2, 82.1, 80.8, 80.5, 80.4, 74.8, 73.8, 73.49, 72.48, 71.40, 71.35, 70.9, 70.7, 70.2, 69.92, 69.90, 69.8, 69.6, 69.4, 68.6, 68.2, 68.1, 68.0, 67.8, 67.60, 67.58, 67.2, 64.2, 64.0, 63.7, 63.6, 62.7, 62.10, 62.05,

61.54, 61.52, 37.8 ($CH_2\underline{C}H_2C(O)CH_3$), 29.5 ($CH_2CH_2C(O)\underline{C}H_3$), 27.9 ($\underline{C}H_2CH_2C(O)CH_3$), 18.0 ($OCH_2\underline{C}H_2Si$), -1.6 ($Si(CH_3)_3$); HRMS–ESI–TOF calcd for $[M+2Na]^{2+}$ $C_{219}H_{191}N_3Na_2O_{65}Si$: 1988.0643. Found 1988.0701.



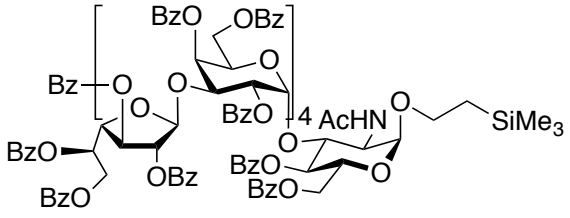
2-(Trimethylsilyl)ethyl 2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2-azido-2-deoxy-4,6-di-*O*-benzoyl-α-D-glucopyranoside (3.13). To a solution of **3.38** (658 mg; 0.167 mmol) in CH_2Cl_2–CH_3OH (15.0 mL, 2:1 v/v) was added H_2NNH_2·AcOH (35.0 mg, 0.380 mmol). The reaction mixture was stirred for 3.5 h at rt, before being diluted with CH_2Cl_2 (20.0 mL) and washed with H_2O. The organic layer was dried with Na_2SO_4, filtered, and concentrated. The crude residue was purified by silica gel column chromatography (1:1 hexanes–EtOAc) to yield **3.13** (619 mg, 97%) as a fluffy, white solid. R_f 0.35 (1:1 hexanes–EtOAc) ; $[\alpha]_D$ +44 (*c* 0.10, $CHCl_3$); ^{1}H NMR (700 MHz, $CDCl_3$, δ_H) 8.09 – 8.04 (m, 6 H, ArH), 8.03 – 7.90 (m, 18 H, ArH), 7.89 – 7.84 (m, 2 H, ArH), 7.84 – 7.81 (m, 2 H, ArH), 7.79 – 7.74 (m, 7 H, ArH), 7.73 – 7.68 (m, 4 H, ArH), 7.66 – 7.62 (m, 2 H, ArH), 7.58 – 7.52 (m, 3 H, ArH), 7.52 – 7.48 (m, 1 H, ArH), 7.48 – 6.94 (m, 70 H, ArH), 5.98 (dd, *J* = 3.2, 1.4 Hz, 1 H), 5.89 – 5.86 (m, 1 H), 5.81 – 5.77 (m, 1 H), 5.72 (dd, *J* = 10.4, 4.0 Hz, 1 H), 5.63 (dd, *J* = 10.5, 3.9 Hz, 1 H), 5.60 (dd, *J* = 10.5, 3.8 Hz, 1 H), 5.58 (d, *J* = 3.8 Hz, 1 H, H-$1_{Gal\,p}$), 5.55

(ddd, J = 7.8, 2.8, 2.8 Hz, 1 H), 5.49 – 5.46 (m, 1 H), 5.44 (ddd, J = 8.2, 2.9, 2.9 Hz, 1 H), 5.39 (d, J = 4.0 Hz, 1 H, H-$1_{Gal\textit{p}}$), 5.38 (d, J = 3.9 Hz, 1 H, H-$1_{Gal\textit{p}}$), 5.36 (s, 1 H, H-$1_{Gal\textit{f}}$), 5.35 – 5.32 (m, 2 H, H-$1_{Gal\textit{p}}$, H-$1_{Gal\textit{f}}$), 5.32 (s, 1 H, H-$1_{Gal\textit{f}}$), 5.31 – 5.30 (m, 1 H), 5.28 (dd, J = 10.6, 3.9 Hz, 1 H), 5.24 (dd, J = 9.4, 8.7 Hz, 1 H), 5.22 – 5.20 (m, 1 H), 5.18 – 5.14 (m, 1 H), 5.15 – 5.11 (m, 1 H), 5.06 (d, J = 3.7 Hz, 1 H, H-1_{Glc}), 4.97 – 4.93 (m, 1 H), 4.73 (dd, J = 5.7, 2.5 Hz, 1 H), 4.71 – 4.61 (m, 5 H), 4.61 – 4.51 (m, 3 H), 4.45 – 4.40 (m, 2 H), 4.37 – 4.22 (m, 8 H), 4.22 – 4.17 (m, 1 H), 4.15 (ddd, J = 10.1, 6.4, 3.7 Hz, 1 H), 4.12 – 4.05 (m, 5 H), 4.04 – 3.98 (m, 2 H), 3.95 (dd, J = 11.1, 5.8 Hz, 1 H), 3.89 (dd, J = 11.1, 7.2 Hz, 1 H), 3.79 – 3.71 (m, 2 H), 3.71 – 3.65 (m, 1 H), 3.59 – 3.52 (m, 1 H), 3.34 (dd, J = 10.2, 3.7 Hz, 1 H, H-2_{Glc}), 1.79 (d, J = 5.5 Hz, 1 H, OH), 0.99 (ddd, J = 13.6, 12.1, 5.2 Hz, 1 H, $OCH_2C\underline{H}_2Si$), 0.93 (ddd, J = 13.5, 12.1, 5.8 Hz, 1 H, $OCH_2C\underline{H}_2Si$), -0.05 (s, 9 H, $Si(CH_3)_3$); ^{13}C NMR (126 MHz, $CDCl_3$, δ_C) 166.5 (C=O), 166.24 (C=O), 166.19 (C=O), 166.04 (C=O), 165.96 (C=O), 165.93 (C=O), 165.86 (C=O), 165.85 (C=O), 165.69 (C=O), 165.67 (C=O), 165.64 (C=O), 165.62 (C=O), 165.56 (C=O), 165.54 (C=O), 165.50 (C=O), 165.4 (C=O), 165.2 (C=O), 164.8 (C=O), 164.4 (C=O), 164.3 (C=O), 164.0 (C=O), 133.62 (Ar), 133.60 (Ar), 133.4 (Ar), 133.23 (Ar), 133.18 (Ar), 133.16 (Ar), 133.00 (Ar), 132.98 (Ar), 132.95 (Ar), 132.92 (Ar), 132.88 (Ar), 132.83 (Ar), 132.79 (Ar), 132.6 (Ar), 132.50 (Ar), 132.45 (Ar), 108.3 (2 x C-$1_{Gal\textit{f}}$), 107.6 (C-$1_{Gal\textit{f}}$), 99.5 (C-$1_{Gal\textit{p}}$), 99.4 (C-$1_{Gal\textit{p}}$), 99.3 (C-$1_{Gal\textit{p}}$), 98.1 (C-$1_{Gal\textit{p}}$), 96.8 (C-1_{Glc}), 85.4, 85.3, 84.9, 82.4, 82.2, 82.1, 81.5, 80.8, 80.5, 74.8, 73.9, 73.7, 72.5, 71.43, 71.35, 71.1, 71.0, 70.88, 70.87, 70.2, 70.1, 70.0, 69.7, 69.6, 69.4, 68.1, 68.0, 67.64, 67.61, 67.4, 67.0, 66.2 ($O\underline{C}H_2CH_2Si$), 64.2, 64.0, 63.7, 63.6, 62.7, 62.1, 62.02, 61.95, 61.5 (C-2_{Glc}), 18.0 ($OCH_2\underline{C}H_2Si$), -1.6 ($Si(CH_3)_3$); HRMS–ESI–TOF calcd for $[M+2Na]^{2+}$ $C_{214}H_{185}N_3Na_2O_{63}Si$: 1939.0459. Found 1939.0418.



2-(Trimethylsilyl)ethyl 2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2-azido-2-deoxy-4,6-di-*O*-benzoyl-α-D-glucopyranoside (3.6). Compounds **3.12**[151] (230 mg; 0.30 mmol) and **3.13** (99 mg; 0.026 mmol) were dissolved in CH_2Cl_2–toluene and were concentrated to dryness on high vacuum for 1 h. Dry CH_2Cl_2 (5.0 mL) and 4 Å M.S. were added to the flask, and the mixture was stirred for 30 min at rt. The suspension was cooled to 0 °C, and TBSOTf (4.0 μL; 0.02 mmol) was added. The reaction mixture was stirred for 2 h, while warming to rt, before being neutralized by the addition of Et_3N, filtered, and concentrated. The crude residue was purified by silica gel column chromatography (3:2→1:1 hexanes–EtOAc) to give **3.6** (111 mg, 97%) as a white, fluffy solid. R_f 0.29 (9:1 toluene–EtOAc); $[\alpha]_D$ +53 (*c* 0.10, $CHCl_3$); ^{1}H NMR (700 MHz, $CDCl_3$, δ_H) 8.10 – 8.04 (m, 6 H, ArH), 8.04 – 7.91 (m, 18 H, ArH), 7.87 (d, *J* = 7.4 Hz, 2 H, ArH), 7.84 (d, *J* = 7.4 Hz, 2 H, ArH), 7.78 (d, *J* = 7.4 Hz, 2 H, ArH), 7.76 – 7.67 (m, 10 H, ArH), 7.66 – 7.63 (m, 4 H, ArH), 7.54 (d, *J* = 7.4 Hz, 2 H, ArH), 7.51 – 6.92 (m, 89 H, ArH), 6.08 (ddd, *J* = 6.8, 3.2, 3.2 Hz, 1 H), 6.00 – 5.95 (m, 1 H), 5.82 – 5.75 (m, 3 H), 5.69 – 5.56 (m, 5 H, H-1$_{Gal p}$), 5.49 – 5.40 (m, 5 H, H-1$_{Gal p}$), 5.40 – 5.34 (m, 4 H, 2 x H-1$_{Gal p}$, 2 x H-1$_{Gal f}$), 5.32 (s, 1 H, H-1$_{Gal f}$), 5.29 – 5.20 (m, 4 H), 5.16 (s, 1 H, H-1$_{Gal f}$), 5.12 (d, *J* = 2.5 Hz, 1 H), 5.06 (d, *J* =

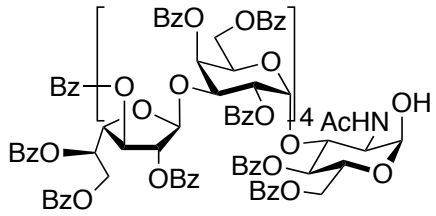
3.6 Hz, 1 H, H-1_{Glc}), 4.98 – 4.90 (m, 2 H), 4.90 – 4.82 (m, 1 H), 4.82 – 4.76 (m, 1 H), 4.68 – 4.61 (m, 5 H), 4.61 – 4.53 (m, 3 H), 4.45 – 4.37 (m, 3 H), 4.37 – 4.11 (m, 10 H), 4.11 – 4.04 (m, 3 H), 4.03 – 3.96 (m, 3 H), 3.96 – 3.90 (m, 1 H), 3.89 – 3.69 (m, 4 H), 3.60 – 3.51 (m, 1 H), 3.34 (dd, *J* = 10.2, 3.6 Hz, 1 H, H-2_{Glc}), 1.03 – 0.90 (m, 2 H, $OCH_2C\underline{H}_2Si$), -0.05 (s, 9 H, $Si(CH_3)_3$); ^{13}C NMR (126 MHz, $CDCl_3$, δ_C) 166.2 (C=O), 166.00 (C=O), 165.96 (C=O), 165.93 (C=O), 165.87 (C=O), 165.82 (C=O), 165.79 (C=O), 165.67 (C=O), 165.64 (C=O), 165.58 (C=O), 165.56 (C=O), 165.52 (C=O), 165.45 (C=O), 165.3 (C=O), 165.1 (C=O), 164.8 (C=O), 164.6 (C=O), 164.34 (C=O), 164.30 (C=O), 164.0 (C=O), 133.6 (Ar), 133.34 (Ar), 133.32 (Ar), 133.2 (Ar), 133.1 (Ar), 132.9 (Ar), 132.8 (Ar), 132.7 (Ar), 132.6 (Ar), 132.5 (Ar), 132.4 (Ar), 129.89 (Ar), 129.86 (Ar), 129.75 (Ar), 129.72 (Ar), 129.67 (Ar), 129.58 (Ar), 129.51 (Ar), 129.46 (Ar), 129.40 (Ar), 128.8 (Ar), 128.6 (Ar), 128.5 (Ar), 128.33 (Ar), 128.26 (Ar), 128.22 (Ar), 128.16 (Ar), 128.1 (Ar), 128.0 (Ar), 127.9 (Ar), 127.8 (Ar), 108.4 (C-$1_{Gal\mathit{f}}$), 108.3 (C-$1_{Gal\mathit{f}}$), 108.2 (C-$1_{Gal\mathit{f}}$), 107.5 (C-$1_{Gal\mathit{f}}$), 99.5 (2 x C-$1_{Gal\mathit{p}}$), 99.4 (C-$1_{Gal\mathit{p}}$), 98.1 (C-$1_{Gal\mathit{p}}$), 96.7 (C-1_{Glc}), 85.3, 85.0, 84.9, 82.3, 82.4, 82.2, 82.0, 81.8, 80.8, 80.5, 80.3, 77.8, 74.8, 73.7, 73.5, 72.4, 71.4, 71.3, 70.9, 70.8, 70.7, 70.1, 69.8, 69.7, 69.53, 69.46, 69.37, 68.04, 67.98, 67.63, 67.55, 66.20 ($O\underline{C}H_2CH_2Si$), 64.14, 63.98, 63.68, 63.51, 62.67, 62.04, 61.96, 61.89, 61.5 (C-2_{Glc}), 18.0 ($OCH_2\underline{C}H_2Si$), -1.6 ($Si(CH_3)_3$); HRMS–ESI–TOF calcd for $[M+2NH_4]^{2+}$ $C_{248}H_{219}N_5O_{72}Si$: 2223.1694. Found 2223.1705.



2-(Trimethylsilyl)ethyl 2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-

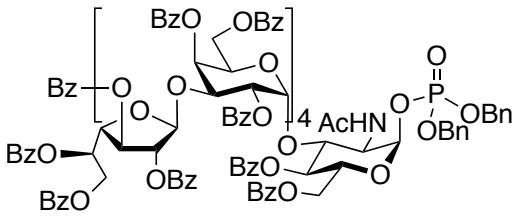
2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-4,6-di-*O*-benzoyl-α-D-glucopyranoside (3.39). To a solution of **3.6** (110 mg; 0.025 mmol) in THF–H_2O (10.0 mL; 3:2 v/v), was added 1 M Me_3P in THF (0.05 mL), followed by 1 M $NaOH_{(aq)}$ (0.016 mL). The pH was ~ 8, and the reaction mixture was stirred at rt. After 16 h, the reaction was incomplete, so the mixture was heated to 50 °C and stirred for an additional 4 h, before being diluted with brine (20.0 mL) and extracted twice with EtOAc (25.0 mL, 25.0 mL). The combined organic layers were dried with Na_2SO_4, filtered, and concentrated. The crude residue (130 mg) was dissolved in pyridine (6.00 mL), and cooled to 0 °C. Ac_2O (0.05 mL, 0.5 mmol) was added slowly dropwise, and the reaction mixture was stirred for 21 h, while warming to rt, before being diluted with EtOAc (20.0 mL), and washed with H_2O. The organic layer was washed, in succession, with solutions of 1 N HCl three times, saturated sodium bicarbonate, and brine, before being dried with Na_2SO_4, filtered, and concentrated. The crude residue was purified by silica gel column chromatography (gradient of 6:1→4:1 toluene–EtOAc) to yield **3.39** (87.0 mg, 78% over 2 steps) as white solid. R_f 0.24 (6:1 toluene–EtOAc); $[\alpha]_D$ +17 (*c* 0.10, $CHCl_3$); ^{1}H NMR (700 MHz, $CDCl_3$, δ_H) 8.10 – 7.91 (m, 27 H, ArH), 7.88 – 7.81 (m, 4 H, ArH), 7.76 – 7.68 (m, 16 H, ArH), 7.66 – 7.61 (m, 4 H, ArH), 7.52 – 6.91 (m, 84 H, ArH), 6.07 (ddd, *J* = 7.1, 3.0, 3.0 Hz, 1 H), 5.91 (d, *J* = 9.8 Hz, 1 H, NH), 5.87 – 5.84 (m, 1 H), 5.82 – 5.77 (m, 2 H), 5.74 (dd, *J* = 3.2, 1.1 Hz, 1 H), 5.68 – 5.58 (m, 4 H, H-$1_{Gal p}$), 5.50 (dd, *J* = 10.6, 3.7 Hz, 1 H), 5.47 – 5.40 (m, 5 H, H-$1_{Gal p}$), 5.37 (d, *J* = 4.1 Hz, 1 H, H-$1_{Gal p}$), 5.36 (s, 1 H, H-$1_{Gal f}$), 5.35 – 5.31 (m, 3 H, H-$1_{Gal f}$, H-$1_{Gal p}$), 5.25 – 5.22 (m, 2 H), 5.21 – 5.18 (m, 3 H, H-$1_{Gal f}$), 5.17 (s, 1 H, H-$1_{Gal f}$), 4.93 (dd, *J* = 5.6, 2.8 Hz, 1 H), 4.88 – 4.81 (m, 3 H, H-1_{Glc}), 4.79 (dd, *J* = 12.1, 3.5 Hz, 1 H), 4.74 –

4.70 (m, 1 H), 4.68 – 4.54 (m, 5 H), 4.54 – 4.47 (m, 3 H), 4.46 – 4.33 (m, 5 H), 4.33 – 4.13 (m, 8 H), 4.10 – 4.03 (m, 4 H), 4.02 – 3.95 (m, 3 H), 3.95 – 3.90 (m, 1 H), 3.86 (dd, *J* = 11.0, 7.2 Hz, 1 H), 3.83 – 3.78 (m, 1 H), 3.78 – 3.72 (m, 1 H), 3.66 (ddd, *J* = 12.2, 10.6, 5.3 Hz, 1 H), 3.47 (ddd, *J* = 12.2, 10.6, 5.3 Hz, 1 H), 1.98 (s, 3 H, $C(O)CH_3$), 0.89 (ddd, *J* = 13.8, 12.5, 5.1 Hz, 1 H, $OCH_2C\underline{H}_2Si$), 0.79 (ddd, *J* = 13.5, 12.2, 5.3 Hz, 1 H, $OCH_2C\underline{H}_2Si$), -0.06 (s, 9 H, $Si(CH_3)_3$); ^{13}C NMR (126 MHz, $CDCl_3$, δ_C) 170.2 (C=O), 166.5 (C=O), 166.2 (C=O), 166.04 (C=O), 166.00 (C=O), 165.9 (C=O), 165.83 (C=O), 165.81 (C=O), 165.71 (C=O), 165.69 (C=O), 165.60 (C=O), 165.56 (C=O), 165.53 (C=O), 165.49 (C=O), 165.41 (C=O), 165.38 (C=O), 165.36 (C=O), 164.63 (C=O), 164.60 (C=O), 164.4 (C=O), 164.3 (C=O), 164.2 (C=O), 133.7 (Ar), 133.40 (Ar), 133.35 (Ar), 133.33 (Ar), 133.28 (Ar), 133.2 (Ar), 133.12 (Ar), 133.08 (Ar), 133.0 (Ar), 132.91 (Ar), 132.86 (Ar), 132.8 (Ar), 132.7 (Ar), 132.6 (Ar), 132.5 (Ar), 132.4 (Ar), 130.0 (Ar), 129.9 (Ar), 129.83 (Ar), 129.78 (Ar), 129.7 (Ar), 129.63 (Ar), 129.55 (Ar), 129.50 (Ar), 129.45 (Ar), 129.41 (Ar), 129.40 (Ar), 129.32 (Ar), 129.27 (Ar), 129.0 (Ar), 128.8 (Ar), 128.73 (Ar), 128.71 (Ar), 128.61 (Ar), 128.56 (Ar), 128.49 (Ar), 128.44 (Ar), 128.42 (Ar), 128.37 (Ar), 128.34 (Ar), 128.30 (Ar), 128.26 (Ar), 128.24 (Ar), 128.19 (Ar), 128.16 (Ar), 128.12 (Ar), 128.08 (Ar), 128.06 (Ar), 128.04 (Ar), 127.96 (Ar), 127.8 (Ar), 108.3 (2 x C-$1_{Gal\mathit{f}}$), 108.2 (C-$1_{Gal\mathit{f}}$), 108.1 (C-$1_{Gal\mathit{f}}$), 99.5 (C-$1_{Gal\mathit{p}}$), 99.42 (C-$1_{Gal\mathit{p}}$), 99.38 (C-$1_{Gal\mathit{p}}$), 97.0 (C-$1_{Gal\mathit{p}}$), 96.4 (C-1_{Glc}), 85.1, 84.9, 82.5, 82.3, 81.94, 81.85, 80.7, 80.42, 80.39, 77.8, 73.9, 73.7, 73.5, 72.9, 72.6, 71.3, 70.9, 70.7, 69.9, 69.8, 69.7, 69.5, 69.4, 69.3, 68.2, 67.7, 67.6, 67.5, 67.2, 65.5 ($O\underline{C}H_2CH_2Si$), 64.2, 64.0, 63.9, 63.5, 63.1, 62.00, 61.99, 61.93, 61.91, 52.0 (C-2_{Glc}), 23.3 ($C(O)\underline{C}H_3$), 17.9 ($OCH_2\underline{C}H_2Si$), -1.5 ($Si(CH_3)_3$); HRMS–ESI–TOF calcd for $[M+H+NH_4]^{2+}$ $C_{250}H_{220}N_2NaO_{73}Si$: 2222.6662. Found 2222.6659.



2,3,5,6-Tetra-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-4,6-di-*O*-benzoyl-α-D-glucopyranose (3.40). To a solution of **3.39** (84 mg, 10 μmol) in CH_2Cl_2 (2.2 mL) and H_2O (0.05 mL) that was cooled to 0 °C, was added TFA (4.0 mL) slowly dropwise, and the mixture was stirred for 26 h while warming to rt. The solution was concentrated, dissolved in CH_2Cl_2 (25 mL), and washed with sodium bicarbonate until the organic layer reached neutral pH. The organic layer was separated, dried with Na_2SO_4, filtered, and concentrated to give crude oxazoline **3.40***, which was treated with a solution TFA–H_2O (1.0 mL, 9:1 v/v) and stirred for 5 min at rt, before being neutralized by the addition of saturated sodium bicarbonate (5.0 mL), and extracted with EtOAc (10 mL). The organic layer was dried with Na_2SO_4, filtered, and concentrated. The crude residue was purified by silica gel column chromatography (3:2 toluene–EtOAc) to yield **3.40** (61 mg, 74%) as a white solid. R_f 0.28 (3:2 toluene–EtOAc); $[\alpha]_D$ +41 (*c* 0.10, $CHCl_3$); ^{1}H NMR (700 MHz, $CDCl_3$, δ_H) 8.10 – 7.92 (m, 27 H, ArH), 7.85 – 7.81 (m, 4 H, ArH), 7.77 – 7.68 (m, 16 H, ArH), 7.66 – 7.62 (m, 4 H, ArH), 7.52 – 6.92 (m, 84 H, ArH), 6.15 (d, *J* = 9.4 Hz, 1 H, NH), 6.08 (ddd, *J* = 6.6, 3.0, 3.0 Hz, 1 H), 5.85 – 5.82 (m, 1 H), 5.82 – 5.77 (m, 2 H), 5.76 – 5.73 (m, 1 H), 5.68 – 5.59 (m, 4 H, H-1$_{Gal}$*p*), 5.52 – 5.34 (m, 11 H, 3 x H-1$_{Gal}$*p*, 2

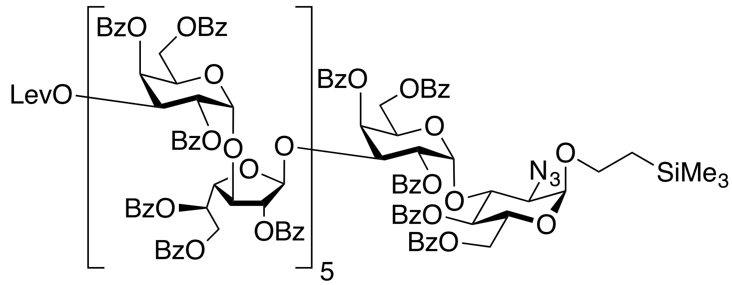
x H-1$_{Galf}$), 5.26 – 5.16 (m, 5 H, H-1$_{Glc}$, 2 x H-1$_{Galf}$), 4.93 (dd, *J* = 5.4, 2.7 Hz, 1 H), 4.86 (dd, *J* = 12.1, 7.4 Hz, 1 H), 4.82 – 4.75 (m, 2 H), 4.69 – 4.61 (m, 3 H), 4.60 – 4.55 (m, 2 H), 4.54 – 4.48 (m, 2 H), 4.47 – 4.34 (m, 8 H), 4.30 – 4.13 (m, 7 H), 4.11 – 3.92 (m, 7 H), 3.89 – 3.84 (m, 1 H), 3.81 (dd, *J* = 10.9, 7.2 Hz, 1 H), 3.75 (dd, *J* = 10.9, 6.9 Hz, 1 H), 3.18 (br s, 1 H, OH), 1.94 (s, 3 H, C(O)CH$_3$); ^{13}C NMR (126 MHz, $CDCl_3$, δ_C) 170.5 (C=O), 166.6 (C=O), 166.2 (C=O), 166.1 (C=O), 166.0 (C=O), 165.91 (C=O), 165.85 (C=O), 165.84 (C=O), 165.82 (C=O), 165.71 (C=O), 165.66 (C=O), 165.60 (C=O), 165.56 (C=O), 165.53 (C=O), 165.51 (C=O), 165.49 (C=O), 165.40 (C=O), 165.36 (C=O), 164.6 (C=O), 164.5 (C=O), 164.34 (C=O), 164.33 (C=O), 164.2 (C=O), 133.8 (Ar), 133.44 (Ar), 133.38 (Ar), 133.3 (Ar), 133.2 (Ar), 133.12 (Ar), 133.08 (Ar), 132.97 (Ar), 132.96 (Ar), 132.9 (Ar), 132.8 (Ar), 132.6 (Ar), 132.5 (Ar), 132.4 (Ar), 130.0 (Ar), 129.9 (Ar), 129.83 (Ar), 129.79 (Ar), 129.77 (Ar), 129.74 (Ar), 129.70 (Ar), 129.66 (Ar), 129.6 (Ar), 129.51 (Ar), 129.49 (Ar), 129.44 (Ar), 129.39 (Ar), 129.3 (Ar), 129.2 (Ar), 129.0 (Ar), 128.8 (Ar), 128.72 (Ar), 128.70 (Ar), 128.66 (Ar), 128.62 (Ar), 128.56 (Ar), 128.5 (Ar), 128.43 (Ar), 128.41 (Ar), 128.36 (Ar), 128.33 (Ar), 128.30 (Ar), 128.25 (Ar), 128.19 (Ar), 128.15 (Ar), 128.11 (Ar), 128.05 (Ar), 128.0 (Ar), 127.8 (Ar), 108.3 (C-1$_{Galf}$), 108.22 (C-1$_{Galf}$), 108.20 (C-1$_{Galf}$), 108.1 (C-1$_{Galf}$), 99.5 (C-1$_{Galp}$), 99.42 (C-1$_{Galp}$), 99.35 (C-1$_{Galp}$), 96.9 (C-1$_{Galp}$), 92.1 (C-1$_{Glc}$), 85.1, 85.0, 84.9, 84.8, 82.51, 82.45, 82.3, 82.0, 81.8, 80.6, 80.5, 80.4, 77.8, 73.73, 73.68, 73.5, 72.9, 72.8, 72.6, 71.3, 70.9, 70.7, 69.9, 69.7, 69.5, 69.4, 69.30, 69.28, 68.3, 67.7, 67.6, 67.5, 67.3, 64.2, 64.0, 63.8, 63.5, 63.2, 62.0, 61.9, 52.6 (C-2$_{Glc}$), 23.1 (C(O)<u>C</u>H$_3$); HRMS–ESI–TOF calcd for $[M+2Na]^{2+}$ $C_{245}H_{203}NNa_2O_{73}$: 2186.0994. Found 2186.0999.



2,3,5,6-Tetra-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-4,6-di-*O*-benzoyl-α-D-glucopyranose dibenzylphosphate (3.41). To a solution of **3.40** (26 mg, 6.0 μmol) and tetrazole (9.0 mg, 0.13 mmol) in dry CH_2Cl_2 (3.0 mL) that was cooled to 0 °C, was added *i*-$Pr_2NP(OBn)_2$ (20 μL, 0.06 mmol) dropwise. The reaction mixture was stirred at rt for 2 h, before being cooled to –78 °C, and *m*-CPBA (22 mg, 0.13 mmol) was added. The mixture was warmed to rt and stirred for an additional 2 h, before being diluted with CH_2Cl_2 (25 mL), washed with saturated sodium bicarbonate and with brine. The organic layer was dried with Na_2SO_4, filtered, and concentrated. The crude residue was purified by silica gel column chromatography (gradient of 60%→100% EtOAc–hexanes) to yield **3.41** (20 mg, 73% over 2 steps) as a beige, off-white solid. R_f 0.23 (2:3 hexanes–EtOAc); $[\alpha]_D$ +38 (*c* 0.20, $CHCl_3$); ^{1}H NMR (700 MHz, $CDCl_3$, δ_H) 8.10 – 7.91 (m, 26 H, ArH), 7.85 – 7.79 (m, 4 H, ArH), 7.75 – 7.68 (m, 13 H, ArH), 7.67 – 7.62 (m, 4 H, ArH), 7.60 – 7.56 (m, 2 H, ArH), 7.51 – 7.02 (m, 88 H, ArH), 7.02 – 6.92 (m, 8 H, ArH), 6.07 (ddd, *J* = 6.9, 3.2, 3.2 Hz, 1 H), 6.03 (d, *J* = 9.4 Hz, 1 H, NH), 5.88 – 5.84 (m, 1 H), 5.82 – 5.77 (m, 2 H), 5.75 – 5.72 (m, 1 H), 5.69 (dd, *J* = 6.1, 3.4 Hz, 1 H, H-1_{Glc}), 5.67 – 5.59 (m, 3 H), 5.54 (d, *J* = 3.7 Hz, 1 H, H-$1_{Gal\,p}$), 5.51 – 5.48 (m, 1 H), 5.47 – 5.36 (m, 7 H, 2 x

H-1$_{Gal\,p}$), 5.34 (s, 1 H, H-1$_{Gal\,f}$), 5.33 – 5.30 (m, 2 H, H-1$_{Gal\,p}$, H-1$_{Gal\,f}$), 5.25 – 5.22 (m, 2 H), 5.21 (d, *J* = 1.1 Hz, 1 H), 5.19 – 5.14 (m, 3 H, 2 x H-1$_{Gal\,f}$), 5.10 – 4.97 (m, 5 H), 4.93 (dd, *J* = 5.5, 2.8 Hz, 1 H), 4.88 – 4.83 (m, 1 H), 4.79 (dd, *J* = 12.1, 3.6 Hz, 1 H), 4.68 – 4.54 (m, 7 H), 4.53 – 4.49 (m, 1 H), 4.47 – 4.30 (m, 6 H), 4.29 – 4.24 (m, 2 H), 4.23 – 4.09 (m, 7 H), 4.09 – 4.03 (m, 3 H), 4.01 – 3.94 (m, 3 H), 3.94 – 3.89 (m, 1 H), 3.89 – 3.84 (m, 1 H), 3.83 – 3.78 (m, 1 H), 3.78 – 3.74 (m, 1 H), 1.72 (s, 3 H, C(O)CH$_3$); ^{13}C NMR (126 MHz, $CDCl_3$, δ_C) 170.6 (C=O), 166.5 (C=O), 166.1 (C=O), 166.0 (C=O), 165.90 (C=O), 165.89 (C=O), 165.84 (C=O), 165.83 (C=O), 165.81 (C=O), 165.70 (C=O), 165.68 (C=O), 165.60 (C=O), 165.55 (C=O), 165.53 (C=O), 165.49 (C=O), 165.45 (C=O), 165.36 (C=O), 165.35 (C=O), 164.6 (C=O), 164.4 (C=O), 164.34 (C=O), 164.32 (C=O), 164.1 (C=O), 135.3 (d, *J* = 6.5 Hz, Ar), 135.1 (d, *J* = 6.6 Hz, Ar), 133.7 (Ar), 133.5 (Ar), 133.4 (Ar), 133.3 (Ar), 133.22 (Ar), 133.15 (Ar), 133.12 (Ar), 133.07 (Ar), 133.0 (Ar), 132.89 (Ar), 132.86 (Ar), 132.84 (Ar), 132.78 (Ar), 132.6 (Ar), 132.51 (Ar), 132.45 (Ar), 130.0 (Ar), 129.9 (Ar), 129.8 (Ar), 129.74 (Ar), 129.67 (Ar), 129.64 (Ar), 129.62 (Ar), 129.60 (Ar), 129.55 (Ar), 129.50 (Ar), 129.45 (Ar), 129.41 (Ar), 129.39 (Ar), 129.34 (Ar), 129.27 (Ar), 129.2 (Ar), 128.90 (Ar), 128.87 (Ar), 128.79 (Ar), 128.76 (Ar), 128.7 (Ar), 128.61 (Ar), 128.55 (Ar), 128.48 (Ar), 128.44 (Ar), 128.36 (Ar), 128.30 (Ar), 128.25 (Ar), 128.2 (Ar), 128.14 (Ar), 128.12 (Ar), 128.08 (Ar), 128.06 (Ar), 128.04 (Ar), 127.97 (Ar), 127.83 (Ar), 127.80 (Ar), 127.6 (Ar), 108.30 (C-1$_{Gal\,f}$), 108.28 (C-1$_{Gal\,f}$), 108.22 (C-1$_{Gal\,f}$), 108.15 (C-1$_{Gal\,f}$), 99.53 (C-1$_{Gal\,p}$), 99.41 (C-1$_{Gal\,p}$), 99.38 (C-1$_{Gal\,p}$), 97.46 (C-1$_{Gal\,p}$), 96.51 (d, *J* = 6.4 Hz, C-1$_{Glc}$), 85.1, 84.92, 84.90, 82.51, 82.47, 82.3, 81.9, 81.8, 80.6, 80.4, 77.8, 73.73, 73.65, 73.5, 72.6, 71.8, 71.0, 70.8, 70.7, 70.1, 70.03, 69.96, 69.92, 69.86, 69.8, 69.7, 69.5, 69.4, 69.3, 67.7, 67.6, 67.5, 67.2, 64.2, 64.0, 63.81, 62.76, 62.4, 61.96, 61.92, 52.1 (d, *J* = 8.0 Hz, C-2$_{Glc}$), 22.8 (C(O)<u>C</u>H$_3$);

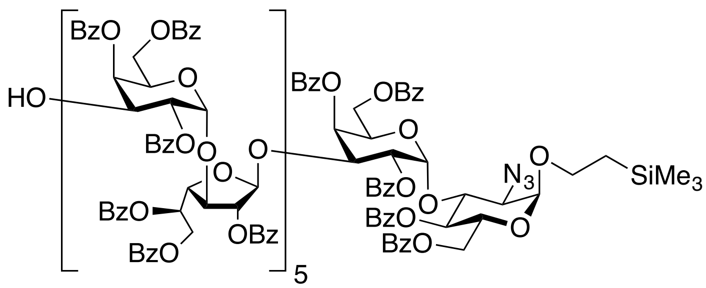
^{31}P NMR (162 MHz, $CDCl_3$, δ_P) -2.36; HRMS–ESI–TOF calcd for $[M+2Na]^{2+}$ $C_{259}H_{216}NNaO_{76}P$: 2316.1295. Found 2316.1274.



2-(Trimethylsilyl)ethyl 2,4,6-tri-*O*-benzoyl-3-*O*-levulinoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2-azido-2-deoxy-4,6-di-*O*-benzoyl-α-D-glucopyranoside (3.49). Compounds **3.13** (850 mg; 0.222 mmol) and **3.18** (680 mg; 0.311 mmol) were dissolved in CH_2Cl_2–toluene and were concentrated to dryness on high vacuum for 1 h. Dry CH_2Cl_2 (2.7 mL) and 4 Å M.S. were added to the flask, and the mixture was stirred for 30 min at rt. The suspension was cooled to 0 °C, and TBSOTf (10 µL; 0.044 mmol) was added. The reaction mixture was stirred for 1 h at 0 °C, before being neutralized by the addition of Et_3N, filtered, and concentrated. The crude residue was purified by silica gel column chromatography (1:1→2:3 hexanes–EtOAc) to give **3.49** (1.13 g, 87%) as a white, fluffy solid. R_f 0.15 (1:1 hexanes–EtOAc); $[\alpha]_D$ +41 (*c* 0.70, $CHCl_3$); ^1H NMR (700 MHz, $CDCl_3$, δ_H) 8.10 – 7.84 (m, 41 H, ArH), 7.79 – 7.66 (m, 23 H, ArH), 7.65 – 7.62 (m, 2 H, ArH), 7.55 – 6.90 (m, 109 H, ArH),

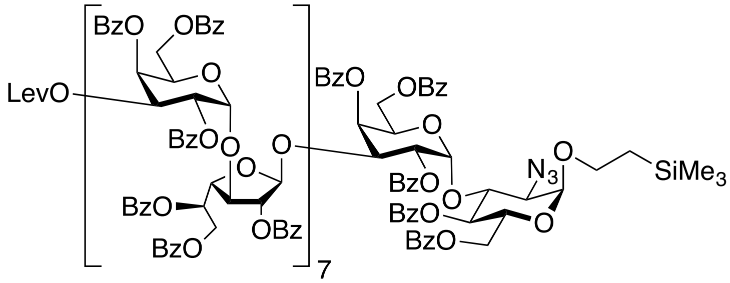
5.98 – 5.96 (m, 1 H), 5.80 – 5.75 (m, 4 H), 5.67 – 5.56 (m, 6 H, H-$1_{Gal\textit{p}}$), 5.55 – 5.39 (m, 9 H, 2 x H-$1_{Gal\textit{p}}$), 5.39 – 5.35 (m, 4 H, 3 x H-$1_{Gal\textit{p}}$), 5.35 (s, 1 H, H-$1_{Gal\textit{f}}$), 5.32 (s, 1 H, H-$1_{Gal\textit{f}}$), 5.27 (d, *J* = 2.9 Hz, 1 H), 5.25 – 5.20 (m, 5 H, H-$1_{Gal\textit{f}}$), 5.17 (s, 1 H, H-$1_{Gal\textit{f}}$), 5.13 (s, 1 H, H-$1_{Gal\textit{f}}$), 5.12 (d, *J* = 2.6 Hz, 1 H), 5.06 (d, *J* = 3.7 Hz, 1 H, H-1_{Glc}), 4.94 (ddd, *J* = 6.4, 6.4, 1.4 Hz, 1 H), 4.76 – 4.72 (m, 1 H), 4.67 – 4.53 (m, 10 H), 4.51 – 4.46 (m, 1 H), 4.45 – 4.35 (m, 4 H), 4.35 – 4.18 (m, 10 H), 4.18 – 4.11 (m, 4 H), 4.10 – 3.82 (m, 15 H), 3.79 – 3.68 (m, 2 H), 3.55 (ddd, *J* = 11.9, 10.3, 5.2 Hz, 1 H), 3.34 (dd, *J* = 10.2, 3.7 Hz, 1 H, H-2_{Glc}), 2.57 (dt, *J* = 18.1, 7.1 Hz, 1 H, $CH_2C\underline{H}_2C(O)CH_3$), 2.48 (dt, *J* = 18.3, 6.8 Hz, 1 H, $CH_2C\underline{H}_2C(O)CH_3$), 2.42 – 2.28 (m, 2 H, $C\underline{H}_2CH_2C(O)CH_3$), 1.93 (s, 3 H, $CH_2CH_2C(O)C\underline{H}_3$), 1.02 – 0.96 (m, 1 H, $OCH_2C\underline{H}_2Si$), 0.96 – 0.90 (m, 1 H, $OCH_2C\underline{H}_2Si$), -0.05 (s, 9 H, $Si(CH_3)_3$); ^{13}C NMR (126 MHz, $CDCl_3$, δ_C) 205.9 (C=O), 171.0 (C=O), 166.2 (C=O), 166.03 (C=O), 165.96 (C=O), 165.90 (C=O), 165.88 (C=O), 165.84 (C=O), 165.83 (C=O), 165.70 (C=O), 165.68 (C=O), 165.62 (C=O), 165.55 (C=O), 165.51 (C=O), 165.48 (C=O), 165.38 (C=O), 165.35 (C=O), 165.3 (C=O), 165.2 (C=O), 164.8 (C=O), 164.38 (C=O), 164.35 (C=O), 164.33 (C=O), 164.0 (C=O), 133.61 (Ar), 133.59 (Ar), 133.37 (Ar), 133.35 (Ar), 133.33 (Ar), 133.31 (Ar), 133.23 (Ar), 133.20 (Ar), 133.15 (Ar), 133.1 (Ar), 133.0(Ar) , 132.9 (Ar), 132.83 (Ar), 132.79 (Ar), 132.77 (Ar), 132.70 (Ar), 132.66 (Ar), 132.53 (Ar), 132.48 (Ar), 132.4 (Ar), 129.93 (Ar), 129.89 (Ar), 129.84 (Ar), 129.75 (Ar), 129.71 (Ar), 129.68 (Ar), 129.65 (Ar), 129.62 (Ar), 129.59 (Ar), 129.54 (Ar), 129.48 (Ar), 129.43 (Ar), 129.37 (Ar), 129.28 (Ar), 129.25 (Ar), 129.2 (Ar), 128.8 (Ar), 128.6 (Ar), 128.5 (Ar), 128.44 (Ar), 128.42 (Ar), 128.37 (Ar), 128.34 (Ar), 128.28 (Ar), 128.26 (Ar), 128.24 (Ar), 128.21 (Ar), 128.17 (Ar), 128.15 (Ar), 128.10 (Ar), 128.05 (Ar), 128.0 (Ar), 127.9 (Ar), 127.8 (Ar), 108.4 (C-$1_{Gal\textit{f}}$), 108.3 (2 x C-$1_{Gal\textit{f}}$), 108.2 (C-$1_{Gal\textit{f}}$), 107.6 (C-$1_{Gal\textit{f}}$), 99.52 (C-$1_{Gal\textit{p}}$), 99.48 (C-$1_{Gal\textit{p}}$), 99.44 (C-$1_{Gal\textit{p}}$), 99.39 (C-$1_{Gal\textit{p}}$), 99.1 (C-$1_{Gal\textit{p}}$), 98.1 (C-$1_{Gal\textit{p}}$), 96.8 (C-1_{Glc}), 85.3, 85.0, 84.9, 84.8, 82.5,

82.4, 82.2, 82.1, 80.8, 80.5, 80.4, 80.3, 76.8, 74.8, 73.9, 73.64, 73.55, 72.5, 71.4, 71.3, 70.9, 70.7, 70.2, 69.92, 69.89, 69.8, 69.7, 69.6, 69.4, 68.6, 68.2, 68.0, 67.8, 67.6, 67.2, 66.2 (O<u>C</u>H_2CH_2Si), 64.2, 64.0, 63.7, 63.6, 62.7, 62.0, 61.53, 61.51 (C-2_{Glc}), 37.8 (CH_2<u>C</u>$H_2C(O)CH_3$), 29.5 ($CH_2CH_2C(O)$<u>C</u>H_3), 27.9 (<u>C</u>$H_2CH_2C(O)CH_3$), 18.0 (OCH_2C<u>H_2</u>Si), -1.6 ($Si(CH_3)_3$); LRMS–MALDI–TOF calcd for $[M+Na]^+$ $C_{327}H_{279}N_3NaO_{97}Si$: 5853.8. Found 5853.0.



2-(Trimethylsilyl)ethyl 2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2-azido-2-deoxy-4,6-di-*O*-benzoyl-α-D-glucopyranoside (3.50). To a solution of **3.49** (1.12 g; 0.192 mmol) in CH_2Cl_2–CH_3OH (30.0 mL, 2:1 v/v) was added $H_2NNH_2 \cdot AcOH$ (36.2 mg, 0.393 mmol). The reaction mixture was stirred for 4 h at rt, before being diluted with CH_2Cl_2 (50.0 mL) and washed with H_2O. The organic layer was dried with Na_2SO_4, filtered, and concentrated. The crude residue was purified by silica gel column chromatography (1:1→2:3 hexanes–EtOAc) to yield **3.50** (916 mg, 83%) as a fluffy, white solid. R_f 0.20 (1:1 hexanes–EtOAc); $[\alpha]_D$ +40 (*c* 0.5, $CHCl_3$); ^1H NMR (700 MHz,

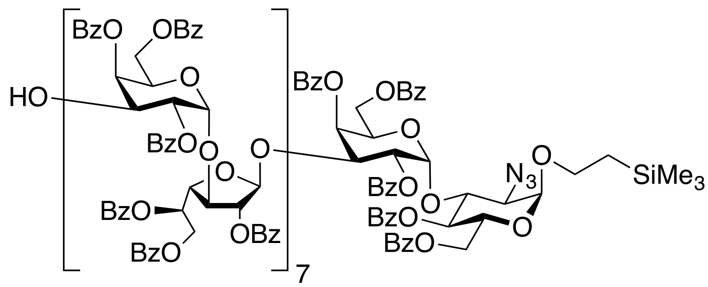
$CDCl_3$, δ_H) 8.09 – 8.04 (m, 6 H, ArH), 8.03 – 7.91 (m, 30 H, ArH), 7.88 – 7.81 (m, 4 H, ArH), 7.79 – 7.66 (m, 18 H, ArH), 7.65 – 7.62 (m, 2 H, ArH), 7.58 – 6.92 (m, 115 H, ArH), 6.00 – 5.95 (m, 1 H), 5.88 – 5.84 (m, 1 H), 5.80 – 5.75 (m, 3 H), 5.72 (dd, *J* = 10.4, 3.8 Hz, 1 H), 5.69 – 5.56 (m, 5 H), 5.56 – 5.52 (m, 1 H), 5.47 – 5.41 (m, 4 H), 5.40 – 5.31 (m, 8 H, 3 x H-$1_{Gal f}$, 5 x H-$1_{Gal p}$), 5.30 – 5.20 (m, 5 H), 5.19 (s, 1 H, H-$1_{Gal f}$), 5.16 (d, *J* = 2.6 Hz, 1 H), 5.14 (s, 1 H, H-$1_{Gal f}$), 5.12 (d, *J* = 2.6 Hz, 1 H), 5.06 (d, *J* = 3.6 Hz, 1 H, H-1_{Glc}), 4.96 – 4.91 (m, 1 H), 4.72 (dd, *J* = 5.6, 2.4 Hz, 1 H), 4.70 – 4.49 (m, 12 H), 4.46 – 4.37 (m, 4 H), 4.35 – 4.11 (m, 14 H), 4.11 – 4.03 (m, 6 H), 4.02 – 3.95 (m, 4 H), 3.95 – 3.83 (m, 4 H), 3.78 – 3.64 (m, 3 H, $OC\underline{H}_2CH_2Si$), 3.58 – 3.51 (m, 1 H, $OC\underline{H}_2CH_2Si$), 3.34 (dd, *J* = 10.2, 3.7 Hz, 1 H, H-2_{Glc}), 1.79 (d, *J* = 5.5 Hz, 1 H, OH), 1.02 – 0.96 (m, 1 H, $OCH_2C\underline{H}_2Si$), 0.96 – 0.90 (m, 1 H, $OCH_2C\underline{H}_2Si$), -0.05 (s, 9 H, $Si(CH_3)_3$); ^{13}C NMR (126 MHz, $CDCl_3$, δ_C) 166.5 (C=O), 166.23 (C=O), 166.20 (C=O), 166.03 (C=O), 165.96 (C=O), 165.9 (C=O), 165.62 (C=O), 165.57 (C=O), 165.5 (C=O), 165.4 (C=O), 165.2 (C=O), 164.83 (C=O), 164.78 (C=O), 164.4 (C=O), 164.0 (C=O), 133.6 (Ar), 133.4 (Ar), 133.2 (Ar), 133.0 (Ar), 132.8 (Ar), 132.6 (Ar), 132.5 (Ar), 132.4 (Ar), 129.9 (Ar), 129.7 (Ar), 129.6 (Ar), 129.52 (Ar), 129.50 (Ar), 129.4 (Ar), 129.3 (Ar), 128.8 (Ar), 128.62 (Ar), 128.55 (Ar), 128.5 (Ar), 128.40 (Ar), 128.35 (Ar), 128.3 (Ar), 128.1 (Ar), 128.0 (Ar), 127.9 (Ar), 127.8 (Ar), 108.4 (C-$1_{Gal f}$), 108.3 (2 x C-$1_{Gal f}$), 108.2 (C-$1_{Gal f}$), 107.6 (C-$1_{Gal f}$), 99.5 (C-$1_{Gal p}$), 99.4 (2 x C-$1_{Gal p}$), 99.3 (2 x C-$1_{Gal p}$), 98.1 (C-$1_{Gal p}$), 96.8 (C-1_{Glc}), 85.4, 85.3, 85.0, 84.90, 84.87, 82.5, 82.4, 82.3, 82.1, 81.5, 80.8, 80.5, 80.4, 74.8, 73.9, 73.84, 73.83, 73.6, 72.5, 71.42, 71.35, 71.1, 71.0, 70.9, 70.2, 70.1, 69.9, 69.7, 69.6, 69.4, 68.1, 68.0, 67.6, 67.4, 67.0, 66.2 ($O\underline{C}H_2CH_2Si$), 64.2, 64.0, 63.5, 62.7, 62.0, 61.5 (C-2_{Glc}), 18.0 (OCH_2CH_2Si), -1.6 ($Si(CH_3)_3$); LRMS–MALDI–TOF calcd for $[M+Na]^+$ $C_{322}H_{273}N_3NaO_{95}Si$: 5755.7. Found 5755.3.



2-(Trimethylsilyl)ethyl 2,4,6-tri-*O*-benzoyl-3-*O*-levulinoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2-azido-2-deoxy-4,6-di-*O*-benzoyl-α-D-glucopyranoside (3.44). Compounds **3.50** (899 mg; 0.157 mmol) and **3.18** (480 mg; 0.220 mmol) were dissolved in CH_2Cl_2–toluene and were concentrated to dryness on high vacuum for 1 h. Dry CH_2Cl_2 (2.00 mL) and 4 Å M.S. were added to the flask, and the mixture was stirred for 30 min at rt. The suspension was cooled to 0 °C, and TBSOTf (4.0 µL; 17 µmol) was added. The reaction mixture was stirred for 2 h at 0 °C, before being neutralized by the addition of Et_3N, filtered, and concentrated. The crude residue was purified by silica gel column chromatography (gradient of 55%→70% EtOAc–hexanes) to give **3.44** (995 mg, 82%) as a white, fluffy solid. R_f 0.33 (2:3 hexanes–EtOAc); $[\alpha]_D$ +41 (*c* 0.60, $CHCl_3$); ^1H NMR (700 MHz, $CDCl_3$, δ_H) 8.10 – 8.07 (m, 2 H, ArH), 8.07 – 8.04 (m, 2 H, ArH), 8.04 – 7.85

(m, 58 H, ArH), 7.79 – 7.66 (m, 35 H, ArH), 7.65 – 7.62 (m, 2 H, ArH), 7.55 – 6.90 (m, 136 H, ArH), 5.98 – 5.96 (m, 1 H), 5.81 – 5.74 (m, 6 H), 5.68 – 5.56 (m, 7 H, H-$1_{Gal\textit{p}}$), 5.54 – 5.39 (m, 13 H, 3 x H-$1_{Gal\textit{p}}$), 5.39 – 5.33 (m, 7 H, 4 x H-$1_{Gal\textit{p}}$, H-$1_{Gal\textit{f}}$), 5.31 (s, 1 H, H-$1_{Gal\textit{f}}$), 5.27 – 5.20 (m, 8 H, H-$1_{Gal\textit{f}}$), 5.16 (s, 3 H, 3 x H-$1_{Gal\textit{f}}$), 5.14 – 5.10 (m, 2 H, H-$1_{Gal\textit{f}}$), 5.06 (d, J = 3.6 Hz, 1 H, H-1_{Glc}), 4.96 – 4.92 (m, 1 H), 4.74 (dd, J = 7.4, 1.7 Hz, 1 H), 4.67 – 4.53 (m, 14 H), 4.52 – 4.47 (m, 1 H), 4.45 – 4.18 (m, 17 H), 4.18 – 3.83 (m, 27 H), 3.78 – 3.68 (m, 2 H), 3.58 – 3.52 (m, 1 H), 3.34 (dd, J = 10.2, 3.6 Hz, 1 H, H-2_{Glc}), 2.61 – 2.52 (m, 1 H, $CH_2C\underline{H}_2C(O)CH_3$), 2.52 – 2.44 (m, 1 H, $CH_2C\underline{H}_2C(O)CH_3$), 2.41 – 2.28 (m, 2 H, $C\underline{H}_2CH_2C(O)CH_3$), 1.93 (s, 3 H,), 1.02 – 0.96 (m, 1 H, $OCH_2C\underline{H}_2Si$), 0.96 – 0.90 (m, 1 H, $OCH_2C\underline{H}_2Si$), -0.05 (s, 9 H, $Si(CH_3)_3$); ^{13}C NMR (126 MHz, $CDCl_3$, δ_C) 205.9 (C=O), 171.0 (C=O), 166.2 (C=O), 166.03 (C=O), 165.96 (C=O), 165.90 (C=O), 165.86 (C=O), 165.8 (C=O), 165.70 (C=O), 165.68 (C=O), 165.61 (C=O), 165.55 (C=O), 165.51 (C=O), 165.46 (C=O), 165.37 (C=O), 165.35 (C=O), 165.3 (C=O), 165.2 (C=O), 164.8 (C=O), 164.38 (C=O), 164.35 (C=O), 164.3 (C=O), 164.0 (C=O), 133.6 (Ar), 133.4 (Ar), 133.33 (Ar), 133.31 (Ar), 133.24 (Ar), 133.15 (Ar), 133.1 (Ar), 133.0 (Ar), 132.9 (Ar), 132.82 (Ar), 132.80 (Ar), 132.69 (Ar), 132.65 (Ar), 132.6 (Ar), 132.53 (Ar), 132.48 (Ar), 132.43 (Ar), 132.41 (Ar), 132.3 (Ar), 129.9 (Ar), 129.84 (Ar), 129.75 (Ar), 129.7 (Ar), 129.64 (Ar), 129.59 (Ar), 129.53 (Ar), 129.48 (Ar), 129.44 (Ar), 129.37 (Ar), 129.28 (Ar), 129.25 (Ar), 129.2 (Ar), 128.8 (Ar), 128.6 (Ar), 128.5 (Ar), 128.4 (Ar), 128.34 (Ar), 128.25 (Ar), 128.21 (Ar), 128.17 (Ar), 128.09 (Ar), 128.05 (Ar), 128.0 (Ar), 127.9 (Ar), 127.8 (Ar), 108.4 (C-$1_{Gal\textit{f}}$), 108.3 (C-$1_{Gal\textit{f}}$), 108.24 (2 x C-$1_{Gal\textit{f}}$), 108.18 (2 x C-$1_{Gal\textit{f}}$), 107.6 (C-$1_{Gal\textit{f}}$), 99.51 (C-$1_{Gal\textit{p}}$), 99.46 (3 x C-$1_{Gal\textit{p}}$), 99.4 (C-$1_{Gal\textit{p}}$), 99.1 (2 x C-$1_{Gal\textit{p}}$), 98.1 (C-$1_{Gal\textit{p}}$), 96.8 (C-1_{Glc}), 85.3, 85.0, 84.9, 84.8, 82.48, 82.46, 82.2, 82.1, 80.5, 80.41, 80.35, 80.3, 74.8, 73.8, 73.67, 73.65, 73.6, 72.5, 71.4, 71.3, 70.9, 70.7, 70.2, 69.8, 69.7, 69.6, 69.4, 68.6, 68.2, 68.1, 68.0, 67.8, 67.6, 67.2, 66.2

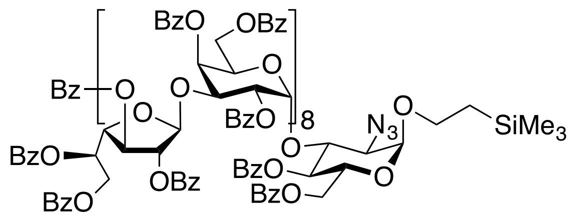
($O\underline{C}H_2CH_2Si$) , 64.2, 64.0, 63.7, 63.5, 62.7, 62.1, 62.0, 61.5 (C-2_{Glc}), 37.8 ($CH_2\underline{C}H_2C(O)CH_3$), 29.7 ($CH_2CH_2C(O)\underline{C}H_3$), 27.9 ($\underline{C}H_2CH_2C(O)CH_3$), 18.0 ($OCH_2\underline{C}H_2Si$), -1.6 ($Si(CH_3)_3$); LRMS–MALDI–TOF calcd for $[M+Na]^+$ $C_{435}H_{367}N_3NaO_{129}Si$: 7751.7. Found 7749.9.



2-(Trimethylsilyl)ethyl 2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2-azido-2-deoxy-4,6-di-*O*-benzoyl-α-D-glucopyranoside (3.14). To a solution of **3.44** (977 mg; 0.126 mmol) in CH_2Cl_2–CH_3OH (36.0 mL, 2:1 v/v) was added $H_2NNH_2·AcOH$ (24.5 mg, 0.266 mmol). The reaction mixture was stirred for 6 h at rt, before being diluted with CH_2Cl_2 (50.0 mL) and washed with H_2O. The organic layer was dried with Na_2SO_4, filtered, and concentrated. The crude residue was purified by silica gel column chromatography (9:11→2:3 hexanes–EtOAc) to yield **3.14** (716 mg, 74%) as a fluffy, white solid. R_f 0.41 (2:3 hexanes–EtOAc); $[\alpha]_D$ +33 (*c*

0.80, CH_2Cl_2); ^{1}H NMR (700 MHz, $CDCl_3$, δ_H) 8.09 – 8.03 (m, 5 H, ArH), 8.03 – 7.81 (m, 40 H, ArH), 7.79 – 7.74 (m, 4 H, ArH), 7.74 – 7.66 (m, 26 H, ArH), 7.65 – 7.61 (m, 2 H, ArH), 7.58 – 6.92 (m, 156 H, ArH), 5.98 – 5.96 (m, 1 H), 5.88 – 5.85 (m, 1 H), 5.80 – 5.74 (m, 4 H), 5.72 (dd, *J* = 10.3, 3.9 Hz, 1 H), 5.69 – 5.64 (m, 4 H), 5.63 – 5.56 (m, 3 H, H-$1_{Gal\textit{p}}$), 5.56 – 5.52 (m, 1 H), 5.47 – 5.30 (m, 16 H, 7 x H-$1_{Gal\textit{p}}$, 4 x H-$1_{Gal\textit{f}}$), 5.30 – 5.20 (m, 8 H), 5.18 (s, 1 H, H-$1_{Gal\textit{f}}$), 5.17 (s, 4 H, 2 x H-$1_{Gal\textit{f}}$), 5.13 (s, 1 H, H-$1_{Gal\textit{f}}$), 5.12 – 5.10 (m, 1 H), 5.06 (d, *J* = 3.6 Hz, 1 H, H-1_{Glc}), 4.96 – 4.92 (m, 1 H), 4.74 – 4.71 (m, 1 H), 4.70 – 4.51 (m, 16 H), 4.44 – 4.35 (m, 6 H), 4.35 – 3.82 (m, 38 H), 3.78 – 3.65 (m, 3 H), 3.58 – 3.51 (m, 1 H), 3.34 (dd, *J* = 10.1, 3.6 Hz, 1 H, H-2_{Glc}), 1.79 (d, *J* = 5.7 Hz, 1 H, OH), 1.01 – 0.96 (m, 1 H, $OCH_2C\underline{H}_2Si$), 0.96 – 0.90 (m, 1 H, $OCH_2C\underline{H}_2Si$), -0.05 (s, 9 H, $Si(CH_3)_3$); ^{13}C NMR (126 MHz, $CDCl_3$, δ_C) 166.5 (C=O), 166.24 (C=O), 166.18 (C=O), 166.03 (C=O), 165.96 (C=O), 165.91 (C=O), 165.87 (C=O), 165.8 (C=O), 165.7 (C=O), 165.64 (C=O), 165.61 (C=O), 165.55 (C=O), 165.51 (C=O), 165.47 (C=O), 165.46 (C=O), 165.4 (C=O), 165.2 (C=O), 164.8 (C=O), 164.4 (C=O), 164.3 (C=O), 164.0 (C=O), 133.62 (Ar), 133.59 (Ar), 133.34 (Ar), 133.25 (Ar), 133.2 (Ar), 133.0 (Ar), 132.8 (Ar), 132.6 (Ar), 132.5 (Ar), 132.4 (Ar), 129.92 (Ar), 129.87 (Ar), 129.78 (Ar), 129.75 (Ar), 129.68 (Ar), 129.66 (Ar), 129.6 (Ar), 129.53 (Ar), 129.48 (Ar), 129.4 (Ar), 129.31 (Ar), 129.28 (Ar), 128.8 (Ar), 128.63 (Ar), 128.55 (Ar), 128.52 (Ar), 128.48 (Ar), 128.44 (Ar), 128.40 (Ar), 128.35 (Ar), 128.3 (Ar), 128.2 (Ar), 128.10 (Ar), 128.05 (Ar), 128.02 (Ar), 127.97 (Ar), 127.9 (Ar), 127.8 (Ar), 108.41(C-$1_{Gal\textit{f}}$), 108.35(C-$1_{Gal\textit{f}}$), 108.31(C-$1_{Gal\textit{f}}$), 108.26 (C-$1_{Gal\textit{f}}$), 108.24 (C-$1_{Gal\textit{f}}$), 108.19 (C-$1_{Gal\textit{f}}$), 107.6 (C-$1_{Gal\textit{f}}$), 99.52 (2 x C-$1_{Gal\textit{p}}$), 99.47 (2 x C-$1_{Gal\textit{p}}$), 99.4 (2 x C-$1_{Gal\textit{p}}$), 99.3 (C-$1_{Gal\textit{p}}$), 98.1 (C-$1_{Gal\textit{p}}$), 96.8 (C-1_{Glc}), 85.4, 85.3, 85.0, 84.92, 84.87, 82.48, 82.45, 82.3, 82.1, 81.5, 80.4, 80.3, 74.8, 73.9, 73.8, 73.7, 73.54, 72.47, 71.41, 71.35, 71.1, 71.0, 70.9, 70.2, 70.1, 69.9, 69.69, 69.65, 69.4, 68.1, 68.0, 67.63, 67.55, 67.4, 67.0, 66.2 ($O\underline{C}H_2CH_2Si$), 64.2,

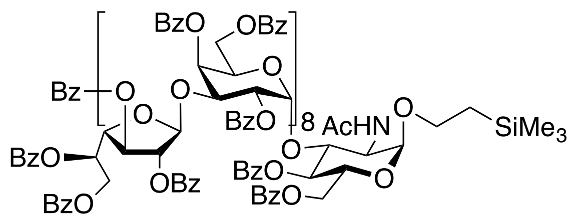
64.0, 63.7, 63.5, 62.7, 62.1, 62.0, 61.5 (C-2_{Glc}), 18.0 ($OCH_2\underline{C}H_2Si$), -1.6 ($Si(CH_3)_3$); LRMS–MALDI–TOF calcd for $[M+Na]^+$ $C_{430}H_{361}N_3NaO_{127}Si$: 7653.6. Found 7652.9.



2-(Trimethylsilyl)ethyl 2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2-azido-2-deoxy-4,6-di-*O*-benzoyl-α-D-glucopyranoside (3.7). Compounds **3.12**[151] (156 mg; 0.203 mmol) and **3.14** (99.0 mg; 13.0 μmol) were dissolved in CH_2Cl_2–toluene and were concentrated to dryness on high vacuum for 1 h. Dry CH_2Cl_2 (2.00 mL) and 4 Å M.S. were added to the flask, and the mixture was stirred for 30 min at rt. The suspension was cooled to 0 °C, and TBSOTf (2.0 μL; 8.7 μmol). The reaction mixture was stirred for 1 h while warming to rt, before being neutralized by the addition of Et_3N, filtered, and concentrated. The crude residue was purified by silica gel column chromatography (1:1→2:3 hexanes–EtOAc) to give **3.7** (100 mg, 94%) as a white, fluffy solid. R_f 0.26 (6:1

toluene–EtOAc); $[\alpha]_D$ +30 (*c* 0.2, $CHCl_3$); ^1H NMR (700 MHz, $CDCl_3$, δ_H) 8.09 – 8.04 (m, 6 H, ArH), 8.03 – 7.91 (m, 44 H, ArH), 7.88 – 7.85 (m, 4 H, ArH), 7.85 – 7.81 (m, 4 H, ArH), 7.79 – 7.66 (m, 30 H, ArH), 7.66 – 7.62 (m, 4 H, ArH), 7.56 – 7.52 (m, 2 H, ArH), 7.50 – 6.91 (m, 165 H, ArH), 6.07 (ddd, *J* = 6.8, 3.3, 3.3 Hz, 1 H), 5.99 – 5.96 (m, 1 H), 5.82 – 5.75 (m, 7 H), 5.68 – 5.56 (m, 10 H, H-$1_{Gal\textit{p}}$), 5.47 – 5.34 (m, 18 H, 7 x H-$1_{Gal\textit{p}}$, 2 x H-$1_{Gal\textit{f}}$), 5.32 (s, 1 H, H-$1_{Gal\textit{f}}$), 5.27 – 5.20 (m, 8 H), 5.18 (s, 1 H, H-$1_{Gal\textit{f}}$), 5.16 (s, 3 H, 3 x H-$1_{Gal\textit{f}}$), 5.13 (s, 1 H, H-$1_{Gal\textit{f}}$), 5.12 (d, *J* = 2.7 Hz, 1 H), 5.06 (d, *J* = 3.7 Hz, 1 H, H-1_{Glc}), 4.95 – 4.92 (m, 2 H), 4.86 (dd, *J* = 12.1, 7.3 Hz, 1 H), 4.79 (dd, *J* = 12.1, 3.4 Hz, 1 H), 4.67 – 4.53 (m, 16 H), 4.44 – 4.36 (m, 8 H), 4.35 – 4.17 (m, 18 H), 4.17 – 4.12 (m, 6 H), 4.09 – 4.02 (m, 8 H), 4.01 – 3.90 (m, 8 H), 3.90 – 3.84 (m, 4 H), 3.81 (dd, *J* = 11.0, 7.2 Hz, 1 H), 3.78 – 3.69 (m, 2 H), 3.57 – 3.53 (m, 1 H), 3.34 (dd, *J* = 10.2, 3.7 Hz, 1 H, H-2_{Glc}), 1.02 – 0.96 (m, 1 H, $OCH_2C\underline{H}_2Si$), 0.96 – 0.90 (m, 1 H, $OCH_2C\underline{H}_2Si$), -0.05 (s, 9 H, $Si(CH_3)_3$); ^{13}C NMR (126 MHz, $CDCl_3$, δ_C) 166.2 (C=O), 166.04 (C=O), 166.00 (C=O), 165.97 (C=O), 165.91 (C=O), 165.87 (C=O), 165.8 (C=O), 165.70 (C=O), 165.68 (C=O), 165.62 (C=O), 165.60 (C=O), 165.56 (C=O), 165.52 (C=O), 165.47 (C=O), 165.38 (C=O), 165.36 (C=O), 165.2 (C=O), 164.8 (C=O), 164.6 (C=O), 164.3 (C=O), 164.0 (C=O), 133.34 (Ar), 133.25 (Ar), 133.21 (Ar), 133.15 (Ar), 133.11 (Ar), 133.07 (Ar), 132.83 (Ar), 132.80 (Ar), 132.6 (Ar), 132.5 (Ar), 132.4 (Ar), 129.9 (Ar), 129.8 (Ar), 129.69 (Ar), 129.68 (Ar), 129.6 (Ar), 129.53 (Ar), 129.48 (Ar), 129.4 (Ar), 129.3 (Ar), 128.8 (Ar), 128.73 (Ar), 128.71 (Ar), 128.60 (Ar), 128.55 (Ar), 128.5 (Ar), 128.44 (Ar), 128.35 (Ar), 128.3 (Ar), 128.20 (Ar), 128.17 (Ar), 128.11 (Ar), 128.06 (Ar), 128.0 (Ar), 127.9 (Ar), 127.8 (Ar), 108.4 (C-$1_{Gal\textit{f}}$), 108.3 (3 x C-$1_{Gal\textit{f}}$), 108.2 (3 x C-$1_{Gal\textit{f}}$), 107.6 (C-$1_{Gal\textit{f}}$), 99.52 (2 x C-$1_{Gal\textit{p}}$), 99.46 (3 x C-$1_{Gal\textit{p}}$), 99.4 (2 x C-$1_{Gal\textit{p}}$), 98.1 (C-$1_{Gal\textit{p}}$), 96.8 (C-1_{Glc}), 85.3, 85.0, 84.9, 82.5, 82.3, 82.1, 81.83, 80.80, 80.78, 80.5, 80.4, 77.8, 74.8, 73.8, 73.74, 73.68, 73.65, 73.6, 72.5, 71.42, 71.35, 70.92, 70.88, 70.7, 70.2, 69.92,

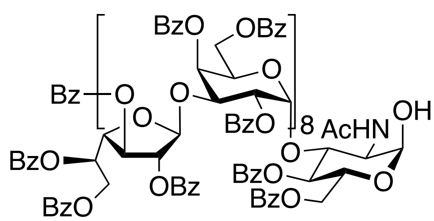
69.85, 69.7, 69.6, 69.5, 69.4, 68.1, 68.0, 67.7, 67.6, 66.2 (O$\underline{C}H_2CH_2$Si), 64.2, 64.0, 63.7, 63.5, 62.7, 62.1, 62.0, 61.9, 61.5 (C-2_{Glc}), 18.0 (OC$H_2\underline{C}H_2$Si), -1.6 (Si($CH_3)_3$); LRMS–MALDI–TOF calcd for $[M+Na]^+$ $C_{464}H_{387}N_3NaO_{136}Si$: 8232.2. Found 8230.5.



2-(Trimethylsilyl)ethyl 2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-4,6-di-*O*-benzoyl-α-D-glucopyranoside (3.51). To a solution of **3.7** (103 mg; 10.0 μmol) in THF–H_2O (5.00 mL; 3:2 v/v), was added a 1 M solution of Me_3P in THF (0.020 mL), followed by a 1 M solution of $NaOH_{(aq)}$ (0.010 mL). The pH was ~8, and the reaction mixture was heated to 50 °C and stirred for 3 h, before being diluted with brine (20.0 mL) and extracted twice with EtOAc (20.0 mL, 20.0 mL). The combined organic layers were dried with Na_2SO_4, filtered, and concentrated. The crude residue (120 mg) was dissolved in pyridine (2.00 mL), and cooled to 0 °C. Ac_2O (0.04 mL,

0.4 mmol) was added slowly dropwise, and the reaction mixture was stirred for 15 h while warming to rt, before being diluted with EtOAc (25.0 mL) and washed with H_2O. The organic layer was washed, in succession, with solutions of 1 N HCl three times, saturated sodium bicarbonate, and brine, before being dried with Na_2SO_4, filtered, and concentrated. The crude residue was purified by silica gel column chromatography (gradient of 6:1→4:1 toluene–EtOAc) to yield **3.51** (86.0 mg, 84% over 2 steps) as white solid. R_f 0.35 (4:1 toluene–EtOAc); $[\alpha]_D$ +23 (*c* 0.20, $CHCl_3$); ^{1}H NMR (700 MHz, $CDCl_3$, δ_H) 8.11 – 7.93 (m, 54 H, ArH), 7.89 – 7.83 (m, 4 H, ArH), 7.77 – 7.69 (m, 33 H, ArH), 7.68 – 7.63 (m, 4 H, ArH), 7.53 – 6.93 (m, 160 H, ArH), 6.11 – 6.08 (m, 1 H), 5.94 (d, *J* = 9.7 Hz, 1 H, NH), 5.89 – 5.87 (m, 1 H), 5.84 – 5.74 (m, 7 H), 5.70 – 5.60 (m, 8 H, H-1$_{Gal\textit{p}}$), 5.54 – 5.50 (m, 1 H), 5.49 – 5.42 (m, 9 H, 3 x H-1$_{Gal\textit{p}}$), 5.40 – 5.33 (m, 9 H, 4 x H-1$_{Gal\textit{p}}$, 2 x H-1$_{Gal\textit{f}}$), 5.28 – 5.16 (m, 13 H, 6 x H-1$_{Gal\textit{f}}$), 4.97 – 4.93 (m, 1 H), 4.90 – 4.83 (m, 2 H, H-1$_{Glc}$), 4.83 – 4.79 (m, 1 H), 4.76 – 4.72 (m, 1 H), 4.69 – 4.56 (m, 13 H), 4.55 – 4.51 (m, 3 H, H-2$_{Glc}$), 4.47 – 4.36 (m, 9 H), 4.35 – 3.85 (m, 38 H), 3.84 – 3.81 (m, 1 H), 3.78 – 3.74 (m, 1 H), 3.70 – 3.65 (m, 1 H), 3.51 – 3.46 (m, 1 H), 2.00 (s, 3 H, C(O)CH_3), 0.93 – 0.87 (m, 1 H, $OCH_2C\underline{H}_2Si$), 0.82 – 0.77 (m, 1 H, $OCH_2C\underline{H}_2Si$), -0.04 (s, 9 H, $Si(CH_3)_3$); ^{13}C NMR (126 MHz, $CDCl_3$, δ_C) 170.3 (C=O), 166.5 (C=O), 166.2 (C=O), 166.1 (C=O), 166.0 (C=O), 165.91 (C=O), 165.88 (C=O), 165.85 (C=O), 165.72 (C=O), 165.71 (C=O), 165.61 (C=O), 165.57 (C=O), 165.55 (C=O), 165.51 (C=O), 165.48 (C=O), 165.4 (C=O), 164.64 (C=O), 164.61 (C=O), 164.4 (C=O), 164.3 (C=O), 164.2 (C=O), 133.7 (Ar), 133.40 (Ar), 133.36 (Ar), 133.3 (Ar), 133.2 (Ar), 133.1 (Ar), 133.0 (Ar), 132.8 (Ar), 132.6 (Ar), 132.50 (Ar), 132.45 (Ar), 130.0 (Ar), 129.9 (Ar), 129.79 (Ar), 129.78 (Ar), 129.7 (Ar), 129.60 (Ar), 129.55 (Ar), 129.49 (Ar), 129.45 (Ar), 129.4 (Ar), 129.34 (Ar), 129.29 (Ar), 129.0 (Ar), 128.8 (Ar), 128.74 (Ar), 128.73 (Ar), 128.62 (Ar), 128.58 (Ar), 128.49 (Ar), 128.45 (Ar), 128.4 (Ar), 128.31 (Ar), 128.25 (Ar),

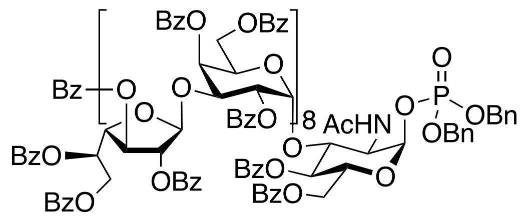
128.2 (Ar), 128.12 (Ar), 128.07 (Ar), 128.04 (Ar), 127.98 (Ar), 127.9 (Ar), 108.3 (7 x C-1_{Galf}), 108.1 (C-1_{Galf}), 99.49 (2 x C-1_{Galp}), 99.48 (2 x C-1_{Galp}), 99.43 (2 x C-1_{Galp}), 99.38 (C-1_{Galp}), 97.0 (C-1_{Galp}), 96.4 (C-1_{Glc}), 85.1, 84.9, 82.5, 82.3, 82.0, 81.9, 80.7, 80.4, 77.9, 73.9, 73.8, 73.7, 73.6, 72.9, 72.63, 72.59, 71.3, 70.94, 70.91, 70.7, 69.9, 69.7, 69.5, 69.3, 68.2, 67.6, 67.5, 67.2, 65.5 (O<u>C</u>H_2CH_2Si), 64.2, 64.0, 63.9, 63.6, 63.1, 62.00, 61.95, 52.1 (C-2_{Glc}), 23.3 (C(O)<u>C</u>H_3), 17.9 (OCH_2<u>C</u>H_2Si), -1.5 (Si$(CH_3)_3$); LRMS–MALDI–TOF calcd for $[M+Na]^+$ $C_{466}H_{391}NNaO_{137}Si$: 8248.2. Found 8249.1.



2,3,5,6-Ttetra-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-4,6-di-*O*-benzoyl-α-D-glucopyranose (3.52). To a solution of **3.51** (85 mg, 0.010 mmol) in CH_2Cl_2 (4.5 mL) and H_2O (1.5 mL) that was cooled to 0 °C, was added TFA (9.0 mL) slowly dropwise. The reaction mixture was stirred for

66 h at rt, before being concentrated, dissolved in CH_2Cl_2 (50 mL), and washed with a solution of saturated sodium bicarbonate until the organic layer reached neutral pH. The organic layer was dried with Na_2SO_4, filtered, and concentrated. The crude residue was purified by silica gel column chromatography (gradient of 2:1→3:2 toluene–EtOAc) to yield **3.52** (46 mg, 55%) as white solid. R_f 0.18 (2:1 toluene–EtOAc); $[\alpha]_D$ +34 (*c* 0.20, $CHCl_3$); ^{1}H NMR (700 MHz, $CDCl_3$, δ_H) 8.10 – 7.91 (m, 54 H, ArH), 7.86 – 7.81 (m, 4 H, ArH), 7.77 – 7.68 (m, 33 H, ArH), 7.66 – 7.62 (m, 4 H, ArH), 7.51 – 6.92 (m, 160 H, ArH), 6.16 (d, *J* = 9.4 Hz, 1 H, NH), 6.08 (ddd, *J* = 6.7, 3.2, 3.2 Hz, 1 H), 5.85 – 5.72 (m, 8 H), 5.68 – 5.59 (m, 8 H, H-1$_{Gal\textit{p}}$), 5.52 – 5.33 (m, 19 H, 3 x H-1$_{Gal\textit{f}}$, 7 x H-1$_{Gal\textit{p}}$), 5.27 – 5.14 (m, 14 H, H-1$_{Glc}$, 5 x H-1$_{Gal\textit{f}}$), 4.94 (dd, *J* = 5.4, 2.7 Hz, 1 H), 4.86 (dd, *J* = 12.1, 7.3 Hz, 1 H), 4.81 – 4.75 (m, 2 H), 4.70 – 3.71 (m, 65 H), 3.31 (br s, 1 H, OH), 1.94 (s, 3 H, C(O)CH_3); ^{13}C NMR (126 MHz, $CDCl_3$, δ_C) 170.5 (C=O), 166.6 (C=O), 166.2 (C=O), 166.1 (C=O), 166.0 (C=O), 165.90 (C=O), 165.87 (C=O), 165.8 (C=O), 165.71 (C=O), 165.66 (C=O), 165.59 (C=O), 165.56 (C=O), 165.49 (C=O), 165.47 (C=O), 165.4 (C=O), 164.6 (C=O), 164.5 (C=O), 164.3 (C=O), 164.2 (C=O), 133.4 (Ar), 133.3 (Ar), 133.2 (Ar), 133.12 (Ar), 133.07 (Ar), 133.0 (Ar), 132.8 (Ar), 132.6 (Ar), 132.5 (Ar), 130.0 (Ar), 129.9 (Ar), 129.79 (Ar), 129.77 (Ar), 129.68 (Ar), 129.66 (Ar), 129.6 (Ar), 129.53 (Ar), 129.48 (Ar), 129.44 (Ar), 129.38 (Ar), 129.3 (Ar), 129.0 (Ar), 128.8 (Ar), 128.73 (Ar), 128.71 (Ar), 128.67 (Ar), 128.62 (Ar), 128.56 (Ar), 128.5 (Ar), 128.44 (Ar), 128.41 (Ar), 128.35 (Ar), 128.31 (Ar), 128.25 (Ar), 128.20 (Ar), 128.18 (Ar), 128.11 (Ar), 128.06 (Ar), 128.03 (Ar), 127.97 (Ar), 127.8 (Ar), 108.4 (2 x C-1$_{Gal\textit{f}}$), 108.3 (2 x C-1$_{Gal\textit{f}}$), 108.2 (2 x C-1$_{Gal\textit{f}}$), 108.12 (C-1$_{Gal\textit{f}}$), 108.08 (C-1$_{Gal\textit{f}}$), 99.54 (C-1$_{Gal\textit{p}}$), 99.48 (2 x C-1$_{Gal\textit{p}}$), 99.47 (2 x C-1$_{Gal\textit{p}}$), 99.4 (C-1$_{Gal\textit{p}}$), 99.3 (C-1$_{Gal\textit{p}}$), 97.0 (C-1$_{Gal\textit{p}}$), 92.1 (C-1$_{Glc}$), 85.1, 85.0, 84.9, 82.5, 82.3, 82.0, 81.9, 80.6, 80.4, 77.9, 77.3, 73.7, 73.6, 72.9, 72.8, 72.6, 71.3, 70.92, 70.89, 70.7, 69.9, 69.73, 69.68, 69.5, 69.4, 68.3, 67.7, 67.6, 67.3,

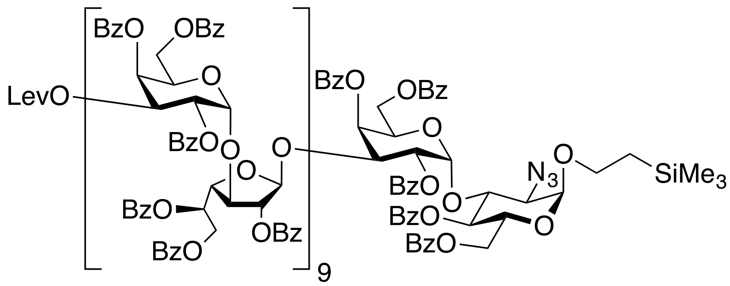
64.2, 64.0, 63.9, 63.5, 63.2, 62.0, 52.6 (C-2_{Glc}), 23.1 (C(O)$\underline{C}H_3$); LRMS–MALDI–TOF calcd for $[M+Na]^+$ $C_{466}H_{391}NNaO_{137}Si$: 8148.0. Found 8150.7.



2,3,5,6-Tetra-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-4,6-di-*O*-benzoyl-α-D-glucopyranose dibenzylphosphate (3.53). To a solution of **3.52** (36 mg, 4.0 μmol) and tetrazole (7.4 mg, 0.11 mmol) in dry CH_2Cl_2 (5.0 mL) that was cooled to 0 °C, was added *i*-$Pr_2NP(OBn)_2$ (0.02 mL, 0.06 mmol) dropwise. The reaction mixture was stirred at rt for 2 h, before being cooled to –78 °C, and *m*-CPBA (22 mg, 0.13 mmol) was added. The mixture was warmed to rt and stirred for an additional 2 h, before being diluted with CH_2Cl_2 (25 mL) and washed with a solution of saturated sodium bicarbonate and with brine. The organic layer was dried with Na_2SO_4, filtered, and concentrated. The crude residue was purified by silica gel column chromatography (gradient of

60%→100% EtOAc–hexanes) to yield **3.53** (31 mg, 83% over 2 steps) as a white solid. R_f 0.14 (2:3 hexanes–EtOAc); $[\alpha]_D$ +34 (*c* 0.10, $CHCl_3$); ^{1}H NMR (700 MHz, $CDCl_3$, δ_H) 8.10 – 8.06 (m, 4 H), 8.06 – 7.92 (m, 46 H), 7.85 – 7.80 (m, 4 H), 7.76 – 7.68 (m, 29 H), 7.67 – 7.63 (m, 4 H), 7.60 – 7.57 (m, 2 H), 7.51 – 6.92 (m, 176 H), 6.12 – 6.06 (m, 2 H), 5.88 – 5.86 (m, 1 H), 5.82 – 5.72 (m, 7 H), 5.69 (dd, *J* = 6.0, 3.5 Hz, 1 H, H-1_{Glc}), 5.68 – 5.59 (m, 7 H), 5.55 (d, *J* = 3.7 Hz, 1 H, H-$1_{Gal\textit{p}}$), 5.51 (dd, *J* = 10.6, 3.7 Hz, 1 H), 5.48 – 5.30 (m, 18 H, 7 x H-$1_{Gal\textit{p}}$, 2 x H-$1_{Gal\textit{f}}$), 5.27 – 5.20 (m, 7 H), 5.19 – 5.13 (m, 7 H, 6 x H-$1_{Gal\textit{f}}$), 5.07 – 4.98 (m, 5 H), 4.94 (dd, *J* = 5.5, 2.7 Hz, 1 H), 4.86 (dd, *J* = 12.1, 7.3 Hz, 1 H), 4.82 – 4.77 (m, 1 H), 4.68 – 4.55 (m, 14 H), 4.54 – 4.50 (m, 1 H), 4.48 – 4.11 (m, 28 H), 4.09 – 4.02 (m, 6 H), 4.01 – 3.85 (m, 14 H), 3.81 (dd, *J* = 10.8, 7.1 Hz, 1 H), 3.79 – 3.74 (m, 1 H), 1.73 (s, 3 H, C(O)CH_3); ^{13}C NMR (126 MHz, $CDCl_3$, δ_C) 170.7 (C=O), 166.5 (C=O), 166.1 (C=O), 166.0 (C=O), 165.91 (C=O), 165.89 (C=O), 165.87 (C=O), 165.8 (C=O), 165.71 (C=O), 165.70 (C=O), 165.60 (C=O), 165.56 (C=O), 165.53 (C=O), 165.49 (C=O), 165.47 (C=O), 165.38 (C=O), 165.37 (C=O), 164.6 (C=O), 164.4 (C=O), 164.34 (C=O), 164.31 (C=O), 164.1 (C=O), 135.3 (d, *J* = 6.3 Hz, Ar), 135.1 (d, *J* = 6.4 Hz, Ar), 133.7 (Ar), 133.5 (Ar), 133.4 (Ar), 133.3 (Ar), 133.21 (Ar), 133.15 (Ar), 133.12 (Ar), 133.08 (Ar), 133.0 (Ar), 132.88 (Ar), 132.86 (Ar), 132.84 (Ar), 132.82 (Ar), 132.75 (Ar), 132.6 (Ar), 132.5 (Ar), 132.4 (Ar), 130.0 (Ar), 129.9 (Ar), 129.76 (Ar), 129.75 (Ar), 129.7 (Ar), 129.6 (Ar), 129.54 (Ar), 129.48 (Ar), 129.4 (Ar), 129.28 (Ar), 129.26 (Ar), 129.2 (Ar), 128.91 (Ar), 128.87 (Ar), 128.78 (Ar), 128.75 (Ar), 128.7 (Ar), 128.6 (Ar), 128.44 (Ar), 128.35 (Ar), 128.30 (Ar), 128.25 (Ar), 128.2 (Ar), 128.14 (Ar), 128.13 (Ar), 128.06 (Ar), 128.03 (Ar), 127.97 (Ar), 127.8 (Ar), 108.3 (7 x C-$1_{Gal\textit{f}}$), 108.1 (C-$1_{Gal\textit{f}}$), 99.5 (5 x C-$1_{Gal\textit{p}}$), 99.42 (C-$1_{Gal\textit{p}}$), 99.37(C-$1_{Gal\textit{p}}$), 97.5(C-$1_{Gal\textit{p}}$), 96.5 (d, *J* = 6.1 Hz, C-1_{Glc}), 85.1, 84.9, 82.5, 82.3, 81.9, 81.8, 80.6, 80.4, 77.9, 73.73, 73.67, 73.5, 72.6, 71.8, 71.0, 70.92, 70.85, 70.69, 70.68, 70.09, 70.05, 70.0, 69.94, 69.86,

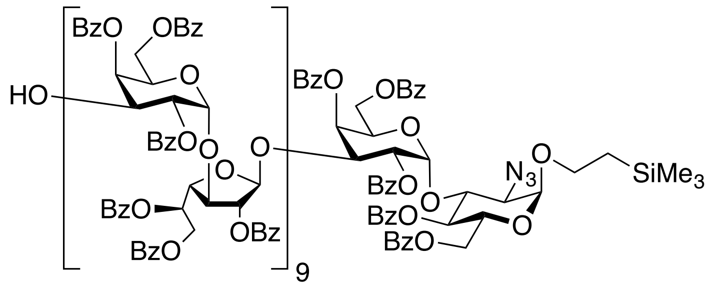
69.74, 69.67, 69.4, 69.3, 67.7, 67.6, 67.5, 67.2, 64.2, 64.0, 63.9, 63.82, 63.81, 62.8, 62.4, 62.0, 61.9, 52.1 (d, J = 8.6 Hz, C-2_{Glc}), 22.8 (C(O)CH_3); ^{31}P NMR (162 MHz, $CDCl_3$, δ_P) -2.23; LRMS–MALDI–TOF calcd for $[M+Na]^+$ $C_{475}H_{392}NNaO_{140}P$: 8408.2. Found 8411.9.



2-(Trimethylsilyl)ethyl 2,4,6-tri-*O*-benzoyl-3-*O*-levulinoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2-azido-2-deoxy-4,6-di-*O*-benzoyl-α-D-glucopyranoside (3.54). Compounds **3.14** (585 mg; 79.0 μmol) and **3.18** (239 mg; 0.110 mmol) were dissolved in CH_2Cl_2–toluene and were concentrated to dryness on high vacuum overnight. Dry CH_2Cl_2 (1.00 mL) and 4 Å M.S. were added to the flask, and the mixture was stirred for 30 min at rt. The

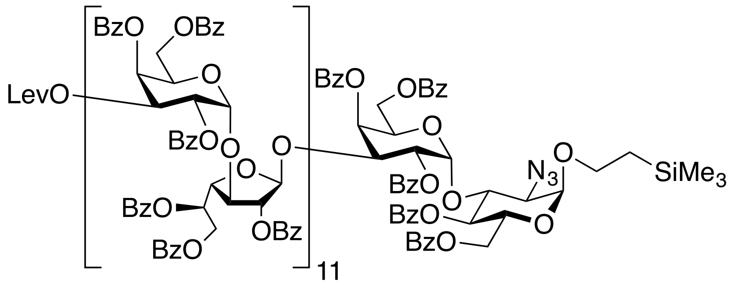
suspension was cooled to 0 °C, and TBSOTf (2 μL; 0.008 mmol) was added. The reaction mixture was stirred for 1 h at 0 °C, before being neutralized by the addition of Et_3N, filtered, and concentrated. The crude residue was purified by silica gel column chromatography (2:3 EtOAc–hexanes) to give **3.54** (640 mg, 82%) as a white, fluffy solid. R_f 0.23 (2:3 hexanes–EtOAc); $[\alpha]_D$ +39 (*c* 0.60, $CHCl_3$); ^{1}H NMR (700 MHz, $CDCl_3$, δ_H) 8.10 – 7.90 (m, 62 H, ArH), 7.88 – 7.85 (m, 2 H, ArH), 7.79 – 7.66 (m, 39 H, ArH), 7.65 – 7.62 (m, 2 H, ArH), 7.55 – 6.89 (m, 190 H, ArH), 5.99 – 5.96 (m, 1 H), 5.81 – 5.74 (m, 8 H), 5.68 – 5.56 (m, 10 H, H-$1_{Gal\textit{p}}$), 5.55 – 5.33 (m, 23 H, 9 x H-$1_{Gal\textit{p}}$, H-$1_{Gal\textit{f}}$), 5.32 (s, 1 H, H-$1_{Gal\textit{f}}$), 5.27 – 5.20 (m, 10 H, 2 x H-$1_{Gal\textit{f}}$), 5.16 (s, 4 H, 4 x H-$1_{Gal\textit{f}}$), 5.13 (s, 1 H, H-$1_{Gal\textit{f}}$), 5.12 (d, *J* = 2.6 Hz, 1 H), 5.06 (d, *J* = 3.7 Hz, 1 H, H-1_{Glc}), 4.96 – 4.93 (m, 1 H), 4.76 – 4.72 (m, 1 H), 4.68 – 4.53 (m, 19 H), 4.51 – 4.48 (m, 1 H), 4.45 – 4.11 (m, 29 H), 4.10 – 3.83 (m, 27 H), 3.78 – 3.69 (m, 2 H), 3.58 – 3.52 (m, 1 H), 3.34 (dd, *J* = 10.2, 3.7 Hz, 1H, H-2_{Glc}), 2.57 (dt, *J* = 18.2, 7.0 Hz, 1 H, $CH_2C\underline{H}_2C(O)CH_3$), 2.49 (dt, *J* = 18.3, 6.7 Hz, 1 H, $CH_2C\underline{H}_2C(O)CH_3$), 2.41 – 2.30 (m, 2 H, $C\underline{H}_2CH_2C(O)CH_3$), 1.93 (s, 3 H, $CH_2CH_2C(O)C\underline{H}_3$), 1.02 – 0.96 (m, 1 H, $OCH_2C\underline{H}_2Si$), 0.96 – 0.90 (m, 1 H, $OCH_2C\underline{H}_2Si$), -0.05 (s, 9 H, $Si(CH_3)_3$); ^{13}C NMR (126 MHz, $CDCl_3$, δ_C) 205.9, 171.0, 166.2, 166.04, 165.97, 165.9, 165.8, 165.71, 165.69, 165.62, 165.56, 165.52, 165.47, 165.4, 165.3, 165.2, 164.8, 164.39, 164.36, 164.3, 164.0, 133.6, 133.32, 133.25, 133.2, 132.0, 132.83, 132.81, 132.7, 132.54, 132.49, 132.4, 129.9, 129.84, 129.75, 129.68, 129.65, 129.6, 129.53, 129.48, 129.43, 129.37, 129.28, 129.25, 129.2, 128.8, 128.6, 128.5, 128.43, 128.35, 128.3, 128.21, 128.18, 128.10, 128.06, 128.0, 127.9, 127.8, 108.4, 108.3, 108.2, 107.6, 99.5, 99.1, 98.1, 96.8, 85.3, 85.0, 84.9, 84.8, 82.5, 82.2, 82.1, 80.8, 80.4, 80.3, 76.8, 74.8, 73.7, 73.6, 72.5, 71.42, 71.35, 70.9, 70.7, 70.2, 69.92, 69.89, 69.8, 69.7, 69.6, 69.4, 68.6, 68.2, 68.1, 68.0, 67.8, 67.6, 67.2, 66.2, 64.2, 64.0, 63.7, 63.5, 62.7,

62.0, 61.5, 37.8, 29.7, 27.9, 18.0, -1.6; LRMS–MALDI–TOF calcd for $[M+Na]^+$ $C_{543}H_{455}N_3NaO_{161}Si$: 9649.6. Found 9650.3.



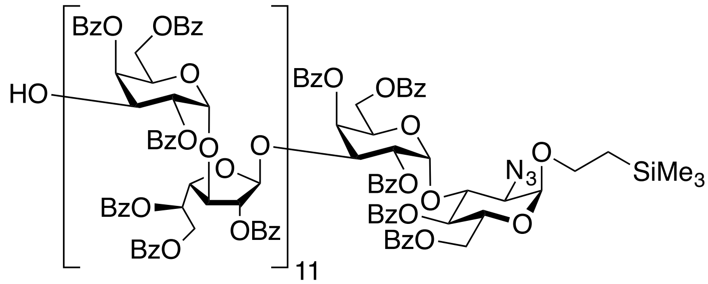
2-(Trimethylsilyl)ethyl 2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2-azido-2-deoxy-4,6-di-*O*-benzoyl-α-D-glucopyranoside (3.55). To a solution of **3.54** (637 mg; 66.0 µmol) in CH_2Cl_2–CH_3OH (18.0 mL, 2:1 v/v) was added $H_2NNH_2{\cdot}AcOH$ (12 mg, 0.13 mmol). The reaction mixture was stirred for 6 h at rt, before being diluted with CH_2Cl_2 (20.0 mL) and washed with H_2O. The organic layer was dried with Na_2SO_4, filtered, and concentrated. The crude residue was purified by silica gel column chromatography (2:3 hexanes–

EtOAc) to yield **3.55** (504 mg, 80%) as a fluffy, white solid. R_f 0.34 (2:3 hexanes–EtOAc); $[\alpha]_D$ +39 (*c* 0.40, $CHCl_3$); ^1H NMR (700 MHz, $CDCl_3$, δ_H) 8.09 – 8.04 (m, 6 H, ArH), 8.03 – 7.92 (m, 60 H, ArH), 7.89 – 7.86 (m, 2 H, ArH), 7.85 – 7.82 (m, 2 H, ArH), 7.79 – 7.75 (m, 8 H, ArH), 7.75 – 7.67 (m, 32 H, ArH), 7.65 – 7.63 (m, 2 H, ArH), 7.58 – 6.93 (m, 183 H, ArH), 5.99 – 5.97 (m, 1 H), 5.88 – 5.86 (m, 1 H), 5.81 – 5.72 (m, 8 H), 5.69 – 5.57 (m, 8 H, H-$1_{Gal\textit{p}}$), 5.56 – 5.54 (m, 1 H), 5.48 – 5.31 (m, 20 H, 3 x H-$1_{Gal\textit{f}}$, 9 x H-$1_{Gal\textit{p}}$), 5.30 – 5.21 (m, 10 H), 5.19 (s, 1 H, H-$1_{Gal\textit{f}}$), 5.17 (s, 4 H, 4 x H-$1_{Gal\textit{f}}$), 5.14 (s, 1 H, H-$1_{Gal\textit{f}}$), 5.13 (d, *J* = 2.4 Hz, 1 H), 5.07 (d, *J* = 3.7 Hz, 1 H, H-1_{Glc}), 4.97 – 4.93 (m, 1 H), 4.75 – 4.72 (m, 1 H), 4.71 – 4.51 (m, 20 H), 4.46 – 3.84 (m, 58 H), 3.79 – 3.66 (m, 3 H), 3.58 – 3.53 (m, 1 H), 3.35 (dd, *J* = 10.2, 3.7 Hz, 1 H, H-2_{Glc}), 1.83 – 1.79 (m, 1 H, OH), 1.02 – 0.97 (m, 1 H, $OCH_2C\underline{H_2}Si$), 0.96 – 0.91 (m, 1 H, $OCH_2C\underline{H_2}Si$), -0.05 (s, 9 H, $Si(CH_3)_3$); ^{13}C NMR (126 MHz, $CDCl_3$, δ_C) 166.5, 166.3, 166.2, 166.04, 165.97, 165.93, 165.85, 165.68, 165.65, 165.63, 165.56, 165.53, 165.47, 165.4, 165.2, 164.8, 164.3, 164.0, 133.6, 133.4, 133.3, 133.21, 133.18, 133.17, 133.0, 132.8, 132.6, 132.5, 132.4, 129.9, 129.79, 129.76, 129.68, 129.67, 129.6, 129.54, 129.48, 129.4, 129.32, 129.28, 128.8, 128.64, 128.56, 128.44, 128.36, 128.3, 128.2, 128.10, 128.06, 128.03, 127.98, 127.9, 127.8, 108.44, 108.36, 108.3, 108.22, 108.17, 107.6, 99.5, 99.4, 99.3, 98.1, 96.8, 85.5, 85.3, 85.0, 84.93, 84.88, 82.5, 82.3, 82.1, 81.5, 80.5, 80.38, 80.37, 74.8, 73.9, 73.8, 73.71, 73.66, 73.6, 72.5, 71.41, 71.36, 71.1, 71.0, 70.9, 70.2, 70.1, 69.9, 69.7, 69.6, 69.4, 68.1, 68.0, 67.63, 67.56, 67.4, 67.0, 66.24, 64.17, 64.0, 63.7, 63.6, 62.7, 62.02, 61.99, 61.5, 18.0, -1.6; LRMS–MALDI–TOF calcd for $[M+Na]^+$ $C_{538}H_{449}N_3NaO_{159}Si$: 9551.5. Found 9554.1.



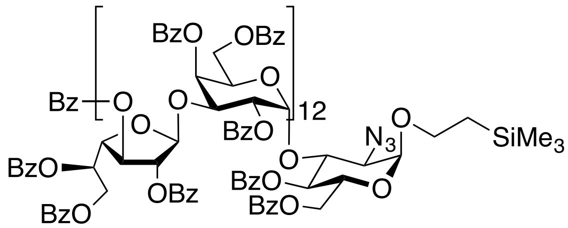
2-(Trimethylsilyl)ethyl 2,4,6-tri-*O*-benzoyl-3-*O*-levulinoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2-azido-2-deoxy-4,6-di-*O*-benzoyl-α-D-glucopyranoside (3.56). Compounds **3.55** (500 mg; 0.05 mmol) and **3.18** (162 mg; 74.0 μmol) were dissolved in CH_2Cl_2–toluene and were concentrated to dryness on high vacuum overnight. Dry CH_2Cl_2 (1.0 mL) and 4 Å M.S. were added to the flask, and the mixture was stirred for 30 min at rt. The suspension was cooled to 0 °C, and TBSOTf (2 μL; 9 μmol) was added. The

reaction mixture was stirred for 1 h at 0 °C, before being neutralized by the addition of Et_3N, filtered, and concentrated. The crude residue was purified by silica gel column chromatography (2:3 hexanes–EtOAc) to give **3.56** (427 mg, 71%) as a white, fluffy solid. R_f 0.13 (2:3 hexanes–EtOAc); $[\alpha]_D$ +41 (*c* 0.70, $CHCl_3$); ^{1}H NMR (700 MHz, $CDCl_3$, δ_H) 8.12 – 8.08 (m, 2 H, ArH), 8.08 – 8.05 (m, 2 H, ArH), 8.05 – 7.90 (m, 65 H, ArH), 7.89 – 7.85 (m, 2 H, ArH), 7.80 – 7.68 (m, 44 H, ArH), 7.66 – 7.63 (m, 2 H, ArH), 7.57 – 6.90 (m, 238 H, ArH), 6.00 – 5.97 (m, 1 H), 5.82 – 5.75 (m, 9 H), 5.72 – 5.58 (m, 12 H), 5.56 – 5.31 (m, 28 H), 5.29 – 5.21 (m, 12 H), 5.17 (s, 6 H), 5.15 (s, 1 H), 5.14 – 5.12 (m, 1 H), 5.07 (d, *J* = 3.7 Hz, 1 H, H-1_{Glc}), 4.97 – 4.94 (m, 1 H), 4.77 – 4.74 (m, 1 H), 4.69 – 4.54 (m, 22 H), 4.53 – 4.49 (m, 1 H), 4.46 – 3.84 (m, 70 H), 3.79 – 3.69 (m, 2 H), 3.59 – 3.52 (m, 1 H), 3.35 (dd, *J* = 10.2, 3.7 Hz, 1 H, H-2_{Glc}), 2.58 (dt, *J* = 18.2, 7.0 Hz, 1 H, $CH_2C\underline{H}_2C(O)CH_3$), 2.49 (dt, *J* = 18.2, 6.7 Hz, 1 H, $CH_2C\underline{H}_2C(O)CH_3$), 2.43 – 2.30 (m, 2 H, $C\underline{H}_2CH_2C(O)CH_3$), 1.94 (s, 3 H, $CH_2CH_2C(O)C\underline{H}_3$), 1.03 – 0.97 (m, 1 H, $OCH_2C\underline{H}_2Si$), 0.97 – 0.90 (m, 1 H, $OCH_2C\underline{H}_2Si$), -0.04 (s, 9 H, $Si(CH_3)_3$); ^{13}C NMR (126 MHz, $CDCl_3$, δ_C) 205.9, 171.1, 166.2, 166.1, 166.0, 165.9, 165.72, 165.70, 165.63, 165.57, 165.53, 165.48, 165.4, 165.3, 165.2, 164.8, 164.4, 164.0, 135.63, 133.61, 133.59, 133.33, 133.26, 133.2, 133.1, 133.0, 132.8, 132.71, 132.66, 132.5, 132.44, 132.39, 129.93, 129.85, 129.8, 129.7, 129.6, 129.54, 129.49, 129.4, 129.28, 129.26, 129.2, 128.8, 128.6, 128.5, 128.44, 128.36, 128.3, 128.2, 128.11, 128.06, 128.0, 127.9, 127.8, 108.3, 99.5, 99.1, 98.1, 96.8, 85.3, 85.0, 84.94, 84.88, 82.5, 82.2, 82.1, 80.8, 80.6, 80.4, 74.8, 73.8, 73.7, 73.6, 72.5, 71.41, 71.37, 70.9, 70.7, 70.2, 70.0, 69.9, 69.8, 69.7, 69.4, 68.6, 68.2, 68.1, 68.0, 67.8, 67.6, 67.5, 67.2, 66.3, 64.3, 64.2, 64.0, 63.7, 63.60, 63.56, 62.7, 62.0, 61.5, 37.8, 29.7, 28.0, 18.0, -1.6; LRMS–MALDI–TOF calcd for $[M+Na]^+$ $C_{651}H_{543}N_3NaO_{193}Si$: 11547.4. Found 11552.6.



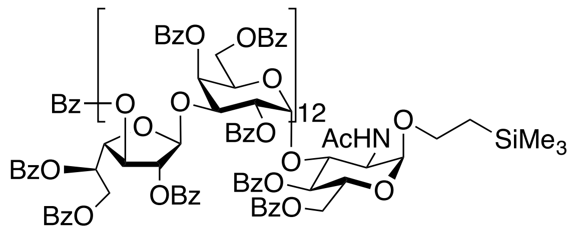
2-(Trimethylsilyl)ethyl 2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2-azido-2-deoxy-4,6-di-*O*-benzoyl-α-D-glucopyranoside (3.15). To a solution of **3.56** (427 mg; 37.0 μmol) in CH_2Cl_2–CH_3OH (10.0 mL, 2:1 v/v) was added H_2NNH_2·AcOH (7.5 mg, 0.081 mmol). The reaction mixture was stirred for 6 h at rt, before being diluted with CH_2Cl_2 (40.0 mL) and washed with H_2O. The organic layer was dried with Na_2SO_4, filtered, and concentrated. The crude residue was purified by silica

gel column chromatography (2:3 hexanes–EtOAc) to yield **3.15** (367 mg, 88%) as a fluffy, white solid. R_f 0.38 (4:1 toluene–EtOAc); $[\alpha]_D$ +35 (*c* 0.40, $CHCl_3$); ^1H NMR (700 MHz, $CDCl_3$, δ_H) 8.10 – 8.05 (m, 6 H, ArH), 8.05 – 7.91 (m, 61 H, ArH), 7.89 – 7.82 (m, 5 H, ArH), 7.80 – 7.75 (m, 7 H, ArH), 7.75 – 7.68 (m, 34 H, ArH), 7.66 – 7.63 (m, 2 H, ArH), 7.59 – 7.53 (m, 3 H, ArH), 7.53 – 6.94 (m, 237 H, ArH), 6.00 – 5.97 (m, 1 H), 5.89 – 5.86 (m, 1 H), 5.82 – 5.72 (m, 9 H), 5.71 – 5.54 (m, 12 H), 5.48 – 5.32 (m, 24 H), 5.30 – 5.22 (m, 12 H), 5.21 – 5.12 (m, 10 H), 5.07 (d, *J* = 3.7 Hz, 1 H, H-1_{Glc}), 4.97 – 4.94 (m, 1 H), 4.76 – 4.72 (m, 1 H), 4.72 – 4.52 (m, 23 H), 4.47 – 3.85 (m, 68 H), 3.79 – 3.66 (m, 3 H), 3.59 – 3.53 (m, 1 H), 3.35 (dd, *J* = 10.2, 3.6 Hz, 1 H, H-2_{Glc}), 1.82 (s, 1 H, OH), 1.03 – 0.97 (m, 1 H, $OCH_2C\underline{H}_2Si$), 0.97 – 0.91 (m, 1 H, $OCH_2C\underline{H}_2Si$), -0.04 (s, 9 H, $Si(CH_3)_3$); ^{13}C NMR (126 MHz, $CDCl_3$, δ_C) 166.5, 166.3, 166.2, 166.1, 166.0, 165.93, 165.85, 165.8, 165.69, 165.66, 165.63, 165.56, 165.53, 165.47, 165.4, 165.3, 165.2, 164.8, 164.4, 164.0, 133.63, 133.60, 133.4, 133.3, 133.19, 133.18, 133.1, 133.0, 132.9, 132.8, 132.6, 132.5, 132.44, 132.42, 129.9, 129.79, 129.76, 129.7, 129.6, 129.54, 129.49, 129.4, 129.32, 129.29, 128.8, 128.63, 128.56, 128.5, 128.41, 128.36, 128.3, 128.2, 128.11, 128.07, 128.03, 127.99, 127.9, 127.8, 108.42, 108.37, 108.34, 108.28, 108.2, 108.1, 107.6, 99.53, 99.47, 99.4, 99.3, 98.1, 96.8, 85.5, 85.3, 85.0, 84.94, 84.88, 82.5, 82.3, 82.1, 81.5, 80.8, 80.6, 80.4, 74.8, 73.8, 73.73, 73.69, 73.67, 73.6, 72.5, 71.4, 71.2, 71.0, 70.9, 70.2, 70.1, 69.7, 69.6, 69.4, 68.1, 68.0, 67.64, 67.57, 67.4, 67.0, 66.24, 64.18, 64.0, 62.7, 62.0, 61.5, 18.0, -1.6; LRMS–MALDI–TOF calcd for $[M+Na]^+$ $C_{646}H_{537}N_3NaO_{191}Si$: 11449.3. Found 11452.2.



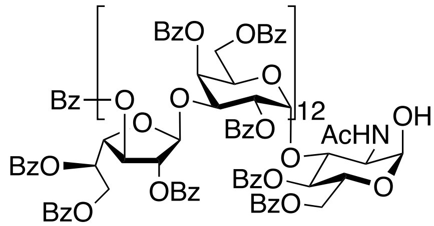
2-(Trimethylsilyl)ethyl 2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2-azido-2-deoxy-4,6-di-*O*-benzoyl-α-D-glucopyranoside (3.8). Compounds **3.12**[151] (72 mg; 0.094 mmol) and **3.15** (104 mg; 9.00 μmol) were dissolved in CH_2Cl_2–toluene and were concentrated to dryness on high vacuum overnight. Dry CH_2Cl_2 (1.4 mL) and 4 Å M.S. were added to the flask, and the mixture was stirred for 30 min at rt. The suspension was cooled to 0 °C, and TBSOTf (0.5 μL; 2 μmol) was added. The reaction mixture was stirred for 3.5 h while warming

to rt, before being neutralized by the addition of Et_3N, filtered, and concentrated. The crude residue was purified by silica gel column chromatography (2:3→1:2 hexanes–EtOAc) to give **3.8** (102 mg, 93%) as a white, fluffy solid. R_f 0.31 (4:1 toluene–EtOAc); $[\alpha]_D$ +45 (*c* 0.20, $CHCl_3$); ^1H NMR (700 MHz, $CDCl_3$, δ_H) 8.10 – 8.05 (m, 6 H, ArH), 8.04 – 7.92 (m, 60 H, ArH), 7.89 – 7.82 (m, 6 H, ArH), 7.80 – 7.63 (m, 45 H, ArH), 7.56 – 7.53 (m, 2 H, ArH), 7.51 – 6.93 (m, 256 H, ArH), 6.08 (ddd, *J* = 6.4, 3.2, 3.2 Hz, 1 H), 5.99 – 5.97 (m, 1 H), 5.83 – 5.75 (m, 10 H), 5.69 – 5.57 (m, 13 H), 5.48 – 5.31 (m, 25 H), 5.29 – 5.21 (m, 13 H), 5.20 – 5.12 (m, 10 H), 5.07 (d, *J* = 3.6 Hz, 1 H, H-1_{Glc}), 4.97 – 4.92 (m, 2 H), 4.87 (dd, *J* = 12.1, 7.2 Hz, 1 H), 4.82 – 4.77 (m, 1 H), 4.69 – 4.54 (m, 24 H), 4.46 – 3.69 (m, 73 H), 3.56 (ddd, *J* = 11.7, 11.7, 5.3 Hz, 1 H), 3.35 (dd, *J* = 10.2, 3.6 Hz, 1 H, H-2_{Glc}), 1.03 – 0.97 (m, 1 H, $OCH_2C\underline{H}_2Si$), 0.96 – 0.91 (m, 1 H, $OCH_2C\underline{H}_2Si$), -0.05 (s, 9 H, $Si(CH_3)_3$); ^{13}C NMR (126 MHz, $CDCl_3$, δ_C) 166.2, 166.04, 166.01, 165.97, 165.91, 165.85, 165.71, 165.69, 165.62, 165.61, 165.56, 165.53, 165.47, 165.4, 165.2, 164.8, 164.6, 164.3, 164.0, 133.34, 133.32, 133.26, 133.2, 133.13, 133.12, 133.08, 133.0, 132.8, 132.6, 132.5, 129.9, 129.8, 129.7, 129.6, 129.53, 129.48, 129.45, 129.4, 129.3, 128.8, 128.73, 128.72, 128.6, 128.5, 128.44, 128.36, 128.31, 128.26, 128.2, 128.11, 128.06, 128.0, 127.9, 127.8, 108.3, 107.5, 99.5, 99.4, 98.1, 96.8, 85.3, 84.9, 82.5, 82.4, 82.3, 82.1, 81.8, 80.8, 80.5, 80.4, 77.9, 74.8, 73.7, 73.6, 72.5, 71.41, 71.36, 70.9, 70.73, 70.70, 70.68, 70.2, 69.9, 69.8, 69.7, 69.6, 69.43, 69.35, 68.1, 68.0, 67.7, 67.6, 66.2, 64.2, 64.0, 63.7, 63.6, 62.7, 62.0, 61.9, 61.5, 18.0, -1.6; LRMS–MALDI–TOF calcd for $[M+Na]^+$ $C_{680}H_{563}N_3NaO_{200}Si$: 12027.9. Found 12030.7.



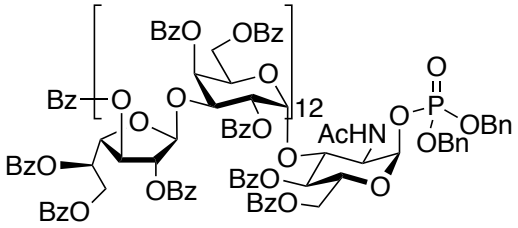
2-(Trimethylsilyl)ethyl 2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-4,6-di-*O*-benzoyl-α-D-glucopyranoside (3.57). To a solution of **3.8** (99 mg; 8.0 μmol) in THF–H_2O (10 mL; 4:1 v/v), was added 1 M Me_3P in THF (15 μL), followed by 1 M $NaOH_{(aq)}$ (5.0 μL). The pH was ~8, and the reaction mixture was heated to 50 °C and stirred for 4 h, before being diluted with brine (25.0 mL) and extracted twice with EtOAc (30.0 mL, 30.0 mL). The combined organic layers were dried with Na_2SO_4, filtered, and concentrated. The

crude residue (104 mg) was dissolved in pyridine (2.0 mL), and cooled to 0 °C. Ac_2O (0.05 mL, 0.5 mmol) was added slowly dropwise, and the reaction mixture was stirred for 19 h while warming to rt, before being diluted with EtOAc (30.0 mL) and washed with H_2O. The organic layer was washed, in succession, with solutions of 1 N HCl three times, saturated sodium bicarbonate, and brine, before being dried with Na_2SO_4, filtered, and concentrated. The crude residue was purified by silica gel column chromatography (4:1 toluene–EtOAc) to yield **3.57** (81.0 mg, 82% over 2 steps) as white solid. R_f 0.31 (4:1 toluene–EtOAc); $[\alpha]_D$ +33 (*c* 0.20, $CHCl_3$); ^{1}H NMR (700 MHz, $CDCl_3$, δ_H) 8.10 – 7.93 (m, 90 H, ArH), 7.88 – 7.82 (m, 7 H, ArH), 7.76 – 7.68 (m, 57 H, ArH), 7.67 – 7.62 (m, 5 H, ArH), 7.52 – 6.92 (m, 216 H, ArH), 6.09 (ddd, *J* = 6.7, 3.3, 3.3 Hz, 1 H), 5.93 (d, *J* = 9.7 Hz, 1 H, NH), 5.88 – 5.85 (m, 1 H), 5.83 – 5.73 (m, 11 H), 5.70 – 5.59 (m, 12 H), 5.53 – 5.31 (m, 26 H), 5.29 – 5.14 (m, 22 H), 4.96 – 4.92 (m, 1 H), 4.89 – 4.83 (m, 2 H, H-1_{Glc}), 4.82 – 4.78 (m, 1 H), 4.75 – 4.70 (m, 1 H), 4.69 – 4.49 (m, 24 H), 4.47 – 3.72 (m, 73 H), 3.69 – 3.63 (m, 1 H), 3.51 – 3.44 (m, 1 H), 1.99 (s, 3 H, $C(O)CH_3$), 0.93 – 0.87 (m, 1 H, $OCH_2C\underline{H}_2Si$), 0.82 – 0.77 (m, 1 H, $OCH_2C\underline{H}_2Si$), -0.05 (s, 9 H, $Si(CH_3)_3$); ^{13}C NMR (126 MHz, $CDCl_3$, δ_C) 170.2, 166.5, 166.2, 166.04, 166.01, 165.9, 165.71, 165.65, 165.60, 165.56, 165.50, 165.47, 165.4, 164.63, 164.60, 164.3, 164.2, 133.4, 133.34, 133.26, 133.14, 133.11, 133.08, 133.0, 132.8, 132.6, 132.5, 130.0, 129.9, 129.78, 129.77, 129.7, 129.6, 129.54, 129.49, 129.45, 129.4, 129.33, 129.28, 129.0, 128.8, 128.74, 128.72, 128.62, 128.56, 128.5, 128.4, 128.3, 128.24, 128.18, 128.11, 128.06, 128.0, 127.85, 127.84, 108.3, 99.5, 97.0, 96.4, 84.9, 82.5, 82.3, 81.9, 81.8, 80.6, 80.4, 77.9, 73.9, 73.7, 72.9, 72.6, 71.3, 70.9, 70.7, 69.9, 69.74, 69.67, 69.5, 69.4, 69.3, 68.2, 67.6, 67.2, 65.5, 65.4, 64.2, 64.0, 63.5, 63.1, 62.0, 61.8, 52.1, 23.3, 17.9, -1.5; LRMS–MALDI–TOF calcd for $[M+Na]^+$ $C_{682}H_{567}NNaO_{201}Si$: 12043.9. Found 12044.3.



2,3,5,6-Tetra-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-4,6-di-*O*-benzoyl-α-D-glucopyranose (3.58). To a solution of **3.57** (78 mg, 10 μmol) in CH_2Cl_2 (3.0 mL) and H_2O (1.0 mL) that was cooled to 0 °C, was added TFA (6.0 mL) slowly dropwise. The reaction mixture was stirred for 113 h at rt, before being concentrated, dissolved in CH_2Cl_2 (25 mL), and washed with a solution of saturated sodium bicarbonate until the organic layer reached neutral pH. The organic layer was dried with Na_2SO_4, filtered, and concentrated.

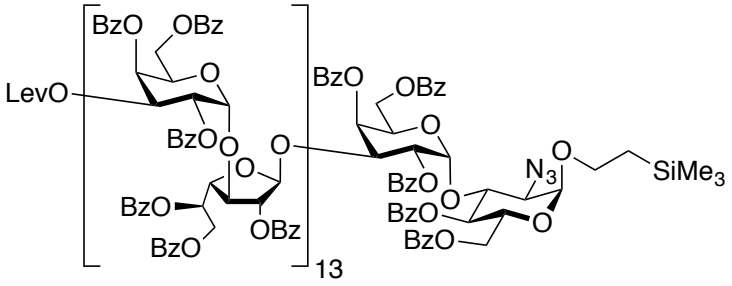
The crude residue was purified by silica gel column chromatography (7:13→3:7 hexanes–EtOAc) to yield **3.58** (31 mg, 41%) as white solid. R_f 0.11 (7:13 hexanes–EtOAc); $[\alpha]_D$ +44 (*c* 0.10, $CHCl_3$); ^{1}H NMR (700 MHz, $CDCl_3$, δ_H) 8.09 – 8.03 (m, 10 H, ArH), 8.03 – 7.91 (m, 71 H, ArH), 7.85 – 7.81 (m, 6 H, ArH), 7.77 – 7.68 (m, 51 H, ArH), 7.66 – 7.62 (m, 5 H, ArH), 7.50 – 6.92 (m, 232 H, ArH), 6.13 (d, *J* = 9.4 Hz, 1 H, NH), 6.09 – 6.04 (m, 1 H), 5.85 – 5.82 (m, 1 H), 5.82 – 5.70 (m, 10 H), 5.69 – 5.58 (m, 12 H), 5.52 – 5.47 (m, 1 H), 5.47 – 5.32 (m, 24 H), 5.30 – 5.11 (m, 22 H), 4.94 (dd, *J* = 5.5, 2.7 Hz, 1 H), 4.86 (dd, *J* = 12.2, 7.4 Hz, 1 H), 4.81 – 4.75 (m, 2 H), 4.75 – 4.71 (m, 1 H), 4.70 – 4.47 (m, 22 H), 4.46 – 4.33 (m, 15 H), 4.31 – 3.71 (m, 62 H), 3.01 (br s, 1 H, OH), 1.94 (s, 3 H, C(O)CH_3); ^{13}C NMR (126 MHz, $CDCl_3$, δ_C) 170.4, 166.6, 166.2, 166.1, 166.0, 165.8, 165.70, 165.65, 165.6, 165.5, 165.4, 164.6, 164.5, 164.3, 164.2, 133.5, 133.4, 133.3, 133.0, 132.9, 132.8, 132.6, 132.5, 130.0, 129.9, 129.83, 129.78, 129.76, 129.7, 129.6, 129.53, 129.48, 129.44, 129.37, 129.3, 129.2, 129.0, 128.8, 128.73, 128.71, 128.67, 128.62, 128.55, 128.5, 128.44, 128.35, 128.30, 128.25, 128.2, 128.10, 128.06, 128.03, 127.97, 127.8, 108.3, 108.1, 99.5, 96.9, 92.1, 84.9, 82.5, 82.3, 82.0, 81.8, 80.6, 80.4, 77.9, 73.7, 72.8, 72.7, 72.6, 71.3, 70.9, 70.7, 69.9, 69.7, 69.4, 69.3, 68.3, 67.6, 67.3, 64.2, 64.0, 63.8, 63.7, 63.6, 63.2, 62.0, 52.6, 23.3; LRMS–MALDI–TOF calcd for $[M+Na]^+$ $C_{677}H_{555}NNaO_{201}$: 11943.7. Found 11952.7.



2,3,5,6-Tetra-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-

benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-4,6-di-*O*-benzoyl-α-D-glucopyranose dibenzylphosphate (3.59). To a solution of **3.58** (16 mg, 1.3 μmol) and tetrazole (1.6 mg, 23 μmol) in dry CH_2Cl_2 (2.0 mL) that was cooled to 0 °C, was added *i*-$Pr_2NP(OBn)_2$ (12 μL, 36 μmol) dropwise. The reaction mixture was stirred at rt for 2 h, before being cooled to –78 °C, and *m*-CPBA (6.2 mg, 36 μmol) was added. The mixture was warmed to rt and stirred for an additional 2 h, before being diluted with CH_2Cl_2 (3.0 mL) and washed with a solution of saturated sodium bicarbonate and with brine. The organic layer was dried with Na_2SO_4, filtered, and concentrated. The crude residue was purified by silica gel column chromatography (gradient of 60%→100% EtOAc–hexanes) to yield **3.59** (14 mg, 86% over 2 steps) as a beige, off-white solid. R_f 0.31 (3:7 hexanes–EtOAc); $[\alpha]_D$ +28 (*c* 0.80, $CHCl_3$); ^1H NMR (700 MHz, $CDCl_3$, δ_H) 8.13 – 7.89 (m, 73 H, ArH), 7.87 – 7.79 (m, 4 H, ArH), 7.77 –

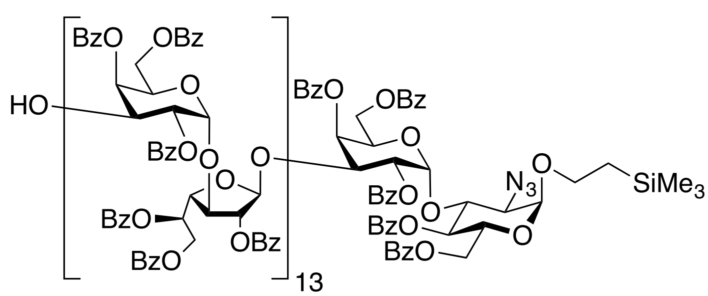
7.67 (m, 44 H, ArH), 7.67 – 7.63 (m, 4 H, ArH), 7.60 – 7.56 (m, 2 H, ArH), 7.51 – 6.86 (m, 258 H, ArH), 6.07 (ddd, *J* = 6.9, 3.3, 3.3 Hz, 1 H), 6.00 (d, *J* = 9.4 Hz, 1 H, NH), 5.89 – 5.83 (m, 1 H), 5.83 – 5.71 (m, 9 H), 5.71 – 5.57 (m, 12 H), 5.54 (d, *J* = 3.7 Hz, 1 H), 5.52 – 5.33 (m, 25 H), 5.33 – 5.29 (m, 2 H), 5.27 – 5.21 (m, 10 H), 5.19 – 5.11 (m, 10 H), 5.07 – 4.95 (m, 4 H), 4.94 (dd, *J* = 5.5, 2.7 Hz, 1 H), 4.91 – 4.83 (m, 1 H), 4.82 – 4.76 (m, 1 H), 4.74 – 3.71 (m, 101 H), 1.72 (s, 3 H, C(O)CH_3); ^{13}C NMR (126 MHz, $CDCl_3$, δ_C) 170.6, 166.5, 166.1, 166.0, 165.8, 165.69, 165.68, 165.54, 165.48, 165.45, 165.4, 164.6, 164.4, 164.3, 164.1, 135.3 (d, *J* = 6.4 Hz), 135.1 (d, *J* = 6.6 Hz), 133.5, 133.3, 133.2, 133.0, 132.9, 132.8, 132.5, 130.0, 129.9, 129.74, 129.68, 129.6, 129.53, 129.48, 129.4, 129.3, 129.2, 128.90, 128.86, 128.8, 128.7, 128.6, 128.44, 128.35, 128.30, 128.25, 128.2, 128.14, 128.12, 128.05, 128.02, 127.97, 127.83, 127.80, 127.6, 108.3, 108.1, 99.5, 99.4, 97.5, 96.5 (d, *J* = 8.0 Hz), 84.9, 81.9, 81.8, 80.6, 80.4, 77.8, 73.7, 73.6, 72.6, 71.8, 71.0, 70.9, 70.7, 70.1, 70.0, 69.91, 69.86, 69.74, 69.66, 69.5, 69.4, 69.2, 67.6, 64.2, 62.8, 62.4, 62.0, 52.1 (d, *J* = 10.1 Hz), 22.8; ^{31}P NMR (162 MHz, $CDCl_3$, δ_P) -2.23; LRMS–MALDI–TOF calcd for $[M+Na]^+$ $C_{691}H_{566}NNaO_{204}P$: 12203.9. Found 12212.2.



2-(Trimethylsilyl)ethyl 2,4,6-tri-*O*-benzoyl-3-*O*-levulinoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-

2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2-azido-2-deoxy-4,6-di-*O*-benzoyl-α-D-glucopyranoside (3.60). Compounds **3.15** (261 mg; 23.0 μmol) and **3.18** (101 mg; 46.0 μmol) were dissolved in CH_2Cl_2–toluene and were concentrated to dryness on high vacuum overnight. Dry CH_2Cl_2 (1.2 mL) and 4 Å M.S. were added to the flask, and the mixture was stirred for 30 min at rt. The suspension was cooled to 0 °C, and TBSOTf (2 μL; 9 μmol) was added. The reaction mixture was stirred for 1 h at 0 °C, before being neutralized by the addition of Et_3N, filtered, and concentrated. The crude residue was purified by silica gel column chromatography (2:3→7:13 hexanes–EtOAc) to give **3.60** (225 mg, 73%) as a white, fluffy solid. R_f 0.18 (4:1 toluene–EtOAc); $[\alpha]_D$ +35 (*c* 0.30, $CHCl_3$); ^1H NMR (700 MHz, $CDCl_3$, δ_H) 8.11 – 7.90 (m, 79 H, ArH), 7.88 – 7.85 (m, 3 H, ArH), 7.79 – 7.67 (m, 51 H, ArH), 7.65 – 7.62 (m, 2 H, ArH), 7.55 – 6.90 (m, 280 H, ArH), 5.99 – 5.96 (m, 1 H), 5.81 – 5.75 (m, 11 H), 5.68 – 5.57 (m, 14 H), 5.55 – 5.31 (m, 32 H), 5.28 – 5.20 (m, 14 H), 5.18 – 5.11 (m, 11 H), 5.06

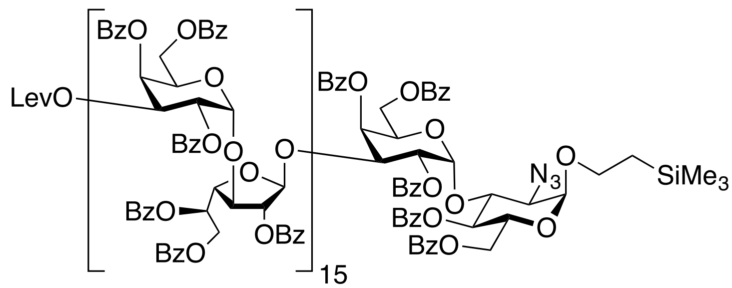
(d, J = 3.7 Hz, 1 H, H-1_{Glc}), 4.95 (dd, J = 8.7, 4.7 Hz, 1 H), 4.96 – 4.93 (m, 1 H), 4.68 – 4.53 (m, 26 H), 4.52 – 4.49 (m, 1 H), 4.45 – 3.83 (m, 81 H), 3.78 – 3.69 (m, 2 H), 3.56 (ddd, J = 11.9, 5.2 Hz, 1 H), 3.35 (dd, J = 10.1, 3.7 Hz, 1 H, H-2_{Glc}), 2.57 (dt, J = 18.2, 7.0 Hz, 1 H, $CH_2C\underline{H}_2C(O)CH_3$), 2.49 (dt, J = 18.2, 6.7 Hz, 1 H, $CH_2C\underline{H}_2C(O)CH_3$), 2.42 – 2.30 (m, 2 H, $C\underline{H}_2CH_2C(O)CH_3$), 1.93 (s, 3 H, $CH_2CH_2C(O)C\underline{H}_3$), 1.02 – 0.97 (m, 1 H, $OCH_2C\underline{H}_2Si$), 0.96 – 0.91 (m, 1 H, $OCH_2C\underline{H}_2Si$), -0.05 (s, 9 H, $Si(CH_3)_3$); ^{13}C NMR (126 MHz, $CDCl_3$, δ_C) 205.9, 171.0, 166.2, 166.03, 165.96, 165.84, 165.78, 165.74, 165.70, 165.68, 165.62, 165.55, 165.5, 165.4, 165.3, 165.2, 164.8, 164.4, 164.3, 164.0, 133.32, 133.25, 133.2, 133.0, 132.8, 132.7, 132.5, 132.4, 129.9, 129.84, 129.75, 129.7, 129.6, 129.53, 129.48, 129.4, 129.28, 129.26, 129.2, 128.8, 128.6, 128.5, 128.44, 128.35, 128.3, 128.2, 128.10, 128.06, 128.0, 127.9, 127.8, 108.3, 99.5, 99.1, 96.7, 85.3, 84.93, 84.85, 82.5, 82.1, 80.8, 80.5, 80.4, 74.8, 73.7, 73.6, 71.41, 71.37, 70.7, 70.2, 70.0, 69.9, 69.7, 69.4, 68.6, 68.2, 68.1, 68.0, 67.8, 67.6, 67.2, 66.2, 64.2, 64.0, 63.5, 62.7, 62.0, 61.5, 37.8, 29.5, 27.9, 18.0, -1.6; LRMS–MALDI–TOF calcd for $[M+Na]^+$ $C_{759}H_{631}N_3NaO_{225}Si$: 13445.3. Found 13440.9.



2-(Trimethylsilyl)ethyl 2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-

benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2-azido-2-deoxy-4,6-di-*O*-benzoyl-α-D-glucopyranoside (3.61). To a solution of **3.60** (222 mg; 17.0 μmol) in THF–CH_2Cl_2–CH_3OH (60.0 mL, 1:1:1 v/v/v) was added H_2NNH_2·AcOH (15 mg, 0.17 mmol). The reaction mixture was heated to 60 ºC and stirred for 3 h, before being cooled to rt, diluted with CH_2Cl_2 (30.0 mL), and washed with H_2O. The organic layer was dried with Na_2SO_4, filtered, and concentrated. The crude residue was purified by silica gel column chromatography (2:3→3:7 hexanes–EtOAc) to yield **3.61** (135 mg, 61%) as a fluffy, white solid. R_f 0.50 (7:13 hexanes–EtOAc); $[\alpha]_D$ +32 (*c* 0.10, $CHCl_3$); ^1H NMR (700 MHz, $CDCl_3$, δ_H) 8.10 – 7.90 (m, 79 H, ArH), 7.88 – 7.81 (m, 5 H, ArH), 7.79 – 7.66 (m, 49 H, ArH), 7.65 – 7.62 (m, 2 H, ArH), 7.58 – 6.90 (m, 280 H, ArH), 5.99 – 5.95 (m, 1 H), 5.88 – 5.85 (m, 1 H), 5.81 – 5.70 (m, 10 H), 5.69 – 5.52 (m, 14 H), 5.47 – 5.30 (m, 25 H), 5.30 – 5.20 (m, 13 H), 5.20 – 5.10 (m, 11 H), 5.07 – 5.05 (m, 1 H), 4.97 – 4.92 (m, 1 H), 4.75 – 4.71 (m, 1 H), 4.70 – 4.49 (m, 25 H), 4.45 – 3.81 (m, 90 H), 3.78

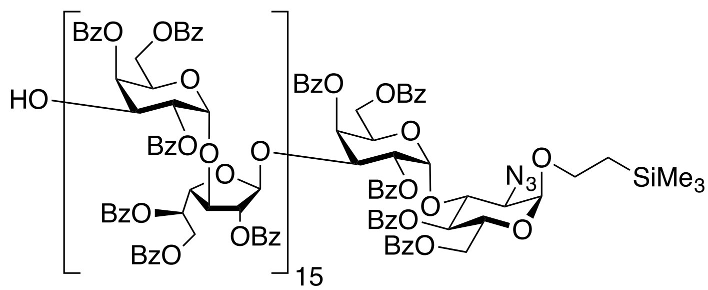
– 3.64 (m, 3 H), 3.58 – 3.52 (m, 1 H), 3.36 – 3.32 (m, 1 H), 1.78 (d, J = 5.1 Hz, 1H, OH), 1.02 – 0.96 (m, 1 H, $OCH_2C\underline{H}_2Si$), 0.96 – 0.90 (m, 1 H, $OCH_2C\underline{H}_2Si$), -0.05 (s, 9 H, $Si(CH_3)_3$); ^{13}C NMR (126 MHz, $CDCl_3$, δ_C) 165.8, 165.7, 165.61, 165.55, 165.5, 165.4, 165.2, 164.3, 164.0, 133.33, 133.26, 133.0, 132.8, 132.7, 132.5, 132.4, 129.9, 129.8, 129.7, 129.6, 129.53, 129.48, 129.4, 128.8, 128.64, 128.55, 128.44, 128.35, 128.3, 128.2, 128.1, 128.02, 127.97, 127.9, 127.8, 108.3, 99.5, 96.8, 84.9, 82.5, 81.5, 80.4, 73.7, 72.5, 71.4, 71.14, 71.09, 70.9, 69.7, 69.4, 68.1, 67.5, 67.4, 66.2, 64.2, 62.0, 18.0, -1.6; LRMS–MALDI–TOF calcd for $[M+Na]^+$ $C_{754}H_{625}N_3NaO_{223}Si$: 13347.2. Found 13357.4.



2-(Trimethylsilyl)ethyl 2,4,6-tri-*O*-benzoyl-3-*O*-levulinoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-

(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2-azido-2-deoxy-4,6-di-*O*-benzoyl-α-D-glucopyranoside (3.62). Compounds **3.61** (135 mg; 11.0 μmol) and **3.18** (82 mg; 0.038 mmol) were dissolved in CH_2Cl_2–toluene and were concentrated to dryness on high vacuum overnight. Dry CH_2Cl_2 (1.0 mL) and 4 Å M.S. were added to the flask, and the mixture was stirred for 30 min at rt. The suspension was cooled to 0 °C, and TBSOTf (1 μL; 4 μmol) was added. The reaction mixture was stirred for 2 h at 0 °C, before being neutralized by the addition of Et_3N, filtered, and concentrated. The crude residue was purified by silica gel column chromatography (2:3→1:3 hexanes–EtOAc) to give **3.62** (121 mg, 77%) as a white, fluffy solid. R_f 0.23 (7:13 hexanes–EtOAc); $[\alpha]_D$ +23 (*c* 0.10, $CHCl_3$); ^1H NMR (700 MHz, $CDCl_3$, δ_H) 8.12 – 8.09 (m, 2 H, ArH), 8.09 – 8.05 (m, 2 H, ArH), 8.05 – 7.91 (m, 86 H, ArH), 7.89 – 7.86 (m, 3 H, ArH), 7.80 – 7.68 (m, 58 H, ArH), 7.67 – 7.63 (m, 2 H, ArH), 7.57 – 7.15 (m, 220 H, ArH), 7.14 – 6.91 (m, 102 H, ArH), 6.00 – 5.96 (m, 1 H), 5.83 – 5.74 (m, 13 H), 5.71 – 5.57 (m, 16 H), 5.57 – 5.31 (m, 35 H), 5.30 – 5.21 (m, 16 H), 5.21 – 5.11 (m, 13 H), 5.07 (d, J = 3.6 Hz, 1 H, H-1_{Glc}), 4.98 – 4.93 (m, 1 H), 4.78 – 4.73 (m, 1 H), 4.70 – 4.54 (m, 30 H), 4.53 – 4.48 (m, 1 H), 4.47 – 3.82 (m, 94 H), 3.80 – 3.69 (m, 2 H), 3.60 – 3.52 (m, 1 H), 3.35 (dd, J = 10.2, 3.6 Hz, 1 H, H-2_{Glc}), 2.58 (dt, J = 18.2, 7.0 Hz, 1 H,

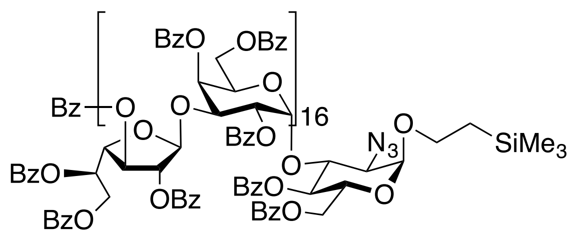
$\underline{C}H_2CH_2C(O)CH_3$), 2.50 (dt, J = 18.2, 6.7 Hz, 1 H, $\underline{C}H_2CH_2C(O)CH_3$), 2.43 – 2.29 (m, 2 H, $C\underline{H}_2CH_2C(O)CH_3$), 1.94 (s, 3 H, $CH_2CH_2C(O)C\underline{H}_3$), 1.03 – 0.97 (m, 1 H, $OCH_2C\underline{H}_2Si$), 0.97 – 0.91 (m, 1 H, $OCH_2C\underline{H}_2Si$), -0.04 (s, 9 H, $Si(CH_3)_3$); ^{13}C NMR (126 MHz, $CDCl_3$, δ_C) 205.9, 171.0, 166.04, 165.97, 165.9, 165.7, 165.62, 165.56, 165.52, 165.47, 165.4, 165.3, 165.2, 164.8, 164.3, 164.0, 133.33, 133.26, 133.0, 132.83, 132.81, 132.7, 132.5, 132.4, 129.93, 129.86, 129.8, 129.7, 129.60, 129.55, 129.5, 129.4, 129.29, 129.27, 129.2, 128.8, 128.6, 128.49, 128.45, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 108.3, 99.5, 96.8, 85.3, 85.0, 82.5, 82.2, 82.1, 80.4, 74.8, 73.7, 72.5, 71.4, 70.9, 69.9, 69.7, 69.4, 68.6, 68.2, 68.0, 67.84, 67.77, 67.6, 67.2, 66.24, 64.18, 63.6, 62.7, 62.10, 62.05, 62.0, 61.5, 37.8, 29.5, 28.0, 18.0, -1.6; LRMS–MALDI–TOF calcd for $[M+Na]^+$ $C_{867}H_{719}N_3NaO_{257}Si$: 15343.1. Found 15351.8.



2-(Trimethylsilyl)ethyl 2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-

galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2-azido-2-deoxy-4,6-di-*O*-benzoyl-α-D-glucopyranoside (3.16). To a solution of **3.62** (120 mg; 8.00 μmol) in THF–CH_2Cl_2–CH_3OH (30.0 mL, 1:1:1 v/v/v) was added H_2NNH_2·AcOH (7.6 mg, 80 μmol). The reaction mixture was heated to 60 ºC and stirred for 5 h, before being diluted with CH_2Cl_2 (50.0 mL), and washed with H_2O. The organic layer was dried with Na_2SO_4, filtered, and concentrated. The crude residue was purified by silica gel column chromatography (2:3→3:7 hexanes–EtOAc) to yield **3.16** (110 mg, 92%) as a fluffy, white solid. R_f 0.41 (7:13 hexanes–EtOAc); $[\alpha]_D$ +16 (*c* 0.20, $CHCl_3$); ^1H NMR (700 MHz, $CDCl_3$, δ_H) 8.11 – 8.05 (m, 4 H, ArH), 8.05 – 7.91 (m, 85 H, ArH), 7.89 – 7.86 (m, 2 H, ArH), 7.86 – 7.83 (m, 2 H, ArH), 7.81 – 7.75 (m, 5 H, ArH), 7.76 – 7.67 (m, 57 H, ArH), 7.67 – 7.63 (m, 2 H, ArH), 7.59 – 7.54 (m, 2 H, ArH), 7.53 – 6.93 (m, 316 H, ArH), 6.00 – 5.97 (m, 1 H), 5.89 – 5.86 (m, 1 H), 5.82 – 5.72 (m, 14 H), 5.71 – 5.54 (m, 18 H), 5.49 – 5.32 (m, 30 H), 5.31 – 5.12 (m, 30 H), 5.09 – 5.06 (m, 1 H, H-1_{Glc}), 4.98 – 4.94 (m, 1 H), 4.76 – 4.73 (m, 1 H), 4.72 – 4.52 (m, 30 H), 4.46 – 3.82 (m, 94 H), 3.80 – 3.66 (m, 3 H), 3.59 – 3.53 (m, 1 H), 3.36 (dd, *J* = 10.1, 3.6 Hz, 1 H, H-2_{Glc}), 1.80 (d, *J* =

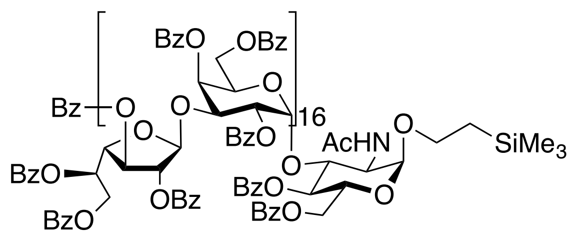
5.4 Hz, 1 H, OH), 1.04 – 0.97 (m, 1 H, $OCH_2C\underline{H}_2Si$), 0.97 – 0.90 (m, 1 H, $OCH_2C\underline{H}_2Si$), -0.04 (s, 9 H, $Si(CH_3)_3$); ^{13}C NMR (126 MHz, $CDCl_3$, δ_C) 166.5, 166.3, 166.04, 165.97, 165.9, 165.7, 165.63, 165.56, 165.5, 165.4, 165.2, 164.8, 164.3, 164.0, 133.34, 133.32, 133.27, 133.20, 133.16, 133.0, 132.8, 132.6, 132.5, 132.4, 129.9, 129.8, 129.67, 129.60, 129.55, 129.5, 129.4, 129.3, 128.8, 128.6, 128.5, 128.4, 128.3, 128.2, 128.1, 128.04, 127.99, 127.9, 127.8, 108.3, 99.5, 98.1, 96.8, 85.4, 85.0, 82.5, 82.1, 81.5, 80.4, 74.8, 73.7, 72.5, 71.4, 71.2, 70.9, 70.2, 69.7, 69.4, 68.1, 68.0, 67.6, 67.4, 66.3, 64.2, 63.6, 62.7, 62.0, 61.5, 18.0, -1.6; LRMS–MALDI–TOF calcd for $[M+Na]^+$ $C_{862}H_{713}N_3NaO_{255}Si$: 15245.0. Found 15256.9.



2-(Trimethylsilyl)ethyl 2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-

(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2-azido-2-deoxy-4,6-di-*O*-benzoyl-α-D-glucopyranoside (3.9). Compounds **3.12**[151] (111 mg; 0.145 mmol) and **3.16** (110 mg; 7.00 μmol) were dissolved in CH_2Cl_2–toluene and were concentrated to dryness on high vacuum overnight. Dry CH_2Cl_2 (1.2 mL) and 4 Å M.S. were added to the flask, and the mixture was stirred for 30 min at rt. The suspension was cooled to 0 °C, and TBSOTf (0.4 μL; 1 μmol) was added. The reaction mixture was stirred for 2.5 h while warming to rt, before being neutralized by the addition of Et_3N, filtered, and concentrated. The crude residue was purified by silica gel column chromatography (2:3→3:7 hexanes–EtOAc) to give **3.6** (102 mg, 89%) as a white, fluffy solid. R_f 0.29 (4:1 toluene–EtOAc); $[\alpha]_D$ +35 (*c* 0.40, $CHCl_3$); ^1H NMR (700 MHz, $CDCl_3$, δ_H) 8.10 – 7.92 (m, 88 H, ArH), 7.89 – 7.83 (m, 6 H, ArH), 7.80 – 7.77 (m, 2 H, ArH), 7.76 – 7.67 (m, 56 H, ArH), 7.67 – 7.63 (m, 4 H, ArH), 7.56 – 7.53 (m, 2 H, ArH), 7.52 – 6.92 (m, 337 H, ArH), 6.08 (ddd, *J* = 6.7, 3.3, 3.3 Hz, 1 H), 6.00 – 5.96 (m, 1 H), 5.83 – 5.74 (m, 14 H), 5.70 – 5.57 (m, 17 H), 5.48 – 5.31 (m, 32 H), 5.29 – 5.20 (m, 16 H), 5.20 – 5.11 (m, 14 H), 5.07 (d, *J* = 3.6 Hz, 1 H, H-1_{Glc}), 4.97 – 4.92 (m, 2 H), 4.87 (dd, *J* = 12.1, 7.3 Hz, 1 H), 4.80 (dd, *J* = 12.0, 3.3 Hz, 1 H), 4.69 – 4.53 (m, 30 H), 4.46 – 3.68 (m, 101 H), 3.59 – 3.53 (m, 1 H), 3.35 (dd, *J* = 10.1, 3.6 Hz, 1 H H-2_{Glc}), 1.03 – 0.96 (m, 1 H, $OCH_2C\underline{H}_2Si$), 0.96 – 0.91 (m, 1H, $OCH_2C\underline{H}_2Si$), -

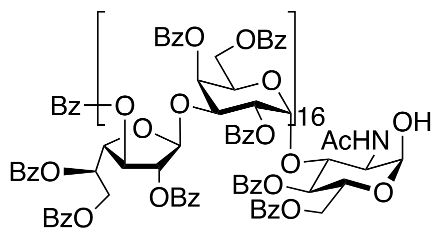
0.04 (s, 9 H, $Si(CH_3)_3$); ^{13}C NMR (126 MHz, $CDCl_3$, δ_C) 166.2, 166.04, 166.00, 165.97, 165.8, 165.71, 165.68, 165.62, 165.60, 165.56, 165.5, 165.4, 165.3, 165.2, 164.8, 164.6, 164.3, 164.0, 133.33, 133.26, 133.1, 133.0, 132.8, 132.6, 132.5, 129.9, 129.8, 129.7, 129.6, 129.54, 129.48, 129.45, 129.4, 129.3, 128.8, 128.7, 128.6, 128.44, 128.36, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 108.3, 99.5, 98.1, 96.8, 84.9, 82.5, 80.4, 77.9, 74.8, 73.7, 72.5, 71.4, 70.9, 70.7, 70.2, 69.9, 69.7, 69.4, 68.1, 67.6, 66.24, 64.17, 63.6, 62.0, 61.5, 18.0, -1.6; LRMS–MALDI–TOF calcd for $[M+Na]^+$ $C_{896}H_{739}N_3NaO_{264}Si$: 15812.4. Found 15843.9.



2-(Trimethylsilyl)ethyl 2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-

galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-4,6-di-*O*-benzoyl-α-D-glucopyranoside (3.63). To a solution of **3.9** (98 mg; 6.3 μmol) in THF–H_2O (10 mL; 4:1 v/v), was added 1 M Me_3P in THF (19 μL), followed by 1 M $NaOH_{(aq)}$ (6.0 μL). The pH was ~8, and the reaction mixture was heated to 50 °C and stirred for 26 h, before being diluted with brine (5.0 mL) and extracted twice with EtOAc (10 mL, 10 mL). The combined organic layers were dried with Na_2SO_4, filtered, and concentrated. The crude residue (106 mg) was dissolved in pyridine (2.0 mL), and cooled to 0 °C. Ac_2O (0.10 mL, 1.1 mmol) was added slowly dropwise, and the reaction mixture was stirred for 17 h while warming to rt, before being diluted with EtOAc (20 mL) and washed with H_2O. The organic layer was washed, in succession, with solutions of 1 N HCl three times, saturated sodium bicarbonate, and brine, before being dried with Na_2SO_4, filtered, and concentrated. The crude residue was purified by silica gel column chromatography (2:3→3:7 hexanes–EtOAc) to yield **3.63** (80 mg, 82% over 2 steps) as white solid. R_f 0.14 (4:1 toluene–EtOAc); $[\alpha]_D$ +40 (*c* 0.10, $CHCl_3$); ^1H NMR (700 MHz, $CDCl_3$, δ_H) 8.12 – 7.92 (m, 102 H, ArH), 7.89 – 7.82 (m, 8 H, ArH), 7.78 – 7.68 (m, 67 H, ArH), 7.67 – 7.62 (m, 4 H, ArH), 7.52 – 6.92 (m, 314 H, ArH), 6.09 (ddd, *J* = 6.7, 3.3, 3.3 Hz, 1 H), 5.92 (d, *J* = 9.8 Hz, 1 H, NH), 5.89 – 5.84 (m, 1 H), 5.84 – 5.72 (m, 14 H), 5.72 – 5.57 (m, 16 H), 5.54 – 5.31 (m, 35 H), 5.30 – 5.11 (m, 28 H), 4.95 (dd, *J* = 5.4, 2.6 Hz, 1 H), 4.90 – 4.83 (m, 2 H), 4.80 (dd,

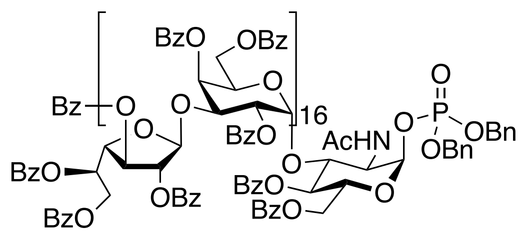
J = 12.0, 3.3 Hz, 1 H), 4.76 – 4.70 (m, 1 H), 4.70 – 4.55 (m, 28 H), 4.55 – 4.49 (m, 2 H), 4.49 – 3.79 (m, 100 H), 3.79 – 3.73 (m, 1 H), 3.70 – 3.63 (m, 1 H), 3.51 – 3.44 (m, 1 H), 1.99 (s, 3 H, C(O)CH_3), 0.93 – 0.87 (m, 1 H, $OCH_2C\underline{H}_2Si$), 0.83 – 0.77 (m, 1 H, $OCH_2C\underline{H}_2Si$), -0.05 (s, 9 H, $Si(CH_3)_3$); ^{13}C NMR (126 MHz, $CDCl_3$, δ_C) 170.2, 166.5, 166.2, 166.1, 166.0, 165.9, 165.7, 165.6, 165.5, 165.4, 164.63, 164.60, 164.3, 164.2, 133.34, 133.26, 133.1, 133.0, 132.8, 132.5, 129.9, 129.8, 129.7, 129.6, 129.54, 129.49, 129.45, 129.4, 129.3, 128.8, 128.7, 128.62, 128.56, 128.5, 128.4, 128.2, 128.1, 128.0, 127.8, 108.3, 99.5, 97.0, 84.9, 82.5, 82.3, 81.9, 80.4, 77.9, 73.7, 72.9, 72.6, 71.3, 70.9, 70.7, 69.9, 69.7, 69.4, 68.2, 67.6, 65.5, 64.2, 63.5, 62.0, 52.1, 23.3, 17.9, -1.5; LRMS–MALDI–TOF calcd for $[M+Na]^+$ $C_{898}H_{743}NNaO_{265}Si$: 15828.4. Found 15851.4.



2,3,5,6-Tetra-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-

galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-4,6-di-*O*-benzoyl-α-D-glucopyranose (3.64). To a solution of **3.63** (80 mg, 5.1 μmol) in CH_2Cl_2 (3.0 mL) and H_2O (1.0 mL) that was cooled to 0 °C, was added TFA (6.0 mL) slowly dropwise. The reaction mixture was stirred for 31 h at rt, before being concentrated, dissolved in CH_2Cl_2 (20 mL), and washed with a solution of saturated sodium bicarbonate until the organic layer reached neutral pH. The organic layer was dried with Na_2SO_4, filtered, and concentrated. The crude residue was purified by silica gel column chromatography (2:3→1:3 hexanes–EtOAc) to yield **3.64** (33 mg, 41%) as white solid. R_f 0.18 (1:2 hexanes–EtOAc); $[\alpha]_D$ +33 (*c* 0.10, $CHCl_3$); ^1H NMR (700 MHz, $CDCl_3$, δ_H) 8.10 – 7.91 (m, 104 H, ArH), 7.85 – 7.82 (m, 8 H, ArH), 7.78 – 7.68 (m, 80 H, ArH), 7.67 – 7.62 (m, 6 H, ArH), 7.52 – 6.92 (m, 297 H, ArH), 6.15 (d, *J* = 9.4 Hz, 1 H, NH), 6.08 (ddd, *J* = 6.4, 3.1, 3.1 Hz, 1 H), 5.86 – 5.71 (m, 16 H), 5.70 – 5.58 (m, 18 H), 5.53 – 5.30 (m, 32 H), 5.29 – 5.11 (m, 30 H), 4.96 – 4.92 (m, 1 H), 4.86 (dd, *J* = 12.1, 7.3 Hz, 1 H), 4.82 – 4.71 (m, 2 H), 4.71 – 4.47 (m, 30 H), 4.47 – 3.71 (m, 103 H), 3.20 (br s, 1 H, OH), 1.94 (s, 3 H, $C(O)CH_3$); ^{13}C NMR (126 MHz, $CDCl_3$, δ_C) 170.5, 166.6, 166.5, 166.2, 166.1, 166.0, 165.9,

165.71, 165.68, 165.6, 165.5, 165.4, 164.5, 164.3, 164.2, 133.34, 133.26, 133.1, 133.0, 132.9, 132.8, 132.7, 132.6, 132.5, 130.0, 129.92, 129.85, 129.8, 129.7, 129.6, 129.53, 129.48, 129.44, 129.37, 129.3, 128.8, 128.7, 128.62, 128.56, 128.44, 128.36, 128.2, 128.1, 128.0, 127.8, 108.3, 99.5, 97.0, 92.1, 84.9, 82.5, 82.3, 81.9, 80.4, 77.9, 73.7, 72.8, 72.6, 70.9, 70.7, 69.7, 69.4, 67.6, 64.2, 64.0, 63.2, 62.0, 52.6, 23.4; LRMS–MALDI–TOF calcd for $[M+Na]^+$ $C_{893}H_{731}NNaO_{265}$: 15728.4. Found 15740.0.



2,3,5,6-Tetra-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-

galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-4,6-di-*O*-benzoyl-α-D-glucopyranose dibenzylphosphate (3.65). To a solution of **3.64** (33 mg, 2.0 μmol) and tetrazole (3 mg, 0.05 mmol) in dry CH_2Cl_2 (3.0 mL) that was cooled to 0 °C, was added *i*-$Pr_2NP(OBn)_2$ (20 μL, 0.06 mmol) dropwise. The reaction mixture was stirred at rt for 2 h, before being cooled to –78 °C, and *m*-CPBA (19 mg, 0.11 mmol) was added. The mixture was warmed to rt and stirred for an additional 2 h, before being diluted with CH_2Cl_2 (10 mL) and washed with a solution of saturated sodium bicarbonate and with brine. The organic layer was dried with Na_2SO_4, filtered, and concentrated. The crude residue was purified by preparative TLC (2:1 toluene–EtOAc then 1:1 hexanes–EtOAc) to yield **3.65** (1.2 mg, 4% over 2 steps) as a beige, off-white solid. R_f 0.33 (3:7 hexanes–EtOAc); $[\alpha]_D$ +20 (*c* 0.1, $CHCl_3$); ^{1}H NMR (700 MHz, $CDCl_3$, δ_H) 8.09 – 7.90 (m, 119 H, ArH), 7.85 – 7.79 (m, 6 H, ArH), 7.75 – 7.67 (m, 75 H, ArH), 7.66 – 7.62 (m, 6 H, ArH), 7.59 – 7.56 (m, 2 H, ArH), 7.51 – 6.91 (m, 297 H, ArH), 6.09 – 6.05 (m, 1 H), 6.02 (d, *J* = 9.7 Hz, 1 H, NH), 5.88 – 5.84 (m, 1 H), 5.82 – 5.71 (m, 15 H), 5.70 – 5.58 (m, 17 H), 5.54 (d, *J* = 4.0 Hz, 1 H), 5.49 (dd, *J* = 10.5, 4.3 Hz, 1 H), 5.47 – 5.30 (m, 30 H), 5.26 – 5.19 (m, 15 H), 5.19 – 5.11 (m, 15 H), 5.06 – 4.97 (m, 4 H), 4.94 – 4.92 (m, 1 H), 4.85 (dd, *J* = 11.9, 6.8 Hz, 1 H), 4.81 – 4.77 (m, 1 H), 4.68 – 4.49 (m, 30 H), 4.48 – 3.70 (m, 101 H), 1.71 (s, 3 H, $C(O)CH_3$); ^{13}C NMR (126 MHz, $CDCl_3$, δ_C) 165.8, 165.5, 165.44, 165.36, 164.3, 133.32, 133.25, 133.0, 132.83, 132.80, 132.7, 132.5, 129.9, 129.78, 129.75, 129.74, 129.67, 129.6,

129.52, 129.47, 129.43, 129.36, 128.7, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 108.3, 99.5, 84.9, 82.5, 80.4, 73.7, 70.9, 69.7, 69.4, 68.2, 67.5, 64.2, 62.0, 22.7; ^{31}P NMR (162 MHz, $CDCl_3$, δ_P) -2.36; LRMS–MALDI–TOF calcd for $[M+Na]^+$ $C_{907}H_{744}NNaO_{268}P$: 15988.4. Found 16014.0.

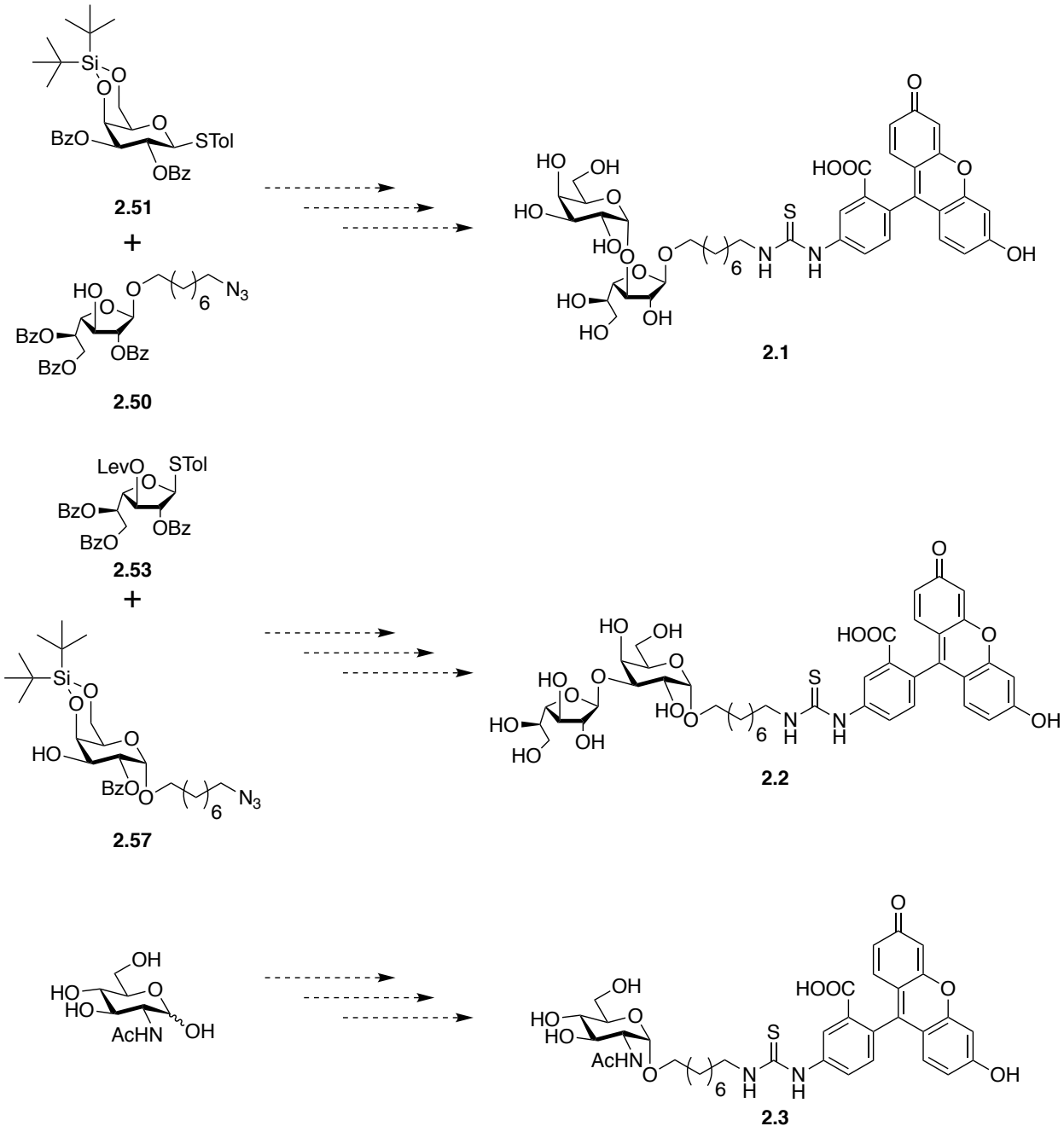
Chapter 4: Summary and Future Work

4.1 Summary and Future Work

In this thesis, I reported the chemical synthesis of molecular probes related to D-galactan I from *K. pneumoniae* O2a. The work includes the synthesis of small molecule glycosyltransferase probes (Chapter 2) and the synthesis of lipid-linked oligosaccharide probes (Chapter 3).

4.1.1 Synthesis of Small Molecule Glycosyltransferase Probes

In Chapter 2, I accomplished the synthesis of three fluorescein-tagged probes **2.1**–**2.3**. Initially, I planned to obtain a conserved building block that could be used in the synthesis of the lipid-linked oligosaccharide probes **3.1**–**3.5**. However, I was unable to obtain the desired conserved building block **2.33**. This was because thiogalactofuranoside acceptor **2.35** was a poor nucleophile when used as the acceptor in glycosylations, likely due to the conformation of the galactofuranose ring. After redesigning the synthetic route, targets **2.1** and **2.2** were obtained successfully (Scheme 4.1). The 1,2-*trans*-β glycosidic linkages were installed using 2-*O*-benzoylated galactofuranosyl donor (**2.53**), as neighboring group participation from the 2-*O*-benzoyl group gave exclusive β-selectivity. The more challenging 1,2-*cis*-α glycosidic linkages were installed with complete stereoselectivity using DTBS-protected galactopyranosyl donors (**2.51** and **2.58**). The third target, **2.3**, was obtained after a thermodynamically-controlled glycosylation with 8-azido-1-octanol with *N*-acetyl-glucosamine, followed by conjugation to FITC. Low yields resulted from difficulties during purification.



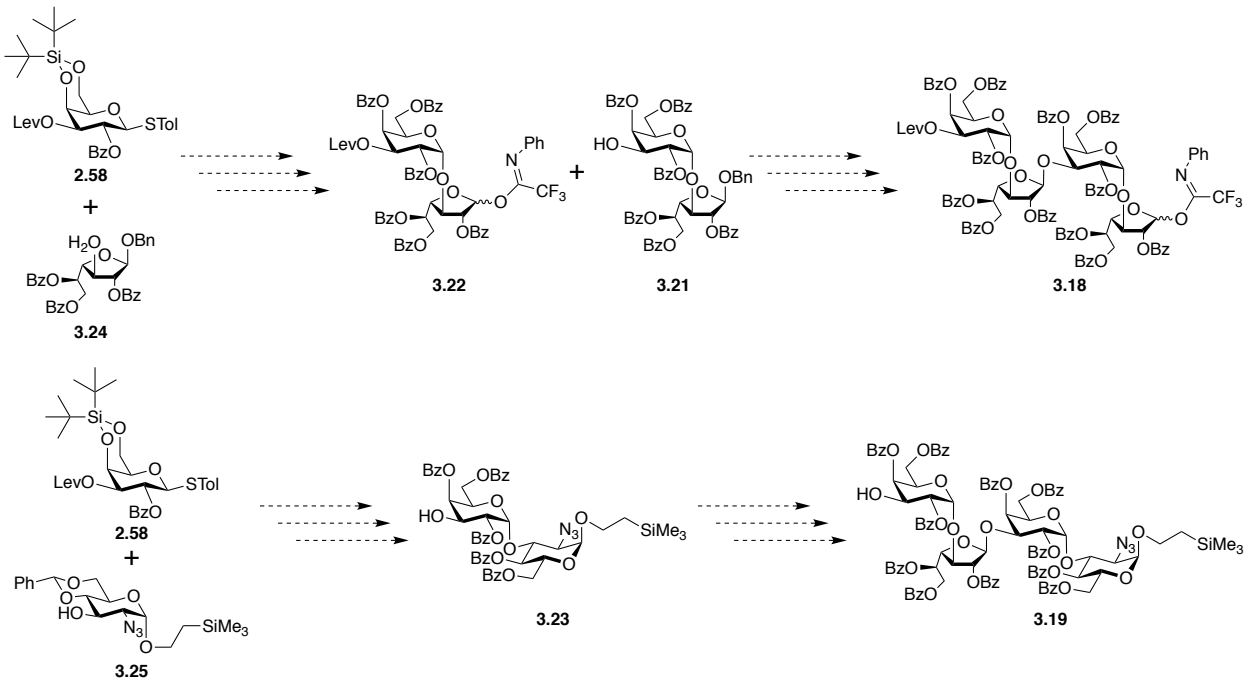
Scheme 4.1 Key monosaccharides converted to targets **2.1**, **2.2** and **2.3**.

These compounds are currently being studied with the enzymes – WbbM, WbbO, and WbbN – from the O-PS biosynthetic pathway in *K. pneumoniae* O2a, in collaboration with the Whitfield and Kimber labs at the University of Guelph. Preliminary results from their labs

suggest that WbbM is a bifunctional GTase responsible for the polymerization of the O-PS in *K. pneumoniae* O2a. Additional preliminary results also suggest that WbbO and WbbN are GTases accountable for the initiation of the synthesis of the O-PS. Ultimately, these synthetic acceptors are clarifying the roles of the GTases. However, more work remains to be done to further elucidate the O-PS biosynthetic pathway, particularly in regards to how the chain length is controlled and the mechanism of O-PS recognition and export.

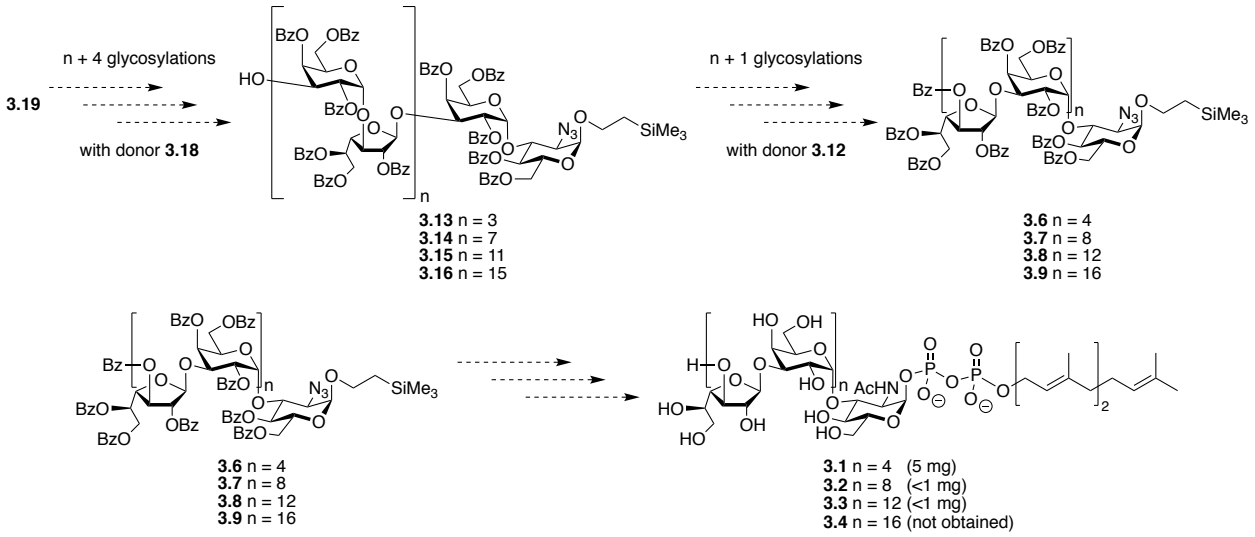
4.1.2 Synthesis of Oligosaccharide Probes to Establish the Chain-Length Control and Export Mechanism in *K. pneumoniae* O2a O-PS Biosynthesis

In Chapter 3, I devised a synthetic strategy that allowed synthesis of small library of lipid-linked oligosaccharides related to D-galactan I from *K. pneumoniae* O2a. Starting from galactofuranoside building block **3.23** (Scheme 4.2) and glucosamine-based building block **3.24**, and in conjunction with galactopyranoside donor **2.58**, the 1,2-*cis*-α glycosides (**3.29** and **3.33**) were synthesized in high yields. These disaccharides could be easily converted to acceptors **3.20** and **3.22**, but obtaining the 2-*O*-benzoylated donor **3.21** was more difficult. A method that uses Raney-Ni as the reductant led to access of **3.21** in good yield. Using 2 + 2 glycosylations with donor **3.21** and either **3.20** or **3.22** led to the key tetrasaccharide building blocks **3.18** and **3.19** in good yields.



Scheme 4.2 Key intermediates in the synthesis of tetrasaccharides **3.18** and **3.19**.

With these key tetrasaccharides, an n + 4 glycosylation strategy was used to obtain crucial oligosaccharide acceptors **3.13**–**3.16** (Scheme 4.3). Beginning with **3.19** as the first acceptor in this series of glycosylations, the 2-*O*-benzoylated donor **3.18** was activated in the presence of the appropriate acceptor to give the desired glycosylation product with complete β-selectivity. Removal of the levulinoyl esters gave the desired alcohols, which were glycosylated until the synthetic glycans reached the appropriate length. These repeated reactions all proceeded smoothly in moderate to good yields. Once **3.13**–**3.16** were acquired, the target backbones **3.6**–**3.9** were isolated in high yields via n + 1 glycosylations with donor **3.12**. Target backbones **3.6**–**3.8** were successfully converted to oligosaccharide targets **3.1**–**3.3**, albeit **3.2** and **3.3** were obtained as impure mixtures in sub-milligram quantities. Up to this point, targets **3.4** and **3.5** have not been obtained, due to a lack of material of 32-mer **3.16** and 33-mer **3.59**.



Scheme 4.3 Key oligosacchride intermediates in the synthesis of targets **3.1**–**3.3**.

Despite the lack of success in obtaining **3.4** and **3.5**, this synthetic strategy efficiently prepared the desired protected target backbones **3.6**–**3.9** with high yields and selectivities. However, the late-stage protecting group manipulations were disappointingly low yielding and resulted in a substantial loss of material, as **3.2** and **3.3** were obtained in sub-milligram quantities as partially purified mixtures and **3.59** could not be converted to target **3.4**. Despite this issue, this strategy does allow the coupling of the native undecaprenol lipid to the glycan in future studies, as the longer lipid might be necessary to get a true understanding of the processes in O-PS biosynthesis.

As the synthesis of large oligosaccharides is lengthy and time-consuming, optimizing these late-stage protecting group manipulations is a challenge. The low yields of the removal of the TMSEt group might have been due to the many (up to 99) benzoyl esters on my compounds, as the inductive effects of these electron-withdrawing groups might have prevented the deprotection from taking place effectively. Because the yields dropped as the molecules got larger, there seems to be an inverse correlation between molecular size and the yield of this

reaction. Subtle polarity differences between oligosaccharide products and by-products is also a difficult challenge to overcome, as in the conversion of **3.58** to **3.59**. It is possible that one might have to use preparative TLC or HPLC to overcome this problem, as the cheaper, more efficient, and more common methods (like column chromatography) do not separate these compounds effeciently. The phosphate–phosphate coupling and global deprotection steps also need to be optimized further. While the coupling reaction is a traditionally low-yielding reaction,[116,150] the deprotection can be further improved. It may be necessary to change protecting groups from benzoyl esters to acetate esters, if modification of the reaction conditions does not improve the removal of the benzoyl groups. This is the strategy used by a former colleague.[149] These issues will need to be addressed to achieve the synthesis of target molecules **3.4** and **3.5**.

Compounds **3.1**–**3.3** will be sent to lab of Professor Chris Whitfield at the University of Guelph as part of our collaboration. Using these molecules, we hope to further clarify the O-PS chain length control and export mechanisms from *K. pneumoniae* O2a.

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