Studies Towards Understanding the Biosynthesis of Methyl Phosphoramidates Found in *Campylobacter jejuni*

by

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Abstract

The human pathogen *Campylobacter jejuni* is the leading cause of food-borne gastroenteritis, causing more foodborne illnesses than *Salmonella*, *Shigella*, and *Listeria* combined. Of the approximate 5.3 million people infected every year in North America alone, approximately 1/1000 will develop the post-infectious paralytic disorder Guillain–Barré syndrome. In addition, the Public Health Agency of Canada has recently reported that approximately 90% of all chickens in supermarkets are infected with *C. jejuni*.

C. jejuni produces a capsular polysaccharide (CPS) that is an important virulence factor involved in colonization and invasion. The structure of *C. jejuni* CPS varies widely from strain to strain and is often functionalized with a range of phase-variable modifications. One of these unique and rare modifications is an *O*-methyl phosphoramidate (MeOPN) motif, which is found in over 70% of *C. jejuni* strains. The role of these MeOPNs is unknown, but recent studies have demonstrated that MeOPN-functionalized CPS plays a significant role in modulating several aspects of the immune response. Thus, there is good evidence for a biological role for the MeOPN group. Access to these MeOPN motifs could lead to the development of vaccines against *C. jejuni* and targeting the enzymes involved in its biosynthesis could lead to new therapeutic agents for the treatment of *C. jejuni* infections. However, before such agents can be identified, better characterization of these biosynthetic enzymes and their substrates are required.

This thesis will focus on the MeOPN motif in two parts. The first part describes the development of a reliable method for the synthesis of the MeOPN motif and assess its stability through various chemical transformations. This will enable access to these rare and unique modifications for the potential development of vaccines against *C. jejuni*. Part two of this thesis

will describe work directed at unraveling the biosynthesis of the MeOPN motifs. More specifically, we will describe work to demonstrate enzyme activity for the two phosphoramidate transferases found in the *C. jejuni* strain 11168H. This work, when complete, will provide insights that could be used to development of inhibitors against these proteins and possible new therapeutic agents against *C. jejuni*.

Preface

Chapter 2 – Part of this chapter was published:

Ashmus, Roger A.; Lowary, T. L. *Org. Lett.* **2014**, *16*, 2518–2521.

Professor Lowary and I wrote the paper together. I was the only experimentalist on this work.

van Alphen, Lieke B.; Wenzel, Cory Q.; Richards, Michele R.; Fodor, Christopher; Ashmus, Roger A.; Stahl, Martin; Karlyshev, Andrey V.; Wren, Brendan W.; Stintzi, Alain; Miller, William G.; Lowary, Todd L.; Szymanski, Christine M. *PLoS ONE* **2014**, *9*, e87051.

The paper was written by our collaborators from the Szymanski laboratory and edited by Professor Lowary and I. My contributions to this paper include the preparation of samples used for insecticidal activity testing.

Chapter 3 – Part of this chapter was published:

Ashmus, Roger A.; Jayasuriya, Anushka B.; Lim, Ying-Jie; O'Doherty, George A.; Lowary, Todd L. *J. Org. Chem.* **2016**, *81*, 3058–3063.

Professor Lowary and I wrote the paper together. I performed approximately 90% of the work in this paper.

Chapter 4 – Part of this chapter was published:

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Professor Lowary, Mr. Lin, and I wrote the paper together. I performed approximately 40% of the experimental work.

The rest of the work presented in this thesis is unpublished and was carried out solely by me unless otherwise indicated in the text.

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List of Abbreviations

2,2-DMP	2,2-dimethoxypropane
2-N_3-AcOH	2-azidoacetic acid
6d-Hep*p*	6-deoxy-α-D-*altro*-heptopyranose
4Å MS	4 Ångstrom molecular sieves
Ac_2O	acetic anhydride
AgOTf	silver trifluoromethanesulfonate
ADP	adenosine diphosphate
AMP	adenosine monophosphate
AMP-PNP	adenyl-5'-yl imidodiphosphate
APS	adenosine-5'-phosphosulfate
ATP	adenosine triphosphate
BAIB	[bis(acetoxy)iodo]benzene
Bz_2O	benzoic anhydride
BzCl	benzoyl chloride
BLAST	Basic Local Alignment Search Tool
BnBr	benzyl bromide
BnCl	benzyl chloride
BnN_3	benzylazide
$BnNH_2$	benzylamine
Bn-O(C=NH)CCl_3	benzyl 2,2,2-trichloroacetimidate
BSA	bovine serum albumin

cj1421-H$_6$	clone of gene *cj1421* to contain a C-terminal His$_6$-tag
cj1422-H$_6$	clone of gene *cj1422* to contain a C-terminal His$_6$-tag
Cj1421-His$_6$	*cj1421* expressed C-terminal His$_6$-tag protein (putative phosphoramidate transferase for Gal*f*NAc residue)
C. jejuni	*Campylobacter jejuni*
CAN	ceric ammonium nitrate
CDI	carbonyl diimidazole
CDP	cytidine diphosphate
CDP-choline	cytidine diphosphate-choline
Cm	chloramphenicol
CMP	cytidine monophosphate
COSY	correlation spectroscopy
CPS	capsular polysaccharide
CTP	cytidine triphosphate
DBU	1,8-diazabicycloundec-7-ene
DCC	*N,N'*-dicyclohexylcarbodiimide
(DHQD)$_2$PHAL	(*S*)-hydroquinidine 1,4-phthalazinediyl diether
DIAD	diisopropyl azodicarboxylate
DIPEA	diisopropylethylamine
DMAP	4-dimethylaminopyridine
DMC	2-chloro-1,3-dimethylimidazoliunium chloride
DMF	dimethylformamide
DMPSH	2,6-dimethylbenzenethiol

DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DTT	dithiothreitol
E. coli	*Escherichia coli*
EDTA	Ethylenediaminetetraacetic acid
ESI	electrospray ionization
Et_2O	diethyl ether
Et_3N	triethylamine
Et_3SiH	triethylsilane
EtOAc	ethyl acetate
EtOH	ethanol
EtSH	ethanethiol
GBS	Guillain–Barré Syndrome
GDP	guanosine diphosphate
Gal	D-galactopyranose
Gal*f*	D-galactofuranose
Gal*f*N	Gal*f*NAc analogue
Gal*f*NAc	2-acetamido-2-deoxy-β-D-galactofuranose
GalNAc	2-acetamido-2-deoxy-D-galactopyranose
Glc	α-D-glucopyranoside
GlcA	α-D-glucopyranuronic acid
GlcU	α-D-glucopyranuronamide
GM1/GM2	gangliosides

GMP	guanosine monophosphate
H_6-cj1415	clone of gene *cj1415* to contain an N-terminal His_6-tag
H_6-cj1422	clone of gene *cj1422* to contain an N-terminal His_6-tag
Hep*p*	6-*O*-methyl-D-*glycero*-L-*gluco*-heptopyranose
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
His_6	hexahistidine
His_6-Cj1415	*cj1415* expressed N-terminal His_6-tag protein (homology to APS kinase)
HMW	high molecular weight
HPLC	high-performance liquid chromatography
HR-MAS	high resolution magic angle spinning
HS	heat stable
HSQC	heteronuclear single quantum coherence
IBS	irritable bowel syndrome
IBX	2-iodoxybenzoic acid
Im	imidazole
IPTG	isopropyl 1-thio-β-D-galactopyranoside
Kan	Kanamycin
Kdo	2-keto-3-deoxy-octulosonic acid
LB	Luria Bertani
LevOH	levulinic acid
LOS	lipooligosaccharide
LPS	lipopolysaccharide

MCPBA	*meta*-chloroperoxybenzoic acid
MeOPN	methyl phosphoramidate
MS	mass spectrometry
MW	molecular weight
NBS	*N*-bromosuccinimide
nBu$_2$SnO	dibutyltin(IV) oxide
nBu$_4$NOH	tetra-*n*-butylammonium hydroxide
NDP	nucleoside diphosphate
NHS	*N*-hydroxysuccinimide
Ni^{2+}-NTA	Nickel-nitrilotriacetic acid
NIS	*N*-iodosuccinimide
NMI	*N*-methylimidazole
NMO	4-methylmorpholine *N*-oxide
NMP	nucleoside monophosphate
NMR	nuclear magnetic resonance
OD	optical density
ONBNH$_2$	*ortho*-nitrobenzylamine
PAPS	3'phosphoadenosine-5'-phosphosulfate
PBS	phosphate buffer solution
PCR	polymerase chain reaction
PhthCl$_2$	phthaloyl chloride
*p*I	isoelectric point
PivCl	pivaloyl chloride

$PMBNH_2$	*para*-methoxybenzylamine
PPh_3	triphenylphosphine
PTSA	*para*-toluenesulfonic acid
Py	pyridine
$(PyS)_2$	2,2'-dithiodipyridine
ReA	reactive arthritis
Rib*f*	β-D-ribofuranoside
rSAP	recombinant shrimp alkaline phosphatase
SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
STD-NMR	saturation transfer differential nuclear magnetic resonance
STEC	Shiga toxin-producing *E. coli*
T.V.	total volume
TBAF	tetra-*n*-butylammonium fluoride
TBAI	tetra-*n*-butylammonium iodide
TBDPSCl	*t*ert-butyldiphenylsilyl chloride
TBSOTf	*tert*-butyldimethylsilyl trifluoromethanesulfonate
*t*BuOH	*tert*-butyl alcohol
TCACl	trichloroacetyl chloride
TCICA	trichloroisocyanuric acid
TEMPO	2,2,6,6-tetramethyl-1-piperidinyloxy
THF	tetrahydrofuran
TiPSCl	2,4,6-triisopropylsulfonyl chloride
Tf_2O	trifluoromethanesulfonic anhydride

TfOH	trifluoromethanesulfonic acid
TLC	thin-layer chromatography
T_m	melting temperature
TMSBr	trimethylsilyl bromide
TMSOTf	trimethylsilyl trifluoromethanesulfonate
$(TolS)_2$	4-methylbenzene disulfide
TolSBr	*para*-toluenesulfenyl bromide
TolSH	4-methylbenzenethiol
Tris	tris(hydroxymethyl)aminomethane
$TrSBCl_6$	triphenylcarbenium hexachloroantimonate
TsCl	4-toluenesulfonyl chloride
UDP	uridine diphosphate
UDP-GlcA	uridine disphospho-α-D-glucopyranosuronic acid
UDP	uridine diphosphate
VCD	vibrational circular dichroism
UV	ultraviolet
WHO	World Health Organization
WT	wild-type

Chapter 1

Campylobacter jejuni: Background, Cell Wall Structure, Virulence Factors, and Research Overview

1.1 Background

Campylobacter is a genus of Gram-negative bacteria that belongs to the epsilon class of proteobacteria. It is microaerophilic (requires oxygen to survive) and helical-shaped. *Campylobacter jejuni* (*C. jejuni*), a species of *Campylobacter*, have small genomes (1.6–2.1 megabases) and can establish long-term associations with their hosts. *C. jejuni* exist as commensals in the gastrointestinal tract of wild and domestic birds; chickens are a common reservoir.[1] Figure 1-1 shows a scanning electron micrograph of *C. jejuni* cells. They are usually 3–4 μm in length and 0.2–0.5 μm in width and contain two flagella on opposite ends.

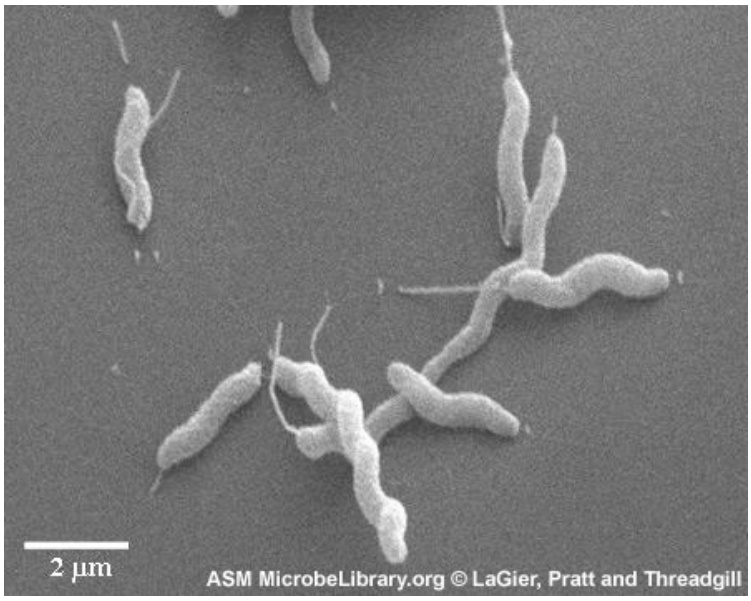


Figure 1-1: Scanning electron micrograph of *C. jejuni*. Reprinted with permission from American Society for Microbiology: microbelibrary.org.

1.1.1 Disease burden

Campylobacter is the leading cause of food-borne gastroenteritis, causing more foodborne illnesses than *Salmonella*, *Shigella*, and *Listeria* combined.[2] *Campylobacter* infects

~5.3 million people every year in North America alone and 400–500 million cases worldwide.[3] It is estimated 5–15% of these cases are represented by travelers.[4]

An increasing number of studies are highlighting *Campylobacter*-associated chronic health conditions that are the consequence of a previous infection (**Table 1-1**). Up to one third of Guillain–Barré Syndrome (GBS) cases can be attributed to past *Campylobacter* infections. GBS is a disorder in which the body's immune system attacks parts of the peripheral nervous system, which can lead to paralysis. The potential link between campylobacter infections and GBS is further discussed in Section 1.3.1 of this chapter. In addition to GBS, reactive arthritis (ReA), inflammatory bowel disease (particularly Crohn's Disease), and irritable bowel syndrome are also recognized as post-campylobacteriosis attributable risks in industrialized populations.

Table 1-1: Summary of post-*Campylobacter* infection risk of selected chronic health consequences.

Post-infective attributable risks		Comment
GBS	1 per 1,000	14–32% of GBS cases can be attributed to *C. jejuni*
Reactive arthritis (ReA)	1–5%	5% of *C. jejuni* ReA may be chronic or relapsing
Inflammatory bowel disease	3–4 per 10,000	*C. jejuni* can breach the intestinal barrier and lead to chronic inflammatory responses in susceptible individuals
Irritable bowel syndrome	1–10%	IBS developed in 36% of patients associated with outbreak of *Campylobacter* and STEC in Walkerton, ON, Canada. Symptoms persist in approximately 40–50% of these patients 5–7 years later.

Table adapted from *Front. Cell Infect. Microbiol.* **2012**, *2*, 1–11.[5] STEC = Shiga toxin-producing *E. coli*.

In a recent ranking of disease burden of pathogens in food sources, *Campylobacter* was estimated to cost the USA over $1.7 billion each year.[6] Annual *Campylobacter* estimated costs were approximately ~$2.69 billion in Europe[7] and $86 million in New Zealand.[8] Taken together,

these data highlight the clear importance of *Campylobacter* in global populations in terms of disease incidences.

1.1.2 Transmission

Several environmental reservoirs can lead to human infection by *C. jejuni*. The majority of the cases, however, are associated with handling raw poultry, consumption of raw or undercooked poultry meat or cross-contamination between raw and cooked foods.[9] The Public Health Agency of Canada has recently reported that approximately 90% of all chickens in supermarkets are infected with *C. jejuni*.[2] Other reservoirs that have contributed to human infection include drinking a contaminated water supply[10] or unpasteurized milk.[11,12] These infections likely occurred due to fecal cross-contamination.

1.1.3 Infection

To establish an infection, *C. jejuni* must bypass the mechanical and immunological barriers of the gastrointestinal tract. The first line of defense by the gastrointestinal wall is the mucus layer. It keeps the commensal bacteria at bay; however, pathogenic bacteria like *C. jejuni* have evolved various molecular strategies to penetrate the mucus layer. Figure 1-2 summarizes the various pathways *C. jejuni* can employ once reaching the epithelial layer. Pathway A shows the bacteria attaching to the epithelial cells (this process is known as adherence). At this stage, the organism can proliferate and form biofilms. Pathway B shows the bacteria invading the epithelial cell at which point it can continue to travel to its target organ, the colon. In addition, *C. jejuni*, like many Gram-negative bacteria, can produce toxins that affect epithelial cells (Pathway

C). The factors that allow *C. jejuni* to invade and infect are known as virulence factors and will be further discussed in Section 1.4.

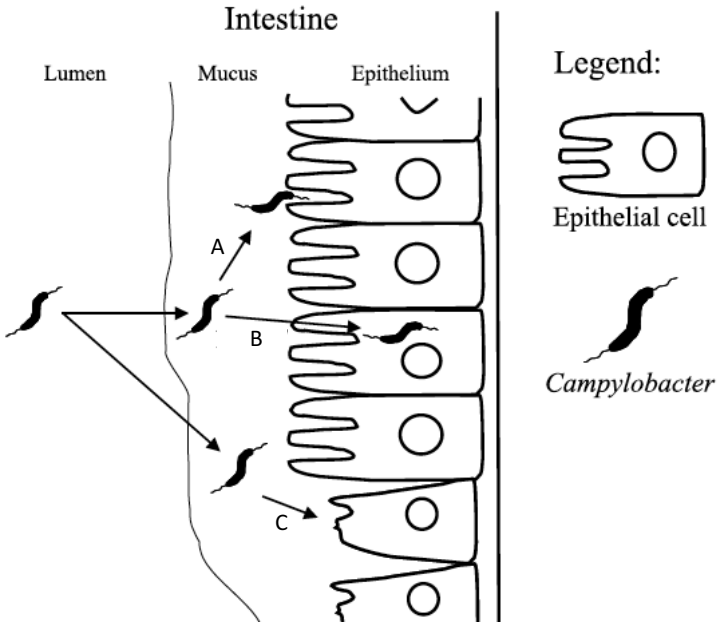


Figure 1-2: Overview of *C. jejuni* colonization of the intestine. Modified and reprinted with permission from John Wiley and Sons: *J. Appl. Bacteriol.*, *90*, 45S–56S, copyright 2001.[13]

1.1.4 Treatment and antibiotic resistance

Campylobacter infections are usually self-limited and rarely cause mortality. Most patients will recover without any specific treatment other than replacing lost fluids and electrolytes. In more severe cases, antibiotics are given to help clear the bacteria. *C. jejuni* antibiotic resistance is emerging globally and has already been recognized by the World Health Organization (WHO) as a problem of public health importance.[14,15] More specifically, *C. jejuni* strains have commonly developed resistance to tetracycline, fluoroquinolones, and erythromycin.[16,17] This is in part caused by an indiscriminate use of antibiotics in animal production to treat, control, and prevent infections.[16]

1.1.5 Prevention

Segregation of *Campylobacter*-positive chicken flocks from negative chicken flocks at the slaughterhouse has been an effective method of reducing the spread of contamination.[18] In Denmark, this approach produces *Campylobacter*-free poultry.[19] Strict cleaning practices in processing plants can also help reduce the spread of *Campylobacter* on surface areas. Perhaps the most practical approach is the use of sufficient cooking temperatures and times to eliminate the organisms, as long as working surfaces and utensils are not cross-contaminated.

Another preventative strategy is the potential use of vaccines. Although there is currently no vaccine against *C. jejuni* for humans, Monteiro and Guerry have shown the effectiveness of capsular polysaccharide (CPS)-based vaccines that prevent diarrheal disease in animal models. In their study, purified CPS from *C. jejuni* strain 81-176 (HS:23/36) conjugated to the CRM_{197} carrier protein was able to protect New World monkeys from diarrheal disease.[20] As a result, a CPS conjugate vaccine against strain 81-176 (HS:23/36) is currently in clinical trials (ClinicalTrials.gov identifier: NCT02067676). Aside from the tedious purification and conjugation methods required for the generation of CPS-based vaccines, a major drawback is that this strategy can only protect against a single serotype of *C. jejuni*. To date, there are over 60 serotypes of *C. jejuni*; therefore, a vaccine based on a single CPS is not practical in terms of broad coverage.

1.2 Cell wall structure

Similar to many Gram-negative bacteria, the cell wall contains an inner membrane and outer membrane with the periplasm and peptidoglycan layer in between the two membranes. Embedded in the outer membrane are lipooligosaccharides (LOSs) and capsular polysaccharides (CPSs). These polysaccharides have vital roles in *C. jejuni* biology, particularly host–bacterium interactions.

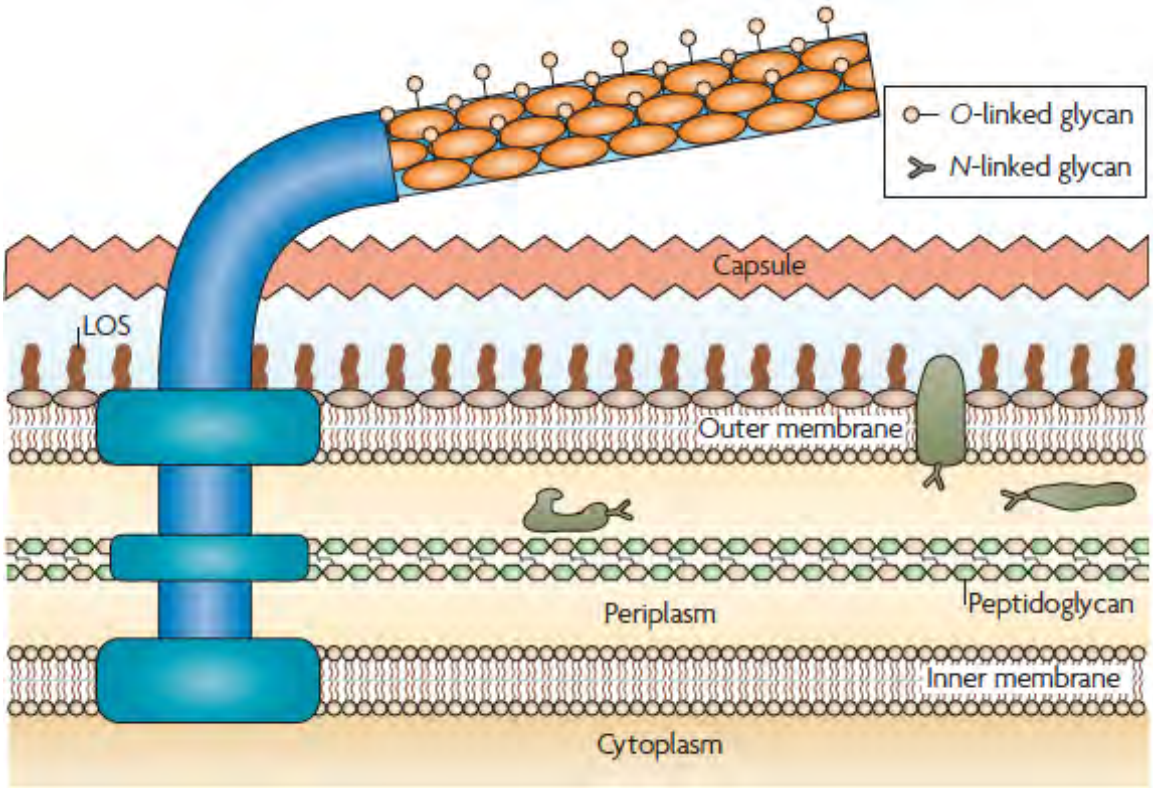


Figure 1-3: *Campylobacter jejuni* cell wall. Reprinted with permission from Nature Publishing Group: *Nat. Rev. Micro.*, *5*, 665–679, copyright 2007.[21]

1.2.1 Lipooligosaccharides (LOSs)

In addition to maintaining the structural integrity of the bacterium, the LOS is an important virulence factor (see Section 1.4.2). The structures of LOS are highly variable due to the LOS gene cluster possessing several homopolymeric nucleotide tracts (short DNA sequences

that contain the same nucleotide). The presence of the homopolymeric nucleotide tracts in genes is prone to phase variation that causes random mutation. To date, the structure of only a handful of LOS has been completely elucidated. One of these them, from *C. jejuni* NCTC11168, is shown in Figure 1-4.[22,23]

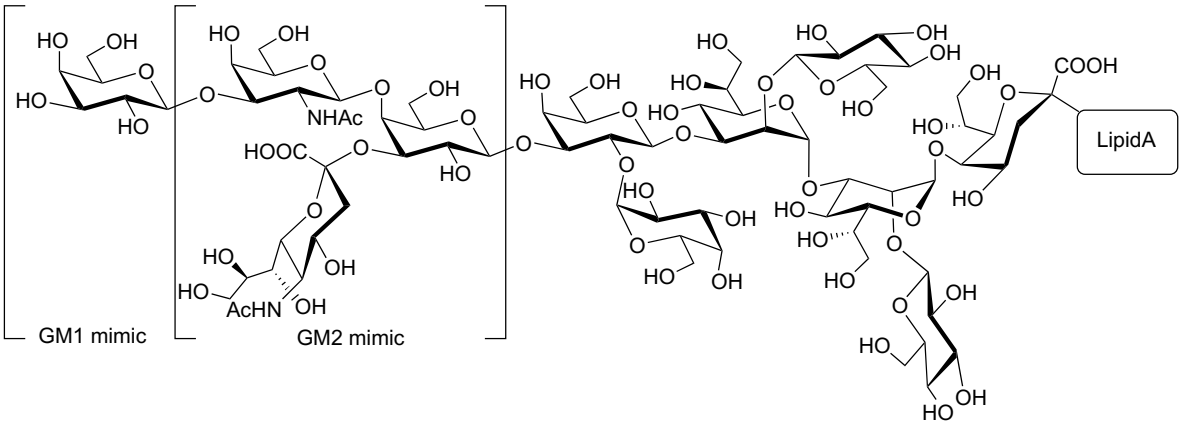


Figure 1-4: Lipooligosaccharide structure found in *C. jejuni* NCTC11168.[24]

Interestingly, *C. jejuni* are the only bacteria reported to be capable of incorporating sialic acid into its LOS. This unique feature allows the bacterial LOSs to mimic human gangliosides in structure (e.g. GM1 and GM2 mimics shown in Figure 1-4). It is this mimicry that can cause the antibodies generated against the LOS to cross-react with host gangliosides and lead to the development of GBS as well as Miller–Fisher syndrome (a nerve disease variant of GBS).[25-28]

1.2.2 Capsular polysaccharides (CPSs)

The *C. jejuni* CPSs were once recognized as high-molecular weight lipopolysaccharides (HMW-LPS). This mistake was, in part, due to the challenges of distinguishing CPS from LPS as they are often found associated with each other. Despite the difficulties, some of this earlier work postulated the presence of CPS instead of LPS.

Some initial speculation was based on the work of Jann and Schmidt on characterizing CPS antigens and analysis of the lipid moiety of *E. coli* K12 and K82 strains.[29] This study found that CPSs contained 1,2-dipalmitoyl glycerolphosphate as a labile lipid moiety. The labile linkage was observed by Savage and coworkers when elucidating the chemical structure of the HMW-LPS found in *C. jejuni* strain 176.83 (HS:41), and led them to speculate that LPS was rather a CPS.[30] In addition, some ambiguities were reported when serotyping *C. jejuni* strains. Serotyping of *C. jejuni* is based on a technique developed by Penner et al.[31] The method, in general, involves extracting HMW-LPS by heating the suspension of bacteria in saline to 100 °C, and centrifugation. The supernatant can then be used in passive hemagglutination techniques for the detection of antibodies. In serotyping *C. jejuni*, however, Rowe and coworkers noticed and reported some unusual observations.[32] First, after using the extraction protocol developed by Penner, the extracted "HMW-LPS" was not visible using silver staining (a method to visualize LPS) on a sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) gel. In addition, lack of visualization by immunoblotting with anti-LPS rabbit antiserum suggested that the polysaccharide extracted was not LPS. In addition, the antigenic material could readily be eluted from the bacterial surface by incubation at 50 °C whereas LPS must be incubated at 100 °C for this to occur. Finally, its resistance to proteolytic digestion suggested the material was a CPS. These results, unfortunately, were regarded as technical problems[33] and went largely unnoticed until the *C. jejuni* NCTC11168 genome was sequenced in 2000.[34]

The first genetic evidence for the presence of CPS emerged when Wren and coworkers constructed a random library of primers to clone various portions of the chromosomal DNA of *C. jejuni* NCTC11168. Approximately 100 amplified regions were cloned and sequenced. Database searches of these sequences resulted in the identification of genes encoding proteins similar to

those involved in CPS biosynthesis (e.g., the kps gene cluster).[35] The sequencing of the genome confirmed the initial data and a gene cluster with significant sequence similarity to CPS transport genes of *E. coli* was found.[34] Biochemical evidence was later established by mutation of the newly identified CPS transport genes *kpsM*, *kpsS*, and *kpsC*, which showed the loss of the thermostable *O*-antigen.[36] In addition, the *C. jejuni* strain 81-176 *kpsM* gene was shown to complement or restore the function of a deleted gene similar in homology in *E. coli*.[37]

The presence of CPS were later supported by staining with Alcian blue dye[38] (known to stain bacterial CPSs) and electron microscopy to visualize the presence and absence of CPSs in *C. jejuni*.[39] Figure 1-5a*i* shows the staining of extracted CPS from the wild-type strain (Lanes 1 and 4) and the absence of the CPS from a *kpsM* knockout mutant (Lanes 2 and 3). Figure 1-5a*ii* shows the presence of the CPS and LPS after staining with the Alcian blue dye and silver staining. Figure 1-5b shows an electron micrograph of the wild type straining possessing the CPS (Figure 1-5b*i*) and the *kpsM* mutant that lacks the CPS on its cell surface. Further evidence was given by the identification of the phospholipid anchor in three CPS types (HS:3, HS:6, and HS:23/36) as dipalmitoyl-glycerophosphate.[40] LPS possess Lipid A as the phospholipid anchor (Figure 1-6a); a glycerophosphate as the phospholipid anchor strongly suggests the presence of a CPS.[41]

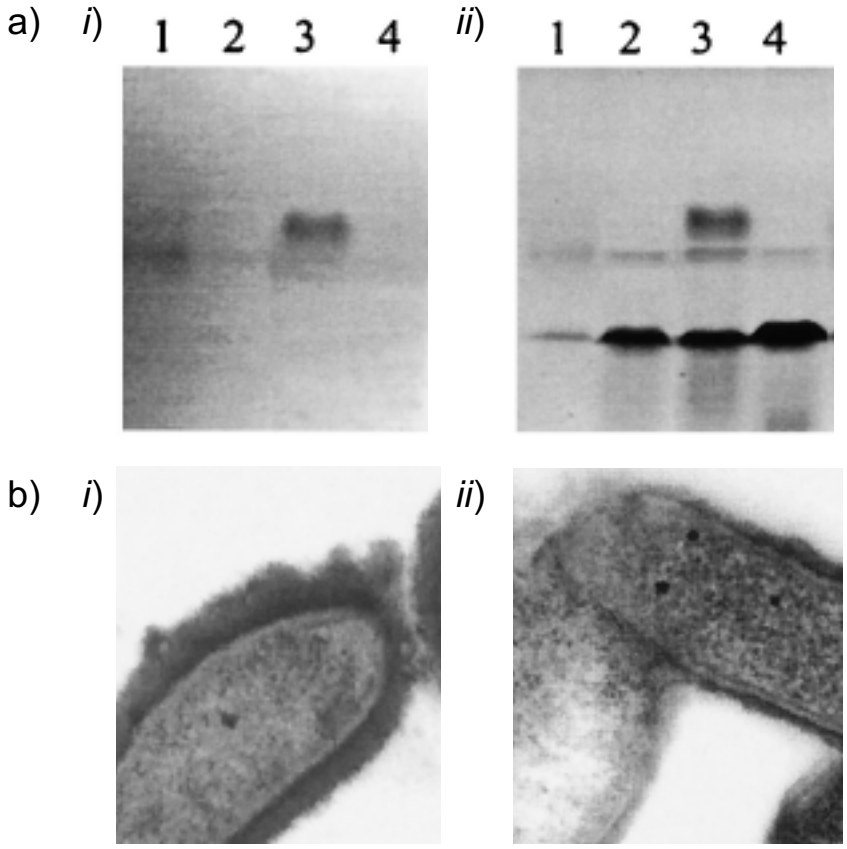


Figure 1-5: Visualization methods of *C. jejuni* CPS. a) SDS-PAGE gel[38] *i*) strained with Alcian blue dye *ii*) strained with Alcian blue dye and silver. Lanes 1 & 3 are wild-type strain. Lanes 2 & 4 are *kpsM* mutants. b) Electron microscopy[39] *i*) wild-type strain *ii*) *kpsM* mutant (lacks CPS transport protein). Reprinted with permission from American Society For Microbiology: *J. Clin. Microbiol.*, *39*, 279–284, copyright 2001 and *Infect. Immun.*, *69*, 5921–5924, copyright 2001.

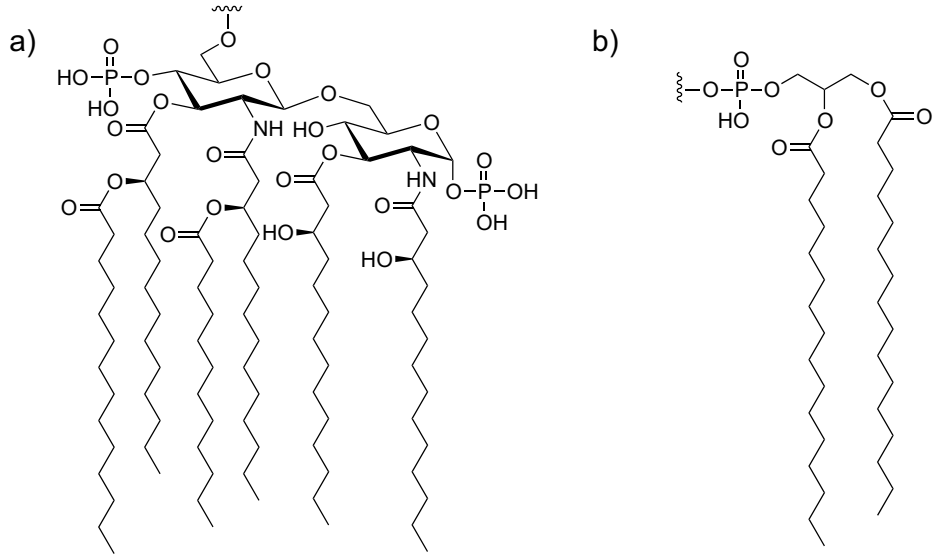


Figure 1-6: Common lipid anchors a) Lipid A (LPS) b) Dipalmitoyl glycerophosphate (CPS).

The CPS can act as a barrier to substances entering or leaving the cell. Unlike the other glycans (LOS, *O*-glycans, *N*-glycans) found in *C. jejuni*, the CPS is constructed of regular repeating subunits of 1–6 monosaccharides that can extend up to 200 sugar residues.[42] The CPS forms a layer around the cell and can extend anywhere from 100–400 nm. Similar to LOS, the CPS is a virulence factor (see Section 1.4.4) and is highly variable.

To date, there are over 60 serotypes of *C. jejuni* and it is predicted that each serotype possesses its own unique CPS structure varying in sugar composition and linkage. The high variation of CPS structures is caused by exchange of entire clusters by horizontal transfer, gene duplication, deletion, fusion and gene variation.[43] To date, only a handful of the CPS structures have been elucidated – HS:1,[44] HS:2,[22,45] HS:3,[28,46] HS:4,[47] HS:10,[48], HS:13,[49] HS:15,[50] HS:19,[51] HS:23/36,[52,53] HS:41,[30] and HS:53.[5,54] The structures of the repeating units from two CPS are shown in Figure 1-7.

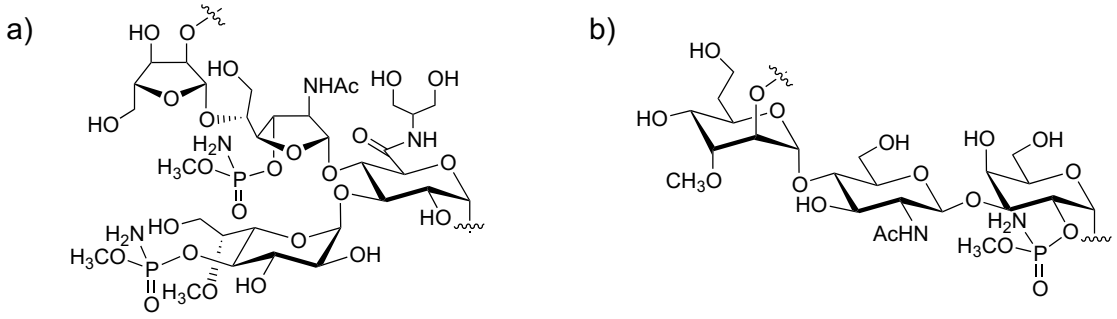


Figure 1-7: CPS structure of *C. jejuni* a) 11168H (HS:2) b) 81-176 (HS:23/36).

Of particular relevance to this thesis is the CPS from *C. jejuni* 11168H (Figure 1-7a), which possesses a repeating tetrasaccharide unit consisting of the sugar residues α-D-glucopyranuronic acid (GlcA) amidated with serinol, 2-acetamido-2-deoxy-β-D-galactofuranose (Gal*f*NAc), β-D-ribofuranose (Rib*f*), and a D-*glycero*-L-*gluco*-heptopyranose (Hep*p*).[22,45] On the other hand, the *C. jejuni* 81-176 (HS:23/36) CPS contains a trisaccharide repeating unit consisting of sugar residues α-D-galactopyranose (Gal), a 2-acetamido-2-deoxy-β-D-glucopyranose (GlcNAc), and a 6-deoxy-α-D-*altro*-heptopyranose (6d-Hep*p*).[52,53]

Despite the variability of sugar composition and linkages, one of the key structural markers of the CPS in *Campylobacter* is the presence of heptose residues in several configurations (Figure 1-7).[5,43] The structural complexity of the heptoses is further enhanced by the introduction of a deoxy function at the C-6 position. An example of these residues is found in the repeating trisaccharide unit in *C. jejuni* 81-176 (Figure 1-7b). The different configurations of the heptose residues present in *C. jejuni* will be discussed further in Chapter 3 of this thesis.

In addition to the various sugar composition, the high variability of the CPS structures is also due to the presence of several phase variable modifications (Figure 1-7).[44,45] Some of these modifications include various amines (e.g. 2-deoxy-2-amino-glycerol, ethanolamine) that amidate the glycuronic acid residues, various *O*-methylation, and the presence of a methyl

phosphoramidate (MeOPN) motif that has not been observed anywhere else in nature.[45] Similar to the heptose residues, the MeOPN motifs are another key structural marker for *Campylobacter* species. Although rare in nature, an estimated 70% of *C. jejuni* strains possess this unique moiety.[55]

The presence of these labile MeOPN moieties was serendipitously discovered by the use of high resolution magic angle spinning (HR-MAS) NMR spectroscopy in examining the CPS of intact *C. jejuni* cells.[45] Traditional CPS extraction methods (the hot phenol and water method)[56] caused a loss of the MeOPN motif.[45] The MeOPN structure was deduced by analysis through mass spectrometry and ^{1}H NMR (methyl signals in MeOPN) and ^{31}P NMR (chemical shift consistent with phosphoramidate) spectroscopy of whole campylobacter, which had not undergone these extraction protocols.[57] Further support of the structure was added by the ^{1}J (^{15}N–^{31}P) = 39 Hz, which is consistent with other reports,[58-61] after growing *C. jejuni* in $^{15}NH_4Cl$-enriched media.[62] NMR spectroscopy shows these MeOPN moieties exist as a single stereoisomer; however, to date, the stereochemistry of the MeOPN is unknown. Interestingly, these modifications alone have shown to be a virulence factors (see Section 1.4.4.1).

1.3 Pathogenicity and virulence factors

For bacteria to be pathogenic, they must have the capacity to infect and invade before colonizing. To infect, the bacteria must possess the cellular components that can contribute to pathogenicity; these components are known as virulence factors. Virulence factors can include the ability for adherence to host cells, the production of toxins, invasive capacity, and the ability to evade the host's immune system.[13,63] Although specific virulence mechanisms have not been

clearly elucidated for *C. jejuni*, the presence of cellular components that can contribute to these include the flagella, LOSs, and CPSs.[10]

1.3.1 Flagella

The physical motility of bacteria is important to evade parts of the host's immune system as well as colonization. Like many bacteria, *C. jejuni* regulates motility through a chemotaxis system in which chemical stimuli direct its movements away from repellants and towards attractants.[64,65] In addition to colonization through mobility, the flagella were found to be important for the secretion of effector proteins (e.g., CiaB, CiaC) similar to a type 3 secretion system.[66] This injection of effector proteins in epithelial cells influences bacteria–host interactions and helps *C. jejuni* invade host cells through a "trigger" mechanism described by Croinin and Backert.[3] Lastly, the glycosylation of flagella is predicted to help the organism evade the host innate immune system by potentially masking its protein receptors (e.g., TLR5) as well as to modulate autoagglutination and facilitate biofilm formation.[64,67]

1.3.2 Lipooligosaccharides (LOSs)

The LOSs plays an important role in maintaining the structural integrity of the bacterium by acting as a barrier to substances entering or leaving the cell. Its high variation in structure could potentially play a role in its adaption to harsh microenvironments as well as avoidance of the innate immune response. Variation in LOS structure will present the host with a shifting set of antigens, some of which have been shown to have similarity to host structures. For instance, the ability for *C. jejuni* to produce sialic acid was shown to prevent killing by the host immune system. On the other hand, knockout mutants that lack sialic acid residues showed greater

immunoreactivity and decreases in killing by host serum.[68,69] As described earlier, the similarity of LOS structures to host gangliosides can lead to cross-reactivity of the generated antibodies for the development of GBS (see Section 1.3.1). The role of LOS is also important for *C. jejuni* invasion capacity. Some studies have shown that bacteria with truncated LOS structures through mutation of some biosynthetic genes exhibited decreases in adherence[69] and colonization.[70]

1.3.3 Capsular polysaccharides (CPSs)

As the most external polysaccharide of *C. jejuni*, the CPS is important in the first interactions between pathogens, their host, and the environment. They have been found to play important roles in bacteria survival as well as colonization. Like the LOS, the CPS is a barrier to substances entering or leaving the cell. In addition, because the CPS is highly hydrated, they can protect the bacteria from harmful effects of desiccation. This results in the increased survival outside the host compared to non-encapsulated bacteria, which in turn, helps promote transmission.[71,72]

CPSs, like the LOSs and *O*-glycans found on the flagella, can promote adherence to surfaces and to each other.[37,73,74] This often facilitates the formation of biofilms. Biofilms are advantageous as they could provide protection from phagocytes.[75] In addition, biofilms have also been shown to act as a barrier to antibiotics.[75]

The CPS is responsible for offering resistance to host immunity.[76] Although most CPSs can elicit an immune response, they are, like most carbohydrates, generally poor immunogens due to their inability to stimulate T-cell activation. Like the LOSs, the extensive variation in CPS structure can present the host with a shifting set of antigens. In addition, the CPS can partially mask underlying structures such as the LOSs and other antigenic components (e.g., Lipid A).

This masking can prevent the binding of serum proteins involved in the formation of membrane attack complexes.[77] Some studies suggest that the CPS can also prevent phagocytosis, by blocking access of phagocytes.[72,78] Furthermore, some CPS are known to modulate the release of cytokines by the host.[79] For instance, Dallman and coworkers demonstrated that *C. jejuni* NCTC11168 mutants that lack CPS showed increased production of interleukin-6, interleukin-10, and tumor necrosis factor-alpha from murine dendritic cells.[80] These cytokines are important for cell signaling for the activation of the innate and complement immune system.

Studies have also shown the importance of the CPS in invasion. A *kpsM* mutant of *C. jejuni* strain 81-176, which lacks to the CPS on its cell surface, showed a reduction in invasion of intestinal epithelial cells *in vitro*.[37] Similar results were obtained using non-encapsulated mutants of other strains.[81] In addition, encapsulated strains have shown to colonize chickens better than mutants lacking the CPS.[74] Although their modes of action are not known, these results collectively show the importance of the CPS for *C. jejuni*. Interestingly, disrupting the biosynthesis of one of the phase variable modifications found on the CPS, such as the MeOPN motif, has also been shown to be an important biological factor for *C. jejuni* when present (See section 1.3.3.1).

1.3.3.1 Role of MeOPN as a virulence factor

Little is known about the precise biological role of these unique MeOPN modifications; however, studies suggest that *C. jejuni* strains that contain MeOPN motifs on their cell surface appear to be more virulent than those that do not. On a study of 63 strains, MeOPN was observed for 82% of strains that cause gastrointestinal enteritis.[82] In addition, 80% of the GBS and 100%

of the Miller–Fisher syndrome-associated strains express the MeOPN motif on their CPS, which suggests their role in *C. jejuni* biology is important.

Champion et al. reported that the larvae of the wax moth, *Galleria mellonella*, are suitable infection model for *C. jejuni*. In their study (Figure 1-8), they demonstrated that wild-type strains (11168H) that synthesize MeOPN on its CPS had significant insecticidal activity when compared to mutants (e.g. *cj1416*) that lack the motif.[83] Reintroduction of the gene (cj1416+) returned the killing of the wax moth larvae. This activity could be potentially attributed to the MeOPN motif, which is structurally similar to several organophosphate pesticides (e.g. methamidophos, propetamphos oxon).[84,85] These pesticides work by inhibiting acetylcholinesterase, which is an enzyme essential for nerve functioning. However, the relevance of the insecticidal activity of MeOPN to its role in disease caused by *C. jejuni* infections, remains to be established.

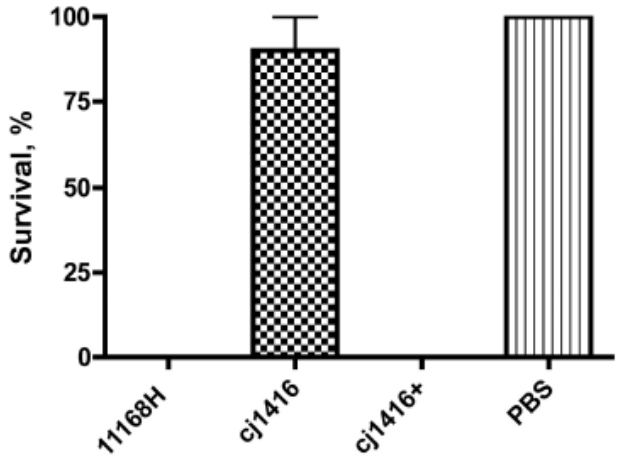


Figure 1-8: Killing of *Galleria mellonella* larvae by infection with *C. jejuni* 11168H, the *cj1416* mutant, which lacks the MeOPN motif and complementation of *cj1416* at 24 h after challenge. Phosphate buffer solution (PBS) was used as a control. Groups of 10 larvae were used in each challenge study. Reprinted with permission from Oxford University Press: *J. Infect. Dis.*, *201*, 776–782, copyright 2010.[83]

In addition, some studies suggest that the MeOPN motifs play significant roles in reduced killing by the complement system as well as in colonization. Guerry and coworkers demonstrated

the role of MeOPN motifs in killing by treating the *C. jejuni* strain 81-176 (HS:23/36) with normal human serum. Figure 1-9 shows that the wild type strain (black line) has survival rates greater than 60% after exposure to normal human serum over 60 min. The *kpsM* mutant (green line), which lacks a gene that is involved in CPS transport across the lipid membrane, shows significant sensitivity towards normal human serum. Similarly, the *mpnC* mutant (red line), which lacks a gene essential for MeOPN biosynthesis, also shows a significant increase in sensitivity to normal human serum. Returning this gene in a complementation experiment results in resistance to normal human serum (blue line), which suggests the MeOPN could be important in preventing killing by the complement system.

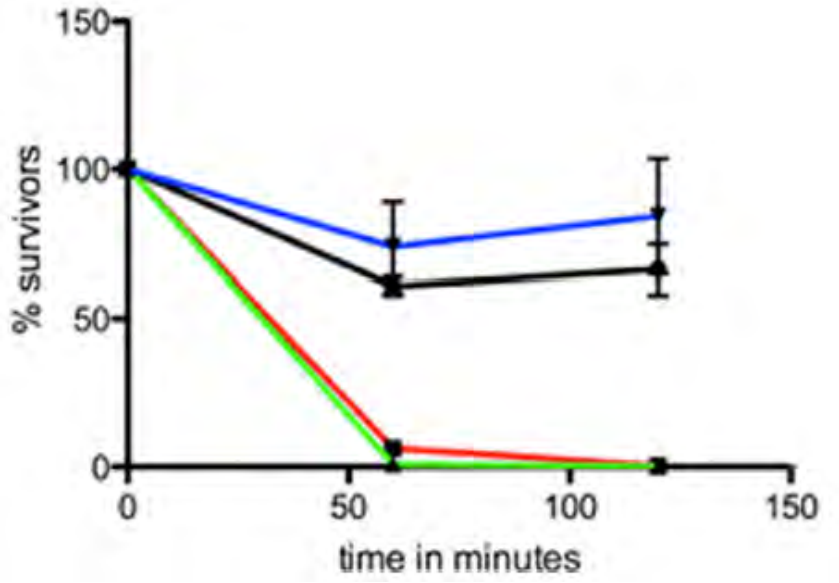


Figure 1-9: MeOPN prevents killing by normal human serum in *C. jejuni* 81-176. Black, wild-type strain 81-176; red, *kpsM* mutant; green, *mpnC* mutant; blue, *mpnC* mutant complement. Reprinted with permission from American Society for Microbiology: *Infect. Immun.*, *81*, 665–672, copyright 2013.[55]

Figure 1-10 compares the colonization of the wild type *C. jejuni* 81-176 strain and two mutant strains, one lacking the *kpsM* gene and the other lacking the *mpnC* gene, in mice. Figure 1-10a and 1-10b show a high level of colonization in mice up to 15 days post-infection with the wild type *C. jejuni* strain (solid line). On the other hand, mice infected with *kpsM* mutant had a

short duration of colonization; the majority of the *kpsM*-infected mice cleared the infection by day 18. In a parallel experiment, the colonization capacity of the *mpnC* mutant was similar to the wild type strain up to 15 days post-infection. Although the *mpnC* mutant did not demonstrate a significant reduction in colonization as observed in the *kpsM* mutant, the general trend supports the hypothesis that MeOPN motifs synthesized on *C. jejuni* cell surfaces may be involved in bacterial colonization.

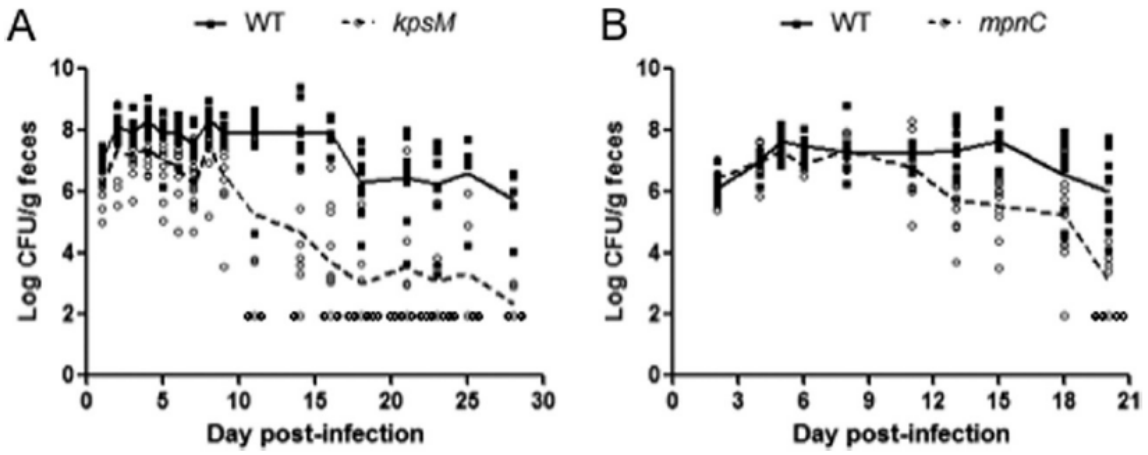


Figure 1-10: MeOPN affects colonization. Reprinted with permission from American Society for Microbiology: *Infect. Immun.*, *81*, 665–672, copyright 2013.[55]

Furthermore, Wren and coworkers have shown that *C. jejuni* 11168H mutants that lack the MeOPN motif on their CPS show an increased production of cytokines important for the activation of the host immune system.[80] In their study (Figure 1-11), they compare production of the cytokines interleukin-6, tumor necrosis factor-alpha, and interleukin-10 of the wild type strain and two mutants, one that lacks the CPS (*cj1439*) and another that lacks the MeOPN on its CPS (*cj1417*). In each mutant study, there was an increase in cytokine production compared to that of the wild type strain. Notably, the increased production interleukin-6 and interleukin-10 of the mutant lacking the MeOPN group on its CPS was similar to that of the mutant lacking the CPS altogether, which suggests the importance of the MeOPN motif to this effect.[80]

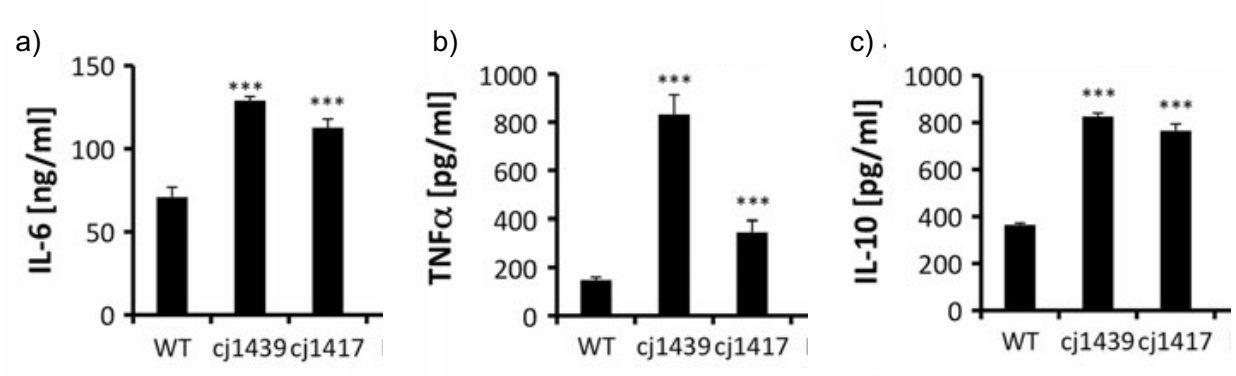


Figure 1-11: MeOPN affects cytokine production in *C. jejuni* 11168H. a) Interleukin-6, b) Tumor necrosis factor-α, c) Interleukin-10. Reprinted with permission from Springer: *Med. Microbiol. Immunol.*, *201*, 137–144, copyright 2012.[80]

Collectively, these studies support a significant role of the MeOPN motif for *C. jejuni* in colonization, resistance to killing by human serum, and cytokine production. This suggests targeting the proteins involved in the MeOPN biosynthesis could potentially be used as an approach for the development of new therapeutic agents against *C. jejuni*.

1.4 Overview of thesis research

Since the sequencing of *C. jejuni* NCTC11168 genome, significant progress has been made in identifying genes and the putative function of their encoded proteins based on homology studies. Large contributions have also been made to understand the role of specific virulence factors by the generation of knock out mutants (e.g., the CPS role in invasion and cytokine production). Despite these advancements, little progress has been made to develop strategies to combat future *C. jejuni* infections. The only strategy that is currently being developed is CPS-based vaccines. This has been shown to protect New World monkey against diarrheal disease. This approach, however, is currently only beneficial for one *C. jejuni* serostrain. There are, to

date, over 60 serostrains that have been identified. Specific to the CPS gene cluster, little effort has been made to characterize the genes encoding biological function. For instance, the CPS glycosyltransferases and proteins for MeOPN biosynthesis have been putatively identified, but not characterized. This is in part due to the difficulty in working with the CPS. Unlike the LOS and the glycans found on the flagella and proteins, the CPS contains a repeating unit; therefore, knockout mutant studies often abolishes the CPS altogether. This makes it difficult to correlate the gene to its encoded protein as well as to make any inferences. Therefore, to characterize these proteins, a biochemical approach is needed.

With respect to this thesis, we focus on the MeOPN motifs. The first part of this thesis will focus on obtaining the MeOPN motifs through chemical synthesis. Conjugation of these MeOPN-containing substrates to proteins will lead to materials that can be used as antigens for immunological studies. The second part of this thesis will focus on developing probes to help unravel the biosynthetic steps involved in adding the MeOPN motif to the CPS. Unraveling the biosynthesis of the MeOPN motifs could potentially aid in the development of new therapeutic agents that can specifically target *C. jejuni*. As described earlier, these structurally unique motifs are rare in nature (only found in *Campylobacter* species). Although rare in nature, these modifications have been found in approximately 70% of *C. jejuni* strains. Moreover, from an ultimate drug-development standpoint, while targeting the MeOPN will not affect all strains, this approach can target the more pathogenic strains (82% of the enteritis strains possessed the MeOPN motif).

1.4.1 Research objective 1 – synthesis of MeOPN motifs

Obtaining the MeOPN motif through natural sources is not feasible due to the modification being labile (particularly to acid). Therefore, a more suitable route to obtain quantities of MeOPN motifs for use in biochemical/biological investigations is through chemical synthesis. Although the synthesis of phosphoramidates has been achieved, when I started this work there were no reported chemical syntheses of MeOPN motifs. In addition, due to the MeOPN being labile, it was necessary to assess the motif's stability through various transformations that would be required in multi-step chemical synthesis. Therefore, this research objective contains two parts that are summarized in Figure 1-12.

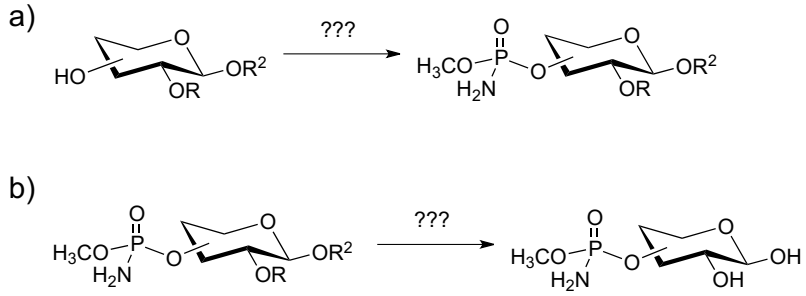


Figure 1-12: Research objective 1 summary. a) Develop method to synthesize MeOPN, b) Assess stability of MeOPN motif.

The first aim of the research objective 1 was to develop a reliable method to synthesize the MeOPN motif. The second aim was to assess the stability of the MeOPN motif through various chemical transformations. This will enable the synthesis and conjugation of MeOPN-containing substrates to carrier proteins for immunological experiments.

1.4.2 Research objective 2 – unravel the biosynthetic steps for the construction of the MeOPN motif

The *C. jejuni* 11168H CPS gene cluster and structure are shown in Figure 1-13. By using high resolution magic angle spinning (HR-MAS) NMR spectroscopy as a high throughput screening method, Szymanski and coworkers were able to identify subtle changes in the CPS structure of intact *C. jejuni* cells. Using this method with a library of *C. jejuni* knockout mutants, they were able to identify the genes involved in the biosynthesis of the MeOPN motif.

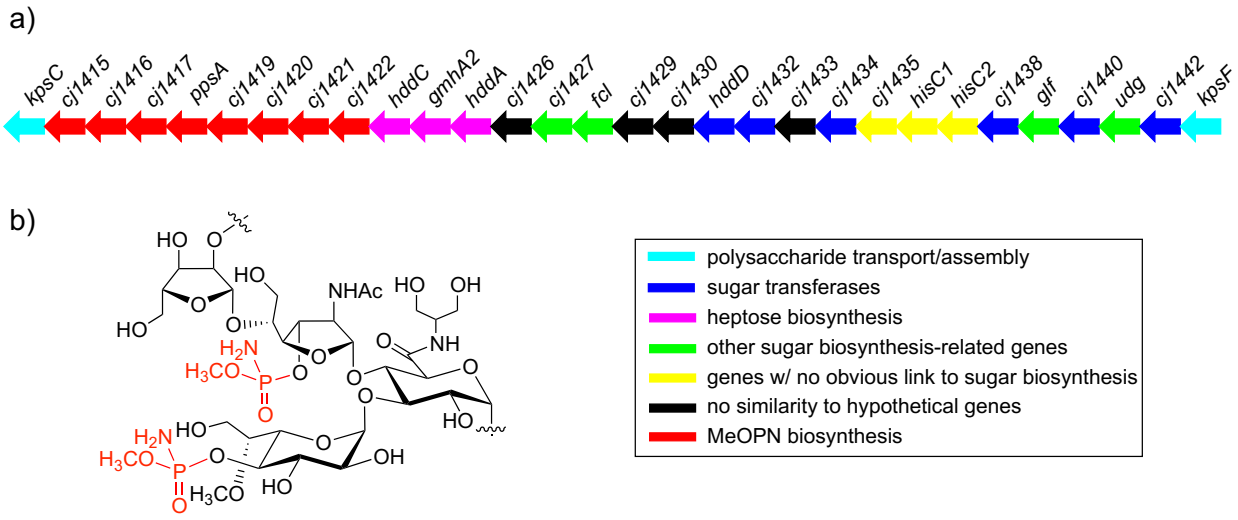


Figure 1-13: *C. jejuni* 11168H CPS a) Gene cluster and putative functions b) Structure.

The genes colored in red (Figure 1-13a) were identified as genes involved in MeOPN biosynthesis. Through the knockout mutant studies, Szymanski and coworkers identified *cj1421* and *cj1422* as the phosphoramidate transferases for the Gal*f*NAc and Hep*p* residues, respectively. In this research objective, the goal is to demonstrate enzyme activity for these two proteins. In doing so, the donor in which the transferases use would be identified. To achieve this, however, several components are required to determine enzyme activity. First, substrate(s)

that can mimic the core CPS structure is required. Second, the phosphoramidate transferases are needed. Last, the phosphoramidate donor is needed.

Aim 1. Synthesize a tetrasaccharide (**1-1**, Figure 1-14) substrate to mimic core CPS structure. The tetrasaccharide repeating unit could be an appropriate acceptor substrate for the phosphoramidate transferases. Unfortunately, the repeat unit itself has not been isolated; therefore, its chemical synthesis is needed. A retrosynthetic scheme for its synthesis is shown in Figure 1-14. The core tetrasaccharide **1-1** could be obtained from the four building blocks – Rib*f* donor **1-2**, Gal*f*NAc analogue **1-3**, Hep*p* residue **1-4**, and Glc acceptor **1-5**.

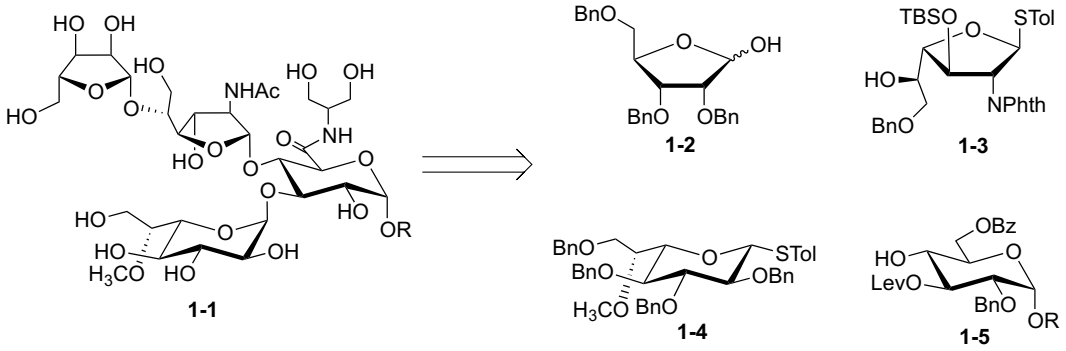
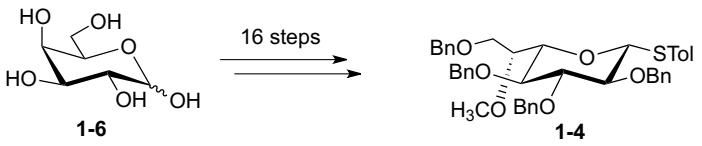


Figure 1-14: Retrosynthetic analysis of the tetrasaccharide found in *C. jejuni* 11168H CPS repeating unit.

Building blocks **1-2**, **1-3** and **1-5** could be achieved through traditional carbohydrate synthesis using commercially available monosaccharides. For **1-4**, the required D-*glycero*-L-*gluco*-heptose, however, is not commercially available and must be synthesized. Our group has previously reported the synthesis of the Hep*p* donor **1-4** in 16 steps from D-galactose (Figure 1-15); therefore, this work will be reproduced. In addition, an alternative *de novo* asymmetric strategy for its synthesis will also be developed for a more expediency.

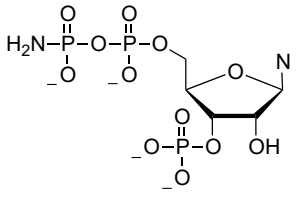


1-6 1-4

Figure 1-15: Reported synthesis of D-*glycero*-L-*gluco*-heptopyranoside analogue **1-4**.[86]

Aim 2. Clone and express the proteins encoded for *cj1415*, *cj1421*, and *cj1422*. The genes will be cloned using standard approaches. Each gene will be incorporated into pET28a or pET30a vectors. The plasmids carrying sequences for His_6 tags will then be transformed into BL21(DE3) competent cells for protein expression. Purification of the His_6-tagged proteins will be carried out using nickel-nitrolotriacetic acid (Ni^{2+}-NTA) columns.

Aim 3. Obtain the phosphoramidate donor. Based on homology studies of gene *cj1415* to an APS-kinase, we postulate that the phosphoramidate donor is **1-9** (Figure 1-16). Unfortunately, the nucleobase has not been identified; therefore, analogues of adenine, cytidine, guanine, and uridine will be needed.



1-9
N = A, C, G, U

Figure 1-16: Postulated phosphoramidate donor. A = adenine, C = cytodine, G = guanine, U = uridine.

Once the postulated donor is obtained, we will work to identify the nucleobase by either saturated transfer differential nuclear magnetic resonance (STD-NMR) or measuring for enzyme activity. Once identified, **1-9** and acceptor substrate **1-1** will then be incubated with the

expressed proteins from *cj1421* and *cj1422*. The activity will be measured through a combination of HPLC and mass spectrometry. If successful, these studies will represent the first detailed investigations of the biosynthetic proteins involved in the assembly of the MeOPN motif.

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Chapter 2

Synthesis of Carbohydrate Methyl Phosphoramidates

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2.1 Background

As described in Chapter 1, there are more than 60 serostrains reported for *C. jejuni* and it is predicted that each one produces a capsular polysaccharide (CPS) with a different structure.[1] The broad diversity of structures is due in part to the addition of phase-variable modifications such as *O*-methyl, ethanolamine, aminoglycerol, and *O*-methyl phosphoramidate (MeOPN) groups.[2-4] Of these modifications, perhaps the structurally most interesting is the MeOPN motif, which is found in over 70% all *C. jejuni* strains.[5] As examples, Figure 2-1 show the repeating core structures of two *C. jejuni* strains that possess MeOPN moieties in their CPS.[2,6-9] The role of these MeOPN moieties remains unclear; however, recent studies suggest they play roles in serum resistance and colonization[5] and they have also been shown to have insecticidal activity,[10] although this latter activity has been questioned.[11]

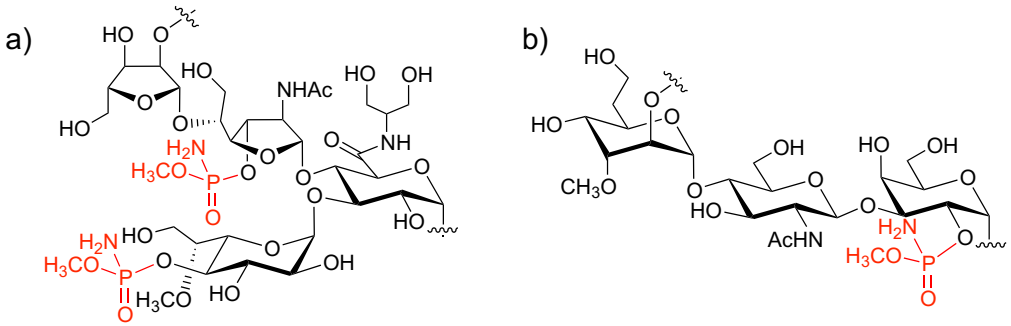


Figure 2-1: Repeating unit structures of the CPS. (a) *C. jejuni* 11168H and (b) *C. jejuni* 81-176.

Guerry and coworkers have shown that a 81-176 CPS CRM_{197} protein conjugate can serve as a vaccine, protecting non-human primates against *C. jejuni* diarrhea.[12] One drawback to this vaccine is that this CPS conjugate only protects against the *C. jejuni* strain 81-176. Because, there are more than 60 *C. jejuni* serostrains with each producing a different CPS structure,

developing a conjugate for each is not feasible. Due to the rarity of the MeOPN motif, which exists as a single, although currently unknown, stereochemistry on phosphorus,[2,5] its antigenicity could be explored for the development of vaccines.[12,13] A vaccine specific to the MeOPN motif could potentially target more than 70% of *C. jejuni* strains. In addition, access to MeOPN-functionalized carbohydrates could help unravel the biosynthetic pathway by which these groups are assembled and lead to future development of new therapeutic agents against *C. jejuni*. However, such studies require a method for the synthesis of the MeOPN motifs.

To date, there are several approaches that have been reported for the synthesis of phosphoramidates, which are summarized in Figure 2-2. For example, dialkyl/diaryl phosphates (e.g., **2-1**) can be converted to the phosphoramidate **2-8** by treatment with an activating reagent such as 4-toluenesulfonyl chloride or *N*,*N*'-dicyclohexylcarbodiimide and then the addition of an amine nucleophile.[14,15] Phosphochloridate **2-2** can be treated directly with an amine nucleophile to generate the phosphoramidate.[16,17] These functionalities can also be prepared from *H*-phosphonates (e.g., **2-3**) via oxidization by halogenating agents in the presence an amine nucleophile[18] or alternatively by oxidative cross-coupling with amines/amides.[19] Boranophosphates (e.g., **2-4**) can also serve as a precursor to phosphoramidates, through treatment with iodine in the presence of an amine nucleophile.[20] Treatment of phosphoryl azides (e.g., **2-5**) with an amine[21] leads to phosphoramidates as does their couplings via a C–H activation approach.[22] P(III) phosphorus reagents can also be used for the synthesis of phosphoramidates. For example, phosphoramidites (e.g., **2-6**) can be directly oxidized to phosphoramidates.[23] In addition, phosphites (e.g., **2-7**) can be treated with alkyl/aryl azides to generate a phosphoramidate in a Staudinger–phosphite reaction.[24,25]

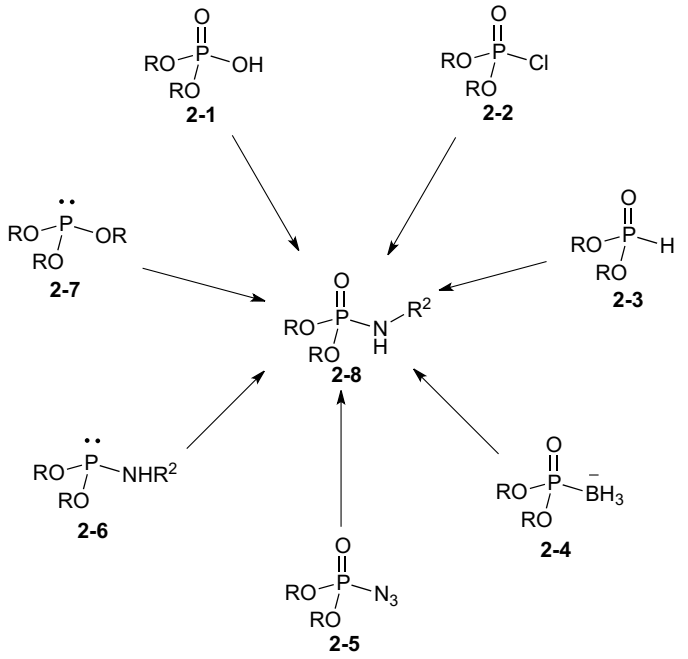
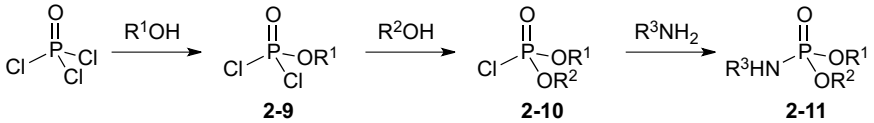


Figure 2-2: Approaches for the synthesis of phosphoramidates.

Of the synthetic approaches provided in Figure 2-2, the most often used route goes through a phosphochloridate intermediate (i.e., **2-2**). These phosphochloridates can be obtained from phosphoryl chloride through carefully controlled stepwise additions of nucleophiles. Obtaining a phosphoramidate containing two different alkoxy groups (such as the MeOPN group in *C. jejuni* CPS) requires treating phosphoryl chloride in succession with two different alcohols followed by an amine (Scheme 2-1). In some cases, the second alcohol and the amine can be sequentially added to the product of the initial reaction. However, in many cases, purification of these highly reactive intermediates is used to avoid a mixture of products and/or difficult purification of the final products.

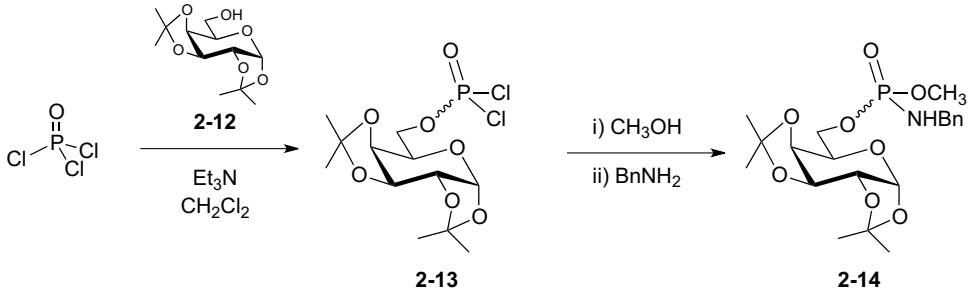


Scheme 2-1: Route for the synthesis of phosphoramidates from $POCl_3$.

This chapter will discuss the development of a two-step route in the synthesis of the MeOPN motif, present its application to the preparation of several carbohydrate substrates, and describe a study of the stability of the MeOPN group towards various deprotection strategies.

2.2 Method development for the synthesis of MeOPN

The goal of this project was to develop an efficient and reliable method to synthesize carbohydrates containing MeOPN motifs. Given the precedence, phosphoryl chloride was initially explored as the phosphorylating reagent (Scheme 2-2).



Scheme 2-2: Attempted synthesis of MeOPN-functionalized 1,2:4,6-di-*O*-isopropylidene-galactopyranose through sequential nucleophilic additions to $POCl_3$.

Thus, $POCl_3$ was treated with 1,2:3,4-di-*O*-isopropylidene-galactopyranose (**2-12**) in the presence of Et_3N (Scheme 2-2). Upon the consumption of **2-12**, as monitored by thin layer chromatography (TLC), methanol was subsequently added followed by benzylamine. Unfortunately, this approach led to several reaction products and purification of the desired phosphoramidate was difficult. Therefore, the use of phosphoryl chloride as the phosphorylating reagent was abandoned.

With the failure of the phosphoryl chloride approach, other commercially available phosphorylating reagents (Figure 2-3), methyl dichlorophosphate (**2-15**), and 2-cyanoethyl *N,N*-diisopropylidenechlorophosphoramidite (**2-16**), and diphenyl phosphite (**2-17**), were explored.

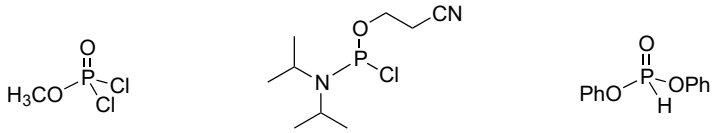
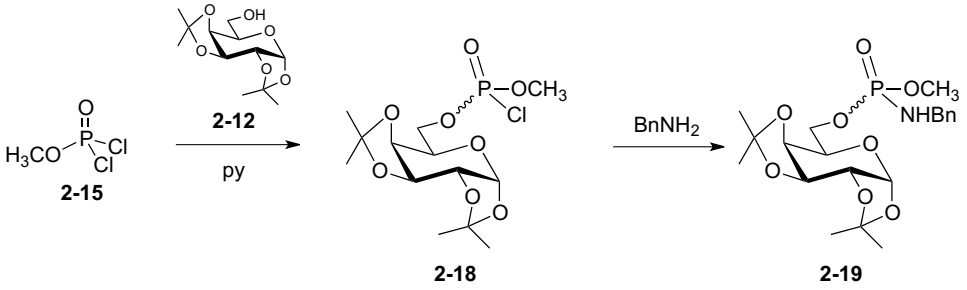


Figure 2-3: Other commercially available phosphorylating reagents explored for the synthesis of the MeOPN motif in 1,2:3,4-di-*O*-isopropylidene-galactopyranose.

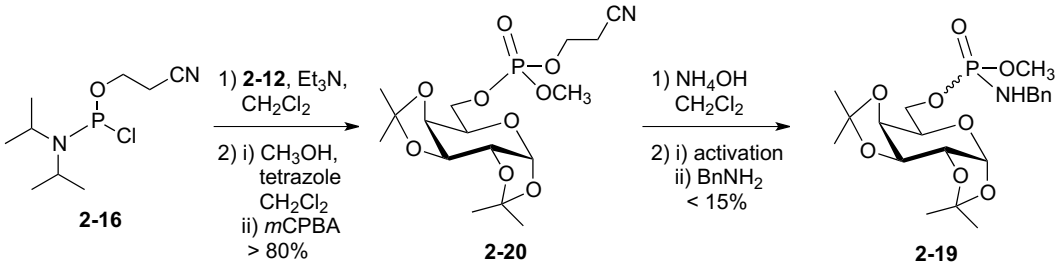
Methyl dichlorophosphate (**2-15**) was first explored as the phosphorylating reagent. We envisioned a double displacement of the chloride groups, first with a bulky carbohydrate alcohol and then with the benzylamine, could lead to the protected MeOPN motif and suppress the formation of other by-products. Unfortunately, treating the dichlorophosphate **2-15** with the sugar alcohol **2-12** and then benzylamine did not furnish the desired compound **2-19** (Scheme 2-3). Instead, a significant amount of the alcohol **2-12** remained unreactive and a polar adduct, presumably due to hydrolysis of the phosphorylating reagent, was formed.



Scheme 2-3: Attempted synthesis of MeOPN-functionalized 1,2:3,4-di-*O*-isopropylidene-galactopyranose using methyl phosphodichloridate.

Given the lack of success with methyl dichlorophosphate, *N,N*-di-isopropyl-chloro-phosphoramidite (**2-16**) was next explored. Because this reagent has two substituents with

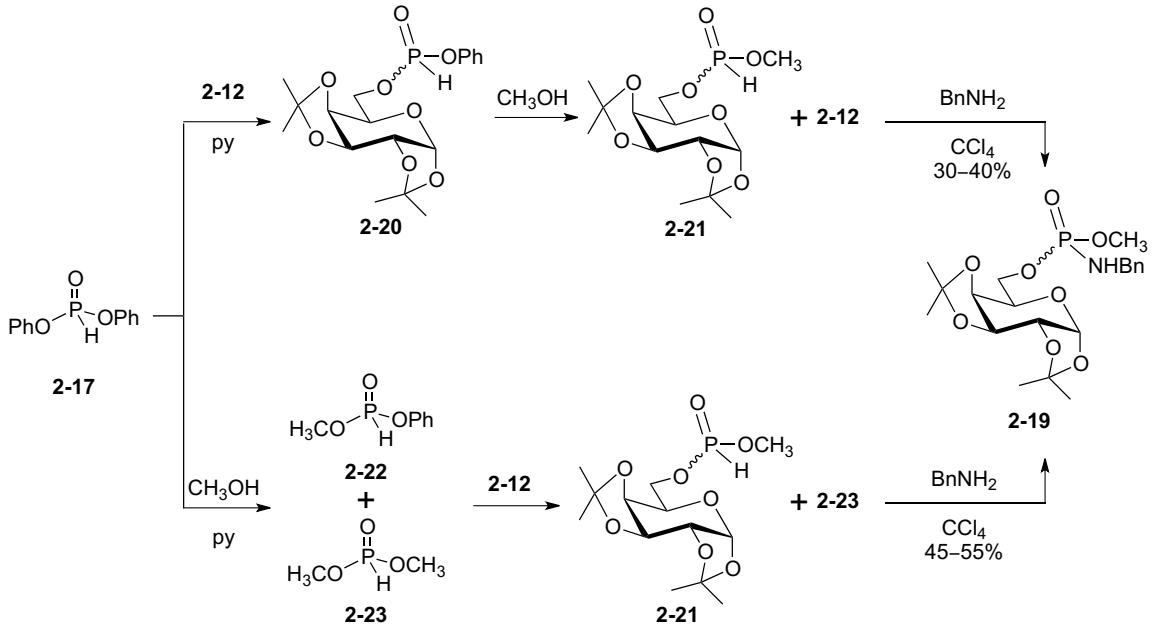
different leaving group ability, it is possible to add each nucleophile selectively, thus preventing the production of a mixture of products. Using this strategy, phosphoramidite **2-16** was first treated with alcohol **2-12** in the presence of triethylamine. This adduct was then treated methanol in dichloromethane in the presence of tetrazole, and the resulting adduct was next oxidized with *meta*-chloroperoxybenzoic acid to afford phosphate **2-20** in >80% yield. Phosphate **2-20** was treated with tetra-*n*-butylammonium hydroxide to remove the cyanoethyl group. Activation of the phosphate salt by treatment with either 2,2'-dipyridyl disulfide in the presence of triphenylphosphine or 2,4,6-triisopropylsulfonyl chloride,[14,26] then addition of benzylamine led to the desired MeOPN-containing saccharide **2-19**, albeit in low yields. Obtaining the MeOPN-containing saccharide **2-19** using **2-16** was possible; however, the low yield in the introduction of the amine made this approach less than desirable.



Scheme 2-4: Synthesis of MeOPN-functionalized 1,2:3,4-di-*O*-isopropylidene-galactopyranose employing *N,N*-di-isopropyl-chloro-phosphoramidite.

Lastly, we explored diphenyl phosphite (**2-17**) as the phosphorylating reagent. This approach involved the preparation of an *H*-phosphonate intermediate and then oxidation to the phosphoramidate. With regard to the formation of the *H*-phosphonate, Kraszewski and co-workers reported that dialkyl *H*-phosphonates could be obtained from diphenyl phosphite by treatment with an excess of alcohol in pyridine.[27] We envisioned that reaction of a slight excess of the diphenyl phosphite with the sugar alcohol **2-12** could form the phenyl sugar *H*-

phosphonate intermediate **2-20** in good yield (Scheme 2-5). The bulkiness of the sugar was anticipated to prevent a second addition of **2-12**. Addition of excess methanol could then convert **2-20** to the methyl sugar *H*-phosphonate **2-21**. Using this approach, diphenyl phosphite in pyridine was first treated with **2-12**, which led to a new spot, presumably the *H*-phosphonate **2-20**, on TLC. Upon subsequent addition of excess methanol, a new adduct, which was later characterized as **2-21** was observed, together with the reformation of sugar alcohol **2-12**. The mixture of products, unstable toward silica gel, was then converted to the MeOPN-containing monosaccharide **2-19** in modest yields by stirring in carbon tetrachloride in the presence of benzylamine. Alternatively, treating diphenyl phosphite (**2-17**) with methanol gave a mixture of phosphites **2-22** and **2-23**. Addition of the sugar alcohol produced **2-21** and a subsequent oxidation using carbon tetrachloride in the presence of benzylamine furnished the MeOPN-containing monosaccharide **2-19** in slightly improved yields, albeit only after increasing the equivalence of the phosphorylating reagent **2-17**. These experiments suggest the methyl substituent should be present prior to the phosphorylation of the sugar alcohol **2-12**.



Scheme 2-5: Synthesis of MeOPN-functionalized 1,2:3,4-di-*O*-isopropylidene-galactopyranose using diphenyl phosphite.

Given the positive results obtained using diphenyl phosphite (**2-17**), other *H*-phosphonate-based reagents already possessing the methyl substituent were explored to see if the yield could be improved (Figure 2-4). Given the precedence of alkyl *H*-phosphonate salts for synthesizing various *H*-phosphonate compounds,[28,29] we envisioned **2-24** and **2-25** could be used to introduce this group into carbohydrates. These compounds, in turn can be obtained from a methyl *H*-phosphonate salt **2-27** (Scheme 2-6).

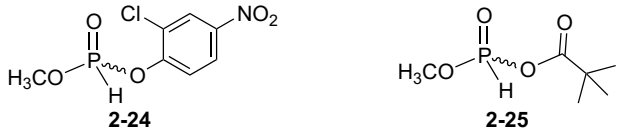
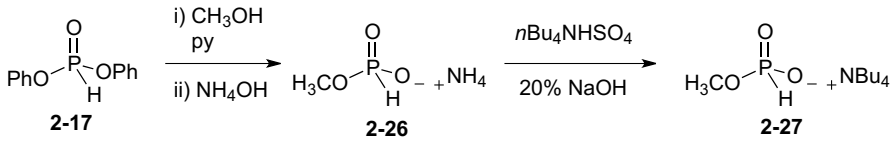


Figure 2-4: Synthetic phosphorylating reagents explored for the synthesis of the MeOPN motif in 1,2:3,4-di-*O*-isopropylidene-galactopyranose.

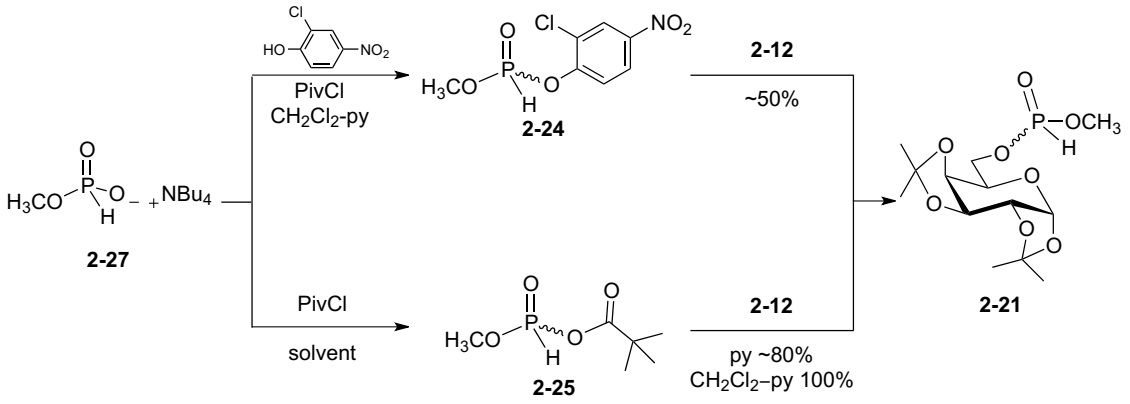
Based on Kraszewski and co-workers' work on the preparation of alkyl *H*-phosphonate monoesters,[27] the methyl *H*-phosphonate ammonium salt (**2-26**) was obtained by treatment of

diphenyl phosphite (**2-17**) with methanol in pyridine and then addition of ammonium hydroxide. The *H*-phosphonate salt **2-26** was then treated with tetra-*n*-butylammonium hydrogen sulfonate in an aqueous 20% sodium hydroxide solution to afford the *H*-phosphonate salt **2-27**,[30] which is more soluble in organic solvents.



Scheme 2-6: Synthesis of methyl *H*-phosphonate salt **2-27**.

With the *H*-phosphonate salt **2-27** in hand, the synthesis of the methyl containing phosphorylating reagents was explored as was their ability to phosphorylate **2-12**. Based on Stawiński and Kraszewski's work on using electron withdrawing phenolic substituents,[31] the *H*-phosphonate reagent **2-24** can be synthesized cleanly, as indicated by ^{31}P NMR spectroscopy, by treatment of **2-27** with pivaloyl chloride in the presence of 2-chloro-4-nitrophenol. Subsequent addition of the sugar alcohol **2-12** showed ~50% conversion to **2-21** by TLC analysis. Increasing the equivalents of the phosphorylating reagent from three to nine showed higher conversion; however, **2-12** was never completely consumed.



Scheme 2-7: Synthesis and phosphorylation tests of synthetic methyl *H*-phosphonate reagents.

In a second experiment, the *H*-phosphonate salt **2-27** was first treated with pivaloyl chloride in pyridine to give **2-25**.[32] Subsequent addition of **2-12** led to the formation of **2-21** in ~80% conversion as indicated by TLC analysis. Switching the solvent from pyridine to a mixture of 9:1 dichloromethane–pyridine, however, showed complete consumption of the sugar alcohol **2-12**. Treatment of *H*-phosphonates with pivaloyl chloride in pyridine has been shown to produce a mixture of phosphorus products (e.g. diacyl phosphites, trimetaphosphites).[32-34] The use of a mixture of solvents (e.g. CH_3CN–pyridine or CH_2Cl_2–pyridine), however, is reported to prevent the formation of other phosphorus products and exclusively form the *H*-phosphonate **2-25**.

With a phosphorylating reagent and conditions that efficiently produced **2-21** identified, oxidative approaches to convert the *H*-phosphonate into the corresponding MeOPN-containing saccharide **2-19** were evaluated (Table 2-1). Initially, we explored Atherton–Todd conditions (Entry 1) using carbon tetrachloride as the oxidant and solvent in the presence of excess benzylamine. These conditions showed clean conversion of **2-21** to **2-19** in an isolated yield of 85%. The addition of triethylamine to the reaction mixture reduced the number of equivalents of benzylamine required (from 10 to 1.5) without affecting the product yield (Entry 2). Other oxidants – trichloroisocyanuric acid[35] and bromotrichloromethane[36,37] – were also tested. Switching to trichloroisocyanuric acid (Entries 3 and 4) was inferior to carbon tetrachloride, while employing bromotrichloromethane (Entries 5 and 6) provided product yields comparable to when carbon tetrachloride was used. Other oxidants (e.g., carbon tetrabromide, iodoform, iodine, *N*-bromosuccinimide and *N*-chlorosuccinimide) have been previously used in the Atherton–Todd reaction;[38-42] however, these reagents were not explored.

The oxidation reaction of the *H*-phosphonate to a phosphoramidate is known as the Atherton–Todd reaction, which proceeds from the *H*-phosphonate phosphite tautomeric form, in this case, **2-21b**. The phosphite reacts with carbon tetrachloride to produce the phosphochloridate **2-28**, which then reacts with benzylamine to afford the corresponding MeOPN-containing saccharide **2-19**.

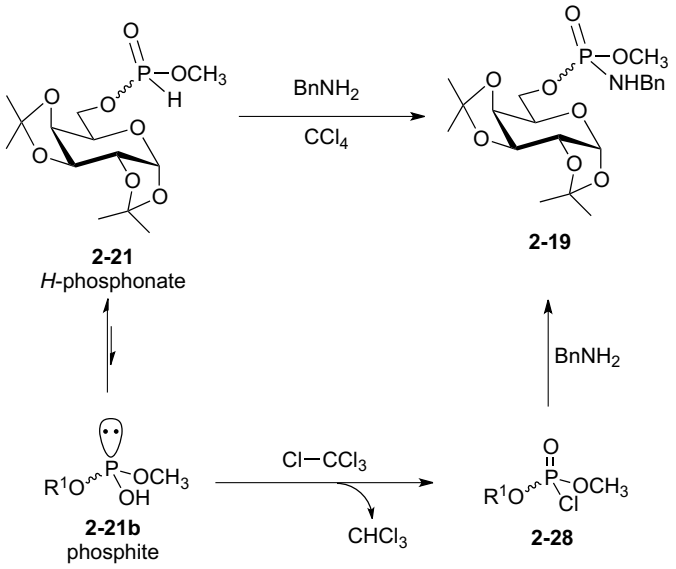
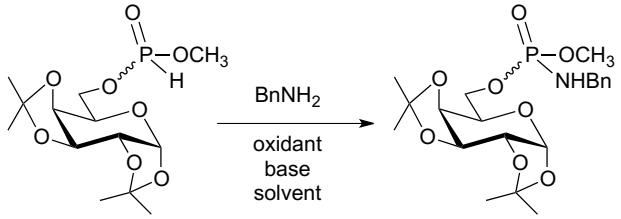


Figure 2-5: Mechanism of the Atherton–Todd reaction.

Table 2-1: Optimization of the oxidation of *H*-phosphonate **2-21** into phosphoramidate **2-19**.



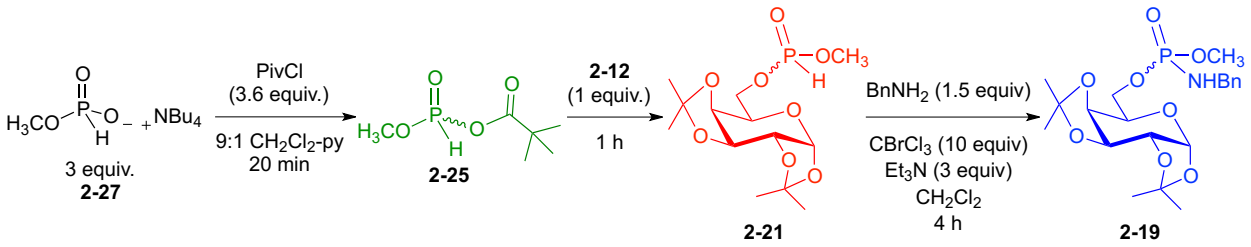
2-21 **2-19**

entry	equiv. $BnNH_2$	equiv. oxidant	equiv. base	solvent	conversion
1	10	CCl_4	---	CCl_4	100%[a], 85%[b]
2	1.5	CCl_4	Et_3N (3)	CCl_4	100%[a]
3	1.5	TCICA (1)	Et_3N (3)	CH_3CN	< 30%[a]
4	1.5	TCICA (3)	Et_3N (3)	CH_3CN	~50%[a]
5	1.5	$CBrCl_3$ (10)	Et_3N (3)	CH_2Cl_2	100%[a], 79%[b]
6	1.5	$CBrCl_3$ (5)	Et_3N (3)	CH_2Cl_2	100%[a], 76%[b]

[a] TLC conversion. [b] isolated yield. * TCICA = trichloroisocyanuric acid.

Using the optimized conditions for the synthesis of the *H*-phosphonate and its subsequent oxidation, we investigated the ability to follow the reaction by ^{31}P NMR spectroscopy, if desired. Figure 2.5 shows a representative reaction sequence with monitoring by ^{31}P NMR spectroscopy. *H*-phosphonate salt **2-27** was consumed within 20 minutes after addition of pivaloyl chloride to generate a new product, which had a ^{31}P chemical shift consistent with **2-25**.[32-34] After the formation of **2-25** was complete, alcohol **2-12** was added and, after being stirred for one hour, the reaction was worked up. ^{31}P NMR spectroscopy of the product shows two new signals in an ~1:1 ratio, indicative of the two diastereomeric methyl sugar *H*-phosphonates **2-21**. Without further purification, **2-21** was converted to **2-19** via an Atherton–Todd reaction with benzylamine and bromotrichloromethane in the presence of triethylamine. After four hours, the

reaction was worked up and ^{31}P NMR spectroscopy of the product revealed two new signals for **2-19**, corresponding to an ~1:1 ratio of diastereomers that were inseparable.



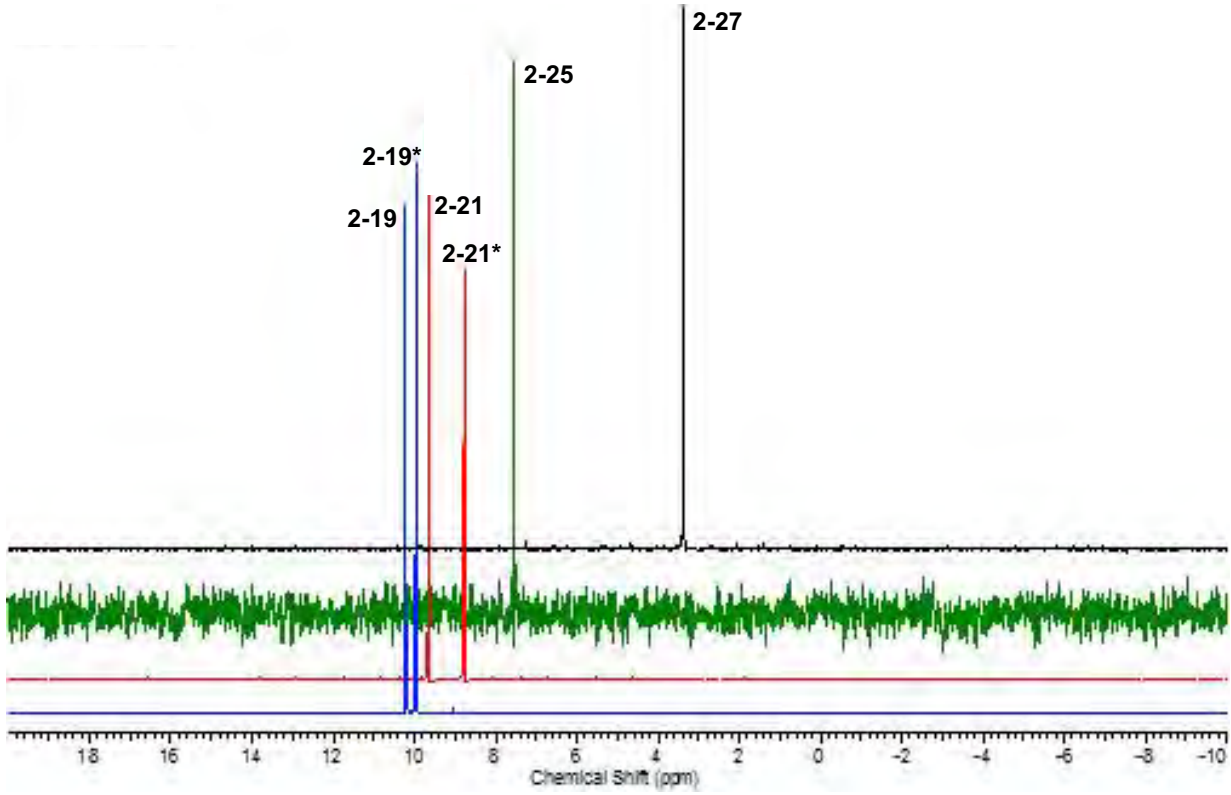


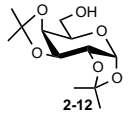
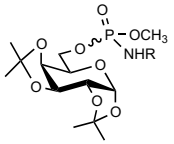
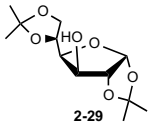
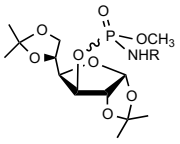
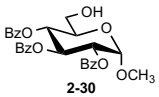
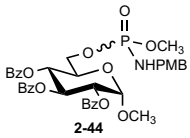
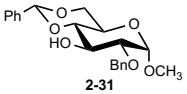
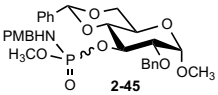
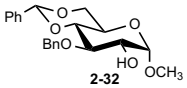
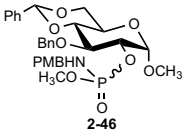
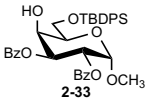
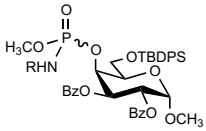
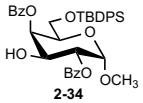
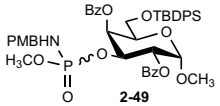
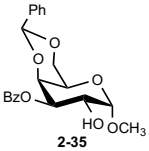
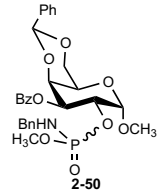
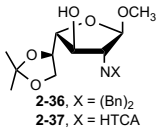
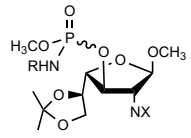
Figure 2-6: Monitoring the two-step synthesis of **2-19** from **2-27** by ^{31}P NMR spectroscopy.

2.3 Substrate scope

After having developed the optimized conditions, a panel of substrates was evaluated to explore the scope of the method (Table 2-2). As indicated by Entries 1 and 2, following

introduction of the *H*-phosphonate, the oxidation can be done using various amine nucleophiles including benzylamine (Entries 1, 2, 6, 8, and 9), *para*-methoxybenzylamine (Entries 1–7 and 9), *ortho*-nitrobenzylamine (Entries 1 and 2), and ammonia (Entry 2) generated from ammonium hydroxide. When benzylamine, *para*-methoxybenzylamine and *ortho*-nitrobenzylamine were used in the Atherton–Todd reaction, the yields were consistent with all but one acceptor (Entry 9). Ammonia can also be used as the nucleophile to produce the unprotected MeOPN-containing saccharide **2-43**; however, the yields are modest (Entry 2).

Table 2-2: Introduction of MeOPN moieties onto carbohydrates.

Entry	MeOPN acceptor	Product	Yield
1	 **2-12**		**2-19**, R = Bn, 79% **2-38**, R = PMB, 81% **2-39**, R = ONB*, 73%
2	 **2-29**		**2-40**, R = Bn, 75% **2-41**, R = PMB, 71% **2-42**, R = ONB*, 71% **2-43**, R = H, 56%[a]
3	 **2-30**	 **2-44**	78%
4	 **2-31**	 **2-45**	82%
5	 **2-32**	 **2-46**	80%
6	 **2-33**		**2-47**, R = Bn, 73% **2-48**, R = PMB, 75%
7	 **2-34**	 **2-49**	75%
8	 **2-35**	 **2-50**	77%
9	 **2-36**, X = $(Bn)_2$ **2-37**, X = HTCA		X = $(Bn)_2$ **2-51**, R = Bn, 52%[b] **2-52**, R = PMB, 48%[b] X = HTCA **2-53**, R = Bn, 55%[b]

Conditions: 1) i) *H*-phosphonate **2-27** (3 equiv), PivCl (3.6 equiv), 9:1 CH_2Cl_2–pyridine, 20 min, ii) acceptor (1 equiv), 1 h; 2) amine (1.5 equiv), $CBrCl_3$ (10 equiv), Et_3N (3 equiv), CH_2Cl_2, 4 h. [a] NH_3 obtained from NH_4OH. [b] Used 5 equiv. and 6 equiv. of phosphorylating reagent and PivCl, respectively. *ONB = *ortho*-nitrobenzyl; TCA = trichloroacetyl.

In addition to those trends, the data in Table 2-2 demonstrates that the method is compatible with primary (Entries 1 and 3) and secondary hydroxyl groups (Entries 2, 4–9) in either the pyranose (Entries 1, 3–8) or furanose (Entries 2 and 9) ring configurations. Notably, the method was capable of producing protected precursors of the motifs **2-50** and **2-51**–**2-53** found in the CPS of *C. jejuni* strains 11168H and 81-176 (Figure 2-1).

This data also reveals that the conditions are sufficiently mild so that a variety of protecting groups can be used as substrates. These groups include benzylidene acetals (Entries 4, 5, and 8), benzyl ethers (Entries 4, 5, and 9), benzoate esters (Entries 3, 6–8), isopropylidene ketals (Entries 1, 2, and 9), and silyl ethers (Entries 6 and 7). In each case, the product was produced as an ~1:1 mixture of diastereomers, which in most cases was inseparable. It is important to note that two substrates, **2-54** and **2-55**, were found to be incompatible with this approach (Figure 2-7).

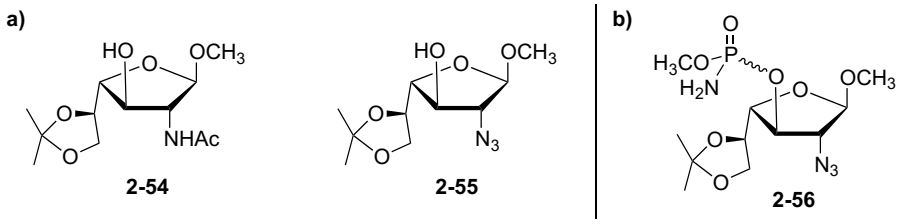
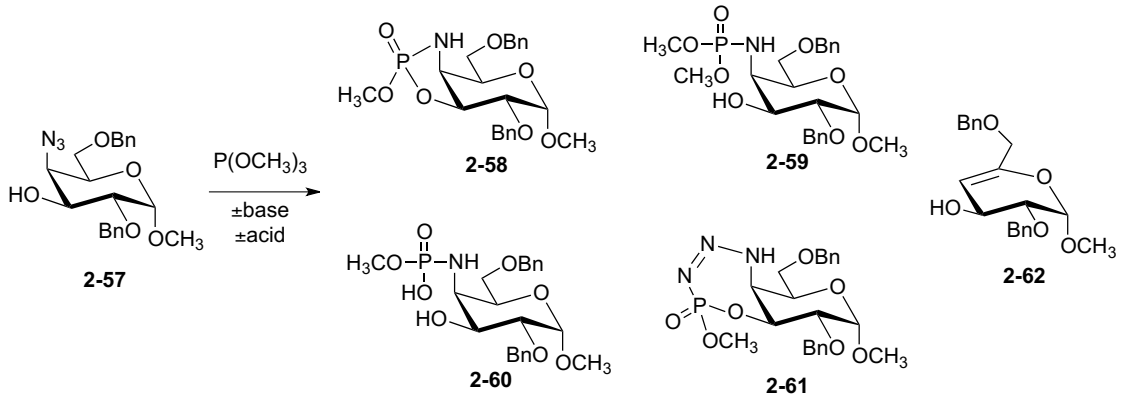


Figure 2-7: a) Two incompatible substrates for the phosphorylation by the *H*-phosphonate/Atherton–Todd approach. b) Isolated MeOPN-containing saccharide **2-56**, produced from **2-55** upon application of the *H*-phosphonate/Atherton–Todd approach.

In the reaction of *N*-acetylated substrate **2-54** with the phosphorylating reagent, a product of unknown structure was formed; this compound was resistant to the Atherton–Todd reaction. In the case of the azido substrate **2-55**, treatment with the phosphorylating reagent presumably led to a Staudinger-type reaction. Interestingly, a small percent (10–15%) of the unprotected MeOPN-containing saccharide **2-56** was also isolated. This type of reaction, to our knowledge, has not been observed previously, although Guo and coworkers have reported product mixtures

resulting from the treatment of azido-carbohydrates with phosphoramidites.[43] In their efforts to help unravel the complications in phosphorylating azido-carbohydrates with a P(III) reagent, they treated azido-carbohydrate **2-57** with trimethylphosphite (Scheme 2-8). Most of the adducts (e.g. **2-58**, **2-59**, and **2-60**) generated are products found in the Staudinger–phosphite reaction (various forms of phosphoramidates).[44,45] In the presence of an acid, however, they reported the formation of phosphotriazene **2-61** and an elimination product **2-62**. The isolation of the phosphotriazene is interesting and may be involved in the mechanistic pathway in the formation of the MeOPN-containing saccharide **2-56** as detailed below.



Scheme 2-8: Guo's reported product mixtures upon treating azido-carbohydrate **2-54** with trimethylphosphite.[43]

In a search for papers describing phosphotriazene syntheses, we discovered work by Raines and coworkers on the synthesis of diazo compounds from azides.[46,47] They reported that during Staudinger reactions/ligations[48,49] the intermediate phosphazides can be trapped before the loss of nitrogen if a highly electrophilic ester (e.g., *N*-hydroxysuccimide ester) is present in the phosphine reagent (Figure 2-8). Subsequent hydrolysis of the acyl triazenophosphonium salt leads to the formation of the acyl triazene, which then results in the formation of a diazo compound. This work builds upon earlier studies by Baumgarten,[50,51] which demonstrated that acyl triazenes could fragment to form the diazo substrate and a primary amide.[46,47]

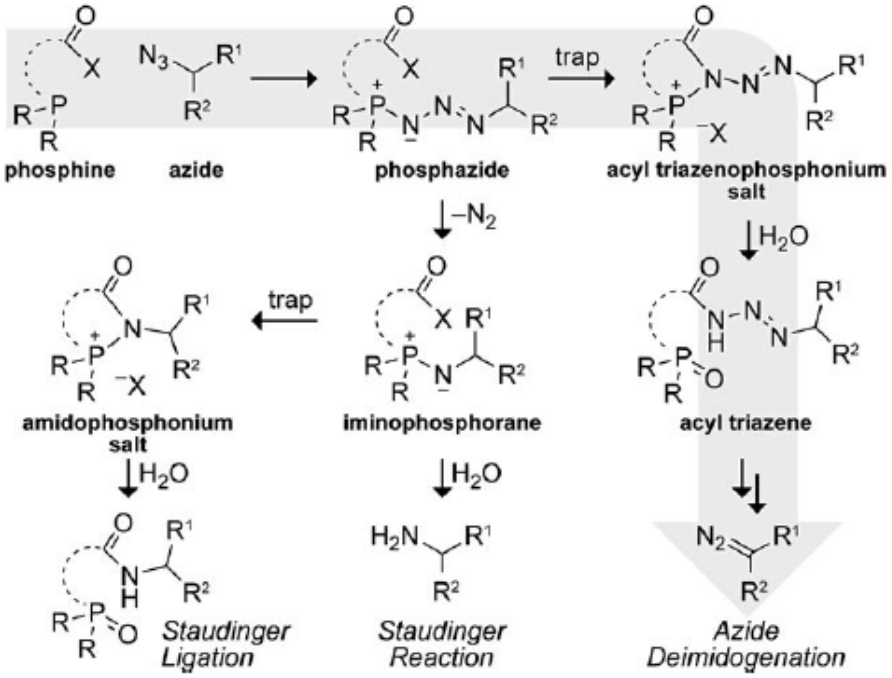


Figure 2-8: Reactions of phosphines and azides. Reprinted with permission from John Wiley and Sons: *Angew. Chem. Int. Ed.*, *48*, 2359–2363, copyright 2009.[47]

Taking the discussion above into consideration, we propose that the MeOPN-containing sugar **2-56** is formed from **2-55** via the mechanistic pathway outlined in Figure 2-9. First, **2-55** is phosphorylated by **2-28** to form *H*-phosphonate **2-63a**, which, through its phosphite tautomeric form, reacts with the azide of another molecule of **2-55** to form phosphazide **2-64**. Transfer of a proton would lead to the phosphotriazene **2-65**, which could undergo scission of the N–N bond,[52] producing the MeOPN-containing saccharide **2-56** and the diazo compound **2-66**.

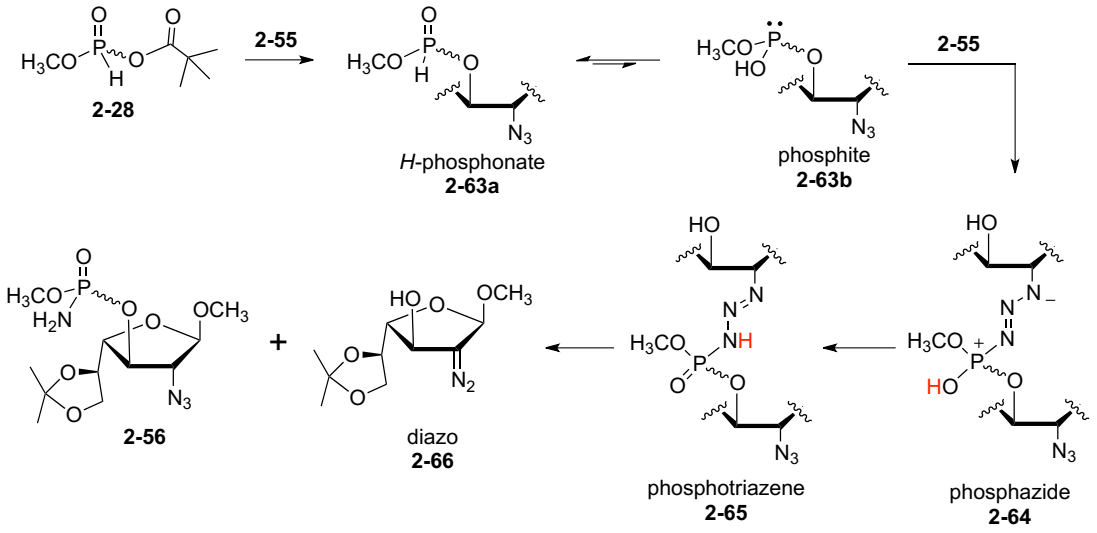
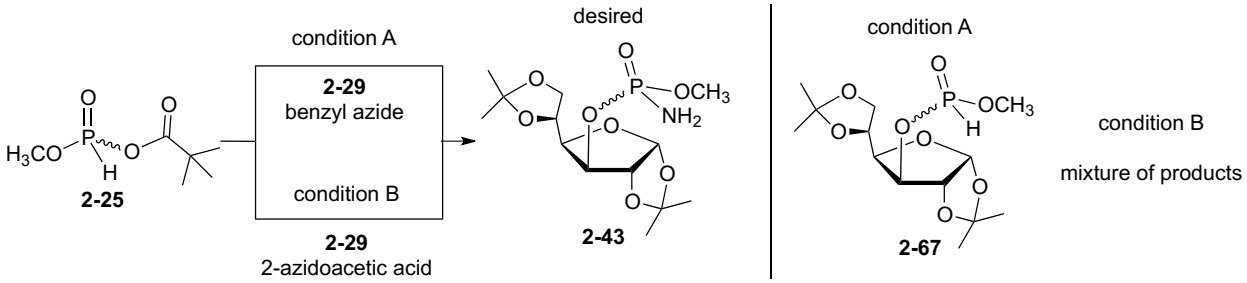


Figure 2-9: Proposed pathway for the formation of **2-56** from **2-55** upon treatment with **2-28**.

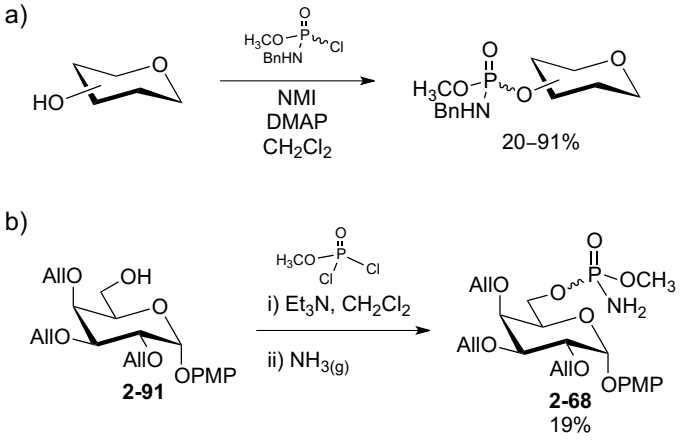
To exploit this mechanistic pathway, we attempted the phosphorylation of **2-29** in the presence of benzyl azide or 2-azidoacetic acid. In the presence of benzyl azide (condition A), the only product that formed was the methyl sugar *H*-phosphonate **2-67**. When attempting the phosphorylation of **2-29** in the presence of the 2-azidoacetic acid (condition B), a complex mixture of products was formed. Therefore, further exploration of this unusual transformation was not performed.



Scheme 2-9: Attempted synthesis of MeOPN-containing saccharide **2-43** via exploit of the azide deimidogenation pathway.

In regard to the synthesis of MeOPN motifs on carbohydrates, it is important to note that two alternate methods were published after our report (Scheme 2-10).[53,54] Wang and coworkers

reported that treatment of carbohydrates with *N*-benzylphosphoramidochloridate (obtained by reaction of dichlorophosphate **2-15** with benzylamine) in the presence of 4-dimethylaminopyridine and excess *N*-methylimidazole produced the MeOPN-containing saccharides in yields of 20–91% (Scheme 2-10a).[53,54] In a later report, Monteiro and coworkers reported a one-pot/two-step synthesis of a MeOPN-containing saccharide by first treating the carbohydrate alcohol with methyl dichlorophosphate **2-15** in dichloromethane in the presence of triethylamine and then bubbling ammonia through the reaction mixture (Scheme 2-10b). This protocol produced the MeOPN-containing saccharide **2-68** in 19% yield.[52]



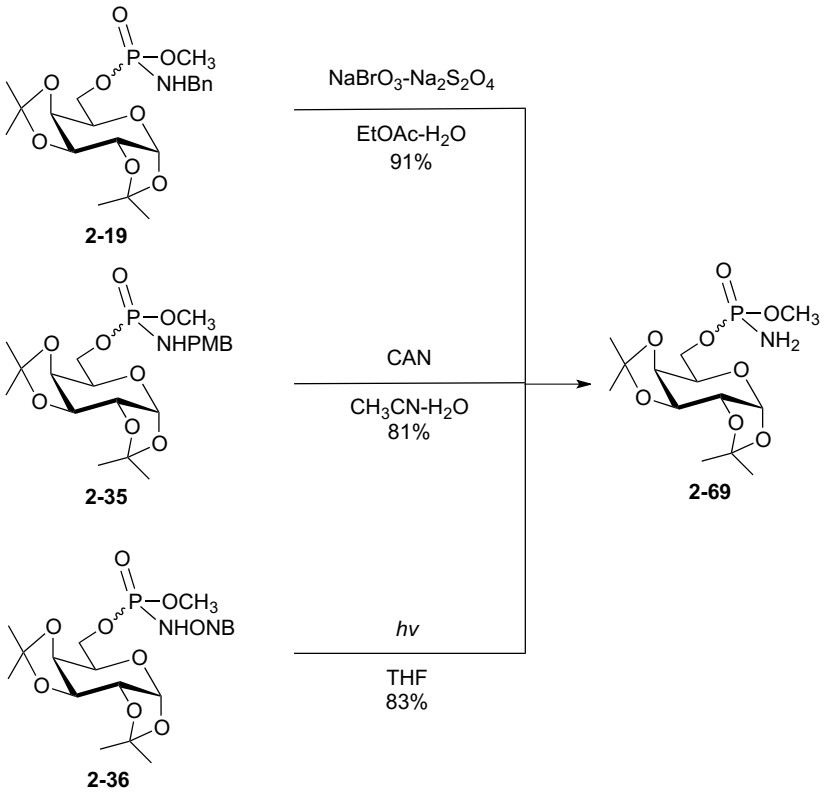
Scheme 2-10: Reported syntheses of MeOPN-containing saccharides. a) Using *N*-benzyl phosphoramidochloridate by Wang and coworkers.[51] b) Using methyl dichlorophosphate by Monteiro and coworkers.[52]

The key difference between these routes and our initial approach with methyl dichlorophosphate (**2-15**) was the use of *N*-methylimidazole and triethylamine over pyridine in the phosphorylation step. Reports have shown dealkylation of phosphate esters can occur by nucleophilic displacement with an amine or a base such as pyridine, ammonia, or 1,8-diazabicycloundec-7-ene.[55] *O*-Methyl and *O*-benzyl substituents found on phosphates are notoriously susceptible to these dealkylation processes.[55-57] It is possible that our initial approach

in using methyl dichlorophosphate in pyridine caused the loss of the methyl group during the phosphorylation step. Wang's approach, which uses *N*-benzyl phosphoramidochloridate, has the advantage of a one-step synthesis of protected MeOPN-containing saccharides. However, the phosphorylation reagent was reported to be highly unstable (it must be used within hours of its synthesis) and the products yields were highly variable.

2.4 MeOPN stability towards removal of protecting groups

With the success of synthesizing the protected MeOPN-containing saccharides on a variety of substrates, the stability of the MeOPN motif was examined using various deprotection strategies. The removal of the nitrogen-protecting group on the MeOPN motif was first studied. We found that *N*-benzyl and *N*-*para*-methoxybenzyl groups in substrates **2-19** and **2-35** could not be removed by standard hydrogenolysis conditions in the presence of palladium on carbon (Pd–C). They, however, can be removed oxidatively. The *N*-benzyl group can be removed from **2-19** using sodium bromate and sodium hydrosulfite,[58] without affecting the MeOPN motif (Scheme 2-11). Similarly, the *N*-*para*-methoxybenzyl group on **2-35** can also be removed using ceric ammonium nitrate. The *N*-*ortho*-nitrobenzyl group on **2-36** could be removed by photolysis in polar aprotic solvents.

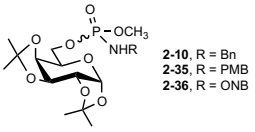
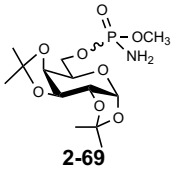
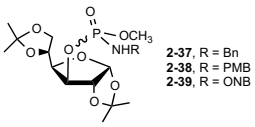
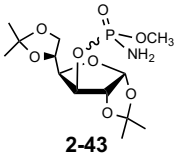
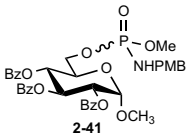
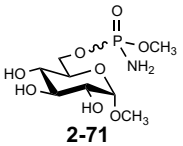
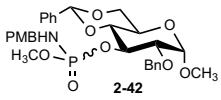
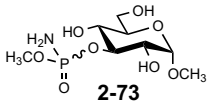
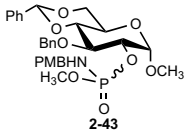
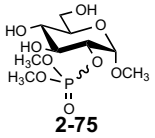
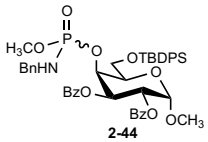
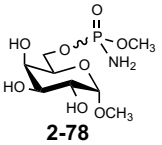
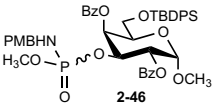
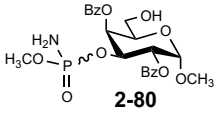
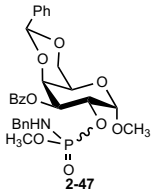
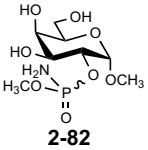
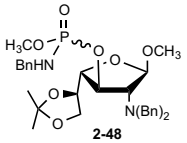
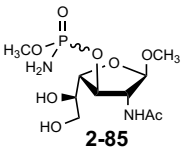


Scheme 2-11: Conditions to remove P–*N*-protecting groups.

With the removal of the *N*-protecting groups on the MeOPN motif established, we next focused on the oxygen protecting groups (Table 2-3). Benzylidene acetals and benzyl ethers can be reductively cleaved by hydrogenolysis in the presence of Raney nickel (Entries 4 and 5). Hydrogenolysis in the presence of Pd–C led to incomplete cleavage of the benzylidene acetals even after long reaction times and the use of high pressure. In addition, benzylidene acetals can be opened oxidatively to produce a benzoate ester. For instance, Entry 8 demonstrates the simultaneous removal of an *N*-benzyl group and regioselective oxidative ring-opening of the benzylidene acetal to form **2-77**. Benzoate and acetate esters (Entries 3, 6, 8 and 9) can be removed using a 7:2:1 mixture of methanol–water–triethylamine. The use of sodium methoxide results in significant (often complete) cleavage of the phosphoramidate from the carbohydrate. It

is also worth noting that MeOPN transfer occurred from the C-4 hydroxyl group to O-6 in the final deprotection step of removing the benzoates (Entry 6). Silyl ethers (Entries 6 and 7) can be removed using hydrogen fluoride–pyridine or tetra-*n*-butylammonium fluoride. Isopropylidene ketals (Entry 9) can be removed with iodine in methanol. The use of standard hydrolysis (80% acetic acid in water heated to 60 °C) to cleave isopropylidene ketals or benzylidene acetals led to significant loss of the phosphoramidate motif.

Table 2-3: Deprotection studies of MeOPN-containing carbohydrates.

entry	MeOPN acceptor	product	conditions, yield
1	 **2-10**, R = Bn **2-35**, R = PMB **2-36**, R = ONB	 **2-69**	R = Bn, $NaBrO_3$–$Na_2S_2O_3$, 91% R = PMB, CAN, 81% R = ONB, *hv*, 83%
2	 **2-37**, R = Bn **2-38**, R = PMB **2-39**, R = ONB	 **2-43**	R = Bn, $NaBrO_3$–$Na_2S_2O_3$, 88% R = ONB, *hv*, 74%
3	 **2-41**	 **2-71**	1) CAN, 83% (**2-70**)* 2) CH_3OH–H_2O–Et_3N, 79% (**2-71**)
4	 **2-42**	 **2-73**	1) CAN, 76% (**2-72**)* 2) H_2, Raney Ni, 61% (**2-73**)
5	 **2-43**	 **2-75**	1) CAN, 73% (**2-74**)* 2) H_2, Raney Ni, 65% (**2-75**)
6	 **2-44**	 **2-78**	1) HF·py, 85% (**2-76**)* 2) $NaBrO_3$–$Na_2S_2O_3$, 74% (**2-77**)* 3) CH_3OH–H_2O–Et_3N, 44% (**2-78**)
7	 **2-46**	 **2-80**	1) HF·py, 80% (**2-79**)* 2) CAN, 74% (**2-80**)
8	 **2-47**	 **2-82**	1) $NaBrO_3$–$Na_2S_2O_3$, 89% (**2-81**)* 2) CH_3OH–H_2O–Et_3N, 81% (**2-82**)
9	 **2-48**	 **2-85**	1) 7.5% I_2–CH_3OH, 66% (**2-83**)* 2) H_2, Pd–C 3) Ac_2O, 74% (**2-84**)* 5) $NaBrO_3$–$Na_2S_2O_3$ 6) CH_3OH–H_2O–Et_3N, 45% (**2-85**)

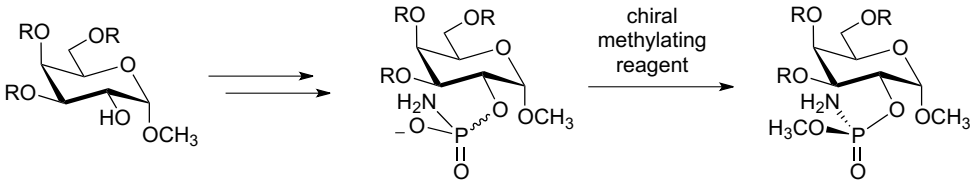
*all compounds without structures can be found in experimental

2.5 Summary and future directions

In summary, the work described in this chapter provides an efficient two-step route for the synthesis of MeOPN-containing carbohydrates. The method was found to be compatible with primary and secondary alcohols in the presence of a variety of protecting groups. Various deprotection strategies were explored in studying the stability of the MeOPN motifs. As described earlier, the developed method produces the MeOPN-containing saccharides in ~1:1 ratio of diastereomers. In some cases, the diastereomers are separable by flash chromatography; however, in others they are not. As part of a collaborative project, another group member (Sicheng Lin) and I have synthesized two MeOPN-containing saccharides and separated the two diastereomers. These compounds have been characterized by NMR spectroscopy and crystallization of these saccharides is currently being attempted. Professor Yunjie Xu's group (University of Alberta) is currently investigating these compounds by vibrational circular dichroism (VCD). Previous studies have reported the determination of phosphorus stereochemistry using VCD and ab initio calculations.[59] We hope to be able to correlate NMR data, e.g., ^{31}P or ^{13}C chemical shifts (of the OCH_3 group), with phosphorus stereochemistry. This would, in turn allow us to determine the stereochemistry of the phosphorus center in the naturally occurring CPS.

In other work, our developed two-step route has been modified for the synthesis of *O*-benzyl protected phosphoramidates. Standard hydrogenolysis can remove the *O*-benzyl group. With this substrate, Sicheng Lin is currently investigating a route to synthesize the MeOPN diastereoselectively by use of chiral methylating reagents. A successful diastereoselective

synthesis could potentially aid in identifying the stereochemistry of the phosphorus center in the native CPS and ultimately its biological role.



Scheme 2-12: Attempted diastereoselective synthesis of MeOPN-containing saccharides by using chiral methylating reagents.

2.6 Experimental

2.6.1 General experimental methods

All reagents were purchased from commercial sources and were used without further purification unless noted. Solvents used in reactions were purified by successive passage through columns of alumina and copper under argon. Unless stated otherwise, all reactions were carried out at room temperature and monitored by TLC on Silica Gel G-25 F_{254} (0.25 mm). TLC spots were detected under UV light and/or by charring with a solution of *p*-anisaldehyde in ethanol, acetic acid and H_2SO_4. Column chromatography was performed on Silica Gel 60 (40–60 μm). Solvents were evaporated under reduced pressure on a rotary evaporator. ^{1}H NMR spectra were recorded using 400, 500, or 600 MHz NMR instruments and were referenced to residual proton signal of $CDCl_3$ (7.26 ppm) or CD_3OD (3.30 ppm). ^{13}C NMR spectra were recorded using 126 MHz (cold probe) NMR instrument and were referenced to residual ^{13}C signals of $CDCl_3$ (77 ppm) or CD_3OD (49 ppm). ^{31}P NMR spectra were collected using 202 or 162 MHz NMR instruments and were referenced to an external 85% H_3PO_4 standard (0.00 ppm). ^{1}H NMR data are reported as though they were first order, and peak assignments were made on the basis of 2D-NMR (^{1}H –^{1}H COSY and HSQC) experiments. For ^{1}H NMR data, diastereomer proton signals

were indicated by an asterisk (*). In all cases, the phosphoramidates were obtained in an ~1:1 mixture of diastereomers. ESI-MS spectra (time-of-flight analyzer) were recorded on samples dissolved in THF or CH_3OH and added NaCl. Optical rotations were measured at 22 ± 2 °C at the sodium D line (589 nm) and are in a microcell (10 cm, 1 mL) in units of deg·mL(dm·g)$^{-1}$.

2.6.2 Experimental, spectroscopic, and analytical data

General procedure for MeOPN synthesis.

To thoroughly dried (dried under vacuum for 3 h) tetra-*N*-butylammonium methyl-*H*-phosphonate **2-24** (0.90 mmol, 3 equiv), an anhydrous 9:1 CH_2Cl_2–pyridine solution (10 mL) was added. To this stirring solution, PivCl (1.08 mmol, 3.6 equiv) was then added and, after stirring for 20 min, the MeOPN acceptor (0.30 mmol, 1 equiv) was added and stirring was continued for 1 h. The reaction mixture was then poured into a saturated aqueous $NaHCO_3$ solution and extracted with CH_2Cl_2 (2x). The organic layers were combined, washed with brine, dried over $MgSO_4$, filtered, and concentrated. The crude mixture was dissolved in anhydrous CH_2Cl_2 (12 mL). To this solution, $CBrCl_3$ (3.00 mmol, 10 equiv), Et_3N (1.20 mmol, 4 equiv), and the amine nucleophile (0.60 mmol, 2 equiv) were added and the mixture was stirred for 4 h. The reaction mixture was then poured over a saturated aqueous $NaHCO_3$ solution and extracted with CH_2Cl_2 (2x). The organic layers were combined, washed with brine, dried over $MgSO_4$, filtered, and concentrated. The crude mixture was then purified by flash chromatography in the appropriate eluent.

General procedure for P*N*-benzyl cleavage.

To the *N*-benzyl-protected MeOPN-containing saccharide (0.30 mmol) in EtOAc (4 mL), a solution of $NaBrO_3$ (136 mg, 0.90 mmol) in H_2O (3 mL) was added via a Pasteur pipette. To this vigorously stirred mixture, a solution of $Na_2S_2O_4$ (157 mg, 0.90 mmol) in H_2O (6 mL) was added dropwise via an addition funnel over 10–15 min. After complete addition, the reaction mixture was vigorously stirred for 45 min. The reaction mixture was then diluted with EtOAc and washed with a saturated aqueous $Na_2S_2O_3$ solution and brine. The organic layer was dried over $MgSO_4$, filtered, and concentrated. The crude material was purified by flash chromatography using the appropriate eluent.

General procedure for P*N*-*para*-methoxybenzyl cleavage.

To the *N*-*para*-methoxybenzyl-protected MeOPN-containing saccharide (0.20–0.30 mmol, 1 equiv) in a 9:1 CH_3CN–H_2O solution (10 mL), CAN (3 equiv) was added and the mixture was stirred at room temperature until TLC showed complete consumption of the starting material (30–60 min). The reaction mixture was then poured into H_2O and extracted with EtOAc (1×). The organic layer was then washed with H_2O, saturated aqueous $NaHCO_3$ solution, and brine, dried over $MgSO_4$, filtered, and concentrated. The crude material was then purified by flash chromatography using the appropriate eluent.

General procedure for P*N*-*o*-nitrobenzyl cleavage.

The *N*-*o*-nitrobenzyl protected MeOPN-containing saccharide (0.10 mmol) in anhydrous THF (1.5 mL) was transferred to a Quartz test tube and the solution was irradiated at 254 nm in a

photochemical reactor until TLC showed complete consumption of the starting material (4–6 h). The reaction mixture was concentrated and resulting residue was purified by flash chromatography using the appropriate eluent.

General procedure for acetate and benzoate ester cleavage.

To the benzoate/acetate protected MeOPN-containing saccharide (0.08–0.11 mmol) was added a solution of 7:2:1 CH_3OH–H_2O–Et_3N (3 mL). The mixture was stirred at room temperature, while being monitored closely by TLC until consumption of starting material (2–5 h). The solution was concentrated and the resulting residue was purified by flash chromatography using the appropriate eluent.

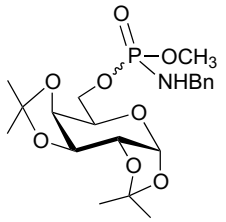
General procedure for hydrogenation with Raney Nickel.

To the MeOPN-containing saccharide (0.11–0.15 mmol) in EtOH (5 mL) was added Raney Ni (~250 μL). The reaction mixture was then flushed with H_2 and stirred at room temperature until TLC showed reaction completion. The mixture was then filtered through Fisherbrand filter paper and the filtrate was concentrated. The residue was then purified by flash chromatography using the appropriate eluent.

General procedure for TBDPS cleavage.

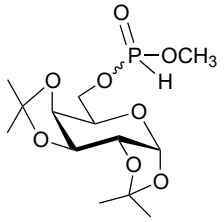
To the MeOPN-containing saccharide (1 equiv.) in a centrifuge tube under argon, anhydrous CH_3CN (1 mL/0.03 mmol saccharide) was added followed by anhydrous pyridine (20 equiv) and 70% HF·pyridine (10 equiv, based on HF). The mixture was stirred for 12 h and then poured into a saturated aqueous $NaHCO_3$ solution and extracted with EtOAc (2×). The organic

layers were combined, washed with brine, dried over $MgSO_4$, filtered, and concentrated. The crude product was purified by flash chromatography using the appropriate eluent.



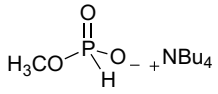
1,2:3,4-di-*O*-isopropylidene-6-*O*-(methyl *N*-benzylphosphoramidyl)-D-galactopyranose (2-19)

General procedure for MeOPN synthesis was used and the reaction was carried out on **2-8** (78 mg, 0.30 mmol). Purified by flash chromatography (4:1→6:1 EtOAc–hexanes containing 1% Et_3N) to afford **2-10** (105 mg, 79%) as a clear oil. R_f 0.20 (4:1 EtOAc–hexanes); ^{1}H NMR (500 MHz; $CDCl_3$): δ 7.36–7.18 (m, 10H, Ar), 5.54 (d, 1H, J = 4.6 Hz, H-1), 5.53 (d, 1H, J = 4.7 Hz, H-1*), 4.63 (dd, 1H, J = 2.3, 2.3 Hz, H-3), 4.61 (dd, 1H, J = 2.3, 2.3 Hz, H-3*), 4.34 (dd, 2H, J = 4.9, 2.3 Hz, H-2, H-2*), 4.26–4.08 (m, 12H, H-4, H-4*, H-5, H-5*, H-6, H-6' H-6*, H-6'*, PhC$\underline{H_2}$N, PhC$\underline{H_2}$N*), 3.73 (d, 3H, J = 11.2 Hz, POC$\underline{H_3}$), 3.72 (d, 3H, J = 11.2 Hz, POC$\underline{H_3}$*), 3.13–3.06 (m, 2H, N$\underline{H}$, N$\underline{H}$*), 1.55 (s, 3H, C(C$\underline{H_3}$)$_2$), 1.53 (s, 3H, C(C$\underline{H_3}$)$_2$), 1.43 (s, 3H, C(C$\underline{H_3}$)$_2$), 1.42 (s, 3H, C(C$\underline{H_3}$)$_2$), 1.33 (s, 6H, 2 × C(C$\underline{H_3}$)$_2$), 1.32 (s, 3H, C(C$\underline{H_3}$)$_2$), 1.31 (s, 3H, C(C$\underline{H_3}$)$_2$); ^{13}C NMR (126 MHz, $CDCl_3$): δ 139.7, 139.6 (3C), 128.6 (2C), 127.4 (2C), 127.3, 109.6, 109.5, 108.8 (2C), 96.3, 70.8, 70.7 (2C), 70.6, 70.5 (2C), 67.4 (2C), 66.8 (2C), 65.5, 65.4 (2C), 64.9, 53.3 (2C), 53.2, 45.3, 44.5, 44.4, 26.0 (2C), 25.9, 25.0 (2C), 24.5 (2C); ^{31}P NMR (162 MHz, $CDCl_3$): δ 10.22, 9.97; HRMS (ESI) *m/z* (M + Na^+) Calc. for $C_{20}H_{30}NNaO_8P$: 466.1601. Found 466.1595.



1,2:3,4-di-*O*-isopropylidene-6-*O*-(methyl *H*-phosphonyl)-D-galactopyranose (2-21)

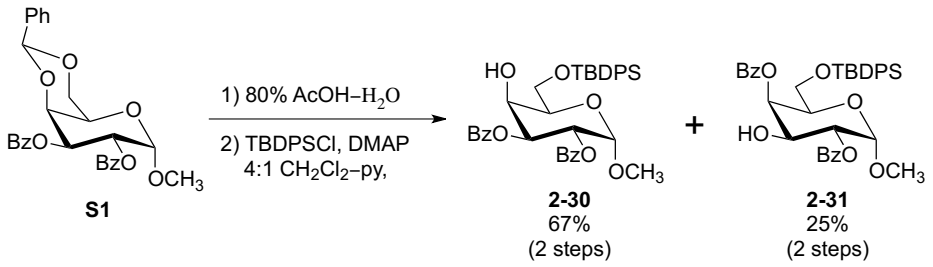
H-phosphonate **2-15** was obtained as an intermediate before the reaction under the modified Atherton–Todd reaction in the general procedure for MeOPN synthesis. R_f 0.20 (2:1 EtOAc–hexanes); ^{1}H NMR (500 MHz; $CDCl_3$): δ 6.87 (d, 1H, J = 709.2 Hz, PH), 6.84 (d, 1H, J = 709.2 Hz, PH), 5.53 (d, 1H, J = 5.2 Hz, H-1), 5.52 (d, 1H, J = 5.1 Hz, H-1*), 4.63–4.59 (m, 2H, H-3, H-3*), 4.33 (dd, 1H, J = 5.0, 2.6 Hz, H-2), 4.32 (dd, 1H, J = 5.0, 2.5 Hz, H-2*), 4.25–4.19 (m, 6H, H-4, H-4*, H-6, H-6', H-6*, H-6'*), 4.06–4.01 (m, 2H, H-5, H-5*), 3.78 (d, 3H, J = 12.0 Hz, POCH$_3$), 3.77 (d, 3H, J = 12.0 Hz, POCH$_3$), 1.52 (s, 6H, 2 × CCH$_3$), 1.43 (s, 3H, CCH$_3$), 1.42 (s, 3H, CCH$_3$), 1.32 (s, 9H, 3 × CCH$_3$), 1.31 (s, 3H, CCH$_3$); ^{13}C NMR (126 MHz; $CDCl_3$): δ 109.8 (2C) 108.9 (2C), 96.3, 70.7 (2C), 70.6, 70.4, 70.4 (2C), 67.4, 67.1, 67.0, 65.0 (2C), 64.6 (2C), 52.1, 52.0, 51.9 (2C), 26.0, 25.9, 24.9, 24.4; HRMS (ESI) Calc. for (M + Na) $C_{13}H_{23}NaO_8P$: 361.1023. Found 361.1015.



Tetra-*N*-butylammonium methyl *H*-phosphonate (2-28)

Tetra-*n*-butyl ammonium hydrogen sulfate (16.30 g, 48.00 mmol) was dissolved in H_2O (11 mL) and the solution was cooled to 0 °C. A 20% aqueous NaOH solution (12 mL) was added dropwise and then the mixture was warmed to room temperature. Ammonium methyl *H*-phosphonate (**2-23**) in H_2O (7.2 mL) was then added dropwise. Additional H_2O (2 mL) was used

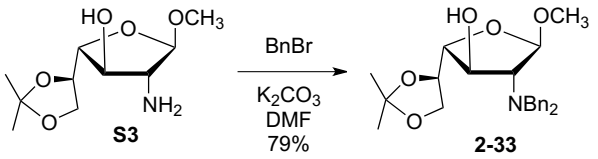
to rinse the flask containing the ammonium methyl *H*-phosphonate salt. The reaction mixture was vigorously stirred for 20 min before being extracted with CH_2Cl_2 (8 × 50 mL). The organic layers were combined, dried over $MgSO_4$, filtered, and concentrated to afford **2-24** (16.01 g, quantitative yield) as a pale yellowish viscous oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.89 (d, 1H, J = 685.6 Hz, PH), 3.51 (d, 3H, J = 11.7 Hz, $POCH_3$), 3.39–3.27 (m, 8H, 4 × NCH_2), 1.75–1.56 (m, 8H, 4 × NCH_2CH_2), 1.53–1.31 (m, 8H, 4 × $CH_2CH_2CH_3$), 0.99 (t, J = 7.3 Hz, 12H, 4 × CH_2CH_3); ^{13}C NMR (126 MHz, $CDCl_3$): δ 58.7, 51.7 (2×), 24.1, 19.7, 13.7. ^{31}P NMR (162 MHz, $CDCl_3$): δ 3.37; HRMS (ESI) Calc. for (M –) CH_4O_3P: 94.9904. Found 94.9904.



Methyl 2,3-di-*O*-benzoyl-6-*O*-*tert*-butyldiphenylsilyl-α-D-galactopyranoside (2-30) and **Methyl 2,4-di-*O*-benzoyl-6-*O*-*tert*-butyldiphenylsilyl-α-D-galactopyranside (2-31)**

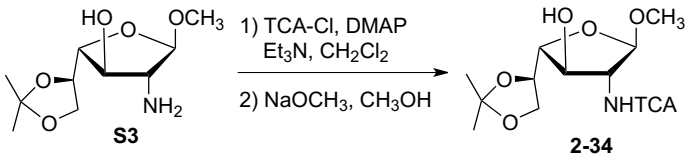
Compound **S1**[60,61] (461 mg, 0.94 mmol) was dissolved in 4:1 AcOH–H_2O (25 mL) and the reaction mixture was stirred at 85 °C for 14 h. The solution was then cooled and concentrated to dryness before DMAP (60 mg, 0.49 mmol) was added and the flask was flushed with argon. A 4:1 CH_2Cl_2–pyridine solution (15 mL) was added and the mixture was cooled to 0 °C. TBDPSCl was then added dropwise via syringe and the solution was stirred at 0 °C for 10 min before warming to room temperature. After stirring for an additional 10 h, the reaction mixture was diluted with CH_2Cl_2 and then washed with a saturated aqueous $NaHCO_3$ solution (1×), water (1×), and brine (1×). The organic layer was dried over $MgSO_4$, filtered, concentrated, and the

resulting residue was purified by flash chromatography (6:1→4:1 hexanes–EtOAc) to afford **2-30** (404 mg, 67%) and **2-31** (150 mg, 25%). Data for **2-30**.White amorphous solid. R_f 0.42 (4:1 hexanes–EtOAc); $[\alpha]_D$ +103.7 (*c* 2.1, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.08–7.99 (m, 4H, Ar), 7.81–7.71 (m, 4H, Ar), 7.58–7.35 (m, 12H, Ar), 5.77–5.66 (m, 2H, H-2, H-3), 5.23 (d, 1H, *J* = 3.0 Hz, H-1), 4.52 (br. s, 1H, H-4), 4.09–3.94 (m, 3H, H-5, H-6, H-6'), 3.40 (s, 3H, OC$\underline{H}_3$), 2.94 (d, 1H, *J* = 3.2 Hz, C-4-O$\underline{H}$), 1.12 (s, 9H, SiC(C$\underline{H}_3$)$_3$); ^{13}C NMR (126 MHz, $CDCl_3$): δ 166.1 (C=O), 165.9 (C=O), 135.7 (Ar), 135.6 (Ar), 133.2 (2C, 2 × Ar), 132.9 (Ar), 132.7 (Ar), 129.9 (2C, 2 × Ar), 129.8 (Ar), 129.6 (Ar), 129.5 (Ar), 128.4 (2C, 2 × Ar), 127.8 (2C, 2 × Ar), 127.7 (Ar), 97.5 (C-1), 71.2 (C-2), 69.3, 69.2, 69.1, 63.9 (C-6), 55.4 (O$\underline{C}H_3$), 26.8 (SiC($\underline{C}H_3$)$_3$), 19.2 (SiC($\underline{C}H_3$)$_3$); HRMS (ESI) Calc. for (M + Na) $C_{37}H_{40}NaO_8Si$: 663.2385. Found 663.2376. Data for **2-31**. Clear oil. R_f 0.28 (4:1 hexanes–EtOAc); $[\alpha]_D$ +80.9 (*c* 1.3, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.14–8.07 (m, 4H, Ar), 7.72–7.67 (m, 2H, Ar), 7.66–7.54 (m, 4H, Ar), 7.53–7.32 (m, 8H, Ar), 7.26–7.19 (m, 2H, Ar), 5.86 (dd, 1H, *J* = 3.4, 1.1 Hz, H-4), 5.34 (dd, 1H, *J* = 10.4, 3.7 Hz, H-2), 5.17 (d, 1H, *J* = 3.7 Hz, H-1), 4.55 (ddd, 1H, *J* = 10.3, 4.6, 3.7 Hz, H-3), 4.19 (ddd, 1H, *J* = 7.4, 6.8, 1.0 Hz, H-5), 3.84 (dd, 1H, *J* = 10.2, 7.1 Hz, H-6), 3.79 (dd, 1H, *J* = 10.2, 6.6 Hz, H-6'), 3.42 (s, 3H, OC$\underline{H}_3$), 2.44 (d, 1H, *J* = 4.8 Hz, C-3–O$\underline{H}$), 1.06 (s, 9H, SiC(C$\underline{H}_3$)$_3$); ^{13}C NMR (126 MHz, $CDCl_3$): δ 166.7 (2×, C=O), 135.6 (Ar), 135.5 (Ar), 133.3 (2×, Ar), 133.1 (Ar), 132.9 (Ar), 130.0 (Ar), 129.9 (Ar), 129.8 (Ar), 129.7 (Ar), 129.6 (Ar), 129.5 (Ar), 128.5 (Ar), 128.4 (Ar), 127.7 (Ar), 127.6 (Ar), 97.6 (C-1), 72.4 (C-2), 71.4 (C-4), 69.3 (C-5), 67.8 (C-3), 61.9 (C-6), 55.6 (O$\underline{C}H_3$), 26.7 (SiC($\underline{C}H_3$)$_3$), 19.1 (SiC($\underline{C}H_3$)$_3$); HRMS (ESI) Calc. for (M + Na) $C_{37}H_{40}NaO_8Si$: 663.2385. Found 663.2382.



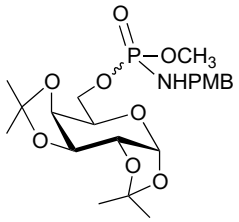
Methyl 2-di-*N,N'*-benzyl-2-deoxy-5,6-*O*-isopropylidene-β-D-galactofuranoside (2-33)

To **S3** (218 mg, 0.94 mmol) and K_2CO_3 (517 mg, 3.74 mmol), anhydrous DMF (10 mL) was added followed by BnBr (333 μL, 2.80 mmol). The reaction mixture was stirred for 24 h and then concentrated, diluted with EtOAc, before being washed with H_2O (1×) and brine (1×), dried over $MgSO_4$, and concentrated. The crude product was purified by flash chromatography (3:1 hexanes–acetone) to afford **2-33** (306 mg, 79%) as a clear oil. R_f 0.34 (3:1 hexanes–acetone); $[\alpha]_D$ −9.5 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz; $CDCl_3$): δ 7.39–7.25 (m, 10H, Ar), 4.98 (d, 1H, *J* = 2.1 Hz, H-1), 4.28 (ddd, 1H, *J* = 6.8, 6.6, 5.9 Hz, H-5), 4.19–4.12 (m, 1H, H-3), 4.04 (dd, 1H, *J* = 8.4, 6.7 Hz, H-6), 3.94 (dd, 1H, *J* = 8.4, 6.9 Hz, H-6'), 3.82 (dd, 1H, *J* = 7.7, 5.7 Hz, H-4), 3.78 (d, 2H, *J* = 14.5 Hz, 2 × PhC$\underline{H}_2$N), 3.68 (d, 2H, *J* = 14.1 Hz, 2 × PhC$\underline{H}_2$O), 3.37 (s, 3H, OC$\underline{H}_3$), 3.30 (dd, 1H, *J* = 5.2, 2.1 Hz, H-2), 1.87 (d, 1H, *J* = 6.0 Hz, C-3–O$\underline{H}$), 1.42 (s, 3H, C(C$\underline{H}_3$)$_2$), 1.38 (s, 3H, C(C$\underline{H}_3$)$_2$); ^{13}C NMR (126 MHz, $CDCl_3$): δ 139.1 (Ar), 128.7 (Ar), 128.4 (Ar), 127.1 (Ar), 109.6 ($O_2\underline{C}(CH_3)_2$), 104.8 (C-1), 81.5 (C-4), 75.8 (C-5), 74.6 (C-2), 74.2 (C-3), 65.3 (C-6), 55.5 (2×, 2 × Ph$\underline{C}H_2$O), 55.0 (O$\underline{C}H_3$), 26.4 (C($\underline{C}H_3$)$_2$), 25.4 (C($\underline{C}H_3$)$_2$); HRMS (ESI) Calc. for (M + H) $C_{24}H_{32}NO_5$: 414.2275. Found 414.2268.



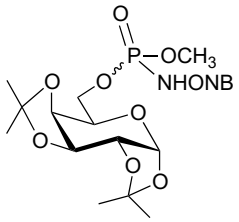
Methyl 2-deoxy-5,6-*O*-isopropylidene-2-*N*-trichloroacetamido-β-D-galactofuranoside (2-34)

To a suspended solution of **S3** (160 mg, 0.69 mmol) and DMAP (8 mg, 0.69 mmol) in anhydrous CH_2Cl_2 (7 mL) was added Et_3N (287 μL, 2.06 mmol). The mixture was cooled to 0 °C, then TCA-Cl (200 μL, 1.78 mmol) was added dropwise via syringe over followed by 2–3 min. The reaction mixture was warmed to room temperature and stirred for 3 h. The mixture was diluted with CH_2Cl_2, then washed with a saturated aqueous solution of NH_4Cl (1×), H_2O (1×), and a saturated aqueous solution of $NaHCO_3$ (1×). The organic layer was dried over $MgSO_4$, filtered, and concentrated. After drying under reduce pressure, the residue was diluted with anhydrous CH_3OH (5 mL) and placed under a positive pressure of argon. To this stirring solution, small slivers of Na^0 was added until the pH of the reaction mixture measured ~9 by pH paper. The reaction mixture was then stirred for 1 h. The mixture was then concentrated and the residue was purified by flash chromatography (3:2→1:1 hexanes–EtOAc) to afford **2-34** (213 mg, 82%) as a clear oil. R_f 0.35 (1:1 hexanes–EtOAc); $[\alpha]_D$ −15.2 (*c* 1.0, $CHCl_3$; ^{1}H NMR (600 MHz; $CDCl_3$): δ 7.84 (d, 1H, *J* = 8.8 Hz, N<u>H</u>), 4.95 (s, 1H, H-1), 4.41 (d, 1H, *J* = 9.0 Hz, H-2), 4.36 (dd, 1H, *J* = 8.8, 6.5 Hz, H-5), 4.14–4.10 (m, 3H, H-3, H-4, H-6), 3.97 (dd, 1H, *J* = 8.6, 8.6 Hz, H-6'), 3.44 (s, 3H, OC<u>H</u>$_3$), 3.07 (d, 1H, *J* = 10.9 Hz, C-3-O<u>H</u>), 1.46 (s, 3H, C(C<u>H</u>$_3$)$_2$), 1.41 (s, 3H, C(C<u>H</u>$_3$)$_2$); ^{13}C NMR (126 MHz; $CDCl_3$): δ 161.6 (C=O), 110.4 (O_2<u>C</u>$(CH_3)_2$), 108.2 (C-1), 84.9, 78.2, 75.9 (C-5), 65.8 (C-6), 61.5 (C-2), 55.2 (O<u>C</u>H$_3$), 26.3 (C(<u>C</u>H$_3$)$_2$), 25.9 (C(<u>C</u>H$_3$)$_2$); HRMS (ESI) Calc. for (M + Na) $C_{12}H_{18}Cl_3NNaO_6$: 400.0092. Found 400.0085.



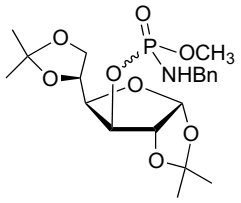
1,2:3,4-di-*O*-isopropylidene-6-*O*-(methyl *N*-*p*-methoxybenzylphosphoramidyl)-D-galactopyranose (2-35)

General procedure for MeOPN synthesis was used and the reaction was carried out on **2-8** (78 mg, 0.30 mmol). Purified by flash chromatography (4:1→6:1 EtOAc–hexanes with 1% Et_3N) to afford **2-35** (115 mg, 81%) as a clear oil. R_f 0.18 (4:1 EtOAc–hexanes); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.28–7.20 (m, 4H, Ar), 6.88–6.80 (m, 4H, Ar), 5.52 (d, 1H, *J* = 5.0 Hz, H-1), 5.51 (d, 1H, *J* = 5.0 Hz, H-1*), 4.61 (dd, 1H, *J* = 7.8, 2.3 Hz, H-3), 4.59 (dd, 1H, *J* = 7.8, 2.3 Hz, H-3*), 4.31 (dd, 2H, *J* = 5.0, 2.5 Hz, H-2, H-2*), 4.23 (dd, 1H, *J* = 7.9, 2.0 Hz, H-4), 4.22 (dd, 1H, *J* = 7.7, 1.9 Hz, H-4*), 4.20–4.02 (m, 10H, H-5, H-5*, H-6, H-6', H-6*, H-6'*, ArC$\underline{H_2}$N, ArC$\underline{H_2}$N*), 3.78 (s, 6H, ArOC$\underline{H_3}$, ArOC$\underline{H_3}$*), 3.70 (d, 3H, *J* = 3.2 Hz, POC$\underline{H_3}$), 3.69 (d, 3H, *J* = 3.1 Hz, POC$\underline{H_3}$), 2.99 (app. quin., 2H, *J* = 6.4 Hz, N$\underline{H}$, N$\underline{H}$*), 1.53 (s, 3H, C(C$\underline{H_3}$)$_2$), 1.51 (s, 3H, C(C$\underline{H_3}$)$_2$), 1.41 (s, 3H, C(C$\underline{H_3}$)$_2$), 1.40 (s, 3H, C(C$\underline{H_3}$)$_2$), 1.31 (s, 6H, 2 × C(C$\underline{H_3}$)$_2$), 1.30 (s, 3H, C(C$\underline{H_3}$)$_2$), 1.29 (s, 3H, C(C$\underline{H_3}$)$_2$); ^{13}C NMR (126 MHz, $CDCl_3$): δ 158.9 (2C), 131.8 (3C), 131.7, 128.8, 114.0, 113.9, 109.6, 109.5, 108.8 (2C), 96.3, 70.9, 70.7 (2C), 70.5 (2C), 67.4 (2C), 66.8 (2C), 65.4 (2C), 64.9 (2C), 55.3 (3C), 29.7, 26.0 (2C), 25.9, 25.0 (2C), 24.5; ^{31}P NMR (162 MHz, $CDCl_3$): δ 10.17, 9.94; HRMS (ESI) Calc. for (M + Na) $C_{21}H_{32}NNaO_9P$: 469.1707. Found 496.1689.



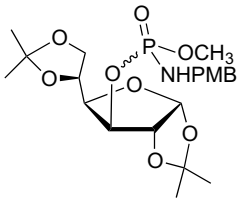
1,2:3,4-di-*O*-isopropylidene-6-*O*-(methyl *N*-*o*-nitrobenzylphosphoramidyl)-D-galactopyranose (18)

General procedure for MeOPN synthesis was used and the reaction was carried out on **2-8** (78 mg, 0.30 mmol). Purified by flash chromatography (4:1→6:1 EtOAc–hexanes with 1% Et_3N) to afford **18** (108 mg, 73%) as a clear oil. R_f 0.20 (4:1 EtOAc–hexanes); ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.06–8.04 (m, 2H, Ar), 7.75–7.72 (m, 2H, Ar), 7.67–7.63 (m, 2H, Ar), 7.48–7.44 (m, 2H, Ar), 5.53 (d, 2H, J = 5.0 Hz, H-1, H-1*), 4.63–4.60 (m, 2H, H-3, H-3*), 4.43–4.39 (m, 4H, ArC$\underline{H_2}$N, ArC$\underline{H_2}$N*), 4.33 (dd, 1H, J = 4.8, 2.3 Hz, H-2), 4.33 (dd, 1H, J = 4.9, 2.5 Hz, H-2*), 4.23 (dd, 1H, J = 7.9, 1.9 Hz, H-4), 4.20 (dd, 1H, J = 8.0, 1.7 Hz, H-4*), 4.15–4.06 (m, 6H, H-5, H-5*, H-6, H-6', H-6*, H-6'*), 3.71–3.64 (m, 8H, N$\underline{H}$, N$\underline{H}$*, POC$\underline{H_3}$, POC$\underline{H_3}$*), 1.53 (s, 3H, C(C$\underline{H_3}$)$_2$), 1.52 (s, 3H, C(C$\underline{H_3}$)$_2$), 1.42 (s, 3H, C(C$\underline{H_3}$)$_2$), 1.41 (s, 3H, C(C$\underline{H_3}$)$_2$), 1.32 (s, 9H, 3 × C(C$\underline{H_3}$)$_2$), 1.31 (s, 3H, C(C$\underline{H_3}$)$_2$); ^{13}C NMR (126 MHz, $CDCl_3$): δ 148.3, 148.2, 135.5, 135.4, 134.0, 133.9, 131.4 (2C), 128.5 (2C), 125.0 (2C), 109.7, 109.6, 108.8 (2C), 96.3 (2C), 77.3 (2C), 77.0, 76.8, 70.8, 70.7, 70.6, 70.4 (2C), 67.5, 67.4, 66.8, 66.7, 65.6 (2C), 65.1 (2C), 53.3, 53.2 (3C), 43.2 (2C), 26.0 (2C), 25.9, 25.0, 24.9, 24.5, 24.4; ^{31}P NMR (202 MHz, $CDCl_3$): δ 10.12, 9.78; HRMS (ESI) *m/z* (M + Na^+) Calc. for $C_{20}H_{29}N_2NaO_{10}P$: 511.1452. Found 511.1445.



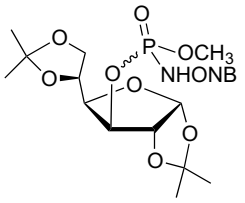
1,2:5,6-di-*O*-isopropylidene-3-*O*-(methyl *N*-benzylphosphoramidyl)-D-glucopyranose (2-37)

General procedure for MeOPN synthesis was used and the reaction was carried out on **2-26** (78 mg, 0.30 mmol). Purified by flash chromatography (4:1→6:1 EtOAc–hexanes with 1% Et_3N) to afford **2-37** (100 mg, 75%) as a clear oil. R_f 0.27 (4:1 EtOAc–hexanes); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.36–7.26 (m, 10H, Ar), 5.91 (d, 1H, *J* = 3.5 Hz, H-1), 5.84 (d, 1H, *J* = 3.6 Hz, H-1*), 4.86 (d, 1H, *J* = 3.5 Hz, H-2), 4.81–4.78 (m, 2H, H-3, H-3*), 4.72 (d, 1H, *J* = 3.6 Hz, H-2*), 4.25–4.05 (m, 10H, H-4, H-4*, H-5, H-5*, H-6, H-6*, PhC$\underline{H_2}$N, PhC$\underline{H_2}$N*), 4.00–3.96 (m, 2H, H-6', H-6'*), 3.76 (d, 3H, *J* = 11.2 Hz, POC$\underline{H_3}$), 3.72 (d, 3H, *J* = 11.4 Hz, POC$\underline{H_3}$*), 3.44 (app. quin., 1H, *J* = 6.3 Hz, N$\underline{H}$), 3.34 (app. quin., 1H, *J* = 6.3 Hz, N$\underline{H}$*), 1.50 (s, 3H, C(C$\underline{H_3}$)$_2$), 1.48 (s, 3H, C(C$\underline{H_3}$)$_2$), 1.34 (s, 3H, C(C$\underline{H_3}$)$_2$), 1.31 (s, 3H, C(C$\underline{H_3}$)$_2$), 1.28 (s, 3H, C(C$\underline{H_3}$)$_2$), 1.23 (s, 3H, C(C$\underline{H_3}$)$_2$), 1.21 (s, 3H, C(C$\underline{H_3}$)$_2$), 1.16 (s, 3H, C(C$\underline{H_3}$)$_2$); ^{13}C NMR (126 MHz, $CDCl_3$): δ 139.4 (2C), 139.3 (2C), 128.7 (2C), 127.6, 127.5, 112.4, 112.3, 109.5, 109.4, 105.3, 105.2, 83.8, 83.6, 80.7 (2C), 80.6 (2C), 79.5 (2C), 78.4, 78.3, 72.4, 72.2, 67.6, 67.4, 53.6, 53.5 (2C), 53.5, 45.3, 45.2, 26.8 (2C), 26.7, 26.3, 26.2, 25.2; ^{31}P NMR (202 MHz, $CDCl_3$): δ 9.93, 8.58; HRMS (ESI) *m/z* (M + Na^+) Calc. for $C_{20}H_{30}NNaO_8P$: 466.1601. Found 466.1595.



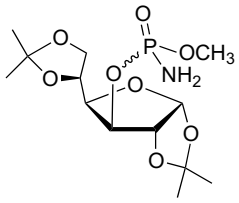
1,2:5,6-di-*O*-isopropylidene-3-*O*-(methyl *N*-*p*-methoxybenzylphosphoramidyl)-D-glucopyranose (2-38)

General procedure for MeOPN synthesis was used and the reaction was carried out on **2-26** (156 mg, 0.60 mmol). Purified by flash chromatography (4:1→6:1 EtOAc–hexanes with 1% Et_3N) to afford **2-38** (201 mg, 71%) as a clear oil. R_f 0.27 (4:1 EtOAc–hexanes); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.25–7.21 (m, 4H, Ar), 6.88–6.84 (m, 4H, Ar), 5.90 (d, 1H, *J* = 3.5 Hz, H-1), 5.85 (d, 1H, *J* = 3.6 Hz, H-1*), 4.85 (d, 1H, *J* = 3.5 Hz, H-2), 4.79-4.76 (m, 2H, H-3, H-3*), 4.72 (d, 1H, *J* = 3.6 Hz, H-2*), 4.27–4.18 (m, 2H, H-5, H-5*), 4.13–4.03 (m, 8H, H-4, H-4*, H-6, H-6*, ArC$\underline{H_2}$N, ArC$\underline{H_2}$N*), 3.99 (dd, 1H, *J* = 4.9, 1.9 Hz, H-6'), 3.97 (dd, 1H, *J* = 5.0, 1.8 Hz, H-6'*), 3.79 (s, 3H, ArOC$\underline{H_3}$), 3.79 (s, 3H, ArOC$\underline{H_3}$*), 3.75 (d, 3H, *J* = 11.2 Hz, POC$\underline{H_3}$), 3.71 (d, 3H, *J* = 11.4 Hz, POC$\underline{H_3}$*), 3.35 (app. quin., 1H, *J* = 6.0 Hz, N$\underline{H}$), 3.24 (app. quin., 1H, *J* = 6.3 Hz, N$\underline{H}$*), 1.50 (s, 3H, C(C$\underline{H_3}$)$_2$), 1.48 (s, 3H, C(C$\underline{H_3}$)$_2$), 1.35 (s, 3H, C(C$\underline{H_3}$)$_2$), 1.31 (s, 3H, C(C$\underline{H_3}$)$_2$), 1.28 (s, 3H, C(C$\underline{H_3}$)$_2$), 1.25 (s, 3H, C(C$\underline{H_3}$)$_2$), 1.22 (s, 3H, C(C$\underline{H_3}$)$_2$), 1.18 (s, 3H, C(C$\underline{H_3}$)$_2$); ^{13}C NMR (126 MHz, $CDCl_3$): δ 131.6 (2C), 131.5, 131.4, 128.8, 128.7, 114.1, 114.0, 112.4, 112.3, 109.5, 109.4, 105.3, 105.2, 83.8, 83.6, 80.8, 80.7 (2C), 80.6, 79.5, 79.4, 78.3 (2C), 72.4, 72.2, 67.6, 67.4, 55.3, 53.5 (2C), 53.5, 53.4, 44.8, 44.7, 26.8 (2C), 26.8, 26.7, 26.3, 25.2; ^{31}P NMR (202 MHz, $CDCl_3$): δ 9.88, 8.56; HRMS (ESI) *m/z* (M + Na^+) Calc. for $C_{21}H_{32}NNaO_9P$: 496.1707. Found 496.1700.



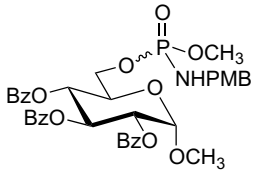
1,2:5,6-di-*O*-isopropylidene-3-*O*-(methyl *N*-*o*-nitrobenzylphosphoramidyl)-D-glucopyranose (2-39)

General procedure for MeOPN synthesis was used and the reaction was carried out on **2-26** (78 mg, 0.30 mmol). Purified by flash chromatography (4:1→6:1 EtOAc–hexanes with 1% Et_3N) to afford **2-39** (105 mg, 71%) as a clear oil. R_f 0.21 (4:1 EtOAc–hexanes); ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.09–8.06 (m, 2H, Ar), 7.73–7.65 (m, 4H, Ar), 7.52–7.48 (m, 2H, Ar), 5.92 (d, 1H, *J* = 3.5 Hz, H-1), 5.85 (d, 1H, *J* = 3.6 Hz, H-1*), 4.84 (d, 1H, *J* = 3.5 Hz, H-2), 4.78 (dd, 1H, *J* = 7.4, 2.7 Hz, H-3), 4.79–4.72 (m, 2H, H-2*, H-3*), 4.47–4.41 (m, 4H, H-6, H-6', H-6*, H-6'*), 4.22–3.98 (m, 9H, H-4, H-4*, H-5, H-5*, NH, ArCH_2N, ArCH_2N*), 3.84 (app. quin., 1H, *J* = 7.5 Hz, NH*), 3.69 (d, 3H, *J* = 11.3 Hz, POCH_3), 3.61 (d, 3H, *J* = 11.5 Hz, POCH_3*), 1.50 (s, 3H, C(CH_3)$_2$), 1.49 (s, 3H, C(CH_3)$_2$), 1.41 (s, 3H, C(CH_3)$_2$), 1.38 (s, 3H, C(CH_3)$_2$), 1.31 (s, 3H, C(CH_3)$_2$), 1.31 (s, 3H, C(CH_3)$_2$), 1.29 (s, 3H, C(CH_3)$_2$), 1.28 (s, 3H, C(CH_3)$_2$); ^{13}C NMR (126 MHz, $CDCl_3$): δ 148.3, 148.2, 135.3 (2C), 135.1 (2C), 134.1, 133.9, 131.7, 131.3, 125.2, 125.0, 112.4, 112.3, 109.7, 109.5, 105.3, 105.1, 83.8 (2C), 83.5 (2C), 80.6 (2C), 80.6, 80.5, 79.6 (2C), 78.5, 78.4, 77.3, 77.2, 77.0, 76.8, 72.4, 72.2, 67.5, 67.4, 53.5, 53.4, 53.4, 53.3, 43.2 (2C), 26.9, 26.8 (2C), 26.7, 26.3, 26.2, 25.2, 25.1; ^{31}P NMR (202 MHz, $CDCl_3$): δ 9.92, 8.42; HRMS (ESI) *m/z* (M + Na^+) Calc. for $C_{20}H_{29}N_2NaO_{10}P$: 511.1452. Found 511.1442.



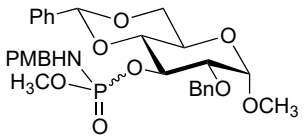
1,2:5,6-di-*O*-isopropylidene-3-*O*-(methyl phosphoramidyl)-D-glucopyranose (2-40)

General procedure for MeOPN synthesis was used and the reaction was carried out on **2-26** (78 mg, 0.30 mmol). Purified by flash chromatography (20:1 CH_2Cl_2–CH_3OH with 1% Et_3N) to afford **2-40** (59 mg, 56%) as a white solid. R_f 0.19 (20:1 CH_2Cl_2–CH_3OH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.91 (d, 2H, J = 3.5 Hz, H-1, H-1*), 4.86 (dd, 1H, J = 9.9, 2.7 Hz, H-3), 4.79–4.75 (m, 3H, H-2, H-2*, H-3*), 4.31–4.25 (m, 1H, H-5), 4.24–4.19 (m, 1H, H-5*), 4.15–4.08 (m, 4H, H-4, H-4*, H-6, H-6*), 4.02 (dd, 2H, J = 8.8, 4.4 Hz, H-6', H-6'*), 3.80 (d, 3H, J = 11.4 Hz, POC$\underline{H}_3$), 3.75 (d, 3H, J = 11.6 Hz, POC$\underline{H}_3$*), 3.44 (d, 2H, J = 4.1 Hz, PN$\underline{H}_2$), 3.31 (s, 2H, PN$\underline{H}_2$*), 1.48 (s, 6H, 2 × C(C$\underline{H}_3$)$_2$), 1.42 (s, 6H, 2 × C(C$\underline{H}_3$)$_2$), 1.33 (s, 3H, C(C$\underline{H}_3$)$_2$), 1.32 (s, 3H, C(C$\underline{H}_3$)$_2$), 1.30 (s, 3H, C(C$\underline{H}_3$)$_2$), 1.29 (s, 3H, C(C$\underline{H}_3$)$_2$); ^{13}C NMR (126 MHz, $CDCl_3$): δ 112.4 (2C), 109.6, 109.5, 105.3, 105.2, 84.0 (2C), 83.8, 83.7, 80.7, 80.6 (2C), 80.5, 79.6 (2C), 78.5 (2C), 72.6, 72.3, 67.7, 67.4, 53.6 (2C), 53.5 (2C), 26.9 (2C), 26.8 (2C), 26.3, 26.2, 25.4, 25.3; ^{31}P NMR (202 MHz, $CDCl_3$) δ 11.29, 9.98; HRMS (ESI) m/z (M + Na^+) Calc. for $C_{13}H_{24}NNaO_8P$: 376.1132. Found 376.1124.



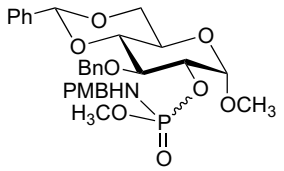
Methyl 2,3,4-tri-*O*-benzoyl-6-*O*-(methyl *N*-benzylphosphoramidyl)-α-D-glucopyranoside (2-41)

General procedure for MeOPN synthesis was used and the reaction was carried out on **2-27**[62,63] (152 mg, 0.30 mmol). Purified by flash chromatography (4:1→6:1 EtOAc–hexanes with 1% Et_3N) to afford **2-41** (168 mg, 78%) as a clear oil. R_f 0.24 (4:1 EtOAc–hexanes); ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.00–7.81 (m, 12H, Ar), 7.53–7.47 (m, 4H, Ar), 7.44–7.23 (m, 10H, Ar), 7.30–7.24 (m, 6H, Ar), 7.18 (d, 2H, *J* = 8.6 Hz, Ar), 6.89–6.84 (m, 2H, Ar), 6.82–6.77 (m, 2H, Ar), 6.17 (dd, 1H, *J* = 9.6, 9.6 Hz, H-3), 6.15 (dd, 1H, *J* = 9.7, 9.7 Hz, H-3*), 5.62 (dd, 1H, *J* = 9.8, 9.7 Hz, H-4), 5.58 (dd, 1H, *J* = 9.8, 9.9 Hz, H-4*), 5.25 (dd, 1H, *J* = 3.2, 3.2 Hz, H-2), 5.24–5.18 (m, 3H, H-1, H-1*, H-2*), 4.28–4.19 (m, 5H, H-5, H-5*, H-6, H-6', H-6*), 4.13 (dd, 1H, *J* = 11.5, 5.0 Hz, H-6'*), 4.10–4.06 (m, 2H, ArC$\underline{H}_2$N, ArC$\underline{H}_2$N*), 4.00 (dd, 2H, *J* = 6.8, 2.0 Hz, ArC$\underline{H}_2$N, ArC$\underline{H}_2$N*), 3.79 (s, 3H, ArOC$\underline{H}_3$), 3.77 (s, 3H, ArOC$\underline{H}_3$*), 3.76 (d, 3H, *J* = 11.2 Hz, POC$\underline{H}_3$), 3.64 (d, 3H, *J* = 11.2 Hz, POC$\underline{H}_3$*), 3.47 (s, 3H, C-1–OC$\underline{H}_3$), 3.45 (s, 3H, C-1–OC$\underline{H}_3$*), 3.03–2.95 (m, 2H, N$\underline{H}$, N$\underline{H}$*); ^{13}C NMR (126 MHz, $CDCl_3$): δ 165.8 (2C), 165.7, 165.5, 165.2, 159.0, 158.9, 133.5, 133.4, 133.1 (2C), 131.7 (3C), 131.6, 129.9 (2C), 129.8, 129.7 (2C), 129.2 (2C), 129.0 (2C), 128.8, 128.7 (2C), 128.5, 128.4 (2C), 128.3, 114.0 (3C), 97.1, 97.0, 72.0 (2C), 70.5, 70.4, 69.0, 68.9, 68.4 (2C), 68.3 (2C), 64.5, 64.4 (2C), 64.3, 55.7, 55.3 (2C), 53.4 (2C), 53.3 (2C), 44.8 (2C); ^{31}P NMR (202 MHz, $CDCl_3$): δ 10.06, 9.70; HRMS (ESI) *m/z* ($M + Na^+$) Calc. for $C_{37}H_{38}NNaO_{12}P$: 742.2024. Found 742.2018.



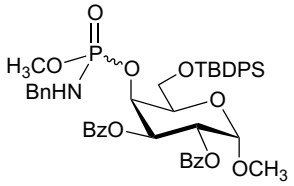
Methyl 2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-(methyl *N*-*p*-methoxybenzylphosphoramidyl)-α-D-glucopyranoside (2-42)

General procedure for MeOPN synthesis was used and the reaction was carried out on **2-28**[64] (225 mg, 0.60 mmol). Purified by flash chromatography (2:1→4:1 EtOAc–hexanes with 1% Et_3N) to afford **2-42** (287 mg, 82%) as a clear oil. R_f 0.22 (2:1 EtOAc–hexanes); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.46–7.21 (m, 20H, Ar), 7.03 (d, 2H, J = 8.7 Hz, Ar), 6.98 (d, 2H, J = 8.7 Hz, Ar), 6.79–6.73 (m, 4H, Ar), 5.47 (s, 1H, PhC$\underline{H}O_2$), 5.45 (s, 1H, PhC$\underline{H}O_2$*), 4.81 (d, 1H, J = 11.9 Hz, PhC$\underline{H_2}$O), 4.74 (d, 1H, J = 9.0 Hz, PhC$\underline{H_2}$O*), 4.73–4.57 (m, 6H, H-1, H-1*, H-3, H-3*, PhC$\underline{H_2}$O, PhC$\underline{H_2}$O*), 4.25 (app. qt, 2H, J = 10.3, 5.1 Hz, H-6, H-6*), 3.95–3.76 (m, 12H, H-5, H-5*, ArC$\underline{H_2}$N, ArC$\underline{H_2}$N*, ArOC$\underline{H_3}$, ArOC$\underline{H_3}$*), 3.70 (dd, 1H, J = 10.3, 2.0 Hz, H-6'), 3.68 (dd, 1H, J = 10.3, 2.0 Hz, H-6'*), 3.66–3.56 (m, 7H, H-2, H-2*, H-4, H-4*, POC$\underline{H_3}$), 3.53 (d, 3H, J = 11.3 Hz, POC$\underline{H_3}$*), 3.38–3.29 (m, 8H, C-1–OC$\underline{H_3}$, C-1–OC$\underline{H_3}$*, N$\underline{H}$, N$\underline{H}$*); ^{13}C NMR (126 MHz, $CDCl_3$): δ 158.7 (2C), 137.6, 137.4, 136.8, 136.6, 132.1 (2C), 132.1 (2C), 129.3, 129.2, 128.6 (2C), 128.6, 128.3 (2C), 128.3, 128.2 (2C), 128.2, 126.3, 126.1, 113.7 (2C), 102.1, 101.9, 98.9, 98.7, 80.5, 80.4 (2C), 80.3, 78.6 (2C), 78.6 (2C), 75.8, 75.7, 75.3 (2C), 73.7, 73.3, 68.9, 62.2, 62.1, 55.4, 55.3, 53.7 (2C), 53.6 (2C), 44.5; ^{31}P NMR (202 MHz, $CDCl_3$): δ 9.26, 8.53; HRMS (ESI) *m/z* ($M + Na^+$) Calc. for $C_{30}H_{36}NNaO_9P$: 608.2020. Found 608.2006.



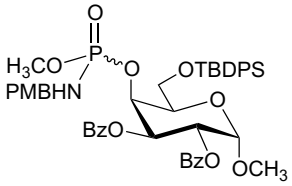
Methyl 3-*O*-benzyl-4,6-*O*-benzylidene-2-*O*-(methyl *N*-*p*-methoxybenzylphosphoramidyl)-α-D-glucopyranoside (2-43)

General procedure for MeOPN synthesis was used and the reaction was carried out on **2-29**[65] (112 mg, 0.30 mmol). Purified by flash chromatography (2:1→6:1 EtOAc–hexanes with 1% Et_3N) to afford **2-43** (140 mg, 80%) as a clear oil. R_f 0.25 (4:1 EtOAc–hexanes); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.49–7.32 (m, 4H, Ar), 7.40–7.32 (m, 8H, Ar), 7.30–7.18 (m, 10H, Ar), 7.05–7.02 (m, 2H, Ar), 6.87–6.83 (m, 2H, Ar), 6.82–6.77 (m, 2H, Ar), 5.58 (s, 1H, PhC$\underline{H}O_2$), 5.57 (s, 1H, PhC$\underline{H}O_2$*), 5.05 (d, 1H, *J* = 3.8 Hz, H-1), 5.01 (d, 1H, *J* = 3.8 Hz, H-1*), 4.95 (d, 1H, *J* = 11.2 Hz, PhC$\underline{H}_2$O), 4.91 (d, 1H, *J* = 11.4 Hz, PhC$\underline{H}_2$O*), 4.73 (d, 1H, *J* = 11.4 Hz, PhC$\underline{H}_2$O), 4.65 (d, 1H, *J* = 11.2 Hz, PhC$\underline{H}_2$O*), 4.39 (dddd, 1H, *J* = 8.1, 8.1, 3.8, 3.8 Hz, H-2), 4.33–4.26 (m, 3H, H-2*, H-6, H-6*), 4.07–3.98 (m, 4H, H-3, H-3*, ArC$\underline{H}_2$N, ArC$\underline{H}_2$N*), 3.92–3.84 (m, 4H, H-5, H-5*, ArC$\underline{H}_2$N, ArC$\underline{H}_2$N*), 3.80–3.73 (m, 8H, H-6', H-6'*, ArOC$\underline{H}_3$, ArOC$\underline{H}_3$*), 3.69–3.64 (m, 5H, H-4, H-4*, POC$\underline{H}_3$), 3.63 (d, 3H, *J* = 11.3 Hz, POC$\underline{H}_3$*), 3.47 (s, 3H, C-1–OC$\underline{H}_3$), 3.40 (s, 3H, C-1–OC$\underline{H}_3$*), 2.98–2.90 (m, 2H, N$\underline{H}$, N$\underline{H}$*); ^{13}C NMR (126 MHz, $CDCl_3$): δ 159.0, 158.8, 138.4, 138.2, 137.3 (2C), 131.8, 131.7, 131.6 (2C), 129.0 (2C), 128.7 (2C), 128.4, 128.3, 128.2, 128.0, 127.8 (2C), 127.6, 126.0 (2C), 114.0, 113.8, 101.4, 99.2, 98.9, 82.5, 82.1, 76.0 (2C), 75.3, 75.0, 74.9, 74.8, 69.0, 62.3, 55.6, 55.4, 55.3 (2C), 53.5 (2C), 53.4, 53.3, 44.7; ^{31}P NMR (202 MHz, $CDCl_3$): δ 10.48, 9.04; HRMS (ESI) *m/z* (M + Na^+) Calc. for $C_{30}H_{36}NNaO_9P$: 608.2020. Found 608.2017.



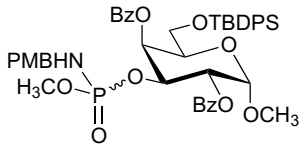
Methyl 2,3-di-*O*-benzoyl-6-*O*-*tert*-butyldiphenylsilyl-4-*O*-(methyl *N*-benzylphosphoramidyl)-α-D-galactopyranoside (2-44)

General procedure for MeOPN synthesis was used and the reaction was carried out on **2-30** (383 mg, 0.60 mmol). Purified by flash chromatography (2:1→1:1 hexanes–EtOAc with 1% Et_3N) to afford **2-44** (360 mg, 73%) as a clear oil. R_f 0.17 & 0.26 (2:1 hexanes–EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.05–7.98 (m, 8H, Ar), 7.76–7.69 (m, 8H, Ar), 7.56–7.16 (m, 34H, Ar), 5.77–5.64 (m, 4H, H-2, H-2*, H-3, H-3*), 5.20 (d, 1H, *J* = 3.6 Hz, H-1), 5.19–5.14 (m, 3H, H-1*, H-4, H-4*), 4.17–4.13 (m, 2H, H-5, H-5*), 4.09–3.87 (m, 8H, H-6, H-6', H-6*, H-6'*, PhC$\underline{H_2}$N, PhC$\underline{H_2}$N*), 3.43 (d, 3H, *J* = 11.4 Hz, POC$\underline{H_3}$), 3.43 (s, 3H, C-1-OC$\underline{H_3}$), 3.41 (s, 3H, C-1-OC$\underline{H_3}$*), 3.41 (d, 3H, *J* = 11.4 Hz, POC$\underline{H_3}$*), 2.77 (m, 2H, N$\underline{H}$, N$\underline{H}$*), 1.11 (s, 9H, SiC(C$\underline{H_3}$)$_3$), 1.10 (s, 9H, SiC(C$\underline{H_3}$)$_3$*); ^{13}C NMR (126 MHz, $CDCl_3$): δ 166.1 (2C), 165.9 (2C), 139.4 (2C), 139.4, 139.3, 135.7, 135.6, 135.5, 133.3 (4C), 133.1, 133.0, 129.9 (2C), 129.8 (2C), 129.7 (3C), 129.5, 129.4, 129.1, 128.5 (2C), 128.4 (3C), 128.3, 128.2, 127.8 (4C), 127.7, 127.4, 127.3 (3C), 125.3, 97.3 (2C), 73.5, 73.4, 73.2 (2C), 70.1 (4C), 69.7 (2C), 69.5 (2C), 68.9, 68.8, 63.3, 63.1, 55.3, 55.2, 53.3, 53.2 (3C), 45.4, 45.3, 26.8 (2C), 19.2; ^{31}P NMR (202 MHz, $CDCl_3$): δ 9.64, 9.30; HRMS (ESI) *m/z* (M + Na^+) Calc. for $C_{45}H_{50}NNaO_{10}PSi$: 846.2834. Found 846.2820.



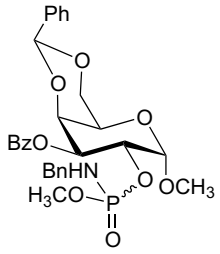
Methyl 2,3-di-*O*-benzoyl-6-*O*-*tert*-butyldiphenylsilyl-4-*O*-(methyl *N*-*p*-methoxybenzylphosphoramidyl)-α-D-galactopyranoside (2-45)

General procedure for MeOPN synthesis was used and the reaction was carried out on **2-30** (189 mg, 0.30 mmol). Purified by flash chromatography (2:1→3:2 hexanes–EtOAc with 1% Et_3N) to afford **2-45** (199 mg, 77%) as a clear oil. R_f 0.13 & 0.21 (2:1 hexanes–EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.99 (app. t, 6H, *J* = 7.2 Hz, Ar), 7.94 (d, 2H, *J* = 7.7 Hz, Ar), 7.73–7.65 (m, 8H, Ar), 7.56–7.22 (m, 24H, Ar), 7.08 (d, 2H, *J* = 8.4 Hz, Ar), 7.05 (d, 2H, *J* = 8.5 Hz, Ar), 6.78 (app. t, 4H, *J* = 8.2 Hz, Ar), 5.74–5.57 (m, 4H, H-2, H-2*, H-3, H-3*), 5.17 (d, 1H, *J* = 3.5 Hz, H-1), 5.15 (d, 1H, *J* = 3.2 Hz, H-1*), 5.14–5.09 (m, 2H, H-4, H-4*), 4.15–4.07 (m, 2H, H-5, H-5*), 4.03–3.79 (m, 8H, H-6, H-6', H-6*, H-6'*, ArC$\underline{H_2}$N, ArC$\underline{H_2}$N*), 3.78 (s, 3H, ArOC$\underline{H_3}$), 3.77 (s, 3H, ArOC$\underline{H_3}$*), 3.40–3.35 (m, 12H, C-1–OC$\underline{H_3}$, C-1–OC$\underline{H_3}$*, POC$\underline{H_3}$, POC$\underline{H_3}$*), 2.69–2.58 (m, 2H, N$\underline{H}$, N$\underline{H}$*) 1.08 (s, 9H, SiC(C$\underline{H_3}$)$_3$), 1.07 (s, 9H, SiC(C$\underline{H_3}$)$_3$*); ^{13}C NMR (126 MHz, $CDCl_3$): δ 166.1, 166.0, 165.9, 165.8, 158.9 (2C), 135.7, 135.6, 135.5, 133.3, 133.0 (2C), 131.6 (3C), 131.5, 129.9, 129.8, 129.7 (3C), 129.5, 129.4, 128.7, 128.6, 128.4 (2C), 128.3, 127.8 (3C), 113.9 (2C), 97.3, 97.2, 73.4, 73.3, 73.2, 73.1, 70.2, 70.1 (3C), 69.7 (2C), 69.5 (2C), 69.0, 68.8, 63.3, 63.2, 55.3 (2C), 55.2, 53.2 (3C), 53.1, 44.9, 44.8, 26.8 (2C), 19.2; ^{31}P NMR (202 MHz, $CDCl_3$): δ 9.65, 9.30; HRMS (ESI) *m/z* (M + Na^+) Calc. for $C_{46}H_{52}NNaO_{11}PSi$: 876.2939. Found 876.2926.



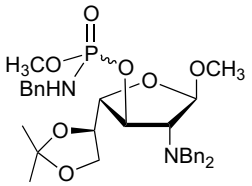
Methyl 2,4-di-*O*-benzoyl-6-*O*-*tert*-butyldiphenylsilyl-3-*O*-(methyl *N*-*p*-methoxybenzylphosphoramidyl)-α-D-galactopyranoside (2-46)

General procedure for MeOPN synthesis was used and the reaction was carried out on **2-31** (192 mg, 0.30 mmol). Purified by flash chromatography (2:1→3:2 hexanes–EtOAc with 1% Et_3N) to afford **2-46** (192 mg, 75%) as a clear oil. R_f 0.30 & 0.38 (2:1 hexanes–EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.21–8.14 (m, 4H, Ar), 8.05–8.01 (m, 4H, Ar), 7.67–7.21 (m, 32H, Ar), 7.14 (app. t, 2H, *J* = 7.6 Hz, Ar), 6.90 (d, 2H, *J* = 8.6 Hz, Ar), 6.82–6.81 (m, 2H, Ar), 6.64–6.62 (m, 2H, Ar), 5.99 (d, 1H, *J* = 3.1 Hz, H-4), 5.89 (d, 1H, *J* = 3.2 Hz, H-4*), 5.39 (dd, 1H, *J* = 10.3, 3.6 Hz, H-2), 5.34 (dd, 1H, *J* = 10.4, 3.6 Hz, H-2*), 5.27–5.16 (m, 4H, H-3, H-3*, H-1, H-1*), 4.17–4.07 (m, 4H, H-5, H-5*, ArC$\underline{H}_2$N, ArC$\underline{H}_2$N*), 3.75–3.71 (m, 12H, H-6, H-6', H-6*, H-6'*, ArC$\underline{H}_2$N, ArC$\underline{H}_2$N*, ArOC$\underline{H}_3$, ArOC$\underline{H}_3$*), 3.69 (d, 3H, *J* = 11.4 Hz, POC$\underline{H}_3$), 3.35 (s, 3H, C-1–OC$\underline{H}_3$), 3.34 (s, 3H, C-1–OC$\underline{H}_3$*), 3.31 (d, 3H, *J* = 11.4 Hz, POC$\underline{H}_3$*), 2.69 (app. t, 1H, *J* = 5.3 Hz, N$\underline{H}$), 2.67 (app. t, 1H, *J* = 5.4 Hz, N$\underline{H}$*), 1.03 (s, 9H, SiC(C$\underline{H}_3$)$_3$), 0.99 (s, 9H, SiC(C$\underline{H}_3$)$_3$*); ^{13}C NMR (126 MHz, $CDCl_3$): δ 166.3 (2C), 166.2, 165.5, 158.8, 158.7, 135.6 (2C), 135.5, 135.4, 133.4, 133.3, 133.2, 133.1, 132.9, 132.9, 132.7, 132.2, 132.1, 131.5, 131.4, 130.2, 130.1, 130.0, 129.9, 129.8, 129.7 (2C), 129.6, 129.5 (2C), 129.4, 128.7, 128.6, 128.5 (3C), 128.4, 127.8, 127.7 (2C), 127.6, 113.9, 113.7, 97.5, 97.3, 71.7 (2C), 71.2 (2C), 70.7 (2C), 70.4 (2C), 70.2, 70.1 (4C), 69.5, 68.7, 62.0, 61.5, 55.5, 55.4, 55.3, 55.2 (3C), 53.4, 53.3, 44.7, 44.6, 36.7, 29.7, 26.7 (2C), 24.7, 19.1, 19.0; ^{31}P NMR (202 MHz, $CDCl_3$): δ 10.56, 9.99; HRMS (ESI) *m/z* (M + Na^+) Calc. for $C_{46}H_{52}NNaO_{11}PSi$: 876.2939. Found 876.2930.



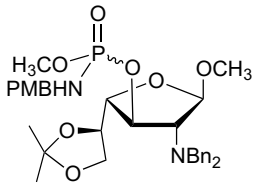
Methyl 3-*O*-benzoyl-4,6-*O*-benzylidene-2-*O*-(methyl *N*-benzylphosphoramidyl)-α-D-galactopyranoside (2-47)

General procedure for MeOPN synthesis was used and the reaction was carried out on **2-32**[61,66] (116 mg, 0.30 mmol). Purified by flash chromatography (2:1→4:1 EtOAc–hexanes with 1% Et_3N) to afford **2-47** (139 mg, 77%) as a clear oil. R_f 0.29 (4:1 EtOAc–hexanes); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.15–8.09 (m, 2H, Ar), 8.03–7.96 (m, 2H, Ar), 7.60–7.07 (m, 26H, Ar), 5.61 (d, 1H, *J* = 10.4 Hz, H-3), 5.59 (d, 1H, *J* = 10.4 Hz, H-3*), 5.52 (s, 2H, PhC$\underline{H}O_2$, PhC$\underline{H}O_2$*), 5.29 (d, 1H, *J* = 3.6 Hz, H-1), 5.17 (d, 1H, *J* = 3.6 Hz, H-1*), 5.13–5.06 (m, 2H, H-2, H-2*), 4.59 (s, 1H, H-4), 4.58 (s, 1H, H-4*), 4.34 (dd, 1H, *J* = 12.6, 1.4 Hz, H-6), 4.31 (dd, 1H, *J* = 12.6, 1.3 Hz, H-6*), 4.13–4.00 (m, 4H, H-6', H-6'*, PhC$\underline{H}_2$N, PhC$\underline{H}_2$N*), 3.97–3.91 (m, 2H, PhC$\underline{H}_2$N, PhC$\underline{H}_2$N*), 3.86 (dd, 2H, *J* = 10.0, 1.0 Hz, H-5, H-5*), 3.69 (d, 3H, *J* = 11.3 Hz, POC$\underline{H}_3$), 3.53 (s, 3H, C-1-OC$\underline{H}_3$), 3.49 (d, 3H, *J* = 11.3 Hz, POC$\underline{H}_3$*), 3.42 (s, 3H, C-1-OC$\underline{H}_3$*), 2.90 (app. dt, 1H, *J* = 11.3, 6.5 Hz, N$\underline{H}$), 2.77 (app. dt, 1H, *J* = 11.3, 6.5 Hz, N$\underline{H}$*); ^{13}C NMR (126 MHz, $CDCl_3$): δ 166.1, 160.9, 158.0, 139.3 (2C), 139.2 (2C), 139.1, 137.6 (2C), 133.3, 130.0, 129.8, 129.7 (2C), 128.9, 128.8, 128.7, 128.6, 128.5 (2C), 128.4, 128.2, 128.1, 127.8, 127.7, 127.5, 127.4 (2C), 127.3, 127.2 (2C), 126.2 (2C), 100.8 (2C), 99.2, 98.9, 74.4, 74.3, 71.1, 71.0, 70.6, 70.5, 70.1, 70.0, 69.9 (2C), 69.1 (2C), 62.2, 55.9, 55.6, 53.3 (2C), 45.4, 45.3, 44.6, 42.2, 35.5; ^{31}P NMR (162 MHz, $CDCl_3$): δ 9.65, 8.75; HRMS (ESI) *m/z* (M + Na^+) Calc. for $C_{29}H_{32}NNaO_9P$: 592.1707. Found 592.1697.



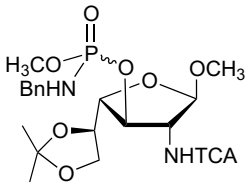
Methyl 2-amino-2-*N*,*N*-di-benzyl-2-deoxy-5,6-*O*-isopropylidene-3-*O*-(methyl *N*-benzylphosphoramidyl)-β-D-galactofuranoside (2-48)

General procedure for MeOPN synthesis was used and the reaction was carried out on **2-33** (120 mg, 0.29 mmol). Purified by flash chromatography (2:1 hexanes–acetone with 1% Et_3N) to afford **2-48** (90 mg, 52%) as a clear oil. R_f 0.44 & 0.39 (2:1 hexanes–acetone); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.40–7.21 (m, 30H, Ar), 5.09 (d, 1H, J = 0.2 Hz, H-1), 5.05–5.00 (m, 3H, H-1*, H-3, H-3*), 4.52–4.46 (m, 2H, H-5, H-5*), 4.12 (dd, 1H, J = 8.1, 7.0 Hz, H-6), 4.07–3.84 (m, 13H, H-4, H-4*, H-6*, H-6', H-6'*, PhC$\underline{H_2}$N, PhC$\underline{H_2}$N*, PhC$\underline{H_2}$N, PhC$\underline{H_2}$N*), 3.69 (d, 3H, J = 11.3 Hz, POC$\underline{H_3}$), 3.64–3.56 (m, 9H, H-2, H-2*, PhC$\underline{H_2}$N, PhC$\underline{H_2}$NC*, POC$\underline{H_3}$*), 3.41 (s, 2H, C-1-OC$\underline{H_3}$), 3.38 (s, 3H, C-1-OC$\underline{H_3}$*), 3.00 (app. dt, 1H, J = 11.2, 6.1 Hz, N$\underline{H}$), 2.77 (app. dt, 1H, J = 11.7, 6.3 Hz, N$\underline{H}$*), 1.40 (s, 3H, C(C$\underline{H_3}$)$_2$), 1.38 (s, 3H, C(C$\underline{H_3}$)$_2$), 1.37 (s, 3H, C(C$\underline{H_3}$)$_2$), 1.35 (s, 3H, C(C$\underline{H_3}$)$_2$); ^{13}C NMR (126 MHz, $CDCl_3$): 139.4 (2C), 139.0, 129.0 (2C), 128.6 (2C), 128.6, 128.3 (2C), 127.7, 127.5 (2C), 127.4 (2C), 127.1 (2C), 109.6 (2C), 104.2, 103.9, 80.9 (2C), 80.5 (2C), 78.9, 78.8, 78.7, 78.6, 74.8, 74.5, 72.9 (2C), 72.7 (2C), 65.7, 55.2, 55.1, 54.8 (2C), 53.3 (2C), 53.2, 53.1, 45.4, 45.3, 45.0, 44.6, 41.3, 26.2, 26.1, 25.4, 13.9; ^{31}P NMR (202 MHz, $CDCl_3$): δ 10.12, 9.78; HRMS (ESI) *m/z* (M + H^+) Calc. for $C_{32}H_{42}N_2O_7P$: 597.2724. Found 597.2709.



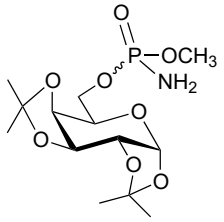
Methyl 2-amino-2-*N*,*N*-di-benzyl-2-deoxy-5,6-*O*-isopropylidene-3-*O*-(methyl *N*-*p*-methoxybenzylphosphoramidyl)-β-D-galactofuranoside (2-49)

General procedure for MeOPN synthesis was used and the reaction was carried out on **2-33** (120 mg, 0.29 mmol). Purified by flash chromatography (2:1 hexanes–acetone with 1% Et_3N) to afford **2-49** (88 mg, 48%) as a clear oil. R_f 0.45 & 0.50 (2:1 hexanes–acetone); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.39 (app. t, *J* = 7.8 Hz, 8H, Ar), 7.32–7.19 (m, 16H, Ar), 6.90–6.86 (m, 4H, Ar), 5.08 (s, 1H, H-1), 5.04–5.00 (m, 3H, H-1, H-3, H-3*), 4.54–4.47 (m, 2H, H-5, H-5*), 4.12 (dd, 1H, *J* = 8.1, 7.0 Hz, H-6), 4.06 (dd, 1H, *J* = 8.1, 7.1 Hz, H-6*), 4.00–3.81 (m, 18H, H-4, H-4*, H-6', H-6'*, ArC$\underline{H}_2$N, ArC$\underline{H}_2$N*, PhC$\underline{H}_2$N, PhC$\underline{H}_2$N*, ArOC$\underline{H}_3$, ArOC$\underline{H}_3$*), 3.68 (d, *J* = 11.3 Hz, 3H, POC$\underline{H}_3$), 3.64–3.56 (m, 9H, H-2, H-2*, POC$\underline{H}_3$*, PhC$\underline{H}_2$N, PhC$\underline{H}_2$N*), 3.40 (s, 3H, C-1-OC$\underline{H}_3$), 3.37 (s, 3H, C-1-OC$\underline{H}_3$*), 2.95 (app. dt, 1H, *J* = 10.7, 6.8 Hz, N$\underline{H}$), 2.74 (app. dt, 1H, *J* = 10.7, 6.8 Hz, N$\underline{H}$*), 1.40 (s, 3H, C(C$\underline{H}_3$)$_2$), 1.38 (s, 3H, C(C$\underline{H}_3$)$_2$), 1.37 (s, 3H, C(C$\underline{H}_3$)$_2$), 1.35 (s, 3H, C(C$\underline{H}_3$)$_2$); ^{13}C NMR (126 MHz, $CDCl_3$): δ ; ^{31}P NMR (202 MHz, $CDCl_3$): δ 9.10, 8.96; HRMS (ESI) *m/z* (M + H^+) Calc. for $C_{33}H_{44}N_2O_8P$: 627.2830. Found 627.2818.



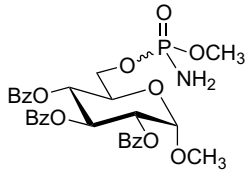
Methyl 2-deoxy-5,6-*O*-isopropylidene-3-*O*-(methyl *N*-benzylphosphoramidyl)-2-*N*-trichloroacetamido-β-D-galactofuranoside (2-50)

General procedure for MeOPN synthesis was used and the reaction was carried out on **2-34** (101 mg, 0.30 mmol). Purified by flash chromatography (1:1 hexanes–acetone with 1% Et_3N) to afford **2-50** (92 mg, 55%) as a clear oil. R_f 0.58 (1:1 hexanes–acetone); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.72 (d, 1H, *J* = 9.2 Hz, C-2-NH), 7.68 (d, 1H, *J* = 9.2 Hz, C-2-NH*), 7.38–7.27 (m, 10H, Ar), 4.95 (s, 1H, H-1), 4.94 (s, 1H, H-1*), 4.74 (dd, 1H, *J* = 7.7, 2.2 Hz, H-3), 4.67 (dd, 1H, *J* = 7.8, 2.2 Hz, H-3*), 4.62 (d, 1H, *J* = 9.1 Hz, H-2), 4.52 (d, 1H, *J* = 9.1 Hz, H-2*), 4.43 (app. t, 1H, *J* = 8.0 Hz, H-5), 4.39 (br. s, 1H, H-4), 4.32 (app. t, 1H, *J* = 7.7 Hz, H-5*), 4.28 (br. s, 1H, H-4*), 4.17–4.11 (m, 5H, H-6, PhCH_2N, PhCH_2N*), 4.06 (dd, 1H, *J* = 8.2, 6.6 Hz, H-6*), 3.98 (dd, 1H, *J* = 8.3, 8.3 Hz, H-6'), 3.94 (dd, 1H, *J* = 8.3, 8.3 Hz, H-6'*), 3.75 (d, 3H, *J* = 11.4 Hz, POCH_3), 3.74 (d, 3H, *J* = 11.3 Hz, POCH_3*), 3.43 (s, 3H, C-1-OCH_3), 3.42 (s, 3H, C-1-OCH_3*), 3.20–3.12 (m, 2H, PNHCH_2, PNHCH_2*), 1.47 (s, 3H, C(CH_3)$_2$), 1.46 (s, 3H, C(CH_3)$_2$), 1.41 (s, 3H, C(CH_3)$_2$), 1.40 (s, 3H, C(CH_3)$_2$); ^{13}C NMR (126 MHz, $CDCl_3$): δ 161.4 (2C), 139.2 (2C), 139.1, 139.0, 128.7 (2C), 127.5, 127.4, 110.2 (2C), 108.7, 108.6, 92.3 (2C), 83.4, 83.3, 82.9, 82.8, 81.1 (2C), 80.6 (2C), 75.6 (2C), 65.6 (2C), 61.1 (2C), 61.0, 60.9, 55.2, 55.1, 53.5 (2C), 53.4, 53.3, 45.4 (2C), 26.1 (2C), 26.0 (2C); ^{31}P NMR (202 MHz; $CDCl_3$): δ 9.6, 9.3; HRMS (ESI) *m/z* (M + Na^+) Calc. for $C_{20}H_{28}Cl_3N_2NaO_8P$: 583.0541. Found 583.0541.



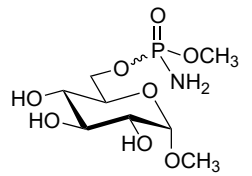
1,2:3,4-di-*O*-isopropylidene-6-*O*-(methyl phosphoramidyl)-D-galactopyranose (2-82)

General procedure for P-*N*-benzyl cleavage was used and carried out on **2-10** (133 mg, 0.30 mmol) to afford **2-82** (96 mg, 91%). General procedure for *N*-*p*-methoxybenzyl cleavage was used and carried out on **2-35** (104 mg, 0.22 mmol) to afford **2-82** (63 mg, 81%). General procedure for *N*-*o*-nitrobenzyl cleavage was carried out on **2-36** (50 mg, 0.10 mmol) to afford **2-82** (29 mg, 83%). Purified by flash chromatography (20:1 CH_2Cl_2–CH_3OH with 1% Et_3N). Clear oil. R_f 0.58 (9:1 CH_2Cl_2–CH_3OH); ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.52 (d, 2H, J = 5.0 Hz, H-1, H-1*), 4.60 (dd, 1H, J = 7.9, 2.2 Hz, H-3), 4.58 (dd, 1H, J = 7.9, 2.2 Hz, H-3*), 4.31 (dd, 1H, J = 5.0, 2.4 Hz, H-2), 4.29 (dd, 1H, J = 5.0, 2.5 Hz, H-2*), 4.24 (dd, 1H, J = 8.0, 1.9 Hz, H-4), 4.22 (dd, 1H, J = 7.9, 1.9 Hz, H-4*), 4.19–4.01 (m, 6H, H-5, H-5*, H-6, H-6', H-6*, H-6'*), 3.72 (d, 3H, J = 11.3 Hz, POC$\underline{H}_3$), 3.70 (d, 3H, J = 11.3 Hz, POC$\underline{H}_3$*), 3.16 (d, 2H, J = 3.7 Hz, PN$\underline{H}_2$), 3.13 (d, 2H, J = 4.1 Hz, PN$\underline{H}_2$*), 1.51 (s, 3H, C(C$\underline{H}_3$)$_2$), 1.50 (s, 3H, C(C$\underline{H}_3$)$_2$), 1.41 (s, 6H, 2 × C(C$\underline{H}_3$)$_2$), 1.30 (s, 12H, 4 × C(C$\underline{H}_3$)$_2$); ^{13}C NMR (126 MHz, $CDCl_3$): δ 109.7 (2C), 109.6 (2C), 108.9, 96.3 (4C), 70.8 (2C), 70.7 (3C), 70.4 (2C), 67.5 (2C), 67.4 (2C), 66.9 (2C), 65.6, 65.5 (2C), 65.4, 65.3 (2C), 53.3 (4C), 53.2, 25.9, 25.0, 24.9, 24.5, 24.4; ^{31}P (202 MHz, $CDCl_3$): δ 11.40, 11.01; HRMS (ESI) *m/z* (M + Na^+) Calc. for $C_{13}H_{24}NNaO_8P$: 376.1132. Found 376.1130.



Methyl 2,3,4-tri-*O*-benzoyl-6-*O*-(methyl phosphoramidyl)-α-D-glucopyranoside (2-83)

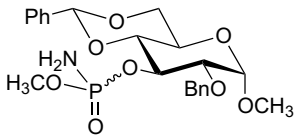
General procedure for *N*-*p*-methoxybenzyl cleavage was used and carried on **2-41** (148 mg, 0.21 mmol). Purified by flash chromatography (20:1 CH_2Cl_2–CH_3OH with 1% Et_3N) to afford **2-83** (103 mg, 83%) as a clear oil. R_f 0.26 (20:1 CH_2Cl_2–CH_3OH); ^1H NMR (500 MHz, $CDCl_3$): δ 8.00–7.95 (m, 4H, Ar), 7.95–7.90 (m, 4H, Ar), 7.88–7.82 (m, 4H, Ar), 7.55–7.48 (m, 4H, Ar), 7.45–7.34 (m, 10H, Ar), 7.31–7.25 (m, 4H, Ar), 6.16 (dd, 1H, *J* = 9.6, 9.6 Hz, H-3), 6.15 (dd, 1H, *J* = 9.5, 9.5 Hz, H-3*), 5.64 (d, 1H, *J* = 9.8 Hz, H-4), 5.62 (d, 1H, *J* = 9.7 Hz, H-4*), 5.28–5.23 (m, 4H, H-1, H-1*, H-2, H-2*), 4.29–4.19 (m, 6H, H-5, H-5*, H-6, H-6', H-6*, H-6'*), 3.82 (d, 3H, *J* = 11.4 Hz, POC$\underline{H}_3$), 3.67 (d, 3H, *J* = 11.3 Hz, POC$\underline{H}_3$*), 3.48 (s, 6H, C-1-OC$\underline{H}_3$, C-1-OC$\underline{H}_3$*), 2.93 (d, 2H, *J* = 4.4 Hz, PN$\underline{H}_2$), 2.91 (d, 2H, *J* = 4.7 Hz, PN$\underline{H}_2$*); ^{13}C NMR (126 MHz, $CDCl_3$): δ 165.8 (2C), 165.7 (2C), 165.4, 133.6, 133.5, 133.4, 133.1 (2C), 130.0 (2C), 129.8, 129.7 (2C), 129.2 (2C), 129.0 (2C), 128.8, 128.5, 128.4 (2C), 128.3, 97.2 (2C), 72.0 (2C), 70.4 (2C), 69.0, 68.9, 68.4, 68.3, 68.2, 64.7 (2C), 64.3 (2C), 55.8 (2C), 53.5 (2C), 53.4, 53.3, 29.7; ^{31}P NMR (202 MHz, $CDCl_3$): δ 10.84, 10.40; HRMS (ESI) *m/z* (M + Na^+) Calc. for $C_{29}H_{30}NNaO_{11}P$: 622.1449. Found 622.1440.



Methyl 6-*O*-(methyl phosphoramidyl)-α-D-glucopyranoside (2-84)

General procedure for acetate and benzoate esters cleavage was used and carried out on **2-83** (50 mg, 0.08 mmol). Purified by flash chromatography (6:1 CH_2Cl_2–CH_3OH with 1% Et_3N)

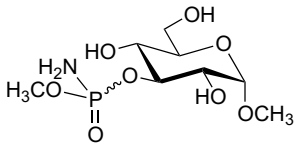
to afford **2-84** (19 mg, 79%) as a clear oil. R_f 0.18 (6:1 CH_2Cl_2–CH_3OH); ^{1}H NMR (500 MHz, CD_3OD) δ 4.66 (d, 2H, *J* = 3.7 Hz, H-1, H-1*), 4.23 (dd, 1H, *J* = 11.1, 2.0 Hz, H-6), 4.21 (dd, 1H, *J* = 11.1, 2.0 Hz, H-6*), 4.14–4.09 (m, 2H, H-6', H-6'*), 3.70 (d, 3H, *J* = 11.4 Hz, POC$\underline{H}_3$), 3.69 (d, 3H, *J* = 11.4 Hz, POC$\underline{H}_3$*), 3.68–3.64 (m, 2H, H-5, H-5*), 3.61 (d, 1H, *J* = 9.3 Hz, H-3), 3.59 (d, 1H, *J* = 9.3 Hz, H-3*), 3.40–3.36 (m, 8H, H-2, H-2*, C-1–OC$\underline{H}_3$, C-1–OC$\underline{H}_3$*), 3.34–3.30 (m, 2H, H-4, H-4*); ^{13}C NMR (126 MHz, CD_3OD) δ 99.9 (2C), 73.6, 72.0 (2C), 70.6 (4C), 69.9, 69.8, 65.5, 65.4 (3C), 54.3, 52.4, 52.3 (2C); ^{31}P NMR (202 MHz, CD_3OD) δ 13.65; HRMS (ESI) *m/z* (M + Na^+) Calc. for $C_8H_{18}NNaO_8P$: 310.0662. Found 310.0657.



Methyl 2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-(methyl phosphoramidyl)-α-D-glucopyranoside (2-85)

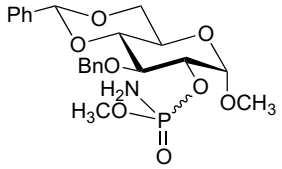
General procedure for *N*-*p*-methoxybenzyl cleavage was used and carried out on **2-42** (150 mg, 0.26 mmol). Purified by flash chromatography (20:1 CH_2Cl_2–CH_3OH with 1% Et_3N) to afford **2-85** (90 mg, 76%) as a clear oil. R_f 0.30 (20:1 CH_2Cl_2–CH_3OH); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.49–7.44 (m, 2H, Ar), 7.42–7.28 (m, 8H, Ar), 5.52 (s, 1H, PhC$\underline{H}O_2$), 5.50 (s, 1H, PhC$\underline{H}O_2$*), 4.80 (d, 2H, *J* = 11.8 Hz, PhC$\underline{H}_2$O, PhC$\underline{H}_2$O*), 4.71–4.63 (m, 5H, H-1, H-3, H-3*, PhC$\underline{H}_2$O, PhC$\underline{H}_2$O*), 4.61 (d, 1H, *J* = 3.6 Hz, H-1*), 4.27 (dd, 2H, *J* = 10.3, 4.8 Hz, H-6, H-6*), 3.87–3.79 (m, 2H, H-5, H-5*), 3.74–3.58 (m, 9H, H-2, H-2*, H-4, H-4*, H-6', H-6'*, POC$\underline{H}_3$), 3.55 (d, 3H, *J* = 11.4 Hz, POC$\underline{H}_3$*), 3.38 (s, 6H, C1-OC$\underline{H}_3$, C1-OC$\underline{H}_3$*), 2.93 (d, 2H, *J* = 3.4 Hz, PN$\underline{H}_2$), 2.89 (d, 2H, *J* = 2.7 Hz, PN$\underline{H}_2$*); ^{13}C NMR (126 MHz, $CDCl_3$): δ 137.5, 137.3, 136.8, 136.7, 129.3, 128.7, 128.6, 128.5, 128.4, 128.3 (4C), 126.2, 126.1, 102.0, 101.8, 98.8, 98.7, 80.5,

80.4, 80.3 (2C), 78.6, 78.5, 78.4 (2C), 75.7, 75.6, 75.4, 75.3, 73.7, 73.5, 68.9 (2C), 62.2, 62.1, 55.5, 53.5 (2C), 53.5 (2C); ^{31}P NMR (202 MHz, $CDCl_3$): δ 10.05, 9.58; HRMS (ESI) *m/z* ($M + Na^+$) Calc. for $C_{22}H_{28}NNaO_8P$: 488.1445. Found 488.1437.



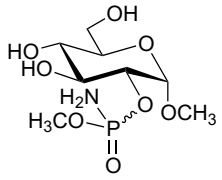
Methyl 3-*O*-(methyl phosphoramidyl)-α-D-glucopyranoside (2-86)

General procedure for hydrogenation with Raney Nickel was used and carried out on **2-85** (70 mg, 0.15 mmol). Purified by flash chromatography (6:1 CH_2Cl_2–CH_3OH with 1% Et_3N) to afford **2-86** (26 mg, 61%) as a clear oil. R_f 0.23 (6:1 CH_2Cl_2–CH_3OH); ^{1}H NMR (500 MHz, CD_3OD): δ 4.69 (d, 1H, *J* = 3.5 Hz, H-1), 4.68 (d, 1H, *J* = 3.5 Hz, H-1*), 4.33–4.26 (m, 2H, H-3, H-3*), 3.78 (dd, 2H, *J* = 11.9, 2.2 Hz, H-6, H-6*), 3.72 (d, 3H, *J* = 11.5 Hz, POC$\underline{H_3}$), 3.71 (d, 3H, *J* = 11.5 Hz, POC$\underline{H_3}$*), 3.67 (dd, 2H, *J* = 11.9, 5.0 Hz, H-6', H-6'*), 3.56–3.42 (m, 6H, H-2, H-2*, H-4, H-4*, H-5, H-5*), 3.39 (s, 3H, C1-COC$\underline{H_3}$), 3.39 (s, 3H, C1-OC$\underline{H_3}$*); ^{13}C NMR (126 MHz, CD_3OD): δ 99.7 (2C), 81.1, 81.0 (2C), 80.9, 71.9 (2C), 71.0, 70.9 (2C), 69.1, 69.0 (2C), 60.9, 54.2 (2C), 52.7 (2C), 52.6; ^{31}P NMR (202 MHz, CD_3OD): δ 14.35, 14.21; HRMS (ESI) *m/z* ($M + Na^+$) Calc. for $C_8H_{18}NNaO_8P$: 310.0662. Found 310.0655.



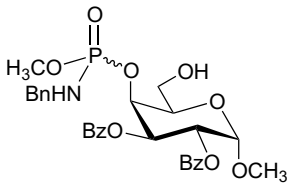
Methyl 3-*O*-benzyl-4,6-*O*-benzylidene-2-*O*-(methyl phosphoramidyl)-α-D-glucopyranoside (2-87)

General procedure for *N*-*p*-methoxybenzyl cleavage was used and carried out on **2-43** (176 mg, 0.30 mmol. Purified by flash chromatography (20:1 CH_2Cl_2–CH_3OH with 1% Et_3N) to afford **2-87** (102 mg, 73%) as a clear oil. R_f 0.22 & 0.27 (20:1 CH_2Cl_2–CH_3OH); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.50–7.20 (m, 10H, Ar), 5.59 (s, 1H, PhC$\underline{H}O_2$), 5.57 (s, 1H, PhC$\underline{H}O_2$*), 5.04–4.89 (m, 4H, H-1, H-1*, PhC$\underline{H_2}$O, PhC$\underline{H_2}$O*), 4.73 (d, 1H, *J* = 11.4 Hz, PhC$\underline{H_2}$O), 4.65 (d, 1H, *J* = 11.2 Hz, PhC$\underline{H_2}$O*), 4.40–4.26 (m, 4H, H-2, H-2*, H-6, H-6*), 4.05 (dd, 1H, *J* = 9.1, 8.8 Hz, H-3), 4.03 (dd, 1H, *J* = 9.2, 9.0 Hz, H-3*), 3.94–3.83 (m, 2H, H-6', H-6'*), 3.82–3.60 (m, 10H, H-4, H-4*, H-5, H-5*, POC$\underline{H_3}$, POC$\underline{H_3}$*), 3.46 (s, 3H, C1-OC$\underline{H_3}$), 3.45 (s, 3H, C1-OC$\underline{H_3}$*), 2.87 (s, 2H, PN$\underline{H_2}$), 2.77 (s, 2H, PN$\underline{H_2}$*); ^{13}C NMR (126 MHz, $CDCl_3$): δ 138.3, 138.2, 137.2 (2C), 129.1, 129.0, 128.6, 128.3 (2C), 128.1, 128.0 (2C), 127.7, 126.0 (2C), 101.4, 99.1, 98.9, 82.4, 82.1, 77.5 (2C), 76.0 (2C), 75.4, 75.1, 74.8 (2C), 69.0 (2C), 62.3 (2C), 55.6, 55.5, 53.6 (2C), 53.5, 53.4, 29.7; ^{31}P NMR (202 MHz, $CDCl_3$): δ 11.48, 9.88; HRMS (ESI) *m/z* (M + Na^+) Calc. for $C_{22}H_{28}NNaO_8P$: 488.1445. Found 488.1434.



Methyl 2-*O*-(methyl phosphoramidyl)-α-D-glucopyranoside (2-88)

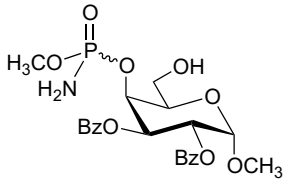
General procedure for hydrogenation with Raney Nickel was used and carried out on **2-87** (51 mg, 0.11 mmol). Purified by flash chromatography (6:1 CH_2Cl_2–CH_3OH with 1% Et_3N) to afford **2-88** (20 mg, 65%) as a clear oil. R_f 0.15 (6:1 CH_2Cl_2–CH_3OH); ^{1}H NMR (400 MHz, CD_3OD): δ 4.90 (d, 1H, J = 3.6 Hz, H-1, H-1*), 4.04–3.98 (m, 2H, H-2, H-2*), 3.81 (dd, 2H, J = 11.9, 2.3 Hz, H-6, H-6*), 3.79–3.72 (m, 8H, H-3, H-3*, POC$\underline{H_3}$, POC$\underline{H_3}$*), 3.67 (dd, 2H, J = 11.9, 5.6 Hz, H-6', H-6'*), 3.56 (dd, 1H, J = 5.5, 2.3 Hz, H-5), 3.53 (dd, 1H, J = 5.5, 2.3 Hz, H-5*), 3.408 (s, 3H, C1-OC$\underline{H_3}$); 3.406 (s, 3H, C1-OC$\underline{H_3}$); 3.35–3.32 (m, 2H, H-4, H-4*); ^{13}C NMR (126 MHz, CD_3OD): δ 98.1, 97.9, 76.5 (2C), 76.2 (2C), 72.0 (4C), 71.9 (2C), 70.3 (2C), 61.1, 54.2 (2C), 52.6 (2C), 52.5 (2C); ^{31}P NMR (162 MHz, CD_3OD): δ 13.68, 13.22; HRMS (ESI) *m/z* ($M + Na^+$) Calc. for $C_8H_{18}NNaO_8P$: 310.0662. Found 310.0659.



Methyl 2,3-di-*O*-benzoyl-4-*O*-(methyl *N*-benzylphosphoramidyl)-α-D-galactopyranoside (2-89)

General procedure for TBDPS cleavage was used and carried out on **2-44** (346 mg, 0.42 mmol). Purified by flash chromatography (30:1→20:1 CH_2Cl_2–CH_3OH with 1% Et_3N) to afford **2-89** (210 mg, 85%) as a clear oil. R_f 0.46 & 0.49 (20:1 CH_2Cl_2–CH_3OH); ^{1}H NMR (500 MHz,

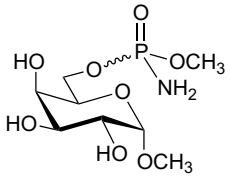
$CDCl_3$): δ 8.05–7.99 (m, 6H, Ar), 7.91 (dd, 2H, *J* = 8.4, 1.3 Hz, Ar), 7.56–7.48 (m, 4H, Ar), 7.43–7.28 (m, 14H, Ar), 7.20–7.17 (m, 2H, Ar), 6.87–6.85 (m, 2H, Ar), 5.80 (ddd, 1H, *J* = 10.8, 3.1, 1.9 Hz, H-4), 5.72–5.64 (m, 3H, H-4*, H-3, H-3*), 5.21 (dd, *J* = 10.6, 3.3 Hz, H-2), 5.16–5.13 (m, 3H, H-1, H-1*, H-2*), 4.25–4.07 (m, 4H, H-5, H-5*, PhC$\underline{H_2}$N, PhC$\underline{H_2}$N*), 3.91–3.69 (m, 9H, H-6, H-6', H-6*, H-6'*, PhC$\underline{H_2}$N, PhC$\underline{H_2}$N*, POC$\underline{H_3}$), 3.46 (s, 6H, C1-OC$\underline{H_3}$, C1-OC$\underline{H_3}$*), 3.21 (d, 3H, *J* = 11.2 Hz, POC$\underline{H_3}$*), 3.15 (app. dt, 1H, *J* = 11.6, 7.0 Hz, PN$\underline{H}$), 2.63 (app. dt, 1H, *J* = 10.3, 7.0 Hz, PN$\underline{H}$*); ^{13}C NMR (126 MHz, $CDCl_3$): δ 166.1 (2C), 165.7, 165.3, 138.9 (4C), 133.5, 133.4, 133.3, 133.1, 129.9, 129.8 (2C), 129.6, 129.4 (2C), 129.2, 129.1, 128.8, 128.7, 128.5 (2C), 128.3, 127.6, 127.4 (2C), 127.2, 97.6, 97.5, 71.9, 71.7 (2C), 69.5 (2C), 69.3 (2C), 68.8 (2C), 68.5 (2C), 68.4 (2C), 59.3, 59.1, 55.6, 54.1 (2C), 53.3 (2C), 45.6, 45.5, 41.3, 13.9; ^{31}P NMR (162 MHz, $CDCl_3$): δ 12.59, 12.21; HRMS (ESI) *m/z* (M + Na) Calc. for $C_{29}H_{32}NNaO_{10}P$: 608.1656. Found 608.1641.



Methyl 2,3-di-*O*-benzoyl-4-*O*-(methyl phosphoramidyl)-α-D-galactopyranoside (2-90)

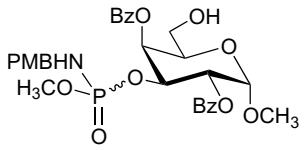
General procedure for *N*-benzyl cleavage was used and carried out on **2-89** (190 mg, 0.32 mmol). Purified by flash chromatography (20:1 CH_2Cl_2–CH_3OH with 1% Et_3N) to afford **2-90** (119 mg, 74%) as a clear oil. R_f 0.29 & 0.34 (20:1 CH_2Cl_2–CH_3OH); ^{1}H NMR (500 MHz; $CDCl_3$): δ 8.04–7.98 (m, 8H, Ar), 7.56–7.52 (m, 4H, Ar), 7.44–7.37 (m, 8H, Ar), 5.79 (ddd, 1H, *J* = 10.9, 3.2, 1.7 Hz, H-4), 5.71–5.63 (m, 3H, H-4*, H-3, H-3*), 5.20 (dd, 1H, *J* = 10.9, 3.3 Hz, H-2), 5.16 (dd, 1H, *J* = 10.8, 3.3 Hz, H-2*), 5.13 (d, 1H, *J* = 3.6 Hz, H-1), 5.11 (d, 1H, *J* = 3.4

Hz, H-1*), 4.20–4.14 (m, 5H, H-6, H-6*, POC$\underline{H}_3$), 3.87–3.82 (m, 2H, H-6', H-6'*), 3.45 (s, 3H, C1-OC$\underline{H}_3$), 3.44 (s, 3H, C1-OC$\underline{H}_3$*), 3.32 (d, 3H, *J* = 11.3 Hz, POC$\underline{H}_3$*), 3.17 (s, 2H, PN$\underline{H}_2$), 2.71 (d, 2H, *J* = 4.7 Hz, PN$\underline{H}_2$*); ^{13}C NMR (126 MHz, $CDCl_3$): δ 174.1, 166.1 (2C), 165.8, 165.4, 133.6, 133.4 (2C), 133.1, 129.9, 129.8, 129.7 (2C), 129.4, 129.3 (2C), 129.2, 128.9, 128.5, 128.3, 97.5 (2C), 72.0 (2C), 71.7 (2C), 69.4 (2C), 69.2 (2C), 68.6 (2C), 68.5 (2C), 68.4, 59.3, 59.1, 55.6, 54.1, 54.0, 53.6 (2C), 20.6; ^{31}P NMR (162 MHz, $CDCl_3$): δ 13.47, 13.05; HRMS (ESI) *m/z* (M + Na) Calc. for $C_{22}H_{26}NNaO_{10}P$: 518.1187. Found 518.1174.



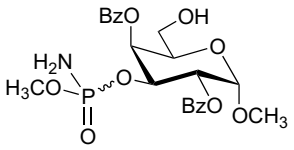
Methyl 6-*O*-(methyl phosphoramidyl)-α-D-galactopyranoside (2-91)

General procedure for acetate and benzoate esters cleavage was used and carried out on **2-90** (99 mg, 0.20 mmol). Purified by flash chromatography (6:1 CH_2Cl_2–CH_3OH with 1% Et_3N) to afford **41** (25 mg, 44%) as a clear oil. R_f 0.10 (6:1 CH_2Cl_2–CH_3OH); ^{1}H NMR (500 MHz, CD_3OD) δ 4.69 (d, 2H, *J* = 3.5 Hz, H-1, H-1*), 4.10 (m, 4H, H-6, H-6', H-6*, H-6'*), 3.98–3.93 (m, 2H, H-5, H-5*), 3.87 (d, 2H, *J* = 3.0 Hz, H-4, H-4*), 3.78–3.68 (m, 10H, H-2, H-2*, H-3, H-3*, POC$\underline{H}_3$, POC$\underline{H}_3$*), 3.39 (s, 6H, C1-OC$\underline{H}_3$, C1-OC$\underline{H}_3$*); ^{13}C NMR (126 MHz, CD_3OD) δ 100.1, 69.8, 69.3, 69.2 (2C), 69.1, 68.7, 65.4 (2C), 65.3 (2C), 65.2, 54.3, 52.3, 52.2; ^{31}P NMR (202 MHz, CD_3OD) δ 13.91, 13.86; HRMS (ESI) *m/z* (M + Na) Calc. for $C_8H_{18}NNaO_8P$: 310.0662. Found 310.0659.



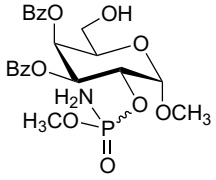
Methyl 2,4-di-*O*-benzoyl-6-*O*-*tert*-butyldiphenylsilyl-3-*O*-(methyl *N*-phosphoramidyl)-α-D-galactopyranoside (2-92)

General procedure for TBDPS cleavage was used and carried out on **2-46** (71 mg, 0.08 mmol). Purified by flash chromatography (20:1 CH_2Cl_2–CH_3OH with 1% Et_3N) to afford **2-92** (41 mg, 80%) as a clear oil. R_f 0.27 (20:1 CH_2Cl_2–CH_3OH); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.20–8.09 (m, 4H, Ar), 7.65–7.40 (m, 16H, Ar), 7.14–7.10 (m, 2H, Ar), 6.92–6.88 (m, 2H, Ar), 6.78–6.74 (m, 2H, Ar), 6.67–6.64 (m, 2H, Ar), 5.86 (d, 1H, J = 2.8 Hz, H-4), 5.70 (d, 1H, J = 3.0 Hz, H-4*), 5.55 (dd, 1H, J = 10.4, 3.7 Hz, H-2), 5.51 (dd, 1H, J = 10.4, 3.6 Hz, H-2*), 5.25–5.19 (m, 4H, H-1, H-1*, H-3, H-3*), 4.20 (dd, 1H, J = 7.0, 7.0 Hz, H-5), 4.13 (dd, J = 6.9, 6.9 Hz, H-5*), 3.94–3.90 (m, 2H, ArC$\underline{H}_2$N, ArC$\underline{H}_2$N*), 3.76–3.71 (m, 10H, H-6, H-6*, ArC$\underline{H}_2$N, ArC$\underline{H}_2$N*, C1-OC$\underline{H}_3$, C1-OC$\underline{H}_3$*), 3.63–3.55 (m, 5H, H-6', H-6'*, POC$\underline{H}_3$), 3.43 (s, 3H, ArOC$\underline{H}_3$), 3.42 (s, 3H, ArOC$\underline{H}_3$*) 3.37 (d, 3H, J = 11.3 Hz, POC$\underline{H}_3$*), 3.12 (app. dt, 1H, J = 9.9, 6.7 Hz, PN$\underline{H}$), 2.68 (app. dt, 1H, J = 11.3, 6.7 Hz, PN$\underline{H}$*); ^{13}C NMR (126 MHz, $CDCl_3$): 167.2, 167.0, 166.2 (2C), 158.9, 158.8, 133.8 (2C), 133.5, 133.4, 131.7 (2C), 131.1 (2C), 130.1 (4C), 129.4 (2C), 129.0, 128.9, 128.7 (2C), 128.6, 128.5 (2C), 128.4, 113.9, 113.8, 97.6, 97.5, 71.2 (2C), 71.1 (3C), 71.0, 70.9 (2C), 70.4 (2C), 70.2, 70.1, 69.0, 60.9, 60.6, 55.7, 55.6, 55.3, 55.2, 53.3 (2C), 53.2, 44.8, 44.6, 29.7 (2C); ^{31}P NMR (162 MHz, $CDCl_3$): δ 9.80, 9.68; HRMS (ESI) *m/z* (M + Na) Calc. for $C_{30}H_{34}NNaO_{11}P$: 638.1762. Found 638.1760.



Methyl 2,4-di-*O*-benzoyl-3-*O*-(methyl *N*-phosphoramidyl)-α-D-galactopyranoside (2-93)

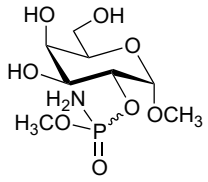
General procedure for *N*-*p*-methoxybenzyl cleavage was used and carried out on **2-92** (33 mg, 0.05 mmol). Purified by flash chromatography (20:1→15:1 CH_2Cl_2–CH_3OH with 1% Et_3N) to afford **2-93** (20 mg, 74%) as a clear oil. R_f 0.16 (20:1 CH_2Cl_2–CH_3OH); ^{1}H NMR (500 MHz; $CDCl_3$): δ 8.17–8.10 (m, 8H, Ar), 7.63–7.55 (m, 4H, Ar), 7.50–7.43 (m, 8H, Ar), 5.88 (d, 1H, *J* = 3.1 Hz, H-4), 5.84 (d, 1H, *J* = 3.2 Hz, H-4*), 5.52 (dd, 1H, *J* = 10.4, 3.6 Hz, H-2), 5.45 (dd, 1H, *J* = 10.4, 3.6 Hz, H-2*), 5.29–5.19 (m, 4H, H-1, H-1*, H-3, H-3*), 4.24–4.18 (m, 2H, H-5, H-5*), 3.80–3.57 (m, 7H, H-6, H-6', H-6*, H-6'*, POC$\underline{H_3}$), 3.42 (s, 3H, C1-OC$\underline{H_3}$), 3.41 (s, 3H, C1-OC$\underline{H_3}$*), 3.38 (d, 3H, *J* = 11.5 Hz, POC$\underline{H_3}$*), 3.13 (d, 2H, *J* = 4.2 Hz, PN$\underline{H_2}$), 2.70 (d, 2H, *J* = 4.2 Hz, PN$\underline{H_2}$*), 2.52 (s, 1H, C6–O$\underline{H}$); ^{13}C NMR (126 MHz, $CDCl_3$): δ 167.0 (2C), 166.2, 166.1, 133.8 (2C), 133.6, 133.4, 130.1 (2C), 130.0, 129.4, 129.3, 129.0 (2C), 128.7 (3C), 128.5, 97.5, 97.4, 71.4, 71.3, 71.2 (2C), 71.0 (2C), 70.6, 70.4, 70.3, 70.2 (2C), 69.1, 69.0, 61.0, 60.7, 55.6, 53.5, 53.4 (3C), 53.3 (2C), 29.7; ^{31}P NMR (162 MHz, $CDCl_3$): δ 10.81, 10.48; HRMS (ESI) *m/z* (M + Na) Calc. for $C_{22}H_{26}NNaO_{10}P$: 518.1187. Found 518.1177.



Methyl 3,4-di-*O*-benzoyl-2-*O*-(methyl phosphoramidyl)-α-D-galactopyranoside (2-94)

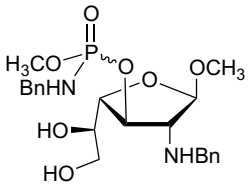
To **2-47** (110 mg, 0.19 mmol) in EtOAc (4 mL), a solution of $NaBrO_3$ (175 mg, 1.16 mmol) in H_2O (4 mL) was added dropwise. To this vigorously stirred mixture, a solution of

$Na_2S_2O_4$ (202 mg, 1.16 mmol) in H_2O (8 mL) was added dropwise via an addition funnel over 10–15 min. After complete addition, the reaction mixture was vigorously stirred for 1.5 h. The reaction mixture was then diluted with EtOAc and washed with a saturated aqueous $Na_2S_2O_3$ solution and brine. The organic layer was dried over $MgSO_4$ and concentrated. The resulting crude product was purified by flash chromatography (20:1→15:1 CH_2Cl_2–CH_3OH with 1% Et_3N) to afford **2-94** (85 mg, 89%) as a clear oil. R_f 0.14 & 0.20 (20:1 CH_2Cl_2–CH_3OH); ^1H NMR (500 MHz, $CDCl_3$): δ 8.07–8.03 (m, 4H, Ar), 7.93–7.87 (m, 4H, Ar), 7.63–7.59 (m, 2H, Ar), 7.51–7.44 (m, 6H, Ar), 7.33–7.28 (m, 4H, Ar), 5.80 (d, 1H, *J* = 3.2 Hz, H-4), 5.78 (d, 1H, *J* = 3.1 Hz, H-4*), 5.74 (dd, 1H, *J* = 10.0, 3.2 Hz, H-3), 5.71 (dd, 1H, *J* = 10.3, 3.4 Hz, H-3*), 5.24 (d, 1H, *J* = 3.6 Hz, H-1), 5.18 (d, 1H, *J* = 3.7 Hz, H-1*), 5.07–5.02 (m, 2H, H-2, H-2*), 4.28–4.23 (m, 2H, H-5, H-5*), 3.77–3.70 (m, 5H, H-6, H-6*, POC$\underline{H}_3$), 3.61–3.57 (m, 2H, H-6', H-6'*), 3.52 (s, 3H, C1-OC$\underline{H}_3$), 3.51 (s, 3H, C1-OC$\underline{H}_3$*), 3.42 (d, 3H, *J* = 11.5 Hz, POC$\underline{H}_3$*), 2.95 (d, 2H, *J* = 3.8 Hz, PN$\underline{H}_2$), 2.65 (d, 2H, *J* = 4.7 Hz, PN$\underline{H}_2$*), 2.55 (s, 1H, C6-O$\underline{H}$), 2.49 (s, 1H, C6-O$\underline{H}$*); ^{13}C NMR (126 MHz, $CDCl_3$): δ 166.6 (2C), 165.4, 165.2, 133.7, 133.4, 133.3, 130.0 (3C), 129.3 (2C), 128.9 (2C), 128.7, 128.5, 128.3, 98.6 (2C), 98.3 (2C), 71.8 (2C), 71.4 (2C), 70.0 (2C), 69.3, 69.2 (2C), 69.1, 69.0 (2C), 60.9, 55.7, 55.6, 53.5 (2C), 53.4 (2C); ^{31}P NMR (202 MHz, $CDCl_3$): δ 10.96, 10.21; HRMS (ESI) *m/z* (M + Na) Calc. for $C_{22}H_{26}NNaO_{10}P$: 518.1187. Found 518.1179.



Methyl 2-*O*-(methyl phosphoramidyl)-α-D-galactopyranoside (2-95)

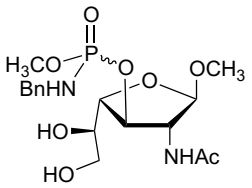
General procedure for acetate and benzoate esters cleavage was used and carried out on **2-94** (55 mg, 0.11 mmol). Purified by flash chromatography (6:1 CH_2Cl_2–CH_3OH with 1% Et_3N) to afford **2-95** (26 mg, 81%) as a clear oil. R_f 0.13 (6:1 CH_2Cl_2–CH_3OH); ^{1}H NMR (500 MHz, CD_3OD): δ 4.92 (d, 1H, *J* = 3.4 Hz, H-1), 4.92 (d, 1H, *J* = 3.3 Hz, H-1*), 4.42 (app. dd, 1H, *J* = 9.8, 3.8 Hz, H-2), 4.40 (app. dd, 1H, *J* = 9.8, 3.8 Hz, H-2*), 3.91 (s, 2H, H-4, H-4*), 3.87 (dd, 2H, *J* = 10.0, 3.4 Hz, H-3, H-3*), 3.78 (dd, 2H, *J* = 6.0, 6.0 Hz, H-5, H-5*), 3.74–3.65 (m, 10H, H-6, H-6', H-6*, H-6'*, POC$\underline{H}_3$, POC$\underline{H}_3$*), 3.40 (s, 6H, C1-OC$\underline{H}_3$, C1-OC$\underline{H}_3$*); ^{13}C NMR (126 MHz, CD_3OD): δ 98.4, 98.3, 74.4, 74.3, 74.0 (2C), 70.8, 69.8, 68.5, 68.4 (2C), 61.2 (2C), 54.2, 52.6, 52.5 (3C); ^{31}P NMR (202 MHz, CD_3OD): δ 14.04, 13.70; HRMS (ESI) *m/z* (M + Na) Calc. for $C_8H_{18}NNaO_8P$: 310.0662. Found 310.0657.



Methyl 2-amino-*N*-benzyl-2-deoxy-3-*O*-(methyl *N*-benzylphosphoramidyl)-β-D-galactofuranoside (2-96)

To **2-95** (184 mg, 0.31 mmol), a 7.5% I_2–CH_3OH solution (20 mL, *w/v*) was added and the mixture stirred for 36 h. The excess I_2 was quenched by addition of finely crushed $Na_2S_2O_3$ to a point where the solution became a pale yellow color. Excess $Na_2S_2O_3$ was filtered off and

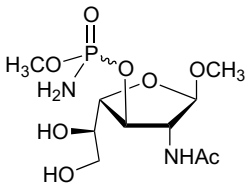
the filtrate was concentrated, then diluted with EtOAc and washed with H_2O. The aqueous layer was extracted with EtOAc several times. Organic layer was combined, washed with brine (minimal), dried over $MgSO_4$, and concentrated. The crude product was purified by flash chromatography (15:1→9:1 CH_2Cl_2–CH_3OH with 1% Et_3N) to afford **2-96** (91 mg, 66%) as a clear oil. R_f 0.41 (9:1 CH_2Cl_2–CH_3OH); ^1H NMR (500 MHz, $CDCl_3$): δ 7.36–7.25 (m, 20H, Ar), 4.87 (s, 2H, H-1, H-1*), 4.80–4.73 (m, 2H, H-3, H-3*), 4.30 (dd, 1H, J = 4.2, 2.7 Hz, H-4), 4.23 (dd, 1H, J = 4.5, 2.5 Hz, H-4*), 4.07–4.02 (m, 4H, PhC$\underline{H_2}$N, PhC$\underline{H_2}$N*), 3.97–3.94 (m, 1H, H-5), 3.86–3.63 (m, 16H, H-5*, H-6, H-6', H-6*, H-6'*, PN$\underline{H}$CH_2, PhC$\underline{H_2}$N, PhC$\underline{H_2}$NC*, POC$\underline{H_3}$, POC$\underline{H_3}$*), 3.53–3.48 (m, 1H, PN$\underline{H}$*), 3.42–3.41 (m, 1H, H-2), 3.37–3.35 (m, 7H, H-2*, C1-OC$\underline{H_3}$, C1-OC$\underline{H_3}$*), 3.22 (s, 4H, C5–O$\underline{H}$, C5–O$\underline{H}$*, C6–O$\underline{H}$, C6–O$\underline{H}$*); ^{13}C NMR (126 MHz, $CDCl_3$): δ 139.4, 139.3 (3C), 138.6, 138.5, 128.6 (2C), 128.5, 128.4, 127.5, 127.4 (2C), 108.5, 108.2, 84.3, 84.2, 83.9, 83.8, 79.5, 70.3, 70.2, 68.7 (2C), 68.5 (2C), 64.3 (2C), 55.1, 55.0, 53.3, 51.6, 45.3, 45.2; ^{31}P NMR (202 MHz, $CDCl_3$): δ 10.20, 10.07; HRMS (ESI) *m/z* (M + H) Calc. for $C_{22}H_{32}N_2O_7P$: 467.1942. Found 467.1931. *Note – compound at R_f 0.59 (9:1 CH_2Cl_2–CH_3OH) is a byproduct containing 2 *N*-benzyl moieties at the C-2 position.



Methyl 2-*N*-acetamido-2-deoxy-3-*O*-(methyl *N*-benzylphosphoramidyl)-β-D-galactofuranoside (2-97)

To **2-96** (112 mg, 0.221 mmol), CH_3OH (8 mL) was added followed by 10% Pd–C (40 mg). The reaction mixture was then placed under a positive pressure of $H_{2(g)}$ and stirred for 4 h.

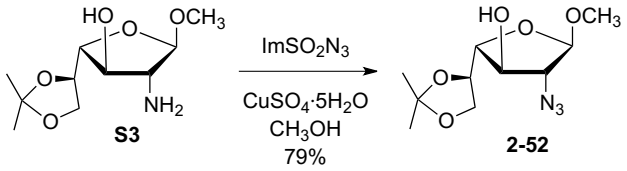
The Pd–C was filtered off using Fisherbrand filter paper and the filtrate was concentrated. A 10:1:1 CH_3OH–Ac_2O–Et_3N solution (12 mL) was then added and the solution was stirred for 16 h. The reaction mixture was concentrated and the crude product was purified by flash chromatography (9:1 CH_2Cl_2–CH_3OH with 1% Et_3N) to afford **2-97** (68 mg, 74%) as a clear oil. R_f 0.29 (9:1 CH_2Cl_2–CH_3OH); ^{1}H NMR (500 MHz, CD_3OD): δ 7.36–7.29 (m, 8H, Ar), 7.24–7.21 (m, 2H, Ar), 4.79 (s, 1H, H-1), 4.78 (d, 1H, J = 0.9 Hz, H-1*), 4.67–4.63 (m, 2H, H-3, H-3*), 4.39 (dd, 1H, J = 2.7, 1.2 Hz, H-2), 4.35 (dd, 1H, J = 2.4, 0.9 Hz, H-2*), 4.21 (dd, 1H, J = 4.9, 2.1 Hz, H-4), 4.15 (dd, 1H, J = 5.1, 2.2 Hz, H-4*), 4.08–4.05 (m, 4H, PhC$\underline{H_2}$N, PhC$\underline{H_2}$N*), 3.86 (ddd, 1H, J = 6.5, 6.5, 2.1 Hz, H-5), 3.80 (ddd, 1H, J = 6.4, 6.4, 2.1 Hz, H-5*), 3.66–3.60 (m, 10H, H-6, H-6', H-6*, H-6'*, POC$\underline{H_3}$, POC$\underline{H_3}$*), 3.35 (s, 3H, C1-OC$\underline{H_3}$), 3.34 (s, 3H, C1-OC$\underline{H_3}$*), 1.95 (s, 3H, COC$\underline{H_3}$), 1.90 (s, 3H, COC$\underline{H_3}$); ^{13}C NMR (126 MHz, CD_3OD): δ 171.3, 171.2, 140.1, 140.0 (3C), 128.1, 128.0, 127.2, 127.1, 126.8, 107.7 (2C), 82.7, 82.6, 82.4 (2C), 80.3 (2C), 80.2 (2C), 70.1, 62.8, 61.2, 61.1, 61.0 (2C), 53.7, 53.6, 52.6, 52.5 (2C), 44.6, 44.5; ^{31}P NMR (202 MHz, CD_3OD): δ 10.92, 9.72; HRMS (ESI) *m/z* (M + Na) Calc. for $C_{17}H_{27}N_2NaO_8P$: 441.1397. Found 441.1390.



Methyl 2-*N*-acetamido-2-deoxy-3-*O*-(methyl phosphoramidyl)-β-D-galactofuranoside (2-98)

General procedure for *N*-benzyl cleavage was used and carried out on **2-97** (65 mg, 0.16 mmol). Purified by flash chromatography (6:1 EtOAc–CH_3OH with 1% Et_3N) to afford **2-98** (23 mg, 45%) as a clear oil. R_f 0.12 (6:1 EtOAc–CH_3OH); ^{1}H NMR (500 MHz, CD_3OD): δ 4.82 (2H,

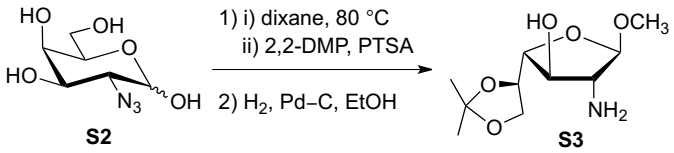
H-1, H-1*, masked by H_2O peak) 4.68–4.63 (m, 2H, H-3, H-3*), 4.36 (d, 1H, *J* = 1.3 Hz, H-2), 4.34 (d, 1H, *J* = 1.3 Hz, H-2*), 4.20–4.18 (m, 2H, H-4, H-4*), 3.87–3.84 (m, 2H, H-5, H-5*), 3.71–3.63 (m, 10H, H-6, H-6', H-6*, H-6'*, POC$\underline{H}_3$, POC$\underline{H}_3$*), 3.35 (s, 3H, C1-OC$\underline{H}_3$), 3.35 (s, 3H, C1-OC$\underline{H}_3$*), 1.96 (s, 6H, COC$\underline{H}_3$, COC$\underline{H}_3$*); ^{13}C NMR (126 MHz, $CDCl_3$): δ 171.5, 171.4, 107.7 (2C), 82.6, 82.5, 80.3, 80.2, 80.1, 80.0, 61.3 (2C), 61.2 (2C), 53.7, 53.6, 52.6, 52.5 (2C), 52.4; ^{31}P NMR (201 MHz, CD_3OD): δ 13.04, 12.94; HRMS (ESI) *m/z* (M + H) Calc. for $C_{10}H_{21}N_2NaO_8P$: 351.0928. Found 351.0920.



Methyl 2-azido-2-deoxy-5,6-*O*-isopropylidene-β-D-galactofuranoside (2-52)

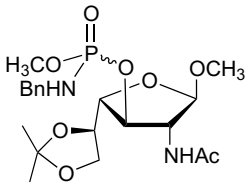
To **S3** (147 mg, 0.63 mmol), K_2CO_3 (267 mg, 1.93 mmol), and $CuSO_4·5H_2O$ (2 mg, 0.01 mmol) in anhydrous CH_3OH (5 mL) was added imidazole-1-sulfonyl-azide (270 mg, 1.29 mmol). The reaction mixture was stirred for 6 h before being concentrated and then diluted with EtOAc. The organic solution was washed with water, brine, dried over $MgSO_4$, and concentrated. The crude product was purified by flash chromatography (3:1 hexanes–acetone) to afford **50** (129 mg, 79%) as a clear oil. R_f 0.63 (9:1 CH_2Cl_2–CH_3OH); $[\alpha]_D$ −64.2 (*c* 2.7, $CHCl_3$); ^{1}H NMR (500 MHz; $CDCl_3$): δ 4.91 (d, 1H, *J* = 0.9 Hz, H-1), 4.25 (ddd, 1H, *J* = 6.5, 6.5, 6.5 Hz, H-5), 4.05 (dd, 1H, *J* = 8.5, 6.8 Hz, H-6), 3.96 (dd, 1H, *J* = 6.2, 4.8 Hz, H-4), 3.92–3.87 (m, 3H, H-2, H-3, H-6'), 3.41 (s, 3H, OC$\underline{H}_3$), 2.62 (d, 1H, *J* = 8.3 Hz, O$\underline{H}$), 1.44 (s, 3H, C(C$\underline{H}_3$)$_2$), 1.36 (s, 3H, C(C$\underline{H}_3$)$_2$); ^{13}C NMR (126 MHz, $CDCl_3$): δ 110.0 (C(CH_3)$_2$), 106.6 (C-1), 85.1 (C-4), 76.5 (C-2), 75.4 (C-5), 71.2 (C-3), 65.4 (C-6), 55.4 (OCH_3), 26.4 (C(CH_3)$_2$), 25.2 (C(CH_3)$_2$); IR

(KBr): 2107 cm^{-1} (N_3); HRMS (ESI) Calc. for (M + Na) $C_{10}H_{17}N_3NaO_5$: 282.1060. Found 282.1054.



Methyl 2-amino-2-deoxy-5,6-*O*-isopropylidene-β-D-galactofuranoside (s3)

To **S2**[67] (1.97 g, 9.60 mmol) in anhydrous 1,4-dioxane (24 mL) heated to 80 °C, 2,2-DMP (6.5 mL, 53.05 mmol) was added slowly followed by PTSA (643 mg, 3.38 mmol). The reaction mixture was continually stirred at 80 °C for 1 h before being cooled to room temperature and then Et_3N (1 mL) was added. The mixture was diluted with EtOAc, and washed with H_2O and brine. The organic layer was dried over $MgSO_4$ and concentrated. The crude product was partially purified by flash chromatography in 3:1 hexane–acetone. To this product, EtOH (30 mL) was added followed by 10% Pd–C (160 mg). The mixture was placed under a positive pressure of $H_{2(g)}$ and stirred for 2 h. The Pd–C was filtered using Fisherbrand filter paper and the filtrate was concentrated. The crude product was purified by flash chromatography (15:1→9:1 CH_2Cl_2–CH_3OH) to afford **S3** (455 mg, 20% over 2 steps) as a yellowish oil. R_f 0.31 (9:1 CH_2Cl_2–CH_3OH); $[\alpha]_D$ −88.1 (*c* 1.7, CH_3OH); ^1H NMR (400 MHz; CD_3OD): δ 4.71 (d, 1H, *J* = 2.2 Hz, H-1), 4.25 (ddd, 1H, *J* = 7.3, 6.8, 3.9 Hz, H-5), 4.06 (dd, 1H, *J* = 8.2, 6.7 Hz, H-6), 3.91 (dd, 1H, *J* = 8.2, 7.5 Hz, H-6'), 3.82 (dd, 1H, *J* = 5.8, 4.1 Hz, H-4), 3.78 (dd, 1H, *J* = 5.8, 3.8 Hz, H-3), 3.37 (s, 3H, OC$\underline{H_3}$), 3.09 (dd, 1H, *J* = 3.8, 2.2 Hz, H-2), 1.38 (s, 3H, C(C$\underline{H_3}$)$_2$), 1.35 (s, 3H, C(C$\underline{H_3}$)$_2$); ^{13}C NMR (126 MHz, CD_3OD): δ 109.9 (C-1), 109.2 (C$\underline{C}H_3$), 83.1 (C-4), 78.5 (C-3), 76.0 (C-5), 65.1 (C-6), 64.0 (C-2), 54.0 (O$\underline{C}H_3$), 25.1 (C($\underline{C}H_3$)$_2$), 24.5 (C($\underline{C}H_3$)$_2$); HRMS (ESI) Calc. for (M + H) $C_{10}H_{20}NO_5$: 234.1336. Found 234.1332.



Methyl 2-*N*-acetamido-2-deoxy-5,6-*O*-isopropylidene-3-*O*-(methyl *N*-benzylphosphoramidyl)-β-D-galactofuranoside (S4)

To **2-50** (22 mg, 0.39 mmol) in benzene (1.5 mL) was added AIBN (1 mg, 0.01 mmol) and nBu$_3$SnH (47 uL, 0.18ffff mmol). The mixture was heated to reflux for 6 h then concentrated to afford crude **S4** as a clear oil. R_f 0.58 (1:1 hexanes–acetone); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.37–7.27 (m, 10H, Ar), 6.56 (d, 1H, J = 8.9 Hz, C-2-NH), 6.50 (d, 1H, J = 9.0 Hz, C-2-NH*), 4.86 (s, 1H, H-1), 4.85 (s, 1H, H-1*), 4.64 (dd, 1H, J = 7.7, 2.6 Hz, H-3), 4.59–4.55 (m, 2H, H-2, H-3*), 4.52 (d, 1H, J = 9.0 Hz, H-2*), 4.46 (ddd, 1H, J = 7.1, 7.1 1.9 Hz, H-5), 4.35 (ddd, 1H, J = 7.1, 7.1, 1.8 Hz, H-5*), 4.29 (dd, 1H, J = 2.4, 2.4 Hz, H-4), 4.21 (dd, 1H, J = 2.2, 2.2 Hz, H-4*), 4.16–4.11 (m, 5H, H-6, PhCH_2N, PhCH_2N*), 4.07 (dd, 1H, J = 8.2, 7.0 Hz, H-6*), 4.02 (dd, 1H, J = 8.1, 7.4 Hz, H-6'), 3.98 (dd, 1H, J = 8.1, 7.5 Hz, H-6'*), 3.73 (d, 3H, J = 11.4 Hz, POCH_3), 3.71 (d, 3H, J = 11.3 Hz, POCH_3*), 3.39 (s, 3H, C1-OCH_3), 3.38 (s, 3H, C1-OCH_3*), 3.25 (ddd, 1H, J = 11.0, 7.0, 7.0 Hz, PNHCH$_2$), 3.17 (ddd, 1H, J = 11.0, 7.0, 7.0 Hz, PNHCH$_2$*), 1.98 (s, 3H, COCH_3), 1.96 (s, 3H, COCH_3*); ^{13}C NMR (126 MHz, $CDCl_3$): δ 169.0 (2C), 139.4 (3C), 139.3, 128.7, 128.6, 127.5 (2C), 109.6 (2C), 109.0, 108.8, 83.4 (2C), 83.0 (2C), 81.1, 81.0, 80.7 (2C), 75.3, 75.2, 65.8, 65.6, 59.3 (4C), 55.0 (2C), 53.5, 53.4, 53.3 (2C), 45.4, 45.3, 26.0 (2C), 25.5 (2C); HRMS (ESI) *m/z* (M + Na) Calc. for $C_{20}H_{31}N_2NaO_8P$: 481.1710. Found 481.1715.

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Chapter 3

Synthesis of a 6-*O*-Me-D-*glycero*-L-*gluco*-heptopyranose-derived Thioglycoside

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A part of this chapter has been published:

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3.1 Background

Heptose residues are commonly found in the capsular polysaccharide (CPS), lipopolysaccharide (LPS), and lipooligosaccharides (LOS) of a number of bacterial species including *Campylobacter*, *Yersinia*, *Burkholderia*, *Eubacterium*, *Plesiomonas*, and *E. coli*. To date, there are five heptose (Figure 3-1) and six 6-deoxyheptose residues found in bacterial polysaccharides.[1,2] The precise molecular role of heptoses is not known; however, some studies suggest they play important roles in antibiotic resistance and capsular function.[3-5]

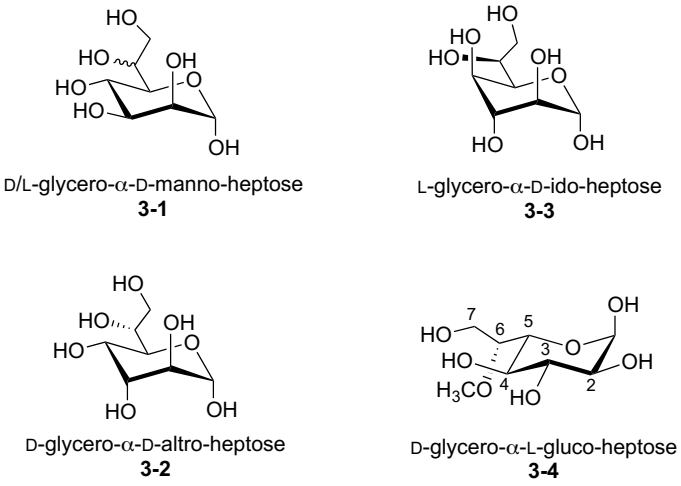


Figure 3-1: Heptose residues found in bacterial CPS/LPS.

The majority of the *Campylobacter* species contain heptose residues in their CPS. Of particular interest to this thesis, the CPS found in *C. jejuni* 11168H strain possesses a heptose residue with the D-*glycero*-L-*gluco* stereochemistry (Figure 3-1 & 3-2).[6,7] This is the first naturally occurring carbohydrate residue with this stereochemistry. Due to its rarity, glycoconjugates containing this monosaccharide could be used towards the development of vaccines or diagnostics. However, these applications require ready access to such species. In addition, access to this heptose residue as a glycosyl donor could be used in the synthesis of

substrates to probe biosynthetic enzymes involved in assembling the CPS. Such studies require significant quantities of the heptose residue. Unfortunately, this and most other heptose residues are not widely available from natural sources; therefore, its chemical synthesis is needed.

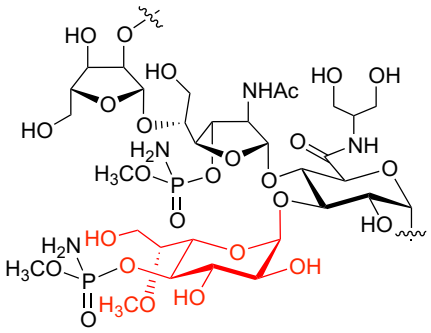


Figure 3-2: Repeating unit found in the CPS of *C. jejuni* 11168H. The heptose residue is highlighted in red.

This chapter describes two approaches for the synthesis of a 6-*O*-methyl-D-*glycero*-L-*gluco*-heptopyranose-derived thioglycoside donor. The first synthesis is a slightly modified version of a route from D-galactose, which was previously published by the group.[8] The second synthesis entails a new asymmetric *de novo* approach starting from furfural.

3.2 Reported approaches to heptose residues

The conventional synthesis of heptose residues involves elongation of an oxidized hexose derivative possessing an aldehyde at C-6.[2,9] Three synthetic approaches using this strategy are shown in Figure 3-3.

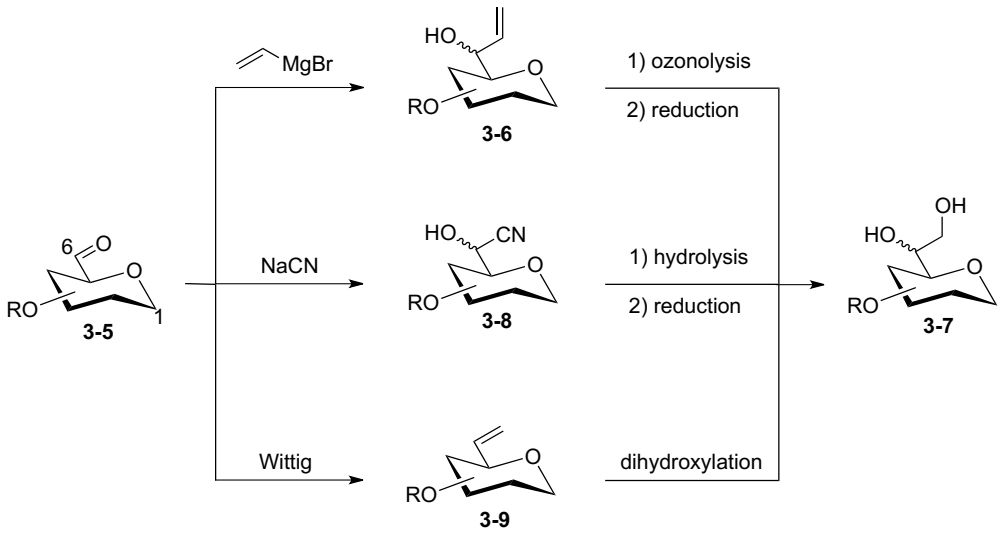
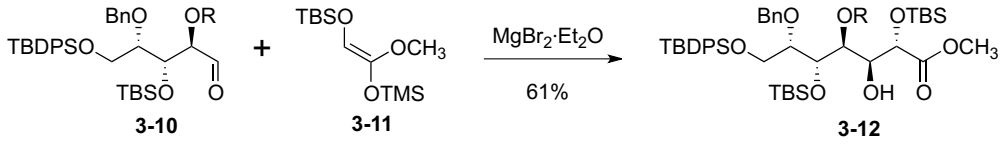


Figure 3-3: Traditional syntheses of heptose residues from a C-6 oxidized hexose (e.g., **3-5**).

Elongation by nucleophilic attack of vinyl magnesium bromide onto the aldehyde can produce vinyl adduct **3-6**. Subsequent ozonolysis and reduction of the resulting aldehyde can furnish a heptose residue (e.g., **3-7**).[10] Alternatively, nucleophilic addition by cyanide onto **3-5** can produce **3-8**. Hydrolysis of the cyano group to the acid followed by reduction can furnish **3-7**.[11-13] Finally, C-6 chain extension can be achieved by a Wittig olefination to generate alkene **3-9**. Subsequent dihydroxylation of the olefin can lead to the desired heptose residue.[14,15] A drawback of the described routes is the production of a diastereomeric mixture of products at C-6, which in some cases can lead to tedious purification steps.[14]

These routes have been commonly used for the synthesis of D/L-*glycero*-D-*manno*-heptopyranosides. However, the heptose residue found in *C. jejuni* strain 11168H possesses the D-*glycero*-L-*gluco* stereochemistry (Figure 3-2). The C-6 elongation approach would require large quantities of L-glucose, which, although commercially available, is quite costly; therefore, a more practical synthetic route is needed.

As an alternative approach, elongation at the C-1 aldehyde of a carbohydrate residue is less common, but reported.[12,16] For example, Seeberger and coworkers reported the use of this approach in their synthesis of L-*glycero*-D-*manno*-heptose (Scheme 3-1).[17] The core L-*glycero*-D-*manno*-heptose analogue, in its linear form, was achieved by a Mukaiyama aldol addition of a protected L-lyxose derivative **3-10** with the silyl enol ether **3-11**.[18] Although the yield was modest, this aldol addition proceeded in excellent 2,3-*anti*-3,4-*syn*-selectivity.



Scheme 3-1: Key step in the synthesis of L-*glycero*-D-manno-heptose analogue reported by Seeberger and coworkers.[17]

Inspired by some of the work involving C-1 elongation, our group reported the first synthesis of the D-*glycero*-L-*gluco*-heptose-derived thioglycoside **3-13** and trichloroacetimidate **3-14** (Figure 3-4).[8] Two key steps for its synthesis involved a diastereoselective divinylzinc addition to aldehyde **3-15** (obtained from D-galactose) followed by ozonolysis of **3-16** to produce the core structure of the heptose residue. Subsequent modifications led to **3-13** and **3-14**. The preparation of heptose donors **3-13** and **3-14** were achieved in 16 and 18 steps, respectively, from D-galactose.

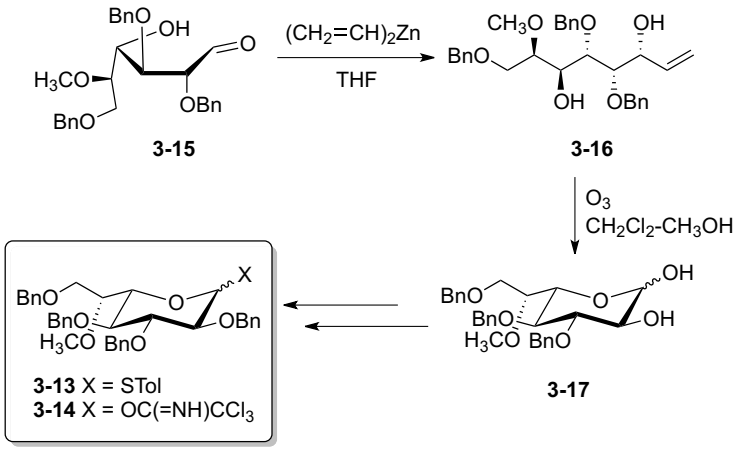
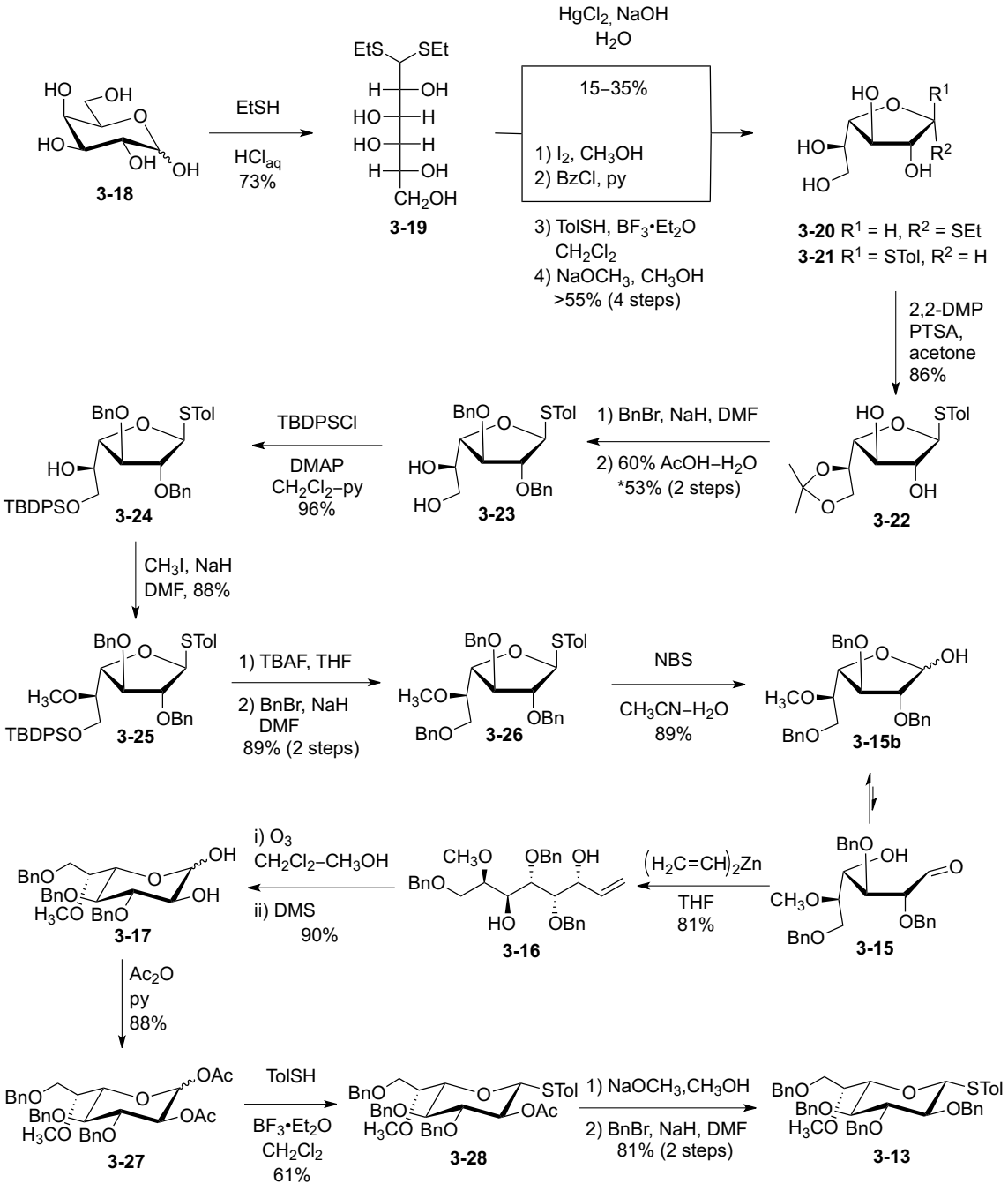


Figure 3-4: Key steps for the synthesis of D-*glycero*-L-*gluco*-heptose-based glycosyl donors **3-13** and **3-14** developed by Peng et al.[8]

3.3 Optimized synthesis of D-*glycero*-L-*gluco*-heptose-derived thioglycoside from galactose

In need of the heptose donors **3-13** or **3-14**, their reported synthesis was reproduced (Scheme 3-2). The synthesis began by treating D-galactose (**3-18**) with ethanethiol in concentrated hydrochloric acid to furnish dithioacetal **3-19** in 73% yield. The next step was a mercury (II) chloride-catalyzed cyclization of dithioacetal **3-19** to produce the galactofuranose (Gal*f*) thioglycoside **3-20**. The results of this reaction were, however, highly variable and afforded the product in low yields. Given these complications, we decided to focus in obtaining the Gal*f* thioglycoside **3-21**, which was previously synthesized by our group.[19] Compounds **3-20** and **3-21** differ in the identity of the aglycone (SEt in **3-20** and STol in **3-21**). Thus, dithioacetal **3-19** was treated with 2% iodine in methanol followed by acylation with benzoyl chloride in pyridine. Subsequent glycosylation with *para*-methylbenzenethiol and debenzoylation furnished thioglycoside **3-21** in >55% yield over the four steps. Although three additional steps were

added, this route consistently gave the product in much-improved yields and only one additional purification step via flash chromatography was required.



Scheme 3-2: Synthesis of the heptose thioglycoside donor **3-13** from D-galactose.

With thioglycoside **3-21** in hand, the C-5 and C-6 hydroxyl groups were protected with an isopropylidene acetal by mixing 2,2-dimethoxypropane in the presence of *para*-toluenesulfonic acid to afford Gal*f* derivative **3-22** in 86% yield. Subsequent benzylation with benzyl bromide and sodium hydride in dimethylformamide and treatment of the resulting product with 60% acetic acid in water at 60 °C afforded the diol **3-23**, in 53% yield over the two steps. The primary hydroxyl group was then protected as a *tert*-butyldiphenylsilyl ether to afford **3-24** in 96% yield. Subsequently, alcohol **3-24** was methylated by treatment with methyl iodide and sodium hydride to afford **3-25** in 88% yield. Next, the silyl group in **3-25** was exchanged with a benzyl group by first treatment with tetra-*n*-butylammonium fluoride and then benzylation with benzyl bromide and sodium hydride to produce the fully protected Gal*f* derivative **3-26** in 89% over two steps. The thioglycoside **3-26** was then hydrolyzed to the hemiacetal **3-15b** by mixing with *N*-bromosuccimide in a mixture of water and acetonitrile. The key adduct, **3-15b**, was treated with divinylzinc to afford the olefin **3-16** in 81% yield and excellent diastereoselectivity. The use of divinylzinc instead of vinyl magnesium bromide was imperative for high selectivity, presumably as a result of tighter chelation of the organometallic reagent with the aldehyde **3-15**. The Cram–chelate model as shown in Figure 3-5, can rationalize the selectivity.

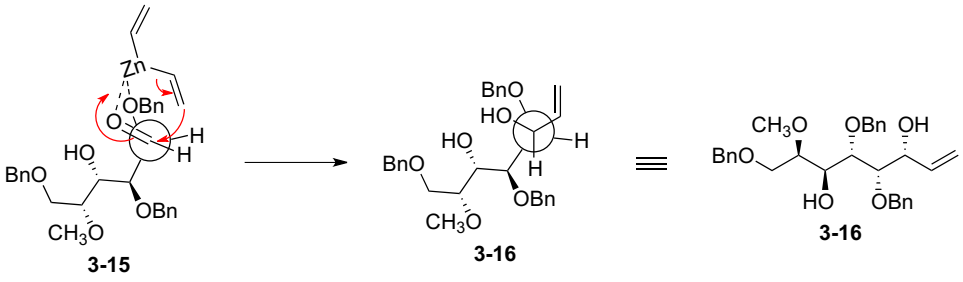


Figure 3-5: Proposed Cram-chelate model for divinylzinc-mediated vinyl addition to **3-15**.

Subsequent ozonolysis of olefin **3-16** led to the formation of the core heptose **3-17**. Heptose **3-17** was then acetylated in 88% yield with acetic anhydride in pyridine (to afford **3-27**) and glycosylated with *para*-methylbenzenethiol in the presence of boron trifluoride etherate to afford thioglycoside **3-28** in 61% yield. Finally, the C-2 acetyl group of **3-28** was converted to a benzyl group by first treatment with sodium methoxide in methanol and then with benzyl bromide and sodium hydride in dimethylformamide to afford the D-*glycero*-L-*gluco*-hepto-derived thioglycoside **3-13**.

The synthesis of the D-*glycero*-L-*gluco*-heptose-derived thioglycoside **3-13** was successfully achieved in 19 steps. Although three additional steps were needed from the original publication,[8] we find this route was more robust in producing the intermediate thioglycoside **3-21**.

3.4 Synthesis of D-*glycero*-L-*gluco*-heptose-derived thioglycoside from furfural

Although the approach described in the previous section could be used to prepare gram quantities of heptose donor **3-13**, the large number of synthetic (16 from published work and 19 from the modified route shown above) and purification steps are less than desirable. This led us to consider other, more efficient, approaches.

Alternative to the more traditional C-6 and C-1 elongation of carbohydrate residues, there are a number of *de novo* asymmetric approaches for the synthesis of hexopyranoses. Representative examples are summarized in Figure 3-6. Based on earlier work by List and Barbas,[20-22] proline-catalyzed aldol reactions have frequently been used for the syntheses of several natural and unnatural pentoses and hexoses from ketal **3-29** (Figure 3-6a).[23-31]

Alternatively, asymmetric syntheses of hexoses from furfural (**3-32**) have been reported (Figure 3-6b). Ogasawara and coworkers have shown that diol **3-33**, obtained from furfural, can easily be converted to **3-34** and its enantiomer. Subsequent modifications can lead to several D- and L-hexopyranoses.[32,33] On the other hand, O'Doherty and coworkers have shown alcohol **3-35**, also obtained from furfural, can be converted to the pyranone **3-36**. Through selective reduction and oxidation chemistry, pyranone **3-36** has been used to synthesize various D- and L-hexopyranosides.[34-36]

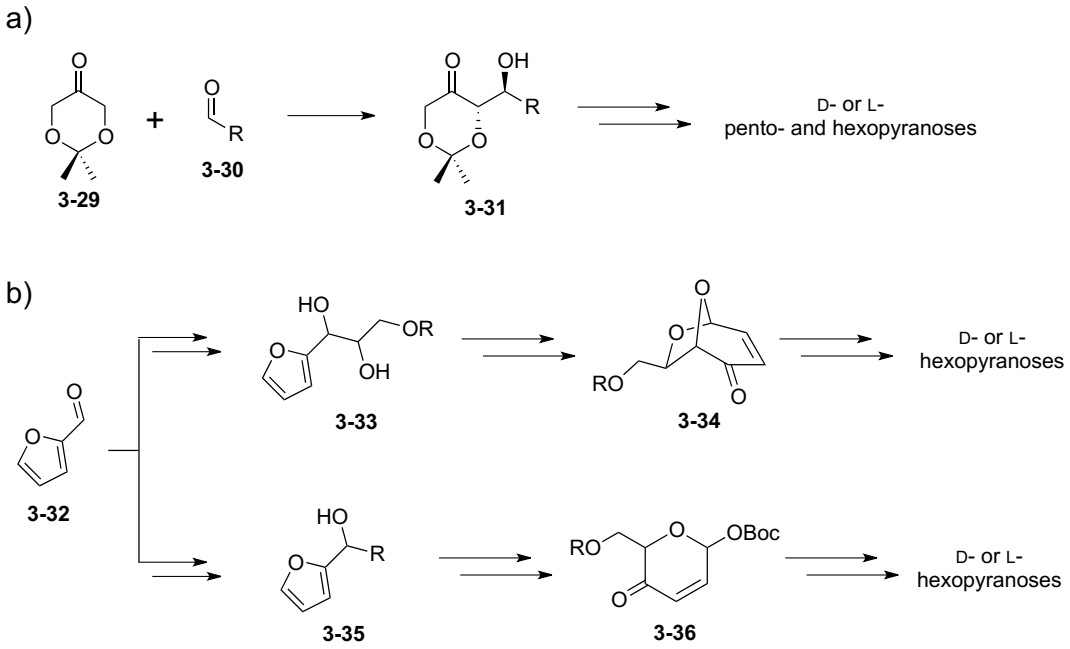


Figure 3-6: Asymmetric synthesis of carbohydrates from a) keto-diols and b) furfural.

We envisioned that heptose thioglycoside **3-13** could be synthesized from the diol **3-37**, already seven carbons in length, via one of two paths (Figure 3-7). In the first route, thioglycoside **3-13** could be obtained from the bicyclic adduct **3-38** after a selective ring opening and subsequent methylation. The bicyclic adduct **3-38** could be obtained through selective reduction and oxidation chemistry from enone **3-39**, which can be synthesized from diol **3-**

37.[32,33] In the second route, the thioglycoside **3-13** could be obtained through selective reductive and oxidative chemistry of pyranone **3-40**. The pyranone **3-40** could be obtained from diol **3-37** after a regioselective methylation, an Achmatowicz reaction, and selective pivaloylation. Both routes were explored.

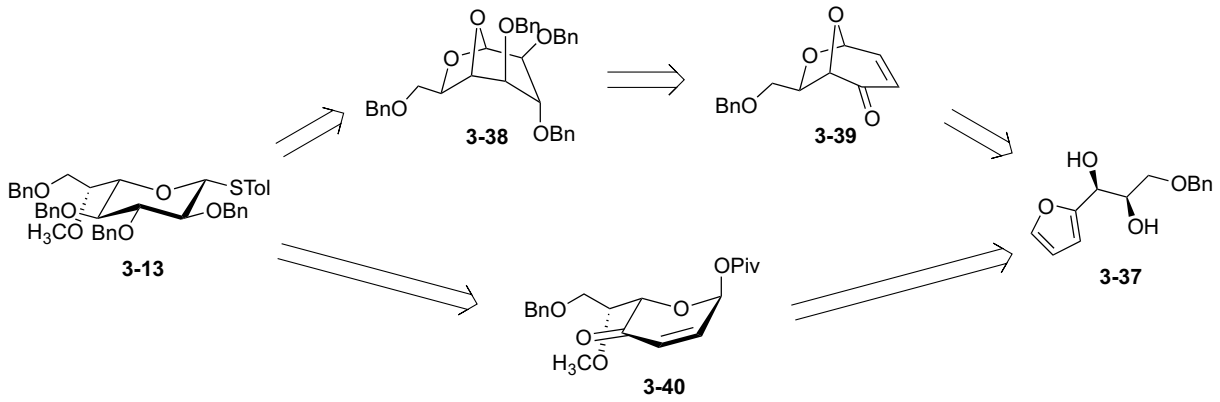
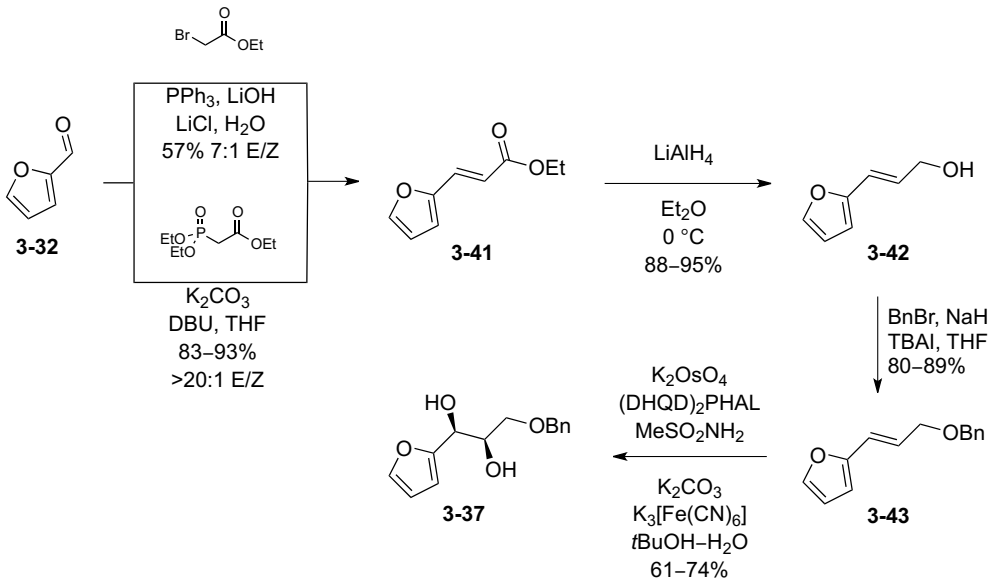


Figure 3-7: Two retrosynthetic routes towards heptose thioglycoside **3-13** from diol **3-37**.

The synthesis of diol **3-37** is illustrated in Scheme 3-3. Initially, a one-pot Wittig reaction[37,38] was used in the synthesis of the furan-ester **3-41**; however, these conditions produced the *trans* olefin in modest yields and selectivity. Switching to a Horner–Wadsworth–Emmons reaction using triethyl phosphonoacetate in the presence of 1,8-diazabicycloundec-7-ene and potassium carbonate produced furan-ester **3-41** in good yields and with excellent *trans* selectivity.[39,40] Reduction of ester **3-41** by treatment with lithium aluminum hydride (to afford **3-42**) followed by benzylation with benzyl bromide and sodium hydride in the presence of tetra-*n*-butyl-ammonium iodide led to **3-43** in good yields. A Sharpless asymmetric dihydroxylation of olefin **3-43** afforded diol **3-37** in reasonable yields and with excellent enantioselectivity. With **3-37** in hand, the two routes for the synthesis of **3-13** were explored.



Scheme 3-3: Synthesis of diol **3-37** from furfural (**3-32**).

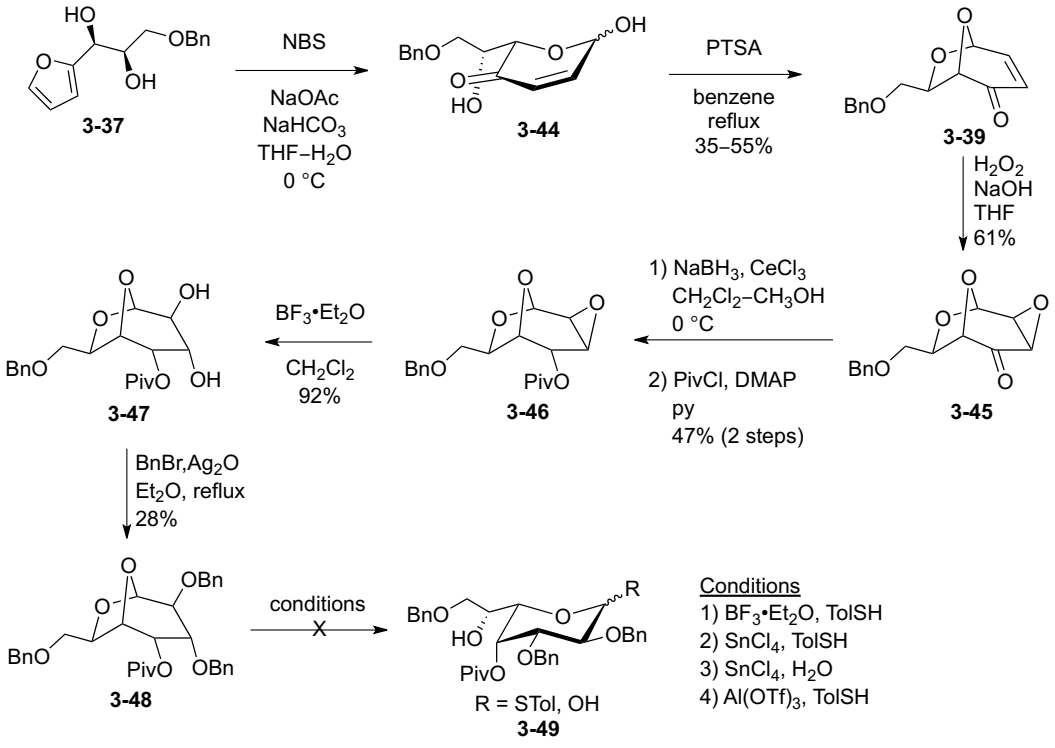
3.4.1 Efforts towards the synthesis of heptose donor via bicyclic enone

The work detailed in this section was conducted together with Dr. Anushka B. Jayasuriya, a former graduate student in the group.

Given the precedence for the synthesis of several hexopyranoses from enone **3-34**, we envisioned the synthesis of the heptose donor **3-13** to be straightforward. If successful, this approach would shorten the synthesis of the heptose donor **3-13** to nine steps from diol **3-37**.

The synthesis began by converting the diol **3-37** to the bicyclic enone **3-39** by first treatment with an *N*-bromosuccinimide catalyzed Achmatowicz reaction (to form **3-44**) followed by a *para*-toluenesulfonic acid catalyzed ring closure. The yield over the two steps was modest, but the compound could be obtained. With the bicyclic enone **3-39** in hand, we initially explored the reduction of the ketone and subsequent epoxidation of the olefin, however we faced several complications (e.g. poor selectivity, low yields) in this approach; therefore we treated enone **3-39** with hydrogen peroxide in the presence of sodium hydroxide affording epoxide **3-45** in 61%

yield. Reduction of the ketone with sodium borohydride and subsequent acylation with pivaloyl chloride led to the epoxide **3-46** in modest (47%) yields over two steps. The epoxide **3-46** was successfully opened using boron trifluoride etherate in dichloromethane to give the diol **3-47** in 92% yield. Subsequent benzylation with benzyl bromide in the presence of silver(I) oxide afforded **3-44** in 28% yield. The use of standard benzylation conditions with sodium hydride led to a mixture of products. We then examined the selective ring opening of the fully-protected adduct **3-48**. Unfortunately, all attempts to open the bicyclic ring in **3-48** to produce the advanced intermediate **3-49** failed, even in the presence of strong Lewis acids (e.g., tin chloride, aluminum trifluoromethanesulfonate). Elevated temperatures led to the degradation of the bicyclic adduct **3-48**. Due to our inability to produce **3–49** and the low to moderate yields in some of the earlier steps, this route was abandoned.

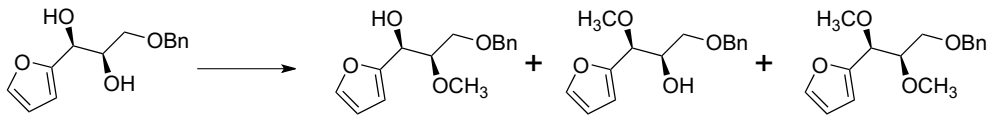


Scheme 3-4: Attempted synthesis of heptose thioglycoside donor via bicyclic enone **3-39**.

3.4.2 Synthesis of the heptose donor via a pyranone intermediate

As an alternative route, we envisioned the heptose donor **3-13** could also be obtained from the pyranone **3-40**. Its synthesis began by exploring various conditions for regioselective methylation of the diol **3-37** (Table 1). The 2-aminoethyl diphenylborinic acid catalyzed alkylation, described by Taylor and coworkers, proved to be the optimal method (Scheme 3-5).[41,42] This approach showed complete (TLC) conversion of **3-37** to **3-50a** and **3-50b**, albeit in an ~1:1 inseparable mixture. Other protecting groups (e.g. *tert*-butyldimethyl silyl, *p*-methoxybenzyl, and benzoyl) were also explored for the regioselective protection, however the selectivity remained unchanged. Subsequent Achmatowicz reaction of **3-50a** and **3-50b** using *N*-bromosuccinimide as the oxidant in the presence of sodium acetate and sodium bicarbonate, followed by an α-selective pivaloylation afforded the pyranone **3-40** in 38% yield over three steps (average yield of 72% per step). The structure of the pyranone **3-40** is supported by $J_{1,2}$ coupling constant value of 3.7 Hz, which closely matches the 1,2-allylic coupling constants in similar pyranones.[35,43]

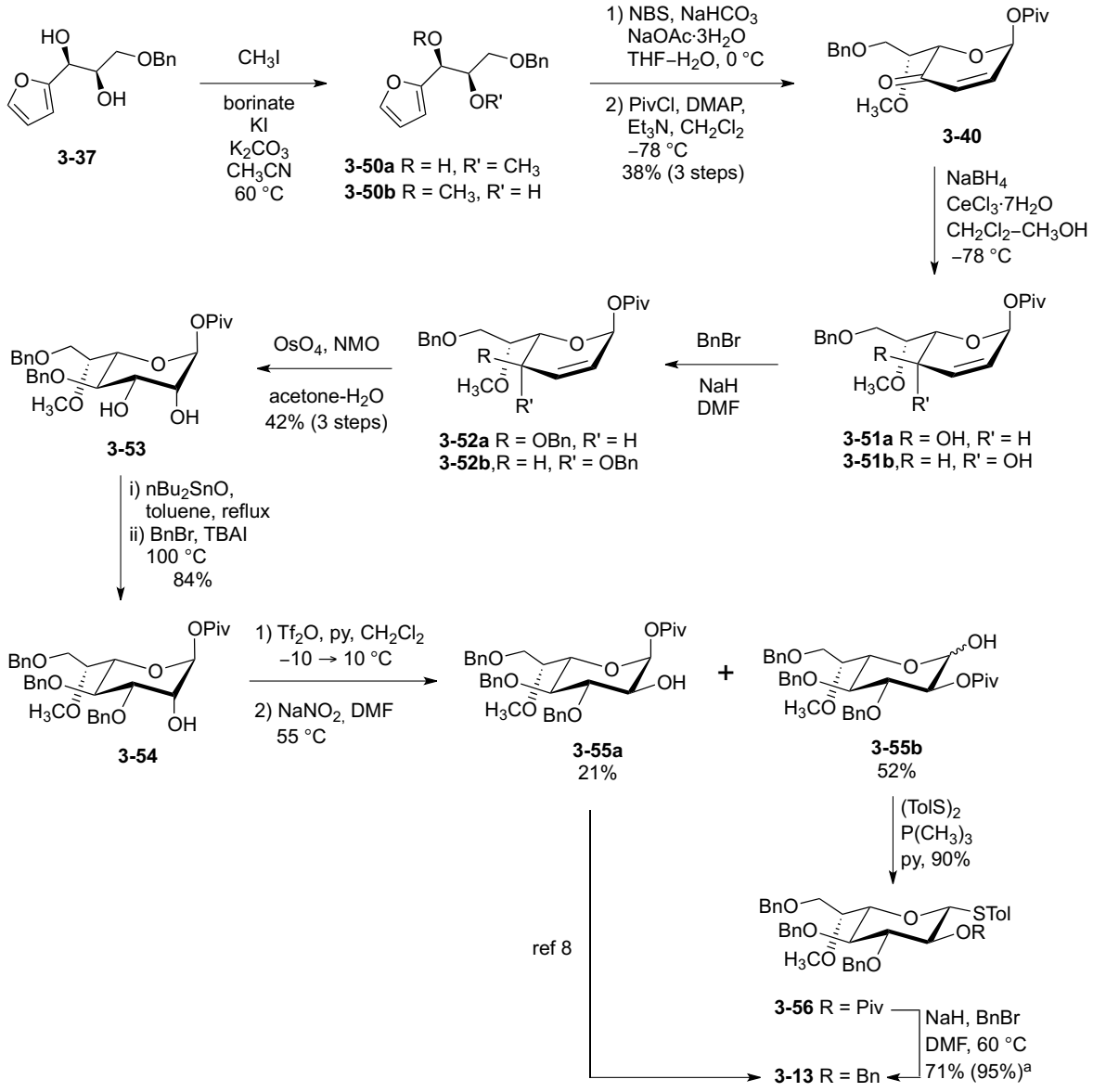
Table 3-1: Conditions explored for the regioselective methylation of **3-37**.



3-37 **3-50a** **3-50b** **3-50c**

entry	conditions	Product 3-50 a:b:c	Yield (%)
1	i) nBu$_2$SnO, toluene, reflux ii) CH_3I, 40 °C	---	0
2	i) nBu$_2$SnO, toluene, reflux ii) CH_3OTf	0:1:0[a]	~10[b,c]
3	i) nBu$_2$SnO, toluene, reflux ii) CH_3OTf, 0 °C	~1:4:0[a]	55[b]
4	AgO, CH_3I, CH_3CN, reflux	~2:3:0[a]	~22[d]
5	CH_3I, NaH, THF	~0:0:1[e]	n.d.
6	CH_3I, K_2CO_3, borinate,[f] CH_3CN, 60 °C	~1:1:0[a]	95[b]

[a] Product ratio determined by ^{1}H NMR spectroscopy. [b] Isolated yield. [c] Unknown impurity present. [d] Yield based on ^{1}H NMR spectroscopy. [e] TLC analysis showed dimethylation to be favored adduct with little to no mono-methylation product. [f] borinate = 2-aminoethyl diphenylborinate.

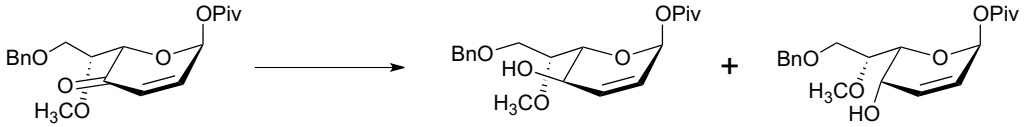


Scheme 3-5: Synthetic scheme for the asymmetric *de novo* synthesis of **3-13**. [a]Yield based on recovered starting material and product lacking a Piv group.

The stereoselective reduction of pyranone **3-40** was then explored (Table 2-2). Despite precedence for the stereoselective reduction of similar substrates,[35,36,43-46] the reduction of **3-40** suffered from modest stereoselectivity albeit in favor of the desired diastereomers **3-51a**. Although complete stereoselective reduction was achieved using L-selectride, substantial amounts of the 1,4-conjugate addition product were also observed. It was subsequently

discovered that the best results were obtained by Luche reduction of **3-40** at −78 °C. Under these conditions, an ~2:1 inseparable mixture of diastereomeric alcohols **3-51a**/**3-51b**, in which the desired isomer **3-51a** predominated, was formed.

Table 3-2: Conditions explored for the stereoselective reduction of **3-40.**



3-40 **3-51a** **3-51b**

entry	conditions (equiv.)	solvent	temp	product[d] 3:51 a:b	yield
1	$NaBH_4$ (1) $CeCl_3 \cdot 7H_2O$ (0.5)	1:1 CH_2Cl_2–CH_3OH	0 °C	~1:2	86%[a]
2	$NaBH_4$ (1) $CeCl_3 \cdot 7H_2O$ (0.5)	1:1 CH_2Cl_2–CH_3OH	–30 °C	~1:1	90%[a]
3	$NaBH_4$ (1) $CeCl_3 \cdot 7H_2O$ (0.5)	1:1 CH_2Cl_2–CH_3OH	–78 °C	~2:1	89%[a]
4	$NaBH_4$ (1) $CeCl_3 \cdot 7H_2O$ (0.2)	1:1 CH_2Cl_2–CH_3OH	–78 °C	~7:2	~55%[a,b,c]
5	L-Selectride (1.3)	THF	–78 °C	1:0	~40%[a,b,c]
6	$NaBH_4$ (1)	THF	–78 °C	~4:1	~35%[a,b,c]

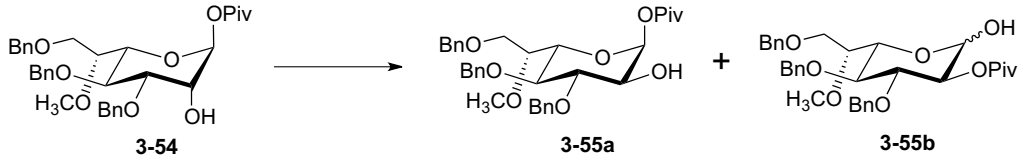
[a] Isolated yield. [b] 1,4-Conjugate addition observed. [c] Impurity of unknown structure present. [d] Product ratio determined by ^{1}H NMR spectroscopy.

Benzylation of the **3-51a**/**3-51b** mixture provided **3-52a/3-52b**. Although purification of the diastereomers can be achieved at this step, we instead carried the mixture forward. Dihydroxylation proceeded selectively from the face opposite that of the pivalate ester to afford **3-53** in 42% yield over three steps from pyranone **3-40** (average yield of 75% per step). Importantly, only diastereomer **3-52a** underwent dihydroxylation, presumably due to the pseudoaxial orientation of the groups at C-1 and C-4 in **3-52b**. This fortuitous selectivity significantly eased purification of the desired product. Tin catalyzed benzylation of diol **3-53**

afforded tribenzylated adduct **3-54** in 84% yield. The $J_{3,4}$ and $J_{4,5}$ coupling constant values of 9.5 and 9.5 Hz support the correct configuration of C-2, C-3, and C-4 stereocenters in alcohol **3-54**.

Subsequent stereoinversion of C-2 was achieved by triflation followed by treatment with sodium nitrite in dimethylformamide at 60 °C. These conditions produced a 73% yield of an ~2:5 mixture of **3-55a** and **3-55b**, which was formed through migration of the pivalate ester from O-1 to O-2. The respective $J_{1,2}$ coupling constant values of 3.6, 3.7, and 8.3 Hz of alcohols **3-54a**, **3-55bα**, and **3-55bβ** supports the stereoinversion at C-2. Other conditions (Table 3-3) were explored to either prevent or further promote the migration of the pivalate group, but these conditions also afforded a mixture of regioisomers. Alternative stereoinversion approaches, such as Mitsunobu reaction or oxidation to the ketone and subsequent reduction, either did not produce the desired compound, or were poorly selective. Regioisomers **3-55a** and **3-55b** were then treated with *para*-tolyl disulfide and trimethylphosphine to afford **3-56** (**3-55b** remained unreactive) in 90% yield, based on the amount of **3-55b** in the mixture. Thioglycoside **3-56** was then treated with excess sodium hydride in the presence of benzyl bromide at 60 °C to simultaneously remove the pivalate group and protect the resulting alcohol with a benzyl group to afford, in 95% yield, the heptose thioglycoside **3-13**, data for which matched that previously reported. The minor adduct formed in the C-2 inversion step, **3-55a**, could also be converted to **3-13** following the original protocol published after acetylation of the alcohol.[8]

Table 3-3: Conditions explored to invert C-2 stereochemistry in **3-54**.



entry	conditions	product obtained	yield
1	1) Swern oxidation 2) $NaBH_4$, CH_2Cl_2–CH_3OH, –78 °C	~3:2[b] **55a**–**54**	88%[a]
2	1) Tf_2O, pyridine, CH_2Cl_2, –10 to 10 °C 2) $NaNO_2$, DMF, 60 °C	~2:5[b] **55a**–**55b**	73%[a]
3	1) Tf_2O, pyridine, CH_2Cl_2, –10 to 10 °C 2) H_2O, DMF	~4:1[b] **55a**–**55b**	79%[a]

[a] Isolated yield. [b] Product ratio determined by ^1H NMR spectroscopy.

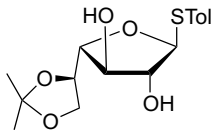
In summary, this section describes the first *de novo* asymmetric synthesis of D-*glycero*-L-*gluco*-hepto-derived thioglycoside **3-13**. This route provides the heptose donor more efficiently than the other previously reported synthesis of this compound.[8] Thioglycoside **3-13** was produced in 11 steps from the easily prepared benzyl protected diol **3-37** (15 steps from furfural). Importantly, the route described here significantly reduced the number of purification steps required. From **3-37**, only six purification steps (via flash chromatography) were used, compared to 15 steps required by the previously reported route from D-galactose. The reduction of number of steps, and more importantly the number of purifications, significantly facilitates the large-scale synthesis of heptose thioglycoside donor **3-13**.

3.5 Experimental

3.5.1 General experimental methods

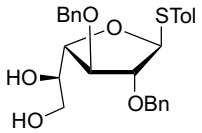
All reagents were purchased from commercial sources and were used without further purification unless noted. Solvents used in reactions were purified by successive passage through columns of alumina and copper under argon. Unless stated otherwise, all reactions were carried out at room temperature and monitored by TLC on Silica Gel G-25 F_{254} (0.25 mm). TLC spots were detected under UV light and/or by charring with a solution of *p*-anisaldehyde in ethanol, acetic acid and sulfuric acid. Column chromatography was performed on Silica Gel 60 (40–60 μm). Solvents were evaporated under reduced pressure on a rotary evaporator. ^{1}H NMR spectra were recorded using 400, 500, or 600 MHz NMR instruments and were referenced to residual proton signal of $CDCl_3$ (7.26 ppm). ^{13}C NMR spectra were recorded using 126 MHz (cold probe) NMR instrument and were referenced to residual ^{13}C signals of $CDCl_3$ (77 ppm). ^{1}H NMR data are reported as though they were first order, and peak assignments were made on the basis of 2D-NMR (^{1}H –^{1}H COSY and HSQC) experiments. ESI-MS spectra (time-of-flight analyzer) were recorded on samples dissolved in THF or CH_3OH and added NaCl. Optical rotations were measured at 22 ± 2 °C at the sodium D line (589 nm) and are in a microcell (10 cm, 1 mL) in units of deg·mL(dm·g)$^{-1}$.

3.5.2 Experimental, spectroscopic, and analytical data



Tolyl 5,6-*O*-isopropylidene-1-thio-beta-D-galactofuranoside (3-18)

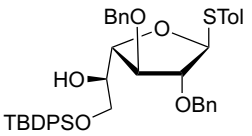
To a solution of **3-17** (3.70 g, 12.92 mmol) and 2,2-dimethoxypropane (3.2 mL, 26.08 mmol) in anhydrous acetone (100 mL) was added PTSA (248 mg, 1.30 mmol). The reaction mixture was stirred for 8 h and then Et_3N (300 uL) was added. The mixture was concentrated and the resulting residue was purified by flash chromatography using a gradient of 3:1→1:1 CH_2Cl_2−EtOAc to afford **3-18** (3.63 g, 86%) as a clear viscous oil. R_f 0.16 (3:1 CH_2Cl_2−CH_3OH); $[\alpha]_D$ −90.3 (*c* 2.1, $CHCl_3$); ^{1}H NMR (500 MHz; $CDCl_3$): δ 7.42−7.39 (m, 2H, Ar), 7.17−7.15 (m, 2H, Ar), 5.48 (d, 1H, *J* = 1.3 Hz, H-1), 4.38 (dd, 1H, *J* = 7.1, 7.1 Hz, H-5), 4.23 (br. s, 2H, H-3, H-4), 4.18 (br. s, 1H, H-2), 4.10 (dd, 1H, *J* = 8.3, 6.9 Hz, H-6), 4.09 (br. s, 1H, O<u>H</u>), 4.02 (dd, 1H, *J* = 8.1, 7.4 Hz, H-6'), 2.83 (br. s, 1H, O<u>H</u>), 2.36 (s, 3H, ArC<u>H</u>$_3$), 1.42 (s, 3H, C(C<u>H</u>$_3$)$_2$), 1.41 (s, 3H, C(C<u>H</u>$_3$)$_2$); ^{13}C NMR (126 MHz; $CDCl_3$): δ 138.0 (Ar), 132.6 (Ar), 129.9 (Ar), 129.4 (Ar), 110.1 (O_2<u>C</u>$(CH_3)_2$), 93.6 (C-1), 84.7 (C-3/C-4), 81.0 (C-2), 79.4 (C-3/C-4), 75.8 (C-5), 65.6 (C-6), 25.7 (Ar<u>C</u>H$_3$), 21.2 (2C, C(<u>C</u>H$_3$)$_2$); HRMS (ESI) Calc. for (M + Na) $C_{16}H_{22}NaO_5S$: 349.1086. Found 349.1082.



Tolyl 2,3-di-*O*-benzyl-1-thio-beta-D-galactofuranoside (3-19)

To a solution of **3-18** (9.09 g, 27.85 mmol) in anhydrous DMF (210 mL) under argon, was added BnBr (9.3 mL, 78.19 mmol) via syringe. The mixture was cooled to 0 ºC, then NaH (3.34 g, 83.55 mmol) was then added in portions over ~40 min. After complete addition of the NaH, the reaction mixture was stirred at 0 ºC for ~30 min, then gradually warmed to room temp and stirred for 14 h. The excess NaH was quenched by the addition of CH_3OH (~10 mL) dropwise over ~20 min after cooling the mixture to 0 ºC. The mixture was concentrated, diluted with EtOAc (300 mL), and then washed with water (100 mL) and brine (50 mL). The organic layer was concentrated and the resulting residue was suspended in a 60% AcOH−H_2O solution (150 mL) and stirred at 70 ºC for 10 h. The mixture was then concentrated and then co-evaporated with toluene. The resulting residue was then purified by flash chromatography using a gradient of 6:1→3:1 CH_2Cl_2−EtOAc to afford **3-19** (6.83 g, 53%) as a viscous oil. R_f 0.15 (8:1 CH_2Cl_2−CH_3OH); $[\alpha]_D$ −151.8 (*c* 1.4, $CHCl_3$); ^{1}H NMR (500 MHz; $CDCl_3$): δ 7.37−7.27 (m, 12H, Ar), 7.12−7.10 (m, 2H, Ar), 5.47 (d, 1H, *J* = 2.4 Hz, H-1), 4.62 (d, 1H, *J* = 11.7 Hz, PhC$\underline{H}_2$O), 4.58 (d, 1H, *J* = 11.7 Hz, PhC$\underline{H}_2$O), 4.52 (d, 1H, *J* = 11.5 Hz, PhC$\underline{H}_2$O), 4.50 (d, 1H, *J* = 11.6 Hz, PhC$\underline{H}_2$O), 4.26 (dd, 1H, *J* = 6.8, 3.5 Hz, H-4), 4.12 (dd, 1H, *J* = 6.8, 3.1 Hz, H-3), 4.09 (dd, 1H, *J* = 3.1, 2.5 Hz, H-2), 3.81−3.76 (m, 1H, H-5), 3.72−3.63 (m, 2H, H-6, H-6'), 2.40 (d, 1H *J* = 7.7 Hz, C5-O$\underline{H}$), 2.33 (s, 3H, ArC$\underline{H}_3$), 2.05 (dd, 1H, *J* = 8.6, 4.4 Hz, C6-O$\underline{H}$); ^{13}C NMR (126 MHz; $CDCl_3$): δ 138.0 (Ar), 137.4 (Ar), 137.0 (Ar), 132.6 (Ar), 130.3 (Ar), 129.9 (Ar), 128.6 (Ar), 128.5 (Ar), 128.1 (2C, 2 × Ar), 128.0 (Ar), 127.9 (Ar), 91.1 (C-1), 87.7 (C-2),

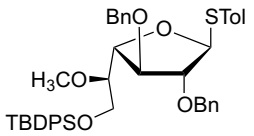
83.5 (C-3), 82.1 (C-4), 72.6 (Ph<u>C</u>H_2O), 72.2 (Ph<u>C</u>H_2O), 70.7 (C-5), 64.7 (C-6), 21.2 (Ar<u>C</u>H_3); HRMS (ESI) Calc. for (M + Na) $C_{27}H_{30}NaO_5S$: 489.1706. Found 489.1701.



Tolyl 2,3-di-*O*-benzyl-6-*O*-*tert*-butyldiphenylsilyl-1-thio-beta-D-galactofuranoside (3-20)

To a solution of **3-19** (6.83 g, 14.64 mmol) and DMAP (357 mg, 2.93 mmol) in anhydrous 6:1 CH_2Cl_2−pyridine s (70 mL) under argon at 0 ºC was added TBDPSCl (4.1 mL, 16.10 mmol) dropwise over ~5 min via syringe. After complete addition of TBDPSCl, the reaction mixture was warmed to room temp and stirred for 14 h. The reaction mixture was then diluted with CH_2Cl_2 (130 mL) and washed with a 1 M aqueous HCl solution (2 × 80 mL), dried over $MgSO_4$, filtered, and concentrated. The resulting residue was then purified by flash chromatography (dry loading) using a gradient of 10:1→7:1 hexanes−EtOAc to afford **3-20** (9.91 g, 96%) as a viscous oil. R_f 0.36 (7:1 hexanes−EtOAc); $[\alpha]_D$ −84.0 (*c* 4.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.70−7.68 (m, 4H, Ar), 7.45−7.42 (m, 2H, Ar), 7.40−7.30 (m, 16H, Ar), 7.09−7.07 (m, 2H, Ar), 5.51 (d, 1H, *J* = 2.5 Hz, H-1), 4.64 (d, 1H, *J* = 11.8 Hz, PhC<u>H</u>$_2$O), 4.60 (d, 1H, *J* = 11.8 Hz, PhC<u>H</u>$_2$O), 4.55 (d, 1H, *J* = 11.8 Hz, PhC<u>H</u>$_2$O), 4.52 (d, 1H, *J* = 11.8 Hz, PhC<u>H</u>$_2$O), 4.46 (dd, 1H, *J* = 6.6, 2.6 Hz, H-4), 4.18 (dd, 1H, *J* = 6.7, 3.1 Hz, H-3), 4.13 (dd, 1H, *J* = 2.8, 2.8 Hz, H-2), 3.88 (qd, 1H, *J* = 6.6, 2.7 Hz, H-5), 3.76 (dd, 1H, *J* = 10.1, 6.7 Hz, H-6), 3.72 (dd, 1H, *J* = 10.2, 6.5 Hz, H-6'), 2.38 (d, 1H, *J* = 6.8 Hz, C5-O<u>H</u>), 2.35 (s, 3H, ArC<u>H</u>$_3$), 1.09 (s, 9H, SiC(C<u>H</u>$_3$)$_3$); ^{13}C NMR (126 Hz; $CDCl_3$) δ 137.7 (Ar), 137.5 (Ar), 137.2 (Ar), 135.6 (2C, 2 × Ar), 133.3 (2C, 2 × Ar), 132.4 (Ar), 130.8 (Ar), 129.7 (3C, 3 × Ar), 128.5 (Ar), 128.4 (Ar), 128.0 (2C, 2 × Ar), 127.8 (3C, 3 × Ar), 91.0 (C-1), 87.9 (C-2), 83.4 (C-3), 80.4 (C-4), 72.4

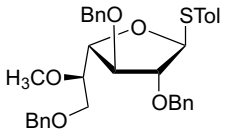
(Ph<u>C</u>H_2O), 72.1 (Ph<u>C</u>H_2O), 70.9 (C-5), 64.9 (C-6), 26.9 (C(<u>C</u>H_3)$_3$), 21.1 (Ar<u>C</u>H_3), 19.3 (Si<u>C</u>(CH_3)$_3$); HRMS (ESI) Calc. for (M + Na) $C_{43}H_{48}NaO_5SSi$: 727.2884. Found 727.2874.



Tolyl 2,3-di-*O*-benzyl-6-*O*-*tert*-butyldiphenylsilyl-5-*O*-methyl-1-thio-beta-D-galactofuranoside (3-21)

To a solution of **3-20** (13.45 g, 19.08 mmol) and CH_3I (1.54 mL, 24.80 mmol) in anhydrous DMF (100 mL) cooled to 0 ºC was added NaH (1.14 g, 28.62 mmol) in portions over ~40 min. After complete addition of the NaH, the reaction mixture was stirred at 0 ºC for ~30 min and then gradually warmed to room temp and stirred for an additional 3 h. The excess NaH was quenched by addition of CH_3OH (5 mL) drop wise over ~20 min after cooling the mixture to 0 ºC. The mixture was then concentrated, diluted with EtOAc (300 mL), and washed with water (100 mL) and brine (50 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated. The resulting residue was then purified by flash chromatography (dry loading) using a gradient of 12:1→8:1 hexanes−EtOAc to afford **3-21** (12.05 g, 88%) as a viscous oil. R_f 0.46 (7:1 hexanes−EtOAc); $[\alpha]_D$ −86.4 (*c* 3.0, $CHCl_3$); ^{1}H NMR (500 MHz; $CDCl_3$): δ 7.76−7.68 (m, 4H, Ar), 7.47−7.30 (m, 18H, Ar), 7.05 (d, 2H, *J* = 7.9 Hz, Ar), 5.50 (d, 1H, *J* = 2.9 Hz, H-1), 4.69 (d, 1H, *J* = 11.8 Hz, PhC<u>H</u>$_2$O), 4.61 (d, 1H, *J* = 11.9 Hz, PhC<u>H</u>$_2$O), 4.52 (d, 1H, *J* = 11.8, PhC<u>H</u>$_2$O), 4.51 (d, 1H, *J* = 11.9 Hz, PhC<u>H</u>$_2$O), 4.37 (dd, 1H, *J* = 7.3, 2.7 Hz, H-4), 4.13−4.10 (m, 2H, H-2, H-3), 3.84 (dd, 1H, *J* = 10.5, 6.5 Hz, H-6), 3.80 (dd, 1H, *J* = 10.6, 6.1 Hz, H-6'), 3.43 (ddd, 1H, *J* = 6.3, 6.3, 2.8 Hz, H-5), 3.33 (s, 3H, OC<u>H</u>$_3$), 2.33 (s, 3H, ArC<u>H</u>$_3$), 1.08 (s, 9H, C(C<u>H</u>$_3$)$_3$); ^{13}C NMR (126 MHz; $CDCl_3$): δ 137.9 (Ar), 137.5 (Ar), 137.2 (Ar), 135.6

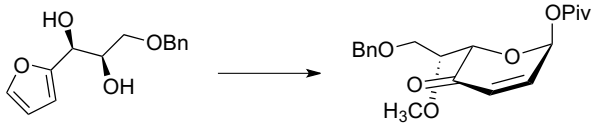
(2C, 2 × Ar), 134.8 (Ar), 133.5 (Ar), 133.4 (Ar), 132.1 (Ar), 130.9 (Ar), 129.7 (Ar), 129.6 (Ar), 128.4 (2C, 2 × Ar), 128.1 (Ar), 128.0 (Ar), 127.9 (Ar), 127.8 (Ar), 127.7 (2C, 2 × Ar), 90.2 (C-1), 88.6 (C-2), 82.5 (C-3), 79.9 (C-4), 79.2 (C-5), 72.3 (Ph<u>C</u>H_2O), 72.2 (Ph<u>C</u>H_2O), 63.2 (C-6), 59.6 (O<u>C</u>H_3), 26.8 (C(<u>C</u>H_3)$_3$), 21.1 (Ar<u>C</u>H_3), 19.2 (Si<u>C</u>(CH_3)$_3$); HRMS (ESI) Calc. for (M + Na) $C_{44}H_{50}NaO_5SSi$: 741.3040. Found 741.3032.



Tolyl 2,3,6-tri-*O*-benzyl-5-*O*-methyl-1-thio-beta-D-galactofuranoside (3-22)

To **3-21** (10.61 g, 14.76 mmol) in anhydrous THF (75 mL) under argon was added a 1 M TBAF solution in THF (22 mL) via syringe. The reaction mixture was stirred for 15 h. The reaction mixture was then diluted with EtOAc (200 mL), washed with a saturated aqueous $NaHCO_3$ solution (100 mL), dried over $MgSO_4$, concentrated, and placed under vacuum overnight. The crude residue, under argon, was then diluted with anhydrous DMF (75 mL). To the mixture was added BnBr (2.3 mL, 19.35 mmol) via syringe and the solution was cooled to 0 ºC. NaH (900 mg, 22.50 mmol) was then added in portions over ~20 min. After complete addition of NaH, the reaction mixture was gradually warmed to room temp and stirred for 6 h. Additional BnBr (1.75 mL, 14.75 mmol) and NaH (590 mg, 14.75 mmol) were added after cooling to 0 ºC. The reaction mixture was gradually warmed to room temp and stirred for an additional 3 h. The excess NaH was quenched by addition of CH_3OH (~5 mL) dropwise. The mixture was concentrated, diluted with EtOAc (200 mL), and then washed with H_2O (100 mL) and brine (100 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated. The resulting residue was purified by flash chromatography (dry loading) using a gradient of

7:1→5:1 hexanes–EtOAc to afford **3-22** (7.47 g, 89%) as a viscous oil. R_f 0.47 (4:1 hexanes−EtOAc); $[\alpha]_D$ −116.5 (*c* 3.1, $CHCl_3$); ^{1}H NMR (500 Hz, $CDCl_3$) δ 7.41−7.29 (m, 17H, Ar), 7.11 (d, 2H, *J* = 7.9 Hz, Ar), 5.55 (d, 1H, *J* = 3.0 Hz, H-1), 4.70 (d, 1H, *J* = 11.8 Hz, PhC$\underline{H}_2$O), 4.62 (d, 1H, *J* = 11.9 Hz, PhC$\underline{H}_2$O), 4.56−4.51 (m, 4H, 4 × PhC$\underline{H}_2$O), 4.33 (dd, 1H, *J* = 7.5, 3.1 Hz, H-4), 4.14 (dd, 1H, *J* = 7.5, 3.6 Hz, H-3), 4.11 (dd, 1H, *J* = 3.3, 3.3 Hz, H-2), 3.69 (dd, 1H, *J* = 10.0, 6.6 Hz, H-6), 3.64 (dd, 1H, *J* = 10.0, 5.0 Hz, H-6'), 3.59 (ddd, 1H, *J* = 6.5, 5.0, 3.2 Hz, H-5), 3.43 (s, 3H, OC$\underline{H}_3$), 2.35 (s, 3H, ArC$\underline{H}_3$); ^{13}C NMR (126 Hz, $CDCl_3$) δ 138.3 (Ar), 137.8 (Ar), 137.5 (Ar), 137.3 (Ar), 132.0 (Ar), 130.9 (Ar), 129.6 (Ar), 128.5 (Ar), 128.4 (2C, 2 × Ar), 128.1 (Ar), 128.0 (Ar), 127.9 (2C, 2 × Ar), 127.6 (2C, 2 × Ar), 90.3 (C-1), 88.6 (C-2), 82.5 (C-3), 80.1 (C-4), 78.3 (C-5), 73.5 (Ph$\underline{C}H_2$O), 72.3 (Ph$\underline{C}H_2$O), 72.2 (Ph$\underline{C}H_2$O), 70.2 (C-6), 59.4 (O$\underline{C}H_3$), 21.1 (Ar$\underline{C}H_3$); HRMS (ESI) Calc. for (M + Na) $C_{35}H_{38}NaO_5S$: 593.2332. Found 593.2324.

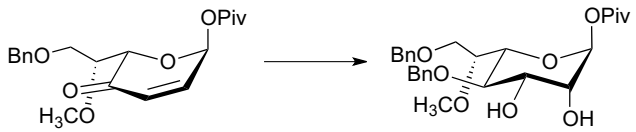


(*R*)-2-(benzyloxy)-1-((2*S*,6*S*)-3-oxo-6-(pivaloyloxy)-3,6-dihydro-2*H*-pyran-2-yl)ethyl pivalate (3-36).

To benzyl protected diol **3-33** (1.00 g, 4.03 mmol) under argon were sequentially added 2-aminoethyl diphenylborinate (181 mg, 0.81 mmol), K_2CO_3 (613 mg, 4.43 mmol), anhydrous CH_3CN (40 mL), and CH_3I (5.55 mL, 12.09 mmol). The reaction mixture was heated to 60 °C and stirred for 16 h. The mixture was poured over a solution of brine (20 mL) and H_2O (20 mL), then extracted with EtOAc (3 × 40 mL). The organic layers were combined, dried over Na_2SO_4, filtered, and concentrated. To the resulting residue were sequentially added $NaHCO_3$ (677 mg,

8.06 mmol), NaOAc·3H_2O (548 mg, 4.03 mmol), and a 4:1 THF–H_2O solution (40 mL). The mixture was cooled to 0 °C and NBS (932 mg, 5.24 mmol) was added in portions over ~20 min. For optimal results either newly purchased or freshly recrystallized (from H_2O) NBS should be used. After complete addition of the NBS, the reaction mixture was stirred at 0 °C for 1.5 h. The excess NBS was quenched by addition of a saturated aqueous $Na_2S_2O_3$ solution (20 mL), stirred for ~20 min, poured over a saturated aqueous $NaHCO_3$ solution (20 mL), and then extracted with EtOAc (3 × 40 mL). The organic layers were combined and then washed with a minimal amount of brine. The organic layer was dried over Na_2SO_4, filtered, and concentrated. The residue was dried under vacuum in the presence of P_2O_5 for ~2 h. To the residue under argon were added anhydrous CH_2Cl_2 (20 mL), Et_3N (1.69 mL, 12.09 mmol) and DMAP (25 mg, 0.20 mmol). The mixture was cooled to –78 °C, then PivCl (744 μL, 6.05 mmol) was added dropwise over ~3 min. The reaction mixture was stirred at –78 °C for 1.5 h. The reaction mixture was poured over a saturated aqueous NH_4Cl solution (30 mL) and extracted with CH_2Cl_2 (100 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated. The residue was purified by flash chromatography using a gradient of 4:1→3:1 hexanes–EtOAc to afford **3-36** (555 mg, 38%) as a pale yellow oil. R_f 0.37 (3:1 hexanes–EtOAc); $[\alpha]_D$ +55.4 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz; $CDCl_3$): δ 7.38–7.29 (m, 5H, Ar), 6.95 (dd, 1H, *J* = 10.3, 3.7 Hz, H-2), 6.63 (d, 1H, *J* = 3.7 Hz, H-1), 6.30 (d, 1H, *J* = 10.3 Hz, H-3), 4.71 (d, 1H, *J* = 1.8 Hz, H-5), 4.57 (d, 1H, *J* = 12.4 Hz, PhC$\underline{H}_2$O) 4.55 (d, 1H, *J* = 12.3 Hz, PhC$\underline{H}_2$O), 4.19 (ddd, 1H, *J* = 8.0, 5.9, 2.0 Hz, H-6), 3.71 (dd, 1H, *J* = 9.4, 5.7 Hz, H-7), 3.66 (dd, 1H, *J* = 9.4, 8.2 Hz, H-7'), 3.43 (s, 3H, OC$\underline{H}_3$), 1.20 (s, 9H, C(C$\underline{H}_3$)$_3$); ^{13}C NMR (126 MHz; $CDCl_3$): δ 194.1 (C4=O), 176.7 (C=O), 142.2 (C-2), 138.0 (Ar), 129.2 (C-3), 128.4 (Ar), 127.6 (Ar), 127.4 (Ar), 87.0 (C-1), 77.8 (C-6), 75.8 (C-5), 73.4 (Ph$\underline{C}H_2$O), 67.3 (C-7), 59.8 (O$\underline{C}H_3$), 39.2 ($\underline{C}(CH_3)_3$, 27.0 (C($\underline{C}H_3$)$_3$; HRMS (ESI) Calc. for (M +

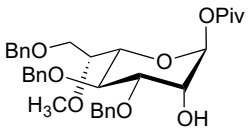
Na) $C_{20}H_{26}NaO_6$: 385.1622. Found 385.1620. *Note: When scale was increased to 30 mmol, the yield decreased to 26% over 3 steps.



4,7-Di-*O*-benzyl-6-*O*-methyl-1-*O*-pivaloyl-D-*glycero*-α-L-*manno*-heptopyranose (3-49).

To pyrenone **3-36** (1.3 g, 3.59 mmol) was added an anhydrous 1:1 CH_2Cl_2–CH_3OH solution (18 mL); $CeCl_3 \cdot 7H_2O$ (670 mg, 1.80 mmol) was then added. The mixture was stirred, with sonication if necessary, until a homogenous solution was achieved. The mixture was cooled to –78 °C and then $NaBH_4$ (136 mg, 3.59 mmol) was added in five portions over ~5 min. The reaction mixture was stirred at –78 °C for 30 min before being diluted with Et_2O (10 mL). A saturated aqueous $NaHCO_3$ solution (10 mL) was added and the mixture was then extracted with Et_2O (3 × 40 mL). The organic layers were combined, washed with brine, dried over Na_2SO_4, filtered, and concentrated. The residue was dried under vacuum in the presence of P_2O_5 for ~3 h. To the residue under argon was added anhydrous DMF (26 mL) and BnBr (1.12 mL, 7.18 mmol). The mixture was stirred and NaH (215 mg, 5.39 mmol) was added in three portions over 0.5 h before being stirred for an additional 1.5 h. Excess NaH was quenched by addition of HOAc (150 μL). The mixture was concentrated and the resulting residue was diluted with EtOAc (100 mL) and washed with a solution of brine (15 mL) and then H_2O (15 mL). The organic layer was then concentrated. To the residue were sequentially added a 5:1 acetone–H_2O solution (30 mL), NMO (630 mg, 5.39 mmol), and a 5% OsO_4 in *t*BuOH (600 μL, ~3 mol%). The reaction mixture was stirred for 30 h and the mixture was poured over a solution of saturated aqueous $Na_2S_2O_3$ solution diluted with equal volume of H_2O (60 mL), then extracted with EtOAc (3 × 50

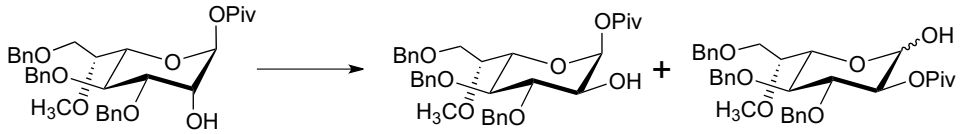
mL). The organic layers were combined, dried over Na_2SO_4, filtered, and concentrated. The resulting residue was purified by flash chromatography using a gradient of 1:1→2:1 EtOAc–hexanes to afford diol **3-49** (739 mg, 42%) as a clear oil. R_f 0.43 (2:1 EtOAc–hexanes); $[\alpha]_D$ –26.6 (*c* 1.0, $CHCl_3$); ^1H NMR (500 MHz; $CDCl_3$): δ 7.43–7.29 (m, 10H, Ar), 6.12 (d, 1H, *J* = 1.7 Hz, H-1), 4.90 (d, 1H, *J* = 11.3 Hz, PhC$\underline{H}_2$O), 4.75 (d, 1H, *J* = 11.3 Hz, PhC$\underline{H}_2$O), 4.53 (app. s, 2H, PhC$\underline{H}_2$O), 3.97–3.89 (m, 3H, H-2, H-3, H-4), 3.83 (ddd, 1H, *J* = 6.0, 6.0, 1.7, H-6), 3.81 (dd, 1H, *J* = 9.2, 1.3 Hz, H-5), 3.75 (dd, 1H, *J* = 9.7, 6.0 Hz, H-7), 3.60 (dd, 1H, *J* = 9.7, 6.1 Hz, H-7'), 3.49 (s, 3H, OC$\underline{H}_3$), 2.98 (br s, 1H, C2-O$\underline{H}$), 2.63 (d, 1H, *J* = 6.2 Hz, C3-O$\underline{H}$), 1.16 (s, 9H, C(C$\underline{H}_3$)$_3$); ^{13}C NMR (126 MHz; $CDCl_3$): δ 176.1 (C=O), 138.2 (Ar), 138.1 (Ar), 128.7 (Ar), 128.4 (Ar), 128.1 (2C, 2 × Ar), 127.6 (Ar), 127.5 (Ar), 93.0 (C-1), 76.5 (C-6), 75.0 (Ph$\underline{C}H_2$O), 74.9 (C-3/C-4), 73.4 (Ph$\underline{C}H_2$O), 72.9 (C-5), 72.2 (C-3/C-4), 70.1 (C-2), 69.3 (C-7), 58.6 (O$\underline{C}H_3$), 39.0 ($\underline{C}(CH_3)_3$), 27.0 (C($\underline{C}H_3$)$_3$; HRMS (ESI) Calc. for (M + Na) $C_{27}H_{36}NaO_8$: 511.2302. Found 511.2299.



3,4,7-Tri-*O*-benzyl-6-*O*-methyl-1-*O*-pivolyl-D-*glycero*-α-L-*manno*-heptopyranose (3-50).

To diol **3-49** (1.5 g, 3.07 mmol) in anhydrous toluene (30 mL), *n*Bu_2SnO (841 mg, 3.38 mmol) was added. A Dean–Stark apparatus was attached, filled w/ toluene, and the mixture was heated to reflux for 8 h. The mixture was cooled to room temperature, the Dean–Stark apparatus was removed and then *n*Bu_4NI (1.25 g, 3.38 mmol) and BnBr (450 μL, 3.79 mmol) were added. The reaction mixture was heated to 100 °C and stirred for 17 h, before being cooled and then poured over a solution of saturated aqueous $Na_2S_2O_4$ solution (30 mL) and H_2O (30 mL) and

then extracted with EtOAc (120 mL). The organic layer was washed with brine (20 mL), dried over Na_2SO_4, filtered, and concentrated. The residue was then purified by flash chromatography using a gradient of 3:2→1:1 hexanes–EtOAc to afford **3-50** (1.5 g, 84%) as clear viscous oil. R_f 0.50 (1:1 hexanes–EtOAc); $[\alpha]_D$ –41.3 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz; $CDCl_3$): δ 7.39–7.28 (m, 15H, Ar), 6.14 (d, 1H, *J* = 1.8 Hz, H-1), 4.98 (d, 1H, *J* = 10.9 Hz, PhC$\underline{H}_2$O), 4.75 (d, 1H, *J* = 11.8 Hz, PhC$\underline{H}_2$O), 4.73 (d, 1H, *J* = 11.7 Hz, PhC$\underline{H}_2$O), 4.69 (d, 1H, *J* = 10.9 Hz, PhC$\underline{H}_2$O), 4.52 (app. s, 2H, PhC$\underline{H}_2$O), 4.12 (dd, 1H, *J* = 9.5, 9.5 Hz, H-4), 3.90–3.80 (m, 4H, H-2, H-3, H-5, H-6), 3.75 (dd, 1H, *J* = 9.7, 6.0 Hz, H-7), 3.59 (dd, 1H, *J* = 9.7, 6.1 Hz, H-7'), 3.47 (s, 3H, OC$\underline{H}_3$), 2.56 (d, 1H, *J* = 2.6 Hz, C2-O$\underline{H}$), 1.13 (s, 9H, C(C$\underline{H}_3$)$_3$); ^{13}C NMR (126 MHz; $CDCl_3$): δ 175.9 (C=O), 138.3 (Ar), 138.2 (Ar), 137.5 (Ar), 128.6 (Ar), 128.5 (Ar), 128.3 (Ar), 128.2 (Ar), 128.1 (Ar), 128.0 (Ar), 127.9 (Ar), 127.5 (2C, 2 × Ar), 92.8 (C-1), 79.7, 76.3, 75.4 (Ph$\underline{C}H_2$O), 73.4 (Ph$\underline{C}H_2$O), 73.2, 73.1, 72.1 (Ph$\underline{C}H_2$O), 69.3 (C-7), 67.7 (C-2), 58.6 (O$\underline{C}H_3$), 39.0 ($\underline{C}(CH_3)_3$), 27.0 (C($\underline{C}H_3$)$_3$; HRMS (ESI) Calc. for (M + Na) $C_{34}H_{42}NaO_8$: 601.2772. Found 601.2768.



3-51a 21%

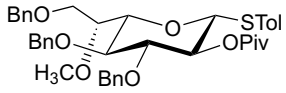
3-51b 52%

3,4,7-Tri-*O*-benzyl-6-*O*-methyl-1-*O*-pivolyl-D-*glycero*-α-L-*gluco*-heptopyranose (3-51a) and **3,4,7-tri-*O*-benzyl-6-*O*-methyl-2-*O*-pivolyl-D-*glycero*-α/β-L-*gluco*-heptopyranose (3-51b).**

To **3-50** (1.5 g, 2.59 mmol) in anhydrous CH_2Cl_2 (26 mL) under argon was added anhydrous pyridine (1.9 mL, 23.31 mmol). The mixture was cooled to –10 °C and Tf_2O (1.3 mL, 7.77 mmol) was added dropwise via syringe over ~7 min. The reaction mixture was gradually warmed to 10 °C over 2.5 h. After which, the reaction mixture was poured over an ice cold 1M

HCl solution (25 mL) and extracted with CH_2Cl_2 (75 mL). The organic layer was then sequentially washed with ice cold H_2O (25 mL), a saturated aqueous $NaHCO_3$ solution (25 mL), and brine (25 mL). The organic layer was dried over Na_2SO_4, filtered, and concentrated. The residue was dried under vacuum in the presence of P_2O_5 for 1 h. To the residue under argon was added anhydrous DMF (18 mL) and $NaNO_2$ (894 mg, 12.95 mmol). The reaction mixture was heated to 60 °C for 10 h. The mixture was concentrated, diluted with EtOAc (120 mL), and then washed with brine (25 mL). The organic layer was dried over Na_2SO_4, filtered, and concentrated. The resulting residue was purified by flash chromatography using a gradient of 3:1→2:1 hexanes–EtOAc to afford isomers **3-51a** (320 mg, 21%) and **3-51b** (780 mg, 52%). **Data for 3-51a**: Pale yellow oil. R_f 0.45 (2:1 hexanes–EtOAc); $[\alpha]_D$ –50.4 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz; $CDCl_3$): δ 7.41...7.29 (m, 15H, Ar), 6.20 (d, 1H, *J* = 3.6 Hz, H-1), 4.98 (d, 1H, *J* = 10.9 Hz, PhC$\underline{H}_2$O), 4.95 (d, 1H, *J* = 11.3 Hz, PhC$\underline{H}_2$O), 4.87 (d, 1H, *J* = 11.3 Hz, PhC$\underline{H}_2$O), 4.70 (d, 1H, *J* = 10.9 Hz, PhC$\underline{H}_2$O), 4.53 (s, 2H, PhC$\underline{H}_2$O), 3.90–3.79 (m, 5H, H-2, H-3, H-4, H-5, H-6), 3.73 (dd, 1H, *J* = 9.7, 5.9 Hz, H-7), 3.58 (dd, 1H, *J* = 9.7, 6.2 Hz, H-7'), 3.48 (d, 3H, *J* = 1.7 Hz, OC$\underline{H}_3$), 1.88 (s, 1H, O$\underline{H}$), 1.19 (s, 9H, C(C$\underline{H}_3$)$_3$); ^{13}C NMR (126 MHz; $CDCl_3$): δ 176.7 (C=O), 138.3 (Ar), 138.0 (2C, 2 × Ar), 128.6 (2C, 2 × Ar), 128.4 (Ar), 128.1 (Ar), 128.0 (3C, 3 × Ar), 127.6 (Ar), 127.5 (Ar), 91.6 (C-1), 82.5, 76.9, 76.2, 75.3 (Ph$\underline{C}H_2$O), 75.2 (Ph$\underline{C}H_2$O), 73.4 (Ph$\underline{C}H_2$O), 72.8, 71.7, 69.3 (C-7), 58.7 (O$\underline{C}H_3$), 39.2 ($\underline{C}(CH_3)_3$), 27.1 (C($\underline{C}H_3$)$_3$); HRMS (ESI) Calc. for (M + Na) $C_{34}H_{42}NaO_8$: 601.2772. Found 601.2768. **Data for 3-51b**: (~2:1 α/β mixture) clear oil. R_f 0.63 & 0.57 (2:1 hexanes–EtOAc); ^{1}H NMR (500 MHz; $CDCl_3$): δ 7.38–7.28 (m, 30H, Ar), 5.38 (d, 1H, *J* = 3.7 Hz, H-1α), 4.94 (d, 1H, *J* = 11.1 Hz, PhC$\underline{H}_2$O), 4.93–4.78 (m, 7H, H-2α, H-2β, 5 × PhC$\underline{H}_2$O), 4.66 (d, 2H, *J* = 11.2 Hz, 2 × PhC$\underline{H}_2$O), 4.60 (d, 1H, *J* = 12.0 Hz, PhC$\underline{H}_2$O), 4.59 (d, 1H, *J* = 12.1 Hz, PhC$\underline{H}_2$O), 4.55 (d, 1H, *J* = 11.9 Hz, PhC$\underline{H}_2$O), 4.51 (d, 1H, *J*

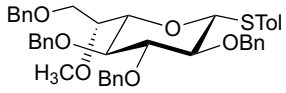
= 12.0 Hz, PhC$\underline{H}_2$O), 4.51 (d, 1H, *J* = 8.3 Hz, H-1β), 4.11 (dd, 1H, *J* = 9.5, 9.5 Hz, H-3α), 4.05 (dd, 1H, *J* = 10.0, 1.4 Hz, H-5α), 3.92 (dd, 1H, *J* = 9.7, 9.0 Hz, H-4β), 3.87–3.73 (m, 7H, H-3β, H-4α, H-6α, H-6β, H-7α, H-7β, H-7'β), 3.67 (dd, 1H, *J* = 9.7, 6.3 Hz, H-7'α), 3.55 (dd, 1H, *J* = 9.8, 1.4 Hz, H-5β), 3.48 (s, 6H, 2 × OC$\underline{H}_3$), 1.23 (s, 18H, 2 × C(C$\underline{H}_3$)$_3$); ^{13}C NMR (126 MHz; $CDCl_3$): δ 179.2 (C=O), 177.8 (C=O), 138.4 (2C, 2 × Ar), 138.2 (2C, 2 × Ar), 138.1 (Ar), 138.0 (Ar), 128.5 (2C, 2 × Ar), 128.4 (3C, 3 × Ar), 128.1 (2C, 2 × Ar), 127.8 (2C, 2 × Ar), 127.9 (Ar), 127.7 (3C, 3 × Ar), 127.6 (Ar), 127.5 (Ar), 127.4 (Ar), 96.6 (C-1β), 90.3 (C-1α), 82.8 (C-3β), 80.1 (C-3α), 77.3, 77.1, 76.3, 76.2, 76.1, 75.3 (Ph$\underline{C}$H$_2$O), 75.1 (Ph$\underline{C}$H$_2$O), 74.9 (3C, C-5β, 2 × Ph$\underline{C}$H$_2$O), 74.0, 73.5 (Ph$\underline{C}$H$_2$O), 73.3 (Ph$\underline{C}$H$_2$O), 70.0 (C-5α), 69.3 (2C, C-7α, C-7β), 58.9 (OCH_3), 58.8 (OCH_3), 39.0 ($\underline{C}(CH_3)_3$), 38.8 ($\underline{C}(CH_3)_3$), 27.2 (C($\underline{C}H_3$)$_3$), 27.1 (C($\underline{C}H_3$)$_3$); HRMS (ESI) Calc. for (M + Na) $C_{34}H_{42}NaO_8$: 601.2772. Found 601.2769.



***p*-Tolyl 3,4,7-tri-*O*-benzyl-6-*O*-methyl-2-*O*-pivloyl-1-thio-D-*glycero*-β-L-*gluco*-heptopyranoside (3-52).**

To **3-51b** (799 mg, 1.38 mmol) in anhydrous pyridine (7.5 mL) under argon were sequentially added $(TolS)_2$ (680 mg, 2.76 mmol) and 1M solution of $P(CH_3)_3$ in THF (2.76 mL, 2.76 mmol). The reaction mixture was stirred for 21 h and then poured over a solution of brine (5 mL) and H_2O (5 mL) and then extracted with EtOAc (2 × 40 mL). The organic layers were combined, dried over Na_2SO_4, filtered and concentrated. The resulting residue was purified by flash chromatography (dry loading) in 4:1 hexanes–EtOAc to afford **16** (850 mg, 90%) as a pale yellow viscous oil. R_f 0.56 (3:1 hexanes–EtOAc); $[\alpha]_D$ –9.7 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz; $CDCl_3$): δ 7.39–7.26 (m, 17H, Ar), 7.04 (d, 2H, *J* = 7.9 Hz, Ar), 5.15 (dd, 1H, *J* = 10.1, 9.2 Hz,

H-2), 4.89 (d, 1H, *J* = 11.0 Hz, PhC$\underline{H}_2$O), 4.77 (d, 1H, *J* = 11.0 Hz, PhC$\underline{H}_2$O), 4.73 (d, 1H, *J* = 11.0 Hz, PhC$\underline{H}_2$O), 4.65 (d, 1H, *J* = 11.0 Hz, PhC$\underline{H}_2$O), 4.57 (d, 1H, *J* = 10.2 Hz, H-1), 4.44 (d, 1H, *J* = 11.8 Hz, PhC$\underline{H}_2$O), 4.42 (d, 1H, *J* = 11.8 Hz, PhC$\underline{H}_2$O), 3.94 (dd, 1H, *J* = 9.4, 9.4 Hz, H-4), 3.83–3.80 (m, 2H, H-6, H-7a), 3.78 (dd, 1H, *J* = 9.1, 9.1 Hz, H-3), 3.63 (dd, 1H, *J* = 11.5, 9.1 Hz, H-7'), 3.51 (dd, 1H, *J* = 9.8, 1.7 Hz, H-5), 3.46 (s, 3H, OC$\underline{H}_3$), 2.29 (s, 3H, (ArC$\underline{H}_3$)), 1.26 (s, 9H, C(C$\underline{H}_3$)$_3$); ^{13}C NMR (126 MHz; $CDCl_3$): δ 176.8 (C=O), 138.3 (Ar), 138.1(4) (Ar), 138.1(1) (Ar), 137.8 (Ar), 132.7 (Ar), 130.4 (Ar), 129.6 (Ar), 128.4 (3C, 3 × Ar), 127.7 (2C, 2 × Ar), 127.6 (2C, 2 × Ar), 127.5 (Ar), 127.4 (Ar), 88.2 (C-1), 85.1 (C-3), 78.6 (C-5), 76.9 (C-4), 76.2 (C-6), 75.1 (Ph$\underline{C}$H$_2$O), 74.9 (Ph$\underline{C}$H$_2$O), 73.4 (Ph$\underline{C}$H$_2$O), 72.0 (C-2), 68.9 (C-7), 58.4 (O$\underline{C}$H$_3$), 38.8 ($\underline{C}$(CH$_3$)$_3$), 27.2 (C($\underline{C}$H$_3$)$_3$), 21.1 (Ar$\underline{C}$H$_3$); HRMS (ESI) Calc. for (M + NH_4) $C_{41}H_{52}NO_7S$: 702.3459. Found 702.3445.



***p*-Tolyl 2,3,4,7-tetra-*O*-benzyl-6-*O*-methyl-1-thio-D-*glycero*-β-L-*gluco*-heptopyranoside (3-9).**

To **3-52** (800 mg, 1.17 mmol) in anhydrous DMF (12 mL) under argon were sequentially added BnBr (280 μL, 2.34 mmol) and NaH (94 mg, 2.34 mmol). The reaction mixture was stirred for 0.5 h, then heated to 60 °C and stirred for another 3 h. The mixture was then cooled to room temperature and additional NaH (94 mg, 2.34 mmol) was added before the mixture was heated again 60 °C and stirred for another 3 h. After cooling to room temperature, excess NaH was quenched by addition of CH_3OH (~1 mL). The mixture was then concentrated. The residue was diluted with EtOAc (100 mL) and washed with a solution of brine (20 mL) and then H_2O (20 mL). The organic layer was dried over Na_2SO_4, filtered, and concentrated. The resulting residue

was purified by flash chromatography (dry loading) using a gradient of 10:1→4:1 hexanes–EtOAc to afford **3-9** (575 mg, 71%, 95% based on recovered **3-52** and substrate lacking Piv group) as a pale yellow viscous oil. Obtained $[\alpha]_D$ –17.8 (*c* 0.7, $CHCl_3$); reported $[\alpha]_D$ –15.9 (0.7 *c*, $CHCl_3$); ^1H and ^{13}C NMR data matched that reported previously.

3.6 References

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Chapter 4

Efforts Towards the Synthesis of the Tetrasaccharide Repeating Unit found in *C. jejuni* 11168H Capsular Polysaccharide

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A part of this chapter has been published:

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4.1 Background

As part of Research Objective 2 of this thesis, the tetrasaccharide repeating unit found in *C. jejuni* 11168H (HS:2) lacking the methyl phosphoramidate (MeOPN) motifs is needed. The CPS structure of the HS:2 serotype is shown in Figure 4-1.

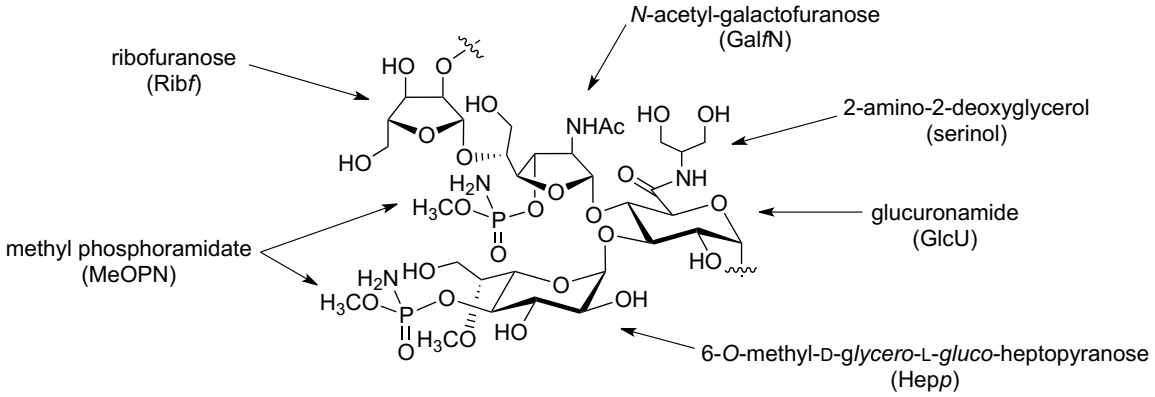


Figure 4-1: *C. jejuni* 11168H capsular polysaccharide structure.

The CPS structure of the *C. jejuni* 11168H (HS:2), absent of the methyl phosphoramidate, was first elucidated by Monteiro and coworkers in 2002.[1] The repeating unit consists of an α-D-glucuronic acid (GlcA) that is amidated with a serinol motif, a 2-acetamido-2-deoxy-β-D-galactofuranose (Gal*f*NAc), a β-D-ribofuranose (Rib*f*), and a 6-*O*-methyl-D-*glycero*-α-L-*gluco*-heptose (Hep*p*). The presence of the MeOPN moieties was not originally found due to their lability in standard CPS extraction protocols (e.g., hot phenol and water).[2] The first MeOPN motif (on the Gal*f*NAc) was serendipitously discovered by examining the CPS of intact cells of NCTC11168 using high resolution magic angle spinning (HR-MAS) NMR spectroscopy.[3] The presence of a second MeOPN motif (on the Hep*p* residue) was later found in the 11168H strain.[4]

The CPS of NCTC11168/11168H is structurally interesting, as it possesses two rare sugar residues Hep*p* and Gal*f*NAc. The Hep*p* residue, as described in Chapter 3 of this thesis, was the first reported to possess the D-*glycero*-L-*gluco* stereochemistry.[1] The 2-acetamido-2-deoxy-D-galactose residue (GalNAc) is common; however, it being found in its furanose ring configuration is rare. This is the second reported example of Gal*f*NAc in nature. The first was its presence in a lipopolysaccharide (LPS) of the Gram-negative bacteria *Proteus penneri*.[5]

As a substrate for the phosphoramidate transferases, the tetrasaccharide shown in Figure 4-2 is our target molecule as it can potentially mimic the CPS found in *C. jejuni* 11168H. Its synthesis will present challenges. First, an appropriate method to obtain GalNAc in its furanose ring configuration needs to be developed. Second, the timing of the serinol introduction needs to be established. Finally, the tetrasaccharide possess two 1,2-*cis* glycosidic linkages, the formation of which still remains a challenge. Another challenge is preparing a Hep*p* glycosyl donor, which was reported in Chapter 3.

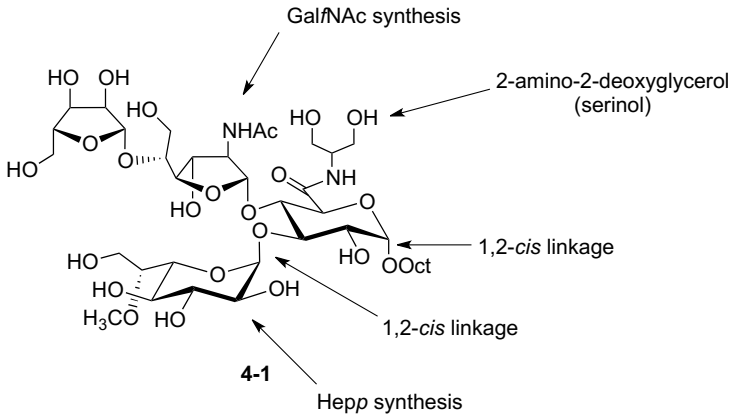


Figure 4-2: Target molecule with postulated synthetic challenges.

The retrosynthetic analysis of **4-1** is shown in Figure 4-3. Given the problems commonly associated with glycosylations in the presence of an amide (i.e., poor to modest yields),[6-8] a late stage oxidation–amidation approach will be implemented and will be followed by a global deprotection. The core tetrasaccharide **4-2** could be obtained by a 2 + 2 glycosylation of the thioglycoside **4-3** and disaccharide acceptor **4-4**. The disaccharide donor **4-3** could be synthesized by a dehydrative glycosylation of 1-hydroxy Rib*f* **4-5** and thioglycoside **4-6**. The disaccharide acceptor **4-4** could be obtained from a glycosylation of Hep*p* thioglycoside donor **4-7** and the glucose acceptor **4-8**. Establishing the two 1,2-*cis* glycosidic linkages early on could help overcome any purification difficulties. In addition, the choice of an octyl algycone was done to help facilitate purification of the deprotected molecule, particularly after enzymatic reactions, via a C_{18} column.[9]

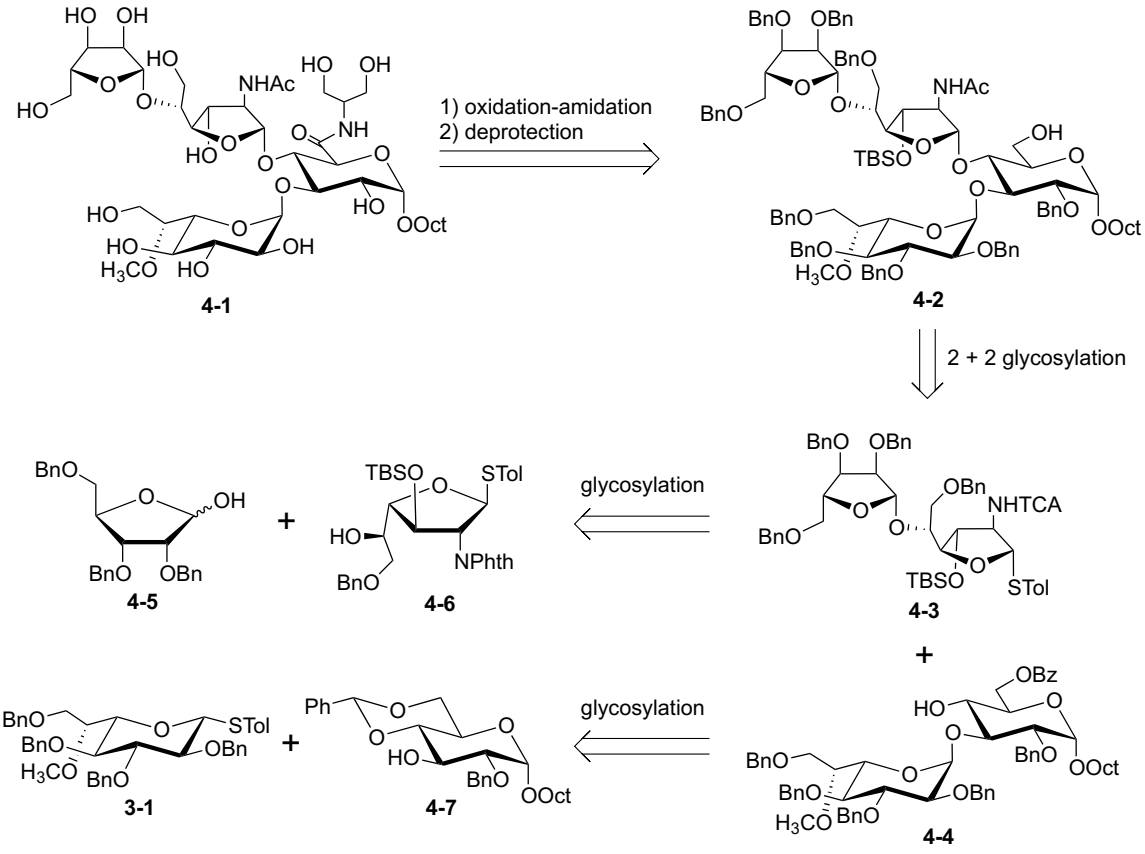


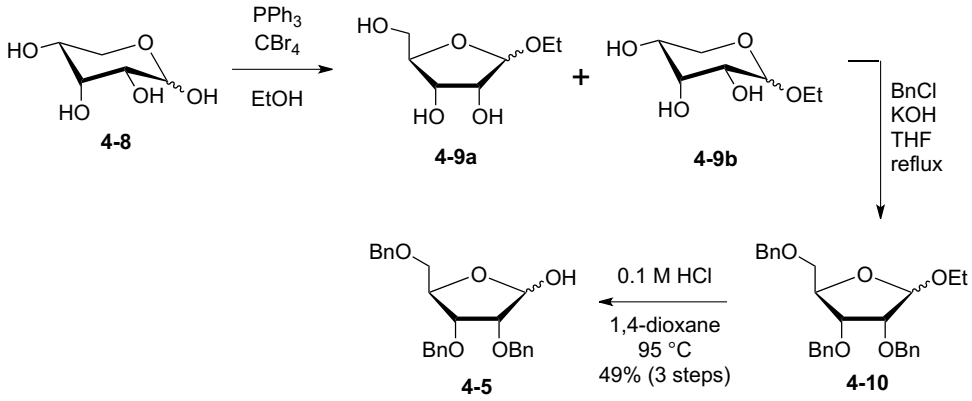
Figure 4-3: Retrosynthetic analysis of tetrasaccharide **4-1**.

4.2 Synthesis of monosaccharide building blocks

4.2.1 Synthesis of ribofuranose building block

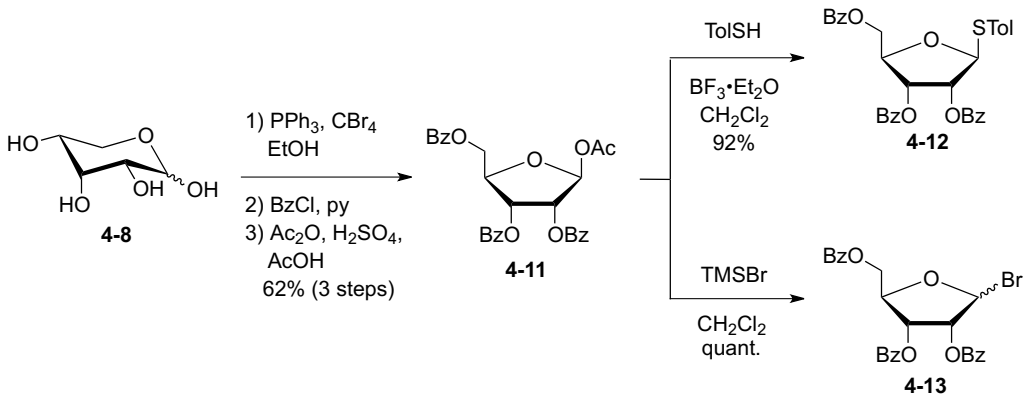
The synthesis of Rib*f* derivative **4-5** began by treating D-ribose (**4-8**) with ethanol in the presence of triphenylphosphine and carbon tetrabromide to afford a mixture of ethyl glycosides **4-9a** and **4-9b**. This method, developed by Schmalisch and Mahrwald,[10] produced an ~9:1 mixture of furanoside **4-9a** and pyranoside **4-9b** compared to an ~3:1 mixture by traditional acid-catalyzed Fisher glycosylation of D-ribose (**4-8**).[11,12] The mixture of **4-9a** and **4-9b** was treated with benzyl chloride in the presence of potassium

hydroxide at reflux to give pure **4-10**, after passage through a silica plug to remove the pyranoside adduct. Treatment of **4-10** with a 0.1 M solution of hydrochloric acid at 95 °C furnished **4-5**[11,12] in 49% yield over three steps.



Scheme 4-1: Synthesis of Rib*f* derivative **4-5**.

As an alternative, two additional ribofuranose donors, **4-13** and **4-14** (Scheme 4-2), were synthesized to address problems that appeared in the assembly of the target (see discussion below). Similar to above, D-ribose (**4-8**) was treated with ethanol in the presence of triphenylphosphine and carbon tetrabromide to afford a mixture of the furanoside **4-9a** and pyranoside **4-9b**. The mixture was treated with benzoyl chloride in pyridine and then with acetic anhydride in the presence of sulfuric acid to afford **4-11** in 62% yield over three steps. Compound **4-11** was converted to thioglycoside **4-12** by glycosylation with *para*-methylbenzene thiol in the presence of boron trifluoride etherate in 92% yield. Alternatively, **4-11** was treated with bromotrimethylsilane to afford the ribofuranosyl bromide **4-13** in quantitative yield.



Scheme 4-2: Synthesis of ribofuranose thioglycoside **4-12** and ribofuranosyl bromide **4-13**.

4.2.2 Synthesis of 2-acetamido-2-deoxy-galactofuranose building block

Little work has been focused on the synthesis of Gal*f*NAc and its analogues. Two reported syntheses of GalNAc in its furanose ring configuration are shown in Figure 4-4. In the first synthesis, GalNAc (**4-14**) was treated with 2,2-dimethoxypropane in the presence of *para*-toluenesulfonic acid at 80 °C to furnish the Gal*f*NAc analogue **4-15** in 72% yield.[13] This product, however, is not suitable for conversion to a glycosyl donor due to the presence of the isopropylidene group, which would be cleaved under conditions needed to activate the methyl glycoside. In the second synthesis, a methanolic solution of GalNAc (**4-14**) was heated to reflux in the presence of freshly prepared acidic zeolites.[14,15] These conditions afforded the product **4-16** in 67% yield. A caveat to these approaches, however, is the presence of the 2-acetamido group, which often causes problems in glycosylation reactions.[16-18] Therefore, an alternative strategy to obtain GalNAc in its furanose ring configuration was sought.

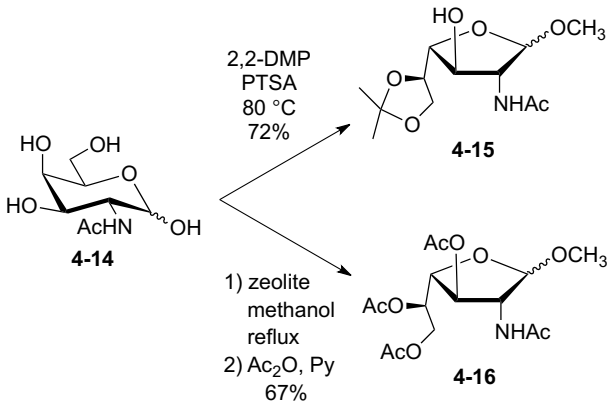


Figure 4-4: Reported syntheses of 2-acetamido-2-deoxy-galactose in the furanose ring configuration.[13-15]

Although the synthesis of other Gal*f*NAc analogues has not been reported, there are several reported syntheses of galactofuranosides (Gal*f*) from D-galactose (**4-17**).[19] Some of these strategies are summarized in Figure 4-5. One approach involves acylation of reducing sugars at elevated temperatures to form a peracylated substrate (e.g., **4-18**).[20,21] A key disadvantage, however, is the unavoidable production of the peracylated pyranoses (e.g., **4-19**), which can complicate purification. As an alternative approach, dithioacetals such as **4-20**, made by treating D-galactose (**4-13**) with ethanethiol in the presence of hydrochloric acid, can cyclize into furanosides. Two methods have been developed for this transformation. In one, the dithioacetal **4-20** is mixed with iodine in methanol to give the thiofuranoside **4-21**.[22] Alternatively, the dithioacetal **4-20** is treated with mercuric salts to give the furanoside **4-22**.[23-25] The main advantage of these transformations is that the cyclization proceeds to give only the furanoside, thus significantly easing purification. Thus I chose to pursue the dithioacetal approach as illustrated in Scheme 4-3.

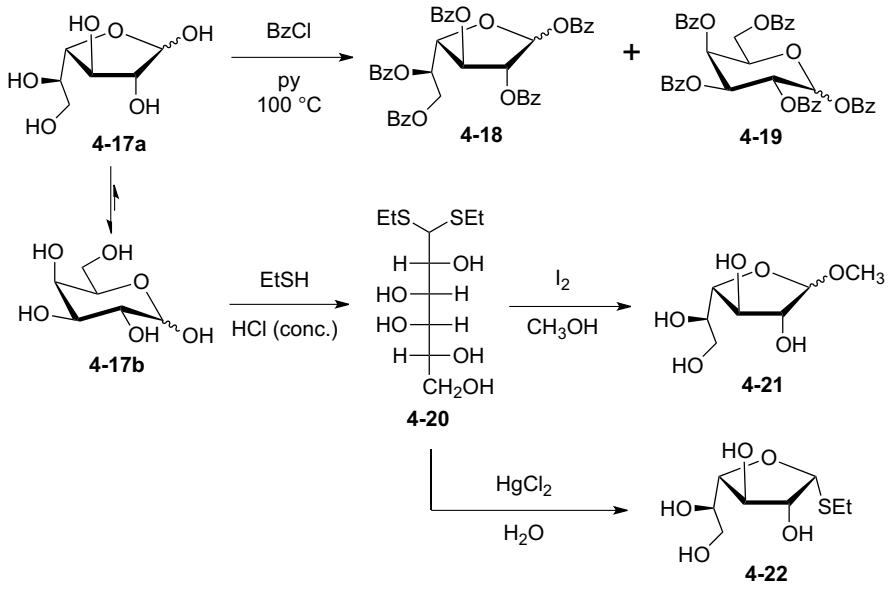
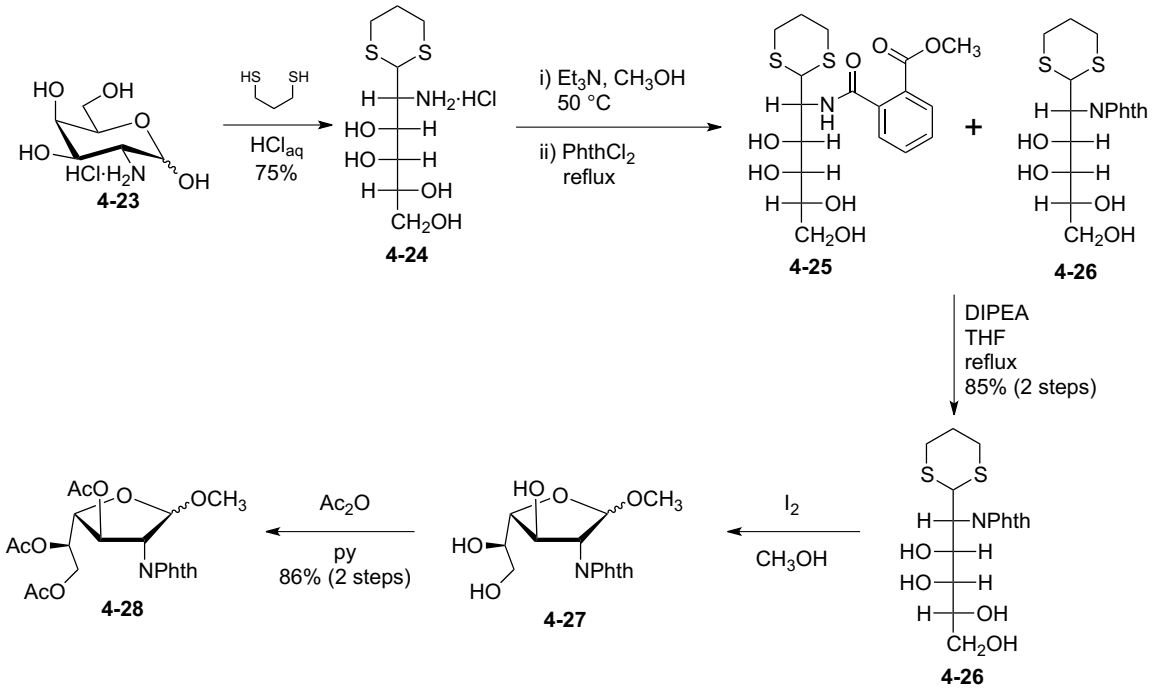


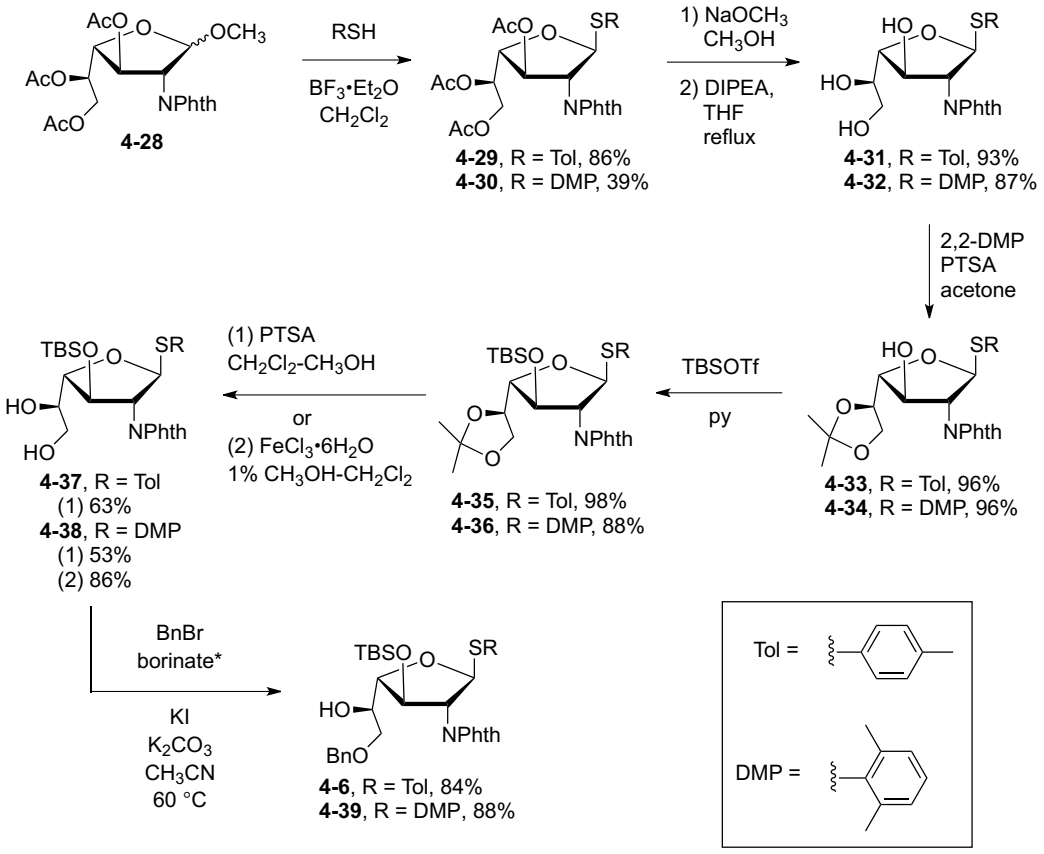
Figure 4-5: Reported approaches for converting galactose into its furanoside form.[19]

Given the precedence for dithioacetal formation from glucosamine hydrochloride,[26] galactosamine hydrochloride (**4-23**) was mixed with 1,3-dithiopropane in concentrated hydrochloric acid to give dithioacetal **4-24** in 75% yield. The amino group of **4-24** was then protected as a phthalimide by first treatment with triethylamine at 50 °C and then addition of phthaloyl chloride followed by heating at reflux in methanol. Although this gave a mixture of products **4-25** and **4-26**, treatment with diisopropylethylamine at reflux can convert the former into the latter. This two-step protocol furnished **4-26** in 85% yield from **4-24**. The mixing of **4-26** with iodine in methanol (to give **4-27**) followed by acylation with acetic anhydride in pyridine afforded furanoside **4-28** in 86% over two steps. The furanoside ring form in **4-28** is supported by the downfield shift of H-5 to 5.35 ppm due to the presence of the electron withdrawing substituent.



Scheme 4-3: Synthesis of methyl 3,5,6-*O*-acetyl-2-deoxy-2-*N*-phthalamido-galactofuranoside.

Furanoside **4-28** was converted into thioglycoside **4-29** in 86% yield when mixed with *para*-methylbenzenethiol in the presence of boron trifluoride etherate. The acetate groups of **4-29** were then removed by treatment with sodium methoxide to give a mixture of **4-31** and a byproduct in which the phthalimido group was opened (similar to **4-25**). The mixture was stirred with diisopropylethylamine to re-close the phthalimido group to give **4-31** in 93% yield. The C-5 and C-6 hydroxyl groups of **4-31** were then protected with an isopropylidene ketal using 2,2-dimethoxypropane in the presence of *para*-toluenesulfonic acid to afford **4-33** in 96% yield. Alcohol **4-33** was next treated with *tert*-butyldimethylsilyl trifluoromethanesfulonate to give, in 98% yield, silyl ether **4-35**. The isopropylidene ketal was then selectively removed in the presence of the *tert*-butyldimethyl silyl group by treatment with a 1:1 methanol–dichloromethane solution in the presence of *para*-toluenesulfonic acid to give diol **4-37** in 63% yield.

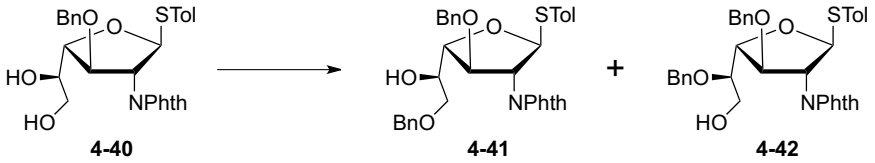


Scheme 4-4: Synthesis of Gal*f*NAc-derived thioglycosides **4-6** and **4-39**. *borinate = 2-aminoethyl diphenylborinate

With diol **4-37** in hand, conditions were explored to selectively protect the C-6 alcohol with a benzyl group (Table 4-1). In exploring different methods, a C-3 benzyloxy analogue (**4-40**) was used due to more complex mixtures forming when the silyl group was present. I first investigated the protection of the C-5 and C-6 hydroxyl groups as a benzylidene acetal followed by regioselective reductive ring opening. Several conditions (Entries 1–5), involving varying the Lewis acid and hydride source, were explored to produce a compound with a benzyl group at the C-6 hydroxyl group (e.g., **4-41**).[27-30] These attempts led to either complete hydrolysis of the benzylidene acetal (Entries 5 and

6) or produced the desired compound in modest selectivity and yield (Entries 1 and 2); therefore, other conditions were explored (Entries 6–9).

Table 4-1: Conditions explored to regioselectively benzylate the C-6 hydroxl group in **4-40**.



Entry	Conditions	R	Product[a] 4-41:4-42	Yield[b]
1	1) $PhCH(OCH_3)_2$, PTSA 2) $BH_3{\cdot}N(CH_3)_3$, $AlCl_3$, THF, 4Å MS	Bn	2:1[c]	45%
2	1) $PhCH(OCH_3)_2$, PTSA 2) $BH_3{\cdot}S(CH_3)_2$, $AlCl_3$, THF, 4Å MS	Bn	1:0[c]	~22%[d]
3	1) $PhCH(OCH_3)_2$, PTSA 2) Et_3SiH, I_2, CH_3CN, 4Å MS	Bn	1:1[c]	~47%[d]
4	1) $PhCH(OCH_3)_2$, PTSA 2) Et_3SiH, $BF_3{\cdot}Et_2O$, CH_3CN, 4Å MS	Bn	0:0[c]	---
5	1) $PhCH(OCH_3)_2$, PTSA 2) Et_3SiH, $AlCl_3$, CH_3CN, 4Å MS	Bn	0:0[c]	---
6	Bn-OTCA, TMSOTf, CH_2Cl_2, 0 °C	Bn	2:1[c]	~20%[d]
7	BnBr, $AgCO_3$, toluene, 60 °C	Bn	4:1[c]	~35%[d]
8	i) $n$$Bu_2SnO$, toluene, reflux ii) BnBr, TBAI, 100 °C	Bn	5:1	64%
9	BnBr, borinate, KI, K_2CO_3, CH_3CN, 60 °C	Bn	6:1	82%

[a] Product ratio determined by ^{1}H NMR spectroscopy. [b] Isolated yield. [c] Major product is recovered starting material. [d] Impurity present.

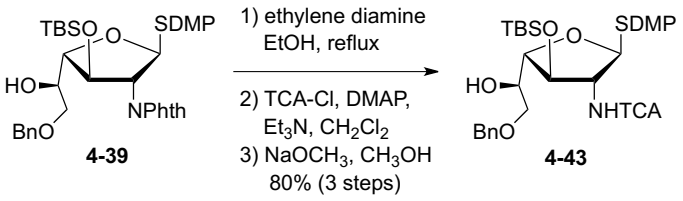
I then moved on to trying a regioselective alkylation of the diol in **4-40**. In a first attempt, benzyl 2,2,2-trichloroacetimidate in the presence of trimethylsilyl chloride was

explored (Entry 6). These conditions furnished a ~2:1 mixture of products in favor of the desired adduct **4-41**; however, a significant amount of the starting diol remained. Selective diol alkylation has been reported using silver and tin catalysts.[31,32] The use of silver carbonate to selectively benzylate the C-6 hydroxyl group was investigated first (Entry 7). These conditions showed reasonable selectivity but the reaction was sluggish. Prolonged reaction times led to the formation of other products (e.g., hydrolysis of the thioglycoside). The first promising result was achieved by treating **4-40** with dibutyltin oxide at reflux using a Dean–Stark apparatus and then addition of benzyl bromide in the presence of tetra-*n*-butylammonium iodide (Entry 8). These conditions resulted in an ~5:1 selectivity in favor of **4-41** and a 64% yield. Treating the diol **4-40** under the conditions described by Taylor and coworkers (benzyl bromide, potassium carbonate, potassium iodide, and 2-aminoethyl diphenylborinate stirred at 60 °C)[33,34] improved the selectivity to 6:1 and the yield to 82% (Entry 9). Having optimized these conditions on **4-40**, they were applied to diol **4-37**. I was pleased to find that complete regioselective benzylation of the C-6 hydroxyl group resulted, furnishing the Gal*f*NAc substrate **4-6** in 84% yield.

In addition to **4-6**, an analogue in which the 4-methylbenzene sulfide (STol) aglycone was replaced with 2,6-dimethylbenzene sulfide (SDMP) was synthesized under similar conditions (Scheme 4-4). The reason for its synthesis will be described in Section 4.3 of this chapter. The reactions, for the most part, proceeded in a manner comparable to the substrate possessing the STol aglycone. The only major difference was the conversion of glycoside **4-28** into thioglycoside **4-30**, which proceeded in lower yield. This is likely due to the steric congestion near the nucleophilic site in the 2,6-dimethylbenzene

thiophenol acceptor. The unreacted starting material, however, can be recovered and re-subjected to the reaction conditions.

As an alternative, the Gal*f*NAc analogue possessing an *N*-trichloroacetamide (e.g., **4-41**) instead of the *N*-phthalimide was also synthesized. We found that the removal of the phthalimide will affect an orthogonal protecting group strategy and the conversion to the *N*-trichloroacetamide group later resulted in modest yields (see Section 4.3). This conversion was achieved by first mixing the glycoside **4-39** with ethylenediamine at reflux to remove the phthalimido group. The product was treated with trichloroacetyl chloride in the presence of triethylamine and 4-dimethylaminopyridine and then with sodium methoxide to furnish **4-43** in 80% over the three steps.



Scheme 4-5: Synthesis of Gal*f*NAc-derived thioglycoside **4-43**.

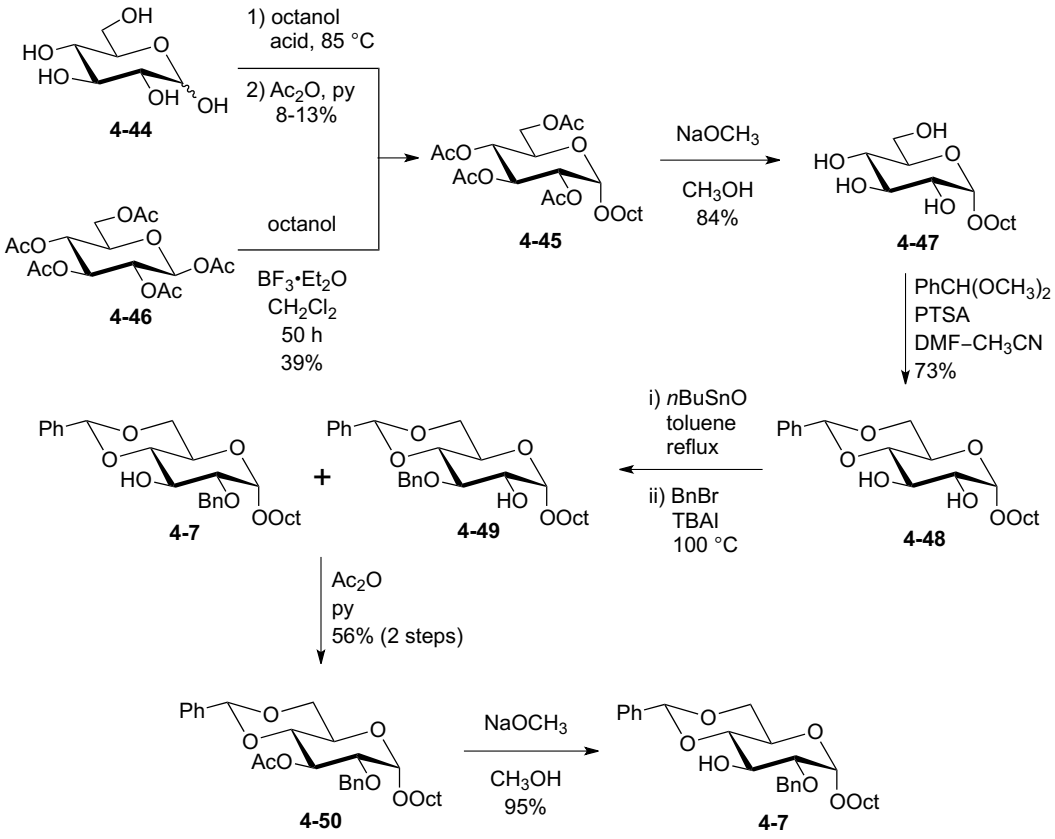
This section described the first synthesis of a glycosyl donor of GalNAc in its furanose ring configuration. The routes developed allowed the desired Gal*f*NAc analogues **4-6**, **4-39**, and **4-43** to be obtained on multi-gram scale.

4.2.3 Synthesis of glucose building block

As described earlier, the GlcA residue found in the *C. jejuni* 11168H CPS contains an α-1,2-*cis* linkage. To minimize the difficulties associated in selectively producing it on complex oligosaccharide substrates, we sought to establish the 1,2-*cis*

glycosidic bond early in the synthetic route. The most traditional method to achieve this, for simple alcohol aglycones, is Fischer glycosylation.

Under standard Fischer-type conditions (Scheme 4-6), D-glucose (**4-44**) was stirred in octanol in the presence of a strong acid (either Amberlyst 15 ion exchange resin or boron trifluoride etherate) and heated to 85 °C. Subsequent acylation with acetic anhydride in pyridine was performed to facilitate the separation of the anomers. These conditions, unfortunately, produced the desired α-anomer **4-45** in poor yields (8–13%). As an alternative, there have been several reports of converting β-glycosides to the α-glycoside by treatment with a Lewis acid over extended periods of time.[35,36] Taking this idea, peracetylated β-D-glucose (**4-46**) was mixed with octanol in the presence of boron trifluoride etherate. As expected, the β-glycoside was predominantly formed. However, as the reaction mixture was stirred (24–48 hours), the α-glycoside became the dominant product. Purification afforded the desired product **4-45** in 39% yield. It was found stirring the reaction longer than 50 hours did not improve the α-selectivity.

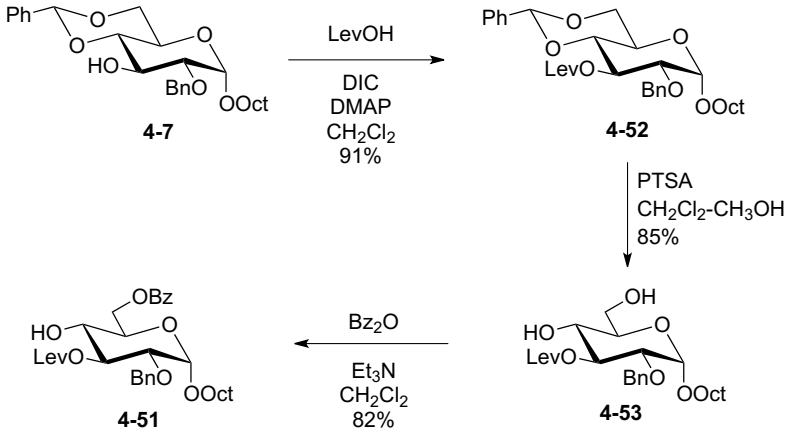


Scheme 4-6: Synthesis of glucose acceptor **4-7**.

With **4-45** in hand, the acetate groups were removed by treatment with sodium methoxide to afford **4-47** in 84% yield. The C-4 and C-6 hydroxyl groups of **4-45** were then protected with a benzylidene acetal by mixing with benzaldehyde dimethyl acetal in the presence of *para*-toluenesulfonic acid to furnish **4-48** in 73% yield. Glycoside **4-48** was first reacted with dibutyltin oxide at reflux with Dean–Stark apparatus, then benzyl bromide and tetra-*n*-butylammonium iodide were added and the mixture was stirred at 100 °C to afford an inseparable ~5:2 ratio of regioisomers **4-7** and **4-49**, albeit in favor of the desired product. The mixture of regioisomers **4-7** and **4-49** was then treated with acetic anhydride in pyridine to facilitate purification and afford **4-50** in 56% yield over two steps. Other methods to selectively benzylate the C-2 hydroxyl group of **4-48** (e.g.,

Nickel catalyzed benzylation,[37] phase transfer alkylation[38,39]) were also explored; however, these approaches were inferior either with regard to regioselectivity and/or yield. Finally, the acetate group of **4-50** was then removed by treatment with sodium methoxide to afford **4-7** in 95% yield.

In addition to glycoside **4-7**, the glucose acceptor **4-51** was synthesized due to synthetic challenges that will be further discussed in Section 4.5. The C-3 hydroxyl group of **4-7** was protected as a levulinate ester by mixing levulinic acid and diisopropyl carbodiimide in the presence of 4-dimethylaminopyridine to afford **4-52** in 91% yield. The benzylidene acetal of **4-52** was removed by *para*-toluenesulfonic acid catalyzed methanolysis to give diol **4-53** in 85% yield. The C-6 hydroxyl group of **4-53** was then selectively protected with a benzoate group by treatment with benzoic anhydride in the presence of triethylamine to afford the glucose acceptor **4-51** in 82% yield.



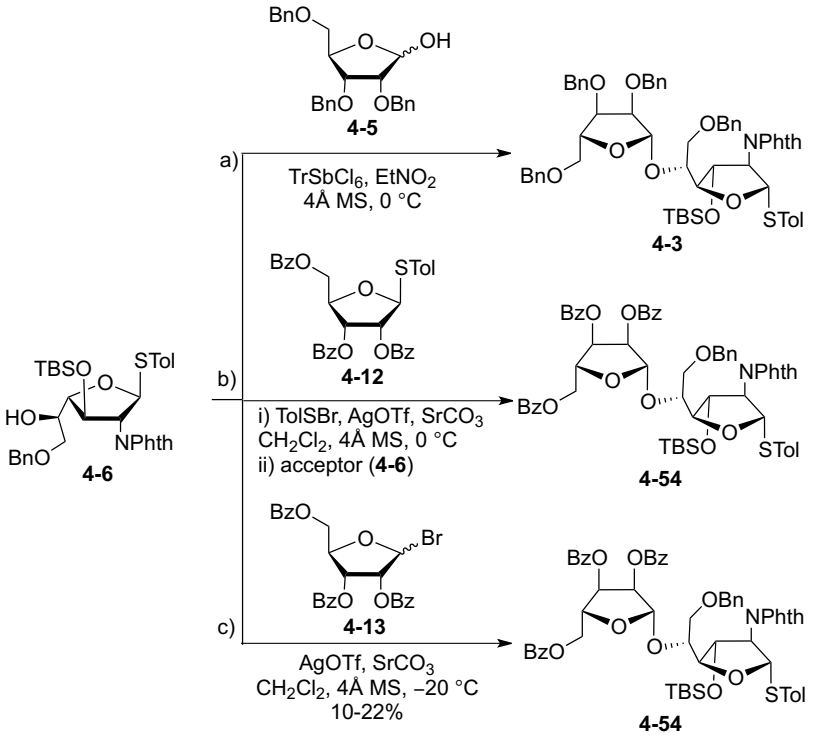
Scheme 4-7: Synthesis of glucose acceptor **4-51**.

In summary, two glucose substrates, **4-7** and **4-51**, were successfully synthesized from D-glucose in good yields in six and nine steps, respectively. These substrates will be used as acceptor building blocks in the glycosylation reactions described below.

4.3 Synthesis of Rib*f*-Gal*f*NAc disaccharide donor 4-3

With the all the necessary building blocks constructed, the next step was to synthesize the disaccharide donor **4-3**. Mukaiyama and coworkers previously reported a β-selective dehydrative glycosylation of the ribofuranose derivative **4-5** using trityl salts.[40] Given these results, our initial attempt at synthesizing disaccharide donor **4-3** involved treating **4-5** with the Gal*f*NAc analogue **4-6** in the presence of triphenylcarbenium hexachloroantimonate at 0 °C. Unfortunately, using these conditions did not lead to the production of the disaccharide even when increasing the catalyst loading from 10 mol% to 100 mol%. These results led us to synthesize the Rib*f* donors **4-12** and **4-13**.

With regard to the use of donor **4-12**, the idea was to pre-activate this species and then add the acceptor **4-6** to the reaction mixture. Several pre-activation glycosylation strategies have been developed for the synthesis of complex glycans.[41,42] Using this pre-activation strategy, the ribofuranoside donor **4-12** was treated with *para*-toluenesulfenyl bromide (made by mixing *para*-tolyl disulfide and bromine in dichloroethane) in the presence of silver trifluoromethanesulfonate and strontium carbonate at 0 °C for 15 minutes[43] before **4-6** was added. These conditions, unfortunately, produced a complex mixture of products.



Scheme 4-8: Ribofuranose glycosylation of Gal*f*NAc analogue **4-6**.

Finally, ribofuranosyl bromide **4-13** was treated with the Gal*f*NAc analogue **4-6** in the presence of silver trifluoromethanesulfonate and strontium carbonate at –20 °C. The acceptor **4-6** was consumed by monitoring the reaction mixture via thin layer chromatography (TLC); however, three major products were observed. In addition to the desired disaccharide **4-54**, the products **4-55** and **4-12** were observed (Figure 4-6). The loss of the *tert*-butyldimethylsilyl group in **4-55** was presumably due to the acidity of the glycosylation conditions despite the presence of strontium carbonate. Addition of excess strontium carbonate could potentially prevent this from occurring.

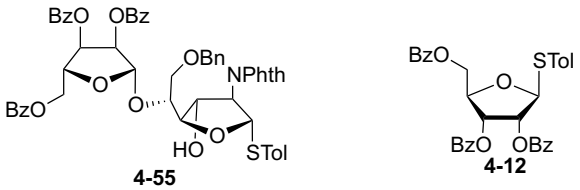


Figure 4-6: Byproducts observed from the glycosylation of Gal*f*NAc analogue **4-6** with ribofuranosyl bromide **4-13**.

Byproduct **4-12** forms by a process known as aglycone transfer.[44-49] Figure 4-7 depicts the process. In this example, the donor **4-13**, upon addition of a promoter, can generate the oxacarbenium ion **4-56**. From here, there are two pathways that are possible. Pathway A, is nucleophilic attack of the alcohol (**4-6**) onto **4-56** leading to the desired disaccharide (**4-54**). If the alcohol is less nucleophilic, perhaps by the presence of electron withdrawing substituents (e.g., benzoate groups, acetate groups) or due to steric congestion, the oxacarbenium ion **4-56** can react instead with the sulfide group of the thioglycoside, leading to a sulfonium ion (**4-57**, pathway B). The sulfonium ion can break down to reform the oxacarbenium ion acceptor. Alternatively, it can break down by transfer of the aglycone to produce a new thioglycoside (**4-12**) along with a new oxacarbenium ion (**4-58**). The driving force of the aglycone transfer is the production of a more stable oxacarbenium ion (**4-56** vs **4-58**). Li and Gildersleeve have carried out mechanistic studies of this process.[50] In their investigations they found that use of 2,6-dimethylthiophenol as the aglycone of the acceptor can prevent aglycone transfer.[50] It was for this reason the Gal*f*NAc analogue **4-39** was synthesized.

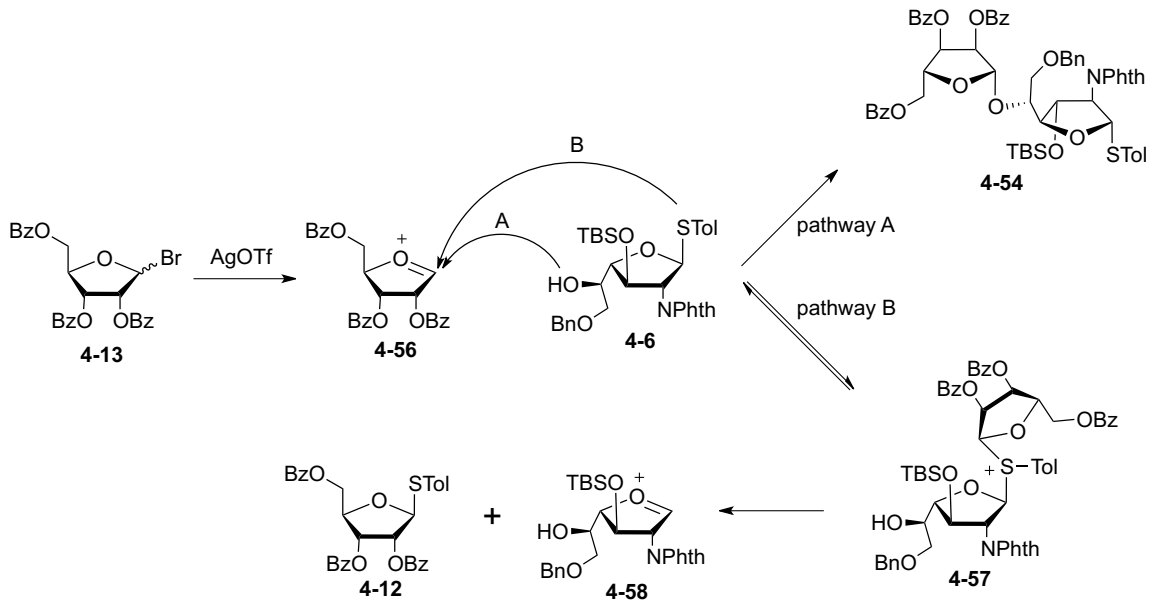
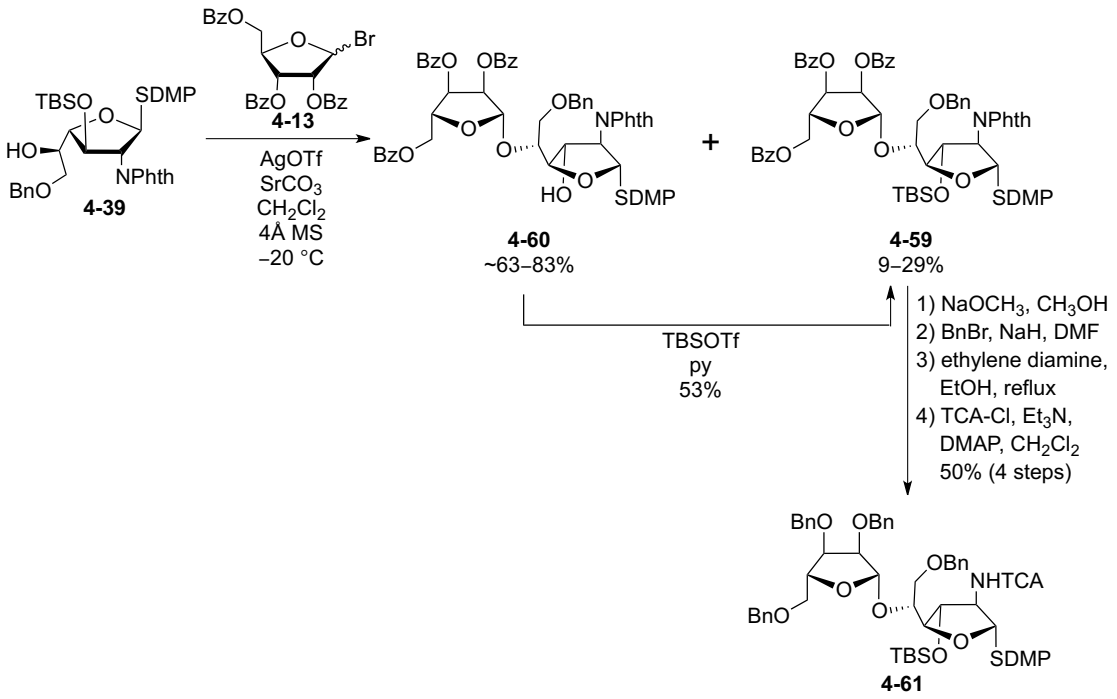


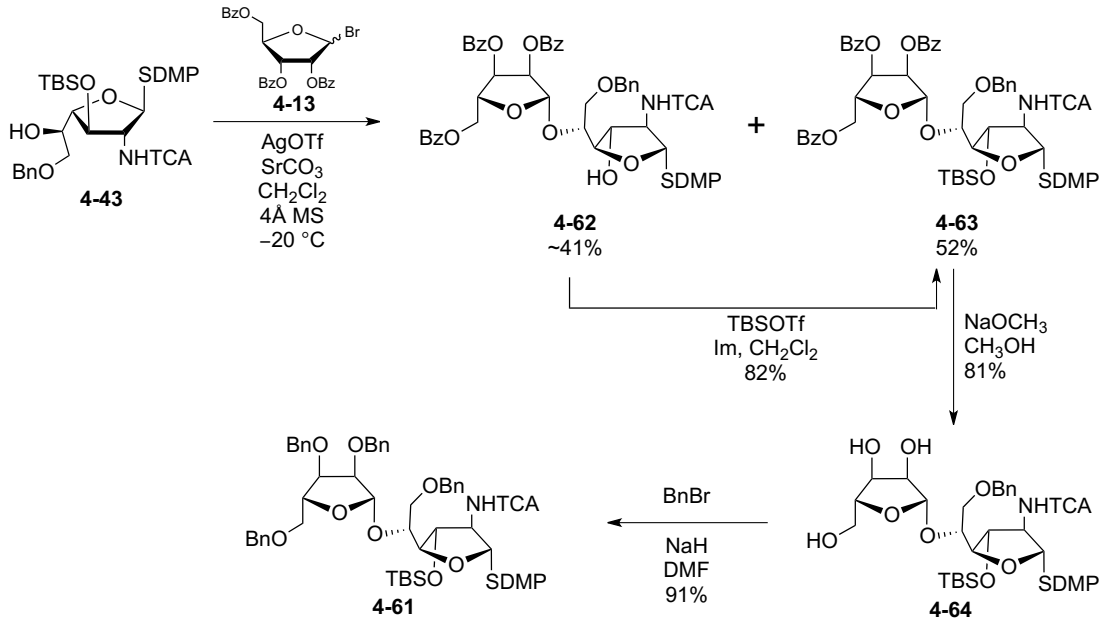
Figure 4-7: Schematic representation of aglycone transfer.

With thioglycoside alcohol **4-39** in hand, glycosylation with ribosyl bromide **4-13** was carried out (Scheme 4-9) to give a mixture of the desired product **4-59** and the desilylated derivative **4-60** in excellent combined yield (>90%). Importantly, no aglycone transfer was observed for the glycosylation. Although the formation of **4-60** is undesired, the C-3 hydroxyl group can be re-protected by treatment with *tert*-butyldimethylsilyl trifluoromethanesulfonate to produce **4-59**, albeit in a modest yield (53%). Having a developed a route to disaccharide **4-59** it was then converted to the **4-61** in 50% over four steps by sequential treatment with sodium methoxide, benzyl bromide in the presence of sodium hydride, ethylenediamine at reflux, and trichloroacetyl chloride in the presence of 4-dimethylaminopyridine and triethylamine. The exchange of the phthalimide to the trichloroacetamido group was performed due to the lability of the Gal*f*NAc glycosidic bond (further described in Section 4.7).



Scheme 4-9: Synthesis of disaccharide donor **4-61**.

In an alternative approach, the use of trichloroacetamide containing analogue **4-43** for the synthesis of the disaccharide was explored (Scheme 4-10). Similar to above, thioglycoside **4-43** was glycosylated with ribofuranosyl bromide **4-13** to give a mixture of **4-62** and **4-63** in excellent yields (>85% based on combined yields). Unfortunately, the addition of excess strontium carbonate did not prevent the loss of the *tert*-butyldimethyl silyl group during the glycosylation. Protection of **4-62** was achieved by treatment with *tert*-butyldimethylsilyl trifluoromethanesulfonate in the presence of imidazole[51] afforded **4-63** in a yield of 82%. The benzoate groups of **4-63** were removed by mixing with sodium methoxide to give **4-64** in 81% yield. The disaccharide donor **4-61** was then obtained in 91% yield by treating **4-64** with benzyl bromide in the presence of sodium hydride.



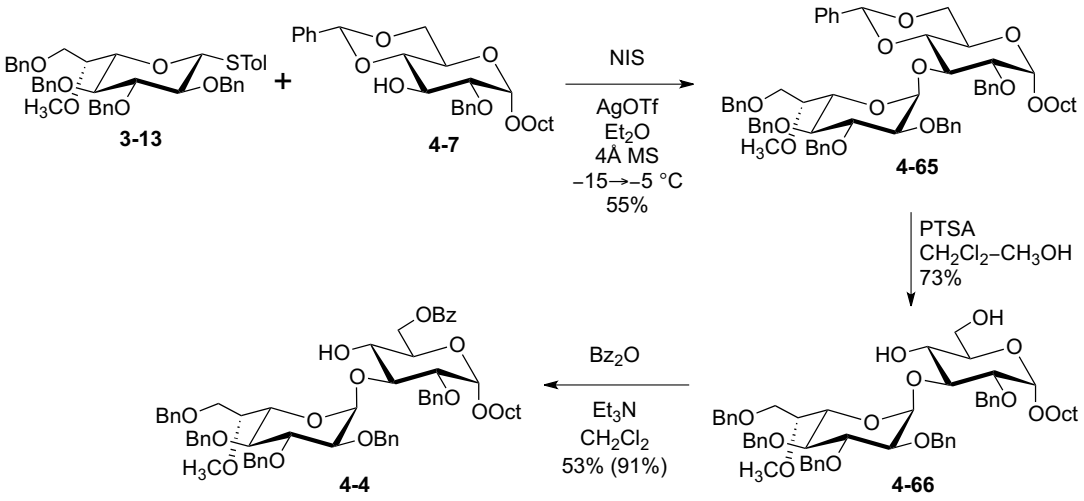
Scheme 4-10: Alternative synthesis of disaccharide donor **4-61**. * Im = imidazole.

4.4 Synthesis of the Hep*p*-Glc*p* disaccharide acceptor 4-4

With the disaccharide donor completed, we next focused on obtaining the Hep*p*-Glc*p* disaccharide acceptor **4-4**. The first step in its synthesis requires an α-1,2-*cis* selective glycosylation. Although multiple elegant strategies have been made to promote the α-1,2-*cis* glycosylation,[52-60] we initially investigated a simple approach: running the reaction in diethyl ether. Ethereal solvents are known to promote α-selective glycosylations presumably through solvent participation.[61,62]

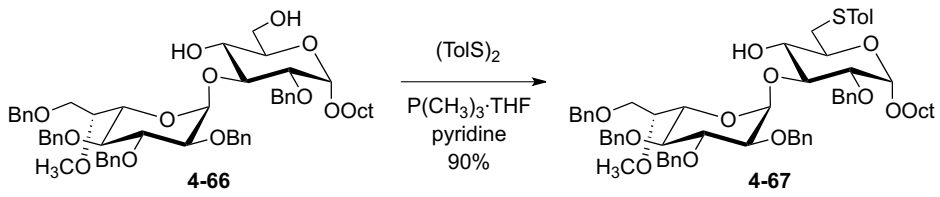
With this in mind, the first step towards the synthesis of the disaccharide **4-4** involved the glycosylation of **4-7** with the Hep*p* donor **3-13** (its synthesis was reported in Chapter 3) in the presence of *N*-iodosuccinimide and silver trifluoromethanesulfonate in diethyl ether (Scheme 4-11). These conditions furnished **4-65** in 55% yield with ~3:1 α:β

selectivity. The benzylidene acetal of **4-65** was removed by a *para*-toluenesulfonic acid-catalyzed methanolysis to give **4-66** in 73% yield. The C-6 hydroxyl group of **4-66** was then selectively protected with a benzoate group using benzoic anhydride in the presence of triethylamine to give the disaccharide acceptor **4-4** in 53% yield (91% based on recovered starting material).



Scheme 4-11: Synthesis of disaccharide acceptor **4-4**.

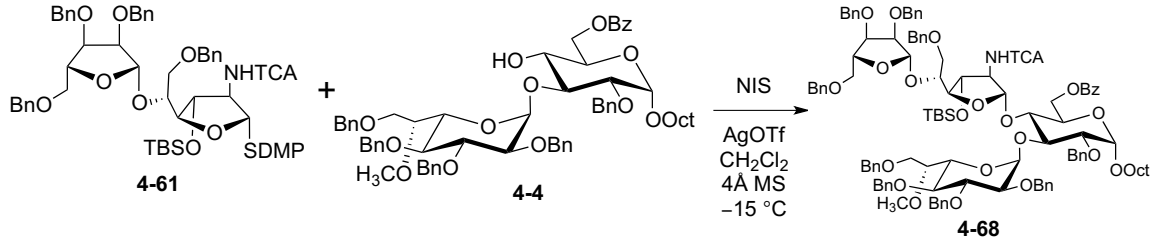
As an alternative substrate, disaccharide **4-67**, which possesses a C-6 thioether was also synthesized (Scheme 4-12). This compound could potentially improve glycosylation yields and shorten the synthesis of the tetrasaccharide through a novel oxidation–amidation method described in Section 4.8. The selective thioetherification was achieved by treating diol **4-66** with *para*-methylbenzene disulfide and trimethylphosphine to give **4-67** in 90% yield.



Scheme 4-12: Synthesis of disaccharide acceptor **4-67**.

4.5 Synthesis of tetrasaccharide via a 2 + 2 glycosylation

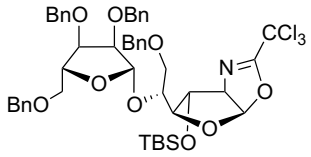
With the disaccharide donor and acceptor in hand, the 2 + 2 glycosylation was explored (Scheme 4-13). The disaccharide donor **4-61** was treated with the acceptor **4-4** in the presence of *N*-iodosuccinimide and silver trifluoromethanesulfonate. A new product was observed via thin layer chromatography (TLC) analysis; however, isolation and characterization did not support its structure as the tetrasaccharide **4-68**. Instead, ^{1}H NMR spectroscopy and mass spectrometric analysis indicated it was oxazoline **4-69** (Figure 4-8).



Scheme 4-13: Synthesis of tetrasaccharide **4-68**.

Oxazolines are a common byproduct in glycosylation reactions when the glycosyl donor contains an acetamido group at its C-2 position;[63-65] however, isolation of this oxazoline was unexpected. The use of C-2 acetamides with electron-withdrawing

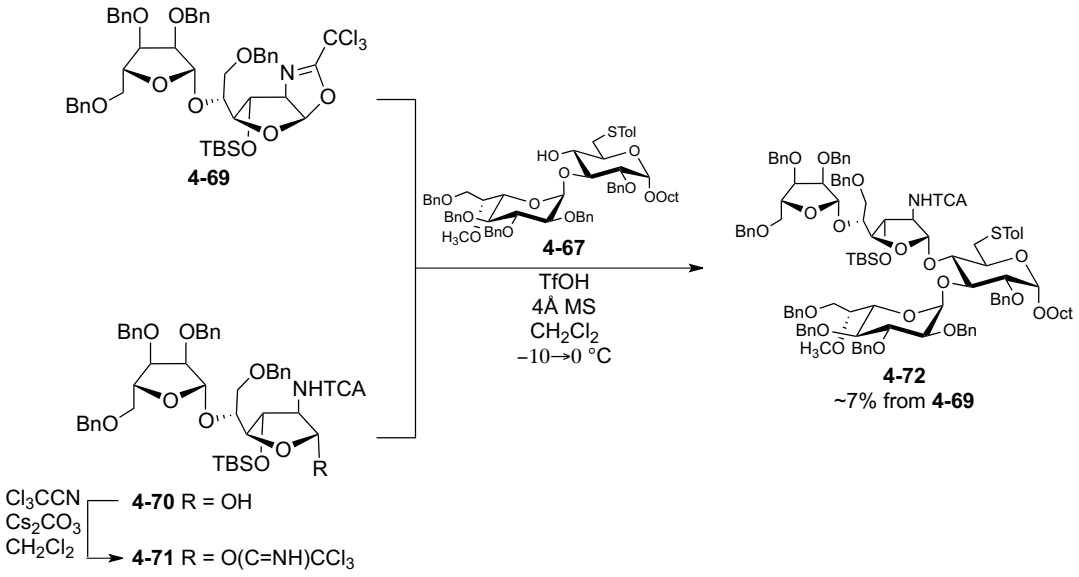
substituents (e.g., trifluoroacetamides or trichloroacetamides), in general, should prevent the formation of the oxazoline. Its formation usually occurs when either no nucleophile is present, or the nucleophile has low reactivity. In this case, the poor nucleophilicity of the C-4 hydroxyl group in **4-4** may arise from the presence of the benzoate ester at C-6 and steric congestion from the heptose residue. On a positive note, oxazolines have been previously used as glycosyl donors; therefore, its formation could be used to our advantage.



4-69

Figure 4-9: Byproduct isolated in the 2 + 2 glycosylation of **4-61** and **4-4**.

To allow of more detailed investigation of this glycosyation, in addition to the oxazoline **4-69**, trichloroacetimidate **4-71** was used as a donor in the glycosylation of **4-67** in the presence of trifluoromethanesulfonic acid (Scheme 4-14). Compound **4-71** was made by treating the hydrolyzed donor **4-70** with trichloroacetonitrile in the presence of cesium carbonate. Using the oxazoline donor **4-69**, tetrasaccharide **4-72**, was obtained but only in ~7% yield. The imidate donor **4-71** showed similar results to the oxazoline donor **4-69**. The major product of both reactions was a dimer of the donor, tetrasaccharide **4-73** (Figure 4-9).



Scheme 4-14: Synthesis of tetrasaccharide **4-72**.

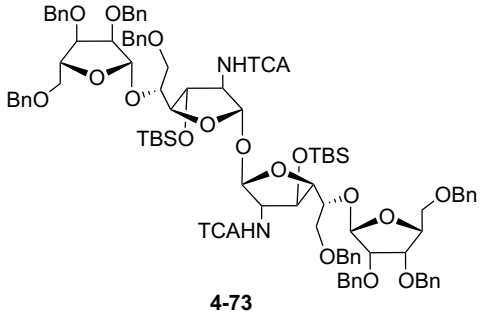


Figure 4-9: Byproduct isolated in the 2 + 2 glycosylation of donors **4-69** and **4-71** with acceptor **4-67**.

Due to the difficulty in synthesizing the tetrasaccharide via the 2 + 2 glycosylation, an alternative strategy was sought. In some cases, the order of addition of the sugar residues can be used to overcome difficult glycosylations. Therefore, we investigated the possibility of a 3 + 1 coupling.

4.6 Synthesis of tetrasaccharide via 3 + 1 glycosylation

Given the synthesis of the tetrasaccharide could not be achieved successfully via a 2 + 2 glycosylation, we envisioned a 3 + 1 glycosylation of the donor **3-13** with acceptor **4-74** could be used to obtain the core tetrasaccharide **4-68** (Figure 4-10).

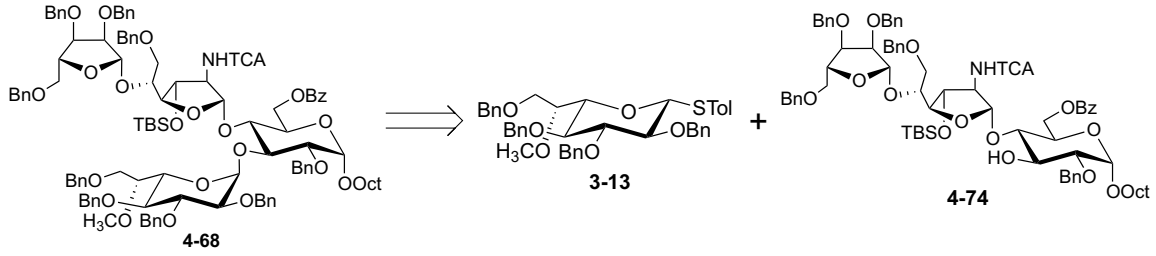
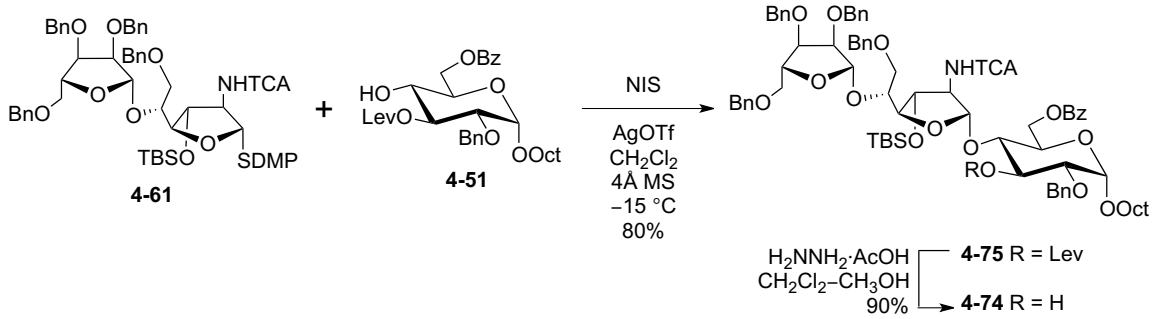


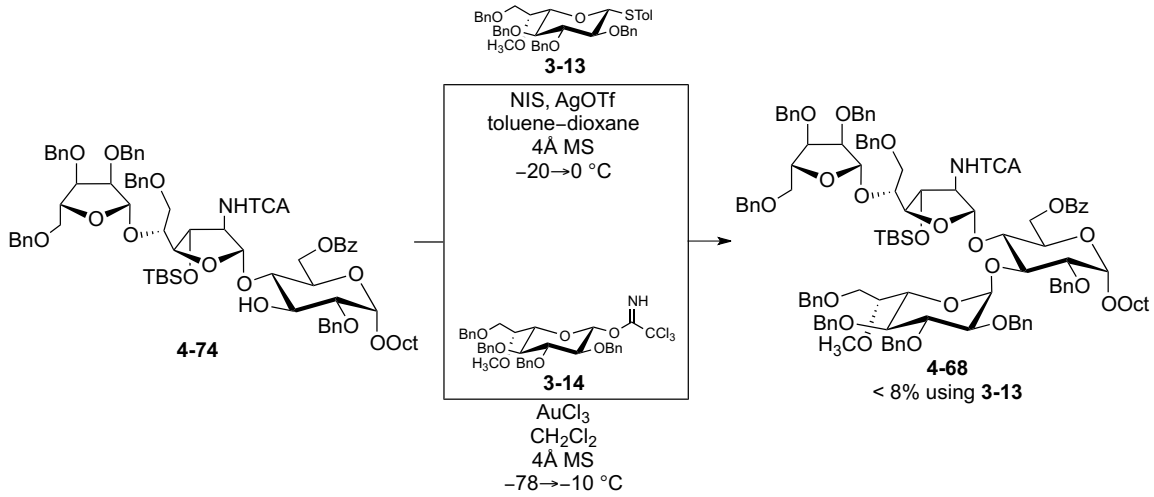
Figure 4-10: Retrosynthetic analysis of tetrasaccharide **4-68** via a 3+1 approach.

With this in mind, trisaccharide **4-75** was synthesized in 80% yield by glycosidation of the **4-61** with **4-51** in the presence of *N*-iodosuccinimide and silver trifluoromethanesulfonate (Scheme 4-15). The levulinate group of **4-75** was then selectively removed by using hydrazine acetate to give the trisaccharide **4-74** in 90% yield.



Scheme 4-15: Synthesis of trisaccharide acceptor **4-74**.

After accessing acceptor **4-74** in hand, the glycosidation of the **3-13** and **3-14** was examined (Scheme 4-16). First, the thioglycoside **3-13** was treated with trisaccharide **4-79** in the presence of *N*-iodosuccinimide and silver trifluoromethanesulfonate in a 3:2 toluene–1,4-dioxane solution. The switch to this solvent mixture was chosen to accelerate the reaction; moreover 1,4-dioxane has been shown to be more effective in promoting the α-glycosylations.[61] Unfortunately, the reaction still proceeded poorly in furnishing the tetrasaccharide **4-68** in less than 8% yield.



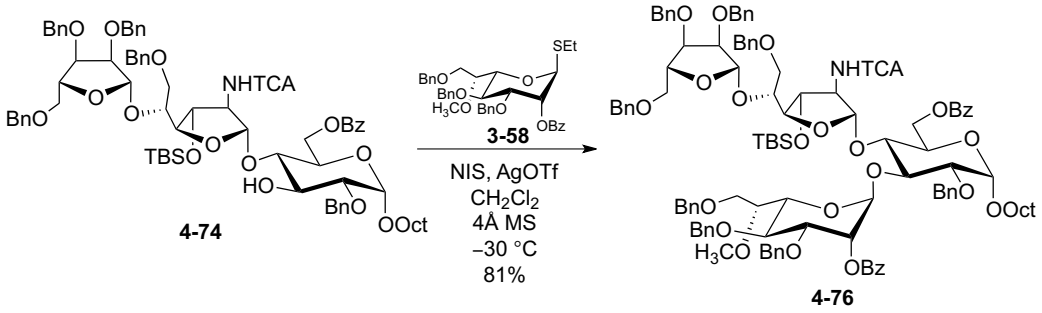
Scheme 4-16: Synthesis of tetrasaccharide **4-68** via 3 + 1 glycosylation.

In searching for a solution, I found a report by Peng and Schmidt describing a highly α-selective glycosylation using β-trichloroacetimidate donors in the presence of gold trichloride.[66] Using this idea and under the conditions described in the paper, alcohol **4-74** was first mixed with gold trichloride and then **3-14** was added at –78 °C. Unfortunately, the product was not observed under these reaction conditions.

Given the difficulty in the ethereal solvent-mediated and gold-catalyzed glycosylation approaches described above, another strategy was explored. One approach

to overcome the difficult α-1,2-*cis* glycosylation is to take advantage of the neighboring group participation strategy with a diastereomeric donor. This strategy requires a donor with an acyl substituent at C-2 in the opposite stereochemistry (e.g., **3-58** shown in Scheme 4-17). The presence of this acyl group will promote the α-1,2-*trans* glycosylation. Subsequent steps as after can invert the C-2 stereochemistry producing the α-1,2-*cis* product.[67]

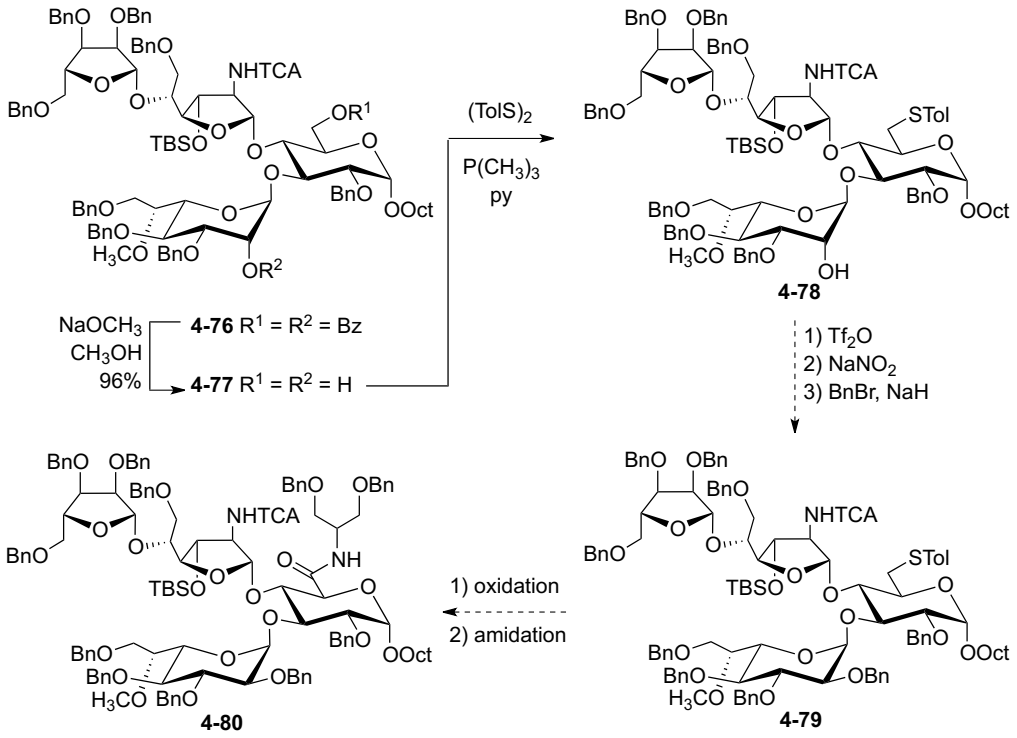
With this in mind, trisaccharide **4-74** was glycosylated with the **3-58** which possesses a C-2 acyl group with the opposite stereochemistry, in the presence of *N*-iodosuccinimide and silver trifluoromethanesulfonate (Scheme 4-17). The synthesis of **3-58** was described in Chapter 3; conveniently, it is an intermediate in the route starting with furfural. Using **3-58**, tetrasaccharide **4-76** was obtained in 81% yield from trisaccharide **4-74**. These conditions furnished the α-linkage, supported by a $J_{1,2}$ coupling constant 1.8 Hz. No β-glycoside product was observed.



Scheme 4-17: Synthesis of tetrasaccharide **4-76** via 3 + 1 glycosylation.

Having a route to tetrasaccharide **4-76** in place, the next step was to invert the C-2 stereochemistry of the heptose residue (Scheme 4-18). Thus, tetrasaccharide **4-76** was first treated with sodium methoxide to remove the benzoate groups to give **4-77** in 96%

yield. An attempt to selectively convert the primary alcohol of **4-78** to its corresponding thioether using 4-methylbenzene disulfide and trimethylphosphine however, failed. Two unknown products were formed in the reaction mixture.



Scheme 4-18: Efforts for the synthesis of tetrasaccharide **4-80**.

Given the difficulties facing the synthesis of the tetrasaccharide unit, we moved to synthesize smaller fragments (described in Chapter 5 of this thesis) of the tetrasaccharide unit for the purpose of MeOPN biosynthetic studies. Before shifting our focus, we did however develop a novel oxidation–amidation method that was to be applied in the synthesis of the tetrasaccharide.

4.7 A novel oxidation–amidation strategy for the preparation of glycuronamides

Because the desired tetrasaccharide possesses a glycuronamide, an oxidation–amidation strategy was needed. The conventional approach that is employed in glycuronamide synthesis is shown in Figure 4-11. The strategy first involves oxidation of the alcohol (**4-81**) into the corresponding carboxylic acid (**4-82**). The acid is then converted to an activated intermediate (**4-83**) and then addition of an amine nucleophile can produce the glycuronamide (**4-84**).[6,68-72] Alternative amidation approaches have been described;[7,8,73,74] however, these have not been employed on complex glycans and in some cases are limited to carbohydrate lactones.

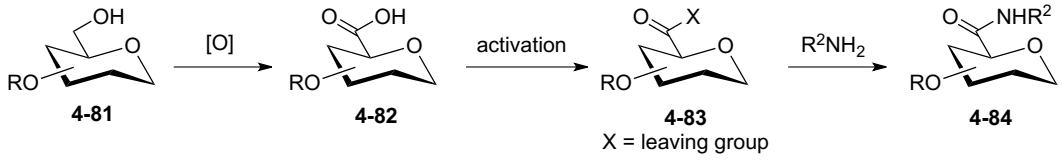
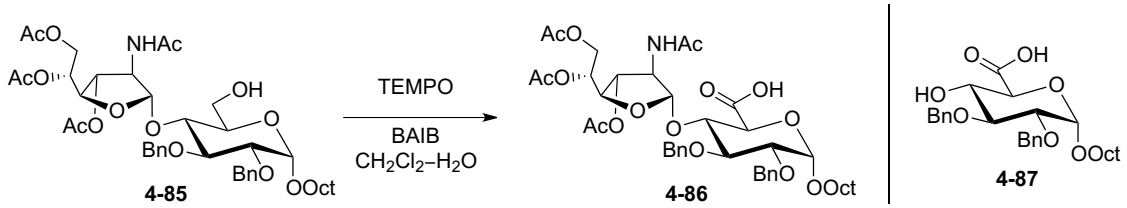


Figure 4-11: Traditional oxidation–amidation synthesis of complex glycans containing uronamide residues. Activation is generally achieved by use of a peptide-coupling reagent such as dicyclohexylcarbodiimide.

The conventional strategy was explored using disaccharide **4-85** as a model substrate. Thus, **4-85** was treated with 2,2,6,6-tetramethyl-1-piperidinyloxy and bis(acetoxy)iodobenzene (Scheme 4-19). These oxidative conditions, however, did not afford the desired disaccharide **4-86**. Instead, the major product was the monosaccharide **4-87**, which was isolated in 63% yield. These results were a surprise, as most glycosidic bonds remain intact under mildly acidic conditions (e.g., in the presence of catalytic

trifluoroacetic acid or 80% acetic acid in water at 80 °C) and these conditions were anticipated to be more mild.



Scheme 4-19: Oxidation attempt of disaccharide **4-85**. Gal*f*NAc glycoside bond is labile.

We rationalized that this hydrolysis occurred because of the Gal*f*NAc residue present in **4-86**. In the presence of an acid, such as acetic acid generated in the oxidation of **4-85** (Scheme 4-19), the acetamido group of the Gal*f*NAc residue is in a favorable position to partake in the neighboring group participation and leading to the hydrolysis of the Gal*f*NAc glycoside bond (Figure 4-12). Alternatively, the acid of the GlcA residue may be involved in an intramolecular hydrogen bond that could facilitate the hydrolysis of the Gal*f*NAc. A similar type of intramolecular hydrogen bonding was found in sialosides and promoted hydrolysis of the residue.[75]

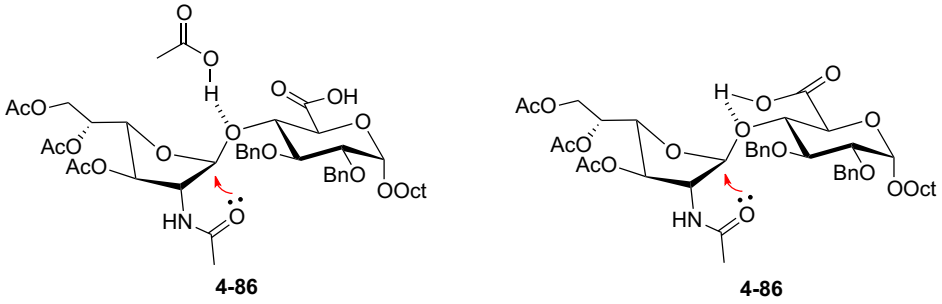
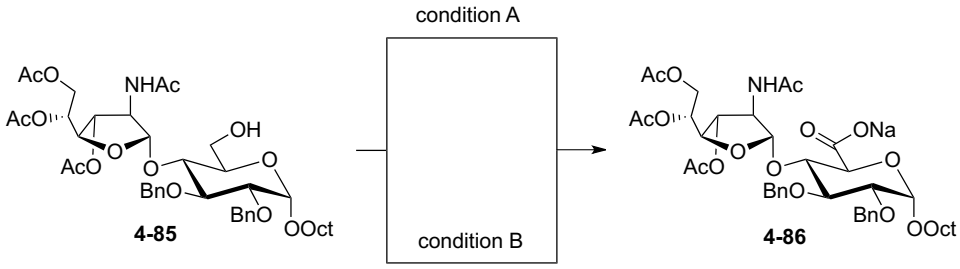


Figure 4-12: Proposed rationale for the hydrolysis of the disaccharide **4-86**.

To overcome the hydrolysis issues, the oxidation of **4-85** was performed under basic conditions (Scheme 4-20). Unfortunately, buffering the solution under condition A

(2,2,6,6-tetramethyl-1-piperidinyloxy and bis(acetoxy)iodobenzene) with triethylamine or sodium bicarbonate led to poor conversion. Oxidation under condition B (sodium hypochlorite and 2,2,6,6-tetramethyl-1-piperidinyloxy in a sodium bicarbonate solution) showed better conversion (by consumption of starting material as indicated by TLC analysis), however the crude product was difficult to assess by TLC analysis and ^{1}H NMR spectroscopy. To overcome this inconvenience, a one-step oxidation–amidation approach, where there was no carboxylic acid intermediate formed, was desired.



Scheme 4-20: Alternative oxidation attempts of disaccharide **4-85**. Condition A: TEMPO, BAIB, Base (Et_3N or $NaHCO_3$), CH_2Cl_2–H_2O. Condition B: NaOCl, TEMPO, $NaHCO_3$(aq).

Milstein and coworkers achieved the first direct oxidation–amidation of primary alcohols in 2007 using a ruthenium based catalyst.[76] Since then, several other one-pot oxidation–amidation syntheses have been achieved.[77-82] The common theme in these methods, which could suit our needs, is summarized in Figure 4-13.[83-85] This approach involves the oxidation of the primary alcohol **4-88** to the labile ester **4-89** using iodine-based reagents (e.g., 2-acetoxybenzoic acid, iodine) in the presence of *N*-hydroxysuccinimide; subsequent addition of the amine produces the amide **4-90**.

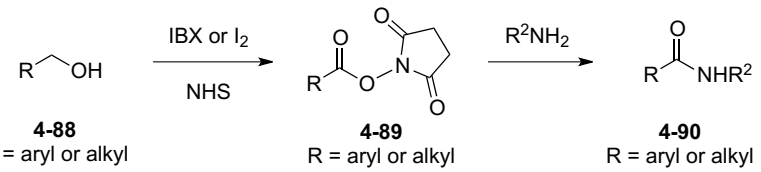
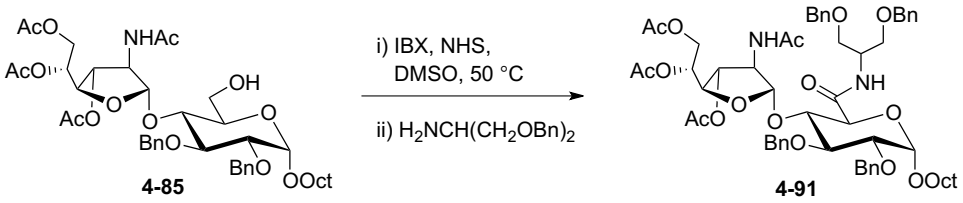


Figure 4-13: Reported one-pot oxidation-amidation approaches to be explored in glycuronamide synthesis.

Given these strategies, disaccharide **4-85** was treated with 2-iodoxybenzoic acid in the presence of *N*-hydroxysuccinimide for 4–16 hours and then 1,3-di-*O*-benzyl-serinol was added (Scheme 4-21). These conditions produced a mixture of products that were difficult to assess; however, mass spectrometry showed the presence of a product consistent with **4-91**. Optimization was carried out using a monosaccharide analogue; however, these results were unacceptable in terms of yield and ease of product purification.



Scheme 4-21: IBX mediated oxidation–amidation of disaccharide **4-85**.

We then turned our attention to a report by Yu and coworkers that described the preparation of glycuronates from 6-*S*-phenyl-glycosides (Figure 4-14a).[86,87] In this approach, the first step involves treatment of the sulfide **4-92** with sulfuryl chloride to generate the α,α-dichlorosulfide **4-93**, which can then be converted to the methyl glycuronate **4-94** in the presence of mercuric chloride and methanol. In other work,

Fortes and coworkers showed that keto-α,α-dichlorosulfides (e.g., **4-96**), when treated with water in the presence of sodium carbonate, can give α-keto-thioesters (e.g., **4-97**).[88]

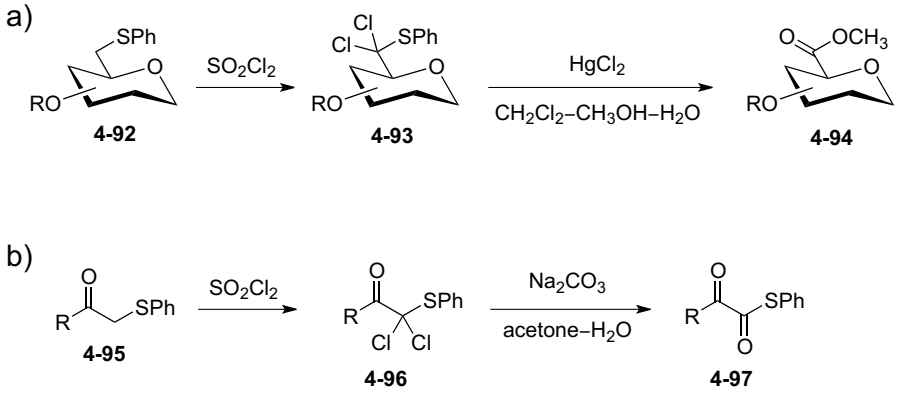


Figure 4-14: Reported syntheses of methyl glycuronates and α-keto-thioesters from thiosulfides. a) Synthesis of methyl glycuronate by Yu and coworkers.[86,87] b) Synthesis of α-keto-thioesters by Fortes and coworkers.[88]

These investigations prompted us to combine these approaches and we explored the possibility of preparing thiouronates (e.g., **4-98**) from a 6-*S*-thioether (e.g., **4-92**) and, in turn, glycuronamides (e.g., **4-99**) by addition of an amine (Figure 4-15).

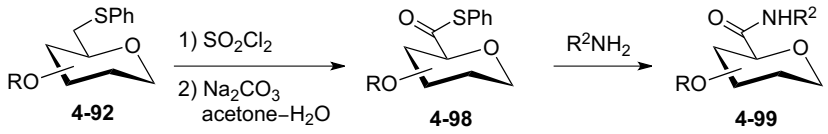
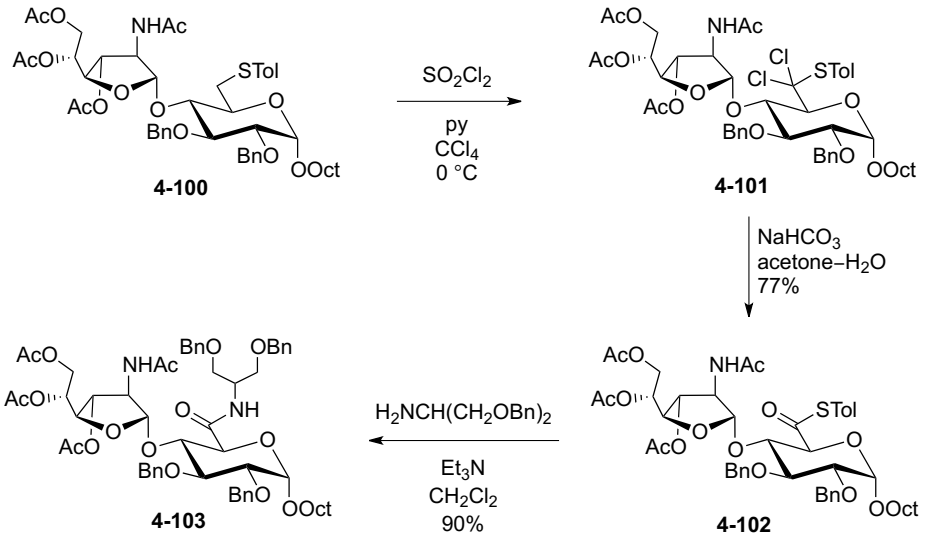


Figure 4-15: Proposed synthesis of glycuronamides from 6-*S*-thioethers.

To explore this possibility, the 6-*S*-*p*-tolyl-glycoside **4-100** (its preparation is described in Section 5.3 of this thesis) was treated with sulfuryl chloride in the presence of pyridine to give **4-101** (Scheme 4-22). Without purification, the α,α-dichlorosulfide **4-101** was then treated with water in the presence of sodium bicarbonate to give the thioester **4-102** in 77% yield over two steps. The formation of the thioester was

confirmed by a signal in the ^{13}C NMR spectrum at 196.2 ppm and a strong band at 1702 cm^{-1} in the IR spectrum. Both of these features are indicative of thioester carbonyls.[89] The amidation of **4-102** to give glycuronamide **4-103** proceeded in 90% yield, upon mixing with 1,3-di-*O*-benzyl-serinol in the presence of triethylamine. This three-step strategy represents a novel approach for the synthesis of glycuronamides and, in this case, could be applied to overcome the difficulties associated with the hydrolysis of the Gal*f*NAc residue upon a conventional oxidation protocols. Although this approach was suitable for several substrates (further described in Chapter 5), formation of the thioether at the tetrasaccharide was problematic, presumably due to the presence of the trichloroacetamido group on the Gal*f*NAc residue as shown in Scheme 4-18.



Scheme 4-22: Synthesis of glycuronamide **4-103** via a three-step protocol.

In summary, although the synthesis of the NCTC11168 CPS tetrasaccharide repeating unit was not complete, this chapter described work that overcame several problems in its synthesis. First, an effective route to obtain the Gal*f*NAc donor was developed. Second, it appears the best approach to obtain the tetrasaccharide core, albeit

with the C-2 position of the heptose containing the wrong stereocenter, is a 3 + 1 glycosylation. A new strategy in the oxidation–amidation approach was developed to overcome the problems associated with the hydrolysis of the Gal*f*NAc residue by avoiding the common carboxylic intermediate.

4.8 Future Work

With many of the problems encountered and solved, it is reasonable the synthesis of the tetrasaccharide could be completed in the near future, however by another member in the group due to time constraints of my PhD studies. Several building blocks (e.g., glycosyl acceptors and donors) for the construction of the tetrasaccharide are still in hand. Figure 4-16 shows the synthetic route I would implement for the synthesis of tetrasaccharide **4-106**. Briefly, the glycosidation of **4-69** with **4-104** could produce the trisaccharide **4-105** after selective removal of the levulinate group. The tetrasaccharide **4-72** could be obtained by glycosidation of **3-58** with **4-105** and inverting the stereochemistry at C-2 of the heptose residue. With the 6-*S*-thioether already present in **4-72**, the novel oxidation–amidation approach can be used for the synthesis of the tetrasaccharide **4-106**.

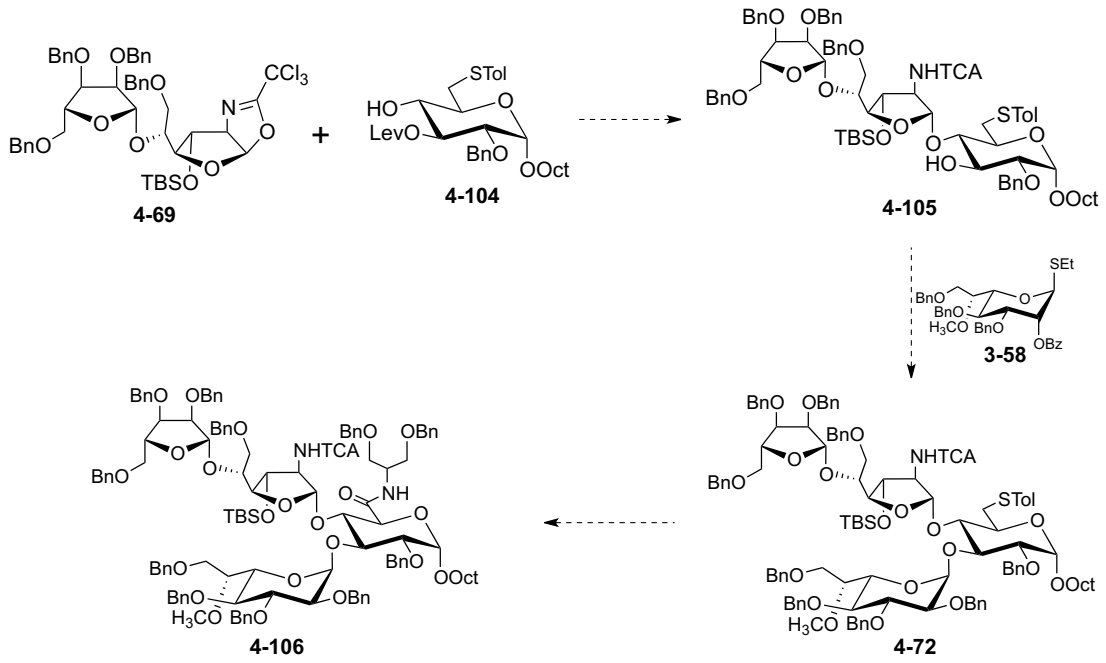


Figure 4-16: Synthetic route to employ for the construction of tetrasaccharide **4-106**.

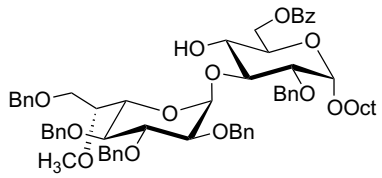
4.9 Experimental

4.9.1 General experimental methods

All reagents were purchased from commercial sources and were used without further purification unless noted. Solvents used in reactions were purified by successive passage through columns of alumina and copper under argon. Unless stated otherwise, all reactions were carried out at room temperature and monitored by TLC on Silica Gel G-25 F_{254} (0.25 mm). TLC spots were detected under UV light and/or by charring with a solution of *p*-anisaldehyde in ethanol, acetic acid and H_2SO_4. Column chromatography was performed on Silica Gel 60 (40–60 μm). Solvents were evaporated under reduced pressure on a rotary evaporator. ^{1}H NMR spectra were recorded using 400, 500, or 600 MHz NMR instruments and were referenced to residual proton signal of $CDCl_3$ (7.26

ppm) or CD_3OD (3.30 ppm). ^{13}C NMR spectra were recorded using 126 MHz (cold probe) NMR instrument and were referenced to residual ^{13}C signals of $CDCl_3$ (77 ppm) or CD_3OD (49 ppm). ^1H NMR data are reported as though they were first order, and peak assignments were made on the basis of 2D-NMR (^1H –^1H COSY and HSQC) experiments. ESI-MS spectra (time-of-flight analyzer) were recorded on samples dissolved in THF or CH_3OH and added NaCl. Optical rotations were measured at 22 ± 2 °C at the sodium D line (589 nm) and are in a microcell (10 cm, 1 mL) in units of deg·mL(dm·g)$^{-1}$.

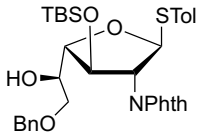
4.9.2 Experimental, spectroscopic, and analytical data



Octyl 2,3,4,7-tetra-*O*-benzyl-6-*O*-methyl-D-*glycero*-α-L-*gluco*-heptopyranosyl-(1→3)-6-*O*-benzoyl-2-*O*-benzyl-α-D-glucopyranoside (4-4)

To **4-78** (60 mg, 0.06 mmol) in CH_2Cl_2 (1 mL) were added Et_3N (88 μL, 0.63 mmol) and Bz_2O (29 mg, 0.13 mmol). The reaction mixture was stirred at room temperature for 22 h before being diluted with CH_2Cl_2 and washed with a saturated $NaHCO_3$ solution (2×). The organic layers were combined, dried over Na_2SO_4, filtered, and concentrated. The resulting residue was purified by flash chromatography using a gradient of 4:1→3:1 hexanes–EtOAc to give **4-4** (35 mg, 53%, 91% based on recovered **4-78**) as a clear oil. R_f 0.43 (3:1 hexanes–EtOAc); $[\alpha]_D$ −4.1 (*c* 1.0, $CHCl_3$); ^1H NMR

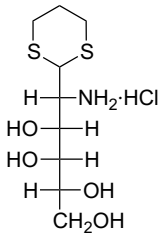
(498 MHz, $CDCl_3$) δ 8.07–8.06 (m, 2H, Ar), 7.60–7.56 (m, 1H, Ar), 7.48–7.44 (m, 2H, Ar), 7.38–7.22 (m, 25H, Ar), 5.52 (d, 1H, *J* = 3.5 Hz, H-1_{Hep}), 5.04 (d, 1H, *J* = 10.9 Hz, PhC$\underline{H}_2$O), 5.00 (d, 1H, *J* = 11.2 Hz, PhC$\underline{H}_2$O), 4.84 (d, 1H, *J* = 10.9 Hz, PhC$\underline{H}_2$O), 4.80 (d, 1H, *J* = 3.5 Hz, H-1_{Glc}), 4.74 (d, 1H, *J* = 12.0 Hz, PhC$\underline{H}_2$O), 4.67 (d, 1H, *J* = 12.2, PhC$\underline{H}_2$O), 4.66 (d, 1H, *J* = 12.2 Hz, PhC$\underline{H}_2$O), 4.63 (d, 1H, *J* = 11.2 Hz, PhC$\underline{H}_2$O), 4.57 (s, 2H, PhC$\underline{H}_2$O), 4.54 (d, 1H, *J* = 12.1 Hz, PhC$\underline{H}_2$O), 4.41 (dd, 1H, *J* = 11.9, 2.0 Hz, H-6_{Glc}), 4.35–4.31 (m, 2H, H-5_{Hep}, H-6_{Glc}), 4.13 (dd, 1H, *J* = 9.4, 9.4 Hz, H-3_{Hep}), 4.04 (dd, 1H, *J* = 9.1, 9.1 Hz, H-3_{Glc}), 3.88 (dd, 1H, *J* = 9.7, 7.3 Hz, H-7_{Hep}), 3.79–3.52 (m, 9H, H-2_{Glc}, H-2_{Hep}, H-4_{Glc}, H-4_{Hep}, H-5_{Glc}, H-6_{Hep}, H-7_{Hep}, OC$\underline{H}_2CH_2$, O$\underline{H}$), 3.41–3.37 (m, 4H, OC$\underline{H}_3$, OC$\underline{H}_2CH_2$), 1.69–1.63 (m, 2H, $OCH_2C\underline{H}_2$), 1.41–1.29 (m, 10H, 5 × C$\underline{H}_2$), 0.91 (t, 3H, *J* = 7.0 Hz, $CH_2C\underline{H}_3$); ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.5 (C=O), 138.8 (Ar), 138.6 (Ar), 138.5 (Ar), 138.0 (Ar), 137.4 (Ar), 133.0 (Ar), 130.0 (Ar), 129.7 (Ar), 128.5 (Ar), 128.4 (3×, 3 × Ar), 128.3 (Ar), 128.0 (2×, 2 × Ar), 127.9 (Ar), 127.8 (Ar), 127.7 (Ar), 127.6 (3×, 3 × Ar), 127.5 (Ar), 127.4 (Ar), 97.5 (C-1_{Hep}), 96.5 (C-1_{Glc}), 81.8 (C-3_{Hep}), 79.8 (C-2_{Glc}), 79.3 (C-2_{Hep}), 79.2 (C-3_{Glc}), 76.1 (2×, C-4_{Hep}, C-6_{Hep}), 75.6 (Ph$\underline{C}H_2$O), 74.9 (Ph$\underline{C}H_2$O), 73.5 (Ph$\underline{C}H_2$O), 72.5 (Ph$\underline{C}H_2$O), 72.4 (Ph$\underline{C}H_2$O), 70.3 (C-5_{Hep}), 70.0 (C-5_{Glc}), 69.7 (C-4_{Glc}), 68.3 (C-7_{Hep}), 68.2 (O$\underline{C}H_2CH_2$), 63.9 (C-6_{Glc}), 58.7 (O$\underline{C}H_3$), 31.9 ($\underline{C}H_2$), 29.5 (2×, 2 × $\underline{C}H_2$), 29.3 ($\underline{C}H_2$), 26.3 ($\underline{C}H_2$), 22.7 ($\underline{C}H_2$), 14.1 ($CH_2\underline{C}H_3$); HRMS (ESI) Calc. for (M + Na) $C_{64}H_{76}NaO_{13}$: 1075.5178. Found 1075.5174.



Tolyl 6-*O*-benzyl-3-*O*-*tert*-butyldimethylsilyl-2-deoxy-2-*N*-phthalimido-1-thio-β-D-galactofuranoside (4-6)

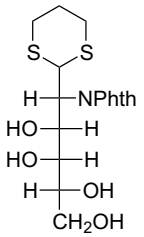
To **4-36** (1.6 g, 3.16 mmol), 2-aminoethyl diphenylborinate (142 mg, 0.63 mmol), KI (526 mg, 3.17 mmol), and K_2CO_3 (482 mg, 3.48 mmol) under argon were added CH_3CN (30 mL) and BnBr (565 μL, 4.75 mmol). The reaction mixture was stirred at 60 °C for 18 h. The mixture was then poured over brine, diluted with an equal volume of H_2O (40 mL) and extracted with EtOAc (3×). The organic layers were combined, dried over Na_2SO_4, filtered, and concentrated. The resulting residue was purified by flash chromatography (dry loading) using a gradient of 20:1→5:2 hexanes–EtOAc to give **4-6** (1.58 g, 84%) as a viscous oil. R_f 0.28 (4:1 hexanes–EtOAc); $[\alpha]_D$ −77.5 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz; $CDCl_3$): δ 7.87 (dd, 2H, *J* = 5.7, 2.5 Hz, Ar), 7.76 (dd, 2H, *J* = 5.8, 2.6 Hz, Ar), 7.39–7.28 (m, 7H, Ar), 7.06 (d, 2H, *J* = 7.9 Hz, Ar), 5.67 (d, 1H, *J* = 7.3 Hz, H-1), 5.06 (dd, 1H, *J* = 8.0, 8.0 Hz, H-3), 4.67 (dd, 1H, *J* = 7.6, 7.6 Hz, H-2), 4.64 (d, 1H, *J* = 12.0 Hz, PhC$\underline{H}_2$O), 4.60 (d, 1H, *J* = 12.0 Hz, PhC$\underline{H}_2$O), 4.02 (dd, 1H, *J* = 8.3, 1.5 Hz, H-4), 3.97–3.91 (m, 1H, H-5), 3.69 (dd, 1H, *J* = 9.6, 7.4 Hz, H-6), 3.62 (dd, 1H, *J* = 9.6, 5.6 Hz, H-6'), 2.39 (d, 1H, *J* = 8.0 Hz, C5-O$\underline{H}$), 2.30 (s, 3H, ArC$\underline{H}_3$), 0.78 (s, 9H, SiC(C$\underline{H}_3$)$_3$), –0.02 (s, 3H, SiC$\underline{H}_3$), –0.24 (s, 3H, SiC$\underline{H}_3$); ^{13}C NMR (126 MHz; $CDCl_3$): δ 167.5 (C=O), 138.1 (Ar), 238.0 (Ar), 134.4 (Ar), 133.1 (Ar), 131.60 (Ar), 129.6 (Ar), 129.4 (Ar), 128.5 (Ar), 127.8 (2×, Ar), 123.6 (Ar), 86.1 (C-1), 81.4 (C-4), 73.5 (Ph$\underline{C}H_2$O), 71.9 (C-6), 71.1 (C-3), 67.7 (C-5), 62.2 (C-2), 25.5 (SiC($\underline{C}H_3$)$_3$), 21.1 (Ar$\underline{C}H_3$), 17.6

($Si\underline{C}(CH_3)_3$), –4.81 ($Si\underline{C}H_3$), –4.86 ($Si\underline{C}H_3$); HRMS (ESI) Calc. for (M + Na) $C_{34}H_{41}NNaO_6SSi$: 642.2316. Found 642.2304.



2-Amino-2-deoxy-D-galactose propane-1,3-diyldithioacetal hydrochloride (4-24)

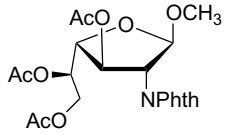
To D-galactosamine hydrochloride (**4-23**, 10.0 g, 46.40 mmol), 1,3-propanedithiol (5.3 mL, 48.70 mmol) and concentrated HCl (80 mL) were added. The suspended mixture was vigorously stirred at room temperature for 96 h. The solvent was evaporated under reduced pressure and the remaining solid was recrystallized from EtOH (2×) to afford **4-24** (12.4 g, 87%) as a white crystalline solid. R_f 0.63 (7:2:1 isopropanol–H_2O–NH_4OH_{aq}); $[\alpha]_D$ –7.9 (*c* 1.0, H_2O); ^{1}H NMR (500 MHz, D_2O): δ 4.59 (d, 1H, *J* = 6.4 Hz, H-3), 4.28 (d, 1H, *J* = 10.6, H-2), 4.10 (d, 1H, *J* = 10.7 Hz, H-1), 3.97–3.94 (m, 1H, H-5), 3.89 (dd, 1H, *J* = 6.6, 2.2 Hz, H-4), 3.77–3.72 (m, 2H, H-6, H-6'), 3.14–3.03 (m, 2H, $C\underline{H}_2$), 2.84–2.77 (m, 2H, $C\underline{H}_2$), 2.20–2.02 (m, 2H, $C\underline{H}_2$); ^{13}C NMR (126 MHz, D_2O): δ 72.4 (C-4), 71.5 (C-5), 67.8 (C-3), 63.8 (C-6), 53.7 (C-2), 42.7 (C-1), 26.1 ($S\underline{C}H_2$), 25.8 ($S\underline{C}H_2$), 25.2 ($\underline{C}H_2$). HRMS (ESI) Calc. for (M + Na) $C_9H_{19}NO_4S_2$: 292.0648. Found: 292.0650.



2-Deoxy-2-*N*-phthalimido-D-galactose propane-1,3-diyldithioacetal (4-26)

To **4-24** (6.1 g, 19.95 mmol) under argon, anhydrous CH_3OH (70 mL) and Et_3N (14 mL, 100.00 mmol) were added. The suspended mixture was heated at reflux for 2 h at which point the solution became clear. The mixture was then cooled to room temperature and $PhthCl_2$ (3.75 mL, 26.03 mmol) was added dropwise over ~5 min. The reaction mixture was then stirred at reflux for 14 h before being cooled and concentrated. Excess salts were removed by recrystallization from THF (2×). The filtrate was concentrated and dried under vacuum for 1 h. To the residue were added THF (80 mL) and DIPEA (5 mL). The mixture was heated at reflux for 15 h. The mixture was cooled, concentrated and the resulting residue was purified by flash chromatography in 20:1→14:1 CH_2Cl_2–CH_3OH to afford **4-26** (6.74 g, 85%) as a white solid. R_f 0.45 (10:1 CH_2Cl_2–CH_3OH); $[\alpha]_D$ –35.7 (*c* 1.0, CH_3OH); ^{1}H NMR (400 MHz; CD_3OD): δ 7.90–7.82 (m, 4H, Ar), 5.25 (dd, 1H, *J* = 11.9, 3.3 Hz, H-2), 4.65 (dd, 1H, *J* = 9.7, 3.3 Hz, H-3), 4.51 (d, 1H, *J* = 11.8 Hz, H-1), 3.93 (ddd, 1H, *J* = 6.6, 6.6, 1.4 Hz, H-5), 3.56 (dd, 1H, *J* = 9.9, 5.4 Hz, H-6), 3.52 (dd, 1H, *J* = 9.9, 5.6 Hz, H-6'), 3.27 (dd, 1H, *J* = 9.7, 1.4 Hz, H-4), 3.15 (ddd, 1H, *J* = 14.2, 10.6, 3.2 Hz, S-C$\underline{H}_2$), 3.00 (ddd, 1H, *J* = 14.0, 10.7, 3.1 Hz, S-C$\underline{H}_2$), 2.66 (ddd, 1H, *J* = 14.3, 5.9, 3.1 Hz, S-C$\underline{H}_2$), 2.47 (ddd, 1H, *J* = 14.2, 5.9, 3.1 Hz, S-C$\underline{H}_2$), 2.07–1.91 (m, 2H, S-$CH_2C\underline{H}_2$); ^{13}C NMR (126 MHz; CD_3OD): δ 169.5 (C=O), 134.4 (Ar), 131.6 (Ar), 123.2 (Ar), 71.3 (C-4), 69.8 (C-5), 68.8 (C-3), 63.0 (C-6), 54.1 (C-2), 41.2 (C-1), 25.8

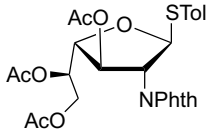
(<u>C</u>H$_2$), 25.2 (<u>C</u>H$_2$), 24.8 (<u>C</u>H$_2$); HRMS (ESI) Calc. for (M + Na) $C_{17}H_{21}NO_6S_2$: 422.0702. Found: 422.0701.



Methyl 3,5,6-tri-*O*-acetyl-2-deoxy-2-*N*-phthalimido-β-D-galactofuranoside (4-28)

To a stirring solution of 2% I_2 in anhydrous CH_3OH (100 mL), **4-26** (3.0 g, 7.51 mmol) was added and the reaction mixture was stirred at room temperature. A portion of powdered I_2 (2.0 g) was added every 2 d until the starting material disappeared completely. After the stirring was continued for 8 d the reaction was complete as indicated by a new spot by TLC (R_f 0.37, 10:1 CH_2Cl_2–CH_3OH), which was presumably the cyclized product. At this point, Na_2CO_3 and $Na_2S_2O_3$ solids were added to neutralize the solution and quench the excess iodine. The excess solids were removed by filtration and the filtrate was concentrated and dried under vacuum for 10 h. The resulting residue was dissolved in pyridine (100 mL) and Ac_2O (30 mL) was added dropwise to the solution at 0 °C over ~20 min. After complete addition, the reaction mixture was warmed to room temperature and stirred for 20 h. The excess Ac_2O was quenched by the addition of CH_3OH (30 mL). The mixture was concentrated and then co-evaporated with toluene (2×). The resulting residue was diluted with CH_2Cl_2 and washed sequentially with 1 M HCl (2×), H_2O (1×), saturated $NaHCO_3$ solution (1×), and brine (1×). The organic layer was dried over Na_2SO_4, filtered, and concentrated. The resulting residue was purified by flash chromatography in 5:2 hexanes–EtOAc to afford **4-28** (2.90 g, 86%, over two steps) as a white solid. R_f 0.45 (1:1 hexanes–EtOAc); Data for β-anomer. $[\alpha]_D$ –24.6 (*c* 1.2,

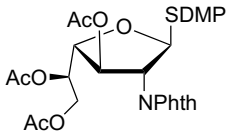
CH_2Cl_2); ^1H NMR (300 MHz, $CDCl_3$): δ 7.81–7.68 (m, 4H, Ar), 5.53 (dd, 1H, *J* = 8.4, 6.9 Hz, H-3), 5.35 (ddd, 1H, *J* = 7.5, 3.0 Hz, H-5), 5.27 (d, 1H, *J* = 3.6 Hz, H-1), 4.66 (dd, 1H, *J* = 6.9, 3.6 Hz, H-2), 4.40–4.38 (m, 2H, H-4, H-6), 4.30 (dd, 1H, *J* = 11.7, 7.5 Hz, H-6'), 3.34 (s, 3H, OCH_3), 2.17 (s, 3H, $COCH_3$), 2.03 (s, 3H, $COCH_3$), 1.95 (s, 3H, $COCH_3$); ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.4 (C=O), 170.3 (C=O), 170.2 (C=O), 167.3 (C=O), 134.1 (Ar), 131.5 (Ar), 123.4 (Ar), 104.7 (C-1), 77.5 (C-4), 72.9 (C-3), 68.5 (C-5), 62.5 (C-6), 61.1 (C-2), 55.6 (OCH_3), 20.6 ($COCH_3$), 20.5 ($COCH_3$), 20.2 ($COCH_3$). HRMS (ESI) Calc. for (M + Na) $C_{21}H_{23}NO_{10}$: 472.1214. Found: 472.1216.



Tolyl 3,5,6-tri-*O*-acetyl-2-deoxy-2-*N*-phthalimido-1-thio-β-D-galactofuranoside (4-29)

To a solution of **4-28** (1.34 g, 2.98 mmol) and 4-methylbenzenethiol (445 mg, 3.58 mmol) in CH_2Cl_2 (36 mL) under argon was added $BF_3 \cdot OEt_2$ (551 uL, 4.47 mmol) dropwise via syringe over 5 min at 0 °C. The reaction mixture was warmed to room temperature and stirred for 12 h. Et_3N (~1 mL) was added and the mixture was then diluted with CH_2Cl_2 and washed with H_2O (2×) and brine (1×). The organic layer was dried over Na_2SO_4, filtered, and concentrated. The resulting residue was purified by flash chromatography using a gradient of 2:1→3:2 hexanes–EtOAc to afford **4-29** (1.39 g, 86%) as a white solid. R_f 0.55 (1:1 hexanes–EtOAc); $[\alpha]_D$ –37.1 (*c* 2.0, CH_2Cl_2); ^1H NMR (498 MHz; $CDCl_3$): δ 7.85 (dd, 2H, *J* = 5.5, 3.0 Hz, Ar), 7.73 (dd, 2H, *J* = 5.5, 3.1 Hz, Ar), 7.38 (d, 2H, *J* = 8.1 Hz, Ar), 7.13 (d, 2H, *J* = 7.9 Hz, Ar), 5.77 (d, 1H, *J* = 8.1 Hz, H-1), 5.70 (dd, 1H, *J* = 7.5, 7.5 Hz, H-3), 5.40 (ddd, 1H, *J* = 7.3, 4.5, 2.9 Hz, H-5),

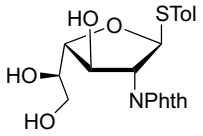
4.64 (dd, 1H, J = 8.0, 7.6 Hz, H-2), 4.39 (dd, 1H, J = 11.7, 4.7 Hz, H-6), 4.33 (dd, 1H, J = 7.4, 3.1 Hz, H-4), 4.29 (dd, 1H, J = 11.7, 7.2 Hz, H-6'), 2.33 (s, 3H, ArC$\underline{H}_3$), 2.23 (s, 3H, COC$\underline{H}_3$), 2.07 (s, 3H, COC$\underline{H}_3$), 1.97 (s, 3H, COC$\underline{H}_3$); ^{13}C NMR (126 MHz; $CDCl_3$): δ 170.6 (C=O), 170.5 (C=O), 170.4 (C=O), 167.4 (C=O), 138.5 (Ar), 134.3 (Ar), 133.4 (Ar), 131.7 (Ar), 129.7 (Ar), 128.0 (Ar), 123.7 (Ar), 85.9 (C-1), 78.7 (C-4), 72.4 (C-3), 69.1 (C-5), 62.7 (C-6), 59.2 (C-2), 21.2 (Ar$\underline{C}H_3$), 20.9 (CO$\underline{C}H_3$), 20.8 (CO$\underline{C}H_3$), 20.5 (CO$\underline{C}H_3$); HRMS (ESI) Calc. for (M + Na) $C_{27}H_{27}NO_9S$: 564.1299. Found: 564.1296.



2,6-Dimethylphenyl 3,5,6-tri-*O*-acetyl-2-deoxy-2-*N*-phthalimido-1-thio-β-D-galactofuranoside (4-30)

To a solution of **4-28** (4.59 g, 10.21 mmol) and 2,6-dimethylthiophenol (1.8 mL, 13.27 mmol) in CH_2Cl_2 (50 mL) under argon was added $BF_3{\cdot}OEt_2$ (3.8 mL, 30.63 mmol) dropwise via syringe over ~10 min at 0 °C. The reaction mixture was warmed to room temperature and stirred for 16 h before Et_3N (~5 mL) was added. The mixture was then diluted with CH_2Cl_2 and washed with H_2O (2×) and brine (1×). The organic layer was dried over Na_2SO_4, filtered, and concentrated. The resulting residue was purified by flash chromatography using a gradient of 2:1→1:1 hexanes–EtOAc to afford **4-30** (2.21 g, 39%) as a white solid. R_f 0.53 (1:1 hexanes–EtOAc); $[\alpha]_D$ –28.9 (c 1.0, $CHCl_3$); ^{1}H NMR (498 MHz; $CDCl_3$): δ 7.89 (dd, 2H, J = 5.8, 3.1 Hz, Ar), 7.77 (dd, 2H, J = 5.8, 3.0 Hz, Ar), 7.14–7.06 (m, 3H, Ar), 5.63 (dd, 1H, J = 7.5, 7.5 Hz, H-3), 5.60 (d, 1H, J = 7.5 Hz, H-1), 5.39 (ddd, 1H, J = 7.0, 4.9, 3.4 Hz, H-5), 4.81 (dd, 1H, J = 7.4, 7.4 Hz, H-2), 4.50

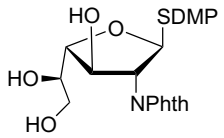
(dd, 1H, J = 7.7, 3.4 Hz, H-4), 4.35 (dd, 1H, J = 11.7, 4.9 Hz, H-6), 4.21 (dd, 1H, J = 11.7, 7.0 Hz, H-6'), 2.42 (s, 6H, 2 × ArC$\underline{H}_3$), 2.16 (s, 3H, COC$\underline{H}_3$), 2.08 (s, 3H, COC$\underline{H}_3$), 2.03 (s, 3H, COC$\underline{H}_3$); ^{13}C NMR (126 MHz; $CDCl_3$): δ 170.5 (C=O), 170.4 (2C, 2 × C=O), 167.3 (C=O), 143.5 (Ar), 134.4 (Ar), 131.6 (Ar), 130.9 (Ar), 129.2 (Ar), 128.2 (Ar), 123.7 (Ar), 88.2 (C-1), 78.3 (C-4), 72.6 (C-3), 68.8 (C-5), 62.5 (C-6), 60.7 (C-2), 22.1 (Ar$\underline{C}H_3$), 20.7 (2C, 2 × CO$\underline{C}H_3$), 20.5 (CO$\underline{C}H_3$); HRMS (ESI) Calc. for (M + Na) $C_{28}H_{29}NNaO_9S$: 578.1455. Found 578.1449.



Tolyl 2-deoxy-2-*N*-phthalimido-1-thio-β-D-galactofuranoside (4-31)

To a solution of **4-29** (3.91 g, 7.22 mmol) in CH_3OH (80 mL) was added K_2CO_3 (100 mg, 0.72 mmol). The mixture was stirred for 2.5 h and then the base was neutralized by the addition of Amberlyst 15 ion exchange resin. The resin was removed by filtration and the filtrate was concentrated. To the resulting residue was added THF (72 mL) and DIPEA (7.2 mL). The mixture was heated at reflux for 12 h, cooled and then concentrated. The resulting residue was purified by flash chromatography using a gradient of 15:1→10:1 CH_2Cl_2–CH_3OH to give **4-31** (2.8 g, 93%) as a white solid. R_f 0.44 (10:1 CH_2Cl_2–CH_3OH); $[\alpha]_D$ –74.5 (*c* 1.0, CH_3OH); ^{1}H NMR (600 MHz; CD_3OD): δ 7.86 (dd, 2H, J = 5.5, 3.0 Hz, Ar), 7.81 (dd, 1H, J = 5.6, 3.0 Hz, Ar), 7.41 (d, 2H, J = 8.1 Hz, Ar), 7.11 (d, 2H, J = 7.9 Hz, Ar), 5.72 (d, 1H, J = 8.4 Hz, H-1), 4.91 (dd, 1H, J = 8.7, 8.7 Hz, H-3), 4.57 (dd, 1H, J = 8.6 Hz, H-2), 3.85 (dd, 1H, J = 8.6, 2.9 Hz, H-4), 3.74 (ddd, 1H, J = 6.4, 6.4, 2.9 Hz, H-5), 3.66 (d, 2H, J = 6.4 Hz, H-6, H-6'), 2.28 (s, 3H,

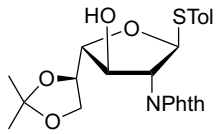
ArC<u>H</u>$_3$); ^{13}C NMR (126 MHz; CD_3OD): δ 167.8 (C=O), 138.2 (Ar), 134.3 (Ar), 133.6 (Ar), 131.6 (Ar), 129.2 (Ar), 128.4 (Ar), 122.9 (Ar), 84.6 (C-1), 81.3 (C-4), 70.4 (C-5), 69.6 (C-3), 63.0 (C-6), 61.5 (C-2), 19.8(Ar<u>C</u>H$_3$); HRMS (ESI) Calc. for (M + Na) $C_{21}H_{21}NO_6S$: 438.0982. Found: 438.0982.



2,6-Dimethylphenyl 2-deoxy-2-*N*-phthalimido-1-thio-β-D-galactofuranoside (4-32)

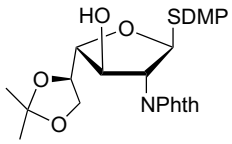
To a solution of **4-30** (16.81 g, 30.26 mmol) in a 10:1 CH_3OH–CH_2Cl_2 solution (220 mL) was added small Na pieces the solution was pH was 8–9 as determined by wet pH paper. The reaction mixture was stirred for 3 h and then the base was neutralized by the addition of Amberlyst 15 ion exchange resin. The resin was removed by filtration and the filtrate was concentrated and dried under vacuum for 2 h. To the resulting residue were added anhydrous THF (200 mL) and DIPEA (10 mL). The mixture was heated at reflux for 14 h, cooled and then concentrated. The resulting residue was purified by flash chromatography using a gradient of 20:1→15:1 CH_2Cl_2–CH_3OH to give **4-32** (11.27 g, 87%) as a white solid. R_f 0.50 (10:1 CH_2Cl_2–CH_3OH); $[\alpha]_D$ –6.2 (*c* 1.0, CH_3OH); ^{1}H NMR (498 MHz; CD_3OD): δ 7.90–7.88 (m, 2H, Ar), 7.86–7.83 (m, 2H, Ar), 7.04–7.01 (m, 3H, Ar), 5.56 (d, 1H, *J* = 8.3 Hz, H-1), 4.86 (dd, 1H, *J* = 8.7, 8.7 Hz, H-3), 4.69 (dd, 1H, *J* = 8.5, 8.5 Hz, H-2), 4.05 (dd, 1H, *J* = 8.6, 2.7 Hz, H-4), 3.74 (ddd, 1H, *J* = 7.1, 5.7, 2.6 Hz, H-5), 3.65–3.59 (m, 2H, H-6, H-6'), 2.37 (s, 6H, 2 × ArC<u>H</u>$_3$); ^{13}C NMR (126 MHz; $CDCl_3$): δ 169.1 (C=O), 144.6 (Ar), 135.8 (Ar), 132.9 (Ar), 132.8 (Ar), 130.1 (Ar), 129.2 (Ar), 124.4 (Ar), 88.2 (C-1), 82.6 (C-4), 71.6 (C-5), 71.3 (C-3), 64.6 (C-6), 64.0

(C-2), 22.5 (Ar<u>C</u>H$_3$); HRMS (ESI) Calc. for (M + Na) $C_{22}H_{23}NNaO_6S$: 452.1138. Found 452.1142. *Note: The opened phthalimide adduct (0.9 g, 7%) was also collected.



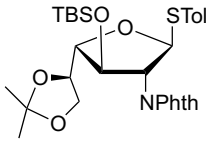
Tolyl 2-deoxy-5,6-*O*-isopropylidene-2-*N*-phthalimido-1-thio-β-D-galactofuranoside (4-33)

To **4-31** (6.14 g, 14.79 mmol) in acetone (75 mL) under argon, 2,2-dimethoxy propane (3.6 mL, 29.58 mmol) and PTSA (422 mg, 2.22 mmol) were added. The reaction mixture was stirred for 2 h and then the PTSA was quenched by addition of Et_3N (~3 mL). The mixture was concentrated and resulting residue was diluted with EtOAc and washed with H_2O (1×) and brine (1×). The organic layer was dried over Na_2SO_4, filtered, and concentrated. The resulting residue was purified by flash chromatography using a gradient of 2:1→5:3 hexanes–EtOAc to afford **4-33** (6.47 g, 96%) as a white solid. R_f 0.28 (2:1 hexanes–EtOAc); $[\alpha]_D$ −58.1 (*c* 2.5, $CHCl_3$); ^{1}H NMR (400 MHz; $CDCl_3$): δ 7.85 (dd, 2H, *J* = 5.4, 3.1 Hz, Ar), 7.74 (dd, 2H, *J* = 5.5, 3.0 Hz, Ar), 7.42 (d, 2H, *J* = 8.1 Hz, Ar), 7.11 (d, 2H, *J* = 8.0 Hz, Ar), 5.75 (d, 1H, *J* = 8.1 Hz, H-1), 4.86 (dd, *J* = 8.3, 8.3 Hz, H-3), 4.64 (dd, 1H, *J* = 8.3, 8.3 Hz, H-2), 4.42 (ddd, 1H, *J* = 6.5, 6.5, 4.9 Hz, H-5), 4.13–4.07 (m, 2H, H-6, H-6'), 3.97 (dd, 1H, *J* = 8.2, 4.7 Hz, H-4), 2.32 (s, 3H, Ar<u>C</u>H$_3$), 1.45 (s, 3H, C<u>C</u>H$_3$), 1.39 (s, 3H, C<u>C</u>H$_3$); ^{13}C NMR (126 MHz; $CDCl_3$): δ 167.7 (C=O), 138.4 (Ar), 134.4 (Ar), 133.7 (Ar), 131.6 (Ar), 129.7 (Ar), 128.3 (Ar), 123.6 (Ar), 109.7 (O_2<u>C</u>$(CH_3)_2$), 85.1 (C-1), 80.9 (C-4), 75.0 (C-5), 71.7 (C-3), 65.0 (C-6), 61.3 (C-2), 26.2 (C<u>C</u>H$_3$), 25.1 (C<u>C</u>H$_3$), 21.2 (Ar<u>C</u>H$_3$); HRMS (ESI) Calc. for (M + Na) $C_{24}H_{25}NNaO_6S$: 478.1295. Found 478.1293.



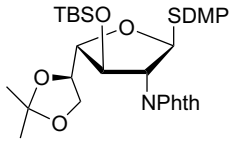
2,6-Dimethylphenyl 2-deoxy-5,6-*O*-isopropylidene-2-*N*-phthalimido-1-thio-β-D-galactofuranoside (4-34)

To **3-32** (2.0 g, 4.66 mmol) in acetone (25 mL) under argon were added 2,2-dimethoxy propane (1.14 mL, 9.32 mmol) and PTSA (90 mg, 0.47 mmol). The reaction mixture was stirred for 2 h and then the PTSA was quenched by addition of Et_3N (~500 uL). The mixture was concentrated and resulting residue was diluted with EtOAc and washed with H_2O (1×) and brine (1×). The organic layer was dried over Na_2SO_4, filtered, and concentrated. The resulting residue was purified by flash chromatography in 1:1 hexanes–EtOAc to afford **4-34** (2.10 g, 96%) as a white solid. R_f 0.50 (1:1 hexanes–EtOAc); $[\alpha]_D$ –29.45 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz; $CDCl_3$): δ 7.89 (dd, 2H, *J* = 5.5, 3.0 Hz, Ar), 7.77 (dd, 2H, *J* = 5.5, 3.0 Hz, Ar), 7.12–7.05 (m, 3H, Ar), 5.62 (d, 1H, *J* = 7.3 Hz, H-1), 4.81–4.78 (m, 2H, H-2, H-3), 4.42 (ddd, 1H, *J* = 6.6, 6.6, 4.8 Hz, H-5), 4.21 (dd, 1H, *J* = 8.1, 4.7 Hz, H-4), 4.11–4.06 (m, 2H, H-6, H-6'), 2.54 (s, 1H, C3-O<u>H</u>), 2.44 (s, 6H, 2 × Ar<u>CH</u>$_3$), 1.38 (s, 6H, 2 × C<u>CH</u>$_3$); ^{13}C NMR (126 MHz; $CDCl_3$): δ 167.6 (C=O), 143.6 (Ar), 134.4 (Ar), 131.6 (Ar), 131.3 (Ar), 129.0 (Ar), 128.2 (Ar), 123.6 (Ar), 109.7 (<u>C</u>$(CH_3)_2$, 87.2 (C-1), 80.6 (C-4), 75.0 (C-5), 72.7 (C-3), 65.0 (C-6), 63.0 (C-2), 26.0 (Ar<u>C</u>H_3), 25.1 (C<u>C</u>H_3), 22.3 (C<u>C</u>H_3); HRMS (ESI) Calc. for (M + NH_4) $C_{25}H_{31}N_2O_6S$: 487.1897. Found 487.1900.



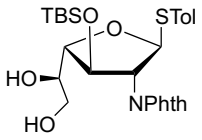
Tolyl 3-*O*-*tert*-butyldimethylsilyl-2-deoxy-5,6-*O*-isopropylidene-2-*N*-phthalimido-1-thio-β-D-galactofuranoside (4-35)

To **4-33** (1.54 g, 3.38 mmol) in pyridine (34 mL) was added TBSOTf (1.2 mL, 5.07 mmol) at 0 °C. The reaction mixture was stirred for 10 min before CH_3OH (1 mL) was added. The mixture was concentrated, the residue was dissolved in EtOAc and then washed with H_2O (1×) and brine (1×). The organic layer was dried over Na_2SO_4, filtered, and concentrated. The resulting residue was purified by flash chromatography (dry loading) in 6:1 hexane–EtOAc to give **4-35** (1.69 g, 88%) as a white solid. R_f 0.35 (6:1 hexanes–EtOAc); $[\alpha]_D$ –46.2 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz; $CDCl_3$): δ 7.87 (dd, 2H, *J* = 5.5, 3.0 Hz, Ar), 7.75 (dd, 2H, *J* = 5.4, 3.1 Hz, Ar), 7.41–7.39 (m, 2H, Ar), 7.09 (d, 2H, *J* = 7.9 Hz, Ar), 5.72 (d, 1H, *J* = 8.6 Hz, H-1), 5.04 (dd, 1H, *J* = 8.2, 8.2 Hz, H-3), 4.64 (dd, 1H, *J* = 8.5, 8.5 Hz, H-2), 4.25 (ddd, 1H, *J* = 7.1, 7.1, 2.9 Hz, H-5), 4.09–4.02 (m, 2H, H-6, H-6'), 3.83 (dd, 1H, *J* = 7.9, 2.9 Hz, H-4), 2.30 (s, 3H, ArC$\underline{H}_3$), 1.48 (s, 3H, CC$\underline{H}_3$), 1.41 (s, 3H, CC$\underline{H}_3$), 0.77 (s, 9H, SiC(C$\underline{H}_3$)$_3$), –0.03 (s, 3H, SiC$\underline{H}_3$), –0.26 (s, 3H, SiC$\underline{H}_3$); ^{13}C NMR (126 MHz; $CDCl_3$): δ 168.6 (C=O), 137.8 (Ar), 134.3 (Ar), 132.8 (Ar), 131.6 (Ar), 129.6 (Ar), 129.4 (Ar), 123.5 (Ar), 109.6 ($O_2\underline{C}(CH_3)_2$), 94.8 (C-1), 84.8 (C-4), 78.8 (C-5), 75.5 (C-3), 65.8 (C-6), 62.2 (C-2), 26.0 (C$\underline{C}H_3$), 25.8 (C$\underline{C}H_3$), 25.7 (SiC($\underline{C}H_3$)$_3$), 21.1 (Ar$\underline{C}H_3$), 17.9 (Si$\underline{C}$(CH$_3$)$_3$, –4.6 (Si$\underline{C}H_3$), –4.9 (Si$\underline{C}H_3$); HRMS (ESI) Calc. for (M + Na) $C_{30}H_{39}NNaO_6SSi$: 592.2160. Found 592.2156.



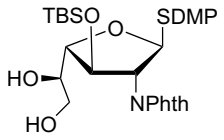
2,6-Dimethylphenyl 3-*O*-*tert*-butyldimethylsilyl-2-deoxy-5,6-*O*-isopropylidene-2-*N*-phthalimido-1-thio-β-D-galactofuranoside (4-36)

To **4-34** (2.75 g, 5.85 mmol) in pyridine (30 mL) was added TBSOTf (1.6 mL, 7.03 mmol) at 0 °C. The reaction mixture was stirred for 20 min before CH_3OH (2 mL) was added. The mixture was concentrated and then co-evaporated with toluene (2×). The residue was dissolved in EtOAc, washed with H_2O (1×) and brine (1×). The organic layer was dried over Na_2SO_4, filtered, and concentrated. The resulting residue was purified by flash chromatography (dry loading) in 6:1 hexane–EtOAc to give **4-36** (3.02 g, 88%) as a white solid. R_f 0.33 (6:1 hexanes–EtOAc); $[\alpha]_D$ –19.3 (*c* 1.0, $CHCl_3$); ^{1}H NMR (498 MHz; $CDCl_3$): δ 7.90 (dd, 2H, *J* = 5.8, 2.5 Hz, Ar), 7.81–7.77 (m, 2H, Ar), 7.09–7.02 (m, 3H, Ar), 5.50 (d, 1H, *J* = 8.1 Hz, H-1), 4.98 (dd, 1H, *J* = 8.0, 8.0 Hz, H-3), 4.77 (dd, *J* = 8.1, 8.1 Hz, H-2), 4.26 (ddd, 1H, *J* = 7.5, 6.7, 3.1 Hz, H-5), 4.07 (dd, 1H, *J* = 7.9, 6.7 Hz, H-6), 4.01 (dd, 1H, *J* = 7.9, 3.1 Hz, H-4), 3.97 (dd, 1H, *J* = 7.8, 7.8 Hz, H-6'), 2.41 (s, 6H, 2 × Ar$C\underline{H}_3$), 1.38 (s, 3H, $CC\underline{H}_3$), 1.31 (s, 3H, $CC\underline{H}_3$), 0.82 (s, 9H, $SiC(C\underline{H}_3)_3$), –0.01 (s, 3H, $SiC\underline{H}_3$), –0.23 (s, 3H, $SiC\underline{H}_3$); ^{13}C NMR (126 MHz; $CDCl_3$): δ 167.5 (C=O), 143.5 (Ar), 134.4 (Ar), 131.7 (Ar), 131.5 (Ar), 128.8 (Ar), 128.1 (Ar), 123.5 (Ar), 109.5 ($O_2\underline{C}(CH_3)_2$, 87.4 (C-1), 80.6 (C-4), 74.1 (C-5), 72.0 (C-3), 65.4 (C-6), 63.0 (C-2), 25.9 ($C\underline{C}H_3$), 25.8 ($C\underline{C}H_3$), 25.5 ($SiC(\underline{C}H_3)_3$, 22.2 ($Ar\underline{C}H_3$), 17.6 ($Si\underline{C}(CH_3)_3$, –4.7 ($Si\underline{C}H_3$), –5.0 ($Si\underline{C}H_3$); HRMS (ESI) Calc. for (M + NH_4) $C_{31}H_{45}N_2O_6SSi$: 601.2762. Found 601.2756.



Tolyl 3-*O*-*tert*-butyldimethylsilyl-2-deoxy-2-*N*-phthalimido-1-thio-β-D-galactofuranoside (4-37)

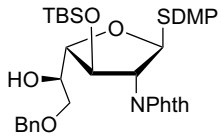
To **4-35** (1.69 g, 2.97 mmol) in a 1:1 CH_2Cl_2–CH_3OH solution (30 mL) was added PTSA (282 mg, 1.48 mmol). The reaction mixture was stirred for 2 h with frequent monitoring by TLC analysis. When the reaction was complete, the acid was quenched by the addition of Et_3N (~1 mL). The residue was poured over brine, diluted with an equal volume of H_2O and then extracted with EtOAc (3×). The organic layers were combined, dried over Na_2SO_4, filtered, and concentrated. The resulting residue was purified by flash chromatography in 1:1 hexanes–EtOAc to give **4-37** (990 mg, 63%) as a white solid. R_f 0.40 (1:1 hexanes–EtOAc); $[\alpha]_D$ –76.9 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz; $CDCl_3$): δ 7.88 (dd, 2H, *J* = 5.5, 3.0 Hz, Ar), 7.76 (dd, 2H, *J* = 5.5, 3.1 Hz, Ar), 7.39–7.35 (m, 2H, Ar), 7.11 (dd, 2H, *J* = 8.4, 0.6 Hz, Ar), 5.64 (d, 1H, *J* = 7.2 Hz, H-1), 5.02 (dd, 1H, *J* = 8.0, 8.0 Hz, H-3), 4.66 (dd, 1H, *J* = 7.6, 7.3 Hz, H-2), 3.97 (dd, 1H, *J* = 8.2, 1.3 Hz, H-4), 3.87–3.74 (m, 3H, H-5, H-6, H-6'), 2.61 (s, 1H, O<u>H</u>), 2.32 (s, 3H, ArC<u>H</u>$_3$), 2.21 (s, 1H, O<u>H</u>), 0.78 (s, 9H, SiC(C<u>H</u>$_3$)$_3$), –0.01 (s, 3H, SiC<u>H</u>$_3$), –0.23 (s, 3H, SiC<u>H</u>$_3$); ^{13}C NMR (126 MHz; $CDCl_3$): δ 167.5 (C=O), 138.5 (Ar), 134.5 (Ar), 133.4 (Ar), 131.5 (Ar), 129.8 (Ar), 129.0 (Ar), 123.6 (Ar), 86.4 (C-1), 83.2 (C-4), 71.5 (C-3), 68.6 (C-5), 65.4 (C-6), 61.9 (C-2), 25.5 (SiC(<u>C</u>H$_3$)$_3$), 21.2 (Ar<u>C</u>H$_3$), 17.6 (Si<u>C</u>(CH$_3$)$_3$), -4.8 (Si<u>C</u>H$_3$), -4.9 (Si<u>C</u>H$_3$); HRMS (ESI) Calc. for (M + Na) $C_{27}H_{35}NNaO_6SSi$: 552.1847. Found 552.1845.



2,6-Dimethylphenyl 3-*O*-*tert*-butyldimethylsilyl-2-deoxy-2-*N*-phthalimido-1-thio-β-D-galactofuranoside (4-38)

Conditions 1: To **4-36** (11.57 g, 24.64 mmol) in a 1:1 CH_2Cl_2–CH_3OH solution (250 mL) was added PTSA (2.34 g, 12.32 mmol). The reaction mixture was stirred for 2 h with frequent monitoring by TLC analysis. When the reaction was complete, the acid was quenched by the addition of Et_3N (~3.5 mL). The residue was concentrated and the resulting residue was then purified by flash chromatography using a gradient of 2:1→1:1 hexanes–EtOAc to give **4-38** (7.15 g, 53%). Conditions 2: To **4-36** (860 mg, 1.47 mmol) in a 1% CH_3OH in CH_2Cl_2 solution (24 mL) was added $FeCl_3 \cdot 6H_2O$ (80 mg, 0.30 mmol). The reaction mixture was stirred for 40 min and then a saturated $NaHCO_3$ solution (20 mL) was added. The mixture was extracted with CH_2Cl_2 (3×). The organic layers were combined, dried over Na_2SO_4, filtered, and concentrated. The resulting residue was purified by flash chromatography using a gradient of 3:2→1:1 hexanes–EtOAc to give **4-38** (687 mg, 86%). White solid. R_f 0.21 (2:1 hexanes–EtOAc); $[\alpha]_D$ –39.3 (*c* 1.0, $CHCl_3$); ^{1}H NMR (498 MHz; $CDCl_3$): δ 7.92–7.90 (m, 2H, Ar), 7.80 (dd, 2H, *J* = 5.4, 3.0 Hz, Ar), 7.14–7.07 (m, 3H, Ar), 5.50 (d, 1H, *J* = 7.1 Hz, H-1), 4.99 (dd, 1H, *J* = 7.8, 7.8 Hz, H-3), 4.80 (dd, 1H, *J* = 7.2, 7.2 Hz, H-2), 4.15 (d, 1H, *J* = 7.8 Hz, H-4), 3.81–3.71 (m, 3H, H-5, H-6, H-6'), 2.54 (s, 1H, O<u>H</u>), 2.43 (s, 6H, 2 × Ar<u>CH</u>$_3$), 2.14 (s, 1H, O<u>H</u>), 0.83 (s, 9H, SiC(<u>CH</u>$_3$)$_3$), 0.01 (s, 3H, Si<u>CH</u>$_3$), –0.20 (s, 3H, Si<u>CH</u>$_3$); ^{13}C NMR (126 MHz; $CDCl_3$): δ 167.4 (C=O), 143.3 (Ar), 134.5 (Ar), 131.4 (2C, 2 × Ar), 129.1 (Ar), 128.3 (Ar), 123.7 (Ar), 87.8 (C-1), 83.2 (C-4), 71.8 (C-3), 68.6 (C-5), 65.4 (C-6), 62.9 (C-2), 25.5

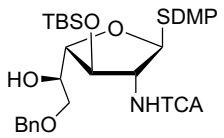
(SiC($\underline{C}$H$_3$)$_3$), 22.1 (Ar$\underline{C}$H$_3$), 17.6 (Si$\underline{C}$(CH$_3$)$_3$), –4.8 (Si$\underline{C}$H$_3$), –4.9 (Si$\underline{C}$H$_3$); HRMS (ESI) Calc. for (M + Na) $C_{28}H_{37}NNaO_6SSi$: 566.2003. Found 566.2001.



2,6-Dimethylphenyl 6-*O*-benzyl-3-*O*-*tert*-butyldimethylsilyl-2-deoxy-2-*N*-phthalimido-1-thio-β-D-galactofuranoside (4-39)

To **4-38** (1.0 g, 1.84 mmol), 2-aminoethyl diphenylborinate (83 mg, 0.37 mmol), KI (305 mg, 1.84 mmol), and K_2CO_3 (280 mg, 2.02 mmol) under argon were added CH_3CN (18 mL) and BnBr (330 μL, 2.76 mmol). The reaction mixture was stirred at 60 °C for 20 h. The mixture was then cooled, poured over brine, diluted with an equal volume of H_2O and extracted with EtOAc (3×). The organic layers were combined, dried over Na_2SO_4, filtered, and concentrated. The resulting residue was purified by flash chromatography (dry loading) using a gradient of 1:0→5:2 hexanes–EtOAc to give **4-39** (1.02 g, 88%) as a viscous oil. R_f 0.36 (3:1 hexanes–EtOAc); $[\alpha]_D$ –43.8 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz; $CDCl_3$): δ 7.92 (dd, 2H, *J* = 5.5, 3.0 Hz, Ar), 7.79 (dd, 2H, *J* = 5.4, 3.1 Hz, Ar), 7.39–7.33 (m, 5H, Ar), 7.13–7.04 (m, 3H, Ar), 5.51 (d, 1H, *J* = 7.1 Hz, H-1), 5.00 (dd, 1H, *J* = 8.0, 8.0 Hz, H-3), 4.81 (dd, 1H, *J* = 7.7, 7.2 Hz, H-2), 4.61 (d, 1H, *J* = 11.9 Hz, PhC$\underline{H}_2$O), 4.60 (d, 1H, *J* = 11.9 Hz, PhC$\underline{H}_2$O), 4.18 (dd, 1H, *J* = 8.3, 1.4 Hz, H-4), 3.96 (dddd, 1H, *J* = 8.0, 7.7, 7.6, 6.0, 1.5 Hz, H-5), 3.62 (dd, 1H, *J* = 9.6, 7.4 Hz, H-6), 3.58 (dd, 1H, *J* = 9.6, 5.7 Hz, H6'), 2.40 (s, 6H, 2 × Ar$\underline{C}$H$_3$), 2.34 (d, 1H, *J* = 8.0 Hz, C5-O$\underline{H}$), 0.83 (s, 9H, SiC(C$\underline{H}_3$)$_3$), 0.01 (s, 3H, Si$\underline{C}$H$_3$), –0.21 (s, 3H, Si$\underline{C}$H$_3$); ^{13}C NMR (126 MHz; $CDCl_3$): δ 167.4 (C=O), 143.5 (Ar), 138.0 (Ar), 134.4 (Ar), 131.7 (Ar), 131.5 (Ar),

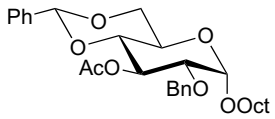
128.9 (Ar), 128.5 (Ar), 128.1 (Ar), 127.8 (2C, 2 × Ar), 123.6 (Ar), 87.7 (C-1), 81.4 (C-4), 73.5 (Ph<u>C</u>H_2O), 71.9 (C-6), 71.4 (C-3), 67.5 (C-5), 63.2 (C-2), 25.5 (SiC(<u>C</u>H_3)$_3$), 22.2 (Ar<u>C</u>H_3), 17.7 (Si<u>C</u>$(CH_3)_3$), –4.8 (Si<u>C</u>H_3), –4.9 (Si<u>C</u>H_3); HRMS (ESI) Calc. for (M + Na) $C_{35}H_{43}NNaO_6SSi$: 656.2473. Found 656.2473.



2,6-Dimethylphenyl 6-*O*-benzyl-3-*O*-*tert*-butyldimethylsilyl-2-deoxy-2-trichloroacetamido-1-thio-β-D-galactofuranoside (4-43)

To **4-39** (1 g, 1.578 mmol) was added a 10% solution of ethylenediamine in EtOH (11 mL). The reaction mixture was heated at reflux for 10 h. The mixture was then cooled, concentrated, co-evaporated with toluene (2×), and dried under vacuum for 8 h. The residue was dissolved in EtOAc, then washed with H_2O (1×) and brine (1×). The organic layer was dried over Na_2SO_4, filtered, concentrated, and dried under vacuum for 3 h. To the residue were added CH_2Cl_2 (16 mL), Et_3N (1.3 mL, 9.47 mmol), and DMAP (39 mg, 0.32 mmol). The mixture was cooled to 0 °C and then trichloroacetyl chloride (530 μL, 4.73 mmol) dropwise via syringe over ~2 min. The reaction mixture warmed to room temperature and stirred for 2 h. The excess trichloroacetyl chloride was quenched by the addition of CH_3OH (~500 μL). The mixture was diluted with CH_2Cl_2, washed with a saturated NH_4Cl solution (1×), H_2O (1×), and brine (1×). The organic layer was dried over Na_2SO_4, filtered, concentrated, and dried under vacuum for 2 h. The residue was then dissolved in CH_3OH (12 mL) and small sodium pieces were added until the pH of the solution was 8–9 as determined by wet pH paper. The reaction mixture was stirred for

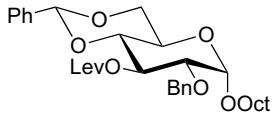
20 min and then AcOH (3 drops) was added and the mixture was concentrated. The resulting residue was purified by flash chromatography in 4:1 hexanes–EtOAc to afford **4-43** (818 mg, 80% over three steps) as a clear oil. R_f 0.45 (2:1 hexanes–EtOAc); $[\alpha]_D$ –59.3 (*c* 1.0, $CHCl_3$); ^{1}H NMR (498 MHz; $CDCl_3$): δ 8.25 (d, 1H, *J* = 8.6 Hz, NH), 7.41–7.33 (m, 5H, Ar), 7.16–7.11 (m, 3H, Ar), 5.13 (s, 1H, H-1), 4.58 (d, 1H, *J* = 11.7 Hz, PhCH_2O), 4.57 (d, 1H, *J* = 11.7 Hz, PhCH_2O), 4.53 (ddd, 1H, *J* = 8.7, 0.9, 0.9 Hz, H-2), 4.34–4.32 (m, 1H, H-3), 4.26–4.26 (m, 1H, H-4), 4.12–4.09 (m, 1H, H-5), 3.63 (dd, 1H, *J* = 9.6, 3.7 Hz, H-6), 3.58 (dd, 1H, *J* = 9.4, 9.4 Hz, H-6'), 2.77 (dd, 1H, *J* = 2.3, 1.8 Hz, C5-OH), 2.56 (s, 6H, 2 × ArCH_3), 0.99 (s, 9H, SiC(CH_3)$_3$), 0.20 (s, 3H, SiCH_3), 0.15 (s, 3H, SiCH_3); ^{13}C NMR (126 MHz; $CDCl_3$): δ 161.4 (C=O), 143.6 (Ar), 137.5 (Ar), 133.5 (Ar), 128.7 (Ar), 128.6 (Ar), 128.1 (Ar), 128.1 (Ar), 127.8 (Ar), 95.0 (C-1), 92.5 (COCCl$_3$), 86.6 (C-4), 78.3 (C-3), 73.6 (PhCH$_2$O), 71.4 (C-6), 70.0 (C-5), 64.4 (C-2), 25.7 (SiC(CH_3)$_3$), 22.5 (ArCH$_3$), 17.9 (SiC(CH$_3$)$_3$), –4.71 (SiCH$_3$), –4.79 (SiCH$_3$); HRMS (ESI) Calc. for (M + Na) $C_{29}H_{40}Cl_3NNaO_5SSi$: 670.1358. Found 670.1369.



Octyl 3-*O*-acetyl-2-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranoside (4-50)

To **4-48** (2.54 g, 6.68 mmol) and *n*-Bu_2SnO (1.83 g, 7.35 mmol) was added toluene (68 mL). The mixture was heated at reflux with Dean–Stark apparatus for 8 h. After cooling to room temperature, the Dean–Stark apparatus was removed and to the mixture were added BnBr (950 μL, 8.01 mmol) and TBAI (2.71 g, 7.34 mmol). The reaction mixture was then stirred at 100 °C for 14 h. The mixture was poured over a

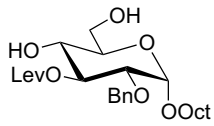
saturated solution of $Na_2S_2O_3$ and extracted with EtOAc. The organic layer was dried over Na_2SO_4, filtered, concentrated and dried under vacuum for 2 h. The residue was then dissolved in pyridine (4 mL) and Ac_2O (1 mL) was added. The reaction mixture was stirred at room temperature for 3 h. The excess Ac_2O was quenched by the addition of CH_3OH (1 mL), concentrated, and co-evaporated with toluene (2×). The residue was diluted with EtOAc, washed with H_2O (1×) and brine (1×). The organic layer was dried over Na_2SO_4, filtered, and concentrated. The resulting residue was purified by flash chromatography (dry loading) in 4:1 hexanes−EtOAc to afford **4-50** (1.91 g, 53%) as a white solid. R_f 0.50 (4:1–EtOAc); $[\alpha]_D$ +40.4 (*c* 1.0, $CHCl_3$); ^1H NMR (500 MHz; $CDCl_3$): δ 7.48–7.46 (m, 2H, Ar), 7.39–7.31 (m, 8H, Ar), 5.60 (dd, 1H, *J* = 9.7, 9.7 Hz, H-3), 5.49 (s, 1H, PhC<u>H</u>O_2), 4.83 (d, 1H, *J* = 3.6 Hz, H-1), 4.69 (d, 1H, *J* = 12.4 Hz, PhC<u>H</u>$_2$O), 4.65 (d, 1H, *J* = 12.4 Hz, PhC<u>H</u>$_2$O), 4.28 (dd, 1H, *J* = 10.2, 4.9 Hz, H-6), 3.95 (ddd, 1H, *J* = 9.9, 9.9, 4.8 Hz, H-5), 3.71 (d, 1H, *J* = 10.1, 10.1 Hz, H-6'), 3.69 (ddd, 1H, *J* = 9.7, 7.0, 7.0 Hz, OC<u>H</u>$_2$$CH_2$), 3.60 (dd, 1H, *J* = 9.7, 3.6 Hz, H-2), 3.55 (dd, 1H, *J* = 9.6, 9.6 Hz Hz, H-4), 3.43 (ddd, 1H, *J* = 9.6, 6.8, 6.8 Hz, OC<u>H</u>$_2$$CH_2$), 2.08 (s, 3H, COC<u>H</u>$_3$), 1.68–1.65 (m, 2H, OCH_2C<u>H</u>$_2$), 1.41–1.27 (m, 10H, 5 × C<u>H</u>$_2$), 0.91 (t, 3H, *J* = 7.0 Hz, CH_2C<u>H</u>$_3$); ^{13}C NMR (126 MHz; $CDCl_3$): δ 169.8 (C=O), 138.1 (Ar), 137.1, (Ar) 129.0 (Ar), 128.4 (Ar), 128.2 (Ar), 127.9 (Ar), 127.8 (Ar), 126.2 (Ar), 101.4 (Ph<u>C</u>HO_2), 97.8 (C-1), 79.8 (C-4), 77.7 (C-2), 72.6 (Ph<u>C</u>H_2O), 70.6 (C-3), 69.1 (C-6), 68.7 (O<u>C</u>H_2CH_2), 62.4 (C-5), 31.9 (<u>C</u>H_2), 29.5 (<u>C</u>H_2), 29.4 (<u>C</u>H_2), 29.2 (<u>C</u>H_2), 26.2 (<u>C</u>H_2), 22.7 (<u>C</u>H_2), 21.1 (CO<u>C</u>H_3), 14.1 (CH_2<u>C</u>H_3); HRMS (ESI) Calc. for (M + Na) $C_{30}H_{40}NaO_7$: 535.2666. Found 535.2658.



Octyl 2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-levulinoyl-α-D-glucopyranoside (4-52)

To **4-7** (1.41 g, 3.00 mmol) in CH_2Cl_2 (30 mL) were added DMAP (183 mg, 1.50 mmol), diisopropylcarbodiimide (600 μL, 3.90 mmol), and LevOH (400 μL, 3.90 mmol). The reaction mixture was stirred at room temperature for 20 h. The reaction mixture was diluted with CH_2Cl_2 and washed with H_2O (1×) and brine (1×). The organic layer was dried over Na_2SO_4, filtered, and concentrated. The residue was suspended in Et_2O and the solids were removed by filtration. The filtrate was concentrated and the resulting residue was purified by flash chromatography using a gradient of 5:2→2:1 hexanes–EtOAc to give **4-52** (1.55 g, 91%) as a white solid. R_f 0.43 (1:1 hexanes–EtOAc); $[\alpha]_D$ +30.5 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz; $CDCl_3$): δ 7.48–7.45 (m, 2H, Ar), 7.40–7.31 (m, 8H, Ar), 5.59 (dd, 1H, *J* = 9.7, 9.7 Hz, H-3), 5.49 (s, 1H, PhC$\underline{H}O_2$), 4.79 (d, 1H, *J* = 3.6 Hz, H-1), 4.72 (d, 1H, *J* = 12.4 Hz, PhC$\underline{H}_2$O), 4.64 (d, 1H, *J* = 12.4 Hz, PhC$\underline{H}_2$O), 4.28 (dd, 1H, *J* = 10.2, 4.9 Hz, H-6), 3.93 (ddd, 1H, *J* = 9.9, 9.9, 4.8 Hz, H-5), 3.72 (dd, 1H, *J* = 10.2, 10.2 Hz, H-6'), 3.68 (ddd, 1H, *J* = 9.6, 6.8, 6.8 Hz, OC$\underline{H}_2$CH$_2$), 3.59 (dd, 1H, *J* = 9.8, 3.7 Hz, H-2), 3.56 (dd, 1H, *J* = 9.8, 9.8 Hz, H-4), 3.41 (ddd, 1H, *J* = 9.6, 6.7, 6.7 Hz, OC$\underline{H}_2$CH$_2$), 2.76–2.73 (m, 2H, C$\underline{H}_2$), 2.64–2.61 (m, 2H, C$\underline{H}_2$), 2.15 (s, 3H, COC$\underline{H}_3$), 1.69–1.63 (m, 2H, OCH$_2$C$\underline{H}_2$), 1.42–1.30 (m, 10H, 5 × C$\underline{H}_2$), 0.91 (t, 3H, *J* = 7.0 Hz, CH$_2$C$\underline{H}_3$); ^{13}C NMR (126 MHz; $CDCl_3$): δ 206.4 (C=O), 171.7 (C=O), 138.1 (Ar), 137.1 (Ar), 128.9 (Ar), 128.4 (Ar), 128.2 (Ar), 127.9 (2C, 2 × Ar), 126.2 (Ar), 101.4 (Ph$\underline{C}HO_2$), 97.8 (C-1), 79.7 (C-4), 77.7 (C-2), 72.7 (Ph$\underline{C}H_2$O), 71.0 (C-3), 69.0 (C-6), 68.7 (O$\underline{C}H_2$CH$_2$), 62.4 (C-5), 38.1 ($\underline{C}H_2$), 31.9 ($\underline{C}H_2$), 29.9 (CO$\underline{C}H_3$), 29.5 ($\underline{C}H_2$), 29.4 ($\underline{C}H_2$), 29.2 ($\underline{C}H_2$), 28.1

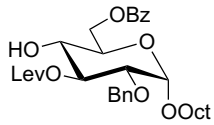
($\underline{C}H_2$), 26.2 ($\underline{C}H_2$), 22.7 ($\underline{C}H_2$), 14.1 ($CH_2\underline{C}H_3$); HRMS (ESI) Calc. for (M + Na) $C_{33}H_{44}NaO_8$: 591.2928. Found 591.2927.



Octyl 2-*O*-benzyl-3-*O*-levulinoyl-α-D-glucopyranoside (4-53)

To **4-52** (916 mg, 1.61 mmol) in a 1:1 mixture of CH_2CH_2–CH_3OH (16 mL) was added PTSA (306 mg, 1.61 mmol). The reaction mixture was stirred for 18 h, at which point additional PTSA (100 mg, 0.54 mmol) was added. The reaction mixture was stirred for an additional 16 h. The reaction mixture was poured over a saturated $NaHCO_3$ solution and extracted with EtOAc (3×). The organic layers were combined, dried over Na_2SO_4, filtered, and concentrated. The resulting residue was purified by flash chromatography using a gradient of 20:1→15:1 CH_2Cl_2–CH_3OH to give **4-53** (655 mg, 85%) as a clear oil. R_f 0.31 (15:1 CH_2Cl_2–CH_3OH); $[\alpha]_D$ +60.1 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz; $CDCl_3$): δ 7.37–7.28 (m, 5H, Ar), 5.33 (dd, 1H, *J* = 9.8, 9.1 Hz, H-3), 4.78 (d, 1H, *J* = 3.6 Hz, H-1), 4.67 (d, 1H, *J* = 12.4 Hz, PhC$\underline{H}_2$O), 4.61 (d, 1H, *J* = 12.4 Hz, PhC$\underline{H}_2$O), 3.87–3.80 (m, 2H, H-6, H-6'), 3.72 (ddd, *J* = 9.8, 3.8, 3.8 Hz, H-5), 3.69–3.62 (m, 2H, H-4, OC$\underline{H}_2CH_2$), 3.50 (d, 1H, *J* = 9.9, 3.6 Hz, H-2), 3.39 (ddd, 1H, *J* = 9.6, 6.7, 6.7 Hz, OC$\underline{H}_2CH_2$), 3.30 (br. s, 1H, O$\underline{H}$), 2.90–2.70 (m, 1H, C$\underline{H}_2$), 2.74 (dt, 1H, *J* = 18.5, 5.9 Hz, C$\underline{H}_2$), 2.62 (ddd, 1H, *J* = 16.7, 8.3, 5.3 Hz, C$\underline{H}_2$), 2.53 (ddd, 1H, *J* = 16.7, 6.3, 5.8 Hz, OC$\underline{H}_2$), 2.19 (s, 3H, COC$\underline{H}_3$), 2.10 (s, 1H, O$\underline{H}$), 1.66–1.61 (m, 2H, OCH$_2$C$\underline{H}_2$), 1.39–1.26 (m, 10H, 5 × C$\underline{H}_2$), 0.90 (t, 3H, *J* = 7.0 Hz, $CH_2C\underline{H}_3$); ^{13}C NMR (126 MHz; $CDCl_3$): δ 207.6 (C=O), 173.6 (C=O), 138.2 (Ar), 128.4 (Ar), 127.9 (Ar), 127.8 (Ar), 96.8 (C-1),

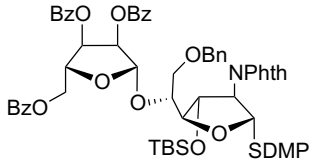
75.8 (2C, C-2, C-3), 72.6 (Ph<u>C</u>H_2O), 70.9 (C-5), 70.4 (C-4), 68.4 (O<u>C</u>$H_2$$CH_2$), 62.3 (C-6), 38.4 (<u>C</u>H_2), 31.9 (<u>C</u>H_2), 29.8 (CO<u>C</u>H_3), 29.4 (2C, 2 × <u>C</u>H_2), 29.2 (<u>C</u>H_2), 28.3 (<u>C</u>H_2), 26.2 (<u>C</u>H_2), 22.7 (<u>C</u>H_2), 14.1 (CH_2<u>C</u>H_3); HRMS (ESI) Calc. for (M + Na) $C_{26}H_{40}NaO_8$: 503.2615. Found 503.2615.



Octyl 6-*O*-benzoyl-2-*O*-benzyl-3-*O*-levulinoyl-α-D-glucopyranoside (4-51)

To **4-53** (629 mg, 1.31 mmol) in CH_2Cl_2 (13 mL) were added Et_3N (1.8 mL, 13.09 mmol) and Bz_2O (444 mg, 1.96 mmol). The reaction mixture was stirred at room temperature for 26 h at which point additional Bz_2O (222 mg, 0.98 mmol) was added. The reaction mixture was stirred for an additional 20 h. The reaction mixture was diluted with CH_2Cl_2, washed with a saturated solution of NH_4Cl (1×), H_2O (1×), and a saturated solution of $NaHCO_3$ (2×). The organic layer was dried over Na_2SO_4, filtered, and concentrated. The resulting residue was purified by flash chromatography in 20:1 CH_2Cl_2–CH_3OH to give **4-51** (624 mg, 82%) as a clear oil. R_f 0.46 (20:1 CH_2Cl_2–CH_3OH); $[\alpha]_D$ +58.5 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz; $CDCl_3$): δ 8.07 (dt, 2H, *J* = 8.3, 1.5 Hz, Ar), 7.60–7.58 (m, 1H, Ar), 7.47–7.44 (m, 2H, Ar), 7.37–7.29 (m, 5H), 5.36 (dd, 1H, *J* = 9.5, 9.5 Hz, H-3), 4.82 (d, 1H, *J* = 3.6 Hz, H-1), 4.68 (d, 1H, *J* = 12.4 Hz, PhC<u>H</u>$_2$O), 4.63–4.60 (m, 3H, H-6, H-6', PhC<u>H</u>$_2$O), 4.01 (ddd, 1H, *J* = 10.0, 5.2, 2.5 Hz, H-5), 3.68 (ddd, 1H, *J* = 9.7, 7.0, 7.0 Hz, OC<u>H</u>$_2$$CH_2$), 3.61 (ddd, 1H, *J* = 9.6, 9.6, 3.2 Hz, H-4), 3.55 (dd, 1H, *J* = 9.8, 3.6 Hz, H-2), 3.42 (ddd, 1H, *J* = 9.7, 6.7, 6.7 Hz, OC<u>H</u>$_2$$CH_2$), 3.30 (d, 1H, *J* = 3.4 Hz, C4-O<u>H</u>), 2.89–2.74 (m, 2H, C<u>H</u>$_2$), 2.67–2.53 (m, 2H, C<u>H</u>$_2$), 2.19

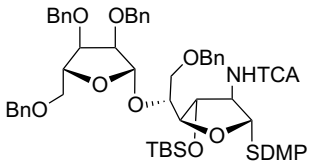
(s, 3H, $COC\underline{H}_3$), 1.68–1.62 (m, 2H, $OCH_2C\underline{H}_2$), 1.37–1.25 (m, 10H, 5 × $C\underline{H}_2$), 0.90 (t, 3H, J = 7.0 Hz, $CH_2C\underline{H}_3$); ^{13}C NMR (126 MHz; $CDCl_3$): δ 207.4 (C=O), 173.6 (C=O), 166.7 (C=O), 138.2 (Ar), 133.1 (Ar), 129.9 (Ar), 129.8 (Ar), 128.4 (2C, 2 × Ar), 127.9 (Ar), 127.8 (Ar), 96.7 (C-1), 76.8 (C-2), 75.6 (C-3), 72.7 ($Ph\underline{C}H_2O$), 70.1 (C-4), 69.7 (C-5), 68.5 ($O\underline{C}H_2CH_2$), 63.8 (C-6), 38.4 ($\underline{C}H_2$), 31.8 ($\underline{C}H_2$), 29.8 ($CO\underline{C}H_3$), 29.4 (2C, 2 × $\underline{C}H_2$) 29.3 ($\underline{C}H_2$), 28.3 ($\underline{C}H_2$), 26.2 ($\underline{C}H_2$), 22.7 ($\underline{C}H_2$), 14.1 ($CH_2\underline{C}H_3$); HRMS (ESI) Calc. for (M + Na) $C_{33}H_{44}NaO_9$: 607.2878. Found 607.2879.



2,6-Dimethylphenyl 2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl-(1→5)-6-*O*-benzyl-2-deoxy-2-*N*-phthalimido-3-*O*-*tert*-butyldimethylsilyl-1-thio-β-D-galactofuranoside (4-59)

To **4-39** (460 mg, 0.73 mmol) and 4Å MS (400 mg) under argon was added CH_2Cl_2 (3 mL). The mixture was stirred at room temperature for 2 h and cooled to –30 °C before AgOTf (224 mg, 0.87 mmol) and $SrCO_3$ (731 mg, 4.95 mmol) were added. To this mixture was added a solution of **4-13** (0.83 mmol) in CH_2Cl_2 (4 mL) dropwise over ~2 min. The reaction mixture was stirred for 20 min before Et_3N (~200 μL) was added. The mixture was passed through a bed of Celite and the filtrate was concentrated. The resulting residue was purified by flash chromatography (dry loading) using a gradient of 3:1→2:1 hexanes–acetone to give **4-59** (331 mg, 42%) as a clear oil. R_f 0.32 (3:1 hexanes–acetone); $[\alpha]_D$ −18.2 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.01–7.99 (m, 3H, Ar), 7.90–7.83 (m, 2H, Ar), 7.74–7.72 (m, 2H, Ar), 7.60–7.40 (m, 2H, Ar),

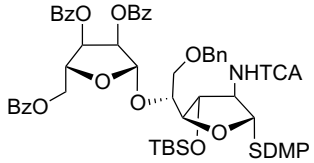
7.60–7.44 (m, 6H, Ar), 7.34–7.20 (m, 9H, Ar), 7.07–7.02 (m, 3H, Ar), 5.90 (dd, 1H, J = 4.6, 2.3 Hz, H-3$_{Rib\mathit{f}}$), 5.79 (dd, 1H, J = 4.8, 1.3 Hz, H-2$_{Rib\mathit{f}}$), 5.74 (d, 1H, J = 1.2 Hz, H-1$_{Rib\mathit{f}}$), 5.48 (d, 1H, J = 7.5 Hz, H-1$_{Gal\mathit{f}N}$), 5.13 (dd, 1H, J = 7.6, 7.6 Hz, H-3$_{Gal\mathit{f}N}$), 4.77 (dd, 1H, J = 7.5, 7.5 Hz, H-2$_{Gal\mathit{f}N}$), 4.69–4.67 (m, 3H, H-4$_{Rib\mathit{f}}$, H-5$_{Rib\mathit{f}}$, H-5'$_{Rib\mathit{f}}$), 4.61 (d, 1H, J = 11.8 Hz, PhC$\underline{H}_2$O), 4.55 (d, 1H, J = 11.8 Hz, PhC$\underline{H}_2$O), 4.21 (dd, 1H, J = 7.7, 2.3 Hz, H-4$_{Gal\mathit{f}N}$), 4.15 (ddd, 1H, J = 7.7, 4.0, 2.4 Hz, H-5$_{Gal\mathit{f}N}$), 3.83 (dd, 1H, J = 10.0, 7.8 Hz, H-6$_{Gal\mathit{f}N}$), 3.75 (dd, 1H, J = 10.1, 4.1 Hz, H-6'$_{Gal\mathit{f}N}$), 2.40 (s, 6H, 2 × ArC$\underline{H}_3$), 0.84 (s, 9H, SiC(C$\underline{H}_3$)$_3$), 0.05 (s, 3H, SiC$\underline{H}_3$), –0.23 (s, 3H, SiC$\underline{H}_3$); ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.3 (C=O), 166.1 (C=O), 165.2 (C=O), 165.1 (C=O), 143.5 (Ar), 137.9 (Ar), 134.2 (Ar), 133.2 (2C, 2 × Ar), 132.9 (Ar), 131.5 (2C, 2 × Ar), 130.0 (Ar), 129.8 (3C, 3 × Ar), 129.7 (Ar), 129.5 (Ar), 129.2 (Ar), 128.8 (Ar), 128.4 (Ar), 128.3 (2C, 2 × Ar), 128.2 (Ar), 128.1 (Ar), 127.7 (Ar), 127.6 (Ar), 123.6 (Ar), 106.4 (C-1$_{Rib\mathit{f}}$), 87.7 (C-1$_{Gal\mathit{f}N}$), 82.2 (C-4$_{Gal\mathit{f}N}$), 78.7 (C-4$_{Rib\mathit{f}}$), 76.2 (C-5$_{Gal\mathit{f}N}$), 76.0 (C-2$_{Rib\mathit{f}}$), 73.6 (Ph$\underline{C}$H$_2$O), 72.7 (C-3$_{Rib\mathit{f}}$), 71.9 (C-3$_{Gal\mathit{f}N}$), 71.5 (C-6$_{Gal\mathit{f}N}$), 66.2 (C-5$_{Rib\mathit{f}}$), 63.5 (C-2$_{Gal\mathit{f}N}$), 25.6 (SiC($\underline{C}$H$_3$)$_3$), 22.2 (Ar$\underline{C}$H$_3$), 17.7 (Si$\underline{C}$(CH$_3$)$_3$), –4.7 (Si$\underline{C}$H$_3$), –4.8 (Si$\underline{C}$H$_3$); HRMS (ESI) Calc. for (M + Na) $C_{61}H_{63}NNaO_{13}SSi$: 1100.3682. Found 1100.3664.



2,6-Dimethylphenyl 2,3,5-tri-*O*-benzyl-β-D-ribofuranosyl-(1→5)-6-*O*-benzyl-2-deoxy-2-*N*-trichloroacetamido-3-*O*-*tert*-butyldimethylsilyl-1-thio-β-D-galactofuranoside (4-61)

To **4-59** (466 mg, 0.426 mmol) in a solution of 2:1 CH_3OH–CH_2Cl_2 (5 mL) was added a 0.10 M solution of $NaOCH_3$ in CH_3OH dropwise until the solution was a pH of 8–9 as determined by wet pH paper. The reaction mixture was stirred for 18 h and then concentrated. The resulting residue was passed through short silica plug using a gradient of 2:1→1:2 hexanes–EtOAc to give **4-72**. To this residue were added DMF (5 mL), BnBr (405 μL, 3.41 mmol) and then NaH (170 mg, 4.25 mmol). The reaction mixture was stirred at room temperature for 2 h before the excess NaH was quenched by the addition of a 10% solution of AcOH in CH_3OH (1 mL). The mixture was concentrated and then diluted with CH_2Cl_2. The organic layer was washed with brine (1×), dried over Na_2SO_4, filtered, and concentrated. The resulting residue was purified by flash chromatography (dry loading) in 5:1→4:1 hexanes–EtOAc to give **4-61** (442 mg, 91%) as a clear oil. R_f 0.50 (4:1 hexanes–EtOAc); $[\alpha]_D$ −30.7 (*c* 1.0, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.32–7.22 (m, 20H, Ar), 7.15–7.10 (m, 3H, Ar), 5.36 (d, 1H, *J* = 3.1 Hz, H-$1_{Rib/}$), 5.06 (s, 1H, H-$1_{Gal/N}$), 4.57–4.40 (m, 11H, H-$2_{Gal/N}$, H-$3_{Gal/N}$, H-$4_{Gal/N}$, 8 × PhC$\underline{H}_2$O), 4.23 (ddd, 1H, *J* = 6.3, 4.8, 2.8 Hz, H-$4_{Rib/}$), 4.16–4.12 (m, 1H, H-$5_{Gal/N}$), 3.93 (dd, 1H, *J* = 5.8, 5.8 Hz, H-$3_{Rib/}$), 3.82 (dd, 1H, *J* = 5.5, 3.1 Hz, H-$2_{Rib/}$), 3.71 (dd, 1H, *J* = 10.3, 6.4 Hz, H-$6_{Gal/N}$), 3.63 (dd, 1H, *J* = 10.3, 4.9 Hz, H-6'$_{Gal/N}$), 3.57 (dd, 1H, *J* = 10.5, 4.4 Hz, H-$5_{Rib/}$), 3.52 (dd, 1H, *J* = 10.5, 5.2 Hz, H-$5_{Rib/}$), 2.54 (s, 6H, 2 × ArC$\underline{H}_3$), 0.96 (s, 9H, SiC(C$\underline{H}_3$)$_3$),

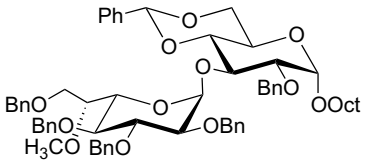
0.13 (s, 6H, 2 × SiC$\underline{H}_3$); ^{13}C NMR (126 MHz, $CDCl_3$) δ 161.4 (C=O), 143.7 (Ar), 137.7 (3C, 3 × Ar), 137.6 (Ar), 133.3 (Ar), 128.7 (Ar), 128.5, 128.4 (2C, 2 × Ar), 128.1 (Ar), 127.9 (2C, 2 × Ar), 127.8 (2C, 2 × Ar), 127.7 (Ar), 127.6 (Ar), 106.8 (C-$1_{Rib\mathit{f}}$), 94.0 (C-$1_{Gal\mathit{f}N}$), 92.3 (CO$\underline{C}Cl_3$), 85.7 (C-$4_{Gal\mathit{f}N}$), 80.2 (C-$4_{Rib\mathit{f}}$), 79.4 (C-$2_{Rib\mathit{f}}$), 76.8 (C-$3_{Rib\mathit{f}}$), 76.3 (2C, C-$3_{Gal\mathit{f}N}$, C-$5_{Gal\mathit{f}N}$), 73.9 (Ph$\underline{C}H_2O$), 73.2 (Ph$\underline{C}H_2O$), 72.2 (Ph$\underline{C}H_2O$), 71.9 (Ph$\underline{C}H_2O$), 70.3 (C-$5_{Rib\mathit{f}}$), 70.0 (C-$6_{Gal\mathit{f}N}$), 65.7 (C-$2_{Gal\mathit{f}N}$), 25.7 (SiC($\underline{C}H_3)_3$), 22.4 (Ar$\underline{C}H_3$), 17.8 (Si$\underline{C}(CH_3)_3$), –4.61 (Si$\underline{C}H_3$), –4.75 (Si$\underline{C}H_3$); HRMS (ESI) Calc. for (M + Na) $C_{55}H_{66}Cl_3NNaO_9SSi$: 1072.3185. Found 1072.3200.



2,6-Dimethylphenyl 2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl-(1→5)-6-*O*-benzyl-2-deoxy-2-trichloroacetamido-3-*O*-*tert*-butyldimethylsilyl-1-thio-β-D-galactofuranoside (4-63)

To **4-43** (400 mg, 0.62 mmol) and 4Å MS (400 mg) under argon was added CH_2Cl_2 (3 mL). The mixture was stirred at room temperature for 2 h and then cooled to –30 °C before AgOTf (205 mg, 0.80 mmol) and $SrCO_3$ (731 mg, 4.95 mmol) were added. To this mixture was added a solution of **4-13** (0.800 mmol) in CH_2Cl_2 (4 mL) dropwise over ~2 min. The reaction mixture was stirred for 20 min before Et_3N (~200 μL) was added. The mixture was passed through a bed of Celite and the filtrate was concentrated. The resulting residue was purified by flash chromatography (dry loading) using a gradient of 7:2→2:1 hexanes–EtOAc to give **4-63** (353 mg, 52%) as a clear oil. $[\alpha]_D$ −20.8 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.03–8.01 (m, 2H, Ar), 7.96–7.89

(m, 5H, N<u>H</u>$_{Gal/N}$, Ar), 7.59–7.53 (m, 2H, Ar), 7.50–7.47 (m, 1H, Ar), 7.41–7.23 (m, 11H, Ar), 7.13–7.07 (m, 3H, Ar), 5.75 (dd, 1H, J = 5.8, 5.8 Hz, H-3$_{Rib/}$), 5.69 (d, 1H, J = 1.7 Hz, H-1$_{Rib/}$), 5.61 (dd, 1H, J = 5.3, 1.7 Hz, H-2$_{Rib/}$), 4.95 (d, 1H, J = 3.8 Hz, H-1$_{Gal/N}$), 4.73 (dd, 1H, J = 11.5, 3.7 Hz, H-5$_{Rib/}$), 4.69–4.65 (m, 1H, H-4$_{Rib/}$), 4.60 (dd, 1H, J = 11.5, 5.3 Hz, H-5$_{Rib/}$), 4.57–4.53 (m, 3H, H-2$_{Gal/N}$, H-3$_{Gal/N}$, PhC<u>H</u>$_2$O), 4.50 (d, 1H, J = 11.9 Hz, PhC<u>H</u>$_2$O), 4.29 (dd, 1H, J = 6.3, 2.8 Hz, H-4$_{Gal/N}$), 4.15 (ddd, 1H, J = 7.0, 4.3, 2.8 Hz, H-5$_{Gal/N}$), 3.71 (dd, 1H, J = 10.4, 7.1 Hz, H-6$_{Gal/N}$), 3.64 (dd, 1H, J = 10.4, 4.5 Hz, H-6'$_{Gal/N}$), 2.50 (s, 6H, 2 × ArC<u>H</u>$_3$), 0.95 (s, 9H, SiC(C<u>H</u>$_3$)$_3$), 0.18 (s, 3H, SiC<u>H</u>$_3$), 0.17 (s, 3H, SiC<u>H</u>$_3$); ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.4 (C=O), 165.7 (C=O), 165.3 (C=O), 161.8 (C=O), 143.6 (Ar), 137.7 (Ar), 133.6 (Ar), 133.5 (Ar), 133.3 (Ar), 132.5 (Ar), 129.8 (Ar), 129.7 (2C, 2 × Ar), 129.6 (Ar), 129.0 (Ar), 128.9 (Ar), 128.8 (Ar), 128.5 (Ar), 128.4 (3C, 3 × Ar), 128.1 (Ar), 127.7 (2C, 2 × Ar), 105.5 (C-1$_{Rib/}$), 92.5 (CO<u>C</u>Cl$_3$), 92.3 (C-1$_{Gal/N}$), 83.4 (C-4$_{Gal/N}$), 79.3 (C-4$_{Rib/}$), 76.1 (C-2$_{Rib/}$), 75.6 (C-3$_{Gal/N}$), 75.5 (C-5$_{Gal/N}$), 73.6 (Ph<u>C</u>H$_2$O), 72.1 (C-3$_{Rib/}$), 70.2 (C-6$_{Gal/N}$), 65.1 (C-2$_{Gal/N}$), 64.9 (C-5$_{Rib/}$), 25.7 (SiC(<u>C</u>H$_3$)$_3$), 22.3 (Ar<u>C</u>H$_3$), 17.9 (Si<u>C</u>(CH$_3$)$_3$), –4.56 (Si<u>C</u>H$_3$), –4.70 (Si<u>C</u>H$_3$); HRMS (ESI) Calc. for (M + Na) $C_{55}H_{60}Cl_3NNaO_{12}SSi$: 1114.2563. Found 1114.2577.

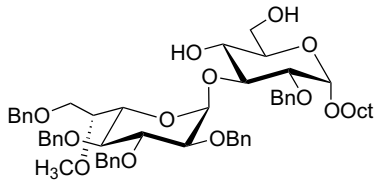


Octyl 2,3,4,7-tetra-*O*-benzyl-6-*O*-methyl-D-*glycero*-α-L-*gluco*-heptopyranosyl-(1→3)-2-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranoside (4-65)

To **3-13** (119 mg, 0.17 mmol) and **4-7** (97 mg, 0.21 mmol) under argon were added CH_2Cl_2 (3.5 mL) and 4Å MS (130 mg). The mixture was stirred at room

temperature for 2 h before cooling to –15 °C. NIS (77 mg, 0.34 mmol) and AgOTf (9 mg, 0.03 mmol) were added and the reaction mixture was stirred for 5.5 h. Et_3N (~100 μL) was added and the mixture was then passed through a bed of Celite. The filtrate was washed with a saturated solution of $Na_2S_2O_3$ (1×), H_2O (1×), and brine (1×). The organic layer was dried over Na_2SO_4, filtered, and concentrated. The resulting residue was then purified by flash chromatography (dry loading) using a gradient of 5:1→4:1 hexanes–EtOAc to afford **4-65** (100 mg, 55%) as a clear oil. R_f 0.33 (4:1 hexanes–EtOAc); $[\alpha]_D$ −9.9 (*c* 1.0, $CHCl_3$); ^{1}H NMR (600 MHz; $CDCl_3$): δ 7.45 (d, 2H, *J* = 7.3 Hz, Ar), 7.38–7.18 (m, 25H, Ar), 7.13–7.07 (m, 3H, Ar), 5.64 (d, 1H, *J* = 3.6 Hz, H-1$_{Hepp}$), 5.58 (s, 1H, PhC$\underline{H}O_2$), 5.04 (d, 1H, *J* = 10.9 Hz, PhC$\underline{H}_2$O), 4.96 (d, 1H, *J* = 11.2 Hz, PhC$\underline{H}_2$O), 4.84 (d, 1H, *J* = 10.6 Hz, PhC$\underline{H}_2$O), 4.83 (d, 1H, *J* = 3.8 Hz, H-1$_{Glc}$), 4.66 (d, 1H, *J* = 11.7 Hz, PhC$\underline{H}_2$O), 4.63 (d, 1H, *J* = 12.0 Hz, PhC$\underline{H}_2$O), 4.59 (d, 1H, *J* = 11.8 Hz, PhC$\underline{H}_2$O), 4.59 (d, 1H, *J* = 11.2 Hz, PhC$\underline{H}_2$O), 4.47 (d, 1H, *J* = 12.0 Hz, PhC$\underline{H}_2$O), 4.31 (dd, 1H, *J* = 10.2, 4.7 Hz, H-6$_{Glc}$), 4.28–4.24 (m, 2H, H-3$_{Glc}$, H-5$_{Hepp}$), 4.15–4.08 (m, 3H, H-3$_{Hepp}$, 2 × PhC$\underline{H}_2$O), 3.81–3.66 (m, 7H, H-2$_{Glc}$, H-4$_{Glc}$, H-4$_{Hepp}$, H-5$_{Glc}$, H-6'$_{Glc}$, H-7$_{Hepp}$, OC$\underline{H}_2$CH$_2$), 3.54–3.53 (m, 2H, H-2$_{Hepp}$, H-6$_{Hepp}$), 3.47–3.44 (m, 4H, H-7'$_{Hepp}$, OC$\underline{H}_3$), 3.34 (ddd, 1H, *J* = 9.7, 6.8, 6.8 Hz, OC$\underline{H}_2$CH$_2$), 1.66–1.61 (m, 2H, OCH$_2$C$\underline{H}_2$), 1.39–1.23 (m, 10H, 5 × C$\underline{H}_2$), 0.89 (t, 3H, *J* = 7.0 Hz, CH$_2$C$\underline{H}_3$); ^{13}C NMR (126 MHz, $CDCl_3$): δ 139.0 (2C, 2 × Ar), 138.7 (Ar), 138.1 (Ar), 137.9 (Ar), 137.0 (Ar), 128.7 (Ar), 128.4 (Ar), 128.3 (2C, 2 × Ar), 128.2 (2C, 2 × Ar), 127.8 (2C, 2 × Ar), 127.7 (Ar), 127.6 (Ar), 127.5 (Ar), 127.4 (2C, 2 × Ar), 127.2 (Ar), 125.8 (Ar), 101.2 (Ph$\underline{C}HO_2$), 97.0 (C-1$_{Glc}$), 96.0 (C-1$_{Hepp}$), 82.1 (C-3$_{Hepp}$), 81.6, 79.8, 79.1, 77.6, 75.5 (Ph$\underline{C}H_2$O), 74.7 (Ph$\underline{C}H_2$O), 73.8 (C-7$_{Hepp}$), 72.8 (Ph$\underline{C}H_2$O), 72.2 (Ph$\underline{C}H_2$O), 71.9 (Ph$\underline{C}H_2$O), 71.5, 70.9, 69.0 (C-6$_{Glc}$), 68.5 (O$\underline{C}H_2$CH$_2$),

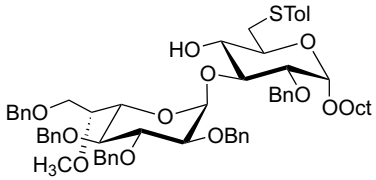
62.8, 59.3 ($O\underline{C}H_3$), 31.9 ($\underline{C}H_2$), 29.5 ($\underline{C}H_2$), 29.4 ($\underline{C}H_2$), 29.3 ($\underline{C}H_2$), 26.1 ($\underline{C}H_2$), 22.7 ($\underline{C}H_2$), 14.1 ($CH_2\underline{C}H_3$); HRMS (ESI) Calc. for (M + NH_4) $C_{64}H_{80}NO_{12}$: 1054.5675. Found 1054.5660.



Octyl 2,3,4,7-tetra-*O*-benzyl-6-*O*-methyl-D-*glycero*-α-L-*gluco*-heptopyranosyl-(1→3)-2-*O*-benzyl-α-D-glucopyranoside (4-66)

To **4-65** (100 mg, 0.96 mmol) in a solution of 1:1 CH_2Cl_2–CH_3OH (2 mL) was added PTSA (16 mg, 0.09 mmol). The reaction mixture was stirred at room temperature for 21 h. The mixture was poured over brine, diluted with equal parts of a saturated $NaHCO_3$ solution and extracted with EtOAc (3×). The organic layers were combined, dried over Na_2SO_4, filtered, and concentrated. The resulting residue was purified by flash chromatography using a gradient of 3:2→1:1 hexanes-EtOAc to give **4-66** (66 mg, 73%) as a clear oil. R_f 0.29 (3:2 hexanes–EtOAc); $[\alpha]_D$ +0.6 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz; $CDCl_3$): δ 7.38–7.27 (m, 25H), 5.43 (d, 1H, *J* = 3.5 Hz, H-1_{Hepp}), 5.03 (d, 1H, *J* = 11.1 Hz, PhC$\underline{H}_2$O), 5.01 (d, 1H, *J* = 11.9 Hz, PhC$\underline{H}_2$O), 4.84 (d, 1H, *J* = 10.8 Hz, PhC$\underline{H}_2$O), 4.77 (d, 1H, *J* = 3.7 Hz, H-1_{Glc}), 4.76 (d, 1H, *J* = 12.2 Hz, PhC$\underline{H}_2$O), 4.67 (d, 1H, *J* = 12.2 Hz, PhC$\underline{H}_2$O), 4.66 (d, 1H, *J* = 12.1 Hz, PhC$\underline{H}_2$O), 4.63 (d, 1H, *J* = 11.3 Hz, PhC$\underline{H}_2$O), 4.62 (d, 1H, *J* = 12.2 Hz, PhC$\underline{H}_2$O), 4.57 (d, 1H, *J* = 12.1 Hz, PhC$\underline{H}_2$O), 4.56 (d, 1H, *J* = 12.2 Hz, PhC$\underline{H}_2$O), 4.26 (dd, 1H, *J* = 9.9, 1.5 Hz, H-5_{Hepp}), 4.13 (dd, 1H, *J* = 9.4, 9.4 Hz, H-3_{hepp}), 4.00 (dd, 1H, *J* = 8.9, 8.9 Hz, H-3_{Glc}), 3.82 (dd, 1H, *J* = 9.5, 7.5 Hz,

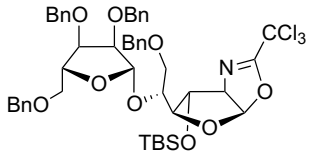
H-7$_{Hepp}$), 3.79–3.73 (m, 2H, H-4$_{Hepp}$, H-6$_{Hepp}$), 3.69–3.64 (m, 3H, H-6$_{Glc}$, H-7'$_{Hepp}$, OC$\underline{H}_2$CH$_2$), 3.61–3.53 (m, 4H, H-2$_{Hepp}$, H-2$_{Glc}$, H-6$_{Glc}$, O$\underline{H}$), 3.52–3.49 (m, 2H, H-4$_{Glc}$, H-5$_{Glc}$), 3.40–3.35 (m, 4H, OC$\underline{H}_2$CH$_2$, OC$\underline{H}_3$), 1.71–1.63 (m, 2H, OCH$_2$C$\underline{H}_2$), 1.43–1.30 (m, 10H, 5 × C$\underline{H}_2$), 0.91 (t, 3H, J = 7.0 Hz, CH$_2$C$\underline{H}_3$); ^{13}C NMR (126 MHz, CDCl$_3$): δ 138.8 (Ar), 138.6 (Ar), 138.5 (Ar), 138.1 (Ar), 137.5 (Ar), 128.5 (Ar), 128.4 (2C, 2 × Ar), 128.3 (2C, 2 × Ar), 128.0 (Ar), 127.9 (2C, 2 × Ar), 127.8 (Ar), 127.6 (3C, 3 × Ar), 127.5 (2C, 2 × Ar), 127.4 (Ar), 97.8 (C-1$_{Hepp}$), 96.7 (C-1$_{Glc}$), 81.7 (C-3$_{Hepp}$), 80.5 (C-3$_{Glc}$), 79.4 (C-2), 79.3 (C-2), 77.2 (C-4$_{Glc}$), 76.0 (C-6$_{Hepp}$), 75.6 (Ph$\underline{C}$H$_2$O), 74.8 (Ph$\underline{C}$H$_2$O), 73.4 (Ph$\underline{C}$H$_2$O), 72.7 (Ph$\underline{C}$H$_2$O), 72.4 (Ph$\underline{C}$H$_2$O), 71.1 (C-4$_{Glc}$/C-5$_{Glc}$), 70.6 (C-4$_{Glc}$/C-5$_{Glc}$), 70.4 (C-5$_{Hepp}$), 68.2 (O$\underline{C}$H$_2$CH$_2$), 67.7 (C-7$_{Hepp}$), 62.8 (C-6$_{Glc}$), 58.7 (O$\underline{C}$H$_3$), 31.9 ($\underline{C}$H$_2$), 29.5 (2C, 2 × $\underline{C}$H$_2$), 29.3 ($\underline{C}$H$_2$), 26.2 ($\underline{C}$H$_2$), 22.7 ($\underline{C}$H$_2$), 14.1 (CH$_2\underline{C}$H$_3$); HRMS (ESI) Calc. for (M + Na) $C_{57}H_{72}NaO_{12}$: 971.4916. Found 917.4905.



Octyl 2,3,4,7-tetra-*O*-benzyl-6-*O*-methyl-D-*glycero*-α-L-*gluco*-heptopyranosyl-(1→3)-2-*O*-benzyl-6-thio-6-*S*-tolyl-α-D-glucopyranoside (4-67)

To **4-66** (160 mg, 0.18 mmol) under argon in pyridine (2 mL) were sequentially added (TolS)$_2$ (169 mg, 0.68 mmol) and a 1.0 M solution of P(CH$_3$)$_3$ in THF (676 μL, 0.68 mmol). The reaction mixture was stirred at room temperature for 20 h. The mixture was poured over brine, diluted with an equal part of H$_2$O and then extracted with EtOAc (3×). The organic layers were combined, dried over Na$_2$O$_4$, filtered, and concentrated.

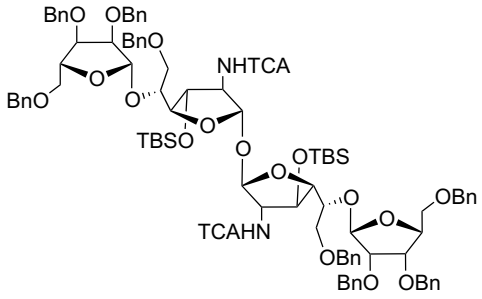
The resulting residue was purified by flash chromatography using a gradient of 4:1→3:1 hexanes–EtOAc to give **4-67** (161 mg, 90%) as a clear oil. R_f 0.53 (3:1 hexanes–EtOAc); $[\alpha]_D$ –0.3 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz; $CDCl_3$): δ 7.37–7.23 (m, 27H, Ar), 7.03–7.02 (m, 2H, Ar), 5.46 (d, 1H, *J* = 3.6 Hz, H-1_{Hepp}), 5.04 (d, 1H, *J* = 10.8 Hz, PhC$\underline{H}_2$O), 5.01 (d, 1H, *J* = 11.3 Hz, PhC$\underline{H}_2$O), 4.83 (d, 1H, *J* = 10.8 Hz, PhC$\underline{H}_2$O), 4.76 (d, 1H, *J* = 3.5 Hz, H-1_{Glc}), 4.74 (d, 1H, *J* = 12.1 Hz, PhC$\underline{H}_2$O), 4.67 (d, 1H, *J* = 12.0 Hz, PhC$\underline{H}_2$O), 4.65 (d, 1H, *J* = 12.4 Hz, PhC$\underline{H}_2$O), 4.62 (d, 1H, *J* = 11.2 Hz, PhC$\underline{H}_2$O), 4.54 (d, 1H, *J* = 12.1 Hz, PhC$\underline{H}_2$O), 4.51 (d, 1H, *J* = 12.2 Hz, PhC$\underline{H}_2$O), 4.47 (d, 1H, *J* = 12.2 Hz, PhC$\underline{H}_2$O), 4.25 (dd, 1H, *J* = 9.9, 1.6 Hz, H-5_{Hepp}), 4.13 (dd, 1H, *J* = 9.4, 9.4 Hz, H-3_{Hepp}), 3.96 (dd, 1H, *J* = 9.1, 9.1 Hz, H-3_{Glc}), 3.79–3.62 (m, 6H, H-4_{Hepp}, H-5_{Glc}, H-6_{Hepp}, H-7_{Hepp}, H-7'$_{Hepp}$, OC$\underline{H}_2$CH_2), 3.60 (dd, 1H, *J* = 9.1, 3.6 Hz, H-2_{Glc}), 3.58 (dd, 1H, *J* = 9.6, 3.6 Hz, H-2_{Hepp}), 3.41–3.32 (m, 6H, H-4_{Glc}, H-6_{Glc}, OC$\underline{H}_2$CH_2, OC$\underline{H}_3$), 2.78 (dd, 1H, *J* = 13.4, 8.7 Hz, H-6'$_{Glc}$), 2.25 (s, 3H, ArC$\underline{H}_3$), 1.61–1.56 (m, 2H, OCH_2C$\underline{H}_2$), 1.37–1.27 (m, 10H, 5 × C$\underline{H}_2$), 0.91 (t, 3H, *J* = 7.0 Hz, CH_2C$\underline{H}_3$); ^{13}C NMR (126 MHz, $CDCl_3$): δ 138.8 (Ar), 138.6 (2C, 2 × Ar), 138.1 (Ar), 137.4 (Ar), 135.7 (Ar), 133.1 (Ar), 129.6 (Ar), 129.4 (Ar), 128.5 (Ar), 128.4 (2C, 2 × Ar), 128.3 (2C, 2 × Ar), 128.0 (2C, 2 × Ar), 127.9 (Ar), 127.7 (Ar), 127.6 (2C, 2 × Ar), 127.5 (2C, 2 × Ar), 127.4 (Ar), 97.7 (C-1_{Hepp}), 96.4 (C-1_{Glc}), 81.7 (C-3_{Hepp}), 80.1 (C-3_{Glc}), 79.5 (C-2), 79.4 (C-2), 77.2 (C-4_{Hepp}), 76.0 (C-6_{Hepp}), 75.6 (Ph$\underline{C}H_2$O), 74.8 (Ph$\underline{C}H_2$O), 73.3 (Ph$\underline{C}H_2$O), 73.1 (C-4_{Glc}), 72.6 (Ph$\underline{C}H_2$O), 72.4 (Ph$\underline{C}H_2$O), 70.3 (C-5_{Glc}), 70.2 (C-5_{Hepp}), 67.9 (O$\underline{C}H_2$CH_2), 67.4 (C-7_{Hepp}), 58.6 (O$\underline{C}H_3$), 36.4 (C-6_{Glc}), 31.9 ($\underline{C}H_2$), 29.6 ($\underline{C}H_2$), 29.5 ($\underline{C}H_2$), 29.4 ($\underline{C}H_2$), 26.3 ($\underline{C}H_2$), 22.7 ($\underline{C}H_2$), 20.9 (Ar$\underline{C}H_3$), 14.1 (CH_2C$\underline{H}_3$); HRMS (ESI) Calc. for (M + Na) $C_{64}H_{78}NaO_{11}S$: 1077.5157. Found 1077.5146.



2,3,5-tri-*O*-benzyl-β-D-ribofuranosyl-(1→5)-2-amino-6-*O*-benzyl-3-*O*-*tert*-butyldimethylsilyl-1-*O*,2-*N*-(trichloroetylylidene)-α-D-galactofuranose (4-69)

To **4-61** (263 mg, 0.25 mmol) and 4Å MS (300 mg) under argon was added CH_2Cl_2 (5 mL). The mixture was stirred at room temperature for 2 h before cooling to –20 °C and then NIS (73 mg, 0.32 mmol) and AgOTf (8 mg) were then sequentially added. The reaction mixture was stirred for 40 min and then Et_3N (~100 μL) was added. The mixture was passed through a bed of Celite and the filtrate was washed with a saturated $Na_2S_2O_3$ solution (1×) and brine (1×). The organic layer was dried over Na_2SO_4, filtered, and concentrated. The resulting residue was purified by flash chromatography to give **4-81** (169 mg, 64%) as a clear oil. R_f 0.55 (4:1 hexanes–EtOAc); $[\alpha]_D$ –3.0 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz; $CDCl_3$): δ 7.38–7.29 (m, 20H, Ar), 6.42 (d, 1H, *J* = 5.4 Hz, H-$1_{Galf/NAc}$), 5.32 (s, 1H, H-1_{Ribf}), 4.72 (br. s, 1H, H-$3_{Galf/NAc}$), 4.65–4.50 (m, 8H, H-$2_{Galf/NAc}$, 8 × PhC$\underline{H}_2$O), 4.37 (app. q, 1H, *J* = 6.0 Hz, H-4_{Ribf}), 4.18 (dd, 1H, *J* = 5.0, 2.0 Hz, H-$4_{Galf/NAc}$), 4.02 (dd, 1H, *J* = 6.4, 5.1 Hz, H-3_{Ribf}), 3.93–3.88 (m, 2H, H-2_{Ribf}, H-$5_{Galf/NAc}$), 3.72 (ddd, 2H, *J* = 4.9, 4.2, 3.3 Hz, H-5_{Ribf}, H-5'$_{Ribf}$), 3.64 (dd, 1H, *J* = 10.4, 4.6 Hz, H-$6_{Galf/NAc}$), 3.57 (dd, 1H, *J* = 10.4, 5.9 Hz, H-6'$_{Galf/NAc}$), 0.91 (s, 9H, SiC(C$\underline{H}_3$)$_3$), 0.17 (s, 3H, SiC$\underline{H}_3$), 0.11 (s, 3H, SiC$\underline{H}_3$); ^{13}C NMR (126 MHz, $CDCl_3$): δ 161.4 (C=N), 138.4 (Ar), 138.0 (2C, 2 × Ar), 137.9 (Ar), 128.4 (Ar), 128.3 (3C, 3 × Ar), 127.8 (2C, 2 × Ar), 127.7 (2C, 2 × Ar), 127.6 (2C, 2 × Ar), 127.4 (Ar), 110.8 (C-$1_{Galf/NAc}$), 105.1 (C-1_{Ribf}), 89.6 (C-$4_{Galf/NAc}$), 86.5 (CO$\underline{C}$Cl$_3$), 81.0 (C-$2_{Galf/NAc}$), 80.4 (C-4_{Ribf}), 79.9 (C-2_{Ribf}), 78.7 (C-3_{Ribf}), 76.0 (C-$3_{Galf/NAc}$), 75.3 (C-$5_{Galf/NAc}$), 73.4 (Ph$\underline{C}$H$_2$O), 73.1 (Ph$\underline{C}$H$_2$O), 72.2 (Ph$\underline{C}$H$_2$O), 71.9

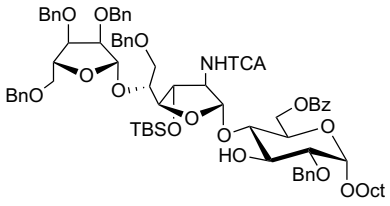
(2C, C-5_{Ribf}, Ph<u>C</u>H_2O), 69.8 (C-$6_{Galf NAc}$), 25.7 (SiC(<u>C</u>H_3)$_3$), 17.8 (Si<u>C</u>$(CH_3)_3$), –4.7 (2C, 2 × Si<u>C</u>H_3); HRMS (ESI) Calc. for (M + Na) $C_{47}H_{56}Cl_3NNaO_9Si$: 934.2694. Found 934.2675. *Note: the hydrolyzed donor **4-70** (33 mg, 14%) was also isolated.



2,3,5-tri-*O*-benzyl-β-D-ribofuranosyl-(1→5)-3-*O*-*tert*-butyldimethylsilyl-2-deoxy-2-trichloroacetamido-β-D-galactofuranosyl 3-*O*-*tert*-butyldimethylsilyl-2-deoxy-2-trichloroacetamido-β-D-galactofuranosyl-(5→1)-2,3,5-tri-*O*-benzyl-β-D-ribofuranoside (4-73)

By-product **4-73** isolated during the 2 + 2 glycosylation of **4-67** and **4-69**. ^{1}H NMR (700 MHz; $CDCl_3$): 7.31–7.22 (m, 40H, Ar), 7.03 (d, 2H, *J* = 8.4 Hz, N<u>H</u>$_{Galf NAc}$), 5.40 (d, 2H, *J* = 2.9 Hz, H-1_{Ribf}), 5.12 (s, 2H, H-$1_{Galf NAc}$), 4.57 (d, 2H, *J* = 12.0 Hz, PhC<u>H</u>$_2$O), 4.53–4.46 (m, 14H, H-$3_{Galf NAc}$, 6 × PhC<u>H</u>$_2$O), 4.43 (d, 2H, *J* = 11.7 Hz, PhC<u>H</u>$_2$O), 4.21 (app. q, 2H, *J* = 5.5 Hz, H-4_{Ribf}), 4.17–4.15 (m, 4H, H-$2_{Galf NAc}$, H-$4_{Galf NAc}$), 4.09 (ddd, 2H, *J* = 6.6, 3.3, 3.3 Hz, H-$5_{Galf NAc}$), 3.90 (dd, 2H, *J* = 5.9, 5.9 Hz, H-3_{Ribf}), 3.84 (dd, 2H, *J* = 5.5, 3.0 Hz, H-2_{Ribf}), 3.77 (dd, 2H, *J* = 10.7, 6.9 Hz, H-$6_{Galf NAc}$), 3.69 (dd, 2H, *J* = 10.7, 3.6 Hz, H-6'$_{Galf NAc}$), 3.56–3.51 (m, 4H, H-5_{Ribf}, H-5'$_{Ribf}$), 0.87 (s, 18H, SiC(C<u>H</u>$_3$)$_3$), 0.06 (s, 6H, 2 × SiC<u>H</u>$_3$); ^{13}C NMR (126 MHz; $CDCl_3$): δ 161.3, 137.8, 137.8 (2C), 128.5, 128.4 (3C), 128.3, 127.9, 127.8, 127.7 (5C), 127.6, 106.8, 102.0, 92.4, 85.8,

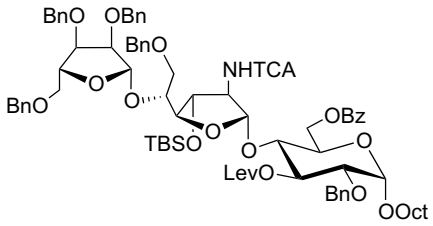
79.8, 79.5, 77.1, 76.9, 76.3, 73.8, 73.1, 72.2, 71.8, 71.0, 70.6, 64.8, 53.4, 25.8, 17.9, –4.67, –4.72; LRMS (ESI) Calc. for (M + Na) $C_{94}H_{114}Cl_6N_2NaO_{19}Si_2$: 1863.6. Found 1862.6.



Octyl 2,3,5-tri-*O*-benzyl-β-D-ribofuranosyl-(1→5)-6-*O*-benzyl-3-*tert*-butyldimethylsilyl-2-deoxy-2-trichloroacetamido-β-D-galactofuranosyl-(1→4)-6-*O*-benzoyl-2-*O*-benzyl-α-D-glucopyranoside (4-74)

To **4-75** (109 mg, 0.07 mmol) in a 1:1 mixture of CH_2Cl_2–CH_3OH (1.5 mL) was added a freshly prepared 1 M solution of H_2NNH_2 in a 1:1 mixture AcOH–pyridine (220 μL, 0.22 mmol). The reaction mixture was stirred at room temperature for 2.5 h. The mixture was poured over brine, diluted with equal parts H_2O and extracted with EtOAc (3×). The organic layers were combined, dried over Na_2SO_4, filtered, and concentrated. The resulting residue was purified by flash chromatography using a gradient of 3:1→5:2 hexanes–EtOAc to give **4-74** (92 mg, 90%) as a clear oil. R_f 0.43 (3:1 hexanes–EtOAc); $[\alpha]_D$ +7.1 (*c* 1.0, $CHCl_3$); ^{1}H NMR (600 MHz; $CDCl_3$): δ 8.04 (dd, 2H, *J* = 8.3, 1.3 Hz, Ar), 7.54–7.51 (m, 1H, Ar), 7.41–7.25 (m, 27H, Ar), 7.19 (d, 1H, *J* = 8.2 Hz, N$\underline{H}_{Galf\text{NAc}}$), 5.33 (d, 1H, *J* = 3.0 Hz, H-1_{Ribf}), 5.04 (d, 1H, *J* = 0.9 Hz, H-$1_{Galf\text{NAc}}$), 4.87 (d, 1H, *J* = 12.3 Hz, PhC$\underline{H}_2$O), 4.75 (d, 1H, *J* = 3.7 Hz, H-1_{Glc}), 4.67 (d, 1H, *J* = 12.3 Hz, PhC$\underline{H}_2$O), 4.62 (dd, 1H, *J* = 12.0, 1.9 Hz, H-6_{Glc}), 4.59–4.52 (m, 8H, H-$3_{Galf\text{NAc}}$, H-6_{Glc}, 6 × PhC$\underline{H}_2$O), 4.50 (d, 1H, *J* = 12.2 Hz, PhC$\underline{H}_2$O), 4.46 (d, 1H, *J* = 11.8 Hz, PhC$\underline{H}_2$O), 4.35 (dd, 1H, *J* = 4.3, 2.7 Hz, H-$4_{Galf\text{NAc}}$), 4.26–4.23 (m, 2H, H-$2_{Galf\text{NAc}}$, H-4_{Ribf}), 4.08–4.06 (m,

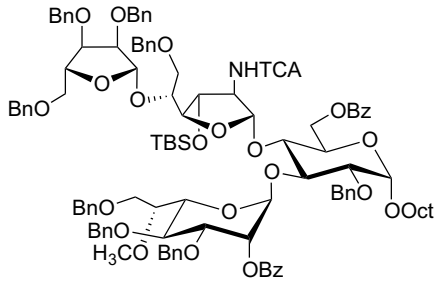
2H, H-3_{Glc}, H-$5_{Galf NAc}$), 4.00–3.95 (m, 3H, H-3_{Ribf}, H-5_{Glc}, C3-O$\underline{H}_{Glc}$), 3.85 (dd, 1H, J = 5.5, 3.1 Hz, H-2_{Ribf}), 3.75 (dd, 1H, J = 10.4, 6.5 Hz, H-$6_{Galf NAc}$), 3.68 (dd, 1H, J = 10.4, 4.8 Hz, H-6'$_{Galf NAc}$), 3.64 (ddd, 1H, J = 9.7, 7.0, 7.0 Hz, OC$\underline{H}_2$CH_2), 3.60–3.57 (m, 2H, H-4_{Glc}, H-5_{Ribf}), 3.54 (dd, 1H, J = 10.7, 5.3 Hz, H-5'$_{Ribf}$), 3.44 (dd, 1H, J = 9.6, 3.7 Hz, H-2_{Glc}), 3.37 (ddd, 1H, J = 9.7, 6.7 Hz, OC$\underline{H}_2$CH_2), 1.66–1.61 (m, 2H, OCH_2C$\underline{H}_2$), 1.36–1.27 (m, 10H, 5 × C$\underline{H}_2$), 0.90 (s, 9H, SiC(C$\underline{H}_3$)$_3$), 0.10 (s, 6H, 2 × SiC$\underline{H}_3$); ^{13}C NMR (126 MHz; $CDCl_3$): δ 166.1 (C=O), 161.7 (C=O), 138.7 (Ar), 137.7 (2C, 2 × Ar), 137.6 (2C, 2 × Ar), 132.9 (Ar), 130.1 (Ar), 129.7 (Ar), 128.5 (2C, 2 × Ar), 128.4 (Ar), 128.3 (2C, 2 × Ar), 127.9 (3C, 3 × Ar), 127.8 (3C, 3 × Ar), 127.6 (Ar), 107.2 (C-$1_{Galf NAc}$), 106.8 (C-1_{Ribf}), 97.0 (C-1_{Glc}), 92.1 (CO$\underline{C}Cl_3$), 85.7 (C-$4_{Galf NAc}$), 80.0 (C-4_{Ribf}), 79.5 (C-2_{Ribf}), 78.8 (2C, C-2_{Glc}, C-4_{Glc}), 76.9 (C-3_{Ribf}), 76.4 (C-$5_{Galf NAc}$), 75.9 (C-$3_{Galf NAc}$), 73.9 (Ph$\underline{C}H_2$O), 73.1 (2C, 2 × Ph$\underline{C}H_2$O), 72.3 (C-3_{Glc}), 72.2 (Ph$\underline{C}H_2$O), 72.0 (Ph$\underline{C}H_2$O), 70.3 (C-5_{Ribf}), 70.0 (C-$6_{Galf NAc}$), 68.4 (O$\underline{C}H_2$CH_2), 68.0 (C-5_{Glc}), 64.8 (C-$2_{Galf NAc}$), 63.3 (C-6_{Glc}), 31.9 ($\underline{C}H_2$), 29.4 (2C, 2 × $\underline{C}H_2$), 29.3 ($\underline{C}H_2$), 26.2 ($\underline{C}H_2$), 25.7 (SiC($\underline{C}H_3$)$_3$), 22.7 ($\underline{C}H_2$), 17.8 (Si$\underline{C}$(CH_3)$_3$), 14.1 (C$H_2\underline{C}H_3$), –4.70 (2C, 2 × Si$\underline{C}H_3$); HRMS (ESI) Calc. for (M + Na) $C_{75}H_{94}Cl_3NNaO_{16}Si$: 1420.5300. Found 1421.5293.



Octyl 2,3,5-tri-*O*-benzyl-β-D-ribofuranosyl-(1→5)-6-*O*-benzyl-3-*tert*-butyldimethylsilyl-2-deoxy-2-trichloroacetamido-β-D-galactofuranosyl-(1→4)-6-*O*-benzoyl-2-*O*-benzyl-3-*O*-levulinoyl-α-D-glucopyranoside (4-75)

To **4-61** (210 mg, 0.18 mmol) and **4-51** (90 mg, 0.15 mmol) under argon were added CH_2Cl_2 (2 mL) and 4Å MS (200 mg). The mixture was stirred at room temperature for 2 h before cooling to –20 °C. NIS (54 mg, 0.24 mmol) and AgOTf (6 mg, 0.02 mmol) were added and the reaction mixture was stirred for 1 h. Additional NIS (27 mg, 0.12 mmol) and AgOTf (3 mg, 0.01 mmol) were added and the reaction mixture was stirred for another 30 min. Et_3N (~100 μL) was added and the mixture was then passed through a bed of Celite. The filtrate was washed with a saturated solution of $Na_2S_2O_3$ (1×), H_2O (1×), and brine (1×). The organic layer was dried over Na_2SO_4, filtered, and concentrated. The resulting residue was then purified by flash chromatography (dry loading) using a gradient of 3:1→5:2 hexanes–EtOAc to afford **4-75** (184 mg, 80%) as a clear oil. R_f 0.48 (5:2 hexanes–EtOAc); $[\alpha]_D$ +12.4 (*c* 1.0, $CHCl_3$); ^{1}H NMR (600 MHz; $CDCl_3$): δ 8.06–8.05 (m, 2H, Ar), 7.55–7.52 (m, 1H, Ar), 7.42–7.40 (m, 2H, Ar), 7.34–7.24 (m, 25H, Ar), 7.23 (d, 1H, J = 7.9 Hz, N$\underline{H}_{Galf\text{NAc}}$), 5.52 (dd, 1H, J = 9.5, 9.5 Hz, H-3$_{Glc}$), 5.37 (d, 1H, J = 2.9 Hz, H-1$_{Ribf}$), 5.02 (s, 1H, H-1$_{Galf\text{NAc}}$), 4.76–4.74 (m, 2H, H-1$_{Glc}$, H-6$_{Glc}$), 4.63 (d, 1H, J = 12.5 Hz, PhC$\underline{H}_2$O), 4.59–4.51 (m, 10H, H-3$_{Galf\text{NAc}}$, H-6'$_{Glc}$, 8 × PhC$\underline{H}_2$O), 4.47 (d, 1H, J = 11.8 Hz, PhC$\underline{H}_2$O), 4.26 (app. q, 1H, J = 5.4 Hz, H-4$_{Ribf}$), 4.15 (dd, 1H, J = 3.9, 3.0 Hz, H-4$_{Galf\text{NAc}}$), 4.10–4.09 (m, 1H, H-2$_{Galf\text{NAc}}$), 4.06–4.03 (m, 2H, H-5$_{Galf\text{NAc}}$, H-5$_{Glc}$), 3.94

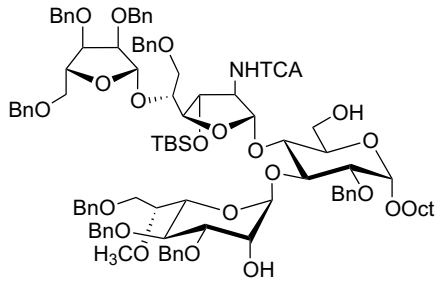
(dd, 1H, J = 5.8, 5.8 Hz, H-3$_{Rib\textit{f}}$), 3.85 (dd, 1H, J = 5.5, 2.9 Hz, H-2$_{Rib\textit{f}}$), 3.78–3.74 (m, 2H, H-4$_{Glc}$, H-6$_{Gal\textit{f}NAc}$), 3.69 (dd, 1H, J = 10.8, 4.0 Hz, H-6$_{Gal\textit{f}NAc}$), 3.62 (ddd, 1H, J = 9.6, 7.0, 7.0 Hz, OC$\underline{H}_2$CH$_2$), 3.58–3.57 (m, 2H, H-5$_{Rib\textit{f}}$, H-5'$_{Rib\textit{f}}$), 3.45 (dd, 1H, J = 9.9, 3.5 Hz, H-2$_{Glc}$), 3.37 (ddd, 1H, J = 9.7, 6.8, 6.8 Hz, OC$\underline{H}_2$CH$_2$), 2.69–2.56 (m, 4H, 2 × C$\underline{H}_2$), 2.08 (s, 3H, COC$\underline{H}_3$), 1.67–1.61 (m, 2H, OCH$_2$C$\underline{H}_2$), 1.33–1.24 (m, 10H, 5 × C$\underline{H}_2$), 0.90 (t, 3H, J = 7.1 Hz, CH$_2$C$\underline{H}_3$), 0.87 (s, 9H, SiC(C$\underline{H}_3$)$_3$), 0.06 (s, 6H, 2 × SiC$\underline{H}_3$); ^{13}C NMR (126 MHz; CDCl$_3$): δ 206.4 (C=O), 171.4 (C=O), 166.0 (C=O), 161.6 (C=O), 138.2 (Ar), 137.8 (3C, 3 × Ar), 137.7 (Ar), 132.9 (Ar), 130.2 (Ar), 129.8 (Ar), 128.5 (Ar), 128.4 (3C, 3 × Ar), 128.3 (2C, 2 × Ar), 127.9 (Ar), 127.8 (4C, 4 × Ar), 127.7 (4C, 4 × Ar), 107.7 (C-1$_{Gal\textit{f}NAc}$), 106.6 (C-1$_{Rib\textit{f}}$), 96.4 (C-1$_{Glc}$), 92.2 (CO$\underline{C}$Cl$_3$), 86.3 (C-4$_{Gal\textit{f}NAc}$), 80.0 (C-4$_{Rob\textit{f}}$), 79.6 (C-2$_{Rib\textit{f}}$), 77.8 (C-2$_{Glc}$), 77.2 (C-3$_{Rib\textit{f}}$), 76.4 (2C, C-4$_{Glc}$, C-5$_{Gal\textit{f}NAc}$), 75.6 (C-3$_{Gal\textit{f}NAc}$), 73.8 (Ph$\underline{C}$H$_2$O), 73.1 (Ph$\underline{C}$H$_2$O), 72.6 (Ph$\underline{C}$H$_2$O), 72.5 (C-3$_{Glc}$), 72.2 (Ph$\underline{C}$H$_2$O), 71.9 (Ph$\underline{C}$H$_2$O), 70.6 (C-5$_{Rib\textit{f}}$), 70.1 (C-6$_{Gal\textit{f}NAc}$), 68.6 (C-5$_{Glc}$), 68.5 (O$\underline{C}$H$_2$CH$_2$), 65.3 (C-2$_{Gal\textit{f}NAc}$), 63.1 (C-6$_{Glc}$), 37.9 ($\underline{C}$H$_2$), 31.9 ($\underline{C}$H$_2$), 29.9 (CO$\underline{C}$H$_3$), 29.4 (2C, 2 × $\underline{C}$H$_2$), 29.3 ($\underline{C}$H$_2$), 28.1 ($\underline{C}$H$_2$), 26.1 ($\underline{C}$H$_2$), 25.7 (SiC($\underline{C}$H$_3$)$_3$), 22.7 ($\underline{C}$H$_2$), 17.8 (Si$\underline{C}$(CH$_3$)$_3$), 14.1 (CH$_2\underline{C}$H$_3$), –4.6 (Si$\underline{C}$H$_3$), –4.7 (Si$\underline{C}$H$_3$); HRMS (ESI) Calc. for (M + Na) $C_{80}H_{100}Cl_3NNaO_{18}Si$: 1518.5667. Found 1520.5660.



Octyl 2,3,5-tri-*O*-benzyl-β-D-ribofuranosyl-(1→5)-6-*O*-benzyl-3-*tert*-butyldimethylsilyl-2-deoxy-2-trichloroacetamido-β-D-galactofuranosyl-(1→4)-[2-*O*-benzoyl-3,4,7-tri-*O*-benzyl-6-*O*-methyl-D-*glycero*-L-*manno*-heptopyranosyl-(1→3)]-6-*O*-benzoyl-2-*O*-benzyl-α-D-glucopyranoside (4-76)

To **4-74** (168 mg, 0.12 mmol) and **3-58** (100 mg, 0.16 mmol) under argon were added 4Å MS (180 mg). The mixture was stirred at room temperature for 2 h before cooling to –30 °C. NIS (54 mg, 0.20 mmol) and AgOTf (5 mg, 0.02 mmol) were added and the reaction mixture was stirred for 30 min. Et_3N (~100 μL) was added and the mixture was then passed through a bed of Celite. The filtrate was washed with a saturated solution of $Na_2S_2O_3$ (1×) and brine (1×). The organic layer was dried over Na_2SO_4, filtered, and concentrated. The resulting residue was then purified by flash chromatography (dry loading) using a gradient of 4:1→3:2 hexanes–EtOAc to afford **4-76** (192 mg, 81%) as a clear oil. R_f 0.43 (3:1 hexanes–EtOAc); $[\alpha]_D$ +31.0 (*c* 1.0, $CHCl_3$); ^{1}H NMR (600 MHz; $CDCl_3$): δ 8.15–8.13 (m, 2H, Ar), 8.06–8.03 (m, 2H, Ar), 7.58–7.50 (m, 3H, N$\underline{H}_{Gal/NAc}$, Ar), 7.45–7.09 (m, 44H, Ar), 5.83 (d, 1H, *J* = 1.3 Hz, H-$1_{Hep/p}$), 5.75 (dd, 1H, *J* = 2.8, 1.9 Hz, H-$2_{Hep/p}$), 5.38 (d, 1H, *J* = 1.8 Hz, H-$1_{Gal/NAc}$), 5.23 (d, 1H, *J* = 2.5 Hz, H-$1_{Rib/f}$), 4.89–4.84 (m, 3H, H-6_{Glc}, 2 × PhC$\underline{H}_2$O), 4.75 (dd, 1H, *J* = 4.0, 2.9 Hz, H-$3_{Gal/NAc}$), 4.70–4.65 (m, 4H, H-1_{Glc}, 3 × PhC$\underline{H}_2$O), 4.59–4.57 (m, 1H, H-6'$_{Glc}$), 4.56 (d, 1H, *J* = 12.0 Hz, PhC$\underline{H}_2$O), 4.51 (d, 1H, *J* = 11.6 Hz, PhC$\underline{H}_2$O), 4.48–4.43 (m, 5H, 5 ×

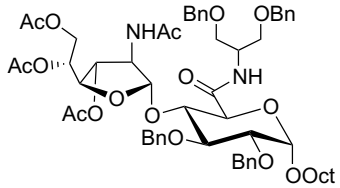
PhC$\underline{H}_2$O), 4.39–4.35 (m, 4H, H-4$_{Galf\underline{N}Ac}$, H-5$_{Hepp}$, 2 × PhC$\underline{H}_2$O), 4.31 (ddd, 1H, *J* = 7.7, 2.1, 2.1 Hz, H-2$_{Galf\underline{N}Ac}$), 4.27–4.22 (m, 4H, H-3$_{Glc}$, H-4$_{Ribf}$, 2 × PhC$\underline{H}_2$O), 4.15 (dd, 1H, *J* = 9.6, 9.6 Hz, H-4$_{Hepp}$), 4.04 (dd, 1H, *J* = 9.5, 3.1 Hz, H-3$_{Hepp}$), 4.01–3.97 (m, 3H, H-6$_{Hepp}$, H-7$_{Hepp}$, H-7'$_{Hepp}$), 3.95–3.87 (m, 4H, H-3$_{Ribf}$, H-4$_{Glc}$, H-5$_{Glc}$, H-5$_{Galf\underline{N}Ac}$), 3.83 (dd, 1H, *J* = 5.2, 2.6 Hz, H-2$_{Ribf}$), 3.77 (dd, 1H, *J* = 11.5, 5.9 Hz, H-6$_{Galf\underline{N}Ac}$), 3.63 (dd, 1H, *J* = 9.6, 3.4 Hz, H-2$_{Glc}$), 3.60–3.54 (m, 4H, OC$\underline{H}_2$CH$_2$, OC$\underline{H}_3$), 3.52–3.45 (m, 3H, H-5$_{Ribf}$, H-5'$_{Ribf}$, H-6'$_{Galf\underline{N}Ac}$), 3.23 (ddd, 1H, *J* = 9.7, 6.9, 6.9 Hz, OC$\underline{H}_2$CH$_2$), 1.59–1.54 (m, 2H, OCH$_2$C$\underline{H}_2$), 1.32–1.25 (m, 10H, 5 × C$\underline{H}_2$), 0.89 (t, 3H, *J* = 7.1 Hz, CH$_2$C$\underline{H}_3$), 0.83 (s, 9H, SiC(C$\underline{H}_3$)$_3$), 0.08 (s, 3H, SiC$\underline{H}_3$), 0.04 (s, 3H, SiC$\underline{H}_3$); ^{13}C NMR (126 MHz; $CDCl_3$): δ 166.2 (C=O), 165.5 (C=O), 162.0 (C=O), 139.0 (Ar), 138.9 (Ar), 138.3 (Ar), 137.9 (Ar), 137.8 (4C, 4 × Ar), 130.1 (2C, 2 × Ar), 130.0 (2C, 2 × Ar), 128.4 (2C, 2 × Ar), 128.3 (3C, 3 × Ar), 128.2 (5C, 5 × Ar), 128.0 (Ar), 127.8 (3C, 3 × Ar), 127.7 (3C, 3 × Ar), 127.5 (Ar), 127.4 (2C, 2 × Ar), 127.2 (Ar), 107.8 (C-1$_{Galf\underline{N}Ac}$), 106.4 (C-1$_{Ribf}$), 97.9 (C-1$_{Hepp}$), 96.0 (C-1$_{Glc}$), 92.4 (CO$\underline{C}$Cl$_3$), 86.8 (C-4$_{Galf\underline{N}Ac}$), 81.4 (C-2$_{Glc}$), 80.0, 79.8, 79.5, 77.5 (2C), 75.7, 75.5, 75.2 (Ph$\underline{C}$H$_2$O), 74.2, 73.7 (Ph$\underline{C}$H$_2$O), 73.4 (Ph$\underline{C}$H$_2$O), 72.9 (Ph$\underline{C}$H$_2$O), 72.7 (2C, 2 × Ph$\underline{C}$H$_2$O), 72.6, 72.1 (Ph$\underline{C}$H$_2$O), 72.0 (Ph$\underline{C}$H$_2$O), 71.7 (C-5$_{Ribf}$), 70.7 (C-6$_{Galf\underline{N}Ac}$), 69.1, 68.8, 68.3 (O$\underline{C}$H$_2$CH$_2$), 64.7 (C-2$_{Galf\underline{N}Ac}$), 62.3 (C-6$_{Glc}$), 58.9 (O$\underline{C}$H$_3$), 31.9 ($\underline{C}$H$_2$), 29.4 ($\underline{C}$H$_2$), 29.3 ($\underline{C}$H$_2$), 26.2 ($\underline{C}$H$_2$), 25.9 (SiC($\underline{C}$H$_3$)$_3$), 22.7 ($\underline{C}$H$_2$), 18.1 (Si$\underline{C}$(CH$_3$)$_3$), 14.1 (CH$_2\underline{C}$H$_3$), –4.5 (Si$\underline{C}$H$_3$), –4.6 (Si$\underline{C}$H$_3$); HRMS (ESI) Calc. for (M + NH$_4$) $C_{111}H_{134}Cl_3N_2O_{23}Si$: 1995.8207. Found 1995.8214.



Octyl 2,3,5-tri-*O*-benzyl-β-D-ribofuranosyl-(1→5)-6-*O*-benzyl-3-*tert*-butyldimethylsilyl-2-deoxy-2-trichloroacetamido-β-D-galactofuranosyl-(1→4)-[3,4,7-tri-*O*-benzyl-6-*O*-methyl-D-*glycero*-L-*manno*-heptopyranosyl-(1→3)]-2-*O*-benzyl-α-D-glucopyranoside (4-77)

To **4-76** (189 mg, 0.10 mmol) in a solution of 2:1 CH_3OH–CH_2Cl_2 (3 mL) was added a 0.1 M solution of $NaOCH_3$ in CH_3OH dropwise until the solution was a pH of 8–9 as determined by wet pH paper. The reaction mixture was stirred for 14 h and then concentrated. The resulting residue was purified by flash chromatography in 2:1 hexanes–EtOAc to give **4-77** (162 mg, 96%) as a clear oil. R_f 0.39 (2:1 hexanes–EtOAc); $[\alpha]_D$ +21.3 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz; $CDCl_3$): δ 7.40–7.17 (m, 41H, N$\underline{H}_{Galf\text{NAc}}$, Ar), 5.62 (d, 1H, *J* = 1.1 Hz, H-1_{Hepp}), 5.24 (d, 1H, *J* = 1.4 Hz, H-$1_{Galf\text{NAc}}$), 5.21 (d, 1H, *J* = 2.7 Hz, H-1_{Ribf}), 4.87–4.85 (m, 2H, 2 × PhC$\underline{H}_2$O), 4.71-4.57 (m, 6H, H-1_{Glc}, H-$3_{Galf\text{NAc}}$, 4 × PhC$\underline{H}_2$O), 4.53–4.42 (m, 8H, 8 × PhC$\underline{H}_2$O), 4.31–4.21 (m, 7H, H-$2_{Galf\text{NAc}}$, H-3_{Glc}, H-$4_{Galf\text{NAc}}$, H-4_{Ribf}, H-5_{hepp}, 2 × PhC$\underline{H}_2$O), 4.04 (dd, 1H, *J* = 9.5, 9.5 Hz, H-4_{Hepp}), 3.97–3.78 (m, 9H, H-2_{Ribf}, H-3_{Ribf}, H-3_{Hepp}, H-4_{Glc}, H-5_{Glc}, H-$5_{Galf\text{NAc}}$, H-6_{Hepp}, H-7_{Hepp}, H-7'$_{Hepp}$), 3.73 (dd, 1H, H-$6_{Galf\text{NAc}}$), 3.65–3.61 (m, 2H, H-6_{Glc}, H-$6_{Galf\text{NAc}}$), 3.59–3.53 (m, 3H, H-2_{Glc}, H-2_{Hepp}, H-6'$_{Glc}$), 3.52–3.47 (m, 5H, H-5_{Ribf}, H-5'$_{Ribf}$, OC$\underline{H}_3$), 3.30 (ddd, 1H, *J* = 9.8, 6.7, 6.7 Hz, OC$\underline{H}_2$CH$_2$), 1.62–1.55 (m, 2H, C$\underline{H}_2$), 1.34–1.23 (m, 10H, 5 × C$\underline{H}_2$), 0.90–0.89 (m, 12H, SiC(C$\underline{H}_3$)$_3$, CH$_2$C$\underline{H}_3$), 0.11 (s, 3H, SiC$\underline{H}_3$), 0.10 (s, 3H, SiC$\underline{H}_3$); ^{13}C NMR

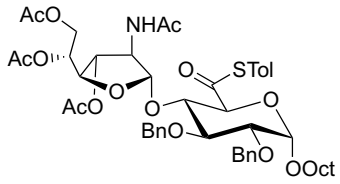
(126 MHz; $CDCl_3$): δ 162.1 (C=O), 147.5 (Ar), 145.2 (Ar), 138.8 (2C, 2 × Ar), 138.1 (Ar), 137.8 (Ar), 137.8 (Ar), 137.7 (Ar), 128.5 (Ar), 128.4 (3C, 3 × Ar), 128.3 (2C, 2 × Ar), 128.2 (Ar), 128.0 (Ar), 127.9 (Ar), 127.8 (5C, 5 × Ar), 127.7 (Ar), 127.6 (2C, 2 × Ar), 127.5 (2C, 2 × Ar), 127.3 (Ar), 123.7 (Ar), 108.4 (C-$1_{Galf/NAc}$), 106.4 (C-1_{Ribf}), 99.0 (C-1_{Hepp}), 95.8 (C-1_{Glc}), 92.3 ($CO\underline{C}Cl_3$), 86.6 (C-$4_{Galf/NAc}$), 81.6 (C-2_{Glc}), 80.8, 79.8 (2C), 77.4 (2C), 75.9, 75.2 ($Ph\underline{C}H_2O$), 74.7, 74.0 ($Ph\underline{C}H_2O$), 73.9 ($Ph\underline{C}H_2O$), 73.6 ($Ph\underline{C}H_2O$), 73.4 ($Ph\underline{C}H_2O$), 72.9 ($Ph\underline{C}H_2O$), 72.5, 72.1 ($Ph\underline{C}H_2O$), 72.0 (2C, $Ph\underline{C}H_2O$), 71.8 (C-5_{Ribf}), 70.7 (3C, C-$6_{Galf/NAc}$), 69.9, 68.5, 68.3 ($O\underline{C}H_2CH_2$), 64.7, 61.1 (C-6_{Glc}), 58.9 ($O\underline{C}H_3$), 31.9 ($\underline{C}H_2$), 29.4 (2C, 2 × $\underline{C}H_2$), 29.3 ($\underline{C}H_2$), 26.2 ($\underline{C}H_2$), 25.9 ($SiC(\underline{C}H_3)_3$), 22.7 ($\underline{C}H_2$), 18.1 ($Si\underline{C}(CH_3)_3$), 14.1 ($CH_2\underline{C}H_3$), –4.6 (2C, 2 × $Si\underline{C}H_3$).



2-*N*-[2-acetamido-3,5,6-tri-*O*-acetyl-2-deoxy-β-D-galactofuranosyl-(1→4)-(octyl 2,3-di-*O*-benzyl-α-D-glucopyranosid)]uronoyl-1,3-di-*O*-benzyl-2-deoxy-glycerol (4-91)

To **4-102** (26 mg, 0.03 mmol) in CH_2Cl_2 (1 mL) were added 1,3-di-*O*-benzyl-2-aminopropane (11 mg, 0.04 mmol) and Et_3N (12 μL, 0.08 mmol). The reaction mixture was stirred 20 h, before additional 2-amino-1,3-di-*O*-benzyl-propane (11 mg, 0.04 mmol) and Et_3N (12 μL, 0.08 mmol) were added and stirred for 24 h. The reaction mixture was concentrated and then co-evaporated with toluene. The resulting residue was purified by flash chromatography in 20:1 CH_2Cl_2–CH_3OH to afford **4-91** (27 mg, 90%) as a clear oil. R_f = 0.53 (15:1 CH_2Cl_2–CH_3OH); $[\alpha]_D$ −13.6 (1.0 *c*, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$)

δ 7.40–7.28 (m, 20H, Ar), 6.73 (d, 1H, J = 8.7 Hz, CONHCH), 6.48 (d, 1H, J = 6.3 Hz, $NH_{Galf/N}$), 5.21 (d, 1H, J = 3.3 Hz, H-$1_{Galf/N}$), 5.11 (ddd, 1H, J = 7.6, 3.9, 3.9 Hz, H-$5_{Galf/N}$), 4.95–4.87 (m, 3H, H-$3_{Galf/N}$, $PhCH_2O$, $PhCH_2O$), 4.78–4.72 (m, 2H, H-1_{Glc}, $PhCH_2O$), 4.63 (d, 1H, J = 12.1 Hz, $PhCH_2O$), 4.59–4.51 (m, 4H, 4 × $PhCH_2O$), 4.34–4.30 (m, 2H, H-$2_{Galf/N}$, $NHCH(CH_2)_2$), 4.19 (dd, 1H, J = 5.6, 4.7 Hz, H-$4_{Galf/N}$), 4.08 (d, 1H, J = 9.8 Hz, H-5_{Glc}), 4.03 (dd, 1H, J = 12.0, 7.5 Hz, H-$6_{Galf/N}$), 3.96 (dd, 1H, J = 12.0, 3.7 Hz, H-$6'_{Galf/N}$), 3.89 (dd, 1H, J = 9.2, 9.2 Hz, H-3_{Glc}), 3.75 (dd, 1H, J = 9.3, 9.3 Hz, H-4_{Glc}), 3.67–3.51 (m, 6H, H-2_{Glc}, OCH_2CH_2, 4 × $CHCH_2O$), 3.40 (ddd, 1H, J = 9.7, 6.9, 6.9 Hz, OCH_2CH_2), 2.09 (s, 3H, $COCH_3$), 2.01 (s, 3H, $COCH_3$), 1.98 (s, 3H, $COCH_3$), 1.95 (s, 3H, $COCH_3$), 1.67–1.61 (m, 2H, OCH_2CH_2), 1.35–1.25 (m, 10H, 5 × CH_2), 0.92 (t, 3H, J = 6.9 Hz, CH_2CH_3); ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.4 (C=O), 170.3 (C=O), 170.2 (C=O), 170.0 (C=O), 169.2 (C=O), 138.9 (Ar), 138.2 (Ar), 137.9 (2C, 2 × Ar), 128.5 (Ar), 128.4 (2C, 2 × Ar), 128.3 (Ar), 128.0 (Ar), 127.9 (Ar), 127.8 (2C, 2 × Ar), 127.7 (2C, 2 × Ar), 127.5 (2C, 2 × Ar), 105.5 (C-$1_{Galf/N}$), 96.9 (C-1_{Glc}), 79.6 (C-3_{Glc}), 79.5 (C-$4_{Galf/N}$), 79.2 (C-2_{Glc}), 77.9 (C-4_{Glc}), 76.6 (C-$3_{Galf/N}$), 75.6 ($PhCH_2O$), 73.3 ($PhCH_2O$), 73.2 ($PhCH_2O$), 73.1 ($PhCH_2O$), 70.6 (C-5_{Glc}), 69.9 (C-$5_{Galf/N}$), 69.0 (OCH_2CH_2), 68.6 ($CHCH_2O$), 68.5 ($CHCH_2O$), 62.8 (C-$6_{Galf/N}$), 60.8 (C-$2_{Galf/N}$), 48.3 ($NHCH(CH_2)_2$), 31.9 (CH_2), 29.4 (2C, CH_2, CH_2), 29.2 (CH_2), 26.1 (CH_2), 23.1 ($COCH_3$), 22.7 (CH_2), 20.9 ($COCH_3$), 20.8 ($COCH_3$), 20.7 ($COCH_3$), 14.1 (CH_2CH_3); HRMS (ESI) Calc. for (M + Na) $C_{59}H_{76}N_2NaO_{16}$: 1091.5087. Found 1091.5078.



_S_-_p_-Tolyl thio[2-acetamido-3,5,6-tri-_O_-acetyl-2-deoxy-β-D-galactofuranosyl-(1→4)-(octyl 2,3-di-_O_-benzyl-α-D-glucopyranosid)]uronate (4-102)

To **4-100** (100 mg, 0.11 mmol) in CCl_4 (2.5 mL) under argon was added anhydrous pyridine (36 μL, 0.44 mmol) via syringe. The mixture was cooled to 0 °C, then SO_2Cl_2 (27 μL, 0.33 mmol) was added via Rayonette pipette. The reaction mixture was stirred for 5 h before being diluted with CH_2Cl_2 and washed with H_2O (1×) and brine (1×). The organic layer was dried over Na_2SO_4, filtered, and concentrated. The resulting residue was diluted with a 3:2 acetone–H_2O solution (2.5 mL) and $NaHCO_3$ (37 mg, 0.440 mmol) was added. The suspended reaction mixture was stirred at room temperature for 28 h, then diluted with H_2O and extracted with EtOAc (3×). The organic layers were combined, washed with brine (1×), dried over Na_2SO_4, filtered, and concentrated. The resulting residue was purified by flash chromatography using a gradient of 1:1→3:2 EtOAc–hexanes to afford **4-102** (78 mg, 77%) as a white solid. R_f = 0.34 (3:2 EtOAc–hexanes); $[\alpha]_D$ +13.7 (1.0 *c*, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.36–7.29 (m, 12H, Ar), 7.24 (d, 2H, *J* = 8.0 Hz, Ar), 5.91 (d, 1H, *J* = 8.3 Hz, N$\underline{H}_{Gal/N}$), 5.11 (ddd, 1H, *J* = 7.0, 4.5, 4.5 Hz, H-$5_{Gal/N}$), 5.07 (s, 1H, H-$1_{Gal/N}$), 5.01 (d, 1H, *J* = 10.9 Hz, PhC$\underline{H}_2$O), 4.85 (d, 1H, *J* = 3.6 Hz, H-1_{Glc}), 4.81 (d, 1H, *J* = 10.9 Hz, PhC$\underline{H}_2$O), 4.78–4.74 (m, 1H, H-$3_{Gal/N}$, PhC$\underline{H}_2$O), 4.63 (d, 1H, *J* = 12.0 Hz, PhC$\underline{H}_2$O), 4.44 (br. d, 1H, *J* = 8.0 Hz, H-$2_{Gal/N}$), 4.37 (d, 1H, *J* = 9.2 Hz, H-5_{Glc}), 4.20 (dd, 1H, *J* = 4.6, 4.6 Hz, H-$4_{Gal/N}$), 4.03–3.94 (m, 3H, H-4_{Glc}, H-$6_{Gal/N}$, H-6'$_{Gal/N}$), 3.92 (dd, 1H, *J* = 9.1, 9.1 Hz, H-3_{Glc}), 3.76 (ddd, 1H, *J* = 9.8, 7.0,

7.0 Hz, OC$\underline{H_2}$CH$_2$), 3.61 (dd, 1H, *J* = 9.2, 3.6 Hz, H-2$_{Glc}$), 3.51 (ddd, 1H, *J* = 9.8, 6.8, 6.8 Hz, OC$\underline{H_2}$CH$_2$), 2.40 (s, 3H, ArC$\underline{H_3}$), 2.07 (s, 3H, COC$\underline{H_3}$), 2.04 (s, 3H, COC$\underline{H_3}$), 1.97 (s, 3H, COC$\underline{H_3}$), 1.91 (s, 3H, COC$\underline{H_3}$), 1.75–1.69 (m, 2H, OCH$_2$C$\underline{H_2}$), 1.45–1.28 (m, 10H, 5 × C$\underline{H_2}$), 0.92 (t, 3H, *J* = 6.9 Hz, CH$_2$C$\underline{H_3}$); ^{13}C NMR (126 MHz, $CDCl_3$) δ 196.2 (C6$_{Glc}$=O), 170.4 (C=O), 170.2 (2C, 2 × C=O), 169.2 (C=O), 140.0 (Ar), 138.7 (Ar), 138.0 (Ar), 134.5 (Ar), 130.1 (Ar), 128.5 (Ar), 128.4 (Ar), 128.1 (Ar), 128.0 (Ar), 127.5 (Ar), 127.3 (Ar), 123.0 (Ar), 106.5 (C-1$_{Gal/N}$), 97.3 (C-1$_{Glc}$), 80.6 (C-4$_{Gal/N}$), 79.6 (2C, C-2$_{Glc}$, C-3$_{Glc}$), 77.8 (C-3$_{Gal/N}$), 76.2 (C-4$_{Glc}$), 75.4 (2C, C-5$_{Glc}$, Ph$\underline{C}$H$_2$O), 73.3 (Ph$\underline{C}$H$_2$O), 70.1 (C-5$_{Gal/N}$), 69.2 (O$\underline{C}$H$_2$CH$_2$), 62.7 (C-6$_{Gal/N}$), 60.1 (C-2$_{Gal/N}$) 31.9 ($\underline{C}$H$_2$), 29.4 (2C, $\underline{C}$H$_2$, $\underline{C}$H$_2$), 29.3 ($\underline{C}$H$_2$), 26.2 ($\underline{C}$H$_2$), 23.1 (CO$\underline{C}$H$_3$), 22.7 ($\underline{C}$H$_2$), 21.4 (Ar$\underline{C}$H$_3$), 20. 9(CO$\underline{C}$H$_3$), 20.7 (2C, CO$\underline{C}$H$_3$, CO$\underline{C}$H$_3$), 14.1 (CH$_2\underline{C}$H$_3$); HRMS (ESI) Calc. for (M + Na) $C_{49}H_{63}NNaO_{14}S$: 944.3861. Found 944.3860.

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Chapter 5

Efforts Towards Understanding MeOPN Biosynthesis in *C. jejuni* 11168H

5.1 Background

As described earlier, approximately 70% of *C. jejuni* strains synthesize the MeOPN motif in their capsular polysaccharide (CPS).[1] The precise role of the MeOPN motif remains unknown; however, studies suggest they play roles in colonization and resistance to killing by human serum by evading the immune system and affecting cytokine production.[2,3] These experiments were described further in Section 1.4.4.1 of this thesis and collectively, suggest that targeting the enzymes involved in the biosynthesis of the MeOPN motif could lead to the development of therapeutic agents specific to *C. jejuni*. However, before these agents can be explored, a better understanding of MeOPN biosynthesis is needed.

5.2 MeOPN Biosynthesis

Since the sequencing of the *C. jejuni* NCTC11168 genome,[4] extensive work has gone into annotating the function of the proteins encoded by the individual genes.[1,5,6] Figure 1a shows the gene cluster involved in the CPS assembly in *C. jejuni* 11168H; Figure 1b shows the structure of the repeating tetrasaccharide unit. The structure of the tetrasaccharide was originally elucidated in 2002,[7] albeit without the MeOPN motifs. These motifs were later identified by high resolution magic angle spinning (HR-MAS) nuclear magnetic resonance (NMR) spectroscopy directly from live campylobacter cells.[8] The lability of the MeOPN to normal CPS extraction and purification methods was presumably the reason that these groups had not been previously detected. Using HR-MAS NMR spectroscopy, Szymanski and coworkers showed the

commonality of the MeOPN in *C. jejuni*.[1] In addition, they identified the genes involved in the biosynthesis of the MeOPN motif found in *C. jejuni* 11168H (shown in red in Figure 5-1a).

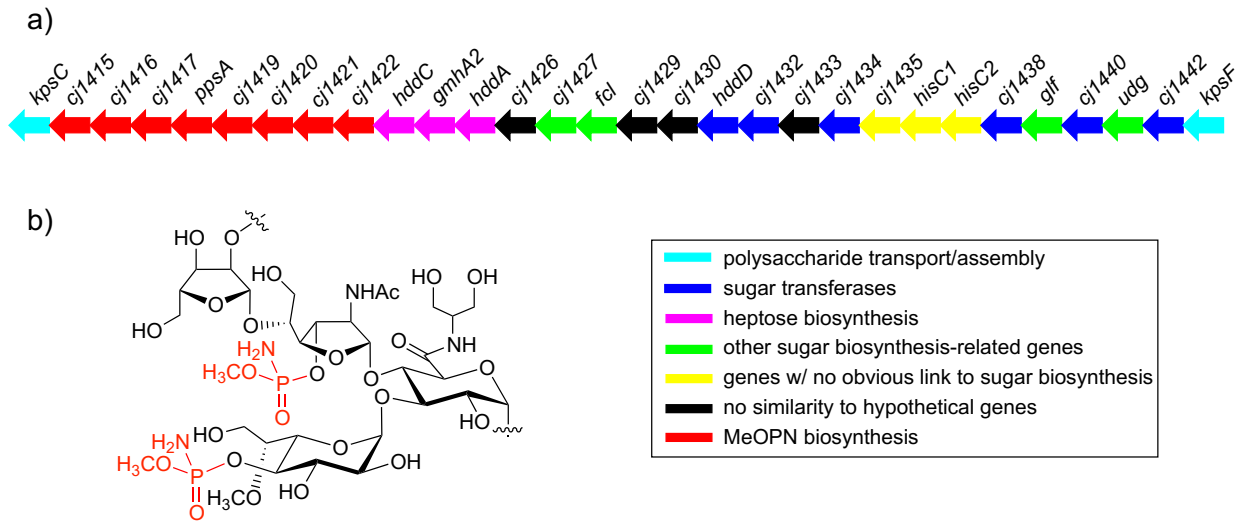


Figure 5-1: *C. jejuni* 11168H CPS a) Gene cluster, b) Repeating tetrasaccharide unit.

To identify the MeOPN biosynthetic genes, Szymanski and coworkers created a library of knockout mutants and examined the CPS of the intact cells using HR-MAS NMR spectroscopy (Figure 5-2). The HR-MAS NMR screen of the wild type (WT) 11168H showed that the methyl groups of the MeOPN motifs are distinctive. The loss of all MeOPN methyl group was observed for the *cj1415–1418* knockouts. On the other hand, the *cj1421* and *cj1422* knockouts showed the loss of just one MeOPN methyl signal. Complementation studies concluded that the *cj1421* and *cj1422* genes encode for MeOPN transferases. It was suggested that the protein encoded by the *cj1421* gene adds the MeOPN to the β-D-Gal*f*NAc residue, whereas that encoded by *cj1422* adds the MeOPN to the D-*glycero*-α-L-*gluco*-heptose residue. In the same investigation, the protein products of *cj1419* and *cj1420* were suggested to have no observed role in the biosynthesis of the MeOPN motif although the ^{1}H NMR spectrum of the *cj1420* knockout is similar to that of the *cj1421* knockout. However, upon revaluating the HR-

MAS NMR spectrum of the *cj1419* and *cj1420* knockouts, Szymanski and coworkers have now putatively assigned these proteins as the methyltransferase involved in the synthesis of the MeOPN motif (Szymanski unpublished) as detailed below. Based on the results of their screen and homology studies, the genes associated with the MeOPN biosynthesis were putatively assigned protein functions. These results are summarized in Table 5-1.

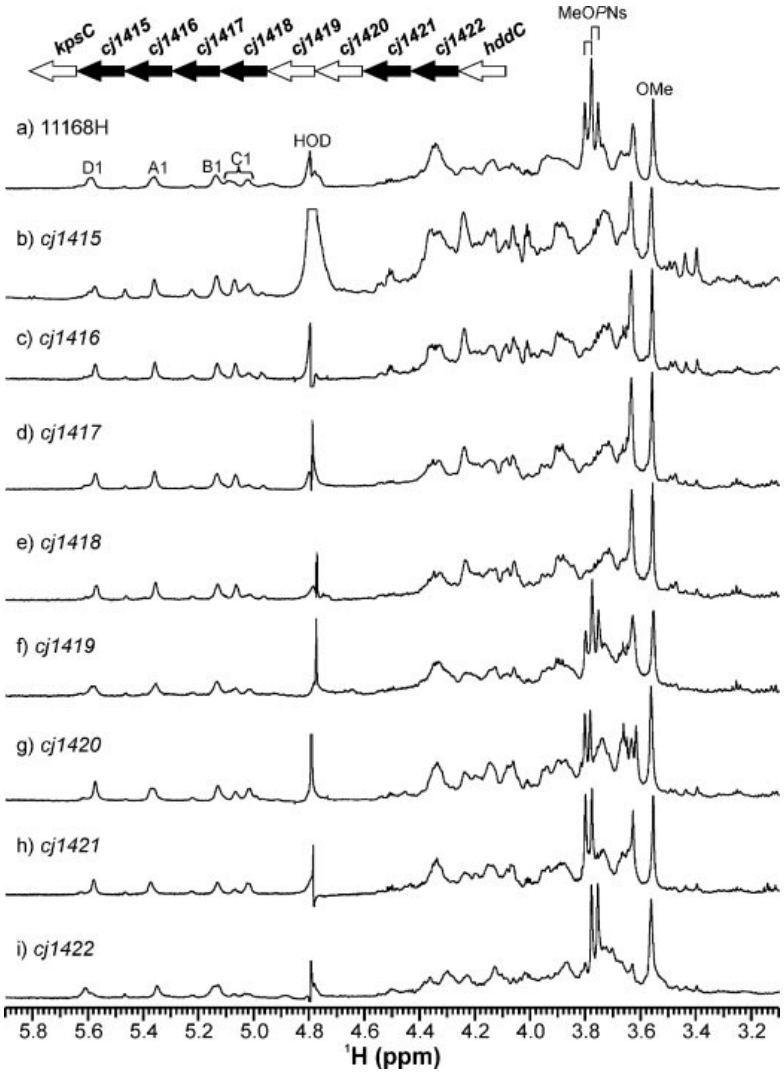


Figure 5-2: Screening intact cells of *C. jejuni* CPS mutant library for the presence of MeOPN using high resolution magic angle spinning nuclear magnetic resonance spectroscopy. a) wild type *C. jejuni* 11168H. *C. jejuni* knockout mutants: a) *cj1415*, a) *cj1416*, a) *cj1417*, a) *cj1418*, a) *cj1419*, a) *cj1420*, a) *cj1421*, a) *cj1422*. Reprinted with permission from the American Society for Biochemistry and Molecular Biology: *J. Chem. Biol.*, *282*, 28566–28576, copyright 2007.

Table 5-1: Summary of gene encoded proteins involved in MeOPN biosynthesis.

Gene	CPS phenotype	Homology	Function annotation
cj1415	Loss of MeOPN	Adenosine-5'-phosphosulfate kinase	Putative adenylylsulfate kinase
cj1416	Loss of MeOPN	Cytidylyltransferase	Putative nucleotidyltransferase
cj1417	Loss of MeOPN	Glutamine amidotransferase	Putative phosphoramidation
cj1418	Loss of MeOPN	Pyruvate phosphate dikinase	Phosphate biosynthesis
cj1419	No observation	No correlation	Putative methyltransferase
cj1420	No observation	No correlation	Putative methyltransferase
cj1421	Loss of MeOPN (Gal*f*NAc)	Glycosyltransferase	Putative MeOPN transferase (Gal*f*NAc)
cj1422	Loss of MeOPN (Hep*p*)	Glycosyltransferase	Putative MeOPN transferase (Hep*p*)

Currently, there are no known substrates for the biosynthesis of the MeOPN motifs. Despite this, inferences can be made for the phosphoramidate donor based on the presence of specific genes and homology studies. First, the knockout studies (e.g. *cj1419* and *cj1420*) performed by Szymanski and coworkers and the presence of two methyltransferases in *C. jejuni* 11168H suggest the methyl group on the MeOPN motif is added after the phosphoramidate group is transferred to the CPS. In addition, the putatively assigned functions for proteins encoded by genes *cj1415* and *cj1416* show homology to an adenosine-5'-phosphosulfate kinase (APS kinase) and cytidylyltransferase (LicC), respectively. The reactions carried out by these two proteins are shown in Figure 5-3.

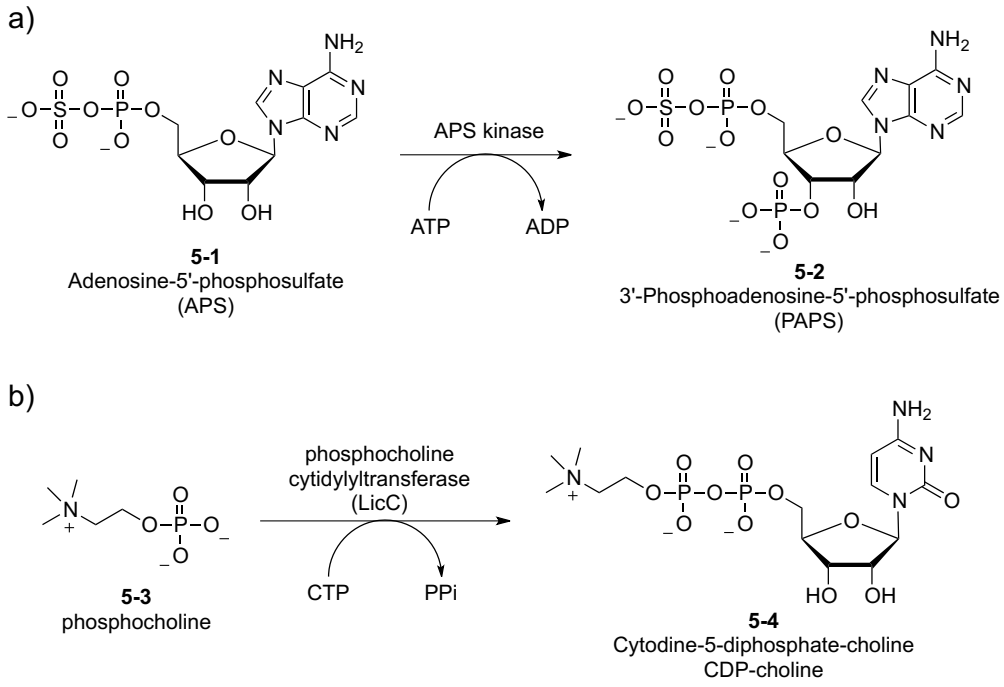


Figure 5-3: Enzymatic reactions. a) APS-Kinase. b) Cytidylyltransferase.

The APS kinase (Figure 5-3a) phosphorylates the 3-OH of the ribofuranose (Rib*f*) residue in APS (**5-1**) using adenosine triphosphate (ATP) to produce 3'-phosphoadenosine-5'-phosphosulfate (PAPS, **5-2**). PAPS (**5-2**) is the substrate that the sulfotransferase uses to add the sulfate moiety to its target of interest. The phosphocholine cytidylyltransferase (Figure 5-3b), on the other hand, uses phosphocholine (**5-3**) to displace the pyrophosphate group in cytidine triphosphate (CTP) to give cytosine-5'-diphosphate choline (CDP-choline, **5-4**). Based on these reactions, we propose the following biosynthetic pathway for the phosphoramidate donor (Figure 5-4).

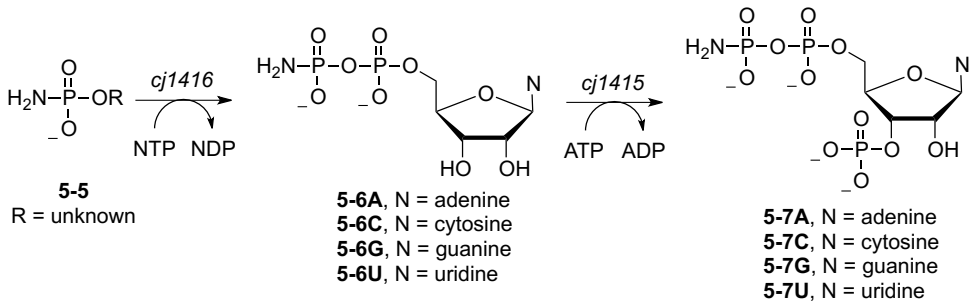


Figure 5-4: Proposed protein functions for *cj1416* and *cj1415*.

Similar to LicC (32% identity to *cj1416*), the protein encoded for *cj1416* could use the phosphoramidate motif of an unknown substrate to displace the pyrophosphate group of nucleotide triphosphate to give **5-6**. The protein encoded for *cj1415*, which shows 70% identity to APS kinase, could then use ATP to phosphorylate the 3'-OH of **5-6** to give the postulated phosphoramidate donor **5-7**. Unfortunately, the nucleobase has not been identified.

Figure 5-5 summarizes the proposed final biosynthetic steps of the MeOPN in *C. jejuni* 11168H. We propose the protein encoded for *cj1415* phosphorylates the 3-OH of the Rib*f* residue in **5-6** to produce the phosphoramidate donor **5-7**. The phosphoramidate transferases (encoded for *cj1421* and *cj1422*) use **5-7** to transfer the phosphoramidate onto the Gal*f*NAc and Hep*p* residues onto the growing CPS. The methyltransferases (encoded for *cj1419* and *cj1420*) then methylates the phosphoramidate motifs completing the MeOPN biosynthesis.

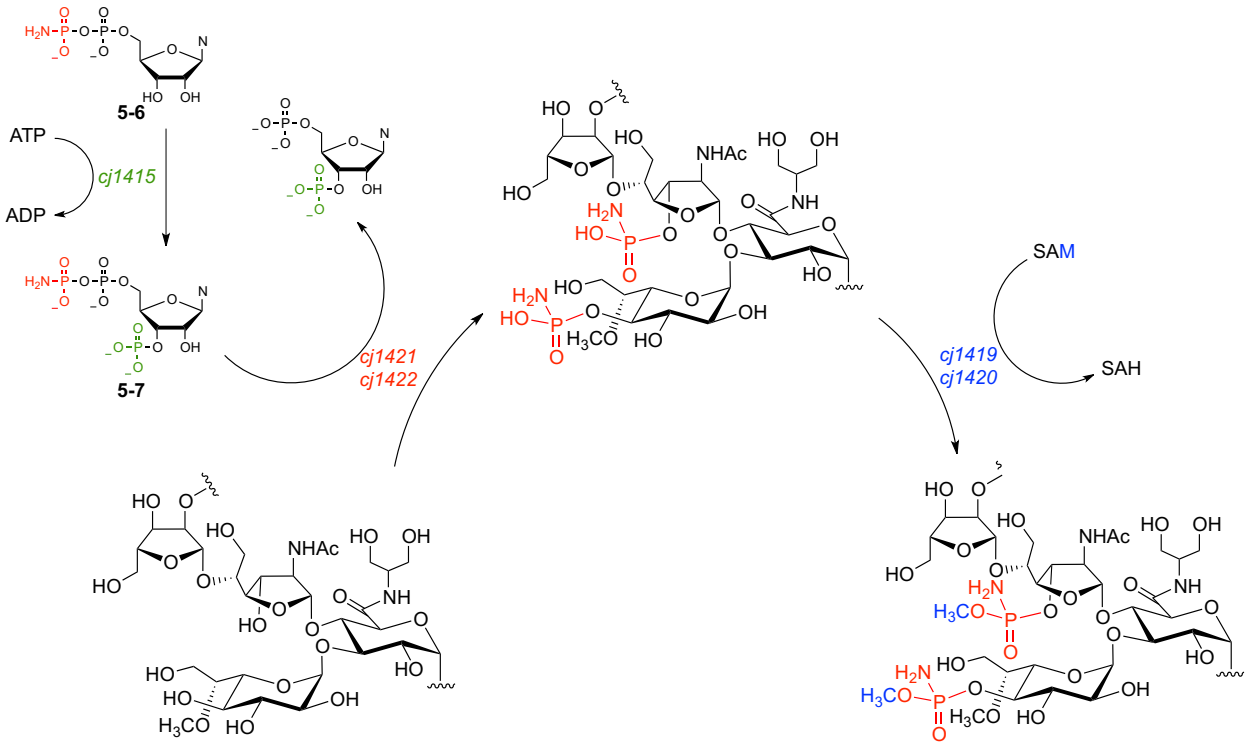


Figure 5-5: Postulated final biosynthetic steps for the assembly of the MeOPN motif in *C. jejuni* 11168H.

To test the proposed biosynthetic steps, we will use a biochemical approach to demonstrate enzyme activity for proteins encoded by *cj1415*, *cj1421*, and *cj1422*. To achieve this three aims are needed: 1) acceptor substrates that can mimic the CPS, 2) the phosphoramidate transferases, and 3) the putative phosphoramidate donor **5-7**.

This chapter will discuss the synthesis of substrates that can mimic the CPS, the cloning and expression of *cj1415*, *cj1421*, and *cj1422*, and efforts towards obtaining the potential substrate for *cj1415*.

5.3 Synthesis of substrates that mimic the CPS

The core tetrasaccharide repeating unit would be the ideal substrate for the phosphoramidate transferases; however, as described in Chapter 4 of this thesis, its total synthesis remains incomplete. Therefore, we sought to use simpler substrates that can mimic the CPS structure. Such fragments of the more complex structure would also allow probing the substrate specificity of the enzyme. These substrates are shown in Figure 5-6. It should be noted that the stage at which the glucuronamide is constructed in the biosynthesis has not been identified. Its assembly could occur either before or after the phosphoramidate is introduced. Therefore, a substrate that lacks and a substrate that includes the serinol motif are included in the target molecules. These CPS fragments include monosaccharides (**5-8** and **5-12**) as well as disaccharides (**5-9**, **5-10**, **5-11**, **5-13**, **5-14**) in the group of molecules to be synthesized.

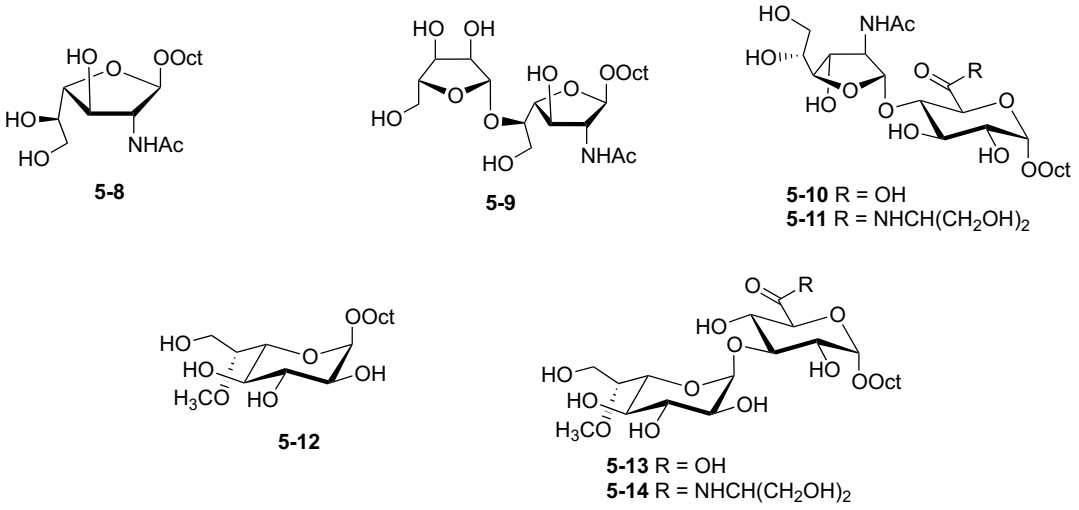
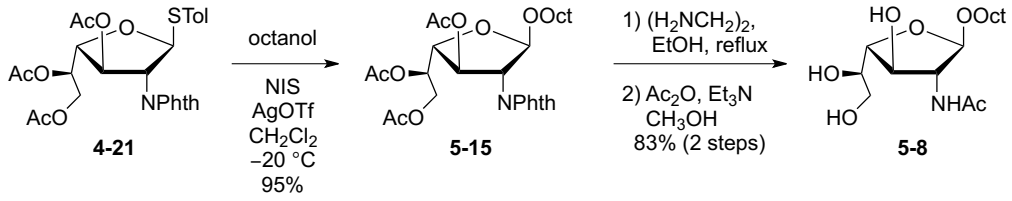


Figure 5-6: Target phosphoramidate acceptor substrates.

5.3.1 Synthesis of Gal*f*NAc containing substrates

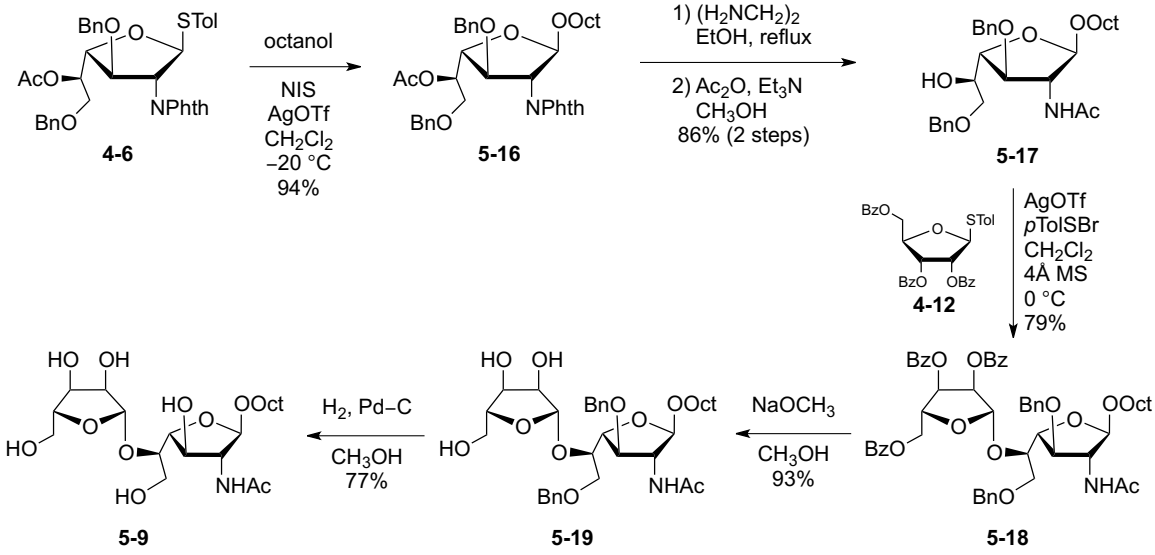
The synthesis of the first Gal*f*NAc substrate **5-8** began by treating **4-21** (its preparation was described in Section 4.2.2 of this thesis) with *N*-iodosuccinimide and silver trifluoromethanesulfonate in the presence of octanol to afford the glycosylated product **5-15** in 95% yield (Scheme 5-1). Glycoside **5-15** was treated with ethylenediamine to remove the acetyl and *N*-phthalamido protecting groups, and then with acetic anhydride in the presence of triethylamine to selectively acylate the amine to give the first monosaccharide substrate, **5-8**, in 83% yield over two steps.



Scheme 5-1: Synthesis of monosaccharide acceptor **5-8**.

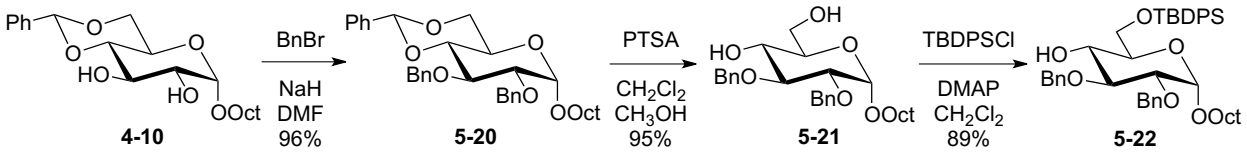
The synthesis of disaccharide **5-9** began by treating **4-6** (its preparation was described in Section 4.2.2 of this thesis) with *N*-iodosuccinimide and silver trifluoromethanesulfonate in the presence of octanol to give **5-16** in 94% yield (Scheme 5-2). The glycoside **5-17** was obtained in 86% yield over two steps by mixing **5-16** with ethylenediamine and then with acetic anhydride in the presence of triethylamine. The glycoside **5-17** was glycosylated with Rib*f* donor **4-12** in the presence of silver trifluoromethanesulfonate and *para*-methylbenzenesulfenyl bromide to afford disaccharide **5-18** in 79% yield. These somewhat unusual conditions were used as traditional promoters (e.g., NIS/AgOTf) failed to activate the Rib*f* donor. The benzoate esters in **5-18** were cleaved by treatment with sodium methoxide to give **5-19** in 93% yield. The benzyl groups in **5-**

19 were then removed by hydrogenolysis in the presence of Pd–C to give the disaccharide substrate **5-9** in 77% yield.



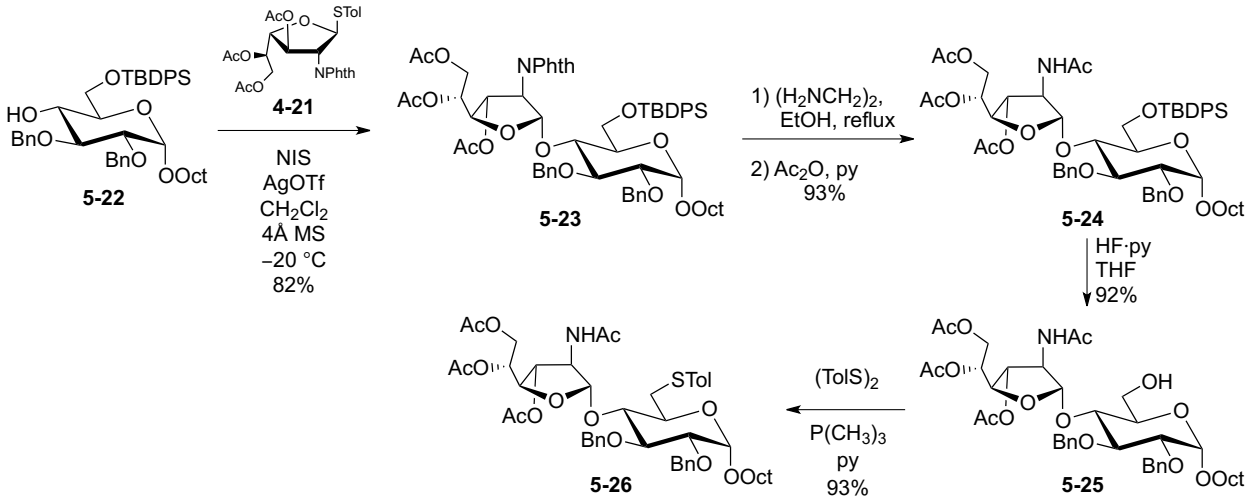
Scheme 5-2: Synthesis of disaccharide acceptor **5-9**.

For the construction of disaccharides **5-10** and **5-11**, an appropriate glycosyl acceptor was first needed. Thus, glucoside **4-10** (its preparation was described in Section 4.2.3 of this thesis) was benzylated with benzyl bromide in the presence of sodium hydride to give **5-20** in 96% yield (Scheme 5-3). The benzylidene acetal in **5-20** was removed by a *para*-toluenesulfonic acid catalyzed methanolysis to afford **5-21** in 95% yield and then the resulting diol was treated with *tert*-butyldiphenylsilyl chloride in the presence of 4-dimethylaminopyridine to give the Glc acceptor **5-22**. Compound **5-22** was obtained in 89% yield from **5-21**.



Scheme 5-3: Synthesis of glucose glycosyl acceptor **5-22**.

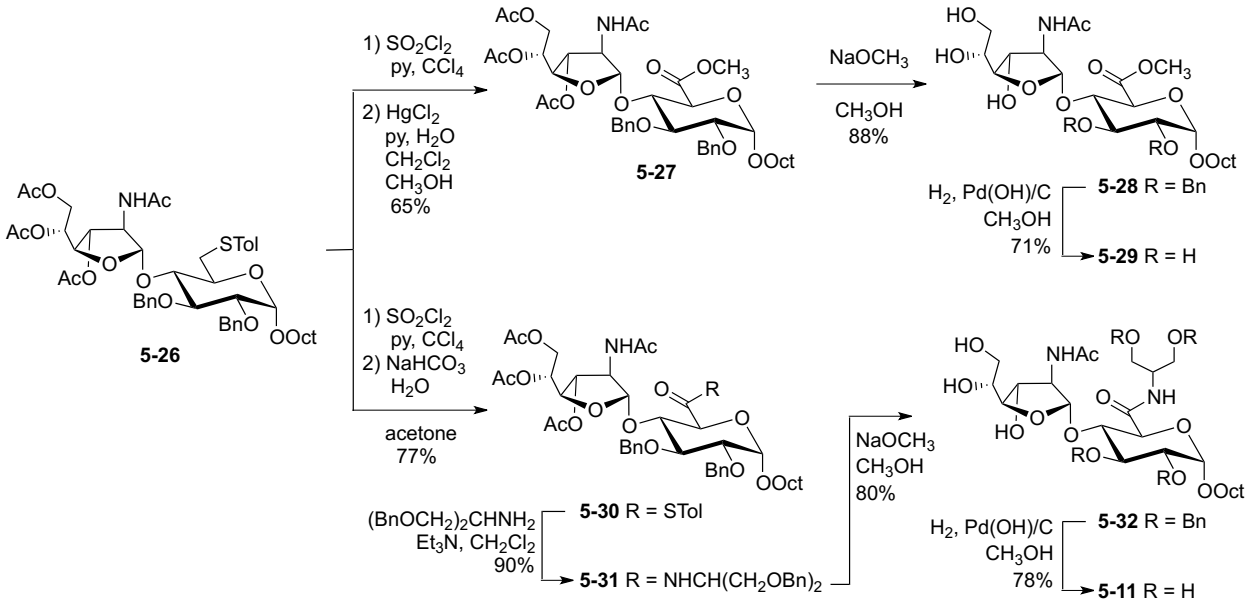
The Glc acceptor **5-22** was glycosylated with the donor **4-21** (its preparation was described in Section 4.2.2 of this thesis) in the presence of *N*-iodosuccinimide and silver trifluoromethanesulfonate to afford, in 82% yield, disaccharide **5-23** (Scheme 5-4). The *N*-pthalimido group of **5-23** was converted to an *N*-acetyl group by first treatment with ethylenediamine and then acetic anhydride in pyridine to afford **5-24** in 93% yield over two steps. The *tert*-butyldiphenylsilyl group in **5-23** was removed by use of a hydrogen fluoride–pyridine complex in tetrahydrofuran to give the alcohol **5-25** in 92% yield. Then, **5-25** was then converted to its thioether counterpart **5-26** in 93% yield by mixing with *para*-methylbenzene disulfide and trimethyl phosphine.



Scheme 5-4: Synthesis of thioether intermediate **5-26**.

With the thioether **5-26** in hand, two separate routes were used for the synthesis of **5-10** and **5-11** (Scheme 5-5). The first route involved treating **5-26** with sulfuryl chloride in the presence of pyridine and then with mercuric chloride in the presence of pyridine and methanol to afford the methyl glycuronate **5-27** in yields of 65%. The *O*-acetyl groups of **5-27** were removed by stirring with sodium methoxide to afford **5-28** in yields of 88%. Lastly, the benzyl groups

were removed by hydrogenolysis in the presence of palladium hydroxide on carbon to give **5-29** in 71% yield. This adduct was kept as the methyl glycuronate until needed to avoid any decomposition. In the second route for the preparation of glycuronamide **5-11**, thioether **5-26** was treated with sulfuryl chloride in the presence of pyridine and then with water in the presence of sodium bicarbonate to give the thioester **5-30** in yields of 77%. The thioester **5-30**, being more labile than the methyl ester, was then stirred with the 1,3-di-*O*-benzyl-serinol in the presence of triethylamine to give the glycuronamide **5-31** in 90% yield. Similar to the conditions described in the first route, the glycuronamide **5-31** was treated with sodium methoxide to remove the *O*-acetyl groups (to afford **5-32** in 80% yield) and then by hydrogenolysis in the presence of palladium hydroxide on carbon to give the disaccharide acceptor **5-11** in 78% yield.



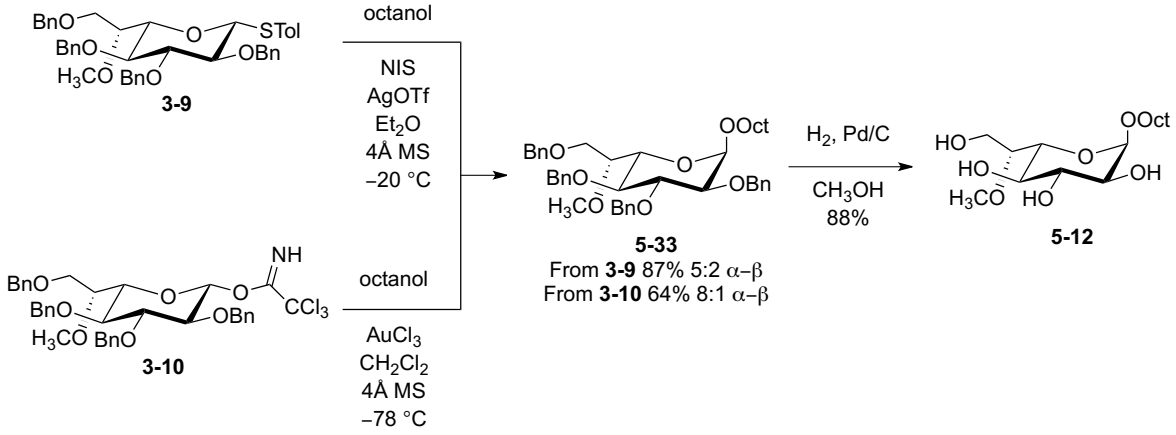
Scheme 5-5: Synthesis of a **5-10** derivative and disaccharide acceptor **5-11**.

This work showed the successful syntheses of four potential substrates for the phosphoramidate transferase specific to the Gal*f*NAc residue. At least one, if not all, of these

structurally different substrates should be applicable in screening for phosphoramidate transferase activity.

5.3.2 Synthesis of Hep*p* containing substrates

The synthesis of **5-12** began by treating the heptose donor **3-9** (its preparation was described in Chapter 3 of this thesis) with octanol in the presence of *N*-iodosuccinimide and silver trifluoromethanesulfonate in diethyl ether to give **5-33** in 87% yield in an ~3:1 α/β ratio. As described in Section 4.4, the use of diethyl ether was used to help promote the formation of α-glycosylated product. In an alternative approach described by Peng and Schmidt,[9] treating the β-imidate **3-10** (its preparation was described in Chapter 3 of this thesis) was glycosylated with octanol in the presence of gold chloride to give **5-33** in 64% yield, but in a higher (~8:1) α/β ratio. Hydrogenolysis of **5-33** in the presence of Pd–C gave the heptose acceptor **5-12** in 88% yield.



Scheme 5-6: Synthesis of monosaccharide acceptor **5-12**.

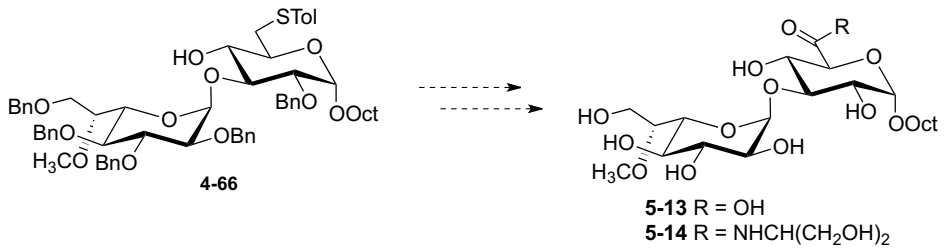


Figure 5-7: Future work needed for the synthesis of disaccharide acceptors **5-13** and **5-14**.

The synthesis of the advanced intermediate **4-66** that will be used for the synthesis of **5-13** and **5-14** (Figure 5-7) was described in Section 4.4 of this thesis. At this point, however, we began focusing on the obtaining the phosphoramidate transferases and phosphoramidate donor, thereby Hep*p* containing substrates **5-13** and **5-14** were not completed. Although these substrates were not complete, we were able to successfully synthesize the Hep*p*-containing substrate **5-12** for the phosphoramidate transferase specific to the Hep*p* residue.

5.4 Cloning and expression of phosphoramidate biosynthetic proteins

Our collaborator Professor Christine Szymanski provided the pET28a vector containing a gene sequence of an N-terminal His_6-tagged *cj1415* (*H_6-cj1415*). The cloning and expression of *cj1421*, *cj1422*, and *cj1415* were performed with the aid and supervision of Cory Wenzel, a technician in Professor Szymanski's laboratory in the Department of Biological Sciences at the University of Alberta.

This section of the chapter will focus on cloning the genes encoding for the phosphoramidate transferases (*cj1421* and *cj1422*). The vectors containing the desired gene *cj1415* (provided by the Szymanski lab), *cj1421*, and *cj1422* as well a sequence that encodes for

a terminal His_6-tag will be transformed and expressed in *E. coli* cells. The addition of the His_6-tag will allow for the purification of the expressed protein by immobilized metal affinity chromatography using a Nickel-nitrolotriacetic acid (Ni^{2+}-NTA) agarose column. As it is widely observed that the position of the His_6-tag may affect the expression, stability, and even the function of the recombinant protein, clones with the His_6-tag in the N- and C- termini will be generated. Table 5-2 provides the size of the genes in base pairs, their predicted size of the encoded protein in kDa, and their theoretical *p*I values as determined by ExPASy, a bioinformatics resource website.

Table 5-2: Summary of protein information pertaining to genes *cj1415*, *cj1421*, and *cj1422*.

Gene	Number base pairs	Size of protein (kDa)	*p*I
cj1415	510	41.6	5.13
cj1421	1836	73.4	8.86
cj1422	1875	74.2	8.92

[a] Size of protein and *p*I generated from ProtParam (http://web.expasy.org/protparam/).

The pET28a vector was used for the *N*-terminal expression constructs while the pET30a was employed for the *C*-terminal expression constructs. The *cj1421* and *cj1422* genes were amplified from the DNA genome of *C. jejuni* NCTC11168 by polymerase chain reaction (PCR) using the primers shown in Table 5-3.

Table 5-3: Primers, vectors, and appropriate restriction sites used for *N*- and *C*-terminal His_6 clones for *cj1421* and *cj1422*.

Primer	Vector	Sequence (5'→3')	Restriction site	T_m (°C)
cj1421[1]	pET28a/30a	GGATCGTCATATGCTCAACCCAAATTC	NdeI	62
H_6-cj1421[2]	pET28a	CAAATCCTCGAGCTAAATATCACCATCC	XhoI	62
cj1421-H_6[2]	pET30a	CTTACTCGAGAATATCACCATCCAAC	XhoI	62
cj1422[1]	pET28a/30a	GTAAAGGTGACATATGCAAGCAATAATAC	NdeI	62
H_6-cj1422[2]	pET28a	GAAAACTCTCGAGTCAATTATTGATTAGAAAATG	XhoI	62
***c**j1422-H_6*[2]	pET30a	GAAATTATTACTCGAGATTATTGATTAGAAAATG	XhoI	62

[1] Forward [2] Reverse [3] T_m = melting temperature. [*] Nucleotides underlined indicate restriction endonuclease sites.

The initial attempt to clone the genes of interest under standard PCR conditions produced multiple products. To overcome the non-specific binding of the primer, a combination of hot start and touchdown PCR was used. This modified form of the PCR uses an initial annealing temperature higher than the melting temperature (T_m) of the primers and then incrementally lowers it over subsequent cycles until a "touchdown temperature" or optimal annealing temperature is reached. The use of temperatures higher than the calculated T_m decreases the non-specific binding of the primers, thereby only high primer–template complementarity amplicons are extended. The subsequent lowering of the annealing temperature, over iterative cycles, increases the yield of the PCR products.

The combination of hot start and touchdown PCR was successful in amplifying *cj1421*; however, *cj1422* still produced a mixture of products. In a closer examination of the *C. jejuni* NCTC11168 chromosomal DNA, we found the presence of a truncated gene duplication of *cj1422*. This presence causes the non-specific binding of the primer and causes a mixture of products. Given this information, several PCRs were run, combined, and purified to provide

enough DNA for *cj1422*. Following PCR, the amplified genes (*cj1421* and *cj1422*) and pET vectors were each digested with the appropriate restriction endonucleases (Table 5-3). The digested genes and pET vectors were ligated and transformed into DH5α *E. coli* cells. Isolation of plasmid DNA from resultant colonies was carried out and analytical digests were performed on the isolated plasmids. The putative expression constructs including plasmid DNA of *cj1415* (Figure 5-8), were identified and correlated with the expected size of gene inserts (Figure 5-8).

The analytical digests of *cj1415* containing a sequence that encodes for an N-terminal His_6-tag (*H_6-cj1415*, Figure 5-8a), *cj1421* containing a sequence that encodes for a C-terminal His_6-tag (*cj1421-H_6*, Figure 5-8b), *cj1422* containing a sequence that encodes for an N-terminal His_6-tag (*H_6-cj1422*, Figure 5-8c), and *cj1422* containing a sequence that encodes for a C-terminal His_6-tag (*cj1422-H_6*, Figure 5-8d) were identified and correlated with the expected size of gene inserts (Table 5-2). DNA sequencing confirmed the correct sequences for *H_6-cj1415* and *cj1421-H_6*. DNA sequencing for *H_6-cj1422* and *cj1422-H_6* showed a single point mutation on a poly-G tract section of the gene; therefore, further cloning optimization is needed in the future.

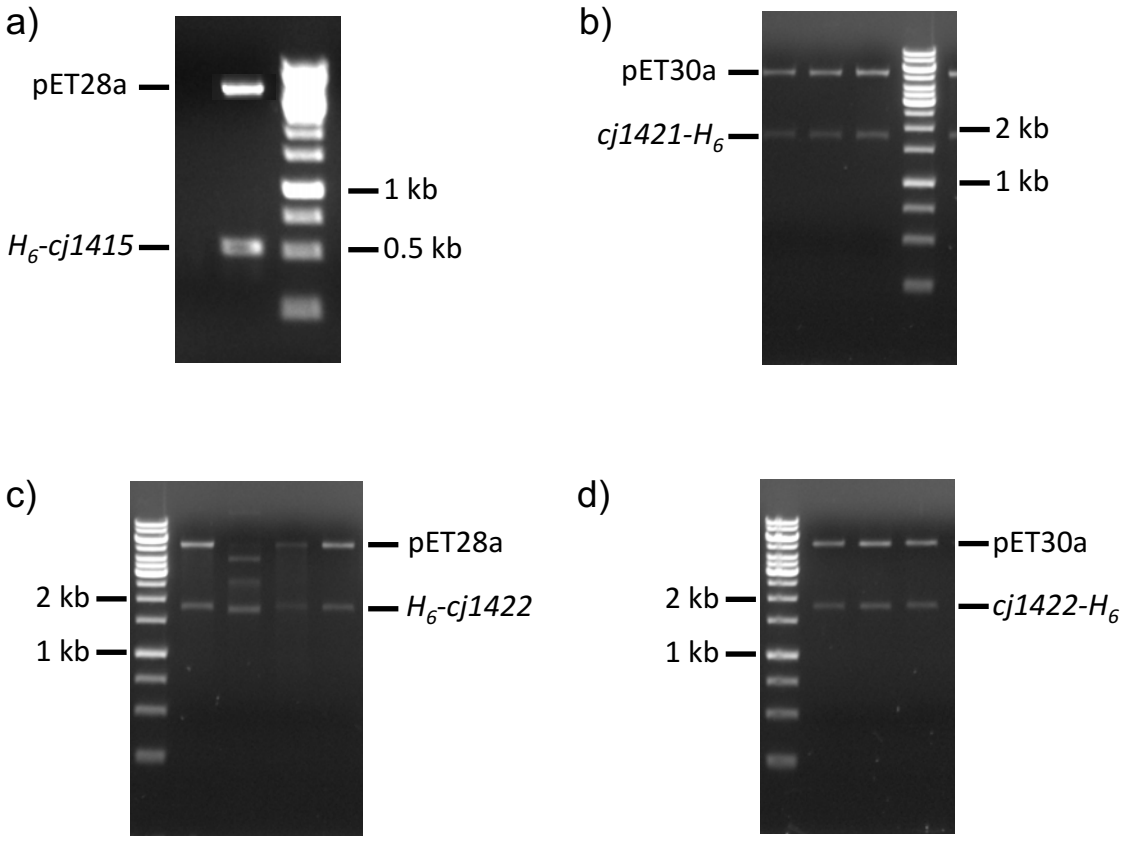


Figure 5-8: Agarose gels of analytical digests for DNA plasmids a) N-terminal His_6-tagged *cj1415* (*H_6-cj1415*/pET28a), b) C-terminal His_6-tagged *cj1421* (*cj1421-H_6*/pET30a), c) N-terminal His_6-tagged *cj1422* (*H_6-cj1422*/pET28a), and d) C-terminal His_6-tagged *cj1422* (*cj1422*-H/pET30a).

To help identify the nucleotidyl base in the phosphoramidate donor, the expression of the *H_6-cj1415* was performed. The plasmid DNA containing *H_6-cj1415* was transformed into *E. coli* BL21(DE3) competent cells and grown in LB growth medium at 37 °C until OD_{600nm} = 0.6, followed by induction of isopropyl 1-thio-β-D-galactopyranoside (IPTG). Purification of the expressed N-terminal His_6-tagged protein (His_6-Cj1415) was achieved by using a Ni^{2+}–NTA column with most of the protein eluting at 250 mM imidazole in 4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid (HEPES) buffer (Figure 5-9a). The expected protein band was observed on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gel and anti-His_6 Western immunoblot (Figure 5-9b).

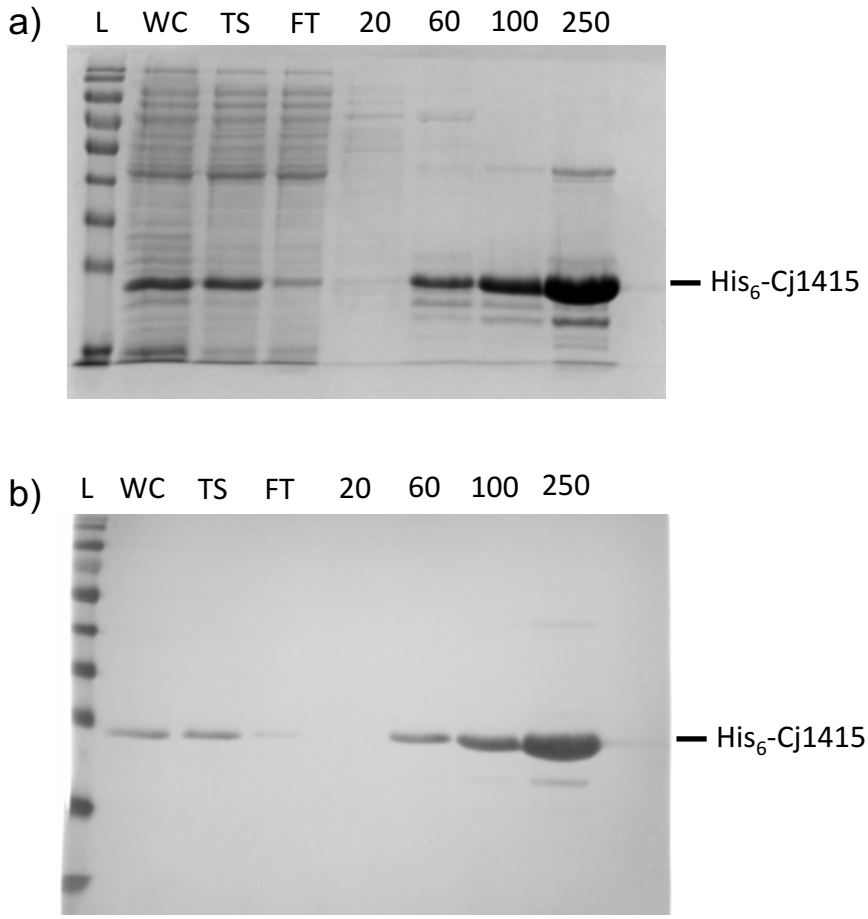


Figure 5-9: a) SDS-PAGE of His_6-Cj1415 purification. b) SDS-PAGE of Anti-His_6 Western immunoblotting for His_6-Cj1415. L = ladder, WC = whole cell lysate, TS = total soluble fractions, FT = flow-through fraction, 20 = elution with 20 mM imidazole, 60 = elution with 60 mM imidazole, 100 = elution with 100 mM imidazole, 250 = elution with 250 mM imidazole.

The goal of this section was to obtain plasmid/gene constructs for *cj1421* and *cj1422* (genes encoding for the phosphoramidate transferases) that also contain a sequence that encodes for an N- or C-terminal His_6-tag. These constructs and the *H_6-cj1415*/pET28a vector, provided by the Szymanski group, were then to be expressed in *E. coli* cells. In summary, we were able to obtain a *cj1421-H_6*/pET30a construct that encodes for a C-terminal His_6-tagged phosphoramidate transferase specific for the Gal*f*NAc residue (Cj1421-His_6). In addition, we were able to successfully express a putative N-terminal His_6-tagged phosphokinase (His_6-Cj1415). Additional

work is needed to obtain a plasmid/gene construct possessing the gene *cj1422*. The expression of this construct as well as *cj1421-H*$_6$/pET30a is also needed to obtain the putative phosphoramidate transferases.

5.5 Efforts towards the synthesis of the phosphoramidate substrate

With the expression and purification of His$_6$-Cj1415 a success, we focused our attention on synthesizing **5-6** (Figure 5-10). This substrate will then be incubated with His$_6$-Cj1415 to potentially produce the postulated phosphoramidate donor **5-7**. One caveat, however, is that the nucleobase for the phosphoramidate donor **5-7** has not been identified; therefore the chemical synthesis of **5-6** that possess the nucleobases adenine (**5-6A**), cytosine (**5-6C**), guanine (**5-6G**), and uridine (**5-6U**) is needed. These substrates will then be incubated with His$_6$-Cj1415 and measured for enzyme activity under the assumption that only one of these compounds will bind and undergo phosphorylation at the C-3 alcohol position of the ribose residue. In an alternative strategy, saturation transfer difference NMR (STD-NMR) could potentially be used to identify the correct nucleobase found in the phosphoramidate donor.

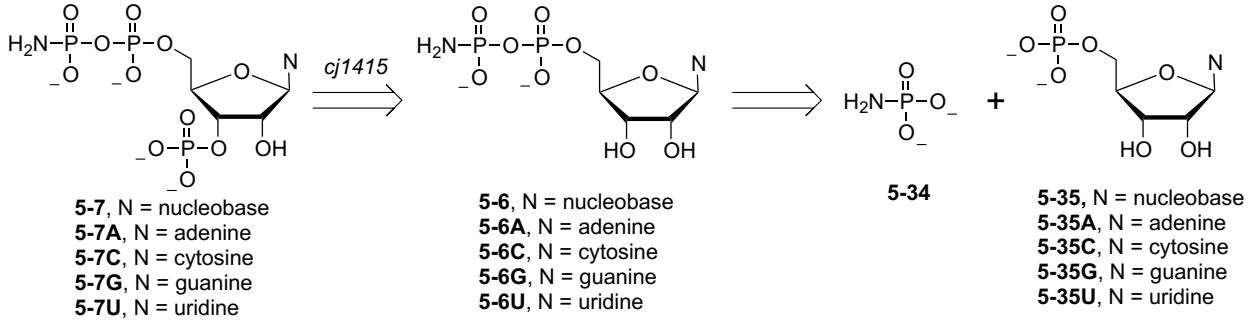


Figure 5-10: Retrosynthesis of **5-7** from NMP (**5-35**).

The retrosynthetic analysis of the postulated phosphoramidate donor (**5-7**) is shown in Figure 5-10. With the high homology to APS kinase (Figure 5-4), we envision that the protein encoding for *cj1415* could be used to obtain **5-7** from **5-6**. Although **5-6**, has not been previously synthesized, we envisioned that coupling phosphoramidate **5-34** and a nucleotide-5'-monophosphate (NMP, **5-35**) could be used for its synthesis.

The coupling of two phosphates is commonly done in the chemical synthesis of sugar nucleotides.[10,11] The most traditional approach for the synthesis of sugar nucleotides (e.g. **5-37**) involves the activation of the NMP (**5-35**) and then addition of a sugar-1-phosphate (e.g. **5-36**) as shown in Figure 5-11a. Although this approach has not been carried out with a phosphoramidate (e.g. **5-34**), we envisioned the coupling could still yield the desired **5-6** (Figure 5-11b). To test this, we first need access to the phosphoramidate **5-34**.

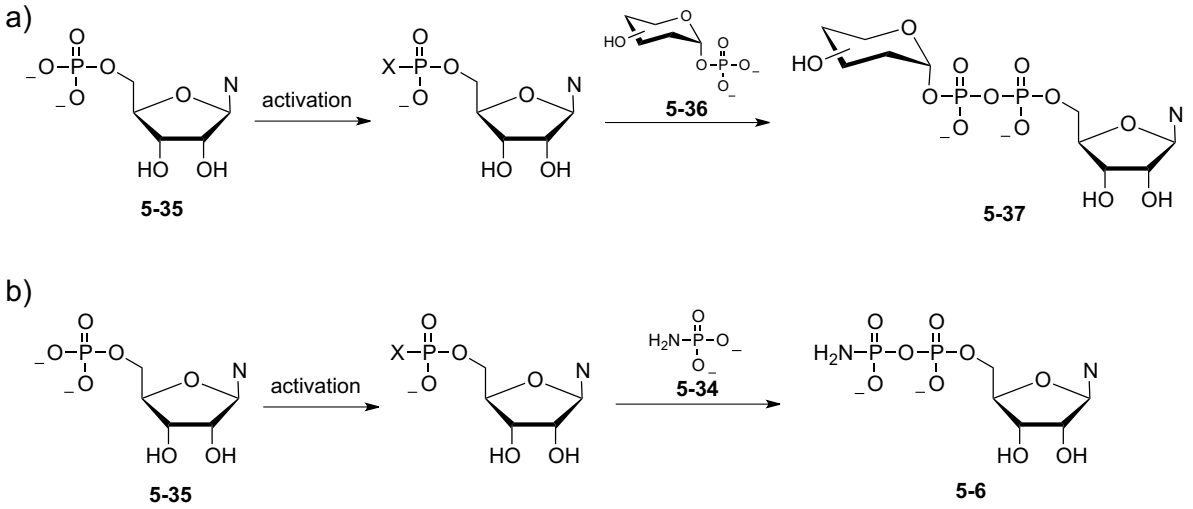
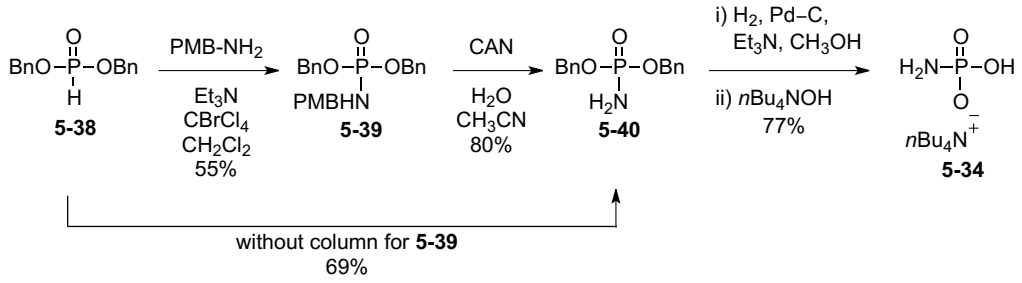


Figure 5-11: Phosphate coupling reactions. a) Phosphate coupling for the synthesis of sugar nucleotides. b) Potential phosphate coupling for the synthesis of **5-6**.

Given our previous success with the Atherton–Todd reaction, dibenzyl phosphite (**5-38**) was treated with bromotrichloromethane in the presence of *para*-methoxybenzylamine and triethylamine to give the protected phosphoramidate **5-39** in a modest 55% yield (Scheme 5-7).

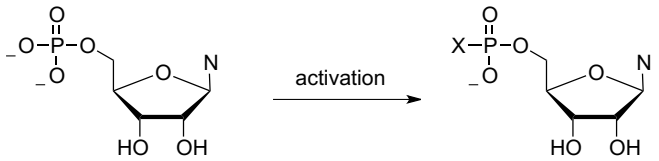
The *para*-methoxybenzyl group of **5-39** was removed oxidatively by the use of ceric ammonium nitrate to afford **5-40** in 80% yield. We later found that treating **5-39** with ceric ammonium nitrate without purification by flash chromatography could furnish **5-40** in an improved 69% yield compared to 44% over two steps. Finally, the *O*-benzyl groups of **5-40** were removed by hydrogenolysis in the presence of palladium on carbon and triethylamine. A subsequent cationic salt exchange was performed by addition of tetra-*n*-butylammonium hydroxide to furnish tetra-*n*-butylammonium salt of **5-34** in 77% yield after lyophilization. The triethylammonium salt of **5-34** (the product before cationic salt exchange) was insoluble in dimethylformamide, the solvent used in subsequent transformations.



Scheme 5-7: Synthesis of phosphoramidate **5-34**.

With the phosphoramidate **5-34** in hand, we then examined the different activation strategies. There are a number of methods for the activation of phosphates. Some of the more common strategies are shown in Table 5-3. These include the generation of phosphoric anhydrides or diphosphate species (Entry 1),[12,13] sulfonates (Entries 2 and 3),[14] urea precursors (Entries 4 and 5),[15,16] and active phosphoramidate derivatives (Entries 6–9).[16-18] In addition, several additives such as $MnCl_2$,[19,20] $MgBr_2/MgCl_2$,[19-21] tetrazole,[17,22-25] imidazole,[16] and *N*-methylimidazole[26,27] have often been employed to accelerate and improve the yields of phosphate coupling reactions.

Table 5-4: Phosphate activation methods.



5-35
N = A, C, G, or U

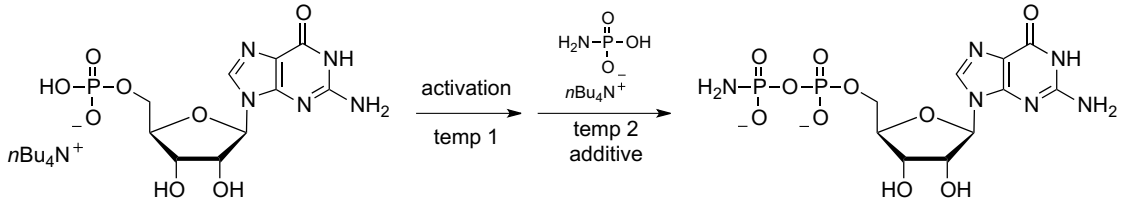
N = A, C, G, or U

entry	activation	X
1	$(PhO)_2POCl$[13]	**5-41**
2	TiPSCl[14]	**5-42**
3	TCICA[14]	**5-43**
4	DMC[16]	**5-44**
5	DCC, morpholine[15]	**5-45**
6	$(PyS)_2$, PPh_3, piperidine[19,28]	**5-46**
7	CDI[29-34]	**5-47**
8	Tf_2O, NMI[18,27,35]	**5-48**

***** TiPSCl = 2,4,6-triisopropylphenylsulfonyl chloride, TCICA = trichloroisocyanuric acid, DMC = 2-chloro-1,3-dimethylimidazolinium chloride, DCC = *N*,*N*'-dicyclohexylcarbodiimide, $(PyS)_2$ = 2,2'-dithiodipyridine, CDI = 1,1'-carbonyldiimidazole, Tf_2O = trifluoromethanesulfonic anhydride, NMI = *N*-methylimidazole.

With the phosphoramidate **5-34** in hand, we explored some of the phosphate coupling strategies for the synthesis of **5-6G**, in which the nucleoside base is guanosine. These results are summarized in Table 5-4. We initially explored using 2,4,6-triisopropylbenzenesulfonyl chloride (Entry 1) and *N,N'*-dicyclohexylcarbodiimide in the presence of morpholine (Entry 2); however, these conditions led to incomplete consumption of the starting material (GMP, **5-35G**) via ^{31}P NMR spectroscopy. Treatment of GMP (**5-35G**) with dimethylimidazolinium chloride in the presence of imidazole in water (Entry 4) led to production of two new phosphorus signals indicative of phosphorimidazolide **5-47G** and presumably a self-coupled GMP analogue as seen by Hindsgaul and coworkers.[16] Switching solvents from water (Entry 3) to dimethylformamide (Entry 4) showed complete consumption of GMP (**5-35G)**, however the self-coupled GMP became more prominent. Regardless, the phosphoramidate **5-34** was added. To our delight, two new ^{31}P NMR signals, indicative of the successful phosphate coupling (the presence of two doublet ^{31}P NMR signals with J = ~20 Hz), were observed. Switching to the carbonyldiimadazole as the activation strategy showed complete consumption of GMP (**5-35G**) without the formation of any other byproducts. The phosphoramidate **5-34** was added under a variety of conditions (Entry 5–8). Of these conditions, the most optimal protocol was Entry 6 where after addition of the phosphoramidate **5-34**, the reaction was stirred at 40 °C for four hours.

Table 5-5: Phosphate coupling conditions explored for the synthesis of **5-6**.



5-35G **5-6G**

entry	activation	solvent	temp 1	additive	temp 2	product observed[a]
1	TiPSCl	pyridine	rt	----	----	no[b]
2	DCC, morpholine	DMF	rt	----	----	no[b]
3	DMC, imidazole	H_2O	40 °C	----	40 °C	no[b]
4	DMC, imidazole	DMF	40 °C	----	40 °C	yes
5	CDI	DMF	rt	----	rt	yes
6	CDI	DMF	rt	----	40 °C	yes
7	CDI	DMF	rt	$MgBr_2$	rt	yes
8	CDI	DMF	rt	NMI	rt	yes

[a] Product observed was based on the presence of two ^{31}P NMR signals described in Figure 5-11b. [b] The phosphoramidate was not added.

Figure 5-12 shows a representative reaction sequence with monitoring by ^{31}P NMR spectroscopy. GMP (**5-35G**) was consumed within one hour after addition of carbonyldiimidazole (6 equiv.) to generate a new ^{31}P NMR signal that was later characterized as the phosphorimidazolide **5-47G** (Figure 5-12). The phosphoramidate **5-34** was then added and the reaction mixture was stirred at 40 °C for four hours. Although ^{31}P NMR spectroscopy showed a mixture of products, two doublet ^{31}P NMR signals were found that had ^{31}P–^{31}P

coupling constants of 19.8 and 20.2 Hz. These scalar coupling constants support the formation of the P–O–P bond. Analytical HPLC was used to screen for any new product; however, these results were inconclusive.

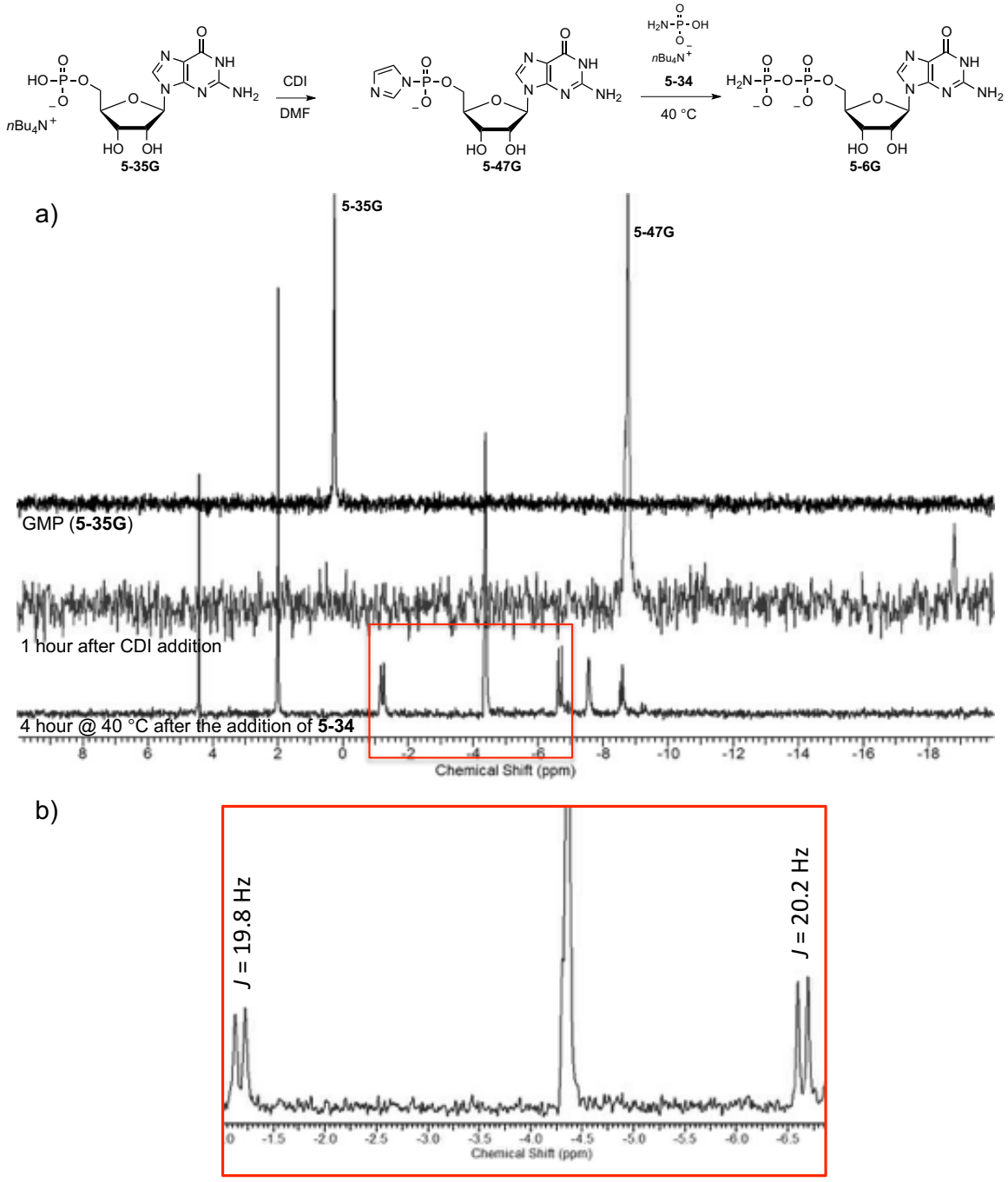
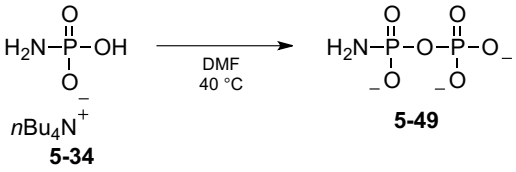


Figure 5-12: a) Monitoring the GMP and phosphoramidate coupling reaction via ^{31}P NMR spectroscopy. b) Expansion of the ^{31}P NMR spectrum with scalar coupling of the ^{31}P signals.

Upon more careful examination of the product of this reaction (Entry 6, Table 5-5), we later found that the set of two ^{31}P NMR signals were not indicative of the P–O–P bond found in **5-6G**, but rather **5-49** (Figure 5-13), the product of the phosphoramidate **5-34** self-coupling with itself. As a control experiment, phosphoramidate **5-34** was placed in DMF and stirred at 40 °C for four hours. Examination of the reaction by ^{31}P NMR spectroscopy showed the same set of two ^{31}P NMR signals, which suggests the formation of **5-49** rather than **5-6G**.



5-34 5-49

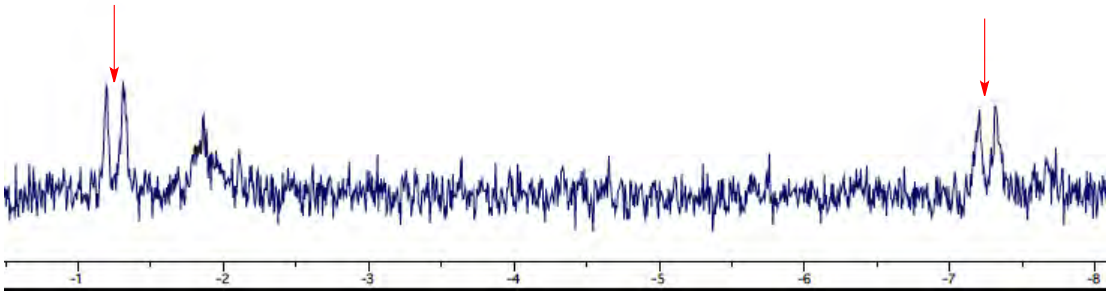
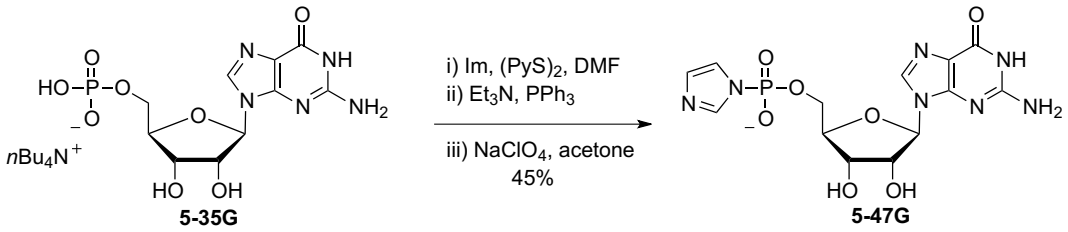


Figure 5-13: ^{31}P NMR spectrum of the product formed by heating **5-34** in dimethylformamide at 40 °C for four hours. The product peaks indicated by the arrows correspond to those found in the attempted synthesis **5-6G**.

Given this lack of success, we thought simplifying the coupling to a single step could potentially aid in the formation of the desired adduct. Following Jemielity and coworkers, we synthesized, in modest yields, the phosphorimidazolide **5-47G** (Scheme 5-8) by treatment of GMP (**5-35G**) with 2,2'-dithiodipyridine and triphenylphosphine in the presence of imidazole

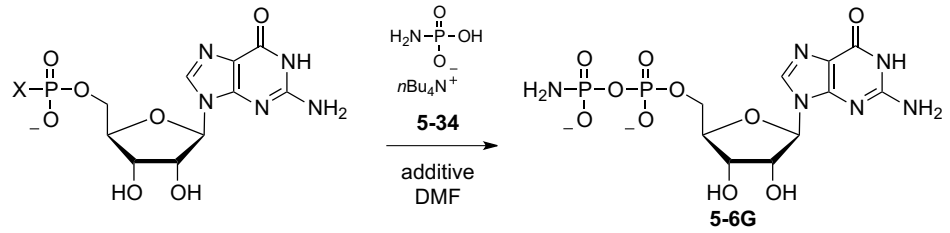
and triethylamine and then precipitation by addition of sodium hypochlorite in acetone (Scheme 5-8).[21]

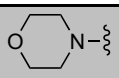
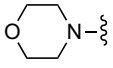
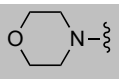
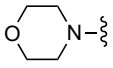
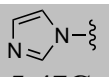
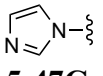
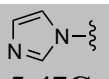


Scheme 5-8: Synthesis and isolation of phosphorimidazolide **5-47G**.

The phosphorimidazolide **5-47G** and commercially available phosphormorpholidate **5-45G**, were then examined in the coupling with phosphoramidate **5-34** (Table 5-6). As described earlier, additives can greatly accelerate the phosphate coupling; therefore, *N*-methylimidazole, magnesium bromide, and tetrazole were tested to promote the formation of **5-6G**. Unfortunately, these results all led to a mixture of products and nothing indicative of the desired phosphoramidate product **5-6G**.

Table 5-6: Phosphate coupling conditions explored with additives for the synthesis of **5-6G**.

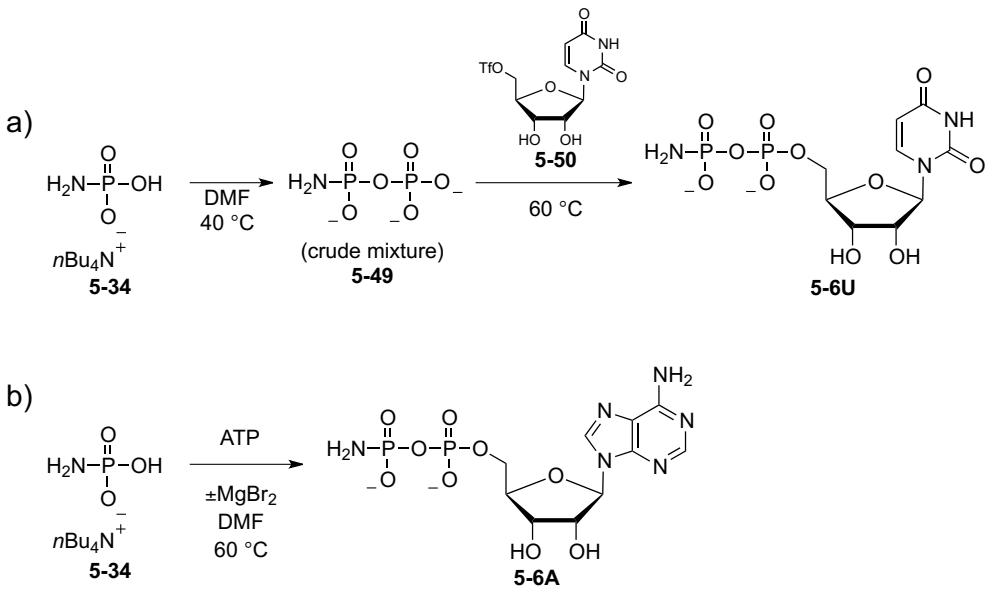


entry	X	additive	product observed[a]
1	 **5-45G**	----	no
2	 **5-45G**	NMI	no
3	 **5-45G**	$MgBr_2$	no
4	 **5-45G**	tetrazole	no
5	 **5-47G**	NMI	no
6	 **5-47G**	$MgBr_2$	no
7	 **5-47G**	tetrazole	no

[a] Product observed was based on the presence of two different ^{31}P NMR signals as described in Figure 5-12 and 5-13.

In a final effort to synthesize **5-6**, we attempted a nucleophilic displacement of either a triflate group or a pyrophosphate of adenosine-5'-triphosphate (ATP, Scheme 5-9). The crude phosphoramidate-phosphate mixture, made by heating phosphoramidate **5-34** in DMF at 40 °C for four hours, was added to the triflate **5-50** and heated to 60 °C with monitoring by ^{31}P NMR spectroscopy over 0.5–4 hours. This reaction did not afford the desired adduct. As an alternative strategy, the pyrophosphate group in ATP could potentially be displaced. ATP was mixed with

excess phosphoramidate **5-34** (10–50 equiv.) in the presence and absence of $MgBr_2$ and heated to 60 °C. These results showed a complex mixture of products and it was difficult to assess whether **5-6A** was formed. With the lack of success in generating **5-6**, it was necessary to abandon my efforts to characterize the phosphoramidate transferases.



Scheme 5-9: Attempted synthesis of **5-6** a) Via nucleophilic displacement of ribose 5'-*O*-triflate **5-50**. b) Via nucleophilic displace of pyrophosphate from adenosine-5'-triphosphate (ATP).

5.6 Efforts to identify the nucleobase in the phosphoramidate donor

Although we did not obtain conclusive evidence for the formation of the **5-6**, we were able to express and purify the N-terminal His_6-tagged protein for *cj1415* (His_6-Cj1415). Because of this, compounds that could mimic **5-6** could be incubated with His_6-Cj1415 and examined for protein activity.

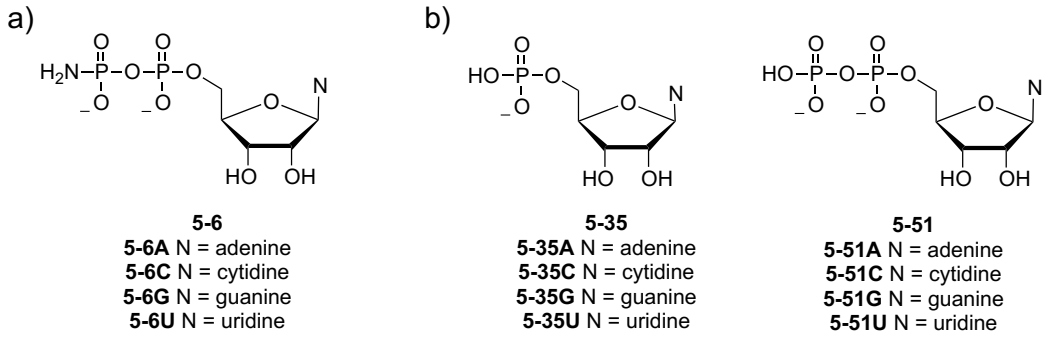
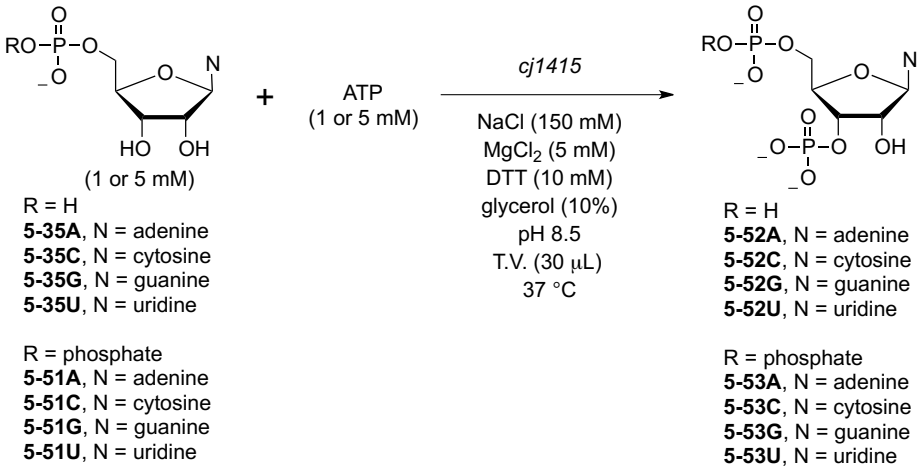


Figure 5-14: Substrates for His_6-Cj1415. a) Postulated substrate. b) Commercially available substrates that could mimic **5-6**.

Figure 5-14 shows the postulated substrate (**5-6**) for His_6-Cj1415 as well as two commercially available substrates, nucleotide monophosphates (NMP, **5-35**) and nucleotide diphosphates (NDP, **5-51**), that could potentially mimic **5-6**. The NMPs (**5-35**) lack the phosphoramidate motif while the NDPs (**5-51**) replace the amine of the phosphoramidate group with a hydroxyl group. Due to their structural similarities, it is reasonable to suggest that NMPs and NDPs could mimic **5-6** and serve as substrates for this enzyme. With this in mind, the four NMPs (**5-35**) and four NDPs (**5-51**) were incubated with His_6-Cj1415 in the presence of ATP under conditions that were optimized for APS kinase (70% homology to His_6-Cj1415) described by Schriek and Schwenn (Scheme 5-10).[36]



Scheme 5-10: Incubation of NMP (1 mM and 5 mM) and ATP (1 mM and 5 mM) with His_6-Cj1415 under conditions optimized for APS kinase. *DTT = dithiothreitol. T.V. = total volume.

For the enzyme assay, 16 reactions were setup using AMP (**5-35A**), CMP (**5-35C**), GMP (**5-35G**), UMP (**5-35U**), ADP (**5-51A**), CDP (**5-51C**), GDP (**5-51G**), and UDP (**5-51U**) as a substrate with ATP. The substrates, at either 1 mM or 5 mM final concentration, were incubated with His_6-Cj1415 at 37 °C for 24 hours. The reactions were terminated by heat shock at 95 °C, centrifuged, and then analyzed by HPLC. Interestingly, the only substrates that clearly showed a new adduct were CMP (**5-35C**) and CDP (**5-51C**). The HPLC trace for the incubation of CMP and ATP with His_6-Cj1415 is shown in Figure 5-17.

Figure 5-17a shows the HPLC trace for the CDP standard, which also contains small quantities of CMP. Under the reaction conditions, a new adduct (indicated by red arrow), eluting between 10–12 minutes, was observed. Although further investigations and controls are needed, this new adduct may be **5-52C** (Scheme 5-10).

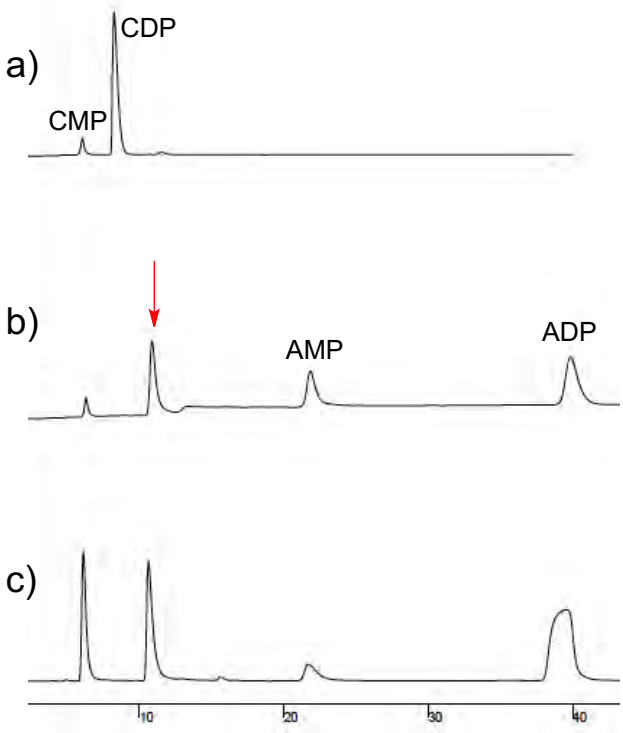
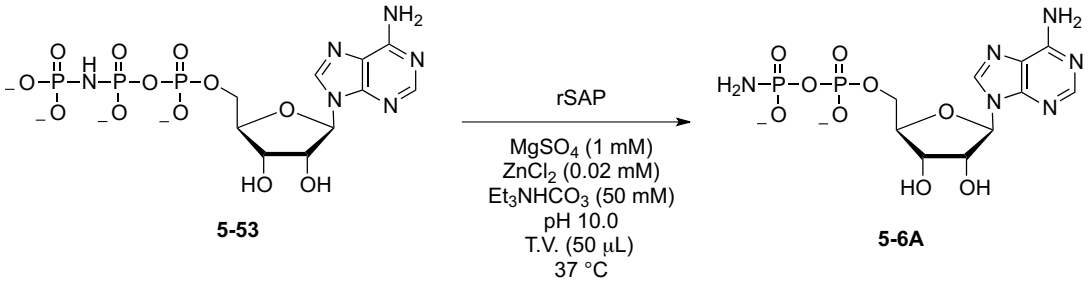


Figure 5-15: HPLC trace for CMP and ATP incubated with His_6-Cj1415. a) CDP standard. b) Incubation of CMP (1 mM) and ATP (1 mM) with His_6-Cj1415. c) Incubation of CMP (5 mM) and ATP (5 mM) with His_6-Cj1415. CMP = Cytodine-5'-monophosphate. CDP = cytodine-5'-diphosphate, AMP = adenine-5'-monophosphate. ADP = adenine-5'-diphosphate.

As detailed above, based on homology, the *cj1416* gene was putatively assigned as a phosphocholine cytidydyltransferase (LicC) and was speculated to be involved in constructing **5-6** (Figure 5-4). Given this homology data, and the preliminary results shown in Figure 5-15, the nucleobase for the phosphoramidate donor may be cytosine. Although further investigations and characterization data are needed, due to time constraints for my PhD studies, my involvement in this area did not continue.

5.7 A final attempt and future work

In addition to the preliminary data that may suggest the nucleobase of the phosphoramidate donor is cytosine, a strategy to produce the postulated substrate for the protein encoded for the gene *cj1415* was potentially found. In examining ATP formation from adenyl-5'-yl imidodiphosphate (AMP-PNP, **5-53**), Penningroth and coworkers reported incubating AMP-PNP (**5-53**) with an alkaline phosphatase produces **5-6A** (the substrate that I could not chemically synthesize).[37] Unfortunately, the formation **5-6A** was only speculative because data (e.g., NMR spectroscopy, mass spectrometry) for characterization were not performed. In lieu of this, we sought to reproduce these results for the formation of **5-6A** (Scheme 5-11).



Scheme 5-11: Incubation of AMP-PNP (**5-53**) with shrimp alkaline phosphatase (rSAP). T.V. = total volume.

Newly purchased AMP-PNP (**5-53**) was incubated with shrimp alkaline phosphatase (rSAP) at 37 °C for 24 hours under the conditions described by Penningroth and coworkers (Scheme 5-11).[37] After passage through an ultra centrifugal filter, the reaction mixture was monitored by HPLC. Unfortunately, these results via HPLC were inconclusive. Liquid chromatography mass spectrometry, however, showed a mass peak that correlated to **5-6A**. This

preliminary data may offer a strategy to finally obtain **5-6**; however, the nucleobase analogues of AMP-PNP (**5-53**) will need to be chemically synthesized, as they are not commercially available.

Although the research project involved in unraveling the biosynthesis of the MeOPN motifs was not successful, the preliminary findings warrant further investigation to characterize the products from the enzymatic assays of His_6-Cj1415 with CMP (**5-35C**) and rSAP with AMP-PNP (**5-53**). Provided these results are reproducible, identifying the phosphoramidate donor and characterizing the phosphoramidate transferases could still be achieved.

5.8 Experimental

5.8.1 General methods

5.8.1.1 General experimental methods involving chemical synthesis

All reagents were purchased from commercial sources and were used without further purification unless noted. Solvents used in reactions were purified by successive passage through columns of alumina and copper under argon. Unless stated otherwise, all reactions were carried out at room temperature and monitored by TLC on Silica Gel G-25 F_{254} (0.25 mm). TLC spots were detected under UV light and/or by charring with a solution of *p*-anisaldehyde in ethanol, acetic acid and H_2SO_4. Column chromatography was performed on Silica Gel 60 (40–60 μm). Solvents were evaporated under reduced pressure on a rotary evaporator. ^{1}H NMR spectra were recorded using 400, 500 or 600 MHz NMR instruments and were referenced to residual proton signal of $CDCl_3$ (7.26 ppm) or CD_3OD (3.30 ppm). ^{13}C NMR spectra were recorded using 126 MHz (cold probe) NMR instrument and were referenced to residual ^{13}C signals of $CDCl_3$ (77

ppm) or CD_3OD (49 ppm). ^{31}P NMR spectra were collected using 202 or 162 MHz NMR instruments and were referenced to an external 85% H_3PO_4 standard (0.00 ppm). ^{1}H NMR data are reported as though they were first order, and peak assignments were made on the basis of 2D-NMR (^{1}H –^{1}H COSY and HSQC) experiments. ESI-MS spectra (time-of-flight analyzer) were recorded on samples dissolved in THF or CH_3OH and added NaCl. Optical rotations were measured at 22 ± 2 °C at the sodium D line (589 nm) and are in a microcell (10 cm, 1 mL) in units of $deg \cdot mL(dm \cdot g)^{-1}$.

5.8.1.2 General cloning procedures

Plasmid DNA was isolated fusing Qiaprep Spin Miniprep Kit (Qiagen Inc., Mussussauga, ON) as described by the manufacturers. DNA restriction endonucleases were used as recommended by the manufacturer (Invitrogen Canada Inc.). Ligation of restriction endonuclease-digested vectors and insert DNA was performed using T4 DNA ligase (New England Biolabs, Ltd., Mississauga, ON) at 15 °C for 16 h prior to transformation into E. coli strains. Cloning of PCR products into pET28 and pET30a was performed using the corresponding cloning kits (Novagen, Madison, WI) as described by the manufacturer. Purification of DNA from agarose gels was performed using the UltraClean 15 DNA Purification Kit (Mo Mio Laboratories, Inc., Solana Beach, CA).

5.8.1.3 General PCR amplification

The oligonucleotide primers were designed and analyzed using DNA analysis software GENERUNNER for Windows (Hastings Software, NY; www.generunner.com), based on the nucleotide sequences from the complete *C. jejuni* NCTC11168 genome. The oligonucleotide

primers used are listed in Table 5-3. All restriction endonucleases were obtained from New England Biolabs (Mississauga, ON, Canada). PCR-amplification reactions were performed with a Gene Amp 2400 PCR system thermocycler (PerkinElmer Canada, Woodbridge, ON) using Taq or PWO DNA polymerase (Roche Diagnostics Corporation, Indianapolis IN) according to the manufacturers specifications. All PCR reactions were performed with HPLC-grade water (Fischer Scientific Ltd, Nepean, ON).

Hot start with touchdown PCR reaction: The template DNA was denatured by incubation at 95 °C for 5 min with the forward primer, prior to addition of Taq or PWO DNA polymerase. The reaction then cycled through a series of denaturation (90 °C), annealing (58 °C), and polymerization/extension (68 °C) steps for 3 cycles (2.5 min extension time), before adding the reverse primer. Lastly, the reverse primer was added, and the reaction cycled through a series of denaturation (90 °C), annealing (58 °C to 50 °C), and polymerization/extension (68 °C) steps for 27 cycles (2.5 min extension time), followed by a final 7 min polymerization/extension (68 °C) step. PCR reactions were analyzed by agarose gel electrophoresis as follows: 5 μL of DNA tracking dye (0.1% bromophenol blue, 15% ficoll) was loaded into a 0.8%–1.0% agarose-TAE gel (0.8%–1.0% agarose, 20 mM acetic acid, 1 mM ethylenediaminetetraacetic acid (EDTA), 40 mM tris(hydroxymethyl)aminomethane (Tris) and electrophoresed at 80–100 V. Gels were stained in a 0.002% ethidium bromide solution and visualized under ultraviolet (UV) light. Products were purified using QIAquick PCR Purification Kit (Qiagen Inc) prior to restriction endonuclease digestion.

5.8.1.4 General screening of potential clones

Screening of potential clones for those containing plasmid DNA with the desired insert was performed by the E-lyse method.[38] Colonies to be screened were patched onto the appropriate selective media and incubated overnight at 37 °C. Patched cells were scraped using a sterile loop and resuspended in 15 μL of TE buffer (1 mM EDTA, 10 mM Tris-HCl, pH 8.0) and combined with 15 μL of SRL lysis solution (25% sucrose, 2 units/mL RNAse, and 1 mg/mL lysozyme in TBE buffer (90 mM boric acid, 2.5 mM EDTA, 90 mM Tris-HCl). Samples were then loaded into a 1% agarose–TBE gel (1% agarose, 0.2% SDS in TBE buffer) that was submerged in TBE buffer (45 mM Tris-borate, 1 mM EDTA, pH = 8.3), and allowed to stand in the wells for 10 min. Electrophoresis was performed at 120 V for 1–2 h. The gel was stained in a 0.002% ethidium bromide bath for 20 min, and then visualized under UV light.

5.8.1.5 General DNA sequencing

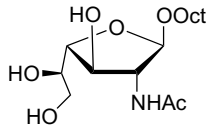
DNA samples were prepared as described above (5.7.1.2) and DNA sequencing was performed by the Molecular Biology Service Unit (MBSU), Department of Biological Sciences, University of Alberta. The resultant DNA sequences were analyzed using the analyzed using DNA analysis software GENERUNNER for Windows (Hastings Software, NY; htttp://www.generunner.com), and DNA and protein database searches were performed using the National Centre for Biotechnology Institute (NCBI) Basic Local Alignment Search Tool (BLAST) network server.

5.8.1.6 General Protein Methods

Unless otherwise stated, all reagents were obtained from Sigma–Aldrich Canada Ltd. (Oakville, ON), all media were obtained from Difco Laboratories (Detroit, MI), and all solutions were prepared using water purified by a Milli-Q PF Ultra-Pure Water System (Millipore (Canada) Ltd., Missisauga, ON). The plasmids were used as listed in Table 5-3.

5.8.2 Experimental, spectroscopic, and analytical data

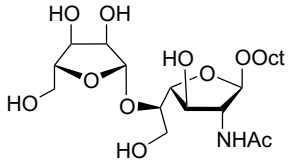
*The synthesis of compounds **5-26**, **5-30**, and **5-31** are described in Chapter 4 of this thesis.



Octyl 2-acetamido-2-deoxy-β-D-galactofuranoside (5-8)

Phthalimide **5-15** (315 mg, 0.58 mmol) was dissolved in a solution of 20% ethylene diamine in ethanol (10 mL). The reaction mixture was stirred at reflux for 16 hours, concentrated, co-evaporated with toluene, and dried under vacuum for 12 h. To the resulting residue was added a solution of 10:1:1 CH_3OH–Et_3N–Ac_2O (10 mL) and the reaction mixture was stirred for 18 hours. The reaction mixture was concentrated and the resulting residue was purified by flash chromatography in 9:1 CH_2Cl_2–CH_3OH to afford **5-8** (160 mg, 83%) as a clear oil. R_f 0.42 (9:1 CH_2Cl_2–CH_3OH); $[\alpha]_D$ −73.9 (*c* 1.0, CH_3OH); ^{1}H NMR (600 MHz; CD_3OD): δ 4.83 (d, 1H, *J* = 2.3 Hz, H-1), 4.13 (dd, 1H, *J* = 4.5, 2.2 Hz, H-2), 4.03 (dd, 1H, *J* = 6.6, 4.5 Hz, H-3), 3.94 (dd, 1H, *J* = 6.6, 2.9 Hz, H-4), 3.72 (ddd, 1H, *J* = 6.3, 6.3, 2.8 Hz, H-5), 3.67 (ddd,

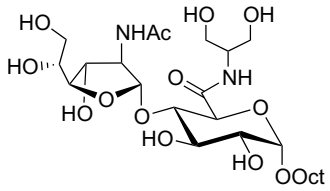
1H, *J* = 9.6, 6.7, 6.7 Hz, OC$\underline{H}_2$CH$_2$), 3.62–3.61 (m, 2H, H-6, H-6'), 3.40 (ddd, *J* = 9.6, 6.6, 6.6 Hz, OC$\underline{H}_2$CH$_2$), 1.95 (s, 3H, COC$\underline{H}_3$), 1.58–1.55 (m, 2H, OCH$_2$C$\underline{H}_2$), 1.37–1.26 (m, 10H, 5 × C$\underline{H}_2$), 0.89 (t, 3H, *J* = 7.0 Hz, CH$_2$C$\underline{H}_3$); ^{13}C NMR (126 MHz; CD$_3$OD): δ 171.6 (C=O), 106.5 (C-1), 82.8 (C-4), 76.1 (C-3), 70.8 (C-5), 67.5 (O$\underline{C}$H$_2$CH$_2$), 63.2 (C-2), 63.1 (C-6), 31.6 ($\underline{C}$H$_2$), 29.2 ($\underline{C}$H$_2$), 29.1 ($\underline{C}$H$_2$), 29.0 ($\underline{C}$H$_2$), 25.8 ($\underline{C}$H$_2$), 22.3 ($\underline{C}$H$_2$), 21.2 (CO$\underline{C}$H$_3$), 13.0 (CH$_2\underline{C}$H$_3$); HRMS (ESI) Calc. for (M + Na) $C_{16}H_{31}NNaO_6$: 356.2044. Found 356.2038.



Octyl β-D-ribofuranosyl-(1→5)-2-acetamido-2-deoxy-β-D-galactofuranoside (5-9)

To **5-19** (61 mg, 0.94 mmol) in CH$_3$OH (3 mL) was added Pd(OH)$_2$–C (12 mg). The mixture was placed under a positive pressure of H$_2$ and stirred for 115 h. The palladium was removed by filtration through filter paper and the filtrate was concentrated. The residue was dissolved in methanol and was passed through a 0.22 μm filter unit. The filtrate was concentrated to afford **5-9** (34 mg, 77%) as a clear oil. R$_f$ 0.29 (6:1 CH$_2$Cl$_2$–CH$_3$OH); [α]$_D$ −69.7 (1.0 *c*, CH$_3$OH); ^{1}H NMR (400 MHz, CD$_3$OD): δ 5.15 (s, 1H, H-1$_{Rib\mathit{f}}$), 4.80 (s, 1H, H-1$_{Gal\mathit{f}N}$), 4.17 (dd, 1H, *J* = 7.1, 4.7 Hz, H-3$_{Rib\mathit{f}}$), 4.10 (dd, 1H, *J* = 6.8, 4.7 Hz, H-3$_{Gal\mathit{f}N}$), 4.06 (app. dd, 1H, *J* = 4.6, 2.1 Hz, H-2$_{Gal\mathit{f}N}$), 3.97 (d, 1H, *J* = 4.6 Hz, H-2$_{Rib\mathit{f}}$), 3.95–3.90 (m, 2H, H-4$_{Gal\mathit{f}N}$, H-4$_{Rib\mathit{f}}$), 3.82–3.78 (m, 1H, H-5$_{Gal\mathit{f}N}$), 3.78 (dd, 1H, *J* = 12.3, 2.7 Hz, H-5$_{Rib\mathit{f}}$), 3.69 (app. d, 2H, *J* = 5.6 Hz, H-6$_{Gal\mathit{f}N}$, H-6'$_{Gal\mathit{f}N}$), 3.65–3.59 (m, 2H, H-5$_{Rib\mathit{f}}$, OC$\underline{H}_2$CH$_2$), 3.38 (ddd, 1H, *J* = 9.6, 6.5, 6.5 Hz, OC$\underline{H}_2$CH$_2$), 1.94 (s, 3H, COC$\underline{H}_3$), 1.59–1.50 (m, 2H, OCH$_2$C$\underline{H}_2$), 1.35–1.28 (m, 10H, 5 × C$\underline{H}_2$), 0.87 (t, 3H, *J* = 6.8 Hz, CH$_2$C$\underline{H}_3$); ^{13}C NMR (126 MHz, CD$_3$OD): δ 171.8 (C=O), 107.0 (C-1$_{Rib\mathit{f}}$), 106.1 (C-1$_{Gal\mathit{f}N}$), 83.4 (C-4), 81.8 (C-4), 77.3 (C-5$_{Gal\mathit{f}N}$), 75.8 (C-3$_{Gal\mathit{f}N}$), 75.3 (C-2$_{Rib\mathit{f}}$), 70.0 (C-

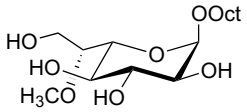
3$_{Rib\textit{f}}$), 67.6 (O<u>C</u>H$_2$CH$_2$), 63.5 (C-2$_{Gal\textit{f}N}$), 61.8 (C-5$_{Rib\textit{f}}$), 61.3 (C-6$_{Gal\textit{f}N}$), 31.6 (<u>C</u>H$_2$), 29.2 (<u>C</u>H$_2$), 29.1 (<u>C</u>H$_2$), 29.0 (<u>C</u>H$_2$), 25.8 (<u>C</u>H$_2$), 22.3 (<u>C</u>H$_2$), 21.2 (CO<u>C</u>H$_3$), 21.2 (CH$_2$<u>C</u>H$_3$); HRMS (ESI) Calc. for (M + Na) $C_{21}H_{39}NNaO_{10}$: 488.2466. Found 488.2459.



2-*N*-[2-Acetamido-2-deoxy-β-D-galactofuranosyl-(1→4)-(octyl α-D-glucopyranosiduronoyl)]-1,3-di-*O*-benzyl-2-deoxy-glycerol (5-11)

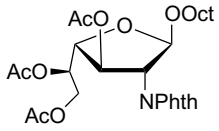
To **5-32** (29 mg, 0.03 mmol) in CH_3OH (1 mL) was added $Pd(OH)_2$–C (15 mg). The mixture was placed under a positive pressure of H_2 and stirred for 48 h. The palladium was removed by filtration through filter paper and the filtrate was concentrated. The resulting residue was purified by C_{18} flash chromatography using a gradient of 1:0→3:2 H_2O–CH_3OH to afford **5-11** (14 mg, 78%) as a clear oil. $[\alpha]_D$ +11.5 (1.0 *c*, CH_3OH); ^{1}H NMR (500 MHz, CD_3OD) δ 5.02 (d, 1H, *J* = 1.9 Hz, H-1$_{Gal\textit{f}N}$), 4.83 (d, 1H, *J* = 3.8 Hz, H-1$_{Glc}$), 4.17 (dd, 1H, *J* = 5.9, 2.7 Hz, H-4$_{Gal\textit{f}N}$), 4.14 (dd, 1H, *J* = 4.0, 2.0 Hz, H-2$_{Gal\textit{f}N}$), 4.10 (d, 1H, *J* = 9.7 Hz, H-5$_{Glc}$), 4.04 (dd, 1H, *J* = 5.9, 4.0 Hz, H-3$_{Gal\textit{f}N}$), 3.94 (app q, 1H, *J* = 5.6 Hz, NC<u>H</u>(CH$_2$OH)$_2$), 3.75 (dd, 1H, *J* = 9.1, 9.1 Hz, H-3$_{Glc}$), 3.74–3.58 (m, 9H, H-4$_{Glc}$, H-5$_{Gal\textit{f}N}$, H-6$_{Gal\textit{f}N}$, H-6'$_{Gal\textit{f}N}$, OC<u>H$_2$</u>CH$_2$, CHC<u>H$_2$</u>CH$_2$OH, CHC<u>H$_2$</u>OH), 3.49 (dd, 1H, *J* = 9.4, 3.8 Hz, H-2$_{Glc}$), 3.44 (ddd, 1H, *J* = 9.7, 6.8, 6.8 Hz, OC<u>H$_2$</u>CH$_2$), 1.96 (s, 3H, COC<u>H$_3$</u>), 1.66–1.61 (m, 2H, OCH$_2$C<u>H$_2$</u>), 1.40–1.28 (m, 10H, 5 × C<u>H$_2$</u>), 0.89 (t, 3H, *J* = 7.1 Hz, CH$_2$C<u>H$_3$</u>); ^{13}C NMR (126 MHz, CD_3OD) δ 173.2 (C=O), 171.9 (C=O), 107.1 (C-1$_{Gal\textit{f}N}$), 100.4 (C-1$_{Glc}$), 85.0 (C-4$_{Gal\textit{f}N}$), 78.8 (C-4$_{Glc}$), 77.7 (C-5$_{Gal\textit{f}N}$), 73.1, 73.0 (2C), 72.3, 72.0 (C-5$_{Glc}$), 70.0 (O<u>C</u>H$_2$CH$_2$), 64.5 (2C, C-6$_{Gal\textit{f}N}$, C-2$_{Gal\textit{f}N}$), 61.9 (CH<u>C</u>H$_2$OH), 61.8

(CH<u>C</u>H$_2$OH), 54.5 (N<u>C</u>H(CH$_2$OH)$_2$), 33.0 (<u>C</u>H$_2$), 30.7 (<u>C</u>H$_2$), 30.6 (<u>C</u>H$_2$), 30.4 (<u>C</u>H$_2$), 27.2 (<u>C</u>H$_2$), 23.7 (<u>C</u>H$_2$), 22.8 (CO<u>C</u>H$_3$), 14.4 (CH$_2$<u>C</u>H$_3$); HRMS (ESI) Calc. for (M + Na) $C_{25}H_{46}N_2NaO_{13}$: 605.2892. Found 605.2883.



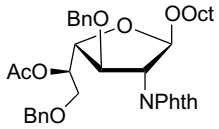
Octyl 6-*O*-methyl- D-glycero-α- L-gluco-heptopyranoside (5-12)

To a solution of **5-33** (51 mg, 0.07 mmol) in methanol (2 mL) was added Pd–C (8 mg). The reaction was stirred under H_2(g) for 66 h. The palladium was removed by filtration through filter paper and the filtrate was concentrated. The residue was diluted with methanol and then passed through a 0.22 μm filter. The filtrate was concentrated to afford **5-12** (22 mg, 88%) as a clear oil. R_f = 0.65 (6:1 CH_2Cl_2–CH_3OH); $[\alpha]_D$ –0.0 (*c* 1.0, CH_3OH); ^{1}H NMR (500 MHz, CD_3OD) δ 4.72 (d, 1H, *J* = 3.7 Hz, H-1), 3.70–3.57 (m, 5H, H-3, H-5, H-6, H-7, OC<u>H</u>$_2$CH$_2$), 3.53 (s, 3H, OC<u>H</u>$_3$), 3.51–3.43 (m, 1H, H-4), 3.41–3.32 (m, 2H, H-7', H-2), 1.78–1.44 (m, 2H, OCH$_2$C<u>H</u>$_2$), 1.44–1.15 (m, 10H, 5 × C<u>H</u>$_2$), 0.88 (t, 3H, *J* = 6.8 Hz, CH$_2$C<u>H</u>$_3$); ^{13}C NMR (126 MHz, CD_3OD): δ 100.2 (C-1), 80.5 (C-5), 75.4 (C-3), 73.5 (C-2), 72.0 (C-6), 71.1 (C-4), 69.1 (C-7), 62.5 (O<u>C</u>H$_2$CH$_2$), 60.3 (O<u>C</u>H$_3$), 30.6 (<u>C</u>H$_2$), 30.5 (<u>C</u>H$_2$), 30.2 (<u>C</u>H$_2$), 29.8 (<u>C</u>H$_2$), 25.7 (<u>C</u>H$_2$), 22.1 (<u>C</u>H$_2$), 21.2 (CH$_2$<u>C</u>H$_3$); HRMS (ESI) Calc. for (M + Na) $C_{16}H_{32}NaO_7$: 359.2046. Found 359.2040.



Octyl 3,5,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-galactofuranoside (5-15)

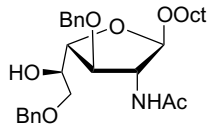
To **4-11** (350 mg, 0.65 mmol) and octanol (153 μL, 0.97 mmol) in anhydrous CH_2Cl_2 (7 mL) was added 4Å MS (300 mg). The mixture was stirred for 7 h at room temperature, then cooled to –15 °C. NIS (218 mg, 0.97 mmol) and AgOTf (25 mg, 0.10 mmol) were then added and the reaction mixture was stirred for 1.5 h. Et_3N (100 μL) was then added. The mixture was passed through a bed of Celite and the filtrate was washed with a saturated $Na_2S_2O_3$ solution (1×), H_2O (1×), and brine (1×). The organic layer was then dried over $MgSO_4$, filtered, and concentrated. The resulting residue was purified by flash chromatography in 2:1 hexanes–EtOAc to afford **5-15** (335 mg, 95%) as a clear oil. R_f = 0.31 (2:1 hexanes–EtOAc); $[\alpha]_D$ −43.0 (1.0 *c*, $CHCl_3$); ^{1}H NMR (400 MHz; $CDCl_3$): δ 7.86 (dd, 2H, *J* = 5.5, 3.0 Hz, Ar), 7.73 (dd, 2H, *J* = 5.4, 3.1 Hz, Ar), 5.52 (dd, 1H, *J* = 8.5, 7.0 Hz, H-3), 5.39–5.35 (m, 2H, H-1, H-5), 4.69 (dd, 1H, *J* = 7.0, 3.8 Hz, H-4), 4.43–4.39 (m, 2H, H-2, H-6), 4.31 (dd, 1H, *J* = 11.8, 7.3 Hz, H-6'), 3.70 (ddd, 1H, *J* = 9.6, 6.7, 6.7 Hz, OC$\underline{H}_2$CH$_2$), 3.41 (ddd, 1H, *J* = 9.6, 6.6 Hz, OC$\underline{H}_2$CH$_2$), 2.22 (s, 3H, COC$\underline{H}_3$), 2.07 (s, 3H, COC$\underline{H}_3$), 2.00 (s, 3H, COC$\underline{H}_3$), 1.60–1.52 (m, 2H, OCH$_2$C$\underline{H}_2$), 1.32–1.21 (m, 10H, 5 × C$\underline{H}_2$), 0.86 (t, 3H, *J* = 6.9 Hz, CH$_2$C$\underline{H}_3$); ^{13}C NMR (126 MHz; $CDCl_3$): δ 170.5 (C=O), 170.4 (C=O), 167.5 (C=O), 141.4 (Ar) 134.2 (Ar), 131.8 (Ar), 123.6 (Ar), 103.9 (C-1), 77.5 (C-2), 73.1 (C-3), 68.9 (O$\underline{C}$H$_2$CH$_2$), 68.7 (C-5), 62.8 (C-6), 61.5 (C-4), 31.8 ($\underline{C}$H$_2$), 29.5 ($\underline{C}$H$_2$), 29.3 ($\underline{C}$H$_2$), 29.2 ($\underline{C}$H$_2$), 26.0 ($\underline{C}$H$_2$), 22.6 ($\underline{C}$H$_2$), 20.8 (2C, 2 × CO$\underline{C}$H$_3$), 20.5 (CO$\underline{C}$H$_3$), 14.1 (CH$_2\underline{C}$H$_3$); HRMS (ESI) Calc. for (M + Na) $C_{28}H_{37}NNaO_{10}$: 570.2310. Found 570.2296.



Octyl 5-*O*-acetyl-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-galactofuranoside (5-16)

To **4-12** (270 mg, 0.42 mmol) and octanol (83 mg, 0.64 mmol) in anhydrous CH_2Cl_2 (4.5 mL) was added 4Å MS (280 mg). The mixture was stirred for 1.5 h at room temperature, then cooled to –15 °C. NIS (124 mg, 0.55 mmol) and AgOTf (14 mg, 0.06 mmol) were then added and the reaction mixture was stirred for 40 min. Et_3N (100 μL) was then added. The mixture was passed through a bed of Celite and the filtrate was washed with a saturated $Na_2S_2O_3$ solution (1×), H_2O (1×), and brine (1×). The organic layer was then dried over Na_2SO_4, filtered, and concentrated. The resulting residue was purified by flash chromatography in 3:1 hexanes–EtOAc to afford **5-16** (255 mg, 94%) as a clear oil. R_f = 0.30 (3:1 hexanes–EtOAc); $[\alpha]_D$ −24.7 (1.0 *c*, $CHCl_3$); ^{1}H NMR (500 MHz; $CDCl_3$): δ 7.81–7.79 (m, 2H, Ar), 7.76–7.73 (m, 2H, Ar), 7.38–7.35 (m, 4H, Ar), 7.32–7.29 (m, 1H, Ar), 7.14–7.12 (m, 2H, Ar), 7.03–7.00 (m, 2H, Ar), 6.92–6.89 (m, 1H, Ar), 5.54 (ddd, 1H, *J* = 7.3, 5.4, 2.1 Hz, H-5), 5.21 (d, 1H, *J* = 4.0 Hz, H-1), 4.72 (dd, 1H, *J* = 7.1, 4.0 Hz, H-2), 4.62 (d, 1H, *J* = 12.0 Hz, PhC$\underline{H_2}$O), 4.59 (d, 1H, *J* = 12.0 Hz, PhC$\underline{H_2}$O), 4.52 (d, 1H, *J* = 11.9 Hz, PhC$\underline{H_2}$O), 4.48 (dd, 1H, *J* = 8.2, 7.2 Hz, H-3), 4.41 (d, 1H, *J* = 11.9 Hz, PhC$\underline{H_2}$O), 4.36 (dd, 1H, *J* = 8.3, 2.2 Hz, H-4), 3.80 (dd, 1H, *J* = 10.3, 7.2 Hz, H-6), 3.75 (dd, 1H, *J* = 10.3, 5.5 Hz, H-6'), 3.67 (ddd, 1H, *J* = 9.6, 6.7, 6.7 Hz, OC$\underline{H_2}$CH$_2$), 3.36 (ddd, *J* = 9.6, 6.7, 6.7 Hz, OC$\underline{H_2}$CH$_2$), 2.26 (s, 3H, COC$\underline{H_3}$), 1.56–1.51 (m, 2H, OCH$_2$C$\underline{H_2}$), 1.29–1.20 (m, 10H, 5 × C$\underline{H_2}$), 0.87 (t, 3H, *J* = 7.0 Hz, CH$_2$C$\underline{H_3}$); ^{13}C NMR (126 MHz; $CDCl_3$): δ 171.0 (C=O), 167.2 (C=O), 138.0 (Ar), 137.6 (Ar), 134.0 (Ar), 131.8 (Ar), 128.4 (Ar), 128.2 (2C, 2 × Ar) 127.6 (3C, 3 × Ar), 123.3 (Ar), 103.9 (C-1), 79.1 (C-4), 78.2 (C-3), 73.2 (Ar$\underline{C}$H$_2$O), 72.9 (Ar$\underline{C}$H$_2$O), 69.8 (C-5), 69.0 (C-6), 68.5 (O$\underline{C}$H$_2$CH$_2$), 61.8 (C-2), 31.8 ($\underline{C}$H$_2$), 29.6 ($\underline{C}$H$_2$), 29.3

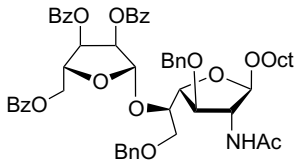
($\underline{C}H_2$), 29.2 ($\underline{C}H_2$), 26.0 ($\underline{C}H_2$), 22.6 ($\underline{C}H_2$), 21.1 ($CO\underline{C}H_3$), 14.1 ($CH_2\underline{C}H_3$); HRMS (ESI) Calc. for (M + Na) $C_{38}H_{45}NNaO_8$: 666.3037. Found 666.3031.



Octyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-galactofuranoside (5-17)

Phthalimide **5-16** (255 mg, 0.37 mmol) was dissolved in a solution of 10% ethylene diamine in ethanol (7 mL). The mixture was stirred at reflux for 16 h. The mixture was concentrated, co-evaporated with toluene, and dried under vacuum for 12 h. To the resulting residue was added a solution of 8:1:1 CH_3OH–Ac_2O–Et_3N (7 mL) and the reaction mixture was stirred for 20 h. The solution was then concentrated and the resulting residue was purified by flash chromatography using a gradient of 3:1→4:1 EtOAc–hexanes to afford **5-17** (164 mg, 86%) as a clear oil. R_f = 0.37 (3:1 EtOAc–hexanes); $[\alpha]_D$ −78.3 (1.0 *c*, $CHCl_3$); ^{1}H NMR (498 MHz; $CDCl_3$): δ 7.39–7.28 (m, 10H, Ar), 6.43 (d, 1H, *J* = 9.0 Hz, N$\underline{H}$), 4.94 (s, 1H, H-1), 4.79 (d, 1H, *J* = 12.1 Hz, PhC$\underline{H}_2$O), 4.60 (d, 1H, *J* = 11.8 Hz, PhC$\underline{H}_2$O), 4.57–4.50 (m, 3H, H-2, PhC$\underline{H}_2$O, PhC$\underline{H}_2$O), 4.13 (dd, 1H, *J* = 3.3, 1.9 Hz, H-4), 3.96–3.93 (m, 1H, H-5), 3.90 (d, 1H, *J* = 3.4 Hz, H-3), 3.67–3.58 (m, 3H, H-6, H-6', OC$\underline{H}_2CH_2$), 3.44 (ddd, 1H, *J* = 9.6, 6.6, 6.6 Hz, OC$\underline{H}_2CH_2$), 2.81 (s, 1H, C-5-O$\underline{H}$), 1.95 (s, 3H, COC$\underline{H}_3$), 1.63–1.57 (m, 2H, $OCH_2C\underline{H}_2$), 1.36–1.28 (m, 10H, 5 × C$\underline{H}_2$), 0.90 (t, 3H, *J* = 7.0 Hz, $CH_2C\underline{H}_3$); ^{13}C NMR (126 MHz; $CDCl_3$): δ 169.2 (C=O), 137.8 (Ar), 137.7 (Ar), 128.5 (Ar), 128.4 (Ar), 128.0 (Ar), 127.8 (2C, 2 × Ar), 127.7 (Ar), 108.1 (C-1), 85.2 (C-3), 83.6 (C-4), 73.5 (Ph$\underline{C}H_2$O), 71.8, 71.7, 70.3 (C-5), 67.6 (O$\underline{C}H_2CH_2$), 57.7 (C-2), 31.9 ($\underline{C}H_2$), 29.6 ($\underline{C}H_2$), 29.4 ($\underline{C}H_2$), 29.3 ($\underline{C}H_2$), 26.1 ($\underline{C}H_2$), 23.3

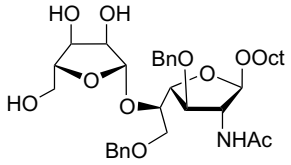
(CO<u>C</u>H$_3$), 22.7 (<u>C</u>H$_2$), 14.1 (CH$_2$<u>C</u>H$_3$); HRMS (ESI) Calc. for (M + Na) $C_{30}H_{43}NNaO_6$: 536.2983. Found 536.2978.



Octyl 2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl-(1→5)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-galactofuranoside (5-18)

A mixture of **5-17** (30 mg, 0.06 mmol), **4-13** (66 mg, 0.12 mmol), $SrCO_3$ (34 mg, 0.23 mmol) and 4Å molecular sieves (100 mg) in anhydrous CH_2Cl_2 (1 mL) under argon was stirred at room temperature for 2 h. The mixture was then cooled to 0 °C before AgOTf (30 mg, 0.12 mmol) and a freshly prepared 2.0 M solution of *p*-TolSBr (60 μL, 0.12 mmol) were sequentially added. The reaction mixture was continually stirred at 0 °C for 15 min, at which point Et_3N (100 μL) was added. The mixture was passed through a bed of Celite. The filtrate was concentrated and resulting residue was purified by flash chromatography using a gradient of 2:1→3:2 hexane−EtOAc to afford **5-18** (44 mg, 79%) as a clear oil. R_f 0.25 (2:1 hexanes–EtOAc); $[\alpha]_D$ −41.5 (1.0 *c*, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.06–8.00 (m, 4H, Ar), 7.91–7.89 (m, 2H, Ar), 7.63–7.52 (m, 3H, Ar), 7.46–7.17 (m, 16H, Ar), 6.55 (d, 1H, *J* = 8.5 Hz, N<u>H</u>$_{Galf\text{N}}$), 5.80 (dd, 1H, *J* = 6.7, 4.9 Hz, H-3$_{Ribf}$), 5.72 (dd, 1H, *J* = 4.9, 1.1 Hz, H-2$_{Ribf}$), 5.49 (d, 1H, *J* = 0.8 Hz, H-1$_{Ribf}$), 4.87 (s, 1H, H-1$_{Galf\text{N}}$), 4.80 (d, 1H, *J* = 11.8 Hz, PhC<u>H</u>$_2$O), 4.76 (dd, 1H, H-5$_{Ribf}$), 4.69–4.65 (m, 1H, H-4$_{Ribf}$), 4.59 (dd, 1H, *J* = 11.5, 3.7 Hz, H-5'$_{Ribf}$), 4.54–4.50 (m, 3H, H-2$_{Galf\text{N}}$, PhC<u>H</u>$_2$O, PhC<u>H</u>$_2$O), 4.47 (d, 1H, *J* = 12.0 Hz, PhC<u>H</u>$_2$O), 4.13–4.07 (m, 2H, H-4$_{Galf\text{N}}$, H-5$_{Galf\text{N}}$), 3.85 (dd, 1H, *J* = 5.1, 1.4 Hz, H-3$_{Galf\text{N}}$), 3.69 (dd, 1H, *J* = 10.6, 7.3 Hz, H-6$_{Galf\text{N}}$), 3.63 (ddd, 1H, *J* = 9.7, 6.8, 6.8 Hz, OC<u>H</u>$_2$CH$_2$), 3.56 (dd, 1H, *J* = 10.5, 3.4 Hz, H-6'$_{Galf\text{N}}$), 3.42 (ddd, 1H, *J* = 9.7,

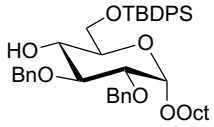
6.7, 6.7 Hz, OC$\underline{H}_2$CH$_2$), 2.00 (s, 3H, COC$\underline{H}_3$), 1.63–1.56 (m, 2H, OCH$_2$C$\underline{H}_2$), 1.36–1.28 (m, 10H, 5 × C$\underline{H}_2$), 0.89 (t, 3H, J = 6.9 Hz, CH$_2$C$\underline{H}_3$); ^{13}C NMR (126 MHz; $CDCl_3$): δ 169.6 (C=O), 166.5 (C=O), 165.3 (2C, C=O, C=O), 137.9 (Ar), 137.8 (Ar), 133.5 (2C, 2 × Ar), 133.4 (Ar), 129.8 (3C, 3 × Ar), 129.5 (Ar), 129.2 (Ar), 128.9 (Ar), 128.5 (2C, 2 × Ar), 128.4 (2C, 2 × Ar), 128.3 (Ar), 128.1 (Ar), 127.6 (3C, 3 × Ar), 107.2 (C-1$_{Galf\text{N}}$), 105.4 (C-1$_{Ribf}$), 84.6 (C-3$_{Galf\text{N}}$), 82.3 (C-4$_{Galf\text{N}}$), 79.2 (C-4$_{Ribf}$), 75.6 (2C, C-5$_{Galf\text{N}}$, C-2$_{Ribf}$), 73.5 (Ph$\underline{C}$H$_2$O), 72.2 (C-3$_{Ribf}$), 71.8 (Ph$\underline{C}$H$_2$O), 70.8 (C-6$_{Galf\text{N}}$), 67.6 (O$\underline{C}$H$_2$CH$_2$), 65.2 (C-5$_{Ribf}$), 59.5 (C-2$_{Galf\text{N}}$), 31.9 ($\underline{C}$H$_2$), 29.5 ($\underline{C}$H$_2$), 29.4 ($\underline{C}$H$_2$), 29.3 ($\underline{C}$H$_2$), 26.1 ($\underline{C}$H$_2$), 23.1 (CO$\underline{C}$H$_3$), 22.7 ($\underline{C}$H$_2$), 14.1 (CH$_2\underline{C}$H$_3$); HRMS (ESI) Calc. for (M + Na) $C_{56}H_{63}NNaO_{13}$: 980.4192. Found 980.4184. *Note: increasing scale to 0.247 mmol (acceptor) under same conditions afforded the product (108 mg, 46%) in decreased yields.



Octyl β-D-ribofuranosyl-(1→5)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-galactofuranoside (5-19)

To a solution of **5-18** (148 mg, 0.15 mmol) in anhydrous CH_3OH (3 mL) under argon was added a 0.10 mmol $NaOCH_3$ solution in CH_3OH dropwise until a piece of wet pH paper read 8–9. The reaction mixture was stirred for 1.5 h and was then concentrated. The resulting residue was purified by flash chromatography in 20:1→15:1 CH_2Cl_2–CH_3OH to afford **5-19** (92 mg, 93%) as a clear oil. R_f 0.40 (10:1 CH_2Cl_2–CH_3OH); $[\alpha]_D$ −84.9 (1.0 *c*, CH_3OH); ^{1}H NMR (498 MHz, CD_3OD) δ 7.32–7.24 (m, 10H, Ar), 5.18 (s, 1H, H-1$_{Ribf}$), 4.86 (s, 1H, H-1$_{Galf\text{N}}$), 4.69 (d, 1H, J = 11.8 Hz, PhC$\underline{H}_2$O), 4.49 (d, 1H, J = 12.3 Hz, PhC$\underline{H}_2$O), 4.48 (d, 1H, J = 11.7 Hz,

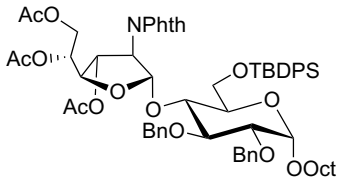
PhC$\underline{H}_2$O), 4.47 (d, 1H, J = 12.4 Hz, PhC$\underline{H}_2$O), 4.26 (d, 1H, J = 1.5 Hz, H-2$_{Gal\mathit{f}N}$), 4.17 (dd, 1H, J = 7.1, 4.7 Hz, H-3$_{Rib\mathit{f}}$), 4.04 (dd, 1H, J = 5.6, 5.6 Hz, H-4$_{Gal\mathit{f}N}$), 3.98–3.93 (m, 4H, H-3$_{Gal\mathit{f}N}$, H-5$_{Gal\mathit{f}N}$, H-2$_{Rib\mathit{f}}$, H-4$_{Rib\mathit{f}}$), 3.73 (dd, 1H, J = 12.1, 2.8 Hz, H-5$_{Rib\mathit{f}}$), 3.66–3.60 (m, 2H, H-5'$_{Rib\mathit{f}}$, OC$\underline{H}_2$CH$_2$), 3.55 (d, 2H, J = 5.5 Hz, H-6$_{Gal\mathit{f}N}$, H-6'$_{Gal\mathit{f}N}$), 3.40 (ddd, 1H, J = 9.6, 6.3 Hz, OC$\underline{H}_2$CH$_2$), 1.93 (s, 3H, COC$\underline{H}_3$), 1.59–1.53 (m, 2H, OCH$_2$C$\underline{H}_2$), 1.36–1.27 (m, 10H, 5 × C$\underline{H}_2$), 0.88 (t, 3H, J = 7.0 Hz, CH$_2$C$\underline{H}_3$); ^{13}C NMR (126 MHz, CD$_3$OD): δ 171.1 (C=O), 138.1 (Ar), 138.0 (Ar), 128.0 (Ar), 127.9 (Ar), 127.7 (Ar), 127.4 (Ar), 127.3 (2C, 2 × Ar), 107.0 (C-1$_{Rib\mathit{f}}$), 106.6 (C-1$_{Gal\mathit{f}N}$), 84.5, 83.4, 81.8 (C-4$_{Gal\mathit{f}N}$), 75.9, 75.4, 73.0 (Ph$\underline{C}$H$_2$O), 71.6 (Ph$\underline{C}$H$_2$O), 70.1 (C-3$_{Rib\mathit{f}}$), 69.9 (C-6$_{Gal\mathit{f}N}$), 67.1 (O$\underline{C}$H$_2$CH$_2$), 61.8 (C-5$_{Rib\mathit{f}}$), 60.7 (C-2$_{Gal\mathit{f}N}$), 31.6 ($\underline{C}$H$_2$), 29.2 ($\underline{C}$H$_2$), 29.0 (2C, $\underline{C}$H$_2$, $\underline{C}$H$_2$), 25.9 ($\underline{C}$H$_2$), 22.3 ($\underline{C}$H$_2$), 21.2 (CO$\underline{C}$H$_3$), 13.1 (CH$_2\underline{C}$H$_3$); HRMS (ESI) Calc. for (M + Na) $C_{35}H_{51}NNaO_{10}$: 668.3405. Found 668.3397.



Octyl 4,6-*O*-benzylidene-6-*O*-*tert*-butyldiphenylsilyl-α-D-glucopyranoside (5-22)

To **5-21**[39] (730 mg, 1.88 mmol) in an anhydrous 4:1 CH_2Cl_2–pyridine solution (10 mL) under argon was added TBDPSCl (590 μL, 2.26 mmol) dropwise over ~2 min at 0 °C. After complete addition, the reaction mixture was gradually warmed to room temperature and stirred for 14 h. The reaction mixture was then re-cooled to 0 °C and additional TBDPSCl (200 μL, 0.77 mmol) was added. The reaction mixture was warmed to room temperature and stirred for an additional 3 h before the excess TBDPSCl was quenched by the addition of CH_3OH (1 mL). The mixture was then concentrated and purified by flash chromatography in 8:1 hexanes−EtOAc to afford **5-22** (1.05 g, 89%) as a clear viscous oil. R_f 0.37 (8:1 hexanes−EtOAc); $[\alpha]_D$ +30.8 (2.3 *c*,

$CHCl_3$); ^1H NMR (498 MHz; $CDCl_3$): δ 7.76−7.70 (m, 4H, Ar), 7.45−7.32 (m, 16H, Ar), 5.02 (d, 1H, *J* = 11.3 Hz, PhC$\underline{H}_2$O), 4.80 (d, 1H, *J* = 3.4 Hz, H-1), 4.80 (d, 1H, *J* = 11.2 Hz, PhC$\underline{H}_2$O), 4.79 (d, 1H, *J* = 11.9 Hz, PhC$\underline{H}_2$O), 4.69 (d, 1H, *J* = 12.0 Hz, PhC$\underline{H}_2$O), 3.90 (dd, 1H, *J* = 10.9, 3.8 Hz, H-6), 3.87−3.84 (m, 2H, H-3, H-6'), 3.75−3.71 (m, 1H, H-5), 3.66 (ddd, 1H, *J* = 9.8, 7.1, 7.1 Hz, OC$\underline{H}_2$CH$_2$), 3.61 (dd, 1H, *J* = 9.2, 9.2 Hz, H-4), 3.53 (dd, 1H, *J* = 9.6, 3.6 Hz, H-2), 3.43 (ddd, 1H, *J* = 9.8, 6.7, 6.7 Hz, OC$\underline{H}_2$CH$_2$), 2.42 (s, 1H, C4-O$\underline{H}$), 1.69-1.63 (m, 2H, OCH$_2$C$\underline{H}_2$), 1.40−1.27 (m, 10H, 5 × C$\underline{H}_2$), 1.08 (s, 9H, SiC(C$\underline{H}_3$)$_3$), 0.91 (t, 3H, *J* = 7.0 Hz, CH$_2$C$\underline{H}_3$); ^{13}C NMR (126 MHz; $CDCl_3$): δ 139.0 (Ar), 138.4 (Ar), 135.7 (2C, 2 × Ar), 134.8 (Ar), 133.3 (2C, 2 × Ar), 129.7 (2C, 2 × Ar), 128.5 (Ar), 128.4 (Ar), 128.0 (2C, 2 × Ar), 127.8 (Ar), 127.7 (2C, 2 × Ar), 96.5 (C-1), 81.7 (C-3), 79.9 (C-2), 75.5 (Ph$\underline{C}$H$_2$O), 72.9 (Ph$\underline{C}$H$_2$O), 71.6 (C-4), 71.0 (C-5), 68.0 (O$\underline{C}$H$_2$CH$_2$), 64.3 (C-6), 31.9 ($\underline{C}$H$_2$), 29.5 ($\underline{C}$H$_2$), 29.4 ($\underline{C}$H$_2$), 29.3 ($\underline{C}$H$_2$), 26.6 (Si$\underline{C}$(CH$_3$)$_3$), 26.2 ($\underline{C}$H$_2$), 22.7 ($\underline{C}$H$_2$), 19.3 (SiC($\underline{C}$H$_3$)$_3$), 14.1 (CH$_2\underline{C}$H$_3$); HRMS (ESI) Calc. for (M + Na) $C_{44}H_{58}NaO_6Si$: 733.3895. Found 733.3887.

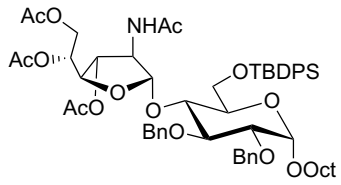


Octyl 3,5,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-galactofuranosyl-(1→4)-2,3-di-*O*-benzyl-6-*O*-*tert*-butyldiphenylsilyl-α-D-glucopyranoside (5-23)

Compounds **5-21** (429 mg, 0.68 mmol) and **4-11** (445 mg, 0.82 mmol) were dried under vacuum in the presence of P_2O_5 for ~10 h and then dissolved in anhydrous CH_2Cl_2 (7 mL) before 4Å MS (400 mg) were added. The mixture was stirred for 5 h at room temperature, then cooled to –15 °C. NIS (277 mg, 1.23 mmol) and AgOTf (32 mg, 0.12 mmol) were then added and the

reaction mixture was stirred for 1 h. Et_3N (250 μL) was then added. The mixture was passed through a bed of Celite and the filtrate was washed with a saturated $Na_2S_2O_3$ solution (1×), H_2O (1×), and brine (1×). The organic layer was then dried over Na_2SO_4, filtered, and concentrated. The resulting residue was purified by flash chromatography using a gradient of 5:2→2:1 hexanes–EtOAc to afford **5-23** (630 mg, 82%) as a clear oil. R_f = 0.50 (2:1 hexanes–EtOAc); $[\alpha]_D$ +4.5 (1.1 *c*, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.80 (dd, 2H, *J* = 5.5, 3.0 Hz, Ar), 7.72 (dd, 2H, *J* = 5.5, 3.0 Hz, Ar), 7.60 (dd, 2H, *J* = 8.0, 1.4 Hz, Ar), 7.56 (dd, 2H, *J* = 8.0, 1.4 Hz, Ar), 7.44–7.42 (m, 2H, Ar), 7.37–7.22 (m, 14H, Ar), 5.62 (d, 1H, *J* = 3.3 Hz, H-1$_{Galf N}$), 5.49 (dd, 1H, *J* = 8.6, 6.9 Hz, H-3$_{Galf N}$), 5.12 (ddd, 1H, *J* = 7.7, 3.4, 3.4 Hz, H-5$_{Galf N}$), 5.02 (d, 1H, *J* = 10.9 Hz, PhC$\underline{H}_2$O), 4.85 (d, 1H, *J* = 10.8 Hz, PhC$\underline{H}_2$O), 4.75 (d, 1H, *J* = 11.9 Hz, PhC$\underline{H}_2$O), 4.68 (d, 1H, *J* = 3.7 Hz, H-1$_{Glc}$), 4.67 (dd, 1H, *J* = 7.0, 3.4 Hz, H-2$_{Galf N}$), 4.65 (d, 1H, *J* = 11.9 Hz, PhC$\underline{H}_2$O), 4.37 (dd, 1H, *J* = 8.6, 3.0 Hz, H-4$_{Galf N}$), 4.13 (dd, 1H, *J* = 12.0, 7.8 Hz, H-6$_{Galf N}$), 4.02 (dd, 1H, *J* = 12.0, 3.7 Hz, H-6'$_{Galf N}$), 3.93 (dd, 1H, *J* = 9.3, 9.3 Hz, H-3$_{Glc}$), 3.84 (dd, 1H, *J* = 9.2, 9.2 Hz, H-4$_{Glc}$), 3.75–3.72 (m, 1H, H-6$_{Glc}$), 3.68–3.65 (m, 2H, H-5$_{Glc}$, H-6'$_{Glc}$), 3.52 (dd, 1H, *J* = 9.6, 3.7 Hz, H-2), 3.55 (ddd, 1H, *J* = 14.2,7.0, 7.0 Hz, OC$\underline{H}_2$CH$_2$), 3.33 (ddd, 1H, *J* = 13.8, 6.9, 6.9 Hz, OC$\underline{H}_2$CH$_2$), 2.12 (s, 3H, COC$\underline{H}_3$), 2.00 (s, 3H, COC$\underline{H}_3$), 1.98 (s, 3H, COC$\underline{H}_3$), 1.63–1.57 (m, 2H, OCH$_2$C$\underline{H}_2$), 1.33–1.24 (m, 10H, 5 × C$\underline{H}_2$), 0.96 (s, 9H, SiC(C$\underline{H}_3$)$_3$), 0.89 (t, 3H, *J* = 7.1 Hz, CH$_2$C$\underline{H}_3$); ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.6 (C=O), 170.4 (2C, C=O, C=O), 167.3 (C=O), 139.0 (Ar), 138.4 (Ar), 135.8 (Ar), 135.6 (Ar), 134.1 (Ar), 133.5 (2C, 2 × Ar), 131.7 (Ar), 129.4 (2C, 2 × Ar), 128.4 (Ar), 128.2 (Ar), 128.0 (Ar), 127.9 (Ar), 127.8 (Ar), 127.4 (2C, 2 × Ar), 127.3 (Ar), 123.6 (Ar), 103.4 (C-1$_{Galf N}$), 96.1 (C-1$_{Glc}$), 80.8 (C-2$_{Glc}$), 79.7 (C-3$_{Glc}$), 77.8 (C-4$_{Galf N}$), 75.4 (Ph$\underline{C}$H$_2$O), 74.6 (C-4$_{Glc}$), 73.2 (C-3$_{Galf N}$), 73.0 (Ph$\underline{C}$H$_2$O), 71.1 (C-5$_{Glc}$), 68.8 (C-5$_{Galf N}$), 67.9 (O$\underline{C}$H$_2$CH$_2$), 63.1 (C-6$_{Galf NAc}$), 63.0 (C-6$_{Glc}$), 62.4 (C-2$_{Galf N}$), 31.9 ($\underline{C}$H$_2$), 29.4 ($\underline{C}$H$_2$),

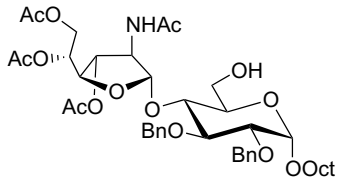
29.3 (2C, $\underline{C}H_2$, $\underline{C}H_2$), 26.7 (Si$\underline{C}(CH_3)_3$), 26.2 ($\underline{C}H_2$), 22.7 ($\underline{C}H_2$), 20.8 (CO$\underline{C}$H3), 20.7 (CO$\underline{C}$H3), 20.4 (CO$\underline{C}$H3), 19.3 (SiC($\underline{C}H_3)_3$), 14.1 ($CH_2\underline{C}H_3$); HRMS (ESI) Calc. for (M + Na) $C_{64}H_{77}NNaO_{15}Si$: 1150.4955. Found 1150.4943.



Octyl 2-acetamido-3,5,6-tri-*O*-acetyl-2-deoxy-β-D-galactofuranosyl-(1→4)-2,3-di-*O*-benzyl-6-*O*-*tert*-butyldiphenylsilyl-α-D-glucopyranoside (5-24)

Compound **5-23** (630 mg, 0.56 mmol) was dissolved in a solution of 20% ethylenediamine in ethanol (15 mL). The mixture was stirred at reflux for 17 h. The mixture was concentrated, then co-evaporated with toluene, and dried under vacuum for 24 h. To the resulting residue was added pyridine (8 mL) and Ac_2O (4 mL). The reaction mixture was stirred for 19 h before the excess Ac_2O was quenched by the addition of CH_3OH (~5 mL). The mixture was concentrated, then co-evaporated with toluene. The crude residue was dissolved EtOAc, then washed with an aqueous 20% $CuSO_4$ solution (1×), H_2O (1×), and brine (1×). The organic layer was dried over Na_2SO_4, filtered, and concentrated. The resulting residue was purified by flash chromatography using a gradient of 2:1→1:1 hexanes–EtOAc to afford **5-24** (540 mg, 93%) as a clear oil. R_f = 0.38 (1:1 hexanes–EtOAc); $[\alpha]_D$ +17.9 (1.5 *c*, $CHCl_3$); ^{1}H NMR (498 MHz, $CDCl_3$) δ 7.72–7.68 (m, 4H, Ar), 7.43–7.28 (m, 16H, Ar), 5.82 (d, 1H, *J* = 8.0 Hz, N$\underline{H}_{Galf/N}$), 5.29 (s, 1H, H-$1_{Galf/N}$), 5.10 (ddd, 1H, *J* = 8.1, 3.9, 3.9 Hz, H-$5_{Galf/N}$), 5.05 (d, 1H, *J* = 10.9 Hz, PhC$\underline{H}_2$O), 4.77–4.75 (m, 3H, H-1, 2 × PhC$\underline{H}_2$O), 4.71 (dd, 1H, *J* = 4.6, 2.1 Hz, H-$3_{Galf/N}$), 4.68 (d, 1H, *J* = 11.9 Hz, PhC$\underline{H}_2$O), 4.36 (dd, 1H, *J* = 8.0, 1.9 Hz, H-$2_{Galf/N}$), 4.20 (dd, 1H, *J* = 4.6, 4.6 Hz,

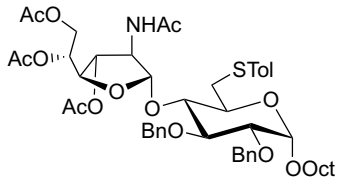
H-4$_{Galf N}$), 4.03 (dd, 1H, *J* = 12.1, 7.9 Hz, H-6$_{Galf N}$), 3.97–3.92 (m, 3H, H-4$_{Glc}$, H-6'$_{Galf N}$, H-6$_{Glc}$), 3.89–3.85 (m, 2H, H-3$_{Glc}$, H-6'$_{Glc}$), 3.69–3.66 (m, 1H, H-5$_{Glc}$), 3.58–3.53 (m, 2H, H-2$_{Glc}$, OC$\underline{H}_2$CH$_2$), 3.37 (ddd, *J* = 9.9, 6.8, 6.8 Hz, C$\underline{H}_2$CH$_2$), 2.08 (s, 3H, COC$\underline{H}_3$), 2.05 (s, 3H, COC$\underline{H}_3$), 1.98 (s, 3H, COC$\underline{H}_3$), 1.88 (s, 3H, COC$\underline{H}_3$), 1.65–1.58 (m, 2H, OCH$_2$C$\underline{H}_2$), 1.33–1.27 (m, 10H, 5 × C$\underline{H}_2$), 1.07 (s, 9H, SiC(C$\underline{H}_3$)$_3$), 0.89 (t, 3H, *J* = 6.9 Hz, CH$_2$C$\underline{H}_3$); ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.4 (C=O), 170.3 (C=O), 170.2 (C=O), 169.3 (C=O), 139.0 (Ar), 138.4 (Ar), 135.9 (Ar), 135.7 (Ar), 133.9 (Ar), 133.3 (Ar), 129.6 (2C, 2 × Ar), 128.4 (2C, 2 × Ar), 127.9 (Ar), 127.8 (Ar), 127.6 (Ar), 127.5 (3C, 3 × Ar), 106.0 (C-1$_{Galf N}$), 96.3 (C-1$_{Glc}$), 80.8 (C-2$_{Glc}$), 80.1, 80.0, 78.1 (C-3$_{Galf N}$), 75.3 (Ph$\underline{C}$H$_2$O), 73.5 (C-4$_{Glc}$), 73.0 (Ph$\underline{C}$H$_2$O), 71.1 (C-5$_{Glc}$), 70.2 (C-5$_{Galf N}$), 68.0 (O$\underline{C}$H$_2$CH$_2$), 63.0 (C-6), 62.7 (C-6), 60.2 (C-2$_{Galf N}$), 31.9 ($\underline{C}$H$_2$), 29.4 (2C, $\underline{C}$H$_2$, $\underline{C}$H$_2$), 29.2 ($\underline{C}$H$_2$), 26.8 (SiC($\underline{C}$H$_3$)$_3$), 26.2 ($\underline{C}$H$_2$), 23.1 (CO$\underline{C}$H$_3$), 22.7 ($\underline{C}$H$_2$), 20.9 (CO$\underline{C}$H$_3$), 20.7 (2C, CO$\underline{C}$H$_3$, CO$\underline{C}$H$_3$), 19.4 (Si$\underline{C}$(CH$_3$)$_3$), 14.1 (CH$_2\underline{C}$H$_3$); HRMS (ESI) Calc. for (M + Na) $C_{58}H_{77}NNaO_{14}Si$: 1062.5006. Found 1062.5000.



Octyl 2-acetamido-3,5,6-tri-*O*-acetyl-2-deoxy-β-D-galactofuranosyl-(1→4)-2,3-di-*O*-benzyl-α-D-glucopyranoside (5-25)

To **5-24** (530 mg, 0.51 mmol) in 1:1 THF–pyridine solution (10 mL) under argon was added HF·pyridine complex (650 μL). The reaction mixture was stirred at room temperature for 6 h. The mixture was poured over a saturated aqueous $NaHCO_3$ solution (1×) and extracted with EtOAc (3×). The organic layers were combined, then washed with an aqueous 20% $CuSO_4$

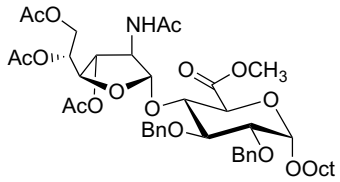
solution (1×) and brine (1×). The organic layer was dried over Na_2SO_4, filtered, and concentrated. The resulting residue was purified by flash chromatography using a gradient of 30:1→25:1 CH_2Cl_2–CH_3OH to afford **5-25** (374 mg, 92%) as a white solid. R_f = 0.30 (20:1 CH_2Cl_2–CH_3OH); $[\alpha]_D$ +25.6 (2.0 *c*, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37–7.25 (m, 10H, Ar), 6.16 (d, 1H, *J* = 6.3 Hz, N$\underline{H}_{Galf\mathrm{N}}$), 5.22 (s, 1H, H-$1_{Galf\mathrm{N}}$), 5.16–5.13 (m, 1H, H-$5_{Galf\mathrm{N}}$), 4.99 (d, 1H, *J* = 10.6 Hz, PhC$\underline{H}_2$O), 4.77 (d, 1H, *J* = 10.6 Hz, PhC$\underline{H}_2$O), 4.73–4.69 (m, 3H, H-1_{Glc}, H-$3_{Galf\mathrm{N}}$, PhC$\underline{H}_2$O), 4.61 (d, 1H, *J* = 12.0 Hz, PhC$\underline{H}_2$O), 4.29 (dd, 1H, *J* = 6.1, 4.1 Hz, H-$4_{Galf\mathrm{N}}$), 4.18 (dd, 1H, *J* = 6.2, 3.7 Hz, H-$2_{Galf\mathrm{N}}$), 4.04 (d, 2H, *J* = 5.9 Hz, H-$6_{Galf\mathrm{N}}$, H-6'$_{Galf\mathrm{N}}$), 3.96–3.91 (m, 1H, H-6_{Glc}), 3.88 (d, 1H, *J* = 9.2 Hz, H-3_{Glc}), 3.79–3.73 (m, 2H, H-4_{Glc}, H-6'$_{Glc}$), 3.67–3.58 (m, 2H, H-5_{Glc}, OC$\underline{H}_2CH_2$), 3.52 (dd, 1H, *J* = 9.4, 3.7 Hz, H-2_{Glc}), 3.40 (ddd, 1H, *J* = 9.9, 6.8, 6.8 Hz, OC$\underline{H}_2CH_2$), 2.84 (dd, 1H, *J* = 6.5, 6.5 Hz, C-6-O$\underline{H}$), 2.10 (s, 3H, COC$\underline{H}_3$), 2.05 (s, 3H, COC$\underline{H}_3$), 1.99 (s, 3H, COC$\underline{H}_3$), 1.97 (s, 3H, COC$\underline{H}_3$), 1.67–1.60 (m, 2H, $OCH_2C\underline{H}_2$), 1.37–1.29 (m, 10H, 5 × C$\underline{H}_2$), 0.89 (t, 3H, *J* = 6.9 Hz, $CH_2C\underline{H}_3$); ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.8 (C=O), 170.4 (2C, 2 × C=O), 170.1 (C=O), 139.0 (Ar), 138.2 (Ar), 128.4 (Ar), 128.3 (Ar), 128.1 (Ar), 127.9 (Ar), 127.7 (Ar), 127.5 (Ar), 108.0 (C-$1_{Galf\mathrm{N}}$), 96.7 (C-1_{Glc}), 80.5, 80.4, 79.1 (C-$4_{Galf\mathrm{N}}$), 77.2 (C-$3_{Galf\mathrm{N}}$), 75.2 (2C, Ph$\underline{C}H_2O$, C-4_{Glc}), 73.1 (Ph$\underline{C}H_2O$), 70.6 (C-5_{Glc}), 69.8 (C-$5_{Galf\mathrm{N}}$), 68.3 (O$\underline{C}H_2CH_2$), 62.9 (C-$6_{Galf\mathrm{NAc}}$), 62.3 (H-$2_{Galf\mathrm{NAc}}$), 61.0 (C-6_{Glc}), 31.9 ($\underline{C}H_2$), 29.4 (2C, $\underline{C}H_2$, $\underline{C}H_2$), 29.3 ($\underline{C}H_2$), 26.2 ($\underline{C}H_2$), 23.1 (CO$\underline{C}H_3$), 22.7 ($\underline{C}H_2$), 20.9 (CO$\underline{C}H_3$), 20.7 (2C, CO$\underline{C}H_3$, CO$\underline{C}H_3$), 14.1 ($CH_2\underline{C}H_3$); HRMS (ESI) Calc. for (M + Na) $C_{42}H_{59}NNaO_{14}$: 824.3828. Found 824.3826.



Octyl 2-acetamido-3,5,6-tri-*O*-acetyl-2-deoxy-β-D-galactofuranosyl-(1→4)-2,3-di-*O*-benzyl-6-thio-6-*S*-*p*-tolyl-α-D-glucopyranoside (5-26)

To **5-25** (504 mg, 0.63 mmol) in pyridine (9 mL) under argon were added $(TolS)_2$ (774 mg, 3.14 mmol) and a 1.0 M solution of $P(CH_3)_3$ in THF (3.2 mL). The reaction mixture was stirred at room temperature for 24 h before H_2O (5 mL) was added. The mixture was concentrated and then co-evaporated with toluene. The residue was diluted with EtOAc and then washed with H_2O (1×) and brine (1×). The organic layer was dried over Na_2SO_4, filtered, and concentrated. The resulting residue was purified by flash chromatography using a gradient of 1:1→3:2 EtOAc–hexanes to afford **5-26** (530 mg, 93%) as a clear oil. R_f = 0.31 (3:2 EtOAc–hexanes); $[\alpha]_D$ +15.5 (1.0 *c*, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.36–7.29 (m, 12H, Ar), 7.10 (d, 2H, *J* = 8.0 Hz, Ar), 5.97 (d, 1H, *J* = 7.4 Hz, N$\underline{H}_{Gal\mathit{f}N}$), 5.26 (s, 1H, H-$1_{Gal\mathit{f}N}$), 5.13 (ddd, 1H, *J* = 7.7, 3.9, 3.9 Hz, H-$5_{Gal\mathit{f}N}$), 5.04 (d, 1H, *J* = 10.8 Hz, PhC$\underline{H}_2$O), 4.76–4.72 (m, 4H, H-1_{Glc}, H-$3_{Gal\mathit{f}N}$, PhC$\underline{H}_2$O, PhC$\underline{H}_2$O), 4.63 (d, 1H, *J* = 12.0 Hz, PhC$\underline{H}_2$O), 4.35 (dd, 1H, *J* = 7.4, 2.1 Hz, H-$2_{Gal\mathit{f}N}$), 4.26 (dd, 1H, *J* = 4.7, 4.7 Hz, H-$4_{Gal\mathit{f}N}$), 4.07 (dd, 1H, *J* = 12.1, 7.5 Hz, H-$6_{Gal\mathit{f}N}$), 4.00 (dd, 1H, *J* = 12.1, 3.8 Hz, H-$6'_{Gal\mathit{f}N}$) 3.96 (ddd, 1H, *J* = 9.8, 7.8, 2.6 Hz, H-5_{Glc}), 3.89 (dd, 1H, *J* = 9.2, 9.2 Hz, H-3_{Glc}), 3.66 (ddd, 1H, *J* = 9.6, 7.0, 7.0 Hz, OC$\underline{H}_2$CH$_2$), 3.62 (dd, 1H, *J* = 9.5, 9.5 Hz, H-4_{Glc}), 3.57 (dd, 1H, *J* = 9.5, 3.7 Hz, H-2_{Glc}), 3.53 (dd, 1H, *J* = 13.1, 2.7 Hz, H-6_{Glc}), 3.39 (ddd, 1H, *J* = 9.7, 6.8, 6.8 Hz, OC$\underline{H}_2$CH$_2$), 3.09 (dd, 1H, *J* = 13.1, 7.7 Hz, H-$6'_{Glc}$), 2.32 (s, 3H, ArC$\underline{H}_3$), 2.08 (s, 3H, COC$\underline{H}_3$), 2.03 (s, 3H, COC$\underline{H}_3$), 2.01 (s, 3H, COC$\underline{H}_3$), 1.94 (s, 3H, COC$\underline{H}_3$), 1.63–1.57 (m, 2H, OCH$_2$C$\underline{H}_2$), 1.35–1.30 (m, 10H, 5 × C$\underline{H}_2$), 0.91 (t, 3H, *J* = 7.0,

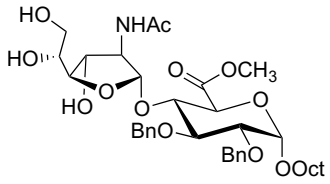
7.0 Hz, $CH_2C\underline{H}_3$); ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.5 (C=O), 170.4 (C=O), 170.1 (C=O), 169.6 (C=O), 138.9 (Ar), 138.1 (Ar), 135.8 (Ar), 132.8 (Ar), 129.6 (Ar), 129.4 (Ar), 128.4 (2C, 2 × Ar), 128.0 (Ar), 127.9 (Ar), 127.6 (Ar), 127.5 (Ar), 107.2 (C-$1_{Gal/N}$), 96.3 (C-1_{Glc}), 80.5 (C-2_{Glc}), 80.4 (C-$4_{Gal/N}$), 80.1 (C-3_{Glc}), 77.7 (C-$3_{Gal/N}$), 77.4 (C-4_{Glc}), 75.3 (Ph$\underline{C}H_2O$), 73.0 (Ph$\underline{C}H_2O$), 70.1 (C-$5_{Gal/N}$), 69.1 (C-5_{Glc}), 68.2 (O$\underline{C}H_2CH_2$), 62.9 (C-$6_{Gal/N}$), 60.8 (C-$2_{Gal/N}$), 35.9 (C-6_{Glc}), 31.9 ($\underline{C}H_2$), 29.5 ($\underline{C}H_2$), 29.4 ($\underline{C}H_2$), 29.3 ($\underline{C}H_2$), 26.2 ($\underline{C}H_2$), 23.1 (CO$\underline{C}H_3$), 22.7 ($\underline{C}H_2$), 21.0 (Ar$\underline{C}H_3$), 20.9 (CO$\underline{C}H_3$), 20.7 (CO$\underline{C}H_3$), 20.6 (CO$\underline{C}H_3$), 14.1 ($CH_2\underline{C}H_3$); HRMS (ESI) Calc. for (M + Na) $C_{49}H_{65}NNaO_{13}S$: 930.4069. Found 930.4062.



Methyl [2-acetamido-3,5,6-tri-*O*-acetyl-2-deoxy-β-D-galactofuranosyl-(1→4)-(octyl 2,3-di-*O*-benzyl-α-D-glucopyranosid)]-uronate (5-27)

To **5-26** (165 mg, 0.18 mmol) in CCl_4 (3.6 mL) under argon was added pyridine (59 μL, 0.73 mmol) via syringe. The mixture was cooled to 0 °C, then SO_2Cl_2 (44 μL, 0.55 mmol) was added via a pipette. The reaction mixture was stirred for 5 h before being diluted with CH_2Cl_2 and washed with H_2O (1×) and brine (1×). The organic layer was dried over Na_2SO_4, filtered, and concentrated. The resulting residue was diluted with a 2:1:1 CH_3OH–CH_2Cl_2–H_2O solution (3.6 mL), then pyridine (59 μL, 0.73 mmol) and $HgCl_2$ (494 mg, 1.82 mmol) were added. The suspended reaction mixture was stirred at room temperature for 48 h and then filtered through a bed of Celite. The filtrate, diluted with a saturated $NaHCO_3$ solution, was extracted with EtOAc (2×). The organic layers were combined, washed with brine (1×), dried over Na_2SO_4, filtered,

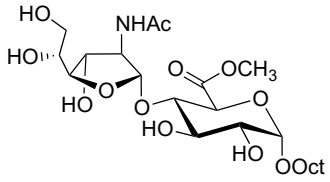
and concentrated. The resulting residue was purified by flash chromatography using a gradient of 2:1→3:1 EtOAc–hexanes to afford **5-27** (98 mg, 65%) as a clear oil. R_f = 0.26 (3:1 EtOAc–hexanes); $[\alpha]_D$ +8.4 (1.0 *c*, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.36–7.29 (m, 10H, Ar), 5.98 (d, 1H, *J* = 8.3 Hz, N$\underline{H}_{Galf\mathrm{N}}$), 5.12 (ddd, 1H, *J* = 7.8, 4.0, 4.0 Hz, H-5$_{Galf\mathrm{N}}$), 5.02 (d, 1H, *J* = 10.8 Hz, PhC$\underline{H}_2$O), 4.99 (s, 1H, H-1$_{Galf\mathrm{N}}$), 4.77 (d, 1H, *J* = 10.5 Hz, PhC$\underline{H}_2$O), 4.76 (d, 1H, *J* = 3.9 Hz, H-1$_{Glc}$), 4.73 (d, 1H, *J* = 12.0 Hz, PhC$\underline{H}_2$O), 4.71 (dd, 1H, *J* = 4.4, 2.2 Hz, H-3$_{Galf\mathrm{N}}$), 4.61 (d, 1H, *J* = 12.0 Hz, PhC$\underline{H}_2$O), 4.37 (ddd, 1H, *J* = 8.4, 2.0, 0.6 Hz, C-2$_{Galf\mathrm{N}}$), 4.27–4.23 (m, 1H, H-4$_{Glc}$), 4.21 (dd, 1H, *J* = 4.5, 4.5 Hz, H-4$_{Galf\mathrm{N}}$), 4.02 (dd, 1H, *J* = 12.1, 7.6 Hz, H-6$_{Galf\mathrm{N}}$), 3.96 (dd, 1H, *J* = 12.1, 3.8 Hz, H-6$_{Galf\mathrm{N}}$), 3.89–3.87 (m, 2H, H-3$_{Glc}$, H-5$_{Glc}$), 3.80 (s, 3H, OC$\underline{H}_3$), 3.68 (ddd, 1H, *J* = 9.8, 7.0, 7.0 Hz, OC$\underline{H}_2$CH$_2$), 3.60–3.58 (m, 1H, H-2$_{Glc}$), 3.43 (ddd, 1H, *J* = 8.3, 6.9, 6.9 Hz, OC$\underline{H}_2$CH$_2$), 2.10 (s, 3H, COC$\underline{H}_3$), 2.09 (s, 3H, COC$\underline{H}_3$), 1.98 (s, 3H, COC$\underline{H}_3$), 1.98 (s, 3H, COC$\underline{H}_3$), 1.69–1.64 (m, 2H, OCH$_2$C$\underline{H}_2$), 1.40–1.28 (m, 10H, 5 × C$\underline{H}_2$), 0.92 (t, 3H, *J* = 7.0 Hz, CH$_2$C$\underline{H}_3$); ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.4 (C=O), 170.3 (C=O), 170.1 (2C, 2 × C=O), 169.1 (C=O), 138.7 (Ar), 137.9 (Ar), 128.5 (Ar), 128.4 (Ar), 128.1 (Ar), 128.0 (Ar), 127.6 (Ar), 127.3 (Ar), 106.7 (C-1$_{Galf\mathrm{N}}$), 97.2 (C-1$_{Glc}$), 80.6 (C-4$_{Galf\mathrm{N}}$), 79.7 (C-2$_{Glc}$), 79.4, 78.0 (C-3$_{Galf\mathrm{N}}$), 76.3, 75.4 (Ph$\underline{C}$H$_2$O), 73.3 (Ph$\underline{C}$H$_2$O), 70.2 (C-5$_{Galf\mathrm{N}}$), 70.0 (C-4$_{Glc}$), 68.9 (O$\underline{C}$H$_2$CH$_2$), 62.8 (C-6$_{Galf\mathrm{N}}$), 60.0 (C-2$_{Galf\mathrm{N}}$), 52.8 (O$\underline{C}$H$_3$), 31.9 ($\underline{C}$H$_2$), 29.4 ($\underline{C}$H$_2$), 29.3 ($\underline{C}$H$_2$), 29.2 ($\underline{C}$H$_2$), 26.1 ($\underline{C}$H$_2$), 23.2 (CO$\underline{C}$H$_3$), 22.7 ($\underline{C}$H$_2$), 20.9 (CO$\underline{C}$H$_3$), 20.8 (CO$\underline{C}$H$_3$), 20.6 (CO$\underline{C}$H$_3$), 14.1 (CH$_2$$\underline{C}H_3$); HRMS (ESI) Calc. for (M + Na) $C_{43}H_{59}NNaO_{15}$: 852.3777. Found 852.3769.



Methyl [2-acetamido-2-deoxy-β-D-galactofuranosyl-(1→4)-(octyl 2,3-di-*O*-benzyl-α-D-glucopyranosid)]-uronate (5-28)

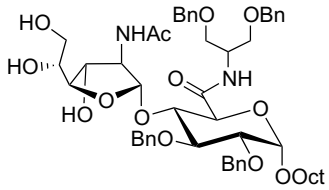
To **5-27** (70 mg, 0.08 mmol) in CH_3OH (1.5 mL) under argon was added 0.10 M $NaOCH_3$ solution in CH_3OH dropwise via syringe until a wet pH paper read 8–9. The reaction mixture was stirred for 30 min and then AcOH (3 drops) was then added via syringe and the mixture was concentrated. The resulting residue was purified by flash chromatography in 10:1 CH_2Cl_2–CH_3OH to afford **5-32** (52 mg, 88%) as a clear oil. R_f = 0.43 (10:1 CH_2Cl_2–CH_3OH); $[\alpha]_D$ +8.0 (1.0 *c*, $CHCl_3$); ^{1}H NMR (600 MHz, CD_3OD) δ 7.38 (d, 2H, *J* = 7.1 Hz, Ar), 7.31–7.23 (m, 8H), 4.95 (d, 1H, *J* = 2.1 Hz, H-1$_{Galf/N}$), 4.92 (d, 1H, *J* = 11.0 Hz, PhC$\underline{H}_2$O), 4.86 (d, 1H, *J* = 3.5 Hz, H-1$_{Glc}$), 4.83 (d, *J* = 10.5 Hz, PhC$\underline{H}_2$O), 4.65 (d, 1H, *J* = 11.7 Hz, PhC$\underline{H}_2$O), 4.62 (d, 1H, *J* = 11.6 Hz, PhC$\underline{H}_2$O), 4.19 (d, 1H, *J* = 9.3 Hz, H-5$_{Glc}$), 4.15 (dd, 1H, *J* = 4.1, 2.1 Hz, H-2$_{Galf/N}$), 4.03 (dd, 1H, *J* = 5.9, 4.2 Hz, H-3$_{Galf/N}$), 4.00 (dd, 1H, *J* = 6.0, 2.4 Hz, H-4$_{Galf/N}$), 3.88–3.83 (m, 2H, H-3$_{Glc}$, H-4$_{Glc}$), 3.75 (s, 3H, OC$\underline{H}_3$), 3.68 (ddd, 1H, *J* = 9.8, 6.5, 6.5 Hz, OC$\underline{H}_2$OCH$_2$), 3.59 (ddd, 1H, *J* = 7.5, 4.7, 2.6 Hz, H-5$_{Galf/N}$), 3.54 (dd, 1H, *J* = 9.3, 3.5 Hz, H-2$_{Glc}$), 3.43–3.39 (m, 2H, H-6$_{Galf/N}$, OC$\underline{H}_2$CH$_2$), 3.28 (dd, 1H, *J* = 11.4, 4.6 Hz, H-6'$_{Galf/N}$), 1.92 (s, 3H, COC$\underline{H}_3$), 1.65–1.59 (m, 2H, OCH$_2$C$\underline{H}_2$), 1.43–1.28 (m, 10H, 5 × C$\underline{H}_2$), 0.90 (t, 3H, *J* = 7.0 Hz, CH$_2$C$\underline{H}_3$); ^{13}C NMR (126 MHz, CD_3OD) δ 172.7 (C=O), 171.7 (C=O), 140.3 (Ar), 139.6 (Ar), 129.4 (Ar), 129.2 (Ar), 129.1 (Ar), 129.0 (Ar), 128.9 (Ar), 128.5 (Ar), 108.2 (C-1$_{Galf/N}$), 98.5 (C-1$_{Glc}$), 85.6 (C-4$_{Galf/N}$), 81.1 (C-2$_{Glc}$), 80.5, 78.1, 77.4 (C-3$_{Galf/N}$), 76.4 (Ph$\underline{C}$H$_2$O), 74.0 (Ph$\underline{C}$H$_2$O), 72.3 (C-5$_{Galf/N}$), 71.6 (C-5$_{Glc}$), 69.8 (O$\underline{C}$H$_2$CH$_2$), 64.9 (C-6$_{Galf/N}$), 64.2 (C-2$_{Galf/N}$), 53.2 (O$\underline{C}$H$_3$), 33.1 ($\underline{C}$H$_2$), 30.5 (2C,

$\underline{C}H_2$, $\underline{C}H_2$), 30.4 ($\underline{C}H_2$), 27.4 ($\underline{C}H_2$), 23.8 ($\underline{C}H_2$), 22.8 ($CO\underline{C}H_3$), 14.5 ($CH_2\underline{C}H_3$); HRMS (ESI) Calc. for (M + Na) $C_{37}H_{53}NNaO_{12}$: 726.3460. Found 726.3454.



Methyl [2-acetamido-2-deoxy-β-D-galactofuranosyl-(1→4)-(octyl α-D-glucopyranosid)]-uronate (5-29)

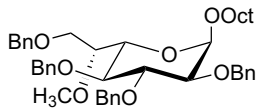
To **5-28** (42 mg, 0.06 mmol) in CH_3OH (1 mL) was added $Pd(OH)_2$–C (15 mg). The mixture was placed under a positive pressure of H_2 and stirred for 48 h. The palladium was removed by filtration through filter paper and the filtrate was concentrated. The resulting residue was purified by C_{18} flash chromatography using a gradient of 1:0→3:2 H_2O–CH_3OH to afford **5-29** (22 mg, 71%) as a clear oil. $[\alpha]_D$ +45.1 (1.0 *c*, CH_3OH); ^{1}H NMR (500 MHz, CD_3OD) δ 4.89 (d, 1H, *J* = 2.2 Hz, H-$1_{Galf\underline{N}}$), 4.79 (d, 1H, *J* = 3.6 Hz, H-1_{Glc}), 4.17–4.11 (m, 3H, H-$2_{Galf\underline{N}}$, H-$4_{Galf\underline{N}}$, H-4_{Glc}), 4.06 (dd, 1H, *J* = 5.8, 5.8 Hz, H-$3_{Galf\underline{N}}$), 3.74–3.59 (m, 9H, H-3_{Glc}, H-$5_{Galf\underline{N}}$, H-5_{Glc}, H-$6_{Galf\underline{N}}$, H-6'$_{Galf\underline{N}}$, $OC\underline{H}_2CH_2$, $OC\underline{H}_3$), 3.50–3.44 (m, 2H, H-2_{Glc}, $OC\underline{H}_2CH_2$), 1.95 (s, 3H, $COC\underline{H}_3$), 1.66–1.57 (m, 2H, $OCH_2C\underline{H}_2$), 1.39–1.28 (m, 10H, 5 × $C\underline{H}_2$), 0.90 (t, 3H, *J* = 6.9 Hz, $CH_2C\underline{H}_3$); ^{13}C NMR (126 MHz, CD_3OD) δ 172.8 (C=O), 171.5 (C=O), 108.3 (C-$1_{Galf\underline{N}}$), 100.6 (C-1_{Glc}), 84.4 (C-$4_{Galf\underline{N}}$), 80.1, 76.7 (C-$3_{Galf\underline{N}}$), 73.0, 71.8, 71.3, 69.9 ($O\underline{C}H_2CH_2$), 64.5 (C-$6_{Galf\underline{N}}$), 64.0 (C-$2_{Galf\underline{N}}$), 53.1 ($O\underline{C}H_3$), 33.0 ($\underline{C}H_2$), 30.6 ($\underline{C}H_2$), 30.5 ($\underline{C}H_2$), 30.4 ($\underline{C}H_2$), 27.3 ($\underline{C}H_2$), 23.7 ($\underline{C}H_2$), 22.8 ($CO\underline{C}H_3$), 14.5 ($CH_2\underline{C}H_3$); HRMS (ESI) Calc. for (M + Na) $C_{23}H_{41}NNaO_{12}$: 523.2521. Found 523.2521.



2-*N*-[2-acetamido-2-deoxy-β-D-galactofuranosyl-(1→4)-(octyl 2,3-di-*O*-benzyl-α-D-glucopyranosid)]uronoyl-1,3-di-*O*-benzyl-2-deoxy-glycerol (5-32)

To **5-31** (50 mg, 0.05 mmol) in CH_3OH (1 mL) under argon was added 0.10 M $NaOCH_3$ solution in CH_3OH dropwise via syringe until a wet pH paper read 8–9. The reaction mixture was stirred for 30 min. AcOH (3 drops via syringe) was then added and the mixture was concentrated. The resulting residue was purified by flash chromatography in 10:1 CH_2Cl_2–CH_3OH to afford **5-32** (35 mg, 80%) as a clear oil. R_f = 0.50 (10:1 CH_2Cl_2–CH_3OH); $[\alpha]_D$ +12.9 (1.0 *c*, CH_3OH); ^{1}H NMR (600 MHz, CD_3OD) δ 7.39–7.23 (m, 20H, Ar), 5.03 (d, 1H, *J* = 2.0 Hz, H-$1_{Galf/N}$), 4.91 (d, 1H, *J* = 10.9 Hz, PhC$\underline{H}_2$O), 4.85 (dd, 1H, *J* = 11.0, 6.2 Hz, H-1_{GlcA}), 4.84 (d, 1H, *J* = 11.2 Hz, PhC$\underline{H}_2$O), 4.66 (d, 1H, *J* = 11.7 Hz, PhC$\underline{H}_2$O), 4.63 (d, 1H, *J* = 11.7 Hz, PhC$\underline{H}_2$O), 4.51–4.50 (m, 4H, 4 × PhC$\underline{H}_2$O), 4.28 (dt, 2H, *J* = 11.1, 5.5 Hz, CON$\underline{H}$($CH_2O)_2$), 4.13–4.09 (m, 2H, H-$2_{Galf/N}$, H-5_{GlcA}), 4.04 (dd, 1H, *J* = 5.9, 2.7 Hz, H-$4_{Galf/N}$), 3.96 (dd, 1H, *J* = 5.9, 3.9 Hz, H-$3_{Galf/N}$), 3.87 (dd, 1H, *J* = 6.8, 6.6 Hz, H-4_{GlcA}), 3.85 (dd, 1H, *J* = 6.8, 6.4 Hz, H-3_{GlcA}), 3.66 (ddd, 1H, *J* = 10.0, 6.6, 6.6 Hz, OC$\underline{H}_2CH_2$), 3.41 (dd, *J* = 11.3, 7.7 Hz, 1H), 3.61–3.54 (m, 6H, H-2_{GlcA}, H-$5_{Galf/N}$, 4 × CHC$\underline{H}_2$O), 3.41 (dd, 1H, *J* = 11.4, 7.7 Hz, H-$6_{Galf/N}$), 3.36 (ddd, 1H, *J* = 9.8, 6.7, 6.7 Hz, OC$\underline{H}_2CH_2$), 3.28 (dd, 1H, *J* = 11.4, 4.5 Hz, H-$6_{Galf/N}$), 1.87 (s, 3H, COC$\underline{H}_3$), 1.61–1.56 (m, 2H, $OCH_2C\underline{H}_2$), 1.36–1.22 (m, 10H, 5 × C$\underline{H}_2$), 0.88 (t, 3H, *J* = 6.9 Hz, $CH_2C\underline{H}_3$); ^{13}C NMR (126 MHz, CD_3OD) δ 172.9 (C=O), 171.6 (C=O), 140.2 (Ar), 139.7 (Ar), 139.6 (Ar), 139.5 (Ar), 129.4 (3C, 3 × Ar), 129.2 (2C, 2 × Ar), 129.1 (Ar), 128.9 (2C, 2 × Ar), 128.8 (Ar), 128.7 (Ar), 128.5 (Ar), 107.1 (C-$1_{Galf/N}$), 98.3 (C-1_{Glc}), 85.6 (C-$4_{Galf/N}$), 81.1 (C-2_{Glc}),

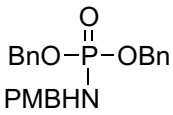
80.6, 77.9, 77.4, 76.3 (Ph$\underline{C}H_2O$), 74.2 (Ph$\underline{C}H_2O$), 74.1 (Ph$\underline{C}H_2O$), 74.0 (Ph$\underline{C}H_2O$), 72.4, 69.8 (3C, O$\underline{C}H_2CH_2$, CH$\underline{C}H_2O$, CH$\underline{C}$'H_2O), 64.9 (C-$6_{Gal/N}$), 64.6, 50.3 (NH$\underline{C}$H($CH_2O)_2$), 33.1 ($\underline{C}H_2$), 30.6 ($\underline{C}H_2$), 30.5 (2C, 2 × $\underline{C}H_2$), 27.3 ($\underline{C}H_2$), 23.8 ($\underline{C}H_2$), 22.8 (CO$\underline{C}H_3$), 14.5 ($CH_2\underline{C}H_3$); HRMS (ESI) Calc. for (M + Na) $C_{53}H_{70}N_2NaO_{13}$: 965.4770. Found 965.4771.



Octyl 2,3,4,7-tetra-*O*-benzyl-6-*O*-methyl-D-*glycero*-α-L-*gluco*-heptopyranoside (5-33)

Condition 1. To **3-9** (104 mg, 0.15 mmol) and octanol (31 μL, 0.20 mmol) in Et_2O (3 mL) under argon was added 4Å MS (100 mg). The mixture was stirred at room temperature for 1 h, then cooled to –60 °C. NIS (44 mg, 0.20 mmol) and AgOTf (5 mg, 0.02 mmol) were then added and the reaction mixture was stirred for 2.5 h. Et_3N (50 μL) was added. The mixture was then passed through a bed of Celite and the filtrate was diluted with CH_2Cl_2, washed with a saturated $Na_2S_2O_3$ solution (1×), H_2O (1×), and brine (1×). The organic layer was dried over Na_2SO_4, filtered, and concentrated. The resulting residue was purified by flash chromatography in 4:1 hexanes–EtOAc to afford **5-33** (65 mg, 62%) as a white solid. **Condition 2.** A mixture of octanol (12 μL, 0.08 mmol), $AuCl_3$ (3 mg, 0.01 mmol), and 4Å MS (100 mg) in CH_2Cl_2 (1 mL) under argon was stirred at room temperature for 1 h. The mixture was cooled to –78 °C, then a solution of **3-10** (37 mg, 0.05 mmol) in CH_2Cl_2 (1 mL) dropwise via syringe over ~5 min. The reaction mixture was stirred for 30 min. Et_3N (25 μL) was added. The mixture was passed through a bed of Celite and the filtrate was diluted with CH_2Cl_2, washed with a saturated $Na_2S_2O_3$ solution (1×), H_2O (1×), and brine (1×). The organic layer was dried over Na_2SO_4, filtered, and concentrated. The resulting residue was purified by flash chromatography in 4:1

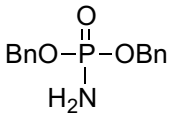
hexanes–EtOAc to afford **5-33** (20 mg, 56%) as a white solid. R_f = 0.32 (4:1 hexanes–EtOAc); $[\alpha]_D$ –13.7 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, CD_3OD) δ 7.38–7.29 (m, 20H, Ar), 5.04 (d, 1H, *J* = 10.9 Hz, PhC$\underline{H}_2$O), 4.99 (d, 1H, *J* = 11.0 Hz, PhC$\underline{H}_2$O), 4.84 (d, 1H, *J* = 10.9 Hz, PhC$\underline{H}_2$O), 4.78 (d, 1H, *J* = 12.1 Hz, PhC$\underline{H}_2$O), 4.74 (d, 1H, *J* = 3.6 Hz, H-1), 4.66 (d, 1H, *J* = 12.2 Hz, PhC$\underline{H}_2$O), 4.65 (d, 1H, *J* = 11.0 Hz, PhC$\underline{H}_2$O), 4.54 (s, 2H, PhC$\underline{H}_2$O, PhC$\underline{H}_2$O), 4.05 (dd, 1H, *J* = 9.2, 9.2 Hz, H-3), 3.84–3.80 (m, 2H, H-5, H-6), 3.78–3.73 (m, 2H, H-4, H-7), 3.65 (dd, 1H, *J* = 9.5, 5.9 Hz, H-7'), 3.59–3.55 (m, 2H, H-2, OC$\underline{H}_2$CH$_2$), 3.46 (s, 3H, OC$\underline{H}_3$), 3.33 (ddd, 1H, *J* = 9.6, 6.8, 6.8 Hz, OC$\underline{H}_2$CH$_2$), 1.60–1.56 (m, 2H, OCH$_2$C$\underline{H}_2$), 1.34–1.27 (m, 10H, 5 × C$\underline{H}_2$), 0.91 (t, 3H, *J* = 7.0 Hz, CH$_2$C$\underline{H}_3$); ^{13}C NMR (125 MHz, CD_3OD): δ 138.9 (Ar), 138.5 (Ar), 138.4 (Ar), 138.1 (Ar), 128.5 (Ar), 128.4 (3C, 3 x Ar), 128.1 (Ar), 128.0 (Ar), 127.8 (Ar), 127.7 (Ar), 127.6 (Ar), 127.5 (2C, 2 × Ar), 96.7 (C-1), 82.3 (C-3), 80.0 (C-2), 77.4 (C-4), 76.6, 75.6 (Ph$\underline{C}$H$_2$O), 75.0 (Ph$\underline{C}$H$_2$O), 73.4 (Ph$\underline{C}$H$_2$O), 73.1 (Ph$\underline{C}$H$_2$O), 70.1 (2C, C-7), 68.0 (O$\underline{C}$H$_2$CH$_2$), 59.0 (O$\underline{C}$H$_3$), 31.9 ($\underline{C}$H$_2$), 29.5 ($\underline{C}$H$_2$), 29.4 ($\underline{C}$H$_2$), 29.3 ($\underline{C}$H$_2$), 26.2 ($\underline{C}$H$_2$), 22.7 ($\underline{C}$H$_2$), 14.1 (CH$_2\underline{C}$H$_3$); HRMS (ESI) Calc. for (M + Na) $C_{44}H_{56}NaO_7$: 719.3922. Found 719.3918.



Dibenzyl *N*-*p*-methoxybenzylphosphoramidate (5-42)

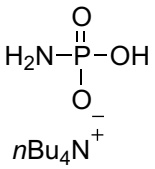
To **5-41** (1 mL, 4.53 mmol) in CH_2Cl_2 (25 mL) were added $CBrCl_3$ (2.3 mL, 23.32 mmol), Et_3N (1.3 mL, 9.33 mmol), and PMB-NH_2 (900 μL, 6.89 mmol). The reaction mixture was stirred at room temperature for 5 h, poured over a saturated aqueous $NaHCO_3$ solution, and extracted with CH_2Cl_2 (2×). The organic layers were combined, dried over $MgSO_4$, filtered, and concentrated. The resulting residue was purified by flash chromatography using a gradient of

2:1→3:1 EtOAc–hexanes to afford **5-42** (993 mg, 55%) as a yellowish oil. R_f = 0.27 (2:1 EtOAc–hexanes); ^{1}H NMR (498 MHz, $CDCl_3$) δ 7.36–7.30 (m, 10H, Ar), 7.14 (d, 2H, *J* = 8.7 Hz, Ar), 6.81 (d, 2H, *J* = 8.7 Hz, Ar), 5.08–5.00 (m, 4H, PhC$\underline{H_2}$O, PhC$\underline{H_2}$O), 3.98 (dd, 2H, *J* = 9.5, 6.8 Hz, ArC$\underline{H_2}$N), 3.78 (s, 3H, P–OC$\underline{H_3}$), 2.86 (dt, 1H, *J* = 11.0, 6.6 Hz, P–N$\underline{H}$CH$_2$); ^{13}C NMR (125 MHz, CD_3OD): δ 136.3 (2C, 2 × Ar), 128.6 (Ar), 128.4 (Ar), 127.9 (Ar), 68.3 (Ph$\underline{C}$H$_2$O), 68.2 (Ar$\underline{C}$H$_2$N); ^{31}P NMR (202 MHz, $CDCl_3$) δ 8.7; HRMS (ESI) Calc. for (M + H) $C_{22}H_{25}NO_4$: 398.1521. Found 398.1528.



Dibenzyl phosphoramidate (5-43)

To **5-42** (982 mg, 2.47) in a 9:1 CH_3CN–H_2O solution (25 mL) was added CAN (4.06 g, 7.41 mmol). The reaction mixture was stirred for 1 h at room temperature, poured over H_2O, and extracted with EtOAc (2×). The organic layers were combined, washed with an aqueous saturated $NaHCO_3$ solution (1×), dried over Na_2SO_4, filtered, and concentrated. The resulting residue was purified by flash chromatography using a gradient of 4:1→8:1 EtOAc–hexanes to afford **5-42** (554 mg, 80%) as a white solid. R_f = 0.21 (4:1 EtOAc–hexanes); ^{1}H NMR (498 MHz, $CDCl_3$) δ 7.37–7.31 (m, 10H), 5.10–5.03 (m, 4H, PhC$\underline{H_2}$O, PhC$\underline{H_2}$O), 2.82 (s, 2H, P–N$\underline{H_2}$); ^{13}C NMR (126 MHz, $CDCl_3$): δ 136.3 (2C, 2 × Ar), 128.6 (Ar), 128.4 (Ar), 127.9 (Ar), 68.3 (2C, 2 × Ph$\underline{C}$H$_2$O); ^{31}P NMR (202 MHz, $CDCl_3$) δ 8.6; HRMS (ESI) Calc. for (M + H) $C_{14}H_{17}NO_3$: 278.0946. Found 278.0940.



Phosphoramidate mono tetra-*N*-butylammonium salt (5-44)

To **5-43** (860 mg, 3.10 mmol) in a 20:1 CH_3OH–Et_3N solution (42 mL) was added Pd–C (86 mg). The mixture was place under a positive pressure of H_2(g) and stirred for 2 h. The palladium was removed by filtration through filter paper. To the filtrate was added a solution of 40% nBu$_4$NOH in H_2O (1.35 mL, 3.10 mmol). The mixture was concentrated, diluted with H_2O, and then lyophilized to afford **5-44** (808 mg, 77%) as a hygroscopic white solid. ^{31}P NMR (202 MHz, D_2O) δ 1.5.

5.7.3 Expression of His$_6$-Cj1415

Newly transformed *E. coli* BL21(DE3) competent cells (Novagen Biosciences, La Jolla, CA) carrying recombinant plasmids were cultured while shaking at 200 rpm overnight at 37 °C in 5 mL LB broth supplemented with kanamycin (50 ug/mL final concentration). Overnight cultures were used to inoculate 250 mL of LB broth supplemented with kanamycin in a 1 L culture flask and incubated as described above. Cultures were grown to an OD_{600} = 0.6, and then induced by addition of IPTG to a final concentration of 0.1 mM. Cultures were incubated for another 3 h at 37 °C, at which point the cells were harvested by centrifugation (5,000 × *g*, 10 min at 4 °C). Cell pellets were stored at –20 °C until needed.

Cell pellets were resuspended in 10 mL of binding buffer (100 mM HEPES, pH 7.5, 125 mM NaCl, and containing 10% glycerol + 5 mM imidazole) and lysed by double passage through a pre-chilled bench top cell disrupter (Constant Systems Incorporated, Sanford, NC) set to 35,000 psi. The cell lysate was centrifuged at 105,000 × *g* for 60 min. The supernatant

containing soluble proteins was decanted and purified by Ni–NTA affinity chromatography as per manufacturer's directions using 2.5 mL bed-volume of resin. The protein of interest was eluted with 250 mM imidazole in purification buffer (100 mM HEPES, pH 7.5,125 mM NaCl, and containing 10% glycerol).

5.7.4 Visualization of protein His_6-Cj1415

Protein sample was first combined with 4× SDS-PAGE sample buffer (8% 2-mercaptoethanol, 40% glycerol, 8% SDS, 250 mM Tris-HCl, pH 6.8), boiled for 10 min and separated by discontinuous SDS-PAGE using 12.5% polyacrylamide gels with Tris-glycine running buffer, according to the standard method of Laemmli.[40] Proteins were visualized by staining with Coomassie Brilliant Blue R250 for 1 h at room temperature, followed by extensive destaining in 10% acetic acid, 25% ethanol. Alternative, SDS-PAGE protein gels were used for Western immunoblotting analysis according to the method described by Kievit and Lam,[41] with minor modifications. Briefly, samples separated by SDS-PAGE were transferred electrophoretically to BioTrace nitrocellulose membrane (Geman Sciences Inc., Ann Arbor, MI). The membrane was temporarily stained with Ponceau S to note the location of the molecular weight marker bands, blocked with 3% bovine serum albumin (BSA) in Tris-buffered saline (150 mM NaCl, 10 mM Tris-HCl, pH 7.5), and incubated with Penta-His (Qiagen Inc.) mouse anti-His_6 antibody. The immunoblot was washed successively with Tween-Triton-TBS (0.05% Tween 20, 0.2% Triton X-100, 500 mM NaCl, 20 mM Tris-HCl, pH 7.5) and TBS, then incubated with alkaline phosphatase-conjugated goat anti-mouse Fab fragments (Jackson ImmunoResearch Laboratories, Inc., Westgrove, PA) in 3% BSA-TBS for 30 min. The membrane was washed successively with Tween-Triton-TBS and TBS, and developed with

0.033% NBT, 0.016% BCIP, 100 mM sodium bicarbonate buffer, pH 9.8.

5.7.5 His_6-Cj1415 assay with NMP/NDP

NMP/NDP (1 or 5 mM) and ATP (1 or 5 mM) were dissolved 10% glycerol in water in a 50 μL centrifuge tube containing NaCl buffer (150 mM, pH 8.5), $MgSO_4$ (5 mM) and DTT (10 mM). After adding appropriate amounts of His_6Cj1415 (600–900 nM), water was added to bring the final volume of the reaction mixture to 30 μL. The reaction mixture was incubated at 37 °C on a rotisserie tube rotator for 24 h. The reaction was terminated by heat shock at 95 °C for 5 min and centrifuged. The reactions were analyzed by HPLC.

5.7.12 rSAP assay with AMP-PNP (5-59A)

The preparation of **5-6A** was carried out as described by Penningroth and coworkers.[37] AMP-PNP (0.5 mM) was dissolved in water in a 100 μL centrifuge tube containing Et_3NHCO_3 buffer (50 mM, pH 10.0), $MgSO_4$ (1 mM), and $ZnCl_2$ (0.02 mM). After adding appropriate amounts of rSAP (5 U), water was added to bring the final volume of the reaction mixture to 50 μL. The reaction mixture was incubated at 37 °C on a rotisserie tube rotator for 24 h. The protein was removed by passage through a centrifugal filter (Amicon Ultra). The filtrate was then monitored for any protein activity by HPLC. In a parallel experiment, the buffer from the filtrate was removed by lyophilization. The residue was then submitted for mass spec analysis.

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Chapter 6

Summary and Future Work

6.1 Introduction

As described earlier, this thesis, which revolves around the methyl phosphoramidate (MeOPN) motifs found in the capsular polysaccharide (CPS) of *C. jejuni*, contains two parts. The first part of this thesis was to develop a reliable method to synthesize the MeOPN motif and assess its stability through various transformations. The second part focused on unraveling parts of MeOPN biosynthesis. More specifically, demonstrating protein activity for the putative phosphoramidate transferases (encoded by genes *cj1421* and *cj1422*) and a putative kinase (encoded by the gene *cj1415*).

6.2 Summary

6.2.1 Method to synthesize MeOPN motifs

We developed a two-step method to reliably synthesize MeOPN-containing carbohydrates (Figure 6-1a). The protocol involves the phosphorylation of the sugar alcohol **6-1** with methyl pivaloyl *H*-phosphonate (**6-2**) followed by an Atherton–Todd reaction. The method was shown to be effective on 17 examples (10 carbohydrate substrates with four different amine nucleophiles) with yields of 48–82%. We then demonstrated the nitrogen protecting groups on the MeOPN motif (e.g. **6-4**) can be removed without affecting the phosphoramidate (Figure 6-2b). Finally, the stability of the MeOPN modification was evaluated through various transformations upon removing the carbohydrate protecting groups (Figure 6-1c).

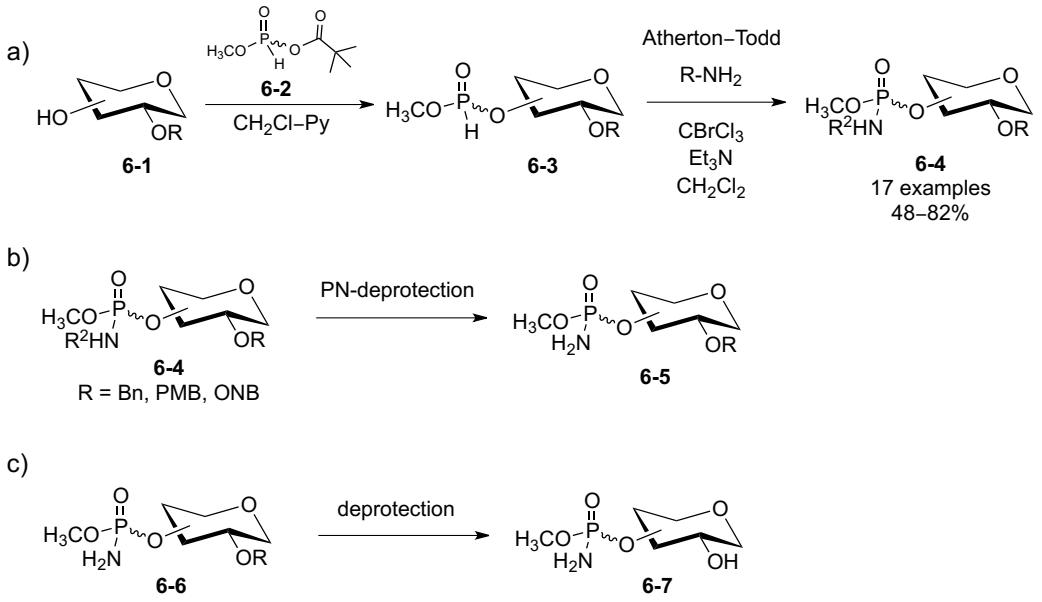


Figure 6-1: Summary of part one of this thesis.

Although the synthesis of other phosphoramidates has been reported, this is the first synthesis of the MeOPN motif. The subsequent evaluation of its stability could also provide valuable information pertaining to the lability of this and other phosphoramidate motifs in complex molecule synthesis. The developed protocol allows access to MeOPN-containing substrates that can be used for immunological experiments for the potential development of vaccines against *C. jejuni*.

6.2.2 MeOPN biosynthesis

In the second part of this thesis, we focused our attention on the biosynthesis of MeOPN motifs. Based on homology studies of the genes that encode for the proteins involved in the addition of MeOPN groups onto the CPS, we postulated the biosynthetic steps shown in Figure 6-2. We believed the protein encoded by the gene *cj1415* would phosphorylate **6-8** to give the phosphoramidate donor **6-9**. The donor **6-9** is then used by the putative phosphoramidate

transferases, encoded by the genes *cj1421* and *cj1422*, to phosphorylate the growing CPS mimic **6-10** to give **6-11**. In order to confirm our hypothesis, we needed a CPS mimic (e.g. **6-10**), the putative proteins encoded by genes *cj1415*, *cj1421*, and *cj1422*, and the phosphoramidate substrate **6-8.**

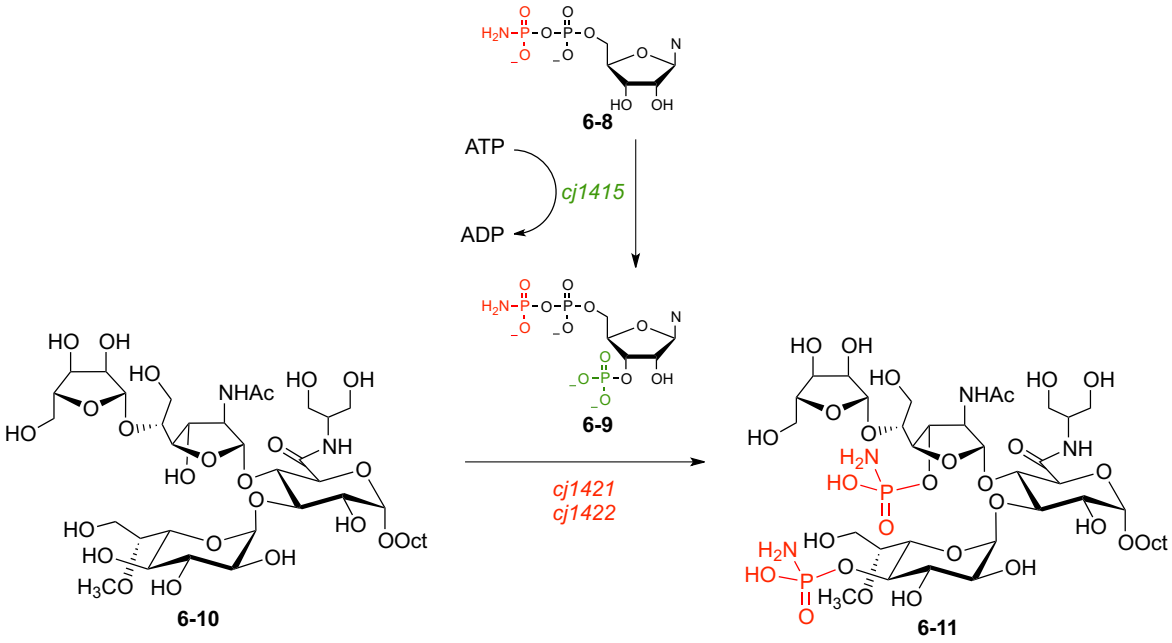


Figure 6-2: Goal of part two of this thesis – confirm postulated biosynthetic steps of the MeOPN motif.

To synthesize the tetrasaccharide **6-10**, four building blocks were needed. The syntheses of these building blocks are summarized in Figure 6-3. Briefly, the Hep*p* donor **6-13** was synthesized from D-galactose (**6-12**) in 19 steps as well as from furfural (**6-14**) in 15 steps. The Rib*f* donor was synthesized from D-ribose in four steps. The Gal*f*NAc donor analogue **6-18** was synthesized from D-galactosamine (**6-17**) in 12 steps and the Glc acceptor **6-20** was synthesized from D-glucose (**6-19**) in nine steps.

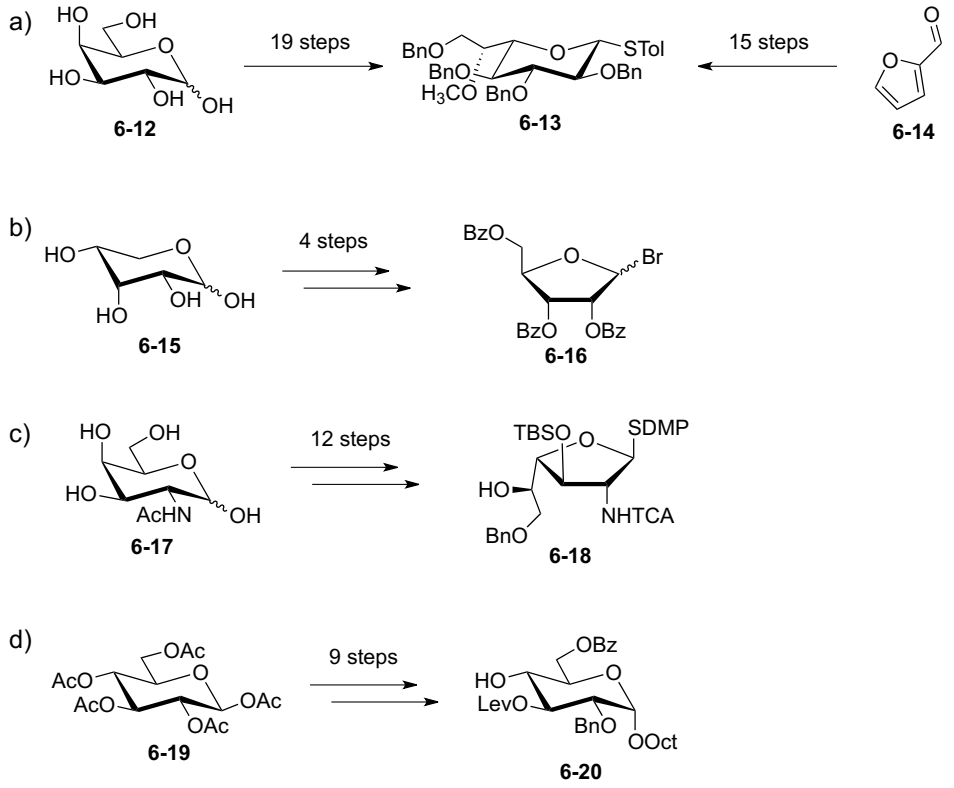


Figure 6-3: Summary of the synthesis of the monosaccharide building blocks. a) Hep*p* donor **6-13** from D-galactose and from furfural. b) Rib*f* donor from D-ribose. c) Gal*f*NAc acceptor/donor from D-galactosamine. d) Synthesis of the Glc acceptor from D-glucose.

With these building blocks (and others), a significant amount of effort was made to synthesize the tetrasaccharide **6-10**. The most optimal route we developed on route to the tetrasaccharide **6-10** is summarized in Figure 6-4. It involves the glycosidation of Rib*f* **6-16** with Gal*f*NAc analogue **6-18**. Subsequent steps after lead to the synthesis of the disaccharide donor **6-21**. The glycosidation of the donor **6-21** with Glc **6-20** followed by the removal of the levulinate group gave the trisaccharide **6-22**. Finally, the glycosylation of the trisaccharide **6-22** with the heptose donor **6-23**, that possesses the opposite stereochemistry at C-2, followed by the removal of the benzoate groups gave the tetrasaccharide **6-24**.

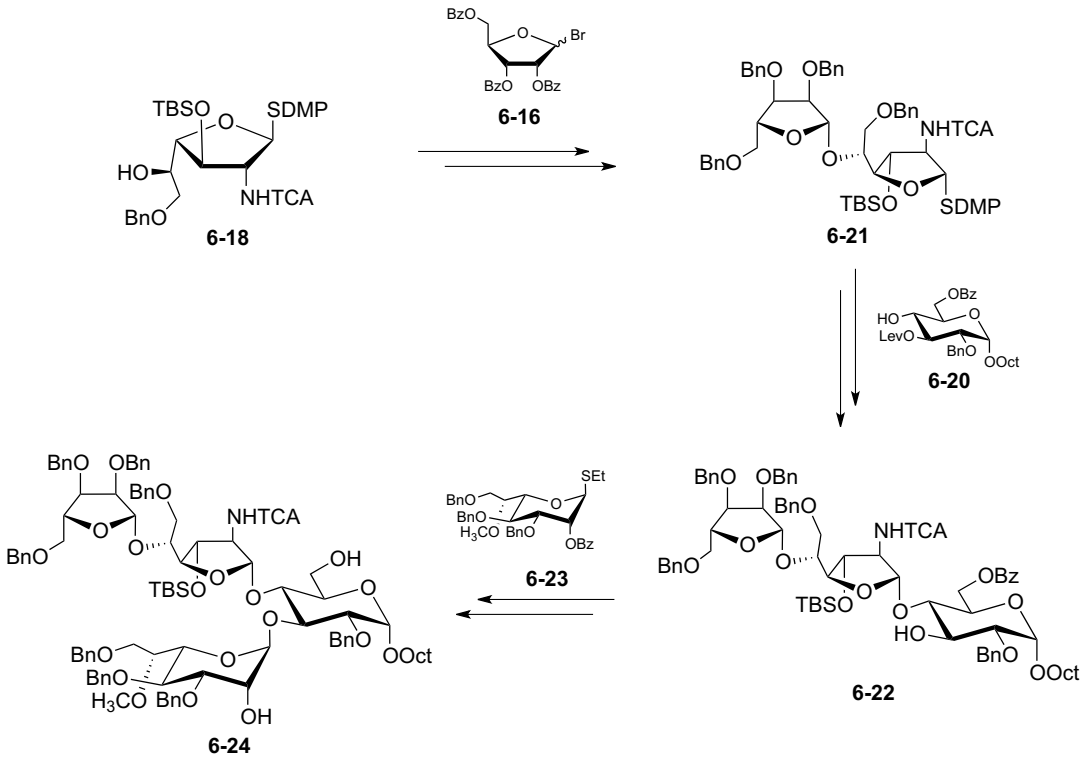


Figure 6-4: Summary for the construction of the tetrasaccharide.

Although the synthesis of tetrasaccharide **6-10** from **6-24** was not complete, the synthesis of five other substrates that could mimic parts of the CPS was accomplished. These substrates are shown in Figure 6-5. During this process, we also developed a novel oxidation–amidation approach for the synthesis of glycuronamides to overcome the difficulties associated with the lability of the Gal*f*NAc glycosidic bond. This strategy is summarized in Figure 6-6 and involves the oxidation of the 6-*S*-tolyl-glycoside **6-30** to the thioester **6-31** and then subsequent addition of an amine leading to the formation of the glycuronamide **6-32**.

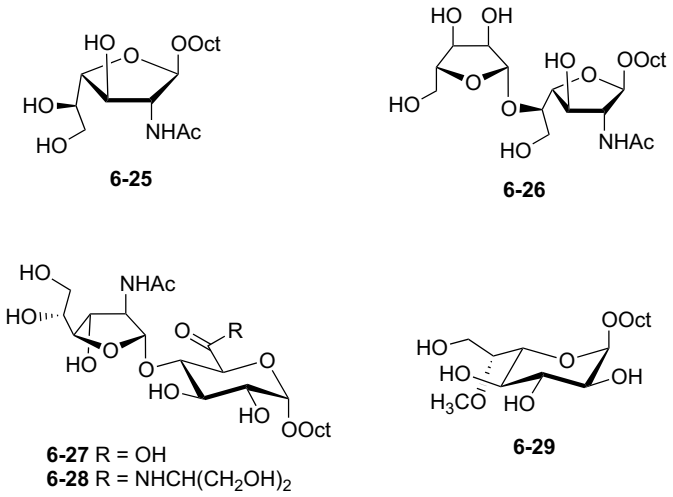


Figure 6-5: Substrates synthesized to mimic CPS.

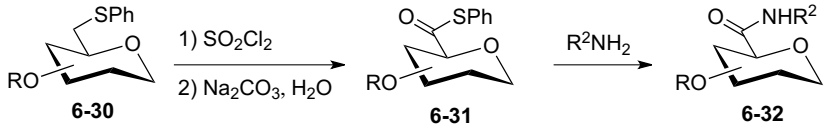


Figure 6-6: Novel method for the synthesis of glycuronamides.

We next focused on obtaining the proteins encoded by genes *cj1415*, *cj1421*, and *cj1422*. A pET30a construct possessing the gene *cj1421* was successfully obtained. The putative N-terminal His_6-tagged phosphokinase encoded by gene *cj1415* (His_6-Cj1415) was also expressed in *E. coli*. With this protein in hand, we next focused on obtaining the phosphoramidate substrate to be incubated with His_6-Cj1415.

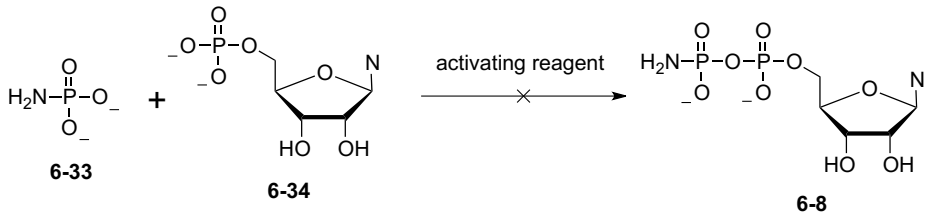
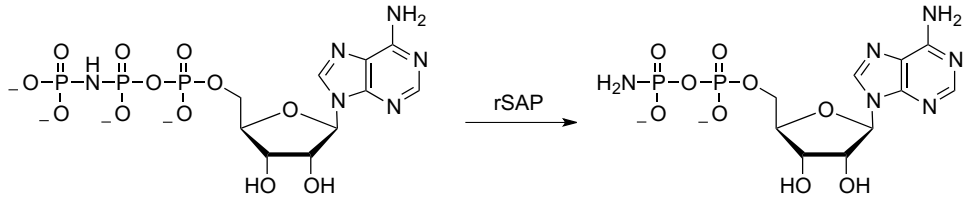


Figure 6-7: Attempted synthesis of phosphoramidate substrate **6-8**.

Unfortunately, our attempts to chemically synthesize **6-8** from phosphoramidate **6-33** and nucleoside monosphosphate **6-34** failed to show any conclusive evidence of the product being formed. Despite these setbacks, NMPs (e.g. AMP, CMP, GMP, UMP) and NDPs (e.g ADP, CDP, GDP, UDP) were used as mimics of **6-8** and were incubated with His_6-Cj1415. Our preliminary findings in these studies may suggest the nucleobase of the postulated phosphoramidate donor is cytosine.

Although the work involved in the second research project did not go as planned, progress has been made. CPS mimics were synthesized, a vector possessing the gene *cj1421* was obtained, and the expression of His_6-Cj1415 was a success. In addition, mass spectrometry analysis of the product(s) in the incubation of **6-35** with recombinant shrimp alkaline phosphatase (rSAP) showed a mass signal that correlates to **6-36A**, the phosphoramidate substrate in which I could not synthesize.



6-35 **6-36A**

Figure 6-8: Incubation of **6-35** with shrimp alkaline phosphatase (rSAP). A possible strategy to obtain the phosphoramidate substrate **6-36**.

6.3 Future work

Our developed two-step method for the synthesis of MeOPN motif has ongoing projects. Our method produces the MeOPN-containing saccharides in ~1:1 ratio of diastereomers. In some

cases, these diastereomers are separable by flash chromatography. As part of a collaborating project, a group member, Sicheng Lin, and I have re-synthesized two MeOPN-containing saccharides and separated their diastereomers. These compounds have been characterized by flash chromatography. Professor Yunjie Xu's group (University of Alberta) is currently investigating these compounds by vibrational circular dichroism (VCD). By using a combination of VCD, ab initio calculations, and NMR spectroscopy, we hope to determine the stereochemistry of the phosphorus center in naturally occurring CPSs. In addition, this two-step has been modified to contain an *O*-benzyl group in place of the *O*-methyl group. This enables the removal of the benzyl group to produce a substrate that is currently being investigated for the synthesis of diastereoselective MeOPN motifs by use of a chiral methylating reagent. If successful, this approach could potentially aid in determining the stereochemistry of the phosphorus center.

With respect to the work involved the MeOPN biosynthesis, the work described here warrants further investigations both in the synthesis of the tetrasaccharide substrate and the biosynthesis of the MeOPN motifs. With many of the problems solved on model substrates, we envision the synthesis of the tetrasaccharide substrate could be complete in the near future. With work directly involved in the MeOPN biosynthesis, our preliminary findings in incubating CMP and CDP with His_6-Cj1415 and in incubating **6-35** with rSAP warrant further investigation to characterize their products. Provided these results are reproducible and data is obtained to support the postulated products, identifying the phosphoramidate donor and demonstrating phosphoramidate transferase activity could still be achieved. In addition, the CPS mimics synthesized in Figure 6-5 could also be used as probes for other biosynthetic proteins (e.g. glycosyltransferases).

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