Studies Towards the Synthesis of 6-Deoxy-heptopyranose Containing *Campylobacter* Glycans

by

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A thesis submitted in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

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Abstract

Campylobacters are the leading cause of food-borne gastrointestinal illness worldwide. *C. jejuni* is the most commonly involved in human disease followed by *C. coli*. Although these infections usually resolve in a few days, they sometimes lead to the neurological auto-immune disorder, Guillain–Barré Syndrome. The cell surface of these pathogens is decorated by polysaccharides that contribute to virulence.

The *C. jejuni* capsular polysaccharide (CPS) differentiates the 47 different serotypes identified to date. Although, currently there is no commercially-available vaccine against *C. jejuni* for humans, a CPS conjugate vaccine against *C. jejuni* 81-176 protects against diarrheal disease in non-human primates. Because of the large variety of serotypes, a practical vaccine must contain CPS from many different strains; however, the number required is not known.

The structure of *C. jejuni* CPS varies widely from strain to strain and is often functionalized with a range of phase-variable modifications such as the addition of methyl, ethanolamine, aminoglycerol, and *O*-methyl phosphoramidate (MeOPN) groups. These CPSs possess a number of unusual structural motifs and prominent among these are the 6-deoxy-heptoses and the MeOPN group. The MeOPN phosphorus atom in these CPS is stereogenic and is found as a single stereoisomer.

Although the CPS conjugate vaccine has 100% efficacy against diarrheal disease in animal models, a drawback to this vaccine is that it protects only against the 81-176 strain. The addition of serotypes from additional strains is necessary for better protection. However, the isolation of CPS in pure form from bacteria is difficult and the heterogeneity in the carbohydrate is not ideal for use in vaccines. Therefore, an alternate approach to access this material –

synthetic chemistry – is necessary. Chemically synthesized CPSs will be well-defined and homogeneous, which can be used as antigens for vaccine development.

This thesis was focused on the synthesis of CPS fragments from selected serotypes that contain 6-deoxy-heptopyranoses in the D-configuration. The synthesis of thioglycoside donors of 6-deoxy-D-heptopyranosides was focused in the first part. The second part of the thesis will focus on assembling the monosaccharide building blocks, including the 6-deoxy-D-heptopyranoside thioglycosides, into targeted CPS fragments.

Preface

The work described in this thesis is an original work carried out by V Narasimharao (Narasimha) Thota which includes carrying out all the experiments, obtaining, analyzing and documenting the data. Mass spectroscopic data of all compounds were collected by Dr. Randy Whittal and his colleagues (majority of the samples were collected by Dr. Angelina Morales-Izquierdo (Angie)) in the mass spectrometric lab. Optical rotation data were recorded by Wayne Moffat and his colleagues in the analytical Lab.

The work described in **Chapter 2** was carried out solely by me and has not been published at the time of writing. A part of this Chapter will be soon published as Thota, V. N. and Lowary, T. L. (*Manuscript in preparation*).

Chapter 3–A part of this Chapter was published:

Professor Lowary and I wrote the manuscript together. I was the only experimentalist on this work. M. J. Ferguson carried out X-ray analysis on compound **(*R*)-3-54**. R. P. Sweeney made the compound **3-46** first in the lab which I reproduced and used in the synthesis. Mark Miskolzie from the University of Alberta NMR facility carried out the high temperature NMR experiments on **(*R*)-3-54** and **(*S*)-3-54**.

Thota, V. N.; Ferguson, M. J.; Sweeney, R. P.; Lowary, T. L. *Angew*. *Chem*., *Int*. *Ed*. **2018**, *57*,

15592–15596.

The work described in **Chapter 4** was carried out solely by me and has not been published at the time of writing. A part of this Chapter will be soon published as Thota, V. N. and Lowary, T. L. (*Manuscript in preparation*).

Dedication

I want to thank my parents for upbringing and making the person what I am today by providing me education, unconditional love, respect and support. I sincerely dedicate this thesis to my beloved mother (Veera Venkata Nagendramani Thota), my beloved father (Lakshmanarao Thota), and my beloved brother (Rama Venkata Satyanarayana Thota). I also would like to dedicate this thesis to my love and soon to be fiancée Ms. Swetha Akula.

Acknowledgements

First and foremost, I would like to thank my supervisor Professor Todd L. Lowary, for his help, guidance, encouragement, and constant support throughout the last six years. He has created a beautiful environment to work, to express myself, to learn and I am thankful for all the opportunities given to me. I am also thankful to him for his insightful scientific discussions, and suggestions in my projects. I express my sincere gratitude for his numerous edits of my thesis, abstracts, posters, CV, scholarship, and job applications. I am very fortunate to have carried out my graduate research under his extraordinary mentorship and I could not have asked for a better supervisor and a better place while the time I am here in the University of Alberta.

Next, I would like to thank my supervisory committee, candidacy committee and PhD defense committee members: Professors Jonathan G. C. Veinot, Christopher W. Cairo, Frederick G. West, Derrick L. J. Clive. I would also like to thank my thesis external examiner Professor Steven J. Sucheck, from The University of Toledo, OH, USA. Their suggestions for this thesis are greatly appreciated.

I would also like to thank all the Scientific Services Staff of the Department of Chemistry, University of Alberta. I would like to express my gratitude to the people of the NMR facility namely Nupur Dabral (for NMR training) and Mark Miskolzie for their help and suggestions, especially Mark because he is always willing to help me with the instruments and show me all the tricks and shortcuts (postscript file modifications, obtaining the data for mg quantity samples, and digitization of the data etc.) irrespective of what time of the day it was. I would also like to thank the people of the Mass Spectrometry facility namely Béla Reiz, Jing Zheng and Dr. Angelina “Angie” Morales-Izquierdo for their help and suggestions. I wish to express my special

gratitude to “Angie” because she is always willing to help me with the HRMS of my research samples despite being submitted my research samples very late in the day (after 5 PM). I would also like to thank the people of the Analytical and Instrumentation Laboratory facility namely Dr. Wayne Moffat and his colleagues, especially Jennifer Jones for their help in recording optical rotation data. I would also like to thank Michael J. Ferguson from X-ray Crystallography Laboratory facility for conducting X-ray analysis on the most important research sample in my graduate studies.

I am also grateful to the Technical staff members Jason Dibbs, Dirk Kelm and Paul Crothers for their help especially to Jason Dibbs for his extraordinary skills in my research glassware repair, and making the required dimensions of the glassware with in short time. I am also thankful to the Support staff members Anita Weiler, Esther Moibi, and Bernie Hippel (retired). I would like to express sincere gratitude to the gradute pragramme assistant Anita Weiler for her help for keeping me in line with the paper work, meeting the deadlines, and informing the necessary things when required for my gradute studies. Special thanks also go to Laura Pham for her help during my stay as a graduate student in the Chemistry Department, University of Alberta. Other special thanks also go to Dr. Hayley Wan, Nada Djokic and Connor Part for their help in finishing my Teaching Assistant duties.

I have been fortunate to have the opportunity to work with a great group of people during the course of my graduate research studies and I would like to thank all the members of the Lowary group, past (especially Dr. Junfeng Zhang, Dr. Roger Ashmus, Dr. Claude Larrivée Aboussafy, Dr. Tzuting-Kao and Dr. Ryan Sweeney) and present, for their help and support throughout my PhD studies. Without them, the research phase of my graduate studies would not have been so enjoyable. Very special thanks go to Dr. Maju Joe for his help, suggestions, and helpful

discussions-laughs in and out of Chemsitry. I would like to express my sincere gratitude to both Maju and Vitor Cunha for their help in proof reading my thesis (text) chapters and providing me useful tips and comments. Special thanks also go to Chun-Jui ("Anthony") Chu for proof reading couple of my experimental parts in my thesis and also for his generous nature of lending his things during my Chem 502 exam. I also thank Ying-Jie Lim (Winston) for his helpful scientific discussions. Very special thanks go to Dr. Junfeng Zhang for making me (who is very lazy to go to gym but not to sports) to have fun and joyful time outside the Chemistry lab by teaching me the squash sport and make me run harder to beat him in almost all closely fought games. Special thanks also go to Dr. Tzuting-Kao for organizing the weekly badminton matches and to all my badminton team members: Boshun-Huang, Mikel Jason Allas, Tzuting, Pei-Jhen, Manas Jana, Posen-Tseng, Ying-Jie Lim (Winston), and Sicheng Lin (one time player). Without all these people, my graduate research career would be incomplete. I would also like to thank all of my other friends, well-wishers in the department and my family for all of their unconditional love and support during my stay in Canada.

Out side of my lab, very special thanks go to Dr. Anindya Swarnakar for his valuable support during first few weeks of my arrival in Canada, to Justin Thuss for mentoring the Chem 261 lab in the first semester, to Christine Dunbar for her help in opening the student lockers in Chem 261 lab before I go to lab (I am late because of the class just before the lab), to Dr. Venkata Pavan Kumar Kondapi for his help during my 502 preparation, for mentoring Chem 263 lab and all other suggestions he gave during my graduate studies, to Dr. Ronnie Perez for encouraging me to find a girl friend in Canada (which I could not find as I was attracted to Swetha, a close family friends daughter of my parents), to Dr. Vijay Kumar Hinge for being a well-wisher, to my housemate Luthfur Rahman for playing weekly badminton matches.

I would also like to thank all my childhood friends especially Bellamkonda Adinarayana (Hari), Jyothi Oduri, Sivakrishna kumari (Krishna) Thota, Kiran kumar Ande, Ramesh Kunche, Hanumathurao Konidela (Raja), Satti raja venkannababu Vemana (Satya), Anjaneyulu Achanta, Durgaprasad Tadi, Kumar raja Yenumula, Venkata reddy naidu (Dheeraj) Kommula, Raju Tenneti, Sivakrishna Koppada, Uma maheswararao (Bobby) Yenumula, Prasad Undapalli, Durgaprasad Kadali for making my childhood so colorful with their friendship. I would also like to thank all my batchelors friends especially Rajaram Surimenu, Kishore Balla, Sattiraju Adabala, Damodar Addala, Rajesh Arukapalli, Phani kumar Rudra, Praveen, Ramakrishna (Lakkavaram), Ramakrishna (Rams), Srinu (narasapuram) for all their friendship all these years. I would also like to thank all my masters (from IIT-Bombay) friends especially Venkataiah (Venkat Anna) Husnabad, Tirupathiah (Thiru) Avullala, Raju Kolupula, Yernaidu Reddi, Ganapathi Gedala, Ramu Pedamajji, Ajay Kumar Paliki, Durganna Nallabathula, Madanakrishna Katari, Krishnendu Maity, Raj kumar Kolipaka, Srinivasarao (Srinu) Alamanda, Suresh babu Guduguntla, Rambabu Tursham, Ramesh Banoth, Govindaraju Singappagudem, Ravi Joriga, for all their friendship all these years. Without all these people, my graduate studies would not have been so enjoyable outside the non-research time.

I would like to express my sincere gratitude to my master's supervisor Prof. Suvarn S. Kulkarni for writing an open recommendation letter to enter into the University of Alberta graduate studies as a PhD student. Lastly, I would like to thank my parents and my brother for all their help and support to achieve mental stability during the PhD research phase of my career.

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List of Schemes

List of Abbreviations

$[\alpha]_D$	specific rotation (sodium D line)
Å	angstrom
Ac_2O	acetic anhydride
AcOH	acetic acid
AgOTf	silver trifluoromethanesulfonate
AllOH	allyl alcohol
app t	apparent triplet
APT	attached proton test
ArH	aromatic hydrogen
ATCC	American Type Culture Collection
BAIB	bis(acetoxy)iodobenzene
$BH_3 \cdot THF$	borane tetrahydrofuran complex
BnBr	benzyl bromide
br s	broad singlet
BzCl	benzoyl chloride
C. coli	*Campylobacter coli*
C. jejuni	*Campylobacter jejuni*
CDP	cytidine diphosphate
CFU	colony forming unit
COSY	correlation spectroscopy
CPS	capsular polysaccharide
CSA	camphorsulfonic acid

DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
Ddmh	putative heptose-6-dehydratase enzyme involved in the 6-deoxy-D-*manno*-heptose biosynthesis
DiBAL-H	diisobutylaluminium hydride
DIPEA	*N*,*N*-diisopropylethylamine
DMP	2,2-dimethoxypropane
DMSO	dimethyl sulfoxide
DTBMP	2,6-di-tert-butyl-4-methyl-pyridine
$DTBS(OTf)_2$	di-tert-butylsilyl bis(trifluoromethanesulfonate)
EDC·HCl	*N*-(3-dimethylaminopropyl)-*N*'-ethylcarbodiimide hydrochloride
ESI-MS	electrospray ionization mass spectrometry
Et_3SiH	triethylsilane
FGI	functional group interconversion
GBS	Guillain–Barré Syndrome
GDP	guanosine diphosphate
HMBC	heteronuclear multiple bond correlation
HMPA	hexamethylphosphoric triamide
HMW	high molecular weight
HRMS	high-resolution mass spectrometry
HS	heat-stable
HSQC	heteronuclear single quantum coherence
IAD	intramolecular aglycone delivery
IDCP	iodonium di-sym-collidine perchlorate

IgG	immunoglobulin G
KO*t*-Bu	potassium *tert*-butoxide
LDA	lithium diisopropylamide
$LiEt_3BH$	lithium triethylborohydride
LiHMDS	lithium bis(trimethylsilyl)amide
LOS	lipooligosaccharide
LPS	lipopolysaccharide
MeOPN	*O*-methyl phosphoramidate
MsCl	methanesulfonyl chloride
NBS	*N*-bromosuccinimide
NCTC	national collection of type cultures
NIS	*N*-iodosuccinimide
NMR	nuclear magnetic resonance
PCR	polymerase chain reaction
PivCl	pivaloyl chloride
p-TSA	*p*-toluenesulfonic acid
SDS-PAGE	sodium dodecyl sulfate-polyacrylamide gel electrophoresis
TBAF	tetra-n-butylammonium fluoride
TBDPS	*tert*-butyldiphenylsilyl
TBS	*tert*-butyldimethylsilyl
TEMPO	(2,2,6,6-tetramethylpiperidin-1-yl)oxyl
Tf_2O	trifluoromethanesulfonic anhydride
TFA	trifluoroacetic acid

TfOH	trifluoromethanesulfonic acid
TLC	thin layer chromatography
TMSOTf	trimethylsilyl trifluoromethanesulfonate
TOCSY	total correlation spectroscopy
TROESY	transverse rotating frame overhauser effect spectroscopy

Chapter 1

Introduction to *Campylobacters* and their cell wall structure, glycoconjugate vaccines and research overview

1.1 Introduction

1.1.1 History of *Campylobacters*

In 1886, Theodor Escherich (who also discovered *Escherichia coli*) observed the organisms that today are called *Campylobacters* in the feces of children who had died of diarrhea. He named the disease as "*cholera infantum*" or "*summer complaint*".[1,2] Several years later, in 1913, MacFayden and Stockman described *Vibrio*-like organisms (called so as they are related *Vibrio*) in fetal tissues of aborted sheep.[3] However, because of the difficulties in the growth and characterization of these organisms, they remained overlooked and were referred to as "*Vibrio*-like organisms" for several years.[4] It was not until 1963 that Sebald and Véron named the genus *Campylobacter*, based on their curved shape; the name is derived from Greek words for curved (kampylos) and rod (baktron).[5] Later developments led to the isolation of *Campylobacters* in humans, animals, food and water and they are currently regarded as common human pathogens.[6]

1.1.2 Characteristics of *Campylobacters*

The *Campylobacter* genus belongs to the family of Gram-negative bacteria *Campylobacteraceae*, which also includes the genera *Arcobacter*, *Sulfurospirillum* and *Bacteroides*. *Campylobacter* require oxygen to survive and the optimal growth temperature is 42 °C.[2] They are slender, curved, and S-shaped or spiral rod shaped and they contain two flagella at opposite ends of the cell. Figure 1-1 shows both scanning electron micrograph and transmission electron micrographs of *Campylobacter jejuni* ATCC33560.[7] Recently, the structure of *C. jejuni* cells was reported using electron cryotomography.[8] They are typically motile at above 37 °C and move in a corkscrew-like motion with the help of the flagella.

A B

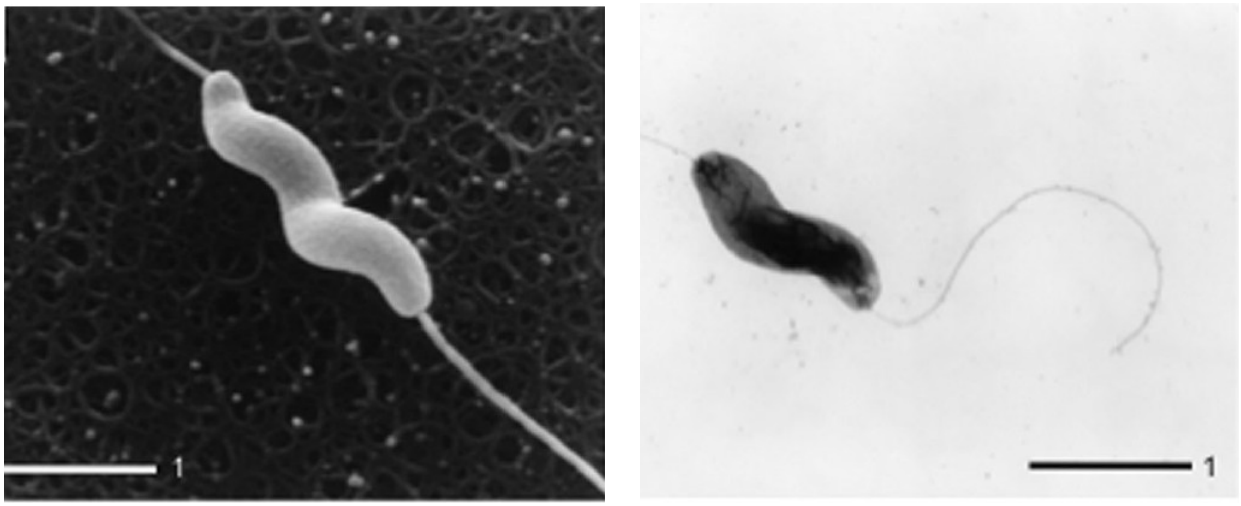

Figure 1-1: Scanning electron micrograph (A) and transmission electron micrograph (B) of *C. jejuni* ATCC33560 cells showing shapes and flagella. Reprinted with permission from: *Microbiol Immunol 57*, 83–90, copyright 2013.[7]

1.1.3 *Campylobacters* infection in humans

Because of its relative recent discovery compared to other organisms affecting the intestine – *Salmonella*, *Vibrio* and *Shigella* – *Campylobacter* was not considered as human pathogen until the 1970's. Once its isolation methods and growing media were established, this genus has been given increasing attention and *Campylobacters* are the leading cause of food-borne gastrointestinal illness worldwide.[9] Currently, there are 29 species and 11 subspecies assigned to the genus *Campylobacter*[10] of which *C. jejuni* is the most commonly involved in human disease followed by *C. coli*, *C. upsaliensis*, and *C. lari*.[11–13] The most widely used biochemical assay for distinguishing pathogenic *C. jejuni* from other species in the genus is the hippurate (*N*-benzoyl-glycine) test.[14] Because of the presence of the *hip*O gene, *C. jejuni* can produce an enzyme that hydrolyses hippurate to benzoic acid and glycine. The products can be

identified by a ninhydrin test, thin layer chromatography or gas-liquid chromatography.[15,16] Later, several methods were developed for strain characterization and have been used for epidemiological typing of *Campylobacter* species.[11,17,18]

1.1.3.1 Gastroenteritis and epidemiology of *Campylobacter* infection

Campylobacters are commonly found in the gastrointestinal tract of animals, especially birds. They were also found in the intestine of many mammals including humans. *C. jejuni* is a chicken commensal; the bacteria asymptomatically colonize its gastrointestinal tract.[19] Human infection is primarily caused by eating or handling of contaminated poultry but infections have also resulted from inadequately pasteurised milk[20] and contaminated water.[21] For example, poultry-contaminated food was responsible for 60% of *Campylobacter* infections in a 2010–2013 case study (Figure 1-2).[22]

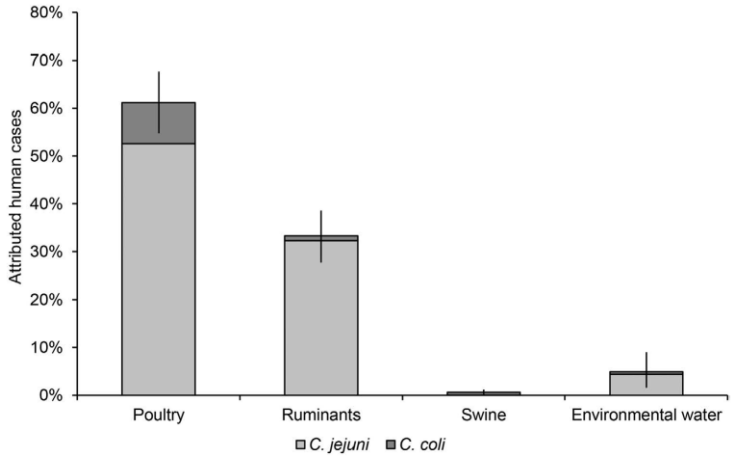

Figure 1-2: A case study showing the source of campylobacteriosis and percentage of human cases attributed to animal and environmental sources in Luxembourg. Reprinted with permission from: *Sci. Rep. 6*, 1–12, copyright 2016.[22]

Only a few reports are available on dose of *Campylobacter* needed for an infection. Infectious doses can be as low as 500–800 colony forming unit (CFU).[23,24] However, it has been speculated that the dose can be even lower, 360 CFU.[25] In the past decade there has been increase in the global incidence of the disease.[26] There are 14 and 65 cases/100,000/year in US and Europe, respectively. In Canada, there are 35 cases/100,000 and in developing countries, in particular Southeast Asia, the incidence is projected to be at least 10 times higher.[26,27] It is estimated that 5–15% of all these cases are travellers.[28] The number of cases of these infections is under-reported as people with mild symptoms often do not seek medical care and not all laboratory confirmed cases are reported.[10]

1.1.3.2 Outcomes of *Campylobacter* infections

Symptoms of *Campylobacter* infection are often indistinguishable from those caused by other food-borne pathogens such as *Salmonella* and *Shigella*.[13] Once *Campylobacter* has been ingested, symptoms appear usually 2–5 days later. The result of infection varies and depends on the infecting strain and susceptibility of the individual, ranging from mild abdominal pain and diarrhea, nausea, fever, headache, and vomiting. Patients with more severe cases can have bloody diarrhea that can last for up to a week.[10] *Campylobacter* infections are usually last less than one week but may last longer. In most cases, people recover without antibiotics.

Campylobacter infections have been associated with chronic diseases such as reactive arthritis, irritable bowel syndrome, Guillain–Barré Syndrome (GBS) and Miller Fisher syndrome, a variant of GBS. The most serious of them is GBS, which is an auto immune neurological disorder that can lead to paralysis. The incidence rate is less than 1 in 1000; however, because *C. jejuni* infection rates are high, this is a significant number. *C. jejuni* is one of a few bacteria that incorporate sialic acid into their surface glycans as part of their

lipooligosaccharides (LOSs). These glycans have similar structures to the gangliosides on human peripheral nerves.[12] Thus, antibodies that are generated against *C. jejuni* LOS can also cross-react with host gangliosides leading to GBS (Figure 1-3).[29–31] The fatality rate from *C. jejuni* infections can be as high as 9%.[13]

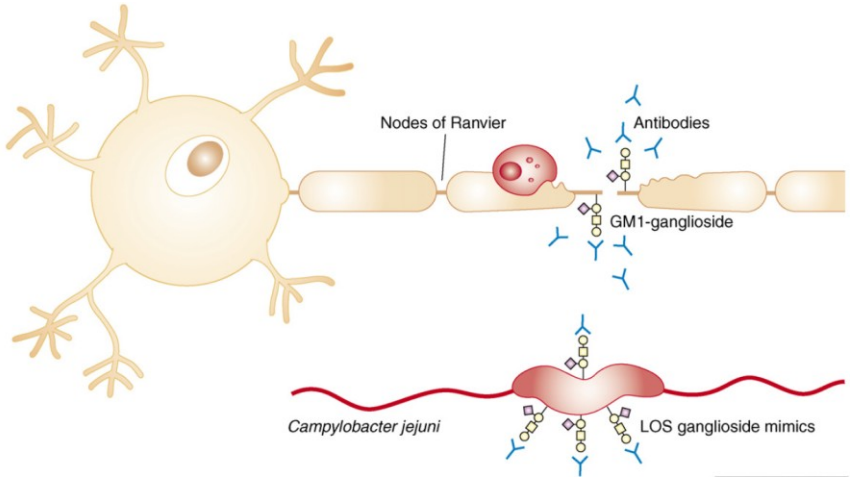

Figure 1-3: *C. jejuni*-induced autoimmunity. Reprinted with permission from: *Trends Microbiol. 16*, 428–435, copyright 2008.[12]

1.1.3.3 Treatment of *Campylobacter* infection

Most people with *Campylobacter* infections recover without treatment. Antibiotics are needed only for patients who are very ill or who have weakened immune systems or those that are undergoing chemotherapy. Macrolides and fluoroquinolones can be used for treatment of severe *Campylobacter* infections. However, the widespread use of antibiotics as therapeutics and in animal production to treat, control and prevent infection have led to resistance.[32,33] Infections by antibiotic-resistant bacteria are often harder to treat; the infections can last longer, and may result in more severe illness.

1.1.3.4 Prevention of *Campylobacter* infection

A number of strategies can be used to prevent *Campylobacter* infections from including the use of control measures from production on a farm, to processing, manufacturing and the preparation of foods.[10] For example, segregation of *Campylobacter* infected from non-infected chickens at the slaughterhouse is an effective method of reducing transmission of the contamination.[34] Education to prevent the transfer of *Campylobacter* from, raw foods is also an effective prevention measure.

Another way of controlling *Campylobacter* infection would be to vaccinate chickens or humans against infection. Although, currently there is no commercially-available vaccine against *C. jejuni* for humans, Monteiro, Guerry and co-workers have shown the effectiveness of a conjugate vaccine candidate, derived from antigens present in *C. jejuni* strains.[35] This conjugate vaccine prevented diarrhea in animal models (discussed in section 1.3 of this Chapter).

1.2 Cell wall structure of *Campylobacter jejuni*

Surface polysaccharides are present on all bacterial cell surfaces and they act as an interface between the organism and its host or the environment. Similar to Gram-negative bacteria, the cell wall of *C. jejuni* has an inner membrane and an outer membrane with the periplasm and peptidoglycan between them.[36] Historically, *C. jejuni* was believed to produce either high molecular weight lipopolysaccharides (HMW LPSs) or lipooligosaccharides (LOSs), and in some cases both, as part of their surface polysaccharides. However, later studies showed that what were thought to be the HMW LPSs are actually capsular polysaccharides (CPSs).[37–39] The genome sequence of *C. jejuni* strain NCTC11168 revealed four distinct carbohydrate gene clusters: genes for the synthesis of LOSs, CPSs, and *O*- and *N*-linked protein glycosylation

systems. These glycolipids and glycoproteins are on the outer membrane of the *C. jejuni* cell wall. LOSs possess Lipid A as the phospholipid anchor as shown in Figure 1-4. The glycolipids that are relevant to this thesis are the CPSs and are discussed further in section 1.2.1.

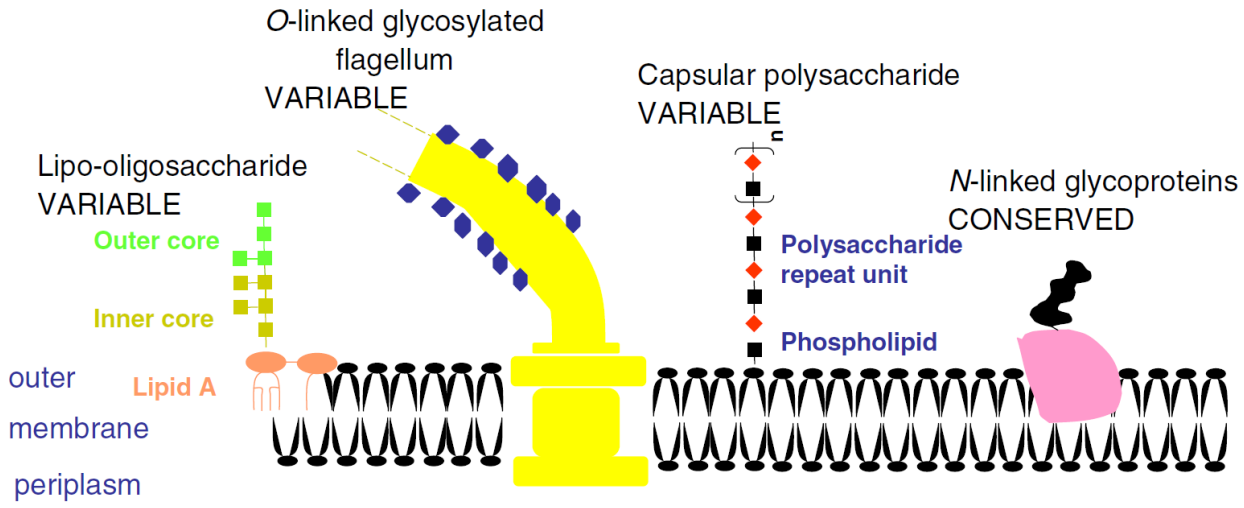

Figure 1-4: *C. jejuni* surface glycans on the outer membrane. Reprinted with permission from: *FEMS Microbiol. Rev. 29*, 377–390, copyright 2005.[40]

1.2.1 Capsular polysaccharides (CPSs)

As described above, *C. jejuni* CPSs were once thought to be HMW LPSs. This misconception was, in part, due to the challenges in distinguishing CPS from LPS as they are often found associated with each other.

1.2.1.1 Discovery of capsular polysaccharides (CPSs) in *Campylobacters*

The first evidence pointing to the existence of CPSs in *Campylobacter* was observed by Aspinall and co-workers in 1995.[41,42] In their structural analysis of *C. lari* strains PC 637 and ATCC 35221, the soluble polysaccharide obtained from hot water/phenol extraction (the standard extraction protocol for LPS extraction) was not attached to lipid A or LOS regions.

Also, the absence of usual LPS core sugar components (D- or L-*glycero*-heptoses or D-hexosamines) and of ladder-like banding patterns on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) led them to speculate that the isolated polysaccharide was not LPS and was instead a CPS. They further hypothesized that the isolated polysaccharides might have lipid termini such as di-*O*-acyl glycerol units. Similar properties were observed from hot water/phenol extracted polysaccharides by Moran and co-workers[43] and by Savage and co-workers in the structural analysis of *C. jejuni* strain 176.83.[44] In 1996, Chart and co-workers also hypothesized the presence of CPS in *Campylobacter* while examining the serotyping antigens (discussed in section 1.2.1.3) expressed by *C. jejuni* strains through SDS-PAGE and immunoblotting.[45] Following these observations, the first *C. jejuni* genome sequence (for strain NCTC 11168) published in 2000 also suggested the production of CPS by *Campylobacter*.[37]

The first unambiguous demonstration of the presence of CPS in *Campylobacter* was reported by Karlyshev and co-workers through genetic and biochemical evidence.[38,39] They sequenced the *C. jejuni* strain NCTC 11168 and subsequent analysis of the data resulted in the identification of the genes encoding for proteins similar to those involved in CPS biosynthesis in *Escherichia coli*.[38] Biochemical evidence was later established by mutation of the newly identified CPS transport genes *kpsM*, *kpsS*, and *kpsC*, which showed the loss of the CPS.[39]

In addition, phospholipase treatment of the extracted polysaccharide (assumed to be HMW LPS) through hot water/phenol extraction resulted in complete loss of HMW LPS as detected by Western blotting. Their explanation of the loss of HMW LPS upon phospholipase treatment is that, without the lipid moiety, the polysaccharide can no longer bind SDS and does not enter the gel during electrophoresis. Because Lipid A, a component of all LPSs, is not attached to the core oligosaccharide through a phosphodiester linkage (Figure 1-5A) it is

unreactive to treatment with phospholipase. The reactivity of this polysaccharide to phospholipase indicates that it is attached to another phospholipid. This confirms that the polysaccharide is a CPS not HMW LPS. The attachment of a phospholipid to the CPS was further confirmed by Moran and co-workers in 2006 in three *C. jejuni* strains: 81-176, 81116, and ATCC 43431.[46] In their study, a mild extraction protocol allowed them to isolate the lipid-linked *Campylobacter* polysaccharide for the first time. Structural characterization revealed that the lipid anchor is dipalmitoyl-glycerophosphate. The general lipid anchors present in LPS (other Gram-negative bacteria) and CPS (*Campylobacter*) are shown in Figure 1-5.

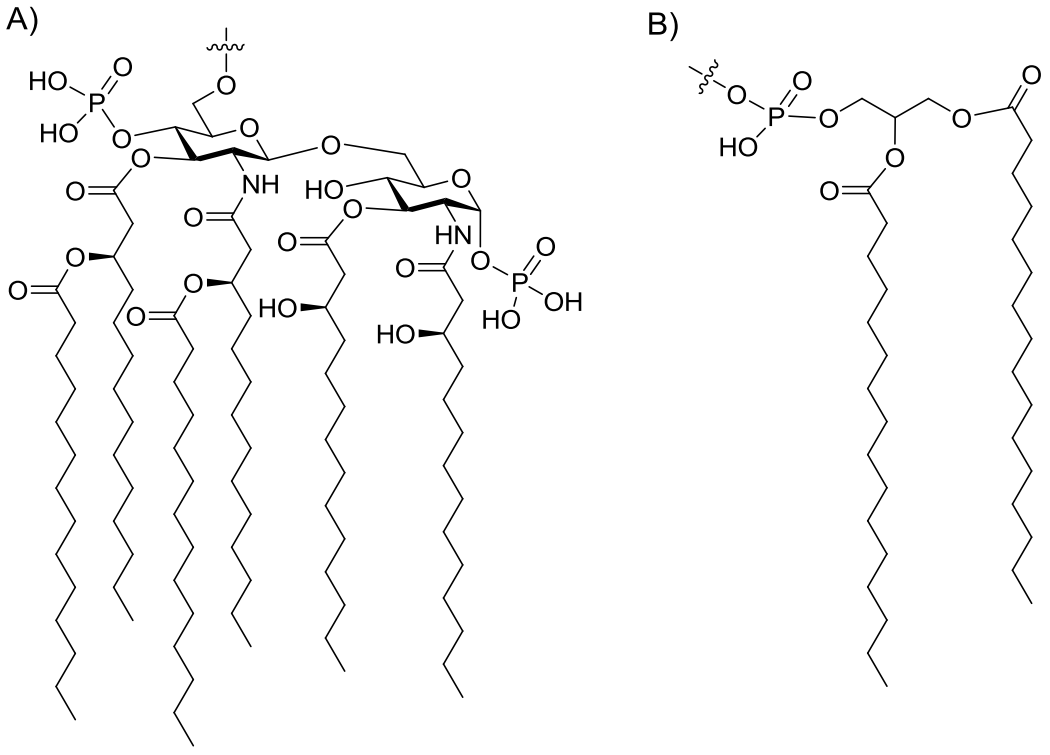

Figure 1-5: Common lipid anchors in gram-negative cell surface polysaccharides: A) Lipid A present in LOS and LPS of most Gram-negative bacteria. B) Dipalmitoyl glycerophosphate present in *Campylobacter* CPS.

1.2.1.2 Visualization of capsular polysaccharides (CPSs) in *Campylobacters*

After demonstrating the genetic and biochemical evidence of CPS in *Campylobacters*, Karlyshev and Wren[47] further confirmed the presence of CPS through staining using Alcian blue dye (known to stain CPSs from other bacterial species[48]). In Figure 1-6A, the staining of extracted CPS at 50 °C (Lane 2) and lysate (Lane 3) can be observed, as well as the absence of the CPS from supernatant of cells washed with saline (Lane 1). Both CPS and LOS can be stained with Alcian blue, but the CPS stains more intensely than LOS (Figure 1-6A) when compared to Western blot using antiserum (Figure 1-6B). The intensity can be increased by staining with silver; however, this causes a significant increase in the background (Figure 1-6C).

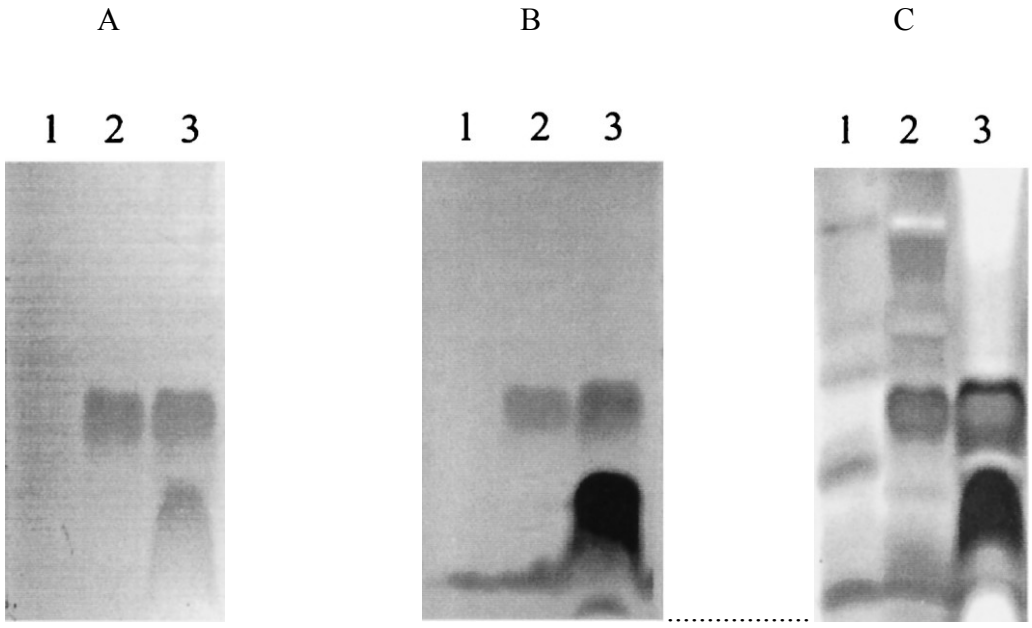

Figure 1-6: Visualization of *C. jejuni* CPS through SDS-PAGE gel: A) stained with Alcian blue dye. B) antiserum C) Stained with Alcian blue dye and silver. Lanes 1, supernatant of cells washed with saline. Lane 2, 50 °C extract. Lane 3, lysate. Reprinted with permission from: *J. Clin. Microbiol.*, *39*, 279–284, copyright 2001.

Later Wren and co-workers[49] reported the visualization of the CPS through electron microscopy in *C. jejuni* cells after staining with Alcian blue dye. Figure 1-7 shows an electron micrograph of the wild type staining possessing the CPS (Figure 1-7A) and the *kpsM* mutant, which lacks the cell surface CPS (Figure 1-7B).

A B

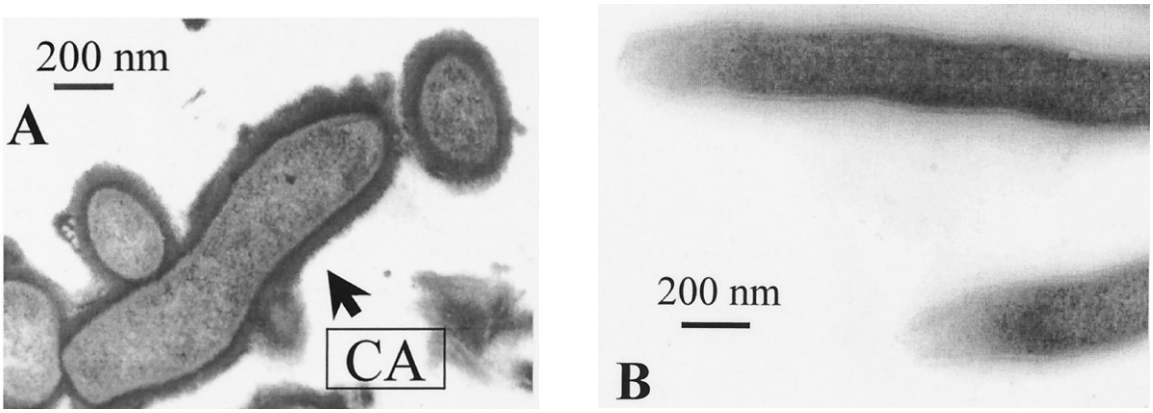

Figure 1-7: Visualization of *C. jejuni* CPS through electron microscopy after staining with Alcian blue: A) wild-type strain. B) *kpsM* mutant (that lacks cell surface CPS). CA = capsule. Reprinted with permission from: *Infect. Immun.*, *69*, 5921–5924, copyright 2001.[49]

1.2.1.3 Serotyping of *Campylobacters*

In 1980, a serological typing method of *Campylobacter* species was introduced by Penner and Hennessy, which remains the gold standard.[17] This method is based on hemagglutination using soluble antigen extracts of isolates and specific antisera raised (by vaccinated rabbits) to *Campylobacter* antigens. The antigenic materials were extracted from *Campylobacter* stains by heating a bacterial suspension in saline at 100 °C followed by centrifugation. The Penner serotyping method has been widely used in studies of *Campylobacter* outbreaks all over the world.[50] The antigens isolated were stable at 100 °C (or heat-stable, HS) and hence this

serotyping method is also known as HS serotyping. As described earlier, these antigens were mistakenly assumed to be LPSs or the *O*-antigen components of LPSs. Hence, earlier publications often incorrectly refer to them as *O*-serotypes. The CPS, not the LPS, is the major serodeterminant of Penner serotyping. In the original publication, 23 serotypes were described by Penner and Hennessy, but this number quickly increased. Now, Penner serotyping recognizes 47 *C. jejuni* serotypes. Because of similarities in CPS structure (based on cross reacting Penner antisera), these 47 serotypes can be collapsed to 35 groups.[51] Due to limitations in maintaining serum banks (especially cost), Penner serotyping has been gradually replaced by other approaches such as multiplex polymerase chain reaction (PCR) methods.[18]

1.2.1.4 *Campylobacter* capsule: Structural features and importance

Campylobacter CPS consist of regular repeating units of 1–6 monosaccharides and can extend up to several sugar residues. To date, there are more than 60 *C. jejuni* serostrains and it is predicted that each possesses its own unique CPS structure varying in monosaccharide composition and linkage. The high variation of CPS is caused by exchange of biosynthetic genes.[52] To date, only a few elucidated CPS structures from *Campylobacter* have been published and the majority of them are from *C. jejuni*.[53] Some of the elucidated structures include serotypes HS1,[54] HS2,[55,56] HS3,[57,58] HS4,[59] HS10,[53] HS13,[53] HS15,[60] HS19,[61] HS23/36,[62,63] HS30,[64] HS41,[44] and HS53[65] and these all vary in sugar composition and linkage. The CPS structures that are relevant to this thesis are shown in Figure 1-8. The repeating unit in CPS from the HS30 serotype (also known as the O:30 serotype) is a trisaccharide that consists of an unusual 6-deoxy-heptose with the D-*talo*- stereochemistry, *N*-acetyl-D-glucosamine and ribitol phosphate (Figure 1-8A). On the other hand, the repeating unit in the HS4 serotype (CG8486 strain) CPS is a disaccharide that contains *N*-acetyl-β-D-glucoamine and 6-deoxy-heptose with the D-*ido*-

stereochemistry (Figure 1-8B). Finally, the repeating unit in the HS23/36 serotype (81-176 strain) CPS is a trisaccharide containing a 6-deoxy-heptose with the D-*altro*- stereochemistry, *N*-acetyl-D-glucosamine and D-galactose. In this latter structure, three variants are found, arising from phase-variable modifications (see discussion below): the addition of a methyl phosphoramidate group and/or a methyl group (Figure 1-8C to 1-8E).

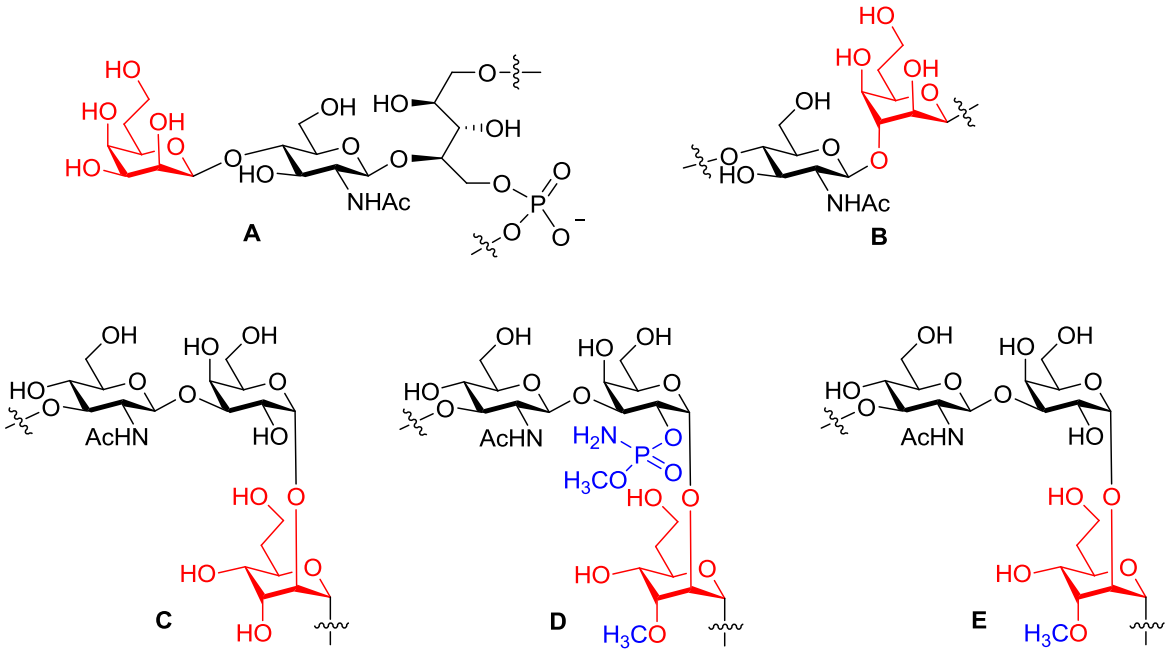

Figure 1-8: CPS structures of *Campylobacter*: A) *C. coli* HS30 (O:30). B) *C. jejuni* CG8486 (HS4). C), D) and E) *C. jejuni* 81-176 (HS23/36). 6-Deoxy-D-heptoses are shown in red and phase variable methyl and MeOPN motif are in blue.

Despite the variability of sugar composition and linkages, a key structural marker of *Campylobacter* CPS is the presence of heptose residues of unusual configurations (i.e., *altro*, *ido*, *talo*, *gulo*).[66] The structural complexity of the heptoses is further enhanced by deoxygenation at C-6 (Figure 1-8).[53] These deoxy-heptoses are found both in the D- and L-configurations.

However, this thesis includes work on only 6-deoxy-heptoses in the D-configuration. A more detailed discussion of 6-deoxy-heptoses is found in section 2.1 of Chapter 2.

Campylobacter produces variable structures as a result of genetic differences. These structural features are further broadened due to the presence of phase variation.[56,67] Phase variation results in CPS biosynthetic enzymes being turned on and off, which not only affects the expression of CPS structural modifications but also the production of the CPS itself.[68–70] This ability may be advantageous for the pathogen to express the polysaccharide only at specific times during its life cycle and supports its role in virulence. Some of the phase variable modifications observed on CPS include the presence of amines (e.g., 2-deoxy-2-amino-glycerol, ethanolamine), *O*-methylation (Figure 1-8D and Figure 1-8E), and the presence of a labile *O*-methyl phosphoramidate (MeOPN, Figure 1-8D) motif that has not been observed in glycans anywhere else in nature.[56] This structural diversity is consistent with CPS being the major serodeterminant in the Penner serotyping scheme.

Similar to the heptose residues, the MeOPN motif is a key structural feature of *Campylobacter* CPS. Although rare in nature, ~70% of *C. jejuni* strains possess this unique modification in their CPS. Its presence was serendipitously discovered by Brisson and co-workers in 2005 when examining live *Campylobacter* cells using high resolution magic angle spinning NMR spectroscopy.[56] This discovery led to investigations of a few serotypes and resulted in two new CPS structures – for *C. jejuni* HS1[52,54] and HS2.[56] The use of traditional hot water/phenol CPS extraction method for these serotypes resulted in the loss of the MeOPN motif, which indicates its labile nature. However, in contrast, Guerry and co-workers reported the presence of the MeOPN motif in wild-type strain 81-176 (HS23/36 serotype, Figure 1-8D) CPS in which the polysaccharide was extracted by the traditional hot phenol/water method.[63]

The phosphorus atom in the MeOPN substituent is stereogenic and it occurs as a single stereoisomer in the CPSs in which it is found. Recent work has established the biosynthesis of the MeOPN group: the immediate precursor is an activated cytidine diphosphate derivative and the amino group is derived from glutamine.[71–74] Despite these advances, the stereochemistry on phosphorus was unknown before the work described in this thesis. The MeOPN transferase genes are not well conserved between strains, which would be expected given that the MeOPN motif can be attached to different monosaccharide hydroxyl groups[59] or to different monosaccharide residues (e.g., galactose, fructose or glucosamine).[54,56,61]

The CPS serves as an interface between bacteria and the environment. Mutants of *C. jejuni* that do not produce CPS have reduced ability to invade host cells, to colonize chickens and to cause diarrhea disease in ferrets.[69,75,76] Strong support for the involvement of CPS in disease progression is that a CPS conjugate vaccine[35] (further described in section 1.3) protects against diarrheal disease in non-human primates. These results collectively show the importance of *Campylobacter* CPS in the pathogenesis of the organism.

1.2.1.5 Biosynthesis of 6-deoxyheptoses

Biosynthesis of 6-deoxyheptoses proceeds through guanosine diphosphate (GDP)-linked intermediates. All strains of *C. jejuni* that produce heptoses share four highly conserved enzymes that convert D-sedoheptulose 7-phosphate **1-1** to the key intermediate GDP-D-glycero-D-*manno*-heptose **1-2** (Figure 1-9). With the exception of HS2 (NCTC11168), all strains that have a heptose in their CPS produce a putative heptose-6-dehydratase enzyme (Ddmh) to generate the 6-deoxyheptose units. As shown in Figure 1-9, so far only the biosynthesis of GDP-6-deoxy-D-*manno*-heptose **1-3**[77] and GDP-6-deoxy-D-*altro*-heptose **1-4**[78] has been elucidated. Future

sequencing and CPS structural analysis will possibly reveal additional heptose isomers and the enzymes involved in their biosynthesis.

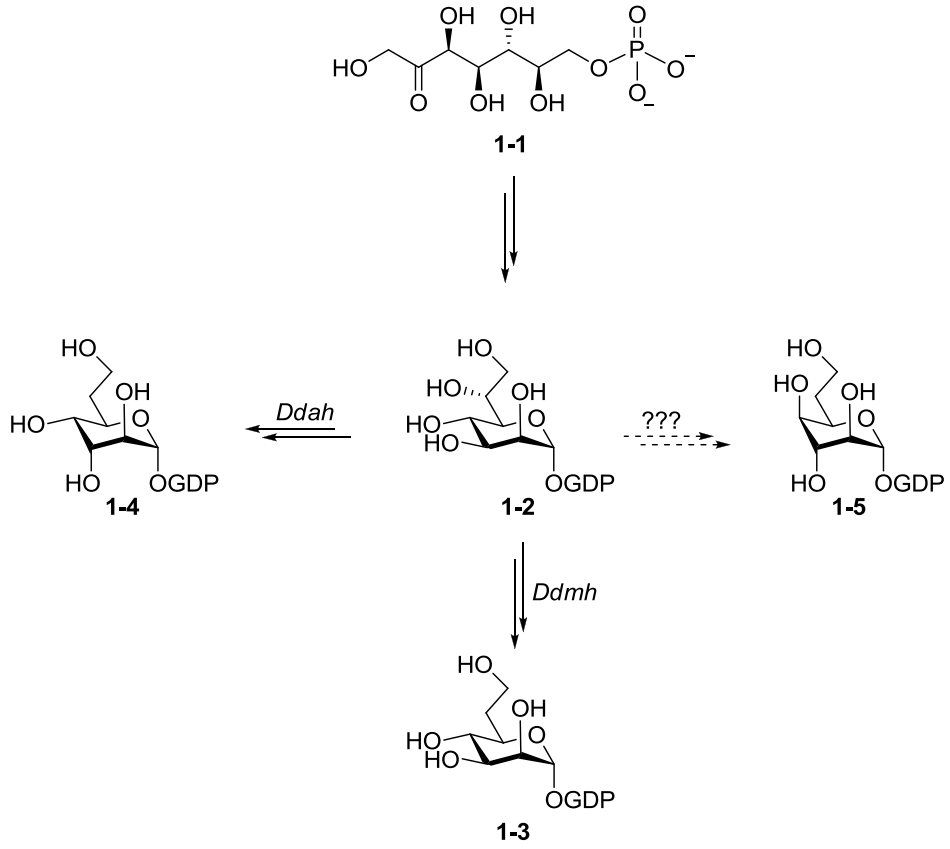

Figure 1-9: Synthesis of GDP-6-deoxy-D-*manno*-heptose **1-3** and GDP-6-deoxy-D-*altro*-heptose **1-4** in *C. jejuni* 81-176 strain (HS23/36) via GDP-D-glycero-D-*manno*-heptose **1-2**.

1.3 Glycoconjugate vaccines

Since Edward Jenner's discovery in 1796 that inoculation with cowpox protects against smallpox infection, vaccines have become hugely important in combatting infectious diseases.[79] The unique polysaccharides on the surfaces of infectious pathogens serve as an attractive target for vaccine development. However, polysaccharide-based vaccines are poorly immunogenic and have limited efficacy, due to their inability to generate immune B-cell memory.[80,81]

Polysaccharide immunogenicity can be greatly enhanced by conjugation to an immunogenic carrier protein, i.e., by forming a glycoconjugate. Unlike polysaccharide-only vaccines, glycoconjugate vaccines lead to T-cell recruitment and immune B-cell memory that is directed to carbohydrates. The result is high-affinity immunoglobulin G (IgG) antibodies that result in long-lived immune responses and protection against disease.[80,81] The success of such a vaccine against the bacterium *Haemophilus influenzae* type b was a key breakthrough that showed the potential of glyconjugate vacccines.[81] Several glycoconjugate versions of polysaccharide vaccines are in clinical use or in development.[79]

1.3.1 *Campylobacter jejuni* glycoconjugate vaccines

A significant problem to vaccine development using live or attenuated *C. jejuni* strains is the association of these infections with GBS. As described previously, LOS core structures of most of *Campylobacter* strains contain sialic acid with molecular similarity to human gangliosides. This is thought to be responsible for the GBS association with *C. jejuni* as it leads to an autoimmune response from the host and that can eventually result in paralysis. Although humans have been vaccinated with *C. jejuni* strain 81-176,[24,82] which contains the LOS that mimics human gangliosides,[35,83] these studies occurred before the association of *C. jejuni* with GBS was known. Therefore, the use of any whole cell vaccine approach to prevent *Campylobacter* infections, pose safety concerns. However, given the success of CPS-based conjugate vaccines approach against other bacteria[79–81] (e.g., *Streptococcus pneumoniae*, *Neisseria meningiditis*, and *Hemophilus influenzae*), a similar approach was evaluated for *C. jejuni* by Guerry and co-workers.[35]

1.3.1.1 *Campylobacter jejuni* CPS glycoconjugate vaccines

As discussed previously, *C. jejuni* CPSs have unique structures that could be used to develop anti-*C. jejuni* vaccines. Importantly, there has been no ganglioside mimicry associated with any *C. jejuni* CPS and thus no association with GBS would be expected.[68,83] In 2009, Guerry and co-workers reported two prototype *C. jejuni* vaccines based on the well characterized strains 81-176 (HS23/36 serotype) and CG8486 (HS4 serotype) CPS.[35] In their initial studies, the CPS was purified and conjugated to the carrier protein, CRM_{197} using reductive amination (Figure 1-10). The CRM_{197} protein is a non-toxic diphtheria toxin mutant that has been safe and effective in numerous clinical trials. CRM_{197} also has the advantage of commercial availability and contains a large number of lysine groups that can be used for conjugation. As shown in Figure 1-10A, CPS **1-6** isolated from 81-176 strain, was subjected to periodate oxidation to generate aldehyde functionalities (to form **1-7**), which served as an attachment point to CRM_{197} via reductive amination. This resulted in $CPS_{81\text{-}176}$-CRM_{197} conjugate **1-8**; mass spectrometry data suggested that the $CPS_{81\text{-}176}$-CRM_{197} conjugate contained 2–5 CPSs per CRM_{197}. A similar strategy on the CPS isolated from CG8486 strain **1-10** furnished the CPS_{8486}-CRM_{197} conjugate **1-11** (Figure 1-10B) with a similar loading of glycan on the protein.

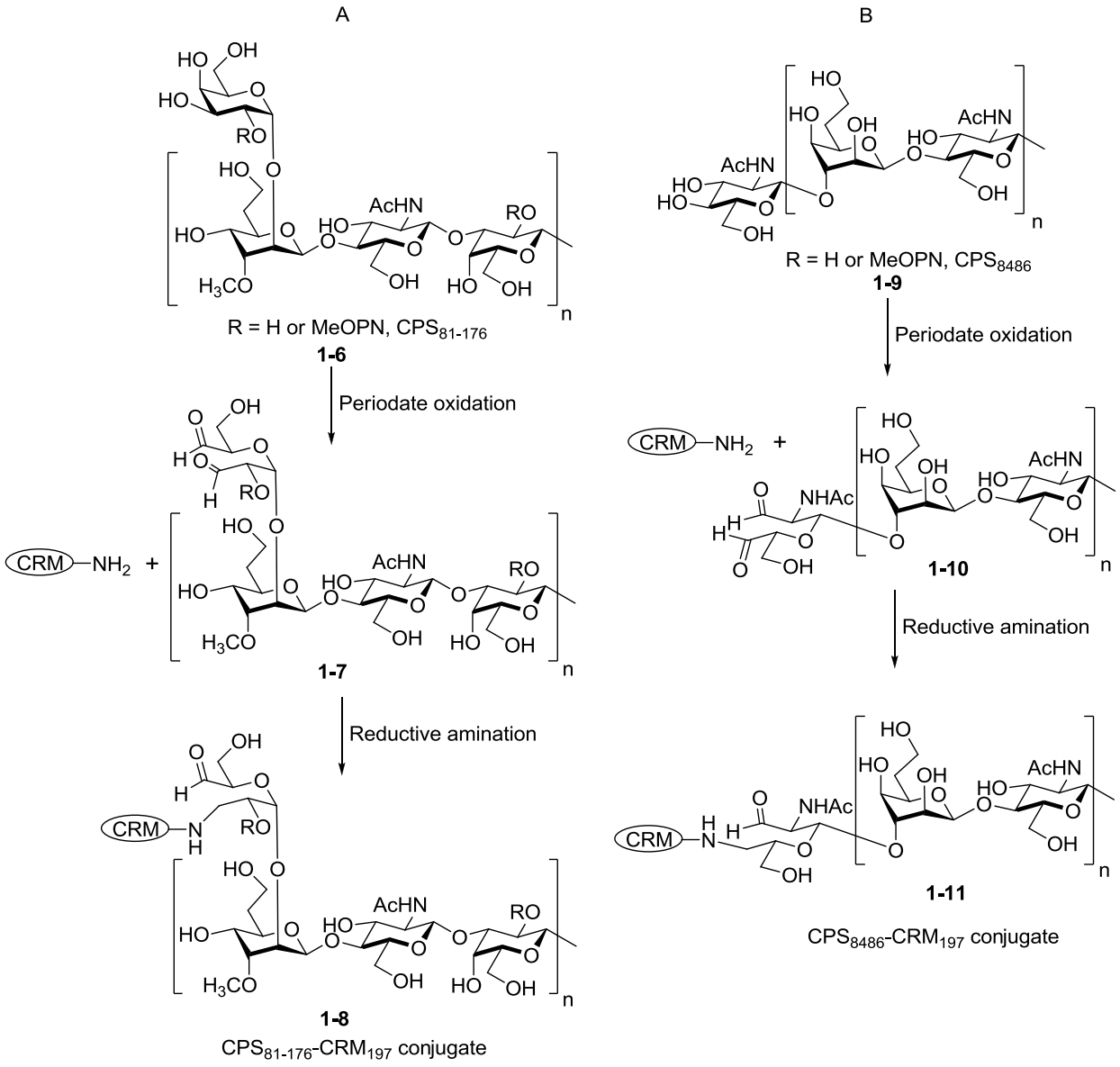

Figure 1-10: Chemical structures and reductive amination conjugation strategy used in the synthesis of $CPS_{81\text{-}176}$-CRM_{197} glycoconjugate **1-8** and CPS_{8486}-CRM_{197} glycoconjugate **1-11** reported by Guerry and co-workers.

Glycoconjugates **1-8** and **1-11** were tested for their immunogenicity in mice by vaccination with different amounts (1, 5 or 25 μg) of conjugate. Three separate studies were done where mice were immunized at 2-, 4- or 6- week intervals. As an example, results from study

where the mice were vaccinated every four weeks are shown in Figure 1-11. Regardless of the dose (with the exception of 1 μg dose of the CPS_{8486}-CRM_{197} glycoconjugate, Figure 1-11B), all immunized mice showed significant anti-CPS IgG responses. In addition, the response persisted up to 26 weeks after the final immunization (Figure 1-11A).

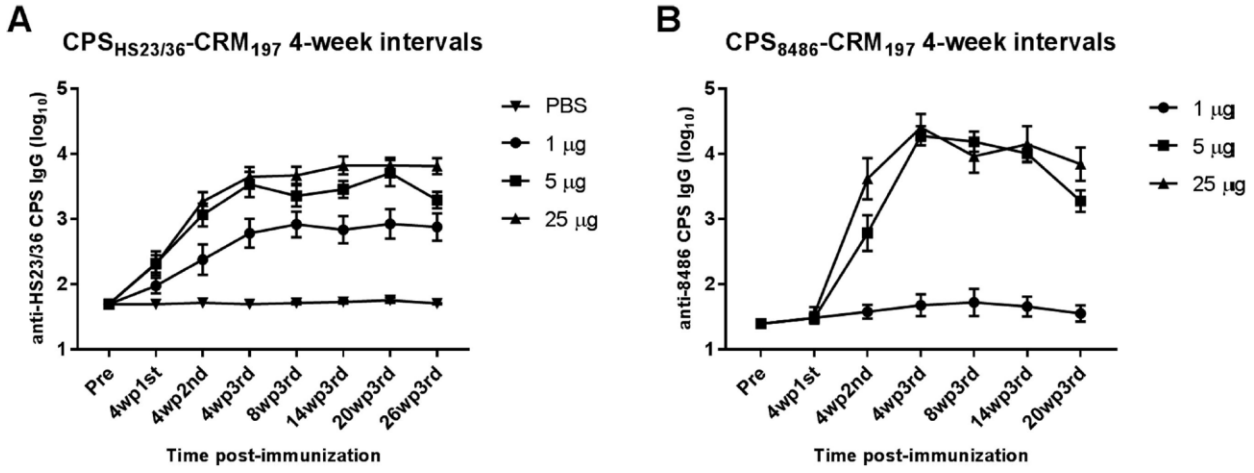

Figure 1-11: A. Immunogenicity of $CPS_{81\text{-}176}$-CRM_{197} glycoconjugate **1-8** B. Immunogenicity of CPS_{8486}-CRM_{197} glycoconjugate **1-11**. PBS, phosphate buffered saline; Pre, pre immunization; 4wp1st, four weeks after first dose. Reprinted with permission from: *ACS Symp. Ser.*, *1290*, 249–271, copyright 2018.

After obtaining a successful immune response in mice, Guerry and co-workers tested the ability of **1-8** and **1-11** to prevent the diarrhea in the New World monkey (*Aotus nancymaae*) as it exhibits similar symptoms of diarrhea as observed in humans.[83] First, they immunized the monkeys every six weeks with 1, 5 and 25 μg of the $CPS_{81\text{-}176}$-CRM_{197} conjugate and observed the induction of IgG antibodies. After nine weeks following the last immunization, all monkeys were treated with *C. jejuni* 81-176 strain. The vaccine showed 100% efficacy in protection.

Following these two prototype conjugate vaccines, Monteiro and co-workers prepared another two vaccine candidates, for the HS15[60] and HS53 serotypes.[68] The CPS_{HS15}-CRM_{197} conjugate was immunogenic in mice whereas the exploration of the CPS_{HS53}-CRM_{197} conjugate is currently underway. Of all the glycoconjugates described, the $CPS_{81\text{-}176}$-CRM_{197} conjugate is the most studied and it has been advanced to a Phase I human clinical trial.[68]

Because of the large variety of serotypes (47 based on the Penner serodeterminant CPS), a practical vaccine must contain CPS from any different strains; however, the number required is not known. There are ~10 serotypes that are frequently associated with human disease with HS1, HS2 and HS4 being the most prevalent around the globe.[68,83] Thus, an ideal CPS conjugate vaccine would have ten of these structures.

1.4 Research overview

One of the main characteristics of *C. jejuni* CPS is the presence of 6-deoxy-heptoses and numerous structural variations arising from different sugar sequences and also phase-variable modifications such as the attachment of *O*-methyl, *N*-ethanolamine, and *O*-methyl phosphoramidate (MeOPN) groups. Although the CPS conjugate vaccine against *C. jejuni* 81-176 has 100% efficacy against diarrheal disease in New World monkeys, a drawback to this vaccine is that it protects only against the 81-176 strain. The addition of serotypes from additional strains is necessary for better protection. However, the isolation of CPS in pure form from bacteria is difficult and the heterogeneity in the carbohydrate is not ideal for use in vaccines. Therefore, an alternate approach to access this material – synthetic chemistry – is necessary. Chemically synthesized CPSs will be well-defined and homogeneous, which can be used as antigens for vaccine development.

With respect to this thesis, we focused on the chemical synthesis of some CPS fragments that contain 6-deoxy-heptoses in the D-configuration and in the pyranose form. The first part of this thesis will focus on the synthesis of thioglycoside donors of 6-deoxy-D-heptopyranosides. The second part of the thesis will focus on assembling the monosaccharide building blocks, including the 6-deoxy-D-heptopyranoside thioglycosides, into targeted CPS fragments. We focused on synthesizing these CPS fragments with a suitable linker to allow for further conjugation to protein.

1.4.1 Research objective 1: Synthesis of 6-deoxy-D-heptopyranoside thioglycosides

To synthesize CPS structures that contain rare 6-deoxy-D-heptoses, the preparation of appropriate glycosyl donors is essential. Most 6-deoxyheptose residues are not readily available from natural sources; therefore, they must be obtained through chemical synthesis. Prior to starting this work, many groups had developed methods to obtain the 6-deoxy-D-heptoses either starting from hexose or pentose derivatives with the majority starting from hexoses. The C-6 homologation was achieved by Wittig reaction, nucleophilic addition or nucleophilic substitution. However, most of the 6-deoxy heptoses were synthesized as methyl glycosides, confined to a single natural 6-deoxy-D-heptose and involved permanent protecting groups at C-2, C-3 and C-4. This limits their use in the synthesis of CPSs. Moreover, the homologation step often employs toxic reagents such as potassium cyanide (KCN) and mercury salts. To address these issues, we planned to develop a method for the synthesis of as many 6-deoxy-D-heptoses as possible from the commercially available methyl α-D-mannopyranoside **1-12**. The preparation of different 6-deoxy-D-heptoses as thioglycoside donors would enable the synthesis of the desired CPS structures. Research objective 1 is outlined in Figure 1-12.

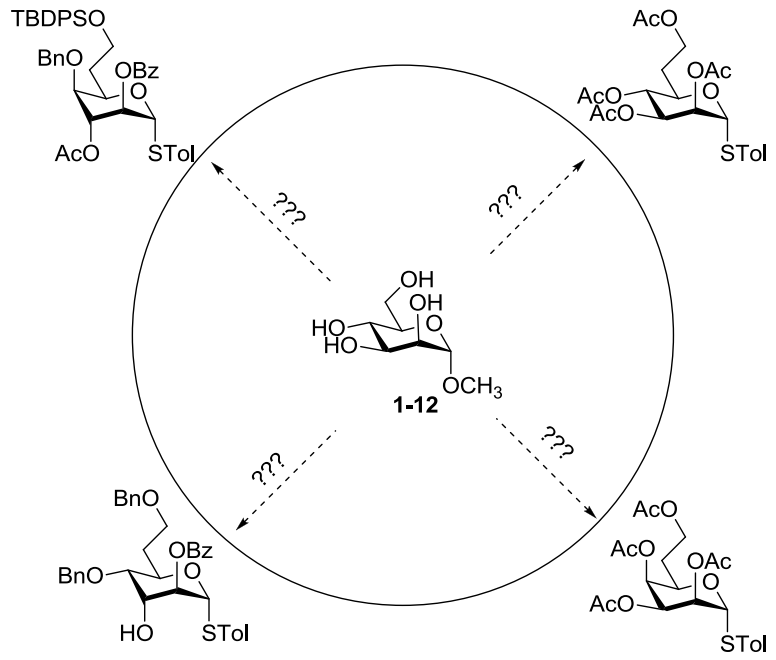

Figure 1-12: Research objective 1: Develop a method for rapid access of the 6-deoxy-D-heptopyranoside thioglycosides from commercially available methyl α-D-mannopyranoside **1-12**.

1.4.2 Research objective 2: Synthesis of the 6-deoxy-D-heptopyranoside containing CPS fragments

We chose repeating units from three different *Campylobacter* CPS serotypes that are structurally complex and have challenging sugar linkages (e.g., 1,2-*cis*-β/1,2-*cis*-α type). Those chosen are: 1) *C. coli* HS30 (O:30 serotype, Figure 1-13A); 2) *C. jejuni* HS23/36 complex (81-176 strain, Figure 1-13B); and 3) *C. jejuni* HS4 (CG8486 strain, Figure 1-13C).

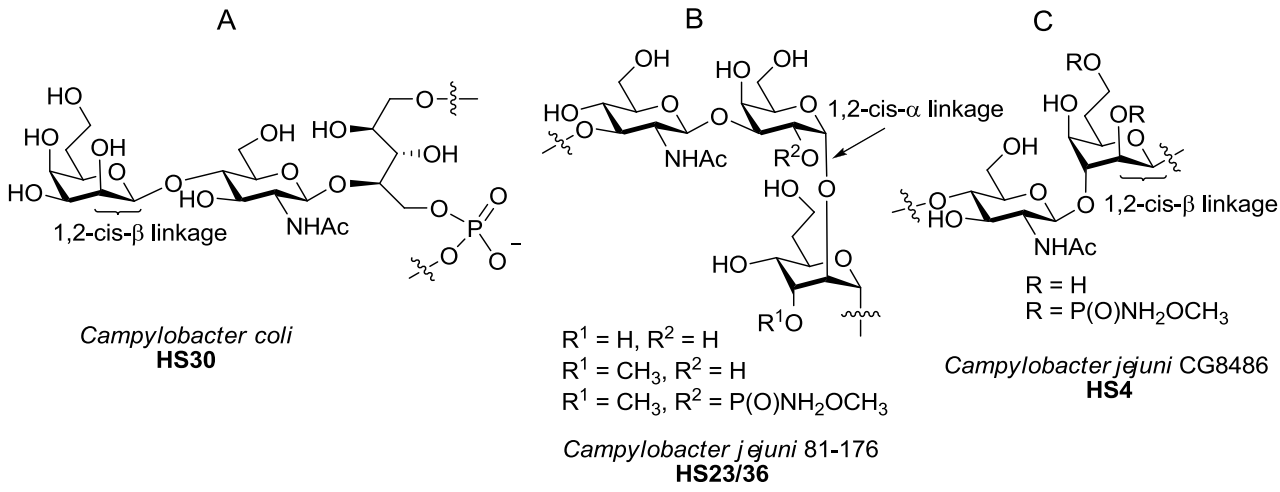

Figure 1-13: Research objective 2:. Develop a strategy to make CPS repeating unit fragments from *C. jejuni* serotypes HS30 (A), HS23/36 (B) and HS4 (C).

In Chapter 2, our strategy to make the trisaccharide repeating unit from *C. coli* HS30 serotype will be described. Chapter 3, describes the synthesis of the three trisaccharide repeating units from *C. jejuni* HS23/36 serotype (81-176 strain). Perhaps, the most interesting target of the three molecules is the one that contains both methyl and MeOPN phase variable groups. Our group was the first to report a method for introducing MeOPN groups onto the carbohydrates. However, prior to my PhD studies, no chemically synthesized *Campylobacter* CPS fragments that possess a MeOPN motif had been reported. In addition, the stereochemistry on the phosphorous atom in this strain remained unknown. Finally, in Chapter 4, the synthesis of the two disaccharide repeating units from *C. jejuni* HS4 serotype (CG8486 strain) is described; one of the structures contains two MeOPN motifs.

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Chapter 2

Synthesis of *p*-tolyl 6-deoxy-D-heptopyranose thioglycosides *en route* to the total synthesis of the trisaccharide repeating unit of the *Campylobacter coli* serotype O:30 capsular polysaccharide

2.1 Background

Campylobacter species produce specific capsular polysaccharides (CPSs) on their surfaces. The structure of these CPSs vary from strain to strain resulting from the exchange of biosynthetic genes.[1] Heptoses are one of the most important constituents of cell wall anchored CPSs, lipopolysaccharides (LPSs) and lipooligosaccharides (LOSs). These heptoses are rare higher-carbon sugars that occur in numerous structural variations. To date, there are five heptoses and six monodeoxy-heptose residues found in bacterial polysaccharides.[2,3] Of the six possible monodeoxy-heptoses, only 6-deoxy-heptose sugars (Figure 2-1) are present in nature and they are found in polysaccharides of Gram-negative bacteria e.g., *Campylobacter*, *Yersinia*, *Burkholderia*, *Eubacterium*, *Plesiomonas* and *Escherichia* species.

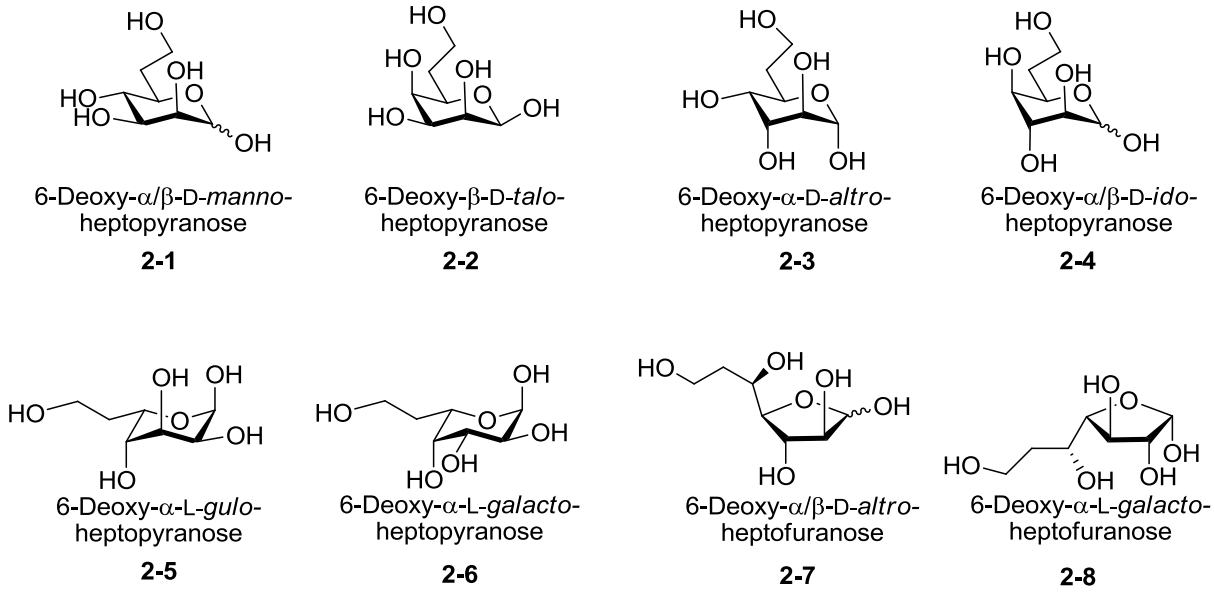


Figure 2-1: 6-Deoxyheptose residues isolated from pathogenic microorganisms.

To date, the pyranose form of only six out of 16 possible stereoisomers (excluding the differences in anomeric configuration) of 6-deoxy-heptoses have been reported. This includes 6-

deoxy-heptoses in the D-configuration (*manno*-, *talo*-, *altro*- and *ido*-, **2-1**–**2-4**) and 6-deoxy heptoses in the L-configuration (*gulo*- and *galacto*-, **2-5** and **2-6**). The 6-deoxy-D-*altro*- and L-*galacto*-heptoses are also found in the furanose form (**2-7** and **2-8**). These heptoses occur as sugar units within the polysaccharide chain as well as branches along the chain.[3] The majority of the CPSs isolated from *Campylobacter* contain at least one 6-deoxy-heptose. In this thesis, we are particularly interested in CPSs containing 6-deoxy-D-heptoses in the pyranose form (**2-9**–**2-11**, Figure 2-2).

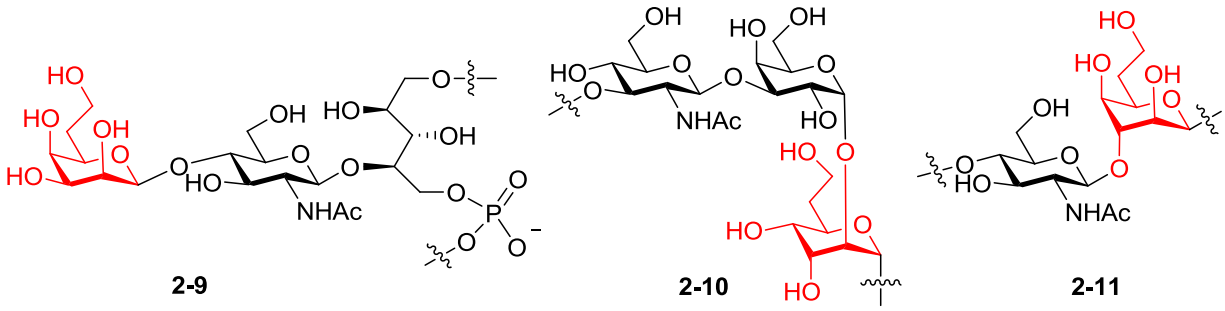


Figure 2-2: Repeating units found in selected CPSs of *Campylobacter* species. 6-Deoxy-heptose residues are highlighted in red.

Besides structural variations of 6-deoxy-heptoses, CPSs contain phase variable modifications[4,5] such as the addition of *O*-methyl,[6] *N*-ethanolamine,[7] and *O*-methyl phosphoramidate groups.[3,6] Due to these phase variable modifications, isolated CPSs are heterogeneous. Therefore, chemical synthesis is required for the production of homogeneous and well-defined CPS structures if they are to be used as antigens for vaccine development. However, these applications require straightforward access to such compounds. This, in turn, requires routes to 6-deoxy-heptose glycosyl donors that can be used to assemble fragments of the

natural CPS. Unfortunately, no 6-deoxy-heptoses are available from natural sources in multi-milligram quantities. Therefore, chemical synthesis of 6-deoxy-heptose donors is pivotal.

This chapter contains two parts. The first part describes two approaches for the synthesis of 6-deoxy-heptoses as *p*-tolyl thioglycoside donors. In the second part, the first total synthesis of the trisaccharide repeating unit of the *Campylobacter coli* serotype O:30 (also known as HS30) CPS[8] is described.

2.2 Reported approaches to 6-deoxy-D-heptose residues

Most of the literature methods to synthesize 6-deoxy-D-heptoses involve either hexose or pentose derivatives. Starting from the first chemical synthesis of 6-deoxy-α-D-*manno*-heptose reported by Garegg and coworkers[9a] many research groups have developed different methodologies to access 6-deoxy-D-heptoses. These approaches are summarized in Figure 2-3. A key feature is carbon chain homologation of the pentose or hexose by Wittig reaction[9] or nucleophilic addition[10] onto a C-6 aldehyde, or nucleophilic substitution at C-6.[11]

Homologation via C-6 of a hexose derived aldehyde (e.g., **2-12a**) can be achieved by Wittig olefination to generate alkene **2-14** or vinyl ether **2-15**. Alkene **2-14** can be converted to 6-deoxy-heptose **2-16**, either by hydroboration followed by oxidation, or dihydroxylation followed by C-6 deoxygenation.[9b,9c] On the other hand, when vinyl ether **2-15** is subjected to hydrolysis followed by reduction,[9a] 6-deoxy-heptose **2-16** is produced. Alternatively, nucleophilic addition of a lithiated acetyliron complex, or propargyl bromide and Zn, onto the furanose derived aldehyde **2-13** can lead to iron complex **2-17** or homo propargylic alcohol **2-18**, respectively. Iron complex **2-17**, upon decomplexation followed by reduction,[10a] can lead to 6-deoxy heptofuranose **2-19**. Propargylic alcohol **2-18**, when subjected to ozonolysis followed by

reduction,[10b] can generate the 6-deoxy-heptofuranose **2-19**. Finally, nucleophilic substitution of either iodide **2-12b** or 6-*O*-trifluoromethanesulfonate **2-12c** by cyanide ion can generate nitrile **2-20**, which, when reduced to the imine followed by hydrolysis and subsequent reduction,[11a-11d,11g] can furnish 6-deoxy-heptose **2-16**. Chain extension via C-6 can also be achieved by nucleophilic substitution of 6-*O*-trifluoromethanesulfonate **2-12c** by a lithiated dithioacetal to give dithioacetal **2-21**, which, when hydrolyzed and reduced, produces **2-16**.

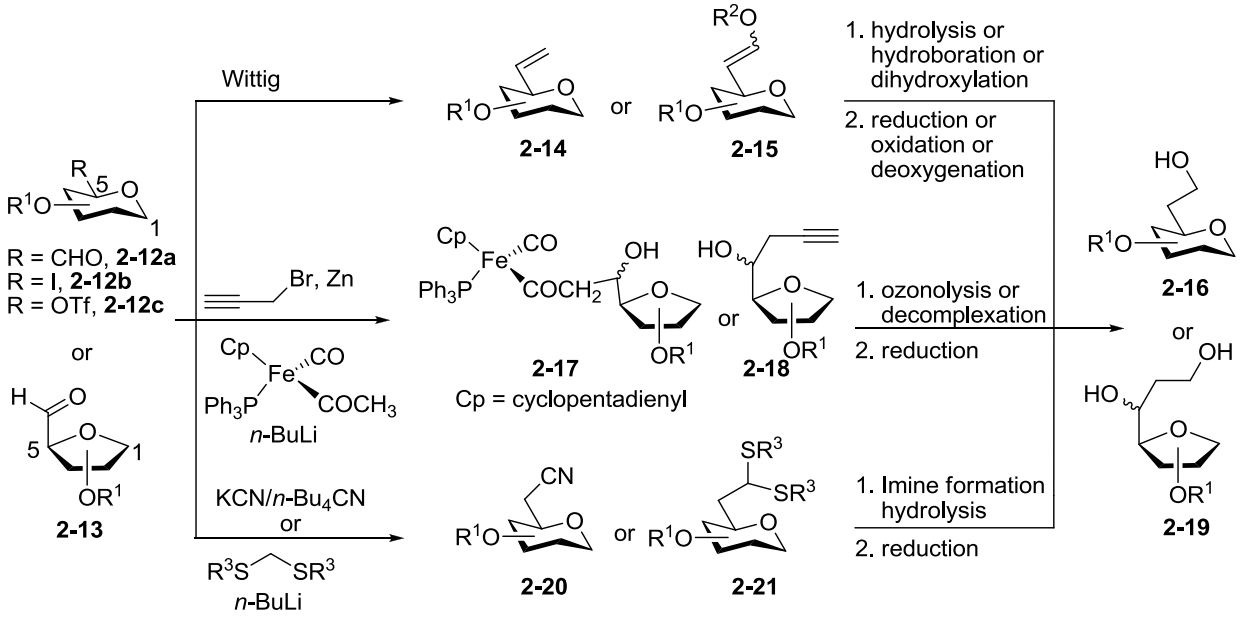
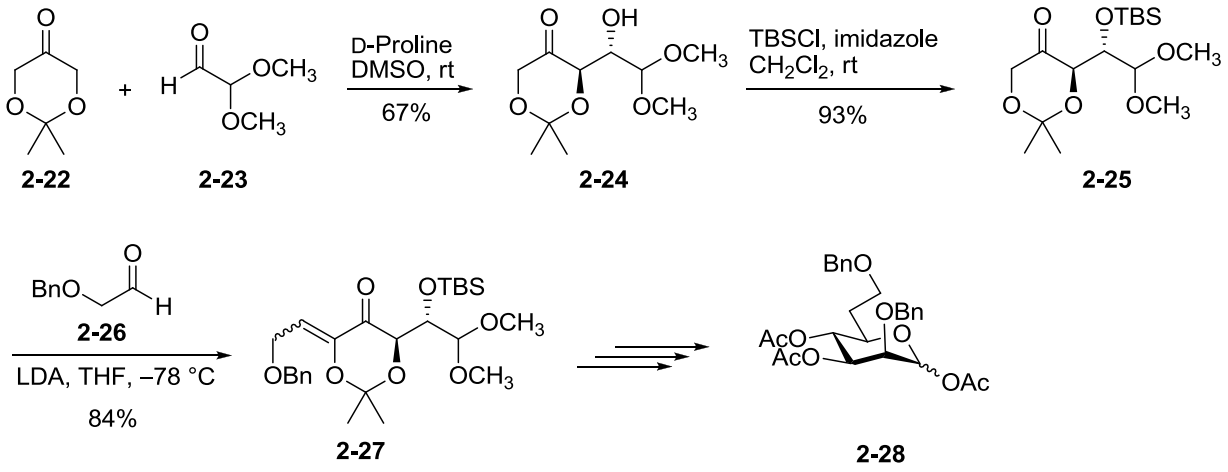


Figure 2-3: Various approaches to access 6-deoxy-D-heptose building blocks.

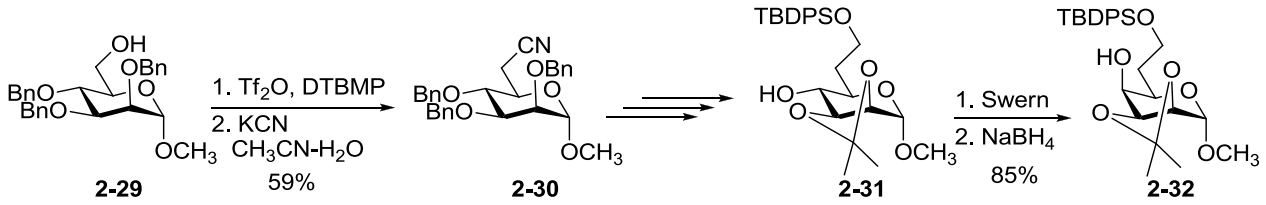
De novo approaches for 6-deoxy-D-heptose synthesis were not reported until recently. In 2019, Yang and co-workers[12] reported the *de novo* synthesis of a 6-deoxy-D-*manno*-heptose building block using D-proline catalysis and lithium diisopropylamide (LDA)-promoted aldol reactions as key steps (Scheme 2-1). D-Proline-catalyzed aldol reaction of ketone **2-22** with 2,2-dimethoxyacetaldehyde **2-23** in dimethyl sulfoxide (DMSO) afforded 2,3-*anti*-β-hydroxy-ketone **2-24** in 67% yield. After *tert*-butyldimethylsilyl (TBS) protection, a second LDA-promoted aldol

reaction of ketone **2-25** with benzyloxyacetaldehyde **2-26** in THF at –78 °C furnished homologated heptose **2-27** in the open chain form. Protecting group manipulations of ketone **2-27** furnished 6-deoxy-D-*manno*-heptose **2-28**. It is interesting to note that the LDA-promoted aldol reaction furnished an α,β-unsaturated ketone **2-27** as a single isomer instead of the expected β-hydroxy ketone. Because of the complex trisubstituted alkene system, the authors could not assign the stereochemistry of the newly formed double bond by NMR spectroscopy.



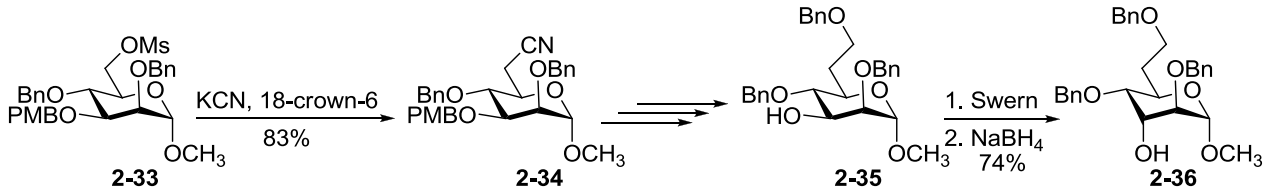
Scheme 2-1: Key steps involved in the *de novo* synthesis of 6-deoxy-D-*manno*-heptose **2-28** reported by Yang and co-workers.[12]

Over the past four and a half decades, only a very few research groups have reported the synthesis of more than one 6-deoxy-D-heptose from a single commercially available starting material. For example, Aspinall and co-workers[11b] reported the synthesis of methyl glycosides of both 6-deoxy-D-*manno*-heptose **2-31** and 6-deoxy-D-*talo*-heptose **2-32** (Scheme 2-2) from 2,3,4-tri-*O*-benzyl-α-D-mannopyranoside (**2-29**). Homologation was achieved by nucleophilic displacement of the 6-*O*-triflate derivative of **2-29** with KCN. Swern oxidation of alcohol **2-31**, followed by reduction using sodium borohydride ($NaBH_4$) furnished the *talo*- isomer **2-32**.



Scheme 2-2: Synthesis of methyl glycosides of 6-deoxy-D-*manno*- and 6-deoxy-D-*talo*-heptoses **2-31** and **2-32**, respectively, by Aspinall and co-workers.[11b]

In another example, Nam shin and co-workers[11c] reported the synthesis of methyl glycosides of 6-deoxy-D-*manno*-heptose **2-35** and 6-deoxy-D-*altro*-heptose **2-36** (Scheme 2-3) both from methyl glycoside **2-33**. Chain elongation was achieved by nucleophilic displacement of the sulfonate ester in **2-33** with KCN. Swern oxidation of alcohol **2-35** followed by reduction with $NaBH_4$ afforded *altro* isomer **2-36**.



Scheme 2-3: Synthesis of methyl glycosides of 6-deoxy-D-*manno*- and 6-deoxy-D-*altro*-heptoses **2-35** and **2-36,** respectively, by Nam shin and co-workers.[11c]

Despite these examples, the majority of the routes to 6-deoxy-D-heptoses lead to methyl glycosides,[5] and provide a single isomer with permanent (e.g., benzyl ether) protecting groups at the C-2, C-3 and C-4 positions.[9d,11g,13] These features complicate their use in the synthesis of CPSs fragments. Additionally, the homologation step often employs toxic reagents such as KCN

and mercury salts.[5,11a-11d,11g] To address some of these issues, we chose to explore the synthesis of as many 6-deoxy-D-heptoses as possible from single commercially-available monosaccharide.

2.3 Approach towards the synthesis of 6-deoxy-D-heptose residues

The synthesis of different 6-deoxy-D-heptoses as thioglycosides would enable more straightforward synthesis of many CPS-related oligosaccharides. This motivated us to develop a route to these compounds and we explored Wittig olefination and nucleophilic substitution at the C-6 position of hexoses as the key steps for chain homologation.

2.3.1 Attempted synthesis of 6-deoxy-D-heptoses by homologation of hexoses using Wittig olefination

As shown in Figure 2-4, the *talo*- and *altro*-heptoses (**2-41** and **2-42**, respectively) are the C-4 and C-3 epimers of *manno*-heptose **2-40**; the *ido*-heptose **2-43** is a diastereomer of *manno*-heptose with inversion of configuration at both C-3 and C-4. A detailed retrosynthetic analysis to these heptoses is shown in Figure 2-4. We envisioned that commercially available methyl α-D-mannopyranoside (**2-37**) would be an ideal starting material to access *manno*-, *talo*-, *altro*- and *ido*-D-heptopyranosides, via carbon chain elongation at C-6 by Wittig olefination. Regioselective protecting group manipulations of **2-37** would generate alcohol **2-38** and diol **2-39**. The preparation of 6-deoxy-1-thio-α-D-*manno*-heptopyranoside derviative **2-40** could be achieved by C-6 oxidation of **2-38** followed by Wittig olefination and functional group interconversion (FGI). Similarly, 6-deoxy-1-thio-α-D-*talo*-heptopyranoside **2-41** could be obtained by C-6 oxidation of **2-39** followed by Wittig olefination, FGI and C-4 inversion. Alternatively, oxidation of **2-38** followed by Wittig olefination, FGI and C-3 inversion would give access to 6-deoxy-1-thio-α-D-

altro-heptopyranoside **2-42**. Lastly, oxidation of **2-39** followed by Wittig olefination, FGI and double inversion at C-3 and C-4 would generate 6-deoxy-1-thio-α-D-*ido*-heptopyranoside **2-43**.

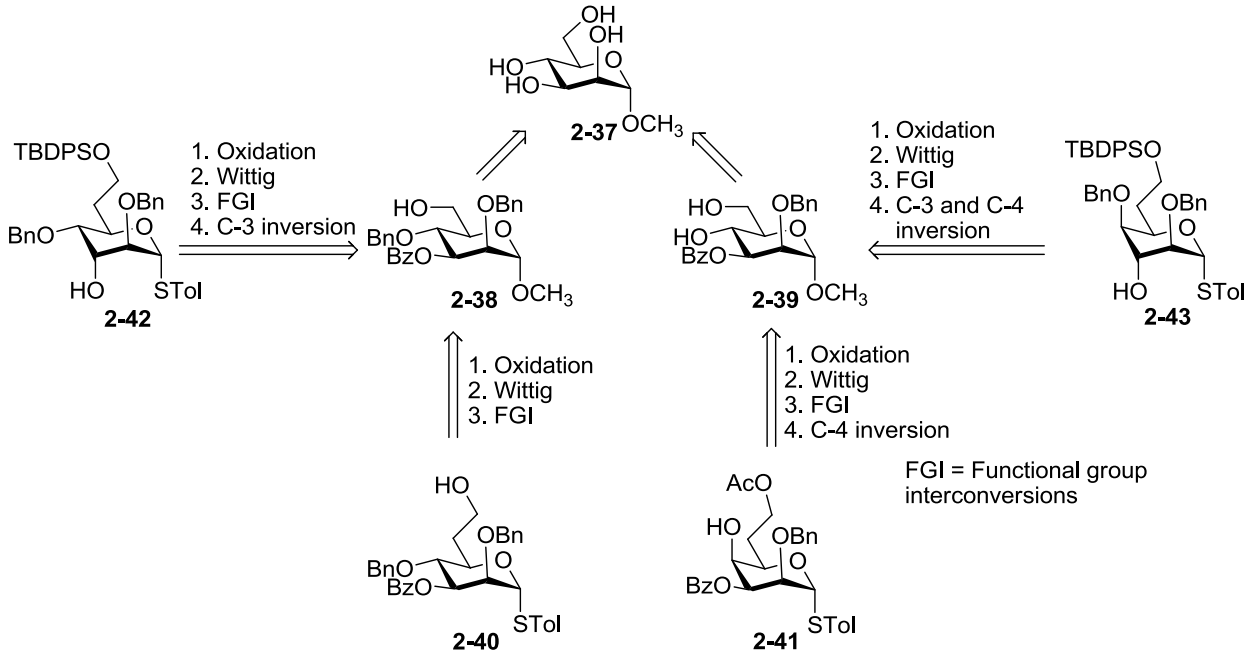
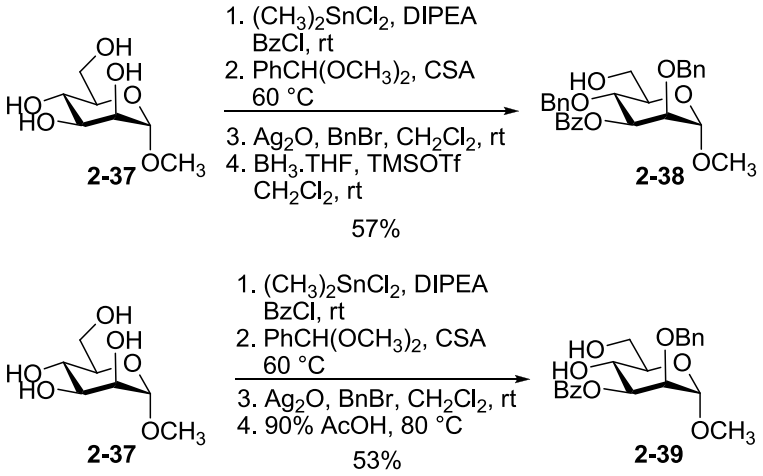


Figure 2-4: Retrosynthetic analysis to access 6-deoxy-D-heptoses via C-6 homologation of methyl α-D-mannopyranosides (**2-37**) using Wittig olefination.

2.3.1.1 Synthesis of alcohol 2-38 and diol 2-39

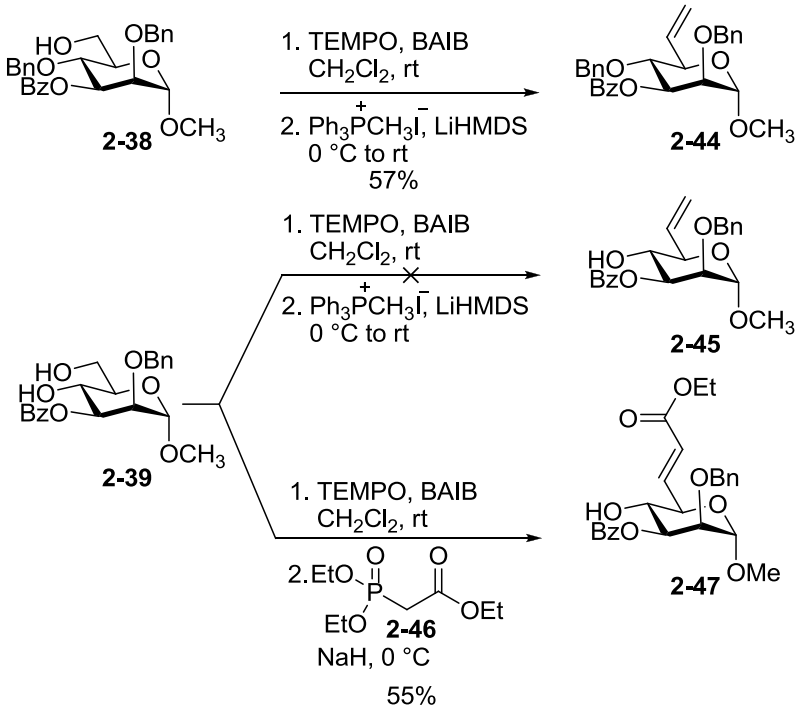
With this plan in mind, the syntheses of alcohol **2-38** and diol **2-39** was carried out (Scheme 2-4). Regioselective benzoylation[14] of **2-37** using dimethyltin dichloride and benzoyl chloride followed by benzylidene formation, benzylation using silver oxide and benzyl bromide (BnBr) and regioselective reductive ring opening using borane tetrahydrofuran complex[15] afforded alcohol **2-38** in 57% yield over the four steps. Similarly, regioselective benzoylation of **2-37**[14] followed by benzylidene formation, benzylation and hydrolysis of the benzylidene acetal using 90% acetic acid furnished diol **2-38** in 53% yield over the four steps.



Scheme 2-4: Synthesis of alcohol **2-38** and diol **2-39** from methyl α-D-mannopyranoside (**2-37**).

2.3.1.2 Attempted Wittig homologation using methylenetriphenylphosphorane

The attempted Wittig homologation at C-6 of alcohol **2-38** and diol **2-39** using methylenetriphenylphosphorane is illustrated in Scheme 2-5. Alcohol **2-38** was oxidized using (2,2,6,6-tetramethylpiperidin-1-yl)oxyl (TEMPO) and bis(acetoxy)iodobenzene (BAIB).[16] The crude aldehyde obtained was subjected to Wittig olefination using methylenetriphenylphosphorane, generated by treating methyltriphenylphosphonium iodide with lithium bis(trimethylsilyl)amide.[17] This sequence afforded alkene **2-44** in 57% yield over the two steps. Diol **2-39** was then subjected to selective oxidation using TEMPO and BAIB.[16] The crude aldehyde obtained was carried forward using the same Wittig olefination conditions but we could not isolate the desired alkene **2-45**. To test for the existence of an aldehyde in the selective oxidation of **2-39**, we subjected the crude aldehyde to a Horner–Wadsworth–Emmons reaction using triethyl phosphonoacetate **2-46**. Gratifyingly, we could isolate unsaturated ester **2-47** in 55% yield indicating that the aldehyde is formed. We then moved on to different Wittig olefination conditions.

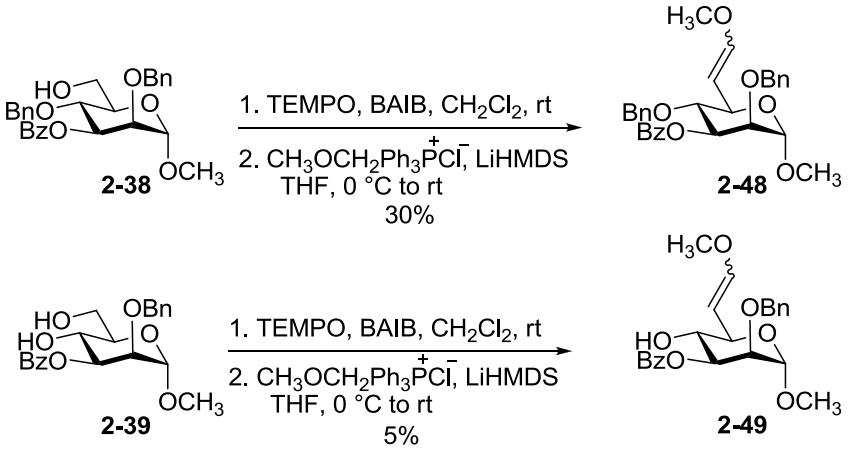


Scheme 2-5: C-6 homologation of alcohol **2-38** and attempted C-6 homologation of diol **2-39**.

2.3.1.3 Attempted Wittig homologation using methoxymethylenetriphenylphosphorane

We next attempted the Wittig homologation of **2-38** and **2-39** using methoxymethylenetriphenylphosphorane (Scheme 2-6). Alcohol **2-38** was oxidized using TEMPO and BAIB[16] and the crude aldehyde was treated with methoxymethylenetriphenyl-phosphorane to afford alkene **2-48** in a modest 30% yield over the two steps. When subjected to selective oxidation using TEMPO and BAIB followed by Wittig olefination under the same conditions, diol **2-39** furnished alkene **2-49** in very low yield (5%) over the two steps. Our attempted Wittig olefination reactions (Scheme 2-5 and Scheme 2-6) suggest that the 4-hydroxyl group in **2-39** might be interfering with the homologation reaction. However, even when this hydroxyl group is protected (**2-38**), only modest yields of the desired alkene products (**2-44** and **2-48**) were isolated. Low yields of Wittig olefinations have been reported earlier in the synthesis

of 6-deoxy-D-heptoses.[9a] Due to the practical inapplicability of Wittig olefination to access our target molecules, we abandoned this route.



Scheme 2-6: Homologation of **2-38** and **2-39** with methoxymethylenetriphenylphosphorane.

2.3.2 Synthesis of 6-deoxy-D-heptoses by homologation using dithioacetals

As an alternate approach to extend the chain length, we explored nucleophilic substitution at C-6 of hexoses using dithioacetals. Lithiated 1,3-dithiane, bis(methylthio)methane and bis(phenylthio)methane[18] have been used as nucleophiles previously in the synthesis of 6-deoxy-heptoses through the opening of epoxides[19] and cyclic sulfates,[20] and via the displacement of sulfonate esters.[11f,21] The synthesis of the appropriate precursors, however, requires a significant number of steps. To our surprise, nucleophilic displacement of halogens using dithioacetals has never been explored in the synthesis of 6-deoxy-D-heptoses. Therefore, similar to the earlier route, we envisioned that commercially available methyl α-D-mannopyranoside (**2-37**) would be ideal to access the targets. The retrosynthetic plan to explore this approach is shown in Figure 2-5. The preparation of methyl 6-deoxy-6-iodo-2,3-*O*-isopropylidene-α-D-mannopyranoside **2-50**,[22] which can be obtained from **2-37** in two steps, significantly reduces the

number of steps (compared to literature methods[5]). Nucleophilic substitution of 6-iodo compound **2-50** by a lithiated dithioacetal would generate **2-51**; the 6-deoxy-1-thio-α-D-*manno*-heptopyranoside **2-52** can be obtained by hydrolysis followed by FGI. Conversion of **2-51** into 6-deoxy-1-thio-α-D-*talo*-heptopyranoside **2-53**, 6-deoxy-1-thio-α-D-*altro*-heptopyranoside **2-54** and 6-deoxy-1-thio-α-D-*ido*-heptopyranoside **2-55** could be achieved using tranformations similar to those described in Section 2.3.1.

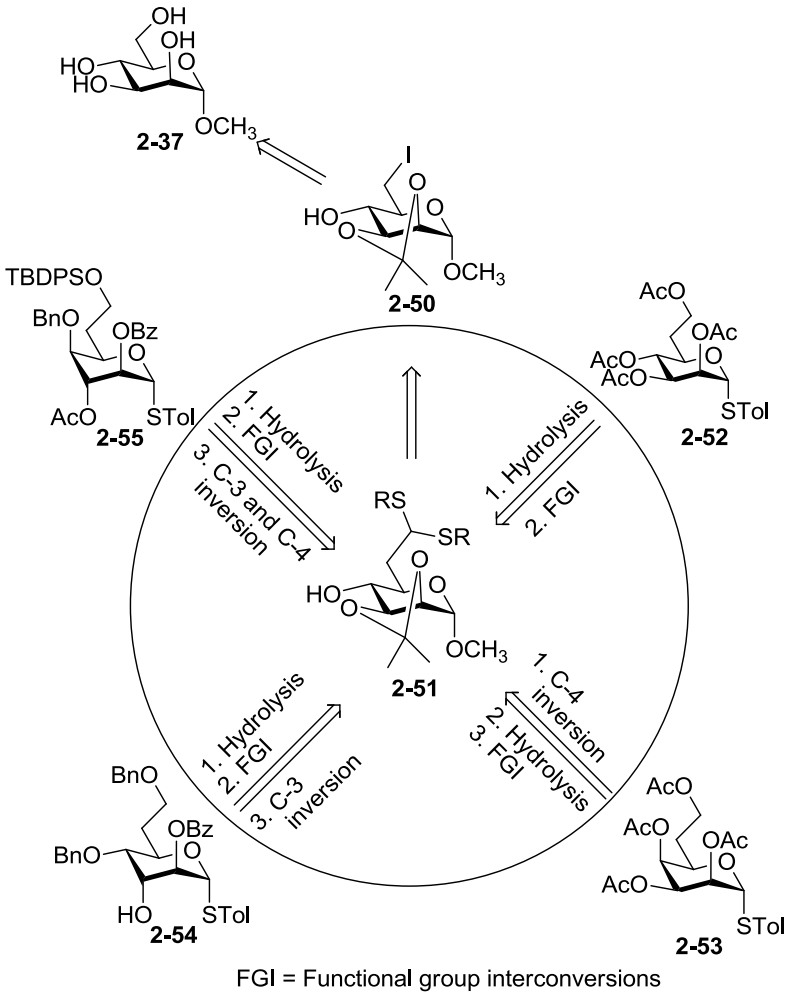
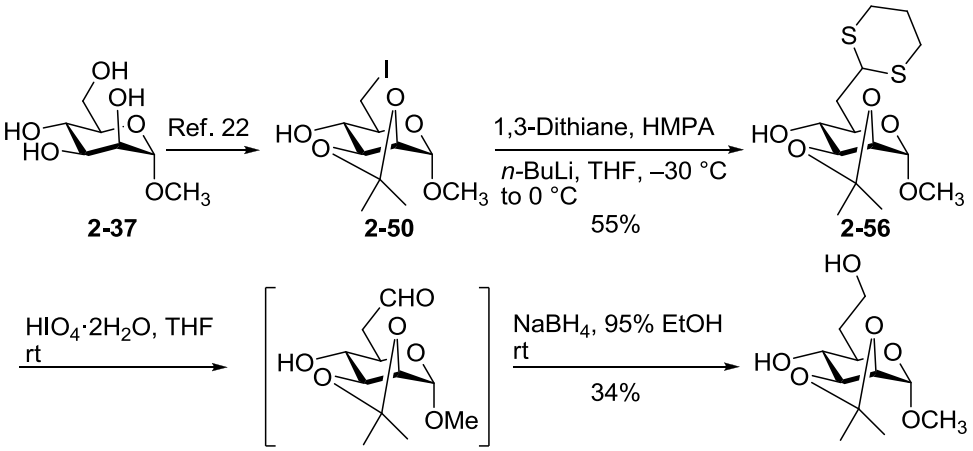


Figure 2-5: Retrosynthetic analysis to access 6-deoxy-D-heptose residues via C-6 homologation of hexoses using dithioacetals.

2.3.2.1 Synthesis of 6-deoxy-D-heptoses by C-6 homologation of hexoses with lithiated 1,3-dithiane

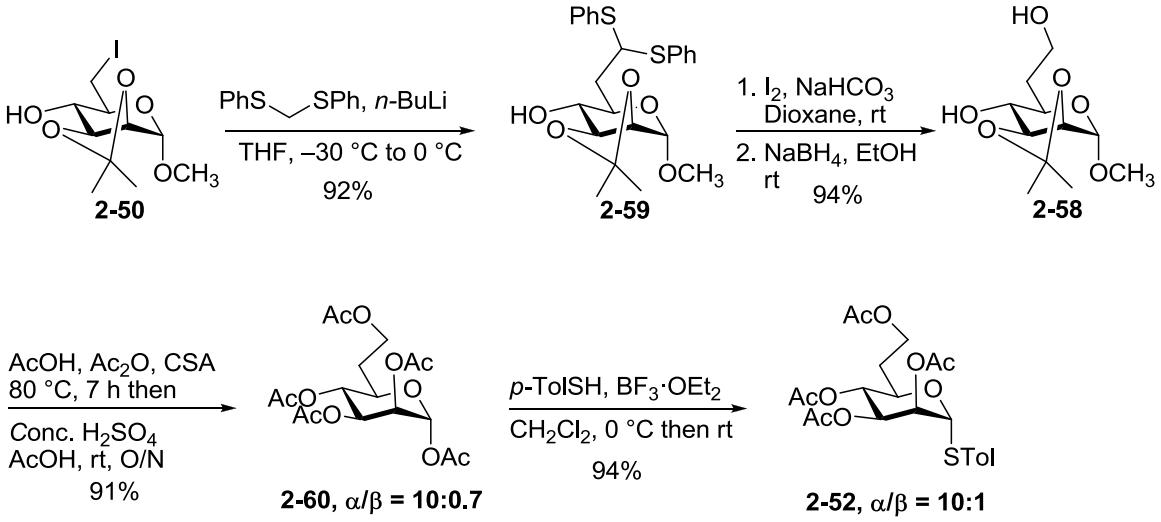
Diol **2-58**[20a] was synthesized as shown in Scheme 2-7, from **2-50**, which was prepared from methyl α-D-mannopyranoside (**2-37**) in two steps as reported.[22] Nucleophilic displacement of **2-50** using lithiated 1,3-dithiane, prepared by treating 1,3-dithiane with *n*-BuLi in THF containing hexamethylphosphoric triamide (HMPA) generated the desired dithioacetal **2-56** in 55% yield. When HMPA was not used, much lower yields (≤40%) were obtained possibly due to the lower stability of lithiated 1,3-dithiane.[23] Hydrolysis of **2-56** was attempted (to obtain aldehyde **2-57**) using known dithioacetal cleavage protocols.[24] However, we obtained low yields (10–34%) of desired diol **2-58**[20a] after $NaBH_4$ reduction of the aldehyde intermediate. The best result was obtained when dithioacetal **2-56** was treated with periodic acid dihydrate[25] and then $NaBH_4$ reduction of the resulting aldehyde; this process gave **2-58** in 34% yield.



Scheme 2-7: Synthesis of **2-58** via lithiated 1,3-dithiane homologation.

2.3.2.2 Improved homologation and synthesis of *p*-tolyl 6-deoxy-1-thio-α/β-D-*manno*-heptopyranoside 2-52

Given the low yields obtained with the approach shown in Scheme 2-7, we envisioned that using a dithioacetal with a lower pK_a than 1,3-dithiane (pK_a = 39 in DMSO) might improve the yield. Accordingly, we explored bis(phenylthio)methane (pK_a = 30.8 in DMSO) as a precursor to the nucleophile.[26] To our delight, nucleophilic displacement of **2-50** using lithiated bis(phenylthio)methane furnished dithioacetal **2-59** in 92% yield (Scheme 2-8). It is noteworthy to mention that this reaction does not require the addition of HMPA, given the enhanced acidity of bis(phenylthio)methane compared to 1,3-dithiane. Bis(phenylthio)methane has two additional advantages. First, it is not a malodorous reagent. Second, the excess reagent can be isolated and recrystallized after the reaction is completed. This recrystalized material can be used again for the displacement reaction without any erosion in yield.



Scheme 2-8: Synthesis of **2-58** via homologation with lithiated bis(phenylthio)methane and synthesis of *p*-tolyl-2,3,4,7-tetra-*O*-acetyl-6-deoxy-1-thio-α/β-D-*manno*-heptopyranoside (**2-52**).

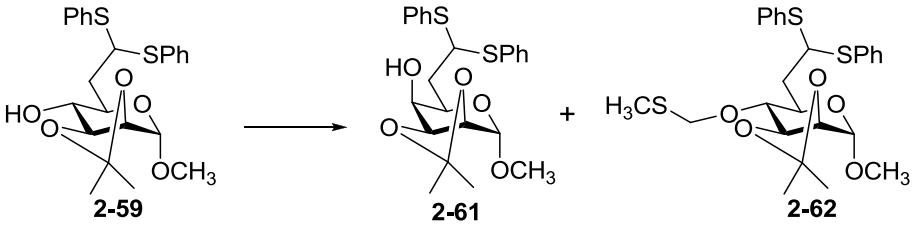
Hydrolysis of dithioacetal **2-59** was initially tested with *N*-bromosuccinimide (NBS). The use of this reagent to hydrolyze **2-59**, followed by reduction of the resulting aldehyde with $NaBH_4$, furnished desired known diol **2-58** in 60% yield. However, when attempted in scales above 800 milligrams, this sequence produced significantly lower yields. Alternatively, hydrolysis of **2-59** using iodine and sodium bicarbonate, and then treatment of the crude product with $NaBH_4$, afforded **2-58**[20a] in 94% yield. Using a slightly modified version of a previously-reported procedure,[27] acetolysis of diol **2-58** with acetic anhydride, acetic acid, camphorsulfonic acid (CSA) and concentrated sulfuric acid furnished 1,2,3,4,7-penta-*O*-acetyl-6-deoxy-D-*manno*-heptopyranose (**2-60**) in 91% yield and a 10:0.7 α/β ratio. This protocol gives access to **2-60** in fewer steps compared to literature protocols.[5,20a] To facilitate the assembly of oligosaccharides containing this monosaccharide, **2-60** was converted to thioglycoside **2-52** upon treatment with *p*-thiocresol and $BF_3 \cdot OEt_2$, in 94% yield.

2.3.2.3 Synthesis of *p*-tolyl 6-deoxy-1-thio-α/β-D-*talo*-heptopyranoside donor 2-53

The free C-4 hydroxyl group in **2-59** is advantageous for configurational inversion to obtain the 6-deoxy-D-*talo*-heptose derivative **2-53**. Its synthesis began with exploring different oxidation conditions of **2-59** and subsequent reduction to achieve configurational inversion (Table 2-1). Attempted TEMPO oxidation[28] of **2-59** gave multiple spots on thin layer chromatography (TLC). Oppenauer oxidation using aluminum isopropoxide and oxidation using potassium *tert*-butoxide (KO*t*-Bu) and benzophenone,[29] resulted in reisolation of unreacted starting material. Oxidation using DMSO and acetic anhydride[30] at room temperature again resulted in no conversion of the substrate; when heated at 52 °C, undesired byproduct **2-62** was produced. Eventually, success was achieved using a Parikh–Doering oxidation[31] with DMSO and sulfur trioxide and pyridine complex. Oxidation of **2-59** followed by $NaBH_4$ reduction afforded a

~7:1 ratio of **2-61** and **2-59** in 83% yield. Alternatively, Parikh–Doering oxidation of **2-59** followed by treatment with lithium triethylborohydride ($LiEt_3BH$) furnished **2-61** in 87% yield as the only isolated product.

Table 2-1: Conditions explored for the C-4 configurational inversion of **2-59**



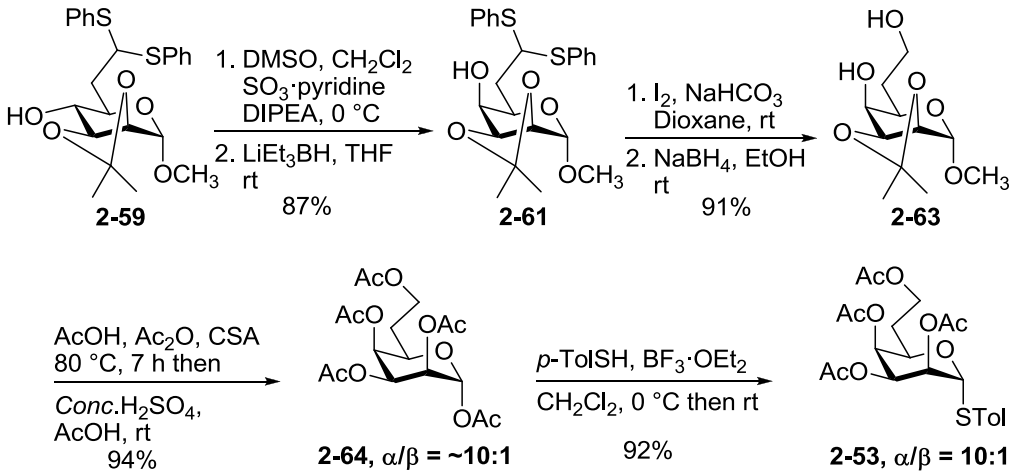
Entry	Conditions	Product ratio 2-61:2-59	Yield[a]	Byproduct 2-62
1	TEMPO, TCICA, NaBr, $NaHCO_3$, Acetone, rt	–	–[b]	–
2	$Al(OiPr)_3$, Acetone, 40 °C	–	–[c]	–
3	KO*t*-Bu, PhC(O)Ph, toluene, 80 °C	–	–[c]	–
4	DMSO, Ac_2O, rt	–	–[c]	–
5	DMSO, Ac_2O, 52 °C	–	–[d]	85%
6	1) DMSO, SO_3·pyridine, DIPEA, 0 °C 2) $NaBH_4$, 95% EtOH, rt	7.14:1[e]	83%	<5%
7	1) DMSO, SO_3·pyridine, DIPEA, 0 °C 2) $LiEt_3BH$, THF, rt	1:0[e]	87%	<5%

[a]Isolated yield. [b]multiple spots on TLC. [c]Starting material recovered. [d]Very small amount of oxidized product formed. [e]Ratio determined by ^1H NMR spectroscopy of the crude reaction mixture.

The configurational change at C-4 was confirmed by analyzing the ^1H NMR spectrum of **2-61**, notably the signal for H-4, and comparing it to that in the spectrum of **2-59**. For **2-61**, the

signal for H-4 appears as an apparent doublet ($J_{3,4}$ = 4.0 Hz and $J_{4,5}$ = 0 Hz), which is a dramatic change from that seen for **2-59** (doublet of doublet of doublets with $J_{3,4}$ = 5.6 Hz, $J_{4,5}$ = 9.1 Hz and $J_{4,4\text{-OH}}$ = 5.0 Hz). These changes indicate that inversion of the configuration at C-4 had occurred. It is interesting to note that in **2-61** long range coupling (W-coupling) was observed between 4-OH and H-5 ($J_{4\text{-OH},5}$ = 1.5 Hz). In addition, a cross peak between H-2 and H-4 was observed in the COSY spectrum, which is a characteristic of the *talo*- or *ido*- ring configuration and suggestive of a second W-coupling.

Having worked out the C-4 inversion protocol, we proceeded to synthesize thioglycoside **2-53** as illustrated in Scheme 2-9. Unmasking the dithioacetal **2-61** using iodine and sodium bicarbonate and then $NaBH_4$ reduction of the resulting aldehyde afforded diol **2-63** in 91% yield. Acetolysis of **2-63** cleanly afforded 1,2,3,4,7-penta-*O*-acetyl-6-deoxy-D-*talo*-heptopyranose (**2-64**) in 94% yield. Glycosyl acetate **2-64** was then converted, in 92% yield, to thioglycoside **2-53** by treatment with *p*-thiocresol and $BF_3 \cdot OEt_2$.

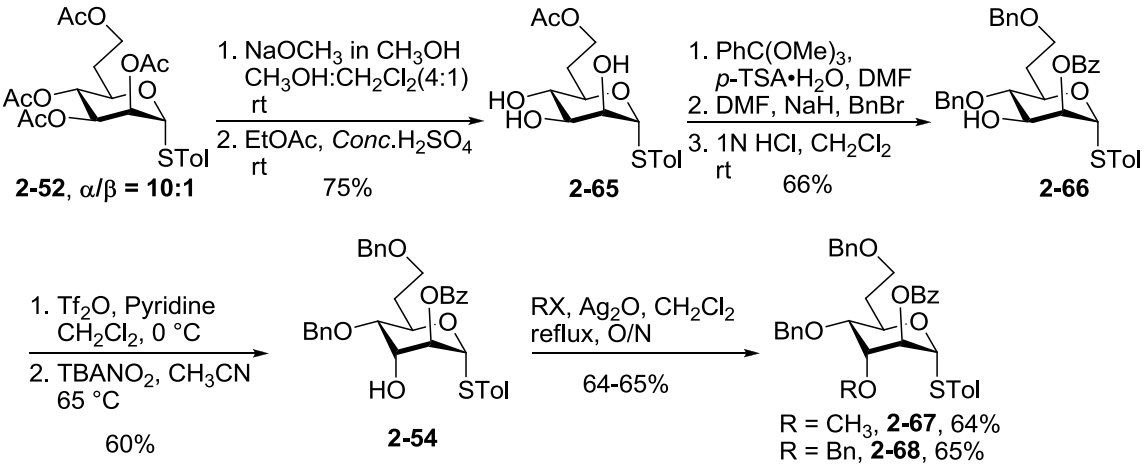


Scheme 2-9: Synthesis of *p*-tolyl-2,3,4,7-tetra-*O*-acetyl-6-deoxy-1-thio-α/β-D-*talo*-heptopyranoside (**2-53**) through oxidation and stereoselective reduction of the C-4-hydroxyl group in **2-59**.

2.3.2.4 Synthesis of D-*altro*-heptopyranoside donors 2-67 and 2-68

Thiogycoside **2-52** served as a convenient starting material for the synthesis of the C-3 epimer, which has the *altro* configuration (Scheme 2-10). Zemplén deacetylation of **2-52** using sodium methoxide in methanol afforded the tetrol, which was subjected to transesterification using ethyl acetate[32] and concentrated sulfuric acid to afford the 7-*O*-acetyl thioglycoside **2-65** in 75% yield over the two steps. Orthoester formation using trimethyl orthobenzoate and *p*-toluenesulfonic acid (*p*-TSA), benzylation, and then regioselective orthoester opening[33] furnished the C-3 free hydroxyl group compound **2-66** in 66% yield over the sequence. Triflation and nucleophilic inversion of **2-66** was our approach for obtaining the *altro* isomer. Accordingly, triflation of **2-66** using trifluoromethanesulfonic anhydride and pyridine followed by treatment with tetrabutylammonium nitrite gave **2-54** in 60% yield over the two steps. The configurational change at C-3 was confirmed by analyzing the changes in the ^{1}H NMR spectrum of **2-54**,

compared to that for **2-66**. A particular focus was the signal for H-3, which was an apparent triplet with $J_{2,3}$ = 3.0 Hz and $J_{3,4}$ = 3.0 Hz in the spectrum of **2-54**. In comparison, the signal for H-3 in the spectrum of **2-66** had a $J_{3,4}$ value of 9.5 Hz and a $J_{2,3}$ value of 3.0 Hz. These changes indicate that inversion of configuration had occurred at C-3. Alkylation of **2-54** using methyl iodide or BnBr and silver oxide afforded thioglycosides **2-67** and **2-68** in 64% and 65% yield, respectively. Use of **2-67** or **2-68** in the synthesis of a target CPS trisaccharide is discussed in Chapter 3.



Scheme 2-10: Synthesis of *p*-tolyl 6-deoxy-1-thio-α/β-D-*altro*-heptopyranoside donors **2-67** and **2-68** through triflation and inversion of **2-66**.

2.3.2.5 Alteration of approach to D-*ido*-heptopyranoside thioglycoside donor 2-55

We initially also planned to synthesize 6-deoxy-D-*ido*-heptopyranoside derivative **2-55** from methyl α-D-mannopyranoside (**2-37**). However, we realized doing so would require >15 linear steps. Moreover, in 2017 Ling and co-workers[9d] published a synthesis of 6-deoxy-D-*ido*-heptopyranoside **2-71** in 11 steps from commercially available 1,2:3,4-di-*O*-isopropylidene-α-D-

galactopyranose (**2-69**) through a key 2,3-epoxide intermediate **2-70** (Figure 2-6). Our approach to the synthesis of 6-deoxy-D-*ido*-heptopyranoside derivatives are discussed in Chapter 4.

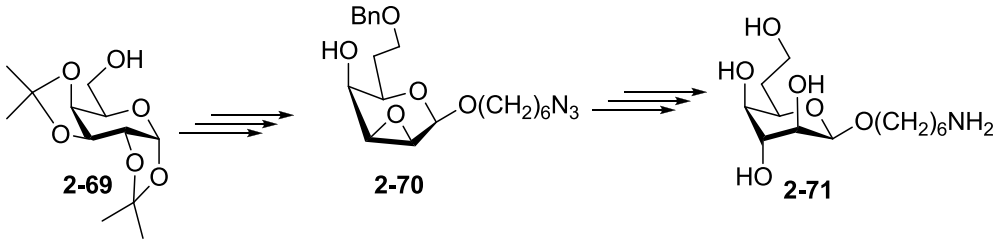


Figure 2-6: Key epoxide intermediate **2-70** involved in the synthesis of 6-deoxy-D-*ido*-heptopyranose **2-71** reported by Ling and co-workers.

2.4 Synthesis of trisaccharide repeating unit of *C. coli* serotype O:30

As an application of our methodology and also as part of Research Objective 2 of this thesis, we turned our attention to the synthesis of the trisaccharide repeating unit of *C. coli* serotype O:30 CPS. The structure of this polysaccharide (**2-9**, Figure 2-7) was determined by Aspinall and co-workers.[8] The repeating unit consists of 6-deoxy-D-*talo*-heptopyranose, *N*-acetyl-D-glucosamine and ribitol phosphate, with the latter serving as the backbone and pendant *O*-glycosyl substituents at the *O*-4 position of the ribitol moiety. The structure is unusual in Gram-negative bacteria and instead resembles cell wall teichoic acids of Gram-positive organisms. The absolute configuration of the ribitol moiety was not determined with respect to the *O*-glycosyl substitution but it was presumed that the unit might have been derived from cytidine-5'-phospho-ribitol.[8,34]

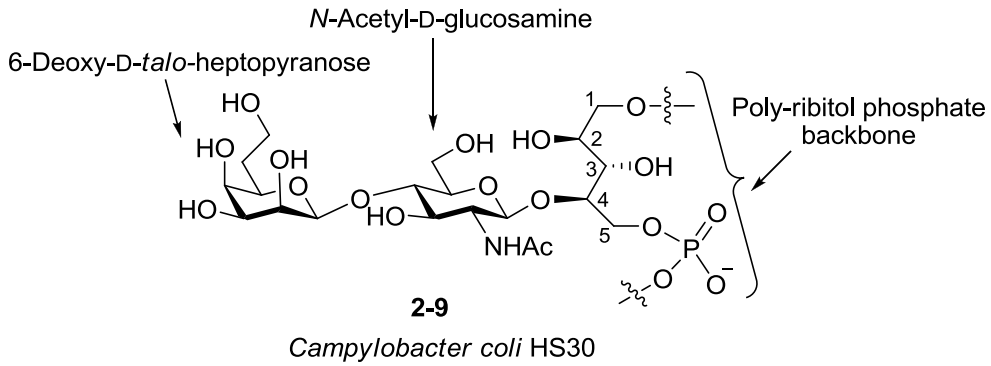


Figure 2-7: *C. coli* HS30 CPS repeating trisaccharide structure.

We selected trisaccharide (**2-9a**, Figure 2-8) for synthesis. We anticipated that the major challenges would be 1) the synthesis of the 6-deoxy-D-*talo*-heptopyranose glycosyl donor, which was described in Section 2.3.2.3; 2) the stereoselective installation of the 6-deoxy-β-*talo*-heptoside residue in a 1,2-*cis*-fashion; 3) the preparation of a suitably-protected ribitol derivative; and 4) the introduction of the ribitol containing phosphodiester linkage. We envisioned that the formation of the 1,2-*cis*-heptosidic linkage would be the most challenging of all to address. This linkage is stereochemically equivalent to β-mannoside linkages, for which a number of methods have been developed. Two approaches to these 1,2-*cis*-heptosidic linkages are described below, after a summary of important methods for making bonds of this type.

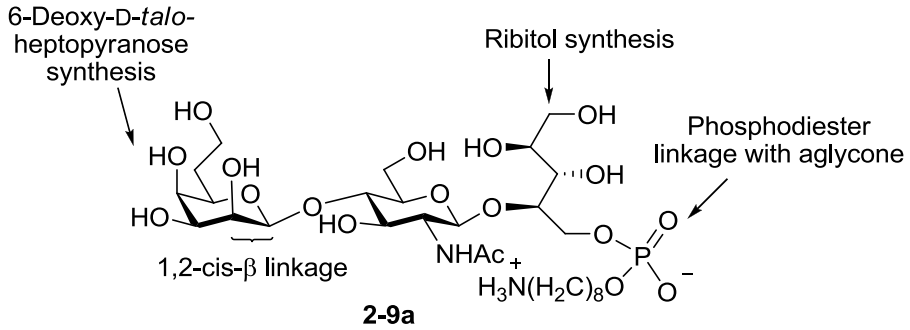


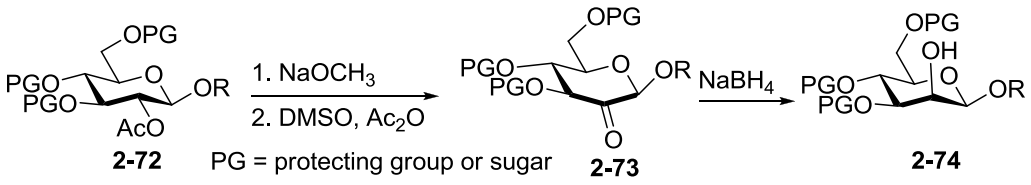
Figure 2-8: Target molecule with presupposed synthetic challenges.

2.4.1 Reported methods for the construction of 1,2-cis-β-linkages in hexopyranosides

Construction of 1,2-*cis*-β (β-*manno*) glycosidic linkages is arguably the most challenging one for the synthetic chemist. To overcome this challenge, researchers around the world have developed both indirect and direct approaches. Some of these are discussed, in the order they were reported, in the following sections.

2.4.1.1 Formation of β-mannosides by oxidation–reduction of β-glucosides

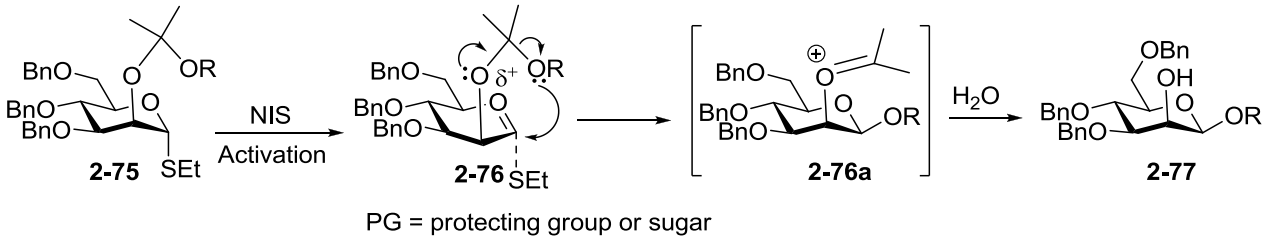
In 1980, Jeanloz and co-workers[35] reported an indirect approach for the formation of 1,2-*cis*-β-mannosidic linkages via oxidation–reduction of β-glucosides (Scheme 2-11). De-*O*-acetylation of **2-72** and oxidation affords 2-uloside **2-73**, which, upon reduction, produces the 1,2-β-mannoside **2-74**. The reduction usually proceeds with high *manno* selectivity.



Scheme 2-11: Formation 1,2-*cis*-β-mannosides by oxidation–reduction of β-glucosides.

2.4.1.2 Synthesis of β-mannosides by intramolecular aglycone delivery

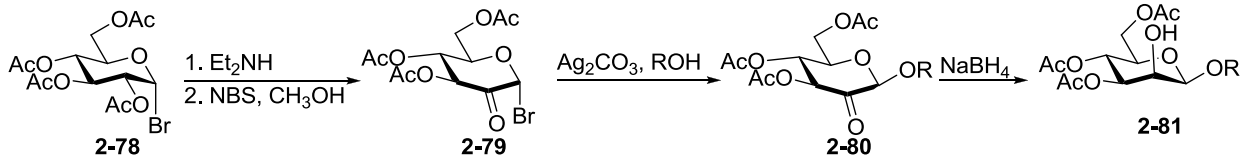
In 1991, Hindsgaul and co-workers[36] reported the formation of 1,2-*cis*-β-mannosidic linkages via intramolecular aglycone delivery (IAD, Scheme 2-12). This method requires coupling of donor and acceptor together before glycosylation to form mixed acetal **2-75**. Upon activation of **2-75**, the acceptor oxygen selectively traps the transient oxocarbenium ion **2-76** from the β-face to form **2-76a**, generating the β-mannoside **2-77** upon hydrolysis. This approach has been further explored by other using other linkers to make the mixed acetal.[37]



Scheme 2-12: Formation 1,2-*cis*-β-mannosides by intramolecular aglycon delivery.

2.4.1.3 Synthesis of β-mannosides via ulosyl bromide glycosylation and reduction

In 1992, Lichtenthaler and co-workers[38] reported an indirect approach for the formation of β-mannosides, starting from 2-oxoglycosyl bromides (or ulosyl bromides, Scheme 2-13). Glycosyl bromide **2-78**, when treated with diethylamine followed by NBS and methanol, generates ulosyl bromide **2-79**. Activation of **2-79** in the presence of acceptor alcohol generates the 2-uloside **2-80**, which, upon reduction, provides the mannoside **2-81**. It is believed that the glycosylation proceeds in an S_N2 fashion as oxocarbenium ion formation at C-1 is disfavored by the adjacent carbonyl group. Reduction normally proceeds with >10:1 *manno*:*gluco* selectivity.

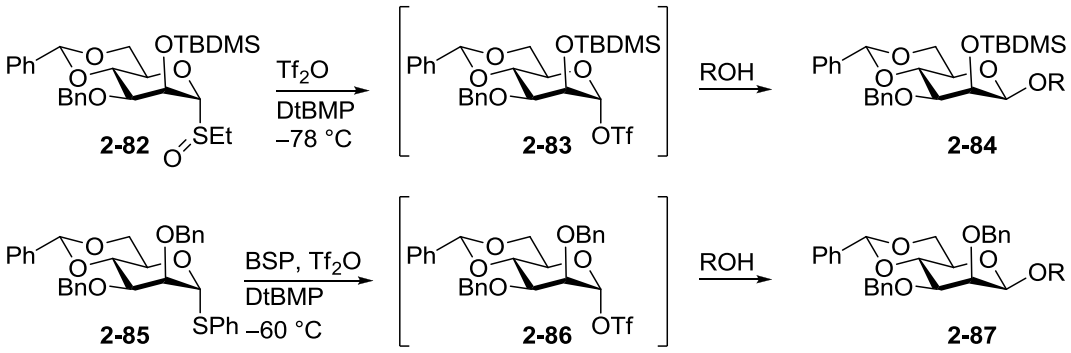


Scheme 2-13: Formation 1,2-*cis*-β-mannosides from ulosyl bromide **2-79**.

2.4.1.4 Synthesis of β-mannosides by preactivation of mannosyl sulfoxides or thioglycosides

In 1996 and 2001, Crich and co-workers[39] reported a direct approach for the formation of β-mannosides via preactivation of mannosyl sulfoxides or thioglycosides (Scheme 2-14). Sulfoxide **2-82**, upon activation with triflic anhydride at –78 °C can form glycosyl triflate **2-83**,

which reacts with an acceptor alcohol to afford β-mannoside **2-84**. Similarly, thioglycoside **2-85**, upon reaction with 1-benzenesulfinyl piperidine and triflic anhydride at –60 °C, can form glycosyl triflate **2-86** and then β-mannoside **2-87**. Among the methods reported, the Crich protocol is a notable breakthrough and is widely used in β-mannoside formation. This method generally proceeds with high selectivity but it requires the use of 4,6-*O*-benzylidene-protected donors. After Crich's protocol was published, a wide range of 4,6-*O*-benzylidene-protected donors[40] have been used in the synthesis of β-mannosides. In addition, Bols, Pedersen and co-workers[41] have replaced the 4,6-*O*-benzylidene acetal by a 4,6-*O*-(di-*tert*-butylsilylene) acetal and successfully used them in the synthesis of β-mannosides.

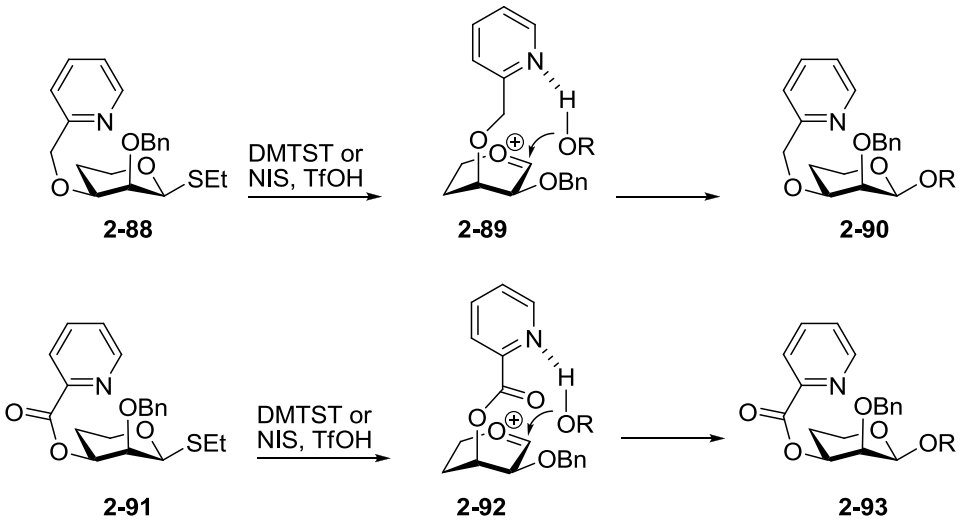


Scheme 2-14: Formation of β-mannosides from mannosyl sulfoxides or thioglycosides by preactivation.

2.4.1.5 Synthesis of β-mannosides by hydrogen-bond mediated aglycon delivery

In 2012, Demchenko and co-workers[42] reported a direct approach for the formation of β-mannosides via picolinyl **2-88** or picoloyl **2-91** donors (Scheme 2-15). These groups, when remotely placed on the donor, can form intermolecular hydrogen bonds with acceptors (e.g., **2-89** and **2-92**) upon activation by dimethyl(methylthio)sulfonium trifluoromethanesulfonate or *N*-iodosuccinimide (NIS) and trifluoromethanesulfonic acid (TfOH). When these groups are

strategically placed on the ring, this tethering via hydrogen bonding affords β-mannosides (e.g., **2-90** and **2-93**).



Scheme 2-15: Formation β-mannosides from picolinyl or picoloyl-protected donors (**2-88** and **2-91**, respectively) by intermolecular hydrogen bonding.

2.4.2 β-Selective (1,2-*cis*-type) glycosylation with 6-deoxy-D-heptosyl donors

β-Selective (1,2-*cis*-type) glycosylation with 6-deoxy-D-heptosyl donors has been less explored.[9b,11g,12,43] Because of the lack of a C-6 hydroxyl group, the Crich approach cannot be used and other methods often require a significant number of synthetic steps.[36,39-42] In 2014, Gauthier and co-workers[11g] used the IAD approach for the construction of 6-deoxy-β-*manno*-heptosidic linkages. In 2015, Scott and co-workers[43] used both triflation and inversion, as well as oxidation and reduction approaches, to construct 6-deoxy-β-*manno*-heptosidic linkages from 6-deoxy-β-*gluco*-heptosides. In 2019, Yang and co-workers[12] prepared a 6-deoxy-D-*manno*-heptosyl *o*-hexynylbenzoate donor and constructed the 6-deoxy-β-*manno*-heptosidic linkage with moderate β-selectivity (up to 5:1). For us to use IAD, a significant number of steps are needed to

prepare the donor (Figure 2-9) from **2-64**. In addition, we anticipated we might face regioselective protection issues because of the *cis*-relationship of the hydroxyl groups at C-2 and C-4 in **2-64**. The synthesis of *o*-hexynylbenzoate donor **2-95** would takes at least four steps from **2-64** and requires an expensive gold(I) catalyst for activation.[40d,40e] Alternatively, the ulosyl bromide approach requires only three steps to make the donor **2-96** from **2-64**. Also, to date, there is no precedence for using ulosyl bromides derived from heptoses as donors in the construction of 6-deoxy-β-heptosidic linkages. Therefore, we explored this approach.

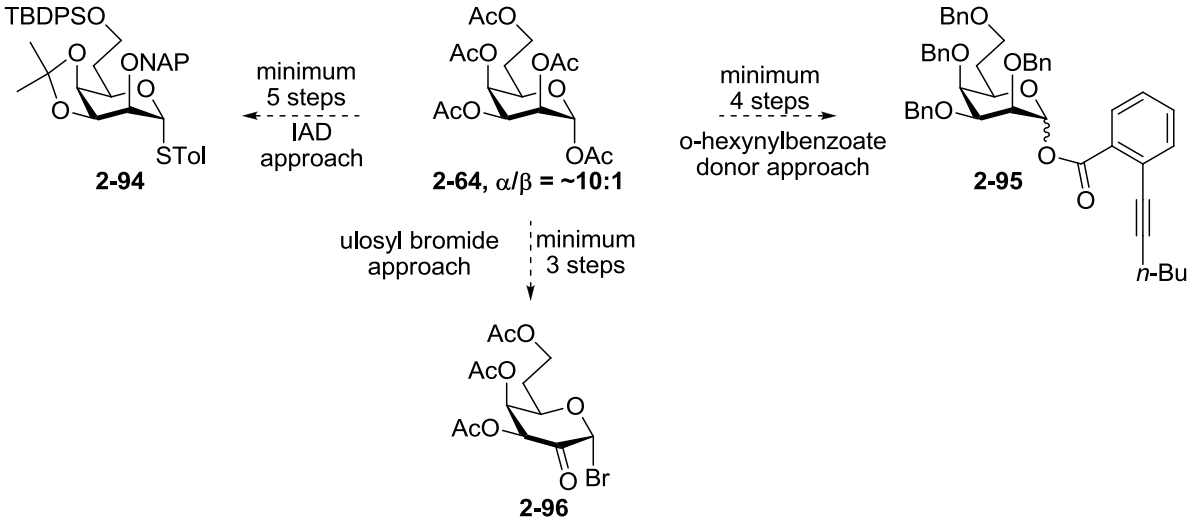


Figure 2-9: Different approaches for 6-deoxy-D-heptosyl donors **2-94**, **2-95** and **2-96**.

2.4.3 Stereoselective synthesis of 6-deoxy-β-*talo*-heptosidic linkages in trisaccharide 2-9a through the ulosyl bromide approach

2.4.3.1 Retrosynthetic analysis of trisaccharide 2-9a involving ulosyl bromide 2-96

The initial retrosynthetic analysis of **2-9a** is outlined in Figure 2-10. An aminooctyl aglycone was incorporated into the target through a phosphodiester linkage to allow facile conjugation of the molecule to proteins. We envisaged that a [1 + 2] glycosylation would

facilitate the synthesis. Glycosylation of disaccharide acceptor **2-97** with ulosyl bromide **2-96** followed by stereoselective reduction of the resulting uloside would generate the desired β-heptosidic linkage. Ulosyl bromide **2-96** can be obtained from 2-acetoxyglycal **2-98**, which in turn can be obtained from **2-64** (shown in Figure 2-9) in two steps first by treatment with HBr and then dehydrobromination. Disaccharide **2-97** can be obtained from thioglycoside **2-99** and ribitol derivative **2-100**. Finally, we envisioned that the phosphate could be introduced into the trisaccharide after [1 + 2] glycosylation and *tert*-butyldiphenylsilyl (TBDPS) deprotection.

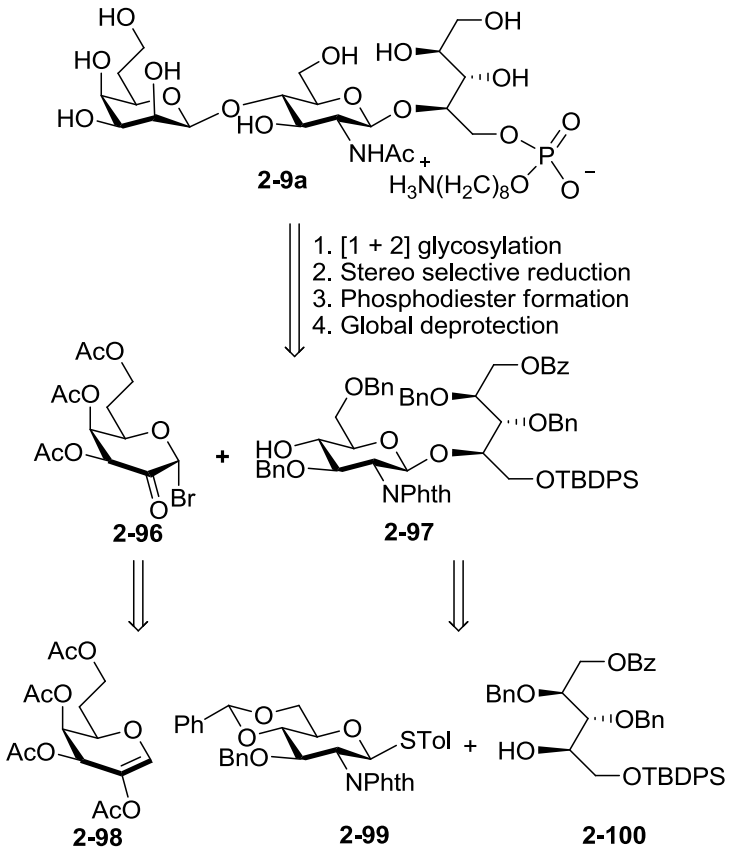
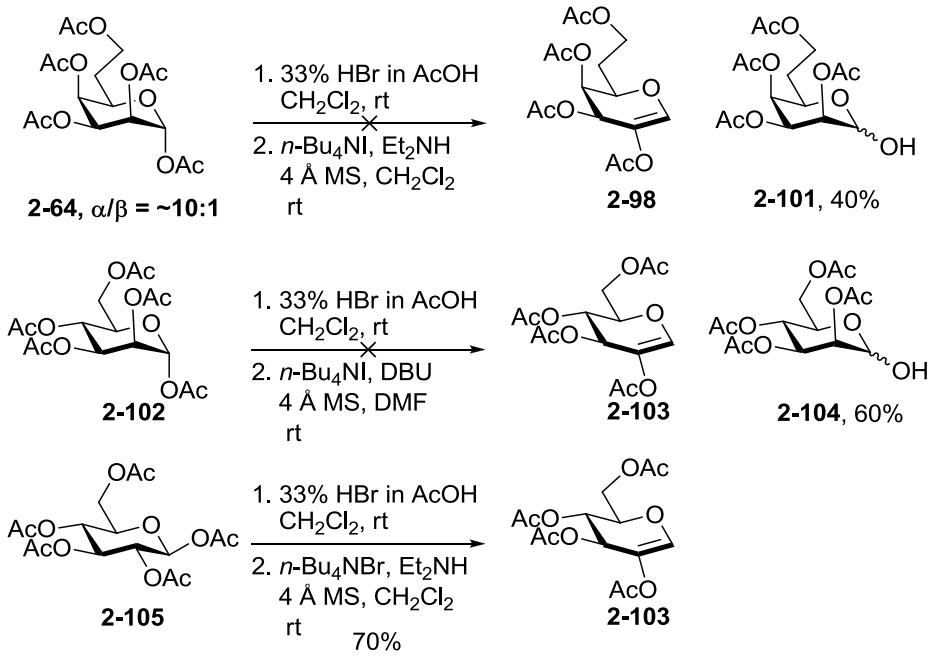


Figure 2-10: Retrosynthetic analysis of trisaccharide **2-9a** involving a [1 + 2] glycosylation approach with ulosyl bromide **2-96** and disaccharide acceptor **2-97**.

2.4.3.2 Attempted synthesis of 2-aceoxy glycal 2-98 from 2-64

The synthesis of the trisaccharide began by attempting to prepare glycal **2-98** from **2-64** (Scheme 2-16). Pentaacetate **2-64** was treated with HBr in acetic acid to afford the corresponding glycosyl bromide. Treating this glycosyl bromide with tetra-*n*-butylammonium iodide (presumably to improve the yield via *in situ* anomerization to the more reactive β-bromide[44]) and diethylamine gave a compound in 40% yield that lacked an acetyl group in ^{1}H NMR spectrum. However, NMR analysis revealed that the product was hemiacetal **2-101**, not the desired 2-acetoxy glycal **2-98**.



Scheme 2-16: Attempted synthesis of 2-acetoxy glycal **2-98**.

To further investigate this process, commercially available penta-*O*-acetyl-D-mannose **2-102** and penta-*O*-acetyl-D-glucose **2-105** were subjected to similar reaction conditions. We were able to isolate 2-acetoxyglucal **2-103** (preparation of **2-103** was done by a reported protocol[44b]

from **2-105**) only when the starting material was the glucose-derived pentaacetate. We isolated hemiacetal **2-104** in 60% yield when the starting material was the mannose derived compound. Therefore, it appears that this E2 elimination reaction has a strict requirement for an antiperiplanar arrangement of the H and Br, which is possible only when the C-2 substituent is equatorial. This is in agreement with Lemieux and co-workers[45] discovery that the rate of 2-acetoxyglycal formation from glucosyl bromides is faster than that of *in situ* anomerization in the presence of amine. Our findings, and Lemiuex's earlier work, call into question the role of the tetra-*n*-butylammonium salt in this process.

2.4.3.3 Successful route to 2-acetoxy glycal 2-98

Because the C-2 stereocenter is lost during the formation of **2-98**, we envisioned that the C-2 epimer of **2-64** i.e., 1,2,3,4,7-penta-*O*-acetyl-6-deoxy-α/β-D-*galacto*-heptopyranose (**2-106**) would give the desired compound (Figure 2-11).

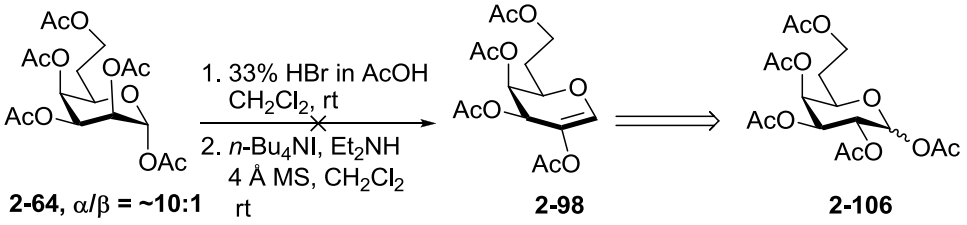
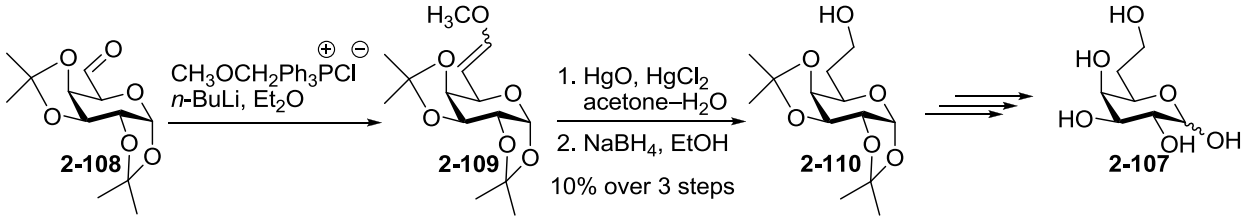


Figure 2-11: Plausible solution to obtain 2-acetoxy glycal **2-98**.

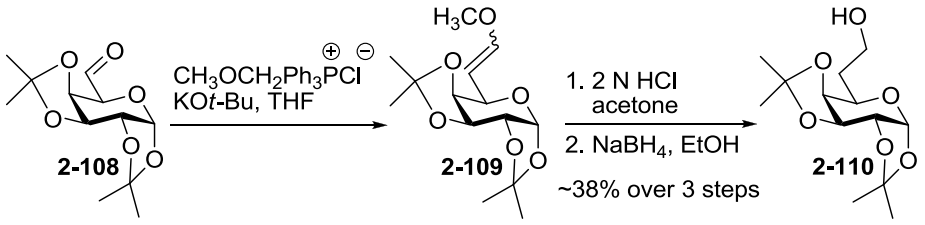
6-Deoxy-D-*galacto*-heptopyranose (**2-107**) is not a natural 6-deoxy-D-heptose, but its synthesis has been reported.[46] In 1974, Garegg and co-workers[46a] described its preparation via Wittig homologation (Scheme 2-17). Thus, when **2-108** was treated with methoxymethylenetriphenylphosphorane, methyl vinyl ether **2-109** was obtained. Hydrolysis

using mercuric oxide (HgO) and mercuric chloride ($HgCl_2$) followed by reduction of the resulting aldehyde with $NaBH_4$ furnished alcohol **2-110** in 10% overall yield.



Scheme 2-17: Key steps in the synthesis of **2-107** reported by Garegg co-workers.[46a]

In 2003, an improved synthesis of **2-110** was reported by Wünsch and co-workers.[46b] (Scheme 2-18). Treatment of **2-108** with methoxymethylenetriphenylphosphorane furnished methyl vinyl ether **2-109**, which, upon acid hydrolysis and reduction, provided **2-110** in 38% overall yield.



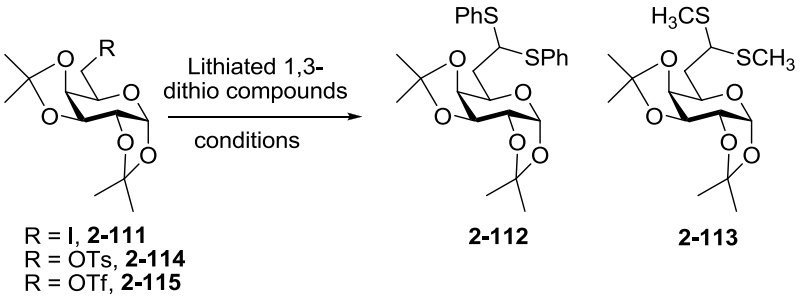
Scheme 2-18: Improved synthesis of **2-110** by Wünsch and co-workers.[46b]

2.4.3.4 C-6 Homologation trials and successful synthesis of 2-acetoxy glycal 2-98

Because our successful homologation approach for the synthesis of 6-deoxy-D-heptoses (above) involves nucleophilic substitution at C-6 by lithiated dithioacetals, we explored nucleophilic substitution at C-6 position of 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (**2-69**, Table 2-2) instead of the Wittg approach. Accordingly, two derivatives of **2-69** were

prepared (**2-111** and **2-114**).[46c,47] When 6-iodo compound **2-111**[45c] was subjected to nucleophilic displacement using a lithiated bis(phenylthio)methane, the desired dithioacetal **2-112** was afforded in only 12% yield and unreacted starting material was recovered. Alternatively, when the 6-iodo compound **2-111** was subjected to nucleophilic displacement using lithiated bis(methylthio)methane, only unreacted starting material was isolated and none of the desired dithioacetal **2-113** was detected. Similar results were observed with tosylate **2-114**[47] in the reaction with lithiated bis(phenylthio)methane. However, treatment of triflate **2-115** with lithiated bis(phenylthio)methane, furnished the desired dithioacetal **2-112** in 50% yield over the two steps from **2-69**. This displacement of triflate by lithiated bis(phenylthio)methane was reported earlier by Zeller and co-workers[21b] but yields were not shown.

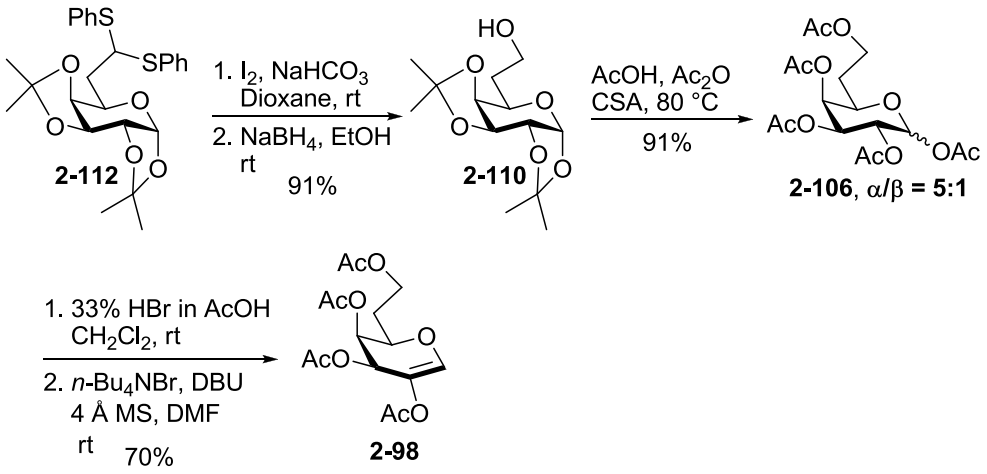
Table 2-2: Conditions explored for the C-6 homologation of **2-69**.



Entry	Compound	Conditions	Product, yield[a]
1	**2-111**	$PhSCH_2SPh$, *n*-BuLi, THF –30 °C to 0 °C	**2-112**, 12%[b]
2	**2-111**	$H_3CSCH_2SCH_3$, HMPA, *n*-BuLi, THF –30 °C to 0 °C	**2-113**, 0%[b]
3	**2-114**	$PhSCH_2SPh$, *n*-BuLi, THF –30 °C to 0 °C	**2-112**, 0%[b]
4	**2-115**	$PhSCH_2SPh$, *n*-BuLi, THF –30 °C to 0 °C	**2-112**, 50% from **2-69**

[a] Isolated yield. [b] Starting material recovered.

Elaboration of dithioacetal **2-112** into 1,2,3,4,7-penta-*O*-acetyl-6-deoxy-α/β-D-*galacto*-heptopyranose (**2-106**) and successful synthesis of 2-acetoxy glycal **2-98** is illustrated in Scheme 2-19. Unmasking of dithioacetal **2-112** using iodine and sodium bicarbonate and then $NaBH_4$ reduction of the resulting aldehyde afforded **2-110** in 91% yield. Acetolysis of **2-110** using acetic anhydride, acetic acid and CSA cleanly afforded **2-106**, also in 91% yield. Pentaacetate **2-106**, when subjected to HBr in acetic acid followed by treatment with tetra-*n*-tetrabutylammonium bromide (*n*-Bu_4NBr) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in DMF, gave the desired 2-acetoxyglycal **2-98** in 70% yield over the two steps. This reaction supported our hypothesis that the hydrogen and bromine atoms must be antiperipalanar for the dehydrobromination reaction to occur.

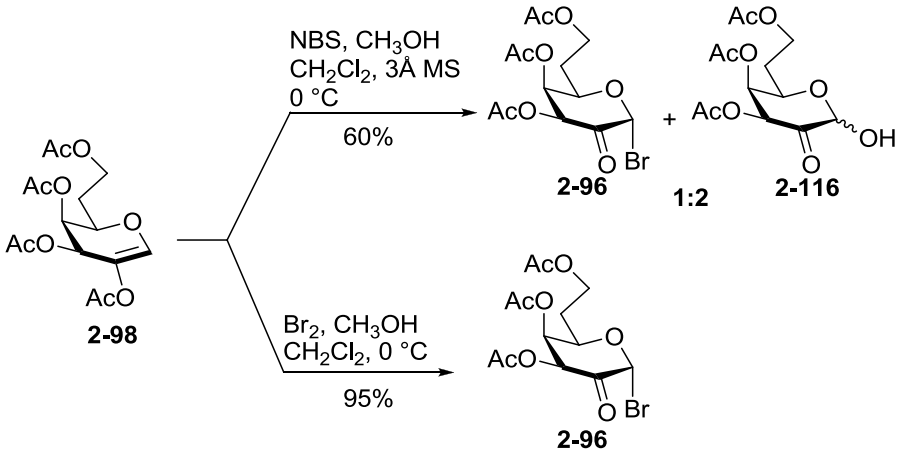


Scheme 2-19: Successful synthesis of 2-acetoxy glycal **2-98**.

2.4.3.5 Synthesis of ulosyl bromide donors 2-96 and 2-117

Transformation of 2-acetoxy glycal **2-98** into ulosyl bromide donor **2-96** is outlined in Scheme 2-20. Glycal **2-98**, when subjected to NBS and methanol at 0 °C, furnished an inseparable mixture of the desired ulosyl bromide **2-96** and the undesired hemiacetal **2-116** in a

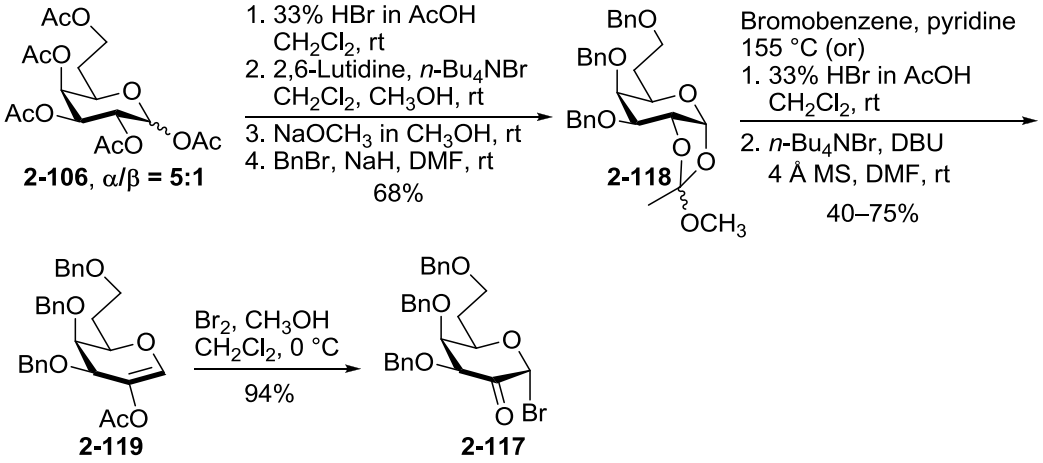
2:1 ratio in 60% yield. The ^{1}H NMR spectrum was inconclusive for the formation of **2-116**, but high-resolution mass spectrometry (HRMS) indicated its presence in the reaction mixture. We could not detect ulosyl bromide **2-96** through HRMS analysis. After the NBS reaction, the mixture was generally treated with cold water to remove the succinimide byproduct. Hence, **2-96** might have hydrolyzed during this cold aqueous workup. Therefore, to avoid hydrolysis, we switched to anhydrous workup conditions by changing the brominating agent from NBS to molecular bromine. Accordingly, glycal **2-98**, when treated with bromine and methanol at 0 °C furnished the desired ulosyl bromide **2-96** in 95% yield with a negligible amount of hydrolysis. The ulosyl bromide is of high purity without the need for any aqueous workup as both bromine and methanol can be removed on a rotary evaporator.



Scheme 2-20: Synthesis of ulosyl bromide donor **2-96**.

We also prepared 3,4,7-tri-*O*-benzyl ulosyl bromide **2-117** to evaluate it as a glycosyl donor (Scheme 2-21). Pentaacetate **2-106** was first converted to orthoester **2-118** by treatment with 33% HBr in acetic acid followed by reaction with *n*-Bu_4NBr and methanol in the presence of 2,6-lutidine to furnish an acetylated 1,2-orthoester intermediate. This compound was subjected

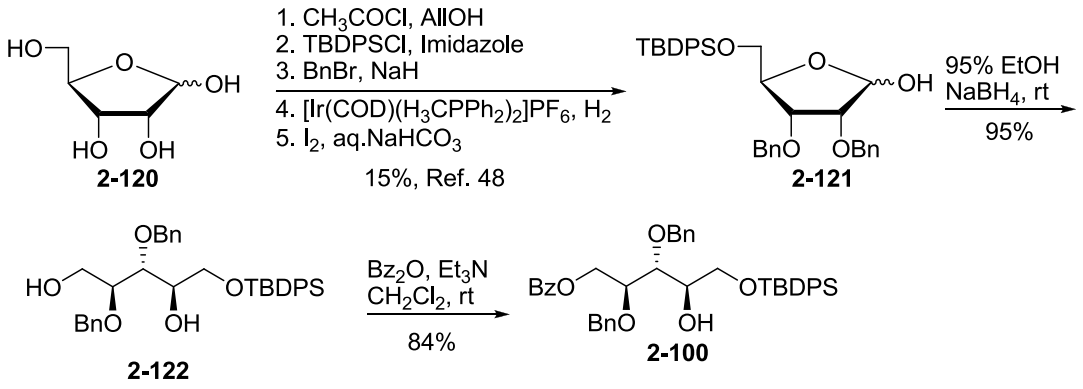
to Zemplén deacetylation followed by benzylation to afford **2-118** in 68% yield over the four steps. Thermal rearrangement of orthoester **2-118** using pyridine and bromobenzene at 155 °C, furnished benzylated 2-acetoxy glycal **2-119** in 40% yield. The low yield of **2-119** is presumably because of its instability at higher temperatures. Alternatively, **2-119** was obtained in 75% yield over two steps when **2-118** was subjected to 33% HBr in acetic acid followed by elimination with *n*-Bu_4NBr and DBU in DMF. Glycal **2-119**, when treated with bromine and methanol at 0 °C, furnished the desired ulosyl bromide **2-117** in 94% yield with high purity.



Scheme 2-21: Synthesis of ulosyl bromide donor **2-117** via 1,2-orthoacetate **2-118**.

2.4.3.6 Synthesis of ribitol acceptor 2-100

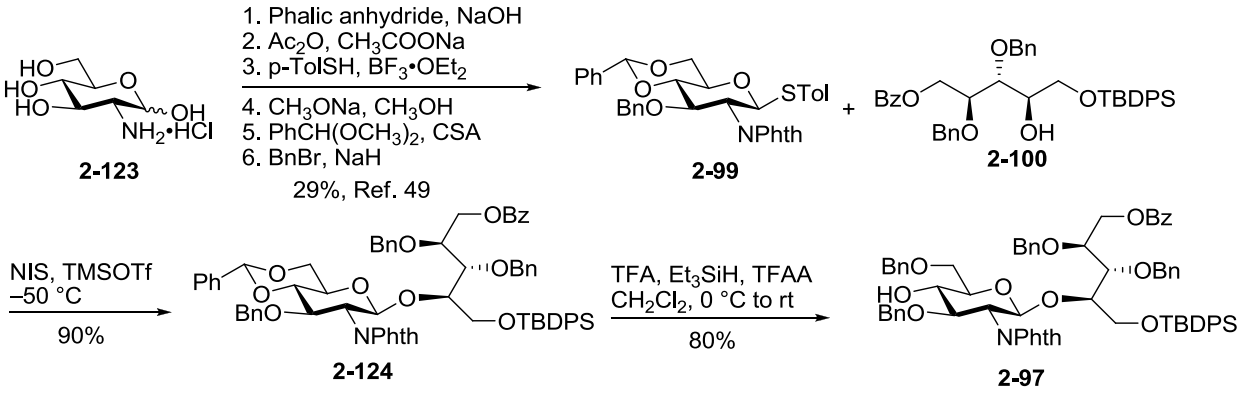
The synthesis of ribitol acceptor **2-100** is outlined in Scheme 2-22. First, D-ribose (**2-120**) was converted, in five steps and 15% overall yield, to the known hemiacetal **2-121** using a literature protocol.[48] Reduction of **2-121** using $NaBH_4$ in 95% ethanol afforded diol **2-122**, which was regioselectively benzoylated with benzoic anhydride and triethylamine to give **2-100** in 84% yield.



Scheme 2-22: Synthesis of ribitol acceptor **2-100**.

2.4.3.7 Synthesis of D-glucosamine–ribitol acceptor 2-97

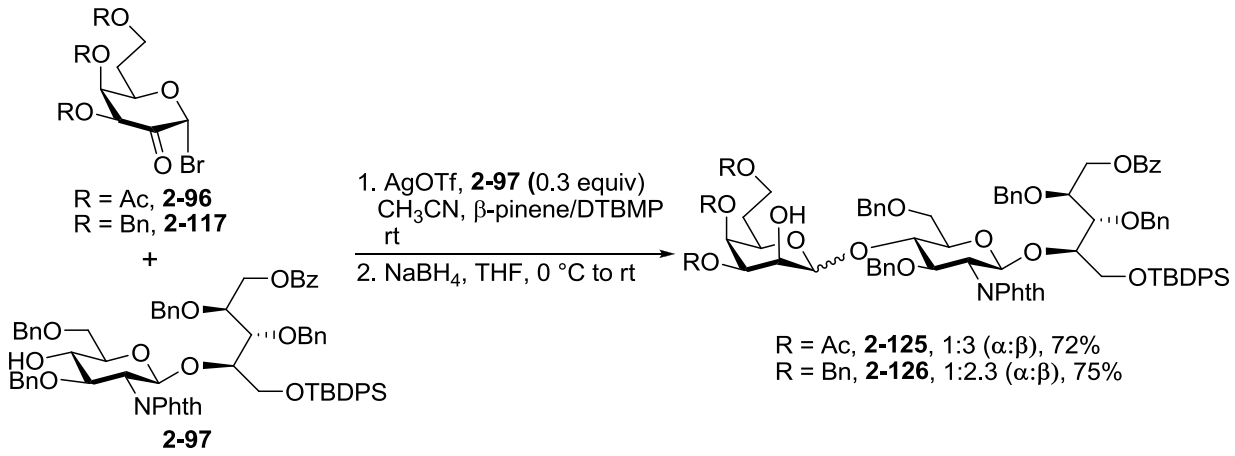
Scheme 2-23 shows the synthesis of D-glucosamine–ribitol acceptor **2-97** from D-glucosamine hydrochloride (**2-123**). Using a literature method, **2-123** was converted to thioglycoside **2-99** in six steps and 29% overall yield.[49] Glycosylation of **2-100** with thioglycoside **2-99**[49] promoted by NIS and trimethylsilyl trifluoromethanesulfonate (TMSOTf) at –50 °C afforded a 90% yield of the β-linked disaccharide **2-124** (β-linkage, δ_H 5.56 ppm, $J_{1,2}$ = 8.5 Hz). Regioselective reductive ring opening of **2-124** with trifluoroacetic acid and triethylsilane furnished **2-97** in 80% yield.



Scheme 2-23: Synthesis of D-glucosamine–ribitol acceptor **2-97**.

2.4.3.8 [1 + 2] Glycosylation of disaccharide acceptor 2-97 with ulosyl bromide donors 2-96 or 2-117

With the building blocks **2-96**, **2-97** and **2-117** in hand, we attempted the synthesis of trisaccharides **2-125** and **2-126** (Scheme 2-24). Silver trifluoromethanesulfonate-promoted glycosylation of ulosyl bromide **2-96** with disaccharide **2-97** in the presence of β-pinene[50] in acetonitrile, followed by reduction of the resulting uloside with $NaBH_4$ gave the desired trisaccharide **2-125** (α:β = 1:3) in 72% yield. Reduction of the uloside proceeded with high diastereoselectivity; no *galacto* isomer was isolated. Using this approach, products corresponding to both anomers of *talo*-heptoside were formed (α-linkage, δ_H 5.39 ppm, $^1J_{C\text{-}1,H\text{-}1}$ = 172.9 Hz, β-linkage, δ_H 4.56 ppm, $^1J_{C\text{-}1,H\text{-}1}$ = 159.7 Hz). Fortuitously, they could be separated by column chromatography. Similar treatment of ulosyl bromide **2-117** with disaccharide **2-97,** this time using 2,6-di-*tert*-butyl-4-methyl-pyridine (DTBMP) as the base in the glycosylation step, gave the trisaccharide **2-126** as an inseparable mixture of anomers (α:β = 1:2.3, α-linkage, δ_H 5.48 ppm, $^1J_{C\text{-}1,H\text{-}1}$ = 174.1 Hz, β-linkage, δ_H 4.46 ppm, $^1J_{C\text{-}1,H\text{-}1}$ = 155.6 Hz) in 75% yield after purification. As in the products produced from the acetylated ulosyl bromide, the reduction proceeded with high diastereocontrol.



Scheme 2-24: [1 + 2] Glycosylation of **2-97** with ulosyl bromide donors **2-96** and **2-117**.

To the best of our knowledge, these glycosylations constitute the first examples of using heptose derived ulosyl bromides as glycosyl donors. However, because the stereoselectivity of [1 + 2] glycosylations with ulosyl bromide approach produced, unexpectedly, the desired β-linked trisaccharides with moderate selectivity, we explored an oxidation–reduction approach for the synthesis of trisaccharide **2-126**. The high selectivity seen in the ketone reduction of glycosylation products suggested this approach would be successful.

2.4.4 Stereoselective synthesis of 2-9a through an oxidation–reduction approach

2.4.4.1 Retrosynthetic analysis involving glycosyl bromide 2-127

The revised retrosynthetic plan for the synthesis of trisaccharide **2-9a** is outlined in Figure 2-12. We envisioned taking advantage of the completely stereoselective reduction described above, by switching from the ulosyl bromide approach to a C-2 oxidation–reduction approach. This approach was facilitated by the fact that the required heptosyl donor, glycosyl bromide **2-127**, can be accessed from the previously-prepared 1,2-orthoacetate **2-118** in one step. Glycosylation of acceptor **2-97** (prepared above) with glycosyl bromide **2-127** followed by C-2

inversion using an oxidation–reduction approach would generate the 1,2-*cis*-β-heptosidic linkage. Finally, we envisioned that the phosphate could be introduced after the [1 + 2] glycosylation and TBDPS deprotection.

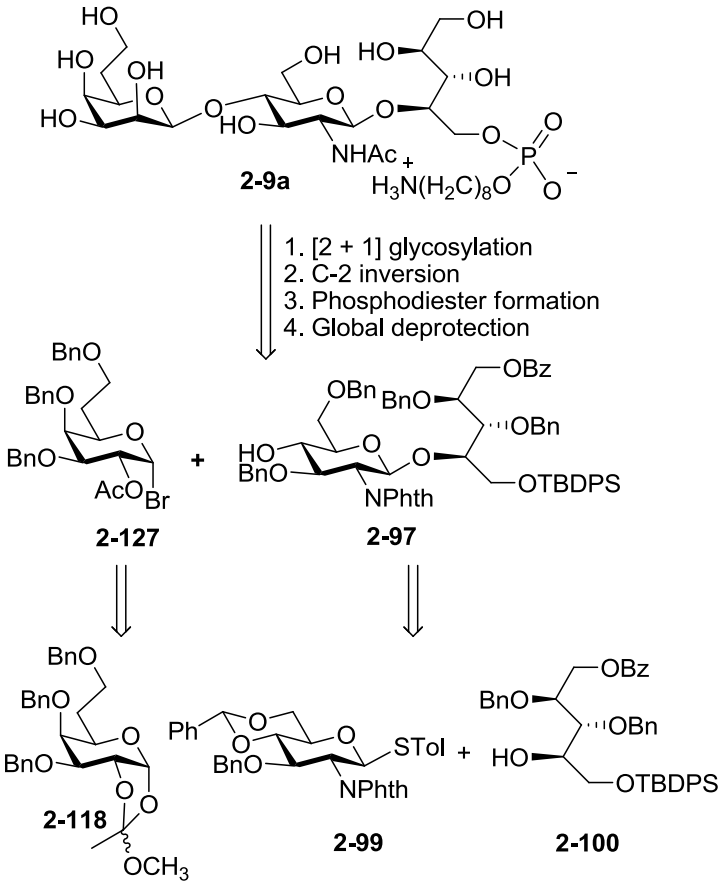
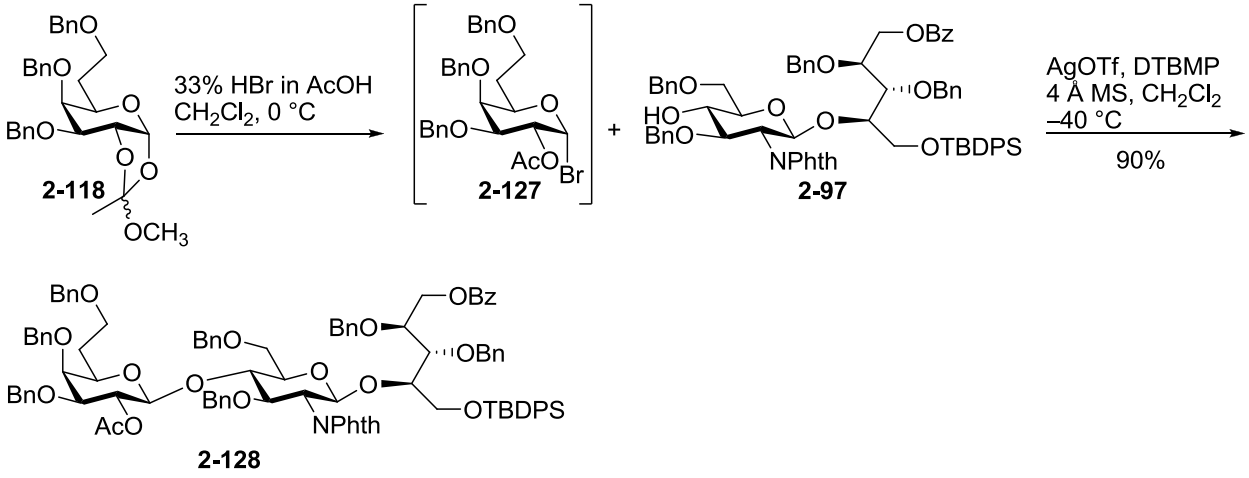


Figure 2-12: Retrosynthetic analysis of **2-9a** involving a [1 + 2] glycosylation approach.

2.4.4.2 Synthesis of glycosyl bromide 2-127 and successful [1 + 2] glycosylation

Transformation of 1,2-orthoacetate **2-118** into glycosyl bromide donor **2-127** and successful [1 + 2] glycosylation is outlined in Scheme 2-25. The synthesis of disaccharide acceptor **2-97** is described above in Section 2.4.3.7. When treated with HBr and acetic acid, 1,2-orthoacetate **2-118** afforded glycosyl bromide **2-127**, which was then used immediately to

glycosylate **2-97** employing silver triflate at –40 °C. This reaction furnished the β-linked trisaccharide **2-128** in 90% yield over the two steps (β-linkage, δ_H 4.39 ppm, $J_{1,2}$ = 7.7 Hz).



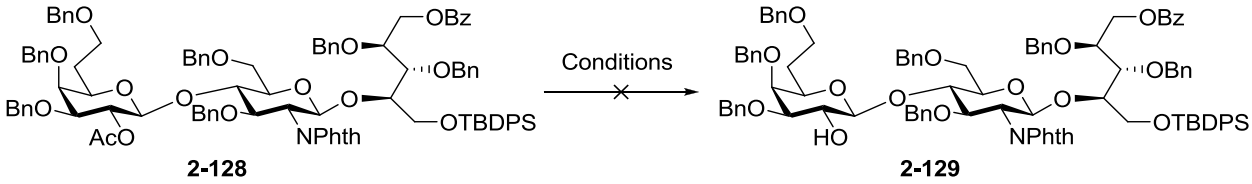
Scheme 2-25: Synthesis of glycosyl bromide **2-127** and successful [1 + 2] glycosylation.

2.4.4.3 Attempted oxidation–reduction of 2-128 to obtain β-*talo*-heptosidic linkage

To apply the oxidation–reduction approach to trisaccharide **2-128**, we first needed to remove the 2-*O*-acetyl protecting group selectively in the presence of the primary benzoate and TBDPS protecting groups. The attempted de-*O*-acetylation conditions tested are outlined in Table 2-3. Treatment of trisaccharide **2-128** with DBU and methanol[51], *p*-TSA and methanol[52] or tetrafluoroboric acid and methanol[53] resulted in loss of both the acetate and benzoate protecting groups. The use of acetyl chloride and methanol[54] cleaved both the acetate and the benzoate protecting groups, as well as the TBDPS ether. The unsuccessful selective de-*O*-acetylation is likely because the acetate protecting group is on a secondary carbon whereas the benzoate protecting group is on a primary carbon. Hence, the benzoate is similarly reactive to the acetate

towards the deprotection conditions we attempted. Due to our inability to produce alcohol **2-129**, we abandoned this route.

Table 2-3: Conditions explored for selective de-*O*-acetylation of trisaccharide **2-128**.



2-128 **2-129**

Entry	Conditions	Yield	Result	Comments
1	DBU, CH_3OH	0%	diol	both acetate (OAc) and benzoate (OBz) were cleaved
2	*p*-TSA, CH_3OH	0%[a]	diol	reaction is slow
3	HBF_4, CH_3OH	0%	diol	both OAc and OBz cleaved
4	CH_3COCl, CH_3OH	0%	diol plus triol mixture	both OAc and OBz cleaved and TBDPS also effected

[a] Starting material (80%) recovered after 24 h.

2.4.4.4 Revised retrosynthetic analysis of trisaccharide 2-9a from glycosyl bromide 2-127

Given the difficulties described above, another strategy was explored (Figure 2-13). One approach to overcome the selective de-*O*-acetylation problem is to change the [1 + 2] glycosylation to a [2 + 1] approach. This strategy requires building the non-reducing end disaccharide **2-130** first, rather than the reducing end disaccharide **2-97** (shown in Figure 2-12). The revised retrosynthetic plan is provided in Figure 2-13. Disaccharide **2-130** can be obtained from glycosyl bromide **2-127** and acceptor **2-131**. The desired β-*talo*-heptosidic linkage could be obtained by C-2 inversion of **2-130**. The trisaccharide could be obtained by glycosylation of the

C-2 inverted disaccharide **2-130** and ribitol acceptor **2-100**. Finally, we envisioned that the phosphate could be introduced into the differentially protected ribitol derivative after removal of the TBDPS protecting group in **2-9b**.

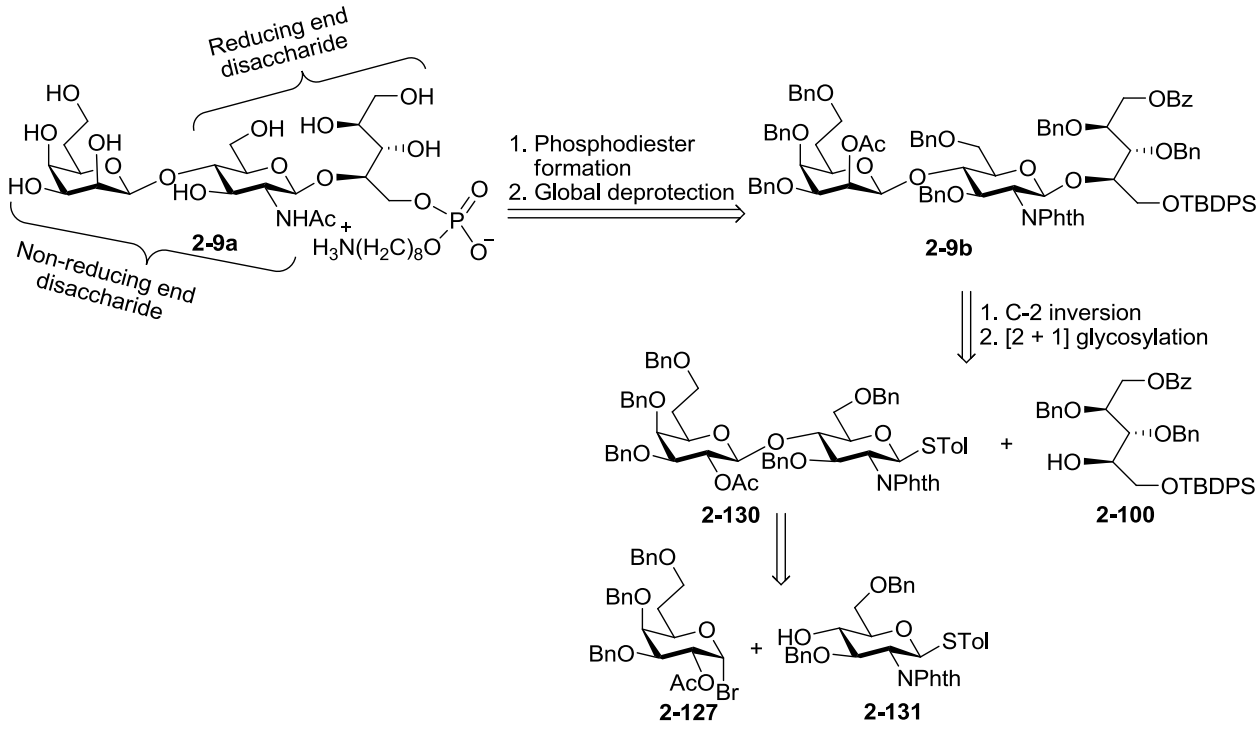
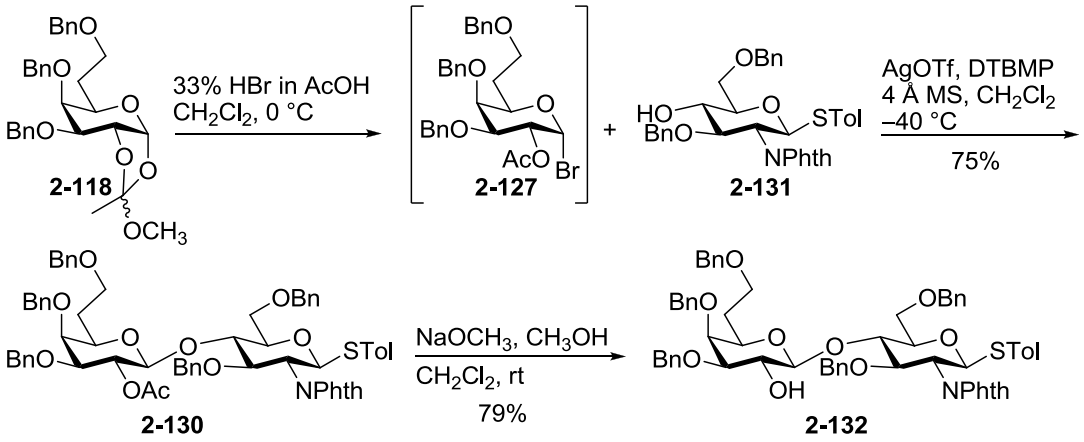


Figure 2-13: Retrosynthetic analysis of trisaccharide **2-9a** involving a [2 + 1] glycosylation.

2.4.4.5 Synthesis of non-reducing end disaccharide 2-130

The synthesis of disaccharide **2-130** is outlined in Scheme 2-26. The preparation of D-glucosamine acceptor **2-131** was done starting from thioglycoside **2-99** (shown in Scheme 2-22) using a reported protocol.[55] Glycosyl bromide **2-127** was generated from 1,2-orthoacetate **2-118** upon treatment with HBr in acetic acid and then used immediately to glycosylate **2-131** promoted by silver triflate at –40 °C. This reaction afforded the β-linked disaccharide **2-130** in 75% yield over the two steps (β-linkage, δ_H 5.49 ppm, $J_{1,2}$ = 10.0 Hz). Zemplén deacetylation of

2-130 using sodium methoxide in methanol furnished disaccharide **2-132**, which contains a free hydroxyl group, in 79% yield. This approach circumvented the selective deprotection problems seen in the earlier route.

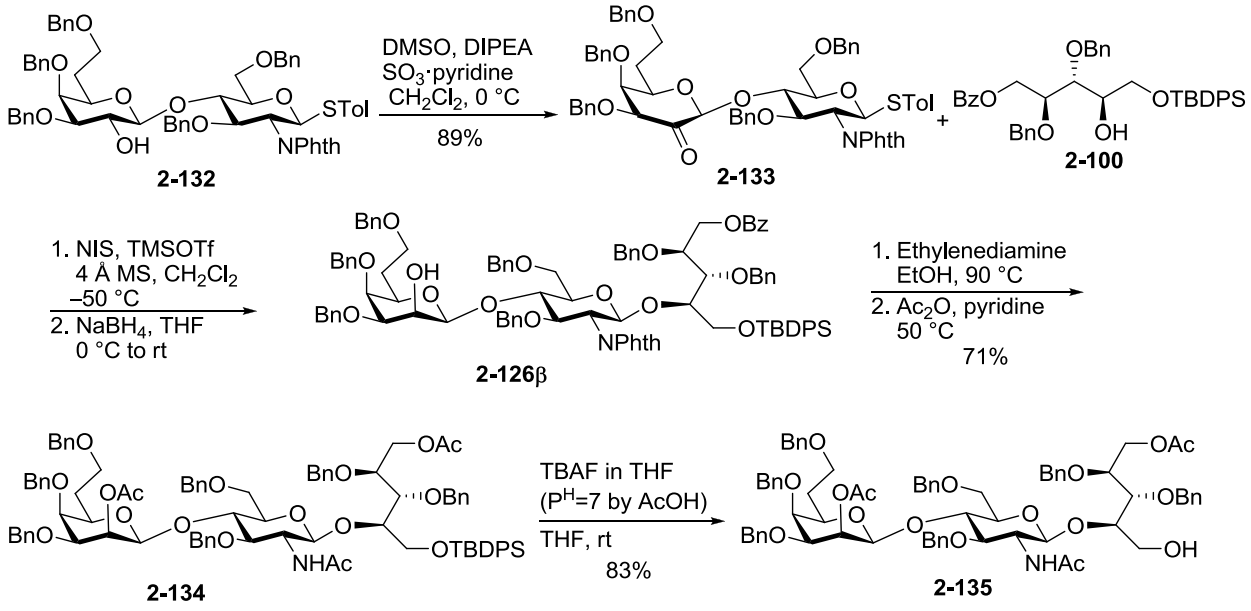


Scheme 2-26: Synthesis of disaccharide **2-130**.

2.4.4.6 Successful oxidation–reduction to obtain β-*talo*-heptosidic linkage

Having synthesized **2-132**, Parikh-Doering oxidation[31] cleanly generated 2-uloside **2-133** in 89% yield (Scheme 2-27, β-linkage, δ_H ~4.92 ppm, $^1J_{C\text{-}1,H\text{-}1}$ = 155.1 Hz). It is interesting to note that **2-133** exists as an inseparable mixture of ketone (uloside) and geminal diol (hydrate) in a ~5:1 ratio as determined by ^{1}H NMR spectroscopy through integration of the H-1$_{GlcN}$ signal. To reduce one protection step, we performed the subsequent glycosylation with the ribitol acceptor **2-100** without reducing the ketone. This was done by reaction of **2-133** and **2-100** promoted by NIS and TMSOTf at –50 °C. After glycosylation, reduction of the uloside trisaccharide using $NaBH_4$ was carried out. Gratifyingly, this sequence yielded trisaccharide **2-126** (β-linkage$_{TalHep}$, δ_H 4.47 ppm, $^1J_{C\text{-}1,H\text{-}1}$ = 156.9 Hz, β-linkage$_{GlcN}$, δ_H 5.48 ppm, $J_{1,2}$ = 8.5 Hz) in 80% yield. Treatment of **2-126** with ethylenediamine at 90 °C cleaved both phthalimide and the benzoate

groups. Acetylation of the intermediate product was achieved by reaction with acetic anhydride and pyridine at 50 °C. This sequence yielded trisaccharide **2-134** in 71% yield over the two steps. Deprotection of the TBDPS protecting group in **2-134** was achieved by treatment with buffered tetrabutylammonium fluoride solution, giving **2-135** in 83% yield.



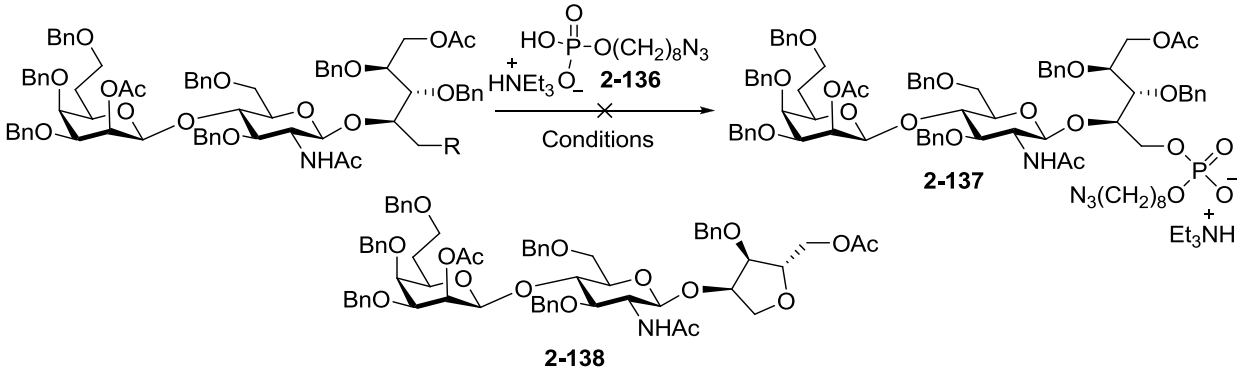
Scheme 2-27: Stereoselective β-*talo*-heptosidic linkage formation using an oxidation–reduction approach on disaccharide **2-132**.

2.4.4.7 Attempted phosphodiester formation on trisaccharide 2-135

We planned to incorporate an azidooctyl linker through the phosphate. Because trisaccharide **2-135** is more valuable than azidooctanol, we phosphorylated azidooctanol using $POCl_3$ to generate **2-136** in 90% yield with >90% purity and planned to use that in excess in the coupling reaction. The conditions explored for phosphodiester formation between trisaccharide **2-135** and phosphate **2-136** are illustrated in Table 2-4. Carbodiimides and arylsulfonyl chlorides were introduced by Khorana and coworkers as effective agents for phosphodiester formation.[56]

We initially explored coupling **2-136** with trisaccharide **2-135** using *N*-(3-dimethylaminopropyl)-*N′*-ethylcarbodiimide hydrochloride and triethylamine, but under these conditions no product was formed; only the starting material was isolated. The use of 2,4,6-triisopropylbenzenesulfonyl chloride as the condensing agent also resulted in the reisolation of **2-135**. Therefore, trisaccharide **2-135** was converted to methanesulfonate ester **2-139** in an anticipation of S_N2 reaction between phosphate **2-136** and sulfonate **2-139**. Under these conditions, no product was formed at room temperature in acetonitrile. Reflux at 80 °C resulted in a new polar spot on TLC, which after isolation showed the loss of a benzyl group. NMR analysis showed that the product formed was cyclic ether **2-138**, not the desired phosphodiester **2-137**. Debenzylative cycloetherifications are well precedented in the literature[57] and are common in systems when sulfonate esters and benzyloxy groups are in 1,4-relationship. Because the phosphorylation of azidooctanol followed by coupling to the trisaccharide did not yield the desired phosphodiester, we envisioned phosphorylating trisaccharide **2-135** and then coupling with azidooctanol. The use of $POCl_3$ as a phosphorylation agent for phosphorylation of trisaccharide **2-135** again resulted in the isolation of cyclized product **2-138** in 40% yield along with the corresponding phosphate. Given these failures, we explored the H-phosphonate approach for phosphodiester formation.[58]

Table 2-4: Conditions explored for phosphodiester formation on trisaccharide **2-135**.



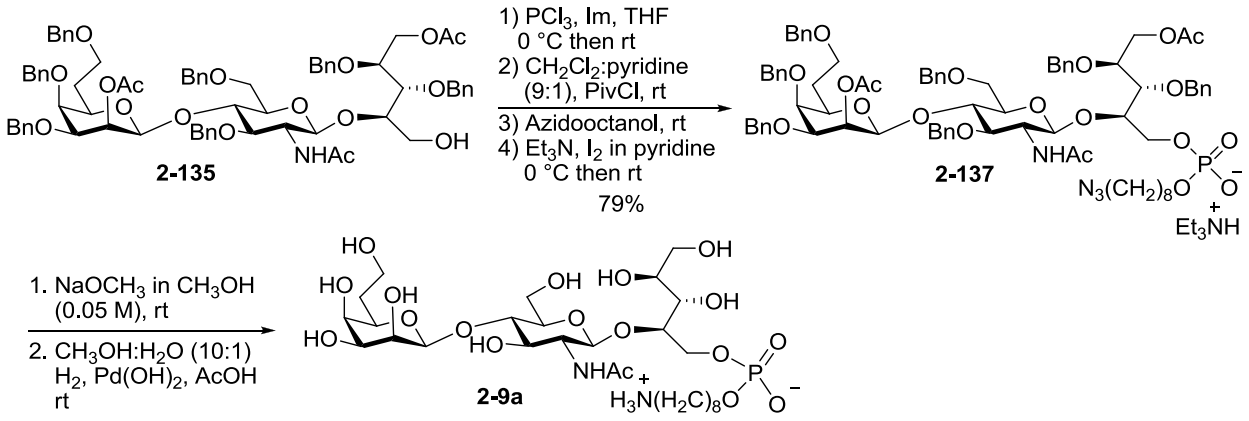
Entry	R	Conditions	Product, yield[a]
1	OH, **2-135**	**2-136**, EDC·HCl, Et_3N, THF, reflux	**2-137**, 0%[b]
2	OH, **2-135**	**2-136**, 2,4,6-triisopropylbenzenesulfonyl chloride, CH_2Cl_2, pyridine	**2-137**, 0%[b]
3	OMs, **2-139**	**2-136**, CH_3CN, rt	**2-137**, 0%[b]
4	OMs, **2-139**	**2-136**, CH_3CN, 80 °C	**2-138**, 62%
5	OH, **2-135**	$POCl_3$, CH_2Cl_2, 0 °C to rt	**2-138**, 40%

[a] Isolated yield. [b] Starting material recovered.

2.4.4.8 Successful phosphodiester formation on trisaccharide 2-135, elaboration into target trisaccharide 2-9a and comparison to the natural CPS

The successful phosphodiester formation on **2-135** and elaboration into trisaccharide **2-9a** is illustrated in Scheme 2-28. Trisaccharide **2-135**, when treated with phosphorus trichloride and imidazole, furnished the desired H-phosphonate. Activation of this intermediate H-phophonate with pivaloyl chloride (PivCl)[59] in a solution of dichloromethane and pyridine followed by treatment with azidooctanol afforded the corresponding dialkyl phosphite (H-phosphonate).

Oxidation of with iodine and pyridine[58a] gave trisaccharide **2-137** containing a phosphodietser linkage in 79% yield over the four steps. Zemplén deacetylation of **2-137** furnished a diol that was then subjected to simultaneous hydrogenolysis and azide reduction using $Pd(OH)_2$ to afford target trisaccharide **2-9a** in 65% yield over two steps.



Scheme 2-28: Successful phosphodiester formation and elaboration into target compound **2-9a**.

With **2-9a** in hand, to allow the determination of the stereochemistry of the ribitol moiety in the natural CPS, comparison of the NMR data with that previously reported for the CPS[8] repeating unit was carried out. We note the significant differences in the chemical shifts of the ribitol C-2 and C-3 in ^{13}C NMR and phosphorous in ^{31}P NMR. The ^{31}P NMR spectrum of **2-9a** showed a signal at 0.92 ppm and the resonance for this atom in the CPS is showed at 2.5 ppm. Similarly, the ^{13}C NMR spectrum of **2-9a** showed a signal at 72.5 ppm for C-3 ribitol, at 72.7 ppm for C-3 ribitol, and the resonance for these atoms in the CPS is showed at 70.1 ppm for C-2 and 70.6 ppm for C-3 respectively. This data suggests that the stereochemistry of ribitol moiety proposed by Aspinall and co-workers is incorrect. Therefore, we hypothesized that the

stereochemistry of the ribitol moiety would be the enantiomer of what they proposed as shown in the Figure 2-14 below.

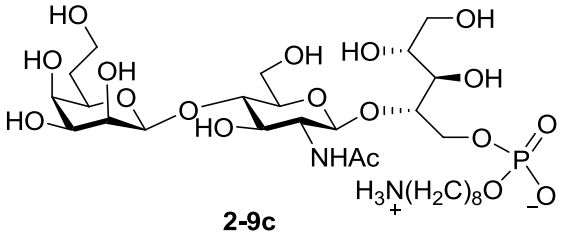


Figure 2-14: Proposed structure of the trisaccharide repeating unit of *C. coli* serotype O:30 CPS.

2.5 Summary

In summary, the work described in this chapter provides an efficient and rapid methodology to access *p*-tolyl 6-deoxy-1-thio-D-*manno*- (**2-52**), 6-deoxy-1-thio-D-*talo*- (**2-53**), 6-deoxy-1-thio-D-*altro*-heptopyranosides (**2-67** and **2-68**) through C-6 homologation by bis(phenylthio)methane from commercially available methyl α-D-mannopyranoside **2-37**. Compound **2-52** can be obtained in seven steps in 55% overall yield, compound **2-53** can be obtained in nine steps in 47% overall yield, and **2-68** can be obtained in 15 steps in 10% overall yield. These thioglycosides[60] can act as either donors or acceptors and can be used to access glycoconjugates. In addition, we extended this methodology to the first total synthesis of the trisaccharide repeating unit of the *C. coli* serotype O:30 CPS (**2-9a**) in a convergent manner. In developing this route, introduction of the β-D-*talo*-heptopyranoside linkage with the ulosyl bromide approach gave moderate overall diastereoselectivity. A different approach, using glycosyl bromides and oxidation–reduction of disaccharide **2-132**, was developed to obtain the β-D-*talo*-heptopyranoside stereoselectively using a [2 + 1] glycosylation. The phosphodiester linkage was introduced using the H-phosphonate derived from trisaccharide **2-135**, activation

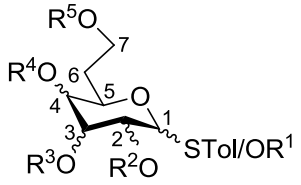
using PivCl and oxidation using iodine and pyridine. Trisaccharide **2-9a** was synthesized in a form suitable for conjugation to proteins and/or probes and hence its immunogenicity can be tested.

2.6 Experimental

2.6.1 General experimental methods

8-Azido-1-octanol was prepared according to the literature protocol.[61] All reactions were carried out in oven-dried glassware. All reagents used 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 under a positive pressure of argon and were 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 at 40 °C. ^{1}H NMR spectra were recorded at 500 or 700 MHz and were referenced to the residual proton signal of $CDCl_3$ (7.26 ppm), CD_3OD (3.30 ppm) or HOD (4.79 ppm). $^{13}C\{^1H\}$ NMR spectra were recorded at 125 (cold probe) or 175 MHz and were referenced to the ^{13}C signals of $CDCl_3$ (77.06 ppm) or CD_3OD (49 ppm). $^{31}P\{^1H\}$ NMR spectra were collected at 202 or 162 MHz and were referenced to an external 85% H_3PO_4 standard (0.00 ppm). ^{1}H NMR data are reported as if they were first order, and peak assignments were made on the basis of 2D NMR (^1H–^1H COSY, APT, TOCSY, HSQC and HMBC) experiments. ESI-MS spectra (time-of-flight analyzer) were recorded on samples dissolved in THF or CH_3OH with added NaCl or NH_4Cl. Optical rotations were measured at 22 ± 2 °C at the

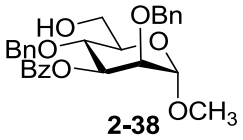
sodium D line (589 nm) in a microcell (10 cm, 1 mL) and are in units of deg·mL(dm·g)$^{-1}$. IR spectra were recorded at room temperature on a Thermo Nicolet 8700 FT-IR spectrometer with a continuum FT-IR microscope attached using IR-transparent silicone wafer cast films. Carbon or proton signals in the 6-deoxy-heptose sugars are numbered as shown in the figure below.



2-140

Figure 2-15: Legend for numbering proton or carbon signals in the 6-deoxy-heptose sugar.

2.6.2 Experimental, spectroscopic and analytical data



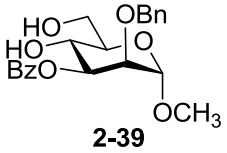
2-38

Methyl 3-*O*-benzoyl-2,4-di-*O*-benzyl-α-D-mannopyranoside (2-38)

To a stirred solution of tetrol **2-37** (795 mg, 4.09 mmol) in THF (9 mL) and H_2O (1 mL) was added dimethyltin dichloride (45.0 mg, 0.205 mmol), DIPEA (1.45 mL, 8.32 mmol) followed by benzoyl chloride (0.55 mL, 4.7 mmol). The mixture was stirred for 2.5 h before a 3% aqueous HCl solution (20 mL) was added and the solution was extracted with EtOAc (2 × 20 mL). The combined organic phases were washed with brine (20 mL), dried over anhydrous Na_2SO_4, filtered and concentrated and the resulting crude product was purified by column chromatography (80% EtOAc in hexane) to afford known methyl 3-*O*-benzoyl-2,4-di-*O*-benzyl-α-D-mannopyranoside.[14] The data for this material matched that reported.[14] This compound was

dissolved in CH_3CN (12 mL), before camphorsulfonic acid (230 mg, 0.990 mmol) and benzaldehyde dimethyl acetal (0.75 mL, 5.0 mmol) were added and the reaction mixture was heated at 60 °C for 2 h. The solution was cooled to room temperature over 20 min. The camphorsulfonic acid was quenched by the addition of Et_3N (0.5 mL) and the solution was concentrated. The resulting crude product was dissolved in CH_2Cl_2 (15 mL) and freshly prepared Ag_2O[62] (1.80 g, 7.78 mmol) was added and the solution was stirred for 9 h. The reaction mixture was diluted with CH_2Cl_2 (50 mL) and filtered through a pad of Celite-545. The filtrate was concentrated and the resulting crude product was purified by column chromatography (25% EtOAc in hexane) to afford known methyl 3-*O*-benzoyl-2-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside.[63] The data for this material matched that reported.[63] This compound was dissolved in CH_2Cl_2 (15 mL) and TMSOTf (70 μL, 0.39 mmol) was added followed by BH_3·THF (13 mL, 1.0 M in THF, 13 mmol). After stirring for 1.5 h, excess BH_3·THF was quenched by the addition of Et_3N (0.5 mL) and the solution was concentrated. The resulting crude product was purified by column chromatography (30% EtOAc in hexane) to afford **2-38** (1.12 g, 57% from **2-37**) as a colorless viscous liquid: $[\alpha]^{22}_D$ –14.5 (*c* 0.70, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.09–8.06 (m, 2 H, ArH), 7.64–7.20 (m, 13 H, ArH), 5.56 (dd, *J* = 9.5, 3.0 Hz, 1 H, H-3), 4.78, 4.69 (ABq, *J* = 11.0 Hz, 2 H, 2 × OC*H*$_2$Ph), 4.78 (d, *J* = 1.5 Hz, 1 H, H-1), 4.67–4.62 (m, 2 H, 2 × OC*H*$_2$Ph), 4.24 (app t, *J* = 9.5, 9.5 Hz, 1 H, H-4), 4.03 (dd, *J* = 3.0, 1.5 Hz, 1 H, H-2), 3.92 (dd, *J* = 12.0, 3.0 Hz, 1 H, H-6a), 3.86 (dd, *J* = 12.0, 4.0 Hz, 1 H, H-6b), 3.80 (app dt, *J* = 9.5, 4.0, 3.5 Hz, 1 H, H-5), 3.40 (s, 3 H, OC*H*$_3$), 2.18 (s, 1 H, O*H*); ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.7 (*C*OPh), 137.9 (Ar), 137.6 (Ar), 133.2 (Ar), 130.0 (Ar), 129.8 (Ar), 128.5 (Ar), 128.39 (Ar), 128.38 (Ar), 128.3 (Ar), 128.0 (Ar), 127.9 (Ar), 127.8 (Ar), 127.7 (Ar), 99.2 (C-1), 76.1 (C-2), 75.0 (O*C*H$_2$Ph), 74.6 (C-3), 73.31 (O*C*H$_2$Ph), 73.28 (C-4), 71.9 (C-5), 62.1 (C-

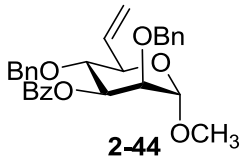
6), 55.0 (O<u>*C*</u>H$_3$); IR (cast film, CHCl$_3$) ν 3494, 3063, 3032, 2930, 2836, 1721, 1496, 1452, 1097, 714 cm^{-1}; HRMS (ESI-TOF) m/z: [M + Na]$^+$ Calcd for $C_{28}H_{30}O_7Na$ 501.1884; Found 501.1880.



2-39

Methyl 3-*O*-benzoyl-2-*O*-benzyl-α-D-mannopyranoside (2-39)

Known methyl 3-*O*-benzoyl-2-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside[63] (1.50 g, 3.13 mmol, prepared according to the procedure described in the synthesis of **2-38**) was dissolved in 90% AcOH (20 mL) and the reaction mixture was heated at 80 °C for 2.5 h, after which time the solution was cooled to room temperature over 30 min. Excess AcOH was concentrated immediately after the addition of Et$_3$N (0.5 mL). The resulting crude product was purified by column chromatography (50% EtOAc in hexane) to afford **2-39** (1.03 g, 53% from **2-37**) as a colorless viscous liquid: $[\alpha]^{22}_D$ +27.0 (*c* 0.55, CHCl$_3$); ^{1}H NMR (500 MHz, CDCl$_3$) δ 8.09–8.03 (m, 2 H, ArH), 7.60–7.20 (m, 8 H, ArH), 5.35 (dd, *J* = 10.0, 3.5 Hz, 1 H, H-3), 4.76 (d, *J* = 1.5 Hz, 1 H, H-1), 4.65 (s, 2 H, 2 × OC<u>*H*</u>$_2$Ph), 4.26 (app t, *J* = 10.0, 9.5 Hz, 1 H, H-4), 3.96 (dd, *J* = 3.5, 1.5 Hz, 1 H, H-2), 3.93 (dd, *J* = 11.5, 3.5 Hz, 1 H, H-6a), 3.89 (dd, *J* = 11.5, 4.5 Hz, 1 H, H-6b), 3.74–3.70 (m, 1 H, H-5), 3.38 (s, 3 H, OC<u>*H*</u>$_3$), 2.67 (s, 1 H, O<u>*H*</u>), 2.27 (s, 1 H, O<u>*H*</u>); ^{13}C NMR (125 MHz, CDCl$_3$) δ 166.8 (<u>*C*O</u>Ph), 137.7 (Ar), 133.4 (Ar), 129.9 (Ar), 129.7 (Ar), 128.5 (Ar), 128.4 (Ar), 127.9 (Ar), 127.8 (Ar), 99.2 (C-1), 76.0 (C-2), 75.0 (C-3), 73.4 (O<u>*C*</u>H$_2$Ph), 72.4 (C-5), 66.7 (C-4), 62.6 (C-6), 55.0 (O<u>*C*</u>H$_3$); IR (cast film, CHCl$_3$) ν 3469, 3067, 3032, 2925, 2832, 1719, 1496, 1452, 1279, 715 cm^{-1}; HRMS (ESI-TOF) m/z: [M + Na]$^+$ Calcd for $C_{21}H_{24}O_7Na$ 411.1414; Found 411.1408.

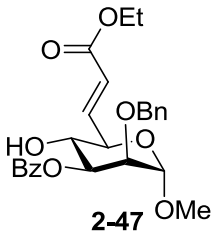


2-44

Methyl 3-*O*-benzoyl-2,4-di-*O*-benzyl-6,7-dideoxy-α-D-*manno*-hept-6-enopyranoside (2-44)

To a stirred solution of **2-38** (110 mg, 0.230 mmol) in CH_2Cl_2 (2 mL) was added TEMPO (4.0 mg, 0.026 mmol) and bis(acetoxy)iodobenzene (93 mg, 0.29 mmol). The mixture stirred for 8 h and then diluted with CH_2Cl_2 (20 mL) before being washed with saturated aqueous $Na_2S_2O_3$ solution (10 mL) and saturated aqueous $NaHCO_3$ solution (2 × 10 mL). The organic layer was separated and the aqueous layer was further extracted with CH_2Cl_2 (2 × 20 mL). The combined organic phases were washed with brine (15 mL), dried over anhydrous Na_2SO_4, filtered and concentrated and the resulting crude aldehyde product was dissolved in THF (3 mL). Separately, methyltriphenylphosphonium iodide (215 mg, 0.529 mmol) was dissolved in dry THF (3 mL) and cooled to 0 °C. LiHMDS (1.0 M in THF, 0.45 mL, 0.45 mmol) was added and the solution was stirred at 0 °C for 45 min, before the crude aldehyde described above in THF was added dropwise. The ice bath was removed and the reaction mixture was warmed to room temperature over 15 h. Excess methylenetriphenylphosphorane was quenched by the addition of saturated aqueous NH_4Cl solution (10 mL). The reaction mixture was extracted with CH_2Cl_2 (20 mL) and the aqueous layer was separated and further extracted with CH_2Cl_2 (2 × 20 mL). The combined organic phases were washed with brine (10 mL), dried over anhydrous Na_2SO_4, filtered and concentrated and the resulting crude product was purified by column chromatography (25% EtOAc in hexane) to afford **2-44** (62 mg, 57% over the two steps) as a colorless viscous liquid: $[\alpha]^{22}_D$ –15.2 (*c* 0.20, $CHCl_3$); ^{1}H NMR (700 MHz, $CDCl_3$) δ 8.09–8.02 (m, 2 H, ArH), 7.59–7.14 (m, 13 H, ArH), 6.03 (ddd, J = 17.2, 10.5, 7.0 Hz, 1 H, C*H*=CH_2), 5.53–5.48 (m, 2 H, H-3 and

CH=C$\underline{H_2}$), 5.31 (d, J = 10.5 Hz, 1 H, CH=C$\underline{H_2}$), 4.75 (s, 1 H, H-1), 4.67–4.56 (m, 4 H, 4 × OC$\underline{H_2}$Ph), 4.17 (app t, J = 9.1, 7.0 Hz, 1 H, H-5), 3.96 (s, 1 H, H-2), 3.91 (app t, J = 9.8, 9.1 Hz, 1 H, H-4), 3.37 (s, 3 H, OC$\underline{H_3}$); ^{13}C NMR (175 MHz, $CDCl_3$) δ 165.7 ($\underline{C}$OPh), 138.0 (Ar), 137.9 (Ar), 135.2 ($\underline{C}$H=CH_2), 133.1 (Ar), 130.1 (Ar), 129.8 (Ar), 128.54 (Ar), 128.46 (Ar), 128.41 (Ar), 128.35 (Ar), 128.3 (Ar), 128.09 (Ar), 128.06 (Ar), 127.9 (Ar), 127.79 (Ar), 127.77 (Ar), 127.6 (Ar), 127.5 (Ar), 118.6 (CH=$\underline{C}H_2$), 99.0 (C-1), 77.3 (C-4), 76.1 (C-2), 74.9 (O$\underline{C}H_2$Ph), 74.1 (C-3), 73.2 (O$\underline{C}H_2$Ph), 72.7 (C-5), 55.0 (O$\underline{C}H_3$); IR (cast film, $CHCl_3$) ν 3062, 3031, 2960, 2927, 2871, 1721, 1452, 1099, 1067, 714 cm^{-1}; HRMS (ESI-TOF) m/z: [M + Na]$^+$ Calcd for $C_{29}H_{30}O_6Na$ 497.1935; Found 497.1934.

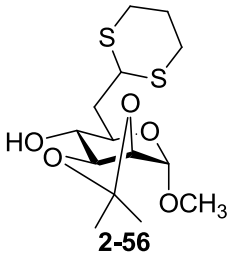


2-47

Ethyl [methyl (*E*)-3-*O*-benzoyl-2,4-di-*O*-benzyl-6,7-dideoxy-α-D-*manno*-oct-6-enopyranoside]uronate (2-47)

To a stirred solution of **2-39** (125 mg, 0.322 mmol) in CH_2Cl_2 (2.5 mL) was added TEMPO (5.0 mg, 0.032 mmol) and bis(acetoxy)iodobenzene (130 mg, 0.404 mmol). The mixture and stirred for 8 h and then diluted with CH_2Cl_2 (20 mL) and washed with saturated aqueous $Na_2S_2O_3$ solution (10 mL) and saturated aqueous $NaHCO_3$ solution (2 × 10 mL). The organic layer was separated and the aqueous layer was further extracted with CH_2Cl_2 (2 × 20 mL). The combined organic phases were washed with brine (15 mL), dried over anhydrous Na_2SO_4, filtered and concentrated to afford the crude aldehyde, which was dissolved in toluene (3 mL). In a separate flask, sodium hydride (60% in mineral oil, 30 mg, 0.75 mmol) was

dissolved in toluene (3 mL) and cooled to °C before triethyl phosphonoacetate **2-46** (0.15 mL, 0.76 mmol) was added. The so formed solution was added dropwise to the aldehyde solution in toluene and stirred at 0 °C for 20 min. Excess NaH was quenched by the addition of saturated aqueous NH_4Cl solution (10 mL). The reaction mixture was extracted with CH_2Cl_2 (20 mL) and the aqueous layer was separated and further extracted with CH_2Cl_2 (2 × 20 mL). The combined organic phases were washed with brine (10 mL), dried over anhydrous Na_2SO_4, filtered and concentrated and the resulting crude product was purified by column chromatography (30% EtOAc in hexane) to afford **2-47** (62 mg, 57% over the two steps) as a colorless viscous liquid: $[\alpha]^{22}_D$ +43.3 (*c* 0.98, $CHCl_3$); ^{1}H NMR (700 MHz, $CDCl_3$) δ 8.08–7.98 (m, 2 H, ArH), 7.60–7.15 (m, 9 H, 8 × ArH and C*H*=CHCO$_2$Et), 6.25 (d, *J* = 15.4 Hz, 1 H, CH=C*H*CO$_2$Et), 5.36 (dd, *J* = 9.8, 2.8 Hz, 1 H, H-3), 4.79 (s, 1 H, H-1), 4.65, 4.62 (ABq, *J* = 12.2 Hz, 2 H, 2 × OC*H*$_2$Ph), 4.27 (dd, *J* = 9.8, 3.5 Hz, 1 H, H-5), 4.17 (app q, *J* = 8.4, 7.0, 7.0 Hz, 2 H, OC*H*$_2$CH$_3$), 4.05 (app t, *J* = 9.8, 9.8 Hz, 1 H, H-4), 3.96 (s, 1 H, H-2), 3.36 (s, 3 H, OC*H*$_3$); ^{13}C NMR (175 MHz, $CDCl_3$) δ 166.7 (*C*O), 166.5 (*C*O), 143.9 (*C*H=CHCO$_2$Et), 137.7 (Ar), 133.3 (Ar), 129.9 (Ar), 129.8 (Ar), 129.6 (Ar), 128.6 (Ar), 128.4 (Ar), 128.3 (Ar), 127.8 (Ar), 127.7 (Ar), 122.4 (CH=*C*HCO$_2$Et), 99.2 (C-1), 75.8 (C-2), 74.8 (C-3), 73.3 (O*C*H$_2$Ph), 71.2 (C-5), 69.2 (C-4), 60.5 (O*C*H$_2$CH$_3$), 55.0 (O*C*H$_3$), 14.2 (OCH$_2$*C*H$_3$); IR (cast film, $CHCl_3$) *v* 3482, 3062, 3031, 2981, 2908, 1721, 1452, 1276, 1121, 1070, 714 cm^{-1}; HRMS (ESI-TOF) m/z: $[M + Na]^+$ Calcd for $C_{25}H_{28}O_8Na$ 479.1676; Found 479.1674.

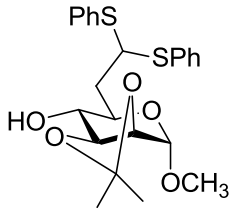


2-56

Methyl 6-deoxy-6-(2′-dithianyl)-2,3-*O*-isopropylidene-α-D-*manno*pyranoside (2-56)

To a stirred solution of 1,3-dithiane (893 mg, 7.43 mmol) and hexamethylphosphoric triamide (0.30 mL, 1.7 mmol) in THF (20 mL) at –30 °C was added *n*-butyllithium in hexane (1.60 M, 4.36 mL, 6.98 mmol). The resulting solution was stirred for 30 min at –30 °C. To this solution was then added a solution of **2-50** (500 mg, 1.45 mmol) in THF (7 mL) dropwise and the reaction mixture was stirred between –30 °C to 0 °C for 3 h. Excess lithiated 1,3-dithiane was quenched by the dropwise addition of saturated aqueous NH_4Cl solution (5 mL) and the reaction mixture was extracted with CH_2Cl_2 (100 mL). The aqueous layer was separated and further extracted with CH_2Cl_2 (2 × 100 mL) and then the combined organic phases were washed with brine (30 mL), dried over anhydrous Na_2SO_4, filtered and concentrated. The resulting crude product was purified by column chromatography (25% EtOAc in hexane) to afford **2-56** (269 mg, 55%) as a colorless viscous liquid: $[\alpha]^{22}_D$ +33.5 (*c* 0.27, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.81 (s, 1 H, H-1), 4.27 (dd, J = 10.5, 4.0 Hz, 1 H, C*H*S$_2$), 4.13–4.07 (m, 2 H, H-2 and H-3), 3.87 (app td, J = 9.5, 9.0, 3.0 Hz, 1 H, H-5), 3.50–3.43 (m, 1 H, H-4), 3.44 (s, 3 H, OC*H*$_3$), 2.89–2.74 (m, 4 H, 2 × SC*H*$_2$CH$_2$), 2.45 (d, J = 5.0 Hz, 1 H, O*H*), 2.34 (ddd, J = 14.1, 10.5, 3.0 Hz, 1 H, H-6a), 2.13–2.05 (m, 1 H, SCH$_2$C*H*$_2$), 1.98–1.86 (m, 2 H, H-6b and SCH$_2$C*H*$_2$), 1.51 (s, 3 H, ((C*H*$_3$)$_2$C)), 1.35 (s, 3 H, ((C*H*$_3$)$_2$C)); ^{13}C NMR (125 MHz, $CDCl_3$) δ 109.6 ((CH$_3$)$_2$*C*)), 98.3 (C-1), 78.3 (C-3), 75.6 (C-2), 72.9 (C-4), 66.4 (C-5), 55.5 (O*C*H$_3$), 42.8 (*C*HS$_2$), 37.8 (C-6), 29.9 (S*C*H$_2$CH$_2$), 29.4 (S*C*H$_2$CH$_2$), 28.0 ((*C*H$_3$)$_2$C)), 26.2 ((*C*H$_3$)$_2$C)), 26.0 (*C*H$_2$); IR (cast film,

$CHCl_3$) ν 3457, 2987, 2910, 1382, 1139, 1086, 756 cm^{-1}; HRMS (ESI-TOF) m/z: $[M + Na]^+$ Calcd for $C_{14}H_{24}O_5S_2Na$ 359.0957; Found 359.0958.

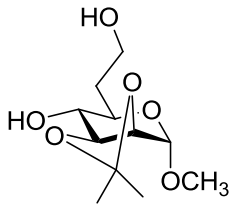


2-59

Methyl 6-deoxy-2,3-*O*-isopropylidene-α-D-*manno*-heptadialdo-1,5-pyranoside-7-(diphenyl dithioacetal) (2-59)

To a stirred solution of bis(phenylthio)methane (21.5 g, 92.6 mmol) in THF (200 mL) at –30 °C was added *n*-butyllithium in hexane (1.60 M, 54.5 mL, 87.2 mmol). The resulting solution was stirred for 30 min at –30 °C. To this solution was then added a solution of **2-50** (9.10 g, 14.5 mmol) in THF (50 mL) dropwise over 20 min and the mixture was stirred between –30 °C to 0 °C for 3 h. Excess lithiated diphenyldithiomethane was quenched by the addition of saturated aqueous NH_4Cl solution (30 mL). Approximately two-thirds of the volatiles were evaporated and the reaction mixture was extracted with CH_2Cl_2 (150 mL). The aqueous layer was separated and further extracted with CH_2Cl_2 (2 × 100 mL). The combined organic phases were washed with brine (30 mL), dried over anhydrous Na_2SO_4, filtered, concentrated and the resulting crude product was purified by column chromatography (25% EtOAc in hexane) to afford **2-59** (10.9 g, 92%) as a pale yellow solid: $[\alpha]^{22}_D$ –15.0 (*c* 0.71, $CHCl_3$); ^1H NMR (700 MHz, $CDCl_3$) δ 7.52 (d, J = 7.7 Hz, 2 H, ArH), 7.46 (d, J = 7.7 Hz, 2 H, ArH), 7.32–7.24 (m, 6 H, ArH), 4.84 (s, 1 H, H-1), 4.76 (dd, J = 10.5, 4.2 Hz, 1 H, C*H*S_2), 4.13–4.10 (m, 2 H, H-2 and H-3), 4.05 (app td, J = 9.8, 9.1, 2.8 Hz, 1 H, H-5), 3.45 (ddd, J = 9.1, 5.6, 5.0 Hz, 1 H, H-4), 3.35 (s, 3 H, OC*H*$_3$), 2.41 (d, J = 5.0 Hz, 1 H, O*H*), 2.30–2.26 (m, 1 H, H-6a), 2.18–2.14 (m, 1 H, H-

6b), 1.45 (s, 3 H, (C$\underline{H}_3$)$_2$C), 1.34 (s, 3 H, (C$\underline{H}_3$)$_2$C); ^{13}C NMR (125 MHz, $CDCl_3$) δ 134.2 (Ar), 133.4 (Ar), 132.4 (Ar), 132.3 (Ar), 128.9 (Ar), 128.8 (Ar), 127.6 (Ar), 127.5 (Ar), 109.5 ((CH$_3$)$_2\underline{C}$)), 98.3 (C-1), 78.2 (C-3), 75.4 (C-2), 72.5 (C-4), 67.2 (C-5), 55.5 (O$\underline{C}$H$_3$), 53.1 ($\underline{C}$HS$_2$), 38.4 (C-6), 27.8 (($\underline{C}$H$_3$)$_2$C)), 26.0 (($\underline{C}$H$_3$)$_2$C)); IR (cast film, $CHCl_3$) ν 3461, 3058, 2988, 2933, 2835, 1582, 1480, 1439, 1086, 750 cm^{-1}; HRMS (ESI-TOF) m/z: [M + Na]$^+$ Calcd for $C_{23}H_{28}O_5S_2Na$ 471.1270; Found 471.1266.

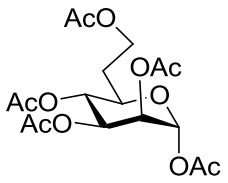


2-58

Methyl 6-deoxy-2,3-*O*-isopropylidene-α-D-*manno*-heptopyranoside (2-58)

To a stirred solution of dithioacetal **2-59** (9.235 g, 20.59 mmol) and solid $NaHCO_3$ (10.4 g, 124 mmol) in 1,4-dioxane (100 mL) and H_2O (4 mL) was added iodine (21.4 g, 84.3 mmol) in several portions. The resulting mixture was stirred for 12 h. Excess iodine was quenched by the addition of solid $Na_2S_2O_3$ (13 g) and the solution was filtered to separate solids. The reaction flask was washed with EtOAc (3 × 10 mL) to remove material that adhered to the walls. The organic layer was washed with H_2O (15 mL) and the aqueous layer was separated and further extracted with EtOAc (2 × 100 mL). The combined organic phases were washed with brine (30 mL), dried over anhydrous Na_2SO_4, filtered and concentrated to obtain the crude aldehyde as a pale-yellow viscous liquid. Without further purification, this crude aldehyde was dissolved in EtOH (100 mL) and cooled to 0 °C. $NaBH_4$ (1.56 g, 41.2 mmol) was added portionwise to the reaction mixture and then the ice bath was removed and the reaction mixture was warmed to

room temperature over 2 h. Excess $NaBH_4$ was quenched by the dropwise addition of saturated aqueous NH_4Cl solution until no effervescence was observed. The reaction mixture was diluted with EtOAc (75 mL) and the aqueous layer was separated and further extracted with EtOAc (3 × 100 mL). The combined organic phases were dried over anhydrous Na_2SO_4, filtered and concentrated and the resulting crude product was purified by column chromatography (75% EtOAc in hexane) to afford **2-58** (4.8 g, 94%) as a colorless viscous liquid. The ^1H and ^{13}C NMR data of **2-58** matched that reported.[20a]

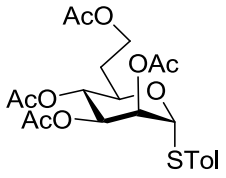


2-60, α/β = 10:0.7

1,2,3,4,7-Penta-*O*-acetyl-6-deoxy-α/β-D-*manno*-heptopyranose (2-60)

To a stirred solution of diol **2-58** (5.0 g, 20 mmol) in AcOH (22 mL) and Ac_2O (11 mL) was added camphorsulfonic acid (5.20 g, 22.4 mmol). The reaction mixture was heated at 80 °C for 7 h, after which time the solution was cooled to room temperature over 20 min. To this solution, concentrated H_2SO_4 (4.5 mL) dissolved in AcOH (4 mL) was added dropwise over 5 min and the resulting solution was stirred for 7 h and then diluted with EtOAc (50 mL). The mixture was transferred to a separatory funnel and saturated aqueous $NaHCO_3$ solution was added until no effervescence was observed. The resulting solution was extracted with EtOAc (150 mL) and the aqueous layer was separated and further extracted with EtOAc (2 × 150 mL). The combined organic phases were washed with brine (50 mL), dried over anhydrous Na_2SO_4, filtered, concentrated and the resulting crude product was purified by column chromatography

(50% EtOAc in hexane) to afford **2-60** (7.4 g, 91%) as a pale yellow viscous liquid. The ^{1}H and ^{13}C NMR data of **2-60** matched that reported.[10a,20a]

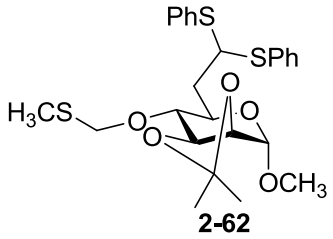


2-52, α/β = 10:1

***p*-Tolyl 2,3,4,7-tetra-*O*-acetyl-6-deoxy-1-thio-α/β-D-*manno*-heptopyranoside (2-52)**

To a stirred solution of pentaacetate **2-60** (8.43 g, 20.8 mmol) and *p*-toluenethiol (3.40 g, 27.4 mmol) in CH_2Cl_2 (30 mL) at 0 °C was added $BF_3{\cdot}OEt_2$ (5.10 mL, 40.2 mmol) dropwise over a period of 2 min. The ice bath was removed and the reaction mixture was stirred for 20 h. Excess $BF_3{\cdot}OEt_2$ was quenched by the addition of saturated aqueous $NaHCO_3$ solution and the reaction mixture was extracted with CH_2Cl_2 (75 mL). The aqueous layer was separated and further extracted with CH_2Cl_2 (2 × 75 mL). The combined organic phases were washed with brine (30 mL), dried over anhydrous Na_2SO_4, filtered, concentrated and the resulting crude product was purified by column chromatography (45% EtOAc in hexane) to afford **2-52** (9.2 g, 94%) as a pale yellow viscous liquid (10:1 diastereomeric ratio): ^{1}H NMR (500 MHz, $CDCl_3$, major isomer) δ 7.32 (m, 2 H, ArH), 7.11 (m, 2 H, ArH), 5.49 (dd, J = 3.5, 1.0 Hz, 1 H, H-2), 5.36 (d, J = 1.0 Hz, 1 H, H-1), 5.30 (dd, J = 9.5, 3.5 Hz, 1 H, H-3), 5.17 (app t, J = 9.5, 9.5 Hz, 1 H, H-4), 4.37 (app td, J = 9.5, 9.5, 2.5 Hz, 1 H, H-5), 4.11 (ddd, J = 11.0, 6.5, 5.0 Hz, 1 H, H-7a), 3.98 (ddd, J = 11.0, 9.0, 6.0 Hz, 1 H, H-7b), 2.32 (s, 3 H, C$\underline{H_3}$C$_6$H$_4$S), 2.14 (s, 3 H, 3 × COC$\underline{H_3}$), 2.08 (s, 3 H, 3 × COC$\underline{H_3}$), 2.00 (s, 3 H, 3 × COC$\underline{H_3}$), 1.99 (s, 3 H, 3 × COC$\underline{H_3}$), 1.95–1.86 (m, 1 H, H-6a), 1.86–1.78 (m, 1 H, H-6b); ^{13}C NMR (125 MHz, $CDCl_3$, major isomer) δ 170.87 ($\underline{C}$OCH$_3$), 169.99 ($\underline{C}$OCH$_3$), 169.96 ($\underline{C}$OCH$_3$), 169.88 ($\underline{C}$OCH$_3$), 138.4 (Ar), 132.3 (Ar), 130.0 (Ar), 129.0 (Ar), 85.9 (C-1), 71.2 (C-2), 69.8 (C-4), 69.5 (C-3), 67.8 (C-5), 60.2 (C-7),

30.4 (C-6), 21.1 (<u>C</u>H$_3$C$_6$H$_4$S), 20.93 (CO<u>C</u>H$_3$), 20.91 (CO<u>C</u>H$_3$), 20.8 (CO<u>C</u>H$_3$), 20.7 (CO<u>C</u>H$_3$); HRMS (ESI-TOF) m/z: $[M + Na]^+$ Calcd for $C_{22}H_{28}O_9SNa$ 491.1346; Found 491.1339.

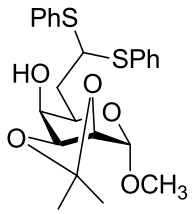


2-62

Methyl 6-deoxy-2,3-*O*-isopropylidene-4-*O*-methylthiomethyl-α-D-*manno*-heptadialdo-1,5-pyranoside-7-(diphenyl dithioacetal) (2-62)

A stirred solution of dithioacetal **2-59** (195 mg, 0.435 mmol) in THF (2 mL) was added DMSO (0.2 mL, 2.8 mmol) followed by Ac_2O (0.25 mL, 2.6 mmol). The reaction mixture was heated at 52 °C for 5 h, after which time the solution was cooled to room temperature over 20 min. The reaction mixture was diluted with CH_2Cl_2 (50 mL) and washed with saturated aqueous $NaHCO_3$ solution (20 mL). The aqueous layer was separated and further extracted with CH_2Cl_2 (2 × 20 mL). The combined organic phases were washed with brine (15 mL), dried over anhydrous Na_2SO_4, filtered, concentrated and the resulting crude product was purified by column chromatography (15% EtOAc in hexane) to afford **2-62** (188 mg, 85%) as a colorless viscous liquid: $[\alpha]^{22}_D$ +100.2 (*c* 0.19, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.56–7.46 (m, 4 H, ArH), 7.35–7.25 (m, 6 H, ArH), 4.92–4.87 (m, 2 H, H-1 and (CH$_3$)SC<u>H$_2$</u>O), 4.80–4.75 (m, 2 H, H-7 and (CH$_3$)SC<u>H$_2$</u>O), 4.24 (app t, *J* = 6.5, 5.5 Hz, 1 H, H-3), 4.11 (d, *J* = 5.5 Hz, 1 H, H-2), 4.08 (app td, *J* = 10.0, 10.0, 2.8 Hz, 1 H, H-5), 3.62 (ddd, *J* = 10.0, 6.5 Hz, 1 H, H-4), 3.36 (s, 3 H, OC<u>H$_3$</u>), 2.29–2.22 (m, 1 H, H-6a), 2.17–2.10 (m, 1 H, H-6b), 1.99 (s, 3 H, (C<u>H$_3$</u>)SCH$_2$O), 1.54 (s, 3 H, (C<u>H$_3$</u>)$_2$C), 1.34 (s, 3 H, (C<u>H$_3$</u>)$_2$C); ^{13}C NMR (125 MHz, $CDCl_3$) δ 133.9 (Ar), 133.1 (Ar), 132.55 (Ar), 132.5 (Ar), 128.9 (Ar), 128.7 (Ar), 127.57 (Ar), 127.55 (Ar), 109.4 (CH$_3$)$_2$<u>C</u>), 98.0

(C-1), 78.2 (C-3), 75.7 (C-2), 75.4 (C-4), 74.6 (CH_3)S<u>C</u>H_2O), 65.3 (C-5), 55.5 (O<u>C</u>H_3), 53.2 (C-7), 38.4 (C-6), 27.8 (<u>C</u>H_3)$_2$C), 26.3 (<u>C</u>H_3)$_2$C), 14.2 (<u>C</u>H_3)SCH_2O); IR (cast film, $CHCl_3$) ν 3073, 3057, 2986, 2912, 2853, 1479, 1438, 1382, 1094, 1061, 691 cm^{-1}; HRMS (ESI-TOF) m/z: $[M + Na]^+$ Calcd for $C_{25}H_{32}O_5S_3Na$ 531.1304; Found 531.1303.

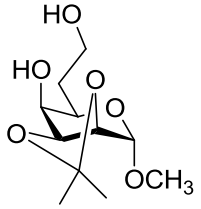


2-61

Methyl 6-deoxy-2,3-*O*-isopropylidene-α-D-*talo*-heptadialdo-1,5-pyranoside-7-(diphenyl dithioacetal) (2-61)

A stirred solution of dithioacetal **2-59** (176 mg, 0.392 mmol) in CH_2Cl_2 (1 mL) was cooled to 0 °C. DMSO (0.30 mL, 4.2 mmol) followed by DIPEA (0.35 mL, 2.0 mmol) were added sequentially to the reaction mixture before SO_3·pyridine (188 mg, 1.18 mmol) was added portionwise. The reaction was stirred at 0 °C for 15 min and then diluted with CH_2Cl_2 (50 mL) and washed with saturated aqueous $Na_2S_2O_3$ solution (10 mL). The aqueous layer was separated and further extracted with CH_2Cl_2 (2 × 50 mL). The combined organic phases were washed with brine (15 mL), dried over anhydrous Na_2SO_4, filtered, concentrated and the resulting crude product was purified by column chromatography (20% EtOAc in hexane) to afford the corresponding ketone, which was dissolved in THF (3 mL) and cooled to 0 °C. Lithium triethylborohydride in THF (1.0 M, 2.0 mL, 2.0 mmol) was added dropwise and the mixture was stirred at 0 °C for 1 h. Excess lithium triethylborohydride was quenched by the addition of saturated aqueous NH_4Cl solution and the reaction mixture was extracted with EtOAc (50 mL). The aqueous layer was separated and further extracted with EtOAc (2 × 50 mL). The combined

organic phases were washed with brine (15 mL), dried over anhydrous Na_2SO_4, filtered, concentrated and the resulting crude product was purified by column chromatography (25% EtOAc in hexane) to afford **2-61** (153 mg, 87% over the two steps) as a colorless viscous liquid: $[\alpha]^{22}_D$ –5.3 (*c* 0.03, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.52–7.49 (m, 2 H, ArH), 7.48–7.45 (m, 2 H, ArH), 7.33–7.25 (m, 6 H, ArH), 4.92 (d, *J* = 0.5 Hz, 1 H, H-1), 4.67 (dd, *J* = 10.0, 4.5 Hz, 1 H, H-7), 4.22 (d, *J* = 6.0, 4.0 Hz, 1 H, H-3), 4.18 (ddd, *J* = 9.0, 4.0, 1.5 Hz, 1 H, H-5), 4.05 (dd, *J* = 6.0, 0.5 Hz, 1 H, H-2), 3.59 (app t, *J* = 4.0, 4.0 Hz, 1 H, H-4), 3.34 (s, 3 H, OC$\underline{H}_3$), 2.55 (ddd, *J* = 15.0, 9.0, 4.5 Hz, 1 H, H-6a), 2.23 (d, *J* = 4.0 Hz, 1 H, CHO$\underline{H}$), 1.99 (ddd, *J* = 15.0, 10.0, 4.0 Hz, 1 H, H-6b), 1.55 (s, 3 H, (C$\underline{H}_3$)$_2$C), 1.37 (s, 3 H, (C$\underline{H}_3$)$_2$C); ^{13}C NMR (125 MHz, $CDCl_3$) δ 133.7 (Ar), 133.6 (Ar), 132.8 (Ar), 132.1 (Ar), 129.0 (Ar), 128.9 (Ar), 127.8 (Ar), 127.6 (Ar), 109.4 (CH$_3$)$_2\underline{C}$), 98.7 (C-1), 73.6 (C-2), 72.7 (C-3), 66.3 (C-5), 66.0 (C-4), 55.7 (O$\underline{C}$H$_3$), 54.0 (C-7), 37.6 (C-6), 25.8 ($\underline{C}$H$_3$)$_2$C), 25.1 ($\underline{C}$H$_3$)$_2$C); IR (cast film, $CHCl_3$) *v* 3539, 3077, 3058, 2987, 2930, 1583, 1480, 1438, 1139, 1089, 750 cm^{-1}; HRMS (ESI-TOF) m/z: [M + Na]$^+$ Calcd for $C_{23}H_{28}O_5S_2Na$ 471.1270; Found 471.1264.

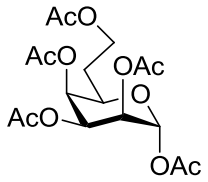


2-63

Methyl 6-deoxy-2,3-*O*-isopropylidene-α-D-*talo*-heptopyranoside (2-63)

The synthesis of diol **2-63** was achieved starting from **2-61** (659 mg, 1.47 mmol) using solid $NaHCO_3$ (750 mg, 8.93 mmol), iodine (1.50 g, 5.91 mmol) and $NaBH_4$ (166 mg, 4.39 mmol), following the procedure described for **2-58** to obtain a crude product, which was purified by column chromatography (75% EtOAc in hexane) to afford diol **2-63** (332 mg, 91%) as a

colorless viscous liquid: $[\alpha]^{22}_{D}$ +39.1 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.96 (s, 1 H, H-1), 4.23 (dd, *J* = 6.0, 5.0 Hz, 1 H, H-3), 4.05 (dd, *J* = 6.0, 0.5 Hz, 1 H, H-2), 3.92 (dd, *J* = 9.0, 4.0 Hz, 1 H, H-5), 3.82 (m, 2 H, H-7a and H-7b), 3.64 (d, *J* = 5.0 Hz, 1 H, H-4), 3.43 (s, 3 H, OC*H*$_3$), 2.37 (br s, 2 H, CH*OH* and CH_2*OH*), 2.19–2.11 (m, 1 H, H-6a), 1.81 (dddd, *J* = 14.9, 7.3, 4.3, 4.0 Hz, 1 H, H-6b), 1.58 (s, 3 H, (C*H*$_3$)$_2$C), 1.38 (s, 3 H, (C*H*$_3$)$_2$C); ^{13}C NMR (125 MHz, $CDCl_3$) δ 109.5 $(CH_3)_2C$), 98.7 (C-1), 73.5 (C-2), 72.7 (C-3), 66.7 (C-5), 66.2 (C-4), 59.8 (C-7), 55.4 (O*C*H$_3$), 33.9 (C-6), 25.9 (*C*H$_3$)$_2$C), 25.2 (*C*H$_3$)$_2$C); IR (cast film, $CHCl_3$) *v* 3450, 2984, 2934, 2836, 1382, 1089, 753 cm^{-1}; HRMS (ESI-TOF) m/z: $[M + Na]^+$ Calcd for $C_{11}H_{20}O_6Na$ 271.1152; Found 271.1151.

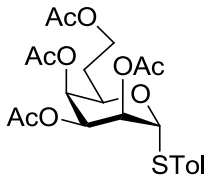


2-64, α/β = ~10:1

1,2,3,4,7-Penta-*O*-acetyl-6-deoxy-α/β-D-*talo*-heptopyranose (2-64)

The synthesis of pentaacetate **2-64** was achieved starting from **2-63** (101.6 mg, 0.4092 mmol) using AcOH (2 mL), Ac_2O (1 mL), camphorsulfonic acid (475 mg, 2.04 mmol) and concentrated H_2SO_4 (0.1 mL), following the procedure described for **2-60** to obtain a crude product, which was purified by column chromatography (50% EtOAc in hexane) to afford pentaacetate **2-64** (156 mg, 94%) as a colorless viscous liquid as a ~10:1 diastereomeric ratio: ^{1}H NMR (700 MHz, $CDCl_3$, major isomer) δ 6.12 (d, *J* = 1.5 Hz, 1 H, H-1), 5.32 (app t, *J* = 4.2, 3.5 Hz, 1 H, H-3), 5.24 (d, *J* = 3.5 Hz, 1 H, H-4), 5.09 (ddd, *J* = 4.2, 1.5, 0.7 Hz, 1 H, H-2), 4.20–4.16 (m, 2 H, H-5 and H-7a), 4.13–4.08 (m, 1 H, H-7b), 2.15 (s, 3 H, 3 × COC*H*$_3$), 2.14 (s, 3 H, 3 × COC*H*$_3$), 2.13 (s, 3 H, 3 × COC*H*$_3$), 2.02 (s, 3 H, 3 × COC*H*$_3$), 1.97 (s, 3 H, 3 × COC*H*$_3$),

1.96 (app ddt, J = 14.7, 9.8, 4.9, 4.9 Hz,1 H, H-6a), 1.77–1.72 (m, 1 H, H-6b); ^{13}C NMR (175 MHz, $CDCl_3$, major isomer) δ 170.7 (<u>C</u>OCH_3), 170.3 (<u>C</u>OCH_3), 169.73 (<u>C</u>OCH_3), 169.72 (<u>C</u>OCH_3), 168.1 (<u>C</u>OCH_3), 91.4 (C-1), 68.2 (C-5), 67.8 (C-4), 66.3 (C-2), 65.6 (C-3), 60.3 (C-7), 30.1 (C-6), 20.9 (CO<u>C</u>H_3), 20.84 (CO<u>C</u>H_3), 20.82 (CO<u>C</u>H_3), 20.7 (CO<u>C</u>H_3), 20.6 (CO<u>C</u>H_3); HRMS (ESI-TOF) m/z: $[M + Na]^+$ Calcd for $C_{17}H_{24}O_{11}Na$ 427.1211; Found 427.1211.

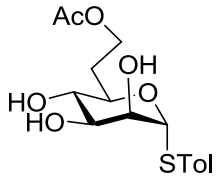


2-53, α/β = 10:1

***p*-Tolyl 2,3,4,7-tetra-*O*-acetyl-6-deoxy-1-thio-α/β-D-*talo*-heptopyranoside (2-53)**

The synthesis of thioglycoside **2-53** was achieved starting from **2-64** (154 mg, 0.381 mmol) using *p*-toluenethiol (62 mg, 0.50 mmol) and $BF_3{\cdot}OEt_2$ (70 μL, 0.57 mmol), following the procedure described for **2-52** to obtain the crude product, which was purified by column chromatography (45% EtOAc in hexane) to afford thioglycoside **2-53** (164 mg, 92%) as a pale yellow viscous liquid which exhibits ~10:1 diastereomeric ratio, as determined by ^{1}H NMR spectroscopy in $CDCl_3$ at 27 °C: ^{1}H NMR (700 MHz, $CDCl_3$, major isomer) δ 7.31 (d, J = 8.4 Hz, 2 H, ArH), 7.11 (d, J = 8.4 Hz, 2 H, ArH), 5.53 (s, 1 H, H-1), 5.34 (d, J = 3.5 Hz, 1 H, H-2), 5.29 (app t, J = 3.5, 3.5 Hz, 1 H, H-3), 5.26 (d, J = 3.5 Hz, 1 H, H-4), 4.62 (dd, J = 9.8, 2.1 Hz, 1 H, H-5), 4.07 (ddd, J = 9.8, 5.6, 5.6 Hz, 1 H, H-7a), 3.90 (app td, J = 9.8, 9.8, 4.9 Hz, 1 H, H-7b), 2.32 (s, 3 H, C<u>H_3</u>C_6H_4S), 2.16 (s, 3 H, 3 × COC<u>H_3</u>), 2.14 (s, 3 H, 3 × COC<u>H_3</u>), 2.01 (s, 3 H, 3 × COC<u>H_3</u>), 1.98 (s, 3 H, 3 × COC<u>H_3</u>), 1.97–1.92 (m, 1 H, H-6a), 1.78–1.73 (m, 1 H, H-6b); ^{13}C NMR (175 MHz, $CDCl_3$, major isomer) δ 170.8 (<u>C</u>OCH_3), 170.3 (<u>C</u>OCH_3), 169.9 (<u>C</u>OCH_3), 169.6 (<u>C</u>OCH_3), 138.2 (Ar), 131.9 (Ar), 130.0 (Ar), 128.9 (Ar), 86.3 (C-1), 68.3 (C-2), 68.5 (C-4), 66.8 (C-5), 66.4 (C-3), 60.3 (C-7), 29.9 (C-6), 21.1 (<u>C</u>$H_3C_6H_4S$), 20.98 (CO<u>C</u>H_3), 20.86

(CO<u>*C*</u>H$_3$), 20.7 (CO<u>*C*</u>H$_3$), 20.6 (CO<u>*C*</u>H$_3$); HRMS (ESI-TOF) m/z: [M + Na]$^+$ Calcd for $C_{22}H_{28}O_9SNa$ 491.1346; Found 491.1342.

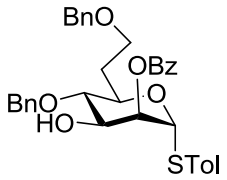


2-65

***p*-Tolyl 7-*O*-acetyl-6-deoxy-1-thio-α-D-*manno*-heptopyranoside (2-65)**

To a stirred solution of thioglycoside **2-52** (3.0 g, 6.4 mmol) in CH_3OH (24 mL) and CH_2Cl_2 (6 mL) was added sodium methoxide in CH_3OH (0.556 M, 5.50 mL, 3.06 mmol). After stirring for 2 h, Amberlite IR-120 resin (H^+ form) was added to neutralize the solution and, following filtration, the filtrate was concentrated to give the tetrol as a pale yellow solid, which was used directly in the next step. Concentrated H_2SO_4 (0.20 mL, 3.8 mmol) was added dropwise over a period of 5 min to a stirred solution of the crude tetrol in EtOAc (150 mL) and the resulting solution was stirred for 17 h and then transferred to a separatory funnel and saturated aqueous $NaHCO_3$ solution was added until no effervescence was observed. The organic layer was separated and the aqueous layer was further extracted with EtOAc (2 × 100 mL). The combined organic phases were washed with brine (30 mL), dried over anhydrous Na_2SO_4, filtered, concentrated and the resulting crude product was purified by column chromatography (5% CH_3OH in CH_2Cl_2) to afford **2-65** (1.64 g, 75%) as a white solid: ^{1}H NMR (500 MHz, $CDCl_3$, major isomer) δ 7.32 (d, J = 8.0 Hz, 2 H, ArH), 7.11 (d, J = 8.0 Hz, 2 H, ArH), 5.37 (d, J = 1.5 Hz, 1 H, H-1), 4.09–4.02 (m, 3 H, H-2, H-5 and H-7a), 4.11 (ddd, J = 11.0, 9.5, 5.5 Hz, 1 H, H-7b), 3.66 (dd, J = 9.5, 3.5 Hz, 1 H, H-3), 3.50 (dd, J = 10.0, 9.5 Hz, 1 H, H-4), 2.30 (s, 3 H, C<u>*H*$_3$</u>C$_6$H$_4$S), 2.24 (dddd, J = 14.8, 8.4, 5.5, 2.5 Hz 1 H, H-6a), 1.94 (s, 3 H, 3 × COC<u>*H*$_3$</u>), 1.72

(dddd, J = 14.8, 9.5, 5.3, 4.5 Hz, 1 H, H-6b); ^{13}C NMR (125 MHz, CD_3OD) δ 172.9 ($\underline{C}O$CH$_3$), 138.9 (Ar), 132.9 (Ar), 131.8 (Ar), 130.8 (Ar), 90.0 (C-1), 73.5 (C-2), 73.1 (C-3), 72.6 (C-4), 71.0 (C-5), 62.4 (C-7), 31.7 (C-6), 21.1 ($\underline{C}$H$_3$C$_6$H$_4$S), 20.8 (CO$\underline{C}$H$_3$); IR (cast film, CH_3OH) v 3410, 3021, 2922, 1737, 1493, 1426, 1384, 1068, 790 cm^{-1}; HRMS (ESI-TOF) m/z: [M + Na]$^+$ Calcd for $C_{16}H_{22}O_6SNa$ 365.1029; Found 365.1028.

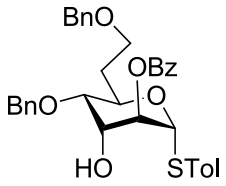


2-66

***p*-Tolyl 2-*O*-benzoyl-4,7-di-*O*-benzyl-6-deoxy-1-thio-α-D-*manno*-heptopyranoside (2-66)**

To a stirred solution of triol **2-65** (2.5 g, 7.3 mmol) in DMF (10 mL) was added trimethyl orthobenzoate (10 mL, 58 mmol) followed by *p*-TSA·H_2O (0.350 g, 1.84 mmol). The resultant solution was stirred for 2 h, after which time additional DMF (15 mL) was added followed by sodium hydride (60% in mineral oil, 1.4 g, 35 mmol). After 5 min, the suspension was cooled to 0 °C and benzyl bromide (3.90 mL, 32.8 mmol) was added dropwise over 5 min. The ice bath was removed and the reaction mixture was warmed to room temperature over 5 h. Excess NaH was quenched by the addition of saturated aqueous NH_4Cl solution and the reaction mixture was extracted with CH_2Cl_2 (100 mL). The aqueous layer was separated and further extracted with CH_2Cl_2 (2 × 100 mL). The combined organic phases were concentrated to a volume of ~75 mL. To this solution, 1 M HCl (150 mL) was added and the solution was stirred vigorously for 2 h. The organic layer was separated and the aqueous layer was further extracted with CH_2Cl_2 (2 × 100 mL). The combined organic phases were washed with brine (30 mL), dried over anhydrous Na_2SO_4, filtered and concentrated and the resulting crude product was purified by column

chromatography (17% EtOAc in hexane) to afford **2-66** (2.82 g, 66%) as a colorless viscous liquid: $[\alpha]^{22}_{D}$ +103.2 (*c* 0.45, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.04 (d, *J* = 7.5 Hz, 2 H, ArH), 7.62–7.24 (m, 15 H, ArH), 7.08 (d, *J* = 8.0 Hz, 2 H, ArH), 5.61 (d, *J* = 3.0 Hz, 1 H, H-2), 5.53 (s, 1 H, H-1), 4.85, 4.78 (ABq, *J* = 11.2 Hz, 2 H, 2 × OC*H*$_2$Ph), 4.45 (s, 2 H, 2 × OC*H*$_2$Ph), 4.33 (app td, *J* = 9.5, 2.0 Hz, 1 H, H-5), 4.23 (dd, *J* = 9.5, 3.0 Hz, 1 H, H-3), 3.66 (app t, *J* = 9.5 Hz, 1 H, H-4), 3.56–3.49 (m, 2 H, H-7a and H-7b), 2.32 (s, 3 H, C*H*$_3$C$_6$H$_4$S), 2.36 (s, 1 H, CH*OH*), (2.35–2.21 (m, 2 H, H-6a), 1.95–1.86 (m, 1 H, H-6b); ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.1 (*C*OPh), 138.5 (Ar), 138.1 (Ar), 137.9 (Ar), 133.4 (Ar), 132.4 (Ar), 129.9 (Ar), 129.88 (Ar), 129.85 (Ar), 129.7 (Ar), 128.6 (Ar), 128.5 (Ar), 128.4 (Ar), 128.1 (Ar), 128.0 (Ar), 127.6 (Ar), 127.5 (Ar), 86.1 (C-1), 80.2 (C-4), 75.1 (O*C*H$_2$Ph), 74.7 (C-2), 73.1 (O*C*H$_2$Ph), 71.3 (C-3), 69.7 (C-5), 66.8 (C-7), 32.0 (C-6), 21.1 (*C*H$_3$C$_6$H$_4$S); IR (cast film, $CHCl_3$) *v* 3442, 3031, 2920, 1721, 1601, 1494, 1452, 1096, 745 cm^{-1}; HRMS (ESI-TOF) m/z: $[M + NH_4]^+$ Calcd for $C_{35}H_{40}NO_6S$ 602.2571; Found 602.2580.

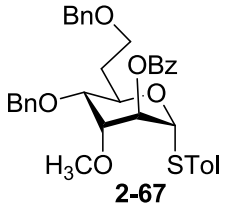


2-54

***p*-Tolyl 2-*O*-benzoyl-4,7-di-*O*-benzyl-6-deoxy-1-thio-α-D-*altro*-heptopyranoside (2-54)**

To a stirred solution of Tf_2O (0.250 mL, 1.49 mmol) in dry CH_2Cl_2 (5 mL) at 0 °C was added pyridine (0.60 mL, 7.4 mmol) dropwise over 3 min. Then, a solution of alcohol **2-66** (0.48 g, 0.82 mmol) in dry CH_2Cl_2 (3 mL) was added dropwise. The reaction mixture was stirred at 0 °C for 45 min, then diluted with CH_2Cl_2 (60 mL) and washed with saturated aqueous $NaHCO_3$ solution (20 mL). The organic layer was separated and the aqueous layer was further extracted

with CH_2Cl_2 (2 × 75 mL). The combined organic phases were washed with brine (30 mL), dried over anhydrous Na_2SO_4, filtered, concentrated and the resulting crude product was quickly purified by column chromatography (20% EtOAc in hexane) to obtain the corresponding triflate, which dissolved in CH_3CN (6 mL). Tetrabutylammonium nitrite (1.20 g, 4.16 mmol) was added and the reaction mixture was heated at 60 °C for 18 h, after which time the solution was cooled to room temperature over 20 min. The reaction mixture was diluted with EtOAc (100 mL) and washed with water (30 mL). The organic layer was separated and the aqueous layer was further extracted with EtOAc (2 × 100 mL). The combined organic phases were washed with brine (30 mL), dried over anhydrous Na_2SO_4, filtered, concentrated and the resulting crude product was purified by column chromatography (18% EtOAc in hexane) to afford **2-54** (0.29 g, 60%) as a pale yellow solid: $[\alpha]^{22}_D$ +98.9 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.00 (d, J = 7.0 Hz, 2 H, ArH), 7.62–7.25 (m, 15 H, ArH), 7.07 (d, J = 8.0 Hz, 2 H, ArH), 5.55 (dd, J = 3.0, 1.5 Hz, 1 H, H-2), 5.41 (d, J = 1.5 Hz, 1 H, H-1), 4.66, 4.60 (ABq, J = 11.4 Hz, 2 H, 2 × OC$\underline{H_2}$Ph), 4.50, 4.46 (ABq, J = 11.9 Hz, 2 H, 2 × OC$\underline{H_2}$Ph), 4.62 (app td, J = 9.5, 2.5 Hz, 1 H, H-5), 4.22 (app t, J = 3.0, 3.0 Hz, 1 H, H-3), 3.68 (dd, J = 9.5, 3.0 Hz, 1 H, H-4), 3.64–3.56 (m, 2 H, H-7a and H-7b), 2.73 (s, 1 H, CH$O\underline{H}$), 2.32 (s, 3 H, C$\underline{H_3}C_6H_4S$), 2.32–2.25 (m, 1 H, H-6a), 1.88–1.80 (m, 1 H, H-6b);^{13}C NMR (125 MHz, $CDCl_3$) δ 165.0 ($\underline{C}$OPh), 138.5 (Ar), 137.24 (Ar), 137.16 (Ar), 133.4 (Ar), 132.7 (Ar), 131.7 (Ar), 129.8 (Ar), 129.7 (Ar), 129.5 (Ar), 128.6 (Ar), 128.5 (Ar), 128.4 (Ar), 128.21 (Ar), 128.15 (Ar), 127.7 (Ar), 127.5 (Ar), 85.3 (C-1), 76.2 (C-4), 73.4 (C-2), 73.2 (O$\underline{C}H_2$Ph), 71.5 (O$\underline{C}H_2$Ph), 66.8 (C-7), 65.9 (C-3), 64.7 (C-5), 31.7 (C-6), 21.1 ($\underline{C}H_3C_6H_4S$); IR (cast film, $CHCl_3$) ν 3454, 3030, 2921, 1723, 1601, 1584, 1493, 1094, 809 cm^{-1}; HRMS (ESI-TOF) m/z: $[M + Na]^+$ Calcd for $C_{35}H_{36}O_6SNa$ 607.2125; Found 607.2126.

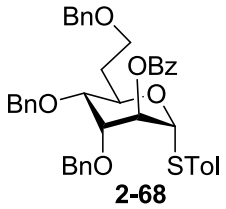


2-67

***p*-Tolyl 2-*O*-benzoyl-4,7-di-*O*-benzyl-6-deoxy-3-*O*-methyl-1-thio-α-D-*altro*-heptopyranoside (2-67)**

To a stirred solution of alcohol **2-54** (114 mg, 0.195 mmol) in dry CH_2Cl_2 (4 mL) was added CH_3I (60 μL, 0.96 mmol) followed by freshly prepared Ag_2O[62] (220 mg, 0.949 mmol). The reaction mixture was heated at reflux for 12 h, after which time the solution was cooled to room temperature over 20 min. The reaction mixture was diluted with CH_2Cl_2 (100 mL) and filtered over a pad of Celite-545. The filtrate was concentrated and the resulting crude product was purified by column chromatography (14% EtOAc in hexane) to afford **2-67** (75 mg, 64%) as a colorless viscous liquid: $[\alpha]^{22}_D$ +116.3 (*c* 0.41, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.97 (d, J = 7.0 Hz, 2 H, ArH), 7.63–7.25 (m, 15 H, ArH), 7.08 (d, J = 8.0 Hz, 2 H, ArH), 5.61 (d, J = 3.5 Hz, 1 H, H-2), 5.40 (s, 1 H, H-1), 4.74–4.66 (m, 1 H, H-5), 4.66, 4.60 (ABq, J = 11.8 Hz, 2 H, 2 × OC$\underline{H_2}$Ph), 4.52, 4.50 (ABq, J = 12.2 Hz, 2 H, 2 × OC$\underline{H_2}$Ph), 3.74 (app t, J = 3.0 Hz, 1 H, H-3), 3.68–3.59 (m, 3 H, H-4, H-7a and H-7b), 3.65 (s, 3 H, OC$\underline{H_3}$), 2.42–2.35 (m, 1 H, H-6a), 2.33 (s, 3 H, C$\underline{H_3}$C$_6$H$_4$S), 1.88–1.81 (m, 1 H, H-6b); ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.1 ($\underline{C}$OPh), 138.6 (Ar), 137.6 (Ar), 137.1 (Ar), 133.5 (Ar), 133.3 (Ar), 131.7 (Ar), 131.4 (Ar), 129.74 (Ar), 129.67 (Ar), 129.6 (Ar), 129.4 (Ar), 128.5 (Ar), 128.43 (Ar), 128.39 (Ar), 128.3 (Ar), 128.2 (Ar), 127.9 (Ar), 127.7 (Ar), 127.6 (Ar), 127.4 (Ar), 86.2 (C-1), 75.7 (C-4), 74.6 (C-3), 73.0 (O$\underline{C}$H$_2$Ph), 71.34 (O$\underline{C}$H$_2$Ph), 71.25 (C-2), 67.2 (C-7), 65.7 (C-5), 58.9 (O$\underline{C}$H$_3$), 31.7 (C-6), 21.1

($\underline{C}H_3C_6H_4S$); IR (cast film, $CHCl_3$) ν 3032, 2927, 1722, 1601, 1493, 1452, 1108, 811 cm^{-1}; HRMS (ESI-TOF) m/z: $[M + Na]^+$ Calcd for $C_{36}H_{38}O_6SNa$ 621.2281; Found 621.2276.

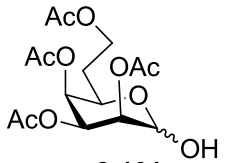


2-68

***p*-Tolyl 2-*O*-benzoyl-3,4,7-tri-*O*-benzyl-6-deoxy-1-thio-α-D-*altro*-heptopyranoside (2-68)**

The synthesis of thioglycoside **2-68** was achieved starting from **2-54** (123 mg, 0.210 mmol) using benzyl bromide (75 μL, 0.63 mmol) and freshly prepared Ag_2O[62] (194 mg, 0.837 mmol), following the procedure described for **2-67** to obtain crude product, which was purified by column chromatography (12% EtOAc in hexane) to afford thioglycoside **2-68** (92 mg, 65%) as a colorless viscous liquid: $[\alpha]^{22}_D$ +134.6 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.98 (dd, *J* = 8.0, 1.0 Hz, 2 H, ArH), 7.61–7.23 (m, 20 H, ArH), 7.09 (d, *J* = 8.0 Hz, 2 H, ArH), 5.70 (d, *J* = 3.5 Hz, 1 H, H-2), 5.44 (s, 1 H, H-1), 4.98 (d, *J* = 12.5 Hz, 1 H, OC$\underline{H_2}$Ph), 4.75 (app td, *J* = 9.5 Hz, 2.5 Hz, 1 H, H-5), 4.75 (d, *J* = 12.0 Hz, 1 H, OC$\underline{H_2}$Ph), 4.53, 4.51 (ABq, *J* = 12.0 Hz, 2 H, 2 × OC$\underline{H_2}$Ph), 4.45, 4.35 (ABq, *J* = 11.7 Hz, 2 H, 2 × OC$\underline{H_2}$Ph), 3.99 (app t, *J* = 2.5 Hz, 1 H, H-3), 3.70–3.65 (m, 2 H, H-7a and H-7b), 3.63 (dd, *J* = 9.5, 2.5 Hz, 1 H, H-4), 2.44–2.38 (m, 1 H, H-6a), 2.34 (s, 3 H, C$\underline{H_3}C_6H_4S$), 1.89–1.82 (m, 1 H, H-6b); ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.1 ($\underline{C}$OPh), 138.6 (Ar), 137.7 (Ar), 137.6 (Ar), 137.2 (Ar), 133.5 (Ar), 133.4 (Ar), 131.8 (Ar), 129.8 (Ar), 129.6 (Ar), 129.4 (Ar), 128.5 (Ar), 128.4 (Ar), 128.30 (Ar), 128.26 (Ar), 128.1 (Ar), 127.8 (Ar), 127.73 (Ar), 127.71 (Ar), 127.4 (Ar), 86.3 (C-1), 75.7 (C-4), 73.1 (O$\underline{C}H_2$Ph), 72.2 (O$\underline{C}H_2$Ph), 71.7 (C-2), 71.1 (C-3), 70.9 (O$\underline{C}H_2$Ph), 67.2 (C-7), 65.8 (C-5), 31.7 (C-6), 21.1

($\underline{C}H_3C_6H_4S$); IR (cast film, $CHCl_3$) ν 3031, 2922, 1721, 1601, 1494, 1452, 1107, 743 cm^{-1}; HRMS (ESI-TOF) m/z: $[M + NH_4]^+$ Calcd for $C_{42}H_{46}NO_6S$ 692.3040; Found 692.3033.

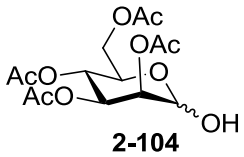


2-101

2,3,4,7-Tetra-*O*-acetyl-6-deoxy-1-α/β-D-*talo*-heptopyranose (2-101)

To a stirred solution of pentaacetate **2-64** (88 mg, 0.22 mmol) in CH_2Cl_2 (1 mL) was added 33% HBr in AcOH (0.15 mL). The mixture was stirred for 3.5 h and then diluted with CH_2Cl_2 and washed with cold water (2 × 50 mL). The organic layer was separated and treated with cold saturated aqueous $NaHCO_3$ solution until a neutral pH was observed. The aqueous layer was separated and further extracted with CH_2Cl_2 (2 × 50 mL). The combined organic phases were washed with cold brine solution (10 mL), dried over anhydrous Na_2SO_4, filtered, concentrated and the resulting crude bromide was used in the next step without further purification. The crude bromide was dissolved in CH_2Cl_2 (1.5 mL) and then molecular sieves (4 Å, 200 mg) were added. After stirring for 5 min, *n*-Bu_4NI (97 mg, 0.26 mmol) followed by Et_2NH (110 μL, 1.06 mmol) were added to the reaction mixture and stirring was continued for 5 h. The reaction mixture was diluted with CH_2Cl_2 (20 mL), washed with 1N HCl (5 mL) and the organic layer was separated and washed with water (5 mL). The aqueous layer was separated and further extracted with CH_2Cl_2 (2 × 25 mL). The combined organic phases were washed with brine (15 mL), dried over anhydrous Na_2SO_4, filtered, concentrated and the resulting crude product was purified by column chromatography (60% EtOAc in hexane) to afford **2-101** (32 mg, 40%) as a colorless viscous liquid, as a 20:3 diastereomeric ratio: ^1H NMR (500 MHz,

$CDCl_3$, major isomer) δ 5.35 (app t, J = 3.5, 3.5 Hz, 1 H, H-3), 5.27 (s, 1 H, H-1), 5.21 (d, J = 3.5 Hz, 1 H, H-4), 5.13 (d, J = 3.5 Hz, 1 H, H-2), 4.44–4.26 (m, 2 H, H-5 and H-7a), 4.11 (ddd, J = 10.8, 5.3, 5.0 Hz, 1 H, H-7b), 3.53 (br s, 1 H, CH*O*H), 2.15 (s, 3 H, 3 × COC*H*$_3$), 2.14 (s, 3 H, 3 × COC*H*$_3$), 2.05 (s, 3 H, 3 × COC*H*$_3$), 1.99 (s, 3 H, 3 × COC*H*$_3$), 1.99–1.90 (m, 1 H, H-6a), 1.78–1.70 (m, 1 H, H-6b); ^{13}C NMR (125 MHz, $CDCl_3$, major isomer) δ 171.7 (*C*OCH_3), 170.5 (*C*OCH_3), 170.2 (*C*OCH_3), 169.7 (*C*OCH_3), 93.2 (C-1), 68.6 (C-4), 67.8 (C-2), 65.8 (C-3), 65.3 (C-5), 60.6 (C-7), 30.3 (C-6), 21.05 (CO*C*H$_3$), 21.00 (CO*C*H$_3$), 20.8 (CO*C*H$_3$), 20.7 (CO*C*H$_3$); HRMS (ESI-TOF) m/z: $[M + Na]^+$ Calcd for $C_{15}H_{22}O_{10}Na$ 385.1105; Found 385.1105.

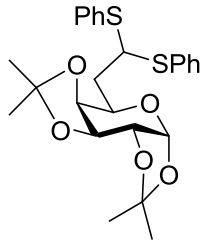


2-104

2,3,4,6-Tetra-*O*-acetyl-α/β-D-mannopyranose (2-104)

To a stirred solution of pentaacetate **2-102** (211 mg, 0.541 mmol) in CH_2Cl_2 (1 mL) was added 33% HBr in AcOH (1.5 mL). The mixture was stirred for 3.5 h, diluted with CH_2Cl_2 and then washed with cold water (2 × 20 mL). The organic layer was separated and treated with cold saturated aqueous $NaHCO_3$ solution until a neutral pH was observed. The aqueous layer was separated and further extracted with CH_2Cl_2 (2 × 20 mL). The combined organic phases were washed with cold brine solution (10 mL), dried over anhydrous Na_2SO_4, filtered, concentrated and the resulting crude bromide was used in the next step without further purification. The crude bromide was dissolved in DMF (1.5 mL) and molecular sieves (4 Å, 430 mg) were added. After stirring for 5 min, TEABr (125 mg, 0.595 mmol) followed by Et_2NH (0.35 mL, 3.4 mmol) were added to the reaction mixture and stirring was continued for 5 h. The reaction mixture was diluted with CH_2Cl_2 (20 mL) and washed with 1N HCl (5 mL) and then the organic layer was

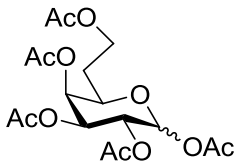
separated and washed with water (5 mL). The aqueous layer was separated and further extracted with CH_2Cl_2 (2 × 25 mL). The combined organic phases were washed with brine (15 mL), dried over anhydrous Na_2SO_4, filtered, concentrated and the resulting crude product was purified by column chromatography (60% EtOAc in hexane) to afford **2-104** (113 mg, 60%) as a colorless viscous liquid: The ^1H and ^{13}C NMR data of **2-104** matched that reported.[64]



2-112

6-Deoxy-1,2:3,4-di-*O*-isopropylidene-α-D-*galacto*-heptadialdo-1,5-pyranose-7-(diphenyl dithioacetal) (2-112)

Compound **2-112** was prepared according to the literature protocol[21b] in 50% yield starting from 1,2:3,4-di-*O*-isopropylidene-α-D-galactose **2-110**. The ^1H and ^{13}C NMR data of **2-112** matched that reported.[21b]



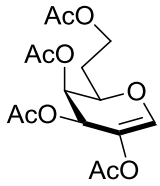
2-106, **α/β = 5:1**

1,2,3,4,7-Penta-*O*-acetyl-6-deoxy-α/β-D-*galacto*-heptopyranose (2-106)

To a stirred solution of known dithioacetal **2-112**[21b] (1.677 g, 3.533 mmol) and solid $NaHCO_3$ (1.78 g, 21.2 mmol) in 1,4-dioxane (20 mL) and H_2O (1 mL) was added iodine (3.59 g, 14.1 mmol) in several portions. The resulting mixture was stirred for 12 h. Excess iodine was

quenched by the addition of solid $Na_2S_2O_3$ (2.5 g) and the solution was filtered to separate solids. The flask was washed with EtOAc (3 × 10 mL) to remove material adhered to the side walls and filtered. The filtrate was washed with H_2O (10 mL) and the aqueous layer was separated and further extracted with EtOAc (2 × 75 mL). The combined organic phases were washed with brine (20 mL), dried over anhydrous Na_2SO_4, filtered, concentrated to obtain the crude aldehyde as a pale yellow viscous liquid. Without further purification, this crude aldehyde was dissolved in EtOH (15 mL) and cooled to 0 °C. $NaBH_4$ (265 mg, 7.01 mmol) was added portionwise to the reaction mixture and then the ice bath was removed and the reaction mixture warmed to room temperature over 2 h. Excess $NaBH_4$ was quenched by the addition of saturated aqueous NH_4Cl solution until no effervescence was observed. The reaction mixture was diluted with EtOAc (75 mL) and the aqueous layer was separated and further extracted with EtOAc (2 × 75 mL). The combined organic phases were dried over anhydrous Na_2SO_4, filtered, concentrated and the resulting crude product was purified by column chromatography (40% EtOAc in hexane) to afford the corresponding known alcohol[45] (882 mg, 91%) as a colorless viscous liquid. This compound (471 mg, 1.72 mmol) was dissolved in AcOH (3 mL) and Ac_2O (1.5 mL). Camphorsulfonic acid (1.60 g, 6.89 mmol) was added to the reaction mixture and the solution was heated at 80 °C for 7 h. The solution was then cooled to room temperature over 20 min and then diluted with EtOAc (20 mL). The mixture was transferred to a separatory funnel and saturated aqueous $NaHCO_3$ solution was added until no effervescence was observed. The resulting solution was extracted with EtOAc (50 mL) and the aqueous layer was separated and further extracted with EtOAc (2 × 30 mL). The combined organic phases were washed with brine (20 mL), dried over anhydrous Na_2SO_4, filtered, concentrated and the resulting crude product was purified by column chromatography (50% EtOAc in hexane) to afford pentaacetate **2-106**

(632 mg, 91%) as a colorless viscous liquid as a 5:1 (α:β) diastereomeric ratio: ^{1}H NMR (500 MHz, $CDCl_3$, major isomer) δ 6.33 (d, J = 3.5 Hz, 1 H, H-1), 5.39–5.38 (m, 1 H, H-4), 5.33–5.31 (m, 2 H, H-2 and H-3), 4.20 (dd, J = 9.0, 4.0 Hz, 1 H, H-5), 4.17–4.12 (m, 1 H, H-7a), 4.10–4.04 (m, 1 H, H-7b), 2.16 (s, 3 H, 3 × COC$\underline{H_3}$), 2.14 (s, 3 H, 3 × COC$\underline{H_3}$), 2.03 (s, 3 H, 3 × COC$\underline{H_3}$), 2.01 (s, 3 H, 3 × COC$\underline{H_3}$), 1.99 (s, 3 H, 3 × COC$\underline{H_3}$), 1.89–1.82 (m, 1 H, H-6a), 1.76–1.68 (m, 1 H, H-6b); ^{13}C NMR (125 MHz, $CDCl_3$, major isomer) δ 170.8 ($\underline{C}OCH_3$), 170.4 ($\underline{C}OCH_3$), 170.2 ($\underline{C}OCH_3$), 169.9 ($\underline{C}OCH_3$), 169.0 ($\underline{C}OCH_3$), 89.7 (C-1), 69.9 (C-4), 68.3 (C-5), 67.8 (C-3), 66.5 (C-2), 60.4 (C-7), 29.8 (C-6), 20.88 (CO$\underline{C}H_3$), 20.86 (CO$\underline{C}H_3$), 20.69 (CO$\underline{C}H_3$), 20.65 (CO$\underline{C}H_3$), 20.6 (CO$\underline{C}H_3$); HRMS (ESI-TOF) m/z: $[M + NH_4]^+$ Calcd for $C_{17}H_{28}NO_{11}$ 422.1657; Found 422.1658.

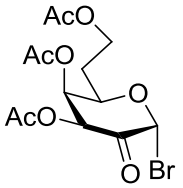


2-98

1,5-Anhydro-2,3,4,7-tetra-*O*-acetyl-6-deoxy-D-*lyxo*-hept-1-enitol (2-98)

To a stirred solution of pentaacetate **2-106** (137.6 mg, 0.3403 mmol) in CH_2Cl_2 (1.5 mL) was added 33% HBr in AcOH (1.5 mL). The solution was stirred for 3.5 h and then diluted with CH_2Cl_2 and washed with cold water (2 × 10 mL). The organic layer was separated and treated with cold saturated aqueous $NaHCO_3$ solution until a neutral pH was observed. The aqueous layer was separated and further extracted with CH_2Cl_2 (2 × 50 mL). The combined organic phases were washed with cold brine solution (10 mL), dried over anhydrous Na_2SO_4, filtered, concentrated and the resulting crude product was used in the next step without further purification. The crude bromide was dissolved in DMF (2 mL) and molecular sieves (4 Å, 200

mg) were added. The mixture was stirred for 5 min before *n*-Bu_4NBr (121 mg, 0.375mmol) followed by DBU (70 µL, 0.47 mmol) were added in the reaction mixture. Stirring was continued for 5 h and then the reaction mixture was diluted with CH_2Cl_2 (25 mL) and washed with 1N HCl (5 mL). The organic layer was separated and washed with water (2 × 15 mL) and the aqueous layer was separated and further extracted with CH_2Cl_2 (2 × 50 mL). The combined organic phases were washed with brine (15 mL), dried over anhydrous Na_2SO_4, filtered, concentrated and the resulting crude product was purified by column chromatography (33% EtOAc in hexane) to afford **2-98** (82 mg, 70%) as a pale yellow viscous liquid: $[\alpha]^{22}_D$ +8.7 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.60 (d, J = 1.5 Hz, 1 H, H-1), 5.87 (m, 1 H, H-3), 5.38 (dd, J = 5.0, 1.5 Hz, 1 H, H-4), 4.26 (dd, J = 9.5, 4.0 Hz, 1 H, H-5), 4.23–4.18 (m, 1 H, H-7a), 4.16–4.10 (m, 1 H, H-7b), 2.13 (s, 3 H, 3 × COC$\underline{H_3}$), 2.09 (s, 3 H, 3 × COC$\underline{H_3}$), 2.04 (s, 3 H, 3 × COC$\underline{H_3}$), 2.01 (s, 3 H, 3 × COC$\underline{H_3}$), 2.08–2.00 (m, 1 H, H-6a), 1.89–1.82 (m, 1 H, H-6b); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.8 ($\underline{C}OCH_3$), 170.3 ($\underline{C}OCH_3$), 169.9 ($\underline{C}OCH_3$), 169.4 ($\underline{C}OCH_3$), 139.1 (C-1), 127.1 (C-2), 72.7 (C-5), 65.6 (C-4), 64.4 (C-3), 60.1 (C-7), 29.6 (C-6), 20.9 (CO$\underline{C}H_3$), 20.61 (CO$\underline{C}H_3$), 20.56 (CO$\underline{C}H_3$), 20.4 (CO$\underline{C}H_3$); HRMS (ESI-TOF) m/z: $[M + Na]^+$ Calcd for $C_{15}H_{20}O_9Na$ 367.1000; Found 367.1001.

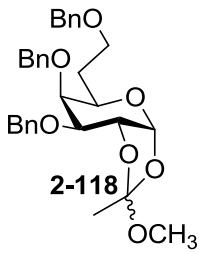


2-96

3,4,7-Tri-*O*-acetyl-6-deoxy-α-D-*lyxo*-heptopyranos-2-ulosyl bromide (2-96)

To a 0 °C solution of 2-acetoxyglycal **2-98** (271.7 mg, 0.7891 mmol) in CH_2Cl_2 (5 mL) was added bromine (85.0 µL, 1.66 mmol) and CH_3OH (35 µL, 0.86 mmol) and the mixture was

stirred for 4 min. The soultion was then concentrated and co-evaporated with toluene (3 × 1 mL) to obtain **2-96** (286 mg, 95%) as a pale yellow viscous liquid, which was used in the next step without further purification: ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.39 (s, 1 H, H-1), 6.19 (d, J = 3.5 Hz, 1 H, H-3), 5.64 (d, J = 3.5 Hz, 1 H, H-4), 4.67 (dd, J = 9.0, 4.5 Hz, 1 H, H-5), 4.18–4.10 (m, 2 H, H-7a and H-7b), 2.11 (s, 3 H, 3 × COC$\underline{H_3}$), 2.09 (s, 3 H, 3 × COC$\underline{H_3}$), 2.05 (s, 3 H, 3 × COC$\underline{H_3}$), 1.95–1.87 (m, 2 H, H-6a and H-6b); ^{13}C NMR (125 MHz, $CDCl_3$) δ 189.3 (C-2), 170.7 ($\underline{C}OCH_3$), 169.8 ($\underline{C}OCH_3$), 169.1 ($\underline{C}OCH_3$), 84.6 (C-1), 72.1 (C-4), 71.2 (C-5), 69.6 (C-3), 59.7 (C-7), 29.2 (C-6), 20.8 (CO$\underline{C}H_3$), 20.4 (CO$\underline{C}H_3$), 20.2 (CO$\underline{C}H_3$).

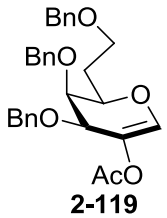


3,4,7-Tri-*O*-benzyl-6-deoxy-1,2-*O*-(methoxyethylidene)-α-D-*galacto*-heptopyranose (2-118)

To a stirred solution of pentaacetate **2-106** (6.086 g, 15.05 mmol) in CH_2Cl_2 (7 mL) was added 33% HBr in AcOH (25 mL). The solution was stirred for 3.5 h and then diluted with CH_2Cl_2 (100 mL) and washed with cold water (2 × 20 mL). The organic layer was separated and treated with cold saturated aqueous $NaHCO_3$ solution until a neutral pH was observed. The aqueous layer was separated and further extracted with CH_2Cl_2 (2 × 75 mL). The combined organic phases were washed with cold brine solution (20 mL), dried over anhydrous Na_2SO_4, filtered, concentrated and the resulting crude product was used immediately in the next step without further purification. The crude bromide was dissolved in CH_2Cl_2 (20 mL) and then 2,6-lutidine (5.00 mL, 42.9 mmol), n-Bu_4NBr (4.90 g, 15.2 mmol) and CH_3OH (2.30 mL, 56.9

mmol) were added. The reaction mixture was stirred for 12 h and then diluted with CH_2Cl_2 (100 mL) and washed with saturated aqueous $NaHCO_3$. The aqueous layer was separated and further extracted with CH_2Cl_2 (2 × 75 mL). The combined organic phases were washed with brine (30 mL), dried over anhydrous Na_2SO_4, filtered and concentrated to obtain a crude product that was quickly purified by column chromatography (20% EtOAc in hexane with 1% Et_3N) to afford the corresponding 3,4,7-tri-*O*-acetyl orthoester, which was dissolved in CH_3OH (40 mL) and CH_2Cl_2 (10 mL). Sodium methoxide in CH_3OH (0.60 M, 8.3 mL, 5.0 mmol) was added and the mixture was stirred for 2 h. Amberlite IR-120 resin (H^+ form) was added to neutralize the solution and, following filtration, the filtrate was concentrated to give the triol as a pale yellow viscous liquid, which was used directly in the next step. To a cooled solution of this triol in DMF (10 mL) was added sodium hydride (60% in mineral oil, 3.0 g, 75 mmol). After 5 min, benzyl bromide (7.10 mL, 59.7 mmol) was added dropwise over 5 min. The ice bath was removed and the reaction mixture was warmed to room temperature over 5 h. Excess NaH was quenched by the addition of saturated aqueous NH_4Cl solution and the reaction mixture was extracted with CH_2Cl_2 (100 mL). The aqueous layer was separated and further extracted with CH_2Cl_2 (2 × 100 mL). The combined organic phases were washed with brine (30 mL), dried over anhydrous Na_2SO_4, filtered and concentrated and the resulting crude product was purified by column chromatography (9% acetone in hexane with 1% Et_3N) to afford **2-118** (5.33 g, 68% over the four steps) as a colorless viscous liquid in a 20:3 diastereomeric ratio: ^1H NMR (500 MHz, $CDCl_3$, major isomer) δ 7.46–7.30 (m, 15 H, ArH), 5.79 (d, J = 4.0 Hz, 1 H, H-1), 4.98 (d, J = 11.5 Hz, 1 H, OC$\underline{H_2}$Ph), 4.86, 4.73 (ABq, J = 11.9 Hz, 2 H, 2 × OC$\underline{H_2}$Ph), 4.66 (d, J = 11.5 Hz, 1 H, OC$\underline{H_2}$Ph), 4.55–4.45 (m, 3 H, 2 × OC$\underline{H_2}$Ph and H-2), 4.10–4.04 (m, 1 H, H-5), 3.80–3.78 (m, 1 H, H-4), 3.69 (dd, J = 6.5, 2.0 Hz, 1 H, H-3), 3.61 (ddd, J = 9.0, 9.0, 5.0 Hz, 1 H, H-7a), 3.55–3.49 (m, 1 H, H-7b), 3.32 (s,

3 H, $CH_3C(OC\underline{H}_3)$), 2.12–2.05 (m, 1 H, H-6a), 1.85–1.76 (m, 1 H, H-6b), 1.64 (s, 3 H, $C\underline{H}_3C(OCH_3)$); ^{13}C NMR (125 MHz, $CDCl_3$, major isomer) δ 138.39 (Ar), 138.35 (Ar), 138.3 (Ar), 138.1 (Ar), 128.47 (Ar), 128.37 (Ar), 128.34 (Ar), 128.29 (Ar), 128.2 (Ar), 128.1 (Ar), 127.9 (Ar), 127.8 (Ar), 127.7 (Ar), 127.65 (Ar), 127.56 (Ar), 127.5 (Ar), 121.7 ($CH_3\underline{C}(OCH_3)$), 97.7 (C-1), 80.7 (C-3), 79.3 (C-2), 74.5 (C-4), 74.3 ($O\underline{C}H_2Ph$), 73.0 ($O\underline{C}H_2Ph$), 71.4 ($O\underline{C}H_2Ph$), 71.3 (C-5), 66.4 (C-7), 49.6 ($CH_3C(O\underline{C}H_3)$), 31.0 (C-6), 24.4 ($\underline{C}H_3C(OCH_3)$); HRMS (ESI-TOF) m/z: $[M + Na]^+$ Calcd for $C_{31}H_{36}O_7Na$ 543.2353; Found 543.2350.



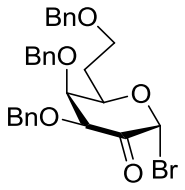
2-119

2-*O*-Acetyl-1,5-anhydro-3,4,7-tri-*O*-benzyl-6-deoxy-D-*lyxo*-hept-1-enitol (2-119)

Procedure A: To a stirred solution of orthoacetate **2-118** (2.982 g, 5.728 mmol) in bromobenzene (104 mL) was added pyridine (0.70 mL, 8.7 mmol). The reaction mixture was heated at reflux at 160 °C for 1.5 h, after which time the solution was cooled to room temperature over 20 min. The solution was concentrated using a vacuum pump to obtain a syrup that was purified by column chromatography (20% EtOAc in hexane) to afford **2-119** (1.12 g, 40%) as a white solid.

Procedure B: A mixture of **2-118** (2.10 g, 4.03 mmol), 4 Å molecular sieves (800 mg) and CH_2Cl_2 (20 mL) was stirred for 30 min and then cooled to 0 °C. To this solution, 33% HBr in AcOH (3.6 mL) was added and the mixture was stirred for 20 min. The reaction mixture was diluted with CH_2Cl_2 (100 mL) and filtered over a pad of Celite-545. The filtrate was concentrated to obtain crude bromide that was used in the next step without further purification. The crude

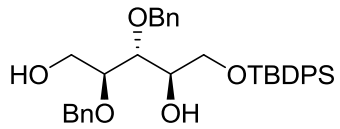
bromide was dissolved in DMF (12 mL), molecular sieves (4 Å, 500 mg) were added and the solution was stirred for 5 min before *n*-Bu_4NBr (1.31 g, 4.06 mmol) followed by DBU (0.75 mL, 5.02 mmol) were added to the reaction mixture. After stirring for 5 h, the solution was diluted with CH_2Cl_2 (75 mL) and washed with 1N HCl (5 mL). The organic layer was separated and washed with water (3 × 15 mL) and the aqueous layer was separated and further extracted with CH_2Cl_2 (2 × 75 mL). The combined organic phases were washed with brine (20 mL), dried over anhydrous Na_2SO_4, filtered, concentrated and the resulting crude product was purified by column chromatography (20% EtOAc in hexane) to afford **2-119** (1.48 g, 75%) as a white solid: $[\alpha]^{22}_D$ – 19.5 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.39–7.29 (m, 15 H, ArH), 6.52 (d, *J* = 1.0 Hz, 1 H, H-1), 4.92 (d, *J* = 11.5 Hz, 1 H, OC*H*$_2$Ph), 4.74, 4.63 (ABq, *J* = 12.0 Hz, 2 H, 2 × OC*H*$_2$Ph), 4.64 (d, *J* = 11.5 Hz, 1 H, OC*H*$_2$Ph), 4.61 (app dt, *J* = 4.5, 1.0, 1.0 Hz, 1 H, H-3), 4.51, 4.47 (ABq, *J* = 11.9 Hz, 2 H, 2 × OC*H*$_2$Ph), 4.31–4.27 (m, 1 H, H-5), 3.89 (dd, *J* = 4.5, 2.5 Hz, 1 H, H-4), 3.58–3.52 (m, 1 H, H-7a), 3.49–3.45 (m, 1 H, H-7b), 2.22–2.15 (m, 1 H, H-6a), 2.10 (s, 3 H, 3 × COC*H*$_3$), 1.97–1.90 (m, 1 H, H-6b); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.1 (*C*OCH$_3$), 138.4 (Ar), 138.2 (Ar), 138.1 (Ar), 137.0 (C-1), 129.9 (C-2), 128.4 (Ar), 128.34 (Ar), 128.32 (Ar), 128.1 (Ar), 127.9 (Ar), 127.8 (Ar), 127.7 (Ar), 127.64 (Ar), 127.56 (Ar), 73.8 (C-5), 73.5 (O*C*H$_2$Ph), 73.1 (O*C*H$_2$Ph), 72.91 (O*C*H$_2$Ph), 72.86 (C-3), 72.4 (C-4), 66.3 (C-7), 30.1 (C-6), 20.6 (CO*C*H$_3$); HRMS (ESI-TOF) m/z: $[M + Na]^+$ Calcd for $C_{30}H_{32}O_6Na$ 511.2091; Found 511.2091.



2-117

3,4,7-Tri-*O*-benzyl-6-deoxy-α-D-*lyxo*-heptopyranos-2-ulosyl bromide (2-117)

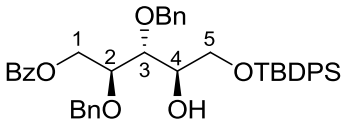
To a 0 °C solution of acetoxyglycal **2-119** (150.3 mg, 0.3076 mmol) in CH_2Cl_2 (4 mL) was added bromine (35 μL, 0.68 mmol) and CH_3OH (15 μL, 0.37 mmol) The solution was stirred for 4 min and then concentrated and then co-evaporated with toluene (3 × 1 mL) to obtain **2-117** (152 mg, 94%) as a colorless viscous liquid, which was used in the next step without further purification: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.42–7.27 (m, 15 H, ArH), 6.43 (s, 1 H, H-1), 5.03 (d, J = 12.0 Hz, 1 H, OC$\underline{H_2}$Ph), 4.97–4.94 (m, 2 H, OC$\underline{H_2}$Ph and H-3), 4.64 (d, J = 11.5 Hz, 1 H, OC$\underline{H_2}$Ph), 4.60 (d, J = 12.0 Hz, 1 H, OC$\underline{H_2}$Ph), 4.50–4.47 (app t, J = 5.5, 3.0 Hz, 1 H, H-5), 4.47, 4.43 (ABq, J = 11.6 Hz, 2 H, 2 × OC$\underline{H_2}$Ph), 4.02 (d, J = 2.5 Hz, 1 H, H-4), 3.48–3.43 (m, 1 H, H-7a), 3.41–3.37 (m, 1 H, H-7b), 2.06–2.01 (m, 1 H, H-6a), 1.78–1.71 (m, 1 H, H-6b); ^{13}C NMR (125 MHz, $CDCl_3$) δ 195.3 (C-2), 138.1 (Ar), 137.6 (Ar), 137.2 (Ar), 128.67 (Ar), 128.64 (Ar), 128.5 (Ar), 128.4 (Ar), 128.3 (Ar), 128.2 (Ar), 128.0 (Ar), 127.98 (Ar), 127.91 (Ar), 127.81 (Ar), 127.76 (Ar), 87.6 (C-1), 78.96 (C-4), 78.91 (C-3), 74.6 (O$\underline{C}$H$_2$Ph), 73.8 (C-5), 73.4 (O$\underline{C}$H$_2$Ph), 73.1 (O$\underline{C}$H$_2$Ph), 65.6 (C-7), 30.9 (C-6).



2-122

2,3-Di-*O*-benzyl-5-*O*-*tert*-butyldiphenylsilyl-D-ribitol (2-122)

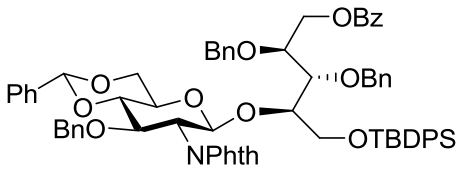
To a 0 °C solution of known hemiacetal[47] **2-121** (527.5 mg, 0.9274 mmol) in 95% EtOH was added $NaBH_4$ (75.0 mg, 1.98 mmol). The reaction mixture was then warmed to room temperature over a period of 2 h. Excess $NaBH_4$ was quenched by the addition of saturated aqueous NH_4Cl solution until no effervescence was observed. The reaction mixture was extracted with EtOAc (50 mL) and the aqueous layer was separated and further extracted with EtOAc (2 × 30 mL). The combined organic phases were washed with brine (20 mL), dried over anhydrous Na_2SO_4, filtered, concentrated and the resulting crude product was purified by column chromatography (32% EtOAc in hexane) to afford **2-122** (502 mg, 95%) as a colorless viscous liquid: $[\alpha]^{22}_D$ –2.7 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.69–7.64 (m, 4 H, ArH), 7.48–7.25 (m, 14 H, ArH), 7.22–7.16 (m, 2 H, ArH), 4.74 (d, J = 11.0 Hz, 1 H, OC$\underline{H_2}$Ph), 4.62, 4.60 (ABq, J = 11.6 Hz, 2 H, 2 × OC$\underline{H_2}$Ph), 4.54 (d, J = 11.0 Hz, 1 H, OC$\underline{H_2}$Ph), 3.95–3.91 (m, 1 H), 3.88–3.76 (m, 6 H), 2.81 (br s, 1 H, O$\underline{H}$), 2.50 (br s, 1 H, O$\underline{H}$), 1.10 (s, 9 H, C$\underline{H_3}$)$_3$CSi(Ph)$_2$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.1 (Ar), 137.9 (Ar), 135.5 (Ar), 133.1 (Ar), 133.0 (Ar), 129.8 (Ar), 128.4 (Ar), 128.3 (Ar), 127.9 (Ar), 127.8 (Ar), 127.75 (Ar), 127.70 (Ar), 127.67 (Ar), 127.64 (Ar), 79.34, 79.32, 73.8 (O$\underline{C}$H$_2$Ph), 71.9 (O$\underline{C}$H$_2$Ph), 71.6, 64.9, 61.1, 26.9 (($\underline{C}$H$_3$)$_3$CSi(Ph)$_2$), 19.2 ((CH$_3$)$_3\underline{C}$Si(Ph)$_2$); HRMS (ESI-TOF) m/z: $[M + NH_4]^+$ Calcd for $C_{35}H_{46}NO_5Si$ 588.3140; Found 588.3138.



2-100

1-*O*-Benzoyl-2,3-di-*O*-benzyl-5-*O*-*tert*-butyldiphenylsilyl-D-ribitol (2-100)

To a stirred solution of diol **2-122** (225.6 mg, 0.3952 mmol) in CH_2Cl_2 (3 mL) was added Bz_2O (135 mg, 0.597 mmol). After stirring for 9 h, the solution was concentrated and the residue was purified by column chromatography (13% EtOAc in hexane) to afford **2-100** (224 mg, 84%) as a colorless viscous liquid: $[\alpha]^{22}_D$ –9.1 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.06 (d, *J* = 7.5 Hz, 2 H, ArH), 7.70–7.19 (m, 23 H, ArH), 4.80–4.74 (m, 3 H, 2 × OC*H*$_2$Ph and H-1a), 4.68 (d, *J* = 12.0 Hz, 1 H, OC*H*$_2$Ph), 4.59 (d, *J* = 11.0 Hz, 1 H, OC*H*$_2$Ph), 4.56 (dd, *J* = 12.0, 6.5 Hz, 1 H, H-1b), 4.18 (ddd, *J* = 6.5, 3.5, 3.0 Hz, 1 H, H-2), 4.02–3.96 (m, 1 H, H-4), 3.92 (dd, *J* = 10.5, 3.5 Hz, 1 H, H-5a), 3.87 (dd, *J* = 7.5, 3.5 Hz, 1 H, H-3), 3.83 (dd, *J* = 10.5, 6.0 Hz, 1 H, H-5b), 2.76 (d, *J* = 4.5 Hz, 1 H, *OH*), 1.12 (s, 9 H, C*H*$_3$)$_3$CSi(Ph)$_2$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.5 (*C*OPh), 138.1 (Ar), 138.0 (Ar), 135.7 (Ar), 135.6 (Ar), 133.2 (Ar), 133.0 (Ar), 132.9 (Ar), 130.2 (Ar), 129.89 (Ar), 129.87 (Ar), 129.7 (Ar), 128.4 (Ar), 128.39 (Ar), 128.34 (Ar), 128.0 (Ar), 127.9 (Ar), 127.8 (Ar), 127.70 (Ar), 127.68 (Ar), 78.9 (C-3), 77.9 (C-2), 73.8 (O*C*H$_2$Ph), 72.4 (O*C*H$_2$Ph), 71.6 (C-4), 65.0 (C-5), 64.3 (C-1), 26.9 ((*C*H$_3$)$_3$CSi(Ph)$_2$), 19.3 ((CH$_3$)$_3$*C*Si(Ph)$_2$); HRMS (ESI-TOF) m/z: $[M + NH_4]^+$ Calcd for $C_{42}H_{50}NO_6Si$ 692.3402; Found 692.3399.

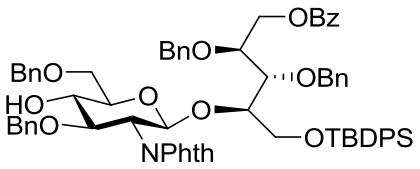


2-124

3-*O*-Benzyl-4,6-*O*-benzylidene-2-deoxy-2-*N*-phthalimido-β-D-glucopyranosyl-(1→4)-1-*O*-benzoyl-2,3-di-*O*-benzyl-5-*O*-*tert*-butyldiphenylsilyl-D-ribitol (2-124)

A solution of known thioglycoside **2-99**[49] (1.20 g, 2.02 mmol) and alcohol **2-100** (1.63 g, 2.42 mmol) in dry CH_2Cl_2 (25 mL) was stirred with 4 Å molecular sieves (1.5 g) for 30 min. The reaction mixture was cooled to –50 °C and then NIS (950 mg, 4.22 mmol) and TMSOTf (40 µL, 0.22 mmol) dissolved in CH_2Cl_2 (1 mL) were sequentially added to the solution. After stirring at –50 °C for 1 h, the TMSOTf was quenched by the addition of Et_3N (0.2 mL) and the reaction mixture was diluted with CH_2Cl_2 (50 mL) and filtered over a pad of Celite-545. The filtrate was washed with saturated aqueous $Na_2S_2O_3$ solution (25 mL) and the organic layer was separated and the aqueous layer was further extracted with CH_2Cl_2 (2 × 75 mL). The combined organic phases were washed with brine (20 mL), dried over anhydrous Na_2SO_4, filtered, concentrated and the resulting crude product was purified by column chromatography (14% acetone in hexane) to afford **2-124** (2.08 g, 90%) as a colorless viscous liquid: $[\alpha]^{22}_D$ –23.0 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.04 (d, J = 8.0 Hz, 2 H, ArH), 7.68–6.95 (m, 37 H, ArH), 5.59 (s, 1 H, C*H*Ph), 5.52 (d, J = 8.0 Hz, 1 H, H-1$_{GlcN}$), 4.88 (d, J = 12.0 Hz, 1 H, OC*H*$_2$Ph), 4.72 (dd, J = 12.0, 2.0 Hz, 1 H, H-1a$_{Rib\text{-}ol}$), 4.65–4.52 (m, 5 H, 4 × OC*H*$_2$Ph and H-3$_{GlcN}$), 4.46–4.41 (m, 2 H, OC*H*$_2$Ph and H-1b$_{Rib\text{-}ol}$), 4.37 (dd, J = 10.0, 8.0 Hz, 1 H, H-2$_{GlcN}$), 4.33 (dd, J = 9.5, 4.5 Hz, 1 H, H-6a$_{GlcN}$), 4.22 (app q, J = 4.6, 4.5, 4.5 Hz,1 H, H-4$_{Rib\text{-}ol}$), 4.04–4.01 (m, 1 H, H-2$_{Rib\text{-}ol}$), 3.91 (dd, J = 4.5, 3.5 Hz, 1 H, H-3$_{Rib\text{-}ol}$), 3.86 (dd, J = 11.5, 4.6 Hz, 1 H, H-5a$_{Rib\text{-}ol}$), 3.82 (app t, J = 9.5,

9.0 Hz, 1 H, H-4$_{GlcN}$), 3.77 (app t, J = 9.5, 9.0 Hz, 1 H, H-6b$_{GlcN}$), 3.64–3.58 (m, 2 H, H-5$_{GlcN}$, and H-5b$_{Rib\text{-}ol}$), 0.98 (s, 9 H, C$\underline{H}_3$)$_3$CSi(Ph)$_2$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 167.6 ($\underline{C}$OPhth), 166.4 (2C, $\underline{C}$OPhth and $\underline{C}$OPh), 138.3 (Ar), 138.1 (Ar), 138.0 (Ar), 137.4 (Ar), 135.6 (Ar), 135.5 (Ar), 133.7 (Ar), 133.2 (Ar), 132.9 (Ar), 132.8 (Ar), 131.5 (Ar), 130.3 (Ar), 129.7 (Ar), 129.6 (Ar), 129.0 (Ar), 128.33 (Ar), 128.29 (Ar), 128.19 (Ar), 128.17 (Ar), 128.0 (Ar), 127.9 (Ar), 127.8 (Ar), 127.7 (Ar), 127.6 (Ar), 127.5 (Ar), 127.4 (Ar), 127.3 (Ar), 126.1 (Ar), 123.3 (Ar), 101.3 ($\underline{C}$HPh), 97.9 (C-1$_{GlcN}$), 82.9 (C-4$_{GlcN}$), 78.4 (C-4$_{Rib\text{-}ol}$), 77.8 (C-3$_{Rib\text{-}ol}$), 77.3 (C-2$_{Rib\text{-}ol}$), 74.9 (C-3$_{GlcN}$), 74.2 (O$\underline{C}$H$_2$Ph), 73.3 (O$\underline{C}$H$_2$Ph), 72.0 (O$\underline{C}$H$_2$Ph), 68.7 (C-6$_{GlcN}$), 65.9 (C-5$_{GlcN}$), 63.9 (C-1$_{Rib\text{-}ol}$), 63.7 (C-5$_{Rib\text{-}ol}$), 56.3 (C-2$_{GlcN}$), 26.8 (($\underline{C}$H$_3$)$_3$CSi(Ph)$_2$), 19.0 ((CH$_3$)$_3\underline{C}$Si(Ph)$_2$); IR (cast film, $CHCl_3$) ν 3067, 3031, 2931, 2858, 1776, 1717, 1257, 1106, 699 cm^{-1}; HRMS (ESI-TOF) m/z: [M + NH_4]$^+$ Calcd for $C_{70}H_{73}N_2O_{12}Si$ 1161.4927; Found 1161.4918.

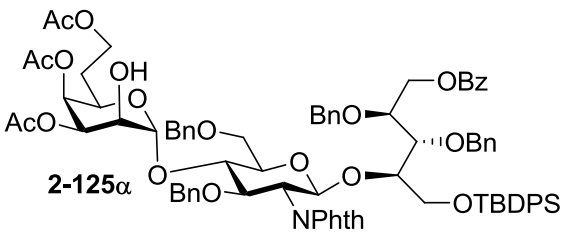


2-97

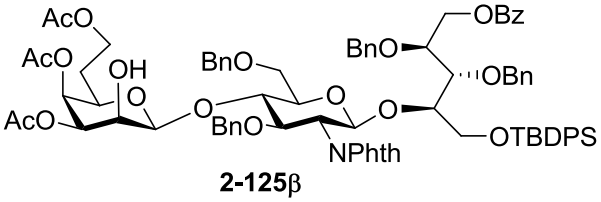
3,6-Di-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-2-*N*-phthalimido-β-D-glucopyranosyl-(1→4)-1-*O*-benzoyl-2,3-di-*O*-benzyl-5-*O*-*tert*-butyldiphenylsilyl-D-ribitol (2-97)

To a 0 °C solution of disaccharide **2-124** (1.937 g, 1.693 mmol) in CH_2Cl_2 (25 mL) was added trifluoroacetic anhydride (0.50 mL, 3.6 mmol), Et_3SiH (2.80 mL, 17.5 mmol) and TFA (1.30 mL, 16.9 mmol) sequentially. The ice bath was removed and the reaction mixture was warmed to room temperature over 3 h. Excess Et_3SiH and TFA were quenched by the addition of saturated aqueous $NaHCO_3$ solution until no effervescence was observed. The reaction mixture was diluted with CH_2Cl_2 (75 mL) and the aqueous layer was separated and further extracted with

CH_2Cl_2 (2 × 75 mL). The combined organic phases were washed with brine (20 mL), dried over anhydrous Na_2SO_4, filtered, concentrated and the resulting crude product was purified by column chromatography (22% acetone in hexane) to afford **2-97** (1.55 g, 80%) as a white foam: $[\alpha]^{22}_D$ – 31.2 (*c* 2.38, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.99–7.95 (m, 2 H, ArH), 7.63–6.97 (m, 37 H, ArH), 5.43 (d, J = 8.5 Hz, 1 H, H-1$_{GlcN}$), 4.82 (d, J = 12.5 Hz, 1 H, OC$\underline{H_2}$Ph), 4.64 (dd, J = 12.5, 2.5 Hz, 1 H, H-1a$_{Rib\text{-}ol}$), 4.59–4.53 (m, 3 H, 3 × OC$\underline{H_2}$Ph), 4.49 (d, J = 11.0 Hz, 1 H, OC$\underline{H_2}$Ph), 4.46–4.34 (m, 5 H, 3 × OC$\underline{H_2}$Ph, H-1b$_{Rib\text{-}ol}$ and H-3$_{GlcN}$), 4.26 (dd, J = 11.0, 8.5 Hz, 1 H, H-2$_{GlcN}$), 4.19–4.15 (m, 1 H, H-4$_{Rib\text{-}ol}$), 4.00 (app td, J = 6.0, 6.0, 2.5 Hz, 1 H, H-2$_{Rib\text{-}ol}$), 3.89 (dd, J = 6.0, 4.0 Hz, 1 H, H-3$_{Rib\text{-}ol}$), 3.84–3.80 (m, 2 H, H-4$_{GlcN}$ and H-5a$_{Rib\text{-}ol}$), 3.74 (dd, J = 10.0, 4.5 Hz, 1 H, H-6a$_{GlcN}$), 3.63–3.55 (m, 3 H, H-5$_{GlcN}$, H-6b$_{GlcN}$ and H-5b$_{Rib\text{-}ol}$), 3.14 (s, 1 H, CH$\underline{OH}$), 0.94 (s, 9 H, (C$\underline{H_3}$)$_3$CSi(Ph)$_2$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 167.9 ($\underline{C}$OPhth), 167.8 ($\underline{C}$OPhth), 166.4 ($\underline{C}$OPh), 138.4 (Ar), 138.3 (Ar), 138.1 (Ar), 137.5 (Ar), 135.6 (Ar), 135.5 (Ar), 133.7 (Ar), 133.3 (Ar), 133.0 (Ar), 132.8 (Ar), 130.3 (Ar), 129.7 (Ar), 129.6 (Ar), 129.5 (Ar), 128.5 (Ar), 128.3 (Ar), 128.2 (Ar), 128.14 (Ar), 128.11 (Ar), 127.94 (Ar), 127.89 (Ar), 127.8 (Ar), 127.7 (Ar), 127.68 (Ar), 127.67 (Ar), 127.6 (Ar), 127.4 (Ar), 127.3 (Ar), 123.3 (Ar), 97.5 (C-1$_{GlcN}$), 78.7 (C-3$_{GlcN}$), 78.3 (C-4$_{Rib\text{-}ol}$), 77.9 (C-3$_{Rib\text{-}ol}$), 77.3 (C-2$_{Rib\text{-}ol}$), 75.4 (C-4$_{GlcN}$), 74.3 (O$\underline{C}$H$_2$Ph), 73.8 (O$\underline{C}$H$_2$Ph), 73.2 (O$\underline{C}$H$_2$Ph), 72.8 (C-5$_{GlcN}$), 72.0 (O$\underline{C}$H$_2$Ph), 71.4 (C-6$_{GlcN}$), 64.0 (C-1$_{Rib\text{-}ol}$), 63.7 (C-5$_{Rib\text{-}ol}$), 55.8 (C-2$_{GlcN}$), 26.8 (($\underline{C}$H$_3$)$_3$CSi(Ph)$_2$), 19.0 ((CH$_3$)$_3\underline{C}$Si(Ph)$_2$); IR (cast film, $CHCl_3$) ν 3478, 3065, 3031, 2929, 2857, 1776, 1715, 1428, 1275, 1112, 667 cm^{-1}; HRMS (ESI-TOF) m/z: $[M + Na]^+$ Calcd for $C_{70}H_{71}NO_{12}SiNa$ 1168.4638; Found 1168.4626.



2-125α



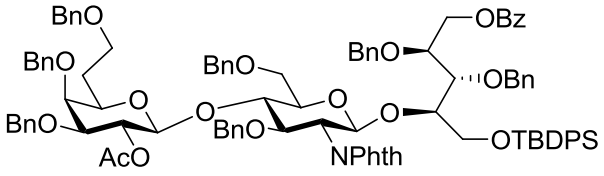
2-125β

3,4,7-Tri-*O*-acetyl-6-deoxy-α-D-*talo*-heptopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-*N*-phthalimido-β-D-glucopyranosyl)-(1→4)-1-*O*-benzoyl-2,3-di-*O*-benzyl-5-*O*-*tert*-butyldiphenylsilyl-D-ribitol (2-125α) and **3,4,7-Tri-*O*-acetyl-6-deoxy-β-D-*talo*-heptopyranosyl)-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-*N*-phthalimido-β-D-glucopyranosyl-(1→4)-1-*O*-benzoyl-2,3-di-*O*-benzyl-5-*O*-*tert*-butyldiphenylsilyl-D-ribitol (2-125β)**

A solution of ulosyl bromide **2-96** (286 mg, 0.750 mmol), alcohol **2-97** (176.9 mg, 0.1543 mmol), and (–)-β-pinene (0.25 mL, 1.59 mmol) in dry CH_3CN (5 mL) was stirred with 4 Å molecular sieves (304 mg) for 30 min. AgOTf (215 mg, 0.837 mmol) was added to the solution and the mixture was stirred for 3 h before being diluted with CH_2Cl_2 (50 mL) and filtered over a pad of Celite-545. The filtrate was concentrated and the resulting crude product was dissolved in THF (5 mL) and cooled to 0 °C. $NaBH_4$ (20 mg, 0.53 mmol) was added portionwise to the reaction mixture and then the ice bath was removed and the reaction mixture was warmed to room temperature over 2 h. Excess $NaBH_4$ was quenched by the dropwise addition of saturated aqueous NH_4Cl solution until no effervescence was observed. The reaction mixture was diluted with EtOAc (30 mL) and the aqueous layer was separated and further extracted with EtOAc (2 × 30 mL). The combined organic phases were dried over anhydrous Na_2SO_4, filtered and concentrated and the resulting crude product was purified by column chromatography (50% EtOAc in hexane) to afford **2-125α** (40 mg, 18%) as a pale yellow viscous liquid and **2-125β** (121 mg, 54%) as a pale yellow viscous liquid: Data for **2-125β**: $[\alpha]^{22}_D$ –3.3 (*c*

1.0, $CHCl_3$); ^{1}H NMR (700 MHz, $CDCl_3$) δ 7.94–7.92 (m, 2 H, ArH), 7.60–6.85 (m, 37 H, ArH), 5.41 (d, J = 9.1 Hz, 1 H, H-1_{GlcN}), 5.11 (d, J = 2.5 Hz, 1 H, H-4_{TalHep}), 4.89 (d, J = 8.5 Hz, 1 H, OC$\underline{H}_2$Ph), 4.65–4.62 (m, 2 H, H-3_{TalHep} and H-$1a_{Rib\text{-}ol}$), 4.58–4.55 (m, 3 H, H-1_{TalHep} and 2 × OC$\underline{H}_2$Ph), 4.53–4.46 (m, 4 H, H-3_{GlcN} and 3 × OC$\underline{H}_2$Ph), 4.39 (dd, J = 8.5, 4.0 Hz, 1 H, H-$1b_{Rib\text{-}ol}$), 4.32–4.22 (m, 4 H, H-2_{GlcN}, H-$4_{Rib\text{-}ol}$ and 2 × OC$\underline{H}_2$Ph), 4.08–4.03 (m, 2 H, H-4_{GlcN} and H-$7a_{TalHep}$), 4.02–3.97 (m, 1 H, H-$7b_{TalHep}$), 3.95–3.92 (m, 1 H, H-$2_{Rib\text{-}ol}$), 3.87 (dd, J = 7.0, 3.5 Hz, 1 H, H-$3_{Rib\text{-}ol}$), 3.81–3.73 (m, 3 H, H-$5a_{Rib\text{-}ol}$, H-2_{TalHep} and H-$6a_{GlcN}$), 3.61–3.54 (m, 3 H, H-5_{GlcN}, H-$6b_{GlcN}$ and H-$5b_{Rib\text{-}ol}$), 3.40 (dd, J = 8.4, 5.6 Hz, 1 H, H-5_{TalHep}), 2.40 (br s, 1 H, CHO$\underline{H}$), 2.08 (s, 3 H, 3 × COC$\underline{H}_3$), 2.07 (s, 3 H, 3 × COC$\underline{H}_3$), 1.96 (s, 3 H, 3 × COC$\underline{H}_3$), 1.66–1.55 (m, 2 H, H-$6a_{TalHep}$ and H-$6b_{TalHep}$), 0.91 (s, 9 H, (C$\underline{H}_3$)$_3$CSi(Ph)$_2$); ^{13}C NMR (175 MHz, $CDCl_3$) δ 170.7 ($\underline{C}$OCH$_3$), 169.9 ($\underline{C}$OCH$_3$), 169.6 ($\underline{C}$OCH$_3$), 167.9 ($\underline{C}$OPhth), 167.6 ($\underline{C}$OPhth), 166.4 ($\underline{C}$OPh), 138.8 (Ar), 138.3 (Ar), 138.2 (Ar), 137.9 (Ar), 135.6 (Ar), 135.5 (Ar), 133.6 (Ar), 133.2 (Ar), 133.0 (Ar), 132.8 (Ar), 130.3 (Ar), 129.6 (Ar), 129.5 (Ar), 128.52 (Ar), 128.48 (Ar), 128.3 (Ar), 128.1 (Ar), 127.93 (Ar), 127.86 (Ar), 127.8 (Ar), 127.65 (Ar), 127.61 (Ar), 127.3 (Ar), 126.9 (Ar), 123.2 (Ar), 100.4 (C-1_{TalHep}), 97.7 (C-1_{GlcN}), 78.7 (C-$4_{Rib\text{-}ol}$), 78.6 (C-4_{GlcN}), 78.4 (C-$3_{Rib\text{-}ol}$), 77.34, 77.31 (C-3_{GlcN} and C-$2_{Rib\text{-}ol}$), 74.5 (O$\underline{C}$H$_2$Ph), 74.3 (C-5_{GlcN}), 73.7 (O$\underline{C}$H$_2$Ph), 73.3 (O$\underline{C}$H$_2$Ph), 72.2 (O$\underline{C}$H$_2$Ph), 71.1 (C-5_{TalHep}), 69.9 (C-3_{TalHep}), 69.0 (C-4_{TalHep}), 68.8 (C-2_{TalHep}), 68.6 (C-6_{GlcN}), 63.9, 63.8 (C-$5_{Rib\text{-}ol}$ and C-$1_{Rib\text{-}ol}$), 60.4 (C-7_{TalHep}), 56.3 (C-2_{GlcN}), 29.9 (C-6_{TalHep}), 26.8 (($\underline{C}$H$_3$)$_3$CSi(Ph)$_2$), 20.9 (CO$\underline{C}$H$_3$), 20.8 (CO$\underline{C}$H$_3$), 20.7 (CO$\underline{C}$H$_3$), 19.0 ((CH$_3$)$_3\underline{C}$Si(Ph)$_2$); IR (cast film, $CHCl_3$) ν 3577, 3067, 3031, 2931, 2858, 1776, 1744, 1716, 1453, 1427, 1113, 1071, 753 cm^{-1}; HRMS (ESI-TOF) m/z: [M + Na]$^+$ Calcd for $C_{83}H_{89}NO_{20}SiNa$ 1470.5639; Found 1470.5649: Data for **2-125α**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.95–7.93 (m, 2 H, ArH), 7.61–6.86 (m, 37 H, ArH), 5.43 (dd, J = 5.0, 1.5 Hz, 1 H, H-3_{TalHep}),

5.42–5.38 (m, 2 H, H-1_{GlcN} and H-1_{TalHep}), 5.26 (d, J = 1.5 Hz, 1 H, H-4_{TalHep}), 4.75–4.00 (m, 16 H), 4.00–3.64 (m, 4 H), 3.62–3.48 (m, 5 H), 2.80 (br s, 1 H, CH$\underline{OH}$), 2.18 (s, 3 H, 3 × COC$\underline{H_3}$), 2.14 (s, 3 H, 3 × COC$\underline{H_3}$), 2.06 (s, 3 H, 3 × COC$\underline{H_3}$), 1.94–1.72 (m, 2 H, H-$6a_{TalHep}$ and H-$6b_{TalHep}$), 0.95 (s, 9 H, $(C\underline{H_3})_3CSi(Ph)_2$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.7 ($\underline{C}OCH_3$), 169.9 ($\underline{C}OCH_3$), 169.5 ($\underline{C}OCH_3$), 167.8 ($\underline{C}O$Phth), 167.5 ($\underline{C}O$Phth), 166.4 ($\underline{C}O$Ph), 139.2 (Ar), 138.3 (Ar), 138.1 (Ar), 137.1 (Ar), 135.6 (Ar), 133.8 (Ar), 133.2 (Ar), 132.9 (Ar), 132.8 (Ar), 130.2 (Ar), 129.6 (Ar), 128.3 (Ar), 128.1 (Ar), 127.8 (Ar), 127.5 (Ar), 127.2 (Ar), 126.9 (Ar), 100.5 (C-1_{TalHep}), 97.5 (C-1_{GlcN}), 80.4, 78.6, 78.4, 78.0, 75.1, 74.9, 73.7, 73.2, 72.7, 72.1, 70.7, 70.5, 67.6, 67.3, 65.7, 64.4, 63.9, 63.8, 60.7, 60.1, 56.6 (C-2_{GlcN}), 29.9 (C-6_{TalHep}), 26.8 ($(\underline{C}H_3)_3CSi(Ph)_2$), 20.93 (CO$\underline{C}H_3$), 20.88 (CO$\underline{C}H_3$), 20.7 (CO$\underline{C}H_3$), 19.0 ($(CH_3)_3\underline{C}Si(Ph)_2$); HRMS (ESI-TOF) m/z: $[M + Na]^+$ Calcd for $C_{83}H_{89}NO_{20}SiNa$ 1470.5639; Found 1470.5648.



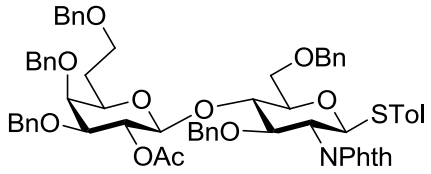
2-128

2-*O*-Acetyl-3,4,7-tri-*O*-benzyl-6-deoxy-β-D-*galacto*-heptopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-*N*-phthalimido-β-D-glucopyranosyl-(1→4)-1-*O*-benzoyl-2,3-di-*O*-benzyl-5-*O*-*tert*-butyldiphenylsilyl-D-ribitol (2-128)

A mixture of **2-118** (42.9 mg, 0.0824 mmol), 4 Å molecular sieves (190 mg), and CH_2Cl_2 (2 mL) was stirred for 30 min and then cooled to 0 °C. To this solution, 33% HBr in AcOH (0.1 mL) was added dropwise. After stirring for 20 min, the reaction mixture was diluted with CH_2Cl_2 (50 mL) and filtered over a pad of Celite-545. The filtrate was concentrated to obtain crude bromide **2-127** as a white foam, which was used in the next step without further purification. A

solution of glycosyl bromide **2-127** (44 mg, 0.077 mmol), DTBMP (24.0 mg, 0.117 mmol) and disaccharide acceptor **2-97** (52.9 mg, 0.0461 mmol) in dry CH_2Cl_2 (2.5 mL) was stirred with 4 Å molecular sieves (250 mg) for 30 min. The reaction mixture was cooled to –40 °C and AgOTf (24 mg, 0.093 mmol) was added and the solution was stirred at –40 °C for 1.5 h. The AgOTf was quenched by the addition of Et_3N (0.1 mL) and the reaction mixture was diluted with CH_2Cl_2 (20 mL) and filtered over a pad of Celite-545. The filtrate was washed with saturated aqueous $NaHCO_3$ solution (15 mL) and the organic layer was separated and the aqueous layer was further extracted with CH_2Cl_2 (2 × 20 mL). The combined organic phases were washed with brine (15 mL), dried over anhydrous Na_2SO_4, filtered, concentrated and the resulting crude product was purified by column chromatography (28% EtOAc in hexane) to afford **2-128** (68 mg, 75% over the two steps) as a white foam: $[\alpha]^{22}_D$ –6.3 (*c* 0.49, $CHCl_3$); ^{1}H NMR (700 MHz, $CDCl_3$) δ 7.93 (m, 2 H, ArH), 7.65–6.79 (m, 52 H, ArH), 5.35 (d, J = 8.4 Hz, 1 H, H-1$_{GlcN}$), 5.32 (dd, J = 9.8, 7.7 Hz, 1 H, H-2$_{GalHep}$), 4.93 (d, J = 11.2 Hz, 1 H, OC$\underline{H_2}$Ph), 4.90 (d, J = 11.9 Hz, 1 H, OC$\underline{H_2}$Ph), 4.67–4.63 (m, 2 H, H-1a$_{Rib\text{-}ol}$ and OC$\underline{H_2}$Ph), 4.60–4.46 (m, 6 H, 6 × OC$\underline{H_2}$Ph), 4.42 (dd, J = 12.6, 5.6 Hz, 1 H, H-1b$_{Rib\text{-}ol}$), 4.39 (d, J = 7.7 Hz, 1 H, H-1$_{GalHep}$), 4.39–4.28 (m, 6 H, 5 × OC$\underline{H_2}$Ph and H-3$_{GlcN}$), 4.26–4.20 (m, 2 H, H-2$_{GlcN}$ and H-4$_{Rib\text{-}ol}$), 4.00–3.97 (m, 1 H, H-2$_{Rib\text{-}ol}$), 3.93 (dd, J = 7.0, 3.5 Hz, 1 H, H-3$_{Rib\text{-}ol}$), 3.85 (app t, J = 9.8, 8.4 Hz, 1 H, H-4$_{GlcN}$), 3.79 (dd, J = 10.5, 4.2 Hz, 1 H, H-5a$_{Rib\text{-}ol}$), 3.70 (dd, J = 10.5, 4.2 Hz, 1 H, H-6a$_{GlcN}$), 3.61–3.56 (m, 3 H, H-5b$_{Rib\text{-}ol}$, H-6b$_{GlcN}$ and H-4$_{GalHep}$), 3.42–3.35 (m, 3 H, H-5$_{GlcN}$, H-5$_{GalHep}$ and H-7a$_{GalHep}$), 3.34 (dd, J = 9.8, 2.8 Hz, 1 H, H-3$_{GalHep}$), 3.19 (ddd, J = 9.3, 4.9, 4.6 Hz, 1 H, H-7b$_{GalHep}$), 1.99 (s, 3 H, 3 × COC$\underline{H_3}$), 1.77 (dddd, J = 13.0, 9.6, 4.6, 4.2 Hz, 1 H, H-6a$_{GalHep}$), 1.53 (dddd, J = 13.0, 9.3, 4.9, 4.6 Hz, 1 H, H-6b$_{GalHep}$), 0.90 (s, 9 H, (C$\underline{H_3}$)$_3$CSi(Ph)$_2$); ^{13}C NMR (175 MHz, $CDCl_3$) δ 169.3 ($\underline{C}$OCH$_3$), 167.9 ($\underline{C}$OPhth), 167.8 ($\underline{C}$OPhth), 166.5 ($\underline{C}$OPh), 139.2 (Ar), 138.7 (Ar), 138.5 (Ar), 138.3 (Ar),

138.1 (Ar), 135.6 (Ar), 135.5 (Ar), 133.5 (Ar), 133.2 (Ar), 133.1 (Ar), 132.7 (Ar), 131.8 (Ar), 131.5 (Ar), 130.3 (Ar), 129.7 (Ar), 129.5 (Ar), 128.5 (Ar), 128.3 (Ar), 128.1 (Ar), 127.9 (Ar), 127.6 (Ar), 127.4 (Ar), 126.6 (Ar), 123.2 (Ar), 100.8 (C-1_{GalHep}), 97.7 (C-1_{GlcN}), 80.9 (C-3_{GalHep}), 78.6 (C-$4_{Rib\text{-}ol}$), 78.4 (C-$3_{Rib\text{-}ol}$), 78.0 (C-4_{GlcN}), 77.5 (C-$2_{Rib\text{-}ol}$), 77.1 (C-3_{GlcN}), 75.1 (2C, C-5_{GlcN} and C-4_{GalHep}), 74.5 (O$\underline{C}H_2$Ph), 74.3 (O$\underline{C}H_2$Ph), 73.6 (O$\underline{C}H_2$Ph), 73.3 (O$\underline{C}H_2$Ph), 73.1 (O$\underline{C}H_2$Ph), 72.3 (C-2_{GalHep}), 72.1 (O$\underline{C}H_2$Ph), 71.9 (O$\underline{C}H_2$Ph), 71.3 (C-5_{GalHep}), 67.9 (C-6_{GlcN}), 66.1 (C-7_{GalHep}), 64.0 (C-$1_{Rib\text{-}ol}$), 63.5 (C-$1_{Rib\text{-}ol}$), 56.3 (C-2_{GlcN}), 31.4 (C-6_{GalHep}), 26.8 (($\underline{C}H_3$)$_3$CSi(Ph)$_2$), 21.1 (CO$\underline{C}H_3$), 19.0 ((CH$_3$)$_3\underline{C}$Si(Ph)$_2$); IR (cast film, $CHCl_3$) ν 3063, 3031, 2929, 2859, 1751, 1716, 1496, 1389, 1365, 1095, 699 cm^{-1}; HRMS (ESI-TOF) m/z: $[M + NH_4]^+$ Calcd for $C_{100}H_{107}N_2O_{18}Si$ 1651.7283; Found 1651.7271.



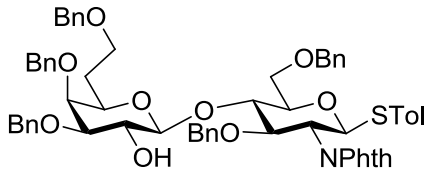
2-130

***p*-Tolyl 2-*O*-acetyl-3,4,7-tri-*O*-benzyl-6-deoxy-β-D-*galacto*-heptopyranosyl-(1→4)-4,6-di-*O*-benzyl-2-deoxy-2-*N*-phthalimido-1-thio-β-D-glucopyranoside (2-130)**

A mixture of **2-118** (440 mg, 0.615 mmol), 4 Å molecular sieves (350 mg), and CH_2Cl_2 (7 mL) was stirred for 30 min and then cooled to 0 °C. To this solution, 33% HBr in AcOH (0.8 mL) was added dropwise and the mixture was stirred for 20 min. The reaction mixture was then diluted with CH_2Cl_2 (50 mL) and filtered over a pad of Celite-545. The filtrate was concentrated to obtain crude bromide **2-127** as a white foam, which was used in the next step without further purification. A solution of **2-127** (480 mg, 0.843 mmol), DTBMP (232 mg, 1.13 mmol) and known thioglycoside **2-131**[55] (307.1 mg, 0.5155 mmol) in dry CH_2Cl_2 (10 mL) was stirred with 4

Å molecular sieves (650 mg) for 30 min. The reaction mixture was cooled to –40 °C and AgOTf (245 mg, 0.992 mmol) was added and the solution was stirred at –40 °C for 1.5 h. The AgOTf was quenched by the addition of Et_3N (0.1mL) and the reaction mixture was diluted with CH_2Cl_2 (50 mL) and filtered over a pad of Celite-545. The filtrate was washed with saturated aqueous $NaHCO_3$ solution (15 mL) and the organic layer was separated and the aqueous layer was further extracted with CH_2Cl_2 (2 × 50 mL). The combined organic phases were washed with brine (20 mL), dried over anhydrous Na_2SO_4, filtered, concentrated and the resulting crude product was purified by column chromatography (32% EtOAc in hexane) to afford **2-130** (419 mg, 75%) as a white foam: $[\alpha]^{22}_D$ +54.2 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.85–7.83 (m, 1 H, ArH), 7.73–7.65 (m, 3 H, ArH), 7.43–7.20 (m, 22 H, ArH), 7.00–6.95 (m, 4 H, ArH), 6.86–6.81 (m, 3 H, ArH), 5.49 (d, J = 10.0 Hz, 1 H, H-1_{GlcN}), 5.37 (dd, J = 10.0, 8.5 Hz, 1 H, H-2_{GalHep}), 4.97 (d, J = 11.5 Hz, 1 H, OC$\underline{H_2}$Ph), 4.88 (d, J = 12.0 Hz, 1 H, OC$\underline{H_2}$Ph), 4.71 (d, J = 11.5 Hz, 1 H, OC$\underline{H_2}$Ph), 4.69 (d, J = 12.0 Hz, 1 H, OC$\underline{H_2}$Ph), 4.55 (s, 2 H, 2 × OC$\underline{H_2}$Ph), 4.54 (d, J = 12.0 Hz, 1 H, OC$\underline{H_2}$Ph), 4.45–4.39 (m, 4 H, 3 × OC$\underline{H_2}$Ph and H-1_{GalHep}), 4.30–4.22 (m, 2 H, H-2_{GlcN} and H-3_{GlcN}), 3.86–3.82 (m, 2 H, H-4_{GlcN} and H-$6a_{GlcN}$), 3.78 (dd, J = 11.0, 4.0 Hz, 1 H, H-$6b_{GlcN}$), 3.65 (d, J = 2.0 Hz, 1 H, H-4_{GalHep}), 3.61 (dd, J = 10.0, 2.0 Hz, 1 H, H-5_{GlcN}), 3.48–3.40 (m, 3 H, H-3_{GalHep}, H-5_{GalHep} and H-$7a_{GalHep}$), 3.19 (ddd, J = 9.5, 5.0, 4.5 Hz, 1 H, H-$7b_{GalHep}$), 2.26 (s, 3 H, C$\underline{H_3}C_6H_4S$), 2.04 (s, 3 H, 3 × COC$\underline{H_3}$), 1.86–1.78 (m, 1 H, H-$6a_{GalHep}$), 1.62–1.54 (m, 1 H, H-$6b_{GalHep}$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.3 ($\underline{C}OCH_3$), 167.9 ($\underline{C}O$Phth), 167.4 ($\underline{C}O$Phth), 138.8 (Ar), 138.7 (Ar), 138.4 (Ar), 138.2 (Ar), 138.0 (Ar), 137.8 (Ar), 133.8 (Ar), 133.7 (Ar), 132.9 (Ar), 131.7 (Ar), 131.6 (Ar), 129.5 (Ar), 128.6 (Ar), 128.5 (Ar), 128.44 (Ar), 128.41 (Ar), 128.40 (Ar), 128.11 (Ar), 128.09 (Ar), 127.95 (Ar), 127.93 (Ar), 127.89 (Ar), 127.88 (Ar), 127.79 (Ar), 127.77 (Ar), 127.74 (Ar), 127.71 (Ar), 127.6 (Ar), 127.4 (Ar), 126.7

(Ar), 123.31 (Ar), 123.27 (Ar), 100.8 (C-1_{GalHep}), 83.8 (C-1_{GlcN}), 80.8 (C-3_{GalHep}), 79.4 (C-5_{GlcN}), 77.9 (2C, C-3_{GlcN} and C-4_{GlcN}), 75.0 (C-4_{GalHep}), 74.7 (O<u>*C*</u>H_2Ph), 74.3 (O<u>*C*</u>H_2Ph), 73.4 (O<u>*C*</u>H_2Ph), 73.1 (O<u>*C*</u>H_2Ph), 72.0 (C-2_{GalHep}), 71.9 (O<u>*C*</u>H_2Ph), 71.2 (C-5_{GalHep}), 68.1 (C-6_{GlcN}), 65.9 (C-7_{GalHep}), 56.0 (C-2_{GlcN}), 31.3 (C-6_{GalHep}), 21.0 (2C, <u>*C*</u>$H_3C_6H_4S$ and CO<u>*C*</u>H_3); IR (cast film, $CHCl_3$) *v* 3087, 3063, 3029, 2924, 2867, 1775, 1750, 1715, 1495, 1454, 1232, 1089, 698 cm^{-1}; HRMS (ESI-TOF) m/z: $[M + Na]^+$ Calcd for $C_{65}H_{65}NO_{12}SNa$ 1106.4120; Found 1106.4126.

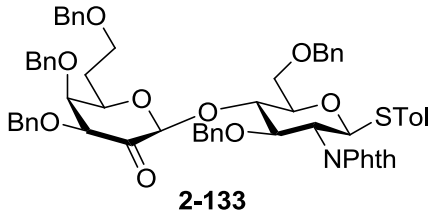


2-132

***p*-Tolyl 3,4,7-tri-*O*-benzyl-6-deoxy-β-D-*galacto*-heptopyranosyl-(1→4)-4,6-di-*O*-benzyl-2-deoxy-2-*N*-phthalimido-1-thio-β-D-glucopyranoside (2-132)**

To a stirred solution of thioglycoside **2-130** (1.560 g, 1.439 mmol) in CH_3OH (15 mL) and CH_2Cl_2 (5 mL) was added sodium methoxide in CH_3OH (0.60 M, 1.7 mL, 1.0 mmol). The resultant solution was stirred for 24 h and then Amberlite IR-120 resin (H^+ form) was added to neutralize the solution. The mixture was filtered and the resin was washed with CH_3OH (50 mL) and the resulting filtrate was concentrated to obtain a crude product that was purified by column chromatography (8% EtOAc in toluene) to afford **2-132** (1.19 g, 79%) as a colorless viscous liquid: $[\alpha]^{22}_D$ +52.3 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.81–7.79 (m, 1 H, ArH), 7.70–7.61 (m, 3 H, ArH), 7.39–7.19 (m, 22 H, ArH), 7.00–6.93 (m, 4 H, ArH), 6.83–6.78 (m, 3 H, ArH), 5.45 (d, *J* = 11.0 Hz, 1 H, H-1_{GlcN}), 4.92 (d, *J* = 12.0 Hz, 1 H, OC<u>*H*</u>$_2$Ph), 4.84 (d, *J* = 12.5 Hz, 1 H, OC<u>*H*</u>$_2$Ph), 4.76–4.68 (m, 3 H, 3 × OC<u>*H*</u>$_2$Ph), 4.61 (d, *J* = 12.0 Hz, 1 H, OC<u>*H*</u>$_2$Ph),

4.56 (d, J = 12.0 Hz, 1 H, OC$\underline{H_2}$Ph), 4.52 (d, J = 7.5 Hz, 1 H, H-1$_{GalHep}$), 4.44 (d, J = 12.0 Hz, 1 H, OC$\underline{H_2}$Ph), 4.36 (app t, J = 9.5, 9.0 Hz, 1 H, H-3$_{GlcN}$), 4.30, 4.27 (ABq, J = 12.0 Hz, 2 H, 2 × OC$\underline{H_2}$Ph), 4.22 (app t, J = 11.0, 9.5 Hz, 1 H, H-2$_{GlcN}$), 4.03 (dd, J = 11.5, 3.5 Hz, 1 H, H-6a$_{GlcN}$), 3.93 (app t, J = 9.0, 8.5 Hz, 1 H, H-4$_{GlcN}$), 3.93 (app t, J = 9.0, 7.5 Hz, 1 H, H-2$_{GalHep}$), 3.87 (d, J = 11.5 Hz, 1 H, H-6b$_{GlcN}$), 3.69 (m, 1 H, H-5$_{GlcN}$), 3.60 (d, J = 1.5 Hz, 1 H, H-4$_{GalHep}$), 3.47 (dd, J = 8.0, 3.5 Hz, 1 H, H-5$_{GalHep}$), 3.41–3.33 (m, 2 H, H-3$_{GalHep}$, and H-7a$_{GalHep}$), 3.21 (ddd, J = 9.0, 5.0, 4.5 Hz, 1 H, H-7b$_{GalHep}$), 3.12 (br s, 1 H, CH$\underline{OH}$), 2.26 (s, 3 H, C$\underline{H_3}$C$_6$H$_4$S), 1.90–1.80 (m, 1 H, H-6a$_{GalHep}$), 1.65–1.59 (m, 1 H, H-6b$_{GalHep}$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 168.0 ($\underline{C}$OPhth), 167.4 ($\underline{C}$OPhth), 138.8 (Ar), 138.6 (Ar), 138.4 (Ar), 138.3 (Ar), 138.1 (Ar), 138.0 (Ar), 133.8 (Ar), 133.7 (Ar), 133.3 (Ar), 131.8 (Ar), 131.7 (Ar), 129.6 (Ar), 128.5 (Ar), 128.43 (Ar), 128.38 (Ar), 128.23 (Ar), 128.15 (Ar), 128.1 (Ar), 127.81 (Ar), 127.78 (Ar), 127.7 (Ar), 127.4 (Ar), 126.9 (Ar), 123.4 (Ar), 123.2 (Ar), 103.4 (C-1$_{GalHep}$), 83.7 (C-1$_{GlcN}$), 82.4 (C-3$_{GalHep}$), 79.3 (C-3$_{GlcN}$), 79.2 (C-5$_{GlcN}$), 78.0 (C-4$_{GlcN}$), 75.2 (C-4$_{GalHep}$), 74.7 (O$\underline{C}$H$_2$Ph), 74.3 (O$\underline{C}$H$_2$Ph), 73.6 (O$\underline{C}$H$_2$Ph), 73.0 (O$\underline{C}$H$_2$Ph), 72.6 (O$\underline{C}$H$_2$Ph), 72.3 (C-2$_{GalHep}$), 71.7 (C-5$_{GalHep}$), 68.4 (C-6$_{GlcN}$), 66.1 (C-7$_{GalHep}$), 55.1 (C-2$_{GlcN}$), 31.5 (C-6$_{GalHep}$), 21.1 ($\underline{C}$H$_3$C$_6$H$_4$S); IR (cast film, $CHCl_3$) ν 3519, 3087, 3062, 3029, 2922, 2865, 1776, 1714, 1495, 1454, 1387, 1087, 751 cm^{-1}; HRMS (ESI-TOF) m/z: [M + Na]$^+$ Calcd for $C_{63}H_{63}NO_{11}SNa$ 1064.4014; Found 1064.4042.

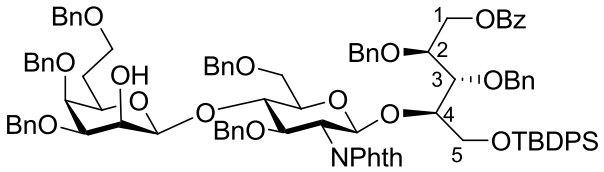


2-133

***p*-Tolyl 3,4,7-tri-*O*-benzyl-6-deoxy-β-D-*lyxo*-heptopyranos-2-ulosyl-(1→4)-4,6-di-*O*-benzyl-2-deoxy-2-*N*-phthalimido-1-thio-β-D-glucopyranoside (2-133)**

A stirred solution of alcohol **2-132** (1.118 g, 1.073 mmol) in CH_2Cl_2 (5.5 mL) was cooled to 0 °C. DMSO (0.80 mL, 11 mmol) and DIPEA (0.75 mL, 4.3 mmol) were added sequentially to the reaction mixture. SO_3·pyridine (515 mg, 3.24 mmol) was then added portionwise and the reaction mixture was stirred at 0 °C for 50 min. The reaction mixture was diluted with CH_2Cl_2 (50 mL) and washed with saturated aqueous $Na_2S_2O_3$ solution (15 mL) and the aqueous layer was separated and further extracted with CH_2Cl_2 (2 × 50 mL). The combined organic phases were washed with brine (20 mL), dried over anhydrous Na_2SO_4, filtered, concentrated and the resulting crude product was purified by column chromatography (10% EtOAc in toluene) to afford **2-133** (989 mg, 89%) as a colorless viscous liquid consisting of β-uloside **2-133** and its hydrate in an ~5:1 ratio: Data for β-uloside: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.82–7.79 (m, 1 H, ArH), 7.70–7.61 (m, 3 H, ArH), 7.40–7.20 (m, 22 H, ArH), 7.00–6.91 (m, 4 H, ArH), 6.82–6.77 (m, 3 H, ArH), 5.46 (d, J = 10.5 Hz, 1 H, H-1_{GlcN}), 4.94 (d, J = 11.5 Hz, 1 H, OC$\underline{H_2}$Ph), 4.92 (s, 1 H, H-1_{GalHep}), 4.88 (d, J = 12.5 Hz, 1 H, OC$\underline{H_2}$Ph), 4.80 (d, J = 12.5 Hz, 1 H, OC$\underline{H_2}$Ph), 4.67 (d, J = 12.0 Hz, 1 H, OC$\underline{H_2}$Ph), 4.57 (d, J = 12.0 Hz, 1 H, OC$\underline{H_2}$Ph), 4.47–4.39 (m, 4 H, 3 × OC$\underline{H_2}$Ph and H-3_{GlcN}), 4.35 (s, 2 H, 2 × OC$\underline{H_2}$Ph), 4.22 (app t, J = 10.5, 10.5 Hz, 1 H, H-2_{GlcN}), 3.99–3.90 (m, 4 H, H-3_{GalHep}, H-4_{GalHep}, H-4_{GlcN} and H-$6a_{GlcN}$), 3.86–3.82 (m, 2 H, H-5_{GalHep} and H-$6b_{GlcN}$), 3.78 (ddd, J = 10.0, 3.5, 1.5 Hz, 1 H, H-5_{GlcN}), 3.40–3.35 (m, 1 H, H-$7a_{GalHep}$), 3.17–

3.13 (m, 1 H, H-7b$_{GalHep}$), 2.27 (s, 3 H, C$\underline{H}_3$C$_6$H$_4$S), 1.89–1.81 (m, 1 H, H-6a$_{GalHep}$), 1.67–1.58 (m, 1 H, H-6b$_{GalHep}$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 199.2 (C-2$_{GalHep}$), 167.9 ($\underline{C}$OPhth), 167.4 ($\underline{C}$OPhth), 138.6 (Ar), 138.3 (Ar), 138.2 (Ar), 138.1 (Ar), 137.6 (Ar), 133.8 (Ar), 133.7 (Ar), 133.6 (Ar), 133.4 (Ar), 131.8 (Ar), 131.6 (Ar), 129.6 (Ar), 128.6 (Ar), 128.5 (Ar), 128.4 (Ar), 128.34 (Ar), 128.28 (Ar), 128.21 (Ar), 128.15 (Ar), 128.09 (Ar), 128.05 (Ar), 128.0 (Ar), 127.93 (Ar), 127.86 (Ar), 127.79 (Ar), 127.76 (Ar), 127.71 (Ar), 127.67 (Ar), 127.6 (Ar), 126.9 (Ar), 123.3 (Ar), 100.6 (C-1$_{GalHep}$), 83.60 (C-1$_{GlcN}$), 83.55 (C-3$_{GalHep}$), 81.0 (C-4$_{GalHep}$), 80.0 (C-4$_{GlcN}$), 78.8 (C-5$_{GlcN}$), 78.4 (C-3$_{GlcN}$), 74.9 (O$\underline{C}$H$_2$Ph), 74.2 (O$\underline{C}$H$_2$Ph), 73.3 (O$\underline{C}$H$_2$Ph), 73.1 (O$\underline{C}$H$_2$Ph), 72.4 (O$\underline{C}$H$_2$Ph), 71.9 (C-5$_{GalHep}$), 68.7 (C-6$_{GlcN}$), 65.9 (C-7$_{GalHep}$), 55.1 (C-2$_{GlcN}$), 31.1 (C-6$_{GalHep}$), 21.1 ($\underline{C}$H$_3$C$_6$H$_4$S); IR (cast film, $CHCl_3$) ν 3088, 3062, 3030, 2918, 2869, 1775, 1757, 1714, 1495, 1453, 1386, 1074, 698 cm^{-1}; HRMS (ESI-TOF) m/z: [M + Na]$^+$ Calcd for $C_{63}H_{61}NO_{11}SNa$ 1062.3858; Found 1062.3859.



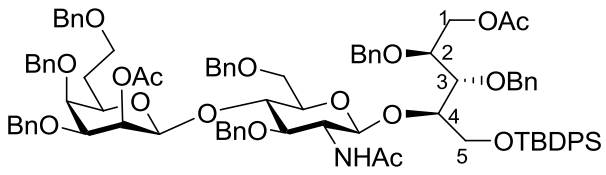
2-126β

3,4,7-Tri-*O*-benzyl-6-deoxy-β-D-*talo*-heptopyranosyl-(1→4)-(3,6-di-*O*-benzyl-2-deoxy-2-*N*-phthalimido-β-D-glucopyranosyl)-(1→4)-1-*O*-benzoyl-2,3-di-*O*-benzyl-5-*O*-*tert*-butyldiphenylsilyl-D-ribitol (2-126β)

A solution of thioglycoside **2-133** (1.028 g, 0.9882 mmol) and alcohol **2-100** (833.7 mg, 1.235 mmol) in dry CH_2Cl_2 (27 mL) was stirred with 4 Å molecular sieves (2.1 g) for 30 min. The reaction mixture was cooled to –50 °C before NIS (445 mg, 1.98 mmol) and TMSOTf (20 μL, 0.11 mmol) dissolved in CH_2Cl_2 (1 mL) were sequentially added to the solution. The

mixture was stirred at –50 °C for 1.5 h. TMSOTf was quenched by the addition of Et_3N (0.1 mL) and the reaction mixture was diluted with CH_2Cl_2 (75 mL) and filtered over a pad of Celite-545. The filtrate was washed with saturated aqueous $Na_2S_2O_3$ solution (20 mL) and the organic layer was separated and the aqueous layer was further extracted with CH_2Cl_2 (2 × 50 mL). The combined organic phases were washed with brine (20 mL), dried over anhydrous Na_2SO_4, filtered and concentrated and the resulting crude product was quickly purified by column chromatography (50% EtOAc in hexane) to afford the corresponding uloside as a white foam. This uloside (1.321 g, 0.8303 mmol) was dissolved in THF (15 mL) and cooled to 0 °C. $NaBH_4$ (63.0 mg, 1.67 mmol) was added portionwise to the reaction mixture and the ice bath was removed and allowed to warm to room temperature over a period of 2 h. Excess $NaBH_4$ was quenched by the addition of saturated aqueous NH_4Cl solution and the reaction mixture was extracted with EtOAc (30 mL). The aqueous layer was separated and further extracted with EtOAc (2 × 30 mL). The combined organic phases were washed with brine (20 mL), dried over anhydrous Na_2SO_4, filtered and concentrated and the resulting crude product was purified by column chromatography (45% EtOAc in hexane) to afford **2-126β** (1.26 g, 80% over the two steps) as a colorless viscous liquid: $[\alpha]^{22}_D$ –10.9 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.97 (d, *J* = 8.0 Hz, 2 H, ArH), 7.69–7.50 (m, 9 H, ArH), 7.43–7.13 (m, 38 H, ArH), 7.00–6.93 (m, 2 H, ArH), 6.83–6.78 (m, 3 H, ArH), 5.48 (d, *J* = 8.5 Hz, 1 H, H-1_{GlcN}), 5.04 (d, *J* = 11.5 Hz, 1 H, OC$\underline{H}_2$Ph), 4.97 (d, *J* = 12.5 Hz, 1 H, OC$\underline{H}_2$Ph), 4.75 (d, *J* = 12.0 Hz, 1 H, OC$\underline{H}_2$Ph), 4.73–4.68 (m, 1 H, H-$1a_{Rib\text{-}ol}$), 4.61–4.52 (m, 7 H, 6 × OC$\underline{H}_2$Ph and H-3_{GlcN}), 4.49–4.42 (m, 3 H, OC$\underline{H}_2$Ph, H-$1b_{Rib\text{-}ol}$ and H-1_{TalHep}), 4.39–4.35 (m, 4 H, 4 × OC$\underline{H}_2$Ph), 4.34–4.26 (m, 2 H, H-2_{GlcN} and H-$4_{Rib\text{-}ol}$), 4.10–4.06 (m, 1 H, H-2_{TalHep}), 4.04–3.92 (m, 4 H, H-4_{GlcN}, H-$2_{Rib\text{-}ol}$, H-$3_{Rib\text{-}ol}$ and CH$\underline{OH}$), 3.89 (dd, *J* = 11.5, 3.5 Hz, 1 H, H-$6a_{GlcN}$), 3.85 (dd, *J* = 11.0, 4.5 Hz, 1 H, H-$5a_{Rib\text{-}ol}$),

3.71–3.60 (m, 4 H, H-4$_{TalHep}$, H-5b$_{Rib\text{-}ol}$, H-5$_{GlcN}$, and H-6b$_{GlcN}$), 3.21 (app td, *J* = 9.1, 9.0, 4.0 Hz, 1 H, H-7a$_{TalHep}$), 3.42 (dd, *J* = 9.0, 4.0 Hz, 1 H, H-5$_{TalHep}$), 3.32–3.24 (m, 2 H, H-3$_{TalHep}$ and H-7b$_{TalHep}$), 1.96–1.89 (app ddt, *J* = 13.8, 9.1, 4.5, 4.5 Hz, 1 H, H-6a$_{TalHep}$), 1.68–1.61 (m, 1 H, H-6b$_{TalHep}$), 0.98 (s, 9 H, (C*H*$_3$)$_3$CSi(Ph)$_2$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 167.8 (*C*OPhth), 167.7 (*C*OPhth), 166.4 (*C*OPh), 139.0 (Ar), 138.3 (Ar), 138.2 (Ar), 138.0 (Ar), 137.8 (Ar), 135.6 (Ar), 135.5 (Ar), 133.4 (Ar), 133.3 (Ar), 133.1 (Ar), 132.7 (Ar), 130.3 (Ar), 129.6 (Ar), 129.53 (Ar), 129.52 (Ar), 128.4 (Ar), 128.3 (Ar), 128.2 (Ar), 128.1 (Ar), 128.0 (Ar), 127.8 (Ar), 127.7 (Ar), 127.5 (Ar), 127.3 (Ar), 126.7 (Ar), 123.2 (Ar), 123.1 (Ar), 101.8 (C-1$_{TalHep}$), 97.6 (C-1$_{GlcN}$), 78.7 (C-4$_{GlcN}$), 78.5 (C-4$_{Rib\text{-}ol}$), 78.4 (C-3$_{Rib\text{-}ol}$), 77.6 (C-3$_{GlcN}$), 77.4 (2C, C-2$_{Rib\text{-}ol}$ and C-3$_{TalHep}$), 77.0 (C-4$_{TalHep}$), 75.3 (O*C*H$_2$Ph), 74.6 (O*C*H$_2$Ph), 74.5 (C-5$_{GlcN}$), 73.4 (O*C*H$_2$Ph), 73.2 (O*C*H$_2$Ph), 73.1 (O*C*H$_2$Ph), 72.20 (O*C*H$_2$Ph), 72.15 (C-5$_{TalHep}$), 69.6 (O*C*H$_2$Ph), 68.7 (C-6$_{GlcN}$), 68.5 (C-2$_{TalHep}$), 66.1 (C-7$_{TalHep}$), 64.0 (C-1$_{Rib\text{-}ol}$), 63.8 (C-5$_{Rib\text{-}ol}$), 56.4 (C-2$_{GlcN}$), 31.4 (C-6$_{TalHep}$), 26.8 ((*C*H$_3$)$_3$CSi(Ph)$_2$), 19.0 ((CH$_3$)$_3$*C*Si(Ph)$_2$); IR (cast film, $CHCl_3$) ν 3490, 3060, 3030, 2928, 2857, 1776, 1716, 1453, 1428, 1112, 1065, 699 cm^{-1}; HRMS (ESI-TOF) m/z: $[M + Na]^+$ Calcd for $C_{98}H_{101}NO_{17}SiNa$ 1614.6731; Found 1614.6711.

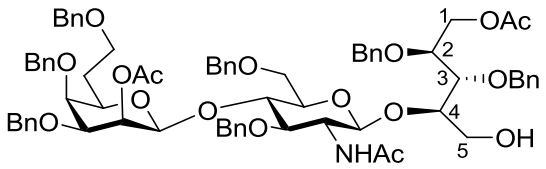


2-134

2-*O*-Acetyl-3,4,7-tri-*O*-benzyl-6-deoxy-β-D-*talo*-heptopyranosyl-(1→4)-(2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→4)-1-*O*-acetyl-2,3-di-*O*-benzyl-5-*O*-*tert*-butyldiphenylsilyl-D-ribitol (2-134)

To a stirred solution of trisaccharide **2-126β** (269 mg, 0.169 mmol) in ethanol (2.5 mL) was added ethylenediamine (0.50 mL, 7.5 mmol). The reaction mixture was heated at 90 °C for 48 h, after which time the solution was cooled to room temperature over 20 min. The solution was concentrated and the resulting crude product was dissolved in EtOAc (30 mL) and washed with water (2 × 5 mL). The organic layers were separated and the aqueous layer was further extracted with EtOAc (2 × 50 mL), dried over anhydrous Na_2SO_4, filtered and concentrated to obtain the crude amino alcohol as a pale yellow viscous liquid, which was used directly in the next step. This crude amino alcohol was dissolved in pyridine (3 mL), Ac_2O (0.50 mL, 5.3 mmol) was added and the reaction mixture was heated at 50 °C for 48 h, after which time the solution was cooled to room temperature over 15 min. Toluene (5 mL) was added and the resulting solution was concentrated. Co-concentration with toluene (4 × 5 mL) produced a crude product that was purified by column chromatography (20% EtOAc in toluene) to afford **2-134** (178 mg, 71% over the two steps) as a white foam: $[\alpha]^{22}_D$ –26.3 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.68–7.62 (m, 4 H, ArH), 7.47–7.15 (m, 41 H, ArH), 5.45 (d, J = 3.0 Hz, 1 H, H-2_{TalHep}), 5.17 (d, J = 8.0 Hz, 1 H, $N\underline{H}COCH_3$), 4.99 (d, J = 11.0 Hz, 1 H, OC$\underline{H}_2$Ph), 4.97 (d, J = 11.5 Hz, 1 H, OC$\underline{H}_2$Ph), 4.82 (d, J = 7.5 Hz, 1 H, H-1_{GlcN}), 4.65 (s, 2 H, 2 × OC$\underline{H}_2$Ph), 4.63–4.53

(m, 7 H, 6 × OC$\underline{H_2}$Ph and H-1$_{TalHep}$), 4.47 (dd, J = 11.5, 2.5 Hz, 1 H, H-1a$_{Rib\text{-}ol}$), 4.42 (d, J = 12.0 Hz, 1 H, OC$\underline{H_2}$Ph), 4.41 (d, J = 12.0 Hz, 1 H, OC$\underline{H_2}$Ph), 4.65 (s, 2 H, 2 × OC$\underline{H_2}$Ph), 4.27 (dd, J = 11.5, 6.0 Hz, 1 H, H-1b$_{Rib\text{-}ol}$), 4.16 (app dt, J = 6.0, 4.3, 4.0 Hz, 1 H, H-4$_{Rib\text{-}ol}$), 4.04–3.95 (m, 3 H, H-4$_{GlcN}$, H-2$_{Rib\text{-}ol}$ and H-5a$_{Rib\text{-}ol}$), 3.93–3.85 (m, 2 H, H-3$_{Rib\text{-}ol}$ and H-5b$_{Rib\text{-}ol}$), 3.75–3.64 (m, 4 H, H-2$_{GlcN}$, H-3$_{GlcN}$, H-6a$_{GlcN}$ and H-6b$_{GlcN}$), 3.53 (d, J = 2.1 Hz, 1 H, H-4$_{TalHep}$), 3.49–3.37 (m, 3 H, H-5$_{GlcN}$, H-5$_{TalHep}$ and H-7a$_{TalHep}$), 3.33–3.28 (m, 2 H, H-3$_{TalHep}$ and H-7b$_{TalHep}$), 2.07 (s, 3 H, 3 × COC$\underline{H_3}$), 2.07–2.00 (m, 1 H, H-6a$_{TalHep}$), 1.93 (s, 3 H, 3 × COC$\underline{H_3}$), 1.82–1.72 (m, 1 H, H-6b$_{TalHep}$), 1.62 (s, 3 H, 3 × NHCOC$\underline{H_3}$), 1.10 (s, 9 H, (C$\underline{H_3}$)$_3$CSi(Ph)$_2$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.1 ($\underline{C}$O$\underline{C}$H$_3$), 171.0 ($\underline{C}$O$\underline{C}$H$_3$), 169.9 ($\underline{C}$O$\underline{C}$H$_3$), 139.3 (Ar), 139.0 (Ar), 138.5 (Ar), 138.3 (Ar), 138.2 (Ar), 138.0 (Ar), 135.7 (Ar), 135.6 (Ar), 133.6 (Ar), 133.0 (Ar), 129.9 (Ar), 128.5 (Ar), 128.4 (Ar), 128.3 (Ar), 128.1 (Ar), 128.0 (Ar), 127.8 (Ar), 127.7 (Ar), 127.6 (Ar), 127.5 (Ar), 127.4 (Ar), 127.2 (Ar), 100.4 (C-1$_{GlcN}$), 98.7 (C-1$_{TalHep}$), 79.5 (C-3$_{GlcN}$), 78.5 (C-4$_{Rib\text{-}ol}$), 78.4 (C-3$_{Rib\text{-}ol}$), 77.9 (2C, C-2$_{Rib\text{-}ol}$ and C-3$_{TalHep}$), 76.7 (C-4$_{GlcN}$), 74.5 (C-4$_{TalHep}$), 74.4 (C-5$_{GlcN}$), 74.2 (O$\underline{C}$H$_2$Ph), 73.9 (O$\underline{C}$H$_2$Ph), 73.4 (2C, 2 × O$\underline{C}$H$_2$Ph), 72.9 (O$\underline{C}$H$_2$Ph), 72.6 (C-5$_{TalHep}$), 72.2 (O$\underline{C}$H$_2$Ph), 70.6 (O$\underline{C}$H$_2$Ph), 69.1 (C-6$_{GlcN}$), 66.9 (C-2$_{TalHep}$), 66.4 (C-7$_{TalHep}$), 64.4 (C-5$_{Rib\text{-}ol}$), 63.6 (C-1$_{Rib\text{-}ol}$), 55.4 (C-2$_{GlcN}$), 31.2 (C-6$_{TalHep}$), 27.0 (($\underline{C}$H$_3$)$_3$CSi(Ph)$_2$), 23.4 (NHCO$\underline{C}$H$_3$), 21.2 (CO$\underline{C}$H$_3$), 20.9 (CO$\underline{C}$H$_3$), 19.3 ((CH$_3$)$_3\underline{C}$Si(Ph)$_2$); IR (cast film, $CHCl_3$) ν 3398, 3065, 3030, 2928, 2858, 1739, 1678, 1454, 1366, 1112, 1069, 739 cm^{-1}; HRMS (ESI-TOF) m/z: [M + Na]$^+$ Calcd for $C_{89}H_{101}NO_{17}SiNa$ 1506.6731; Found 1506.6765.

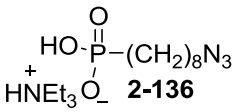


2-135

2-*O*-Acetyl-3,4,7-tri-*O*-benzyl-6-deoxy-β-D-*talo*-heptopyranosyl-(1→4)-(2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→4)-1-*O*-acetyl-2,3-di-*O*-benzyl-D-ribitol (2-135)

To a stirred solution of trisaccharide **2-134** (66 mg, 0.044 mmol) in THF (2 mL) was added TBAF (0.20 mL, 0.20 mmol, buffered with 30 μL AcOH). The reaction mixture was stirred for 24 h and then concentrated. The resulting crude product was purified by column chromatography (75% EtOAc in hexane) to afford alcohol **2-135** (46 mg, 83%) as a colorless viscous liquid: $[\alpha]^{22}_{D}$ –20.0 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.42–7.18 (m, 35 H, ArH), 5.87 (d, J = 8.0 Hz, 1 H, $N\underline{H}COCH_3$), 5.44 (d, J = 2.5 Hz, 1 H, H-2_{TalHep}), 5.00 (d, J = 7.0 Hz, 1 H, H-1_{GlcN}), 4.97 (d, J = 11.0 Hz, 1 H, OC$\underline{H}_2$Ph), 4.93 (d, J = 11.0 Hz, 1 H, OC$\underline{H}_2$Ph), 4.78–4.54 (m, 8 H, 8 × OC$\underline{H}_2$Ph), 4.50–4.39 (m, 6 H, 4 × OC$\underline{H}_2$Ph, H-1_{TalHep} and H-$1a_{Rib\text{-}ol}$), 4.27 (dd, J = 11.5, 6.0 Hz, 1 H, H-$1b_{Rib\text{-}ol}$), 4.04 (app t, J = 7.5, 7.0 Hz, 1 H, H-4_{GlcN}), 3.98–3.91 (m, 3 H, H-$2_{Rib\text{-}ol}$, H-$3_{Rib\text{-}ol}$ and H-$4_{Rib\text{-}ol}$), 3.89 (app t, J = 7.5, 7.5 Hz, 1 H, H-3_{GlcN}), 3.78–3.66 (m, 4 H, H-$5a_{Rib\text{-}ol}$, H-$5b_{Rib\text{-}ol}$, H-$6a_{GlcN}$ and H-$6b_{GlcN}$), 3.65–3.54 (m, 3 H, H-2_{GlcN}, H-5_{GlcN} and H-4_{TalHep}), 3.52–3.46 (m, 1 H, H-$7a_{TalHep}$), 3.44 (dd, J = 8.5, 5.0 Hz, 1 H, H-5_{TalHep}), 2.85 (br s, 1 H, CH$O\underline{H}$), 3.38–3.33 (m, 2 H, H-3_{TalHep} and H-$7b_{TalHep}$), 2.09–2.00 (m, 1 H, H-$6a_{TalHep}$), 2.05 (s, 3 H, 3 × COC$\underline{H}_3$), 1.96 (s, 3 H, 3 × COC$\underline{H}_3$), 1.86 (s, 3 H, 3 × NHCOC$\underline{H}_3$), 1.86–1.76 (m, 1 H, H-$6b_{TalHep}$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.96 ($\underline{C}O$CH$_3$), 170.94 ($\underline{C}O$CH$_3$), 170.8 ($\underline{C}O$CH$_3$), 139.1 (Ar), 138.6 (Ar), 138.4 (Ar), 138.1 (Ar), 138.0 (Ar), 137.91 (Ar), 137.87 (Ar), 128.5 (Ar), 128.42 (Ar), 128.38 (Ar), 128.33 (Ar), 128.31 (Ar), 128.2 (Ar), 128.05 (Ar), 128.03 (Ar), 127.94 (Ar), 127.87 (Ar), 127.74 (Ar), 127.66 (Ar), 127.60 (Ar), 127.58 (Ar), 127.5 (Ar), 127.4, 127.33

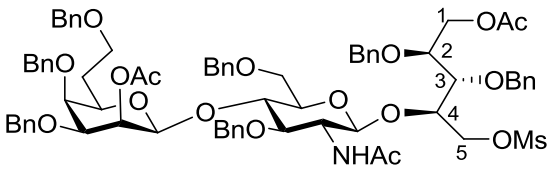
(Ar), 127.29 (Ar), 100.5 (C-1_{GlcN}), 98.3 (C-1_{TalHep}), 79.5 (C-$4_{Rib\text{-}ol}$), 78.7 (2C, C-$3_{Rib\text{-}ol}$ and C-3_{GlcN}), 77.6 (C-3_{TalHep}), 77.1 (C-$2_{Rib\text{-}ol}$), 76.1 (C-4_{GlcN}), 74.8 (C-5_{GlcN}), 74.2 (2C, C-4_{TalHep} and O$\underline{C}H_2$Ph), 74.0 (O$\underline{C}H_2$Ph), 73.8 (O$\underline{C}H_2$Ph), 73.4 (O$\underline{C}H_2$Ph), 72.9 (O$\underline{C}H_2$Ph), 72.6 (C-5_{TalHep}), 72.3 (O$\underline{C}H_2$Ph), 70.6 (O$\underline{C}H_2$Ph), 69.2 (C-6_{GlcN}), 66.9 (C-2_{TalHep}), 66.3 (C-7_{TalHep}), 63.3 (C-$1_{Rib\text{-}ol}$), 61.7 (C-$5_{Rib\text{-}ol}$), 55.8 (C-2_{GlcN}), 31.2 (C-6_{TalHep}), 23.3 (NHCO$\underline{C}H_3$), 21.1 (CO$\underline{C}H_3$), 20.9 (CO$\underline{C}H_3$); IR (cast film, $CHCl_3$) ν 3313, 3088, 3063, 3030, 2923, 2866, 1738, 1657, 1496, 1454, 1069, 737 cm^{-1}; HRMS (ESI-TOF) m/z: $[M + Na]^+$ Calcd for $C_{73}H_{83}NO_{17}Na$ 1268.5553; Found 1268.5569.



Triethylammonium 8-azidooctyl-1-phosphate (2-136)

To a 0 °C solution of 8-azido-1-octanol[61] (260.8 mg, 1.524 mmol) in THF (3.5 mL) was added Et_3N (0.75 mL, 5.4 mmol) and $POCl_3$ (215 μL, 2.31 mmol). The ice bath was removed and the reaction mixture was warmed to room temperature over 1 h with stirring. Water (2 mL) was added and the reaction mixture was extracted with CH_2Cl_2 (30 mL). The aqueous layer was separated and further extracted with CH_2Cl_2 (2 × 20 mL). The combined organic phases were washed with brine (10 mL), dried over anhydrous Na_2SO_4, filtered and concentrated and the resulting crude product (483 mg, 90%) was used in the next step without further purification: ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.58 (m, 2 H, O(PO)(O$\underline{H}$)$_2$), 4.20–3.99 (m, 2 H, OC$\underline{H_2}$CH$_2$), 3.26 (t, J = 7.0 Hz, 2 H, 2 × C$\underline{H_2}$N$_3$), 1.73–1.63 (m, 2 H, C$\underline{H_2}$), 1.63–1.55 (m, 2 H, C$\underline{H_2}$), 1.42–1.29 (m, 8 H, (C$\underline{H_2}$)$_4$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 68.1 ($\underline{C}$H$_2$O(PO)(O$\underline{H}$)$_2$), 51.5 ($\underline{C}$H$_2$N$_3$), 30.1 ($\underline{C}$H$_2$),

29.02 (<u>C</u>H$_2$), 28.99 (<u>C</u>H$_2$), 28.8 (<u>C</u>H$_2$), 26.6 (<u>C</u>H$_2$), 25.3 (<u>C</u>H$_2$); ^{31}P NMR (202 MHz, $CDCl_3$) δ 1.23; HRMS (ESI-TOF) m/z: $[M]^-$ Calcd for $C_8H_{17}N_3O_4P$ 250.0962; Found 250.0960.

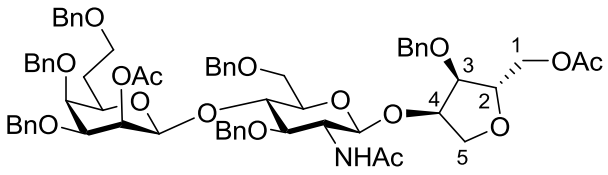


2-139

2-*O*-Acetyl-3,4,7-tri-*O*-benzyl-6-deoxy-β-D-*talo*-heptopyranosyl-(1→4)-(2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→4)-1-*O*-acetyl-2,3-di-*O*-benzyl-5-*O*-methanesulfonyl-D-ribitol (2-139)

To a 0 °C solution of trisaccharide **2-135** (16.1 mg, 0.0129 mmol) in CH_2Cl_2 (1.5 mL) was sequentially added pyridine (20 µL, 0.25 mmol) and methanesulfonyl chloride (15 µL, 0.19 mmol). The ice bath was removed and the reaction mixture warmed to room temperature over 3 h with stirring. The reaction mixture was diluted with CH_2Cl_2 (20 mL) and washed with saturated aqueous $NaHCO_3$ solution (5 mL). The organic layer was separated and the aqueous layer was further extracted with CH_2Cl_2 (2 × 15 mL). The combined organic phases were washed with brine (10 mL), dried over anhydrous Na_2SO_4, filtered and concentrated and the resulting crude product was purified by column chromatography (75% EtOAc in hexane) to afford **2-139** (14 mg, 82%) as a colorless viscous liquid: $[\alpha]^{22}_D$ –22.6 (*c* 0.23, $CHCl_3$); ^{1}H NMR (700 MHz, $CDCl_3$) δ 7.36–7.14 (m, 35 H, ArH), 5.72 (d, *J* = 9.1 Hz, 1 H, *N<u>H</u>*COCH$_3$), 5.41 (d, *J* = 2.8 Hz, 1 H, H-2$_{TalHep}$), 4.94 (d, *J* = 11.2 Hz, 1 H, OC<u>*H*$_2$</u>Ph), 4.92 (d, *J* = 11.2 Hz, 1 H, OC<u>*H*$_2$</u>Ph), 4.83 (d, *J* = 7.7 Hz, 1 H, H-1$_{GlcN}$), 4.71–4.68 (m, 2 H, OC<u>*H*$_2$</u>Ph and H-1a$_{Rib\text{-}ol}$), 4.65 (d, *J* = 11.2 Hz, 1 H, OC<u>*H*$_2$</u>Ph), 4.64 (d, *J* = 11.9 Hz, 1 H, OC<u>*H*$_2$</u>Ph), 4.55–4.51 (m, 4 H, 4 × OC<u>*H*$_2$</u>Ph), 4.47 (s, 1 H,

H-1$_{TalHep}$), 4.42–4.35 (m, 3 H, OC$\underline{H_2}$Ph, H-1b$_{Rib\text{-}ol}$ and H-5a$_{Rib\text{-}ol}$), 4.33–4.29 (m, 4 H, 4 × OC$\underline{H_2}$Ph), 4.21–4.17 (m, 2 H, H-4$_{Rib\text{-}ol}$ and H-5b$_{Rib\text{-}ol}$), 3.97 (app t, J = 8.4, 7.7 Hz, 1 H, H-4$_{GlcN}$), 3.88–3.81 (m, 3 H, H-2$_{Rib\text{-}ol}$, H-3$_{Rib\text{-}ol}$ and H-3$_{GlcN}$), 3.68–3.65 (m, 2 H, H-2$_{GlcN}$ and H-6a$_{GlcN}$), 3.63 (dd, J = 11.2, 3.5 Hz, 1 H, H-6b$_{GlcN}$), 3.58–3.53 (m, 1 H, H-5$_{GlcN}$), 3.50–3.48 (m, 1 H, H-4$_{TalHep}$), 3.42 (ddd, J = 8.9, 6.5, 4.9 Hz, 1 H, H-7a$_{TalHep}$), 3.38 (dd, J = 8.4, 4.9 Hz, 1 H, H-5$_{TalHep}$), 3.38 (app t, J = 3.5, 2.8 Hz, 1 H, H-3$_{TalHep}$), 3.29–3.25 (m, 1 H, H-7b$_{TalHep}$), 2.90 (s, 3 H, OSO$_2$C$\underline{H_3}$), 2.06–1.97 (m, 1 H, H-6a$_{TalHep}$), 2.02 (s, 3 H, 3 × COC$\underline{H_3}$), 1.92 (s, 3 H, 3 × COC$\underline{H_3}$), 1.89 (s, 3 H, 3 × NHCOC$\underline{H_3}$), 1.77–1.72 (m, 1 H, H-6b$_{TalHep}$); ^{13}C NMR (175 MHz, $CDCl_3$) δ 171.2 ($\underline{C}OCH_3$), 170.8 ($\underline{C}OCH_3$), 170.6 ($\underline{C}OCH_3$), 139.2 (Ar), 138.8 (Ar), 138.5 (Ar), 138.03 (Ar), 138.01 (Ar), 137.9 (Ar), 137.8 (Ar), 128.53 (Ar), 128.48 (Ar), 128.4 (Ar), 128.3 (Ar), 128.2 (Ar), 128.1 (Ar), 128.0 (Ar), 127.85 (Ar), 127.78 (Ar), 127.7 (Ar), 127.6 (Ar), 127.4 (Ar), 127.3 (Ar), 100.8 (C-1$_{GlcN}$), 98.4 (C-1$_{TalHep}$), 79.4 (C-3$_{GlcN}$), 78.5 (C-3$_{Rib\text{-}ol}$), 77.8 (C-3$_{TalHep}$), 76.78 (C-2$_{Rib\text{-}ol}$), 76.71 (C-4$_{Rib\text{-}ol}$), 76.3 (C-4$_{GlcN}$), 74.9 (C-5$_{GlcN}$), 74.3 (C-4$_{TalHep}$), 74.2 (O$\underline{C}H_2$Ph), 74.0 (O$\underline{C}H_2$Ph), 73.9 (O$\underline{C}H_2$Ph), 73.5 (O$\underline{C}H_2$Ph), 73.0 (O$\underline{C}H_2$Ph), 72.7 (C-5$_{TalHep}$), 72.5 (O$\underline{C}H_2$Ph), 70.6 (C-1$_{Rib\text{-}ol}$), 69.9 (O$\underline{C}H_2$Ph), 69.2 (C-6$_{GlcN}$), 66.9 (C-2$_{TalHep}$), 66.4 (C-7$_{TalHep}$), 62.8 (C-5$_{Rib\text{-}ol}$), 55.6 (C-2$_{GlcN}$), 37.3 (OSO$_2\underline{C}H_3$), 31.2 (C-6$_{TalHep}$), 23.5 (NHCO$\underline{C}H_3$), 21.2 (CO$\underline{C}H_3$), 20.9 (CO$\underline{C}H_3$); IR (cast film, $CHCl_3$) ν 3327, 3089, 3062, 3030, 2959, 2925, 2854, 1738, 1667, 1497, 1454, 1068, 748 cm^{-1}; HRMS (ESI-TOF) m/z: $[M + H]^+$ Calcd for $C_{74}H_{86}NO_{19}S$ 1324.5509; Found 1324.5509.



2-138

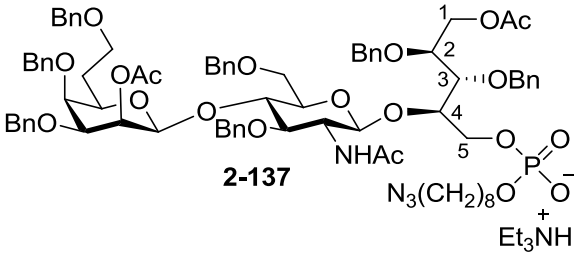
2-*O*-Acetyl-3,4,7-tri-*O*-benzyl-6-deoxy-β-D-*galacto*-heptopyranosyl-(1→4)-2-acetamido-4,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl-(1→4)-1-*O*-acetyl-2,5-anhydro-3-*O*-benzyl-D-ribitol (2-138)

Procedure A: To a stirred solution of trisaccharide **2-139** (14.0 mg, 0.0106 mmol) in CH_3CN (1.5 mL), was added phosphate **2-136** (16.5 mg, 0.0468 mmol). The resulting mixture was heated at reflux for 12 h at 80 °C, after which time the solution was cooled to room temperature over 20 min. The reaction mixture was diluted with EtOAc (30 mL) and washed with water (5 mL). The organic layer was separated and the aqueous layer was further extracted with EtOAc (2 × 15 mL). The combined organic phases were washed with brine (10 mL), dried over anhydrous Na_2SO_4, filtered and concentrated and the resulting crude product was purified by column chromatography (90% EtOAc in hexane) to afford **2-138** (7.5 mg, 62%) as a colorless viscous liquid.

Procedure B: To a stirred solution of trisaccharide **2-135** (14.1 mg, 0.0113 mmol) in THF (1.5 mL) was added Et_3N (10 μL, 0.072 mmol) and $POCl_3$ (5.0 μL, 0.054 mmol). The ice bath was removed and the reaction mixture was warmed to room temperature over 1.5 h with stirring. Water (1 mL) was added and the reaction mixture was extracted with CH_2Cl_2 (20 mL). The aqueous layer was separated and further extracted with CH_2Cl_2 (2 × 20 mL). The combined organic phases were washed with brine (10 mL), dried over anhydrous Na_2SO_4, filtered and concentrated and the resulting crude product was purified by column chromatography (90%

EtOAc in hexane) to afford the corresponding phosphate (35%) along with **2-138** (5.2 mg, 40%) as a colorless viscous liquid: Data for **2-138:** $[\alpha]^{22}_{D}$ –233.9 (*c* 0.01, $CHCl_3$); ^{1}H NMR (700 MHz, $CDCl_3$) δ 7.37–7.13 (m, 30 H, ArH), 5.70 (d, J = 7.7 Hz, 1 H, $N\underline{H}COCH_3$), 5.41 (d, J = 2.8 Hz, 1 H, H-2_{TalHep}), 5.12 (d, J = 7.7 Hz, 1 H, H-1_{GlcN}), 5.03 (d, J = 10.5 Hz, 1 H, OC$\underline{H_2}$Ph), 4.94 (d, J = 11.2 Hz, 1 H, OC$\underline{H_2}$Ph), 4.82 (d, J = 11.2 Hz, 1 H, OC$\underline{H_2}$Ph), 4.71 (d, J = 11.9 Hz, 1 H, OC$\underline{H_2}$Ph), 4.55 (d, J = 11.9 Hz, 1 H, OC$\underline{H_2}$Ph), 4.54 (d, J = 11.2 Hz, 1 H, OC$\underline{H_2}$Ph), 4.50–4.45 (m, 3 H, 2 × OC$\underline{H_2}$Ph and H-1_{TalHep}), 4.43–4.37 (m, 3 H, 2 × OC$\underline{H_2}$Ph and H-4_{Rib}), 4.34 (s, 2 H, 2 × OC$\underline{H_2}$Ph), 4.23 (dd, J = 11.9, 3.5 Hz, 1 H, H-$1a_{Rib}$), 4.20 (app t, J = 9.1, 8.4 Hz, 1 H, H-3_{GlcN}), 4.12–4.08 (m, 1 H, H-2_{Rib}), 4.01 (dd, J = 11.9, 5.6 Hz, 1 H, H-$1b_{Rib}$), 3.97 (dd, J = 9.8, 4.2 Hz, 1 H, H-$5a_{Rib}$), 3.95 (app t, J = 9.1, 8.4 Hz, 1 H, H-4_{GlcN}), 3.85 (dd, J = 9.8, 2.8 Hz, 1 H, H-$5b_{Rib}$), 3.76–3.69 (m, 3 H, H-3_{Rib}, H-$6a_{GlcN}$ and H-$6b_{GlcN}$), 3.64–3.60 (m, 1 H, H-5_{GlcN}), 3.52–3.49 (m, 1 H, H-4_{TalHep}), 3.44 (ddd, J = 9.1, 9.1, 4.2 Hz, 1 H, H-$7a_{TalHep}$), 3.40 (dd, J = 8.4, 4.9 Hz, 1 H, H-5_{TalHep}), 3.32–3.26 (m, 2 H, H-3_{TalHep} and H-$7b_{TalHep}$), 3.21 (app q, J = 8.4, 7.7, 7.7 Hz, 1 H, H-2_{GlcN}), 2.05–1.97 (m, 1 H, H-$6a_{TalHep}$), 2.00 (s, 3 H, 3 × COC$\underline{H_3}$), 1.99 (s, 3 H, 3 × COC$\underline{H_3}$), 1.84 (s, 3 H, 3 × NHCOC$\underline{H_3}$), 1.77 (dddd, J = 13.7, 9.1, 4.9, 4.9 Hz, 1 H, H-$6b_{TalHep}$); ^{13}C NMR (175 MHz, $CDCl_3$) δ 171.1 ($\underline{C}OCH_3$), 170.9 ($\underline{C}OCH_3$), 170.6 ($\underline{C}OCH_3$), 139.3 (Ar), 138.9 (Ar), 138.5 (Ar), 138.0 (Ar), 137.9 (Ar), 137.8 (Ar), 128.54 (Ar), 128.50 (Ar), 128.46 (Ar), 128.2 (Ar), 128.1 (Ar), 127.95 (Ar), 127.86 (Ar), 127.82 (Ar), 127.80 (Ar), 127.7 (Ar), 127.5 (Ar), 127.4 (Ar), 127.3 (Ar), 98.5 (C-1_{TalHep}), 98.4 (C-1_{GlcN}), 78.6 (C-2_{Rib}), 77.94 (C-3_{GlcN}), 77.88 (C-3_{Rib}), 77.86 (C-3_{TalHep}), 77.0 (C-4_{GlcN}), 75.5 (C-4_{Rib}), 74.7 (O$\underline{C}H_2$Ph), 74.5 (C-5_{GlcN}), 74.4 (C-4_{TalHep}), 74.3 (O$\underline{C}H_2$Ph), 73.5 (O$\underline{C}H_2$Ph), 73.0 (O$\underline{C}H_2$Ph), 72.6 (C-5_{TalHep}), 71.8 (O$\underline{C}H_2$Ph), 71.6 (C-1_{Rib}), 70.6 (O$\underline{C}H_2$Ph), 69.2 (C-6_{GlcN}), 66.9 (C-2_{TalHep}), 66.4 (C-7_{TalHep}), 64.5 (C-5_{Rib}), 57.4 (C-2_{GlcN}), 31.3 (C-6_{TalHep}), 23.5 (NHCO$\underline{C}H_3$), 21.1 (CO$\underline{C}H_3$), 20.8 (CO$\underline{C}H_3$); IR (cast film, $CHCl_3$) ν 3287,

3089, 3063, 3030, 2953, 2921, 2851, 1739, 1657, 1454, 1070, 699 cm^{-1}; HRMS (ESI-TOF) m/z: $[M + Na]^+$ Calcd for $C_{66}H_{75}NO_{16}Na$ 1160.4978; Found 1160.4973.

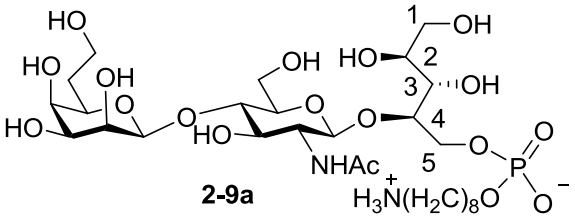


2-*O*-Acetyl-3,4,7-tri-*O*-benzyl-6-deoxy-β-D-*talo*-heptopyranosyl-(1→4)-(2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→4)-triethylammonium 1-*O*-acetyl-2,3-di-*O*-benzyl-D-ribitol-5-(8-azidooctyl phosphate) (2-137)

To a 0 °C solution of PCl_3 (20 μL, 0.23 mmol) in CH_2Cl_2 (3.2 mL) was added imidazole (63.0 mg, 0.925 mmol) in several portions and the solution was stirred for 30 min. A solution of trisaccharide **2-135** (38.2 mg, 0.0306 mmol) in CH_2Cl_2 (3 mL) was added dropwise to the above reaction mixture and the mixture was stirred for 2 h. Water (0.5 mL) was added, the solution was stirred for 5 min and then the mixture was diluted with CH_2Cl_2 (30 mL) and washed with 0.5 M NH_4Cl solution (10 mL). The organic layer was separated and the aqueous layer was further extracted with CH_2Cl_2 (2 × 20 mL). The combined organic phases were washed with brine (15 mL), dried over anhydrous Na_2SO_4, filtered and concentrated and the resulting crude product was quickly purified by column chromatography (30% CH_3OH in EtOAc with 1% Et_3N) to afford the corresponding H-phosphonate. This H-phosphonate (41.5 mg, 0.0293 mmol) was dissolved in CH_2Cl_2 (1.8 mL) and pyridine (0.2 mL). pivaloyl chloride (5.0 μL, 0.041 mmol) was added and stirred for 1 h. 8-Azido-octanol[61] (12.6 mg, 0.0736 mmol) in CH_2Cl_2 (2.5 mL) was added to the above mixture and the solution was stirred for 3 h. The reaction mixture was cooled

to 0 °C and then Et_3N (25 μL, 0.18 mmol) followed by iodine (12 mg, 0.047 mmol) in pyridine (1 mL) were added. The ice bath was removed and the reaction mixture was warmed to room temperature over 3 h. Excess iodine was quenched by the addition of saturated aqueous $Na_2S_2O_3$ solution (5 mL) and the reaction mixture was extracted with CH_2Cl_2 (25 mL). The aqueous layer was separated and further extracted with CH_2Cl_2 (2 × 15 mL). The combined organic phases were washed with brine (10 mL), dried over anhydrous Na_2SO_4, filtered, concentrated and the resulting crude product was purified by column chromatography (25% CH_3OH in EtOAc with 1% Et_3N) to afford **2-137** (38.2 mg, 79%) as a pale yellow viscous liquid: $[\alpha]^{22}_D$ –21.8 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.84–7.10 (m, 30 H, ArH), 5.39 (d, J = 3.0 Hz, 1 H, H-2_{TalHep}), 5.01 (d, J = 11.0 Hz, 1 H, OC$\underline{H_2}$Ph), 4.92 (d, J = 11.5 Hz, 1 H, OC$\underline{H_2}$Ph), 4.90 (d, J = 8.5 Hz, 1 H, H-1_{GlcN}), 4.75–4.50 (m, 10 H, 9 × OC$\underline{H_2}$Ph and H-1_{TalHep}), 4.42–4.27 (m, 7 H, 5 × OC$\underline{H_2}$Ph, H-$1a_{Rib\text{-}ol}$ and H-$1b_{Rib\text{-}ol}$), 4.20–4.11 (m, 2 H, H-$3_{Rib\text{-}ol}$ and H-$5a_{Rib\text{-}ol}$), 4.10–4.00 (m, 3 H, H-2_{GlcN}, H-$2_{Rib\text{-}ol}$ and H-$5b_{Rib\text{-}ol}$), 3.97 (app t, J = 9.5, 9.0 Hz, 1 H, H-4_{GlcN}), 3.86–3.80 (m, 3 H, $OP(O_2)OC\underline{H_2}CH_2$ and H-$4_{Rib\text{-}ol}$), 3.72 (dd, J = 11.0, 5.6 Hz, 1 H, H-$6a_{GlcN}$), 3.63 (dd, J = 11.0, 2.0 Hz, 1 H, H-$6b_{GlcN}$), 3.54 (app t, J = 9.5, 9.0 Hz, 1 H, H-3_{GlcN}), 3.46–3.45 (m, 1 H, H-4_{TalHep}), 3.44–3.38 (m, 1 H, H-5_{GlcN}), 3.37–3.29 (m, 2 H, H-5_{TalHep} and H-$7a_{TalHep}$), 3.27–3.16 (m, 4 H, H-3_{TalHep}, H-$7b_{TalHep}$ and 2 × C$\underline{H_2}N_3$), 3.04 (q, J = 7.5 Hz, 6 H, $(CH_3C\underline{H_2})_3NH$), 2.01 (s, 3 H, 3 × COC$\underline{H_3}$), 2.00 (s, 3 H, 3 × COC$\underline{H_3}$), 1.95–1.88 (m, 1 H, H-$6a_{TalHep}$), 1.89 (s, 3 H, 3 × NHCOC$\underline{H_3}$), 1.75–1.67 (m, 1 H, H-$6b_{TalHep}$), 1.64–1.52 (m, 4 H, 4 × C$\underline{H_2}$), 1.38–1.22 (m, 17 H, 8 × C$\underline{H_2}$ and 3 × $(C\underline{H_3}CH_2)_3NH$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.1 ($\underline{CO}CH_3$), 170.91 ($\underline{CO}CH_3$), 170.89 ($\underline{CO}CH_3$), 139.3 (Ar), 138.5 (Ar), 138.2 (Ar), 138.0 (Ar), 128.3 (Ar), 128.0 (Ar), 127.9 (Ar), 127.7 (Ar), 127.6 (Ar), 127.3 (Ar), 126.8 (Ar), 126.6 (Ar), 100.7 (C-1_{GlcN}), 98.8 (C-1_{TalHep}), 82.3 (C-3_{GlcN}), 78.6 (C-$4_{Rib\text{-}ol}$), 77.8 (C-3_{TalHep}), 77.4 (2C, C-$2_{Rib\text{-}ol}$ and C-$3_{Rib\text{-}ol}$), 76.6

(C-4_{GlcN}), 74.8 (C-5_{GlcN}), 74.4 (C-4_{TalHep}), 74.1 (O$\underline{C}H_2$Ph), 74.0 (O$\underline{C}H_2$Ph), 73.7 (O$\underline{C}H_2$Ph), 73.3 (O$\underline{C}H_2$Ph), 72.8 (O$\underline{C}H_2$Ph), 72.7 (C-5_{TalHep}), 72.4 (O$\underline{C}H_2$Ph), 70.5 (O$\underline{C}H_2$Ph), 69.2 (C-6_{GlcN}), 66.8 (C-2_{TalHep}), 66.4 (2C, C-7_{TalHep} and C-$5_{Rib\text{-}ol}$), 65.6 (d, J = 6.1 Hz, OP(O_2)O$\underline{C}H_2$), 63.5 (C-$1_{Rib\text{-}ol}$), 54.7 (C-2_{GlcN}), 51.4 ($\underline{C}H_2N_3$), 45.8 ((NH$\underline{C}H_2CH_3)_3$), 31.0 (C-6_{TalHep}), 29.3($\underline{C}H_2$), 29.2 ($\underline{C}H_2$), 29.1 ($\underline{C}H_2$), 28.8 ($\underline{C}H_2$), 26.6 ($\underline{C}H_2$), 25.7 ($\underline{C}H_2$), 23.4 (NHCO$\underline{C}H_3$), 21.1 (CO$\underline{C}H_3$), 20.9 (CO$\underline{C}H_3$), 8.6 ((NHCH$_2\underline{C}H_3)_3$); ^{31}P NMR (162 MHz, $CDCl_3$) δ –0.17; IR (cast film, $CHCl_3$) ν 3280, 3062, 3030, 2931, 2859, 2624, 2604, 2498, 2096, 1738, 1675, 1496, 1454, 1069, 740 cm^{-1}; HRMS (ESI-TOF) m/z: [M]$^-$ Calcd for $C_{81}H_{98}N_4O_{20}P$ 1477.6518; Found 1477.6526.



6-deoxy-β-D-*talo*-heptopyranosyl-(1→4)-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→4)-D-ribitol-5-(8-aminooctyl phosphate) (2-9a)

To a stirred solution of trisaccharide **2-137** (38.2 mg, 0.0242 mmol) in CH_3OH (1.5 mL) was added sodium methoxide in CH_3OH (0.60 M, 25 μL, 0.015 mmol). After stirring for 21 h, Amberlite IR-120 resin (H^+ form) was added to neutralize the solution and, following filtration, the solution was concentrated from the filtrate. The resulting crude product was quickly purified by column chromatography (30% CH_3OH in EtOAc with 1% Et_3N) to obtain the corresponding diol. This diol (32.5 mg, 0.0217 mmol) was dissolved in CH_3OH (4 mL) and H_2O (0.4 mL) and stirred under an argon balloon. To this solution was added AcOH (10 μL) followed by $Pd(OH)_2$–C (20 wt%, 65 mg, 0.46 mmol) under an argon atmosphere. The argon balloon was replaced with a H_2 balloon (1 atm) and the solution was flushed with H_2 for 10 sec. The reaction mixture was

stirred under H_2 balloon for 24 h and then the H_2 balloon was replaced with argon balloon and flushed with argon for 1 min. The reaction mixture was diluted with CH_3OH (75 mL) and filtered over a pad of Celite-545. The filtrate was concentrated and the resulting crude product was purified by column chromatography on reversed phase (C_{18}) silica gel (2% CH_3OH in water). The solvent was evaporated and the product was redissolved in H_2O, followed by lyophilization to afford trisaccharide **2-9a** (11.6 mg, 65%) as white foam: $[\alpha]^{22}_D$ +6.0 (*c* 0.01, $CHCl_3$); ^{1}H NMR (500 MHz, D_2O) δ 4.82–4.78 (m, 1 H, H-1_{GlcN}), 4.69 (s, 1 H, H-1_{TalHep}), 4.15–4.12 (m, 2 H, H-$5a_{Rib\text{-}ol}$ and H-$4_{Rib\text{-}ol}$), 4.04 (d, J = 3.0 Hz, 1 H, H-2_{TalHep}), 3.96–3.89 (m, 4 H, H-$5b_{Rib\text{-}ol}$, H-$6a_{GlcN}$, OP(O_2)OC$\underline{H_2}$$CH_2$), 3.89–3.73 (m, 12 H, H-2_{GlcN}, H-3_{GlcN}, H-4_{GlcN}, H-$6b_{GlcN}$, H-$2_{Rib\text{-}ol}$, H-$3_{Rib\text{-}ol}$, H-$1a_{Rib\text{-}ol}$, H-3_{TalHep}, H-4_{TalHep}, H-5_{TalHep} and H-7a,$7b_{TalHep}$), 3.69–3.63 (m, 1 H, H-$1b_{Rib\text{-}ol}$), 3.63–3.59 (m, 1 H, H-5_{GlcN}), 3.02 (app t, J = 7.8, 7.2 Hz, 2 H, 2 × C$\underline{H_2}$$NH_2$), 2.11 (s, 3 H, 3 × NHCOC$\underline{H_3}$), 2.07–2.00 (m, 1 H, H-$6a_{TalHep}$), 1.90–1.80 (m, 1 H, H-$6b_{TalHep}$), 1.72–1.60 (m, 4 H, 4 × C$\underline{H_2}$), 1.43–1.30 (m, 8 H, 8 × C$\underline{H_2}$); ^{13}C NMR (125 MHz, D_2O) δ 175.9 (NH$\underline{CO}$$CH_3$), 102.3 (C-$1_{GlcN}$), 101.7 (C-$1_{TalHep}$), 80.4 (d, J = 7.4 Hz, C-$4_{Rib\text{-}ol}$), 80.1 (C-4_{GlcN}), 75.4 (C-5_{GlcN}), 73.8 (C-5_{TalHep}), 73.3 (C-3_{GlcN}), 72.7 (C-$3_{Rib\text{-}ol}$), 72.5 (C-$2_{Rib\text{-}ol}$), 71.7 (C-2_{TalHep}), 71.4 (C-4_{TalHep}), 69.4 (C-3_{TalHep}), 67.3 (d, J = 5.7 Hz, OP(O_2)O$\underline{C}$$H_2CH_2$), 65.9 (d, J = 5.4 Hz, C-$5_{Rib\text{-}ol}$), 63.6 (C-$1_{Rib\text{-}ol}$), 61.1 (C-6_{GlcN}), 59.1 (C-7_{TalHep}), 56.3 (C-2_{GlcN}), 40.5 ($\underline{C}$$H_2N_3$), 33.5 (C-$6_{TalHep}$), 30.7(d, J = 6.9 Hz, OP(O_2)$OCH_2\underline{C}H_2$), 29.0 (2C, $\underline{C}$$H_2$), 27.6 ($\underline{C}$$H_2$), 26.4 ($\underline{C}$$H_2$), 25.8 ($\underline{C}$$H_2$), 23.3 (NHCO$\underline{C}$$H_3$); ^{31}P NMR (162 MHz, $CDCl_3$) δ 0.92; IR (cast film, $CHCl_3$) ν 3281, 2927, 2859, 1651, 1571, 1375, 1207, 1049, 766 cm^{-1}; HRMS (ESI-TOF) m/z: $[M]^-$ Calcd for $C_{28}H_{55}N_2O_{18}P$ 761.3080; Found 761.3075.

2.7 References

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Chapter 3

Synthesis of the *Campylobacter jejuni* 81-176 strain capsular polysaccharide repeating unit reveals the absolute configuration of its *O*-methyl phosphoramidate motif

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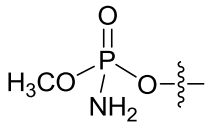
A part of this Chapter has been published:

Thota, V. N.; Ferguson, M. J.; Sweeney, R. P.; Lowary, T. L. *Angew*. *Chem*., *Int*. *Ed*. **2018**, *57*, 15592–15596.

3.1 Background

Campylobacter jejuni (*C. jejuni*) is a food-borne pathogen that causes significant gastrointestinal illness worldwide.[1] Although these infections usually resolve in a few days, they sometimes lead to the neurological disease, GBS.[2] An important *C. jejuni* virulence factor is its CPS, which differentiates the 47 different serotypes identified to date.[3] The structure of *C. jejuni* CPS varies widely from strain to strain and is often functionalized with a range of phase-variable modifications such as the incorporation of *O*-methyl,[4] *N*-ethanolamine,[5] aminoglycerol[6] and *O*-methyl phosphoramidate (MeOPN) groups.[4,6] These CPSs possess a number of unusual structural motifs and prominent among these is the MeOPN group (**3-1**, Figure 3-1). This motif is a labile non-stoichiometric substituent found in more than 70% of all *C. jejuni* strains and is not observed anywhere else in nature. The presence of this labile MeOPN motif was discovered serendipitously by examining live *Campylobacter* using high resolution magic angle spinning NMR spectroscopy.[7] The MeOPN group is believed to contribute importantly to the virulence of the organism[8] and a vaccine currently in development for the prevention of *C. jejuni* infections incorporates CPS bearing this functionality.[9]



3-1

Figure 3-1: MeOPN motif present in CPS of *Campylobacter* species.

The phosphorus atom in the MeOPN substituent is stereogenic and it occurs as a single stereoisomer in the CPSs in which it is found. Recent work has established the biosynthesis of the MeOPN group: the immediate precursor is an activated cytidine diphosphate (CDP)

derivative and the amino group is derived from glutamine.[10] Despite these advances, the stereochemistry on phosphorus remains unknown.

Mutational studies have demonstrated that the CPS plays a key role in virulence.[8,11] In addition, Guerry and co-workers work on a CPS-based conjugate vaccine[9] against *C. jejuni* 81-176 has shown that it is immunogenic in mice and provides 100% protection against diarrheal disease in the New World monkey *Aotus nancymaae*. These findings further indicate that this structure is both a virulence determinant and a protective antigen. However, a drawback to this vaccine is that it only protects against the *C. jejuni* strain 81-176. There are more than 60 serostrains reported for *C. jejuni* and it is anticipated that each strain produces a CPS with a different structure. Therefore, it is difficult to make conjugate vaccine for each strain. Because the MeOPN motif is present in more than 70% of *C. jejuni* strains, the antigenic nature of MeOPN could be used for the generation of vaccines that could potentially target the majority of the strains.

This Chapter describes the synthesis of the three repeating units (Figure 3-2, **2-10**, **3-2** and **3-3**) found in the CPS of *C. jejuni* 81-176 strain including one that contains a MeOPN group (**3-3**). In executing these synthetic investigations, we established, for the first time, that the stereochemistry on phosphorus in the MeOPN motif is *R*.

Campylobacter jejuni 81-176 is a highly virulent strain that has been studied as a model of *C. jejuni* infection.[12] Serologically, *C. jejuni* 81-176 belongs to HS23 and HS36 serogroups. Structures of the CPS repeating units present in *C. jejuni* strain 81-176 CPS are shown in Figure 3-2. Aspinall and co-workers[13] showed that the lipooligosaccharides (LOSs) of *C. jejuni* HS23 and HS36 serostrains were indistinguishable, and that each serodeterminant CPSs (Figure 3-2)

repeating unit consists of *N*-acetyl-D-glucosamine, D-galactose and an unusual 6-deoxy-heptose[14] with the D-*altro*-stereochemistry. The heptose is present either in its unmethylated (**2-10**) or *O*-3 methylated form (**3-2**). In 2006, Monteiro and co-workers[15] reported the presence of the MeOPN motif in wild-type strain 81-176 CPS extracted by the traditional hot water/phenol method. The MeOPN group is found on *O*-2 of the galactose residue[9b,15] (**3-3**) and the structure of the glycan was further supported by fast atom bombardment mass spectrometry. However, the stability of the labile MeOPN motif in *Campylobacter jejuni* 81-176 during the hot water/phenol extraction is not well understood as opposed to the loss of the MeOPN motif in other CPSs using the same extraction procedure.[6,7,16] The methyl and MeOPN groups are both phase-variable modifications.[7,17] Trisaccharide **2-10**, lacking both of these functionalities, has been synthesized previously by Nam Shin and coworkers.[18] (described in section 3.2.1)

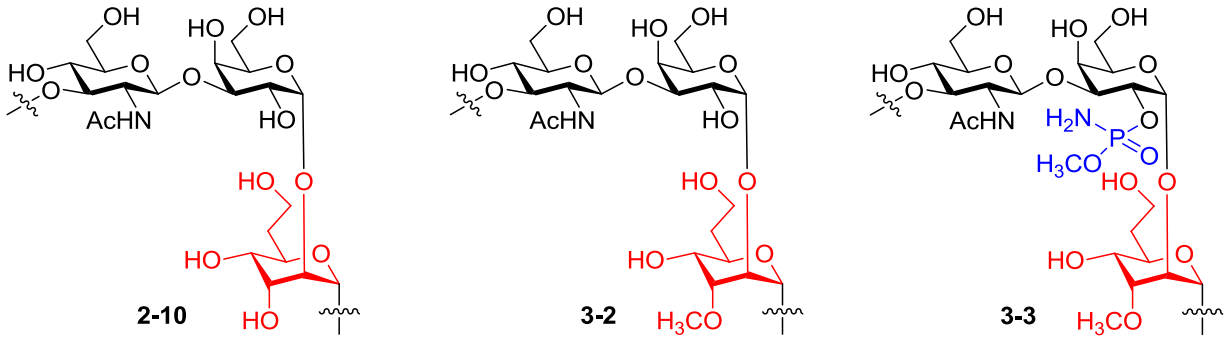


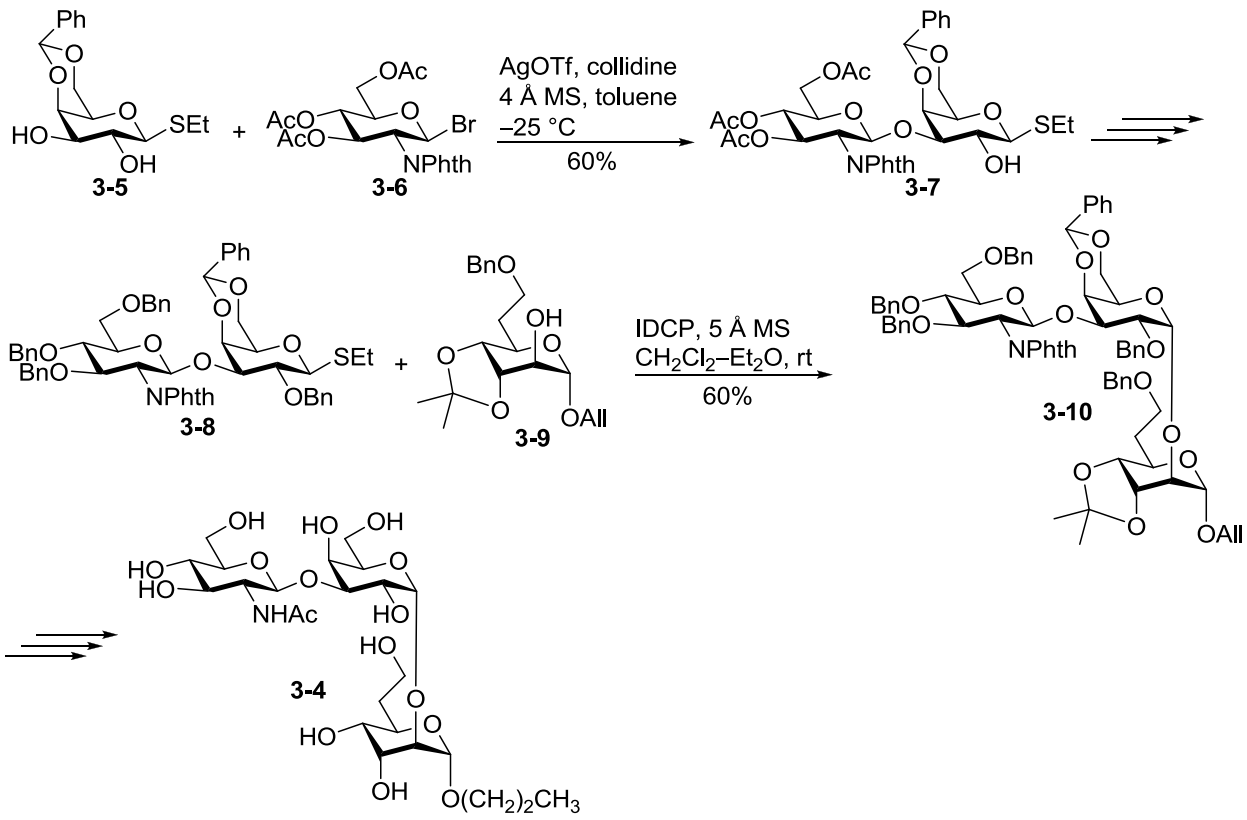
Figure 3-2: Structures of the CPS repeating units present in *C. jejuni* strain 81-176 CPS. MeOPN motif is shown in blue.

3.2 Reported syntheses of trisaccharide repeating unit 2-10

Nam Shin and coworkers[18] synthesized all the possible repeating trisaccharides of **2-10**, which lacks both the methyl and the MeOPN phase variable functionalities.

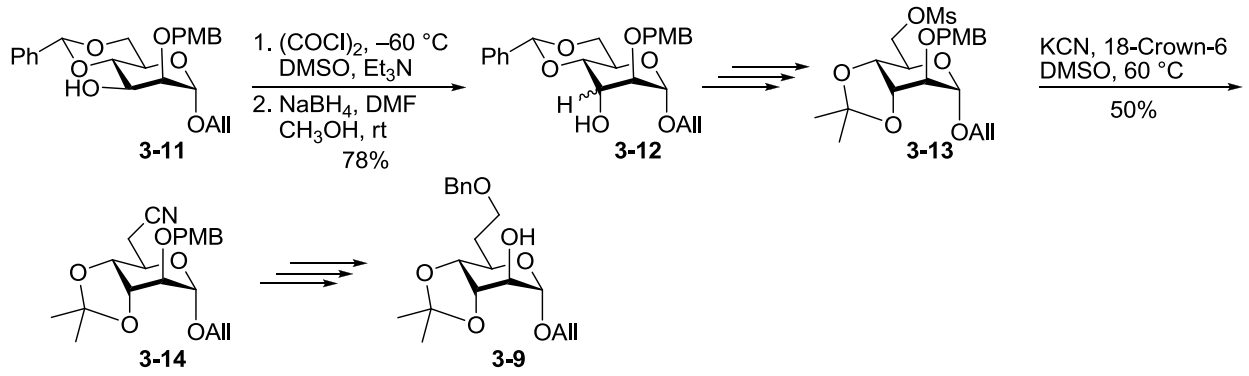
3.2.1. Reported synthesis of the trisaccharide β-D-GlcNAc-(1→3)-α-D-Gal*p*-(1→2)-6-deoxy-α-D-*altro*-Hep*p*-(1→*O*-propyl): 3-4

In 2004, Nam Shin and coworkers[18a] reported the first synthesis of the trisaccharide repeating unit β-D-GlcNAc-(1→3)-α-D-Gal*p*-(1→2)-6-deoxy-α-D-*altro*-Hep*p*-(1→*O*-propyl) **3-4** as a propyl glycoside. The key steps in the synthesis are outlined in Scheme 3-1. The regio- and stereoselective glycosylation of thioglycoside acceptor **3-5** with glycosyl bromide **3-6** (derived from D-glucosamine) in the presence of silver trifluoromethanesulfonate (AgOTf) and collidine as an acid scanvenger at –25 °C furnished disaccharide **3-7** in 60% yield. Protecting group manipulations of **3-7** furnished thioglycoside donor **3-8**. Iodonium di-*sym*-collidine perchlorate (IDCP)-promoted glycosylation of the 6-deoxy-*altro*-heptoside acceptor **3-9** with thioglycoside **3-8** afforded a 60% yield of trisaccharide **3-10**. The trisaccharide **3-10** furnished propyl glycoside **3-4** upon global deprotection.



Scheme 3-1: Key steps involved in the synthesis of trisaccharide **3-4**.[18a]

The synthesis of 6-deoxy-*altro*-heptoside acceptor **3-9** was achieved via homologation by nucleophilic displacement of a 6-*O*-sulfonate ester at the C-6 position of a hexose (Scheme 3-2). Swern oxidation of allyl mannopyranoside derivative **3-11** at –60 °C followed by reduction with sodium borohydride ($NaBH_4$) furnished a mixture of C-3 epimerized compounds (combined 78% yield) in 4:1 ratio in favor of the desired *altro* isomer **3-12**. Protecting group manipulations of **3-12** furnished 6-*O*-methanesulfonate ester **3-13**. Homologation of **3-13** by nucleophilic displacement with KCN in the presence of 18-crown-6 in DMSO generated nitrile **3-14**. Protecting group manipulation and fuctional group transformation of **3-14** furnished the 6-deoxy-*altro*-heptoside acceptor **3-9**.

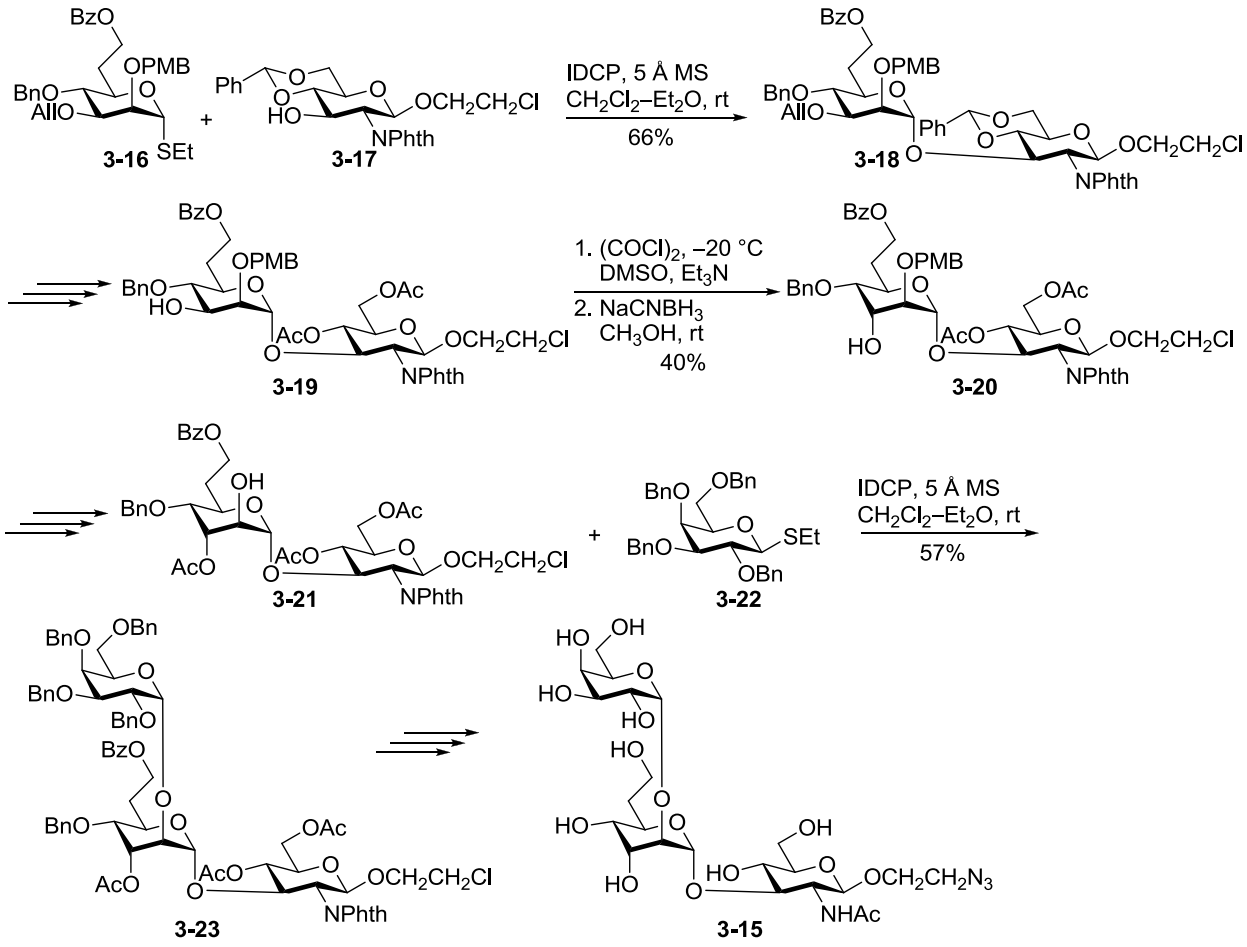


Scheme 3-2: Key steps involved in the synthesis of 6-deoxy-*altro*-heptoside acceptor **3-9**.[18a]

3.2.2 Reported synthesis of trisaccharide α-D-Gal*p*-(1→2)-6-deoxy-α-D-*altro*-Hep*p*-(1→3)-β-D-GlcNAc: 3-15

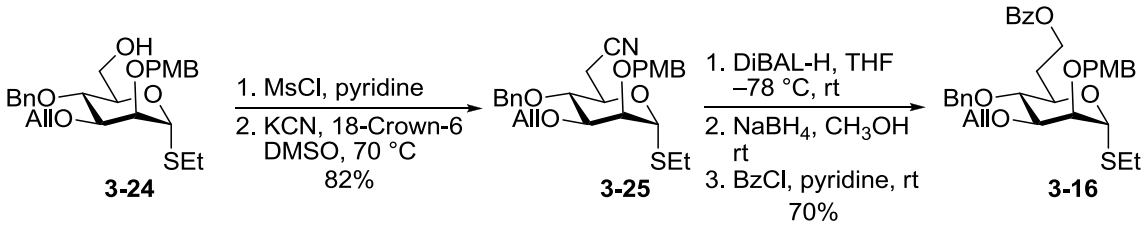
Nam Shin and coworkers[18b] also reported the synthesis of the β-D-GlcNAc-(1→3)-α-D-Gal*p*-(1→2)-6-deoxy-α-D-*altro*-Hep*p* trisaccharide as a 2'-azidoethyl glycoside (**3-15**, Scheme 3-3). The stereoselective formation of the α-*altro*-heptosidic linkage was the most challenging step because of the 1,3-*syn*-diaxial stereoelectronic repulsion of the *altro*-heptopyranoside ring system. Because of the bulky axial substituent at C-3 of the *altro*-heptose, the incoming nucleophile (acceptor) will experience steric hindrance in α-glycoside formation. When the nucleophile is a sugar, this becomes more severe. In addition, the lone pairs on O-3 causes repulsion to the incomining nucleophile. Therefore, to achieve this linkage, an indirect approach, involving C-3 epimerization of an α-*manno*-heptopyranosyl derivative, was used. Direct α-*altro*-heptosidic linkage formation with various glycosidic acceptors under known activation conditions proceeded with low α-selectivities and yields as expected. The indirect approach involves IDCP promoted glycosylation of *N*-phthaloyl glucoside acceptor **3-17** with the thioglycoside **3-16** to afford a 66% yield of disaccharide **3-18**. Protecting group manipulations of **3-18** furnished the *manno*-heptosyl derivative **3-19**. Swern oxidation of **3-19** at –20 °C followed

by reduction with sodium cyanoborohydride ($NaCNBH_3$) furnished a mixture of the C-3 epimerized compound (combined 59% yield) in 1:2 ratio in favor of the desired *altro* isomer **3-20**. Fortuitously, the two diastereomers can be separated by column chromatography and they isolated the desired alcohol **3-20** in 40% yield along with the undesired starting material **3-19** in 19% yield. Protecting group manipulations of disaccharide **3-20** furnished the 6-deoxy-*altro*-heptoside acceptor **3-21**. IDCP-promoted glycosylation of **3-21** with thioglycoside **3-22** afforded a 57% yield of **3-23**. Trisaccharide **3-23**, upon protecting group manipulations, nucleophilic displacement with azide and global deprotection furnished 2'-azidoethyl glycoside **3-15**.



Scheme 3-3: Key steps involved in the synthesis of trisaccharide **3-15**.[18b]

The synthesis of 6-deoxy-*manno*-heptoside donor **3-16** was achieved via homologation by nucleophilic displacement of a hexose 6-*O*-sulfonate ester at (Scheme 3-4). Conversion of ethylthio mannopyranoside **3-24** to nitrile **3-25** was achieved by treatment of **3-24** with methanesulfonyl chloride (MsCl) in pyridine followed by nucleophilic displacement with KCN in the presence of 18-Crown-6 in DMSO. Reduction of **3-25** to the imine with diisobutylaluminium hydride (DiBAL-H) at –78 °C followed by hydrolysis and subsequent reduction using $NaBH_4$ and finally benzoylation with benzoyl chloride in pyridine furnished the 6-deoxy-*altro*-heptoside donor **3-16** in 70% yield.

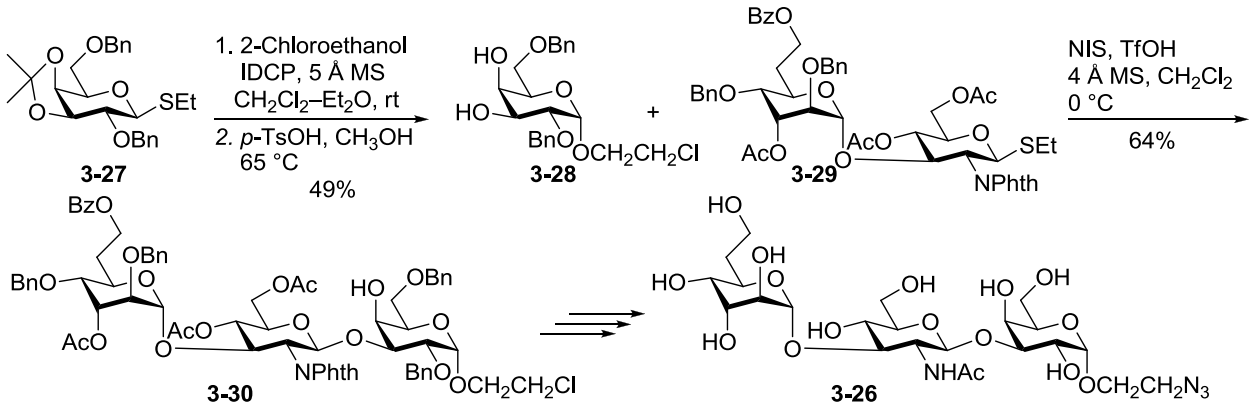


Scheme 3-4: Key steps involved in the synthesis of 6-deoxy-*altro*heptoside donor **3-16**.[18b]

3.2.3 Reported synthesis of trisaccharide 6-deoxy-α-D-*altro*-Hep*p*-(1→3)-β-D-GlcNAc-(1→3)-α-D-Gal*p*: 3-26

In 2008, Nam Shin and coworkers[18c] reported the synthesis of the trisaccharide 6-deoxy-α-D-*altro*-Hep*p*-(1→3)-β-D-GlcNAc-(1→3)-α-D-Gal*p*-(1-*O*$CH_2CH_2N_3$) as a 2'-azidoethyl glycoside (Scheme 3-5). Similar to the previous syntheses of the related trisaccharides by this group, homologation was achieved by nucleophilic displacement of a 6-*O*-sulfonate ester. Likewise, as detailed in the previous section (3.2.2), the challenging stereoselective formation of the α-*altro*-heptosidic linkage present in the **3-26** was achieved via an indirect approach from an α-*manno*-heptopyranosyl derivative. IDCP-promoted glycosylation of 2-chloroethanol acceptor

with the thioglycoside **3-27** to afford 2'-chloroethyl galactoside (~5:1 α:β anomeric ratio) followed by treatment with *p*-TSA furnished the α-linked 2'-chloroethyl galactoside **3-28** in 49% yield. Regioselective glycosylation of diol **3-28** with thioglycoside **3-29** promoted by NIS and TfOH afforded a 64% yield of trisaccharide **3-30**. Protecting group manipulations of **3-30**, nucleophilic displacement with azide and global deprotection furnished the 2'-azidoethyl glycoside **3-26**.



Scheme 3-5: Key steps involved in the synthesis of trisaccharide **3-26**.[18c]

3.3 Our approach towards the syntheses of the *C. jejuni* 81-176 CPS trisaccharides

Although, Nam Shin and coworkers syntheses of these trisaccharides proceeded in a reasonable number of steps, the glycosylations produced the desired compounds in modest yields. In addition, the C-3 oxidation–reduction approach from a *manno*-heptose/mannose derivative (**3-11** or **3-19**) to obtain the *altro* isomer gave poor diastereoselectivity. Finally trisaccharide with the phase variable methyl and MeOPN functionalities were not synthesized. To address these issues, we chose to explore the possibility of obtaining all three trisaccharides (shown in Figure 3-2) via a single strategy (described in Section 3.3.1 and 3.3.2).

Trisaccharides **2-10a**, **3-2a** and **3-3a** (Figure 3-3) are our targets. We anticipated the major synthetic challenges to be: 1) stereoselective installation of the 1,2-*cis*-α-D-galactoside residue; 2) the preparation of the 6-deoxy-heptose with the D-*altro*-stereochemistry; and 3) the introduction of the MeOPN moiety. We chose to address the first challenge through the use of an α-selective donor with a di-*tert*-butyl silyl acetal on *O*-4 and *O*-6.[19] Triflation and inversion of an advanced intermediate (**2-66** shown in Scheme 2-10, Chapter 2) synthesized from 6-deoxy-D-*manno*-heptopyranoside **2-58**[20] (Scheme 2-8, Chapter 2) was our approach in addressing the second. Finally, we envisioned that the MeOPN group could be introduced from one of the three methods previously developed for introducing this structure onto carbohydrates.[21]

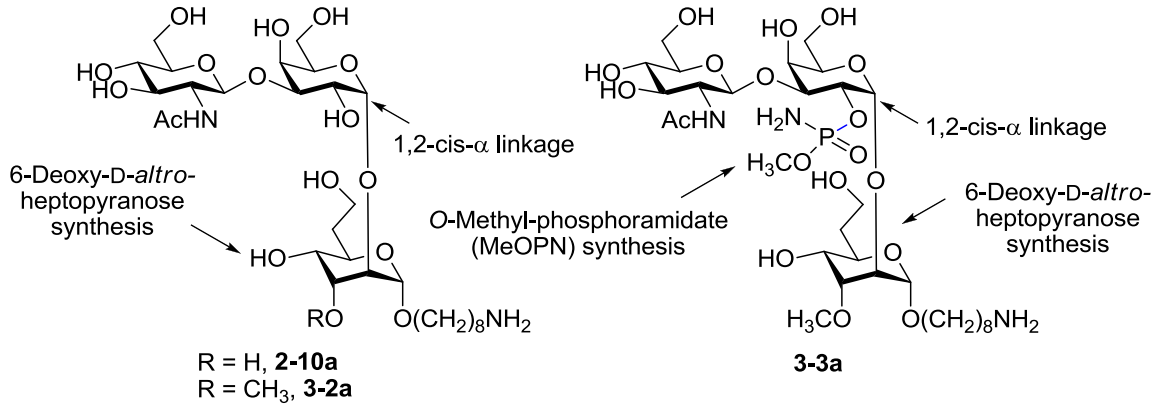


Figure 3-3: Target trisaccharides **2-10a**, **3-2a** and **3-3a** with anticipated synthetic challenges

3.3.1 Retrosynthetic analysis of the *C. jejuni* 81-176 CPS trisaccharides

The retrosynthetic analysis of *C. jejuni* 81-176 CPS trisaccharides **2-10a, 3-2a** and **3-3a** is outlined in Figure 3-4. An aminooctyl aglycone was incorporated into the target trisaccharides to allow facile conjugation to either proteins or surfaces. For a convergent synthesis of all trisaccharides, we envisaged that a [2 + 1] glycosylation would speed up the synthesis. Glycosylation of the acceptor **3-33** (or **3-34**) with disaccharide donor **3-31** would generate the

desired 1,2-*cis*-α-D-galactosidic linkage. The disaccharide **3-31** could obtained from acceptor **3-36** and known thioglycoside **3-35**[22] (prepared according to the literature methods). 6-Deoxy-*altro*-heptoside acceptors **3-33** or **3-34** could be accessed from thioglycosides **2-67** or **2-68** and 8-azido-1-octanol **3-37**.[23] Finally, we envisioned that the MeOPN group could be introduced from H-phosphonate **3-32** or one of two other methods previously developed for introducing this structure onto carbohydrates.[21b,21c] The synthesis of **2-67** and **2-68** is described in section 2.3.2.4 of Chapter 2.

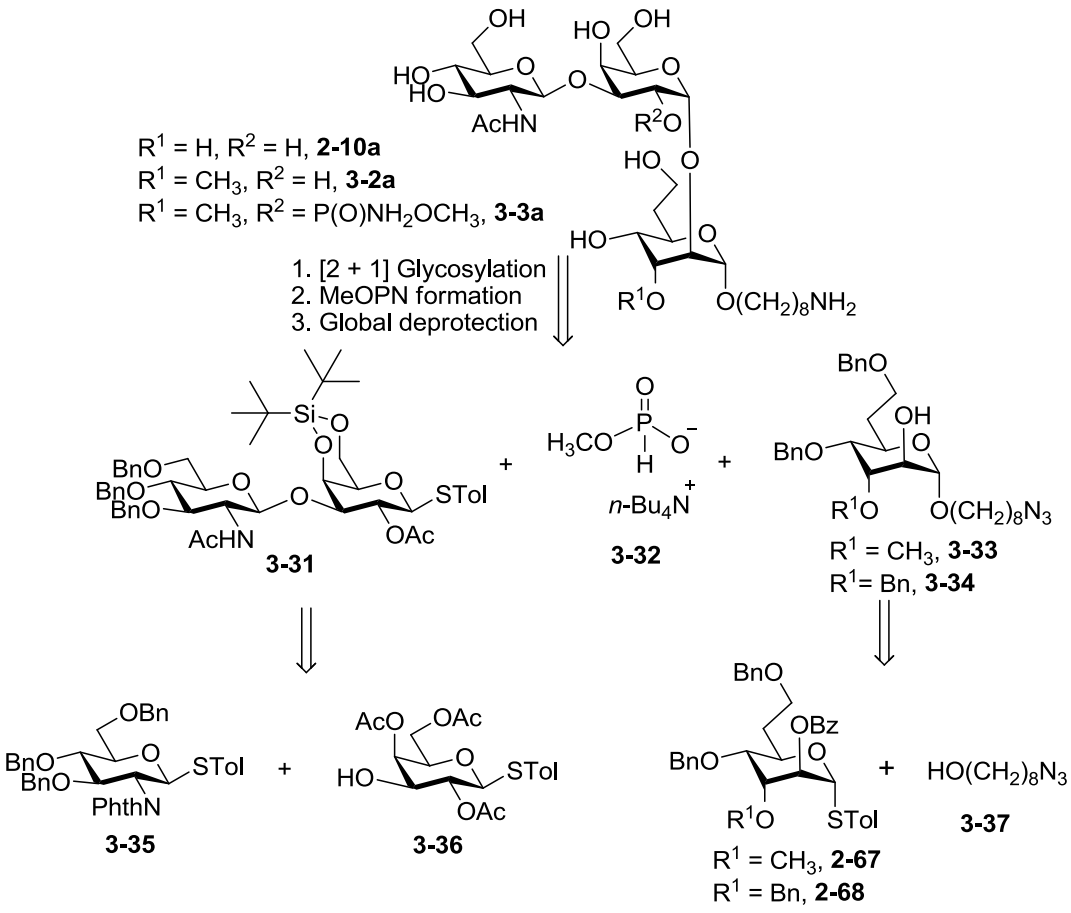
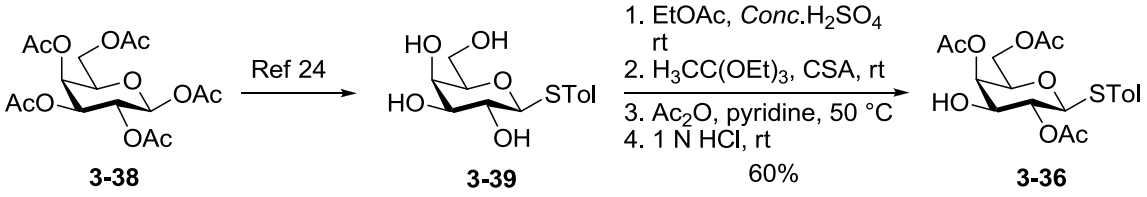


Figure 3-4: Retrosynthetic analysis of trisaccharides **2-10a**, **3-2a** and **3-3a**.

3.3.1.1 Synthesis of D-galactose acceptor 3-36

The synthesis of D-galactose acceptor **3-36** is outlined in Scheme 3-6. First, 1,2,3,4,6-penta-*O*-acetyl-D-galactopyranose **3-38** was converted to thioglycoside **3-39** as reported.[24,25] Treatment of **3-39** with ethyl acetate and sulfuric acid followed by orthoacetate formation, acetylation and hydrolysis of the orthoacetate using 1 N HCl furnished the D-galactose acceptor **3-36** in 60% yield over the four steps.

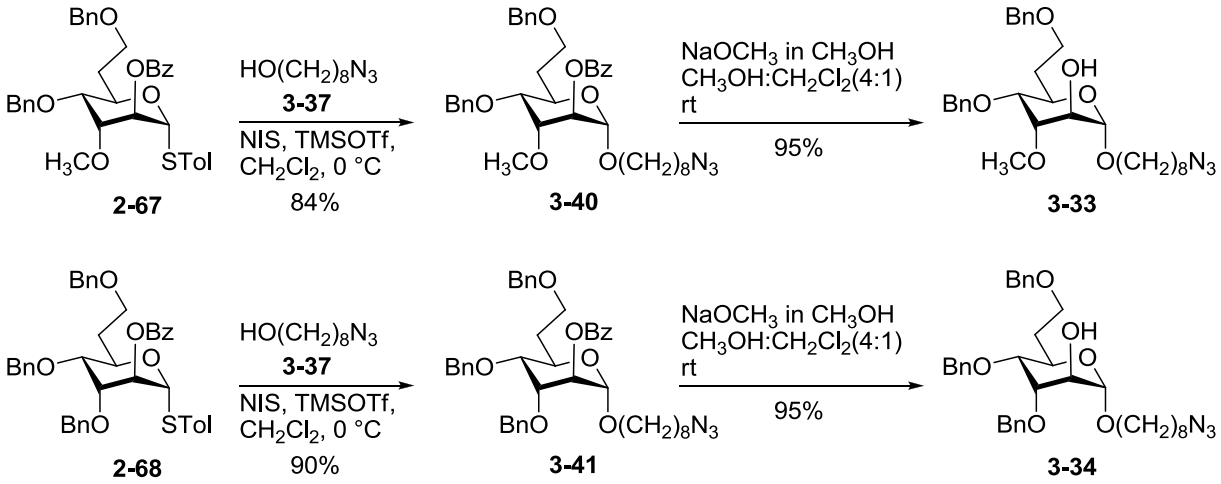


3-38 **3-39** **3-36**

Scheme 3-6: Synthesis of D-galactose acceptor **3-36**.

3.3.1.2 Synthesis of 6-deoxy-*altro*-heptoside acceptors 3-33 and 3-34

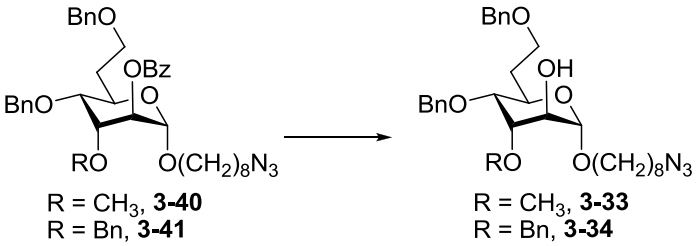
The synthesis of 6-deoxy-*altro*-heptoside acceptors **3-33** and **3-34** are outlined in Scheme 3-7. The glycosylation of azidooctanol (**3-37**) with thioglycoside **2-67** (synthesis described in section 2.3.2.4 of Chapter 2) furnished α-linked 6-deoxy-*altro*-heptoside **3-40** in 84% yield. Zemplén deacylation of **3-40** afforded 6-deoxy-*altro*-heptoside acceptor **3-33** in 95% yield. Similarly, glycosylation of azidooctanol **3-37** with thioglycoside **2-68** (synthesis described in section 2.3.2.4 of Chapter 2) furnished α-linked 6-deoxy-*altro*-heptoside **3-41** in 90% yield. Zemplén deacylation of **3-41** afforded 6-deoxy-*altro*-heptoside acceptor **3-34** in 95% yield.



Scheme 3-7: Synthesis of 6-deoxy-*altro*-heptoside acceptors **3-33** and **3-34**.

When comparing the NMR spectroscopic data of **3-40** and **3-41** with those of **3-33** and **3-34**, we were intrigued by the differences (Table 3-1). When the benzoate ester is present at the C-2 position (**3-40** and **3-41**), the anomeric proton multiplicity is a singlet with $^{1}J_{C\text{-}1,H\text{-}1}$ values within the anticipated range for an α-glycoside (~170 Hz). In contrast, when the benzoate was deprotected (to form **3-33** and **3-34**), the anomeric proton multiplicity appears as a doublet (J = 4.0 or 4.5 Hz) and the $^{1}J_{C\text{-}1,H\text{-}1}$ value is close to 160 Hz, a value typical for β-glycosides. In addition, $J_{2,3}$ and $J_{4,5}$ values are significantly different from the values of $J_{2,3}$ and $J_{4,5}$ in **3-40** and **3-41**.. Therefore, we hypothesized that when the benzoate is present at C-2, the 6-deoxy-*altro*-heptosides (**3-40** and **3-41**) exist predominantly in $^{4}C_{1}$ conformation whereas when the benzoate is deprotected, they do not adopt $^{4}C_{1}$ conformation. Coupling constants suggest that both **3-33** and **3-34** do not exist in the $^{1}C_{4}$ conformation either. Instead, we postulate that there 2–3 different conformations that rapidly interconvert and the J values are averaged on an NMR time scale. Another possibility is that the ring adopts a single different conformation (e.g., a twist-boat).

Table 3-1: Spectroscopic data for Zemplén deacylation of **3-40** and **3-41**.

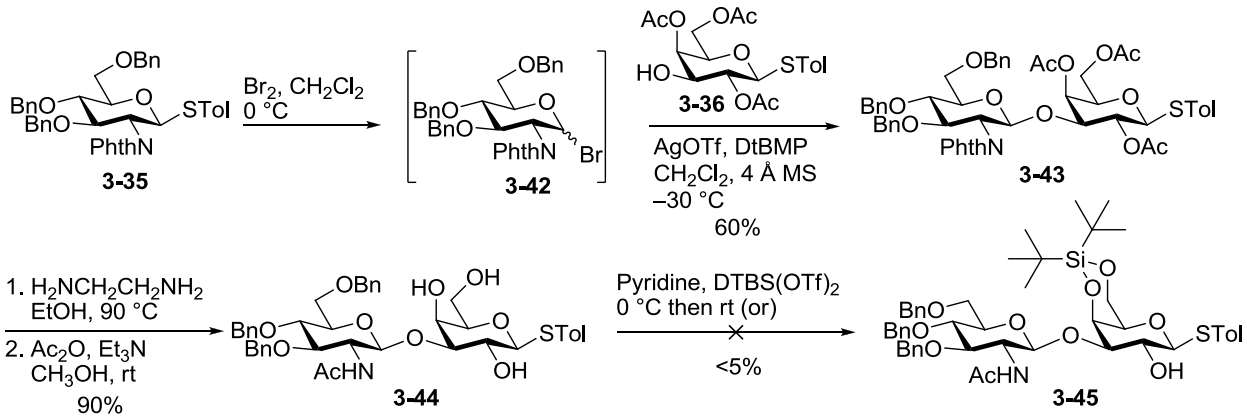


Entry	3-40 and 3-41			3-33 and 3-34		
	R	**multiplicity** (H-1)	$^1J_{C\text{-}1,H\text{-}1}$ (Hz)	**R**	**multiplicity** (H-1) $J_{1,2}$	$^1J_{C\text{-}1,H\text{-}1}$ (Hz)
1	CH_3	singlet	168.5	CH_3	doublet J = 4.5 Hz	162.9
2	Bn	singlet	168.4	Bn	doublet J = 4.0 Hz	162.8

3.3.1.3 Attempted synthesis of disaccaride donor precursor 3-45

The attempted synthesis of disaccharide donor precursor **3-45** is outlined in Scheme 3-8. Thioglycoside **3-35**[22] was converted to bromide **3-42**, which was then used immediately to glycosylate galactose acceptor **3-36** employing silver trifluoromethanesulfonate (AgOTf) as the activator. This reaction furnished the β-linked disaccharide **3-43** in 60% yield (δ_H 5.23, $J_{1,2}$ = 8.0 Hz). Treatment of **3-43** with ethylenediamine at 90 °C cleaved both the phthalimide and the acetate esters; selective *N*-acetylation of the intermediate product was achieved by reaction with acetic anhydride, triethylamine and methanol. This sequence yielded disaccharide **3-44** in 90% yield over the two steps. We then examined the regioselective protection of triol **3-44** to produce alcohol **3-45**. Unfortunately, all attempts to install the di-*tert*-butylsilylene acetal leading to **3-45** in appreciable yields failed. This is presumably because of steric hindrance caused by the

glucosamine residue at O-3. For example, **3-44**, when treated with pyridine and di-*tert*-butylsilyl bis(trifluoromethanesulfonate) (DTBS(OTf)$_2$), furnished <5% yield of the desired alcohol **3-45**. Addition of excess DTBS(OTf)$_2$ caused over silylation. Closing the di-*tert*-butylsilylene ring with galactose O-4 would bring one of the tert-butyl group to be occupied over the galactose ring. We propose this leads to a steric clash with the glucosamine residue already present at galactose O-3. Due to our inability to produce alcohol **3-45** in a desirable yield, we abandoned this route.



Scheme 3-8: Attempted synthesis of disaccharide donor precursor **3-45**.

3.3.1.4 Plausible solution to obtain di-*tert*-butylsilylene acetal

The installation of the di-*tert*-butylsilylene acetal is essential for the construction of 1,2-*cis*-α-D-galactosidic linkage.[19] Given the difficulty in the di-*tert*-butylsilylene acetal formation on the disaccharide **3-44**, another strategy was explored (Figure 3-5). One approach is to pre-form the di-*tert*-butylsilylene acetal on the monosaccharide **3-39.** Accordingly, the galactose acceptor **3-46** is our choice instead of the galactose acceptor **3-36**. Alcohol **3-46** can be used to make a disaccharide **3-47** containing the di-*tert*-butylsilylene acetal on the galactose residue. Moreover, similar to **3-36**, generation of **3-46** requires four steps from tetrol **3-39**.

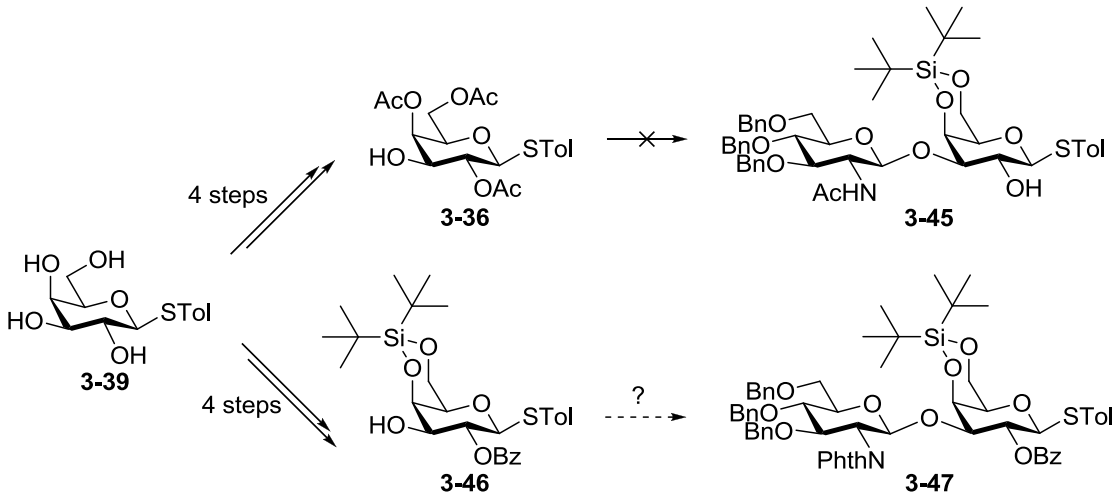


Figure 3-5: Plausible solution to introduce the di-*tert*-butylsilylene acetal.

3.3.2 Revised retrosynthetic analysis of the *C. jejuni* 81-176 CPS trisaccharides

Based on the alternate strategy described above, a revised retrosynthetic plan is shown in Figure 3-6. The glycosylation of disaccharide donor **3-47** and the acceptor **3-33** (or **3-34**) would generate the desired 1,2-cis-α-D-galactosidic linkage. The disaccharide **3-47** can be obtained from the known thioglycoside **3-35**[22] and the acceptor **3-46**. The other steps are similar to those proposed in the earlier retrosynthetic analysis (Figure 3-4).

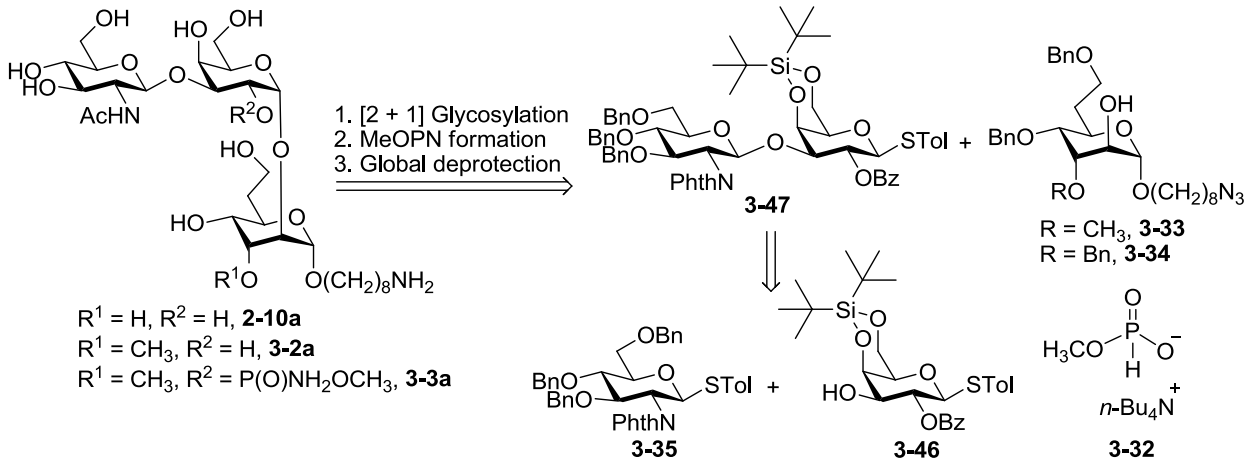
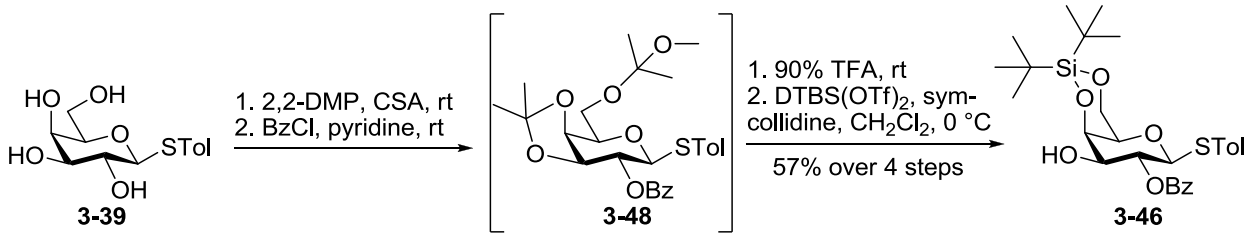


Figure 3-6: Revised retrosynthetic analysis of trisaccharides **2-10a**, **3-2a** and **3-3a**.

3.3.2.1 Synthesis of galactose acceptor 3-46

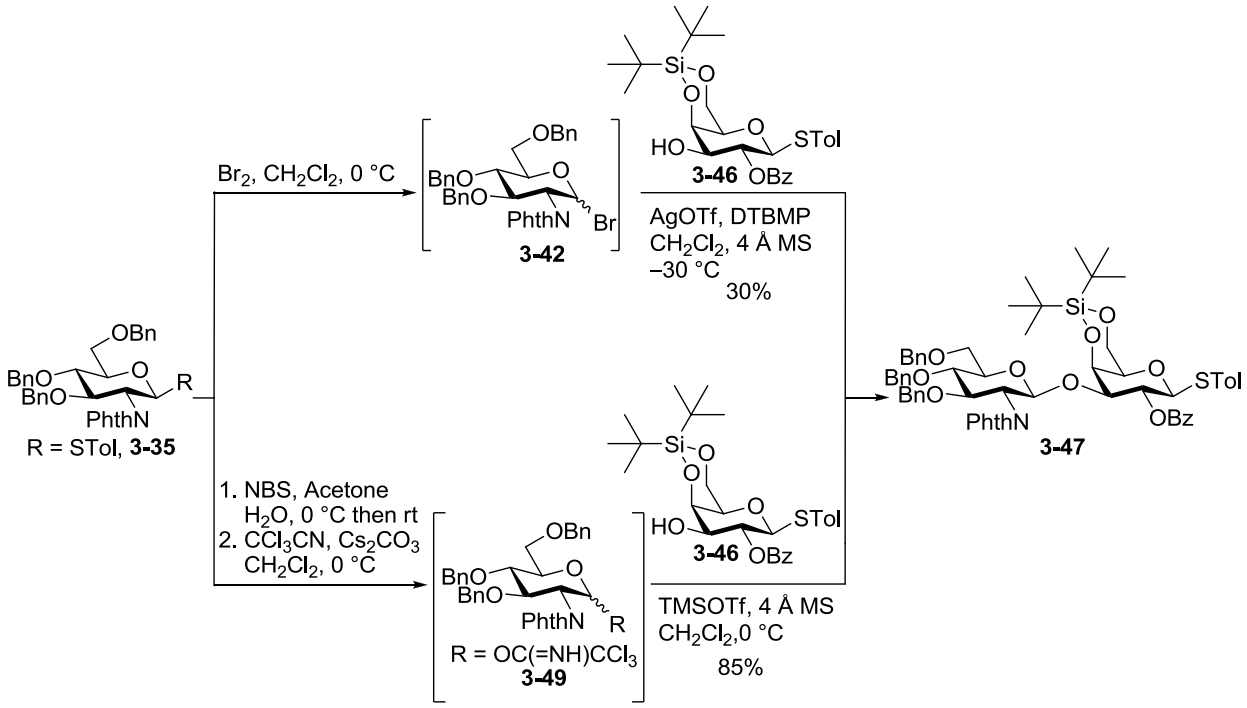
The synthesis of galactose acceptor **3-46** is outlined in Scheme 3-9. Treatment of thioglycoside **3-39**[24] with 2,2-dimethoxypropane (DMP) and CSA followed by benzoylation using benzoyl chloride resulted in crude compound **3-48**. The crude benzoate was treated with aqueous 90% trifluoroacetic acid (TFA) followed by DTBS(OTf)$_2$ and *sym*-collidine. This four-step reaction sequence generated the desired di-*tert*-butylsilylene acetal **3-46** in 57% yield.



Scheme 3-9: Synthesis of galactose acceptor **3-46**.

3.3.2.2 Synthesis of disaccharide 3-47 containing the di-*tert*-butylsilylene acetal

Scheme 3-10 illustrates the synthesis of disaccharide **3-47**, containing a di-*tert*-butylsilylene acetal. Thioglycoside **3-35**[22] was converted to bromide **3-42**, which was then used immediately to glycosylate **3-46** employing AgOTf as the activator. This reaction furnished the β-linked disaccharide **3-47** in a modest 30% yield (δ_H 5.36, $J_{1,2}$ = 7.5 Hz). Alternatively, thioglycoside **3-35**[22] was converted to trichloroacetimidate **3-49**, which was then used immediately to glycosylate **3-46** employing trimethylsilyl trifluoromethanesulfonate (TMSOTf) as the activator. This reaction furnished the same β-linked disaccharide **3-47** but in 85% yield.

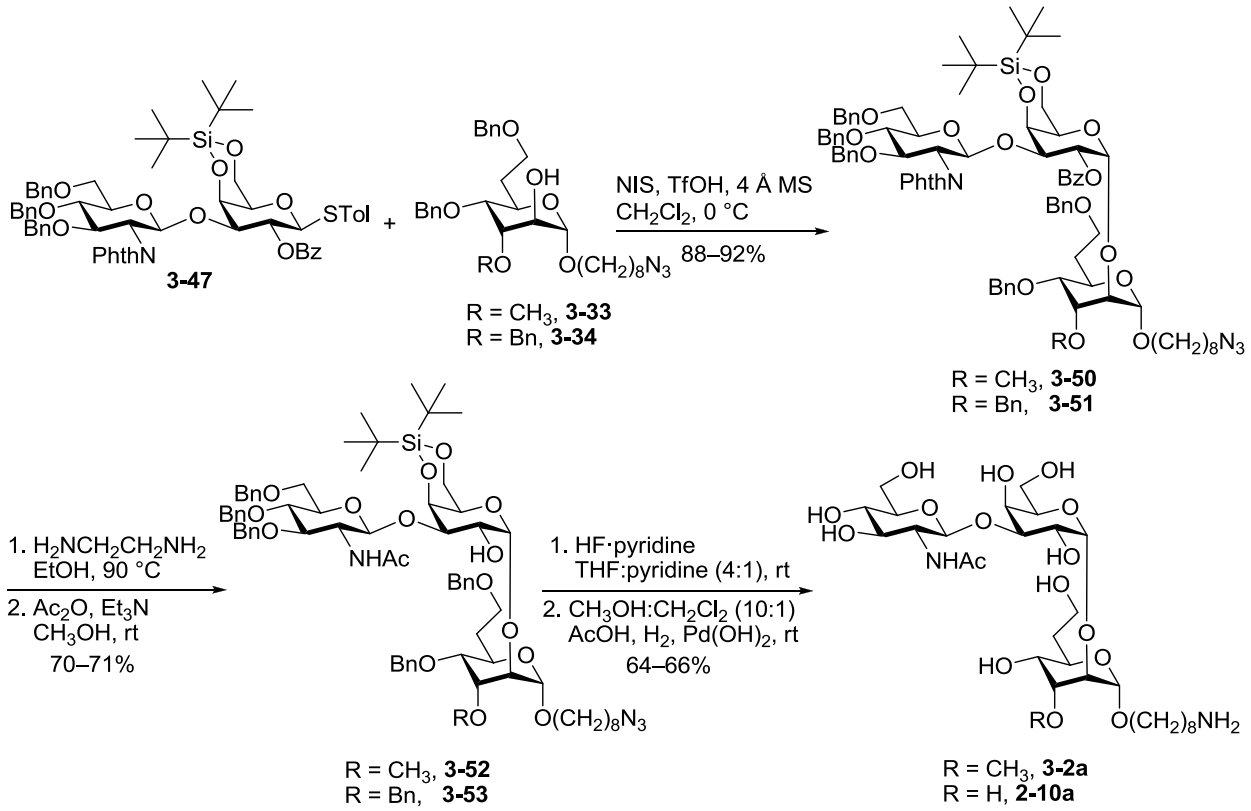


Scheme 3-10: Synthesis of disaccharide **3-47** containing a di-*tert*-butylsilylene acetal.

3.3.2.3 Synthesis of parent trisaccharide 2-10a and its derivative 3-2a containing the phase variable methyl group

The synthesis of parent trisaccharide **2-10a** and its derivative **3-2a** containing the phase variable methyl group is outlined in Scheme 3-11. The glycosylation of the 6-deoxy-*altro*-heptoside acceptor **3-34** with disaccharide donor **3-47**, promoted by NIS and TfOH, afforded an 88% yield of α-linked trisaccharide **3-51** (δ_H 5.08, $J_{1,2}$ = 4.0 Hz). Treatment of **3-51** with ethylenediamine at 90 °C cleaved the phthalimide and the benzoate ester. Subsequent acetylation of the intermediate product could be done selectively up on the nitrogen by reaction with acetic anhydride, triethylamine and methanol. This sequence yielded trisaccharide **3-53** in 70% yield over the two steps. Deprotection of **3-53** was achieved by first treatment with hydrogen fluoride in pyridine to give a triol that was then subjected to hydrogenolysis and azide reduction using $Pd(OH)_2$ to afford **2-10a** in 64% yield over the two steps.

The synthesis of **3-2a**, which contains the methyl group, was achieved by first glycosylation of the 6-deoxy-*altro*-heptoside acceptor **3-33** with disaccharide donor **3-47** to afford a 92% yield of α-linked trisaccharide **3-50** ($^1J_{C\text{-}1,H\text{-}1}$ = 174.6 Hz). Next, application of the same functional group transformations and deprotection reactions used for synthesizing **2-10**, provided **3-2a** in 47% overall yield from **3-50**.

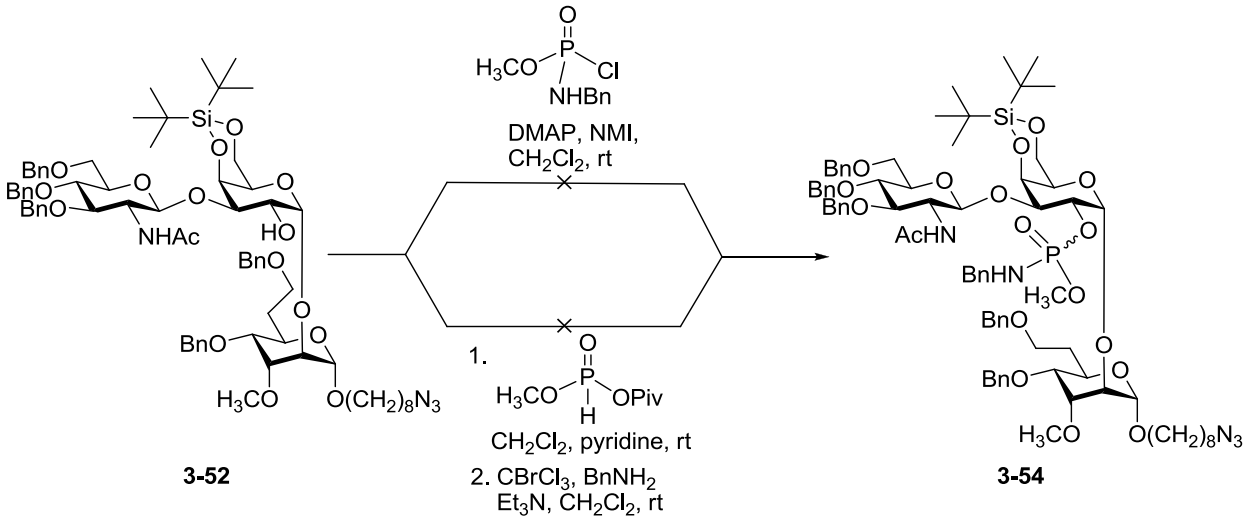


Scheme 3-11: Synthesis of parent trisaccharide **2-10a** and its derivative **3-2a** containing the phase variable methyl group.

3.3.2.4 Attempted synthesis of the methyl phosphoramidate trisaccharide 3-54

Next, we turned our attention to the synthesis of **3-3a** (Figure 3-6), which contains both of the phase-variable modifications: the methyl and MeOPN groups. The approach (Scheme 3-12) was to introduce the MeOPN group onto **3-52**, which already contains the methyl group. We initially explored two previously reported methods to carry out this transformation. We first investigated using methyl benzylphosphoramidochloridate;[21b] however, under these conditions, no product was formed; only the starting material (**3-52**) was isolated. The use of a two-step approach[21a] involving an activated *O*-methyl *H*-phosphonate pivalate ester (obtained by treatment of the parent *O*-methyl *H*-phosphonate with PivCl immediately before addition of the

alcohol) and subsequent oxidation via the Atherton–Todd reaction,[26] again resulted only in the re-isolation of **3-52**.

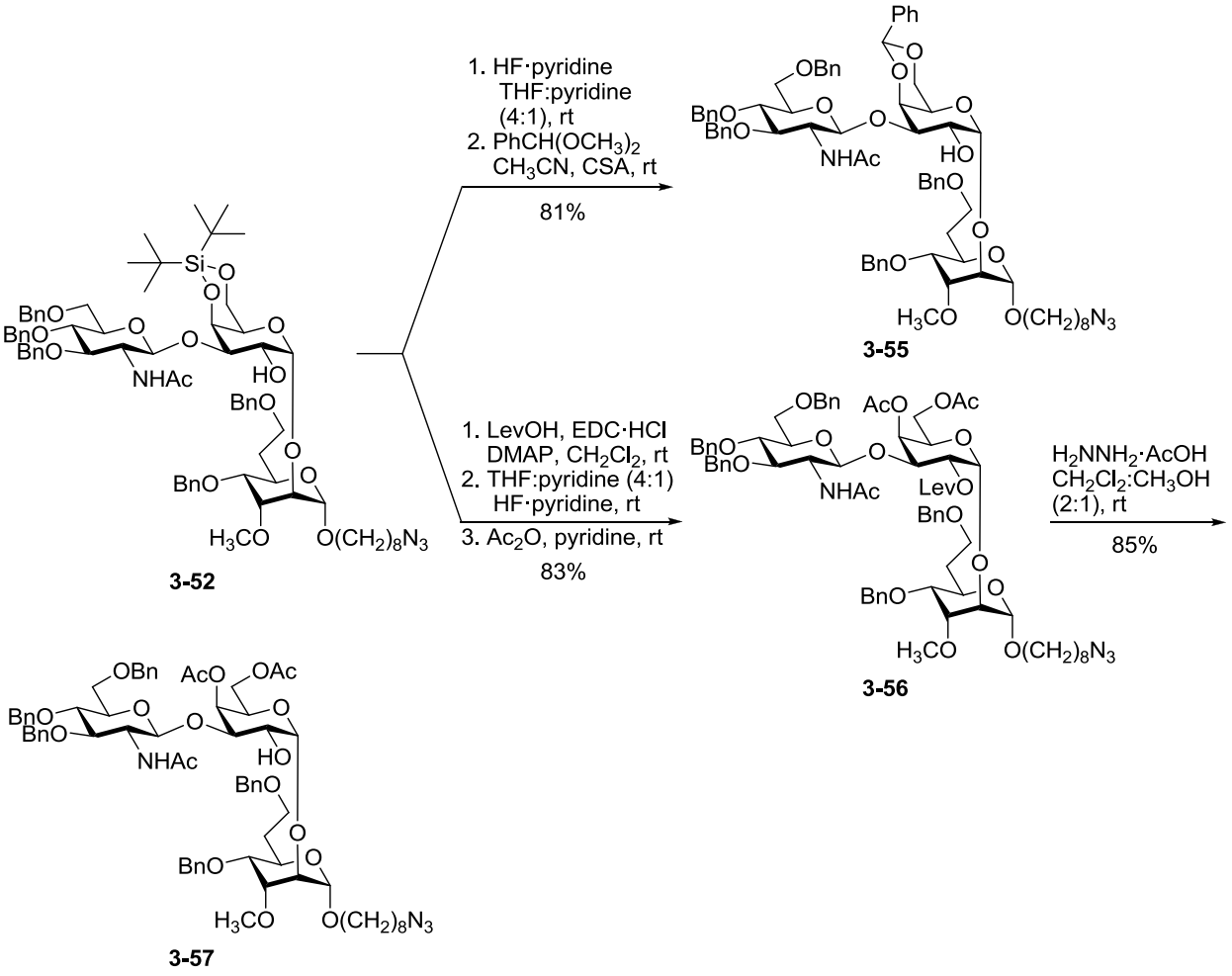


Scheme 3-12: Attempted synthesis of phosphoramidate **3-54** from **3-52** using two reported methods.[21a,21b]

3.3.2.5 Synthesis of derivatives of 3-52 with the di-*tert*-butylsilylene acetal replaced

Because of the steric bulk of the 4,6-*O*-di-*tert*-butylsilylene acetal, we envisioned that derivatives of **3-52** in which this protecting group was replaced with a benzylidene acetal (**3-55**) or acetate esters (**3-57**) would be better substrates to attempt the incorporation of the MeOPN (Scheme 3-13). Accordingly, trisaccharide **3-52**, when treated with hydrogen fluoride in pyridine, produced a triol that was then subjected to benzylidene acetal formation using benzaldehyde dimethylacetal to afford an 81% yield of **3-55** over the two steps. On the other hand, the treatment of **3-52** with levulinic acid and *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC·HCl) followed by reaction with hydrogen fluoride in pyridine produced a diol that was then subjected to acetylation to furnish diacetate **3-56** in 83%

yield over the three steps. Treatment of **3-56** with hydrazine acetate furnished alcohol **3-57** in 85% yeld.



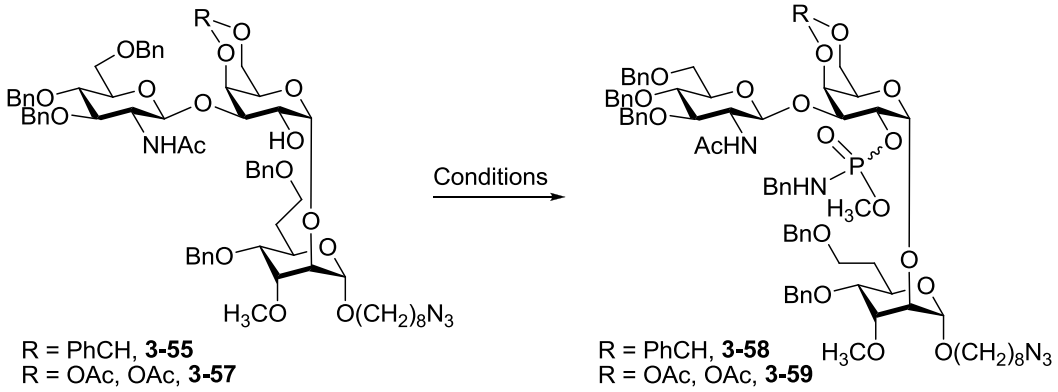
Scheme 3-13: Synthesis of di-*tert*-butylsilylene acetal replaced derivatives of **3-52**.

3.3.2.6 Attempted synthesis of the methyl phosphoramidate trisaccharides 3-58 and 3-59

With **3-55** and **3-57** synthesized, we explored the same two methods[21a,21b] attempted with **3-52** to install the MeOPN group (Table 3-2). We first studied the transformation with the benzylidene-actatl protected susbtrate **3-55**. With methyl benzylphosphoramidochloridate,[21b] no product was formed; only the starting material was isolated. The use of a two-step approach[21a] –

formation of an activated *O*-methyl *H*-phosphonate pivalate ester and subsequent Atherton–Todd reaction[26] – again resulted only in the re-isolation of **3-55**. Similarly, when we attempted to introduce the MeOPN onto **3-57**, which contatains two acetate esters, the use of methyl benzylphosphoramidochloridate,[21b] led to re-isolation of the starting material. However, the use of the two-step approach on **3-55**[21a] furnished the desired methyl phosphoramidate in 22% yield along with 68% recovered starting material. These experiments suggest that there is much steric congestion around the galactose C-2 position in **3-52**, **3-55** and **3-57**.

Table 3-2: Conditions explored for the incorporation of the MeOPN group onto **3-55** and **3-57**



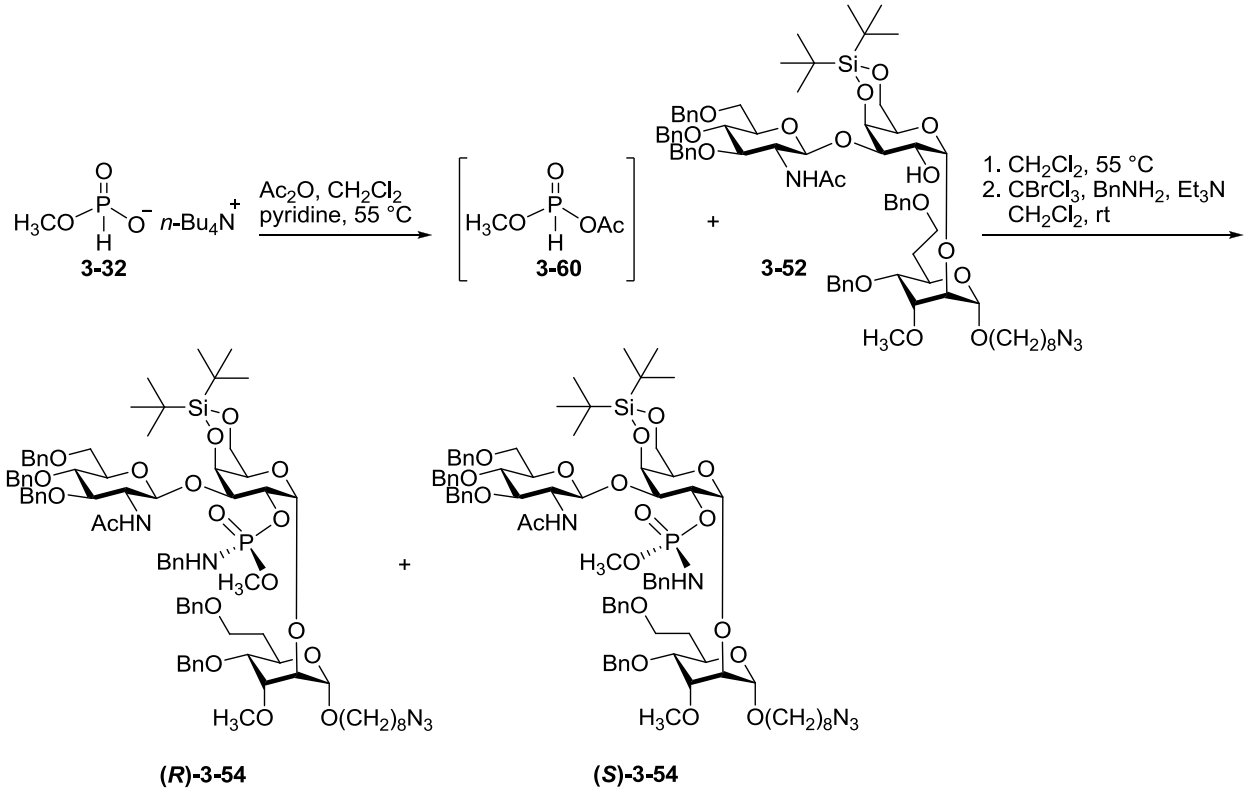
Entry	R	Conditions	Product, yield[a]
1	PhCH, **3-55**	$H_3COP(O)(Cl)NHBn$, DMAP, NMI, CH_2Cl_2, rt, 2-4 days	**3-58**, 0%[b]
2	PhCH, **3-55**	i) $H_3COP(O)(H)OPiv$, CH_2Cl_2, pyridine, rt 2–3 days ii) $CBrCl_3$, $BnNH_2$, Et_3N, CH_2Cl_2, rt, 4 h	**3-58**, 0%[b]
3	OAc, OAc **3-57**	$H_3COP(O)(Cl)NHBn$, DMAP, NMI, CH_2Cl_2, rt, 2-4 days	**3-59**, 0%[b]
4	OAc, OAc **3-57**	i) $H_3COP(O)(H)OPiv$, CH_2Cl_2, pyridine, rt 2-3 days ii) $CBrCl_3$, $BnNH_2$, Et_3N, CH_2Cl_2, rt, 4 h	**3-59**, 22%[b]

[a] Isolated yield. [b] Starting material recovered.

3.3.2.7 Successful introduction of the methyl phosphoramidate onto the trisaccharide 3-52

Because of, at best, very low yields of the desired methyl phosphoramidate product with **3-52** and their derivatives (**3-55** and **3-57**) using the activated *O*-methyl *H*-phosphonate pivalate ester, we postulated that the lack of success was due to steric hindrance near the C-2 hydroxyl group in the galactose residue. We further surmised that using a reagent smaller than those shown in Scheme 3-12, and more forcing conditions, would provide the desired target. With this

approach in mind, **3-52** was subjected to a modified version of the H-phosphonate formation (Scheme 3-14).



Scheme 3-14: Successful introduction of the methyl phosphoramidate onto trisaccharide **3-52**.

Activation of the precursor H-phosphonate (**3-32**) was done using acetic anhydride instead of PivCl, and the coupling was done at 55 °C instead of room temperature. Under these conditions, a new product was formed that, when subjected to the Atherton–Todd reaction,[26] furnished the desired *O*-methyl phosphoramidate in 65% yield. As in other examples using this approach,[21a] products corresponding to both diastereomers on phosphorus were formed: (***R***)-**3-54** and (***S***)-**3-54**. While these two diastereomers are often inseparable, in this case they could be separated. Fortuitously, one of the diastereomers [(***R***)-**3-54**] was a solid and we obtained an X-ray structure of the compound (Figure 3-7).[27] Analysis of the structure allowed us to assign the

stereochemistry at phosphorous in (***R***)-**3-54** as *R*.[28] By inference then, the phosphorus atom in (***S***)-**3-54** has the *S* stereochemistry.

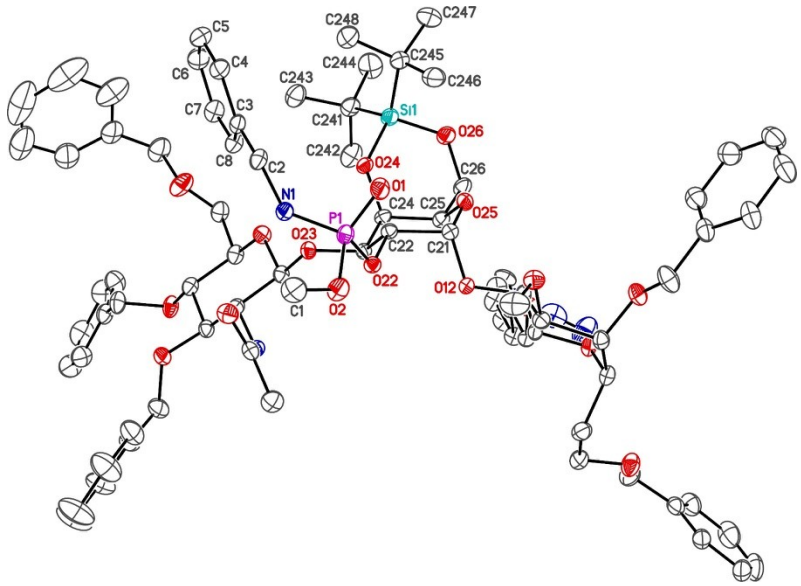


Figure 3-7: ORTEP of (***R***)-**3-54**, showing the *R*-stereochemistry on phoshorus.

In further characterizing (***R***)-**3-54** we were surprised to find that the ^{31}P NMR spectrum of the compound showed two signals instead of the anticipated single peak (ratio 4:1, Figure 3-8); a similar thing was seen in the ^{31}P spectrum of (***S***)-**3-54** (ratio 2:1, Figure 3-9). Given the difficulty we had in installing the MeOPN group, presumably due to steric congestion, we hypothesized that these two resonances arise from restricted rotation around one of the phosphoramidate bonds. To test this, we measured the ^{31}P spectrum on (***R***)-**3-54** and (***S***)-**3-54** at 60 °C. Under these conditions, the two ^{31}P signals for both compounds changed in relative intensity, leading to spectra that contained one major signal (Figure 3-10); in the case of (***S***)-**3-54** a single resonance was observed (Figure 3-11). This experiment provides support for restricted rotation leading to the doubling of the signals in these NMR spectra.

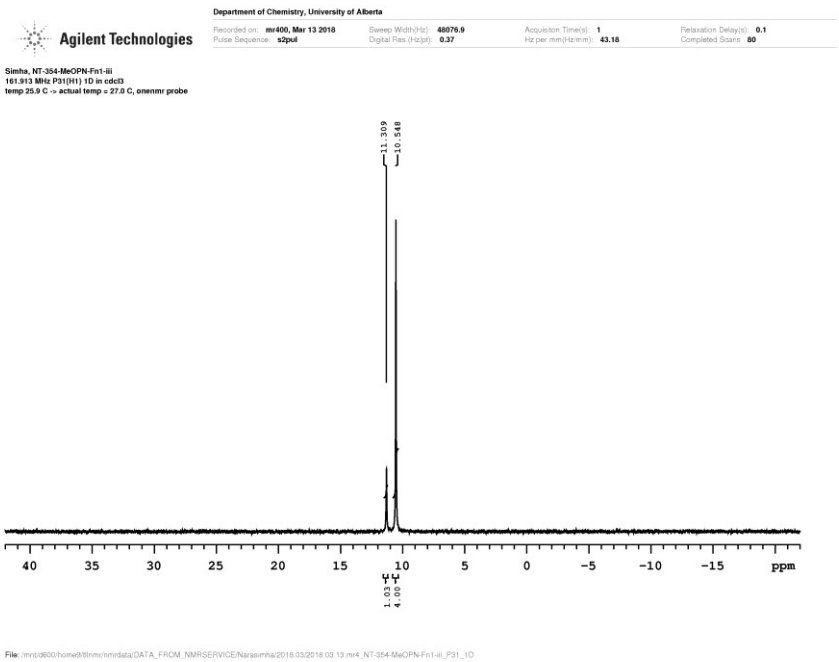


Figure 3-8: ^{31}P NMR spectrum of (***R***)-**3-54** at 27 °C.

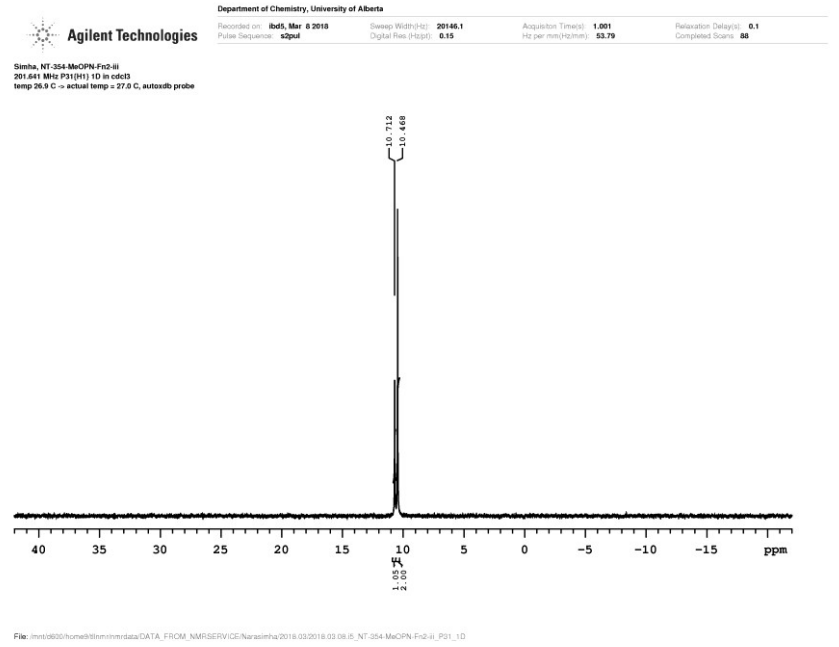


Figure 3-9: ^{31}P NMR spectrum of (***S***)-**3-54** at 27 °C.

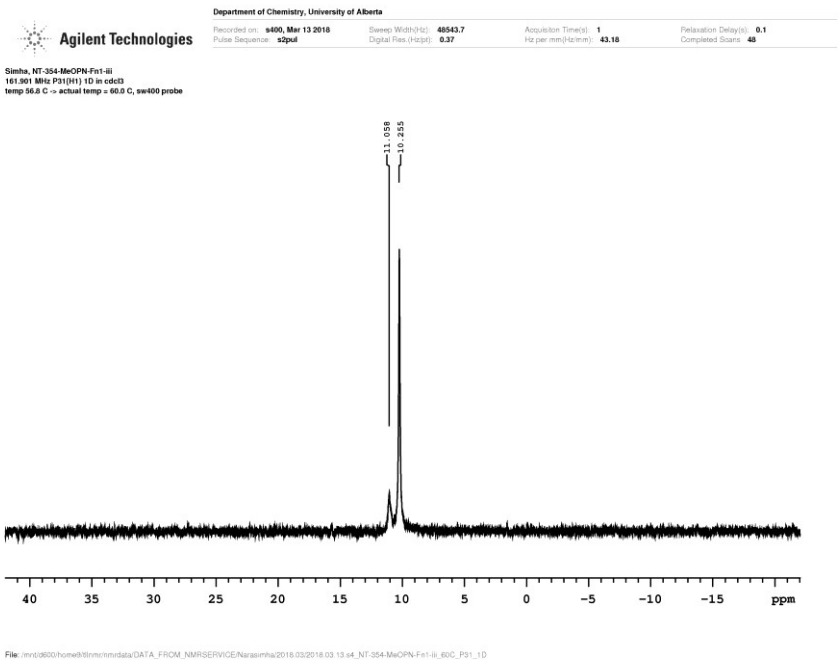


Figure 3-10: ^{31}P NMR spectrum of (***R***)-**3-54** at 60 °C.

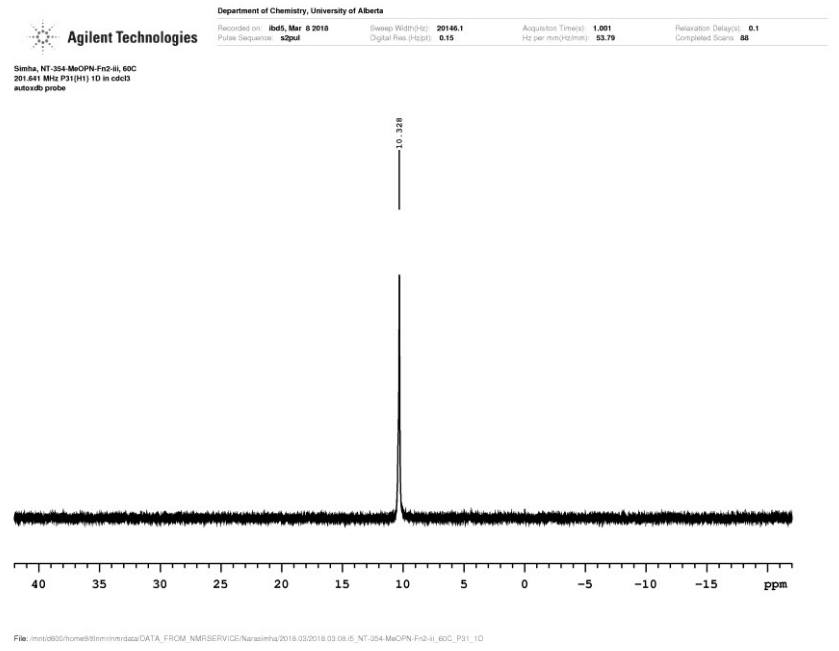


Figure 3-11: ^{31}P NMR spectrum of (***S***)-**3-54** at 60 °C.

Unfortunately, the solvent used ($CDCl_3$) limited the temperature at which we could heat the sample and thus it was never possible to obtain spectra of (***R***)-**3-54** that had a single resonance, indicative of free rotation of the phosphoramidate bond in this molecule. Nevertheless, this observation, and the difficulty with which it is to install the MeOPN group onto the molecule, provides insight into the sterically hindered nature of this region of the molecule. With this modified route established for the introduction of MeOPN, by the application of this route on trisaccharides (**3-55** and **3-57**, shown in Scheme 3-13), we were able to successfully introduce the MeOPN group to afford **3-58** and **3-59** in 70% and 60% yield respectively.

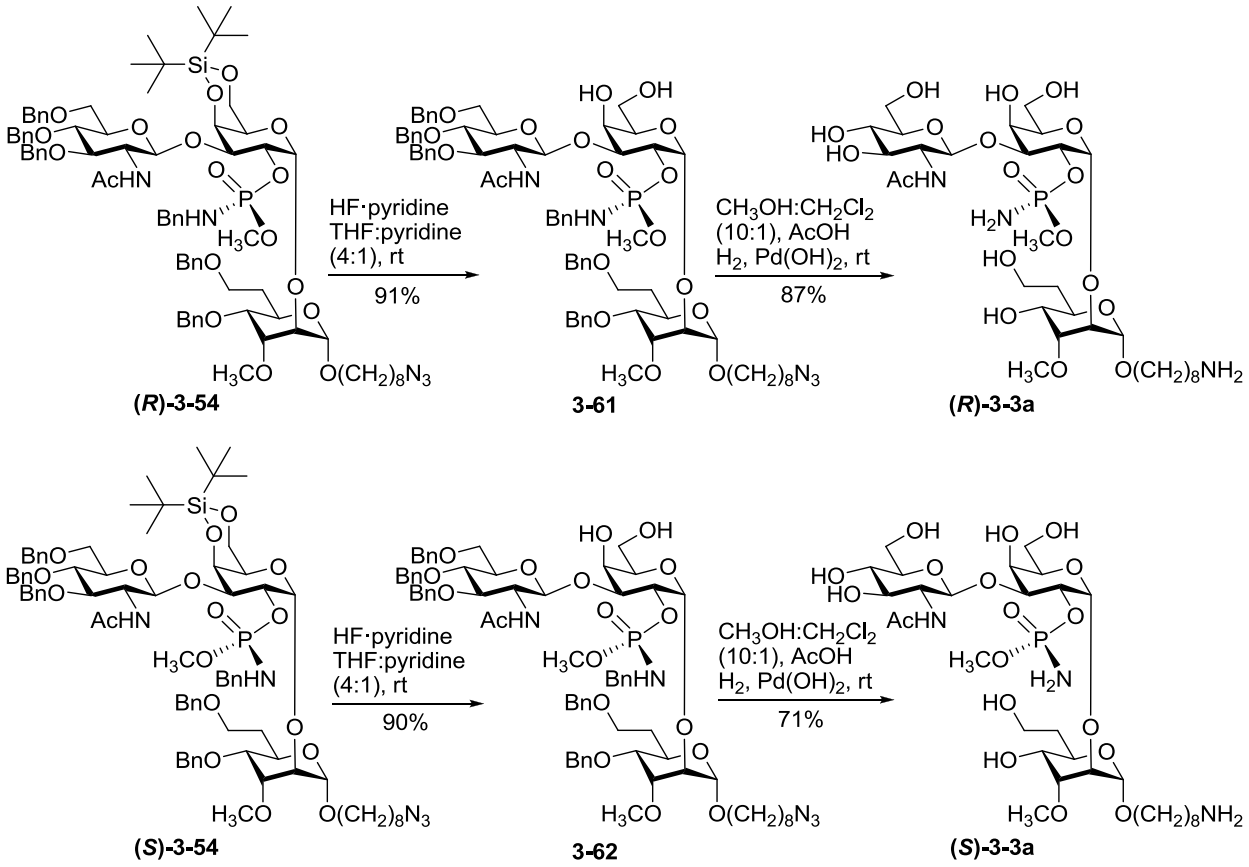
3.3.2.8 Determination of the phosphorous stereochemistry in the natural CPS through the syntheses of natural and unnatural methyl phosphoramidate trisaccharides

To allow the determination of the phosphorous stereochemistry in the natural CPS, both (***R***)-**3-54** and (***S***)-**3-54** were deprotected (Scheme 3-15). Treatment of (***R***)-**3-54** with HF·pyridine furnished the diol **3-61** in 91% yield. Similar treatment of (***S***)-**3-54** provided the diol **3-62** in 90% yield. It is interesting to note that ^{31}P NMR spectrum of **3-61** showed single peak as expected. But, in contrast, the ^{31}P NMR spectrum of **3-62** showed two signals instead of the anticipated single peak (ratio 10:1). We hypothesized that these two resonances arise from restricted rotation around one of the phosphoramidate bonds as before. We also found that the hydrogenolysis needed significant optimization (Table 3-3) and the best results were obtained when a small amount of acetic acid was added to the reaction mixture. Without acetic acid we found that the majority of the MeOPN group was cleaved (to form **3-2a**, shown in Scheme 3-11).

Table 3-3: Optimization of Hydrogenolysis of **3-61**

Compound	Hydrogenolysis Conditions	(*R*)-3-3a:3-2a Ratio
3-61	C_2H_5OH, CH_2Cl_2, $Pd(OH)_2$–C, H_2, rt, 9 h	3.45:1[a]
3-61	CH_3OH, CH_2Cl_2, $Pd(OH)_2$–C, AcOH (3-5 drops), H_2, rt, 6 h	>14:1[b]
3-61	CH_3OH, CH_2Cl_2, $Pd(OH)_2$–C, AcOH (0.5 mL), H_2, rt, 12 h	20:1[b]

[a]Crude ratio as determined by ^{1}H NMR spectroscopy. [b]Isolated ratio as determined by ^{1}H NMR spectroscopy.



Scheme 3-15: Syntheses of natural and unnatural MeOPN trisaccharides **(*R*)-3-3a** and **(*S*)-3-3a**.

The deprotected compounds were also found to have limited stability in D_2O; most of the MeOPN group was cleaved within few hours during storage in D_2O. The lability of this group in the native CPS was not reported previously,[4] although we note that the NMR spectra of the polysaccharide were recorded in the presence of 1% acetic acid. It thus appears that the presence of a small amount of acid is required for the molecule to be stable in solution. This is also consistent with our finding (above) that in the absence of acetic acid in the solvent mixture, the hydrogenolysis led to cleavage of the MeOPN group.

With both **(*R*)-3-3a** and **(*S*)-3-3a** in hand, comparison of the NMR data with that previously reported for the CPS[4,9b] was carried out (Table 3-4). It should be noted that for these deprotected compounds the restricted rotation seen in (***R***)-**3-54** and (***S***)-**3-54** was not observed; thus, in all NMR spectra only single resonances were present. The ^{31}P NMR spectrum of **(*R*)-3-3a** showed a signal at 14.29 ppm and the resonance for this atom in the CPS is in close agreement (14.26 ppm). On the other hand, the ^{31}P NMR spectrum of **(*S*)-3-3a** showed a resonance at 13.85 ppm. Both spectra contained a single resonance, which suggests that there was no loss of stereochemical integrity on phosphorus during the deprotection. Had scrambling of the phosphorus stereochemistry occurred, a mixture of the two diastereomers would be expected, not complete conversion to the opposite stereoisomer. When considering the H-2 signal of galactose residue in the ^{1}H NMR spectra, the data for **(*R*)-3-3a** (4.54 ppm) is in close agreement with that reported for the CPS (4.52 ppm), whereas the data for **(*S*)-3-3a** differs. Based on these comparisons, we conclude that MeOPN group in the *C. jejuni* 81-176 CPS is *R*.

Table 3-4: Comparison of the NMR data for ***(R)*-3-3a** and ***(S)*-3-3a** with natural CPS.

Compound	^{31}P NMR[a]	^{1}H NMR[a,b]
***(R)*-3-3a**	14.291	4.54
***(S)*-3-3a**	13.849	4.48
CPS	14.257[4]	4.52[9b]

[a]Chemical shifts in ppm. [b]Chemical shift of H-2 in galactose residue.

3.4 Summary

In summary, the work described in this chapter provides an efficient synthesis of the three repeating units present in CPS (**2-10a**, **3-2a** and **3-3a**) produced by *C. jejuni* 81-176, including one that contains a MeOPN motif (**3-3a**) via a single strategy using building blocks **3-32**–**3-35** and **3-46**. While carrying out these syntheses we obtained a crystal structure of a key intermediate – (***R*)-3-54** – that, following its deprotection and comparison with data for the polysaccharide, established the phosphorous stereochemistry of the MeOPN in the native CPS as *R*. This represents the first unambiguous determination of the stereochemistry of a *Campylobacter* CPS MeOPN group, which exists naturally as a single stereoisomer. It remains to be determined if MeOPN-functionalized CPS produced by other *Campylobacter* have the same stereochemistry. However, we note that the biosynthesis of the group is done enzymatically,[10] which, depending on the similarities of enzymes across species, could suggest it is the same in all *Campylobacter*s. The use of **(*R*)-3-3a** and its enantiomer **(*S*)-3-3a** can be used to probe the specificity of sera raised against vaccination with the native CPS. These compounds will be useful probes in studies of MeOPN function and assembly. We anticipate this study will also motivate the development of methods for the diastereoselective introduction of MeOPN groups

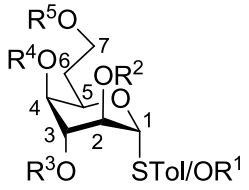
onto carbohydrates. The structurally well-defined antigenic trisaccharides (**2-10a**, **3-2a** and **3-3a**) were synthesized in a form suitable for conjugation to appropriate proteins and/or probes and hence can be tested for its immunogenicity.

3.5 Experimental

3.5.1 General experimental methods

8-Azido-1-octanol was prepared according to literature protocol.[23] All reactions were carried out in oven-dried glassware. All reagents used 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 under a positive pressure of argon and were 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 at 40 °C. ^{1}H NMR spectra were recorded at 500, or 700 MHz and were referenced to the residual proton signal of $CDCl_3$ (7.26 ppm), CD_3OD (3.30 ppm) or HOD (4.79 ppm). $^{13}C\{^1H\}$ NMR spectra were recorded at 125 (cold probe) or 175 MHz and were referenced to ^{13}C signals of $CDCl_3$ (77.06 ppm) or CD_3OD (49 ppm). $^{31}P\{^1H\}$ NMR spectra were collected at 202 or 162 MHz and were referenced to an external 85% H_3PO_4 standard (0.00 ppm). ^{1}H NMR data are reported as if they were first order, and peak assignments were made on the basis of 2D NMR (^{1}H–^{1}H COSY, APT, TOCSY, HSQC and HMBC) experiments. For the ^{1}H and ^{13}C NMR data, signals from rotamers, arising due to restricted rotation in (***R***)-**3-54** and (***S***)-**3-54** were indicated by an asterisk (*). ESI-MS spectra (time-of-

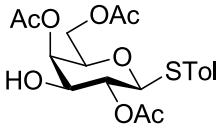
flight analyzer) were recorded on samples dissolved in THF or CH_3OH with added NaCl or NH_4Cl. Optical rotations were measured at 22 ± 2 °C at the sodium D line (589 nm) in a microcell (10 cm, 1 mL) and are in units of deg·mL(dm·g)$^{-1}$. IR spectra were recorded at room temperature on a Thermo Nicolet 8700 FT-IR spectrometer with a continuum FT-IR microscope attached using IR-transparent silicone wafer cast films. Carbon or proton signals in the 6-deoxy-*altro*-heptose sugar is numbered as shown in the figure below.



3-63

Figure 3-12: Legend for numbering proton or carbon signals in the 6-deoxy-*altro*-heptose sugar.

3.5.2 Experimental, spectroscopic and analytical data



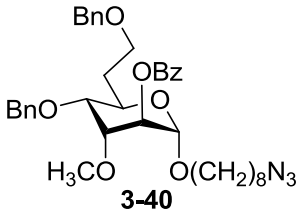
3-36

p-Tolyl 2,4,6-tri-*O*-acetyl-1-thio-β-D-galactopyranoside (3-36)

Concentrated H_2SO_4 (0.45 mL, 8.4 mmol) was added dropwise over 3 min to a stirred solution of thioglycoside **3-39** (2.10 g, 7.33 mmol) in EtOAc (150 mL). The resulting solution was stirred for 17 h and then transferred to a separatory funnel and saturated aqueous $NaHCO_3$ solution was added until no further effervescence was observed. The organic layer was separated and the aqueous layer was further extracted with EtOAc (2 × 50 mL). The combined organic

phases were washed with brine (30 mL), dried over anhydrous Na_2SO_4, filtered, concentrated and the resulting crude product was quickly purified by column chromatography (5% CH_3OH in EtOAc) to afford the known triol.[24] To a stirred solution of the resulting triol (1.81 g, 5.51 mmol) in CH_3CN (25 mL) was added triethyl orthoacetate (5.0 mL, 0.027 mmol) followed by camphorsulfonic acid (310 mg, 1.33 mmol). The resulting solution was stirred for 45 min. Camphorsulfonic acid was quenched by the addition of Et_3N (0.2 mL) and resulting solution was concentrated and re-dissolved in pyridine (10 mL). Acetic anhydride (5.0 mL, 0.053 mmol) was added and the mixture was stirred at 50 °C for 2 h before being cooled to room temperature over 15 min. Toluene (5 mL) was added and the resulting solution was concentrated. Co-concentration with toluene (4 × 5 mL) produced a crude product that was re-dissolved in CH_2Cl_2 (10 mL). 1 M HCl (50 mL) was added and the resulting solution was stirred vigorously for 1 h. The organic layer was separated and the aqueous layer was further extracted with CH_2Cl_2 (2 × 50 mL). The combined organic phases were washed with brine (20 mL), dried over anhydrous Na_2SO_4, filtered, concentrated and the resulting crude product was purified by column chromatography (50% EtOAc in hexane) to afford **3-36** (1.81 g, 60% over the four steps) as a white solid: $[\alpha]^{22}_D$ +9.8 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.41–7.37 (m, 2 H, ArH), 7.12–7.08 (m, 2 H, ArH), 5.32 (d, J = 3.0 Hz, 1 H, H-4), 4.98 (app t, J = 10.0, 9.5 Hz, 1 H, H-2), 4.59 (d, J = 10.0 Hz, 1 H, H-1), 4.16–4.11 (m, 2 H, H-6a and H-6b), 3.86–3.81 (m, 2 H, H-3 and H-5), 2.75 (d, J = 4.5 Hz, CH*OH*), 2.33 (s, 3 H, C*H*$_3$C$_6$H$_4$S), 2.15 (s, 3 H, 3 × COC*H*$_3$), 2.13 (s, 3 H, 3 × COC*H*$_3$), 2.04 (s, 3 H, 3 × COC*H*$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.0 (*C*OCH_3), 170.9 (*C*OCH_3), 170.5 (*C*OCH_3), 138.3 (Ar), 133.0 (Ar), 129.6 (Ar), 128.9 (Ar), 86.5 (C-1), 74.8 (C-5), 72.3 (C-3), 70.8 (C-2), 69.9 (C-4), 62.2 (C-6), 21.1 (*C*H$_3$C$_6$H$_4$S), 21.0 (CO*C*H$_3$), 20.8 (CO*C*H$_3$), 20.7 (CO*C*H$_3$); IR (cast film, $CHCl_3$) ν 3467, 3036, 2959, 2862, 1742, 1226, 1057,

804 cm^{-1}; HRMS (ESI-TOF) m/z: $[M + Na]^+$ Calcd for $C_{19}H_{24}O_8SNa$ 435.1084; Found 435.1085.

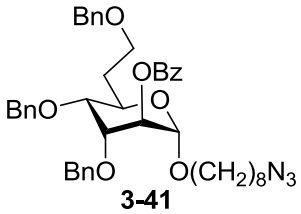


3-40

8-Azidooctyl 2-*O*-benzoyl-4,7-di-*O*-benzyl-6-deoxy-3-*O*-methyl-α-D-*altro*-heptopyranoside (3-40)

A solution of thioglycoside **2-67** (616 mg, 1.03 mmol) and 8-azidooctanol **3-37** (350 mg, 2.05 mmol) in dry CH_2Cl_2 (15 mL) was stirred with 4 Å molecular sieves (0.64 g) for 30 min. The reaction mixture was cooled to 0 °C and NIS (324 mg, 1.44 mmol) and TMSOTf (30 µL, 0.16 mmol) were added sequentially and the solution was stirred for 1.5 h. The TMSOTf was quenched by the addition of Et_3N (0.25 mL) and the reaction mixture was diluted with CH_2Cl_2 (50 mL) and filtered over a pad of Celite-545. The filtrate was washed with saturated aqueous $Na_2S_2O_3$ solution (20 mL). The organic layer was separated and the aqueous layer was further extracted with CH_2Cl_2 (2 × 75 mL). The combined organic phases were washed with brine (20 mL), dried over anhydrous Na_2SO_4, filtered, concentrated and the resulting crude product was purified by column chromatography (14% EtOAc in hexane) to afford **3-40** (558 mg, 84%) as a colorless viscous liquid: $[\alpha]^{22}_D$ +39.0 (*c* 0.99, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.96 (dd, *J* = 8.0, 1.0 Hz, 2 H, ArH), 7.63–7.25 (m, 13 H, ArH), 5.33 (dd, *J* = 3.0, 0.5 Hz, 1 H, H-2), 4.75 (d, *J* = 0.5 Hz, 1 H, H-1), 4.64, 4.55 (ABq, *J* = 11.9 Hz, 2 H, 2 × OC*H*$_2$Ph), 4.52 (s, 2 H, 2 × OC*H*$_2$Ph), 4.30 (app td, *J* = 9.5, 3.0 Hz, 1 H, H-5), 3.69 (app t, *J* = 3.0 Hz, 1 H, H-3), 3.68–3.63 (m, 3 H, H-7a, H-7b and OC*H*$_2$CH$_2$), 3.56 (dd, *J* = 9.5, 3.0 Hz, 1 H, H-4), 3.56 (s, 3 H, OC*H*$_3$),

3.35 (dt, J = 9.5, 6.0 Hz, 1 H, OC$\underline{H}_2$CH$_2$), 3.25 (t, J = 7.0 Hz, 2 H, 2 × C$\underline{H}_2$N$_3$), 2.36–2.29 (m, 1 H, H-6a), 1.79–1.72 (m, 1 H, H-6b), 1.62–1.50 (m, 4 H, 4 × C$\underline{H}_2$), 1.38–1.25 (m, 8 H, 8 × C$\underline{H}_2$); ^{13}C NMR (125 MHz, CDCl$_3$) δ 165.3 ($\underline{C}$OPh), 138.6 (Ar), 137.7 (Ar), 133.4 (Ar), 129.8 (Ar), 129.6 (Ar), 128.5 (Ar), 128.4 (Ar), 128.32 (Ar), 128.28 (Ar), 127.9 (Ar), 127.6 (Ar), 127.5 (Ar), 97.3 (C-1), 75.4 (C-4), 74.8 (C-3), 73.0 (O$\underline{C}$H$_2$Ph), 71.1 (O$\underline{C}$H$_2$Ph), 69.1 (C-2), 67.7 (O$\underline{C}$H$_2$CH2), 67.1 (C-7), 64.3 (C-5), 58.1 (O$\underline{C}$H$_3$), 51.5 ($\underline{C}$H$_2$N$_3$), 31.7 (C-6), 29.4 ($\underline{C}$H$_2$), 29.3 ($\underline{C}$H$_2$), 29.1 ($\underline{C}$H$_2$), 28.9 ($\underline{C}$H$_2$), 26.7 ($\underline{C}$H$_2$), 26.1 ($\underline{C}$H$_2$); IR (cast film, CHCl$_3$) ν 3030, 2932, 2096, 1724, 1601, 1496, 1453, 1112, 741 cm^{-1}; HRMS (ESI-TOF) m/z: [M + Na]$^+$ Calcd for C$_{37}$H$_{47}$N$_3$O$_7$Na 668.3306; Found 668.3298.

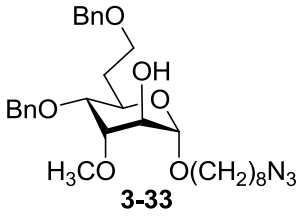


3-41

8-Azidooctyl 2-*O*-benzoyl-3,4,7-tri-*O*-benzyl-6-deoxy-α-D-*altro*-heptopyranoside (3-41)

A solution of thioglycoside **2-68** (90.7 mg, 0.134 mmol) and 8-azidooctanol **3-37** (46 mg, 0.27 mmol) in dry CH$_2$Cl$_2$ (3 mL) was stirred with 4 Å molecular sieves (0.25 g) for 30 min. The reaction mixture was cooled to 0 °C and NIS (45 mg, 0.20 mmol) and TMSOTf (5 µL, 0.03 mmol) were added sequentially and the solution was stirred for 1.5 h. The TMSOTf was quenched by the addition of Et$_3$N and the reaction mixture was diluted with CH$_2$Cl$_2$ (50 mL) and filtered over a pad of Celite-545. The filtrate was washed with saturated aqueous Na$_2$S$_2$O$_3$ solution (20 mL). The organic layer was separated and the aqueous layer was further extracted with CH$_2$Cl$_2$ (2 × 75 mL). The combined organic phases were washed with brine (20 mL), dried over anhydrous Na$_2$SO$_4$, filtered, concentrated and the resulting crude product was purified by

column chromatography (13% EtOAc in hexane) to afford **3-41** (87 mg, 90%) as a colorless viscous liquid: $[\alpha]^{22}_{D}$ +49.4 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.97 (d, J = 7.0, 1.0 Hz, 2 H, ArH), 7.62–7.25 (m, 18 H, ArH), 5.42 (d, J = 2.5 Hz, 1 H, H-2), 4.85, 4.67 (ABq, J = 12.0 Hz, 2 H, 2 × OC$\underline{H_2}$Ph), 4.79 (s, 1 H, H-1),4.53 (s, 2 H, 2 × OC$\underline{H_2}$Ph), 4.51, 4.39 (ABq, J = 11.4 Hz, 2 H, 2 × OC$\underline{H_2}$Ph), 4.42–4.38 (m, 1 H, H-5), 3.91 (app t, J = 3.0, 2.5 Hz, 1 H, H-3), 3.72–3.65 (m, 3 H, H-7a, H-7b and OC$\underline{H_2}CH_2$), 3.56 (dd, J = 9.0, 3.0 Hz, 1 H, H-4), 3.37 (dt, J = 9.0, 6.0 Hz, 1 H, OC$\underline{H_2}CH_2$), 3.23 (t, J = 7.0 Hz, 2 H, 2 × C$\underline{H_2}N_3$), 2.37–2.30 (m, 1 H, H-6a), 1.81–1.74 (m, 1 H, H-6b), 1.65–1.52 (m, 4 H, 4 × C$\underline{H_2}$), 1.39–1.23 (m, 8 H, 8 × C$\underline{H_2}$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.3 ($\underline{C}$OPh), 138.6 (Ar), 138.2 (Ar), 137.8 (Ar), 133.4 (Ar), 130.9 (Ar), 129.8 (Ar), 129.6 (Ar), 128.9 (Ar), 128.5 (Ar), 128.3 (Ar), 128.2 (Ar), 128.1 (Ar), 127.9 (Ar), 127.7 (Ar), 127.6 (Ar), 127.54 (Ar), 127.46 (Ar), 97.5 (C-1), 75.4 (C-4), 73.0 (O$\underline{C}H_2$Ph), 71.9 (C-3), 71.5 (O$\underline{C}H_2$Ph), 70.6 (O$\underline{C}H_2$Ph), 69.4 (C-2), 67.8 (O$\underline{C}H_2CH_2$), 67.2 (C-7), 64.5 (C-5), 51.5 ($\underline{C}H_2N_3$), 31.7 (C-6), 29.5 ($\underline{C}H_2$), 29.4 ($\underline{C}H_2$), 29.1 ($\underline{C}H_2$), 28.8 ($\underline{C}H_2$), 26.7 ($\underline{C}H_2$), 26.2 ($\underline{C}H_2$); IR (cast film, $CHCl_3$) ν 3031, 2931, 2095, 1723, 1601, 1496, 1453, 1112, 737 cm^{-1}; HRMS (ESI-TOF) m/z: $[M + Na]^+$ Calcd for $C_{43}H_{51}N_3O_7Na$ 744.3619; Found 744.3617.

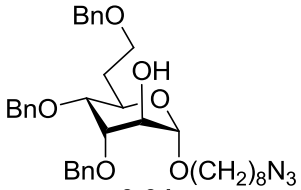


3-33

8-Azidooctyl 4,7-di-*O*-benzyl-6-deoxy-3-*O*-methyl-α-D-*altro*-heptopyranoside (3-33)

To a stirred solution of glycoside **3-40** (517 mg, 0.801 mmol) in CH_3OH (9 mL) and CH_2Cl_2 (1 mL) was added sodium methoxide in methanol (0.55 M, 1.8 mL, 0.99 mmol). The resultant solution was stirred for 2 h and then Amberlite IR-120 resin (H^+ form) was added to

neutralize the solution, solution was filtered, the resin was washed with CH_3OH (25 mL) and the resulting filtrate was concentrated to obtain crude product that was purified by column chromatography (30% EtOAc in hexane) to afford **3-33** (412 mg, 95%) as a colorless viscous liquid: $[\alpha]^{22}_D$ +33.9 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.36–7.26 (m, 10 H, ArH), 4.64, 4.60 (ABq, *J* = 12.1 Hz, 2 H, 2 × OC*H*$_2$Ph), 4.54 (d, *J* = 4.5 Hz, 1 H, H-1), 4.48 (s, 2 H, 2 × OC*H*$_2$Ph), 4.24–4.21 (m, 1 H, H-5), 3.96 (dd, *J* = 7.0, 5.0 Hz, 1 H, H-2), 3.69 (dt, *J* = 9.5, 7.0 Hz, 1 H, OC*H*$_2$CH$_2$), 3.64 (dd, *J* = 6.0, 3.5 Hz, 1 H, H-4), 3.62–3.55 (m, 2 H, H-7a and H-7b), 3.48 (dd, *J* = 7.0, 3.5 Hz, 1 H, H-3), 3.39 (s, 3 H, OC*H*$_3$), 3.35 (dt, *J* = 9.5, 7.0 Hz, 1 H, OC*H*$_2$CH$_2$), 3.24 (t, *J* = 7.0 Hz, 2 H, 2 × C*H*$_2$N$_3$), 2.12 (br s, CHO*H*), 1.99 (dddd, *J* = 14.3, 8.9, 6.0, 5.6 Hz, 1 H, H-6a), 1.82 (dddd, *J* = 14.3, 7.5, 7.3, 4.5 Hz, 1 H, H-6b), 1.61–1.53 (m, 4 H, 4 × C*H*$_2$), 1.38–1.23 (m, 8 H, 8 × C*H*$_2$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.4 (Ar), 138.2 (Ar), 128.38 (Ar), 128.36 (Ar), 128.0 (Ar), 127.7 (Ar), 127.65 (Ar), 127.60 (Ar), 99.9 (C-1), 78.7 (C-3), 74.8 (C-4), 73.1 (O*C*H$_2$Ph), 71.6 (O*C*H$_2$Ph), 69.5 (C-2), 68.6 (C-5), 68.5 (O*C*H$_2$CH$_2$), 66.9 (C-7), 57.9, 51.5 (*C*H$_2$N$_3$), 31.4 (C-6), 29.5 (*C*H$_2$), 29.3 (*C*H$_2$), 29.1 (*C*H$_2$), 28.8 (*C*H$_2$), 26.7 (*C*H$_2$), 26.0 (*C*H$_2$); IR (cast film, $CHCl_3$) *v* 3456, 3031, 2929, 2095, 1496, 1454, 1364, 1100, 737 cm^{-1}; HRMS (ESI-TOF) m/z: $[M + Na]^+$ Calcd for $C_{30}H_{43}N_3O_6Na$ 564.3044; Found 564.3043.

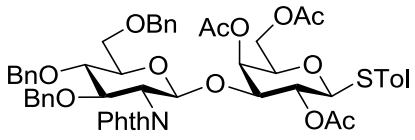


3-34

8-Azidooctyl 3,4,7-tri-*O*-benzyl-6-deoxy-α-D-*altro*-heptopyranoside (3-34)

To a stirred solution of glycoside **3-41** (80 mg, 0.11 mmol) in CH_3OH (1.8 mL) and CH_2Cl_2 (0.2 mL) was added sodium methoxide in methanol (0.55 M, 0.35 mL, 0.19 mmol). The

resultant solution was stirred for 2 h and then Amberlite IR-120 resin (H^+ form) was added to neutralize the solution, solution was filtered, the resin was washed with CH_3OH (50 mL) and the resulting filtrate was concentrated to obtain crude product that was purified by column chromatography (25% EtOAc in hexane) to afford **3-34** (65 mg, 95%) as a colorless viscous liquid: $[\alpha]^{22}_D$ +36.5 (*c* 0.91, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.35–7.26 (m, 15 H, ArH), 4.61 (s, 2 H, 2 × OC$\underline{H_2}$Ph), 4.60, 4.55 (ABq, J = 11.8 Hz, 2 H, 2 × OC$\underline{H_2}$Ph), 4.55 (d, J = 4.5 Hz, 1 H, H-1), 4.48 (s, 2 H, 2 × OC$\underline{H_2}$Ph), 4.25 (ddd, J = 9.5, 6.0, 4.5 Hz, 1 H, H-5), 4.02 (dd, J = 7.0, 4.5 Hz, 1 H, H-2), 3.73–3.68 (m, 2 H, OC$\underline{H_2}$CH$_2$ and H-3), 3.61 (dd, J = 6.0, 3.5 Hz, 1 H, H-4), 3.59–3.53 (m, 2 H, H-7a and H-7b), 3.35 (dt, J = 9.5, 6.5 Hz, 1 H, OC$\underline{H_2}$CH$_2$), 3.23 (t, J = 7.0 Hz, 2 H, 2 × C$\underline{H_2}$N$_3$), 2.06 (br s, 1 H, CHO$\underline{H}$), 1.98 (dddd, J = 12.3, 7.5, 7.3, 4.0 Hz, 1 H, H-6a), 1.79 (dddd, J = 12.3, 8.9, 6.0, 5.8 Hz, 1 H, H-6b), 1.59–1.54 (m, 4 H, 4 × C$\underline{H_2}$), 1.37–1.23 (m, 8 H, 8 × C$\underline{H_2}$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.4 (Ar), 138.32 (Ar), 138.25 (Ar), 128.39 (Ar), 128.37 (Ar), 128.35 (Ar), 127.9 (Ar), 127.8 (Ar), 127.7 (Ar), 127.6 (Ar), 100.0 (C-1), 76.2 (C-3), 75.5 (C-4), 73.1 (O$\underline{C}$H$_2$Ph), 71.9 (O$\underline{C}$H$_2$Ph), 71.6 (O$\underline{C}$H$_2$Ph), 69.8 (C-2), 68.6 (C-5), 68.5 (O$\underline{C}$H$_2$CH$_2$), 66.9 (C-7), 51.5 ($\underline{C}$H$_2$N$_3$), 31.4 (C-6), 29.6 ($\underline{C}$H$_2$), 29.4 ($\underline{C}$H$_2$), 29.1 ($\underline{C}$H$_2$), 28.9 ($\underline{C}$H$_2$), 26.7 ($\underline{C}$H$_2$), 26.1 ($\underline{C}$H$_2$); IR (cast film, $CHCl_3$) ν 3449, 3028, 2856, 2095, 1496, 1454, 1363, 1099, 738 cm^{-1}; HRMS (ESI-TOF) m/z: $[M + Na]^+$ Calcd for $C_{36}H_{47}N_3O_6Na$ 640.3357; Found 640.3356.

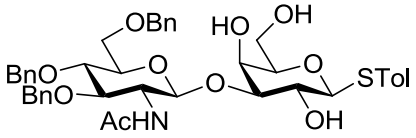


3-43

***p*-Tolyl 3,4,6-tri-*O*-benzyl-2-deoxy-2-*N*-phthalimido-β-D-glucopyranosyl-(1→3)- 2,4,6-tri-*O*-acetyl-1-thio-β-D-galactopyranoside (3-43)**

To a 0 °C solution of thioglycoside **3-35**[22] (301 mg, 0.439 mmol) in CH_2Cl_2 (5 mL) was added bromine (45.0 μL, 0878 mmol) and the mixture was stirred for 75 min. The reaction mixture was concentrated and co-evaporated with toluene (3 × 1 mL) to obtain crude the glycosyl bromide **3-42** as a pale yellow viscous liquid, which was used in the next step without further purification. A solution of **3-42** (268 mg, 0.417 mmol) and thioglycoside **3-36** (217 mg, 0.526 mmol) in dry CH_2Cl_2 (8 mL) was stirred with 4 Å molecular sieves (520 mg) for 30 min. The reaction mixture was cooled to –30 °C and AgOTf (121 mg, 0.471 mmol) was added and the solution was stirred for 1.5 h. The AgOTf was quenched by the addition of Et_3N (0. 1 mL) and the reaction mixture was diluted with CH_2Cl_2 (75 mL) and filtered over a pad of Celite-545. The filtrate was washed with saturated aqueous $NaHCO_3$ solution (25 mL) and the organic layer was separated and the aqueous layer was further extracted with CH_2Cl_2 (2 × 50 mL). The combined organic phases were washed with brine (25 mL), dried over anhydrous Na_2SO_4, filtered, concentrated and the resulting crude product was purified by column chromatography (38% EtOAc in hexane) to afford **3-43** (256 mg, 60% over the two steps) as a white solid: $[\alpha]^{22}_D$ +43.7 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.74–7.66 (m, 3 H, ArH), 7.43–7.24 (m, 13 H, ArH), 7.10–7.05 (m, 2 H, ArH), 7.02–6.98 (m, 2 H, ArH), 6.93–6.85 (m, 3 H, ArH), 5.46 (dd, J = 3.5, 0.5 Hz, 1 H, H-4_{Gal}), 5.23 (d, J = 8.0 Hz, 1 H, H-1_{GlcN}), 4.98 (app t, J = 10.0, 10.0 Hz, 1 H, H-2_{Gal}), 4.84, 4.67 (ABq, J = 10.9 Hz, 2 H, 2 × OC$\underline{H_2}$Ph), 4.79 (d, J = 12.0 Hz, 1 H, OC$\underline{H_2}$Ph),

4.72, 4.63 (ABq, J = 12.2 Hz, 2 H, 2 × OC$\underline{H_2}$Ph), 4.46 (d, J = 10.0 Hz, 1 H, H-1$_{Gal}$), 4.43 (d, J = 12.0 Hz, 1 H, OC$\underline{H_2}$Ph), 4.36 (app t, J = 10.5, 8.5 Hz, 1 H, H-3$_{GlcN}$), 4.15 (dd, J = 12.0, 5.0 Hz, 1 H, H-6a$_{Gal}$), 4.13–4.04 (m, 2 H, H-6b$_{Gal}$ and H-2$_{GlcN}$), 3.84 (dd, J = 10.0, 3.5 Hz, 1 H, H-3$_{Gal}$), 3.82–3.78 (m, 2 H, H-6a$_{GlcN}$ and H-6b$_{GlcN}$), 3.76–3.70 (m, 2 H, H-4$_{GlcN}$ and H-5$_{Gal}$), 3.66 (ddd, J = 10.0, 4.5, 2.0 Hz, 1 H, H-5$_{GlcN}$), 2.32 (s, 3 H, C$\underline{H_3}$C$_6$H$_4$S), 2.11 (s, 3 H, 3 × COC$\underline{H_3}$), 2.02 (s, 3 H, 3 × COC$\underline{H_3}$), 1.78 (s, 3 H, 3 × COC$\underline{H_3}$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.3 ($\underline{C}$OCH$_3$), 170.1 ($\underline{C}$OCH$_3$), 169.1 ($\underline{C}$OCH$_3$), 138.4 (Ar), 137.92 (Ar), 137.86 (Ar), 137.8 (Ar), 133.7 (Ar), 132.3 (Ar), 131.6 (Ar), 129.7 (Ar), 129.5 (Ar), 128.42 (Ar), 128.36 (Ar), 127.97 (Ar), 127.96 (Ar), 127.8 (Ar), 127.6 (Ar), 127.51 (Ar), 127.49 (Ar), 127.3 (Ar), 123.1 (Ar), 98.3 (C-1$_{GlcN}$), 87.2 (C-1$_{Gal}$), 79.5 (C-4$_{GlcN}$), 78.8 (C-3$_{GlcN}$), 76.6 (C-3$_{Gal}$), 75.2 (C-5$_{GlcN}$), 75.0 (C-5$_{Gal}$), 74.9 (O$\underline{C}$H$_2$Ph), 74.7 (O$\underline{C}$H$_2$Ph), 73.4 (O$\underline{C}$H$_2$Ph), 69.5 (C-4$_{Gal}$), 69.0 (C-2$_{Gal}$), 68.8 (C-6$_{GlcN}$), 62.6 (C-6$_{Gal}$), 55.8 (C-2$_{GlcN}$), 21.0 ($\underline{C}$H$_3$C$_6$H$_4$S), 20.62 (CO$\underline{C}$H$_3$), 20.57 (CO$\underline{C}$H$_3$), 20.56 (CO$\underline{C}$H$_3$); IR (cast film, $CHCl_3$) v 3063, 3030, 2923, 2868, 1776, 1750, 1714, 1495, 1454, 1429, 1070, 753 cm^{-1}; HRMS (ESI-TOF) m/z: [M + Na]$^+$ Calcd for $C_{54}H_{55}NO_{14}SNa$ 996.3235; Found 996.3243.

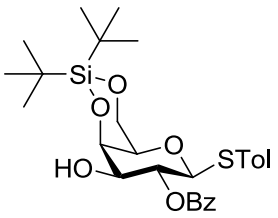


3-44

***p*-Tolyl 3,4,6-tri-*O*-benzyl-2-deoxy-2-*N*-phthalimido-β-D-glucopyranosyl-(1→3)-1-thio-β-D-galactopyranoside (3-44)**

To a stirred solution of disaccharide **3-43** (239 mg, 0.245 mmol) in ethanol (5 mL) was added ethylenediamine (0.80 mL, 12 mmol). The reaction mixture was heated at 90 °C for 48 h, after which time the solution was cooled to room temperature over 20 min. The solution was concentrated and the resulting crude product was dissolved in EtOAc (50 mL) and washed with

water (2 × 5 mL). The organic layers were separated and the aqueous layer was further extracted with EtOAc (2 × 30 mL), dried over anhydrous Na_2SO_4, filtered and concentrated to obtain crude amino alcohol as a pale yellow viscous liquid, which was used directly in the next step. This crude amino alcohol was dissolved in CH_3OH (6 mL). Et_3N (1.2 mL) followed by Ac_2O (0.10 mL, 1.06 mmol) were the solution added and stirred for 3.5 h. The solution was concentrated and the resulting crude product was purified by column chromatography (10% EtOAc in CH_3OH) to afford **3-44** (168 mg, 70% over the two steps) as a colorless viscous liquid: $[\alpha]^{22}_D$ –14.4 (*c* 1.0, CH_3OH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.45–7.41 (m, 2 H, ArH), 7.35–7.26 (m, 13 H, ArH), 7.22–7.18 (m, 2 H, ArH), 7.10–7.06 (m, 2 H, ArH), 5.71 (d, J = 7.5 Hz, 1 H, $N\underline{H}COCH_3$), 4.93 (d, J = 8.0 Hz, 1 H, H-1_{GlcN}), 4.82, 4.65 (ABq, J = 11.7 Hz, 2 H, 2 × OC$\underline{H_2}$Ph), 4.78, 4.54 (ABq, J = 10.9 Hz, 2 H, 2 × OC$\underline{H_2}$Ph), 4.53, 4.48 (ABq, J = 11.9 Hz, 2 H, 2 × OC$\underline{H_2}$Ph), 4.47 (d, J = 9.5 Hz, 1 H, H-1_{Gal}), 4.06 (d, J = 2.5 Hz, 1 H, H-4_{Gal}), 3.91–3.83 (m, 2 H, H-$6a_{Gal}$ and H-3_{GlcN}), 3.76 (app t, J = 9.5, 9.0 Hz, 1 H, H-2_{Gal}), 3.68–3.53 (m, 2 H, H-2_{GlcN}, H-4_{GlcN}, H-5_{GlcN}, H-$6a_{GlcN}$, H-$6b_{GlcN}$, H-3_{Gal} and H-$6b_{Gal}$), 4.36 (app t, J = 5.5, 5.0 Hz, 1 H, H-5_{Gal}), 3.18 (br s, 1 H, CH$O\underline{H}$), 2.31 (s, 3 H, C$\underline{H_3}C_6H_4S$), 1.79 (s, 3 H, 3 × NHCOC$\underline{H_3}$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.5 (NH$\underline{C}\underline{O}CH_3$), 138.2 (Ar), 138.1 (Ar), 137.80 (Ar), 137.79 (Ar), 133.0 (Ar), 129.8 (Ar), 128.6 (Ar), 128.52 (Ar), 128.51 (Ar), 128.3 (Ar), 128.04 (Ar), 127.99 (Ar), 127.88 (Ar), 127.85 (Ar), 101.2 (C-1_{GlcN}), 88.4 (C-1_{Gal}), 83.9 (C-3_{Gal}), 80.8 (C-3_{GlcN}), 78.6 (C-4_{GlcN}), 78.1 (C-5_{Gal}), 74.9 (C-5_{GlcN}), 74.8 (O$\underline{C}H_2$Ph), 73.5 (O$\underline{C}H_2$Ph), 68.9 (C-6_{GlcN}), 68.8 (C-4_{Gal}), 68.3 (C-2_{Gal}), 62.7 (C-6_{Gal}), 56.7 (C-2_{GlcN}), 23.5 (NHCO$\underline{C}H_3$), 21.2 ($\underline{C}H_3C_6H_4S$); IR (cast film, $CHCl_3$) ν 3422, 3279, 3062, 3030, 2922, 2856, 1649, 1551, 1494, 1453, 1071, 696 cm^{-1}; HRMS (ESI-TOF) m/z: $[M + Na]^+$ Calcd for $C_{42}H_{49}NO_{10}SNa$ 782.2969; Found 782.2971.

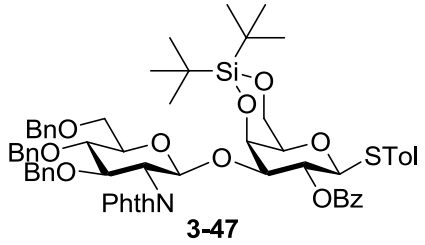


3-46

***p*-Tolyl 2-*O*-benzoyl-4,6-*O*-(di-*tert*-butylsilylene)-1-thio-β-D-galactopyranoside (3-46)**

To a stirred solution of thioglycoside **3-39**[24] (2.00 g, 6.98 mmol) in 2,2-dimethoxypropane (25 mL) was added camphorsulfonic acid (405 mg, 1.74 mmol). The mixutre was stirred for 36 h, the camphorsulfonic acid was quenched by the addition of Et_3N (1 mL) and the solution was concentrated. The resulting crude product was dissolved in pyridine (10 mL) and cooled to 0 °C. BzCl (1.60 mL, 13.8 mmol) was added dropwise over 2 min. The ice bath was removed and the reaction mixture was stirred for 12 h. Toluene (5 mL) was added and the resulting solution was concentrated and co-concentrated with toluene (4 × 5 mL) to obtain a crude product that was dissolved in 90% TFA (10 mL) over 5 min. The reaction mixture was further stirred for 10 min and then the TFA was quenched by the portionwise addition of solid $NaHCO_3$. The reaction mixture was diluted with EtOAc (100 mL) and washed with water (30 mL). The organic layer was separated and the aqueous layer was further extracted with EtOAc (2 × 150 mL). The combined organic phases were washed with brine (30 mL), dried over anhydrous Na_2SO_4, filtered, concentrated and the resulting crude product was dissolved in dry CH_2Cl_2 (25 mL) and cooled to 0 °C. Sym-collidine (2.30 mL, 17.4 mmol) and $DTBS(OTf)_2$ (2.7 mL, 8.3 mmol) were added sequentially at 0 °C. The ice bath was removed and the reaction mixture was stirred for 2 h before being diluted with CH_2Cl_2 (100 mL) and washed with saturated aqueous $NaHCO_3$ solution (25 mL). The organic layer was separated and the aqueous layer was further extracted with CH_2Cl_2 (2 × 100 mL). The combined organic phases were

washed with brine (25 mL), dried over anhydrous Na_2SO_4, filtered, concentrated and the resulting crude product was purified by column chromatography (23% EtOAc in hexane) to afford **3-46** (2.1 g, 57% over the four steps) as a white foam: $[\alpha]^{22}_D$ +24.6 (*c* 0.43, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.08 (d, *J* = 7.0 Hz, 2 H, ArH), 7.58 (app t, *J* = 7.5 Hz, 1 H, ArH), 7.46 (app t, *J* = 7.0 Hz, 2 H, ArH), 7.36 (d, *J* = 8.0 Hz, 2 H, ArH), 7.06 (d, *J* = 8.0 Hz, 2 H, ArH), 5.43 (app t, *J* = 9.5 Hz, 1 H, H-2), 4.74 (d, *J* = 9.5 Hz, 1 H, H-1), 4.50 (d, *J* = 3.5 Hz, 1 H, H-4), 4.29 (m, 2 H, H-6a and H-6b), 3.73 (ddd, *J* = 11.0, 9.5, 3.5 Hz, 1 H, H-3), 3.51 (s, 1 H, H-5), 2.76 (d, *J* = 11.0 Hz, 1 H, CH*OH*), 2.31 (s, 3 H, C*H*$_3$C$_6$H$_4$S), 1.13 (s, 9 H, SiC(C*H*$_3$)$_3$), 1.07 (s, 9 H, SiC(C*H*$_3$)$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.2 (*C*OPh), 138.0 (Ar), 133.18 (Ar), 133.16 (Ar), 130.0 (Ar), 129.9 (Ar), 129.7 (Ar), 128.4 (Ar), 87.5 (C-1), 75.2 (C-5), 73.9 (C-3), 73.1 (C-4), 71.7 (C-2), 67.1 (C-6), 27.6 (SiC(*C*H$_3$)$_3$), 27.5 (SiC(*C*H$_3$)$_3$), 23.4 (Si*C*(CH$_3$)$_3$), 21.2 (*C*H$_3$C$_6$H$_4$S), 20.8 (Si*C*(CH$_3$)$_3$); IR (cast film, $CHCl_3$) *v* 3557, 3021, 2933, 1724, 1492, 1474, 1451, 1084, 710 cm^{-1}; HRMS (ESI-TOF) m/z: $[M + Na]^+$ Calcd for $C_{28}H_{38}O_6SSiNa$ 553.2051; Found 553.2050.



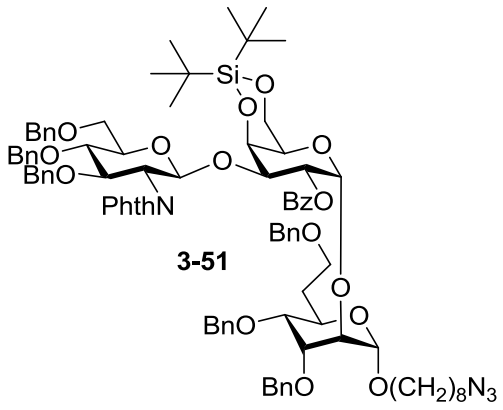
3-47

***p*-Tolyl 3,4,6-tri-*O*-benzyl-2-deoxy-2-*N*-phthalimido-β-D-glucopyranosyl-(1→3)-2-*O*-benzoyl-4,6-*O*-(di-*tert*-butylsilylene)-1-thio-β-D-galactopyranoside (3-47)**

A solution of thioglycoside **3-35**[22] (1.159 g, 1.690 mmol) in acetone (13.5 mL) and water (1.5 mL) was cooled to 0 °C. *N*-bromosuccinimide (905 mg, 5.08 mmol) was added, the ice bath

was removed and the reaction mixture was stirred for 1.5 h. The solution was diluted with CH_2Cl_2 (75 mL) and washed with saturated aqueous $Na_2S_2O_3$ solution (30 mL). The organic layer was separated and the aqueous layer was further extracted with CH_2Cl_2 (2 × 100 mL). The combined organic phases were washed with brine (25 mL), dried over anhydrous Na_2SO_4, filtered, concentrated and the resulting crude product was purified by column chromatography (23% EtOAc/hexane) to afford the known hemiacetal[29] (847 mg, 86%) as a colorless viscous liquid. A solution of the hemiacetal (178 mg, 0.307 mmol) in dry CH_2Cl_2 (3 mL) was cooled to 0 °C. CCl_3CN (0.45 mL, 4.49 mmol) and Cs_2CO_3 (205 mg, 0.629 mmol) were added sequentially at 0 °C and the solution was stirred for 1.5 h. The reaction mixture was diluted with CH_2Cl_2 (75 mL) and filtered over a pad of Celite-545. The filtrate was concentrated to obtain the known imidate **3-49**[30] as white foam, which was used directly in the next step. A solution of **3-49** (222 mg, 0.307 mmol) and thioglycoside **3-46** (85 mg, 0.16 mmol) in dry CH_2Cl_2 (5 mL) was stirred with 4 Å molecular sieves (0.25 g) for 30 min. The reaction mixture was cooled to 0 °C and TMSOTf (6.0 μL, 0.033 mmol) was added at 0 °C and stirred for 1.5 h. The TMSOTf was quenched by the addition of Et_3N (0. 1 mL) and the reaction mixture was diluted with CH_2Cl_2 (75 mL) and filtered over a pad of Celite-545. The filtrate was washed with saturated aqueous $NaHCO_3$ solution (25 mL) and the organic layer was separated and the aqueous layer was further extracted with CH_2Cl_2 (2 × 100 mL). The combined organic phases were washed with brine (25 mL), dried over anhydrous Na_2SO_4, filtered, concentrated and the resulting crude product was purified by column chromatography (25% EtOAc in hexane) to afford **3-47** (149 mg, 85% over the two steps) as a white foam: $[\alpha]^{22}_D$ +61.5 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.61 (d, J = 7.0 Hz, 2 H, ArH), 7.49 (app t, J = 7.5 Hz, 1 H, ArH), 7.36–7.17 (m, 18 H, ArH), 6.95 (d, J = 8.0 Hz, 2 H, ArH), 6.91–6.82 (m, 5 H, ArH), 5.40 (app t, J = 9.5 Hz, 1 H, H-2_{Gal}), 5.36 (d, J

= 7.5 Hz, 1 H, H-1_{GlcN}), 4.80 (d, *J* = 11.0 Hz, 1 H, OC*H*$_2$Ph), 4.79–4.78 (m, 1 H, H-4_{Gal}), 4.69, 4.57 (ABq, *J* = 11.4 Hz, 2 H, 2 × OC*H*$_2$Ph), 4.59 (d, *J* = 10.0 Hz, 1 H, H-1_{Gal}), 4.55, 4.48 (ABq, *J* = 11.7 Hz, 2 H, 2 × OC*H*$_2$Ph), 4.32 (d, *J* = 11.0 Hz, 1 H, OC*H*$_2$Ph), 4.25 (app t, *J* = 7.5, 7.5 Hz, 1 H, H-2_{GlcN}), 4.28–4.21 (m, 1 H, H-5_{GlcN}), 4.17 (dd, *J* = 12.5, 1.5 Hz, 1 H, H-$6a_{Gal}$), 4.07 (dd, *J* = 12.5, 2.5 Hz, 1 H, H-$6b_{Gal}$), 3.79–3.77 (m, 2 H, H-$6a_{GlcN}$ and H-3_{Gal}), 3.71–3.61 (m, 3 H, H-$6b_{GlcN}$, H-4_{GlcN} and H-3_{GlcN}), 3.33 (s, 1 H, H-5_{Gal}), 2.25 (s, 3 H, C*H*$_3$C$_6$H$_4$S), 1.07 (s, 9 H, SiC(C*H*$_3$)$_3$), 1.06 (s, 9 H, SiC(C*H*$_3$)$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 167.6 (*C*OPhth), 167.3 (*C*OPhth), 164.8 (*C*OPh), 138.1 (Ar), 137.84 (Ar), 137.77 (Ar), 137.7 (Ar), 133.2 (Ar), 132.8 (Ar), 132.6 (Ar), 130.6 (Ar), 129.7 (Ar), 129.5 (Ar), 128.5 (Ar), 128.4 (Ar), 128.1 (Ar), 127.99 (Ar), 127.91 (Ar), 127.7 (Ar), 127.6 (Ar), 127.5 (Ar), 127.3 (Ar), 122.9 (Ar), 99.6 (C-1_{GlcN}), 88.3 (C-1_{Gal}), 81.0 (C-3_{Gal}), 79.9 (C-3_{GlcN}), 79.3 (C-5_{GlcN}), 75.2 (C-5_{Gal}), 75.0 (O*C*H$_2$Ph), 74.8 (O*C*H$_2$Ph), 74.7 (C-4_{GlcN}), 73.2 (O*C*H$_2$Ph), 72.8 (C-4_{Gal}), 69.7 (C-2_{Gal}), 69.6 (C-6_{GlcN}), 67.2 (C-6_{Gal}), 55.9 (C-2_{GlcN}), 27.6 (SiC(*C*H$_3$)$_3$), 27.5 (SiC(*C*H$_3$)$_3$), 23.3 (Si*C*(CH$_3$)$_3$), 21.1 (*C*H$_3$C$_6$H$_4$S), 20.6 (Si*C*(CH$_3$)$_3$); IR (cast film, $CHCl_3$) *v* 3030, 2932, 1775, 1713, 1494, 1473, 1453, 1087, 828 cm^{-1}; HRMS (ESI-TOF) m/z: [M + Na]$^+$ Calcd for $C_{63}H_{69}NO_{12}SSiNa$ 1114.4202; Found 1114.4208.

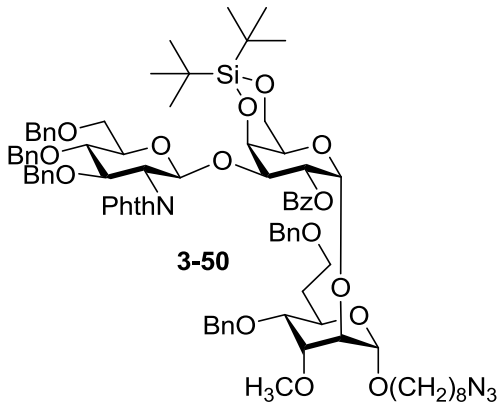


3-51

8-Azidooctyl 3,4,6-tri-*O*-benzyl-2-deoxy-2-*N*-phthalimido-β-D-glucopyranosyl-(1→3)-2-*O*-benzoyl-4,6-*O*-(di-*tert*-butylsilylene)-α-D-galactopyranosyl-(1→2)-3,4,7-tri-*O*-benzyl-6-deoxy-α-D-*altro*-heptopyranoside (3-51)

A solution of thioglycoside **3-47** (164 mg, 0.150 mmol) and azidooctyl glycoside **3-34** (72.9 mg, 0.118 mmol) in dry CH_2Cl_2 (4 mL) was stirred with 4 Å molecular sieves (0.3 g) for 30 min. The reaction mixture was cooled to 0 °C before NIS (50 mg, 0.27 mmol) and TfOH (3.0 μL, 0.034 mmol) were added sequentially. The solution was stirred at the same temperature for 1.5 h before the TfOH was quenched by the addition of Et_3N (0.1 mL). The reaction mixture was diluted with CH_2Cl_2 (75 mL) and filtered over a pad of Celite-545. The filtrate was washed with saturated aqueous $Na_2S_2O_3$ solution (25 mL) and the organic layer was separated and the aqueous layer was further extracted with CH_2Cl_2 (2 × 100 mL). The combined organic phases were washed with brine (25 mL), dried over anhydrous Na_2SO_4, filtered, concentrated and the resulting crude product was purified by column chromatography (23% EtOAc in hexane) to afford **3-51** (165 mg, 88%) as a colorless viscous liquid: $[\alpha]^{22}_D$ +116.3 (*c* 0.29, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.72 (d, J = 7.0 Hz, 2 H, ArH), 7.45–7.15 (m, 30 H, ArH), 6.98–6.80 (m, 7 H, ArH), 5.46 (d, J = 8.5 Hz, 1 H, H-1_{GlcN}), 5.18 (dd, J = 10.5, 4.0 Hz, 1 H, H-2_{Gal}), 5.08 (d, J = 4.0 Hz, 1 H, H-1_{Gal}), 4.82, 4.61 (ABq, J = 10.8 Hz, 2 H, 2 × OC$\underline{H_2}$Ph), 4.80 (d, J = 2.9 Hz, 1 H,

H-4$_{Gal}$), 4.74 (d, J = 11.5 Hz, 1 H, OC$\underline{H_2}$Ph), 4.59, 4.50 (ABq, J = 12.2 Hz, 2 H, 2 × OC$\underline{H_2}$Ph), 4.57 (s, 1 H, H-1$_{AltHep}$), 4.49 (s, 2 H, 2 × OC$\underline{H_2}$Ph), 4.43 (d, J = 12.0 Hz, 1 H, OC$\underline{H_2}$Ph), 4.37 (d, J = 11.5 Hz, 1 H, OC$\underline{H_2}$Ph), 4.38–4.33 (m, 2 H, H-3$_{GlcN}$ and OC$\underline{H_2}$Ph), 4.22 (dd, J = 10.8, 8.5 Hz, 1 H, H-2$_{GlcN}$), 4.13 (app td, J = 10.0, 3.0 Hz, 1 H, H-5$_{AltHep}$), 4.10–4.03 (m, 3 H, H-3$_{Gal}$, H-6a$_{Gal}$ and H-6b$_{Gal}$), 3.84–3.66 (m, 6 H, H-2$_{AltHep}$, H-5$_{GlcN}$, H-4$_{GlcN}$, H-6a$_{GlcN}$, H-6b$_{GlcN}$ and OC$\underline{H_2}$Ph), 3.65–3.56 (m, 4 H, H-5$_{Gal}$, H-7a$_{AltHep}$, H-7b$_{AltHep}$ and OC$\underline{H_2}$CH$_2$), 3.47 (d, J = 11.0 Hz, 1 H, OC$\underline{H_2}$Ph), 3.48–3.46 (m, 1 H, H-3$_{AltHep}$), 3.27 (dt, J = 9.5, 6.5 Hz, 1 H, OC$\underline{H_2}$CH$_2$), 3.21 (t, J = 7.0 Hz, 2 H, 2 × C$\underline{H_2}$N$_3$), 3.17 (dd, J = 9.5, 3.0 Hz, 1 H, H-4$_{AltHep}$), 2.22–2.16 (m, 1 H, H-6a$_{AltHep}$), 1.71–1.62 (m, 1 H, H-6b$_{AltHep}$), 1.58–1.49 (m, 4 H, 4 × C$\underline{H_2}$), 1.33–1.17 (m, 8 H, 8 × C$\underline{H_2}$), 1.06 (s, 9 H, SiC(C$\underline{H_3}$)$_3$), 1.04 (s, 9 H, SiC(C$\underline{H_3}$)$_3$); ^{13}C NMR (125 MHz, CDCl$_3$) δ 167.8 ($\underline{C}$OPhth), 167.5 ($\underline{C}$OPhth), 165.2 ($\underline{C}$OPh), 138.6 (Ar), 138.3 (Ar), 138.1 (Ar), 137.9 (Ar), 133.2 (Ar), 133.1 (Ar), 129.8 (Ar), 129.3 (Ar), 128.5 (Ar), 128.3 (Ar), 128.0 (Ar), 127.9 (Ar), 127.6 (Ar), 127.5 (Ar), 127.4 (Ar), 127.3 (Ar), 122.8 (Ar), 99.5 (C-1$_{GlcN}$), 98.9 (C-1$_{AltHep}$), 95.8 (C-1$_{Gal}$), 80.0 (C-4$_{GlcN}$), 79.5 (C-3$_{GlcN}$), 76.6 (C-4$_{AltHep}$), 75.9 (C-3$_{Gal}$), 75.1 (O$\underline{C}$H$_2$Ph), 74.9 (O$\underline{C}$H$_2$Ph), 74.7 (C-5$_{GlcN}$), 73.27 (O$\underline{C}$H$_2$Ph), 73.25 (C-4$_{Gal}$), 72.9 (O$\underline{C}$H$_2$Ph), 72.6 (C-2$_{AltHep}$), 71.8 (O$\underline{C}$H$_2$Ph), 71.5 (C-3$_{AltHep}$), 70.2 (O$\underline{C}$H$_2$Ph), 69.7 (C-2$_{Gal}$), 69.6 (C-6$_{GlcN}$), 67.8 (C-5$_{Gal}$), 67.5 (O$\underline{C}$H$_2$CH2), 67.3 (C-7$_{AltHep}$), 66.9 (C-6$_{Gal}$), 64.4 (C-5$_{AltHep}$), 56.0 (C-2$_{GlcN}$), 51.4 ($\underline{C}$H$_2$N$_3$), 31.8 (C-6$_{AltHep}$), 29.5 ($\underline{C}$H$_2$), 29.4 ($\underline{C}$H$_2$), 29.1 ($\underline{C}$H$_2$), 28.8 ($\underline{C}$H$_2$), 27.6 (SiC($\underline{C}$H$_3$)$_3$), 27.2 (SiC($\underline{C}$H$_3$)$_3$), 26.7 ($\underline{C}$H$_2$), 26.2 ($\underline{C}$H$_2$), 23.4 (Si$\underline{C}$(CH$_3$)$_3$), 20.6 (Si$\underline{C}$(CH$_3$)$_3$); IR (cast film, CHCl$_3$) ν 3031, 2932, 2095, 1777, 1717, 1496, 1470, 1454, 1110, 795 cm^{-1}; HRMS (ESI-TOF) m/z: [M + Na]$^+$ Calcd for C$_{92}$H$_{108}$N$_4$O$_{18}$SiNa 1607.7320; Found 1607.7358.

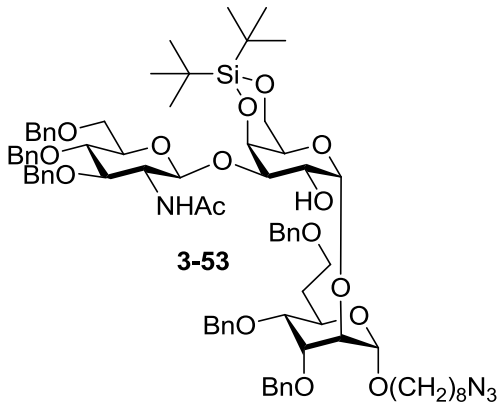


3-50

8-Azidooctyl 3,4,6-tri-*O*-benzyl-2-deoxy-2-*N*-phthalimido-β-D-glucopyranosyl-(1→3)-2-*O*-benzoyl-4,6-*O*-(di-*tert*-butylsilylene)-α-D-galactopyranosyl-(1→2)-4,7-di-*O*-benzyl-6-deoxy-3-*O*-methyl-α-D-*altro*-heptopyranoside (3-50)

A solution of thioglycoside **3-47** (367 mg, 0.336 mmol) and azidooctyl glycoside **3-33** (151.4 mg, 0.2795 mmol) in dry CH_2Cl_2 (10 mL) was stirred with 4 Å molecular sieves (0.9 g) for 30 min. The reaction mixture was cooled to 0 °C and NIS (113 mg, 0.502 mmol) and then TfOH (6.0 μL, 0.068 mmol) were added and the solution was stirred at 0 °C for 1.5 h. The TfOH was quenched by the addition of Et_3N (0.1 mL) and the reaction mixture was diluted with CH_2Cl_2 (100 mL) and filtered over a pad of Celite-545. The filtrate was washed with saturated aqueous $Na_2S_2O_3$ solution (25 mL) and the organic layer was separated and the aqueous layer was further extracted with CH_2Cl_2 (2 × 100 mL). The combined organic phases were washed with brine (25 mL), dried over anhydrous Na_2SO_4, filtered, concentrated and the resulting crude product was purified by column chromatography (23% EtOAc in hexane) to afford **3-50** (388 mg, 92%) as a colorless viscous liquid: $[\alpha]^{22}_D$ +89.8 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.76 (d, J = 7.5 Hz, 2 H, ArH), 7.44 (app t, J = 7.5 Hz, 1 H, ArH), 7.43–7.22 (m, 24 H, ArH), 7.03–6.79 (m, 7 H, ArH), 5.47 (d, J = 8.5 Hz, 1 H, H-1_{GlcN}), 5.22–5.20 (m, 2 H, H-1_{Gal} and H-2_{Gal}), 4.81, 4.60 (ABq, J = 11.1 Hz, 2 H, 2 × OC$\underline{H_2}$Ph), 4.80 (d, J = 4.0 Hz, 1 H, H-4_{Gal}), 4.73 (d,

J = 12.0 Hz, 1 H, OC$\underline{H}_2$Ph), 4.58, 4.50 (ABq, J = 11.8 Hz, 2 H, 2 × OC$\underline{H}_2$Ph), 4.55 (s, 1 H, H-1_{AltHep}), 4.48 (s, 2 H, 2 × OC$\underline{H}_2$Ph), 4.36 (d, J = 12.0 Hz, 1 H, OC$\underline{H}_2$Ph), 4.34 (dd, J = 11.0, 8.5 Hz, 1 H, H-3_{GluN}), 4.22 (dd, J = 11.0, 8.5 Hz, 1 H, H-2_{GlcN}), 4.09–4.03 (m, 4 H, H-3_{Gal}, H-5_{AltHep}, H-$6a_{Gal}$ and H-$6b_{Gal}$), 3.92 (d, J = 11.5, 1 H, OC$\underline{H}_2$Ph), 3.84–3.76 (m, 3 H, H-5_{GlcN}, H-2_{AltHep} and H-$6a_{GlcN}$), 3.73–3.65 (m, 3 H, H-4_{GluN}, H-5_{Gal} and H-$6b_{GlcN}$), 3.64–3.55 (m, 4 H, H-$7a_{AltHep}$, H-$7b_{AltHep}$, OC$\underline{H}_2$CH$_2$ and OC$\underline{H}_2$Ph), 3.29–3.19 (m, 5 H, H-3_{AltHep}, H-4_{AltHep}, OC$\underline{H}_2$CH$_2$ and 2 × C$\underline{H}_2$N$_3$), 3.10 (s, 3 H, OC$\underline{H}_3$), 2.16–2.09 (m, 1 H, H-$6a_{AltHep}$), 1.70–1.63 (m, 1 H, H-$6b_{AltHep}$), 1.59–1.19 (m, 12 H, (C$\underline{H}_2$)$_6$), 1.05 (s, 9 H, SiC(C$\underline{H}_3$)$_3$), 1.02 (s, 9 H, SiC(C$\underline{H}_3$)$_3$); ^{13}C NMR (125 MHz, CDCl$_3$) δ 167.8 ($\underline{C}$OPhth), 167.5 ($\underline{C}$OPhth), 165.4 ($\underline{C}$OPh), 138.5 (Ar), 138.3 (Ar), 138.0 (Ar), 137.9 (Ar), 133.1 (Ar), 129.8 (Ar), 129.4 (Ar), 128.5 (Ar), 128.4 (Ar), 128.3 (Ar), 128.0 (Ar), 127.9 (Ar), 127.6 (Ar), 127.5 (Ar), 127.3 (Ar), 99.6 (C-1_{GlcN}), 98.8 (C-1_{AltHep}), 95.9 (C-1_{Gal}), 80.0 (C-4_{GlcN}), 79.5 (C-3_{GlcN}), 76.4 (C-4_{AltHep}), 75.9 (C-3_{Gal}), 75.09 (O$\underline{C}$H$_2$Ph), 75.07 (C-3_{Gal}), 74.9 (O$\underline{C}$H$_2$Ph), 74.7 (C-5_{GlcN}), 73.30 (O$\underline{C}$H$_2$Ph), 73.29 (C-4_{Gal}), 73.0 (O$\underline{C}$H$_2$Ph), 72.6 (C-2_{AltHep}), 70.5 (O$\underline{C}$H$_2$Ph), 69.9 (C-2_{Gal}), 69.7 (C-6_{GlcN}), 67.8 (C-5_{Gal}), 67.6 (O$\underline{C}$H$_2$CH$_2$), 67.3 (C-7_{AltHep}), 66.9 (C-6_{Gal}), 65.0 (C-5_{AltHep}), 57.9 (O$\underline{C}$H$_3$), 56.1 (C-2_{GlcN}), 51.5 ($\underline{C}$H$_2$N$_3$), 31.8 (C-6_{AltHep}), 29.4 ($\underline{C}$H$_2$), 29.3 ($\underline{C}$H$_2$), 29.1 ($\underline{C}$H$_2$), 28.8 ($\underline{C}$H$_2$), 27.7 (SiC($\underline{C}$H$_3$)$_3$), 27.2 (SiC($\underline{C}$H$_3$)$_3$), 26.7 ($\underline{C}$H$_2$), 26.1 ($\underline{C}$H$_2$), 23.4 (Si$\underline{C}$(CH$_3$)$_3$), 20.6 (Si$\underline{C}$(CH$_3$)$_3$); IR (cast film, CHCl$_3$) ν 3031, 2932, 2095, 1777, 1717, 1496, 1469, 1453, 1109, 826 cm^{-1}; HRMS (ESI-TOF) m/z: [M + Na]$^+$ Calcd for $C_{86}H_{104}N_4O_{18}SiNa$ 1531.7007; Found 1531.7030.

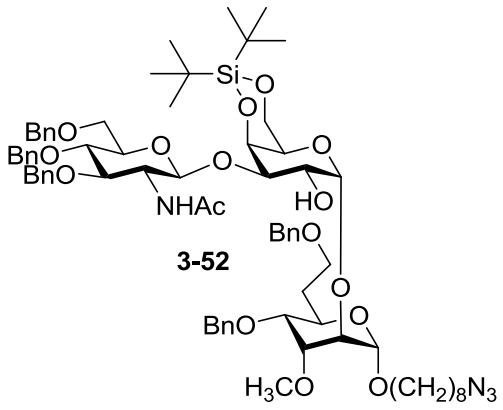


3-53

8-Azidooctyl 2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranosyl-(1→3)-4,6-*O*-(di-*tert*-butylsilylene)-α-D-galactopyranosyl-(1→2)-3,4,7-tri-*O*-benzyl-6-deoxy-α-D-*altro*-heptopyranoside (3-53)

To a stirred solution of trisaccharide **3-51** (163 mg, 0.103 mmol) in ethanol (2 mL) was added ethylenediamine (0.40 mL, 5.98 mmol). The reaction mixture was stirred at 90 °C for 48 hbefore being cooled to room temperature over 20 min. The solution was concentrated and the resulting crude product was dissolved in EtOAc (50 mL) and washed with water (2 × 5 mL). The organic layers were separated and the aqueous layer was further extracted with EtOAc (2 × 50 mL), dried over anhydrous Na_2SO_4, filtered and concentrated to obtain crude amino alcohol as a pale yellow viscous liquid, which was used directly in the next step. This crude amino alcohol was dissolved in CH_3OH (4 mL) and CH_2Cl_2 (0.4 mL). Et_3N (0.8 mL) and then Ac_2O (0.10 mL, 1.06 mmol) were added and stirred for 3.5 h. The solution was concentrated and the resulting crude product was purified by column chromatography (45% EtOAc in hexane) to afford **3-53** (100 mg, 70%) as a colorless viscous liquid: $[\alpha]^{22}_D$ +85.3 (*c* 0.12, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.35–7.17 (m, 30 H, ArH), 5.66 (d, J = 7.5 Hz, 1 H, $N\underline{H}COCH_3$), 5.01 (d, J = 8.0 Hz, 1 H, H-1_{GlcN}), 4.95 (d, J = 4.0 Hz, 1 H, H-1_{Gal}), 4.83, 4.69 (ABq, J = 11.6 Hz, 2 H, 2 × OC$\underline{H}_2$Ph), 4.79 (d, J = 11.0 Hz, 1 H, OC$\underline{H}_2$Ph), 4.62 (d, J = 12.0 Hz, 1 H, OC$\underline{H}_2$Ph), 4.59–4.54 (m, 6 H, H-

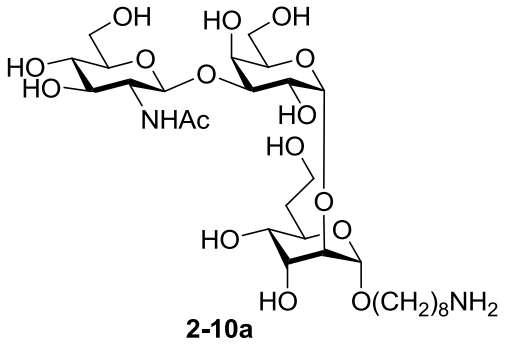
4_{Gal}, H-1_{AltHep} and 4 × OC$\underline{H_2}$Ph), 4.49–4.46 (m, 4 H, 4 × OC$\underline{H_2}$Ph), 4.35–4.21 (m, 1 H, H-5_{AltHep}), 4.16–4.06 (m, 2 H, H-$6a_{Gal}$ and H-$6b_{Gal}$), 4.04 (dd, J = 10.0, 4.0 Hz, 1 H, H-2_{Gal}), 4.00 (dd, J = 5.0, 2.5 Hz, 1 H, H-2_{AltHep}), 3.93 (dd, J = 9.5, 8.5 Hz, 1 H, H-3_{GlcN}), 3.81 (dd, J = 4.5, 3.5 Hz, 1 H, H-3_{AltHep}), 3.74–3.56 (m, 11 H, H-5_{Gal}, H-3_{Gal}, H-$6a_{GlcN}$, H-$6b_{GlcN}$, H-4_{GlcN}, H-5_{GlcN}, H-2_{GlcN}, H-4_{AltHep}, H-$7a_{AltHep}$, H-$7b_{AltHep}$ and OC$\underline{H_2}$$CH_2$), 3.28 (dt, J = 8.5, 6.0 Hz, 1 H, OC$\underline{H_2}$$CH_2$), 3.23 (t, J = 7.0 Hz, 2 H, 2 × C$\underline{H_2}$$N_3$), 2.15–2.08 (m, 2 H, H-$6a_{AltHep}$ and CH$O\underline{H}$), 1.84 (s, 3 H, 3 × NHCOC$\underline{H_3}$), 1.76–1.67 (m, 1 H, H-$6b_{AltHep}$), 1.60–1.45 (m, 4 H, 4 × C$\underline{H_2}$), 1.35–1.25 (m, 8 H, 8 × C$\underline{H_2}$), 1.03 (s, 18 H, Si(C(C$\underline{H_3}$)$_3$)$_2$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.1 (NH$\underline{CO}$$CH_3$), 138.51 (Ar), 138.45 (Ar), 138.2 (Ar), 138.1 (Ar), 138.0 (Ar), 130.9 (Ar), 128.9 (Ar), 128.5 (Ar), 128.3 (Ar), 128.0 (Ar), 127.98 (Ar), 127.89 (Ar), 127.8 (Ar), 127.6 (Ar), 127.5 (Ar), 101.5 (C-1_{GluN}), 99.5 (C-1_{Gal}), 99.3 (C-1_{AltHep}), 81.3 (C-3_{GlcN}), 79.8 (C-3_{Gal}), 78.7, 75.7, 74.9, 74.8 (O$\underline{C}$$H_2$Ph), 74.6 (O$\underline{C}$$H_2$Ph), 74.3 (C-$2_{AltHep}$), 73.5 (C-$3_{AltHep}$), 73.4 (O$\underline{C}$$H_2$Ph), 72.9 (O$\underline{C}$$H_2$Ph), 71.7 (O$\underline{C}$$H_2$Ph), 71.3 (O$\underline{C}$$H_2$Ph), 69.5 (C-$6_{GlcN}$), 68.2 (C-$5_{Gal}$), 67.9, 67.2 (C-$2_{Gal}$), 67.13, 67.08, 66.4 (C-5_{AltHep}), 57.1 (C-2_{GlcN}), 51.5 ($\underline{C}$$H_2$$N_3$), 31.8 (C-$6_{AltHep}$), 29.6 ($\underline{C}$$H_2$), 29.4 ($\underline{C}$$H_2$), 29.1 ($\underline{C}$$H_2$), 28.8 ($\underline{C}$$H_2$), 27.7 (SiC($\underline{C}$$H_3$)$_3$), 27.4 (SiC($\underline{C}$$H_3$)$_3$), 26.7 ($\underline{C}$$H_2$), 26.2 ($\underline{C}$$H_2$), 23.6 (NHCO$\underline{C}$$H_3$), 23.4 (Si$\underline{C}$($CH_3$)$_3$), 20.7 (Si$\underline{C}$($CH_3$)$_3$); IR (cast film, $CHCl_3$) ν 3297, 3030, 2931, 2095, 1662, 1545, 1473, 1088, 738 cm^{-1}; HRMS (ESI-TOF) m/z: $[M + Na]^+$ Calcd for $C_{79}H_{104}N_4O_{16}SiNa$ 1415.7109; Found 1415.7092.



8-Azidooctyl 2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranosyl-(1→3)-4,6-*O*-(di-*tert*-butylsilylene)-α-D-galactopyranosyl-(1→2)-4,7-di-*O*-benzyl-6-deoxy-3-*O*-methyl-α-D-*altro*-heptopyranoside (3-52)

To a stirred solution of trisaccharide **3-50** (309 mg, 0.205 mmol) in ethanol (3 mL) was added ethylenediamine (0.80 mL, 12 mmol). The reaction mixture was stirred at 90 °C for 48 h before being cooled to room temperature over 20 min. The solution was concentrated and the resulting crude product was dissolved in EtOAc (50 mL) and washed with water (2 × 5 mL). The organic layers were separated and the aqueous layer was further extracted with EtOAc (2 × 50 mL), dried over anhydrous Na_2SO_4, filtered and concentrated to obtain crude amino alcohol as a pale yellow viscous liquid, which was used directly in the next step. This crude amino alcohol was dissolved in CH_3OH (4 mL) and CH_2Cl_2 (0.4 mL). Et_3N (0.8 mL) and then Ac_2O (0.10 mL, 1.06 mmol) were added and stirrred for 3.5 h. The solution was concentrated and the resulting crude product was purified by column chromatography (60% EtOAc in hexane) to afford **3-52** (191 mg, 71%) as a white foam: $[\alpha]^{22}_D$ +74.5 (*c* 0.28, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.34–7.19 (m, 25 H, ArH), 5.74 (d, J = 7.5 Hz, 1 H, $N\underline{H}COCH_3$), 5.08 (d, J = 8.0 Hz, 1 H, H-1_{GluN}), 5.04 (d, J = 4.0 Hz, 1 H, H-1_{Gal}), 4.83, 4.69 (ABq, J = 11.6 Hz, 2 H, 2 × OC$\underline{H}_2$Ph), 4.79 (d, J = 11.0 Hz, 1 H, OC$\underline{H}_2$Ph), 4.64 (d, J = 12.0 Hz, 1 H, OC$\underline{H}_2$Ph), 4.60–4.53 (m, 5 H, H-4_{Gal},

H-1_{AltHep} and 3 × OC$\underline{H_2}$Ph), 4.48 (d, J = 11.5 Hz, 1 H, OC$\underline{H_2}$Ph), 4.47 (s, 2 H, 2 × OC$\underline{H_2}$Ph), 4.19–4.04 (m, 4 H, H-2_{Gal}, H-5_{AltHep}, H-$6a_{Gal}$ and H-$6b_{Gal}$), 3.99 (dd, J = 9.5, 8.5 Hz, 1 H, H-3_{GlcN}), 3.94 (dd, J = 4.5, 2.5 Hz, 1 H, H-2_{AltHep}), 3.75–3.65 (m, 4 H, H-5_{Gal}, H-3_{Gal}, H-$6a_{GlcN}$ and H-$6b_{GlcN}$), 3.64–3.52 (m, 8 H, H-4_{GlcN}, H-5_{GlcN}, H-2_{GlcN}, H-3_{AltHep}, H-4_{AltHep}, H-$7a_{AltHep}$, H-$7b_{AltHep}$ and OC$\underline{H_2}$$CH_2$), 3.39 (s, 3 H, OC$\underline{H_3}$), 3.30–3.25 (m, 1 H, OC$\underline{H_2}$$CH_2$), 3.24 (t, J = 7.0 Hz, 2 H, 2 × C$\underline{H_2}$$N_3$), 2.16 (br s, 1 H, CHO$\underline{H}$), 2.13–2.05 (m, 1 H, H-$6a_{AltHep}$), 1.86 (s, 3 H, 3 × NHCOC$\underline{H_3}$), 1.76–1.66 (m, 1 H, H-$6b_{AltHep}$), 1.60–1.44 (m, 4 H, 4 × C$\underline{H_2}$), 1.36–1.22 (m, 8 H, 8 × C$\underline{H_2}$), 1.03 (s, 9 H, SiC(C$\underline{H_3}$)$_3$), 1.02 (s, 9 H, SiC(C$\underline{H_3}$)$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.2 (NH$\underline{CO}$$CH_3$), 138.5 (Ar), 138.2 (Ar), 138.1 (Ar), 138.0 (Ar), 128.5 (Ar), 128.3 (Ar), 128.2 (Ar), 128.1 (Ar), 127.99 (Ar), 127.86 (Ar), 127.8 (Ar), 127.70 (Ar), 127.65 (Ar), 127.6 (Ar), 127.5 (Ar), 101.4 (C-1_{GlcN}), 100.0 (C-1_{Gal}), 99.1 (C-1_{AltHep}), 81.2 (C-3_{GlcN}), 79.9 (C-3_{Gal}), 78.8, 75.4, 74.9, 74.8 (O$\underline{C}$$H_2$Ph), 74.7 (C-$2_{AltHep}$), 74.6 (O$\underline{C}$$H_2$Ph), 73.6 (C-$4_{Gal}$), 73.4 (O$\underline{C}$$H_2$Ph), 72.9 (O$\underline{C}$$H_2$Ph), 71.5 (O$\underline{C}$$H_2$Ph), 69.5 (C-$6_{GlcN}$), 68.3 (C-$5_{Gal}$), 67.9 (O$\underline{C}$$H_2$CH2), 67.3 (C-$2_{Gal}$), 67.1 (2C, C-$6_{Gal}$ and C-7_{AltHep}), 66.7 (C-5_{AltHep}), 57.9 (O$\underline{C}$$H_3$), 57.3 (C-$2_{GlcN}$), 51.5 ($\underline{C}$$H_2$$N_3$), 31.8 (C-$6_{AltHep}$), 29.5 ($\underline{C}$$H_2$), 29.4 ($\underline{C}$$H_2$), 29.1 ($\underline{C}$$H_2$), 28.9 ($\underline{C}$$H_2$), 27.7 (SiC($\underline{C}$$H_3$)$_3$), 27.4 (SiC($\underline{C}$$H_3$)$_3$), 26.7 ($\underline{C}$$H_2$), 26.1 ($\underline{C}$$H_2$), 23.7 (NHCO$\underline{C}$$H_3$), 23.4 (Si$\underline{C}$($CH_3$)$_3$), 20.7 (Si$\underline{C}$($CH_3$)$_3$); IR (cast film, $CHCl_3$) ν 3030, 2932, 2095, 1664, 1549, 1454, 1088, 796 cm^{-1}; HRMS (ESI-TOF) m/z: [M + Na]$^+$ Calcd for $C_{73}H_{100}N_4O_{16}SiNa$ 1339.6796; Found 1339.6800.

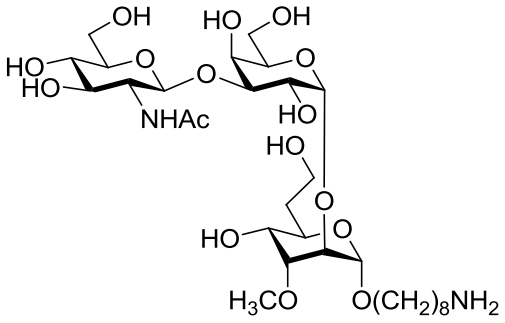


2-10a

8-Aminooctyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-α-D-galactopyranosyl-(1→2)-6-deoxy-α-D-*altro*-heptopyranoside (2-10a)

To a stirred solution of trisaccharide **3-53** (100 mg, 0.0717 mmol) in THF (2 mL) and pyridine (0.5 mL) was added 70% HF·pyridine (50 μL, 1.9 mmol) and the reaction mixture was stirred for 6 h. Excess HF was quenched by the addition of saturated aqueous $NaHCO_3$ solution (20 mL) and the reaction mixture was extracted with EtOAc (75 mL). The aqueous layer was separated and further extracted with EtOAc (2 × 75 mL). The combined organic phases were washed with brine (10 mL), dried over anhydrous Na_2SO_4, filtered, concentrated and the resulting crude product was quickly purified by column chromatography (5% CH_3OH in EtOAc) to afford the corresponding triol (82 mg, 91%) as a colorless viscous liquid. A solution of this triol (25.6 mg, 0.0204 mmol) in CH_3OH (4 mL) and CH_2Cl_2 (0.4 mL) was stirred under an argon balloon. AcOH (0.1 mL) followed by $Pd(OH)_2$–C (20 wt%, 60% wet, 51 mg, 0.029 mmol) were added to the reaction mixture under an argon atmosphere and then the argon balloon was replaced with a H_2 balloon (1 atm). The solution was flushed with H_2 for 10 sec and the reaction mixture was stirred under H_2 for 12 h. At this point, the H_2 balloon was replaced with argon balloon and flushed argon for 1 min. The reaction mixture was diluted with CH_3OH (75 mL) and the solution was filtered over a pad of Celite-545. The filtrate was concentrated and the resulting

crude product was purified by column chromatography on reversed-phase (C_{18}) silica gel (20% CH_3OH in water). Solvents were evaporated to dryness and the product was redissolved in H_2O, followed by lyophilization afforded trisaccharide **2-10a** (9.8 mg, 70%) as a white foam: $[\alpha]^{22}_D$ +97.8 (*c* 0.09, CH_3OH); ^1H NMR (500 MHz, D_2O) δ 5.13 (d, J = 3.0 Hz, 1 H, H-1_{Gal}), 4.92 (s, 1 H, H-1_{AltHep}), 4.73 (d, J = 8.5 Hz, 1 H, H-1_{GlcN}), 4.23 (s, 1 H, H-4_{Gal}), 4.11–4.08 (m, 2 H, H-3_{AltHep} and H-5_{AltHep}), 4.00 (app t, J = 6.0 Hz, 1 H, H-5_{Gal}), 3.96–3.88 (m, 4 H, H-2_{Gal}, H-3_{Gal}, H-2_{AltHep} and H-$6a_{GlcN}$), 3.86–3.72 (m, 8 H, H-$6b_{GlcN}$, H-2_{GlcN}, H-4_{AltHep}, H-$7a_{AltHep}$, H-$7b_{AltHep}$, H-$6a_{Gal}$, H-$6b_{Gal}$ and OC$\underline{H_2}$CH$_2$), 3.60 (dd, J = 10.0, 8.5 Hz, 1 H, H-3_{GlcN}), 3.57–3.44 (m, 3 H, H-4_{GlcN}, H-5_{GlcN} and OC$\underline{H_2}$CH$_2$), 2.94 (t, J = 4.5 Hz, 2 H, 2 × C$\underline{H_2}$NH$_2$), 2.16–2.06 (m, 1 H, H-$6a_{AltHep}$), 2.07 (s, 3 H, 3 × NHCOC$\underline{H_3}$), 1.83–1.74 (m, 1 H, H-$6b_{AltHep}$), 1.68–1.60 (m, 4 H, 4 × C$\underline{H_2}$), 1.44–1.28 (m, 8 H, 8 × C$\underline{H_2}$); ^{13}C NMR (125 MHz, D_2O) δ 175.9 (NH$\underline{C}\underline{O}CH_3$), 103.7 (C-$1_{GlcN}$), 99.9 (C-$1_{Gal}$), 99.2 (C-$1_{AltHep}$), 79.8 (C-$3_{Gal}$), 76.8 (C-$2_{AltHep}$), 76.6 (C-$5_{GlcN}$), 74.6 (C-$3_{GlcN}$), 72.0 (C-$5_{Gal}$), 70.7 (C-$4_{GlcN}$), 70.1 (C-$4_{Gal}$), 69.6 (O$\underline{C}H_2CH_2$), 69.1 (2C, C-$3_{AltHep}$ and C-4_{AltHep}), 68.1 (C-2_{Gal}), 66.7 (C-5_{AltHep}), 62.1 (C-6_{Gal}), 61.5 (C-6_{GlcN}), 59.3 (C-7_{AltHep}), 56.7 (C-2_{GlcN}), 40.7 ($\underline{C}$H$_2$NH$_2$), 34.1 (C-6_{AltHep}), 29.3 ($\underline{C}$H$_2$), 29.2 ($\underline{C}$H$_2$), 29.1 ($\underline{C}$H$_2$), 28.7 ($\underline{C}$H$_2$), 26.5 ($\underline{C}$H$_2$), 26.2 ($\underline{C}$H$_2$), 23.2 (NHCO$\underline{C}$H$_3$); IR (cast film, $CHCl_3$) ν 3306, 2927, 1645, 1567, 1409, 1073 cm^{-1}; HRMS (ESI-TOF) m/z: $[M + H]^+$ Calcd for $C_{29}H_{55}N_2O_{16}$ 687.3546; Found 687.3554.

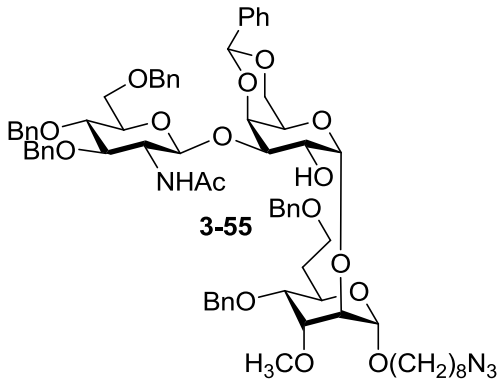


3-2a

8-Aminooctyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-α-D-galactopyranosyl-(1→2)-6-deoxy-3-*O*-methyl-α-D-*altro*-heptopyranoside (3-2a)

To a stirred solution of trisaccharide **3-52** (34.5 mg, 0.0262 mmol) in THF (2 mL) and pyridine (0.5 mL) was added 70% HF·pyridine (50 μL, 1.9 mmol based on HF). The reaction mixture was stirred at the room temperature for 6 h and then excess HF was quenched by the addition of saturated aqueous $NaHCO_3$ solution (25 mL) before the solution was extracted with EtOAc (75 mL). The aqueous layer was separated and further extracted with EtOAc (2 × 75 mL). The combined organic phases were washed with brine (10 mL), dried over anhydrous Na_2SO_4, filtered, concentrated and the resulting crude product was quickly purified by column chromatography (5% CH_3OH in EtOAc) to afford the corresponding triol (29 mg, 94%) as a colorless viscous liquid. A solution of this triol (16.3 mg, 0.0138 mmol) in CH_3OH (4 mL) and CH_2Cl_2 (0.4 mL) was stirred under an argon balloon. AcOH (0.1 mL) followed by $Pd(OH)_2$–C (20 wt%, 60% wet, 32 mg, 0.018 mmol) were to the reaction mixture under an argon atmosphere. The argon balloon was replaced with a H_2 balloon (1 atm), the solution was flushed with H_2 for 10 sec and then the reaction mixture was stirred under a H_2 balloon for 12 h. The H_2 balloon was then replaced with argon balloon and the solution was flushed with argon for 1 min. The reaction mixture was diluted with CH_3OH (75 mL) and filtered over a pad of Celite-545.

The filtrate was concentrated and the resulting crude product was purified by column chromatography on reversed phase (C_{18}) silica gel (20% CH_3OH in water). Solvents were evaporated to dryness and the product was redissolved in H_2O, followed by lyophilization afforded trisaccharide **3-2a** (6.8 mg, 70%) as a white foam: $[\alpha]^{22}_D$ +96.8 (*c* 0.1, CH_3OH); ^1H NMR (500 MHz, D_2O) δ 5.15 (d, J = 2.0 Hz, 1 H, H-1_{Gal}), 4.88 (s, 1 H, H-1_{AltHep}), 4.73 (d, J = 8.5 Hz, 1 H, H-1_{GlcN}), 4.23 (s, 1 H, H-4_{Gal}), 4.05–4.00 (m, 3 H, H-2_{AltHep}, H-5_{AltHep} and H-5_{Gal}), 3.95–3.88 (m, 3 H, H-2_{Gal}, H-3_{Gal}, and H-$6a_{GlcN}$), 3.85 (dd, J = 9.0, 3.0 Hz, 1 H, H-4_{AltHep}), 3.82–3.66 (m, 8 H, H-$6b_{GlcN}$, H-2_{GlcN}, H-$7a_{AltHep}$, H-$7b_{AltHep}$, H-3_{AltHep}, H-$6a_{Gal}$, H-$6b_{Gal}$ and OC$\underline{H_2}$CH$_2$), 3.60 (dd, J = 10.5, 8.0 Hz, 1 H, H-3_{GlcN}), 3.59–3.44 (m, 3 H, H-4_{GlcN}, H-5_{GlcN} and OC$\underline{H_2}$CH$_2$), 3.49 (s, 3 H, OC$\underline{H_3}$), 2.96 (t, J = 7.0 Hz, 2 H, 2 × C$\underline{H_2}$NH$_2$), 2.14–2.02 (m, 1 H, H-$6a_{AltHep}$), 2.07 (s, 3 H, 3 × NHCOC$\underline{H_3}$), 1.82–1.72 (m, 1 H, H-$6b_{AltHep}$), 1.70–1.58 (m, 4 H, 4 × C$\underline{H_2}$), 1.44–1.28 (m, 8 H, 8 × C$\underline{H_2}$); ^{13}C NMR (125 MHz, D_2O) δ 175.9 (NH$\underline{C}$O$\underline{C}$H$_3$), 103.7 (C-1_{GlcN}), 99.9 (C-1_{Gal}), 99.2 (C-1_{AltHep}), 79.8 (C-3_{Gal}), 78.9 (C-3_{AltHep}), 76.6 (C-5_{GlcN}), 74.6 (C-3_{GlcN}), 73.9 (C-2_{AltHep}), 71.9 (C-5_{Gal}), 70.7 (C-4_{GlcN}), 70.1 (C-4_{Gal}), 69.7 (O$\underline{C}$H$_2$CH$_2$), 68.5 (C-4_{AltHep}), 68.1 (C-2_{Gal}), 67.6 (C-5_{AltHep}), 61.9 (C-6_{Gal}), 61.5 (C-6_{GlcN}), 59.2 (C-7_{AltHep}), 59.1 (O$\underline{C}$H$_3$), 56.7 (C-2_{GlcN}), 40.6 ($\underline{C}$H$_2$NH$_2$), 34.0 (C-6_{AltHep}), 29.3 ($\underline{C}$H$_2$), 29.2 ($\underline{C}$H$_2$), 29.1 ($\underline{C}$H$_2$), 28.3 ($\underline{C}$H$_2$), 26.5 ($\underline{C}$H$_2$), 26.2 ($\underline{C}$H$_2$), 23.2 (NHCO$\underline{C}$H$_3$); IR (cast film, $CHCl_3$) ν 3312, 2931, 1646, 1568, 1409, 1072, 947 cm^{-1}; HRMS (ESI-TOF) m/z: $[M + H]^+$ Calcd for $C_{30}H_{57}N_2O_{16}$ 701.3703; Found 701.3701.

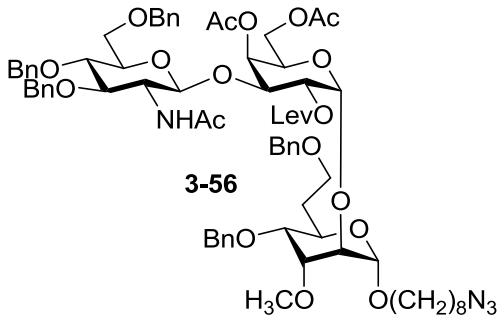


3-55

8-Azidooctyl 2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranosyl-(1→3)-4,6-*O*-benzylidene-α-D-galactopyranosyl-(1→2)-4,7-di-*O*-benzyl-6-deoxy-3-*O*-methyl-α-D-*altro*-heptopyranoside (3-55)

To a stirred solution of trisaccharide **3-52** (34.5 mg, 0.0262 mmol) in THF (2 mL) and pyridine (0.5 mL) was added 70% HF·pyridine (50 μL, 1.9 mmol based on HF). The reaction mixture was stirred at the room temperature for 6 h and then excess HF was quenched by the addition of saturated aqueous $NaHCO_3$ solution (20 mL) before the solution was extracted with EtOAc (50 mL). The aqueous layer was separated and further extracted with EtOAc (2 × 50 mL). The combined organic phases were washed with brine (10 mL), dried over anhydrous Na_2SO_4, filtered, concentrated and the resulting crude product was quickly purified by column chromatography (5% CH_3OH in EtOAc) to afford the corresponding triol (29 mg) as a colorless viscous liquid. To a stirred solution of this triol (28.9 mg, 0.0245 mmol) in CH_3CN (2 mL) was added camphorsulfonic acid (4.0 mg, 0.017 mmol) and the reaction mixture was stirred for 2 h. The camphorsulfonic acid was quenched by the addition of Et_3N (0. 1 mL) and the solution was concentrated. The resulting crude product was purified by column chromatography (65% EtOAc in hexane) to afford **3-55** (26.7 mg, 81% over the two steps) as a colorless viscous liquid: $[\alpha]^{22}_D$ +100.4 (*c* 0.15, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.51–7.20 (m, 30 H, ArH), 5.51 (d, *J* =

7.0 Hz, 1 H, $N\underline{H}COCH_3$), 5.46 (s, 1 H, C$\underline{H}$Ph), 5.15 (d, J = 3.5 Hz, 1 H, H-1_{Gal}), 5.14 (d, J = 8.0 Hz, 1 H, H-1_{GlcN}), 4.83 (d, J = 11.0 Hz, 1 H, OC$\underline{H_2}$Ph), 4.80 (d, J = 10.5 Hz, 1 H, OC$\underline{H_2}$Ph), 4.69–4.48 (m, 7 H, H-1_{AltHep} and 6 × OC$\underline{H_2}$Ph), 4.49 (s, 2 H, 2 × OC$\underline{H_2}$Ph), 4.33 (s, 1 H, H-4_{Gal}), 4.26–4.06 (m, 3 H, H-5_{AltHep}, H-2_{Gal} and H-$6a_{Gal}$), 4.02 (dd, J = 9.0, 8.5 Hz, 1 H, H-3_{GlcN}), 4.00–3.88 (m, 3 H, H-2_{AltHep}, H-3_{Gal} and H-$6b_{Gal}$), 3.78–3.55 (m, 9 H, H-4_{GlcN}, H-5_{GlcN}, H-$6a_{GlcN}$, H-$6b_{GlcN}$, H-$7a_{AltHep}$, H-$7b_{AltHep}$, H-3_{AltHep}, H-4_{AltHep} and OC$\underline{H_2}CH_2$), 3.49 (dd, J = 9.5, 8.5 Hz, 1 H, H-2_{GlcN}), 3.39 (s, 3 H, 3 × OC$\underline{H_3}$), 3.34–3.28 (m, 1 H, OC$\underline{H_2}CH_2$), 3.25 (t, J = 7.0 Hz, 2 H, 2 × C$\underline{H_2}N_3$), 2.96 (br s, 1 H, CH$\underline{OH}$), 2.18–2.10 (m, 1 H, H-$6a_{AltHep}$), 1.83 (s, 3 H, 3 × NHCOC$\underline{H_3}$), 1.80–1.71 (m, 1 H, H-$6b_{AltHep}$), 1.60–1.50 (m, 4 H, 4 × C$\underline{H_2}$), 1.38–1.24 (m, 8 H, 8 × C$\underline{H_2}$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.2 (NH$\underline{CO}CH_3$), 138.5 (Ar), 138.2 (Ar), 138.1 (Ar), 137.9 (Ar), 137.5 (Ar), 129.9 (Ar), 129.3 (Ar), 128.9 (Ar), 128.4 (Ar), 128.1 (Ar), 127.9 (Ar), 127.7 (Ar), 127.6 (Ar), 126.3 (Ar), 101.3 (C-1_{GlcN}), 100.9 ($\underline{C}$HPh), 100.1 (C-1_{Gal}), 99.0 (C-1_{AltHep}), 81.2 (C-3_{GlcN}), 78.7 (C-4_{GlcN}), 76.5 (2C, C-4_{Gal} and C-3_{AltHep}), 75.5, 74.9, 74.8 (O$\underline{C}H_2$Ph), 73.5 (O$\underline{C}H_2$Ph), 72.9 (2C, 2 × O$\underline{C}H_2$Ph), 71.6 (O$\underline{C}H_2$Ph), 69.5 (C-6_{GlcN}), 69.3 (C-6_{Gal}), 67.8 (O$\underline{C}H_2CH_2$), 67.6 (C-2_{Gal}), 67.1 (C-7_{AltHep}), 66.6 (C-5_{AltHep}), 63.5 (C-5_{Gal}), 57.9 (O$\underline{C}H_3$), 57.4 (C-2_{GlcN}), 51.5 ($\underline{C}H_2N_3$), 31.7 (C-6_{AltHep}), 29.5 ($\underline{C}H_2$), 29.4 ($\underline{C}H_2$), 29.1 ($\underline{C}H_2$), 28.8 ($\underline{C}H_2$), 26.7 ($\underline{C}H_2$), 26.1 ($\underline{C}H_2$), 23.6 (NHCO$\underline{C}H_3$); IR (cast film, $CHCl_3$) ν 3386, 3031, 2925, 1645, 1534, 1454, 1086, 757 cm^{-1}; HRMS (ESI-TOF) m/z: $[M + Na]^+$ Calcd for $C_{72}H_{88}N_4O_{16}Na$ 1287.6088; Found 1287.6102.

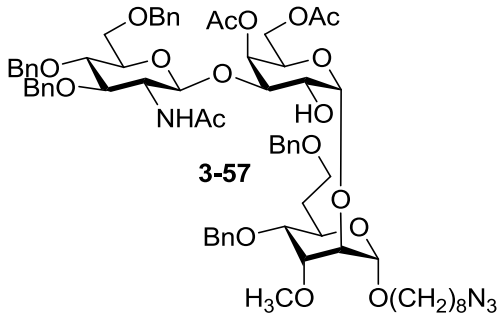


8-Azidooctyl 2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranosyl-(1→3)-4,6-di-*O*-acetyl-2-*O*-levulinoyl-α-D-galactopyranosyl-(1→2)-4,7-di-*O*-benzyl-6-deoxy-3-*O*-methyl-α-D-*altro*-heptopyranoside (3-56)

To a stirred solution of trisaccharide **3-52** (31.8 mg, 0.0241 mmol) in CH_2Cl_2 (2 mL) was added levulinic acid (10 μL, 0.098 mmol) followed by *N*,*N*-dimethylaminopyridine (4 mg, 0.03 mmol) and *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (10 mg, 0.052 mmol). The solution was stirred for 15 h and then diluted with CH_2Cl_2 (50 mL) and washed with saturated aqueous $NaHCO_3$ solution (15 mL). Organic layer was separated and the aqueous layer was further extracted with CH_2Cl_2 (2 × 50 mL). The combined organic phases were washed with brine (15 mL), dried over anhydrous Na_2SO_4, filtered, concentrated and the resulting crude product was purified by column chromatography (50% EtOAc in hexane) to afford the corresponding levulinate ester as a colorless viscous liquid. This compound which was dissolved in THF (2 mL) and pyridine (0.5 mL), and then 70% HF·pyridine (50 μL, 1.9 mmol based on HF) was added and the reaction mixture was stirred for 6 h. Excess HF was quenched by the addition of saturated aqueous $NaHCO_3$ solution (15 mL) and the reaction mixture was extracted with EtOAc (50 mL). The aqueous layer was separated and further extracted with EtOAc (2 × 50 mL). The combined organic phases were washed with brine (15 mL), dried over anhydrous Na_2SO_4, filtered and concentrated to obtain the crude diol as a pale yellow viscous liquid.

Without further purification, this crude diol was dissolved in pyridine (2 mL) and Ac_2O (0.10 mL, 1.1 mmol). The reaction mixture was stirred for 13 h before toluene (5 mL) was added and the resulting solution was concentrated. Co-concentration with toluene (4 × 5 mL) produced a crude product that was purified by column chromatography (55% EtOAc in hexane) to afford **3-56** (27.4 mg, 83% over the three steps) as a colorless viscous liquid: $[\alpha]^{22}_D$ +60.5 (*c* 0.23, $CHCl_3$); ^{1}H NMR (700 MHz, $CDCl_3$) δ 7.38–7.18 (m, 25 H, ArH), 6.50 (d, J = 8.4 Hz, 1 H, $N\underline{H}COCH_3$), 5.47 (d, J = 3.5 Hz, 1 H, H-4_{Gal}), 5.17–5.14 (m, 2 H, H-1_{Gal} and H-2_{Gal}), 4.81 (d, J = 7.7 Hz, 1 H, H-1_{GlcN}), 4.79, 4.71 (ABq, J = 11.5 Hz, 2 H, 2 × OC$\underline{H}_2$Ph), 4.77 (d, J = 11.2 Hz, 1 H, OC$\underline{H}_2$Ph), 4.67 (d, J = 2.1 Hz, 1 H, H-1_{AltHep}), 4.65 (d, J = 11.9 Hz, 1 H, OC$\underline{H}_2$Ph), 4.62 (d, J = 11.9 Hz, 1 H, OC$\underline{H}_2$Ph), 4.59–4.53 (m, 3 H, 3 × OC$\underline{H}_2$Ph), 4.48 (s, 2 H, 2 × OC$\underline{H}_2$Ph), 4.23 (dd, J = 7.0, 4.9 Hz, 1 H, H-5_{Gal}), 4.21 (dd, J = 9.8, 3.5 Hz, 1 H, H-3_{Gal}), 4.19–4.15 (m, 1 H, H-5_{AltHep}), 4.13 (dd, J = 11.2, 4.9 Hz, 1 H, H-6a$_{Gal}$), 3.98 (dd, J = 11.2, 7.0 Hz, 1 H, H-6b$_{Gal}$), 3.93–3.88 (m, 2 H, H-3_{GlcN} and H-2_{AltHep}), 3.78–3.69 (m, 3 H, H-6a$_{GlcN}$, H-6b$_{GlcN}$ and H-2_{GlcN}), 3.65 (dd, J = 9.8, 8.4 Hz, 1 H, H-4_{GlcN}), 3.64–3.58 (m, 3 H, H-7a$_{AltHep}$, H-7b$_{AltHep}$ and OC$\underline{H}_2CH_2$), 3.57–3.51 (m, 3 H, H-5_{GlcN}, H-3_{AltHep} and H-4_{AltHep}), 3.37 (s, 3 H, 3 × OC$\underline{H}_3$), 3.27 (dt, J = 9.1, 6.3 Hz, 1 H, OC$\underline{H}_2CH_2$), 3.23 (t, J = 7.7 Hz, 2 H, 2 × C$\underline{H}_2N_3$), 2.91–2.85 (m, 1 H, OCOC$\underline{H}_2CH_2$), 2.64–2.58 (m, 2 H, $OCOCH_2C\underline{H}_2$), 2.34–2.29 (m, 1 H, OCOC$\underline{H}_2CH_2$), 2.18 (s, 3 H, 3 × $CH_2COC\underline{H}_3$), 2.16–2.11 (m, 1 H, H-6a$_{AltHep}$), 2.10 (s, 3 H, 3 × COC$\underline{H}_3$), 2.01 (s, 3 H, 3 × COC$\underline{H}_3$), 1.83–1.74 (m, 1 H, H-6b$_{AltHep}$), 1.80 (s, 3 H, 3 × NHCOC$\underline{H}_3$), 1.60–1.48 (m, 4 H, 4 × C$\underline{H}_2$), 1.34–1.22 (m, 8 H, 8 × C$\underline{H}_2$); ^{13}C NMR (175 MHz, $CDCl_3$) δ 208.1 ($CH_2\underline{C}OCH_3$), 171.6 ($\underline{C}OCH_3$), 170.4 ($\underline{C}OCH_3$), 170.2 ($\underline{C}OCH_3$), 138.7 (Ar), 138.5 (Ar), 138.4 (Ar), 138.3 (Ar), 138.2 (Ar), 129.8 (Ar), 129.6 (Ar), 128.3 (Ar), 128.1 (Ar), 128.0 (Ar), 127.8 (Ar), 127.6 (Ar), 100.2 (C-1_{GlcN}), 98.7 (C-1_{AltHep}), 96.5 (C-1_{Gal}), 81.7 (C-3_{GlcN}), 78.1 (C-4_{GlcN}), 76.8 (C-3_{AltHep}), 76.2 (C-

4_{AltHep}), 75.1 (C-5_{GlcN}), 74.8 (O<u>C</u>H_2Ph), 74.4 (O<u>C</u>H_2Ph), 74.2 (C-2_{AltHep}), 73.4 (O<u>C</u>H_2Ph), 73.0 (O<u>C</u>H_2Ph), 71.6 (O<u>C</u>H_2Ph), 70.93, 70.88, 69.7 (C-4_{Gal}), 69.1 (C-6_{GlcN}), 67.9 (O<u>C</u>H_2CH_2), 67.4 (C-5_{Gal}), 67.1 (C-7_{AltHep}), 66.3 (C-5_{AltHep}), 62.6 (C-6_{Gal}), 57.8 (O<u>C</u>H_3), 55.9 (C-2_{GlcN}), 51.5 (<u>C</u>H_2N_3), 37.6 (OCO<u>C</u>H_2CH_2), 31.9 (C-6_{AltHep}), 29.9 (CH_2CH_2CO<u>C</u>H_3), 29.6 (<u>C</u>H_2), 29.4 (<u>C</u>H_2), 29.1 (<u>C</u>H_2), 28.8 (<u>C</u>H_2), 27.8 (OCOCH_2<u>C</u>H_2), 26.7 (<u>C</u>H_2), 26.1 (<u>C</u>H_2), 23.2 (NHCO<u>C</u>H_3), 20.71 (CO<u>C</u>H_3), 20.65 (CO<u>C</u>H_3); IR (cast film, $CHCl_3$) ν 3378, 3030, 2929, 2096, 1747, 1719, 1667, 1541, 1454, 1072, 752 cm^{-1}; HRMS (ESI-TOF) m/z: $[M + Na]^+$ Calcd for $C_{74}H_{94}N_4O_{20}Na$ 1381.6354; Found 1381.6343.

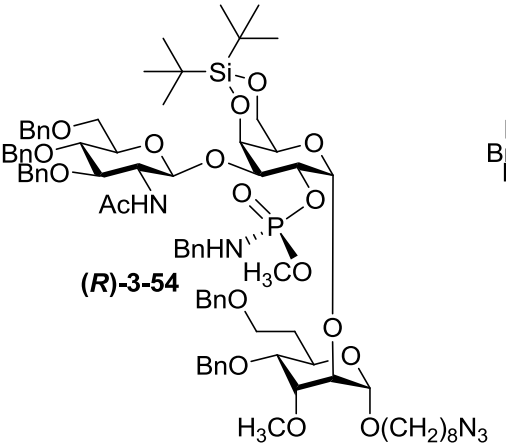


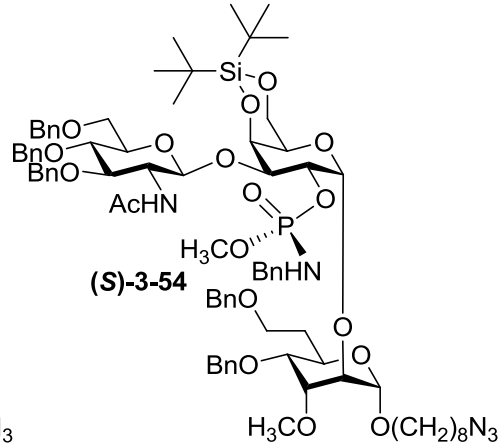
8-Azidooctyl 2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranosyl-(1→3)-4,6-di-*O*-acetyl-α-D-galactopyranosyl-(1→2)-4,7-di-*O*-benzyl-6-deoxy-3-*O*-methyl-α-D-*altro*-heptopyranoside (3-57)

Trisaccharide **3-56** (27.4 mg, 0.0202 mmol) was dissolved in CH_2Cl_2 (2 mL) and CH_3OH (1 mL). A solution of hydrazine acetate (6.2 mg, 0.067 mmol) dissolved in 1:1 CH_2Cl_2-CH_3OH (1 mL) was added dropwise over 2 min and the reaction mixture was stirred for 6 h. The reaction mixture was diluted with CH_2Cl_2 (50 mL) and washed with water (15 mL) and the organic layer was separated and the aqueous layer was further extracted with CH_2Cl_2 (2 × 50 mL). The combined organic phases were washed with brine (15 mL), dried over anhydrous Na_2SO_4, filtered, concentrated and the resulting crude product was purified by column chromatography

(70% EtOAc in hexane) to afford **3-57** (21.7 mg, 85%) as a colorless viscous liquid: $[\alpha]^{22}_{D}$ +97.3 (*c* 0.11, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.03–7.10 (m, 25 H, ArH), 5.51 (d, *J* = 7.5 Hz, 1 H, *N*H$\underline{}$COCH$_3$), 5.39 (d, *J* = 2.5 Hz, 1 H, H-4$_{Gal}$), 5.03 (d, *J* = 4.0 Hz, 1 H, H-1$_{Gal}$), 4.85 (d, *J* = 8.0 Hz, 1 H, H-1$_{GlcN}$), 4.82, 4.66 (ABq, *J* = 11.5 Hz, 2 H, 2 × OC$\underline{H_2}$Ph), 4.77 (d, *J* = 11.0 Hz, 1 H, OC$\underline{H_2}$Ph), 4.68–4.58 (m, 4 H, H-1$_{AltHep}$ and 3 × OC$\underline{H_2}$Ph), 4.55 (d, *J* = 12.0 Hz, 1 H, OC$\underline{H_2}$Ph), 4.55 (d, *J* = 12.0 Hz, 1 H, OC$\underline{H_2}$Ph), 4.48 (s, 2 H, 2 × OC$\underline{H_2}$Ph), 4.19–4.09 (m, 3 H, H-5$_{AltHep}$, H-5$_{Gal}$ and H-6a$_{Gal}$), 3.98–3.89 (m, 3 H, H-2$_{AltHep}$, H-2$_{Gal}$ and H-6b$_{Gal}$), 3.85 (dd, *J* = 10.0, 3.0 Hz, 1 H, H-3$_{Gal}$), 3.80 (dd, *J* = 9.0, 9.0 Hz, 1 H, H-3$_{GlcN}$), 3.79–3.61 (m, 2 H, H-6a$_{GlcN}$ and H-6b$_{GlcN}$), 3.68 (dd, *J* = 9.0, 9.0 Hz, 1 H, H-4$_{GlcN}$), 3.67–3.48 (m, 7 H, H-2$_{GlcN}$, H-5$_{GlcN}$, H-7a$_{AltHep}$, H-7b$_{AltHep}$, H-3$_{AltHep}$, H-4$_{AltHep}$ and OC$\underline{H_2}$CH$_2$), 3.38 (s, 3 H, 3 × OC$\underline{H_3}$), 3.35–3.28 (m, 1 H, OC$\underline{H_2}$CH$_2$), 3.23 (t, *J* = 6.5 Hz, 2 H, 2 × C$\underline{H_2}$N$_3$), 2.93 (br s, 1 H, CH*O*$\underline{H}$) 2.16–2.08 (m, 1 H, H-6a$_{AltHep}$), 2.05 (s, 3 H, 3 × COC$\underline{H_3}$), 2.01 (s, 3 H, 3 × COC$\underline{H_3}$), 1.82 (s, 3 H, 3 × NHCOC$\underline{H_3}$), 1.77–1.68 (m, 1 H, H-6b$_{AltHep}$), 1.60–1.48 (m, 4 H, 4 × C$\underline{H_2}$), 1.36–1.20 (m, 8 H, 8 × C$\underline{H_2}$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.1 (*C*O$\underline{}$CH$_3$), 170.5 (*C*O$\underline{}$CH$_3$), 170.2 (*C*O$\underline{}$CH$_3$), 138.5 (Ar), 138.3 (Ar), 138.2 (Ar), 137.9 (Ar), 132.9 (Ar), 129.9 (Ar), 129.6 (Ar), 128.5 (Ar), 128.4 (Ar), 128.2 (Ar), 128.1 (Ar), 127.9 (Ar), 127.8 (Ar), 127.6 (Ar), 101.4 (C-1$_{GlcN}$), 99.4 (C-1$_{Gal}$), 99.0 (C-1$_{AltHep}$), 80.9 (C-3$_{GlcN}$), 78.2 (C-4$_{GlcN}$), 76.4 (C-3$_{Gal}$), 75.6 (C-3$_{AltHep}$), 75.1 (2C, C-4$_{AltHep}$ and C-5$_{GlcN}$), 74.9 (C-2$_{Gal}$), 74.8 (O*C*H$_2$Ph), 74.4 (O*C*H$_2$Ph), 73.4 (O*C*H$_2$Ph), 73.0 (O*C*H$_2$Ph), 71.5 (O*C*H$_2$Ph), 69.8 (C-4$_{Gal}$), 68.9 (C-6$_{GlcN}$), 68.4 (C-2$_{AltHep}$), 67.99 (O*C*H$_2$CH$_2$), 67.96 (C-5$_{Gal}$), 67.1 (C-7$_{AltHep}$), 66.4 (C-5$_{AltHep}$), 62.8 (C-6$_{Gal}$), 57.8 (O*C*H$_3$), 56.4 (C-2$_{GlcN}$), 51.5 (*C*H$_2$N$_3$), 31.8 (C-6$_{AltHep}$), 29.6 (*C*H$_2$), 29.4 (*C*H$_2$), 29.1 (*C*H$_2$), 28.8 (*C*H$_2$), 26.7 (*C*H$_2$), 26.1 (*C*H$_2$), 23.6 (NHCO*C*H$_3$), 22.7 (CO*C*H$_3$), 20.7 (CO*C*H$_3$); IR (cast film, $CHCl_3$) *v* 3301, 3030, 2929, 2095,

1745, 1651, 1554, 1453, 1073, 738 cm^{-1}; HRMS (ESI-TOF) m/z: $[M + Na]^+$ Calcd for $C_{69}H_{88}N_4O_{18}Na$ 1283.5986; Found 1283.5976.





8-Azidooctyl 2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranosyl-(1→3)-4,6-*O*-(di-*tert*-butylsilylene)-(2*R*)-2-*O*-(methyl *N*-benzylphosphoramidyl)-α-D-galactopyranosyl-(1→2)-4,7-di-*O*-benzyl-6-deoxy-3-*O*-methyl-α-D-*altro*-heptopyranoside [(*R*)-**3-54**] and **8-Azidooctyl 2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranosyl-(1→3)-4,6-*O*-(di-*tert*-butylsilylene)-(2*S*)-2-*O*-(methyl *N*-benzylphosphoramidyl)-α-D-galactopyranosyl-(1→2)-4,7-di-*O*-benzyl-6-deoxy-3-*O*-methyl-α-D-*altro*-heptopyranoside** [(*S*)-**3-54**]

To a stirred solution of tetra-*N*-butylammonium methyl *H*-phosphonate **3-32**[21a] (589 mg, 1.75 mmol) in CH_2Cl_2 (4.5 mL) and pyridine (0.5 mL) was added Ac_2O (180 μL, 1.90 mmol). The reaction mixture was stirred at 55 °C for 1 h before being cooled to room temperature over 5 min using an ice bath. A solution of trisaccharide **3-52** (230 mg, 0.175 mmol) in CH_2Cl_2 (4 mL) was added dropwise to the above cooled reaction mixture over 5 min and the reaction mixture was stirred at 55 °C for for 15 h before being cooled to room temperature over 15 min. The reaction mixture was concentrated and the resulting crude product was quickly purified by column chromatography (70% EtOAc in hexane) to obtain the corresponding sugar *H*-

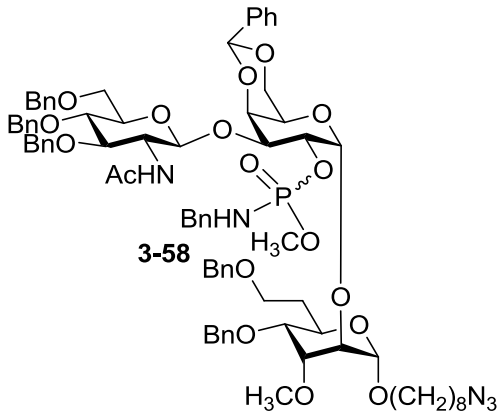
phosphonate. This compound was dissolved in dry CH_2Cl_2 (4 mL) and to this stirring solution, Et_3N (80 μL, 0.57 mmol), $CBrCl_3$ (180 μL, 1.83 mmol) and $BnNH_2$ (40 μL, 0.37 mmol) were added sequentially and stirred for 4 h. The reaction mixture was diluted with CH_2Cl_2 (75 mL) and washed with saturated aqueous $NaHCO_3$ solution (25 mL). The organic layer was separated and the aqueous layer was further extracted with CH_2Cl_2 (2 × 100 mL). The combined organic phases were washed with brine (25 mL), dried over anhydrous Na_2SO_4, filtered, concentrated and the resulting crude product was purified by column chromatography (40% acetone in hexane) to obtain a mixture of methyl *N*-benzylphosphoramidates (170 mg, 65% yield over the two steps) in 1:1.2 ratio. This mixture of phosphoramidates was purified five times using chromatography (45% EtOAc in hexane) to obtain each of the diastereomers ([(***R***)-**3-54**] and [(***S***)-**3-54**]) as pure compounds.

Data for (***R***)-**3-54** (81 mg, 31%): White solid which exhibits 4:1 rotameric ratio, as determined by NMR spectroscopy in $CDCl_3$ at 27 °C: mp 104–108 °C; $[\alpha]^{22}_D$ +70.1 (*c* 0.37, $CHCl_3$); ^{1}H NMR (700 MHz, $CDCl_3$) δ 7.41–7.15 (m, 40 H, ArH), 6.20 (d, J = 7.7 Hz, 1 H, $N\underline{H}COCH_3$), 5.41 (d, J = 3.5 Hz, 1 H, H-1*$_{Gal}$), 5.30 (d, J = 3.5 Hz, 1 H, H-1$_{Gal}$), 4.87 (d, J = 7.7 Hz, 1 H, H-1$_{GlcN}$), 4.82–4.58 (m, 10 H, H-2$_{Gal}$, H-2*$_{Gal}$, H-4$_{Gal}$, H-4*$_{Gal}$, H-1$_{AltHep}$, H-1*$_{AltHep}$, OC$\underline{H}_2$Ph and OC$\underline{H}_2$Ph*), 4.53–4.45 (m, 7 H, OC$\underline{H}_2$Ph and OC$\underline{H}_2$Ph*), 4.34 (d, J = 8.4 Hz, 1 H, H-1*$_{GlcN}$), 4.22–4.12 (m, 4 H), 4.11–4.01 (m, 3 H), 3.97 (dd, 1 H, J = 3.5, 2.1 Hz, H-2$_{AltHep}$), 3.93–3.91 (m, 3 H, H-3$_{GlcN}$, H-3$_{Gal}$, H-3*$_{Gal}$), 3.77 (app t, J = 3.5 Hz, 1 H, H-3$_{AltHep}$), 3.75–3.53 (m, 15 H), 3.60 (d, 3 H, J = 10.5 Hz, 1 H, POC$\underline{H}_3$), 3.54 (d, 3 H, J = 11.2 Hz, 1 H, POC$\underline{H}_3$*), 3.49 (s, 3 H, OC$\underline{H}_3$*), 3.49 (s, 3 H, 3 × OC$\underline{H}_3$), 3.27 (dt, J = 9.8, 6.3 Hz, 2 H, OC$\underline{H}_2CH_2$ and OC$\underline{H}_2CH_2$*), 3.23 (t, J = 7.0 Hz, 4 H, 2 × C$\underline{H}_2N_3$ and 2 × C$\underline{H}_2N_3$*), 2.29–2.24 (m, 1 H, H-6a*$_{AltHep}$), 2.19–2.13 (m, 1 H, H-6a$_{AltHep}$), 2.02 (s, 3 H, 3 × NHCOC$\underline{H}_3$*), 1.79 (s, 3 H, 3 ×

NHCOC$\underline{H}_3$), 1.72–1.66 (m, 2 H, H-6b$_{AltHep}$ and H-6b*$_{AltHep}$), 1.59–1.46 (m, 8 H, 4 × C$\underline{H}_2$ and 4 × C$\underline{H}_2$*), 1.36–1.22 (m, 16 H, 8 × C$\underline{H}_2$ and 8 × C$\underline{H}_2$*), 1.04 (s, 18 H, SiC(C$\underline{H}_3$)$_3$ and SiC(C$\underline{H}_3$)$_3$*), 1.02 (s, 18 H, SiC(C$\underline{H}_3$)$_3$ and SiC(C$\underline{H}_3$)$_3$*); ^{13}C NMR (175 MHz, $CDCl_3$) δ 175.8 (NH$\underline{C}\underline{O}CH_3$*), 171.1 (NH$\underline{C}\underline{O}CH_3$), 139.8 (Ar), 138.5 (Ar), 138.43 (Ar), 138.38 (Ar), 138.1 (Ar), 137.9 (Ar), 128.7 (Ar), 128.4 (2C, Ar), 128.37 (Ar), 128.31 (Ar), 128.3 (Ar), 127.97 (Ar), 127.94 (Ar), 127.59 (Ar), 127.56 (Ar), 127.5 (Ar), 102.8 (C-1$_{GlcN}$*), 101.8 (C-1$_{GlcN}$), 98.9 (C-1$_{AltHep}$), 98.8 (C-1$_{AltHep}$*), 98.4 (C-1$_{Gal}$), 98.3 (C-1$_{Gal}$*), 82.3 (*), 81.2 (C-3$_{GlcN}$), 78.5, 76.73 (*), 76.70 (*), 76.3, 76.2 (*), 75.7, 75.5 (*), 75.0 (*), 74.9 (*), 74.75, 74.68 (O$\underline{C}H_2$Ph), 74.6, 74.4 (O$\underline{C}H_2$Ph), 73.5 (*), 73.4 (C-4$_{Gal}$), 73.2 (O$\underline{C}H_2$Ph), 72.9 (O$\underline{C}H_2$Ph), 72.1 (d, J = 4.9 Hz, C-2$_{Gal}$), 71.1 (O$\underline{C}H_2$Ph), 70.6 (*), 69.5, 68.7 (*), 68.0 (C-5$_{Gal}$), 67.9 (*), 67.7, 67.5 (*), 67.4 (*), 67.2, 66.8, 65.5 (C-5$_{AltHep}$), 57.9 (O$\underline{C}H_3$), 56.57 (*), 56.55 (C-2$_{GlcN}$), 53.1 (d, J = 5.1 Hz, PO$\underline{C}H_3$), 51.5 ($\underline{C}H_2N_3$), 46.2 (*), 45.8 (NH$\underline{C}H_2$Ph), 31.9, 31.8 (*), 29.5, 29.4, 29.1, 28.8, 27.6 (SiC($\underline{C}H_3$)$_3$), 27.4 (SiC($\underline{C}H_3$)$_3$), 26.7, 26.1, 23.41 (NHCO$\underline{C}H_3$), 23.37 (Si$\underline{C}$(CH$_3$)$_3$), 20.7 (Si$\underline{C}$(CH$_3$)$_3$); ^{31}P NMR (162 MHz, $CDCl_3$, 27 °C) δ 11.31, 10.55 (major); ^{31}P NMR (162 MHz, $CDCl_3$, 65 °C) δ 11.05; IR (cast film, $CHCl_3$) ν 3262, 3030, 2931, 2095, 1664, 1496, 1454, 1073, 735 cm^{-1}; HRMS (ESI-TOF) m/z: $[M + Na]^+$ Calcd for $C_{81}H_{110}N_5O_{18}PSiNa$ 1522.7245; Found 1522.7250.

Data for (***S***)-**3-54** (78 mg, 30%): Colorless viscous liquid which exhibits 2:1 rotameric ratio, as determined by NMR spectroscopy in $CDCl_3$ at 27 °C: $[\alpha]^{22}_D$ +59.9 (*c* 0.65, $CHCl_3$); ^{1}H NMR (700 MHz, $CDCl_3$) δ 7.38–7.14 (m, 55 H, ArH), 6.78 (d, J = 7.7 Hz, 1 H, $N\underline{H}COCH_3$), 6.19 (d, J = 6.3 Hz, 1 H, $N\underline{H}COCH_3$*), 5.29 (d, J = 3.5 Hz, 1 H, H-1*$_{Gal}$), 5.25 (d, J = 3.5 Hz, 1 H, H-1$_{Gal}$), 4.99 (d, J = 7.0 Hz, 1 H, H-1$_{GlcN}$), 4.82 (d, J = 11.2 Hz, 1 H, OC$\underline{H}_2$Ph*), 4.76–4.68 (m, 7 H, H-2$_{Gal}$, H-2*$_{Gal}$, OC$\underline{H}_2$Ph and OC$\underline{H}_2$Ph*), 4.68–4.44 (m, 18 H, H-4$_{Gal}$, H-4*$_{Gal}$, H-1$_{AltHep}$, H-1*$_{AltHep}$, H-1*$_{GlcN}$, OC$\underline{H}_2$Ph and OC$\underline{H}_2$Ph*), 4.20–3.92 (m, 16 H), 3.90–3.84 (m, 1 H),

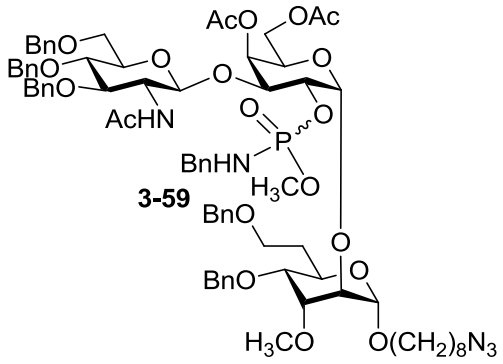
3.80–3.76 (m, 4 H), 3.75–3.52 (m, 18 H), 3.63 (d, 3 H, J = 11.9 Hz, 1 H, POC$\underline{H}_3$), 3.58 (d, 3 H, J = 11.2 Hz, 1 H, POC$\underline{H}_3$*), 3.48–3.42 (m, 2 H*), 3.46 (s, 3 H, OC$\underline{H}_3$*), 3.40–3.36 (m, 3 H*), 3.38 (s, 3 H, OC$\underline{H}_3$), 3.29–3.24 (m, 2 H, OC$\underline{H}_2$CH$_2$ and OC$\underline{H}_2$CH$_2$*), 3.23 (t, J = 7.0 Hz, 4 H, 2 × C$\underline{H}_2$N$_3$ and 2 × C$\underline{H}_2$N$_3$*), 2.24–2.17 (m, 1 H, H-6a*$_{AltHep}$), 2.14–2.08 (m, 1 H, H-6a$_{AltHep}$), 2.07 (s, 3 H, 3 × NHCOC$\underline{H}_3$*), 1.83 (s, 3 H, 3 × NHCOC$\underline{H}_3$), 1.73–1.67 (m, 2 H, H-6b$_{AltHep}$ and H-6b*$_{AltHep}$), 1.60–1.44 (m, 8 H, 4 × C$\underline{H}_2$ and 4 × C$\underline{H}_2$*), 1.36–1.20 (m, 16 H, 8 × C$\underline{H}_2$ and 8 × C$\underline{H}_2$*), 1.18–0.96 (m, 36 H, Si(C(C$\underline{H}_3$)$_3$)$_2$ and Si(C(C$\underline{H}_3$)$_3$)$_2$*); ^{13}C NMR (175 MHz, $CDCl_3$) δ 175.7 (NH$\underline{C}$OCH$_3$*), 171.2 (NH$\underline{C}$OCH$_3$), 140.2, 138.52, 138.48, 138.4, 138.2, 138.1, 128.7, 128.41, 128.36, 128.31, 128.26, 127.87, 127.85, 127.8, 127.58, 127.57, 127.3, 102.4 (C-1*$_{GlcN}$), 100.8 (C-1$_{GlcN}$), 98.9 (C-1$_{AltHep}$), 98.8 (C-1*$_{AltHep}$), 98.5 (d, J = 2.8 Hz, C-1$_{Gal}$), 98.3 (d, J = 1.2 Hz, C-1*$_{Gal}$), 82.9 (*), 80.8, 78.3 (*), 78.0, 76.5 (*), 76.0 (*), 75.89 (*), 75.86 (*), 75.84, 75.78 (*), 74.9 (*), 74.8 (*), 74.7, 74.6, 74.5, 74.3, 74.0 (*), 73.7 (*), 73.5 (d, J = 8.1 Hz), 73.3, 73.1 (*), 73.07 (*), 72.9, 71.1, 68.9 (*), 69.7, 67.9, 67.8 (*), 67.7, 67.3 (*), 67.2, 66.2 (C-5$_{AltHep}$), 58.0 (O$\underline{C}$H$_3$*), 57.8 (O$\underline{C}$H$_3$), 56.2 (C-2$_{GlcN}$), 53.3 (d, J = 5.6 Hz, PO$\underline{C}$H$_3$), 45.5 (NH$\underline{C}$H$_2$Ph), 45.4 (NH$\underline{C}$H$_2$Ph*), 31.9 (C-6$_{AltHep}$), 29.7, 29.4, 29.1, 28.8, 27.6, 27.4 (SiC($\underline{C}$H$_3$)$_3$), 27.3 (SiC($\underline{C}$H$_3$)$_3$), 26.7, 23.5 (NHCO$\underline{C}$H$_3$), 23.4 (Si$\underline{C}$(CH$_3$)$_3$), 20.7 (Si$\underline{C}$(CH$_3$)$_3$); ^{31}P NMR (202 MHz, $CDCl_3$, 27 °C) δ 10.71, 10.47 (major); ^{31}P NMR (202 MHz, $CDCl_3$, 60 °C) δ 10.33; IR (cast film, $CHCl_3$) ν 3273, 3031, 2931, 2096, 1660, 1497, 1454, 1071, 738 cm^{-1}; HRMS (ESI-TOF) m/z: $[M + Na]^+$ Calcd for $C_{81}H_{110}N_5O_{18}PSiNa$ 1522.7245; Found 1522.7241.



8-Azidooctyl 2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranosyl-(1→3)-4,6-*O*-benzylidene-2-*O*-(methyl *N*-benzylphosphoramidyl)-α-D-galactopyranosyl-(1→2)-4,7-di-*O*-benzyl-6-deoxy-3-*O*-methyl-α-D-*altro*-heptopyranoside (3-58)

The synthesis of phosphoramidate **3-58** was achieved starting from the trisaccharide **3-55** (20 mg, 0.016 mmol) following the procedure described for phosphoramidate diastereomer (***R***)-**3-54** to obtain the crude product, which was purified by column chromatography (65% EtOAc in Hexane) to afford an inseparable mixture of phosphoramidates **3-58** (16.1 mg, 70%) as a colorless viscous liquid: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.58–7.14 (m, 70 H, ArH), 6.60 (d, J = 7.5 Hz, 1 H), 6.34 (d, J = 8.0 Hz, 1 H), 5.44 (s, 2H), 5.39 (dd, J = 5.5, 4.0 Hz, 2 H), 5.31 (s, 1 H), 5.09 (d, J = 7.0 Hz, 1 H), 4.90 (d, J = 7.5 Hz, 1 H), 4.84–4.33 (m, 22 H), 4.24–3.44 (m, 54 H), 3.40 (s, 3 H), 3.36 (s, 3 H), 3.34–3.27 (m, 2 H), 3.25 (t, J = 7.0 Hz, 4 H), 2.22–2.12 (m, 2 H), 1.84 (s, 3 H, 3 × NHCOC$\underline{H}_3$), 1.82 (s, 3 H, 3 × NHCOC$\underline{H}_3$), 1.77–1.64 (m, 2 H), 1.63–1.44 (m, 8 H), 1.40–1.10 (m, 16 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.4, 171.2, 140.05, 140.01, 139.74, 139.69, 139.6, 138.6, 138.1, 137.8, 129.9, 128.5, 127.8, 127.3, 126.3, 101.4, 100.7, 100.61, 100.58, 98.8, 98.74, 98.68, 98.67, 98.45, 81.2, 80.0, 78.3, 78.1, 76.3, 76.0, 75.9, 75.7, 74.87, 74.85, 74.8, 74.7, 74.5, 74.4, 74.3, 73.4, 73.0, 71.2, 71.1, 69.8, 69.6, 69.1, 68.8, 67.78, 67.69, 67.29, 67.23, 65.8, 65.4, 63.34, 63.26, 58.1, 57.9, 56.1, 53.23, 53.21, 53.19, 53.0, 51.5, 45.7,

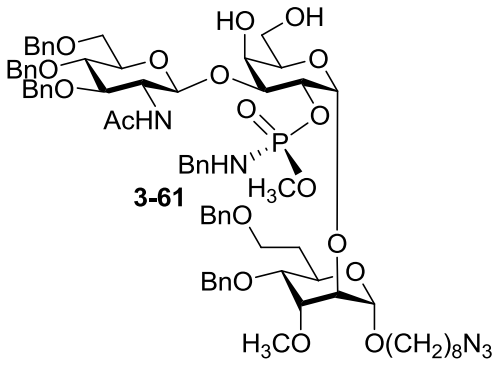
45.6, 31.97, 31.91, 29.7, 29.4, 29.1, 28.9, 26.7, 26.1, 23.5, 23.4; ^{31}P NMR (162 MHz, $CDCl_3$) δ 11.03, 10.53, 10.36, 10.12; HRMS (ESI-TOF) m/z: $[M + H]^+$ Calcd for $C_{80}H_{99}N_5O_{18}P$ 1448.6717; Found 1448.6701.



8-Azidooctyl 2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranosyl-(1→3)-4,6-di-*O*-acetyl-2-*O*-(methyl *N*-benzylphosphoramidyl)-α-D-galactopyranosyl-(1→2)-4,7-di-*O*-benzyl-6-deoxy-3-*O*-methyl-α-D-altro-heptopyranoside (3-59)

The synthesis of phosphoramidate **3-59** was achieved starting from the trisaccharide **3-57** (11.7 mg, 0.0347 mmol) following the procedure described for phosphoramidate diastereomer (***R***)-**3-54** to obtain crude product, which was purified by column chromatography (60% EtOAc in Hexane) to afford an inseparable mixture of phosphoramidates **3-59** (8.7 mg, 65%) as a colorless viscous liquid: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.08–7.18 (m, 60 H, ArH), 6.38 (d, J = 8.0 Hz, 1 H), 6.06 (d, J = 8.0 Hz, 1 H), 5.39 (d, J = 8.5 Hz, 1 H), 5.47–5.43 (m, 2 H), 5.47–5.43 (m, 2 H), 5.34 (app t, J = 4.0 Hz, 1 H), 4.92 (d, J = 6.0 Hz, 1 H), 4.83–4.42 (m, 22 H), 4.28–3.54 (m, 46 H), 4.28–3.54 (m, 46 H), 3.51–3.20 (m, 11 H), 4.28–3.54 (m, 11 H), 3.24 (app t, J = 6.5 Hz, 4 H), 2.22–2.12 (m, 1 H), 2.21–1.96 (m, 1 H), 2.04–1.94 (m, 12 H), 2.02 (s, 3 H, 3 × COC$\underline{H}_3$), 1.02–1.81 (m, 1 H), 1.82 (s, 3 H, 3 × NHCOC$\underline{H}_3$), 1.77 (s, 3 H, 3 × NHCOC$\underline{H}_3$), 1.77–1.68 (m, 1 H), 1.65–1.48 (m, 8 H), 1.36–1.14 (m, 16 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.9, 170.8,

170.5, 170.4, 170.2, 170.0, 139.97, 139.91, 139.7, 138.4, 138.0, 129.7, 128.3, 127.6, 101.2, 101.2, 100.1, 98.8, 98.7, 98.7, 97.9, 81.0, 80.2, 78.1, 77.6, 76.5, 76.0, 75.9, 75.5, 75.2, 74.9, 74.8, 74.6, 74.3, 74.2, 73.8, 73.3, 73.2, 72.9, 72.6, 71.2, 71.1, 70.1, 69.4, 68.9, 67.8, 67.79, 67.75, 67.6, 67.22, 67.20, 65.8, 65.3, 63.0, 62.5, 58.0, 57.8, 55.1, 53.33, 53.29, 52.97, 52.93, 51.5, 45.8, 45.6, 31.9, 28.9, 26.7, 26.2, 23.4, 22.7, 20.8; ^{31}P NMR (162 MHz, $CDCl_3$) δ 10.99, 10.43, 10.36, 10.30; HRMS (ESI-TOF) m/z: $[M + Na]^+$ Calcd for $C_{77}H_{98}N_5O_{20}PNa$ 1466.6435; Found 1466.6454.

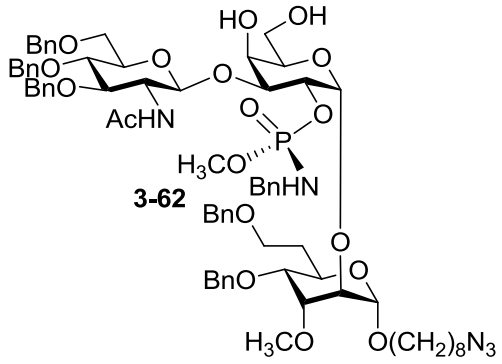


8-Azidooctyl 2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranosyl-(1→3)-(2*R*)-2-*O*-(methyl *N*-benzylphosphoramidyl)-α-D-galactopyranosyl-(1→2)-4,7-di-*O*-benzyl-6-deoxy-3-*O*-methyl-α-D-*altro*-heptopyranoside (3-61)

To a stirred solution of phosphoramidate (***R*)-3-54** (144 mg, 0.0959 mmol) in THF (2 mL) and pyridine (4 mL) was added 70% HF·pyridine (15 μL, 0.58 mmol based on HF). The reaction mixture stirred for 6 h, excess HF was quenched by the addition of saturated aqueous $NaHCO_3$ solution (25 mL) and the reaction mixture was extracted with EtOAc (100 mL). The aqueous layer was separated and further extracted with EtOAc (2 × 100 mL). The combined organic phases were washed with brine (25 mL), dried over anhydrous Na_2SO_4, filtered, concentrated and the resulting crude product was purified by column chromatography (5%

CH_3OH in EtOAc) to afford diol **3-61** (119 mg, 91%) as a colorless viscous liquid: $[\alpha]^{22}_D$ +61.2 (*c* 0.19, $CHCl_3$); ^{1}H NMR (700 MHz, $CDCl_3$) δ 7.35–7.19 (m, 30 H, ArH), 6.24 (d, J = 7.0 Hz, 1 H, $N\underline{H}COCH_3$), 5.42 (d, J = 4.0 Hz, 1 H, H-1_{Gal}), 4.77, 4.66 (ABq, J = 11.4 Hz, 2 H, 2 × O$C\underline{H}_2$Ph), 4.77 (d, J = 11.2 Hz, 1 H, O$C\underline{H}_2$Ph), 4.75 (s, 1 H, H-1_{AltHep}), 4.67 (d, J = 8.4 Hz, 1 H, H-1_{GlcN}), 4.61 (d, J = 11.9 Hz, 1 H, O$C\underline{H}_2$Ph), 4.56 (ddd, J = 9.5, 9.3, 4.0 Hz, 1 H, H-2_{Gal}), 4.53–4.49 (m, 3 H, 3 × O$C\underline{H}_2$Ph), 4.55 (d, J = 11.9 Hz, 1 H, O$C\underline{H}_2$Ph), 4.55 (s, 2 H, 2 × O$C\underline{H}_2$Ph), 4.16–4.11 (m, 4 H, H-5_{AltHep}, H-4_{Gal} and 2 × NH$C\underline{H}_2$Ph), 3.94 (dd, J = 3.5, 1.4 Hz, 1 H, H-2_{AltHep}), 3.91–3.88 (m, 2 H, H-3_{Gal} and H-5_{Gal}), 3.85–3.81 (m, 2 H, H-3_{GlcN} and H-$6a_{Gal}$), 3.77 (dd, J = 8.4, 8.4 Hz, 1 H, H-2_{GlcN}), 3.73 (app t, J = 3.5 Hz, 1 H, H-3_{AltHep}), 3.69–3.64 (m, 2 H, H-$6b_{Gal}$ and H-$6a_{GlcN}$), 3.61–3.51 (m, 7 H, H-$6b_{GlcN}$, H-4_{GlcN}, H-5_{GlcN}, H-4_{AltHep}, H-$7a_{AltHep}$, H-$7b_{AltHep}$ and O$C\underline{H}_2CH_2$), 3.57 (d, J = 11.2 Hz, 3 H, POC$\underline{H}_3$), 3.33 (s, 3 H, OC$\underline{H}_3$), 3.28 (dt, J = 9.1, 6.3 Hz, 1 H, O$C\underline{H}_2CH_2$), 3.22 (t, J = 7.0 Hz, 2 H, 2 × $C\underline{H}_2N_3$), 2.54 (br s, 1 H, CH$O\underline{H}$), 2.17 (dddd, J = 14.2, 7.4, 7.0, 2.8 Hz, 1 H, H-$6a_{AltHep}$), 1.83 (s, 3 H, 3 × NHCOC$\underline{H}_3$), 1.69–1.63 (m, 1 H, H-$6b_{AltHep}$), 1.58–1.46 (m, 4 H, 4 × $C\underline{H}_2$), 1.32–1.21 (m, 8 H, 8 × $C\underline{H}_2$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.6 (NH$\underline{C}\underline{O}CH_3$), 140.0 (Ar), 139.9 (Ar), 138.6 (Ar), 138.3 (Ar), 138.2 (Ar), 137.8 (Ar), 137.7 (Ar), 128.7 (Ar), 128.5 (Ar), 128.3 (Ar), 128.0 (Ar), 127.98 (Ar), 127.93 (Ar), 127.8 (Ar), 127.6 (Ar), 127.5 (Ar), 101.1 (C-1_{GlcN}), 98.5 (C-1_{AltHep}), 97.9 (d, J = 1.4 Hz, C-1_{Gal}), 81.0 (C-3_{GlcN}), 78.2 (C-4_{GlcN}), 77.2 (d, J = 6.2 Hz, C-3_{Gal}), 76.4 (C-4_{AltHep}), 75.6 (C-3_{AltHep}), 74.88 (O$\underline{C}H_2$Ph), 74.86 (C-2_{AltHep}), 74.8 (C-5_{GlcN}), 74.5 (O$\underline{C}H_2$Ph), 73.5 (O$\underline{C}H_2$Ph), 72.9 (O$\underline{C}H_2$Ph), 72.1 (d, J = 5.2 Hz, C-2_{Gal}), 71.1 (O$\underline{C}H_2$Ph), 69.8 (C-4_{Gal}), 69.6 (C-5_{Gal}), 68.9 (C-6_{GlcN}), 67.8 (O$\underline{C}H_2CH_2$), 67.2 (C-7_{AltHep}), 65.2 (C-5_{AltHep}), 62.9 (C-6_{Gal}), 58.0 (O$\underline{C}H_3$), 55.8 (C-2_{GlcN}), 52.9 (d, J = 4.9 Hz, PO$\underline{C}H_3$), 51.5 ($\underline{C}H_2N_3$), 45.8 (NH$\underline{C}H_2$Ph), 31.9 (C-6_{AltHep}), 29.5 ($\underline{C}H_2$), 29.3 ($\underline{C}H_2$), 29.1 ($\underline{C}H_2$), 28.8 ($\underline{C}H_2$), 26.7 ($\underline{C}H_2$), 26.1 ($\underline{C}H_2$), 23.4 (NHCO$\underline{C}H_3$); ^{31}P NMR (162 MHz,

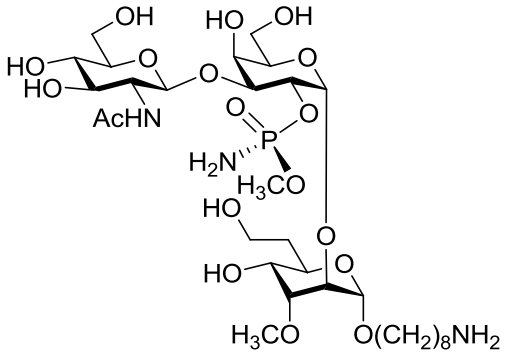
$CDCl_3$) δ 10.47; IR (cast film, $CHCl_3$) ν 3288, 3031, 2928, 2095, 1663, 1554, 1454, 1072, 737 cm^{-1}; HRMS (ESI-TOF) m/z: $[M + Na]^+$ Calcd for $C_{73}H_{94}N_5O_{18}PNa$ 1382.6224; Found 1382.6225.



8-Azidooctyl 2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranosyl-(1→3)-(2*S*)-2-*O*-(methyl *N*-benzylphosphoramidyl)-α-D-galactopyranosyl-(1→2)-4,7-di-*O*-benzyl-6-deoxy-3-*O*-methyl-α-D-altro-heptopyranoside (3-62)

The synthesis of **3-62** was done starting from [(***S***)-**3-54**] (141 mg, 0.0939 mmol) following the procedure described for the preparation of **3-61** to obtain diol **3-62** (115 mg, 90%) as a colorless viscous liquid, which exhibits 10:1 rotameric ratio, as determined by NMR spectroscopy in $CDCl_3$ at 27 °C: $[\alpha]^{22}_D$ +83.3 (*c* 0.06, $CHCl_3$); ^1H NMR (700 MHz, $CDCl_3$, major rotamer) δ 7.34–7.18 (m, 35 H, ArH), 6.67 (d, J = 7.7 Hz, 1 H, $N\underline{H}COCH_3$), 5.22 (d, J = 4.0 Hz, 1 H, H-1_{Gal}), 4.93 (d, J = 7.7 Hz, 1 H, H-1_{GlcN}), 4.77, 4.68 (ABq, J = 11.3 Hz, 2 H, 2 × OC$\underline{H}_2$Ph), 4.76 (d, J = 11.2 Hz, 1 H, OC$\underline{H}_2$Ph), 4.69 (d, J = 2.1 Hz, 1 H, H-1_{AltHep}), 4.63 (d, J = 11.9 Hz, 1 H, OC$\underline{H}_2$Ph), 4.57 (ddd, J = 9.1, 9.1, 4.0 Hz, 1 H, H-2_{Gal}), 4.54–4.48 (m, 4 H, 4 × OC$\underline{H}_2$Ph), 4.46 (s, 2 H, 2 × OC$\underline{H}_2$Ph), 4.18–4.02 (m, 5 H, H-5_{AltHep}, H-4_{Gal}, H-3_{GlcN} and 2 × NHC$\underline{H}_2$Ph), 3.98 (dd, J = 9.1, 2.8 Hz, 1 H, H-3_{Gal}), 3.96–3.93 (m, 2 H, H-2_{AltHep} and H-5_{Gal}), 3.84 (dd, J = 11.9, 5.6 Hz, 1 H, and H-$6a_{Gal}$), 3.75 (dd, J = 4.9, 3.5 Hz, 1 H, H-3_{AltHep}), 3.72–3.64

(m, 2 H, H-6b$_{Gal}$ and H-6a$_{GlcN}$), 3.63–3.50 (m, 8 H, H-6b$_{GlcN}$, H-2$_{GlcN}$, H-4$_{GlcN}$, H-5$_{GlcN}$, H-4$_{AltHep}$, H-7a$_{AltHep}$, H-7b$_{AltHep}$ and OC$\underline{H_2}$CH$_2$), 3.61 (d, J = 11.2 Hz, 3 H, POC$\underline{H_3}$), 3.34 (s, 3 H, OC$\underline{H_3}$), 3.30 (dt, J = 9.1, 7.0 Hz, 1 H, OC$\underline{H_2}$CH$_2$), 3.22 (t, J = 7.0 Hz, 2 H, 2 × C$\underline{H_2}$N$_3$), 2.55 (br s, 1 H, CH$\underline{OH}$), 2.10 (dddd, J = 14.2, 7.4, 7.0, 3.5 Hz, 1 H, H-6a$_{AltHep}$), 1.83 (s, 3 H, 3 × NHCOC$\underline{H_3}$), 1.74–1.66 (m, 1 H, H-6b$_{AltHep}$), 1.58–1.46 (m, 4 H, 4 × C$\underline{H_2}$), 1.34–1.20 (m, 8 H, 8 × C$\underline{H_2}$); ^{13}C NMR (175 MHz, CDCl$_3$, major rotamer) δ 171.8 (NH$\underline{CO}$CH$_3$), 139.67 (Ar), 139.64 (Ar), 138.5 (Ar), 138.4 (Ar), 138.3 (Ar), 137.9 (Ar), 137.8 (Ar), 128.8 (Ar), 128.7 (Ar), 128.5 (Ar), 128.48 (Ar), 128.47 (Ar), 128.33 (Ar), 128.29 (Ar), 128.0 (Ar), 127.96 (Ar), 127.83 (Ar), 127.8 (Ar), 127.6 (Ar), 127.4 (Ar), 100.0 (C-1$_{GlcN}$), 98.9 (C-1$_{AltHep}$), 98.2 (d, J = 2.5 Hz, C-1$_{Gal}$), 80.6 (C-3$_{GlcN}$), 78.3 (C-4$_{GlcN}$), 76.7 (C-3$_{AltHep}$), 76.2 (d, J = 5.5 Hz, C-3$_{Gal}$), 75.8 (C-4$_{AltHep}$), 75.2 (C-2$_{AltHep}$), 74.75 (C-5$_{GlcN}$), 74.74 (O$\underline{C}$H$_2$Ph), 74.7 (O$\underline{C}$H$_2$Ph), 73.4 (O$\underline{C}$H$_2$Ph), 73.2 (d, J = 5.8 Hz, C-2$_{Gal}$), 72.9 (O$\underline{C}$H$_2$Ph), 71.3 (O$\underline{C}$H$_2$Ph), 70.2 (C-4$_{Gal}$), 69.5 (C-5$_{Gal}$), 69.0 (C-6$_{GlcN}$), 67.9 (O$\underline{C}$H$_2$CH$_2$), 67.1 (C-7$_{AltHep}$), 66.5 (C-5$_{AltHep}$), 62.9 (C-6$_{Gal}$), 57.8 (O$\underline{C}$H$_3$), 56.8 (C-2$_{GlcN}$), 53.3 (d, J = 5.3 Hz, PO$\underline{C}$H$_3$), 51.5 ($\underline{C}$H$_2$N$_3$), 45.6 (NH$\underline{C}$H$_2$Ph), 32.1 (C-6$_{AltHep}$), 29.5 ($\underline{C}$H$_2$), 29.4 ($\underline{C}$H$_2$), 29.1 ($\underline{C}$H$_2$), 28.8 ($\underline{C}$H$_2$), 26.7 ($\underline{C}$H$_2$), 26.1 ($\underline{C}$H$_2$), 23.5 (NHCO$\underline{C}$H$_3$); ^{31}P NMR (162 MHz, CDCl$_3$) δ 10.59, 9.78 (major); IR (cast film, CHCl$_3$) ν 3285, 3029, 2921, 2095, 1660, 1550, 1454, 1071, 737 cm^{-1}; HRMS (ESI-TOF) m/z: [M + Na]$^+$ Calcd for C$_{73}$H$_{94}$N$_5$O$_{18}$PNa 1382.6224; Found 1382.6218.

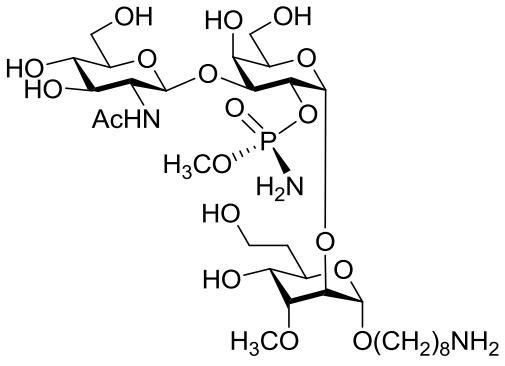


(*R*)-3-3a

8-Aminooctyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-(2*R*)-2-*O*-(methyl phosphoramidyl)-α-D-galactopyranosyl-(1→2)-6-deoxy-3-*O*-methyl-α-D-*altro*-heptopyranoside (*R*)-3-3a

A solution of phosphoramidate diol **3-61** (25.80 mg, 0.01896 mmol) in CH_3OH (4 mL) and CH_2Cl_2 (0.4 mL) was stirred under an argon balloon. To this solution was added AcOH (0.5 mL) followed by $Pd(OH)_2$–C (20 wt%, 60% wet, 52 mg, 0.030 mmol) under an argon atmosphere. The argon balloon was replaced with a H_2 balloon (1 atm) and the solution was flushed with H_2 for 10 sec. The reaction mixture was stirred under H_2 balloon for 12 h and then the H_2 balloon was replaced with argon balloon and flushed with argon for 1 min. The reaction mixture was diluted with CH_3OH (75 mL) and filtered over a pad of Celite-545. The filtrate was concentrated and the resulting crude product was purified by column chromatography on reversed phase (C_{18}) silica gel (50% CH_3OH in water). The solvents were evaporated to dryness and the product was redissolved in H_2O, followed by lyophilization afforded an inseparable mixture of trisaccharide **(*R*)-3-3a** (13.2 mg, 87%) and **3-2a** in 20:1 ratio, as determined by ^1H NMR spectroscopy in D_2O at 27 °C as a white foam: Data for **(*R*)-3-3a**: ^1H NMR (700 MHz, D_2O) δ 5.36 (d, J = 3.5 Hz, 1 H, H-1_{Gal}), 4.87 (s, 1 H, H-1_{AltHep}), 4.69 (d, J = 9.1 Hz, 1 H, H-1_{GlcN}), 4.54 (ddd, J = 9.5, 9.3, 3.5 Hz, 1 H, H-2_{Gal}), 4.33 (s, 1 H, H-4_{Gal}), 4.09 (dd, J = 9.5, 2.1

Hz, 1 H, H-3_{Gal}), 4.05–4.00 (m, 3 H, H-2_{AltHep}, H-5_{AltHep} and H-5_{Gal}), 3.94–3.90 (m, 1 H, H-$6a_{GlcN}$), 3.83 (d, J = 11.2 Hz, 3 H, POC$\underline{H}_3$), 3.81–3.66 (m, 9 H, H-$6b_{GlcN}$, H-2_{GlcN}, , H-$7a_{AltHep}$, H-$7b_{AltHep}$, H-3_{AltHep}, H-4_{AltHep}, H-$6a_{Gal}$, H-$6b_{Gal}$ and OC$\underline{H}_2$CH$_2$), 3.59–3.44 (m, 4 H, H-3_{GlcN} H-4_{GlcN}, H-5_{GlcN} and OC$\underline{H}_2$CH$_2$), 3.49 (s, 3 H, OC$\underline{H}_3$), 2.96 (br s, 2 H, 2 × C$\underline{H}_2$NH$_2$), 2.12–2.06 (m, 1 H, H-$6a_{AltHep}$), 2.10 (s, 3 H, 3 × NHCOC$\underline{H}_3$), 1.76–1.69 (m, 1 H, H-$6b_{AltHep}$), 1.67–1.59 (m, 4 H, 4 × C$\underline{H}_2$), 1.42–1.28 (m, 8 H, 8 × C$\underline{H}_2$); ^{13}C NMR (125 MHz, D$_2$O) δ 175.8 (NH$\underline{C}$OCH$_3$), 102.9 (C-1_{GlcN}), 99.1 (C-1_{AltHep}), 98.0 (C-1_{Gal}), 78.7 (C-3_{AltHep}), 78.5 (d, J = 7.2 Hz, C-3_{Gal}), 76.7 (C-5_{GlcN}), 74.8 (C-3_{GlcN}), 73.7 (C-2_{AltHep}), 72.4 (d, J = 4.7 Hz, C-2_{Gal}), 72.2 (C-5_{Gal}), 70.5 (C-4_{GlcN}), 69.8 (C-4_{Gal}), 69.6 (O$\underline{C}$H$_2$CH$_2$), 68.6 (C-4_{AltHep}), 67.0 (C-5_{AltHep}), 61.9 (C-7_{AltHep}), 61.4 (C-6_{GlcN}), 59.3 (O$\underline{C}$H$_3$), 59.2 (C-6_{Gal}), 56.6 (C-2_{GlcN}), 54.6 (d, J = 5.4 Hz, PO$\underline{C}$H$_3$), 40.7 ($\underline{C}$H$_2$NH$_2$), 34.2 (C-6_{AltHep}), 29.24 (2C, $\underline{C}$H$_2$), 29.15 ($\underline{C}$H$_2$), 26.6 ($\underline{C}$H$_2$), 26.2 (2C, $\underline{C}$H$_2$), 23.3 (NHCO$\underline{C}$H$_3$); ^{31}P NMR (162 MHz, CDCl$_3$) δ 14.29; HRMS (ESI-TOF) m/z: $[M + H]^+$ Calcd for $C_{31}H_{61}N_3O_{18}P$ 794.3682; Found 794.3686.



(*S*)-3-3a

8-Aminooctyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-(2*S*)-2-*O*-(methyl phosphoramidyl)-α-D-galactopyranosyl-(1→2)-6-deoxy-3-*O*-methyl-α-D-altro-heptopyranoside (*S*)-3-3a

The synthesis of **(*S*)-3-3a** was achieved starting from the **3-62** (55.2 mg, 0.0406 mmol) following the procedure described for the prepraration of **(*R*)-3-3a** to obtain the crude product, which was purified by column chromatography on reversed phase silca gel (50% CH_3OH in H_2O). The solvents were evaporated to dryness and the product was redissolved in H_2O, followed by lyophilization afforded an inseparable mixture of trisaccharide **(*S*)-3-3a** (23 mg, 71%) and **3-2a** in 20:1 ratio, as determined by ^1H NMR spectroscopy in D_2O at 27 °C as a white foam: Data for **(*S*)-3-3a**: ^1H NMR (700 MHz, D_2O) δ 5.36 (d, J = 3.5 Hz, 1 H, H-1_{Gal}), 4.86 (s, 1 H, H-1_{AltHep}), 4.71 (d, J = 8.4 Hz, 1 H, H-1_{GlcN}), 4.48 (ddd, J = 9.8, 9.5, 3.5 Hz, 1 H, H-2_{Gal}), 4.31 (s, 1 H, H-4_{Gal}), 4.11 (dd, J = 9.8, 2.1 Hz, 1 H, H-3_{Gal}), 4.05–3.96 (m, 3 H, H-2_{AltHep}, H-5_{AltHep} and H-5_{Gal}), 3.91 (dd, J = 9.8, 5.6 Hz, 1 H, H-$6a_{GlcN}$), 3.82 (d, J = 11.9 Hz, 3 H, POC$\underline{H_3}$), 3.80–3.66 (m, 9 H, H-$6b_{GlcN}$, H-2_{GlcN}, , H-$7a_{AltHep}$, H-$7b_{AltHep}$, H-3_{AltHep}, H-4_{AltHep}, H-$6a_{Gal}$, H-$6b_{Gal}$ and OC$\underline{H_2}$CH$_2$), 3.59–3.44 (m, 4 H, H-3_{GlcN} H-4_{GlcN}, H-5_{GlcN} and OC$\underline{H_2}$CH$_2$), 3.49 (s, 3 H, OC$\underline{H_3}$), 2.94 (br s, 2 H, 2 × C$\underline{H_2}$NH$_2$), 2.15–2.06 (m, 1 H, H-$6a_{AltHep}$), 2.11 (s, 3 H, 3 × NHCOC$\underline{H_3}$), 1.78–1.70 (m, 1 H, H-$6b_{AltHep}$), 1.68–1.58 (m, 4 H, 4 × C$\underline{H_2}$), 1.42–1.30 (m, 8 H, 8 × C$\underline{H_2}$); ^{13}C

NMR (125 MHz, D_2O) δ 175.8 (NH<u>*C*O</u>CH_3), 102.9 (C-1_{GlcN}), 99.1(C-1_{AltHep}), 97.8 (C-1_{Gal}), 78.7 (C-3_{AltHep}), 78.0 (d, J = 7.4 Hz, C-3_{Gal}), 76.6 (C-5_{GlcN}), 74.9 (C-3_{GlcN}), 73.7 (C-2_{AltHep}), 73.2 (d, J = 5.9 Hz, C-2_{Gal}), 72.1 (C-5_{Gal}), 70.5 (C-4_{GlcN}), 70.2 (C-4_{Gal}), 69.6 (O<u>*C*</u>H_2CH_2), 68.5 (C-4_{AltHep}), 67.1 (C-5_{AltHep}), 61.9 (C-7_{AltHep}), 61.4 (C-5_{GlcN}), 59.3 (O<u>*C*</u>H_3), 59.2 (C-6_{Gal}), 56.7 (C-2_{GlcN}), 55.0 (d, J = 5.4 Hz, PO<u>*C*</u>H_3), 40.7 (<u>*C*</u>H_2NH_2), 34.2 (C-6_{AltHep}), 29.25 (2C, <u>*C*</u>H_2), 29.2 (<u>*C*</u>H_2), 26.6 (2C, <u>*C*</u>H_2), 26.2 (<u>*C*</u>H_2), 23.4 (NHCO<u>*C*</u>H_3); ^{31}P NMR (162 MHz, $CDCl_3$) δ 13.85; HRMS (ESI-TOF) m/z: $[M + H]^+$ Calcd for $C_{31}H_{61}N_3O_{18}P$ 794.3682; Found 794.3679.

3.6 References

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(27) Crystallographic data for [(***R***)-**3-54**]: $C_{81}H_{110}N_5O_{18}PSi$, M_r = 1500.79, colourless needle, 0.38 x 0.06 x 0.05 mm, orthorhombic, $P2_12_12_1$ (No. 19), a = 15.1429(3) Å, b = 20.0680(4) Å, c = 26.8195(6) Å, V = 8150.1(3) Å^3, Z = 4, ρ_{calcd} = 1.223 g cm^{-3}, μ = 1.008 mm^{-1}, Cu Kα (1.54178), T = –100 °C, 2θ limit = 144.77°, no. of measured (48945) and independent (15989) reflections, R_{int} = 0.0513, R_1 = 0.0436, wR_2 = 0.1137, largest difference peak and

hole 0.282 and –0.333 e $Å^{-3}$. Data were collected on a Bruker D8 diffractometer, equipped with a Cu microfocus source and an APEX II CCD detector, using 1.0º ω and ϕ scans and 15 s exposures. Face-indexed absorption corrections were performed using SADABS-2016. The structure was solved using intrinsic phasing method of SHELXT-2014 and refined with the program SHELXL-2018. The CIF has been deposited at the Cambridge Crystallographic Data Centre (CCDC 1866111) and can be obtained free of charge via https://www.ccdc.cam.ac.uk/structures/

(28) A common mistake in assigning Cahn–Ingold–Prelog priorities is to view P^{+}–O^{-} as P=O. (a) Cahn, R. S.; Ingold, C.; Prelog, V. *Angew*. *Chem*., *Int*. *Ed*. **1966**, *5*, 385–415. (b) Malla, R. K.; Bandyopadhyay, S.; Spilling, C. D.; Dutta, S.; Dupureur, C. M. *Org*. *Lett*. **2011**, *13*, 3094–3097.

(29) (a) Pinto, B. M.; Reimer, K. B.; Tixidre, A. *Carbohydr*. *Res*. **1991**, *210*, 199–219. (b) Bernardes, G. J. L.; Gamblin, D. P.; Davis, B. G. *Angew*. *Chem*., *Int*. *Ed*. **2006**, *45*, 4007–4011.

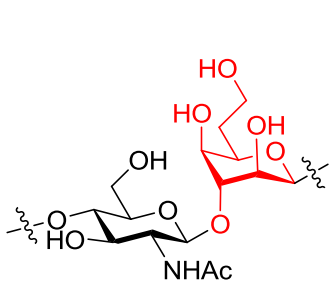
(30) Yamazaki, F.; Nukada, T.; Ito, Y.; Sato, S.; Ogawa, T. *Tetrahedron Lett*. **1989**, *30*, 4417–4420.

Chapter 4

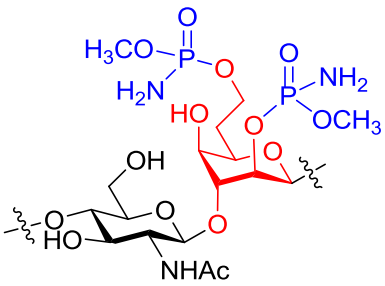
Synthesis of the *Campylobacter jejuni* CG8486 strain (HS4 serotype) capsular polysaccharide repeating unit

4.1 Background

As mentioned in Chapters 1 and 3, there are more than 60 *C. jejuni* serostrains identified to date and it is predicted that each one produces a capsular polysaccharide (CPS) with a different structure.[1-3] These CPSs possess a number of unusual structural motifs and prominent among these are 6-deoxy-heptoses[4,5] (shown in Figure 2-1, Chapter 2) and the *O*-methyl phosphoramidate (MeOPN) group (**3-1**, shown in Figure 3-1, Chapter 3). As an example, Figure 4-1 shows the repeating unit structure of *C. jejuni* CG8486 strain, which possesses both the MeOPN and the 6-deoxyheptose moieties. The MeOPN group is believed to contribute importantly to the virulence of the organism[6] and a vaccine currently in development for the prevention of *C. jejuni* infections incorporates CPS bearing this functionality.[7]



2-11



4-1

Figure 4-1: Repeating units found in CPS of *C. jejuni* CG8486 strain. The 6-deoxyheptose residue is highlighted in red and the MeOPN residue is highlighted in blue.

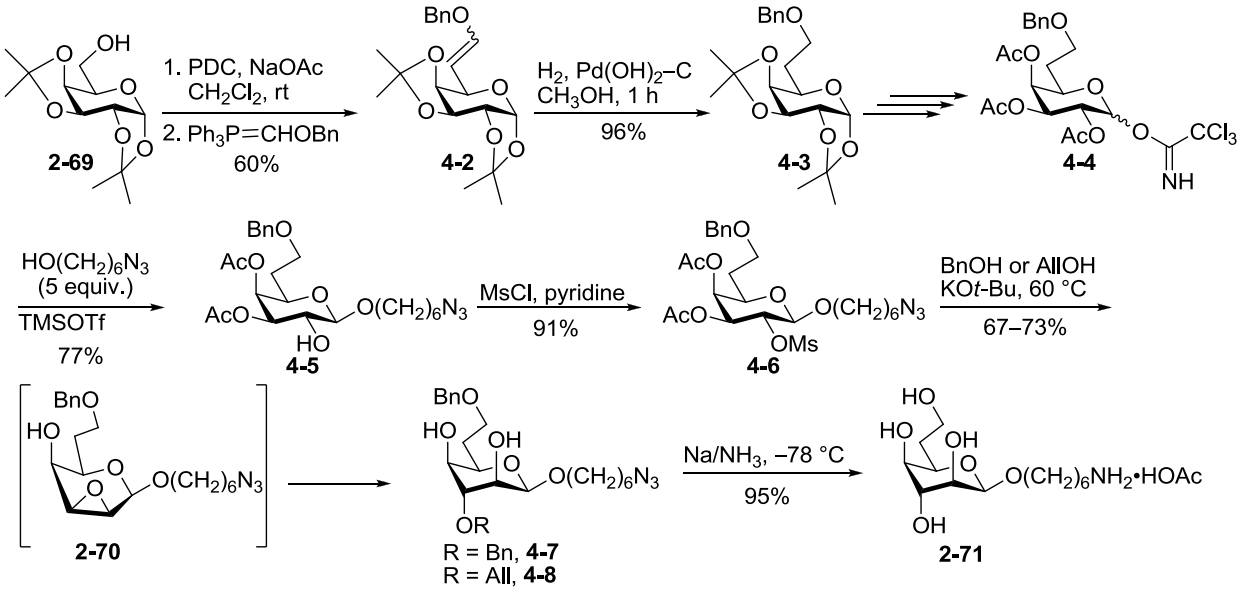
Serologically, the *C. jejuni* CG8486 strain belongs to HS4 serotype. The CPS structutre of this strain was first elucidated by Monteiro, Guerry and co-workers[8] in 2008 and it is composed of approximately 17 disaccharide repeating blocks of 4-substituted *N*-acetyl-β-D-

glucopyranosamine and 3-substituted 6-deoxy-β-D-*ido*-heptopyranose (Figure 4-1). A small number of 6-deoxy-β-D-*ido*-heptopyranose units were observed to carry the MeOPN moieties at the *O*-2 and/or *O*-7 position. Because of the expected antigenic nature of the 6-deoxy-β-D-*ido*-heptopyranose and the MeOPN residues, glycoconjugates containing these disaccharide repeating units could be used towards the development of vaccines or in the generation of antibodies for use in diagnostics. However, these applications require ready access to such compounds. Isolation of CPS from bacteria is difficult and the heterogeneity in the carbohydrate is not ideal for use in vaccines. Therefore, chemical synthesis is needed for the production of homogeneous and well-defined CPS structures to use them as antigens. This Chapter describes the synthesis of the two repeating units (**2-11** and **4-1** in Figure 4-1) found in the CPS of *C. jejuni* CG8486 strain (HS4 serotype) including the one that contains two MeOPN motifs (**4-1**).

4.2 Reported synthesis of 6-deoxy-D-*ido*-heptopyranose

Ling and co-workers[9] reported the first and only synthesis of the 6-deoxy-D-*ido*-heptopyranose as its 6-amino-hexyl glycoside (**2-71**) in 11 steps from commercially available 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose **2-69** through a key 2,3-anhydro-β-D-*talo*-heptopyranoside intermediate **2-70** (Scheme 4-1). Oxidation of **2-69** followed by homologation via Wittig olefination using benzyloxymethylenetriphenylphosphorane generated vinyl ether **4-2** in 60% yield over the two steps. Hydrogenation of alkene **4-2** (to form **4-3**) followed by protecting group manipulations furnished the trichloacetimidate donor **4-4**. Glycosylation of five equivalents of 6-azido-1-hexanol acceptor with **4-4** employing trimethylsilyl trifluoromethanesulfonate (TMSOTf) furnished the 2-*O*-deacetylated β-linked alcohol **4-5**. The use of an excess of the acceptor is essential for the 2-*O*-deacetylation to occur. A similar 2-*O*-deacetylation was observed earlier by Bundle and co-workers,[10] when activating the 1,2-

orthoester with $BF_3 \cdot OEt_2$ in the presence of excess allyl alcohol (AllOH). Mesylation of **4-5** furnished the 2-*O*-mesylate **4-6**. Double inversion at C-2 and C-3 was achieved by the treatment of **4-6** with either benzyl alcohol and KO*t*-Bu or AllOH and KO*t*-Bu at 60 °C to furnish the diols **4-7** or **4-8** in 67% and 73% yield, respectively. The formation of the *ido*-configured product is achieved via 2,3-anhydro-β-D-*talo*-heptopyranoside intermediate **2-70**. Only diol **4-7** was subjected to hydrogenolysis using the Birch reduction conditions. Treatment of **4-7** with sodium in liquid ammonia at –78 °C furnished the 6-deoxy-D-*ido*-heptopyranoside **2-71** in 95% yield.



Scheme 4-1: Key steps involved in the synthesis of 6-deoxy-D-*ido*-heptopyranose **2-71** reported by Ling and co-workers.[9]

4.2.1 Drawbacks of Ling's protocol for the synthesis of *C. jejuni* CG8486 strain CPS fragments

Although Ling's approach[9] for synthesizing the 6-deoxy-D-*ido*-heptopyranose **2-71** involves a small number of steps, the approach has some drawbacks. Firstly, they only reported

the synthesis of the 6-deoxy-D-*ido*-heptopyranose **2-71** and did not report any CPS-related oligosaccharide containing this motif. However, benzylating diol **4-8** (to form **4-9**, Figure 4-2) and subsequent de-allylation would generate alcohol that could be used as an acceptor in the glycosylation to make oligosaccharides. In contrast, to introduce the MeOPN motifs onto the heptose at the C-2 and/or C-7 positions, one needs to have a building block (e.g., **4-10**) with orthogonal protection on O-2, O-3 and O-7. This poses a second and more significant limitation. Differentiating the C-2 and C-4 hydroxyl groups in diol **4-8** is difficult considering that they are in 1,3-cis relation. In addition, installing the temporary protecting group at C-7 in **4-8** (to introduce the MeOPN) is highly difficult as we would need to remove the benzyl group in the presence of allyl protecting group at C-3 and azide in the linker. Therefore, to address these issues, we aimed at synthesizing both the building blocks (**4-9** and **4-10**) required for the synthesis of the CPS fragments **2-11** and **4-1** by a different approach (described in section 4.3).

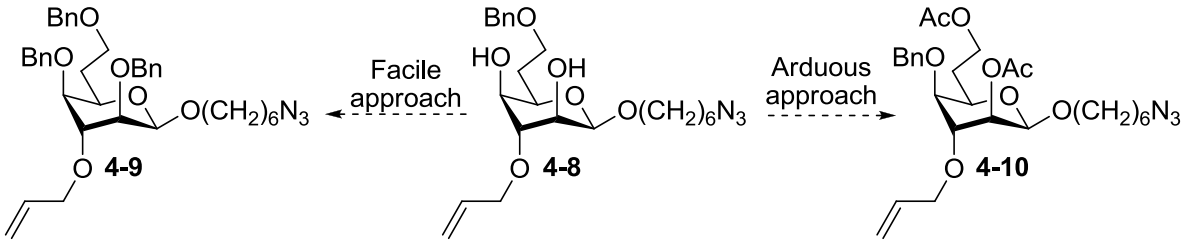


Figure 4-2: Advantages and difficulties associated with Ling's approach[9] for the synthesis of building blocks needed for the assembly of CPS fragments from *C. jejuni* CG8486 strain.

4.3 Our approach for the synthesis of the *C. jejuni* CG8486 strain CPS repeating disaccharide

In designing the synthesis of the *C. jejuni* CG8486 strain CPS disaccharide, we wanted to take advantage of the highly regioselective opening of the 2,3-anhydro-*talo*-heptoside in

obtaining the *ido*-ring configuration using Ling's protocol,[9] while at the same time modifying it to allow the regioselective protecting required for the targets. Our plan was to synthesize both disaccharides (**2-11a** and **4-1a,** Figure 4-3) via a single strategy by exploring the different strategies to differentiate the C-2 and C-7 positions to install the MeOPN groups onto the disaccharide.

The major challenges in the synthesis of **2-11a** and **4-1a** are: 1) the preparation of the 6-deoxyheptose with the D-*ido*-stereochemistry; 2) the stereoselective installation of the 1,2-*cis*-β-D-*ido*-heptoside residue; and 3) the introduction of the MeOPN moiety. We chose to address the first challenge using the regioselective opening of a 2,3-anhydro-*talo*-heptoside using Ling's protocol.[9] Glycosylation with the β-D-*galacto*-heptoside followed by C-2 inversion was our approach in addressing the second. Finally, we envisioned that the MeOPN group could be introduced from one of the three methods previously developed.[11]

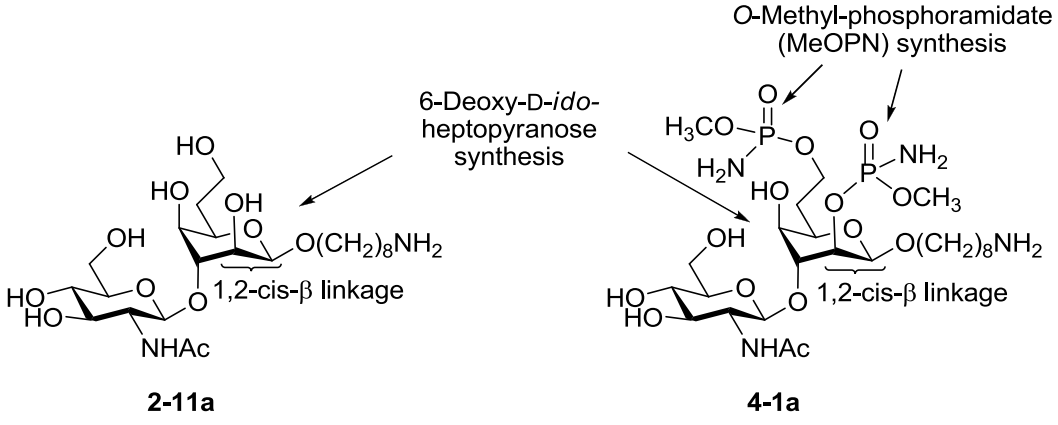


Figure 4-3: Target disaccharides **2-11a** and **4-1a** with anticipated synthetic challenges.

4.3.1 Retrosynthetic analysis of the *C. jejuni* CG8486 strain CPS disaccharides

The retrosynthetic analysis of disaccharides **2-11a** and **4-1a** is outlined in Figure 4-4. Glycosylation of **4-11** (or **4-12**) with the thioglycoside **3-35**[12] would generate the desired β-linked disaccharide. Acceptors **4-11** and **4-12** could be obtained from 2,3-anhydro-β-D-*talo*-heptopyranosides **4-13** and **4-14**, respectively. These anhydrosugars could be accessed from **2-106** via glycosylation of **3-37** and protecting group manipulations. We envisioned that **2-106** could be synthesized from dithioacetal **2-112**, which could be obtained via C-6 homologation of **2-69** (described in section 2.4.3.4 of Chapter 2) using lithium bis(phenylthio)methanide. Finally, the MeOPN group could be introduced from **3-32**.[11b,11c] The synthesis of **2-112** and **2-106** are described in section 2.4.3.4 of Chapter 2.

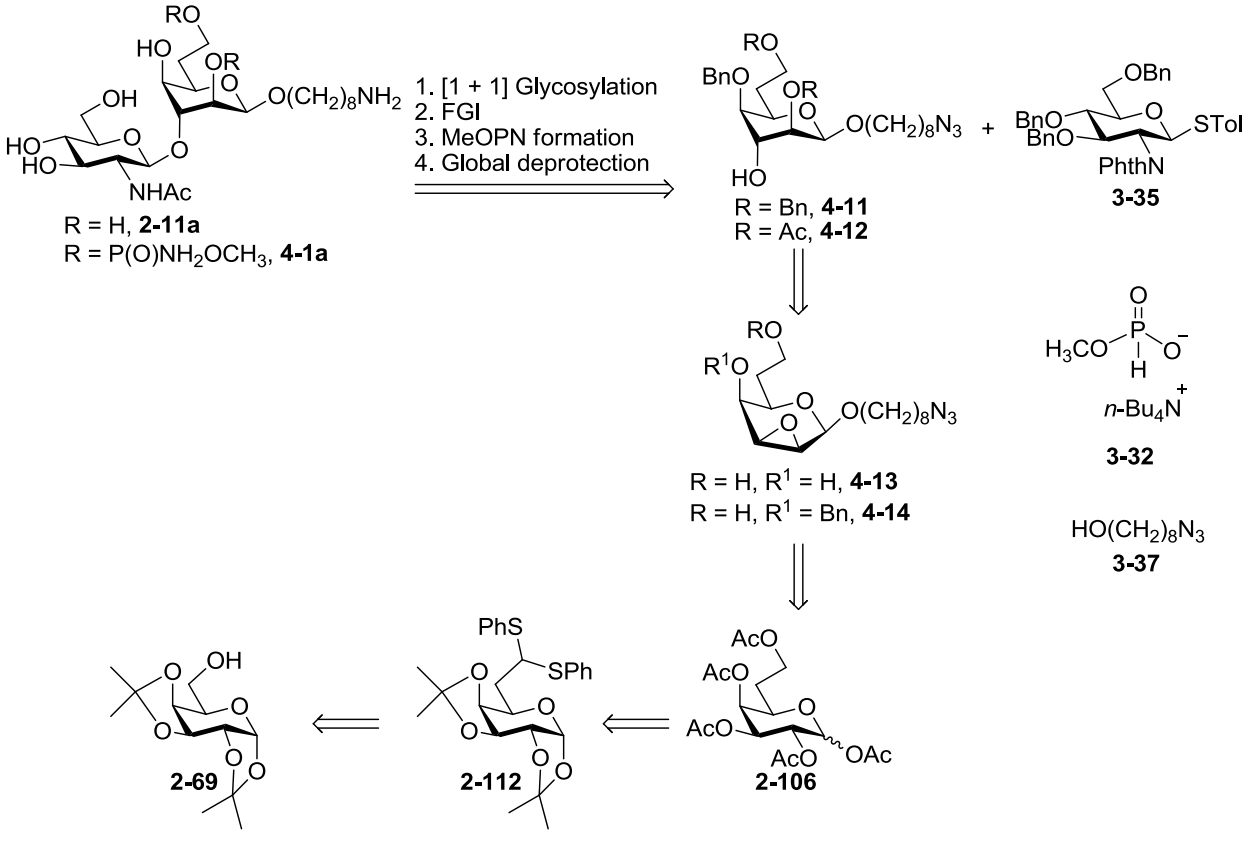


Figure 4-4: Retrosynthetic analysis of the disaccharides **2-11a** and **4-1a**.

4.3.2 Attempted synthesis of the 6-deoxy-*ido*-heptopyranoside acceptor 4-12

Because differentiating the O-2 and O-7 positions in the 6-deoxy-*ido*-heptopyranoside residue is the biggest challenge, we initially focused on the synthesis of acceptor **4-12**. We envisioned that to differentiate O-2 and O-7 positions in **4-12**, we needed to install a benzyl protecting group on O-4. The retrosynthesis of **4-12** is outlined in Figure 4-5. The acceptor **4-12** could be obtained from the diacetate **4-15** by allyl deprotection. Diacetate **4-15** could be obtained from mesylate **4-16** via the 2,3-anhydro-*talo*-heptoside intermediate **4-14**. Mesylate **4-16** could be obtained **4-17**, which, in turn, can be produced from **4-18**. Alcohol **4-18** can be obtained by the glycosylation of 8-azidooctanol (**3-37**) with **4-19**.

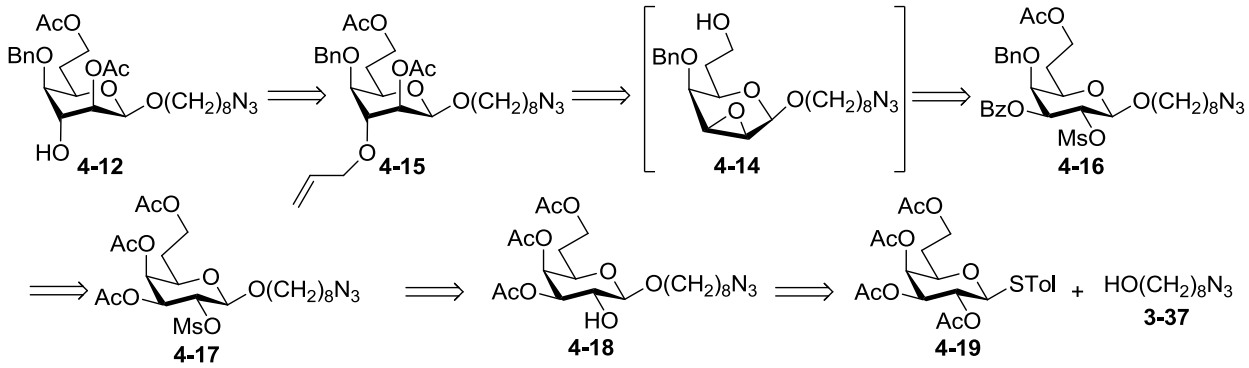
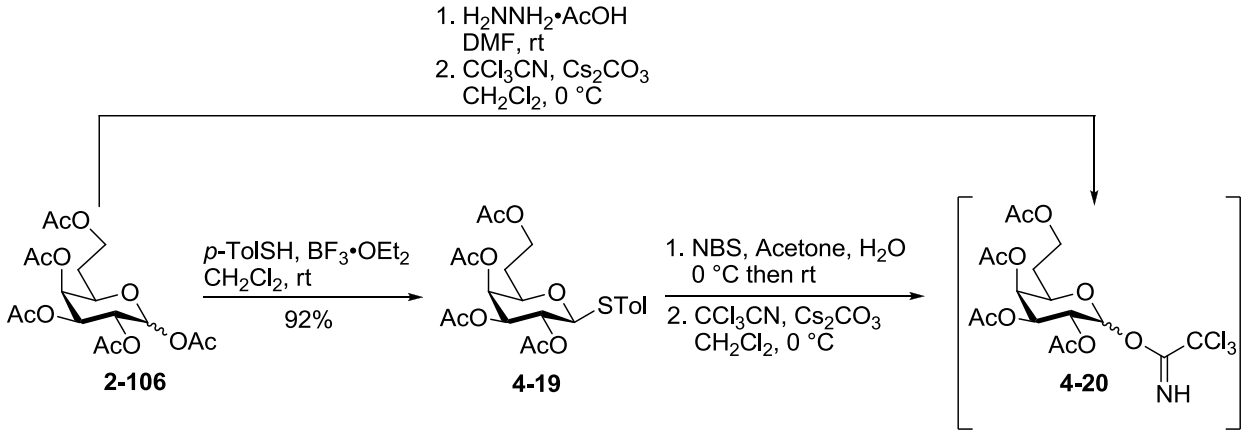


Figure 4-5: Detalied retrosynthetic analysis of the 6-deoxy-*ido*-heptopyranoside acceptor **4-12**

4.3.2.1 Synthesis of the β-linked 6-deoxy-D-*galacto*-heptopyranoside 4-18

The synthesis of **4-18** began with preparing thioglycoside donor **4-19** and trichloroacetimidate donor **4-20** (Scheme 4-2). Initially, 1,2,3,4,7-penta-*O*-acetyl-6-deoxy-α/β-D-*galacto*-heptopyranose **2-106** (its synthesis is described in section 2.4.3.4 of Chapter 2) was converted to *p*-tolyl-1-thioglycoside donor **4-19** in 92% yield using *p*-thiocresol and $BF_3 \cdot OEt_2$. Treatment of **4-19** with *N*-bromosuccinimide followed by the reaction with trichloroacetonitrile

furnished the trichloroacetimidate **4-20**, which was directly used in the glycosylation. Alternatively, treatment of the **2-106** with hydrazinium acetate, followed by reaction with CCl_3CN, furnished the same trichloroacetimidate **4-20** which was used without purification.

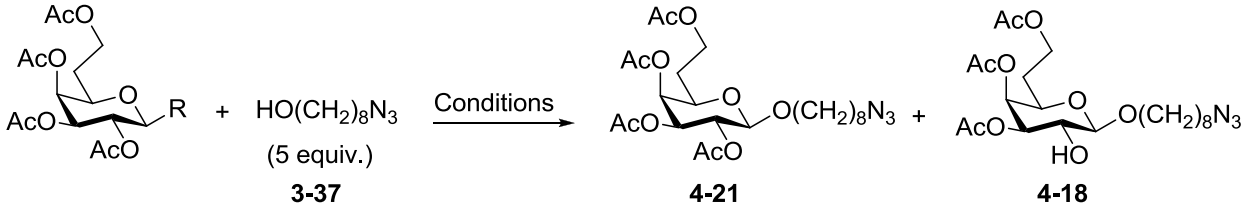


Scheme 4-2: Synthesis of thioglycoside donor **4-19** and trichloroacetimidate donor **4-20**.

Next, we explored glycosylation using donors **4-19** and **4-20** (Table 4-1). Glycosylation of five equivalents of azidooctanol with **4-19**, promoted by NIS and AgOTf afforded the desired β-linked 6-deoxy-D-*galacto*-heptopyranoside **4-18** in only 14% isolated yield. The undesired tetraacetate **4-21** was isolated as the major product in 53% yield. Similar treatment of **3-37** with **4-19** employing NIS and TMSOTf as the activator also furnished **4-18** and **4-21**, both in 42% yield. In contrast, reaction of **3-37** and thioglycoside **4-19** using NIS and TfOH furnished the desired alcohol **4-18** in 37% yield along with the undesired tetraacetate **4-21** in only 8% yield. Alternatively, glycosylation of **3-37** with trichloroacetimidate donor **4-20**, promoted by TMSOTf, afforded the desired alcohol **4-18** in 42% yield along with the **4-21** in only 32% yield over the three steps. The products obtained here using **3-37** are in contrast to the glycosylation of

6-azido-1-hexanol with a glycosyl imidate by Ling.[9] Using five equivalents of the acceptor alcohol, they isolated only the 2-*O*-deacetylated compound **4-5** (shown in Scheme 4-1).

Table 4-1: Conditions explored for the glycosylation of **3-37** with donors **4-19** and **4-20**.



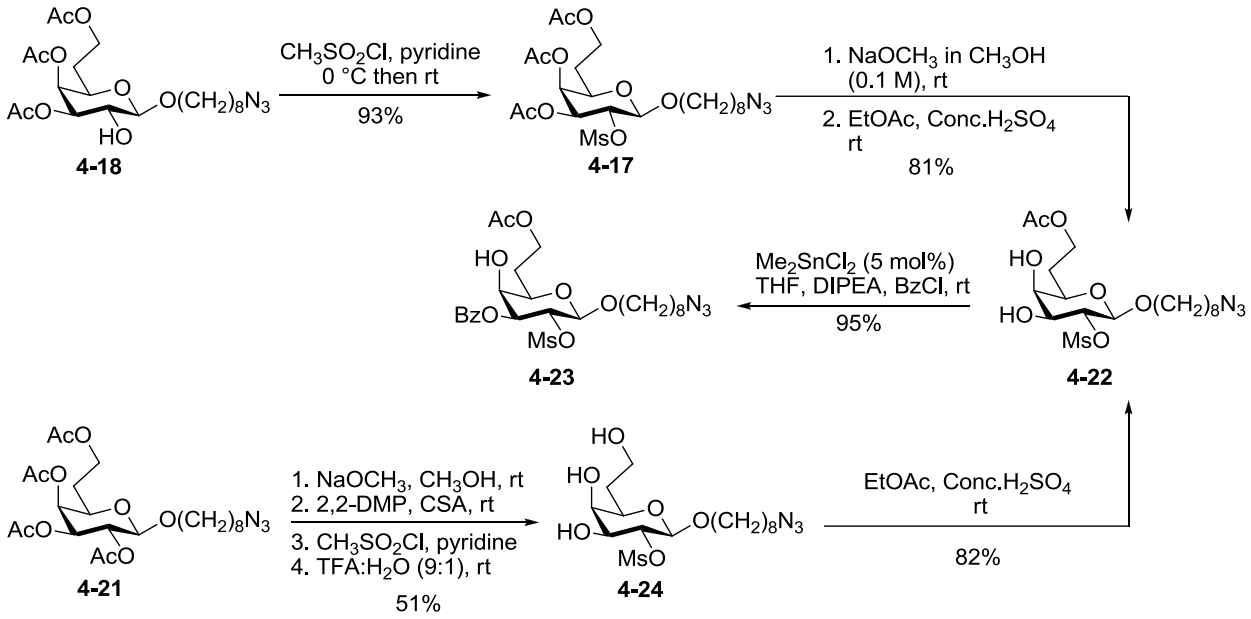
Entry	R	Conditions	**Product** and **yield**[a]	
			4-21	**4-18**
1	STol, **4-19**	NIS, AgOTf, 4 Å MS CH_2Cl_2, –20 °C to 0 °C, 2 h	53%	14%
2	STol, **4-19**	NIS, TMSOTf, 4 Å MS CH_2Cl_2, –20 °C to 0 °C, 2 h	42%	42%
3	STol, **4-19**	NIS, TfOH, 4 Å MS CH_2Cl_2, –20 °C to 0 °C, 2 h	8%	37%
4	$O(C{=}NH)CCl_3$, **4-20**	TMSOTf, 4 Å MS CH_2Cl_2, 0 °C, 2 h	42%	32%

[a]Isolated yield.

4.3.2.2 Attempted synthesis of orthogonally protected β-D-*galacto*-heptopyranoside 4-16.

The first attempt to synthesize orthogonally protected β-D-*galacto*-heptopyranoside **4-16** began by converting the triacetate **4-18** and tetraacetate **4-21** into **4-22** (Scheme 4-3). Treatment of the triacetate **4-18** with MsCl furnished the 2-*O*-mesylate **4-17** in 93% yield. Zemplén deacetylation of **4-17** followed by transesterification using EtOAc[13] and concentrated sulfuric acid afforded the diol **4-22** in 81% yield over the two steps. Alternatively, Zemplén deacetylation of **4-21**, followed by the treatment of the resulting tetrol with 2,2-dimethoxypropane, mesylation using MsCl and hydrolysis of the resulting compound using aqueous 90% trifluoroacetic acid

furnished 2-*O*-mesylate **4-24** in 51% yield over the four steps. Triol **4-24** when subjected to same transesterification reaction conditions as before, affording **4-22** in 82% yield. Regioselective benzoylation of **4-22** furnished alcohol **4-23** in 95% yield.

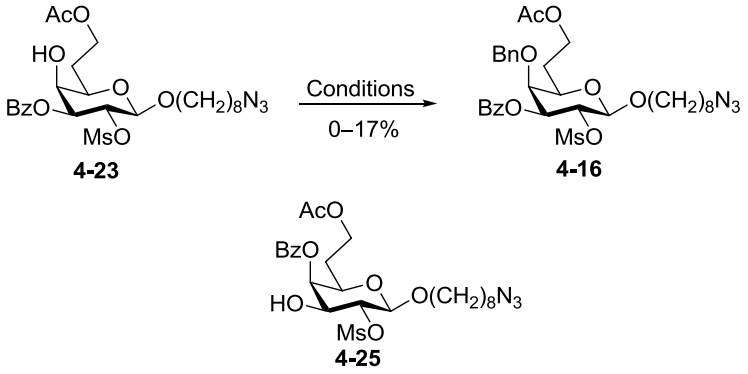


Scheme 4-3: Synthesis of the 6-deoxy-β-D-*galacto*-heptopyranoside derivative **4-23** via **4-22** obtained from either triacetate **4-18** or tetraacetate **4-21**.

Next, we explored different conditions to benzylate the C-4-hydroxyl group in **4-23** (Table 4-2). We first attempted the reaction using silver oxide and BnBr, but under these conditions no desired product **4-16** was formed. The product resulting from benzoate migration to O-4 (**4-25**) was isolated in 35% yield along with the recovered starting material **4-23** in 35% yield. The use of Dudley's neutral benzylation conditions[14] involving the treatment of 2-benzyloxy-1-methylpyridinium triflate and magnesium oxide again resulted in only 10% isolated yield of the desired product **4-16**. In this case also, the benzoate migrated product **4-25** was

isolated in 22% yield along with recovered **4-23** in 43% yield. The use of benzyl trichloroacetimidate[15] furnished the desired product **4-16** in only 17% isolated yield. Due to our inability to produce **4-16** in desirable yield, we abandoned this route.

Table 4-2: Conditions explored for benzylation of **4-23**.



Entry	Conditions	**Product** and **yield**[a]	
		4-16	**4-25**
1	Ag_2O, BnBr, CH_2Cl_2 rt, 14 h	0%[b]	35%
2	2-Benzyloxy-1-methylpyridinium triflate, MgO, toluene, 85 °C, 24 h	10%[c]	22%
3	$BnOC(=NH)CCl_3$, TfOH cyclohexane:CH_2Cl_2 (2:1), rt, 4 h	17%	0%

[a]Isolated yield. [b]35% of starting material (SM) recovered. [c]43% of SM recovered.

4.3.3 Successful synthesis of the 6-deoxy-*ido*-heptopyranoside acceptors 4-11 and 4-12

4.3.3.1 Revised retrosynthetic analysis of the 6-deoxy-*ido*-heptopyranoside acceptor 4-12

Given the difficulty in the benzylation of the C-4 hydroxyl group in **4-23**, another strategy was explored (Figure 4-6) to synthesize **4-12**. The approach investigated was formation of the 2,3-anhydro-*talo*-heptoside intermediate **4-13** before differentiating the O-4 and O-7 hydroxyl groups. This strategy requires controlling of the 2,3-anhydro-*talo*-heptoside formation

from the 2-*O*-mesylate **4-17**. If successful, **4-13** could also be used to generate 6-deoxy-*ido*-heptopyranoside acceptor **4-11** via the tri-*O*-benzyl *ido*-heptoside **4-26**.

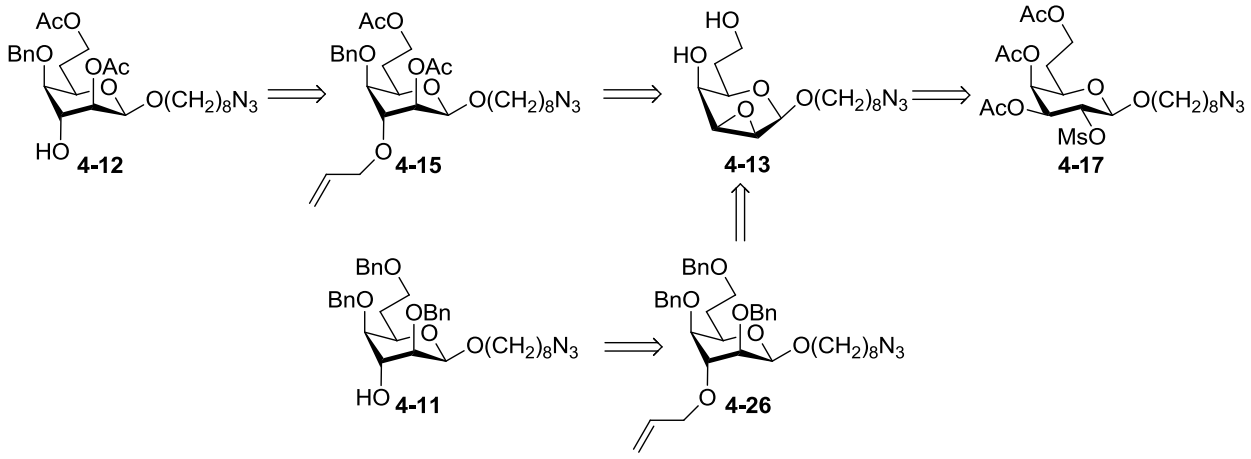
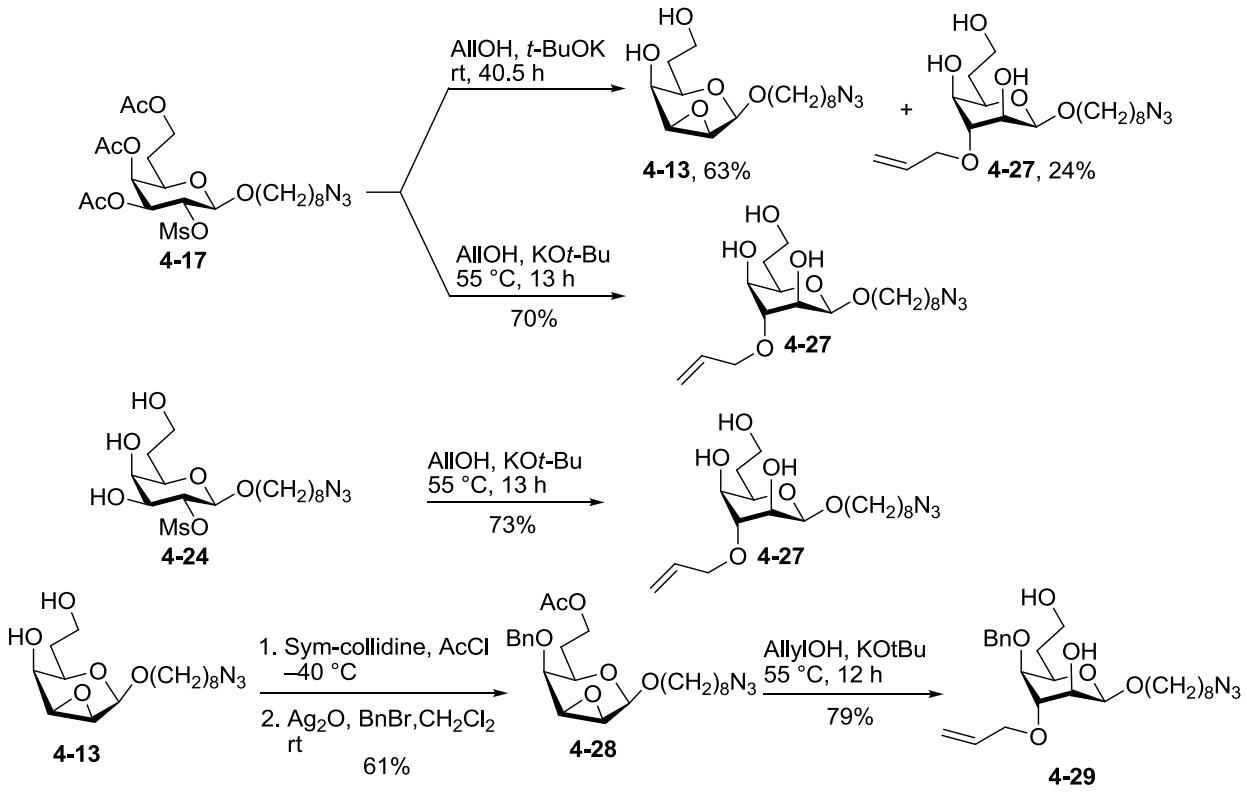


Figure 4-6: Revised retrosynthetic analysis of the 6-deoxy-*ido*-heptopyranoside acceptor **4-12**.

4.3.3.2 Successful formation of the *ido*-heptopyranoside ring and elaboration into the 6-deoxy-*ido*-heptopyranoside acceptors 4-11 and 4-12

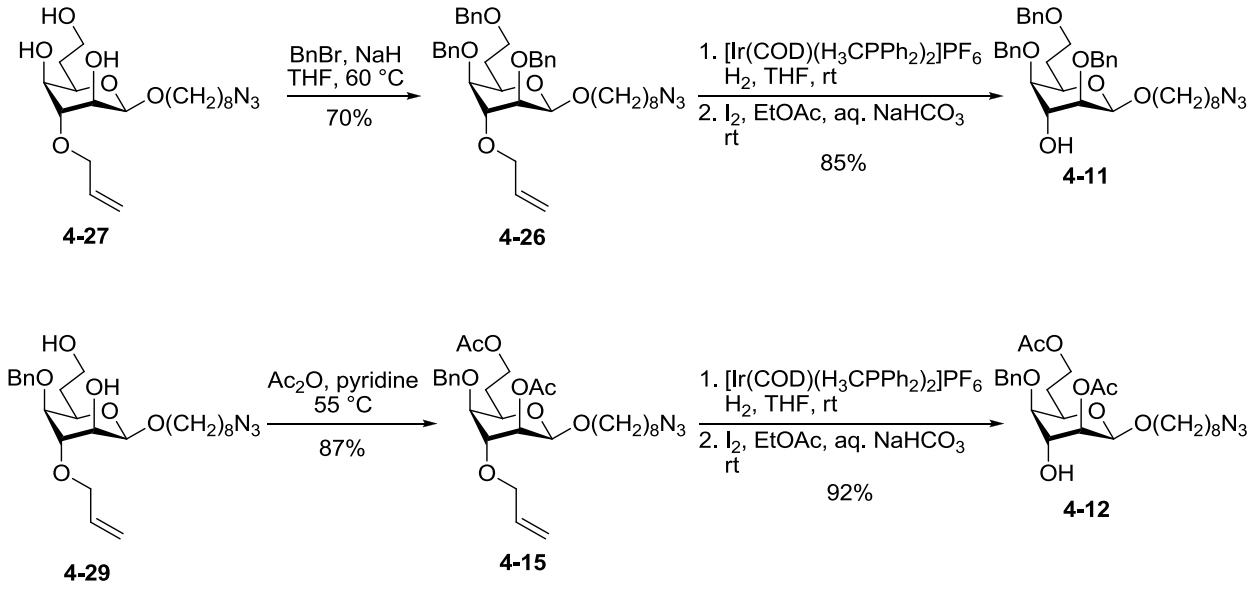
The synthesis of the *ido*-heptopyranoside ring began by exploring different conditions for the 2,3-anhydro-*talo*-heptoside **4-13** formation (Scheme 4-4). Treatment of the 2-*O*-mesylate **4-17** with AllOH and KO*t*-Bu at room temperature for ~41 h resulted in isolation of the desired 2,3-anhydro-*talo*-heptoside diol **4-13** in 63% yield. The triol **4-27** with the *ido*-heptopyranoside ring configuration was also formed in 24% yield. This is in contrast to the observations of Ling and co-workers that under similar conditions only the 2,3-epoxide was isolated.[9] Treatment of 2-*O*-mesylate **4-17** with AllOH and KO*t*-Bu at 55 °C furnished the triol **4-27** with the *ido*-heptopyranoside ring configuration in 70% yield. No 2,3-anhydro-*talo*-heptoside **4-13** was isolated under these conditions. The lower yield (70%) of the latter reaction, compared to the combined yield (87%) of the reaction when conducted at rt, is presumably because of the

decomposition of the epoxide at higher temperatures. Alternatively, subjecting triol **4-24** to similar conditions produced *ido*-heptopyranoside **4-27** in 73% yield. Treatment of diol **4-13** with *sym*-collidine and acetyl chloride at –40 °C followed by benzylation of the resulting primary acetate afforded 4-*O*-benzyl-2,3-anhydro-*talo*-heptoside **4-28** in 61% yield. It is interesting to note that benzylation of the O-4 hydroxyl group proceeded smoothly when the *sym*-collidine contamination (carried over from the acetylation step) is present in the reaction mixture; in its absence, the reaction proceeded significantly slower. Treatment of **4-28** with AllOH and KO*t*-Bu at 55 °C furnished the diol **4-29** with the *ido*-heptopyranoside ring configuration in 79% yield. The sterochemistry was confirmed by analyzing the ^{1}H NMR spectra, which indicated small coupling constants for vicinal protons (^{3}J). For example, for compound **4-27** the anomeric proton was observed at δ_H 4.70 as a singlet, H-4 was observed at δ_H 3.46 as a broad singlet, H-5 was observed at δ_H 3.97 as a doublet of doublets ($J_{5,6a}$ = 9.0 Hz, $J_{5,6a}$ = 4.0 Hz,) and H-2 and H-3 were observed as part of a multiplet at δ_H 3.86–3.73. The 1,2-*cis*-β-linkage at the anomeric center was confirmed from the $^{1}J_{C\text{-}1,H\text{-}1}$ (160.7 Hz). Similarly, for compound **4-29** the anomeric proton was observed at δ_H 4.66 as a singlet, H-2 was observed at δ_H 3.71 as a doublet of doublets ($J_{2,OH}$ = 10.5 Hz, $J_{2,3}$ = 3.0 Hz), H-3 was observed at δ_H 3.88 as an apparent triplet ($J_{2,3}$ = 3.0 Hz, $J_{3,4}$ = 3.0 Hz) and H-4 was observed at δ_H 3.26 as a broad triplet ($J_{3,4}$ = 3.0 Hz, $J_{4,5}$ = 1.5 Hz). The $^{1}J_{C\text{-}1,H\text{-}1}$ was 158.8 Hz, consistent with the 1,2-*cis*-β-linkage.



Scheme 4-4: Successful formation of 6-deoxy-*ido*-heptopyranosides **4-27** and **4-29** via 2,3-anhydro-*talo*-heptoside **4-13**.

With the building blocks **4-27** and **4-29** in hand, we converted them into the desired 6-deoxy-*ido*-heptopyranoside acceptors **4-11** and **4-12** (Scheme 4-5). Benzylation of **4-27** furnished the tri-*O*-benzylated compound **4-26** in 70% yield. Treatment of **4-26** with (1,5-cyclooctadiene)bis(methyldiphenylphosphine)iridium(I) hexafluorophosphate[16] followed by hydrolysis of the resulting enol ether using iodine and aqueous sodium bicarbonate[17] furnished the desired acceptor **4-11** in 85% yield over the two steps. Similarly, acetylation of the diol **4-29** afforded the diacetate **4-15** in 87% yield. Allyl deprotection of **4-15** under same conditions furnished the acceptor **4-12** in 92% yield over the two steps.

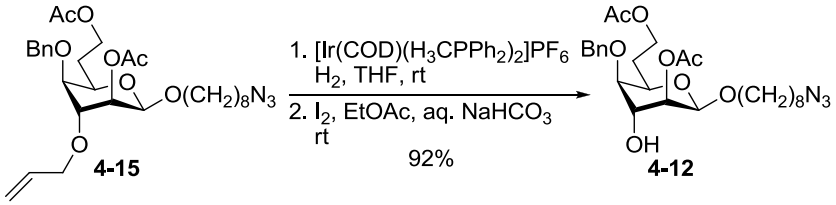


Scheme 4-5: Synthesis of the 6-deoxy-*ido*-heptopyranoside acceptors **4-11** and **4-12**.

We were intrigued by the spectroscopic data of **4-11** and **4-12**. The ^{1}H NMR spectra of **4-26** and **4-15** revealed that when the allyl group is present on O-3, the ^{3}J for all the sugar protons are in the range 0–1 Hz. In contrast, after removal of allyl group, the ^{3}J are between 2.8–8.2 Hz. A comparison of the coupling constants of **4-15** and **4-12** is provided in Table 4-3. Similar changes were observed in the NMR spectra for the allyl deprotection of **4-26**. We hypothesize that when the allyl group is present on O-3, the 6-deoxy-*ido*-heptosides (**4-26** and **4-15**) exist predominantly in the $^{4}C_{1}$ ring conformation. On the other hand, when this hydroxyl group is unprotected, they do not adopt this shape. The ^{3}J observed for the allyl deprotected compounds (**4-11** and **4-12**) are inconsistent with these molecules existing in the $^{1}C_{4}$ conformation. Instead, we propose that they adopt either another ring form (e.g., twist boat, half-chair) or that they exist as a mixture of conformers that equilibrate quickly on the NMR time scale. We note that the idopyranose ring is conformationally flexible[18] and one would anticipate 6-deoxy-*ido*-heptosides

would also able to achieve a number of energetically similar ring forms, given the ring stereochemistry. Further study is necessary to better understand the conformation of these molecules with more certainty.

Table 4-3: Spectroscopic data for allyl deprotection of **4-15**.



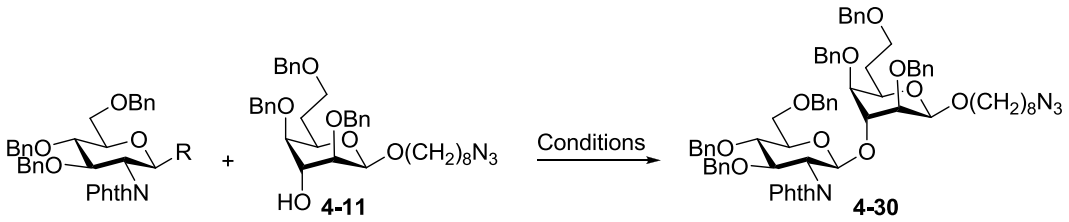
4-15				**4-12**		
Entry	**Proton**	**multiplicity** (^3J in Hz)	$^1J_{C\text{-}1,H\text{-}1}$ (Hz)	**Proton**	**multiplicity** (^3J in Hz)	$^1J_{C\text{-}1,H\text{-}1}$ (Hz)
1	H-1	doublet (1.0)	157.9	H-1	doublet (2.8)	163.9
2	H-2	broad singlet	-	H-2	doublet of doublets (5.6, 2.8)	-
3	H-3	part of multiplet	-	H-3	part of multiplet	-
4	H-4	broad singlet	-	H-4	apparent triplet (4.9, 4.2)	-
5	H-5	part of multiplet	-	H-5	apparent doublet of triplets (10.0, 4.2, 3.5)	-

4.3.4 Glycosylation of 6-deoxy-*ido*-heptopyranoside acceptor 4-11 with thioglycoside donors 3-35 and 3-49

Initially, we focused on the synthesis of the parent disaccharide **2-11a** (shown in Figure 4-4) by exploring (Table 4-4) different glycosylation conditions using acceptor **4-11** and thioglycosides **3-35**[12] and **3-49**[19]. Glycosylation of **4-11** with **3-35**, promoted by NIS and

TMSOTf, did not afford the desired β-linked disaccharide **4-30**. Under these conditions, only unidentified compounds were isolated along with the unreacted acceptor **4-11** in 30% yield. Similar treatment of **4-11** with the thioglycoside **3-35** employing NIS and TfOH as the activator furnished desired β-linked disaccharide **4-30** only in 19% yield together with unreacted acceptor **4-11** in 63% yield. Fortunately, glycosylation of the 6-deoxy-*ido*-heptopyranoside acceptor **4-11** with the trichloroacetimidate donor **3-49**, promoted by TMSOTf, afforded the desired β-linked disaccharide **4-30** in 75% isolated yield.

Table 4-4: Conditions explored for the glycosylation of acceptor **4-11** with donors **3-35** and **3-49**.



Entry	R	Conditions	Product (4-30) yield[a]
1	STol, **3-35**	NIS, TMSOTf, 4 Å MS CH_2Cl_2, –50 °C to 0 °C, 5 h	0%[b]
2	STol, **3-35**	NIS, TfOH, 4 Å MS CH_2Cl_2, 0 °C, 2 h	19%[c]
4	O(C=NH)CCl_3, **3-49**	TMSOTf, 4 Å MS CH_2Cl_2, 0 °C, 2 h	75%

[a]Isolated yield. [b]30% Acceptor recovered. [c]63% Acceptor recovered.

The newly formed β-linkage in disaccharide **4-30** was confirmed by analyzing the NMR spectra. The anomeric proton for glucosamine was observed at δ_H 4.95 as a doublet with large coupling constant ($J_{1,2}$ = 8.5 Hz). The most noticeable feature of the ^{1}H NMR spectrum of **4-30** is an unusual upfield signal at δ_H 2.63, which was assigned to H-4 of the 6-deoxy-*ido*-heptose residue (Figure 4-7). We speculate that H-4 of the heptose is situated in the shelding cone of one

of the phthalmide carbonyl groups leading to the up-field shift. In support of this, 1D-TROESY analysis of **4-30** indicated that H-1 of the glucosamine is in close proximity to H-3 (as expected) as well as H-4 of the heptose sugar (Figure 4-8). This is possible only if the 6-deoxy-*ido*-heptopyranoside sugar exists in the $^4C_1$ ring conformation (Figure 4-9). This observation is consistent with the results discussed previously for **4-15** and **4-26**. Similar correlations were observed by Monteiro, Guerry and co-workers in the the spectrum of the isolated CPS with the **2-11** repeating unit.[8]

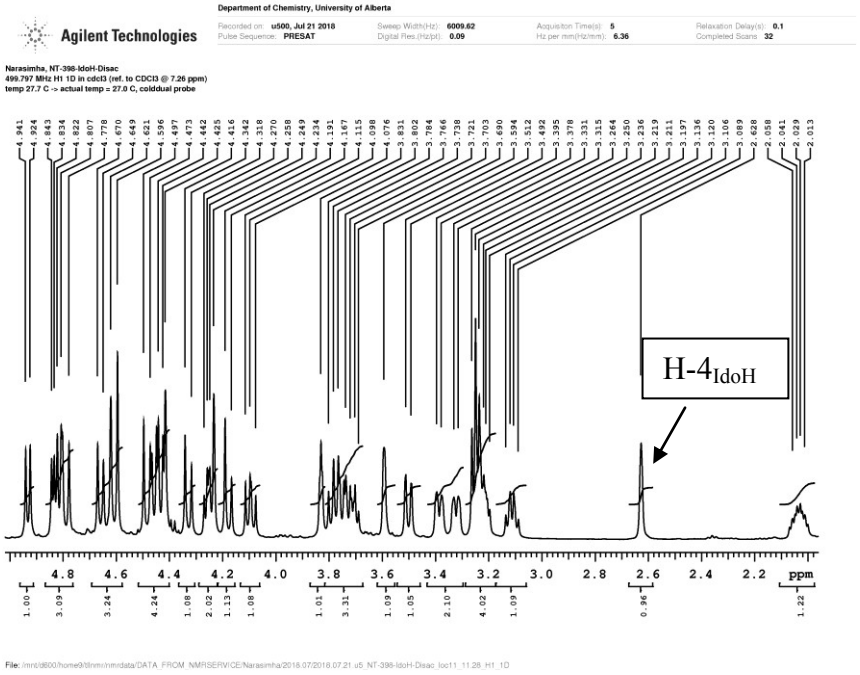


Figure 4-7: ^{1}H NMR spectrum of **4-30** showing the unusual signal at δ_H 2.63 for the *ido*-heptose H-4 signal.

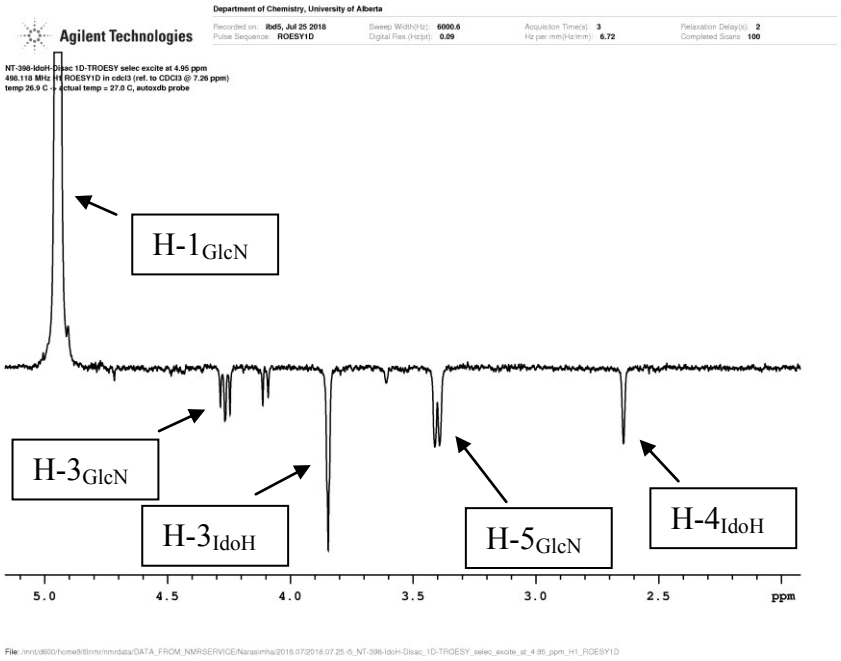
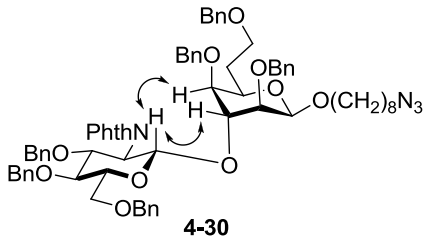


Figure 4-8: 1D-TROESY spectrum of **4-30** irradiated at glucosamine H-1 at δ_H 4.95.



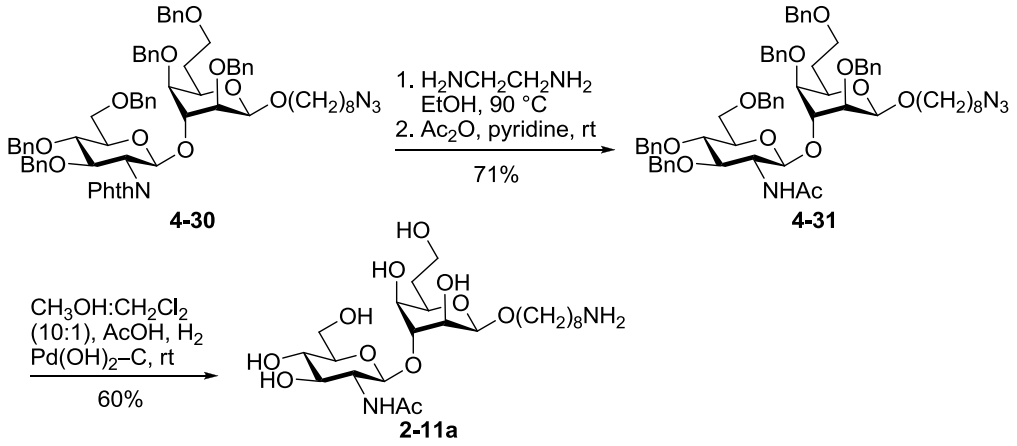
4-30

Figure 4-9: Most likely conformation of **4-30** showing the key correlations observed in the1D-TROESY spectrum.

The $^4C_1$ ring conformation of the *ido*-heptose was further confirmed by the small coupling constants for vicinal protons (^3J): the anomeric proton was observed at δ_H 4.42 as a singlet; H-2 was observed at δ_H 3.59 as a broad singlet; H-3 was observed at δ_H 3.83 as an apparent triplet ($J_{2,3}$ = 2.5 Hz, $J_{3,4}$ = 2.5 Hz) and H-4 was observed at δ_H 2.63 as a broad singlet;

4.3.5 Synthesis of the *C. jejuni* CG8486 strain CPS disaccharide 2-11a

With disaccharide **4-30** in hand, we focused on synthesizing the *C. jejuni* CG8486 strain CPS disaccharide **2-11a** (Scheme 4-6). Treatment of **4-30** with ethylenediamine at 90 °C cleaved the phthalimide; *N*-acetylation was achieved by reaction with acetic anhydride and pyridine. This sequence yielded the disaccharide **4-31** in 71% yield over the two steps. Analysis of the ^{1}H NMR spectrum of **4-31** revealed that H-4 of the 6-deoxy-*ido*-heptose residue was observed at δ_H 3.05, downfield compared to the same proton (δ_H 2.63) signal in the starting material (**4-30**). This observation supports our presumption that the unusual upfield signal of this hydrogen in the spectrum of **4-30** is due to the phthaloyl protecting group. Hydrogenolysis and azide reduction of **4-31** using $Pd(OH)_2$ afforded the parent disaccharide **2-11a** in 60% yield over the two steps.

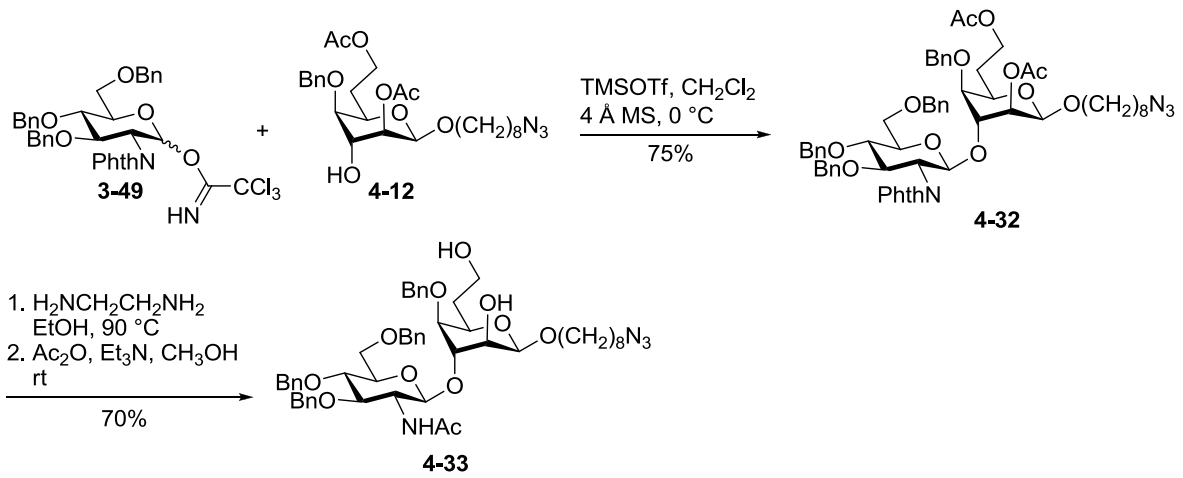


Scheme 4-6: Synthesis of the *C. jejuni* CG8486 strain CPS disaccharide **2-11a**.

4.3.6 Synthesis of the *C. jejuni* CG8486 strain CPS disaccharide 4-1a

4.3.6.1 Synthesis of the diol 4-33 required for the introduction of the MeOPN group

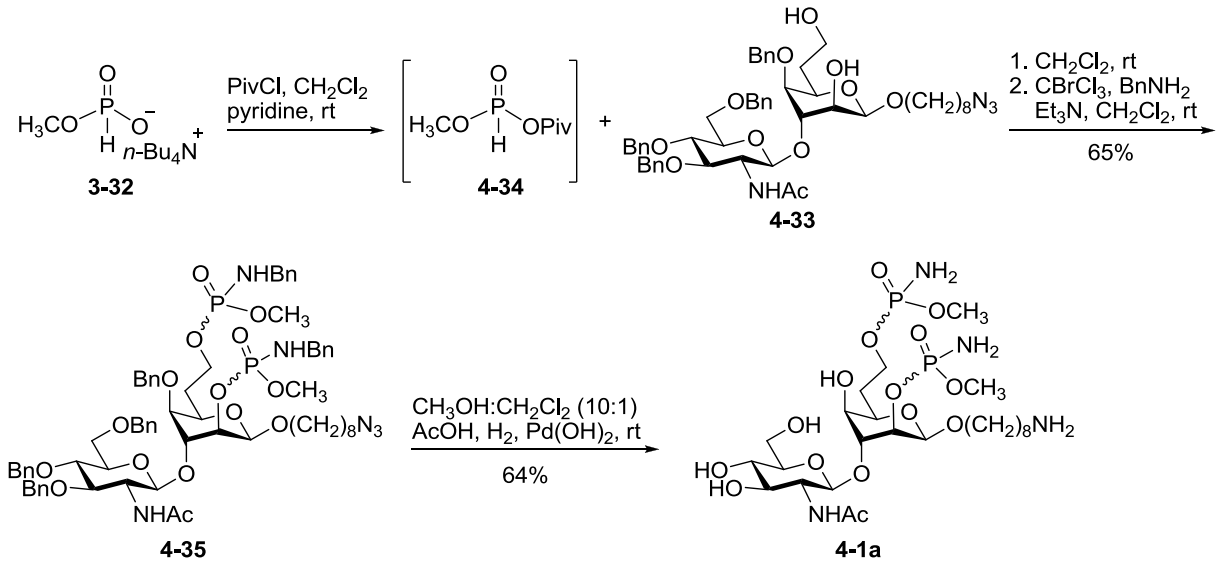
With the route established for the synthesis of the parent disaccharide, we next focused on the synthesis of the disaccharide **4-1a** (shown in Figure 4-4 and Scheme 4-8), which contains the phase variable MeOPN group. The synthesis began with the preparation of the precursor diol **4-33** required for the introduction of the MeOPN group (Scheme 4-7). With the optimized glycosylation conditions established for the synthesis of the disaccharide **4-30** described in section 4.3.4, the preparation of the desired β-linked disaccharide **4-32** (β-linkage, δ_H 5.21, $J_{1,2}$ = 8.5 Hz) was achieved in 75% isolated yield by glycosylation of the 6-deoxy-*ido*-heptopyranoside acceptor **4-12** with the trichloroacetimidate donor **3-49**, promoted by TMSOTf. Similar to disaccharide **4-30**, analysis of the ^{1}H NMR spectrum of **4-32** revealed an upfield signal at δ_H 2.67 for the H-4 of the 6-deoxy-*ido*-heptose residue. Also, similar to **4-30**, 1D-TROESY analysis revealed that H-1 of glucosamine in **4-32** is in close proximity to both H-3 and H-4 of the heptose sugar supporting the $^4C_1$ ring conformation for the 6-deoxy-*ido*-heptopyranoside ring. Treatment of **4-32** with ethylenediamine at 90 °C cleaved both the phthalimide and acetate esters. Next, selective *N*-acetylation of the resulting amine was achieved by reaction with acetic anhydride, triethylamine and methanol. This sequence yielded disaccharide **4-33** in 70% yield over the two steps. Simliar to **4-31**, in the ^{1}H NMR spectrum of **4-33**, H-4 of the 6-deoxy-*ido*-heptose sugar was observed at δ_H 3.20, downfield compared to the same proton (δ_H 2.67) in **4-32**.



Scheme 4-7: Synthesis of the precursor diol **4-33** required for the introduction of the MeOPN group.

4.3.6.2 Installation of the methyl phosphoramidate groups onto 4-33 and elaboration into the target disaccharide 4-1a

With the diol **4-33** in hand, we attempted to install the MeOPN group using the two-step approach[11a] reported earlier by our group (Scheme 4-8). Treatment of the *O*-methyl *H*-phosphonate **3-32** with PivCl afforded an activated *O*-methyl *H*-phosphonate pivalate ester **4-34**. Coupling of **4-34** with the diol **4-33** and subsequent oxidation via the Atherton–Todd reaction,[20] furnished the desired di-*O*-methyl phosphoramidate **4-35** in 65% yield.



3-32 4-34 4-33 4-35 4-1a

Scheme 4-8: Introduction of the MeOPN group onto **4-33** and elaboration into the target disaccharide **4-1a**.

The ^{31}P NMR spectrum of **4-35** showed 12 signals (7 major peaks) instead of the anticipated four peaks for two MeOPN groups, which are both produced as a mixture of stereoisomers on phosphorus. A greater than excepted number of peaks was also seen in the ^{31}P NMR spectra of the MeOPN diastereomers (***R***)-**3-54** and (***S***)-**3-54** described in section 3.3.2.7 of Chapter 3. We assume that, like all of the other O-3 substitued 6-deoxy-*ido*-heptopyranose rings we have studied in the project, this residue adopts the $^4C_1$ conformation. If that is the case, then presumably compound **4-35** shows severe 1,3-diaxial interactions. We hypothesized that these 12 resonances arise from steric congestion leading to restricted rotation around one of the phosphoramidate bonds in both MeOPN groups. To test this, we measured the ^{31}P spectrum on **4-35** at 70 °C. Under these conditions, the twelve ^{31}P signals changed in relative intensity, leading to spectra that contained three major signals with one being broad in appearance. This experiment provides support for restricted rotation leading to the doubling of the signals.

Hydrogenolysis and azide reduction of **4-35** using $Pd(OH)_2$ afforded the disaccharide **4-1a** in 64% yield over the two steps. Similar to the MeOPN diastereomers (***R***)-**3-3** and (***S***)-**3-3** (described in section 3.3.2.8 of Chapter 3), the deprotected compound **4-1a** showed limited stability in D_2O; most of the MeOPN group was cleaved within few hours during storage in this solvent. For this deprotected compound, the restricted rotation seen in **4-35** was not observed; thus in the ^{31}P NMR spectrum only four resonances, corresponding to two MeOPN groups, were present.

4.4 Summary

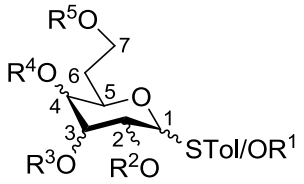
In summary, the work described in this chapter provides an efficient syntheses of the two repeating disaccharide units present in the CPS (**2-11a** and **4-1a**) produced by *C. jejuni* CG8486 strain, including one that contains two MeOPN motifs (**4-1a**) via a single strategy using building blocks **3-32**, **3-35** and **4-11**–**4-13**. While carrying out these syntheses, we found that the 6-deoxy-*ido*-heptopyranoside is in the $^4C_1$ ring conformation when O-3 is substituted by alkylation or glycosylation. When O-3 is unsubstituted, the ring adopts a different conformer, or rapidly-equilibrating mixture of conformers. The structurally well-defined antigenic trisaccharides (**2-11a** and **4-1a**) were synthesized in a form for conjugation to appropriate proteins and/or probes and hence can be tested for their immunogenicity.

4.5 Experimental

4.5.1 General experimental methods

8-Azido-1-octanol was prepared as described.[21] All reactions were carried out in oven-dried glassware. All reagents used were purchased from commercial sources and were used

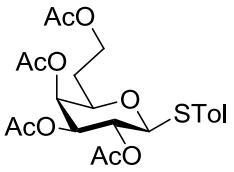
without further purification unless noted. Solvents used in reactions were purified by successive passage through columns of alumina and copper under argon. Unless stated otherwise, all reactions were carried out at room temperature under a positive pressure of argon and were monitored by TLC on Silica 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 at 40 °C. ^{1}H NMR spectra were recorded using 500, or 700 MHz and were referenced to the residual proton signal of $CDCl_3$ (7.26 ppm), CD_3OD (3.30 ppm) or HOD (4.79 ppm). $^{13}C\{^1H\}$ NMR spectra were recorded at 125 (cold probe) or 175 MHz and were referenced to the residual ^{13}C signals of $CDCl_3$ (77.06 ppm) or CD_3OD (49 ppm). $^{31}P\{^1H\}$ NMR spectra were collected at 162 MHz NMR 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, APT, TOCSY, HSQC and HMBC) experiments. ESI-MS spectra (time-of-flight analyzer) were recorded on samples dissolved in THF or CH_3OH with added NaCl or NH_4Cl. Optical rotations were measured at 22 ± 2 °C at the sodium D line (589 nm) in a microcell (10 cm, 1 mL) and are in units of $deg \cdot mL(dm \cdot g)^{-1}$. IR spectra were recorded at room temperature on a Thermo Nicolet 8700 FT-IR spectrometer with a continuum FT-IR microscope attached using IR-transparent silicone wafer cast films. Carbon or proton signals in the 6-deoxy-heptose sugar are numbered as shown in the figure below.



2-140

Figure 4-10: Legend for numbering proton or carbon signals in the 6-deoxy-heptose sugar

4.5.2 Experimental, spectroscopic and analytical data

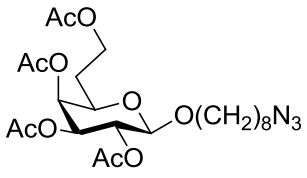


4-19

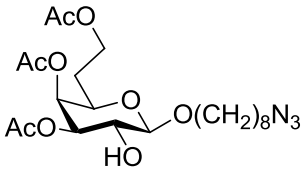
***p*-Tolyl 2,3,4,7-tetra-*O*-acetyl-6-deoxy-1-thio-β-D-*galcto*-heptopyranoside (4-19)**

To a stirred solution of pentaacetate **2-106** (634.1 mg, 1.568 mmol) and *p*-toluenethiol (320 mg, 2.58 mmol) in CH_2Cl_2 (8 mL) at 0 °C was added 48% $BF_3{\cdot}OEt_2$ (0.40 mL, 3.24 mmol) dropwise over 2 min. The reaction mixture was brought to room temperature and stirred for 20 h. Excess $BF_3{\cdot}OEt_2$ was quenched by the addition of saturated aqueous $NaHCO_3$ solution (5 mL) and the reaction mixture was extracted with CH_2Cl_2 (40 mL). The aqueous layer was separated and further extracted with CH_2Cl_2 (2 × 30 mL). The combined organic phases were washed with brine (20 mL), dried over anhydrous Na_2SO_4, filtered, concentrated and the resulting crude product was purified by column chromatography (45% EtOAc in hexane) to afford **4-19** (676 mg, 92%) as a pale yellow viscous liquid: $[\alpha]^{22}_D$ +40.4 (*c* 0.89, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.38–7.35 (m, 2 H, ArH), 7.12–7.09 (m, 2 H, ArH), 5.29 (d, J = 3.0 Hz, 1 H, H-4), 5.20 (app t, J = 10.0, 10.0 Hz, 1 H, H-2), 5.03 (dd, J = 10.0, 3.0 Hz, 1 H, H-3), 4.62 (d, J = 10.0 Hz, 1 H, H-1), 4.18–4.07 (m, 2 H, H-7a and H-7b), 3.75 (dd, J = 9.5, 4.0 Hz, 1 H, H-5), 2.32 (s, 3 H,

C<u>*H*</u>$_3$C$_6$H$_4$S), 2.12 (s, 3 H, 3 × COC<u>*H*</u>$_3$), 2.08 (s, 3 H, 3 × COC<u>*H*</u>$_3$), 1.99 (s, 3 H, 3 × COC<u>*H*</u>$_3$), 1.96 (s, 3 H, 3 × COC<u>*H*</u>$_3$), 1.95–1.90 (m, 1 H, H-6a), 1.77–1.70 (m, 1 H, H-6b); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.7 (<u>*C*</u>OCH$_3$), 170.4 (<u>*C*</u>OCH$_3$), 170.1 (<u>*C*</u>OCH$_3$), 169.5 (<u>*C*</u>OCH$_3$), 138.3 (Ar), 132.9 (Ar), 129.7 (Ar), 128.9 (Ar), 87.0 (C-1), 73.8 (C-5), 72.3 (C-3), 69.6 (C-4), 67.5 (C-2), 60.5 (C-7), 30.0 (C-6), 21.1 (<u>*C*</u>H$_3$C$_6$H$_4$S), 20.9 (CO<u>*C*</u>H$_3$), 20.8 (CO<u>*C*</u>H$_3$), 20.65 (CO<u>*C*</u>H$_3$), 20.58 (CO<u>*C*</u>H$_3$); HRMS (ESI-TOF) m/z: $[M + Na]^+$ Calcd for $C_{22}H_{28}O_9SNa$ 491.1346; Found 491.1345.



4-21



4-18

8-Azidooctyl 2,3,4,7-tetra-*O*-acetyl-6-deoxy-β-D-*galacto*-heptopyranoside (4-21) and **8-Azidooctyl 3,4,7-tri-*O*-acetyl-6-deoxy-β-D-*galacto*-heptopyranoside (4-18)**

Procedure A: A solution of the thioglycoside **4-19** (108.1 mg, 0.2307 mmol) and 8-azidooctanol (198 mg, 1.16 mmol) in dry CH_2Cl_2 (5 mL) was stirred with 4 Å molecular sieves (410 mg) for 30 min. The reaction mixture was cooled to –20 °C and NIS (77 mg, 0.34 mmol) and AgOTf (15 mg, 0.058 mmol) were added sequentially and the solution was stirred between –20 °C and 0 °C for 1.5 h. The AgOTf was quenched by the addition of Et_3N (0.1 mL) and the reaction mixture was diluted with CH_2Cl_2 (30 mL) and filtered over a pad of Celite-545. The filtrate was washed with saturated aqueous $Na_2S_2O_3$ solution (2 × 10 mL). The organic layer was separated and the aqueous layer was further extracted with CH_2Cl_2 (2 × 40 mL). The combined organic phases were washed with brine (20 mL), dried over anhydrous Na_2SO_4, filtered, concentrated and the resulting crude product was purified by column chromatography (17.5% acetone in hexane) to

afford **4-21** (63.5 mg, 53%) as a pale yellow viscous liquid and **4-18** (40% acetone in hexane, 15 mg, 14%) as a pale yellow viscous liquid.

Procedure B: Thioglycoside **4-19** (29.2 mg, 0.0623 mmol) and 8-azidooctanol (54 mg, 0.32 mmol) were coupled using NIS (21 mg, 93 μmol) and TMSOTf (2.0 μL, 11 μmol) in the presence of 4 Å molecular sieves (155 mg) in CH_2Cl_2 (3 mL) between –20 °C and 0 °C for 1.5 h. Following the workup procedure described for NIS and AgOTf (Procedure A), the reaction afforded **4-21** (13.5 mg, 42%) as a pale yellow viscous liquid and **4-18** (12.5 mg, 42%) as a pale yellow viscous liquid.

Procedure C: Thioglycoside **4-19** (44.1 mg, 0.0941 mmol) and 8-azidooctanol (82 mg, 0.48 mmol) were coupled using NIS (31 mg, 0.14 mmol) and TfOH (2.0 μL, 23 μmol) in the presence of 4 Å molecular sieves (165 mg) in CH_2Cl_2 (3 mL) between –20 °C and 0 °C for 1.5 h. Following the workup procedure described for NIS and AgOTf system (Procedure A), the reaction afforded **4-21** (4 mg, 8%) as a pale yellow viscous liquid and **4-18** (16.5 mg, 37%) as a colorless viscous liquid.

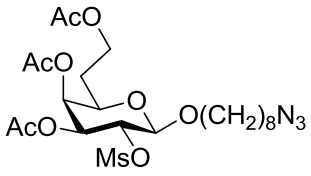
Procedure D: A solution of thioglycoside **4-19** (133.1 mg, 0.2841 mmol) in acetone (2.7 mL) and water (0.3 mL) was cooled to 0 °C. *N*-bromosuccinimide (158 mg, 0.888 mmol) was added, the ice bath was removed and the reaction mixture was stirred at room temperature for 1.5 h. The solution was diluted with CH_2Cl_2 (40 mL) and washed with saturated aqueous $Na_2S_2O_3$ solution (10 mL). The organic layer was separated and the aqueous layer was further extracted with CH_2Cl_2 (2 × 30 mL). The combined organic phases were washed with brine (20 mL), dried over anhydrous Na_2SO_4, filtered, concentrated and the resulting crude product was purified by column chromatography (55% EtOAc in hexane) to afford the corresponding hemiacetal (85 mg, 83%)

as a colorless viscous liquid. A solution of the hemiacetal (61.5 mg, 0.169 mmol) in dry CH_2Cl_2 (2 mL) was cooled to 0 °C. CCl_3CN (0.25 mL, 2.49 mmol) and Cs_2CO_3 (116 mg, 0.356 mmol) were added sequentially at 0 °C and the solution was stirred for 1.5 h. The reaction mixture was diluted with CH_2Cl_2 (30 mL) and filtered over a pad of Celite-545. The filtrate was concentrated to obtain the imidate **4-20** as white foam, which was used directly in the next step. A solution of **4-20** (86.0 mg, 0.170 mmol) and 8-azidooctanol (172 mg, 1.01 mmol) in dry CH_2Cl_2 (5 mL) was stirred with 4 Å molecular sieves (400 mg) for 30 min. The reaction mixture was cooled to 0 °C, TMSOTf (5.0 μL, 0.028 mmol) was added, and the solution was stirred for 1.5 h. The TMSOTf was quenched by the addition of Et_3N (0.1 mL) and the reaction mixture was diluted with CH_2Cl_2 (40 mL) and filtered over a pad of Celite-545. The filtrate was washed with saturated aqueous $NaHCO_3$ solution (15 mL) and the organic layer was separated and the aqueous layer was further extracted with CH_2Cl_2 (2 × 30 mL). The combined organic phases were washed with brine (20 mL), dried over anhydrous Na_2SO_4, filtered, concentrated and the resulting crude product was purified by column chromatography (17.5% acetone in hexane) to afford **4-21** (37 mg, 42%) as a pale yellow viscous liquid and **4-18** (40% acetone in hexane, 25 mg, 32%) as a pale yellow viscous liquid.

Data for **4-21**: $[\alpha]^{22}_D$ +8.5 (*c* 0.61, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.27 (d, J = 3.5 Hz, 1 H, H-4), 5.17 (dd, J = 10.0, 8.0 Hz, 1 H, H-2), 5.01 (dd, J = 10.0, 3.5 Hz, 1 H, H-3), 4.40 (d, J = 8.0 Hz, 1 H, H-1), 4.22–4.11 (m, 2 H, H-7a and H-7b), 3.87 (app dt, J = 9.5, 6.5, 6.3 Hz, 1 H, OC$\underline{H_2}$CH$_2$), 3.72 (dd, J = 9.0, 3.5 Hz, 1 H, H-5), 3.44 (dt, J = 9.5, 7.0, 7.0 Hz, 1 H, OC$\underline{H_2}$CH$_2$), 3.24 (t, J = 7.0 Hz, 2 H, 2 × C$\underline{H_2}$N$_3$), 2.14 (s, 3 H, 3 × COC$\underline{H_3}$), 2.04 (s, 3 H, 3 × COC$\underline{H_3}$), 2.03 (s, 3 H, 3 × COC$\underline{H_3}$), 1.97 (s, 3 H, 3 × COC$\underline{H_3}$), 1.97–1.90 (m, 1 H, H-6a), 1.80–1.72 (m, 1 H, H-6b), 1.61–1.50 (m, 4 H, 4 × C$\underline{H_2}$), 1.38–1.20 (m, 8 H, 8 × C$\underline{H_2}$); ^{13}C NMR (125

MHz, $CDCl_3$) δ 170.9 (<u>C</u>OCH_3), 170.5 (<u>C</u>OCH_3), 170.3 (<u>C</u>OCH_3), 169.4 (<u>C</u>OCH_3), 101.3 (C-1), 71.3 (C-3), 70.1 (O<u>C</u>H_2CH_2), 69.9 (C-5), 69.6 (C-4), 69.1 (C-2), 60.5 (C-7), 51.5 (<u>C</u>H_2N_3), 30.0 (C-6), 29.4 (<u>C</u>H_2), 29.2 (<u>C</u>H_2), 29.16 (<u>C</u>H_2), 29.07 (<u>C</u>H_2), 26.6 (<u>C</u>H_2), 25.7 (<u>C</u>H_2), 20.9 (CO<u>C</u>H_3), 20.8 (CO<u>C</u>H_3), 20.7 (CO<u>C</u>H_3), 20.6 (CO<u>C</u>H_3); IR (cast film, $CHCl_3$) ν 2933, 2858, 2097, 1750, 1225, 1059, 948 cm^{-1}; HRMS (ESI-TOF) m/z: $[M + Na]^+$ Calcd for $C_{23}H_{37}N_3O_{10}Na$ 538.2371; Found 538.2371.

Data for **4-18**: $[\alpha]^{22}_D$ +21.9 (*c* 0.45, $CHCl_3$); ^1H NMR (700 MHz, $CDCl_3$) δ 5.27 (d, *J* = 3.5 Hz, 1 H, H-4), 4.92 (dd, *J* = 9.8, 3.5 Hz, 1 H, H-3), 4.29 (d, *J* = 7.7 Hz, 1 H, H-1), 4.21–4.17 (m, 1 H, H-7a), 4.15–4.11 (m, 1 H, H-7b), 3.91 (app dt, *J* = 9.3, 7.0, 6.7 Hz, 1 H, OC<u>*H*</u>$_2CH_2$), 3.77 (dd, *J* = 9.8, 7.7 Hz, 1 H, H-2), 3.73 (dd, *J* = 9.8, 4.2 Hz, 1 H, H-5), 3.53 (app dt, *J* = 9.3, 7.4, 7.0 Hz, 1 H, OC<u>*H*</u>$_2CH_2$), 3.24 (app t, *J* = 7.7, 6.3 Hz, 2 H, 2 × C<u>*H*</u>$_2N_3$), 2.33 (br s, 1 H, CH*O*<u>*H*</u>), 2.12 (s, 3 H, 3 × COC<u>*H*</u>$_3$), 2.05 (s, 3 H, 3 × COC<u>*H*</u>$_3$), 2.04 (s, 3 H, 3 × COC<u>*H*</u>$_3$), 1.93 (dddd, *J* = 14.4, 9.8, 4.9, 4.9 Hz, 1 H, H-6a), 1.78–1.73 (m, 1 H, H-6b), 1.67–1.56 (m, 4 H, 4 × C<u>*H*</u>$_2$), 1.36–1.31 (m, 8 H, 8 × C<u>*H*</u>$_2$); ^{13}C NMR (175 MHz, $CDCl_3$) δ 170.9 (<u>C</u>OCH_3), 170.4 (2C, 2 × <u>C</u>OCH_3), 103.2 (C-1), 72.9 (C-3), 70.4 (O<u>C</u>H_2CH_2), 70.0 (C-5), 69.7 (C-4), 69.2 (C-2), 60.5 (C-7), 51.5 (<u>C</u>H_2N_3), 30.0 (C-6), 29.5 (<u>C</u>H_2), 29.2 (<u>C</u>H_2), 29.0 (<u>C</u>H_2), 28.8 (<u>C</u>H_2), 26.6 (<u>C</u>H_2), 25.8 (<u>C</u>H_2), 20.9 (CO<u>C</u>H_3), 20.8 (CO<u>C</u>H_3), 20.7 (CO<u>C</u>H_3); IR (cast film, $CHCl_3$) ν 3476, 2933, 2858, 2097, 1746, 1370, 1245, 1070, 947 cm^{-1}; HRMS (ESI-TOF) m/z: $[M + Na]^+$ Calcd for $C_{21}H_{35}N_3O_9Na$ 496.2266; Found 496.2260.

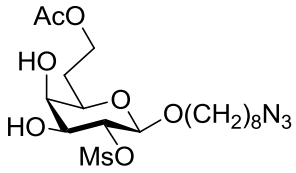


4-17

8-Azidooctyl 3,4,7-tri-*O*-acetyl-6-deoxy-2-*O*-methanesulfonyl-β-D-*galacto*-heptopyranoside (4-17)

To a 0 °C solution of triacetate **4-18** (425 mg, 0.898 mmol) in pyridine (4 mL) was added methanesulfonyl chloride (0.14 mL, 1.8 mmol). The reaction mixture was warmed to room temperature over 3 h and then diluted with CH_2Cl_2 (30 mL) before being washed with saturated aqueous $NaHCO_3$ solution (10 mL). The organic layer was separated and the aqueous layer was further extracted with CH_2Cl_2 (2 × 20 mL). The combined organic phases were washed with brine (10 mL), dried over anhydrous Na_2SO_4, filtered, concentrated and the resulting crude product was purified by column chromatography (37.5% EtOAc in hexane) to afford **4-17** (460 mg, 93%) as a colorless viscous liquid: $[\alpha]^{22}_D$ +6.7 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.31 (d, J = 3.5 Hz, 1 H, H-4), 5.06 (dd, J = 10.0, 3.5 Hz, 1 H, H-3), 4.67 (dd, J = 10.0, 8.0 Hz, 1 H, H-2), 4.48 (d, J = 8.0 Hz, 1 H, H-1), 4.23–4.17 (m, 1 H, H-7a), 4.15–4.10 (m, 1 H, H-7b), 3.90 (app dt, J = 9.5, 7.0, 7.0 Hz, 1 H, OC$\underline{H_2}$CH$_2$), 3.74 (dd, J = 9.0, 3.5 Hz, 1 H, H-5), 3.54 (app dt, J = 9.5, 7.0, 7.0 Hz, 1 H, OC$\underline{H_2}$CH$_2$), 3.24 (t, J = 7.0 Hz, 2 H, 2 × C$\underline{H_2}$N$_3$), 3.06 (s, 3 H, OSO$_2$C$\underline{H_3}$), 2.15 (s, 3 H, 3 × COC$\underline{H_3}$), 2.05 (s, 3 H, 3 × COC$\underline{H_3}$), 2.04 (s, 3 H, 3 × COC$\underline{H_3}$), 1.96–1.88 (m, 1 H, H-6a), 1.81–1.73 (m, 1 H, H-6b), 1.65–1.54 (m, 4 H, 4 × C$\underline{H_2}$), 1.41–1.26 (m, 8 H, 8 × C$\underline{H_2}$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.9 (C$\underline{O}$CH$_3$), 170.3 (C$\underline{O}$CH$_3$), 170.0 (C$\underline{O}$CH$_3$), 100.4 (C-1), 77.4 (C-2), 70.6 (C-3), 70.3 (O$\underline{C}$H$_2$CH$_2$), 70.1 (C-5), 69.9 (C-4), 60.3 (C-7), 51.4 ($\underline{C}$H$_2$N$_3$), 39.0 (OSO$_2\underline{C}$H$_3$), 29.9 (C-6), 29.5 ($\underline{C}$H$_2$), 29.2 ($\underline{C}$H$_2$), 29.0 ($\underline{C}$H$_2$), 28.8 ($\underline{C}$H$_2$), 26.6

($\underline{C}H_2$), 25.8 ($\underline{C}H_2$), 20.9 (CO$\underline{C}H_3$), 20.7 (CO$\underline{C}H_3$), 20.6 (CO$\underline{C}H_3$); IR (cast film, $CHCl_3$) ν 3033, 2931, 2857, 2097, 1748, 1240, 1050, 846 cm^{-1}; HRMS (ESI-TOF) m/z: $[M + NH_4]^+$ Calcd for $C_{22}H_{41}N_4O_{11}S$ 569.2487; Found 569.2482.

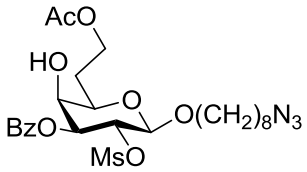


4-22

8-Azidooctyl 7-*O*-acetyl-6-deoxy-2-*O*-methanesulfonyl-β-D-*galacto*-heptopyranoside (4-22)

To a stirred solution of thioglycoside **4-17** (40 mg, 0.073 mmol) in CH_3OH (1.8 mL) and CH_2Cl_2 (0.2 mL) was added sodium methoxide in methanol (0.556 M, 0.350 mL, 0.195 mmol). After stirring for 2 h, Amberlite IR-120 resin (H^+ form) was added to neutralize the solution and, following filtration, the solution was concentrated from the filtrate to give the triol as a pale yellow viscous liquid, which was used directly in the next step. Concentrated H_2SO_4 (10 μL, 0.19 mmol) was added to a stirred solution of the crude triol in EtOAc (5 mL) and the resulting solution was stirred for 17 h. The reaction mixture was diluted with EtOAc (20 mL) and transferred to a separatory funnel and saturated aqueous $NaHCO_3$ solution was added until no further effervescence was observed. The organic layer was separated and the aqueous layer was further extracted with EtOAc (2 × 30 mL). The combined organic phases were washed with brine (20 mL), dried over anhydrous Na_2SO_4, filtered, concentrated and the resulting crude product was purified by column chromatography (60% EtOAc in hexane) to afford **4-22** (27.5 mg, 81%) as a colorless viscous liquid: $[\alpha]^{22}_D$ +0.2 (*c* 0.37, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 4.46 (dd, *J* = 9.0, 8.0 Hz, 1 H, H-2), 4.37 (d, *J* = 8.0 Hz, 1 H, H-1), 4.25–4.15 (m, 2 H, H-7a and H-7b), 3.90–3.84 (m, 2 H, H-4 and OC$\underline{H_2}CH_2$), 3.80–3.74 (m, 1 H, H-3), 3.65 (d, *J* = 5.5 Hz, 1 H,

CH*O*<u>*H*</u>), 3.55 (dd, *J* = 9.0, 4.0 Hz, 1 H, H-5), 3.50 (app dt, *J* = 9.4, 6.6, 6.5 Hz, 1 H, OC<u>*H*</u>$_2$CH$_2$), 3.24 (app t, *J* = 7.0, 6.5 Hz, 2 H, 2 × C<u>*H*</u>$_2$N$_3$), 3.16–3.08 (br s, 1 H, CH*O*<u>*H*</u>), 3.12 (s, 3 H, OSO$_2$C<u>*H*</u>$_3$), 2.16 (app ddt, *J* = 14.5, 9.6, 5.0, 5.0 Hz, 1 H, H-6a), 2.05 (s, 3 H, 3 × COC<u>*H*</u>$_3$), 1.96–1.88 (m, 1 H, H-6b), 1.63–1.54 (m, 4 H, 4 × C<u>*H*</u>$_2$), 1.38–1.27 (m, 8 H, 8 × C<u>*H*</u>$_2$); ^{13}C NMR (125 MHz, CDCl$_3$) δ 171.1 (<u>*C*</u>O<u>C</u>H$_3$), 100.0 (C-1), 81.7 (C-2), 72.0 (C-3), 71.1 (C-5), 70.8 (C-4), 70.0 (O<u>*C*</u>H$_2$CH$_2$), 61.0 (C-7), 51.4 (<u>*C*</u>H$_2$N$_3$), 38.5 (OSO$_2$<u>*C*</u>H$_3$), 29.8 (C-6), 29.5 (<u>*C*</u>H$_2$), 29.2 (<u>*C*</u>H$_2$), 29.0 (<u>*C*</u>H$_2$), 28.8 (<u>*C*</u>H$_2$), 26.6 (<u>*C*</u>H$_2$), 25.8 (<u>*C*</u>H$_2$), 21.0 (CO<u>*C*</u>H$_3$); IR (cast film, CHCl$_3$) *v* 3476, 2930, 2857, 2096, 1737, 1178, 1054, 859 cm^{-1}; HRMS (ESI-TOF) m/z: [M + Na]$^+$ Calcd for C$_{18}$H$_{33}$N$_3$O$_9$SNa 490.1830; Found 490.1824.

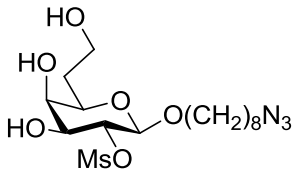


4-23

8-Azidooctyl 7-*O*-acetyl-3-*O*-benzoyl-6-deoxy-2-*O*-methanesulfonyl-β-D-*galacto*-heptopyranoside (4-23)

To a stirred solution of diol **4-22** (31.9 mg, 0.0682 mmol) in THF (2 mL) was added Me$_2$SnCl$_2$ (2.0 mg, 9.1 μmol), DIPEA (0.10 mL, 0.57 mmol) and BzCl (30 μL, 0.26 mmol). The solution was stirred for 3.5 h before 0.5 M HCl (2 mL) was added and the mixture was extracted with EtOAc (20 mL). The aqueous layer was separated and further extracted with EtOAc (2 × 20 mL). The combined organic phases were washed with brine (20 mL), dried over anhydrous Na$_2$SO$_4$, filtered, concentrated and the resulting crude product was purified by column chromatography (40% EtOAc in hexane) to afford **4-23** (37.1 mg, 95%) as a colorless viscous liquid: $[\alpha]^{22}_D$ +42.5 (*c* 0.6, CHCl$_3$); ^{1}H NMR (700 MHz, CDCl$_3$) δ 8.14–8.10 (m, 2 H, ArH),

7.59–7.56 (m, 1 H, ArH), 7.46–7.43 (m, 2 H, ArH), 5.14 (dd, *J* = 9.8, 3.5 Hz, 1 H, H-3), 4.92 (dd, *J* = 9.8, 7.7 Hz, 1 H, H-2), 4.53 (d, *J* = 7.7 Hz, 1 H, H-1), 4.26–4.22 (m, 1 H, H-7a), 4.18–4.15 (m, 1 H, H-7b), 4.14 (d, *J* = 3.5 Hz, 1 H, H-4), 3.92 (dt, *J* = 9.1, 7.0, 7.0 Hz, 1 H, OC$\underline{H}_2$CH$_2$), 3.71 (dd, *J* = 9.8, 4.2 Hz, 1 H, H-5), 3.56 (dt, *J* = 9.1, 7.0, 7.0 Hz, 1 H, OC$\underline{H}_2$CH$_2$), 3.25 (t, *J* = 7.0, 7.0 Hz, 2 H, 2 × C$\underline{H}_2$N$_3$), 3.03 (s, 3 H, OSO$_2$C$\underline{H}_3$), 2.17 (app ddt, *J* = 14.7, 9.8, 4.9, 4.9 Hz, 1 H, H-6a), 2.06 (s, 3 H, 3 × COC$\underline{H}_3$), 1.93–1.87 (m, 1 H, H-6b), 1.65–1.55 (m, 4 H, 4 × C$\underline{H}_2$), 1.36–1.30 (m, 8 H, 8 × C$\underline{H}_2$); ^{13}C NMR (175 MHz, CDCl$_3$) δ 171.1 ($\underline{C}$OCH$_3$), 165.9 ($\underline{C}$OPh), 133.7 (Ar), 130.2 (Ar), 129.0 (Ar), 128.6 (Ar), 100.4 (C-1), 77.6 (C-2), 74.1 (C-3), 71.1 (C-5), 70.1 (O$\underline{C}$H$_2$CH$_2$), 69.4 (C-4), 60.8 (C-7), 51.4 ($\underline{C}$H$_2$N$_3$), 39.0 (OSO$_2$$\underline{C}H_3$), 29.8 (C-6), 29.5 ($\underline{C}H_2$), 29.2 ($\underline{C}H_2$), 29.0 ($\underline{C}H_2$), 28.8 ($\underline{C}H_2$), 26.6 ($\underline{C}H_2$), 25.8 ($\underline{C}H_2$), 21.0 (CO$\underline{C}H_3$); IR (cast film, CHCl$_3$) *v* 3503, 3070, 3028, 2935, 2858, 2097, 1724, 1273, 1071, 715 cm^{-1}; HRMS (ESI-TOF) m/z: [M + Na]$^+$ Calcd for C$_{25}$H$_{37}$N$_3$O$_{10}$SNa 594.2092; Found 594.2078.

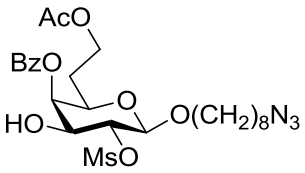


4-24

8-Azidooctyl 6-deoxy-2-*O*-methanesulfonyl-β-D-*galacto*-heptopyranoside (4-24)

To a stirred solution of thioglycoside **4-21** (151.7 mg, 0.2942 mmol) in CH$_3$OH (2 mL) and CH$_2$Cl$_2$ (0.5 mL) was added sodium methoxide in methanol (0.556 M, 0.350 mL, 0.194 mmol). After stirring for 2 h, Amberlite IR-120 resin (H$^+$ form) was added to neutralize the solution and, following filtration, the solution was concentrated from the filtrate to give the tetrol as a pale yellow viscous liquid, which was used directly in the next step. To a stirred solution of this tetrol (105.9 mg, 0.3048 mmol) in 2,2-dimethoxypropane (3 mL) was added

camphorsulfonic acid (18 mg, 0.077 mmol) and the mixture was stirred for 36 h. The camphorsulfonic acid was quenched by the addition of Et_3N (0.2 mL) and the solution was concentrated. The resulting crude product was dissolved in pyridine (2 mL) and cooled to 0 °C. methanesulfonyl chloride (50 μL, 0.65 mmol) was added dropwise and the ice bath was removed and the reaction mixture was stirred for 3.5 h. Toluene (5 mL) was added and the resulting solution was concentrated and then co-concentrated with toluene (4 × 5 mL) to obtain a crude product that was dissolved in 90% TFA (aqueous, 2.5 mL) over 5 min. The reaction mixture was further stirred for 10 min and then the TFA was quenched by the portionwise addition of solid $NaHCO_3$. The reaction mixture was diluted with EtOAc (40 mL) and washed with water (20 mL). The organic layer was separated and the aqueous layer was further extracted with EtOAc (2 × 30 mL). The combined organic phases were washed with brine (20 mL), dried over anhydrous Na_2SO_4, filtered, concentrated and the resulting crude product was purified by column chromatography (5% CH_3OH in EtOAc) to afford **4-24** (64 mg, 51% over the four steps) as a white solid: $[\alpha]^{22}_D$ –8.8 (*c* 0.43, $CHCl_3$); ^{1}H NMR (500 MHz, CD_3OD) δ 4.47 (dd, J = 9.5, 7.5 Hz, 1 H, H-2), 4.42 (d, J = 7.5 Hz, 1 H, H-1), 3.85 (app dt, J = 9.5, 7.0, 6.6 Hz, 1 H, OC$\underline{H}_2$CH$_2$), 3.74–3.64 (m, 5 H, H-7a, H-7b, H-3, H-4 and H-5), 3.57 (app dt, J = 9.5, 6.5, 6.5 Hz, 1 H, OC$\underline{H}_2$CH$_2$), 3.27 (app t, J = 7.0, 6.5 Hz, 2 H, 2 × C$\underline{H}_2$N$_3$), 3.14 (s, 3 H, OSO$_2$C$\underline{H}_3$), 1.97 (app ddt, J = 14.4, 9.1, 5.5, 5.5 Hz, 1 H, H-6a), 1.76 (app dtd, J = 14.4, 7.0, 7.0, 4.5 Hz, 1 H, H-6b), 1.64–1.54 (m, 4 H, 4 × C$\underline{H}_2$), 1.42–1.30 (m, 8 H, 8 × C$\underline{H}_2$); ^{13}C NMR (125 MHz, CD_3OD) δ 101.9 (C-1), 83.1 (C-2), 73.1, 72.8, 72.6 (C-3, C-4 and C-5), 70.7 (O$\underline{C}$H$_2$CH$_2$), 59.3 (C-7), 52.4 ($\underline{C}$H$_2$N$_3$), 39.4 (OSO$_2\underline{C}$H$_3$), 34.6 (C-6), 30.7 ($\underline{C}$H$_2$), 30.2 ($\underline{C}$H$_2$), 30.1 ($\underline{C}$H$_2$), 29.8 ($\underline{C}$H$_2$), 27.7 ($\underline{C}$H$_2$), 26.9 ($\underline{C}$H$_2$); IR (cast film, $CHCl_3$) ν 3406, 3297, 3045, 2919, 2855, 2095, 1216, 1063, 866 cm^{-1}; HRMS (ESI-TOF) m/z: $[M + Na]^+$ Calcd for $C_{16}H_{31}N_3O_8SNa$ 448.1724; Found 448.1717.

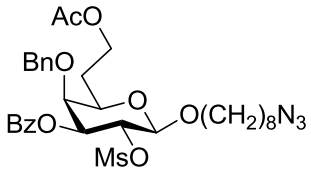


4-25

8-Azidooctyl 7-*O*-acetyl-4-*O*-benzoyl-6-deoxy-2-*O*-methanesulfonyl-β-D-*galacto*-heptopyranoside (4-25)

To a stirred solution of diol **4-22** (27.8 mg, 0.0486 mmol) in dry CH_2Cl_2 (2 mL) was added benzyl bromide (40 μL, 0.34 mmol) followed by freshly prepared Ag_2O (60.0 mg, 0.259 mmol). The reaction mixture was stirred for 14.5 h and diluted with CH_2Cl_2 (30 mL) and filtered over a pad of Celite-545. The filtrate was concentrated and the resulting crude product was purified by column chromatography (55% EtOAc in hexane) to afford **4-25** (9.8 mg, 35%) as a colorless viscous liquid along with recovered **4-23** (10 mg, 36%): $[\alpha]^{22}_D$ +14.5 (*c* 0.5, $CHCl_3$); ^{1}H NMR (700 MHz, $CDCl_3$) δ 8.10–8.07 (m, 2 H, ArH), 7.61–7.57 (m, 1 H, ArH), 7.50–7.44 (m, 2 H, ArH), 5.53 (d, J = 3.5 Hz, 1 H, H-4), 4.57 (dd, J = 9.8, 7.7 Hz, 1 H, H-2), 4.49 (d, J = 7.7 Hz, 1 H, H-1), 4.23–4.19 (m, 1 H, H-7a), 4.18–4.14 (m, 1 H, H-7b), 4.05 (ddd, J = 9.8, 4.9, 3.5 Hz, 1 H, H-3), 3.94 (app dt, J = 9.1, 6.7, 6.3 Hz, 1 H, OC$\underline{H_2}$CH$_2$), 3.78 (dd, J = 9.8, 4.2 Hz, 1 H, H-5), 3.57 (dt, J = 9.1, 7.0, 7.0 Hz, 1 H, OC$\underline{H_2}$CH$_2$), 3.26 (t, J = 7.0, 7.0 Hz, 2 H, 2 × C$\underline{H_2}$N$_3$), 3.14 (s, 3 H, OSO$_2$C$\underline{H_3}$), 2.78 (d, J = 4.9 Hz, 1 H, CH$\underline{OH}$), 2.05 (s, 3 H, 3 × COC$\underline{H_3}$), 1.99–1.93 (m, 1 H, H-6a), 1.92–1.86 (m, 1 H, H-6b), 1.68–1.57 (m, 4 H, 4 × C$\underline{H_2}$), 1.42–1.30 (m, 8 H, 8 × C$\underline{H_2}$); ^{13}C NMR (175 MHz, $CDCl_3$) δ 171.0 ($\underline{C}$OCH$_3$), 166.6 ($\underline{C}$OPh), 133.7 (Ar), 130.1 (Ar), 129.0 (Ar), 128.7 (Ar), 100.3 (C-1), 81.3 (C-2), 72.7 (C-4), 71.2 (C-3), 70.5 (C-5), 70.4 (O$\underline{C}$H$_2$CH$_2$), 60.6 (C-7), 51.5 ($\underline{C}$H$_2$N$_3$), 38.8 (OSO$_2\underline{C}$H$_3$), 30.2 (C-6), 29.6 ($\underline{C}$H$_2$), 29.2 ($\underline{C}$H$_2$), 29.1 ($\underline{C}$H$_2$), 28.8 ($\underline{C}$H$_2$), 26.7 ($\underline{C}$H$_2$), 25.8 ($\underline{C}$H$_2$), 21.0 (CO$\underline{C}$H$_3$); IR (cast film, $CHCl_3$) ν 3481, 2934, 2858, 2097,

1726, 1271, 1069, 713 cm^{-1}; HRMS (ESI-TOF) m/z: $[M + Na]^+$ Calcd for $C_{25}H_{37}N_3O_{10}SNa$ 594.2092; Found 594.2087.

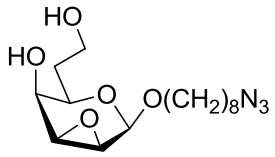


4-16

8-Azidooctyl 7-*O*-acetyl-3-*O*-benzoyl-4-*O*-benzyl-6-deoxy-2-*O*-methanesulfonyl-β-D-*galacto*-heptopyranoside (4-16)

To a stirred solution of alcohol **4-23** (37.1 mg, 0.0649 mmol) in cyclohexane (2 mL) and dichloromethane (1 mL) was added $BnOC(=NH)CCl_3$ (60 μL, 0.32 mmol) and TfOH (1.0 μL, 0.011 mmol). The mixture was stirred for 4 h, the TfOH was quenched by the addition of Et_3N (0.1 mL) and then the reaction mixture was diluted with CH_2Cl_2 (20 mL) and washed with H_2O. The aqueous layer was separated and further extracted with CH_2Cl_2 (2 × 20 mL). The combined organic phases were washed with brine (15 mL), dried over anhydrous Na_2SO_4, filtered, concentrated and the resulting crude product was purified by column chromatography (30% EtOAc in hexane) to afford **4-16** (7.8 mg, 18%) as a colorless viscous liquid: $[\alpha]^{22}_D$ +25.9 (*c* 0.31, $CHCl_3$); ^{1}H NMR (700 MHz, $CDCl_3$) δ 8.17–8.15 (m, 2 H, ArH), 7.60–7.56 (m, 1 H, ArH), 7.48–7.44 (m, 2 H, ArH), 7.25–7.19 (m, 5 H, ArH), 5.17 (dd, *J* = 10.5, 2.8 Hz, 1 H, H-3), 5.04 (dd, *J* = 10.5, 8.4 Hz, 1 H, H-2), 4.74 (d, *J* = 11.2 Hz, 1 H, OC$\underline{H_2}$Ph), 4.52 (d, *J* = 8.4 Hz, 1 H, H-1), 4.48 (d, *J* = 11.2 Hz, 1 H, OC$\underline{H_2}$Ph), 4.18–4.14 (m, 1 H, H-7a), 4.08–4.04 (m, 1 H, H-7b), 4.00 (d, *J* = 2.8 Hz, 1 H, H-4), 3.93 (dt, *J* = 9.1, 7.0, 7.0 Hz, 1 H, OC$\underline{H_2}$CH$_2$), 3.65 (dd, *J* = 9.1, 3.5 Hz, 1 H, H-5), 3.54 (dt, *J* = 9.1, 7.0, 7.0 Hz, 1 H, OC$\underline{H_2}$CH$_2$), 3.25 (t, *J* = 7.0, 7.0 Hz, 2 H, 2 × C$\underline{H_2}$N$_3$), 3.06 (s, 3 H, OSO$_2$C$\underline{H_3}$), 2.11 (ddt, *J* = 14.7, 9.8, 4.9, 4.9 Hz, 1 H, H-6a), 2.07 (s, 3 H,

3 × COC$\underline{H}_3$), 1.70–1.64 (m, 1 H, H-6b), 1.64–1.54 (m, 4 H, 4 × C$\underline{H}_2$), 1.40–1.23 (m, 8 H, 8 × C$\underline{H}_2$); ^{13}C NMR (175 MHz, $CDCl_3$) δ 171.0 ($\underline{C}OCH_3$), 166.2 ($\underline{C}OPh$), 137.1 (Ar), 133.7 (Ar), 130.3 (Ar), 129.0 (Ar), 128.7 (Ar), 128.5 (Ar), 128.4 (Ar), 128.1 (Ar), 100.5 (C-1), 78.0 (C-2), 75.7 (C-4), 75.2 (O$\underline{C}H_2Ph$), 74.4 (C-3), 71.2 (C-5), 70.1 (O$\underline{C}H_2CH_2$), 60.7 (C-7), 51.5 ($\underline{C}H_2N_3$), 39.1 ($OSO_2\underline{C}H_3$), 30.2 (C-6), 29.6 ($\underline{C}H_2$), 29.3 ($\underline{C}H_2$), 29.1 ($\underline{C}H_2$), 28.8 ($\underline{C}H_2$), 26.7 ($\underline{C}H_2$), 25.9 ($\underline{C}H_2$), 21.0 ($CO\underline{C}H_3$); IR (cast film, $CHCl_3$) v 3032, 2927, 2856, 2096, 1727, 1601, 1495, 1453, 1270, 1069, 714 cm^{-1}; HRMS (ESI-TOF) m/z: $[M + Na]^+$ Calcd for $C_{32}H_{43}N_3O_{10}SNa$ 684.2561; Found 684.2562.

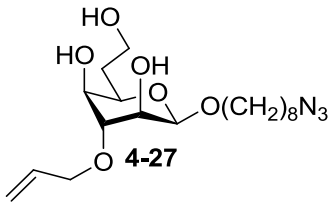


4-13

8-Azidooctyl 2,3-anhydro-6-deoxy-β-D-*talo*-heptopyranoside (4-13)

To a stirred solution of compound **4-17** (459.9 mg, 0.8337 mmol) in allyl alcohol (8 mL) was added potassium *tert*-butoxide (318 mg, 2.834 mmol). The reaction mixture was stirred for 40.5 h and then diluted with EtOAc (40 mL) and washed with brine (20 mL). The aqueous layer was separated and further extracted with EtOAc (2 × 20 mL). The combined organic layer was dried over anhydrous Na_2SO_4, filtered, concentrated and the resulting crude product was purified by column chromatography (100% EtOAc) to afford epoxide **4-13** (172 mg, 63%) as a colorless viscous liquid: $[\alpha]^{22}_D$ –36.8 (*c* 0.47, $CHCl_3$); ^{1}H NMR (700 MHz, $CDCl_3$) δ 4.80 (s, 1 H, H-1), 4.54 (app dt, J = 9.5, 7.0, 6.6 Hz, 1 H, OC$\underline{H}_2CH_2$), 3.79–3.72 (m, 3 H, H-4, H-7a and H-7b), 3.58 (dd, J = 5.6, 3.5 Hz, 1 H, H-3), 3.55–3.51 (m, 2 H, H-5 and OC$\underline{H}_2CH_2$), 3.31 (d, J = 3.5 Hz, 1 H, H-2), 3.25 (app t, J = 7.0, 6.3 Hz, 2 H, 2 × C$\underline{H}_2N_3$), 2.53 (d, J = 11.9 Hz, 1 H, CH$O\underline{H}$), 1.99

(dddd, J = 14.8, 8.6, 6.6, 4.9 Hz, 1 H, H-6a), 1.90 (br s, 1 H, $CH_2O\underline{H}$), 1.79 (dddd, J = 14.8, 7.4, 7.0, 4.9, 4.5 Hz, 1 H, H-6b), 1.70–1.56 (m, 4 H, 4 × $C\underline{H}_2$), 1.42–1.29 (m, 8 H, 8 × $C\underline{H}_2$); ^{13}C NMR (175 MHz, $CDCl_3$) δ 98.4 (C-1), 75.6 (C-5), 70.0 ($O\underline{C}H_2CH_2$), 63.1 (C-4), 59.6 (C-7), 54.2 (C-3), 52.8 (C-2), 51.5 ($\underline{C}H_2N_3$), 32.4 (C-6), 29.6 ($\underline{C}H_2$), 29.2 ($\underline{C}H_2$), 29.0 ($\underline{C}H_2$), 28.8 ($\underline{C}H_2$), 26.6 ($\underline{C}H_2$), 25.9 ($\underline{C}H_2$); IR (cast film, $CHCl_3$) ν 3432, 2932, 2857, 2096, 1099, 1041, 912 cm^{-1}; HRMS (ESI-TOF) m/z: $[M + Na]^+$ Calcd for $C_{15}H_{27}N_3O_5Na$ 352.1843; Found 352.1842.

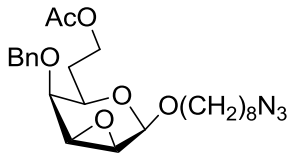


8-Azidooctyl 3-*O*-allyl-6-deoxy-β-D-*ido*-heptopyranoside (4-27)

Procedure A: To a stirred solution of compound **4-17** (106.5 mg, 0.1931 mmol) in allyl alcohol (3 mL) was added potassium *tert*-butoxide (72 mg, 0.64 mmol). The reaction mixture was heated at 55 °C for 12 h, after which time the solution was cooled to room temperature over 10 min. The reaction mixture was diluted with EtOAc (40 mL) and washed with brine (20 mL). The organic layer was separated, dried over anhydrous Na_2SO_4, filtered, concentrated and the resulting crude product was purified by column chromatography (75% EtOAc in hexane) to afford triol **4-27** (52 mg, 70%) as a colorless viscous liquid:

Procedure B: The synthesis of triol **4-27** was achieved starting from the compound **4-24** (62.5 mg, 0.147 mmol) following the procedure described for the preparation of **4-27** from **4-17**. The crude product was purified by column chromatography (75% EtOAc in Hexane) affording triol **4-27** (41.5 mg, 73%) as a colorless viscous liquid: $[\alpha]^{22}_D$ –23.2 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.90–5.82 (m, 1 H, $OCH_2C\underline{H}=CH_2$), 5.27 (dddd, J = 17.0, 1.5, 1.5, 1.5 Hz, 1 H,

$OCH_2CH{=}C\underline{H_2}$), 5.21 (dddd, J = 10.5, 1.5, 1.3, 1.0 Hz, 1 H, $OCH_2CH{=}C\underline{H_2}$), 4.70 (s, 1 H, H-1), 4.12–4.02 (m, 2 H, 2 × $OC\underline{H_2}CH{=}CH_2$), 3.97 (dd, J = 9.0, 4.0 Hz, 1 H, H-5), 3.89 (app dt, J = 9.5, 6.8, 6.5 Hz, 1 H, $OC\underline{H_2}CH_2$), 3.86–3.73 (m, 4 H, H-2, H-3, H-7a and H-7b), 3.63 (br s, 1 H, $CHO\underline{H}$), 3.89 (app dt, J = 9.5, 6.8, 6.5 Hz, 1 H, $OC\underline{H_2}CH_2$), 3.46 (br s, 1 H, H-4), 3.25 (t, J = 7.0, 7.0 Hz, 2 H, 2 × $C\underline{H_2}N_3$), 2.84 (br s, 1 H, $CHO\underline{H}$), 2.35 (br s, 1 H, $CHO\underline{H}$), 2.13 (dddd, J = 14.5, 7.9, 7.5, 4.5 Hz, 1 H, H-6a), 1.80 (ddt, J = 14.5, 7.0, 4.0, 4.0 Hz, 1 H, H-6b), 1.63–1.55 (m, 4 H, 4 × $C\underline{H_2}$), 1.40–1.26 (m, 8 H, 8 × $C\underline{H_2}$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 134.1 ($OCH_2\underline{C}H{=}CH_2$), 117.6 ($OCH_2CH{=}\underline{C}H_2$), 98.4 (C-1), 75.9 (C-3), 72.9 (C-5), 71.4 ($O\underline{C}H_2CH{=}CH_2$), 69.4 ($O\underline{C}H_2CH_2$), 68.8 (C-2), 68.4 (C-4), 60.0 (C-7), 51.5 ($\underline{C}H_2N_3$), 33.5 (C-6), 29.6 ($\underline{C}H_2$), 29.2 ($\underline{C}H_2$), 29.0 ($\underline{C}H_2$), 28.8 ($\underline{C}H_2$), 26.6 ($\underline{C}H_2$), 25.9 ($\underline{C}H_2$); IR (cast film, $CHCl_3$) v 3448, 2932, 2858, 2096, 1081, 1040, 933 cm^{-1}; HRMS (ESI-TOF) m/z: $[M + Na]^+$ Calcd for $C_{18}H_{33}N_3O_6Na$ 410.2262; Found 410.2261.

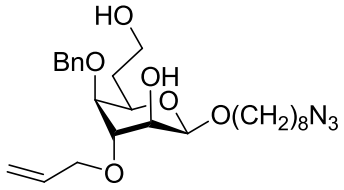


4-28

8-Azidooctyl 7-*O*-acetyl-2,3-anhydro-4-*O*-benzyl-6-deoxy-β-D-*talo*-heptopyranoside (4-28)

A stirred solution of diol **4-13** (127.3 mg, 0.3865 mmol) in *sym*-collidine (1.5 mL) was cooled to –40 °C. Acetyl chloride (45 µL, 0.63 mmol) was added and the solution was stirred for 2 h at –40 °C before CH_3OH (0.5 mL) was added and all the volatiles were evaporated. The resulting residue was quickly purified by column chromatography (50% EtOAc in hexane) to afford the corresponding 7-*O*-acetyl compound. This material was dissolved in CH_2Cl_2 (2 mL) and then benzyl bromide (230 µL, 1.93 mmol) and freshly prepared Ag_2O (220 mg, 0.949 mmol)

were added and the solution was stirred for 48 h. The reaction mixture was diluted with CH_2Cl_2 (30 mL) and filtered over a pad of Celite-545. The filtrate was concentrated and the resulting crude product was purified by column chromatography (25% EtOAc in hexane) to afford **4-28** (109 mg, 61% over the two steps) as a colorless viscous liquid: $[\alpha]^{22}_D$ –69.3 (*c* 0.15, $CHCl_3$); ^{1}H NMR (700 MHz, $CDCl_3$) δ 7.38–7.27 (m, 5 H, ArH), 4.64, 4.55 (ABq, J = 12.3 Hz, 2 H, 2 × OC$\underline{H_2}$Ph), 4.72 (s, 1 H, H-1), 4.21–4.16 (m, 1 H, H-7a), 4.11 (ddd, J = 11.2, 5.6, 5.6 Hz, 1 H, H-7b), 3.92 (app dt, J = 9.1, 6.6, 6.3 Hz, 1 H, OC$\underline{H_2}$CH$_2$), 3.54 (app t, J = 4.2, 3.5 Hz, 1 H, H-4), 3.92 (dt, J = 9.1, 7.0, 7.0 Hz, 1 H, OC$\underline{H_2}$CH$_2$), 3.48–3.43 (m, 1 H, H-5), 3.42 (app t, J = 4.2, 4.2 Hz, 1 H, H-3), 3.25–3.21 (m, 3 H, H-2 and 2 × C$\underline{H_2}$N$_3$), 2.20 (ddt, J = 14.7, 9.8, 4.9, 4.9 Hz, 1 H, H-6a), 2.02 (s, 3 H, 3 × COC$\underline{H_3}$), 1.85–1.80 (m, 1 H, H-6b), 1.64–1.55 (m, 4 H, 4 × C$\underline{H_2}$), 1.40–1.28 (m, 8 H, 8 × C$\underline{H_2}$); ^{13}C NMR (175 MHz, $CDCl_3$) δ 171.0 ($\underline{C}$OCH$_3$), 137.8 (Ar), 128.1 (Ar), 127.9 (Ar), 127.8 (Ar), 98.3 (C-1), 73.0 (C-5), 70.7 (O$\underline{C}$H$_2$Ph), 69.4 (O$\underline{C}$H$_2$CH$_2$), 68.6 (C-4), 61.2 (C-7), 51.4 ($\underline{C}$H$_2$N$_3$), 51.3, 51.2 (C-2 and C-3), 29.5 (C-6), 29.2 ($\underline{C}$H$_2$), 29.1 ($\underline{C}$H$_2$), 29.0 ($\underline{C}$H$_2$), 28.8 ($\underline{C}$H$_2$), 26.6 ($\underline{C}$H$_2$), 25.9 ($\underline{C}$H$_2$), 20.9 (CO$\underline{C}$H$_3$); IR (cast film, $CHCl_3$) ν 2919, 2850, 2095, 1736, 1097, 1042, 728 cm^{-1}; HRMS (ESI-TOF) m/z: $[M + Na]^+$ Calcd for $C_{24}H_{35}N_3O_6Na$ 484.2418; Found 484.2418.



4-29

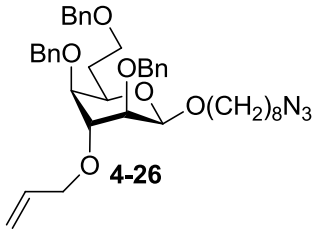
8-Azidooctyl 3-*O*-allyl-4-*O*-benzyl-6-deoxy-β-D-*ido*-heptopyranoside (4-29)

Procedure A: To a stirred solution of compound **4-16** (7.8 mg, 0.012 mmol) in allyl alcohol (2 mL) was added potassium *tert*-butoxide (10 mg, 0.089 mmol). The reaction mixture

was heated at 70 °C 12 h, after which time the solution was cooled to room temperature over 10 min. The reaction mixture was diluted with EtOAc (20 mL) and washed with brine (10 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered, concentrated and the resulting crude product was purified by column chromatography (60% EtOAc in hexane) to afford diol **4-29** (1.8 mg, 32%) as a colorless viscous liquid.

Procedure B: To a stirred solution of compound **4-28** (90.2 mg, 0.195 mmol) in allyl alcohol (3 mL) was added potassium *tert*-butoxide (60.0 mg, 0.535 mmol). The reaction mixture was heated at 55 °C for 12 h, after which time the solution was cooled to room temperature over 10 min. The reaction mixture was diluted with EtOAc (40 mL) and washed with brine (20 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered, concentrated and the resulting crude product was purified by column chromatography (60% EtOAc in hexane) to afford diol **4-29** (74 mg, 79%) as a colorless viscous liquid: $[\alpha]^{22}_{D}$ –25.1 (*c* 0.87, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.36–7.27 (m, 5 H, ArH), 5.88–5.79 (m, 1 H, $OCH_2C\underline{H}=CH_2$), 5.25 (dd, J = 17.0, 1.5 Hz, 1 H, $OCH_2CH=C\underline{H_2}$), 5.25 (dd, J = 10.0, 1.5 Hz, 1 H, $OCH_2CH=C\underline{H_2}$), 4.70, 4.48 (ABq, J = 11.5 Hz, 2 H, 2 × $OC\underline{H_2}Ph$), 4.66 (s, 1 H, H-1), 4.07–3.96 (m, 3 H, H-5 and 2 × $OC\underline{H_2}CH=CH_2$), 3.88 (app t, J = 3.0, 3.0 Hz, 1 H, H-3), 3.85 (dt, J = 9.8, 7.0, 7.0 Hz, 1 H, $OC\underline{H_2}CH_2$), 3.75 (app t, J = 6.0, 5.5 Hz, 2 H, H-7a and H-7b), 3.71 (dd, J = 10.5, 3.0 Hz, 1 H, H-2), 3.51 (dt, J = 9.8, 7.0, 7.0 Hz, 1 H, $OC\underline{H_2}CH_2$), 3.32 (d, J = 10.5 Hz, 1 H, $CHO\underline{H}$), 3.26 (brt, J = 3.0, 1.5 Hz, 1 H, H-4), 3.23 (t, J = 7.0, 7.0 Hz, 2 H, 2 × $C\underline{H_2}N_3$), 2.23–2.14 (m, 1 H, H-6a), 2.06 (br s, 1 H, $CH_2O\underline{H}$), 1.66–1.53 (m, 5 H, H-6b and 4 × $C\underline{H_2}$), 1.38–1.27 (m, 8 H, 8 × $C\underline{H_2}$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 136.9 (Ar), 134.1 ($OCH_2\underline{C}H=CH_2$), 128.5 (Ar), 128.4 (Ar), 128.2 (Ar), 117.7 ($OCH_2CH=\underline{C}H_2$), 99.6 (C-1), 75.2 (C-4), 73.6 (C-3), 73.0 (C-5), 72.7 ($O\underline{C}H_2Ph$), 71.3 ($O\underline{C}H_2CH=CH_2$), 69.8 ($O\underline{C}H_2CH_2$), 68.2 (C-2), 60.5 (C-7), 51.4 ($\underline{C}H_2N_3$), 33.3 (C-6), 29.6

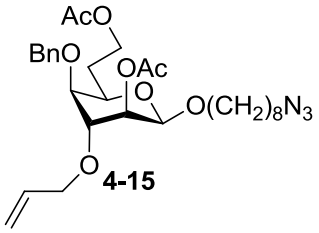
($\underline{C}H_2$), 29.2 ($\underline{C}H_2$), 29.0 ($\underline{C}H_2$), 28.8 ($\underline{C}H_2$), 26.6 ($\underline{C}H_2$), 25.9 ($\underline{C}H_2$); IR (cast film, $CHCl_3$) ν 3508, 2932, 2858, 2095, 1497, 1455, 1372, 1076, 1029, 699 cm^{-1}; HRMS (ESI-TOF) m/z: $[M + Na]^+$ Calcd for $C_{25}H_{39}N_3O_6Na$ 500.2731; Found 500.2732.



8-Azidooctyl 3-*O*-allyl-2,4,7-tri-*O*-benzyl-6-deoxy-β-D-*ido*-heptopyranoside (4-26)

To a 0 °C solution of triol **4-27** (60.7 mg, 0.157 mmol) in THF (4 mL) was added sodium hydride (60% in mineral oil, 54.0 mg, 1.35 mmol). After 5 min, benzyl bromide (0.150 mL, 1.26 mmol) was added dropwise over 5 min. The reaction mixture was heated at reflux after which time the solution was cooled to room temperature over 15 min. Excess NaH was quenched by the addition of saturated aqueous NH_4Cl solution and the reaction mixture was extracted with CH_2Cl_2 (20 mL). The aqueous layers were separated and further extracted with CH_2Cl_2 (2 × 20 mL). The combined organic phases were washed with brine (10 mL), dried over anhydrous Na_2SO_4, filtered, concentrated and the resulting crude product was purified by column chromatography (15% EtOAc in hexane) to afford **4-13** (72 mg, 70%) as a colorless viscous liquid: $[\alpha]^{22}_D$ –47.9 (*c* 0.78, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.40–7.27 (m, 15 H, ArH), 5.76 (dddd, J = 16.9, 10.9, 6.0, 5.5 Hz, 1 H, $OCH_2C\underline{H}=CH_2$), 5.17–5.08 (m, 2 H, 2 × $OCH_2CH=C\underline{H_2}$), 4.86 (d, J = 13.0 Hz, 1 H, $OC\underline{H_2}Ph$), 4.70–4.66 (m, 2 H, 2 × $OC\underline{H_2}Ph$), 4.61 (d, J = 2.0 Hz, 1 H, H-1), 4.53 (d, J = 12.0 Hz, 1 H, $OC\underline{H_2}Ph$), 4.50, 4.48 (ABq, J = 11.9 Hz, 2 H, 2 × $OC\underline{H_2}Ph$), 3.97 (app dt, J = 9.6, 3.0, 3.0 Hz, 1 H, H-5), 3.94–3.88 (m, 2 H, 2 × $OC\underline{H_2}CH=CH_2$), 3.84 (dt, J = 9.5, 6.5, 6.5 Hz, 1 H, $OC\underline{H_2}CH_2$), 3.71 (app t, J = 3.5, 3.0 Hz, 1 H,

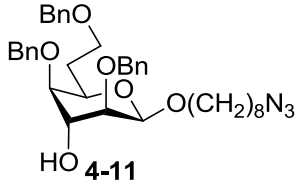
H-3), 3.66–3.61 (m, 1 H, H-7a), 3.58–3.53 (m, 1 H, H-7b), 3.43 (dd, J = 3.5, 2.0 Hz, 1 H, H-2), 3.36 (dt, J = 9.5, 7.0, 6.8 Hz, 1 H, OC$\underline{H}_2$CH$_2$), 3.25 (t, J = 7.0, 7.0 Hz, 2 H, 2 × C$\underline{H}_2$N$_3$), 3.22 (app t, J = 3.0, 3.0 Hz, 1 H, H-4), 2.27 (ddt, J = 14.4, 9.8, 5.0, 5.0 Hz, 1 H, H-6a), 1.90–1.82 (m, 1 H, H-6b), 1.62–1.56 (m, 4 H, 4 × C$\underline{H}_2$), 1.42–1.30 (m, 8 H, 8 × C$\underline{H}_2$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 139.2 (Ar), 138.7 (Ar), 138.4 (Ar), 134.5 (OCH$_2\underline{C}$H=CH$_2$), 128.3 (Ar), 128.2 (Ar), 128.1 (Ar), 127.9 (Ar), 127.8 (Ar), 127.7 (Ar), 127.63 (Ar), 127.59 (Ar), 127.5 (Ar), 127.4 (Ar), 117.2 (OCH$_2$CH=$\underline{C}$H$_2$), 100.1 (C-1), 75.2 (C-4), 74.8 (C-2), 74.4 (C-3), 73.7 (O$\underline{C}$H$_2$Ph), 73.0 (O$\underline{C}$H$_2$Ph), 72.1 (O$\underline{C}$H$_2$Ph), 71.5 (O$\underline{C}$H$_2$CH=CH$_2$), 71.4 (C-5), 69.1 (O$\underline{C}$H$_2$CH$_2$), 67.0 (C-7), 51.5 ($\underline{C}$H$_2$N$_3$), 30.8 (C-6), 29.7 ($\underline{C}$H$_2$), 29.3 ($\underline{C}$H$_2$), 29.1 ($\underline{C}$H$_2$), 28.8 ($\underline{C}$H$_2$), 26.7 ($\underline{C}$H$_2$), 26.1 ($\underline{C}$H$_2$); IR (cast film, $CHCl_3$) ν 3091, 3064, 3030, 2930, 2858, 2095, 1496, 1454, 1361, 1097, 697 cm^{-1}; HRMS (ESI-TOF) m/z: [M + Na]$^+$ Calcd for $C_{39}H_{51}N_3O_6Na$ 680.3670; Found 680.3633.



8-Azidooctyl 2,4-di-*O*-acetyl-3-*O*-allyl-4-*O*-benzyl-6-deoxy-β-D-*ido*-heptopyranoside (4-15)

To a stirred solution of diol **4-29** (76.60 mg, 0.1604 mmol) in pyridine (2.5 mL) was added Ac_2O (0.15 mL, 1.6 mmol) and the reaction mixture was heated at 55 °C for 16 h, after which time the solution was cooled to room temperature over 15 min. Toluene (5 mL) was added and the resulting solution was concentrated. Co-concentration with toluene (4 × 5 mL) produced a crude product that was purified by column chromatography (20% EtOAc in hexane) to afford **4-15** (78 mg, 87%) as a colorless viscous liquid: $[\alpha]^{22}_D$ –22.0 (c 0.38, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.35–7.27 (m, 5 H, ArH), 5.87 (ddt, J = 17.0, 10.5, 6.0, 6.0 Hz,1 H,

$OCH_2C\underline{H}=CH_2$), 5.29 (dd, J = 17.0, 1.5 Hz, 1 H, $OCH_2CH=C\underline{H_2}$), 5.22 (d, J = 10.5 Hz, 1 H, $OCH_2CH=C\underline{H_2}$), 4.86 (br s, 1 H, H-2), 4.75 (d, J = 1.0 Hz, 1 H, H-1), 4.58, 4.48 (ABq, J = 11.7 Hz, 2 H, 2 × $OC\underline{H_2}Ph$), 4.28–4.12 (m, 3 H, H-7a, H-7b and $OC\underline{H_2}CH=CH_2$), 4.08 (dd, J = 12.5, 6.0 Hz, 1 H, $OC\underline{H_2}CH=CH_2$), 3.91–3.84 (m, 3 H, H-3, H-5 and $OC\underline{H_2}CH_2$), 3.46 (dt, J = 9.5, 7.0, 7.0 Hz, 1 H, $OC\underline{H_2}CH_2$), 3.25 (t, J = 7.0, 7.0 Hz, 2 H, 2 × $C\underline{H_2}N_3$), 3.21 (br s, 1 H, H-4), 2.31 (ddt, J = 14.5, 9.5, 5.0, 5.0 Hz,1 H, H-6a), 2.06 (s, 3 H, 3 × $COC\underline{H_3}$), 2.05 (s, 3 H, 3 × $COC\underline{H_3}$), 1.79 (dddd, J = 14.5, 9.0, 5.6, 3.5 Hz,1 H, H-6b), 1.62–1.54 (m, 4 H, 4 × $C\underline{H_2}$), 1.40–1.28 (m, 8 H, 8 × $C\underline{H_2}$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.0 ($\underline{C}OCH_3$), 170.9 ($\underline{CO}CH_3$), 137.7 (Ar), 134.0 ($OCH_2\underline{C}H=CH_2$), 128.3 (Ar), 128.0 (Ar), 127.9 (Ar), 117.8 ($OCH_2CH=\underline{C}H_2$), 97.8 (C-1), 74.6 (C-4), 72.34 ($O\underline{C}H_2Ph$), 72.31 (C-3), 71.6 ($O\underline{C}H_2CH=CH_2$), 70.9 (C-5), 69.6 ($O\underline{C}H_2CH_2$), 68.2 (C-2), 61.2 (C-7), 51.4 ($\underline{C}H_2N_3$), 29.8 (C-6), 29.4 ($\underline{C}H_2$), 29.2 ($\underline{C}H_2$), 29.0 ($\underline{C}H_2$), 28.8 ($\underline{C}H_2$), 26.6 ($\underline{C}H_2$), 25.8 ($\underline{C}H_2$), 21.1 ($CO\underline{C}H_3$), 21.0 ($CO\underline{C}H_3$); IR (cast film, $CHCl_3$) ν 3064, 3031, 2932, 2859, 2096, 1739, 1455, 1371, 1240, 1046, 700 cm^{-1}; HRMS (ESI-TOF) m/z: $[M + Na]^+$ Calcd for $C_{29}H_{43}N_3O_8Na$ 584.2942; Found 584.2938.

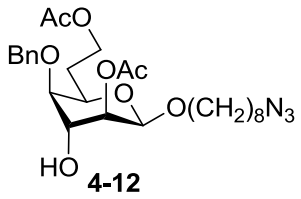


4-11

8-Azidooctyl 2,4,7-tri-*O*-benzyl-6-deoxy-β-D-*ido*-heptopyranoside (4-11)

To a 0 °C solution of compound **4-26** (70.5 mg, 0.107 mmol) in THF (3 mL) was added $[Ir(COD)(Ph_2PCH_3)_2]PF_6$ (1.5 mg, 1.8 μmol) under an argon atmosphere. After 5 min, solution was degassed and replaced with a H_2 balloon (1 atm) and flushed with H_2 for 2 min. The solution was degassed again and flushed with H_2 for 1 min, during which time solution turned clear and

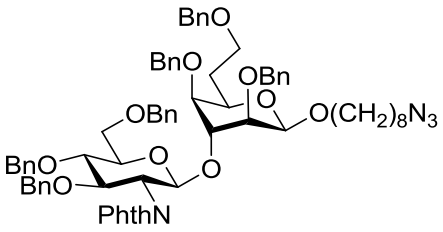
colorless. The solution was then degassed once more and replaced with argon balloon, after which time the solution turned a slight pink color. The reaction mixture was stirred at room temperature for 13 h and then concentrated and the residue was redissolved in EtOAc (3 mL). Next, iodine (41 mg, 0.16 mmol) followed by saturated aqueous $NaHCO_3$ (1 mL) was added and the mixture stirred for 5 h before the excess iodine was quenched by the addition of saturated aqueous $Na_2S_2O_3$ (5 mL). The reaction mixture was diluted with EtOAc (30 mL) and the aqueous layer was separated and further extracted with EtOAc (2 × 20 mL). The combined organic phases were dried over anhydrous Na_2SO_4, filtered, concentrated and the resulting crude product was purified by column chromatography (20% EtOAc in hexane) to afford **4-11** (65 mg, 85%) as a colorless viscous liquid: $[\alpha]^{22}_D$ –19.6 (*c* 0.52, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.38–7.26 (m, 15 H, ArH), 4.74 (d, J = 3.5 Hz, 1 H, H-1), 4.70 (s, 2 H, 2 × OC$\underline{H_2}$Ph), 4.65, 4.61 (ABq, J = 11.8 Hz, 2 H, 2 × OC$\underline{H_2}$Ph), 4.51 (s, 2 H, 2 × OC$\underline{H_2}$Ph), 4.19–4.14 (m, 1 H, H-5), 4.15 (app t, J = 8.0, 8.0 Hz, 1 H, H-3), 3.73 (app dt, J = 9.3, 6.8, 6.5 Hz, 1 H, OC$\underline{H_2}$CH$_2$), 3.67–3.58 (m, 2 H, H-7a and H-7b), 3.47 (dd, J = 8.0, 5.5 Hz, 1 H, H-4), 3.39 (dd, J = 8.0, 3.5 Hz, 1 H, H-2), 3.32 (app dt, J = 9.3, 6.8, 6.5 Hz, 1 H, OC$\underline{H_2}$CH$_2$), 3.24 (t, J = 7.0, 7.0 Hz, 2 H, 2 × C$\underline{H_2}$N$_3$), 2.39 (br s, 1 H, CH$\underline{OH}$), 2.49 (ddt, J = 14.8, 9.9, 5.0, 5.0 Hz, 1 H, H-6a), 2.12–2.03 (m, 1 H, H-6b), 1.62–1.54 (m, 4 H, 4 × C$\underline{H_2}$), 1.40–1.25 (m, 8 H, 8 × C$\underline{H_2}$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.7 (Ar), 138.5 (Ar), 138.1 (Ar), 128.40 (Ar), 128.38 (Ar), 128.3 (Ar), 127.94 (Ar), 127.89 (Ar), 127.8 (Ar), 127.6 (Ar), 127.5 (Ar), 98.7 (C-1), 79.0 (C-2), 78.6 (C-4), 73.0 (O$\underline{C}$H$_2$Ph), 72.9 (O$\underline{C}$H$_2$Ph), 72.6 (O$\underline{C}$H$_2$Ph), 71.2 (C-5), 69.4 (O$\underline{C}$H$_2$CH$_2$), 68.2 (C-3), 67.4 (C-7), 51.5 ($\underline{C}$H$_2$N$_3$), 30.0 (C-6), 29.5 ($\underline{C}$H$_2$), 29.3 ($\underline{C}$H$_2$), 29.1 ($\underline{C}$H$_2$), 28.8 ($\underline{C}$H$_2$), 26.7 ($\underline{C}$H$_2$), 26.1 ($\underline{C}$H$_2$); IR (cast film, $CHCl_3$) ν 3472, 3063, 3030, 2929, 2857, 2095, 1496, 1454, 1362, 1096, 697 cm^{-1}; HRMS (ESI-TOF) m/z: $[M + Na]^+$ Calcd for $C_{36}H_{47}N_3O_6Na$ 640.3357; Found 640.3353.



4-12

8-Azidooctyl 2,4-di-*O*-acetyl-4-*O*-benzyl-6-deoxy-β-D-*ido*-heptopyranoside (4-12)

The synthesis of alcohol **4-12** was achieved starting from compound **4-15** (77.9 mg, 0.139 mmol) following the procedure described for the synthesis of **4-11**. The crude product was purified by column chromatography (33% EtOAc in Hexane) to afford **4-12** (66.6 mg, 92%) as a colorless viscous liquid: $[\alpha]^{22}_{D}$ –25.7 (*c* 0.3, $CHCl_3$); ^{1}H NMR (700 MHz, $CDCl_3$) δ 7.35–7.27 (m, 5 H, ArH), 4.89 (d, *J* = 2.8 Hz, 1 H, H-1), 4.73 (dd, *J* = 5.6, 2.8 Hz, 1 H, H-2), 4.60, 4.54 (ABq, *J* = 11.8 Hz, 2 H, 2 × OC*H*$_2$Ph), 4.24–4.19 (m, 3 H, H-3, H-7a and H-7b), 4.01 (app dt, *J* = 10.0, 4.2, 3.5 Hz, 1 H, H-5), 3.83 (dt, *J* = 9.8, 7.0, 7.0 Hz, 1 H, OC*H*$_2$CH$_2$), 3.43 (dt, *J* = 9.8, 7.0, 7.0 Hz, 1 H, OC*H*$_2$CH$_2$), 3.36 (dd, *J* = 4.9, 4.2 Hz, 1 H, H-4), 3.24 (t, *J* = 7.0, 7.0 Hz, 2 H, 2 × C*H*$_2$N$_3$), 2.74 (br s, 1 H, CH*OH*), 2.27 (dddd, *J* = 14.7, 10.2, 5.3, 4.9 Hz,1 H, H-6a), 2.06 (s, 3 H, 3 × COC*H*$_3$), 2.04 (s, 3 H, 3 × COC*H*$_3$), 1.93–1.87 (m, 1 H, H-6b), 1.60–1.50 (m, 4 H, 4 × C*H*$_2$), 1.38–1.26 (m, 8 H, 8 × C*H*$_2$); ^{13}C NMR (175 MHz, $CDCl_3$) δ 171.2 (*C*OCH$_3$), 171.1 (*C*OCH$_3$), 137.7 (Ar), 128.5 (Ar), 128.01 (Ar), 127.99 (Ar), 97.4 (C-1), 77.2 (C-4), 72.7 (O*C*H$_2$Ph), 71.8 (C-2), 70.7 (C-5), 69.7 (O*C*H$_2$CH$_2$), 66.0 (C-3), 61.5 (C-7), 51.5 (*C*H$_2$N$_3$), 29.4 (2C, C-6 and *C*H$_2$), 29.2 (*C*H$_2$), 29.0 (*C*H$_2$), 28.8 (*C*H$_2$), 26.6 (*C*H$_2$), 25.8 (*C*H$_2$), 21.1 (CO*C*H$_3$), 21.0 (CO*C*H$_3$); IR (cast film, $CHCl_3$) *v* 3471, 3065, 3031, 2933, 2858, 2096, 1738, 1497, 1455, 1433, 1242, 1045, 699 cm^{-1}; HRMS (ESI-TOF) m/z: $[M + NH_4]^+$ Calcd for $C_{26}H_{43}N_4O_8$ 539.3075; Found 539.3077.

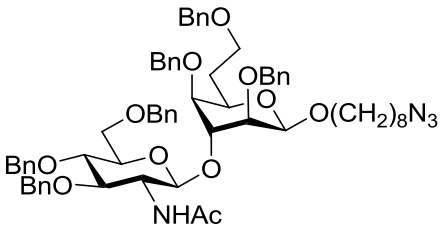


4-30

8-Azidooctyl 3,4,6-tri-*O*-benzyl-2-deoxy-2-*N*-phthalimido-β-D-glucopyranosyl-(1→3)-2,4,7-tri-*O*-benzyl-6-deoxy-β-D-*ido*-heptopyranoside (4-30)

A solution of **3-49** (80 mg, 0.11 mmol) and alcohol **4-11** (34 mg, 0.055 mmol) in dry CH_2Cl_2 (3 mL) was stirred with 4 Å molecular sieves (200 mg) for 30 min. The reaction mixture was cooled to 0 °C and TMSOTf (3.0 μL, 0.017 mmol) was added at 0 °C and stirred for 1.5 h. The TMSOTf was quenched by the addition of Et_3N (0.1 mL) and the reaction mixture was diluted with CH_2Cl_2 (40 mL) and filtered over a pad of Celite-545. The filtrate was washed with saturated aqueous $NaHCO_3$ solution (10 mL) and the organic layer was separated and the aqueous layer was further extracted with CH_2Cl_2 (2 × 30 mL). The combined organic phases were washed with brine (20 mL), dried over anhydrous Na_2SO_4, filtered, concentrated and the resulting crude product was purified by column chromatography (30% EtOAc in hexane) to afford **4-30** (49 mg, 75% over the two steps) as a white foam: $[\alpha]^{22}_D$ –4.1 (*c* 0.98, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.68–7.52 (m, 2 H, ArH), 7.40–7.16 (m, 27 H, ArH), 7.02–6.98 (m, 2 H, ArH), 6.91–6.83 (m, 3 H, ArH), 4.93 (d, J = 8.5 Hz, 1 H, H-1_{GlcN}), 4.83 (d, J = 10.5 Hz, 1 H, OC$\underline{H}_2$Ph), 4.82 (d, J = 12.5 Hz, 1 H, OC$\underline{H}_2$Ph), 4.79 (d, J = 12.0 Hz, 1 H, OC$\underline{H}_2$Ph), 4.66 (d, J = 10.5 Hz, 1 H, OC$\underline{H}_2$Ph), 4.64–4.59 (m, 2 H, 2 × OC$\underline{H}_2$Ph), 4.49 (d, J = 12.0 Hz, 1 H, OC$\underline{H}_2$Ph), 4.45 (d, J = 12.0 Hz, 1 H, OC$\underline{H}_2$Ph), 4.44 (d, J = 12.0 Hz, 1 H, OC$\underline{H}_2$Ph), 4.42 (br s, 1 H, H-1_{IdoHep}), 4.33 (d, J = 12.0 Hz, 1 H, OC$\underline{H}_2$Ph), 4.28–4.16 (m, 3 H, H-3_{GlcN} and 2 × OC$\underline{H}_2$Ph), 4.10 (dd, J = 10.5, 8.5 Hz, 1 H, H-2_{GlcN}), 3.83 (app t, J = 2.5, 2.5 Hz, 1 H, H-3_{IdoHep}), 3.81–3.68 (m, 3

H, H-4$_{GlcN}$, H-6a$_{GlcN}$ and OC$\underline{H_2}$CH$_2$), 3.59 (br s, 1 H, H-2$_{IdoHep}$), 3.50 (d, J = 10.0 Hz, 1 H, H-6b$_{GlcN}$), 3.41–3.36 (m, 1 H, H-5$_{GlcN}$), 3.34–3.30 (m, 1 H, H-5$_{IdoHep}$), 3.25 (t, J = 7.0, 7.0 Hz, 2 H, 2 × C$\underline{H_2}$N$_3$), 3.27–3.19 (m, 2 H, OC$\underline{H_2}$CH$_2$ and H-7a$_{IdoHep}$), 3.11 (app q, J = 8.5, 7.8, 7.6 Hz, 1 H, H-7b$_{IdoHep}$), 2.63 (br s, 1 H, H-4$_{IdoHep}$), 2.04 (dddd, J = 14.1, 9.1, 6.0, 5.3 Hz, 1 H, H-6a$_{IdoHep}$), 1.65–1.50 (m, 4 H, 4 × C$\underline{H_2}$), 1.40–1.10 (m, 9 H, H-6b$_{IdoHep}$ and 8 × C$\underline{H_2}$); ^{13}C NMR (125 MHz, CDCl$_3$) δ 168.2 ($\underline{C}$OPhth), 167.5 ($\underline{C}$OPhth), 139.4 (Ar), 138.7 (Ar), 138.1 (Ar), 138.0 (Ar), 133.9 (Ar), 131.4 (Ar), 131.0 (Ar), 128.5 (Ar), 128.4 (Ar), 128.2 (Ar), 128.1 (Ar), 128.0 (Ar), 127.7 (Ar), 127.5 (Ar), 127.2 (Ar), 123.5 (Ar), 123.1 (Ar), 100.1 (C-1$_{IdoHep}$), 98.8 (C-1$_{GlcN}$), 79.3 (C-4$_{GlcN}$), 79.1 (C-3$_{GlcN}$), 75.14 (C-5$_{GlcN}$), 75.07 (O$\underline{C}$H$_2$Ph), 74.9 (C-3$_{IdoHep}$), 74.8 (O$\underline{C}$H$_2$Ph), 74.7 (C-2$_{IdoHep}$), 74.6 (C-4$_{IdoHep}$), 73.8 (O$\underline{C}$H$_2$Ph), 73.7 (O$\underline{C}$H$_2$Ph), 72.6 (O$\underline{C}$H$_2$Ph), 72.3 (O$\underline{C}$H$_2$Ph), 70.9 (C-5$_{IdoHep}$), 69.1 (O$\underline{C}$H$_2$CH$_2$), 68.3 (C-6$_{GlcN}$), 66.7 (C-7$_{IdoHep}$), 55.9 (C-2$_{GlcN}$), 51.5 ($\underline{C}$H$_2$N$_3$), 30.6 (C-6$_{IdoHep}$), 29.7 ($\underline{C}$H$_2$), 29.3 ($\underline{C}$H$_2$), 29.2 ($\underline{C}$H$_2$), 28.9 ($\underline{C}$H$_2$), 26.7 ($\underline{C}$H$_2$), 26.1 ($\underline{C}$H$_2$); IR (cast film, CHCl$_3$) ν 3083, 3063, 3030, 2922, 2854, 2095, 1775, 1714, 1496, 1454, 1388, 1073, 697 cm^{-1}; HRMS (ESI-TOF) m/z: [M + NH$_4$]$^+$ Calcd for C$_{71}$H$_{82}$N$_5$O$_{12}$ 1196.5954; Found 1196.5953.

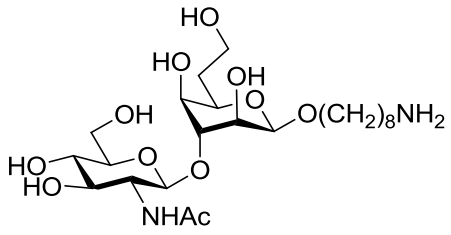


4-31

8-Azidooctyl 2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranosyl-(1→3)-2,4,7-tri-*O*-benzyl-6-deoxy-β-D-*ido*-heptopyranoside (4-31)

To a stirred solution of disaccharide **4-30** (67.0 mg, 0.0568 mmol) in ethanol (2 mL) was added ethylenediamine (0.40 mL, 6.0 mmol). The reaction mixture was heated at 90 °C for 48 h, and then the solution was cooled to room temperature over 20 min. The solution was concentrated and the resulting crude product was dissolved in EtOAc (30 mL) and washed with water (2 × 5 mL). The organic layers were separated and the aqueous layer was further extracted with EtOAc (2 × 20 mL), dried over anhydrous Na_2SO_4, filtered and concentrated to obtain the crude amine as a pale yellow viscous liquid, which was used directly in the next step. This crude amine was dissolved in pyridine (3 mL). Ac_2O (0.250 mL, 2.64 mmol) was added and the solution was stirred for 12 h. Toluene (5 mL) was added and the resulting solution was concentrated. Co-concentration with toluene (4 × 5 mL) produced a crude product that was purified by column chromatography (55% EtOAc in hexane) to afford **4-31** (44 mg, 71% over the two steps) as a colorless viscous liquid: $[\alpha]^{22}_D$ –16.7 (*c* 0.09, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.41–7.00 (m, 30 H, ArH), 5.23 (d, J = 7.5 Hz, 1 H, $N\underline{H}COCH_3$), 4.86 (d, J = 13.0 Hz, 1 H, $OC\underline{H}_2Ph$), 4.80 (d, J = 11.0 Hz, 1 H, $OC\underline{H}_2Ph$), 4.78 (d, J = 10.0 Hz, 1 H, $OC\underline{H}_2Ph$), 4.70 (d, J = 8.0 Hz, 1 H, H-1_{GlcN}), 4.66 (d, J = 13.0 Hz, 1 H, $OC\underline{H}_2Ph$), 4.64–4.42 (m, 9 H, H-1_{IdoHep} and 8 × $OC\underline{H}_2Ph$), 4.03 (app t, J = 9.5, 9.0 Hz, 1 H, H-3_{GlcN}), 3.98 (app t, J = 3.5, 2.0 Hz, 1 H, H-3_{IdoHep}), 3.89–3.82 (m, 2 H, H-5_{IdoHep} and $OC\underline{H}_2CH_2$), 3.68 (dd, J = 9.5, 4.0 Hz, 1 H, H-6a$_{GlcN}$),

3.66–3.58 (m, 3 H, H-2_{IdoHep}, H-4_{GlcN}, and H-$7a_{IdoHep}$), 3.54 (dd, J = 10.0, 5.0 Hz, 1 H, H-$7b_{IdoHep}$), 3.49 (d, J = 9.5 Hz, 1 H, H-$6b_{GlcN}$), 3.39–3.30 (m, 2 H, H-5_{GlcN} and OC$\underline{H_2}CH_2$), 3.26 (t, J = 7.0, 7.0 Hz, 2 H, 2 × C$\underline{H_2}N_3$), 3.12 (ddd, J = 9.0, 8.0, 7.5 Hz, 1 H, H-2_{GlcN}), 3.05 (br s, 1 H, H-4_{IdoHep}), 2.32–2.23 (m, 1 H, H-$6a_{IdoHep}$), 1.76–1.54 (m, 5 H, H-$6b_{IdoHep}$ and 4 × C$\underline{H_2}$), 1.64 (s, 3 H, 3 × NHCOC$\underline{H_3}$), 1.43–1.20 (m, 8 H, 8 × C$\underline{H_2}$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.3 (NH$\underline{C}$OCH_3), 139.5 (Ar), 138.7 (Ar), 138.5 (Ar), 138.1 (Ar), 137.8 (Ar), 130.4 (Ar), 130.0 (Ar), 128.5 (Ar), 128.3 (Ar), 127.9 (Ar), 127.7 (Ar), 127.2 (Ar), 126.4 (Ar), 125.7 (Ar), 124.5 (Ar), 100.3 (C-1_{IdoHep}), 100.0 (C-1_{GlcN}), 80.2 (C-3_{GlcN}), 78.6 (C-4_{GlcN}), 75.0 (C-2_{IdoHep}), 74.9 (C-5_{GlcN}), 74.8 (O$\underline{C}H_2$Ph), 74.7 (O$\underline{C}H_2$Ph), 74.5 (C-4_{IdoHep}), 74.4 (C-3_{IdoHep}), 73.8 (O$\underline{C}H_2$Ph), 73.6 (O$\underline{C}H_2$Ph), 73.1 (O$\underline{C}H_2$Ph), 72.1 (O$\underline{C}H_2$Ph), 70.8 (C-5_{IdoHep}), 69.2 (O$\underline{C}H_2CH_2$), 68.5 (C-6_{GlcN}), 66.9 (C-7_{IdoHep}), 57.7 (C-2_{GlcN}), 51.5 ($\underline{C}H_2N_3$), 31.0 (C-6_{IdoHep}), 29.7 ($\underline{C}H_2$), 29.4 ($\underline{C}H_2$), 29.2 ($\underline{C}H_2$), 28.9 ($\underline{C}H_2$), 26.7 ($\underline{C}H_2$), 26.1 ($\underline{C}H_2$), 23.3 (NHCO$\underline{C}H_3$); IR (cast film, $CHCl_3$) ν 3263, 3089, 3063, 3030, 2925, 2855, 2094, 1648, 1566, 1496, 1454, 1072, 737 cm^{-1}; HRMS (ESI-TOF) m/z: $[M + Na]^+$ Calcd for $C_{65}H_{78}N_4O_{11}Na$ 1113.5559; Found 1113.5560.

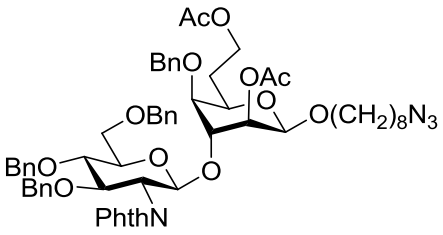


2-11a

8-Aminooctyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-6-deoxy-α-D-*altro*-heptopyranoside (2-11a)

A solution of disaccharide **4-31** (17.3 mg, 0.0159 mmol) in CH_3OH (4 mL) and CH_2Cl_2 (0.4 mL) was stirred under an argon balloon. AcOH (0.05 mL) followed by $Pd(OH)_2$–C (20 wt%,

35 mg) were added to the reaction mixture under an argon atmosphere and then the argon balloon was replaced with a H_2 balloon (1 atm). The solution was flushed with H_2 for 10 sec and the reaction mixture was stirred under H_2 for 12 h. At this point, the H_2 balloon was replaced with argon balloon and the solution was flushed argon for 1 min. The reaction mixture was diluted with CH_3OH (40 mL) and the solution was filtered over a pad of Celite-545. The filtrate was concentrated and the resulting crude product was purified by column chromatography on reversed-phase (C_{18}) silica gel (50% CH_3OH in water). The solvent was evaporated and the product was redissolved in H_2O, followed by lyophilization to afford trisaccharide **2-11a** (5.0 mg, 60%) as a white foam: $[\alpha]^{22}_D$ –10.0 (*c* 0.03, CH_3OH); ^{1}H NMR (700 MHz, D_2O) δ 4.76 (s, 1 H, H-1_{IdoHep}), 4.64 (d, J = 8.4 Hz, 1 H, H-1_{GlcN}), 4.15 (app t, J = 3.5, 2.8 Hz, 1 H, H-3_{IdoHep}), 3.97 (dd, J = 11.9, 1.4 Hz, 1 H, H-6a$_{GlcN}$), 3.92–3.84 (m, 3 H, H-2_{IdoHep}, H-5_{IdoHep} and OC$\underline{H}_2$CH$_2$), 3.81–3.74 (m, 3 H, H-7a$_{IdoHep}$, H-7b$_{IdoHep}$ and H-6b$_{GlcN}$), 3.74–3.69 (m, 2 H, H-2_{GlcN} and OC$\underline{H}_2$CH$_2$), 3.58 (app t, J = 9.8, 9.1 Hz, 1 H, H-3_{GlcN}), 3.53–3.45 (m, 3 H, H-4_{GlcN}, H-5_{GlcN} and H-4_{IdoHep}), 3.02 (t, J = 7.7, 7.7 Hz, 2 H, 2 × C$\underline{H}_2$NH$_2$), 2.07 (s, 3 H, 3 × NHCOC$\underline{H}_3$), 2.03 (ddt, J = 14.7, 9.8, 4.9, 4.9 Hz,1 H, H-6a$_{IdoHep}$), 1.79–1.73 (m, 1 H, H-6b$_{IdoHep}$), 1.71–1.62 (m, 4 H, 4 × C$\underline{H}_2$), 1.44–1.32 (m, 8 H, 8 × C$\underline{H}_2$); ^{13}C NMR (175 MHz, D_2O) δ 175.3 (NH$\underline{C}$O$\underline{C}$H$_3$), 101.4 (C-1_{GlcN}), 99.8 (C-1_{IdoHep}), 77.2 (C-3_{IdoHep}), 76.8 (C-5_{GlcN}), 74.3 (C-3_{GlcN}), 72.1 (C-5_{IdoHep}), 70.9 (O$\underline{C}$H$_2$CH$_2$), 70.7 (C-4_{GlcN}), 69.3 (C-2_{IdoHep}), 68.6 (C-4_{IdoHep}), 61.5 (C-6_{GlcN}), 58.8 (C-7_{IdoHep}), 56.3 (C-2_{GlcN}), 40.4 ($\underline{C}$H$_2$NH$_2$), 33.6 (C-6_{IdoHep}), 29.5 ($\underline{C}$H$_2$), 29.0 ($\underline{C}$H$_2$), 28.9 ($\underline{C}$H$_2$), 27.5 ($\underline{C}$H$_2$), 26.3 ($\underline{C}$H$_2$), 25.8 ($\underline{C}$H$_2$), 23.0 (NHCO$\underline{C}$H$_3$); IR (cast film, CH_3OH) ν 3293, 2928, 2856, 1658, 1077, 1036, 717 cm^{-1}; HRMS (ESI-TOF) m/z: $[M + H]^+$ Calcd for $C_{23}H_{45}N_2O_{11}$ 525.3018; Found 525.3018.

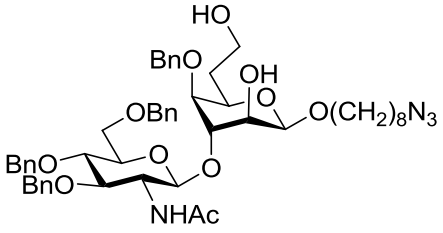


4-32

8-Azidooctyl 3,4,6-tri-*O*-benzyl-2-deoxy-2-*N*-phthalimido-β-D-glucopyranosyl-(1→3)-2,7-di-*O*-acetyl-4-*O*-benzyl-6-deoxy-β-D-*ido*-heptopyranoside (4-32)

A solution of **3-49** (184 mg, 0.254 mmol) and alcohol **4-12** (66.0 mg, 0.127 mmol) in dry CH_2Cl_2 (5 mL) was stirred with 4 Å molecular sieves (250 mg) for 30 min. The reaction mixture was cooled to 0 °C and TMSOTf (8.0 μL, 0.044 mmol) was added and the solution was stirred at 0 °C for 1.5 h. The TMSOTf was quenched by the addition of Et_3N (0.2 mL) and the reaction mixture was diluted with CH_2Cl_2 (30 mL) and filtered over a pad of Celite-545. The filtrate was washed with saturated aqueous $NaHCO_3$ solution (20 mL) and the organic layer was separated and the aqueous layer was further extracted with CH_2Cl_2 (2 × 20 mL). The combined organic phases were washed with brine (20 mL), dried over anhydrous Na_2SO_4, filtered, concentrated and the resulting crude product was purified by column chromatography (60% EtOAc in hexane) to afford **4-32** (107 mg, 78% over the two steps) as a colorless viscous liquid: $[\alpha]^{22}_D$ +12.3 (*c* 0.3, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.83–7.60 (m, 2 H, ArH), 7.42–7.22 (m, 15 H, ArH), 7.17–7.12 (m, 2 H, ArH), 7.02–6.99 (m, 2 H, ArH), 6.92–6.83 (m, 3 H, ArH), 5.21 (d, J = 8.5 Hz, 1 H, H-1_{GlcN}), 5.10 (br s, 1 H, H-2_{IdoHep}), 4.86, 4.71 (ABq, J = 10.8 Hz, 2 H, 2 × OC$\underline{H_2}$Ph), 4.81 (d, J = 12.0 Hz, 1 H, OC$\underline{H_2}$Ph), 4.73, 4.60 (ABq, J = 12.1 Hz, 2 H, 2 × OC$\underline{H_2}$Ph), 4.63 (d, J = 1.0 Hz, 1 H, H-1_{IdoHep}), 4.46 (d, J = 12.0 Hz, 1 H, OC$\underline{H_2}$Ph), 4.37, 4.23 (ABq, J = 11.8 Hz, 2 H, 2 × OC$\underline{H_2}$Ph), 4.31 (d, J = 10.5, 8.5 Hz, 1 H, H-3_{GlcN}), 4.18 (dd, J = 10.5, 8.5 Hz, 1 H, H-2_{GlcN}), 3.99 (app t, J = 3.0, 2.5 Hz, 1 H, H-3_{IdoHep}), 3.90–3.76 (m, 6 H, H-4_{GlcN}, H-$6a_{GlcN}$, H-

6b$_{GlcN}$, H-7a$_{IdoHep}$, H-7b$_{IdoHep}$ and OC$\underline{H_2}$CH$_2$), 3.66–3.62 (m, 1 H, H-5$_{GlcN}$), 3.45–3.36 (m, 1 H, H-5$_{IdoHep}$ and OC$\underline{H_2}$CH$_2$), 3.25 (t, J = 7.0, 7.0 Hz, 2 H, 2 × C$\underline{H_2}$N$_3$), 2.67 (br s, 1 H, H-4$_{IdoHep}$), 2.10–2.01 (m, 1 H, H-6a$_{IdoHep}$), 2.03 (s, 3 H, 3 × COC$\underline{H_3}$), 1.97 (s, 3 H, 3 × COC$\underline{H_3}$), 1.62–1.49 (m, 4 H, 4 × C$\underline{H_2}$), 1.38–1.22 (m, 9 H, H-6b$_{IdoHep}$ and 8 × C$\underline{H_2}$); ^{13}C NMR (125 MHz, CDCl$_3$) δ 170.8 ($\underline{C}$OCH$_3$), 170.5 ($\underline{C}$OCH$_3$), 168.1 ($\underline{C}$OPhth), 167.4 ($\underline{C}$OPhth), 138.3 (Ar), 138.02 (Ar), 137.98 (Ar), 137.4 (Ar), 128.5 (Ar), 128.4 (Ar), 128.3 (Ar), 128.13 (Ar), 128.10 (Ar), 128.0 (Ar), 127.91 (Ar), 127.88 (Ar), 127.6 (Ar), 127.4 (Ar), 99.2 (C-1$_{GlcN}$), 97.7 (C-1$_{IdoHep}$), 79.4 (C-4$_{GlcN}$), 79.1 (C-3$_{GlcN}$), 75.5 (C-5$_{GlcN}$), 75.1 (O$\underline{C}$H$_2$Ph), 74.8 (O$\underline{C}$H$_2$Ph), 74.3 (C-4$_{IdoHep}$), 73.8 (O$\underline{C}$H$_2$Ph), 73.6 (C-3$_{IdoHep}$), 72.3 (O$\underline{C}$H$_2$Ph), 70.5 (C-5$_{IdoHep}$), 69.7 (O$\underline{C}$H$_2$CH$_2$), 68.5 (C-2$_{IdoHep}$), 68.4 (C-6$_{GlcN}$), 61.0 (C-7$_{IdoHep}$), 56.1 (C-2$_{GlcN}$), 51.5 ($\underline{C}$H$_2$N$_3$), 29.7 (C-6$_{IdoHep}$), 29.5 ($\underline{C}$H$_2$), 29.3 ($\underline{C}$H$_2$), 29.1 ($\underline{C}$H$_2$), 28.9 ($\underline{C}$H$_2$), 26.7 ($\underline{C}$H$_2$), 25.9 ($\underline{C}$H$_2$), 21.1 (CO$\underline{C}$H$_3$), 20.9 (CO$\underline{C}$H$_3$); IR (cast film, CHCl$_3$) ν 3090, 3063, 3031, 2926, 2095, 1776, 1738, 1715, 1496, 1454, 1388, 1074, 699 cm^{-1}; HRMS (ESI-TOF) m/z: [M + NH$_4$]$^+$ Calcd for C$_{61}$H$_{74}$N$_5$O$_{14}$ 1100.5227; Found 1100.5231.



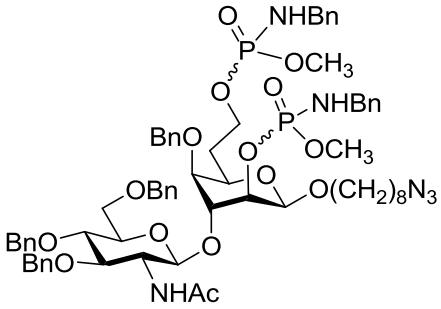
4-33

8-Azidooctyl 2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranosyl-(1→3)-4-*O*-benzyl-6-deoxy-β-D-*ido*-heptopyranoside (4-33)

To a stirred solution of disaccharide **4-32** (96.9 mg, 0.0895 mmol) in ethanol (3 mL) was added ethylenediamine (0.60 mL, 9.0 mmol). The reaction mixture was heated at 90 °C for 48 h, after which time the solution was cooled to room temperature over 20 min. The solution was

concentrated and the resulting crude product was dissolved in EtOAc (30 mL) and washed with water (2 × 5 mL). The organic layers were separated and the aqueous layer was further extracted with EtOAc (2 × 20 mL), dried over anhydrous Na_2SO_4, filtered and concentrated to obtain the crude amine as a pale yellow viscous liquid, which was used directly in the next step. This crude amine was dissolved in CH_3OH (3 mL) before Et_3N (0.6 mL) and Ac_2O (0.50 mL, 5.3 mmol) were sequentially added. The mixture was stirred for 4 h and then concentrated before the resulting crude product was purified by column chromatography (70% EtOAc in hexane) to afford **4-33** (57 mg, 70% over the two steps) as a colorless viscous liquid: $[\alpha]^{22}_D$ +6.8 (*c* 0.31, $CHCl_3$); ^{1}H NMR (700 MHz, $CDCl_3$) δ 7.38–7.19 (m, 20 H, ArH), 5.46 (d, J = 7.7 Hz, 1 H, $N\underline{H}COCH_3$), 4.83–4.81 (m, 2 H, H-1_{GlcN} and OC$\underline{H}_2$Ph), 4.78 (d, J = 10.5 Hz, 1 H, OC$\underline{H}_2$Ph), 4.67, 4.49 (ABq, J = 11.5 Hz, 2 H, 2 × OC$\underline{H}_2$Ph), 4.65 (s, 1 H, H-1_{IdoHep}), 4.62, 4.59 (ABq, J = 12.0 Hz, 2 H, 2 × OC$\underline{H}_2$Ph), 4.62–4.59 (m, 2 H, 2 × OC$\underline{H}_2$Ph), 4.16 (app t, J = 3.5, 2.8 Hz, 1 H, H-3_{IdoHep}), 3.94–3.87 (m, 2 H, H-3_{GlcN} and H-5_{IdoHep}), 3.85–3.82 (m, 2 H, H-2_{IdoHep} and OC$\underline{H}_2CH_2$), 3.76–3.66 (m, 4 H, H-4_{GlcN}, H-$6a_{GlcN}$, $6b_{GlcN}$ and H-$7a_{IdoHep}$), 3.63–3.59 (m, 1 H, H-$7b_{IdoHep}$), 3.49–3.45 (m, 2 H, H-5_{GlcN} and OC$\underline{H}_2CH_2$), 3.32–3.26 (m, 2 H, H-2_{GlcN} and CH$O\underline{H}$), 3.24 (t, J = 7.0, 7.0 Hz, 2 H, 2 × C$\underline{H}_2N_3$), 3.20 (br s, 1 H, H-4_{IdoHep}), 2.49 (br s, 1 H, $CH_2O\underline{H}$), 2.12–2.04 (m, 1 H, H-$6a_{IdoHep}$), 1.73 (s, 3 H, 3 × NHCOC$\underline{H}_3$), 1.66–1.54 (m, 5 H, H-$6b_{IdoHep}$ and 4 × C$\underline{H}_2$), 1.37–1.20 (m, 8 H, 8 × C$\underline{H}_2$); ^{13}C NMR (175 MHz, $CDCl_3$) δ 170.5 (NH$\underline{C}\underline{O}CH_3$), 138.3 (Ar), 137.9 (Ar), 136.9 (Ar), 128.6 (Ar), 128.54 (Ar), 128.50 (Ar), 128.48 (Ar), 128.3 (Ar), 128.1 (Ar), 128.0 (Ar), 127.9 (Ar), 127.8 (Ar), 99.69 (C-1_{IdoHep}), 99.66 (C-1_{GlcN}), 80.0 (C-3_{GlcN}), 78.4 (C-4_{GlcN}), 75.0 (C-5_{GlcN}), 74.7 (O$\underline{C}H_2$Ph), 74.6 (O$\underline{C}H_2$Ph), 73.9 (C-4_{IdoHep}), 73.7 (O$\underline{C}H_2$Ph), 73.5 (C-3_{IdoHep}), 72.7 (O$\underline{C}H_2$Ph), 72.2 (C-5_{IdoHep}), 69.9 (O$\underline{C}H_2CH_2$), 69.3 (C-2_{IdoHep}), 68.5 (C-6_{GlcN}), 59.8 (C-7_{IdoHep}), 57.2 (C-2_{GlcN}), 51.5 ($\underline{C}H_2N_3$), 33.3 (C-6_{IdoHep}), 29.6 ($\underline{C}H_2$), 29.3

($\underline{C}H_2$), 29.1 ($\underline{C}H_2$), 28.8 ($\underline{C}H_2$), 26.7 ($\underline{C}H_2$), 25.9 ($\underline{C}H_2$), 23.4 (NHCO$\underline{C}H_3$); IR (cast film, $CHCl_3$) ν 3481, 3299, 3065, 3030, 2929, 2858, 2096, 1656, 1557, 1497, 1454, 1073, 699 cm^{-1}; HRMS (ESI-TOF) m/z: $[M + Na]^+$ Calcd for $C_{51}H_{66}N_4O_{11}Na$ 933.4620; Found 933.4630.

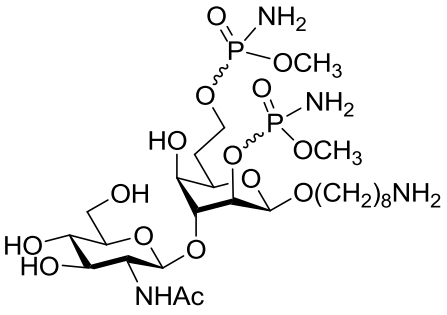


4-35

8-Azidooctyl 2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranosyl-(1→3)-4-*O*-benzyl-6-deoxy-2,7-di-*O*-(methyl *N*-benzylphosphoramidyl)-β-D-*ido*-heptopyranoside (4-35)

To a stirred solution of tetra-*N*-butylammonium methyl *H*-phosphonate, **3-32**[11a] (66.0 mg, 0.196 mmol) in CH_2Cl_2 (1.8 mL) and pyridine (0.2 mL) was added pivaloyl chloride (30 μL, 0.24 mmol). The reaction mixture was stirred for 1 h and then a solution of disaccharide **4-33** (17.7 mg, 0.0194 mmol) in CH_2Cl_2 (2 mL) was added dropwise to the above reaction mixture over 5 min. The reaction mixture was stirred for 4 h, concentrated and the resulting crude product was quickly purified by column chromatography (5% CH_3OH in EtOAc) to obtain the corresponding sugar *H*-phosphonate (18.1 mg). This *H*-phosphonate was dissolved in dry CH_2Cl_2 (1.5 mL) and to this stirring solution, Et_3N (15.0 μL, 0.108 mmol), $CBrCl_3$ (25 μL, 0.254 mmol) and $BnNH_2$ (12.0 μL, 0.110 mmol) were added sequentially, After stirring at room temperature for 4 h, the solution was diluted with CH_2Cl_2 (30 mL) and washed with saturated aqueous $NaHCO_3$ solution (15 mL). The organic layer was separated and the aqueous layer was further extracted with CH_2Cl_2 (2 × 20 mL). The combined organic phases were washed with

brine (20 mL), dried over anhydrous Na_2SO_4, filtered, concentrated and the resulting crude product was purified by column chromatography (3% CH_3OH in EtOAc) to obtain **4-35** as an inseparable mixture of methyl *N*-benzylphosphoramidates (21.9 mg, 88% yield over the two steps) as a colorless viscous liquid: ^{1}H NMR (700 MHz, $CDCl_3$) δ 7.60–7.10 (m, 120 H, ArH), 6.34 (d, J = 7.7 Hz, 1 H, $N\underline{H}COCH_3$), 6.23 (d, J = 7.7 Hz, 1 H, $N\underline{H}COCH_3$), 6.14 (br s, 1 H, $N\underline{H}COCH_3$), 6.08 (br s, 1 H, $N\underline{H}COCH_3$), 4.86–4.80 (m, 3 H), 4.80–4.65 (m, 12 H), 4.65–4.52 (m, 13 H), 4.51–4.33 (m, 14 H), 4.17–3.92 (m, 28 H), 3.90–3.70 (m, 12 H), 3.70–3.58 (m, 21 H), 3.57–3.48 (m, 12 H), 3.48–3.30 (m, 8 H), 3.30–3.10 (m, 16 H), 2.80–2.14 (m, 3 H), 2.02–1.78 (m, 5 H), 1.76 (s, 3 H, 3 × NHCOC$\underline{H}_3$), 1.72 (s, 6 H, 6 × NHCOC$\underline{H}_3$), 1.70 (s, 3 H, 3 × NHCOC$\underline{H}_3$), 1.60–1.04 (m, 48 H); ^{13}C NMR (175 MHz, $CDCl_3$) δ 170.60 (NH$\underline{C}O$CH$_3$), 170.58 (NH$\underline{C}O$CH$_3$), 170.56 (NH$\underline{C}O$CH$_3$), 170.53 (NH$\underline{C}O$CH$_3$), 139.9, 139.5, 138.5, 138.1, 137.8, 128.7, 128.5, 128.3, 128.1, 127.9, 127.5, 127.3, 127.1, 100.6, 100.5, 100.41, 100.36, 98.5, 98.23, 98.20, 80.7, 78.5, 75.2, 74.7, 73.6, 73.0, 71.9, 71.3, 70.7, 69.8, 68.9, 63.3, 59.9, 56.6, 56.4, 56.2, 56.0, 53.4, 53.0, 51.5, 45.4, 45.2, 41.1, 31.9, 31.5, 31.3, 30.2, 29.7, 29.3, 29.1, 28.8, 26.7, 25.9, 23.3, 22.7; ^{31}P NMR (162 MHz, $CDCl_3$, 26 °C) δ 12.33, 12.19, 11.25, 11.20, 10.81, 10.32, 10.15, 9.94, 9.87, 9.84, 9.80, 9.29; ^{31}P NMR (162 MHz, $CDCl_3$, 70 °C) δ 10.99, 10.11, 9.73; HRMS (ESI-TOF) m/z: $[M + Na]^+$ Calcd for $C_{67}H_{86}N_6O_{15}P_2Na$ 1299.5519; Found 1299.5518.



4-1a

8-Aminooctyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-6-deoxy-2,7-di-*O*-(methyl phosphoramidyl)-β-D-*ido*-heptopyranoside (4-1a)

A solution of disaccharide **4-35** (42.4 mg, 0.0332 mmol) in CH_3OH (5 mL) and CH_2Cl_2 (1 mL) was stirred under an argon balloon. AcOH (0.25 mL) followed by $Pd(OH)_2$–C (20 wt%, 85 mg) were added to the reaction mixture under an argon atmosphere and then the argon balloon was replaced with a H_2 balloon (1 atm). The solution was flushed with H_2 for 10 sec and the reaction mixture was stirred under H_2 for 14 h. At this point, the H_2 balloon was replaced with argon balloon and the solution was flushed argon for 1 min. The reaction mixture was diluted with CH_3OH (40 mL) and the solution was filtered over a pad of Celite-545. The filtrate was concentrated and the resulting crude product was purified by column chromatography on reversed-phase (C_{18}) silica gel (60% CH_3OH in water). The solvent was evaporated and the product was redissolved in H_2O and then lyophilizated to afford trisaccharide **4-1a** (15 mg, 64%) as a white foam: ^{1}H NMR (700 MHz, D_2O) δ 4.79–4.77 (m, 2 H), 4.62–4.57 (m, 2 H), 4.44–4.41 (m, 2 H), 4.34 (s, 1 H), 4.22–4.12 (m, 6 H), 3.91–3.81 (m, 7 H), 3.76–3.58 (m, 22 H), 3.53–3.48 (m, 4 H), 3.48–3.40 (m, 6 H), 3.34–3.29 (m, 2 H), 2.96–2.84 (m, 3 H), 2.10–1.98 (m, 10 H), 1.90–1.84 (m, 2 H), 1.63–1.50 (m, 9 H), 1.38–1.20 (m, 20 H); ^{13}C NMR (175 MHz, D_2O) δ 174.5 (NH*C*OCH$_3$), 174.4 (NH*C*OCH$_3$), 101.4, 101.3, 97.93, 97.90, 97.87, 76.0, 73.5, 73.4, 72.1,

72.0, 71.7, 71.0, 70.82, 70.79, 70.75, 70.72, 70.3, 70.0, 69.8, 69.5, 69.2, 67.33, 67.26, 63.4, 63.3, 60.6, 60.4, 55.5, 54.19, 54.16, 54.0, 53.9, 53.7, 53.64, 53.61, 53.58, 43.1, 39.7, 30.88, 30.80, 30.75, 30.7, 28.7, 28.5, 28.22, 28.19, 28.18, 27.7, 26.6, 26.5, 25.6, 25.14, 25.05, 22.19, 22.17, 21.9, 18.6; ^{31}P NMR (162 MHz, $CDCl_3$) δ 14.64, 14.46, 14.43, 14.02; HRMS (ESI-TOF) m/z: $[M + H]^+$ Calcd for $C_{25}H_{53}N_4O_{15}P_2$ 711.2977; Found 711.2976.

4.6 References

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Chapter 5

Summary and Future work

5.1 Introduction

This thesis describes the synthesis of fragments of 6-deoxy-D-heptopyranoside-containing capsular polysaccharides (CPS) of *Campylobacter* species and contains two parts. The first part is focused on developing an efficient route to synthesize 6-deoxy-D-heptopyranosides as 1-thio-glycoside donors (Research Objective 1: Chapter 2, part-1). The second part is focused on the synthesis of 6-deoxy-D-heptopyranoside containing CPS fragments isolated from different *Campylobacter* species (Research Objective 2: Chapter 2, part-2, Chapter 3 and Chapter 4).

5.2 Summary

5.2.1 Synthesis of 6-deoxy-D-*hepto*pyranosides as *p*-tolyl-1-thio glycoside donors

In Chapter 2, part-1, we developed an efficient route to *p*-tolyl 6-deoxy-1-thio-D-heptopyranosides (Figure 5-1) from readily available methyl α-D-mannopyranoside **2-37**.

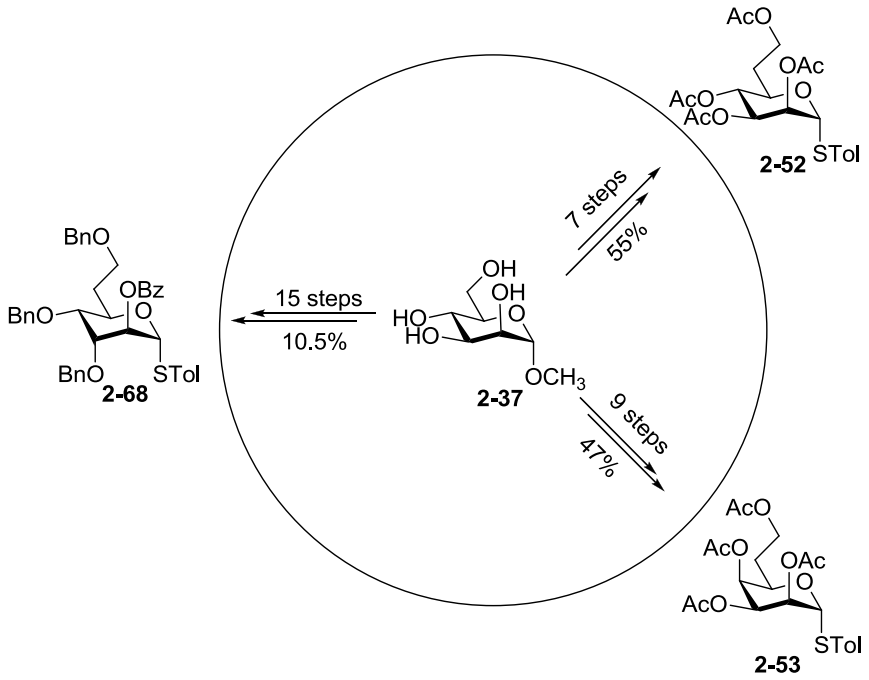

Figure 5-1: Summary of the synthesis of *p*-tolyl 6-deoxy-1-thio-D-heptopyranosides.

The key homologation step in the approach is the nucleophilic displacement of the 6-iodo compound **2-50** using lithium bis(phenylthio)methanide (**5-1**) followed by hydrolysis of dithioacetal **2-59** and reduction of the resulting aldehyde (Figure 5-2). After homologation, **2-59** could be converted into **2-52** and the C-4 and C-3 configurational isomers **2-53** and **2-67**/**2-68**. The developed protocol allows the synthesis of differentially protected 6-deoxy-D-heptopyranosides that can be used in the synthesis of other CPS fragments.

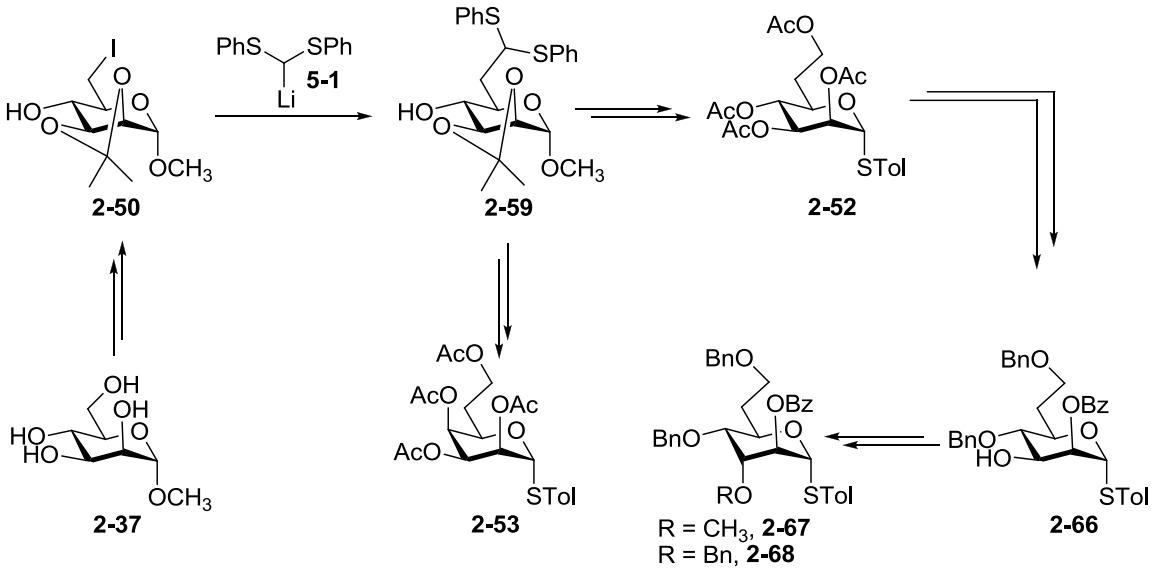

Figure 5-2: Key intermediates in the synthesis of *p*-tolyl 6-deoxy-1-thio-D-heptopyranosides.

5.2.2 Synthesis of trisaccharide repeating unit of *Campylobacter coli* serotype O:30

The second part of this thesis (Research Objective 2) describes the synthesis of CPS fragments. In Chapter 2, part-2 we focused on the synthesis of the trisaccharide (**2-9a**) present in *Campylobacter coli* serotype O:30. The optimal route developed for the synthesis of **2-9a** is summarized in Figure 5-3. We initially used a [1 + 2] glycosylation approach using the ulosyl bromide **2-117** and the disaccharide **2-97** followed by highly diastereoselective reduction of the uloside to generate the β-D-*talo*-heptopyranosidic linkage present in trisaccharide **2-126**. This

approach gave the moderate diastereoselectivity during the glycosylation. To improve the selectivity, we explored an oxidation–reduction approach with a [1 + 2] glycosylation strategy using the glycosyl bromide **2-127** and the disaccharide **2-97** (to form **2-128**). However, we could not deprotect the acetate in presence of benzoate in **2-128**, which was necessary for the oxidation–reduction approach. Finally, a different oxidation–reduction approach was developed, involving glycosylation between bromide **2-127** and the thioglycoside **2-131** to obtain β-D-*talo*-heptopyranoside disaccharide that was coupled to ribitol derivative **2-100**. The phosphodiester linkage in **2-9a** was achieved through the H-phosphonate derived from **2-135**, activation using PivCl and oxidation using iodine and pyridine and finally subjecting the resulting phosphodiester to global deprotection.

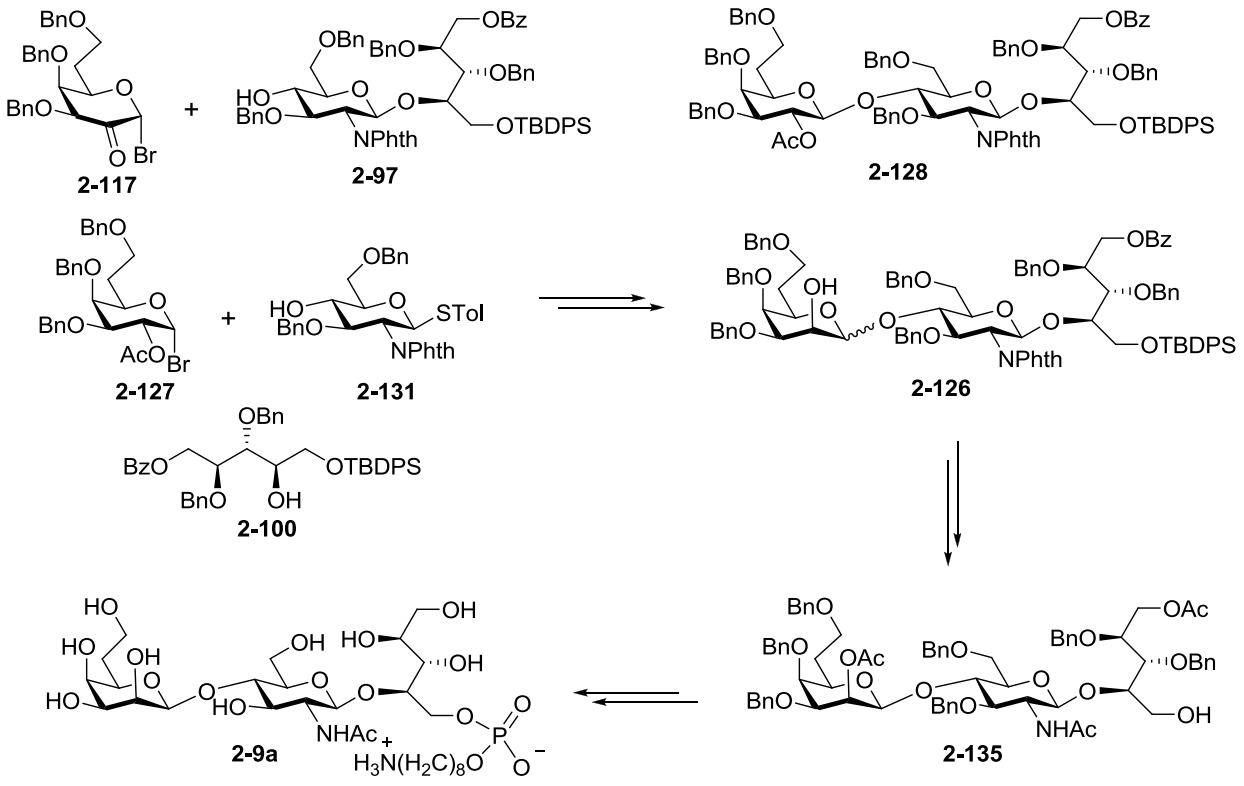

Figure 5-3: Key building blocks and intermediates in the synthesis of the CPS trisaccharide **2-9a**.

5.2.3 Syntheses of the *Campylobacter jejuni* 81-176 CPS trisaccharides

In Chapter 3, we focused on the syntheses of the three trisaccharides (**2-10a**, **3-2a** and **3-3a**) produced by *Campylobacter jejuni* 81-176 strain. The optimal route we developed for these trisaccharides, including one that contains a MeOPN motif (**3-3a**), is summarized in Figure 5-4. Their synthesis began with the formation of the β-linked disaccharide **3-47**. The 1,2-*cis*-α-glycosidic linkage was installed via the coupling of the 4,6-*O*-di-*tert*-butylsilylene acetal containing thioglycoside donor **3-35** and the 6-deoxy-*altro*-heptopyranoside acceptors **3-33** or **3-34**. Subsequent steps led to the syntheses of the key intermediates **3-52** and **3-53**. Deprotection of **3-52** and **3-53** produced the parent trisaccharide **2-10a** and trisaccharide **3-2a**, containing the methyl modification. Successful installation of the MeOPN group on the trisaccharide was achieved by reaction with an activated *O*-methyl *H*-phosphonate acetate ester (**3-60**) followed by oxidation using the Atherton–Todd reaction. Using this approach, products corresponding to both diastereomers on phosphorus were formed – (***R***)-**3-54** and (***S***)-**3-54**. We were fortunate to separate the two diastereomers. Fortuitously, (***R***)-**3-54** was a solid and we obtained an X-ray structure of the compound. Analysis of the structure allowed us to assign the stereochemistry at phosphorous in (***R***)-**3-54** as *R*. The diastereomer (***R***)-**3-54**, following its deprotection (to form (***R***)-**3-3a**) and comparison with data for the polysaccharide, established the phosphorous stereochemistry of the MeOPN in the native CPS as *R*. This represents the first unambiguous determination of the stereochemistry of a *Campylobacter* CPS MeOPN group, which exists naturally as a single stereoisomer. It remains to be determined if MeOPN-functionalized CPS produced by other *Campylobacter* have the same stereochemistry. Similarly, deprotection of the diastereomer (***S***)-**3-54** produced unnatural CPS trisaccharide **(*S*)-3-3a**. We anticipate this study

will lead to the development of methods for the diastereoselective introduction of the MeOPN groups onto carbohydrates.

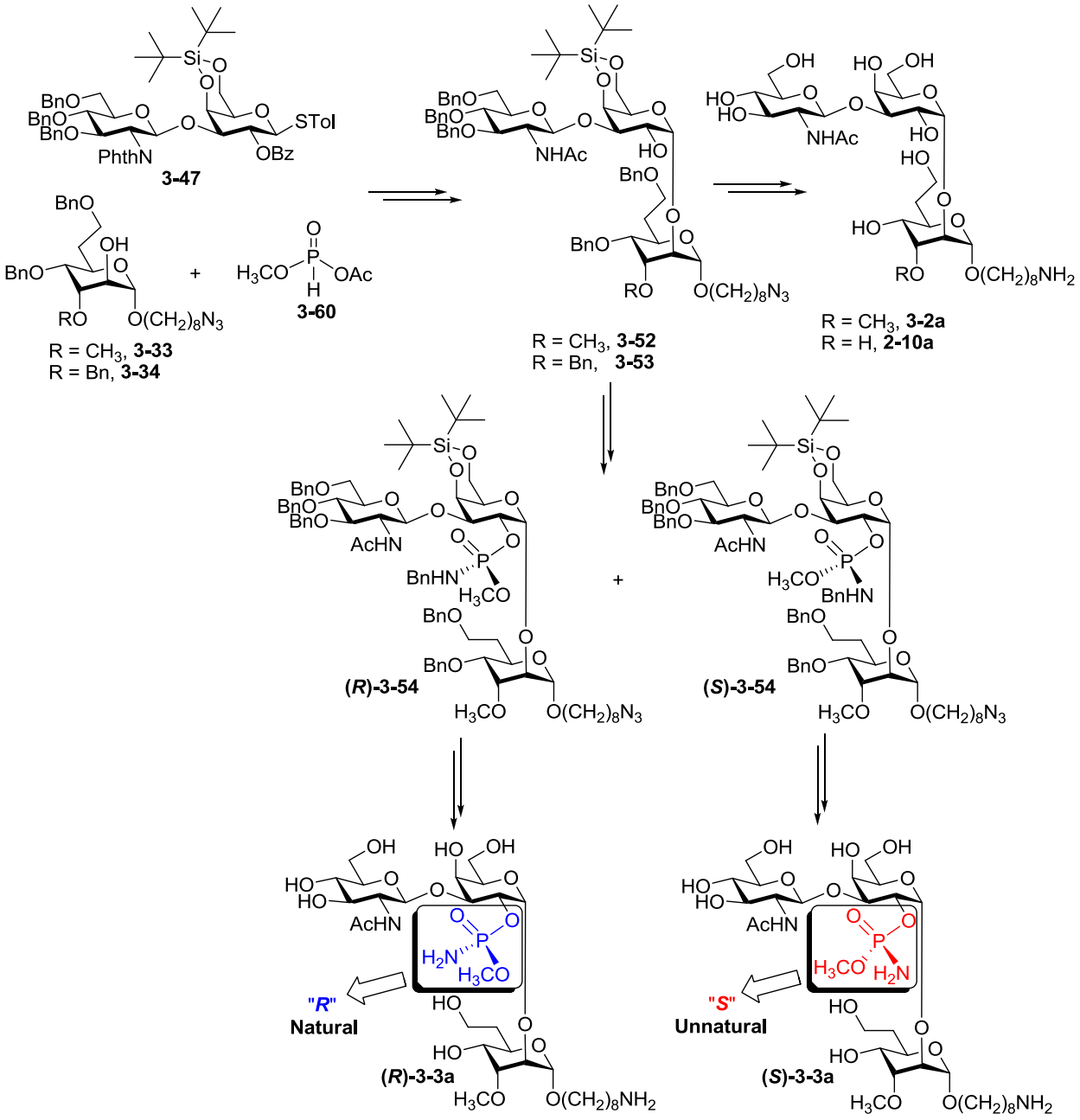

Figure 5-4: Key building blocks and intermediates in the syntheses of the CPS trisaccharides **2-10a**, **3-2a** and **3-3a**.

5.2.4 Syntheses of the *Campylobacter jejuni* CG8486 strain (HS4 serotype) CPS disaccharides

In Chapter 4, we focused on the syntheses of the two disaccharides (**2-11a** and **4-1a**) produced by *Campylobacter jejuni* CG8486 strain (Figure 5-5). Their synthesis began with the preparation of 6-deoxy-D-*galacto*-heptopyranoside donors, **4-19** and **4-20** from **2-69**. The C-6 homologation of **2-69** and subsequent steps led to the syntheses of donors **4-19** and **4-20**. The 1,2-*trans*-β-glycosidic linkage was installed using donors **4-19** and **4-20**. Further steps using Ling's protocol[1] led to the syntheses of the key 2,3-anhydro-β-D-*talo*-heptopyranoside intermediates **4-13** and **4-14**. Protecting group manipulations and C-3 inversion gave β-D-*ido*-heptopyranoside acceptors **4-11** and **4-12**. Glycosylation of **4-11** or **4-12** with the trichloroacetimidate donor **3-49** followed by protecting group manipulations provided disaccharides **4-31** and **4-33**. Deprotection of **4-31** afforded **2-11**. Successful installation of the MeOPN onto the disaccharide **4-33** was achieved by reacting the disaccharide with an activated *O*-methyl *H*-phosphonate pivalate ester **4-34** followed by oxidation using the Atherton–Todd reaction. Deprotection of the resulting disaccharide led to the synthesis of the di-MeOPN containing disaccharide **4-1a**.

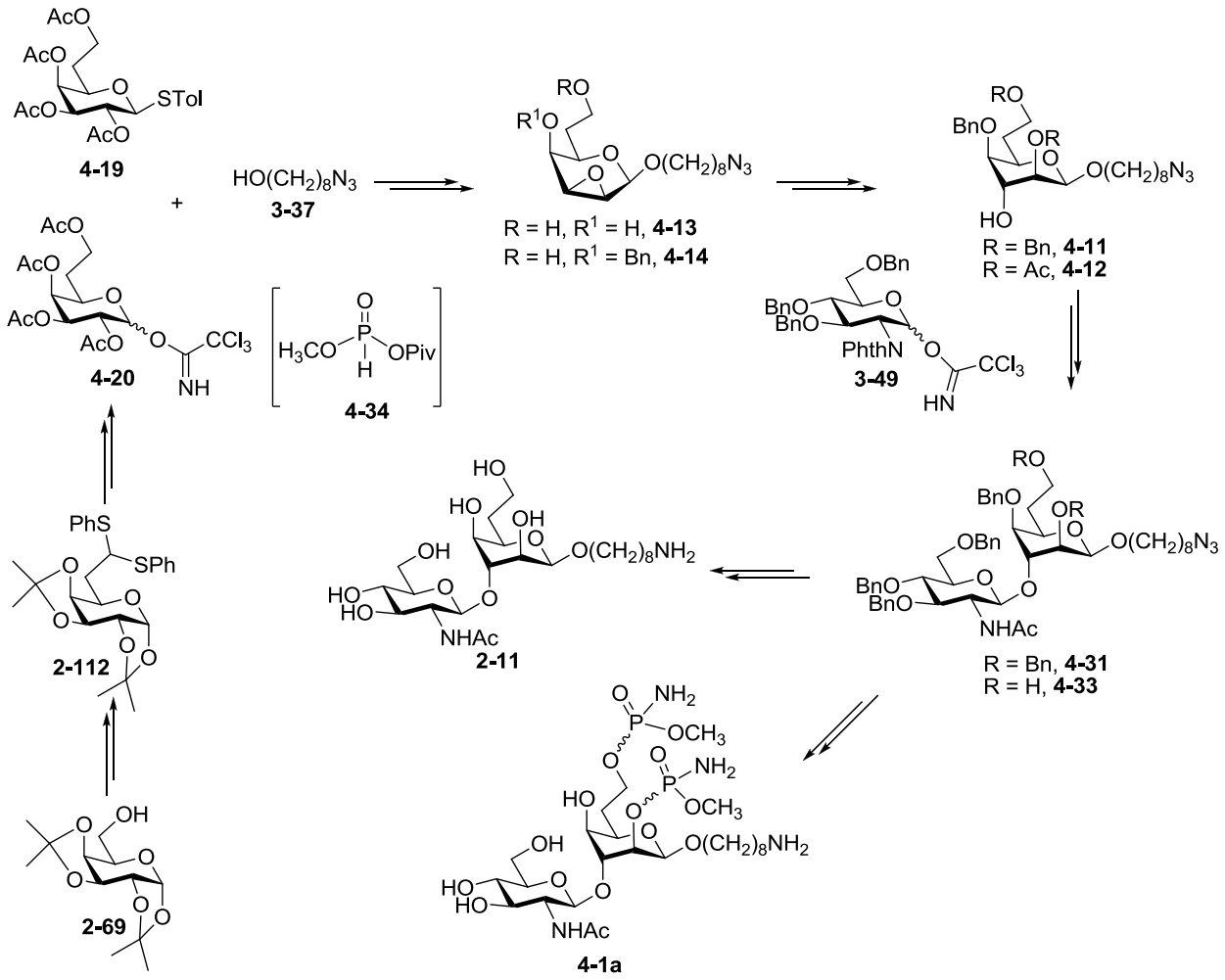

Figure 5-5: Key building blocks and intermediates in the syntheses of the CPS trisaccharides **2-11a**, and **4-1a**.

5.3 Future work

Our developed homologation approach for the synthesis of 6-deoxy-D-heptopyranosides resulted in the preparation of multigram quantities of *p*-tolyl 2,3,4,7-tetra-*O*-acetyl-6-deoxy-1-thio-α/β-D-*manno*-heptopyranoside **2-52**. This compound will be used in the synthesis of the pentasaccharide repeating unit (**5-2**, Figure 5-6) of the CPS produced by *C. jejuni* RM1221 strain

(HS53 serotype). This work will be continued by another member in the group, Boshun-Huang due to time constraints of my Ph.D. studies.

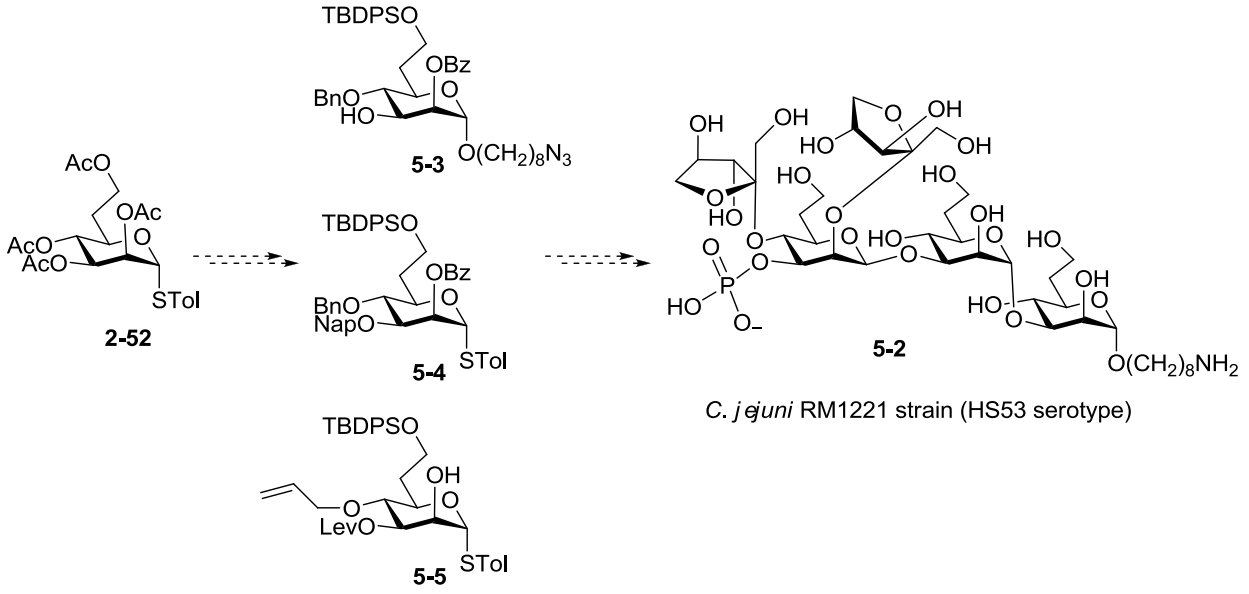

Figure 5-6: Future work with respect to synthetic CPS antigen **5-2**.

The antigenic trisaccharides (**2-9a**, **2-10a**, **2-11a** and **3-2a**) were synthesized in a form for conjugation to appropriate proteins and/or probes (Figure 5-7). Future work involves the synthesis of bovine serum albumin (BSA) conjugates and evaluation of antibody generation through collaboration for **2-10a**, **2-11a**, and **3-2a**. Unfortunately, because of the time constraints of my Ph.D. studies, I could not make these BSA conjugates. For the trisaccharide **2-9a**, future work involves the determination of the absolute stereochemistry of ribitol moiety through the synthesis of the trisaccharide with the proposed ribitol moiety (**2-9c**) stereochemistry and comparison to the natural CPS.[2]

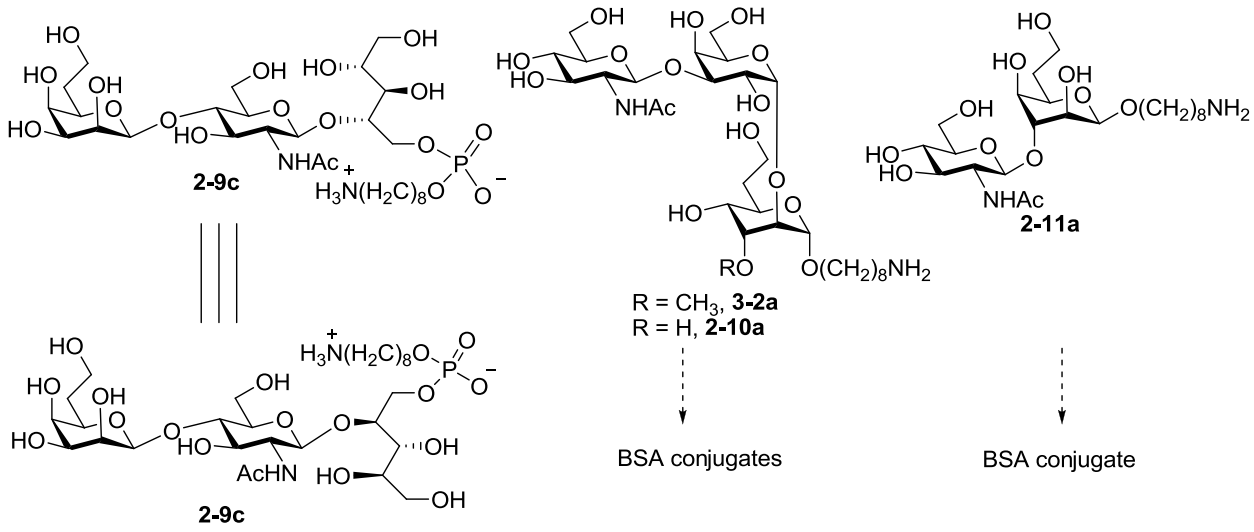

Figure 5-7: Future work with respect to synthetic CPS antigens **2-9a**, **2-10a**, **2-11a**, and **3-2a**.

With respect to trisaccharides (***R***)**-3-3a** and (***S***)**-3-3a**, the future work involves the synthesis of BSA conjugates using a di-*p*-nitrophenyl-adipate linker (Figure 5-8). They will be tested for the specificity of sera raised against vaccination with the native CPS in collaboration with Professor Mario Monteiro at the University of Guelph. Unfortunately, because of the time constraints of my Ph.D. studies, I could not make these BSA conjugates.

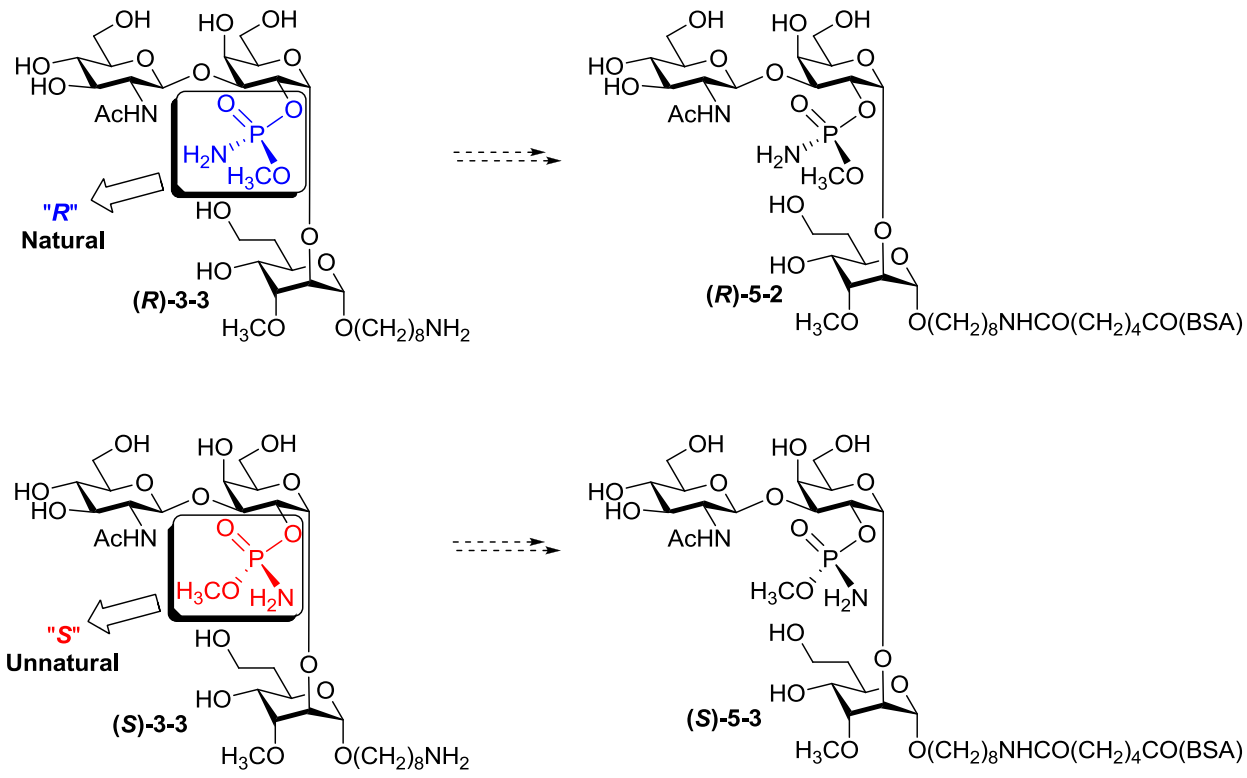

Figure 5-8: Preparation of BSA conjugates of (***R***)**-3-3a** and (***S***)**-3-3a**.

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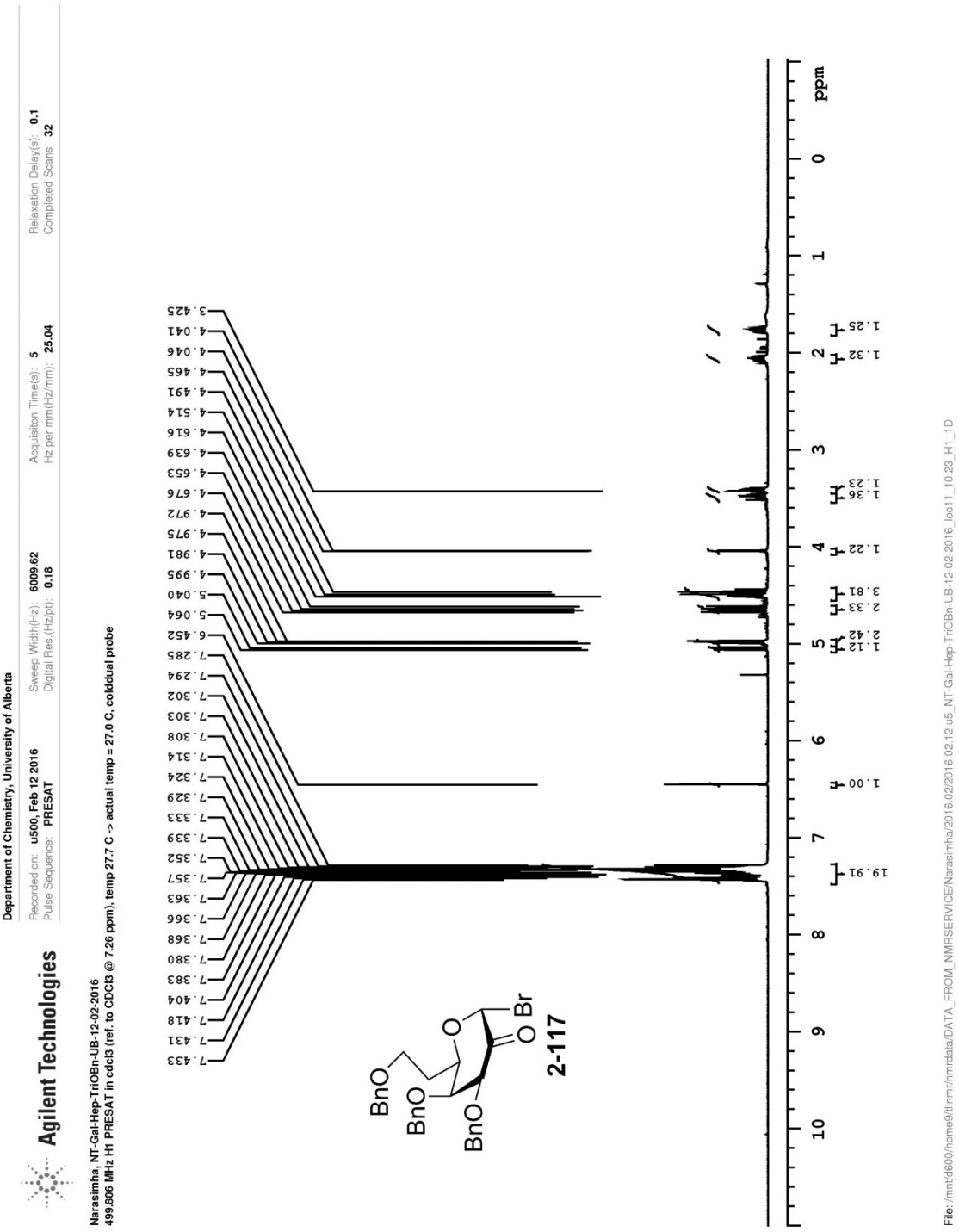
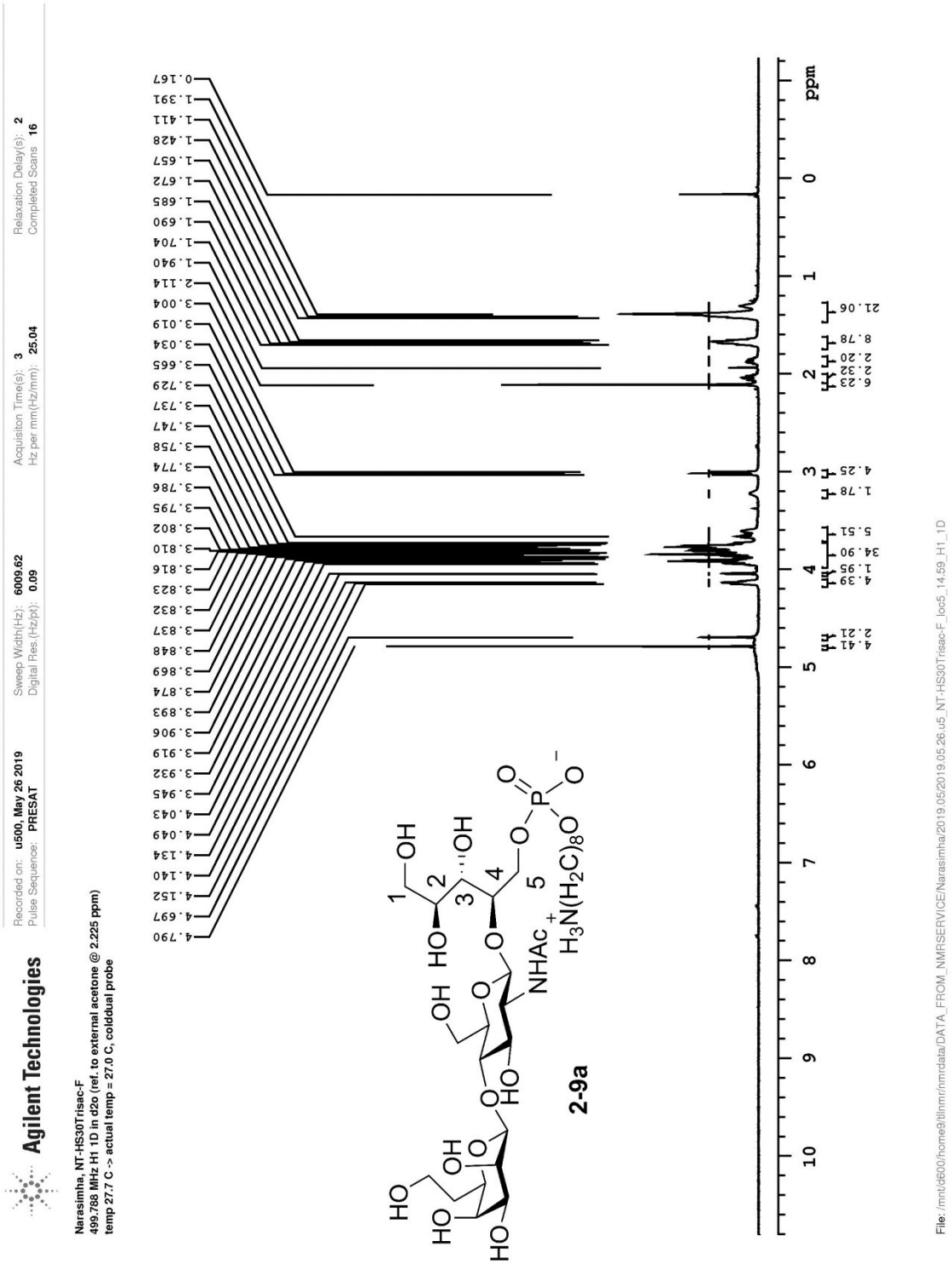
Appendix: Selected copies of NMR spectra

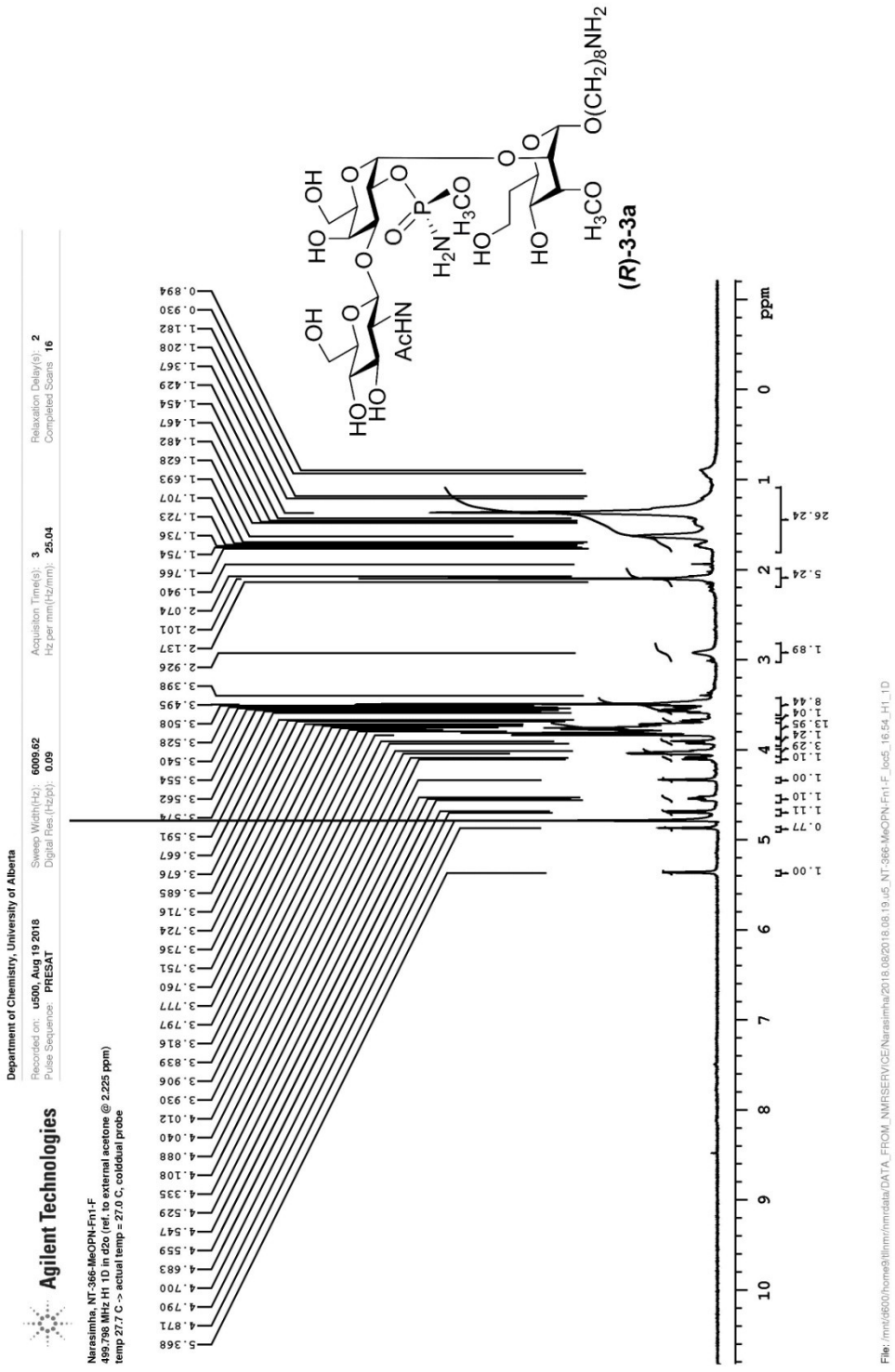
^{1}H NMR spectrum of compund **2-117** (500 MHz, $CDCl_3$)



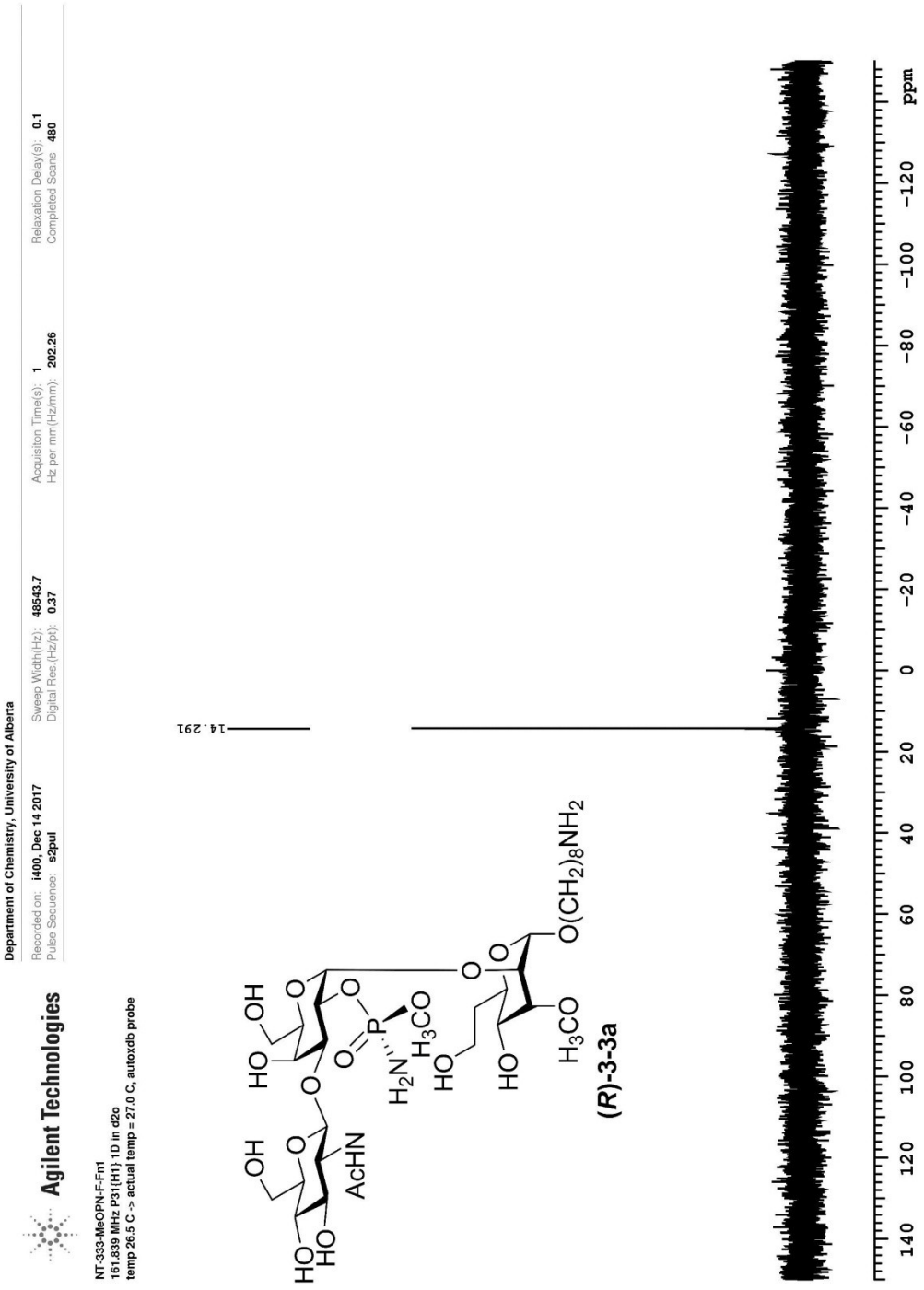
^{1}H NMR spectrum of compund **2-9a** (500 MHz, D_2O)



^{1}H NMR spectrum of compund **(*R*)-3-3a** (500 MHz, D_2O)



^{31}P NMR spectrum of compund **(*R*)-3-3a** (162 MHz, D_2O)



^{1}H NMR spectrum of compund **2-11a** (500 MHz, D_2O)