

Chemical Synthesis of Lipopolysaccharide Derived from *Klebsiella pneumoniae* for Conjugate Vaccine Development

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Chemical Synthesis of Lipopolysaccharide Derived from *Klebsiella pneumoniae* for Conjugate Vaccine Development

**Chemische Synthese van *Klebsiella pneumoniae*
Lipopolysaccharide Derivaten voor de Ontwikkeling
van Geconjugeerde Vaccins**
(met een samenvatting in het Nederlands)

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List of Abbreviations

Ac	Acetyl
All	Allyl
BAIB	(Diacetoxyiodo)benzene
Bn	Benzyl
Bu	Butyl
BSA	Bovine Serum Albumin
Bz	Benzoyl
Cbz	Carboxybenzyl
CPS	Capsular Polysaccharide
CRM ₁₉₇	Cross Reacting Material 197
CSA	Camphor-10-sulfonic acid
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DCC	<i>N,N'</i> -Dicyclohexylcarbodiimide
DCM	Dichloromethane
DDQ	2,3-Dichloro-5,6-dicyano-1,4-benzoquinone
DIPEA	<i>N,N</i> -Diisopropylethylamine
DMAP	4-Dimethylaminopyridine
DMF	Dimethylformamide
DTT	Dithiothreitol
EA	Ethyl Acetate
EDCI	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
ESI-TOF	Electrospray Ionization – Time of Flight
Et	Ethyl
Gal	Galactose
GalA	Galacturonic Acid
Glc	Glucose
GlcN	Glucosamine

GlcNAc	<i>N</i> -acetyl-D-glucosamine
Hep	<i>L-glycero</i> -D- <i>manno</i> -heptose
HRMS	High Resolution Mass Spectrometry
Kdo	3-Deoxy-D- <i>manno</i> -2-octulosonic acid
LC-MS	Liquid Chromatography – Mass Spectrometry
Lev	Levulinoyl
LPS	Lipopolysaccharide
Man	D-Mannose
Me	Methyl
MALDI-Tof	Matrix-Assisted Laser Desorption/Ionization–Time of Flight
4 Å MS	4 Angstrom Molecular Sieves
MS	Mass Spectrometry
Nap	2-Methylnaphthyl
NIS	<i>N</i> -Iodosuccinimide
NMR	Nuclear Magnetic Resonance
PBS	Phosphate-Buffered Saline
PE	Petroleum Ether
Ph	Phenyl
RT	Room Temperature
SAMA-OPfp	Perfluorophenyl 2-(acetylthio)acetate
SMCC	Succinimidyl- <i>trans</i> -4-(<i>N</i> -maleimidylmethyl)cyclohexane-1-carboxylate
TBAB	Tetrabutylammonium Bromide
TBAF	Tetrabutylammonium Fluoride
TBDPS	<i>tert</i> -Butyldiphenylsilyl
TBS	(<i>tert</i> -Butyl)dimethylsilyl
TEMPO	(2,2,6,6-Tetramethylpiperidin-1-yl)oxyl
TMSOTf	Trimethylsilyl Trifluoromethanesulfonate

TLC	Thin Layer Chromatography
Tf	Trifluoromethanesulfonyl
TCA	Trichloroacetyl
TFA	Trifluoroacetyl
THF	Tetrahydrofuran
Tol	Toluene

Chapter 1

General Introduction

1.1 *Klebsiella pneumoniae*

Klebsiella pneumoniae (*K. pneumoniae*) is a rod-shaped, nonmotile Gram-negative bacteria (GNB), and a common nosocomial pathogen.^{1, 2} The most frequently disease caused by *K. pneumoniae* is pneumonia, which has a mortality rate of ~50%. These bacteria develop easily drug resistance.³ The strains produced by enhanced-spectrum β -lactamase (ESBL) are even more problematic due to their resistance to carbapenems, which are widely used drugs for management of infections caused by *K. pneumoniae*. In Europe, more than 90,000 infections and 7,000 deaths occur annually accounting for these *K. pneumoniae* strains.⁴ In Southeast Asia and South Africa, patients infected by a high virulence *K. pneumoniae*, which expresses a hypermucoid K1 capsular polysaccharide, was observed, and these *K. pneumoniae* infections can cause diverse diseases such as pyogenic liver abscess, meningitis and endophthalmitis.⁵ Despite antibiotics are becoming less effective, there is still no vaccine for *K. pneumoniae*.⁶ A number of vaccines have reached clinical trials, but have failed due to the poor vaccine coverage or low immunogenicity.⁷ Hence, there is an urgent need to develop a vaccine for *K. pneumoniae*.^{8, 9}

1.2 Virulence factors of *Klebsiella pneumoniae*

Numerous virulence factors of *K. pneumoniae*, including capsular polysaccharide (CPS), lipopolysaccharide (LPS), siderophores and fimbriae (also called pili), have been identified.² These factors play important roles in growth and survival of *K. pneumoniae* after infection.¹⁰ After infection, *K. pneumoniae* will be recognized by the immune system of host, and the bacteria has to protect itself to survive by escaping from host immune defenses.¹¹ LPS and CPS are two factors

of *K. pneumoniae* that contribute to these processes.¹⁰

1.2.1 Lipopolysaccharide (LPS, endotoxin)

LPS, a major component of outer membrane of Gram-negative bacteria, protects it from immune attack but can also activate host immune responses during infection. For *K. pneumoniae*, LPS may be covered by a layer of CPS, and these surface structures may be able to deactivate complement. However, LPS may also be exposed on cell surface by reaching through the CPS layer in certain *K. pneumoniae* serotypes.¹² Similar to other Gram negative bacteria such as *Helicobacter pylori*, *Yersinia pestis* and *Porphyromonas gingivalis*, LPS of *K. pneumoniae* can also be modified to avoid recognition by immune receptors in certain strains.¹³

To date, nine serotypes of *K. pneumoniae* LPS have been identified and they consist of three parts-lipid A that anchors the structure to the outer membrane, an oligosaccharide core and repeating polysaccharide chain called O-antigen (Fig. 1)¹⁴. Generally, lipid A exerts high endotoxic activity by binding to Toll-like receptor 4 (TLR4) causing strong activation of innate immunity. Lipid A is an important binding target of polymyxins, and an increased susceptibility to these antibiotics for *K. pneumoniae* strains with under-acylated lipid A has been observed.¹⁵ The core oligosaccharide links lipid A to O-antigen, and can be divided into an inner- and outer core (Fig. 2A). The LPS core shows high similarity between bacterial species especially the inner core region. There are only two types of core oligosaccharide described, and core type 1 is the most common one.¹⁶ Unlike other *Enterobacteriaceae*, *K. pneumoniae* lacks phosphoryl modifications and instead contains galacturonic acid (GalA) and Kdo residues that provide negative charge to stabilize the outer membrane.¹⁷ The most common clinical O-antigen isolates are subdivided into four serogroups (O1, O2, O3 and O5) and all of their polysaccharide chains consist of galactans or mannans.¹⁸ O1 expresses two O-antigens, D-Gal I and D-Gal II, while D-Gal II is

GlcNAc, *N*-acetylglucosamine; Kdo, 3-deoxy-D-*manno*-2-octulosonic acid, L,D-Hep, L-*glycero*-D-*manno*-heptose; GlcN, glucosamine; GalA, galacturonic acid; Glc, glucose; Gal, galactose; Galf, galactofuranose; Man, mannose; CR, core region.

1.2.2 Capsular polysaccharide (CPS, K-antigen)

CPS, a complex acidic polysaccharide that coats the cell, is produced by almost all *Enterobacteriaceae* and is another type of polysaccharide of *K. pneumoniae* cell surface besides LPS.^{10, 23} It plays important roles in protecting bacteria against phagocytosis by covering the cell with thick fibrillous structures.²⁴ To date, 79 serotypes of K-antigen for *K. pneumoniae* have been identified.²⁵ The high diversity of chemical structures makes it difficult to develop a universal vaccine for *K. pneumoniae*.²⁶ A few of the 79 serotypes, such as K1 and K2, show higher virulence than others due to an increase of CPS production.²⁷ However, 10 - 70% isolates are not typeable as they are non-capsulated or express a novel capsule.²⁸ Furthermore, the encapsulated *K. pneumoniae* strains are more difficult to kill by serum complement than non-capsule strains due to the ability of CPS to block phagocytosis.^{29, 30} Based on these observations, only several serotypes of K-antigens have been studied systemically.¹² With a very high mortality rate (30-70%), infections caused by Carbapenem-resistant *Klebsiella pneumoniae* (CR-*Kp*) have been a major cause of hospital deaths in recent years.³¹ The majority of CR-*Kp* clinical isolates are designated as ST258, which comprise two distinct clades-CPS1 and CPS2.³² Infections with CPS2 have a higher mortality and are more common (encompasses up to 88% of ST258 strains) than those with CPS1. The chemical structure of CPS2 is similar to CPS of K19 and K34, and it consists of a hexasaccharide repeating unit (Fig. 3).^{33, 34}

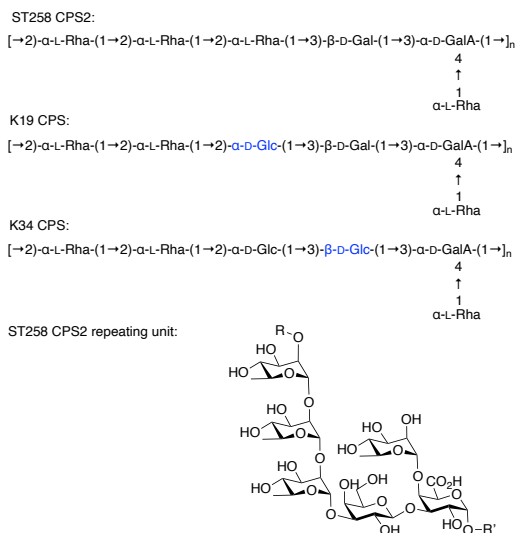


Figure 3. Chemical structure of oligosaccharide of ST258 CPS2, K19 and K34. Rha, rhamnose; Gal, galactose; GalA, galacturonic acid, Glc, glucose.

1.2.3 Siderophores and fimbriae (pili)

Siderophores are small iron-chelating molecules that are produced by bacteria to take up iron from the host and transport ferric ions, which are essential for almost all forms of life including Gram negative bacteria, into bacterial cells by binding the iron-complex to an outer-membrane receptor.³⁵ Therefore, siderophores are critical for bacterial growth and establishing infections.³⁶ It has been found that *K. pneumoniae* can produce one or more of four siderophores: enterobactin (primary siderophore), salmochelin, yersiniabactin and aerobactin.²

Fimbriae, generally comprised of repeating protein subunits and adhesive subunits, are produced by bacteria to facilitate surface adhesion, cell-to-cell interaction and biofilm formation.^{37, 38} *K. pneumoniae* produce two major fimbriae: type 1 and type 3 fimbriae.³⁹ Type 1 fimbriae are on the bacterial cell surface and expressed by 90% of *K. pneumoniae*, and they have the ability to bind D-mannosylated glycoproteins.² Type 3 fimbriae, on the other hand, are unable to bind mannose and were found to be expressed by almost all *K. pneumoniae*

strains.² Vaccines based on siderophores or fimbriae are still at earlier stages of development.⁴⁰

1.3 Vaccinations against *Klebsiella pneumoniae*

It is challenging to control infections caused by *K. pneumoniae* due to the emergence of Carbapenem-resistant strains and the high variability of strains.^{40,}

⁴¹ Currently, epitope-based vaccines have drawn attention for treatment of bacterial infections.⁴² These vaccines, consist of highly immunogenic epitopes, can stimulate T cells that introduce protective immune response in infectious diseases.⁴³ However, no licensed vaccine is available to date despite many experimental vaccines have been explored since 1990s.⁶

1.3.1 Whole cell vaccines

Whole cell vaccines, also called the first-generation vaccines, consist of whole inactivated bacterial cell that can induce immune responses. The inactivated/killed vaccines were prepared by killing bacterial cells using formalin or drying the cells with acetone.^{44, 45} However, the inactivated/killed vaccines failed to reach the market because of limited safety.⁸ A safer method to produce whole cell vaccines was developed by using bacterial cell lysate to prepare cell-free vaccine that contains all immunogenic components of cells. The vaccine prepared by treating *K. pneumoniae* strain 204 with hydroxylamine showed high immunogenicity and the ability to reduce reactogenic response.⁴⁶

1.3.2 Glycoconjugate vaccines

Among pathogen factors of *K. pneumoniae*, CPS and LPS are attractive for developing vaccines.¹² In 1980s, a 24-valent CPS based vaccine was examined to protect humans from *K. pneumoniae* infections. It elicited high antibody titers, however, the vaccine was not released to market because of the low coverage (less than 70%) of *K. pneumoniae* strains and its production was complex.^{47, 48} LPS, on the other hand, is regarded as a more attractive vaccine candidate because

it has fewer serotypes and the antibodies targeting LPS are beneficial.⁴⁹ The main disadvantage of LPS being a vaccine is toxicity, which can be reduced by detoxification of LPS such as liposomal preparations or alkali treatments.⁸

A polysaccharide alone elicits thymus-independent immune responses, which provides only short-term protection due to a lack of T cell epitopes (Fig. 4A).⁵⁰ Polysaccharides can easily be recognized and bound by antibodies expressed on the surface of B cells, however, cannot be presented by the major histocompatibility complex.⁵¹ By conjugation of a polysaccharide such as LPS or CPS to a carrier protein, proteolytic peptides derived from the carrier protein can be placed in the cavity of the major histocompatibility complex and can then be presented to T cells. Therefore, T cells will be engaged that will lead to the production of memory B cells and plasma cells (Fig. 4B).⁵² As a consequence, conjugate vaccine can elicit thymus-dependent immune response, which provides long-term protection. A vaccine for *Haemophilus influenzae* type B has been approved for human use that illustrates the potential use of organic synthesis in the development of glycoconjugate vaccines.⁵³ However, the conjugation of polysaccharide to carrier protein is still challenging due to lack of functional groups on the sugar for controlled conjugation.

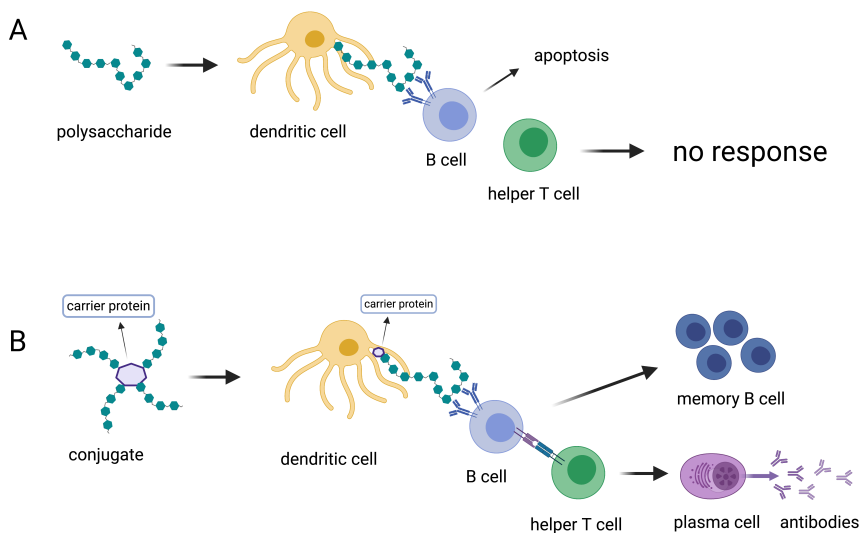


Figure 4. Interactions of polysaccharide (A) and conjugate (B) vaccines with dendritic cell, B cell and T cell.

As a carrier for conjugate vaccines, the protein should be safe (nontoxic), well established and immunogenic. To date, there are five carrier proteins that have been used in licensed conjugate vaccines (Table 1.): tetanus toxoid (TT), diphtheria toxoid (DT), cross-reactive material 197 (CRM₁₉₇), *Neisseria meningitides* outer membrane protein complexes (OMPC) and non-typeable *Haemophilus influenzae* derived protein D (PD).⁵⁴ Other carrier proteins, such as bovine serum albumin (BSA) and keyhole limpet hemocyanin (KLH), are not clinically acceptable despite they have been widely used for development of conjugate vaccines because the data generated from the studies of clinical acceptable carrier proteins and those may have large difference.⁵⁵ All the five carrier proteins are derived from pathogens: TT (140 kDa) and DT (58 kDa) are prepared by chemical inactivation of tetanus and diphtheria toxin, respectively; CRM₁₉₇, 58 kDa, is a nontoxic mutant of diphtheria toxin that does not need to be detoxified; OMPC, 37 kDa, is derived from *Neisseria meningitides* serogroup B strain 11; PD, 42 kDa, is a cell-surface protein found in all *Haemophilus influenzae*.^{56, 57} Comparison studies of similar polysaccharides conjugated to the

various carrier proteins demonstrated that all have similar safety and immunogenicity profiles.⁵⁸ Additionally, carrier protein conjugated vaccines can result in immune response to the pathogens from which the carriers are derived.⁵⁹

Table 1. Carrier proteins used for licensed conjugate vaccines.

Adapted from Broker et al.^{59, 60}

Carrier protein	Polysaccharide(s)	Brand names
TT	Hib	Hiberix, ActHIB
	MenA	MenAfriVac
	MenC	NeisVac-C
	MenACWY	Nimenrix
	MenC-Hib	Menitorix
DT	Hib	ProHIBIT
	MenACWY	Menactra
CRM ₁₉₇	Hib	VaxemHib
	7 pneumococcal serotypes	Prenvar
	13 pneumococcal serotypes	Prenvar
	MenC	Meningitec™, Menjugate
	MenACWY	Menveo
OMPC	Hib	PedvaxHIB
	7 pneumococcal serotypes	PNEUMOVAX
PD	11 pneumococcal serotypes	Infanrix hexa
	10 pneumococcal serotypes	PHiD-CV

Hib, *Haemophilus influenzae* type b; MenA, meningococcal capsular group A; MenACWY meningococcal capsular groups A, C, W, Y; MenC, meningococcal capsular group C; MenCY, meningococcal capsular groups C, Y

1.4 Isolation and extraction strategies of glycans for preparing conjugate vaccines

Most of the licensed conjugate vaccines are prepared from isolated/extracted polysaccharides.⁶¹ Isolation/extraction of polysaccharides from bacteria is conceptionally simple, however, it is still fraught with difficulties of culturing every bacterial strain.⁶² There are three commonly use methods for the isolation/extraction: hot water⁶³, dilute alkali-water solution⁶⁴ and enzymolysis⁶⁵. The selection of isolation/extraction method needs to be performed carefully since the polysaccharides can degrade during the procedure. Additionally, the purification is complicated as the isolated product inevitably contains many impurities, such as undesired saccharides, proteins and salts, that increases the difficulty of preparing homogeneous polysaccharide antigen.^{62, 66} Furthermore, it is still difficult to obtain polysaccharides in sufficient quantities.^{67, 68} Therefore, the isolation/extraction methods are still challenging to meet the quality control and safety standards for drug preparation.⁶⁹

1.5 Chemical synthesis of conjugate vaccines

Despite enzymatic synthesis methods are step-economical for oligosaccharides preparation, it is still challenging to express the required enzymes for synthetic purposes. Compared to the diverse glycan structures, the number of defined enzymes is still limited.⁷⁰ On the other hand, chemical synthesis is a well-developed method and attractive to produce oligosaccharides because of its reliability and scalability though it is time-consuming because no general glycosylation protocols are available.^{6, 71} A large number of monosaccharide units are employed to assemble complex oligosaccharides and the need for stereoselective glycosylations complicated the synthesis.⁷² Generally, synthesis of monosaccharide building blocks is the first step for chemically preparing carbohydrate-based vaccines, which is followed by their assembly into an oligosaccharide. The next step involves the removal of all protecting (blocking)

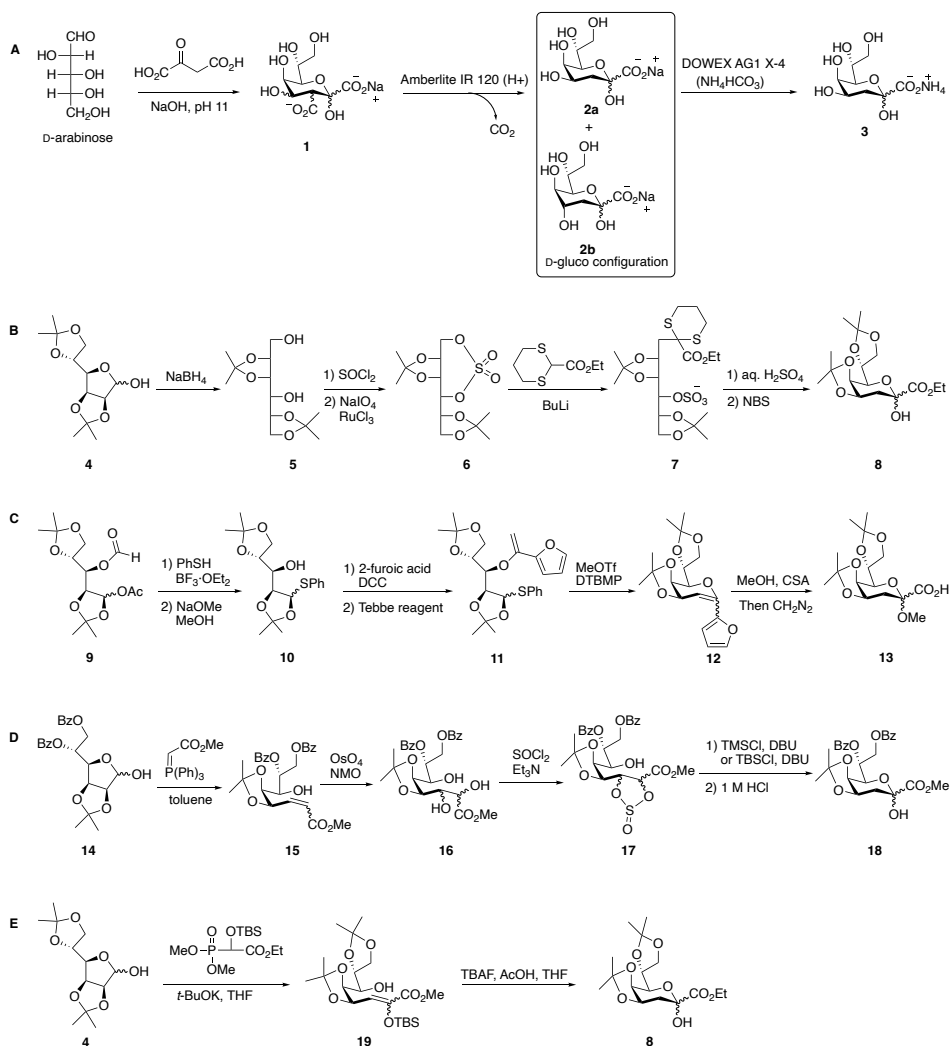
groups, which is followed by conjugation of the complex carbohydrate to a carrier protein chemically. Quimi-Hib, a synthetic conjugate vaccine for *H. influenza* type b, was licensed in several countries. This success encouraged researchers to explore the development of chemical methods to produce conjugate vaccines for bacteria, such as *K. pneumoniae*, *N. meningitidis*, *S. pneumoniae*, *F. tularensis* and *B. anthracis*.^{6, 73-76} Generally, synthetic glycan targets are selected based on knowledge of chemical structure of CPS, O-antigen, core region and glycolipids.⁶¹ Starting from 1990s, glycans derived from various bacteria have been synthesized and conjugated to carrier proteins to provide an alternative for conjugate vaccines preparation.⁷⁷⁻⁸⁰

1.5.1 Synthesis of Kdo and L,D-heptose

Kdo (3-deoxy-D-manno-2-octulosonic acid) and L,D-heptose (L-glycero-D-manno-heptose) are common components of LPS core region of GNBs, however, they are extremely expensive (€58 / mg for unprotected Kdo and €6.7 / mg for fully acetylated L,D-heptose, Sigma-Aldrich) due to the difficulty of isolation from natural sources.^{81, 82} The synthesis of complex carbohydrates requires large quantities of monosaccharides, thus, purchasing of Kdo or L,D-heptose as starting material is not economical. Chemical synthesis of Kdo and L,D-heptose from easily available sugars have been developed to reduce the cost of preparing oligosaccharides.⁸³ However, the additional synthetic steps of these approaches may lead to a low overall yield and are time-consuming.⁸⁴

Reliable methods for the chemical synthesizing Kdo are often carbohydrate-based *via* three- or two-carbon elongation of five- or six-carbon chiral monosaccharide (Scheme 1).⁸⁵ As a member of 3-deoxy-2-ulosonic acid family, Kdo was firstly synthesized following a strategy for preparing sialic acid in 1960s that uses D-arabinose and oxaloacetic acid as starting materials (Scheme 1A).⁸⁶ After aldol condensation, the resulting intermediate **1** was decarboxylated under mild condition to form Kdo **3**. The low yield and formation of epimeric D-*gluco*-

configured Kdo **2b** limited the application of this method.⁸⁷ To avoid the formation of unwanted D-*gluco* epimer, a synthetic route was developed that using D-mannitol derivative **5** as the starting material (Scheme 1B).⁸⁸ Treatment of di-*O*-isopropylidene protected D-mannitol **5** with thionyl chloride resulted in the formation of cyclic sulfate intermediate **6**, which was then attacked by a nucleophile to give the sulfate **7**. After removal of the sulfate ester and dithioacetal, Kdo derivative **8** was formed as a mixture of anomers. Another synthetic method starting from protected D-arabinose **9** (four steps from D-mannose) was developed (Scheme 1C).⁸⁹ A thiol ether was installed at the anomeric center, and the formyl ester was removed under basic condition to give **10**. By reaction with 2-furoic acid, the free alcohol converted into an ester which then underwent Tebbe olefination to form **11**. Methyl trifluoromethanesulfonate (MeOTf) was employed as promoter for the cyclization to form six-member ring **12**. The double bond was methanolized and the furan was oxidized to give Kdo derivative **13**. To reduce the number of synthetic steps of modification of Kdo derivatives, a modified D-mannofuranose **14** was employed as starting material (Scheme 1D).⁹⁰ An unsaturated ester **15** was obtained by two-carbon elongation by a Wittig reaction, and the double bond was then oxidized into triol **16**, which was treated with thionyl chloride to yield cyclic sulfite **17**. The free hydroxyl was protected as trimethylsilyl (TMS) ether, which was removed by aqueous HCl and meanwhile the ring closed to provide the modified Kdo monosaccharide **18**.^{85, 90} By changing the Wittig reagent into a phosphate ester, the *t*-butyldimethylsilyl (TBS)-protected unsaturated ester **19** was formed *via* Horner-Wadsworth-Emmons reaction from diacetone D-mannofuranose **4**. After deprotection of the TBS group with tetra-*n*-butylammonium fluoride (TBAF), Kdo derivative **8** was obtained in an overall yield of 80% (Scheme 1E).⁹¹



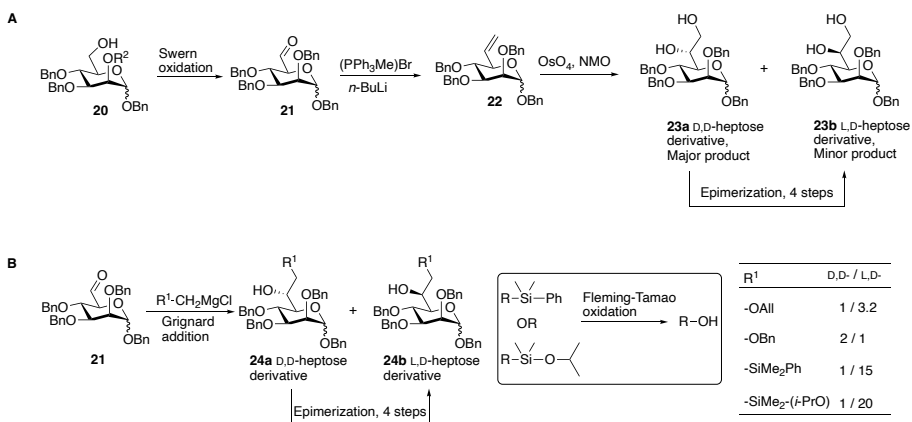
Scheme 1. Synthetic routes for preparing Kdo.

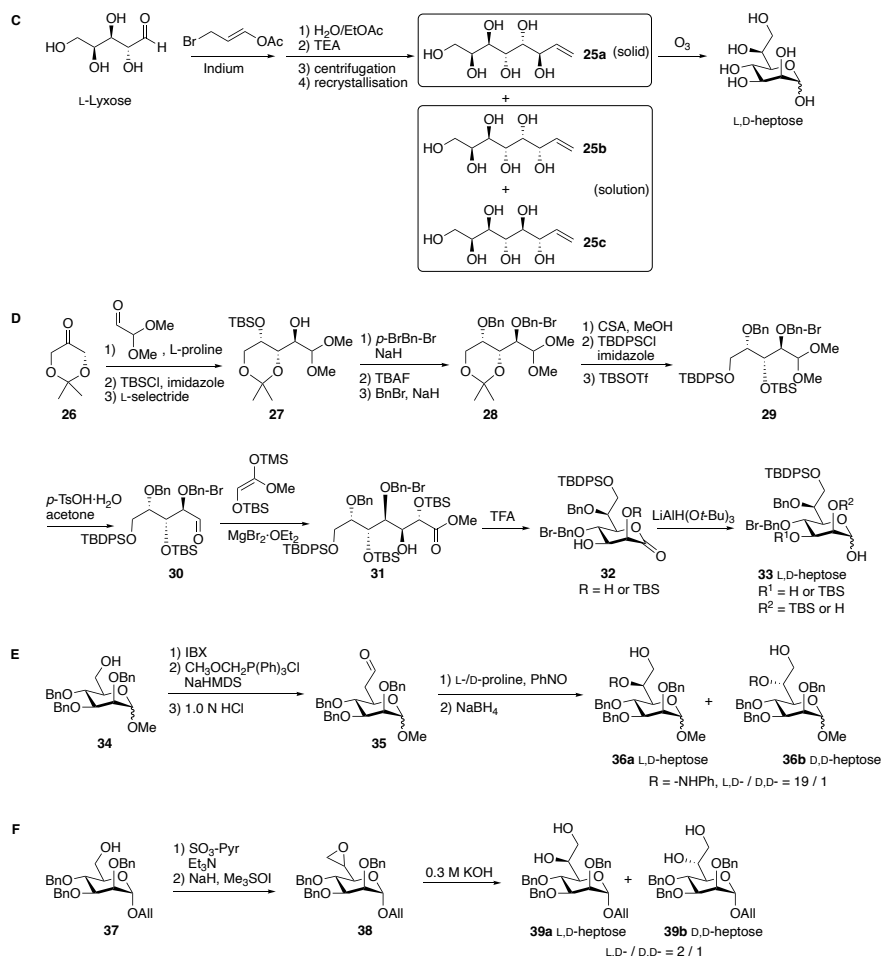
Chemical synthesis of L,D-heptose usually relies on carbon elongation of D-mannose or L-lyxose. Among D-mannoside-based approaches, dihydroxylation of olefin and Grignard addition of aldehyde have been widely used.⁹² The first is that unstable C-6 aldehyde **21**, formed after Swern oxidation of primary alcohol **20**, was subjected to Wittig olefination, and the resulting alkene **22** was then treated with osmium tetroxide (OsO_4) and 4-methylmorpholine *N*-oxide (NMO)

to yield 6,7-diols of D,D- and L,D-heptose (**23a** and **23b**) in 2.4:1 ratio, and the D,D-diastereoisomer can be epimerized to the desired L,D-isomer by a 4 step procedure (Scheme 2A).^{93, 94} The second approach is based on reaction of C-6 aldehyde with alkoxymethyl Grignard reagent, such as benzyl- and allyloxymethyl magnesium chloride, to give products as an epimeric mixture of D,D- and L,D-heptose derivatives (**24a** and **24b**, Scheme 2B).⁹⁵⁻⁹⁷ Although these approaches avoid the usage of toxic OsO₄ and are one step shorter than the first one, their L-stereoselectivity was still poor (D,D-/L,D-heptoside, 1/3.2 for allyl- and 2/1 for benzyl-Grignard reagent). The most appealing strategy to synthesize L,D-heptosides is the reaction of the C-6 aldehyde **21** with silylated Grignard reagent, such as PhMe₂SiCH₂MgCl or (*i*-PrO)-Me₂SiCH₂MgCl, to form **24a** and **24b**. Fleming–Tamao oxidation was employed to convert the silyl group into hydroxyl. Despite the high L-diastereoselectivity (L,D-/D,D-heptoside, 15/1 to 20/1), ester protecting groups are not tolerated by Grignard reactions.⁹⁸⁻¹⁰⁰

A shorter synthetic method is based on using L-lyxose (Scheme 2C).⁹² First, L-lyxose was treated with 3-bromopropenyl acetate and indium using Barbier-type procedure, and the resulting enitols were worked up, deacetylated with triethylamine (TEA), and recrystallized to give pure *manno*-configuration **25a** as solid in approx. 50% yield leaving the unwanted *gluco*- and *allo*-configured products (**25b** and **25c**) in the mother liquid. L,D-heptose was obtained after ozonolysis of **25a** in 95% yield. Additionally, D,D-heptose can be prepared with this approach by using D-lyxose as starting material.¹⁰¹ An L-diastereoselective synthetic method *via* aldol reaction has been reported (Scheme 2D).¹⁰² First, ketone **26** was reacted with dimethoxyacetaldehyde, and the aldol product was TBS-protected which underwent a 1,3-migration during reduction with L-selectride. The resulting alcohol **27** was protected by a *p*-bromobenzyl ether, and the silyl group was exchanged to benzyl ether to yield **28**. The isopropylidene acetal was selectively removed, and the obtained primary alcohol was protected by reaction with *tert*-butyldiphenylchlorosilane (TBDPSCl), while the secondary

alcohol was converted into TBS ether to give **29**. Aldehyde **30** was obtained by hydrolysis of the acetal, and a second aldol reaction was performed to afford **31**. The TBS ether was cleaved by treatment with TFA that resulted in lactonization to form **32** which was then reduced by $\text{LiAl}(\text{O}^t\text{Bu})_3$ to give L,D-heptose **33**. This strategy employed a number of orthogonal protecting groups and obtained excellent L-diastereoselectivity for L,D-heptose preparation. A one-carbon elongation method for L,D-heptose synthesis was communicated in 2015 (Scheme 2E).¹⁰³ The C-6 primary alcohol **34**, prepared from D-mannose, was oxidized by 2-iodoxybenzoic acid (IBX), and the resulting product was used in a Wittig olefination and then treated with acid to form aldehyde **35**. After proline-catalyzed aminooxylation of **35**, a mixture of L,D- and D,D-heptose (**36a** and **36b**) was obtained in a ratio of 19:1. Beside these methods, an approach based on Corey-Chaykovsky epoxidation reaction was reported (Scheme 2F).¹⁰⁴ The alcohol of **37** was oxidized into aldehyde by SO_3 -pyridine, after which NaH and Me_3SOI were employed to react with the aldehyde to give the epoxide **38**. After treatment with base, the heptose was formed as a mixture of L,D- and D,D-figuration in a ratio of 2:1.





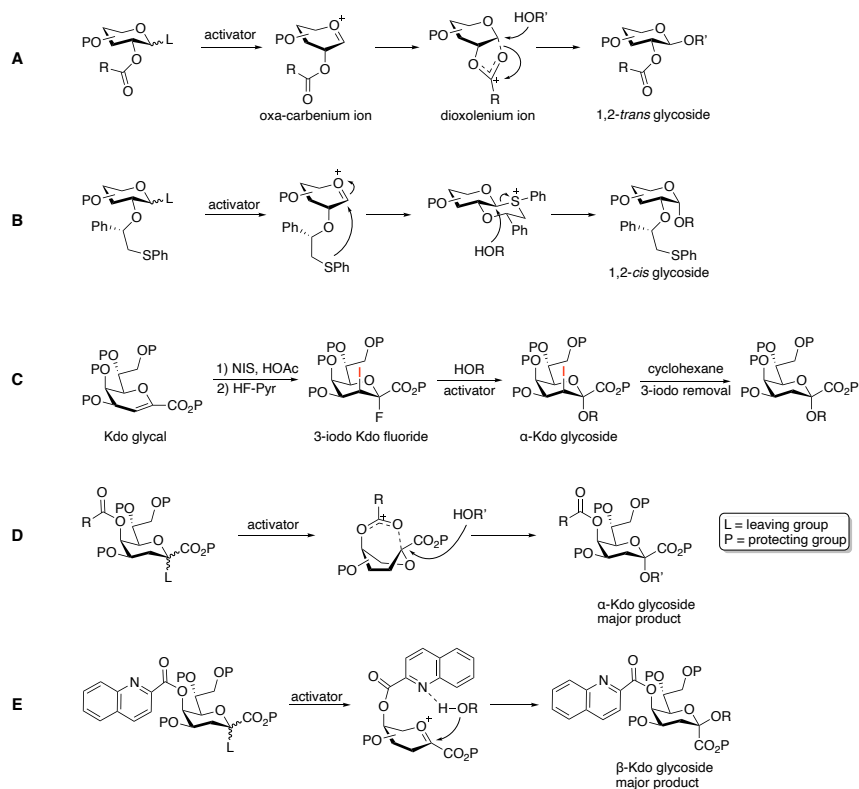
Scheme 2. Synthetic routes for the preparation of L,D-heptose.

1.5.2 Chemical strategies control stereoselectivity of glycosidic linkages formation

Assembly of complex carbohydrates is generally achieved through glycosylations of glycosyl donors having a leaving group at anomeric center and glycosyl acceptors with free hydroxyl(s). The non-desired hydroxyls of donor and acceptor should be protected (blocked) to avoid these from participating in glycosylations. The stereoselectivity during glycosidic linkage formation is a major challenge in carbohydrate chemistry. However, many protocols of stereoselective

glycosylations have been developed in recent years.^{105, 106} Neighboring group participation is widely used to install 1,2-*trans* (β -) linkages of galactose or glucose and 1,2-*trans* (α -) linkages for mannose or *manno*-heptose (Scheme 3A). In these reactions, an ester protecting group at C-2 of the glycosyl donor is necessary. An oxocarbenium ion formed after departure of the leaving group which is followed by the formation of a dioxolenium ion that gives β -linked glycoside. An α -linked glycoside can be produced by modifying C-2 of a glycosyl donor with (S)-(phenylthiomethyl)benzyl moiety, which leads to the formation of *trans*-decalin fused intermediate (Scheme 3B).¹⁰⁷

However, this strategy is not possible for 3-deoxy-D-*manno*-oct-2-ulosonic acid (Kdo) as a glycosyl donor because of the absence of a functionality at C-3. Furthermore, the anomeric center will be deactivated by the electron-withdrawing effect of the carboxylic ester, which can also lead to α,β -unsaturated ester formation during glycosylation.¹⁰⁸ Several protocols for controlling the stereoselectivity of Kdo glycosylations have been developed in recent years. In order to obtain the α -linkage, a stereo-directing group at C-3 (3-iodo) of the Kdo donor has been employed (Scheme 3C).¹⁰⁹ Despite only α -linkage product was obtained through this strategy, the larger number of synthetic steps (elimination reaction to provide Kdo glycal, 3-iodo installation and 3-iodo removal after glycosylation reaction) led to a lower total conversion of Kdo donor. In addition, Kdo glycal is the main product of glycosylation reaction when allyl aglycon exists.¹¹⁰ In recent years, another method was developed to control the stereoselectivity of Kdo glycosylations.¹¹¹ Similar to galactosyl donors¹¹², an ester such as acetyl or benzoyl ester at the axial C-5 alcohol of Kdo can provide remote neighboring group participation that improves the α -anomeric selectivity (Scheme 3D). Furthermore, by changing the ester to 2-quinolinecarbonyl group at C-5 of Kdo donor, β -linked Kdo glycosides were obtained as major product by hydrogen bond mediated delivery of the glycosyl acceptor (Scheme 3E).

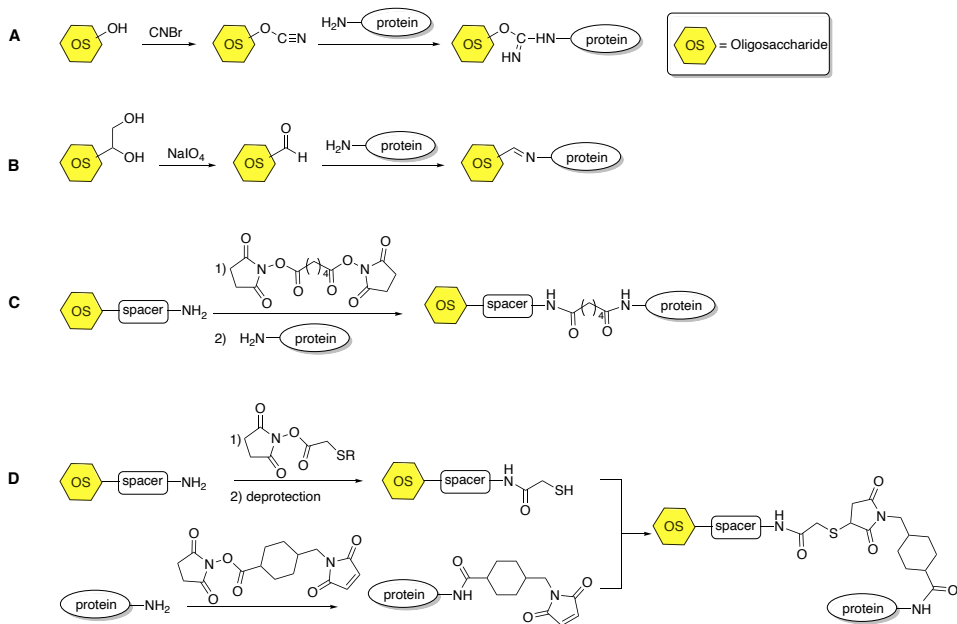


Scheme 3. General strategies for controlling stereoselectivity of chemical glycosylations.

1.6 Chemical conjugation of carbohydrate with carrier protein

There are generally two approaches to covalently link an oligosaccharide to carrier protein: i) random activation of hydroxyls of a carbohydrate followed by conjugation to a carrier protein; ii) and the use of an oligosaccharide having a linker with unique reactivity for controlled conjugation to a carrier protein.¹¹³ For example, after random cyanate ester(s) formation by treatment with cyanogen bromide, an oligosaccharide can react with amines of a carrier protein to give a glycoconjugate (Scheme 4A). Hydroxyl(s) of carbohydrates can also be oxidized to aldehyde(s) by sodium periodate and then couple with a carrier protein by reductive amination (Scheme 4B). However, the drawback of these protocols is

the formation of cross-linked products resulting in very heterogeneous products. To better control the chemical structure of glycoconjugates, a chemical spacer having for example a primary amino group can be installed at the reducing terminus of a carbohydrate. The modified saccharide can be reacted with a diester linker and conjugated to a carrier protein (Scheme 4C). Alternatively, a thiol group can be introduced at the spacer and coupled with a maleimide-activated carrier protein (Scheme 4D).^{114, 115}



Scheme 4. General protocols for conjugation of carbohydrates to carrier proteins.

1.7 Scope and outline

This thesis describes the synthesis of oligosaccharides derived from the core of LPS of *K. pneumoniae* and their conjugation to carrier proteins for the development of a broad acting vaccine. It is anticipated that the glycoconjugate vaccine will provide an alternative for antibiotic treatment. The core of *K. pneumoniae* LPS was selected as a target for vaccine development because its

structure is rather conserved. We employed CRM₁₉₇ as the carrier protein because it is non-toxic and has been used in many licensed vaccines. To avoid reactions of the free amine of the outer core oligosaccharide, thiol-maleimide coupling was employed for the preparation of glycoconjugates.

The chemical synthesis of an outer core tetrasaccharide is described in **Chapter 2**. The compound contains three amino groups in different forms, a Kdo residue which is a common component of inner core oligosaccharides, and a thiol-containing linker at the reducing end for protein conjugation. A two-carbon extension strategy was employed to synthesize a Kdo building block. The installation of challenging α -galactoside/ α -Kdo linkages was accomplished by remote neighboring group participation. The subsequent **Chapter 3** describes the synthesis of a pentasaccharide derived from the outer core of *K. pneumoniae* containing a nonstoichiometric L,D-heptose substituent. A crowded 4,5-branched Kdo residue was installed by a block synthetic strategy, and a heptosyl donor was prepared from D-mannose with excellent L-diastereoselectivity by using a one-carbon elongation approach. The immunological evaluation of the two conjugates is ongoing and will reveal the role of the heptoside in eliciting relevant immune responses.

In **Chapter 4**, the synthesis of an inner core pentasaccharide derived from *K. pneumoniae* is described. Compared to the diversity of CPS, the inner core has only one type among *K. pneumoniae* making it attractive for the development of a broad acting vaccine. Galacturonic acid is expressed by the bacteria to provide negative charge since the phosphoryl modifications are absent in the inner core. To reveal the effects of the acidic substituent for eliciting immune response, a substructure lacking galacturonic acid was also synthesized containing a crowded 3,4-branched L,D-heptoside. The tetra- and pentasaccharide have been conjugated to carrier proteins, and future studies will focus on examining antigenic properties by mouse immunizations.

Future perspective. New methods will become available to improve the regio-/stereo-selectivity of glycosylations, thereby shortening glycan synthesis and increasing overall yields. Carrier proteins may be replaced by synthetic carriers, such as peptides, glycolipids, and gold nanoparticles, to conjugate with glycans that may avoid the need for adjuvant and reduce the difficulty of conjugate vaccines production. The synthetic vaccine candidates may be combined with isolated structures to prepare multivalent vaccines to provide more therapeutic options for drug resistant bacteria.

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Chapter 2

Chemical Synthesis of an Outer Core Tetrasaccharide Derived from *Klebsiella pneumoniae* for Conjugate Vaccine Development

Introduction

Klebsiella pneumoniae (*K. pneumoniae*), a member of Gram-negative bacterium (GNB) belonging to *Enterobacteriaceae* family, is a commonly isolated nosocomial pathogens that can cause pneumonia, bloodstream infections and sepsis.¹⁻⁴ Infections caused by carbapenem-resistant *K. pneumoniae* (CR-*Kp*) are particularly problematic due to a lack of therapeutic options leading to a 50% mortality.⁵⁻⁷ Furthermore, these strains are increasingly being isolated from patients worldwide and for example, the proportion of CR-*Kp* isolates in Greece increased to 68% in 2011 from 28% in 2005.⁸ No vaccines for *K. pneumoniae* is available, which is a pressing goal to control infections caused by this bacteria.⁹⁻

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Capsular polysaccharides (CPS) and lipopolysaccharides (LPS) are promising targets for the development of vaccines for *K. pneumoniae*.¹² Compared to 9 serotypes of LPS, more than 79 serotypes of CPS have currently been identified and the high structural diversity complicates the development of CPS-based vaccine with broad coverage.^{13, 14} LPS can be divided into three parts: an O-antigen that consists of repeating sugar units; a lipid A moiety which is the main endotoxic factor among GNB; and a core region that connects the lipid A and O-antigen.¹⁵

Similar to other *Enterobacteriaceae*, the core oligosaccharide of *K. pneumoniae* can be subdivided into two regions: an inner core that is linked to lipid A, and an outer core connecting the O-antigen.² The major difference of core

oligosaccharides of *K. pneumoniae* and other *Enterobacteriaceae* such as *Escherichia coli* (*E. coli*) or *Salmonella* is the absence of phosphoryl modifications. Galacturonic acids (GalA) and 3-deoxy-D-manno-2-octulosonic acid (Kdo) are incorporated instead of phosphoryl substitutions to provide negative charges to improve the stability of *K. pneumoniae* outer membrane.¹⁶ Unlike the high structural similarity of the inner core region, the outer core is structurally more diversity among *Enterobacteriaceae*.¹⁷ However, only two outer core oligosaccharide types (type 1 and type 2) have been observed for *K. pneumoniae* (Fig. 1).¹⁸ According to the data of isolates from patients,¹⁹ outer core type 1 is much more common than the other making it attractive target for the development of a vaccine. The LPS outer core type 1 contains a Kdo and a nonstoichiometric substitution of its C-4 by L,αD-heptose, and both of these are confined to the inner core generally.²⁰ Kdo residues expressed in the inner core region link the lipid A and core region, while the outer core region Kdo residue of *K. pneumoniae* provides O-antigen polysaccharide an attachment site.^{20, 21}

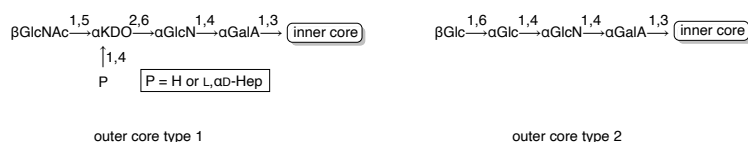


Figure 1. Two types of *K. pneumoniae* LPS outer core region.

Many studies have shown that glycoconjugate vaccines can provide long-term protection against various diseases, including those caused by antibiotic resistant bacteria.^{22, 23} However, the conjugation of short oligosaccharides to a carrier protein is difficult to control to produce a structure without affecting immunological domains.²⁴ Despite glycan fragments can be isolated from LPS, it is difficult to obtain homogeneous oligosaccharide antigens.^{25, 26} Chemical synthesis of glycoconjugates is an attractive approach for vaccine development.²⁷ Herein, we report the chemical synthesis of tetrasaccharide **1** derived from *K. pneumoniae* LPS outer core region, which was conjugated to carrier proteins to

examine its antigenic properties.

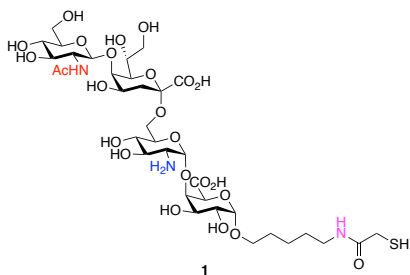


Figure 2. Chemical structure of target tetrasaccharide **1**.

Results and Discussion

The preparation of outer core oligosaccharide **1** of *K. pneumoniae* is challenging due to the need to install glycosidic linkages, including α -linked Kdo, α -linked glucosamine and α -galactoside, that usually give mixtures of anomeric products.²⁸⁻³¹ Furthermore, the target compound has three types of amino substitutions that require the selection of amino masking or protecting groups that can individually be modulated, and allow the introduction of glycosidic linkages with appropriate anomeric configuration. Additionally, the free amine together with the two carboxylic acids of the target tetrasaccharide makes conjugation to carrier proteins challenging.³²

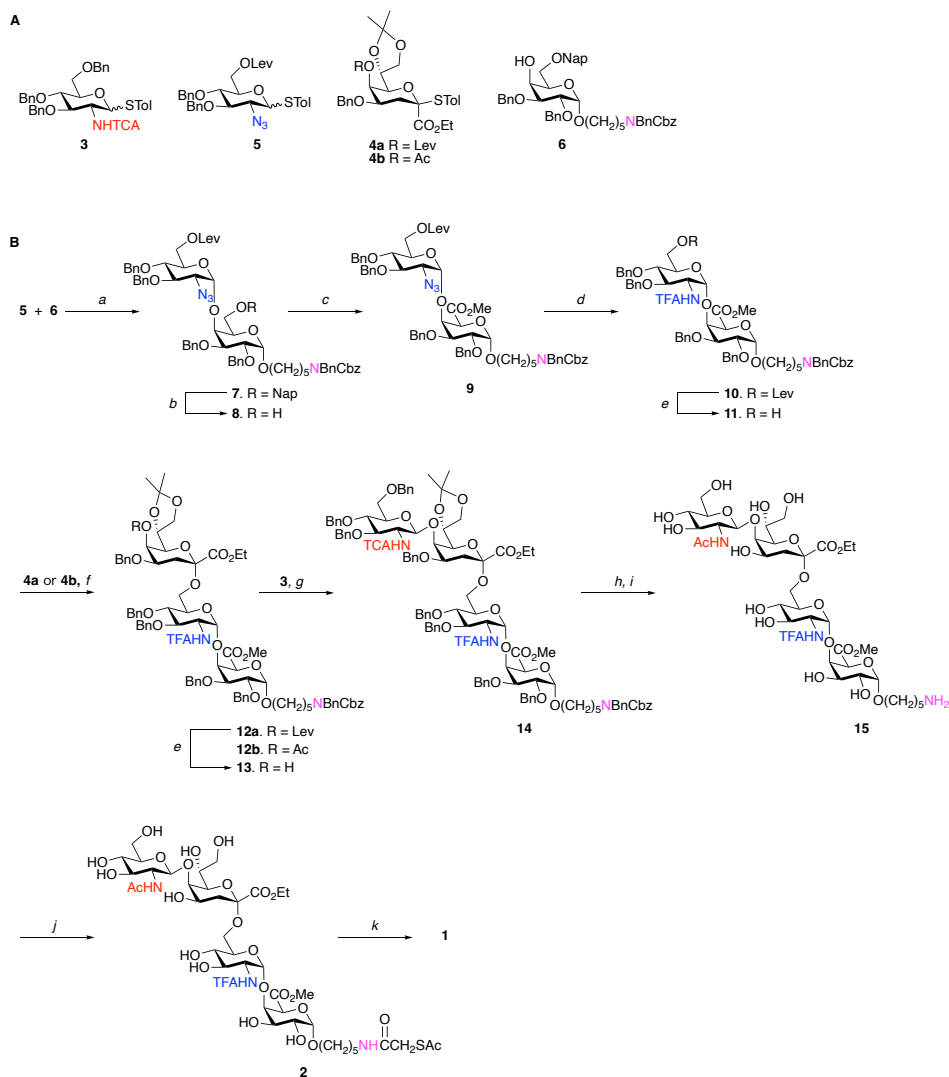
To assemble the LPS outer core derived from *K. pneumoniae*, a strategy was designed employing monosaccharide building blocks **3**, **4a**, **5** and **6** (Scheme 1A). The C-2 amine of glycosyl donor **3** is modified as trichloroacetamide because during glycosylation it will control the anomeric selectivity by neighboring group participation to provide a 1,2-*trans*-glycoside without causing oxazoline formation. The C-3, C-4, and C-6 hydroxyls of **3** are protected as benzyl ethers which at the end of the synthesis can easily be removed by hydrogenation with concomitant reduction of the trichloroacetyl (TCA) moiety to give the required *N*-acetyl-glucosamine residue. Kdo was prepared from D-mannose by a reported

procedure,³³ and subjected to protecting group manipulation to provide **4a** (see Scheme **S4** for details). The levulinoyl (Lev) ester of **4a** can selectively be removed to give an acceptor for further glycosylation. Furthermore, the ester at C-5 of the Kdo donor was expected to improve the α -anomeric selectivity of the glycosylation.^{8,34} The C-2 azido moiety of **5**, which does not perform neighboring group participation during glycosylations, allowed the installation of an α -glucosidic linkage, which subsequently can be reduced to an amine and temporary protected as a trifluoroacetamide. At the very end of the synthetic route, saponification will remove trifluoroacetyl (TFA) protecting group to provide the required glucosamine moiety. Galactosyl acceptor **6** is equipped with an α -linked aminopentyl moiety that is doubly protected by benzyl and benzyloxycarbonyl (Cbz), which at a late stage of the synthesis can be removed by hydrogenation to give an amine, which is modified by a thioacetyl moiety for conjugation to a carrier protein.²⁴ The 2-methylnaphthyl (Nap) ether of **6** can at an appropriate stage be removed to give an alcohol that can be oxidized to a carboxylic acid.

The assembly of tetrasaccharide **1** started with a glycosylation of 2-azidoglucosyl donor **5** with galactosyl acceptor **6** in the presence of *N*-iodosuccinimide (NIS) and trimethylsilyl trifluoromethanesulfonate (TMSOTf)³⁵ to give the disaccharide **7** as only the α -anomer ($\delta_{\text{Glc H1}}$ 4.99, $J_{\text{Glc H1-H2}}$ = 3.7 Hz) in a moderate yield of 55%. The 2-methylnaphthyl (Nap) ether of **7** could easily be removed by using 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) in wet dichloromethane (DCM)³⁶ to afford alcohol **8**, which was oxidized to a carboxylic acid using (2,2,6,6-tetramethylpiperidin-1-yl)oxyl (TEMPO) and (diacetoxyiodo)benzene (BAIB).³⁷ Subsequent treated with (diazomethyl)trimethylsilane (TMSCHN₂) in tetrahydrofuran (THF) gave methyl ester **9**. The azido moiety of the latter compound was reduced with zinc in acetic acid and THF to give an amine that was protected as trifluoroacetamide by reaction with trifluoroacetic anhydride providing **10**. Disaccharide acceptor **11** was obtained by treatment of **10** with hydrazine acetate in DCM and methanol

resulting in clean removal of the Lev ester. Next, coupling of the Kdo donor **4a** with **11** using NIS and trifluoromethanesulfonic acid (TfOH) as the promoter system and acetonitrile as the solvent resulted in the formation of trisaccharide **12a** in yield of 72% as mainly the separable α -anomer ($\alpha:\beta = 4:1$; α Kdo, $J_{C1-H3ax} < 1$ Hz).^{29, 38} Kdo derivative **4b**, which is protected by an acetyl ester at C-5, was also examined as donor, and a glycosylation under similar conditions provided trisaccharide **12b** as a separable mixture of α/β -isomers (α , 70%; β , 19%). Removal of the ester without affecting other groups was, however, challenging and did not provide a reliable route to the target compound (see SI). Thus, the Lev ester of **12a** was removed using hydrazine acetate, and the resulting trisaccharide acceptor **13** was glycosylated with glucosamine donor **3** using NIS/TfOH as the activator system in a mixture of DCM and acetonitrile at 0 °C to provide **14** in an acceptable yield of 56% as only the β -anomer (confirmed in the next step by NMR). Some hydrolysis of the donor was observed probably due to the low reactivity of the Kdo acceptor.^{29, 38, 39}

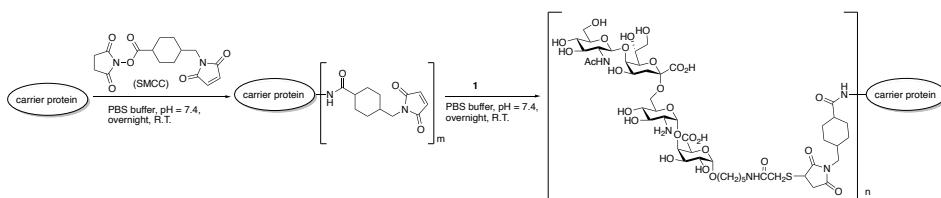
The isopropylidene acetal of **14** was cleaved in a mixture of trifluoroacetic acid, DCM, and water. The resulting compound was subjected to hydrogenation over Pd(OH)₂/C to remove the benzyl ethers and convert the trichloroacetamide into an acetamide to give **15** in a yield of 62% ($\delta_{GlcNAc H1} = 4.63$, $J_{H1-H2} = 8.5$ Hz). The hydrogenation also removed the protecting groups of the anomeric aminopentyl linker, and the resulting amine was modified as an (acetyl)thioacetate by reaction with perfluorophenyl 2-(acetylthio)acetate (SAMA-OPfp) to give **2**. Finally, the methyl, ethyl and acetyl esters and the trifluoroacetamide were saponified with aqueous NaOH to give, after purification by bio-gel P2 size exclusion column chromatography, tetrasaccharide **1** in 90% yield. The thiol of the resulting compounds provided a reactive handle for coupling to a maleimide-modified carrier protein. The developed synthetic strategy made it possible to install an acetamido and free amine in the oligosaccharide and provided a derivative for selective conjugation to a protein.



Scheme 1. Monosaccharide building blocks (A) and their assembly for preparation of target pentasaccharide **1** (B). Reagents and conditions: *a*) NIS, TMSOTf, DCM, 55%. *b*) DDQ, DCM/H₂O, 76%. *c*) i. TEMPO, BAIB, HOAc, DCM/H₂O, ii. TMSCHN₂, THF, 80%. *d*) i. Zn, HOAc/THF, ii. (CF₃CO)₂O, THF, 79%. *e*) NH₂NH₂•HOAc, DCM/MeOH. 82% for **10**; 99% for **12a**. *f*) NIS, TfOH, MeCN, **12a**: α:β = 4:1, 72% of α anomer; **12b**: α:β = 3.7:1, 70% of α anomer. *g*) NIS, TfOH, DCM/MeCN, 56%. *h*) TFA/H₂O/DCM. *i*) H₂, Pd(OH)₂/C, *t*-

BuOH/H₂O, 62% over 2 steps. j) SAMA-OPfp, DIPEA, DMF. k) 0.1 M NaOH, H₂O, 90% over 2 steps.

Target tetrasaccharide **1** was conjugated to carrier proteins by using thiol-maleimide coupling chemistry to give glycoconjugates, which will be used to explore its antigenicity in animal models.⁴⁰ First, the carrier proteins Bovine Serum Albumin (BSA) and CRM₁₉₇ were treated with succinimidyl-*trans*-4-(*N*-maleimidylmethyl)cyclohexane-1-carboxylate (SMCC) in PBS buffer (pH 7.4), and the resulting maleimide-activated proteins were purified by using spin filtration and analysis of the products by MALDI-TOF mass spectrometry^{40, 41} indicated the presence of seventeen maleimide moieties to BSA and fifteen to CRM₁₉₇, respectively. Finally, tetrasaccharide **1** was treated with D,L-dithiothreitol (DTT) to cleave the disulfide bond and purified by bio-gel P2 column to give a free thiol contained oligosaccharide **1**, which was mixed with the maleimide activated carrier proteins to produce glycoproteins: BSA-**1** and CRM-**1** having an average loading of approximately seven and four glycans (Scheme 2), respectively, as determined by MALDI-TOF mass spectrometry.



Scheme 2. Modification of carrier proteins and their conjugations with target tetrasaccharide **1**. *BSA, $m = 17$, $n = 7.0$; CRM₁₉₇, $m = 15$, $n = 4.0$.

Conclusion

Tetrasaccharide **1**, which is derived from the outer core of *K. pneumoniae* LPS, was synthesized chemically, and the strategy employed remote neighboring group participation to form challenging 1,2-*cis*-galactoside and α -Kdo linkage. The three amino substitutions were remained as different types by masking with

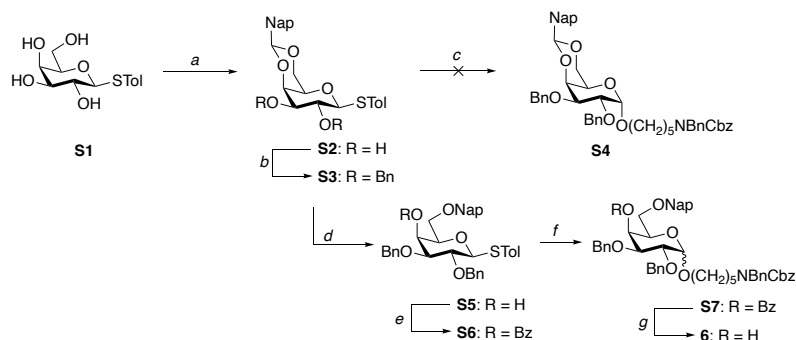
orthogonal protecting groups, which can be removed individually at appropriate synthetic stage. The thiol-maleimide coupling chemistry was adopted to prepare the glycoconjugates, which can be explored for the development of a universal vaccine against *K. pneumoniae*. The study of serum antibody screening and immunization with CRM-1 is ongoing that will provide an insight in the potency of a new treatment approach of infections caused by *K. pneumoniae*.

Experimental section

General procedures

All chemicals were purchased from Sigma-Aldrich, Fisher Scientific or Biosynth Carbosynth. BSA was purchased from Sigma-Aldrich and CRM₁₉₇ was purchased from Scarab Genomics LLC. NMR spectra, including ¹H, ¹³C, COSY, HSQC, HMBC and EXSIDE, were recorded on Agilent 400-MR or Bruker 600 MHz with chemical shifts reported in part per million (ppm) relative to CDCl₃ or D₂O. ¹H NMR data are presented in the order: Chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, dd = doublet of doublets, m = multiplet) and coupling constants (J) are reported in Hertz (Hz). Mass spectra of proteins were recorded on a Kratos Analytical Maxima-CFR MALDI-TOF system with sinapinic acid in acetonitrile/water (0.3/0.7, v/v) as matrix, and High-resolution mass spectrometry (HRMS) was recorded on an Agilent technologies 6560 Ion mobility Q-TOF. Chromatographic purifications were performed on silica gel G60 (Silicycle 60 – 200 µm, 60 Å), and size exclusion chromatography was performed on Bio-Gel P-2 (45-90 µm) by using 5 % *n*-butanol in water as eluent. TLC analysis was conducted on Silica gel 60 F254 (EMD Chemicals Inc.) and detected by using UV light (254 nm), staining by 5 % sulfuric acid in ethanol, *p*-anisaldehyde solution or an aqueous solution of Ce(NH₄)₂(NO₃)₆ / (NH₄)₆MoO₂₄·4H₂O in 5 % sulfuric acid. 4 Å molecular sieves were flame-activated under vacuum before use. Carrier proteins were purified by ultrafiltration using ultrafilter (Amicon Ultra-0.5 Centrifugal Filter Unit, 10K) before and after reactions.

Synthetic protocols



Scheme S1. Synthesis of galactosyl acceptor **6**. Reagents and conditions: *a*) 2-Naphthaldehyde, *p*-TsOH, MeCN. *b*) NaH, BnBr, DMF, 84% over 2 steps. *c*) activator, benzyl-(5-hydroxy-pentyl)-carbamic acid benzyl ester, NIS, 4Å M.S., solvents. *d*) Et₃SiH, TfOH, DCM, 92%. *e*) BzCl, Pyridine, 95%. *f*) benzyl-(5-hydroxy-pentyl)-carbamic acid benzyl ester, NIS, TMSOTf, toluene/1,4-dioxane, 71% of α (α : β = 5:1). *g*) NaOMe, MeOH/THF, reflux, 96%.

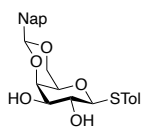
Table S1. Conditions of glycosylation of donor **S3** and benzyl-(5-hydroxy-pentyl)-carbamic acid benzyl ester acceptor.

Activator	Solvent(s)	Temperature	Anomer of product	Yield
TMSOTf	DCM/Et ₂ O (3/1)	-20 °C	β	55%
TMSOTf	DCM/Et ₂ O (3/1)	-20 °C to 0 °C	β	83%
TMSOTf	DCM	-20 °C	β	46%
TfOH	DCM/Et ₂ O (3/1)	0 °C	β	89%
TfOH	DCM/Et ₂ O (1/3)	0 °C	β	85%
TMSOTf	toluene/1,4-dioxane (1/3)	0 °C	β	67%

Glycosylation of galactoside donor **S3** with linker was not successful during

which only formed β -linked product and no desired α -linked product **S4** obtained (Table S1.). To synthesize α -linked galactoside building block, remote neighboring group participation effect of an ester protecting group was employed. Firstly, the 4,6-naphthylidene acetal was cleaved regio-selectively to give **S5** with free hydroxyl at C4, which was protected by benzoyl ester, and the resulted galactosyl donor **S6** was glycosylated with a protected aminopenyl linker to form **S7** as a mixture of α - and β - anomers. An acetyl protected C4 of galactoside donor was also used for glycosylation with the aminopentyl linker, however, the anomeric selectivity was poorer ($\alpha/\beta = 2/1$). The desired α -anomer was treated with sodium methoxide to give galactosyl acceptor **6**.

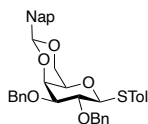
4-Methylphenyl 4,6-O-(2-naphthyl) methylene-1-thio- β -D-galactopyranoside (S2): Compound **S1**⁴² (21.4 g, 75 mmol) was dissolved in



acetonitrile (400 mL), which was followed by the addition of 2-naphthaldehyde (23.4 g, 150 mmol) and *p*-toluenesulfonic acid (*p*-TsOH, 1.42 g, 7.5 mmol). After stirring for 3 h at room temperature,

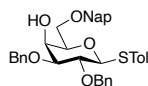
the reaction mixture was neutralized with triethylamine and concentrated under reduced pressure. The resulting residue was partially dissolved in acetone with an apparition of solid left and the mixture was heated until all solid dissolved, after which petroleum ether was added, and the clouded mixture was cooled to -20 °C and stored for 15 min. The solid was then filtered and washed with petroleum ether to give **S2**, which was used for the next step without further purification. ¹H NMR (400 MHz, Chloroform-*d*) δ 7.98 – 7.79 (m, 4H, Ar), 7.65 – 7.56 (m, 2H, Ar), 7.54 – 7.46 (m, 3H, Ar), 7.12 – 7.06 (m, 2H, Ar), 5.68 (s, 1H, Nap-CH), 4.48 (d, $J = 9.1$ Hz, 1H, H-1), 4.43 (dd, $J = 12.4, 1.8$ Hz, 1H, H-6a), 4.30 – 4.26 (m, 1H, H-4), 4.09 (dd, $J = 12.5, 1.9$ Hz, 1H, H-6b), 3.77 – 3.65 (m, 2H, H-2, H-3), 3.62 – 3.56 (bs, 1H, H-5), 2.55 (bs, 2H, -OH), 2.31 (s, 3H, PhCH₃). ¹³C NMR (101 MHz, cdcl₃) δ 134.29, 129.90, 128.47, 128.20, 127.87, 126.64, 126.32, 126.07, 124.20, 101.59, 87.30, 75.58, 73.95, 70.22, 69.53, 68.89, 21.33.

***p*-Methylphenyl 2,3-di-*O*-benzyl-4,6-*O*-(2-naphthyl) methylene-1-thio- β -D-galactopyranoside (**S3**):**



was slowly added to a solution of **S2** (28.7 g, 67.8 mmol) in dry DMF (200 mL) at 0 °C. The reaction mixture was stirred for 20 min at 0 °C, which was followed by the addition of benzyl bromide (34.2 mL, 271.1 mmol). The resulting reaction mixture was stirred for 3 h at room temperature. After TLC analysis showed consumption of the starting material, the reaction was quenched with dropwise addition of MeOH (5 mL). The solvents were removed under reduced pressure, and the resulting residue was dissolved in EtOAc (200 mL) and washed with sat. aq. NaHCO₃. The aqueous layer was back extracted with EtOAc (3 × 50 mL), and the combined organic phases were dried (Na₂SO₄), filtered, and the filtrate was concentrated *in vacuo*. The resulting residue was purified by silica gel column chromatography (PE/EA = 5/1, v/v) to give **S3** (38.3 g, 84% over 2 steps) as light-yellow solid. ¹H NMR (400 MHz, Chloroform-*d*) δ 8.02 – 7.86 (m, 4H, Ar), 7.68 – 7.60 (m, 3H, Ar), 7.55 – 7.48 (m, 2H, Ar), 7.44 (dd, *J* = 7.7, 1.6 Hz, 2H, Ar), 7.39 – 7.24 (m, 8H, Ar), 6.98 (d, *J* = 7.9 Hz, 2H, Ar), 5.66 (s, 1H, Nap-CH), 4.73 (m, 4H, PhCH₂ × 2), 4.60 (d, *J* = 9.5, 1H, H-1), 4.43 (dd, *J* = 12.3, 1.6 Hz, 1H, H-6), 4.22 (d, *J* = 3.4 Hz, 1H, H-4), 4.05 (dd, *J* = 12.3, 1.6 Hz, 1H, H-6), 3.90 (t, *J* = 9.4, 1H, H-2), 3.66 (dd, *J* = 9.2, 3.4, 1H, H-3), 3.45 (t, *J* = 1.3 Hz, 1H, H-5), 2.24 (s, 3H, PhCH₃). ¹³C NMR (101 MHz, cdcl₃) δ 138.74, 138.29, 137.77, 135.48, 133.95, 133.50, 133.11, 129.81, 128.90, 128.58, 128.56, 128.49, 128.35, 128.12, 127.98, 127.96, 127.86, 127.83, 126.44, 126.13, 126.02, 124.54, 101.56 (C-1), 86.84, 81.63, 75.56, 75.49, 73.92, 69.97, 69.71, 21.21. ESI HRMS (*m/z*): [*M* + Na⁺] calcd for C₃₈H₃₆NaO₅S, 627.2176 found 627.2111.

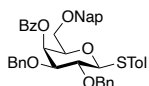
***p*-Methylphenyl 2,3-di-*O*-benzyl-6-*O*-(2-naphthyl) methyl-1-thio- β -D-galactopyranoside (**S5**):**



in DCM (20 mL) was added triethylsilane (1.62 mL, 10.24 mmol) and TfOH (0.81 mL, 9.25 mmol) sequentially. The reaction was

stirred at -78 °C for 1 h, after which it was quenched with MeOH (2 mL) and triethylamine (2 mL). The resulting mixture was washed with sat. aq. NaHCO₃. The aqueous layer was extracted with DCM (2 × 10 mL), and the combined organic layers were dried (Na₂SO₄), filtered, and the filtrate was concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography (PE/EA = 4/1, v/v) to give **S5** (1.8 g, 90%, 85% for 42 g scale) as light brown solid. ¹H NMR (400 MHz, Chloroform-*d*) δ 7.88 – 7.76 (m, 4H, Ar), 7.53 – 7.26 (m, 15H, Ar), 7.06 – 6.99 (m, 2H, Ar), 4.85 (d, *J* = 10.3 Hz, 1H, ArCHH), 4.79 – 4.64 (m, 5H, ArCHH₂, ArCH₂), 4.60 (d, *J* = 9.7 Hz, 1H, H-1), 4.15 – 4.08 (m, 1H, H-4), 3.90 – 3.78 (m, 2H, H-6a/b), 3.72 (dd, *J* = 9.7, 8.9 Hz, 1H, H-2), 3.65 – 3.58 (m, 1H, H-5), 3.58 (dd, *J* = 8.9, 3.2 Hz, 1H, H-3), 2.53 (dd, *J* = 2.6, 0.9 Hz, 1H, OH-4), 2.26 (s, 3H, PhCH₃). ¹³C NMR (101 MHz, cdCl₃) δ 138.38, 137.81, 137.72, 135.56, 133.41, 133.17, 132.66, 130.01, 129.78, 128.68, 128.52, 128.42, 128.36, 128.14, 128.06, 128.04, 127.95, 127.83, 126.76, 126.25, 126.07, 125.94, 88.10, 82.74, 77.21, 77.11, 75.87, 73.98, 72.25, 69.68, 67.10, 21.20. ESI HRMS (*m/z*): [M + Na⁺] calcd for C₃₈H₃₈NaO₅S, 629.2332 found 629.2263.

***p*-Methylphenyl 2,3-di-*O*-benzyl-4-*O*-benzoyl-6-*O*-(2-naphthyl) methyl-1-thio-β-D-galactopyranoside (**S6**):** Benzoyl chloride (1.0 mL, 8.7 mmol) was

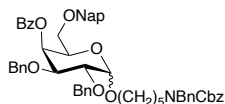


added into a cooled (0 °C) solution of **S5** (1.7g, 2.9 mmol) in pyridine (20 mL). The resulting reaction mixture was stirred

overnight at room temperature, after which it was diluted with water, and the aqueous layer was extracted with EtOAc (50 mL). The organic layer was washed with aq. HCl (1.0 M), brine and was dried (Na₂SO₄), filtered, and the filtrate was concentrated *in vacuo*. The resulting residue was purified by silica gel column chromatography (PE/EA = 5/1, v/v) to give **S6** (2.0 g, 100%) as yellow oil. ¹H NMR (400 MHz, Chloroform-*d*) δ 8.05 – 7.96 (m, 2H, Ar), 7.83 – 7.66 (m, 4H, Ar), 7.66 – 7.20 (m, 18H, Ar), 7.09 (d, *J* = 7.9 Hz, 2H, Ar), 5.93 (d, *J* = 2.9 Hz, 1H, H-5), 4.87 (d, *J* = 11.2 Hz, 1H, ArCHH), 4.78 – 4.62 (m, 5H, ArCHH, ArCH₂),

ArCHH, H-1), 4.54 (d, $J = 11.1$ Hz, 1H, ArCHH), 3.92 (t, $J = 6.1$ Hz, 1H, H-3), 3.81 – 3.60 (m, 4H, H-2, H-4, H-6a,b), 2.34 (s, 3H, PhCH₃). ¹³C NMR (101 MHz, cdcl₃) δ 165.85, 138.49, 137.95, 137.77, 135.25, 133.82, 133.51, 133.34, 133.25, 133.16, 130.72, 130.32, 130.13, 129.92, 129.76, 129.20, 129.02, 128.61, 128.45, 128.44, 128.43, 128.37, 128.30, 128.06, 127.84, 127.79, 126.91, 126.15, 125.99, 87.55 (C-1), 81.64, 76.67, 76.44, 75.75, 73.95, 71.97, 68.56, 67.58, 21.33. ESI HRMS (m/z): [M + Na⁺] calcd for C₄₅H₄₂NaO₆S, 733.2594 found 733.2595.

***N*-(Benzyl)-*N*-benzyloxycarbonyl-5-aminopentyl 2,3-di-*O*-benzyl-4-*O*-benzoyl-6-*O*-(2-naphthyl) methyl- α -D-galactopyranoside (S7):** Benzyl-(5-

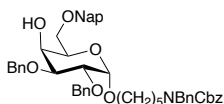


hydroxy-pentyl)-carbamic acid benzyl ester⁴³ (7.7 g, 23.6 mmol) and donor **S6** (11.1 g, 15.7 mmol) were co-evaporated with toluene (3 times), and were dissolved in

1,4-dioxane/toluene (120 mL/40 mL) and placed under an atmosphere of Argon. NIS (9 g, 31.4 mmol) and 4 Å molecular sieves (flame activated) were added to the solution. The resulting reaction mixture was cooled to 0 °C and was stirred at this temperature for 15 min, which was followed by the addition of TMSOTf (724 μ L, 3.93 mmol). The reaction mixture was stirred for additional 1 h at 0 °C and was then quenched with triethylamine (2 mL). After removing all solvents under reduced pressure, the resulting residue was dissolved in DCM (200 mL) and washed with brine (100 mL), and then water (100 mL). The organic layer was dried (Na₂SO₄), filtered, and the filtrate was concentrated *in vacuo*. The crude product was purified by silica gel column chromatography (PE/EA = 6/1, v/v) to give **S7** (α : 10.2 g, 71%, β : 1.9 g, 14%) as oils. α -anomer: ¹H NMR (400 MHz, Chloroform-*d*) δ 8.04 (dd, $J = 7.3, 2.3$ Hz, 2H, Ar), 7.82 – 7.70 (m, 4H, Ar), 7.57 (dd, $J = 8.2, 6.7$ Hz, 1H, Ar), 7.49 – 7.15 (m, 22H, Ar), 5.92 (d, $J = 3.1$ Hz, 1H, H-4), 5.29 – 5.12 (m, 2H, CH₂-Cbz), 4.87 (d, $J = 2.5$ Hz, 1H, H-1), 4.86 – 4.78 (m, 2H, ArCH₂), 4.73 – 4.57 (m, 4H, ArCH₂ \times 2), 4.50 (d, $J = 11.9$ Hz, 2H, ArCH₂), 4.28 – 4.21 (m, 1H, H-5), 4.11 (d, $J = 10.1$ Hz, 1H, H-3), 3.94 (dt, $J =$

10.0, 3.1 Hz, 1H, H-2), 3.74 – 3.36 (m, 4H, H-6a/b, Linker-CH₂), 3.25 (m, 2H, Linker-CH₂), 1.81 – 1.15 (m, 6H, Linker-CH₂ × 3). ¹³C NMR (101 MHz, cdcl₃) δ 165.89, 138.68, 138.38, 138.05, 135.34, 133.28, 133.07, 130.18, 129.94, 128.63, 128.55, 128.43, 128.37, 128.27, 128.26, 128.08, 128.01, 127.97, 127.94, 127.75, 127.71, 127.49, 127.38, 126.64, 126.10, 125.91, 125.81, 98.02 (C-1), 77.48, 77.16, 76.84, 76.61, 75.48, 73.81, 73.52, 72.04, 68.94, 68.39, 68.22, 67.25, 50.34, 47.28, 46.29, 29.79, 29.22, 28.07, 27.66, 23.57. ESI HRMS (m/z): [M + Na⁺] calcd for C₅₈H₅₉NNaO₉, 936.4082 found 936.4080.

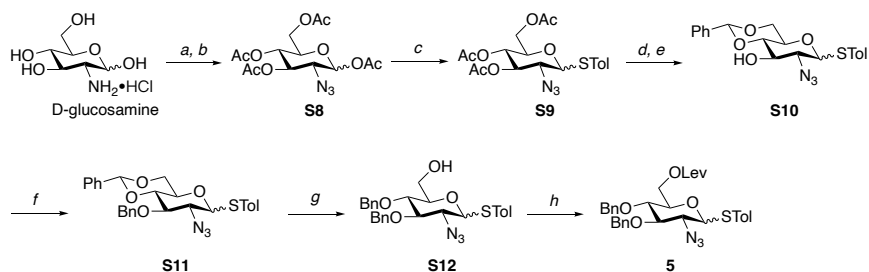
***N*-(Benzyl)-*N*-benzyloxycarbonyl-5-aminopentyl-2,3-di-*O*-benzyl-6-*O*-(2-naphthyl) methyl- α -D-galactopyranoside (6):** Compound **S7** (10.1 g, 11.1



mmol) was dissolved in MeOH/THF (80 mL/40 mL), which was followed by the addition of sodium methoxide (0.5 M in methanol, 520 μ L, 2.78 mmol). The resulting

reaction mixture was stirred at 50 °C for 30 h. After TLC analysis showed consumption of the starting material, the reaction mixture was cooled to room temperature and was neutralized with Amberlite 120 H⁺. The resulting mixture was filtered immediately, and the filtrate was concentrated *in vacuo*. The resulting residue was purified by silica gel column chromatography (PE/EA = 3/1, v/v) to give the galactoside acceptor **6** (7.3 g, 83%) as a light-yellow oil. ¹H NMR (400 MHz, Chloroform-*d*) δ 7.85 – 7.74 (m, 4H, Ar), 7.46 (m, 3H, Ar), 7.41 – 7.11 (m, 17H, Ar), 5.17 (d, *J* = 8.1 Hz, 2H, CH₂-Cbz), 4.79 (m, 3H, H-1, ArCH₂), 4.75 – 4.67 (m, 3H, ArCH₂, ArCHH), 4.64 (d, *J* = 12.1 Hz, 1H, ArCHH), 4.47 (d, *J* = 12.9 Hz, 2H, ArCH₂), 4.09 (s, 1H, H-4), 3.94 (d, *J* = 7.8 Hz, 1H, H-5), 3.87 (m, H-2, H-3), 3.78 (dd, *J* = 10.0, 5.4 Hz, 1H, H-6a), 3.71 (dd, *J* = 10.0, 6.3 Hz, 1H, H-6b), 3.65 – 3.56 (m, 1H, CHH-Linker), 3.44 – 3.32 (m, 1H, CHH-Linker), 3.21 (m, 2H, CH₂-Linker), 2.62 (bs, 1H, HO-4), 1.56 (m, 4H, CH₂-Linker × 2), 1.40 – 1.19 (m, 2H, CH₂-Linker). ¹³C NMR (101 MHz, cdcl₃) δ 138.66, 138.38, 138.05, 135.64, 133.36, 133.11, 128.65, 128.58, 128.48, 128.29, 128.04, 128.01, 127.97, 127.94, 127.89, 127.84, 127.81, 127.38, 126.60, 126.23, 126.01, 125.80,

97.49 (C-1), 77.82, 76.01, 73.84, 73.39, 72.83, 69.78, 68.60, 68.17, 67.26, 29.24, 23.59. ESI HRMS (m/z): $[M + Na^+]$ calcd for $C_{51}H_{55}NNaO_8$, 832.3820 found 832.3817.



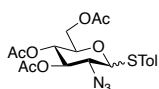
Scheme S2. Synthesis of Glc- N_3 building block. Reagents and Conditions: *a)* imidazole-1-sulfonyl azide hydrogen sulfate, $CuSO_4 \cdot 5H_2O$, K_2CO_3 , MeOH. *b)* Ac_2O , DMAP, pyridine, 59% over 2 steps. *c)* *p*-thiocresol, $BF_3 \cdot Et_2O$, DCM, 80%. *d)* NaOMe, MeOH. *e)* $PhCH(OMe)_2$, *p*-TsOH $\cdot H_2O$, MeCN. *f)* BnBr, NaH, DMF, 63% over 3 steps. *g)* Et_3SiH , $PhBCl_2$, DCM, 98%. *h)* LevOH, DMAP, EDC, DCM, 94%.

1,3,4,6-Tetra-*O*-acetyl-2-azido-2-deoxy-D-glucopyranose (S8):

D-glucosamine hydrochloride (10.3 g, 47.9 mmol) and imidazole-1-sulfonyl azide hydrogen sulfate⁴⁴ (10.0 g, 36.9 mmol) were dissolved in MeOH, after which K_2CO_3 (9.6 g, 70.1 mmol) and $CuSO_4 \cdot 5H_2O$ (0.48 mmol, 120 mg) were added to the solution. The reaction mixture was stirred at room temperature overnight, and all the solvents were removed under reduced pressure. The resulting residue was purified by silica gel column chromatography (DCM/MeOH = 5/1, v/v) to give the 2-azido-2-deoxy-D-glucose as a yellow oil, which was then dissolved in pyridine. Ac_2O (70 mL, 0.73 mol) and 4-(dimethylamino)pyridine (DMAP, 0.45 g, 3.69 mmol) were added to the solution, and the reaction was stirred overnight at room temperature. The reaction mixture was concentrated under reduced pressure, and the residue

was dissolved in EtOAc (100 mL) and washed with brine (3×50 mL). The organic layer was combined, dried over Na_2SO_4 , and the filtrate was concentrated. the resulting residue was purified by silica gel column chromatography (PE/EA = 2/1, v/v) to give **S8** as colorless oil (8.1 g, 59%, $\beta/\alpha = 2.5/1$). ^1H NMR (400 MHz, Chloroform-*d*) δ 6.31 (d, $J = 3.7$ Hz, 0.4H, α H-1), 5.60 (d, $J = 8.5$ Hz, 1H, β H-1), 5.45 (dd, $J = 10.5, 9.4$ Hz, 0.4H, α H-3), 5.17 – 5.01 (m, 2.4H, α H-4, β H-3, β H-4), 4.30 (m, 1.4H α H-6a, β H-6a), 4.15 – 4.01 (m, 2.4H, α H-5, α H-6b, β H-6b), 3.86 (ddd, $J = 10.0, 4.5, 2.2$ Hz, 1H, β H-5), 3.78 – 3.64 (m, 1.4H, α H-2, β H-2), 2.19 (s, 4.2H, α, β -OAc), 2.11 (s, 1.2H, α -OAc), 2.09 (s, 3H, β -OAc), 2.07 (s, 4.2H, α, β -OAc), 2.05 (s, 1.2H, α -OAc), 2.04 (s, 1.2H, α -OAc), 2.02 (s, 3H, β -OAc). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 170.31, 169.85, 169.57, 169.45, 169.36, 168.40, 168.35, 92.37, 89.84, 72.49, 70.60, 69.61, 67.78, 67.71, 62.46, 61.31, 61.29, 60.18, 60.12, 20.71, 20.67, 20.48, 20.46, 20.42, 20.35. ESI HRMS (*m/z*): [*M* + Na^+] calcd for $\text{C}_{14}\text{H}_{19}\text{N}_3\text{NaO}_9$, 396.1014 found 396.1019.

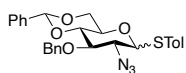
***p*-Methylphenyl 2-azido-2-deoxy-3, 4, 6-tri-*O*-acetyl-1-thio-D-glucopyranoside (S9):** To a cooled (0 °C) solution of **S8** (23.4 g, 62.6 mmol) in



dry DCM (100 mL) was added *p*-thiocresol (11.7 g, 94 mmol) and $\text{BF}_3 \cdot \text{Et}_2\text{O}$ (77.3 mL, 0.62 mol). The reaction mixture was stirred for 2 days at room temperature, after which it was cooled to 0 °C and was then quenched with sat. aq. NaHCO_3 . The two-phase reaction mixture was separated, and the organic layer was washed with sat. aq. NaHCO_3 (3×100 mL), dried (Na_2SO_4), filtered, and the filtrate was concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography (PE/EA = 10/1, v/v) to give **S9** as light-yellow oil (19.7 g, 72%, $\alpha/\beta = 2/1$). ^1H NMR (400 MHz, Chloroform-*d*) δ 7.48 (d, $J = 8.0$ Hz, 0.9H, β Ar), 7.41 – 7.36 (m, 2H, α Ar), 7.15 (dd, $J = 11.0, 7.8$ Hz, 3H, α, β Ar), 5.57 (d, $J = 5.5$ Hz, 1H, α H-1), 5.34 (dd, $J = 10.5, 9.2$ Hz, 1H, α H-3), 5.10 – 4.99 (m, 1.5H, α H-4, β H-3), 4.95 – 4.85 (m, 0.5H, β H-4), 4.62 (ddd, $J = 10.3, 5.2, 2.2$ Hz, 1H, α H-5), 4.43 (dd, $J = 10.1, 0.5$ Hz, 1H, β H-1), 4.32 – 4.13 (m, 1H, α H-6a), 4.23 – 4.15 (m,

1H, β H-6a/b), 4.09 – 4.00 (m, 2H, α H-2, α H-6b), 3.71 – 3.63 (m, 0.5H, β H-5), 3.37 (td, J = 10.0, 0.9 Hz, 0.5H, β H-2), 2.38 (s, 1.5H, β Ph-CH₃), 2.34 (s, 3H, α Ph-CH₃), 2.10 (s, 3H, α OAc), 2.09 (s, 1.5H, β OAc), 2.06 (s, 4.5H, α , β OAc), 2.04 (s, 3H, α OAc), 2.01 (s, 1.5H, β OAc). ¹³C NMR (101 MHz, Chloroform-*d*) δ 170.62, 169.97, 169.91, 169.79, 139.46, 138.59, 134.80, 132.95, 130.14, 130.00, 128.68, 126.26, 87.01, 85.89, 75.83, 74.59, 72.15, 68.90, 68.52, 68.20, 62.58, 62.15, 62.10, 61.75, 21.34, 21.25, 20.84, 20.78, 20.75, 20.73, 20.68. ESI HRMS (m/z): [M + Na⁺] calcd for C₁₉H₂₃N₃NaO₇S, 460.1149 found 460.1155.

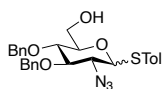
***p*-Methylphenyl 2-azido-2-*O*-deoxy-3-*O*-benzyl-4, 6-*O*-benzylidene-1-thio-D-glucopyranoside (S11):** Compound **S9** (19.7 g, 45 mmol) was dissolved in dry



MeOH (200 mL). NaOMe (30% in methanol, 0.4 mL) was added, and the reaction was stirred overnight at room temperature, after which it was neutralized with Amberlite (H⁺, washed with MeOH before use). The resin was filtered off, and the filtrate was concentrated in *vacuo* to give the crude product which was used for next step without additional purification. It was co-evaporated with toluene (3 \times 50 mL) before being dissolved in dry MeCN (250 mL). Benzaldehyde dimethyl acetal (13.5 mL, 90 mmol) and *p*-toluenesulfonic acid monohydrate (856 mg, 4.5 mmol) were added to the solution. After stirring for 3 h at room temperature, the reaction was quenched with triethylamine (3 mL). The solvents were removed under reduced pressure, and the resulting residue was dissolved in EtOAc (150 mL) and washed with brine (3 \times 100 mL). The organic layer was dried (Na₂SO₄), filtered, and the filtrate was concentrated to give **S10**, which was co-evaporated with toluene (3 \times 100 mL) and used for next step without further purification. To the cooled (0 $^{\circ}$ C) solution of **S10** in dry DMF (100 mL) was added sodium hydride (60% dispersion in mineral oil, 2.7 g, 67.5 mmol). The suspension was stirred for 30 min at 0 $^{\circ}$ C, after which benzyl bromide (8.0 mL, 67.5 mmol) was added, and the reaction mixture was stirred for 2 h at room temperature. After TLC analysis showed consumption of starting material, the reaction was cooled to 0 $^{\circ}$ C and quenched

with MeOH (10 mL). All the solvents were removed in *vacuo*, and the residue was dissolved in EtOAc (150 mL) and washed with brine (3 × 100 mL). The organic layer was dried (Na₂SO₄), filtered, and the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (PE/EA = 10/1, v/v) to give **S11** as white solid (13.8 g, 63% over 3 steps). ¹H NMR (400 MHz, cdcl₃) δ 7.51 (dd, *J* = 7.4, 2.3 Hz, 2H, Ar), 7.45 (d, *J* = 8.3 Hz, 1H, Ar), 7.43 – 7.36 (m, 7H, Ar), 7.36 – 7.29 (m, 5H, Ar), 7.14 (t, *J* = 6.1 Hz, 2H, Ar), 5.60 (s, 1H, CHPh), 5.56 (s, 0.2H, CHPh), 5.49 (d, *J* = 4.8 Hz, 1H, α H-1), 4.97 (d, *J* = 10.9 Hz, 1H, CHHPh Ph), 4.90 (d, *J* = 10.9 Hz, 0.2H CHHPh), 4.83 (d, *J* = 10.9 Hz, 1H, CHHPh), 4.77 (d, *J* = 10.9 Hz, 0.2H, CHHPh), 4.50 – 4.34 (m, 1.4H, α H-5, β H-1, β H-5), 4.23 (dd, *J* = 10.3, 4.9 Hz, 1H, α H-6a), 4.02 – 3.90 (m, 2H, α H-2, α H-3), 3.76 (m, 2.2H, α H-4, β H-6a, α H-6b), 3.68 – 3.56 (m, 0.4H, β H-3, β H-6b), 3.43 (td, *J* = 9.4, 4.9 Hz, 0.2H, β H-4), 3.32 (dd, *J* = 10.1, 8.5 Hz, 0.2H, β H-2), 2.35 (s, 0.6H, -SPhCH₃), 2.34 (s, 3H, -SPhCH₃). ¹³C NMR (101 MHz, cdcl₃) δ 138.51, 137.80, 137.26, 134.68, 133.26, 130.14, 130.06, 129.24, 129.18, 128.58, 128.49, 128.47, 128.45, 128.39, 128.18, 128.10, 126.15, 126.10, 101.63, 101.39, 88.30, 86.73, 82.90, 81.45, 81.15, 77.97, 75.37, 75.34, 70.60, 68.76, 64.64, 63.85, 63.73, 21.30. ESI HRMS (*m/z*): [*M* + Na⁺] calcd for C₂₇H₂₇N₃NaO₄S, 512.1614 found 512.1603.

***p*-Methylphenyl 2-azido-6-*O*-hydroxyl-2-*O*-deoxy-3, 4-di-*O*-benzyl-1-thio-D-glucopyranoside (S12):** To a cooled (-78 °C) solution of **S11** (13.8 g, 28.20

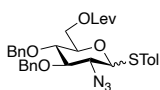


mmol) in DCM (150 mL) was added triethylsilane (6.85 mL, 42.30 mmol) and dichlorophenylborane (6.22 mL, 47.94 mmol)

sequentially. After stirring for 1 h at -78 °C, the reaction was quenched with MeOH (12 mL) and triethylamine (12 mL). The resulting mixture was diluted with DCM (200 mL) and washed with sat. aq. NaHCO₃ (300 mL). The aqueous layer was extracted with DCM (3 × 50 mL), and the combined organic layers were dried (Na₂SO₄), filtered, and the filtrate was concentrated *in vacuo*. The resulting residue was purified by silica gel column chromatography (PE/EA = 5/1,

v/v) to give **S12** (13.6 g, 98%, $\alpha/\beta=1/0.4$) as colorless oil. ^1H NMR (400 MHz, Chloroform-*d*) δ 7.43 – 7.14 (m, 17H, α/β Ar), 7.14 (d, $J = 7.7$ Hz, 3H, α/β Ar), 5.50 (d, $J = 4.6$ Hz, 1H, α H-1), 4.93 (m, 2.8H, α $\underline{\text{CH}_2\text{Ph}}$, β $\underline{\text{CH}_2\text{Ph}}$), 4.91 – 4.86 (m, 1.8H, α $\underline{\text{CHHPh}}$, β $\underline{\text{CH}_2\text{Ph}}$), 4.70 (d, $J = 11.1$ Hz, 1H, α $\underline{\text{CHHPh}}$), 4.40 (d, $J = 10.2$ Hz, 0.4H, β H-1), 4.28 (dt, $J = 10.1, 3.3$ Hz, 1H, α H-5), 3.94 – 3.85 (m, 2H, α H-2, α H-3), 3.81 – 3.74 (m, 2H, α H-6a/b), 3.69 – 3.62 (m, 1H, α H-4), 2.35 (s, 3H SPhCH_3), 1.63 (s, 1 H, $\underline{\text{OH-6}}$). ^{13}C NMR (101 MHz, cdCl_3) δ 138.43, 137.85, 137.75, 134.30, 133.23, 133.14, 130.11, 130.05, 128.71, 128.69, 128.67, 128.65, 128.36, 128.29, 128.19, 128.18, 128.12, 128.06, 87.59 (α C-1), 81.78, 78.07, 75.88, 75.27, 72.46, 64.29, 61.63, 21.27 (CH_3). ESI HRMS (m/z): $[\text{M} + \text{Na}^+]$ calcd for $\text{C}_{27}\text{H}_{29}\text{N}_3\text{NaO}_4\text{S}$, 514.1771 found 514.1777.

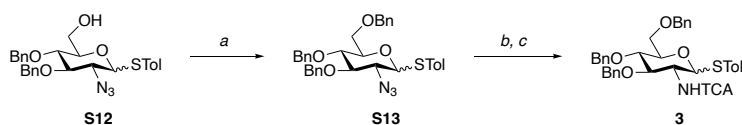
***p*-Methylphenyl 2-azido-6-*O*-levulinoyl-2-*O*-deoxy-3, 4-di-*O*-benzyl-1-thio-D-glucopyranoside (5):** Compound **S12** (13.5 g, 27.6 mmol) was dissolved in



DCM (300 mL), and levulinic acid (LevOH, 4.58 g, 39.5 mmol), *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDC·HCl, 8.57 g, 45.12 mmol) and 4-

(dimethylamino)pyridine (DMAP, 340 mg, 2.82 mmol) were added into the solution. After stirring overnight at room temperature, the reaction mixture was diluted with DCM (200 mL) and washed with sat. aq. NaHCO_3 . The aqueous layer was back extracted with DCM (3×50 mL), and the combined organic layers were dried (Na_2SO_4), filtered, and the filtrate was concentrated *in vacuo*. The resulting residue was purified with silica gel column chromatography (PE/EA = 5/1, v/v) to give **5** (14.8 g, 91%, $\alpha/\beta = 3/1$) as a white solid. ^1H NMR (400 MHz, Chloroform-*d*) δ 7.51 – 7.26 (m, 15H, α/β Ar), 7.13 (m, 3H, α/β Ar), 5.51 (d, $J = 5.2$ Hz, 1H, α H-1), 4.98 – 4.79 (m, 4.2H, α $\underline{\text{CH}_2\text{Ph}}$, α $\underline{\text{CHHPh}}$, β $\text{PhCH}_2 \times 2$), 4.61 (d, $J = 10.8$ Hz, 1H, α $\underline{\text{CHHPh}}$), 4.45 (ddd, $J = 10.0, 4.8, 2.2$ Hz, 1H, α H-5), 4.34 – 4.30 (m, 1.3H, α H-6a, β H-1), 4.23 (dd, $J = 12.0, 2.3$ Hz, 1H, α H-6b), 3.93 (dd, $J = 10.3, 5.3$ Hz, 1H, α H-2), 3.89 – 3.82 (m, 1H, α H-3), 3.58 (dd, $J = 10.0, 8.5$ Hz, 1H, α H-4), 3.53 – 3.41 (m, 1H), 2.75 – 2.68 (m, 2H, α/β $\underline{\text{CH}_2\text{-Lev}}$), 2.53

(t, $J = 6.6$ Hz, 2H, α/β $\underline{\text{CH}_2\text{-Lev}}$), 2.33 (s, 3H, $\underline{\text{CH}_3\text{-Lev}}$), 2.16 (s, 3H, $\text{Ph}\underline{\text{CH}_3}$). ^{13}C NMR (101 MHz, cdCl_3) δ 171.64, 137.65, 134.47, 132.91, 132.89, 130.07, 130.05, 129.87, 128.70, 128.68, 128.35, 128.30, 128.28, 128.18, 128.15, 128.13, 87.42 (α , C-1), 81.88, 78.27, 75.92, 75.31, 75.24, 70.18, 64.22, 63.09, 37.95, 29.95, 27.88, 21.24. ESI HRMS (m/z): $[\text{M} + \text{NH}_4^+]$ calcd for $\text{C}_{32}\text{H}_{35}\text{N}_3\text{O}_6\text{SNH}_4^+$, 607.2585 found 607.2517.

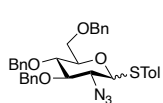


Scheme S3. Synthesis of GlcNAc donor **3**. Conditions and reagents: *a*) BnBr, NaH, DMF, 63%. *b*) Zn, HOAc/THF. *c*) TCACl, NaHCO₃, THF, 67% over 2 steps.

p-Methylphenyl

2-azido-2-*O*-deoxy-3,4,6-tri-*O*-benzyl-1-thio-D-

glucopyranoside (S13): To a cooled (0 °C) solution of **S12** (2.0 g, 4.0 mmol) in

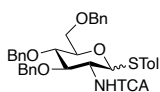


DMF (20 mL) was added NaH (60% dispersion in mineral oil, 240 mg, 6.0 mmol). The suspension was stirred for 30 min at 0 °C, after which benzyl bromide (714 μL , 6.0 mmol) was added,

and the reaction mixture was stirred for another 2 h at room temperature. After TLC analysis showed consumption of starting material, the reaction was quenched with MeOH (2 mL) and concentrated under reduced pressure. The residue was dissolved in EtOAc (50 mL) and washed with brine (3×50 mL), and the aqueous layer was back extracted with EtOAc (3×50 mL). The combined organic layer was dried (Na_2SO_4), filtered, and the filtrate was concentrated in *vacuo*. The resulting residue was purified with silica gel column chromatography (PE/EA = 6/1, v/v) to give **S13** (1.5 g, 63%) as a white solid. ^1H NMR (600 MHz, CDCl_3) δ 7.49 – 7.03 (m, 25H, Ar), 5.53 (d, $J = 5.4$ Hz, 1H, α H-1), 4.91 (d, $J = 10.6$ Hz, 1H, α $\underline{\text{CHHPh}}$), 4.87 (d, $J = 10.6$ Hz, 1H, α $\underline{\text{CHHPh}}$), 4.86 – 4.76 (m, 1.9H, α $\underline{\text{CHHPh}}$, β $\underline{\text{CH}_2\text{Ph}}$, β $\underline{\text{CHHPh}}$), 4.63 – 4.57 (m, 1.6H, α $\underline{\text{CHHPh}}$, β $\underline{\text{CHHPh}}$, β $\underline{\text{CHHPh}}$), 4.55 – 4.52 (m, 1.3H, α $\underline{\text{CHHPh}}$, β $\underline{\text{CHHPh}}$), 4.44 (d, $J = 11.9$ Hz, 1H,

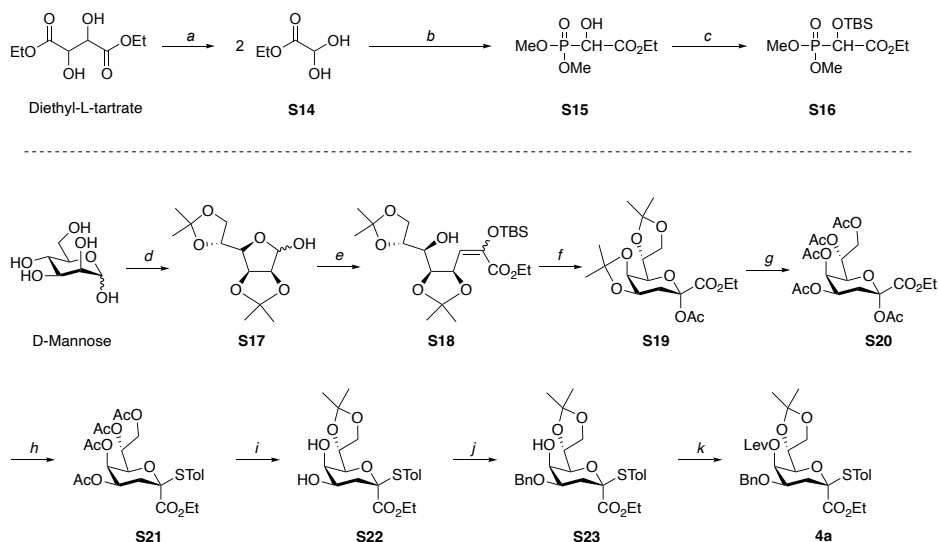
α CHHPPh), 4.38 (ddd, $J = 10.0, 3.9, 1.9$ Hz, 1H, α H-5), 4.35 (d, $J = 10.1$ Hz, 0.3H, β H-1), 3.93 (dd, $J = 10.2, 5.4$ Hz, 1H, α H-2), 3.82 (dd, $J = 10.1, 8.8$ Hz, 1H, α H-3), 3.80 – 3.78 (m, 1H, α H-6a), 3.77 – 3.71 (m, 1.6H, α H-4, β H-6a/b), 3.64 (dd, $J = 10.8, 2.0$ Hz, 1H, α H-6b), 3.58 (t, $J = 9.4$ Hz, 0.3H, β H-4), 3.50 (t, $J = 9.2$ Hz, 0.3H β H-3), 3.45 (ddd, $J = 9.8, 4.0, 2.1$ Hz, 0.3H, β H-5), 3.29 (t, $J = 9.7$ Hz, 0.3H, β H-2), 2.31 (s, 3.9H, α/β -SPhCH₃). ¹³C NMR (151 MHz, CDCl₃) δ 138.04, 138.00, 137.96, 137.83, 134.48, 132.78, 130.44, 129.99, 129.91, 129.74, 128.66, 128.64, 128.61, 128.51, 128.50, 128.37, 128.28, 128.16, 128.10, 128.00, 127.98, 127.90, 127.86, 127.73, 127.71, 87.72, 86.01, 85.22, 81.95, 79.46, 78.46, 77.68, 76.04, 75.87, 75.22, 75.18, 73.59, 73.57, 71.86, 68.87, 68.49, 65.01, 64.28, 21.26. ESI HRMS (m/z): $[M + Na^+]$ calcd for C₃₄H₃₅N₃NaO₄S, 604.2240 found 604.2223.

***p*-Methylphenyl 2-trichloroacetamido-2-*O*-deoxy-3, 4, 6-tri-*O*-benzyl-1-thio-D-glucopyranoside (3):** Compound **S13** (1.5 g, 2.58 mmol) was dissolved in a



mixture of AcOH/THF (5 mL/ 20mL), and Zn (2.5 g, activated by 1M HCl) was then added to the solution. After attiring overnight, all solids were filtered off, and the filtrate was concentrated under reduced pressure. The resulting residue was co-evaporated with toluene (10 mL \times 4) and was dissolved in dry THF (20 mL). After the addition of anhydrous NaHCO₃ (1.3 g, 15.5 mmol), the suspension was cooled to 0 °C and placed under an atmosphere of Argon. Trichloroacetyl chloride (TCACl, 576 μ L, 5.16 mmol) was then added to the mixture dropwise. The reaction mixture was stirred for 1 h at 0 °C until TLC analysis showed consumption of starting material. The reaction was quenched with MeOH (5 mL), and all salts were filtered off. The filtrate was concentrated under reduced pressure, and the resulting residue was dissolved in DCM (20 mL) and washed with water (10 mL). The organic layer was dried (Na₂SO₄), filtered, and the filtrate was concentrated *in vacuo*. The crude product was purified by flash chromatography over silica gel (PE/EA = 6/1, v/v) to give **3** (1.2 g, 67%) as a light-yellow solid. α -anomer: ¹H

NMR (400 MHz, Chloroform-*d*) δ 7.38 – 7.25 (m, 15H, Ar), 7.20 (dd, J = 7.4, 2.2 Hz, 2H, Ar), 7.07 (d, J = 7.9 Hz, 2H, Ar), 6.97 (d, J = 8.5 Hz, 1H, NHTCA), 5.55 (d, J = 4.9 Hz, 1H, H-1), 4.86 – 4.72 (m, 3H, PhCH₂, PhCHH), 4.64 (d, J = 12.0 Hz, 1H, PhCHH), 4.58 (d, J = 10.9 Hz, 1H, PhCHH), 4.51 (d, J = 12.0 Hz, 1H, PhCHH), 4.49 – 4.36 (m, 2H, H-2, H-5), 3.87 (dd, J = 10.9, 4.2 Hz, 1H, H-6a), 3.82 (t, J = 8.8 Hz, 1H, H-4), 3.77 – 3.65 (m, 2H, H-3, H-6b), 2.32 (s, 3H, PhCH₃). ¹³C NMR (101 MHz, cdcl₃) δ 161.64, 138.44, 138.00, 137.83, 137.64, 132.76, 130.44, 130.13, 129.77, 128.80, 128.63, 128.55, 128.51, 128.17, 128.16, 128.09, 127.99, 127.87, 127.00, 88.91 (C-1), 79.80, 78.16, 75.09, 75.02, 73.63, 72.92, 68.43, 54.82, 21.26. ESI HRMS (*m/z*): [M + Na⁺] calcd for C₃₆H₃₆Cl₃NNaO₅S, 722.1272 found 722.1268.



Scheme S4. Synthesis of Kdo donor **4a**. Reagents and conditions: *a*) NaIO₄, DCM/H₂O. *b*) dimethyl phosphite, Et₃N, toluene. *c*) TBSCl, imidazole, DMF, 71% over 3 steps. *d*) TsOH•H₂O, 2,2-dimethoxypropane, DMF, rt. *e*) **S16**, LiHMDS, THF, 55°C. *f*) i. TBAF, AcOH/H₂O, 0 °C. ii. Ac₂O, DMAP, pyridine 59% from D-mannose. *g*) i. TFA/DCM/H₂O, 0 °C. ii. Ac₂O, DMAP, Pyridine, 72% over 2 steps. *h*) *p*-toluenethiol, BF₃•Et₂O, DCM, 75%. *i*) i. NaOMe, MeOH. ii. 2-methoxypropene, CSA, DMF/1,4-dioxane, 48% over 2 steps. *j*) i. Bu₂SnO,

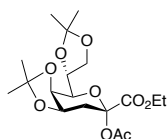
toluene, 110°C. ii. BnBr, CsF, DMF, rt, 82% over 2 steps. k) LevOH, DMAP, DCC, DCM, reflux, 99%.

Ethyl ester α -(dimethoxyphosphinyl) glycolate *t*-butyldimethylsilyl Ether

(S16): Diethyl-L-tartrate (10 g, 48.4 mmol) was dissolved in DCM (100 mL) and the solution was stirred rapidly at room temperature, which was followed by the addition of NaIO₄ (20.8 g, 97 mmol) and H₂O (20 mL). The resulting biphasic reaction mixture was heated to reflux for 2 h, and during this period a thick white precipitate was formed in the solution. After TLC analysis showed consumption of the starting material, the reaction mixture was cooled to room temperature and was added Na₂SO₄. The resulting suspension was stirred for 30 min and all salts were filtered. The filtrate was concentrated under reduced pressure to give **S14**, which can be used for the next step without further purification. Triethylamine (46 mL, 290 mmol) was added to a solution of dimethyl phosphite (10.6 mL, 116.2 mmol) in dry toluene (100 mL) at 0 °C. After stirring for 15 min at this temperature, the solution of **S14** in dry toluene (20 mL) was added. The reaction was warmed to room temperature slowly and was stirred for 3 h. After TLC analysis showed consumption of the starting material, the reaction mixture was concentrated under reduced pressure to give **S15**. Imidazole (15.6 g, 230 mmol) and *tert*-butyldimethylsilyl chloride (TBSCl, 25.9 g, 270 mmol) were added into a solution of the **S15** in DMF (120 mL). The reaction mixture was stirred overnight at room temperature, after which it was concentrated *in vacuo*. The resulting residue was dissolved in EtOAc (200 mL) and washed with sat. aq. NaHCO₃ (100 mL) and water (100 mL). The organic layer was dried (Na₂SO₄), filtered, and the filtrate was concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography (PE/EA = 1/1, v/v) to form **S16**³³ (23.5 g, 71%) as a colorless liquid. ¹H NMR (400 MHz, Chloroform-*d*) δ 4.58 (d, *J* = 18.1 Hz, 1H), 4.24 (p, *J* = 7.1 Hz, 2H), 3.82 (d, *J* = 6.6 Hz, 3H), 3.79 (d, *J* = 6.6 Hz, 3H), 1.32 – 1.22 (m, 3H), 0.89 (s, 9H), 0.09 (s, 3H), 0.08 (s, 3H). ¹³C

NMR (101 MHz, cdCl_3) δ 168.46 (d, $J = 2.7$ Hz, P split), 70.67 (d, $J = 162.1$ Hz, P split), 61.90, 54.13 (t, $J = 6.5$ Hz, P split), 25.60, 18.42, 14.15, -5.28, -5.45.

Ethyl [2-*O*-acetyl-4,5:7,8-di-*O*-isopropylidene-3-deoxy-D-manno-oct-2-ulo-pyranosyl]onate (S19): 2,2-Dimethoxypropane (3.8 mL, 30.8 mmol) was added



to the solution of D-mannose (3.4 g, 19 mmol) and *p*-toluenesulfonic acid monohydrate ($\text{TsOH} \cdot \text{H}_2\text{O}$, 217 mg, 1.14 mmol) in dry DMF (30 mL). After stirring for 6 h at room temperature, another portion of 2,2-dimethoxypropane (3.1 mL,

25 mmol) was added and the reaction mixture was stirred overnight. The reaction was quenched with sat. aq. NaHCO_3 (3 mL), and all the solvents were removed *in vacuo*. The resulting residue was dissolved in EtOAc (100 mL) and washed with sat. aq. NaHCO_3 (50 mL). The aqueous layer was extracted with EtOAc (20 mL \times 5), and the combined organic layers were dried (Na_2SO_4) and filtered. The filtrate was concentrated under reduced pressure to give the crude product **S17**, which was co-evaporated with toluene (30 mL \times 3) and can be used for the next step without further purification.

Lithium bis(trimethylsilyl)amide (LiHMDS , 38 mL, 38 mmol, 1 M in THF) was added to dry THF (20 mL) and the solution was placed under an atmosphere of Argon, which was followed by the addition of a solution of **S16** (9.3 g, 28.5 mmol) in dry THF (20 mL). The reaction mixture was stirred for 15 min at room temperature, after which a solution of **S17** in dry THF (20 mL) was added. The reaction mixture was heated to 55 °C gradually and stirred for 1 h at this temperature. The reaction was then cooled down to room temperature and quenched with 20 mL of sat. aq. NH_4Cl . Most of the organic solvents were removed under reduced pressure, and the resulting mixture was dissolved in EtOAc (50 mL) and washed with brine (20 mL). The aqueous layer was extracted with EtOAc (20 mL \times 3), and the combined organic layers were dried (Na_2SO_4), filtered, and the filtrate was concentrated under reduced pressure. The resulting

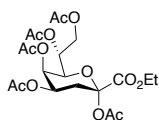
crude product **S18** was used for the next step without further purification.

AcOH (20 % in H₂O, 40 mL) was added in the cooled (0 °C) solution of **S18** in THF (30 mL), and TBAF (30 mL, 30 mmol, 1.0 M in THF) was then added. The reaction mixture was stirred for 3 h. After TLC analysis showed consumption of starting material, the reaction was neutralized with NaHCO₃ and filtered. The filtrate was concentrated under reduced pressure. The resulting residue was dissolved in EtOAc (100 mL) and washed with sat. aq. NaHCO₃ (50 mL). The organic layer was dried (Na₂SO₄), filtered, and the filtrate was concentrated *in vacuo*. After treatment of the product-with a free hydroxyl at C2-with acid to remove all the isopropylidene acetals, the sugar ring was opened and closed into a five-member-ring which is no longer Kdo. To avoid the formation of by-product, we decided to protect the free alcohol with acetyl immediately.

The residue obtained above was dissolved in pyridine (40 mL), and Ac₂O (9 mL, 95 mmol) and 4-(dimethylamino)pyridine (DMAP, 232 mg, 1.9 mmol) were added to the solution. After stirred overnight, the reaction mixture was concentrated under reduced pressure. The resulting residue was dissolved in EtOAc (50 mL) and washed with sat. aq. NaHCO₃ (20 mL), and the aqueous phase was extracted with EtOAc (3 × 20 mL). The combined organic layers were dried (Na₂SO₄), filtered, and the filtrate was concentrated *in vacuo*. The resulting residue was purified by silica gel column chromatography (PE/EA = 2/1, v/v) to give **S19**⁴⁵ (4.35 g, 59% from D-Mannose) as a light-yellow oil. ¹H NMR (400 MHz, Chloroform-*d*) δ 4.51 (dt, *J* = 7.9, 3.4 Hz, 1H, H-4), 4.35 (m, 1H, H-7), 4.31 (dd, *J* = 7.8, 1.8 Hz, 1H, H-5), 4.20 (q, *J* = 7.1 Hz, 2H, CO₂CH₂CH₃), 4.07 (ddd, *J* = 9.1, 6.1, 1.4 Hz, 1H, H-8a), 3.84 (ddd, *J* = 9.1, 3.6, 1.4 Hz, 1H, H-8b), 3.58 (dd, *J* = 8.4, 1.8 Hz, 1H, H-6), 2.65 (ddd, *J* = 15.6, 3.8, 1.5 Hz, 1H, H_{eq}-3), 2.08 – 1.97 (m, 4H, H_{ax}-3, -OOCCH₃), 1.46 (s, 3H, C(CH₃)₂), 1.39 (s, 3H, C(CH₃)₂), 1.32 (d, *J* = 1.5 Hz, 3H, C(CH₃)₂), 1.29 (s, 3H, C(CH₃)₂), 1.25 (tt, *J* = 7.1, 1.1 Hz, 3H, CO₂CH₂CH₃). ¹³C NMR (101 MHz, cdcl₃) δ 169.01, 168.10,

109.85, 109.54, 96.93 (C-2), 73.35, 72.93, 71.24, 69.53, 67.16, 62.13, 32.12, 27.11, 25.59, 25.15, 24.82, 21.09, 13.91. ESI HRMS (m/z): [M + Na⁺] calcd for C₁₈H₂₈NaO₉, 411.1626 found 411.1625.

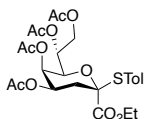
Ethyl [2,4,5,7,8-penta-*O*-acetyl-3-deoxy-D-*manno*-oct-ulo-pyranoside]onate



(S20): Compound **S19** (7.8 g) was dissolved in a cooled (0 °C) mixture of DCM (90 mL) and aq. TFA (60% in H₂O, 10 mL), and the reaction mixture was stirred for 30 min at 0 °C. After TLC

showed consumption of the starting material, the reaction mixture was diluted with toluene (150 mL) and concentrated under reduced pressure. After co-evaporated with toluene (3 × 50 mL), the crude product was dissolved in pyridine (100 mL). Ac₂O (41 mL, 440 mmol) and 4-(dimethylamino)pyridine (DMAP, 161 mg, 1.32 mmol) were added, and the reaction mixture was stirred overnight at room temperature. The solvents were removed under reduced pressure, and the residue was dissolved in EtOAc (150 mL) and washed with sat. aq. NaHCO₃ (100 mL). The organic layer was dried (over Na₂SO₄), filtered, and the filtrate was concentrated *in vacuo*. The resulting residue was purified by silica gel column chromatography (PE/EA = 1/1, v/v) to give **S20**⁴⁶ (7.5 g, 72%) as a light-yellow oil. ¹H NMR (400 MHz, Chloroform-*d*) δ 5.37 (d, *J* = 3.0 Hz, 1H, H-5), 5.31 (ddd, *J* = 11.4, 5.9, 3.1 Hz, 1H, H-4), 5.21 (ddd, *J* = 9.8, 4.0, 2.3 Hz, 1H, H-7), 4.46 (dd, *J* = 12.4, 2.3 Hz, 1H, H-8a), 4.25 (q, *J* = 7.1 Hz, 2H, CH₂CH₃), 4.16 (dd, *J* = 9.8, 1.4 Hz, 1H, H-6), 4.11 (dd, *J* = 12.4, 4.0 Hz, 1H, H-8b), 2.25 – 2.17 (m, 2H, H_{eq/ax}-3), 2.12 (s, 3H, OAc), 2.09 (s, 3H, OAc), 2.03 (s, 3H, OAc), 1.99 (s, 3H, OAc), 1.98 (s, 3H, OAc), 1.28 (t, *J* = 7.2 Hz, 3H, CH₂CH₃). ¹³C NMR (101 MHz, cdcl₃) δ 170.55, 170.39, 170.12, 169.68, 168.02, 166.12, 97.61, 69.76, 67.43, 66.07, 64.06, 62.48, 62.25, 30.95, 20.86, 20.78, 20.75, 14.04. ESI HRMS (m/z): [M + Na⁺] calcd for C₂₀H₂₈NaO₁₃, 499.1422 found 499.1430.

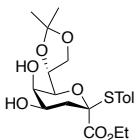
Ethyl [*p*-methylphenyl 4,5,7,8-tetra-*O*-acetyl-3-deoxy-2-thio-*D*-manno-octulopyranoside]onate (S21**):** *p*-Toluenethiol (3.1 g, 25 mmol) and BF₃·Et₂O (6.2



mL, 50 mmol) were added in a cooled (0 °C) solution of **S20** (8 g, 16.8 mmol) in dry DCM (100 mL). The reaction mixture was stirred for 24 h at room temperature. After TLC showed consumption of starting material, the reaction was cooled to 0 °C

again and was then quenched by adding sat. aq. NaHCO₃ (50 mL) slowly. The bi-phase system was separated, and the organic layer was washed with brine, dried (over Na₂SO₄), filtered, and the filtrate was concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography (PE/EA = 4/1, v/v) to give **S21** (5.3 g, 58%) as a light-yellow oil. ¹H NMR (600 MHz, CDCl₃) δ 7.45 – 7.42 (m, 2H, Ar), 7.14 (d, *J* = 7.9 Hz, 2H, Ar), 5.28 (dd, *J* = 2.8, 1.3 Hz, 1H, H-5), 5.22 (ddd, *J* = 9.5, 5.5, 2.3 Hz, 1H, H-7), 4.88 (ddd, *J* = 12.5, 4.8, 2.9 Hz, 1H, H-4), 4.57 (dd, *J* = 12.2, 2.3 Hz, 1H, H-8a), 4.04 (m, 2H, H-8b, CO₂CHHCH₃), 3.95 (dq, *J* = 10.7, 7.1 Hz, 1H, -CO₂CHHCH₃), 3.89 (dd, *J* = 9.6, 1.3 Hz, 1H, H-6), 2.58 (dd, *J* = 12.6, 4.8 Hz, 1H, H-3_{eq}), 2.35 (s, 3H, SPhCH₃), 2.19 (t, *J* = 12.6 Hz, 1H, H-3_{ax}), 2.08 (s, 3H, OAc), 2.04 (s, 3H, OAc), 1.99 (s, 3H, OAc), 1.97 (s, 3H, OAc), 1.10 (t, *J* = 7.1 Hz, 3H, CO₂CH₂CH₃). ¹³C NMR (151 MHz, CDCl₃) δ 170.55, 170.48, 169.85, 169.70, 167.62, 140.24, 136.37, 129.50, 125.26, 87.44, 72.41, 67.91, 67.36, 63.84, 62.86, 62.05, 32.01, 21.30, 20.74, 20.66, 13.85. ESI HRMS (*m/z*): [*M* + Na⁺] calcd for C₂₅H₃₂NaO₁₁S, 563.1558 found 563.1568.

Ethyl [*p*-methylphenyl 7,8-*O*-isopropylidene-3-deoxy-2-thio-*D*-manno-octulopyranoside]onate (S22**):** Compound **S21** (2.0 g, 3.7 mmol) was dissolved in

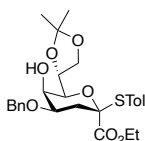


MeOH (20 mL), and the solution was adjusted to pH = 11 by sodium methoxide (0.5 M in methanol). After stirring for 15 min at room temperature, the reaction was neutralized with Amberlite (H⁺, washed with MeOH before use). The resin was washed with MeOH

(3 mL × 5) before filtered off, and the combined filtrate was concentrated under

reduced pressure. The crude product was co-evaporated 3 times with toluene (20 mL) and was dissolved in dry DMF/1,4-dioxane (10 mL/5 mL), and the solution was placed under an atmosphere of Argon. 2-Methoxy propene (425 μ L, 4.4 mmol) and camphor-10-sulfonic acid (CSA, 171 mg, 0.74 mmol) were then added, and the reaction mixture was stirred for 16 h at room temperature. After TLC analysis showed consumption of starting material, the reaction was quenched with triethylamine (1 mL). All the solvents were removed to give the crude product, which was purified by silica gel column chromatography (PE/EA = 2/1, v/v) to give **S22** (712 mg, 47%) as a white solid. ^1H NMR (400 MHz, Chloroform-*d*) δ 7.39 (d, J = 8.1 Hz, 2H, Ar), 7.11 (d, J = 7.9 Hz, 2H, Ar), 4.35 (ddd, J = 8.6, 6.2, 4.7 Hz, 1H, H-7), 4.14 (dd, J = 8.7, 6.2 Hz, 1H, H-8a), 4.11 – 4.01 (m, 1H, COOCHHCH₃), 3.97 (dd, J = 8.7, 4.7 Hz, 1H, H-8b), 3.95 (d, J = 3.0 Hz, 1H, H-5), 3.83 (dq, J = 10.8, 7.1 Hz, 1H, CO₂CHHCH₃), 3.71 – 3.60 (m, 1H, H-4), 3.27 (dd, J = 8.6, 1.2 Hz, 1H, H-6), 2.64 (dd, J = 12.7, 5.0 Hz, 1H, H_{eq}-3), 2.35 (s, 3H, SPh-CH₃), 1.95 (t, J = 12.2 Hz, 1H, H_{ax}-3), 1.36 (s, 3H, C(CH₃)₂), 1.35 (s, 3H, C(CH₃)₂), 1.06 (t, J = 7.1 Hz, 3H, COOCH₂CH₃). ^{13}C NMR (101 MHz, cdcl₃) δ 168.50, 140.17, 136.44, 129.44, 125.94, 109.83, 87.28 (C-2), 77.53, 73.11, 67.55, 67.37, 66.34, 61.91, 35.33, 27.01, 25.31, 21.44, 13.94. ESI HRMS (m/z): [M + Na⁺] calcd for C₂₀H₂₈NaO₇S, 435.1448 found 435.1450.

Ethyl [*p*-methylphenyl 4-*O*-benzyl-7,8-*O*-isopropylidene-3-deoxy-2-thio-D-manno-oct-ulopyranoside]onate (S23**):** Compound **S22** (600 mg, 1.45 mmol)

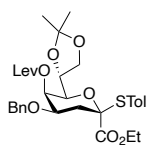


and dibutyltin oxide (Bu₂SnO, 434 mg, 1.75 mmol) were dissolved in toluene (10 mL). The suspension was heated with a pre-heated oil bath (110 °C) and kept reflux for 3 h, during which period the reaction mixture became clear solution. The reaction

was cooled to room temperature and concentrated under reduced pressure. The resulting residue was dissolved in DMF (10 mL), and benzyl bromide (BnBr, 208 μ L, 1.75 mmol) and CsF (661 mg, 4.35 mmol) were added. The reaction mixture was stirred for 16 h at room temperature. After TLC showed consumption of

starting material, the reaction was diluted with EtOAc (50 mL) and washed with H₂O (20 mL). The aqueous layer was extracted with EtOAc (20 mL × 5), and the combined organic layer was dried (Na₂SO₄) and filtered. The filtrate was concentrated under reduced pressure, and the resulting residue was purified by silica gel column chromatography (PE/EA = 3/1, v/v) to give **S23** (712 mg, 97%) as a colorless oil. ¹H NMR (600 MHz, Chloroform-*d*) δ 7.41 – 7.27 (m, 7H, Ar), 7.12 – 7.08 (m, 2H, Ar), 4.65 – 4.57 (m, 2H, PhCH₂), 4.45 (ddd, *J* = 8.7, 6.3, 4.9 Hz, 1H, H-7), 4.13 (td, *J* = 8.9, 6.7 Hz, 1H, H-8a), 4.07 (d, *J* = 2.8 Hz, 1H, H-5), 4.03 – 3.98 (m, 1H, CO₂CHHCH₃), 3.96 (dt, *J* = 8.7, 4.7 Hz, 1H, H-8b), 3.78 (dq, *J* = 10.7, 7.1 Hz, 1H, CO₂CHHCH₃), 3.48 (ddd, *J* = 11.8, 4.8, 2.9 Hz, 1H, H-4), 3.20 (dd, *J* = 8.8, 1.2 Hz, 1H, H-6), 2.68 (dd, *J* = 12.7, 4.8 Hz, 1H, H_{eq}-3), 2.34 (s, 3H, SPhCH₃), 2.24 (d, *J* = 2.2 Hz, 1H, HO-5), 2.10 (dd, *J* = 12.6, 11.7 Hz, 1H, H_{ax}-3), 1.37 (s, 3H, C(CH₃)₂), 1.35 (s, 3H, C(CH₃)₂), 1.00 (t, *J* = 7.1 Hz, 3H, CO₂CH₂CH₃). ¹³C NMR (151 MHz, CDCl₃) δ 168.71, 140.06, 137.58, 136.46, 129.38, 128.69, 128.15, 127.89, 109.66, 87.30 (C-2), 77.48, 74.07, 72.92, 70.31, 67.62, 63.83, 61.77, 32.22, 26.99, 25.33, 21.43, 13.88. ESI HRMS (*m/z*): [M + Na⁺] calcd for C₂₇H₃₄NaO₇S, 525.1917 found 525.1919.

Ethyl [*p*-methylphenyl 4-*O*-benzyl-5-*O*-levulinoyl-7, 8-*O*-isopropylidene-3-deoxy-2-thio-*D*-manno-oct-ulopyranoside] onate (4a**):** *N*, *N'*-

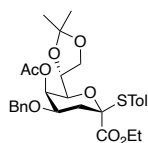


Dicyclohexylcarbodiimide (DCC, 706 mg, 3.42 mmol), levulinic acid (LevOH, 397 mg, 3.42 mmol) and 4-(dimethylamino)pyridine (DMAP, 418 mg, 3.42 mmol) were added to a solution of **S23** (860 mg, 1.71 mmol) in DCM (20 mL), and the reaction mixture was

stirred under reflux for 2 h. After TLC analysis showed consumption of starting material, the reaction was cooled to 0 °C, washed with sat. aq. NaHCO₃ (20 mL) and extracted with cooled DCM (0 °C, 20 mL × 3). The cooled combined organic layer was dried (Na₂SO₄), filtered, and the filtrate was concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography (DCM/acetone, 20/1, v/v) to give Kdo donor **4a** (1.02 g, 99%)

as a colorless oil. ^1H NMR (600 MHz, Chloroform-*d*) δ 7.41 – 7.37 (m, 2H, Ar), 7.35 – 7.26 (m, 5H, Ar), 7.13 – 7.09 (m, 2H, Ar), 5.53 (dd, J = 2.9, 1.4 Hz, 1H, H-5), 4.70 (d, J = 11.7 Hz, 1H, PhCH $\underline{\text{H}}$), 4.43 (d, J = 11.7 Hz, 1H, PhC $\underline{\text{H}}$ H), 4.20 – 4.08 (m, 2H, H-7, H-8a), 3.98 (m, 2H, H-8b, CO $_2$ CH $\underline{\text{H}}$ CH $_3$), 3.80 (dq, J = 10.7, 7.1 Hz, 1H, CO $_2$ C $\underline{\text{H}}$ HCH $_3$), 3.48 (ddd, J = 11.9, 4.7, 2.8 Hz, 1H, H-4), 3.39 – 3.30 (m, 1H, H-6), 2.84 – 2.58 (m, 5H, CH $_2$ -Lev \times 2, H $_{\text{eq}}$ -3), 2.34 (s, 3H, SPhC $\underline{\text{H}}$ CH $_3$), 2.19 (s, 3H, Lev), 2.07 (t, J = 12.4 Hz, 1H, H $_{\text{ax}}$ -3), 1.34 (s, 3H, C($\underline{\text{C}}\text{H}_3$) $_2$), 1.33 (s, 3H, C($\underline{\text{C}}\text{H}_3$) $_2$), 1.01 (t, J = 7.1 Hz, 3H, CO $_2$ CH $_2$ C $\underline{\text{H}}$ CH $_3$). ^{13}C NMR (151 MHz, CDCl $_3$) δ 206.44, 171.86, 168.50, 140.10, 137.80, 136.42, 132.86, 130.04, 129.39, 128.49, 128.39, 127.82, 127.67, 125.85, 109.76, 87.55 (C-2), 76.60, 73.03, 72.85, 70.48, 67.33, 64.69, 61.87, 38.20, 33.67, 30.03, 28.21, 26.97, 25.30, 21.38, 13.85. ESI HRMS (m/z): [$M + \text{Na}^+$] calcd for C $_{32}$ H $_{40}$ NaO $_9$ S, 623.2285 found 622.2290.

Ethyl [*p*-methylphenyl 4-*O*-benzyl-5-*O*-acetyl-7, 8-*O*-isopropylidene-3-deoxy-2-thio-D-*manno*-oct-ulopyranoside] onate (4b): Acetic anhydride (100

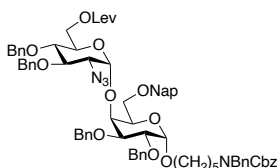


μL , 1.07 mmol) and 4-(dimethylamino)pyridine (DMAP, 8.5 mg, 0.07 mmol) were added to a solution of **S23** (180 mg, 0.35 mmol) in pyridine (5 mL). The reaction was stirred overnight at room temperature and was then concentrated under reduced pressure.

The resulting residue was dissolved in EtOAc (30 mL) and washed with brine (10 mL). The organic layer was dried (Na $_2$ SO $_4$), filtered, and the filtrate was concentrated *in vacuo*. The crude product was purified by silica gel column chromatography (PE/EA = 2/1, v/v) to give **4b**.⁴⁵ ^1H NMR (400 MHz, cdcl $_3$) δ 7.46 – 7.03 (m, 9H, Ar), 5.52 (dd, J = 5.6, 2.7 Hz, 1H, H-5), 4.72 (d, J = 11.7 Hz, 1H, PhCH $\underline{\text{H}}$), 4.42 (d, J = 11.7 Hz, 1H, PhC $\underline{\text{H}}$ H), 4.16 – 4.05 (m, 2H, H-7, H-8a), 4.04 – 3.92 (m, 2H, H-8b, CO $_2$ CH $\underline{\text{H}}$ CH $_3$), 3.78 (dq, J = 10.8, 7.2 Hz, 1H, CO $_2$ C $\underline{\text{H}}$ HCH $_3$), 3.52 – 3.41 (m, 1H, H-4), 3.35 (d, J = 8.1 Hz, 1H, H-6), 2.60 (dd, J = 12.6, 4.6 Hz, 1H, H-3 $_{\text{eq}}$), 2.32 (s, 3H, PhCH $_3$), 2.15 – 2.01 (m, 4H, Ac, H-3 $_{\text{ax}}$), 1.33 (s, 3H, C($\underline{\text{C}}\text{H}_3$) $_2$), 1.31 (s, 3H, C($\underline{\text{C}}\text{H}_3$) $_2$), 1.00 (td, J = 7.2, 1.3 Hz, 3H, CO $_2$ CH $_2$ C $\underline{\text{H}}$ CH $_3$). ^{13}C NMR (101 MHz, cdcl $_3$) δ 170.23, 168.49, 140.11, 137.83,

136.41, 129.43, 128.58, 127.88, 125.94, 109.83, 87.76, 76.65, 73.23, 73.00, 70.66, 67.41, 64.45, 61.92, 33.71, 27.01, 25.42, 21.43, 21.12, 13.91.

***N*-(Benzyl)-*N*-benzyloxycarbonyl-5-aminopentyl-2-azido-6-*O*-levulinoyl-2-*O*-deoxy-3, 4-di-*O*-benzyl- α -D-glucopyranosyl-(1 \rightarrow 4)-2,3-di-*O*-benzyl-6-*O*-(2-naphthyl) methyl- α -D-galactopyranoside (**7**):** Acceptor **6** (2.3 g, 2.8 mmol)

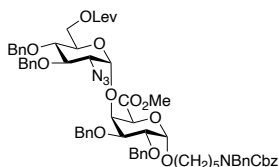


and donor **5** (1.1 g, 1.89 mmol) were co-evaporated with toluene (3×10 mL), and then dissolved in DCM (10 mL) and placed under an atmosphere of Argon. *N*-iodosuccinimide (NIS, 1 g, 4.72 mmol) and 4 Å molecular sieves (flame activated) were added to the

solution. The resulting mixture was cooled to 0 °C and stirred at this temperature for 15 min, which was followed by the addition of trimethylsilyl trifluoromethanesulfonate (85.5 μ L, 0.47 mmol). The reaction mixture was warmed slowly to room temperature and stirred for another 4 h, and then quenched with triethylamine (1 mL). After removing all solvents under reduced pressure, the resulting residue was dissolved in DCM (200 mL) and washed with brine and water. The organic layer was dried (Na_2SO_4), filtered, and the filtrate was concentrated *in vacuo*. The crude product was purified by silica gel column chromatography (PE/EA = 3/1, v/v) to give the disaccharide **7** (1.32 g, 55%, α only) as a light-yellow oil. ^1H NMR (400 MHz, Chloroform-*d*) δ 7.88 – 7.76 (m, 4H, Ar), 7.53 – 7.44 (m, 3H, Ar), 7.43 – 7.11 (m, 28H, Ar), 5.20 (d, J = 8.4 Hz, 2H, $\underline{\text{CH}_2}$ -Cbz), 4.99 (d, J = 3.7 Hz, 1H, Glc-H-1), 4.93 – 4.68 (m, 6H, Gal-H-1, $\text{ArCH}_2 \times 2$, ArCHH), 4.77 – 4.69 (m, 4H, ArCHH , ArCH_2 , ArCHH), 4.55 (d, J = 10.8 Hz, 1H, ArCHH), 4.49 (d, J = 11.1 Hz, 2H, ArCH_2), 4.39 (dd, J = 10.2, 2.3 Hz, 1H, Glc-H-5), 4.24 (d, J = 2.8 Hz, 1H, Gal-H-4), 3.99 – 3.85 (m, 6H, Gal-H-2, Gal-H-3, Gal-H-5, Gal-H-6a, Glc-H-3, Glc-H-6a), 3.74 (dd, J = 12.6, 2.3 Hz, 1H, Glc-H-6b), 3.67 – 3.50 (m, 3H, Gal-H-6b, Glc-H-4, CHH -Linker), 3.47 – 3.34 (m, 1H, CHH -Linker), 3.30 (dd, J = 10.3, 3.6 Hz, 1H, Glc-H-2), 3.27 – 3.13 (m, 2H, $\underline{\text{CH}_2}$ -Linker), 2.80 – 2.64 (m, 2H, $\underline{\text{CH}_2}$ -Lev), 2.58 – 2.47 (m, 2H, $\underline{\text{CH}_2}$ -

2.16 (s, 3H, $\underline{\text{CH}_3\text{-Lev}}$), 1.53 (m, 4H, $\underline{\text{CH}_2\text{-Linker}} \times 2$), 1.41 – 1.32 (m, 2H, $\underline{\text{CH}_2\text{-Linker}}$). ^{13}C NMR (101 MHz, cdCl_3) δ 206.36, 172.32, 138.45, 138.37, 137.96, 137.77, 128.66, 128.59, 128.56, 128.53, 128.50, 128.46, 128.19, 128.13, 128.10, 127.99, 127.92, 127.87, 127.83, 127.63, 127.48, 127.45, 127.26, 98.28 (Glc-C-1), 97.36 (Gal-C-1), 80.35, 78.06, 75.75, 75.53, 75.05, 74.73, 73.35, 72.94, 70.87, 69.24, 67.49, 67.22, 64.28, 62.41, 60.14, 50.40, 47.54, 37.90, 29.88, 28.97, 27.86, 27.10, 23.43, 14.28. ESI HRMS (m/z): $[\text{M} + \text{Na}^+]$ calcd for $\text{C}_{65}\text{H}_{74}\text{N}_4\text{NaO}_{14}$, 1157.5094 found 1157.5094.

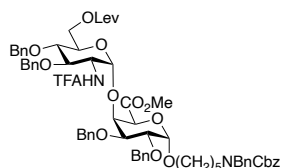
***N*-(Benzyl)-*N*-benzyloxycarbonyl-5-aminopentyl-2-deoxy-2-azido-6-*O*-levulinoyl-3, 4-di-*O*-benzyl- α -D-glucopyranosyl-(1 \rightarrow 4)-methyl (2,3-di-*O*-benzyl)- α -D-galactopyranosyluronate (9):** Compound **8** (1.8 g, 1.6 mmol) was



dissolved in a two-phase solvent of DCM (12 mL) and H_2O (6 mL), which was followed by the addition of 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO, 50 mg, 0.32 mmol) and (diacetoxyiodo)benzene (BAIB, 1.28 g, 4.0 mmol). After stirring for 6 h at room temperature, the reaction was quenched with aq. $\text{Na}_2\text{S}_2\text{O}_3$ (20%, 40 mL). The two-phase mixture was diluted with DCM (30 mL) and separated. The organic layer was dried (Na_2SO_4), filtered, and the filtrate was concentrated under reduced pressure. The resulting residue was co-evaporated 3 times with toluene before being dissolved in THF (16 mL). The solution was cooled to 0°C and was stirred for 15 min at this temperature, after which trimethylsilyldiazomethane (TMSCHN_2 , 2.0 M in ether, 4 mL, 8 mmol) was added dropwise via syringe over 15 min. After TLC analysis showed consumption of the starting material, the reaction was quenched with AcOH (5% in MeOH, 10 mL). The reaction mixture was concentrated *in vacuo*, and the resulting residue was purified by flash chromatography over silica gel (PE/EA = 1/1, v/v) to give **9** (1.5 g, 80% over 2 steps) as a light-yellow oil. ^1H NMR (400 MHz, $\text{Chloroform-}d$) δ 7.46 – 7.14 (m, 30H, Ar), 5.20 (d, $J = 9.0$ Hz, 2H, $\underline{\text{CH}_2\text{-Cbz}}$), 4.97 (d, $J = 3.6$ Hz, 1H, Glc-H-1),

4.92 (bs, 1H, GalA-H-1), 4.88 (d, $J = 2.5$ Hz, 2H, PhCH₂), 4.85 (d, $J = 3.4$ Hz, 1H, PhCHH), 4.82 – 4.79 (m, 3H, CHH, PhCH₂), 4.71 (d, $J = 12.2$ Hz, 1H, PhCHH), 4.57 (d, $J = 10.6$ Hz, 1H, PhCHH), 4.55 – 4.48 (m, 3H, GalA-H-4, PhCH₂), 4.33 (split by Cbz, 1H, GalA-H-5), 4.31 – 4.24 (m, 1H, Glc-H-5), 4.06 (dd, $J = 12.3, 2.3$ Hz, 1H, Glc-H-6a), 3.94 – 3.88 (m, 3H, GalA-H-3, GalA-H-2, Glc-H-3), 3.85 (s, 3H, GalA-CO₂CH₃), 3.77 (d, $J = 12.3$ Hz, 1H, Glc-H-6b), 3.61 (t, $J = 9.5$ Hz, 1H, Glc-H-4), 3.68 – 3.54 (m, 1H, CHH-Linker), 3.45 (q, $J = 7.0, 6.5$ Hz, 1H, CHH-Linker), 3.39 (dd, $J = 10.3, 3.5$ Hz, 1H, Glc-H-2), 3.26 – 3.12 (m, 2H, CH₂-Linker), 2.71 (qt, $J = 18.3, 6.4$ Hz, 2H, CH₂-Lev), 2.53 (td, $J = 6.4, 2.1$ Hz, 2H, CH₂-Lev), 2.17 (s, 3H, CH₃-Lev), 1.63 – 1.44 (m, 4H, CH₂-Linker \times 2), 1.40 – 1.19 (m, 2H, CH₂-Linker). ¹³C NMR (101 MHz, cdcl₃) δ 206.17, 172.18, 169.06, 138.04, 137.99, 137.92, 137.89, 128.53, 128.48, 128.47, 128.45, 128.43, 128.35, 128.24, 128.04, 128.02, 127.97, 127.93, 127.90, 127.88, 127.82, 127.75, 127.52, 127.29, 127.15, 99.00 (Glc-C-1), 97.45 (GalA-C-1), 80.29, 77.70, 76.20, 75.42, 75.03, 74.44, 73.16, 73.05, 70.06, 69.51, 68.65, 67.15, 63.90, 62.15, 52.58, 37.79, 29.79, 29.01, 27.74, 23.32. ESI HRMS (m/z): [M + Na⁺] calcd for C₆₆H₇₄N₄NaO₁₅, 1185.5043 found 1185.5050.

***N*-(Benzyl)-*N*-benzyloxycarbonyl-5-aminopentyl-2-deoxy-2-trifluoroacetamido-6-*O*-levulinoyl-3, 4-di-*O*-benzyl- α -D-glucopyranosyl-(1 \rightarrow 4)-methyl (2,3-di-*O*-benzyl)- α -D-galactopyranosyluronate (10):** To a

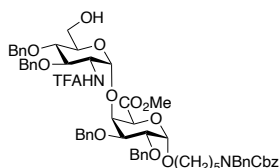


solution of **9** (315 mg, 0.27 mmol) in THF/AcOH (8 mL/2 mL) was added Zn (washed by 1.0 M HCl, H₂O and acetone sequentially, 0.5 g). The reaction suspension was stirred overnight at room temperature, after which it was filtered, and the filtrate was

concentrated *in vacuo*. The resulting residue was co-evaporated with toluene (4 \times 5 mL), and then was dissolved in dry THF (5 mL). The solution was cooled to 0 °C, and then added trifluoroacetic anhydride (375 μ L, 2.71 mmol). After stirring for 1 h at 0 °C, the reaction mixture was diluted with toluene (20 mL) and was

concentrated under reduced pressure. The resulting residue was dissolved in EtOAc (30 mL) and washed with aq. sat. NaHCO₃ (10 mL), the organic layer was dried (Na₂SO₄), filtered, and the filtrate was concentrated *in vacuo*. The crude product was purified by flash chromatography over silica gel (PE/EA = 1.5/1, v/v) to give **10** (264 mg, 79%) as a colorless oil. ¹H NMR (600 MHz, Chloroform-*d*) δ 7.44 – 7.09 (m, 30H), 6.62 (t, *J* = 10.2 Hz, 1H, CF₃CONH), 5.16 (d, *J* = 18.4 Hz, 2H, CH₂-Cbz), 4.89 (bs, 1H, GalA-H-1), 4.84 – 4.66 (m, 7H, PhCH₂ × 3, Glc-H-1), 4.61 (dd, *J* = 11.0, 4.6 Hz, 1H, PhCHH), 4.54 (dd, *J* = 10.6, 4.2 Hz, 1H, PhCHH), 4.47 (d, *J* = 15.5 Hz, 2H, PhCH₂), 4.40 (s, 1H, GalA-H-4), 4.32 – 4.25 (m, 2H, GalA-H-5, Glc-H-5), 4.25 – 4.18 (m, 1H, Glc-H-2), 3.99 – 3.93 (m, 1H, Glc-H-6a), 3.89 (d, *J* = 11.5 Hz, 1H, GalA-H-3), 3.84 – 3.78 (m, 2H, GalA-H-2, Glc-H-6b), 3.74 – 3.65 (m, 2H, Glc-H-3, Glc-H-4), 3.65 (s, 3H, CO₂CH₃), 3.63 – 3.50 (m, 1H, CHH-Linker), 3.46 – 3.33 (m, 1H, CHH-Linker), 3.25 – 3.12 (m, 2H, CH₂-Linker), 2.71 (qt, *J* = 18.3, 6.4 Hz, 2H, CH₂-Lev), 2.53 (q, *J* = 6.5 Hz, 2H, CH₂-Lev), 2.16 (s, 3H, CH₃-Lev), 1.61 – 1.45 (m, 4H, CH₂-Linker × 2), 1.32 – 1.13 (m, 2H, CH₂-Linker). ¹³C NMR (151 MHz, CDCl₃) δ 206.33, 172.38, 138.10, 137.93, 137.65, 128.67, 128.61, 128.57, 128.36, 128.09, 128.06, 128.04, 128.00, 127.95, 127.67, 127.28, 98.10 (Glc-C-1), 97.51 (GalA-C-1), 79.29, 77.83, 76.14, 75.55, 75.20, 73.62, 73.10, 70.17, 69.47, 68.97, 67.30, 62.05, 53.39, 52.46, 37.94, 29.94, 29.15, 27.90, 23.42. ESI HRMS (*m/z*): [*M* + Na⁺] calcd for C₆₈H₇₅F₃N₂NaO₁₆, 1255.4961 found 1255.4958.

***N*-(Benzyl)-*N*-benzyloxycarbonyl-5-aminopentyl-2-deoxy-2-trifluoroacetamido-3, 4-di-*O*-benzyl-α-D-glucopyranosyl-(1→4)-methyl (2,3-di-*O*-benzyl)-α-D-galactopyranosyl-uronate (**11**):** Hydrazine acetate (35 mg,

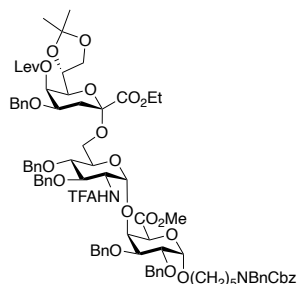


0.374 mmol) was added to the solution of **10** (230 mg, 0.187 mmol) in DCM/MeOH (2 mL/0.2 mL), and the mixture was stirred for 2 h at room temperature. After

TLC analysis showed consumption of the starting material, the reaction mixture was concentrated *in vacuo*. The resulting residue

was dissolved in DCM (50 mL) and washed with water (3 × 30 mL), and the organic phase was dried (Na₂SO₄), filtered, and the filtrate was concentrated *in vacuo*. The resulting crude product was purified by silica gel column chromatography (DCM/acetone, 15/1, v/v) to give the disaccharide acceptor **11** (175 mg, 83%) as a colorless oil. ¹H NMR (600 MHz, Chloroform-*d*) δ 7.30 (m, 30H, Ar), 6.69 (d, *J* = 10.2 Hz, 1H, TFANH), 4.88 (bs, GalA-H-1), 4.85 – 4.77 (m, 5H, Glc-H-1, CH₂-Cbz, PhCH₂), 4.73 (d, *J* = 11.9 Hz, 1H, PhCHH), 4.67 (t, *J* = 14.0 Hz, 2H, PhCHH, PhCHH), 4.61 (d, *J* = 11.0 Hz, 1H, PhCHH), 4.48 (d, *J* = 15.2 Hz, 2H, PhCH₂), 4.40 (bs, 1H, GalA-H-4), 4.30 (split by Cbz, GalA-H-5), 4.19 (td, *J* = 9.6, 3.4 Hz, 1H, Glc-H-2), 4.12 (d, *J* = 8.0 Hz, 1H, Glc-H-5), 3.87 (td, *J* = 9.4, 2.8 Hz, 1H, GalA-H-3), 3.82 (dd, *J* = 10.2, 2.8 Hz, 1H, GalA-H-2), 3.66 (s, 3H, CO₂CH₃), 3.68 – 3.66 (m, 2H, Glc-H-3, Glc-H-4), 3.64 – 3.51 (m, 1H, CHH-Linker), 3.46 – 3.33 (m, 3H, Glc-H-6, CHH-Linker, CHH-Linker), 3.28 – 3.12 (m, 2H, CH₂-Linker), 1.60 – 1.48 (m, 4H, CH₂-Linker × 2), 1.29 – 1.22 (m, 2H, CH₂-Linker). ¹³C NMR (151 MHz, CDCl₃) δ 169.15, 138.09, 137.79, 128.68, 128.66, 128.64, 128.61, 128.57, 128.22, 128.14, 128.08, 128.04, 127.95, 127.60, 127.48, 127.28, 98.22 (Glc-C-1), 97.58 (GalA-C-1), 79.18, 77.91, 76.44, 75.45, 75.31, 75.14, 73.60, 73.22, 72.38, 69.56, 68.98, 67.30, 60.95, 53.64, 52.55, 29.16, 27.59, 23.45. ESI HRMS (*m/z*): [M + Na⁺] calcd for C₆₃H₆₉F₃N₂NaO₁₄, 1157.4593 found 1157.4594.

***N*-(Benzyl)-*N*-benzyloxycarbonyl-5-aminopentyl-ethyl (4-*O*-benzyl-5-*O*-levulinoyl-7,8-*O*-isopropylidene-3-deoxy- α -D-manno-oct-2-
ulopyranoside)onate(2→6)-2-deoxy-2-trifluoroacetamido-3, 4-di-*O*-benzyl-**



α -D-glucopyranosyl-(1→4)-methyl (2, 3-di-*O*-benzyl)- α -D-galactopyranosyl urinate (12a):

Disaccharide acceptor **11** (86 mg, 0.076 mmol) and Kdo donor **4a** (92 mg, 0.152 mmol) were co-evaporated with toluene (3 × 10 mL), and were dissolved in dry acetonitrile (2 mL) and placed under

an atmosphere of Argon. *N*-iodosuccinimide (NIS, 52 mg, 0.228 mmol) and 4 Å molecular sieves (flame-activated) were added. The reaction mixture was cooled to 0 °C and kept stirred for 15 min, after which trifluoromethanesulfonic acid (TfOH, 1.3 µL, 0.015 mmol) was added. The reaction was stirred for 5 h at 0 °C and then quenched with triethylamine (0.5 mL). The molecular sieves were filtered off, and the filtrate was concentrated under reduced pressure. The resulting residue was dissolved in EtOAc (20 mL) and washed with brine (10 mL), and the organic layer was dried (Na₂SO₄), filtered, and the filtrate was concentrated in *vacuo*. The resulting yellow oil was purified by flash chromatography over silica gel (toluene/EA, 3/1, v/v) to give the trisaccharide **12a** (88 mg, 72% of α anomer, $\alpha:\beta = 4:1$) as a light-yellow oil. **α -anomer:** ¹H NMR (600 MHz, CDCl₃) δ 7.39 – 7.10 (m, 35H, Ar), 6.56 (d, $J = 8.5$ Hz, 1H, GlcNHTFA), 5.52 – 5.44 (m, 1H, Kdo H-5), 5.16 (d, $J = 17.0$ Hz, 2H, CH₂-Cbz), 4.85 (d, $J = 3.5$ Hz, 1H, GalA H-1), 4.79 (d, $J = 12.1$ Hz, 1H, PhCHH), 4.75 (d, $J = 12.4$ Hz, 1H, PhCHH), 4.75 – 4.72 (m, 2H, GlcNTFA H-1, PhCHH), 4.71 – 4.60 (m, 4H, PhCHH, PhCH₂, PhCHH), 4.56 (d, $J = 10.9$ Hz, 1H, PhCHH), 4.52 – 4.43 (m, 3H, PhCH₂, PhCHH), 4.39 (dd, $J = 14.5, 11.5$ Hz, 1H, PhCHH), 4.37 – 4.32 (m, 1H, GalA H-4), 4.30 – 4.26 (m, 1H, GlcNTFA H-5), 4.24 – 4.10 (m, 4H, GalA H-5, GlcNTFA H-2, CO₂CH₂CH₃), 4.11 – 4.05 (m, 1H, Kdo H-7), 4.00 (dd, $J = 8.5, 6.2$ Hz, 1H, Kdo H-8a), 3.83 – 3.78 (m, 2H, Kdo H-4, GalA H-3), 3.76 (dd, $J = 10.1, 3.5$ Hz, 1H, GalA H-2), 3.72 (dd, $J = 8.5, 5.8$ Hz, 1H, Kdo H-8b), 3.65 (m, 4H, CO₂CH₃, GlcNTFA H-3), 3.62 – 3.46 (m, 4H, Kdo H-6, GlcNTFA H-4, GlcNTFA H-6a, CHH-Linker), 3.41 (m, 2H, GlcNTFA H-6b, CHH-Linker), 3.27 – 3.12 (m, 2H, CH₂-Linker), 2.80 – 2.55 (m, 4H, CH₂-Lev \times 2), 2.22 (dd, $J = 13.1, 4.8$ Hz, 1H, Kdo H-3_{eq}), 2.14 (s, 3H, CH₃-Lev), 1.92 (t, $J = 12.5$ Hz, 1H, Kdo H-3_{ax}), 1.61 – 1.45 (m, 4H, CH₂-Linker \times 2), 1.37 – 1.17 (m, 11H, C(CH₃)₂, CO₂CH₂CH₃, CH₂-Linker). ¹³C NMR (151 MHz, CDCl₃) δ 206.29, 177.15, 176.72, 172.43, 172.00, 171.57, 169.00, 167.29, 166.00, 157.86, 156.57, 147.14, 138.57, 138.14, 137.72, 137.72, 137.29, 136.86, 129.14, 128.29,

127.86, 127.43, 127.00, 112.43 , 109.43, 109.00, 108.57, 106.00, 98.71, 97.43, 79.00, 79.00, 78.14, 77.71, 77.28, 76.86, 76.43, 76.00, 75.57, 75.14, 74.71, 73.43, 73.00, 72.57, 72.14, 70.86, 70.43, 65.71, 65.28, 64.86, 62.28, 61.43, 58.43, 53.28, 52.43, 47.28, 38.28, 37.85, 34.85, 33.14, 31.85, 31.43, 30.14, 29.71, 29.28, 28.00, 26.71, 25.43, 23.28, 22.85, 22.00, 14.28, 13.85. EXSIDE NMR (Figure S1.) was employed to measure the glycosidic linkage between Kdo and GlcNHTFA by calculate the coupling constant between H3_{ax} and C1 of Kdo, and the value is less than 1 Hz for α -linked Kdo.^{29, 38} ESI HRMS (m/z): [M + Na⁺] calcd for C₈₈H₁₀₁F₃N₂NaO₂₃, 1633.6639 found 1633.6638.

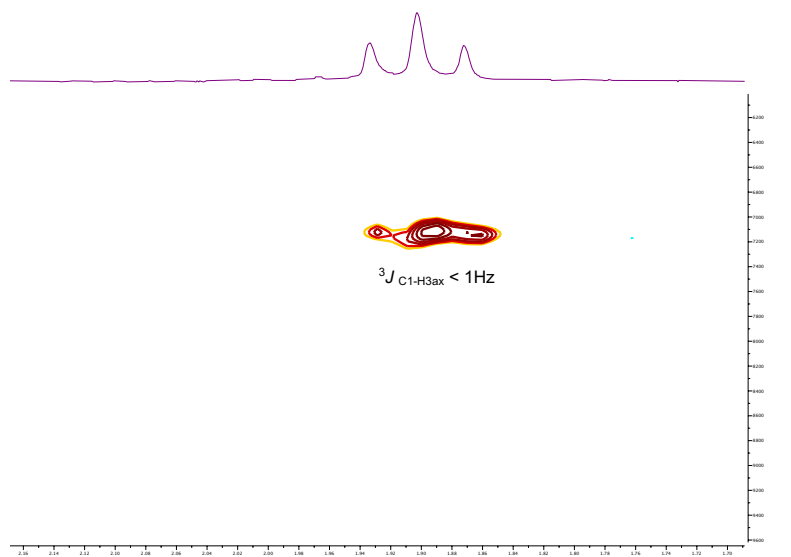
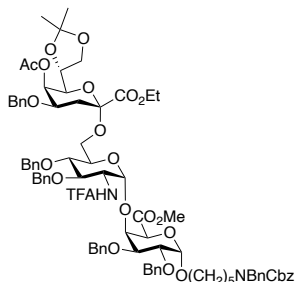


Figure S1. EXSIDE NMR of trisaccharide **12a**.

***N*-(Benzyl)-*N*-benzyloxycarbonyl-5-aminopentyl-ethyl (4-*O*-benzyl-5-*O*-acetyl-7,8-*O*-isopropylidene-3-deoxy- α -D-manno-oct-2-ulopyranoside)onate(2 \rightarrow 6)-2-deoxy-2-trifluoroacetamido-3, 4-di-*O*-benzyl- α -D-glucopyranosyl-(1 \rightarrow 4)-methyl (2, 3-di-*O*-benzyl)- α -D-galactopyranosyl urinate (**12b**):** Kdo donor **4b** (73.7 mg, 0.065 mmol) was glycosylated with



disaccharide acceptor **11** (71.2 mg, 0.13 mmol) to give **12b** (71 mg, 70% of α anomer, $\alpha:\beta = 3.7:1$)

following the procedure for preparation of **12a**. α -

anomer of 12b: ^1H NMR (400 MHz, cdCl_3) δ 7.47 – 7.10 (m, 35H, Ar), 6.49 (d, $J = 9.9$ Hz, 1H, GlcNHTFA), 5.49 (t, $J = 1.7$ Hz, 1H, Kdo H-5), 5.16 (d, $J = 9.0$ Hz, 2H, $\text{CH}_2\text{-Cbz}$), 4.86 (bs, 1H, GalA H-

1), 4.82 – 4.75 (m, 2H, PhCH_2), 4.74 – 4.69 (m, 5H, Glc H-1, $\text{PhCH}_2 \times 2$), 4.55 (d, $J = 10.9$ Hz, 1H, PhCHH), 4.51 – 4.42 (m, 3H, PhCHH , PhCH_2), 4.40 (dd, $J = 15.5, 11.4$ Hz, 2H, PhCH_2), 4.33 (d, $J = 2.8$ Hz, 1H, GalA H-4), 4.29 – 4.20 (m, 2H, GlcNHTFA H-5, GalA H-5), 4.20 – 4.05 (m, 4H, GlcNHTFA H-2, Kdo H-7, $\text{CO}_2\text{CH}_2\text{CH}_3$), 3.97 (dd, $J = 8.7, 6.1$ Hz, 1H, Kdo H-8a), 3.86 – 3.78 (m, 2H, Kdo H-4, GalA H-3), 3.74 (m, 2H, GalA H-2, Kdo H-8b), 3.66 (s, 3H, CO_2CH_3), 3.63 (d, $J = 10.2$ Hz, 1H, GlcNHTFA H-3), 3.60 – 3.48 (m, 4H, Kdo H-6, GlcNHTFA H-4, GlcNHTFA H-6a, CHH-Linker), 3.41 – 3.38 (m, 2H, GlcNHTFA H-6b, CHH-Linker), 3.27 – 3.14 (m, 2H, $\text{CH}_2\text{-Linker}$), 2.22 (dd, $J = 13.1, 4.7$ Hz, 1H, Kdo H-3_{eq}), 2.11 (s, 3H, Ac), 1.94 (t, $J = 12.6$ Hz, 1H, Kdo H-3_{ax}), 1.52 (m, 4H, $\text{CH}_2\text{-Linker} \times 2$), 1.33 – 1.19 (m, 11H, $\text{C}(\text{CH}_3)_2$, $\text{CO}_2\text{CH}_2\text{CH}_3$, $\text{CH}_2\text{-Linker}$). ^{13}C NMR (101 MHz, cdCl_3) δ 178.17, 170.15, 169.23, 167.23, 138.01, 137.85, 128.68, 128.66, 128.63, 128.12, 128.04, 127.97, 127.73, 127.37, 109.46, 98.91, 97.62, 97.37, 79.19, 78.26, 76.46, 75.50, 75.10, 74.93, 73.57, 73.26, 72.93, 72.58, 71.18, 70.78, 70.49, 69.31, 69.01, 67.32, 66.99, 64.97, 62.38, 61.67, 53.43, 52.54, 50.41, 33.32, 29.20, 26.74, 25.56, 23.43, 21.09, 14.22.

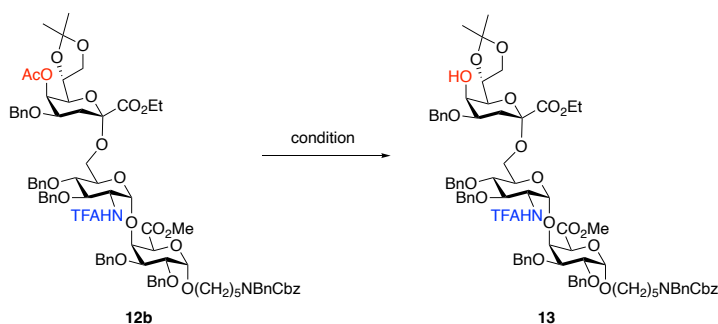
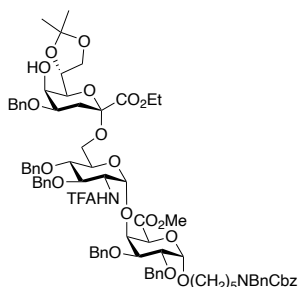


Table S2. Conditions of cleavage of acetyl group.

Entry	Conditions	Result
1	NaOMe/MeOH, pH = 10, 2 h	TFA- removed, Ac-remained
2	NaOMe/MeOH, pH = 8, 3 h	TFA- removed, Ac-remained
3	1 equiv. <i>t</i> -BuOK in EtOH, overnight	~70% 12b and ~30% 13
4	Et ₃ N/MeOH = 1/1, reflux, 3 h	no reaction
5	0.1M NaOMe in MeOH (30 μ L)/MeOH (1 mL), 24 h	54% 13 , TFA- removed (46%)

*Different conditions were examined to remove the acetyl group of **12b**, however, the TFA protecting group of glucosamine is even less stable than the acetyl of Kdo.

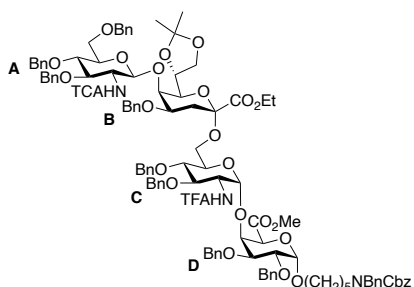
***N*-(Benzyl)-*N*-benzyloxycarbonyl-5-aminopentyl-ethyl (4-*O*-benzyl-7, 8-*O*-isopropylidene-3-deoxy- α -D-*manno*-oct-2-ulopyranoside)onate(2 \rightarrow 6)-2-deoxy-2-trifluoroacetamido-3, 4-di-*O*-benzyl- α -D-glucopyranosyl-(1 \rightarrow 4)-methyl (2,3-di-*O*-benzyl)- α -D-galactopyranosyl urinate (**13**):**



Hydrazine acetate (8.6 mg, 0.094 mmol) was added to a solution of **12a** (76 mg, 0.047 mmol) in DCM/MeOH (3 mL/0.3 mL). The reaction mixture was stirred at room temperature for 2 h, after which all solvents were removed under reduced pressure, and the resulting residue was dissolved in DCM (10 mL) and

washed with H₂O (10 mL). The organic layer was dried (Na₂SO₄), filtered, and the filtrate was concentrated in *vacuo*. The crude product was purified by flash chromatography over silica gel (DCM/acetone, 15/1, v/v) to give the trisaccharide acceptor **13** (71 mg, 99%) as a colorless oil. ¹H NMR (600 MHz, Chloroform-*d*) δ 7.43 – 7.09 (m, 35H, Ar), 6.72 (s, 1H, TFANH), 5.16 (d, *J* = 17.9 Hz, 2H, CH₂-Cbz), 4.85 (bs, 1H, GalA-H-1), 4.83 – 4.76 (m, 1H, PhCHH), 4.76 – 4.63 (m, 6H, PhCHH, PhCH2 × 2, Glc-H-1), 4.60 – 4.52 (m, 3H, PhCH2, PhCHH), 4.47 (d, *J* = 16.2 Hz, 2H, PhCHH, PhCHH), 4.41 (d, *J* = 11.0 Hz, 1H, PhCHH), 4.34 (q, *J* = 6.2, 5.5 Hz, 2H, GalA-H-4, Kdo -H-7), 4.30 – 4.19 (m, 3H, GalA-H-5, Glc-H-5, Glc-H-2), 4.15 (q, *J* = 7.2 Hz, 2H, CO₂CH2CH₃), 4.04 (dd, *J* = 8.8, 6.3 Hz, 1H, Kdo -H-8a), 4.00 (s, 1H, Kdo -H-5), 3.83 (d, *J* = 9.9 Hz, 1H, GalA-H-3), 3.78 (dd, *J* = 10.0, 3.5 Hz, 2H, GalA-H-2, Kdo -H-4), 3.73 (dd, *J* = 9.0, 6.1 Hz, 1H, Kdo H-8b), 3.66 (s, 3H, CO₂CH3), 3.69 – 3.63 (overlapping signal, 1H, Glc-H-3), 3.61 (d, *J* = 9.4 Hz, 1H, Glc-H-4), 3.58 – 3.48 (m, 1H, CHH-Linker), 3.51 (d, *J* = 11.3 Hz, 1H, Glc-H-6a), 3.42 (d, *J* = 11.3 Hz, 1H, Glc-H-6b), 3.45 – 3.34 (m, 2H, Kdo H-6, CHH-Linker), 3.25 – 3.12 (m, 2H, CH₂-Linker), 2.25 (dd, *J* = 13.1, 4.8 Hz, 1H, Kdo H_{eq}-3), 1.96 (t, *J* = 13.06 Hz, 1H, Kdo H_{ax}-3), 1.62 – 1.45 (m, 4H, CH₂-Linker × 2), 1.34 (s, 3H, C(CH₃)₂), 1.30 – 1.17 (m, 8H, C(CH₃)₂, CH₂-Linker, CO₂CH₂CH₃). ¹³C NMR (151 MHz, CDCl₃) δ 169.12, 167.36, 138.17, 138.01, 137.71, 137.53, 128.71, 128.65, 128.60, 128.58, 128.54, 128.18, 128.05, 127.99, 127.95, 127.92, 127.86, 127.77, 127.66, 127.37, 127.25, 109.25, 98.95, 97.63, 97.56, 79.13, 78.10, 76.27, 75.44, 75.23, 74.93, 74.81, 73.71, 73.34, 73.19, 72.89, 72.25, 70.80, 70.02, 69.35, 68.95, 67.28, 64.27, 62.21, 61.60, 53.43, 52.48, 31.65, 29.64, 29.13, 26.66, 25.44, 23.38, 14.20. ESI HRMS (*m/z*): [*M* + Na⁺] calcd for C₈₈H₁₀₁F₃N₂NaO₂₁, 1535.6272 found 1535.6274.

***N*-(Benzyl)-*N*-benzyloxycarbonyl-5-aminopentyl-2-deoxy-2-trichloroacetamido-3,4,6-tri-*O*-benzyl- β -D-glucopyranosyl-(1 \rightarrow 5)-ethyl (4-*O*-benzyl-7, 8-*O*-isopropylidene-3-deoxy- α -D-manno-oct-2-ulopyranoside)onate(2 \rightarrow 6)-2-deoxy-2-trifluoroacetamido-3, 4-di-*O*-benzyl- α -D-glucopyranosyl-(1 \rightarrow 4)-methyl (2,3-di-*O*-benzyl)- α -D-galactopyranosyl urinate (**14**):** Trisaccharide acceptor **13** (71 mg, 0.047 mmol) and donor **3** (65

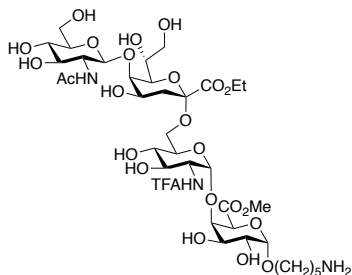


mg, 0.94 mmol) were co-evaporated with toluene (10 mL \times 3) and then dissolved in dry DCM/MeCN (1.2 mL/0.3 mL). *N*-iodosuccinimide (NIS, 32 mg, 0.143 mmol) and 4 Å molecular sieves (flame-activated) were added and the solution was placed under an atmosphere of Argon. The reaction

mixture was cooled to 0 °C and kept stirred for 15 min, after which trifluoromethanesulfonic acid (TfOH, 0.8 μ L, 0.0094 mmol) was added. The reaction was stirred for 5 h at 0 °C and then quenched with triethylamine (0.5 mL). The molecular sieves were filtered off, and the filtrate was concentrated under reduced pressure. The resulting residue was dissolved in EtOAc (20 mL) and washed with brine (10 mL), and the organic layer was dried (Na₂SO₄), filtered, and the filtrate was concentrated *in vacuo*. The resulting yellow oil was purified by flash chromatography over silica gel (toluene/EA, 2.5/1, v/v) to give the tetrasaccharide **14** (55 mg, 56%) as a white solid. ¹H NMR (600 MHz, Chloroform-*d*) δ 7.38 – 7.10 (m, 50H, Ar), 6.90 (d, *J* = 8.1 Hz, 1H, A-TCANH), 6.67 (d, *J* = 9.7 Hz, 1H, C-TFANH), 5.16 (d, *J* = 16.8 Hz, 2H, CH₂-Cbz), 4.86 (bs, 1H, D-H-1), 4.81 – 4.75 (m, 4H, A-H-1, C-H-1, PhCH₂), 4.75 – 4.64 (m, 6H, PhCH₂ \times 3), 4.64 – 4.50 (m, 6H, PhCH₂ \times 3), 4.47 (d, *J* = 15.4 Hz, 2H, PhCH₂), 4.41 (d, *J* = 11.3 Hz, 2H, PhCH₂), 4.33 (s, 1H, D-H-4), 4.31 – 4.21 (m, 4H, C-H-5, A-H-5, C-H-2, B-H-7), 4.12 – 4.05 (m, 3H, CO₂CH₂CH₃, B-H-8a), 3.94 (s, 1H, B-H-5), 3.92 – 3.84 (m, 2H, B-H-8b, A-H-2), 3.86 – 3.74 (m, 3H, B-H-4, D-H-3,

D-H-2), 3.74 – 3.69 (m, 2H, A-H-6a/b), 3.69 – 3.64 (m, 5H, C-H-3, C-H-4, CO₂CH₃), 3.62 – 3.59 (m, 2H, B-H-6, C-H-6a), 3.59 – 3.45 (m, 5H, A-H-4, A-H-3, C-H-6b, CHH-Linker), 3.41 – 3.35 (m, 1H, CHH-Linker), 3.23 – 3.14 (m, 2H, CH₂-Linker), 2.28 (dd, $J = 13.4, 4.6$ Hz, 1H, B-H_{eq}-3), 2.02 (t, $J = 12.4$ Hz, 1H, B-H_{ax}-3), 1.58 – 1.45 (m, 4H, CH₂-Linker $\times 2$), 1.31 (s, 3H, C(CH₃)₂), 1.27 – 1.14 (m, 8H, CH₂-Linker, C(CH₃)₂, CO₂CH₂CH₃). ¹³C NMR (151 MHz, CDCl₃) δ 168.84, 167.13, 161.56, 161.13, 137.99, 137.56, 128.13, 127.70, 108.42, 100.71, 98.56, 97.78, 75.85, 74.99, 74.56, 73.28, 72.73, 72.43, 71.14, 69.86, 68.99, 65.99, 61.28, 57.00, 53.14, 32.14, 29.76, 28.96, 27.67, 26.22, 26.07, 23.32, 14.14. ESI HRMS (m/z): [M + Na⁺] calcd for C₁₁₂H₁₂₃Cl₃F₃N₃NaO₂₆, 2110.7305 found 2110.7309.

5-Amino-pentyl-2-deoxy-2-acetamine- β -D-glucopyranosyl-(1 \rightarrow 5)-ethyl (3-deoxy- α -D-manno-oct-2-ulopyranoside)onate-(2 \rightarrow 6)-2-deoxy-2-trifluoroacetamido- α -D-glucopyranosyl-(1 \rightarrow 4)-methyl- α -D-galactopyranosyl urinate (15): To a cooled (0 °C) solution of **14** (34 mg, 0.016



mmol) in DCM (1.8 mL) was added aqueous TFA (60 %, 0.2 mL, 0 °C). The reaction mixture was stirred for 2 h at 0 °C, after which it was diluted with toluene (10 mL) and concentrated under reduced pressure. The resulting residue was co-evaporated 3 times with toluene (10 mL), and the crude product was used for next step

without additional purification.

To a solution of the crude product obtained above in *t*-BuOH/H₂O (4 mL/1 mL) was added digussa type Pd(OH)₂/C (34 mg), and the reaction mixture was stirred under an atmosphere of H₂ (1 atm) for 24 h at room temperature. The reaction was filtered, and the filtrate was concentrated under reduced pressure. The resulting crude product **15** was used for next step without further purification.

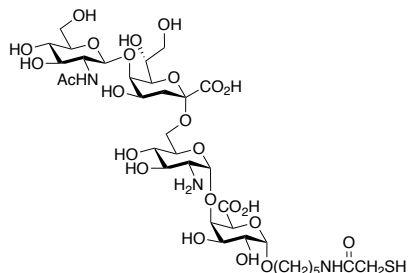
Table S3. ^1H NMR spectrum of **15**

	H1	H2	H3	H4	H5	H6(a)	H7(6b)
βGlcNAc	4.72 (d, J = 8.5 Hz)	3.79	3.57	3.47	3.50	3.96	3.75
αKdo	---	---	1.81 ^{ax} /2.16 ^{eq}	4.24	4.17	3.67	4.06
αGlcN	4.88 (d, J = 3.9 Hz)	3.96	3.83	3.59	4.34	3.78	3.42
αGalA	4.96 (d, J = 3.9 Hz)	3.89	4.00	4.43	4.63	---	---
Linker	1.43(x2), 1.65(x2), 1.67(x2), 2.98(x2), 3.55, 3.71						
Other	4.31/4.34(CH_2CH_3), 1.33(CH_2CH_3), 3.64(CO_2CH_3), 3.59/3.92(H8 Kdo)						

Table S4. ^{13}C NMR spectrum of **15**.

	C1	C2	C3	C4	C5	C6	C7	C8
βGlcNAc	102.36	55.52	73.71	70.17	75.48	60.83	---	---
αKdo	---	---	33.63	65.66	73.87	71.78	69.04	62.61
αGlcN	96.89	54.40	70.33	69.53	70.17	61.56	---	---
αGalA	98.50	67.60	68.08	77.25	69.37	---		
Linker	22.36, 26.39, 28.15, 39.26, 68.56							
Other	63.41(CH_2CH_3), 13.35(CH_2CH_3), 22.36(Ac), 52.79(CO_2CH_3)							

***N*-(2-mercaptoacetamide)-5-carboxylamino-pentyl-2-deoxy-2-acetamine- β -D-glucopyranosyl-(1 \rightarrow 5)-3-deoxy- α -D-manno-oct-2-ulopyranosid-(2 \rightarrow 6)-2-deoxy-2-amino- α -D-glucopyranosyl-(1 \rightarrow 4)- α -D-galactopyranosyluronic**



acid(1): The tetrasaccharide **15** was co-evaporated with toluene (3 \times 10 mL) before dissolved in dry DMF (0.5 mL) and the solution was placed under an atmosphere of Argon, which was followed by the addition of S-acetylthioglycolic acid

pentafluorophenyl ester (SAMA-OPfp, 6.2 mg, 19.9 μ mol) and *N,N*-diisopropylethylamine (DIPEA, 3.48 μ L, 19.9 μ mol). The reaction was stirred for 3 h at room temperature, after which it was quenched with water (0.2 mL). The resulting mixture was concentrated in *vacuo*, and the residue was dissolved in MeOH (0.5 mL). The solution was cooled to 0 °C, and aqueous 0.2 M NaOH (0.5 mL) was added. The reaction was stirred for 3 h at 0 °C, after which it was neutralized with Amberlite (H⁺, washed with MeOH, aq. 1 M NaOH, H₂O, aq. 1 M HCl, H₂O and MeOH sequentially) till pH = 7. The resin was filtered off, and the filtrate was treated with D,L-dithiothreitol (DTT, 0.2 mg). After stirred for 30 min at 40 °C, the reaction mixture was concentrated under reduced pressure, and the resulting residue was purified by bio-gel P2 column using 5% *n*-BuOH in H₂O (de-oxygen before use) as eluent to give the final product **1** as white powder (4.5 mg, 30% over 4 steps). ESI HRMS (m/z): [M + Na⁺] calcd for C₃₅H₅₉N₃NaO₂₄S, 960.3101 found 960.3127.

Table S5. ¹H NMR spectrum of **1**.

	H1	H2	H3	H4	H5	H6(a)	H7(6b)	H8(Ac)
βGlcNAc	4.76	3.76	3.61	3.48	3.52	3.77	3.95	2.02
αKdo	—	—	1.70/2.12	4.24	4.16	3.61	4.06	3.6/3.97
αGlcN	5.18	3.33	3.87	3.55	4.30	3.43	3.62	—
αGalA	5.01	3.88	4.03	4.39	4.30	—	—	—
Linker	1.40	1.56	1.64	3.25	3.72	3.58	3.47 (CH ₂ SH)	

*Disulfide of compound **1**.

Table S6. C¹³ NMR spectrum of **1**. (Collected from the F1 dimension in the HSQC spectra.)

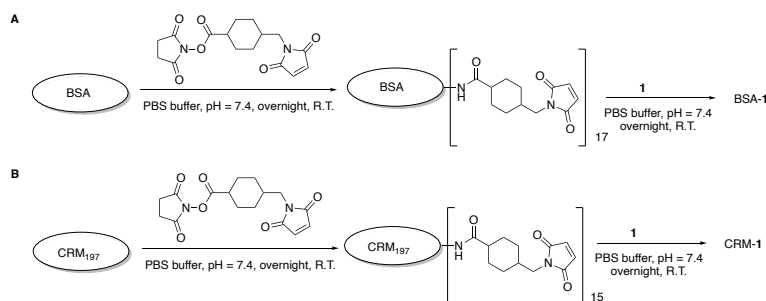
	C1	C2	C3	C4	C5	C6	C7(Ac)	C8
βGlcNAc	102.2	55.8	73.7	70.2	75.6	60.8	22.3	
αKdo	—	—	34.2	66.0	74.3	71.3	69.3	63.0

α GlcN	96.1	53.9	67.9	69.7	70.6	61.1	—	—
α GalA	98.2	69.7	68.7	79.5	70.6	—	—	—
Linker	22.8	27.9	28.3	39.6	68.4	68.4	41.0 ($\underline{\text{CH}_2\text{SH}}$)	

*Disulfide of compound **1**.

Conjugation of tetrasaccharide **1** to carrier proteins (BSA and CRM₁₉₇).

The carrier protein solution (450 μ L, 2.5 mg/mL in pH 7.4 HEPES buffer for CRM₁₉₇; 450 μ L, 2.5 mg/mL in pH 7.4 PBS buffer for BSA) was purified by ultrafiltration (Amicon Ultra-0.5 Centrifugal Filter Unit, 10K) 3 times use pH 7.4 PBS buffer as eluent. The residue was dissolved in pH 7.4 PBS buffer (0.5 mL), and succinimidyl 4-(*N*-maleimidomethyl)cyclohexane-1-carboxylate (SMCC, 1.5 mg) was added. The reaction suspension was shaken overnight at room temperature, after which it was centrifuged, and the supernatant was transferred to a centrifugal filter (Amicon Ultra-0.5 Centrifugal Filter Unit, 10K) and purified 5 times. The resulting maleimide-activated carrier protein was diluted with pH 7.4 PBS buffer to ~10 mg/mL using nano-drop. Tetrasaccharide **1** (~1 mg) was dissolved in H₂O (100 μ L) and added into the protein solution. The mixture was shaken gently overnight at room temperature and purified by ultrafiltration (Amicon Ultra-0.5 Centrifugal Filter Unit, 10K) 5 times. The resulting glycoconjugate was diluted to ~1 mg/mL with pH 7.4 PBS buffer.



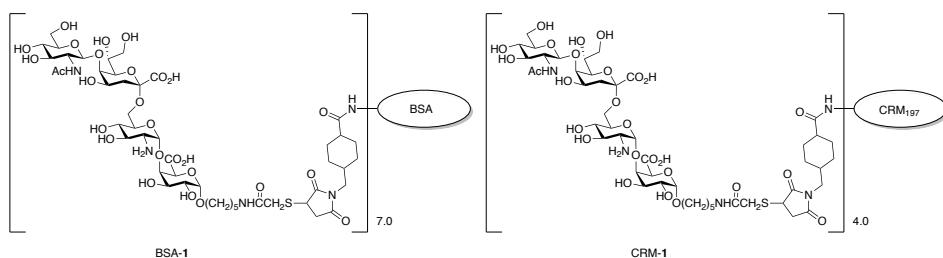


Figure S2. (A) Conjugation of tetrasaccharide **1** with BSA. (B) Conjugation of tetrasaccharide **1** with CRM₁₉₇.

MALDI-TOF analysis of modified proteins

5 mg of sinapinic acid was dissolved in 500 μ L CH₃CN/H₂O/TFA (3/7/0.01), and the mixture was treated with ultrasonic and then centrifuged. The resulting supernatant was used as matrix.⁴⁰ The carrier proteins or glycoconjugates were desalted with C18 ZipTip (Millipore, 10 μ L) using manufacturer's protocol, and the concentration was adjusted to \sim 1 mg/mL with water. 1 μ L of desalted carrier protein or glycoconjugate was mixed with 2 μ L of freshly prepared matrix solution, and 1 μ L of the resulting mixture was loaded on MALDI plate. After the spot was dried by air, the sample was measured using Linear positive mode.

Table S7. MALDI-TOF data of Proteins, maleimide-modified carrier proteins and glycoconjugates.

	BSA	CRM ₁₉₇	BSA-M	CRM-M	BSA- 1	CRM- 1
Mass	66715.6	58764.5	70517.0	62163.2	77078.6	65920.9
Loading number	—	—	17	15	7	4

*BSA-M = maleimide-modified BSA; CRM-M = maleimide-modified CRM₁₉₇.

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Chapter 3

Synthesis of a Branched, Heptose-Containing Pentasaccharide Derived from *Klebsiella pneumoniae* for Conjugate Vaccine Development

Introduction

Klebsiella pneumoniae (*K. pneumoniae*) is a Gram-negative bacteria that commonly causes hospital acquired infections.^{1, 2} The frequency of multi-drug resistant (MDR) strains, especially strains resistant to carbapenem (drugs of last resort for antibacterial treatment), have dramatically increased worldwide, and these strains have become a major threat to healthcare because of their high morbidity and mortality.^{3, 4} The lack of therapeutic options for MDR-*K. pneumoniae* needs urgent addressing.^{5, 6} Vaccination is an attractive option to control infectious diseases and has been proven effective for bacteria such as *N. meningitidis*, *S. typhi* and *S. pneumoniae*.^{4, 7}

In the 1970s, a whole cell vaccine was developed to prevent infections by *K. pneumoniae* but it failed to be licensed due to safety concerns.^{8, 9} To date, no marketed vaccine for *K. pneumoniae* is available.¹⁰ The development of vaccines, diagnostics and immune-therapeutics for *K. pneumoniae* needs a detailed understanding of glycan structures that can be recognized by protective antibodies.¹¹ A carrier protein conjugated oligosaccharide is also required for immunization studies to establish structural motifs that can provide protection.¹² Capsular polysaccharide (CPS) and lipopolysaccharide (LPS) are major pathogenicity factors of *K. pneumoniae*.¹³ The main advantage of LPS as vaccine candidate compared to CPS is the number of serotypes as the former has only 9, which is much less than 79 of the latter.^{3, 14}

K. pneumoniae LPS is composed of lipid A, a core oligosaccharide and an O-antigen.^{15, 16} The most common O-antigens are composed of galactans or mannans repeating units, and classified into four serogroups (O1, O2, O3 and O5).¹⁷ On the other hand, there are only two types of core oligosaccharide expressed by *K. pneumoniae*.^{18, 19} Structural studies have shown the most common outer core contains an L-glycero-D-manno-heptose (L,D-heptose) substituted at C-4 of 3-deoxy-D-manno-2-octulosonic acid (Kdo) in nonstoichiometric quantities (Fig. 1).^{20, 21} Both of these residues are commonly found in the inner core of *Enterobacteriaceae*.²²⁻²⁴ Additionally, the L,αD-Hep-(1→4)-αKdo-(2→ residue, which exist in LPS outer core of *K. pneumoniae* and certain strains of *H. alvei*, has been found to be a common target for mannose-binding lectins.²⁵ Furthermore, studies have shown some structures containing L,D- or D,D-heptose bind very well to cross-reactive antibacterial monoclonal antibody.²⁶⁻²⁸

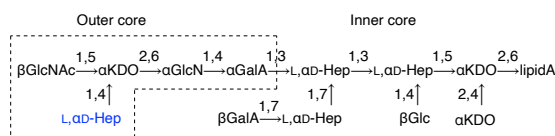


Figure 1. Chemical structure of *K. pneumoniae* LPS core (most common type).

Although most of licensed conjugate vaccines are based on polysaccharides obtained from cultured bacteria, it is still challenging to obtain purified glycans or to meet the quality standard for vaccine preparation.^{29, 30} Synthetic approaches provide an attractive alternative for the development of conjugate vaccines.^{12, 31} Additionally, it can determine the minimal requirements of an epitope for protective immunity by providing substructures.¹² In chapter 2, we have described the successful synthesis of outer core tetrasaccharide derived from *K. pneumoniae*, in this chapter, we report the synthesis of pentasaccharide **1** that is equipped with a nonstoichiometric L,αD-heptoside moiety substituted at C-4 of Kdo. The synthetic glycoconjugate is attractive for immunodominant epitope determination and vaccine development.

Results and Discussion

The synthetic route for preparation of pentasaccharide **1** was designed based on a similar approach described Chapter 2. Although the two compounds share a tetrasaccharide part structure, the synthesis of saccharide **1** is still challenging due to the low reactivity of heptosyl glycosyl donors, the need to install glycosidic bonds at a crowded 4,5-branched Kdo and the additional synthetic steps required for the stereo-selectively preparation of an L,D-heptosyl building block.^{32, 33} Furthermore, the free amine and carboxylic acid of the pentasaccharide caused challenges in conjugation of the glycan to carrier proteins. Finally, the three differently modified amines of **1** required the selection of orthogonal groups that are compatible with the glycosylation strategy.

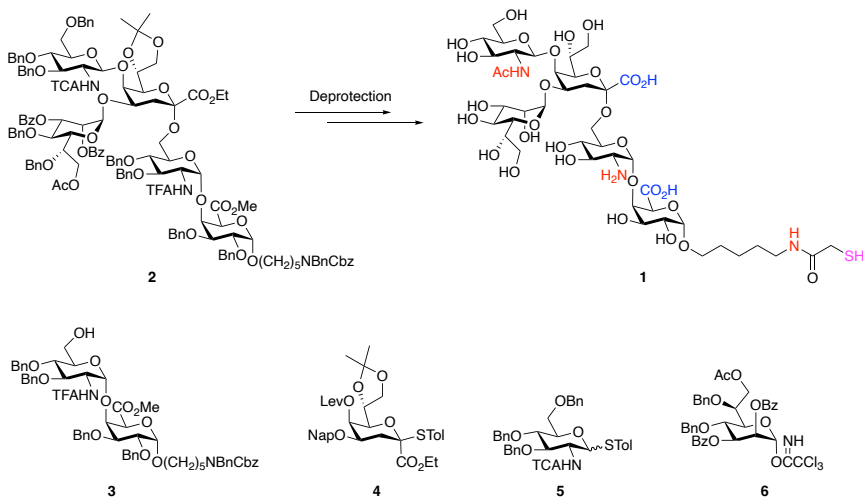


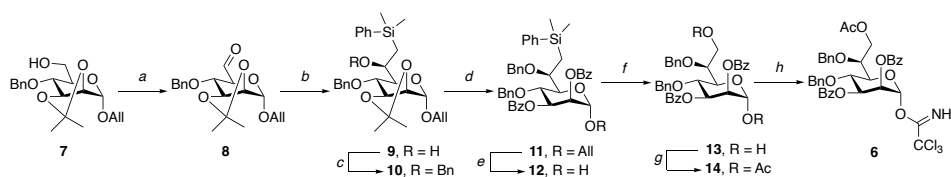
Figure 2. Target pentasaccharide **1** and the building blocks requires for its assembly.

The target compound **1** was prepared from protected pentasaccharide **2**, which in turn was assembled from mono- and disaccharide building blocks **3-6** (Fig. 2). Disaccharide acceptor **3** has a free hydroxyl at C-6' for glycosylation with Kdo donor **4**. Furthermore, the anomeric aminopentyl linker of this building block is doubly protected by a benzyl (Bn) and benzyloxycarbamate (Cbz) moiety, which

at a late stage of synthesis can be removed by hydrogenation. The amine at C-2' of **3** is protected as a trifluoroacetamide, which is stable under hydrogenation, but can be removed under mild basic conditions after modification of the amine of the anomeric linker as a thioacetyl moiety. The thiol of the linker allowed selective conjugation to carrier proteins modified by maleimides. Kdo donor **4** is modified by the orthogonal protecting groups, 2-methylnaphthyl (Nap) ether and levulinoyl (Lev) ester, which can be removed by oxidation with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) or treatment with hydrazine acetate, respectively and it was expected these conditions will not impact any of the other protecting groups. The resulting hydroxyls at C-4 and C-5 of Kdo can then be glycosylated with glycosyl donors **6** and **5**, respectively to give the target pentasaccharide **2**. The C-2 amine of donor **5** is protected as a trichloroacetamide, which will provide neighboring group participation during glycosylation to selectively install β -glycosidic product. Furthermore, during hydrogenation to remove Bn and Cbz protecting groups, the trichloroacetamide moiety will be reduced to an acetamide to install the required *N*-acetyl glucosamine (GlcNAc) moiety. Finally, the heptosyl donor **6** contains benzoyl esters at C-2 which will perform neighboring group participation during glycosylation to provide the required α -anomer.

Disaccharide acceptor **3** was prepared from glucosamine and galactose (see chapter 2 for details). The Kdo building block **4** was synthesized by a similar approach described in Chapter 2 (see Scheme S1 for details).³⁴ Glycosyl donor **5** was obtained by modification of glucosamine following the protocol described in chapter 2. The preparation of L,D-heptosyl donor **6** commenced with known modified mannoside **7**,³⁵ which was treated with Dess-Martin periodinane in DCM to give aldehyde **8**. Subsequent Grignard reaction with freshly prepared (phenyldimethylsilyl)-methylmagnesium chloride resulting in the formation of 7-(Si-dimethylphenyl-silyl)-7-deoxy-L-*glycero*-D-heptoside **9** in 85% yield over two steps.^{36, 37} Only the L-*glycero*-isomer was observed because the chosen silyl

Grignard reagent is sufficiently bulky to sterically block the *D*-glycero-face.^{38, 39} A benzyl (Bn) ether was then installed at C-6 using benzyl bromide and sodium hydride in DMF to afford compound **10** in 88% yield. The isopropylidene acetal was removed by heating in aqueous acetic acid, and the resulting diol was masked as benzoyl (Bz) ester through treatment with benzoyl chloride in pyridine gave compound **11** in an overall yield of 92%. The allyl (All) ether will be oxidized by peracetic acid,⁴⁰ and therefore it was first removed using palladium chloride as catalyst to form the heptosyl precursor **12**, which was subjected to a Fleming-Tamao oxidation^{41, 42} to convert the C-7 phenylsilyl to a hydroxyl using potassium bromide and sodium acetate in a mixture of peracetic acid and acetic acid to afford L,D-heptoside derivative **13** in 88% yield. The resulting free hydroxyls were protected as acetyl (Ac) ester by treatment with acetic anhydride and 4-dimethylaminopyridine (DMAP) in pyridine to provide compound **14**. The anomeric acetyl ester was selectively removed using hydrazine acetate in DMF,⁴³ and L,D-heptosyl donor **6** was obtained by installation of trichloroacetimidate by treatment of trichloroacetonitrile and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in DCM in an overall yield of 90%.

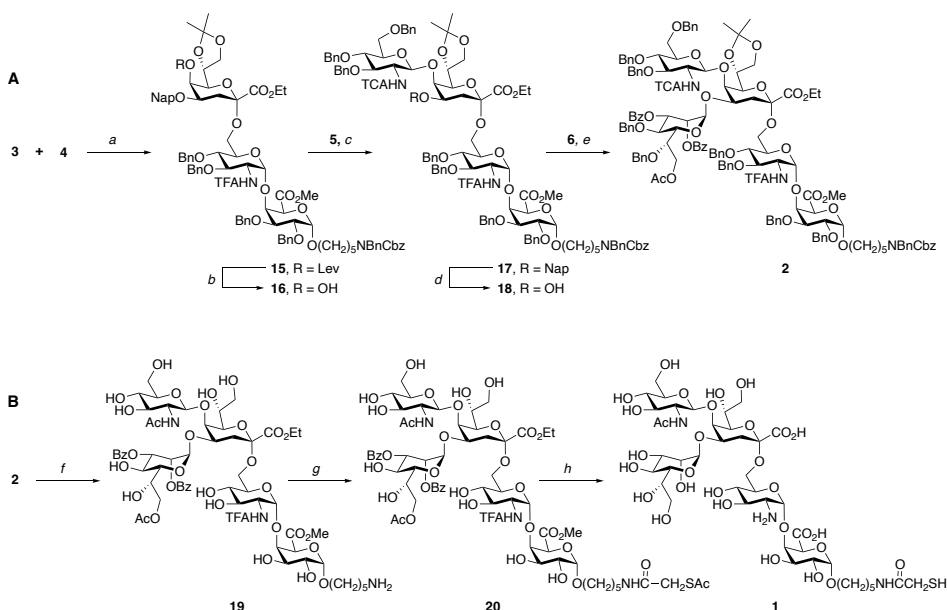


Scheme 1. Preparation of L,D-heptosyl building block **6**. Conditions and reagents: *a*) Dess-Martin periodinane, DCM. *b*) (phenyldimethylsilyl)methylmagnesium chloride, THF, 85%. *c*) BnBr, NaH, DMF, 88%. *d*) i. AcOH/H₂O (4/1, v/v), 80°C. ii. BzCl, pyridine, 92%. *e*) PdCl₂, MeOH, 85%. *f*) KBr, NaOAc, AcOOH, AcOH, 88%. *g*) Ac₂O, DMAP, pyridine, 90%. *h*) i. NH₂NH₂·AcOH, DMF. ii. CCl₃CN, DBU, DCM, 90%.

Having building blocks **3-6** in hand, the protected pentasaccharide **2** was assembled by a sequence of glycosylations shown in Scheme 1A. Disaccharide acceptor **3** was glycosylated with Kdo donor **4** using *N*-iodosuccinimide (NIS) and trifluoromethanesulfonic acid (TfOH)⁴⁴ as the promoter system and acetonitrile as the solvent at 0°C to give trisaccharide **7** as a separable mixture of α/β -anomers ($\alpha/\beta = 4/1$) in a yield of 71% of the desired α -linked product (configuration conformed by coupling constant between C-1 and H-3_{ax} of Kdo^{32, 45, 46}, $J_{C1-H3ax} < 1$ Hz). We chose for a strategy to first glycosylate the axial C-5 hydroxyl of the Kdo moiety of **15** because it has a lower glycosyl accepting reactivity compared to the equatorial hydroxyl at C-4. Thus, treatment of **15** with hydrazine acetate remove the Lev ester to provide trisaccharide acceptor **16** in 70% yield. The latter compound was coupled with the thioglycosyl donor **5** using TfOH/NIS as the promotor system⁴⁷ in a mixture of DCM and acetonitrile at 0°C to afford tetrasaccharide **17** in yield of 76%. Only the β -anomer was obtained due to neighboring group participation of the TCA protecting group. Next, the Nap ether of **17** was oxidatively cleaved with DDQ using β -pinene as the proton scavenger⁴⁸ to give tetrasaccharide **18** in 72% yield (configuration confirmed by ¹H NMR, ($\delta_{\beta\text{Glc H1}} = 5.04$, d, $J_{H1-H2} = 7.8$ Hz). The glycosylation of acceptor **18** with heptosyl donor **6** was mediated by a catalytic amount of TMSOTf in DCM at 0°C resulting in the formation of pentasaccharide **2** in a yield of 70%, and the configuration was confirmed using coupling constants between C-1 and H-1 ($J_{\alpha\text{Hep C1-H1}} = 174$ Hz, $J_{\alpha\text{GalA C1-H1}} = 176$ Hz, $J_{\alpha\text{GlcNHTFA C1-H1}} = 177$ Hz, $J_{\beta\text{GlcNHTCA C1-H1}} = 162$ Hz).

The deprotection of pentasaccharide **2** started with the cleavage of isopropylidene acetal using TFA in wet DCM which was followed by hydrogenation over Pd(OH)₂/C in a mixture of *t*-BuOH and H₂O to remove the benzyl ethers and protecting groups of the amino pentyl linker. Under these conditions, the trichloroacetamide was reduced to *N*-acetyl moiety to give pentasaccharide **19**. The aminopentyl linker of the later compound was reacted with perfluorophenyl

2-(acetylthio)acetate (SAMA-OPfp) in the presence of *N,N*-diisopropylethylamine (DIPEA) in dry DMF to give, after purification by bio-gel P2 size exclusion column chromatography, compound **20** in a yield of 70%. Next, the benzoyl, acetyl, methyl, ethyl esters and trifluoroacetamide were saponified using aqueous NaOH in MeOH at 0°C to afford, after purification by bio-gel P2 size exclusion column chromatography, the target compound **1**.



Scheme 2. (A) Synthesis of protected pentasaccharide **2**. Conditions and reagents: *a*) TfOH, NIS, MeCN, $\alpha/\beta = 4/1$, 71% of α -anomer. *b*) $\text{NH}_2\text{NH}_2 \cdot \text{HOAc}$, DCM/MeOH, 70%. *c*) TfOH, NIS, DCM/MeCN, 76%. *d*) DDQ, β -pinene, DCM/ H_2O , 72%. *e*) TMSOTf, DCM, 70%. (B) Deprotection and thiol installation of target pentasaccharide **1**. Conditions and reagents: *f*) i. TFA/DCM/ H_2O ii. $\text{Pd}(\text{OH})_2/\text{C}$, H_2 , *t*-BuOH/ H_2O , 69%. *g*) SAMA-OPfp, DIPEA, DMF, 70%. *h*) 0.2M NaOH/MeOH = 1/1, quant.

The carrier proteins BSA and CRM₁₉₇ were activated by treatment with succinimidyl 4-(*N*-maleimidomethyl)cyclohexane-1-carboxylate (SMCC) in phosphate buffered saline (PBS, pH 7.4) to give, after purification by

ultrafiltration, maleimide-modified proteins. After treatment with D,L-dithiothreitol (DTT) and purification by bio-gel P2 size exclusion column chromatography, the thiol-containing pentasaccharide **1** was reacted with maleimide-modified carrier proteins in PBS buffer (pH 7.4) to give, after purification by ultrafiltration, the responding BSA and CRM₁₉₇ conjugates (BSA-**1**, CRM-**1**). MALDI-TOF mass spectrometry showed that BSA-**1** contains an average of 4.9 pentasaccharide molecules per BSA, and the number for CRM-**1** is 4.5 (SI).

Conclusions

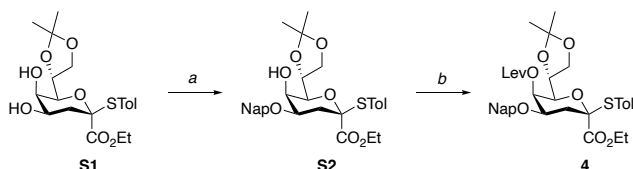
In summary, pentasaccharide **1** containing nonstoichiometric L, α D-heptoside derived from outer core of *K. pneumoniae* LPS has been synthesized chemically use a block coupling strategy, and the crowded 4,5-branched Kdo was obtained using sequential glycosylations in appropriate order that involves modification of Kdo building block with orthogonal protecting groups. Thiol-maleimide coupling chemistry was employed for the conjugation of oligosaccharide **1** to BSA and CRM₁₉₇. The successful synthesis of **1** provides opportunities for preparing many other oligosaccharides containing 4,5-branched Kdo moieties. The synthetic glycoconjugates will be further examined as vaccine candidates against *K. pneumoniae*.

General procedures

All chemicals were purchased from Sigma-Aldrich, Fisher Scientific or Biosynth Carbosynth. BSA was purchased from Sigma-Aldrich and CRM₁₉₇ was purchased from Scarab Genomics LLC. NMR spectra, including ¹H, ¹³C, COSY, HSQC, HMBC, EXSIDE and bsHSQCAD, were recorded on Agilent 400-MR or Bruker 600 MHz with chemical shifts reported in part per million (ppm) relative to CDCl₃ or D₂O. ¹H NMR data are presented in the order: Chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, dd = doublet of doublets, m = multiplet) and coupling constants (J) are reported in Hertz (Hz). Mass spectra of

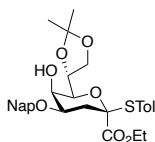
proteins were recorded on a Kratos Analytical Maxima-CFR MALDI-TOF system with sinapinic acid in acetonitrile/water (0.3/0.7, v/v) as matrix, and High-resolution mass spectrometry (HRMS) was recorded on an Agilent technologies 6560 Ion mobility Q-TOF. Chromatographic purifications were performed on silica gel G60 (Silicycle 60 – 200 μm , 60 \AA), and size exclusion chromatography was performed on Bio-Gel P-2 (45-90 μm) by using 5 % *n*-butanol in water as eluent. TLC analysis was conducted on Silica gel 60 F254 (EMD Chemicals Inc.) and detected by using UV light (254 nm), staining by 5 % sulfuric acid in ethanol, *p*-anisaldehyde solution or an aqueous solution of $\text{Ce}(\text{NH}_4)_2(\text{NO}_3)_6$ / $(\text{NH}_4)_6\text{MoO}_{24} \cdot 4\text{H}_2\text{O}$ in 5 % sulfuric acid. 4 \AA molecular sieves were flame-activated under vacuum before use. Carrier proteins were purified by ultrafiltration using ultrafilter (Amicon Ultra-0.5 Centrifugal Filter Unit, 10K) before and after reactions.

Synthetic Protocols



Scheme S1. Synthesis of Kdo donor **4**. Conditions and reagents: *a*) i. Bu_2SnO , toluene, 110 $^\circ\text{C}$. ii. NapBr , CsF , DMF , 96%. *b*) LevOH , DMAP , DCC , DCM , 92%.

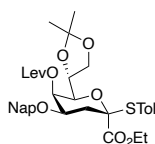
Ethyl [p-methylphenyl 4-O-(2-naphthyl)methyl-7,8-O-isopropylidene-3-deoxy-2-thio-D-manno-oct-ulopyranoside]onate (S2): Compound **S1**³⁴ (the



synthesis has been described in Chapter 2, 500 mg, 1.21 mmol) and dibutyltin oxide (363 mg, 1.46 mmol) were added to dry toluene (5 mL), and the suspension was heated to reflux (110 $^\circ\text{C}$). After stirring for 3 h at this temperature, the reaction was cooled to room temperature and concentrated to dryness *in vacuo*. The resulting residue

was dissolved in dry DMF (5 mL), and 2-(bromomethyl)naphthalene (NapBr, 321 mg, 1.46 mmol) and CsF (552 mg, 3.63 mmol) were added to the solution. The reaction mixture was stirred for 16 h at room temperature, after which it was diluted with EtOAc (50 mL) and washed with H₂O (30 mL). The aqueous layer was backextracted with EtOAc (30 mL) 5 times, and the combined organic layer was dried (Na₂SO₄), filtered, and the filtrate was concentrated *in vacuo*. The resulting crude product was purified by silica gel column chromatography (PE/Ea = 3/1, v/v) to give **S2** (641 mg, 96%) as a colorless oil. ¹H NMR (600 MHz, CDCl₃) δ 7.83 (m, 4H, Ar), 7.48 (m, 3H, Ar), 7.43 – 7.37 (m, 2H, Ar), 7.10 (d, *J* = 8.0 Hz, 2H, Ar), 4.77 (d, *J* = 2.3 Hz, 2H, NapCH₂), 4.45 (ddd, *J* = 8.7, 6.3, 5.0 Hz, 1H, H-7), 4.15 (dd, *J* = 8.6, 6.3 Hz, 1H, H-8a), 4.10 – 4.08 (m, 1H, H-5), 4.00 – 3.94 (m, 2H, H-8b, OCH₂HCH₃), 3.78 – 3.67 (m, 1H, OCH₂HCH₃), 3.53 (ddd, *J* = 11.7, 4.8, 2.8 Hz, 1H, H-4), 3.22 (d, *J* = 8.7 Hz, 1H, H-6), 2.74 (dd, *J* = 12.6, 4.7 Hz, 1H, H_{eq}-3), 2.34 (s, 3H, SPhCH₃), 2.16 (t, *J* = 12.2 Hz, 1H, H_{ax}-3), 1.38 (s, 3H, C(CH₃)₂), 1.35 (s, 3H, C(CH₃)₂), 0.95 (t, *J* = 7.1 Hz, 3H, OCH₂CH₃). ¹³C NMR (151 MHz, CDCl₃) δ 168.63, 139.98, 136.41, 134.98, 133.30, 133.17, 132.53, 129.98, 129.32, 128.48, 127.97, 127.82, 127.80, 126.70, 126.35, 126.18, 125.99, 125.73, 125.70, 109.60, 87.26, 77.49, 73.97, 72.88, 70.32, 67.56, 63.91, 61.69, 32.18, 26.92, 25.29, 21.37, 13.75. ESI HRMS (*m/z*): [M + Na⁺] calcd for C₃₁H₃₆NaO₇S, 575.2074 found 575.2073.

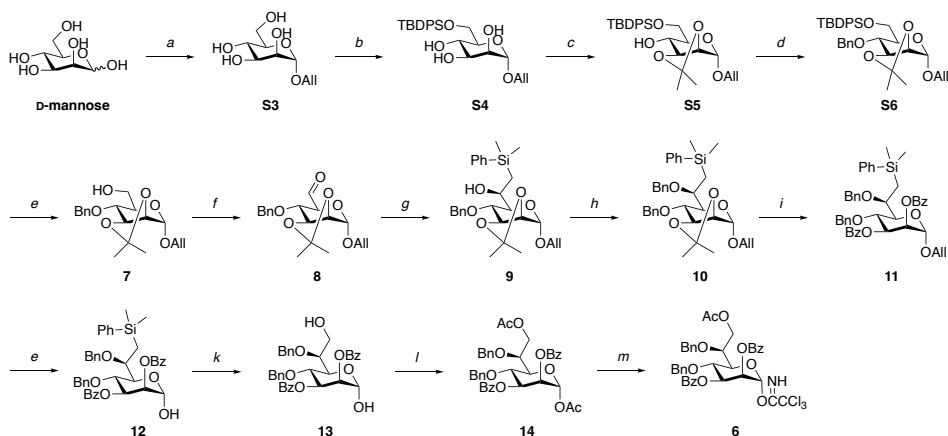
Ethyl [p-methylphenyl 4-O-(2-naphthyl)methyl-5-O-levulinoyl-7, 8-O-isopropylidene-3-deoxy-2-thio-D-manno-oct-ulopyranoside] onate (4): A



solution of **S2** (457 mg, 0.82 mmol) in DCM (10 mL) was added levulinic acid (LevOH, 190 mg, 1.64 mmol), *N,N'*-dicyclohexylcarbodiimide (DCC, 338 mg, 1.64 mmol) and 4-dimethylaminopyridine (DMAP, 200 mg, 1.64 mmol). The

reaction mixture was stirred under reflux (45°C) for 2 h, after which it was cooled to room temperature, diluted with DCM (40 mL), and washed with sat. aq. NaHCO₃ (30 mL). The aqueous layer was backextracted with DCM (3 × 20 mL),

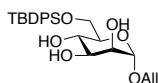
and the combined organic phase was dried (Na_2SO_4), filtered, and the filtrate was concentrated *in vacuo*. The resulting crude product was purified by silica gel column chromatography (DCM/acetone, 20/1, v/v) to give **4** (492 mg, 92%) as a colorless oil. ^1H NMR (600 MHz, CDCl_3) δ 7.89 – 7.79 (m, 3H, Ar), 7.75 (d, J = 1.7 Hz, 1H, Ar), 7.52 – 7.36 (m, 5H, Ar), 7.14 – 7.07 (m, 2H, Ar), 5.59 (dt, J = 2.5, 1.2 Hz, 1H, H-5), 4.85 (t, J = 11.8 Hz, 1H, NapCHH), 4.61 (d, J = 11.8 Hz, 1H, NapCHH), 4.18 – 4.11 (m, 2H, H-7, H-8a), 4.03 – 3.92 (m, 2H, H-8b, OCHHCH $_3$), 3.81 – 3.68 (m, 1H, OCHHCH $_3$), 3.55 (ddd, J = 11.9, 4.7, 2.7 Hz, 1H, H-4), 3.40 – 3.35 (m, 1H, H-6), 2.84 – 2.63 (m, 5H, H $_{\text{eq}}$ -3, CH $_2$ CH $_2$ -Lev), 2.34 (s, 3H, SPhCH $_3$), 2.16 (s, 3H, CH $_3$ -Lev), 2.15 – 2.09 (m, 1H, H $_{\text{ax}}$ -3), 1.35 (s, 3H, C(CH $_3$) $_2$), 1.35 (s, 3H, C(CH $_3$) $_2$), 0.94 (t, J = 7.1 Hz, 3H, OCH $_2$ CH $_3$). ^{13}C NMR (151 MHz, CDCl_3) δ 206.34, 171.85, 168.42, 140.05, 136.37, 135.26, 133.31, 133.06, 129.34, 128.43, 128.22, 128.04, 127.94, 127.75, 127.72, 127.35, 126.59, 126.35, 126.31, 126.14, 125.96, 125.87, 125.82, 125.76, 109.72, 87.51, 76.57, 72.98, 72.83, 70.52, 67.30, 64.71, 61.81, 38.14, 33.67, 29.94, 28.18, 26.93, 25.28, 21.33, 13.72. ESI HRMS (m/z): $[\text{M} + \text{Na}^+]$ calcd for $\text{C}_{36}\text{H}_{42}\text{NaO}_9\text{S}$, 673.2442 found 673.2447.



Scheme S2. Preparation of L,D-heptose glycosyl donor **6**. Conditions and reagents: *a*) AlOH, TfOH. *b*) TBDPSCl, imidazole, DMF, 63% over 2 steps. *c*) 2,2-Dimethoxypropane, CSA, acetone, 78%. *d*) BnBr, NaH, DMF, 94%. *e*)

TBAF, THF, quant. *f*) Dess-Martin periodinane, DCM. *g*) (phenyldimethylsilyl)-methylmagnesium chloride, THF, 85%. *h*) BnBr, NaH, DMF, 88%. *i*) i. AcOH/H₂O (4/1, v/v), 80°C. ii. BzCl, pyridine, 92%. *j*) PdCl₂, MeOH, 85%. *k*) KBr, NaOAc, AcOOH, AcOH, 88%. *l*) Ac₂O, DMAP, pyridine, 90%. *m*) i. NH₂NH₂·AcOH, DMF. ii. CCl₃CN, DBU, DCM, 90%.

Allyl 6-*O*-*tert*-butyldiphenylsilyl- α -D-mannopyranoside (S4):



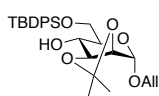
Trifluoromethanesulfonic acid (TfOH, 0.5 mL) was added to a solution of D-mannose (6.6 g, 36.6 mmol) in allyl alcohol (50 mL), and the reaction mixture was stirred under reflux (110°C)

for 3 h. After TLC analysis showed consumption of starting material, the reaction was cooled to room temperature and then quenched with Et₃N (2 mL). The dark brown solution was concentrated *in vacuo* to give the crude product **S3**, which was used for next step without further purification.

Imidazole (3.74 g, 55 mmol) was added to a solution of compound **S3** in dry DMF (40 mL), and the solution was cooled to 0°C. After stirred for 10 min at this temperature, *tert*-butyldiphenylchlorosilane (TBDPSCl, 11.4 mL, 44 mmol) was added dropwise. The reaction was slowly warmed up to room temperature and stirred for 6 h. After TLC analysis showed consumption of starting material, the reaction was quenched with H₂O (2 mL). The solvents were removed under reduced pressure, and the resulting residue was dissolved in DCM (100 mL) and washed with 1M HCl (50 mL) and H₂O (50 mL) sequentially. The organic phase was dried (Na₂SO₄), filtered, and the filtration was concentrated *in vacuo*. The resulting crude product was purified by silica gel column chromatography (DCM/MeOH = 50/1, v/v) to afford **S4** (10.6 g, 63% over 2 steps) as light-yellow oil. ¹H NMR (600 MHz, CD₃OD_SPE) δ 7.77 – 7.70 (m, 4H, Ar), 7.44 – 7.35 (m, 6H, Ar), 5.96 (ddt, *J* = 16.4, 10.8, 5.6 Hz, 1H, CH₂-CH=CH₂), 5.28 (dt, *J* = 17.2, 1.6 Hz, 1H, CH₂-CH=CH₂), 5.17 (dt, *J* = 10.4, 1.4 Hz, 1H, CH₂-CH=CH₂), 4.85 (d, *J* = 1.6 Hz, 1H, H-1), 4.32 – 4.26 (m, 1H, CH-CH=CH₂), 4.08 – 4.01 (m,

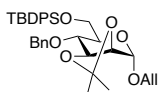
2H, CHH-CH=CH_2 , H-6a), 3.86 – 3.81 (m, 2H, H-2, H-6b), 3.72 (m, 2H, H-3, H-5), 3.56 (t, $J = 9.7$ Hz, 1H, H-4), 1.07 – 1.03 (m, 9H, CH_3 -*t*-Bu \times 3). ^{13}C NMR (151 MHz, $\text{CD}_3\text{OD-SPE}$) δ 136.77, 136.75, 135.50, 134.81, 134.76, 128.71, 117.45, 100.50, 75.29, 72.80, 72.05, 68.85, 68.65, 65.38, 27.31, 20.09. ESI HRMS (m/z): $[\text{M} + \text{Na}^+]$ calcd for $\text{C}_{25}\text{H}_{34}\text{NaO}_6\text{Si}$, 481.2017 found 481.2013.

Allyl 6-*O*-*tert*-butyldiphenylsilyl-2,3-*O*-isopropylidene- α -D-mannopyranoside (S5): Compound **S4** (3.12 g, 6.8 mmol), camphorsulfonic



acid (CSA, 95 mg, 0.4 mmol) and 2,2-dimethoxypropane (1.3 mL, 10.2 mmol) were dissolved in dry acetone (15 mL). The reaction was stirred for 3 h at room temperature, after which it was quenched with Et_3N (1 mL). The solvents were removed under reduced pressure, and the resulting residue was purified by silica gel column chromatography (PE/EA = 8/1, v/v) to afford **S5** (2.65 g, 78%) as a colorless oil. ^1H NMR (600 MHz, CDCl_3) δ 7.75 – 7.71 (m, 4H, Ar), 7.46 – 7.37 (m, 6H, Ar), 5.90 – 5.84 (m, 1H, CH_2 - $\underline{\text{CH}}=\text{CH}_2$), 5.33 – 5.25 (m, 1H, CH_2 - $\text{CH}=\underline{\text{CH}}\text{H}$), 5.20 (dq, $J = 10.4$, 1.3 Hz, 1H, CH_2 - $\text{CH}=\underline{\text{CH}}\text{H}$), 5.08 (s, 1H, H-1), 4.21 – 4.17 (m, 3H, H-2, H-3, CHH-CH=CH_2), 3.99 (ddt, $J = 12.8$, 6.3, 1.3 Hz, 1H, $\underline{\text{CH}}\text{H-CH=CH}_2$), 3.95 (dd, $J = 10.8$, 4.5 Hz, 1H, H-6a), 3.90 (dd, $J = 10.8$, 5.2 Hz, 1H, H-6b), 3.82 – 3.77 (m, 1H, H-4), 3.70 (dt, $J = 9.6$, 4.8 Hz, 1H, H-5), 3.01 (d, $J = 3.8$ Hz, 1H, -OH), 1.52 (s, 3H, $\text{C}(\underline{\text{CH}}_3)_2$), 1.36 (s, 3H, $\text{C}(\underline{\text{CH}}_3)_2$), 1.09 (s, 9H, CH_3 -*t*-Bu \times 3). ^{13}C NMR (151 MHz, CDCl_3) δ 135.76, 135.68, 133.62, 133.25, 129.89, 127.86, 127.82, 117.94, 109.57, 96.23, 78.45, 75.53, 70.53, 69.82, 67.90, 64.54, 28.00, 26.90, 26.23, 19.31. ESI HRMS (m/z): $[\text{M} + \text{Na}^+]$ calcd for $\text{C}_{28}\text{H}_{38}\text{NaO}_6\text{Si}$, 521.2330 found 521.2296.

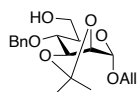
Allyl 6-*O*-*tert*-butyldiphenylsilyl-4-*O*-benzyl-2,3-*O*-isopropylidene- α -D-mannopyranoside (S6): Compound **S5** (2.65 g, 5.3 mmol) and benzyl bromide



(BnBr, 1.6 mL, 10.6 mmol) were dissolved in dry DMF (30 mL), and the solution was cooled to 0°C . NaH (60% dispersion in

mineral oil, 424 mg, 10.6 mmol) was then added slowly. After stirred for 3 h at room temperature, the reaction was quenched with sat. aq. NH_4Cl (1 mL). The reaction mixture was concentrated *in vacuo*, and the resulting residue was dissolved in DCM (100 mL) and washed with brine (50 mL). The aqueous layer was back extracted with DCM (3×10 mL), and the combined organic phase was dried (Na_2SO_4), filtered, and the filtration was concentrated *in vacuo*. The resulting residue was purified by silica gel column chromatography (PE/EA = 25/1, v/v) to afford **S6** (2.95 g, 94%) as a colorless oil. ^1H NMR (600 MHz, CDCl_3) δ 7.71 (m, 5H, Ar), 7.45 – 7.34 (m, 8H, Ar), 7.28 – 7.18 (m, 2H, Ar), 5.94 – 5.83 (m, 1H, $\text{CH}_2\text{-CH=CH}_2$), 5.27 (dt, $J = 17.2, 1.6$ Hz, 1H, $\text{CH}_2\text{-CH=CHH}$), 5.18 (dt, $J = 10.3, 1.4$ Hz, 1H, $\text{CH}_2\text{-CH=CHH}$), 5.12 (s, 1H, H-1), 4.88 (d, $J = 11.4$ Hz, 1H, PhCHH), 4.58 – 4.53 (d, $J = 11.4$ Hz, 1H, PhCHH), 4.36 (t, $J = 6.4$ Hz, 1H, H-3), 4.25 – 4.18 (m, 2H, H-2, CHH-CH=CH_2), 4.00 (ddd, $J = 12.8, 6.4, 1.4$ Hz, 1H, CHH-CH=CH_2), 3.94 (dd, $J = 11.0, 1.5$ Hz, 1H, H-6a), 3.86 (dd, $J = 11.0, 5.2$ Hz, 1H, H-6b), 3.73 (dd, $J = 10.4, 5.0$ Hz, 1H, H-5), 3.63 (dd, $J = 10.2, 6.9$ Hz, 1H, H-4), 1.53 (s, 3H, $\text{C}(\text{CH}_3)_2$), 1.39 (s, 3H, $\text{C}(\text{CH}_3)_2$), 1.05 (m, 9H, $\text{CH}_3\text{-}t\text{-Bu} \times 3$). ^{13}C NMR (151 MHz, CDCl_3) δ 138.43, 135.99, 135.76, 135.65, 133.86, 133.78, 133.45, 129.74, 129.70, 129.68, 128.54, 128.38, 127.96, 127.92, 127.85, 127.82, 127.78, 127.70, 127.65, 117.95, 109.41, 96.11, 79.15, 76.09, 75.87, 73.09, 69.84, 67.65, 63.44, 28.12, 26.90, 26.55, 19.42. ESI HRMS (m/z): $[\text{M} + \text{Na}^+]$ calcd for $\text{C}_{35}\text{H}_{44}\text{NaO}_6\text{Si}$, 611.2799 found 611.2790.

Allyl 4-O-benzyl-2,3-O-isopropylidene- α -D-mannopyranoside (7): TBAF

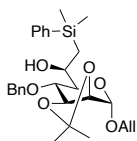


(1.0M in THF, 10 mL, 10.0 mmol) was added to a solution of **S6** (2.95 g, 5.0 mmol) in THF (20 mL), and the reaction mixture was stirred for 6 h at room temperature. The solvent was removed under

reduced pressure, and the residue was dissolved in DCM (100 mL) and washed with H_2O (50 mL). The organic phase was dried (Na_2SO_4), filtered, and the filtration was concentrated *in vacuo*. The resulting residue was purified by silica gel column chromatography (PE/EA = 3/1, v/v) to afford **7** (1.75 g, quant.) as a

white solid. ^1H NMR (600 MHz, CDCl_3) δ 7.37 – 7.26 (m, 5H, Ar), 5.93 – 5.86 (m, 1H, $\text{CH}_2\text{-}\underline{\text{CH}}=\text{CH}_2$), 5.30 (dq, $J = 17.2, 1.6$ Hz, 1H, $\text{CH}_2\text{-CH=CH}\underline{\text{H}}$), 5.21 (dq, $J = 10.4, 1.3$ Hz, 1H, $\text{CH}_2\text{-CH=CH}\underline{\text{H}}$), 5.08 (d, $J = 0.7$ Hz, 1H, H-1), 4.90 (d, $J = 11.5$ Hz, 1H, $\text{PhCH}\underline{\text{H}}$), 4.63 (d, $J = 11.5$ Hz, 1H, $\text{PhCH}\underline{\text{H}}$), 4.35 (dd, $J = 7.0, 5.7$ Hz, 1H, H-3), 4.23 – 4.14 (m, 2H, H-2, $\text{CH}\underline{\text{H}}\text{-CH=CH}_2$), 3.99 (ddd, $J = 12.7, 6.2, 1.3$ Hz, 1H, $\text{CH}\underline{\text{H}}\text{-CH=CH}_2$), 3.85 (ddd, $J = 11.7, 5.5, 3.3$ Hz, 1H, H-6a), 3.75 (ddd, $J = 11.9, 7.6, 4.7$ Hz, 1H, H-6b), 3.68 (ddd, $J = 10.1, 4.7, 3.3$ Hz, 1H, H-5), 3.55 (dd, $J = 10.1, 7.0$ Hz, 1H, H-4), 1.97 (dd, $J = 7.7, 5.6$ Hz, 1H, -OH), 1.51 (s, 3H, $\text{C}(\underline{\text{CH}}_3)_2$), 1.38 (s, 3H, $\text{C}(\underline{\text{CH}}_3)_2$). ^{13}C NMR (151 MHz, CDCl_3) δ 138.20, 133.54, 128.52, 128.17, 127.92, 118.12, 109.51, 96.42, 78.80, 76.14, 75.96, 72.99, 68.64, 68.21, 62.66, 28.12, 26.47. ESI HRMS (m/z): $[\text{M} + \text{Na}^+]$ calcd for $\text{C}_{19}\text{H}_{26}\text{NaO}_6$, 373.1622 found 373.1619.

Allyl 4-*O*-benzyl-2,3-*O*-isopropylidene-7-(*Si*-dimethylphenyl-silyl)-*L*-glycero- α -D-manno-heptopyranoside (9): Dess-Martin periodinane (1.8 g, 4.27



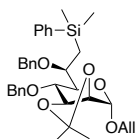
mmol) was washed with cooled ($-20\text{ }^\circ\text{C}$) diethyl ether (10 mL \times 5) before adding to the solution of **7** (1.0 g, 2.85 mmol) in DCM (10 mL) at $0\text{ }^\circ\text{C}$, and the reaction mixture was stirred for 2 h at room temperature. After TLC analysis showed consumption of starting material (DCM/acetone, 30/1, v/v), the reaction was diluted with DCM (40 mL) and quenched with sat. aq. $\text{Na}_2\text{S}_2\text{O}_3$ (5 mL). The two-phase reaction mixture was separated, and the organic phase was washed with sat. aq. NaHCO_3 (30 mL), and then H_2O (20 mL). The organic layer was dried (Na_2SO_4), filtered, and the filtration was concentrated *in vacuo*. After co-evaporated with toluene (3×10 mL), the resulting aldehyde **8** was used for the next step without additional purification.

Magnesium (1.0 g) was washed with 1.0M HCl (10 mL \times 5), H_2O (10 mL \times 3) and acetone (10 mL \times 3). After dried *in vacuo*, the activated Mg was added to dry THF (10 mL), and the solution was placed under an atmosphere of Argon. The

resulting suspension was heated to reflux (70 °C), and (phenyldimethylsilyl)methyl chloride (200 μ L, 1.2 mmol) was added. The reaction was initiated by 1,2-dibromoethane (100 μ L), and the second portion of (phenyldimethylsilyl)methyl chloride ($\text{PhSi}(\text{CH}_3)_2\text{CH}_2\text{Cl}$, 800 μ L, 4.8 mmol) was added dropwise. The reaction was refluxed for 1.5 h and then cooled to 0 °C to give the Grignard reagent as a dark grey suspension. The aldehyde **8** in dry THF (10 mL) was then added to the Grignard reagent suspension dropwise at 0 °C, and the resulting reaction mixture was stirred for 2 h at this temperature, after which it was warmed to room temperature and stirred overnight. The reaction was cooled to 0 °C again and then quenched with sat. aq. NH_4Cl (10 mL). The solids were filtered off, and the organic solvents were removed under reduced pressure. The resulting aqueous was extracted with EtOAc (3 \times 30 mL), and the resulting organic phase was washed with H_2O (50 mL). The organic layer was dried (Na_2SO_4), filtered, and the filtration was concentrated *in vacuo*. The resulting residue was purified by silica gel column chromatography (PE/EA = 10/1, v/v) to afford **9** (1.21 g, 85%) as a colorless oil. ^1H NMR (600 MHz, CDCl_3) δ 7.60 – 7.53 (m, 2H, Ar), 7.42 – 7.35 (m, 3H, Ar), 7.34 – 7.27 (m, 5H, Ar), 5.88 (m, 1H, $\text{CH}_2\text{-CH=CH}$), 5.28 (dq, J = 17.2, 1.6 Hz, 1H, $\text{CH}_2\text{-CH=CH}$), 5.21 (dq, J = 10.4, 1.3 Hz, 1H, $\text{CH}_2\text{-CH=CH}$), 5.11 (s, 1H, H-1), 4.87 (d, J = 11.2 Hz, 1H, PhCH), 4.60 (d, J = 11.2 Hz, 1H, PhCH), 4.33 (dd, J = 7.1, 5.7 Hz, 1H, H-3), 4.19 (ddt, J = 12.8, 5.2, 1.5 Hz, 1H, CH-CH=CH_2), 4.16 (d, J = 5.7 Hz, 1H, H-2), 4.10 (ddd, J = 13.3, 10.6, 3.5 Hz, 1H, H-6), 3.99 (ddt, J = 12.8, 6.2, 1.3 Hz, 1H, CH-CH=CH_2), 3.66 (dd, J = 10.0, 7.0 Hz, 1H, H-4), 3.42 (dd, J = 10.1, 1.6 Hz, 1H, H-5), 1.80 (d, J = 10.5 Hz, 1H, -OH), 1.52 (s, 3H, $\text{C}(\text{CH}_3)_2$), 1.38 (s, 3H, $\text{C}(\text{CH}_3)_2$), 1.33 (dd, J = 14.8, 11.2 Hz, 1H, H-7a), 0.93 (dd, J = 14.8, 3.6 Hz, 1H, H-7b), 0.37 (s, 3H, $\text{Si}(\text{CH}_3)_2$), 0.36 (s, 3H, $\text{Si}(\text{CH}_3)_2$). ^{13}C NMR (151 MHz, CDCl_3) δ 139.35, 138.27, 133.79, 129.01, 128.50, 128.30, 127.92, 117.99, 96.42, 79.14, 76.17, 75.83, 73.39, 72.08, 68.07, 67.48, 28.13, 26.52, 21.69, -1.97 ($\text{Si}(\text{CH}_3)_2$), -

2.16 (Si(CH₃)₂). ESI HRMS (m/z): [M + Na⁺] calcd for C₂₈H₃₈NaO₆Si, 521.2330 found 521.2328.

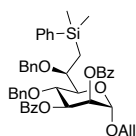
Allyl 4,6-di-*O*-benzyl-2,3-*O*-isopropylidene-7-(Si-dimethylphenyl-silyl)-*L*-glycero- α -D-manno-heptopyranoside (10): Compound **9** (1.21 g, 2.43 mmol)



and benzyl bromide (BnBr, 577 μ L, 4.86 mmol) were dissolved in dry DMF (20 mL), and the solution was cooled to 0°C. NaH (60% dispersion in mineral oil, 195 mg, 4.86 mmol) was then added slowly. The reaction mixture was stirred for 3 h at room temperature, after which it was quenched with sat. aq. NH₄Cl (1 mL) and was concentrated *in vacuo*. The resulting residue was dissolved in DCM (100 mL) and washed with H₂O (50 mL). The aqueous layer was back extracted with DCM (3 \times 10 mL), and the organic layer was combined, dried (Na₂SO₄), filtered, and the filtrate was concentrated *in vacuo*. The resulting residue was purified by silica gel column chromatography (PE/EA = 10/1, v/v) to afford **10** (1.26 g, 88%) as a light-yellow oil. ¹H NMR (600 MHz, CDCl₃) δ 7.50 (dt, *J* = 6.7, 1.5 Hz, 2H, Ar), 7.40 – 7.35 (m, 1H, Ar), 7.32 (dd, *J* = 7.8, 6.5 Hz, 2H, Ar), 7.28 – 7.21 (m, 3H, Ar), 7.20 – 7.13 (m, 5H, Ar), 7.11 – 7.07 (m, 2H, Ar), 5.90 – 5.82 (m, 1H, CH₂-CH=CH₂), 5.26 (dq, *J* = 17.2, 1.6 Hz, 1H, CH₂-CH=CH₂), 5.18 (dq, *J* = 10.5, 1.4 Hz, 1H, CH₂-CH=CH₂), 5.15 (d, *J* = 1.2 Hz, 1H, H-1), 4.77 (d, *J* = 11.6 Hz, 1H, PhCH₂), 4.46 (d, *J* = 11.8 Hz, 1H, PhCH₂), 4.29 (t, *J* = 6.4 Hz, 1H, H-3), 4.20 (ddt, *J* = 12.9, 5.1, 1.6 Hz, 1H, CH₂-CH=CH₂), 4.16 – 4.09 (m, 3H, H-2, PhCH₂), 4.00 (ddt, *J* = 13.0, 6.1, 1.4 Hz, 1H, CH₂-CH=CH₂), 3.97 (ddd, *J* = 7.5, 5.8, 1.4 Hz, 1H, H-6), 3.66 (dd, *J* = 9.9, 6.7 Hz, 1H, H-4), 3.48 (dd, *J* = 9.9, 1.4 Hz, 1H, H-5), 1.50 (s, 3H, C(CH₃)₂), 1.37 (dd, *J* = 6.9, 2.6 Hz, 2H, H-7a/b), 1.35 (s, 3H, C(CH₃)₂), 0.32 (s, 3H, Si(CH₃)₂), 0.31 (s, 3H, Si(CH₃)₂). ¹³C NMR (151 MHz, CDCl₃) δ 138.80, 138.76, 138.63, 133.80, 133.67, 129.16, 128.25, 128.22, 128.14, 128.01, 127.58, 127.47, 127.39, 117.79, 109.42, 96.86, 79.24, 75.93, 75.86, 73.41, 72.04, 71.75, 71.01, 68.50, 27.98, 26.46, 17.98, -1.92 (Si(CH₃)₂), -

2.22 ($\text{Si}(\text{CH}_3)_2$). ESI HRMS (m/z): $[\text{M} + \text{Na}^+]$ calcd for $\text{C}_{35}\text{H}_{44}\text{NaO}_6\text{Si}$, 611.2799 found 611.2792.

Allyl 4,6-di-*O*-benzyl-2,3-di-*O*-benzoyl-7-(*Si*-dimethylphenyl-silyl)-*L*-glycero- α -D-manno-heptopyranoside (11**):** Compound **10** (465 mg, 0.79 mmol)

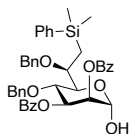


was dissolved in aqueous AcOH (80%, 5 mL), and the solution was stirred at 80 °C for 2 h. The reaction mixture was then cooled to room temperature and concentrated under reduced pressure. The resulting residue was co-evaporated with toluene (5×5 mL) to give the diol, which was dissolved in dry pyridine (5 mL), and the solution was cooled to 0 °C. Benzoyl chloride (367 μL , 3.16 mmol) was added dropwise to the solution, and the reaction mixture was stirred at room temperature overnight. The reaction was quenched with MeOH (2 mL) and concentrated under reduced pressure. The residue was dissolved in EtOAc and washed with sat. aq. NaHCO_3 , and the organic layer was dried (Na_2SO_4) and filtered. The filtrate was concentrated *in vacuo*, and the resulting residue was purified by silica gel column chromatography (PE/EA = 10/1, v/v) to give **11** (548 mg, 92%) as a light-yellow oil. ^1H NMR (600 MHz, Chloroform-*d*) δ 8.06 – 7.98 (m, 2H, Ar), 7.88 (dd, J = 8.2, 1.4 Hz, 2H, Ar), 7.58 – 7.46 (m, 4H, Ar), 7.39 – 7.29 (m, 7H, Ar), 7.25 (m, 5H, Ar), 7.14 (m, 3H, Ar), 6.99 (dd, J = 6.6, 2.9 Hz, 2H, Ar), 5.90 – 5.84 (m, $\text{CH}_2\text{-CH=CH}_2$), 5.75 (dd, J = 9.4, 3.2 Hz, 1H, H-3), 5.60 (dd, J = 3.2, 2.0 Hz, 1H, H-2), 5.31 (dq, J = 17.2, 1.6 Hz, 1H, $\text{CH}_2\text{CH=CHH}$), 5.22 (dq, J = 10.4, 1.5 Hz, 1H, $\text{CH}_2\text{CH=CHH}$), 5.10 (d, J = 2.0 Hz, 1H, H-1), 4.65 (d, J = 11.7 Hz, 1H, PhCHH), 4.51 (d, J = 11.0 Hz, 1H, PhCHH), 4.31 (t, J = 9.5 Hz, 1H, H-4), 4.24 – 4.16 (m, 3H, PhCH_2 , CHH-CH=CH_2), 4.07 (ddd, J = 9.3, 4.5, 1.3 Hz, 1H, H-6), 4.02 (ddt, J = 13.0, 6.2, 1.4 Hz, 1H, CHH-CH=CH_2), 3.78 (dd, J = 9.6, 1.3 Hz, 1H, H-5), 1.54 – 1.43 (m, 2H, H-7a/b), 0.35 (s, 3H, $\text{SiPh}(\text{CH}_3)_2$), 0.35 (s, 3H, $\text{SiPh}(\text{CH}_3)_2$). ^{13}C NMR (151 MHz, CDCl_3) δ 165.58, 165.57, 138.74, 138.55, 138.02, 133.79, 133.38, 133.35, 133.16, 130.01, 129.95, 129.81, 129.76, 129.31, 128.58, 128.46, 128.31, 128.11, 127.91, 127.78, 127.66, 127.54, 118.05, 96.71

(C-1), 74.67, 74.46, 74.01, 73.17, 73.15, 70.94, 70.10, 68.68, 17.03, -1.88 (SiPh(CH₃)₂), -2.30 (SiPh(CH₃)₂). ESI HRMS (m/z): [M + Na⁺] calcd for C₄₆H₄₈NaO₈Si, 779.3011 found 779.3012.

4,6-Di-*O*-benzyl-2,3-di-*O*-benzoyl-7-(Si-dimethylphenyl-silyl)-L-glycero- α -

D-manno-heptopyra-noside (12**):** A suspension of **11** (538 mg, 0.71 mmol) and



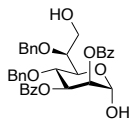
PdCl₂ (25.1 mg, 0.142 mmol) in MeOH (5 mL) was stirred overnight, after which the reaction mixture was diluted with diethyl ether (10 mL). The mixture was filtered over celite, and the filtrate was concentrated under reduced pressure. The resulting residue was

purified by silica gel column chromatography (PE/EA = 5/1, v/v) to afford **12**

(431 mg, 85%) as a light-yellow solid. ¹H NMR (600 MHz, Chloroform-*d*) δ 8.02 – 7.97 (m, 2H, Ar), 7.88 – 7.84 (m, 2H, Ar), 7.62 (dd, *J* = 7.2, 2.0 Hz, 2H, Ar), 7.57 – 7.53 (m, 1H, Ar), 7.51 – 7.46 (m, 1H, Ar), 7.43 – 7.37 (m, 5H, Ar), 7.36 – 7.29 (m, 7H, Ar), 7.18 – 7.13 (m, 3H, Ar), 7.05 (dd, *J* = 6.6, 2.9 Hz, 2H, Ar), 5.65 (dd, *J* = 9.7, 3.2 Hz, 1H, H-3), 5.44 (dd, *J* = 3.2, 2.0 Hz, 1H, H-2), 5.12 (t, *J* = 2.6 Hz, 1H, H-1), 4.79 (d, *J* = 11.6 Hz, 1H, PhCHH), 4.59 (d, *J* = 11.1 Hz, 1H, PhCHH), 4.43 – 4.37 (m, 2H, H-4, PhCHH), 4.31 (d, *J* = 11.6 Hz, 1H, PhCHH), 4.16 – 4.11 (m, 1H, H-6), 3.73 (dd, *J* = 9.5, 1.5 Hz, 1H, H-5), 1.70 (dd, *J* = 14.2, 11.4 Hz, 1H, H-7a), 1.45 (dd, *J* = 14.2, 4.1 Hz, 1H, H-7b), 1.21 (d, *J* = 3.1 Hz, 1H, -OH), 0.41 (s, 3H, SiPh(CH₃)₂), 0.31 (s, 3H, SiPh(CH₃)₂). ¹³C NMR (151 MHz, CDCl₃) δ 165.57, 165.47, 139.50, 139.01, 138.18, 134.12, 133.88, 133.30, 133.09, 130.15, 130.01, 129.98, 129.83, 129.76, 129.53, 128.65, 128.57, 128.50, 128.48, 128.45, 128.42, 128.34, 128.08, 128.05, 127.63, 127.59, 127.53, 127.49, 92.13 (C-1), 74.81, 74.01, 74.00, 73.38, 72.56, 70.91, 69.93, 16.48, -0.74 (SiPh(CH₃)₂), -3.75 (SiPh(CH₃)₂). ESI HRMS (m/z): [M + Na⁺] calcd for C₄₃H₄₄NaO₈Si, 739.2698 found 739.2691.

4,6-Di-*O*-benzyl-2,3-di-*O*-benzoyl-*L*-glycero-*D*-manno-heptopyranoside (**13**):

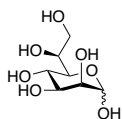
A solution of **12** (425 mg, 0.59 mmol) in acetic acid (5 mL) was added sodium



acetate (593 mg) and potassium bromide (85 mg, 0.71 mmol). The suspension was stirred vigorously until all NaOAc was dissolved, and the mixture was then cooled to 10 °C. The solution was placed

under darkness and peracetic acid (ca. 35wt.% sol. in diluted acetic acid, 3.2 mL) was added dropwise to the mixture at 10 °C, and during the addition gas was liberated. The reaction mixture was stirred at room temperature for 3 h, and then quenched with aqueous solution of Na₂S₂O₃ (20%, 10 mL). The mixture was extracted with EtOAc (10 mL × 5), and the combined organic layers were washed with sat. aq. NaHCO₃ (30 mL) and H₂O (20 mL). The organic phase was dried (Na₂SO₄), filtered, and the filtrate was concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography (PE/EA = 1/1, v/v) to give **13** (290 mg, 82%) as a white solid. ¹H NMR (600 MHz, CDCl₃) δ 8.07 – 7.99 (m, 2H, Ar), 7.93 – 7.86 (m, 2H, Ar), 7.58 (tt, *J* = 7.3, 1.3 Hz, 1H, Ar), 7.50 (ddt, *J* = 8.7, 7.2, 1.3 Hz, 1H, Ar), 7.45 – 7.30 (m, 9H, Ar), 7.16 (m, 3H, Ar), 7.09 (dd, *J* = 7.2, 2.5 Hz, 2H, Ar), 5.85 (dd, *J* = 9.4, 3.3 Hz, 1H, H-3), 5.63 (dd, *J* = 3.3, 2.0 Hz, 1H, H-2), 5.41 (d, *J* = 2.5 Hz, 1H, H-1), 4.84 (d, *J* = 11.5 Hz, 1H, PhCHH), 4.65 (d, *J* = 11.0 Hz, 1H, PhCHH), 4.51 (d, *J* = 11.6 Hz, 1H, PhCHH), 4.43 (m, 2H, H-4, PhCHH), 4.32 (dd, *J* = 9.7, 1.6 Hz, 1H, H-5), 4.07 – 3.98 (m, 2H, H-6, H-7a), 3.92 (dd, *J* = 10.6, 3.2 Hz, 1H, H-7b). ¹³C NMR (151 MHz, CDCl₃) δ 165.64, 138.46, 137.78, 133.46, 133.27, 130.03, 129.80, 129.76, 128.68, 128.64, 128.53, 128.50, 128.48, 127.90, 127.82, 127.80, 92.52 (C-1), 75.45, 74.75, 73.44, 72.75, 72.73, 72.04, 71.28, 61.98. HRMS (*m/z*): [*M* + Na⁺] calcd for C₃₅H₃₄NaO₉, 621.2095 found 621.2094.

***L*-glycero- α/β -*D*-manno-heptose:** Compound **13** (10 mg, 0.016 mmol) was

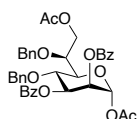


dissolved in MeOH (2 mL), and NaOH (aqueous 0.2 M) was added to the solution. The reaction mixture was stirred for 3 h at room temperature, after which it was neutralized with Amberlite (H⁺,

washed with MeOH, aq. 1 M NaOH, H₂O, aq. 1 M HCl, H₂O and MeOH sequentially) till pH = 7. The resin was filtered off, and the filtrate was concentrated under reduced pressure. The residue was co-evaporated with toluene (3 × 5 mL) and then dissolved in a mixture of MeOH and water (5 mL, 1/1, v/v), and the solution was placed under an atmosphere of H₂. Pd(OH)₂/C was added to the solution, and the reaction was stirred overnight at room temperature, after which the solid was filtered off, and the filtrate was concentrated *in vacuo*. The resulting residue was dissolved in water (2 mL) and then lyophilized to give L,D-heptose as a white foam (2.9 mg, 86% α/β = 2/1). NMR data consistent with the literature.⁴⁹ ¹H NMR (600 MHz, Deuterium Oxide) δ 5.18 (d, J = 1.8 Hz, 1H, α H-1), 4.87 (d, J = 0.9 Hz, 0.5 H, β H-1), 4.04 (ddd, J = 7.3, 5.4, 1.6 Hz, 1H, α H-6), 4.01 – 3.97 (m, 0.5H, β H-6), 3.95 – 3.91 (m, 1.5H, α H-2, β H-2), 3.88 – 3.83 (m, 2H, α H-3, α H-4), 3.80 (t, J = 9.8 Hz, 0.5H, β H-4), 3.78 – 3.74 (m, 1H, α H-5), 3.73 – 3.64 (m, 3.5H, β H-7a/b, α H-7a/b, β H-3), 3.34 (dd, J = 9.9, 1.7 Hz, 0.5H, β H-5). ¹³C NMR (151 MHz, D₂O) δ 94.16, 93.90, 74.59, 73.24, 71.18, 70.96, 70.61, 70.52, 68.77, 68.66, 66.19, 65.84, 62.99, 62.71.

1,7-Di-*O*-acetyl-4,6-di-*O*-benzyl-2,3-di-*O*-benzoyl-L-glycero-D-manno-

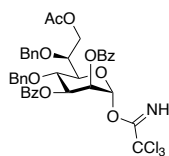
heptopyranoside (14): A solution of **13** (245 mg, 0.41 mmol) in dry pyridine (5 mL) was added acetic anhydride (Ac₂O, 309 μ L, 3.27 mmol) and 4-(dimethylamino)pyridine (DMAP, 3 mg, 0.024 mmol), and the mixture was stirred overnight at room temperature. The reaction mixture was then concentrated under reduced pressure, and the resulting residue was dissolved in EtOAc (20 mL) and washed with sat. aq. NaHCO₃ (10 mL). The organic layer was dried (Na₂SO₄), filtered, and the filtrate was concentrated *in vacuo*. The resulting residue was purified by silica gel column chromatography (PE/EA = 3/1, v/v) to give **14** (253 mg, 90%) as a colorless oil. ¹H NMR (600 MHz, Chloroform-*d*) δ 8.06 – 8.01 (m, 2H, Ar), 7.92 – 7.86 (m, 2H, Ar), 7.60 (tt, J = 7.5, 1.3 Hz, 1H, Ar), 7.52 (tt, J = 7.4, 1.3 Hz, 1H, Ar), 7.48 – 7.28 (m, 9H, Ar), 7.22 – 7.13 (m, 3H, Ar), 7.10 – 7.05 (m, 2H, Ar),



6.30 (d, $J = 2.2$ Hz, 1H, H-1), 5.79 (dd, $J = 9.4, 3.3$ Hz, 1H, H-3), 5.64 (dd, $J = 3.3, 2.3$ Hz, 1H, H-2), 4.89 (d, $J = 11.7$ Hz, 1H, PhCHH), 4.62 (d, $J = 10.9$ Hz, 1H, PhCHH), 4.58 – 4.52 (m, 2H, H-7a, PhCHH), 4.41 (t, $J = 9.5$ Hz, 1H, H-4), 4.36 (d, $J = 10.9$ Hz, 1H, PhCHH), 4.29 (dd, $J = 11.4, 6.6$ Hz, 1H, H-7b), 4.10 (td, $J = 6.3, 1.8$ Hz, 1H, H-6), 4.06 (dd, $J = 9.7, 1.8$ Hz, 1H, H-5), 2.19 (s, 3H, OAc), 2.07 (s, 3H, OAc). ^{13}C NMR (151 MHz, CDCl_3) δ 170.70, 168.34, 165.64, 165.26, 138.27, 137.49, 133.64, 133.46, 130.10, 129.82, 129.54, 129.41, 128.70, 128.66, 128.58, 128.54, 128.49, 128.04, 127.96, 127.88, 127.82, 127.76, 90.99 (C-1), 74.96, 73.86, 73.29, 72.85, 72.68, 72.46, 69.59, 62.65, 21.05, 21.02. HRMS (m/z): $[\text{M} + \text{Na}^+]$ calcd for $\text{C}_{39}\text{H}_{38}\text{NaO}_{11}$, 705.2306 found 705.2305.

2,3-Di-*O*-benzoyl-4,6-di-*O*-benzyl-7-*O*-acetyl-L-glycero-D-manno-

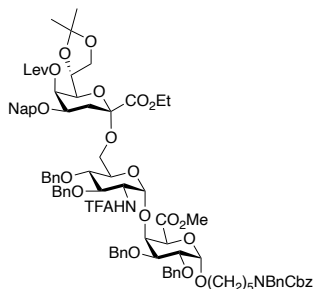
heptopyranosyl trichloroacetimidate (**6**): A solution of **14** (125 mg, 0.183 mmol) in dry DMF (1 mL) was added hydrazine acetate (33 mg, 0.366 mmol) under an atmosphere of Argon, and the reaction mixture was stirred at room temperature for 2 h. The reaction mixture was then filtered over celite and evaporated to dryness.



The residue was dissolved in DCM (30 mL) and washed with brine (10 mL). The aqueous layer was back extracted with DCM (3×10 mL), and the organic layer was combined, dried (Na_2SO_4), filtered, and the filtrate was concentrated *in vacuo*. The resulting residue was co-evaporated with toluene (3×3 mL) and dissolved in dry DCM (1 mL) and the solution was placed under an atmosphere of Argon, which was followed by the addition of trichloroacetonitrile (300 μL , 3.0 mmol) and a catalytic amount of DBU (5.5 μL , 0.036 mmol). The reaction mixture was stirred at room temperature for 2 h and then concentrated *in vacuo*. The resulting residue was purified by silica gel column chromatography (PE/EA = 3/1, v/v) to afford the heptosyl donor **6** (128 mg, 90%) as a white solid. ^1H NMR (600 MHz, CDCl_3) δ 8.77 (s, 1H, =NH), 8.06 – 8.02 (m, 2H, Ar), 7.91 – 7.87 (m, 2H, Ar), 7.61 (tt, $J = 7.4, 1.3$ Hz, 1H, Ar), 7.52 (tt, $J = 7.4, 1.3$ Hz, 1H, Ar), 7.44 – 7.33 (m, 9H, Ar), 7.18 (m, 3H, Ar), 7.09 (dd, $J = 6.7, 2.9$ Hz, 2H, Ar), 6.49 (d, $J = 1.7$

Hz, 1H, H-1), 5.88 – 5.83 (m, 2H, H-2, H-3), 4.89 (d, $J = 11.6$ Hz, 1H, PhCHH), 4.66 (d, $J = 10.9$ Hz, 1H, PhCHH), 4.57 (dd, $J = 11.2, 5.9$ Hz, 1H, H-7a), 4.54 (d, $J = 11.6$ Hz, 1H, PhCHH), 4.50 (t, $J = 9.3$ Hz, 1H, H-4), 4.42 (d, $J = 10.9$ Hz, 1H, PhCHH), 4.27 (dd, $J = 11.2, 7.1$ Hz, 1H, H-7b), 4.22 (dd, $J = 9.7, 1.7$ Hz, 1H, H-5), 4.15 (ddd, $J = 7.4, 5.9, 1.5$ Hz, 1H, H-6), 2.04 (s, 3H, -OAc). ^{13}C NMR (151 MHz, CDCl_3) δ 170.70, 165.51, 165.22, 160.02, 138.29, 137.46, 133.67, 133.41, 130.11, 129.79, 129.57, 129.37, 128.71, 128.65, 128.58, 128.55, 128.09, 127.99, 127.92, 127.66, 94.97, 75.05, 73.80, 73.59, 72.70, 72.50, 69.21, 62.39, 21.08. HRMS (m/z): $[\text{M} + \text{Na}^+]$ calcd for $\text{C}_{39}\text{H}_{36}\text{Cl}_3\text{NNaO}_{10}$, 806.1297 found 806.1296.

***N*-(Benzyl)-*N*-benzyloxycarbonyl-5-aminopentyl-ethyl (4-*O*-(2-methylnaphthyl)-5-*O*-levulinoyl-7,8-*O*-isopropylidene-3-deoxy- α -D-manno-oct-2-ulopyranoside)onate(2 \rightarrow 6)-2-deoxy-2-trifluoroacetamido-3, 4-di-*O*-benzyl- α -D-glucopyranosyl-(1 \rightarrow 4)-methyl (2, 3-di-*O*-benzyl)- α -D-galactopyranosyl urinate (15):** Disaccharide acceptor **3** (471 mg, 0.41 mmol)



and Kdo donor **4** (540 mg, 0.82 mmol) were co-evaporated with toluene (3×5 mL) and were dissolved in dry acetonitrile (5 mL) and the solution was placed under an atmosphere of Argon. *N*-iodosuccinimide (284 mg, 1.26 mmol) and flame activated 4Å M.S. were then added. The mixture was stirred for 15 min at 0°C, after which trifluoromethanesulfonic acid (TfOH, 7.3 μL , 0.082 mmol) was added, and the reaction was stirred for 2 h at 0°C. After TLC analysis showed consumption of disaccharide acceptor, the reaction was quenched with Et_3N (0.5 mL). The molecular sieves were filtered off, and the solution was concentrated *in vacuo*. The resulting residue was purified by silica gel column chromatography (toluene/EA = 3/1, v/v) to give **15** (487 mg, 71% of α -anomer) as a light-yellow oil. ^1H NMR (600 MHz, CDCl_3) δ 7.83 – 7.69 (m, 4H, Ar), 7.42 – 7.16 (m, 31H, Ar), 7.09 (dd, $J = 7.4, 2.2$ Hz, 2H, Ar), 5.52 (dd, $J = 2.7, 1.4$ Hz, 1H, Kdo-H-5),

5.16 (d, $J = 15.7$ Hz, 2H, CH₂-Cbz), 4.84 (bs, 1H, GalA-H-1), 4.78 (dd, $J = 12.0$, 6.7 Hz, 2H, PhCH₂), 4.74 – 4.67 (m, 3H, GlcNTFA-H-1, PhCH₂), 4.67 – 4.63 (m, 1H, PhCHH), 4.60 (d, $J = 12.5$ Hz, 1H, PhCHH), 4.55 (dd, $J = 13.0$, 11.4 Hz, 2H, PhCH₂), 4.51 (d, $J = 11.0$ Hz, 1H, PhCHH), 4.49 – 4.44 (m, 2H, PhCHH, PhCHH), 4.33 – 4.29 (m, 2H, GalA-H-4, PhCHH), 4.27 – 4.20 (m, 2H, GlcNTFA-H-5, GalA-H-5), 4.19 (dt, $J = 6.8$, 2.6 Hz, 1H, GlcNTFA-H-2), 4.17 – 4.12 (m, 2H, CO₂CH₂CH₃), 4.10 (dt, $J = 7.4$, 5.9 Hz, 1H, Kdo-H-7), 4.01 (dd, $J = 8.6$, 6.1 Hz, 1H, Kdo-H-8a), 3.87 (ddd, $J = 11.7$, 4.8, 2.8 Hz, 1H, Kdo-H-4), 3.79 – 3.68 (m, 3H, Kdo-H-8b, GalA-H-2, GalA-H-3), 3.65 (s, 3H, CO₂CH₃), 3.65 – 3.50 (m, 5H, GlcNTFA-H-3, GlcNTFA-H-4, GlcNTFA-H-6a, Kdo-H-6, CHH-Linker), 3.41 (m, 2H, GlcNTFA-H-6b, CHH-Linker), 3.25 – 3.13 (m, 2H, CH₂-Linker), 2.78 – 2.60 (m, 4H, CH₂CH₂-Lev), 2.29 (ddd, $J = 13.1$, 4.9, 1.2 Hz, 1H, Kdo-H_{eq}-3), 2.11 (s, 3H, CH₃-Lev), 1.96 (dd, $J = 13.2$, 11.9 Hz, 1H, Kdo-H_{ax}-3), 1.58 – 1.43 (m, 2H, CH₂-Linker), 1.31 (s, 3H, C(CH₃)₂), 1.29 – 1.22 (m, 12H, CH₂-Linker, CHH-Linker, CO₂CH₂CH₃, C(CH₃)₂). 0.89 (q, $J = 7.3$ Hz, 1H, CHH-Linker). ¹³C NMR (151 MHz, CDCl₃) δ 206.32, 171.96, 167.23, 138.09, 137.70, 135.38, 133.37, 133.12, 128.69, 128.67, 128.63, 128.61, 128.58, 128.36, 128.12, 128.02, 127.97, 127.82, 127.79, 127.70, 127.35, 126.75, 126.25, 126.01, 125.83, 109.44, 98.93, 97.60, 97.44, 79.09, 78.31, 76.38, 75.44, 75.07, 74.82, 73.51, 72.85, 72.58, 71.40, 70.83, 70.56, 66.98, 65.32, 62.45, 61.68, 53.42, 52.53, 47.33, 38.18, 33.34, 29.91, 29.71, 28.21, 26.75, 25.58, 25.53, 22.77, 14.25, 11.93. HRMS (m/z): [M + Na⁺] calcd for C₉₂H₁₀₃F₃N₂NaO₂₃, 1683.6796 found 1683.6800.

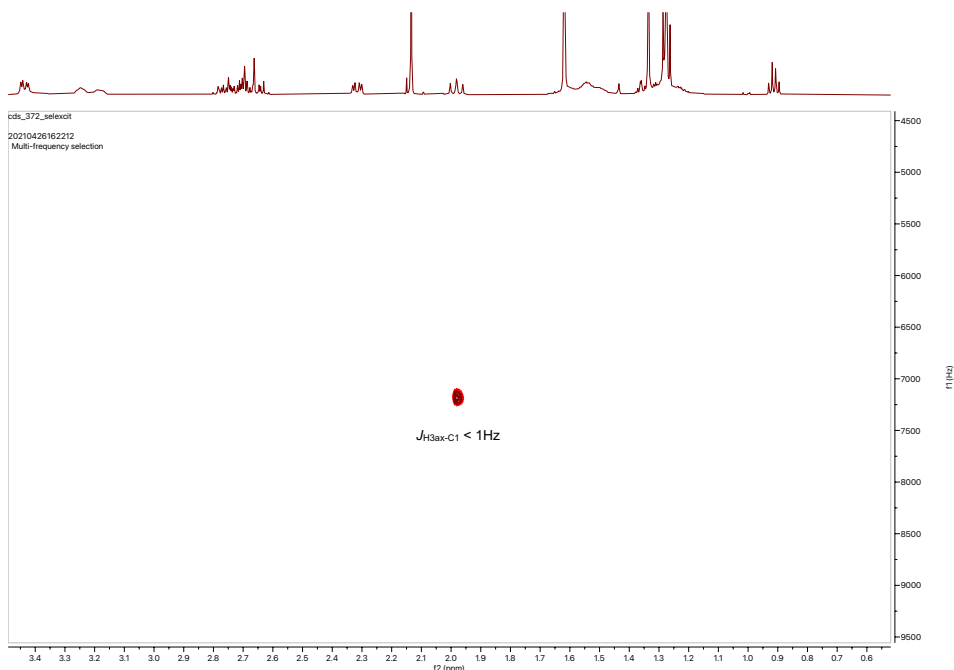
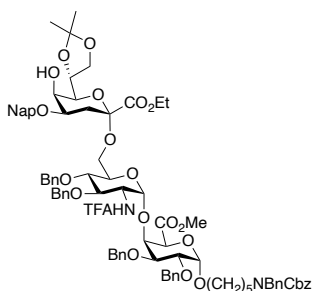


Figure S1. EXSIDE NMR of trisaccharide **15**.

***N*-(Benzyl)-*N*-benzyloxycarbonyl-5-aminopentyl-ethyl (4-*O*-(2-methylnaphthyl)-7,8-*O*-isopropylidene-3-deoxy- α -D-manno-oct-2-ulopyranoside)onate-(2 \rightarrow 6)-2-deoxy-2-trifluoroacetamido-3,4-di-*O*-benzyl- α -D-glucopyranosyl-(1 \rightarrow 4)-methyl (2, 3-di-*O*-benzyl)- α -D-galactopyranosyl urinate (**16**):** Trisaccharide **15** (480 mg, 0.29 mmol) and $\text{NH}_2\text{NH}_2 \cdot \text{HOAc}$ (54 mg,

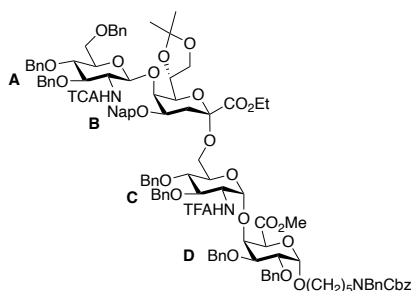


0.58 mmol) were dissolved in a mixture of DCM/MeOH (10 mL / 1 mL), and the reaction was stirred for 3 h at room temperature. After TLC analysis showed consumption of starting material, the reaction mixture was concentrated *in vacuo*. The obtained residue was dissolved in DCM (20 mL) and washed with H_2O (10 mL). The organic

layer was dried (Na_2SO_4), filtered, and the filtration was concentrated *in vacuo*. The resulting residue was purified by silica gel column chromatography

(DCM/acetone = 15/1, v/v) to give the trisaccharide acceptor **16** (317 mg, 70%) as a light-yellow oil. ^1H NMR (600 MHz, CDCl_3) δ 7.79 (d, $J = 8.4$ Hz, 1H, Ar), 7.76 – 7.70 (m, 3H, Ar), 7.42 (dd, $J = 8.4, 1.7$ Hz, 1H, Ar), 7.40 – 7.13 (m, 30H), 7.09 (dd, $J = 7.3, 2.2$ Hz, 2H, Ar), 5.16 (d, $J = 16.5$ Hz, 2H, $\text{CH}_2\text{-Cbz}$), 4.85 (bs, 1H, GalA-H-1), 4.79 (d, $J = 12.1$ Hz, 1H, PhCHH), 4.75 – 4.72 (m, 2H, GlcNTFA-H-1, PhCHH), 4.71 (m, 2H, PhCH_2), 4.69 – 4.63 (m, 3H, PhCH_2 , PhHH), 4.58 (d, $J = 11.0$ Hz, 1H, PhCHH), 4.53 (d, $J = 11.0$ Hz, 1H, PhHH), 4.47 (m, 2H, PhCHH , PhCHH), 4.35 (m, 3H, Kdo-H-7, GalA-H-4, PhCHH), 4.27 – 4.20 (m, 3H, GlcNTFA-H-2, GlcNTFA-H-5, GalA-H-5), 4.16 (q, $J = 7.1$ Hz, 2H, $\text{CO}_2\text{CH}_2\text{CH}_3$), 4.06 – 4.01 (m, 2H, Kdo-H-5, Kdo-H-8a), 3.86 – 3.79 (m, 2H, Kdo-H-4, GalA-H-3), 3.74 (m, 2H, Kdo-H-8b, GalA-H-2), 3.65 (s, 3H, CO_2CH_3), 3.64 – 3.56 (m, 2H, GlcNTFA-H-2, GlcNTFA-H-4), 3.56 – 3.48 (m, 1H, GlcNTFA-H-6a), 3.45 – 3.34 (m, 4H, Kdo-H-6, GlcNTFA-H-6b, $\text{CH}_2\text{-Linker}$), 3.26 – 3.13 (m, 2H, $\text{CH}_2\text{-Linker}$), 2.32 (dd, $J = 13.2, 4.9$ Hz, 1H, Kdo- $\text{H}_{\text{eq}}\text{-3}$), 2.00 (t, $J = 13.0$, 1H, Kdo- $\text{H}_{\text{ax}}\text{-3}$), 1.59 – 1.44 (m, 4H, $\text{CH}_2\text{-Linker} \times 2$), 1.34 (s, 3H, $\text{C}(\text{CH}_3)_2$), 1.31 – 1.20 (m, 11H, $\text{CH}_2\text{-Linker}$, $\text{CO}_2\text{CH}_2\text{CH}_3$, $\text{C}(\text{CH}_3)_2$). ^{13}C NMR (151 MHz, CDCl_3) δ 177.01, 167.35, 138.24, 138.08, 137.74, 135.11, 133.34, 133.18, 128.69, 128.67, 128.63, 128.57, 128.11, 128.04, 128.01, 127.97, 127.94, 127.84, 127.81, 127.66, 127.41, 126.68, 126.45, 126.20, 125.68, 109.30, 99.02, 97.59, 79.18, 78.24, 76.35, 75.48, 75.20, 75.04, 74.78, 73.77, 73.36, 73.20, 72.90, 72.59, 70.90, 70.30, 69.36, 69.00, 67.33, 64.41, 62.29, 61.64, 53.43, 52.51, 31.72, 29.19, 26.71, 25.49, 23.44, 14.26. HRMS (m/z): $[\text{M} + \text{Na}^+]$ calcd for $\text{C}_{87}\text{H}_{97}\text{F}_3\text{N}_2\text{NaO}_{21}$, 1585.6428 found 1585.6429.

***N*-(Benzyl)-*N*-benzyloxycarbonyl-5-aminopentyl-2-deoxy-2-trichloroacetamido-3,4,6-tri-*O*-benzyl- β -D-glucopyranosyl-(1 \rightarrow 5)-ethyl (4-*O*-(2-methylnaphthyl)-7, 8-*O*-isopropylidene-3-deoxy- α -D-manno-oct-2-ulopyranoside)onate(2 \rightarrow 6)-2-deoxy-2-trifluoroacetamido-3, 4-di-*O*-benzyl- α -D-glucopyranosyl-(1 \rightarrow 4)-methyl (2,3-di-*O*-benzyl)- α -D-galactopyranosyl urinate (9):** Trisaccharide acceptor **16** (264 mg, 0.169 mmol) and

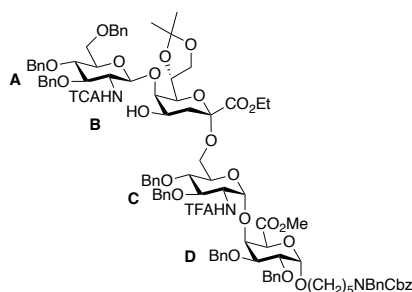


monosaccharide donor **5** (234 mg, 0.34 mmol) were co-evaporated with toluene (5 mL \times 3) and were dissolved in DCM/MeCN (2 mL / 0.5 mL) under an atmosphere of Argon. NIS (112.5 mg, 0.5 mmol) and flame activated 4Å M.S. were added. The suspension was stirred for 15 min at 0°C,

after which was added TfOH (3.0 μ L, 0.034 mmol), and the reaction mixture was stirred for 6 h at 0°C. The reaction was quenched with Et₃N (0.5 mL), and the molecular sieves were filtered off. The obtained solution was concentrated *in vacuo*, and the resulting residue was purified by silica gel column chromatography (PE/EA = 3/1, v/v) to give **17** (274 mg, 76%) as a light-yellow oil. ¹H NMR (600 MHz, CDCl₃) δ 7.84 – 7.69 (m, 4H, Ar), 7.43 – 7.10 (m, 48H, Ar), 6.86 (d, *J* = 8.2 Hz, 1H, NHTCA), 6.60 (s, 1H, NHTFA), 5.17 (d, *J* = 16.4 Hz, 2H, CH₂-Cbz), 4.89 – 4.85 (bs, 1H, D-H-1), 4.83 – 4.77 (m, 3H, A-H-1, C-H-1, PhCHH), 4.77 – 4.73 (m, 2H, PhCHH), 4.72 – 4.64 (m, 4H, PhCH₂ \times 2), 4.62 – 4.51 (m, 6H, PhCH₂ \times 3), 4.51 – 4.45 (m, 2H, PhCH₂), 4.40 (d, *J* = 10.8 Hz, 1H, PhCHH), 4.37 – 4.33 (m, 2H, D-H-4, PhCHH), 4.31 (dd, *J* = 6.8, 5.1 Hz, 1H, C-H-5), 4.25 (m, 3H, A-H-5, C-H-2, D-H-5), 4.14 – 4.06 (m, 3H, B-H-8a, CO₂CH₂CH₃), 4.00 – 3.96 (m, 1H, B-H-5), 3.95 – 3.89 (m, 1H, B-H-8b), 3.89 – 3.80 (m, 3H, A-H-2, B-H-4, D-H-3), 3.77 (dd, *J* = 10.1, 3.4 Hz, 1H, D-H-2), 3.74 – 3.69 (m, 2H, A-H-6a/b), 3.67 (s, 3H, CO₂CH₃), 3.66 – 3.60 (m, 4H, B-H-6, C-H-3, C-H-4, C-H-6a), 3.59 – 3.55 (m, 2H, C-H-6b, CHH-Linker), 3.49 (ddd, *J* =

9.5, 4.2, 2.6 Hz, 1H, A-H-4), 3.38 (m, 2H, A-H-3, CHH-Linker), 3.25 – 3.14 (m, 2H, CH₂-Linker), 2.40 – 2.33 (m, 1H, B-H_{eq}-3), 2.07 (t, *J* = 12.3 Hz, 1H, B-H_{ax}-3), 1.61 – 1.44 (m, 4H, CH₂-Linker × 2), 1.32 (s, 3H, C(CH₃)₂), 1.30 – 1.13 (m, 11H, CH₂-Linker, CO₂CH₂CH₃, C(CH₃)₂). ¹³C NMR (151 MHz, CDCl₃) δ 167.25, 161.51, 157.73, 138.32, 138.22, 138.12, 138.07, 137.92, 137.73, 134.82, 133.26, 128.88, 128.65, 128.64, 128.62, 128.59, 128.56, 128.53, 128.51, 128.40, 128.09, 128.06, 128.00, 127.96, 127.95, 127.91, 127.80, 127.75, 127.74, 127.71, 127.43, 127.27, 127.06, 126.77, 126.48, 125.78, 116.79, 114.88, 108.56, 100.93, 98.91, 97.77, 97.55, 92.71, 83.03, 79.30, 78.29, 78.17, 76.06, 75.82, 75.52, 75.26, 75.12, 75.01, 74.84, 74.80, 74.00, 73.76, 73.12, 72.99, 72.86, 72.42, 71.10, 70.94, 69.39, 69.24, 68.95, 67.29, 65.91, 62.23, 61.66, 57.39, 53.45, 52.50, 32.31, 29.15, 26.24, 26.17, 23.40, 14.20. HRMS (*m/z*): [M + NH₄⁺] calcd for C₁₁₆H₁₂₉Cl₃F₃N₄O₂₆, 2155.7907 found 2155.7880.

***N*-(Benzyl)-*N*-benzyloxycarbonyl-5-aminopentyl-2-deoxy-2-trichloroacetamido-3,4,6-tri-*O*-benzyl-β-D-glucopyranosyl-(1→5)-ethyl 7,8-*O*-isopropylidene-3-deoxy-α-D-*manno*-oct-2-ulopyranoside)onate(2→6)-2-deoxy-2-trifluoroacetamido-3, 4-di-*O*-benzyl-α-D-glucopyranosyl-(1→4)-methyl (2,3-di-*O*-benzyl)-α-D-galactopyranosyl urinate (18):** β-Pinene (37 μL,

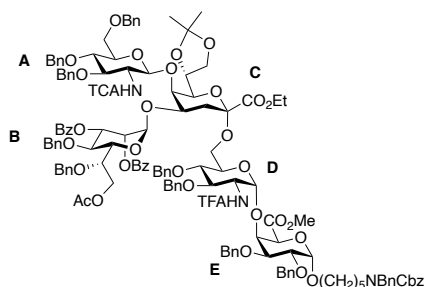


0.234 mmol) and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ, 27 mg, 0.117 mmol) were added to a mixture of **17** (148 mg, 0.069 mmol) in DCM/H₂O (8 mL / 0.4 mL) under the exclusion of light. The reaction mixture was stirred for 2 h at room temperature, after which it was

diluted with DCM (20 mL) and was quenched with sat. aq. NaHCO₃ (10 mL). The organic layer was dried (Na₂SO₄), filtered, and the filtration was concentrated *in vacuo*. The resulting residue was purified by silica gel column chromatography (PE/EA = 2/1, v/v) to give the tetrasaccharide acceptor **18** (100 mg, 72%) as a

white amorphous solid. ^1H NMR (600 MHz, CDCl_3) δ 7.46 – 7.12 (m, 52H, Ar), 6.64 (s, 1H, NHTFA), 5.19 (d, J = 17.4 Hz, 2H, $\text{CH}_2\text{-Cbz}$), 5.04 (d, J = 7.8 Hz, 1H, A-H-1), 4.91 (bs, 1H, D-H-1), 4.86 – 4.80 (m, 4H, C-H-1, PhCH_2 , PhCHH), 4.79 – 4.74 (m, 4H, PhCHH , PhCH_2 , PhCHH), 4.69 (m, 2H, PhCHH , PhCHH), 4.63 – 4.58 (m, 4H, PhCHH , PhCH_2 , PhCHH), 4.55 (d, J = 12.0 Hz, 1H, PhCHH), 4.49 (d, J = 14.3 Hz, 2H, PhCH_2), 4.45 – 4.43 (m, 1H, D-H-4), 4.38 – 4.30 (m, 2H, A-H-5, B-H-7), 4.27 (td, J = 4.5, 1.6 Hz, 1H, D-H-5), 4.22 (td, J = 10.1, 3.5 Hz, 1H, C-H-2), 4.17 – 4.09 (m, 3H, $\text{CO}_2\text{CH}_2\text{CH}_3$, B-H-8a), 4.08 – 4.00 (m, 1H, B-H-4), 3.96 (dd, J = 8.8, 6.2 Hz, 1H, B-H-8b), 3.94 – 3.90 (m, 2H, B-H-5, C-H-4), 3.88 (d, J = 9.5 Hz, 1H, D-H-3), 3.81 (dd, J = 9.8, 3.5 Hz, 1H, D-H-2), 3.79 – 3.68 (m, 5H, A-H-2, A-H-4, B-H-6, C-H-3), 3.67 (m, 5H, A-H-6a/b, CO_2CH_3), 3.66 – 3.54 (m, 5H, A-H-3, C-H-5, C-H-6a, $\text{CH}_2\text{-Linker}$), 3.47 – 3.37 (m, 1H, C-H-6b), 3.29 – 3.15 (m, 2H, $\text{CH}_2\text{-Linker}$), 2.57 – 2.48 (m, 1H, -OH), 2.00 (dd, J = 13.0, 4.5 Hz, 1H, B- H_{eq} -3), 1.88 (t, J = 12.3 Hz, 1H, B- H_{ax} -3), 1.64 – 1.46 (m, 4H, $\text{CH}_2\text{-Linker} \times 2$), 1.35 – 1.17 (m, 11H, $\text{CH}_2\text{-Linker}$, $\text{CO}_2\text{CH}_2\text{CH}_3$, $\text{C}(\text{CH}_3)_2$). ^{13}C NMR (151 MHz, CDCl_3) δ 176.52, 167.41, 161.90, 138.31, 138.26, 138.23, 137.94, 137.92, 137.71, 137.65, 128.69, 128.66, 128.62, 128.59, 128.56, 128.09, 128.06, 128.02, 128.01, 127.98, 127.97, 127.95, 127.83, 127.81, 127.70, 127.44, 114.87, 99.78, 98.73, 92.65, 79.10, 78.87, 77.78, 76.16, 75.43, 75.24, 75.19, 75.12, 74.88, 74.68, 73.75, 73.11, 72.85, 71.09, 70.68, 69.48, 69.34, 69.11, 66.81, 66.16, 63.17, 62.69, 61.54, 57.49, 53.52, 52.54, 35.37, 32.06, 29.83, 29.71, 29.17, 26.51, 26.13, 22.83, 22.76, 14.26, 14.21, 14.09, 11.93. HRMS (m/z): $[\text{M} + \text{Na}^+]$ calcd for $\text{C}_{105}\text{H}_{117}\text{Cl}_3\text{F}_3\text{N}_3\text{NaO}_{26}$, 2020.6835 found 2020.6885.

***N*-(Benzyl)-*N*-benzyloxycarbonyl-5-aminopentyl-[2,3-di-*O*-benzoyl-4,6-di-*O*-benzyl-7-*O*-acetyl- α -D-manno-heptopyranosyl]-(1 \rightarrow 4)-[2-deoxy-2-trichloroacetamido-3,4,6-tri-*O*-benzyl- β -D-glucopyranosyl]-(1 \rightarrow 5)-ethyl (7, 8-*O*-isopropylidene-3-deoxy- α -D-manno-oct-2-ulopyranoside)onate(2 \rightarrow 6)-2-deoxy-2-trifluoroacetamido-3, 4-di-*O*-benzyl- α -D-glucopyranosyl-(1 \rightarrow 4)-methyl (2,3-di-*O*-benzyl)- α -D-galactopyranosyl urinate (2):** Tetrasaccharide acceptor **18** (100 mg, 0.05 mmol) and heptosyl



donor **6** (79 mg, 0.10 mmol) were co-evaporated with toluene (5 mL \times 3) and were dissolved in dry DCM (3 mL) and the solution was placed under an atmosphere of Argon. Flame activated 4Å M.S. were added, and the obtained suspension was stirred for 15 min at 0°C.

Trimethylsilyl trifluoromethanesulfonate (TMSOTf, 1.8 μ L, 0.01 mmol) was then added, and the reaction mixture was stirred for 6 h at 0°C. The reaction was quenched with Et₃N (0.5 mL), and the molecular sieves were filtered off. The solvents were removed *in vacuo*, and the resulting residue was purified by silica gel column chromatography (PE/EA = 2.5/1, v/v) to give pentasaccharide **2** (92 mg, 70%) as a white solid. NMR data showed the heptose is α -linked (J_{C1-H1} = 174 Hz). ¹H NMR (600 MHz, CDCl₃) δ 8.03 – 7.94 (m, 2H, Ar), 7.81 – 7.76 (m, 2H, Ar), 7.71 (d, J = 9.7 Hz, 1H, Ar), 7.58 (t, J = 7.4 Hz, 1H, Ar), 7.50 (t, J = 7.2 Hz, 1H, Ar), 7.42 (d, J = 6.6 Hz, 2H, Ar), 7.38 – 7.14 (m, 54H, Ar), 7.11 – 7.06 (m, 2H, Ar), 6.37 (d, J = 9.5 Hz, 1H, NHTFA), 5.72 (dd, J = 9.7, 2.8 Hz, 1H, B-H-3), 5.54 (t, J = 2.5 Hz, 1H, B-H-2), 5.16 (d, J = 15.3 Hz, 2H, CH₂-Cbz), 5.11 (d, J = 2.1 Hz, 1H, B-H-1), 5.06 (d, J = 10.8 Hz, 1H, PhCHH), 4.93 (d, J = 10.9 Hz, 1H, PhCHH), 4.84 (m, 3H, E-H-1, PhCH₂), 4.81 – 4.56 (m, 13H, A-H-1, D-H-1, B-H-7a, PhCH₂ \times 5), 4.53 (d, J = 11.7 Hz, 2H, PhCH₂), 4.50 – 4.40 (m, 4H, B-H-4, PhCH₂, PhCHH), 4.35 (m, 2H, E-H-4, PhCHH), 4.32 – 4.16 (m, 7H, A-

H-2, B-H-7b, C-H-7, C-H-8a, D-H-2, E-H-5, CO₂CHHCH₃), 4.15 – 4.02 (m, 4H, B-H-6, C-H-4, C-H-6, CO₂CHHCH₃), 4.03 – 3.94 (m, 2H, A-H-3, B-H-5), 3.90 (t-like, $J = 1.6$ Hz, 1H, C-H-5), 3.84 (d, $J = 7.1$ Hz, 1H, E-H-3), 3.80 – 3.66 (m, 6H, A-H-5, A-H-6a/b, C-H-6, D-H-5, E-H-2), 3.65 (s, 4H, CO₂CH₃, CHH-Linker), 3.64 – 3.50 (m, 4H, A-H-4, D-H-3, D-H-6a, CHH-Linker), 3.43 – 3.31 (m, 1H, D-H-6b), 3.25 – 3.13 (m, 2H, CH₂-Linker), 2.28 – 2.17 (m, 2H, C-H-3_{eq/ax}), 2.13 (s, 3H, OAc), 1.60 – 1.43 (m, 2H, CH₂-Linker), 1.37 – 1.13 (m, 13H, CH₂-Linker \times 2, CO₂CH₂CH₃, C(CH₃)₂). ¹³C NMR (151 MHz, CDCl₃) δ 170.85, 169.11, 167.22, 167.18, 165.68, 161.96, 138.48, 138.30, 137.98, 137.73, 133.71, 130.05, 129.76, 129.24, 129.10, 128.73, 128.69, 128.64, 128.63, 128.61, 128.57, 128.51, 128.49, 128.45, 128.11, 128.06, 128.02, 127.98, 127.92, 127.82, 127.77, 127.67, 127.63, 127.53, 127.50, 116.80, 108.37, 101.82, 98.78, 97.63, 97.55, 97.21, 93.17, 82.67, 79.14, 78.58, 77.97, 76.06, 75.84, 75.68, 75.27, 75.09, 74.99, 74.88, 74.81, 74.02, 73.56, 73.17, 72.84, 72.73, 72.54, 72.46, 72.00, 70.91, 70.60, 70.23, 69.38, 69.05, 67.30, 65.57, 61.63, 61.12, 57.58, 53.15, 52.48, 33.77, 32.08, 29.85, 29.51, 29.15, 26.36, 26.11, 23.41, 22.84, 21.02, 14.27, 14.18. HRMS (m/z): [M + Na⁺] calcd for C₁₄₂H₁₅₁Cl₃F₃N₃NaO₃₅, 2642.9038 found 2642.9104.

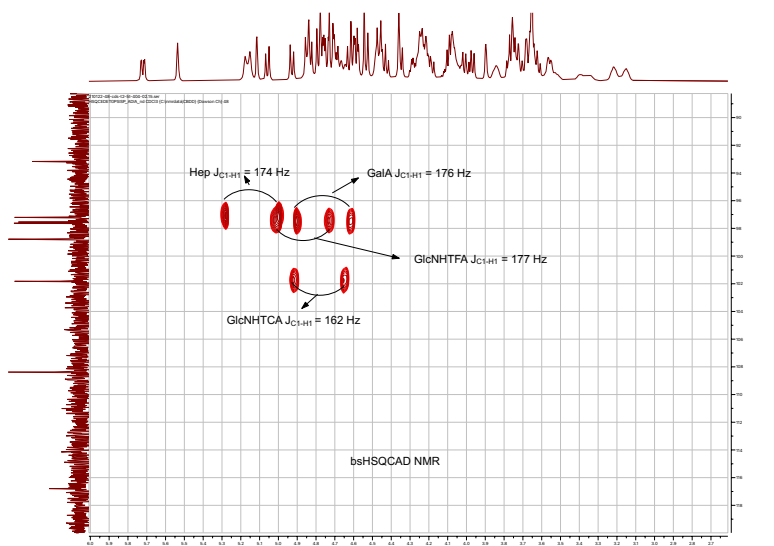
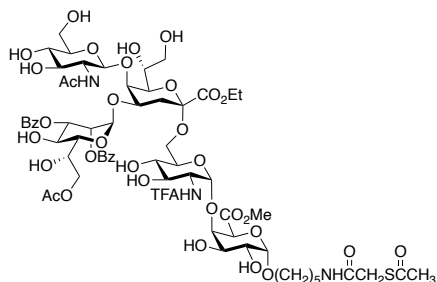


Figure S2. bsHSQCAD NMR

***N*-(acetylthiolmethyl)-5-carbonylamino-pentyl-[2,3-di-*O*-benzoyl-7-*O*-acetyl-L-glycero- α -D-manno-heptopyranosyl]-(1 \rightarrow 4)-[2-deoxy-2-acetamine- β -D-glucopyranosyl]-(1 \rightarrow 5)-ethyl (3-deoxy- α -D-manno-oct-2-ulopyranoside)onate-(2 \rightarrow 6)-2-deoxy-2-trifluoroacetamido- α -D-glucopyranosyl-(1 \rightarrow 4)-methyl- α -D-galactopyranosyl urinate (**20**):** A solution

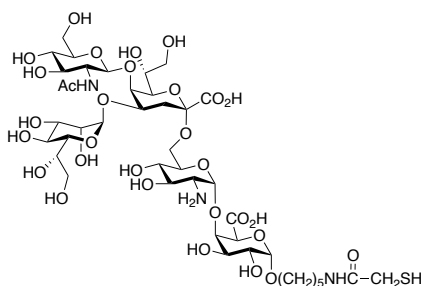


of pentasaccharide **2** (41 mg, 0.015 mmol) in DCM/TFA/H₂O (1.8 mL / 0.18 mL / 0.02 mL) was stirred for 30 min at 0°C. After TLC analysis showed consumption of starting material, the reaction was diluted with toluene (10 mL) and concentrated *in vacuo*. The obtained

residue was dissolved in *t*-BuOH/H₂O (4 mL / 1 mL), and Pd(OH)₂/C (20 mg) and AcOH (20 μ L) were added. The reaction was stirred for 24 h at room temperature under an atmosphere of H₂. The reaction mixture was filtered through celite and was concentrated *in vacuo* to give crude product **19**, which was used for the next step directly.

A solution of pentasaccharide **19** in dry DMF (1.0 mL) was added S-acetylthioglycolic acid pentafluorophenyl ester (SAMA-OPfp, 9.4 mg, 0.031 mmol) and *N,N*-diisopropylethylamine (DIPEA, 5.4 μ L, 0.031 mmol). The reaction was stirred for 3 h at room temperature, after which it was quenched with H₂O (1 mL). The reaction mixture was concentrated *in vacuo*, and the resulting residue was purified by size-exclusion chromatography (bio-gel P2, eluent: 5% *n*-BuOH in H₂O) to give compound **20** (14.4 mg, 49%) was a white solid.

***N*-(2-mercaptoacetamide)-5-carboxylamino-pentyl-[L-glycero- α -D-manno-heptopyranosyl]-(1 \rightarrow 4)-[2-deoxy-2-acetamine- β -D-glucopyranosyl]-(1 \rightarrow 5)-3-deoxy- α -D-manno-oct-2-ulopyranosid-(2 \rightarrow 6)-2-deoxy-2-amino- α -D-glucopyranosyl-(1 \rightarrow 4)- α -D-galactopyranosyluronic acid (**1**):** Pentasaccharide **20** (10 mg, 0.0064 mmol) was dissolved in MeOH (1 mL), and the solution was



cooled to 0°C. NaOH (aqueous, 0.2 M, 1 mL) was then added, and the reaction mixture was stirred for 3 h at 0 °C. After LC-MS analysis showed complete conversion of starting material, the reaction was neutralized with Amberlite

(H⁺, washed with MeOH, aq. 1 M NaOH, H₂O, aq. 1 M HCl, H₂O and MeOH sequentially) till pH = 7. The resin was filtered off, and the filtrate was treated with D,L-dithiothreitol (DTT, 0.2 mg). The obtained solution was stirred for 30 min at 40 °C, after which it was concentrated under reduced pressure. The resulting residue was purified by Bio-Gel P2 column using 5% *n*-BuOH in H₂O (de-oxygen before use) as eluent to give the final product **1** (7.1 mg, quant.) as white powder. HRMS (m/z): [M + H⁺] calcd for C₄₂H₇₂N₃O₃₀S, 1130.3916 found 1130.3934.

Table S2. H¹ NMR spectrum of **1**.

	H1	H2	H3	H4	H5	H6a	H7(6b)	H8(Ac)
βGlcNAc	4.58, d, 7.4 Hz	3.68	3.56	3.47	3.51	3.96	3.76	2.01
αKdo	—	—	1.85/2.07	4.19	4.38	3.61	3.81	3.58/3.97
αGlcN	5.18, d, 3.4 Hz	3.39	3.85	3.55	4.32	3.62	3.29	—
αGalA	5.01, d, 3.0 Hz	3.85	4.05	4.36	4.21	—	—	—
αHep	5.10, s	4.00	3.82	3.88	3.57	4.02	3.72	—
Linker	1.25×2, 1.50×2, 2.20/2.28, 3.17×2, 3.47, 3.63						3.38×2 (CH ₂ SH)	

^{13}C NMR (151 MHz, D_2O) δ 102.07, 98.20, 97.56, 96.27, 79.51, 75.32, 72.74, 71.94, 70.97, 70.65, 70.49, 70.33, 70.17, 70.00, 69.52, 69.04, 68.55, 68.39, 67.91, 66.14, 63.72, 63.56, 62.75, 60.98, 60.34, 55.99, 54.05, 41.00, 39.71, 35.04, 34.39, 33.43, 27.95, 24.89, 22.79, 22.31, 19.73

Conjugation of tetrasaccharide **1 to BSA and CRM₁₉₇**

Carrier protein (450 μL , 2.5 mg/mL in pH 7.4 HEPES buffer for CRM₁₉₇; 450 μL , 2.5 mg/mL in pH 7.4 PBS buffer for BSA) was purified by ultrafiltration (Amicon Ultra-0.5 Centrifugal Filter Unit, 10K) 3 times use pH 7.4 PBS buffer as eluent. The concentrated protein was dissolved in PBS buffer (pH 7.4, 0.5 mL), and succinimidyl 4-(*N*-maleimidomethyl)cyclohexane-1-carboxylate (SMCC, 1.5 mg) was added, and the reaction mixture was shaken overnight at room temperature. The suspension was then centrifuged, and the supernatant was transferred to a centrifugal filter (Amicon Ultra-0.5 Centrifugal Filter Unit, 10K). After purified 5 times with PBS buffer (Ph 7.4) as eluent, the concentrated protein was diluted with PBS buffer (pH 7.4) till ~10 mg/mL (concentration detected by nano-drop). Pentasaccharide **1** (1.0 mg) in water (100 μL) was added to the solution, and the reaction mixture was shaken overnight at room temperature. The solution was then transferred to a centrifugal filter (0.5 mL, 10K), and purified 5 times using PBS buffer (pH 7.4) as eluent to give the glycoconjugate, which was directly diluted to ~1 mg/mL with pH 7.4 PBS buffer.

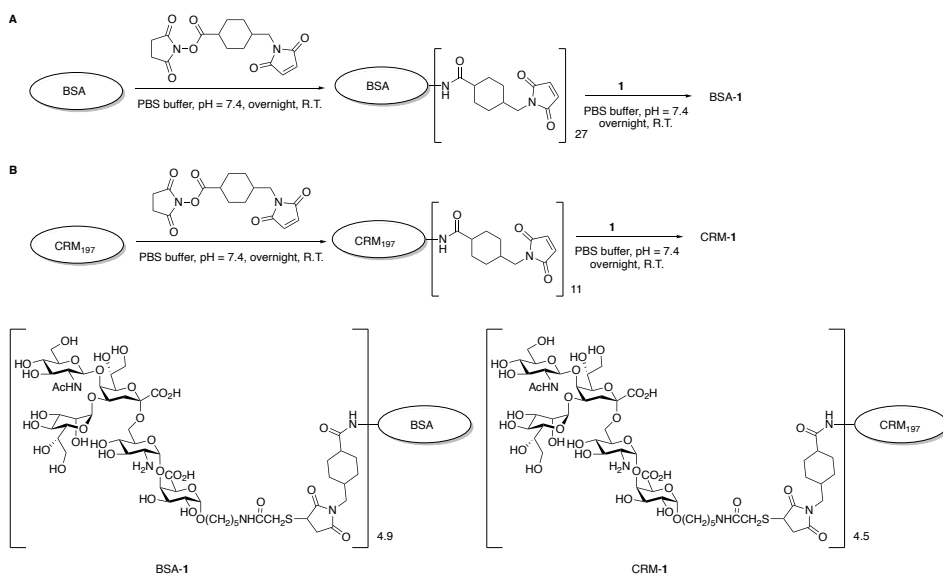


Figure S3. (A) Maleimide modified BSA. (B) Maleimide modified CRM₁₉₇. (C) Conjugation of pentasaccharide **1** with maleimide-activated BSA. (D) Conjugation of pentasaccharide **1** with maleimide-activated CRM₁₉₇.

Sinapinic acid (5 mg) was dissolved in a mixture of acetonitrile/water/trifluoroacetic acid (3/7/0.01, v/v/v), and the suspension was treated by ultrasonification, then filtered through a PTFE syringe filter (Acrodisc, 0.2 μm), and the filtrate was used as a matrix for MALDI-TOF measurement.⁵ The proteins (BSA, CRM₁₉₇, maleimide-modified BSA and maleimide-modified CRM₁₉₇) and glycoconjugate (BSA-**1** or CRM-**1**) were desalted with C18 ZipTip (Millipore, 10 μL) followed manufacturer's protocol, and the concentration was adjusted to ~ 1 mg/mL with milli-Q water. 1 μL of the desalted protein solution and 2 μL of the fresh prepared matrix were mixed, and 1 μL of the mixture were loaded on MALDI plate. The sample spots were dried by air, and then measured by MALDI-TOF using Linear positive mode.

Table S3. MALDI-TOF data of proteins, maleimide-modified proteins and glycoconjugates.

	BSA	CRM ₁₉₇	BSA-M	CRM-M	BSA-1	CRM-1
Mass	66715.6	58764.5	72714.0	61232.1	78231.3	66285.7
Loading number	—	—	27	11	4.9	4.5

*BSA-M = maleimide-modified BSA; CRM-M = maleimide-modified CRM₁₉₇.

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Chapter 4

Chemical Synthesis of Inner Core Oligosaccharides of *Klebsiella pneumoniae* for Conjugate Vaccine Development

Introduction

Klebsiella pneumoniae (*K. pneumoniae*), which is an encapsulated Gram-negative bacterium that causes nosocomial infections with a mortality of ~50%, expresses a capsular and lipopolysaccharide (LPS) on its cell surface.¹⁻⁴ LPS is a major virulence factor of *K. pneumoniae*, and consists of an O-antigen, core region and lipid A. The core region can be further subdivided into inner- and outer core.⁵ Among *Enterobacteriaceae*, the inner core is well conserved because it plays an important role in stabilizing the bacterial outer membrane.⁶

Inner core regions have attracted attention as vaccine candidate for pathogenic bacteria. Previous studies showed that protective antibodies elicited by inner core saccharide are able to recognize the antigen expressed by bacteria, such as *Y. pestis*⁷, *H. influenza*⁸, *N. meningitidis*⁹ and *F. tularensis*¹⁰, that indicated inner core is an attractive vaccine candidate. Due to high structural similarity of inner core regions, they may elicit immunity that provide cross-protective antibodies against different bacteria.¹¹⁻¹⁴

The core region of *K. pneumoniae* differs from those of other *Enterobacteriaceae* by lacking phosphoryl substitutions and instead contains galacturonic acid (GalA) and 3-deoxy-D-manno-2-octulosonic acid (Kdo) moieties to provide a negative charge for bacterial outer membrane stabilization.^{6, 15-17} Two types of LPS outer cores have been identified that both share the same inner core (Fig. 1A).^{18, 19} The partial structure of the inner core backbone (L,αD-Hep-(1→7)-L,αD-Hep-(1→3)-L,αD-Hep), however, is expressed by many bacteria, such as *S. enterica*, *E. coli*, *P. penneri*, *Yersinia* and *S. marcescens*.²⁰ Compared to 79 serotypes of capsular

polysaccharide (CPS) and 9 serotypes of O-antigen, the core of *K. pneumoniae* is a more conserved and therefore is an attractive target for the development of a vaccine with broad coverage.^{5, 21} Currently, there is no licensed vaccine for *K. pneumoniae* and there is an urgent to develop such vaccines.^{22, 23}

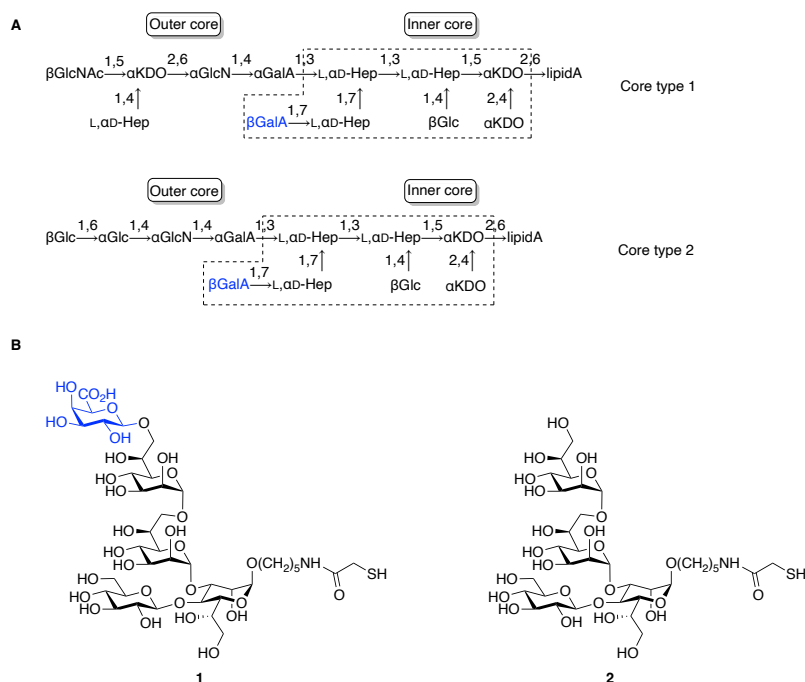


Figure 1. (A) Chemical structure of core type 1 and core type 2. (B) Chemical structure of target pentasaccharide **1** and tetrasaccharide **2**.

The development of broad acting carbohydrate-based vaccine requires conjugation of a well-defined and non-toxic core oligosaccharide to a carrier protein, which is important for providing T-cell-dependent immunity.^{10, 24} Most of the marketed conjugate vaccines are based on polysaccharides isolated from bacteria, however, such an approach is often challenging to meet safety standards or obtain desired saccharides in sufficient quantities for controlled conjugation to a carrier protein.^{25, 26} Synthetic approaches provide an attractive means to address these problems.^{10, 27} They allow the installation of a reactive linker for controlled

conjugation to a protein. Additionally, that makes it possible to explore the relationship between part-structures and immunity.^{28, 29}

In chapters 2 and 3, attention is focused on the preparation of outer-core oligosaccharides derived from the LPS of *K. pneumoniae*. Herein, we report the chemical synthesis of pentasaccharide **1** and tetrasaccharide **2**, which are derived from the inner core of *K. pneumoniae* (Fig. 1B) and their conjugations to carrier proteins. The glycoconjugates are attractive to explore immunological properties of the core oligosaccharide of *K. pneumoniae*, and in particular, make it possible to explore the influence of the nonstoichiometric β GalA residue on immune responses. The synthesis of 3,4-branched trisaccharide residue (L, α D-HepB-(1 \rightarrow 3)-[β Glc-(1 \rightarrow 4)]-L, α D-HepA) derived from LPS inner core have been reported that employed the formation of 1,6-anhydro bridge of L,D-heptosyl acceptor (HepA) to change the confirmation from 4C_1 to 1C_4 , or non-bulky blocking groups (acetyl ester) protected glucosyl donor to reduce steric hindrance during glycosylation.³⁰⁻³³

Results and Discussion

The chemical synthesis of inner core saccharide derived from *K. pneumoniae* is challenging because of need to install a sterically crowded 3,4-branched heptoside, the preparation of L,D-heptose building blocks, and the regio-selective installation/removal of protecting groups.³¹⁻³⁴ It was envisaged that the fully protected pentasaccharide **14**, which is a precursor of target compound **1**, could be prepared by a convergent [2+3] strategy using disaccharide donor **4** and a trisaccharide acceptor **3** (Figure 2A). In addition, it was expected that the fully protected tetrasaccharide **15**, which is a precursor for target compound **2**, could be synthesized using a [1+3] strategy employing monosaccharide donor **5** and the same acceptor **3** (Fig. 2B).

Monosaccharide building blocks **5**, **6**, **7**, **8** and **9** were employed to assemble the targets (Fig. 2C). Heptosyl donor **5** is modified with levulinoyl (Lev) ester at C-

7 that can be easily removed to provide an acceptor for further extension, and C-2, C-3 and C-4 hydroxyls are protected as benzoyl (Bz) esters, which at a late stage of synthesis can be removed by saponification. Heptosyl acceptor **6** is equipped with a temporary 2-methylnaphthyl (Nap) ether protecting group at C-4 that can be removed by oxidation with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) to provide a glycosyl acceptor. In addition, it contains an α -linked aminopentyl that is doubly protected by benzyl and benzyloxycarbonyl (Cbz), which can be removed at a late synthetic stage to give a free amine for further modification as a thioacetyl moiety for the conjugation to a carrier protein (See Scheme S1 for the synthesis of heptosyl donor **6**). The C-2, C-6 and C-7 hydroxyls are protected as benzyl ethers, which can be removed by hydrogenation at the very end of the synthesis sequence. The glucosyl trichloroacetimidate **7** is per-*O*-acylated to reduce steric hindrance during glycosylation to form the crowded 3,4-branched heptoside.^{32, 33} The C-4 and C-6 of galactoside donor **8** is protected with a benzylidene, which at an appropriate stage of synthesis can be removed under mild acidic conditions to give a diol, and the primary alcohol can selectively be oxidized to a carboxylic acid. The C-1, C-2, C-3, and C-4 hydroxyls of heptosyl acceptor **9** are protected as Bz esters, and the anomeric hydroxyl can be selectively unmasked and then installed a trichloroacetimidate to give a disaccharide donor, which is used for the [2+3] glycosylation. Both targets contain 1,2-*trans*-linkages (α -heptoside and β -glucoside/-galactoside), which were selectively installed by neighboring participation of a C-2 ester protecting group of a glycosyl donor.

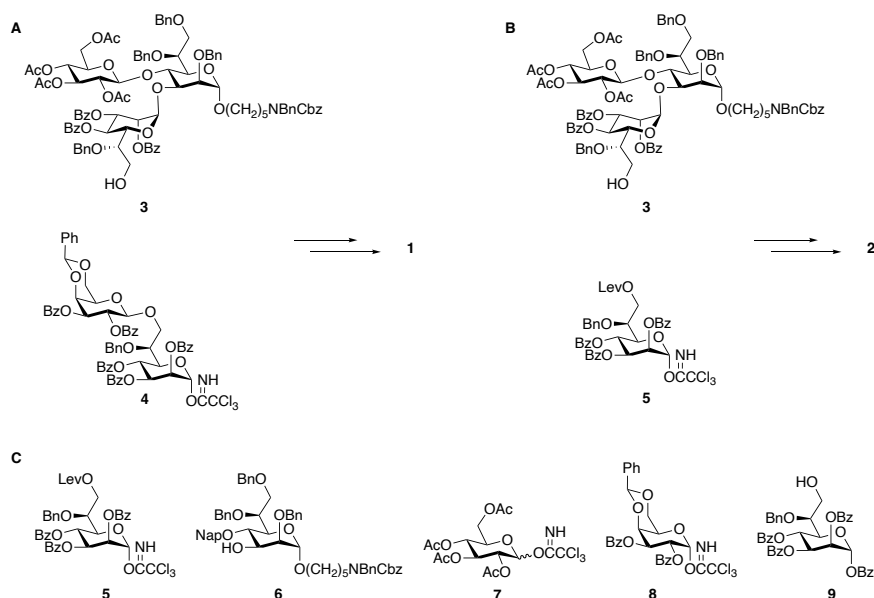
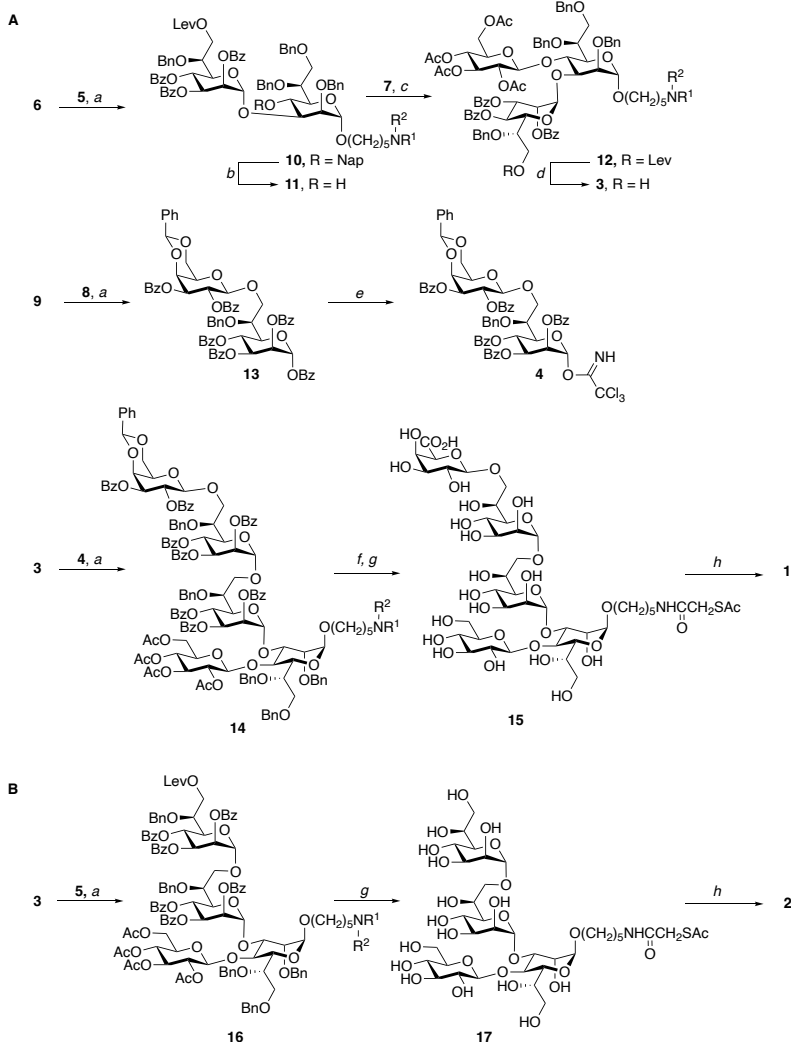


Figure 2. Chemical synthesis of inner core pentasaccharide **1** (A), tetrasaccharide **2** (B) and building blocks for targets assembly (C).

The assembly of pentasaccharide **1** started with a glycosylation of heptosyl acceptor **6** with heptosyl donor **5** using trimethylsilyl trifluoromethanesulfonate (TMSOTf) as the promoter to provide disaccharide **10** in a yield of 92%. Oxidative removal of the Nap ether of **10** by DDQ/ β -pinene in wet DCM afforded disaccharide acceptor **11**,³⁵ which after purification by silica gel column chromatography was coupled with glucosyl donor **7** to give the 3,4-branched trisaccharide **12** in an acceptable yield of 53%. The anomeric configuration of the newly installed glycosidic linkages was confirmed by NMR using the coupling constant between C-1 and H-1 ($J_{\text{aHepA C1-H1}} = 170$ Hz, $J_{\text{aHepB C1-H1}} = 178$ Hz, $J_{\beta\text{Glc C1-H1}} = 160$ Hz).^{36, 37} Trisaccharide acceptor **3** was obtained by treating **12** with hydrazine acetate in a yield of 90%. Next, the heptosyl acceptor **9** and trichloroacetimidate donor **8** were coupled using TMSOTf as the catalyst to provide disaccharide **13** in a yield of 98% as only the β -anomer ($J_{\text{Gal C1-H1}} = 8.0$ Hz). The anomeric benzoyl ester of **13** was selectively removed using

hydrazine acetate in DMF under heating,^{38, 39} and the resulting lactol was converted into trichloroacetimidate **4** by reaction with trichloroacetonitrile in the presence of 1,8-diazadicycloundec-7-ene (DBU) in DCM.³² Glycosylation of trisaccharide acceptor **3** with disaccharide donor **4** using TMSOTf as the activator gave pentasaccharide **14** in a yield of 79% as only α -anomer, which was confirmed by NMR ($J_{\beta\text{Gal C1-H1}} = 156$ Hz, $J_{\beta\text{Glc C1-H1}} = 162$ Hz, $J_{\alpha\text{HepA C1-H1}} = 174$ Hz, $J_{\alpha\text{HepB C1-H1}} = 178$ Hz, $J_{\alpha\text{HepC C1-H1}} = 174$ Hz). Treatment of **14** with trifluoroacetic acid in wet DCM at 0°C resulted in the cleavage of benzylidene. The primary alcohol of the resulting compound was oxidized to a carboxylic acid using (2,2,6,6-tetramethylpiperidin-1-yl)oxyl (TEMPO) and (diacetoxyiodo)benzene (BAIB), which gave a mixture of carboxylic acid and aldehyde. The resulting aldehyde was further oxidized by Pinnick oxidation using sodium chlorite and sodium dihydrogen phosphate to give the corresponding carboxylic acid.⁴⁰⁻⁴² Global deprotection of the pentasaccharide started with saponification of the esters using aqueous NaOH in a mixture of 1,4-dioxane and methanol, which was followed by hydrogenation over Pd(OH)₂/C in *t*-BuOH/H₂O under an atmosphere of H₂ to remove the benzyl ethers and unmask the anomeric aminopenyl moiety. The free amine of the pentyl linker was then modified as thioacetyl using S-acetylthioglycolic acid pentafluorophenyl ester (SAMA-OPfp) and *N,N*-diisopropylethylamine (DIPEA) in DMF to give, after purification by bio-gel P2 size exclusion column chromatography, pentasaccharide **15** which was then treated with ammonia in water to remove the acetyl moiety to afford the target compound **1**.



Scheme 1. Assembly of pentasaccharide **1** (A) and tetrasaccharide **2** (B).

Conditions and reagents: *a*) TMSOTf, DCM, 0°C, 6 h, 92% for **10**, 98% for **13**, 79% for **14** and 58% for **15**. *b*) DDQ, β -pinene, DCM/H₂O, 97%. *c*) TMSOTf, DCM, 0°C-R.T., overnight, 53%. *d*) NH₂NH₂·HOAc, DCM/MeOH, 90%. *e*) i. NH₂NH₂·HOAc, DMF, 60°C, 3 h. ii. CCl₃CN, DBU, DCM, 2 h, 49%. *f*) i. TFA/H₂O/DCM (0.9/0.1/9, v/v/v), 30 min. ii. TEMPO, BAIB, DCM/H₂O. iii. NaClO₂, NaH₂PO₄, DCM/*t*-BuOH, 83% *g*) i. 1M NaOH, 1,4-dioxane, MeOH. ii.

H₂, Pd(OH)₂/C, *t*-BuOH/H₂O. iii. SAMA-OPfp, DIPEA, DMF, 63% for **1** over 3 steps, 71% for **2** over 3 steps. h) ammonia (7 N in methanol), H₂O, Ar. *R¹ = -Bn, R² = -Cbz.

A similar strategy was employed to prepare tetrasaccharide **2** starting with a glycosylation of heptosyl donor **5** with trisaccharide acceptor **3** under aforementioned conditions to provide the fully protected tetrasaccharide **16** in a yield of 58%. Next, the Lev, acetyl and benzoyl esters were saponified using aqueous NaOH, which was followed by hydrogenation using similar conditions as described for compound **1**. A thioacetyl group was installed at the pentyl linker by reaction with SAMA-OPfp in the presence of DIPEA in DMF to give, after purification by bio-gel P2 size exclusion column chromatography, tetrasaccharide **17**. The configuration of the anomeric linkages was confirmed by NMR using the coupling constant between C-1 and H-1 ($J_{\beta\text{Glc C1-H1}} = 156$ Hz, $J_{\alpha\text{HepA C1-H1}} = 168$ Hz, $J_{\alpha\text{HepB C1-H1}} = 174$ Hz, $J_{\alpha\text{HepC C1-H1}} = 174$ Hz). Finally, the thiol was unmasked by treatment of **17** with ammonia in water to give tetrasaccharide **2**.

Thiol-maleimide coupling chemistry was employed to conjugate the synthetic oligosaccharides, pentasaccharide **1** and tetrasaccharide **2**, to carrier proteins. Ultrafiltration purified BSA or CRM₁₉₇ were reacted with succinimidyl 4-(*N*-maleimidomethyl)cyclohexane-1-carboxylate (SMCC) in phosphate buffered saline (PBS, pH 7.4) to give, after purification by ultrafiltration, the corresponding maleimide-activated carrier proteins, which were conjugated with pentasaccharide **1** and tetrasaccharide **2** in PBS (pH 7.4), respectively. Glycoconjugates (BSA-**1**, BSA-**2**, CRM-**1**, and CRM-**2**) were obtained after purification by ultrafiltration, and their glycan contents were determined by MALDI-TOF mass spectrometry: 6.1 for BSA-**1**; 4.9 for BSA-**2**; 5.1 for CRM-**1**; and 4.1 for CRM-**2** (saccharides per protein molecule, SI).

Conclusion

The pentasaccharide **1** and tetrasaccharide **2** derived from LPS inner core of *K*.

pneumoniae were synthesized chemically. A convergent [2+3] oligosaccharide assembly strategy was employed to prepare the pentasaccharide, which contains a nonstoichiometric modification of β GalA moiety to provide negative charge for the molecule. The synthetically challenging crowded 3,4-branched heptoside was obtained by sequential glycosylations in appropriate order that using a per-acetylated glucosyl donor to reduce the steric hindrance. Additionally, the selective oxidation of primary alcohol into carboxylic acid was performed on the pentasaccharide using a two-stage oxidation approach. Both target compounds were conjugated to carrier proteins by a thiol-maleimide coupling chemistry. The neoglycoconjugates will be used in immunization studies to provide a better understanding of the effect of β GalA for eliciting immune responses.

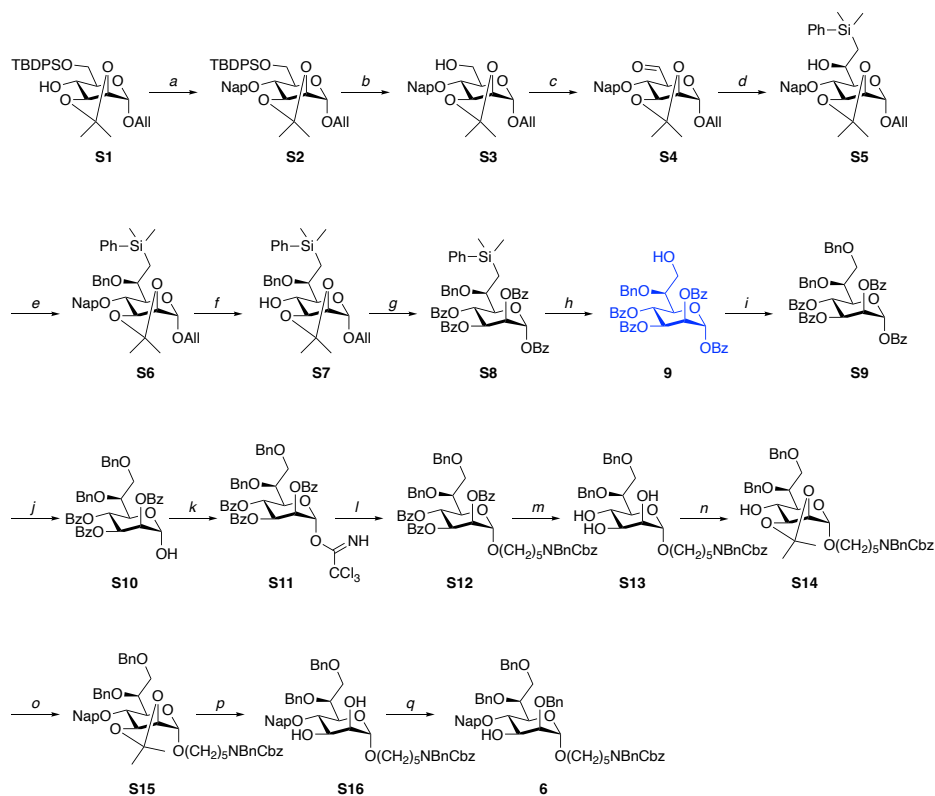
Experimental section

General procedures

All chemicals were purchased from Sigma-Aldrich, Fisher Scientific or Biosynth Carbosynth. BSA was purchased from Sigma-Aldrich and CRM₁₉₇ was purchased from Scarab Genomics LLC. NMR spectra, including ^1H , ^{13}C , COSY, HSQC, HMBC and bsHSQCAD, were recorded on Agilent 400-MR or Bruker 600 MHz with chemical shifts reported in part per million (ppm) relative to CDCl_3 or D_2O . ^1H NMR data are presented in the order: Chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, dd = doublet of doublets, m = multiplet) and coupling constants (J) are reported in Hertz (Hz). Mass spectra of proteins were recorded on a Kratos Analytical Maxima-CFR MALDI-TOF spectromete, a Bruker Microflex MALDI-TOF spectrometer or a Bruker ultrafleXtreme MALDI-TOF spectrometer with sinapinic acid in acetonitrile/water (0.3/0.7, v/v) as matrix, and High-resolution mass spectrometry (HRMS) was recorded on an Agilent technologies 6560 Ion mobility Q-TOF. Chromatographic purifications were performed on silica gel G60 (Silicycle 60 – 200 μm , 60 \AA), and size exclusion chromatography was performed on Bio-Gel P-2 (45-90 μm) by using 5 % *n*-

butanol in water as eluent. TLC analysis was conducted on Silica gel 60 F254 (EMD Chemicals Inc.) and detected by using UV light (254 nm), staining by 5 % sulfuric acid in ethanol, *p*-anisaldehyde solution or an aqueous solution of $\text{Ce}(\text{NH}_4)_2(\text{NO}_3)_6 / (\text{NH}_4)_6\text{MoO}_{24} \cdot 4\text{H}_2\text{O}$ in 5 % sulfuric acid. 4 Å molecular sieves were flame-activated under vacuum before use. Carrier proteins were purified by ultrafiltration using ultrafilter (Amicon Ultra-0.5 Centrifugal Filter Unit, 10K) before and after reactions.

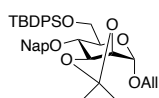
Synthetic Protocols



Scheme S1. Synthesis of heptose acceptor **6**. Conditions and reagents: *a*) NapBr, NaH, DMF, 97%. *b*) TBAF, THF, 95%. *c*) Dess-Martin periodinane, DCM. *d*) $\text{PhSi}(\text{Me})_2\text{CH}_2\text{Cl}$, Mg, THF, 86% over 2 steps. *e*) BnBr, NaH, DMF, 72%. *f*) DDQ, β -pinene, DCM/ H_2O (20/1, v/v). *g*) i. PdCl_2 , MeOH. ii. 80% AcOH in H_2O , 80%.

iii. BzCl, pyridine, 44% over 4 steps. *h*) KBr, NaOAc, AcOOH, AcOH, 79%. *i*) Benzyl 2,2,2-trichloroacetimidate, TMSOTf, DCM, 87%. *j*) MeNH₂, THF/EtOH (4/1, v/v), 90%. *k*) CCl₃CN, DBU, DCM, 88%. *l*) (5-hydroxy-pentyl)-carbamic acid benzyl ester, TMSOTf, DCM, 80%. *m*) NaOMe, MeOH, reflux. *n*) 2,2-Dimethoxypropane, CSA, acetone, 83% over 2 steps. *o*) NapBr, NaH, DMF, 99%. *p*) TFA/H₂O/DCM (0.9/0.1/9, v/v/v). *q*) BnBr, TBAB, aq. NaOH, DCM, 88% over 2 steps.

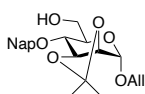
Allyl 6-*O*-*tert*-butyldiphenylsilyl-4-*O*-(2-methylnaphthyl)-2,3-*O*-isopropylidene- α -D-mannopyranoside (S2): S1³² (the synthesis has been



described in Chapter 3, 9.2 g, 18.4 mmol) and 2-(bromomethyl)naphthalene (NapBr, 6.12 g, 27.7 mmol) were dissolved in dry DMF (80 mL). The solution was stirred at 0°C for 5 min, after which it was slowly added sodium hydride (60% dispersion in mineral oil, 1.1 g, 27.7 mmol). The reaction mixture was warmed to room temperature and stirred for 3 h. After TLC analysis showed consumption of starting material, the reaction was quenched with sat. aq. NH₄Cl (3 mL). The solvents were removed *in vacuo*, and the resulting residue was dissolved in DCM (200 mL) and washed with water (100 mL). The organic layer was dried (Na₂SO₄), filtered, and the filtrate was concentrated *in vacuo*. The resulting residue was purified by silica gel column chromatography (PE/EA = 20/1, v/v) to give S2 (11.4 g, 97%) as a light-yellow oil. ¹H NMR (600 MHz, CDCl₃) δ 7.82 – 7.67 (m, 8H, Ar), 7.46 – 7.30 (m, 9H, Ar), 5.92 – 5.85 (m, 1H, CH₂-CH=CH₂), 5.27 (dq, *J* = 17.2, 1.6 Hz, 1H, CH₂-CH=CH₂), 5.21 – 5.15 (m, 1H, CH₂-CH=CH₂), 5.13 (s, 1H, H-1), 5.04 (d, *J* = 11.6 Hz, 1H, CH₂HNap), 4.74 (d, *J* = 11.6 Hz, 1H, CH₂HNap), 4.41 (dd, *J* = 6.9, 5.8 Hz, 1H, H-3), 4.26 – 4.19 (m, 2H, H-2, CH₂-CH=CH₂), 4.04 – 3.95 (m, 2H, CH₂-CH=CH₂, H-6a), 3.89 (dd, *J* = 11.0, 5.2 Hz, 1H, H-6b), 3.77 (ddd, *J* = 10.2, 5.2, 1.8 Hz, 1H, H-5), 3.69 (dd, *J* = 10.2, 6.9 Hz, 1H, H-4), 1.53 (s, 3H, C(CH₃)₂), 1.40 (s, 3H, C(CH₃)₂), 1.05 (s, 9H, *t*-Bu-CH₃ \times 3). ¹³C NMR (151 MHz, CDCl₃) δ 135.97, 135.84, 135.72, 133.83, 133.75, 133.42,

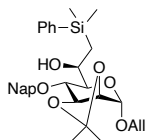
133.30, 133.04, 129.70, 129.67, 128.13, 128.01, 127.77, 127.76, 127.69, 126.74, 126.10, 126.06, 125.88, 117.96, 109.41, 96.08, 79.16, 76.08, 75.76, 73.12, 69.82, 67.64, 63.45, 28.12, 26.89, 26.55. HRMS (m/z): $[M + Na^+]$ calcd for $C_{39}H_{46}NaO_6Si$, 661.2961 found 661.2949.

Allyl 4-*O*-(2-methylnaphthyl)-2,3-*O*-isopropylidene- α -D-mannopyranoside (S3): Tetra-*n*-butylammonium fluoride (TBAF, 1.0 M in THF, 35.6 mL, 35.6



mmol) was added to a solution of **S2** (11.4 g, 17.8 mmol) in THF (100 mL). The reaction was stirred for 6 h at room temperature, after which it was concentrated *in vacuo*. The obtained residue was dissolved in DCM (200 mL) and washed with water (100 mL). The organic layer was dried (Na_2SO_4), filtered, and the filtrate was concentrated *in vacuo*. The resulting residue was purified by silica gel column chromatography (PE/EA = 5/1, v/v) to give **S3** (6.8 g, 95%) as a white solid. 1H NMR (600 MHz, $CDCl_3$) δ 7.87 – 7.80 (m, 4H, Ar), 7.53 – 7.44 (m, 3H, Ar), 5.93 – 5.86 (m, 1H, $CH_2-\underline{CH}=\underline{CH}_2$), 5.31 (dq, $J = 17.2, 1.6$ Hz, 1H, $CH_2-\underline{CH}=\underline{CH}\underline{H}$), 5.22 (dq, $J = 10.4, 1.3$ Hz, 1H, $CH_2-\underline{CH}=\underline{CH}\underline{H}$), 5.09 (s, 1H, H-1), 5.06 (dd, $J = 11.7, 0.8$ Hz, 1H, $\underline{CH}\underline{H}Nap$), 4.81 (dd, $J = 11.6, 0.8$ Hz, 1H, $\underline{CH}\underline{H}Nap$), 4.40 (dd, $J = 7.0, 5.7$ Hz, 1H, H-3), 4.19 (m, 2H, H-2, $\underline{CH}\underline{H}-\underline{CH}=\underline{CH}_2$), 4.00 (ddt, $J = 12.7, 6.2, 1.4$ Hz, 1H, $\underline{CH}\underline{H}-\underline{CH}=\underline{CH}_2$), 3.89 (ddd, $J = 11.7, 5.2, 3.2$ Hz, 1H, H-6a), 3.78 (ddd, $J = 11.8, 7.3, 4.7$ Hz, 1H, H-6b), 3.75 – 3.69 (m, 1H, H-5), 3.60 (dd, $J = 10.1, 7.1$ Hz, 1H, H-4), 2.08 – 1.96 (m, 1H, -OH), 1.50 (s, 3H, $C(\underline{CH}_3)_2$), 1.39 (s, 3H, $C(\underline{CH}_3)_2$). ^{13}C NMR (151 MHz, $CDCl_3$) δ 135.58, 133.53, 133.33, 133.12, 128.31, 128.01, 127.81, 127.03, 126.22, 126.10, 126.03, 118.13, 109.53, 96.40, 78.80, 76.05, 75.97, 73.02, 68.66, 68.20, 62.66, 28.11, 26.46. HRMS (m/z): $[M + Na^+]$ calcd for $C_{23}H_{28}NaO_6$, 423.1784; found 423.1775.

Allyl 4-*O*-(2-methylnaphthyl)-2,3-*O*-isopropylidene-7-(Si-dimethylphenylsilyl)-L-glycero- α -D-manno-heptopyranoside (S5**):** Dess-Martin periodinane



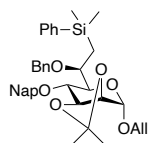
(10.8 g, 25 mmol) was washed with cooled (-20 °C) diethyl ether (20 mL \times 5) before adding to a solution of **S3** (6.8 g, 17.0 mmol) in DCM (50 mL) at 0 °C. The reaction mixture was stirred for 2 h at room temperature. After TLC analysis showed consumption of

starting material (DCM/acetone, 30/1, v/v), the reaction mixture was diluted with DCM (300 mL) and quenched with sat. aq. Na₂S₂O₃ (50 mL). The two-phasic mixture was separated, and the organic phase was washed with sat. aq. NaHCO₃ (200 mL), and then H₂O (200 mL). The organic layer was dried (Na₂SO₄), filtered, and the filtrate was concentrated *in vacuo*. After co-evaporated with toluene (50 mL \times 3), the resulting aldehyde **S4** was used in the next step without additional purification.

Magnesium (5.0 g) was washed with 1.0M HCl (20 mL \times 5), H₂O (30 mL \times 3) and acetone (30 mL \times 3), and then dried *in vacuo*. The activated Mg was added to dry THF (50 mL) and placed under an atmosphere of Argon. The resulting suspension was heated to reflux (70 °C), and (phenyldimethylsilyl)methyl chloride (1.1 mL, 6.8 mmol) was added. The reaction was initiated by 1,2-dibromoethane (100 μ L), and the second portion of (phenyldimethylsilyl)methyl chloride (4.6 mL, 27.2 mmol) was added dropwise. The reaction mixture was heated under reflux for 1.5 h and then cooled to 0 °C to give a Grignard reagent as a dark grey suspension. The aldehyde **21** in dry THF (20 mL) was then added to the Grignard reagent dropwise at 0 °C, and the resulting reaction mixture was stirred for 2 h at this temperature, after which it was warmed to room temperature and kept stirring overnight. The reaction mixture was cooled to 0 °C again and then quenched with sat. aq. NH₄Cl (30 mL). The solids were filtered off, and the organic solvents were removed under reduced pressure. The resulting mixture was diluted with EtOAc (300 mL) and washed with H₂O (100 mL \times 3). The aqueous layer was backextracted with EtOAc (50 mL \times 3), and the combined

organic layer was dried (Na₂SO₄), filtered, and the filtrate was concentrated *in vacuo*. The resulting residue was purified by silica gel column chromatography (PE/EA = 6/1, v/v) to afford **S5** (8.0 g, 86%) as a colorless oil. ¹H NMR (600 MHz, CDCl₃) δ 7.87 – 7.76 (m, 4H, Ar), 7.57 – 7.51 (m, 2H, Ar), 7.51 – 7.45 (m, 2H, Ar), 7.39 (dd, *J* = 8.4, 1.7 Hz, 1H, Ar), 7.36 – 7.30 (m, 3H, Ar), 5.90 – 5.84 (m, 1H, CH₂-CH=CH₂), 5.28 (dd, *J* = 17.3, 1.6 Hz, 1H, CH₂-CH=CHH), 5.21 (dd, *J* = 10.4, 1.4 Hz, 1H, CH₂-CH=CHH), 5.11 (s, 1H, H-1), 5.03 (d, *J* = 11.3 Hz, 1H, CHHNap), 4.76 (d, *J* = 11.3 Hz, 1H, CHHNap), 4.40 – 4.34 (m, 1H, H-3), 4.20 – 4.13 (m, 3H, CHH-CH=CH₂, H-2, H-6), 3.99 (ddt, *J* = 12.8, 6.2, 1.4 Hz, 1H, CHH-CH=CH₂), 3.71 (dd, *J* = 10.0, 7.1 Hz, 1H, H-4), 3.45 (dd, *J* = 10.0, 1.6 Hz, 1H, H-5), 1.83 (d, *J* = 10.5 Hz, 1H, -OH), 1.51 (s, 3H, C(CH₃)₂), 1.38 (s, 3H, C(CH₃)₂), 1.35 – 1.30 (m, 1H, H-7a), 0.94 (dd, *J* = 14.8, 3.7 Hz, 1H, H-7b), 0.36 (s, 6H, Si(CH₃)₂). ¹³C NMR (151 MHz, CDCl₃) δ 139.40, 135.74, 133.79, 133.50, 133.40, 133.16, 129.04, 128.30, 128.09, 127.94, 127.82, 127.08, 126.27, 126.16, 126.01, 118.03, 109.51, 96.47, 79.17, 76.27, 75.89, 73.47, 72.13, 68.13, 67.57, 29.85, 28.15, 26.55, 21.75, -1.97 (Si(CH₃)₂), -2.15 (Si(CH₃)₂). HRMS (*m/z*): [M + Na⁺] calcd for C₃₂H₄₀NaO₆Si, 571.2486; found 571.2489.

Allyl 6-*O*-benzyl-4-*O*-(2-methylnaphthyl)-2,3-*O*-isopropylidene-7-(Si-dimethylphenyl-silyl)-L-glycero- α -D-manno-heptopyranoside (S6**):**

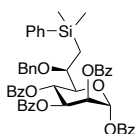


Compound **S5** (4.78 g, 8.7 mmol) and benzyl bromide (BnBr, 1.5 mL, 12.86 mmol) were dissolved in dry DMF (50 mL), and the solution was stirred for 5 min at 0°C. Sodium hydride (60% dispersion in mineral oil, 515 mg, 12.86 mmol) was added slowly

at this temperature, after which the reaction mixture was stirred for 3 h at room temperature. After TLC analysis showed consumption of starting material, the reaction was quenched with sat. aq. NH₄Cl (2 mL). The reaction mixture was concentrated *in vacuo*, and the resulting residue was dissolved in DCM (200 mL) and washed with water (100 mL). The organic layer was dried (Na₂SO₄), filtered, and the filtrate was concentrated *in vacuo*. The obtained residue was purified by

silica gel column chromatography (PE/EA = 10/1, v/v) to afford **S6** (4.0 g, 72%) as a light-yellow oil. ^1H NMR (600 MHz, CDCl_3) δ 7.82 – 7.79 (m, 1H, Ar), 7.74 (dd, J = 8.9, 2.3 Hz, 2H, Ar), 7.64 (d, J = 1.7 Hz, 1H, Ar), 7.52 – 7.49 (m, 2H, Ar), 7.48 – 7.43 (m, 2H, Ar), 7.37 – 7.25 (m, 4H, Ar), 7.12 (dd, J = 5.1, 1.9 Hz, 3H, Ar), 7.07 – 7.02 (m, 2H, Ar), 5.91 – 5.84 (m, 1H, $\text{CH}_2\text{-CH=CH}_2$), 5.27 (dq, J = 17.2, 1.6 Hz, 1H, $\text{CH}_2\text{-CH=CHH}$), 5.19 (dq, J = 10.5, 1.4 Hz, 1H, $\text{CH}_2\text{-CH=CHH}$), 5.17 (d, J = 1.2 Hz, 1H, H-1), 4.93 (d, J = 11.9 Hz, 1H, CHHNap), 4.47 (d, J = 11.8 Hz, 1H, CHHPh), 4.34 (t, J = 6.4 Hz, 1H, H-3), 4.31 – 4.28 (m, 1H, CHHNap), 4.20 (ddt, J = 12.9, 5.1, 1.5 Hz, 1H, CHH-CH=CH_2), 4.15 (dd, J = 6.0, 1.2 Hz, 1H, H-2), 4.10 (d, J = 11.9 Hz, 1H, CHHPh), 4.05 – 3.98 (m, 2H, H-6, CHH-CH=CH_2), 3.72 (dd, J = 9.9, 6.8 Hz, 1H, H-4), 3.52 (dd, J = 9.9, 1.4 Hz, 1H, H-5), 1.52 (s, 3H, $\text{C}(\text{CH}_3)_2$), 1.42 – 1.36 (m, 2H, H-7a/b), 1.37 (s, 3H, $\text{C}(\text{CH}_3)_2$), 0.33 (s, 6H, $\text{Si}(\text{CH}_3)_2$). ^{13}C NMR (151 MHz, CDCl_3) δ 138.83, 138.58, 136.24, 133.78, 133.68, 133.34, 132.96, 129.18, 128.25, 128.10, 128.03, 128.02, 127.99, 127.89, 127.78, 127.46, 126.19, 126.05, 125.92, 125.80, 117.82, 109.49, 96.89, 79.28, 75.94, 75.90, 73.39, 72.12, 71.74, 70.96, 68.53, 28.01, 26.49, 17.90, -1.89 ($\text{Si}(\text{CH}_3)_2$), -2.20 ($\text{Si}(\text{CH}_3)_2$). HRMS (m/z): $[\text{M} + \text{Na}^+]$ calcd for $\text{C}_{39}\text{H}_{46}\text{NaO}_6\text{Si}$, 661.2956; found 661.2954.

1,2,3,4-tetra-*O*-benzoyl-6-*O*-benzyl-7-(Si-dimethylphenyl-silyl)-L-glycero-D-manno-heptopyranoside (S8**):** β -Pinene (8.4 mL, 53.3 mmol) and 2,3-dichloro-

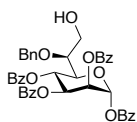


5,6-dicyano-1,4-benzoquinone (DDQ, 7.1 g, 31.1 mmol) were added to the solution of **23** (10.0 g, 15.6 mmol) in a mixture of DCM/ H_2O (200 mL / 10 mL) under exclusion of light. The reaction mixture was stirred for 2 h at room temperature, after which it was

quenched with sat. aq. NaHCO_3 (100 mL). The two-phasic mixture was separated, and the organic layer was dried (Na_2SO_4), filtered, and the filtrate was concentrated *in vacuo*. The resulting residue was purified by silica gel column chromatography (PE/EA = 5/1, v/v) to give **S7** as a red solid, which was used for next step without further purification.

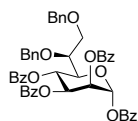
PdCl_2 (414 mg, 2.34 mmol) was added to a solution of **S7** in MeOH (100 mL), and the reaction mixture was stirred overnight at room temperature, after which it was diluted with Et_2O (200 mL) and filtered through celite. The filtrate was concentrated *in vacuo*, and the residue was dissolved in EtOAc (300 mL) and washed with sat. aq. NaHCO_3 (100 mL) and water (100 mL). The organic layer was dried (Na_2SO_4), filtered, and the filtrate was concentrated *in vacuo*. The resulting residue was dissolved in a mixture of AcOH (16 mL) and H_2O (4 mL). The reaction mixture was stirred for 2 h at 80°C , after which it was concentrated *in vacuo*. The resulting residue was co-evaporated with toluene (50 mL \times 4) and then dissolved in pyridine (100 mL). Benzoyl chloride (18 mL, 156 mmol) was added to the solution dropwise at 0°C . After stirring overnight at room temperature, the reaction was quenched with MeOH (30 mL) and stirred for another 30 min, and then concentrated *in vacuo*. The resulting residue was dissolved in EtOAc (200 mL) and washed with sat. aq. NaHCO_3 (100 mL), and the organic layer was dried (Na_2SO_4), filtered, and the filtrate was concentrated *in vacuo*. The resulting residue was purified by silica gel column chromatography (PE/EA = 5/1, v/v) to give **S8** (5.7 g, 44% over 4 steps) as a white solid. ^1H NMR (600 MHz, CDCl_3) δ 8.25 – 7.26 (m, 24H, Ar), 7.22 (m, 4H, Ar), 7.16 – 7.09 (m, 2H, Ar), 6.66 (d, J = 2.1 Hz, 1H, H-1), 6.41 (t, J = 10.0 Hz, 1H, H-4), 5.95 (dd, J = 10.2, 3.2 Hz, 1H, H-3), 5.85 (dd, J = 3.2, 2.1 Hz, 1H, H-2), 4.49 (d, J = 10.7 Hz, 1H, CHHPh), 4.32 (d, J = 10.7 Hz, 1H, CHHPh), 4.16 (dd, J = 9.7, 1.7 Hz, 1H, H-5), 3.73 (ddd, J = 9.9, 4.7, 1.7 Hz, 1H, H-6), 1.57 (dd, J = 14.5, 9.9 Hz, 1H, H-7a), 1.44 (dd, J = 14.5, 4.7 Hz, 1H, H-7b), 0.18 (d, J = 6.8 Hz, 6H, $\text{Si}(\text{CH}_3)_2$). ^{13}C NMR (151 MHz, CDCl_3) δ 165.96, 165.39, 165.28, 164.08, 138.04, 138.02, 134.06, 133.58, 133.50, 133.41, 133.34, 130.29, 130.17, 130.15, 129.92, 129.87, 129.82, 129.36, 129.17, 129.09, 129.07, 128.89, 128.68, 128.58, 128.51, 128.49, 128.47, 128.38, 128.34, 127.96, 127.51, 91.54, 74.36, 73.96, 71.34, 70.67, 69.45, 66.43, 16.30, -1.72 ($\text{Si}(\text{CH}_3)_2$), -2.45 ($\text{Si}(\text{CH}_3)_2$). HRMS (m/z): $[\text{M} + \text{Na}^+]$ calcd for $\text{C}_{50}\text{H}_{46}\text{NaO}_{10}\text{Si}$, 857.2752; found 857.2747.

1,2,3,4-tetra-*O*-benzoyl-6-*O*-benzyl-L-glycero-D-manno-heptopyranoside (9):



A solution of **S8** (1.7g, 2.0 mmol) in acetic acid (15 mL) was added NaOAc (2.0 g) and KBr (250 mg, 2.4 mmol) and placed under exclusion of light. The suspension was stirred vigorously until all NaOAc were dissolved, and then cooled to 10 °C. Peracetic acid (ca. 35wt.% sol. in diluted acetic acid, 10 mL) was added dropwise to the mixture at 10 °C, and during the addition gas was liberated. The reaction mixture was stirred at room temperature for 3 h, and then quenched with aqueous solution of Na₂S₂O₃ (20%, 30 mL). The mixture was extracted with EtOAc (20 mL × 5), and the combined organic layers were washed with sat. aq. NaHCO₃ (30 mL) and H₂O (30 mL). The organic phase was dried (Na₂SO₄), filtered, and the filtrate was concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography (PE/EA = 3/1, v/v) to give **9** (1.13 g, 79%) as a colorless oil. ¹H NMR (600 MHz, CDCl₃) δ 8.14 (dd, *J* = 7.9, 1.4 Hz, 2H, Ar), 7.95 (dd, *J* = 8.1, 1.4 Hz, 2H, Ar), 7.93 – 7.85 (m, 4H, Ar), 7.63 – 7.56 (m, 1H, Ar), 7.53 (q, *J* = 7.7 Hz, 2H, Ar), 7.46 (t, *J* = 7.3 Hz, 1H, Ar), 7.43 – 7.21 (m, 13H, Ar), 6.35 (t, *J* = 9.9 Hz, 1H, H-4), 6.23 (d, *J* = 1.0 Hz, 1H, H-1), 6.10 (d, *J* = 3.1 Hz, 1H, H-2), 5.71 (dd, *J* = 10.0, 3.1 Hz, 1H, H-3), 4.60 (q, *J* = 10.8 Hz, 2H, CHHPh), 4.31 (dd, *J* = 9.9, 2.1 Hz, 1H, H-5), 3.96 (td, *J* = 10.5, 6.2 Hz, 2H, H-7a/b), 3.81 (td, *J* = 6.2, 2.1 Hz, 1H, H-6), 2.45 (s, 1H, -OH). ¹³C NMR (151 MHz, CDCl₃) δ 165.79, 165.73, 165.34, 164.92, 137.83, 134.00, 133.61, 133.59, 133.49, 130.31, 130.27, 130.03, 129.94, 129.90, 129.42, 129.22, 128.89, 128.76, 128.63, 128.62, 128.52, 128.49, 128.44, 127.91, 92.11, 75.56, 74.81, 73.41, 72.66, 69.50, 66.06, 61.18. HRMS (*m/z*): [*M* + Na⁺] calcd for C₄₂H₃₆NaO₁₁, 739.2150; found 739.2144.

1,2,3,4-tetra-*O*-benzoyl-6,7-di-*O*-benzyl-L-glycero-D-manno-



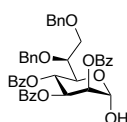
heptopyranoside (S9): A solution of benzyl alcohol (1.0 mL, 9.6 mmol) in dry DCM (10 mL) was added trichloroacetonitrile (4.8 mL, 48 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 287

μL , 1.92 mmol). The reaction mixture was stirred for 2 h at room temperature. After TLC analysis showed consumption of starting material, the reaction was diluted with Et_2O (10 mL), filtered and the filtrate was concentrated *in vacuo*. The resulting residue was purified by silica gel column chromatography (PE/EA = 10/1, v/v) to give benzyl 2,2,2-trichloroacetimidate⁴³ (2.1 g, 88%) as a light yellow liquid. ^1H NMR (600 MHz, CDCl_3) δ 8.39 (s, 1H, =NH), 7.44 – 7.42 (m, 2H, Ph), 7.40 – 7.36 (m, 2H, Ph), 7.35 – 7.32 (m, 1H, Ph), 5.34 (s, 2H, CH_2HPh). ^{13}C NMR (151 MHz, CDCl_3) δ 162.70, 135.57, 128.65, 128.40, 127.82, 91.50, 70.84.

Compound **9** (1.1 g, 1.53 mmol) and benzyl 2,2,2-trichloroacetimidate (771 mg, 3.06 mmol) were co-evaporated with toluene (5 mL \times 3) and then dissolved in dry DCM (10 mL), and the solution was placed under an atmosphere of Argon. Flame-activated 4Å MS were added to the solution, and the mixture was stirred for 15 min at 0°C, which was followed by the addition of trimethylsilyl trifluoromethanesulfonate (TMSOTf, 55 μL , 0.3 mmol). The reaction mixture was slowly warmed to room temperature and stirred overnight, after which it was quenched with Et_3N (0.5 mL), and the solids were filtered off. The filtrate was concentrated *in vacuo*, and the resulting residue was purified by silica gel column chromatography (PE/EA = 3/1, v/v) to give **S9** (1.1 g, 87%) as a white solid. ^1H NMR (600 MHz, CDCl_3) δ 8.15 – 8.10 (m, 2H, Ar), 7.97 – 7.94 (m, 2H, Ar), 7.93 – 7.90 (m, 2H, Ar), 7.89 – 7.85 (m, 2H, Ar), 7.59 (dd, J = 8.2, 6.7 Hz, 1H, Ar), 7.55 – 7.43 (m, 6H, Ar), 7.42 – 7.27 (m, 15H, Ar), 6.36 (t, J = 10.0 Hz, 1H, H-4), 6.30 (s, 1H, H-1), 6.07 (d, J = 3.1 Hz, 1H, H-2), 5.71 (dd, J = 10.1, 3.1 Hz, 1H, H-3), 4.69 (d, J = 10.5 Hz, 1H, CH_2HPh), 4.64 – 4.52 (m, 3H, CH_2HPh , CH_2HPh), 4.27 – 4.19 (m, 1H, H-5), 3.91 – 3.78 (m, 3H, H-6, H-7a/b). ^{13}C NMR (151 MHz, CDCl_3) δ 165.64, 133.32, 130.19, 130.15, 129.91, 129.83, 128.61, 128.48, 128.44, 128.41, 128.36, 128.34, 127.98, 127.77, 127.67, 91.67, 74.66, 74.34, 73.84, 72.55, 69.57, 65.90. HRMS (m/z): $[\text{M} + \text{Na}^+]$ calcd for $\text{C}_{49}\text{H}_{42}\text{NaO}_{11}$, 829.2619; found 829.2616.

2,3,4-Tri-*O*-benzoyl-6,7-di-*O*-benzyl-L-glycero-D-manno-heptopyranoside

(S10): A solution of **S9** (1.8 g, 2.23 mmol) in a mixture of THF (60 mL) and

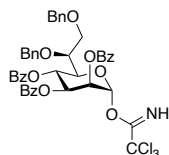


EtOH (15 mL) was added methylamine (2.0 M in THF, 7.5 mL, 15 mmol). The reaction mixture was stirred overnight at room temperature, after which it was concentrated *in vacuo*. The resulting

residue was purified by silica gel column chromatography (DCM/acetone = 30/1, v/v) to give **S10** (1.4 g, 90%) as a white solid. ^1H NMR (600 MHz, CDCl_3) δ 8.04 – 7.92 (m, 5H, Ar), 7.87 – 7.82 (m, 2H, Ar), 7.56 – 7.25 (m, 18H, Ar), 6.29 (t, J = 10.1 Hz, 1H, H-4), 5.91 (dd, J = 10.1, 3.2 Hz, 1H, H-3), 5.68 (dd, J = 3.3, 1.9 Hz, 1H, H-2), 5.41 (dd, J = 3.9 Hz 1.9 Hz, 1H, H-1), 4.68 (d, J = 10.3 Hz, 1H, CHHPh), 4.63 – 4.55 (m, 2H, CHHPh , CHHPh), 4.52 – 4.46 (m, 2H, H-5, CHHPh), 3.85 – 3.78 (m, 3H, H-6, H-7a/b), 3.03 (d, J = 3.9 Hz, 1H, -OH). ^{13}C NMR (151 MHz, CDCl_3) δ 165.50, 163.42, 137.99, 133.36, 133.30, 133.11, 129.96, 129.83, 129.79, 129.33, 128.59, 128.52, 128.50, 128.46, 128.38, 128.30, 128.21, 127.95, 127.72, 92.23, 74.70, 73.98, 73.45, 70.69, 70.44, 69.57, 68.70, 66.50. HRMS (m/z): $[\text{M} + \text{Na}^+]$ calcd for $\text{C}_{42}\text{H}_{38}\text{NaO}_{10}$, 725.2357; found 725.2355.

2,3,4-Tri-*O*-benzoyl-6,7-di-*O*-benzyl-L-glycero-D-manno-heptopyranosyl

trichloroacetimidate (S11): A solution of **S10** (1.26 g, 1.8 mmol) in dry DCM



(20 mL) was added trichloroacetonitrile (1.8 mL, 18 mmol) and DBU (54 μL , 0.36 mmol), and stirred for 2 h at room temperature.

After TLC analysis showed consumption of starting material, the reaction was diluted with Et_2O (20 mL), filtered and the filtrate

was concentrated *in vacuo*. The resulting residue was purified by silica gel column chromatography (PE/EA = 4/1, v/v) to give **S11** (1.34 g, 88%) as a white solid. ^1H NMR (600 MHz, CDCl_3) δ 8.81 (s, 1H, =NH), 8.07 – 8.01 (m, 2H, Ar), 8.00 – 7.97 (m, 2H, Ar), 7.88 – 7.82 (m, 2H, Ar), 7.58 – 7.27 (m, 19H, Ar), 6.56 (d, J = 1.8 Hz, 1H, H-1), 6.42 (t, J = 10.0 Hz, 1H, H-4), 5.97 – 5.89 (m, 2H, H-2, H-3), 4.70 (d, J = 10.4 Hz, 1H, CHHPh), 4.55 (m, 3H, H-5, CHHPh , CHHPh),

46.23, 31.95, 29.73, 29.39, 29.10, 28.05, 27.60, 23.39, 22.72, 14.15. HRMS (m/z): [M + Na⁺] calcd for C₆₂H₆₁NNaO₁₂, 1034.4086; found 1034.4078. The configuration was confirmed by coupling constant between C-1 and H-1 (J_{C1-H1} = 172.0 Hz).³²

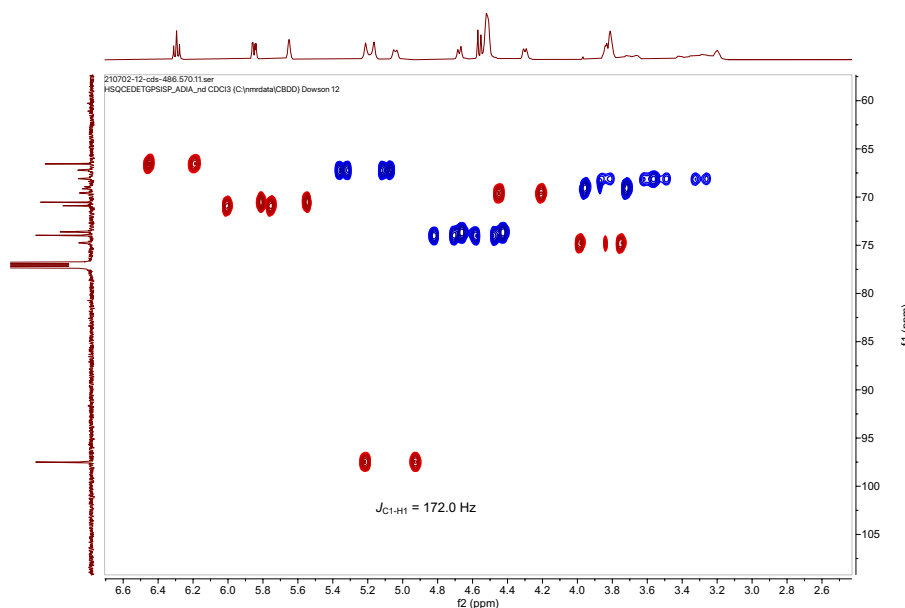
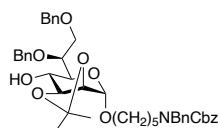


Figure S1. bsHSQCAD NMR data of **S12**.

***N*-(Benzyl)-*N*-benzyloxycarbonyl-5-aminopentyl 2,3-*O*-isopropylidene-6,7-di-*O*-benzyl-L-glycero- α -D-manno-heptopyranoside (**S14**):** A solution of **S12**

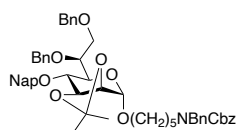


(710 mg, 0.7 mmol) in a mixture of MeOH (8 mL) and THF (4 mL) was added NaOMe (30% in methanol, 0.1 mL). The reaction mixture was stirred overnight at 60°C, after which it was neutralized with Amberlite 120 H⁺ (washed with MeOH before use). The resin was filtered off, and the filtrate was concentrated *in vacuo* to give the crude product **S13** as a white foam.

Compound **S13** was co-evaporated with toluene (5 mL \times 3) and then dissolved in dry acetone (5 mL). 2,2-Dimethoxypropane (130 μ L) and (+)-camphor-10-sulfonic acid (CSA, 16 mg, 0.07 mmol) were added, and the reaction mixture was stirred for 3 h at room temperature, after which it was quenched with Et₃N (0.1 mL) and then concentrated *in vacuo*. The resulting residue was purified by silica gel column chromatography (DCM/acetone = 30/1, v/v) to give **S14** (430 mg, 83%) as a colorless oil. ¹H NMR (600 MHz, CDCl₃) δ 7.40 – 7.11 (m, 20H, Ar), 5.24 – 5.12 (m, 2H, CH₂-Cbz), 4.98 (split by Cbz, 1H, H-1), 4.80 (d, *J* = 11.9 Hz, 1H, CHHPh), 4.60 (d, *J* = 10.8 Hz, 1H, CHHPh), 4.56 – 4.42 (m, 4H, CH₂Ph \times 2), 4.07 – 4.03 (m, 2H, H-2, H-3), 3.96 – 3.89 (m, 1H, H-5), 3.78 – 3.68 (m, 3H, H-4, H-7a, CHH-Linker), 3.65 – 3.52 (m, 2H, H-6, CHH-Linker), 3.35 – 3.08 (m, 3H, H-7b, CH₂-Linker), 1.51 (s, 3H, C(CH₃)₂), 1.49 – 1.39 (m, 4H, CH₂-Linker \times 2), 1.33 (s, 3H, C(CH₃)₂), 1.25 – 1.13 (m, 2H, CH₂-Linker). ¹³C NMR (151 MHz, CDCl₃) δ 138.32, 138.00, 137.88, 136.87, 136.70, 128.96, 128.89, 128.74, 128.54, 128.48, 128.43, 128.39, 128.11, 127.95, 127.84, 127.77, 127.65, 127.45, 127.30, 127.16, 109.36, 97.23, 78.53, 75.72, 73.36, 69.04, 67.19, 29.70, 28.98, 28.10, 26.32. HRMS (*m/z*): [M + Na⁺] calcd for C₄₄H₅₃NNaO₉, 762.3612; found 762.3613.

***N*-(Benzyl)-*N*-benzyloxycarbonyl-5-aminopentyl 4-*O*-(2-methylnaphthyl)-2,3-*O*-isopropylidene-6,7-di-*O*-benzyl-L-glycero- α -D-manno-**

heptopyranoside (S15): Compound **S14** (430 mg, 0.58 mmol) and 2-



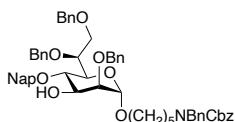
(bromomethyl)naphthalene (NapBr, 265 mg, 1.2 mmol)

were dissolved in dry DMF (5 mL). The solution was stirred for 5 min at 0°C, after which it was slowly added sodium hydride (60% dispersion in mineral oil, 48 mg, 1.2

mmol). The reaction mixture was warmed to room temperature and stirred for 3 h. After TLC analysis showed consumption of starting material, the reaction was quenched with sat. aq. NH₄Cl (0.5 mL). The solvents were removed *in vacuo*, and the resulting residue was dissolved in DCM (50 mL) and washed with water

(20 mL). The organic layer was dried (Na₂SO₄), filtered, and the filtrate was concentrated *in vacuo*. The resulting residue was purified by silica gel column chromatography (PE/EA = 3/1, v/v) to give **S15** (508 mg, 99%) as a light-yellow oil. ¹H NMR (600 MHz, CDCl₃) δ 7.82 – 7.69 (m, 4H, Ar), 7.45 (td, *J* = 6.4, 3.4 Hz, 2H, Ar), 7.40 – 7.08 (m, 17H, Ar), 5.19 – 5.13 (m, 2H, CH₂-Cbz), 5.04 (split by Cbz, 1H, H-1), 5.01 (d, *J* = 11.5 Hz, 1H, CHHPh), 4.74 (d, *J* = 11.8 Hz, 1H, CHHPh), 4.53 – 4.41 (m, 6H, CH₂Ph × 3), 4.37 (t, *J* = 6.4 Hz, 1H, H-2), 4.13 – 4.09 (m, 2H, H-5, H-6), 3.84 (dd, *J* = 10.0, 6.9 Hz, 1H, H-3), 3.81 – 3.64 (m, 3H, H-4, H-7a/b), 3.64 – 3.55 (m, 1H, CHH-Linker), 3.33 – 3.10 (m, 3H, CHH-Linker, CH₂-Linker), 1.55 (s, 3H, C(CH₃)₂), 1.46 (m, 4H, CH₂-Linker × 2), 1.39 (s, 3H, C(CH₃)₂), 1.24 – 1.11 (m, 2H, CH₂-Linker). ¹³C NMR (151 MHz, CDCl₃) δ 128.76, 128.12, 127.96, 127.96, 127.31, 126.51, 126.35, 126.03, 125.87, 125.06, 124.42, 97.21, 79.19, 75.48, 75.16, 73.55, 73.39, 73.07, 72.42, 72.26, 70.17, 68.08, 67.43, 67.11, 50.37, 50.21, 29.12, 28.16, 28.00, 26.55, 25.10, 23.49. HRMS (*m/z*): [*M* + Na⁺] calcd for C₅₅H₆₁NNaO₉, 902.4239; found 902.4234.

***N*-(Benzyl)-*N*-benzyloxycarbonyl-5-aminopentyl 4-*O*-(2-methylnaphthyl)-2,6,7-tri-*O*-benzyl-L-glycero- α -D-manno-heptopyranoside (**6**):** Compound



S15 (500 mg, 0.57 mol) was dissolved in a mixture of trifluoroacetic acid (0.6 mL), H₂O (0.4 mL) and DCM (9 mL), and the reaction mixture was stirred for 1 h at 0°C.

After TLC analysis showed consumption of starting material, the reaction mixture was diluted with toluene (20 mL) and concentrated *in vacuo*. to give **S16**, which was used in the next step without further purification.

A solution of **S16** in DCM (6 mL) was added NaOH (5% aqueous, 4.6 mL), tetra-*n*-butylammonium bromide (TBAB, 37 mg, 0.11 mmol) and BnBr (82 μ L, 0.68 mmol), and the reaction mixture was stirred for 5 h under reflux (45°C). The reaction mixture was then cooled to room temperature, diluted with DCM (50 mL), and washed with water (30 mL). The organic layer was dried (Na₂SO₄),

filtered, and the filtrate was concentrated *in vacuo*. The resulting residue was purified by silica gel column chromatography (PE/EA = 3/1, v/v) to give **6** (469 mg, 88%) as a colorless oil. ^1H NMR (600 MHz, CDCl_3) δ 7.81 – 7.71 (m, 3H, Ar), 7.67 (s, 1H, Ar), 7.43 (dt, $J = 7.0, 3.5$ Hz, 2H, Ar), 7.40 – 7.11 (m, 26H, Ar), 5.20 – 5.14 (m, 2H, CHHPh-Cbz), 5.02 (d, $J = 11.3$ Hz, 1H, CHHPh), 4.94 (split by Cbz, 1H, H-1), 4.83 (d, $J = 11.9$ Hz, 1H, CHHPh), 4.81 – 4.77 (m, 1H, CHHPh), 4.56 – 4.43 (m, 7H, CHHPh , $\text{CH}_2\text{Ph} \times 3$), 4.6 (t, $J = 6.1$ Hz, 1H, H-6), 4.01 – 4.03 (m, 1H, H-3), 3.92 (t, $J = 9.4$ Hz, 1H, H-4), 3.81 (t, $J = 7.8$ Hz, 1H, H-7a), 3.77 – 3.68 (m, 3H, H-2, H-5, H-7b), 3.62 – 3.51 (m, 1H, CHH-Linker), 3.31 – 3.06 (m, 3H, CHH-Linker , $\text{CH}_2\text{-Linker}$), 2.37 (d, $J = 9.8$ Hz, 1H, -OH), 1.53 – 1.35 (m, 5H, $\text{CH}_2\text{-Linker} \times 2$, CHH-Linker), 1.23 – 1.10 (m, 1H, CHH-Linker). ^{13}C NMR (151 MHz, CDCl_3) δ 138.69, 138.12, 137.80, 133.30, 132.93, 128.56, 128.46, 128.38, 128.30, 128.09, 127.97, 127.94, 127.86, 127.74, 127.67, 127.63, 127.57, 127.48, 127.19, 126.32, 125.98, 125.94, 125.77, 96.63, 78.55, 76.30, 74.54, 73.38, 72.83, 72.70, 72.29, 70.63, 67.21, 50.49, 47.07, 46.12, 29.17, 23.45. HRMS (m/z): $[\text{M} + \text{Na}^+]$ calcd for $\text{C}_{59}\text{H}_{63}\text{NNaO}_9$, 952.4395; found 952.4392. Compound **6** was confirmed by ^1H - ^1H COSY NMR.

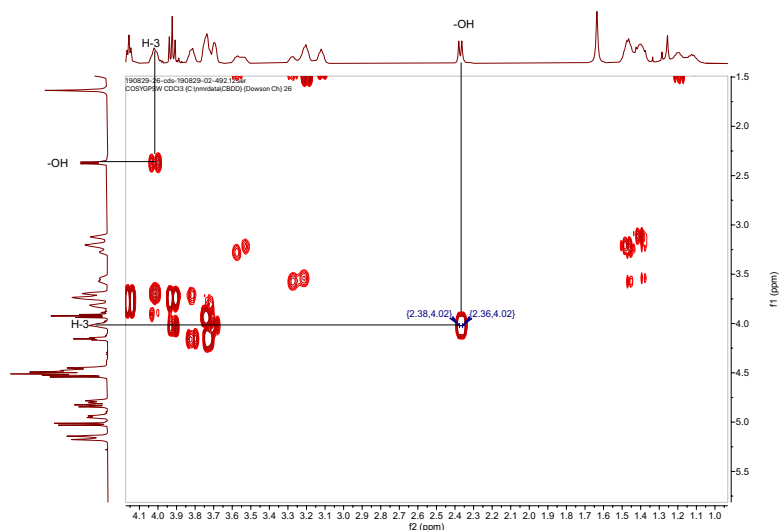
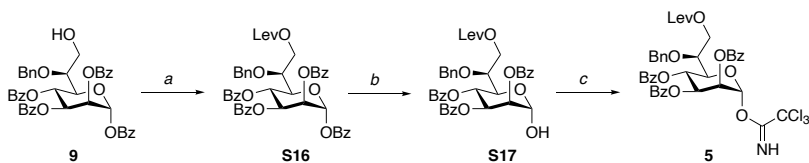


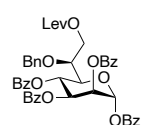
Figure S2. ^1H - ^1H COSY of heptosyl acceptor **6**.



Scheme S2. Synthesis of heptose donor **5**. Conditions and reagents: *a*) LevOH, EDC·HCl, DMAP, DCM, 97%. *b*) MeNH₂, THF/EtOH, 79%. *c*) CCl₃CN, DBU, DCM, 70%

1,2,3,4-Tetra-*O*-benzoyl-6-*O*-benzyl-7-*O*-levulinoyl-L-glycero-D-manno-

heptopyranoside (S16): A solution of **9** (680 mg, 0.95 mmol) in DCM (10 mL)



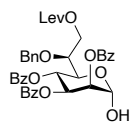
was added levulinic acid (220 mg, 1.9 mmol), *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDC·HCl, 364 mg, 1.9 mmol) and 4-dimethylaminopyridine

(DMAP, 116 mg, 0.95 mmol), and the mixture was stirred for 3 h at room temperature. After TLC analysis showed consumption of starting material, the reaction mixture was diluted with DCM (20 mL) and washed with sat. aq. NaHCO₃ (20 mL). The organic layer was dried (Na₂SO₄), filtered, and the filtrate was concentrated *in vacuo*. The resulting residue was purified by silica gel column chromatography (DCM/acetone = 30/1, v/v) to give **34** (750 mg, 97%) as a white solid. ¹H NMR (600 MHz, CDCl₃) δ 8.12 (dt, *J* = 8.3, 1.3 Hz, 2H, Ar), 7.98 (dt, *J* = 8.3, 1.3 Hz, 2H, Ar), 7.93 – 7.83 (m, 4H, Ar), 7.61 – 7.43 (m, 8H, Ar), 7.42 – 7.27 (m, 11H, Ar), 6.35 (t, *J* = 9.9 Hz, 1H, H-4), 6.31 (s, 1H, H-1), 6.08 (d, *J* = 3.1 Hz, 1H, H-2), 5.74 (dd, *J* = 10.0, 3.1 Hz, 1H, H-3), 4.69 (d, *J* = 10.6 Hz, 1H, CHHPh), 4.58 (d, *J* = 10.6 Hz, 1H, CHHPh), 4.53 (dd, *J* = 11.3, 5.9 Hz, 1H, H-7a), 4.41 (dd, *J* = 11.2, 6.7 Hz, 1H, H-7b), 4.22 (dd, *J* = 9.9, 1.9 Hz, 1H, H-5), 3.91 (td, *J* = 6.4, 1.8 Hz, 1H, H-6), 2.77 (q, *J* = 6.3 Hz, 2H, CH₂-Lev), 2.62 (td, *J* = 6.7, 2.7 Hz, 2H, CH₂-Lev), 2.17 (s, 3H, CH₃-Lev). ¹³C NMR (151 MHz, CDCl₃) δ 206.51, 172.50, 165.64, 165.61, 165.27, 164.14, 137.68, 133.70, 133.48, 133.42, 133.36, 130.18, 130.15, 129.91, 129.87, 129.33, 129.11, 128.80, 128.64, 128.56, 128.51, 128.48, 128.42, 128.40, 128.37, 127.80, 91.60, 74.22,

73.73, 73.62, 72.48, 69.50, 65.83, 62.66, 38.01, 29.81, 27.96. HRMS (m/z): [M + Na⁺] calcd for C₄₇H₄₂NaO₁₃, 837.2518; found 837.2518.

2,3,4-Tri-*O*-benzoyl-6-*O*-benzyl-7-*O*-levulinoyl-L-glycero-D-manno-

heptopyranoside (S17): A solution of **S16** (320 mg, 0.39 mmol) in a mixture of

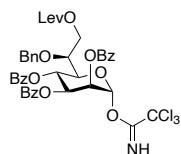


THF (4 mL) and EtOH (1 mL) was added methylamine (2.0 M in THF, 785 μ L, 1.57 mmol), and the mixture was stirred overnight at room temperature. After TLC analysis showed consumption of

starting material, the reaction mixture was concentrated *in vacuo*, and the resulting residue was purified by silica gel column chromatography (DCM/acetone = 25/1, v/v) to give **S17** (220 mg, 79%) as a colorless oil. ¹H NMR (600 MHz, CDCl₃) δ 8.04 (dd, *J* = 8.4, 1.3 Hz, 2H, Ar), 7.99 (dd, *J* = 8.4, 1.3 Hz, 2H, Ar), 7.85 (dd, *J* = 8.4, 1.3 Hz, 2H, Ar), 7.76 (dd, *J* = 8.4, 1.4 Hz, 2H, Ar), 7.57 – 7.27 (m, 12H, Ar), 6.31 (t, *J* = 10.1 Hz, 1H, H-4), 5.99 (dd, *J* = 10.1, 3.3 Hz, 1H, H-3), 5.73 (dd, *J* = 3.3, 1.9 Hz, 1H, H-2), 5.55 (d, *J* = 2.3 Hz, 1H, H-1), 4.77 (d, *J* = 3.5 Hz, 1H, -OH), 4.61 (bs, 2H, CH₂Ph), 4.59 – 4.53 (m, 2H, H-5, H-7a), 4.18 (dd, *J* = 10.8, 5.3 Hz, 1H, H-7b), 3.90 (ddd, *J* = 8.5, 5.2, 1.7 Hz, 1H, H-6), 2.96 – 2.87 (m, 1H, CHH-Lev), 2.75 – 2.61 (m, 2H, CHH-Lev, CHH-Lev), 2.48 (ddd, *J* = 16.9, 6.5, 4.0 Hz, 1H, CHH-Lev), 2.22 (s, 3H, CH₃-Lev). ¹³C NMR (151 MHz, CDCl₃) δ 208.64, 172.47, 165.57, 137.59, 133.34, 133.23, 133.03, 131.40, 130.01, 129.82, 129.80, 129.51, 129.40, 129.30, 128.60, 128.52, 128.48, 128.44, 128.26, 127.88, 126.82, 92.45, 73.60, 73.19, 70.91, 70.71, 68.75, 66.69, 61.28, 38.20, 29.93, 27.88, 26.88. HRMS (m/z): [M + Na⁺] calcd for C₄₀H₃₈NaO₁₂, 733.2255; found 733.2251.

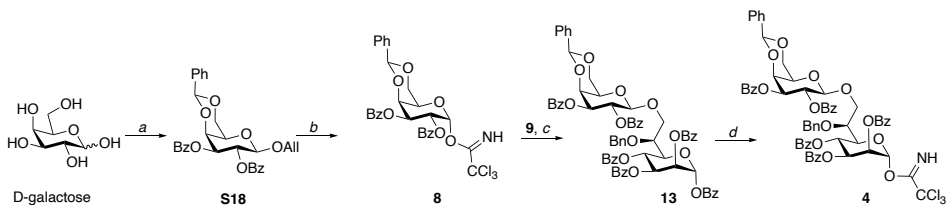
2,3,4-Tri-*O*-benzoyl-6-*O*-benzyl-7-*O*-levulinoyl-L-glycero-D-manno-

heptopyranosyl trichloroacetimidate (5): A solution of **S17** (256 mg, 0.36 mmol) in dry DCM (5 mL) was added trichloroacetonitrile (360



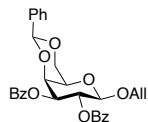
μ L, 3.6 mmol) and DBU (5.4 μ L, 0.036 mmol), and the mixture was stirred for 2 h at room temperature. After TLC analysis

showed consumption of starting material, the reaction was diluted with Et₂O (10 mL), filtered and the filtrate was concentrated *in vacuo*. The resulting residue was purified by silica gel column chromatography (PE/EA = 2/1, v/v) to give **5** (201 mg, 70%) as a white solid, which was directly used for next coupling step. HRMS (*m/z*): [*M* + NH₄⁺] calcd for C₄₂H₄₂Cl₃N₂O₁₂, 871.1798; found 871.1750.



Scheme S3. Synthesis of disaccharide donor **4**. Conditions and reagents: *a*) i. AlIOH, TfOH, reflux. ii. PhCH(OMe)₂, *p*-TsOH, DMF. iii. BzCl, pyridine, DCM, 28% over 3 steps. *b*) i. PdCl₂, MeOH/DCM. ii. CCl₃CN, DBU, DCM, 50%. *c*) TMSOTf, DCM, 98%. *d*) i. NH₂NH₂·HOAc, DMF, 60°C. ii. CCl₃CN, DBU, DCM, 49%.

Allyl 2,3-di-O-benzoyl-4,6-O-benzylidene-β-D-galactopyranoside (S18): A



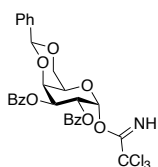
solution of D-(+)-galactose (27.7 g, 153 mmol) in allyl alcohol (200 mL) was added trifluoromethanesulfonic acid (TfOH, 2 mL), and the mixture was stirred for 6 h under reflux (110°C). After

TLC analysis showed consumption of starting material, the reaction was quenched with Et₃N (5 mL) and then concentrated *in vacuo*. The resulting residue was co-evaporated with toluene (50 mL × 3) and dissolved in dry DMF, which was followed by the addition of *p*-toluenesulfonic acid monohydrate (*p*-TsOH·H₂O, 2.9 g, 15.3 mmol) and benzaldehyde dimethyl acetal (25 mL, 168 mmol). The reaction mixture was stirred for 3 h at room temperature and then neutralized with Et₃N. The mixture was concentrated *in vacuo*, and the obtained residue was dissolved in DCM (400 mL). Benzoyl chloride (70 mL, 612 mmol) and pyridine (123 mL) were added to the solution at 0°C. After stirring overnight at room temperature, the reaction was quenched with MeOH (100 mL) and then

concentrated *in vacuo*, and the resulting residue was purified by silica gel column chromatography (PE/EA = 3/1, v/v) to give **S18** (4g, 28% over 3 steps) as a white solid. ^1H NMR (600 MHz, CDCl_3) δ 8.03 – 7.95 (m, 4H, Ar), 7.57 – 7.46 (m, 4H, Ar), 7.42 – 7.33 (m, 7H, Ar), 5.91 (dd, J = 10.4, 8.0 Hz, 1H, H-2), 5.86 – 5.76 (m, 1H, $\text{CH}_2\text{-CH=CH}_2$), 5.56 (s, 1H, CHPh), 5.37 (dd, J = 10.4, 3.6 Hz, 1H, H-3), 5.24 (dq, J = 17.3, 1.7 Hz, 1H, $\text{CH}_2\text{-CH=CHH}$), 5.12 (dq, J = 10.4, 1.4 Hz, 1H, $\text{CH}_2\text{-CH=CHH}$), 4.81 (d, J = 8.0 Hz, 1H, H-1), 4.60 (dd, J = 3.7, 1.1 Hz, 1H, H-4), 4.45 – 4.38 (m, 2H, H-6a, CHH-CH=CH_2), 4.18 (ddt, J = 13.3, 6.3, 1.4 Hz, 1H, CHH-CH=CH_2), 4.15 (dd, J = 12.4, 1.8 Hz, 1H, H-6b), 3.67 – 3.66 (m, 1H, H-5). ^{13}C NMR (151 MHz, CDCl_3) δ 166.24, 165.25, 137.48, 133.74, 133.36, 133.04, 129.95, 129.72, 129.14, 128.94, 128.46, 128.40, 128.30, 128.11, 126.29, 117.44, 100.88 (CHPh), 99.85 (C1), 73.60, 72.84, 69.47, 69.08, 68.97, 66.52. HRMS (m/z): $[\text{M} + \text{Na}^+]$ calcd for $\text{C}_{30}\text{H}_{28}\text{NaO}_8$, 539.1676; found 539.1680.

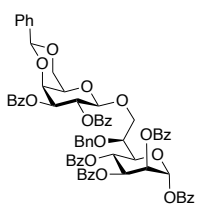
2,3-Di-*O*-benzoyl-4,6-*O*-benzylidene- β -D-galactopyranosyl

trichloroacetimidate (8): A solution of **S18** (1.35 g, 2.61 mmol) in a mixture of MeOH (10 mL) and DCM (10 mL) was added PdCl₂ (93 mg, 0.52 mmol), and the reaction mixture was stirred overnight. After TLC analysis showed consumption of starting material, the reaction was diluted with Et₂O (50 mL), and the solids were filtered off through celite. The filtrate was concentrated *in vacuo*, and the resulting residue was dissolved in dry DCM (10 mL). CCl₃CN (2.6 mL, 26 mmol) and DBU (78 μL , 0.52 mmol) were added to the solution, and the reaction mixture was stirred for 3 h at room temperature, after which it was concentrated *in vacuo*, and the resulting residue was purified by silica gel column chromatography (PE/EA = 3/1, v/v) to give **8** (798 mg, 50% over 2 steps) as a white solid. ^1H NMR (600 MHz, CDCl_3) δ 8.59 (s, 1H, =NH), 8.03 – 7.90 (m, 4H, Ar), 7.58 – 7.45 (m, 5H, Ar), 7.43 – 7.32 (m, 6H, Ar), 6.88 (d, J = 3.5 Hz, 1H, H-1), 6.05 (dd, J = 10.7, 3.5 Hz, 1H, H-2), 5.88 (dd, J = 10.7, 3.4 Hz, 1H, H-3), 5.59 (s, 1H, CHPh), 4.77 (dd, J = 3.5, 1.2 Hz, 1H, H-4), 4.41 (dd, J = 12.6, 1.6 Hz, 1H, H-6a), 4.22 – 4.10 (m, 2H,



H-5, H-6b). ^{13}C NMR (151 MHz, CDCl_3) δ 166.20, 165.52, 160.62, 137.34, 133.44, 133.39, 129.93, 129.90, 129.83, 129.24, 129.06, 129.04, 129.00, 128.47, 128.38, 128.22, 126.17, 126.15, 100.80, 94.73, 73.73, 69.18, 68.84, 67.46, 64.97. HRMS (m/z): $[\text{M} + \text{Na}^+]$ calcd for $\text{C}_{39}\text{H}_{24}\text{Cl}_3\text{NNaO}_8$, 642.0460; found 642.0456.

***N*-(Benzyl)-*N*-benzyloxycarbonyl-5-aminopentyl-2,3-di-*O*-benzoyl-4,6-*O*-benzylidene- β -D-galactopyranosyl-(1 \rightarrow 7)-1,2,3,4-tetra-*O*-benzoyl-6-*O*-benzyl-L-glycero-D-manno-heptopyranoside (**13**):** Galactosyl donor **8** (138 mg,

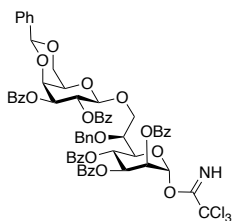


0.22 mmol) and heptosyl acceptor **9** (80 mg, 0.11 mmol) were co-evaporated with toluene (3 mL \times 3) and then dissolved in dry DCM (3 mL) and placed under an atmosphere of Argon. Flame-activated 4Å MS were added to the solution, and the reaction mixture was cooled to 0°C. After stirring for 15 min

at this temperature, TMSOTf (4 μL , 0.02 mmol) was added. The reaction was stirred for 6 h at 0°C, and then quenched with Et_3N (0.5 mL). The solids were filtered off, and the filtrate was concentrated *in vacuo*. The resulting residue was purified by silica gel column chromatography (PE/EA = 3/1, v/v) to give **13** (127 mg, 98%) as a white solid. ^1H NMR (600 MHz, CDCl_3) δ 8.33 – 8.26 (m, 2H, Ar), 8.10 – 8.02 (m, 4H, Ar), 7.94 – 7.90 (m, 2H, Ar), 7.86 – 7.81 (m, 2H, Ar), 7.71 – 7.63 (m, 3H, Ar), 7.62 – 7.27 (m, 27H, Ar), 6.18 (t, J = 10.0 Hz, 1H, Hep-H-4), 5.96 (dd, J = 10.4, 8.0 Hz, 1H, Gal-H-2), 5.92 – 5.86 (m, 2H, Hep-H-2, CHPh), 5.72 (dd, J = 10.4, 3.7 Hz, 1H, Gal-H-3), 5.58 (s, 1H, Hep-H-1), 5.07 (d, J = 8.0 Hz, 1H, Gal-H-1), 4.93 – 4.86 (m, 1H, Hep-H-3), 4.72 (dd, J = 3.7, 1.0 Hz, 1H, Gal-H-4), 4.52 – 4.44 (m, 2H, CHHPh), 4.38 (dd, J = 12.4, 1.6 Hz, 1H, Gal-H-6a), 4.22 (dd, J = 10.1, 4.5 Hz, 1H, Hep-H-7a), 4.15 (dd, J = 12.5, 1.8 Hz, 1H, Gal-H-6b), 3.87 (dd, J = 10.0, 1.6 Hz, 1H, Hep-H-5), 3.84 – 3.78 (m, 2H, Hep-H-7b, Gal-H-5), 3.69 (ddd, J = 10.3, 4.6, 1.5 Hz, 1H, Hep-H-6). ^{13}C NMR (151 MHz, CDCl_3) δ 166.27, 166.26, 165.55, 165.18, 165.07, 164.51, 137.62, 134.22, 133.82, 133.43, 133.34, 130.16, 130.10, 130.00, 129.94, 129.83, 129.75, 129.32, 129.04, 128.89, 128.55, 128.53, 128.45, 128.43, 128.38, 128.32, 128.09,

127.78, 126.28, 101.81, 100.87, 91.32, 73.90, 73.79, 73.72, 72.56, 69.64, 69.17, 69.01, 66.48, 65.06. HRMS (m/z): $[M + Na^+]$ calcd for $C_{69}H_{58}NaO_{18}$, 1197.3515; found 1197.3507.

***N*-(Benzyl)-*N*-benzyloxycarbonyl-5-aminopentyl-2,3-di-*O*-benzoyl-4,6-*O*-benzylidene- β -D-galactopyranosyl-(1 \rightarrow 7)-2,3,4-tri-*O*-benzoyl-6-*O*-benzyl-L-glycero-D-manno-heptopyranosyl trichloroacetimidate (**4**):** $NH_2NH_2 \cdot HOAc$

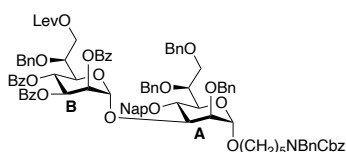


(18.4 mg, 0.2 mmol) was added to a solution of **13** (120 mg, 0.1 mmol) in DMF (5 mL), and the reaction was stirred for 3 h at 60°C. After TLC analysis showed consumption of starting material, the reaction mixture was concentrated *in vacuo*, and the resulting residue was dissolved in DCM (30 mL) and washed with water (20

mL). The organic layer was dried (Na_2SO_4), filtered, and the filtrate was concentrated *in vacuo*. The resulting crude product were dissolved in dry DCM, and CCl_3CN (100 μ L, 1.0 mmol) and DBU (4.5 μ L, 0.3 mmol) (5 mL) were added. The reaction mixture was stirred for 3 h at room temperature, and then diluted with Et_2O (10 mL), filtered, and the filtrate was concentrated *in vacuo*. The resulting residue was purified by silica gel column chromatography (PE/EA = 1/1, v/v) to give the disaccharide donor **4** (59 mg, 49% over 2 steps) as a light-yellow oil. 1H NMR (600 MHz, $CDCl_3$) δ 8.84 (s, 1H, =NH), 8.13 – 7.95 (m, 7H, Ar), 7.87 – 7.80 (m, 2H, Ar), 7.66 (dd, J = 8.3, 1.4 Hz, 2H, Ar), 7.59 – 7.22 (m, 24H, Ar), 6.57 (d, J = 2.0 Hz, 1H, Hep-H-1), 6.39 (t, J = 10.2 Hz, 1H, Hep-H-4), 5.91 (dd, J = 3.2, 2.1 Hz, 1H, Hep-H-2), 5.89 – 5.85 (m, 2H, Gal-H-2, Hep-H-3), 5.53 (s, 1H, CHPh), 5.26 (dd, J = 10.4, 3.6 Hz, 1H, Gal-H-3), 4.79 (d, J = 8.0 Hz, 1H, Gal-H-1), 4.73 (d, J = 10.3 Hz, 1H, CHHPh), 4.57 (dd, J = 3.6, 1.1 Hz, 1H, Gal-H-4), 4.52 – 4.45 (m, 2H, Hep-H-5, CHHPh), 4.31 (dd, J = 12.4, 1.6 Hz, 1H, Gal-H-6a), 4.23 (dd, J = 10.9, 5.3 Hz, 1H, Hep-H-7a), 4.08 (dd, J = 12.4, 1.8 Hz, 1H, Gal-H-6b), 4.00 (dd, J = 10.9, 7.0 Hz, 1H, Hep-H-7b), 3.80 (ddd, J = 7.0, 5.2, 1.5 Hz, 1H, Hep-H-6), 3.63 (m, 1H, Gal-H-5). ^{13}C NMR (151 MHz, $CDCl_3$) δ 166.18,

165.53, 165.27, 165.19, 165.18, 160.15, 137.89, 137.54, 133.49, 133.39, 133.34, 133.28, 133.22, 130.06, 129.99, 129.78, 129.71, 129.60, 129.10, 128.97, 128.95, 128.92, 128.87, 128.61, 128.58, 128.53, 128.42, 128.39, 128.34, 128.31, 128.14, 127.62, 126.31, 101.54, 100.92, 95.46, 90.71, 74.64, 73.69, 73.54, 72.80, 72.73, 70.41, 69.19, 68.79, 68.52, 67.75, 66.61, 65.34. HRMS (m/z): [M + Na⁺] calcd for C₆₄H₅₄Cl₃NNaO₁₇, 1236.2350; found 1236.2326.

***N*-(Benzyl)-*N*-benzyloxycarbonyl-5-aminopentyl 2,3,4-tri-*O*-benzoyl-6-*O*-benzyl-7-*O*-levulinoyl-*L*-glycero- α -*D*-manno-heptopyranosyl-(1 \rightarrow 3)-4-*O*-(2-methylnaphthyl)-2,6,7-tri-*O*-benzyl-*L*-glycero- α -*D*-manno-heptopyranoside (10):** Heptosyl donor **5** (169 mg, 0.23 mmol) and heptosyl acceptor **6** (144 mg,

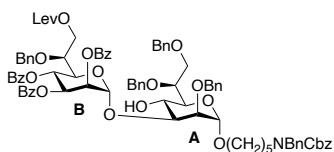


0.155 mmol) were co-evaporated with toluene (3 mL \times 3) and then dissolved in dry DCM (3 mL), and the solution was placed under an atmosphere of Argon. Flame-activated 4Å MS were added,

and the suspension was cooled to 0°C. After stirring for 15 min at this temperature, TMSOTf (5.6 μ L, 0.028 mmol) was added. The reaction mixture was stirred for 6 h at 0°C, after which it was quenched with Et₃N (0.5 mL). The solids were filtered off, and the filtrate was concentrated *in vacuo*. The resulting residue was purified by silica gel column chromatography (PE/EA = 1.5/1, v/v) to give **10** (233 mg, 93%) as a colorless oil. ¹H NMR (600 MHz, CDCl₃) δ 7.95 – 7.87 (m, 2H, Ar), 7.85 – 7.73 (m, 4H, Ar), 7.67 – 7.09 (m, 46H), 6.23 (t, *J* = 10.1 Hz, 1H, B-H-4), 6.03 (dd, *J* = 10.3, 3.1 Hz, 1H, B-H-3), 5.87 (s, 1H, B-H-2), 5.52 (s, 1H, B-H-1), 5.20 – 5.09 (m, 2H, CH₂-Cbz), 5.06 – 4.78 (m, 6H, A-H-1, CHHAr, CHHAr \times 2), 4.64 (d, *J* = 10.4 Hz, 1H, CHHAr), 4.57 – 4.41 (m, 8H, A-H-6, A-H-7a, CHHAr \times 3), 4.30 (s, 2H, B-H-5, B-H-6), 4.11 (bs, 2H, A-H-4, A-H-7b), 3.87 (m, 2H, A-H-2, B-H-7a), 3.76 (s, 2H, A-H-3, B-H-7b), 3.73 – 3.67 (m, 1H, A-H-5), 3.60 – 3.49 (m, 1H, CHH-Linker), 3.32 – 3.06 (m, 3H, CHH-Linker, CHH-Linker), 2.56 (d, *J* = 9.5 Hz, 2H, CH₂-Lev), 2.26 (m, 2H, CH₂-Lev), 2.07 (s, 3H, CH₃-Lev), 1.51 – 1.36 (m, 4H, CHH-Linker \times 2), 1.21 – 1.03 (m, 2H,

CHH-Linker). ^{13}C NMR (151 MHz, CDCl_3) δ 172.17, 165.67, 165.45, 164.95, 138.67, 138.51, 138.10, 138.01, 137.78, 136.82, 135.46, 133.42, 133.19, 133.13, 132.78, 129.88, 129.83, 129.29, 129.22, 129.12, 128.59, 128.55, 128.51, 128.48, 128.44, 128.42, 128.39, 128.32, 128.15, 128.01, 127.92, 127.83, 127.74, 127.67, 127.62, 127.58, 127.43, 127.22, 126.02, 125.67, 125.56, 75.31, 74.77, 74.20, 73.87, 73.39, 72.37, 72.15, 70.52, 70.38, 70.14, 67.37, 67.13, 66.56, 50.48, 50.16, 47.11, 46.20, 37.73, 29.80, 29.75, 29.22, 28.03, 27.55, 27.44, 23.43. HRMS (m/z): $[\text{M} + \text{Na}^+]$ calcd for $\text{C}_{99}\text{H}_{99}\text{NNaO}_{12}$, 1644.6653; found 1644.6647.

***N*-(Benzyl)-*N*-benzyloxycarbonyl-5-aminopentyl 2,3,4-tri-*O*-benzoyl-6-*O*-benzyl-7-*O*-levulinoyl-*L*-glycero- α -D-manno-heptopyranosyl-(1 \rightarrow 3)-2,6,7-tri-*O*-benzyl-*L*-glycero- α -D-manno-heptopyranoside (**11**):** β -Pinene (123 μL ,

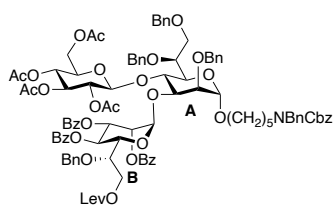


0.78 mmol) and DDQ (100 mg, 0.44 mmol) were added to a mixture of **10** (370 mg, 0.22 mmol) in $\text{DCM}/\text{H}_2\text{O}$ (40 mL / 2 mL) under the exclusion of light. The reaction mixture was stirred for 2 h at

room temperature, after which it was quenched with sat. aq. NaHCO_3 (20 mL). The two-phasic mixture was separated, and the organic layer was dried (Na_2SO_4), filtered, and the filtrate was concentrated *in vacuo*. The resulting residue was purified by silica gel column chromatography ($\text{DCM}/\text{acetone} = 30/1$, v/v) to give **11** (318 mg, 97%) as colorless oil. ^1H NMR (600 MHz, CDCl_3) δ 7.99 (dd, $J = 7.7, 3.6$ Hz, 2H, Ar), 7.96 – 7.92 (m, 2H, Ar), 7.91 – 7.84 (m, 2H, Ar), 7.59 – 7.49 (m, 4H, Ar), 7.48 – 7.11 (m, 35H, Ar), 6.26 (t, $J = 10.0$ Hz, 1H, B-H-4), 6.00 (t, $J = 9.5$ Hz, 1H, B-H-3), 5.88 – 5.80 (m, 1H, B-H-2), 5.62 – 5.46 (bs, 1H, B-H-1), 5.17 – 5.06 (m, 2H, $\text{CH}_2\text{-Cbz}$), 4.98 – 4.92 (m, 1H, CHHPh), 4.88 – 4.86 (m, 2H, A-H-1, CHHPh), 4.81 (d, $J = 12.0$ Hz, 1H, CHHPh), 4.68 – 4.59 (m, 2H, CHHPh , CHHPh), 4.58 – 4.48 (m, 3H, A-H-7a, CHHPh , CHHPh), 4.49 – 4.39 (m, 2H, CHHPh), 4.27 – 4.23 (m, 2H, A-H-5, B-H-5), 4.15 – 4.08 (m, 1H, A-H-7b), 4.07 – 3.94 (m, 2H, A-H-3, B-H-6), 3.79 – 3.70 (m, 4H, A-H-2, A-H-6, B-H-7a/b), 3.65 – 3.42 (m, 3H, A-H-4, CHH-Linker), 3.32 – 3.07 (m, 2H, CHH-Linker).

Linker), 2.56 (q, $J = 6.9$ Hz, 2H, CH₂-Lev), 2.33 – 2.25 (m, 1H, CHH-Lev), 2.17 (m, 1H, CHH-Lev), 2.07 (s, 3H, CH₃-Lev), 1.51 – 1.34 (m, 4H, CHH-Linker \times 2), 1.22 – 1.01 (m, 2H, CHH-Linker). ¹³C NMR (151 MHz, CDCl₃) δ 172.18, 165.65, 165.45, 165.32, 138.69, 137.94, 137.82, 133.36, 133.11, 129.96, 129.83, 129.41, 129.34, 129.27, 129.06, 128.54, 128.52, 128.50, 128.45, 128.37, 128.32, 128.25, 127.84, 127.70, 127.58, 127.52, 127.21, 125.32, 99.12, 97.90, 74.58, 74.14, 73.78, 73.38, 73.24, 72.50, 71.77, 70.57, 70.24, 69.90, 67.82, 67.15, 67.10, 66.60, 50.21, 47.29, 46.15, 37.74, 29.76, 27.44, 23.51. HRMS (m/z): [M + Na⁺] calcd for C₈₈H₉₁NNaO₂₀, 1504.6027; found 1504.6028.

***N*-(Benzyl)-*N*-benzyloxycarbonyl-5-aminopentyl [2,3,4-tri-*O*-benzoyl-6-*O*-benzyl-7-*O*-levulinoyl-*L*-glycero- α -D-manno-heptopyranosyl]-(1 \rightarrow 3)-[2,3,4,6-tetra-*O*-acetyl- β -D-glucopyranosyl]-(1 \rightarrow 4)-2,6,7-tri-*O*-benzyl-*L*-glycero- α -D-manno-heptopyranoside (12):** Glucosyl donor **7**⁴⁵ (208 mg, 0.45

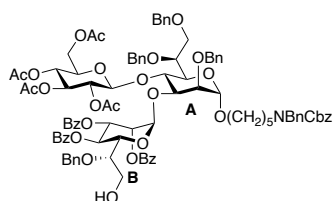


mmol) and disaccharide acceptor **11** (338 mg, 0.228 mmol) were co-evaporated with toluene (3 mL \times 3) and then dissolved in dry DCM (3 mL), and the solution was placed under an atmosphere of Argon. Flame-activated 4Å MS were added, and

the suspension was cooled to 0°C. After stirring for 15 min at this temperature, TMSOTf (41 μ L, 0.228 mmol) was added. The reaction was stirred for 1 h at 0°C, and another portion of glucosyl donor **7** (156 mg, 0.342 mmol) was then added, after which the reaction mixture was slowly warmed to room temperature and kept stirring overnight. The reaction was then quenched with Et₃N (0.5 mL), and the solids were filtered off. The filtrate was concentrated *in vacuo*, and the resulting residue was purified by silica gel column chromatography (DCM/acetone = 35/1, v/v) to give **12** (220 mg, 53%) as a light-yellow solid. ¹H NMR (600 MHz, CDCl₃) δ 8.05 (dd, $J = 8.1, 1.4$ Hz, 2H, Ar), 7.95 – 7.90 (m, 2H, Ar), 7.82 – 7.80 (m, 2H, Ar), 7.58 (d, $J = 7.2$ Hz, 2H, Ar), 7.52 (t, $J = 7.4$ Hz, 2H, Ar), 7.45 (d, $J = 7.3$ Hz, 4H, Ar), 7.40 – 7.13 (m, 31H, Ar), 6.30 (t, $J = 10.0$ Hz,

1H, B-H-4), 6.07 (td, $J = 11.2, 2.5$ Hz, 1H, B-H-3), 5.90 (s, 1H, B-H-2), 5.48 (s, 1H, B-H-1), 5.21 – 5.11 (m, 2H, $\underline{\text{CH}_2}$ -Cbz), 5.09 – 4.81 (m, 6H, A-H-1, A-H-4, Glc-H-2, Glc-H-3, Glc-H-4, $\underline{\text{CHHPh}}$), 4.64 (d, $J = 10.6$ Hz, 1H, $\underline{\text{CHHPh}}$), 4.60 – 4.42 (m, 9H, B-H-7a, $\underline{\text{CHHPh}} \times 4$), 4.38 (m, 1H, Glc-H-6a), 4.31 – 4.22 (m, 1H, B-H-5), 4.17 – 4.15 (m, 2H, Glc-H-1, A-H-3), 4.14 – 4.01 (m, 2H, A-H-6, B-H-7b), 3.97 (dd, $J = 12.2, 2.1$ Hz, 1H, Glc-H-6b), 3.91 (m, 1H, A-H-7a), 3.83 (s, 1H, A-H-2), 3.76 (dd, $J = 9.2, 6.4$ Hz, 1H, A-H-7b), 3.72 – 3.67 (m, B-H-6), 3.66 – 3.63 (m, 1H, A-H-5), 3.62 – 3.45 (m, 1H, $\underline{\text{CHH-Linker}}$), 3.29 – 3.11 (m, 5H, $\underline{\text{CHH-Linker}}$, $\underline{\text{CHH-Linker}}$, CH_2 -Lev), 2.83 – 2.70 (m, 1H, Glc-H-5), 2.58 – 2.50 (m, 2H, CH_2 -Lev), 2.02 (s, 3H, CH_3 -Lev), 1.94 (s, 6H, $\text{Ac} \times 2$), 1.93 (s, 3H, Ac), 1.62 (s, 3H, Ac), 1.51 – 1.38 (m, 5H, $\underline{\text{CHH-Linker}} \times 2$, $\underline{\text{CHH-Linker}}$), 1.19 – 1.03 (m, 1H, $\underline{\text{CHH-Linker}}$). ^{13}C NMR (151 MHz, CDCl_3) δ 170.46, 169.28, 168.98, 165.40, 138.66, 137.93, 132.95, 130.02, 129.79, 129.75, 128.77, 128.54, 128.51, 128.45, 128.42, 128.34, 128.29, 128.27, 127.79, 127.77, 127.55, 104.14, 98.99, 98.34, 77.09, 75.48, 75.00, 74.03, 73.71, 73.55, 73.39, 72.42, 71.94, 71.78, 71.62, 71.30, 70.49, 70.17, 69.37, 68.08, 67.43, 67.11, 66.95, 66.79, 65.02, 64.54, 62.12, 61.96, 61.48, 61.32, 50.85, 50.21, 47.15, 46.34, 39.26, 37.81, 37.17, 37.01, 35.72, 34.11, 33.79, 33.63, 33.30, 32.82, 32.02, 31.53, 30.25, 29.76, 28.96, 28.80, 28.64, 28.47, 28.15, 27.99, 27.67, 27.35, 27.19, 26.70, 24.77, 23.65, 23.32, 22.84, 22.68, 22.20, 20.59, 20.26, 20.10, 19.78, 19.14, 18.65, 14.15, 12.70, 10.77. ($J_{\alpha\text{Hep},\text{A}} \text{C1-H1} = 169.9$ Hz, $J_{\alpha\text{Hep},\text{B}} \text{C1-H1} = 178.0$ Hz, $J_{\beta\text{Glc}} \text{C1-H1} = 159.6$ Hz). HRMS (m/z): $[\text{M} + \text{Na}^+]$ calcd for $\text{C}_{102}\text{H}_{109}\text{NNaO}_{29}$, 1834.6977; found 1834.6978.

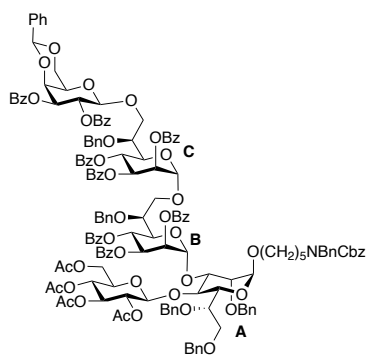
***N*-(Benzyl)-*N*-benzyloxycarbonyl-5-aminopentyl [2,3,4-tri-*O*-benzoyl-6-*O*-benzyl-L-glycero- α -D-manno-heptopyranosyl]-(1 \rightarrow 3)-[2,3,4,6-tetra-*O*-acetyl- β -D-glucopyranosyl]-(1 \rightarrow 4)-2,6,7-tri-*O*-benzyl-L-glycero- α -D-manno-**



heptopyranoside (3): $\text{NH}_2\text{NH}_2 \cdot \text{HOAc}$ (20 mg, 0.22 mmol) was added to a solution of **12** (200 mg, 0.11 mmol) in a mixture of DCM (5 mL) and MeOH (0.5 mL), and the reaction mixture was

stirred for 3 h at room temperature, after which it was concentrated *in vacuo*. The resulting residue was dissolved in DCM (50 mL) and washed with water (30 mL). The organic layer was dried (Na₂SO₄), filtered, and the filtrate was concentrated *in vacuo*. The resulting residue was purified by silica gel column chromatography (DCM/acetone = 35/1, v/v) to give **3** (170 mg, 90%) as white solid. ¹H NMR (600 MHz, CDCl₃) δ 8.15 – 7.08 (m, 45H, Ar), 6.29 (t, *J* = 9.9 Hz, 1H, B-H-4), 5.99 (dd, *J* = 10.2, 3.0 Hz, 1H, B-H-3), 5.85 (s, 1H, B-H-2), 5.50 (s, 1H, B-H-1), 5.21 – 5.09 (m, 2H, CH₂-Cbz), 5.03 (d, *J* = 12.6 Hz, 1H, CHH-Ph), 5.00 – 4.79 (m, 7H, A-H-1, Glc-H-2, Glc-H-3, Glc-H-4, CHHPh, CHHPh), 4.70 – 4.40 (m, 7H, A-H-4, CHHPh × 3), 4.34 – 4.28 (m, 1H, Glc-H-6a), 4.20 – 4.09 (m, 2H, Glc-H-1, A-H-3), 4.03 (m, 3H, A-H-6, B-H-6, Glc-H-6b), 3.99 – 3.87 (m, 3H, A-H-2, A-H-7a, B-H-7a), 3.86 – 3.69 (m, 2H, A-H-7b, B-H-7b), 3.70 – 3.47 (m, 2H, A-H-5, CHH-Linker), 3.36 – 3.06 (m, 3H, CHH-Linker, CHH-Linker), 2.85 – 2.74 (m, 1H, Glc-H-5), 1.99 – 1.92 (m, 12H, Ac × 4), 1.50 – 1.02 (m, 6H, CHH-Linker × 3). ¹³C NMR (151 MHz, CDCl₃) δ 170.85, 168.96, 169.28, 165.44, 165.37, 137.80, 132.96, 130.08, 129.75, 128.79, 128.55, 128.50, 128.43, 128.26, 127.84, 127.78, 127.62, 127.52, 100.44, 98.50, 98.34, 78.22, 77.09, 76.61, 74.03, 73.71, 73.55, 73.39, 72.91, 72.26, 71.78, 71.62, 71.46, 70.49, 69.69, 69.37, 68.24, 67.27, 66.79, 61.64, 50.21, 47.15, 46.99, 46.18, 37.33, 37.17, 33.63, 32.82, 32.02, 30.25, 30.08, 29.76, 29.60, 28.64, 28.31, 28.15, 27.51, 27.19, 26.86, 23.32, 23.16, 22.84, 22.68, 20.59. HRMS (*m/z*): [*M* + Na⁺] calcd for C₉₇H₁₀₃NNaO₂₇, 1736.6610; found 1736.6619.

***N*-(Benzyl)-*N*-benzyloxycarbonyl-5-aminopentyl [2,3-di-*O*-benzoyl-4,6-*O*-benzylidene- β -D-galactopyranosyl-(1 \rightarrow 7)-2,3,4-tri-*O*-benzoyl-6-*O*-benzyl-L-glycero- α -D-manno-heptopyranosyl-(1 \rightarrow 7)-2,3,4-tri-*O*-benzoyl-6-*O*-benzyl-L-glycero- α -D-manno-heptopyranosyl]-(1 \rightarrow 3)-[2,3,4,6-tetra-*O*-acetyl- β -D-glucopyranosyl]-(1 \rightarrow 4)-2,6,7-tri-*O*-benzyl-L-glycero- α -D-manno-heptopyranoside (**14**):** Disaccharide donor **4** (56 mg, 0.046 mmol) and

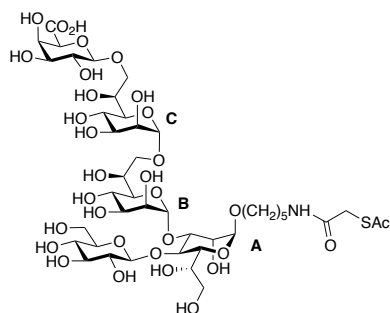


trisaccharide acceptor **3** (40 mg, 0.023 mmol) were co-evaporated with toluene (3 mL \times 3) and then dissolved in dry DCM (2 mL), and the solution was placed under an atmosphere of Argon. Flame-activated 4Å MS was added, and the suspension was cooled to 0°C. After stirring for 15 min at this temperature, TMSOTf (0.8 μ L, 0.0046 mmol) was added,

and the reaction mixture was stirred for 6 h at 0°C and then quenched with Et₃N (0.1 mL). The solids were filtered off and the filtrate was concentrated *in vacuo*. The resulting residue was purified by silica gel column chromatography (DCM/acetone = 40/1, v/v) to give **14** (50 mg, 79%) as a white solid. ¹H NMR (600 MHz, CDCl₃) δ 8.18 – 6.84 (m, 80H, Ar), 6.24 – 6.19 (m, 2H, B-H-4, C-H-4), 6.08 (dd, *J* = 10.1, 2.5 Hz, 1H, B-H-3), 5.98 (s, 1H, B-H-2), 5.83 (dd, *J* = 10.4, 7.9 Hz, 1H, Gal-H-2), 5.66 – 5.49 (m, 3H, B-H-1, C-H-2, C-H-3), 5.47 (s, 1H, CHPh), 5.25 – 5.17 (m, 2H, A-H-1, Gal-H-3), 5.14 – 4.98 (m, 8H, C-H-1, Glc-H-2, Glc-H-4, CH₂-Cbz, CH₂Ph, CHHPh), 4.87 (t, *J* = 9.4 Hz, 1H, Glc-H-3), 4.73 (d, *J* = 10.2 Hz, 1H, CHHPh), 4.67 – 4.55 (m, 4H, Gal-H-1, CH₂Ph, CHHPh), 4.54 – 4.49 (m, 3H, CHHPh, CH₂Ph), 4.47 (d, *J* = 3.7 Hz, 1H, Gal-H-4), 4.46 – 4.36 (m, 4H, B-H-5, Glc-H-6a, CH₂Ph), 4.27 – 4.09 (m, 7H, A-H-3, A-H-4, A-H-5, C-H-5, Glc-H-1, B-H-7a, C-H-7a), 4.03 – 3.89 (m, 3H, A-H-2, Glc-H-6b, A-H-7a, C-H-7b), 3.86 – 3.68 (m, 5H, A-H-6, B-H-6, C-H-6, A-H-7b, B-H-7b), 3.49 – 3.24 (m, 3H, Gal-H-5, CHH-Linker), 3.03 – 2.60 (m, 3H, Glc-H-5, CHH-

Linker), 2.07 – 1.87 (m, 9H, Ac \times 3), 1.57 (s, 3H, Ac), 1.51 – 0.79 (m, 6H, CHH-Linker \times 3). ^{13}C NMR (151 MHz, CDCl_3) δ 170.72, 170.29, 170.29, 169.43, 166.00, 165.59, 165.56, 165.40, 165.36, 165.33, 138.09, 133.01, 129.87, 129.77, 129.58, 129.21, 128.73, 128.50, 128.46, 128.37, 128.22, 128.09, 127.85, 127.45, 127.32, 126.24, 100.76 (CHPh), 100.60 (Gal-C1, $J_{\text{C1-H1}}$ = 156 Hz), 100.44 (Glc-C1, $J_{\text{C1-H1}}$ = 162 Hz), 99.15 (HepB-C1, $J_{\text{C1-H1}}$ = 178 Hz), 98.18 (HepA-C1, $J_{\text{C1-H1}}$ = 174 Hz), 96.41 (HepC-C1, $J_{\text{C1-H1}}$ = 174 Hz), 78.54, 76.29, 74.68, 73.87, 73.87, 73.71, 73.55, 73.55, 73.23, 73.07, 72.75, 72.10, 71.78, 71.62, 71.62, 71.46, 70.81, 70.65, 70.17, 70.17, 70.01, 70.01, 70.01, 70.01, 69.04, 69.04, 68.72, 68.72, 67.76, 67.76, 67.59, 67.59, 67.11, 66.79, 66.31, 66.31, 60.99, 60.99, 50.05, 46.83, 46.02, 31.53, 30.41, 30.25, 30.25, 29.44, 29.12, 27.51, 27.51, 23.16, 23.16, 20.75, 20.59, 20.10, 14.15. HRMS (m/z): $[\text{M} + \text{Na}^+]$ calcd for $\text{C}_{159}\text{H}_{155}\text{NNaO}_{43}$, 2788.9865; found 2788.9874.

***N*-thioacetylacetyl-5-amino-pentyl-[β -D-galactopyranosyluronate-(1 \rightarrow 7)-L-glycero- α -D-manno-heptopyranosyl-(1 \rightarrow 7)-L-glycero- α -D-manno-heptopyranosyl]-(1 \rightarrow 3)-[β -D-glucopyranosyl]-(1 \rightarrow 4)-L-glycero- α -D-manno-heptopyranoside (15):** Pentasaccharide **14** (50 mg, 0.018 mmol) was dissolved



in a mixture of TFA (0.9 mL), H_2O (0.1 mL) and DCM (9 mL) at 0°C , and the reaction mixture was stirred for 30 min at this temperature. After TLC analysis showed consumption of starting material, the reaction mixture was diluted with toluene (20 mL) and concentrated *in vacuo*. The resulting residue was co-evaporated with toluene (5 mL \times 3)

and was then dissolved in a mixture of DCM (2 mL) and H_2O (1 mL). TEMPO (2,2,6,6-Tetramethyl-1-piperidinyloxy, free radical, 0.56 mg, 0.0036 mmol) and BAIB ((diacetoxyiodo)benzene, 14.5 mg, 0.045 mmol) were added to the two-phasic solution, and the reaction was stirred for 4 h at room temperature. However,

TLC analysis (PE/EA = 1/2, v/v) showed the starting material converted to a mixture of aldehyde and desired carboxylic acid, the reaction was then quenched with sat. aq. $\text{Na}_2\text{S}_2\text{O}_3$ (5 mL), and the mixture was extracted with DCM (20 mL \times 3). The organic layer was dried (Na_2SO_4), filtered, and the filtrate was concentrated *in vacuo*. The resulting residue was dissolved in a mixture of *t*-BuOH/ H_2O /DCM (1 mL/0.5 mL/0.2 mL). NaClO_2 (6.5 mg, 0.072 mmol) and NaH_2PO_4 (13 mg, 0.108 mmol) were added to the solution, and the reaction was stirred for 30 min at room temperature, after which it was diluted with water (10 mL) and extracted with EtOAc (20 mL \times 5). The organic layer was dried (Na_2SO_4), filtered, and the filtrate was concentrated *in vacuo*. The resulting residue was purified by silica gel column chromatography (DCM/MeOH = 10/1, v/v) to give the desired acid, which was used in the next step without additional purification.

The obtained acid was dissolved in a mixture of NaOH (1M aqueous, 0.5 mL), 1,4-dioxane (1.5 mL) and MeOH (0.5 mL), and the reaction mixture was stirred overnight at room temperature, after which it was neutralized with Amberlite 120 H^+ (washed with 1 M NaOH, H_2O , 1 M HCl, H_2O and acetone sequentially before use). The resin was filtered off, and the filtrate was concentrated *in vacuo*. The resulted residue was co-evaporated with toluene (5 mL \times 3) and dissolved in a mixture of *t*-BuOH (3 mL) and H_2O (2 mL). $\text{Pd}(\text{OH})_2/\text{C}$ (digussa type, 30 mg) was added to the solution, and the reaction suspension was stirred for 24 h at room temperature under an atmosphere of H_2 (1 atm). The solids were filtered off, and the filtrate was concentrated *in vacuo*. The resulting residue was dissolved in dry DMF (2 mL). S-acetylthioglycolic acid pentafluorophenyl ester (SAMA-OPfp, 10.8 mg, 0.036 mmol) and *N,N*-diisopropylethylamine (DIPEA, 6.2 μL , 0.036 mmol) were added, and the solution was placed under an atmosphere of Argon. The reaction was stirred for 3 h at room temperature, and then quenched with water (0.1 mL) and concentrated *in vacuo*. The resulting residue was purified by bio-gel P2 column using 5% *n*-BuOH in water as the eluent to give the

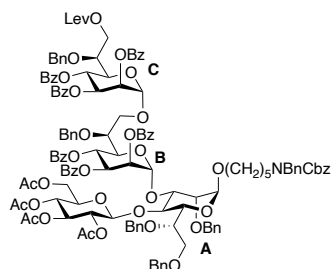
pentasaccharide **15** (7.5 mg, 37% over 5 steps) as a white solid. HRMS (m/z): $[M-H]^+$ calcd for $C_{42}H_{70}NO_{32}S$, 1132.3607; found 1132.3598.

Table S1. 1H NMR spectrum of **15**.

	H1	H2	H3	H4	H5	H6	H7
β GalA	4.37, d, $J = 8.4$ Hz	3.57	3.71	4.21	4.27	—	—
β Glc	4.54, d, $J = 7.8$ Hz	3.31	3.51	3.35	3.47	3.98/3.72	—
α Hep-A	4.81	3.93	4.01	4.23	4.05	NA	NA
α Hep-B	5.27	4.13	3.85	3.74	3.60	NA	NA
α Hep-C	4.85	3.98	3.88	3.89	3.63	NA	NA
Linker	1.37	1.40	1.54	1.61	3.22	3.66	2.42 (Ac)

^{13}C NMR (151 MHz, D_2O) δ 199.43, 174.14, 170.71, 102.53, 102.05, 101.72, 100.27, 99.31, 76.29, 75.48, 75.16, 74.20, 73.71, 72.75, 72.10, 71.94, 71.62, 70.65, 70.49, 70.33, 70.17, 70.01, 69.69, 69.37, 68.88, 67.92, 67.27, 67.11, 65.98, 62.76, 61.48, 43.29, 39.74, 32.82, 29.60, 28.47, 28.15, 23.16.

***N*-(Benzyl)-*N*-benzyloxycarbonyl-5-aminopentyl [2,3,4-tri-*O*-benzoyl-6-*O*-benzyl-7-*O*-levulinoyl-L-glycero- α -D-manno-heptopyranosyl-(1 \rightarrow 7)-2,3,4-tri-*O*-benzoyl-6-*O*-benzyl-L-glycero- α -D-manno-heptopyranosyl]-(1 \rightarrow 3)-[2,3,4,6-tetra-*O*-acetyl- β -D-glucopyranosyl]-(1 \rightarrow 4)-2,6,7-tri-*O*-benzyl-L-glycero- α -D-manno-heptopyranoside (**16**):** Heptosyl donor **5** (105 mg, 0.12

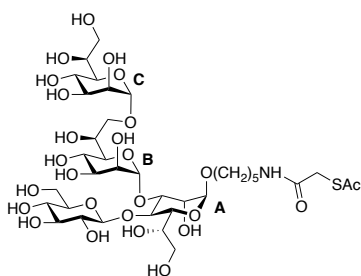


mmol) and trisaccharide acceptor **3** (90 mg, 0.052 mmol) were co-evaporated with toluene (3 mL \times 3) and dissolved in dry DCM (2 mL), and the solution was placed under an atmosphere of Argon. Flame-activated 4Å MS was added and the suspension was cooled to 0°C. After stirred for 15 min at this temperature, TMSOTf (1.9 μ L, 0.0104

mmol) was added. The reaction was stirred for 6 h at 0°C, after which it was

quenched with Et₃N (0.1 mL). The solid was filtered off, and the filtrate was concentrated *in vacuo*. The resulting residue was purified by silica gel column chromatography (DCM/acetone = 15/1, v/v) to give **16** (62 mg, 50%) as a white solid. ¹H NMR (600 MHz, CDCl₃) δ 8.35 – 6.80 (m, 65H, Ar), 6.34 – 6.23 (m, 2H, C-H-1, B-H-4), 6.06 (d, *J* = 9.8 Hz, 1H, C-H-3), 5.95 (s, 1H, C-H-2), 5.80 (dd, *J* = 10.2, 3.2 Hz, 1H, B-H-3), 5.56 (m, 2H, B-H-2, B-H-1), 5.25 – 4.83 (m, 10H, A-H-1, C-H-1, Glc-H-2, Glc-H-3, Glc-H-4, CHHPh-Cbz, CHHPh, CHHPh), 4.76 (d, *J* = 9.8 Hz, 1H, CHHPh), 4.71 – 4.45 (m, 7H, CHHPh × 3, CHHPh), 4.44 – 4.13 (m, 11H, A-H-3, A-H-4, A-H-7a, B-H-5, B-H-7a/b, C-H-5, C-H-7a, Glc-H-1, Glc-H-6a, CHHPh), 4.12 – 3.73 (m, 8H, A-H-2, A-H-5, A-H-6, A-H-7b, C-H-6, C-H-7b, Glc-H-6b), 3.68 (d, *J* = 9.6 Hz, 1H, B-H-6), 3.63 – 3.33 (m, 2H, CHH-Linker), 3.21 – 2.80 (m, 2H, CHH-Linker), 2.78 – 2.29 (m, 5H, Glc-H-5, CH₂-Lev, CHH-Linker), 2.05 – 1.86 (m, 11H, CH₂-Lev, Ac × 3), 1.57 (bs, 10H, CH₃-Lev, Ac, CHH-Linker × 2), 1.39 – 0.83 (m, 2H, CHH-Linker). ¹³C NMR (151 MHz, CDCl₃) δ 170.46, 169.27, 168.96, 165.44, 165.50, 165.40, 165.38, 165.26, 165.01, 164.75, 133.28, 133.28, 133.28, 133.12, 132.96, 130.06, 129.90, 129.90, 129.74, 129.74, 138.84, 137.85, 133.14, 132.98, 129.97, 129.85, 129.82, 129.75, 129.34, 129.24, 128.72, 128.49, 128.47, 128.44, 128.41, 128.37, 128.34, 128.30, 128.27, 128.22, 127.75, 127.54, 127.30, 100.27, 99.15, 98.02, 97.06, 75.80, 74.52, 74.03, 73.87, 73.71, 73.55, 72.75, 71.94, 71.62, 70.65, 70.49, 70.17, 70.01, 69.53, 69.53, 67.92, 67.11, 66.95, 66.31, 61.32, 50.05, 37.81, 31.53, 30.25, 29.76, 29.60, 29.28, 27.83, 27.67, 23.32, 20.59, 20.26. HRMS (*m/z*): [*M* + Na⁺] calcd for C₁₃₇H₁₃₉NNaO₃₈, 2428.8867; found 2428.8855.

***N*-thioacetylacetyl-5-amino-pentyl-[L-glycero- α -D-manno-heptopyranosyl-(1 \rightarrow 7)-L-glycero- α -D-manno-heptopyranosyl]-(1 \rightarrow 3)-[β -D-glucopyranosyl]-(1 \rightarrow 4)-L-glycero- α -D-manno-heptopyranoside (**17**):** The tetrasaccharide **16** (50



mg, 0.02 mmol) was dissolved in a mixture of NaOH (1M aqueous, 0.5 mL), 1,4-dioxane (1.5 mL) and MeOH (0.5 mL), and the reaction mixture was stirred overnight at room temperature, after which it was neutralized with Amberlite 120 H⁺ (washed with 1 M NaOH, H₂O, 1 M HCl, H₂O and acetone sequentially before

use). The resin was filtered off, and the filtrate was concentrated *in vacuo*. The resulting residue was co-evaporated with toluene (5 mL \times 3) and then dissolved in a mixture of *t*-BuOH (3 mL) and H₂O (2 mL). Pd(OH)₂/C (digussa type, 30 mg) was added to the solution, and the reaction suspension was stirred for 24 h at room temperature under an atmosphere of H₂ (1 atm). The solids were filtered off, and the filtrate was concentrated *in vacuo*. The resulting residue was dissolved in dry DMF (2 mL) and placed under an atmosphere of Argon. SAMA-OPfp (12 mg, 0.04 mmol) and DIPEA (6.9 μ L, 0.04 mmol) were added to the solution, and the reaction was stirred for 3 h at room temperature and then quenched with water (0.1 mL). All the solvents were removed *in vacuo*, and the resulting residue was purified by bio-gel P2 column using 5% *n*-BuOH in water as the eluent to give the tetrasaccharide **17** (13 mg, 68%) as a white solid. HRMS (*m/z*): [M + Na⁺] calcd for C₃₆H₆₃NNaO₂₆S, 980.3251; found 980.3247.

Table S2. H¹ NMR spectrum of **17**.

	H1	H2	H3	H4	H5	H6	H7
β Glc	4.56, d, <i>J</i> = 8.0 Hz	3.33	3.51	3.34	3.47	3.98/3.74	—
α Hep-A	4.83	3.95	4.01	4.23	4.01	NA	NA
α Hep-B	5.27	4.14	3.88	3.75	3.61	NA	NA

α Hep-C	4.87	3.98	3.85	3.88	3.59	NA	NA
Linker	1.37	1.42	1.55	1.64	3.22	3.66	2.42 (Ac)

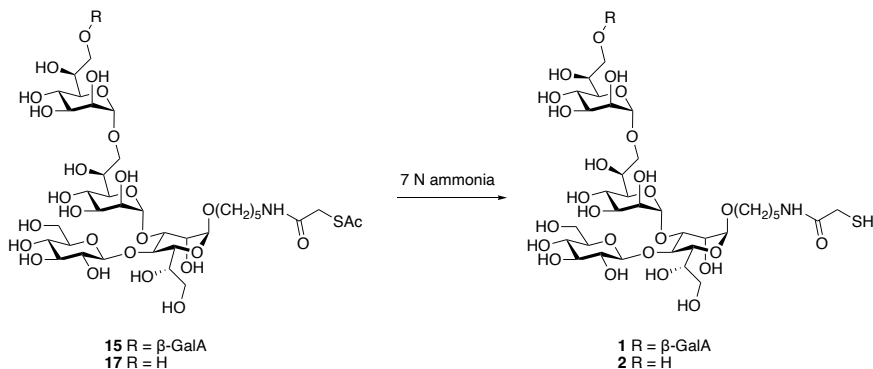
^{13}C NMR (151 MHz, D_2O) δ 199.43, 170.72, 102.57 (Glc-C1, $J_{\text{C1-H1}}$ = 156 Hz), 101.77 (HepB-C1, $J_{\text{C1-H1}}$ = 174 Hz), 100.16 (HepC-C1, $J_{\text{C1-H1}}$ = 174 Hz), 99.35 (HepA-C1, $J_{\text{C1-H1}}$ = 168 Hz), 76.33, 76.17, 75.53, 74.40, 73.75, 73.59, 72.79, 71.66, 71.50, 70.70, 70.37, 70.21, 70.05, 69.73, 68.93, 68.76, 67.80, 67.32, 67.15, 65.87, 62.97, 62.81, 61.52, 54.28, 42.52, 39.79, 34.47, 33.19, 33.03, 32.86, 31.90, 29.64, 28.52, 28.36, 28.20, 23.04.

Conjugation of tetrasaccharide **1** to carrier proteins (BSA and CRM₁₉₇).

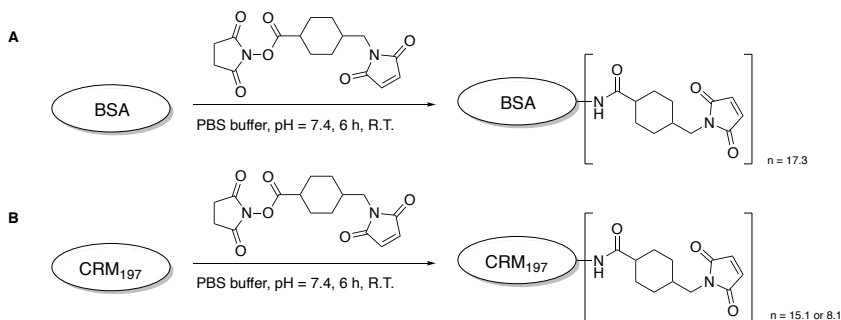
The carrier protein (BSA or CRM₁₉₇, 1 mg) was spin filtered with ultrafilter (Amicon Ultra-0.5 Centrifugal Filter Unit, 10K) 3 times using pH 7.4 PBS buffer as the eluent, and the concentrated protein was diluted with PBS buffer (pH 7.4, 0.5 mL). Succinimidyl 4-(*N*-maleimidomethyl)cyclohexane-1-carboxylate (SMCC, 1.5 mg) was added to the aqueous, and the reaction mixture was shaken at room temperature overnight. It was then centrifuged, and the supernatant was transferred to an ultrafilter (Amicon Ultra-0.5 Centrifugal Filter Unit, 10K) and then purified 5 times using PBS buffer (pH 7.4) as eluent to give the maleimide-activated protein, which was then diluted to ~10 mg/mL (detected by nano-drop) with PBS buffer (pH 7.4) and used for the conjugation directly.

Ammonia (7 N in methanol, 300 μL) was added to a solution of thioacetate-containing oligosaccharide (**15** or **17**, 1.0 mg for each) in Milli-Q water (100 μL) under an atmosphere of Argon. The reaction was stirred for 3 h at room temperature, after which it was concentrated *in vacuo* to give the thiol-containing oligosaccharide (**1** or **2**), which was then dissolved in Milli-Q water (100 μL) and added to the solution of freshly prepared maleimide-activated carrier protein (maleimide-activated-BSA or -CRM₁₉₇). The reaction mixture was shaken at room temperature overnight, and then transferred to an ultrafilter (Amicon Ultra-

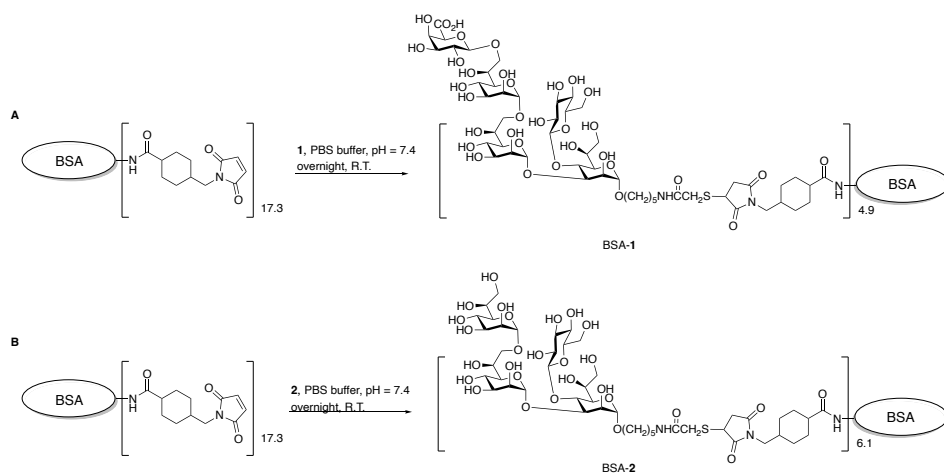
0.5 Centrifugal Filter Unit, 10K). After purified 5 times using PBS buffer (pH 7.4) as eluent, the concentrated glycoconjugate was diluted to ~1 mg/mL with PBS buffer (pH 7.4).



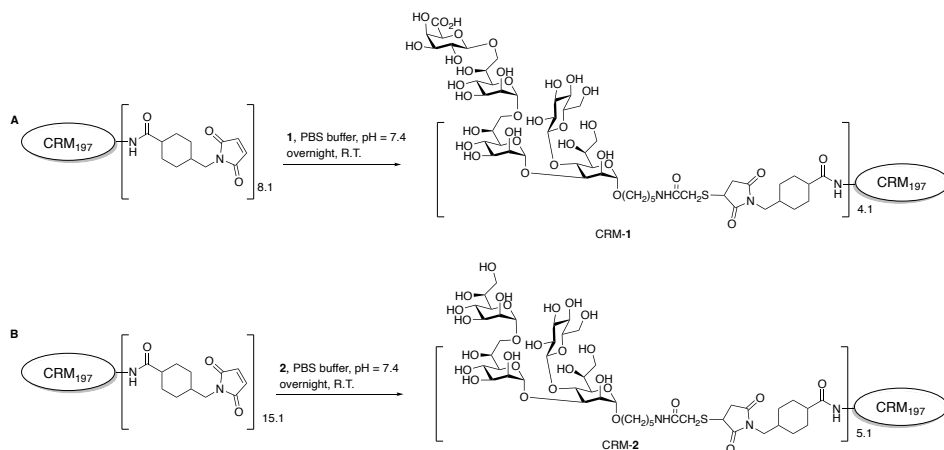
Scheme S4. S-deacetylation of **15** and **17**.



Scheme S5. Conditions of carrier protein modification (A) Modification of BSA with maleimide. (B) Modification of CRM₁₉₇ with maleimide.



Scheme S6. (A) Conjugation of pentasaccharide **1** with maleimide-activated BSA. (B) Conjugation of tetrasaccharide **2** with maleimide-activated BSA.



Scheme S7. (A) Conjugation of pentasaccharide **1** with maleimide-activated CRM₁₉₇. (B) Conjugation of tetrasaccharide **2** with maleimide-activated CRM₁₉₇.

MALDI-TOF analysis of modified proteins

Sinapinic acid was dissolved in CH₃CN/H₂O/TFA (500 μL, 3/7/0.01, v/v/v), and the mixture was treated with ultrasonic and then centrifuged. The resulting supernatant was used as matrix for MALDI-TOF measurement.²⁷ The carrier

proteins or glycoconjugates were desalted with C18 ZipTip (Millipore, 10 μ L) using manufacturer's protocol, and the concentration was adjusted to \sim 1 mg/mL with Milli-Q water. 1 μ L of desalted carrier protein or glycoconjugate was mixed with 2 μ L of freshly prepared matrix solution, and 1 μ L of the resulting mixture was loaded on MALDI plate. After the spot was dried by air, the sample was measured using Linear positive mode.

Table S3. MALDI-TOF data of maleimide-modified carrier proteins.

	BSA ^a	CRM ₁₉₇ ^b	CRM ₁₉₇ ^c	BSA-M ^a	CRM-M ^b	CRM-M ^c
Mass	66715.6	58394.6	57804	70517.0	60159.2	61112
Loading number	—	—	—	17.3	15.1	8.1

^aMeasured on a Kratos Analytical Maxima-CFR MALDI-TOF system, and the modified protein was used for conjugation with compound **1** and **2**; ^bMeasured on a Bruker ultrafleXtreme MALDI-TOF mass spectrometer, and the modified protein was used for the conjugation with compound **1**; ^cMeasured on a Bruker Microflex MALDI-TOF spectrometer by Dr. Lin Liu in The University of Georgia, USA. The modified proteins were used for conjugation with compound **2**. *BSA-M = maleimide-activated BSA, CRM-M = maleimide-activated CRM₁₉₇.

Table S4. MALDI-TOF data of glycoconjugates.

	BSA-1 ^a	BSA-2 ^a	CRM-1 ^b	CRM-2 ^c
Mass	75878.4	76099.5	64644.9	65760
Loading number	4.9	6.1	4.1	5.1

^aMeasured on a Kratos Analytical Maxima-CFR MALDI-TOF system; ^bMeasured on a Bruker ultrafleXtreme MALDI-TOF mass spectrometer; ^cMeasured on a Bruker Microflex MALDI-TOF spectrometer by Dr. Lin Liu in The University of Georgia, USA.

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Chapter 5

Summary and Future Perspectives

Summary

Capsular polysaccharide (CPS) and lipopolysaccharide (LPS) are two virulence factors of *Klebsiella pneumoniae* (*K. pneumoniae*) that provide attractive starting points for vaccine development. The large number (79) of serotypes, however, has complicated CPS vaccine development. In contrast, only nine serotypes of LPS have been identified to date. In addition, only two core types have been identified making the LPS core an attractive target for the development of a broad acting vaccine for *K. pneumoniae*.

Chapter 1 introduces the virulence factors of *K. pneumoniae*, strategies for development of conjugate vaccines and synthetic strategies for complex carbohydrate synthesis. The development of conjugate vaccines relies on the conjugation of a well-defined and non-toxic saccharide to a carrier protein to provide T-cell-dependent immunity. Carrier proteins, isolated from cultured bacterial strains, are not compatible with organic solvents nor harsh reaction conditions which complicates conjugation with glycans. The development of new carriers, such as peptides, glycolipid, or nanoparticle, is still at an early stage but offer possibilities to replace protein carriers in the future. Despite most of licensed conjugate vaccine are based on extracted/isolated glycans, it is still difficult to obtain a correct polysaccharide in sufficient quantities, and it is difficult to culture every bacterial strain. Enzymatic synthesis methods are step-economical; however, it is challenging to express the required enzymes in sufficient quantities. On the other hand, chemical synthesis is as a well-developed method that can address these issues. Additionally, it is possible to determine the minimal length of glycan chain to elicit protective antibodies by preparation of substructure of

pathogens.

Despite many approaches have been explored, the control of regio-/stereo-selectivity is still challenging for the assembly of complex carbohydrates. The control of regio-selectivity requires a careful choice of appropriate protecting groups and the order to install protecting groups during the synthesis of monosaccharide building blocks. The control of stereo-selectivity relies on electronic and steric nature of the glycosyl donor and the choice of reaction condition for glycosylation reactions. Another challenge is the preparation of “rare” sugars, which are difficult to obtain by isolation from natural sources that leads to the requirement of additional synthetic steps. 3-Deoxy-D-*manno*-2-octulosonic acid (Kdo) and L-*glycero*-D-*manno*-heptose (L,D-heptose) are very common moieties expressed by LPS core of Gram-negative bacteria, and a variety of approaches have been developed, however, their preparation is still time consuming.

Two types of LPS core in *K. pneumoniae* have been identified, and core type 1 is most common. The outer core is different from that of other Gram-negative bacteria by expressing α Kdo and L, α D-heptose, which are generally found in the inner core region. The synthesis of an outer core tetrasaccharide and its conjugation to carrier proteins are described in **Chapter 2**. Our synthesis started with the installation of protecting groups to prepare the required monosaccharides as building blocks, and the challenging Kdo donor was synthesized using a two-carbon extension strategy from D-mannose in a high overall yield. The target tetrasaccharide was then assembled through sequential glycosylations, and the challenging 1,2-*cis* galactoside and α Kdo linkages were installed with excellent anomeric selectivity by employing remote neighboring group participation. To reduce the undesired immunogenic responses, we installed an aminopentyl linker at the reducing end of the target oligosaccharide that can be used for conjugation to carrier proteins. At a late stage of synthesis, a thiol group was installed on the

linker to avoid interference of the free amine from glucosamine during conjugation. BSA and CRM₁₉₇ were chosen as the carriers for vaccine preparation because they are safe and widely used, and maleimide was installed to their free amines chemically. The thiol-maleimide coupling chemistry was employed to obtain the required glycoconjugates.

K. pneumoniae LPS outer core type 1 nonstoichiometrically expresses L, α D-heptose, which substitutes the C-4 of α Kdo. The L, α D-Hep-(1 \rightarrow 4)- α Kdo-(2 \rightarrow residue, also exists in LPS outer core of certain strains of *H. alvei*, has been found to be a common target for mannose-binding lectins. To understand the role L,D-heptose plays in eliciting immune response, we report the synthesis of the outer core pentasaccharide derived from *K. pneumoniae* in **Chapter 3**. Firstly, the L,D-heptose was synthesized with excellent L-stereoselectivity using a one-carbon elongation approach from D-mannose. The C-4 and C-5 hydroxyls of Kdo were orthogonally protected and could be removed at appropriate stage to provide desired acceptor, which could be coupled with glycosyl/heptosyl donor to give the challenging 4,5-branched Kdo. The synthetic pentasaccharide was chemically conjugated to maleimide-modified BSA/CRM₁₉₇. The synthetic glycoprotein will be used for immunological evaluation, and the results will be compared with those from the glycoconjugates prepared in chapter 2.

Chapter 4 describes the synthesis of inner core oligosaccharides as well as their conjugation with carrier proteins. The inner core is well conserved among *Enterobacteriaceae*, therefore, conjugate vaccines based on LPS inner core may elicit immunity that provide cross-protective antibodies against different bacteria. The major difference of inner core between *K. pneumoniae* and other Gram-negative bacteria is the absence of phosphate, which provides negative charge for outer membrane stability. Instead, *K. pneumoniae* expresses galacturonic acid that substitutes at C7 of heptose in nonstoichiometric quantity. To determine the necessity of negative charge in eliciting immune response, the inner core

oligosaccharides, with- and without galacturonic acid, were synthesized chemically. The required L,D-heptosyl monosaccharides, prepared following the successful approach described in former chapter, equipped ester protecting groups at their C-2 position to provide neighboring participation effect for stereochemical control during glycosylation reactions. The steric hindrance can be reduced by using a per-*O*-acylated glucosyl donor for glycosylation to form the challenging crowded 3,4-branched heptoside, which is followed by the assembly of pentasaccharide using a [2+3] strategy. The inner core tetrasaccharide was chemically assembled using a similar approach. To compare with outer core-based vaccines, the inner core targets are conjugated with the same carrier proteins (maleimide-activated BSA and CRM₁₉₇) using thiol-maleimide coupling chemistry. Immunization studies of these glycoconjugates are ongoing and the results will provide an insight in the potency of a new treatment approach to prevent infections caused by *K. pneumoniae*.

Future Perspectives

Glycan microarray analysis. The development of bacterial conjugate vaccines requires a knowledge of whether a glycan can elicit immune responses to provide antibodies, whether these antibodies can recognize and kill bacteria, their coverage of bacterial strains and the minimal length of pathogens.¹ Microarray is a strategy for assessing the antigenicity of glycans by detecting the binding of glycans with the serum samples. Our synthetic core oligosaccharides derived from *K. pneumoniae* will be printed on activated glass slides, and the sub-arrays will be incubated with a range of plant lectins. The antiserum, collected from immunized mice that pre-challenged with various *K. pneumoniae* strains, will be assayed on microarray. The data will reveal whether the synthetic core oligosaccharides have elicited IgG antibody responses. The microarray can also be employed to examine immune responses elicited by various *K. pneumoniae* serotypes. This will reveal whether infections with *K. pneumoniae* elicit immune

responses to the core region and will establish which subunits are antigenic. This information in turn will facilitate the design of antigens for vaccine development.

Opsonophagocytic killing (OPK) assay. This assay will give an “opsonic index”, demonstrating the serum dilution to kill 50% of bacteria that can be used to examine the ability of conjugate vaccines to provide protection.² The test sera will be collected from glycoprotein immunized animal, and then incubated with *K. pneumoniae* bacteria. Human promyelocytic leukemia cells (HL-60) are differentiated into neutrophil like cells and will be mixed with the bacterial cells and test sera. The remaining viable *K. pneumoniae* cells will be quantified by colony-forming unit (CFU) assay. It is expected that the serum from glycoprotein immunized animal will mediate killing of *K. pneumoniae* bacteria indicating our synthetic conjugate vaccines are able to elicit immune responses to generate protecting antibodies.

Animal challenge experiments. After determination of the immunogenicity of glycoconjugates, attention will be focused on their immuno-protective properties against *K. pneumoniae*. Animals, such as mice or rabbits, will be immunized with the synthetic glycoconjugates three times at 14 days intervals using Alum as adjuvant. These immunized animals and naïve ones will be infected with certain strain of *K. pneumoniae*, after which the disease progression will be monitored. Bacterial burden will be quantified at appropriate time point to indicate the antibacterial defense, and bacteremia in those animals will also be detected to determine the immuno-protectivity of glycoconjugates.

Synthesis of core octasaccharide derived from *K. pneumoniae* for conjugate vaccine development. Currently, it is not known which core glycan constitutes the ideal structure to elicit relevant antigenic responses.¹ The most common core type 1 exists in most clinical relevant strains, including O1, O2a, O2ac, O3, O4, O5, O8 and O12 of *K. pneumoniae*, making it an attractive vaccine candidate.³ Therefore, it is necessary to synthesize the complete LPS core, to identify a

leading antigen. The chemical structure of the complete core of *K. pneumoniae* has been identified (Fig. 1), and it is unique among Gram-negative bacteria.^{4, 5} The synthesis of outer- and inner core oligosaccharides have been described in chapter 2, 3 and 4. Future attention should focus on the synthesis of the complete core of *K. pneumoniae* LPS.

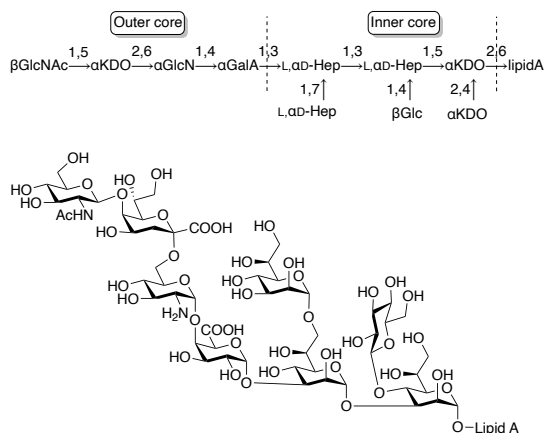
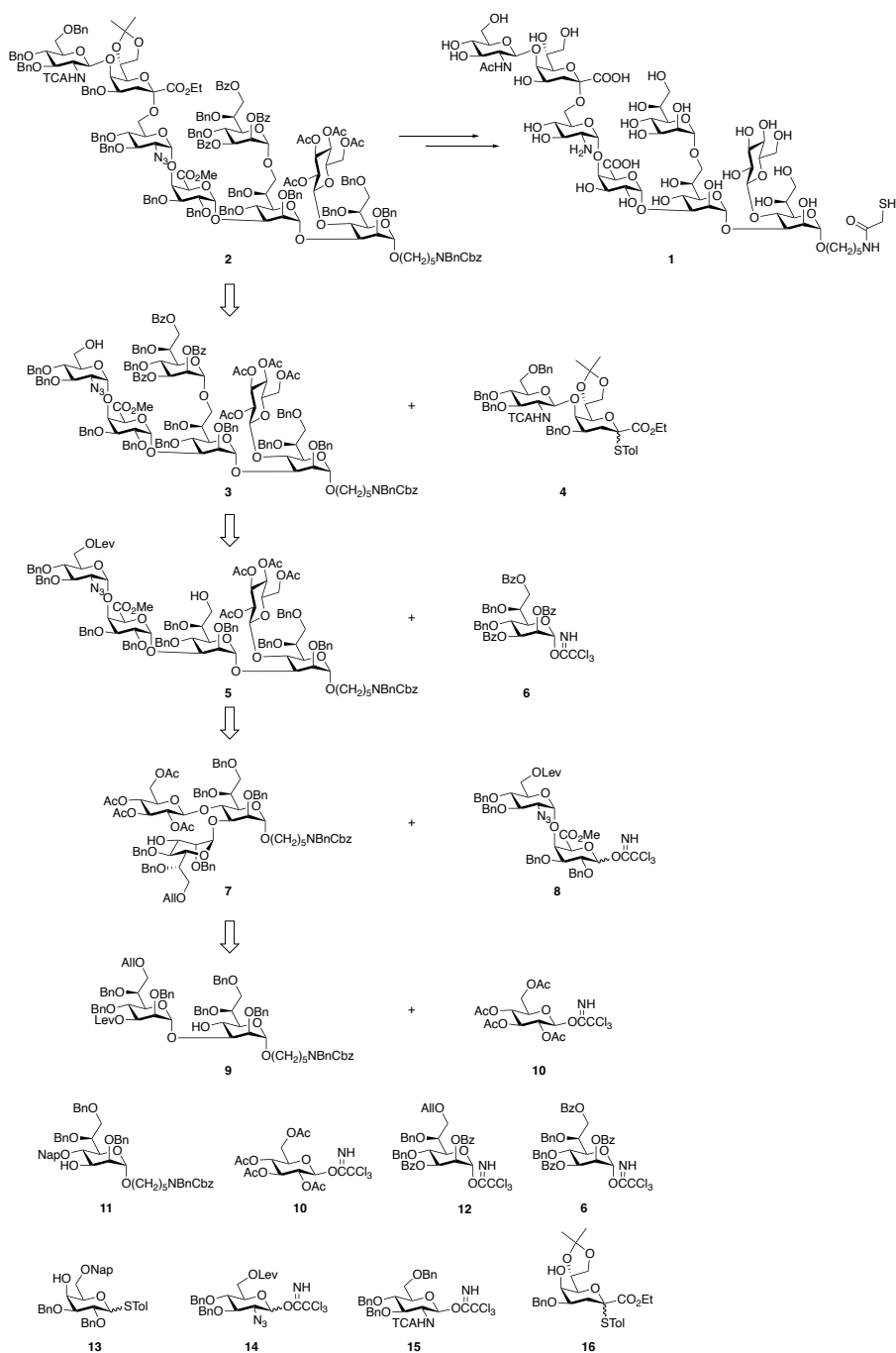


Figure 1. Chemical structure of *K. pneumoniae* LPS core type 1.

It is challenging to synthesize the octasaccharide chemically due to its complex structure, the presence of “rare” sugars, the three different types of amino groups, the 1,2-*cis* glucoside/αKdo linkages, the regio-selective installation of protecting groups on the disaccharide and the low reactivity of axial hydroxyl at C-4 of galactosyl-/ C-5 of Kdo acceptor. It is envisaged that the target compound **1** can be prepared from a fully protected octasaccharide **2**, which in turn can be assembled by a convergent [3+2+1+2] strategy using trisaccharide **7**, disaccharide **8**, monosaccharide **6** and disaccharide **4** (Scheme 1).



Scheme 1. Proposed retrosynthesis route to prepare core oligosaccharide and the

building blocks for its assembly

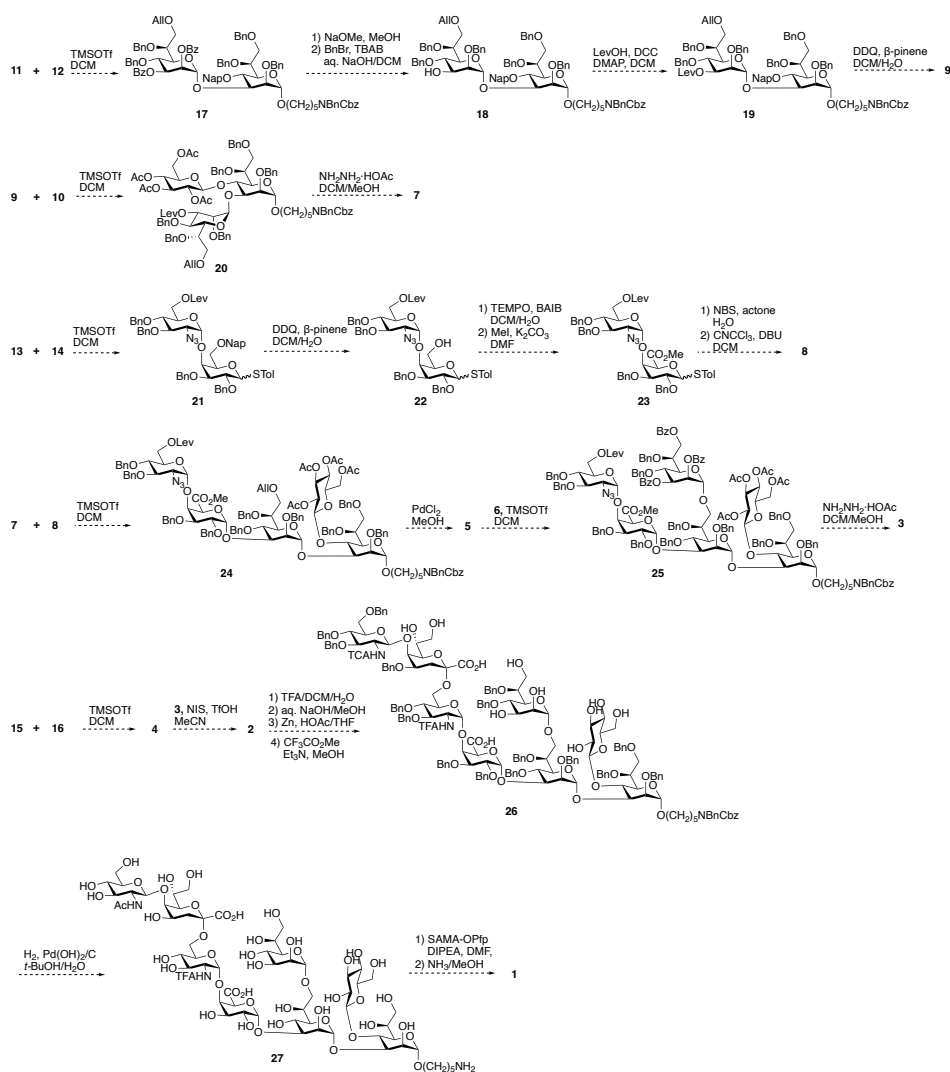
Monosaccharide building blocks (**6**,^{6,7} **10**,⁸ **11**,⁹ **12**,¹⁰ **13**, **14**, **15** and **16**¹¹) will be elaborated for the assembly of target octasaccharide (Scheme 1). Fortunately, most of these can be prepared following strategies described in the chapters of this thesis. Heptosyl acceptor **11** equipped with an α -linked doubly protected aminopentyl linker, which will be used for the conjugation to carrier proteins. The C-4 hydroxyl will be temporarily blocked by 2-methylnaphthyl (Nap) ether, which can be removed by oxidation with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) to give a required acceptor for further extension. The C-7 of heptosyl donor **12** will be protected as allyl (All) ether, which can easily be cleaved using palladium chloride at appropriate stage to provide free hydroxyl for chain elongation. The C-6 hydroxyl of galactosyl acceptor **13** will be temporarily protected as Nap ester which will be removed to give a primary alcohol that can be further oxidized to carboxylic acid and sequentially protected as a methyl ester, and the anomeric hydroxyl will be protected as thiol ether which can be hydrolyzed by treatment with *N*-bromosuccinimide (NBS). The C-2 amine of glucosamine **14** will be modified as 2-azido group, which will be reduced into a free amine at a late synthetic stage, and a levulinoyl (Lev) ester will be installed at C-6 that can be removed by treatment with hydrazine acetate to provide the responding acceptor. To avoid the formation of oxazoline and control the β -anomeric selectivity during glycosylation, the C-2 amino of glucosamine donor **15** will be modified as trichloroacetamide which can be reduced by hydrogenation to provide the required *N*-acetyl-glucosamine residue at the end of synthetic route.

The proposed assembly will start with the coupling of heptosyl acceptor **11** with heptosyl donor **12** using trimethylsilyl trifluoromethanesulfonate (TMSOTf)¹² as catalyst to give disaccharide **17**, which will then be treated with sodium methoxide to remove the benzoyl (Bz) esters. A benzyl (Bn) ether will be

selectively installed at C'-2 of the resulting diol using benzyl bromide and tetrabutyl-ammonium bromide in a mixture of aqueous NaOH and DCM under heating to provide the disaccharide **18**, which may be a separable mixture of C'-2/C'-3 benzylated compound.¹³ The C'-3 hydroxyl will be protected as Lev ester, after which the Nap ether at C-2 will be removed by treatment with DDQ using β -pinene as scavenger to give the disaccharide acceptor **9**.¹⁴ The trisaccharide **20** will be formed by glycosylation of acceptor **9** with glucosyl donor **10** using TMSOTf as promoter. This glycosylation is probably challenging because of the steric hindrance of the crowded 3,4-branched heptoside, and this issue can be addressed by extension of the reaction time or addition of more catalyst.^{12, 15} The Lev ester at C'-3 of compound **20** will be cleaved by using hydrazine acetate in a mixture of DCM and MeOH to provide the trisaccharide acceptor **7**. The glycosylation of galactosyl acceptor **13** with glucosyl donor **14** in DCM catalyzed by TMSOTf will form disaccharide **21**, and the reaction may have a low yield due to the less reactive axial C-4 hydroxyl of the galactosyl acceptor. To address this issue, the reaction will need to be warmed to room temperature and the reaction time will be prolonged. Treatment of the later compound with DDQ will result in clean removal of Nap ether to give primary alcohol **22**, which will be oxidized to a carboxylic acid using (2,2,6,6-tetramethylpiperidin-1-yl)oxyl (TEMPO) and (diacetoxyiodo)benzene (BAIB). Subsequent treated with methyl iodide and potassium carbonate in DMF will give **23**. Due to the interaction of *N*-iodosuccinimide (NIS) with allyl ether of disaccharide acceptor **9**, the thioglycosyl disaccharide **23** will not be used as the donor, instead, it will be treated with *N*-bromosuccinimide (NBS) in a mixture of acetone and water to unmask the anomeric hydroxyl, which will be further modified as trichloroacetimidate to provide the disaccharide **8**. Glycosylation of the later donor with trisaccharide acceptor **7** in promotion of TMSOTf will give the pentasaccharide **24** as a mixture of α - and β - anomers, and the separation of these isomers may be challenging. The removal of allyl ether will be performed using

palladium chloride in methanol to provide the acceptor **5**, which will be subjected to couple with heptosyl donor **6** to give hexasaccharide **25**. Acceptor **3** will form after removal of Lev ester using hydrazine acetate. Because of the low reactivity of C-5 hydroxyl of Kdo, the glycosylation of glucosamine donor **15** with Kdo acceptor **16** using TMSOTf as promoter may need longer reaction time/temperature and additional promoter to improve the yield of disaccharide **4**, which will be reacted with the hexasaccharide acceptor **3** to generate the protected octasaccharide **2** probably as a mixture of α/β -isomers.

The isopropylidene acetyl of **2** will be removed in a mixture of aqueous trifluoroacetic acid and DCM, followed by removal of the esters, including benzoyl-, acetyl-, methyl- and ethyl esters, using aqueous sodium hydroxide in methanol. The resulting compound will be treated with zinc in acetic acid to reduce the 2-azido to a free amine, which will be modified as trifluoroacetamide to form **26**. The benzyl ethers will be removed by hydrogenation, whereby, the doubly protected amino of anomeric linker will be unmasked and the trichloroacetamide will convert to acetamide to give octasaccharide **27**.¹⁶ An (acetyl)thioacetate will be installed to the free amine of pentyl linker by reaction with perfluorophenyl 2-(acetylthio)acetate (SAMA-OPfp),^{17, 18} followed by the treatment of ammonia under an atmosphere of argon to avoid the formation of disulfate, finally, after purification by bio-gel P2 size exclusion column chromatography, the target octasaccharide **1** will be prepared.¹⁹



Scheme 2. Conditions and reagents for proposed assembly of target octasaccharide **1**.

The conjugation of the synthetic oligosaccharide **1** with carrier proteins (BSA and CRM₁₉₇) will be performed following the approaches described in chapters 2,3 and 4,²⁰ and the obtained glycoconjugates will be used for immunological evaluation using glycan microarray, OPK test and in vivo immunogenicity tests. The resulting data will be compared with those from outer-/inner core targets.

Furthermore, the shorter glycans will also be connected to carrier proteins to be tested that will provide us a better understanding of the minimal epitope required for eliciting immune responses and explore the immunogenicity and antigenicity of glycans.

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Nederlands Samenvatting

Capsular polysaccharide (CPS) en lipopolysaccharide (LPS) zijn twee virulentiefactoren van *Klebsiella pneumoniae* (*K. pneumoniae*) en vormen een veelbelovend startpunt voor de ontwikkeling van vaccins. Het grote aantal verschillende serotypes (79) bemoeilijkt de ontwikkeling van CPS-gebaseerde vaccinontwikkeling. In tegenstelling tot CPS zijn er maar negen LPS serotypes geïdentificeerd en in het kerngebied van LPS zijn slechts twee typen geïdentificeerd. Dit maakt het kerngebied van LPS een uitgangspunt met veel potentie voor het ontwikkelen van een breed werkend vaccin.

Hoofdstuk 1 introduceert de virulentiefactoren van *K. pneumoniae*; strategieën voor het ontwikkelen van geconjugeerde vaccins en strategieën voor de synthese van complexe koolhydraten. De ontwikkeling van een geconjugeerd vaccin voor T-cel-afhankelijke immuniteit is afhankelijk van de conjugatie van goed-gedefinieerde en niet-toxische sachariden met een sterk antigeen drager eiwit. Tegenwoordig bevatten erkende vaccins niet-toxische eiwitten als drager die geconjugeerd zijn met het antigen. De dragereiwitten worden geïsoleerd uit gecultiveerde bacteriën en tolereren geen organische oplosmiddelen noch de zware chemische condities die gebruikt worden voor de conjugatie van koolhydraten.

De ontwikkeling van nieuwe dragers zoals peptiden, glycolipiden of nanodeeltjes zijn nog in een vroeg onderzoeksstadium, maar kunnen mogelijk de dragereiwitten vervangen in de toekomst. Hoewel de meeste erkende geconjugeerde vaccins gebaseerd zijn op geïsoleerde koolhydraten, is het nog steeds uitdagend om de correcte polysaccharide in voldoende mate te isoleren en is het onmogelijk om elke bacteriestam te kweken. Enzymatische synthese is een efficiënte methode voor de synthese van koolhydraten maar het is nog niet toereikend om de nodige enzymen in grote hoeveelheden tot expressie te brengen. De chemische synthese die deze probleempunten omzeilt is goed ontwikkeld. Het

is ook mogelijk om door het prepareren van korte delen te bepalen wat de minimaal benodigde lengte van de koolhydraatketen is voor het uitlokken van de ontwikkeling van beschermende antilichamen.

Ondanks het vele onderzoek is de controle over regio- en stereoselectiviteit nog steeds een obstakel voor het assembleren van complexe koolhydraten. Om de regio- en stereoselectiviteit te beïnvloeden is het nodig om de geschikte beschermgroepen in de juiste volgorde te installeren tijdens de synthese van monosacharide bouwstenen. De controle van stereoselectiviteit is afhankelijk van de elektronische en sterische aard van de glycosyldonor en de keuze van de reactieconditie voor glycosyleringsreacties. Een extra uitdaging is de bereiding van ‘zeldzame’ suikers die moeilijk te verkrijgen zijn door isolatie uit natuurlijke bronnen, waardoor meer synthetische stappen nodig zijn. 3-Deoxy-D-manno-2-octulosonic acid (Kdo) en L-*glycero*-D-manno-heptose (L,D-heptose) zijn veel voorkomende structuren die tot expressie worden gebracht in het kerngebied van LPS van Gram-negatieve bacteriën. Hoewel er verschillende benaderingen zijn ontwikkeld, is de synthese hiervan nog steeds zeer gecompliceerd.

Er zijn tot nu toe twee typen van het LPS-kerngebied in *K. pneumoniae* geïdentificeerd, waarvan kerntype 1 vaker voor komt. De buitenste kern verschilt van die van andere Gram-negatieve bacteriën door de expressie van α Kdo en L, α D-heptose, die over het algemeen in het binnenste kerngebied worden aangetroffen.

De synthese van een tetrasacharide van de buitenste kern en de conjugatie ervan aan dragereiwitten worden beschreven in **hoofdstuk 2**. Onze synthese startte met de installatie van beschermende groepen om de vereiste monosacharide bouwstenen te bereiden. De Kdo-donor werd gesynthetiseerd met een hoge totale opbrengst met behulp van een twee-koolstofextensie strategie vanuit D-mannose.

De tetrasacharide werd vervolgens geassembleerd door middel van opeenvolgende glycosylaties. De 1,2-*cis*-galactoside- en α Kdo-koppelingen

werden met een uitstekende anomere selectiviteit gevormd door gebruik te maken van een participatie-effect van naburige groepen. Om ongewenste immunogene reacties te verminderen installeerden we een aminopentyl-linker aan het reducerend uiteinde van de oligosacharide, dat kan worden gebruikt voor verbinding met dragereiwitten. In een laat stadium van de synthese werd een thiol op de linker geïnstalleerd voor de koppeling met de drager. Een koppeling met een thiol heeft geen last van andere vrij amines op het koolhydraat zoals dat van de glucosamine tijdens conjugatie. BSA en CRM197 werden gekozen als dragers voor vaccinbereiding omdat ze veilig zijn en veel worden gebruikt. Aan het eiwit werden maleïmides chemisch aan hun vrije amines geïntroduceerd. De thiol-maleïmide koppelingschemie werd gebruikt om de vereiste glycoconjugaten te verkrijgen.

De buitenste kern van type 1 *K. pneumoniae* LPS brengt niet-stoichiometrische hoeveelheden L, α D-heptose op de C-4 van α Kdo tot expressie. Het L, α D-Hep-(1 \rightarrow 4)- α Kdo-(2 \rightarrow -residu, dat ook voorkomt in de buitenste kern LPS van bepaalde stammen van *H. alvei*, is een vaak voorkomend doelwit gebleken voor mannose-bindende lectines. Om te begrijpen welke rol L,D-heptose speelt bij het opwekken van een immuunrespons, voerden we een synthese uit van de buitenste kern pentasaccharide die afgeleid is van *K. pneumoniae*. Deze synthese wordt gerapporteerd in **Hoofdstuk 3**. Eerst werd de L,D-heptose met uitstekende L-stereoselectiviteit gesynthetiseerd door middel van een één-koolstofextensie strategie vanuit D-mannose. De C-4- en C-5-hydroxylen van Kdo werden orthogonaal beschermd en konden in een geschikt stadium worden verwijderd om de gewenste acceptor te verschaffen. De acceptor kon worden gekoppeld aan glycosyl/heptosyldonoren om de moeilijke 4,5 vertakte Kdo te verkrijgen. De synthetisch opgebouwde pentasaccharide werd chemisch geconjugéerd aan maleïmide-gemodificeerde BSA/CRM197. Het synthetische geglycolyseerde eiwit zal worden gebruikt voor immunologische evaluatie en de resultaten zullen worden vergeleken met die van de glycoconjugaten die in hoofdstuk 2 zijn bereid.

Hoofdstuk 4 beschrijft de synthese van binnenkern-oligosacchariden en de conjugatie met dragereiwitten. De binnenkern is goed geconserveerd bij *Enterobacteriaceae*, daarom kunnen geconjugeerde vaccins op basis van de LPS binnenkern immuniteit opwekken tegen meerdere bacteriestammen. Het belangrijkste verschil in de binnenkern tussen *K. pneumoniae* en andere Gram-negatieve bacteriën is de afwezigheid van fosfaat. De fosfaatgroep zorgt voor een negatieve lading die de stabiliteit van het buitenmembraan verhoogt. In plaats van fosfaat brengt *K. pneumoniae* galacturonzuur tot expressie op de C7 positie van heptose in niet-stoichiometrische hoeveelheden. Om de noodzaak van een negatieve lading voor het opwekken van een immuunrespons te bepalen, werden de binnenkern-oligosacchariden met en zonder galacturonzuur chemisch gesynthetiseerd. De vereiste L,D-heptosylmonosacchariden werden bereid volgens de succesvolle benadering zoals beschreven in het vorige hoofdstuk. Ester-beschermende groepen op de C-2-positie zorgden voor stereochemische controle tijdens de glycosyleringsreacties door het naburige groep participatie-effect. Een teveel aan sterische hinder kon worden vermeden door een per-*O*-geacetylerde glucosyldonor te gebruiken om de zeer sterische bezette 3,4-vertakte heptoside te vormen. De assemblage van pentasaccharide werd voltooid met behulp van een [2+3]-strategie. Het tetrasaccharide in de binnenste kern werd chemisch geassembleerd met behulp van een vergelijkbare benadering. Om de vergelijking met op de buitenste kern gebaseerde vaccins te kunnen maken, werden de binnenkern-oligosacchariden geconjugeerd met dezelfde dragereiwitten (maleïmide-geactiveerde BSA en CRM197) en dezelfde thiol-maleïmide koppelingschemie. Immunisatiestudies van deze glycoconjugaten worden op dit moment uitgevoerd en de resultaten zullen inzichten verschaffen in de potentie van een nieuwe behandelingsaanpak om infecties veroorzaakt door *K. pneumoniae* te voorkomen.

List of Publications

1. **Dushen Chen**; Lin Liu; Akhilesh K. Srivasatava; Tiehai Li; Ivan A. Gagarinov; Margreet A. Wolfert; Jay K. Kolls; Geert-Jan Boons. Chemical synthesis of *Klebsiella pneumoniae* lipopolysaccharide outer core as a vaccine candidate. *in preparation*.
2. **Dushen Chen**; Lin Liu; Akhilesh K. Srivasatava; Tiehai Li; Margreet A. Wolfert; Jay K. Kolls; Geert-Jan Boons. Synthetic *Klebsiella pneumoniae* inner core region for the development of a broad acting vaccine. *in preparation*.
3. **Dushen Chen**; Guangfa Shi; Hang Jiang; Yu Zhang; Yanghui Zhang. Sequential Difunctionalization of Biphenyls by Exploiting the Distinct Reactivities of a Palladacycle and an Acyclic Arylpalladium Species. *Org. Lett.* **2016**, 18, 2130.
4. Guangfa Shi; **Dushen Chen**; Hang Jiang; Yu Zhang; Yanghui Zhang. Synthesis of Fluorenes Starting from 2-Iodobiphenyls and CH₂Br₂ through Palladium-Catalyzed Dual C-C Bond Formation. *Org. Lett.* **2016**, 18, 2958.
5. Yu Zhang; Hang Jiang; **Dushen Chen**; Yanghui Zhang. Amino acid-promoted C-H alkylation with alkylboronic acids using a removable directing group. *Org. Biomol. Chem.* **2016**, 14, 4585.
6. Hang Jiang; Yu Zhang; **Dushen Chen**; Bo Zhou; Yanghui Zhang. An approach to tetraphenylenes via Pd-catalyzed C-H functionalization. *Org. Lett.* **2016**, 18, 2032.
7. Shulei Pan; Hang Jiang; Yu Zhang; **Dushen Chen**; Yanghui Zhang. Synthesis of triphenylenes starting from 2-Iodobiphenyls and Iodobenzenes via palladium-catalyzed dual C-H activation and double C-C bond formation. *Org. Lett.* **2016**, 18, 5192.
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9. Shulei Pan; Hang Jiang; Yanghui Zhang*; Yu Zhang; **Dushen Chen**. Synthesis of 2-substituted tetraphenylenes via transition-metal-catalyzed derivatization of tetraphenylene. *Beilstein J. Org. Chem.* **2016**, 12, 1302.

Curriculum Vitae

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