The synthetic study of inner-core oligosaccharides of lipopoly- and lipooligosaccharides produced by gram-negative bacteria: Construction of 4,5-branched 3-deoxy-D-*manno*-oct-2-ulosonic acid structure

グラム陰性菌が産生するリポ多糖およびリポオリゴ糖の内部コア糖鎖の合成研究：4,5 で分岐した 3-デオキシ-D-マンノオクト-2-ウロン酸の構築

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Abbreviation

Ac_2O	Acetic anhydride
AcOH	Acetic acid
$BF_3 \cdot OEt_2$	Boron trifluoride diethyl etherate
Bn_2SnO	Dibutyltin oxide
Dppb	1,4-Bis(diphenylphosphino)butane
Et_2O	Diethyl ether
GalNAc	*N*-acetyl galactosamine
$GalN_3$	2-Azido-2-deoxy-galatosamine
Glc	Glucose
Hep	L-*Glycero*-D-*manno*-heptopyranose
$HgBr_2$	Mercury(II) bromide
HMBC	Heteronuclear multiple bond correlation
HMQC	Heteronuclear multiple quantum correlation
H_2SO_4	Sulfuric acid
K_2CO_3	Potassium carbonate
Kdo	3-Deoxy-D-*manno*-2-octulosonic acid
Lac	Lactose
LOS	Lipooligosaccharide
LPS	Lipopolysaccharide
Man	Mannose
$MeNO_2$	Nitromethane

MeOH	Methanol
MS 4A	Molecular sieve 4A
MS AW 300	Molecular sieve acid wash 300
NaOH	Sodium hydroxide
N. meningitides	*Neisseria meningitides*
NMR	Nuclear magnetic resonance
OS	Oligosaccharide
P	Phosphite
$Pd(dba)_3$	Tris(dibenzylideneacetone)dipalladium(0)
$Pd(OH)_2/C$	Palladium hydroxide on carbon
PMB	*p*-Methoxybenzyl
PMBCl	*p*-Methoxybenzyl chloride
Ph_3P	Triphenyl phosphite
TBDMSCl	*t*-Butyldimethylsilyl chloride
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TMSOTf	Trimethylsilyl trifluoromethane sulfonate
TLC	Thin-layer chlormatography

Chapter 1

General introduction

1.1 *Neisseria meningitidis*

Neisseria meningitidis is a gram negative bacterium that colonizes and infects only human, and has never been isolated from other animals.[1] *N. meningitidis* is the main cause of bacterial meningitis in children and young adults.[2] Children younger than 5 years are at greatest risk, followed by teenagers of high school age. The World Health Organization estimates that there are 1.2 million cases of meningococcal meningitis worldwide and 135,000 related deaths annually.[3] Especially, the Sub-Sahara African has been plagued by large epidemics of meningococcal meningitis for over a century.[4] Attack rates of 100-800 cases per 100,000 are encountered in this area. The meningococcal disease often progresses very rapidly and is difficult to diagnose and treat.

Without treatment, meningococcal meningitis is almost fatal. Persons with *N. meningitidis* infection should be hospitalized immediately for treatment with antibiotics.[5] However, antibiotic treatment has many important limitations, including drug side effects[6], and the potential for emergence of resistant organisms. Moreover, permanent sequelae such as hearing impairment, mental retardation, or limb loss are common in survivors.[7] Therefore, the prevention of meningococcal meningitis is important.

To prevent the meningococcal disease, especially to stop an outbreak of meningococcal disease, a dose of meningitis vaccine is recommended. The discovery that serum bactericidal antibodies to meningococcal capsular polysaccharides protect against meningococcal disease is the basis for development of meningococcal vaccines.[8] The capsular polysaccharides of *N. meningitidis* are important virulence factors that inhibit host cell protection mechanisms. According to the immunology specificity of capsular polysaccharides, *N. meningitidis* is classified into 13 clinically significant serogroups. Six most important serogroups, A, B, C, Y, W-135, and X, are associated with disease in human. Serogroup A has been the most prevalent in Africa and Asia, but is rare practically absent in North America.[9] In the United States and Europe, serogroup B is the predominant cause of disease and mortality, followed by serogroup C.

Several effective vaccines using bacterial capsular polysaccharides as target to generate bactericidal antibodies against serogroup A, C, Y and W-135 have been developed and used.[10] However, vaccine against serotype B disease is difficult to produce. The capsular polysaccharide on the serotype B bacterium is composed of polysialic acid repeating units that are same with the structures found on human neuronal cells.[11] Antibodies generated against serogroup B capsular polysaccharide are cross-reactive with the polysialic acid moieties expressed on human neural cell adhesion molecules. As a result, serogroup B capsular polysaccharides are poorly immunogenic due to self-tolerance mechanisms. To dissolve this problem, efforts to develop serogroup B vaccine have largely focused on other membrane antigens of *N. meningitides*. One attempted solution is focused on the lipooligosaccharides (LOSs) of *N. meningitides*.

Lipopolysaccharides (LPSs) and lipooligosaccharides (LOSs) are the major component of the outer membrane of gram-negative bacteria.[12] LPS/LOS contributes essentially to the integrity and stability of the outer membrane, and is also the first line of defense for bacteria against a range of environmental factors, including detergents and antimicrobial agents.[13] As a potent virulence factor, LPS/LOS also serves as a surface pathogen-associated antigen for recognition by the host immune system.[14] Therefore, LPS/LOS has attracted much interest for the development of diagnostic tools, therapeutic reagents and vaccine candidates.

1.2 Lipopolysaccharide and lipooligosaccharide

As shown in Figure 1.1[12c], an LPS consists of three domains: a lipophilic moiety termed lipid A, a core oligosaccharide (core OS), and a hydrophilic glycan called O-specific polysaccharide (O-antigen), whereas LOS which is limited to 10 saccharide units lacks an O-antigen polysaccharide. The lipid A moiety, which has a β-(1-6)-linked D-glucosamine disaccharide backbone, is essential for bacterial viability and carries the endotoxic properties of the LPSs/LOSs.[15] The O-antigen polysaccharide is the most variable portion of the LPS and provides serological specificity, which is response for bacterial serotyping.[16] In addition, the core

oligosaccharide (OS) provides useful information for vaccine development that a core oligosaccharide (OS) derived from LOS of pathogenic bacteria strains was reported to be recognized by the human antibody.[17] So core OS is a focused target for vaccine development.

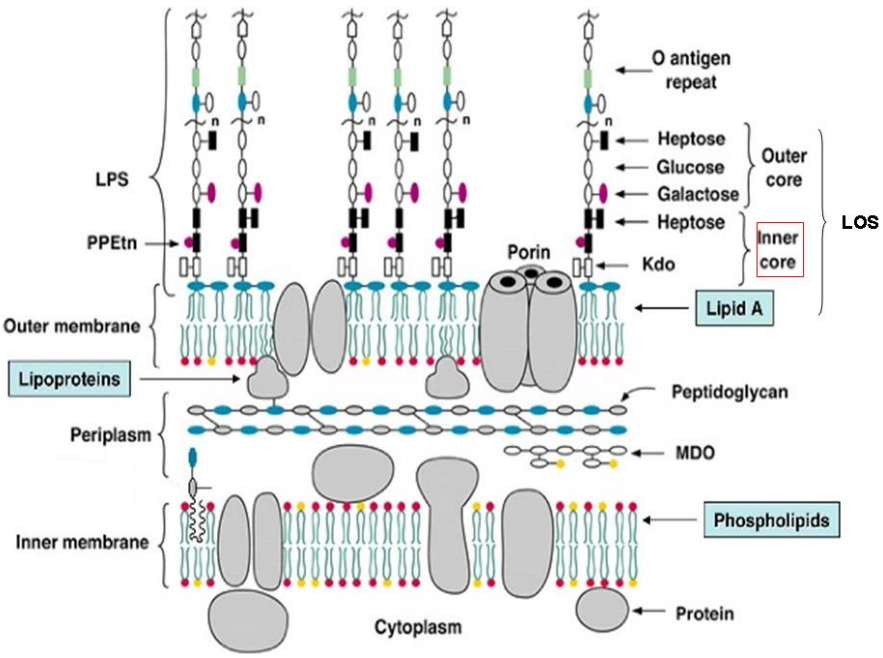


Figure 1.1 The structure of LPS and LOS

1.3 Branched inner core OS

The core OS can be further separated into two regions, one proximal to lipid A (inner-core OS) and the other is distal from lipid A but proximal to the O-antigen (outer-core OS).[12] The inner-core OS is highly conserved in bacterial species. This inner-core OS consists of mostly the unusual higher carbon sugars 3-deoxy-D-*manno*-2-octulosonic acid (Kdo) and L-*glycero*-D-*manno*-heptopyranose (Hep). Kdo is the unique compound of the inner core OS and rarely found in other glycans. Recent studies employing LOS of *Nesseria gonorrhoeae* strain 15,253 as affinity ligand indicated that human antibodies recognized several epitopes including the Kdo region but also the branched heptosyl epitopes.[17] Therefore, the inner-core OS containing these epitopes draws more attention for vaccine research.

Furthermore, the general inner-core OS of LPS/LOS is composed of a

4,5-branched Kdo structure. In Figure 1.2 for example, the inner-core OS of LPSs/LOSs from many gram-negative bacteria, such as *Neisserial*,[18] *Salmonella*,[19] and *haemophilus*[20] and so on, contains a 4,5-branched Hepα(1-3)Hepα(1-5)[Kdoα(2-4)]Kdo tetrasaccharide as the common structure. Moreover, the Hep I moiety could be substituted by other saccharides (such as Man, GalNAc) in some other bacteria, such as *Francisella tularensis*[21] and *Pseudomonas cichorii*[22].

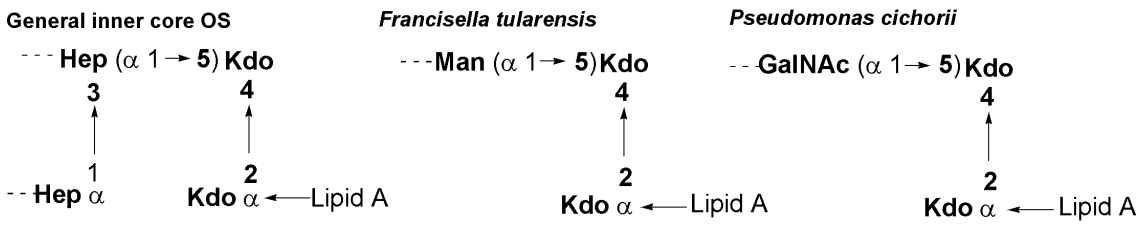


Figure 1.2 The inner-core OS structure of general gram-negative bacteria, *Francisella tularensis* and *Pseudomonas cichorii*.

1.4 The extraction of inner-core OS

Inner-core OS fragments can be extracted from the bacteria strains. For the fragmental extraction, the target bacteria strains are isolated, identified, and grown in tryptic soy broth medium. The LPS/LOS fragments are released from whole bacterial cells by some mild treatments such as strong saline washes[23], the use of chelating agents[24], or aqueous organic solvents.[25] The most general and widely used procedure is the treatment of the whole bacterial cells with hot aqueous phenol, followed by cooling to produce a two-phase system.[26] LPS/LOS in crude, aqueous-phenol extracts are collected and purified. The presence of LPS/LOS in the resulting extract is confirmed by the SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis) analysis.[27]

To obtain released inner-core OS fragments, the collected LPS/LOS is further hydrolyzed under mild acid conditions.[28] After further purification by gel permeation chromatography and high performance liquid chromatography (HPLC), a set of inner core OS fragments with the different lengths could be collected. These fragments are

usually used for structure study.

However, isolation of these oligosaccharides from highly pathogenic bacteria, such as *N. meningitides*, is undesirable. The purification method of extraction and nonselectivity hydrolysis of LPS/LOS also limits the output and purity of these oligosaccharides. Moreover, the vaccine development needs the well defined oligosaccharides to conjugate with carrier proteins for immunizations. It is difficult to conjugate the extracted oligosaccharides to carrier proteins without destroying vital immunological domains.[29]

Fortunately, chemical synthesis can address these issues. Chemical synthesis offers a much more attractive approach to produce multigram amount of highly pure oligosaccharides and makes it possible to incorporate an artificial linker for controlled conjugation to proteins.

1.5 Chemical synthesis of branched inner core OS

Chemical synthetic approaches towards components of the inner-core OS region have to deal with the elaboration of efficient protocols to prepare multigram amounts of the higher carbon aldoses 3-deoxy-D-*manno*-2-octulosonic acid (Kdo) and L-*glycero*-D-*manno*-heptopyranose (Hep) followed by transformation into suitable glycosyl donor and acceptor derivatives. In the past several decades, the synthetic efforts have covered the basic structural units (Kdo[30], Hep[31]) and truncated forms of inner-core OS[32]. However, the chemical synthesis of inner-core OS is still challenging due to its highly branched nature, which complicates the installation of the various glycosidic linkages.

1.6 Recent research of Kdo synthesis in our laboratory

Recently, we reported a useful Kdo intermediate, methyl (7,8-di-*O*-benzoyl-4,5-*O*-isopropylidene-D-*manno*-2-octulopyranosid)onate, for the preparation of 2-4 and 2-8 linked Kdo disaccharides.[33] This Kdo intermediate was prepared from the D-mannose via 8 steps in 53% yield (Figure 1.3). For the synthesis of 2-4 linked Kdo disaccharide, the Kdo intermediate could be converted to the

corresponding glycosyl fluoride donor and the 4,5-diol acceptor with ease. And the glycosylation gave the Kdo(2-4)Kdo disaccharide in a good yield (72%, α/β=5/1).

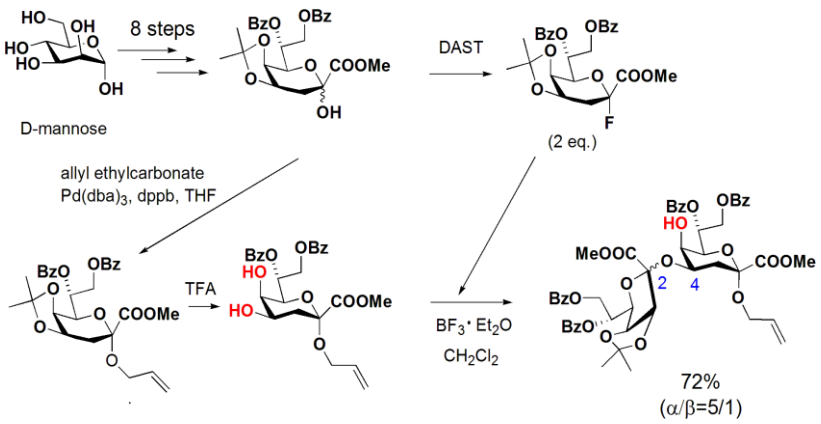


Figure 1.3 The synthesis of Kdo(2-4)Kdo disaccharide.

1.7 Aim of the study

Although many chemical syntheses of linear inner-core OS structures have been described[34], there are few reports for the synthesis of branched inner-core OS. Only Paulsen group reported a synthesis of the 4,5-branched Hepα(1-5)Kdoα(2-4)Kdo trisaccharide (Figure 1.4).[35] In their synthesis, the 4-OH group of Kdo I was firstly protected by a *p*-methoxybenzyl group, and sequentially a Hep donor was coupled with Kdo I to form a Hepα(1-5)Kdo disaccharide. Then the *p*-methoxybenzyl group of Kdo I was deprotected, and a Kdo donor was installed to the formed Hepα(1-5)Kdo disaccharide to gave the desired Hepα(1-5)[Kdoα(2-4)]Kdo trisaccharide. However, the yield of this approach appears to be low (4 steps: only 22%).

In the study of this thesis, it is aimed to develop a new chemical synthetic approach for the 4,5-branched inner-core OSs and to extend the utility of our previous synthetic Kdoα(2-4)Kdo disaccharide. In this research, the 4,5-branched Kdo structures would be synthesized by glycosylation of the 5-OH group of the 2-4 linked Kdo disaccharide. This thesis contains three parts,

1）**The observation of the limitation of (2-4)-linked Kdo glycosylation**

2）**The new route to synthesize 4,5-branched inner-core trisaccharides**

3) **The convergent synthesis of 4,5-branched inner-core OSs**

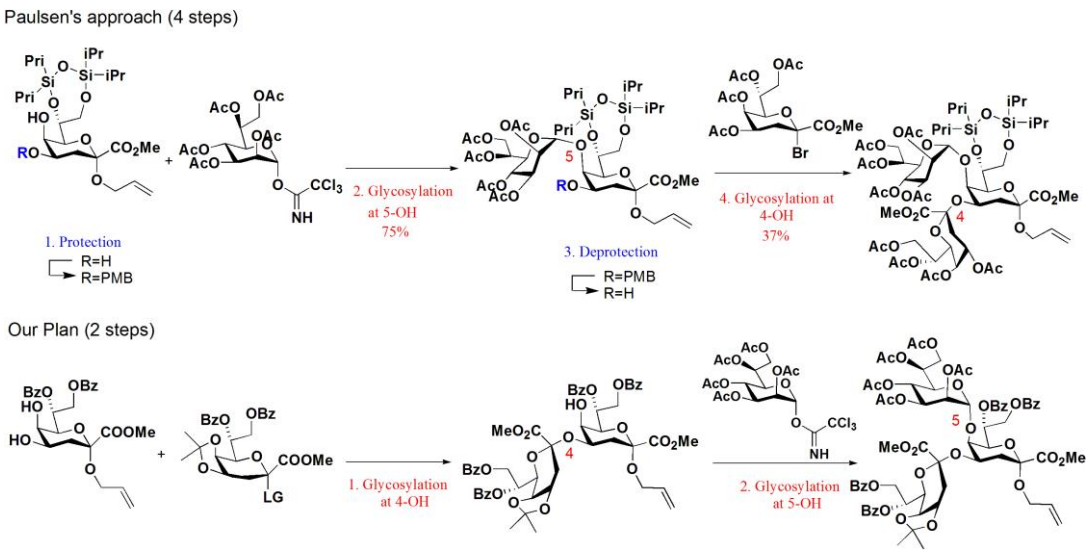


Figure 1.4 Paulsen's method and our plan to synthesize 4,5-branched Kdo trisaccharide.

In Chapter 2, to optimize the reaction condition of Kdo(2-4)Kdo, the glycosidation using several types of Kdo donors and Lewis acid are discussed. These Kdo donors are prepared from a Kdo intermediate, which is designed in our previous research.

In Chapter 3, we focus on the discussion of the glycosylation using the synthetic Kdo(2-4)Kdo in Chapter 2 as the acceptor, to synthesize 4,5-branched inner-core trisaccharides. The reaction conditions of this new route to prepare a Hepα(1-5)[Kdoα(2-4)]Kdo trisaccharide are discussed and the result is compared with Paulsen's method. Moreover, the first synthesis of Manα(1-5)[Kdoα(2-4)]Kdo and GalNAcα(1-5)[Kdoα(2-4)]Kdo by this new approach is also discussed.

In Chapter 4, to further observe the utility of the newly synthetic route discussed in Chapter 3, a convergent synthesis route using the same Kdoα(2-4)Kdo disaccharide as the acceptor to produce more complex inner core OS is discussed. To test the glycosylation conditions and necessary protecting strategy, a lactose donor is chosen as a model compound. Based on the model glycosylation, the corresponding Hep units constructed from the Hep building blocks are coupled with the Kdo moiety to obtain the desired branched inner-core OS.

Chapter 2

The observation of the limitation of

(2-4)-linked Kdo glycosylation

2.1 Introduction

The inner-core OS of gram-negative bacteria consists of at least one of the higher carbon sugar, 3-deoxy-D-*manno*-2-octulosonic acid (Kdo). Kdo is rarely found in other glycans and thus can be considered as a mark for the presence of LPS/LOS.[12] The incorporation of Kdo appears to be a vital step in LPS biosynthesis and in growth of the gram-negative bacteria. Furthermore, the inner core OS of many LPSs/LOSs is composed of a 4,5-branched Kdo structure which contains a Kdoα(2-4)Kdo disaccharide at the reducing end. According to the newly hypothetical route to synthesize the 4,5-branched Kdo structure, the 2-4 linked Kdo disaccharide would be prepared at first.

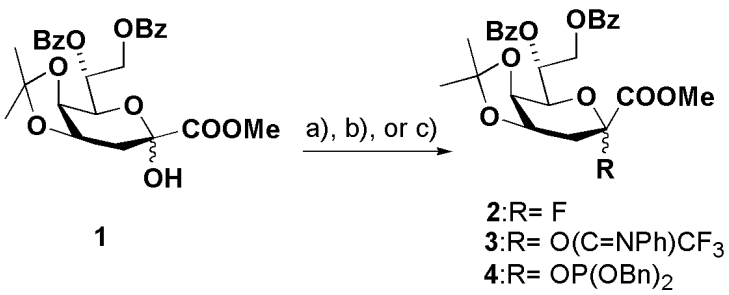
Although many chemical syntheses of Kdoα(2-4)Kdo disaccharide have been reported,[36] there is still no generally accepted high-yielding procedure for the stereoselective preparation of Kdoα(2-4)Kdo disaccharide. In recent, for the synthesis of Kdoα(2-4)Kdo disaccharide, our laboratory reported a useful Kdo intermediate from the D-mannose in 8 steps.[33] This Kdo intermediate could be easily converted to the corresponding glycosyl fluoride donor and 4,5-diol acceptor. And the glycosidation of the fluoride donor with the 4,5-diol acceptor gave the Kdo(2-4)Kdo in 72% yield (α/β=5/1). However, the limitation of this glycosidation was not investigated. Therefore in this Chapter, to optimize the glycosidation conditions of the synthesis of Kdo(2-4)Kdo, several types of Kdo donors and Lewis acid are examined.

2.2 Result and discussion

2.2.1 Preparation of Kdo donors

Three types of Kdo donors **2**, **3**, **4** were synthesized from common Kdo intermediate **1** in Scheme 2.1. Treatment of compound **1** with *N*,*N*-diethylaminosulfur trifluoride at 0 °C gave the fluoride **2** as a mixture of anomers in a good yield (81%).[33] The anomeric ratio of **2** was 3/1, and the major isomer was easily isolated by crystallization from ethyl acetate and hexane. The anomeric configuration of the major product was presumed to be α based on a compare with the NMR data of corresponding Kdo derivatives reported by Imoto.[37] The *N*-phenyl

trifluoroacetimidate **3** was prepared in quantitative yield from compound **1** with 2,2,2-trifluoro-*N*-phenylacetimidoyl chloride in the presence of potassium carbonate.[38] However, the reaction needed 1 week to reach completion. The reaction for preparing the Kdo trichloroacetimidate donor also demanded a week. This showed that the reactivity of the anomeric hydroxyl group of the Kdo derivatives was lower than that of aldose because of steric hindrance and low nucleophilicity. Two isomers could be separated and the major product was presumed to be α. Dibenzyl phosphite **4** was synthesized in moderate yield (56%) from compound **1** with 1*H*-tetrazole, dibenzyl *N*,*N*-diisopropylphosphoramidite (DDP).[39] Also, two isomers were separated and the major product was presumed to be α.



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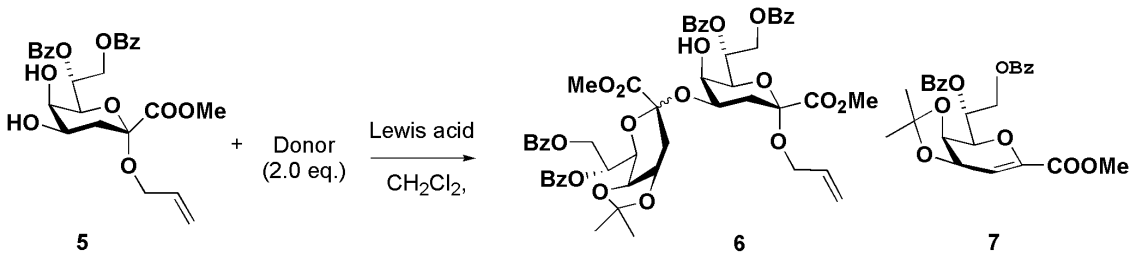
Scheme 2.1 Conditions: (a) DAST, 0 °C, 0.5 h, 81%, α/β = 3/1; (b) *N*-phenyl trifluoroacetimidoyl chloride, K_2CO_3, CH_2Cl_2, rt, 7 days, quant, α/β = 3/2; (c) 1*H*-tetrazole, DDP, CH_2Cl_2, 0 °C→ rt, 3 h, 56%, α/β = 23/1.

2.2.2 The glycosylation of the 4,5-diol acceptor with Kdo donors

Glycosylation of the 4,5-diol acceptor **5**[33], which was also prepared from the Kdo intermediate **1** (Figure 1.4 in Chapter 1), with these Kdo donors **2**, **3**, **4** was examined (Scheme 2.2). The results are summarized in Table 2.1. In entry 1, the glycosidation of Kdo fluoride **2**α in the presence of $BF_3{\cdot}OEt_2$ gave the Kdo(2-4)Kdo **6** in 72% yield (α/β=5/1). When the activator was changed to a combined promoter Cp_2HfCl_2-AgOTf[40], fluoride **2**α was readily converted to glycal **7** (90%, entry 2).



Scheme 2.2 The glycosylation of the 4,5-diol acceptor with Kdo donors

Table 2.1 Glycosylation of Kdo donors **2-4** with the 4,5-diol acceptor **5**.

Entry	Donor	Lewis acid (equiv)	Temp	Time (h)	Yield (%)	α/β	**7**(%)[a]	**5** (%)
1	**2α**	$BF_3{\cdot}OEt_2$ (6.0)	-20 °C	1.5	72	5:1	-	-
2	**2α**	$Cp_2Hf(OTf)_2$(2.2)	-20 °C	1.5	16	3.5:1	90	□□
3	**2β**	$BF_3{\cdot}OEt_2$ (6.0)	-20 °C to rt	10	41	2.5:1	73	□□
4	**2β**	TMSOTf (0.1)	-20 °C to rt	10	-	-	35[b]	□□
5	**3α**	TMSOTf (0.1)	-78 °C	2.0	48	2.4:1	52□□□□	□□
6	**3β**	TMSOTf (0.1)	-78 °C	2.0	61	2.6:1	38□□□□	□□
7	**4α**	TMSOTf (0.1)	-20 °C	2.5	35	3.8:1	61	50
8	**4α**	$BF_3{\cdot}OEt_2$ (1.0)	-20 °C	2.0	30	2.1:1	83	63

[a] The yield was based on the donor.

[b] Twenty four percent of the donor was recovered.

Using β-fluoride **2**β with $BF_3{\cdot}OEt_2$ as an activator gave a moderate yield (41%, entry 3), whereas using TMSOTf as a promoter gave a poor yield (entry 4). The longer reaction time and higher reaction temperature also suggest that the reactivity of β-fluoride was lower than that of α-fluoride. The lower reactivity of β-fluoride might be due to the effect of 4,5-*O*-isopropylidene group. As shown in Figure 2.1, in the glycosidation of fluoride **2**α, the C-F bond is firstly cleaved by $BF_3{\cdot}OEt_2$ to give an oxocarbenium ion intermediate.[41] With the steric hindrance of isopropylidene group on the top face, the acceptor prefers to attack the oxocarbenium ion intermediate from the bottom side to give α-selective glycoside. However, in the glycosidation of the fluoride **2**β, it seems to be difficult to directly form the oxocarbenium ion intermediate. Due to the large electronegativity of the fluorine atom, C-F bond is very

short and stable. In addition, the covalent radius of the fluorine atom is also very small. Therefore, with the steric hindrance of 4,5-*O*-isopropylidene group, the boron atom (Lewis acid) seems to be difficult to attack the fluorine atom to cleave the C-F bond from the top face. The fluoride **2**β might transfer to **2**α to give the glycoside.

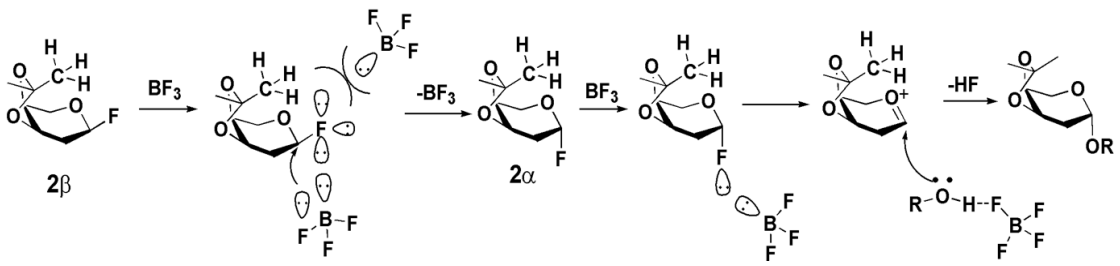


Figure 2.1 The mechanism of the glycosidation of α- and β-fluoride.

The use of *N*-phenyl trifluoroacetimidate **3**α, **3**β (entries 5 and 6) with TMSOTf as an activator gave a good yield, although the stereoselectivity was modest. The use of glycosyl phosphite (entries 7 and 8) with $BF_3{\cdot}OEt_2$ or TMSOTf gave a poor yield and low selectivity. These donors gave glycal **7** as the major product. Comparing the glycosylation results above, Kdo fluoride **2**α (entry 1) with $BF_3{\cdot}OEt_2$ as the activator provided the best yield and α-selectivity. Thus, the reaction of glycosyl fluoride **2**α in the presence of $BF_3{\cdot}OEt_2$ was the most effective for this reaction.

2.3 Conclusion

The change of leaving groups in Kdo donors was not effective on the improvement in the yield for the glycosidation of Kdo(2-4)Kdo dimer. As shown in Figure 2.1, the glycosidation of the Kdo donors with a 4,5-*O*-isopropylidene protecting group tends to proceed in a SN-1 reaction.[41] The leaving groups would be cleaved to form an oxocarbenium ion intermediate. The glycosidation results indicate that the cleaved leaving groups contribute nothing to avoiding the formation of the glycal from the oxocarbenium ion intermediate. The formation of the glycal would decrease the yield of the glycosidation. Moreover, the stereoselectivity was also not

influenced by the type of leaving group. Due to the effect of the 4,5-*O*-isopropylidene group (Figure 2.1), all donors produced the α-glycoside as the main product. These results are consistent with the results reported by Yoshizaki et al. that glycosidation with a 4,5-*O*-isopropylidinene-protected Kdo fluoride donor has a high α-selectivity.[42]

Chapter 3

The new route to synthesize 4,5-branched inner-core trisaccharides

3.1 Introduction

In Chapter 2, the effect of leaving groups and Lewis acid on the yield and stereoselectivity for the glycosylation of 2-4 linked Kdo dimer has been discussed. The result suggests that the reaction of α-fluoride in the presence of $BF_3 \cdot OEt_2$ is the most effective. In this Chapter, we focus on the construction of 4,5-branched Kdo structures using the Kdoα(2-4) Kdo dimer, which was prepared in Chapter 2, as common acceptor.

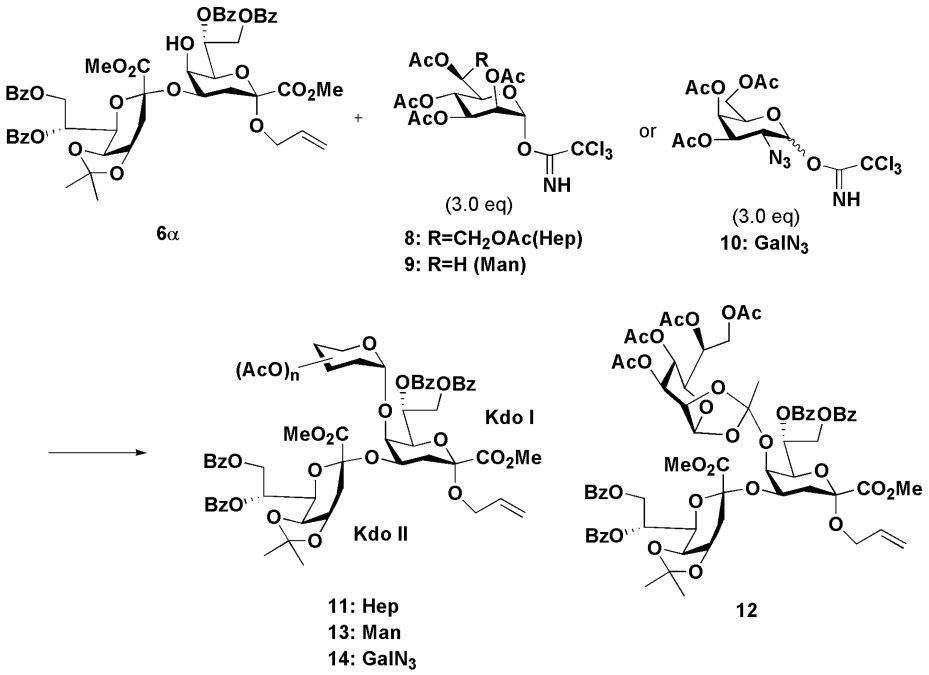
Although many chemical syntheses of linear core LPS/LOS have been reported,[34] only Paulsen et al. described the synthesis of the 4,5-branched Kdo structure.[35] As shown in Figure 1.4 of Chapter 1, they installed the L-*glycero*-D-*manno*-heptopyranosyl donor (Hep) on the 5-OH of the Kdo acceptor to form a Hepα(1-5)Kdo disaccharide and then linked a Kdo donor to the 4-OH of the Kdo moiety to form Hepα(1-5)[Kdoα(2-4)]Kdo trisaccharide. However, some defects limited the application of this approach. Pre-protection and deprotection of 4-OH of the Kdo moiety complicated the reaction. Moreover, due to the steric hindrance of heptose in 5-position and fail of the stereo control, the second glycosidation of Hepα(1-5)Kdo disaccharide with Kdo gave a low yield (37%).

To solve these problems, a new synthetic strategy different from Paulsen's method was proposed. We prepared this 4,5-branched Kdo trisaccharides by glycosylation of the 5-OH group of the 2-4 linked Kdo disaccharide, which was constructed in Chapter 2. Three types of 4,5-branched Kdo trisaccharides were synthesized through this new route.

3.2 Result and discussion

3.2.1 The glycosylation to synthesize 4,5-branched Kdo trisaccharides

Glycosylation of Kdo(2-4)Kdo acceptor **6**α with L-*glycero*-D-*manno*-heptosyl, mannosyl, and 2-azido-2-deoxy-galactosyl imidates **8**–**10** was examined (Scheme 3.1), and the results are presented in Table 3.1.



Scheme 3.1 Glycosylation of the dimeric acceptor **6**α with donors **8**–**10**.

Table 3.1 Glycosylation of the dimeric acceptor **6**α with donors **8**–**10**.

Entry	Donor	Temp (°C)	TMSOTf (equiv)	Time (h)	Product (%)	**12** (%)	**6**α (%)
1	**8** (α)	0	0.04	4	10	35	44
2	**8** (α)	r.t.	0.04	2	28	28	19
3	**8** (α)	r.t.	0.06	2	87	3	9
4	**9** (α)	0	0.04	2	91	-	-
5	**10** (α/β=1:3)	0	0.04	2	56	-	40

3.2.1.1 The glycosidation of Hep donor with Kdoα(2-4)Kdo

The reaction of heptosyl trichloroacetimidate **8**[43] with acceptor **6**α in the presence of 0.04 equiv of TMSOTf at 0 °C gave the corresponding 4,5-branched trisaccharide, Hepα(1-5)[Kdoα(2-4)]Kdo (**11**), in only 10% yield, and orthoester **12** was the major product (35%). To reduce the formation of **12**, the reaction temperature was raised to room temperature (entry 2). Correspondingly, the yield of orthoester **12** reduced to 28%, and that of the target Hepα(1-5)[Kdoα(2-4)]Kdo (**11**) increased to 28%. To suppress the formation of orthoester **12** further, more TMSOTf should be

used, because the orthoester could be transferred to 2-*O*-acyl glycosides in acid.[44] Increasing the amount of TMSOTf (entry 3) afforded trisaccharide **11** in good yield (87%) and only a small amount of orthoester **12** was detected (3%).

The 4,5-branched structure of **11** was determined by 2D NMR analysis (COSY, HMQC, and HMBC). From the COSY and HMBC spectra, we were able to identify the cyclic proton and carbon atoms of each residue of **11**. The newly formed 1-5 linkage was identified by HMBC analysis. Figure 3.1 shows part of the HMBC spectrum. The cross-relay peaks in the HMBC spectrum (Kdo H-5^{I}/Hep C-1^{III}, Hep H-1^{III}/Kdo C-5^{I}) confirmed that heptosyl donor **8** is linked to the 5-position of acceptor **6**α. Moreover, the anomeric configuration was determined from the $^{1}J_{C\text{-}1,H\text{-}1}$ value. The coupling constant between H-1^{III} and C-1^{III} ($^{1}J_{H\text{-}1^{III},C\text{-}1^{III}}$= 178 Hz) of the Hep residue suggested that the newly formed glycosidic bond was an α-linkage.[45] Thus, the trisaccharide, Hepα(1-5)[Kdoα(2-4)]Kdo, was successfully synthesized from the Kdo disaccharide with a heptosyl donor.

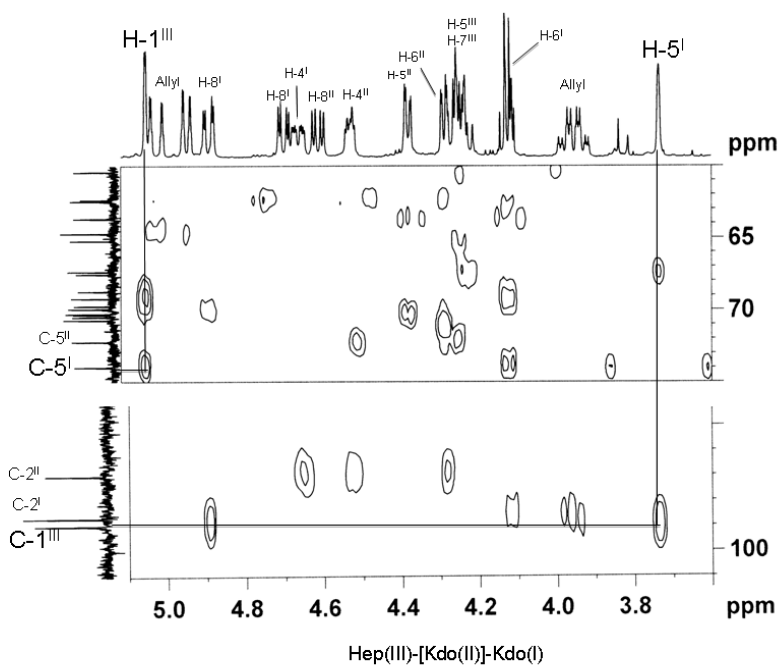


Figure 3.1 Partial HMBC spectra of compound **11** in $CDCl_3$ at 25 °C.

In conjunction with the synthesis of Kdo disaccharide **6**α in Chapter 2, the

preparation of Hepα(1-5)[Kdoα(2-4)Kdo] by our new route was supposed to be more effective than Paulsen's method. Due to a new reaction sequence, the absence of 4-OH protection shortened the reaction steps (from four steps to two steps). Without the disturbance of Hep at the 5-position, the Kdo(2-4)Kdo linkage could be smoothly formed in good yield (72%). Correspondingly, the total yield of Hepα(1-5)[Kdoα(2-4)]Kdo synthesis was improved to 63% (Paulsen: 22%).

3.2.1.2 The glycosidationof Man donor with Kdoα(2-4)Kdo

Following the synthesis of Hepα(1-5)[Kdoα(2-4)]Kdo (**11**), other 4,5-branched Kdo trisaccharides were also synthesized by the same route with **6**α as an acceptor. By coupling **6**α with mannosyl trichloroacetimidate **9**[46], branched trisaccharide **13** was also obtained in good yield (91%) (entry 4). The effect of the participating group (Ac) at the C-2 position meant that only α-isomer, which was identified by the $^{1}J_{\text{H-1}^{\text{III}},\text{C-1}^{\text{III}}}$ value of the Man residue (175 Hz), was isolated.[45] In contrast, for the heptose derivative, mannosylation proceeded smoothly at 0 °C with 0.04 equiv TMSOTf, and no orthoester was detected. These results suggest that mannosyl donor **9** was more active than heptosyl donor **8**.

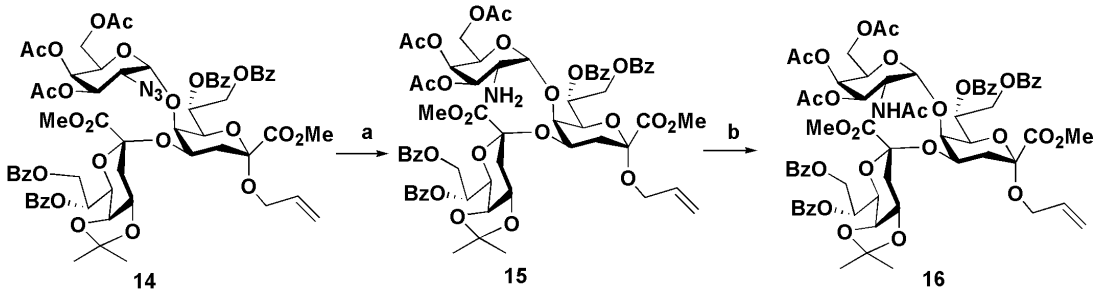
3.2.1.3 The glycosidation of $GalN_3$ donor with Kdoα(2-4)Kdo

Glycosylation of Kdo dimer **6**α with $GalN_3$ trichloroacetimidate **10**[47] was accomplished to give $GalN_3$ containing branched trisaccharide **14** as a single isomer in moderate yield (56%) (entry 5). The coupling constant between $H\text{-}1^{III}$ and $H\text{-}2^{III}$ ($J_{\text{H-1, H-2}}$= 3.4 Hz) of $GalN_3$ residue indicated that an α-glycosidic linkage was formed.[45] The position of azide group meant this glycosylation exploited the anomeric effect to give the α-isomer. In addition, the presence of acetyl groups at the 3- and 4-position was also favorable for α-isomer formation.[48] As observing from the Thin layer chromatography (TLC), only β-anomeric donor was consumed during the glycosidation. After the reaction, α-anomeric donor was almost recovered. These suggested that for $GalN_3$ donor, the β-isomer is more active than the α-isomer.

Therefore, these glycosylation results indicate that it is available to synthesize

the 4,5-branched Kdo trisaccharides by our new approach using Kdo(2-4)Kdo **6**α as common acceptor. Moreover, due to the steric hindrance in the Kdoα(2-4)Kdo acceptor, the Man type donors (Hep **8** and Man **9**) appear to be easier to couple with the Kdo acceptor than then $GalN_3$ donor.

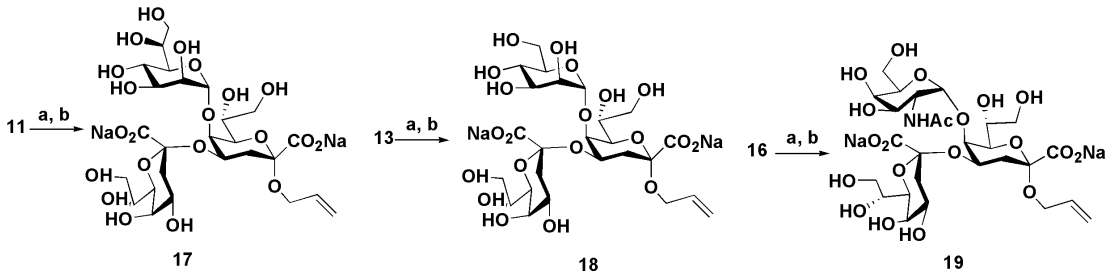
Next the transformation of the azide group to the original acetamide group in trisaccharide **14** was carried out. The azide group could not be converted directly to the acetamide group by thioacetic acid (data not shown).[49] Hence, a stepwise conversion was used (Scheme 3.2). Firstly, the azide group of $GalN_3$ **14** was reduced to the amine group under Staudinger conditions.[50] Then the amine group of **15** was acetylated with anhydrous acetic acid in the presence of *N*,*N*-dimethylaminopyridine (DMAP). Finally, GalNAc trisaccharide **16** was obtained in moderate yield (64%).



Scheme 3.2 Conditions: (a) Ph_3P, THF/H_2O= 19/1, rt, 16 h; (b) Ac_2O, DMAP, pyridine, rt, 17 h, two steps: 64%.

3.2.2 Full deprotection of 4,5-branched Kdo trisaccharides

In Final, the deprotection of all synthetic Kdo trisaccharide **11**, **13**, and **16** was carried out. Acid hydrolysis of the isopropylidene group of Kdo trisaccharide (**11**, **13**, and **16**) with aqueous trifluoroacetic acid and subsequent hydrolysis in 0.1 M sodium hydroxide to remove the ester groups afforded fully deprotected 4,5-branched Kdo trisaccharides **17**–**19** as a disodium salt in good yield (Hep **17**: 87%, Man **18**: 93%, GalNAc **19**: quantitative) (Scheme 3.3).



Scheme 3.3 Conditions: (a) 80% TFA, CH_2Cl_2, rt; (b) 0.1 M NaOH, MeOH, two steps: Hep **17** (87%), Man **18** (93%), GalNAc **19** (quant),.

3.3 Conclusion

A new synthetic strategy using Kdo (2-4)Kdo **6**α as an acceptor was developed for the synthesis of 4,5-branched Kdo trisaccharides. Glycosylation at the 4-OH position of the Kdo acceptor followed by a second glycosylation at 5-OH position produced the heptosyl Kdo dimer, Hepα(1-5)[Kdoα(2-4)]Kdo (**11)**. We also achieved the first synthesis of the 4,5-branched partial inner-core trisaccharides Manα(1-5)[Kdoα(2-4)]Kdo (**13**) from *Francisella tularensis*[21] and $GalN_3$α(1-5)[Kdoα(2-4)]Kdo (**14**) from *Pseudomonas cichorii*[22] in good yield and high α-selectivity. This new route might provide another choice for the synthesis of the inner-core oligosaccharides of LPS/LOS with the Paulsen's method.

Chapter 4

The convergent synthesis of 4,5-branched inner-core OSs

4.1 Introduction

The highly conserved inner-core OS consists of mostly the higher carbon sugars 3-deoxy-D-*manno*-2-octulosonic acid (Kdo) and L-*glycero*-D-*manno*-heptopyranose (Hep). For example in Figure 4.1, the inner core OS of LPSs/LOSs from many gram-negative bacteria contains a 4,5-branched Hepα(1-3)Hepα(1-5)[Kdoα(2-4)]Kdo tetrasaccharide as the common structure.[51] An R (R = Lac, Glc, P) residue is usually substituted at the 4-*O* position of Hep I.[52]

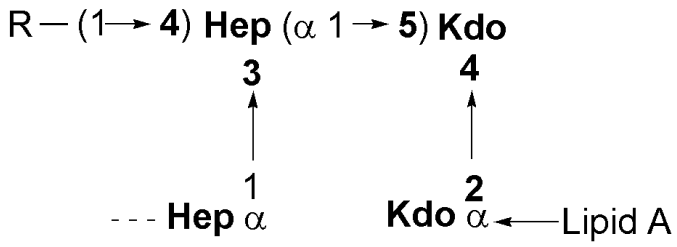


Figure 4.1 General inner-core structure of LPS/LOS.

In Chapter 3, a new synthetic method to prepare 4,5-branched inner-core trisaccharides by coupling monosaccharides (Hep, Man, $GalN_3$) with a common Kdo acceptor **6**α was introduced. To extend the utility of this approach, in this Chapter we prepared more complex 4,5-banched inner core OS structures by using the same Kdo disaccharide **6**α as the acceptor. A lactose donor was initially chosen as a model compound to optimize the glycosylation conditions. Based on the model glycosylation, the corresponding Hep units constructed from the Hep building blocks were coupled with the Kdo moiety to obtain the desired branched inner-core OS.

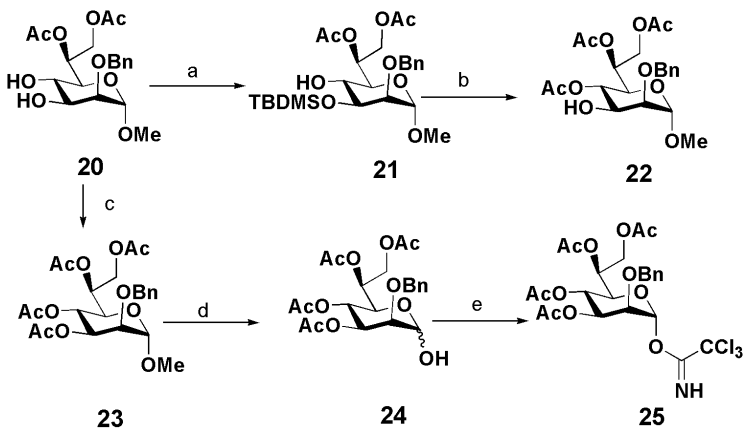
4.2 Result and discussion

4.2.1 Synthesis of Hep units

To install the Kdo moiety, the Hep units, Galβ(1-4)Glcβ(1-4)Hep trisaccharide and Hepα(1-3)Hep disaccharide, were prepared. All the Hep building blocks (**21**, **22**, **25**) required for the Hep units were obtained from known methyl 6,7-di-*O*-acetyl-2-*O*-benzyl-L-*glycero*-D-*manno*-heptopyranoside **20**[53] (Scheme 4.1).

4.2.1.1 Synthesis of Hep building blocks

Treatment of 3,4-diol **20** with *t*-butyldimethylsilyl chloride (TBDMSCl) and 1*H*-imidazole in *N*,*N*-dimethylformamide (DMF) at room temperature gave 3-*O*-TBDMS ether **21** in 94% yield. The acetylation of **21** in acetic anhydride (Ac_2O)/pyridine and subsequent de-*O*-silylation of TBDMS group in aqueous trifluoroacetic acid gave 3-OH product **22** in 88% yield. L-*Glycero*-D-*manno*-heptosyl trichloroacetimidate **25** was also prepared from 3,4-diol **20** in a 69% yield over four steps as follows: sequential acetylation of 3,4-diol **20**, acetolysis of **23**, selective anomeric deacetylation, and treatment of hemiacetal **24** with trichloroacetonitrile in the presence of potassium carbonate.

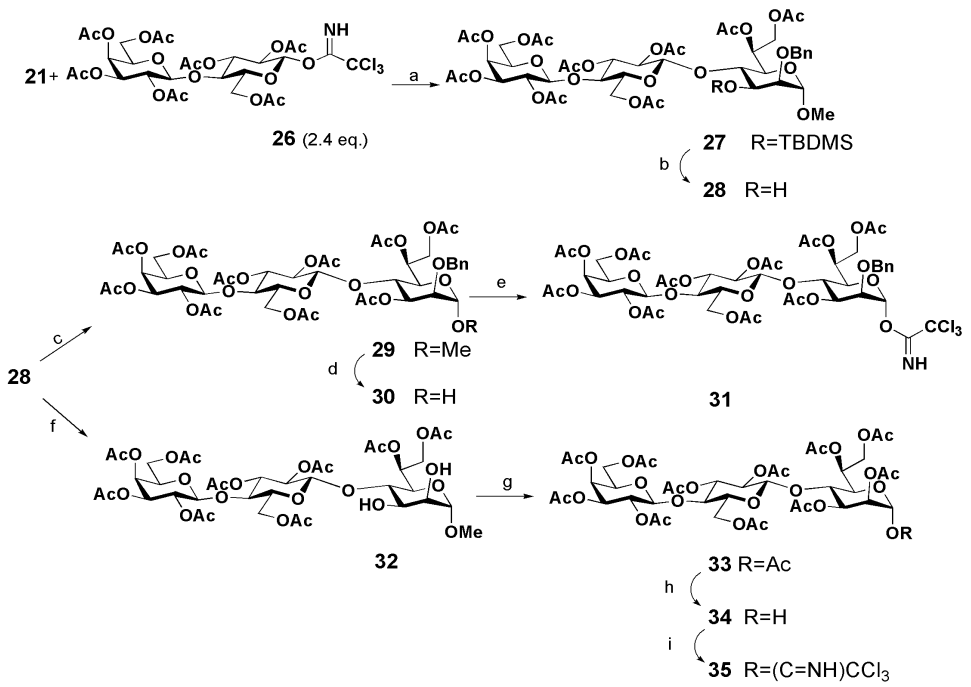


Scheme 4.1 Conditions: (a) TBDMSCl, 1*H*-imidazole, DMF, rt, 4 h, 92%; (b) (i) Ac_2O, DMAP, pyridine, 0 °C→ rt, 17 h, 95%; (ii) 90% TFA aq., rt, 1 h, 93%; (c) Ac_2O, DMAP, pyridine, 0 °C→ rt, 2 h, 94%; (d) (i) H_2SO_4, Ac_2O, AcOH, rt, 2 h, 95%; (ii) hydrazine acetate, 0 °C→ rt, DMF, 2 h, 80%; (e) Cl_3CCN, K_2CO_3, CH_2Cl_2, rt, 22 h, 96%.

4.2.1.2 Synthesis of Lacβ(1-4)Hep unit

Glycosylation of 4-OH Hep building block **21** with hepta-*O*-acetyl-β-lactosyl trichloroacetimidate **26**[54] using TMSOTf as the catalyst in CH_2Cl_2 proceeded smoothly to afford (1-4)-linked Galβ(1-4)Glcβ(1-4)Hep trisaccharide **27**[55] as a Hep unit in 79% yield (Scheme 4.2). The glucosyl-(1-4)-heptose linkage in trisaccharide **27** was assigned as β based on the coupling constant between H-1 and H-2 of the glucose residue ($^3J_{H1,H2}$= 8.0 Hz). Cleavage of the TBDMS group in

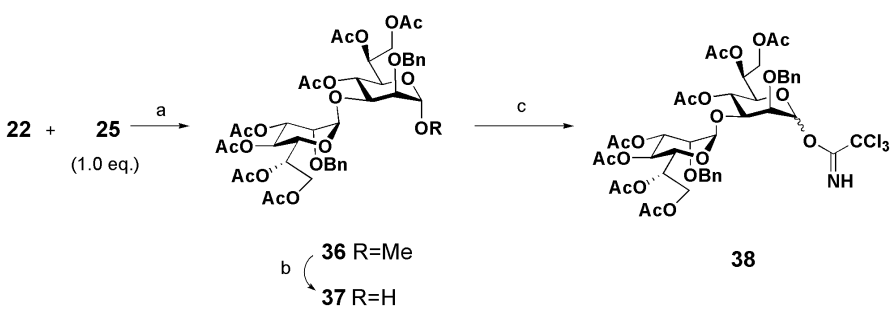
Galβ(1-4)Glcβ(1-4)Hep trisaccharide **27** with aqueous trifluoroacetic acid produced the free 3-OH **28** in 97% yield. To characterize the effect of the protecting group of donor moiety on the glycosidation, two types of Galβ(1-4)Glcβ(1-4)Hep donors (**31** and **35**) with the different protecting groups at C-2 of the Hep residue were prepared from **28**, respectively, as follows. Immediate acetylation of **28** with acetic anhydride in pyridine gave **29** in 73% yield. Acetolysis of **29** in H_2SO_4/Ac_2O/AcOH and subsequent selective anomeric deacetylation afforded hemiacetal **30**. Galβ(1-4)Glcβ(1-4)Hep hemiacetal **30** was transformed in quantitative yield to the corresponding trichloroacetimidate **31**. In addition, to obtain a per-*O*-acetylated Galβ(1-4)Glcβ(1-4)Hep donor, the benzyl group at C-2 of the Hep residue in **28** was removed by hydrogenolysis (10% Pd/C in EtOAc) to give 2,3-diol **32**[55] in 97% yield. Acetylation of **32** with acetic anhydride in pyridine, followed by acetolysis produced **33** in 67% yield. Selective anomeric deacetylation of **33** with hydrazine acetate in DMF at 0 °C gave hemiacetal **34** in 90% yield. Treatment of **34** with trichloroacetonitrile in the presence of K_2CO_3 gave per-*O*-acetylated Galβ(1-4)Glcβ(1-4)Hep trichloroacetimidate **35** in 92% yield. Galβ(1-4)Glcβ(1-4)Hep trichloroacetimidates **31** and **35** were expected to undergo [3+2] coupling with the Kdo moiety.



Scheme 4.2 Conditions: (a) TMSOTf, CH_2Cl_2, MS AW 300 molecular sieves, 0 °C, 3 h, 79%; (b) TFA/H_2O, 9:1, rt, 5 min, 97%; (c) Ac_2O, DMAP, pyridine, rt, 2 h, 73%; (d) (i) H_2SO_4, Ac_2O, AcOH, rt, 3 h, 64%; (ii) hydrazine acetate, DMF, 0 °C, 8 h, 77%; (e) Cl_3CCN, K_2CO_3, CH_2Cl_2, rt, 13 h, quant; (f) 10% Pd/C, H_2, ethyl acetate, rt, 3.5 h, 97%; (g) (i) Ac_2O, pyridine, rt, 24 h; (ii) H_2SO_4, Ac_2O, AcOH, rt, 15 h, two steps: 67%; (h) hydrazine acetate, DMF, 0 °C, 8 h, 90%; (i) Cl_3CCN, K_2CO_3, CH_2Cl_2, rt, 24 h, 92%.

4.2.1.2 Synthesis of Hepα(1-3)Hep unit

The (1-3)-linked heptobiose unit **36** was prepared in 50% yield by glycosidation of imidate **25** with Hep building block **22** by using TMSOTf as a promoter in CH_2Cl_2 (Scheme 4.3). The coupling constant between C-1 and H-1 ($^1J_{C,H}$= 174 Hz) of reducing heptose residue suggested the (1-3) linkage was an α-linkage. No β-isomer was detected. Acetolysis of the methyl ether in **36**, followed by selective cleavage of the anomeric acetyl group with hydrazine acetate in DMF at 0 °C produced disaccharide hemiacetal **37** in 78% yield over two steps. Treatment of **37** with trichloroacetonitrile in the presence of K_2CO_3 gave Hep(1-3)Hep trichloroacetimidate **38**, which was expected to undergo [2+2] coupling with the Kdo moiety.

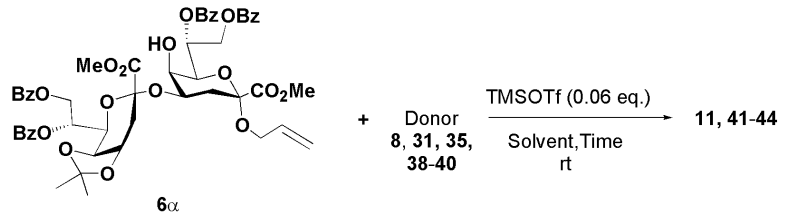


Scheme 4.3 Conditions: (a) TMSOTf, CH_2Cl_2, 4Å molecular sieves, -78 °C→ rt, 2 h, 50%; (b) (i) H_2SO_4, Ac_2O, AcOH, rt, 2 h, (ii) hydrazine acetate, DMF, 0 °C, 7 h, two steps: 78%; (c) Cl_3CCN, K_2CO_3, CH_2Cl_2, rt, 21 h, 80%, α/β=6:1.

4.2.2 Convergent synthesis of 4,5-branched inner-core OS structures

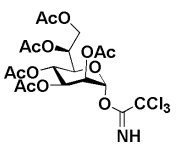
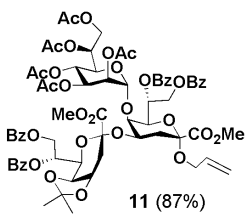
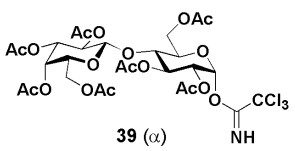
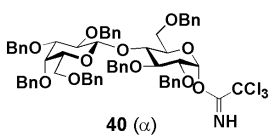
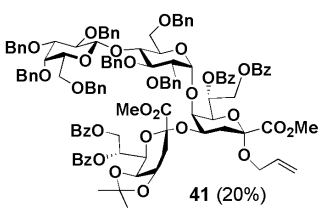
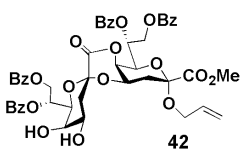
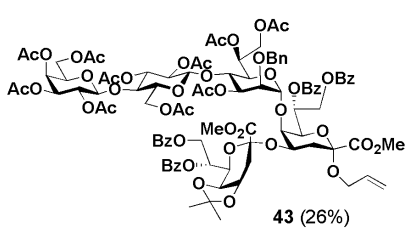
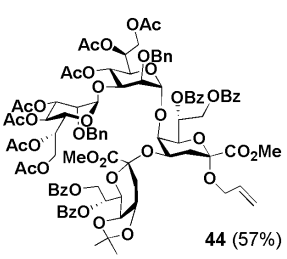
Next, we focused on the glycosidation of the Hep units with the Kdo moiety (Scheme 4.4 and Table 4.1). A model glycosylation using a lactose derivative as a

donor was performed to test this convergent approach.



Scheme 4.4 The convergent synthesis of 4,5-branched inner-core OSs.

Table 4.1 Glycosylation to synthesize 4,5-branched inner-core OSs.

Donor	Solvent	Time	Product	**6**α
 **8** (α)	CH_2Cl_2	2	 **11** (87%)	9%
 **39** (α)	CH_2Cl_2	2	—	90%
 **40** (α)	CH_2Cl_2/Et_2O (3/1)	2	 **41** (20%)	75%
35 (α)	CH_2Cl_2	15	 **42**	-
31(α)	CH_2Cl_2	2	 **43** (26%)	69%
38 (α/β=6/1)	CH_2Cl_2	1	 **44** (57%)	42%

4.2.2.1 Model glycosylation of lactose donor with Kdo disaccharide

Because the glycosylation of per-*O*-acetylated Hep imidate **8** could provide Hepα(1-5)[Kdoα(2-4)]Kdo trisaccharide **11** in good yield (Chapter 3: Table 3.1), per-*O*-acetylated lactosyl imidate **39** was used for coupling with Kdoα(2-4)Kdo acceptor **6**α (Scheme 4.4 and Table 4.1). However, no Lac-Kdo tetrasaccharide was formed. The reactivity of the per-*O*-acetylated lactose donor was too low to form the linkage. Therefore, a more reactive donor, hepta-*O*-benzyl-α-lactosyl trichloroacetimidate **40**[56], was examined. To increase the α-selectivity in the lactosylation, the reaction was carried out in CH_2Cl_2/Et_2O[57] and gave branched Galβ(1-4)Glc(1-5)[Kdoα(2-4)]Kdo tetrasaccharide **41** in only 20% yield as only a single isomer. The coupling constant between H-1 and H-2 of the glucose residue ($^3J_{H1,H2}$= 3.4 Hz) indicated that the (1-5) linkage was an α-linkage. The high stereoselectivity was due to the anomeric effect[58] and the solvent effect[59]. The introduction of benzyl ethers meant that imidate **40** was more effective in providing desired tetrasaccharide **41**, despite the high steric hindrance.

4.2.2.2 The convergent glycosylation of Lacβ(1-4)Hep unit with Kdo disaccharide

Following the model glycosylation, the [3+2] coupling of the Lacβ(1-4)Hep unit with the Kdo moiety was examined. According to the glycosidation results of both Hep donor **8** and **25** giving products in high α-selectivity in CH_2Cl_2, CH_2Cl_2 was used as a solvent for the following heptosylation. The synthesis of Lacβ(1-4)Hepα(1-5)[Kdoα(2-4)]Kdo pentasaccharide by coupling of per-*O*-acetylated Galβ(1-4)Glcβ(1-4)Hep trichloroacetimidate **35** with Kdo acceptor **6**α failed. No branched pentasaccharide was found and mainly imidate **35** was recovered, even though the reaction time was extended to 15 h. In addition, the decomposition of the acceptor **6**α to lactone **42** was observed. The donor was changed to Galβ(1-4)Glcβ(1-4)Hep imidate **31**, which contained a benzyl group at C-2 of the Hep residue, and desired Galβ(1-4)Glcβ(1-4)Hepα(1-5)[Kdoα(2-4)]Kdo pentasaccharide **43** was obtained in a 26% yield as only the α-anomer. The anomeric configuration of the Hep residue in pentasaccharide **43** was confirmed by the coupling

constants between C-1 and H-1 of the heptose residue ($^1J_{C,H}$= 174 Hz). This was consistent with the results of the model glycosylation, which indicated that the reactivity of the donor is important for this convergent approach. The introduction of an benzyl ether at C-2 of the Hep residue increased the reactivity of the Galβ(1-4)Glcβ(1-4)Hep donor to provide the branched pentasaccharide. To increase the pentasaccharide yield further, the strategy of benzylating the 3-OH of the heptose residue was considered. Therefore, in our convergent approach, the sterically crowded heptose unit can be added to the Kdo moiety to produce the desired 4,5-branched core OS structures. This approach was also expected to provide the common inner-core OS structure containing the heptobiose unit.

4.2.2.3 The convergent glycosylation of Hepα(1-3)Hep unit with Kdo dimer

Dibenzyl Hepα(1-3)Hep trichloroacetimidate **38** was coupled with Kdoα(2-4)Kdo acceptor **6**α by using 0.06 equiv of TMSOTf as the activator. As expected, the introduction of the dibenzyl group substantially increased the reactivity of the Hepα(1-3)Hep unit to provide Hepα(1-3)Hep(1-5)[Kdoα(2-4)]Kdo tetrasaccharide **44** in moderate yield (57%) as the α-anomer. The configuration of tetrasaccharide **44** was confirmed by the coupling constants between C-1 and H-1 of the corresponding heptoses ($^1J_{C,H}$= 172, 174 Hz).

Furthermore, all branched structures we synthesized were characterized by analyzing the corresponding 2D NMR spectra (COSY, HMQC, and HMBC). For example, the existence of the (1-5) linkage in Hep(α1-3)Hep(α1-5)[Kdo(α2-4)]Kdo tetrasaccharide **44** was supported by the HMBC analysis. The cross-relay peaks, Kdo H-5^{I}/Hep C-1^{III}, Hep H-1^{III}/Kdo C-5^{I}, in the HMBC spectrum (Figure 4.2) confirmed that the Hep unit is linked to the 5-position of the Kdo moiety.

These results suggest that it is possible to obtain complex 4,5-branched inner core OSs of LPS/LOS using common Kdo dimmer **6**α as an acceptor via a convergent approach. The Lac-Hep imidate **31** with a benzyl group at C-2 of the reducing residue, other than the Lac-Hep peracetate **35**, giving the desired product of glycoside indicates that the effective improvement of the reactivity is supported by the benzyl

group. Meanwhile, the glycosidation of perbenzylated Lac imidate **40** giving less product might indicate that the perbenzylated donor should be too active to obtain the glycoside in good yield. These results suggest that the introduction of appropriate number of benzyl protecting groups appears to be important for the yield of this convergent glycosylation.

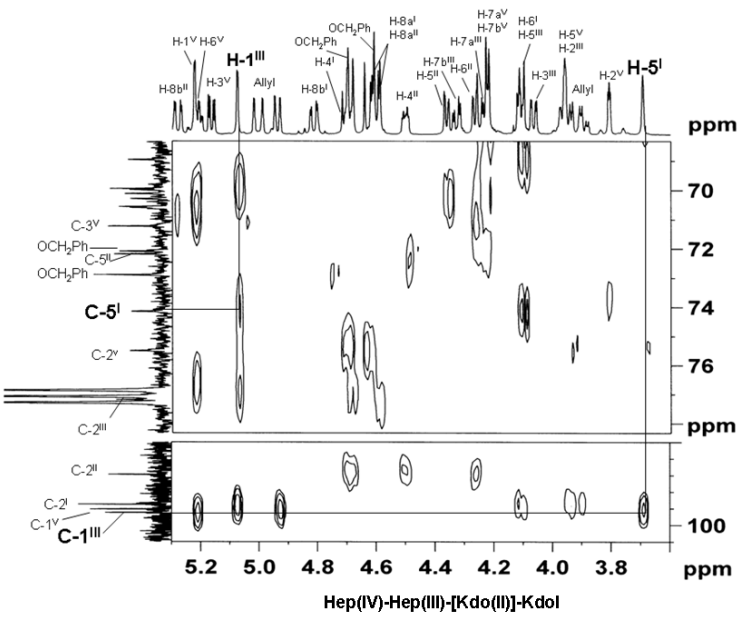


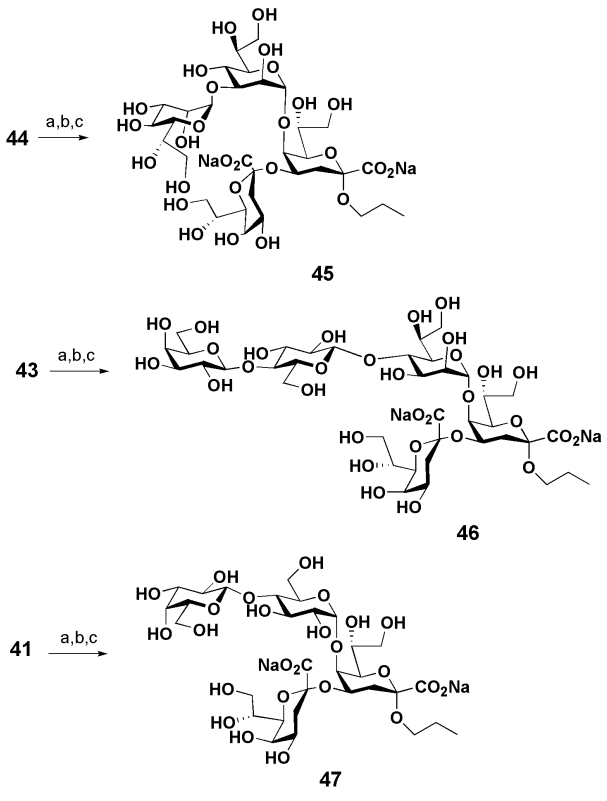
Figure 4.2 Partial HMBC spectrum of tetrasaccharide **44** in $CDCl_3$ at 25 °C.

4.2.3 Full deprotection of 4,5-branched Kdo tetra- and pentasaccharide

Finally, the deprotection of all synthetic Kdo trisaccharide **41**, **43**, and **44** was carried out. As shown in Scheme 4.5, deprotection of Hepα(1-3)Hepα(1-5)[Kdoα(2-4)]Kdo tetrasaccharide **44** was performed over three steps. $Pd(OH)_2$/C-promoted hydrolysis of the benzyl groups, acid hydrolysis of the isopropylidene group with aqueous trifluoroacetic acid, and hydrolysis of the ester group in 0.1 M NaOH produced the target 4,5-branched tetrasaccharide **45** in 90% yield as the disodium salt.

Galβ(1-4)Glcβ(1-4)Hepα(1-5)[Kdoα(2-4)]Kdo pentasaccharide **43** and Galβ(1-4)Glcα(1-5)[Kdoα(2-4)]Kdo tetrasaccharide **41** were subjected to similar deprotection to afford the corresponding deprotected compounds, **46** (53%) and **47**

(60%)



Scheme 4.5 Conditions: (a) $Pd(OH)_2/C$, H_2, MeOH, rt; (b) 80% TFA aq., CH_2Cl_2, rt; (c) 0.1 M NaOH, MeOH, three steps: **41** (90%), **42** (53%), **43** (60%).

4.3 Conclusion

The convergent synthetic strategy using Kdoα(2-4)Kdo as a common acceptor was used to prepare more complex 4,5-branched inner core OS structures. Model glycosylation using a lactose derivative as a test compound suggested that the reactivity of the donor was important for this convergent synthesis, and this was supported by the subsequent glycosylation. Based on the convergent approach, the first synthesis of 4,5-branched inner-core OSs, namely, Galβ(1-4)Glcβ(1-4)Hepα(1-5)[Kdoα(2-4)]Kdo pentasaccharide and a common inner-core Hepα(1-3)Hepα(1-5)[Kdoα(2-4)]Kdo tetrasaccharide, was accomplished by coupling the corresponding Hep units with Kdoα(2-4)Kdo. These results suggested that it is a available to synthesize complex 4,5-branced inner-core OS structures by our new approach and Kdo(2-4)Kdo **6**α is a useful intermediate for these synthesis.

Chapter 5

Conclusion

In this study, a new synthetic approach using a common Kdoα(2-4)Kdo disaccharide as acceptor to synthesize the 4,5-branched inner-core OS structures was described. Using this new approach, several types of 4,5-branced Kdo trisaccharides, tetrasaccharides, and pentasaccharides were successfully synthesized in good yield and high α-selectivity.

In the Chapter 2, to optimize the condition of glycosylation, several types of glycosyl donors with different leaving group and stereoselectivity were prepared from common Kdo intermediate and were glycosylated with 4,5-diol acceptor. The results showed that all donors produced the α-glycoside as the main product and the stereoselectivity was not influenced by the type of leaving group. Moreover, the α-fluoride donor with $BF_3 \cdot OEt_2$ as the activator provided the best yield and α-selectivity of product.

In the Chapter 3, using the constructed Kdoα(2-4)Kdo disaccharide as the common acceptor, three types of 4,5-branched Kdo trisaccharides were successfully synthesized in good yield and high α-selectivity. The glycosylation condition of Hepα(1-5)[Kdoα(2-4)]Kdo was discussed and the result seemed to be better than that of the Paulsen's method in the yield and stereoselectivity. The first synthesis of the 4,5-branched partial inner-core trisaccharides, Manα(1-5)[Kdoα(2-4)]Kdo (**13**) from *Francisella tularensis* and $GalN_3$α(1-5)[Kdoα(2-4)]Kdo (**14**) from *Pseudomonas cichorii*, were also achieved. These results suggest that it is available to synthesize the 4,5-branched Kdo structures by the new reaction sequence of glycosylation at the 4-OH position of the Kdo acceptor followed by a second glycosylation at 5-OH position. This new route should be more effective for the synthesis of the inner-core oligosaccharides of LPS/LOS than Paulsen's method.

In the Chapter 4, to extend the utility of the new synthetic strategy, more complex 4,5-branched inner-core OS structures were synthesized. With the same Kdoα(2-4)Kdo disaccharide as acceptor, three types of 4,5-branched Kdo tetra- and pentasaccharides were synthesized in high α-selectivity. Model glycosylation using a lactose derivative as a test compound suggested that the reactivity of the donor was

important for this convergent synthesis, and this was supported by the subsequent glycosylation. The first synthesis of 4,5-branched inner-core OSs, namely, Galβ(1-4)Glcβ(1-4)Hepα(1-5)[Kdoα(2-4)]Kdo pentasaccharide and a common inner-core Hepα(1-3)Hepα(1-5)[Kdoα(2-4)]Kdo tetrasaccharide, was accomplished by coupling the corresponding Hep units with Kdoα(2-4)Kdo.

In all, the new synthetic approach using Kdoα(2-4)Kdo as an intermediate is useful for the synthesis of 4,5-branched inner-core OS structures including Kdo trisaccharides, Kdo tetrasaccharides and Kdo pentasaccharide. It would provide another synthetic choice to obtain 4,5-branced inner-core OSs, with Paulsen's method.

Chapter 6

Experimental section

6.1 General procedures

Optical rotation was measured with a Horiba SEPA500 polarimeter in $CHCl_3$ and melting point (uncorrected) was measured with a Yanagimoto micro melting point apparatus. All NMR spectra were recorded at 25 °C in $CDCl_3$ or D_2O on a 600 MHz NMR spectrometer (Avance II, Bruker). All NMR chemical shifts (δ) were recorded in parts per million (ppm), and coupling constants (*J*) were reported in hertz (Hz). Mass spectrometry (MS) was performed by positive- and negative-mode electrospray ionization on a Waters LCT Premier spectrometer. For high-precision measurements, the spectra were obtained by scanning the voltage over a narrow mass range at a resolution of 10,000. MALDI-TOF spectra were recorded on a Bruker Daltonics instrument, using 3,5-dihydroxybenzoic acid as the matrix. Elemental analysis was carried out on a performed on Vario ELCUBE and Vario EL III, Elementar. Infrared spectra were determined on a JASCO FT/IR-4100 Spectrometer. Analytical TLC was performed on Merck silica gel 60 F_{254} glass plates. The TLC plates were visualized with UV light and by staining with Hannessian solution (ceric sulfate and ammonium molybdate in aqueous sulfuric acid), and then heating at 200 °C for 3 min. Column chromatography was performed on silica gel 60 (flash column: 0.040–0.063 mm; open column: 0.063–0.200 mm).

6.2 Methyl (7,8-di-*O*-benzoyl-4,5-*O*-isopropylidene-3-deoxy-D-*manno*-2-octulopy -ranosyl *N*-phenyl trifluoroacetimidate)onate (3)

Compound **1** (250.0 mg, 0.5 mmol) was dissolved in dry dichloromethane (5.0 mL) under argon. *N*-phenyl trifluoroacetimidoyl chloride[60] (716.0 μL, 5.0 mmol) and potassium carbonate (113.0 mg, 5.0 mmol) was added into the reaction. After stirring for 1 week, the mixture was filtered through Celite. The solution was concentrated and purified by silica gel column chromatography (ethyl acetate/toluene, 1:5) to give **3** in quantitative yield (343.0 mg, α/β=3:2). α-isomer: $[\alpha]^{25}_{D}$ = +75.6 (*c* 1.0, $CHCl_3$), ^{1}H-NMR (600 MHz, $CDCl_3$): δ 1.24 (s, 3H, Me), 1.50 (s, 3H, Me), 2.33 (1 H, $J_{3a,3e}$=15.6 Hz, $J_{3a,4}$=3.4 Hz, H-3a), 2.78 (dd, 1H, $J_{3a,\,3e}$=15.6 Hz, $J_{3e,4}$=4.0 Hz, H-3e), 3.78 (s, 3H, OMe), 4.37 (dd, 1H, $J_{5,\,6}$=2.0 Hz, $J_{6,7}$=8.6 Hz, H-6), 4.41 (dd, 1H, $J_{4,5}$=7.6

Hz, $J_{5,6}$=2.0 Hz, H-5), 4.62 (ddd, 1H, $J_{3a,4}$=3.4 Hz, $J_{3e,4}$=4.0 Hz, $J_{4,5}$=7.6 Hz, H-4), 4.72 (dd, 1H, $J_{7,8a}$=4.0 Hz, $J_{8a,8b}$=12.4 Hz, H-8a), 5.00 (dd, 1H, $J_{7,8b}$=2.4 Hz, $J_{8a,8b}$=12.4 Hz, H-8b), 5.75 (ddd, 1H, $J_{6,7}$=8.6 Hz, $J_{7,8a}$=4.0 Hz, $J_{7,8b}$=2.4 Hz, H-7), 6.74-6.75 (m, 2H, *N*Ph-Ar), 7.05 (m, 1H, *N*Ph-Ar), 7.24–7.25 (ddd, 2H, *N*Ph-Ar), 7.37-7.44 (m, 4H, Ar), 7.51–7.57 (m, 2H, Ar), 7.98–8.03 (m, 4H, Ar). ^{13}C NMR (150 MHz, $CDCl_3$): δ 24.8, 25.6 (CH_3), 33.0 (C-3), 52.8 (OCH_3), 62.6 (C-8), 69.5 (C-4), 70.1 (C-7), 70.2 (C-6), 70.8 (C-5), 99.0 (C-2), 110.1 (C_{isop}), 116.5 (CF_3), 119.1 (*N*Ph-Ar), 124.3 (*N*Ph-Ar), 128.4, 128.5, 128.7, 129.4, 129.6, 129.7, 129.8, 133.1, 133.3 (14 C, *N*Ph-Ar), 143.2 (C=N), 165.1, 166.2 (C=O), 168.1 (C-1). IR: 1736, 1727, 1229, 1215, 1202 cm^{-1}. ESI-HRMS for $C_{34}H_{32}F_3NO_{10}$: 694.1876 $[M+Na]^+$. Found 694.1873. β-isomer: $[\alpha]^{25}_D$ = +6.6 (*c* 1.0, $CHCl_3$), ^{1}H-NMR (600 MHz, $CDCl_3$): δ 1.29 (s, 3H, Me), 1.54 (s, 3H, Me), 2.34 (dd, 1H, $J_{3a,3e}$=16.2 Hz, $J_{3a,4}$=3.4 Hz, H-3a), 2.78 (dd, 1H, $J_{3a,\ 3e}$=16.2 Hz, $J_{3e,4}$=3.0 Hz, H-3e), 3.66 (s, 3H, OMe), 4.26 (dd, 1H, $J_{5,6}$=1.8 Hz, $J_{6,7}$=8.0 Hz, H-6), 4.44 (dd, 1H, $J_{4,5}$=8.2 Hz, $J_{5,6}$=1.8 Hz, H-5), 4.68 (ddd, 1H, $J_{3a,4}$=3.4 Hz, $J_{3e,4}$=3.0 Hz, $J_{4,5}$=8.2 Hz, H-4), 4.70 (dd, 1H, $J_{7,8a}$=5.2 Hz, $J_{8a,8b}$=12.4 Hz, H-8a), 4.97 (dd, 1H, $J_{7,8b}$=2.4 Hz, $J_{8a,8b}$=12.4 Hz, H-8b), 5.68-5.71 (ddd, 1H, $J_{6,7}$=8.6 Hz, $J_{7,8a}$=5.2 Hz, $J_{7,8b}$=2.4 Hz, H-7), 6.74–6.75 (dd, 2H, *N*Ph-Ar), 7.09 (m, 1H, *N*Ph-Ar), 7.27–7.28 (ddd, 2H, *N*Ph-Ar), 7.39–7.44 (m, 4H, Ar), 7.51–7.57 (m, 2H, Ar), 7.98–8.03 (m, 4H, Ar). ^{13}C NMR (150 MHz, $CDCl_3$): δ 25.6, 25.8 (CH_3), 29.7 (C-3), 52.8 (OCH_3), 62.9 (C-8), 69.2 (C-4), 70.5 (C-7), 70.9 (C-5), 71.5 (C-6), 99.3 (C-2), 110.0 (C_{isop}), 114.6 (CF_3), 119.3 (*N*Ph-Ar), 124.3 (*N*Ph-Ar), 128.4, 128.5, 128.6, 128.8, 129.6, 129.7, 129.8, 129.83, 130.0, 134.0, 133.2 (14 C, *N*Ph-C_{meta} and Ar), 143.2 (C=N), 165.2, 166.1 (C=O), 167.9 (C-1). IR: 1736, 1725, 1230, 1214, 1205 cm^{-1}. ESI-HRMS for $C_{34}H_{32}F_3NO_{10}$: 694.1876 $[M+Na]^+$. Found 694.1855.

6.3 Methyl (dibenzyl-7,8-di-*O*-benzoyl-4,5-*O*-isopropylidene-3-deoxy-D-*manno*-2-octulopyranosyl phosphite)onate (4)

1*H*-tetrazole (112.0 mg, 1.6 mmol) was added to a solution of **1** (200.0 mg, 0.4 mmol) in dry dichloromethane (13.0 mL) under argon. Then the reaction mixture was cooled to 0 ˚C and treated with dibenzyl *N*,*N*-diisopropylphosphoramidite (DDP, 322

μL/0.96 mmol). After stirring for 3 h, the solution was quenched with triethylamine (Et_3N) and concentrated. The residue was purified by silica gel column chromatography (ethyl acetate/ hexane, 2:3+1% Et_3N) to give a mixture of anomers **4** in 56% yield (167.0 mg, α/β=23:1). α-isomer: $[\alpha]^{25}_D$ = +33.8 (*c* 1.1, $CHCl_3$), ^{1}H-NMR (600 MHz, $CDCl_3$): δ 1.20, 1.43 (s, 2H, CH_3), 2.06 (dd, 1H, $J_{3a,3e}$=15.0 Hz, $J_{3a,4}$=3.4 Hz, H-3a), 2.82 (dd, 1H, $J_{3a,3e}$=15.0 Hz, $J_{3e,4}$=5.2 Hz, H-3e), 3.70 (s, 3H, OMe), 4.25 (dd, 1H, $J_{4,5}$=7.0 Hz, $J_{5,6}$=2.0 Hz, H-5), 4.34 (dd, 1H, $J_{5,6}$=2.0 Hz, $J_{6,7}$=7.4 Hz, H-6), 4.47 (ddd, 1H, $J_{3a,4}$=3.4 Hz, $J_{3e,4}$=5.2 Hz, $J_{4,5}$=7.0 Hz, H-4), 4.66 (dd, 1H, $J_{7,8a}$=6.0 Hz, $J_{8a,8b}$=12.4 Hz, H-8a), 4.77 (dd, 1H, *J* =12.4 and 7.8 Hz, OCH_2Ph), 4.81 (d, 2H, *J*=7.8 Hz, OCH_2Ph), 4.83 (dd, 1H, *J*=12.2 and 8.4 Hz, OCH_2Ph), 5.00 (dd, 1H, $J_{7,8b}$=2.4 Hz, $J_{8a,8b}$=12.4 Hz, H-8b), 5.74 (ddd, 1H, $J_{6,7}$=7.4 Hz, $J_{7,8a}$=6.0 Hz, $J_{7,8b}$=2.4 Hz, H-7), 7.18–7.54 (m, 16H, Ar), 7.97–8.03 (m, 4H, Ar). ^{13}C NMR (150 MHz, $CDCl_3$): δ 25.0, 26.0 (CH_3), 33.7 (C-3), 52.7 (OCH_3), 63.3 (C-8), 64.2, 64.20, 64.6, 64.7 (2C, OCH_2), 69.8 (C-4), 70.6 (C-7), 70.7 (C-6), 71.4 (C-5), 97.3, 97.33 (C-2), 109.7 (C_{isop}), 127.6, 127.7, 127.72, 128.1, 128.3, 128.37, 128.4, 128.7, 129.8, 129.9, 130.1, 132.9 133.1, 137.9, 138.0, 138.0 (Ar), 165.3, 166.2 (C=O), 168.7 (C-1). IR: 1749, 1713, 1282, 1252, 1213, 973 cm^{-1}. ESI-HRMS for $C_{40}H_{41}O_{12}P$: 767.2233 $[M+Na]^+$. Found 767.2222. β-isomer: $[\alpha]^{25}_D$ = +23.4 (*c* 1.0, $CHCl_3$), ^{1}H-NMR (600 MHz, $CDCl_3$): δ 1.21, 1.42 (s, 2H, CH_3), 2.40 (dd, 1H, $J_{3a,3e}$=15.2 Hz, $J_{3a,4}$=3.0 Hz, H-3a), 2.94 (dd, 1H, $J_{3a,3e}$=15.2 Hz, $J_{3e,4}$=5.6 Hz, H-3e), 3.73 (s, 3H, OMe), 4.35 (dd, 1H, $J_{4,5}$=7.6 Hz, $J_{5,6}$=2.0 Hz, H-5), 4.52 (ddd, IH, $J_{3a,4}$=3.0 Hz, $J_{3e,4}$=5.6 Hz, $J_{4,5}$=7.6 Hz, H-4), 4.65 (dd, 1H, $J_{5,6}$=2.0 Hz, $J_{6,7}$=7.4 Hz, H-6), 4.66 (dd, 1H, $J_{7,8a}$=7.0 Hz, $J_{8a,8b}$=12.4 Hz, H-8a), 4.95–5.02 (m, 4H, OCH_2), 5.02 (dd, 1H, $J_{7,8b}$=2.4 Hz, $J_{8a,8b}$=12.4 Hz), 5.78 (ddd, 1H, $J_{6,7}$=7.4 Hz, $J_{7,8a}$=7.0 Hz, $J_{7,8b}$=2.4 Hz, H-7), 7.25–7.53 (m, 16H, Ar), 7.98–8.03 (m, 4H, Ar). ^{13}C NMR (150 MHz, $CDCl_3$): δ 24.6, 25.4 (CH_3), 32.2 (C-3), 53.0 (OCH_3), 63.5 (C-8), 69.5 (C-4), 69.55, 69.6, 69.62, 69.7 (2C, OCH_2), 70.3 (C-7), 71.3 (C-5), 72.1 (C-6), 100.0 (C-2), 109.8 (C_{isop}), 128.1, 128.2, 128.3, 128.4 128.42, 128.46, 128.48, 128.5, 129.7, 129.75, 129.8, 130.1, 132.8, 133.1, 135.6 (Ar), 165.3, 166.2 (C=O), 167.2 (C-1). IR: 1748, 1717, 1253, 1213, 954 cm^{-1}. ESI-HRMS for $C_{40}H_{41}O_{12}P$: 767.2233 $[M+Na]^+$. Found 767.2223.

6.4 (2,3,4,6,7-Penta-*O*-acetyl-L-*glycero*-α-D-*manno*-heptopyranosyl)-(1-5)-[methy -l *O*-[methyl (7,8-di-*O*-benzoyl-4,5-*O*-isopropylidene-3-deoxy-α-D-*manno*-2-octu -lopyranosyl)onate]]-(2-4)-(allyl 7,8-di-*O*-benzoyl-3-deoxy-α-D-*manno*-2-octulop-yranosid)onate (11)

A mixture of **6**α (32.0 mg, 32.6 μmol), imidate **8** (55.0 mg, 98.7 μmol), and MS-AW 300 (40.0 mg) was suspended in dichloromethane (1.0 mL). The reaction mixture was stirred for 1 h under argon, and then 0.01 M TMSOTf (196.0 μL, 1.96 μmol) in dichloromethane was added dropwise to the reaction mixture. After stirring for 2 h, the reaction was neutralized by the addition of a few drops of triethylamine and aqueous saturated sodium hydrogen carbonate. The reaction mixture was diluted with dichloromethane and was filtered through Celite. The filtrate was extracted twice with dichloromethane. The combined organic phase was dried over anhydrous sodium sulfate, filtrated, and concentrated. The residue was purified by BioRad S-X3 (toluene/ethyl acetate, 1:1) to give compound **11** (39 mg, 87%) as colorless syrup. Mp 88.2 ˚C, $[\alpha]^{25}_{D}$ = +19.7 (*c* 1.5, $CHCl_3$), ^{1}H-NMR (600 MHz, $CDCl_3$): δ 1.20 (s, 3H, Me), 1.38 (s, 3H, Me), 1.93, 2.02, 2.03, 2.14 (s, 3H x 5, Ac), 2.05 (dd, 1H, $J_{3a,3e}$=12.6 Hz, $J_{3a,4}$=12.0 Hz, H-3a^{I}), 2.12 (dd, 1H, $J_{3a,3e}$=15.4 Hz, $J_{3a,4}$=2.2 Hz, H-3a^{II}), 2.27 (dd, 1H, $J_{3a,3e}$=12.6 Hz, $J_{3e,4}$=4.4 Hz, H-3e^{I}), 2.95 (dd, 1H, $J_{3a,3e}$=15.4 Hz, $J_{3e,4}$=3.6 Hz, H-3e^{II}), 3.43 (s, 3H, OMe^{I}), 3.47 (s, 3H, OMe^{II}), 3.73 (brs, 1H, $J_{4,5}$=2.0 Hz, $J_{5,6}$=1.8 Hz, H-5^{I}), 3.93 (dddd, 1H, *J*=1.6, 1.6, 4.8, 13.0 Hz, OCH_2-), 3.97 (dddd, 1H, *J*=1.4, 1.6, 5.4, 13.0 Hz, OCH_2-), 4.12 (dd, 1H, $J_{5,6}$=1.8 Hz, $J_{6,7}$=10.0 Hz, H-6^{I}), 4.22 (dd, 1H, $J_{4,5}$=10.0 Hz, $J_{5,6}$=8.0 Hz, H-5^{III}), 4.23 (dd, 1H, $J_{6,7a}$=2.4 Hz, $J_{7a,7b}$=12.0 Hz, H-7$_a^{III}$), 4.26 (dd, 1H, $J_{6,7b}$=4.0 Hz, $J_{7a,7b}$=12.0 Hz, H-7$_b^{III}$), 4.28 (dd, 1H, $J_{5,6}$=2.0 Hz, $J_{6,7}$=7.4 Hz, H-6^{II}), 4.37 (dd, 1H, $J_{4,5}$=8.0 Hz, $J_{5,6}$=2.0 Hz, H-5^{II}), 4.52 (ddd, 1H, $J_{3a,4}$=2.2 Hz, $J_{3e,4}$=3.6 Hz, $J_{4,5}$=8.0 Hz, H-4^{II}), 4.60 (dd, 1H, $J_{7,8a}$=5.0 Hz, $J_{8a,8b}$=12.6 Hz, H-8$_a^{II}$), 4.65 (ddd, 1H, $J_{3a,4}$=12.0 Hz, $J_{3e,4}$=4.4 Hz, $J_{4,5}$=2.0 Hz, H-4^{I}), 4.69 (dd, 1H, $J_{7,8a}$=3.6 Hz, $J_{8a,8b}$=12.6 Hz, H-8$_a^{I}$), 4.88 (dd, 1H, $J_{7,8b}$=2.6 Hz, $J_{8a,8b}$=12.6 Hz, H-8$_b^{I}$), 4.93 (dddd, 1H, *J*=1.4, 1.6, 3.0, 10.6 Hz, =CH_2), 5.01 (dddd, 1H, *J*=1.6, 1.6, 3.2, 17.2 Hz, =CH_2), 5.04 (d, 1H, $J_{1,2}$=2.0 Hz, H-1^{III}), 5.24 (dd, 1H, $J_{1,2}$=2.0 Hz, $J_{2,3}$=3.2 Hz, H-2^{III}), 5.28 (dd, 1H, $J_{7,8b}$=2.4 Hz, $J_{8a,8b}$=12.6 Hz, H-8$_b^{II}$), 5.32 (dd, 1H, $J_{3,4}$=10.2 Hz,

$J_{4,5}$=10.0 Hz, H-4^{III}), 5.37 (ddd, 1H, $J_{5,6}$=8.0 Hz, $J_{6,7a}$=2.4 Hz, $J_{6,7b}$=4.0 Hz, H-6^{III}), 5.51 (dd, 1H, $J_{2,3}$=3.2 Hz, $J_{3,4}$=10.2 Hz, H-3^{III}), 5.62-5.68 (m, 1H, -CH=), 5.65 (ddd, 1H, $J_{6,7}$=7.4 Hz, $J_{7,8a}$=5.0 Hz, $J_{7,8b}$=2.4 Hz, H-7^{I}), 5.69 (ddd, 1H, $J_{6,7}$=10.0 Hz, $J_{7,8a}$=3.6 Hz, $J_{7,8b}$=2.6 Hz, H-7^{I}), 7.37–7.45 (m, 8H, Ar^{I}, Ar^{II}), 7.51–7.56 (m, 4H, Ar^{I}, Ar^{II}), 7.93–8.01 (m, 8H, Ar^{I}, Ar^{II}). ^{13}C NMR (150 MHz, $CDCl_3$): δ 20.7, 20.74, 20.8 and 21.1 (Ac-CH_3), 24.7 and 25.1 (Isop-Me), 31.9 (C-3^{II}), 34.4 (C-3^{I}), 52.25 (OMe^{I}), 52.28 (OMe^{II}), 62.4 (C-8^{I}), 62.5 (C-8^{II}), 63.7 (C-7^{III}), 64.7 (OCH_2-), 65.2 (C-4^{III}), 67.4 (C-6^{III}), 67.6 (C-4^{I}), 68.7 (C-3^{III}), 69.2 (C-7^{I}), 69.2 (C-5^{III}), 70.0 (C-4^{II}), 70.3 (C-2^{III}), 70.4 (C-6^{II}), 70.5 (C-6^{I}), 70.7 (C-7^{II}), 72.3 (C-5^{I}), 74.0 (C-5^{II}), 97.0 (C-2^{II}), 98.7 (C-2^{I}), 99.0 (C-1^{III}), 109.7 (C_{isop}), 116.2 (=CH_2), 128.3, 128.4, 128.5, 129.3, 129.6, 129.85, 129.9, 130.2, 132.8, 133.1 (Ar^{I} and Ar^{II}), 133.4 (-CH=), 165.1, 165.2, 165.8, 167.4 (Bz: C=O), 167.4 (C-1^{I}), 169.2 (C-1^{II}), 169.6, 169.7, 169.8, 170.4 and 170.7 (Ac: C=O). IR: 1745, 1725, 1278, 1248, 1218 cm^{-1}. Anal. Calcd for $C_{69}H_{76}O_{30}$: C, 59.82; H, 5.53. Found C, 59.65; H, 5.67. **Ortho ester 12**: Mp 99.0 ˚C, $[\alpha]^{25}_{D}$ = +7.5 (*c* 1.0, $CHCl_3$), ^{1}H-NMR (600 MHz, $CDCl_3$): δ 1.21 (s, 3H, Me), 1.40 (s, 3H, Me), 1.65 (s, 3H, ester-Me), 1.91 (dd, 1H, $J_{3a,3e}$=15.4 Hz, $J_{3a,4}$=2.2 Hz, H-3a^{II}), 1.97, 2.03, 2.04, 2.07 (s, 3H x 4, Ac), 2.31 (dd, 1H, $J_{3a,3e}$=12.6 Hz, $J_{3a,4}$=12.4 Hz, H-3a^{I}), 2.47 (dd, 1H, $J_{3a,3e}$=12.6 Hz, $J_{3e,4}$=4.0 Hz, H-3e^{I}), 2.86 (dd, 1H, $J_{3a,3e}$=15.4 Hz, $J_{3e,4}$=3.6 Hz, H-3e^{II}), 3.38 (dd, 1H, $J_{4,5}$=9.6 Hz, $J_{5,6}$=2.0 Hz, H-5^{III}), 3.43 (s, 3H, OMe^{I}), 3.81 (s, 3H, OMe^{II}), 3.86 (dddd, 1H, *J*=1.6, 1.6, 3.4, 13.4 Hz, OCH_2-), 3.97 (dddd, 1H, *J*=1.6, 1.6, 3.2, 13.4 Hz, OCH_2-), 4.05 (dd, 1H, $J_{5,6}$=nd Hz, $J_{6,7}$=8.6 Hz, H-6^{I}), 4.09 (dd, 1H, $J_{6,7a}$=7.8 Hz, $J_{7a,7b}$=11.6 Hz, H-7a^{III}), 4.11 (brs, 1H, $J_{4,5}$=2.4 Hz, $J_{5,6}$=nd Hz, H-5^{I}), 4.15 (dd, 1H, $J_{6,7b}$=4.8 Hz, $J_{7a,7b}$=11.6 Hz, H-7b^{III}), 4.21 (dd, 1H, $J_{5,6}$=1.8 Hz, $J_{6,7}$=7.0 Hz, H-6^{II}), 4.31 (dd, 1H, $J_{4,5}$=7.6 Hz, $J_{5,6}$=1.8 Hz, H-5^{II}), 4.33 (ddd, 1H, $J_{3a,4}$=12.4 Hz, $J_{3e,4}$=4.0 Hz, $J_{4,5}$=2.4 Hz, H-4^{I}), 4.47 (ddd, 1H, $J_{3a,4}$=2.2 Hz, $J_{3e,4}$=3.6 Hz, $J_{4,5}$=7.6 Hz, H-4^{II}), 4.50 (dd, 1H, $J_{1,2}$=2.2 Hz, $J_{2,3}$=3.8 Hz, H-2^{III}), 4.60 (dd, 1H, $J_{7,8a}$=4.6 Hz, $J_{8a,8b}$=12.4 Hz, H-8a^{II}), 4.64 (dd, 1H, $J_{7,\ 8a}$=3.4 Hz, $J_{8a,\ 8b}$=12.6 Hz, H-8a^{I}), 4.65 (d, 1H, $J_{1,2}$=2.2 Hz, H-1^{III}), 4.94 (dddd, 1H, *J*=1.4, 1.4, 3.0, 10.6 Hz, =CH_2), 5.00 (dd, 1H, $J_{7,\ 8b}$=2.4 Hz, $J_{8a,\ 8b}$= 12.6 Hz, H-8b^{I}), 5.02 (dd, 1H, $J_{2,3}$=3.8 Hz, $J_{3,4}$=10.0 Hz, H-3^{III}), 5.05 (dddd, 1H, *J*=1.6, 1.6, 3.4, 17.2 Hz, =CH_2), 5.11 (dd, 1H, $J_{3,4}$=10.0 Hz, $J_{4,5}$=9.6

Hz, H-4^{III}), 5.14 (ddd, 1H, $J_{5,6}$=2.0 Hz, $J_{6,7a}$=7.8 Hz, $J_{6,7b}$=4.8 Hz, H-6^{III}), 5.24 (dd, 1H, $J_{7,8b}$=2.4 Hz, $J_{8a,8b}$ =12.4 Hz, H-8b^{II}), 5.38 (ddd, 1H, $J_{6,7}$=8.6 Hz, $J_{7,8a}$=3.4 Hz, $J_{7,8b}$=2.4 Hz, H-7^{I}), 5.59–5.64 (m, 1H, -CH=), 5.65 (ddd, 1H, $J_{6,7}$=7.0 Hz, $J_{7,8a}$=4.6 Hz, $J_{7,8b}$=2.4 Hz, H-7^{II}), 7.39-7.47 (m, 8H, Ar^{I}, Ar^{II}), 7.54-7.55 (m, 4H, Ar^{I}, Ar^{II}), 7.95–8.01 (m, 8H, Ar^{I}, Ar^{II}). ^{13}C NMR (150 MHz, $CDCl_3$): δ 20.6, 20.6, 20.7 (Ac-CH_3), 24.7 and 25.3 (Isop-Me), 26.3 (ester-Me), 32.6 (C-3^{II}), 34.3 (C-3^{I}), 52.2 (OMe^{I}), 52.5 (OMe^{II}), 62.2 (C-8^{II}), 62.4 (C-8^{I}), 62.5 (C-7^{III}), 64.2 (OCH_2-), 64.6 (C-4^{III}), 66.8 (C-6^{III}), 67.5 (C-5^{I}), 69.2 (C-4^{I}), 69.8 (C-4^{II}), 70.2 (C-6^{II}), 70.3 (C-3^{III}), 70.4 (C-7^{I}), 70.96 (C-7^{II}), 70.96 (C-6^{I}), 71.1 (C-5^{III}), 72.1 (C-5^{II}), 76.2 (C-2^{II}), 97.2 (C-1^{III}), 98.5 (C-2^{I}), 98.8 (C-2^{II}), 109.7 (C_{isop}), 115.6 (=CH_2), 124.8 (eater-C), 128.40, 128.43, 128.5, 129.5, 129.7, 129.8, 129.9, 130.0, 130.4, 133.0, 133.07, 133.14 (Ar^{I} and Ar^{II}), 133.7 (-CH=), 165.1, 165.3, 165.9, 166.2 (Bz: C=O), 167.5 (C-1^{I}) , 169.4 (C-1^{II}), 170.1, 170.17, 170.24 and 170.6 (Ac: C=O). IR: 1746, 1724, 1279, 1248, 1219 cm^{-1}. ESI-HRMS for $C_{69}H_{76}O_{30}$: 1407.4319 $[M+Na]^+$. Found 1407.4302.

6.5 (2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl)-(1-5)-[methyl *O*-[methyl (7,8-di-*O*-benzoyl-4,5-*O*-isopropylidene-3-deoxy-α-D-*manno*-2-octulopyranosyl)onate]]-(2-4)-(allyl 7,8-di-*O*-benzoyl-3-deoxy-α-D-*manno*-2-octulopyranosid)onate (13)

A reaction mixture of imidate **9** (75.2 mg, 152.6 μmol), **6**α (50.0 mg, 51.0 μmol) and MS-AW 300 (57.8 mg) was suspended in dichloromethane (1.4 mL). The reaction was stirred for 1 h under argon, and then cooled to 0 °C. 0.01 M TMSOTf (200.0 μL, 2.00 μmol) in dichloromethane was added dropwise to the reaction mixture. After stirring for 2 h, the reaction was neutralized by the addition of triethylamine and saturated sodium hydrogen carbonate. The reaction solution was diluted with dichloromethane and then filtered through Celite. The filtrate was extracted twice with dichloromethane. The combined organic layer was dried over Na_2SO_4, filtrated and concentrated. The residue was purified by BioRad S-X3 (toluene/ethyl acetate, 1:1) to give **13** (60.9 mg, 91%) as colorless powder. Mp 85.5 ˚C, $[\alpha]^{25}_D$ = +37.5 (*c* 1.0, $CHCl_3$), ^{1}H-NMR (600 MHz, $CDCl_3$): δ 1.21 (s, 3H, Me), 1.38 (s, 3H, Me), 1.85 (dd, 1H, $J_{3a,3e}$=15.4 Hz, $J_{3a,4}$=2.2 Hz, H-3a^{II}), 2.00, 2.02, 2.05, 2.16 (s, 3H x 4, Ac), 2.23 (dd, 1H, $J_{3a,3e}$=12.6 Hz, $J_{3a,4}$=12.2 Hz, H-3a^{I}), 2.28 (dd, 1H, $J_{3a,3e}$=12.6 Hz, $J_{3e,4}$=4.8

Hz, H-3e^{I}), 2.95 (dd, 1H, $J_{3a,3e}$=15.4 Hz, $J_{3e,4}$=3.6 Hz, H-3e^{II}), 3.42 (s, 3H, OMe^{I}), 3.56 (s, 3H, OMe^{II}), 3.78 (brs, 1H, $J_{4,5}$=2.2 Hz, $J_{5,6}$=1.6 Hz, H-5^{I}), 3.92 (dddd, 1H, J=1.6, 1.6, 5.0, 13.0 Hz, OCH_2-), 3.97 (dddd, 1H, J=1.4, 1.4, 5.4, 13.0 Hz, OCH_2-), 4.09 (dd, 1H, $J_{5,6a}$=2.0 Hz, $J_{6a,6b}$=12.2 Hz, H-6$_a^{III}$), 4.11 (dd, 1H, $J_{5,6}$=1.6 Hz, $J_{6,7}$=9.4 Hz, H-6^{I}), 4.18 (dd, 1H, $J_{5,6}$=1.8 Hz, $J_{6,7}$=7.2 Hz, H-6^{II}), 4.32 (ddd, 1H, $J_{4,5}$=10.0 Hz, $J_{5,6a}$=2.0 Hz, $J_{5,6b}$=3.2 Hz, H-5^{III}), 4.35 (dd, 1H, $J_{4,5}$=7.8 Hz, $J_{5,6}$=1.8 Hz, H-5^{II}), 4.39 (dd, 1H, $J_{5,6b}$=3.2 Hz, $J_{6a,6b}$=12.2 Hz, H-6$_b^{III}$), 4.52 (ddd, 1H, $J_{3a,4}$=2.2 Hz, $J_{3e,4}$=3.6 Hz, $J_{4,5}$=7.8 Hz, H-4^{II}), 4.63 (dd, 1H, $J_{7,8a}$=5.2 Hz, $J_{8a,8b}$ =12.6 Hz, H-8$_a^{II}$), 4.63 (dd, 1H, $J_{7,\ 8a}$=3.8 Hz, $J_{8a,\ 8b}$=12.6 Hz, H-8$_a^{I}$), 4.73 (ddd, 1H, $J_{3a,4}$=11.8 Hz, $J_{3e,4}$=4.8 Hz, $J_{4,5}$=2.2 Hz, H-4^{I}), 4.82 (d, 1H, $J_{1,2}$=2.2 Hz, H-1^{III}), 4.82 (dd, 1H, $J_{7,\ 8b}$=2.4 Hz, $J_{8a,\ 8b}$=12.6 Hz, H-8$_b^{I}$), 4.92 (dddd, 1H, J=1.4, 1.6, 2.8, 10.6 Hz, =CH_2), 5.01 (dddd, 1H, J=1.4, 1.6, 3.2, 17.2 Hz, =CH_2), 5.30 (dd, 1H, $J_{7,8b}$=2.4 Hz, $J_{8a,8b}$ =12.6 Hz, H-8$_b^{II}$), 5.34 (dd, 1H, $J_{3,4}$=3.4 Hz, $J_{4,5}$=10.0 Hz, H-4^{III}), 5.40 (dd, 1H, $J_{2,3}$=11.0 Hz, $J_{3,4}$=3.4 Hz, H-3^{III}), 5.42 (dd, 1H, $J_{1,2}$=3.4 Hz, $J_{2,3}$=11.0 Hz, H-2^{III}), 5.46 (ddd, 1H, $J_{6,7}$=9.4 Hz, $J_{7,8a}$=3.8 Hz, $J_{7,8b}$=2.4 Hz, H-7^{I}), 5.67 (ddd, 1H, $J_{6,7}$=7.2 Hz, $J_{7,8a}$=5.2 Hz, $J_{7,8b}$=2.4 Hz, H-7^{II}), 5.63–570 (m, 1H, -CH=), 7.37–7.44 (m, 8H, Ar^{I}, Ar^{II}), 7.52–7.57 (m, 4H, Ar^{I}, Ar^{II}), 7.94–7.99 (m, 8H, Ar^{I}, Ar^{II}). ^{13}C NMR (150 MHz, $CDCl_3$): δ 20.7, 20.76, 20.78 and 20.9 (Ac-CH_3), 24.6 and 25.1 (Me), 24.6 (C-3^{II}), 25.1 (C-3^{I}), 52.2 (OMe^{I}), 52.4 (OMe^{II}), 61.8 (C-6^{III}), 62.2 (C-8^{II}), 62.3 (C-8^{I}), 64.8 (OCH_2-), 66.0 (C-4^{III}), 67.5 (C-4^{I}), 68.7 (C-5^{III}), 68.9 (C-7^{I}), 69.2 (C-2^{III}), 69.6 (C-4^{II}), 69.8 (C-3^{III}), 70.5 (C-6^{I}), 70.6 (C-6^{II}), 70.8 (C-7^{II}), 72.2 (C-5^{II}), 72.5 (C-5^{I}), 96.7 (C-2^{II}), 98.5 (C-1^{III}), 98.7 (C-2^{I}), 109.8 (C_{isop}), 116.3 (=CH_2), 128.36, 128.41, 128.46, 128.50, 129.0, 129.62, 129.64, 129.7, 129.8, 130.17 and 133.15 (Ar^{I} and Ar^{II}), 133.47 (-CH=), 164.8, 165.3, 165.8, 166.2 (Bz: C=O), 167.3 (C-1^{I}), 168.7 (C-1^{II}), 169.56, 169.61, 169.8 and 170.8 (Ac: C=O). IR (neat): 1746, 1724, 1278, 1249, 1217 cm^{-1}. ESI-HRMS calcd for $C_{66}H_{72}O_{28}$: 1335.4108 $[M+Na]^+$. Found 1335.4097.

6.6 (3,4,6-Tri-*O*-acetyl-2-azido-2-deoxy-α-D-galactopyranosyl)-(1-5)-[methyl *O*-[methyl (7,8-di-*O*-benzoyl-4,5-*O*-isopropylidene-3-deoxy-α-D-*manno*-2-octulopyr-anosyl)onate]]-(2-4)-(allyl 7,8-di-*O*-benzoyl-3-deoxy-α-D-*manno*-2-octulopyrano-sid)onate (14)

A reaction mixture of **6**α (50.0 mg, 51.0 μmol), imidate **10** (72.6 mg, 152.6 μmol) and MS-AW 300 (57.8 mg) in dichloromethane (1.4 mL) was stirred for 1 h under argon and cooled to 0 °C. Then 0.01 M TMSOTf (200 μL, 2.00 μmol) in dichloromethane was added dropwise to the reaction mixture and stirred for 4 h. The solution was neutralized by the addition of triethylamine and saturated $NaHCO_3$ and diluted with dichloromethane. The reaction mixture was filtered through Celite and the filtrate was extracted with dichloromethane. The organic phase was dried over Na_2SO_4, filtered and concentrated. Purification of the residue by BioRad S-X3 (toluene/ethyl acetate, 1:1) obtained compound **14** (38.0 mg, 58%) as colorless powder. Mp 96.0 ˚C, $[\alpha]^{25}_D$ = +51.0 (*c* 1.0, $CHCl_3$), ^{1}H-NMR (600 MHz, $CDCl_3$): δ 1.21 (s, 3H, Me), 1.39 (s, 3H, Me), 1.92 (dd, 1H, $J_{3a,3e}$=15.4 Hz, $J_{3a,4}$=2.2 Hz, H-3a^{II}), 1.96, 2.06, 2.11 (s, 3H x 3, Ac), 2.21 (dd, 1H, $J_{3a,3e}$=12.6 Hz, $J_{3a,4}$=12.4 Hz, H-3a^{I}), 2.27 (dd, 1H, $J_{3a,3e}$=12.6 Hz, $J_{3e,4}$=4.6 Hz, H-3e^{I}), 3.01 (dd, 1H, $J_{3a,3e}$=15.6 Hz, $J_{3e,4}$=3.6 Hz, H-3e^{II}), 3.32 (s, 3H, OMe^{I}), 3.59 (s, 3H, OMe^{II}), 3.74 (dd, 1H, $J_{1,2}$=3.4 Hz, $J_{2,3}$=11.0 Hz, H-2^{III}), 3.83 (brs, 1H, $J_{4,5}$=2.0 Hz, $J_{5,6}$=nd Hz, H-5^{I}), 3.95 (dddd, 1H, *J*=5.4 and 13.0 Hz, OCH_2-), 3.97 (dddd, 1H, *J*=5.0 and 13.0 Hz, OCH_2-), 4.08 (dd, 1H, $J_{5,6a}$=5.5 Hz, $J_{6a,6b}$=10.8 Hz, H-6$_a^{III}$), 4.14 (dd, 1H, $J_{5,6}$=nd Hz, $J_{6,7}$=9.6 Hz, H-6^{I}), 4.15 (dd, 1H, $J_{5,6b}$=9.0 Hz, $J_{6a,6b}$=10.8 Hz, H-6$_b^{III}$), 4.21 (dd, 1H, $J_{5,6}$=1.6 Hz, $J_{6,7}$=7.6 Hz, H-6^{II}), 4.36 (dd, 1H, $J_{4,5}$=7.8 Hz, $J_{5,6}$=1.6 Hz, H-5^{II}), 4.52 (ddd, 1H, $J_{3a,4}$=2.2 Hz, $J_{3e,4}$=3.6 Hz, $J_{4,5}$=7.8 Hz, H-4^{II}), 4.54 (ddd, 1H, $J_{4,5}$=0.8 Hz, $J_{5,6a}$=5.5 Hz, $J_{5,6b}$=9.0 Hz, H-5^{III}), 4.62 (dd, 1H, $J_{7,8a}$=4.2 Hz, $J_{8a,8b}$=12.6 Hz, H-8$_a^{II}$), 4.64 (dd, 1H, $J_{7,8a}$=3.0 Hz, $J_{8a,8b}$=12.0 Hz, H-8$_a^{I}$), 4.79 (ddd, 1H, $J_{3a,4}$=12.4 Hz, $J_{3e,4}$=4.6 Hz, $J_{4,5}$=2.0 Hz, H-4^{I}), 4.91 (dddd, 1H, *J*=10.0 and 1.4 Hz, =CH_2), 4.99 (dddd, 1H, *J*=10.0 and nd Hz, =CH_2), 5.00 (dd, 1H, $J_{7,8b}$=2.6 Hz, $J_{8a,8b}$=12.0 Hz, H-8$_b^{I}$), 5.05 (d, 1H, $J_{1,2}$=3.4 Hz, H-1^{III}), 5.32 (dd, 1H, $J_{2,3}$=11.0 Hz, $J_{3,4}$=3.2 Hz, H-3^{III}), 5.33 (dd, 1H, $J_{7,8b}$=2.5 Hz, $J_{8a,8b}$=12.6 Hz, H-8$_b^{II}$), 5.48 (dd, 1H, $J_{3,4}$=3.2 Hz, $J_{4,5}$=0.8 Hz, H-4^{III}), 5.63-5.67 (m, 1H, -CH=), 5.67 (ddd, 1H, $J_{6,7}$=7.6 Hz, $J_{7,8a}$=4.2 Hz, $J_{7,8b}$=2.5 Hz, H-7^{II}), 5.70(ddd, 1H, $J_{6,7}$=9.6 Hz, $J_{7,8a}$=3.0 Hz, $J_{7,8b}$=2.6 Hz, H-7^{I}), 7.36–7.39 (m, 2H, Ar^{I}, Ar^{II}), 7.42–7.45 (m, 3H, Ar^{I}, Ar^{II}), 7.51–7.58 (m, 4H, Ar^{I}, Ar^{II}), 7.94–8.03 (m, 8H, Ar^{I}, Ar^{II}). ^{13}C NMR (150 MHz, $CDCl_3$): δ 20.6 and 20.7 (Ac-CH_3), 24.6 and 25.1 (Me), 31.9 (C-3^{II}), 34.4 (C-3^{I}), 52.1 (OMe^{II}), 52.2 (OMe^{I}), 58.2 (C-2^{III}), 60.4 (C-6^{III}), 62.0 (C-8^{II}), 62.2 (C-8^{I}), 64.8 (OCH_2-), 66.6 (C-5^{III}), 67.1 (C-4^{III}), 67.7 (C-4^{I}), 68.8 (C-3^{III}), 69.4 (C-7^{I}), 69.9 (C-4^{II}), 70.4 (C-6^{I}, C-6^{II}), 70.7 (C-7^{II}), 72.0 (C-5^{I}), 72.2 (C-5^{II}), 96.6 (C-2^{II}), 97.9 (C-1^{III}), 98.2 (C-2^{I}), 109.8 (C_{isop}), 116.4 (=CH_2), 128.4, 128.47, 128.52, 129.5, 129.65, 129.72,

129.8, 130.2, 132.9, 133.1 and 133.5 (Ar^{I} and Ar^{II}), 133.15 (-CH=), 165.2, 165.8, 166.2 (Bz: C=O), 167.1 (C-1^{I}), 169.0 (C-1^{II}), 169.7, 170.1 and 170.2 (Ac: C=O). IR (neat): 2112, 1748, 1723, 1278, 1251, 1218 cm^{-1}. ESI-HRMS for $C_{64}H_{69}N_3O_{26}$: 1318.4067 [M+Na]. Found 1318.4072.

6.7 (3,4,6-Tri-*O*-acetyl-2-acetamido-2-deoxy-α-D-galactopyranosyl)-(1-5)-[methy -l *O*-[methyl (7,8-di-*O*-benzoyl-4,5-*O*-isopropylidene-3-deoxy-α-D-*manno*-2-octu -lopyranosyl)onate]]-(2-4)-(allyl 7,8-di-*O*-benzoyl-3-deoxy-α-D-*manno*-2-octulop-yranosid)onate (16)

Triphenylphosphine (6.2 mg, 23.7 μmol) was added to a solution of **14** (23.7 mg, 18.3 μmol) dissolved in a mixed solvent (THF/H_2O, 19:1, 0.36 mL). After stirring for 16 h, the reaction mixture was diluted with toluene and concentrated by evaporation to give a residue **15**. Without purification, the residue was directly acetylated with pyridine/Ac_2O (1/0.04, v/v, 190.0 μL) in the presence of a catalytic amount of DMAP over 18 h. After removing the solvent, the residue was purified by TLC (CH_2Cl_2/EtOAc/hexane, 3:3:1) to give compound **16** (15.4 mg, 64%) as colorless powder. Mp 99.0 ˚C, $[\alpha]^{25}_D$ = +62.1 (*c* 1.2, $CHCl_3$), ^{1}H-NMR (600 MHz, $CDCl_3$): δ 1.20 (s, 3H, Me), 1.37 (s, 3H, Me), 1.88 (dd, 1H, $J_{3a,3e}$=15.6 Hz, $J_{3a,4}$=2.0 Hz, H-3a^{II}), 1.97, 2.00, 2.14 (s, 3H x 3, Ac), 2.02 (s, 3H, NHAc), 2.18 (dd, 1H, $J_{3a,4}$=11.8 Hz, $J_{3a,3e}$=13.0 Hz, H-3a^{I}), 2.23 (dd, 1H, $J_{3e,4}$=5.6 Hz, $J_{3a,3e}$=13.0 Hz, H-3e^{I}), 3.02 (dd, 1H, $J_{3a,3e}$=15.6 Hz, $J_{3e,4}$=3.4 Hz, H-3e^{II}), 3.35 (s, 3H, OMe^{I}), 3.43 (s, 3H, OMe^{II}), 3.74 (brs, 1H, $J_{4,5}$=2.0 Hz, $J_{5,6}$=1.6 Hz, H-5^{I}), 3.83 (dddd, 1H, *J*=1.4, 1.6, 4.8 and 11.0 Hz, OCH_2-), 3.90 (dddd, 1H, *J*=1.6, 1.6, 6.0 and 12.4 Hz, OCH_2-), 4.06 (dd, 1H, $J_{5,6a}$=5.0 Hz, $J_{6a,6b}$=10.8 Hz, H-6a^{III}), 4.11 (dd, 1H, $J_{5,6}$=1.6 Hz, $J_{6,7}$=9.8 Hz, H-6^{I}), 4.14 (dd, 1H, $J_{5,6b}$=9.4 Hz, $J_{6a,6b}$=10.8 Hz, H-6b^{III}), 4.19 (dd, 1H, $J_{5,6}$=1.6 Hz, $J_{6,7}$=8.0 Hz, H-6^{II}), 4.36 (dd, 1H, $J_{4,5}$=7.8 Hz, $J_{5,6}$=1.6 Hz, H-5^{II}), 4.53 (ddd, 1H, $J_{3a,4}$=2.0 Hz, $J_{3e,4}$=3.4 Hz, $J_{4,5}$=7.8 Hz, H-4^{II}), 4.53 (ddd, 1H, $J_{4,5}$=2.2 Hz, $J_{5,6a}$=5.0 Hz, $J_{5,6b}$=9.4 Hz, H-5^{III}), 4.53 (dd, 1H, $J_{7,8a}$=4.0 Hz, $J_{8a,8b}$ =12.4 Hz, H-8a^{I}), 4.60 (dd, 1H, $J_{7,\ 8a}$=4.0 Hz, $J_{8a,\ 8b}$=12.6 Hz, H-8a^{II}), 4.71 (dd, 1H, $J_{1,2}$=3.4 Hz, $J_{2,3}$=11.4 Hz, $J_{NH,\ 2}$=10.0 Hz, H-2^{III}), 4.75 (ddd, 1H, $J_{3a,4}$=11.4 Hz, $J_{3e,4}$=5.6 Hz, $J_{4,5}$=2.0 Hz, H-4^{I}), 4.85 (dd, 1H, $J_{7,8b}$=2.6 Hz, $J_{8a,8b}$=12.4 Hz, H-8b^{I}), 4.94 (dddd, 1H, *J*=1.2, 17.6 Hz, =CH_2), 4.96 (dddd, 1H, *J*=1.8, 10.6 Hz, =CH_2), 5.03 (d, 1H, $J_{1,2}$=3.4 Hz, H-1^{III}), 5.34 (dd, 1H, $J_{2,3}$=11.4 Hz, $J_{3,4}$=3.2 Hz, H-3^{III}), 5.34 (dd, 1H, $J_{7,\ 8b}$=2.4 Hz, $J_{8a,\ 8b}$=12.6 Hz, H-8b^{II}), 5.40 (dd, 1H, $J_{3,4}$=3.2 Hz, $J_{4,5}$=2.2 Hz, H-4^{III}), 5.53 (ddd, 1H, $J_{6,7}$=9.8 Hz, $J_{7,8a}$=4.0 Hz, $J_{7,8b}$=2.6 Hz,

H-7^{I}), 5.66 (ddd, 1H, $J_{6,7}$=8.0 Hz, $J_{7,8a}$=4.0 Hz, $J_{7,8b}$=2.4 Hz, H-7^{II}), 5.70 (m, 1H, -CH=), 6.43 (d, 1H, $J_{NH,2}$=10.0Hz. NHAc), 7.37–7.38 (m, 2H, Ar^{I}, Ar^{II}), 7.40–7.46 (m, 6H, Ar^{I}, Ar^{II}), 7.51–7.59 (m, 4H, Ar^{I}, Ar^{II}), 7.93–8.07 (m, 8H, Ar^{I}, Ar^{II}). ^{13}C NMR (150 MHz, $CDCl_3$): δ 20.6, 20.75, 20.84 (Ac-CH_3), 23.0 (NHAc-CH_3), 25.0 and 25.1 (Me), 31.7 (C-3^{II}), 34.9 (C-3^{I}), 47.6 (C-2^{III}), 52.1 (OMe^{II}), 52.4 (OMe^{I}), 60.4 (C-6^{III}), 61.9 (C-8^{II}), 62.4 (C-8^{I}), 65.43 (OCH_2-), 66.45 (C-5^{III}), 66.8 (C-4^{III}), 67.4 (C-4^{I}), 68.5 (C-3^{III}), 68.6 (C-7^{I}), 69.9 (C-4^{II}), 70.2 (C-6^{II}), 70.5 (C-7^{II}), 70.6 (C-5^{I}), 70.7 (C-6^{I}), 72.1 (C-5^{II}), 96.2 (C-2^{II}), 97.9 (C-1^{III}), 99.2 (C-2^{I}), 109.9 (C_{isop}), 117.0 (=CH_2), 128.39, 128.42, 128.5, 128.6, 128.8, 129.3, 129.6, 129.70, 129.73, 129.8, 129.9 and 130.1 (Ar^{I} and Ar^{II}), 133.0 (-CH=), 164.9, 165.1, 165.7, 166.2 (Bz: C=O), 168.3 (C-1^{I}), 168.6 (C-1^{II}), 170.1, 107.3, 170.5 (Ac: C=O), 170.9 (NHAc-C=O). IR(neat): 1746, 1723, 1278, 1249, 1217 cm^{-1}. ESI-HRMS for $C_{66}H_{73}NO_{27}$: 1334.4268 [M+Na]. Found 1334.4253.

6.8 (L-*Glycero*-α-D-*manno*-heptopyranosyl)-(1-5)-[*O*-(sodium 3-deoxy-α-D-*mann*-*o*-2-octulopyranosylonate)]-(2-4)-sodium (allyl 3-deoxy-α-D-*manno*-2-octulopyr-anosid)onate (17)

Aqueous 80% trifluoroacetic acid (TFA, 220 μL) was added to a solution of **11** (21.0 mg, 15.2 μmol) in dichloromethane (2.2 mL) at room temperature. After stirring for 1 h, the solvent was removed by evaporation under an argon stream to give a crude compound that was not subjected to further purification. The crude compound was dissolved in methanol (3.0 mL), and then 0.1 M sodium hydroxide (2.4 mL, 0.24 mmol) was added at room temperature. After stirring for 24 h, the mixture was concentrated by evaporation. The residue was purified by gel filtration chromatography (Biogel P-2) to give compound **17** as colorless powder in 87% yield. $[\alpha]^{25}_{D}$ = +30.2 (*c* 0.5, H_2O), ^{1}H-NMR (600 MHz, D_2O): δ 1.62 (dd, 1H, $J_{3a,3e}$=13.0 Hz, $J_{3a,4}$=12.6 Hz, H-3a^{II}), 1.82 (dd, 1H, $J_{3a,3e}$=12.4 Hz, $J_{3a,4}$=12.8 Hz, H-3a^{I}), 1.91 (dd, 1H, $J_{3a,3e}$=12.8 Hz, $J_{3e,4}$=4.2 Hz, H-3e^{I}), 2.06 (dd, 1H, $J_{3a,3e}$=13.0 Hz, $J_{3e,4}$=4.8 Hz, H-3e^{II}), 3.44 (dd, 1H, $J_{5,6}$=2.4 Hz, $J_{6,7}$=8.8 Hz, H-6^{I}), 3.46 (dd, 1H, $J_{7,8a}$=6.2 Hz, $J_{8a,8b}$=11.6 Hz, H-8a^{II}), 3.54 (dd, 1H, $J_{5,6}$=0.6 Hz, $J_{6,7}$=8.0 Hz, H-6^{II}), 3.59-3.83 (m, 12H, H-8a^{I}, H-8b^{I}, H-7^{I}, H-8b^{II}, H-7^{II}, H-7a^{III}, H-7b^{III}, H-5^{III}, H-4^{III}, H-3^{III}, OCH_2), 3.87-3.90 (m, 3H, H-6^{III}, H-5^{II}, H-2^{III}), 3.96 (ddd, 1H, *J*=3.0, 5.0, 12.2 Hz, H-4^{II}), 4.07

(brs, 1H, H-5^{I}), 4.11 (ddd, 1H, *J*=2.0, 4.2, 12.4 Hz, H-4^{I}), 5.06 (ddd, 1H, *J*=10.4 Hz, =CH_2), 5.17 (d, 1H, *J*=1.8 Hz, H-1^{III}), 5.19 (dddd, 1H, *J*=1.4, 3.2, 17.2 Hz, =CH_2), 5.78-5.85 (m, 1H, -CH=). ^{13}C NMR (150 MHz, D_2O): δ 34.3 (C-3^{I}), 34.5 (C-3^{II}), 62.9 (C-8^{I}), 63.2 (C-8^{II}), 63.9 (C-7^{III}), 64.0 (OCH_2), 66.1 (C-4^{II}), 66.2 (C-5^{II}), 66.3 (C-4^{III}), 69.0 (C-7^{I}), 69.2 (C-6^{III}), 69.5 (C-4^{I}), 70.0 (C-2^{III}), 70.2 (C-7^{II}), 70.4 (C-3^{III}), 71.9 (C-6^{I}), 72.0 (C-6^{II}), 72.6 (C-5^{III}), 72.8 (C-5^{I}), 100.0 (C-2^{I}, C-2^{II}), 101.1 (C-1^{III}), 117.1 (=CH_2), 134.0 (-CH=), 174.87 (C-1^{I}), 174.93 (C-1^{II}). IR (neat): 3346, 3333, 1678, 1623 cm^{-1}. ESI-HRMS for $C_{26}H_{41}O_{21}$: 689.2149 [M-2Na+H]. Found 689.2140.

6.9 (α-D-Mannopyranosyl)-(1-5)-[*O*-(sodium 3-deoxy-α-D-*manno*-2-octulopyran -osylonate]]-(2-4)-sodium (allyl 3-deoxy-α-D-*manno*-2-octulopyranosid)onate (18)

The procedure was similar to that described for compound **17**. The reaction was performed with 51.3 mg of **13**, 80% trifluoro acetic acid (1.7 mL), and 0.1 M sodium hydroxide (5.0 mL) to afford 26.1 mg (93%) of compound **18**. $[\alpha]^{25}_{D}$ = +80.4 (*c* 0.8, H_2O), ^{1}H-NMR (600 MHz, D_2O): δ 1.70 (dd, 1H, $J_{3a,3e}$=12.8 Hz, $J_{3a,4}$=12.0 Hz, H-3a^{II}), 1.90 (dd, 1H, $J_{3a,3e}$=12.2 Hz, $J_{3a,4}$=12.2 Hz, H-3a^{I}), 2.01 (dd, 1H, $J_{3a,3e}$=12.2 Hz, $J_{3e,4}$=4.4 Hz, H-3e^{I}), 2.05 (dd, 1H, $J_{3a,3e}$=12.8 Hz, $J_{3e,4}$=4.6 Hz, H-3e^{II}), 3.49 (dd, 1H, H-6^{I}), 3.51 (dd, 1H, H-8a^{II}), 3.62–3.72 (m, 5H, H-8a^{I}, H-6^{II}, H-7^{I}, OCH_2, H-6a^{III}), 3.77–3.95 (m, 10H, H-4^{III}, H-5^{II}, OCH_2, H-8b^{II}, H-8b^{I}, H-3^{III}, H-7^{II}, H-6b^{III}, H-4^{II}, H-5^{III},) 4.01 (ddd, 1H, H-4^{I}), 4.02 (d, 1H, H-2^{III}), 4.15 (brs, 1H, H-5^{I}), 5.10 (dddd, 1H, =CH_2), 5.13 (d, 1H, $J_{1,2}$=1.6 Hz, H-1^{III}), 5.22 (dddd, 1H, -CH=), 5.82–5.88 (m, 1H, -CH=). ^{13}C NMR (150 MHz, D_2O): δ 34.4 (C-3^{I}), 34.6 (C-3^{II}), 60.5 (C-4^{III}), 62.7 (C-8^{I}), 63.3 (C-8^{II}), 64.0 (OCH_2), 65.9 (C-6^{III}), 66.1 (C-4^{II}), 66.7 (C-5^{II}), 69.2 (C-7^{I}), 70.1 (C-2^{III}), 70.3 (C-3^{III}), 70.5 (C-7^{II}), 70.7 (C-4^{I}), 71.7 (C-6^{II}), 72.2 (C-6^{I}), 72.7 (C-5^{III}), 73.2 (C-5^{I}), 100.00 (C-2^{I}), 100.6 (C-1^{III}), 101.5 (C-2^{II}), 117.0 (=CH_2), 134.0 (-CH=), 175.0 (C-1^{I}), 175.1 (C-1^{II}). IR (neat): 3381, 3274, 1604, 1568 cm^{-1}. ESI-HRMS for $C_{25}H_{39}O_{20}$: 659.2035 [M-2Na+H]. Found 659.2061.

6.10 (2-Acetamido-2-deoxy-α-D-galactopyranosyl)-(1-5)-[*O*-(sodium 3-deoxy-α-D-*manno*-2-octulopyranosylonate]]-(2-4)-sodium (allyl 3-deoxy-α-D-*manno*-2-oct -ulopyranosid)onate (19)

The procedure was similar to that described for compound **17**. The reaction was performed with 27.4 mg of **16**, 80% trifluoro acetic acid (0.9 mL), and 0.1 M sodium hydroxide (2.5 mL) to afford compound **19** (15.6 mg) in quantitative yield. $[\alpha]^{25}_{D}$ = +116.5 (*c* 0.3, H_2O), ^{1}H-NMR (600 MHz, D_2O): δ 1.73 (dd, 1H, $J_{3a,4}$=12.6 Hz, $J_{3a,3b}$=13.0 Hz, H-3a^{II}), 1.94–2.04 (m, 2H, H-3a^{I}, H-3b^{I}), 2.02 (s, 3H, Ac), 2.10 (d, 1H, $J_{3b,4}$=4.4 Hz, $J_{3a,3b}$=13.0 Hz, H-3b^{II}), 3.52 (H-6^{I}), 3.54 (dd, 1H, H-8a^{II}), 3.65–3.77 (m, 6H, H-6^{II}, H-8a^{I}, H-7^{I}, OCH_2, H-6a^{III}, H-6b^{III}), 3.81–3.86 (m, 3H, H-8b^{II}, H-8b^{I}, OCH_2), 3.88–3.91 (m, 1H, H-7^{II}), 3.94–3.99 (m, 4H, H-5^{II}, H-3^{III}, H-4^{II}, H-4^{III}), 4.26 (ddd, 1H, *J*=2.0, 5.4, 11.2 Hz, H-4^{I}), 4.16 (dd, 1H, $J_{1,2}$=3.4 Hz, H-2^{III}), 4.17 (brs, 1H, H-5^{I}), 4.26 (d, 1H, *J*=6.4 Hz, H-5^{III}), 5.14 (dddd, 1H, =CH_2), 5.19 (d, 1H, $J_{1,2}$=3.4 Hz, H-1^{III}), 5.26 (dddd, 1H, =CH_2), 5.86-5.92 (m, 1H, -CH=). ^{13}C NMR (150 MHz, D_2O): δ 22.0 (CH_3), 34.5 (C-3^{I}), 34.6 (C-3^{II}), 50.3 (C-2^{III}), 60.4 (C-6^{III}), 62.8 (C-8^{I}), 63.3 (C-8^{II}), 64.1 (OCH_2), 66.0 (C-4^{II}), 66.6 (C-5^{II}), 67.6 (C-3^{III}), 68.4 (C-4^{III}), 69.2 (C-7^{II}), 70.3 (C-7^{I}), 70.5 (C-5^{III}), 70.9 (C-4^{I}), 71.9 (C-6^{II}), 72.60 (C-6^{I}), 72.63 (C-5^{I}), 97.7 (C-1^{III}), 100.4 (C-2^{II}), 101.2 (C-2^{I}), 117.0 (=CH_2), 134.1 (-CH=), 174.7, 175.0, 175.2 (3C, C-1^{I}, C-1^{II}, C=O). IR (neat): 3295, 1680, 1614 cm^{-1}. ESI-HRMS for $C_{27}H_{42}NO_{20}$: 700.2300 [M-2Na+H]. Found 700.2294.

6.11 Methyl 3,4,6,7-tetra-*O*-acetyl-2-*O*-benzyl-L-*glycero*-α-D-*manno*-heptopyran-oside (23)

A catalytic amount of *N*,*N*-dimethyl-4-aminopyridine (DMAP) was added to a solution of methyl 6,7-di-*O*-acetyl-2-*O*-benzyl-L-*glycero*-D-*manno*-heptopyranoside (**20**; 1.9 g, 4.8 mmol) in acetic anhydride (4.5 mL)/pyridine (9.7 mL) at 0 °C. After stirring for 2 h at room temperature, the mixture was concentrated and purified by silica gel chromatography (ethyl acetate/hexane, 4:5) to give **23** (2.2 g, 94%). $[\alpha]^{25}_{D}$= -19.2 (*c* 3.1, $CHCl_3$), ^{1}H-NMR (600 MHz, $CDCl_3$): δ 1.96, 2.01, 2.05, 2.13 (s, 3H x 4, Ac), 3.35 (s, 3H, OCH_3), 3.82 (dd, 1H, $J_{1,2}$=1.4 Hz, $J_{2,3}$=3.2 Hz, H-2), 3.99 (dd, 1H, $J_{4,5}$=10.2 Hz, $J_{5,6}$=2.0 Hz, H-5), 4.25 (dd, 1H, $J_{6,7a}$=7.6 Hz, $J_{7a,7b}$=11.2 Hz, H-7a), 3.34 (dd, 1H, $J_{6,7b}$=5.8 Hz, $J_{7a,7b}$=11.2 Hz, H-7b), 4.63 (d, 1H, *J*=12.4 Hz, OCH_2Ph), 4.69 (d, 1H, *J*=12.4 Hz, OCH_2Ph), 4.78 (d, 1H, $J_{1,2}$=1.4 Hz, H-1), 5.19 (dd, 1H, $J_{2,3}$=3.2 Hz, $J_{3,4}$=10.2 Hz, H-3), 5.27 (ddd, 1H, $J_{5,6}$=2.0 Hz, $J_{6,7a}$=7.6 Hz, $J_{6,7b}$=5.8

Hz, H-6), 5.44 (dd, 1H, $J_{3,4}$=10.2 Hz, $J_{4,5}$=10.2 Hz, H-4), 7.29–7.37 (m, 5H, Ph). ^{13}C NMR (150 MHz, $CDCl_3$): δ 20.6, 20.7, 20.8, 20.84 (Ac-CH_3), 55.3 (OCH_3), 62.0 (C-7), 65.2 (C-4), 67.1 (C-6), 68.5 (C-5), 71.5 (C-3), 73.2 (OCH_2Ph), 74.9 (C-2), 99.4 (C-1), 128.0, 128.4, 129.9, 137.6 (Ph), 169.6, 170.2, 170.5, 170.51 (Ac: C=O). ESI-HRMS for $C_{23}H_{30}O_{11}$: 505.1686 $[M+Na]^+$. Found 505.1644.

6.12 3,4,6,7-Tetra-*O*-acetyl-2-*O*-benzyl-L-*glycero*-D-*manno*-heptopyranose (24)

A mixture of H_2SO_4/AcOH/Ac_2O (9.0 mL, 2:50:25) was added to a solution of methyl 3,4,6,7-tetra-*O*-acetyl-2-*O*-benzyl-L-*glycero*-α-D-*manno*-heptopyranoside (**23**; 2.2 g, 4.5 mmol) in acetic acid and acetic anhydride (1:2, 10.0 mL). After stirring for 2 h at room temperature, the reaction mixture was neutralized by the addition of sodium acetate (3.5 g), poured into saturated sodium hydrogen carbonate, and extracted with dichloromethane. The combined organic layer was washed with brine, dried over anhydrous sodium sulfate, filtered, and concentrated. The residue was purified by silica gel chromatography (ethyl acetate/hexane, 4:5) to give a syrup (2.2 g, 95%). The syrup was treated with hydrazine acetate (0.5 g, 5.6 mmol) in DMF (30.0 mL) for 2 h at room temperature. The reaction mixture was diluted with ethyl acetate, washed with water and brine, dried over anhydrous sodium sulfate, filtered, and concentrated. The residue was purified by silica gel chromatography (ethyl acetate/hexane, 1:1) to give **24** (1.6 g, 80%). ^{1}H-NMR (600 MHz, $CDCl_3$): δ 1.98, 2.02, 2.06, 2.14 (s, 3H x 4, Ac), 3.86 (dd, 1H, $J_{1,2}$=1.8 Hz, $J_{2,3}$=3.2 Hz, H-2), 4.14 (dd, 1H, $J_{6,7a}$=7.0 Hz, $J_{7a,7b}$=11.4 Hz, H-7a), 4.18 (dd, 1H, $J_{4,5}$=10.0 Hz, $J_{5,6}$=2.0 Hz, H-5), 4.40 (dd, 1H, $J_{6,7b}$=5.4 Hz, $J_{7a,7b}$=11.4 Hz, H-7b), 4.64 (d, 1H, J=12.4 Hz, OCH_2Ph), 4.67 (d, 1H, J=12.4 Hz, OCH_2Ph), 5.23 (ddd, 1H, $J_{5,6}$=2.0 Hz, $J_{6,7a}$=7.0 Hz, $J_{6,7b}$=5.4 Hz, H-6), 5.27 (dd, 1H, $J_{2,3}$=3.2 Hz, $J_{3,4}$=10.2 Hz, H-3), 5.29 (d, 1H, $J_{1,2}$=1.8 Hz, H-1), 5.44 (dd, 1H, $J_{3,4}$=10.2 Hz, $J_{4,5}$=10.0 Hz, H-4), 7.30–7.37 (m, 5H, Ph). ^{13}C NMR (150 MHz, $CDCl_3$): δ 20.7, 20.8, 20.85 (Ac-CH_3), 62.8 (C-7), 65.5 (C-4), 67.2 (C-6), 68.7 (C-5), 71.4 (C-3), 73.2 (OCH_2Ph), 75.6 (C-2), 93.0 (C-1), 127.9, 128.0, 128.4, 137.7 (Ph), 169.8, 170.3, 170.6, 171.3 (Ac: C=O). ESI-HRMS for $C_{22}H_{28}O_{11}$: 491.1529 $[M+Na]^+$. Found 491.1514.

6.13 3,4,6,7-Tetra-*O*-acetyl-2-*O*-benzyl-L-*glycero*-α-D-*manno*-heptopyranosyl tri

-chloroacetimidate (25)

Compound **24** (1.5 g, 3.2 mmol) was dissolved in dry dichloromethane (3.0 mL). Potassium carbonate (2.2 g, 16.0 mmol) was added followed by trichloroacetonitrile (3.2 mL, 32.0 mmol) and the mixture was stirring for 22 h at room temperature. The reaction mixture was filtered through Celite. The solution was concentrated and purified by silica gel column chromatography (ethyl acetate/hexane, 2:3+1% Et_3N) to give **25** (1.9 g, 96%). $[\alpha]^{25}_D$= -13.4 (*c* 3.7, $CHCl_3$), ^{1}H-NMR (600 MHz, $CDCl_3$): δ 1.96, 2.01, 2.02, 2.13 (s, 3H x 4, Ac), 4.06 (dd, 1 H, $J_{1,2}$=1.8 Hz, $J_{2,3}$=3.4 Hz, H-2), 4.18 (dd, 1H, $J_{6,7a}$=7.6 Hz, $J_{7a,7b}$=11.4 Hz, H-7a), 4.20 (dd, 1H, $J_{4,5}$=9.2 Hz, $J_{5,6}$=2.0 Hz, H-5), 4.28 (dd, 1H, $J_{6,7b}$=5.6 Hz, $J_{7a,7b}$=11.4 Hz, H-7b), 4.65 (d, 1H, *J*=12.2 Hz, OCH_2Ph), 4.78 (d, 1H, *J*=12.2 Hz, OCH_2Ph), 5.23 (dd, 1H, $J_{2,3}$=3.4 Hz, $J_{3,4}$=10.2 Hz, H-3), 5.27 (ddd, 1H, $J_{5,6}$=2.0 Hz, $J_{6,7a}$=7.6 Hz, $J_{6,7b}$=5.6 Hz, H-6), 5.54 (dd, 1H, $J_{3,4}$=10.2 Hz, $J_{4,5}$=9.2 Hz, H-4), 6.35 (d, 1H, $J_{1,2}$=1.8 Hz, H-1), 7.27–7.40 (m, 5H, Ph), 8.68 (s, 1H, $OC(NH)CCl_3$). ^{13}C NMR (150 MHz, $CDCl_3$): δ 20.7, 20.8, 20.86 (Ac-CH_3), 62.0 (C-7), 64.6 (C-4), 66.8 (C-6), 71.0 (C-5), 71.3 (C-3), 73.1 (OCH_2Ph), 73.3 (C-2), 90.6 ($OCNHCCl_3$), 95.3 (C-1), 128.1, 128.4, 137.2 (Ph), 160.0 ($OCNHCCl_3$), 169.5, 170.4, 170.5 (Ac: C=O). ESI-HRMS for $C_{24}H_{28}Cl_3NO_{11}$: 634.0626 $[M+Na]^+$. Found 634.0639.

6.14 Methyl (2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1-4)-(2,3,6-tri-*O*-acet -yl-β-D-glucopyranosyl)-(1-4)-6,7-di-*O*-acetyl-2-*O*-benzyl-3-*O*-*tert*-butyldimethyl-silyl-L-*glycero*-α-D-*manno*-heptopyranoside (27)

A mixture of **21** (1.5 g, 2.9 mmol), (2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1-4)-2,3,6-tri-*O*-acetyl-β-D-glucopyran osyl trichloroacetimidate (**26**; 5.6 g, 7.2 mmol), and MS-AW 300 molecular sieves (7.0 g) in dry dichloromethane (50.0 mL) was stirred for 1 h under argon, then cooled to 0 °C. TMSOTf (106.0 μL, 0.6 mmol) in dry dichloromethane (0.5 mL) was added dropwise to the reaction mixture and the mixture was stirred for 3 h. The solution was neutralized by the addition of triethylamine and saturated sodium hydrogen carbonate, and diluted with dichloromethane. The mixture was filtered through Celite and the filtrate was extracted with dichloromethane. The organic phase was dried over

anhydrous sodium sulfate, filtered, and concentrated. Purification of the residue by BioRad S-X1 size exclusion beads (toluene/ethyl acetate, 1:1) and flash column chromatography (toluene/acetone, 5:1) afforded **27** (2.6 g, 79%). $[\alpha]^{25}_{D}$= +1.0 (*c* 1.0, $CHCl_3$), ^{1}H-NMR (600 MHz, $CDCl_3$): δ 0.08, 0.09 [s, 3H x 2, $Si(CH_3)_2C(CH_3)_3$], 0.90 [s, 9H, $Si(CH_3)_2C(CH_3)_3$], 1.96, 1.98, 2.04, 2.05, 2.05, 2.06, 2.09, 2.13, 2.15 (s, 3H x 8, Ac), 3.33 (s, 3H, OCH_3), 3.55 (ddd, 1H, $J_{4,5}$=10.0 Hz, $J_{5,6a}$=5.2 Hz, $J_{5,6b}$=2.0 Hz, H-5^{II}), 3.56 (brs, 1H, $J_{1,2}$=3.4 Hz, $J_{2,3}$=nd Hz, H-2^{I}), 3.64 (dd, 1H, $J_{4,5}$=8.8 Hz, $J_{5,6}$=nd Hz, H-5^{I}), 3.76 (dd, 1H, $J_{3,4}$=9.2 Hz, $J_{4,5}$=10.0 Hz, H-4^{II}), 3.80 (m, 1H, $J_{3,4}$=nd Hz, $J_{4,5}$=8.8 Hz, H-4^{I}), 3.86 (ddd, 1H, $J_{4,5}$=0.8 Hz, $J_{5,6a}$=7.4 Hz, $J_{5,6b}$=6.2 Hz, H-5^{III}), 4.06 (m, 1H, $J_{2,3}$=nd Hz, $J_{3,4}$=nd Hz, H-3^{I}), 4.07 (dd, 1H, $J_{5,6a}$=7.4 Hz, $J_{6a,6b}$=11.2 Hz, H-6a^{III}), 4.09 (dd, 1H, $J_{5,6a}$=5.2 Hz, $J_{6a,6b}$=10.2 Hz, H-6a^{II}), 4.15 (dd, 1H, $J_{5,6b}$=6.2 Hz, $J_{6a,6b}$=11.2 Hz, H-6b^{III}), 4.21 (dd, 1H, $J_{6,7a}$=7.4 Hz, $J_{7a,7b}$=11.2 Hz, H-7a^{I}), 4.33 (dd, 1H, $J_{6,7b}$=5.6 Hz, $J_{7a,7b}$=11.2 Hz, H-7b^{I}), 4.38 (dd, 1H, $J_{5,6b}$=2.0 Hz, $J_{6a,6b}$=10.2 Hz, H-6b^{II}), 4.48 (d, 1H, $J_{1,2}$=8.0 Hz, H-1^{III}), 4.55 (d, 1H, $J_{1,2}$=8.0 Hz, H-1^{II}), 4.63 (d, 1H, *J*=11.8 Hz, OCH_2Ph), 4.72 (d, 1H, $J_{1,2}$=3.4 Hz, H-1^{I}), 4.77 (d, 1H, *J*=11.8 Hz, OCH_2Ph), 4.87 (dd, 1H, $J_{1,2}$=8.0 Hz, $J_{2,3}$=9.4 Hz, H-2^{II}), 4.93 (dd, 1H, $J_{2,3}$=10.4 Hz, $J_{3,4}$=3.4 Hz, H-3^{III}), 5.10 (dd, 1H, $J_{1,2}$=8.0 Hz, $J_{2,3}$=10.4 Hz, H-2^{III}), 5.18 (dd, 1H, $J_{2,3}$=9.4 Hz, $J_{3,4}$=9.2 Hz, H-3^{II}), 5.34 (dd, 1H, $J_{3,4}$=3.4 Hz, $J_{4,5}$=0.8 Hz, H-4^{III}), 5.35 (m, 1H, $J_{5,6}$=nd Hz, $J_{6,7a}$=7.4 Hz, $J_{6,7b}$=5.6 Hz, H-6^{I}), 7.24–7.36 (m, 5H, Ph). ^{13}C NMR (150 MHz, $CDCl_3$): δ -4.8, -4.6 ($Si(CH_3)_2C(CH_3)_3$), 18.1 ($Si(CH_3)_2C(CH_3)_3$), 20.5, 20.6, 20.67, 20.7, 20.85, 20.9, 25.9, 29.7 (Ac-CH_3), 25.8 ($Si(CH_3)_2C(CH_3)_3$), 55.2 (OCH_3), 60.6 (C-6^{III}), 62.3 (C-6^{II}), 62.5 (C-7^{I}), 66.5 (C-4^{III}), 68.7 (C-6^{I}), 69.1 (C-2^{III}), 70.2 (C-5^{I}), 70.6 (C-5^{III}), 70.9 (C-3^{III}), 71.0 (C-3^{I}), 71.9 (C-2^{II}), 72.4 (C-5^{II}), 72.9 (C-3^{II}, CH_2Ph), 76.5 (C-4^{II}), 77.4 (C-4^{I}), 78.3 (C-2^{I}), 100.3 (C-1^{I}, C-1^{II}), 100.9 (C-1^{III}), 127.3, 127.37, 128.1, 138.5 (Ph), 169.0, 169.5, 169.7, 170.0, 170.1, 170.2, 170.23, 170.3, 170.36 (Ac: C=O). Anal. Calcd for $C_{51}H_{74}O_{26}Si$: C, 54.15; H, 6.59. Found: C, 53.94; H, 6.48.

6.15 Methyl (2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1-4)-(2,3,6-tri-*O*-acet-yl-β-D-glucopyranosyl)-(1-4)-6,7-di-*O*-acetyl-2-*O*-benzyl-L-*glycero*-α-D-*manno*-h-eptopyranoside (28)

Compound **27** (486.0 mg, 429.7 μmol) was dissolved in a mixture of trifluoroacetic acid and water (9:1, v/v, 10.0 mL) at room temperature. After stirring for 5 min, the mixture was diluted with toluene and concentrated. The residue was purified by flash column chromatography (dichloromethane/acetone, 9:1) to give **28** (424.0 mg, 97%). $[\alpha]^{25}_{D}$= +8.0 (*c* 1.0, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.97, 2.04, 2.04, 2.05, 2.06, 2.09, 2.12, 2.13, 2.16 (s, 3H x 9, Ac), 3.30 (s, 3H, OCH_3), 3.64 (m, 1H, $J_{4,5}$=9.5 Hz, H-5^{I}), 3.66 (dd, 1H, $J_{3,4}$=9.5 Hz, $J_{4,5}$=9.5 Hz, H-4^{I}), 3.73 (ddd, 1H, $J_{5,6a}$=6.5 Hz, $J_{5,6b}$=2.0 Hz, H-5^{II}), 3.76 (dd, 1H, $J_{3,4}$=9.5 Hz, H-4^{II}), 3.77 (dd, 1H, $J_{1,2}$=2.0 Hz, $J_{2,3}$=3.3 Hz, H-2^{I}), 3.88 (ddd, 1H, $J_{4,5}$=1.5 Hz, $J_{5,6a}$=7.5 Hz, $J_{5,6b}$=6.5 Hz, H-5^{III}), 3.92 (dd, 1H, $J_{2,3}$=3.3 Hz, $J_{3,4}$=9.5 Hz, H-3^{I}), 3.98 (d, 1H, $J_{3\text{-OH},H\text{-}3}$=2.5 Hz, 3-OH), 4.04 (dd, 1H, $J_{5,6a}$=6.5 Hz, $J_{6a,6b}$=12.0 Hz, H-6a^{II}), 4.08 (dd, 1H, $J_{5,6a}$=7.5 Hz, $J_{6a,6b}$=11.0 Hz, H-6a^{III}), 4.13 (dd, 1H, $J_{5,6b}$=6.5 Hz, $J_{6a,6b}$=11.0 Hz, H-6b^{III}), 4.27 (dd, 1H, $J_{6,7a}$=6.5 Hz, $J_{7a,7b}$=11.0 Hz, H-7a^{I}), 4.30 (dd, 1H, $J_{6,7b}$=7.0 Hz, $J_{7a,7b}$=11.0 Hz, H-7b^{I}), 4.49 (d, 1H, $J_{1,2}$=8.0 Hz, H-1^{III}), 4.54 (d, 1H, $J_{1,2}$=8.0 Hz, H-1^{II}), 4.59 (dd, 1H, $J_{5,6b}$=2.0 Hz, $J_{6a,6b}$=12.0 Hz, H-6b^{II}), 4.70 (d, 1H, *J*=12.0 Hz, OCH_2Ph), 4.75 (d, 1H, $J_{1,2}$=2.0 Hz, H-1^{I}), 4.84 (d, 1H, *J*=12.0 Hz, OCH_2Ph), 4.94 (dd, 1H, $J_{1,2}$=8.0 Hz, $J_{2,3}$=9.5 Hz, H-2^{II}), 4.97 (dd, 1H, $J_{2,3}$=10.8 Hz, $J_{3,4}$=3.5 Hz, H-3^{III}), 5.19 (dd, 1H, $J_{1,2}$=8.0 Hz, $J_{2,3}$=10.8 Hz, H-2^{III}), 5.22 (dd, 1H, $J_{2,3}$=9.5 Hz, $J_{3,4}$=9.5 Hz, H-3^{II}), 5.25 (ddd, 1H, $J_{6,7a}$=6.5 Hz, $J_{6,7b}$=7.0 Hz, H-6^{I}), 5.35 (dd, 1H, $J_{3,4}$=3.5 Hz, $J_{4,5}$=1.5 Hz, H-4^{III}), 7.38–7.25 (m, 5H, Ph). ^{13}C NMR (150 MHz, $CDCl_3$): δ 20.3, 20.34, 20.4, 20.5, 20.6, 20.7 (Ac-CH_3), 55.0 (OCH_3), 60.7 (C-6^{III}), 61.8 (C-6^{II}), 62.0 (C-7^{I}), 66.5 (C-4^{III}), 68.0 (C-6^{I}), 68.4 (C-5^{I}), 69.0 (C-2^{III}), 69.8 (C-3^{I}), 70.6 (C-5^{III}), 70.8 (C-3^{III}), 71.3 (C-2^{II}), 72.55 (C-5^{II}), 72.6 (C-3^{II}), 73.0 (OCH_2Ph), 76.0 (C-2^{I}), 76.2 (C-4^{II}), 79.6 (C-4^{I}), 99.0 (C-1^{I}), 100.4 (C-1^{II}), 100.9 (C-1^{III}), 127.3, 127.34, 128.1, 138.4 (Ph), 168.9, 169.3, 169.87, 169.9, 170.0, 170.1, 170.2, 170.23 (Ac: C=O). ESI-HRMS for $C_{45}H_{60}O_{26}$: 1039.3271 $[M+Na]^+$. Found: 1039.3303.

6.16 Methyl (2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl)-(1-4)-(2,3,6-tri-*O*-acet-yl-β-D-glucopyranosyl)-(1-4)-3,6,7-tri-*O*-acetyl-2-*O*-benzyl-L-*glycero*-α-D-*manno*-heptopyranoside (29)

Compound **28** (280.3 mg, 275.8 μmol) was acetylated with pyridine/Ac_2O (1:1,

v/v, 1.6 mL) in the presence of a catalytic amount of *N*,*N*-dimethyl-4-aminopyridine (DMAP) over 2 h. After removing the solvent, the residue was purified by flash column chromatography (dichloromethane/ethyl acetate, 5:2) to give **29** (180.9 mg, 73%). $[\alpha]^{25}_{D}$= +6.1 (*c* 0.8, $CHCl_3$), ^{1}H-NMR (600 MHz, $CDCl_3$): δ 1.96, 1.97, 2.04, 2.05, 2.06, 2.08, 2.09, 2.15, 2.16 (s, 3H x 10, Ac), 3.34 (s, 3H, OCH_3), 3.61 (ddd, 1H, $J_{4,5}$=10.0 Hz, $J_{5,6a}$=4.8 Hz, $J_{5,6b}$=1.8 Hz, H-5^{II}), 3.76 (dd, 1H, $J_{1,2}$=2.4 Hz, $J_{2,3}$=3.4 Hz, H-2^{I}), 3.80 (dd, 1H, $J_{4,5}$=9.6 Hz, $J_{5,6}$=1.0 Hz, H-5^{I}), 3.84 (dd, 1H, $J_{3,4}$=8.6 Hz, $J_{4,5}$=10.0 Hz, H-4^{II}), 3.86 (ddd, 1H, $J_{4,5}$=0.8 Hz, $J_{5,6a}$=7.6 Hz, $J_{5,6b}$=6.6 Hz, H-5^{III}), 3.88 (dd, 1H, $J_{3,4}$=8.4 Hz, $J_{4,5}$=9.6 Hz, H-4^{I}), 4.07 (dd, 1H, $J_{5,6a}$=7.6 Hz, $J_{6a,6b}$=11.4 Hz, H-6a^{III}), 4.10 (dd, 1H, $J_{5,6a}$=5.4 Hz, $J_{6a,6b}$=12.0 Hz, H-6a^{II}), 4.12 (dd, 1H, $J_{5,6b}$=6.6 Hz, $J_{6a,6b}$=11.4 Hz, H-6b^{III}), 4.25 (dd, 1H, $J_{6,7a}$=7.4 Hz, $J_{7a,7b}$=11.2 Hz, H-7a^{I}), 4.32 (dd, 1H, $J_{6,7b}$=6.0 Hz, $J_{7a,7b}$=11.2 Hz, H-7b^{I}), 4.38 (dd, 1H, $J_{5,6b}$=1.8 Hz, $J_{6a,6b}$=12.0 Hz, H-6b^{II}), 4.47 (d, 1H, $J_{1,2}$=8.0 Hz, H-1^{III}), 4.55 (d, 1H, *J*=12.2 Hz, OCH_2Ph), 4.57 (d, 1H, $J_{1,2}$=7.2 Hz, H-1^{II}), 4.64 (d, 1H, *J*=12.2 Hz, OCH_2Ph), 4.77 (d, 1H, $J_{1,2}$=2.4 Hz, H-1^{I}), 4.82 (dd, 1H, $J_{1,2}$=7.2 Hz, $J_{2,3}$=8.2 Hz, H-2^{II}), 4.93 (dd, 1H, $J_{2,3}$=10.4 Hz, $J_{3,4}$=3.4 Hz, H-3^{III}), 5.10 (dd, 1H, $J_{1,2}$=8.0 Hz, $J_{2,3}$=10.4 Hz, H-2^{III}), 5.15 (dd, 1H, $J_{2,3}$=8.2 Hz, $J_{3,4}$=8.6 Hz, H-3^{II}), 5.27 (dd, 1H, $J_{2,3}$=3.4 Hz, $J_{3,4}$=8.4 Hz, H-3^{I}), 5.34 (dd, 1H, $J_{3,4}$=3.4 Hz, $J_{4,5}$=0.8 Hz, H-4^{III}), 5.39 (ddd, 1H, $J_{5,6}$=1.0 Hz, $J_{6,7a}$=7.2 Hz, $J_{6,7b}$=6.0 Hz, H-6^{I}), 7.26–7.33 (m, 5H, Ph). ^{13}C NMR (150 MHz, $CDCl_3$): δ 20.5, 20.6, 20.68, 20.7, 20.74, 20.8, 20.9 (Ac-CH_3), 55.3 (OCH_3), 60.7 (C-6^{III}), 62.2 (C-6^{II}), 62.4 (C-7^{I}), 66.6 (C-4^{III}), 68.1 (C-6^{I}), 69.0 (C-2^{III}), 69.3 (C-3^{I}), 70.6 (C-5^{III}), 70.7 (C-5^{I}), 71.0 (C-3^{III}), 71.9 (C-2^{II}), 72.1 (C-5^{II}), 72.7 (OCH_2Ph), 73.3 (C-3^{II}), 73.9 (C-4^{I}), 75.2 (C-2^{I}), 76.2 (C-4^{II}), 99.1 (C-1^{I}), 99.9 (C-1^{II}), 101.1 (C-1^{III}), 127.8, 127.9, 128.4, 137.7 (Ph), 169.1, 169.7, 169.9, 170.2, 170.3, 170.4, (Ac: C=O). ESI-HRMS for $C_{47}H_{62}O_{27}$: 1067.3376 $[M+Na]^+$. Found 1081.3368.

6.17 (2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-(1-4)-(2,3,6-tri-*O*-acetyl-β-D-g-lucopyranosyl)-(1-4)-3,6,7-tri-*O*-acetyl-2-*O*-benzyl-L-*glycero*-D-*manno*-heptopyranose (30)

A solution of **29** (195.0 mg, 184.1 μmol) in a mixture of H_2SO_4/AcOH/Ac_2O (0.1:6:14, 4.0 mL) was stirred for 3 h at room temperature. The reaction mixture was

neutralized by the addition of sodium acetate, poured into saturated sodium hydrogen carbonate, and extracted with dichloromethane. The combined organic layer was washed with brine, dried over anhydrous sodium sulfate, filtered, and concentrated. The residue was purified by flash column chromatography (ethyl acetate/hexane, 2:1) to give a syrup (124.5 mg, 64%). The syrup was treated with hydrazine acetate (13.0 mg, 120.4 μmol) in DMF (1.0 mL) for 8 h at 0 °C. The reaction mixture was diluted with ethyl acetate, washed with water and brine, dried over anhydrous sodium sulfate, filtered, and concentrated. The residue was purified by silica gel column chromatography (ethyl acetate/hexane, 2:1) to give **30** (89.1 mg, 77%). ^{1}H-NMR (600 MHz, $CDCl_3$): δ 1.96, 1.98, 2.04, 2.06, 2.06, 2.07, 2.15, 2.15 (s, 3H x 10, Ac), 3.37 (brs, 1H, OH), 3.61 (ddd, 1H, $J_{4,5}$=10.0 Hz, $J_{5,6a}$=4.6 Hz, $J_{5,6b}$=2.0 Hz, H-5^{II}), 3.79 (dd, 1H, $J_{1,2}$=2.8 Hz, $J_{2,3}$=3.2 Hz, H-2^{I}), 3.85 (dd, 1H, $J_{3,4}$=9.0 Hz, $J_{4,5}$=10.0 Hz, H-4^{II}), 3.86 (ddd, 1H, $J_{4,5}$=1.2 Hz, $J_{5,6a}$=7.6 Hz, $J_{5,6b}$=6.2 Hz, H-5^{III}), 3.89 (dd, 1H, $J_{3,4}$=8.6 Hz, $J_{4,5}$=9.8 Hz, H-4^{I}), 3.98 (dd, 1H, $J_{4,5}$=9.8 Hz, $J_{5,6}$=nd Hz, H-5^{I}), 4.07 (dd, 1H, $J_{5,6a}$=7.6 Hz, $J_{6a,6b}$=11.0 Hz, H-6a^{III}), 4.09 (dd, 1H, $J_{5,6a}$=4.6 Hz, $J_{6a,6b}$=12.0 Hz, H-6a^{II}), 4.15 (dd, 1H, $J_{5,6b}$=6.2 Hz, $J_{6a,6b}$=11.0 Hz, H-6b^{III}), 4.15 (dd, 1H, $J_{6,7a}$=7.0 Hz, $J_{7a,7b}$=11.0 Hz, H-7a^{I}), 4.38 (dd, 1H, $J_{6,7b}$=6.2 Hz, $J_{7a,7b}$=11.0 Hz, H-7b^{I}), 4.39 (dd, 1H, $J_{5,6b}$=2.0 Hz, $J_{6a,6b}$=12.0 Hz, H-6b^{II}), 4.48 (d, 1H, $J_{1,2}$=8.0 Hz, H-1^{III}), 4.56 (d, 1H, J=12.2 Hz, OCH_2Ph), 4.57 (d, 1H, $J_{1,2}$=7.2 Hz, H-1^{II}), 4.63 (d, 1H, J=12.2 Hz, OCH_2Ph), 4.82 (dd, 1H, $J_{1,2}$=7.2 Hz, $J_{2,3}$=8.4 Hz, H-2^{II}), 4.93 (dd, 1H, $J_{2,3}$=10.4 Hz, $J_{3,4}$=3.4 Hz, H-3^{III}), 5.10 (dd, 1H, $J_{1,2}$=8.0 Hz, $J_{2,3}$=10.4 Hz, H-2^{III}), 5.15 (dd, 1H, $J_{2,3}$=8.4 Hz, $J_{3,4}$=9.0 Hz, H-3^{II}), 5.27 (d, 1H, $J_{1,2}$=2.8 Hz, H-1^{I}), 5.33 (dd, 1H, $J_{3,4}$=3.4 Hz, $J_{4,5}$=1.2 Hz, H-4^{III}), 5.33 (dd, 1H, $J_{2,3}$=3.2 Hz, $J_{3,4}$=8.6 Hz, H-3^{I}), 5.37 (ddd, 1H, $J_{5,6}$=nd Hz, $J_{6,7a}$=7.0 Hz, $J_{6,7b}$=6.2 Hz, H-6^{I}), 7.27–7.33 (m, 5H, Ph). ^{13}C NMR (150 MHz, $CDCl_3$): δ 20.5, 20.6, 20.65, 20.66, 20.7, 20.79, 20.8, 20.9 (Ac-CH_3), 60.7 (C-6^{III}), 62.4 (C-6^{II}), 62.6 (C-7^{I}), 66.6 (C-4^{III}), 68.1 (C-6^{I}), 69.0 (C-2^{III}), 69.3 (C-3^{I}), 70.5 (C-5^{III}), 70.6 (C-5^{I}), 71.0 (C-3^{III}), 71.9 (C-2^{II}), 72.0 (C-5^{II}), 72.7 (OCH_2Ph), 73.2 (C-3^{II}), 74.0 (C-4^{I}), 75.7 (C-2^{I}), 76.2 (C-4^{II}), 92.4 (C-1^{I}), 99.8 (C-1^{II}), 101.1 (C-1^{III}), 127.7, 127.8, 128.4, 137.7 (Ph), 169.2, 169.66, 169.7, 170.0, 170.15, 170.2, 170.24, 170.4, 170.9 (Ac: C=O). ESI-HRMS for $C_{46}H_{60}O_{27}$: 1067.3220 $[M+Na]^+$. Found

1067.3226.

6.18 (2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-(1-4)-(2,3,6-tri-*O*-acetyl-β-D-g-lucopyranosyl)-(1-4)-3,6,7-tri-*O*-acetyl-2-*O*-benzyl-L-*glycero*-α-D-*manno*-heptopyranosyl trichloroacetimidate (31)

Trichloroacetonitrile (85.0 μL, 842.1 μmol) was added to a solution of **30** (88.0 mg, 84.2 μmol) in dry dichloromethane (0.8 mL) under argon. Potassium carbonate (58.0 mg, 421.0 μmol) was added to the reaction mixture. After stirring for 13 h at room temperature, the mixture was filtered through Celite. The solution was concentrated and purified by silica gel column chromatography (ethyl acetate/hexane, 2:1) to give **31** (96.0 mg, 100%). $[\alpha]^{25}_{D}$= -5.7 (*c* 1.3, $CHCl_3$), ^{1}H-NMR (600 MHz, $CDCl_3$): δ 1.96, 1.99, 2.00, 2.06, 2.04, 2.04, 2.04, 2.05, 2.06, 2.07, 2.15, 2.15 (s, 3H x 10, Ac), 3.65 (ddd, 1H, $J_{4,5}$=10.0 Hz, $J_{5,6a}$=4.6 Hz, $J_{5,6b}$=2.0 Hz, H-5^{II}), 3.84 (dd, 1H, $J_{3,4}$=9.2 Hz, $J_{4,5}$=10.0 Hz, H-4^{II}), 3.87 (ddd, 1H, $J_{4,5}$=nd Hz, $J_{5,6a}$=7.6 Hz, $J_{5,6b}$=6.2 Hz, H-5^{III}), 3.88 (dd, 1H, $J_{3,4}$=5.6 Hz, $J_{4,5}$=9.4 Hz, H-4^{I}), 3.94 (dd, 1H, $J_{4,5}$=9.4 Hz, $J_{5,6}$=0.8 Hz, H-5^{I}), 4.04 (dd, 1H, $J_{1,2}$=3.6 Hz, $J_{2,3}$=2.8 Hz, H-2^{I}), 4.07 (dd, 1H, $J_{5,6a}$=7.6 Hz, $J_{6a,6b}$=11.2 Hz, H-6a^{III}), 4.09 (dd, 1H, $J_{5,6a}$=4.8 Hz, $J_{6a,6b}$=12.0 Hz, H-6a^{II}), 4.14 (dd, 1H, $J_{5,6b}$=6.2 Hz, $J_{6a,6b}$=11.2 Hz, H-6b^{III}), 4.16 (dd, 1H, $J_{6,7a}$=7.2 Hz, $J_{7a,7b}$=11.4 Hz, H-7a^{I}), 4.22 (dd, 1H, $J_{6,7b}$=5.8 Hz, $J_{7a,7b}$=11.4 Hz, H-7b^{I}), 4.47 (dd, 1H, $J_{5,6b}$=1.6 Hz, $J_{6a,6b}$=12.0 Hz, H-6b^{II}), 4.49 (d, 1H, $J_{1,2}$=8.0 Hz, H-1^{III}), 4.61 (d, 1H, $J_{1,2}$=7.6 Hz, H-1^{II}), 4.63 (d, 1H, *J*=12.0 Hz, OCH_2Ph), 4.68 (d, 1H, *J*=12.0 Hz, OCH_2Ph), 4.85 (dd, 1H, $J_{1,2}$=7.6 Hz, $J_{2,3}$=8.6 Hz, H-2^{II}), 4.94 (dd, 1H, $J_{2,3}$=10.6 Hz, $J_{3,4}$=3.6 Hz, H-3^{III}), 5.10 (dd, 1H, $J_{1,2}$=8.0 Hz, $J_{2,3}$=10.4 Hz, H-2^{III}), 5.16 (dd, 1H, $J_{2,3}$=8.6 Hz, $J_{3,4}$=9.2 Hz, H-3^{II}), 5.29 (ddd, 1H, $J_{5,6}$=0.8 Hz, $J_{6,7a}$=7.2 Hz, $J_{6,7b}$=6.2 Hz, H-6^{I}), 5.33 (dd, 1H, $J_{3,4}$=3.4 Hz, $J_{4,5}$=nd Hz, H-4^{III}), 5.48 (dd, 1H, $J_{2,3}$=2.8 Hz, $J_{3,4}$=5.6 Hz, H-3^{I}), 6.31 (d, 1H, $J_{1,2}$=3.6 Hz, H-1^{I}), 7.26–7.34 (m, 5H, Ph), 8.68 (NH). ^{13}C NMR (150 MHz, $CDCl_3$): δ 20.5, 20.54, 20.7, 20.74, 20.76, 20.79, 20.8, 20.9, 21.0 (Ac-CH_3), 60.7 (C-6^{III}), 62.1 (C-7^{I} and C-6^{II}), 66.6 (C-4^{III}), 67.9 (C-6^{I}), 69.3 (C-2^{III}), 69.9 (C-3^{I}), 70.6 (C-5^{III}), 71.0 (C-3^{III}), 71.5 (C-2^{II}), 71.6 (C-5^{II}), 72.4 (-OCH_2Ph and C-5^{I}), 73.0 (C-3^{II}), 74.0 (C-4^{I}), 74.9 (C-2^{I}), 76.0 (C-4^{II}), 90.8 ($OCNHCCl_3$), 96.0 (C-1^{I}), 99.2 (C-1^{II}), 101.1 (C-1^{III}), 127.9, 128.0, 128.4, 137.2 (Ph), 160.5

(OCNHCCl$_3$), 169.0, 169.6, 169.8, 169.9, 170.1, 170.13, 170.16, 170.2, 170.24, 170.4 (Ac: C=O). ESI-HRMS for $C_{48}H_{60}Cl_3NO_{27}$: 1210.2316 $[M+Na]^+$. Found 1210.2294.

6.19 Methyl (2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1-4)-(2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl)-(1-4)-6,7-di-*O*-acetyl-L-*glycero*-α-D-*manno*-heptopyranoside (32)

Compound **28** (227.0 mg, 0.2 mmol) was hydrogenated in the presence of 10% Pd/C (100.0 mg) in ethyl acetate (15.0 mL) under atmospheric pressure of hydrogen. After stirring for 3.5 h at room temperature, the reaction mixture was filtered through Celite and concentrated. The residue was purified by flash column chromatography (dichloromethane/acetone, 3:1) to give **32** (198.0 mg, 97%). $[\alpha]^{25}_D$= +26.0 (*c* 1.0, $CHCl_3$), ^{1}H-NMR (600 MHz, $CDCl_3$): δ 1.97, 2.04, 2.04, 2.05, 2.07, 2.12, 2.14, 2.16, 2.17 (s, 3H x 9, Ac), 2.55 (d, 1H, $J_{2\text{-OH,H-2}}$=1.5 Hz, 2-OH), 3.35 (s, 3H, OCH_3), 3.55 (dd, 1H, $J_{3,4}$=8.0 Hz, $J_{4,5}$=9.8 Hz, H-4^I), 3.71 (dd, 1H, $J_{4,5}$=9.8 Hz, $J_{5,6}$=1.0 Hz, H-5^I), 3.72 (ddd, 1H, $J_{4,5}$=10.0 Hz, $J_{5,6a}$=5.5 Hz, $J_{5,6b}$=2.0 Hz, H-5^{II}), 3.78 (dd, 1H, $J_{3,4}$=9.0 Hz, $J_{4,5}$=10.0 Hz, H-4^{II}), 3.81 (dd, 1H, $J_{2,3}$=3.5 Hz, $J_{3,4}$=8.0 Hz, H-3^I), 3.96 (dd, 1H, $J_{1,2}$=1.0 Hz, $J_{2,3}$=3.5 Hz, H-2^I), 3.88 (ddd, 1H, $J_{4,5}$=1.0 Hz, $J_{5,6a}$=7.5 Hz, $J_{5,6b}$=6.5 Hz, H-5^{III}), 4.03 (dd, 1H, $J_{5,6a}$=5.5 Hz, $J_{6a,6b}$=12.0 Hz, H-6a^{II}), 4.08 (dd, 1H, $J_{5,6a}$=7.5 Hz, $J_{6a,6b}$=11.3 Hz, H-6a^{III}), 4.14 (dd, 1H, $J_{5,6b}$=6.5 Hz, $J_{6a,6b}$=11.3 Hz, H-6b^{III}), 4.22 (m, 1H, $J_{5,6}$=1.0 Hz, H-6^I), 4.29 (m, 2H, H-7a^I, H-7b^I), 4.30 (d, 1H, $J_{3\text{-OH,H-3}}$=1.0 Hz, 3-OH), 4.46 (d, 1H, $J_{1,2}$=8.0 Hz, H-1^{II}), 4.51 (d, 1H, $J_{1,2}$=8.5 Hz, H-1^{III}), 4.65 (dd, 1H, $J_{5,6b}$=2.0 Hz, $J_{6a,6b}$=12.0 Hz, H-6b^{II}), 4.80 (d, 1H, $J_{1,2}$=1.0 Hz, H-1^I), 4.93 (dd, 1H, $J_{1,2}$=8.0 Hz, $J_{2,3}$=9.5 Hz, H-2^{II}), 4.97 (dd, 1H, $J_{2,3}$=10.5 Hz, $J_{3,4}$=3.5 Hz, H-3^{III}), 5.11 (dd, 1H, $J_{1,2}$=8.5 Hz, $J_{2,3}$=10.5 Hz, H-2^{III}), 5.22 (dd, 1H, $J_{2,3}$=9.5 Hz, $J_{3,4}$=9.0 Hz, H-3^{II}), 5.35 (dd, 1H, $J_{3,4}$=3.5 Hz, $J_{4,5}$=1.0 Hz, H-4^{III}). ^{13}C NMR (150 MHz, $CDCl_3$): δ 20.4, 20.42, 20.5, 20.54, 20.6, 20.64, 20.9 (Ac-CH_3), 55.2 (OCH_3), 60.7 (C-6^{III}), 61.6 (C-6^{II}), 62.0 (C-7^I), 66.5 (C-4^{III}), 67.7 (C-5^I), 67.9 (C-6^I), 69.0 (C-2^{III}), 69.5 (C-3^I), 69.5 (C-2^I), 70.8 (C-3^{III}), 71.0 (C-5^{III}), 71.2 (C-2^{II}), 72.5 (C-3^{II}), 72.8 (C-5^{II}), 76.0 (C-4^{II}), 79.0 (C-4^I), 100.1 (C-1^I), 100.6 (C-1^{II}), 100.9 (C-1^{III}), 169.0, 169.3, 170.0, 170.04, 170.16, 170.2, 170.26, 170.3 (Ac: C=O). ESI-HRMS for $C_{38}H_{54}O_{26}$: 949.2801 $[M+Na]^+$. Found 949.2766.

6.20 (2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-(1-4)-(2,3,6-tri-*O*-acetyl-β-D-g-lucopyranosyl)-(1-4)-1,2,3,6,7-penta-*O*-acetyl-L-*glycero*-α-D-*manno*-heptopyrano-se (33)

Compound **32** (342.0 mg, 0.4 mmol) was treated with acetic anhydride (1.0 mL) and pyridine (2.0 mL) at room temperature. The reaction mixture was stirred overnight and concentrated. The residue was purified by silica gel column chromatography (dichloromethane/acetone, 4:1) to give a syrup. The syrup was dissolved in a mixture of H_2SO_4/AcOH/Ac_2O (8.0 mL, 0.1:6:14) at room temperature. After stirring for 15 h, the reaction mixture was neutralized by the addition of sodium acetate (0.2 g), poured into saturated sodium hydrogen carbonate, and extracted with chloroform. Combined organic phase was washed with brine, dried over anhydrous sodium sulfate, filtered, and concentrated. The residue was purified by silica gel column chromatography (dichloromethane/acetone, 4:1) to give **33** (257.0 mg, 67%). $[\alpha]^{25}_D$= +29.0 (*c* 1.0, $CHCl_3$), ^{1}H-NMR (600 MHz, $CDCl_3$): δ 1.97, 1.99, 2.03, 2.05, 2.06, 2.07, 2.08, 2.13, 2.14, 2.15, 2.16, 2.18 (s, 3H x 12, Ac), 3.61 (ddd, 1H, $J_{4,5}$=9.5 Hz, $J_{5,6a}$=7.0 Hz, $J_{5,6b}$=5.5 Hz, H-5^{II}), 3.82 (dd, 1H, $J_{3,4}$=8.5 Hz, $J_{4,5}$=9.5 Hz, H-4^{II}), 3.85–3.88 (m, 2H, H-5^{III}, H-4^{I}), 3.91 (dd, 1H, $J_{4,5}$=9.5 Hz, $J_{5,6}$=1.2 Hz, H-5^{I}), 4.05–4.09 (m, 2H, H-6a^{III}, H-7a^{I}), 4.13 (dd, 1H, $J_{5,6b}$=6.2 Hz, $J_{6a,6b}$=11.0 Hz, H-6b^{III}), 4.19 (dd, 1H, $J_{5,6a}$=7.0 Hz, $J_{6a,6b}$=11.5 Hz, H-6a^{II}), 4.24 (dd, 1H, $J_{5,6b}$=5.5 Hz, $J_{6a,,6b}$=11.5 Hz, H-6b^{II}), 4.40 (dd, 1H, $J_{6,7b}$=2.0 Hz, $J_{7a,7b}$=12.0 Hz, H-7b^{I}), 4.49 (d, 1H, $J_{1,2}$=8.0 Hz, H-1^{III}), 4.61 (d, 1H, $J_{1,2}$=7.5 Hz, H-1^{II}), 4.82 (dd, 1H, $J_{1,2}$=7.5 Hz, $J_{2,3}$=8.0 Hz, H-2^{II}), 4.95 (dd, 1H, $J_{2,3}$=10.5 Hz, $J_{3,4}$=3.4 Hz, H-3^{III}), 5.11 (dd, 1H, $J_{1,2}$=8.0 Hz, $J_{2,3}$=10.5 Hz, H-2^{III}), 5.16 (dd, 1H, $J_{2,3}$=8.0 Hz, $J_{3,4}$=8.5 Hz, H-3^{II}), 5.22 (dd, 1H, $J_{1,2}$=2.5 Hz, $J_{2,3}$=9.0 Hz, H-2^{I}), 5.32–5.36 (m, 3H, H-6^{I}, H-4^{III}, H-3^{I}), 6.06 (d, 1H, $J_{1,2}$=2.5 Hz, H-1^{I}). ^{13}C NMR (150 MHz, $CDCl_3$): δ 20.5, 20.51, 20.6, 20.65, 20.7, 20.8, 20.81, 20.9, 21.1 (Ac-CH_3), 60.7 (C-6^{III}), 62.2 (C-6^{II}), 62.3 (C-7^{I}), 66.6 (C-4^{III}), 67.9 (C-3^{I}), 68.4 (C-6^{I}), 68.9 (C-2^{III}), 69.1 (C-2^{I}), 70.6 (C-5^{III}), 71.0 (C-3^{III}), 71.4 (C-5^{I}), 71.9 (C-2^{II}), 72.2 (C-5^{II}), 73.1 (C-3^{II}), 73.2 (C-4^{I}), 76.0 (C-4^{II}), 90.4 (C-1^{I}), 99.8 (C-1^{II}), 101.2 (C-1^{III}), 168.3, 169.1, 169.5, 169.53, 169.6, 169.8, 170.1, 170.14, 170.2, 170.3, 170.4 (Ac: C=O). ESI-HRMS for $C_{43}H_{58}O_{29}$: 1061.2961 $[M+Na]^+$.

Found 1061.2981.

6.21 (2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-(1-4)-(2,3,6-tri-*O*-acetyl-β-D-g-lucopyranosyl)-(1-4)-2,3,6,7-tetra-*O*-acetyl-L-*glycero*-D-*manno*-heptopyranose (34)

Compound **33** (256.6 mg, 247.0 μmol) was treated with hydrazine acetate (45.5 mg, 494.0 μmol) in DMF (3.0 mL) for 8 h at 0 °C. The reaction mixture was diluted with ethyl acetate, washed with water and brine, dried over anhydrous sodium sulfate, filtered, and concentrated. The residue was purified by silica gel column chromatography (acetone/dichloromethane, 1:4) to give **34** (223.4 mg, 90%). ^{1}H-NMR (600 MHz, $CDCl_3$): δ 1.96, 1.98, 2.04, 2.06, 2.07, 2.08, 2.13, 2.16, 2.19 (s, 3H x 11, Ac), 3.27 (brs, 1H, OH), 3.62 (ddd, 1H, $J_{4,5}$=10.0 Hz, $J_{5,6a}$=4.6 Hz, $J_{5,6b}$=1.6 Hz, H-5^{II}), 3.81 (dd, 1H, $J_{3,4}$=9.4 Hz, $J_{4,5}$=10.0 Hz, H-4^{II}), 3.87 (ddd, 1H, $J_{5,6a}$=7.8 Hz, $J_{5,6b}$=6.2 Hz, H-5^{III}), 3.88 (dd, 1H, $J_{4,5}$=9.8 Hz, H-4^{I}), 4.06 (dd, 1H, $J_{5,6a}$=4.6 Hz, $J_{6a,6b}$=12.0 Hz, H-6a^{II}), 4.07 (dd, 1H, $J_{5,6a}$=7.8 Hz, $J_{6a,6b}$=11.2 Hz, H-6a^{III}), 4.09 (dd, 1H, $J_{4,5}$=9.8 Hz, H-5^{I}), 4.12 (dd, 1H, $J_{5,6b}$=6.2 Hz, $J_{6a,6b}$=11.2 Hz, H-6b^{III}), 4.16 (dd, 1H, $J_{6,7a}$=6.8 Hz, $J_{7a,7b}$=11.4 Hz, H-7a^{I}), 4.38 (dd, 1H, $J_{5,6b}$=1.6 Hz, $J_{6a,6b}$=12.0 Hz, H-6b^{II}), 4.39 (dd, 1H, $J_{6,7b}$=5.8 Hz, $J_{7a,7b}$=11.4 Hz, H-7b^{I}), 4.49 (d, 1H, $J_{1,2}$=8.0 Hz, H-1^{III}), 4.60 (d, 1H, $J_{1,2}$=6.8 Hz, H-1^{II}), 4.80 (dd, 1H, $J_{1,2}$=6.8 Hz, $J_{2,3}$=8.2 Hz, H-2^{II}), 4.94 (dd, 1H, $J_{2,3}$=10.4 Hz, $J_{3,4}$=3.4 Hz, H-3^{III}), 5.11 (dd, 1H, $J_{1,2}$=8.0 Hz, $J_{2,3}$=10.4 Hz, H-2^{III}), 5.15 (dd, 1H, $J_{2,3}$=8.2 Hz, $J_{3,4}$=9.4 Hz, H-3^{II}), 5.22–5.23 (m, 2H, H-6^{I}, H-4^{III}), 5.34 (d, 1H, $J_{1,2}$=3.2 Hz, H-1^{I}), 5.40 (dd, 1H, H-3^{I}), 5.41 (dd, 1H, $J_{1,2}$=3.2 Hz, H-2^{I}). ^{13}C NMR (150 MHz, $CDCl_3$): δ 20.5, 20.6, 20.8, 20.9, 20.92 (Ac-CH_3), 60.7 (C-6^{III}), 62.5 (C-6^{II}), 62.8 (C-7^{I}), 66.6 (C-4^{III}), 68.3 (C-6^{I}), 68.96 (C-3^{I}), 69.0 (C-2^{III}), 69.1 (C-2^{I}), 70.4 (C-5^{I}), 70.6 (C-5^{III}), 71.0 (C-3^{III}), 71.9 (C-2^{II}), 71.92 (C-5^{II}), 73.1 (C-3^{II}), 73.3 (C-4^{I}), 76.0 (C-4^{II}), 92.0 (C-1^{I}), 99.1 (C-1^{II}), 101.1 (C-1^{III}), 169.2, 169.7, 169.8, 170.0, 170.1, 170.2, 170.4, 170.5 (Ac: C=O). ESI-HRMS for $C_{41}H_{56}O_{28}$: 1019.2856 $[M+Na]^+$. Found 1019.2848.

6.22 (2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-(1-4)-(2,3,6-tri-*O*-acetyl-β-D-g-lucopyranosyl)-(1-4)-2,3,6,7-tetra-*O*-acetyl-L-*glycero*-α-D-*manno*-heptopyranosyl trichloroacetimidate (35)

Trichloroacetonitrile (57.0 μL, 565.6 μmol) was added to a solution of **34** (57.1 mg, 57.3 μmol) in dry dichloromethane (1.0 mL) under argon. Potassium carbonate (39.3 mg, 284.4 μmol) was added to the reaction mixture. After stirring for 24 h at room temperature, the mixture was filtered through Celite. The solution was concentrated and purified by silica gel column chromatography (ethyl acetate/hexane, 2:1) to give **35** (60.2 mg, 92%). $[\alpha]^{25}_{D}$= +2.4 (*c* 1.0, $CHCl_3$), ^{1}H-NMR (600 MHz, $CDCl_3$): δ 1.97, 2.00, 2.00, 2.04, 2.07, 2.12, 2.15, 2.19 (s, 3H x 11, Ac), 3.63 (ddd, 1H, $J_{4,5}$=10.0 Hz, $J_{5,6a}$=4.0 Hz, $J_{5,6b}$=2.0 Hz, H-5^{II}), 3.84–3.89 (m, 3H, H-4^{II}, H-5^{III}, H-4^{I}), 4.01 (dd, 1H, $J_{4,5}$=9.8 Hz, $J_{5,6}$=1.4 Hz, H-5^{I}), 4.06 (dd, 1H, $J_{5,6a}$=7.2 Hz, $J_{6a,6b}$=11.2 Hz, H-6a^{III}), 4.09 (dd, 1H, $J_{5,6a}$=4.0 Hz, $J_{6a,6b}$=12.2 Hz, H-6a^{II}), 4.14 (dd, 1H, $J_{5,6b}$=6.2 Hz, $J_{6a,6b}$=11.2 Hz, H-6b^{III}), 4.17 (dd, 1H, $J_{6,7a}$=7.2 Hz, $J_{7a,7b}$=11.4 Hz, H-7a^{I}), 4.21 (dd, 1H, $J_{6,7b}$=6.0 Hz, $J_{7a,7b}$=11.4 Hz, H-7b^{I}), 4.47 (dd, 1H, $J_{5,6b}$=2.0 Hz, $J_{6a,6b}$=12.2 Hz, H-6b^{II}), 4.53 (d, 1H, $J_{1,2}$=8.0 Hz, H-1^{III}), 4.61 (d, 1H, $J_{1,2}$=7.0 Hz, H-1^{II}), 4.83 (dd, 1H, $J_{1,2}$=7.0 Hz, $J_{2,3}$=7.8 Hz, H-2^{II}), 4.95 (dd, 1H, $J_{2,3}$=10.6 Hz, $J_{3,4}$=3.4 Hz, H-3^{III}), 5.10 (dd, 1H, $J_{1,2}$=8.0 Hz, $J_{2,3}$=10.6 Hz, H-2^{III}), 5.16 (dd, 1H, $J_{2,3}$=7.8 Hz, $J_{3,4}$=10.4 Hz, H-3^{II}), 5.34 (dd, 1H, $J_{4,5}$=0.8 Hz, $J_{3,4}$=3.4 Hz, H-4^{III}), 5.37 (ddd, 1H, $J_{5,6}$=1.4 Hz, $J_{6,7a}$=7.2 Hz, $J_{6,7b}$=6.0 Hz, H-6^{I}), 5.43 (dd, 1H, $J_{1,2}$=2.2 Hz, $J_{2,3}$=3.4 Hz, H-2^{I}), 5.44 (dd, 1H, $J_{2,3}$=3.4 Hz, H-3^{I}), 6.23 (d, 1H, $J_{1,2}$=2.2 Hz, H-1^{I}), 8.74 (NH). ^{13}C NMR (150 MHz, $CDCl_3$): δ 20.5, 20.57, 20.6, 20.7, 20.74, 20.8 (Ac-CH_3), 60.7 (C-6^{III}), 62.0 (C-6^{II}), 62.1 (C-7^{I}), 66.6 (C-4^{III}), 67.8 (C-2^{I}), 67.9 (C-6^{I}), 69.0 (C-3^{I} and C-2^{III}), 70.6 (C-5^{III}), 71.0 (C-3^{III}), 71.6 (C-2^{II}), 71.7 (C-5^{II}), 72.1 (C-5^{I}), 73.1 (C-3^{II}), 73.3 (C-4^{I}), 75.8 (C-4^{II}), 90.5 (OCNHCCl_3), 94.6 (C-1^{I}), 99.9 (C-1^{II}), 101.1 (C-1^{III}), 160.0 (OCNHCCl_3), 169.1, 169.4, 169.42, 169.67, 169.7, 170.0, 170.1, 170.2, 170.3, 170.4 (Ac: C=O). ESI-HRMS for $C_{43}H_{56}Cl_3NO_{28}$: 1162.1952 $[M+Na]^+$. Found 1162.1940.

6.23 Methyl (3,4,6,7-tetra-*O*-acetyl-2-*O*-benzyl-L-*glycero*-α-D-*manno*-heptopyranosyl)-(1-3)-4,6,7-tri-*O*-acetyl-2-*O*-benzyl-L-*glycero*-α-D-*manno*-heptopyranoside (36)

A mixture of **25** (188.0 mg, 308.0 μmol), and **22** (135.0 mg, 308.0 μmol), and 4 Å molecular sieves (135.0 mg) was suspended in dry dichloromethane (0.9 mL). The reaction mixture was stirred for 1 h under argon, and then cooled to -78 °C.

TMSOTf (2.2 μL, 12.3 μmol) in dichloromethane was added dropwise to the reaction mixture. The reaction was warmed to room temperature and stirred for 2 h. The reaction was neutralized by the addition of a few drops of triethylamine and aqueous saturated sodium hydrogen carbonate. The reaction mixture was diluted with dichloromethane and filtered through Celite. The filtrate was extracted twice with dichloromethane. The combined organic phase was dried over anhydrous sodium sulfate, filtered, and concentrated. The residue was purified by BioRad S-X3 size exclusion beads (toluene/ethyl acetate, 1:1) to give **36** (136.6 mg, 50%). $[\alpha]^{25}_{D}$= +41.2 (*c* 1.0, $CHCl_3$), ^{1}H-NMR (600 MHz, $CDCl_3$): δ 1.77, 1.94, 1.96, 2.04, 2.05, 2.09, 2.13 (s, 3H x 7, Ac), 3.31 (s, 3H, OCH_3), 3.66 (dd, 1H, $J_{1,2}$=1.6 Hz, $J_{2,3}$=3.0 Hz, H-2^{I}), 3.76 (dd, 1H, $J_{4,5}$=10.0 Hz, $J_{5,6}$=1.8 Hz, H-5^{II}), 3.81 (dd, 1H, $J_{1,2}$=1.6 Hz, $J_{2,3}$=3.0 Hz, H-2^{II}), 3.87 (dd, 1H, $J_{4,5}$=10.0 Hz, $J_{5,6}$=2.0 Hz, H-5^{I}), 4.02 (dd, 1H, $J_{6,7a}$=6.4 Hz, $J_{7a,7b}$=11.2 Hz, H-7a^{II}), 4.07 (dd, 1H, $J_{2,3}$=3.0 Hz, $J_{3,4}$=9.8 Hz, H-3^{I}), 4.11 (dd, 1H, $J_{6,7b}$=6.8 Hz, $J_{7a,7b}$=11.2 Hz, H-7b^{II}), 4.23 (dd, 1H, $J_{6,7a}$=7.6 Hz, $J_{7a,7b}$=11.2 Hz, H-7a^{I}), 4.32 (dd, 1H, $J_{6,7b}$=5.6 Hz, $J_{7a,7b}$=11.2 Hz, H-7b^{I}), 4.59, 4.64 (d, 2H, *J*=12.2 Hz, OCH_2Ph), 4.76 (d, 1H, $J_{1,2}$=1.6 Hz, H-1^{I}), 4.82, 4.84 (d, 2H, *J*=12.8 Hz, OCH_2Ph), 5.03 (d, 1H, $J_{1,2}$=1.6 Hz, H-1^{II}), 5.05 (ddd, 1H, $J_{5,6}$=1.8 Hz, $J_{6,7a}$=6.4 Hz, $J_{6,7b}$=6.8 Hz, H-6^{II}), 5.21 (ddd, 1H, $J_{5,6}$=2.0 Hz, $J_{6,7a}$=7.6 Hz, $J_{6,7b}$=5.6 Hz, H-6^{I}), 5.31 (dd, 1H, $J_{2,3}$=3.0 Hz, $J_{3,4}$=10.0 Hz, H-3^{II}), 5.40 (dd, 1H, $J_{3,4}$=9.8 Hz, $J_{4,5}$=10.0 Hz, H-4^{II}), 5.47 (dd, 1H, $J_{3,4}$=9.8 Hz, $J_{4,5}$=10.0 Hz, H-4^{I}), 7.28–7.45 (m, 10H, Ph). ^{13}C NMR (150 MHz, $CDCl_3$): δ 20.4, 20.66, 20.69, 20.7, 20.8, 20.83 (Ac-CH_3), 55.1 (OCH_3), 61.5 (C-7^{II}), 62.0 (C-7^{I}), 65.5 (C-4^{II}), 66.9 (C-6^{I}), 67.1 (C-6^{II}), 67.8 (C-4^{I}), 68.8 (C-5^{I}), 69.2 (C-5^{II}), 70.8 (C-3^{II}), 72.6 (OCH_2Ph), 73.4 (OCH_2Ph), 74.4 (C-3^{I}), 75.1 (C-2^{I}), 75.5 (C-2^{II}), 99.2 (C-1^{II}), 99.4 (C-1^{I}), 128.0, 128.1, 128.2, 128.4, 128.7, 129.0, 137.4, 137.6 (Ph), 169.3, 169.4, 169.7, 170.1, 170.3, 170.5, 170.6 (Ac: O=C). ESI-HRMS for $C_{43}H_{54}O_{20}$: 913.3106 $[M+Na]^+$. Found 913.3093.

6.24 (3,4,6,7-Tetra-*O*-acetyl-2-*O*-benzyl-L-*glycero*-α-D-*manno*-heptopyranosyl)-(1-3)-4,6,7-tri-*O*-acetyl-2-*O*-benzyl-L-*glycero*-D-*manno*-heptopyranose (37)

Compound **36** (158.5 mg, 178.0 μmol) was dissolved in a mixture of H_2SO_4/AcOH/Ac_2O (4.0 mL, 0.1:6:14) and stirred for 2 h at room temperature. The

reaction mixture was neutralized by the addition of sodium acetate (1.2 g), poured into saturated sodium hydrogen carbonate and extracted with dichloromethane. The combined organic layer was washed with brine, dried over anhydrous sodium sulfate, filtered, and concentrated to a syrup (156.5 mg). The crude syrup was treated with hydrazine acetate (20.4 mg, 221.0 μmol) in DMF (1.2 mL) for 7 h at 0 °C. The reaction mixture was diluted with ethyl acetate, washed with water and brine, dried over anhydrous sodium sulfate, filtered, and concentrated. The residue was purified by silica gel column chromatography (ethyl acetate/hexane, 1:1) to give **37** (122.4 mg, 78%). ^{1}H-NMR (600 MHz, $CDCl_3$): δ 1.82, 1.95, 1.96, 2.05, 2.06, 2.10, 2.15 (s, 3H x 7, Ac), 3.03 (s, 1H, OH), 3.70 (dd, 1H, $J_{1,2}$=2.0 Hz, $J_{2,3}$=2.6 Hz, H-2^{I}), 3.72 (dd, 1H, $J_{4,5}$=10.0 Hz, $J_{5,6}$=1.8 Hz, H-5^{II}), 3.81 (dd, 1H, $J_{1,2}$=1.6 Hz, $J_{2,3}$=3.0 Hz, H-2^{II}), 4.07 (dd, 1H, $J_{4,5}$=10.2 Hz, $J_{5,6}$=2.0 Hz, H-5^{I}), 4.10 (dd, 1H, $J_{6,7a}$=5.2 Hz, $J_{7a,7b}$=11.4 Hz, H-7a^{II}), 4.14 (dd, 1H, $J_{6,7b}$=nd Hz, $J_{7a,7b}$=11.4 Hz, H-7b^{II}), 4.14 (dd, 1H, $J_{6,7a}$=nd Hz, $J_{7a,7b}$=11.4 Hz, H-7a^{I}), 4.16 (dd, 1H, $J_{2,3}$=2.6 Hz, $J_{3,4}$=7.0 Hz, H-3^{I}), 4.38 (dd, 1H, $J_{6,7b}$=5.4 Hz, $J_{7a,7b}$=11.4 Hz, H-7b^{I}), 4.59, 4.64 (d, 2H, J=12.2 Hz, OCH_2Ph), 4.82, 4.85 (d, 2H, J=12.8 Hz, OCH_2Ph), 5.04 (ddd, 1H, $J_{5,6}$=1.8 Hz, $J_{6,7a}$=5.2 Hz, $J_{6,7b}$=nd Hz, H-6^{II}), 5.06 (d, 1H, $J_{1,2}$=1.6 Hz, H-1^{II}), 5.18 (ddd, 1H, $J_{5,6}$=2.0 Hz, $J_{6,7a}$=nd Hz, $J_{6,7b}$=5.4 Hz, H-6^{I}), 5.30 (d, 1H, $J_{1,2}$=2.0 Hz, H-1^{I}), 5.31 (dd, 1H, $J_{2,3}$=3.0 Hz, $J_{3,4}$=10.2 Hz, H-3^{II}), 5.40 (dd, 1H, $J_{3,4}$=10.2 Hz, $J_{4,5}$=10.0 Hz, H-4^{II}), 5.47 (dd, 1H, $J_{3,4}$=7.0 Hz, $J_{4,5}$=10.2 Hz, H-4^{I}), 7.30–7.46 (m, 10H, Ph). ^{13}C NMR (150 MHz, $CDCl_3$): δ 20.6, 20.7, 20.75, 20.9, 21.5 (Ac-CH_3), 61.7 (C-7^{II}), 62.6 (C-7^{I}), 65.5 (C-4^{II}), 67.1 (C-6^{I}), 67.16 (C-6^{II}), 68.0 (C-4^{I}), 68.9 (C-5^{I}), 69.3 (C-5^{II}), 70.7 (C-3^{II}), 72.6 (OCH_2Ph), 73.4 (OCH_2Ph), 74.2 (C-3^{I}), 75.4 (C-2^{I}), 75.5 (C-2^{II}), 92.9 (C-1^{I}), 99.4 (C-1^{II}), 128.0, 128.2, 128.25, 128.5, 128.7, 129.1, 137.4, 137.6 (Ph), 169.4, 169.5, 169.8, 170.4, 170.7, 171.1 (Ac: O=C). ESI-HRMS for $C_{42}H_{52}O_{20}$: 899.2950 $[M+Na]^+$. Found 899.2930.

6.25 (3,4,6,7-Tetra-*O*-acetyl-2-*O*-benzyl-L-*glycero*-α-D-*manno*-heptopyranosyl)-(1-3)-4,6,7-tri-*O*-acetyl-2-*O*-benzyl-L-*glycero*-D-*manno*-heptopyranosyl trichloro-acetimidate (38)

Trichloroacetonitrile (192.0 μL, 1.9 mmol) was added to a solution of **37** (139.9

mg, 160.0 μmol) in dry dichloromethane (1.6 mL) under argon. Potassium carbonate (113.0 mg, 0.8 mmol) was added to the reaction. After stirring for 21 h at room temperature, the mixture was filtered through Celite. The solution was concentrated and purified by flash column chromatography (ethyl acetate/hexane, 1:1) to give **38** as a mixture of anomers (129.5 mg, 80%, α/β=6:1). α-isomer: $[\alpha]^{25}_{D}$= +18.6 (*c* 1.5, $CHCl_3$), ^{1}H-NMR (600 MHz, $CDCl_3$): δ 1.90, 1.94, 1.97, 2.01, 2.05, 2.06, 2.13 (s, 3H x 7, Ac), 3.40 (dd, 1H, $J_{4,5}$=10.0 Hz, $J_{5,6}$=2.0 Hz, H-5^{II}), 3.75 (dd, 1H, $J_{1,2}$=1.4 Hz, $J_{2,3}$=3.0 Hz, H-2^{II}), 3.89 (dd, 1H, $J_{6,7a}$=4.0 Hz, $J_{7a,7b}$=11.6 Hz, H-7a^{II}), 3.94 (dd, 1H, $J_{1,2}$=1.6 Hz, $J_{2,3}$=3.2 Hz, H-2^{I}), 4.05 (dd, 1H, $J_{2,3}$=3.2 Hz, $J_{3,4}$=6.6 Hz, H-3^{I}), 4.08 (dd, 1H, $J_{4,5}$=10.0 Hz, $J_{5,6}$=2.0 Hz, H-5^{I}), 4.11 (dd, 1H, $J_{6,7b}$=7.2 Hz, $J_{7a,7b}$=11.6 Hz, H-7b^{II}), 4.16 (dd, 1H, $J_{6,7a}$=7.6 Hz, $J_{7a,7b}$=11.4 Hz, H-7a^{I}), 4.27 (dd, 1H, $J_{6,7b}$=5.4 Hz, $J_{7a,7b}$=11.4 Hz, H-7b^{I}), 4.60, 4.62 (d, 2H, *J*=12.2 Hz, OCH_2Ph), 4.73, 4.91 (d, 2H, *J*=12.4 Hz, OCH_2Ph), 4.98 (ddd, 1H, $J_{5,6}$=2.0 Hz, $J_{6,7a}$=4.0 Hz, $J_{6,7b}$=8.6 Hz, H-6^{II}), 5.02 (d, 1H, $J_{1,2}$=1.4 Hz, H-1^{II}), 5.20 (dd, 1H, $J_{2,3}$=3.0 Hz, $J_{3,4}$=10.2 Hz, H-3^{II}), 5.21 (ddd, 1H, $J_{5,6}$=2.0 Hz, $J_{6,7a}$=7.6 Hz, $J_{6,7b}$=5.4 Hz, H-6^{I}), 5.31 (dd, $J_{3,4}$=10.2 Hz, $J_{4,5}$=10.0 Hz, H-4^{II}), 5.52 (dd, $J_{3,4}$=6.6 Hz, $J_{4,5}$=10.0 Hz, H-4^{I}), 6.40 (d, 1H, $J_{1,2}$=1.6 Hz, H-1^{I}), 7.27–7.50 (m, 10H, Ph), 8.80 (s, 1H, NH). ^{13}C NMR (150 MHz, $CDCl_3$): δ 20.6, 20.7, 20.76, 20.8 (Ac-CH_3), 62.1 (C-7^{I}), 62.6 (C-7^{II}), 65.3 (C-4^{II}), 66.7 (C-6^{I}), 66.9 (C-4^{I}), 67.2 (C-6^{II}), 69.8 (C-5^{II}), 70.8 (C-3^{II}), 71.5 (C-5^{I}), 72.2 (OCH_2Ph), 73.5 (OCH_2Ph), 73.7 (C-2^{I}), 75.3 (C-3^{I}), 75.8 (C-2^{II}), 90.6 ($OCNHCCl_3$), 95.0 (C-1^{I}), 100.2 (C-1^{II}), 127.8, 128.0, 128.2, 128.5, 128.6, 128.9, 136.9, 137.4 (Ph), 159.5 ($OCNHCCl_3$), 169.4, 169.7, 170.2, 170.5 (Ac: O=C). ESI-HRMS for $C_{44}H_{52}Cl_3NO_{20}$: 1042.2046 $[M+Na]^+$. Found 1042.2051. β-isomer: $[\alpha]^{25}_{D}$= -12.6 (*c* 0.3, $CHCl_3$), ^{1}H-NMR (600 MHz, $CDCl_3$): δ 1.75, 1.88, 1.90, 1.97, 1.98, 2.02, 2.05 (s, 3H x 7, Ac), 3.52 (dd, 1H, $J_{4,5}$=9.8 Hz, $J_{5,6}$=2.0 Hz, H-5^{II}), 3.64 (dd, 1H, $J_{4,5}$=10.0 Hz, $J_{5,6}$=2.6 Hz, H-5^{I}), 3.74 (dd, 1H, $J_{1,2}$=2.0 Hz, $J_{2,3}$=3.0 Hz, H-2^{II}), 3.78 (dd, 1H, $J_{2,3}$=2.8 Hz, $J_{3,4}$=9.6 Hz, H-3^{I}), 3.89 (dd, 1H, $J_{1,2}$=nd Hz, $J_{2,3}$=2.8 Hz, H-2^{I}), 4.00 (dd, 1H, $J_{6,7a}$=7.8 Hz, $J_{7a,7b}$=11.6 Hz, H-7a^{I}), 4.03 (dd, 1H, $J_{6,7a}$=6.6 Hz, $J_{7a,7b}$=11.2 Hz, H-7a^{II}), 4.11 (dd, 1H, $J_{6,7b}$=6.4 Hz, $J_{7a,7b}$=11.2 Hz, H-7b^{II}), 4.38 (dd, 1H, $J_{6,7b}$=5.0 Hz, $J_{7a,7b}$=11.6 Hz, H-7b^{I}), 4.53, 4.55 (d, 2H, *J*=12.8 Hz, OCH_2Ph), 4.98 (ddd, 1H, $J_{5,6}$=2.0 Hz, $J_{6,7a}$=6.6

Hz, $J_{6,7b}$=6.4 Hz, H-6^{II}), 4.93, 4.98 (d, 2H, J=12.6 Hz, OCH_2Ph), 4.97 (d, 1H, $J_{1,2}$=2.0 Hz, H-1^{II}), 5.20 (ddd, 1H, $J_{5,6}$=2.0 Hz, $J_{6,7a}$=7.8 Hz, $J_{6,7b}$=5.0 Hz, H-6^{I}), 5.25 (dd, 1H, $J_{2,3}$=3.0 Hz, $J_{3,4}$=10.2 Hz, H-3^{II}), 5.32 (dd, 1H, $J_{3,4}$=10.2 Hz, $J_{4,5}$=9.8 Hz, H-4^{II}), 5.46 (dd, 1H, $J_{3,4}$=9.6 Hz, $J_{4,5}$=10.0 Hz, H-4^{I}), 5.72 (d, 1H, $J_{1,2}$=nd Hz, H-1^{I}), 7.19–7.48 (m, 10H, Ph), 8.69 (s, 1H, NH). ^{13}C NMR (150 MHz, $CDCl_3$): δ 19.5, 19.7, 19.71, 19.8, 19.83, 19.9 (Ac-CH_3), 60.0 (C-7^{I}), 61.3 (C-7^{II}), 64.4 (C-4^{II}), 65.6 (C-6^{I}), 66.0 (C-4^{I}), 66.5 (C-6^{II}), 68.1 (C-5^{II}), 69.5 (C-3^{II}), 72.4 (C-5^{I}), 72.5 (OCH_2Ph), 72.53 (OCH_2Ph), 73.1 (C-2^{I}), 74.5 (C-3^{I}), 75.9 (C-2^{II}), 89.4 ($OCNHCCl_3$), 96.0 (C-1^{I}), 98.4 (C-1^{II}), 126.8, 127.0, 127.0, 127.4, 127.6, 136.3, 136.4 (Ph), 159.7 ($OCNHCCl_3$), 168.2, 168.4, 168.7, 169.1, 169.2, 169.6,169.8 (Ac: O=C). ESI-HRMS for $C_{44}H_{52}Cl_3NO_{20}$: 1042.2046 $[M+Na]^+$. Found 1042.2012.

6.26 (2,3,4,6-Tetra-*O*-benzyl-β-D-galactopyranosyl)-(1-4)-(2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl)-(1-5)-[methyl *O*-[methyl (7,8-di-*O*-benzoyl-4,5-*O*-isopropylidene-3-deoxy-α-D-*manno*-2-octulopyranosyl)onate]]-(2-4)-(allyl 7,8-di-*O*-benzoyl-3-deoxy-α-D-*manno*-2-octulopyranosid)onate (41)

A mixture of **6**α (10.0 mg, 10.2 μmol), and **40** (34.1 mg, 30.5 μmol), and MS-AW 300 molecular sieves (10.0 mg) was suspended in diethyl ether and dichloromethane (3:1, 0.4 mL). The reaction mixture was stirred for 1 h under argon, and cooled to 0 °C. Then, 0.01 M TMSOTf (60.0 μL, 0.6 μmol) in dichloromethane was added dropwise to the reaction mixture. After stirring for 1 h, the reaction was warmed to room temperature and stirred for 1 h. The reaction solution was neutralized by the addition of a few drops of triethylamine and aqueous saturated sodium hydrogen carbonate. The reaction mixture was diluted with dichloromethane and was filtered through Celite. The filtrate was extracted twice with dichloromethane. The combined organic phase was dried over anhydrous sodium sulfate, filtered, and concentrated. The residue was purified by BioRad S-X1 size exclusion beads (toluene/ethyl acetate, 1:1) and TLC chromatography(ethyl acetate/hexane, 1:3) to give **41** (4.0 mg, 20%). $[\alpha]^{25}_D$= +41.0 (*c* 0.5, $CHCl_3$), ^{1}H-NMR (600 MHz, $CDCl_3$): δ 0.98 (dd, 1H, $J_{3a,3b}$=15.4 Hz, $J_{3a,4}$=2.4 Hz, H-3a^{II}), 1.18 (s, 3H, Me), 1.34 (s, 3H, Me), 2.23 (dd, 1H, $J_{3a,3b}$=12.4 Hz, $J_{3a,4}$=5.0 Hz, H-3a^{I}), 2.26 (dd, 1H, $J_{3a,3b}$=12.4 Hz,

$J_{3b,4}$=11.8 Hz, H-3b^{I}), 2.59 (dd, 1H, $J_{3a,3b}$=15.4 Hz, $J_{3b,4}$=3.4 Hz, H-3b^{II}), 3.31 (ddd, 1H, $J_{4,5}$=nd Hz, $J_{5,6a}$=5.0 Hz, $J_{5,6b}$=nd Hz, H-5^{IV}), 3.32 (dd, 1H, $J_{2,3}$=9.8 Hz, $J_{3,4}$=2.8 Hz, H-3^{IV}), 3.38 (dd, 1H, $J_{5,6a}$=5.0 Hz, $J_{6a,6b}$=8.8 Hz, H-6a^{IV}), 3.38 (s, 3H, OMe^{I}), 3.48 (s, 3H, OMe^{II}), 3.51 (dd, 1H, $J_{1,2}$=3.4 Hz, $J_{2,3}$=9.8 Hz, H-2^{III}), 3.56 (dd, 1H, $J_{5,6b}$=nd Hz, $J_{6a,6b}$=8.8 Hz, H-6b^{IV}), 3.64 (dd, 1H, $J_{5,6a}$=1.6 Hz, $J_{6a,6b}$=9.2 Hz, H-6a^{III}), 3.69 (dd, 1H, $J_{4,5}$=3.0 Hz, $J_{5,6}$=1.2 Hz, H-5^{I}), 3.74 (dd, 1H, $J_{1,2}$=7.8 Hz, $J_{2,3}$=9.8 Hz, H-2^{IV}), 3.86 (dd, 1H, $J_{5,6}$=1.6 Hz, $J_{6,7}$=7.6 Hz, H-6^{II}), 3.86 (dddd, 1H, J=1.4, 3.2, 4.8, 13.0 Hz, OCH_2-), 3.90 (dd, 1H, $J_{3,4}$=2.8 Hz, $J_{4,5}$=nd Hz, H-4^{IV}), 3.90 (dd, 1H, $J_{2,3}$=9.8 Hz, $J_{3,4}$=nd Hz, H-3^{III}), 3.94 (dd, 1H, $J_{5,6b}$=nd Hz, $J_{6a,6b}$=9.2 Hz, H-6b^{III}), 3.94 (ddd, 1H, $J_{3a,4}$=2.4 Hz, $J_{3b,4}$=3.4 Hz, $J_{4,5}$=nd Hz, H-4^{II}), 3.95 (dddd, 1H, J=nd Hz, OCH_2-), 4.03 (dd, 1H, $J_{5,6}$=1.2 Hz, $J_{6,7}$=9.4 Hz, H-6^{I}), 4.04 (dd, 1H, $J_{4,5}$=nd Hz, $J_{5,6}$=1.6 Hz, H-5^{II}), 4.05 (dd, 1H, $J_{3,4}$=nd Hz, $J_{4,5}$=10.0 Hz, H-4^{III}), 4.11 (ddd, 1H, $J_{4,5}$=10.0 Hz, $J_{5,6a}$=1.6 Hz, $J_{5,6b}$=nd Hz, H-5^{III}), 4.25, 4.37 (d, 2H, J=11.8 Hz, CH_2Ph), 4.28 (dd, 1H, $J_{7,8a}$=3.4 Hz, $J_{8a,8b}$=12.6 Hz, H-8a^{I}), 4.35 (d, 1H, $J_{1,2}$=7.8 Hz, H-1^{IV}), 4.36, 4.60 (d, 2H, J=12.0 Hz, CH_2Ph), 4.46 (dd, 1H, $J_{7,8b}$=2.6 Hz, $J_{8a,8b}$=12.6 Hz, H-8b^{I}), 4.55, 4.97 (d, 2H, J=11.2 Hz, CH_2Ph), 4.58 (dd, 1H, $J_{7,8a}$=4.6 Hz, $J_{8a,8b}$=12.8 Hz, H-8a^{II}), 4.62, 4.66 (d, 2H, J=12.0 Hz, CH_2Ph), 4.70 (ddd, 1H, $J_{3a,4}$=5.0 Hz, $J_{3b,4}$=11.8 Hz, $J_{4,5}$=3.0 Hz, H-4^{I}), 4.70, 5.01 (d, 2H, J=10.4 Hz, CH_2Ph), 4.77, 5.00 (d, 2H, J=12.4 Hz, CH_2Ph), 4.86, 4.87 (d, 2H, J=nd Hz, CH_2Ph), 4.86 (dddd, 1H, J=nd Hz, =CH_2), 4.88 (d, 1H, $J_{1,2}$=3.4 Hz, H-1^{III}), 4.93 (dddd, 1H, J=1.6, 1.6, 3.2, 17.2 Hz, =CH_2), 5.30 (dd, 1H, $J_{7,8b}$=2.6 Hz, $J_{8a,8b}$=12.8 Hz, H-8b^{II}), 5.58-5.64 (m,1H, -CH=), 5.59 (ddd, 1H, $J_{6,7}$=7.6 Hz, $J_{7,8a}$=4.6 Hz, $J_{7,8b}$=2.6 Hz, H-7^{II}), 5.75 (ddd, 1H, $J_{6,7}$=9.4 Hz, $J_{7,8a}$=3.4 Hz, $J_{7,8b}$=2.6 Hz, H-7^{I}), 7.03–7.56 (m, 47H, Ar), 7.84–8.00 (m, 8H, Ar). ^{13}C NMR (150 MHz, $CDCl_3$): δ 24.6 and 25.1 (Isop-Me), 31.4 (C-3^{II}), 34.4 (C-3^{I}), 52.0 (OMe^{I}), 52.1 (OMe^{II}), 62.1 (C-8^{I}), 62.1 (C-8^{II}), 64.7 (OCH_2-), 67.7 (C-4^{I}), 67.9 (C-6^{III}), 68.1 (C-6^{IV}), 69.3 (C-7^{I}), 69.8 (C-4^{II}), 69.9 (C-6^{II}), 70.66 (C-7^{II}), 70.7 (C-6^{I}), 71.0 (C-5^{III}), 72.03 (C-5^{I}), 72.06 (C-5^{II}), 72.3, 72.6, 73.0, 73.4, 74.4, 74.7, 75.3 (OCH_2Ph), 72.9 (C-5^{IV}), 73.7 (C-4^{IV}), 76.2 (C-4^{III}), 78.9 (C-2^{III}), 80.2 (C-2^{IV}), 80.3 (C-3^{III}), 82.3 (C-3^{IV}), 96.2 (C-2^{II}), 98.3 (C-1^{III}), 98.8 (C-2^{I}), 102.7 (C-1^{IV}), 109.6 (C_{isop}), 116.2 (=CH_2), 126.3, 126.7, 126.9, 127.3, 127.4, 127.5, 127.7, 127.8, 127.9, 128.0, 128.1,

128.14, 128.2, 128.3, 128.31, 128.4, 128.40, 128.45, 128.6, 129.6, 129.65, 129.7, 129.75, 129.8, 130.0, 130.3, 132.9, 133.0, 133.1, 133.4 (Ar), 133.6 (-CH=), 138.14, 138.2, 138.5, 139.0, 139.1, 139.3, 139.9 (Ar), 164.8, 1651, 165.8, 166.1 (Bz: C=O), 167.7 (C-1^{I}), 168.7 (C-1^{II}). MALDI-TOF MS for $C_{113}H_{116}O_{29}$: 1959.750 $[M+Na]^+$. Found 1959.300.

6.27 (2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-(1-4)-(2,3,6-tri-*O*-acetyl-β-D-g-lucopyranosyl)-(1-4)-(3,6,7-tri-*O*-acetyl-2-*O*-benzyl-L-*glycero*-α-D-*manno*-heptop-yranosyl)-(1-5)-[methyl *O*-[methyl (7,8-di-*O*-benzoyl-4,5-*O*-isopropylidene-3-deo-xy-α-D-*manno*-2-octulopyranosyl)onate]]-(2-4)-(allyl 7,8-di-*O*-benzoyl-3-deoxy-α-D-*manno*-2-octulopyranosid)onate (43)

A mixture of **6**α (42.0 mg, 42.7 μmol), **31** (96.0 mg, 84.1 μmol), and MS-AW 300 molecular sieves (43.0 mg) was suspended in dry dichloromethane (1.6 mL). The reaction mixture was stirred for 1 h under argon, and then 0.01 M TMSOTf (260.0 μL, 2.5 μmol) in dichloromethane was added dropwise to the reaction mixture. After stirring for 2 h, the reaction was neutralized by the addition of a few drops of triethylamine and aqueous saturated sodium hydrogen carbonate. The reaction mixture was diluted with dichloromethane and filtered through Celite. The filtrate was extracted twice with dichloromethane. The combined organic phase was dried over anhydrous sodium sulfate, filtered and concentrated. The residue was purified by BioRad S-X1 size exclusion beads (toluene/ethyl acetate, 1:1) and TLC chromatography (ethyl acetate/hexane, 2:1) to give **43** (22.8 mg, 26%). $[\alpha]^{25}_{D}$= +2.9 (*c* 1.9, $CHCl_3$), ^{1}H-NMR (600 MHz, $CDCl_3$): δ 1.22 (s, 3H, Me), 1.41 (s, 3H, Me), 1.90, 1.91, 1.95, 1.99, 2.01, 2.02, 2.03, 2.03, 2.05, 2.14 (s, 3H x 10, Ac), 2.02 (dd, 1H, $J_{3a,3b}$=15.6 Hz, $J_{3a,4}$=2.2 Hz, H-$3a^{II}$), 2.18 (dd, 1H, $J_{3a,3b}$=12.6 Hz, $J_{3a,4}$=12.4 Hz, H-$3a^{I}$), 2.38 (dd, 1H, $J_{3a,3b}$=12.6 Hz, $J_{3b,4}$=4.4 Hz, H-$3b^{I}$), 2.91 (dd, 1H, $J_{3a,3b}$=15.6 Hz, $J_{3b,4}$=3.4 Hz, H-$3b^{II}$), 3.49 (s, 3H, OMe^{I}), 3.55 (s, 3H, OMe^{II}), 3.54 (ddd, 1H, $J_{4,5}$=9.6 Hz, $J_{5,6a}$=5.0 Hz, $J_{5,6b}$=1.6 Hz, H-5^{IV}), 3.79 (dd, 1H, $J_{4,5}$=nd Hz, $J_{5,6}$=nd Hz, H-5^{I}), 3.79 (dd, 1H, $J_{3,4}$=9.0 Hz, $J_{4,5}$=9.6 Hz, H-4^{IV}), 3.82 (ddd, 1H, $J_{4,5}$=0.6 Hz, $J_{5,6a}$=7.2 Hz, $J_{5,6b}$=6.2 Hz, H-5^{V}), 3.82 (dd, 1H, $J_{1,2}$=1.6 Hz, $J_{2,3}$=2.8 Hz, H-2^{III}), 3.85 (dd, 1H, $J_{3,4}$=9.4 Hz, $J_{4,5}$=10.2 Hz, H-4^{III}), 3.91 (dd, 1H, *J*=nd Hz, OCH_2-), 3.92 (dd, 1H,

$J_{6,7a}$=7.8 Hz, $J_{7a,7b}$=11.4 Hz, H-7a^{I}), 3.95 (dd, 1H, $J_{4,5}$=10.2 Hz, $J_{5,6}$=nd Hz, H-5^{III}), 3.97 (dd, 1H, J=nd Hz, OCH_2-), 4.07 (dd, 1H, $J_{5,6a}$=7.2 Hz, $J_{6a,6b}$=11.2 Hz, H-6a^{V}), 4.08 (dd, 1H, $J_{5,6}$=nd Hz, $J_{6,7}$=9.4 Hz, H-6^{I}), 4.08 (dd, 1H, $J_{5,6a}$=5.0 Hz, $J_{6a,6b}$=12.0 Hz, H-6a^{IV}), 4.11 (dd, 1H, $J_{5,6b}$=6.2 Hz, $J_{6a,6b}$=11.2 Hz, H-6b^{V}), 4.22 (dd, 1H, $J_{6,7b}$=5.2 Hz, $J_{7a,7b}$=11.4 Hz, H-7b^{I}), 4.30 (dd, 1H, $J_{5,6}$=1.6 Hz, $J_{6,7}$=6.0 Hz, H-6^{II}), 4.38 (dd, 1H, $J_{4,5}$=7.8 Hz, $J_{5,6}$=1.6 Hz, H-5^{II}), 4.47 (d, 1H, $J_{1,2}$=7.8 Hz, H-1^{IV}), 4.35 (dd, 1H, $J_{5,6b}$=1.6 Hz, $J_{6a,6b}$=12.0 Hz, H-6b^{IV}), 4.43 (d, 1H, $J_{1,2}$=8.0 Hz, H-1^{V}), 4.45, 4.54 (d, 2H, J=12.0 Hz, CH_2Ph), 4.46 (ddd, 1H, $J_{3a,4}$=12.4 Hz, $J_{3b,4}$=4.4 Hz, $J_{4,5}$=nd Hz, H-4^{I}), 4.55 (ddd, 1H, $J_{3a,4}$=2.2 Hz, $J_{3b,4}$=3.4 Hz, $J_{4,5}$=7.8 Hz, H-4^{II}), 4.62 (dd, 1H, $J_{7,8a}$=6.2 Hz, $J_{8a,8b}$=12.4 Hz, H-8a^{II}), 4.63 (dd, 1H, $J_{7,8a}$=3.8 Hz, $J_{8a,8b}$=12.4 Hz, H-8a^{I}), 4.79 (dd, 1H, $J_{1,2}$=7.8 Hz, $J_{2,3}$=8.8 Hz, H-2^{IV}), 4.88 (dd, 1H, $J_{7,8b}$=2.4 Hz, $J_{8a,8b}$=12.4 Hz, H-8b^{I}), 4.91 (dd, 1H, $J_{2,3}$=10.4 Hz, $J_{3,4}$=3.4 Hz, H-3^{V}), 4.93 (dd, 1H, J=1.4, nd Hz, =CH_2), 5.01 (d, 1H, $J_{1,2}$=1.6 Hz, H-1^{III}), 5.05 (dd, 1H, J=1.6, 1.6, 3.2, 17.2 Hz, =CH_2), 5.08 (dd, 1H, $J_{1,2}$=8.0 Hz, $J_{2,3}$=10.4 Hz, H-2^{V}), 5.12 (dd, 1H, $J_{2,3}$=8.8 Hz, $J_{3,4}$=9.0 Hz, H-3^{IV}), 5.18 (dd, 1H, $J_{7,8b}$=2.2 Hz, $J_{8a,8b}$=12.4 Hz, H-8b^{II}), 5.30 (ddd, 1H, $J_{5,6}$=nd Hz, $J_{6,7a}$=7.8 Hz, $J_{6,7b}$=5.2 Hz, H-6^{III}), 5.33 (dd, 1H, $J_{3,4}$=3.4 Hz, $J_{4,5}$=0.6 Hz, H-4^{III}), 5.34 (dd, 1H, $J_{2,3}$=2.8 Hz, $J_{3,4}$=9.4 Hz, H-3^{III}), 5.58 (ddd, 1H, $J_{6,7}$=9.4 Hz, $J_{7,8a}$=3.8 Hz, $J_{7,8b}$=2.4 Hz, H-7^{I}), 5.63-5.69 (m,1H, -CH=), 5.75 (ddd, 1H, $J_{6,7}$=6.0 Hz, $J_{7,8a}$=6.2 Hz, $J_{7,8b}$=2.2 Hz, H-7^{II}), 7.27–7.57 (m, 17H, Ar), 7.94–8.00 (m, 8H, Ar). ^{13}C NMR (150 MHz, $CDCl_3$): δ 20.5, 20.54, 20.7, 20.8, 20.84, 20.9, 23.0, 23.8 (Ac-CH_3), 24.6 and 25.1 (Isop-Me), 32.0 (C-3^{II}), 34.7 (C-3^{I}), 52.2 (OMe^{II}), 52.3 (OMe^{I}), 64.5 (OCH_2-), 60.7 (C-6^{V}), 62.3 (C-6^{IV}), 62.4 (C-8^{I}), 63.2 (C-8^{II}), 63.5 (C-7^{III}), 66.6 (C-4^{V}), 68.2 (C-6^{III}), 68.22 (C-4^{I}), 69.0 (C-2^{V}), 69.67 (C-7^{I}), 69.7 (C-3^{III}), 70.0 (C-4^{II}), 70.5 (C-6^{I}), 70.6 (C-5^{V}), 70.91 (C-7^{II}), 70.9 (C-5^{III}), 71.0 (C-3^{V}), 71.0 (C-6^{II}), 71.4 (C-5^{I}), 71.8 (C-2^{IV}), 72.1 (C-5^{IV}), 72.3 (OCH_2Ph), 72.5 (C-5^{II}), 73.3 (C-3^{IV}), 73.9 (C-4^{III}), 76.9 (C-2^{III}), 76.3 (C-4^{IV}), 97.88 (C-2^{II}), 97.9 (C-1^{III}), 98.7 (C-2^{I}), 100.2 (C-1^{IV}), 101.1 (C-1^{V}), 109.5 (C_{isop}), 116.0 (=CH_2), 127.4, 127.6, 128.2, 128.3, 128.4, 128.5, 128.6, 128.8, 129.4, 129.7, 129.8, 130.1, 130.2, 130.9, 132.8, 133.0, 133.2, 133.5 (Ar), 133.53 (-CH=), 138.4 (Ar), 165.2, 165.3, 166.0, 166.2 (Bz: C=O), 167.6 (C-1^{I}), 169.1 (C-1^{II}), 169.6, 169.6, 169.7, 170.1, 170.2, 170.3, 170.4, 170.4 (Ac: C=O).

MALDI-TOF MS for $C_{98}H_{112}O_{45}$: 2031.638 $[M+Na]^+$. Found 2030.629.

6.28 (3,4,6,7-Tetra-*O*-acetyl-2-*O*-benzyl-L-*glycero*-α-D-*manno*-heptopyranosyl)-(1-3)-(2-*O*-benzyl-4,6,7-tri-*O*-acetyl-L-*glycero*-α-D-*manno*-heptopyranosyl)-(1-5)-[methyl *O*-[methyl (7,8-di-*O*-benzoyl-4,5-*O*-isopropylidene-3-deoxy-α-D-*manno*-2-octulopyranosyl)onate]]-(2-4)-(allyl 7,8-di-*O*-benzoyl-3-deoxy-α-D-*manno*-2-oct-ulopyranosid)onate (44)

A mixture of **6**α (62.3 mg, 63.4 μmol), **38** (129.5 mg, 126.8 μmol, α/β=6:1), and MS-AW 300 molecular sieves (62 mg) was suspended in dichloromethane (2.7 mL). The reaction mixture was stirred for 1 h under argon, and then 0.01 M TMSOTf (380.0 μL, 3.8 μmol) in dichloromethane was added dropwise to the reaction mixture. After stirring for 1 h, the reaction was neutralized by the addition of a few drops of triethylamine and aqueous saturated sodium hydrogen carbonate. The reaction mixture was diluted with dichloromethane and filtered through Celite. The filtrate was extracted twice with dichloromethane. The combined organic phase was dried over anhydrous sodium sulfate, filtered and concentrated. The residue was purified by BioRad S-X1 size exclusion beads (toluene/ethyl acetate, 1:1) and TLC chromatography (ethyl acetate/hexane, 3:2) to give **44** (66.9 mg, 57%). $[\alpha]^{25}_D$= -7.0 (*c* 1.0, $CHCl_3$), ^{1}H-NMR (600 MHz, $CDCl_3$): δ 1.19 (s, 3H, Me), 1.36 (s, 3H, Me), 1.88, 1.93, 1.94, 1.97, 1.99, 2.01, 2.11 (s, 3H x 7, Ac), 2.02 (dd, 1H, $J_{3a,3b}$=15.6 Hz, $J_{3a,4}$=2.6 Hz, H-3a^{II}), 2.08 (dd, 1H, $J_{3a,3b}$=12.4 Hz, $J_{3a,4}$=12.2 Hz, H-3a^{I}), 2.25 (dd, 1H, $J_{3a,3b}$=12.4 Hz, $J_{3b,4}$=4.0 Hz, H-3b^{I}), 2.96 (dd, 1H, $J_{3a,3b}$=15.6 Hz, $J_{3b,4}$=3.6 Hz, H-3b^{II}), 3.38 (s, 3H, OMe^{I}), 3.43 (s, 3H, OMe^{II}), 3.69 (dd, 1H, $J_{4,5}$=2.2 Hz, $J_{5,6}$=nd Hz, H-5^{I}), 3.80 (dd, 1H, $J_{1,2}$=1.8 Hz, $J_{2,3}$=3.0 Hz, H-2^{IV}), 3.89 (dd, 1H, *J*=1.6, 3.0, 4.8, 13.0 Hz, OCH_2-), 3.94 (dd, 1H, *J*=1.4, 2.8, 5.6, nd Hz, OCH_2-), 3.95 (dd, 1H, $J_{4,5}$=9.8 Hz, $J_{5,6}$=8.2 Hz, H-5^{IV}), 3.95 (dd, 1H, $J_{1,2}$=1.6 Hz, $J_{2,3}$=2.4 Hz, H-2^{III}), 4.06 (dd, 1H, $J_{2,3}$=2.4 Hz, $J_{3,4}$=10.0 Hz, H-3^{III}), 4.09 (dd, 1H, $J_{5,6}$=nd Hz, $J_{6,7}$=9.6 Hz, H-6^{I}), 4.10 (dd, 1H, $J_{4,5}$=9.8 Hz, $J_{5,6}$=8.6 Hz, H-5^{III}), 4.21 (dd, 1H, $J_{6,7a}$=6.0 Hz, $J_{7a,7b}$=nd Hz, H-7a^{IV}), 4.21 (dd, 1H, $J_{6,7b}$=2.0 Hz, $J_{7a,7b}$=nd Hz, H-7b^{IV}), 4.24 (dd, 1H, $J_{6,7a}$=5.8 Hz, $J_{7a,7b}$=11.8 Hz, H-7a^{III}), 4.26 (dd, 1H, $J_{5,6}$=1.8 Hz, $J_{6,7}$=7.2 Hz, H-6^{II}), 4.32 (dd, 1H, $J_{6,7b}$=3.4 Hz, $J_{7a,7b}$=11.8 Hz, H-7b^{III}), 4.35 (dd, 1H, $J_{4,5}$=7.8 Hz, $J_{5,6}$=1.8 Hz, H-5^{II}),

4.49 (ddd, 1H, $J_{3a,4}$=2.6 Hz, $J_{3b,4}$=3.6 Hz, $J_{4,5}$=7.8 Hz, H-4^{II}), 4.59 (dd, 1H, $J_{7,8a}$=3.8 Hz, $J_{8a,8b}$=12.4 Hz, H-8a^{I}), 4.61 (dd, 1H, $J_{7,8a}$=4.2 Hz, $J_{8a,8b}$=12.4 Hz, H-8a^{II}), 4.61, 4.68 (d, 2H, *J*=nd Hz, CH_2Ph), 4.62, 4.70 (d, 2H, *J*=nd Hz, CH_2Ph), 4.67 (ddd, 1H, $J_{3a,4}$=12.2 Hz, $J_{3b,4}$=4.0 Hz, $J_{4,5}$=2.2 Hz, H-4^{I}), 4.80 (dd, 1H, $J_{7,8b}$=2.6 Hz, $J_{8a,8b}$=12.4 Hz, H-8b^{I}), 4.93 (dd, 1H, *J*=1.2, 1.6, 2.8, 10.6 Hz, =CH_2), 4.98 (dd, 1H, *J*=1.6, 1.6, 3.2, 17.2 Hz, =CH_2), 5.07 (d, 1H, $J_{1,2}$=1.6 Hz, H-1^{III}), 5.15 (dd, 1H, $J_{2,3}$=3.0 Hz, $J_{3,4}$=10.0 Hz, H-3^{IV}), 5.20 (ddd, 1H, $J_{5,6}$=8.2 Hz, $J_{6,7a}$=6.0 Hz, $J_{6,7b}$=2.0 Hz, H-6^{IV}), 5.21 (d, 1H, $J_{1,2}$=1.8 Hz, H-1^{IV}), 5.27 (dd, 1H, $J_{7,8b}$=2.4 Hz, $J_{8a,8b}$=12.4 Hz, H-8b^{II}), 5.31 (ddd, 1H, $J_{5,6}$=8.6 Hz, $J_{6,7a}$=5.8 Hz, $J_{6,7b}$=3.4 Hz, H-6^{III}), 5.41 (dd, $J_{3,4}$=10.0 Hz, $J_{4,5}$=9.8 Hz, H-4^{IV}), 5.52 (dd, $J_{3,4}$=10.0 Hz, $J_{4,5}$=9.8 Hz, H-4^{III}), 5.57 (ddd, 1H, $J_{6,7}$=9.6 Hz, $J_{7,8a}$=3.8 Hz, $J_{7,8b}$=2.6 Hz, H-7^{I}), 5.63–5.69 (m,1H, -CH=), 5.68 (ddd, 1H, $J_{6,7}$=7.2 Hz, $J_{7,8a}$=4.2 Hz, $J_{7,8b}$=2.4 Hz, H-7^{II}), 7.21–7.60 (m, 22H, Ar), 7.94–8.04 (m, 8H, Ar). ^{13}C NMR (150 MHz, $CDCl_3$): δ 20.6, 20.8, 20.83 and 20.87, 20.9 (Ac-CH_3), 24.6 and 25.1 (Isop-Me), 32.0 (C-3^{II}), 34.5 (C-3^{I}), 52.1 (OMe^{II}), 52.2 (OMe^{I}), 62.4 (C-8^{II}), 62.6 (C-8^{I}), 63.5 (C-7^{IV}), 64.1 (C-7^{III}), 64.8 (OCH_2-), 65.3 (C-4^{IV}), 66.9 (C-4^{III}), 67.6 (C-4^{I}), 67.7 (C-6^{III}), 68.0 (C-6^{IV}), 69.0 (C-7^{I}), 69.9 (C-4^{II}), 70.0 (C-5^{IV}), 70.1 (C-3^{III}), 70.3 (C-6^{I}), 70.3 (C-5^{III}), 70.3 (C-6^{II}), 70.6 (C-7^{II}), 71.2 (C-3^{IV}), 72.1, 72.9 (OCH_2Ph), 72.2 (C-5^{II}), 74.1 (C-5^{I}), 75.5 (C-2^{IV}), 77.2 (C-2^{III}), 97.0 (C-2^{II}), 98.8 (C-2^{I}), 99.0 (C-1^{IV}), 99.2 (C-1^{III}), 109.6 (C_{isop}), 116.4 (=CH_2), 127.3, 127.4, 127.7, 127.8, 128.3, 128.32, 128.4, 128.6, 128.8, 128.9, 129.5, 129.6, 129.7, 129.75, 129.9, 130.1, 132.9, 133.0, 133.3 (Ar), 133.4 (-CH=), 133.8, 137.8, 138.3 (Ar), 165.2, 165.4, 165.8, 166.2 (Bz: C=O), 167.4 (C-1^{I}), 169.4 (C-1^{II}), 169.6, 169.7, 169.8, 170.4, 170.5, 170.7, 170.8 (Ac: C=O). MALDI-TOF MS for $C_{94}H_{104}O_{38}$: 1863.611 $[M+Na]^+$. Found 1862.589.

6.29 (L-*Glycero*-α-D-*manno*-heptopyranosyl)-(1-3)-(L-*glycero*-α-D-*manno*-heptopyranosyl)-(1-5)-[*O*-(sodium 3-deoxy-α-D-*manno*-2-octulopyranosylonate)]-(2-4)-sodium (propyl 3-deoxy-α-D-*manno*-2-octulopyranosid)onate (45)

Compound **44** (10.0 mg, 5.4 μmol) in dry methanol (0.3 mL) was added to a suspension of $Pd(OH)_2$-C (20%, 0.3 mg) in dry methanol (0.2 mL), under a H_2 atmosphere with stirring at room temperature. After stirring for 3 days, insoluble

materials were removed by filtration through Celite and the filtrate was evaporated. The residue was dissolved in dichloromethane (0.7 mL) and aqueous 80% trifluoroacetic acid (80.0 μL) was added at room temperature. After stirring for 1 h, the solvent was removed by evaporation under an argon stream to give a crude compound that was not subjected to further purification. The crude compound was dissolved in methanol (1.0 mL), and then 0.1 M sodium hydroxide (1.3 mL, 0.13 mmol) was added at room temperature. After stirring for 24 h, the mixture was concentrated by evaporation. The residue was purified by gel filtration chromatography (Biogel P-2) and a Sep-Pak C18 column (H_2O) to give **45** (4.5 mg, 90%) as a colorless powder. $[\alpha]^{25}_D$=+127.6 (*c* 0.5, H_2O), ^{1}H-NMR (600 MHz, D_2O): δ 0.92 (t, 3H, *J*=7.4 Hz, CH_3), 1.54–1.61 (m, 2H, CH_2), 1.76 (dd, 1H, $J_{3a,3b}$=13.2 Hz, $J_{3a,4}$=12.6 Hz, H-3a^{II}), 1.93 (dd, 1H, $J_{3a,3b}$=12.6 Hz, $J_{3a,4}$=12.4 Hz, H-3a^{I}), 2.06 (dd, 1H, $J_{3a,3b}$=12.6 Hz, $J_{3b,4}$=4.2 Hz, H-3b^{I}), 2.20 (dd, 1H, $J_{3a,3b}$=13.2 Hz, $J_{3b,4}$=4.8 Hz, H-3b^{II}), 3.21–3.24 (m, 1H, OCH_2), 3.28–3.32 (m, 1H, OCH_2), 3.57 (dd, 1H, $J_{6,7}$=9.4 Hz, H-6^{I}), 3.61 (dd, 1H, $J_{7,8a}$=6.2 Hz, $J_{8a,8b}$=11.8 Hz, H-8a^{II}), 3.66 (dd, 1H, $J_{6,7}$=8.2 Hz, H-6^{II}), 3.68 (dd, 1H, $J_{6,7a}$=5.7 Hz, $J_{7a,7b}$=10.8 Hz, H-7a^{III}), 3.73–3.77 (m, 4H, H-7b^{III}, H-7a^{IV}, H-7b^{IV}, H-5^{III}), 3.81 (dd, 1H, $J_{7,8a}$=6.4 Hz, $J_{8a,8b}$=11.6 Hz, H-8a^{I}), 3.84–3.98 (m, 8H, H-7^{I}, H-3^{III}, H-4^{III}, H-7^{II}, H-8b^{II}, H-4^{IV}, H-5^{IV}, H-8b^{I}), 4.01–4.06 (m, 4H, H-5^{II}, H-3^{IV}, H-6^{III}, H-6^{IV}), 4.07 (dd, 1H, $J_{1,2}$=1.2 Hz, $J_{2,3}$=3.2 Hz, H-2^{III}), 4.09 (ddd, 1H, $J_{3a,4}$=12.6 Hz, $J_{3b,4}$=4.8 Hz, $J_{4,5}$=3.0 Hz, H-4^{II}), 4.13 (dd, 1H, $J_{1,2}$=1.6 Hz, $J_{2,3}$=3.0 Hz, H-2^{IV}), 4.22 (brs, 1H, $J_{4,5}$=2.2 Hz, H-5^{I}), 4.26 (ddd, 1H, $J_{3a,4}$=12.4 Hz, $J_{3b,4}$=4.2 Hz, $J_{4,5}$=2.2 Hz, H-4^{I}), 5.17 (d, 1H, $J_{1,2}$=1.2 Hz, H-1^{III}), 5.29 (d, 1H, $J_{1,2}$=1.6 Hz, H-1^{IV}). ^{13}C NMR (150 MHz, D_2O): δ 10.9 (CH_3), 23.9 (CH_2), 35.2 (C-3^{I}, C-3^{II}), 63.5 (C-7^{IV}, C-8^{I}), 64.0 (C-8^{II}), 64.6 (C-7^{III}), 65.4 (OCH_2,), 66.4 (C-4^{III}), 66.8 (C-4^{II}), 66.81 (C-5^{II}), 67.0 (C-4^{IV}), 69.4 (C-6^{III}), 69.7 (C-7^{I}), 70.0 (C-6^{IV}), 70.1 (C-4^{I}), 70.6 (C-2^{III}), 70.8 (C-2^{IV}), 70.83 (C-7^{II}), 71.1 (C-3^{IV}), 72.4 (C-5^{III}), 72.6 (C-6^{I}), 72.62 (C-6^{II}), 73.3 (C-5^{IV} and C-5^{I}), 79.2 (C-3^{III}), 100.5, 100.7 (C-2^{I}, C-2^{II}), 101.3 (C-1^{III}), 103.0 (C-1^{IV}), 175.6, 175.9 (C-1^{I}, C-1^{II}). ESI-HRMS for $C_{33}H_{55}O_{27}$: 883.2931 $[M-2Na+H]^-$. Found 883.2929.

6.30 (β-D-Galactopyranosyl-(1-4)-β-D-glucopyranosyl)-(1-4)-L-*glycero*-α-D-*manno*-heptopyranosyl-(1-5)-[*O*-(sodium 3-deoxy-α-D-*manno*-2-octulopyranosylonate)]-(2-4)-sodium (propyl 3-deoxy-α-D-*manno*-2-octulopyranosid)onate (46)

Compound **43** (9.0 mg, 4.5 μmol) was hydrogenated in the presence of $Pd(OH)_2$-C (20%, 0.2 mg) in dry methanol (0.5 mL) under atmospheric pressure of hydrogen for 2 days at room temperature. The reaction mixture was filtered through Celite and concentrated. The residue was dissolved in dichloromethane (0.6 mL) and aqueous 80% trifluoroacetic acid (70.0 μL) was added at room temperature. After stirring for 1 h, the solvent was removed by evaporation under an argon stream to give a crude compound that was not subjected to further purification. The crude compound was dissolved in methanol (1.0 mL), and then 0.1 M sodium hydroxide (1.4 mL, 0.14 mmol) was added at room temperature. After stirring for 24 h, the mixture was concentrated by evaporation. The residue was passed through a Bio-Gel P-2 column (2.5 x 100 cm, H_2O) and a Sep-Pak C18 column (H_2O) to give **46** (2.5 mg, 53%) as a colorless powder. $[\alpha]^{25}_D$ = +17.4 (*c* 0.3, H_2O), ^{1}H-NMR (600 MHz, D_2O): δ 0.91 (t, 3H, *J*=7.4 Hz, CH_3), 1.55–1.62 (m, 2H, CH_2), 1.77 (dd, 1H, $J_{3a,3b}$=12.8 Hz, $J_{3a,4}$=12.6 Hz, H-3a^{II}), 1.92 (dd, 1H, $J_{3a,3b}$=12.8 Hz, $J_{3a,4}$=12.0 Hz, H-3a^{I}), 2.07 (dd, 1H, $J_{3a,3b}$=12.8 Hz, $J_{3b,4}$=4.4 Hz, H-3b^{I}), 2.18 (dd, 1H, $J_{3a,3b}$=12.8 Hz, $J_{3b,4}$=4.6 Hz, H-3b^{II}), 3.19–3.24 (m, 1H, OCH_2), 3.26–3.30 (m, 1H, OCH_2), 3.39 (dd, 1H, $J_{1,2}$=8.0 Hz, $J_{2,3}$=8.8 Hz, H-2^{IV}), 3.54 (dd, 1H, $J_{1,2}$=7.8 Hz, $J_{2,3}$=10.0 Hz, H-2^{V}), 3.58 (dd, 1H, $J_{6,7}$=8.4 Hz, H-6^{I}), 3.59 (dd, 1H, $J_{7,8a}$=6.2 Hz, $J_{8a,8b}$=11.6 Hz, H-8a^{II}), 3.64–3.84 (m, 14H, H-3^{V}, H-6^{II}, H-3^{IV}, H-6a^{IV}, H-6b^{IV}, H-5^{V}, H-4^{IV}, H-6a^{V}, H-6b^{V}, H-7a^{III}, H-5^{III}, H-7b^{III}, H-8a^{I}, H-7^{I}), 3.89 (dd, 1H, $J_{7,8b}$=2.6 Hz, $J_{8a,8b}$=11.6 Hz, H-8b^{II}), 3.91–3.99 (m, 4H, H-4^{V}, H-7^{II}, H-5^{IV}, H-8b^{I}), 4.00–4.07 (m, 3H, H-4^{III}, H-3^{III}, H-5^{II}), 4.09 (brs, 1H, $J_{1,2}$=1.6 Hz, H-2^{III}), 4.10 (ddd, 1H, $J_{3a,4}$=12.6 Hz, $J_{3b,4}$=4.6 Hz, $J_{4,5}$=3.0 Hz, H-4^{II}), 4.14 (ddd, 1H, $J_{6,7a}$=7.8 Hz, $J_{6,7b}$=4.8 Hz, H-6^{III}), 4.21 (brs, 1H, $J_{4,5}$=2.2 Hz, H-5^{I}), 4.24 (ddd, 1H, $J_{3a,4}$=12.0 Hz, $J_{3b,4}$=4.4 Hz, $J_{4,5}$=2.2 Hz, H-4^{I}), 4.44 (d, 1H, $J_{1,2}$=7.8 Hz, H-1^{V}), 4.58 (d, 1H, $J_{1,2}$=8.0 Hz, H-1^{IV}), 5.32 (d, 1H, $J_{1,2}$=1.6 Hz, H-1^{III}). ^{13}C NMR (150 MHz, D_2O): δ 10.8 (CH_3), 23.8 (CH_2), 35.0, 35.3 (C-3^{I}, C-3^{II}), 60.6 (C-6^{IV}), 61.6 (C-6^{V}), 63.5 (C-8^{I}), 64.0 (C-8^{II}), 64.6 (C-7^{III}), 65.4 (OCH_2), 66.9 (C-4^{II}), 67.0 (C-5^{II}),

69.2 (C-6^{III}), 69.7 (C-7^{I}), 69.9 (C-4^{V}), 70.2 (C-4^{I}), 70.3 (C3^{III}), 70.5 (C-2^{III}), 70.7 (C-7^{II}), 71.6 (C-5^{III}), 72.0 (C-2^{V}), 72.3 (C-5^{IV}), 72.6 (C-6^{I}), 72.7 (C-6^{II}), 73.1 (C-4^{III}), 73.4 (C-5^{I}), 74.6 (C-3^{IV}), 75.5 (C-2^{IV}), 76.0 (C-3^{V}), 76.5 (C-5^{V}), 78.8 (C-4^{IV}), 100.5, 100.8 (C-2^{I}, C-2^{II}), 101.0 (C-1^{III}), 103.0 (C-1^{IV}), 103.6 (C-1^{V}), 175.5, 175.9 (C-1^{I}, C-1^{II}). ESI-HRMS for $C_{38}H_{63}O_{31}$: 1015.3353 $[M-2Na+H]^-$. Found 1015.3370.

6.31 (β-D-Galactopyranosyl-(1-4)-α-D-glucopyranosyl)-(1-5)-[*O*-(sodium 3-deoxy-α-D-*manno*-2-octulopyranosylonate)]-(2-4)-sodium (propyl 3-deoxy-α-D-*mann*-*o*-2-octulopyranosid)onate (47)

Compound **41** (5.3 mg, 2.7 μmol) was hydrogenated in the presence of $Pd(OH)_2$-C (20%, 1.5 mg) in dry methanol (0.4 mL) under atmospheric pressure of hydrogen for 1 day at room temperature. The reaction mixture was filtered through Celite and concentrated. The residue was treated with aqueous 80% trifluoroacetic acid (40.0 μL) at room temperature. After stirring for 5 min, the solvent was removed by evaporation under an argon stream to give a crude compound that was not subjected to further purification. The crude compound was dissolved in methanol (0.8 mL), and then 0.1 M sodium hydroxide (0.5 mL, 0.05 mmol) was added at room temperature. After stirring for 24 h, the mixture was concentrated by evaporation. The residue was purified by a Bio-Gel P-2 column (2.5 x 100 cm, H_2O) anda Sep-Pak C18 column (H_2O) to give **47** (1.4 mg, 60%) as a colorless powder. $[\alpha]^{25}_D$ = +20.1 (*c* 0.1, H_2O), ^{1}H-NMR (600 MHz, D_2O): δ 0.90 (t, 3H, J=7.4 Hz, CH_3), 1.53–1.60 (m, 2H, CH_2), 1.80 (dd, 1H, $J_{3a,3b}$=12.8 Hz, $J_{3a,4}$=12.6 Hz, H-3a^{II}), 2.02 (dd, 1H, $J_{3a,3b}$=12.6 Hz, H-3a^{I}), 2.06–2.08 (m, 2H, H-3b^{I}, H-3b^{II}), 3.21–3.30 (m, 2H, OCH_2), 3.55 (dd, 1H, $J_{1,2}$=7.8 Hz, $J_{2,3}$=10.2 Hz, H-2^{IV}), 3.56–3.60 (m, 3H, H-2^{III}, H-6^{I}, H-8a^{II}), 3.66 (dd, 1H, $J_{2,3}$=10.2 Hz, $J_{3,4}$=3.4 Hz, H-3^{IV}), 3.72–3.82 (m, 6H, H-6^{II}, H-4^{III}, H-6a^{IV}, H-6b^{IV}, H-5^{IV}, H-8a^{I}), 3.89–4.01 (m, 9H, H-8b^{I}, H-7^{II}, H-4^{IV}, H-6a^{III}, H-6b^{III}, H-3^{III}, H-8b^{II}, H-5^{II}, H-4^{I}), 4.06 (ddd, 1H, $J_{6,7}$=9.0 Hz, $J_{7,8a}$=3.0 Hz, $J_{7,\ 8b}$=2.4 Hz, H-7^{I}), 4.09–4.12 (m, 1H, $J_{3a,4}$=12.6 Hz, $J_{3b,4}$=4.8 Hz, $J_{4,5}$=2.2 Hz, H-4^{II}), 4.23 (brs, 1H, H-5^{I}), 4.23–4.26 (m, 1H, H-5^{III}), 4.47 (d, 1H, $J_{1,2}$=7.8 Hz, H-1^{IV}), 5.28 (dd, 1H, $J_{1,2}$=3.6 Hz, H-1^{III}). ^{13}C NMR (150 MHz, D_2O): δ 10.8 (CH_3), 22.8 (CH_2), 35.2 (C-3^{I}, C-3^{II}), 60.2 (C-6^{III}), 61.7 (C-6^{IV}), 63.3 (C-8^{I}), 64.0 (C-8^{II}), 65.3 (OCH_2), 66.7

(C-4^{II}), 67.5 (C-5^{II}), 69.2 (C-7^{I}), 69.3 (C-4^{IV}), 70.1, 71.3, 71.6, 71.8, 72.0, 72.3, 72.4, 73.0, 73.2 (C-4^{I}, C-7^{II}, C-2^{IV}, C-5^{III}, C-6^{II}, C-6^{I}, C-2^{III}, C-3^{III}, C-5^{I}), 74.0 (C-3^{IV}), 75.9 (C-5^{IV}), 78.5 (C-4^{III}), 99.6, 100.5 (C-2^{I}, C-2^{II}), 102.3 (C-1^{III}), 103.5 (C-1^{IV}), 175.9 and 176.1 (C-1^{I}, C-1^{II}). ESI-HRMS for $C_{31}H_{51}O_{25}$: 823.2719 $[M-2Na+H]^-$. Found 823.2732.

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Summary

Lipopolysaccharides (LPSs) and lipooligosaccharides (LOSs) are the major glycolipds expressed in the outer membrane of gram-negative bacteria and play an important role in the pathogenesis of bacterial infections. LPS is composed of O-specific polysaccharide, a core oligosaccharide (core OS), and lipid A, whereas LOS lacks an O-antigen. The core OS, which is a significant target for vaccine development and diagnostics of pathogenic bacteria, can be further subdivided into the inner core and outer core. The inner core OS is composed of a 4,5-branched 3-deoxy-D-*manno*-2-octulosonic acid (Kdo) structures. However, there are few reports about the synthesis of this branch Kdo structure. To synthesize this branch Kdo structure and extend our previous research, a new synthetic approach was developed.

In this study, chapter 2 described the synthesis of 2–4 linked Kdo disaccharide; chapter 3 showed a new synthetic approach to synthesize 4,5-branched Kdo trisaccharides using Kdo disaccharide as an acceptor; in chapter 4 more complex 4,5-branched Kdo structures were synthesized by using the same Kdo disaccharide as the acceptor; chapter 5 showed the conclusions.

In chapter 2, 2-4 linked Kdo disaccharide was obtained by glycosidation of Kdo donor with 4,5-diol acceptor. To optimize the condition of this reaction, several types of Kdo donors with different leaving groups were prepared from the common Kdo intermediate and were glycosylated with 4,5-diol acceptor. The results showed that all donors produced the α-glycoside as the main product and the stereoselectivity was not influenced by the type of leaving group. Moreover, the α-fluoride donor with $BF_3{\cdot}OEt_2$ as the activator provided the best yield and α-selectivity of product.

In chapter 3, Kdoα(2-4)Kdo as an acceptor was glycosylated with L-*glycero*-D-*manno*-heptosyl (Hep), mannosyl (Man), and 2-azido-2-deoxy-galactosyl imidates ($GalN_3$), respectively, and three corresponding 4,5-branched trisaccharides, Hepα(1-5)[Kdoα(2-4)]Kdo, Manα(1-5)[Kdoα(2-4)]Kdo and $GalN_3$α(1-5)[Kdoα(2-4)]Kdo, were successfully synthesized in good yield and high α-selectivity. These results confirmed that glycosylation at the 4-OH position of the Kdo acceptor followed by a second glycosylation at 5-OH position could produce the

target 4,5-branched Kdo structures and this new synthetic strategy is different from Paulsen's method.

In chapter 4, to extend the utility of the new synthetic strategy, more complex 4,5-branched Kdo structures were synthesized by using the same Kdo disaccharide as the acceptor. To do it, firstly the L-*glycero*-D-*manno*-heptopyranose (Hep) units, Galβ(1-4)Glcβ(1-4)Hep and Hepα(1-3)Hep, for the branched core oligosaccharide were prepared from the corresponding Hep building blocks. Then, the Hep units were glycosylated with the common acceptor Kdoα(2-4)Kdo to afford 4,5-branched core oligosaccharide structures. Three complex 4,5-branched Kdo structures, Galβ(1-4)Glcα(1-5)[Kdoα(2-4)]Kdo and Hepα(1-3)Hepα(1-5)[Kdoα(2-4)]Kdo, Galβ(1-4)Glcβ(1-4)Hepα(1-5)[Kdoα(2-4)]Kdo, were successfully obtained.

In all, the new synthetic approach using Kdoα(2-4)Kdo as an intermediate is useful for the synthesis of 4,5-branched core OS structures including Kdo trisaccharides, Kdo tetrasaccharides and Kdo pentasaccharide.

グラム陰性菌が産生するリポ多糖およびリポオリゴ糖の内部コア糖鎖の合成研究：4,5 で分岐した 3-デオキシ-D-マンノオクト-2-ウロン酸の構築

要旨

リポ多糖(LPS)やリポオリゴ糖(LOS)はグラム陰性細菌が細胞外膜に産生する複合糖脂質であり、細菌感染において重要な役割を持っている。LPS は O 抗原多糖、コアオリゴ糖(コア OS)、そして Lipid A からなり、これに対して LOS は O 抗原を欠損した構造をしている。コア OS は内部コアと外部コアに分けられる。コア OS は細菌の属によって特に糖鎖構造が保存された領域であり、病原性細菌の診断ツールや感染予防のためのワクチン開発の抗原となる標的分子として注目されている。多くの LPS/LOS の内部コア OS には 4,5-分岐した 3-デオキシ-D-マンノオクト-2-ウロン酸(Kdo)構造が存在している。このような分岐構造を有する Kdo 構造を含んだ分岐糖鎖の化学合成法についてはわずかな報告しか存在しない。本論文では、この分岐構造を合成するために、新しい化学合成法を達成した。

本論文では、第 2 章に 2-4 結合を有する Kdo2 糖の合成について述べている。第 3 章ではこの 2 糖を糖受容体として用いた 4,5 分岐構造を有する 3 糖の新しい合成経路での合成について述べている。第 4 章では 4,5-分岐 Kdo 構造を含むより大きな糖鎖の合成法について述べられ、第 5 章でこれらについての総括が述べられている。

第 2 章では、4,5-ジオール Kdo 受容体と様々な Kdo 供与体とのグリコシル化反応について述べている。この反応の反応条件を最適化するために、数種類の脱離基を持った Kdo 誘導体を共通の中間体から調製した。そして 4,5-ジオール受容体とのグリコシルを検討した。その結果すべての供与体においてα-グリコシドを主生成物として得られること、そして BF_3OEt_2 を活性化剤に用いたα体のフッ化糖供与体を用いた反応では最も良い収率とα選択性を与えることが明らかになった。

第 3 章では第 2 章で合成を達成した Kdoα(2-4)Kdo を糖受容体として用い、L-グリセロ-D-マンノヘプトース(Hep)、マンノース (Man)および 2-アジド-2-デオキシガラクトースのイミデート誘導体による 5 位水酸基へのグリコシル化反応の検討を行った。そしてα選択的に良好な収率で合成することに成

功した。この結果は Kdo の 4,5-ジオール誘導体に対してまず 4 位水酸基にへのグリコシル化続く 5 位水酸基へのグリコシル化反応は目的とする 4,5-分岐構造を持つ Kdo の合成が可能であることを示した。この方法はこれまでに唯一報告されている Paulsen らの方法とは異なる方法であり、このような糖鎖の合成における新しい合成の方法論を提供するものである。

第 4 章では 3 章で達成した新しい 4,5-分岐 Kdo 糖鎖合成法の有用性を拡大するために、より複雑な糖鎖の合成を試みた結果について述べた。はじめに Hep を含む供与体ユニット Galβ(1-4)Glcβ(1-4)Hep と Hepα(1-3)Hep の合成を対応する Hep ビルディングブロックから調製した。その後共通の受容体である Kdoα(2-4)Kdo を用いたグリコシル化による 4,5 分岐 Kdo 糖鎖の合成を行った。その結果 3 種類の 4,5 分岐 Kdo 糖鎖である Galβ(1-4)Glcα(1-5)[Kdoα(2-4)]Kdo、 Hepα(1-3)Hepα(1-5)[Kdoα(2-4)]Kdo そして Galβ(1-4)Glcβ(1-4)Hepα(1-5)[Kdoα(2-4)]Kdo の合成を達成した。

Kdoα(2-4)Kdo を中間体として用いる 4,5 分岐 Kdo の新しい合成法はこの構造を有する LPS/LOS の内部コア糖鎖の合成に有用であることを示した。

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List of publications

1. Ruiqin Yi, Atsushi Ogaki, Mayumi Fukunaga, Hiromitsu Nakajima, Tsuyoshi Ichiyanagi. Synthesis of 4,5-disubstituted-3-deoxy-D-*manno*-octulosonic acid (Kdo) derivatives. Tetrahedron 70: 3675-3682. 2014.06 (The corresponding content is in chapter 2 and chapter 3)

2. Ruiqin Yi, Hirofumi Narimoto, Miku Nozoe, Tsuyoshi Ichiyanagi. Convergent synthesis of 4,5-branched inner-core oligosaccharides of lipopoly- and lipooligosaccharide. Bioscience, Biotechnology, and Biochemistry. Accepted at June 13th, 2015 (The corresponding content is in chapter 4)