STRUCTURAL MODIFICATION OF *E. COLI* LIPID A AND KDO-LIPID IVA

by

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(Under the Direction of Geert-Jan Boons)

ABSTRACT

Lipopolysaccharides from gram negative bacteria are responsible for signal transduction for the proinflammatory response in cells by initiating formation of TLR4/MD-2 protein complexes. Excess LPS can lead to septic shock, a condition with often fatal results. Lipid A, the inner diglucosamine region of LPS, is responsible for a majority of LPS endotoxicity. A precursor, lipid IV_A, behaves as an antagonist for LPS binding. Through molecular modeling, specific alterations to the lipid A structure can be designed that may allow tuning of lipid A activity. KDO, a sialic acid derivative naturally covalently linked to lipid A in LPS, can improve endotoxic activity in lipid A. KDO was therefore covalently linked to lipid IV_A to investigate its effects on lipid IV_A antagonistic acitiviy. Analogues of *E. coli* lipid A and KDO-lipid IV_A were synthesized using divergent and partially divergent synthetic schemes.

INDEX WORDS: LIPID, LIPOPOLYSACCHARIDE, *ESCHERICHIA COLI,* TLR4, MD-2

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ABBREVIATIONS

Alloc……..Allyloxycarbonyl

Bn……Benzyl

CD-14…….....Cluster Differentiation 14

CSA……... ……(1*R*)-(−)-10-Camphorsulfonic acid

DAST……(Diethylamino)sulfur trifluoride

DBU……..……1,8-Diazabicyclo[5.4.0]undec-7-ene

DCC……*N,N'*-dicyclohexylcarbodiimide

DCM…….…….....Dichloromethane

DMAP……..…4-(Dimethylamino)pyridine

DMF……...*N,N'*-Dimethylformamide

EDC……*N*-(3-Dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride

Fmoc……..9-Fluorinomethyloxycarbonyl

HMPA……..…….....Hexamethylphosphoramide

HR MS…….…….....High resolution mass spectrometry

KDO…….…...3-Deoxy-D-manno-Octulosonic Acid

LPS…….....……..Lipopolysaccharide

MALDI-ToF……...Matrix assisted laser desorption ionization- time of flight

MyD88…….…… Myeloid differentiation primary response gene (88)

MS……..Molecular sieves

NBS…….*N*-Bromosuccinimide

$RuCl(Binap)_2$…...Dichloro[(R)-(+)-2,2'-bis(diphenylphosphino)-1,1'-binaphthyl]ruthenium(II)

TBAF…….Tetra-*n*-butylammonium fluoride

TDS…….Thexyldimethylsilyl

TLR…..……Toll-like receptor

TNF……Tumor necrosis factor

TRIF……TIR-domain-containing adapter-inducing interferon-β

Troc……2,2,2-Trichloroethyloxycarbonyl

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

Sepsis is the condition wherein immunomodulating molecules such as proinflammatory cytokines and NF-κB are produced in excess, resulting in an excessive proinflammatory response that may result in organ failure or death. Mortality rates for cases of sepsis submitted to hospitals is estimated at up to 18%[1], and some studies estimate that sepsis is responsible for over 9% of deaths in the United States[2]. Currently, no drugs capable of directly treating septic shock are available; the best treatments involve lessening the severity of the illness symptoms, including treatment with antibiotics/antimicrobial agents, vazosuppressors, and fluid resuscitation[3]. A variety of approaches to treatment of sepsis have been investigated in

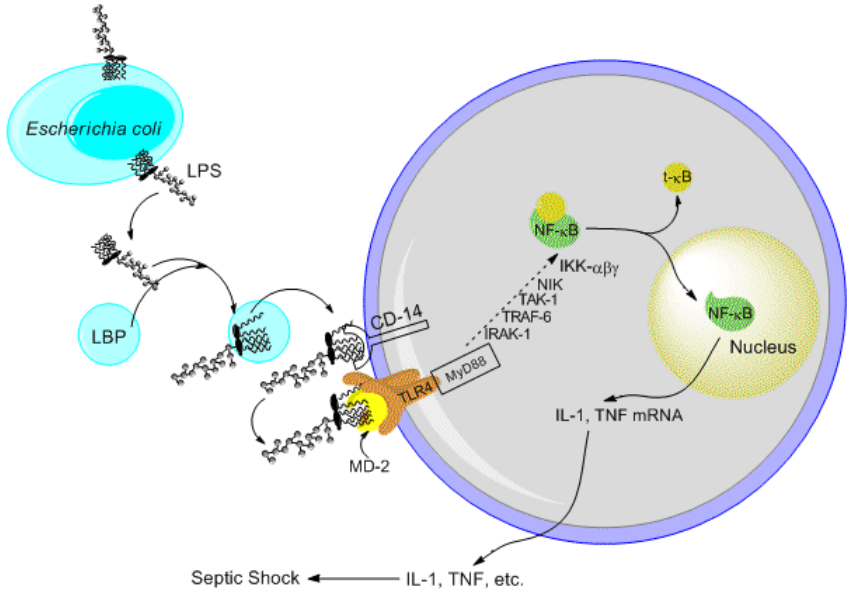

Figure 1.1 From LPS to Septic Shock (adapted from Raetz, et al[4].)

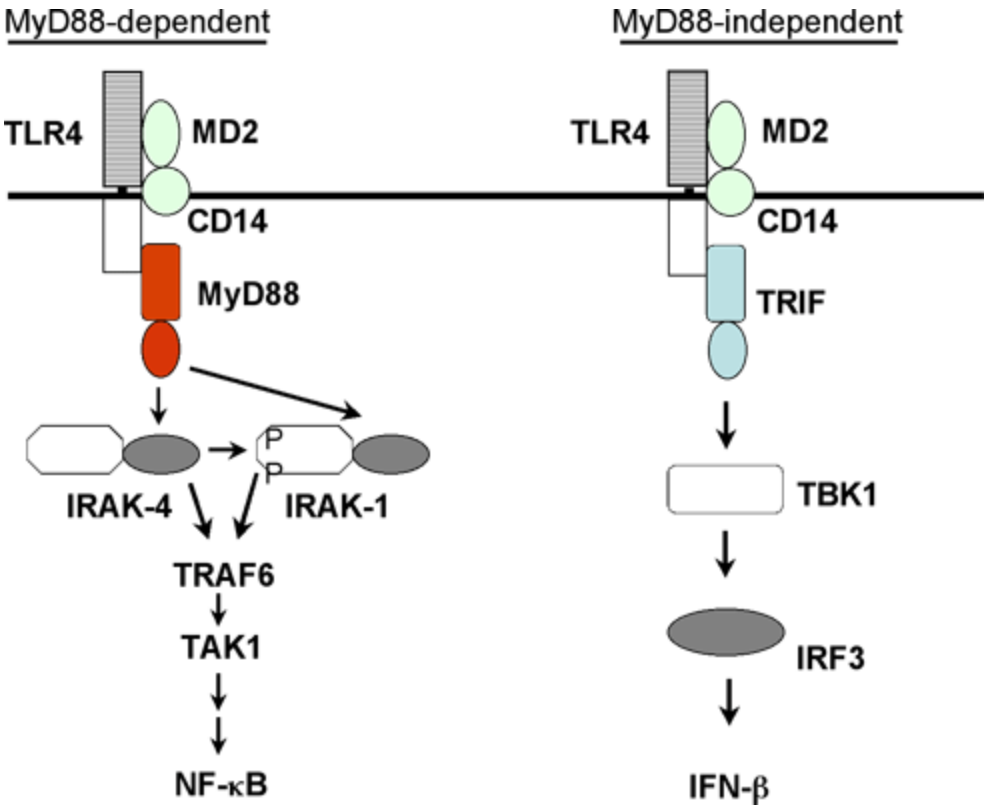

Figure 1.2 MyD88 Dependant and Trif Dependant Cascades[5]

the laboratory, including blocking target cell signaling with antibodies or receptor blockers, blocking intracellular signaling using enzyme inhibitors, and inhibition of release of cell mediators in the cascade[6]. The difficulty in treating sepsis stems from the complexity of the cascades that result in production of the immunomodulating agents, as elimination of one pathway will likely not stem production. A possible solution to this difficulty is to prevent the initiation of the cascades. These cascades are intiated by interaction with an endotoxin released by gram negative bacteria such as *e. coli* (**Figure 1.1**).

1.2 Biology of LPS

Gram-negative bacteria is responsible for a variety of illnesses associated with the immune inflammatory response, including sepsis. One of the key components of gram negative bacteria that significantly impacts its pathogenicity is lipopolysaccharide (LPS) endotoxin that is

present in the outer cell wall. LPS is active in the modulation of the innate immune response in the body through mediating the production of cytokines, necrosis factor, and a variety of other immunomodulating molecules through the initiation of at least two signaling cascades: one MyD88 dependent and one Trif dependent cascade (**Figure 1.2**). In both cascades CD14 transfers LPS to MD-2 on the outer cell wall, which promotes the binding of one TLR-4 unit with one MD-2 unit. Once this binding occurs, the LPS molecule dimerizes through its lipid A region with a second MD-2/ TLR-4 complex. This dimer signals for cytokine production through two cascade pathways, one My-D88 dependent and one which is My-D88 independent. Investigations into cascade initiation in the absence of MD-2 have established that MD-2 is essential for activity of the TLR4 complex[4]. In the myD88 dependent path, TIRAP recruits MyD88 after the formation of the TLR-4/MD-2/lipid A complex, and this binds with IRAK-4, from which the cascade continues, resulting in the signaling for cytokines, MAP kinases and NF-κB. A second pathway, a Trif- dependent pathway, does not require MyD88. In this cascade, TRIF adaptor protein is recruited to the TLR-4/MD-2 complex by TRAM, which binds RIP-1 and continues in the cascade to the production of type 1 interferons and, in greater excesses, NF-κB[7].

1.3 Structure of LPS

LPS is comprised of several regions of carbohydrates (**Figure 1.3**). The outermost region of LPS is an O-polysaccharide chain containing repeating oligosaccharide subunits. This is followed by an outer core of more uncommon sugars and an inner core of conserved sugars including KDO. These core units are O-(1→6) linked to a diglucosamine phospholipid referred to as lipid A. This unit is responsible for much of the immunomodulating activity of LPS, and is

highly conserved through most natural LPS, with a basic structure incorporating a β-(1→6) linked glucosamine backbone, phosphorylation at C1 and C4' positions, and acyl chains coupled

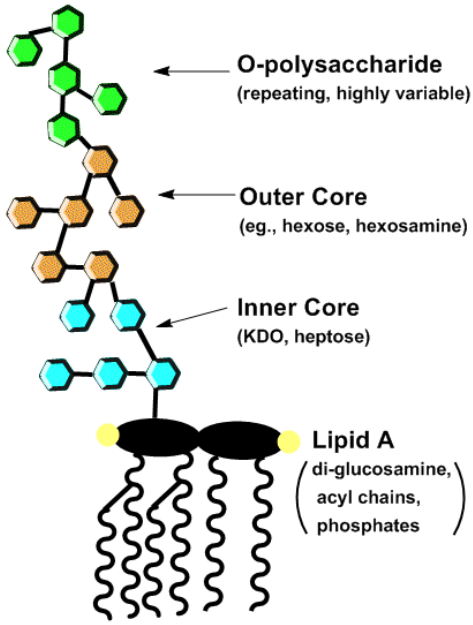

Figure 1.3 Structure of LPS

at C2, C3, C2' and C3' positions. Variation of lipid A occurs primarily at the acyl chain positions and the phosphates. The acyl chains vary in number, having a minimum of four and maximum of seven chains attached to the diglucosamine backbone at C-3, C-3' and both amine positions. The phosphate groups vary in their substitution with other functional groups, including glucosamines, ethanolamine, and D-arabino-furanose.

1.4 Synthesis of Lipid A

Because of its biological significance and the heterogeneity of samples obtained from nature, there is a significant interest in the chemical synthesis of Lipid A and related analogues. Synthetic lipid A provides a number of advantages over its natural counterparts; foremost, synthesizing lipid A allows acquisition of a pure compound. This an accomplishment that is difficult to achieve using natural lipid A isolation, as natural LPS and lipid A are heterogeneous and difficult to separate[8]. Synthesizing lipid A also allows researchers to easily obtain larger

quantities of sample than are currently possible with isolation of the natural product. Finally, synthesis allows for greater variation in analogs of lipid A; modifications can be made at multiple positions during synthesis. For these reasons, significant research has been invested in the development of synthetic strategies for lipid A.

Early lipid A synthetic strategies sought to achieve the most efficient route to a single product, developing monosaccharide building blocks useful for only one result. Early routes to lipid A precursors by Kusumoto involved glycosylation of glucosamine units containing similar or identical protecting groups[9]. In initial strategies to achieve total synthesis of lipid A, single fatty acid chains were coupled to each building block prior to glycosylation, while *N*-positions to be functionalized post-glycosylation were protected by identical groups[10, 11]. While this methodology was rationalized by the decrease in protection steps[12], this method is not useful for efficient synthesis of multiple analogues. Later methodologies utilized building blocks pre-acylated, with orthogonal protecting groups at the β positions of acyl chains. This provided a partially convergent strategy, allowing some variation from an initial synthetic platform[13]. A more convergent synthetic strategy for lipid A was developed by Boons[14], which utilized orthogonality at C2, C3, C2', and C3' positions along the diglucosamine backbone of lipid A, allowing significant structural variation from a single diglucosamine precursor.

1.5 Structural Modification

Significant study has been conducted on the effects of changes to the basic structure of lipid A, with modifications having been made to a number of different naturally occurring varieties of the lipid (**Figure 1.4**). Studies of monosaccharide lipid A analogues, often with multiple acyl chains extending from a carbon sequence at the anomeric center, have shown poor endotoxicity, indicating the diglucosamine backbone is essential to lipid A activity[6]. Two aspects

of the lipid A structure have been the focal points of the most investigation: the acidic phosphate positions and the hydrophobic region consisting of fatty acid chains.

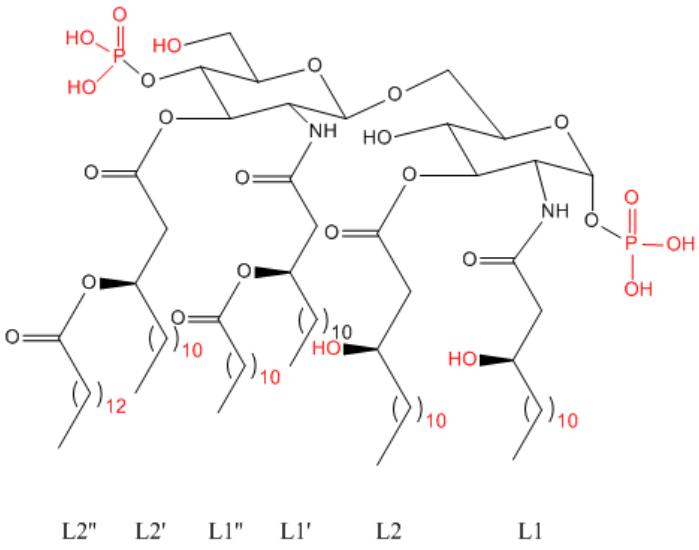

Figure 1.4 E. coli lipid A, with positions of possible modification highlighted in red

One simple but very informative modification of the phosphate positions investigated in early structure-reactivity studies was the omission of one or both phosphate groups from the diglucosamine backbone[8]. Variations studied at this position have found that the absence of both phosphates from the backbone results in significant loss of activity[8]. The C4' phosphate was found to be essential to endotoxic activity, while the anomeric phosphate improves activity but is not essential. Some studies have found that while monophosphoryl lipid A has reduced endotoxic activity, it retains its immunostimulatory activity[15]. Other studies on the importance of the functional group identity at the phosphate positions have investigated the effects of replacing the anomeric phosphate groups with other functional groups, including 2-(phosphonooxy)ethyl, carboxymethyl, [16, 17]. These studies indicated that variation in the functional group has only minor effect on the endotoxic activity of lipid A, provided the acidity of the group is

preserved[18,19]. Conformation of the anomeric phosphate group has also been established as non-essential to endotoxic activity, with α and β conformations being similar in endotoxic activity[16].

The effect of acyl chains on endotoxic activity of a variety of lipid A's has been studied extensively, specifically the number and length of these chains. A majority of the studies on chain length have utilized two or three chain length patterns, either elongating or shortening the acyl chains on one sugar, adjusting the length of all chains on the diglucosamine, or adjusting the lengths of either: 1) the acyl chains directly linked to the diglucosamine backbone, or 2) the acyloxy chains originating at the β positions of the directly linked chains. Through a number of structural studies, general bounds on chain length were established, L3' chains of six carbons or less are incapable of activating the TLR-4/MD-2 complex[20, 21], while lipid A with shortened acyl chains of 8-10 carbons demonstrated improved endotoxic activity. The L3 chain of a hepta-acylated monophosphoryl lipid A was able to vary significantly in length and polarity without significantly affecting its activity as an adjuvant[22].

The number and alignment of acyl chains present on the lipid A analogue has also received significant attention in structure-activity studies. Lipid A structures with greater than six acyl chains indicated a significant decrease in production of several proinflammatory mediators[23, 24]. One of the most studied analogues is the tetraacylated lipid IV_A compound, which contains single 3-hydroxy myristoyloxy chains at *N*, *N'*, C3, and C3' positions. This compound has significantly different activity, and is an antagonist in human systems. The pattern of acyl chains on lipid A also exerts influence over activity. Studies have found that in a series of lipid A structures, the absence of a C3 acyl chain resulted in a minor decrease in activity

1.6 MD-2/TLR4 Crystal Structures:

Recently, crystal structures of lipid IVA/MD-2[25] and lipid A/TLR-4/MD-2[20, 26] complexes have been investigated, and have opened the door to a new approach to lipid A analogue design, allowing the development of analogue structures modified at positions associated with specific interactions predicted from the crystal structures. Using this approach, specific structure-activity relationships can be studied for TLR-4 binding activity. The crystal structures have already been used propose new explanations for some results obtained in previous studies, including findings about the importance of the length and number of acyl chains at the C-3' position. The crystal structure has also established the importance of the phosphate groups and polar functional groups on the non-reducing sugar for binding between the TLR-4 and MD-2 of one unit in the dimer complex. The crystal structures have established the presence of a large hydrophobic pocket, open on one side, but otherwised closed[20, 25]. This may explain the significant effects that lipid chain length has on activity of lipid A: shorter length chains may not hold the glucosamine backbone high enough to interact with TLR4, while longer acyl chains may not fit into the pocket. The open side of the pocket partially exposes the C-2 acyl chain to the outside of the pocket. This opening allows the C-2 acyl chain to interact with the second TLR4 structure[26]. Significant interactions on this lipid chain are likely the large number of hydrophobic interactions along the length of the acyl chain; some interaction is also seen at the C-3_{L2} hydroxy group. This interaction may also explain antagonistic activity of Lipid IV_A; this precursor, having fewer acyl chains, may not fit tightly in the MD-2 hydrophobic pocket and, as a result, may not direct the L2 chain towards the pocket opening, hindering TLR4 dimerization[27]. This observation suggests a study of the L2 acyl chain, specifically altering its length to eliminate individual hydrophobic interactions and altering the hydroxyl functionality to

investigate the significance of the Hydrogen bonding interaction. The crystal structure also indicates limited hydrogen bonding interactions between KDO I and TLR4 and between KDO II and MD2. The significance of the interactions at these positions is not clear, but previous studies have indicated that EC_{50} of *Neisseria meningitidis* lipid A improved significantly, even slightly over LPS[14], when a single KDO molecule is covalently linked to C6' of the lipid A backbone, and the presence of a single KDO I on natural *e. coli* lipid A resulted in improvements in TNF-α production[28].

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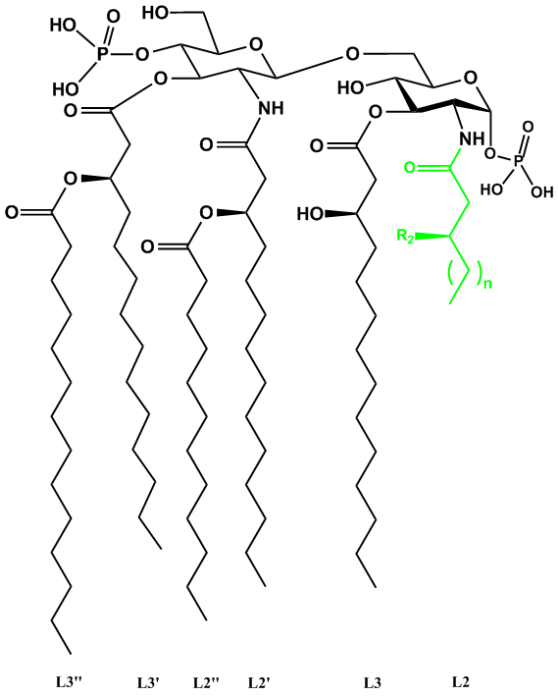
CHAPTER 2

C-2 MODIFICATION OF LIPID A

2.1 Introduction

Sepsis and severe septic shock are a result of overstimulation of the proinflammatory immune response, and are responsible for an estimated 9% of deaths in the United States[1]. Of all sepsis cases reported in hospitals in the United States alone, 18% result in fatalities[2]. One major reason for overstimulation of the proinflammatory response is the release of excess LPS macromolecules from gram negative bacteria cell walls. These macromolecules are delivered to transmembrane MD-2/TLR4 complexes on outer cell membranes in the body by LBP and CD14, and the binding of LPS to this complex induces formation of a dimer-like complex. This complex can induce signaling within the cell through the binding of a sequence of proteins in several different cascades, most commonly either the MyD88 dependent or Trif dependent pathways, which lead to the production of a variety of proinflammatory macromolecules including cytokines, TNF-α[3, 4], NF-κB[5] and IL-1β[6, 7]. The toxicity of LPS is primarily a result of the diglucosamine lipid A covalently linked to the inner core of the molecule[8, 9], and much of the focus in controlling the inflammatory response has centered on modifications of this region of LPS. In previous investigations, several important structural components of lipid A molecules and their biological importance have been studied. Great emphasis has been placed on the implications on activity of shortening or lengthening the groups of fatty acid chains and increasing or decreasing their number. Shortening acyl chains to 8 or 10 carbons improves activity, while significant decreases to 6 carbon chains significantly decreases activity[10]. Studies

comparing *E. coli* and *N. meningitides* lipid As found that even a decrease in chain lengths of two carbons can improve activity[11], while emphasis has also been placed on the biological importance of the phosphate groups at C-1 and C-4'[12]. Previous research has found that the anomeric phosphate improves activity, but similar activity can be achieved with other acidic functionalities. Recently, crystal structure elucidation of the TLR4/MD-2 complex and molecular modeling have provided the possibility of designing specific modifications using predictions based on these models. The focus of this study is the effect of



Analogue	R2	n
A (1)	H	10
B	H	2
C	OH	2
D	NH_2	10
E	NHAc	10
F	OH	18

Figure 2.1 Modifications to *E. coli* lipid A

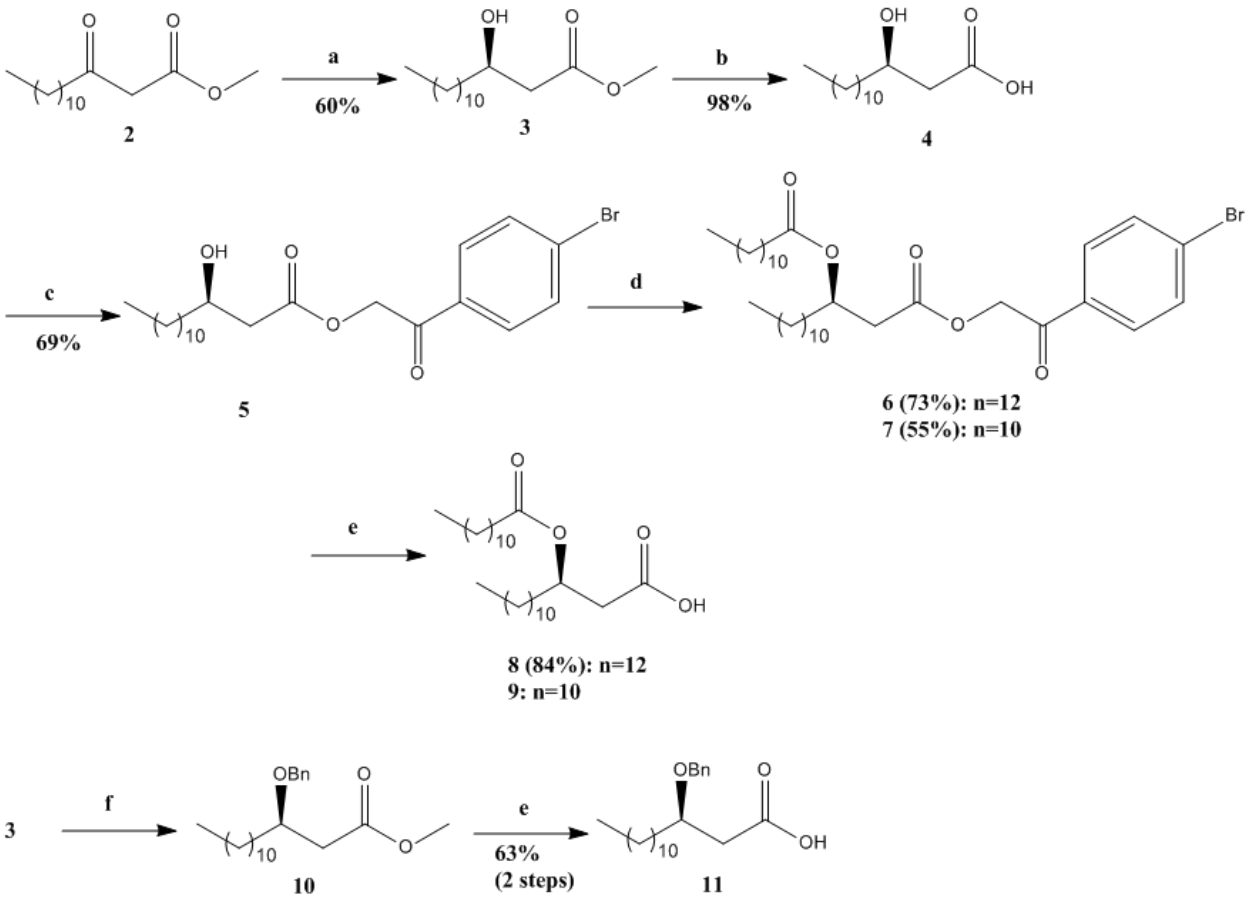
modifications specifically to the N-acyl chain of the reducing glucosamine, which has significant influence over the dimerization of two TLR4/MD-2 units. This lipid is exposed on the side of the MD-2 hydrophobic pocket and, as a result, interacts with TLR4* of the second complex. Based on molecular modeling developed from crystal structures of the MD-2/TLR4 dimer, L2 appears to have two classes of interaction with TLR4*: multiple hydrophobic interactions spaced over

the length of the chain and expected to be the most significant interactions for promoting dimerization, and at least one hydrogen bonding interaction at the C-3 functional group of L2. The primary interest of this initial study is the importance of the functional group at $C3_{L2}$ and the length of this acyl chain, using molecular modeling to propose modifications and predict outcomes. The first structure studied was an analogue having no functional group at the $C\text{-}3_{L2}$ position. While a cursory investigation of this compound was conducted in an early structure-activity relationship study, the study was very limited in scope[13]. Current data leads to the question of how this molecule and others altered at the $C\text{-}3_{L2}$ interact specifically with TLR4.

2.2 Results and Discussion

To determine how the N-acyl chain structure of *E. coli* lipid A affects the interaction of the molecule with the TLR4/MD-2 complex, a series of modifications at L1 was envisioned, synthesized through the development of a diglucosamine core structure that allows a convergent approach to Lipid A analogues modified at the acylated positions C2, C3, and C2''.

The acyl chains for **1** were synthesized from the common intermediate **3** (**Scheme 2.1**), which was obtained through stereoselective reduction of ketoester **2** using (*R)*-$RuCl_2(BINAP)_2$. Compound **2** was synthesized from commercially available reagents. To obtain the diacyl chains **8** and **9**, ester **3** was first hydrolyzed to afford acid **4**, followed by selective protection of the acid by treatment with 2,4'-dibromoacetophenone to obtain **5**. Ester **5** was treated with tetradecanoyl chloride or dodecyl chloride and pyridine to obtain compounds **6** and **7**, respectively. The bromoacetophenone protecting groups were then removed from both **6** and **7** through reduction using zinc dust and acetic acid to afford **8** and **9**. Initially, an excess of tetradecanoyl and dodecyl chloride were used in the synthesis of **6** and **7**, but after inspection of the products in the next step and in initial acylated monosaccharide products, it became clear that complete separation of



Scheme 2.1 (a) Ru(Binap), 1M HCl, H_2 (70 psi), MeOH 45˚C; (b) LiOH, THF/H_2O; (c) 1: DCHA, CH_3CN, Et_3N, reflux; (d) tetradecanoyl chloride, pyridine, DMAP, DCM; (e) LiOH, THF/H_2O; (f) benzaldehyde, $(TMS)_2O$, TMSOTf, THF; then Et_3SiH.

the acid chloride reagent from **6** and **7** was problematic and, as a result, the acyl chloride impurities were carried into subsequent acylation steps to produce a monosaccharide **12** and disaccharide **13** containing single myristate chains at C-3. The simplest solution to this setback was to add an excess of the protected acid to ensure the acid chloride was completely consumed during the coupling, as the difference in R_f values between the protected hydroxy ester and the acylated ester were significant and the excess starting material could be recovered. Benzylated

acid **11** was synthesized by treating methyl (R)-3-hydroxy myristate **3** with benzaldehyde, 1,1,1,3,3,3-hexamethyldisiloxane (TMS_2O), and trimethylsilyl trifluoromethanesulfonate (TMSOTf) to form the benzyloxy methyl ester **10**, followed by hydrolysis of the methyl ester to obtain (*R*)-3-benzyloxy myristic acid **11**.

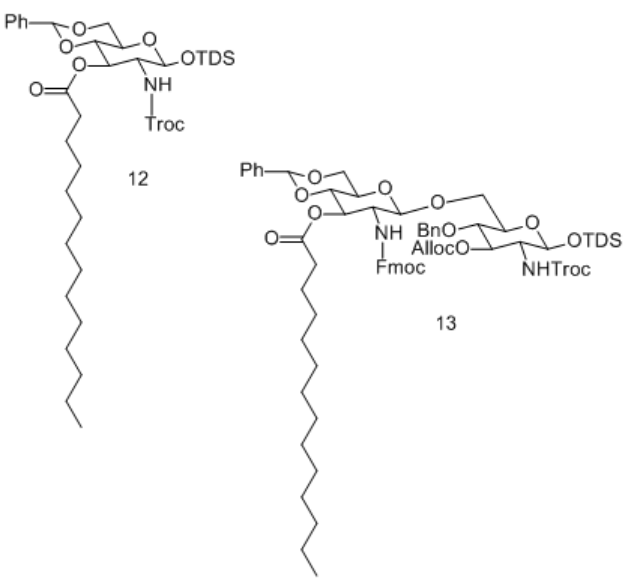
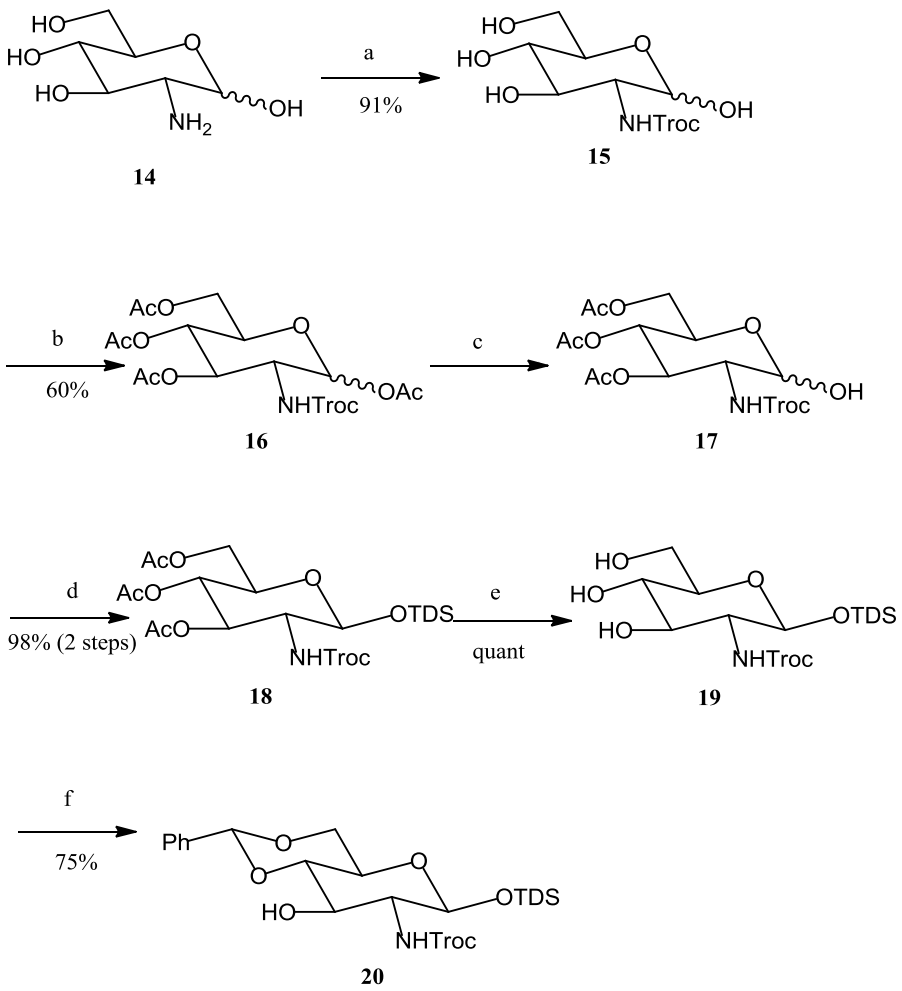


Figure 2.2 Lipid A Side Products

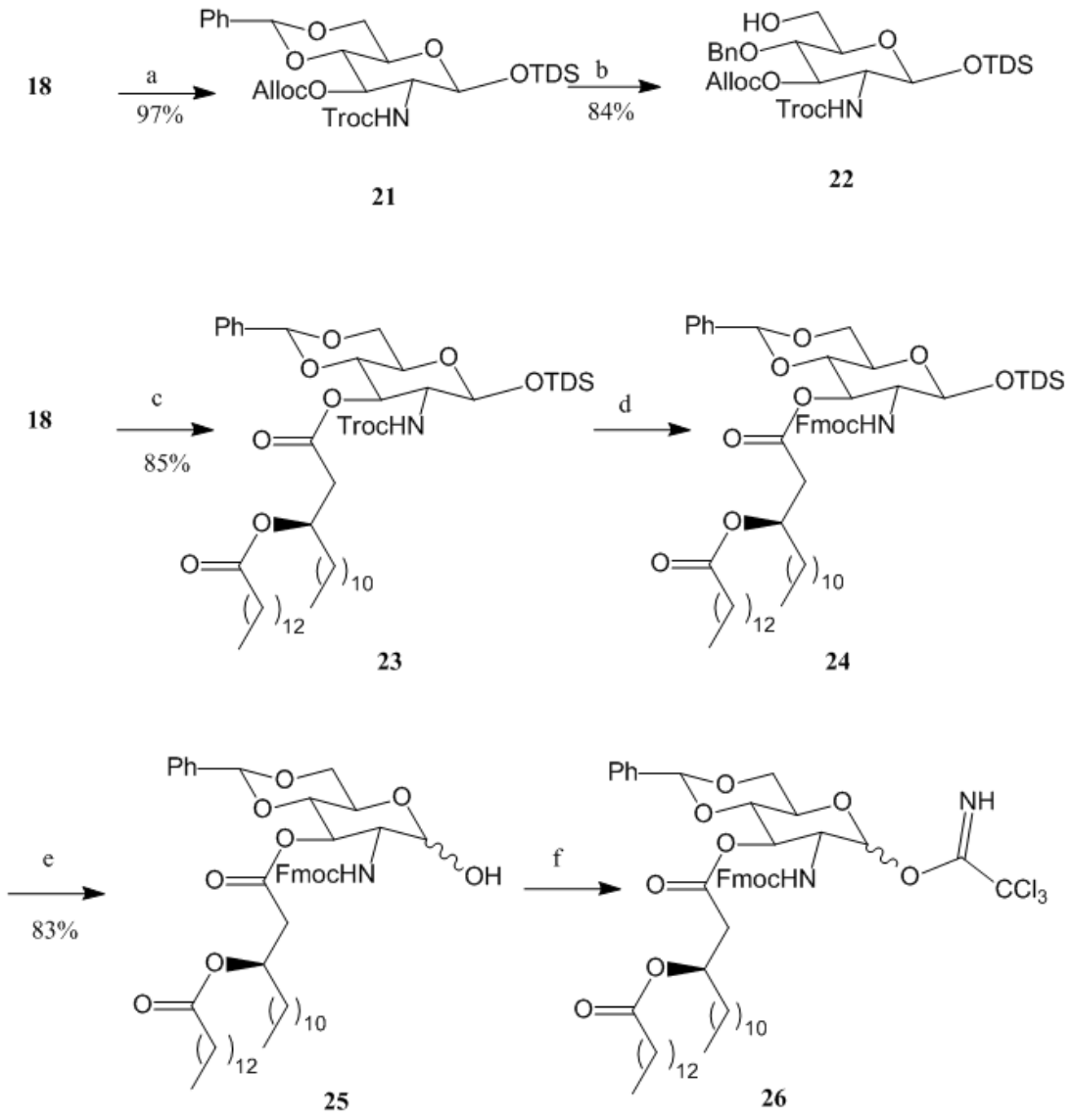
The acceptor, **22**, and the donor, **26**, were synthesized from a common intermediate **20** (**Scheme 2.2)**. **16** was synthesized by treatment of glucosamine hydrochloride **14** with 2,2,2-trichloroethoxy carbonyl (Troc) chloride to obtain the protected glucosamine **15**, followed by subsequent addition of acetic anhydride and pyridine. The anomeric acetyl group was removed via treatment with hydrazine acetate to afford **17**, which was anomerically protected with thexyldimethylsilane to afford **18**. The initial deprotection strategy for deacetylation of **18** required addition of sodium methoxide powder to **18** in methanol. This method led to the formation of a methoxycarbonylamine, through substitution of the trichloroethoxy group of Troc with a methoxy group. This side product was avoided by using a solution of methanol, dichloromethane, 1M methanolic sodium methoxide, and guanidinium chloride. The guanidinium is released from the chloride salt by sodium methoxide, and is used as a milder proton transfer agent for deacetylation[14]. The resulting glucosamine **19** was protected at C-4 and C-6 positions by benzylidine through treatment with benzaldehyde dimethyl acetal and camphor sulfonic acid (CSA) to obtain common intermediate **20**. The acceptor glucosamine **22** was



Scheme 2.2 (a) Troc-Cl, $NaHCO_3/H_2O$, 0˚C, 12h; (b) Ac_2O, pyridine, 0˚C, 12h; (c) hydrazine acetate, DMF, 12h; (d) TDS-Cl, imidazole, DMF, 12h; (e) Guanidine HCl, 1M NaOMe, MeOH/DCM, 2h; (f) benzilidene dimethyl acetal, CSA, DMF, 12h

prepared from the common precursor **20** by protecting the C-3 position with the Alloc, then selectively cleaving the benzylidene ring at C-6 by treatment with dichlorophenylborane and triethylsilane. Synthesis of the donor (**Scheme 2.3**) began with treatment of **20** with tetradecanoyl myristic acid **8** followed by DCC and DMAP to afford **23**. The direct coupling of the acyloxy acid avoids the need for further deprotection of the acyl chain post-coupling using DDQ. This frequently utilized method presents several difficulties, including the addition of two

steps that may result in decreased yields. Coupling the acyloxy acid also eliminates the difficult removal of tetradecanoyl chloride from the acylated product. This acyl chain coupling was followed by deprotection of Troc with zinc dust and acetic acid, then amine protection with 9-fluorenylmethyl carbonyl chloride. The replacement of the Troc group with Fmoc on the donor glucosamine is essential to achieve orthogonality and allow addition of three unique acyl chains in the diglucosamine structure. Use of Fmoc was previously shown to stabilize the imidate of the donor, and also promotes β-selective glycosylation through anchimeric assistance[15]. Following the Fmoc protection of the amine, the resulting sugar **24** was deprotected at the anomeric position by treatment with 70% HF/pyridine to afford the glucopyranose **25 (Table 2.1)**. Initial attempts to achieve this deprotection used TBAF as a fluoride source. These attempts proved unsuccessful, as the procedure resulted in a very slow deprotection over several days, followed by partial removal of the Fmoc group. This likely results from the difficulty in controlling the quality of the TBAF once opened, and the fact that acetic acid must be used to achieve deprotection. Previous studies have found that any water in solution with TBAF can result in deprotection of Fmoc[16]. HF/pyridine proved effective for this purpose, under certain conditions. Buffering the solution with excess pyridine extends the deprotection time to three to four days, so the direct addition of HF/pyridine solution was used. Previous procedures called for the addition of approximately 20 equivalents of HF/pyridine to achieve deprotection. For the purposes of the current study, this procedure resulted in partial deprotection followed by a decrease in reaction rate as HF was consumed. Periodic addition of HF/pyridine in 20 equivalent portions allowed completion of the deprotection within 1.5 days. Attempts to shorten the reaction time further by increasing the



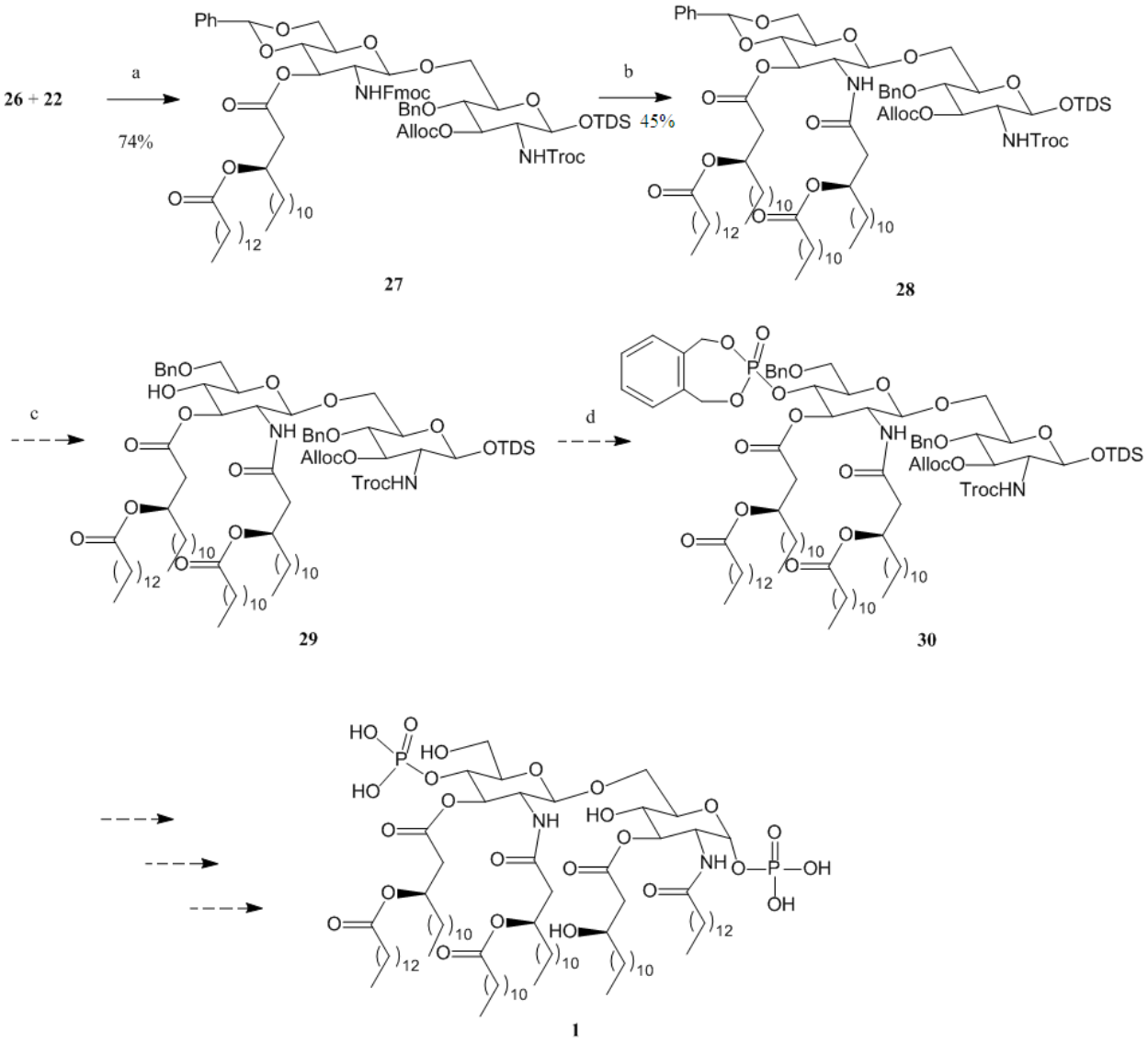
Scheme 2.3 (a) TMEDA, Alloc-Cl, DMF, 12h b) triethylsilane, $PhBCl_2$, DCM, 4Å M.S., -78˚C, 5 min (c) 8, DCC, DMAP, DCM, 0˚C, rt, 12h; d) i. Zn dust, 10% AcOH/DCM; ii. Fmoc-Cl, DIPEA, DCM, 0˚C, rt, 12h; (e) HF/Pyridine, THF, 15h; (f) $CNCCl_3$, $CsCO_3$, DCM, rt, 1h

Table 2.1 Anomeric Deprotection Conditions

Conditions	TBAF, 1M in THF: AcOH (10:1), 1mL THF	TBAF, 1M in THF(2x5eq), AcOH (2x8 eq)	TBAF, 2nd attempt	HF/ Pyridine, 3x 20 eq	HF/Pyridine 4x 20 eq (Every 2 hours, then left overnight)	HF/ Pyridine 40 eq every 2h
Time	Overnight	2 days	2 days	2 days	1.5 days	2h
Major Product	Mixed free C1/Fmoc loss	Some starting material, free C1, and Fmoc removal	Fmoc removal	Free C1	Free C1	Early Fmoc removal

number of equivalents of HF/pyridine per portion to 40 equivalents resulted in partial decomposition of the donor. Imidate **26 w**as prepared by treatment with trichloroacetonitrile and $CsCO_3$. Disaccharide **27** was synthesized by treatment of monosaccharides **22** and **26** with TfOH at -50˚C to give exclusively β-glycosylated product (**Scheme 2.4**). TfOH was used rather than Tf_2O because previous studies showed that the less reactive anomeric conformation did not react completely under Tf_2O conditions[11]. During separation of the disaccharide from excess building blocks, it was found that the acceptor building block eluted within less than 0.1 R_f of the product, and that complete separation was difficult to achieve. Using an excess of donor allowed for the complete reaction of all acceptor, and the hydrolyzed donor could be easily separated from the diglucosamine product by silica gel column chromatography. Fatty acid **9** was coupled to the C-2' position through a two-step process involving the removal of Fmoc from the C-2' amine by treatment with piperidine in DCM (20%, v/v), followed by coupling of the acyl chain using DCC to obtain **28**. This coupling at C2' was carried out prior to the selective deprotection of C4' to avoid loss of Fmoc. Studies on C2 N-Fmoc protected monosaccharide test molecules indicated that the conditions for deprotection of the benzylidene resulted in reduction of Fmoc on the molecule. One cause of this Fmoc reduction might be the use of triethylsilane in the reaction.

Fmoc has been known to undergo cleavage under some stronger reduction conditions[17]. Previous work has established the stability of the N-Troc group and acyl chains to these conditions.



Scheme 2.4 (a) TfOH, 4Å M.S., -50˚C, 1.5h; (b) i. 20% Piperidine, DCM, 30 min; ii. 9, DCC, DCM, 24h; (c) TfOH, Et_3SiH, 4Å M.S., DCM, -78˚C, 5 min; (d) i. 3% tetrazole/ ACN, *O*-xylene-N,N-deithylphosphoramidite, DCM, 45 min; ii. MCPBA, DCM, -20˚C → 0˚C, 1h

2.3 Experimental Section

Column chromatography was performed on silica gel 60 (EM Science, 70-230 mesh). Reactions were monitored by thin-layer chromatography (TLC) on Kieselgel 60 F254 (EM Science), and the compounds were detected by examination under UV light and by charring with 10% sulfuric acid in MeOH. Solvents were removed under reduced pressure at <40˚C. CH_2Cl_2 was distilled from CaH_2 and tetrahydrofuran (THF) was distilled from sodium directly prior to the application. CH_3OH was dried by refluxing with mageniusm methoxide and then was distilled and stored under argon. Pyridine was dried by refluxing with CaH_2 and then was distilled and stored over molecular sieves (3 Å). Molecular sieves (3 and 4 Å), used for reactions, were crushed and activated *in vacuo* at 390˚C during 8 h in the first instance and then for 2-3 h at 390˚C directly prior to application. ^{1}H NMR and ^{13}C NMR spectra were recorded with Varian spectrometers (model Inova500) equipped with Sun workstations. ^{1}H NMR spectra were recorded in CDCl3 and reference to residual CHCl3 at 7.24 ppm, and ^{13}C NMR spectra were referenced to the center peak of CDCl3 at 77.0 ppm. Assignments were made by standard gCOSY and gHSQC. High resolution mass spectra were obtained on a Bruker model Ultraflex MALDI-TOF mass spectrometer. Signals marked with L2 and L3correspond to the monoantennary lipid at C2 and C3, respectively. Signals marked with L2' and L3' correspond to the biantennary lipids at C2' and C3', while signals marked with L2'' and L3'' correspond to the side chains of L2' and L3'.

(R)-3-Hydroxy tetradecanoyl ethanoate (3): 1N HCl (0.01 mL) was added to a solution of ketoester **2** (6.53 g, 25.6 mmol) and dry methanol (40 mL), and the solution was degassed with argon for 15 min. $RuCl(Binap)_2$ (86 mg, 0.10 mmol) was added to the solution, and the reaction mixture was heated at 45˚C under an atmosphere of H_2 (70 psi) overnight. The reaction mixture was filtered over celite, and the solids were washed with ethyl acetate (3x30 mL). The collected

filtrate was concentrated *in vacuo*, and the resulting residue was purified by recrystallization from methanol to afford a yellow solid (6.51 g, 98.5%). *Rf* = 0.16 (hexane/ethyl acetate, 4/1, v.v). ^{1}H (300 MHz, $CDCl_3$) δ 3.77 – 3.70 (3 H, s, C*H*$_3$ of ester), 3.44 (2 H, s, H-2), 2.52 (2 H, t, *J* 7.4, H-4), 1.65 – 1.52 (2 H, m, H-5), 1.25 (16 H, s, H-6 to H-13)), 0.87 (3 H, t, *J* 6.7, H-14). ^{13}C NMR (75 MHz, $CDCl_3$) δ 52.06, 49.05, 43.60, 31.30, 29.31, 14.29. HR MALDI-ToF MS (m/z) calcd for $C_{15}H_{28}O_3$ $[M+Na]^+$ 281.2093; found 281.3538.

(R)-3-Hydroxy myristic acid (4): Lithium hydroxide (1.23 g, 30.50 mmol) was added to ester **3** (7.16 g, 27.33 mmol) in a solution of tetrahydrofuran (30 mL) and water (3 mL) The reaction was stirred vigorously overnight, after which the solvent volume was reduced *in vacuo* to 15 mL, and the mixture was washed with aqueous 1N HCl (2x10 mL), then brine (1x10 mL). The organic layer was dried ($MgSO_4$) and then concentrated *in vacuo*. The residue was purified by silica gel column (hexane/ethyl acetate) to give **4** as a light yellow solid (5.13 mg, 76%). R_f = 0.08 (hexane/ethyl acetate, 2/1, v/v). ^{1}H (300 MHz, $CDCl_3$) δ 3.98 (1 H, t, *J* 7.2, H-3), 3.70 (3 H, s, OCH3), 2.89 (1 H, s, CHOH), 2.45 (2 H, qd, *J* 16.4, 6.1, H-2), 1.57 – 1.41 (2 H, m, H-4), 1.18 (16 H, m, H-(5-13)), 0.87 (3 H, t, *J* 6.6, H-14). ^{13}C NMR (75 MHz, $CDCl_3$) δ 68.14, 51.79, 41.14, 36.65, 36.56, 29.62, 26.32, 25.53, 25.22, 22.86, 22.74, 22.59, 14.05. LR MALDI-ToF MS (m/z) calcd for $C_{14}H_{28}O_3$ $[M+Na]^+$ 267.193; found 267.118

2-(4-bromophenyl)-2-oxoethyl-(*R*)-3-hydroxy myristate (5): A solution of **4** (5.13 g, 20.99 mmol) in dry acetonitrile (20 mL) was heated under reflux. 2,4'-Dibromoacetophenone (6.42 g, 23.09 mmol) was added to the refluxing solution, followed by the slow addition of Et_3N (3.22 mL, 23.09 mmol), and the reaction mixture was stirred under reflux 2h. Product precipitated out of the mixture, and was then filtered. The resulting product was purified by silica gel column chromatography (DCM) to give **5** as a white solid (6.41 g, 69%). R_f = 0.36 (hexane/ethyl acetate,

2/1, v/v). ^{1}H (300 MHz, $CDCl_3$) δ 7.83 – 7.58 (4 H, m, aromatic of phenacetyl), 5.36 (2 H, q, *J* 16.5, C*H*$_2$ of phenacetyl), 4.11 (1 H, m, H-3), 3.28 (1 H, s, CHO*H*), 2.62 (2 H, ddd, *J* 24.3, 15.2, 6.1, H-2), 1.68 – 1.41 (2 H, m, H-4), 1.32 (18 H, m, H-5 through H-13), 0.88 (3 H, t, *J* 6.7, H-14). ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.55, 138.30, 128.51, 127.97, 127.82, 75.87, 71.68, 39.71, 34.32, 32.06, 29.79, 29.77, 29.72, 29.70, 29.49, 25.27, 22.83, 14.26. HR MALDI-ToF MS (m/z) calcd for $C_{22}H_{33}BrO_4$ $[M+Na]^+$ 463.1460; found 463.0466.

2-(4-bromophenyl)-2-oxoethyl-(*R*)-3-tetradecanoyloxy tetradecanoate (6): Tetradecanoyl chloride (0.71 mL, 2.6 mmol) was added dropwise to a solution of myristate **5** (1.5 g, 3.4 mmol) and dry pyridine (1.65 mL, 20.8 mmol) in DCM (2.0 mL) at 0˚C, and then the reaction mixture was allowed to warm to rt overnight. The mixture was then diluted in DCM (50 mL) and washed with 1N aqueous HCl (2x25 mL), then brine (25 mL). The organic layer was dried ($MgSO_4$) and concentrated *in vacuo*, and the residue was then purified by silica gel column chromatography (toluene) to give **6** as a clear solid (1.24 g, 73%). R_f = 0.64 (3/1/0.01, hexane/ethyl acetate/AcOH, v/v/v). ^{1}H (300 MHz, $CDCl_3$) δ 7.81 – 7.59 (3 H, m, H aromatic of phenacetyl), 5.34 – 5.23 (H, m, H-3), 2.82 – 2.65 (2 H, m, H-2), 2.30 (2 H, t, *J* 7.5, H-2'), 1.72 – 1.51 (4 H, m, H-4, H-3'), 1.25 [38 H, s, H-(5-13), H-(4'-13')], 0.88 (6 H, t, *J* 6.7, H-14, H-14'). ^{13}C NMR (300 MHz, $CDCl_3$) δ 132.49 (*C*$(CH)_2$ aromatic), 129.46 (*C*H aromatic) 70.30 (C-3), 39.05 (C-2), 34.59 (C-2'), 34.05 (C-4), 25.13 (C-3'), 29.60 [C-(5-13), C-(4'-13')], 14.15 (C-14, C-14L3'). LR MALDI-ToF MS (m/z) calcd for $C_{36}H_{59}BrO_5$ $[M+Na]^+$ 673.344; found 673.893

2-(4-bromophenyl)-2-oxoethyl-(*R*)-3-dodecyloxy tetradecanoate (7): In a manner similar to the synthesis of **6**, dodecyl chloride (0.60 mL, 2.62 mmol) was added dropwise to a solution of myristate **5** (1.50 g, 3.40 mmol) and dry pyridine (1.66 mL, 20.96 mmol) in DCM (2.0 mL) to afford **7** as a clear solid (1.29 g, 79%). R_f = 0.71 (hexane/ethyl acetate/ AcOH, 3/1/0.01, v/v/v).

^{1}H (300 MHz, acetone) 7.99 – 7.72 (6 H, m, H aromatic), 5.45 (2 H, s, CH_2 phenacetyl), 5.35 – 5.20 (1 H, m, H-3), 2.75 (2 H, d, *J* 6.6, H-2), 2.30 (2 H, t, *J* 7.3, H-2'), 1.78 – 1.52 (4 H, m, H-4, H-3'), 1.28 (32 H, s, H-5 to H-13, H-4'to H-11')], 0.87 (6 H, t, *J* 6.6, H-14, H-12'). ^{13}C NMR (75 MHz, acetone) δ 132.43, 132.40, 129.94, 129.84, 70.06, 66.36, 38.57, 34.05, 33.79, 31.86, 29.50, 24.96, 24.89, 22.56, 13.54. HR MALDI-ToF MS (m/z) calcd for $C_{34}H_{55}BrO_5$ $[M+ Na]^+$ 645.31; found 645.59.

(*R*)-3-tetradecanoyloxy tetradecanoic acid (8): Myristate **6** (0.508 g, 0.781 mmol) was dissolved in glacial acetic acid (15 mL), and zinc (0.495 g, 7.81 mmol) was added to the solution. The reaction mixture was heated under reflux for 2 h, and then the mixture was diluted in DCM (50 mL) and filtered through a pad of celite. The solid residue was washed with DCM (2x25 mL) and then the combined filtrates were concentrated *in vacuo*, and the residue coevaporated with toluene. The residue was purified by silica gel column chromatography (toluene/ethyl acetate, 10/1, v/v) to give **8** as a white solid (0.350, 98%). R_f = 0.20 (2/1, hexane/ethyl acetate, v/v). ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.29 – 5.14 (m, 1 H, H-3), 2.68 – 2.52 (m, 2 H, H-2), 2.27 (t, 2 H, *J* =7.5 Hz, H-2'), 1.60 (m, 4 H, H-4, H-3'), 1.25 (broad, 38 H, H-5 to H-13), H-4'to H-13')], 0.88 (t, 6 H, *J* 6.7, H-14, H-14'); ^{13}C NMR (300 MHz, $CDCl_3$): δ 69.87, 38.72, 34.50, 33.98, 24.99, 29.52, 14.12. HR MALDI-ToF MS (m/z) calcd for $C_{28}H_{54}O_4$ $[M+Na]^+$ 477.3920; found, 477.4179.

(*R*)-3-dodecyloxy tetradecanoic acid (9): In a manner similar to the synthesis of **8**, myristate **6** (1.18 g, 1.89 mmol) was treated with zinc (1.20 g, 1.89 mmol) in acetic acid (30 mL). The residue was purified with silica gel column chromatography (100% toluene) to afford **9** as a clear oil (0.333 g, 41%). R_f = 0.15 (hexane/ethyl acetate, 2/1, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.20 (dd, *J* = 12.6, 6.6 Hz, 1H, H-3), 2.66 – 2.52 (m, 2H, H-2), 2.28 (t, *J* = 7.5 Hz, 2H, H-2'), 1.60 (m,

4H, H-3', H-4), 1.25 (s, 34H, H-5 to H-13, H-4' to H-11'), 0.88 (t, *J* = 6.6 Hz, 6H, H-14, H-12'). ^{13}C NMR (75 MHz, $CDCl_3$) δ 70.42, 39.09, 34.80, 34.74, 34.31, 29.79, 25.40, 14.27. LR MALDI-ToF MS (m/z) calcd for $C_{26}H_{50}O_4$ $[M+Na]^+$ 449.360; found 449.572.

Methyl (*R*)-3-benzyloxy-myristate (10): To a cooled (0˚C) solution of **3** (1.27 g, 4.92 mmol), benzaldehyde (1.50 mL, 14.8 mmol), and TMS_2O (6.27 mL, 29.5 mmol) in dry THF (40 mL) was added dropwise TMSOTf (0.62 mL, 3.4 mmol). After stirring the solution for 15 min, Et_3SiH (2.75 mL, 17.2 mmol) was added dropwise. The reaction mixture was stirred at 0˚C for 1h, and then the reaction allowed to warm to rt overnight. The mixture was then diluted with DCM (30 mL) and washed with saturated aqueous $NaHCO_3$ (2x, 30 mL) then brine (30 mL). The organic phase was dried ($MgSO_4$) and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (hexane/ethyl acetate, 15/1, v/v) to afford **10** as a white solid (1.39 g, 81%). R_f = 0.28 (hexanes/ethyl acetate, 15/1, v/v). ^{1}H (300 MHz, $CDCl_3$) 7.39 – 7.25 (5 H, m, H aromatic), 4.55 (2 H, s, OC*H*$_2$ of benzyl), 3.95 – 3.82 (1 H, m, H-3), 3.68 (3 H, s, OC*H*$_3$), 2.56 (2 H, ddd, *J* 20.4, 15.0, 6.4, H-2), 1.67 – 1.45 (2 H, m, H-4), 1.38 – 1.21 [18 H, m, H-(5-13], 0.89 (3 H, t, *J* 6.7, H-14). ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.42, 138.69, 128.45, 127.90, 127.68, 76.23, 71.67, 51.73, 39.93, 34.55, 32.07, 29.80, 29.78, 29.77, 29.73, 29.71, 29.49, 25.31, 22.84, 14.27. LR MALDI-ToF MS (m/z) calcd for $C_{22}H_{36}O_3$ $[M+Na]^+$ 371.25; found 370.74.

(*R*)-3-Benzyloxy-myristic acid (11): In a manner similar to the synthesis of **4**, LiOH (0.291 g, 12.1 mmol)) was added to a solution of **10** (3.82 g, 11.0 mmol) in THF (30 mL) and H_2O (3 mL). The resulting residue was purified by silica gel column chromatography (hexane/ethyl acetate, 15/1, v/v) to afford **11** as a off-white solid (1.85g, 55%). R_f = 0.1 (hexane/ethyl acetate, 15/1, v/v). δ_H (300 MHz, $CDCl_3$) δ 7.37 – 7.23 (4 H, m aromatic), 4.58 (2 H, s, C*H*$_2$ of benzylidene), 3.89 (1 H, p, *J* 5.9, H-3), 2.60 (2 H, qd, *J* 15.4, 6.2, H-2), 1.72 – 1.51 (2 H, m, H-4), 1.45 – 1.19

(18 H, m, H-5 to H-13), 0.90 (3 H, t, *J* 6.5, H-14). ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.55, 138.30, 128.51, 127.97, 127.82, 75.87, 71.68, 39.71, 34.32, 32.06, 29.79, 29.77, 29.72, 29.70, 29.49, 25.27, 22.83, 14.26. HR MALDI-ToF MS (m/z) calcd for $C_{21}H_{34}O_3$ $[M+Na]^+$ 357.24; found 358.26.

2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranose (15): Glucosamine hydrochloride (34 g, 157.6 mmol) and $NaHCO_3$ (34 g) were dissolved in de-ionized H_2O (500 mL) and the temperature of the reaction mixture was reduced to 0˚C. 2,2,2-Trichloroethoxycarbonyl chloride (31.70 mL, 236 mmol) was added dropwise to the stirring solution which was allowed to stir overnight. The product precipitated from the reaction mixture, and was then filtered and washed with diethyl ether (2x50 mL) to afford **13** as a white solid anomeric mixture (51.05 g, 91%, α/β mixture). LR MALDI-ToF MS (m/z) calcd for $C_9H_{14}Cl_3NO_7$ $[M+ Na]^+$ 375.973; found 376.064.

Acetyl 3,4,6-triacetyloxy-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranoside (16): Acetic anhydride (80 mL, 720 mmol) was dripped slowly into a cooled (0˚C) solution of **15** (51.05 g, 144 mmol) in pyridine (60 mL). The reaction mixture was allowed to warm to rt overnight, after which it was neutralized with 1N HCl. The reaction was diluted in DCM (100 mL) and washed with deionized H_2O (2x75 mL). The organic layer was dried ($MgSO_4$), concentrated *in vacuo*, and the resulting residue was purified by silica gel column chromatography (hexane/ethyl acetate, 2/1, v/v) to give **16** as a white solid (46.01 g, 60%). R_f = 0.68 (hexane/ethyl acetate, 1/1, v/v). ^{1}H (300 MHz, $cdcl_3$) δ 6.23 (5 H, d, *J* 3.7, H-1), 5.74 (1 H, d, *J* 8.8, N*H* of Troc), 5.37 – 5.10 (2 H, m, H-4, H-3), 4.71 (2 H, dd, *J* 58.7, 12.1, CH_2 of Troc), 4.35 – 3.95 (4 H, m, H-6, H-2, H-5), 2.19 (3 H, s, CH_3), 2.12 – 1.88 (9 H, m, 3xCH_3). ^{13}C NMR

(75 MHz, cdcl$_3$) δ 90.59, 74.95, 70.52, 69.90, 67.70, 61.67, 53.42, 21.01, 20.84, 20.76. HR MALDI-ToF MS (m/z) calcd for $C_{17}H_{22}Cl_3NO_{11}$ $[M+Na]^+$ 544.02; found 544.03

3,4,6-triacyloxy-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranose (17): Hydrazine acetate (9.25 g, 100 mmol) was added to a solution of **16** (46.01 g, 87.3 mmol) in DMF (50 mL); the reaction mixture was stirred 12h, after which it was diluted with DCM (30 mL), washed with de-ionized H_2O (2x40 mL) and brine (40 mL). The organic layer was dried ($MgSO_4$) and concentrated *in vacuo* to afford **17** as a light yellow anomeric mixture (43.89 g).

Thexyldimethylsilyl 3,4,6-triacyloxy-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranoside (18): Imidazole (9.63 g, 141.5 mmol) was added to a solution of **17** (34.01 g, 70.76 mmol) in DMF (60 mL). To this soluion thexyldimethylsilyl chloride (15.96 mL, 81.34 mmol) was added. The reaction was stirred 12h; its solvent volume was reduced *in vacuo*, and then the reaction mixture was then diluted with DCM (50 mL) and washed with de-ionized H_2O (2x40 mL). The organic layer was concentrated *in vacuo*, and the resulting residue was purified by re-crystallization from MeOH to afford **18** as a white solid (38.92 g, 98% over 2 steps). R_f = 0.12 (hexane/ethyl acetate, 4/1, v/v). ^{1}H (300 MHz, $CDCl_3$): δ 5.24 (t, 1 H, H-3), 5.02 (t, 2 H, t, *J* 9.6, H-4), 4.85 – 4.56 (m, 3 H, H-1, CH_2 of Troc), 4.16 (qd, H, *J* 12.1, 4.3, H-6), 3.75 – 3.54 (m, 2 H, H-2, H-5), 2.11 – 1.98 (m, 9 H, 3xCH_3 of acyl), 1.66 – 1.51 (m, 1 H, CH(CH_3)$_2$), 0.90 – 0.78 (m, 12 H, 2xC(CH_3)$_2$), 0.15 (d, 5 H, *J* 8.4, Si(CH_3)$_2$). ^{13}C NMR (300 MHz, $CDCl_3$): δ 72.06 (C-3), 69.07 (C-4), 95.93 (C-1), 74.50 (*C*H$_2$ of Troc), 62.49 (C-6), 71.72 (C-5), 58.37 (C-2), 20.80, 20.68, 20.65 (C acyl), 1.60 (*C*H of TDS), 19.98, 19.71, 16.85 (2xC(*C*H$_3$)$_2$), -3.32, -1.98 [Si(*C*H$_3$)$_2$ of TDS]. LR MALDI-ToF MS (m/z) calcd for $C_{23}H_{38}Cl_3NO_{10}Si$ $[M+Na]^+$ 644.12; found 644.31.

Thexyldimethylsilyl 2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)- β-D-glucopyranoside (19): **18** (18.00 g, 29.07 mmol) was dissolved in a solution of methanol/chloroform (9/1, v/v) (50 mL), 1M methanolic NaOMe (1 mL), and guanidine hydrochloride (0.4777 g), and the reaction was stirred for 2h, maintaining pH between 9 and 11. The reaction mixture was then neutralized with Amberlite® IRC-50 weak acid resin, and this was filtered and the solid washed with dichloromethane (3x30 mL). The resulting organic layer was concentrated *in vacuo* and the resulting residue was purified by silica gel column chromatography (DCM/MeOH, 9/1, v/v) to afford **19** as a light yellow solid (14.44 g, quantitative). R_f = 0.25 ($CHCl_3$/MeOH, 9/1, v/v). ^{1}H (300 MHz, acetone): δ 6.78 (1 H, d, *J* 9.3, N*H* of Troc), 4.80 (1 H, d, *J* 8.0, H-1), 4.73 (1 H, s,), 3.87 (1 H, d, *J* 9.0), 3.66 (2 H, ddd, *J* 25.6, 14.2, 6.0), 3.49 – 3.26 (3 H, m), 1.63 (1 H, dq, *J* 13.7, 6.9), 0.89 (12 H, dd, *J* 12.0, 5.1), 0.26 – 0.12 (6 H, m). ^{13}C (300 MHz, acetone): δ 155.56 (*C*=O of Troc), 97.56 (C-1), 77.56 (O*C*H_2 of Troc), 75.58 (C-3), 75.23 (C-2), 72,84 (C-4), 63.19 (C-6), 60.97 (C-5), 34.93 (C(CH_3)$_2$*C*H of TDS), 25. 56, 20.63 (C(CH_3)$_2$C(*C*H_3)$_2$ of TDS), 19.09, 19.07 (C(*C*H_3)$_2$C(CH_3)$_2$ of TDS), -1.37 (Si*C*H_3 of TDS), -3.00 (Si*C*H_3 of TDS). HR MALDI-ToF MS calcd for $[C_{17}H_{32}Cl_3NO_7Si + Na]^+$ 518.0911; found 518.0213.

Dimethylthexylsilyl 4,6-*O*-benzylidene-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranoside (20): To a stirring solution of Dimethylthexylsilyl 2-amino -2-deoxy-2-(2-trichloroethoxycarbonylamino)-β-D-glucopyranoside (13.04g, 26.24 mmol) in anhydrous DMF (50mL) was added benzylidine dimethyl acetal (15.76mL, 105.0 mmol). To this solution was added camphor-10-sulfonic acid (0.6096 g, 2.624 mmol). Light vacuum was applied to the reaction mixture, and this was allowed to stir overnight. Upon completion, the reaction was quenched with triethylamine, then the DMF was removed *in* vacuo and the resulting residue was diluted in dichloromethane and washed with water (2x50 mL), then brine (50 mL). Organic

solvent was removed under vacuum, and the crude residue was purified by silica gel column chromatography (hexane/ ethyl acetate, 5/1) to give **20** as a white solid (11.26g, 75%). R_f = 0.16 (hexane/ethyl acetate, 4/1, v/v). ^{1}H (300 MHz, $CDCl_3$) δ 7.52 – 7.29 (5 H, m, aromatic), 5.54 (1 H, s, C*H* benzylidene), 5.14 (1 H, s, N*H* of Troc), 4.89 (1 H, d, *J* 7.8, H-1), 4.76 – 4.63 (2 H, m, CH_2 of Troc), 4.30 (1 H, dd, *J* 10.5, 4.9, H-6a), 4.11 (1 H, dt, *J* 9.0, 6.7, H-4), 3.79 (1 H, t, *J* 10.2, H-6b), 3.62 – 3.31 (3 H, m, H-5, H-2), 1.71 – 1.55 (1 H, m, C*H* of TDS), 0.92 – 0.83 (12 H, m, 2xC(CH_3)$_2$ of TDS), 0.16 (6 H, d, *J* 4.3, 2xSiCH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 129.02, 126.61, 102.14, 96.36, 96.20, 81.69, 74.92, 70.88, 68.71, 66.24, 60.92, 33.86, 33.81, 19.93, 18.51, -1.89, -3.16. HR MALDI-ToF MS calcd for $[C_{26}H_{40}Cl_3NO_5Si + Na]^+$ 606.1224; found, 606.0707

Dimethylthexylsilyl 3-*O*-allyloxycarbonyl-4,6-*O*-benzylidene-2-deoxy-2-(*N*-trichloroethoxycarbonylamino)-β-D-glucopyranoside (21): To a stirring solution of **20** (1.646g, 2.87 mmol) in DMF was added TMEDA (366µL, 217 mmol). The reaction mixture was cooled to 0˚C, and then allyl chloroformate was added dropwise. Following this addition, the reaction mixture was warmed to room temperature and then stirred overnight. The reaction was then diluted with dichloromethane (50mL) quenched with $NaHCO_3$ (2x40mL), and washed with brine (40mL). The organic phase was dried ($MgSO_4$) and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (hexane/ethyl acetate, 5/1) to give **21** as a white solid (1.7g, 97%). δ_H (300 MHz, $CDCl_3$) 7.39 – 7.20 (m, 5 H, aromatic), 5.77 (m, 1 H, *J* 22.6, 10.9, 5.7, C*H* of Alloc), 5.39 (s, 1 H, C*H* benzylidene), 5.14 (m, 4 H, H-3, H-4, OCH_2CHCH_2), 4.70 (m, 2 H, H-1, CH_2 of Troc), 4.54 – 4.44 (3 H, CH_2 of Troc, OCH_2CH of Alloc), 4.15 (dd, 1 H, *J* 10.5, 4.9, H-6a), 3.74 – 3.47 (2 H, m, H-6b, H-2), 3.38 (td, 1 H, *J* 9.6, 4.9, H-5), 1.56 – 1.41 (m, 1 H, C*H*), 0.81 – 0.63 (m, 12 H, 4xCCH_3), 0.01 (d, 6 H, *J* 7.7, Si(CH_3)$_2$). ^{13}C NMR (75 MHz,

$CDCl_3$) δ 155.08, 154.11, 137.06, 131.23, 129.24, 128.33, 126.35, 119.19, 101.58, 96.68, 78.98, 77.58, 77.16, 76.74, 75.29, 74.81, 69.07, 68.72, 66.35, 59.05, 34.05, 24.91, 20.06, 20.04, 18.63, -1.77, -3.28. MALDI-ToF LR MS (m/z) calcd for $C_{28}H_{40}Cl_3NO_9Si$ $[M+Na]^+$ 690.143; found 690.700

Dimethylthexylsilyl 3-O-allyloxycarbonyl-4-*O*-benzyl-2-(2-trichloroethoxycarbonylamino)-β-D-glucopyranoside (22): A mixture of **21** (2.27g, 3.27 mmol) and 4Å molecular sieves (1.2 g) in DCM (8 mL) was stirred for 1h, then cooled to -78˚C. To this reaction mixture was added $PhBCl_2$ (0.82 mL, 6.73 mmol) followed by triethylsilane (0.89 mL, 5.58 mmol). The reaction mixture was stirred for 5 minutes, then quenched while cold with methanol/pyridine (1/1, v/v, 1 mL). The resulting solution was diluted in dichloromethane (30 mL) and washed with saturated aqueous $NaHCO_3$ (2x25 mL), then brine (25 mL). The organic layer was dried ($MgSO_4$) and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (hexane/ethyl acetate, 5/1, v/v) to give **22** as a white solid (1.08g, 48%). R_f = 0.55 (hexane/ethyl acetate, 3/1, v/v). 1H (300 MHz, $CDCl_3$): δ 7.38 – 7.23 (4 H, m), 5.88 (1 H, ddd, *J* 16.2, 10.9, 5.8, C*H*=CH_2 of Alloc), 5.29 (2 H, ddd, *J* 13.8, 11.5, 1.2, CH=CH_2 of Alloc), 5.15 (1 H, d, *J* 9.2, N*H* of Troc), 5.10 – 4.97 (1 H, m, H-3), 4.86 – 4.51 (7 H, m, H-1, CH_2 of Troc, CHCH_2 of benzyl, CH_2 of Alloc), 3.85 (1 H, ddd, *J* 12.0, 5.6, 2.7, H-6), 3.77 – 3.54 [3 H, m, H-(6_b, 4, 2)], 3.48 – 3.40 (1 H, m, H-5), 1.82 (1 H, dd, *J* 7.8, 5.8, O*H* on C-6), 1.68 – 1.52 (1 H, m, $C(CH_3)_2CH$ of TDS), 0.89 – 0.79 (12 H, m, $C(CH_3)_2C(CH_3)_2$ of TDS), 0.14 (6 H, d, *J* 8.6, $Si(CH_3)_2$ of TDS). ^{13}C NMR (300 MHz, $CDCl_3$): δ 138.42 (C aromatic), 128.25 (C aromatic), 119.68 (C=CH_2 of benzyl), 78.78 (C-3), 96.31(C-1), 74.78 (CH_2 of Troc), 75.01 (CH_2CH of Alloc), 69.07 (CH_2 of benzyl), 62.05 (C-6), 75.78 (H-4), 58.50 (H-2), 75.21 (H-5),

33.92($C(CH_3)_2CH$ of TDS), 19.87-18.46 ($C(CH_3)_2C(CH_3)_2$ of TDS), -1.74,-3.21 ($Si(CH_2)_2$ of TDS). LR MALDI-ToF MS calcd for $C_{28}H_{42}Cl_3NO_9Si$ $[M+Na]^+$ 692.159; found 692.700.

Dimethylthexylsilyl 4,6-*O*-benzylidene-3-*O*-[(R)-3-tetradecanoyloxy-tetradecanoyloxy]-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranoside (23): To a stirring solution of **20** (340 mg, 0.584 mmol) in dry DCM (1.5mL) was added fatty acid **8** (292 mg, 0.642 mmol). The solution was cooled to 0˚C, and then DCC (132mg, 0.642 mmol) and DMAP (7 mg, 0.064 mmol) were added. The reaction mixture was allowed to warm to room temperature and stir overnight, after which it was diluted and the solids were washed with dichloromethane (2x10 mL). The combined filtrates were concentrated *in vacuo*, and the resulting residue was purified by silica gel column chromatography (hexane/ethyl acetate, 20/1→9/1) to give **23** as a white solid (1.90g , %). R_f = 0.44 (hexane/ethyl acetate, 4/1, v/v). ^{1}H NMR (300 MHz, $CDCl_3$): 7.47 – 7.26 (m, 5 H, aromatic), 5.47 (s, 1 H, C*H*Ph), 5.31 (t, 1 H, *J* 6.5, H-3), 5.17 (m, 2 H, N*H*, H-$3_{L3'}$), 4.88 (dd, 1 H, *J* 7.8, H-1), 4.66 (dd, 2 H, *J* 49.4, 12.0, CH_2 of Troc), 4.28 (dd, 1 H, *J* 10.5, 4.9, H-6_a), 3.78 (t, 1 H, *J* 10.3, H-6_b), 3.68 (t, 1 H, *J* 9.4, H-4), 3.62 – 3.43 [m, 2 H, H-(2, 5)], 2.54 (qd, 2 H, *J* 15.2, 6.3, H-$2_{L3'}$), 2.21 – 2.11 (m, 2 H, H-$2_{L3''}$), 1.66 – 1.43 [5 H, m, H-$3_{L3''}$,H-$4_{L3'}$), $C(CH_3)_2CH$ of TDS], 1.34 – 1.09 [38 H, m, H-$5_{L3'}$ to H-$13_{L3'}$, H-$4_{L3''}$ to H-$13_{L3''}$)], 0.91 – 0.77 [18 H, m, H-$14_{L3'}$, H-$14_{L3''}$), 4xC(CH_3) of TDS], 0.13 [6 H, d, *J* 6.7, 2xSiCH_3]. ^{13}C NMR (300 MHz, $CDCl_3$) δ 7.42, 7.33, 5.49, 5.34, 5.17, 4.91, 4.74, 4.61, 3.79, 3.72, 3.69, 3.56, 2.54, 2.18, 2.16, 1.61, 1.53, 1.52, 1.24, 0.87, 0.86, 0.83, 0.15, 0.13. MALDI-ToF HR MS (m/z) calcd for $C_{52}H_{88}Cl_3NO_{10}Si$ $[M+Na]^+$ 1042.5141; found 1042.6793.

Dimethylthexylsilyl 4,6-*O*-benzylidene-3-*O*-[(R)-3-tetradecanoyloxy-tetradecanoyloxy]-2-deoxy-2-(9-fluorenylmethoxycarbonylamino)-β-D-glucopyranoside (24): To a solution of Dimethylthexylsilyl-4,6-*O*-benzylidene-3-*O*-[(R)-3-tetradecanoyloxy-tetradecanoyloxy]-2-

deoxy-2-(2-trichloroethoxycarbonylamino)-β-D-glucopyranoside **23** (756 mg, 0.740 mmol) in acetic acid was added zinc dust (1.41 g, 22.23 mmol). The reaction mixture was heated at 60°C for 2h; it was then diluted with dichloromethane, and the solid was filtered from the solution through celite and washed with dichloromethane (3x 20 mL). The combined filtrates were washed with aqueous $NaHCo_3$ (2x 20 mL). The organic solvent was then removed *in vacuo* to yield a pale yellow oil, which was dried for one hour and then redissolved in dry dichloromethane (3 mL). To this reaction solution was added DIPEA (0.19 mL, 1.02 mmol). The temperature of the solution was reduced to 0°C, and 9-fluorenylmethoxycarbonyl chloride (263 mg, 1.02 mmol) was added. The reaction mixture was stirred at room temperature overnight, after which the mixture was diluted in dichloromethane (15 mL) and washed with water (10 mL), then brine (2x10 mL). The organic layer was concentrated *in vacuo*, and the resulting residue was purified by silica gel column chromatography (hexane/ethyl acetate 20/1→ 10/1) to give **24** as a white solid (474 mg, 60%). R_f = 0.58 (hexane/ethyl acetate, 4/1, v/v) ^{1}H (300 MHz, cdcl$_3$) δ 7.81 – 7.28 (14 H, m, H aromatic), 5.51 (1 H, s, C*H* of benzylidene), 5.40 (1 H, t, *J* 9.8, H-3), 5.26 – 5.15 (1 H, m, H-3$_{L3'}$), 5.09 (1 H, d, *J* 8.8, H-1), 4.95 (1 H, d, *J* 6.9, H-3), 4.42 – 4.16 (4 H, m, C*H*$_2$ Fmoc, H-6a, C*H* of Fmoc), 3.89 – 3.45 (3 H, m, H-6b, H-2, H-4, H-5), 2.57 (2 H, qd, *J* 15.2, 6.4, H-2$_{L3'}$), 2.22 – 2.10 (3 H, m, H-2$_{L3'}$, C*H* of TDS), 1.64 – 1.41 (4 H, m, H-4$_{L3'}$, H-3$_{L3''}$), 1.34 – 1.08 (42 H, m, H-5$_{L3'}$ to H-13$_{L3'}$, H-4$_{L3''}$ to H-13$_{L3''}$), 0.96 – 0.72 (18 H, m, 4xCC*H*$_3$ of TDS, H-14$_{L3'}$, H-14$_{L3''}$), 0.13 (6 H, dd, *J* 14.2, 9.1, 2xSiC*H*$_3$). ^{13}C NMR (75 MHz, cdcl$_3$) δ 129.14, 128.31, 127.64, 126.70, 126.47, 126.07, 125.67, 125.19, 124.17, 120.38, 118.73, 101.89, 97.16, 81.52, 79.30, 71.81, 70.42, 69.17, 69.11, 67.04, 66.97, 59.60, 47.37, 39.65, 34.57, 34.00, 32.20, 29.60, 29.57, 25.32, 25.24, 22.90, 21.12, 20.05, 18.73, 14.25, 14.21, -1.82, -3.05. LR MALDI-ToF MS (m/z) calcd for $C_{64}H_{97}NO_{10}Si$ $[M+Na]^+$ 1090.67; found 1090.20.

Dimethylthexylsilyl 2-Amino-4,6-*O*-benzylidine-2-deoxy-3-*O*-[(R)-3-tetradecanoyloxy-tetradecanoyloxy]-2-(9-fluorenylmethoxycarbonylamino)-D-glucopyranosyl trichloroacetimidate (26): To a solution of dimethylthexylsilyl-4,6-*O*-benzylidene-3-*O*-[(R)-3-tetradecanoyloxy-tetradecanoyloxy]-2-deoxy-2-(2-trichloroethoxycarbonylamino)-β-D-glucopyranoside **24** (100 mg, 0.0937 mmol) in THF (5 mL) was added 70% v/v HF/Pyridine (50 µL, 1.87 mmol). Equal portions of HF/Pyridine were added to the soloution every three hours, to a total of five portions. The reaction was monitored by TLC, and stopped 3 hours after the last HF/Pyridine addition. The mixture was diluted in dichloromethane (20 mL) and washed with aqueous $NaHCO_3$ (2x15 mL), then brine (15 mL). The organic layer was concentrated *in vacuo*, and the residue was purified by silica gel column chromatography (hexane/ethyl acetate 5/1) to yield intermediate **25** as a pale yellow oil (148 mg). R_f = 0.64 (toluene/ethyl acetate, 10/1, v/v). Lactol **25** (148mg, 0.156 mmol) was dissolved in dry dichloromethane (2mL) and trichloroacetonitrile (0.31 mL, 3.11 mmol), and to this solution was added cesium carbonate (28mg, 0.086 mmol). The reaction mixture was stirred at room temperature for 1h, after which the solvent volume was reduced and the resulting residue was passed through a plug of silica gel (ethyl acetate/triethylamine: 1/0.01). The organic solvent was concentrated *in vacuo* to give the crude imidate **26** as a yellow oil (160 mg).

Dimethylthexylsilyl 4,6-O-benzylidene-2-deoxy-2-(9-fluorenylmethylcarbonylamino)-3-*O*-[(*R*)-3-tetradecanoyloxy-tetradecanoyl]-β-D-glucopyranosyl-(1→6)-3-*O*-allyloxycarbonyl-4-*O*-benzyl-2-deoxy-2-(2-trichloroethoxycarbonylamino)-β-D-glucopyranoside (27): Trichloroacetimidate **26** (167 mg, 0.156 mmol) was dissolved in dry dichloromethane was added to a mixture of activated 4Å molecular sieves (247 mg) and glucopyranoside **22** (80 mg, 0.120 mmol). The reaction mixture was stirred at room temperature for 30 min, and its

temperature was reduced to -50˚C. TfOH (2.1 μL, 0.024 mmol) was then added to the solution, which was then stirred at -50˚C for 1.5h. The reaction mixture was quenched at -50˚C with solid $NaHCO_3$, then diluted with dichloromethane (20 mL)and washed with aqueous $NaHCO_3$ (2x15 mL) and brine (15 mL). The organic layer was concentrated *in vacuo*, and the resulting residue was purified by silica gel column (hexanes/ethyl acetate: 5/1) to give **27** as a white foam (150 mg, 79.4%). R_f = 0.64 (hexane/ethyl acetate, 4/1, v/v) ^{1}H (500 MHz, $CDCl_3$): δ 7.63 (2 H, d, *J* 7.3, aromatic), 7.51 – 7.05 (16 H, m, aromatic), 6.33 (1 H, d, *J* 9.6 N*H*), 6.27 (1 H, d, *J* 8.2, N*H'*), 5.78 – 5.65 (1 H, m, OCH_2CHCH_2), 5.41 (1 H, s, C*H* benzylidene), 5.27 (1 H, s, H-3'), 5.18 (1 H, d, *J* 16.8 OCH_2CHCH_{2a}), 5.10 (1 H, d, *J* 10.4 OCH_2CHC_{2b}), 5.07 – 4.98 (1 H, m, H-$3_{L3'}$), 4.82 (1 H, t, *J* 9.9, H-3), 4.73 – 4.56 (3 H, m, H-1, H-1', CH_{2a} Troc), 4.53 – 4.38 (5 H, m, OC$H_2$$CCl_3$, C$H_{2b}$ Troc, CH_2 benzyl), 4.23 (1 H, s, H-6a), 4.12 (1 H, d, *J* 8.2, CH_{2a} Fmoc), 3.98 (2 H, dd, *J* 32.5, 22.2, CH_{2b} Fmoc, H-6a'), 3.81 – 3.56 (3 H, m, H-6b, H-6b', H-5), 3.56 – 3.33 (3 H, m, H-5', H-2, H-2'), 2.40 (2 H, ddd, *J* 20.2, 15.6, 6.2, H-$2_{L3'}$), 1.98 (2 H, s, H-$2_{L3''}$), 1.49 (1 H, dt, *J* 13.6, 6.7, C*H*$(CH_3)_2$), 1.36 (4 H, s, H-$4_{L3'}$, H-$3_{L3''}$), 1.16 (38 H, d, *J* 15.7, H-$5_{L3'}$ to H-$13_{L3'}$, H-$4_{L3''}$ to H-$13_{L3''}$), 0.81 – 0.67 (18 H, m, 2xC(CH_3)$_2$, C-$14_{L3'}$, C-$14_{L3''}$), 0.05 (6 H, d, *J* 15.7, Si(CH_3)$_2$). ^{13}C NMR (126 MHz, $CDCl_3$) δ 133.06, 132.39, 130.84, 129.63, 129.62, 128.82, 127.50, 124.31, 123.69, 121.18, 106.03, 105.80, 100.37, 83.22, 82.81, 80.63, 78.90, 78.77, 78.69, 78.63, 78.16, 75.76, 75.65, 74.24, 72.99, 72.72, 72.70, 72.49, 71.33, 71.17, 70.33, 62.05, 60.64, 53.23, 50.91, 43.04, 43.02, 43.00, 38.22, 37.89, 37.59, 34.61, 33.47, 28.74, 28.64, 26.79, 26.35, 23.60, 22.38, 17.90, 4.79, 1.44. HR MALDI-ToF MS calcd for $C_{84}H_{119}Cl_3N_2O_{18}Si$ $[M+Na]^+$ 1601.7161; found 1602.1129.

Dimethylthexylsilyl 4,6-O-benzylidene-2-deoxy-2-(9-fluorenylmethylcarbonylamino)-3-*O*-[(*R*)-3-tetradecanoyloxy-tetradecanoyl]-β-D-glucopyranosyl-(1→6)-3-*O*-allyloxycarbonyl-4-*O*-benzyl-2-deoxy-2-[(*R*)-3-dodeanoyloxy-tetradecanoylamino]-β-D-glucopyranoside (28): Compound **27** (40.1 mg, 0.025 mmol) was dissolved into a solution of 20% piperidine in DCM (2.5 mL), and this solution was stirred at room temperature for 30 minutes, after which piperidine was removed *in vacuo* by coevaporating with toluene. The resulting residue was purified by silica gel column chromatography (hexanes/ethyl acetate, 6/1→4/1→2/1, v/v). The solvent was concentrated *in vacuo*, and then redissolved in dry dichloromethane (2 mL). The resulting solution was cooled to 0˚C, and (*R*)-3-dodecyloxy-myristic acid (14 mg, 0.0328 mmol) was then added to the reaction solution, followed by DCC (8.4 mg, 0.0406 mmol). This reaction mixture was allowed to stir and warm to room temperature overnight. Solids were then filtered and washed with dichloromethane, and the combined filtrates were concentrated *in vacuo*. The resulting residue was purified by silica gel column chromatography to give the product **28** as a clear oil (20 mg, 45%) R_f = 0.57 (hexane/ethyl acetate, 4/1, v/v) ^{1}H NMR (300 MHz, acetone) δ 7.28 – 7.05 (m, 10H, H aromatic), 6.71 (d, *J* = 9.8 Hz, 1H, N*H*), 5.70 (ddd, *J* = 22.7, 10.8, 5.5 Hz, 1H, OCH_2CHCH_2), 5.45 (s, 1H, C*H* benzylidene), 5.25 – 5.11 (m, 2H, H-3', OCH_2CHCH_{2a}), 5.08 – 4.90 (m, 4H, OCH_2CHCH_{2b}, H-$3_{L3'}$, H-$3_{L2'}$), 4.87 – 4.72 (m, 3H, H-1, H-1', H-3), 4.67 (d, *J* = 12.2 Hz, 1H $OCH_{2a}CHCH_2$), 4.43 (ddd, *J* = 17.7, 13.6, 8.2 Hz, 4H, CH_2 benzyl, $OCH_{2b}CHCH_2$), 4.13 (dd, *J* = 10.2, 4.9 Hz, 1H, H-6a'), 3.91 (d, *J* = 10.0 Hz, 1H, H-6a), 3.84 – 3.51 (m, 5H, H-6b', H-2', H-6b, H-4, H-4'), 3.51 – 3.30 (m, 3H, H-2, H-5, H-5'), 2.40 (qd, *J* = 15.7, 6.5 Hz, 2H, H-$2_{L3'}$), 2.28 – 2.00 (m, 4H, H-$2_{L2'}$, H-$2_{L3''}$), 1.94 (td, *J* = 7.3, 2.6 Hz, 2H, H-$2_{L2'}$), 1.47 – 1.24 (m, 11H, H-$4_{L3'}$, H-$4_{L2'}$, H-$3_{L3''}$, H-$3_{L2''}$), 1.09 (s, 85H, H-$5_{L3'}$ to H-$13_{L3'}$, H-$4_{L3''}$ to H-$13_{L3''}$, H-$5_{L2'}$ to H-$13_{L2'}$, H-$4_{L2''}$ to H-$11_{L2''}$), 0.68 (dd, *J* = 8.4, 5.4 Hz, 26H, H-$14_{L3'}$,

H-14$_{L3''}$, H-14$_{L2'}$, H-12$_{L2''}$, 2xC(C*H*$_3$)$_2$), 0.01 (d, *J* = 8.5 Hz, 6H, Si(C*H*$_3$)$_2$). ^{13}C NMR (75 MHz, acetone) δ 128.47, 118.06, 118.03, 101.82, 101.34, 96.20, 79.39, 79.26, 76.79, 74.60, 74.50, 74.50, 74.44, 74.30, 72.21, 70.19, 68.65, 68.63, 68.49, 68.48, 68.36, 66.56, 58.41, 54.91, 40.96, 40.95, 38.92, 34.20, 34.18, 34.03, 33.88, 32.03, 29.64, 25.09. HR MALDI-ToF MS (m/z) calcd for $C_{95}H_{157}C_{l3}N_2O_{19}Si$ $[M + Na]^+$ 1787.0; found 1787.7.

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CHAPTER 3

KDO MODIFIED LIPID IV_A

3.1 Introduction

Lipid A plays an integral role in the endotoxicity of LPS, and is responsible for TLR4/MD-2 aggregation[1,2]. The resulting dimer-like TLR4/MD-2 complex is responsible for recruiting proteins for the signal transduction pathways that result in production of proinflammatory molecules such as TNF-α and IL-1β[3,4]. Overproduction of these molecules, often due to release of excess LPS, can result in septic shock. Lipid IV_A is a tetraacylated precursor to the hexaacylated *e. coli* lipid A, and while lipid A is considered an endotoxin, lipid IV_A is considered an inhibitor of this endotoxic response. Interestingly, while lipid IV_A is an agonist in murine systems, it functions as an antagonist in humans[5]. Based on crystal structures of lipid IV_A in the pocket of MD-2, binding in the pocket varies from that of lipid A; lipid IV_A may even bind in reverse orientation from natural lipid A, and, significantly, all four chains of lipid IV_A fit inside the MD-2 hydrophobic pocket[6]. The four chains of lipid IV_A do not promote the protrusion of the L2 acyl chain into the

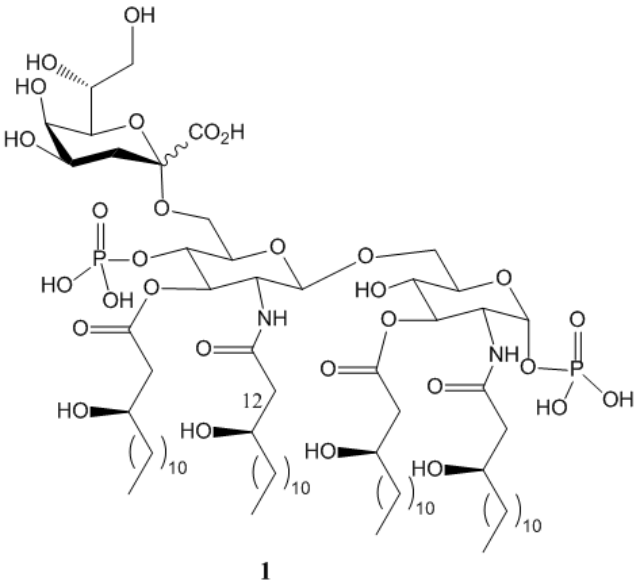

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Figure 3.1 KDO-Lipid IVa

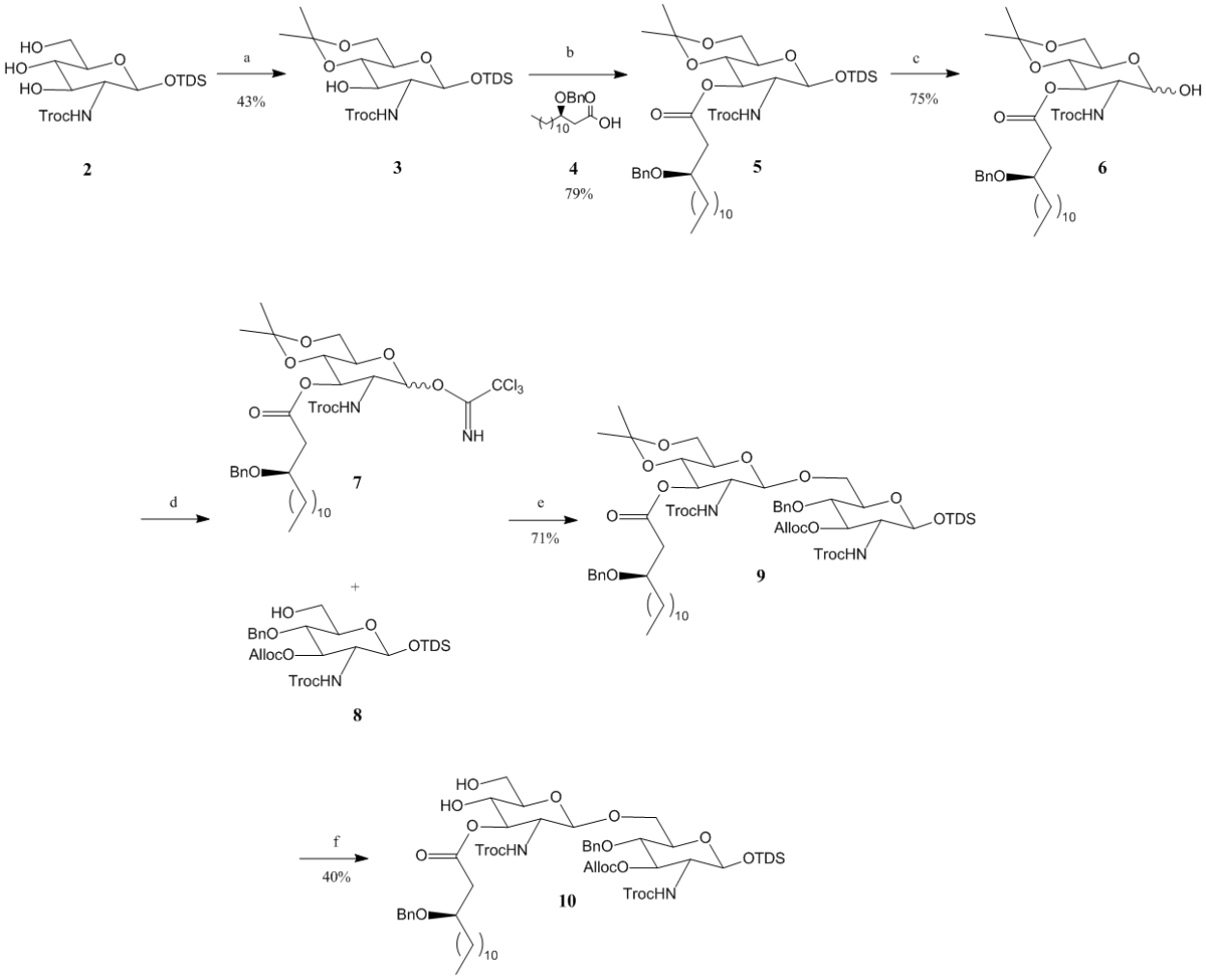
pocket opening, which would lessen the TLR4 clustering necessary for maximum signaling of the cascade and the resulting immunomodulating molecules[7].

In nature, the toxic principle of LPS, lipid A, is covalently linked to the inner core of the LPS oligosaccharide. This inner core consists of several carbohydrates, but the carbohydrates most proximal to the lipid A backbone are two KDO sugars[8, 9]. While not always essential to the activity of lipid A, KDO is required for proper function within bacteria[9], it appears to improve the activity of lipid A. Several studies have looked at the activity of *E. coli* Re LPS, composed of *E. coli* lipid A covalently linked to a KDO_2 unit. These studies have shown an improvement in the production of proinflammatory molecules when compared to *E. coli* lipid A[10]. Limited studies have also indicated that KDO-containing *N. meningitides* lipid A significantly improved activity of the lipid A, based on TNF-α and IFN-β production[11], and KDO linked natural *Rhizobium sin-1* lipid A has also been found to have improved activity over its synthetic counterparts lacking KDO[12]. While these investigations have involved KDO or KDO_2 linked lipid A, it would be interesting to discover whether the addition of a KDO moiety to lipid IV_A would similarly improve the antagonistic activity of the compound, or possibly cause lipid IV_A to behave as an agonist by altering its position in the MD-2 binding pocket. To this end, a synthesis of KDO-lipid IV_A (**Figure 3.1**) was developed employing a glucosamine backbone protected with alloc and troc groups, and a benzyl and isopropylidene protected KDO moiety.

3.2 Results and Discussion

The design of the acceptor and donor (**Scheme 3.1**), follows a design that is only partially convergent, having one of three positions orthogonal. This is not an issue, as lipid IV_A contains only four lipids, all of equal length. Isopropylidene was installed at the C4'/C6' positions, which

allowed a simple and high yielding complete deprotection of these positions once the diglucosamine backbone was obtained. Because KDO would be covalently linked to the C6'

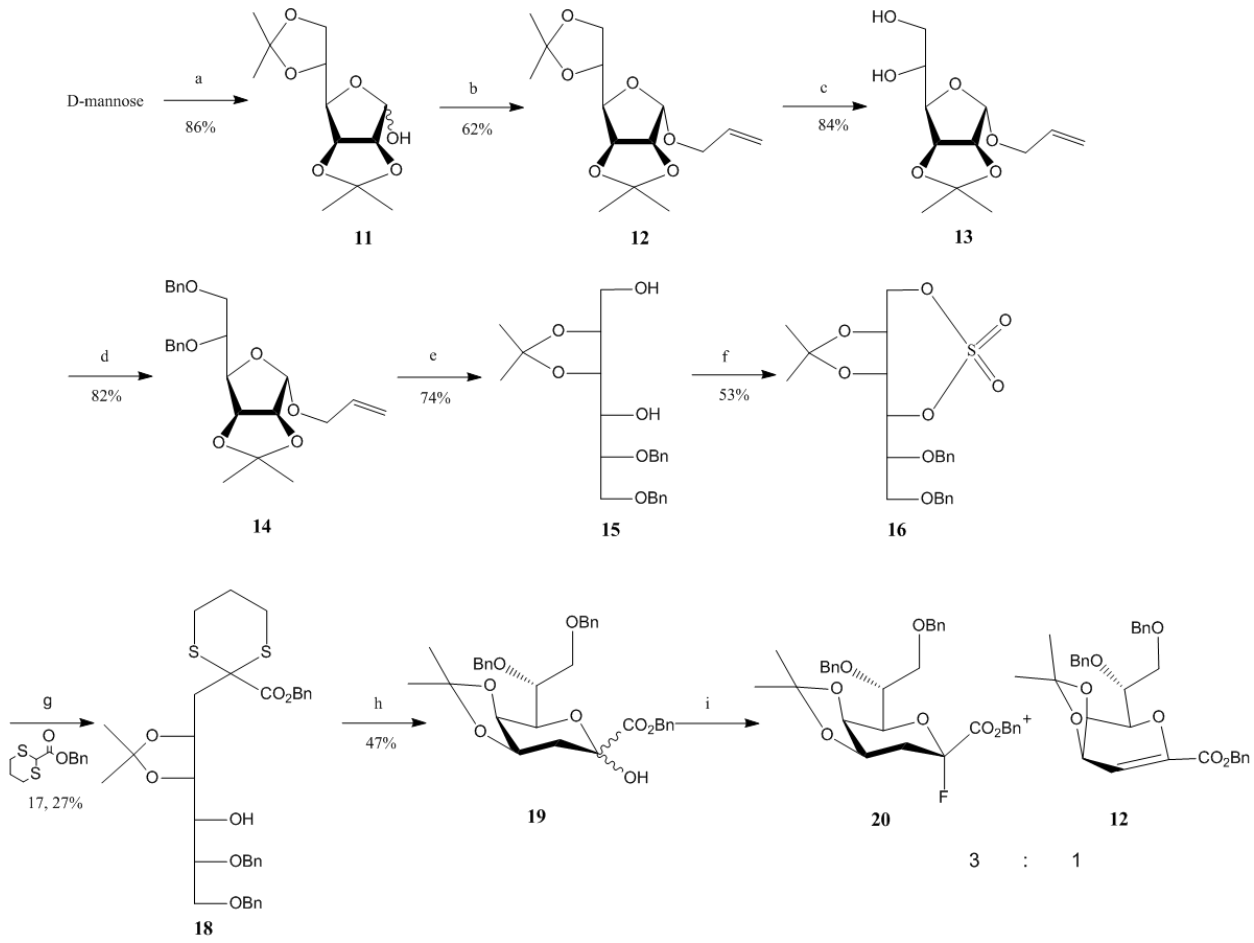


Scheme 3.1 (a) 2,2-dimethoxypropane, CSA, ACN, 12 h (b) DCC, DMAP, DCM, 0˚C –rt, 12 h (c) TBAF, HOAc, THF, 12 h (d) 2,2,2-trichloroacetonitrile, DBU, DCM, 1 h (e) TfOH, MS 4Å, DCM, -50˚C, 1 h (f) 80% TFA, reflux, 2.5 h

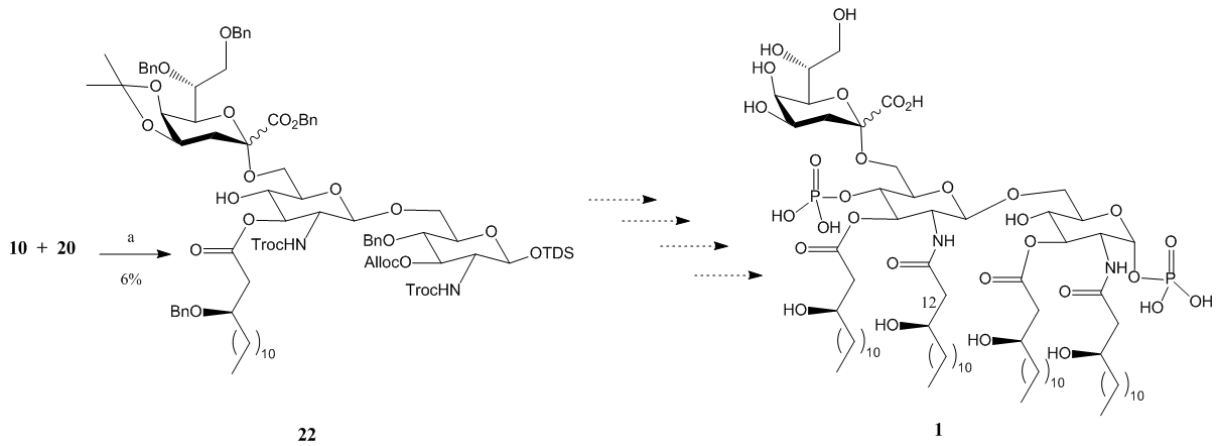
position prior to the addition of the C4' phosphate, the need for selective deprotection of C4' was eliminated[11]. Troc groups protect both C2 and C2' amines, because the acyl chains of lipid IVa are all equivalent. Synthesis of *E. coli* lipid IVa covalently linked with the first KDO of the LPS inner core began with the synthesis of the diglucosamine backbone. From the common precursor,

the donor **3** was synthesized by protection of the C4 and C6 positions through treatment with 2,2-dimethoxypropane and CSA in acetonitrile[13]. Acetonitrile was used preferentially over DMF because it can promote the addition of the protecting group. This isopropylidene protection was followed by coupling at the C3 position with (*R*)-3-benzyloxy myristic acid **4** to provide compound **5**. The anomeric position was deprotected with TBAF/HOAc to provide **6**[14], followed by the formation of the imidate **7** by treatment of **6** with sodium hydride in 3,3,3-trichloroacetonitrile[15]. Compound **7** was then combined with the acceptor **8** in the presence of TfOH and 4Å molecular sieves to obtain disaccharide **9**[11], and the isopropylidene was deprotected using 80% acetic acid to afford disaccharide **10**.

KDO was synthesized from D-mannose based on a method designed by van Boom[16] (**Scheme 3.2**). D-mannose was treated with concentrated sulfuric acid in the presence of acetone, which promotes the conversion of D-mannose into D-mannofuranose and captures this configuration through protection of C5 and C6 with isopropylidene to achieve protected mannofuranose **11**. Allylation of the compound **11** was carried out by treatment with potassium hydroxide and allyl bromide to afford mannofuranoside **12**. The 5,6-isopropylidene was selectively deprotected using 1.3% HCl/MeOH, and the resulting compound **13** was then benzylated at C5 and C6 by treatment with sodium hydride and benzyl bromide to provide compound **14**. The deprotection of the anomeric allyl group was difficult to achieve. Compatable deprotection procedures involve treatment of the compound with Pd/C in methanol under reflux followed by treatment with iodine. This method proved unreliable, with very low to no yields, but it was found that long reflux with Pd/C could result in allyl deprotection, and this procedure was employed to give the desired intermediate mannofuranose. The percent of Pd/C reagent and time are important to the effectiveness of this method. The highest yields were achieved using



Scheme 3.2 (a) i. H_2SO_4, acetone, 0˚C, 12 h; ii. K_2CO_3, H_2O (b) KOH, Allyl-Br, acetone, DMF, 0˚C, 12 h (c) conc. HCl, MeOH, 1.5 h (d) BnBr, NaH, DMF, rt, 10h (e) i. Pd/C (Degussa), CH_3OH, reflux, 48 h; ii. I_2, pyridine, H_2O, THF, 30 min; iii. $NaBH_4$, EtOH, 8h (f) $SOCl_2$, Et_3N, DCM, -15˚C, 20 min; ii. $NaIO_4$, $RuCl_3$, H_2O, CH_3CN, DCM, rt, 20 min (g) i. n-BuLi, THF, HMPA, -45˚C, 2h; ii. H_2SO_4, H_2O, THF, 50˚C, 1h (h) NBS, $NaHCO_3$, H_2O, acetone, rt, 10 min (i) DAST, MS 4Å, DCM, -60˚C-rt, 30 min



Scheme 3.3 (a) $BF_3{\cdot}Et_2O$, MS 4Å, DCM, 0˚C, 1 h;

Degussa 10% Pd/C, a reagent that has a highly regular surface and high surface area, over 48 h The intermediate was then treated with sodium borohydride in ethanol to yield mannitol **15**. Compound **15** was treated with thionyl chloride in the presence of Et_3N, and the intermediate was oxidized using $RuCl_3$ and $NaIO_4$ to give the cyclic sulfate **16**. Previously prepared dithioacetal **17** was coupled with **16** using *n*-BuLi in HMPA/THF to afford **18**. This reaction is very moisture sensitive, and works best in large scale, with dried HMPA. Treatment of the coupling product with NBS in the presence of $NaHCO_3$ afforded **19** as an α\β mixture. Compound **19** was then treated with DAST in the presence of MS 4Å at -60˚C to give the α-glycosyl fluoride **20** and its corresponding glycal **21**[10]. Glycosyl fluoride **20** and diglucosamine **10** were combined and activated with $BF_3{\cdot}Et_2O$ to achieve compound **22** (**Scheme 3.3**), which can be converted after further steps to final target **1**.

3.3 Experimental Section

Column chromatography was performed on silica gel 60 (EM Science, 70-230 mesh). Reactions were monitored by thin-layer chromatography (TLC) on Kieselgel 60 F254 (EM Science), and the compounds were detected by examination under UV light and by charring with 10% sulfuric acid in MeOH. Solvents were removed under reduced pressure at <40˚C. CH_2Cl_2 was distilled

from CaH_2 and tetrahydrofuran (THF) was distilled from sodium directly prior to the application. CH_3OH was dried by refluxing with mageniusm methoxide and then was distilled and stored under argon. Pyridine was dried by refluxing with CaH_2 and then was distilled and stored over molecular sieves (3 Å). Molecular sieves (3 and 4 Å), used for reactions, were crushed and activated *in vacuo* at 390 ˚C during 8 h in the first instance and then for 2-3 h at 390˚C directly prior to application. ^{1}H NMR and ^{13}C NMR spectra were recorded with Varian spectrometers (model Inova500) equipped with Sun workstations. ^{1}H NMR spectra were recorded in CDCl3 and reference to residual $CHCl_3$ at 7.24 ppm, and ^{13}C NMR spectra were referenced to the center peak of $CDCl_3$ at 77.0 ppm. Assignments were made by standard gCOSY and gHSQC. High resolution mass spectra were obtained on a Bruker model Ultraflex MALDI-TOF mass spectrometer. Signals marked with L2 and L3correspond to the monoantennary lipid at C2 and C3, respectively. Signals marked with L2' and L3' correspond to the biantennary lipids at C2' and C3', while signals marked with L2'' and L3'' correspond to the side chains of L2' and L3'.

Thexyldimethylsilyl 2-deoxy-4,6-di-*O*-isopropylidene-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranoside (3): 2,2-dimethoxypropane (10.76 mL, 87.8 mmol) was added to a solution of sugar **2** (6.23 g, 17.6 mmol) in acetonitrile (26 mL). Camphor sulfonic acid (0.408 g, 1.76 mmol) was added to the solution, and the reaction mixture was stirred 24 h. The reaction mixture was then neutralized with triethylamine (1 mL) and the solvent volume was reduced *in vacuo*. The residue was then diluted with DCM (60 mL) and washed with brine (2x25 mL). The organic layer was dried ($MgSO_4$) and concentrated *in vacuo*, and the resulting residue was purified by silica gel column chromatography (hexanes/ethyl acetate, 6/1→ 2/1, v/v) to afford **3** as a light yellow solid (4.01 g, 43%). R_f = 0.18 (hexane/ethyl acetate, 4/1, v/v) ^{1}H NMR (300 MHz, acetone) δ 6.72 (d, *J* = 9.3 Hz, 1H, N*H*), 4.69 (d, *J* = 8.0

Hz, 1H, H-1), 4.58 (s, 2H,), 3.75 – 3.48 (m, 3H, H-6a, CH_{2a} of Troc, H-4), 3.46 – 3.24 (m, 2H), 3.13 – 3.01 (m, 1H), 2.65 (s, 1H), 1.93 – 1.84 (m, 1H), 1.56 – 1.41 (m, 1H), 1.36 (s, 3H), 1.18 (s, 3H), 0.75 – 0.64 (m, 12H), 0.00 (s, 5H). ^{13}C NMR (75 MHz, acetone) δ 206.25, 206.24, 100.06, 97.93, 75.56, 75.25, 72.47, 68.43, 62.90, 61.87, 34.90, 30.73, 30.48, 30.22, 29.96, 29.71, 29.45, 29.19, 25.54, 20.56, 19.54, 19.08, 19.05, -1.54, -2.97. MALDI-ToF HR MS (m/z) calcd for $C_{20}H_{36}Cl_3NO_7Si$ $[M+Na]^+$ 558.1224; found 558.0980.

Thexyldimethylsilyl 3-*O*-[(R)-3-benzyloxy-tetradecanoyloxy]-2-deoxy-4,5-di-*O*-isopropylidene-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranoside (5): Acid **4** (1.22 g, 3.67 mmol) was added to a solution of **3** (1.79 g, 3.33 mmol) in DCM (5.5 mL). EDC (1.02 g, 5.32 mmol) was added to the solution, followed by DMAP (0.002 g, 0.014 mmol). The reaction mixture was stirred 12 h, after which it was diluted in DCM (50 mL) and washed with water (2x25 mL) and brine (25 mL). The organic layer was dried ($MgSO_4$) and concentrated *in vacuo*, and the residue was purified by silica gel column chromatography (hexane/ethyl acetate, 10/1, v/v) to afford **5** as a clear oil (2.25 g, 79%) R_f = 0.39 (hexane/ethyl acetate, 15/1, v/v) ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.25 – 7.09 (m, 5H), 5.04 (t, *J* = 9.9 Hz, 1H), 4.90 (d, *J* = 9.5 Hz, 1H), 4.67 (d, *J* = 7.8 Hz, 1H), 4.50 (dt, *J* = 12.2, 8.3 Hz, 3H), 4.36 (d, *J* = 11.5 Hz, 1H), 3.84 – 3.54 (m, 4H), 3.44 (d, *J* = 8.0 Hz, 1H), 3.23 (dd, *J* = 9.8, 5.3 Hz, 1H), 2.45 (ddd, *J* = 58.2, 15.1, 6.1 Hz, 2H), 1.56 – 1.37 (m, 4H), 1.31 (s, 3H), 1.21 – 1.08 (m, 21H), 0.82 – 0.66 (m, 15H), 0.02 (t, *J* = 6.5 Hz, 5H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 128.35, 127.73, 127.60, 99.65, 77.43, 77.01, 76.58, 75.62, 71.55, 67.50, 62.10, 59.06, 58.98, 34.38, 33.89, 31.93, 29.66, 29.36, 28.95, 25.32, 22.70, 19.92, 18.98, 18.49, 14.13, -0.00. MALDI-ToF HR MS (m/z) calcd for $C_{41}H_{68}C_{l3}NO_9Si$ $[M+Na]^+$ 874.3627; found 874.8670

3-*O*-[(R)-3-benzyloxy-tetradecanoyl]-2-deoxy-4,5-di-*O*-isopropylidene-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranose (6): A mixture of TBAF (1 M, 5.0 mL, 5.00 mmol) and HOAc (0.8 mL) was added to a solution of glucopyranoside **5** (1.28 g, 1.50 mmol) in THF (10 mL), and the reaction was stirred 48 h. The reaction mixture was then diluted in ethyl acetate (30 mL) and washed with saturated aqueous $NaHCO_3$ (2x15 mL), then brine (15 mL). The organic layer was dried ($MgSO_4$) and concentrated *in vacuo*, and the resulting residue was purified by silica gel column chromatography (hexane/ethyl acetate, 2/1, v/v) to afford a white solid (0.8004 g, 75%). R_f = 0.19 (hexane/ethyl acetate, 2/1, v/v) ^{1}H (300 MHz, $CDCl_3$) δ 7.30 (5 H, m, H aromatic), 5.53 – 5.45 (1 H, d, N*H*), 5.36 – 5.21 (2 H, m, H-3, H-1), 4.74 – 4.41 (4 H, m, CH_2 of Troc, CH_2Ph), 3.96 (2 H, dd, *J* 8.8, 4.3, 6b, H-$3_{L3'}$), 3.92 – 3.67 (4 H, m, H-4, H-6a, H-2, H-5), 2.55 (2 H, ddd, *J* 21.1, 15.3, 6.3, H-$2_{L3'}$), 1.44 (3 H, s, CH_3), 1.32 (3 H, s, CH_3), 1.25 (18 H, s, H-$5_{L3'}$ to H-$13_{L3'}$), 0.88 (17 H, t, *J* 6.6, H-$14_{L3'}$). ^{13}C NMR (75 MHz, $CDCl_3$) δ 128.69, 128.53, 128.00, 127.78, 100.02, 74.78, 71.54, 70.27, 64.04, 55.12, 34.70, 32.14, 29.88, 29.57, 29.17, 25.50, 22.91, 19.21, 14.34. MALDI-ToF HR MS (m/z) calcd for $C_{33}H_{50}Cl_3NO_9$ $[M+Na]^+$ 732.2449; found 731.3425.

3-*O*-[(R)-3-benzyloxy-tetradecanoyl]-2-deoxy-4,5-di-*O*-isopropylidene-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranoside trichloroacetimidate (7): Sugar **6** (338 mg, approx 0.476 mmol) was dissolved in DCM (1 mL), followed by the addition of 2,2,2-trichloroacetimidate (0.96 mL, 9.53 mmol). The temperature of the solution was reduced to 0˚C, after which washed NaH (5.7 mg, 0.238 mmol) was added to the cooled solution. The reaction mixture was stirred overnight, after which it was diluted with DCM (10 mL) and washed with saturated aquesous $NaHCO_3$ (2x4 mL) and brine (4 mL). The organic layer was then filtered

through pad of silica gel (hexane/ethyl acetate, 2/1, v/v) and the organic layer was concentrated *in vacuo* to afford **7** as an impure yellow oil (0.521 g).

Dimethylthexylsilyl 3-*O*-[(*R*)-3-benzyloxy-dodecanoyl]-2-deoxy-4,6-di-*O*-isopropylidene-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl-(1→6)-3-*O*-allyloxcarbonyl-4-*O*-benzyloxy-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranoside (9): Trichloroacetimidate **7** (521 mg, 0.610 mmol) and sugar **8** (372 mg, 0.554 mmol) were combined in DCM with activated MS (4Å, 893 mg), and the temperature of the mixture was stirred at rt for 1 h. The mixture was cooled to -50˚C, and then TfOH (9.8 μL, 0.111 mmol) was added to the cooled mixture. The reaction mixture was stirred at -50˚C for 30 min, then allowed to warm to -10˚C and quenched with solid $NaHCO_3$. The mixture was then diluted in DCM and filtered through a pad of celite and the solid washed with DCM (2x10 mL). The filtrate was washed with saturated aqueous $NaHCO_3$ (2x10 mL) and brine (10 mL), and the organic layer was concentrated *in vacuo*. The resulting residue was purified by silica gel column chromatography (hexane/ethyl acetate, 10/1→4/1, v/v) to afford **9** as a white solid (537 mg, 71%). R_f = 0.46 (hexane/ethyl acetate, 4/1, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.36 – 7.24 (m, 10H, aromatic), 5.95 – 5.77 (m, 1H, OCH_2CHCH_2), 5.36 – 5.12 (m, 3H, OCH_2CHCH_2, H-3'), 5.10 – 4.91 (m, 3H, H-1, H-1', H-3), 4.80 (d, *J* = 11.9 Hz, 1H, CH_2Ph), 4.71 – 4.43 (m, 12H, CH_2Ph, 2xOCH_2CCl3, CH_2Ph, OCH_2CHCH_2, H-4, H-4'), 3.94 (dd, *J* = 18.0, 7.5 Hz, 1H, H-6a), 3.84-3.51 (ddt, *J* = 31.2, 18.9, 11.4 Hz, 10H, H-6b, H-$3_{L3'}$, H-6a',H-6b', H-5, H-5', H-2. H-2'), 3.28 (d, *J* = 5.3 Hz, 1H), 2.65 (dd, *J* = 15.1, 6.4 Hz, 1H, H-$2a_{L3'}$), 2.46 (dd, *J* = 15.2, 5.8 Hz, 1H, H-$2b_{L3'}$), 1.64 – 1.48 (m, 5H, H-$4_{L3'}$, H-$5_{L3'}$, $CH(CH_3)_2$), 1.42 (s, 3H, CH_3), 1.31 (s, 3H, CH_3), 1.25 (s, 18H, H-$5_{L3'}$ to H-$13_{L3'}$), 0.90 – 0.79 (m, 15H, 2x$C(CH_3)_2$, H-$14_{L3'}$), 0.15 (d, *J* = 14.0 Hz, 6H, $Si(CH_3)_2$).^{13}C NMR (126 MHz, $CDCl_3$) δ 134.06, 130.67, 130.07, 128.85, 128.60, 96.58, 76.15,

75.40, 69.87, 69.19, 58.06, 34.15, 33.62, 31.14, 28.42, 21.03, 20.22, 18.63, 14.26, 14.25, 11.21, 1.31, -3.03. MALDI-ToF HR MS (m/z) calcd for $C_{61}H_{90}Cl_6N_2O_{17}Si$ $[M+Na]^+$ 1385.4008; found 1385.7169

Dimethylthexylsilyl 3-*O*-[(*R*)-3-benzyloxy-dodecanoyl]-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl-(1→6)-3-*O*-allyloxcarbonyl-4-*O*-benzyloxy-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranoside (10): Disaccharide **9** (0.537 g, 0.394 mmol) was dissolved in aqueous HOAc (80%, 5 mL) and heated under reflux for 2.5 h. Solvent was removed *in vacuo* and the HOAc was removed by coevaporating with toluene. The crude residue was purified by silica gel column chromatography (hexane/ethyl acetate, 2/1, v/v) to afford **10** as a clear oil (210 mg,40%). R_f = 0.19 (hexane/ethyl acetate, 2/1, v/v). ^{1}H (300 MHz, $CDCl_3$) δ 7.27 – 7.08 (10 H, m, H aromatic), 5.84 – 5.66 (1 H, m, OCH_2CHCH_2), 5.15 (3 H, ddd, *J* 27.8, 16.8, 4.2, OCH_2CHCH_2, H-3'), 5.03 – 4.81 (3 H, m, H-1, H-3', H-1), 4.73 – 4.28 (12 H, m, 2xCH_2Ph, OCH_2CHCH_2, 2xOCH_2 of Troc), 3.85 (1 H, d, *J* 10.6, H-6b'), 3.80 – 3.67 (2 H, m, $H\text{-}3_{L3'}$, H-6a'), 3.67 – 3.30 (64 H, m, H-4', H-4, H-6a,b', H-5'), 3.30 – 3.18 (1 H, m, H-5), 2.56 – 2.32 (2 H, m, $H\text{-}2_{L3'}$), 1.47 (2 H, dt, *J* 24.5, 8.9, $H\text{-}4_{L3'}$), 1.17 (18 H, d, *J* 21.2, $H\text{-}5_{L3'}$ to $H\text{-}13_{L3'}$), 0.82 – 0.64 (16 H, m, $H\text{-}14_{L3'}$, 2x$C(CH_3)_2$ of TDS), 0.02 (6 H, dd, *J* 10.9, 4.6, $Si(CH_3)_2$). ^{13}C NMR (75 MHz, $CDCl_3$) δ 128.90, 128.44, 128.23, 126.71, 119.48, 119.43, 119.42, 119.39, 101.21, 96.32, 96.15, 78.90, 76.09, 76.04, 75.63, 75.55, 75.54, 74.87, 74.84, 74.82, 74.81, 74.80, 74.76, 74.58, 71.29, 69.79, 69.11, 68.38, 68.33, 68.29, 62.44, 62.33, 62.28, 58.41, 56.15, 39.74, 39.70, 34.24, 34.10, 34.09, 33.89, 32.05, 29.72, 29.70, 25.03, 20.15, 20.06, 18.64, 14.24, -1.47, -3.22. MALDI-ToF LR MS (m/z) calcd for $C_{58}H_{86}Cl_6N_2O_{17}Si$ $[M+Na]^+$ 1350.37; found 1350.44

2,3-5,6-Diisopropylidene mannofuranose (11): A solution of D-mannose (100 g, 0.555 mol) in acetone (500 mL) was reduced to 0˚C, and conc. H_2SO_4 (25 mL, 0.47 mol) was then dripped into the rapidly stirring solution, and stirred 5 h. The reaction mixture was allowed to warm to rt overnight, and then was poured slowly into stirring potassium carbonate (155 g, 1.12 mol) in deionized H_2O (1 L). Acetone was removed *in vacuo* at low temperature (below 50˚C), resulting in formation of precipitation. The precipitate was filtered, washed with deionized H_2O. The resulting solid was dried *in vacuo* to yield a light tan solid (124.56 g, 86%). R_f = 0.36 (hexane/ethyl acetate, 4/3, v/v) ^{1}H (300 MHz, $CDCl_3$) δ 5.37 (1 H, d, *J* 2.1, H-1), 4.85 – 4.75 (1 H, m, H-3), 4.60 (1 H, d, *J* 5.9, H-2), 4.40 (1 H, dd, *J* 12.1, 5.9, H-4), 4.17 (1 H, dd, *J* 7.1, 3.6, H-6b), 4.12 – 4.00 (2 H, m, H-5, H-6a), 3.20 (1 H, d, *J* 2.3, O*H*), 1.45 (6 H, s, 2xCH_3), 1.37 (3 H, s, CH_3), 1.32 (3 H, s, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 128.69, 128.53, 128.00, 127.78, 100.02, 77.65, 77.23, 76.80, 74.78, 71.54, 70.27, 64.04, 55.12, 34.70, 32.14, 29.88, 29.57, 29.17, 25.50, 22.91, 19.21, 14.34. MALDI-ToF HR MS (m/z) calcd for $C_{12}H_{20}O_6$ $[M+Na]^+$ 283.1158; found 282.9830.

Allyl 2,3,5,6-diisopropylidene mannofuranoside (12): KOH was added to a solution of **11** in anhydrous acetone at 0˚C. After stirring this solution 30 min, allyl bromide was added dropwise and the reaction mixture was allowed to stir 24 h. The reaction mixture was quenched with AcOH, the solvent was removed *in vacuo*, and the resulting residue was purified by silica gel column chromatography (hexane/ethyl acetate, 4/3, v/v) to give **2** as a white solid (12.53 g, 39%). R_f = 0.84 (hexane/ethyl acetate, 4/3, v/v) ^{1}H (300 MHz, $CDCl_3$) δ 5.87 (1 H, dddd, *J* 15.7, 10.4, 6.1, 5.4, OCH_2CHCH_2), 5.31 – 5.13 (2 H, m, OCH_2CHCH_2), 5.01 (1 H, s, H-1), 4.77 (1 H, dd, *J* 5.9, 3.6, H-3), 4.60 (1 H, d, *J* 5.9, H-2), 4.39 (1 H, ddd, *J* 7.6, 6.2, 4.5, H-4), 4.17 – 4.06 (2 H, m, CH_2CHCH_2), 4.02 (1 H, dd, *J* 8.7, 4.4, H-6b), 3.99 – 3.89 (2 H, m, H-6a, H-5), 1.45 (6 H,

d, *J* 2.2, 2xCH_3), 1.37 (3 H, s, CH_3), 1.31 (3 H, s, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 133.93, 117.70, 112.71, 109.34, 105.75, 85.22, 80.47, 79.67, 77.58, 77.16, 76.74, 73.28, 68.13, 67.07, 27.02, 26.02, 25.31, 24.65. MALDI-ToF HR MS (m/z) calcd for $C_{15}H_{24}O_6$ [M+Na], 323.1471; found 323.1858.

Allyl 2,3-di-*O*-isopropylidene-α-D-mannofuranoside (13): Conc. HCl (1.95 mL) was dripped slowly into a stirring solution of **12** (12.53, 41.3 mmol) in anhydrous MeOH (195 mL). The reaction mixture was stirred 1.5 h and then quenched with ammonia (1.95 mL). The quenched solution was coevaporated with anhydrous toluene, then dissolved in ethyl acetate (100 mL) and washed with sat. aqueous $NaHCO_3$ (2x75 mL), the brine (75 mL). The organic layer was dried ($MgSO_4$) and concentrated *in vacuo*, and the resulting residue was purified by silica gel column chromatography (hexane/ethyl acetate, 3/1, v/v) to give **13** as a light yellow oil (9.12 g, 84%). R_f = 0.38 (hexane/ethyl acetate, 6/1, v/v) ^{1}H NMR (300 MHz, CD_3OD) δ 6.01 – 5.85 (m, 1H, OCH_2CHCH_2), 5.24 (dd, *J* = 31.0, 12.9 Hz, 2H, OCH_2CHCH_2), 5.00 (s, 1H, H-1), 4.84 (t, *J* = 3.0 Hz, 1H, H-3), 4.61 (d, *J* = 5.9 Hz, 1H, H-2), 4.21 – 4.08 (m, 1H, $OCH_{2a}CHCH_2$), 4.03 – 3.87 (m, 3H, $OCH_{2b}CHCH_2$, H-4, H-5), 3.82 (dd, *J* = 11.6, 2.5 Hz, 1H, H-6a), 3.61 (dd, *J* = 11.6, 5.5 Hz, 1H, H-6b), 1.45 (s, 3H, CH_3), 1.35 (s, 3H, CH_3). MALDI-ToF HR MS (m/z) for $C_{12}H_{20}O_6$ $[M+Na]^+$ 283.1158; found 282.9830

Allyl 5,6-*O*-dibenzyl-2,3-*O*-isopropylidene-α-D-mannofuranoside (14): NaH (4.2 g washed, 175 mmol) was added in portions to a solution of **13** (9.12 g, 35 mmol) in DMF (30 mL) at 0˚C. The resulting solution was stirred at 0˚C for 30 min, followed by the dropwise addition of benzyl bromide. The reaction mixture was then warmed to room temperature and allowed to stir overnight. The reaction mixture was then quenched with methanol and washed with brine (2x30 mL). The organic layer was dried ($MgSO_4$) and concentrated *in vacuo*, and the residue was then

purified by silica gel column chromatography (hexane/ethyl acetate, 6/1, v/v) to yield **14** as a light yellow oil (12.64 g, 82%). R_f = 0.18 (hexane/ethyl acetate, 10/1, v/v) ^{1}H (300 MHz, $CDCl_3$) δ 7.49 – 7.26 (10 H, m, C*H* aromatic), 5.89 (1 H, dddd, J 16.6, 10.4, 6.1, 5.3, OCH_2CHCH_2), 5.30 – 5.10 (2 H, m, OCH_2CHCH_2), 5.04 (1 H, s, H-1), 4.90 – 4.66 (3 H, m, H-3, 2xCH_2 of Bn), 4.65 – 4.56 (3H, m, H-2, CH_2 of Bn), 4.16 – 4.06 (2 H, m, H-4, $OCH_{2a}CHCH_2$), 4.02 (1 H, ddd, J 9.2, 5.3, 1.9, H-4), 3.98 – 3.83 (2 H, m, H-6a), 3.69 (1 H, dd, J 10.5, 5.4, H-6b), 1.47 (3 H, s, CH_3), 1.36 (3 H, s, C*H*). ^{13}C NMR (75 MHz, $CDCl_3$) δ 138.79, 134.21, 128.66, 127.89, 117.66, 112.42, 105.73, 85.06, 80.13, 78.73, 76.42, 73.75, 73.37, 72.36, 71.31, 67.94, 26.41, 25.21. HR MS (m/z) calcd for $C_{26}H_{32}O_6$ $[M+Na]^+$ 463.2097; found, 463.2118.

5,6-di-*O*-benzyl-2,3-di-*O*-isopropylidene-D-mannitol (15): 1N HCl (0.70 mL) was added to a solution of **14** (4.21 g, 9.57 mmol) in $EtOH/H_2O$ (200 mL, 2/1, v/v). Pd/C (3.36 g, 10% w/v, Degussa) was added, and the reaction was stirred vigorously 24 h. The lactol intermediate (3.86 g, 9.65 mmol) was dissolved in ethanol (58 mL). $NaBH_4$ (0.548 g, 14.48 mmol) was added to the solution, and the reaction mixture was stirred 12 h. The mixture was then cooled to 0˚C and neutralized with acetic acid (15 mL). The neutralized mixture was diluted with ethyl acetate (50 mL) and washed with saturated aqueous $NaHCO_3$ (2x50 mL) and brine (50 mL). The organic layer was dried ($MgSO_4$) and concentrated *in vacuo*, and the residue was purified by silica gel column chromatography (hexane/ethyl acetate, 5/2, v/v) to afford **15** as a white solid (2.86 g, 74%). R_f = 0.27 (hexane/ethyl acetate, 3/1, v/v) ^{1}H (300 MHz, $CDCl_3$) δ 7.41 – 7.24 (10 H, m, aromatic), 4.74 (1 H, d, *J* 11.5, CH_2Ph), 4.58 (2 H, s, CH_2Ph), 4.45 (1 H, dd, *J* 7.1, 2.0, H-3), 4.22 (1 H, dt, *J* 7.0, 4.6, H-2), 3.89 – 3.67 (5 H, m, H-1, H-4, H-5, H-6b), 3.67 – 3.57 (1 H, m, H-6a), 1.50 (3 H, s, CH_3), 1.35 (3 H, s, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 138.28, 138.20, 128.65,

128.63, 128.29, 128.06, 127.92, 108.22, 78.60, 75.46, 73.78, 72.96, 70.09, 68.93, 61.62, 27.26, 25.16, 0.22. MALDI-ToF HR MS (m/z) calcd for $C_{23}H_{30}O_6$ $[M+Na]^+$ 425.1940; found 425.1886.

5,6-di-*O*-benzyl-2,3-di-*O*-isopropylidene-1,4-di-*O*-sulfate-D-mannitol (16): To a cooled (0˚C) solution of diol **17** in DCM (40 mL) was added triethylamine (2.88g, 28.5 mmol). The resulting mixture was cooled to -15˚C followed by the addition of thionyl chloride (0.78 mL, 10.7 mmol). The solution was stirred 30 min at 0˚C, and the reaction mixture was diluted with DCM (40 mL) and washed with saturated aqueous $NaHCO_3$ (2x30 mL) and brine (30 mL). The organic layer was then filtered quickly through a pad of silica gel (eluant: ethyl acetate) and then concentrated *in vacuo*. The crude intermediate was dissolved in DCM/acetonitrile (40 mL, 1/1, v/v). To this solution was added, in sequence, $RuCl_3 \cdot H_2O$ (0.295 g, 1.42 mmol), $NaIO_4$ (2.28 g, 10.7 mmol) and deionized H_2O (26 mL). The reaction mixture was stirred 20 min then diluted with ethyl acetate (60 mL) and washed with saturated aqueous $NaHCO_3$ (2x40 mL) and brine (40 mL). The organic layer was concentrated *in vacuo* and purified by silica gel column chromatography (hexane/ethyl acetate, 10/1, v/v) to yield **17** as a white solid (0.966 g, 53%). R_f = 0.27 (hexane/ethyl acetate, 3/1, v/v) ^{1}H (300 MHz, $CDCl_3$) 7.42 – 7.21 (15 H, m, H aromatic), 4.98 (1 H, d, *J* 9.3, H-4), 4.83 – 4.45 (9 H, m, H-3, 4xCH_2 benzyl,), 4.45 – 4.19 (3 H, m, H-1, H-2), 3.99 – 3.88 (2 H, m, H-5, H-6a), 3.84 – 3.76 (2 H, m, H-6b), 1.51 (3 H, s, CH_3), 1.33 (3 H, s, CH_3) ^{13}C NMR (75 MHz, $CDCl_3$) δ 138.47, 137.71, 128.66, 128.60, 128.55, 128.32, 128.28, 128.23, 128.13, 128.06, 127.98, 127.92, 109.92, 109.04, 76.33, 75.95, 75.42, 69.42, 68.25, 67.88, 66.63, 66.40, 60.61, 27.66, 27.13, 25.40. MALDI-ToF HR MS (m/z) calcd for $C_{23}H_{28}O_8S$ $[M+Na]^+$ 487.1403; found, 487.2287.

Benzyl 2-deoxy-4,5-di-*O*-isopropylidene-7,8-di-*O*-benzyl-D-glycero-D-galacto-octulosonate-1,3-propylene dithioacetal (18): Dry HMPA (1.15 mL) was added to a solution of dithioacetate

17 (966 mg, 3.80 mmol) in THF (2 mL). After stirring the solution 2 h at rt, the solution temperature was reduced to -45˚C and *n*-BuLi (1.64, 4.10 mmol) was added. The reaction mixture was stirred at -45˚C for 2 h, and then a solution of sulfate **16** (1.36 g, 2.93 mmol) in THF (6 mL) was added to the cold reaction mixture. The resulting mixture was allowed to warm to rt and stirred 7 h, after which the reaction was quenched with saturated aqueous NH_4Cl, diluted with DCM (20 mL) and washed with brine (3x10 mL). The organic layer was concentrated *in vacuo*. The resulting intermediate was dissolved in pyridine/dioxane (2 mL, 1/1, v/v) and heated under reflux (90˚C) for 30 min. The reaction was then diluted in DCM (20 mL) and washed with brine (2x15 mL). The organic layer was dried ($MgSO_4$) and concentrated *in vacuo*, and the resulting residue was purified by silica gel column chromatography (hexane/ethyl acetate, 10/1→2/1, v/v) to afford compound **18** as a light yellow oil (500 mg, 27%) R_f = 0.14 (DCM/methanol, 9/1, v/v) ^{1}H (300 MHz, $CDCl_3$) δ 7.43 – 7.21 (15 H, m, aromatic), 5.18 (2 H, dd, *J* 31.5, 12.5, $COOCH_2Ph$), 4.74 (2 H, d, *J* 11.5, C*H2*Ph), 4.64 – 4.49 (4 H, m, 3xCH_2Ph, H-4), 4.39 (1 H, dd, *J* 7.0, 1.4, H-5), 3.85 (1 H, dd, *J* 10.4, 2.9, H-8a), 3.77 – 3.63 (2 H, m, H-6, H-8b), 3.62 – 3.51 (1 H, m, H-7), 3.33 – 3.17 (1 H, m, CH_{2axi} of SCH_2), 3.05 (1 H, dd, *J* 19.0, 7.2, CH'_{2axi} of SCH'_2), 2.66 (3 H, ddd, *J* 14.2, 11.4, 6.5, CH_{2equa} of SCH_2), 2.42 (4 H, dd, *J* 14.9, 2.9, H-3b), 1.39 (3 H, s, CH_3 of isopropylidene), 1.29 (3 H, s, CH_3 of isopropylidene). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.72, 138.58, 138.54, 135.92, 128.72, 128.59, 128.55, 128.36, 128.24, 128.15, 127.99, 127.91, 127.86, 127.80, 107.97, 78.97, 75.99, 73.70, 73.53, 72.92, 70.37, 69.33, 67.61, 52.73, 39.65, 28.07, 27.89, 27.00, 24.83, 24.71. MALDI-ToF HR MS (m/z) calcd for $C_{35}H_{42}O_7S_2$ $[M+Na]^+$, 661.2270; found, 661.1777

Benzyl 3-deoxy-4,5-di-*O*-isopropylidene-7,8-di-*O*-benzyl-α,β-D-manno-2-octulopyranosonate (19): NBS (139 mg, 0.785 mmol) was added to a cooled (0˚C) solution of

18 (100 mg, 0.157 mmol) in acetone/H_2O (1 ml, 97/3), and the reaction mixture was stirred 3 min. The reaction was quenched with aqueous $Na_2S_2O_3$ (15%, 2 mL), diluted in ethyl acetate (10 mL), and washed with saturated aqueous $NaHCO_3$ (2x5 mL) and brine (5 mL). The organic layer was concentrated *in vacuo* and purified by silica gel column chromatography (hexane/ethyl acetate, 6\1, v\v) to afford **20** in an anomeric mixture as a light yellow oil (41 mg, 47%). MALDI-ToF HR MS (m/z) calcd for $C_{32}H_{36}O_8$ [M+Na], 571.2308; found 571.0892.

Benzyl 3-deoxy-4,5-di-*O*-isopropylidene-7,8-di-*O*-benzyl-α,β-D-manno-2-octulopyranosyl fluoride (20): Pyranosate **19** (50 mg, 0.091 mmol) was combined with molecular sieves (4Å, 25 mg) in DCM (1.0 mL), and the mixture was stirred at rt 1 h. The mixture was cooled to -60˚C, and DAST (19 mg, 0.119 mmol) was added dropwise. The reaction mixture was allowed to warm to rt and stirred 1 h; the reaction was then cooled to -30˚C and quenched with acetic acid (11 µL). After stirring 2 min, the mixture was filtered and washed with DCM (10 mL). The filtrate was washed with saturated aqueous $NaHCO_3$ (2x5 mL) and brine (2x5 mL), and the organic layer was concentrated *in vacuo*. The resulting residue was purified by silica gel column chromatography (hexane/ethyl acetate, 6/1, v/v) to give a mixture of **20**/**21** (approximately 3/1) as a light yellow oil (50 mg). MALDI-ToF HR MS (m/z) calcd for $C_{32}H_{35}FO_7$ $[M+Na]^+$ 573.2259; found 573.2516.

Dimethylthexylsilyl benzyl (7,8-di-*O*-benzyl-3-deoxy-4,5-*O*-isopropylidene-α-D-*manno*-oct-2-ulopyranosyl)onate-(2→6)-3-*O*-[(*R*)-3-benzyloxy-tetradecanoyl]-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl-(1→6)-3-*O*-allyloxycarbonyl-4-benzyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranoside (22): Compounds **10** (210 mg, 0.159 mmol) and **20** (50 mg, 0.090 mmol) were combined with activated MS (4Å, 260 mg) in DCM (1 mL). The resulting suspension was stirred 1 h, and then cooled to 0˚C. $BF_3{\cdot}Et_2O$

(13 µL, 0.109 mmol) was added to the suspension, and the reaction mixture was then stirred at 0˚C 1 h. The reaction mixture was quenched with solid $NaHCO_3$ (50 mg), and the quenched mixture was diluted with DCM (10 mL) and washed with saturated aqueous $NaHCO_3$ (2x5 mL) and brine (5 mL). The organic layer was dried ($MgSO_4$) and then concentrated *in vacuo*. The resulting residue was purified by silica gel column chromatography (hexane/ethyl acetate, 10/1→4/1, v/v) to afford **22** as a clear oil (10 mg, 6%). R_f = 0.28 (hexane/ethyl acetate, 4/1, v/v) ^{1}H (500 MHz, $CDCl_3$) δ 7.35 – 7.12 (25 H, m, aromatic), 5.79 (1 H, qd, *J* 11.0, 5.7, OCH_2CHCH_2), 5.25 (1 H, dd, *J* 17.2, 1.1, OCH_2CHCH_{2a}), 5.19 – 5.00 (3 H, m, OCH_2CHCH_{2b}, $COOCH_{2a}Ph$,$COOCH_{2b}Ph$), 4.96 – 4.78 (3 H, m, H-1', H-3', H-3), 4.71 (1H, d, *J* 12.1, H-1), 4.67 – 4.21 (16 H, m, 2xCH_2 Troc, 5xCH_2Ph, H-4'', H-5''), 3.91 – 3.71 (6 H, m, H-6a, H-6", H-7", H-8"a, H-6b, H-$3_{L3'}$), 3.71 – 3.56 (1 H, m, 8"b), 3.55 – 3.25 (6 H, m, H-6'a, H-2', H-6'b, H-4', H-5', H-5), 3.23 – 3.14 (1 H, m, H-2), 2.62 (1 H, dd, *J* 15.2, 4.0, H-3"a), 2.41 (2 H, ddd, *J* 20.4, 15.2, 6.2, H-$2_{L3'}$), 1.66 – 1.45 (3 H, m, H-3"b, H-$4_{L3'}$), 1.20 (21 H, m, H-$5_{L3'}$-H-$13_{L3'}$, CH_3 of isopropylidene), 0.87 – 0.69 (18 H, m, 2x$C(CH_3)_2$ of TDS, $C14_{L3'}$, CH_3 of isopropylidene), 0.08 (6 H, d, *J* 14.2, $Si(CH_3)_2$). ^{13}C NMR (126 MHz, $CDCl_3$) δ 131.28, 131.15, 128.19, 128.17, 128.11, 127.95, 127.79, 119.15, 118.99, 100.98, 95.63, 78.66, 77.26, 76.09, 75.64, 74.04, 73.57, 73.54, 73.22, 73.13, 73.08, 72.00, 71.55, 71.48, 71.21, 71.15, 69.90, 69.63, 69.15, 69.10, 69.07, 69.00, 68.79, 68.05, 68.04, 67.49, 67.09, 62.02, 61.97, 61.94, 58.09, 58.05, 56.02, 39.49, 39.44, 39.40, 33.89, 32.84, 32.84, 32.80, 29.61, 26.52, 26.38, 25.89, 25.59, 25.21, 25.03, 22.64, 22.60, 20.05, 16.86, 13.86, -3.75. MALDI-ToF LR MS (m/z) calcd for $C_{90}H_{120}Cl_6N_2O_{24}Si$ $[M+Na]^+$ 1875.600; found 1876.291

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