SYNTHESIS OF RHIZOBIUM SIN-1 LIPID-A DERIVATIVES AS POTENTIAL THERAPEUTIC AGENTS AGAINST GRAM-NEGATIVE SEPTICEMIA

by

BALAJI SANTHANAM

(Under the Direction of GEERT-JAN BOONS)

ABSTRACT

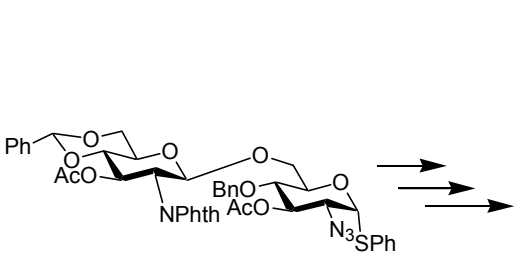
Several studies have shown that structurally novel *Rhizobium sin-1* LPS do not stimulate human monocytes to produce cytokines. They can, however, act as an antagonist and can be of potential therapeutic value for the treatment of gram-negative sepsis. Due to microheterogeneity of R. sin-1 LPS, it is difficult to identify which lipid A moiety makes it an antagonist rather than an agonist.

In this thesis, the development of a highly convergent strategy for the synthesis of several *R. sin-1* lipid A derivatives with varying fatty acid acylation pattern (**2**, **3**, **4**, and **5**) is described. The approach employed the advanced intermediate **1**, which is protected in such a way that the C-2 and C-2' amino groups and C-3 and C-3' hydroxyls can be selectively deprotected and acylated with varying chain lengths of β-hydroxy and acyloxyacyl acids.

Furthermore, it is not known whether the lipid A moiety of *R. sin 1* exists as an aminogluconate or 2-aminogluconolactone and also a significant percentage of lipid A preparations lack a fatty acyl residue at the C-3 position. In order to address these issues, we also have developed highly convergent approach for the facile synthesis of 2-aminogluconate **6** and 2-aminogluconolactone **7,** both lacking C-3 acylation.

Also a general synthetic strategy for long chain ω-1 hydroxy fatty acids (27OHC28:0) that are present in the lipid A of many *Rhizobial* species including R. sin-1 has been developed. The approach employed a key reaction step of an olefin-cross metathesis between ω-unsaturated ester and 3-butene-2-ol.

All the synthetic compounds lack the proinflammatory effects of *E. coli* LPS as indicated by the absence of the production of TNFα protein. In future these compounds can be potential candidates for the treatment of gram-negative sepsis.



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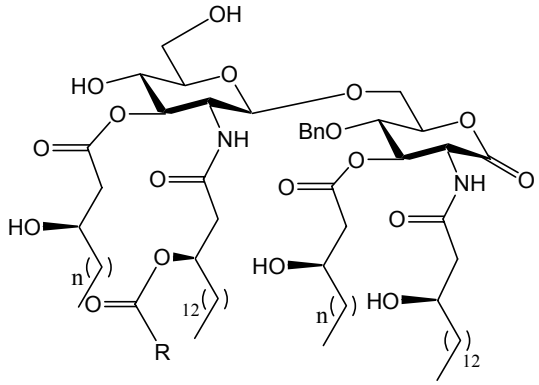
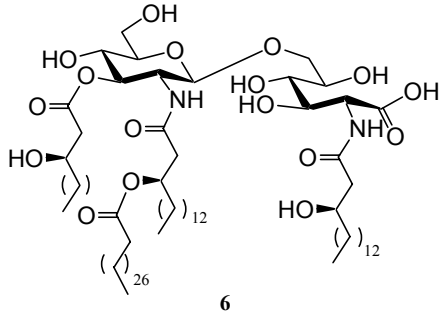


2. R = $C_{27}H_{55}$, n = 12
3. R = $C_{27}H_{55}$, n = 10
4. R = $C_{25}H_{51}(CHOH)CH_3$, n = 10
5. R = $C_{11}H_{23}$, n = 12



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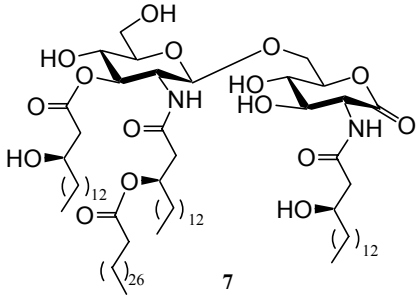

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INDEX WORDS: Carbohydrates, Glycolipid, Drug design, Inhibitors, Cytokines, LPS

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DEDICATION

to

Ram Prakash and Vigneshwara

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I would like to thank Prof. Geert-Jan Boons for giving me the opportunity to work in his research group. He has been very supportive and patient with me, during my time in his lab group. I would also like to thank Prof. Alexei Demchenko, for teaching me how to do carbohydrate chemistry.

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ABBREVIATIONS

Å……Angstrom
Ac……...Acetyl
Ac2O……..Acetic anhydride
AgOTf……..Silver triflate
AMI……Acute Mycocardial Infration
BF3.OEt2……Boron trifluoro diethyl etherate
Bn……...……Benzyl
Br……..broad
t-BuOH……...…..tertiary butanol
CHF……...Congestive Heart Failure
CSA……(±)-10-camphor sulfonic acid
CBr_4……...……...Carbon tetrabromide
DCE……...……...Dichloroethane
DCM……...……Dichloro Methane
DMAP .....*N,N*-Dimethylaminopyridine
DMF……...*N,N*-dimethylformamide
DMSO……...……..Dimethylsulfoxide
eq……Equivalent
Et……..Ethyl
Et_2O……...……...Diethyl ether
EtOH……..……..Ethanol
Et_3SiH……..……..…..Triethyl silane hydride
Fuc……..……...Fucoside
Gal……..……...Galactoside
GlcNAc……..……..*N*-acetyl glucoseamine
h……..……..hour
HPLC……..High Performance Liquid Chromatography
Hz……Hertz
K……...……..Kelvin
m.....Multiplet
m.p……..……Melting point
m/z……...Mass to charge ratio
Man……...……..Mannoside
Me……..……...Methyl
MeOH……..…….Methanol
Min……..…….Minute
mM……...……millimolar
mmol……..……...millimole
MS……..…...Molecular sieves
NaOAc……...……...Sodium acetate
NIS……....*N*-iodosuccinimide
NMR……..Nuclear Magnetic Resonance
PCC……...…Pyridinium Chloro Chromate
Ph……...……Phenyl
Phth……Phthalimido
PPh_3……..……Triphenyl phospine
Ppm……..……..Parts per million
q……..quartet
*R*f……..……....retention factor
s ……...……..singlet
t.....triplet
Tf2O……..……..…triflic anhydride

TFA……...…….trifluoro acetic acid
TfN3……..……...…… Triflic azide
TfOH……...…….Triflic acid
THF……..……Tetrahydrofuran
TLC……..Thin Layer Chromatography
TMSOTf.…….…….Trimethylsilyl trifluoromethane sulfonate
TMS_2O…….……..……Trimethyl disiloxane
TNF……..…… Tumor Necrosis Factor

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CHAPTER 1

INTRODUCTION

Epidemiology

Septicemia commonly known as sepsis is a worldwide affliction estimated to affect 18 million people annually and results in 1,400 deaths each day. It has been estimated that 1% of hospital patients and 20-30% of ICU patients develop sepsis and it is the leading cause of death in patients admitted to non-cardiac intensive care units.[1,2] According to the National Vital Statistics Report,[3] sepsis is the eleventh leading cause of death in the United States, as 750,000 people develop sepsis on an annual basis.[4] About 215,000 of affected Americans die of sepsis each year

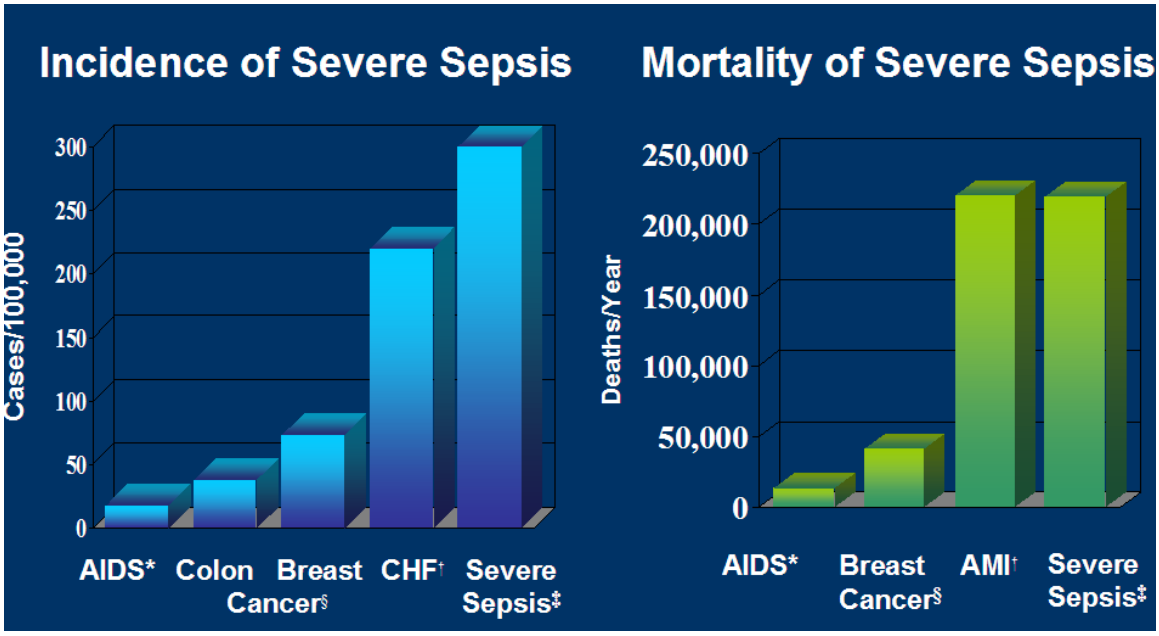

Figure 1.1 Sepsis in comparison with other major diseases

a number equal to the number of Americans who die of coronary heart disease without hospital treatment (Figure 1.1). Data collected from the state hospital discharge records of seven large states by the US Census Bureau, Center for Disease Control and the American Hospital Association reported that the incidence of severe sepsis is 300 cases per 100,000 population. The incidence of sepsis is significantly greater than that of other major diseases such as congestive heart failure (CHF), colon and breast cancer.

Endotoxin (lipopolysaccharide [LPS]) from gram-negative bacteria (*e.g. E. coli*) has been implicated as the major cause of sepsis and accounts for almost half (100,000 in the US) of deaths from the illness.[5] The development of septicemia is often linked to a systemic inflammatory response to LPS in the blood of affected patients.[6-8] The presence of high LPS levels in the blood of affected patients strongly implicates endotoxemia as a potential critical factor in pathogenesis. The biological activity of endotoxin is associated with the LPS and toxicity is linked to the lipid component -Lipid A. Lipid A, the hydrophobic anchor of lipopolysaccharide (endotoxin) is a major component of the outer membrane of Gram-negative bacteria. **(**Figure 1.2**)**

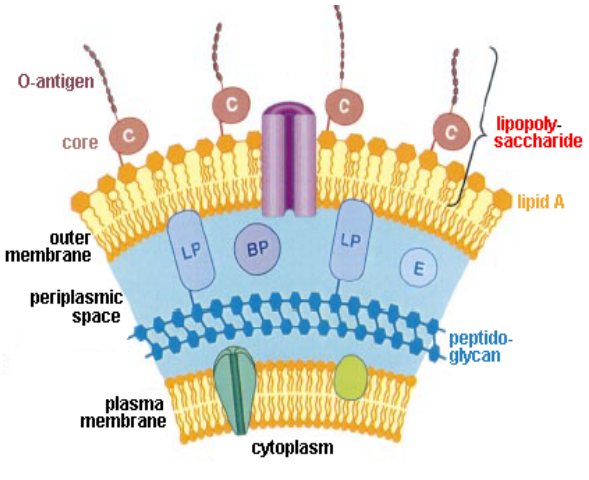

Figure 1.2. Cell envelope of a gram-negative bacterium.

Pathophysiology

The mode of action of endotoxin/LPS-induced pathological effects is now well understood.[9] LPS indirectly harms the body when massive amounts of the toxin are released during severe gram-negative infections. It is the most potent proinflammatory substance known, as its lipid A region initiates the production of multiple host derived inflammatory mediators such as cytokines (*e.g.*, tumor necrosis factor TNFα), arachidonic acid metabolites, and tissue factor. The following stages are important for understanding the pathological effects of LPS:

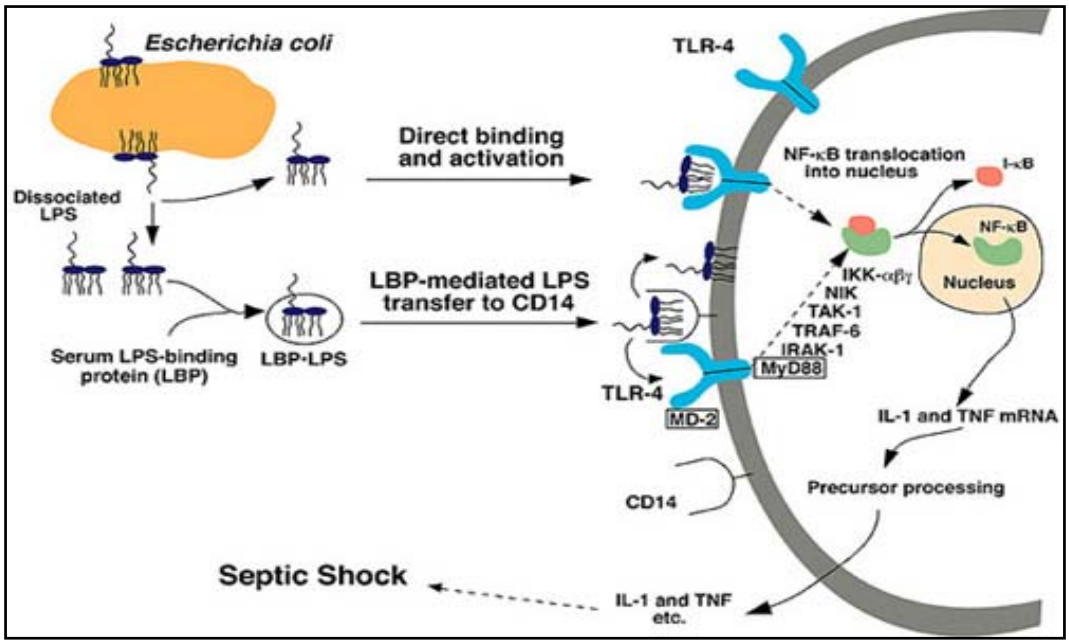

Figure 1.3. Mechanism of Septic Shock

1. Endotoxin (LPS) is shed in small amounts throughout the lifespan of the gram-negative bacteria and is disseminated in large quantities upon cell death and lysis. (Figure 1.3)

2. LPS thus released from the membrane of the gram-negative cell wall binds to a plasma binding protein produced by the body, creating a complex referred to as LPS binding protein (LBP).
3. LBP circulating in the blood binds to CD14 (glycosylphosphatidylinositol-anchored protein) on mononuclear phagocytes, or to soluble CD14 in plasma and then to cells lacking CD14.
4. CD-14 acts as a molecular transporter of LPS or Lipid A that transmits to the Toll-like receptor (TLR4) protein.[10] This protein contains extracellular, ransmembrane, and intracellular domains, and an accessory protein MD-2. Signal transduction takes place after the formation of the muitmeric protein complex (TLR-4*MD-2*LPS).
5. LPS- induced expression of cytokine genes involves an activation of the NF-κB and the MAP kinases. The end result is an up-regulation of more than 120 genes, including those for the cytokines, most notably TNFα, interleukin-1α, and interleukin-1β.
6. This, in turn, stimulates an increase in the pro-inflammatory cytokine levels that are responsible for endotoxic shock (sepsis).

Thus, the endotoxins stimulate the macrophages to produce three groups of powerful mediators (cytokines) [5] (Figure 1.4)

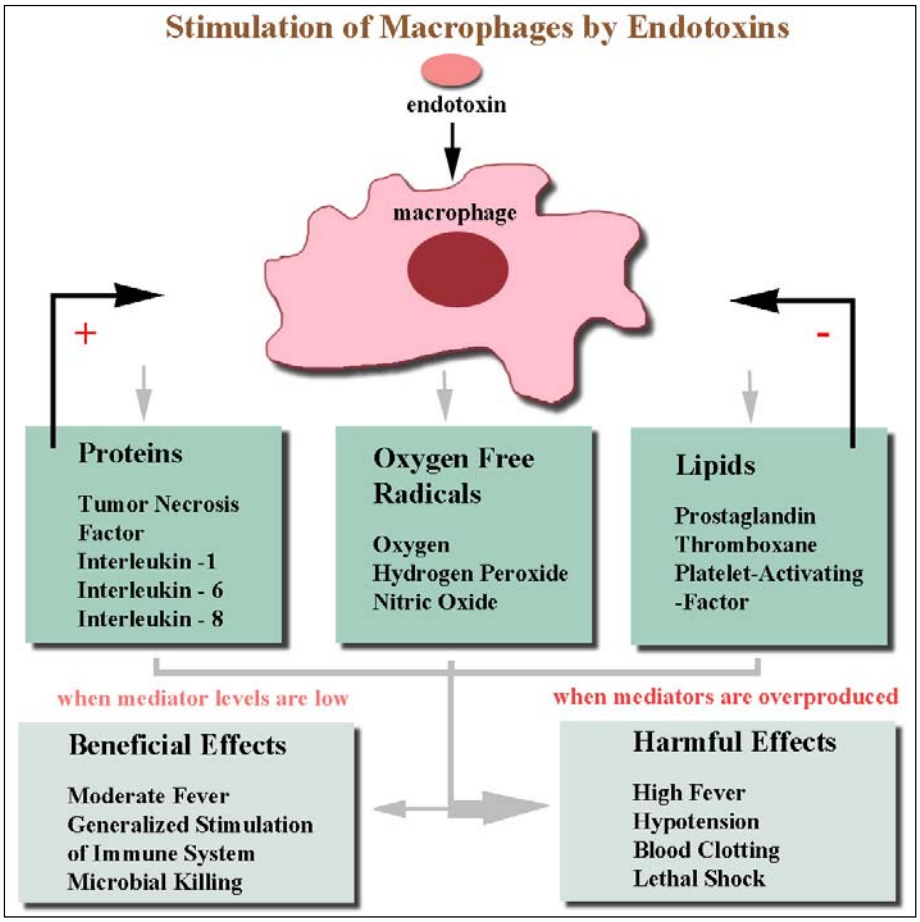

Figure 1.4. Stimulation of Macrophages by endotoxins

- Proteins – TNFα, interleukin-1α, interleukin-1β, interleukin-6, interleukin-8
- Oxygen free radicals - oxygen, hydrogen peroxide, nitric oxide
- Lipids – prostaglandin, tyromboxine, platelet-activating factor.

Mediators may act independently, together or in sequence to engender both positive and negative responses by an organism. For example, if the gram-negative bacteria release a moderate amount of endotoxins, the macrophage products (mediators) help eradicate the immediate infection by generating a desirable, localized and controlled immune response. However, if an infection is severe, large amounts of endotoxin are released into the bloodstream, generating an

overproduction of mediators by the macrophage which leads to the undesirable outcome of septic shock.

General Structure of LPS

The LPS of all gram-negative bacteria consists of two main components a hydrophilic polysaccharide and an O-polysaccharide. The hydrophilic polysaccharide is covalently bound to the hydrophobic lipid A, thus creating an amphiphilic molecule. A further distinction of the polysaccharide is that it can be divided into subdomains- the Core polysaccharide and O-polysaccharide (Figure 1.5).

The O-polysaccharide composes the outermost part of the LPS of all gram-negative bacteria and is therefore the primary antigen targeted by host antibody responses. The responses of the O-polysaccharide can be highly O-chain specific. Often referred to as the O-antigen, it consists of repeating oligosaccharide subunits made up of 2 - 8 sugars. These subunits differ between strains by means of different sugar units, sequence and connectivity. The individual chains vary in length, the greatest length being equal to a repeat of the subunit of fifty times. A single organism can produce a wide range of these lengths as a result of the incomplete synthesis of the chain. The O-polysaccharide is significantly longer than the core polysaccharide and maintains the hydrophilic domain of LPS.

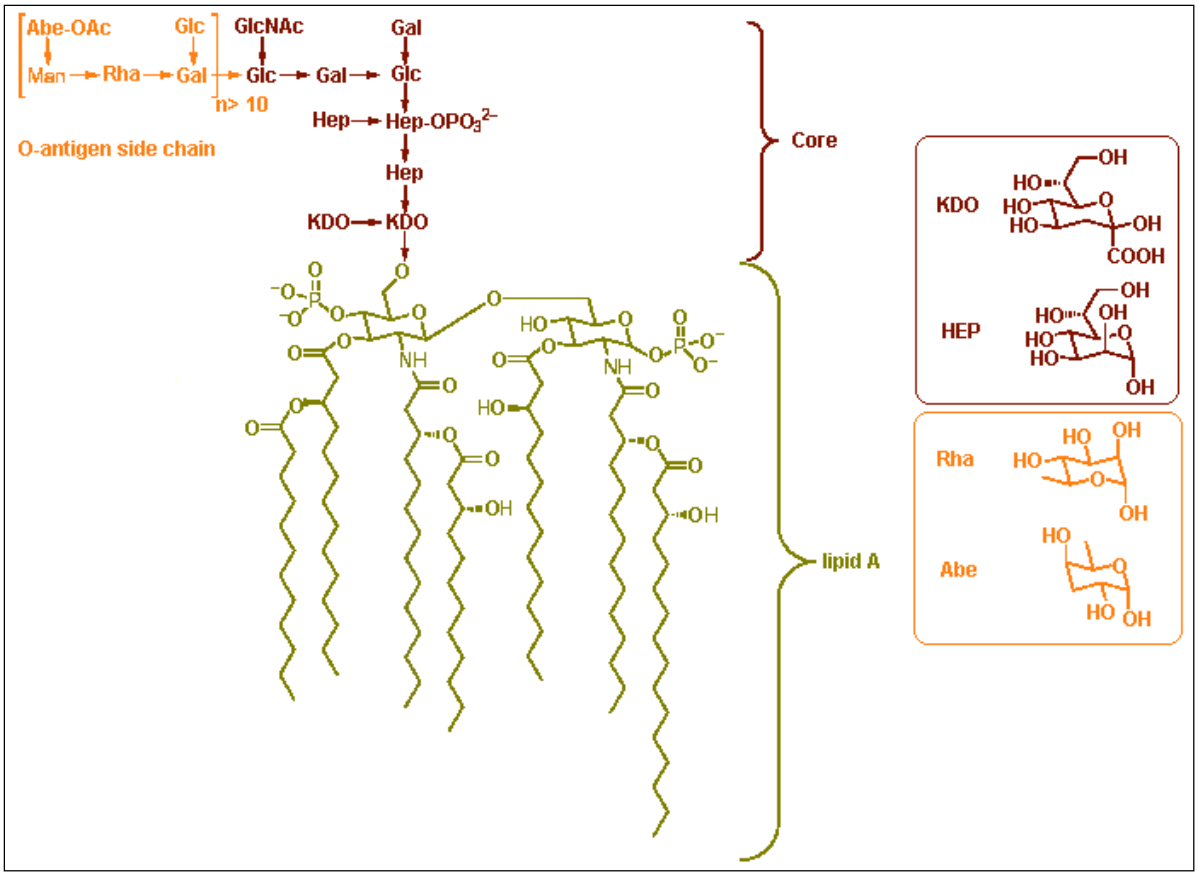

Figure 1.5. Lipopolysaccharide of *Salmonella typhimurium* (GlcNAc- N-acetylglucosamine, KDO- 3-deoxy-D- manno-octulosonic acid, Hep- L-glycero-D-manno-heptose, Rha- (L-rhamnos) 6-deoxy-L- manno-hexose, Abe- (abequose) 3,6-dideoxy-D- xylo-hexose).

The core region of the polysaccharide is much less compared to the O-polysaccharide part. For example, *E. coli* contains only 5 unique core structures but more than 160 different O-chains.

The outer core of the polysaccharide consists of common sugars such as glucose (Glc), galactose (Gal), *N*-acetylglucosamine (GlcNAc) and N-acetyl galactoseamine (GalNAc).

Less variable than the outer core the inner core consists of unusual sugars such as 3-deoxy-D-manno-octulosonic acid (Kdo) and L-glycero-D-manno-heptose (Hep). The Kdo unit is α-bound to the carbohydrate backbone of the lipid A in all instances and very essential for bacteria viability. Hence, drugs targeting Kdo synthesis enzymes would represent a new class of

antibiotics. The bond between the first Kdo unit and the lipid A component is very acid labile, exhibiting a moderate pH of 4.4. The differences between the core polysaccharide and O-polysaccharide extend to sugar types as well.

The Lipid- A region is the hydrophobic and endotoxically active part of the LPS, and is covalently linked to the inner core by the Kdo residue. Structurally, lipid A consists of N-acetylglucosamine dimer covalently bonded through a β (1,6) linkage, carrying two phosphoryl groups at positions 1 and 4'. In many cases, the phosphate group can be further substituted with ethanolamine, ethanolamine phosphate, GlcN, 4-amino-4-deoxy-L-arabino-pyranose and D-arabino-furanose. There are up to four acyl chains attached to this structure by ester and amide linkage,chains which may be substituted further by fatty acids that vary considerably within each species.

Lipid A structure vs. function

In 1954 Otto Westphal and Otto Luderitz postulated that the lipid A component of LPS was primarily responsible for its endotoxicity.[11] Following several years of analytical study beginning in 1954, the complete chemical structure of lipid A (*E. coli & S. enterica sv. Typhimurium*) was elucidated in 1983.[12] Analysis of the chemical structure of Lipid A continued in 1984 with the first total synthesis of *E. coli* by Tetsuo Shiba and Shoichi Kusumoto.[13] A biological analysis of the toxicity, pyrogenicity and activation of monocytes of synthetic lipid A showed that all test systems, were identical to the *E. coli* lipid A. These experiments successfully demonstrated that the endotoxic activity of the large LPS molecule was due to the lipid A component.[14]

During the last decade, lipid A structures derived from several bacterial species have been elucidated and characterized in terms of immunoactivity. Although the lipid A region is often assigned as the conserved part of the LPS of gram-negative bacteria, have been found to express a certain degree of structural diversity with respect to three structural elements:

1. lipid A backbone
2. polar group substituents
3. fatty acid residues linked to the lipid A backbone.

Even the lipid A derived from a single bacterial strain possesses a microheterogenic mixture of several chemical structures with respect to variations in the polar group substituents and acylation pattern. In the following paragraphs, knowledge of the previously mentioned structural elements their contribution to overall toxicity will be discussed.

Disaccharide or lipid A backbone: In a vast majority of the lipid A structures of gram-negative bacteria characterized so far, the general structure of the glycosyl region consists of a β-(1,6) interlinked disaccharide of D-glucosamine (Glc*p*N). Other naturally occurring backbone structures also include; 2,3-diamino-2,3-dideoxy-D-glucopyranose (Glc*p*N3N)-Glc*p*N disaccharide (*e.g. Campylobacter jejuni*), Glc*p*N3N-Glc*p*N3N disaccharide (*e.g. Pseudomonas diminuta*) and Glc*p*N3N monosaccharide backbone (*e.g. R. viridis*).[15] Monosaccharide lipid A backbone generally lacks endotoxicity, suggesting that the disaccharide backbone is required for optimum recognition by for lipid A receptors.

Polar group substituents: The backbone of a lipid A disaccharide contains, in general, two phosphate groups: one α-linked to the glycosylic hydroxyl group at C-1, and the other linked to the hydroxyl group present at C-4'. Structures containing one phosphate (e.g. Bacteroides fragilis) at either 1 or 4' are at least 1000 times less active than *E. coli* lipid A. However,

alteration of the phosphates with phosphono-oxyethyl does not alter the compound's activity, suggesting that charges play an important role in the restoration of activity. Other charged groups identified in naturally occurring lipid A analogs include ethanol amine, phosphoethanolamine,[16] L-4-amino-4-deoxy-arabinopyranose and D-galacturonic.

Fatty acid residue linked to the lipid A backbone: Of all the previously discussed modifications of the lipid A structure, the acylation pattern of the fatty acid is the most critical structural feature that determines endotoxcity. These fatty acids are in the form of (R)-3-hydroxy or (R)-3-hydroxyacyl chains that are linked to the backbone via ester and amide bonds at positions 2 and 3 as well as 2' and 3'.

The number of fatty acid groups present in a molecule has a direct effect on its toxicity. The most common fatty acids in lipid A have 10-16 carbons although longer chains exist (e.g. C_{18} fatty acids in *Helicobacter pylori*,[17] C_{21} in *Chlamydia trachomatis*). Heterogeneity or variation in the degree of fatty acid substitution often results in more than three or four molecular species present in a single preparation due to mutations or defects in fatty-acid-transferases, leading to incomplete biosynthesis. These variations in the degree of fatty acid substitution have garnered much interest as it is now well recognized that fatty acids significantly influence their endotoxic potential when compared to the lipid A derived from wild-type strains. However, some of these compounds exhibit pronounced inhibitory effects. One such compound, compound 506, is a biological precursor of *E. coli* lipid A that has two acyl-substituted fatty acids removed.[11] In a number of biological assays, this analogue not only lacks endotoxic activity but is also an antagonist as it is able to inhibit normal lipid A signaling. Other naturally occurring lipid A indicates that such antagonistic behavior is derived from *R. capsulatus* [16] and *R. sphaeroides* [18]. Although the two species have very similar structures and possess the same of lipid A backbone

obtained from *E. coli* their fatty acyl pattern is quite different. These compounds provide interesting structural leads as endotoxin antagonists.

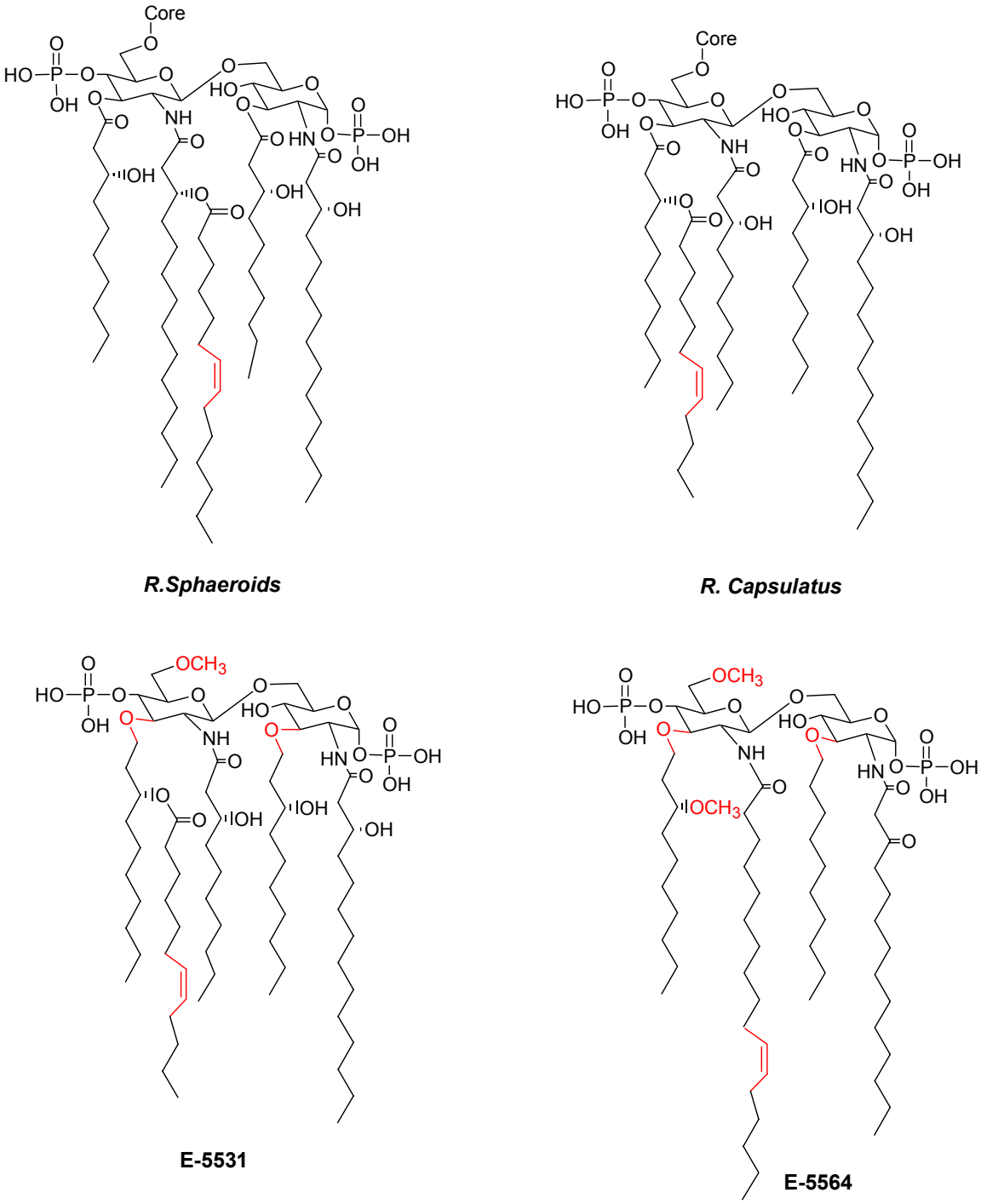

Figure 1.6. Lipid A structures of natural *R. Sphareroids, R. Capsulatus* and the synthetic lipid A, E5531 and E-5564.

In fact, synthetic endotoxin antagonists such as E5531[19,20] and E5564 are structurally modified from *R. capsulatus* and *R. sphaeroides* where the 3 and 3` positions have ether linkages unlike ester fatty acids which were found to undergo degradation in the natural lipid A analogs (Figure 1.6). These compounds exhibit an extraordinary quality in that they possess a unique, undetectable antagonistic activity, a quality which could lead to the creation of a new drug for use in the treatment of gram-negative sepsis. However, the synthetic strategy used to make these analogs is complex and involves many steps and is thus not amenable for the production of the numerous lipid A analogs necessary for an examination of their beneficial properties.

Lipid A from Rhizobial Species:

Studies conducted on horse and human monocytes infected with LPS from three *rhizobial* species (*R. galegae, R. sin-1* and *R. etli*) found that the infected monocytes [21] induced less than 10% of the TNFα activity compared to *E. coli* LPS and, more importantly prevented the induction of TNFα by *E. coli* LPS. These naturally occurring gram-negative bacteria from *rhizobial* species such as *Rhizobium etli,*[22] *Rhizobium sin-1* and *Rhizobium leguminosarum*[23] (Figure 1.7) belong to a family of select microbes that fix nitrogen during symbiosis within the roots.

Structural studies by Carlson and coworkers have shown that the lipid A occurring in these bacteria is very different from other species and perhaps the most unusual lipid A reported to date.[24] The following are the common differences that were found in Lipid A of any rhizobial species:

- the hydrophilic or disaccharide backbone is devoid of phosphate.
- the 4’ phosphate has been replaced in some species by a galacturonosyl residue

- the lipid A backbone contains a 2-aminogluconolactone or 2-aminogluconate
- the lipid-A contains an unusual long fatty acid referred to as the 27-hydroxyoctacosanoic acid which may be esterified by β-hydroxybutyrate.

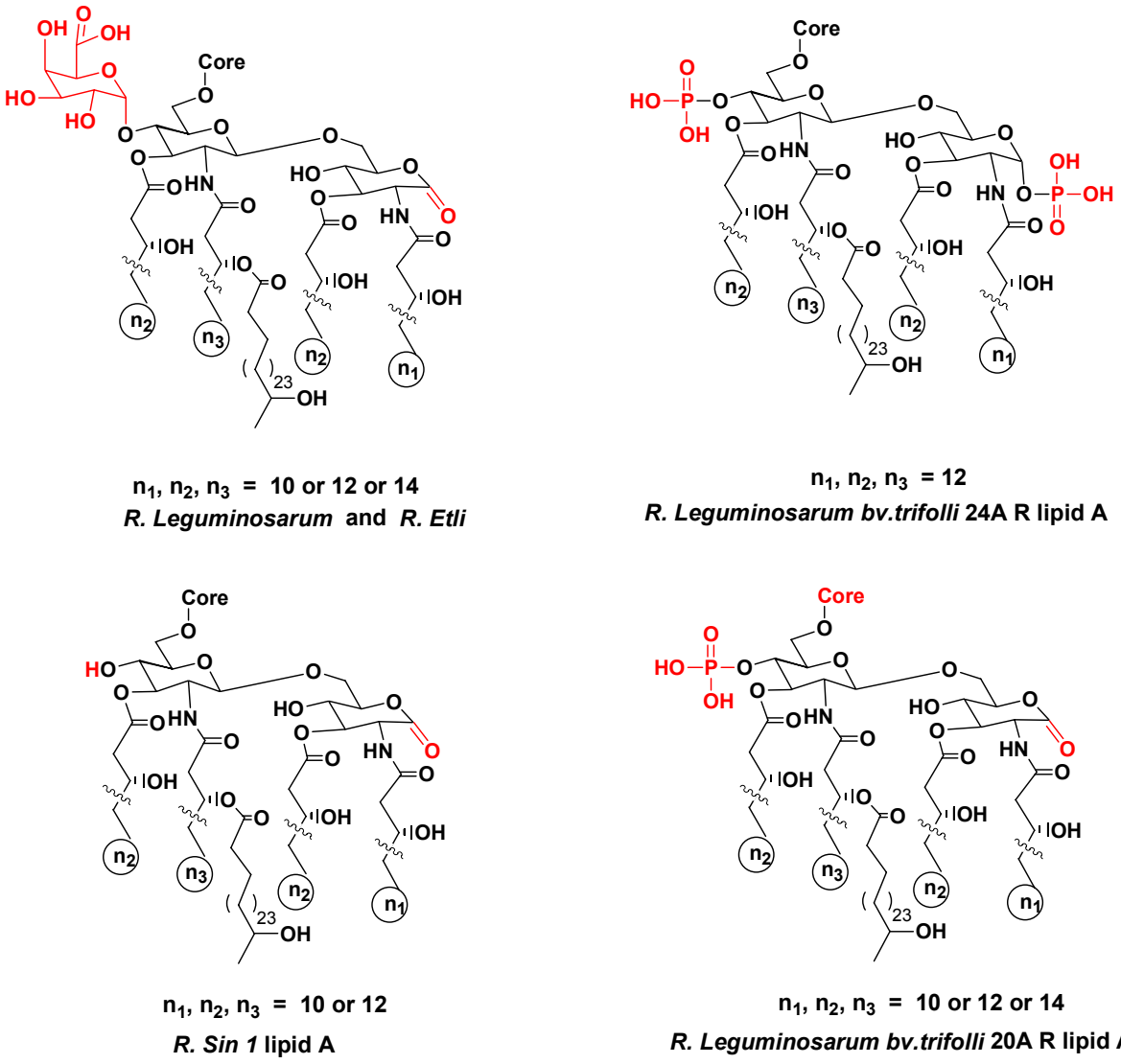

Figure 1.7. Lipid A structures for several *rhizobial* strains and their mutants AR24 and AR20.

As previously discussed, these species differ in all aspects including lipid A backbone and fatty acylation pattern. For example, the lipid A of *R. sin-1* shows considerable microheterogeneity.

The fatty acylation pattern is heterogeneous and consists exclusively of β-hydroxy fatty acids. The *N*-acyl groups can consist of β-hydroxymyristate, β-hydroxypalmitate, or β-hydroxystearate. The *O*-acyl groups are primarily β-hydroxymyristate, but occasionally can also include β-hydroxypentadecanoate. Furthermore, a significant percentage of *R. sin-1* Lipid A lacks a fatty acyl residue at the C-3 position. Due to its inherent molecular heterogeneity within any given rhizobial LPSs, it cannot be developed as a therapeutic agent for Gram-negative septicemia. Furthermore, the inability to separate the different species limits the identification of specific structural features that would identify them as an antagonist as opposed to an agonist.

Based on these findings, rhizobial lipid A provides a unique template upon which to synthesize lipid A analogs that may be potential candidates for the treatment of LPS-mediated diseases. Furthermore, the inability to separate the different species limits identification of specific structural features that makes *R. sin-1* lipid A an antagonist as opposed to an agonist.

Research Outline

Major issues concerning lipid A biosynthesis involves microheterogeneity and isolation of well-defined fragments. These factors limit detailed structure-activity relationship studies to identify the structural features that are responsible for its agonistic or antagonistic properties. Fortunately, recent advances in chemical synthesis of oligosaccharides and fatty acids make it possible to design and prepare pure lipid A derivatives containing a specific structure.

Shiba *et al* described the first successful synthesis of *E. coli* lipid A derivative.[25] In their approach, which involved incorporation of appropriate lipid moieties at C-3 and C-3' and the C-4' phosphate group at the monosaccharide stage, yielded two monosaccharides. The monosaccharides were then coupled to produce a disaccharide derivative, which was then

anomerically phosphorylated. Since then several strategies for the synthesis of lipid A derivatives have been described.[13,25-27] While the reported procedures for lipid A synthesis have established efficient methods for introducing *N*- and *O*- linked lipids and anomeric and non-anomeric phosphate mono-esters, these synthetic routes were directed towards the preparation of specific individual lipid A analogs. No synthetic approach has been developed which enables the facile synthesis of a wide range of lipid A structures. Significant advances in chemical synthesis of oligosaccharides and fatty acids in recent years make it possible to synthesize a series of pure and well-defined lipid A structures ranging from *E. coli* to the rhizobial species. This project will mainly focus on the synthesis of *R. sin-1* lipid A analogs of the rhizobial species.

The specific aims of this project are:

1. To develop a highly convergent strategy for the synthesis of the lipid A of *R. sin-1* with varying fatty acid acylation pattern
2. To developed a highly convergent approach for the facile synthesis of 2-aminogluconate and 2-aminogluconolactone derivatives, both lacking C-3 acylation that are present in a significant percentage of lipid A preparations of *R. sin-1*
3. A general synthetic strategy for long chain ω-1 hydroxy fatty acids (27OHC28:0) that are present in the lipid A of many *Rhizobial* species including *R. sin-1*

CHAPTER 2

SYNTHESIS OF *RHIZOBIUM SIN-1* LIPID A ANALOGS CONTAINING VARYING ACYLATION PATTERN[*]

[*]Balaji Santhanam, Yanghui Zhang, Alexei V. Demchenko, and Geert-Jan Boons

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Abstract

A highly convergent strategy for the synthesis of the lipid A of *Rhizobium sin-1* with varying fatty acid acylation pattern has been developed. The approach employed the advanced intermediate **5**, which is protected in such a way that the C-2 and C-2' amino groups and C-3 and C-3' hydroxyls can be selectively deprotected and acylated with varying chain lengths of β-hydroxy and acyloxyacyl acids. The synthetic strategy was used for the preparation of compounds **12a, 12b** and **18**. By synthesizing these analogs of different chain lengths, one can create a library of compounds for structure-activity relationship studies.

Introduction

Septicemia is a serious worldwide health problem and is associated with mortality rates of 40 - 60%.[1,2] The development of septicemia is often linked to a systemic inflammatory response to lipopolysaccharides (LPS) in the blood of affected patients.[6-8] LPS, a component of Gram-negative bacteria, stimulates host cells of the immune system like the macrophages, resulting in the production of produce powerful mediators such as cytokines, oxygen free radicals, and lipids.[28,29] When the mediators level produced are low, then the immune system is appropriately exhibits beneficial activities. On the other hand, overproduction of the mediators causes endotoxin-related symptoms such as high fever, hypotension, and, in severe cases leads to sepsis. Most of the biological activities of LPS reside in a relatively small portion of the molecule, that is, the terminal disaccharide phospholipids subunit known as lipid A.

Lethality and pyrogenicity are only observed when lipid-A analogs contain a strict set of structural features including a β(1-6)-linked glucosamine disaccharide backbone, biphosphorylation at the anomeric and C-4' position, and a suitable number and location of

appropriately long 3-acyloxyacyl groups per disaccharide.[29,30] If the length of the acyl groups is increased, as occurs in lipid-A of *C. Psittaci*, *B. fragilis* and *L. pneumophilia*, endotoxicity is reduced. [31] In contrast, the chirality of the 3-oxoacyl moieties does not influence biological activity, as evidenced by the fact that lipid-A derivatives having *R*- and *S*-configurations cause similar effects. Distribution of the acyl groups affects the bioactivity of lipid-A.[32] For instance, *Heamophiles influenza* lipid-A, which contains six acyl groups with 14 carbon atoms in an asymmetric distribution, expresses biological properties comparable to that of *E. coli* lipid-A, whereas lipid-A having a symmetric distribution of the same fatty acids has significantly less bioactivity. The least biologically active lipid-A structures are the monosaccharide derivatives or dimeric compounds having four fatty acid side chains.

One of the more appealing methods for preventing the deleterious effects of enteric LPS is by preventing the interaction between lipid A and its receptors on mononuclear phagocytes.[33,34] Interference at this level may prevent initiation of the cellular reactions that lead to systemic inflammatory responses and septic shock. As is often the case, efficacious pharmacological receptor antagonists often are derived by modifying a compound having agonist activity. The most widely studied derivatives of lipid-A are monosacccharide biosynthetic precursors of lipid A [35, 36, 37] and synthetic analogs derived from the lipid-A of *Rhodobacter sphaeroides* or *Rhodobacter capsulatus*, two species having very similar lipid-A structures.[18,38] Although the *R. sphaeroides /R. capsulatus* lipid-A has the same bis-1,4'-phosphorylated glucosamine disaccharide backbone as *E. coli*, its fatty acyl pattern is quite different from that of *E. coli* lipid-A. The *R. sphaeroides /R. capsulatus* lipid-A consists of two 3-oxomyristic acid, two β-hydroxydecanoic acid, and one dodecenoic acid residues. The latter fatty acid is the only acyloxyacyl substituent and is located on the 3'-β-hydroxydecanoic acid residue. The *R.*

sphaeroides /R. capsulatus lipid-A lacks toxic effects, does not induce cytokine synthesis by human monocytes, and is an antagonist of enteric endotoxin.

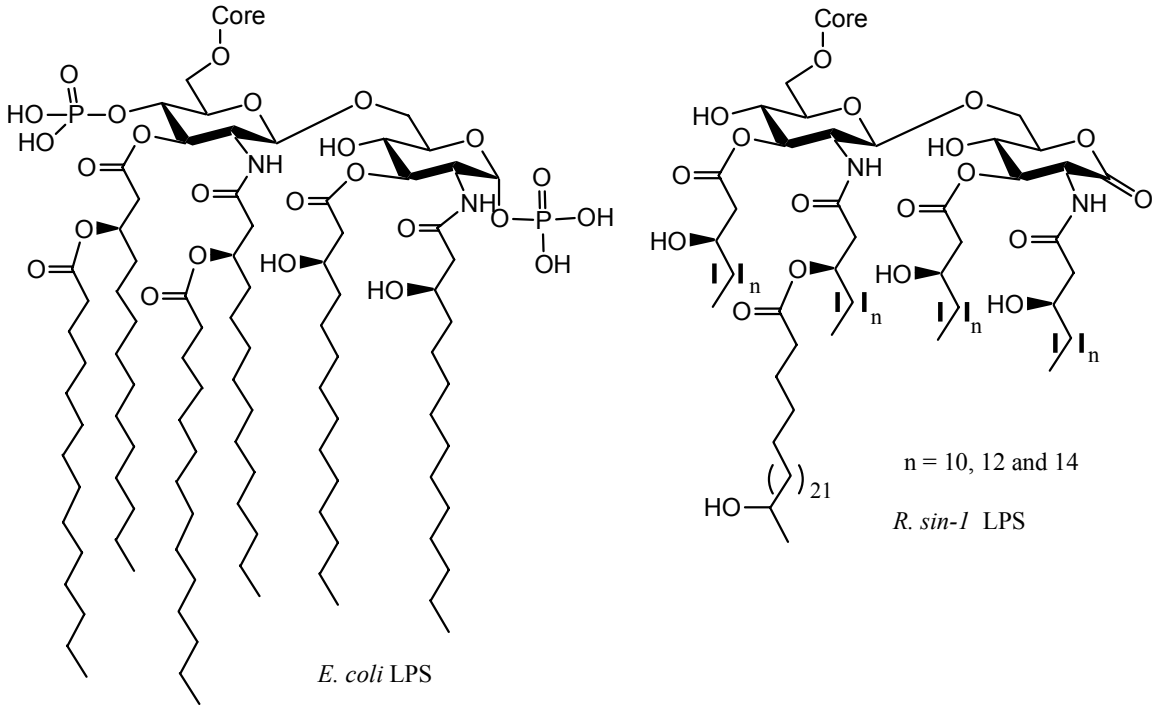


Figure 2.1 Strutcures of *E. coli* and *R. sin 1* LPS

Recent data from our laboratory indicate that LPS from a nitrogen-fixing symbiont, *Rhizobium sin-1*, does not stimulate human monocytes.[21] More importantly, *R. sin-1* LPS significantly inhibits *E. coli* LPS-dependent synthesis of TNF-α by these cells. The lipid-A of *R. sin-1* is perhaps the most structurally unusual lipid-A reported to date and its structure (Figure 2.1) differs in almost every aspect from those known to contribute to the toxicity of enteric LPS.[24] In particular, the disaccharide moiety of rhizobial lipid-A is devoid of phosphate and the glucosamine phosphate is replaced by 2-aminogluconolactone. It is likely that the latter residue can also exist as a 2-aminogluconate, which would contribute to the molecular heterogeneity of *R. sin-1* lipid-A. The fatty acylation pattern is heterogeneous and consists exclusively of β-

hydroxy fatty acids. The *N*-acyl groups can consist of β-hydroxymyristate, β-hydroxypalmitate, or β-hydroxystearate. The *O*-acyl groups are primarily β-hydroxymyristate, but occasionally can also include β-hydroxypentadecanoate. This lipid-A also contains a very long chain fatty acid, 27-hydroxyoctacosanoic acid which, in turn, can be esterified by β-hydroxybutyrate.

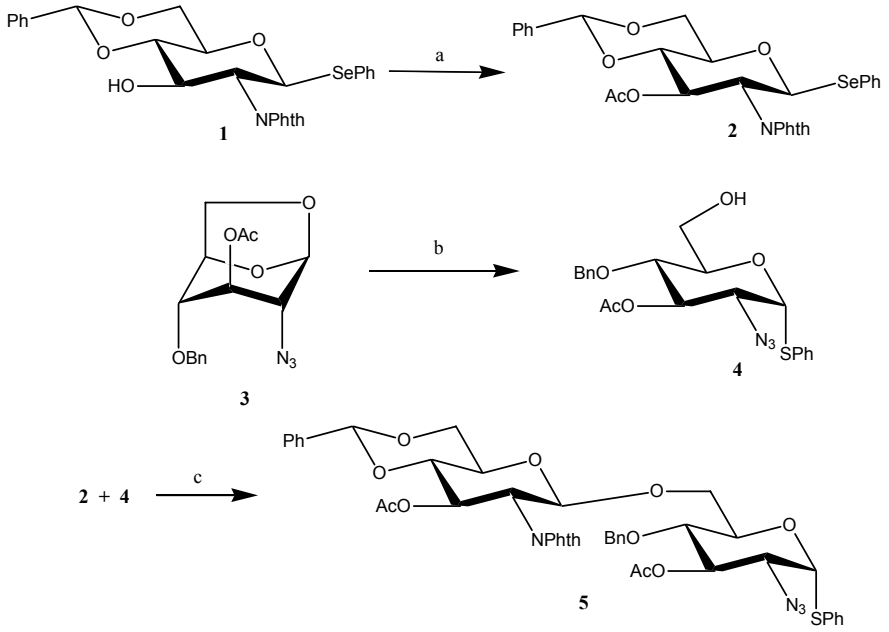
Due to its inherent variations in fatty acid acylation patterns, purified *R. sin-1* lipid-A can not be developed as a therapeutic agent for Gram negative septicemia. Furthermore, the microheterogeneity of rhizobial lipid-A limits the identification of specific structural features that makes it an antagonist rather than an agonist. To address this problem, we have developed a flexible approach for the facile synthesis of a wide range of well-defined lipid-A derivatives based on the structure of *R. sin-1* LPS with variations in fatty acylation pattern. This approach was employed for the preparation of compounds **12a, 12b** and **18** and the biological effects of these derivatives have been determined.

Our synthetic approach uses the advanced intermediate **5** (Scheme 2.1), which is protected in such a way that the anomeric center, the C-2 and C-2' amino groups and C-3 and C-3' hydroxyls can be selectively deprotected and acylated with varying chain lengths of β-hydroxy and acyloxyacyl fatty acids. A key feature of **5** is protection of the anomeric center as a thioglycoside. [39,40] This functionality is stable under a wide range of chemical conditions, but can be readily hydrolyzed under appropriate reaction conditions to give a lactol, which can either be phosphorylated or oxidized to a lactone. Furthermore, the phthalimido and azido functions of **5** offer an attractive set of orthogonal protecting groups that allow selective derivatization of the two amino groups. Removal of the phthalimido group also results in cleavage of the acetyl esters. It was anticipated, however, that the resulting amine and hydroxyls could be selectively derivatized by exploiting the fact that amines are more nucleophilic then hydroxyls.

Results and Discussion

Compound **5** was prepared by a chemoselective coupling of seleno glycoside **2** with thio glycoside **4**.[39] Glycosyl donor **2** was easily obtained by acetylation of known **1** using acetic anhydride in pyridine. Treatment of known **3** with ZnI_2 and TMSSPh[41] followed by short treatment with aqueous TFA gave **4** in a good yield as a separable mixture of anomers (α/β = 6/1). Coupling of glycosyl acceptor **4** with glycosyl donor **3** in the presence of the promoter system AgOTf/K_2CO_3[42] produced the desired disaccharide **5** in an 83% yield (Scheme 2.1). In this coupling, only the β-anomer was formed due to neighboring group participation of the phthalimido group.

Scheme 2.1[a]

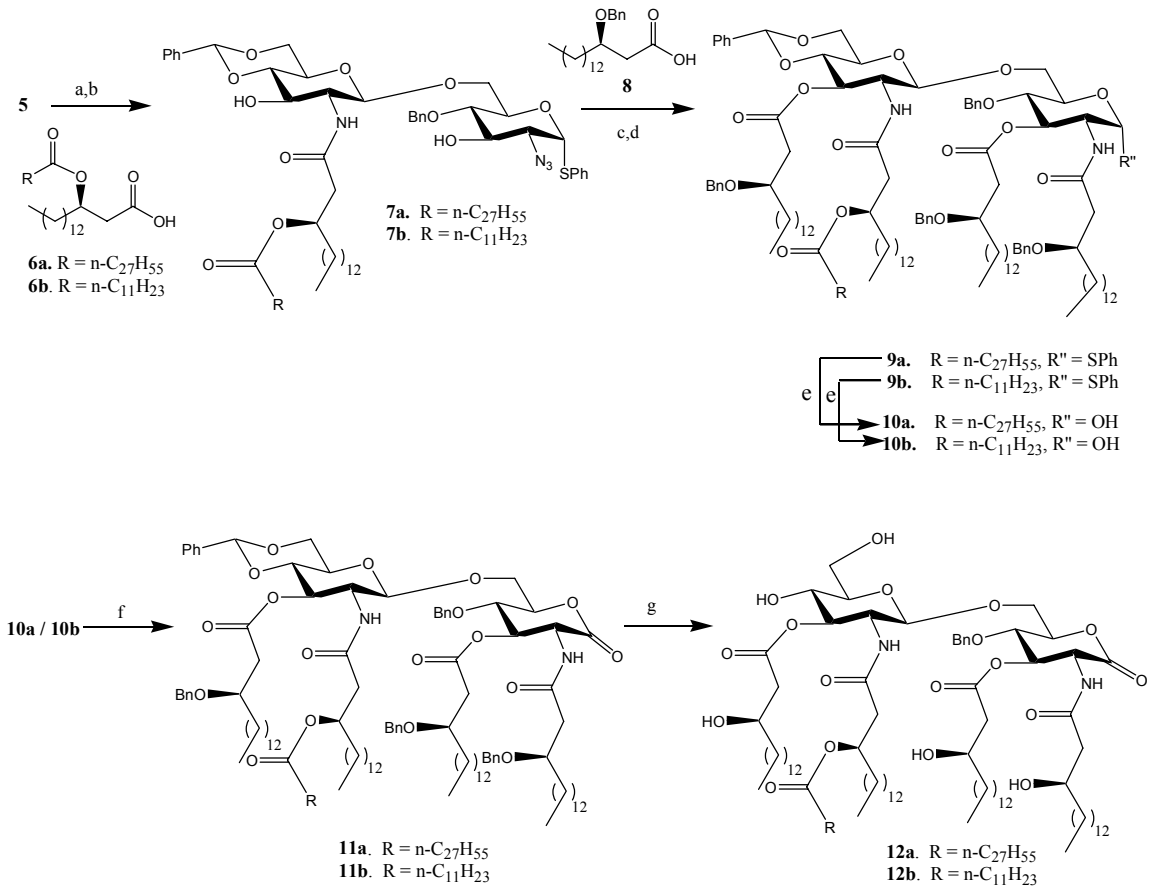


[a]Reagents and conditions: (a) Ac_2O, pyridine; (b) TMSSPh, ZnI_2, DCE, then TFA, H_2O; (c) AgOTf; K_2CO_3, MS 3A, DCM.

Having the requisite key disaccharide **5** in hand, attention was focused on selective introduction of (*R*)-3-hydroxy and alkanoyoxy fatty acid components (Scheme 2.2). Thus, removal of the phthalimido group and acetyl ester of **5** by treatment with ethylene diamine in

refluxing n-butanol[43] followed by selective *N*-acetylation with **6a** and **6b** acyloxyacyl acids in the presence DCC gave **7a** and **7b** in an overall yield of 93% and 78% respectively. Compound **6a** and **6b** were easily obtained by acylation of *p*-bromophenylacyl (*R*)-3-hydroxyhexadecanoate with octacosanoyl and myristoyl chlorides in the presence of DMAP in pyridine followed by removal of the p-bromophenyl acyl ester group using Zn in acetic acid respectively.[44]

Scheme 2.2[a]



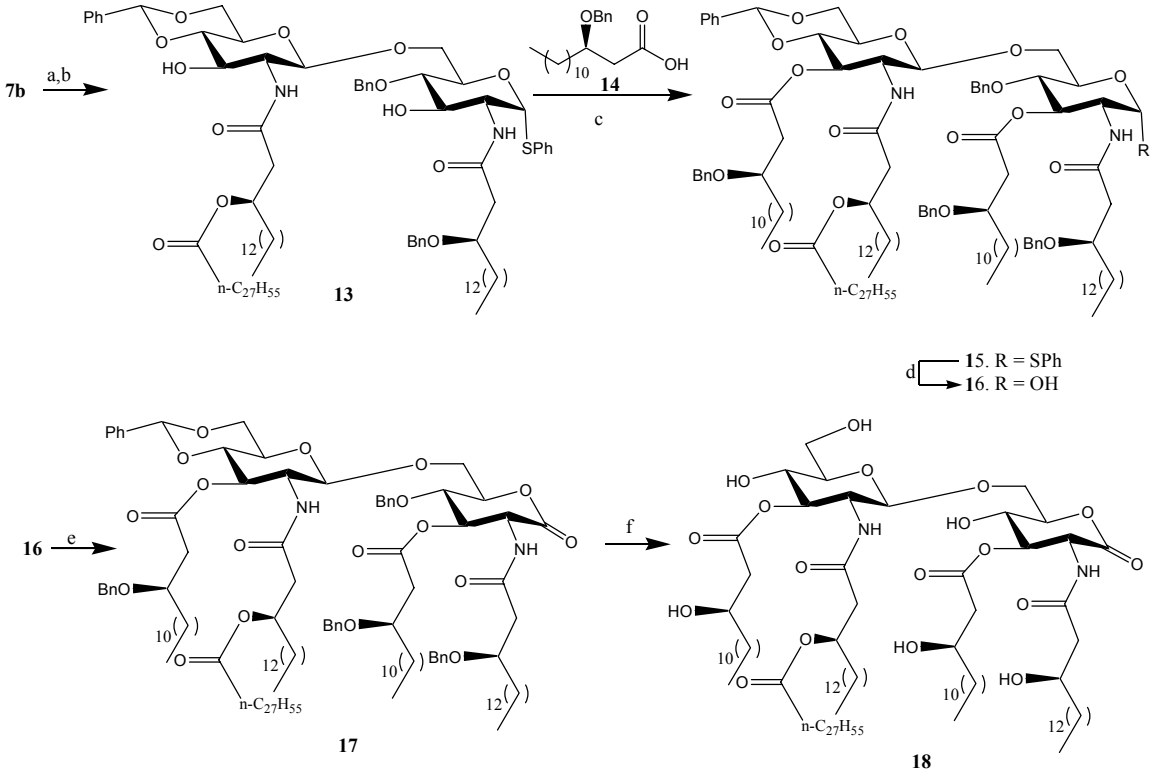
[a]Reagents and conditions: a)N_2H_4, EtOH, reflux; b)**6a/6b** DCC, DCM; c) $HS(CH_2)_2SH$, pyridine, Et_3N, H_2O; d)**8**, DCC, DMAP, DCM; e)NIS, TfOH, DCM, H_2O; f) PCC, MS 3A; DCM; g) Pd/C, H_2, THF, t-BuOH.

Reduction of the azido moieties of **7a** and **7b** was easily accomplished by reaction with propane dithiol[45] in a mixture of pyridine, triethylamine and water and the amine and hydroxyls of the

resulting compound were acylated with **8** using DCC and DMAP as the activation reagents. The end result was **9a** and **9b** in an overall yield of 60% and 58% respectively.

Hydrolysis of the thiophenyl moieties of **9a** and **9b** with NIS and a catalytic amount of TfOH in wet dichloromethane gave corresponding lactols **10a** and **10b** (Scheme 2.2). Oxidation of the anomeric centers was achieved by PCC of the above synthesized lactols to their corresponding lactones **11a** and **11b**. Finally, the benzyl ethers and benzylidene acetal of **11a** and **11b** were removed by catalytic hydrogenation over Pd/C to give the target compound **12a** and **12b.**

Scheme 2.3



[a]Reagents and conditions: a) $HS(CH_2)_2SH$, pyridine, Et_3N, H_2O; b)**8**, DCC, DCM; c)**14**, DCC, DMAP, DCM; d)NIS, TfOH, DCM, H_2O; e) PCC, MS 3A; DCM; f) Pd/C, H_2, THF, t-BuOH.

Selective *N*-acylation was accomplished after azide reduction of **7b** by reacting with **8** in the absence of DMAP to yield **13** in 84% (Scheme 2.3). Further the C-3 and C-3' hydroxyls of **13**

were acylated with a different (*R*)-3-hydroxy fatty acid component **14** using DCC and DMAP as activating agents to yield **15** in 73%. Thus we exploit the nucleophilic nature of amine and hydroxyl group in order to synthesize derivatives having different substitutions at C-2 amine and C-3 and C-3' hydroxyls. Compound **15** was subjected to same reaction conditions as described for **9a** or **9b** to yield the final compound **18** (Scheme 2.3).

Conclusions

A highly convergent strategy for the facile synthesis of several derivatives of *rhizobial sin-1* LPS has been developed. A key aspect of the synthetic strategy was a chemoselective glycosylation of a selenoglycosyl donor with a thioglycosyl acceptor to give selectively protected disaccharide **5**. The anomeric center, the two amino groups and the C-3 and C-3' hydroxyls of **5** could individually be modified, thereby providing a flexible route to produce a wide range of *R. sin-1* lipid A analogs. The synthetic strategy was employed for the preparation of derivatives **12a**, **12b** and **18**.

Experimental Procedures

General synthetic methods: Column chromatography was performed on silica gel 60 (EM Science, 70-230 mesh), size exclusion column chromatography was performed on Sephadex LH-20 (i-PrOH-DCM or MeOH-CH_2Cl_2, 1/1, v/v elution) or Sephadex G-25 (water elution). Reactions were monitored by TLC on Kieselgel 60 F_{254} (EM Science) and the compounds were detected by examination under UV light and by charring with 10% sulfuric acid in methanol. Solvents were removed under reduced pressure at $<40^oC$. CH_2Cl_2, $(ClCH_2)_2$, and MeCN were distilled from CaH_2 (twice) and stored over molecular sieves (3Å). THF was distilled from sodium directly prior to the application. Methanol was dried by refluxing with magnesium

methoxide, distilled and stored under argon. Pyridine was dried by refluxing with CaH_2, then distilled and stored over molecular sieves (3Å). Molecular sieves (3Å and 4Å), 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. Optical rotations were measured with a 'Jasco P-1020' polarimeter. ^{1}H NMR and ^{13}C NMR spectra were recorded with a Varian Inova500 spectrometer and a Varian Inova600 spectrometer equipped with Sun workstations. ^{1}H NMR spectra were recorded in $CDCl_3$ and referenced to residual $CHCl_3$ at 7.24 ppm, and ^{13}C NMR spectra to the central peak of $CDCl_3$ at 77.0 ppm. Assignments were made by standard gCOSY and gHSQC. High-resolution mass spectra were run in a JMS SX/SX102A tandem mass spectrometer, equipped with FAB source. The matrix used was DHB and the internal standards ultramark 1621 and PEG. Abbreviations: $_{L}$ - Signals marked with this symbol belong to the biantennary lipid at C-2' (signals marked as $_{L'}$ belong to the C-28 side chain), $_{S}$ - Signals marked with this symbol belong to the monoantennary lipids at C-2,3, and 3'. Signals marked with (*) might be interchanged

Phenyl 3-*O*-acetyl-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-1-seleno-β-D-glucopyranoside (2). Acetic anhydride (10 mL) was added drop wise to a solution of selenoglycoside **1** (4.48 g, 8.36 mmol) in pyridine (20 mL). After 16 h, the reaction mixture was quenched with MeOH (15 mL), concentrated, co-evaporated with toluene (3 x 15 mL) and dried *in vacuo*. The residue was purified by silica gel column chromatography (10% gradient ethyl acetate in hexane) to afford **2** as a colorless syrup (4.42 g, 92%), which was then crystallized from diethyl ether – hexane mixture: $Rf = 0.51$ (ethyl acetate/hexane, 2/3, v/v); $[\alpha]^{26}_{D}$ +2.6° (c 0.48, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.18-7.90 (m, 14H, aromatic), 6.00 (d, 1H, $J_{1,2}$ = 8.7 Hz, H-1), 5.87 (dd, 1H,

$J_{3,4}$ = 9.2 Hz, H-3), 5.52 (s, 1H, >C*H*Ph), 4.35-4.50 (m, 2H, $J_{2,3}$ = 9.2 Hz, H-2,6a), 3.67-3.86 (m, 3H, H-4,5,6b), 1.85 (s, 3H, $COCH_3$); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 169.8 (C=O), 123.6-136.9 (aromatic), 101.82 (*C*HPh), 101.5 (C-1), 79.4 (C-4), 71.6 (C-3), 70.5 (C-6) 68.6 (H-5), 55.6 (C-2), 20.6 (CO*C*H_3); HR MS (*m*/*z*): calcd for $C_{29}H_{25}NO_7SeNa$ 602.0696, found 602.1697.

Phenyl 3-*O*-acetyl-2-azido-4-*O*-benzyl-2-deoxy-1-thio-α-D-glucopyranoside (4). Phenylthiotrimethylsilane (2.2 ml, 11.67 mmol) and zinc iodide (3.72 g, 11.67 mmol) were added to the stirred solution of 1,6-anhydro glucopyranose **3** (1.24 g, 3.89 mmol) in 1,2-dichloroethane (35 ml). The reaction mixture was stirred for 16 h then diluted with DCM (35 mL), the solids were filtered-off through a pad of celite and the residue was washed with DCM (3 x 10 mL). The combined filtrate was washed with saturated aqueous $NaHCO_3$ (3 x 50 mL) and brine (40 mL). The organic phase was dried ($MgSO_4$), filtered and the filtrate was concentrated *in vacuo*. The residue was treated with a mixture of TFA (5 mL), DCM (25 mL), and water (400 μL) for 10 min at rt then diluted with DCM (75 mL), washed with water (50 mL), saturated aqueous $NaHCO_3$ (2 x 30 mL) and water (3 x 40 mL). The organic phase was dried ($MgSO_4$), filtered, and the filtrate was concentrated *in vacuo.* The residue was purified by silica gel column chromatography (3% gradient ethyl acetate in hexane) to afford **4** as a white foam (1150 mg, 69%): R_f = 0.25 (ethyl acetate/hexane, 3/7, v/v); $[\alpha]^{26}_D$ +99.4^o (c 1.16, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): δ = 7.26-7.51 (m,10H, aromatic), 5.59 (d, 1H, $J_{1,2}$ = 5.5 Hz, H-1), 5.43 (dd, 1H, $J_{3,4}$ = 10.0 Hz, H-3), 4.64 (dd, 2H, *J* = 12.0Hz, CH_2Ph), 4.26-4.34 (m, 1H, H-5), 3.91 (dd, 1H, $J_{2,3}$ = 10.5 Hz, H-2), 3.75-3.82 (m, 2H, H-6a,6b), 3.71 (dd, 1H, $J_{4.5}$ =9.5 Hz, H-4); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 169.8 (C=O), 127.7-137.5 (aromatic), 86.9 (C-1), 75.8 (C-4), 74.7 (*C*H_2Ph), 73.3 (C-3), 72.2 (C-5), 61.2 (C-6), 62.3 (C-2), 20.8 (CO*C*H_3); HR MS (*m*/*z*): calcd for

$C_{21}H_{23}N_3O_5SNa$ 452.1256, found 452.1983. The β-anomer of **4** was also identified and isolated as a white foam (197 mg, 12%): R_f = 0.33 (ethyl acetate/hexane, 3/7, v/v); selected ^{1}H NMR data: 4.55 (d, 1H, $J_{1,2}$ = 10.0 Hz, H-1).

Phenyl 3-*O*-acetyl-6-*O*-(3-*O*-acetyl-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyrano-syl)-2-azido-4-*O*-benzyl-2-deoxy-1-thio-α-D-glucopyranoside (5). A mixture of donor **2** (92 mg, 0.16 mmol), acceptor **4** (59 mg, 0.14 mmol) and activated molecular sieves (4Å, 200 mg) in DCM (1.5 mL) was stirred for 2h under an atmosphere of argon at rt. Then, silver trifluoromethanesulfonate (216 mg, 0.84 mmol) and potassium carbonate (580 mg, 0.84 mmol) were added and the reaction mixture was stirred for 2 h until TLC analysis indicated that reaction had gone to completion. The reaction mixture was diluted with DCM (10 mL), the solids were filtered-off and the residue was washed with DCM (3 x 10 mL). The combined filtrate (40 mL) was washed with aqueous saturated $NaHCO_3$ (15 mL) and H_2O (3 x 20 mL). The organic phase was dried ($MgSO_4$), filtered and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (4% gradient ethyl acetate in toluene) followed by crystallization from diethyl ether – hexane to afford **5** as white crystals (98.5 mg, 83 %): R_f = 0.67 (ethyl acetate/toluene, 3/7, v/v); $[\alpha]^{26}_D$ –48.8^o (c 0.70, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 6.87-7.56 (m, 19H, aromatic), 5.90 (dd, 1H, $J_{3',4'}$ = 9.0 Hz, H-3'), 5.54 (d, 1H, $J_{1,2}$ = 5.5 Hz, H-1), 5.53 (d, 1H, $J_{1',2'}$ = 8.5 Hz, H-1'), 5.52 (s, 1H, >C*H*Ph), 5.27 (dd, 1H, $J_{3,4}$ = 9.2 Hz, H-3), 4.42 (dd, 1H, $J_{2',3'}$ = 10.0 Hz, H-2'), 4.27-4.43 (m, 2H, H-5,5'), 4.08 (dd, 2H, J^2 = 11.0 Hz, CH_2Ph), 4.03 (dd, 1H, $J_{5,6a}$ = 1.7 Hz, $J_{6a,6b}$ = 11.0 Hz, H-6a), 3.69-3.89 (m, 5H, H-2,4',6b,6'a,6'b), 3.47 (dd, 1H, $J_{4.5}$ = 9.6 Hz, H-4), 1.89, 1.91 (2s, 6H, 2 x $COCH_3$); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 123.85-135.43 (aromatic), 101.82 (*C*HPh), 98.98 (C-1'), 87.34 (C-1), 78.67

(C-4'), 76.44(C-4), 75.00 (*C*H_2Ph), 73.51 (C-3), 70.88 (C-5), 70.02 (C-3'), 68.73 (H-5'), 68.28 (C-6), 67.17 (C-6'), 62.12 (C-2), 55.49 (C-2') 21.23 (2 x CO*C*H_3). HR MS (*m*/*z*): calcd for $C_{44}H_{42}N_4O_{12}SNa$ 873.2418, found 873.3286.

(*R*)-3-Octacosanoyloxy-hexadecanoic acid (6a). A mixture of 2-(4-bromophenyl)-2-oxoethyl (*R*)-3-hydroxyhexadecanoate (1.23 g, 2.62 mmol) and octacosanoyl chloride {freshly prepared from commercial octacosanoic acid (2 g, 4.72 mmol) and thionyl chloride (10 ml) 2h at reflux} in pyridine (15 ml) and DMAP (cat.) was stirred for 16 h. Then the reaction was quenched with MeOH (5 mL), concentrated under reduced pressure, the residue was dissolved in DCM (50 mL) and washed with 1M aqueous HCl (2 x 20 mL) and brine (2 x 20 mL). The organic phase was dried ($MgSO_4$), filtered, and the filtrate was concentrated *in vacuo.* The residue was purified by silica gel column chromatography (10% gradient ethyl acetate in DCM) to afford 2-(4-Bromophenyl)-2-oxoethyl (*R*)-3-octacosanoyloxyhexadecanoate as a colorless syrup (2.22 g, 97%): R_f = 0.70 (DCM); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.70 (dd, 4H, aromatic), 5.22-5.35 (m, 3H, H-3, CH_2), 2.64-2.81 (m, 2H, H-2a,2b), 2.30 (pt, 2H, H-2'a,2'b), 1.56-1.71 (m, 4H, H-3'a,3'b,4a,4b), 1.20-1.38 [bs, 70H, H-(5-15),(4'-27')], 0.88 (pt, 6H, H-16,28'). Zinc dust (300 mg, 4.20 mmol) was added portion wise during 30 min to 2-(4-bromophenyl)-2-oxoethyl (*R*)-3-octacosanoyloxyhexadecanoate (147 mg, 0.168 mmol) in acetic acid (2.5 ml) and heated at 60^{o}C. The reaction mixture was stirred for 2 h at 60^{o}C then diluted with DCM (10 mL), the solids were filtered-off through a pad of celite and the residue was washed with DCM (3 x 5 mL). The combined filtrate (~25 mL) was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (5% gradient ethyl acetate in toluene) to afford **6a** as a white solid (110 mg, 97%): R_f = 0.40 (ethyl acetate/toluene, 1/4, v/v); $[\alpha]^{26}_D$ –8.0^o (c 0.64, $CHCl_3$); ^{1}H NMR (300

MHz, $CDCl_3$): δ = 5.21 (m, 1H, H-3), 2.53-2.70 (m, 2H, H-2a,2b), 2.28 (pt, 2H, H-2'a, 2'b) 1.42-1.71 (m, 4H, H-3'a,3'b,4a,4b), 1.27 [bs, 70H, H-(5-15), (4'-27')], 0.88 (pt, 6H, H-16,28).

Phenyl 2-azido-4-*O*-benzyl-6-*O*-{4,6-*O*-benzylidene-2-deoxy-2-[(*R*)-3-octacosanoyloxy-hexadecan] amido-β-D-glucopyranosyl}-2-deoxy-1-thio-α-D-glucopyranoside (7a). To a solution of **5** (100 mg, 0.117 mmol) in *n*-butanol (10 mL), ethylene diamine (2 ml, 30 mmol) was added and stirred for 20 h at 90°C. The reaction mixture was concentrated *in vacuo* to dryness and co-evaporated with toluene (2 x 10 mL). The residue was purified by silica gel column chromatography (2% gradient methanol in DCM) to afford phenyl 6-*O*-(2-amino-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranosyl)-2-azido-4-*O*-benzyl-2-deoxy-1-thio-α-D-glucopyranoside as colorless syrup (67.4 mg, 91 %): R_f = 0.55 (methanol/DCM, 1/9, v/v). Dicyclohexylcarbodiimide (DCC) (87 mg, 0.42 mmol) was added to a solution of **6a** (190.5 mg, 0.281 mmol) in DCM (5 mL) and stirred for 10 min followed by the addition of the above mentioned amino derivative (178.7 mg, 0.281 mmol) in DCM (3 mL). The reaction mixture was stirred for 16 h at rt, the solids were filtered-off and the residue was washed with DCM (2 x 10 mL). The combined filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (4% gradient diethyl ether in DCM) to afford **7a** as a white solid (780 mg, 93%): R_f = 0.35 (diethyl ether/DCM, 3/17, v,v); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.26–7.52 (m, 15H, aromatic), 5.75 (d, 1H, $J_{NH,2'}$ = 6.3 Hz, NH), 5.55 (d, 1H, $J_{1,2}$ = 5.3 Hz, H-1), 5.50 (s, 1H, >C*H*Ph), 4.96 (m, 1H, H-3_L), 4.76 (dd, 2H, J^2 = 11.2 Hz, CH_2Ph), 4.66 (d, 1H, $J_{1',2'}$ = 8.3 Hz, H-1'), 4.34-4.38 (m, 1H, H-5), 4.28 (dd, 1H, $J_{5',6'a}$ = 4.9 Hz, $J_{6'a,6'b}$ = 10.3 Hz, H-6'a), 3.92-4.11 (m, 3H, H-3,3',6a), 3.70-3.82 (m, 3H, H-2,6b,6b'), 3.39-3.53 (m, 4H, H-2',4,4',5'), 2.26 (dd, 2H, $J_{2L',3L'}$ = 7.3 Hz, H-2_L'), 2.23 (dd, 1H, $J_{2L^a,3L}$ = 3.3Hz, $J_{2L^a,2L^b}$ =14.5 Hz, H-$2_L{}^a$), 2.13 (dd,

1H, $J_{2L^b,3L}$ = 4.9 Hz, H-$2_L{}^b$), 1.10-1.60 [m, 74H, H-(4_L-15_L), (3_L'-27_L')], 0.83 (m, 6H, H-16_L,28_L'); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 127.0-133.0 (aromatic), 102.6 (>*C*HPh), 101.5 (C-1'), 87.8 (C-1), 82.0 (C-4'), 79.1 (C-4), 75.4 (*C*H_2Ph), 74.9 (C-3), 72.0 (C-3_L), 71.1 (C-5,3'), 69.2 (C-6a), 68.8 (C-6'), 67.1 (C-5'), 64.7 (C-2), 59.3 (C-2'), 42.8 (C-2_L), 35.0 (C-2_L'), 31.0 [C-(5_L-15_L), (4_L'-27_L')], 25.2 (C-4_L,3_L'), 25.9 (C-16_L, 28_L').

Phenyl 2-azido-4-*O*-benzyl-6-*O*-{4,6-*O*-benzylidene-2-deoxy-2[(R)-3-tetra decanoyloxy-hexadecan]amido-β-D-glucopyranosyl}-1-thio-β-D-glucopyranoside (7b). To a solution of **5** (250 mg, 0.31 mmol) in *n*-butanol (25 mL), ethylenediamine (5 mL, 75 mmol) was added and stirred for 20h at 90C. The reaction mixture was concentrated *in vacuo*, and the residue was dissolved in DCM (30 mL). The solids then were filtered off and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (3.5% MeOH in DCM) to afford phenyl 6-*O*-(2-amino-4,6-O-benzylidene-2-deoxy-β-D-glucopyranosyl)- 2-azido-4-*O*-benzyl-2-deoxy-1-thio-β-D-glucopyranoside as white crystals (180mg, 92%): *Rf* = 0.45(methanol/DCM, 1/10, v/v). DCC (45 mg, 0.218 mmol) was added to a solution of **6b** (70 mg, 0.145 mmol) in DCM (2 mL) and stirred for 20 min, followed by the addition of the previously mentioned amino derivative (58 mg, 0.091 mmol) in DCM (1 mL). The reaction mixture was stirred for 2 h at room temperature, the solids were filtered off, and the residue was washed with DCM (2 x 3ml). The combined filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography to afford **7b** as a white solid (85 mg, 85%): *Rf* = 0.60 (diethyl ether/DCM, 1/6, v/v). ^{1}H NMR (500MHz, CDCl3): δ=7.31-7.57 (m, 15H, aromatic), 5.04 (m, 1H, H-3L), 4.76 (d, 1H, $J_{a,b}$ = 11.5 Hz, $PhCH_{2a}$), 4.73 (d, 1H, $J_{1',2'}$ = 8.0 Hz, H-1'), 4.67 (d, 1H, $J_{b,a}$ = 11.5 Hz, $PhCH_{2b}$), 4.56 (d, 1H, $J_{1,2}$ = 10.0 Hz, H-1), 4.46 (b, 1H, OH'),

4.34 (dd, 1H, $J_{6a',5}$ = 5.0 Hz, $J_{6a',6b'}$=10.0Hz, H-6a'), 4.16 (d, 1H, H-6a), 4.06 (dd, 1H, $J_{3',2}$ = 8.5 Hz, $J_{3',4'}$ = 9.5 Hz, H-3'), 3.79 (dd, 1H, $J_{6b',5}$ = 10.5 Hz, $J_{6b',6a'}$ = 10.0 Hz, H-6b'), 3.60-3.70 (m, 3H, H-3, 5, 6b), 3.58 (dd, 1H, $J_{4',3'}$ = 9.5 Hz, $J_{4',5'}$ = 10.0 Hz, H-4'), 3.52 (m, 1H, H-2'), 3.45 (ddd, 1H, $J_{5',4'}$ = 10.0 Hz, $J_{5',6a'}$ = 5.0 Hz, $J_{5',6b'}$ = 10.0 Hz, H-5'), 3.27 (dd, 1H, $J_{2,1}$ = 9.5 Hz, $J_{2,3}$ = 9.5Hz, H-2), 3.27 (dd, 1H, $J_{4,3}$ = 9.5 Hz, $J_{4,5}$ = 9.5 Hz, H-4), 2.76 (b, 1H, OH), 2,25-2.31 (m, 2H, H-2L), 1.53-1.58 (m, 4H, H-4L, H-3L'), 1.25[m, 42H, H-(5L-15L), H-(4L'-13L')], 0.87-0.90(m, 6H, H-16L, H-14L'). HR MS (m/z) $C_{62}H_{92}N_4O_{11}SNa$: calcd, 1123.6381; found, 1123.32.

(*R*)-3-Octacosanoyloxy-hexadecanoic acid (8). Trifluoromethanesulfonic acid (8.5 μl, 0.10 mmol) was added to a stirred solution of 2-(4-Bromophenyl)-2-oxoethyl (*R*)-3-hydroxyhexadecanoate (300 mg, 0.64 mmol) and benzyl trichloroacetimidate (178 μl, 0.96 mmol) in DCM (3.5 ml) at 0^{o}C. The reaction mixture was stirred for 22 h then diluted with DCM (35 mL) and washed with saturated aqueous $NaHCO_3$ (2 x 20 mL) and water (2 x 20 mL). The organic phase was dried ($MgSO_4$), filtered and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (2% gradient ethyl acetate in toluene) followed by crystallization from diethyl ether to afford 2-(4-Bromophenyl)-2-oxoethyl (*R*)-3-benzyloxyhexadecanoate as white crystals (315 mg, 88 %): R_f = 0.65 (ethyl acetate/toluene, 1/9, v/v); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.20-7.80 (m, 9H, aromatic), 5.26 (ps, 2H, CH_2), 4.56 (dd, 2H, J^2 =11.4 Hz, CH_2Ph), 3.94 (m, 1H, H-3), 2.79 (dd, 1H, $J_{2a,3}$ = 7.1 Hz, $J_{2a,2b}$ = 15.3 Hz, H-2a), 2.65 (dd, 1H, $J_{2b,3}$ = 5.5 Hz, H-2b), 1.55-1.72 (m, 1H, H-4), 1.35-1.45 (m, 2H, H-5,15), 1.30 [bs, 18H, H-(6-14)], 0.88 (pt, 3H, H-16). To the above synthesized benzyloxy-hexadecanoate (295 mg, 0.523 mmol) was subjected to the treatment with Zn/AcOH as described for the synthesis of **6**, and purification by silica gel column chromatography (5%

gradient ethyl acetate in toluene) to afford **8** as a colorless syrup (165 mg, 87%): R_f = 0.40 (ethyl acetate/toluene, 1/3, v/v). $[\alpha]^{26}_D$ –8.2^o (c 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.30-7.40 (m, 5H, aromatic), 4.58 (ps, 2H, CH_2Ph), 3.95 (m, 1H, H-3), 2.58 (m, 2H, H-2a, 2b), 1.30-1.71 (m, 3H, H-4,5,15), 1.28 [bs, 18H, H-(6-14)] 0.85 (pt, 3H, H-16).

Phenyl 4-*O*-benzyl-6-*O*-{4,6-*O*-benzylidene-3-*O*-[(*R*)-3-benzyloxy-hexadecanoyl]-2-deoxy-2-[(*R*)-3-octacosanoyloxy-hexadecan]amido-β-D-glucopyranosyl}-2-[(*R*)-3-benzyloxy-hexadecan]amido-3-*O*-[(*R*)-3-benzyloxy-hexadecanoyl]-2-deoxy-1-thio-α-D-glucopyranoside (9a). Triethylamine (~1.25 ml) was added to a stirred solution of **7a** (755 mg, 0.583 mmol) and propanedithiol (1.2 ml, 11.65 mmol) in pyridine (41 ml) and H_2O (5.8 ml). The reaction mixture was stirred for 16 h at rt then evaporated *in vacuo* to dryness and co-evaporated with toluene (2 x 10 mL) and ethanol (2 x 10 ml). The residue was purified by silica gel column chromatography (2% gradient methanol in DCM) to afford phenyl 2-amino-4-*O*-benzyl-6-*O*-{4,6-*O*-benzylidene-2-deoxy-2-[(*R*)-3-octacosanoyloxy-hexadecan]amido-β-D-glucopyranosyl}-2-deoxy-1-thio-α-D-glucopyranoside as colorless syrup (693 mg, 94 %): R_f = 0.45 (methanol/DCM, 1/9, v/v); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.22 – 7.58 (m, 15H, aromatic), 5.80 (d, 1H, $J_{NH,2}$ = 5.8 Hz, NH), 5.52 (s, 1H, >C*H*Ph), 5.47 (d, 1H, $J_{1,2}$ = 5.4 Hz, H-1), 5.02 (m, 1H, H-3_L), 4.82 (dd, 2H, J^2 = 11.2 Hz, CH_2Ph), 4.73 (d, 1H, $J_{1',2'}$ = 8.3 Hz, H-1'), 4.34-4.40 (m, 1H, H-5), 4.32 (dd, 1H, $J_{5',6'a}$ = 4.9 Hz, $J_{6'a,6'b}$ = 10.2 Hz, H-6'a), 4.15 (dd, 1H, $J_{5,6a}$ = 2.0 Hz, $J_{6a,6b}$ = 11.2 Hz, H-6a), 4.11 (dd, 1H, $J_{3',4'}$ = 9.3 Hz, H-3'), 3.84 (dd, 1H, $J_{5,6b}$ = 5.0 Hz, H-6b), 3.75 (dd, 1H, $J_{5',6'b}$ = 10.2 Hz, H-6'b), 3.40-3.62 (m, 5H, H-2',3,4,4,5'), 3.04 (dd, 1H, $J_{2,3}$ = 10.3 Hz, H-2), 2.27 (dd, 1H, $J_{2L',3L'}$ = 7.8 H, H-2_L'), 2.22 (dd, 1H, $J_{2L^a,3L}$ = 7.8 Hz, $J_{2L^a,2L^b}$ = 14.7 Hz, H-2_L^a), 2.11 (dd, 1H, $J_{2L^b,3L}$ = 4.4 Hz, H-2_L^b), 1.00-1.75 [m, 74H, H-(4_L-15_L), (3_L'-27_L')], 0.83

(m, 6H, Hz-16_L,28_L'); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 125.00-132.00 (aromatic), 101.71 (>*C*HPh), 100.96 (C-1'), 91.88 (C-1), 80.96 (C-4')*, 77.50 (C-4)*, 74.31 (C-3), 73.81(*C*H_2Ph), 71.03 (C-5,3_L), 70.49 (C-3'), 68.28 (C-6), 69.19 (C-6'), 66.11 (C-5'), 56.56 (C-2), 49.02 (C-2'), 42.11 (C-2_L), 34.20 (C-2_L'), 25.12-26.00, 29.64 [C-(4_L-15_L), (3_L'-27_L')], 23.16 (C_L-16_L,28_L'). DCC (43 mg, 0.21 mmol) and dimethylaminopyridine (4.3 mg, 0.035 mmol) were added to a stirred solution of **8** (51 mg, 0.14 mmol) in DCM (3 ml) and stirred for 10 min followed by addition of the above-mentioned amino derivative (45 mg, 0.035 mmol) in DCM (1.5 mL). The reaction mixture was stirred for 16 h at rt, the solids were filtered-off and the residue was washed with DCM (2 x 10 mL). The combined filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (3% gradient ethyl acetate in DCM) to afford **9a** as a white solid (51.2 mg, 63%): R_f = 0.40 (ethyl acetate/DCM, 1/9, v/v). $[\alpha]^{26}_D$ +14.9^o (c 1.4, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.00–7.41 (m, 30H, aromatic), 6.46 (d, 1H, $J_{NH,2}$ = 8.3 Hz, 2-NH), 5.70 (d, 1H, $J_{1,2}$ = 5.3 Hz, H-1), 5.59 (d, 1H, $J_{NH,2'}$ = 8.3 Hz, 2'-NH), 5.37 (s, 1H, >C*H*Ph), 5.37 (dd, 1H, $J_{3',4'}$ = 9.3 Hz, H-3'), 5.24 (dd, 1H, $J_{3,4}$ = 8.8 Hz, H-3), 4.95 (m, 1H, H-3_L), 4.80 (d, 1H, $J_{1',2'}$ = 8.3 Hz, H-1'), 4.37-4.61 (m, 9H, H-2, 4 x C*H*$_2$Ph), 4.33 (m, 1H, H-5), 4.29 (dd, 1H, $J_{5,6'a}$ = 5.5 Hz, $J_{6'a,6'b}$ = 11.0 Hz, H-6'a), 3.64-4.00 (m, 8H, H-2',4,6a,6b,6'b, 3 x H-3_S), 3.60 (dd, 1H, $J_{4',5'}$ = 9.3 Hz, H-4'), 3.48 (m, 1H, H-5'), 1.90-2.64 [m, 10H, H-2_L^a,2_L^b,2_L'a,2_L'b, 3 x H-2_S^a,2_S^b], 1.05-1.63 (m, 146H, H-(4_L-15_L), (3_L'-27_L'), 3 x H-(4_S-15_S)], 0.85 (m, 15H, H-16_L,28_L', 3 x H–16_S); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 125.0-131.5 (aromatic), 101.2 (>*C*HPh), 100.9 (C-1'), 87.0 (C-1), 78.0 (C-4'), 75.9 (C-4, 2 x C-3_S), 75.2 (C_S-3), 74.5 (*C*H_2Ph), 73.0 (C-3), 71.2 (C-5), 70.9 (C-3'), 70.4-70.6 (3 x *C*H_2Ph), 70.3 (C-3_L), 68.2 (C-6'), 67.4 (C-6), 66.0 (C-5'), 55.5 (C-2'), 52.6 (C-2), 32.0-42.2 (C-2_L,2_L', 3 x C-2_S), 29.9 [C-(4_L-

15_L),(3_L'-27_L')3 x H-(4_S-15_S)], 14.3 (C-16_L,28_L', 3 x C-16_S). HR MS (*m*/*z*): calcd for $C_{145}H_{230}N_2O_{17}SNa$ 2326.6847, found 2326.8550.

Phenyl 4-*O*-Benzyl-6-*O*-{4,6-*O*-benzylidene -3-*O*-[(R)-3-benzyloxy-hexadecanoyl] -2-deoxy-2-[(R)-3-tetradecanoyloxy-hexadecan]amido-ß-D-glucopyranosyl}-2-[(R)-3- benzyl oxy-hexadecan]amido-3-*O*-[(R)-3-benzyloxy-hexadecanoyl]-2-deoxy-1-thio-ß-D-glucopyranoside (9b). Triethylamine (0.2 mL) was added to a stirred solution of **7b** (95 mg, 0.086 mmol) and 1,3-propanedithiol (0.18ml, 1.75 mmol) in pyridine (6 mL) and H_2O (0.85 mL). The reaction mixture was stirred for 16h at room temperature and then evaporated *in vacuo* to dryness. The residue was purified by silica gel column chromatography (1.5% methanol in DCM to afford Phenyl 2-amino -4-*O*-benzyl-6-*O*-{4,6-*O*-benzylidene-2-deoxy-2-[(R)-3-tetradecanoyloxy-hexadecan]amido-ß-D glucopyranosyl}-2-deoxy-1-thio-ß-D-glucopyranoside as a white solid (85.5mg, 92.5%): *Rf* = 0.40 (DCM/diethyl ether, 6/1, v/v). ^{1}H NMR(500MHz, CDCl3): δ=7.26-7.53 (m, 15H, aromatic), 6.08 (d, 1H, $J_{NH,2'}$= 5.0 Hz, NH), 5.57 (s, 1H, >CHPh), 5.06 (m, 1H, H-3L), 4.82 (d, 1H, $J_{a,b}$= 11.5 Hz, $PhCH_{2a}$), 4.72 (d, 1H, $J_{1',2'}$= 9.0 Hz), 4.66 (d, 1H, $J_{b,a}$= 11.5 Hz, $PhCH_{2b}$), 4.53 (d, 1H, $J_{1,2}$=9.5 Hz, H-1), 4.34 (dd, 1H, $H_{6a',5'}$= 5.0 Hz, $H_{6a',6b'}$ = 10.5 Hz, H-6a'), 4.20 (d, 1H, H-6a), 4.05 (dd, 1H, $J_{3',4'}$ = 9.0 Hz, $J_{3',2'}$ = 9.5 Hz, H-3'), 3.79 (dd, 1H, $J_{6b',5'}$ = 10.0 Hz, $J_{6b',6a'}$ = 10.5 Hz, H-6b'), 3.64-3.71 (m, 2H, H-5,6b), 3.58 (dd, 1H, $J_{4',3'}$ = 9.0 Hz, $J_{4'5'}$ = 9.0 Hz, H-4'), 3.49-3.52 (m, 2H, H-2',3), 3.45 (ddd, 1H, $J_{5',4'}$ = 9.0 Hz, $J_{5',6a'}$ = 5.0 Hz, $J_{5',6b'}$ = 10.0 Hz, H-5'), 3.26 (dd, 1H, $J_{4,3}$ = 9.0 Hz, $J_{4,5}$ = 9.0 Hz, H-4), 2.63 (dd, 1H, $J_{2,1}$ = 9.5 Hz, $J_{2,3}$ = 10.0 Hz, H-2), 2.27-2.43 (m, 4H, H-2L,2L'), 1.50-1.58 (m, 4H, H-4L,3L'), 1.25 [m, 42H, H-(5L-15L), H-(4L'-13L')], 087-0.90 (m, 6H, H-16L,14L'). ^{13}CNMR (75MHz, CDCl3): =126.60-138.18 (aromatic), 102.18 (>CHPh), 101.45 (C-1'), 89.18 (C-1),

81.73 (C-4'), 78.88 (C-5), 78.55 (C-3), 77.45 (C-4), 74.71 (CH2Ph), 72.04 (C-3'), 71.59 (C-3L), 69.48 (C-6), 68.83 (C-6'), 66.67 (C-5'), 59.49 (C-2'), 56.50 (C-2), 42.39 (C-2L'), 34.79 (C-2L), 22.92-32.15,34.57[C-(4L-15L), (3L'-13L')], 14.35 (C-16L,14L'). DCC(57.5mg, 0.279mmol) was added to a stirred solution of **8** (85 mg, 0.232 mmol) in DCM (3 mL) and stirred for 20 min, followed by addition of DMAP (6.2 mg, 0.051 mmol) and the previously mentioned amino derivative (50 mg, 0.0465 mmol) in DCM (1.5 mL). The reaction mixture was stirred for 5 h at room temperature, the solids were filtered off, and the residue was washed with DCM (2 times, 2ml). The combined filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (20% ethyl acetate in hexane) to afford **9b** as a white solid (62mg, 63.3%): *Rf* = 0.60 (Hexane/Ethyl acetate, 3/1, v/v); ^{1}H NMR (300MHz, CDCl3): δ = 7.14-7.43 (m, 30H, aromatic), 6.46 (d, 1H, $J_{NH,2}$ = 9.6 Hz, 2-NH), 5.42 (s, 1H, >CHPh), 5.36 (d, 1H, $J_{NH,2'}$ = 9.0 Hz, 2'-NH), 5.24 (dd, 1H, $J_{3',2'}$= 9.6Hz, $J_{3',4'}$ = 9.9 Hz, H-3'), 5.10 (dd, 1H, $J_{3,2}$ = 8.7 Hz, $J_{3,4}$ = 10.2 Hz, H-3), 4.96 (m, 1H, H-3L), 4.67 (d, $J_{1',2}$' = 8.4 Hz, H-1'), 4.41-4.61 (m, 9H, H-1, 4 Ph CH_2), 4.32(dd, 1H, $J_{6a,5}$ = 4.8 Hz*, $J_{6a,6b}$ = 10.5 Hz*, H-6a*), 4.04 (m, 1H, H-2), 3.96 (d, 1H, H-6a'*), 3.59-3.84 (m, 7H, H-2',4',6b,6b',3*H-3s), 3.36-3.49 (m, 3H, H-4,5,5'), 2.09-2.69 (m, 10H, H-2La,2Lb,2La',2Lb', 3H-2sa,2sb), 0.98-1.57 [m, 118H, H-(4L-15L), (3L'-13L'), 3H-(4s-15s)], 0.88 (m, 15H, H-16L,14L',3H-16s). ^{13}CNMR (75Hz, CDCl3): δ = 126.34-138.73 (aromatic), 101.62 (>CHPh, C-1'), 86.95 (C-1), 79.68 (C-5 or 5'), 79.13 (C-4'), 75.67-76.30 (C-3, 3*C-3s), 74.67 (C-4), 71.18-71.63 (C-3',3L, 4* $PhCH_2$), 68.86 (C-6 or 6'), 68.33 (C-6 or 6'), 66.43 (C-5 or 5'), 55.24 (C-2'), 53.10 (C-2), 32.16-41.86 (C-2L,2L', 3*C-2S), 22.92-34.34 (C-(4L-15L),(3L'-13L'), 3*C-(4s-15s), 14.35 (C-16L,14L', 3*C-16s). HR MS (m/z) $C_{131}H_{202}N_2O_{17}SNa$: calcd, 2130.4622; found, 2130.6140.

4-*O*-Benzyl-6-*O*-{4,6-*O*-benzylidene-3-*O*-[(*R*)-3-benzyloxy-hexadecanoyl]-2-deoxy-2-[(*R*)-3-octacosanoyloxy-hexadecan]amido-β-D-glucopyranosyl}-2-[(*R*)-3-benzyloxy-hexadecan]amido-3-*O*-[(*R*)-3-benzyloxy-hexadecanoyl]-2-deoxy-α-D-glucopyranose (10a). *N*-Iodosuccinimide (95 mg, 0.42 mmol) and trifluoromethanesulfonic acid (3.5 μL, 0.04 mmol) were added to a stirred solution of **9a** (312 mg, 0.135 mmol) in DCM/H_2O (10 ml, 100:1) at 0°C. The reaction mixture was vigorously stirred for 30 min at 0°C until TLC analysis indicated that reaction had gone to completion. The reaction mixture was diluted with DCM (20 mL), washed with aqueous $Na_2S_2O_3$ (20 %, 20 mL) and water (3 x 20 mL). The organic phase was dried ($MgSO_4$), filtered and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (1% gradient methanol in DCM) to afford **10a** as a white solid (250 mg, 84%): R_f = 0.45 (methanol/DCM, 1/19, v/v); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.00–7.38 (m, 30H, aromatic), 6.26 (d, 1H, $J_{NH,2}$ = 9.8 Hz, 2-NH), 5.90 (d, 1H, $J_{NH,2'}$ = 8.3 Hz, 2'-NH), 5.41 (s, 1H, >C*H*Ph), 5.40-5..36 (m, 2H, H-3, H-3'), 5.18 (d, 1H, $J_{1',2'}$ = 8.3 Hz, H-1'), 4.95 (m, 1H, H-3_L), 4.37-4.59 (m, 9H, H-2, 4 x CH_2Ph), 4.48 (d, 1H, $J_{1,2}$ = 3.4 Hz, H-1), 4.36 (m, 1H, H-6'a), 4.17 (m, 1H, $J_{2,3}$ = 10.3 Hz, H-2), 4.03 (m, 1H, $J_{5,6a}$ = 6.8 Hz, H-5), 3.92 (m, 1H, H-6a), 3.46-3.83 (m, 5H, H-6b,H-6'b,H-4',H-2',H-5'), 3.35 (t, 1H, $J_{4,5}$ = 9.8 Hz, H-4), 1.90-2.64 [m, 10H, H-2_L^a,2_L^b,2_L'a,2_L'b, 3 x H-2_S^a,3x2_S^b], 1.05-1.63 (m, 146H, H-(4_L-15_L), (3_L'-27_L'), 3 x H-(4_S-15_S)], 0.85 (m, 15H, H-16_L,28_L', 3 x H–16_S); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 125.0-131.5 (aromatic), 105.5 (C-1), 101.4 (>*C*HPh), 100.9 (C-1'), 78.1 (C-4'), 75.7 (C-4), 74.48 (*C*H_2Ph), 73.8 (C-3), 72.2 (C-5), 71.2 (C-3'), 70.4-70.7 (3 x *C*H_2Ph), 70.31 (C-3_L), 68.2 (C-6'), 67.2 (C-6), 66.1 (C-5'), 57.0 (C-2'), 53.5 (C-2), 32.00-42.20 (C-2_L,2_L', 3 x C-2_S), 29.88 [C-(4_L-15_L),(3_L'-27_L')3 x H-(4_S-15_S)], 14.33 (C-16_L,28_L', 3 x C-16_S).

4-*O*-Benzyl-6-*O*-{4,6-*O*-benzylidene -3-*O*-[(R)-3-benzyloxy-hexadecanoyl]-2- deoxy-2-[(R)-3-tetradecanoyloxy-hexadecan]amido-ß-D-glucopyranosyl}-2-[(R)-3- benzyl -oxy-hexadecan]-amido-3-*O*-[(R)-3-benzyloxy-hexadecanoyl]-2-deoxy-a-D-glucopyranoside (10b). N-Iodosuccinimide (9.9 mg, 0.044 mmol) and TfOH (0.5 μL, 0.0044 mmol) were added to a stirred solution of **9b** (31 mg, 0.0147 mmol) in DCM/H_2O (4ml, 100/1) at 0C. The reaction mixture was vigorously stirred for about 30 min until TLC analysis indicated that the reaction had gone to completion. The reaction mixture was diluted with DCM and washed with aqueous $Na_2S_2O_3$ (15%) and water (2 times). The organic phase was dried ($MgSO_4$) and filtered, and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (25% ethyl acetate in hexane) to afford **10b** as a white solid (8mg, 27%): *Rf* = 0.2 (Hexane/Ethyl acetate, 2.5/1, v/v). ^{1}HNMR (500MHz, CDCl3): δ=7.18-7.40 (m, 25H, aromatic), 6.28 (d, 1H, $J_{NH,2}$ = 9.0 Hz, NH), 5.95 (d, 1H, $J_{NH',2'}$ = 8.5 Hz, NH'), 5.44 (s, 1H, >CHPh), 5.39-5.43 (m, 2H, H-3,3'), 5.17 (d, 1H, $J_{1',2'}$ = 8.5 Hz, H-1'), 5.09 (m, 1H, H-1), 4.95 (m, 1H, H-3L), 4.40-4.61 (m, 8H, 4* $PhCH_2$), 4.35 (dd, 1H, $J_{6a',5'}$ = 5.0 Hz, $J_{6a',6b'}$ = 10.0 Hz, H-6a'), 4.20 (m, 1H, H-2), 4.07 (m, 1H, H-5), 3.96 (d, 1H, H-6a), 3.74-3.85 (m, 4H, H-3*3S, H-6b'), 3.61-3.68 (m, 2H, H-6b,2',4'), 3.52 (ddd, 1H, $J_{5',6a'}$ = 5.0 Hz, H-5'), 3.37 (t, 1H, $J_{4,3} = J_{4,5}$= 10.0 Hz, H-4), 2.20-2.66 (m, 10H, H-2La,2Lb,2La',2Lb',3H-2sa,2sb), 1.10-1.59 [m, 118H, H-(4L-15L), (3L'-13L')], 3H-(4s-15s), 0.84 (m, 15H, H-16L,14L',3H-16s). ^{13}CNMR (75Hz, CDCl3): δ = 126.0-138.6 (aromatic), 101.5 (>CHPh), 100.4 (C-1'), 91.5 (C-1), 79.0 (C-4'), 76.4 (C-4), 75.5-76.4 (C-3*3S), 74.4 (CH2Ph), 73.5 (C-3 or 3'), 71.6 (C-5), 71.0-71.4 (3*CH2Ph, C-3L, 3 or 3'), 68.7 (C-6'), 67.5 (C-6), 66.5 (C-5'), 31.9-42.0 (C-2L,2L', 3*C-2S), 22.7-31.9 (C-(4L-15L),(3L'-13L'), 3*C-(4s-15s), 14.1 (C-16L,14L'), 3*C-16s), HRMS (m/z) for $C_{125}H_{198}N_2O_{18}Na$: calcd, 2038.4538; found, 2038.3850.

4-*O*-Benzyl-6-*O*-{4,6-*O*-benzylidene-3-*O*-[(*R*)-3-benzyloxy-hexadecanoyl]-2-deoxy-2-[(*R*)-3-octacosanoyloxy-hexadecan]amido-β-D-glucopyranosyl}-2-[(*R*)-3-benzyloxy-hexadecan]amido-3-*O*-[(*R*)-3-benzyloxy-hexadecanoyl]-2-deoxy-D-glucono-1,5-lactone (11a). A mixture of **10a** (14.0 mg, 6.3 μmol) and activated molecular sieves (3Å, 20 mg) in DCM (1.0 mL) was stirred for 2h at rt under atmosphere of argon. Then pyridinium chlorochromate (7 mg, 0.03 mmol) was added and the reaction mixture was stirred for 1 h until TLC analysis indicated that reaction had gone to completion. The reaction mixture was directly placed onto floursil column where it was purified by eluting with *i*-propanol/DCM (1:1) to afford **11a** as colorless film (12.3 mg, 88 %): R_f = 0.55 (diethyl ether/DCM, 1/4, v/v); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.13 –7.31 (m, 25H, aromatic), 5.60 (t, 1H, $J_{3',4'}$ = 9.8 Hz, H-3'), 5.39 (s, 1H, >C*H*Ph), 5.31 (dd, 1H, $J_{3,4}$ = 9.8 Hz, H-3), 5.04 (m, 1H, H-3_L), 4.93 (d, 1H, $J_{1',2'}$ = 8.3 Hz, H-1'), 4.78 (t, 1H, $J_{2,3}$ = 10.3 Hz H-2), 4.36-4.59 (m, 9H, H-5, 4 x CH_2Ph), 4.27 (dd, 1H, $J_{5',6'a}$ = 5.5 Hz, $J_{6'a,6'b}$ = 10.3 Hz, H-6'a), 4.01-4.04 (m, 2H, H-4,6a), 3.55-3.87 (m, 7H, 3 x H_{SL}-3, H-4',6b,6'b,2'), 3.69 (t, 1H, $J_{6'b,5}$ = 9.77 Hz H-6'b), 3.50-3.57 (m, 4H, H-5',2',4',6b), 1.93-2.30 (m, 10H, H-2_L^a,2_L^b,2_L'a,2_L'b, 3 x H-2_S^a,2_S^b), 1.00-1.75 [m, 146H, H-(4_L-15_L),(3_L'-27_L'), 3 x H-(4_S-15_S)], 0.62 (m, 15H, H-16_L,28_L', 3 x H–16_S); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 125.0-128.2 (aromatic), 101.2 (C-1'), 100.8 (>C*H*Ph), 78.3 (C-4',5), 74.9 (3 x C-3_S), 73.5 (*C*H_2Ph,C-4), 71.3 (C-3), 70.6 (C-3',*C*H_2Ph), 70.4 (2 x *C*H_2Ph), 70.0 (C-3_L), 67.9 (C-6), 67.9 (C-6'), 65.4 (C-5'), 54.1 (C-2'), 52.4 (C-2), 37.0-40.3 [C-2_L,2_L', 3 x C-2_S], 20.3-33.8 (C-(4_L-15_L),(3_L'-27_L'), 3 x C-(4_S-15_S)], 13.9 [C-16_L,28_L', 3 x C-16_S].

4-***O*-Benzyl-6-*O*-{4,6-*O*-benzylidene -3-*O*-[(R)-3-benzyloxy-hexadecanoyl]-2-deoxy-2-[(R)-3-tetradecanoyloxy-hexadecan]amido-ß-D-glucopyranosyl}-2-[(R)-3-benzyloxy-hexadecan]-amido-3-*O*-[(R)-3-benzyloxy-hexadecanoyl]-2-deoxy-D-glucono-1,5-lactone (11b)**. A mixture of **10b** (8.0 mg, 4.0 μmol) and activated molecular sieves (3A, 15mg) in DCM (1.0 mL) was stirred for 1h at room temperature under an atmosphere of argon. Then pyridinium chlorochromate (4.3 mg, 0.02 mmol) was added and the reaction mixture was stirred for 1 h until TLC analysis indicated that reaction had gone to completion. The reaction mixture was directly purified by iatrobeads column chromatography (25% ethyl acetate in hexane) to afford **11b** as a white solid (7 mg, 87.5%): $[\alpha]^{23}_{D}$-12.5(c, 1, $CHCl_3$). *Rf* = 0.65 (Hexane/Ethyl acetate, 2.5/1, v/v). ^{1}H NMR (500MHz, CDCl3): δ = 7.20-7.38 (m, 25H, aromatic), 6.82 (d, 1H, $J_{NH,2}$ = 8.5 Hz, NH), 6.61 (d, 1H, $J_{NH',2'}$ = 7.5 Hz, NH'), 5.60 (t, 1H, $J_{3',2'}$ = $J_{3',4'}$ = 10.0 Hz, H-3'), 5.39 (s, 1H, >CHPh), 5.34 (dd, 1H, $J_{3,2}$ = $J_{3,4}$ =10.1 Hz, H-3), 5.07 (m, 1H, H-3L), 4.95 (d, 1H, $J_{1',2'}$ = 8.0 Hz, H-1'), 4.79 (t, 1H, $J_{2,3}$ = 10.0 Hz, H-2), 4.37-4.60 (m, 9H, H-5, 4* Ph CH_2), 4.28 (dd, 1H, $J_{6a',5'}$ = 3.5 Hz, $J_{6a',6b'}$ = 9.5 Hz, H-6a'), 4.02-4.06 (m, 2H, H-4,6a), 3.80-3.85 (3H, 3*3s), 3.75 (t, 1H, $J_{6b',5'}$ = $J_{6b',6a'}$= 10.0 Hz), 3.52-3.60 (m, 4H, H-2',4',5',6a), 2.25-2.68 (m, 10H, H-2La,2Lb,2La',2Lb',3H-2sa,2sb), 1.03-1.60 (m, 118H, H-(4L-15L), (3L'-13L'), 3H-(4s-15s), 0.88 (m, 15H, H-16L,14L',3H-16s). HRMS(m/z) for $C_{125}H_{196}N_2O_{18}Na$: calcd, 2036.4381; found, 2036.8009

2-Deoxy-6-*O*-{2-deoxy-3-*O*-[(*R*)-3-hydroxy-hexadecanoyl]-2-[(*R*)-3-octacosanoyloxy-hexadecan] amido-β-D-glucopyranosyl}-2-[(*R*)-3-hydroxy-hexadecan]amido-3-*O*-[(*R*)-3-hydroxy-hexadecanoyl]-D-glucono-1,5-lactone (12a). The lactone **11a** (8.0 mg, 0.0036 mmol) was dissolved in THF-i-PrOH (1 ml, 1:3) and Pd/C (10 mg) was added. The reaction mixture

was shaken under H_2 (15 psi) for 36 h at rt, then the catalyst was filtered off, washed successively with THF (2 x 10 ml), EtOH (2 x 5 ml), DCM (2 x 10) and the combined filtrate was concentrated. The residue was purified by Florisil column chromatography (2% gradient *i*-propanol in DCM) and size exclusion column chromatography on Sephadex LH-20 (*i*-propanol/DCM, 1:1) to afford **12a** as colorless film (2.1 mg, 33%): R_f = 0.35 (MeOH/DCM, 1/9, v/v). $[\alpha]^{26}_D$ –26.52^o (c 0.22, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ = 5.39 (t, 1H, $J_{3,4}$ = 9.8Hz, H-3), 4.97 (t, 1H, $J_{3',4'}$ = 9.3 Hz, H-3'), 4.55 (1H, H-1'), 4.32 (m, 1H, H-5), 4.17 (m, 1H, $J_{2,3}$ = 10.3Hz H-2), 4.13 (m, 1H, H-6a'), 3.95 (m, 1H, H-4), 3.81-3.85 (m, 2H, H-2', H-6b'), 3.70 (m, 1H, H-6a'), 3.54 [t, 1H, $J_{4',5'}$ = 9.3Hz, H-4'], 3.33 (m, 1H, H-5'). HR MS (*m*/*z*): calcd for $C_{104}H_{196}N_2O_{18}Na$ 1784.4381, found 1784.4787.

2-Deoxy-6-*O*-{2-deoxy-3-*O*-[(R)-3-hydroxy-hexadecanoyl]-2-[(R)-3-tetra decanoyloxy-hexadecan]amido-ß-D-glucopyranosyl}-2-[(R)-3-hydroxy-hexadecan]amido-3-*O*-[(R)-3-hydroxy-hexadecanoyl]-D-glucono-1,5-lactone (12b). Lactone **11b** (7 mg, 0.00348 mmol) was dissolved in THF/*t*-BuOH (1ml, THF/t-BuOH, 1/1, v/v) and Pd/C (10mg) was added. The reaction mixture was shaken under H_2 (15psi) for 24 h at room temperature. The catalyst was filtered off and washed with THF (3 x 3 mL). The combined filtrate was concentrated *in vacuo* to afford **12b** as a white solid (5mg, 91%): ^{1}H NMR (500MHz, $CDCl_3$): δ = 5.34 (t, 1H, $J_{3,2} = J_{3,4}$ =10.0 Hz, H-3), 5.10 (m, 1H, H-3L), 5.00 (t, 1H, $J_{3',2'} = J_{3',4'}$ = 10.0 Hz, H-3'), 4.56 (d, 1H, $J_{1',2'}$ = 8.5Hz, H-1'), 4.26 (m, 1H, H-5), 4.21 (d, 1H, $J_{2,3}$ = 10.0 Hz, H-2), 4.19 (d, 1H, $J_{6a,6b}$ = 11 Hz, H-6a), 3.74-3.92 (m, 7H, H-4,6b,2',6a', 3*3s), 3.63 (m, 1H, H-6b'), 3.47 (dd, 1H, $J_{4',3'}$ = 10.0 Hz, $J_{4',5'}$ = 9.0 Hz, H-4'), 3.27 (m, 1H, H-5'), 2.18-2.27 (m, 10H, H-2La,2Lb,2La',2Lb',3H-

2sa,2sb), 0.96-1.63 [m, 118H, H-(4L-15L), (3L'-13L'), 3H-(4s-15s)], 0.84 (m, 15H, H-16L,14L',3H-16s). HRMS (m/z) for $C_{125}H_{196}N_2O_{18}Na$: calcd, 1588.2190; found, 1588.4548.

Phenyl 4-*O*-benzyl-6-*O*-{4,6-*O*-benzylidene-3-*O*-[(*R*)-3-benzyloxy-tetradecanoyl-2-deoxy-2-[(*R*)-3-octacosanoyloxy-hexadecan]amido-β-D-glucopyranosyl}-2-[(*R*)-3-benzyloxy-hexadecan]amido-3-*O*-[(*R*)-3-benzyloxy-tetradecanoyl]-2-deoxy-1-thio-α-D-glucopyranoside (15): DCC (29 mg, 0.137 mmol) was added to a stirred solution of **14** (37 mg, 0.109 mmol) in DCM (2.5 ml). After stirring for 10 min, a solution of **13** (44.2 mg, 27.4 μmol) and DMAP (4 mg, 27.4 μmol) in DCM (2 mL) was added. The reaction mixture was stirred for 18 h, after which the solids were filtered-off and the residue was washed with DCM (2 x 6 mL). The combined filtrate was concentrated *in vacuo* and the residue was purified by silica gel column chromatography (5% gradient ethyl acetate in toluene) to afford **15** as a white fluffy solid (45 mg, 73%): R_f = 0.64 (10% ethyl acetate in tolune). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.18–7.41 (m, 30H, aromatic), 6.48 (d, 1H, *J* = 8.3 Hz, NH), 5.72 (d, 1H, *J* = 5.3 Hz, H-1), 5.60 (d, 1H, *J* = 8.3 Hz, NH$^{/}$), 5.34 (m, 2H, >C*H*Ph, H-3$^{/}$), 5.26 (dd, 1H, *J* = 8.8 Hz, H-3), 4.99 (m, 1H, H-3$_L$), 4.83 (d, 1H, *J* = 8.3 Hz, H-1$^{/}$), 4.65-4.39 (m, 9H, H-2, 4 x C*H*$_2$Ph), 4.33 (m, 1H, H-5), 4.31 (m, 1H, H-6a$^{/}$), 3.66-4.02 (m, 5H, H-2$^{/}$,4,6a,6b,6b$^{/}$), 3.60 (m, 1H, H-4$^{/}$), 3.48 (m, 1H, H-5$^{/}$); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 173.9, 172.3, 171.4, 171.2, 169.9, 125.0-131.5 (aromatic), 101.2 (>*C*HPh), 100.9 (C-1'), 87.0 (C-1), 78.0 (C-4'), 75.9 (C-4, 2 x C-3$_S$), 75.2 (C$_S$-3), 74.5 (*C*H$_2$Ph), 73.0 (C-3), 71.2 (C-5), 70.9 (C-3'), 70.4-70.6 (3 x *C*H$_2$Ph), 70.3 (C-3$_L$), 68.2 (C-6'), 67.4 (C-6), 66.0 (C-5'), 55.5 (C-2'), 52.6 (C-2). HRMS (*m/z*) for $C_{141}H_{222}N_2O_{17}S$ Na: calcd, 2272.3304; found, 2272.3795.

4-*O*-benzyl-6-*O*-{4,6-*O*-benzylidene-3-*O*-[(*R*)-3-benzyloxy-tetradecanoyl-2-deoxy-2-[(*R*)-3-(octacosanoyloxy-hexadecan]amido-β-D-glucopyranosyl}-2-[(*R*)-3-benzyloxy-hexadecan]amido-3-*O*-[(*R*)-3-benzyloxy-tetradecanoyl]-2-deoxy-D-glucono-1,5-lactone (17): *N*-Iodosuccinimide (7.0 mg, 28.3 μmol) and trifluoromethanesulfonic acid (1.0 μL, 2.65 μmol) were added to a stirred solution of **15** (21.1 mg, 8.91 μmol) in DCM/H_2O (3 ml, 100:1) at 0^{o}C. The reaction mixture was vigorously stirred for 30 min at 0^{o}C until TLC analysis indicated that reaction had gone to completion. The reaction mixture was diluted with DCM (5 mL), washed with aqueous $Na_2S_2O_3$ (10 %, 5 mL) and water (2 x 5 mL). The organic phase was dried ($MgSO_4$), filtered and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (5% gradient ethyl acetate in toluene) to afford the intermediate lactol **16** as a white solid (13.7 mg, 67%). To the lactol **16** (13.7 mg, 6.28 μmol) and activated molecular sieves (3Å, 37 mg) in DCM (2 mL) and was stirred for 1 h at room temperature under an atmosphere of argon. Pyridinium chlorochromate (15 mg, 66.1 μmol) was then added and the reaction mixture was stirred for another 1 hr until TLC analysis indicated completion of the reaction. The reaction mixture was placed onto a column of iatrobeads, and eluted with EtOAc/toluene (1:1) to afford the lactone **17** as a colorless film (10.1 mg, 73%): R_f = 0.73 (25% ethyl acetate in toluene); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.13 –7.31 (m, 25H, aromatic), 5.60 (t, 1H, *J* = 9.8 Hz, H-3'), 5.39 (s, 1H, >C*H*Ph), 5.31 (dd, 1H, *J* = 9.8 Hz, H-3), 5.04 (m, 1H, H-3_L), 4.93 (d, 1H, *J* = 8.3 Hz, H-1'), 4.78 (t, 1H, *J* = 10.3 Hz H-2), 4.36-4.59 (m, 9H, H-5, 4 x CH_2Ph), 4.27 (dd, 1H, *J* = 5.5, 10.3 Hz, H-6'a), 4.01-4.04 (m, 2H, H-4,6a), 3.55-3.87 (m, 7H, 3 x H_{SL}-3, H-4',6b,6'b,2'), 3.69 (t, 1H, *J* = 9.77 Hz, H-6'b), 3.50-3.57 (m, 4H, H-5',2',4',6b); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 125.0-128.2 (aromatic), 101.2 (C-1'), 100.8 (>C*H*Ph), 78.3 (C-4',5), 74.9 (3 x C-3_S), 73.5 (*C*H_2Ph,C-4), 71.3 (C-3), 70.6 (C-3',*C*H_2Ph), 70.4 (2 x *C*H_2Ph), 70.0

(C-3_L), 67.9 (C-6), 67.9 (C-6'), 65.4 (C-5'), 54.1 (C-2'), 52.4 (C-2). HR MS (*m*/*z*): calcd for $C_{135}H_{216}N_2O_{18}Na$ 2178.1520, found 2178.1993.

2-Deoxy-6-*O*-{2-deoxy-3-*O*-[(*R*)-3-hydroxy-tetradecanoyl]-2-[(*R*)-3-octacosanoyloxy-hexadecan]amido-β-D-glucopyranosyl}-2-[(*R*)-3-hydroxy-hexadecan]amido-3-*O*-[(*R*)-3-hydroxy-hexadecanoyl]-D-glucono-1,5-lactone (18). The lactone **17** (10.1 mg, 4.63 μmol) was dissolved in THF/*t*-BuOH (2 ml, 1:3) and Pd/C (10 mg) was added. The reaction mixture was stirred under an atmosphere of H_2 for 36 h at rt, then the catalyst was filtered off, washed successively with THF (2 x 3 ml) and the combined filtrate was concentrated. The residue was purified by size exclusion column chromatography on Sephadex LH-20 (*i*-propanol/DCM, 1:1) to afford **18** as colorless film (7.1 mg, 89%). $[\alpha]^{26}_D$ –26.2° (c 0.34, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3/CD_3OD$, 1:1): δ = 5.39 (t, 1H, *J* = 9.8Hz, H-3), 4.97 (t, 1H, *J* = 9.3 Hz, H-3'), 4.55 (1H, H-1'), 4.32 (m, 1H, H-5), 4.17 (m, 1H, *J* = 10.3Hz H-2), 4.13 (m, 1H, H-6a'), 3.95 (m, 1H, H-4), 3.81-3.85 (m, 2H, H-2', H-6b'), 3.70 (m, 1H, H-6a'), 3.54 [t, 1H, *J* = 9.3Hz, H-4'], 3.33 (m, 1H, H-5'). HR MS (*m*/*z*): calcd for $C_{100}H_{188}N_2O_{18}Na$ 1728.3755, found 1728.5394.

CHAPTER 3

SYNTHESIS AND BIOLOGICAL EVALUATION OF A LIPID A DERIVATIVE THAT CONTAINS AN AMINOGLUCONATE MOIETY*

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Abstract

A highly convergent strategy for the synthesis of several derivatives of the lipid A of *Rhizobium sin-1* has been developed. The synthetic derivatives are 2-aminogluconate **3** and 2-aminogluconolactone **4**, both lacking C-3 acylation. These derivatives were obtained by the preparation of disaccharides in which the two amino groups and the C-3' hydroxyl could be modified individually with acyl- or β-hydroxyl fatty acyl groups. Detailed NMR and MS analysis of **3** and **4** revealed that, even under neutral conditions, the two compounds equilibrate to both forms. The synthetic compounds lack the proinflammatory effects of *E. coli* LPS as indicated by an absence of TNFα protein production. Although that **3** and **4** were able to antagonize *E. coli* LPS, they were significantly less potent than the synthetic compound **2** which is acylated at C-3, and *R. sin-1* LPS, indicating that the β-hydroxyl fatty acyl group at C-3 contributes to the antagonistic potential of *R. sin-1* LPS. Based on a comparison of the biological responses of the synthetic lipid A derivatives, and *R. sin-1* LPS and lipid A, the KDO moieties appear to be important for the optimal antagonization of enteric LPS-induced cytokine production.

Introduction

Septicemia is a life-threatening syndrome for which currently no treatment exists but supportive therapy in an intensive care unit setting.[1,2] The development of Gram-negative sepsis is due to a strong and acute inflammatory response to lipopolysaccharides (LPS) released from the bacterial outer membrane.[6-8] LPS initiates the production of multiple host-derived inflammatory mediators such as tumor necrosis factor (TNF-α), interleukin 1 (IL-1), IL-6, arachidonic acid metabolites and leukotrienes. LPS induces the production of these mediators after binding to the cluster differentiation antigen CD14 on mononuclear phagocytes, or to

soluble CD14 in plasma and then to cells lacking CD14.[28,29,46] The interaction of LPS with CD14 is facilitated by a plasma protein termed LPS binding protein. As CD14 is a glycosylphosphatidylinositol-anchored protein, it lacks transmembrane and cytoplasmic domains, and therefore is unable to directly transmit signals to the interior of the cell. The latter function is performed by the Toll-like receptor 4 (TLR4),[47-49] which contains extracellular, transmembrane and intracellular domains, and an accessory protein MD-2.[50,51] While the precise mechanisms involved in the interactions among LPS, CD14, TLR4, and MD-2 remain to be discovered,[52,53] it is clear that cellular activation leads to the induction of cytokine gene expression, primarily through the activation of NF-κB, and the MAP kinases. The end result is an up-regulation of more than 120 genes, including those for the cytokines, most notably TNFα, interleukin-1α, and interleukin-1β.[54]

LPS consists of an O-chain polysaccharide, a core oligosaccharide and an amphiphilic moiety referred to as Lipid A. Lipid A, obtained by acid hydrolysis of *E. coli* LPS, has lethal toxicity, pyrogenicity, TNF-α and other cytokine-activating properties similar to native LPS, and is thus regarded as the toxic principle of LPS.[30,55] The structure of Lipid A is largely conserved among most enteric bacteria, consisting of a β-(1-6)-linked glucosamine disaccharide backbone with phosphate monoesters at C-1 and C-4' and β-hydroxyl fatty acyl groups and acyloxyacyl residues at positions 2, and 3 and 2' and 3', respectively (Figure 3.1).[56] Small modifications in the acylation pattern of Lipid A are thought to contribute to the virulence of enteric pathogens. For example, fatty acyl components can be present that have shorter chain length, sites of unsaturation or keto functional groups.[15,16,57,58] Other modifications include the addition of a palmitoyl residue, the hydroxylation of a myristoyl substituent and the addition of aminoarabinosyl and phospoethanolamine moieties.[17]

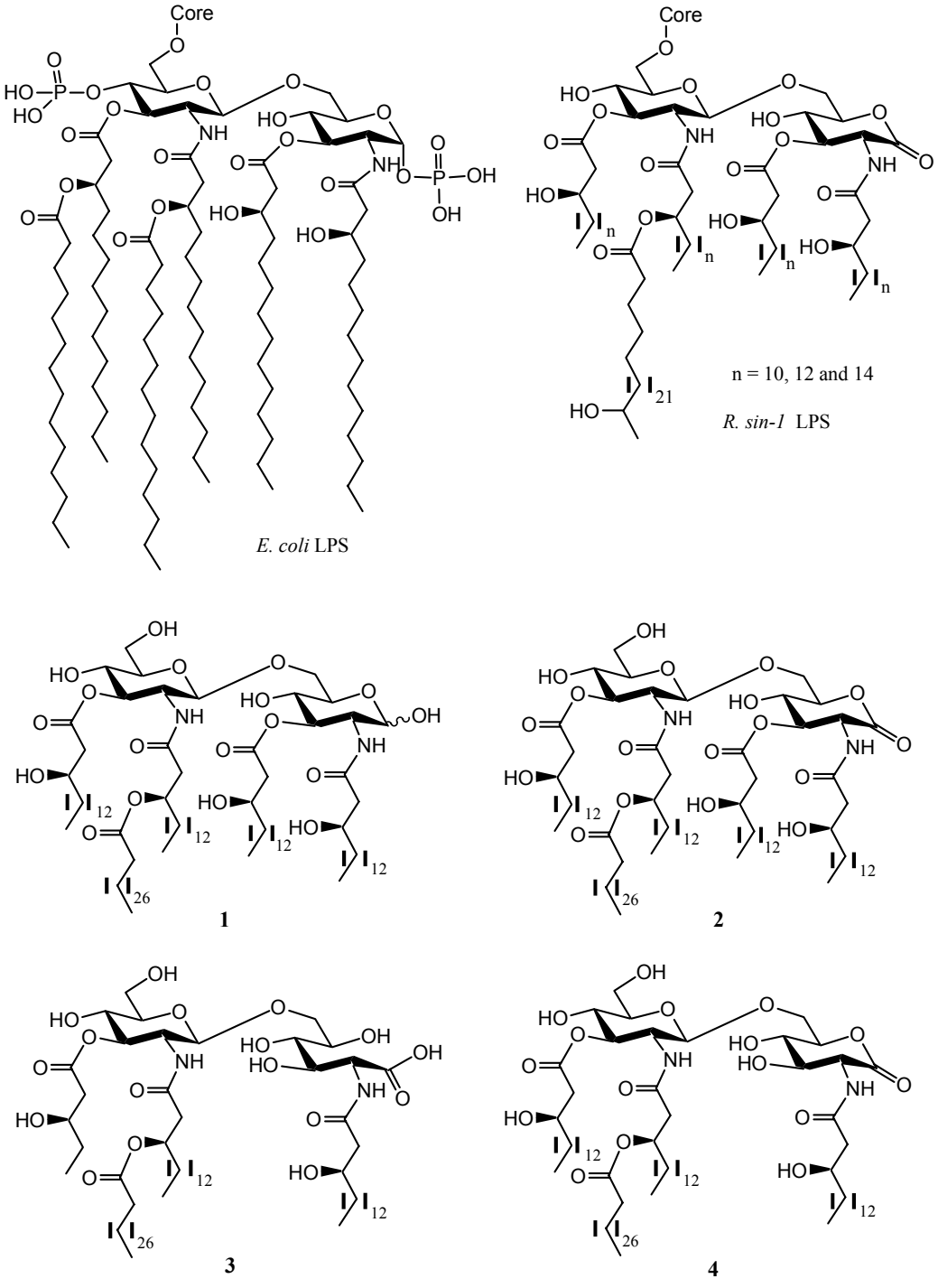


Figure 3.1. Strutcures of E. coli and R. sin 1 LPS and Lipid A

An appealing method for the prevention of the deleterious effects of enteric LPS is to block the interaction of lipid A and its receptors on mononuclear phagocytes.[33,34] Interference at this level may prevent initiation of the cellular reactions that lead to systemic inflammatory responses and septic shock. As is often the case, efficacious pharmacological receptor antagonists often are derived by modifying a compound possessing agonist activity. It has, however, proven difficult to identify lipid A derivatives that possess these properties. The best studied derivatives are monosacccharide biosynthetic precursors of lipid A[35-37] and synthetic analogs derived from the lipid A of *Rhodobacter sphaeroides* or *Rhodobacter capsulatus*, two species having very similar lipid A structures.[18,20,38] Although the *R. sphaeroides /R. capsulatus* lipid A has an identical bis-1,4'-phosphorylated glucosamine disaccharide backbone to that of *E. coli*, its fatty acyl pattern is quite different from *E. coli* lipid A. The *R. sphaeroides /R. capsulatus* lipid A consists of two 3-oxomyristic acid, two (R)-β-hydroxydecanoic acid, and one dodecenoic acid residues. The latter fatty acid is the only acyloxyacyl substituent and is located on the 3'-β-hydroxydecanoic acid residue. The *R. sphaeroides /R. capsulatus* lipid A lacks toxic effects, fails to induce cytokine synthesis by human monocytes, and is an antagonist of enteric endotoxin.

Recent data from our laboratory indicate that LPS from a nitrogen-fixing symbiont, *Rhizobium sin-1*, does not stimulate human monocytes.[21] More importantly, *R. sin-1* LPS significantly inhibits *E. coli* LPS-dependent synthesis of TNFα by these cells. The lipid A of *R. sin-1* is perhaps the most structurally unusual lipid A reported to date, its structure (Figure 3.1) differing in almost every aspect from those known to contribute to the toxicity of enteric LPS.[24] In particular, the disaccharide moiety of rhizobial lipid A is devoid of phosphate and the

glucosamine phosphate is replaced by 2-aminogluconolactone. It contains a very long chain fatty acid, 27-hydroxyoctacosanoic acid which, in turn, can be esterified by β-hydroxybutyrate.

The lipid A of *R. sin-1* shows considerable microheterogeneity. The fatty acylation pattern is heterogeneous and consists exclusively of β-hydroxy fatty acids. The *N*-acyl groups can consist of β-hydroxymyristate, β-hydroxypalmitate, or β-hydroxystearate. The *O*-acyl groups are primarily β-hydroxymyristate, but occasionally can also include β-hydroxypentadecanoate. Furthermore, a significant percentage of *R. sin-1* Lipid A lacks a fatty acyl residue at the C-3 position. It may also be possible that the 2-aminogluconolactone residue exists as a 2-aminogluconate.

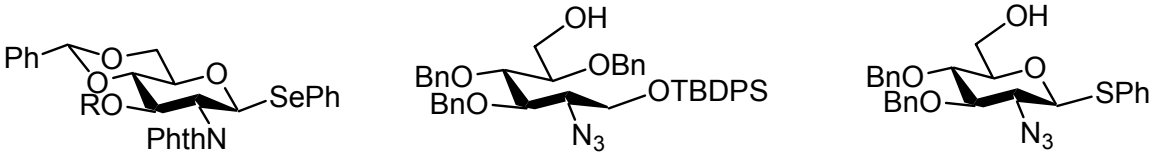
R. sin-1 LPS cannot be developed as a therapeutic agent for Gram-negative septicemia due to its inherent molecular heterogeneity. Furthermore, the inability to separate the different species limits identification of specific structural features that makes *R. sin-1* lipid A an antagonist as opposed to an agonist. To address these problems, we are engaged in a program to develop facile approaches for the synthesis of a wide range of well-defined Lipid A derivatives based on the structure of *R. sin-1* LPS. We have already shown[59] that the synthetic compounds **1** and **2** (Figure 3.1) lack the proinflammatory effects of *E. coli* LPS as indicated by an absence of expression of TNFα mRNA or production of TNFα protein. Furthermore, the synthetic compound **2** was able to antagonize *E. coli* LPS whereas compound **1** was devoid of this activity. Based on the known structure of the lipid A of *R. sin-1*, these results suggest that the gluconolactone moiety of *R. sin-1* LPS is important for this property. This finding was significant as **2** is the first example of a synthetic lipid A derivative that does lack phosphate but can inhibit cytokine production initiated by *E. coli* LPS. In this respect, compounds containing phosphates are less attractive candidates for drug development because of their instabilities.

In this paper, we report efficient approaches for the synthesis of compounds **3** and **4**, which are putative structural elements of *R. sin 1* LPS. As it is unknown whether these derivatives exist as a 2-aminogluconolactone or 2-aminogluconate, both forms were prepared. The proinflammatory properties of compounds **3** and **4** and their capability to inhibit *E. coli* LPS-dependent synthesis of TNFα by human monocytes, have been determined. These studies highlight the importance of C-3 acylation for biological properties. It also uncovered that 2-aminogluconolactone or 2-aminogluconate moieties equilibrate under neutral conditions. In order to determine the importance of KDO moieties for optimal inhibition of enteric LPS-induced cytokine production, the biological responses of the synthetic lipid A derivatives, and *R. sin-1* LPS and lipid A, have been compared.

Results and Discussion

Glycosyl donors **5a,b** and glycosyl acceptors **6** and **7** (Figure 3.2) were used as appropriately protected building blocks for the synthesis of target compounds **3** and **4**. Coupling of the donors with each of the acceptors will give disaccharides (**14**, and **21**, Schemes 3.2 and 3.3) that are appropriately protected for the selective introduction of β-hydroxyl fatty acyl and acyloxyacyl residues. In this respect, the phthalimido moiety of the disaccharides can selectively be cleaved with hydrazine hydrate in refluxing ethanol without affecting the azido group. Under these conditions, the acetyl esters will also be removed. However, resulting amine and hydroxyls can be selectively acylated by exploiting the fact that primary amines are more nucleophilic than hydroxyls. The azido function can be reduced to an amine under mild conditions using propane-1,3-dithiol[45] in a mixture of pyridine, triethylamine and water and these conditions will not affect any of the other functionalities. Finally, at a late stage of the synthesis, the TBDPS and

thiophenyl groups[39,40] at C-1 of the compounds derived from **6** and **7**, respectively, can be selectively removed to install the aminogluconate or lactone moiety.



5a. R = Ac
5b. R = H

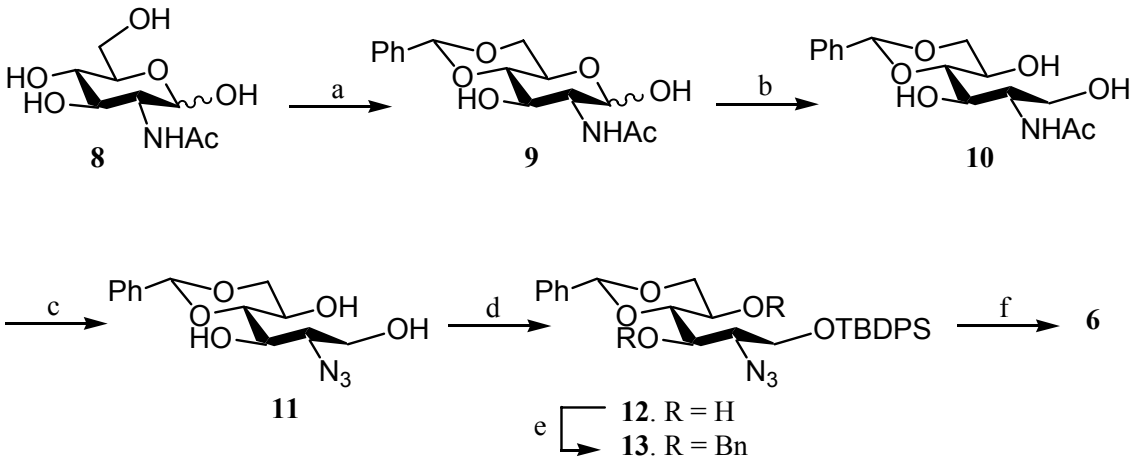
6

7

Figure 3.2. Building blocks for the preparation of compound **3** and **4**

In order to synthesize target compound **3,** an efficient strategy for the preparation of 2-azido-2-deoxy-alditol **6** needed to be developed. It was envisaged that this compound could be obtained by reduction of the anomeric center of an appropriate protected *N*-acetyl glycosamine derivative followed by conversion of the acetamido to an azido group. Thus, the 4,6-diol of commercially available *N*-acetylglucosamine was protected as a benzylidine acetal using freshly distilled benzaldehyde and zinc chloride to give compound **9** (Scheme 3.1).[60] A good yield was achieved when the reaction mixture was sonicated for 2 hrs. Reduction of the hemiacetal of **9** with $NaBH_4$ in methanol at 0^oC gave alditol **10** in a yield of 95%.[61] Treatment of **9** with barium hydroxide converted the acetamido group into an amine, which was transformed into an azido moiety by an azido-transfer reaction with triflic azide to give compound **11** in an excellent overall yield of 85%.[62] Next, the primary alcohol of **11** was selectively protected by reacting with TBDPSCl and imidazole in DMF to give **12**, which was benzylated under standard conditions to afford the fully protected **13**. Finally, regioselective opening of the benzylidine acetal of **13** by treatment with BH_3 (1M THF) and Bu_2BOTf (1M DCM) gave the alditol acceptor **10** in an excellent yield of 85%.[62]

Scheme 3.1[a]

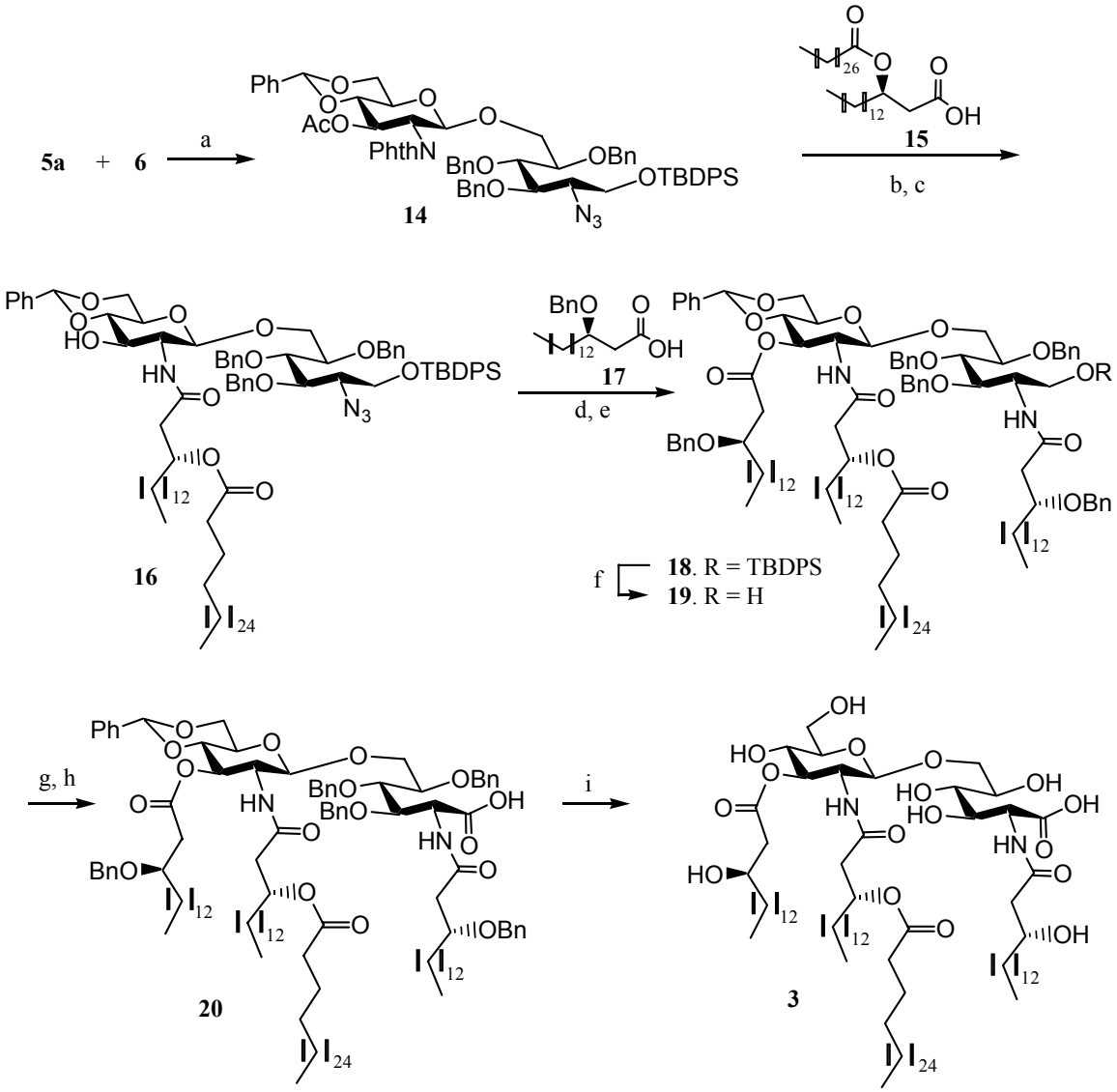


[a]Reagents and conditions: a) PhCHO, $ZnCl_2$; b) $NaBH_4$, MeOH; c) $Ba(OH)_2.8H_2O$, MeOH, H_2O, 90^oC; then Tf_2O, NaN_3, $CuSO_4$; d) TBDPSCl, imidazole, DMF; e) BnBr, NaH, DMF; f) BH_3 (1M in THF), Bu_2BOTf (1M in CH_2Cl_2).

Coupling of the glycosyl donor **5a** with alditol acceptor **6**, using NIS/TMSOTf[63] as the activator, gave disaccharide **14** in excellent yield. In this coupling, only the β-anomer was formed due to neighboring group participation of the phthalimido group. Removal of the phthalimido group of **14** by treatment with hydrazine hydrate in refluxing ethanol followed by selective *N*-acylation with alkanoyoxy fatty acid **15** in the presence DCC gave **16** in a yield of 71%. Reduction of the azido moiety of **16** was easily accomplished by reaction with propane-1,3-dithiol[45] in a mixture of pyridine, triethylamine and water. The C-2 amine and C-3' hydroxyl of the resulting compound were immediately acylated with **17** using 1,3-dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP) as the activation reagents to afford **18** in an overall yield of 60%. It is important to note that selective *N*-acylation could be accomplished by performing the reaction in the absence of DMAP, thereby making it possible to synthesize derivatives having different substituents at C-2 amine and C-3' hydroxyls. The TBDPS group of compound **18** was

removed by treating with tetrabutyl ammonium fluoride in good yields to afford **19** (Scheme 3.2). It was attempted to oxidize the primary alcohol of **19** with a catalytic amount of TEMPO and NaBr and $NaOCl_2$ as the co-oxidant under biphasic conditions to carboxylic acid **20**.[64]

Scheme 3.2[a]

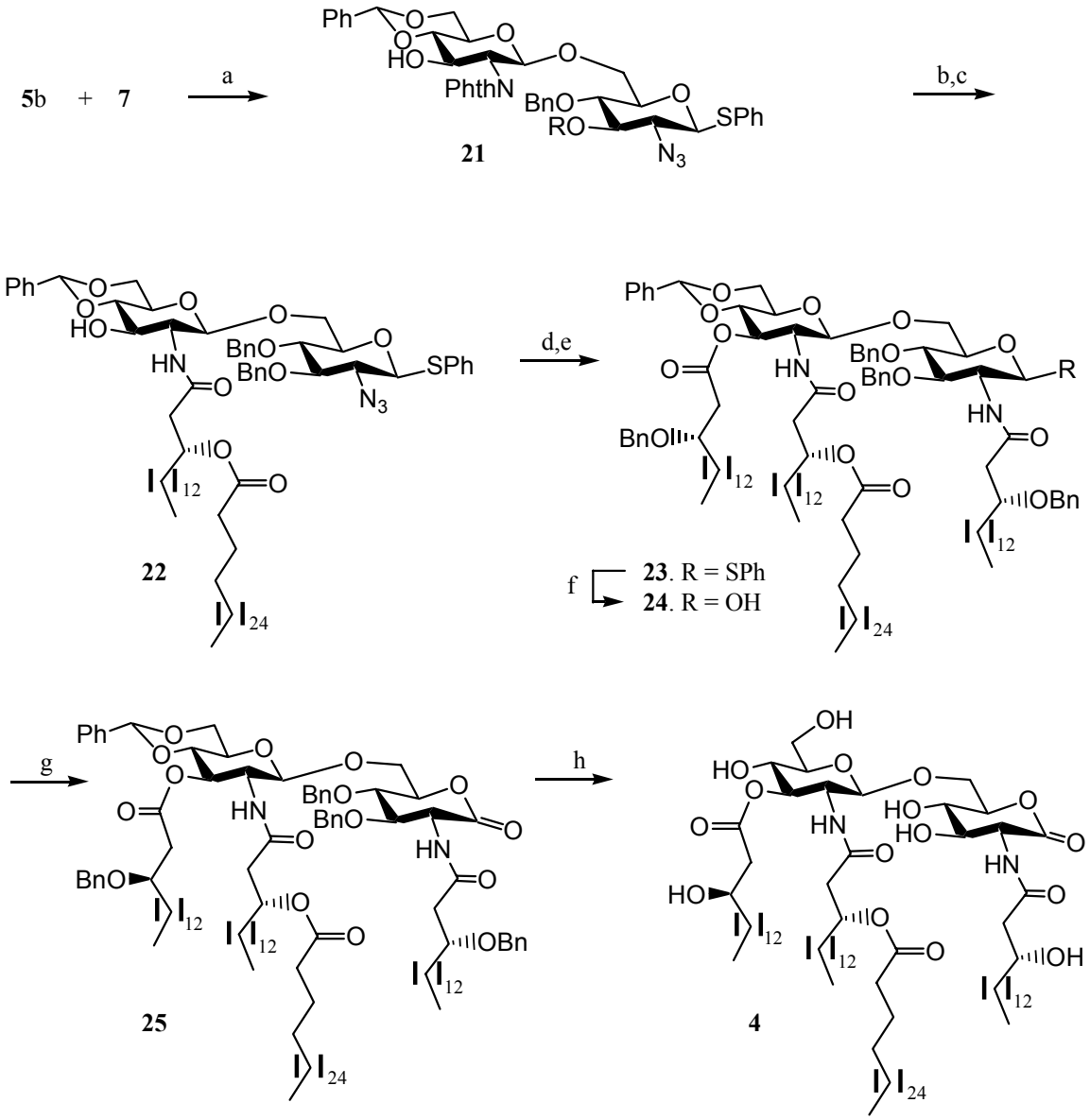


[a]Reagents and conditions: a) NIS, TMSOTf, 4A MS, CH_2Cl_2, -40°C; b) H_2NNH_2, EtOH, Δ, c) DCC, CH_2Cl_2; d) $HS(CH_2)_3SH$, pyridine, Et_3N, H_2O; e) DCC, DMAP, CH_2Cl_2; f) 1M TBAF in THF; g) $(COCl)_2$, DMSO, Et_3N; h) $NaClO_2$, 2-methyl-2-butene, NaH_2PO_4; i) Pd/C, H_2, t-BuOH, THF.

However a low yield of this compound was obtained and the major product was an intermediate aldehyde. PDC/DMF mediated oxidation gave only recovery of the starting material. Fortunately, a two-step procedure involving Swern conditions to give an intermediate aldehyde, which was immediately used without purification in a second oxidation using $NaClO_2$ and sodium dihydrogen phosphate in *t*-butanol to afford **20** in overall 74% yield. Finally, the benzyl ethers and benzylidene acetal of **20** were removed by catalytic hydrogenation over Pd/C to give the target compound **3**.

The preparation of target derivative **4** commenced with a chemoselective glycosylation of selenoglycoside **5b** with thioglycoside **7** using *N*-iodosuccinimate (NIS) / trifluoromethanesulfonic acid (TfOH) as a promoter to give disaccharide **21** (Scheme 3.3). [39,42] Although glycosyl donor **5b** was only partially protected, no products derived from oligomerization were detected. Interestingly, when this donor was used for the glycosylation of **6**, only a low yield of disaccharide was obtained. Apparently, the primary hydroxyl of **7** is significantly more reactive than that of **6**, probably due to its cyclic structure. Disaccharide **21** could be converted into acylated derivative **23** by a similar sequence of reactions as described for **18**. Hydrolysis of the thiophenyl moiety of **23** with NIS and a catalytic amount of TfOH in wet dichloromethane resulted in mixture of **24** and an 1,2-oxazoline derivative. When the above reaction was carried out without TfOH clean hydrolysis was observed to give the **24** in high yield. PCC mediated oxidation of **24** lead, after purification by iatrobeads column chromatography, using ethylacetate/toluene as the eluent, to lactone **25** as a pure compound. Finally, the benzyl ethers and benzylidene acetal of **25** were removed by catalytic hydrogenation of Pd/C to give the first target compound **4**

Scheme 3.3[a]



[a]Reagents and conditions: a) NIS, TMSOTf, 4A MS, CH_2Cl_2, -40°C; b) hydrazine hydrate, ethanol, reflux, c) **15**, DCC, CH_2Cl_2; d) $HS(CH_2)_3SH$, pyridine, Et_3N, H_2O; e) **17**, DCC, DMAP, CH_2Cl_2; f) NIS, aq.THF; g) PCC, MS 3A, CH_2Cl_2; h) Pd/C, H_2, THF, t-BuOH.

Surprisingly, the high-resolution ^1H and spectra of the final compounds **3** and **4** were identical and showed the presence of two derivatives, assigned as a mixture of 2-aminogluconate **3** and 2-aminogluconolactone **4**. Thus, even under neutral conditions, the two compounds equilibrate to the open and closed ring form. Key evidence of the presence of the lactone came

from the chemical shift of H-5 at δ 4.25 ppm, whereas the same proton of the aminogluconate appeared at δ 3.35ppm. The chemical shift of H-4 at 3.54 ppm for both components demonstrates that a 1,4 lactone has not been formed. Integration of H-3' of both compounds indicate that they are present at approximately equal quantities. High resolution MALDI-TOF MS also confirmed the presence of the two compounds.

Biological Evaluation

Compounds **3** and **4** were tested over a wide concentration range for the ability to activate a human monocytic cell line (Mono Mac 6), and to produce TNFα protein. The resulting values were compared with those obtained for *E. coli* and *R. sin-1* LPS, and synthetic compound **2** (Figure 3). Incubation with *E. coli* LPS for 6 hours yielded a clear dose response effect of TNFα production, with maximal supernatant concentrations of TNFα being caused by 10 ng/mL of *E. coli* LPS. The results of these experiments yielded an LPS EC_{50} (concentration producing 50% activity) value of 0.2ng/mL and a Hill slope of 2.5. Neither *R. sin-1* LPS nor synthetic compounds **2**, **3**, and **4** at concentrations up to 10 μg/mL induced significant production of TNFα. To exclude the possibility that any effects observed might be due to the presence of THF, the cells were incubated with concentrations of THF up to 0.5%. These concentrations of THF alone did not cause TNFα production by the cells nor did they alter the response of cells co-incubated with *E. coli* LPS (10 ng/mL) (data not shown).

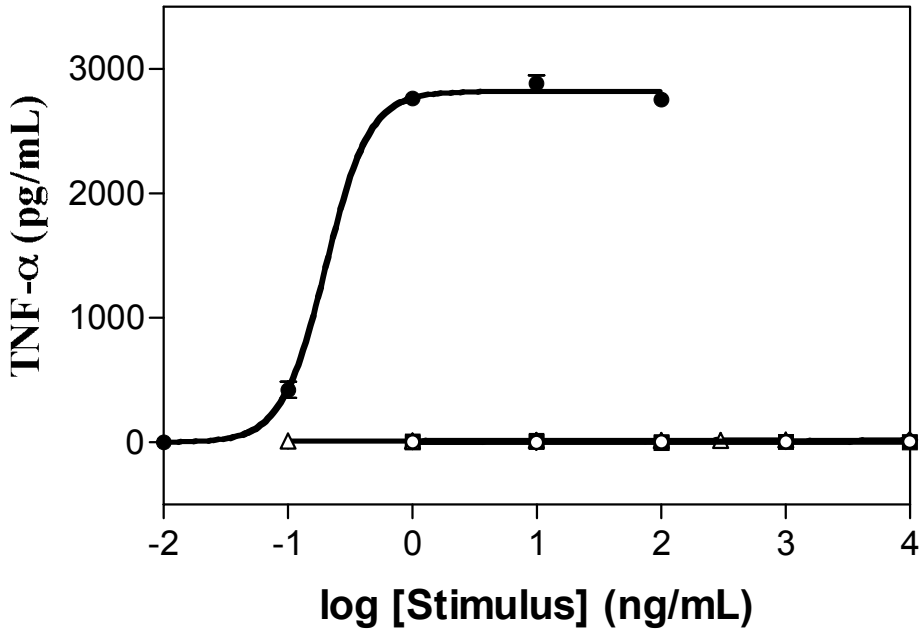


Figure 3.3. Concentration-response curves of *E. coli* LPS, *R. sin-1* LPS, *R. sin*-1 lipid A, and synthetic compounds **2**, **3**, and **4**. Mono Mac 6 cells were incubated for 6 h at 37°C with increasing concentrations of *E. coli* LPS (●), *R. sin-1* LPS (○), *R. sin*-1 lipid A (◆), **2** (Δ), **3** (✎) or **4** (■) as indicated. TNF-α protein in cell supernatants was measured using ELISA. (Please note that *R. sin-1* LPS, *R. sin*-1 lipid A, **2**, **3**, and **4** show background values and therefore overlap in the figure.) Treatment with *E. coli* LPS, *R. sin-1* LPS, *R. sin*-1 lipid A, **2**, **3**, and **4** did not affect cell viability, as judged by cellular exclusion of trypan blue.

Based on their lack of proinflammatory effects, compounds **3** and **4** were tested over a wide concentration range for their ability to antagonize the responses of monocytic cells incubated with *E. coli* LPS (10 ng/mL) (Figure 3.4). At the highest concentration tested, compound **3** and **4** antagonized the effect of *E. coli* LPS by 23%. The synthetic compound **2** and *R. sin-1* LPS were, however, significantly more potent inhibitors with IC_{50} values (concentration producing 50% inhibition) of 13 μmol/mL (7.3 nmol/mL) and 0.21 μg/mL, respectively, whereas **3**/**4** gave an estimated IC_{50} = 330 μg/mL (216 nmol/mL).

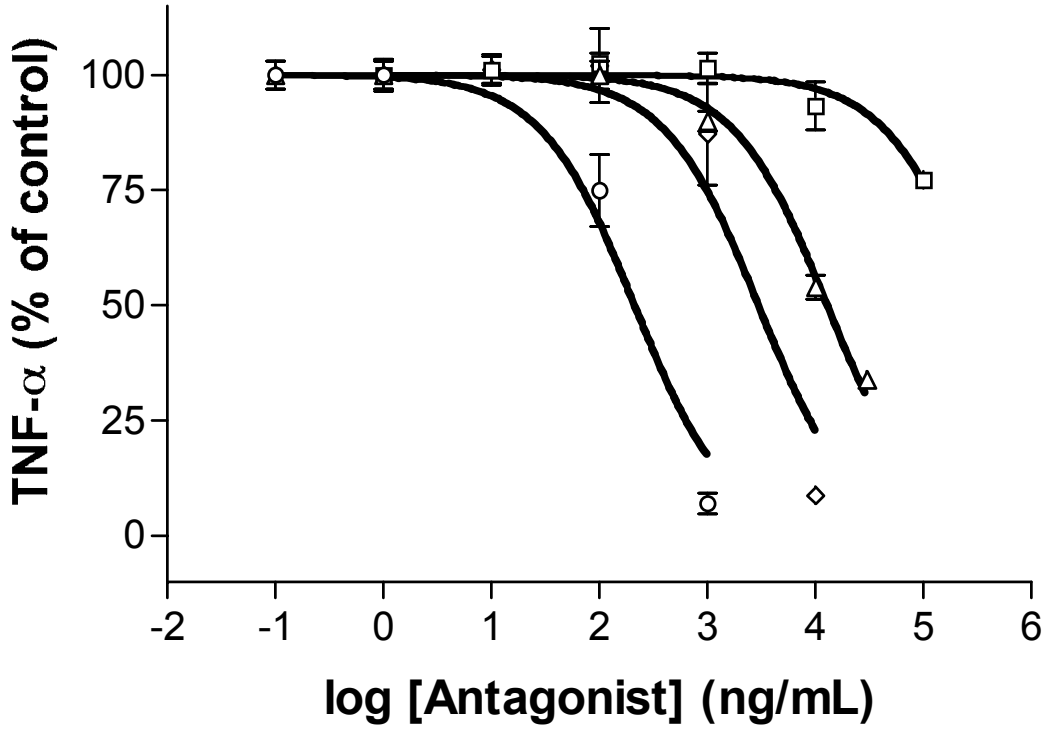


Figure 3.4. Antagonism of *E. coli* LPS by *R. sin*-1 LPS, *R. sin*-1 lipid A, and synthetic compounds **2**, **3**, and **4**. TNF-α concentrations after preincubation of Mono Mac 6 cells with increasing concentrations of *R. sin*-1 LPS (○), *R. sin*-1 lipid A (◆), **2** (Δ), and **3** / **4** (✐) as indicated for 1 h at 37°C, followed by 6 h of incubation with 10 ng/mL *E. coli* LPS. Results are expressed as percentage TNF-α concentration of control cells, which are incubated only with *E. coli* LPS. The effects of **3** and **4** are shown as one curve, since their behavior was exactly the same.

The structure of lipid A of *R. sin-1* shows considerable microheterogeneity.[24] Several major components lack a β-hydroxyl fatty acyl group at C-3. The findings of this study indicate that this fatty acyl moiety is important for antagonistic properties. Although Lipid A derivative **2** is a significantly more potent antagonist than **3** and **4**, it has a sixty fold reduced activity compared to *R. sin-1* LPS. LPS consist of an O-chain polysaccharide and a core oligosaccharide linked to a Lipid A moiety through a dimeric 3-deoxy-D-manno-octulosonic (KDO) moiety. Thus, it may be possible that the KDO residues of the core region contribute to the antagonistic

potential. To explore this possibility, purified *R. sin-1* LPS was subjected to mild acid hydrolysis to cleave the KDO glycosidic linkages. The obtained lipid A preparations were tested for agonistic and antagonist properties. As can be seen in Figure 3.2, the *R. sin-1* lipid A does not stimulate monocytic cells to produce TNFα. However, it could antagonize the effect of *E. coli* LPS with IC_{50} value (3 μg/mL) similar to that of compound **2** (Figure 3.4). Thus, these observations indicate that the oligo(poly)saccharide moiety of LPS contributes to antagonistic potential. In this respect, recent studies[65] have shown that meningococcal lipid A expressed by Meningococci with defects in KDO biosynthesis or transfer has a 10-fold reduction in bioactivity compared to KDO_2 containing *Meningococcal* lipooligosaccharides. Removal of the KDO moieties by mild acidic treatment also dramatically attenuated cellular responses. Thus, it is probable that the cell surface receptors that recognize LPS bind to the lipid A as well as to the KDO moiety of LPS.

The results of previous studies indicate that antagonism of the cell surface receptors that recognize enteric LPS can prevent the production of cytokines.[34] Hence, such compounds have the potential for use as therapeutic interventions for patients with Gram-negative septicemia. Success in this area has been limited and most efforts have been directed towards the synthesis of analogs of lipid A of *R.sphearoides*[18,20] and derivatives of lipid X.[35-37] These compounds, which are either mono- or bis-phosphorylated, may possess metabolic instabilities complicating drug discovery. A unique aspect of our study is that we have identified a lipid A derivative that lacks phosphate and that antagonizes the biological effect of enteric LPS. By comparing the biological responses initiated by the synthetic lipid A derivatives **2**, **3** and **4** and *R. sin-1* LPS and lipid A, the gluconolactone moiety, the β-hydroxyl fatty acyl group at C-3, and the KDO moieties of *R. sin-1* LPS appear to be important for antagonizing enteric LPS-induced cytokine production. The

synthesis of other derivatives to unravel other structural features for optimal importance antagonistic properties is in progress.

Conclusion

Previous studies from our laboratory have shown that LPS of *R. sin 1* can inhibit *E. coli* LPS-dependent synthesis of TNFα by human monocytes. Due to the inherent molecular heterogeneity of lipid A, it cannot be developed as a therapeutic agent for Gram-negative sepsis. Organic synthesis provides an attractive approach for obtaining well-defined derivatives of lipid A of *R. sin-1*. Such compounds will enable us to determine the structural features that account for antagonistic properties. A significant percentage of lipid A derivatives of *R. sin 1* lacks a fatty acyl residue at the C-3 position. Furthermore, it is not known whether the lipid A moiety of R. sin 1 exists as an aminogluconate or 2-aminogluconolactone. In order to address these issues, we have developed highly convergent approaches for the facile synthesis of 2-aminogluconate **3** and 2-aminogluconolactone **4,** both lacking C-3 acylation. A key aspect of the synthesis of **3** was a glycosylation of a properly protected 2-azido-2-deoxy-alditol to give a disaccharide in which the two amino groups and the C-3' hydroxyl could individually be modified with acyl or β-hydroxyl fatty acyl groups. At the end of the synthetic sequence, the C-1 protecting group of the alditol moiety could be selectively removed to give a hydroxyl, which was oxidized to a carboxylic acid using a two-step procedure. 2-Aminogluconolactone **4** could easily be synthesized by a chemoselective glycosylation of a selenoglycosyl donor with a thioglycosyl acceptor to give a disaccharide. The anomeric center, the two amino groups and the C-3' hydroxyls of this compound could individually be modified, thereby giving a flexible route to lipid A analogs.

Detailed NMR and MS analysis of **3** and **4** revealed that even under neutral conditions, the two compounds equilibrate to both forms.

Experimental Procedures

General Synthetic Methods. Column chromatography was performed on silica gel 60 (EM Science, 70-230 mesh), size exclusion column chromatography was performed on Sephadex model LH-20 (*i*-PrOH-DCM or MeOH-CH_2Cl_2, 1/1, v/v elution) or G-25 (water elution) columns. HPLC chromatography was performed on a Prodigy 5*Ì* Silica 100 Å column (250 mm _ 10 mm, CH_2Cl_2-ethyl acetate elution). Reactions were monitored by thin-layer chromatography (TLC) on Kiesel gel 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, $(ClCH_2)_2$, and MeCN were distilled from CaH_2 (twice) and stored over molecular sieves (3Å). Tetrahydrofuran (THF) was distilled from sodium directly prior to the application. MeOH was dried by refluxing with magnesium 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. Optical rotations were measured with a Jasco model P-1020 polarimeter. ^{1}H NMR and ^{13}C NMR spectra were

recorded with Varian spectrometers (models Inova500 and Inova600) equipped with Sun workstations. ^{1}H NMR spectra were recorded in $CDCl_3$ and referenced to residual $CHCl_3$ at 7.24 ppm, and ^{13}C NMR spectra were referenced to the central 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 a subscript L symbol belong to the biantennary lipid at C-2′, whereas signals marked with a subscript L′ symbol belong to the C-28 side chain. Signals marked with a subscript S symbol belong to the mono-antennary lipids at C-2, C-3, and C-3′. Signals marked with an asterisk may be interchangeable.

2-acetamido-4,6-*O*-benzylidene-2-deoxy-D-glucopyranose 9. Freshly distilled benzaldehyde (16.6 mL, 163.3 mmol) was added to *N*-acetyl glucosamine **8** (6.65g, 30.08 mmol) followed by zinc chloride (4.1g, 30.08 mmol). The mixture was sonicated for 2 hours and left stirring overnight. The solid formed was washed with hexanes (3 x 75 mL) followed by water (3 x 75 mL). The filtered solid was washed again with methanol (3 x 50 mL). The white solid thus obtained was dried *in vacuo* to give **9** as a white powder **(**5.4g, 58%). R_f = 0.34 (15% methanol in DCM); ^{1}H NMR (300 MHz, CD_3OD): δ = 7.26 – 7.52 (m, 5H, aromatic), 5.47 (s, 1H, >CHPh), 4.21 (q, J = 5.3 Hz, 1H, H_{6a}), 4.12 (m, 2H, H-2,3), 3.87 (m, 1H, H-5), 3.69-3.54 (m, 4H, H-1a, b, 6b, 4), 1.79 (s, 3H, NHCOCH_3).

2-acetamido-4,6-(R)-*O*-benzylidene-2-deoxy-D-glucitol 10. To a suspension of lactol **9** (5.35g, 17.28 mmol), in methanol (95 mL) at 0° C was added $NaBH_4$ (5.23g, 138.3 mmol) in four equal parts during a period of one hour. The reaction mixture was allowed to stir for 5 hours to give a clear solution. A saturated solution NaH_2PO_4 was added to the reaction mixture until the pH = 7. The mixture was kept in the freezer for 8 hours. The supernatant was decanted and the resulting semi-crystalline mass was washed with MeOH (3 x 75mL). The combined methanolic extracts and supernatant were concentrated under reduced pressure. The solid residue was extracted with

MeOH (4 x 20mL) and the methanolic extracts concentrated to dryness. The resulting white solid was washed with DCM and the residue taken up in methanol. After filtration and evaporation of the methanol pure **10** was obtained (5.22g, 95%): R_f = 0.24 (15% methanol in DCM); ^{1}H NMR (300 MHz, CD_3OD): δ 7.26 – 7.52 (m, 5H, aromatic), 5.47 (s, 1H, >C*H*Ph), 4.21 (q, *J* = 5.3 Hz, 1H, H_{6a}), 4.12 (m, 2H, H-2,3), 3.87 (m, 1H, H-5), 3.69-3.54 (m, 4H, H-1a, b, 6b, 4), 1.79 (s, 3H, NHCOC*H*$_3$). ^{13}C NMR (75 Mhz, CD_3OD): δ 173.1 (NH*C*OCH$_3$*),* 139.0, 129.4, 128.6, 127.1 (Ph), 102.1 (*C*HPh), 83.7 (C-4), 72.2 (C-6), 67.7 (C-3), 62.3 (C-1), 61.6 (C-5), 55.6 (C-2), 22.6 (NHCO*C*H$_3$).

2-azido-4,6-(R)-*O*-benzylidene-2-deoxy-D-glucitol 11. Barium hydroxide octahydrate (6.32 g, 20 mmol) was added to a solution of **10** (2.5g, 8.02 mmol) in a mixture of MeOH/H_2O (1/1, v/v, 60 ml). The reaction mixture was heated under reflux for 14 hours. Sulfuric acid (2.2 mL in 20 mL of H_2O) was added drop wise until the pH=5. The precipitated $BaSO_4$ was removed by centrifugation and filtration. The filtrate was concentrated and then dissolved in minimum amount of MeOH/H_2O (1/1, v/v). The resulting solution was eluted through a column packed with Dowex (550 OH^- resin). The eluant was concentrated under reduced pressure to give a free amine (1.71 g, 79%). Sodium azide (1.14 g, 17.5 mmol) was dissolved in water (3 mL) and cooled to 0°C. Tf_2O in DCM (5 mL) was added and the resulting solution was stirred vigorously for 2 hours. The organic layer was separated and the aq. layer washed with DCM (2 x 5 mL). The combined DCM extracts were washed with a. aqueous satd. $NaHCO_3$ solution. The resulting TfN_3 solution in DCM and catalytic amount of $CuSO_4$ was added to a solution of the free amine (0.47 g, 1.74 mmol) in minimum amount methanol. After stirring the reaction mixture for 2 hours, the solvents were evaporated and the residue coevaporated with toluene (3 x 50mL). The

residue was purified by silica gel column chromatography (50% gradient of ethyl acetate in hexanes) to afford azide **11** (0.47 g, 90%): R_f = 0.65 (100% ethyl acetate); $[\alpha]^{23}_D$ = +38.4^o (c, 1.3, CH_3OH). ^{1}H NMR (300 MHz, CD_3OD): δ 7.31 – 7.55 (m, 5H, aromatic), 5.53 (s, 1H, >C*H*Ph), 4.23 (q, *J* = 5.3 Hz,1H, H_{6a}), 3.99 (d, *J* = 2.5 Hz, 1H, H-3), 3.93 (q, *J* = 4.7 Hz, 1H, H-5), 3.81 (d, *J* = 3.0 Hz, 1H, H-1a), 3.74-3.63 (m, 3H, H-1b, 4, 2), 3.61 (t, 1H, H6b). ^{13}C NMR (75 Mhz, CD_3OD): δ 139.4, 129.8, 129.0, 127.4 (Ph), 102.4 (*C*HPh), 82.7 (C-4), 72.4 (C-6), 70.3 (C-3), 67.9 (C-5), 62.8 (C-1), 61.8 (C-2). HR MS (*m*/*z*) for $C_{13}H_{17}N_3O_5Na$: calcd, 318.1066; found, 318.1006.

2-azido-1-*O*-(t-butyldiphenylsilyl)-2-deoxy-3,5-Di-*O*-benzyl-4,6-*O*-benzylidene-D-glucitol

13. t-Butyldiphenylsilyl chloride (0.3 mL, 1.15 mmol) followed by imidazole (0.157 g, 2.31 mmol) was added to a solution of **11** (0.284 g, 0.963 mmol) in DMF (5 mL). The mixture was stirred at room temperature under an atmosphere of argon. After 12 hours, TLC analysis indicated completion of the reaction. The reaction mixture was diluted with ethyl acetate and hexanes (1/1, v/v, 80 mL) transferred to separatory funnel and washed with ice cold water (2x 25mL). The organic layer was dried ($MgSO_4$) and was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (5% gradient of ethyacetate in hexanes) to afford **12** (0.45 g, 87%). Compound **12** (0.434 g, 0.813 mmol) was dissolved in DMF (6 mL), and sodium hydride (0.078g, 3.25 mmol) added. After stirring at 0**°**C for 30 min., benzyl bromide was added drop-wise. The reaction mixture was allowed warm to room temperature and stirred for 16 hrs. The reaction mixture was diluted with ethyl acetate and hexanes (1:1; 50mL) transferred to separatory funnel and washed with ice-cold water (2x 15mL). The organic layer was dried ($MgSO_4$) and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (3% gradient of ethyacetate in hexanes) to afford **13** (0.401 g, 70%):

R_f = 0.68 (20% ethyl acetate in hexanes); $[\alpha]^{23}_D$ = +18.3° (c, 0.49, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.31 – 7.55 (m, 5H, aromatic), 5.07 (s, 1H, >C*H*Ph), 4.80-4.38(m, 4H, -CH_2Ph), 4.35 (q, 1H, H_{6a}), 4.07 (dd, 1H, H-1a), 3.99 (dd, 1H, H-4), 3.92-3.82 (m, 3H, H-5, 3, H1b), 3.67 (dd, 1H, H-2), 3.52 (t, 1H, H6b), 1.08 (s, 9H, *t*-Bu). ^{13}C NMR (75 Mhz, $CDCl_3$): δ 139.4, 129.8, 129.0, 127.4 (Ph), 102.4 (*C*HPh), 82.7 (C-4), 72.4 (C-6), 70.3 (C-3), 67.9 (C-5), 62.8 (C-1), 61.8 (C-2), 27.0 (-C(*C*H_3)). HR MS (*m*/*z*) for $C_{43}H_{49}N_3O_5SiNa$: calcd, 736.3183; found, 736.3372.

2-azido-1-*O*-(*t*-butyldiphenylsilyl)-2-deoxy-3,4,5-tri-*O*-benzyl-6-*O*-hydroxy-D-glucitol 6: A solution of 1M BH_3 in THF (1.42 mL) was added to the compound **13** (96 mg, 0.134 mmol). After stirring the solution for 10 minutes at 0°C, a solution of 1M Bu_2BOTf in DCM (0.14mL) was added drop-wise. After stirring for another 45 min, TLC analysis showed the completion of the reaction. Triethylamine was added (55 μL) followed by the addition of methanol until the evolution of H_2 gas ceased. The solvents were evaporated under reduced pressure and the residue co-evaporated with methanol (3x 50mL). The residue was purified by silica gel column chromatography (5% ethylacetate gradient in hexanes) to afford acceptor **6** as an oil (83 mg, 85%): R_f = 0.40 (ethyl acetate/toluene, 1:3); $[\alpha]^{25}_D$ = -134.2° (c, 0.64, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.64 – 7.16 (m, 25H, aromatic), 4.51-4.76 (6H, 3 x CH_2Ph), 3.94 (dd, *J* = 5.3, 5.7 Hz, 1H, H4), 3.74-3.84 (m, 4H, H6a, 6b, 2, 1a, H3), 3.6(q, *J* = 4.4 Hz, 1H, H5), 3.56(q, *J* = 4.9 Hz, 1H, H1b), 2.11 (t, 1H, OH), 1.06 (s, 9H, *t*-Bu). ^{13}C NMR (75 Mhz, CD_3OD): δ 127.9-138.3 (Ph), 79.8 (C-5), 79.5 (C-4), 78.5 (C-3), 75.0, 74.9, 71.9 (3 x *C*H_2Ph), 64.0 (C-1), 63.9 (C-2), 61.0 (C-6), 27.0 (C(*C*H_3)). HR MS (*m*/*z*) for $C_{43}H_{49}N_3O_5SiNa$: calcd, 736.3183; found, 736.3372.

1-*O*-(t-butyldiphenylsilyl)- 3-*O*-acetyl-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranosyl) 2-azido-2-deoxy-3,4,5-tri-*O*-benzyl-6-*O*-hydroxy-D-glucitol 14: A suspension of glycosyl donor **5a** (0.443 g, 0.767 mmol) and acceptor **6** (0.457 g, 0.639 mmol) in DCM (20 mL) and 4Å molecular sieves was stirred under and atmosphere of argon for 2 hours. The mixture was cooled to -35°C followed by the addition of NIS (171 mg, 0.767 mmol) and TMSOTf (~7 μL). The reaction mixture was stirred for 1 hour during which the temperature was gradually raised to 0° C. The reaction was quenched by the addition of pyridine (0.15 mL). The reaction mixture was diluted with DCM (35 mL), the molecular sieves removed by filtration through a pad of celite and washed with DCM (3x 50 mL). The combined filtrates was washed with 10% $Na_2S_2O_3$ (2 x 20 mL) and water (2 x 20 mL). The organic phase was dried ($MgSO_4$), filtered and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (5% gradient of ethyl acetate in hexane) to afforded **14** as an oil (0.651 g, 90%): R_f = 0.57 (40% ethyl acetate in hexane); $[\alpha]^{23}_D$ = -6.1° (c, 2.7, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 6.97 – 7.73 (m, 34H, aromatic), 5.92 (dd, *J* = 9.6, 9.3 Hz, 1H, H-3′), 5.53 (s, 1H, >CHPh), 5.51 (d, *J* = 8.5 Hz, 1H, H-1′), 4.23-4.62 (m, 9H, 3 x C*H*$_2$Ph, H6a′, 2′, H6a), 3.60-3.81 (m, 9H, H-4′, 5′, 6b′, 6b, 1a, 2, 3, 4, 5), 3.50 (m, 1H, H1b), 1.91 (s, 3H, COC*H*$_3$), 1.04 (s, 9H, *t*-Bu)). ^{13}C NMR (75 Mhz, $CDCl_3$): δ = 170.4 (*C*OCH_3), (138.3-123.8, Ph), 102.4 (*C*HPh), 82.7 (C-4), 72.4 (C-6), 70.3 (C-3), 67.9 (C-5), 62.8 (C-1), 61.8 (C-2). HR MS (*m*/*z*) for $C_{66}H_{68}N_{412}SiONa$: calcd, 1160.3412; found, 1160.7328.

1-*O*-(t-butyldiphenylsilyl)-6-*O*-{4,6-*O*-benzylidene-2-deoxy-2-[(*R*)-3-octacosanoyloxy hexadecan]amido-β-D-glucopyranosyl}2-azido-2-deoxy-3,4,5-tri-*O*-benzyl-D-glucitol 16: Hydrazine hydrate (0.34 mL, 6.84 mmol) was added to a solution of **14** (0.389 g, 0.342 mmol) in

ethanol (15 mL)). After stirring at 90°C for 15 h, the reaction mixture was cooled and concentrated under reduced. The residue was coevaporated from toluene (3x 25 mL) after which it was purified by silica gel column chromatography (35% ethyl acetate gradient in hexanes) to afford an amine (0.274g, 0.283mmol). Dicyclohexylcarbodiamide (DCC) (0.05g, 0.239mmol) was added to a solution of **15** (0.163g, 0.239mmol) in DCM (5 mL) and stirred for 10 min, followed by the addition of the above described amine (0.193 g, 0.20 mmol) in DCM (10 mL). The reaction mixture was stirred for 16 h at room temperature. The solids were filtered off, and the residue was washed with DCM (2x 10 mL). The combined filtrate was concentrated under reduced pressure. and the residue purified by silica gel column chromatography (10% gradient ethyl acetate in hexanes) to afford **16** as a white solid (0.23 g, 71%): R_f = 0.57 (30% ethyl acetate in hexanes); $[\alpha]^{23}_D$ = +21.3^o (c, 0.60, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.64 – 7.14 (m, 30H, aromatic), 5.98 (d, 1H, *J* = 5.8 Hz, NH), 5.53 (s, 1H, >C*H*Ph), 5.05 (m, 1H, H-3$_L$), 4.71 (d, *J* = 8.3 Hz, 1H, H-1′), 4.25 (dd, 1H, *J* = 5.8, 4.9 Hz H-6a′), 4.17 (d, 1H, *J* = 9.3 Hz, H-6a), 4.08 (t, 1H, *J* = 9.3 Hz, H-3′), 3.92 (m, 1H, H-5), 3.67- 3.79 (m, 6H, H-6a, 1a, 6b′, 4, 3, 2), 3.50-3.56 (m, 3H, H-2′, 4′, 1b), 3.45 (m, 1H, H-5). ^{13}C NMR (75 Mhz, $CDCl_3$): δ 174.4 (NH*C*OCH_3), 171.5 (*C*OCH_3), (138.4-126.6, Ph), 102.1 (*C*HPh), 101.5 (C-1$^/$), 81.7 (C-4$^/$), 79.8 (C-5), 79.5 (C-4), 78.4 (C-3), 75.1-72.4 (3 x *C*H_2Ph), 71.5 (C-3$^/$), 71.4 (C-3L), (C-6), 70.4 (C-3), 68.8 (C-6$^/$), 66.6 (C-5$^/$), 63.9 (C-1, 2), 59.4 (C-2$^/$). HRMS (*m*/*z*) for $C_{100}H_{148}N_4O_{12}SiNa$: calcd, 1649.3401; found, 1649.3158.

1-*O*-(t-butyldiphenylsilyl)-6-*O*-{4,6-*O*-benzylidene-3-*O*-[(*R*)-3-benzyloxy hexadecanoyl]-2-deoxy-2-[(*R*)-3-octacosanoyloxy-hexadecan]-amido-β-D glucopyranosyl}-2-[(*R*)-3-benzyloxy-hexadecan]-amido-3,4,5-tri-*O*-benzyl-D-glucitol 18: A solution of **16** (210 mg,

0.123 mmol) in 1,3-propanedithiol (0.25 mL, 2.46 mmol), pyridine (8.7 mL) and H_2O (1.25 mL) was stirred for 16 h at room temperature. The reaction mixture was concentrated *in vacuo* and the residue coevaporated with toluene (2x 5 mL) and ethanol (2x 5 mL). The residue was purified by silica gel column chromatography (2% gradient methanol in DCM) to afford a free amine as a colorless syrup (189 mg, 95%). DCC (54 mg, 0.261 mmol) and DMAP (20 mg, 0.163 mmol) were added to a stirred solution of **17** (89 mg, 0.244 mmol) in DCM (10 mL). After stirring for 10 min, the amine (131 mg, 0.081 mmol) in DCM (1.5 mL) was added. The reaction mixture was stirred for 16 h at room temperature, after which the solids were filtered off, and the residue washed with DCM (2x 10 mL). The combined filtrates were concentrated *in vacuo* and the residue purified by silica gel column chromatography (3% gradient ethyl acetate in DCM) to afford **18** as a white solid (142 mg, 71%): R_f = 0.43 (20% ethyl acetate in hexanes); $[\alpha]^{23}_D$ = +12.5^o (c, 0.8, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.64 – 7.14 (m, 35H, aromatic), 6.23 (d, 1H, J = 8.3 Hz, NH), 5.78 (d, 1H, NH$^{/}$), 5.39 (s, 1H, >C*H*Ph), 5.38 (t, 1H, J = 9.3 Hz, H-3′), 4.98 (m, 1H, H-3$_L$), 4.74 (1H, H-1′), 4.73 – 4.31 (m, 10H, 5 x C*H*$_2$Ph), 4.29 – 4.26 (m, 2H, H-6a′, 2), 4.13 (d, 1H, J = 9.8 Hz, H-6a), 4.02 (d, 1H, J = 7.8 Hz, H-3), 3.92 – 3.85 (m, 3H, H-5, 6b, 2$^{/}$), 3.78 (m, 2H, H-4), 3.67-3.59 (m, 3H, H-6b′, 4′, 1b), 3.52 (dd, 1H, J = 9.8, 9.3 Hz, H6a), 3.45 (m, 1H, H-5). ^{13}C NMR (75 Mhz, $CDCl_3$): δ 174.4, 172.8 (NH*C*OCH$_3$), 171.5, 170.2 (*C*OCH$_3$), (138.4-126.6, Ph), 102.1 (*C*HPh), 101.6 (C-1$^{/}$), 82.0 (C-4$^{/}$), 79.7 (C-5), 79.3 (C-4), 78.4 (C-3), 76.2-72.3 (5 x *C*H$_2$Ph), 71.8 (C-3$^{/}$), 71.4 (C-3L), 70.8 (C-6), 70.7 (C-3), 68.9 (C-6$^{/}$), 66.5 (C-5$^{/}$), 62.6 (C-1), 55.4 (C-2), 51.5 (C-2$^{/}$). HRMS (*m*/*z*) for $C_{146}H_{222}N_2O_{16}SiNa$: calcd, 2312.1251; found, 2312.1257.

1-*O*-hydroxy-6-O-{4,6-*O*-benzylidene-3-*O*-[(*R*)-3-benzyloxy-hexadecanoyl]-2-deoxy-2-[(*R*)-3-octacosanoyloxy-hexadecan]-amido-β-D-glucopyranosyl}-2-[(*R*)-3-benzyloxy-hexadecan]-amido-3,4,5-tri-*O*-benzyl-D-glucitol 19: 1M TBAF in THF (40 μL, 0.039 mmol) was added to a solution of **18** (76 mg, 0.033 mmol) in THF (2 mL). After stirring for 30 min, TLC analysis indicated the completion of the reaction. The solvent was evaporated *in vacuo* and the oily residue was subjected to purification by silica gel column chromatography (10% gradient of ethylacetate gradient in hexanes) to afford **19** as a fluffy white solid (62 mg, 91%): R_f = 0.38 (30% ethyl acetate in hexanes); $[\alpha]^{25}_D$ = +5.0^o (c, 0.7, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.18 – 7.41 (m, 30H, aromatic), 6.38 (d, 1H, *J* = 8.8 Hz, NH), 6.23 (d, 1H, *J* = 7.32 Hz, NH$^/$), 5.43 (s, 1H, >C*H*Ph), 5.26 (dd, 1H, *J* = 9.3, 8.8 Hz, H-3$^/$), 5.10 (m, 1H, H-3L), 4.52 (m, 1H, H-1$^/$), 4.77 (dd, 1H, *J* = 9.8, 9.3 Hz H-2), 4.83 – 4.35 (m, 10H, 5 x C*H*$_2$Ph), 4.44 (m, 1H, H-5), 4.37-4.32 (m, 2H, H-6a, 6a$^/$), 4.16 (q, 1H, *J* = 7.8, Hz, H-2$^/$), 4.03-3.99 (m, 2H, H-3$^/$, 2), 3.91-3.86 (m, 2H, H-5$^/$, 4$^/$), 3.76 (m, 1H, H-6b), 3.68 (dd, 1H, *J* = 9.1, 9.8 Hz, H-4), 3.58-3.55 (m, 2H, H-6b$^/$, 1a), 3.51-3.47 (m, 2H, H-5, 1b). ^{13}C NMR (75 Mhz, $CDCl_3$): δ 174.2, 171.8 (NH*C*OCH_3), 171.3, 171.2 (*C*OCH_3), 139.5-126.4 (Ph), 102.3 (*C*HPh), 101.7 (C-1$^/$), 82.7, 79.1 (C-5), 78.8 (C-4$^/$), 77.9 (C-3), 76.8 (C-4), 75.7-70.8 (5 x *C*H_2Ph), 71.4 (C-3L), 71.1 (C-3$^/$), 68.8 (C-6), 68.1 (C-6$^/$), 66.8 (C-5$^/$), 62.9, 54.6 (C-2$^/$), 51.9 (C-2). HR MS (*m*/*z*) for $C_{130}H_{204}N_2O_{16}Na$: calcd, 2073.6311; found, 2073.6309.

1-*O*-carboxylicacid-6-*O*-{4,6-*O*-benzylidene-3-*O*-[(*R*)-3-benzyloxy-hexadecanoyl]-2-deoxy-2-[(*R*)-3-octacosanoyloxy-hexadecan]-amido-β-D-glucopyranosyl}-2-[(*R*)-3-benzyloxy-hexadecan]-amido-3,4,5-tri-*O*-benzyl-D-gluconic acid 20. Oxalyl chloride (12μL, 0.136 mmol) was to CH_2Cl_2 (1 mL) cooled to -40 °C under an atmosphere of argon. DMSO (12μL,

0.168 mmol) was added, and the mixture was stirred for 2 min at -40°C. Compound **19** (36 mg, 17.47 μmol) CH_2Cl_2 (1 mL) added drop wise *via* a syringe. Et_3N (50μL, 0.288 mmol) was added after stirring the reaction mixture for 30 min at -40°C. The reaction was allowed to warm to room temperature and stirred for 1 hr. The reaction mixture was then diluted with CH_2Cl_2 (15 mL) and washed with saturated aqueous NH_4Cl solution, and then concentrated under reduced pressure. The residue was dissolved in THF (0.5mL) and $NaClO_2$ (5 mg, 50 μmol), NaH_2PO_4 (3 mg, 2μmol), and 2-methyl-2-butene (110 μL of 2M in THF) in *t*-BuOH (1 mL) and water (0.2 mL) added. After stirring vigorously for 3 h, the reaction mixture was concentrated under reduced pressure, and the residue was purified by silica gel column chromatography (1% MeOH gradient in DCM) to **20** (26 mg, 74%): R_f = 0.61 (10% methanol in DCM); $[\alpha]^{23}_D$ = -9.34^o (c, 0.6, CHCl3). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.18 – 7.41 (m, 30H, aromatic), 7.14 (d, 1H, *J* = 8.8 Hz, NH), 6.36 (d, 1H, *J* = 7.32 Hz, NH$^{/}$), 5.46-5.42 (m, 2H, >C*H*Ph, H-1$^{/}$), 5.26 (dd, 1H, *J* = 9.3, 8.8 Hz, H-3$^{/}$), 5.10 (m, 1H, H-3L), 4.52 (m, 1H, H-1$^{/}$), 4.77 (dd, 1H, *J* = 9.8, 9.3 Hz H-2), 4.83 – 4.35 (m, 10H, 5 x C*H*$_2$Ph), 4.44 (m, 1H, H-5), 4.37-4.32 (m, 2H, H-6a, 6a$^{/}$), 4.16 (q, 1H, *J* = 7.8, Hz, H-2$^{/}$), 4.03-3.99 (m, 2H, H-3$^{/}$, 2), 3.91-3.86 (m, 2H, H-5$^{/}$, 4$^{/}$), 3.76 (m, 1H, H-6b), 3.68 (dd, 1H, *J* = 9.1, 9.8 Hz, H-4), 3.58-3.55 (m, 2H, H-6b$^{/}$, 1a), 3.51-3.47 (m, 2H, H-5, 1b). ^{13}C NMR (75 Mhz, $CDCl_3$): δ 174.2, 171.8 (NH*C*OCH$_3$), 171.3, 171.2 (*C*OCH$_3$), 139.5-126.4 (Ph), 102.3 (*C*HPh), 101.7 (C-1$^{/}$), 82.7, 79.1 (C-5), 78.8 (C-4$^{/}$), 77.9 (C-3), 76.8 (C-4), 75.7-70.8 (5 x *C*H$_2$Ph), 71.4 (C-3L), 71.1 (C-3$^{/}$), 68.8 (C-6), 68.1 (C-6$^{/}$), 66.8 (C-5$^{/}$), 62.9, 54.6 (C-2$^{/}$), 51.9 (C-2). HR MS (*m*/*z*) for $C_{130}H_{202}N_2O_{17}Na$: calcd, 2087.5119; found, 2087.5149.

Debenzylation of compound 20. Pd/C (5 mg) was added to a solution of hydroxy acid **20** (15 mg, 7.26 μmol) was dissolved in a mixture of THF - *t*-BuOH (2 mL, 1/1, v/v). The mixture was

placed under an atmosphere of stirred H_2 and stirred for 24 h at room temperature. The catalyst then was filtered off through a pad of celite and washed subsequently with THF (2x 3 mL) and DCM (2x 3 mL). The combined filtrates were concentrated under reduced pressure and the residue was purified by size exclusion column chromatography using Sephadex LH-20 (*i*-PrOH/DCM) to afford a mixture of **3** and **4** (5 mg, 67%) R_f = 0.41 (methanol:DCM:NH_4OH 15:80:5); ^{1}H NMR (500 MHz, d-THF/MeOD 1:1, v/v): δ = 7.02 (d, 1H, *J* = 8.8 Hz, NH), 6.84 (d, 1H, *J* = 7.32 Hz, NH$^{/}$) Aminogluconate: 5.05 (t, 1H, *J* = 10.4, 9.3 Hz, H-3$^{/}$), 4.71 (d, *J* = 3.6, H-2), 4.54 (d, 1H, *J* = 8.3 Hz, H-1$^{/}$), 4.32 (1H, H-3), 3.82 (1H, H-2$^{/}$), 3.86 (1H, H-6a$^{/}$), 3.70-3.73(1H, H-6a, 6b$^{/}$), 3.55-3.53 (2H, H-4$^{/}$, 6b), 3.54 (1H, H-4), 3.35-3.32 (1H, H-5, 5$^{/}$). HR MS (*m*/*z*) for $C_{88}H_{168}N_2O_{17}Na$: calcd, 1548.2811; found, 1548.2816. Aminogluconolactone: 5.01 (t, 1H, *J* = 10.3, 9.3 Hz, H-3$^{/}$), 4.66 (d, 1H, *J* = 8.3 Hz, H-1$^{/}$), 4.25 (1H, H-2, 5), 3.89 (1H, H-6a), 3.86 (1H, H-2$^{/}$**,** 6a$^{/}$), 3.69 (1H, 6b), 3.54-3.55(1H, H-4, 4$^{/}$), 3.32 (1H, H-5). HR MS (*m*/*z*) for $C_{88}H_{166}N_2O_{16}Na$: calcd, 1530.2135; found, 1530.2786.

Phenyl 3-*O*-benzyl-6-*O*-(3-*O*-hydroxy-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-2-azido-4-*O*-benzyl-2-deoxy-1-thio-β-D-glucopyranoside 21: A mixture of donor **5b** (0.624g, 1.16 mmol) and acceptor **7** (0.463g, 0.97mmol) and activated molecular sieves (4 A, 1.2 g) in DCM (15 mL) was stirred under an atmosphere of argon for 1 hour. The mixture was cooled (-35°C) and NIS (0.26g, 1.16 mmol) and TfOH (~10 μL) added. The reaction mixture was allowed to warm-up to -10°C. After stirring for 45 min, TLC analysis indicated completion of the reaction. The reaction was quenched with pyridine (0.2 mL) and diluted with DCM (50 mL). The molecular sieves were removed by filtration and washed with DCM (3 x 10 mL). The combined filtrates were washed with 10% $Na_2S_2O_3$ (2 x 35 mL) and water (2 x 35

mL). The organic phase was dried ($MgSO_4$) and filtered and the filtrate concentrated *in vacuo*. The residue was purified by silica gel column chromatography (5% gradient of ethyl acetate in toluene), yp afford **21** as a white fluffy solid (0.683 g, 80%): R_f = 0.55 (35% ethyl acetate in hexane); $[\alpha]^{23}_D$ = +36.3^o (c, 2.5, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.08 – 7.78 (m, 24H, aromatic), 5.59 (s, 1H, >CHPh), 5.41 (d, 1H, *J* = 8.79 Hz, H-1′), 4.66 (t, 1H, *J* = 7.82, 8.3 Hz, H-3′), 4.39 (dd, 1H, *J* = 6.35, 1.96 Hz, H-6a′), 4.34 (dd, 1H, *J* = 8.3, 10.26 Hz, H-2′), 4.27 (d, 1H, *J* = 9.77 Hz, H-1), 4.78-4.28 (m, 4H, 2 x C*H*$_2$Ph), 4.11 (dd, 1H, *J* = 1.46, 9.28 Hz, H-6a), 3.85 (dd, 1H, *J* = 10.25, 1.96 Hz, H-6b′), 3.71-3.65 (m, 3H, H6b, 4′, 5′), 3.40-3.36 (m, 2H, H3, 5), 3.28 (dd, 1H, *J* = 9.28, 9.76 Hz, H-4), 3.20 (t, *J* = 9.77 Hz, H-2). ^{13}C NMR (75 Mhz, $CDCl_3$): δ 137.7-123.7 (Ph), 102.2 (*C*HPh), 98.9 (C-1′), 85.1 (C-1), 85.0 (C-5), 82.5 (C-4′), 78.4 (C-3), 77.2 (C-4), 76.0 (*C*H_2Ph), 75.0 (*C*H_2Ph), 68.9 (C-3′, C-6′), 68.2 (C-6), 66.4 (C-5′), 64.9 (C-2), 56.6 (C-2′). HR MS (*m*/*z*) for $C_{47}H_{44}N_4O_{10}SNa$: calcd, 879.2676; found, 873.2520.

Phenyl 2-Azido-4-*O*-benzyl-6-*O*-{4,6-*O*-benzylidene-2-deoxy-2-[(*R*)-3 octacosanoyloxy-hexadecan]amido-β-D-glucopyranosyl}-2-deoxy-3-*O*-benzyl-1-thio-β-D glucopyranoside 22: Hydrazine hydrate (0.30 mL, 6.84 mmol) was added to a solution of **21** (0.273 g, 0.304mmol) in ethanol (15 mL). The reaction mixture was stirred at 90°C for 12 h. After cooling to room temperature, the mixture was concentrated to dryness and the residue coevaporated from toluene (2x 25 mL). The residue was purified by silica gel column chromatography (35% ethyl acetate gradient in hexanes) to afford a free amine (0.195 g, 88%). Dicyclohexylcarbodiamide (DCC) (0.10 g, 0.484 mmol) was added to a solution of **15** (290 mg, 0.431mmol) in DCM (6 mL). After stirring for 10 min, the amine (195 mg, 0.267 mmol) in DCM (4 mL) was added. The reaction mixture was stirred for 16 h, after which the solids were filtered off, and the residue washed with

DCM (2x 10 mL). The combined filtrates were concentrated *in vacuo* and the residue was purified by silica gel column chromatography (10% gradient ethyl acetate in toluene) to afford **22** as a white solid (0.237 g, 65%): R_f = 0.74 (25% ethyl acetate in toluene); $[\alpha]^{23}_{D}$ = +10.65^{o} (c, 0.9, $CHCl_3$). δ 7.26–7.52 (m, 15H, aromatic), 5.75 (d, 1H, *J* = 6.3 Hz, NH), 5.55 (d, 1H, *J* = 5.3 Hz, H-1), 5.50 (s, 1H, >C*H*Ph), 4.96 (m, 1H, H-3_L), 4.76 (dd, 2H, *J* = 11.2 Hz, CH_2Ph), 4.66 (d, 1H, *J* = 8.3 Hz, H-1'), 4.34-4.38 (m, 1H, H-5), 4.28 (dd, 1H, *J* = 4.9, 10.3 Hz, H-6'a), 3.92-4.11 (m, 3H, H-3,3',6a), 3.70-3.82 (m, 3H, H-2,6b,6b'), 3.39-3.53 (m, 4H, H-2',4,4',5'), ^{13}C NMR: δ 127.00-133.00 (Ph), 102.63 (>*C*HPh), 101.50 (C-1'), 87.83 (C-1), 82.00 (C-4'), 79.11 (C-4), 75.41 (*C*H_2Ph), 74.92 (C-3), 72.03 (C-3_L), 71.13 (C-5,3'), 69.24 (C-6a), 68.78 (C-6'), 67.08 (C-5'), 64.69 (C-2), 59.28 (C-2'). HR MS (*m*/*z*) for $C_{83}H_{126}N_4O_{11}SNa$: calcd, 1410.0321; found, 1410.0608.

Phenyl 4-*O*-Benzyl-6-*O*-{4,6-*O*-benzylidene-3-*O*-[(*R*)-3-benzyloxy-hexadecanoyl]-2-deoxy-2-[(*R*)-3-octacosanoyloxy-hexadecan]-amido-β-D glucopyranosyl}-2-[(*R*)-3-benzyloxy-hexadecan]-amido-3-*O*-benzyl-2-deoxy-1-thio-α-D-glucopyranoside 23: A solution of **22** (230 mg, 0.165 mmol) in 1,3-propanedithiol (0.35 mL, 3.32 mmol), pyridine (12 mL) and H_2O (1.8 mL) was stirred for 16 h at room temperature. The mixture was concentrated *in vacuo* and the residue coevaporated with toluene (2x 5 mL) and ethanol (2x 5 mL). The residue was purified by silica gel column chromatography (2% gradient methanol in DCM) to afford a free amine as a colorless syrup (193 mg, 86%). DCC (48 mg, 0.232 mmol) and DMAP (6 mg, 0.046 mmol) were added to a stirred solution of **17** (56 mg, 0.155 mmol) in DCM (5 mL). After stirring for 10 min, the amine (53 mg, 0.039 mmol) in DCM (1.5 mL) was added. The reaction mixture was stirred for 16 h at room temperature, after which the solids were filtered off, and the residue

washed with DCM (2x 5 mL). The combined filtrates were concentrated *in vacuo* and the residue purified by silica gel column chromatography (3% gradient ethyl acetate in toluene) to afford **23** as a white fluffy solid (51 mg, 64%): R_f = 0.68 (10% ethyl acetate in toluene); $[\alpha]^{23}_D$ = -3.8^o (c, 0.52, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.18 – 7.58 (m, 30H, aromatic), 6.62 (d, 1H, *J* = 8.31 Hz, NH), 5.41 (d, 2H, >C*H*Ph, NH$^{/}$), 5.25 (t, 1H, *J* = 9.77 Hz, H-3$^{/}$), 4.98 (m, 1H, H-3L), 4.85 (d, 1H, *J* = 8.79 Hz, H-1), 4.71 (m, 3H, H-1$^{/}$, C*H*$_2$Ph), 4.65 – 4.36 (m, 6H, 3 x C*H*$_2$Ph), 4.32 (dd, 1H, *J* = 4.8, 5.8 Hz, H-6a$^{/}$), 4.02 (d, 1H, *J* = 11.23 Hz, H-6a), 3.85-3.68 (m, 5H, H-2$^{/}$, 2, 6b, 3, 6a$^{/}$), 3.63 (t, 1H, *J* = 9.28 Hz, H-4$^{/}$), 3.50 (dd, 1H, *J* = 8.8, 7.9 Hz, H-5), 3.41 (m, 1H, H-5$^{/}$), 3.33 (t, 1H, *J* = 8.8, 9.3 Hz H-4. ^{13}C NMR (75 MHz, $CDCl_3$): δ 137.7-123.7 (Ph), 101.5 (*C*HPh), 101.4 (C-1$^{/}$), 85.9 (C-1), 82.9, 79.7 (C-5), 79.1 (C-4$^{/}$), 78.5 (C-4), 78.1 (C-3), 74.8-70.9 (4 x *C*H$_2$Ph), 71.5 (C-3L), 68.4 (C-6$^{/}$), 68.2 (C-6), 66.0 (C-5$^{/}$), 54.8 (C-2), 54.6 (C-2$^{/}$). HRMS (*m*/*z*) for $C_{129}H_{200}N_2O_{15}SNa$: calcd, 2073.7321; found, 2073.7354.

4-*O*-Benzyl-6-*O*-{4,6-*O*-benzylidene-3-*O*-[(*R*)-3-benzyloxy-hexadecanoyl]-2-deoxy-2-[(*R*)-3-octacosanoyloxy-hexadecan]amido-β-D-glucopyranosyl}-2-[(*R*)-3-benzyloxy-hexadecan]-amido-3-*O*-benzyl-2-deoxy-D-glucono-1,5-lactone 25. *N*-Iodosuccinimide (14 mg, 62 μmol) was added to a stirred solution of **23** (26 mg, 12.4 μmol) in THF/H_2O (3 mL, 10/1, v/v) at room temperature. The reaction mixture was vigorously stirred for 16 h until TLC analysis indicated that the reaction had gone to completion. The reaction mixture was diluted with DCM (15 mL) and washed with aqueous $Na_2S_2O_3$ (15%, 5 mL) and water (3x 5 mL). The organic phase was dried ($MgSO_4$) and filtered, and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel chromatography (1% gradient MeOH in DCM) to afford the lactol **24** (17 mg, 8.68μmol). A suspension of lactol **24** (14.0 mg, 7.1 μmol) and activated molecular sieves (3Å,

25 mg) in DCM (2 mL) and was stirred for 1 h at room temperature under an atmosphere of argon. Pyridinium chlorochromate (8.1 mg, 37 μmol) was then added and the reaction mixture was stirred for another 1 hr until TLC analysis indicated completion of the reaction. The reaction mixture was placed onto a column of iatrobeads, and eluted with EtOAc/toluene (1:1) to afford the lactone **25** as a colorless film (11.3 mg, 81%): R_f = 0.66 (25% ethyl acetate in toluene); $[\alpha]^{25}_D$ = -13.2^o (c, 0.3, CHCl3). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.18 – 7.38 (m, 25H, aromatic), 7.02 (d, 1H, *J* = 8.8 Hz, NH), 6.84 (d, 1H, *J* = 7.32 Hz, NH$^{/}$), 5.70 (dd, 1H, *J* = 9.3, 8.8 Hz, H-3$^{/}$), 5.39 (s, 1H, >C*H*Ph), 5.08 (m, 1H, H-3L), 5.04 (d, 1H, *J* = 8.3 Hz, H-1$^{/}$), 4.77 (dd, 1H, *J* = 9.8, 9.3 Hz H-2), 4.73 – 4.37 (m, 8H, 4 x CH_2Ph), 4.44 (m, 1H, H-5), 4.28 (m, 1H, H-6a$^{/}$), 4.11 (d, 1H, *J* = 10.7 Hz, H-6a), 3.95 (t, 1H, *J* = 7.3 Hz, H-4) 3.71 (m, 2H, H-3, 6b$^{/}$), 3.57 – 3.52 (m, 3H, H-5$^{/}$, 6b, 4$^{/}$), 3.46 (q, 1H, *J* = 8.3, 9.3 Hz, H-2$^{/}$). ^{13}C NMR (75 Mhz, $CDCl_3$): δ 137.7-123.7 (Ph), 101.7 (*C*HPh), 101.0 (C-1$^{/}$), 79.7 (C-5), 79.1 (C-4$^{/}$, 3), 78.0 (C-4), 74.1-70.9 (4 x *C*H_2Ph), 70.5 (C-3L), 70.3 (C-3$^{/}$), 69.1 (C-6), 68.5 (C-6$^{/}$), 66.0 (C-5$^{/}$), 56.5 (C-2$^{/}$), 52.9 (C-2). HR MS (*m*/*z*) for $C_{123}H_{194}N_2O_{16}Na$: calcd, 1979.8501; found, 1979.8321.

Hydrogenation of compound 25. Pd/C (5 mg) was added to lactone **25** (10 mg, 5.11 μmol) in a mixture of THF - *t*-BuOH (2 mL, 1/1, v/v) and. The reaction mixture was placed under an atmosphere of H2 stirred for 24 h at room temperature. The catalyst then was filtered off through a pad of celite and washed subsequently with THF (2 times, 3 mL) and DCM (2x 3 mL), and the filtrate was concentrated. The residue was purified by size exclusion column chromatography on a Sephadex model LH-20 column (*i*-PrOH/DCM) to afford the a mixture of **3** and **4** (5 mg, 43%) R_f = 0.41 (methanol:DCM:NH_4OH 15:80:5); ^{1}H NMR (500 MHz, d-THF/MeOD 1:1, v/v): δ = 7.02 (d, 1H, *J* = 8.8 Hz, NH), 6.84 (d, 1H, *J* = 7.32 Hz, NH$^{/}$) Aminogluconate: 5.05 (t, 1H, *J* =

10.4, 9.3 Hz, H-3′), 4.71 (d, *J* = 3.6, H-2), 4.54 (d, 1H, *J* = 8.3 Hz, H-1′), 4.32 (1H, H-3), 3.82 (1H, H-2′), 3.86 (1H, H-6a′), 3.70-3.73(1H, H-6a, 6b′), 3.55-3.53 (2H, H-4′, 6b), 3.54 (1H, H-4), 3.35-3.32 (1H, H-5, 5′). HR MS (*m*/*z*) for $C_{88}H_{168}N_2O_{17}Na$: calcd, 1548.2811; found, 1548.2816. Aminogluconolactone: 5.01 (t, 1H, *J* = 10.3, 9.3 Hz, H-3′), 4.66 (d, 1H, *J* = 8.3 Hz, H-1′), 4.25 (1H, H-2, 5), 3.89 (1H, H-6a), 3.86 (1H, H-2′, 6a′), 3.69 (1H, 6b), 3.54-3.55(1H, H-4, 4′), 3.32 (1H, H-5). HR MS (*m*/*z*) for $C_{88}H_{166}N_2O_{16}Na$: calcd, 1530.2135; found, 1530.2786.

Reagents for Biological Experiments. *E. coli* 055:B5 LPS was obtained from List Biologicals, PMA was from Sigma, and *R. sin-1* LPS was kindly provided by Dr. R. Carlson (CCRC, Athens, GA). All data presented in this study were generated using the same batches of *E. coli* 055:B5 LPS and *R. sin-1* LPS. Synthetic compounds were stored lyophilized at –20°C and reconstituted in dry tetrahydrofuran (THF) on the day of the experiment; final concentrations of THF in the biological experiments never exceeded 0.5% to avoid toxic effects.

Cell Maintenance. Mono Mac 6 cells, provided by Dr. H.W.L. Ziegler-Heitbrock (Institute for Inhalationbiology, Munich, Germany), were cultured in RPMI 1640 medium with L-glutamine (BioWhittaker) supplemented with 100 u/mL penicillin, 100 µg/mL streptomycin, 1% OPI supplement (Sigma; containing oxaloacetate, pyruvate and bovine insulin) and 10% fetal calf serum (FCS) (HyClone). The cells were maintained in a humid 5% CO_2 atmosphere at 37°C. New batches of frozen cell stock were grown up every 2 months and growth morphology evaluated. Before each experiment, Mono Mac 6 cells were incubated with 10 ng/mL calcitriol (Sigma) for 2 days to differentiate inot macrophage like cells..

ELISA TNFα. Differentiated cells were harvested by centrifugation and gently suspended (10^6 cells/mL) in prewarmed (37°C) medium. Cells were then incubated with different combinations

of stimuli for 6 hours as described below. Cell supernatants were then collected and stored frozen (-80^{o}C) until assayed for TNFα protein. Concentrations of TNFα protein in culture supernatants were determined in duplicate by a solid phase sandwich ELISA. Briefly, 96-well plates (Nalge Nunc International) were coated with purified mouse anti-human TNFα antibody (Pharmingen). TNFα in standards and samples was allowed to bind to the immobilized antibody for 2 hours at room temperature. Biotinylated mouse anti-human TNFα antibody (Pharmingen) was then added, producing an antibody-antigen-antibody "sandwich". After addition of avidin-horseradish peroxidase conjugate (Pharmingen) and ABTS peroxidase substrate (Kirkegaard & Perry Laboratories), a green color was produced in direct proportion to the amount of TNFα present in the sample. The reaction was stopped by adding peroxidase stop solution (Kirkegaard & Perry Laboratories) and the absorbance was measured at 405 nm using a microplate reader (Dynatech Laboratories). All TNFα data are presented as the means $\pm$ SD of duplicate cultures, with each experiment being repeated three times.

CHAPTER 4

THE PREPARATION OF A LIPID A DERIVAITVE THAT CONTAINS A 27-HYDROXYOCTACOSANOIC ACID MOIETY*

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Abstract

A general synthetic strategy for long chain ω-1 hydroxy fatty acids has been developed that uses as a key reaction step a cross metathesis between ω-unsaturated ester and 3-butene-2-ol. The resulting lipids were employed for the preparation of Lipid A derivatives of *Rhizobium sin-1*, which have the ability to inhibit the *E. coli* LPS-dependent synthesis of tumor necrosis factor (TNFα) by human monocytes.

Introduction

Lipopolysaccharides (LPS), which are important components of the outer leaflet of the outer membrane of Gram-negative bacteria[66], are major virulence factors of pathogenic bacteria[30,55] as well as for symbionts such as the nitrogen-fixing *Rhizobia* of legumes[67]. LPS consists of an O-chain polysaccharide, a core oligosaccharide and an amphiphilic moiety

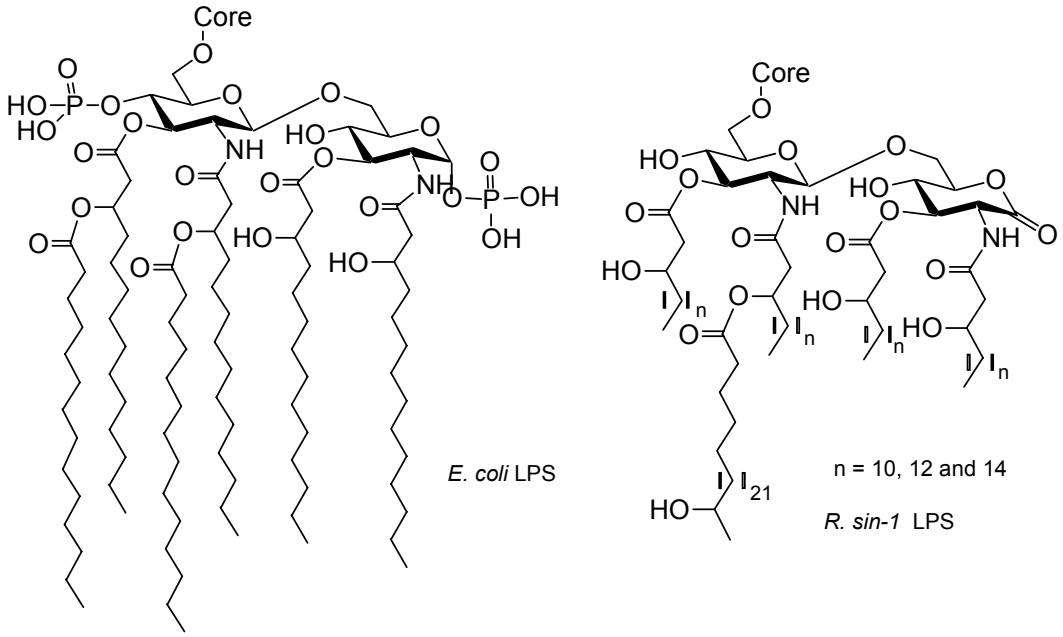


Figure 4.1. *E. coli* and *R-sin1* LPS structures

referred to as Lipid A. The structure of Lipid A is largely conserved among most enteric bacteria, consisting of a β-(1-6)-linked glucosamine disaccharide backbone with phosphate monoesters at C-1 and C-4' and β-hydroxyl fatty acyl groups and acyloxyacyl residues at positions 2, and 3 and 2' and 3', respectively (Figure 4.1). The nitrogen-fixing symbiont, *Rhizobium sin-1*, has a structurally unusual lipid A, (Figure 4.1) differing in almost every aspect from those of enteric LPS[24]. Its disaccharide backbone is devoid of phosphate and the glucosamine phosphate is replaced by 2-aminogluconolactone. Another unique structural feature is the presence of an unusual long chain 27-hydroxyoctacosanoic acid (27OHC28:0) moiety, which in turn can be esterified by β-hydroxybutyrate. It has been suggested that the presence of 27OHC28:0 fatty acid in the lipid A of many Rhizobial species is required for maintaining the stability of the bacterial membrane during endocytotic invasion and is crucial for the survival of the bacterium within the plant-derived symbiosome compartment[68-70]. The 27OHC28:0 fatty acid is also present among a number of facultative intracellular pathogens that cause chronic infections such as *Brucella abortus*, *Bartonella henselae*, and *Legionella pneumophila* [68].

Recently, we demonstrated that LPS from *R. sin-1* inhibits the *E. coli* LPS-dependent synthesis of tumor necrosis factor (TNFα) by human monocytes[21,59]. An LPS mediated overproduction of host-derived inflammatory mediators such as TNF-α may result in septicemia, which is a life-threatening syndrome for which currently no treatment exists but supportive therapy in an intensive care unit setting[1,2]. Thus, compounds such as *R. sin-1* LPS may have the potential to prevent the deleterious effects of enteric LPS. Due to the inherent molecular heterogeneity of *R. sin-1* LPS, it cannot be developed as a therapeutic agent for Gram-negative sepsis. We have, however, demonstrated[59] that a synthetic analog of the lipid A of *R. sin-1* emulates the ability of heterogeneous *R. sin-1* LPS to antagonize enteric LPS, albeit with somewhat higher IC_{50} values.

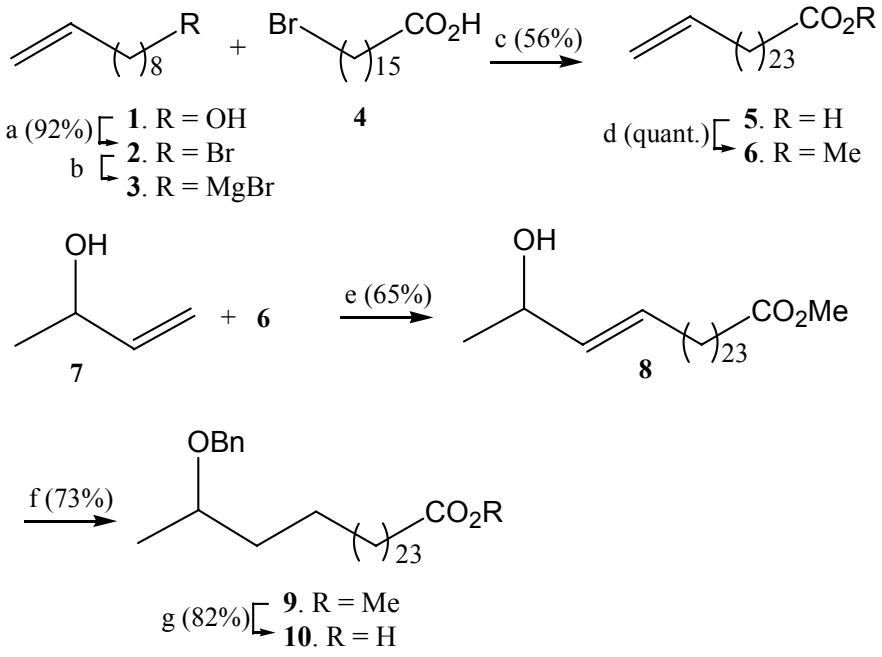
The synthetic compound contained an octacosanoic acid moiety rather than the natural hydroxylated 27-hydroxyoctacosanoic acid.

To determine the contributions of the hydroxylation of the long chain 27-hydroxyoctacosanoic acid moiety for antagonistic properties, an efficient preparation of this fatty acid was required. Furthermore, it was necessary that a synthetic procedure be developed which allowed the introduction of the 27-hydroxyoctacosanoic acid moiety at a late stage of synthesis. The new synthetic approach should allow an easy incorporation of other fatty acids for structure-activity relationship studies.

An efficient approach for the chemical synthesis of long chain ω-1 hydroxy fatty acids (>C18) has not yet been reported. These compounds have been obtained by an enzymatic hydroxylation of inexpensive saturated fatty acid,[71] a procedure that gave, however, mixtures of compounds in which ω-2 and ω-3 were also hydroxylated.

We envisaged that olefin-cross metathesis between an ω-unsaturated ester (*e.g.* **6**, Scheme 4.1**)** and 3-butene-2-ol (**7**) would give access to any ω-1 hydroxyl fatty ester after the reduction of the double bond. In general, high selectivity in olefin cross metathesis can be achieved when the two olefins have significantly different reactivities. In this respect, it has been shown that secondary allylic alcohols are of lower reactivity than terminal olefins[72]. Thus, it was expected that a metathesis reaction of **6** with **7** would give a product in good overall yield.

Scheme 4.1[a]



[a] Reagents and conditions: a) PPh_3, CBr_4; b) **2**, Mg, THF, Δ; c) MeMgBr, -10°, then Li_2CuCl_4 and **3**; d) CH_2CN_2, DCM; e) Grubbs second generalion cat., DCE, 60°C; f) Rh/Al, H_2, THF then C_6H_5CHO, $(TMS)_2O$, TMSOTf, Et_3SiH; g) 2.4 M LiOH, THF then 1M HCl.

Methyl 25-hexacosenoiate (**6**), which is a starting material for the cross metathesis reaction, was prepared from commercially available bifunctional starting materials of appropriate chain lengths (Scheme 4.1). Thus, reaction of 9-decen-1-ol (**1**) with CBr_4 and PPh_3 gave bromide **2** in a yield of 95%, which was converted into a cuprate by reaction with magnesium followed by transmetalation with dilithium tetrachlorocuprate (Li_2CuCl_4). Condensation of the cuprate with bromide **4** gave 25-hexacosenoic acid (**5**)[73], which was transformed into the corresponding methyl ester **6** by treatment with freshly prepared diazomethane. A cross metathesis reaction[72] of ω-unsaturated ester **6** and 3-butene-2-ol (**7**) using Grubbs 1st generation catalyst gave **8** in a low yield of 15%. Fortunately, when Grubbs 2nd generation catalyst (Figure 4.2) was employed, compound **8** was isolated in a much improved yield of 65% as mainly the trans isomer (E/Z =

20:1). A significantly lower yield of the cross metathesis product was obtained when the hydroxyl of **7** was protected as an acetyl ester of benzyl ether. Next, the double bond of **7** was reduced by hydrogenation of Rh/alumina to give **8**. Finally, benzylation of the ω-1 hydroxyl of **8** by treatment with benzaldehyde, TMS_2O, TMSOTf, and Et_3SiH (→ **9**) followed by saponification of the methyl ester with LiOH gave **10** in a 73% overall yield (2 steps).

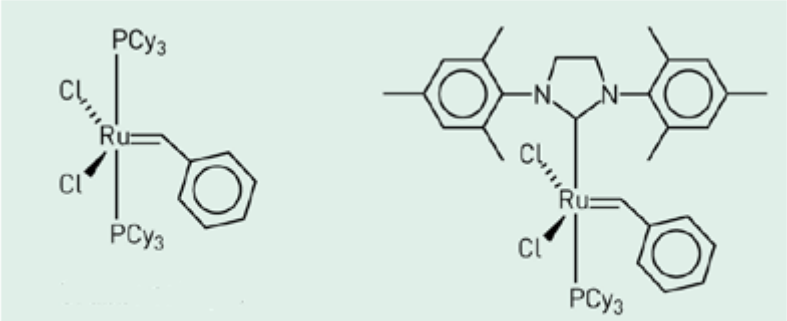
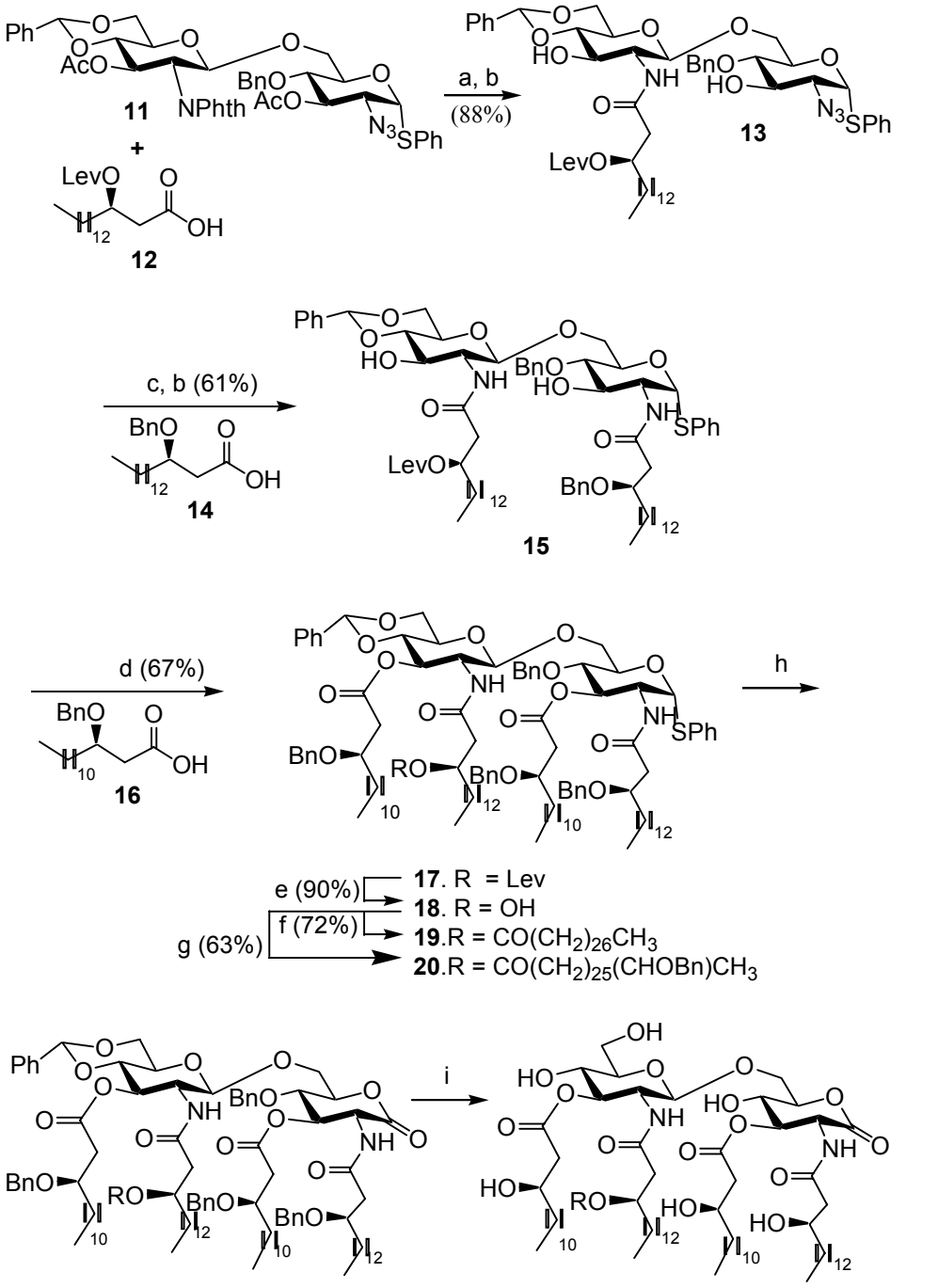


Figure 4.2. Grubbs catalysts for olefin metathesis

Having successfully prepared 27-benzyloxy-octacosanoic acid **10**, attention was focused on the preparation of lipid A derivative **24** (Scheme 4.2), which contains this fatty acid moiety. Lipid A derivatives are usually prepared by first synthesizing β-hydroxy acyl- and acyloxoacyl acids which are then condensed with amines or alcohols of an appropriately protected saccharide. We envisaged that an acylation of the β-hydroxyl of the C-2' myristate moiety of **18** with 27-hydroxyoctacosanoic acid **10** would offer a more convergent approach and efficient use of expensive starting material. Furthermore, the hydroxyl of this advanced intermediate can be acylated with other fatty acids (*e.g.* octacosanic acid leading to compound **23**), thus creating an opportunity for the convenient preparation of a library of compounds for structure-activity relationship studies.

Compound **18** could easily be prepared from the selectively protected disaccharide **11**[59] and benzyl ether of Lev ester protected β-hydroxy fatty acids **12**[74], **14** and **16**[75] (Scheme 4.2). Thus, removal of the phthalimido group and acetyl esters of **11** by treatment with ethylene diamine in refluxing t-butanol followed by selective *N*-acetylation with **12** in the presence of DCC gave **13** in an overall yield of 78%. Reduction of the azido moiety of **13** was easily accomplished by reaction with propane dithiol in a mixture of pyridine, triethylamine and water[45] and the amine of the resulting compound was acylated with **14** using DCC as the activating reagent to give **15** in an overall yield of 83%. The C-3 and C-3' hydroxyls of **15** were acetylated with **16** using DCC and DMAP to yield **17** in 78%. Thus, by employing the DDC-mediated acylation in the presence or absence of DMAP, selectivity between *O*- and *N*-acylation can be accomplished. Selective removal of the Lev ester of **17** could easily be achieved by treatment with hydrazine acetate[76] to give the advanced intermediate **18** in an excellent yield. The hydroxyl of **18** was acylated with octacosanic acid and 27-benzyloxy-octacosanoic acid **10** using DCC and DMAP as the activating reagent to give **19** and **20**, respectively. Hydrolysis of the thiophenyl moiety of **19** and **20** was accomplished with NIS in wet dichloromethane in the presence of TfOH[63] to give the corresponding lactols (α/β mixtures), which were oxidized using PCC to give lactones **21** and **22,** respectively**.** Finally, the benzyl ethers and benzylidene acetal of **21** and **22** were removed by catalytic hydrogenation over Pd/C to afford the target compounds **23** and **24**, respectively.

Scheme 4.2[a]



Reagents and conditions: a) $H_2N(CH_2)_2$, n-BuOH, 90°C; b) DCC, DCM; c) $HS(CH_2)_2SH$, pyridine, Et_3N, H_2O; d) DCC, DMAP, DCM; e) H_2NNH_2. HOAc, DCM; f) $CH_3(CH_2)_{26}COOH$, DCC, DMAP, DCM; g) **10**, DCC, DMAP, DCM; h) NIS, TfOH, DCM, H_2O then PCC, MS 3A; DCM; i) Pd/C, H_2, THF, t-BuOH.

Conclusions

An efficient synthetic approach for ω-unsaturated ester has been developed by olefin-cross metathesis between an ω-unsaturated ester and 3-butene-2-ol followed by a reduction of the double bond of the resulting product. Furthermore, it has been demonstrated that an acylation of the β-hydroxyl of the myristate moiety of a lipid A precursor (*e.g.* **18**) gives easy access to a range of compounds for structure-activity relationship studies. In this study we have employed two different fatty acids, namely the octacosanoic acid and 27- hydroxyoctacosanoic acid, to clarify the effect of the ω-1 hydroxyl group to antagonistic properties of natural *Rhizobial* lipid A. The results of these biological studies will be reported elsewhere.

Experimental Procedures

9-Bromononanal (2): PPh_3 (14.7 g, 56.1 mmol) was added portion wise to an ice cold solution of 9-decen-1-ol **1** (6.67 mL, 37.4mmol) and CBr_4 (12.39 g, 37.4 mmol) in DCM (65 mL). The reaction mixture was vigorously stirred for 2 hours at 0°C and then at rt for 18 h. The reaction mixture was concentrated under reduced pressure and the residue was diluted with hexanes (90 mL). The resulting mixture was filtered through a pad of celite and the washed several times with hexanes. The mixture was concentrated under reduced pressure and the residue was purified by silica gel column chromatography by eluting with hexane to afford **2** (7.54 g, 92%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.88 – 5.47 (m, 1H, CH_2=C*H*-), 5.02 – 4.92 (m, 2H, CH_2=CH-), 3.41 (t, 1H, *J* = 6.8 Hz, -CH_2-Br), 2.04 (q, 2H, *J* = 6.3, 6.9 Hz, CH_2=CH-CH_2-), 1.86 (quintet, 2H, *J* = 7.2, 6.8 Hz, -CH_2-CH_2-Br), 1.42-1.31 (m, 10H, H-3a,3b-7a,7b).

25- Hexacosenoic acid (5): In a dried flask was charged with Mg ribbons (0.322g, 13.2 mmol), THF (2 mL), bromide **2** (1.45 g, 6.6 mmol) and small crystal of iodine. The mixture was stirred and heated under reflux for 3 hours. Excess magnesium was filtered off. In a separate round-bottomed flask, 16-bromo-hexadecanoic acid **4** (2.2g, 6.56 mmol) was dissolved in THF (2 mL) and cooled to -10ºC followed by the addition of Li_2CuCl_4 (0.1 M in THF, 9 mL). The solution was further cooled to -20ºC and stirred vigorously while a solution of **3** was added drop wise. The reaction mixture was stirred for 1.5 hrs at -20º C and then allowed warm-up to rt. After 2hrs of stirring at rt, H_2SO_4 (10%, 10 mL) was added to the dark blue reaction mixture. The mixture was extracted with toluene (2 x 10 mL) and the combined toluene extracts were washed with H_2SO_4 (10%, 10 mL). The organic phase was dried ($MgSO_4$), filtered and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (10% ethyl acetate in hexanes) to afford **5** (1.44 g, 56%). R_f = 0.47 (30% ethyl acetate in hexanes); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.81 (m, 1H, CH_2=C*H*-), 4.98 (d, *J* = 17.0 Hz, CH*H*=C*H*-), 4.92 (d, *J* = 10.1 Hz, C*H*H=C*H*-), 2.34 (dd, *J* = 7.43 Hz, -CH_2-CO_2H), 2.03 (dd, *J* = 7.15, 6.87 Hz, =CH-CH_2-), 1.62 (p, *J* = 7.15, -CH_2-CH2-CO_2H), 1.22-1.56 (m, 42H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 179.8 (-CO2H), 139.5 (CH_2=*C*H-), 114.3 (*C*H_2=*C*H-), 34.2 (=CH-CH_2-), 34.0 (-*C*H_2-CO_2H), 29.9-29.1, 24.9 (-*C*H_2-CH2-CO_2H). HR MS (*m*/*z*): calcd for Me ester $C_{27}H_{52}O_2$ Na 431.6904, found 431.7209.

(E/Z)-27-Hydroxy-25-octacosenoic acid methyl ester (8): 25-Hexacosenoic acid **5** (0.213 g, 0.540 mmol) was treated with freshly prepared ethereal solution of diazomethane. The reaction mixture was stirred for 5h at rt and then concentrated *in vacuo* to dryness to yield **6** (0.217 g, 0.531 mmol). Methyl ester **6** (0.217 g, 0.531 mmol) and 3-butene-2-ol **7** (140 μL, 1.59 mmol)

were added simultaneously to a stirring solution of Grubbs 2nd generation catalyst (35 mg, 42 μmol) in DCE (3 mL). The reaction mixture was refluxed under argon for 16 h then evaporated *in vacuo* to dryness. The residue was purified by silica gel column chromatography (5% gradient ethyl acetate in hexanes) to afford (E)-27-Hydroxy-25-octacosenoic acid methyl ester **8** (0.157 g, 65%). R_f = 0.39 (12% ethyl acetate in hexanes); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.68 – 5.51 (m, 2H, (E) -C*H*=C*H*-), 4.28 (q, 1H, *J* = 6.3 Hz, -C*H*(OH)-), 3.69 (s, 3H, -CO_2CH_3), 2.32 (t, 2H, *J* = 7.8 Hz, -CH_2-CO_2H), 2.03 (dd, *J* = 7.3, 6.8 Hz, =CH-CH_2-), 1.64 (q, 1H, *J* = 7.3, 6.8 Hz, -CH_2-CH2-CO_2H), 1.40-1.28 (m, 44H), 1.21 (d, 3H, *J* = 5.8 Hz, CH_3-CH(OH)-); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 174.6 (-CO_2Me), 134.3 (-CH=*C*H-), 131.5 (-*C*H=CH-), 69.2 (-*C*H(OH)), 51.6 (CO_2Me), 34.3 (=CH-CH_2-), 32.3 (-CH_2-CO_2Me), 29.7-29.2, 25.2 (-CH_2-CH2-CO_2Me), 23.7 (-*C*H_3). HRMS (*m*/*z*): calcd for $C_{29}H_{56}O_3Na$ 475.7429, found 475.8113.

27-Hydroxy-octacosanoic acid methyl ester: To a solution of (E/Z)-27-Hydroxy-25-octacosenoic acid methyl ester **8** (0.134 g, 0.296 mmol) in THF (3 mL) was added 10% Rh/alumina catalyst (80 mg) and stirred under an atmosphere of hydrogen. After stirring the reaction mixture for 8 h, the catalyst was filtered-off through a pad of celite and the residue was washed with DCM (2 x 5mL). The combined filtrate was concentrated *in vacuo* to afford **5** as white solid (0.134 g, 99%). R_f = 0.39 (12% ethyl acetate in hexanes); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 3.81 (m, 1H, -C*H*(OH)-), 3.78 (s, 3H, -CO_2CH_3), 2.32 (t, 2H, *J* = 7.8 Hz, -CH_2-CO_2CH_3), 1.64 (q, 1H, *J* = 7.3, 6.8 Hz, -CH_2-CH2-CO_2CH_3), 1.40-1.28 (m, 44H), 1.21 (d, 3H, *J* = 5.8 Hz, CH_3-CH(OH)-); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 174.6 (-CO_2Me), 68.4 (-*C*H(OH)), 51.6 (CO_2Me), 44.0, 39.6, 34.3 (-CH_2-CO_2Me), 29.7-29.2, 25.2 (-CH_2-CH2-CO_2Me), 23.7 (-*C*H_3). HR MS (*m*/*z*): calcd $C_{29}H_{58}O_3Na$ 477.7588, found 477.6133.

27-Benzyloxy-octacosanoic acid methyl ester (9): Hexamethyldisiloxane (445 μL, 2.07 mmol) and TMSOTf (33 μL, 0.173 mmol) were added to a solution of 27-hydroxy-octacosanoic acid methyl ester (0.157 g, 0.346 mmol) and benzaldehyde (110 μL, 1.03 mmol) in anhydrous THF (3 mL) at 0 °C. After the mixture was stirred for 10 min, triethylsilane (195 μL, 15.1 mmol) was added and stirring was continued for another 6 h. The solution was quenched by the addition of with saturated aq. $NaHCO_3$ and extracted with EtOAc (2 x 15 mL). The organic phase was dried ($MgSO_4$), filtered and the filtrate concentrated *in vacuo* to dryness. The residue was purified by silica gel column chromatography (2% gradient ethyl acetate in hexanes) to afford **9** (0.136 g, 73 %): R_f = 0.68 (20% ethyl acetate in hexanes); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.43-7.22 (m, 5H, aromatic), 4.51 (dd, 2H, *J* = 11.8, 20.0 Hz, -C*H*$_2$Ph), 3.66 (s, 3H, -CO_2C*H*$_3$), 3.50 (m, 1H, -C*H*(OBn)-), 2.30 (t, 2H, *J* = 7.4 Hz, -C*H*$_2$-CO_2CH_3), 1.64 (m, 2H, -C*H*$_2$-CH2-CO_2CH_3), 1.40-1.28 (m, 44H), 1.20 (d, 3H, *J* = 6.0 Hz, C*H*$_3$-CH(OBn)-); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 174.5 (-*C*O_2Me), 139.4 – 127.6 (aromatic), 75.2 (-*C*H_2Ph), 70.5(-*C*H(OBn)), 51.6 (CO_2*Me*), 36.9, 34.4 (-*C*H_2-CO_2Me), 29.7-29.2, 25.8 (-*C*H_2-CH2-CO_2Me), 25.2, 19.9. HR MS (*m*/*z*): calcd for $C_{36}H_{64}O_3Na$ 567.8813, found 567.8779.

27-Benzyloxy-octacosanoic acid (10): LiOH (0.2 ml, 2.4 M) was added to a solution of **9** (0.130g, 0.238 mmol) in THF (3 mL). After stirring vigorously the reaction mixture at rt for 18 h, it was quenched with HCl (1N) and extracted with ether (2 x 10 mL). The ether extracts were combined and dried ($MgSO_4$), filtered and the filtrate concentrated *in vacuo* to dryness. The residue was purified by silica gel column chromatography (10% gradient ethyl acetate in hexanes) to afford **10** (0.103g, 82%) as white solid. R_f = 0.35 (35% ethyl acetate in hexanes); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.43-7.22 (m, 5H, aromatic), 4.51 (dd, 2H, *J* = 11.8, 20.3 Hz, -

C*H*$_2$Ph), 3.50 (m, 1H, -C*H*(OBn)-), 2.34 (t, 2H, *J* = 7.4 Hz, -C*H*$_2$-CO$_2$H), 1.64 (m, 2H, -C*H*$_2$-CH2-CO$_2$H), 1.40-1.28 (m, 44H), 1.19 (d, 3H, *J* = 6.0 Hz, C*H*$_3$-CH(OBn)-); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 179.9 (-*C*O$_2$H), 139.4 – 127.6 (aromatic), 75.2 (-*C*H$_2$Ph), 70.5(-*C*H(OBn)), 36.9, 34.3 (-*C*H$_2$-CO$_2$H), 29.7-29.2, 25.8 (-*C*H$_2$-CH2-CO$_2$H), 24.9, 19.9. HR MS (*m*/*z*): calcd for Me ester $C_{36}H_{64}O_3Na$ 567.8813, found 567.8749.

(*R*)-3-(Levulinoyloxy)-hexadecanoic acid 2-(4-bromo-phenyl)-2-oxo-ethyl ester: A solution of DCC (0.662 g, 3.20 mmol) and DMAP (55 mg, 0.43 mmol) in DCM (10 mL) was added to a stirred solution of 2-(4-bromophenyl)-2-oxoethyl (*R*)-3-hydroxyhexadecanoate (1.00 g, 2.13 mmol) and levulinic acid (0.27 mL, 2.56 mmol) in DCM (10 mL). The reaction mixture became instantaneously cloudy. After 3 hours, TLC indicated completion of the reaction. The solids were filtered-off and the residue was washed with DCM (2 x 10 mL). The combined filtrate was concentrated *in vacuo* and the residue was purified by silica gel column chromatography (5% gradient ethyl aceate in toluene) to afford (*R*)-3-(Levulinoyloxy)-hexadecanoic acid 2-(4-bromo-phenyl)-2-oxo-ethyl ester as a colorless syrup (1.16 g, 96%): R_f = 0.54 (25 % ethyl acetate in hexanes); $[\alpha]^{26}_D$ –3.7^o (c 0.91, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.70 (dd, 4H, aromatic), 5.29-5.27 (m, 3H, H-3, C*H*$_2$ COPh), 2.74 (m, 4H, H-2a,2b, -C*H*$_2$Lev), 2.58 (m, 2H, -C*H*$_2$Lev) 2.17 (s, 3H, -COC*H*$_3$Lev), 1.67 (m, 2H, H-4a,4b), 1.20-1.38 (bs, 22H, H-(5-15)), 0.88 (t, 3H, *J* = 8.3 Hz, H-16); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 206.8 (*C*OCH$_3$Lev), 191.2 (*C*OPh), 172.3 (-*C*OO-Lev), 170.0 (*C*OOCH$_2$COPh), 133.1 – 129.3 (aromatic), 70.9 (*C*2), 66.2 (*C*H$_2$COPh), 39.1 (*C*3), 38.2 (*C*H$_2$ Lev), 34.2 (*C*4), 32.1 (*C*H$_3$ Lev), 30.0 – 22.9 (bs, *C*5-*C*15, *C*H$_2$Lev), 14.3 (*C*16).

(*R*)-3-Levulinoyloxy-hexadecanoic acid (12): Zinc dust (1.26 g, 19.4 mmol) was added portion wise over a period of 30 min to (*R*)-3-(Levulinoyloxy)-hexadecanoic acid 2-(4-bromo-phenyl)-2-oxo-ethyl ester (1.1 g, 1.94 mmol) in acetic acid (8 ml) and the resulting reaction mixture was heated for 2 h at 60^{o}C. The reaction mixture was diluted with DCM (20 mL), the solids were filtered-off through a pad of celite and the residue was washed with DCM (3 x 10 mL). The combined filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (5% gradient methanol in DCM) to afford **12** as a white solid (0.710 g, 98%): R_f = 0.43 (5% methanol in DCM); $[\alpha]^{26}_{D}$ –6.2^{o} (c 0.64, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.22 (m, 1H, H-3), 2.74 (m, 2H, -C*H*$_2$Lev), 2.63(m, 2H, H-2a,2b), 2.58 (m, 2H, -C*H*$_2$Lev), 2.17 (s, 3H, -COC*H*$_3$Lev), 1.62 (m, 2H, H-4a,4b), 1.20-1.38 (bs, 22H, H-(5-15)), 0.88 (t, 3H, *J* = 8.3 Hz, H-16); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 207.0 (*C*OCH_3Lev), 176.0 (*C*OOH), 172.3 (-*C*OO-Lev), 70.8 (*C*2), 39.0 (*C*3), 38.2 (*C*H$_2$ Lev), 34.1 (*C*4), 32.1 (*C*H$_3$ Lev), 30.0 – 22.9 (bs, *C*5-*C*15, *C*H$_2$Lev), 14.3 (*C*16).

2-(4-Bromophenyl)-2-oxoethyl (*R*)-3-benzyloxyhexadecanoate (n = 12): Hexamethyldisiloxane (5.5 mL, 25.6 mmol) and TMSOTf (0.40 mL, 2.13 mmol) were added to a solution of 2-(4-bromophenyl)-2-oxoethyl (*R*)-3-hydroxy hexadecanoate (n = 12) (2.00 g, 4.26 mmol) and benzaldehyde (1.3 mL, 12.8 mmol) in anhydrous THF (30 mL) at 0 °C. After the mixture was stirred for 10 min, triethylsilane (2.45 mL, 15.1 mmol) was added and stirring was continued for another 2 h. The solution was quenched by the addition of saturated aqueous $NaHCO_3$ and extracted with EtOAc (2 x 60 mL). The organic phase was dried ($MgSO_4$), filtered and the filtrate concentrated *in vacuo* to dryness. The residue was purified by silica gel column chromatography (2% gradient ethyl acetate in toluene) followed by crystallization from diethyl

ether to afford 2-(4-bromophenyl)-2-oxoethyl (*R*)-3-benzyloxyhexadecanoate as white crystals (1.98 g, 80 %): R_f = 0.82 (20% ethyl acetate in hexanes); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.20-7.80 (m, 9H, aromatic), 5.26 (ps, 2H, CH_2), 4.56 (dd, 2H, *J* =11.4 Hz, C*H*$_2$Ph), 3.94 (m, 1H, H-3), 2.79 (dd, 1H, *J* = 7.1 Hz, *J* = 15.3 Hz, H-2a), 2.65 (dd, 1H, *J* = 5.5 Hz, H-2b), 1.55-1.72 (m, 1H, H-4), 1.35-1.45 (m, 2H, H-5,15), 1.30 [bs, 18H, H-(6-14)], 0.88 (pt, 3H, H-16).

2-(4-Bromophenyl)-2-oxoethyl (*R*)-3-benzyloxytetradecanoate (n = 10): The benzylation procedure was carried out using 2-(4-bromophenyl)-2-oxoethyl (*R*)-3-hydroxy tertadecanoate (n = 10) (2.3 g, 5.21 mmol) as described for the preparation of hexadecanoate to yield 2-(4-Bromophenyl)-2-oxoethyl (*R*)-3-benzyloxytetradecanoate (2.38 g, 86%). R_f = 0.85 (20% ethyl acetate in hexanes); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.20-7.80 (m, 9H, aromatic), 5.26 (ps, 2H, CH_2), 4.56 (dd, 2H, *J* =11.4 Hz, C*H*$_2$Ph), 3.94 (m, 1H, H-3), 2.79 (dd, 1H, *J* = 7.1 Hz, *J* = 15.3 Hz, H-2a), 2.65 (dd, 1H, *J* = 5.5 Hz, H-2b), 1.55-1.72 (m, 1H, H-4), 1.35-1.45 (m, 2H, H-5,13), 1.30 [bs, 18H, H-(6-12)], 0.88 (pt, 3H, H-14).

(*R*)-3-Benzyloxy- hexadecanoic acid (14) (n = 12): Zinc dust (0.34 g, 5.23 mmol) was added portion over a period of 30 min to 2-(4-bromophenyl)-2-oxoethyl (*R*)-3-benzyloxyhexadecanoate (0.295 g, 0.523 mmol) in acetic acid (2 ml). The reaction mixture was stirred for 2 h at 60^{o}C, and then diluted with DCM (20 mL). The solids were filtered-off through a pad of celite and the residue was washed with DCM (3 x 5 mL). The combined filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (2% gradient methanol in DCM) to afford **14** as a white solid (0.12 g, 64%): R_f = 0.40 (30% ethyl acetate in hexanes). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.30-7.40 (m, 5H, aromatic), 4.58 (ps, 2H, CH_2Ph), 3.95 (m, 1H, H-3),

2.58 (m, 2H, H-2a, 2b), 1.30-1.71 (m, 3H, H-4,5,15), 1.28 [bs, 18H, H-(6-14)] 0.85 (pt, 3H, H-16).

(*R*)-3-Benzyloxy- tetradecanoic acid (15) (n = 10): To the above synthesized 2-(4-bromophenyl)-2-oxoethyl (*R*)-3-benzyloxytetradecanoate (0.520 g, 0.979 mmol) was subjected to Zn/AcOH as described for the synthesis of **14**, and the crude product was purified by silica gel column chromatography (2% gradient methanol in DCM) to afford **15** as a colorless syrup (0.235 g, 72%): R_f = 0.42 (30% ethyl acetate in hexanes). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.30-7.40 (m, 5H, aromatic), 4.58 (ps, 2H, CH_2Ph), 3.95 (m, 1H, H-3), 2.58 (m, 2H, H-2a, 2b), 1.30-1.71 (m, 3H, H-4,5,15), 1.28 [bs, 16H, H-(6-12)] 0.85 (pt, 3H, H-14).

Phenyl 2-azido-4-*O*-benzyl-6-*O*-{4,6-*O*-benzylidene-2-deoxy-2-[(*R*)-3-Levulinoyloxy-hexadecanoicacid] amido-β-D-glucopyranosyl}-2-deoxy-1-thio-α-D-glucopyranoside (13): Hydrazine hydrate (0.48 ml, 9.8 mmol) was added to a solution of **11** (0.416 g, 0.49 mmol) in ethanol (30 mL). After stirring the reaction mixture for 20h at 90^{o}C, it was concentrated *in vacuo* to dryness and co-evaporated with toluene (2 x 10 mL). The residue was purified by silica gel column chromatography (2% gradient methanol in DCM) to afford phenyl 6-*O*-(2-amino-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranosyl)-2-azido-4-*O*-benzyl-2-deoxy-1-thio-α-D-glucopyranoside as colorless syrup (0.297 g, 95 %): R_f = 0.55 (methanol/DCM, 1/9, v/v). Dicyclohexylcarbodiimide (DCC) (0.232 g, 1.12 mmol) was added to a solution of **12** (0.364 g, 0.98 mmol) in DCM (15 mL) and the resulting solution was stirred for 10 min. Next, the above mentioned amino derivative (0.297 g, 0.47 mmol) in DCM (15 mL) was added and the reaction mixture was stirred for 16 h at rt. The solids were filtered-off and the residue was washed with

DCM (2 x 25 mL). The combined filtrate was concentrated *in vacuo* and the residue was purified by silica gel column chromatography (2% gradient methanol in DCM) to afford **13** as a white solid (0.426 mg, 92%): R_f = 0.64 (10% methanol in DCM); $[\alpha]^{23}_D = 4.8^o$ (c, 0.52, $CHCl_3$).^{1}H NMR (500 MHz, $CDCl_3$): δ 7.53 – 7.18 (m, 15H, aromatic), 6.34 (d, 1H, *J* = 6.84 Hz, NH$^{/}$), 5.61 (d, 1H, *J* = 5.38 Hz, H-1), 5.56 (s, 1H, >C*H*Ph), 5.04 (m, 1H, H-3L), 4.91 (d, 1H, *J* = 8.30 Hz, H-1$^{/}$), 4.78 (dd, 2H, *J* = 11.23 Hz, CH_2Ph), 4.37 (d, 1H, *J* = 9.77 Hz, H-5), 4.32 (dd, 1H, *J* = 4.89 Hz, H-6a$^{/}$), 4.23 (t, 1H, *J* = 9.28 Hz, H-3$^{/}$), 4.12 (d, 1H, *J* = 9.76 Hz, H-6a), 3.98 (dd, 1H, *J* = 10.25, 8.79 Hz, H-3), 3.89 – 3.86 (m, 2H, H-2, 6b$^{/}$), 3.79 (dd, 1H, *J* = 10.25, 9.77 Hz, H-6b), 3.63 (t, 1H, *J* = 9.28 Hz, H-4), 3.58 (dd, 1H, *J* = 9.27, 10.26 Hz, H-4$^{/}$), 3.54 – 3.48 (m, 2H, H-2$^{/}$, 5$^{/}$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 137.7-123.7 (Ph), 102.0 (*C*HPh), 100.8 (C-1$^{/}$), 87.6 (C-1), 81.6 (C-4$^{/}$), 78.4 (C-4), 75.2 (*C*H_2Ph), 74.3 (C-3), 72.6 (C-3L), 71.6 (C-5, 3), 71.4 (C-3$^{/}$), 69.0 (C-6$^{/}$), 68.3.3 (C-6), 66.7 (C-5$^{/}$), 64.2 (C-2), 59.4 (C-2$^{/}$). HRMS (*m*/*z*) for $C_{53}H_{72}N_4O_{12}SNa$: calcd, 1012.2143; found, 1012.3649.

Phenyl 4-*O*-benzyl-6-*O*-{4,6-*O*-benzylidene-2-deoxy-2-[(*R*)-3-Levulinoyloxy-hexadecanoicacid]amido-β-D-glucopyranosyl}-2-[(*R*)-3-benzyloxy hexadecan]amido]-2-deoxy-1-thio-α-D-glucopyranoside (15). Triethylamine (~2 ml) was added to a stirred solution of **13** (0.420 g, 0.425 mmol) and propanedithiol (0.85 ml, 8.5 mmol) in pyridine (31 ml) and H_2O (1.5 ml). The reaction mixture was stirred for 16 h at rt. After which it was concentrated *in vacuo* to dryness. The residue was co-evaporated with toluene (2 x 30 mL) and ethanol (2 x 20 ml) and then purified by silica gel column chromatography (4% gradient methanol in DCM) to afford phenyl 2-amino-4-*O*-benzyl-6-*O*-{4,6-*O*-benzylidene-2-deoxy-2-[(*R*)-3-levulinoyloxy-hexadecanoic acid] amido-β-D-glucopyranosyl}-2-deoxy-1-thio-α-D-glucopyranoside as a

colorless syrup (0.291 mg, 71 %): R_f = 0.75 (10% gradient methanol in DCM). DCC (0.111 g, 0.541 mmol) was added to a stirred solution of **14** (0.130 g, 0.361 mmol) in DCM (5 ml). After stirring for 10 min, the above-mentioned amino derivative (0.291 g, 0.301 mmol) in DCM (5 mL) was added. After stirring the mixture for 16 h, the solids were filtered-off and the residue was washed with DCM (2 x 15 mL). The combined filtrate was concentrated *in vacuo* and the residue was purified by silica gel column chromatography (1% methanol in DCM) to afford **15** as a white solid (0.310 g, 78%): R_f = 0.40 (70% ethyl acetate in toluene). $[\alpha]^{26}_{D}$ +17.2^{o} (c 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.86 – 7.27 (m, 20H, aromatic), 6.96 (d, 1H, *J* = 7.81 Hz, NH$^{/}$), 6.96 (d, 1H, *J* = 6.83 Hz, NH), 5.66 (d, 1H, *J* = 5.38 Hz, H-1), 5.43 (s, 1H, >C*H*Ph), 5.07 (m, 1H, H-3L), 4.94 (d, 1H, *J* = 8.79 Hz, H-1$^{/}$), 4.95 – 4.58 (m, 2 x CH_2Ph), 4.40 – 4.23 (m, 4H, *J* = 9.77 Hz, H-5, 2, 6a$^{/}$, 3$^{/}$), 4.14 (d, 1H, *J* = 9.76 Hz, H-6a), 3.88 (dd, 1H, *J* = 4.89, 4.40 Hz, H-6b), 3.76 (m, 1H, H-3), 3.62 – 3.39 (m, 5H, H-6b$^{/}$, 4$^{/}$, 4, 5$^{/}$, 2$^{/}$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 209.2 (*C*OCH_3), 173.1 – 169.6 (5 x -*C*OOR)137.7-123.7 (Ph), 102.0 (*C*HPh), 100.8 (C-1$^{/}$), 87.6 (C-1), 81.6 (C-4$^{/}$), 78.4 (C-4), 75.2 (*C*H_2Ph), 74.3 (C-3), 72.6 (C-3L), 71.6 (C-5, 3), 71.4 (C-3$^{/}$), 69.0 (C-6$^{/}$), 68.3.3 (C-6), 66.7 (C-5$^{/}$), 64.2 (C-2), 59.4 (C-2$^{/}$). HRMS (*m*/*z*) for $C_{76}H_{110}N_2O_{14}SNa$: calcd, 1330.7475; found, 1330.7753.

Phenyl 4-*O*-benzyl-6-*O*-{4,6-*O*-benzylidene-3-*O*-[(*R*)-3-benzyloxy-tetradecanoyl-2-deoxy-2-[(*R*)-3-Levulinoyloxy-hexadecanoic acid]amido-β-D-glucopyranosyl}-2-[(*R*)-3-benzyloxy-hexadecan]amido-3-*O*-[(*R*)-3-benzyloxy-tetradecanoyl]-2-deoxy-1-thio-α-D-glucopyranoside (17): DCC (57 mg, 0.273 mmol) was added to a stirred solution of **16** (74 mg, 0.218 mmol) in DCM (3 ml). After stirring for 10 min, a solution of **15** (72 mg, 54.6 μmol) and

DMAP (7.0 mg, 54.5 μmol) in DCM (3 mL) was added. After stirring the reaction mixture for 16 h, the solids were filtered-off and the residue was washed with DCM (2 x 10 mL). The combined filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (10% gradient ethyl acetate in toluene) to afford **17** as a white solid (70.2 mg, 67%): R_f = 0.43 (10% ethyl acetate in tolune). $[\alpha]^{26}_{D}$ +13.1° (c 1.4, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.38 – 7.20 (m, 30H, aromatic), 6.55 (d, 1H, *J* = 9.28 Hz, NH/), 6.45 (d, 1H, *J* = 8.31 Hz, NH), 5.38 (d, 1H, *J* = 4.89 Hz, H-1), 5.46 – 5.31 (m, 2H, >C*H*Ph, H-3/), 5.25 (t, 1H, *J* = 9.28Hz, H-3), 4.88 (m, 1H, H-3L), 4.84 (d, 1H, *J* = 8.79 Hz, H-1/), 4.63 - 4.36 (m, 9H, 4 x CH_2Ph, H-2), 4.34 (d, 1H, *J* = 9.77 Hz, H-5), 4.30 (dd, 1H, *J* = 4.88, 4.44 Hz, H-6a/), 4.19 (q, 1H, *J* = 9.28 Hz, H-2/), 4.02 (d, 1H, *J* = 10.25 Hz, H-6a), 3.93 (d, 1H, *J* = 3.42 Hz, H-6b), 3.81 (m, 1H, H-4), 3.71 (t, 1H, *J* = 10.26 Hz, H-6a/), 3.63 (t, 1H, *J* = 9.28 Hz, H-4/), 3.49 (m, 1H, H-5/). ^{13}C NMR (75 MHz, $CDCl_3$): δ 209.2 (*C*OCH_3), 173.1 – 169.6 (5 x -*C*OOR), 138.7-126.3 (Ph), 101.9 (*C*HPh), 101.4 (C-1/), 87.9 (C-1), 76.9 (C-4/), 76.1 (C-4), 73.8 (C-3), 71.9 (C-5), 71.4 (C-3/) 74.8-70.9 (4 x *C*H_2Ph), 71.6 (C-3L), 68.7 (C-6/), 67.8 (C-6), 66.6 (C-5/), 54.2 (C-2/), 53.1 (C-2). HRMS (*m*/*z*) for $C_{118}H_{174}N_2O_{18}SNa$: calcd, 1963.7026; found, 1963.7911.

Phenyl 4-*O*-benzyl-6-*O*-{4,6-*O*-benzylidene-3-*O*-[(*R*)-3-benzyloxy-tetradecanoyl-2-deoxy-2-[(*R*)-3-hydroxy-hexadecan]amido-β-D-glucopyranosyl}-2-[(*R*)-3-benzyloxy-hexadecan]amido-3-*O*-[(*R*)-3-benzyloxy-tetradecanoyl]-2-deoxy-1-thio-α-D-glucopyranoside (18): A solution of hydrazine acetate (4 mg, 35.1 μmol) in methanol (0.4 mL) was added to a solution of **17** (62.1 mg, 31.9 μmol) in DCM (3.6 mL). The resulting mixture was stirred for 3 hrs at room temperature after which TLC indicated completion of the reaction. The

reaction mixture was concentrated *in vacuo*, and the residue was purified by silica gel column chromatography (5% gradient ethyl acetate in toluene) to afford **18** as a white solid (53 mg, 90%): R_f = 0.40 (20% ethyl acetate in tolune). $[\alpha]^{23}_D$ = +5.8^o (c, 0.52, $CHCl_3$).^{1}H NMR (500 MHz, $CDCl_3$): δ 7.39 – 7.15 (m, 30H, aromatic), 6.49 (d, 1H, *J* = 8.79 Hz, NH), 5.70 (d, 1H, *J* = 8.79 Hz, NH$^/$), 5.67 (d, 1H, *J* = 4.89 Hz, H-1), 5.46 (dd, 1H, *J* = 9.77, 10.26 Hz, H-3$^/$), 5.42 (s, 1H, >C*H*Ph), 5.27 (dd, 1H, *J* = 9.28, 10.26 Hz, H-3), 4.80 (d, 1H, *J* = 8.31 Hz, H-1$^/$), 4.63 - 4.42 (m, 9H, 4 x C*H*$_2$Ph, H-2), 4.33 (d, 1H, *J* = 9.77 Hz, H-5), 4.30 (dd, 1H, *J* = 4.88, 4.44 Hz, H-6a$^/$), 3.99 (d, 1H, *J* = 10.75 Hz, H-6a), 3.84-3.71 (m, 4H, H-2$^/$, 6b, 4, 6b$^/$), 3.64 (dd, 1H, *J* = 9.28, 9.77 Hz, H-4$^/$), 3.49 (m, 1H, H-5$^/$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 137.7-123.7 (Ph), 101.8 (*C*HPh), 101.0 (C-1$^/$), 87.9 (C-1), 79.0 (C-4$^/$), 76.1 (C-4), 73.8 (C-3), 71.9 (C-5), 71.4 (C-3$^/$) 74.8-70.9 (4 x *C*H$_2$Ph), 71.5 (C-3L), 68.8 (C-6$^/$), 67.3 (C-6), 66.7 (C-5$^/$), 55.7 (C-2$^/$), 53.1 (C-2). HRMS (*m*/*z*) for $C_{113}H_{168}N_2O_{16}SNa$: calcd, 1865.6027; found, 1865.6511.

Phenyl 4-*O*-benzyl-6-*O*-{4,6-*O*-benzylidene-3-*O*-[(*R*)-3-benzyloxy-tetradecanoyl-2-deoxy-2-[(*R*)-3-octacosanoyloxy-hexadecan]amido-β-D-glucopyranosyl}-2-[(*R*)-3-benzyloxy-hexadecan]amido-3-*O*-[(*R*)-3-benzyloxy-tetradecanoyl]-2-deoxy-1-thio-α-D-glucopyranoside (19): DCC (1.3 mg, 5.96 μmol) was added to a stirred solution of octacosanoic acid (2.4 mg, 5.70 μmol) in DCM (1 ml). After stirring for 10 min, a solution of **18** (5 mg, 2.71 μmol) and DMAP (0.4 mg, 3.2 μmol) in DCM (1 mL) was added. The reaction mixture was stirred for 26 h, after which the solids were filtered-off and the residue was washed with DCM (2 x 3 mL). The combined filtrate was concentrated *in vacuo* and the residue was purified by silica gel column chromatography (5% gradient ethyl acetate in toluene) to afford **19** as a white fluffy

solid (4.3 mg, 72%): R_f = 0.64 (10% ethyl acetate in tolune). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.18–7.41 (m, 30H, aromatic), 6.48 (d, 1H, *J* = 8.3 Hz, NH), 5.72 (d, 1H, *J* = 5.3 Hz, H-1), 5.60 (d, 1H, *J* = 8.3 Hz, NH$^{/}$), 5.34 (m, 2H, >C*H*Ph, H-3$^{/}$), 5.26 (dd, 1H, *J* = 8.8 Hz, H-3), 4.99 (m, 1H, H-3_L), 4.83 (d, 1H, *J* = 8.3 Hz, H-1$^{/}$), 4.65-4.39 (m, 9H, H-2, 4 x CH_2Ph), 4.33 (m, 1H, H-5), 4.31 (m, 1H, H-6a$^{/}$), 3.66-4.02 (m, 5H, H-2$^{/}$,4,6a,6b,6b$^{/}$), 3.60 (m, 1H, H-4$^{/}$), 3.48 (m, 1H, H-5$^{/}$); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 173.9, 172.3, 171.4, 171.2, 169.9, 125.0-131.5 (aromatic), 101.2 (>*C*HPh), 100.9 (C-1'), 87.0 (C-1), 78.0 (C-4'), 75.9 (C-4, 2 x C-3_S), 75.2 (C_S-3), 74.5 (*C*H_2Ph), 73.0 (C-3), 71.2 (C-5), 70.9 (C-3'), 70.4-70.6 (3 x *C*H_2Ph), 70.3 (C-3_L), 68.2 (C-6'), 67.4 (C-6), 66.0 (C-5'), 55.5 (C-2'), 52.6 (C-2). HRMS (*m*/*z*) for $C_{141}H_{222}N_2O_{17}S$ Na: calcd, 2272.3304; found, 2272.3795.

Phenyl 4-*O*-benzyl-6-*O*-{4,6-*O*-benzylidene-3-*O*-[(*R*)-3-benzyloxy-tetradecanoyl-2-deoxy-2-[(*R*)-3-(27-benzyloxyoctacosanoyloxy-hexadecan]amido-β-D-glucopyranosyl}-2-[(*R*)-3-benzyloxy-hexadecan]amido-3-*O*-[(*R*)-3-benzyloxy-tetradecanoyl]-2-deoxy-1-thio-α-D-glucopyranoside (20): DCC (11.3 mg, 54.8 μmol) was added to a stirred solution of 27-benzyloxyoctacosanoic acid **10** (27.7 mg, 52.3 μmol) in DCM (1.5 ml). After stirring for 10 min, a solution of **18** (44 mg, 23.8 μmol) and DMAP (3.2 mg, 2.6 μmol) in DCM (2 mL) was added. The reaction mixture was stirred for 30 h, the solids were filtered-off and the residue was washed with DCM (2 x 3 mL). The combined filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (5% gradient ethyl acetate in toluene) to afford **20** as a white fluffy solid (35.7 mg, 63%): R_f = 0.60 (10% ethyl acetate in tolune). ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.20–7.41 (m, 30H, aromatic), 6.51 (d, 1H, *J* = 8.30 Hz, NH), 5.75 (d, 1H, *J* =

4.98 Hz, H-1), 5.63 (d, 1H, J = 8.30 Hz, NH$^{/}$), 5.37 (s, 1H, >C*H*Ph), 5.37 (dd, 1H, J = 9.3 Hz, H-3$^{/}$), 5.24 (dd, 1H, J = 8.8 Hz, H-3), 4.95 (m, 1H, H-3$_L$), 4.91 (d, 1H, J = 8.30 Hz, H-1$^{/}$), 4.37-4.61 (m, 11H, H-2, 5 x CH_2Ph), 4.37 (m, 1H, H-5), 4.34 (dd, 1H, J = 5.5, 11.0 Hz, H-6a$^{/}$), 4.02 - 3.72 (m, 5H, H-2$^{/}$,4, 6a, 6b, 6b$^{/}$), 3.65 (dd, 1H, J = 9.37 Hz, H-4$^{/}$), 3.52 (m, 2H, ω-1$_L$, H-5$^{/}$). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 173.9, 172.3, 171.4, 171.2, 169.9, 139.4-126.3 (aromatic), 101.6 (>*C*HPh), 101.1 (C-1$^{/}$), 87.5 (C-1), 79.0 (C-4$^{/}$), 77.4, 75.9 (C-4), 75.2 (C$_S$-3), 74.5 (*C*H$_2$Ph), 73.0 (C-3), 71.2 (C-5), 70.9 (C-3$^{/}$), 70.4-70.6 (3 x *C*H$_2$Ph), 68.9 (C-6$^{/}$), 67.8 (C-6), 66.5 (C-5$^{/}$), 55.7 (C-2$^{/}$), 53.0 (C-2). HR MS (*m*/*z*): calcd for $C_{148}H_{228}N_2O_{18}SNa$ 2378.4524, found 2378.0593.

4-*O*-benzyl-6-*O*-{4,6-*O*-benzylidene-3-*O*-[(*R*)-3-benzyloxy-tetradecanoyl-2-deoxy-2-[(*R*)-3-(octacosanoyloxy-hexadecan]amido-β-D-glucopyranosyl}-2-[(*R*)-3-benzyloxy-hexadecan]amido-3-*O*-[(*R*)-3-benzyloxy-tetradecanoyl]-2-deoxy-D-glucono-1,5-lactone (21):

N-Iodosuccinimide (7.0 mg, 28.3 μmol) and trifluoromethanesulfonic acid (1.0 μL, 2.65 μmol) were added to a stirred solution of **19** (21.1 mg, 8.91 μmol) in DCM/H_2O (3 ml, 100:1) at 0^{o}C. The reaction mixture was vigorously stirred for 30 min at 0^{o}C until TLC analysis indicated that reaction had gone to completion. The reaction mixture was diluted with DCM (5 mL), washed with aqueous $Na_2S_2O_3$ (10 %, 5 mL) and water (2 x 5 mL). The organic phase was dried ($MgSO_4$), filtered and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (5% gradient ethyl acetate in toluene) to afford the intermediate lactol as a white solid (13.7 mg, 67%). To the lactol (13.7 mg, 6.28 μmol) and activated molecular sieves (3Å, 37 mg) in DCM (2 mL) and was stirred for 1 h at room temperature under an atmosphere of argon. Pyridinium chlorochromate (15 mg, 66.1 μmol) was then added and the

reaction mixture was stirred for another 1 hr until TLC analysis indicated completion of the reaction. The reaction mixture was placed onto a column of iatrobeads, and eluted with EtOAc/toluene (1:1) to afford the lactone **21** as a colorless film (10.1 mg, 73%): R_f = 0.73 (25% ethyl acetate in toluene); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.13 –7.31 (m, 25H, aromatic), 5.60 (t, 1H, *J* = 9.8 Hz, H-3'), 5.39 (s, 1H, >C*H*Ph), 5.31 (dd, 1H, *J* = 9.8 Hz, H-3), 5.04 (m, 1H, H-3_L), 4.93 (d, 1H, *J* = 8.3 Hz, H-1'), 4.78 (t, 1H, *J* = 10.3 Hz H-2), 4.36-4.59 (m, 9H, H-5, 4 x C*H*$_2$Ph), 4.27 (dd, 1H, *J* = 5.5, 10.3 Hz, H-6'a), 4.01-4.04 (m, 2H, H-4,6a), 3.55-3.87 (m, 7H, 3 x H_{SL}-3, H-4',6b,6'b,2'), 3.69 (t, 1H, *J* = 9.77 Hz, H-6'b), 3.50-3.57 (m, 4H, H-5',2',4',6b); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 125.0-128.2 (aromatic), 101.2 (C-1'), 100.8 (>C*H*Ph), 78.3 (C-4',5), 74.9 (3 x C-3_S), 73.5 (*C*H_2Ph,C-4), 71.3 (C-3), 70.6 (C-3',*C*H_2Ph), 70.4 (2 x *C*H_2Ph), 70.0 (C-3_L), 67.9 (C-6), 67.9 (C-6'), 65.4 (C-5'), 54.1 (C-2'), 52.4 (C-2). HR MS (*m*/*z*): calcd for $C_{135}H_{216}N_2O_{18}Na$ 2178.1520, found 2178.1993.

4-*O*-benzyl-6-*O*-{4,6-*O*-benzylidene-3-*O*-[(*R*)-3-benzyloxy-tetradecanoyl-2-deoxy-2-[(*R*)-3-(27-benzyloxyoctacosanoyloxy-hexadecan]amido-β-D-glucopyranosyl}-2-[(*R*)-3-benzyloxy-hexadecan]amido-3-*O*-[(*R*)-3-benzyloxy-tetradecanoyl]-2-deoxy-D-glucono-1,5-lactone (22): *N*-Iodosuccinimide (7.0 mg, 28.3 μmol) and trifluoromethanesulfonic acid (1.0 μL, 2.65 μmol) were added to a stirred solution of **20** (21.1 mg, 8.91 μmol) in DCM/H_2O (3 ml, 100:1) at 0^{o}C. The reaction mixture was vigorously stirred for 30 min at 0^{o}C until TLC analysis indicated that reaction had gone to completion. The reaction mixture was diluted with DCM (5 mL), washed with aqueous $Na_2S_2O_3$ (10 %, 5 mL) and water (2 x 5 mL). The organic phase was dried ($MgSO_4$), filtered and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (5% gradient ethyl acetate in toluene) to afford the intermediate

lactol as a white solid (13.3 mg, 65%). To the lactol (11 mg, 4.81 μmol) and activated molecular sieves (3Å, 25 mg) in DCM (2 mL) and was stirred for 1 h at room temperature under an atmosphere of argon. Pyridinium chlorochromate (6.1 mg, 25 μmol) was then added and the reaction mixture was stirred for another 1 hr until TLC analysis indicated completion of the reaction. The reaction mixture was placed onto a column of iatrobeads, and eluted with EtOAc/toluene (1:1) to afford the lactone **22** as a colorless film (7.7 mg, 70%): R_f = 0.70 (25% ethyl acetate in toluene); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.13 –7.31 (m, 25H, aromatic), 5.60 (t, 1H, $J_{3',4'}$ = 9.8 Hz, H-3'), 5.39 (s, 1H, >C*H*Ph), 5.31 (dd, 1H, *J* = 9.8 Hz, H-3), 5.04 (m, 1H, H-3_L), 4.93 (d, 1H, *J* = 8.3 Hz, H-1'), 4.78 (t, 1H, *J* = 10.3 Hz H-2), 4.36-4.59 (m, 9H, H-5, 4 x C*H*$_2$Ph), 4.27 (dd, 1H, *J* = 5.5 Hz, *J* = 10.3 Hz, H-6'a), 4.01-4.04 (m, 2H, H-4,6a), 3.55-3.87 (m, 7H, 3 x H_{SL}-3, H-4',6b,6'b,2'), 3.69 (t, 1H, *J* = 9.77 Hz H-6'b), 3.50-3.57 (m, 4H, H-5',2',4',6b). ^{13}C NMR (75 MHz, $CDCl_3$): δ =173.9, 172.3, 171.4, 171.2, 169.9, 125.0-128.2 (aromatic), 101.2 (C-1'), 100.8 (>C*H*Ph), 78.3 (C-4',5), 74.9 (3 x C-3_S), 73.5 (*C*H$_2$Ph,C-4), 71.3 (C-3), 70.6 (C-3',*C*H$_2$Ph), 70.4 (2 x *C*H$_2$Ph), 70.0 (C-3_L), 67.9 (C-6), 67.9 (C-6'), 65.4 (C-5'), 54.1 (C-2'), 52.4 (C-2). HR MS (*m*/*z*): calcd for $C_{142}H_{222}N_2O_{19}Na$ 2284.2739, found 2284.3141.

2-Deoxy-6-*O*-{2-deoxy-3-*O*-[(*R*)-3-hydroxy-tetradecanoyl]-2-[(*R*)-3-octacosanoyloxy-hexadecan]amido-β-D-glucopyranosyl}-2-[(*R*)-3-hydroxy-hexadecan]amido-3-*O*-[(*R*)-3-hydroxy-hexadecanoyl]-D-glucono-1,5-lactone (23). The lactone **21** (10.1 mg, 4.68 μmol) was dissolved in THF/*t*-BuOH (2 ml, 1:3) and Pd/C (10 mg) was added. The reaction mixture was shaken under an atmosphere of H_2 for 36 h at rt, then the catalyst was filtered off, washed

successively with THF (2 x 3 ml) and the combined filtrate was concentrated. The residue was purified by size exclusion column chromatography on Sephadex LH-20 (*i*-propanol/DCM, 1:1) to afford **23** as colorless film (7.1 mg, 89%). $[\alpha]^{26}_{D}$ –26.2^{o} (c 0.34, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3/CD_3OD$, 1:1): δ = 5.39 (t, 1H, *J* = 9.8Hz, H-3), 4.97 (t, 1H, *J* = 9.3 Hz, H-3'), 4.55 (1H, H-1'), 4.32 (m, 1H, H-5), 4.17 (m, 1H, *J* = 10.3Hz H-2), 4.13 (m, 1H, H-6a'), 3.95 (m, 1H, H-4), 3.81-3.85 (m, 2H, H-2', H-6b'), 3.70 (m, 1H, H-6a'), 3.54 [t, 1H, *J* = 9.3Hz, H-4'], 3.33 (m, 1H, H-5'). HR MS (*m*/*z*): calcd for $C_{100}H_{188}N_2O_{18}Na$ 1728.3755, found 1728.5394.

2-Deoxy-6-*O*-{2-deoxy-3-*O*-[(*R*)-3-hydroxy-tetradecanoyl]-2-[(*R*)-3-(27-hydroxy-octacosanoyloxy)-hexadecan]amido-β-D-glucopyranosyl}-2-[(*R*)-3-hydroxy-hexadecan]amido-3-*O*-[(*R*)-3-hydroxy-hexadecanoyl]-D-glucono-1,5-lactone (24). The lactone **22** (7.7 mg, 3.41 μmol) was dissolved in THF/*t*-BuOH (2 ml, 1:3) and Pd/C (10 mg) was added. The reaction mixture was shaken under an atmosphere of H_2 for 36 h at rt, then the catalyst was filtered off, washed successively with THF (2 x 3 ml) and the combined filtrate was concentrated. The residue was purified by size exclusion column chromatography on Sephadex LH-20 (*i*-propanol/DCM, 1:1) to afford **24** as colorless film (4.5 mg, 77%). ^{1}H NMR (600 MHz, $CDCl_3/CD_3OD$, 1:1): δ = 5.43 (t, 1H, *J* = 9.8Hz, H-3), 4.99 (t, 1H, *J* = 9.3 Hz, H-3'), 4.64 (1H, H-1'), 4.33 (m, 1H, H-5), 4.29 (m, 1H, *J* = 10.3Hz H-2), 4.17 (m, 1H, H-6a'), 3.97 (m, 1H, H-4), 3.81-3.85 (m, 2H, H-2', H-6b'), 3.70 (m, 2H, H-6a', ω-1-H_{27}), 3.55 [t, 1H, *J* = 9.3Hz, H-4'], 3.33 (m, 1H, H-5'). HR MS (*m*/*z*): calcd for $C_{100}H_{188}N_2O_{19}Na$ 1744.3704, found 1744.5962.

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