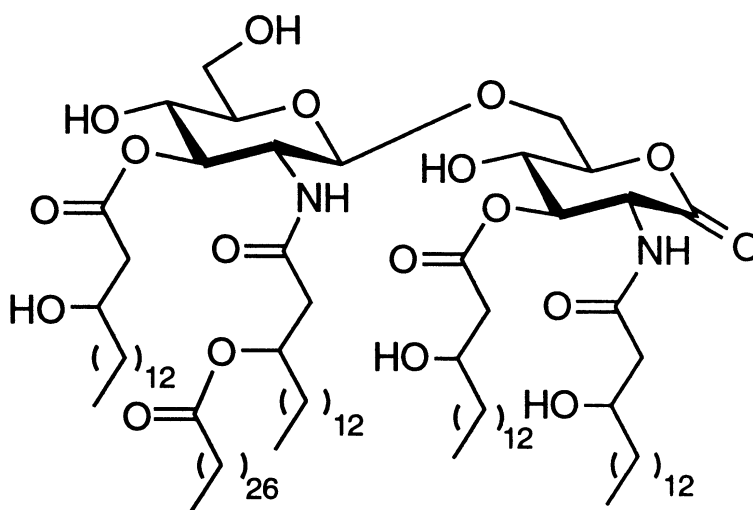Article

Synthesis and Biological Evaluation of *Rhizobium sin*-1 Lipid A Derivatives

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R. sin-1 Lipid A

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Synthesis and Biological Evaluation of *Rhizobium sin*-1 Lipid A Derivatives

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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 approach employed the advanced intermediate 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**), which is protected in such a way that the anomeric center, the C-2 and C-2′ amino groups, and the C-3 and C-3′ hydroxyls can be selectively functionalized. The synthetic strategy was used for the preparation of 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]-α-D-glucopyranose (**11**) and 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 (**13**), which contain an unusual octacosanoic acid moiety and differ in the oxidation state of the anomeric center. The results of biological studies indicate that **11** and **13** lack the proinflammatory effects of *Escherichia coli* lipopolysaccharides (LPS). Furthermore, **13** emulated the ability of heterogeneous *R. sin*-1 LPS to antagonize enteric LPS, providing evidence for the critical role of the gluconolactone moiety of *R. sin*-1 LPS in mediating this antagonistic effect. Compound **13** is the first example of a lipid A derivative that is devoid of phosphate but possesses antagonistic properties, making it an attractive lead compound for development of a drug to use in the treatment of Gram-negative septicemia.

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.[3−5] LPS, a component of Gram-negative bacteria, is among the most potent proinflammatory substances known, with its lipid A region initiating the production of multiple host-derived inflammatory mediators, including cytokines (e.g., tumor necrosis factor TNFα), arachidonic acid metabolites, and tissue factor. LPS causes these effects after binding to cluster differentiation antigen CD14 on mononuclear phagocytes, or to soluble CD14 in plasma and then to cells lacking CD14.[6−8]

The interaction of LPS with CD14 is facilitated by a plasma protein termed the LPS binding protein. Being a glycosylphosphatidylinositol-anchored protein, CD14 lacks transmembrane and cytoplasmic domains and, therefore, is unable to transmit signals directly to the interior of the cell. The latter function is performed by Toll-like receptor 4 (TLR4),[9−11] which contains extracellular, transmembrane, and intracellular domains, and an accessory protein MD-2.[12,13] Although the precise mechanisms involved in the interactions among LPS, CD14, TLR4, and MD-2 remain to be discovered, 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 up-regulation of more than 120 genes, including those for the cytokines, most notably TNFα, interleukin-1α, and interleukin-1β.[14]

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Lethality and pyrogenicity are only observed when lipid A analogues contain a strict set of structural features, including a $\beta(1-6)$-linked glucosamine disaccharide backbone, bisphosphorylation at the anomeric and C-4' position, and a suitable number and location of appropriately long 3-acyloxyacyl groups per disaccharide.[7,15] If the length of the acyl groups is increased, as occurs in lipid A of *Chlamydia psittaci*, *Bacteroides fragilis*, and *Legionella pneumophilia*, endotoxicity is reduced.[16] In contrast, the chirality of the 3-oxoacyl moieties does not influence biological activity, as evidenced by the fact that lipid A derivatives with *R*- and *S*-configurations cause similar effects.[17] Distribution of the acyl groups affects the bioactivity of lipid A. For instance, *Haemophilus influenza* lipid A, which contains 6 acyl groups with 14 carbon atoms in an asymmetric distribution, expresses biological properties comparable to those of *Escherichia coli* lipid A, whereas lipid A, which has 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 that have four fatty acid side chains. All known LPS derivatives that display biological activity contain, however, one or more phosphates, and the current consensus is that these moieties are required for interaction with the cell-surface receptors for LPS.

An appealing method for preventing the deleterious effects of enteric LPS is prevention of the interaction between lipid A and its receptors on mononuclear phagocytes.[18,19] 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 that has agonist activity. It has, however, been difficult to identify lipid A derivatives that possess these properties, and the best-studied derivatives are monosaccharide biosynthetic precursors of lipid A[20-24] and synthetic analogues derived from the lipid A of *Rhodobacter sphaeroides* or *Rhodobacter capsulatus*, which are two species that have very similar lipid A structures.[25-27] Although the *Rh. sphaeroides /Rh. capsulatus* lipid A has the
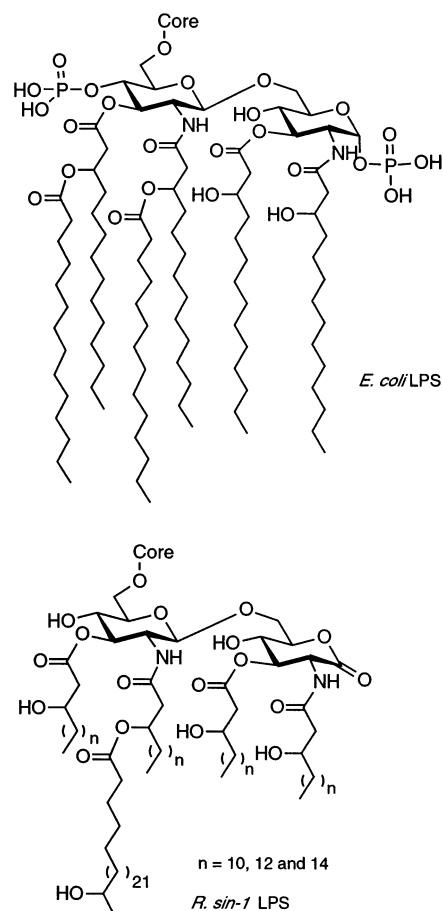

Figure 1. Structures of (a) *E. coli* LPS and (b) *R. sin*-1 LPS.

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 *Rh. sphaeroides /Rh. 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 *Rh. sphaeroides /Rh. capsulatus* lipid A lacks toxic effects, does not 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.[28] More importantly, *R. sin*-1 LPS significantly inhibit *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, and its structure (Figure 1) differs in almost every aspect from those known to contribute to the toxicity of enteric LPS.[29] In particular, the disaccharide moiety is devoid of phosphate and the glucosamine phosphate is replaced by 2-aminogluconolactone. It is possible that the latter residue can also exist as a 2-aminogluconate, which would

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Scheme 1 [a]
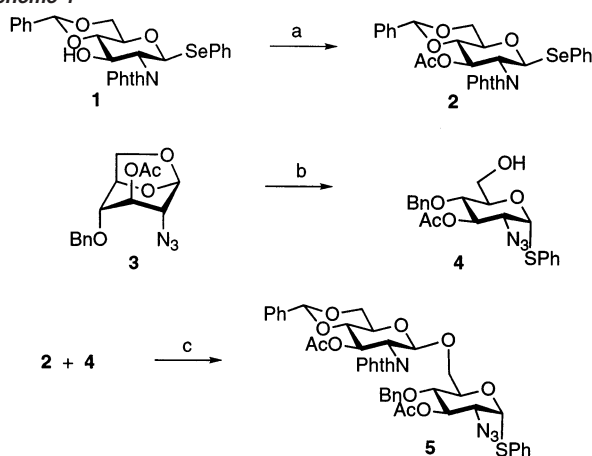
[a] Reagents and conditions: (a) Ac₂O, pyridine; (b) TMSSPh, ZnI₂, DCE, then TFA, H₂O; (c) AgOTf; K₂CO₃, MS 3A, DCM.

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.

Because of its inherent variations in fatty acid acylation patterns, purified *R. sin*-1 lipid A cannot 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 make 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. This approach was employed for the preparation of 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]-α-D-glucopyranose (**11**) and 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 (**13**), and the biological effects of these derivatives have been determined.

Results and Discussion

Synthesis. Our synthetic approach uses an advanced intermediate (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**); see Scheme 1), which is protected in such a way that the anomeric center, the C-2 and C-2′ amino groups, and the C-3 and C-3′ hydroxyls can be selectively functionalized. A key feature of **5** is protection of the anomeric center as a thioglycoside.[30,31] 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

lacton. Furthermore, the phthalimido and azido functions of **5** offer an attractive set of orthogonal protecting groups that allow selective derivatization of either of the two amino groups. Removal of the phthalimido group also results in cleavage of the *O*-acetyl esters. It was anticipated, however, that the resulting amine could be selectively derivatized by exploiting the fact that primary amines are more nucleophilic than hydroxyls.

Compound **5** was prepared by a chemoselective coupling of seleno glycoside **2** with thio glycoside **4**.[30] Glycosyl donor **2** was easily obtained by acetylation of known **1**, using acetic anhydride in pyridine. Treatment of known **3** with ZnI₂ and TMSSPh,[32] followed by short treatment with aqueous TFA, gave **4** in a good yield as a separable mixture of anomers (α/β = 6/1). The coupling of glycosyl acceptor **4** with glycosyl donor **3** in the presence of the promoter system AgOTf/K₂CO₃[33] produced the desired disaccharide **5** in an 83% yield (Scheme 2). In this coupling, only the β-anomer was formed, because of neighboring group participation of the phthalimido group.

Having synthesized the requisite key disaccharide **5**, attention was focused on selective introduction of (*R*)-3-hydroxy and alkanoyoxy fatty acid components (Scheme 2). Thus, removal of the phthalimido group and acetyl ester of **5** by treatment with ethylenediamine in refluxing *n*-butanol,[34] followed by selective *N*-acylation with (*R*)-3-octacosanoyloxy-hexadecanoic acid (**6**) in the presence of 1,3-dicyclohexylcarbodiimide (DCC), gave phenyl 2-azido-4-*O*-benzyl-6-*O*-{4,6-*O*-benzylidene-2-deoxy-2-[(*R*)-3-octacosanoyloxy-hexadecan]amido-β-D-glucopyrano-syl}-1-thio-α-D-glucopyranoside (**7**) in a yield of 93%. Compound **6** was easily obtained by acylation of *p*-bromophenylacyl (*R*)-3-hydroxyhexadecanoate with octacosanoic acid in the presence of 4-(dimethylamino)pyridine (DMAP) in pyridine, followed by removal of the *p*-bromophenyl acyl ester group, using zinc in acetic acid.[35]

Reduction of the azido moiety of **7** was easily accomplished by reaction with propane-1,3-dithiol[36] in a mixture of pyridine, triethylamine, and water, and the amine and hydroxyls of the resulting compound were acylated with (*R*)-3-octacosanoyloxy-hexadecanoic acid (**8**), using DCC and DMAP as the activation reagents to afford 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 (**9**) 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 with different substituents at C-2 amine and C-3 and C-3′ hydroxyls.

Hydrolysis of the thiophenyl moiety of **9** to give 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 (**10**) proved to be more difficult than anticipated. Conventional procedures,

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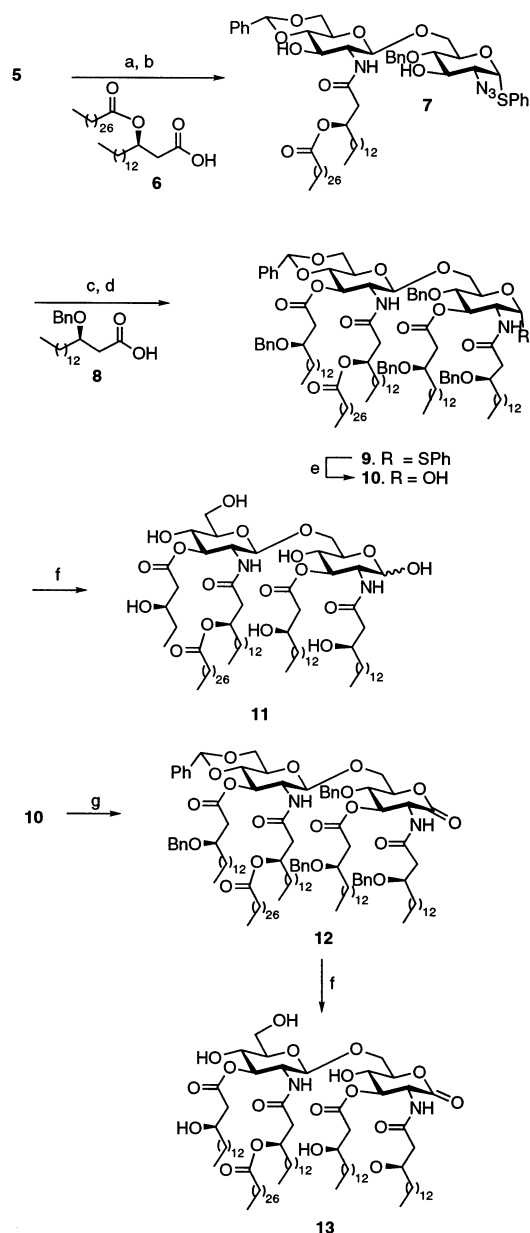(35) Keegan, D. S.; Hagen, S. R.; Johnson, D. A. *Tetrahedron: Asymmetry* **1996**, *7*, 3559−3564.
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Scheme 2 [a]

[a] Reagents and conditions: (a) $H_2N(CH_2)_2$, *n*-BuOH, 90 °C; (b) **6**, DCC, DCM; (c) $HS(CH_2)_2SH$, pyridine, Et_3N, H_2O; (d) **8**, DCC, DMAP, DCM; (e) NIS, TfOH, DCM, H_2O; (f) Pd/C, H_2, THF, EtOH; (g) PCC, MS 3A; DCM.

such as treatment with *N*-bromosuccinimide (NBS) or Hg-(OCOCF$_3$)$_2$, resulted in mixtures of compounds. Conversion of **9** to the corresponding bromide, followed by hydrolysis, also produced side products, as well as the expected compound. Fortunately, treatment of **9** with *N*-iodosuccinimate (NIS) and a catalytic amount of trifluoromethanesulfonic acid (TfOH) in wet dichloromethane (DCM) gave **10** in an excellent yield of 84%. The benzyl ethers and benzylidene acetal of **10** were removed by catalytic hydrogenation of Pd/C to give the first target compound 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]-α-D-glucopyranose (**11**).

Next, we turned our attention to the oxidation of the anomeric center of **11** to give lactone **12**. Moffatt (dimethylsulfoxide
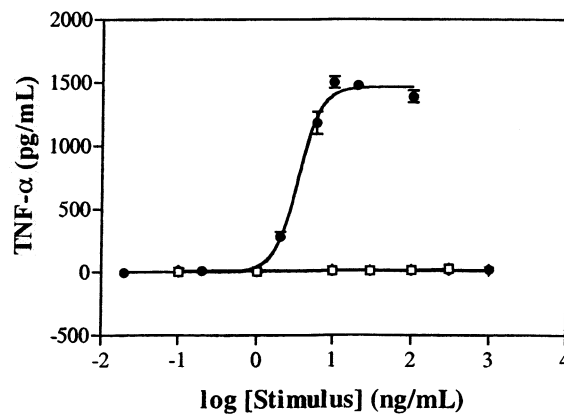
Figure 2. Concentration–response curves of *E. coli* LPS, *R. sin*-1 LPS, and synthetic compounds. Mono Mac 6 cells were incubated for 6 h at 37 °C with increasing concentrations of (●) *E. coli* LPS, (□) *R. sin*-1 LPS, (▼) **11**, or (○) **13**, as indicated. TNFα protein in cell supernatants was measured using ELISA. (Please note that *R. sin*-1 LPS, **11**, and **13** show background values and therefore overlap in the figure.) Treatment with *E. coli* LPS, *R. sin*-1 LPS, **11**, and **13** did not affect cell viability, as judged by cellular exclusion of trypan blue.

(DMSO)/Ac$_2$O) or Swern (DMSO/oxallyl chloride) oxidation led mainly to the formation of a 1,2-oxazoline derivative. Most likely, these reactions proceed through an intermediate compound that has a good anomeric-leaving group, which can be displaced by the acylamido group to give the oxazoline. Oxidation with a catalytic amount of tetrapropylammonium perruthenate (TPAP) in the presence of the co-oxidant 4-methylmorpholine *N*-oxide (NMO) yielded both the expected **12** and an elimination product. An attempt to separate the mixture of compounds by silica gel column chromatography resulted only in recovery of the elimination product. We suspect that the mild acidic conditions of the silica gel promoted the elimination of **12** into a 1,2-dehydro derivative. Fortunately, PCC-mediated oxidation of **10** gave only the formation of **12**, and, in this case, pure product could be obtained by purification over a Sephadex model LH-20 size exclusion column, using methanol/DCM as the eluent. Finally, the benzyl ethers and benzylidene acetal of **12** were removed by catalytic hydrogenation over Pd/C to give the target compound 2-deoxy-6-*O*-{2-deoxy-3-*O*-[(*R*)-3-hydroxy-hexadecanoyl]-2-[(*R*)-3-octacosanoyl-oxy-hexadecan]amido-*β*-D-glucopyranosyl}-2-[(*R*)-3-hydroxy-hexadecan]amido-3-*O*-[(*R*)-3-hydroxy-hexadecanoyl]-D-glucono-1,5-lactone (**13**).

Biological Evaluation. Compounds **11** and **13** were tested over a wide concentration range for agonistic activity in a human monocytic (Mono Mac 6) cell line, with TNFα protein concentration in the supernatant used as a readout; values were compared with those obtained for *E. coli* LPS and *R. sin*-1 LPS (Figure 2). Incubation with *E. coli* LPS for 6 h yielded a clear dose response effect on 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$_{50}$ value of 3.4 ng/mL (where EC$_{50}$ is the concentration producing 50% activity) and a Hill slope of 2.8. In subsequent experiments, *E. coli* LPS concentrations of 2, 6, and 10 ng/mL were used, which approximated concentrations that yielded 33%, 66%, and 100% of maximal supernatant concentration of TNFα. Neither *R. sin*-1 LPS nor **11** and **13** at concentrations up to 1 µg/mL induced significant production of TNFα. To exclude the possibility that any effects observed might be due to the presence
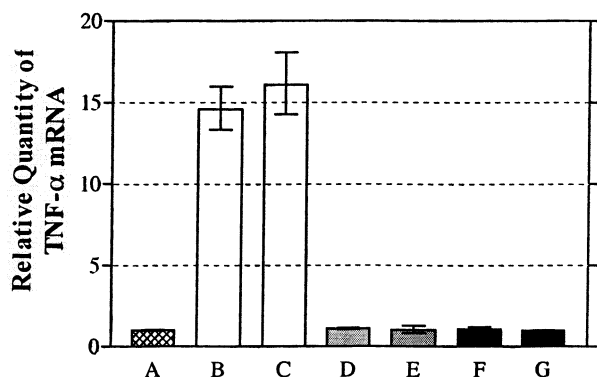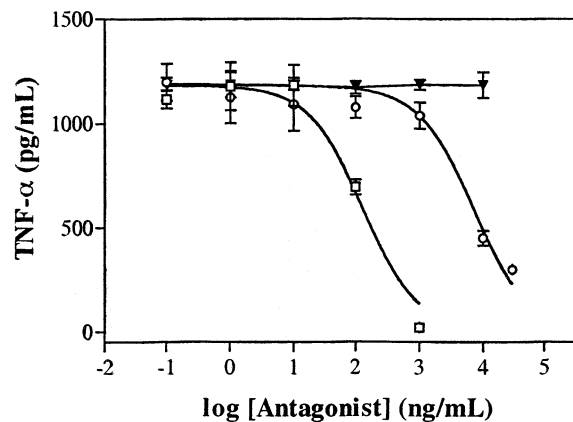
Figure 3. Induction of TNFα mRNA by Mono Mac 6 cells incubated with *E. coli* LPS, *R. sin*-1 LPS, and synthetic compounds. Mono Mac 6 cells were incubated with (A) medium alone and medium containing (B) *E. coli* LPS (2 ng/mL), (C) *E. coli* LPS (6 ng/mL), (D) *R. sin*-1 LPS (1 µg/mL), (E) **11** (1 µg/mL), (F) **13** (1 µg/mL), or (G) **13** (10 µg/mL) for 90 min before RNA was isolated for RT−PCR analysis of TNFα mRNA (40 PCR cycles); mRNA of the 18 S ribosomal gene amplified under the same conditions was used as the internal control for each experiment.
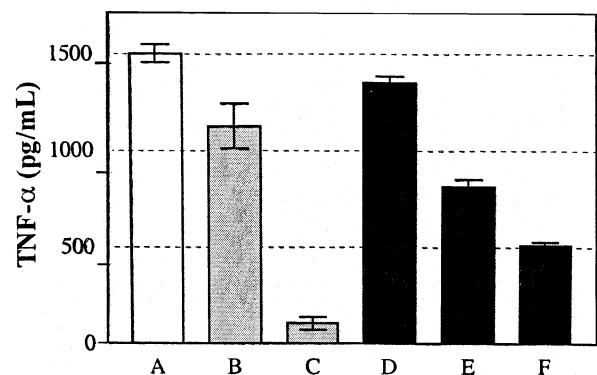
of tetrahydrofuran (THF), the cells were incubated with THF concentrations up to 0.5%. These THF concentrations alone did not cause TNFα production by the cells, nor did they alter the response of cells co-incubated with *E. coli* LPS (10 ng/mL) (data not shown).

Synthesis of the TNFα protein depends on up-regulation of the genes responsible for production of this cytokine, transcription of the message, and then translation of the mRNA.[37−39] Several posttranscriptional factors contribute to the regulation of TNFα mRNA, thereby providing control over the stability of the mRNA and its translation. As a result, it is possible for compounds to induce expression of TNFα mRNA without causing production of TNFα protein. We have identified this effect recently with muramyl dipeptide, another structural component of bacterial cell walls.[40] Therefore, TNFα mRNA expression induced by *E. coli* LPS (2 and 6 ng/mL) was compared to the response to *R. sin*-1 LPS and the synthetic compounds (Figure 3). As expected, incubation of the cells for 90 min with 2 and 6 ng/mL *E. coli* LPS resulted in 14.6- and 16.1-fold increases in TNFα gene expression, compared to control cells. In contrast, expression of TNFα mRNA after incubation of cells with *R. sin*-1 LPS (1 µg/mL) or compounds **11** (1 µg/mL) and **13** (1 and 10 µg/mL) was indistinguishable from that of nonstimulated (control) cells.

On the basis of their lack of proinflammatory effects, compounds **11** and **13** (100 pg/mL to 30 µg/mL) were tested for their ability to antagonize the responses of monocytic cells incubated with *E. coli* LPS (6 ng/mL), and the results were compared with the antagonistic effect of *R. sin*-1 LPS (Figure 4a). Compound **11** did not significantly alter the response to *E. coli* LPS. *R. sin*-1 LPS at 1 µg/mL completely inhibited *E. coli* LPS-induced TNFα production, with an IC$_{50}$ value of 130 ng/mL (where IC$_{50}$ is the concentration producing 50% inhibition). At the highest concentration tested, compound **13** antagonized the effect of *E. coli* LPS by 75%, with an IC$_{50}$ value of 7.1

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(a)

(b)

Figure 4. Antagonism of *E. coli* LPS by *R. sin*-1 LPS and the synthetic compounds. (a) TNFα concentrations after preincubation of Mono Mac 6 cells with increasing concentrations of (□) *R. sin*-1 LPS, (▼) **11**, or (○) **13** as indicated for 1 h at 37 °C, followed by 6 h of incubation with 6 ng/mL *E. coli* LPS. (b) Cells were preincubated with (A) medium alone and medium containing *R. sin*-1 LPS (B) 100 ng/mL and (C) 1 µg/mL, or **13** (D) 1 µg/mL, (E) 10 µg/mL, or (F) 30 µg/mL for 1 h at 37 °C; next, *E. coli* LPS (10 ng/mL) was added, and the cells were further incubated for 6 h, after which point TNFα protein concentrations were determined.

µg/mL. Similar results were obtained when *R. sin*-1 LPS and **13** were used to antagonize the effects of 2 ng/mL *E. coli* LPS; IC$_{50}$ values for *R. sin*-1 LPS and **13** were 27 ng/mL and 3.8 µg/mL, respectively (data not shown). These results, derived from a four-parameter logistic fit of the data, demonstrate that **13** is ~50-fold less potent than *R. sin*-1 LPS in antagonizing *E. coli* LPS. In addition to the previously described experiments, in which Mono Mac 6 cells were incubated with the potential antagonists before the addition of *E. coli* LPS, experiments were also performed in which the potential antagonists were added simultaneously with *E. coli* LPS (data not shown). Simultaneous treatment did not alter the previously described results, suggesting that pretreatment was not necessary for the antagonistic effects of *R. sin*-1 LPS and **13**.
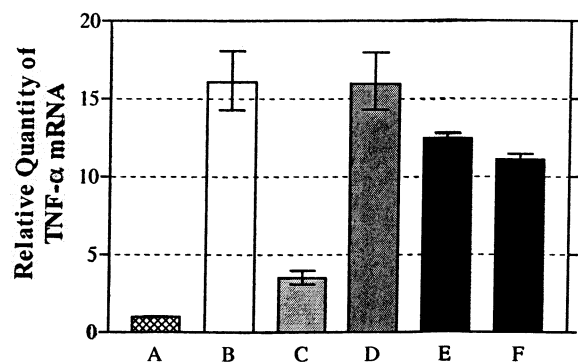
In additional experiments, we examined the effects of *R. sin*-1 LPS and **13** against the lowest concentration of *E. coli* LPS causing maximal TNFα protein production (i.e., 10 ng/mL). At concentrations of 1, 10, and 30 µg/mL, compound **13** decreased *E. coli* LPS-induced TNFα production by 10%, 46%, and 66%, respectively. These findings suggest that **13** acts through the same receptors utilized by *E. coli* LPS (Figure 4b). The finding that **13** did *not* inhibit the effects of phorbol 12-myristate 13-

Figure 5. Induction of TNFα mRNA by Mono Mac 6 cells incubated with different combinations of stimuli. Mono Mac 6 cells were preincubated with (B) medium alone and medium containing (C) *R. sin*-1 LPS (1 μg/ mL), (D) **11** (1 μg/mL), or **13** (E) 1 μg/mL and (F) 10 μg/mL for 1 h at 37 °C; next, *E. coli* LPS (6 ng/mL) was added and the cells were further incubated for 90 min. Total RNA was isolated for RT−PCR analysis of TNFα mRNA (40 PCR cycles); mRNA of the 18 S ribosomal gene amplified under the same conditions was used as the internal control in each experiment. Column A represents data for TNFα mRNA of non-stimulated control cells.

acetate (PMA), a compound that stimulates TNFα production by directly activating protein kinase C, provides evidence that **13** exerts its effect by interacting with more-proximal components of the signal transduction pathway leading to TNFα production (data not shown). On the basis of its structural similarities with *R. sin*-1 LPS, **13** most likely competes with cell receptor-mediated mechanisms utilized by LPS.

The results of previous studies indicate that antagonism of cell surface receptors that recognize enteric LPS can prevent the production of cytokines.[18] Hence, such compounds have the potential to be used as therapeutic interventions for patients with Gram-negative septicemia. Success in this area has been limited, and most efforts have been directed toward the synthesis of analogues of lipid A of *Rh. sphearoides*[26,27] and derivatives of lipid X.[20−22] These compounds, which are either mono- or bis-phosphorylated, may possess metabolic instabilities complicating drug discovery. A unique aspect of the current study is that we have identified a lipid A derivative that lacks phosphate and that antagonizes the biological effect of enteric LPS. Thus, our studies refute the consensus that LPS derivatives require a phosphate species to interact with its cell surface receptors. By comparing the biological responses initiated by the synthetic lipid A derivatives and *R. sin*-1 LPS, the gluconolactone moiety of *R. sin*-1 LPS appears to be important for antagonizing enteric LPS-induced cytokine production. The fatty acylation pattern of natural *R. sin*-1 LPS is heterogeneous,[29] and the fact that the synthetic compound is a less potent antagonist may be due to a fatty acid substitution that is not optimal for this property. Also, the absence of a KDO moiety may reduce the biological activity of the synthetic compound.

At the TNFα mRNA level, the antagonistic effect of **13** was further examined and compared to the antagonist *R. sin*-1 LPS and the inactive compound **11**. In these experiments, Mono Mac 6 cells were preincubated for 1 h with either *R. sin*-1 LPS (1 μg/mL), **11** (1 μg/mL), or **13** (1 or 10 μg/mL), and then exposed to 6 ng/mL *E. coli* LPS (Figure 5). Preincubation of cells with compound **11** resulted in TNFα mRNA levels that were indistinguishable from those of cells incubated with *E. coli* LPS alone. In contrast, compound **13** produced a dose-dependent inhibition of mRNA gene expression. TNFα gene expression

was increased 16.1-fold when cells were incubated with *E. coli* LPS alone but was reduced to 12.4- and 11.1-fold when cells were preincubated with 1 and 10 μg/mL of **13**, respectively. These effects of the two concentrations of **13** correspond with inhibition of *E. coli* LPS-induced TNFα mRNA expression of 23% and 31%, respectively. Preincubation of the cells with 1 μg/mL *R. sin*-1 LPS resulted in mRNA levels that were increased only 3.5-fold over those of control cells. Similar findings were obtained when the stimulatory concentration of *E. coli* LPS was reduced to 2 ng/mL. Preincubation of the cells with *R. sin*-1 LPS at 1 μg/mL decreased the *E. coli* LPS-induced TNFα mRNA expression by 91%, whereas preincubation with **13** at 1 and 10 μg/mL reduced TNFα mRNA expression by 29% and 46%, respectively (data not shown).

Conclusions

We have developed a highly convergent strategy for the facile synthesis of several derivatives of *Rhizobial sin*-1 lipopolysac-charides (LPS), and the biological properties of these compounds have been determined. 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 analogues. The synthetic strategy was employed for the preparation of derivatives **11** and **13**, which differ in regard to the oxidation state of the anomeric center. The synthetic compounds lack the proinflammatory effects of *Escherichia coli* LPS as indicated by the absence of expression of tumor necrosis factor (TNF)α mRNA or production of TNFα protein. Compound **13** was able to antagonize *E. coli* LPS, whereas compound **11** was devoid of this activity. On the basis of 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 is significant, because **13** is the first example of a synthetic lipid A derivative that does lack phosphate and inhibits cytokine production initiated by *E. coli* LPS. In this respect, compounds containing phos-phates are less attractive candidates for drug development, because of their instabilities. Although antagonism of *E. coli* LPS-induced cytokine production was greatest with the natural and heterogeneous *R. sin*-1 LPS, it is to be expected that, by modifying the lipid pattern or addition of a KDO moiety, compounds can be obtained that are equally or more potent than the *R. sin*-1 LPS.

Experimental Section

General Synthetic Methods. Column chromatography was per-formed 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−CH₂Cl₂, 1/1, v/v elution) or G-25 (water elution) columns. HPLC chromatography was performed on a Prodigy 5μ Silica 100 Å column (250 mm × 10 mm, CH₂Cl₂−ethyl acetate elution). Reactions were monitored by thin-layer chromatography (TLC) on Kieselgel 60 F₂₅₄ (EM Science), and the compounds were detected by examination under UV light and by charring with 10% sulfuric acid in MeOH. Solvents were removed under reduced pressure at <40 °C. CH₂Cl₂, (ClCH₂)₂, and MeCN were distilled from CaH₂ (twice) and stored over molecular sieves (3 Å). 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 monoantennary lipids at C-2, C-3, and C-3′. Signals marked with an asterisk may be interchangeable.

Synthesis of Phenyl 3-*O*-Acetyl-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-1-seleno-β-D-glucopyranoside (2). Acetic anhydride (10 mL) was added dropwise 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, coevaporated with toluene (3 times, 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 a diethyl ether−hexane mixture. $R_f = 0.51$ (ethyl acetate/hexane, 2/3, v/v); $[\alpha]^{26}_D$ +2.6° (c 0.48, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 7.18−7.90$ (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, $CDCl_3$): $\delta = 169.8$ (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) for $C_{29}H_{25}NO_7SeNa$: calcd, 602.0696; found, 602.0697. (NMR data given graphically in the Supporting Information.)

Synthesis of 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 ZnI_2 (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 and then diluted with DCM (35 mL); the solids then were filtered-off through a pad of Celite and the residue was washed with DCM (3 times, 10 mL). The combined filtrate was washed with saturated aqueous $NaHCO_3$ (3 times, 50 mL) and brine (40 mL). The organic phase was dried ($MgSO_4$) and 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 mL) for 10 min at room temperature; the mixture then was diluted with DCM (75 mL) and washed with water (50 mL), saturated aqueous $NaHCO_3$ (2 times, 30 mL), and water (3 times, 40 mL). 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 (3% gradient ethyl acetate in hexane) to afford **4** as a white foam (1.15 g, 69%): $R_f = 0.25$ (ethyl acetate/hexane, 3/7, v/v); $[\alpha]^{26}_D$ +99.4° (c, 1.16, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 7.26−7.51$ (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, $J_2 = 12.0$ Hz, CH_2-Ph), 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, $CDCl_3$): $\delta = 169.8$ (C=O), 127.7−137.5 (aromatic), 86.9 (C-1), 75.8 (C-4), 74.7 (*C*H$_2$Ph), 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) for $C_{21}H_{23}N_3O_5SNa$: calcd, 452.1256; found 452.0983. 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). (NMR data given graphically in the Supporting Information.)

Synthesis of Phenyl 3-*O*-Acetyl-6-*O*-(3-*O*-acetyl-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-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 (4 Å, 200 mg) in DCM (1.5 mL) was stirred for 2 h under an atmosphere of argon at room temperature. Silver trifluoromethanesulfonate (216 mg, 0.84 mmol) and potassium carbonate (580 mg, 0.84 mmol) then 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 times, 10 mL). The combined filtrate (40 mL) was washed with aqueous saturated $NaHCO_3$ (15 mL) and H_2O (3 times, 20 mL). 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 (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° (c, 0.70, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 6.87−7.56$ (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 × COCH_3). ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 123.85−135.43$ (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$_2$Ph), 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 × CO*C*H$_3$). HR MS (m/z) for $C_{44}H_{42}N_4O_{12}SNa$: calcd, 873.2418; found, 873.2286. (NMR data given graphically in the Supporting Information.)

Synthesis of (R)-3-Octacosanoyloxy-hexadecanoic Acid (6). 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) refluxed for 2 h, in pyridine (15 mL) and (dimethylamino)-pyridine (DMAP) (cat.), was stirred for 16 h. The reaction then was quenched with MeOH (5 mL) and concentrated under reduced pressure; the residue was dissolved in DCM (50 mL) and washed with 1 M aqueous HCl (2 times, 20 mL) and brine (2 times, 20 mL). 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 (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, $CDCl_3$): $\delta = 7.70$ (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 °C. The reaction mixture was stirred for 2 h at 60 °C and then diluted with DCM (10 mL); the solids were filtered off through a pad of Celite, and the residue was washed with DCM (3 times, 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 **6** as a white solid (110 mg, 97%): $R_f = 0.40$ (ethyl acetate/toluene, 1/4, v/v); $[\alpha]^{26}_D$ −8.0° (c, 0.64, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 5.21$ (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). HR MS (m/z) for $C_{44}H_{86}O_4$: calcd, 678.6526; found, 678.6489. (NMR data given graphically in the Supporting Information.)

Synthesis of 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 (7). To a solution of

5 (100 mg, 0.117 mmol) in *n*-butanol (10 mL), ethylenediamine (2 mL, 30 mmol) was added and stirred for 20 h at 90 °C. The reaction mixture was concentrated in vacuo to dryness and coevaporated with toluene (2 times, 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 a 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 **6** (190.5 mg, 0.281 mmol) in DCM (5 mL) and stirred for 10 min, followed by the addition of the previously mentioned amino derivative (178.7 mg, 0.281 mmol) in DCM (3 mL). The reaction mixture was stirred for 16 h at room temperature, the solids were filtered off, and the residue was washed with DCM (2 times, 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 **7** as a white solid (340 mg, 93%): $R_f = 0.35$ (diethyl ether/DCM, 3/17, v/v). ^{1}H NMR (300 MHz, CDCl$_3$): $\delta = 7.26-7.52$ (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, C*H*$_2$-Ph), 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, $J_{2L^a,3L} = 3.3$ Hz, $J_{2L^a,2L^b} = 14.5$ 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$_3$): $\delta = 127.0-133.0$ (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$_2$Ph), 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$_{L'}$). HR MS (*m/z*) for C$_{76}$H$_{120}$N$_4$O$_{11}$SNa: calcd, 1319.8572; found, 1319.8512. (NMR data given graphically in the Supporting Information.)

Synthesis of (*R*)-3-Octacosanoyloxy-hexadecanoic Acid (8). Trifluoromethanesulfonic acid (TfOH) (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 °C. The reaction mixture was stirred for 22 h and then diluted with DCM (35 mL) and washed with saturated aqueous NaHCO$_3$ (2 times, 20 mL) and water (2 times, 20 mL). 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 (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$_3$): $\delta = 7.20-7.80$ (m, 9H, aromatic), 5.26 (ps, 2H, CH$_2$), 4.56 (dd, 2H, $J_2 = 11.4$ Hz, C*H*$_2$Ph), 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). The previously described synthesized benzyloxy-hexadecanoate (295 mg, 0.523 mmol) was subjected to 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); [α]$^{26}_D$ −8.2° (c 1.0, CHCl$_3$). ^{1}H NMR (300 MHz, CDCl$_3$): $\delta = 7.30-7.40$ (m, 5H, aromatic), 4.58 (ps, 2H, CH$_2$Ph), 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). HR MS (*m/z*) for C$_{23}$H$_{38}$O$_3$: calcd, 362.2821; found, 362.2842. (NMR data given graphically in the Supporting Information.)

Synthesis of 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 (9). Triethylamine (∼1.25 mL) was added to a stirred

solution of **7** (755 mg, 0.583 mmol) and 1,3-propanedithiol (1.2 mL, 11.65 mmol) in pyridine (41 mL) and H$_2$O (5.8 mL). The reaction mixture was stirred for 16 h at room temperature and then evaporated in vacuo to dryness and coevaporated with toluene (2 times, 10 mL) and ethanol (2 times, 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 a colorless syrup (693 mg, 94%): $R_f = 0.45$ (methanol/DCM, 1/9, v/v). ^{1}H NMR (300 MHz, CDCl$_3$): $\delta = 7.22-7.58$ (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, C*H*$_2$Ph), 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$_3$): $\delta = 125.00-132.00$ (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$_2$Ph), 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$_L$-16,28$_{L'}$). DCC (43 mg, 0.21 mmol) and DMAP (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 previously mentioned amino derivative (45 mg, 0.035 mmol) in DCM (1.5 mL). The reaction mixture was stirred for 16 h at room temperature, the solids were filtered off, and the residue was washed with DCM (2 times, 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 **9** as a white solid (51.2 mg, 63%): $R_f = 0.40$ (ethyl acetate/DCM, 1/9, v/v); [α]$^{26}_D$ +14.9° (c 1.4, CHCl$_3$). ^{1}H NMR (300 MHz, CDCl$_3$): $\delta = 7.00-7.41$ (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 × 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 × 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 × H-2$_{Sa}$,2$_{Sb}$], 1.05−1.63 (m, 146H, H-(4$_L$−15$_L$), (3$_{L'}$−27$_{L'}$), 3 × H-(4$_S$−15$_S$)), 0.85 (m, 15H, H-16$_L$,28$_{L'}$, 3 × H-16$_S$). ^{13}C NMR (75 MHz, CDCl$_3$): $\delta = 125.0-131.5$ (aromatic), 101.2 (>*C*HPh), 100.9 (C-1′), 87.0 (C-1), 78.0 (C-4′), 75.9 (C-4, 2 × 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 × *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), 32.0−42.2 (C-2$_L$,2$_{L'}$, 3 × C-2$_S$), 29.9 [C-(4$_L$−15$_L$), (3$_{L'}$−27$_{L'}$), 3 × H-(4$_S$−15$_S$)], 14.3 (C-16$_L$,28$_{L'}$, 3 × C-16$_S$). HR MS (*m/z*) for C$_{145}$H$_{230}$N$_2$O$_{17}$SNa: calcd, 2326.6847; found, 2326.7550. (NMR data given graphically in the Supporting Information.)

Synthesis of 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 (10). *N*-Iodosuccinimide (95 mg, 0.42 mmol) and TfOH (3.5 μL, 0.04 mmol) were added to a stirred solution of **9** (312 mg, 0.135 mmol) in DCM/H$_2$O (10 mL, 100:1) at 0 °C. The reaction mixture was vigorously stirred for 30 min at 0 °C until TLC analysis indicated that the reaction had gone to completion. The reaction mixture was diluted with DCM (20 mL) and washed with aqueous Na$_2$S$_2$O$_3$ (20%, 20 mL) and water (3 times, 20 mL). 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 (1% gradient methanol in DCM) to afford **10** as a white solid (250 mg, 84%): $R_f = 0.45$ (methanol/DCM, 1/19, v/v). ^{1}H NMR (300 MHz, CDCl$_3$): $\delta = 7.00-7.38$ (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, >CHPh), 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 × 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,6'b,4',2',5'), 3.35 (t, 1H, $J_{4,5} = 9.8$ Hz, H-4), 1.90−2.64 [m, 10H, H-2$_{L^a,2L^b,2L'^a,2L'^b}$, 3 × H-2$_{Sa}$, 3 × 2$_{Sb}$], 1.05−1.63 (m, 146H, H-(4$_L$−15$_L$), (3$_{L'}$−27$_{L'}$), 3 × H-(4$_S$−15$_S$)], 0.85 (m, 15H, H-16$_L$,28$_{L'}$, 3 × H-16$_S$). ^{13}C NMR (75 MHz, CDCl$_3$): $\delta = 125.0-131.5$ (aromatic), 105.5 (C-1), 101.4 (>CHPh), 100.9 (C-1'), 78.1 (C-4'), 75.7 (C-4), 74.48 (CH$_2$Ph), 73.8 (C-3), 72.2 (C-5), 71.2 (C-3'), 70.4−70.7 (3 × CH$_2$Ph), 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 × C-2$_S$), 29.88 [C-(4$_L$−15$_L$), (3$_{L'}$−27$_{L'}$), 3 × H-(4$_S$−15$_S$)], 14.33 (C-16$_L$,28$_{L'}$, 3 × C-16$_S$). HR MS (*m/z*) for C$_{139}$H$_{226}$N$_2$O$_{18}$-Na: calcd, 2234.6728; found, 2234.6657. (NMR data given graphically in the Supporting Information.)

Synthesis of 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]-α-D-glucopyranose (11). The lactol **10** (30 mg, 0.135 mmol) was dissolved in THF−EtOH (2 mL, 1/1, v/v) and Pd/C (30 mg) and 2 M aqueous HCl (50 μL) were added. The reaction mixture was stirred under H$_2$ for 40 h at room temperature. The catalyst then was filtered off and washed subsequently with THF (2 times, 10 mL), EtOH (2 times, 5 mL), and DCM (2 times, 10 mL), and the filtrate was concentrated. The residue was purified by silica gel column chromatography (2% gradient methanol in DCM) and size exclusion column chromatography on a Sephadex model LH-20 column (*i*-PrOH/DCM, 1:1, v/v) to afford **11** as a white solid (15.9 mg, 67%): [α]^{26}$_D$ −20.7° (c 0.56, CHCl$_3$). ^{1}H NMR (500 MHz, CDCl$_3$): $\delta = 4.93$ (dd, 1H, $J_{3,4} = 8.8$ Hz, H-3), 4.81−4.90 (m, 2H, H-3$_L$, H-1), 4.71 (d, 1H, $J_{1',2'} = 8.0$ Hz, H-1'), 3.70−3.85 (m, 5H, H-2,5,6a, 2 × H-3$_S$), 3.57−3.65 (m, 3H, H-2',6'a,3$_S$), 3.45−3.52 (m, 2H, H-6b,6b'), 3.60 (dd, 1H, $J_{4',5'} = 9.3$ Hz, H-4'), 3.49 (dd, 1H, $J_{4,5} = 9.3$ Hz, H-4), 3.12−3.19 (m, 1H, H-5'), 1.95−2.33 (m, 10H, H-2$_{L^a,2L^b,2L'^a,2L'^b}$, 3 × H-2$_{Sa,2Sb}$), 1.02−1.73 [m, 146H, H-(4$_L$−15$_L$), (3$_{L'}$−27$_{L'}$), 3 × H-(4$_S$−15$_S$)], 0.63 (m, 15H, H-16$_L$,28$_{L'}$, 3 × 16$_S$). ^{13}C NMR (75 MHz, CDCl$_3$): $\delta = 105.61$ (C-1'), 95.55 (C-1), 80.01 (C-3',5'), 78.36 (C-3), 75.44 (C-3$_L$), 74.61 (C-5), 73.08 (C-6), 72.88 (C-4), 71.50−72.86 (C-4', 3 × C-3$_S$), 66.04 (C-6'), 57.70 (C-2'), 56.39 (C-2), 44.76−46.20 (C-2$_L$,2$_{L'}$, 3 × C-2$_S$), 28.00−37.00 [C-(4$_L$−15$_L$), (3$_{L'}$−27$_{L'}$), 3 × C-(4$_S$−15$_S$)], 18.07 (C-16$_L$,28$_{L'}$, 3 × C-16$_S$). HR MS (*m/z*) for C$_{104}$H$_{198}$N$_2$O$_{18}$Na: calcd, 1786.4537; found, 1786.4683. (NMR data given graphically in the Supporting Information.)

Synthesis of 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 (12). A mixture of **10** (14.0 mg, 6.3 μmol) and activated molecular sieves (3 Å, 20 mg) in DCM (1.0 mL) was stirred for 2 h at room temperature under an atmosphere of argon. Pyridinium chlorochromate (7 mg, 0.03 mmol) then was added and the reaction mixture was stirred for 1 h until TLC analysis indicated that the reaction had gone to completion. The reaction mixture was placed directly onto a Florisil column, where it was purified by elution with *i*-PrOH/DCM (1:1) to afford **12** as a colorless film (12.3 mg, 88%): $R_f = 0.55$ (diethyl ether/DCM, 1/4, v/v). ^{1}H NMR (500 MHz, CDCl$_3$): $\delta = 7.13-7.31$ (m, 25H, aromatic), 5.60 (t, 1H, $J_{3',4'} = 9.8$ Hz, H-3'), 5.39 (s, 1H, >CHPh), 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 × 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 ×

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$_{L^a,2L^b,2L'^a,2L'^b}$, 3 × H-2$_{Sa,2Sb}$), 1.00−1.75 [m, 146H, H-(4$_L$−15$_L$), (3$_{L'}$−27$_{L'}$), 3 × H-(4$_S$−15$_S$)], 0.62 (m, 15H, H-16$_L$,28$_{L'}$, 3 × H-16$_S$). ^{13}C NMR (75 MHz, CDCl$_3$): $\delta = 125.0-128.2$ (aromatic), 101.2 (C-1'), 100.8 (>CHPh), 78.3 (C-4',5), 74.9 (3 × C-3$_S$), 73.5 (CH$_2$Ph, C-4), 71.3 (C-3), 70.6 (C-3', CH$_2$Ph), 70.4 (2 × CH$_2$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), 37.0−40.3 [C-2$_L$,2$_{L'}$, 3 × C-2$_S$], 20.3−33.8 (C-(4$_L$−15$_L$), (3$_{L'}$−27$_{L'}$), 3 × C-(4$_S$−15$_S$)], 13.9 (C-16$_L$,28$_{L'}$, 3 × C-16$_S$). HR MS (*m/z*) for C$_{139}$H$_{224}$N$_2$O$_{18}$Na: calcd, 2232.6572; found, 2232.6703. (NMR data given graphically in the Supporting Information.)

Synthesis of 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 (13). The lactone **12** (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 room temperature. The catalyst then was filtered off and washed successively with THF (2 times, 10 mL), EtOH (2 times, 5 mL), and DCM (2 times, 10 mL), and the combined filtrate was concentrated. The residue was purified by Florisil column chromatography (2% gradient *i*-PrOH in DCM) and size exclusion column chromatography on a Sephadex model LH-20 column (*i*-PrOH/DCM, 1:1, v/v) to afford **13** as a colorless film (2.1 mg, 33%): $R_f = 0.35$ (MeOH/DCM, 1/9, v/v); [α]^{26}$_D$ −26.52° (c 0.22, CHCl$_3$). ^{1}H NMR (600 MHz, CDCl$_3$/CD$_3$OD, 1/1, v/v): $\delta = 5.39$ (t, 1H, $J_{3,4} = 9.8$ Hz, H-3), 5.09 (m, 1H, H-3$_L$), 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, $J_{2,3} = 10.3$ Hz, H-2), 4.13 (m, 1H, H-6a), 3.95 (m, 1H, H-4), 3.81−3.85 (m, 2H, H-2', H-6b'), 3.70−3.76 (m, 1H, H-6a', H-6b), 3.54 (t, 1H, $J_{4',5'} = 9.3$ Hz, H-4'], 3.33 (m, 1H, H-5'), 2.13−2.55 (m, 10H, H-2$_{L^a,2L^b,2L'^a,2L'^b}$, 3 × H-2$_{Sa,Sb}$), 1.02−1.73 [m, 146H, H-(4$_L$−15$_L$), (3$_{L'}$−27$_{L'}$), 3 × H-(4$_S$−15$_S$)], 0.63 (m, 15H, H-16$_L$,28$_{L'}$, 3 × 16$_S$). HR MS (*m/z*) for C$_{104}$H$_{196}$N$_2$O$_{18}$Na: calcd, 1784.4381; found, 1784.4586. (NMR data given graphically in the Supporting Information.)

Reagents for Biological Experiments. *E. coli* 055:B5 LPS was obtained from List Biologicals, and PMA was obtained from Sigma. *R. sin*-1 LPS was kindly provided by Dr. R. Carlson (Complex Carbohydrate Research Center, 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 THF on the day of the experiment; final THF concentrations 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 μg/mL penicillin, 100 μg/mL streptomycin, 1% OPI supplement (containing oxaloacetate, pyruvate, and bovine insulin; supplied by Sigma), and 10% fetal calf serum (FCS; supplied by HyClone). The cells were maintained in a humid 5% CO$_2$ atmosphere at 37 °C. New batches of frozen cell stock were grown every 2 months and the growth morphology was evaluated. Before each experiment, the Mono Mac 6 cells were incubated with 10 ng/mL calcitriol (Sigma) for 2 days, to increase expression of CD14.

ELISA TNFα. Cells were harvested by centrifugation and gently suspended (10^6 cells/mL) in a prewarmed (37 °C) medium. Cells were then incubated with different combinations of stimuli for 6 h, as described below. Cell supernatants were then collected and stored frozen (−80 °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 antihuman TNFα antibody (Pharmingen). TNFα in standards and samples was allowed to bind to the immobilized antibody for 2 h at room temperature. Biotinylated

mouse antihuman TNFα antibodies (Pharmingen) were then added, producing an antibody−antigen−antibody "sandwich". After the addition of avidin−horseradish peroxidase conjugate (Pharmingen) and ABTS peroxidase substrate (Kirkegaard & Perry Laboratories), a green color was produced, the intensity of which was 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 mean values, with the standard deviation (±SD), of duplicate cultures, with each experiment being repeated three times.

Preparation of RNA and Quantification of TNFα mRNA by Real-Time Polymerase Chain Reaction Analysis. Cells were harvested by centrifugation and gently suspended (1.25×10^6 cells/mL) in a prewarmed (37 °C) medium. Cells were then incubated with the indicated concentrations of the stimuli for 1.5 h, after which time cells were harvested and the total RNA was isolated with the Absolutely RNA RT−PCR Miniprep Kit (Stratagene), according to the manufacturer's protocol. TNFα gene expression was quantified in a two-step reverse transcription−polymerase chain reaction (RT−PCR). In the RT step, cDNA was reverse-transcribed from each total RNA sample (625 ng/50 μL) using random hexamers from the TaqMan RT reagents (Applied Biosystems). In the PCR step, products were synthesized from cDNA (22.5 ng/20 μL), using the Taqman universal PCR master mix and TaqMan predeveloped assay reagents for human TNFα (Applied Biosystems). Measurements were performed using the ABI Prism 7900 HT sequence detection system (Applied Biosystems), according to the manufacturer's protocols. As an endogenous control for these PCR quantification studies, 18S ribosomal RNA gene expression was measured using the TaqMan ribosomal RNA control reagents (Applied Biosystems). Results represent mean values (±SD) of triplicate measurements, with each experiment being repeated three times.

Supporting Information Available: Various NMR spectra of **2**, **4**−**13** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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