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Innate Immune Responses of Synthetic Lipid A Derivatives of *Neisseria meningitidis*

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Abstract

Differences in the pattern and chemical nature of fatty acids of lipid A of *Neisseria meningitidis* lipooligosaccharides (LOS) and *Escherichia coli* lipopolysaccharides (LPS) may account for differences in inflammatory properties. Furthermore, there are indications that dimeric 3-deoxy-D-manno-oct-2-ulosonic acid (KDO) moieties of LOS and LPS enhance biological activities. Heterogeneity in the structure of lipid A and possible contaminations with other inflammatory components have made it difficult to confirm these observations. To address these problems, a highly convergent approach for the synthesis of a lipid A derivative containing KDO has been developed, which relies on the ability to selectively remove or unmask in a sequential manner an isopropylidene acetal, 9-fluorenylmethoxycarbonyl (Fmoc), allyloxycarbonate (Alloc), azide, and thexyldimethylsilyl (TDS) ether. The strategy was employed for the synthesis of *N. meningitidis* lipid A containing KDO (**3**). Mouse macrophages were exposed to the synthetic compound and its parent LOS, *E. coli* lipid A (**2**), and a hybrid derivative (**4**) that has the asymmetrical acylation pattern of *E. coli* lipid A, but the shorter lipids of meningococcal lipid A. The resulting supernatants were examined for tumor necrosis factor alpha (TNF- α) and interferon beta (IFN- β) production. The lipid A derivative containing KDO was much more active than lipid A alone and just slightly less active than its parent LOS, indicating that one KDO moiety is sufficient for full activity of TNF- α and IFN- β induction. The lipid A of *N. meningitidis* was a significantly more potent inducer of TNF- α and IFN- β than *E. coli* lipid A, which is due to a number of shorter fatty acids. The compounds did not demonstrate a bias towards a MyD88- or TRIF-dependent response.

Keywords

carbohydrate; glycosylation; innate immunity; KDO; lipid A

Introduction

Neisseria meningitidis is a Gram-negative bacterium that causes fulminant, rapidly fatal sepsis, and meningitis.[1] The morbidity and mortality of meningococcal bacteremia has been linked to a systematic inflammatory response to lipooligosaccharides (LOS) in the blood of affected patients.[2-4] LOS, a major component of the outer membrane of *N. meningitidis*, initiates the production of multiple host-derived inflammatory mediators such as tumor necrosis factor alpha (TNF- α), interferon beta (IFN- β), interleukin 1 (IL-1), IL-6, arachidonic acid metabolites, and leukotrienes. The production of these mediators is initiated by the interaction of LOS with Toll-like receptor 4 (TLR4), a member of the TLR family that participates in pathogen

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recognition.[5,6] Immediately distal to TLR4 activation are two intracellular cascades that regulate signal transduction processes, gene expression, and production of (pro)inflammatory mediators.[5,6] One of these cascades requires a specific intracellular adaptor protein called MyD88, while the other cascade utilizes the TRIF adaptor protein. The MyD88-dependent pathway leads to up-regulation of cytokines and chemokines such as TNF- α , IL-1 β , IL-6, and monocyte chemoattractant protein 1 (MCP-1), whereas the TRIF-dependent pathway leads to the production of IFN- β , which in turn activates the STAT-1 pathway resulting in the production of mediators such as interferon-inducible protein 10 (IP-10) and nitric oxide.[7] Although the initiation of acute inflammatory responses is important for the prevention of infections, over-activation leads to the clinical symptoms of septic shock.

Meningococcal LOS lacks the repeating O-antigen of enteric lipopolysaccharides (LPS) but has a conserved innercore region composed of L-glycero-D-manno-heptosides and a dimeric 3-deoxy-D-manno-oct-2-ulosonic acid (KDO) moiety linked to lipid A.[8,9] Furthermore, the lipid A of *N. meningitidis* is hexa-acylated in a symmetrical fashion (1) whereas enteric bacteria have an asymmetrically hexa-acylated lipid A (2) (Figure 1).[10-13] Also, a number of the fatty acids of *N. meningitidis* are shorter compared to those of *Escherichia coli*. It has been suggested that the differences in the nature and pattern of fatty acid substitution of enteric and meningococcal lipid A affects their biological properties. For example, it has been shown that at low concentrations meningococcal LOS is a potent inducer of MyD88- and TRIF-dependent cytokines, whereas at the same pmole concentrations *E. coli* LPS induced comparable levels of TNF- α , IL-1 β , and MIP-3 α but significantly less IFN- β , nitric oxide, and IP-10.[14]

It has long been thought that the inflammatory properties of LPS and LOS reside in the lipid A moiety.[13,15,16] However, recent studies have shown that lipid A expressed by meningococci with defects in KDO biosynthesis or transfer has significantly reduced bioactivities compared to KDO₂ containing meningococcal LOS.[17] Removal of the KDO moieties of wild type LOS by mild acetic acid treatment also attenuated cellular responses. Interestingly, dendritic cells stimulated with KDO₂-lipid A from meningococci but not lipid A alone stimulated naïve allogeneic CD4⁺ cells to secrete enhanced levels of IFN- γ relative to T-cells primed with immature dendritic cells.[18]

Several other studies have suggested that the KDO moiety of LPS or LOS contributes to inflammatory responses. For example, it has been found that salmonella lipid A is inactive whereas the parent LPS is a potent activator of NF- κ B in a TLR4-dependent manner in a human monocytic cell line.[19] In addition, a synthetic enteric lipid A containing a di-KDO moiety was a more potent elicitor of TNF- α and IL-6 compared to its parent lipid A.[20] Furthermore, LPS from a nitrogen-fixing symbiont, *Rhizobium sin-1* is able to significantly inhibit the *E. coli* LPS-dependent synthesis of TNF- α by human monocytic cells.[21-25] A comparison of the biological responses of synthetic and isolated lipid A derivatives and *R. sin-1* LPS indicated that the KDO moieties are important for optimal antagonistic properties. 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.

Although studies with LOS isolated from meningococci have indicated that it possesses unique immunological properties, heterogeneity in the structure of lipid A and possible contaminations with other inflammatory components of the bacterial cell wall have made it difficult to confirm these results.[14,17,18] Furthermore, the acylation pattern as well as fatty acid length differ between meningococcal and *E. coli* lipid A. Hence, it has been impossible to establish which structural feature is responsible for the unique inflammatory properties. Fortunately, homogeneous lipid A derivatives can be obtained by chemical synthesis,[13,16] an approach which makes it also possible to prepare panels of different compounds to establish which structural features are responsible for particular biological properties.

We report here the preparation of a prototypical lipid A derived from *N. meningitidis* (**1**) and a similar derivative containing a KDO moiety (**3**). Proinflammatory properties of these compounds have been determined in a mouse macrophage cell line and the results compared with similar data for lipid A **2**, which is derived from *E. coli*, and compound **4**, which has the asymmetrical acylation pattern of *E. coli* but fatty acids that are similar in length to those of *N. meningitidis* lipid A. It has been found that the lipid A of *N. meningitidis* (**1**) is a more potent inducer of TNF- α and IFN- β than *E. coli* lipid A (**2**). The greater potency was attributed to the shorter fatty acids of **1** and not to its symmetrical acylation pattern. Furthermore, the KDO moiety of **3** significantly enhanced the potency of proinflammatory responses.

Results and Discussion

Chemical Synthesis

To determine biological properties of *N. meningitidis* lipid A and LOS, we have synthesized compounds **1** and **3** (Figure 1) by a highly convergent approach. Compound **1** is a prototypical lipid A derived from *N. meningitidis* LOS, and is hexa-substituted in a symmetrical fashion. Compound **3** has a structure similar to **1**, except that the C-6' moiety of its lipid A is extended by a KDO moiety. It was envisaged that compound **3** could be prepared from monosaccharides **5** and **6**, KDO donor **7**, [26] and fatty acids **8** and **9** [27] (Scheme 1). Thus, coupling of **5** with **6** will give orthogonally protected disaccharide **15**, which will be subjected to mild acidic conditions to remove the isopropylidene without affecting any of the other protecting groups. The C-6' hydroxyl of the resulting compound can then be regioselectively glycosylated with **7** followed by phosphorylation of the C-4' hydroxyl. Next, the orthogonal protecting groups 9-fluorenylmethoxycarbonyl (Fmoc), allyloxycarbonate (Alloc), and azido will be individually removed, which allows acylation with any lipid at C-2, C-2', and C-3 to give easy access to a panel compounds differing in acylation pattern. At an early stage of the synthesis, the C-3' hydroxyl of **15** was acylated with (*R*)-3-benzyloxy-dodecanoic acid because it was observed that the C-4'-phosphate triester can migrate during acylation of the C-3' hydroxyl. After completion of the acylations, the anomeric hexyldimethylsilyl (TDS) group can be selectively cleaved and phosphorylation of the resulting lactol followed by global deprotection should provide target compound **3**.

Glycosyl acceptor **5** and donor **6** could be prepared from known derivatives **10** [28] and **12**, [29] respectively (Scheme 1). Thus, the C-3 hydroxyl of **10** was protected by an Alloc group by treatment with Alloc chloride in the presence of *N,N,N',N'*-tetramethylethylenediamine (TMEDA) [30] in DCM to give **11** in a yield of 96%. Regioselective reductive opening of the benzylidene acetal of **11** proved more difficult than anticipated. Conventional procedures such as treatment with $\text{BH}_3 \cdot \text{THF}$ /Bu₂BOTf or $\text{BH}_3 \cdot \text{THF}$ /TMSOTf [31] resulted in a loss of the Alloc group. Fortunately, the reaction of **11** with triethyl silane and PhBCl_2 in the presence of molecular sieves at -75°C [32] gave **5** in an excellent yield of 94% as only the C-6 hydroxyl.

The C-3 hydroxyl of **12** was acylated with (*R*)-3-benzyloxy-dodecanoic acid (**8**) using 1,3-dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP) as the activation reagents to give **13** in an excellent yield of 95%. Next, the azido function of **13** was reduced with zinc in a mixture of acetic acid and DCM. The resulting amine was immediately protected as an Fmoc carbamate by reaction with FmocCl in the presence of diisopropylethylamine (DIPEA) to give fully protected **14**. Removal of the anomeric TDS ether of **14** was achieved by treatment with tetrabutylammonium fluoride (TBAF) buffered with acetic acid in THF followed by conversion of the resulting lactol into trichloroacetimidate **6** ($\alpha/\beta \sim 1:1$) by reaction with trichloroacetonitrile in the presence of a catalytic amount of NaH. [33]

A TMSOTf-mediated glycosylation [34] of **5** with **6** in the presence of molecular sieves (4 Å) in DCM at -40°C gave disaccharide **15** in a modest yield of 42%. Surprisingly, only the β -

anomer of trichloroacetimidate **6** had been consumed while the α -anomer remained intact, even when the temperature or amount of TMSOTf was increased. An attempt to prepare selectively the β -anomer of **6** using Cs_2CO_3 as the base resulted unexpectedly in the formation of the α -anomer as the predominant product. Fortunately, the use of trifluoromethanesulfonic acid (TfOH) instead of TMSOTf could drive the glycosylation to completion with consumption of both the α - and β -anomer and provided disaccharide **15** in an excellent yield of 94%. Next, the isopropylidene group of **15** was removed with trifluoroacetic acid (TFA) in wet DCM to yield **16** in an almost quantitative yield.

Glycosylation of **16** with KDO donor **7** was carried out with the aid of $\text{BF}_3 \cdot \text{OEt}_2$ in the presence of MS 4 Å in DCM to afford an inseparable mixture of **17** and its β -anomer ($\alpha/\beta = 9/1$) in a combined yield of 67% (Scheme 2). The anomeric configuration of the KDO glycosides was established by comparing the difference between the chemical shift values of the C-3 methylene protons.[35] In this respect, a larger difference between the chemical shift values of C-3 methylene protons is observed for α -anomers of KDO glycosides that reside in a boat conformation, as compared to similar values for the corresponding β -anomers. In the case of KDO glycosides that adopt a chair form, a large chemical shift difference is observed for β -anomers whereas the α -anomer provides a small difference.[36] After careful examination of the NMR spectra of the glycosylation products, it was found that the chemical shift difference of the C-3 methylene protons of the major product was 0.59 ppm while the corresponding difference was 0.12 ppm for the minor product. Furthermore, the KDO glycosides adopted boat conformations due to protection of the C-4,5 diol as an isopropylidene acetal, which was confirmed by the coupling constants of the C-3 methylene protons with H-4 ($J_{\text{H}3\text{a},\text{H}4} = J_{\text{H}3\text{e},\text{H}4} = 4.8 \text{ Hz}$). Thus, the data imply that the major product has an α -anomeric configuration. To provide additional evidence of the assigned anomeric configurations, the isopropylidene acetal of glycosylation product **16** was removed by treatment with TFA in DCM. NMR data of the resulting compound showed that the chemical shift difference of the C-3 methylene protons of the major product had decreased to 0.08 ppm while the corresponding difference increased to 0.39 ppm for the minor product. Furthermore, the coupling constants of the C-3 methylene protons with H-4 established that the glycosides adopt a chair conformation. Thus, these data provide convincing evidence that the major product has an α -anomeric configuration.

Phosphilation of **17** with *N,N*-diethyl-1,5-dihydro-2,3,4-benzodioxaphosphepin-3-amine in the presence of 1*H*-tetrazole followed by *in-situ* oxidation with *m*-chloroperoxybenzoic acid (mCPBA)[37] gave **18**. Fortunately, at this stage of the synthesis the α - and β -anomer could be separated by silica gel column chromatography. Interestingly, the chemical shift difference of the methylene protons of compound **18** and that of its β -anomer were both very small, an observation that may be due to the deshielding effects of the C-3 methylene protons by the aromatic group of the phosphate diester. As a matter of fact, it has been noted that the empirical rule described above does not apply to all subsequent KDO derivatives protected as 4,5-isopropylidene acetals. To provide additional information, the isopropylidene acetal of **18** and its β -anomer were removed. NMR data showed that the chemical shift difference of the C-3 methylene protons was 0.10 while the corresponding difference increased to 0.37 for the α -anomer, an observation that provided an additional piece of evidence to convincingly show that the major product is an α -anomer, because when the isopropylidene acetal was removed, the KDO existed as a chair form. The chair form was demonstrated by the large coupling constant (12.0 Hz) between H-4 and H-3_{ax}.

Having the advanced trisaccharide **18** in hand, attention was focused on the selective acylation of relevant hydroxyls and amines. Thus, the Fmoc protecting group of **18** was removed using 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in DCM and the resulting amino group acylated with (*R*)-3-dodecanoyl-tetradecanoic acid (**9**) using DCC as the activating agent to give compound **19**. Removal of the Alloc group of **19** was easily accomplished by treatment with

Pd(PPh₃)₄ in the presence of BuNH₂ and HCOOH,[38] and subsequent acylation of the resulting hydroxyl with **8** using DCC and DMAP as activating agent afforded **20**. Next, reduction of the azido function of **20** with zinc in a mixture of acetic acid and DCM followed by acylation of the resulting amine with **9** using standard conditions furnished fully acylated **21**.

Next, the isopropylidene acetal and anomeric TDS of **21** were removed by treatment with TFA/H₂O (3/2, v/v) in DCM and the anomeric hydroxyl of the resulting compound was regioselectively phosphorylated using tetrabenzyl diphosphate in the presence of lithium bis(trimethyl)silylamide in THF at -78°C[39] to give anomeric phosphate **22** as only the α -anomer. Finally, global deprotection of **22** could easily be accomplished by catalytic hydrogenolysis over Pd-black to give target product **3**.

Lipid A derivative **1** could easily be prepared by starting from previously reported[40] compound **23** (Scheme 3). Thus, the azido function of **23** was reduced with activated Zn in a mixture of acetic acid and DCM and the amine of the resulting compound was reacted with (*R*)-2-dodecanoyloxy-tetradecanoic acid (**9**) in the presence of DCC to give **24**. The removal of the Alloc protecting group of **24** could easily be accomplished by treatment with Pd(PPh₃)₄ and the hydroxyl group of the resulting compound **25** was acylated with (*R*)-2-benzyloxy-tetradecanoic acid (**8**) using DCC and DMAP as activating agents to afford fully acylated **26**. The anomeric *tert*-butyldimethylsilyl (TBS) ether of **26** was removed by treatment with HF in pyridine and the resulting anomeric hydroxyl phosphorylated using tetrabenzyl diphosphate in the presence of lithium bis(trimethyl)silylamide in THF at -78°C[39] to give **27**. Global deprotection of **27** by catalytic hydrogenolysis gave the requisite lipid A **1**.

Biological Evaluation

Based on the results of recent studies[5,6] it is clear that LPS-induced cellular activation through TLR4 is complex as many signaling elements are involved. However, it appears that there are two distinct initiation points in the signaling process, one being a specific intracellular adaptor protein called MyD88 and the other an adaptor protein called TRIF, which operates independently of MyD88. It is well established that TNF- α secretion is a prototypical measure for activation of the MyD88-dependent pathway, whereas secretion of IFN- β is commonly used as an indicator of TRIF-dependent cellular activation.

There are some indications that structurally different lipid As can differentially utilize signal transduction pathways leading to complex patterns of proinflammatory responses. For example, it has been suggested that meningococcal LOS is a potent inducer of MyD88- and TRIF-dependent cytokines, whereas at the same pmole concentrations *E. coli* LPS induced comparable levels of MyD88 derived cytokines but significantly less TRIF-associated cytokines.[14,17] In addition, there are indications that the KDO moieties of meningococcal LOS are required for optimal biological properties.[18]

To address these issues, we have examined the well-defined compounds **1-4** and *E. coli* LPS for the ability to initiate production of TNF- α and IFN- β . Compounds **1** and **2**[40] are prototypical lipid As derived from *N. meningitidis* LOS and *E. coli* LPS, respectively. Both compounds are hexa-acylated but differ in the nature and substitution pattern of fatty acids. Compound **3** has a structure similar to **1**, except that the C-6' moiety of its lipid A is extended by a KDO moiety. Compound **4**[40] is a hybrid derivative that has the asymmetrical acylation pattern of *E. coli* lipid A but the shorter lipids of meningococcal lipid.

Mouse macrophages (RAW 264.7 γ NO(-) cells) were exposed over a wide range of concentrations to compounds **1-4** and meningococcal LOS. After 5.5 hours, the supernatants were harvested and examined for mouse TNF- α and IFN- β using a commercial and in-house

developed capture ELISA, respectively. Potencies (EC_{50} , concentration producing 50% activity) and efficacies (maximal level of production) were determined by fitting the dose-response curves to a logistic equation using PRISM software.

As can be seen in Figure 2, synthetic *N. meningitidis* lipid A (**1**) is significantly more potent inducer of TNF- α and IFN- β than *E. coli* lipid A (**2**). Furthermore, the difference in EC_{50} values of the hybrid derivative **4** and *N. meningitidis* lipid A (**1**) is very small (Table 1). Thus, the data suggest that the shorter fatty acids of *N. meningitidis* lipid A are primarily responsible for its greater biological activity.

The EC_{50} values of the KDO containing derivative **3** and meningococcal LOS are very similar whereas these values are significantly smaller than those of lipid A **1**. These results demonstrate the importance of the core region of LOS for biological activity and that one KDO moiety is sufficient for full activity of TNF- α and IFN- β induction by LOS.

Finally, a comparison of the EC_{50} values of TNF- α and IFN- β for each compound indicated that the values for TNF- α are slightly smaller than those of IFN- β (2 - 6 fold), indicating a somewhat higher potency for TNF- α production. No significant differences were observed between efficacies of secreted TNF- α and IFN- β . Thus, it appears that for the compounds tested there is no clear bias towards a MyD88- or TRIF-dependent response. It may be possible that previously observed bias may be due to contaminants or, alternatively, due to minor lipid A components having unique acylation substitutions or patterns.

Conclusion

A convergent approach for the synthesis of a lipid A derivative containing KDO has been developed, which allows for the convenient synthesis of a panel of analogues differing in fatty acid acylation patterns and degree of phosphorylation. The new synthetic approach relies on the ability to selectively remove or unmask in a sequential manner an isopropylidene acetal, a Fmoc carbamate, an Alloc carbonate, azide, and TDS ether. The strategy was employed for the synthesis of *N. meningitidis* lipid A containing KDO (**3**). The compound was tested for cytokine production along with the synthetic *N. meningitidis* lipid A (**1**), its parent LOS, *E. coli* lipid A (**2**), and a hybrid derivative (**4**) that has the asymmetrical acylation pattern of *E. coli* lipid A but the shorter lipids of meningococcal lipid A. Examination of potencies and efficacies of TNF- α and IFN- β production showed that the lipid A derivative containing KDO was much more active than lipid A alone and just slightly less active than its parent LOS, indicating that one KDO moiety is sufficient for full activity of TNF- α and IFN- β production. It still needs to be established whether the increase in activity is due to specific interactions with relevant cell surface receptors or due to alterations in pharmacokinetic properties. It has also been found that the lipid A of *N. meningitidis* is a significantly more potent inducer of TNF- α and IFN- β than *E. coli* lipid A, which is attributed to a number of shorter fatty acids. For each compound, the values for TNF- α were only slightly smaller than those for IFN- β whereas no significant differences were observed between the efficacies. Thus, the compounds tested do not demonstrate a clear bias towards a MyD88- or TRIF-dependent response.

Experimental Section

General synthetic methods

Column chromatography was performed on silica gel 60 (EM Science, 70-230 mesh). Reactions were monitored by thin-layer chromatography (TLC) on Kieselgel 60 F254 (EM Science), and compounds were detected by examination under UV light and by charring with 10% sulfuric acid in MeOH. Solvents were removed under reduced pressure at $< 40^{\circ}\text{C}$. CH_2Cl_2 was distilled from NaH and stored over molecular sieves (3 Å). Tetrahydrofuran (THF)

was distilled from sodium directly prior to application. MeOH was dried by refluxing with magnesium methoxide and then was distilled and stored under argon. Pyridine was dried by heating under refluxing over CaH₂ and then 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 and then for 2-3 h at 390°C directly prior to application. Optical rotations were measured using a Jasco model P-1020 polarimeter. ¹H NMR and ¹³C NMR spectra were recorded on Varian spectrometers (models Inova500 and Inova600) equipped with Sun workstations. ¹H NMR spectra were recorded in CDCl₃ and referenced to residual CHCl₃ at 7.24 ppm, and ¹³C NMR spectra were referenced to the central peak of CDCl₃ at 77.0 ppm. Assignments were made by standard gCOSY and gHSQC. High resolution mass spectra were obtained on a Bruker model Ultraflex MALDI-TOF mass spectrometer. Signals marked with a subscript L belong to the biantennary lipids, whereas signals marked with a subscript L' belong to their side chain. Signals marked with a subscript S symbol belong to the monoantennary lipids.

Dimethylthexylsilyl 3-O-allyloxycarbonyl-2-azido-4,6-O-benzylidene-2-deoxy-β-D-glucopyranoside (**11**)

To a cooled (0°C) solution of compound **10** (1.25 g, 2.87 mmol) and *N,N,N',N'*-tetramethylethylenediamine (TMEDA) (281 μL, 1.87 mmol) in DCM (10 mL) was added dropwise allyl chloroformate (366 μL, 3.44 mmol). The reaction mixture was stirred at room temperature for 3 h, and then diluted with DCM (20 mL) and washed with saturated aqueous NaHCO₃ (2 × 20 mL) and brine (2 × 20 mL). The organic phase was dried (MgSO₄), filtered, and the filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/ethyl acetate, 30/1, v/v) to give **11** as a colorless oil (1.43 g, 96%). *R*_f = 0.60 (hexane/ethyl acetate, 5/1, v/v); [α]_D²⁵ = -34.9° (*c* = 1.0, CHCl₃). ¹H NMR (500 MHz, CDCl₃): δ 7.42-7.32 (m, 5H, aromatic), 5.98-5.86 (m, 1H, OCH₂CH=CH₂), 5.47 (s, 1H, >CHPh), 5.33 (d, 1H, *J* = 17.0 Hz, OCH₂CH=CHH), 5.22 (d, 1H, *J* = 11.0 Hz, OCH₂CH=CHH), 4.87 (t, 1H, *J*_{2,3} = *J*_{3,4} = 10.0 Hz, H-3), 4.69 (d, 1H, *J*_{1,2} = 7.5 Hz, H-1), 4.64 (d, 2H, *J* = 6.0 Hz, OCH₂CH=CH₂), 4.28 (dd, 1H, *J*_{5,6a} = 5.0 Hz, *J*_{6a,6b} = 10.0 Hz, H-6a), 3.76 (dd, 1H, *J*_{5,6b} = *J*_{6a,6b} = 10.5 Hz, H-6b), 3.67 (d, 1H, *J*_{3,4} = *J*_{4,5} = 9.0 Hz, H-4), 3.48-3.40 (m, 2H, H-2, H-5), 1.68-1.63 [m, 1H, CH(CH₃)₂], 0.89-0.87 [m, 12H, SiC(CH₃)₂CH(CH₃)₂], 0.19 (s, 3H, SiCH₃), 0.18 (s, 3H, SiCH₃). ¹³C NMR (75 MHz, CDCl₃): δ 154.15 (C=O), 136.75-126.17 (m, aromatic, OCH₂CH=CH₂), 119.07 (OCH₂CH=CH₂), 101.54 (>CHPh), 97.56 (C-1), 78.57 (C-4), 75.35 (C-3), 68.93 (OCH₂CH=CH₂), 68.57 (C-6), 67.12 (C-2), 66.32 (C-5), 33.82 [SiC(CH₃)₂CH(CH₃)₂], 24.78 [SiC(CH₃)₂CH(CH₃)₂], 19.88, 19.76, 18.46, 18.36 [SiC(CH₃)₂CH(CH₃)₂], -2.21 (SiCH₃), -3.24 (SiCH₃). HR MS (*m/z*) calcd for C₂₅H₃₇N₃O₇Si [M+Na]⁺, 542.2298; found, 542.2475.

Dimethylthexylsilyl 3-O-allyloxycarbonyl-2-azido-4-O-benzyl-2-deoxy-β-D-glucopyranoside (**5**)

A suspension of **11** (1.20 g, 2.31 mmol) and molecular sieve (4 Å, 300 mg) in DCM (20 mL) was stirred at room temperature for 1 h. The mixture was cooled (-75°C) and then triethylsilane (0.55 mL, 3.47 mmol) and PhBCl₂ (0.52 mL, 3.93 mmol) were added dropwise. After stirring the reaction mixture for 1 h, it was quenched by addition of Et₃N (1 mL) and methanol (1 mL). The reaction mixture was warmed up to room temperature and then diluted with ethyl acetate (40 mL). The molecular sieve was removed by filtration, and the filtrate was washed with saturated aqueous NaHCO₃ (30 mL). The organic phase was dried (MgSO₄), filtered, and the filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/ethyl acetate, 10/1, v/v) to give **5** as a colorless oil (1.13 g, 94%). *R*_f = 0.35 (hexane/ethyl acetate, 5/1, v/v); [α]_D²⁵ = -17.1° (*c* = 1.0, CHCl₃); ¹H NMR (300 MHz, CDCl₃): δ 7.28-7.22 (m, 5H, aromatic), 5.99-5.85 (m, 1H, OCH₂CH=CH₂), 5.37 (dd, 1H, *J* = 1.2 Hz, *J* = 15.6 Hz, OCH₂CH=CHH), 5.27 (dd, 1H, *J* = 1.2 Hz, *J* = 11.0 Hz,

OCH₂CH=CHH), 4.80 (dd, 1H, $J_{2,3} = 10.5$ Hz, $J_{3,4} = 9.3$ Hz, H-3), 4.70-2.59 (m, 5H, H-1, CH₂Ph, OCH₂CH=CH₂), 3.84 (dd, 1H, $J_{5,6a} = 2.4$ Hz, $J_{6a,6b} = 11.7$ Hz, H-6a), 3.71 (dd, 1H, $J_{5,6b} = 4.2$ Hz, $J_{6a,6b} = 11.7$ Hz, H-6b), 3.65 (t, 1H, $J_{3,4} = J_{4,5} = 9.6$ Hz, H-4), 3.42-3.37 (m, 1H, H-5), 3.34 (dd, 1H, $J_{1,2} = 7.2$ Hz, $J_{2,3} = 10.5$ Hz, H-2), 1.71-1.62 [m, 1H, CH(CH₃)₂], 0.90-0.88 [m, 12H, SiC(CH₃)₂CH(CH₃)₂], 0.20 (s, 3H, SiCH₃), 0.17 (s, 3H, SiCH₃). ¹³C NMR (75 MHz, CDCl₃): δ 154.24 (C=O), 137.26-127.89 (m, aromatic), 131.17 (OCH₂CH=CH₂), 119.13 (OCH₂CH=CH₂), 96.81 (C-1), 78.52 (C-3), 75.29 (C-4), 74.99 (C-5), 74.66 (OCH₂CH=CH₂), 68.82 (CH₂Ph), 66.49 (C-2), 61.49 (C-6), 33.73 [SiC(CH₃)₂CH(CH₃)₂], 24.69 [SiC(CH₃)₂CH(CH₃)₂], 19.79, 19.71, 18.35, 18.28 [SiC(CH₃)₂CH(CH₃)₂], -2.22 (SiCH₃), -3.32 (SiCH₃). HR MS (m/z) calcd for C₂₅H₃₉N₃O₇Si[M+Na]⁺, 544.2455; found, 544.2548.

Dimethylthexylsilyl 2-azido-3-O-[(*R*)-3-benzyloxy-dodecanoyl]-2-deoxy-4,6-O-isopropylidene-β-D-glucopyranoside (**13**)

A reaction mixture of (*R*)-3-benzyloxy-dodecanoic acid **8** (970 mg, 3.17 mmol) and DCC (949 mg, 4.60 mmol) in DCM (10 mL) was stirred at room temperature for 10 min, and then compound **12** (1.15 g, 2.88 mmol) and DMAP (35 mg, 0.29 mmol) were added. The reaction mixture was stirred at room temperature for 10 h, after which the solids were removed by filtration, and the residue washed with DCM (2 × 1 mL). The combined filtrates were concentrated in vacuo, and the residue was purified by silicon gel column chromatography (eluent: hexane/ethyl acetate, 15/1, v/v) to yield **13** as a syrup (1.82 g, 94%). $R_f = 0.55$ (hexane/ethyl acetate, 8/1, v/v); $[\alpha]_D^{25} = -10.0^\circ$ ($c = 1.0$, CHCl₃); ¹H NMR (300 MHz, CDCl₃): δ 7.34-7.26 (m, 5H, aromatic), 4.91 (t, 1H, $J_{2,3} = J_{3,4} = 9.6$ Hz, H-3), 4.62 (d, 1H, $J_{1,2} = 7.5$ Hz, H-1), 4.60 (d, 1H, $J = 11.4$ Hz, CHHPh), 4.48 (d, 1H, $J = 11.4$ Hz, CHHPh), 3.89-3.83 (m, 2H, H-6a, H-3_S), 3.74 (t, 1H, $J_{5,6b} = J_{6a,6b} = 10.5$ Hz, H-6b), 3.64 (t, 1H, $J_{3,4} = J_{4,5} = 9.6$ Hz, H-4), 3.34-3.23 (m, 2H, H-2, H-5), 2.71 (dd, 1H, $J_{2Sa,2Sb} = 15.0$ Hz, $J_{2Sa,3S} = 6.3$ Hz, H-2_{Sa}), 2.51 (dd, 1H, $J_{2Sa,2Sb} = 15.0$ Hz, $J_{2Sb,3S} = 6.0$ Hz, H-2_{Sb}), 1.69-1.47 [m, 3H, H-4_S, CH(CH₃)], 1.38 (s, 3H, CH₃ of isopropylidene), 1.28 (s, 3H, CH₃ of isopropylidene), 1.24 [bs, 14H, H-(5_S-11_S)], 0.89-0.84 [m, 15H, H-12_S, SiC(CH₃)₂CH(CH₃)₂], 0.19 (s, 3H, SiCH₃), 0.18 (s, 3H, SiCH₃). ¹³C NMR (75 MHz, CDCl₃): δ 170.56 (C=O), 138.50-127.50 (m, aromatic), 99.67 [C(CH₃)₂ of isopropylidene], 97.43 (C-1), 75.70 (C-3_S), 71.63 (C-4), 71.46, 71.41 (C-3, CH₂Ph), 67.46, 67.37 (C-2, C-5), 61.93 (C-6), 39.77 (C-2_S), 34.50-14.07 [m, SiC(CH₃)₂CH(CH₃)₂, CH₃ of isopropylidene, C-(4_S-12_S)], -2.25 (SiCH₃), -3.32 (SiCH₃). HR MS (m/z) calcd for C₃₆H₆₁N₃O₇Si[M+Na]⁺, 698.4176; found, 698.3518.

Dimethylthexylsilyl 3-O-[(*R*)-3-benzyloxy-dodecanoyl]-2-deoxy-2-(9-fluorenylmethoxycarbonyl)-4,6-O-isopropylidene-β-D-glucopyranoside (**14**)

A suspension of compound **13** (1.82 g, 2.70 mmol) and zinc (< 10 micron, 1.75 g, 27.0 mmol) in a mixture of acetic acid (300 μL) and DCM (15 mL) was stirred at room temperature for 5 h, after which it was diluted with ethyl acetate (40 mL). The solids were removed by filtration, and the residue was washed with ethyl acetate (2 × 3 mL). The combined filtrates were washed with saturated aqueous NaHCO₃ (2 × 30 mL) and brine (2 × 20 mL). The organic phase was dried (MgSO₄), filtered, and the filtrate was concentrated in vacuo to afford the crude amine as a pale yellow oil. The resulting amine was dissolved in DCM (15 mL), and then FmocCl (767 mg, 2.97 mmol) and DIPEA (517 μL, 2.97 mmol) were added. The reaction mixture was stirred at room temperature for 2 h, after which it was diluted with DCM (20 mL) and washed with brine (2 × 30 mL). The organic phase was dried (MgSO₄) and concentrated in vacuo. The residue was purified by silica gel column chromatography (eluent: hexane/ethyl acetate, 10/1, v/v) to yield **14** as a colorless syrup (2.02 g, 86%, two steps). $R_f = 0.55$ (hexane/ethyl acetate, 5/1, v/v); $[\alpha]_D^{25} = -2.8^\circ$ ($c = 1.0$, CHCl₃); ¹H NMR (300 MHz, CDCl₃): δ 7.73-7.25 (m, 13H, aromatic), 5.35 (d, 1H, $J_{NH,2} = 9.0$ Hz, NH), 5.27 (t, 1H, $J_{2,3} = J_{3,4} = 9.6$ Hz, H-3), 4.81 (d, 1H, $J_{1,2} = 7.2$ Hz, H-1), 4.55 (d, 1H, $J = 11.1$ Hz, CHHPh), 4.43 (d, 1H, $J = 11.1$ Hz, CHHPh),

4.27-4.13 (m, 3H, OCH_2CH of Fmoc), 3.89-3.69 (m, 5H, H-2, H-4, H-6a, H-6b, H-3_S), 3.47 (bs, 1H, H-5), 2.70 (dd, 1H, $J_{2\text{Sa},2\text{Sb}} = 14.7$ Hz, $J_{2\text{Sa},3\text{S}} = 5.1$ Hz, H-2_{Sa}), 2.46 (dd, 1H, $J_{2\text{Sa},2\text{Sb}} = 14.7$ Hz, $J_{2\text{Sb},3\text{S}} = 6.0$ Hz, H-2_{Sb}), 1.59-1.50 (m, 3H, H-4_S, $\text{CH}(\text{CH}_3)_2$), 1.43 (s, 3H, CH_3 of isopropylidene), 1.34 (s, 3H, CH_3 of isopropylidene), 1.23-1.16 [bs, 14H, H-(5_S-11_S)], 1.16-0.84 [m, 15H, H-12_S, $\text{SiC}(\text{CH}_3)_2\text{CH}(\text{CH}_3)_2$], 0.13 (s, 3H, SiCH_3), 0.10 (s, 3H, SiCH_3). ^{13}C NMR (75 MHz, CDCl_3): δ 171.78 (C=O), 155.82 (C=O), 143.79-119.83 (m, aromatic), 99.45 [$\text{C}(\text{CH}_3)_2$ of isopropylidene], 96.93 (C-1), 75.66 (C-3_S), 72.08 (C-3), 71.83 (C-4), 71.15 (CH_2Ph), 67.07 (C-5, OCH_2 of Fmoc), 62.00 (C-6), 58.55 (C-2), 46.92 (OCH_2CH of Fmoc), 39.76 (C-2_S), 34.41-14.04 [m, $\text{SiC}(\text{CH}_3)_2\text{CH}(\text{CH}_3)_2$, CH_3 of isopropylidene, C-(4_S-12_S)], -2.00 (SiCH_3), -3.42 (SiCH_3). HR MS (m/z) calcd for $\text{C}_{51}\text{H}_{73}\text{NO}_9\text{Si}[\text{M}+\text{Na}]^+$, 894.4952; found, 894.4984.

Dimethylthexylsilyl 3-O-[(*R*)-3-benzyloxy-dodecanoyl]-2-deoxy-2-(9-fluorenylmethoxycarbonyl)-4,6-O-isopropylidene- β -D-glucopyranosyl-(1 $\rightarrow$ 6)-3-O-allyloxycarbonyl-2-azido-4-O-benzyl-2-deoxy- β -D-glucopyranoside (15)

A mixture of Bu_4NF (1 M in THF, 5 mL) and acetic acid (800 μL) was added dropwise to a stirred solution of **14** (1.30 g, 1.49 mmol) in THF (15 mL). After stirring at room temperature for 36 h, the reaction mixture was diluted with DCM (20 mL), and then washed with saturated aqueous NaHCO_3 (2×25 mL) and brine (2×25 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 (eluent: hexane/ethyl acetate, 5/2, v/v) to afford a lactol as a pale yellow oil (978 mg, 90%). The resulting lactol (810 mg, 1.11 mmol) was dissolved in a mixture of trichloroacetonitrile (2.0 mL) and DCM (6 mL), and then Cs_2CO_3 (181 mg, 0.55 mmol) was added. The reaction mixture was stirred at room temperature for 1 h, after which it was diluted with DCM (20 mL), and then washed with saturated aqueous NaHCO_3 (2×25 mL) and brine (2×25 mL). The organic phase was dried (Na_2SO_4), filtered, and the filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography (eluent: hexane/ethyl acetate, 2/1, v/v) to yield **6** as a pale yellow foam (880 mg, 91%). A suspension of trichloroacetimidate **6** (880 mg, 1.01 mmol), acceptor **5** (480 mg, 0.92 mmol) and molecular sieves (4 Å, 500 mg) in DCM (10 mL) was stirred at room temperature for 1 h. The mixture was cooled (-50°C) and then TfOH (4.4 μL , 0.05 mmol) was added. After stirring the reaction mixture for 30 min, it was allowed to warm up to -10°C in 30 min and then quenched with solid NaHCO_3 (50 mg) and diluted with DCM (20 mL). The solution was washed with saturated aqueous NaHCO_3 (2×25 mL) and brine (2×25 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 (eluent: hexane/ethyl acetate, 7/1-4/1, v/v) to yield disaccharide **15** as a colorless solid (1.07 g, 94%). $R_f = 0.50$ (hexane/ethyl acetate, 4/1, v/v); $[\alpha]_D^{24} = -9.6^\circ$ ($c = 1.0$, CHCl_3); ^1H NMR (600 MHz, CDCl_3): δ 7.82-7.19 (m, 18H, aromatic), 6.76 (d, 1H, $J_{\text{NH}',2} = 9.6$ Hz, NH'), 5.88-5.83 (m, 1H, $\text{OCH}_2\text{CH}=\text{CH}_2$), 5.29 (d, $J = 16.8$ Hz, $\text{OCH}_2\text{CH}=\text{CHH}$), 5.23 (t, 1H, $J_{2,3} = J_{3,4} = 9.6$ Hz, H-3'), 5.16 (d, $J = 10.2$ Hz, $\text{OCH}_2\text{CH}=\text{CHH}$), 4.88 (d, 1H, $J_{1',2'} = 8.4$ Hz, H-1'), 4.80-4.69 (m, 2H, H-1, H-3), 4.69 (d, 1H, $J = 10.8$ Hz, CHHPh), 4.60-4.52 (m, 4H, CH_2Ph , $\text{OCH}_2\text{CH}=\text{CH}_2$), 4.39 (d, 1H, $J = 11.4$ Hz, CHHPh), 4.24-4.21 (m, 1H, OCHH of Fmoc), 4.12 (d, 1H, $J_{6a,6b} = 10.8$ Hz, H-6a), 4.08-4.01 (m, 2H, OCHHCH of Fmoc), 3.89-3.84 (m, 2H, H-6'a, H-6b), 3.82-3.79 (m, 2H, H-4', H-6'b), 3.78-3.72 (m, 3H, H-2', H-4, H-3_S), 3.67-3.65 (m, 1H, H-5), 3.40-3.33 (m, 2H, H-2, H-5'), 2.57 (dd, 1H, $J_{2\text{Sa},2\text{Sb}} = 15.6$ Hz, $J_{2\text{Sa},3\text{S}} = 6.0$ Hz, H-2_{Sa}), 2.33 (dd, 1H, $J_{2\text{Sa},2\text{Sb}} = 15.0$ Hz, $J_{2\text{Sb},3\text{S}} = 6.0$ Hz, H-2_{Sb}), 1.71-1.66 [m, 1H, $\text{CH}(\text{CH}_3)_2$], 1.46-1.39 (m, 2H, H-4_S), 1.39-1.11 [m, 20H, CH_3 of isopropylidene, H-(5_S-11_S)], 0.91-0.83 [m, 15H, H-12_S, $\text{SiC}(\text{CH}_3)_2\text{CH}(\text{CH}_3)_2$], 0.24 (s, 3H, SiCH_3), 0.23 (s, 3H, SiCH_3). ^{13}C NMR (75 MHz, CDCl_3): δ 171.30 (C=O), 156.58 (C=O), 154.91 (C=O), 144.85-118.58 (m, $\text{OCH}_2\text{CH}=\text{CH}_2$, aromatic), 102.42 (C-1'), 99.91 [$\text{C}(\text{CH}_3)_2$ of isopropylidene], 97.08 (C-1), 75.66 (C-3_S), 79.23 (C-3), 76.67, 75.97 (C-4, 3_S), 75.05 (CH_2Ph), 74.51 (C-5), 72.96 (C-3'), 73.46 (C-4'), 71.29 (CH_2Ph), 68.93

(OCH₂CH=CH₂), 68.51 (C-6), 67.86, 67.25 (C-2, 5'), 67.08 (OCH₂ of Fmoc), 62.40 (C-6'), 57.27 (C-2'), 47.58 (OCH₂CH of Fmoc), 40.00 (C-2_S), 34.88-14.14 [m, SiC(CH₃)₂CH(CH₃)₂, CH₃ of isopropylidene, C-(4_S-12_S)], -1.81 (SiCH₃), -3.26 (SiCH₃). HR MS (m/z) calcd for C₆₈H₉₂N₄O₁₅Si[M+Na]⁺, 1255.6221; found, 1255.6168.

Dimethylthexylsilyl 3-O-[(R)-3-benzyloxy-dodecanoyl]-2-deoxy-2-(9-fluorenylmethoxycarbonyl)-β-D-glucopyranosyl-(1→6)-3-O-allyloxycarbonyl-2-azido-4-O-benzyl-2-deoxy-β-D-glucopyranoside (16)

TFA/H₂O (3/2, v/v, 250 μL) was added dropwise to a stirred solution of **15** (960 mg, 0.78 mmol) in DCM (15 mL). The reaction mixture was stirred at room temperature for 30 min, after which it was diluted with ethyl acetate (15 mL) and then washed with saturated aqueous NaHCO₃ (2 × 15 mL) and brine (2 × 15 mL). The organic phase was dried (MgSO₄), filtered, and the filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography (eluent: hexane/ethyl acetate, 2/1, v/v) to afford **16** as a pale yellow oil (882 mg, 98%). *R*_f = 0.35 (hexane/ethyl acetate, 1/1, v/v); [α]_D²⁴ = -9.2° (c = 1.0, CHCl₃); ¹H NMR (300 MHz, CD₃COCD₃): δ 7.84-7.18 (m, 18H, aromatic), 6.64 (d, 1H, *J*_{NH',2} = 9.3 Hz, NH'), 5.92-5.79 (m, 1H, OCH₂CH=CH₂), 5.29 (dd, *J* = 1.5 Hz, *J* = 14.4 Hz, OCH₂CH=CHH), 5.22-5.14 (m, 2H, H-3', OCH₂CH=CHH), 4.85-4.79 (m, 3H, H-1, H-1', H-3), 4.74 (d, 1H, *J* = 11.4 Hz, CHHPh), 4.59-4.52 (m, 4H, CH₂Ph, OCH₂CH=CH₂), 4.42 (d, 1H, *J* = 11.7 Hz, CHHPh), 4.21-4.10 (m, 4H, H-6a, OCH₂CH of Fmoc), 3.93-3.84 (m, 2H, H-6b, H-6'a), 3.81-3.62 (m, 6H, H-2', H-4, H-4', H-5', H-6'b, H-3_S), 3.45-3.39 (m, 2H, H-2, H-5), 2.62 (dd, 1H, *J*_{2Sa,2Sb} = 15.6 Hz, *J*_{2Sa,3S} = 6.3 Hz, H-2_{Sa}), 2.41 (dd, 1H, *J*_{2Sa,2Sb} = 15.6 Hz, *J*_{2Sb,3S} = 5.4 Hz, H-2_{Sb}), 1.73-1.64 [m, 1H, CH(CH₃)₂], 1.44-1.39 (m, 2H, H-4_S), 1.28-1.09 [m, 14H, H-(5_S-11_S)], 0.96-0.83 [m, 15H, H-12_S, SiC(CH₃)₂CH(CH₃)₂], 0.23 (s, 3H, SiCH₃), 0.22 (s, 3H, SiCH₃). ¹³C NMR (75 MHz, CD₃COCD₃): δ 171.80 (C=O), 156.48 (C=O), 154.86 (C=O), 144.81-120.51 (m, OCH₂CH=CH₂, aromatic), 118.51 (OCH₂CH=CH₂), 102.01 (C-1'), 97.02 (C-1), 79.21 (C-3), 71.11 (C-5), 76.72, 76.56, 76.07 (C-3', 4, 3_S), 74.93 (CH₂Ph), 74.66 (C-5'), 71.37 (CH₂Ph), 69.66 (C-4'), 68.86 (OCH₂CH=CH₂), 68.46 (C-6), 67.20 (C-2), 67.02 (OCH₂ of Fmoc), 62.40 (C-6'), 56.79 (C-2'), 47.59 (OCH₂CH of Fmoc), 39.98 (C-2_S), 34.83-14.09 [m, SiC(CH₃)₂CH(CH₃)₂, C-(4_S-12_S)], -1.83 (SiCH₃), -3.34 (SiCH₃). HR MS (m/z) calcd for C₆₅H₈₈N₄O₁₅Si[M+Na]⁺, 1215.5913; found, 1215.6797.

Dimethylthexylsilyl benzyl (7,8-di-O-benzyl-3-deoxy-4,5-O-isopropylidene-α/β-D-manno-oct-2-ulopyranosyl)onate-(2→6)-3-O-[(R)-3-benzyloxy-dodecanoyl]-2-deoxy-2-(9-fluorenylmethoxycarbonyl)-β-D-glucopyranosyl-(1→6)-3-O-allyloxycarbonyl-2-azido-4-O-benzyl-2-deoxy-β-D-glucopyranoside (17)

A suspension of **16** (610 mg, 0.51 mmol), **7** (495 mg, 0.91 mmol) and molecular sieves (4 Å, 400 mg) in DCM (8 mL) was stirred at room temperature for 1 h. The mixture was cooled (0° C) and then BF₃·Et₂O (77 μL, 0.61 mmol) was added dropwise. After stirring the reaction mixture for 1 h, it was quenched with solid NaHCO₃ (100 mg) and then diluted with DCM (20 mL). The solids were removed by filtration, and the filtrate was washed with saturated aqueous NaHCO₃ (2 × 25 mL) and brine (2 × 25 mL). The organic phase was dried (MgSO₄), filtered, and the filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography (eluent: hexane/ethyl acetate, 6/1→4/1, v/v) to afford **17** as an amorphous solid (590 mg, 67%). *R*_f = 0.45 (hexane/ethyl acetate, 3/1, v/v); [α]_D²⁴ = -4.9° (c = 1.0, CHCl₃); ¹H NMR of α-anomer (600 MHz, CD₃COCD₃): δ 7.84-7.18 (m, 33H, aromatic), 6.62 (d, 1H, *J*_{NH',2} = 9.0 Hz, NH'), 5.89-5.82 (m, 1H, OCH₂CH=CH₂), 5.30-23 (m, 2H, CO₂CHHPh, OCH₂CH=CHH), 5.20-5.12 (m, 3H, H-3', CO₂CHHPh, OCH₂CH=CHH), 4.84-4.49 (m, 12H, H-1, H-1', H-3, 7 × CHH₂Ph, OCH₂CH=CH₂), 4.43-4.39 (m, 3H, H-4'', H-5'', CHHPh), 4.23-4.18 (m, 1H, OCHH of Fmoc), 4.17-4.10 (m, 3H, H-6a, OCHHCH of Fmoc), 4.00-3.91 (m, 3H, H-6'', H-7'', H-8a''), 3.84 (dd, 1H, *J*_{5',6a'} = 5.4 Hz, *J*_{6a',6b'} = 10.8 Hz,

H-6a'), 3.78-3.77 (m, 2H, H-3_S, H-8b''), 3.73-3.70 (m, 3H, H-4, H-5, H-6b), 3.67-3.65 (m, 2H, H-2', H-6b'), 3.48 (bs, 2H, H-4', H-5'), 3.81 (dd, 1H, $J_{1,2} = 8.4$ Hz, $J_{2,3} = 9.6$ Hz, H-2), 2.62-2.54 (m, 2H, H-3a'', H-2_{Sa}), 2.39 (dd, 1H, $J_{2Sa,2Sb} = 15.6$ Hz, $J_{2Sb,3S} = 5.4$ Hz, H-2_{Sb}), 1.97 (dd, 1H, $J_{3a'',3b''} = 15.0$ Hz, $J_{3b'',4''} = 2.4$ Hz, H-3b''), 1.68-1.62 [m 1H, $CH(CH_3)_2$], 1.43-1.39 (m, 2H, H-4_S), 1.31 (s, 3H, CH_3 of isopropylidene), 1.27 (s, 3H, CH_3 of isopropylidene), 1.16-1.08 [m, 14H, H-(5_S-11_S)], 0.88-0.83 [m, 15H, H-12_S, $SiC(CH_3)_2CH(CH_3)_2$], 0.22 (s, 3H, $SiCH_3$), 0.21 (s, 3H, $SiCH_3$). ¹³C NMR (75 MHz, CD₃COCD₃): δ 171.76 (C=O), 168.49 (C=O), 156.46 (C=O), 154.85 (C=O), 144.81-120.48 (m, OCH₂CH=CH₂, aromatic), 118.59 (OCH₂CH=CH₂), 109.11 (C-2''), 102.02 (C-1'), 98.34 [$C(CH_3)_2$ of isopropylidene], 97.00 (C-1), 79.20 (C-3), 77.87 (C-7''), 76.99 (C-4), 76.48 (C-3'), 76.07 (C-3_S), 75.29 (C-5'), 74.99 (CH₂Ph), 74.85 (C-5), 73.54 (CH₂Ph), 73.47 (CH₂Ph), 72.32 (C-4''), 71.74 (C-6), 71.41 (C-8'', CH₂Ph), 70.71 (C-5''), 70.21 (C-6''), 69.72 (C-4'), 68.90 (OCH₂CH=CH₂), 68.73 (C-6), 67.11 (C-2, CO₂CH₂Ph), 67.00 (OCH₂ of Fmoc), 63.30 (C-6'), 56.74 (C-2'), 47.64 (OCH₂CH of Fmoc), 39.96 (C-2_S), 34.79-14.08 [m, 3'', $SiC(CH_3)_2CH(CH_3)_2$, CH₃ of isopropylidene, C-(4_S-12_S)], -1.72 ($SiCH_3$), -3.29 ($SiCH_3$). HR MS (m/z) calcd for C₉₉H₁₂₆N₄O₂₀Si[M+Na]⁺, 1745.8218; found, 1745.9780.

Dimethylthexylsilyl benzyl (7,8-di-O-benzyl-3-deoxy-4,5-O-isopropylidene-α-D-manno-oct-2-ulopyranosyl)onate-(2→6)-3-O-[(R)-3-benzyloxy-dodecanoyl]-2-deoxy-4-O-(1,5-dihydro-3-oxo-3λ⁵-3H-2,4,3-benzodioxaphosphepin-3-yl)-2-(9-fluorenylmethoxycarbonyl)-β-D-glucopyranosyl-(1→6)-3-O-allyloxycarbonyl-2-azido-4-O-benzyl-2-deoxy-β-D-glucopyranoside (18)

1H-tetrazole (3% wt, 10.0 mmol) in DCM (2.5 mL) was added to a solution of compound **17** (480 mg, 0.28 mmol) and *N,N*-diethyl-1,5-dihydro-3H-2,4,3-benzodioxaphosphepin-3-amine (133 mg, 0.56 mmol) in DCM (8 mL). After the reaction mixture was stirred at room temperature for 40 min, it was cooled (-20°C), stirred for another 10 min and then 3-chloroperoxybenzoic acid (mCPBA) (500 mg, 50-55% wt, 1.12 mmol) was added. The reaction mixture was stirred at -20°C for 20 min, and then quenched by the addition of saturated aqueous NaHCO₃ (20 mL) and diluted with DCM (20 mL). The solution was washed with saturated aqueous NaHCO₃ (2 × 30 mL) and brine (2 × 20 mL). The organic phase was dried (MgSO₄), filtered, and the filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography (eluent: hexane/ethyl acetate, 4/1, v/v) to give **18** as an amorphous solid (470 mg, 88%). $R_f = 0.45$ (hexane/ethyl acetate, 3/1, v/v); $[\alpha]_D^{24} = +6.0^\circ$ ($c = 1.0$, CHCl₃); ¹H NMR (600 MHz, CD₃COCD₃): δ 7.85-7.20 (m, 35H, aromatic), 6.84-6.74 (m, 3H, aromatic × 2, NH'), 6.62 (d, 1H, $J_{NH',2} = 9.0$ Hz, NH'), 5.91-5.85 (m, 1H, OCH₂CH=CH₂), 5.46 (t, 1H, $J_{2',3'} = J_{3',4'} = 9.6$ Hz, H-3'), 5.32-5.23 (m, 3H, CO₂CH₂Ph, OCH₂CH=CHH), 5.18 (dd, 1H, $J = 1.2$ Hz, $J = 10.8$ Hz, OCH₂CH=CHH), 5.08-4.89 (m, 5H, H-1', H-3, 3 × CHHPh), 4.85-4.79 (m, 3H, H-1, 2 × CHHPh), 4.71-4.63 (m, 4H, H-4', H-4'', 2 × CHHPh), 4.61-4.56 (m, 6H, 4 × CHHPh, OCH₂CH=CH₂), 4.46-4.43 (m, 2H, H-5'', CHHPh), 4.20-4.16 (m, 3H, OCH₂CH of Fmoc), 4.10-4.07 (m, 2H, H-6a, H-6''), 4.02-4.01 (m, 2H, H-7'', H-8''), 3.93 (dd, 1H, $J_{5',6a'} = 4.8$ Hz, $J_{6a',6b'} = 11.4$ Hz, H-6a'), 3.85 (dd, 1H, $J_{7'',8a''} = 4.8$ Hz, $J_{8a'',8b''} = 10.8$ Hz, H-8a''), 3.81-3.77 (m, 4H, H-5, H-6a, H-6b', H-3_S), 3.67-3.60 (m, 3H, H-2', H-4, H-5'), 3.35 (dd, 1H, $J_{1,2} = 7.8$ Hz, $J_{2,3} = 10.2$ Hz, H-2), 2.70 (dd, 1H, $J_{2Sa,2Sb} = 16.8$ Hz, $J_{2Sb,3S} = 6.6$ Hz, H-2_{Sb}), 2.53 (dd, 1H, $J_{2Sa,2Sb} = 16.8$ Hz, $J_{2Sb,3S} = 4.8$ Hz, H-2_{Sb}), 2.26 (dd, 1H, $J_{3a'',3b''} = 14.4$ Hz, $J_{3a'',4''} = 7.2$ Hz, H-3a''), 2.12 (dd, 1H, $J_{3a'',3b''} = 14.4$ Hz, $J_{3b'',4''} = 4.8$ Hz, H-3b''), 1.68-1.63 (m 1H, $CH(CH_3)_2$), 1.47-1.43 (m, 2H, H-4_S), 1.37 (s, 3H, CH_3 of isopropylidene), 1.31 (s, 3H, CH_3 of isopropylidene), 1.16-1.08 [m, 14H, H-(5_S-11_S)], 0.89-0.83 [m, 15H, H-12_S, $SiC(CH_3)_2CH(CH_3)_2$], 0.24 (s, 3H, $SiCH_3), 0.23 (s, 3H, $SiCH_3$). ¹³C NMR (75 MHz, CD₃COCD₃): δ 171.78 (C=O), 168.35 (C=O), 156.47 (C=O), 155.03 (C=O), 144.93-120.62 (m, OCH₂CH=CH₂, aromatic), 118.71 (OCH₂CH=CH₂), 109.06 (C-2''), 102.03 (C-1'), 99.13 [$C(CH_3)_2$ of isopropylidene], 97.02 (C-1), 79.25 (C-3), 78.01 (C-7''), 77.49 (C-4), 75.91 (C-5 or 3_S), 74.96, 74.90 (C-5 or 3_S,$

CH₂Ph), 74.68 (C-4'), 73.97 (C-3'), 73.76 (CH₂Ph), 73.42 (C-5', CH₂Ph), 71.80 (C-5''), 71.65 (CH₂Ph), 70.56 (C-4''), 70.35 (C-8''), 69.54 (C-6), 69.19 (C-6''), 69.03 (OCH₂CH=CH₂), 68.82 [2 × (OCH₂)₂Ph], 67.43(CO₂CH₂Ph), 67.34 (C-2), 67.25 (OCH₂ of Fmoc), 62.91 (C-6'), 57.32 (C-2'), 47.77 (OCH₂CH of Fmoc), 39.78 (C-2_S), 34.88-14.20 [m, 3'', SiC(CH₃)₂CH(CH₃)₂, CH₃ of isopropylidene, C-(4_S-12_S)], -1.61 (SiCH₃), -3.18 (SiCH₃). HR MS (m/z) calcd for C₁₀₅H₁₂₉N₄O₂₅Si[M+Na]⁺, 1927.8350; found, 1927.8330.

Dimethylthexylsilyl benzyl (7,8-di-O-benzyl-3-deoxy-4,5-O-isopropylidene- α -D-manno-oct-2-ulopyranosyl)onate-(2→6)-3-O-[(R)-3-benzyloxy-dodecanoyl]-2-deoxy-4-O-(1,5-dihydro-3-oxo-3 λ^5 -3H-2,4,3-benzodioxaphosphepin-3-yl)-2-[(R)-3-dodecanoyloxy-tetradecanoyl]- β -D-glucopyranosyl-(1→6)-3-O-allyloxycarbonyl-2-azido-4-O-benzyl-2-deoxy- β -D-glucopyranoside (19)

DBU (100 μ L) was added dropwise to a stirred solution of **18** (300 mg, 0.16 mmol) in DCM (5 mL). The reaction mixture was stirred at room temperature for 30 min, and then concentrated in vacuo. The residue was purified by silica gel column chromatography (eluent: hexane/ethyl acetate, 5/2, v/v) to yield an amine intermediate as a pale yellow oil (250 mg, 94%). R_f = 0.25 (hexane/ethyl acetate, 5/2, v/v). A reaction mixture of **9** (95 mg, 0.22 mmol) and DCC (62 mg, 0.30 mmol) in DCM (3 mL) was stirred at room temperature for 10 min, and then the above obtained amine (250 mg, 0.15 mmol) was added, and stirring was continued for another 12 h. The insoluble materials were removed by filtration, and the residue was washed with DCM (2 $\times$ 0.5 mL). The combine filtrates were concentrated in vacuo, and the residue was purified by silica gel column chromatography (eluent: hexane/ethyl acetate, 5/1, v/v) to yield **19** as an amorphous solid (280 mg, 89%). R_f = 0.55 (hexane/ethyl acetate, 5/2, v/v); $[\alpha]_D^{25}$ = +7.1° (c = 1.0, CHCl₃); ¹H NMR (600 MHz, CDCl₃): δ 7.36-7.07 (m, 27H, aromatic), 6.69 (d, 1H, J = 7.2 Hz, aromatic), 6.51 (d, 1H, J = 7.2 Hz, aromatic), 5.90-5.84 (m, 1H, OCH₂CH=CH₂), 5.62 (d, 1H, $J_{NH',2}$ = 7.2 Hz, NH'), 5.53 (t, 1H, $J_{2',3'} = J_{3',4'} = 9.6$ Hz, H-3'), 5.20 (d, 1H, J = 17.4 Hz, OCH₂CH=CHH), 5.23-5.16 (m, 3H, CO₂CH₂Ph, OCH₂CH=CHH), 5.03-5.16 (m, 3H, H-1', H-3_L, CHHPh), 4.89-4.81 (m, 2H, H-3, CHHPh), 4.75-4.71 (m, 2H, H-3, CHHPh), 4.68-4.64 (m, 4H, H-4', CHHPh $\times$ 3), 4.61-4.57 (m, 4H, H-1, H-4'', OCH₂CH=CH₂), 4.57-4.47 (m, 6H, 6 $\times$ CHHPh), 4.38 (bs, 1H, H-5''), 4.04 (bs, 1H, H-7''), 3.99 (d, 1H, J = 10.2 Hz, H-6''), 3.89-3.84 (m, 2H, H-8a'', H-3_S), 3.80-3.74 (m, 3H, H-6a, H-6a', H-8a''), 3.63 (d, 1H, $J_{6a',6b'}$ = 10.8 Hz, H-6b'), 3.59-3.54 (m, 2H, H-5, H-6b), 3.80 (dd, 1H, J = 4.8 Hz, J = 9.6 Hz, H-5'), 3.33 (t, 1H, $J_{3,4} = J_{4,5} = 9.0$ Hz, H-4), 3.26 (dd, 1H, $J_{1,2} = 7.8$ Hz, $J_{2,3} = 10.2$ Hz, H-2), 3.11 (dd, 1H, J = 7.8 Hz, H-2'), 2.69-2.63 (m, 2H, H-2_S), 2.32-2.20 (m, 3H, H-2_L, H-2_{L'}), 2.18 (dd, 1H, $J_{3a'',3b''}$ = 14.4 Hz, $J_{3a'',4''}$ = 6.6 Hz, H-3a''), 2.10 (dd, 1H, $J_{3a'',3b''}$ = 14.4 Hz, $J_{3b'',4''}$ = 5.4 Hz, H-3b''), 2.05 (dd, $J_{2La,2Lb} = 15.0$ Hz, $J_{2Lb,3L} = 5.4$ Hz, H-2_{Lb}), 1.66-1.46 [m, 7H, H-4_S, H-4_L, H-3_{L'}, CH(CH₃)₂], 1.38 [s, 3H, CH₃ of isopropylidene], 1.31 [s, 3H, CH₃ of isopropylidene], 1.22 [bs, 48H, H-(5_S-11_S), H-(5_L-13_L), H-(4_L-11_L)], 0.86-0.84 [m, 21H, H-12_S, H-14_L, H-12_L], SiC(CH₃)₂CH(CH₃)₂], 0.16 [s, 6H, Si(CH₃)₂]. ¹³C NMR (75 MHz, CD₃COCD₃): δ 173.59 (C=O), 171.06 (C=O), 169.98 (C=O), 167.78 (C=O), 154.35 (C=O), 138.84-127.46 (m, OCH₂CH=CH₂, aromatic), 119.17 (OCH₂CH=CH₂), 108.66 (C-2''), 99.89 (C-1'), 98.58 [C(CH₃)₂ of isopropylidene], 96.48 (C-1), 78.60 (C-3), 77.00 (C-7''), 76.87 (C-4), 75.54 (C-3_S), 74.46 (CH₂Ph), 74.12 (C-4'), 73.84 (C-5), 73.35 (CH₂Ph), 73.19 (CH₂Ph), 72.56 (C-5'), 71.72 (C-3'), 71.16 (CH₂Ph), 70.83 (C-5''), 70.74 (C-3_L), 69.79 (C-4'', 8''), 68.83 (C-6, OCH₂CH=CH₂), 68.44 (C-6'', CH₂Ph), 68.71 (CH₂Ph), 66.97 (CO₂CH₂Ph), 66.62 (C-2), 61.81 (C-6'), 56.76 (C-2'), 41.62 (C-2_L), 38.91 (C-2_S), 34.47-14.09 [m, 3'', SiC(CH₃)₂CH(CH₃)₂, CH₃ of isopropylidene, C-(4_S-12_S), C-(4_L-14_L), C-(2_L-12_L)], -1.86 (SiCH₃), -3.41 (SiCH₃). HR MS (m/z) calcd for C₁₀₅H₁₂₉N₄O₂₅Si[M+Na]⁺, 2114.1273; found, 2114.2964.

Dimethylthexylsilyl benzyl (7,8-di-O-benzyl-3-deoxy-4,5-O-isopropylidene- α -D-manno-oct-2-ulopyranosyl)onate-(2→6)-3-O-[(R)-3-benzyloxy-dodecanoyl]-2-deoxy-4-O-(1,5-dihydro-3-oxo-3 λ^5 -3H-2,4,3-benzodioxaphosphepin-3-yl)-2-[(R)-3-dodecanoyloxy-tetradecanoyl]- β -D-

glucopyranosyl-(1→6)-2-azido-4-O-benzyl-3-O-[(*R*)-3-benzyloxy-dodecanoyl]-2-deoxy-β-D-glucopyranoside (20)

Tetrakis(triphenylphosphine)palladium (32.5 mg, 0.028 mmol) was added to a solution of **19** (295 mg, 0.141 mmol), *n*-BuNH₂ (28 μL, 0.28 mmol), and HCOOH (11 μL, 0.28 mmol) in THF (5 mL). After stirring the reaction mixture at room temperature for 20 min, it was diluted with DCM (20 mL), and washed successively with water (20 mL), saturated aqueous NaHCO₃ (2 × 20 mL) and brine (2 × 20 mL). The organic phase was dried (MgSO₄), filtered, and the filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/ethyl acetate, 4/1, v/v) to give an alcohol intermediate as a colorless syrup (268 mg, 95%). *R*_f = 0.45 (eluent: hexane/ethyl acetate, 3/1, v/v); ¹H NMR (500 MHz, CDCl₃): δ 7.43-7.14 (m, 27H, aromatic), 6.75 (d, 1H, *J* = 7.5 Hz, aromatic), 6.65 (d, 1H, *J* = 7.0 Hz, aromatic), 5.68 (d, 1H, *J*_{NH',2} = 8.0 Hz, NH'), 5.53 (t, 1H, *J*_{2',3'} = *J*_{3',4'} = 10.0 Hz, H-3'), 5.23 (d, 1H, *J* = 12.5 Hz, CO₂CHHPh), 5.18 (d, 1H, *J* = 12.5, CO₂CHHPh), 5.10-4.92 (m, 4H, H-1', H-3_L, 2 × CHHPh), 4.85-4.50 (m, 13H, H-1, H-4', H-4'', 10 × CHHPh), 4.41 (bs, 1H, H-5''), 4.10-4.08 (m, 1H, H-7''), 4.02 (d, 1H, *J* = 9.5 Hz, H-6''), 3.95-3.89 (m, 3H, H-6a', H-8a'', H-3_S), 3.83-3.79 (m, 2H, H-6a, H-8b''), 3.73 (d, 1H, *J*_{6a,6b} = 10.5 Hz, H-6b), 3.67 (dd, 1H, *J*_{5',6b'} = 5.5 Hz, *J*_{a',b'} = 11.0 Hz, H-6b'), 3.48-3.42 (m, 3H, H-3, H-5, H-5'), 3.33-3.24 (m, 2H, H-2', H-4), 3.15 (dd, 1H, *J*_{1,2} = 8.0 Hz, *J*_{2,3} = 10.0 Hz, H-2), 2.76-2.67 (m, 2H, H-2_S), 2.37-2.27 (m, 4H, H-3a'', H-2_{La}, H-2_{L'}), 2.15-2.08 (2H, H-3b'', H-2_{Lb}), 1.68-1.53 [m, 7H, H-4_S, H-4_L, H-3_L, CH(CH₃)₂], 1.42 (s, 3H, CH₃ of isopropylidene), 1.36 (s, 3H, CH₃ of isopropylidene), 1.28 [bs, 48H, H-(5_S-11_S), H-(5_L-13_L), H-(4_L-11_L)], 0.91-0.90 [m, 21H, H-12_S, H-14_L, H-12_L, SiC(CH₃)₂CH(CH₃)₂], 0.20 [s, 6H, Si(CH₃)₂]. HR MS (*m/z*) calcd for C₁₁₂H₁₆₃N₄O₂₄Si[M+Na]⁺, 2030.1062; found, 2030.4662. A solution of (*R*)-3-benzyloxy-dodecanoic acid **8** (72 mg, 0.234 mmol) and DCC (72 mg, 0.351 mmol) in DCM (5 mL) was stirred at room temperature for 10 min, and then the above obtained intermediate (235 mg, 0.117 mmol) and DMAP (7 mg, 0.06 mmol) were added. The reaction mixture was stirred for another 10 h, after which the solids were removed by filtration and washed with DCM (2 × 2 mL). The combined filtrates were concentrated in vacuo. The residue was purified by silica gel column chromatography (eluent: hexane/ethyl acetate, 5/1-4/1, v/v) to afford **20** as a white solid (228 mg, 84%). *R*_f = 0.60 (hexane/ethyl acetate, 3/1, v/v); [α]_D²⁵ = +7.9° (*c* = 1.0, CHCl₃); ¹H NMR (500 MHz, CDCl₃): δ 7.42-7.12 (m, 32H, aromatic), 6.74 (d, 1H, *J* = 7.5 Hz, aromatic), 6.56 (d, 1H, *J* = 7.0 Hz, aromatic), 5.65 (d, 1H, *J*_{NH',2} = 7.5 Hz, NH'), 5.58 (t, 1H, *J*_{2',3'} = *J*_{3',4'} = 9.5 Hz, H-3'), 5.25 (s, 2H, CO₂CH₂Ph), 5.16 (t, 1H, *J*_{2,3} = *J*_{3,4} = 9.5 Hz, H-3), 5.10-00 (m, 2H, H-3_L, CHHPh), 4.98 (d, 1H, *J*_{1',2'} = 8.5 Hz, H-1'), 4.92 (dd, 1H, *J* = 11.0 Hz, *J* = 14.0 Hz, CHHPh), 4.81-4.47 (m, 15H, H-1, H-4', H-4'', 12 × CHHPh), 4.44 (bs, 1H, H-5''), 4.12-4.10 (m, 1H, H-7''), 4.05 (d, 1H, *J* = 9.5 Hz, H-6''), 3.96-3.89 (m, 3H, H-8a'', 2 × H-3_S), 3.85-3.79 (m, 3H, H-6a, H-6a', H-8b''), 3.68 (d, 1H, *J*_{6a',6b'} = 11.0 Hz, H-6b'), 3.64-3.57 (m, 2H, H-5, H-6b), 3.41 (dd, 1H, *J* = 3.0 Hz, *J* = 10.0 Hz, H-5'), 3.33 (t, 1H, *J*_{3,4} = *J*_{4,5} = 9.0 Hz, H-4), 3.22 (dd, 1H, *J*_{1,2} = 8.0 Hz, *J*_{2,3} = 10.5 Hz, H-2), 3.18 (dd, 1H, *J* = 7.5 Hz, H-2'), 2.76-2.68 (m, 2H, H-2_S), 2.63 (dd, 1H, *J*_{2Sa,2Sb} = 16.0 Hz, *J*_{2Sa,3S} = 7.0 Hz, H-2_{Sa}), 2.50 (dd, 1H, *J*_{2Sa,2Sb} = 16.0 Hz, *J*_{2Sb,3S} = 6.0 Hz, H-2_{Sb}), 2.38-2.26 (m, 3H, H-2_{La}, H-2_{L'}), 2.22 (dd, 1H, *J*_{3a'',3b''} = 14.0 Hz, *J*_{3a'',4''} = 7.5 Hz, H-3a''), 2.16 (dd, 1H, *J*_{3a'',3b''} = 14.0 Hz, *J*_{3b'',4''} = 5.5 Hz, H-3b''), 2.11 (dd, *J*_{2La,2Lb} = 14.5 Hz, *J*_{2Lb,3L} = 5.0 Hz, H-2_{Lb}), 1.69-1.54 [m, 9H, 2 × H-4_S, H-4_L, H-3_L, CH(CH₃)₂], 1.44 (s, 3H, CH₃ of isopropylidene), 1.37 (s, 3H, CH₃ of isopropylidene), 1.22 [bs, 62H, 2 × H-(5_S-11_S), H-(5_L-13_L), H-(4_L-11_L)], 0.92-0.90 [m, 24H, 2 × H-12_S, H-14_L, H-12_L, SiC(CH₃)₂CH(CH₃)₂], 0.22 [s, 6H, Si(CH₃)₂]. ¹³C NMR (75 MHz, CDCl₃): δ 173.56 (C=O), 171.09 (C=O), 170.58 (C=O), 169.92 (C=O), 167.77 (C=O), 138.84-127.43 (m, aromatic), 108.66 (C-2''), 99.90 (C-1'), 98.60 (C(CH₃)₂ of isopropylidene), 96.40 (C-1), 77.43-76.58 (C-3, 7''), 76.58 (C-4), 75.75 (C-3_S), 75.51 (C-3_S), 74.17 (C-5), 73.94 (C-4'), 73.87 (CH₂Ph), 73.36 (CH₂Ph), 73.19 (CH₂Ph), 72.52 (C-5'), 71.74 (C-3'), 71.47 (CH₂Ph), 71.15 (CH₂Ph), 70.83 (C-5''), 70.75 (C-3_L), 69.80 (C-4'', 8''), 68.95 (C-6), 68.40 (C-6'', CH₂Ph), 68.09 (CH₂Ph), 66.97 (CO₂CH₂Ph), 66.90 (C-2), 61.79 (C-6'), 56.70 (C-2'), 41.64 (C-2_L),

39.72 (C-2_S), 38.92 (C-2_S), 34.48-14.09 [m, 3'', SiC(CH₃)₂CH(CH₃)₂, CH₃ of isopropylidene, 2 × C-(4_S-12_S), C-(4_L-14_L), C-(2_L'-12_L'), -1.84 (SiCH₃), -3.42 (SiCH₃). HR MS (m/z) calcd for C₁₃₁H₁₉₁N₄O₂₆Si[M+Na]⁺, 2318.3151; found, 2318.5374.

Dimethylthexylsilyl benzyl (7,8-di-O-benzyl-3-deoxy-4,5-O-isopropylidene- α -D-manno-oct-2-ulopyranosyl)onate-(2→6)-3-O-[(R)-3-benzyloxy-dodecanoyl]-2-deoxy-4-O-(1,5-dihydro-3-oxo-3 λ^5 -H-2,4,3-benzodioxaphosphepin-3-yl)-2-[(R)-3-dodecanoyloxy-tetradecanoyl]- β -D-glucopyranosyl-(1→6)-4-O-benzyl-3-O-[(R)-3-benzyloxy-dodecanoyl]-2-deoxy-2-[(R)-3-dodecanoyloxy-tetradecanoyl]- β -D-glucopyranoside (21)

A suspension of compound **20** (120 mg, 52 μ mol) and zinc (< 10 micron, 210 mg, 3.2 mmol) in a mixture of acetic acid (100 μ L) and DCM (5 mL) was stirred at room temperature for 1 h, after which it was diluted with ethyl acetate (20 mL). The solids were removed by filtration, and the residue was washed with ethyl acetate (2 × 3 mL). The combined filtrates were washed with saturated aqueous NaHCO₃ (2 × 20 mL) and brine (2 × 20 mL). The organic phase was dried (MgSO₄), filtered, and the filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/ethyl acetate, 4/1, v/v) to afford the amine as a pale yellow syrup (108 mg, 92%). R_f = 0.45 (hexane/ethyl acetate, 5/2, v/v). A solution of (R)-3-dodecanoyloxy-dodecanoic acid **9** (43 mg, 100 μ mol) and DCC (31 mg, 150 μ mol) in DCM (5 mL) was stirred at room temperature for 10 min, and then the above obtained intermediate (120 mg, 53 μ mol) was added. The reaction mixture was stirred for another 10 h, after which the solids were removed by filtration and washed with DCM (2 × 1 mL). The combined filtrates were concentrated in vacuo. The residue was purified by silica gel column chromatography (eluent: hexane/ethyl acetate, 5/1-4/1, v/v) to afford **21** as an amorphous solid (126 mg, 89%). R_f = 0.65 (hexane/ethyl acetate, 5/2, v/v); $[\alpha]_D^{25}$ = +4.3° (c = 1.0, CHCl₃); ¹H NMR (600 MHz, CDCl₃): δ 7.47-7.17 (m, 32H, aromatic), 6.76 (d, 1H, J = 7.8 Hz, aromatic), 6.66 (d, 1H, J = 6.0 Hz, aromatic), 5.71-5.69 (m, 2H, NH , NH'), 5.64 (t, 1H, $J_{2',3'} = J_{3',4'} = 9.6$ Hz, H-3'), 5.30 (d, 1H, J = 13.2 Hz, CO₂CHHPh), 5.25 (d, 1H, J = 13.2 Hz, CO₂CHHPh), 5.16 (t, 1H, $J_{2,3} = J_{3,4} = 9.5$ Hz, H-3), 5.15 (t, 1H, $J_{2,3} = J_{3,4} = 9.6$ Hz, H-3), 5.13-5.05 (m, 3H, 2 × H-3_L, CHHPh), 5.04 (d, 1H, $J_{1',2'} = 8.4$ Hz, H-1'), 4.97 (dd, 1H, J = 11.4 Hz, J = 13.8 Hz, CHHPh), 4.87-4.75 (m, 4H, 4 × CHHPh), 4.73-4.69 (m, 1H, H-4'), 4.68 (d, 1H, $J_{1,2} = 7.8$ Hz, H-1), 4.65-4.46 (m, 10H, H-4'', H-5'', 8 × CHHPh), 4.15-4.13 (m, 1H, H-7''), 4.06 (d, 1H, J = 9.6 Hz, H-6''), 3.99-3.93 (m, 3H, H-2, H-8a', H-3_S), 3.89-3.84 (m, 4H, H-6a, H-6a', H-8b'', H-3_S), 3.66 (d, 1H, $J_{6a',6b'} = 10.8$ Hz, H-6b'), 3.62-3.56 (m, 2H, H-5, H-6b), 3.49 (dd, 1H, J = 4.2 Hz, J = 10.2 Hz, H-5'), 3.38 (t, 1H, $J_{3,4} = J_{4,5} = 9.0$ Hz, H-4), 3.19 (dd, 1H, J = 7.8 Hz, H-2'), 2.77 (dd, 1H, $J_{2Sa,2Sb} = 16.2$ Hz, $J_{2Sa,3S} = 7.2$ Hz, H-2_{Sa}), 2.72 (dd, 1H, $J_{2Sa,2Sb} = 16.2$ Hz, $J_{2Sb,3S} = 4.8$ Hz, H-2_{Sb}), 2.61 (dd, 1H, $J_{2Sa,2Sb} = 16.2$ Hz, $J_{2Sa,3S} = 7.2$ Hz, H-2_{Sa}), 2.49 (dd, 1H, $J_{2Sa,2Sb} = 16.2$ Hz, $J_{2Sb,3S} = 5.4$ Hz, H-2_{Sb}), 2.43-2.24 (m, 8H, H-3a'', 2 × H-2_{La}, H-2_{Lb}, 2 × H-2_{L'}), 2.19 (dd, 1H, $J_{3a'',3b''} = 15.0$ Hz, $J_{3b'',4''} = 5.4$ Hz, H-3b''), 2.16 (dd, 1H, $J_{2La,2Lb} = 15.0$ Hz, $J_{2Lb,3L} = 6.0$ Hz, H-2_{Lb}), 1.68-1.58 [m, 15H, 2 × H-4_S, 2 × H-4_L, 2 × H-3_L, CH(CH₃)₂], 1.46 (s, 3H, CH₃ of isopropylidene), 1.39 (s, 3H, CH₃ of isopropylidene), 1.22 [bs, 96H, 2 × H-(5_S-11_S), 2 × H-(5_L-13_L), 2 × H-(4_L'-11_L'), 0.96-0.87 [m, 30H, 2 × H-12_S, 2 × H-14_L, 2 × H-12_L, SiC(CH₃)₂CH(CH₃)₂], 0.21 (s, 3H, SiCH₃), 0.20 (s, 3H, SiCH₃). ¹³C NMR (75 MHz, CDCl₃): δ 173.60 (C=O), 173.54 (C=O), 171.62 (C=O), 171.06 (C=O), 169.95 (C=O), 169.05 (C=O), 168.04 (C=O), 139.00-127.43 (m, aromatic), 108.69 (C-2''), 99.60 (C-1'), 98.43 [C(CH₃)₂ of isopropylidene], 85.85 (C-1), 77.26 (C-7''), 76.90 (C-4), 75.50-75.41 (3C, C-3, C-3_S × 2), 74.22-74.08 (4C, C-4', 5, CH₂Ph × 2), 73.37 (CH₂Ph), 72.52 (C-5'), 71.75 (C-3'), 71.27 (CH₂Ph), 71.21 (CH₂Ph), 70.98 (C-5''), 70.71 (C-3_L × 2), 70.20 (C-8''), 69.88 (C-4''), 68.85 (C-6), 68.62 (C-6''), 68.41 (CH₂Ph), 68.13 (CH₂Ph), 66.98 (CO₂CH₂Ph), 61.90 (C-6'), 56.74 (C-2'), 56.20 (C-2), 41.56 (C-2_L), 41.47 (C-2_L), 39.60 (C-2_S), 38.96 (C-2_S), 34.47-14.09 [m, 3'', SiC(CH₃)₂CH(CH₃)₂, CH₃ of isopropylidene, 2 × C-(4_S-12_S), 2 × C-(4_L-14_L), 2 × C-(2_L'-12_L'), -1.56 (SiCH₃), -3.31 (SiCH₃). HR MS (m/z) calcd for C₁₅₇H₂₄₁N₂O₂₉Si[M+Na]⁺, 2700.6850; found, 2700.6572.

Bis(benzyloxy)phosphoryl benzyl (7,8-di-O-benzyl-3-deoxy- α -D-manno-oct-2-ulopyranosyl) onate-(2 $\rightarrow$ 6)-3-O-[(R)-3-benzyloxydodecanoyl]-2-deoxy-4-O-(1,5-dihydro-3-oxo-3 λ^5 -3H-2,4,3-benzodioxaphosphepin-3-yl)-2-[(R)-3-dodecanoyloxy-tetradecanoyl]- β -D-glucopyranosyl-(1 $\rightarrow$ 6)-4-O-benzyl-3-O-[(R)-3-benzyloxy-dodecanoyl]-2-deoxy-2-[(R)-3-dodecanoyloxy-tetradecanoyl]- α -D-glucopyranose (22)

TFA/H₂O (3/2, v/v, 100 μ L) was added dropwise to a stirred solution of **21** (30 mg, 11 μ mol) in DCM (2 mL). The reaction mixture was stirred at room temperature for 6 h, after which it was diluted with ethyl acetate (10 mL) and then washed with saturated aqueous NaHCO₃ (2 $\times$ 10 mL) and brine (2 $\times$ 10 mL). The organic phase was dried (MgSO₄), filtered, and the filtrate was concentrated in vacuo. The residue was purified by preparative silica gel TLC (eluent: hexane/ethyl acetate, 3/1, v/v) to afford the lactol as a pale yellow syrup (25 mg, 89%). R_f = 0.25 (hexane/acetone, 1/1, v/v); ¹H NMR (600 MHz, CDCl₃): δ 7.42-7.19 (m, 32H, aromatic), 6.82 (d, 1H, J = 7.8 Hz, aromatic), 6.64 (d, 1H, J = 6.0 Hz, aromatic), 5.91 (d, 1H, $J_{2,NH}$ = 9.6 Hz, NH), 5.81 (d, 1H, $J_{2',NH'}$ = 7.8 Hz, NH'), 5.44 (t, 1H, $J_{2',3'} = J_{3',4'} = 9.6$ Hz, H-3'), 5.38 (d, 1H, $J_{1',2'}$ = 7.8 Hz, H-1'), 5.32 (dd, 1H, J = 9.6 Hz, J = 10.2 Hz, H-3), 5.19 (d, 1H, J = 12.6 Hz, CO₂CHHPh), 5.14 (d, 1H, J = 12.6 Hz, CO₂CHHPh), 5.11-5.07 (m, 2H, H-1, H-3_L), 5.01-4.88 (m, 3H, H-3_L, 2 $\times$ CHHPh), 4.83 (dd, 1H, J = 12.2 Hz, J = 13.8 Hz, CHHPh), 4.74-4.67 (m, 3H, 3 $\times$ CHHPh), 4.64-4.59 (m, 1H, H-4'), 4.58-4.41 (m, 10H, 7 $\times$ CHHPh), 4.35-4.31 (m, 2H, H-4'', CHHPh), 4.12-3.94 (m, 5H, H-2, H-5, H-5'', H-6'', H-7''), 3.86-3.72 (m, 7H, H-6a', H-6a'', H-6b', H-8a'', H-8b'', 2 $\times$ H-3_S), 3.66 (d, 1H, $J_{5',6b'}$ = 7.2 Hz, $J_{6a',6b'}$ = 12.0 Hz, H-6b'), 3.46 (bs, 1H, H-5'), 3.24 (t, 1H, $J_{3,4} = J_{4,5} = 9.6$ Hz, H-4), 3.02 (dd, 1H, J = 7.8 Hz, H-2'), 2.68 (dd, 1H, $J_{2Sa,2Sb} = 16.2$ Hz, $J_{2Sa,3S} = 4.8$ Hz, H-2_{Sa}), 2.61 (dd, 1H, $J_{2Sa,2Sb} = 16.2$ Hz, $J_{2Sb,3S} = 6.6$ Hz, H-2_{Sb}), 2.55 (dd, 1H, $J_{2Sa,2Sb} = 15.6$ Hz, $J_{2Sa,3S} = 7.8$ Hz, H-2_{Sa}), 2.41 (dd, 1H, $J_{2Sa,2Sb} = 16.2$ Hz, $J_{2Sb,3S} = 4.8$ Hz, H-2_{Sb}), 2.34-2.22 (m, 7H, 2 $\times$ H-2_{La}, H-2_{Lb}, H-2_{L'} $\times$ 4), 2.18 (dd, 1H, $J_{3a'',3b''} = 12.6$ Hz, $J_{3a'',4''} = 4.6$ Hz, H-3a''), 2.11 (dd, $J_{2La,2Lb} = 15.6$ Hz, $J_{2Lb,3L} = 6.0$ Hz, H-2_{Lb}), 1.96 (t, 1H, $J_{3a'',3b''} = J_{3b'',4''} = 12.6$ Hz, H-3b''), 1.61-1.48 (m, 14H, 2 $\times$ H-4_S, 2 $\times$ H-4_L, 2 $\times$ 3_{L'}), 1.22 [bs, 96H, 2 $\times$ H-(5_S-11_S), 2 $\times$ H-(5_L-13_L), 2 $\times$ H-(4_L-11_L)], 0.96-0.87 (m, 18H, 2 $\times$ H-12_S, 2 $\times$ H-14_L, 2 $\times$ H-12_L). HR MS (m/z) calcd for C₁₄₆H₂₁₉N₂O₂₉Si[M+Na]⁺, 2518.5359; found, 2518.2606. To a cooled (-78°C) solution of the lactol intermediate (23 mg, 9.2 μ mol) and tetrabenzyl diphosphate (10 mg, 18.4 μ mol) in THF (2 mL) was added dropwise lithium bis(trimethylsilyl)amide in THF (1.0 M, 15 μ L, 15 μ mol). The reaction mixture was stirred for 1 h, and then allowed to warm up to -20°C. After stirring the reaction mixture for 1 h at -20°C, it was quenched with saturated aqueous NaHCO₃ (10 mL), and diluted with ethyl acetate (15 mL). The organic phase was washed with brine (2 $\times$ 15 mL), dried (MgSO₄), filtered, and the filtrate was concentrated in vacuo. The residue was purified by Iatrobeds column chromatography (hexane/ethyl acetate, 3/1 $\rightarrow$ 2/1 $\rightarrow$ 1/1 $\rightarrow$ 3/4, v/v) to give **22** as a colorless syrup (19 mg, 75%). R_f = 0.50 (hexane/acetone, 1/1, v/v); ¹H NMR (500 MHz, CDCl₃): δ 7.40-7.14 (m, 42H, aromatic), 6.82 (d, 1H, J = 8.0 Hz, aromatic), 6.69 (d, 1H, J = 7.5 Hz, aromatic), 6.41 (d, 1H, $J_{2',NH'}$ = 8.5 Hz, NH'), 6.04 (d, 1H, $J_{2,NH}$ = 8.5 Hz, NH), 5.65 (bs, 1H, H-1), 5.35 (t, 1H, $J_{2',3'} = J_{3',4'} = 9.5$ Hz, H-3'), 5.24-4.73 (m, 12H, H-1', H-3, 5 $\times$ CHHPh), 4.61-4.36 (m, 11H, H-4', 5 $\times$ CHHPh), 4.38 (d, 1H, J = 11.5 Hz, CHHPh), 4.27-4.23 (m, 1H, H-4''), 4.19-4.14 (m, 1H, H-2), 4.09-3.96 (m, 4H, H-5, H-5'', H-6'', H-7''), 3.91-3.74 (m, 8H, H-6a', H-6a'', H-6b', H-6b'', H-8a'', H-8b'', 2 $\times$ H-3_S), 3.53-3.44 (m, 3H, H-2', H-4, H-5'), 2.70 (dd, 1H, $J_{2Sa,2Sb} = 16.5$ Hz, $J_{2Sa,3S} = 7.0$ Hz, H-2_{Sa}), 2.65 (dd, 1H, $J_{2Sa,2Sb} = 16.5$ Hz, $J_{2Sb,3S} = 5.0$ Hz, H-2_{Sb}), 2.56 (dd, 1H, $J_{2Sa,2Sb} = 16.0$ Hz, $J_{2Sa,3S} = 7.5$ Hz, H-2_{Sa}), 2.48 (dd, 1H, $J_{2Sa,2Sb} = 16.0$ Hz, $J_{2Sb,3S} = 5.0$ Hz, H-2_{Sb}), 2.31-2.18 (m, 7H, 2 $\times$ H-2_{La}, H-2_{Lb}, 2 $\times$ H-2_{L'}), 2.12 (dd, 1H, $J_{3a'',3b''} = 11.0$ Hz, $J_{3a'',4''} = 5.0$ Hz, H-3a''), 2.09 (dd, $J_{2La,2Lb} = 16.5$ Hz, $J_{2Lb,3L} = 6.0$ Hz, H-2_{Lb}), 2.04 (t, 1H, $J_{3a'',3b''} = J_{3b'',4''} = 12.5$ Hz, H-3b''), 1.61-1.49 (m, 14H, 2 $\times$ H-4_S, 2 $\times$ H-4_L, 2 $\times$ 3_{L'}), 1.26 [bs, 96H, 2 $\times$ H-(5_S-11_S), 2 $\times$ H-(5_L-13_L), 2 $\times$ H-(4_L-11_L)], 0.90-0.88 (m, 18H, 2 $\times$ H-12_S, 2 $\times$ H-14_L, 2 $\times$ H-12_L). HR MS (m/z) calcd for C₁₆₀H₂₃₂N₂O₃₂Si[M+Na]⁺, 2778.5961; found, 2778.9185.

(3-Deoxy- α -D-manno-oct-2-ulopyranosyl)onate-(2 $\rightarrow$ 6)-3-O-[(R)-3-hydroxy-dodecanoyl]-2-deoxy-2-[(R)-3-dodecanoyloxy-tetradecanoyl]- β -D-glucopyranosyl-(1 $\rightarrow$ 6)-3-O-[(R)-3-hydroxy-dodecanoyl]-2-deoxy-2-[(R)-3-dodecanoyloxy-tetradecanoyl]- β -D-glucopyranose 1, 4'-biphosphate (3)

A mixture of **22** (9 mg, 3.3 μ mol) and Pd black (15.0 mg) in anhydrous THF (3 mL) was shaken under an atmosphere of H₂ (65 psi) at room temperature for 30 h, after which it was neutralized with triethylamine (10 μ L), and the catalyst was removed by filtration and the residue washed with THF (2 $\times$ 1 mL). The combined filtrates were concentrated in vacuo to afford **3** as a colorless film (5.0 mg, 78%). ¹H NMR (600 MHz, CDCl₃): δ 5.24 (bs, 1H, H-1), 4.91-4.83 (m, 4H, H-3, H-3', 2 $\times$ H-3_L), 3.86 (m, 1H, H-2), 3.82-3.45 (m, 11H, H-5, H-5', H-5'', H-6a, H-6b, H-6'a, H-6'b, H-6'', H-7'', H-8''a, H-8''b), 3.80 (m, 2H, H-4', H-4''), 3.76 (m, 1H, H-3_S), 3.72 (m, 1H, H-3_S), 3.60 (m, 1H, H-2'), 3.29 (m, 1H, H-4), 2.23-2.07 (m, 8H, 2 $\times$ H-2_S, 2 $\times$ H-2_L), 2.04-1.98 (m, 4H, 2 $\times$ H-2_L), 1.76 (dd, 1H, $J_{3a'',3b''} = 15.0$ Hz, $J_{3a'',4''} = 5.4$ Hz, H-3a''), 1.61 (t, 1H, $J_{3a'',3b''} = J_{3b'',4''} = 15.0$ Hz, H-3b''), 1.41-1.19 (m, 14H, 2 $\times$ H-4_S, 2 $\times$ H-4_L, 2 $\times$ H-3_L), 1.12 [bs, 96H, 2 $\times$ H-(5_S-11_S), 2 $\times$ H-(5_L-13_L), 2 $\times$ H-(4_L-11_L)], 0.91-0.88 (m, 18H, 2 $\times$ H-12_S, 2 $\times$ H-14_L, 2 $\times$ H-12_L). HR MS (m/z) (negative) calcd for C₁₆₀H₂₃₂N₂O₃₂Si, 1933.1838; found, 1932.6287[M], 1954.5441[M+Na-H], 1976.4611[M+2Na-2H].

Cell maintenance

RAW 264.7 γ NO(-) cells, derived from the RAW 264.7 mouse monocyte/macrophage cell line, were obtained from ATCC. The cells were maintained in RPMI 1640 medium (ATCC) with L-glutamine (2 mM), adjusted to contain sodium bicarbonate (1.5 g L⁻¹), glucose (4.5 g L⁻¹), HEPES (10 mM), and sodium pyruvate (1.0 mM) and supplemented with penicillin (100 u mL⁻¹) / streptomycin (100 μ g mL⁻¹; Mediatech) and fetal bovine serum (FBS, 10%; Hyclone). Cells were maintained in a humid 5% CO₂ atmosphere at 37°C.

Cytokine induction and ELISAs

RAW 264.7 γ NO(-) cells were plated on the day of the exposure assay as 2 $\times$ 10⁵ cells/well in 96-well tissue culture plates (Nunc). Cells were incubated with different stimuli for 5.5 h in replicates of five. Culture supernatants were then collected, pooled, and stored frozen (-80°C) until assayed for cytokine production.

Cytokine ELISAs were performed in 96-well MaxiSorp plates (Nunc). A DuoSet ELISA Development Kit (R&D Systems) was used for the quantification of mouse TNF- α according to the manufacturer's instructions. The absorbance was measured at 450 nm with wavelength correction set to 540 nm using a microplate reader (BMG Labtech). Concentrations of IFN- β in culture supernatants were determined as follows. ELISA MaxiSorp plates were coated with rabbit polyclonal antibody against mouse IFN- β (PBL Biomedical Laboratories). IFN- β in standards and samples was allowed to bind to the immobilized antibody. Rat anti-mouse IFN- β antibody (USBiological) was then added, producing an antibody-antigen-antibody "sandwich". Next, horseradish peroxidase (HRP) conjugated goat anti-rat IgG (H+L) antibody (Pierce) and a chromogenic substrate for HRP 3,3',5,5'-tetramethylbenzidine (TMB; Pierce) were added. After the reaction was stopped, the absorbance was measured at 450 nm with wavelength correction set to 540 nm. All cytokine values are presented as the means $\pm$ SD of triplicate measurements, with each experiment being repeated three times.

Data analysis

Concentration-response data were analyzed using nonlinear least-squares curve fitting in Prism (GraphPad Software, Inc.). These data were fit with the following four parameter logistic equation: $Y = E_{\max} / (1 + (EC_{50}/X)^{\text{Hill slope}})$, where Y is the cytokine response, X is logarithm

of the concentration of the stimulus, $E_{\max}$ is the maximum response, and EC_{50} is the concentration of the stimulus producing 50% stimulation. The Hill slope was set at 1 to be able to compare the EC_{50} values of the different inducers.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Scheme 1.

Reagents and conditions: (a) AllocCl, TMEDA, DCM; (b) PhBCl₂, Et₃SiH, MS 4 Å, DCM, -75°C; (c) **8**, DCC, DMAP, DCM; (d) Zn/HOAc, DCM; then FmocCl, DIPEA, DCM; (e) Bu₄NF, AcOH, THF; then CCl₃CN, Cs₂CO₃, DCM.

Scheme 2.

Reagents and conditions: (a) TfOH, DCM, -50°C ; (b) TFA, H_2O , DCM; (c) **7**, $\text{BF}_3 \cdot \text{Et}_2\text{O}$, DCM, MS 4 Å, 0°C ; (d) *N,N*-Diethyl-1,5-dihydro-3H-2,3,4-benzodioxaphosphin-3-amine, 1*H*-tetrazole, DCM; then mCPBA, -20°C ; (e) DBU, DCM; then **9**, DCC, DCM; (f) Pd (PPh_3)₄, HCO_2H , *n*-BuNH₂, THF; then **8**, DCC, DMAP, DCM; (g) Zn/HOAc, DCM; then **9**, DCC, DCM; (h) TFA, H_2O , DCM; then tetrabenzyl diphosphate, $\text{LiN}(\text{TMS})_2$, THF, -78°C ; (i) H_2 (65 psi), Pd-black, THF.

Scheme 3.

Reagents and conditions: (a) Zn/HOAc, DCM; then **9**, DCC, DCM; (b) Pd(PPh₃)₄, HCO₂H, n-BuNH₂, THF; (c) **8**, DCC, DMAP, DCM; (d) HF/pyridine; then tetrabenzyl diphosphate, LiN(TMS)₂, THF, -78°C; (e) H₂ (65 psi), Pd-black, THF.

1. *N. meningitidis* lipid A

2. hexa-acylated
E. coli lipid A

3. *N. meningitidis* lipid A
with KDO

4. *E. coli* lipid A with
shorter lipids

Figure 1.
Chemical structures of target lipid As.

Figure 2.

TNF- α and IFN- β production by murine macrophages after stimulation with LOS and lipid A derivatives. Murine RAW γ NO(-) cells were incubated for 5.5 h with increasing concentrations of *N. meningitidis* LOS or lipid A derivatives **1-4** as indicated. TNF- α (a) and IFN- β (b) in cell supernatants were measured using ELISAs.

Table 1EC₅₀ values^[a] (nM) of *N. meningitidis* LOS and lipid A derivatives **1-4**.

	TNF- α		IFN- β	
<i>N. meningitidis</i> LOS	0.085	(0.058 - 0.13)	0.11	(0.085 - 0.15)
lipid A 1	1.5	(1.3 - 1.9)	4.0	(3.6 - 4.5)
lipid A 2	21	(16 - 28)	124	(105 - 147)
lipid A 3	0.092	(0.074 - 0.12)	0.20	(0.17 - 0.49)
lipid A 4	4.1	(2.5 - 6.7)	16	(12 - 23)

^[a] Values of EC₅₀ are reported as best-fit values and as minimum-maximum range (best-fit value $\pm$ std. error).