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Synthesis and Toll-like Receptor 4 (TLR4) Activity of Phosphatidylinositol Dimannoside Analogues

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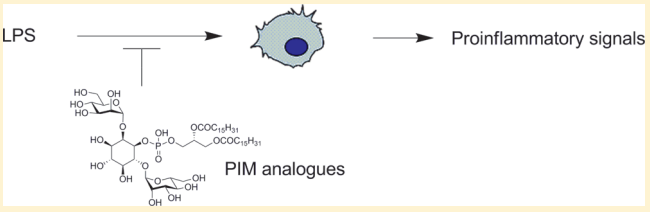
Supporting Information

ABSTRACT: A series of five PIM_2 analogues were synthesized and tested for their ability to activate primary macrophages and modulate LPS signaling. Structural changes included replacement of the fatty acid esters of the phosphatidyl moiety of PIM_2 with the corresponding ether or amide. An $AcPIM_2$ analogue possessing an ether linkage was also prepared. The synthetic methodology utilized an orthogonally protected chiral *myo*-inositol starting material that was conveniently prepared from *myo*-inositol in just two steps. Important steps in the synthetic protocols included the regio- and α-selective glycosylation of inositol O-6 and introduction of the phosphodiester utilizing phosphoramidite chemistry. Replacement of the inositol core with a glycerol moiety gave compounds described as phosphatidylglycerol dimannosides (PGM_2). Biological testing of these PIM compounds indicated that the agonist activity was TLR4 dependent. An ether linkage increased agonist activity. Removal of the inositol ring enhanced antagonist activity, and the presence of an additional lipid chain enhanced LPS-induced cytokine production in primary macrophages. Furthermore, the interruption of the LPS-induced 2:2 TLR4/MD-2 signaling complex formation by PIM_2 represents a previously unidentified mechanism involved in the bioactivity of PIM molecules.



INTRODUCTION

The cell wall of mycobacteria is a rich source of immunomodulatory molecules that includes lipids, glycoplipids, phosphoglycolipids, lipoproteins, and mycolyl-arabinogalactan-peptiglycan motifs.[1] Included among these is the family of mannosylated phosphatidylinositols, commonly known as phosphatidylinositol mannosides or PIMs,[2] that are of great interest because of their biological activity and synthetic accessibility. Biological activity includes the ability to affect T-cell proliferation,[3,4] recruit natural killer T (NKT) cells,[5−7] activate innate receptors,[6,8] and function as immune adjuvants.[9−11] In contrast PIMs have also been shown to suppress allergic airway disease,[12,13] negatively regulate lipopolysaccharide (LPS) signaling in macrophages,[14] and suppress human T-cell proliferation.[15]

In a previous study[16] we synthesized a PIM_2 monoether analogue, PIM_2ME (**1**), where the glyceryl *sn*-2 acyl group ($COC_{15}H_{31}$) had been replaced by an alkyl group ($C_{16}H_{33}$). Our motivation for this change was to overcome the inherent lability of this acyl group toward hydrolysis, giving rise to *lyso*-PIM species. This analogue, **1**, enhanced cytokine production by bone-marrow-derived dendritic cells compared to the natural analogous PIM_2 compound (**2**). Here, we report the syntheses and activity of five novel PIM analogues, **3**, **4**, **5**, **6**, and **7** where the functionality on the glyceryl moiety and the inositol ring has been varied to probe SAR for this class of compound (Figure 1). The targets were specifically designed to gain as much structural information as possible: target **3** probes amide versus ether or ester functionality at the *sn*-2 position; **4** is a positional isomer of **1** (*sn*-1 vs *sn*-2); analogue **5** investigates the effect of introducing a third lipid functionality; targets **6** and **7** probe the importance of the inositol ring. The previously reported diether **8**[16] was also tested to investigate the impact of two ether linkages. These compounds were then assayed and compared for their ability to induce cytokine production and suppress LPS-induced cytokine production in primary macrophages. We also investigated the interactions of PIMs with the TLR4 signaling pathway.

RESULTS AND DISCUSSION

Previous syntheses of PIM compounds have utilized 1-*O*-allyl-3,4,5-tri-*O*-benzyl-D-*myo*-inositol as a convenient starting material.[9,17−19] This compound is generally obtained from α-methyl

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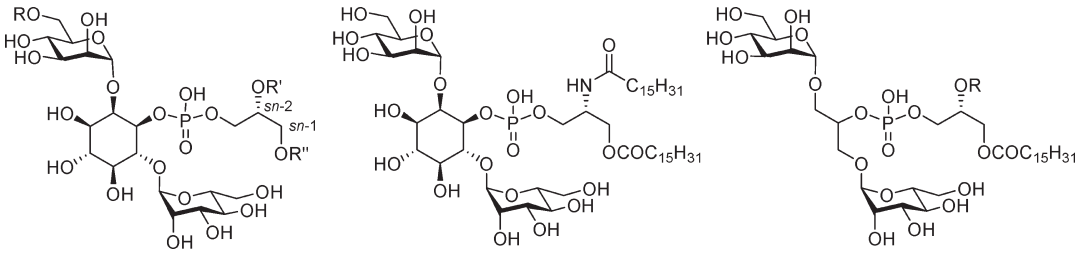


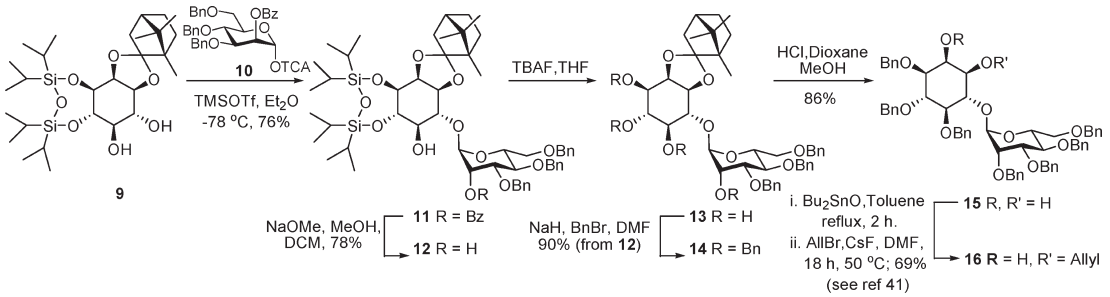
1 R=H, R'=$C_{16}H_{33}$, R''=$COC_{15}H_{31}$
2 R=H, R'=R''=$COC_{15}H_{31}$
4 R=H, R'=$COC_{15}H_{31}$, R''=$C_{16}H_{33}$
5 R=R''=$COC_{15}H_{31}$, R'=$C_{16}H_{33}$
8 R=H, R'=$C_{16}H_{33}$, R''=$C_{16}H_{33}$

3

6 R=$C_{16}H_{33}$
7 R=$COC_{15}H_{31}$

Figure 1. Structures of PIM analogues.

Scheme 1. Synthesis of Common Intermediate 16



D-glucopyranoside by a series of transformations that, although often produce high yields, suffers from the use of stoichiometric amount of a mercuric trifluoroacetate in a carbocyclization reaction.[20] Also, this reaction and the subsequent reduction steps both suffer from the unwanted formation of isomers that are difficult to separate resulting in a relatively unscalable process. For these reasons, we decided to utilize a different approach starting from the known diol **9**[21] that can be prepared from *myo*-inositol via the camphanilydene acetal in a scalable two-step process.[22]

Synthesis of 6-Mannosylated *myo*-Inositol 15. Glycosylation of diol **9** is known to be favored at the C-6 hydroxyl on inositol. Moreover, Martin-Lomas has reported[21] the selective glycosylation of **9** with an 2-azido donor in the synthesis of inositol phosphoglycans (IPGs), and Watanabe demonstrated the synthesis of distearoyl PIM_2 using the analogous racemic cyclohexylidene acetal.[23,24] In the current work, glycosylation of **9** with mannosyl donor **10**[25] gave the desired glycoside **11** as the major product along with smaller amounts of the diglycosylated product. Lowering the temperature of the glycosylation inhibited the formation of the overglycosylated product, resulting in the clean formation of **11**. Routine functional group manipulation via **12** through **14** afforded diol **15**[26] that was regioselectively allylated using a minor modification of the literature transformation to **16** (Scheme 1).[26]

Synthesis of Phosphoramidites 17 and 18. The phosphoramidites **17** and **18** were prepared for the synthesis of targets **3** and **4**, respectively. With this in mind, the known monoether **19**[27] was esterified and the *p*-methoxybenzyl group removed by hydrogenoloysis to afford alcohol **21**[28] in good yield. The phosphoramidite **17** was prepared by reaction with benzyloxy-bis(diisopropylamino)phosphine and 1*H*-tetrazole.

The commercially available (*R*)-(+)-2-amino-3-benzyloxy-1-propanol (**22**) was used as a starting material for the synthesis of the 2-amido containing phosphoramidite **18**. Diacylation with palmitoyl chloride and DMAP in chloroform[29] afforded **23** (Scheme 2). Hydrogenolysis of **23** over Pearlman's catalyst proceeded smoothly to give the desired alcohol **24**. 1*H*-Tetrazole activated reaction of alcohol with benzyloxy-bis(diisopropylamino)phosphine gave the required phosphoramidite **18** in high yield over the three steps.

Synthesis of 3 and 4. Glycosylation of **16** with **10** afforded an inseparable mixture of the anomeric glycosides. This result again demonstrates the propensity of the O-2 inositol hydroxyl to form unexpected β-anomers.[30] Removal of the benzoate directing group allowed separation of the anomers and access to the desired pseudo-trisaccharide **26**. Subsequent benzylation followed by deallylation provided the known inositol dimannoside headgroup **28**.[31] The spectroscopic data collected for compound **28** prepared in this way were identical to those reported previously. Inositol dimannoside **28** was then coupled with phosphoramidite **18** to afford the benzyl protected target molecule **30** in 62% yield (Scheme 3). Hydrogenolysis over Pearlman's catalyst gave the target PIM_2-monoamide analogue that was redissolved in $MeOH/H_2O$ with the aid of triethylamine and then passed through a short column of silica gel. Evaporation and lyophilization of the residue gave **3** as the partial triethylammonium salt in 75% yield. Coupling of **28** with phosphoramidite **17** followed by oxidation afforded the fully protected **29** that was subsequently deprotected to afford the PIM *sn*-1 ether analogue **4** in good purity.

For the synthesis of $AcPIM_2$ monoether (**5**), the thioglycoside donor **31** was employed (Scheme 4). This compound was prepared from diol **32**[32] by regioselective acylation of O-6 with palmitoyl chloride followed by acylation of O-2 with 2-azidomethylbenzoyl

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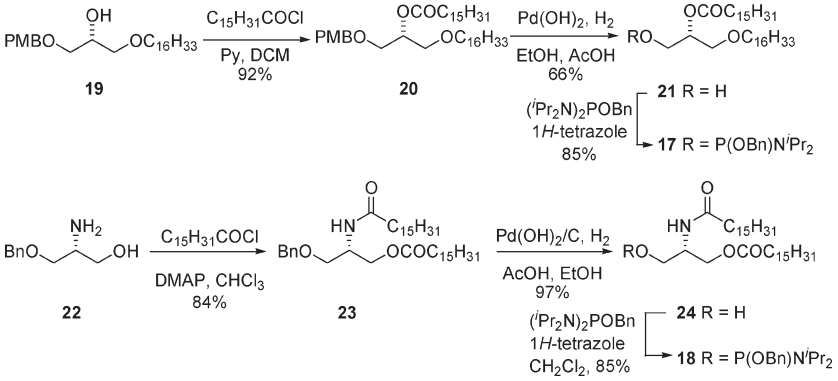
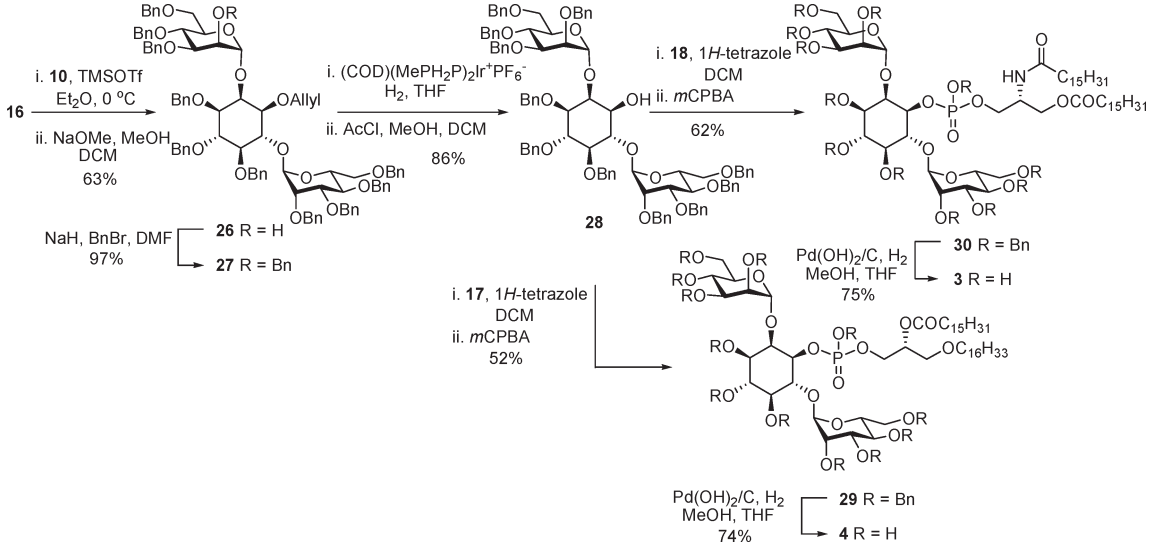
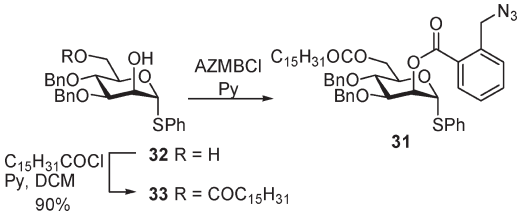
Scheme 2. Synthesis of Phosphoramidites 17 and 18



Scheme 3. Synthesis of 3 and 4



Scheme 4. Synthesis of Donor 31



chloride (AZMBCl).[33] The AZMB protecting group was chosen to provide α-selectivity in the glycosylation, together with an orthogonal mode of deprotection.

Coupling of **16** and **31** using standard thioglycosylation conditions afforded the α,α-product **34** in 34% yield (Scheme 5). The two anomeric ^{13}C NMR signals of **34** were coincident; however, $^{1}J_{CH}$ values for these signals (both 175 Hz) could be extracted from a HSQC experiment run without ^{13}C decoupling during acquisition. As observed previously (vide infra), some β-coupled product was also formed which could be removed by column chromatography.[30] Deallylation followed by installation of the phosphodiester linkage using the known phosphoramidite **36**[16] and deprotection afforded the target compound **5** in high yield and purity.

To probe the effect of the inositol ring in combination with the above changes to the phosphatidyl group, phosphatidylglycerol dimannoside monoether analogue **6** (PGM_2ME), where the inositol ring is replaced by a glycerol unit, was prepared. Preparation of the dimannosylated glycerol derivative was effected by DDQ deprotection of the known PMB ether **39**[12] to give the desired headgroup **40** in 72% yield. Reaction of the dimannosylated glycerol alcohol **40** and phosphoramidite **36** provided the protected lipid **41** (Scheme 6). Hydrogenolytic debenzylation to **41** and selective hydrolysis of the acetates gave the *sn*-2 ether linked PGM_2 analogue **6**. In a similar manner the bis-acylated congener **7** was prepared from dimannosylated glycerol **40** and phosphoramidite **43**.

PIM Analogues Induce Interleukin-6 (IL-6) Production and Negatively Regulate LPS Signaling in Primary Peritoneal Macrophages. TLRs play a key role in the innate immune response of macrophages to exogenous pathogens via recognition of pathogen-associated molecular patterns.[34−36] Whether any specific interaction(s) occur between PIMs and TLRs is currently

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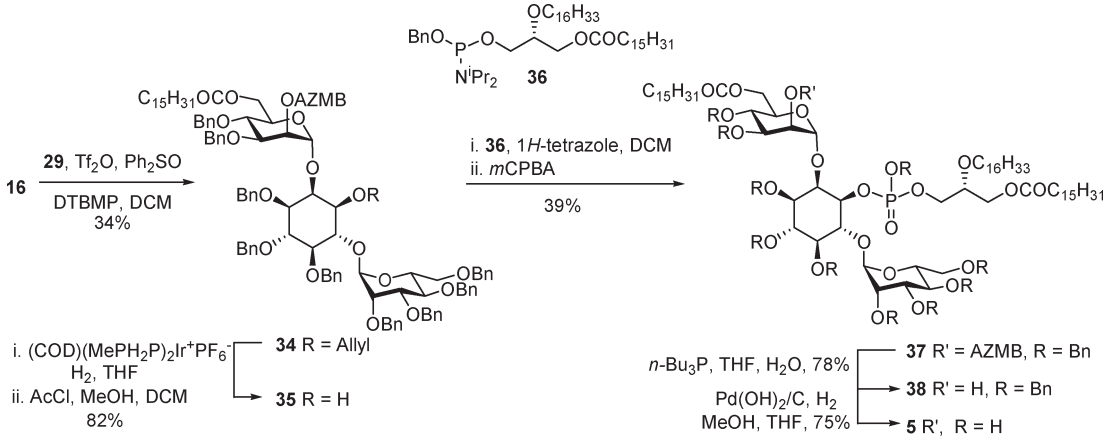
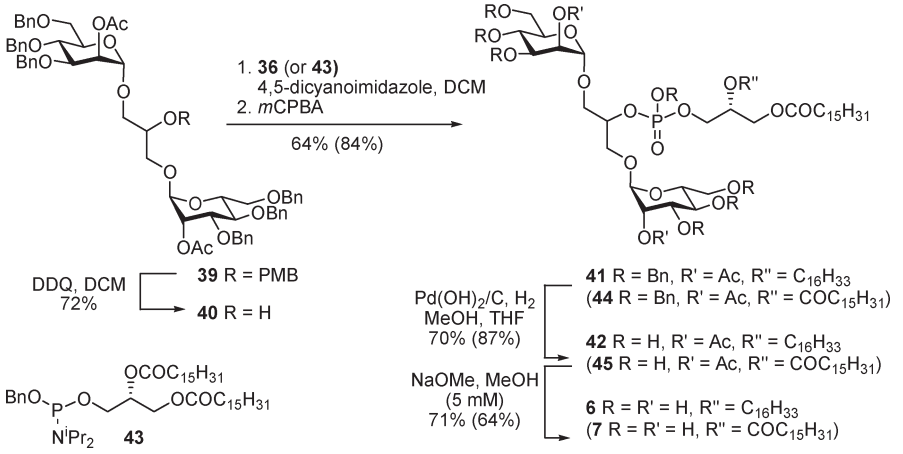
Scheme 5. Synthesis of 5



Scheme 6. Synthesis of Targets 6 and 7



not clear, but PIM molecules have been reported to act as agonists of TLR2 and TLR4[6,8,37,38] and antagonists of LPS[14] signaling in macrophages. For these reasons, we assessed the ability of the synthetic PIM analogues to both directly activate primary macrophages and modulate LPS-induced macrophage activation.

Primary peritoneal macrophages were isolated from mice and the cells treated with synthetic PIM analogues in vitro. Consistent with previous reports of agonist activity of PIM molecules,[8,38] all of the PIM analogues tested induced macrophage IL-6 production (Figure 2B, Table 1). In all cases, peak IL-6 production was observed between 0.6 and 3.1 μM PIM and decreased at higher concentrations without the appearance of cytoxicity[39] (Figure 2B and Supporting Information). Self-regulation of TLR agonist activity has been previously reported for both TLR4 and TLR2.[40] Therefore, it is likely that the observed pattern of cytokine production for the PIM analogues also results from the engagement of negative feedback mechanisms designed to regulate PIM agonist activity via TLRs.

Consistent with our earlier study of dendritic cell responses,[16] the inclusion of an ether rather than an ester group on the *sn*-2 position of glycerol in PIMs increased the induction of IL-6 (Table 1, **1** vs **2** or **6** vs **7**). Moving the ether to the *sn*-1 position resulted in a decrease in IL-6 production (Table 1, **1** vs **4**); however, compound **4** still enhanced IL-6 secretion compared to **2**. In contrast, inclusion of two ether groups, as with compound **8**, or exchanging the ether for an amide group, as with compound **3**, resulted in decreased cytokine production compared to synthetic PIM_2 (**2**). Interestingly, removal of the inositol ring but retention of the natural glycerol diester (**2** vs **7**) resulted in a significant loss of agonist activity that was recovered by introducing the *sn*-2 monoether (**6** vs **7**). Agonist activity was also lost with the addition of a third lipid group, as exemplified by $AcPIM_2ME$ (**5**). Taken together, these results indicate that the inositol ring contributes to the agonist activity and that agonist activity is enhanced with a combination of ester and ether linkages on the phosphatidyl group.

Next, we investigated whether PIM treatment enhanced or suppressed LPS induced IL-6 production by primary macrophages. Previous work on bone-marrow-derived macrophages showed that PIM molecules are able to inhibit LPS-induced cytokine production.[14] Consistent with this finding, the majority of the PIM analogues inhibited LPS-induced macrophage IL-6 production in a dose-dependent manner (Figure 2C and Table 2). In general, suppressive activity fell in the same concentration range as that observed for shutdown of agonist activity. Whether the same regulatory mechanisms are involved in both the inhibition of PIM agonist activity and PIM-dependent inhibition of LPS-induced IL-6 production has yet to be determined.

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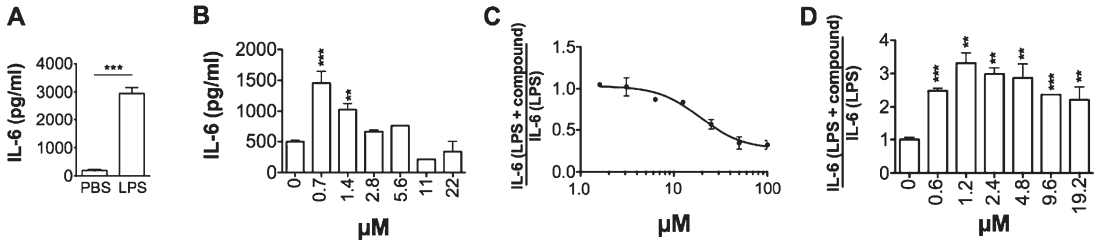

Figure 2. Agonist and antagonist activity of PIM compounds. Thioglycollate-induced peritoneal macrophages were treated with (A) LPS (10 ng/mL) or (B) **1** alone for 18 h. Peritoneal macrophages were treated with LPS (10 ng/mL) in the presence of (C) **1** or (D) **5** for 18 h. Supernatants were collected and the levels of IL-6 determined by ELISA. Data are representative of at least two replicate experiments. Statistical significance was calculated by one-way ANOVA: (**) $p < 0.01$, (***) $p < 0.001$ compared to PBS control.

Table 1. PIM-Induced IL-6 Production by Thioglycollate-Elicited Macrophages

compd	IL-6_{max} (pg/mL)	SE	structural variation
1	1456	161	PIM_2, *sn*-2 ether, *sn*-1 ester
2	817	210	PIM_2, *sn*-2 and *sn*-1 both esters
3	664	332	PIM_2, *sn*-2 amide, *sn*-1 ester
4	1130	33	PIM_2, *sn*-2 ester, *sn*-1 ether
5	275	62	$AcPIM_2$, *sn*-2 ether, *sn*-1 ester
6	923	197	PGM_2, *sn*-2 ether, *sn*-1 ester
7	143	31	PGM_2, *sn*-2 and *sn*-1 both esters
8	494	161	PIM_2, *sn*-2 and *sn*-1 both ethers

Table 2. Inhibition of IL-6 Production by LPS-Stimulated Thioglycollate-Elicited Macrophages

CN	IC_{50} (μM)	SE	structural variation
1	30.3	4.9	PIM_2, *sn*-2 ether, *sn*-1 ester
2	40.2	6.1	PIM_2, *sn*-2 and *sn*-1 both esters
3	22.7	1.8	PIM_2, *sn*-2 amide, *sn*-1 ester
4	28.8	3.8	PIM_2, *sn*-2 ester, *sn*-1 ether
6	42.8	5.1	PGM_2, *sn*-2 ether, *sn*-1 ester
7	12.9	3.0	PGM_2, *sn*-2 and *sn*-1 both esters
8	23.3	5.0	PIM_2, *sn*-2 and *sn*-1 both ethers

Removal of the inositol moiety in **7** (IC_{50} = 12.9 μM) resulted in increased inhibitory activity and decreased agonist activity compared to **2** (IC_{50} = 40.2 μM). Therefore, it appeared that the presence of an inositol ring abrogated inhibitory activity.

Interestingly, only **5** enhanced LPS-induced IL-6 production (Figure 2D). The difference between **1**, which exhibited inhibitory activity, and **5** is the addition of C-16 (palmitic acid) fatty acyl chain to one of the mannosyl moieties. As such, the observed switch from inhibition to enhancement of LPS-induced IL-6 production may be associated with the greater lipophilicity of **5**.

PIM Analogue Activities Act via TLR4. To determine whether PIMs and their analogues may induce cytokine production via TLRs, macrophages from wild type C57 and TLR4-deficient mice were treated with **2**. IL-6 production was abrogated in TLR4-deficient macrophages, indicating that **2** activates macrophages via the TLR4 signaling pathway (Figure 3).

A key event required for LPS signaling via the TLR4 pathway is LPS binding to a heterodimeric 1:1 complex between TLR4 and its co-receptor, myeloid differentiation factor 2 (MD-2), which then induces homodimerization of the 1:1 complex to form a 2:2

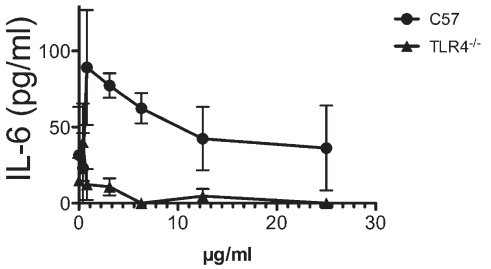

Figure 3. Agonist activity of **2** in thioglycollate-induced macrophages is TLR4 dependent. Data are representative of at least two replicate experiments. Statistical significance between the curves was calculated by two-way ANOVA: $p < 0.0007$.

complex that leads to activation of the intracellular signaling cascade.[41−43] To reveal the molecular basis whereby **2** negatively regulates LPS signaling, we examined the effect of **2** on LPS-mediated formation of the active 2:2 TLR4/MD-2 homodimer. As shown by native PAGE, LPS induces the homodimerization of a purified protein complex between the TLR4 ectodomain (sTLR4) and MD-2 (Figure 4A). This LPS-mediated sTLR4/MD-2 homodimerization was partially inhibited by **2** (Figure 4A).

Given that for TLR4 activation LPS is deeply inserted into the hydrophobic cavity of MD-2, we speculated that **2** would inhibit 2:2 TLR4/MD-2 complex formation by competing with LPS for the MD-2 cavity. However, the direct interaction of MD-2 with LPS or **2** could not be addressed because of technical difficulty in recombinant expression of MD-2 alone. Instead, an MD-2 homologue, MD-1, was used as an MD-2 model system. MD-1 has been implicated in the regulation of LPS-induced immune responses and, like MD-2, houses a large hydrophobic cavity that is able to accommodate LPS and presumably other lipo-gylcan structures such as PIM.[44,45] In native gels, **2** was shown to associate with MD-1 in a dose-dependent manner and to compete with LPS for MD-1 binding (Figure 4B), supporting that **2** directly interacts with MD-2. Taken together, these data suggest that PIM molecules, like MPL,[43] act as partial agonists of TLR4 and likely inhibit LPS signaling by interfering with LPS binding to the TLR4/MD-2 complex.

In summary, we present concise syntheses of PIM analogues **3**, **4**, and **5**, utilizing the readily available mannosylated chiral inositol **16**, and **6** and **7** from a dimannosylated glycerol intermediate. In general, the PIMs and their analogues exhibit partial agonist activity, which is associated with the inositol ring. Furthermore, this activity is enhanced by replacement of a fatty acid ester moiety with that of the corresponding ether on the phosphatidyl

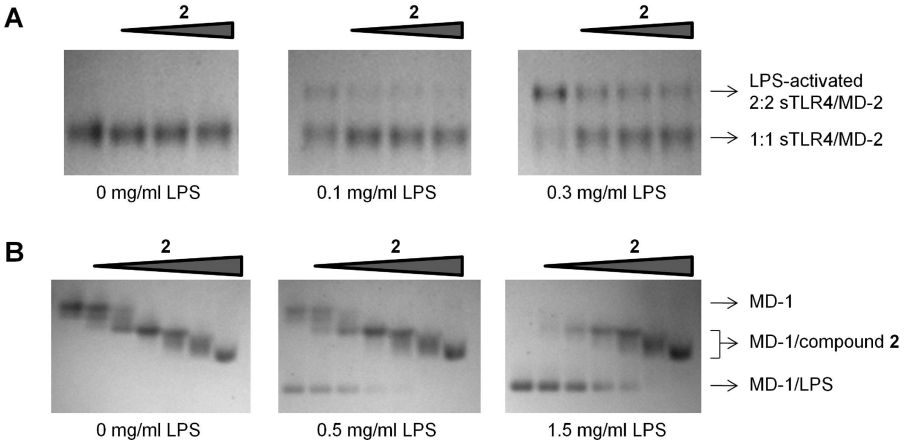

Figure 4. Compound **2** interferes with LPS binding to TLR4/MD-2 (A) and MD-1 (B) as shown by native PAGE. **2** binding to MD-1 results in a band shift (B, left), whereas **2**-induced sTLR4/MD-2 band shift was not observed (A, left) potentially because of the large size of sTLR4/MD-2 (87 kDa) compared to MD-1 (17 kDa).

group. SAR for the inhibition of LPS signaling resulted in two notable observations. First, loss of the inositol ring enhanced the antagonist activity, and somewhat surprisingly, addition of a third lipid moiety may synergize with LPS to enhance proinflammatory activation of macrophages. For the first time, we also show that the activities of PIMs involve regulation of TLR4 signaling, potentially via interactions with TLR4/MD-2. Together, these findings identify key structural features that could be used to direct the synthesis of lipoglycans with optimal agonist, antagonist, or adjuvant activities and identify an important role for TLR4 in the bioactivity of PIMs.

EXPERIMENTAL SECTION

General Experimental Information. NMR spectra are referenced to tetramethylsilane (TMS) (0.0 ppm) or the residual solvent peak (^{1}H $CHCl_3$ δ 7.26; ^{13}C $CDCl_3$ δ 77.0) or to an external reference (^{31}P H_3PO_4 δ 0.0). Anhydrous solvents were sourced commercially and used without further treatment unless otherwise stated. Powdered molecular sieves were flame-dried under vacuum immediately prior to use. Flash column chromatography was carried out using 40−63 μm silica gel unless otherwise stated. All flash chromatography solvents were AR-grade. Petroleum ether used was one with bp 60−80 °C range. All compounds were isolated after silica gel column chromatography, and fractions collected were one spot by thin layer chromatography. Thin layer chromatography (TLC) plates were visualized under an UV lamp and/or with a spray consisting of 5% w/v dodecamolybdophosphoric acid in ethanol with subsequent heating. See Supporting Information for HPLC conditions.

HPLC purities of target compounds are as follows: **1** (97%), **2** (95%), **3** (94%), **4** (96%), **5** (95%), **6** (96%), **7** (95%), and **8** (97%).

6-(2-*O*-Benzoyl-3,4,5-tri-*O*-benzyl-α-D-mannopyranosyl)-3,4-*O*-(1,1,3,3-tetraisopropyldisiloxanedi-1,3-yl)-1,2-*O*-((1*S*,4*S*)-1,7,7-trimethyl-[2.2.1]bicyclohept-6-ylidene)-D-*myo*-inositol (11). TMSOTf (0.20 mL, 1.1 mmol) was added to a mixture of diol **9** (6.00 g, 10.8 mmol), trichloroacetamide **10** (7.53 g, 10.77 mmol), and 4 Å molecular sieves in dry Et_2O (100 mL) at −70 °C. After the mixture was stirred for 45 min, the reaction was quenched with saturated $NaHCO_3$ (100 mL) and extracted with Et_2O (3 × 60 mL) and washed with water (100 mL), dried ($MgSO_4$), filtered and the solvent removed. The crude residue was purified on silica gel (EtOAc/petroleum ether = 1:9) to afford compound **11** (8.95 g, 76%) as a white foam. $[\alpha]^{20}_D$ −1.5 (*c* 0.4, CH_2Cl_2); $[\alpha]^{20}_{365}$ −26 (*c* 0.4, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.11−8.05 (m, 2H), 7.57−7.50 (m, 1H), 7.41−7.15 (m, 17H), 5.71 (dd, *J* = 1.8, 2.7 Hz, 1H), 5.40 (d, *J* = 1.8 Hz, 1H), 4.87−4.74 (m, 3H), 4.60−4.49 (m, 3H), 4.37−4.30 (m, 1H), 4.20 (dd, *J* = 3.9, 5.4 Hz, 1H), 4.16−4.05 (m, 2H), 4.01−3.85 (m, 4H), 3.84−3.70 (2H, m), 3.36 (ddd, *J* = 1.8, 8.7, 10.1 Hz, 1H), 2.57 (d, *J* = 1.8 Hz, 1H), 2.00−1.80 (m, 2H), 1.74−1.57 (m, 2H), 1.46 (d, *J* = 12.9 Hz, 1H), 1.40−1.13 (m, 3H), 1.12−0.94 (m, 30H), 0.82 (s, 3H), 0.86 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.7, 138.9, 138.7, 138.3, 132.9, 130.0, 128.3, 128.24, 128.19, 128.1, 128.0, 127.9, 127.5, 127.4, 127.3, 117.7, 96.8, 79.9, 78.6, 77.4, 77.0, 76.9, 76.6, 76.3, 75.7, 75.1, 74.5, 73.3, 72.9, 72.6, 71.6, 71.5, 69.4, 69.2, 51.5, 48.0, 45.2, 45.1, 29.4, 27.1, 20.4, 20.2, 17.6, 17.4, 17.31, 17.26, 17.2, 17.1, 17.0, 13.0, 12.7, 12.5, 12.3, 12.1, 11.7, 9.7. HRMS-ESI $[M + Na]^+$ calculated for $C_{62}H_{84}O_{13}Si_2Na$: 1115.5348. Found 1115.5365.

6-(3,4,5-Tri-*O*-benzyl-α-D-mannopyranosyl)-3,4-*O*-(1,1,3,3-tetraisopropyldisiloxanedi-1,3-yl)-1,2-*O*-((1*S*,4*S*)-1,7,7-trimethyl[2.2.1]bicyclohept-6-ylidene)-D-*myo*-inositol (12). Sodium methoxide in MeOH (30% solution, 0.58 mL) was added dropwise to a stirred solution of benzoate **11** (3.91 g, 3.58 mmol) in CH_2Cl_2/MeOH (1:1, 50 mL). After 22 h, the mixture was partitioned between Et_2O (100 mL) and saturated NH_4Cl (100 mL), dried ($MgSO_4$), filtered and the solvent removed. The crude residue was purified on silica gel (EtOAc/petroleum ether = 1:9) to afford the title compound **12** (2.75 g, 78%) as a pale yellow oil. $[\alpha]^{20}_D$ +33 (*c* 0.5, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.38−7.15 (m, 15H), 5.38 (d, *J* = 1.2 Hz, 1H), 4.82 (d, *J* = 11.0 Hz, 1H), 4.75−4.61 (m, 3H), 4.59−4.45 (m, 2H), 4.31−4.21 (m, 1H), 4.21−4.15 (m, 1H), 4.15−4.08 (m, 1H), 3.97−3.83 (m, 5H), 3.83−3.71 (m, 2H), 3.71−3.61 (m, 1H), 3.31 (ddd, *J* = 1.5, 8.3, 9.8 Hz, 1H), 2.61−2.56 (m, 1H), 2.50−2.42 (m, 1H), 2.02−1.87 (m, 2H), 1.78−1.64 (m, 2H), 1.49−1.34 (m, 2H), 1.28−1.14 (m, 1H), 1.13−0.82 (m, 37H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 138.7, 138.6, 138.2, 128.4, 128.2, 128.0, 127.9, 127.82, 127.76, 127.4, 127.3, 117.7, 98.2, 80.3, 79.7, 77.4, 77.2, 77.0, 76.9, 76.6, 76.4, 75.8, 74.9, 74.5, 73.2, 73.0, 72.4, 72.0, 70.9, 68.9, 68.8, 51.5, 48.0, 45.4, 45.1, 29.6, 27.1, 20.5, 20.2, 17.6, 17.4, 17.34, 17.28, 17.25, 17.22, 17.1, 17.0, 13.0, 12.7, 12.3, 12.1, 9.7. HRMS-ESI $[M + NH_4]^+$ calculated for $C_{55}H_{80}O_{12}Si_2NH_4$: 1006.5532. Found 1006.5491.

3,4,5-Tri-*O*-benzyl-6-(2,3,4,5-tetra-*O*-benzyl-α-D-mannopyranosyl)-1,2-*O*-((1*S*,4*S*)-1,7,7-trimethyl[2.2.1]bicyclohept-6-ylidene)-D-*myo*-inositol (14). TBAF (1 M, 7.8 mL) was added to a solution of **12** (3.10 g, 3.13 mmol) in THF (50 mL). After the mixture was stirred for 1 h, the solvent was removed. The residue was purified by flash chromatography on silica gel (MeOH/CH_2Cl_2 = 3:97 to 1:24) to afford partially purified **13** (2.8 g). ^{1}H NMR (300 MHz, $CDCl_3$) inter

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alia δ 7.38−7.16 (m, 15H), 5.18 (d, *J* = 1.5 Hz, 1H), 4.81 (d, *J* = 11.0 Hz, 1H), 4.72 (d, *J* = 11.4 Hz, 1H), 4.67 (d, *J* = 11.4 Hz, 1H), 4.61−4.45 (m, 3H), 4.28 (dd, *J* = 4.4, 5.4 Hz, 1H), 3.94−3.84 (m, 2H), 3.82−3.50 (m, 7H), 3.21 (dd, *J* = 3.0, 9.4 Hz, 1H), 3.06 (br s, 1H), 2.89 (br s, 1H), 2.67 (d, *J* = 2.3 Hz, 1H), 2.55 (d, *J* = 7.0 Hz, 1H), 2.06−1.85 (m, 2H), 1.80−1.64 (m, 1H), 1.53−1.33 (m, 2H), 1.28−1.14 (m, 1H), 0.98 (s, 3H), 0.86 (s, 3H), 0.82 (s, 3H). HRMS-ESI $[M + NH_4]^+$ calculated for $C_{43}H_{54}O_{11}NH_4$: 764.4010. Found 764.4013.

BnBr (3.7 mL, 31 mmol) was added dropwise to a stirred solution of the crude tetraol **13** and NaH (0.94 g, 60% in mineral oil, 23.5 mmol) in dry DMF (75 mL) at 0 °C. The mixture was left to slowly warm to room temperature over 80 min before being diluted with Et_2O (300 mL) and quenched by the slow addition of water (150 mL). The aqueous phase was re-extracted with Et_2O (2 × 100 mL), dried ($MgSO_4$), filtered and the solvent removed. The crude residue was purified on silica gel (EtOAc/petroleum ether = 3:7) to afford the title compound **14** (3.12 g, 90%). $[\alpha]^{20}_D$ +19 (*c* 1.7, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.45−7.00 (m, 35H), 5.53 (d, *J* = 1.4 Hz, 1H), 4.89 (d, *J* = 11.0 Hz, 1H), 4.82−4.50 (m, 12H), 4.35 (d, *J* = 12.1 Hz, 1H), 4.25 (dd, *J* = 3.4, 6.4 Hz, 1H), 4.09 (dd, *J* = 8.8, 18.4 Hz, 1H), 4.03−3.95 (m, 2H), 3.90 (dd, *J* = 3.1, 9.4 Hz, 1H), 3.86−3.71 (m, 4H), 3.63 (dd, *J* = 3.7, 11.3 Hz, 1H), 3.50 (dd, *J* = 1.3, 11.3 Hz, 1H), 3.27 (dd, *J* = 7.1, 9.7 Hz, 1H), 2.00−1.87 (m, 2H), 1.80−1.65 (m, 2H), 1.49−1.33 (m, 2H), 1.29−1.12 (m, 1H), 1.06 (s, 3H), 0.88 (s, 3H), 0.86 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 139.1, 138.8, 138.6, 138.5, 138.1, 128.4, 128.3, 128.23, 128.16, 128.1, 127.9, 127.81, 127.77, 127.6, 127.5, 127.4, 127.2, 117.9, 96.1, 81.1, 80.7, 79.6, 77.9, 76.8, 76.4, 75.0, 74.91, 74.85, 74.7, 74.5, 73.8, 73.1, 72.5, 72.1, 71.8, 68.9, 51.6, 47.9, 45.1, 44.9, 29.9, 27.2, 20.6, 20.4, 9.8. HRMS-ESI $[M + NH_4]^+$ calculated for $C_{71}H_{78}O_{11}NH_4$: 1124.5888. Found 1124.5896.

3,4,5-Tri-*O*-benzyl-6-(2,3,4,5-tetra-*O*-benzyl-α-D-manno-pyranosyl)-D-*myo*-inositol (15). HCl (37%, 2 mL) was added to a stirred solution of **9** (322 mg, 0.291 mmol) in dioxane/methanol (2:5, 35 mL) at room temperature. After 48 h, the mixture was diluted with Et_2O (100 mL) and washed with water (100 mL), the aqueous layer was re-extracted with Et_2O (50 mL), the combined organic fractions were washed with saturated $NaHCO_3$ (100 mL), brine (100 mL), dried ($MgSO_4$), and filtered, and the solvent was removed. The crude residue was purified on silica gel (EtOAc/petroleum ether = 1:3 to 2:3) to afford the title compound **15** (245 mg, 86%). Analytical data were consistent with those previously reported.[26]

2-*O*-Hexadecanoyl-1-*O*-hexadecyl-3-*O*-(4-methoxybenzyl)-*sn*-glycerol (20). Palmitoyl chloride (0.300 mL, 0.982 mmol) was added dropwise to a stirred solution of alcohol **19** (270 mg, 0.618 mmol) and dry pyridine (0.300 mL, 3.71 mmol) in dry CH_2Cl_2 (15 mL) at 0 °C. The reaction mixture was allowed to warm to room temperature over 17 h before being quenched with water (100 mL). The mixture was extracted with Et_2O (2 × 150 mL) and the ethereal extract washed with 0.5 M HCl (100 mL), saturated $NaHCO_3$ (100 mL), dried ($MgSO_4$), filtered, and the solvent removed. The crude residue was purified by column chromatography on silica gel (EtOAc/petroleum ether = 0:1 to 1:9) to afford the title compound **20** (384 mg, 92%) as a colorless oil. $[\alpha]^{20}_D$ +1.0 (*c* 7.7, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.27−7.21 (m, 2H), 6.90−6.83 (m, 2H), 5.16 (quintet, *J* = 5.1 Hz, 1H), 4.49−4.43 (m, 2H), 3.79 (s, 3H), 3.60−3.55 (m, 4H), 3.44−3.35 (m, 2H), 2.32 (t, *J* = 7.5 Hz, 2H), 1.64−1.49 (m, 4H), 1.40−1.20 (m, 50H), 0.92−0.83 (m, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.4, 159.3, 130.2, 129.3, 113.8, 73.0, 71.7, 71.4, 69.3, 68.6, 55.3, 34.5, 32.0, 29.8, 29.6, 29.4, 29.2, 26.1, 25.1, 22.8, 14.2. HRMS-ESI $[M + Na]^+$ calculated for $C_{43}H_{78}O_5Na$: 697.5747. Found 697.5734.

2-*O*-Hexadecanoyl-1-*O*-hexadecyl-*sn*-glycerol (21). $Pd(OH)_2$/C (20%, 88 mg) was added to a mixture of **20** (380 mg, 0.563 mmol) in AcOH/EtOH (1:10, 16.5 mL). The mixture was stirred under an H_2 atmosphere for 4 h at room temperature, then filtered through Celite and the solvent removed. The residue was purified by column chromatography on silica gel (EtOAc/CH_2Cl_2 = 1:49 to 1:19) to afford the title compound **21** (207 mg, 66%) as an oil. $[\alpha]^{20}_D$ −2.8 (*c* 0.72, $CHCl_3$); lit.[46] = −2.6 (*c* 2.1, $CHCl_3$); lit.[28] = −1.2. Analytical data were consistent with those previously reported.[28,46]

Benzyl (2-*O*-Hexadecanoyl-1-*O*-hexadecyl-*sn*-glycero)diisopropylphosphoramidite (17). 1*H*-Tetrazole (35 mg, 0.50 mmol) was added to a stirred solution of alcohol **21** (205 mg, 0.369 mmol) and benzyloxy-bis(diisopropylamino)phosphine (262 mg, 0.775 mmol) in dry CH_2Cl_2 (20 mL) at room temperature for 90 min. The solvent was removed and the residue purified by column chromatography on silica gel (Et_3N/EtOAc/petroleum ether = 3:10:90) to afford the title compound **17** (267 mg, 91%) as an oil. $[\alpha]^{20}_D$ +4.9 (*c* 0.72, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.37− 7.23 (m, 5H), 5.13 (quintet, *J* = 5.2 Hz, 1H), 4.74− 4.62 (m, 2H), 3.83−3.35 (m, 8H), 2.30 (t, *J* = 7.3 Hz, 2H), 1.65−1.48 (m, 4H), 1.32−1.16 (m, 62H), 0.90−0.83 (m, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.3, 139.6, 128.3, 127.3, 127.0, 72.2, 71.7, 69.2, 65.5, 65.3, 62.3, 62.1, 61.9, 43.2, 43.1, 34.6, 32.0, 29.8, 29.7, 29.6, 29.4, 29.2, 26.2, 25.1, 24.8, 24.7, 24.6, 22.8, 14.2. ^{31}P NMR (121.5 MHz, $CDCl_3$) δ 149.5, 149.2. HRMS-ESI $[M + H]^+$ calculated for $C_{48}H_{91}NO_5P$: 729.6635. Found 792.6638.

3-*O*-Benzyl-2-deoxy-1-*O*-hexadeconyl-2-hexadeconylamino-*sn*-glycerol (23). Palmitoyl chloride (1.54 mL, 5.08 mmol) was added dropwise to a stirred solution of (*R*)-(+)-2-amino-3-benzyloxy-1-propanol (**22**) (230 mg, 1.27 mmol) and DMAP (621 mg, 5.08 mmol) in dry $CHCl_3$ (25 mL) at 0 °C. After warming to room temperature over 6 h, the mixture was diluted with $CHCl_3$ (30 mL) and washed with H_2O (30 mL), then 0.5 M HCl (30 mL), dried (MgSO4), filtered and the solvent removed. The crude residue was purified by column chromatography (EtOAc/petroleum ether = 1:4) to afford the title compound **23** (705 mg, 84%) as a gummy solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.38−7.26 (m, 5H), 4.79 (d, *J* = 8,6 Hz, 1H), 4.51 (br s, 2H), 4.42−4.31 (m, 1H), 4.25 (dd, *J* = 6.1, 10.9 Hz, 1H), 4.13 (dd, *J* = 6.0, 10.9 Hz, 1H), 3.59 (dd, *J* = 3.4, 9.6 Hz, 1H), 3.48 (dd, *J* = 4.7, 9.6 Hz, 1H), 2.26 (dd, *J* = 7.4, 7.6 Hz, 2H), 2.14 (dd, *J* = 7.4, 7.8 Hz, 2H), 1.65−1.53 (m, 4H), 1.30−1.24 (m, 48H), 0.91−0.85 (m, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ173.8, 172.9, 137.8, 128.5, 128.0, 127.8, 73.4, 68.8, 63.1, 48.0, 36.9, 34.3, 32.0, 29.9, 29.2, 25.8, 25.0, 22.8, 14.2. HRMS-ESI $[M + Na]^+$ calculated for $C_{42}H_{75}NO_4Na$: 680.5594. Found 680.5588.

2-Deoxy-1-*O*-hexadeconyl-2-*N*-hexadeconylamino-*sn*-glycerol (24)[29]. $Pd(OH)_2$/C (20%, 200 mg) was added to a mixture of **23** (663 mg, 1.01 mmol) in AcOH/EtOH (1:10, 33 mL). The mixture was stirred under an H_2 atmosphere for 16 h at room temperature, then filtered through Celite and the solvent removed. The residue was purified by column chromatography on silica gel (EtOAc/CH_2Cl_2 = 3:7) to afford the title compound **24** (555 mg, 0.98 mmol, 97%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.02 (d, *J* = 6.6 Hz, 1H), 4.27−4.12 (m, 3H), 3.72−3.56 (m, 2H), 2.96 (br s, 1H), 2.33 (dd, *J* = 7.6, 7.6 Hz, 2H), 2.19 (dd, *J* = 7.6, 7.6 Hz, 2H), 1.67−1.55 (m, 4H), 1.35−1.19 (m, 48H), 0.91−0.84 (m, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.6, 173.8, 62.5, 62.0, 50.5, 36.8, 34.3, 32.0, 30.0, 29.2, 25.8, 25.0, 22.8. HRMS-ESI $[M + Na]^+$ calculated for $C_{35}H_{69}NO_4Na$: 590.5124. Found 590.5118

2-Deoxy-1-*O*-hexadeconyl-2-*N*-hexadeconylamino-*sn*-glycero-3-*O*-benzyl-(*N*,*N*-diisopropyl)phosphoramidite (18). 1*H*-Tetrazole (28 mg, 0.397 mmol) was added to a stirred solution of alcohol **24** (205 mg, 0.361 mmol) and benzyloxy-bis(diisopropylamino)-phosphine (244 mg, 0.722 mmol) in dry CH_2Cl_2 (10 mL) at room temperature for 60 min. The solvent was removed and the residue purified by column chromatography on silica gel (Et_3N/EtOAc/petroleum ether = 3:10:90) to afford the title compound **18** (280 mg, 96%) as a clear oil that solidified on standing. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.37−7.25 (m, 5H), 4.89−4.58 (m, 2H), 4.38−4.25 (m, 1H), 4.22−4.04 (m, 2H), 3.87−3.56 (m, 2H), 2.28 (dd, *J* = 7.6, 7.6 Hz, 2H), 2.12−2.04 (m, 1H), 1.94−1.87 (m, 1H), 1.64−1.45 (m, 4H), 1.33−1.17 (m, 60H), 0.91−0.85 (m, 6H); ^{31}P NMR (121.5 MHz, $CDCl_3$) δ 150.3, 149.9. HRMS-ESI [M + Na] calculated for $C_{48}H_{89}N_2O_5Na$: 827.6407. Found 827.6407.

1-*O*-Allyl-3,4,5-tri-*O*-benzyl-6-(2,3,4,5-tetra-*O*-benzyl-α-D-mannopyranosyl)-2-*O*-(3,4,5-tri-*O*-benzyl-α-D-mannopyranosyl)-D-*myo*-inositol (26). TMSOTf (12 μL, 0.066 mmol) was added to a mixture of alcohol **16**[26] (0.339 g, 0.39 mmol) and trichloroacetamide **10** (0.46 g, 0.66 mmol) and 4 Å molecular sieves in dry Et_2O (50 mL) at −40 °C. After the mixture was stirred for 1 h, the reaction was quenched with Et_3N, filtered through Celite, and evaporated to dryness. The crude residue was purified on silica gel (EtOAc/petroleum ether = 1:9 to 1:4) to afford the intermediate compound **25** (0.473 g) as an inseparable mixture of anomers. ^{1}H NMR (300 MHz, $CDCl_3$) inter alia δ 5.92 (d, *J* = 2.9 Hz, 0.2H), 5.79−5.60 (m, 2H), 5.58−5.53 (m, 0.8H); ^{13}C NMR (75 MHz, $CDCl_3$) inter alia δ 99.2 ($^1J_{CH}$ = 175 Hz), 98.9 ($^1J_{CH}$ = 173 Hz). HRMS-ESI $[M + Na]^+$ calculated for $C_{98}H_{100}O_{17}Na$: 1571.6858. Found 1571.6818.

Sodium methoxide in MeOH (30% solution, 0.1 mL) was added dropwise to a stirred solution of **25** (323 mg) in CH_2Cl_2/MeOH (1:1, 20 mL). After 24 h, the mixture was quenched by the careful addition of saturated NH_4Cl (50 mL) and extracted with Et_2O (2 × 50 mL), dried ($MgSO_4$), filtered and the solvent removed. The crude residue was purified on silica gel (EtOAc/petroleum ether = 1:9 to 3:7) to afford the title compound **26** (208 mg, 63%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.77 (ddd, *J* = 5.5, 5.5, 10.4, 17.2 Hz, 1H), 4.50 (d, *J* = 1.4 Hz, 1H), 5.22 (dd, *J* = 1.4, 17.2 Hz, 1H), 5.21 (d, *J* = 1.1 Hz, 1H), 5.14 (dd, *J* = 1.2, 10.4 Hz, 1H), 4.95−4.50 (m, 16H), 4.49−4.41 (m, 2H), 4.38−4.28 (m, 2H), 4.24−3.75 (m, 13H), 3.52−3.20 (m, 6H), 3.16 (dd, *J* = 1.6, 9.7 Hz, 1H), 2.36 (d,, *J* = 1.9 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 139.1, 138.8, 138.71, 138.68, 138.6, 138.5, 138.2, 138.1, 138.0, 137.9, 133.9, 128.6, 128.3, 128.23, 128.17, 128.13, 128.05, 128.02, 127.96, 127.91, 127.87, 127.8, 127.6, 127.5, 127.4, 127.32, 127.27, 127.2, 117.7, 100.1, 98.6, 81.6, 81.5, 81.4, 80.1, 79.5, 78.9, 77.4, 77.2, 77.0, 76.6, 76.1, 75.9, 75.6, 75.3, 75.0, 74.9, 74.8, 74.1, 73.4, 73.1, 72.3, 72.2, 72.1, 71.8, 71.0, 70.84, 70.77, 68.7, 68.2. HRMS-ESI $[M + Na]^+$ calculated for $C_{91}H_{96}O_{16}Na$: 1467.6596. Found 1467.6563.

1-*O*-Allyl-3,4,5-tri-*O*-benzyl-2,6-di-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-mannopyranosyl)-D-*myo*-inositol (27). NaH (0.054 g, 60% dispersion in mineral oil, 1.4 mmol) was added to a stirred solution of **26** (0.98 g, 0.68 mmol) and benzyl bromide (0.20 mL, 1.7 mmol) in dry DMF (30 mL) at 0 °C. The mixture was allowed to warm to room temperature over 18 h before being diluted with Et_2O (50 mL) and quenched by the slow addition of water (50 mL). The aqueous phase was re-extracted with Et_2O (2 × 25 mL), dried ($MgSO_4$), filtered and the solvent removed The crude residue was purified on silica gel (EtOAc/petroleum ether = 1:9 to 3:7) to afford the title compound **27** (1.01 g, 97%). Analytical data were consistent with those previously reported.[17,31]

3,4,5-Tri-*O*-benzyl-2,6-di-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-mannopyranosyl)-1-*O*-(2-*O*-hexadecanoyl-1-*O*-hexadecyl-*sn*-glycero-3-benzylphosphoryl)-D-*myo*-inositol (29). 1*H*-Tetrazole (21 mg, 0.30 mmol) was added to a stirred solution of alcohol **28** (90 mg, 0.060 mmol) and phosphoramidite **17** (135 mg, 0.170 mmol) in dry CH_2Cl_2 (7 mL) at 0 °C under argon. After 2 h the reaction mixture was cooled to −40 °C and a dried ($MgSO_4$) solution of *m*-CPBA (∼55%, 90 mg, 0.29 mmol) in CH_2Cl_2 (10 mL) was added to the reaction. After the mixture was warmed to room temperature over 1 h, the reaction was quenched by addition of Na_2SO_3 (10%, 50 mL) and extracted with Et_2O (50 mL). The organic phase was washed with saturated $NaHCO_3$ (50 mL), dried ($MgSO_4$), filtered and the solvent removed. The crude residue was purified by column chromatography on silica gel (EtOAc/petroleum ether = 1:9 to 3:7) followed by further purification on silica gel (EtOAc/CH_2Cl_2 = 1:49 to 1:19) to afford the title compound **29** (68 mg, 52%) as an oil. ^{1}H NMR (300 MHz, $CDCl_3$) mixture of isomers δ 7.38−7.00 (m, 60H), 5.53−5.51 (m, 1H), 5.33−5.32 (m, 1H), 5.05−4.40 (m, 23H), 4.30−3.78 (m, 17H), 3.56−3.20 (m, 9H), 2.23−2.10 (m, 2H), 1.58−1.41 (m, 4H), 1.31−1.15 (m, 50H), 0.89−0.82 (m, 6H). ^{13}C (75 MHz, $CDCl_3$) selected signals δ 173.3, 99.9, 98.9. ^{31}P NMR (121.5 MHz, $CDCl_3$) δ 0.26, 0.00. HRMS-ESI $[M + Na]^+$ calculated for $C_{137}H_{173}O_{22}PNa$: 2224.2054. Found 2224.2051.

2,6-(Di-*O*-α-D-mannopyranosyl)-1-*O*-(2-*O*-hexadecanoyl-1-*O*-hexadecyl-*sn*-glycero-3-phosphoryl)-D-*myo*-inositol (4). $Pd(OH)_2$/C (20%, 25 mg) was added to a stirred solution of **29** (68 mg, 0.031 mmol) in THF/MeOH (2:3, 5 mL). The mixture was stirred under an H_2 atmosphere for 4 h at room temperature, then filtered through Celite and the solvent removed. The residue was purified by column chromatography on silica gel ($CHCl_3$/MeOH/H_2O = 70:40:0 to 70:60:6) to afford the title compound **4** (26 mg, 74%) as a white powder. $[\alpha]^{20}_D$ +39 (*c* 0.40, $CHCl_3$/MeOH/H_2O = 70:60:6); ^{1}H NMR (500 MHz, $CDCl_3$/CD_3OD/D_2O = 70:40:6) δ 5.28−5.20 (m, 1H), 5.15 (br s, 1H), 5.12 (br s, 1H), 4.33 (m, 1H), 4.12−3.96 (m, 7H), 3.87−3.60 (m, 12H), 3.57−3.42 m, 3H), 3.30 (t, *J* = 7.0 Hz, 1H), 2.41 (t, *J* = 7.2 Hz, 2H), 1.65−1.55 (m, 4H), 1.33−1.22 (m, 50H), 0.92−0.85 (m, 6H); ^{13}C (125 MHz, $CDCl_3$/CD_3OD/D_2O = 70:40:6) δ 176.0, 103.0, 102.9, 79.9, 79.8, 78.2, 78.1, 74.8, 74.4, 74.4, 74.2, 73.5, 73.5, 73.0, 72.1, 72.1, 71.8, 71.6, 70.7, 68.4, 68.4, 65.4, 65.4, 62.8, 62.6, 35.7, 33.2, 31.0, 30.9, 30.7, 30.6, 30.4, 27.4, 26.4, 23.9, 15.0, 10.0; ^{31}P NMR (121.5 MHz, $CDCl_3$/CD_3OD/D_2O = 70:40:6) δ 0.63. HRMS-ESI $[M-H]^-$ calculated for $C_{53}H_{100}O_{22}P$: 1119.6444. Found 1119.6456.

3,4,5-Tri-*O*-benzyl-2,6-di-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-mannopyranosyl)-1-*O*-(2-deoxy-3-benzylphosphoryl-1-*O*-hexadeconyl-2-*O*-hexadeconylamino-*sn*-glycero)-D-*myo*-inositol (30). 1*H*-Tetrazole (10 mg, 0.135 mmol) was added to a stirred solution of alcohol **28** (68 mg, 0.045 mmol) and phosphoramidite **18** (109 mg, 0.135 mmol) in dry CH_2Cl_2 (10 mL) at 0 °C under argon. After 1 h, the reaction mixture was cooled to −40 °C and a dried ($MgSO_4$) solution of *m*-CPBA (∼50%, 47 mg, 0.135 mmol) in CH_2Cl_2 (5 mL) was added to the reaction. After the mixture was warmed to room temperature over 2 h the reaction was quenched by addition of Na_2SO_3 (10%, 50 mL) and extracted with Et_2O (50 mL). The organic phase was washed with saturated $NaHCO_3$ (50 mL), dried ($MgSO_4$), filtered and the solvent removed. The crude residue was purified by column chromatography on silica gel (EtOAc/petroleum ether = 1:9 to 1:4) to afford the title compound **30** (61 mg, 62%) as an oil. ^{1}H NMR (300 MHz, $CDCl_3$) mixture of isomers δ 7.48−7.03 (m, 60H), 6.54 (d, *J* = 7.5 Hz, 3H), 6.40 (d, *J* = 8.6 Hz, 7H), 5.48 (br s, 3H), 5.44 (br s, 7H), 5.10−5.42 (m, 21H), 4.51−4.27 (m, 5H), 4.26−3.66 (m, 15H), 3.52−3.12 (m, 6H), 2.37−2.27 (m, 1H), 2.17−2.09 (m, 1H), 2.03−1.95 (m, 2H), 1.71−1.37 (m, 4H), 1.35−1.12 (m, 48H), 0.91−0.84 (m, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) selected signals δ 173.8, 173.5, 173.2, 173.1, 98.8, 98.7, 98.4, 98.1; ^{31}P NMR (121.5 MHz, $CDCl_3$) δ 0.94, −0.45. HRMS-ESI $[M + Na]^+$ calculated for $C_{137}H_{172}NO_5PNa$: 2237.2006. Found 2237.2007.

2,6-(Di-*O*-α-D-mannopyranosyl)-1-*O*-(2-deoxy-1-*O*-hexadecanoyl-2-*N*-hexadecanoylamino-*sn*-glycero-3-phosphoryl)-D-*myo*-inositol (3). $Pd(OH)_2$/C (20%, 40 mg) was added to a stirred solution of **28** (28 mg, 24.8 mmol) in THF/MeOH (2:3, 5 mL). The mixture was stirred under an H_2 atmosphere for 16 h at room temperature, then filtered through Celite and the solvent removed. The residue was purified by column chromatography on silica gel ($CHCl_3$/MeOH/H_2O = 70:40:4) to afford the title compound **3** (21 mg, 75%) as a white powder. $[\alpha]^{20}_D$ +35 (*c* 0.15, $CHCl_3$/MeOH/H_2O = 40:40:10). ^{1}H NMR (500 MHz, $CDCl_3$/CD_3OD/D_2O = 40:40:10) δ 5.15 (d, *J* = 1.6 Hz, 1H), 5.10 (d, *J* = 1.5 Hz, 1H), 4.32−4.28 (m, 2H), 4.15 (dd, *J* = 8.0, 11.4 Hz, 1H), 4.08−4.03 (m, 3H), 4.01−3.93 (m, 4H), 3.85−3.78 (m, 5H), 3.77−3.59 (m, 5H), 3.48 (dd, *J* = 7.6, 10.1 Hz, 1H), 3.30 (dd, *J* = 9.2, 9.3 Hz, 1H), 3.16 (q, *J* = 7.3 Hz, 6H), 2.33 (dd, *J* = 8.3, 6.9 Hz, 2H), 2.24 (dd, *J* = 7.5, 7.5 Hz, 2H), 1.65−1.55 (m, 4H), 1.31 (t, *J* = 7.3 Hz, 9H), 1.29−1.26 (m, 48H), 0.91−0.87 (m, 6H); ^{13}C NMR (125 MHz, $CDCl_3$/CD_3OD/D_2O = 40:40:10) δ176.3, 175.5, 102.4, 102.2, 79.3, 77.55, 77.50, 74.1, 73.8, 73.7, 73.6, 71.5, 71.4, 71.11, 71.07, 70.9, 67.8, 67.7, 65.2, 65.1, 64.2, 62.2, 62.0, 47.4, 37.0, 34.8, 32.6, 30.5, 30.42,

30.37, 30.35, 30.31, 30.30, 30.26, 30.1, 30.01, 30.00, 29.9, 26.7, 25.5, 23.3, 14.5. ^{31}P NMR (121.5 MHz, $CDCl_3$) δ 4.63. HRMS-ESI $[M - H]^-$ calculated for $C_{53}H_{99}NO_{22}P$: 1132.6396. Found 1132.6382.

Phenyl 3,4-Di-*O*-benzyl-6-*O*-hexadecanoyl-1-thio-α-D-mannopyranoside (33). Hexadecanoyl chloride (2.6 mL, 8.5 mmol) was added dropwise to a stirred solution of diol **32**[32] (3.50 g, 7.73 mmol) and pyridine (6.25 mL, 77 mmol) in dry CH_2Cl_2 (30 mL) at 0 °C, and the mixture was allowed to warm to room temperature overnight. The reaction mixture was diluted with CH_2Cl_2 (70 mL), washed with 1 M aqueous HCl (100 mL), saturated aqueous $NaHCO_3$, dried ($MgSO_4$), filtered and the solvent removed. The residue was purified by column chromatography on silica gel (EtOAc/petroleum ether = 1:9 to 3:7) to afford the title compound **33** (4.82 g, 90%) as a yellow oil. $[\alpha]^{20}_D$ +148 (*c* 1.3, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.47−7.43 (m, 2H), 7.39−7.21 (m, 13H), 5.57 (d, *J* = 1.5 Hz, 1H), 4.87 (d, *J* = 10.8 Hz, 1H), 4.71 (s, 2H), 4.58 (d, *J* = 10.8 Hz, 1H), 4.38−4.30 (m, 3H), 4.26−4.23 (m, 1H), 3.90, (dd, *J* = 3.1, 9.0 Hz, 1H), 3.81 (app t, *J* = 9.2 Hz, 1H), 2.65 (d, *J* = 2.5 Hz, 1H), 2.27−2.22 (m, 2H), 1.62−1.52 (m, 2H), 1.33−1.24 (m, 24H), 0.90−0.86 (m, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.5, 137.8, 137.4, 133.6, 131.6, 129.0, 128.6, 128.5, 128.2, 128.01, 127.99, 127.9, 127.5, 87.1, 80.3, 75.2, 74.4, 72.2, 70.5, 69.7, 63.1, 34.1, 31.9, 29.65, 29.62, 29.57, 29.4, 29.3, 29.2, 29.1, 24.8, 22.7, 14.1. HRMS-ESI $[M + Na]^+$ calculated for $C_{42}H_{58}O_6SNa$: 713.3852. Found 713.3881.

Phenyl 2-*O*-(2-Azidomethylbenzoyl)-3,4-di-*O*-benzyl-6-*O*-hexadecanoyl-1-thio-α-D-mannopyranoside (31). 2-Azidomethylbenzoyl chloride (AZMBCl) was prepared as described[47] and used without purification. A solution of **32** (1.35 g, 1.95 mmol) in pyridine (10 mL) was added to ice-cooled AZMBCl (0.49 g, 2.5 mmol), and the stirred mixture was allowed to warm to room temperature overnight. Excess pyridine was concentrated under reduced pressure, and the residue was taken up in EtOAc (150 mL), washed with 1 M aqueous HCl (2 × 50 mL), saturated aqueous $NaHCO_3$ (2 × 50 mL), dried ($MgSO_4$), filtered and the solvent removed. The crude residue was purified by column chromatography on silica gel (EtOAc/petroleum ether = 7.5:92.5) to afford the title compound **31** (1.016 g, 61%) as an oil. $[\alpha]^{20}_D$ +55 (*c* 0.9, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.04, (dd, *J* = 1.2, 7.8 Hz, 1H), 7.58 (dt, *J* = 1.2, 7.4 Hz, 1H), 7.52−7.48 (m, 3H), 7.42−7.24 (m, 14H), 5.83 (dd, *J* = 1.6, 3.0 Hz, 1H), 5.60 (d, *J* = 1.6 Hz, 1H), 4.92 (d, *J* = 10.9 Hz, 1H), 4.80 (d, *J* = 11.3 Hz, 1H), 4.75 (s, 2H), 4.64 (d, *J* = 11.3 Hz, 1H), 4.61 (d, *J* = 10.9 Hz, 1H), 4.48−4.32 (m, 3H), 4.07, (dd, *J* = 3.0, 9.2 Hz, 1H), 3.92 (app t, *J* = 9.3 Hz, 1H), 2.29−2.24 (m, 2H), 1.63−1.53 (m, 2H), 1.32−1.23 (m, 24H), 0.90−0.86 (m, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.4, 165.6, 137.8, 137.6, 137.4, 133.2, 133.0, 132.1, 131.4, 129.7, 129.1, 128.4, 128.2, 128.1, 127.95, 127.1, 86.1, 78.5, 75.3, 74.3, 71.9, 70.9, 70.8, 63.1, 52.9, 34.1, 31.9, 29.7, 29.63, 29.58, 29.5, 29.3, 29.23, 29.20, 24.8, 22.7, 14.1. HRMS-ESI $[M + Na]^+$ calculated for $C_{50}H_{63}N_3O_7SNa$: 872.4284. Found 872.4283.

1-*O*-Allyl-2-*O*-(2-*O*-[2-azidomethylbenzoyl]-3,4-di-*O*-benzyl-6-*O*-hexadecanoyl-α-D-mannopyranosyl)-6-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-mannopyranosyl)-3,4,5-tri-*O*-benzyl-D-*myo*-inositol (34). Acceptor **16**[26] (160 mg, 0.16 mmol) was dissolved in dry CH_2Cl_2 (5 mL) and cooled to −60 °C. In a separate flask, a mixture of donor **31** (125 mg, 0.147 mmol), diphenyl sulfoxide (83 mg, 0.412 mmol), and 2,6-di-*tert*-butyl-4-methylpyridine (DTBMP, 91 mg, 0.441 mmol) was coevaporated from dry CH_2Cl_2 (10 mL). The reagents were redissolved in CH_2Cl_2 (5 mL) and cooled to −60 °C with stirring before the addition of Tf_2O (35 μL, 0.21 mmol). After 5 min, the solution of acceptor was transferred to the reaction vessel (cannula), rinsing with CH_2Cl_2 (2 mL), and the reaction mixture was allowed to warm to 0 °C over 3 h. The reaction was quenched with saturated aqueous $NaHCO_3$ and extracted with CH_2Cl_2. The organic extracts were washed with saturated aqueous $NaHCO_3$, dried ($MgSO_4$), filtered and the solvent removed. The residue was purified by column chromatography on silica gel (EtOAc/petroleum ether = 15:85) followed by a second column (CH_2Cl_2/petroleum ether/diethyl ether = 30:20:1) to afford the title compound **34** (88 mg, 34%). $[\alpha]^{20}_D$ +19 (*c* 0.96, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.03 (dd, *J* = 1.1, 7.8 Hz, 1H), 7.59−7.50 (m, 2H), 7.41−7.08 (m, 46H), 5.82−5.68 (m, 2H), 5.57 (d, *J* = 1.6 Hz, 1H), 5.26−5.19 (m, 2H), 5.08−5.03 (m, 1H), 4.94−4.45 (m, 19H), 4.31−3.82 (m, 15H), 3.40 (dd, *J* = 3.1, 11.3 Hz, 1H), 3.33−3.25 (m, 3H), 3.17 (dd, *J* = 1.7, 9.7 Hz, 1H), 2.24−2.19 (m, 2H), 1.59−1.51 (m, 2H), 1.33−1.21 (m, 24H), 0.90−0.86 (m, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.3, 165.1, 139.1, 138.85, 138.77, 138.6, 138.4, 138.2, 138.1, 137.7, 137.6, 133.8, 132.7, 131.3, 129.4, 128.7, 128.5, 128.4, 128.32, 128.29, 128.2, 128.1, 128.04, 127.95, 127.82, 127.78, 127.7, 127.5, 127.40, 127.35, 127.21, 127.15, 117.4, 98.9, 81.5, 81.4, 80.9, 80.0, 78.5, 75.9, 75.7, 75.3, 75.1, 74.9, 74.8, 73.6, 73.2, 72.55, 72.50, 72.1, 72.0, 71.5, 70.8, 69.8, 69.1, 68.6, 62.8, 52.9, 34.1, 31.9, 29.7, 29.63, 29.59, 29.5, 29.3, 29.23, 29.20, 24.8, 22.7, 14.1. HRMS-ESI $[M + Na]^+$ calculated for $C_{108}H_{125}N_3O_{18}Na$: 1774.8856. Found 1774.8870.

2-*O*-(2-*O*-[2-Azidomethylbenzoyl]-3,4-di-*O*-benzyl-6-*O*-hexadecanoyl-α-D-mannopyranosyl)-6-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-mannopyranosyl)-3,4,5-tri-*O*-benzyl-D-*myo*-inositol (35). (1,5-Cyclooctadiene)bis(methyldiphenylphosphine)iridium(I) hexafluorophosphate (29 mg, 34 mmol) was stirred with a solution of **34** (301 mg, 172 mmol) in THF (10 mL) under Ar at room temperature for 10 min. The mixture was exposed to an atmosphere of H_2 for ∼30 s, during which time the color changed from red to pale yellow, then stirred under Ar for 45 min, at which point TLC (20% EtOAc/petroleum ether) indicated complete consumption of the starting material. The mixture was concentrated under reduced pressure and dissolved in CH_2Cl_2/MeOH (2:1, 12 mL) containing 0.45 mL of AcCl. After 1 h at room temperature, solid $NaHCO_3$ was added and the mixture stirred for a further 5 min. The reaction mixture was diluted with water, extracted with CH_2Cl_2, dried (MgSO4), filtered and the solvent removed. The crude residue was purified by column chromatography on silica gel (EtOAc/petroleum ether = 1:4) to afford the title compound **35** (240 mg, 82%). $[\alpha]^{20}_D$ +32 (*c* 1.1, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.07, (d, *J* = 7.9 Hz, 1H), 7.59−7.50 (m, 2H), 7.39−7.14 (m, 46H), 5.70−5.69 (m, 1H), 5.40 (d, *J* = 1.6 Hz, 1H), 5.30 (d, *J* = 2.0 Hz, 1H), 4.91−4.26 (m, 20H), 4.19−3.76 (m, 11H), 3.64−3.56 (m, 2H), 3.47 (dd, *J* = 6.6, 10.3 Hz, 1H), 3.36−3.27 (m, 3H), 2.26−2.21 (m, 2H), 1.61−1.52 (m, 2H), 1.33−1.21 (m, 24H), 0.90−0.86 (m, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.4, 165.3, 138.53, 138.49, 138.4, 138.2, 138.1, 137.80, 137.76, 137.7, 132.8, 131.4, 129.3, 128.6, 128.44, 128.41, 128.38, 128.30, 128.26, 128.2, 128.0, 127.9, 127.8, 127.7, 127.63, 127.59, 127.54, 127.46, 127.4, 99.0, 96.0, 81.2, 80.4, 80.1, 79.4, 78.2, 76.1, 75.6, 75.3, 75.1, 75.02, 74.97, 74.5, 73.7, 73.3, 72.5, 72.2, 72.14, 72.09, 71.6, 71.1, 69.7, 69.3, 69.2, 62.8, 52.9, 34.1, 31.9, 29.7, 29.62, 29.58, 29.5, 29.3, 29.23, 29.20, 24.8, 22.7, 14.1. HRMS-ESI $[M + Na]^+$ calculated for $C_{105}H_{121}N_3O_{18}Na$: 1734.8543. Found 1734.8586.

2-*O*-(2-*O*-[2-Azidomethylbenzoyl]-3,4-di-*O*-benzyl-6-*O*-hexadecanoyl-α-D-mannopyranosyl)-1-*O*-(1-*O*-hexadecanoyl-2-*O*-hexadecyl-*sn*-glycero-3-benzylphosphoryl)-6-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-mannopyranosyl)-3,4,5-tri-*O*-benzyl-D-*myo*-inositol (37). 1*H*-Tetrazole (13 mg, 0.19 mmol) was added to a stirred solution of alcohol **35** (106 mg, 0.062 mmol) and phosphoramidite **36** (147 mg, 0.186 mmol) in dry CH_2Cl_2 (8 mL) under Ar at 0 °C. After the mixture was stirred at room temperature for 3 h, the reaction mixture was cooled to −40 °C and a solution of *m*-CPBA (50%, 86 mg, 0.25 mmol) in CH_2Cl_2 (10 mL) was transferred by cannula into the reaction mixture. After the mixture was stirred at room temperature for 1 h, the reaction was quenched by addition of 10% aqueous Na_2SO_3 (50 mL) and the combined mixture extracted with Et_2O (100 mL). The ethereal extract was washed with saturated aqueous

$NaHCO_3$ (3 × 50 mL), dried ($MgSO_4$), filtered and the solvent removed. The residue was purified by column chromatography on silica gel (EtOAc/petroleum ether = 1:9 to 1:4) followed by a second column (acetone/toluene = 1:50 to 3:97) to afford the title compound **37** (59 mg, 0.024 mmol, 39%) as an oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.04−7.98 (m, 1H), 7.58−7.43 (m, 2H), 7.36−7.03 (m, 51H), 5.66−5.62 (m, 1H), 5.56−5.62 (m, 1H), 5.33−5.29 (m, 1H), 5.11−5.04 (m, 2H), 4.95−4.39 (m, 20H), 4.36−3.82 (m, 17H), 3.60−3.22 (m, 7H), 2.35−2.13 (m, 4H), 1.61−1.48 (m, 4H), 1.42−1.17 (m, 76H), 0.90−0.86 (m, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) selected signals δ 173.2, 99.6, 98.6; ^{31}P NMR (121 MHz, $CDCl_3$) δ 0.1, 0.0. HRMS-ESI $[M + Na]^+$ calculated for $C_{147}H_{196}N_3O_{24}NaP$: 2441.3845. Found 2441.3855.

2-*O*-(3,4-Di-*O*-benzyl-6-*O*-hexadecanoyl-α-D-mannopyranosyl)-1-*O*-(1-*O*-hexadecanoyl-2-*O*-hexadecyl-*sn*-glycero-3-benzylphosphoryl)-6-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-mannopyranosyl)-3,4,5-tri-*O*-benzyl-D-*myo*-inositol (38). Tri-*n*-butylphosphine (34 μL, 0.14 mmol) was added to a degassed solution of AZMB ester **37** (58 mg, 0.024 mmol) in THF/H_2O (9:1, 10 mL) under Ar. After the mixture was stirred at room temperature for 3 h, toluene (20 mL) was added and the solvent removed in vacuo. The residue was purified by column chromatography on silica gel (EtOAc/petroleum ether = 1:4 to 3:7) to afford the title compound **38** (42 mg, 78%) as an oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40−6.99 (m, 50H), 5.46−5.42 (m, 1H), 5.19 (m, 1H), 5.11−4.39 (m, 20H), 4.19−3.78 (m, 18H), 3.62−3.20 (m, 7H), 2.54−2.49 (m, 1H), 2.28−2.18 (m, 4H), 1.60−1.40 (m, 6H), 1.33−1.22 (m, 74H), 0.90−0.86 (m, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) selected signals δ 173.5, 101.5, 98.4; ^{31}P NMR (121 MHz, $CDCl_3$) δ 0.0, −0.2; HRMS-ESI $[M + Na]^+$ calculated for $C_{139}H_{191}O_{23}PNa$: 2282.3412. Found 2282.3401.

1-*O*-(1-*O*-Hexadecanoyl-2-*O*-hexadecyl-*sn*-glycero-3-phosphoryl)-2-*O*-(6-*O*-hexadecanoyl-α-D-mannopyranosyl)-6-*O*-(α-D-mannopyranosyl)-D-*myo*-inositol (5). $Pd(OH)_2$/C (20%, 36 mg) was added to a stirred solution of compound **38** (42 mg, 0.019 mmol) in THF/MeOH (2:3, 5 mL). The mixture was stirred under a H_2 atmosphere for 3.5 h at room temperature, then filtered through Celite and the solvent removed. The residue was purified by column chromatography on silica gel ($CHCl_3$/MeOH/H_2O = 70:40:0 to 70:40:8), affording the title compound **5** (19 mg, 75%) as a white powder. It was 95% pure by HPLC system 1. $[\alpha]^{20}_D$ +30 (*c* 0.10, $CHCl_3$/CH_3OH/H_2O = 70:40:6); ^{1}H NMR (500 MHz, $CDCl_3$/CD_3OD/D_2O = 70:40:6) δ 5.15 (br s, 1H), 5.10 (br s, 1H), 4.38−4.08 (m, 8H), 4.05 (dd, *J* = 1.7, 3.3 Hz, 1H), 4.01−3.96 (m, 2H), 3.94−3.89 (m, 1H), 3.84−3.73 (m, 6H), 3.70−3.65 (m, 3H), 3.60 (app t, *J* = 9.5 Hz, 1H), 3.55−3.51 (m, 1H), 3.46 (dd, *J* = 2.5, 10 Hz, 1H), 3.29 (t, *J* = 9.3 Hz, 1H), 2.39−2.33 (m, 4H), 1.65−1.54 (m, 6H), 1.37−1.27 (m, 74H), 0.90−0.87 (m, 9H); ^{13}C NMR (126 MHz, $CDCl_3$/CD_3OD/D_2O = 70:40:6) δ 175.7, 175.3, 102.3, 102.2, 79.4, (br d), 79.2, 77.5, 77.4, 74.0, 73.8, 73.5, 71.44, 71.35, 71.3, 71.2, 71.0, 70.9, 70.7, 67.8, 67.6, 64.9, 64.7, (br d), 64.3, 61.9, 34.9, 34.5, 32.51, 30.46, 30.40, 30.37, 30.3, 30.23, 30.18, 30.03, 29.97, 29.9, 29.8, 26.7, 25.6, 25.4, 23.2, 14.5; ^{31}P NMR (202 MHz, $CDCl_3$/CD_3OD/D_2O = 70:40:6) δ −0.3; HRMS-ESI $[M - H]^-$ calculated for $C_{69}H_{130}O_{23}P$: 1357.8741. Found 1357.8729.

1,3-Bis(2-*O*-acetyl-3,4,5-tri-*O*-benzyl-α-D-mannopyranosyl)-2-hydroxypropane (40). DDQ (35 mg, 0.154 mmol) was added to a stirred solution of **39** (149 mg, 0.128 mmol) in CH_2Cl_2/H_2O (9:1, 4 mL). After 50 min the mixture was diluted with Et_2O (80 mL), washed with water (3 × 20 mL), saturated $NaHCO_3$ (3 × 20 mL), dried ($MgSO_4$), filtered and the solvent removed. The crude residue was purified by column chromatography on silica gel (EtOAc/petroleum ether = 2:3) to afford the title compound **40** (96 mg, 72%) as waxy solid. $[\alpha]^{20}_D$ +30 (*c* 0.82, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.35−7.22 (m, 26H), 7.18−7.13 (m, 4H), 5.38−5.35 (m, 2H), 4.87−4.82 (m, 4H), 4.71−4.62 (m, 4H), 4.55−4.50 (m, 2H), 4.50−4.45 (m, 4H), 3.97−3.90 (m, 3H), 3.89−3.78 (m, 4H), 3.78−3.72 (m, 2H), 3.72−3.65 (m, 3H), 3.62 (dd, *J* = 6.2, 10.7 Hz, 1H), 3.56 (dd, *J* = 4.5, 10.7 Hz, 1H), 3.51 (dd, *J* = 6.6, 10.6 Hz, 1H), 2.13 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.3,138.09, 138.07, 137.9, 128.34, 128.28, 128.26, 128.0, 127.8, 127.7, 127.6, 127.5, 98.5 (J^1_{CH} = 170 Hz), 98.3 (J^1_{CH} = 170 Hz), 78.1, 78.0, 75.11, 75.09, 74.2, 73.4, 71.89, 71.85, 71.7, 69.8, 69.6, 69.2, 68.79, 68.77, 68.7, 68.6, 21.0. HRMS-ESI $[M + Na]^+$ calculated for $C_{61}H_{68}O_{15}Na$: 1063.4456. Found 1063.4445.

2-*O*-(Benzyloxy-1-*O*-hexadecanoyl-2-*O*-hexadecyl-*sn*-glycero-3-phosphoryl)-1,3-bis(2-*O*-acetyl-3,4,5-tri-*O*-benzyl-α-D-mannopyranosyl)glycerol (41). A mixture of alcohol **40** (86 mg, 0.083 mmol) and phosphoramidite **36** (98 mg, 0.124 mmol) was coevaporated from dry CH_2Cl_2 (10 mL) and then placed under high vacuum for 1 h. The reagents were dissolved in dry CH_2Cl_2 (2 mL) and stirred for 1 h at room temperature over 4 Å molecular sieves. The solution was cooled to 0 °C before the addition of 4,5-dicyanoimidazole (16 mg, 0.14 mmol) and then warmed to room temperature over 18 h. The solution was then cooled to −15 °C before the addition of a dried ($MgSO_4$) solution of *m*CPBA (∼60%, 50 mg) in CH_2Cl_2. After warming to room temperature over 1 h, the reaction mixture was diluted with Et_2O (30 mL) and washed with $Na_2S_2O_3$ (10%, 30 mL), saturated $NaHCO_3$ (3 × 20 mL), brine (30 mL), dried ($MgSO_4$), filtered and the solvent removed. The crude residue was purified by column chromatography on silica gel (EtOAc/petroleum ether = 1:4 to 2:3) to afford the title compound **41** (93 mg, 64%). ^{1}H NMR (500 MHz, $CDCl_3$) mixture of isomers δ 7.37−7.19 (m, 31H), 7.17−7.10 (m, 4H), 5.11−5.01 (m, 2H), 4.90−4.79 (m, 4H), 4.70−4.57 (m, 5H), 4.52−4.39 (m, 6H), 4.17−4.10 (m, 1H), 4.09−3.73 (m, 10H), 3.71−3.58 (m, 4H), 3.58−3.52 (m, 1H), 3.50−3.37 (m, 2H), 2.26−2.21 (m, 2H), 2.12 (s, 3H), 2.11 (s, 3H), 1.62−1.51 (m, 2H), 1.50−1.41 (m, 2H), 1.35−1.15 (m, 50H), 0.93−0.80 (m, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.3, 170.2, 138.4, 138.2, 137.9, 135.8, 135.75, 135.71, 135.69, 128.6, 128.5, 128.4, 128.32, 128.30, 128.25, 128.2, 128.1, 128.0, 127.81, 127.80, 127.78, 127.7, 127.5, 98.32, 98.31, 98.15, 98.11, 78.20, 78.19, 75.79, 75.77, 75.73, 75.71, 75.4, 75.31, 75.26, 75.10, 75.07, 74.04, 74.00, 73.40, 73.38, 71.92, 71.88, 71.85, 71.83, 70.65, 70.61, 69.42, 69.38, 68.71, 68.66, 68.6, 68.4, 68.1, 66.72, 66.68, 66.42, 66.38, 66.3, 62.5, 34.1, 31.9, 29.9, 29.7, 29.64, 29.62, 29.5, 29.32, 29.28, 29.1, 26.0, 24.8, 22.6, 21.0; ^{31}P NMR (202 MHz, $CDCl_3$) δ −1.44, −1.48. HRMS-ESI $[M + Na]^+$ calculated for $C_{103}H_{143}O_{21}PNa$: 1769.9757. Found 1769.9751 .

2-*O*-(1-*O*-Hexadecanoyl-2-*O*-hexadecyl-*sn*-glycero-3-phosphoryl)-1,3-bis(2-*O*-acetyl-α-D-mannopyranosyl)glycerol (42). A suspension of **41** (90 mg, 51 mmol) and $Pd(OH)_2$ in 2:3 THF/MeOH (5 mL) was stirred under an atmosphere of H_2 at ambient temperature for 4 h. The mixture was filtered through Celite, concentrated, and purified by purified by column chromatography (neat $CHCl_3$ to 2:1 $CHCl_3$/MeOH) to give **42** (40 mg, 70%) as a white powder. $[\alpha]^{20}_D$ +27 (*c* 0.74, 2:1 $CHCl_3$/MeOH); ^{1}H NMR (500 MHz, 2:1 $CDCl_3$/CD_3OD) δ 5.08−5.03 (m, 2H), 4.83 (d, *J* = 0.9 Hz, 1H), 4.81 (d, *J* = 1.1 Hz), 4.54−4.46 (m, 1H), 4.29 (dd, *J* = 2.8, 11.6 Hz), 4.17−4.11 (m, 1H), 4.06−3.86 (m, 8H), 3.80−3.51 (m, 11H), 2.35 (t, *J* = 7.5 Hz, 1H), 2.12 (2 × s, 6H), 1.67−1.52 (m, 4H), 1.38−1.22 (m, 50H), 0.92−0.85 (m, 6H); ^{13}C NMR (125 MHz, 2:1 $CDCl_3$/CD_3OD) δ 174.8, 171.61, 171.58, 98.1, 98.0, 76.8, 76.7, 74.4, 74.3, 73.7, 73.6, 72.72, 72.69, 71.2, 69.8, 68.7, 68.5, 67.0, 65.43, 65.41, 64.0, 62.5, 62.4, 34.6, 32.3, 30.3, 30.05, 30.00, 29.90, 29.87, 29.7, 29.5, 26.4, 25.3, 23.0, 20.9, 14.2; ^{31}P NMR (202 MHz, 2:1 $CDCl_3$/CD_3OD) δ 2.7. HRMS-ESI $[M - H]^-$ calculated for $C_{54}H_{100}O_{21}P$: 1115.6495. Found 1115.6488.

2-*O*-(1-*O*-Hexadecanoyl-2-*O*-hexadecyl-*sn*-glycero-3-phosphoryl)-1,3-bis-*O*-(α-D-mannopyranosyl)glycerol (6). Sodium methoxide in MeOH (0.5 M solution, 50 μL) was added dropwise to a stirred solution of **42** (26 mg, 0.023 mmol) in MeOH (5 mL). After 2 h the mixture was quenched with Dowex 50W8-100 (H^+) resin, filtered, and subsequently made neutral by treatment with Dowex 50W8-100

(Na^+) resin. After filtration and concentration, the crude residue was purified on silica gel ($CHCl_3$/MeOH/H_2O = 70:35:1.75 to 70:35:6) to afford the title compound **6** (17 mg, 71%). $[\alpha]^{20}_D$ +39 (*c* 0.14, $CHCl_3$/MeOH/H_2O = 70:40:6); ^{1}H NMR (500 MHz, $CDCl_3$/CD_3OD/D_2O = 70:35:6) δ 4.87 (br, 1H), 4.84 (br, 1H), 4.39 (br, 1H), 4.30 (dd, *J* = 2.7, 11.8 Hz, 1H), 4.11 (dd, *J* = 7.3, 11.7 Hz, 1H), 3.95−3.83 (m, 8H), 3.77−3.59 (m, 12H), 3.53 (dt, *J* = 7.0, 9.4 Hz, 1H), 2.35 (t, *J* = 7.6 Hz, 2H), 1.65−1.60 (m, 2H), 1.59−1.53 (m, 2H), 1.34−1.27 (m, 50H), 0.90−0.87 (m, 6H); ^{13}C NMR (125 MHz, $CDCl_3$/CD_3OD/D_2O = 70:35:6) δ 175.2, 100.8, 100.7, 77.3, 77.2, 73.5, 73.4, 71.5, 71.3, 70.94, 70.90, 67.8, 67.7, 67.3, 67.1, 64.9, 64.7, 64.6, 61.9, 61.8, 34.8, 32.4, 30.4, 30.23, 30.19, 30.14, 30.08, 30.05, 29.8, 29.7, 26.5, 25.5, 23.1, 14.4; ^{31}P NMR (202 MHz, $CDCl_3$/CD_3OD/D_2O = 70:35:6) δ −0.8. HRMS-ESI $[M\text{-}H]^-$ calculated for $C_{50}H_{96}O_{19}P$: 1031.6283. Found 1031.6288.

Cytokine Release Assay. Thioglycollate-elicited peritoneal murine macrophages were generated as previously described.[48] Macrophages were cultured in 96-well plates at 2×10^5 cells/well in supplemented RPMI-1640 (100 U/mL penicillin−streptomycin, 10% fetal calf serum). Compounds and 10 ng/mL LPS were added to macrophage cultures to a final culture volume of 200 μL/well and incubated at 37 °C, 5% CO_2 for 18 h. Supernatants were collected and IL-6 levels measured by ELISA. Cell viability was measured by trypan blue exclusion.

Expression and Purification of sTLR4/MD-2. A complex between TLR4 ectodomain (sTLR4) and MD-2 was expressed by a baculovirus expression system using a modified dual expression vector, pAcUW51 (BD Biosciences). sTLR4 and MD-2 are appended to a C-terminal His_6-tag and a Strep-tag II, respectively. sTLR4/MD-2 expressing baculovirus was generated by co-transfecting SF9 insect cells with the sTLR4/MD-2 expression vector and linearized baculovirus DNA, BaculoGold (BD Biosciences). For sTLR4/MD-2 expression, Hi5 insect cells were infected with the amplified recombinant virus. The secreted sTLR4/MD-2 protein was initially purified by Ni-NTA and Strep-Tactin affinity chromatography, and its C-terminal tags were removed by thrombin. The resultant protein was further purified by size exclusion chromatography.

Expression and Purification of MD-1. MD-1 was expressed by a baculovirus expression system using a modified pAcGP67 expression vector and purified by three steps including Ni-NTA affinity, anion exchange, and gel filtration chromatography, as described previously.[44]

Native PAGE. Purified sTLR4/MD-2 (or MD-1) protein was mixed with **2** first and then immediately with LPS in 20 mM Hepes, pH 7.4, and 150 mM NaCl, and the mixture was incubated at room temperature for 1 h. The mixture was analyzed by native PAGE, which was performed at room temperature at pH 8.8 using a 4−20% gradient polyacrylamide gel (Bio-Rad).

■ ASSOCIATED CONTENT

Supporting Information. NMR spectra, HPLC data, and viability data. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ABBREVIATIONS USED

TLR, Toll-like receptor; PIM, phosphatidylinositol mannoside; NKT, natural killer T; LPS, lipopolysaccharide; MD-2, myeloid differentiation factor 2

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