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Synthesis of a Monophosphoryl Derivative of *Escherichia coli* Lipid A and Its Efficient Coupling to a Tumor-Associated Carbohydrate Antigen

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Abstract

Monophosphoryl lipid A is a safe and potent immunostimulant and vaccine adjuvant, which is potentially useful for the development of effective carbohydrate-based conjugate vaccines. This paper presented a convergent and efficient synthesis of a monophosphoryl derivative of *E. coli* lipid A having an alkyne functionality at the reducing end, which is suitable for the coupling with various molecules. The coupling of this derivative to an N-modified analog of tumor-associated antigen GM3 by click chemistry is also presented.

Keywords

carbohydrate; lipid A; monophosphoryl lipid A; GM3; glycolipid; glycoconjugate

Introduction

Lipopolysaccharides (LPS), which constitute the major components on the cell surface of Gram-negative bacteria,[1] have been proved to be particularly endotoxic and cause septicemia.[2] It has been further demonstrated that the LPS anchor part, namely, lipid A, is primarily responsible for the endotoxicity of LPS. Lipid A binds to Toll-like receptor 4 (TLR4) to activate a cascade of immunological responses, including the production of a number of cytokines and chemokines such as tumor necrosis factor- α (TNF- α), interleukin-1 β (IL-1 β), IL-6, and interferon- β (IFN- β).[3] Thus, lipid A has become a valuable molecular template in the discovery of new immunostimulants,[4,5] for example vaccine adjuvant, and in the design and development of novel conjugate vaccines.[6]

Lipid A is a highly hydrophobic glycolipid, consisting of a β 1,6-linked di-glucosamine with two phosphate or pyrophosphate groups and 4–8 long lipid chains attached to the 1,4'-O- and 2,2'-N-3,3'-O-positions, respectively (Chart 1). The structures of lipid A derived from different bacteria can vary significantly in terms of the number, structures and locations of their lipids. [1] In order to understand and eventually mitigate the endotoxicity of lipid A for the development of useful immunostimulant, numerous lipid A derivatives have been designed, synthesized, and biologically assayed.[7–10] The structure-activity relationship studies of natural lipid A and various synthetic analogs have shown that both the number and lengths of

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acyl chains and the phosphorylation state of lipid A have a significant impact on its endotoxicity. It appears that the diphosphorylated hexa-acyl form of lipid A, such as *Escherichia coli* lipid A (Chart 1), is optimally recognized by TLR4 to exhibit the full spectrum of endotoxicity.[1] Most importantly, it was observed that the endotoxic activity of lipid A could be significantly reduced after the removal of its anomeric phosphate group,[1] while its immunostimulatory property remained unaffected. For example, although *Salmonella minnesota* lipid A is a potent endotoxin, its 4'-O-monophosphorylated form is essentially nontoxic.[11,12] Monophosphoryl lipid A (MPLA) has been proved to be clinically safe as vaccine adjuvant[13] and even has been explored as potential vaccines against bacteria and cancer.[6]

Meanwhile, the special carbohydrates expressed by bacterial and cancer cells are important targets for the design and development of bacterial and cancer vaccines.[14,15] However, a major problem for carbohydrate antigens, especially the tumor-associated carbohydrate antigens (TACAs), is that they are typically poorly immunogenic.[14] To overcome this problem, much recent effort has been focusing on synthetic multicomponent glycoconjugate vaccines, which consist of a carbohydrate antigen, an immunostimulant, and/or other functional epitopes.[16–22] It has been demonstrated that covalently coupling an immunostimulant to a carbohydrate antigen can remarkably improve the immunogenicity of the latter and generate potent glycoconjugate vaccines. In this regard, MPLA as an extremely potent and nontoxic immunostimulant can be particularly useful.

To exploit the immunostimulatory or adjuvant activity of MPLA for the development of functional conjugate vaccines, we designed a monophosphoryl derivative **1** (Scheme 1) of *E. coli* lipid A,[1] which is suitable for coupling with peptides/proteins and carbohydrates for the assembly of multicomponent glycoconjugates. Although several syntheses of lipid A and MPLA have already been reported,[23–37] the uniqueness of **1** is that it contains an alkyne functionality, enabling its coupling with other molecules by click chemistry. In the meanwhile, an alkyne functionality can be readily transformed into a carboxyl or carbonyl group to facilitate other conjugation methods, such as that based on carboxylic acid-amine condensation reactions or based on reductive amination reactions. This report describes the chemical synthesis of **1** and its conjugation with a TACA by means of click chemistry.

In **1**, the functionality that can be utilized to couple MPLA with other epitopes is located at the disaccharide reducing end. Structure-activity relationship studies of MPLA have revealed that modifying the reducing end of MPLA had little influence on its binding to lipid A receptor and that MPLA derivatives with spacers attached to the reducing end had activities similar to that of MPLA.[1,38] Thus, we anticipate that glycoconjugates derived from **1**, which will have both the carbohydrate antigen and other molecular epitopes linked to the MPLA reducing end, will retain the immunostimulatory activities of MPLA and be highly immunogenic.

Results and Discussion

Depending on the structures and the locations of the acyl chains in the target molecule, there are two general strategies for the synthesis of lipid A and MPLA. One strategy is to assemble the disaccharide backbone first and finally introduce individual lipid chains at proper positions.[23–29] In this case, various positions of the disaccharide unit have to be differentially protected by orthogonal protecting groups, especially when different lipid chains present in the synthetic target. The second strategy is to build the lipidated monosaccharides first and then couple them together via a glycosylation reaction,[30–37] in which case the glycosylation reaction can be challenging, because of increased steric hindrance. Our target molecule **1** has three different types of lipids attached to 2,2'-N-3,3'-O-positions, respectively, and we chose to adopt the second synthetic strategy (Scheme 1). For the monosaccharide **2** at the nonreducing

end, we planned to introduce the lipid moiety at the 3'-O-position at the initial stage, and protect the 2'-N-position by a trichloroethoxycarbonyl (Troc) group, for minimizing the steric hindrance of the glycosyl donor. Meanwhile, Troc can be readily removed later on for the introduction of the lipid moiety, and the acylation of a free amino group should be relatively easy. For the reducing end monosaccharide block **3**, we planned to introduce both lipid chains at the initial stage, as they were expected to have little influence on the relatively reactive primary alcohol as a glycosyl acceptor. It was also designed to have an azidoethyl group, which can be used as molecular handle to perform various chemical transformations, linked to the anomeric position.

The synthesis of **2** is outlined in Scheme 2. After **4** was prepared from glucosamine upon a series of established transformations,[29,39] the phthaloyl group and the acetyl group protecting the 2-N- and 3-O-positions, respectively, were removed by treatment with ethylene diamine, which was followed by regioselective protection of the free amino group by Troc to achieve **5**. The lipidation of **5** by means of (*R*)-3-tetradecanoyloxy-tetradecanoic acid **6** was accomplished with *N,N'*-dicyclohexylcarbodiimide (DCC) as the condensation reagent to afford building block **2** in an excellent yield.

The synthesis of **3** (Scheme 3) started with the preparation of **7** by a reported procedure.[40] Its 4,6-O-positions were then selectively protected with a benzylidene group, followed by deacetylation of the amino group under basic condition. The amino and hydroxyl groups of **9** were simultaneously acylated by (*R*)-3-benzyloxy-tetradecanoic acid **10** to yield **11**, again, with DCC as a condensation reagent. The benzylidene acetal ring was thereafter opened regioselectively with borane as the reducing reagent and trimethylsilyl triflate (TMSOTf) as the catalyst to finally afford building block **3**. The regiochemistry of **3** was confirmed by acetylation of **3**, which resulted in **12** having significantly downfield-shifted NMR signals of H-6a,6b.

For the final stage assembly of the synthetic target **1** (Scheme 4), **3** was glycosylated by **2** using *N*-iodosuccinimide (NIS) and silver triflate (AgOTf) as the promoters. This reaction was stereoselective to afford the desired β -anomer ($J_{1,2} = 3.2$ Hz, $J_{1',2'} = 8.0$ Hz) only, probably owing to the neighboring group participation, and **13** was obtained in a 75% isolated yield. For the regioselective opening of the benzylidene acetal ring of **13**, we were surprised to observe that **13** did not react with sodium cyanoborohydride (NaCNBH₃)/HCl (g), and the starting material was recovered completely. After exploring a number of reactions and conditions, we eventually found that the regioselective ring opening could be accomplished smoothly using triethylsilane (Et₃SiH) and trifluoroborane (BF₃) in diethyl ether to expose the C-4' hydroxyl group. The regiochemistry of the product **14** was also confirmed by an acetylation experiment, which resulted in a significant downfield shift of the NMR signal of its H-4'. Next, **14** was phosphorylated by a two-step-one-pot protocol with dibenzyl phosphoramidite and *t*-BuO₂H as the phosphorylating and oxidizing reagents, respectively, to furnish **15**. We decided to introduce the alkyne functionality at this stage. Thus, the azido group of **15** was reduced by propane-1,3-dithiol, and the free amine **16** was acylated with 4-pentynoic acid **17** using 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC) hydrochloride as the condensation reagent. It is worthy noting that functionalities other than a carbon triple bond can also be introduced at this stage to facilitate alternatively methods for the coupling of MPLA to various structures later on. Moreover, we found that other traditional methods employed to reduce azides, such as that using phosphine or Lindlar catalyst/H₂, did not work for **15**. The former reaction gave a good yield of an iminophosphorane derivative that was difficult to be hydrolyzed, and the latter reaction was slow and inefficient. Finally, the Troc group of **18** was removed by treatment with Zn in AcOH, which was followed by *N*-acylation of **19** by (*R*)-3-dodecanoyloxy-tetradecanoic acid **20** with 2-(7-aza-1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU)/*N,N*-diisopropylethylamine (DIPEA) as

condensation reagents to afford the synthetic target **1** in a 70% yield. We observed that using DCC and EDC for the final condensation reaction could only provide a low yield of the desired product. Compound **1** was positively characterized by NMR and HR MS.

To illustrate that **1** can be conveniently and efficiently coupled to properly modified carbohydrates for investigating multicomponent conjugate vaccines, we then examined the reaction between **1** and an azido derivative **21** of *N*-phenylacetyl GM3 (GM3NPhAc) by means of click reaction[41] (Scheme 5). GM3NPhAc, an unnatural analog of GM3,[42] can form promising vaccines that are presently investigated in our laboratory for cancer immunotherapy. [43,44] To our satisfaction, the reaction between **1** and **21** in the presence of CuI/DIPEA proceeded smoothly in a MeOH/THF mixture to offer a very good yield of the MPLA-GM3NPhAc conjugate **22** (70% isolated yield after silica gel column chromatography).

Conclusion

In short, a highly efficient synthesis of a monophosphoryl derivative **1** of *E. coli* lipid A has been developed. This synthesis is highlighted with the convergent assembly of the MPLA framework by coupling two lipidated monosaccharide building blocks **2** and **3**. The synthetic method can be applicable to similar MPLA structures. The synthetic derivatives of MPLA are useful for the exploration of novel MPLA-derived immunostimulants or immunoadjuvants. Furthermore, these MPLA derivatives can be readily coupled with various structures, as illustrated by the conjugation of **1** and a TACA **21** to form a MPLA-carbohydrate conjugate **22**, for the exploration of effective conjugate vaccines. Finally, glycoconjugate **22** was readily deprotected via Pd-catalyzed hydrogenolysis, and both **22** and its deprotection product are evaluated as cancer vaccines in our laboratory.

Experimental Section

General Methods

NMR spectra were recorded on a 400 or 500 MHz instrument with the chemical shifts reported in ppm (δ) in reference to tetramethylsilane (TMS), if not specified otherwise. Coupling constants (*J*) are reported in hertz (Hz). Optical rotations were obtained with an Autopol III polarimeter. High resolution electron spray ionization mass spectra (HR ESI MS) were recorded with a Waters Micromass-LCTPremier-XE instrument. MALDI-TOF MS were performed on a Bruker Ultraflex Mass Spectrometer. Thin layer chromatography (TLC) was performed on silica gel GF254 plates detected by charring with phosphomolybdic acid or 1% H₂SO₄ in EtOH. Molecular sieves (MS) were dried in high vacuum at 170–180 °C for 6–10 h before use. Commercial anhydrous solvents and other reagents were used without further purification.

Compound 2

To a stirred solution of **6** (200 mg, 0.44 mmol) in dichloromethane (DCM) were added DCC (184 mg, 0.90 mmol) and DMAP (28 mg, 0.23 mmol) at rt, and after the mixture was stirred at rt for 10 min, a solution of **5** (200 mg, 0.36 mmol) in DCM (1.5 mL) was added. The reaction mixture was stirred for another 16 h at rt, and the solid materials were removed by filtration, and the residue was washed with DCM (10 mL). The combined filtrate was concentrated in vacuum, and the residue was purified by silica gel column chromatography to afford **2** as a white solid (330 mg, 93%). $[\alpha]_D^{+14.1}$ (c 1.0, CHCl₃). ¹H NMR (CDCl₃, 400 MHz): δ 7.42–7.26 (m, 7 H), 7.12 (d, *J* = 7.2 Hz, 2 H), 5.51 (d, *J* = 9.6 Hz, 1 H, NH), 5.48 (s, 1 H, PhCH), 5.38 (t, *J* = 9.6 Hz, 1 H, H-3), 5.20–5.14 (m, 1 H, lipid), 4.90 (d, *J* = 10.4 Hz, 1 H, H-1), 4.76 (d, *J* = 4 Hz, 2 H, CH₂CCl₃), 4.34 (dd, *J* = 4.8, 10.4 Hz, 1 H, H-6a), 3.78 (t, *J* = 10.0 Hz, 1 H, H-6b), 3.68–3.60 (m, 2 H, H-4, H-2), 3.56–3.50 (m, 1 H, H-5), 2.62–2.48 (m, 2 H), 2.35 (s, 3

H), 2.17 (t, $J = 7.2$ Hz, 2 H), 1.55-1.52 (m, 4 H), 1.32-1.20 (m, 40 H), 0.88 (t, $J = 7.6$ Hz, 6 H). ^{13}C NMR (CDCl_3 , 100 MHz): δ 173.7, 170.2, 154.3, 138.8, 137.0, 133.7, 130.1, 129.4, 128.5, 126.4, 101.7, 88.1, 78.8, 74.8, 72.5, 70.7, 70.2, 68.7, 56.0, 39.5, 34.6, 34.1, 32.1, 29.9, 29.8, 29.6, 29.6, 29.4, 25.3, 25.2, 22.9, 21.4, 14.4. HR ESI MS (m/z): calcd for $\text{C}_{51}\text{H}_{76}\text{Cl}_3\text{NO}_9\text{SNa}$ ($\text{M}+\text{Na}$) $^+$, 1006.4204; found, 1006.4201

Compound 11

To a stirred solution of **10** (0.70 g, 2.1 mmol) in DCM (10 mL) were added DCC (0.64 g, 3.2 mmol) and DMAP (0.10 g, 0.08 mmol) at rt. After the mixture was stirred for 10 min, a solution of **9** (0.27 g, 0.81 mmol) in DCM (1.5 mL) was added, and the reaction mixture was stirred for another 16 h at rt. The solid materials were then removed by filtration, and the residue was washed with DCM (10 mL). The combined filtrate was concentrated in vacuum, and the residue was purified by silica gel column chromatography to afford **11** as a white solid (0.56 g, 71%). $[\alpha]_{\text{D}}^{25} +26.8$ (c 1.0, CHCl_3). ^1H NMR (CDCl_3 , 400 MHz): δ 7.40-7.21 (m, 15 H), 6.20 (d, $J = 9.6$ Hz, 1 H, NH), 5.46 (s, 1 H, PhCH_2), 5.38 (t, $J = 9.6$ Hz, 1 H, H-3), 4.73 (d, $J = 3.2$ Hz, 1 H, H-1), 4.56-4.35 (m, 5 H, 2PhCH_2 , H-2), 4.25 (dd, $J = 9.6, 4.4$ Hz, 1 H, H-5), 3.92-3.68 (m, 6 H, H-6a, H-6b, H-4, 2 BnOCH_2 , $\text{OCH}_2\text{CH}_2\text{N}_3$), 3.25 (dd, $J = 7.6, 16.4$ Hz, 2 H, $\text{OCH}_2\text{CH}_2\text{N}_3$), 3.15-3.09 (m, 1 H, CH_2N_3), 2.66 (dd, $J = 6.8, 10.8$ Hz, 1H), 2.44-2.36 (m, 1 H), 2.33-2.26 (m, 2 H), 1.47-1.43 (m, 4 H), 1.30-1.19 (m, 36 H), 0.89-0.86 (t, 6 H). ^{13}C NMR (CDCl_3 , 100 MHz): δ 172.0 171.6, 138.9, 138.8, 137.1, 129.3, 128.6, 128.5, 128.4, 128.0, 127.9, 127.8, 127.6, 126.3, 101.8, 98.5, 79.4, 76.7, 75.7, 71.6, 71.3, 70.0, 69.0, 67.3, 63.4, 52.3, 50.4, 42.0, 40.0, 34.8, 34.1, 32.2, 29.9, 29.9, 29.8, 29.6, 25.5, 25.4, 22.9, 14.4. HR ESI MS (m/z): calcd for $\text{C}_{57}\text{H}_{84}\text{N}_4\text{O}_9\text{Na}$ ($\text{M}+\text{Na}$) $^+$, 991.6136; found, 991.6152.

Compound 3

To a stirred solution of compound **11** (365 mg, 0.38 mmol) in BH_3/THF (2 mL, 2.0 mmol) was added dropwise TMSOTf (0.08 mL, 0.46 mmol) in ice-water bath. After 1 h of stirring, TLC showed that the reaction was complete. The reaction was then quenched with triethylamine and MeOH, and the mixture was concentrated, and the residue was purified by silica gel column chromatography to afford **3** as a white solid (213 mg, 58%). ^1H NMR (CDCl_3 , 400 MHz): δ 7.33-7.20 (m, 15 H), 6.13 (d, $J = 8.8$ Hz, 1 H, NH), 5.39-5.34 (m, 1 H, H-3), 4.71 (d, $J = 4.0$ Hz, 1 H, H-1), 4.65-4.43 (m, 6 H, 3 PhCH_2), 4.28-4.22 (m, 1 H, H-2), 3.85-3.62 (m, 7 H, H-6b, H-4, H-5, H-6a, 2 BnOCH_2 , $\text{OCH}_2\text{CH}_2\text{N}_3$), 3.47-3.38 (m, 2 H, $\text{OCH}_2\text{CH}_2\text{N}_3$), 3.25-3.19 (m, 2 H), 3.12-3.07 (m, 1 H, CH_2N_3), 2.56 (dd, $J = 7.2, 16.0$ Hz, 1 H), 2.39 (dd, $J = 5.6, 16.0$ Hz, 1 H), 2.29-2.27 (m, 2 H), 1.66-1.43 (m, 4 H), 1.37-1.16 (m, 36 H), 0.89-0.86 (t, 6 H). ^{13}C NMR (CDCl_3 , 100 MHz): δ 172.3, 171.6, 139.0, 138.8, 137.9, 128.7, 128.6, 128.5, 128.2, 128.1, 128.0, 127.9, 127.9, 127.8, 127.7, 127.6, 97.9, 76.7, 75.7, 75.3, 74.9, 73.3, 71.7, 71.6, 71.5, 70.8, 67.2, 61.7, 52.4, 50.4, 42.1, 40.0, 34.5, 34.2, 34.1, 32.2, 30.0, 29.9, 29.9, 29.8, 29.6, 26.7, 26.6, 25.5, 25.4, 25.2, 22.9, 14.4. HR ESI MS (m/z): calcd for $\text{C}_{57}\text{H}_{86}\text{N}_4\text{O}_9\text{Na}$ ($\text{M}+\text{Na}$) $^+$, 993.6293; found, 993.6284.

Compound 13

A mixture of **2** (162 mg, 0.165 mmol), **3** (150 mg, 0.155 mmol) and 4 Å MS (0.5 g) was stirred in anhydrous DCM (2 mL) at rt for 0.5 h. The reaction mixture was then cooled to -50°C , and NIS (85 mg, 10 mmol) was added. The reaction mixture was allowed to warm to -30°C and stirred for another 1 h before a catalytic amount of AgOTf was added. When TLC showed the glycosyl donor **2** was consumed completely, the reaction mixture was quenched with the addition of triethylamine. MS was removed by filtration through a Celite pad. The filtrate was washed with water, dried, and then evaporated in vacuum. The residue was purified by silica gel column chromatography to give **13** as a white solid (212 mg, 75%). $[\alpha]_{\text{D}}^{25} +11.2$ (c 1.0, CHCl_3); ^1H NMR (CDCl_3 , 400 MHz): δ 7.42-7.20 (m, 20 H), 6.12 (d, $J = 9.6$ Hz, 1 H, NH),

5.49 (s, 1 H), 5.36 (t, $J = 9.2$ Hz, 1 H, H-3), 5.29-5.25 (m, 1 H, H-3'), 5.20-5.17 (m, 1 H, lipid), 4.72 (d, $J = 3.2$ Hz, 1 H, H-1), 4.68-4.46 (m, 8 H, Troc, 3PhCH₂), 4.42 (d, $J = 8.0$ Hz, 1 H, H-1'), 4.33 (dd, $J = 4.8, 10.4$ Hz, 1 H, H-6a'), 4.29-4.23 (m, 1 H, H-2), 4.01 (bd, $J = 10.4$ Hz, 1 H, H-6b), 3.87-3.75 (m, 4 H, 2BnOCH, H-5, H-6b'), 3.69-3.63 (m, 5 H, H-2', H-4, H-4', H-6a, OCH₂CH₂N₃), 3.47-3.40 (m, 2 H, H-5', lipid), 3.25-3.19 (m, 2 H, OCH₂CH₂N₃), 3.12-3.07 (m, 1 H, CH₂N₃), 2.62-2.53 (m, 3 H), 2.41 (dd, $J = 4.8, 16.0$ Hz, 1 H), 2.28 (d, $J = 6.0$ Hz, 2 H), 2.19 (t, $J = 7.2$ Hz, 2 H), 1.62-1.44 (m, 8 H), 1.38-1.25 (m, 86 H), 0.94-0.88 (m, 12 H). ¹³C NMR (CDCl₃, 100 MHz): δ 173.7, 172.2, 171.4, 170.3, 154.3, 138.9, 138.8, 138.2, 137.1, 129.4, 128.8, 128.6, 128.5, 128.2, 128.1, 128.0, 127.7, 126.4, 101.7, 97.8, 95.6, 78.9, 76.7, 75.7, 74.8, 74.5, 73.7, 71.6, 71.3, 70.8, 70.4, 70.2, 68.7, 67.2, 66.6, 57.0, 52.1, 50.4, 42.1, 40.0, 39.5, 34.6, 34.4, 34.3, 34.1, 32.2, 29.9, 29.8, 29.6, 29.4, 26.7, 25.4, 25.2, 22.9, 14.4. HR ESI MS (m/z): calcd for C₁₀₁H₁₅₄Cl₃N₅O₁₈Na (M+Na)⁺, 1853.0252; found, 1853.0221.

Compound 14

To the solution of **13** (70 mg, 0.038 mmol) in DCM (2.0 mL) at 0 °C were added Et₃SiH (15 equiv., 0.10 mL) and BF₃·Et₂O (2 equiv., 10 μ L). After the mixture was warmed to 25 °C over a period of 2 h, it was diluted with DCM (30 mL), washed with NaHCO₃ solution (5 mL), dried and concentrated. The residue was purified by silica gel column chromatography to provide **14** (53 mg, 76%). [α]_D +17.4 (c 1.0, CHCl₃). ¹H NMR (CDCl₃, 400 MHz): δ 7.36-7.17 (m, 20 H), 6.10 (d, $J = 9.6$ Hz, 1 H, NH), 5.31 (dd, $J = 8.8, 10.4$ Hz, 1 H, H-3), 5.12-5.10 (m, 1 H, lipid), 5.09 (d, $J = 8.4$ Hz, 1 H, NH'), 4.91 (t, $J = 9.6$ Hz, 1 H, H-3'), 4.70 (d, $J = 4.0$ Hz, 1 H, H-1), 4.63-4.42 (m, 10 H, Troc, 4PhCH₂), 4.28-4.20 (m, 2 H, H-2, H-1'), 4.06-4.02 (m, 1 H, H-6b), 3.84-3.72 (m, 5 H, 2BnOCH, H-5, H-6a'), 3.67-3.60 (m, 5 H, H-2', H-4, H-4', H-6a, H-6b'), 3.48-3.38 (m, 2 H, H-5', OCH₂CH₂N₃), 3.24-3.17 (m, 2 H, OCH₂CH₂N₃), 3.11-3.05 (m, 1 H, CH₂N₃), 2.60-2.51 (m, 3 H), 2.41 (dd, $J = 4.8, 15.2$ Hz, 1 H), 2.30-2.26 (m, 4 H), 2.19 (1.62-1.44 (m, 8 H), 1.38-1.25 (m, 86 H), 0.94-0.88 (t, 12 H). ¹³C NMR (CDCl₃, 100 MHz): δ 174.6, 172.3, 171.7, 171.5, 154.3, 138.9, 138.7, 138.3, 138.0, 128.7, 128.5, 128.2, 128.0, 127.9, 127.5, 101.3, 97.7, 95.7, 76.7, 75.7, 75.5, 75.4, 74.7, 73.9, 71.6, 71.2, 70.8, 70.5, 70.2, 67.7, 67.2, 55.8, 52.1, 50.4, 42.1, 40.3, 40.1, 36.9, 34.9, 34.7, 34.4, 34.3, 32.4, 32.2, 30.1, 29.9, 29.7, 29.6, 26.7, 25.4, 25.2, 24.9, 23.6, 22.9, 14.4. HR RSI MS (m/z): calcd for C₁₀₁H₁₅₉Cl₃N₅O₁₈Na (M+Na)⁺, 1855.0409; found, 1855.0339.

Compound 15

To a stirred solution of **14** (50 mg, 0.027 mmol) in dry DCM (5 mL) were added 1H-tetrazole (0.45M, 0.5 mL) and dibenzyl diisopropylphosphoramidite (0.05 mL, 0.15 mmol). After the mixture was stirred for 2 h at rt, it was cooled to -30 °C and then *t*-BuOOH (0.4 mmol) was added. The mixture was stirred for another 0.5 h at 0 °C. The solution was washed with saturated aqueous NaHCO₃ and brine. The organic phase was dried and concentrated. The residue was purified by flash chromatography to give **15** as syrup (43 mg, 75%). [α]_D +5.2 (c 0.5, CHCl₃). ¹H NMR (CDCl₃, 400 MHz): δ 7.36-7.22 (m, 30 H), 6.10 (d, $J = 9.6$ Hz, 1 H, NH), 5.45 (d, $J = 8.4$ Hz, 1 H, NH'), 5.38-5.32 (m, 2 H, H-3, H-3'), 5.18 (m, 1 H, lipid), 4.90-4.80 (m, 4 H, 2 PhCH₂), 4.71 (d, $J = 3.2$ Hz, 1 H, H-1), 4.65-4.58 (m, 3 H, H-1', PhCH₂), 4.54-4.38 (m, 8 H, H-4', Troc, PhCH₂), 4.29-4.24 (m, 1 H, H-2), 4.06-4.03 (m, 1 H, H-6b), 3.85-3.77 (m, 4 H, 2 BnOCH, H-5, H-6a'), 3.69-3.56 (m, 5 H, H-4, H-5', H-6a), 3.54-3.45 (m, 1 H, H-2'), 3.44-3.38 (m, 1 H, OCH₂CH₂N₃), 3.26-3.17 (m, 2 H, OCH₂CH₂N₃), 3.10-3.04 (m, 1 H, CH₂N₃), 2.55 (dd, $J = 7.2, 16.0$ Hz, 1 H), 2.47-2.33 (m, 1 H), 2.29-2.21 (m, 5 H), 1.94-1.87 (m, 1 H), 1.62-1.44 (m, 8 H), 1.38-1.25 (m, 86 H), 0.94-0.88 (t, 12 H). ¹³C NMR (CDCl₃, 100 MHz): δ 173.9, 172.2, 171.4, 170.5, 154.2, 138.9, 138.8, 138.3, 138.2, 135.7, 129.7, 128.8, 128.6, 128.3, 128.1, 127.8, 127.6, 100.6, 97.8, 95.5, 76.6, 75.7, 74.7, 74.6, 74.4, 73.6, 72.5, 71.6, 70.8, 70.5, 70.3, 69.8, 68.8, 68.1, 67.6, 67.1, 56.7, 52.1, 50.4, 42.1, 40.0, 39.8, 36.9, 34.7, 34.4, 34.3, 32.2, 29.9, 29.6, 29.4, 26.7, 25.4, 24.9, 23.7, 23.2, 22.9, 14.3. ³¹P NMR (CDCl₃):

δ -0.92. HR ESI MS (m/z): calcd for $C_{115}H_{169}Cl_3N_5O_{21}PNa$ ($M+Na$)⁺, 2115.1011; found, 2115.0942.

Compound 18

After triethylamine (0.2 mL) was added to a stirred solution of **15** (50 mg, 0.024 mmol) and 1,3-propanedithiol (0.15 mL) in pyridine (4 mL) and H₂O (0.2 mL), the mixture was stirred at 0 °C until the starting material disappeared as monitored by TLC. The solvent was then evaporated in vacuum, and the residue was coevaporated with toluene (2 times, 10 mL) and ethanol (2 times, 10 mL). The residue was purified by silica gel column chromatography to give **16**, which was then dissolved in DCM (1.5 mL). To this solution was added EDC (9 mg, 0.048 mmol) and **17** (7 mg, 0.072 mmol) in DCM (3 mL). The reaction mixture was stirred for 2 h at rt. The mixture was diluted with DCM and washed with H₂O and brine. The organic phase was dried, concentrated and the residue was purified by silica gel column chromatography to afford **18** (30 mg, 58%). $[\alpha]_D^{+9.2}$ (c 0.65, CHCl₃). ¹H NMR (CDCl₃, 400 MHz): δ 7.32-7.20 (m, 30 H), 6.28 (d, J = 9.6 Hz, 1 H, NH), 5.94 (s, 1 H, NH), 5.68 (d, J = 7.6 Hz, 1 H, NH'), 5.42 (dd, J = 9.6 Hz, 8.4 Hz, 1 H, H-3'), 5.28 (dd, J = 11.2, 8.4 Hz, 1 H, H-3), 5.17 (m, 1 H, lipid), 4.91-4.88 (m, 4 H, PhCH₂), 4.67-4.39 (m, 13 H, H-1, H-1', H-4', Troc, PhCH₂), 4.27-4.21 (m, 1 H, H-2), 4.06-4.04 (m, 1 H, H-6b), 3.91-3.76 (m, 4 H, 2 BnOCH₂, H-5, H-6a'), 3.63-3.53 (m, 5 H, H-4, H-5', H-6a, OCH₂CH₂NHCO), 3.45-3.41 (m, 1 H, H-2'), 3.14-3.08 (m, 2 H, OCH₂CH₂NHCO), 3.00 (m, 1 H, OCH₂CH₂NHCO), 2.58-2.20 (m, 12 H), 1.65-1.38 (m, 10 H), 1.38-1.25 (m, 90 H), 0.87 (t, 12 H); ¹³C NMR (CDCl₃, 100 MHz): δ 173.8, 172.3, 171.5, 170.4, 154.3, 138.8, 138.4, 138.3, 137.9, 135.7, 128.8, 128.6, 128.3, 127.9, 127.8, 127.7, 127.6, 127.0, 100.4, 97.2, 95.5, 83.5, 77.1, 73.6, 72.3, 71.4, 71.2, 70.5, 70.3, 69.8, 68.8, 68.3, 67.6, 66.8, 56.7, 52.1, 41.9, 39.9, 39.6, 39.0, 35.2, 34.7, 34.4, 34.1, 32.2, 29.9, 29.8, 29.6, 29.4, 25.4, 25.2, 22.9, 15.0, 14.3. ³¹P NMR (CDCl₃): δ -0.91. HR ESI MS (m/z): calcd for $C_{120}H_{175}Cl_3N_5O_{22}PNa$ ($M+Na$)⁺, 2169.1368; found, 2169.1301.

Compound 1

A solution of **18** (52 mg, 0.024 mmol) in acetic acid (5 mL) was stirred with Zn powder (100 mg) at rt for 24 h. The solid materials were removed by filtration over a Celite pad. The filtrate was concentrated and coevaporated with toluene 3 times. The residue was dissolved in CH₂Cl₂ (40 mL) and washed sequentially with saturated aq. NaHCO₃ solution (20 mL), H₂O (10 mL), and brine (10 mL). The organic phase was dried and concentrated. The residue was purified by chromatography on silica gel to afford **19** as a foamy solid. To the solution of **20** (50 mg, 0.121 mmol) and DIPEA (0.02 mL) in DMF (0.5 mL) was added a solution of HATU (50 mg, 0.132 mmol) in DMF (0.2 mL), and the mixture was stirred under N₂ for 30 min. This solution was then added to a stirred solution of **19** in DMF (0.5 mL), and the mixture was stirred under N₂ for 3.5 h at 60 °C. The mixture was diluted with DCM (50 mL) and washed sequentially with saturated aq. NaHCO₃ solution (10 mL), H₂O (10 mL) and brine (3×20 mL). The organic phase was dried, concentrated, and the residue was purified by flash silica gel column chromatography to afford **1** as a white solid (39 mg, 70%). $[\alpha]_D^{+16.4}$ (c 1.0, CHCl₃); ¹H NMR (CDCl₃, 400 MHz): δ 7.31-7.19 (m, 30 H), 6.31 (d, J = 9.2 Hz, 1 H, NH), 6.25 (d, J = 7.2 Hz, 1 H, NH'), 5.41 (t, J = 9.6 Hz, 1 H, H-3'), 5.2 (t, J = 9.6 Hz, 1 H, H-3), 5.16 (m, 1 H, lipid), 5.07 (m, 1 H, lipid), 4.90-4.88 (m, 4 H, PhCH₂), 4.64 (d, J = 4.0 Hz, 1 H, H-1), 4.56-4.41 (m, 10 H, H-1', H-4', 4PhCH₂), 4.28-4.22 (m, 1 H, H-2), 4.06-4.03 (m, 1 H, H-6b), 3.88-3.75 (m, 4 H, 2 ROCH₂, H-5, H-6a'), 3.66-3.48 (m, 6 H, H-4, H-5', H-6a, H-2', OCH₂CH₂NHCO), 3.22-3.10 (m, 3 H, OCH₂CH₂NHCO), 2.56-2.19 (m, 15 H), 2.13 (d, J = 2.0 Hz, 1 H), 1.55-1.42 (m, 12 H), 1.24-1.03 (m, 116 H), 0.98-0.88 (t, 18 H); ¹³C NMR (CDCl₃, 100 MHz): δ 173.8, 172.1, 171.5, 171.3, 170.5, 138.8, 138.5, 138.3, 137.7, 135.8, 128.8, 128.7, 128.5, 128.3, 128.2, 128.0, 127.9, 127.8, 127.7, 100.5, 97.3, 95.5, 83.7, 76.9, 76.1, 75.6, 74.7, 74.4, 73.8, 73.5, 72.7, 71.4, 71.2, 70.7, 70.4, 70.0, 69.8, 68.8, 62.0, 57.9, 52.2, 50.7, 41.9, 39.9, 35.2, 34.7, 34.5, 34.2, 32.2, 29.9, 29.6, 29.5, 29.5, 25.5, 25.4, 25.3, 22.9, 15.1, 14.4. ³¹P NMR

(CDCl₃) : δ -0.91. HR ESI MS (m/z): calcd for C₁₄₃H₂₂₂N₃O₂₃PNa (M+Na)⁺, 2403.5930; found, 2403.5903.

Compound 22

After **1** (10 mg, 0.004mmol), **21** (4 mg, 0.005mmol), and CuI (10 eq) were dissolved in MeOH/THF (2:1, 0.6 mL), DIPEA (10 eq) was added to the solution. After stirring for 24 h at rt, the mixture was filtered through a Celite pad, and the solvent was evaporated in high vacuum. The crude product was then purified by flash silica gel column chromatography (eluent: from AcOEt and hexane from 4:1 to AcOEt and then to MeOH:CH₂Cl₂ 1:5) to produce **22** as a white solid (9 mg, 70%) [α]_D 4.4 (c 0.45, CHCl₃). ¹H NMR (CDCl₃:CD₃OD 3:1, 500 MHz): δ 7.45 (s, 1 H), 7.19-6.90 (m, 35 H), 5.24-5.21 (m, 1 H, H-3'), 5.15-5.08 (m, 1 H, H-3), 4.98 (m, 1 H, lipid), 4.88 (m, 1 H, lipid), 4.72-4.65 (m, 4 H, PhCH₂), 4.43 (d, J = 3.5 Hz, 1 H, H-1), 4.39-4.42 (m, 12 H, H-1', H-4', PhCH₂), 4.09 (d, J = 7.0 Hz, 1 H), 4.28-4.22 (m, 1 H, H-2), 3.96 (m, 1 H, H-6b), 3.78 (m, 1 H), 3.68-3.35 (m, 4 H, 2 ROCH₂, H-5, H-6a'), 2.98-2.92 (m, 2 H), 2.75 (m, 2 H), 2.35-1.99 (m, 12 H), 1.86-1.80 (m, 3 H), 1.55-1.42 (m, 12 H), 1.24-1.03 (m, 116 H), 0.98-0.88 (t, 18 H). ¹³C NMR (CDCl₃:CD₃OD 3:1, 125 MHz): δ 129.0, 128.7, 128.6, 128.4, 128.3, 128.2, 128.1, 128.0, 127.8, 127.7, 127.6, 127.5, 127.0, 123.2, 103.0, 100.2, 97.4, 77.6, 76.7, 75.9, 75.4, 75.0, 74.6, 74.5, 74.0, 73.4, 73.3, 73.1, 72.6, 71.5, 71.2, 70.6, 70.0, 69.8, 68.5, 68.0, 62.0, 57.9, 52.2, 51.7, 50.2, 49.3, 49.1, 49.0, 48.8, 48.6, 41.4, 39.6, 38.8, 35.1, 34.5, 34.2, 31.9, 29.6, 29.3, 29.2, 25.4, 25.1, 25.0, 22.7, 21.2, 13.9. ³¹P NMR (CDCl₃:CD₃OD 3:1): δ -1.50. MALDI-TOF MS (m/z): calcd for C₁₇₄H₂₆₈N₇O₄₂P (M⁺), 3158.9; found 3181.8 (M+Na)⁺, 3203.8 (M-H+2Na)⁺.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Scheme 1.
Structure of the target MPLA derivative and the synthetic plan.

Scheme 2.
Synthesis of monosaccharide building block **2**.

Scheme 3.
Synthesis of monosaccharide building block **3**.

Scheme 4.
Synthesis of the target MPLA derivative **1**.

Scheme 5.
Coupling **1** and GM3NPhAc by click chemistry.

Chart 1.

Structures of selected lipid A derived from different Gram-negative bacteria. Dash lines indicate partial structures as the results of incomplete biosynthesis, and the numbers in parenthesis indicate the potential variation of chain lengths.