

Synthesis and Biological Activity of 3,7-Anhydro-2,4-dideoxy-4-[(*R*)-3-hydroxytetradecanoylamino]-6-*O*-phosphono-5-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-D-*glycero*-D-*ido*-octonic Acid Derivatives

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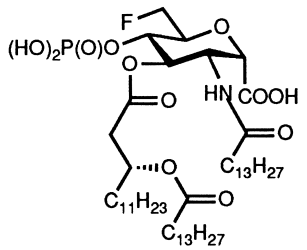
D-Glucosaminylacetic acid derivatives (**10**, **11** and **12**) of GLA-60 were synthesized to investigate the LPS-antagonistic activity toward human monoblastic U937 cells. Compounds **10** and **11** show weak LPS-antagonistic activity, but **12** shows both LPS-antagonistic and -agonistic activities, depending on the concentration.

Lipopolysaccharides (LPS)[1] cover the outer surface membrane of Gram-negative bacteria, and are highly potent stimulators of the immune system.[2] A variety of responses, both beneficial and harmful, can be elicited by LPS. One of these harmful responses is fatal endotoxic shock (bacterial sepsis) caused as a consequence of an acute inflammatory response. This fatal shock has precluded the clinical use of LPS.

Most of the biological activities of LPS reside in a relatively small portion of the molecule, that is, the terminal disaccharide phospholipid subunit, known as lipid A, which is a hydrophobic anchor substance holding an essentially linear polysaccharide chain to the cell wall. Lipid A was first synthesized by Shiba and Kusumoto et al.[3] Analogues of both the non-reducing and reducing sugar parts of Lipid A are also biologically active.[4]

In the early days, endotoxin and its related compounds were mainly investigated for their potential as anti-cancer drugs[5] that function as LPS-agonists by activating macrophages. However, in recent years, endotoxin-related compounds have been studied as LPS-antagonists, which may have potential as immunosuppressants[6] and anti-HIV agents[7] as well as in the therapy of septicemia[8] and autoimmune diseases[6] by deactivating LPS-induced aggressive macrophages. For example, Lipid IVa, which is a biosynthetic precursor of Lipid A, acts as an LPS-antagonist in human systems.[9] Furthermore, Qureshi's group[10] has isolated a lipid A-related compound from *Rhodobacter sphaeroides*, which has shown potent LPS antagonist activity. Recently, an Eisai group has developed a related compound, E5564,[8,11] as a highly potent anti-septicemia drug.

On the other hand, during our investigation of the biological activities of compounds related to GLA-60,[4b] which is a non-reducing monosaccharide analogue of Lipid A, we also found that most of them had LPS-agonistic activity, but a few of them behaved as LPS antagonists. In our previous study,[12] tetrahydropyran-2-carboxylic acid **A** exhibited fairly strong LPS-antagonistic activity toward human U937 cells (Fig. 1). By analogy, we were interested in the biological activity of the anomeric homologues of compound **A**. Therefore, we attempted



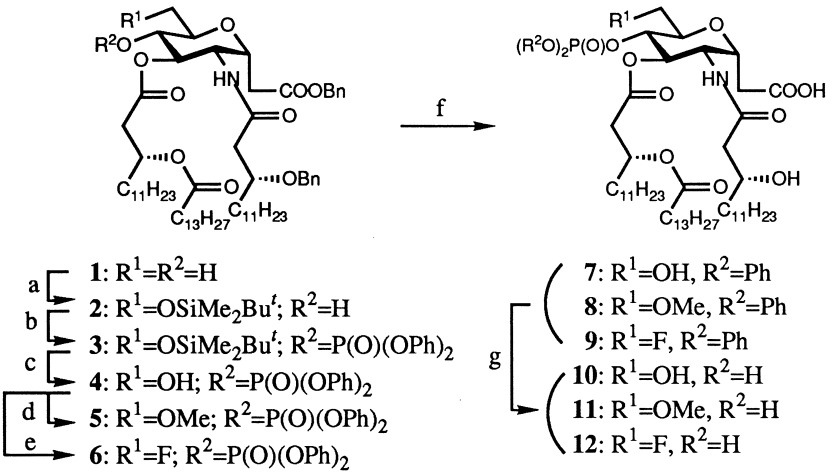
A

Fig. 1. Structure of LPS-antagonist toward human monoblastic U937 cells.

to synthesize the title compound. In this paper, we report that 3,7-anhydro-2,4-dideoxy-4-[(*R*)-3-hydroxytetradecanoylamino]-5-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-D-*glycero*-D-*ido*-octonic acid, and its C8-OMe and C8-F derivatives were synthesized to investigate their biological activities.

Result and Discussion

The starting alcohol **1** synthesized by Vyplel's procedure[13] was converted to *t*-butyldimethylsilyl ether **2** by selective protection at the C8-primary alcohol with *t*-butyldimethylsilyl chloride (TBSCl) using 4-(dimethylamino)pyridine (DMAP) as a base. The treatment of **2** with diphenyl chlorophosphate and DMAP gave diphenyl phosphate **3**. Desilylation of **3** with aqueous HCl gave alcohol **4**. The treatment of **4** with trimethyloxonium tetrafluoroborate using 2,6-di-*t*-butyl-4-methylpyridine as a base gave methyl ether **5**. On the other hand, the treatment of **4** with (diethylaminato)trifluorosulfur (DAST) gave fluoride **6**. Hydrogenolysis of both the benzyl ester and benzyl ether groups of compounds **4**, **5**, and **6** under hydrogen using Pd on carbon or $Pd(OH)_2$ on carbon as catalysts gave acids **7**, **8**, and **9**, respectively. Finally, the diphenyl groups of phosphate esters **7**, **8**, and **9** were deprotected hydrogenetically under hydrogen using PtO_2 as a catalyst to give **10**, **11**, and **12**, respectively (Scheme 1). Thus, we could obtain the target compounds.



Reagents and conditions: (a) TBSCl, DMAP, CH_2Cl_2, rt, 30 min, 99.8%; (b) $(PhO)_2P(O)Cl$, DMAP, CH_2Cl_2, rt, 4.5 h, 95.0%; (c) aq. 3 M HCl, THF, rt, 7 h, 93.6%; (d) $Me_3O{\bullet}BF_4$, CH_2Cl_2, rt, 2.5 h, 88.6%; (e) DAST, CH_2Cl_2, 0 °C ~ rt, 1.5 h, 67.9%; (f) H_2, Pd/C or $Pd(OH)_2/C$, THF, rt, 16 h, 57.2%, 47.9%, and 66.4%, respectively; (g) H_2, PtO_2, THF, rt, 4 h, 98.0%, 97.1% and 94.4%, respectively.

Scheme 1.

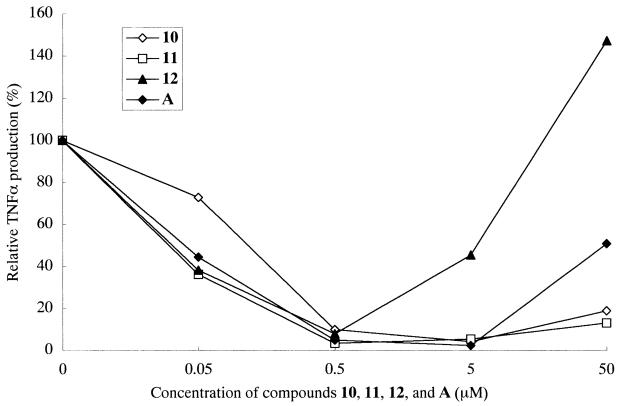


Fig. 2. Relative TNFα release from TPA-treated U937 cells stimulated by LPS (30 ng mL^{-1}) in the presence of compounds **10**, **11**, **12**, and **A**. In the measurement of compounds **10**, **11**, and **12**, TNFα produced by LPS-stimulated U937 cells was 155 pg mL^{-1}, and that was 794 pg mL^{-1} in the case of compound **A**.

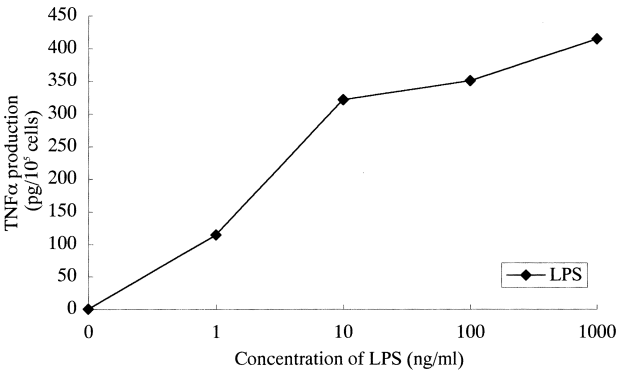


Fig. 3. TNFα-production by U937 cells stimulated with LPS.

Biological Activity. The inhibitory activity of these compounds, **10**, **11**, and **12**, on LPS-induced TNFα production was investigated in vitro using human monoblastic U937 cells, pretreated with TPA, in the absence (LPS agonism) or presence (LPS antagonism) of LPS (30 ng mL^{-1}). Compounds **10** and **11** show activity for LPS-antagonism, but compound **12** shows both LPS-antagonistic and LPS-agonistic activities, depending on the concentration. Namely, compound **12** showed the production of TNFα in the range of $\geq$ 50 μM (Figs. 2 and 3). We can not explain this phenomenon.

Experimental

^{1}H-NMR spectra (400 MHz) were recorded with a JEOL-GSX 400 spectrometer using TMS as the internal standard. IR absorption spectra were measured with an IR A-2 spectrophotometer, and mass spectra were obtained with a JMS-700 mass spectrometer. Separation of the compounds by column chromatography was done with silica-gel 60 (230–400 mesh ASTM) under a slightly elevated pressure (1.1–1.5 atm) for easy elution. The quantity of silica-gel used was 50–100 times the weight charged on the column. THF was distilled in the presence of radical anions generated by benzophenone and sodium. Dichloromethane was passed through an ICN Alumina B-Super I. DMF and pyridine were dried by storage over 4Å molecular sieves.

3,7-Anhydro-4-[(*R*)-3-benzyloxytetradecanoylamino]-8-*O*-*t*-butyldimethylsilyl-2,4-dideoxy-5-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-D-*glycero*-D-*ido*-octonic Acid Benzyl Ester (2). To a solution of **1** (61.1 mg, 0.057 mmol) in CH_2Cl_2 (0.6 mL) were added *tert*-butyldimethylsilyl chloride (18.8 mg, 0.125 mmol) and DMAP (19.1 mmol, 0.156 mmol). The mixture was stirred for 30 min and concentrated in vacuo to give a mixture, which was diluted with EtOAc. This solution was washed with H_2O and brine, dried (Na_2SO_4), filtered, and concentrated to give a residue, which was chromatographed on a silica-gel column. Elution with hexane–EtOAc (1:1) gave **2** (67.5 mg, 99.8%) as a gum. IR ($CHCl_3$) 2956, 2928, 2856, 1734, 1674, 1517, 1466 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 0.04 (3H, s), 0.05 (3H, s), 0.87 (9H, s), 0.88 (9H,

t, J = 6.7 Hz), 1.25 (56H, broad s), 1.40–1.62 (6H, m), 2.26–2.41 (5H, m), 2.50–2.63 (3H, m), 3.41 (1H, d, J = 2.2 Hz, OH), 3.55 (1H, dd, J = 5.9, 10.3 Hz), 3.70 (1H, dt, J = 1.5, 8.8 Hz), 3.76 (1H, dd, J = 4.4, 10.3 Hz), 3.80 (1H, t, J = 5.1 Hz), 4.21 (1H, m), 4.46, 4.53 (2H, AB-q, J = 11.7 Hz), 4.73 (1H, quintet, J = 5.3 Hz), 4.90 (1H, dd, J = 8.4, 10.3 Hz), 5.07 (2H, s), 5.15 (1H, m), 6.52 (1H, d, J = 7.3 Hz, NH), 7.21–7.35 (10H, m). MS (FAB) *m/z* 1178 $(M+H)^+$. HRMS (FAB, positive), calcd. for $C_{70}H_{120}NO_{11}Si$: 1178.8631; found: 1178.8639.

3,7-Anhydro-4-[(*R*)-3-benzyloxytetradecanoylamino]-8-*O*-*t*-butyldimethylsilyl-2,4-dideoxy-6-*O*-diphenylphosphono-5-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-D-*glycero*-D-*ido*-octonic Acid Benzyl Ester (3). To a solution of **2** (48.3 mg, 0.041 mmol) and $(PhO)_2P(O)Cl$ (44.0 mg, 0.164 mmol) in CH_2Cl_2 (0.5 mL) was added DMAP (20.0 mg, 0.164 mmol). The mixture was stirred for 4.5 h at room temperature, diluted with EtOAc, washed with aqueous 0.1 M (1 M = 1 mol dm^{-3}) HCl, sat. $NaHCO_3$ aq, H_2O, and brine, dried (Na_2SO_4), filtered, and concentrated to give a residue, which was chromatographed on a silica-gel column. Elution with hexane–EtOAc (3:1) gave **3** (54.9 mg, 95.0%) as a gum. IR ($CHCl_3$) 2928, 2856, 1735, 1676, 1491 cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.03 (3H, s), 0.04 (3H, s), 0.86 (9H, s), 0.91 (9H, t, J = 6.6 Hz), 1.28 (56H, broad s), 1.44–1.64 (6H, m), 2.23 (2H, t, J = 7.3–8.1 Hz), 2.31–2.33 (2H, m), 2.44–2.64 (4H, m), 3.69 (1H, dd, J = 4.4, 11.0 Hz), 3.77 (1H, dd, J = 4.4, 11.0 Hz), 3.85–3.88 (2H, m), 4.26 (1H, dt, J = 4.4, 8.1 Hz), 4.50, 4.57 (2H, AB-q, J = 11.7 Hz), 4.73 (1H, m), 4.81 (1H, m), 5.09, 5.13 (2H, AB-q, J = 12.4 Hz), 5.19 (1H, m), 5.23 (1H, t, J = 7.3–8.1 Hz), 6.53 (1H, d, J = 8.1 Hz, NH), 7.19–7.39 (20H, m). MS (FAB) *m/z* 1410 $(M+H)^+$, 1448 $(M+K)^+$ (on addition of aq KI). HRMS (FAB, positive), calcd. for $C_{82}H_{128}NO_{14}PSiK$: 1448.8479; found 1448.8480.

3,7-Anhydro-4-[(*R*)-3-benzyloxytetradecanoylamino]-2,4-dideoxy-6-*O*-diphenylphosphono-5-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-D-*glycero*-D-*ido*-octonic Acid Benzyl Ester (4). To a solution of **3** (41.4 mg, 0.029 mmol) in THF (1.0 mL) was added aqueous 3 M HCl (0.1 mL). The mixture was stirred for 7 h at room temperature, and concentrated in vacuo to give a residue, which was chromatographed on a silica-gel column. Elution with hexane–EtOAc (3:2) gave **4** (35.6 mg, 93.6%) as a gum. IR ($CHCl_3$) 3439, 2927, 2855, 1731, 1676, 1591 cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.88 (9H, t, J = 6.6 Hz), 1.24 (56H, broad s), 1.41–1.65 (6H, m), 2.21–2.30 (4H, m), 2.41–2.65 (4H, m), 3.11 (1H, broad s, OH), 3.44 (1H, m), 3.74–3.85 (3H, m), 4.25 (1H, dt, J = 4.4, 8.1 Hz), 4.47, 4.55 (2H, AB-q, J = 11.7 Hz), 4.59 (1H, m), 4.73 (1H, m), 5.06, 5.11 (2H, AB-q, J = 11.7 Hz), 5.12–5.18 (2H, m), 6.54 (1H, d, J = 8.1 Hz, NH), 7.14–7.36 (20H, m). MS (FAB) *m/z* 1296 $(M+H)^+$, 1318$(M+Na)^+$. HRMS (FAB, positive), calcd. for $C_{76}H_{115}NO_{14}P$: 1296.8057; found: 1296.8063.

3,7-Anhydro-4-[(*R*)-3-benzyloxytetradecanoylamino]-2,4-dideoxy-6-*O*-diphenylphosphono-8-*O*-methyl-5-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-D-*glycero*-D-*ido*-octonic Acid Benzyl Ester (5). To a solution of **4** (25.0 mg, 0.019 mmol) and 2,6-di-*t*-butyl-4-methylpyridine (59.8 mg, 0.291 mmol) in CH_2Cl_2 (2.0 mL) was added trimethyloxonium tetrafluoroborate (42.4 mg, 0.287 mmol). The mixture was stirred for 2.5 h under nitrogen at room temperature, and diluted with EtOAc. The solution was washed with sat. $NaHCO_3$ aq and brine, dried (Na_2SO_4), filtered, and concentrated in vacuo to give a residue, which was chromatographed on a silica-gel column. Elution with hexane–EtOAc (3:2) gave **5** (22.4 mg, 88.6%) as a gum. IR ($CHCl_3$) 2729, 2855, 1735, 1676, 1491 cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.88 (9H, t, J = 6.6–7.3 Hz), 1.24 (56H, broad s), 1.40–1.60 (6H, m), 2.20 (2H, t, J = 7.3–8.1 Hz), 2.28–2.30 (2H, m), 2.40–2.59 (4H, m), 3.20 (3H, s), 3.36 (1H, dd, J = 3.7, 11.0 Hz), 3.41 (1H, dd, J = 4.4, 11.0 Hz), 3.83 (1H, m), 3.88 (1H, m), 4.28 (1H, m), 4.46, 4.54 (2H, AB-q, J = 11.7 Hz), 4.71–4.82 (2H, m), 5.08 (2H, s), 5.12 (1H, m), 5.17 (1H, m), 6.47 (1H, d, J = 8.1 Hz, NH), 7.17–7.35 (20H, m). MS (FAB) *m/z* 1310 $(M+H)^+$; 1332 $(M+Na)^+$. HRMS (FAB, positive), calcd. for $C_{77}H_{116}NO_{14}PNa$: 1332.8027; found: 1332.8015.

3,7-Anhydro-4-[(*R*)-3-benzyloxytetradecanoylamino]-6-*O*-diphenylphosphono-8-fluoro-5-*O*-[(*R*)-3-(tetradecanoyloxy)-tetradecanoyl]-2,4,8-trideoxy-D-*glycero*-D-*ido*-octonic Acid Benzyl Ester (6). To a solution of **4** (17.5 mg, 0.014 mmol) in CH_2Cl_2 (1.0 mL) was added DAST (12.5 µL, 0.095 mmol) at 0 °C with stirring. The mixture then was stirred for 1.5 h at room temperature, and diluted with EtOAc. The solution was washed with sat. $NaHCO_3$ aq and brine, dried (Na_2SO_4), filtered, and concentrated in vacuo to give a residue, which was chromatographed on a silica-gel column. Elution with hexane–EtOAc (3:2) gave **6** (11.9 mg, 67.9%) as a gum as well as recovered starting material **4** (2.2 mg, 12.6%). IR ($CHCl_3$) 2927, 2855, 1735, 1677, 1491 cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.88 (9H, t, J = 6.6–7.1 Hz), 1.24 (56H, broad s), 1.40–1.59 (6H, m), 2.20 (2H, t, J = 7.6 Hz), 2.26–2.36 (2M, m), 2.39–2.59 (4H, m), 3.81–3.93 (2H, m), 4.20–4.45 (3H, m), 4.45, 4.55 (2H, AB-q, J = 11.7 Hz), 4.66 (2H, s), 5.10 (1H, m), 5.19 (1H, dd, J = 7.9, 9.2 Hz), 6.51 (1H, d, J = 7.2 Hz, NH), 7.16–7.35 (20H, m). MS (FAB) *m/z* 1298 $(M+H)^+$; 1320 $(M+Na)^+$. HRMS (FAB, positive), calcd. for $C_{76}H_{114}NO_{13}FP$: 1298.8014; found: 1298.8020.

3,7-Anhydro-2,4-dideoxy-6-*O*-diphenylphosphono-4-[(*R*)-3-hydroxytetradecanoylamino]-5-*O*-[(*R*)-3-(tetradecanoyloxy)-tetradecanoyl]-D-*glycero*-D-*ido*-octonic Acid (7). A solution of **4** (39.4 mg, 0.030 mmol) in THF (3 mL) was hydrogenolyzed under hydrogen for 16 h at room temperature using 10% Pd on carbon (41.1 mg) as a catalyst. The mixture was filtered through Celite, which was washed with EtOAc, CH_2Cl_2, and MeOH. The combined solution was concentrated in vacuo to give a residue, which was chromatographed on a silica-gel column. Elution with EtOAc, and then EtOAc–AcOH (99:1) gave **7** (19.4 mg, 57.2%) as a wax. IR ($CHCl_3$) 3691, 3606, 2927, 2855, 1732, 1668, 1601 cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.89 (9H, t, J = 6.5–7.1 Hz), 1.26 (56H, broad s), 1.30–1.61 (6H, m), 2.18–2.71 (8H, m), 3.62 (1H, d, J = 11.8 Hz), 3.78 (1H, m), 3.89 (1H, m), 3.98 (1H, m), 4.30 (1H, m), 4.64 (1H, m), 4.75 (1H, m), 5.12 (1H, m), 5.23 (1H, t, J = 7.8 Hz), 6.89 (1H, d, J = 6.9 Hz, NH), 7.19–7.24 (6H, m), 7.28–7.37 (4H,m). MS (FAB) *m/z* 1116 $(M+H)^+$. High Resolution MS (FAB, positive), calcd. for $C_{62}H_{103}NO_{14}P$: 1116.7112; found: 1116.7116.

3,7-Anhydro-2,4-dideoxy-6-*O*-diphenylphosphono-4-[(*R*)-3-hydroxytetradecanoylamino]-8-*O*-methyl-5-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-D-*glycero*-D-*ido*-octonic Acid (8). Compound **5** (16.0 mg, 0.012 mmol) was treated in the same manner as descibed for the formation of **7** from **4** to give **8** (6.6 mg, 47.9%) as a wax. ^1H NMR (400 MHz, $CDCl_3$) δ 0.89 (9H, t, J = 6.7–7.0 Hz), 1.28 (56H, broad s), 1.41–1.55 (6H, m), 2.18 (2H, t, J = 7.3 Hz), 2.25–2.31 (2H, m), 2.47–2.62 (3H, m), 2.76 (1H, m), 3.20 (3H, s), 3.46–3.48 (2H, m), 3.87–4.00 (2H, m), 4.30 (1H, dd, J = 5.6, 10.3 Hz), 4.53 (1H, m), 4.75 (1H, q, J = 8.4 Hz), 5.13 (1H, m), 5.39 (1H, dd, J = 8.4, 10.2 Hz), 7.20–7.44 (10H,m). MS (FAB) *m/z* 1130 $(M+H)^+$; 1152 $(M+Na)^+$. HRMS, calcd. for $C_{63}H_{104}NO_{14}PNa$: 1152.7092; found: 1152.7101.

3,7-Anhydro-6-*O*-diphenylphosphono-8-fluoro-4-[(*R*)-3-hydroxytetradecanoylamino]-5-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-2,4,8-trideoxy-D-*glycero*-D-*ido*-octonic Acid (9).

A solution of **6** (12.6 mg, 0.010 mmol) in EtOH (2 mL) containing 20% $Pd(OH)_2$ (12.2 mg) as a catalyst was hydrogenolyzed under hydrogen for 16 h at room temperature. The solution was filtered through Celite, and the filtrate was concentrated in vacuo to give a residue, which was chromatographed on a silica-gel column. Elution with CH_2Cl_2–MeOH (1:1) gave a mixture of silica-gel and **9**. This mixture was dissolved in CH_2Cl_2, and washed with aqueous 0.1 M HCl, dried (Na_2SO_4), filtered, and concentrated in vacuo to give **9** (7.2 mg, 66.4%) as a wax. ^{1}H NMR (CD_3OD) δ 0.89 (9H, t, J = 6.7–7.0 Hz), 1.28 (56H, broad s), 1.41–1.56 (6H, m), 2.17–2.29 (4H, m), 2.45 (1H, dd, J = 7.5, 16.3 Hz), 2.54 (1H, dd, J = 4.9, 16.3 Hz), 2.67 (1H, dd, J = 3.9, 15.1 Hz), 2.83 (1H, dd, J = 9.0, 15.1 Hz), 3.90–4.05 (2H, m), 4.34–4.39 (2H, m), 4.49 (1H, m), 5.42 (1H, dd, J = 8.5, 10.4 Hz), 7.14–7.47 (10H, m). MS (FAB) *m/z* 1118 $(M+H)^+$; 1140 $(M+Na)^+$. HRMS (FAB, positive), calcd. for $C_{62}H_{102}NO_{13}FP$: 1118.7073; found: 1118.7081.

3,7-Anhydro-2,4-dideoxy-4-[(*R*)-3-hydroxytetradecanoylamino]-6-*O*-phosphono-5-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-D-*glycero*-D-*ido*-octonic Acid (10). A solution of **7** (20.3 mg, 0.018 mmol) in THF (2.0 mL) was hydrogenolyzed under hydrogen using PtO_2 (15.5 mg) as a catalyst at room temperature for 4 h, filtered through Celite, and concentrated to give a product, which was dissolved in $CHCl_3$. The solution was washed with aqueous 0.1 M HCl, dried (Na_2SO_4), filtered, and concentrated in vacuo to give **10** (17.1 mg, 98.0%) as a powder. IR ($CHCl_3$) 3361 (broad), 2957, 2922, 2852, 1729, 1652, 1542, 1467 cm^{-1}. ^{1}H NMR (CD_3OD) δ 0.90 (9H, t, J = 6.6–7.3 Hz), 1.29 (56H, broad s), 1.45 (2H, broad s), 1.60 (4H, broad s), 2.25–2.37 (4H, m), 2.56–2.77 (4H, m), 3.64 (1H, dd, J = 2.2, 12.5 Hz), 3.78 (1H, m), 3.94–4.00 (2H, m), 4.17–4.27 (2H, m), 4.54 (1H, m), 5.16–5.22 (2H, m). MS (FAB) *m/z* 962 $(M-H)^-$. HRMS (FAB, negative), calcd. for $C_{50}H_{93}NO_{14}P$: 962.6334; found: 962.6349.

3,7-Anhydro-2,4-dideoxy-4-[(*R*)-3-hydroxytetradecanoylamino]-6-*O*-phosphono-8-*O*-methyl-5-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-D-*glycero*-D-*ido*-octonic Acid (11). Compound **8** (5.0 mg, 0.004 mmol) was treated as described above to give **11** (4.2 mg, 97.1%) as a powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.90 (9H, t, J = 6.6–7.3 Hz), 1.29 (56H, broad s), 1.45 (2H, bs), 1.58–1.64 (4H, m), 2.28–2.40 (4H, m), 2.47–2.75 (4H, m), 3.38 (3H, s), 3.70–3.78 (2H, m), 3.93 (1H, m), 4.06 (1H, m), 4.13 (1H, m), 4.21 (1H, dd, J = 4.4, 7.3 Hz), 4.51 (1H, quintet, J = 4.4 Hz), 5.13 (1H, dd, J = 6.6, 7.3 Hz), 5.22 (1H, m). MS (FAB) *m/z* 978 $(M+H)^+$; 1000 $(M+Na)^+$. HR MS (FAB, positive), calcd. for $C_{51}H_{96}NO_{14}PNa$: 1000.6466; found: 1000.6470.

3,7-Anhydro-8-fluoro-4-[(*R*)-3-hydroxytetradecanoylamino]-6-*O*-phosphono-5-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-2,4,8-trideoxy-D-*glycero*-D-*ido*-octonic Acid (12). Compound **9** (3.8 mg, 0.003 mmol) was treated as described above to give **12** (3.1 mg, 94.4%) as a powder. ^{1}H NMR (CD_3OD) δ 0.89 (9H, t, J = 6.8–7.0 Hz), 1.28 (56H, broad s), 1.45–1.66 (6H, m), 2.28–2.36 (4H, m), 2.55 (1H, dd, J = 4.1, 16.0 Hz), 2.62–2.74 (3H, m), 3.93 (1H, m), 4.12 (1H, m), 4.15 (1H, m), 4.22 (1H, m), 4.53 (1H, m), 4.56–4.78 (2H, m), 5.13 (1H, m), 5.20 (1H, m). MS (FAB) *m/z* 966 $(M+H)^+$; 988 $(M+Na)^+$. HRMS (FAB, positive), calcd. for $C_{50}H_{94}NO_{13}FP$: 966.6447; found: 966.6473.

Preparation of Aqueous Solutions of Compounds for Measurement of the Biological Activity. Compound **10** (3.1 mg), obtained as mentioned above, was dissolved in $CHCl_3$ (1 mL), MeOH (2 mL), and 0.1 M HCl (0.8 mL) with stirring at 5–10 °C. Additional $CHCl_3$ (1 mL) and 0.1 M HCl (1 mL) were added to this solution to ensure separation into two phases. The lower chloroform phase was collected, and concentrated to give 3.0 mg of **10**, which was dissolved in aqueous 0.1% Et_3N (v/v) solution for a measurement of the biological activity. Aqueous 0.1% Et_3N solutions of compounds **11** and **12** were prepared as described above in the preparation of a solution of compound **10**.

Method for Biological Activity Measurement. The sources of the materials used in the study were as follows: Lipopolysaccharide (LPS) from *E. coli* serotype 026:B6, 12-*O*-tetradecanoylphorbor acetate (TPA) and prednisolone were from SIGMA, St. Louis, MO. PRMI-6140 medium, fetal bovine serum (FBS), and newborn calf serum (NBCS) were from GIBCO, Grand Island, NY. The human tumor necrosis factor-α enzyme-linked immunosorbent assay (TNFα ELISA) kit was from Genzyme, Cambridge, MA.

Cell culture: Human monoblastic U937 cells were maintained in a RPMI-1640 medium supplemented with 10% FBS, 100 units mL^{-1} of penicillin and 100 μg mL^{-1} of streptomycin (growth medium).

Production of TNFα by U937 cells: U937 cells (1×10^4/200 μL/well) were plated in 96-well plates (Corning, Cambridge, MA), and cultured in the presence of TPA (30 ng/mL) for 72 h at 37 °C. After removing of the supernatant, the cells were incubated in 200 μl of fresh RPMI-1640 medium containing 10% of NBCS, 30 ng/mL of LPS and graded concentrations of compounds in a humidified atmosphere of 5% of CO_2 for 4.5 h at 37 °C. After incubation, the amounts of TNFα produced in the culture supernatants were determined by the TNFα ELISA kits. As a control, the amount of TNFα produced by U937 cells, which were stimulated with 30 ng mL^{-1} of LPS in the absence of compounds (Fig. 3),[14] was used. Since, TNFα produced by LPS-stimulated U937 cells was assessed by bioassay, the amount of TNFα produced varied in each experiment by 100–1000 pg mL^{-1}. All experiments were carried out at least twice.

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