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Synthesis of ceramidated GLA-60 derivatives

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Abstract

Ceramidated GLA-60 derivatives **11** and **11′** were synthesized from **1** via glycosidation of ceramide derivative **12** as a glycosyl acceptor with GLA-60 derivative **5** as a glycosyl donor, and successive conversion. Compound **11′** showed only weak LPS-antagonistic activity without showing any LPS-agonistic activity.

Keywords: Ceramidated GLA-60 derivatives; Glycosidation; Glycosphingolipids; LPS-antagonist

1. Introduction

Glycosphingolipids,[1] a major class of glycoconjugates synthesized by eukaryotic cells, are ubiquitous constituents of cell membranes, and are also blood group antigens or immunologically relevant tumor-associated oligosaccharides. They consist of two distinct components: an extracellular hydrophilic glycan and a membrane located hydrophobic ceramide. On the other hand, GLA-60,[2] a nonreducing component derivative of Lipid A, still shows LPS-agonistic activity such as that of Lipid A itself. We have been interested in the synthesis as well as the biological activity of combined compounds of the ceramide and GLA-60 derivative (Fig. 1). Herein, we report the synthesis of two ceramidated GLA-60 compounds, **11**, which has five long chains in the molecule, and **11′**, which has four long chains. The biological activity of **11′** toward human U937 cells is also reported.

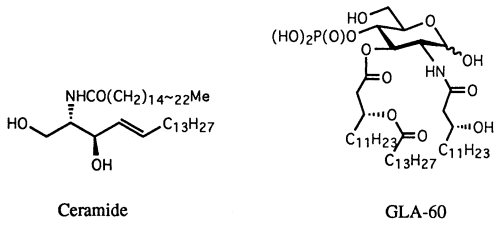

Fig. 1. Structure of ceramide and GLA-60.

2. Results and discussion

Synthesis.—According to retrosynthetic analysis, in the final deprotection step of the phosphate ester of **9**, using procedures such as saponification, acidic hydrolysis, and catalytic hydrogenolysis[3] would have been unsuitable because compound **9** contains easily saponified esters, an acid-labile glycosyl bond and an easily hydrogenated double bond in the molecule, respectively. Therefore, it was necessary to adopt a mild cleavage of diallyl phosphate **9** using tetrakis-(triphenylphosphine)palladium(0) as a catalyst and triphenylphosphine–Et_3N–HCOOH.[4] Using this method, compound **9** could be derived in a few steps from β-oriented ceramide **6**, which was obtained by glycosidation of alcohol **12** with imidate **5**. Imidate **5**, with the amino group protected by the *N*-trichloroethoxycarbonyl group, could be obtained from acetonide **1**. Therefore, we chose allyl *N*-Troc-protected glucosamine derivative **1** as the ideal starting material (Schemes 1 and 2).

Treatment of acetonide **1**[3] with a catalytic amount of *p*-toluenesulfonic acid in methanol, and successive treatment with *tert*-butylchlorodiphenylsilane (TBDPSCl) using imidazole as a base afforded C-6 silyl ether

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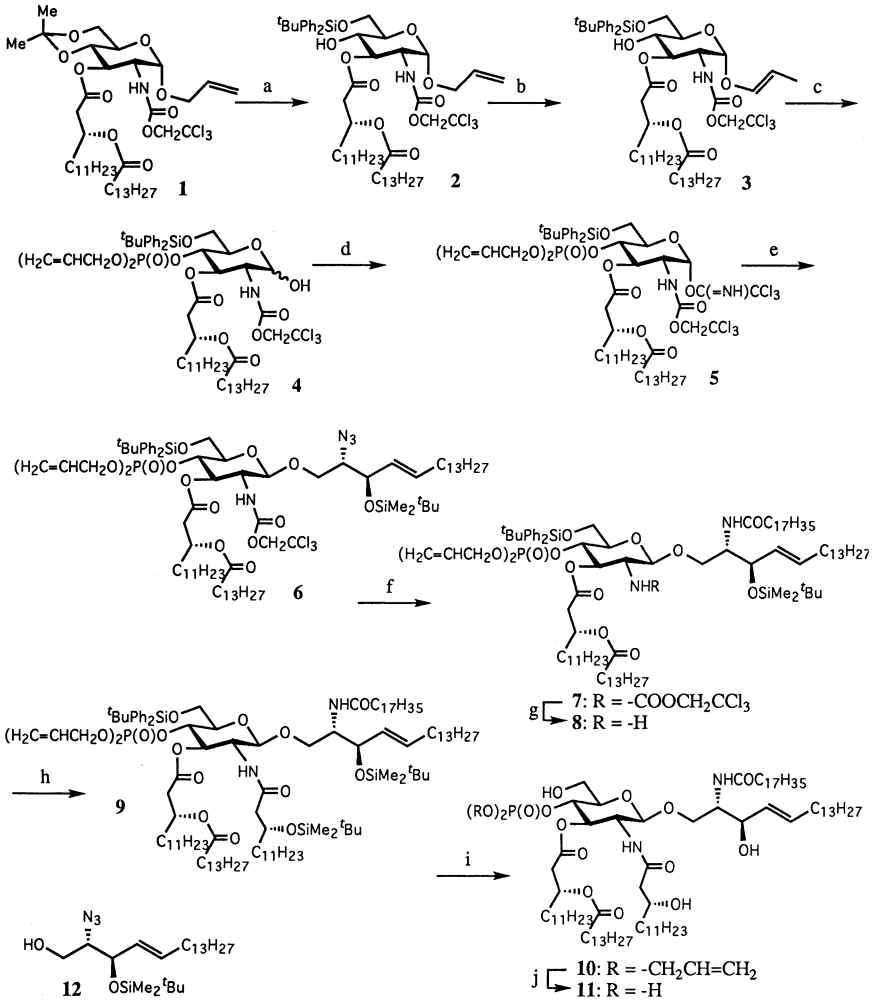
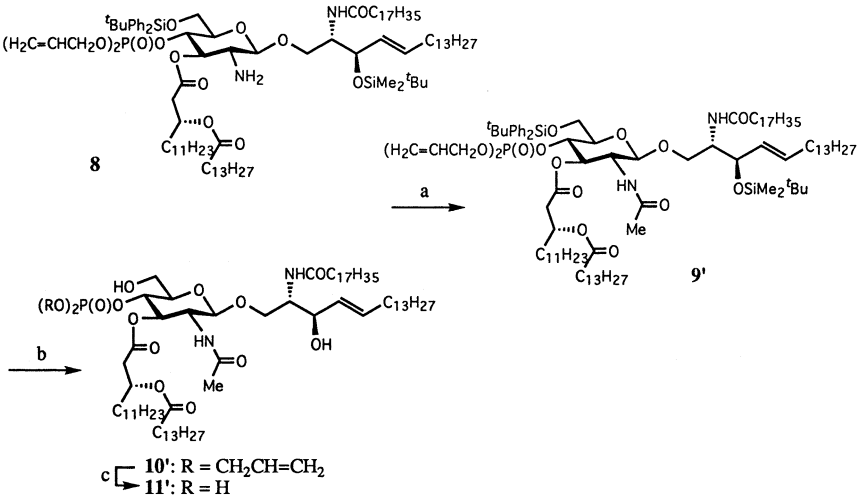


Scheme 1. Reactions and conditions: (a) (1) *p*-TsOH, 2:1 MeOH–THF, rt, 7 h; (2) TBDPSCl, imidazole, DMF, rt, 17 h, two steps 88%; (b) $[Ir(C_8H_{12})(PMePh_2)_2]PF_6$, THF, 40 °C, 1.5 h, 89%; (c) (1) $(H_2C{=}CHCH_2O)_2PN^iPr_2$, 1*H*-tetrazole, THF, rt, 0.5 h, then 30% aq H_2O_2; 0 °C, 0.5 h, and rt, 0.5 h; (2) I_2, H_2O–THF, rt, 2 h, two steps 59%; (d) Cl_3CCN, DBU, CH_2Cl_2, rt, 2 h, 95%; (e) **12**, 4 Å MS, CH_2Cl_2, rt, 2h, then $BF_3{\cdot}OEt_2$, −23 °C, 4 h, 87%; (f) PPh_3, benzene, 60 °C, 7 h, and H_2O, 60 °C, 17 h, then $C_{17}H_{35}COCl$, Et_3N, DMAP, CH_2Cl_2, rt, 2 h, 87%; (g) Zn, sat aq NH_4Cl, DMF, 24 h, rt, 60%; (h) (*R*)-3-(*t*-butyldimethylsilyloxy)tetradecanoic acid, DMAP, DCC, CH_2Cl_2, 50 °C, 24 h, 60%; (i) HF·pyridine, THF, rt, 24 h, 54%; (j) $(Ph_3P)_4Pd$, Ph_3P, Et_3N, HCOOH, THF, 50 °C, 3 h, 78%.

2. Before introduction of the diallyl phosphate group at the C-4 position to differentiate the anomeric allyl group of **2**, the anomeric allyl group was transformed with (1,5-cyclooctadiene)bis(methyldiphenylphosphine)-iridium(I) hexafluorophosphate[5] to give vinylic *E*-olefin **3**. In this reaction, there was no detection of the *Z*-olefin. Treatment of **3** with diallyl diisopropylphosphoramidite and 1*H*-tetrazole in tetrahydrofuran (THF), and successive oxidation of the resulting phosphite with H_2O_2 gave the corresponding phosphate. The anomeric propenyl group of diallylphosphate was deprotected with iodine–water to give compound **4**, and successive treatment of the anomeric hydroxyl group with trichloroacetonitrile using 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) as a base gave exclusively the α-imidate **5**. The configuration of **5** was estimated from the coupling constant (*J* 3.6 Hz) between H-1 and H-2.

Ceramidation of **5** with azide-alcohol **12** using boron trifluoride diethyl etherate as a catalyst gave β-ceramidated glucosamine derivative **6** without formation of the α-anomer. The azide group of **6** was reduced with Ph_3P–water, and the resulting amine was treated with octadecanoyl chloride using 4-dimethylaminopyridine (DMAP) as a base to give amide **7**. The trichloroethoxycarbonyl group of **7** was removed under Zn in satd aq NH_4Cl and *N*,*N*-dimethylformamide (DMF) to give amine **8**. Acylation of **8** with (*R*)-3-(*tert*-butyldimethylsilyloxy)tetradecanoic acid and DMAP using 1,3-dicyclohexylcarbodiimide (DCC) yielded amide **9**, which has the all functional groups of the ceramidated GLA-60.



Scheme 2. Reactions and conditions: (a) AcCl, Et_3N, DMAP, CH_2Cl_2, 24 °C, 3 h, 85%; (b) HF·pyridine, THF, rt, 24 h; (c) $(Ph_3P)_4Pd$, Ph_3P, Et_3N, HCOOH, THF, 50 °C, 3 h, 96%.

Desilylation of two *tert*-butyldimethylsilyl (TBDMS) and one TBDPS group from **9** with HF·pyridine gave triol **10**. Finally, treatment of **10** with Ph_3P, Et_3N, HCOOH and a catalytic amount of $(Ph_3P)_4Pd$ in THF at 50 °C yielded deallylated phosphate **11** as a white wax.

Acetylation of **8** with acetyl chloride using triethylamine and DMAP as bases gave acetamide **9′**. Desilylation of two TBDMS and one TBDPS group from **9′** with HF·pyridine as mentioned above for the preparation of **10** from **9** gave triol **10′**. Finally, the same treatment of **10′** from **10** to **11** yielded deallylated phosphate **11′** as a white powder.

Thus, we were able to synthesize the title compounds **11** and **11′**.

Biological activity.—The inhibitory activities of the compounds **11** and **11′** on LPS-induced TNFα production were investigated in vitro, using human monoblastic U937 cells. However, compound **11** was insoluble in water containing 0.1% Et_3N even under sonication. Therefore, we failed to evaluate the biological activity of **11**. On the other hand, compound **11′** was soluble under the same conditions. The concentration of compound **11′** required to inhibit the LPS-induced TNFα production by U937 cells by 50% (IC_{50}) was 4.7 μM. (The amount of TNFα produced by the U937 cells, which were stimulated with 10 ng/mL of LPS in the absence of **11′**, was used as a control.) Compound **11′** did not show any LPS-agonistic activity on the TNFα production.

3. Experimental

General.—Infrared (IR) spectra were recorded on a Jasco IRA-2 spectrophotometer. High-resolution FAB mass spectra were obtained with a JMS-O1SG mass spectrometer. ^1H NMR spectra were recorded on JEOL EX-400 MHz and EX-270 MHz instruments with tetramethylsilane as an internal reference. Separation of the compounds by column chromatography was carried out with Silica Gel 60 (E. Merck, 230–400 mesh ASTM).

*Allyl 6-O-(*tert*-butyldiphenylsilyl)-2-deoxy-3-O-[(R)-3-(tetradecanoyloxy)tetradecanoyl]-2-[(2,2,2-trichloroethoxycarbonyl)amino]-α-*D*-glucopyranoside* (**2**).—To a solution of 4,6-*O*-acetonide **1** (9.12 g, 10.5 mmol) in MeOH (10 mL) and THF (5 mL) was added *p*-TsOH (27 mg, 0.1 mmol). After stirring for 7 h at rt, Et_3N (2 mL) was added to this reaction mixture, and the solvents were evaporated under reduced pressure. The crude reaction mixture was dissolved in DMF (25 mL) to which were added imidazole (2.1 g, 31.5 mmol) and *tert*-butylchlorodiphenylsilane (2.7 mL, 10.5 mmol). After stirring for 17 h at rt, the reaction mixture was treated with satd $NaHCO_3$ (5 mL) and poured into water and extracted with ether. The organic layer was washed with brine and dried over $MgSO_4$ and concentrated in vacuo to give a crude mixture that was purified by silica-gel column chromatography. Elution with 1:9 EtOAc–hexane mixture afforded a silyl ether **2** (9.46 g, 88%) as a colorless oil. IR ν_{max} ($CHCl_3$): 3502, 3431, 1742, 1515, 1465 cm^{-1}. ^1H NMR (270 MHz, $CDCl_3$): δ 0.88 (t, 6 H, *J* 7.2 Hz), 1.06 (s, 9 H), 1.15–1.40 (m, 38 H), 1.50–1.68 (m, 2 H), 2.27 (t, 2 H, *J* 7.2 Hz), 2.50 (dd, 1 H, *J* 15.1, 5.5 Hz), 2.60 (dd, 2 H, *J* 15.1, 8.2 Hz), 3.16 (d, 1 H, *J* 2.8 Hz), 3.65–3.81 (m, 2 H), 3.82–34.02 (m, 3 H), 4.16 (dd, 1 H, *J* 11.9, 5.5 Hz), 4.68 (d, 1 H, *J* 11.9 Hz), 4.76 (d, 1 H, *J* 11.9 Hz), 4.90 (d, 1 H, *J* 4.9 Hz), 5.08–5.32 (m, 5 H), 5.41 (d, 1 H, *J* 9.8 Hz), 5.87 (ddt, 2 H, *J* 18.1, 11.0, 5.5 Hz), 7.33–7.48 (m, 6 H), 7.62–7.76 (m, 4 H). FABMS (positive-ion) *m/z*: 1090 $[M+Na]^+$ (on addition of

NaI). HRFABMS (positive-ion): Calcd for $C_{56}H_{88}Cl_3NO_{10}SiNa$, 1090.5141; found, 1090.5110.

*(*E*)-1-Propenyl 6-*O*-(*tert*-butyldiphenylsilyl)-2-deoxy-3-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-2-[(2,2,2-trichloroethoxycarbonyl)amino]-α-*D*-glucopyranoside* (**3**).—To a solution of **2** (0.19 g, 0.17 mmol) in THF (0.5 mL) was added $[(cod)Ir(PMePh_2)_2]PF_6$ (1.7 mg, 0.002 mmol). The air in the flask was replaced with hydrogen to activate the Ir complex. After stirring for ca. 3 min, when the red color of the solution had become a pale yellow, the hydrogen was completely replaced with nitrogen. This solution was stirred for 1.5 h at 40 °C. After confirming a double bond shift to an enol ether by ^{1}H NMR spectroscopy, the reaction mixture was treated with water (0.5 mL). After extraction with ether, the organic layer was washed with brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo to give a residue that was chromatographed on a silica gel column. Elution with 1:9 EtOAc–hexane afforded trans enol ether **3** (0.17 g, 89%). $[\alpha]_D^{24}$ +29.9° (*c* 0.95, $CHCl_3$). IR ν_{max} ($CHCl_3$): 3483, 3439, 3348, 1741, 1518, 1465, 1428 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.88 (t, 6 H, *J* 7.3 Hz), 1.06 (s, 9 H), 1.20–1.34 (m, 42 H), 1.52 (dd, 3 H, *J* 1.5, 7.3 Hz), 2.28 (t, 2 H, *J* 8.1 Hz), 2.51 (dd, 1 H, *J* 4.4, 14.6 Hz), 2.59 (dd, 1 H, *J* 7.3, 14.6 Hz), 3.16 (d, 1 H, *J* 2.9 Hz), 3.66–3.88 (m, 4 H), 4.64 (d, 1 H, *J* 11.7 Hz), 4.77 (d, 1 H, *J* 11.7 Hz), 5.08 (d, 1 H, *J* 3.7 Hz), 5.10–5.20 (m, 3 H), 5.43 (d, 1 H, *J* 9.5 Hz), 6.13 (dd, 1 H, *J* 1.5, 12.5 Hz), 7.35–7.46 (m, 6 H), 7.66–7.73 (m, 4 H). FABMS (positive-ion) *m*/*z*: 1090 $[M+Na]^+$ (on addition of NaI). HRFABMS (positive-ion): Calcd for $C_{56}H_{88}Cl_3NO_{10}SiNa$, 1090.5141; found, 1090.5138.

*6-*O*-(*tert*-Butyldiphenylsilyl)-2-deoxy-4-*O*-(diallylphosphono)-3-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-2-[(2,2,2-trichloroethoxycarbonyl)amino]-α-*D*-glucopyranose* (**4**).—(i) To a solution of **3** (110 mg, 0.11 mmol) in THF (0.3 mL) were added diallyl diisopropylphosphoramidite (43.1 mL, 0.16 mmol) and 1*H*-tetrazole (15.4 g, 0.22 mmol). After stirring for 0.5 h at rt, the reaction mixture was cooled to 0 °C, treated with 30% aq H_2O_2 (0.1 mL), and allowed to slowly warm to rt. After stirring for 0.5 h at rt, the reaction mixture was treated with satd aq $Na_2S_2O_3$ solution (0.5 mL) and extracted with ether. The organic layer was washed with brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo to give a residue, which was employed for the next reaction without purification. (ii) This residue was dissolved in THF (0.5 mL) and water (0.2 mL), and to this solution was added I_2 (56 mg, 0.22 mmol). After stirring for 2 h at rt, the reaction mixture was treated with satd aq $Na_2S_2O_3$ (0.5 mL) and extracted with ether. The organic layer was washed with brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo to give a mixture that was chromatographed on a silica gel column. Elution with 1:9 then 1:4 EtOAc–hexane afforded **4** (75.2 mg, two steps 59%) as a colorless gum. IR ν_{max} ($CHCl_3$): 3434, 3327, 1746, 1516, 1465, 1428 cm^{-1}. ^{1}H NMR (270 MHz, $CDCl_3$): δ 0.88 (t, 6 H, *J* 7.7 Hz), 1.07 (s, 9 H), 1.10–1.37 (m, 40 H), 1.48–1.65 (m, 4 H), 2.23 (t, 2 H, *J* 7.7 Hz), 2.57 (dd, 1 H, *J* 16.4, 4.1 Hz), 2.69 (dd, 1 H, *J* 16.4, 7.4 Hz), 2.90 (br, 1 H), 3.80–4.10 (m, 3 H), 4.25–4.50 (m, 4 H), 4.64 (d, 1 H, *J* 12.6 Hz), 4.77 (d, 1 H, *J* 12.6 Hz), 5.10–5.43 (m, 7 H), 5.54 (d, 1 H, *J* 7.1 Hz), 5.68–5.95 (m, 2 H), 7.32–7.47 (m, 6 H), 7.63–7.75 (m, 4 H). FABMS (positive-ion) *m*/*z*: 1210 $[M+Na]^+$ (on addition of NaI). HRFABMS (positive-ion): Calcd for $C_{59}H_{93}Cl_3NO_{13}PSiNa$, 1210.5117; found, 1210.5144.

*6-*O*-(*tert*-Butyldiphenylsilyl)-2-deoxy-4-*O*-(diallylphosphono)-3-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-2-[(2,2,2-trichloroethoxycarbonyl)amino]-α-*D*-glucopyranosyl trichloroacetimidate* (**5**).—A solution of **4** (1.15 g, 0.98 mmol) and trichloroacetonitrile (0.20 mL, 1.95 mmol) in CH_2Cl_2 (3 mL) was stirred for 2 h at rt in the presence of DBU (14.9 mL, 0.10 mmol). The reaction mixture was diluted with ether, washed with water and brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo to give a residue that was chromatographed on a silica gel column. Elution with 3:17 EtOAc–hexane yielded imidate **5** (1.22 g, 95%) as a gum. IR ν_{max} ($CHCl_3$): 3431, 3346, 1749, 1677, 1516, 1465, 1428 cm^{-1}. ^{1}H NMR (270 MHz, $CDCl_3$): δ 0.89(t, 6 H, *J* 7.9 Hz), 1.06 (s, 9 H), 1.12–1.40 (m, 38 H), 1.48–1.67 (m, 4 H), 2.25 (t, 2 H, *J* 7.9 Hz), 2.63 (dd, 1 H, *J* 15.8 Hz, 6.3 Hz), 2.72 (dd, 1 H, *J* 15.8 Hz, 7.6 Hz), 3.83–4.21 (m, 5 H), 4.24–4.50 (m, 5 H), 4.62 (d, 1 H, *J* 12.8 Hz), 4.82 (d, 1 H, 12.8 Hz), 5.10–5.47 (m, 5 H), 5.55 (d, 1 H, *J* 9.2 Hz), 5.66–5.95 (m, 2 H), 6.52 (d, 1 H, *J* 3.6 Hz), 7.30–7.45 (m, 6 H), 7.63–7.75 (m, 4 H), 8.74 (s, 1 H). FABMS (positive-ion) *m*/*z*: 1369 $[M+K]^+$ (on addition of KI). HRFABMS (positive-ion): Calcd for $C_{61}H_{93}Cl_6N_2O_{13}PSiK$, 1369.3953; found, 1369.3949.

*(2*S*,3*R*,4*E*)-2-Azido-3-(tert-butyldimethylsilyloxy)-octadec-4-enyl 6-*O*-(tert-butyldiphenylsilyl)-2-deoxy-4-*O*-(diallylphosphono)-3-*O*-[(*R*)-3-(tetradecanoyloxy)-tetradecanoyl]-2-[(2,2,2-trichloroethoxycarbonyl)amino]-β-*D*-glucopyranoside* (**6**).—To a solution of **5** (0.62 g, 0.47 mmol) and (2*S*,3*R*,4*E*)-2-azido-3-(*tert*-butyldimethylsilyloxy)octadec-4-en-1-ol (**12**, 0.22 g, 0.52 mmol) in CH_2Cl_2 (2 mL) were added powdered 4 Å molecular sieves (0.1 g). After stirring for 2 h at rt, the solution was cooled to −23 °C, and to this solution was added $BF_3{\cdot}OEt_2$ (6.1 μL, 0.05 mmol). After the mixture was stirred for 4 h, the disappearance of the starting material was confirmed by silica gel TLC. The reaction mixture was quenched by satd aq $NaHCO_3$ (0.5 mL), and was filtered through Celite. After extraction of the filtrate with ether, the organic layer was washed with brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo to give a residue that

was chromatographed on a silica gel column. Elution with 1:9 EtOAc–hexane gave β-oriented ceramide **6** (0.65 g, 87%) as a colorless oil. IR ν_{max} (neat): 3350, 2100, 1749, 1538, 1465 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 0.03 (s, 3 H), 0.06 (s, 3 H), 0.88 (t, 9 H, *J* 6.3 Hz), 1.06 (s, 18 H), 1.18–1.32 (m, 68 H), 2.01 (q, 2 H, *J* 6.9 Hz), 2.28 (t, 2 H, *J* 7.6 Hz), 2.58 (dd, 1 H, *J* 15.3, 8.2 Hz), 2.65 (dd, 1 H, 1 H, *J* 14.8, 4.3 Hz), 3.48–3.60 (m, 3 H), 3.85–4.00 (m, 3 H), 4.14–4.19 (m, 1 H), 4.24–4.30 (m, 1 H), 4.32–4.40 (m, 3 H), 4.60 (d, 1 H, *J* 12.4 Hz), 4.81 (d, 1 H, *J* 12.4 Hz), 4.87 (d, 1 H, *J* 8.6 Hz), 5.13 (dd, 1 H, *J* 10.8 Hz, 1.4 Hz), 5.19 (dd, 1 H, *J* 16.6, 1.8 Hz), 5.21 (dd, 1 H, *J* 10.8, 1.4 Hz), 5.29 (dd, 1 H, *J* 17.2, 1.6 Hz), 5.40 (dd, 1 H, *J* 15.6, 7.7 Hz), 5.55 (d, 1 H, *J* 8.4 Hz), 5.59–5.75 (m, 2 H), 5.84 (ddt, 1 H, *J* 16.6, 10.8, 5.2 Hz), 7.34–7.44 (m, 6 H), 7.67–7.75 (m, 4 H). FABMS (positive-ion) *m*/*z*: 1647 $[M + K]^+$ (on addition of KI). HRFABMS (positive-ion): Calcd for $C_{83}H_{140}Cl_3N_4O_{14}PSi_2K$, 1647.8345; found, 1647.8369.

*(2*S*,3*R*,4*E*)-3-(*tert-*Butyldimethylsilyloxy)-2-(octadecanoylamino)octadec-4-enyl 6-*O*-(*tert*-butyldiphenylsilyl)-2-deoxy-4-*O*-(diallylphosphono)-3-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-2-[(2,2,2-trichloroethoxycarbonyl)amino]-β-*D*-glucopyranoside* (**7**).—To a solution of **6** (0.28 g, 0.18 mmol) in benzene (1.0 mL) was added PPh_3 (92.1 mg, 0.35 mmol), and the mixture was stirred for 7 h at 60 °C. Then, water (0.1 mL) was added, and this mixture was stirred for 17 h at 60 °C, and concentrated in vacuo to give a residue, which was dissolved in CH_2Cl_2 (5 mL). To this solution were added DMAP (2.4 mg, 0.02 mmol), Et_3N (74.4 mL, 0.53 mmol), and octadecanoyl chloride (63.6 mg, 0.21 mmol). After stirring for 1 h at rt, the reaction mixture was treated with satd aq $NaHCO_3$ (0.2 mL). After extraction with ether, the organic layer was washed with brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo to give a residue that was chromatographed on a silica gel column. Elution with 2:23, then 1:4 EtOAc–hexane gave amide **7** (0.28 g, 87%) as a gum. IR ν_{max} ($CHCl_3$): 3294, 1740, 1660, 1539, 1460 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 0.01 (s, 3 H), 0.04 (s, 3 H), 0.88 (t, 12 H, *J* 6.4 Hz), 1.07 (s, 18 H), 1.14–1.38 (m, 91 H), 1.50–1.70 (m, 6 H), 1.92 (q, 2 H, *J* 6.9 Hz), 2.09 (dd, 1 H, *J* 15.8, 7.8 Hz), 2.24 (dd, 1 H, *J* 15.8, 7.3 Hz), 2.28 (t, 2 H, *J* 7.3 Hz), 2.62 (d, 2 H, *J* 6.0 Hz), 3.46–3.56 (m, 2 H), 3.70 (dd, 1 H, *J* 11.0, 4.2 Hz), 3.90 (dd, 1 H, *J* 11.6, 5.3 Hz), 3.93–4.03 (m, 2 H), 4.07–4.18 (m, 2 H), 4.21–4.29 (m, 1 H), 4.31–4.41 (m, 2 H), 4.62 (d, 1 H, *J* 11.5 Hz), 4.78 (d, 1 H, *J* 11.5 Hz), 4.81 (d, 1 H, *J* 7.3 Hz), 5.12 (dd, 1 H, *J* 10.7, 1.3 Hz), 5.15–5.35 (m, 6 H), 5.58 (dt, 1 H, *J* 15.4, 6.3 Hz), 5.65–5.78 (m, 2 H), 5.84 (ddt, 1 H, *J* 15.4, 10.7, 5.8 Hz), 7.32–7.45 (m, 6 H), 7.70–7.75 (m, 4 H). FABMS (positive-ion) *m*/*z*: 1872 $[M + Na]^+$ (on addition of NaI). HRFABMS (positive-ion): Calcd for $C_{101}H_{176}Cl_3N_2O_{15}PSi_2Na$, 1872.1310; found, 1872.1379.

*(2*S*,3*R*,4*E*)-3-(*tert-*Butyldimethylsilyloxy)-2-(octadecanoylamino)octadec-4-enyl 2-amino-2-deoxy-4-*O*-(diallylphosphono)-6-*O*-(tert-butyldiphenylsilyl)-3-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-2-[(2,2,2-trichloroethoxycarbonyl)amino]-β-*D*-glucopyranoside* (**8**).—To a solution of **7** (0.19 g, 0.10 mmol) in DMF (1.5 mL) was added satd NH_4Cl (0.2 mL) and zinc dust (0.13 g, 0.002 atom). After stirring for 1 day at rt, the reaction mixture was treated with satd aq potassium sodium tartrate (0.2 mL) and extracted with Et_2O. The organic layer was washed with brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo to give a residue that was chromatographed on a silica gel column. Elution with 1:9 then 1:4 EtOAc–hexane gave amine **8** (99.2 mg, 60%) as colorless oil. IR ν_{max} (neat): 3318, 1738, 1654, 1540, 1465, 1275, 1258 cm^{-1}. ^1H NMR (270 MHz, $CDCl_3$): δ 0.01 (s, 3 H), 0.04 (s, 3 H), 0.88 (t, 12 H, *J* 6.4 Hz), 1.06 (s, 18 H), 1.16–1.36 (m, 98 H), 1.50–1.66 (m, 6 H), 1.96 (q, 2 H, *J* 7.2 Hz), 2.09 (dd, 1 H, *J* 14.7, 8.1 Hz), 2.16 (dd, 1 H, *J* 14.7, 8.0 Hz), 2.26 (t, 2 H, *J* 7.7 Hz), 2.59 (dd, 1 H, *J* 10.2, 8.1 Hz), 2.72 (dd, 1 H, *J* 14.7, 4.2 Hz), 2.85 (dd, 1 H, *J* 10.2, 8.1 Hz), 3.45–3.52 (m, 1 H), 3.64 (d, 1 H, *J* 7.5 Hz), 3.86 (dd, 1 H, *J* 11.0, 5.6 Hz), 3.99 (d, 1 H, *J* 11.0 Hz), 4.07–4.40 (m, 8 H), 4.99 (t, 1 H, *J* 9.9 Hz), 5.10 (dd, 1 H, *J* 10.3, 1.4 Hz), 5.29 (dd, 1 H, *J* 16.9, 1.4 Hz), 5.38 (dd, 1 H, *J* 14.8, 5.8 Hz), 6.12 (d, 1 H, *J* 8.7 Hz), 7.35–7.43 (m, 6 H), 7.67–7.75 (m, 4 H). FABMS (positive-ion) *m*/*z*: 1676 $[M + H]^+$. HRFABMS (positive-ion): Calcd for $C_{98}H_{176}N_2O_{13}PSi_2$, 1676.2449; found, 1676.2445.

*(2*S*,3*R*,4*E*)-3-(*tert-*Butyldimethylsilyloxy)-2-(octadecanoylamino)octadec-4-enyl 2-[(*R*)-3-(tert-butyldimethylsilyloxy)tetradecanoylamino]-6-*O*-(tert-butyldiphenylsilyl)-2-deoxy-4-*O*-(diallylphosphono)-3-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-β-*D*-glucopyranoside* (**9**).—To a solution of **8** (83.0 mg, 0.05 mmol) in CH_2Cl_2 (0.2 mL) were added (*R*)-3-(*tert*-butyldimethylsilyloxy)tetradecanoic acid (26.9 mg, 0.075 mmol), DMAP (9.2 mg, 0.075 mmol), and DCC (15.5 mg, 0.075 mmol). The mixture was stirred for 1 day at 50 °C and treated with satd aq $NaHCO_3$ (0.2 mL). The mixture was extracted with Et_2O, and the organic layer was washed with brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo to give a residue that was chromatographed on a silica gel column. Elution with 7:93 then 3:22 EtOAc–hexane afforded amide **9** (60.3 mg, 60%) as a gum. IR ν_{max} ($CHCl_3$): 3308, 1739, 1683, 1658, 1538, 1465 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 0.00 (s, 3 H), 0.03 (s, 3 H), 0.06 (s, 3 H), 0.08 (s, 3 H), 0.87 (t, 15 H, *J* 7.7 Hz), 1.06 (s, 27 H), 1.16–1.36 (m, 109 H), 1.37–1.46 (m, 2 H), 1.50–1.65 (m, 8 H), 1.86 (q, 2 H, *J* 7.3 Hz), 2.13 (dt, 1 H, *J* 14.8, 7.6 Hz), 2.20–2.28 (m, 4 H), 2.34 (dt, 1 H, *J* 14.8, 7.6 Hz), 2.57 (dd, 1 H, *J* 16.8, 5.8 Hz), 2.69 (dd, 1 H, *J* 16.8, 7.5 Hz), 3.50 (m, 1 H), 3.78–3.98 (m,

4 H), 4.05–4.16 (m, 2 H), 4.21–4.45 (m, 3 H), 4.74 (d, 1 H, 8.8 Hz), 5.10–5.32 (m, H), 5.58 (dt, 1 H, *J* 15.1, 7.6 Hz), 5.71 (ddt, 1 H, *J* 17.6, 11.2, 6.2 Hz), 5.84 (ddt, 1 H, *J* 16.8, 10.9, 5.6 Hz), 5.90 (d, 1 H, *J* 9.0 Hz), 6.61 (d, 1 H, *J* 9.0 Hz), 7.32–7.45 (m, 6 H), 7.67–7.75 (m, 4 H). FABMS (positive-ion) *m*/*z*: 2038 $[M+Na]^+$ (on addition of NaI). HRFABMS (positive-ion): Calcd for $C_{118}H_{215}N_2O_{15}PSi_3Na$, 2038.5066; found, 2038.5096.

*(2*S*,3*R*,4*E*) - 3 - Hydroxy - 2 - (octadecanoylamino)-octadec-4-enyl 2-deoxy-4-*O*-(diallylphosphono)-2-[(*R*)-3 - (hydroxy)tetradecanoylamino] - 3 - O - [(*R*) - 3 - (tetradecanoyloxy)tetradecanoyl]-β-*D*-glucopyranoside* (**10**).—To a solution of **9** (24.3 mg, 0.012 mmol) in THF (0.3 mL) was added HF·pyridine (ca. 20 mg). After stirring for 24 h at rt, the mixture was treated with satd aq $NaHCO_3$ (0.2 mL). The mixture was extracted with Et_2O, and the organic layer was washed with brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo to give a residue that was chromatographed on a silica gel column. Elution with 1:1 EtOAc–hexane, and then with 1:49 MeOH–$CHCl_3$, afforded triol **10** (10.0 mg, 54%) as a white powder. IR ν_{max} ($CHCl_3$): 3298, 1740, 1646, 1550, 1468 cm^{-1}. ^1H NMR (270 MHz, $CDCl_3$): δ 0.88 (t, 15 H, *J* 7.3 Hz), 1.19–1.42 (m, 108 H), 1.50–1.70 (m, 10 H), 2.00–2.08 (m, 1 H), 2.13 (dd, 1 H, *J* 15.1, 10.4 Hz), 2.22 (dt, 1 H, *J* 7.3, 2.0 Hz), 2.28 (t, 2 H, *J* 7.4 Hz), 2.39 (dd, 1 H, *J* 15.1, 2.2 Hz), 2.49–2.54 (m, 2 H), 3.13 (d, 1 H, 10.8 Hz), 3.38 (d, 1 H, *J* 10.3 Hz), 3.55–3.62 (m, 2 H), 3.80–3.98 (m, 3 H), 4.03–4.25 (m, 5 H), 4.38 (d, 1 H, *J* 8.4 Hz), 4.48–4.56 (m, 3 H), 5.08–5.15 (m, 2 H), 5.28 (ddd, 2 H, *J* 11.8, 7.1, 2.3 Hz), 5.38 (ddd, 2 H, *J* 17.9, 5.3, 2.3 Hz), 5.49 (dd, 1 H, *J* 15.9, 7.6 Hz), 5.73 (dt, 1 H, *J* 15.9, 6.8 Hz), 5.85–5.96 (m, 2 H), 6.23 (d, 1 H, *J* 8.5 Hz), 6.83 (d, 1 H, *J* 8.6 Hz). FABMS (positive-ion) *m*/*z*: 1572 $[M+Na]^+$ (on addition of NaI). HRFABMS (positive-ion): Calcd for $C_{90}H_{169}N_2O_{15}PNa$, 1572.2158; found, 1572.2191.

*(2*S*,3*R*,4*E*) - 3 - Hydroxy - 2 - (octadecanoylamino)-octadec-4-enyl 2-[(*R*)-3-(hydroxy)tetradecanoylamino]-2-deoxy-4-*O*-phosphono-3-*O*-[(*R*)-3-(tetradecanoyloxy)-tetradecanoyl]-β-*D*-glucopyranoside* (**11**).—To a solution of **10** (26.4 mg, 0.017 mmol) in THF (0.2 mL) were added Et_3N (11.8 μL, 0.085 mmol), PPh_3 (2.2 mg, 0.009 mmol), HCO_2H (6.4 μL, 0.17 mmol), and $(Ph_3P)_4Pd$ (0.2 mg, 0.0002 mmol). After stirring for 3 h at 50 °C, the crude mixture was concentrated in vacuo to give a residue that was chromatographed on a Sephadex LH20 column. Elution with $CHCl_3$ gave the triethylammonium salt of **11** (21.1 mg) as a white powder. The triethylammonium salt was dissolved in 1:2 $CHCl_3$–MeOH (3 mL), and the solution was treated with 0.1 M aq HCl (1 mL) and extracted with $CHCl_3$. The organic layer was evaporated under reduced pressure to give **11** (19.5 mg, 78%) as a white wax. IR ν_{max} ($CHCl_3$): 3286, 1733, 1650, 1468 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 0.89 (t, 15 H, *J* 6.8 Hz), 1.20–1.48 (m, 106 H), 1.51–1.66 (m, 10 H), 2.03 (q, 2 H, *J* 7.1 Hz), 2.12–2.22 (m, 3 H), 2.31 (t, 2 H, *J* 7.4 Hz), 2.33 (dd, 1 H, *J* 14.6, 2.6 Hz), 2.56 (dd, 1 H, *J* 15.3, 5.4 Hz), 2.66 (dd, 1 H, *J* 15.4, 7.0 Hz), 3.45 (d, 1 H, *J* 9.4 Hz), 3.57 (dd, 1 H, *J* 9.9, 2.7 Hz), 3.80–4.02 (m, 3 H), 4.03–4.14 (m, 2 H), 4.24–4.33 (m, 1 H), 4.44 (d, 1 H, *J* 8.4 Hz), 5.11–5.20 (m, 2 H), 5.43 (dd, 1 H, *J* 15.4, 7.4 Hz), 5.70 (dt, 1 H, *J* 15.4, 6.5 Hz), 7.27 (d, 1 H, *J* 9.2 Hz), 7.63 (d, 1 H, *J* 9.5 Hz). FABMS (positive-ion) *m*/*z*: 1508 $[M+K]^+$ (on addition of KI). HRFABMS (positive-ion): Calcd for $C_{84}H_{161}N_2O_{15}PK$, 1508.1272; found, 1508.1317. Anal. Calcd for $C_{84}H_{161}N_2O_{15}P$ (1470.2): C, 68.62; H, 11.04; N, 1.91. Found: C, 68.54; H, 10.95; N, 1.88.

*(2*S*,3*R*,4*E*)-3-(*tert*-Butyldimethylsilyloxy)-2-(octadecanoylamino)octadec-4-enyl 2-acetylamino-6-*O*-(tert-butyldiphenylsilyl)-2-deoxy-4-*O*-(diallylphosphono)-3-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-β-*D*-glucopyranoside* (**9′**).—To a solution of **8** (0.27 g, 0.16 mmol) in CH_2Cl_2 (0.5 mL) were added DMAP (2.0 mg, 0.016 mmol), Et_3N (67.4 μL, 0.48 mmol), and AcCl (17.1 μL, 0.24 mmol). After stirring for 3 h at rt, the reaction mixture was treated with satd aq $NaHCO_3$ (0.2 mL). The mixture was extracted with Et_2O, and the organic layer was washed with brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo to give a residue that was chromatographed on a silica gel column. Elution with 1:9 then 1:4 EtOAc–hexane afforded **9′** (0.23 g, 85%) as a gum. IR ν_{max} ($CHCl_3$): 3307, 1738, 1669, 1543, 1465 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 0.01 (s, 3 H), 0.04 (s, 3 H), 0.88 (t, 12 H, *J* 6.5 Hz), 1.07 (s, 18 H), 1.17–1.49 (m, 90 H), 1.50–1.67 (m, 6 H), 1.93 (s, 3 H), 2.09–2.23 (m, 2 H), 2.27 (t, 2 H, *J* 7.3 Hz), 2.59–2.65 (m, 2 H), 3.49–3.59 (m, 1 H), 3.73–3.82 (m, 2 H), 3.87–4.00 (m, 4 H), 4.05–4.18 (m, 2 H), 4.22 (t, 1 H, *J* 6.4 Hz), 4.25–4.32 (m, 1 H), 4.34–4.45 (m, 4 H), 4.76 (d, 1 H, *J* 8.0 Hz), 5.13 (dd, 1 H, *J* 10.6, 1.2 Hz), 5.17–5.25 (m, 2 H), 5.26–5.36 (m, 2 H), 5.59 (dt, 1 H, *J* 15.6, 6.6 Hz), 5.72 (ddt, 1 H, *J* 17.2, 10.6, 6.1 Hz), 5.84 (ddt, 1 H, *J* 17.1, 10.6, 6.0 Hz), 5.94 (d, 1 H, *J* 9.0 Hz), 6.17 (d, 1 H, *J* 8.2 Hz), 7.30–7.50 (m, 6 H), 7.62–7.80 (m, 4 H). FABMS (positive-ion) *m*/*z*: 1756 $[M+K]^+$ (on addition of KI). HRFABMS (positive-ion): Calcd for $C_{100}H_{177}Cl_3N_2O_{14}PSi_2K$, 1756.2113; found, 1756.2123.

*(2*S*,3*R*,4*E*) - 3 - Hydroxy - 2 - (octadecanoylamino)octadec-4-enyl 2-acetylamino-2-deoxy-4-*O*-(diallylphosphono)-3-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-β-*D*-glucopyranoside* (**10′**).—To a solution of **9′** (0.13 g, 0.077 mmol) in THF (0.5 mL) was added HF·pyridine (ca. 60 mg). After stirring for 24 h at rt, the mixture was treated with satd aq $NaHCO_3$ (0.2 mL) and extracted with ether. The organic layer was washed with brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo to give a residue that was chromatographed on a silica gel column. Elution with 1:1

EtOAc–hexane, and then 1:49 MeOH–$CHCl_3$ afforded **10′** (64.0 mg, 61%) as a white powder. IR ν_{max} ($CHCl_3$): 3431, 3386, 1743, 1727, 1519, 1467 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 0.88 (t, 12 H, *J* 7.4 Hz), 1.20–1.34 (m, 95 H), 1.52–1.66 (m, 6 H), 1.94 (s, 3 H), 2.03 (q, 2 H, *J* 7.4 Hz), 2.20 (t, 2 H, *J* 7.7 Hz), 2.28 (t, 2 H, *J* 7.5 Hz), 2.51 (dd, 1 H, *J* 15.3, 8.2 Hz), 2.57 (dd, 1 H, *J* 15.3, 4.4 Hz), 3.47 (d, 1 H, *J* 9.9 Hz), 3.70 (d, 1 H, *J* 8.4 Hz), 3.72–3.80 (m, 1 H), 3.81–3.93 (m, 2 H), 3.98–4.08 (m, 2 H), 4.12 (t, 1 H, *J* 6.4 Hz), 4.46–4.58 (m, 3 H), 4.74 (d, 1 H, *J* 8.6 Hz), 5.13–5.32 (m, 3 H), 5.37 (ddt, 2 H, *J* 17.0, 1.7, 1.6 Hz), 5.48 (dd, 1 H, *J* 15.7, 6.8 Hz), 5.72 (dt, 1 H, *J* 15.7, 6.7 Hz), 6.23 (d, 1 H, *J* 7.6 Hz), 6.34 (d, 1 H, *J* 7.7 Hz). FABMS (positive-ion) *m*/*z*: 1388 $[M + Na]^+$ (on addition of NaI). HR-FABMS (positive-ion): Calcd for $C_{78}H_{145}N_2O_{14}PNa$, 1388.0331; found, 1388.0328.

*(2*S*,3*R*,4*E*)-3-Hydroxy-2-(octadecanoylamino)octadec-4-enyl 2-acetylamino-2-deoxy-4*-O*-phosphono-3*-O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-β-*D*-glucopyranoside* (**11′**).—To a solution of **10′** (28.7 mg, 0.021 mmol) in THF (0.2 mL) were added Et_3N (14.6 μL, 0.105 mmol), PPh_3 (2.6 mg, 0.010 mmol), HCO_2H (7.9 μL, 0.210 mmol), and $(Ph_3P)_4Pd$ (0.2 mg, 0.0002 mmol). After stirring for 3 h at 50 °C, the solvents were removed under reduced pressure. The crude mixture was purified by Sephadex LH20 column chromatography. Elution with $CHCl_3$ gave the triethylammonium salt of **11′** (29.0 mg) as a white powder, which was dissolved in 1:2 $CHCl_3$–MeOH (3 mL), treated with 0.1 M aq HCl (1 mL), and extracted with $CHCl_3$. The organic layer was concentrated in vacuo to give **11′** (27.6 mg, 96%) as a powder. IR ν_{max} ($CHCl_3$): 3300, 1737, 1657, 1554, 1467, 1376 cm^{-1}. ^1H NMR (270 MHz, $CDCl_3$): δ 0.88 (t, 12 H, *J* 7.5 Hz), 1.04–1.44 (m, 92 H), 1.50–1.66 (m, 6 H), 1.93 (s, 3 H), 1.97–2.06 (m, 2 H), 2.16 (t, 2 H, *J* 8.0 Hz), 2.30 (t, 2 H, *J* 7.8 Hz), 2.55 (dd, 1 H, *J* 16.5, 6.1 Hz), 2.66 (dd, 1 H, *J* 16.5, 7.2 Hz), 3.37 (m, 1 H), 3.45 (d, 1 H, *J* 9.7 Hz), 3.63 (dd, 1 H, *J* 10.0, 3.3 Hz), 3.82–3.94 (m, 4 H), 4.47 (d, 1 H, *J* 8.6 Hz), 5.10–5.20 (m, 2 H), 5.42 (dd, 1 H, *J* 15.6, 7.7 Hz), 5.71 (dt, 1 H, *J* 15.6, 7.4 Hz). FABMS (positive-ion) *m*/*z*: 1307 $[M + Na]^+$ (on addition of NaI). HR-FABMS (positive-ion): Calcd for $C_{72}H_{137}N_2O_{14}PNa$ $[M + Na]^+$, 1307.9705; found, 1307.9700.

Biological activity studies

Preparation of an aqueous solution of compound **11′** *for measurement of the biological activity*. Compound **11′** (3.1 mg) was dissolved in aq 0.1% Et_3N (0.642 mL) for measurement of the biological activity.

Procedures for measurement of the biological activity.—The sources of the materials used in this study are as follows: Lipopolysaccharide (LPS) from *E. coli* serotype 026:B6, 12-*O*-tetradecanoylphorbolacetate (TPA) and prednisolone were from Sigma Chemical Co., St. Louis, MO. PRMI-1640 medium, fetal bovine serum (FBS), and newborn calf serum (NBCS) were from Gibco, Grand Island, NY. 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 RPMI-1640 medium supplemented with 10% FBS, 100 units/mL of penicillin and 100 μg/mL 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 were cultured in the presence of TPA (30 ng/mL) for 72 h at 37 °C. After removal of the supernatant, the cells were incubated in 200 μL of fresh RPMI-1640 medium containing 10% of NBCS, 10 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 TNFα ELISA kit. As a control, the amount of TNFα produced by the U937 cells, which were stimulated with 10 ng/mL of LPS in the absence of compounds, was used. The relative amounts of TNFα were calculated as percentages of the control amounts.

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