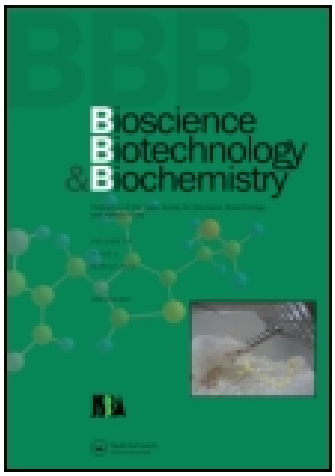

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Syntheses of 1-O-([5-Carboxy)pentanoyl]-2-deoxy-2-(2,2-difluorotetradecanamido)-3-O-[(R)-3-(tetradecanoyloxy)tetradecanoyl]-4-O-phosphono-α-D-glucopyranose and Its Analogues

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Syntheses of 1-*O*-[5-(Carboxy)pentanoyl]-2-deoxy-2-(2,2-difluorotetradecanamido)-3-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-4-*O*-phosphono-α-D-glucopyranose and Its Analogues

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1-*O*-[5-(Carboxy)pentanoyl]-2-deoxy-2-(2,2-difluorotetradecanamido)-3-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-4-*O*-phosphono-α-D-glucopyranose (13) and its analogues (16 and 19) were synthesized. Compound 13 showed strong LPS-agonistic activity.

Lipopolysaccharides (LPS),[1] an outer surface membrane component of such Gram-negative bacterial cells as *Salmonella minnesota*, *Salmonella typhirium*, and *Escherichia coli*, cause fever and lethal shock in septicemia of higher animals, and are also highly potent stimulators of the immune system. An example of the structure of LPS is shown in Fig. 1.[2] The endotoxic shock caused by LPS has precluded clinical used. Most of the biological activities of LPS reside in a relatively small portion of the molecule known as lipid A, which is a hydrophobic anchor substance holding an essentially linear polysaccharide chain to the cell wall[2] and has been chemically synthesized by Imoto *et al.*[3]

In a series of investigations by Hasegawa and Kiso[4] on the relationship between the molecular structure and biological activity of non-reducing sugar subunit analogues of lipid A, it has been demonstrated that several of the biological activities of LPS can be expressed by certain 4-*O*-phosphono-D-glucosamine derivatives such as GLA-60.[4]

We have been investigating the biological activity of compounds related to GLA-60. In this paper, we describe the synthesis of 1-*O*-[5-(carboxy)pentanoyl]-2-deoxy-2-(2,2-difluorotetradecanamido)-3-*O*-[(3*R*)-3-(tetradecanoyloxy)tetradecanoyl]-4-*O*-phosphono-α-D-glucopyranose (**13**) and its analogues (**16** and **19**) shown in Scheme 2 as

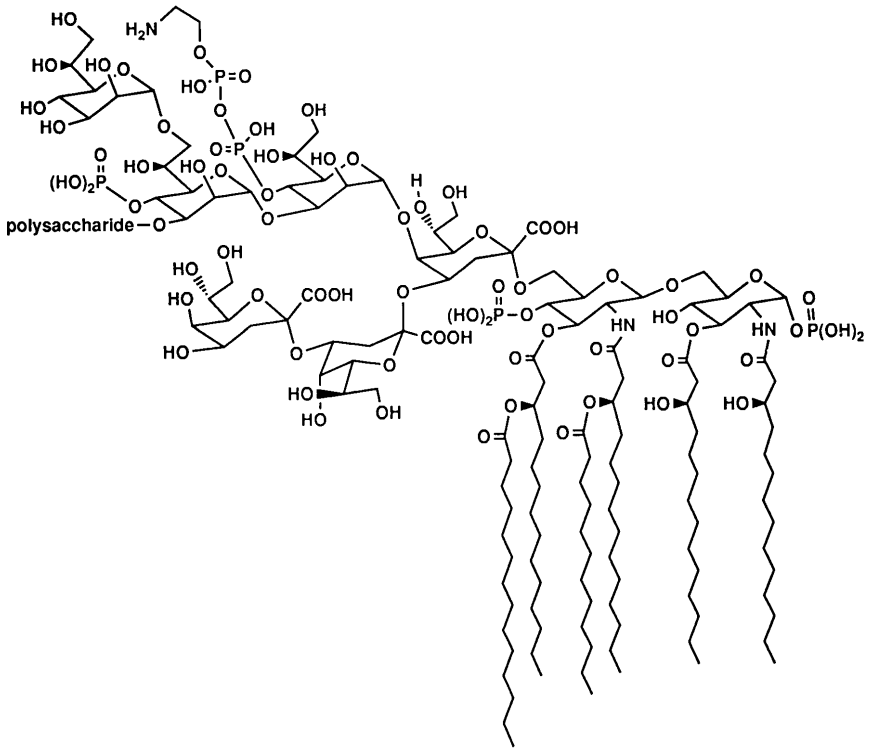

Fig. 1. Structure of Lipopolysaccharides (LPS).[2]

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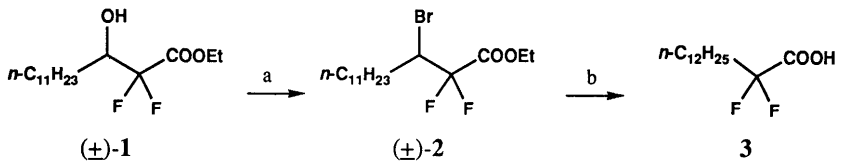
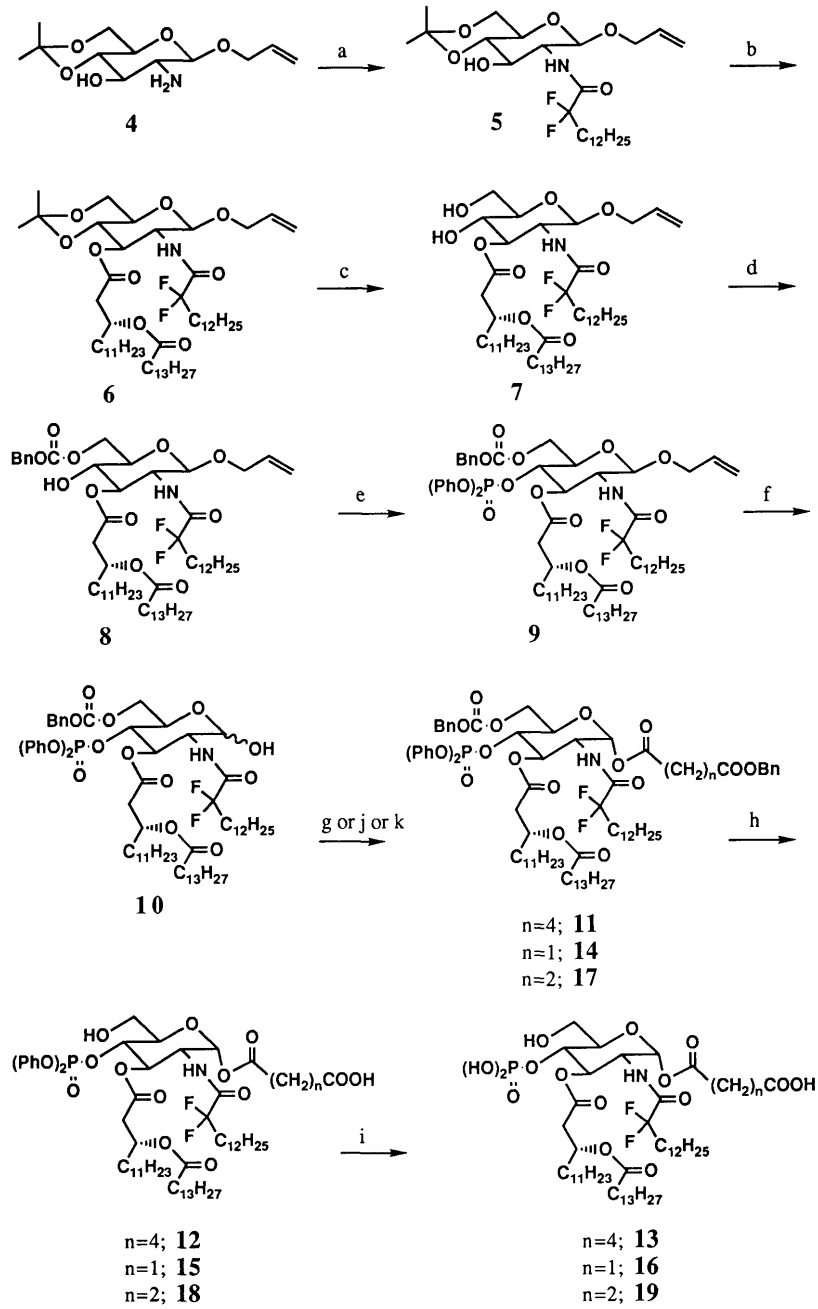


(±)-1 (±)-2 3

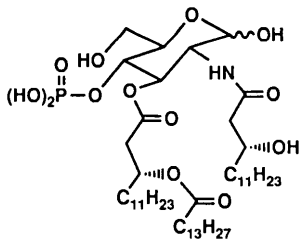
Conditions: (a) CBr_4, Ph_3P-PhH, 50°C, 3 h, 93%; (b) Bu_3SnH, AIBN-PhH, reflux, 8 h, and then 1 M NaOH, 25°C, 5 h, 45%.

Scheme 1.



Conditions: (a) acid chloride of **3**, Et_3N–CH_2Cl_2, 25°C, 1 h, 70%; (b) DCC, DMAP–CH_2Cl_2, 25°C, 2 h, 59%; (c) aq. 85% AcOH, 60°C, 1 h, 86%; (d) ClCOOBn, DMAP–CH_2Cl_2, 24°C, 16 h, 76%; (e) ClP(O)$(OPh)_2$, DMAP–CH_2Cl_2, 24°C, 1 h, 96%; (f) $[C_8H_{12}Ir(PMePh_2)_2]PF_6$–THF, 24°C, 3 h, then pyridine–H_2O–I_2–THF, 24°C, 30 min, 56%; (g) $HOOC(CH_2)_4COOBn$, DCC, DMAP–CH_2Cl_2, 25°C, 1 h, 91% (**11**); (h) H_2, 10% Pd/C–THF, 25°C, 6 h, 6 h, 80% (**12**); 77% (**18**); (i) H_2, PtO_2–THF, 25°C, 5 h, 98% (**13**); 87% (**16** *via* **15** from **14**); 98% (**19**); (j) $HOOCCH_2$–COOBn, DCC, DMAP–CH_2Cl_2, 24°C, 30 min, 38% (**14**, α-anomer)), 29% (**14′**, β-anomer); (k) $HOOCCH_2CH_2COOBn$, DCC, DMAP–CH_2Cl_2, 24°C, 1 h, 90% (**17**).

Scheme 2.



GLA-60

Fig. 2. Structure of GLA-60.

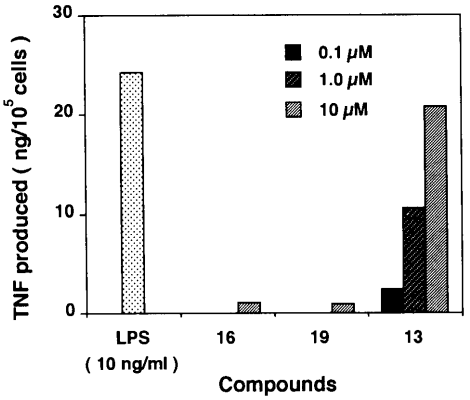


Fig. 3. TNFα-production by J774.1 Cells.

J774.1 cells were cultured in DMEM containing 5% NBBS, 30 ng/ml of TPA and a graded concentration of a compound for 6 h. The amount of TNFα produced by the cells was determined by an ELISA analysis. Data are expressed as the means of duplicate determinations.

compounds containing a 2,2-difluorinated tetradecanoyl group at the 2-amino position, and a carboxypentanoyl, carboxyacetyl or carboxypropionyl group at the anomeric position. The 2,2-difluorinated tetradecanoyl group was expected to improve the biological activity based on our previous work.[5]

Synthesis

The starting allyl 2-amino-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranoside (**4**)[6] was converted to amide **5** with triethyl amine and the acid chloride of **3**, which was synthesized from (±)-ethyl 2,2-difluoro-3-hydroxytetradecanoate (**1**)[5] *via* (i) halo-dehydroxylation of **1** with Ph_3P and CBr_4, (ii) hydro-dehalogenation of **2** with Bu_3SnH, and (iii) successive saponification with aqueous NaOH.

Treatment of **5** with (*R*)-3-(tetradecanoyloxy)tetradecanoic acid, 1,3-dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP) in tetrahydrofuran (THF) gave ester **6**. Deisopropylidenation of **6** with 85% AcOH at 60°C gave **7**, and protection of the primary alcohol of **7** with benzyl chloroformate and DMAP in methylene chloride afforded 6-*O*-benzyloxycarbonyl derivative **8**. Treatment of **8** with diphenyl chlorophosphate and DMAP in methylene chloride yielded **9**. Treatment of **9**, using 1,5-cyclooctadiene-bis(methyldiphenylphosphine)iridium hexafluorophosphate[7] ($[Ir(C_8H_{12})(PMePh_2)_2]PF_6$) as a catalyst in THF to isomerise the double bond of the 1-*O*-allyl group, and successive treatment of the resulting enol ether with iodine-pyridine-water[8] gave **10**. Acylation of **10** with adipic acid monobenzyl ester or succinic acid monobenzyl ester and DCC-DMAP exclusively gave α-anomer **11** or **17**, respectively. However, the same reaction with malonic acid monobenzyl ester gave a 38:29 mixture of the α- and β-anomers (**14** and **14'**). The two venzyl groups of **11**, **14**, and **17** were removed by hydrogenolysis, using 10% Pd on carbon under hydrogen, to yield acid-alcohols **12**, **15**, and **18**, respectively, and successive hydrogenolysis of **12**, **15**, and **18** with PtO_2 afforded **13**, **16**, and **19**, respectively.

Biological activity

Compound **13** ($n=4$) revealed strong LPS-agonist activity toward macrophage-like cell line J774.1, while compounds **16** ($n=1$) and **19** ($n=2$) shown in Fig. 3 did not show such activity. It is obivious that the length of the methylene group at the anomeric position affected the LPS-agonist activity.

Experimental

Melting point (mp) values are uncorrected. ^{1}H-NMR data were recorded at 270 MHz with a JEOL JNN-270 spectrometer, using trimethylsilane as an internal standard. IR absorption spectra were determined with a Jasco IR A-2 spectrophotometer, and mass spectra were obtained with a JMS-O1SG mass spectrometer. Column chromatography was carried out on silica gel-60 (Merck 230–400 mesh ASTM) at a slightly elevated pressure (1.2 atm) for easy elution.

(±)-*Ethyl 3-bromo-2,2-difluorotetradecanoate* (**2**). To a solution of (±)-ethyl 2,2-difluoro-3-hydroxytetradecanoate (**1**; 33.8 g, 0.109 mol) in benzene (300 ml) were added CBr_4 (54.4 g, 0.164 mol) and Ph_3P (43.0 g, 0.164 mol) at 5–10°C, and the mixture was stirred for 3 h at 50°C. The reaction mixture was diluted with hexane and filtered, before the filtrate was concentrated *in vacuo* and chromatographed in a silica gel column. Elution with hexane–EtOAc (9:1) gave **2** (37.8 g, 93% yield). IR ν_{max} ($CHCl_3$): 2910, 2845, 1775, 1465 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t, $J=5.9$–7.3 Hz), 1.20–1.52 (20H, m, containing 3H, t, $J=6.6$–7.3 Hz, at δ 1.38), 1.57–1.76 (1H, m), 1.76–1.94 (1H, m), 1.94–2.08 (1H, m), 4.17–4.34 (1H, m), 4.38 (2H, q, $J=7.0$ Hz); MS *m/z*: 372, 370 (M^+), 344, 342, 291 (M^+-Br), 263, 251, 207. *Anal.* Calcd. for $C_{16}H_{29}O_2F_2Br$ (371.3): C, 51.76; H, 7.87; F, 10.23; Br, 21.52%. Found: C, 51.73; H, 7.90; F, 10.19; Br, 21.33%.

2,2-Difluorotetradecanoic acid (**3**). To a solution of **2** (37.6 g, 0.101 mol) in benzene (500 ml) were added Bu_3SnH (109 ml, 0.405 mol) and AIBN (1.66 g, 10 mmol), and the mixture was refluxed for 8 h under nitrogen. The reaction mixture was diluted with EtOAc, washed with sat. $NaHCO_3$ and brine, dried over $MgSO_4$, filtered and concentrated to give an oily residue, which was then dissolved in EtOH (300 ml). Aqueous 1 M NaOH (200 ml) was added to this solution, and the mixture was stirred for 5 h at 25°C, concentrated *in vacuo* to one third. This concentrated mixture was acidified with 2 M HCl, extracted with EtOAc, washed with water and brine, and concentrated *in vacuo* to give a residue. This residue was chromatographed in a silica gel column, and elution with hexane–EtOAc (9:1, and then 1:1) gave **3** (12.0 g, 45% yield), mp 44–46°C. IR ν_{max} (Nujol): 3540, 3470, 3200–2300 (broad), 1740 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t, $J=5.9$–7.3 Hz), 1.02–1.58 (20H, m), 1.95–2.21 (2H, m), 7.76 (1H, broad); MS *m/z* 264 (M^+). *Anal.* Calcd. for $C_{14}H_{26}O_2F_2$ (264.4): C, 63.61; H, 9.91, F, 14.37%. Found: C, 63.69; H, 9.82; F, 14.31%.

Allyl 2-Deoxy-2-(2,2-difluorotetradecanamido)-4,6-O-isopropylidene-β-D-glucopyranoside (**5**). To a solution of allyl 2-amino-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranoside[5] (**4**, 3.06 g, 11.8 mmol) in CH_2Cl_2 (30 ml) were added a solution of 2,2-difluorotetradecanoyl chloride in CH_2Cl_2 (30 ml) under nitrogen, and then Et_3N (2 ml). (The acid chloride was prepared by treating **3** (3.44 g, 13.0 mmol) with oxalyl chloride (3 ml) and one drop of DMF in CH_2Cl_2 (30 ml) for 1 h at 25°C, the resulting mixture being concentrated *in vacuo* to give an acid chloride, which was employed for the next reaction without further purification.) After 1 h at 25°C, the reaction mixture was diluted with CH_2Cl_2, washed with aq. $NaHCO_3$ and brine, dried over Na_2SO_4, concentrated *in vacuo*, and then chromatographed in a silica gel column. Elution with hexane–EtOAc (5:1, and then 3:1) gave **5** (4.17 g, 70% yield), mp 164–166°C (from CH_2Cl_2–hexane).

IR ν_{max} ($CHCl_3$): 3610, 3460, 2940, 2870, 1705 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t, J=7.3 Hz), 1.19–1.50 (20H, m), 1.44 (3H, s), 1.52 (3H, s), 1.95–2.20 (2H, m), 3.15 (1H, d, J=3.3 Hz, OH), 3.22–3.40 (1H, m), 3.47–3.57 (1H, m), 3.58 (1H, t, J=9.9 Hz), 3.81 (1H, t, J=9.9 Hz), 3.94 (1H, dd, J=5.3, 11.2 Hz), 4.00–4.12 (2H, m), 4.27–4.40 (1H, m), 4.76 (1H, d, J=7.9 Hz), 5.18–5.34 (2H, m), 5.77–5.94 (1H, m), 6.51 (1H, d, J=5.9 Hz, NH); MS *m/z*: 506 (M^++1), 490 (M^+−Me), 448 (M^+−O−allyl). *Anal.* Calcd. for $C_{26}H_{45}NO_6F_2$ (505.6): C, 61.76; H, 8.97; N, 2.77; F, 7.51%. Found: C, 61.62; H, 8.68; N, 2.71; F, 7.62%.

*Allyl 2-Deoxy-2-(2,2-difluorotetradecanamido)-4,6-O-isopropylidene-3-O-[(R)-3-(tetradecanoyloxy)tetradecanoyl]-β-*D*-glucopyranoside* (**6**). To a solution of **5** (2.76 g, 5.46 mmol) and (*R*)-3-(tetradecanoyloxy)tetradecanoic acid (2.73 g, 6.00 mmol) in CH_2Cl_2 (50 ml) were added DCC (1.43 g, 6.94 mmol) and DMAP (0.73 g, 6.00 mmol) under nitrogen. After stirring for 2 h at 25°C, the reaction mixture was filtered, and the filtrate was concentrated *in vacuo* to give a mixture, which was diluted with EtOAc, washed with aq. $NaHCO_3$ and brine, dried over Na_2SO_4, concentrated *in vacuo*, and chromatographed in a silica gel column. Elution with hexane–EtOAc (19 : 1, and then 9 : 1) gave **6** (2.62 g, 59% yield) as a gum. IR ν_{max} ($CHCl_3$): 2920, 2850, 1735, 1720 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 0.88 (9H, t, J=7.3 Hz), 1.20–1.40 (62H, m), 1.37 (3H, s), 1.48 (3H, s), 1.90–2.15 (2H, m), 2.26 (2H, t, J=7.3 Hz), 2.49 (1H, dd, J=6.4, 15.1 Hz), 2.63 (1H, dd, J=6.4, 15.1 Hz), 3.30–3.43 (1H, m), 3.74 (1H, t, J=9.9 Hz), 3.81 (1H, t, J=9.9 Hz), 3.88–4.12 (3H, m), 4.26–4.38 (1H, m), 4.65 (1H, d, J=8.6 Hz), 5.10–5.32 (4H, m), 5.72–5.90 (1H, m), 6.51 (1H, d, J=8.8 Hz, NH); MS *m/z*: 941 (M^+), 926, 884, 755. *Anal.* Calcd. for $C_{54}H_{97}NO_9F_2$ (942.4): C, 68.83; H, 10.38; N, 1.49; F, 4.03%. Found: C, 68.66; H, 10.29; N, 1.58; F, 3.86%.

*Allyl 2-Deoxy-2-(2,2-difluorotetradecanamido)-3-O-[(R)-3-(tetradecanoyloxy)tetradecanoyl]-β-*D*-glucopyranoside* (**7**). A solution of **6** (4.89 g, 5.19 mmol) in AcOH (340 ml) and H_2O (60 ml) was stirred at 60°C for 1 h, and then concentrated *in vacuo* to give a residue, which was chromatographed in a silica gel column. Elution with hexane–EtOAc (3 : 1, and then 2 : 9) gave **7** (4.04 g, 86% yield), mp 109–110°C (CH_2Cl_2–hexane). IR ν_{max} ($CHCl_3$): 3600–3200, 2920, 2850, 1740, 1710 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 0.88 (9H, t, J=6.8 Hz), 1.20–1.50 (62H, m), 1.93–2.10 (2H, m), 2.10–2.17 (1H, br. s, OH), 2.30 (2H, t, J=7.3 Hz), 2.43–2.60 (2H, m), 3.43–3.52 (1H, m), 3.61 (1H, d, J=3.9 Hz, OH), 3.69 (1H, dt, J=3.9, 9.3 Hz), 3.77–4.11 (4H, m), 4.28–4.38 (1H, m), 4.60 (1H, d, J=8.3 Hz), 5.02–5.32 (4H, m), 5.75–5.90 (1H, m), 6.63 (1H, d, J=9.3 Hz, NH). *Anal.* Calcd. for $C_{51}H_{93}NO_9F_2$ (902.3): C, 67.89; H, 10.39; N, 1.55; F, 4.21%. Found: C, 68.09; H, 10.54; N, 1.53; F, 4.24%.

*Allyl 6-O-Benzyloxycarbonyl-2-deoxy-2-(2,2-difluorotetradecanamido)-3-O-[(R)-3-(tetradecanoyloxy)tetradecanoyl]-β-*D*-glucopyranoside* (**8**). To a solution of **7** (1.00 g, 1.11 mmol) and DMAP (136 mg, 1.11 mmol) in CH_2Cl_2 (10 ml) was added dropwise a solution of benzyl chloroformate (473 mg, 2.77 mmol) in CH_2Cl_2 (5 ml). After stirring at 24°C for 16 h, the reaction mixture was diluted with EtOAc, washed with aq. $NaHCO_3$ and brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give a residue, which was then chromatographed in a silica gel column. Elution with cyclohexane–EtOAc (4 : 1) gave **8** (874 mg, 76% yield) as a gum. IR ν_{max} ($CHCl_3$): 3420, 1735, 1710 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 0.88 (9H, t, J=6.6 Hz), 1.10–1.65 (62H, m), 1.90–2.13 (2H, m), 2.24–2.32 (2H, m), 2.41–2.59 (2H, m), 3.51–3.66 (3H, m, containing OH), 3.91–4.08 (2H, m), 4.25–4.46 (2H, m), 4.50–4.60 (2H, m), 4.97–5.29 (6H, m), 5.71–5.88 (1H, m), 6.49 (1H, d, J=9.2 Hz, NH), 7.31–7.44 (5H, m). *Anal.* Calcd. for $C_{59}H_{99}NO_{11}F_2$ (1036.4): C, 68.37; H, 9.63; N, 1.35; F, 3.67%. Found: C, 68.34; H, 9.76; N, 1.39; F, 3.58%.

*Allyl 6-O-Benzyloxycarbonyl-2-deoxy-2-(2,2-difluorotetradecanamido)-4-O-(diphenylphosphoryl)-3-O-[(R)-3-(tetradecanoyloxy)tetradecanoyl]-β-*D*-glucopyranoside* (**9**). To a solution of **8** (2.58 g, 2.49 mmol) and DMAP (610 mg, 4.98 mmol) in CH_2Cl_2 (45 ml) was added dropwise a solution of diphenyl chlorophosphate (1.34 g, 4.98 mmol) in CH_2Cl_2 (5 ml). After stirring at 24°C for 1 h, the reaction mixture was diluted with EtOAc, washed with aq. $NaHCO_3$ and brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give a residue, which was then chromatographed in a silica gel column. Elution with cyclohexane–EtOAc (5 : 1) gave **9** (3.04 g, 96% yield) as a gum. IR ν_{max} (KBr): 1743, 1697 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 0.88 (9H, t, J=5.9–7.3 Hz), 1.08–1.60 (62H, m), 1.88–2.22 (4H, m), 2.31 (1H, dd, J=7.9, 15.8 Hz), 2.40 (1H, dd, J=4.6, 15.8 Hz), 3.68 (1H, td, J=6.6, 9.2 Hz), 3.72–3.83 (1H, m), 4.02 (1H, dd, J=6.6, 12.5 Hz), 4.18–4.41 (3H, m), 4.70 (1H, q, J=8.6–9.2 Hz), 4.98–5.28 (6H, m), 5.59 (1H, dd, J=9.2, 9.9 Hz), 5.73–5.88 (1H, m), 6.76 (1H, d, J=8.6 Hz, NH), 7.10–7.37 (15H, m). *Anal.* Calcd. for $C_{71}H_{108}NO_{14}F_2P$ (1268.6): C, 67.22; H, 8.58; N, 1.10; F, 3.00; P. 2.44%. Found: C, 67.26; H, 8.76; N, 1.16; F, 2.93; P, 2.32%.

*6-O-Benzyloxycarbonyl-2-deoxy-2-(2,2-difluorotetradecanamido)-4-O-(diphenylphosphoryl)-3-O-[(R)-3-(tetradecanoyloxy)tetradecanoyl]-*D*-glucopyranose* (**10**). To a solution of **9** (2.65 g, 2.10 mmol) in THF (30 ml) was added bis(methyldiphenylphosphine)cyclooctadiene iridium(I) hexafluorophosphate, $[C_8H_{12}Ir(PMePh_2)_2]PF_6$ (100 mg). The air in the reaction flask was completely replaced with nitrogen and then further replaced with hydrogen to activate the iridium complex. Immediately after 1 or 2 min, when the red colored solution of the iridium complex had become almost colorless, the hydrogen was completely replaced with nitrogen. After stirring at 24°C for 3 h, to this solution were added H_2O (10 ml), pyridine (1 ml) and iodine (0.50 g). The mixture was stirred for 30 min at 24°C, concentrated *in vacuo*, and diluted with EtOAc. The solution was successively washed with aq. 5% $Na_2S_2O_3$, aq. $NaHCO_3$ and brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give a residue, which was chromatographed in a silica gel column. Elution with cyclohexane–EtOAc (5 : 1) gave **10** (1.89 g, 74% yield) as a gum. IR ν_{max} ($CHCl_3$): 3426, 2956, 2958, 2855, 1749, 1712, 1591 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 0.88 (9H, t, J=6.6 Hz), 1.18–1.60 (62H, m), 1.94–2.18 (4H, m), 2.36–2.54 (2H, m), 3.32 (1H, br. s, OH), 4.16–4.38 (4H, m), 4.75 (1H, q, J=9.2 Hz), 5.01–5.13 (3H, m, containing 2H, AB-q, J=11.9 Hz, at δ 5.04, 5.11), 5.29 (1H, dd, J=3.3, 4.0 Hz), 5.51 (1H, dd, J=9.2, 10.6 Hz), 6.80 (1H, d, J=9.2 Hz, NH), 7.11–7.38 (15H, m); FAB MS (postive) 1228 $(M+H)^+$. *Anal.* Calcd. for $C_{68}H_{104}NO_{14}F_2P$ (1228.5): C, 66.48; H, 8.53; N, 1.14; F, 3.09; P, 2.52%. Found: C, 66.44; H, 8.62; N, 1.17; F, 3.01; P, 2.47%.

*1-O-[5-(Benzyloxycarbonyl)pentanoyl]-6-O-benzyloxycarbonyl-2-deoxy-2-(2,2-difluorotetradecanamido)-4-O-(diphenylphosphoryl)-3-O-[(R)-3-(tetradecanoyloxy)tetradecanoyl]-α-*D*-glucopyranose* (**11**). To a solution of **10** (300 mg, 0.244 mmol), DMAP (45 mg, 0.366 mmol) and adipic acid monobenzyl ester (75 mg, 0.317 mmol) in CH_2Cl_2 (3 ml) was added DCC (151 mg, 0.733 mmol). After stirring at 24°C for 1 h, the reaction mixture was filtered, and the filtrate was concentrated *in vacuo* to give a mixture, which was diluted with EtOAc. The solution was washed with aq. $NaHCO_3$ and brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give a residue, which was chromatographed in a silica gel column. Elution with cyclohexane–EtOAc (5 : 1) gave **11** (322 mg, 91% yield) as a gum. IR ν_{max} ($CHCl_3$): 2910, 2840, 1745, 1735 (shoulder), 1700, 1590 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 0.88 (9H, t, J=6.6 Hz), 1.05–1.59 (62H, m), 1.65–1.73 (4H, m), 1.89–2.22 (4H, m), 2.31–2.51 (6H, m), 4.03–4.12 (1H, m), 4.18–4.43 (3H, m), 4.84 (1H, q, J=9.2 Hz, C4-H), 4.98–5.13 (5H, m), 5.47 (1H, dd, J=9.2, 11.2 Hz), 6.29 (1H, d, J=3.3 Hz, C1-H), 6.89 (1H, d, J=7.9 Hz, NH), 7.11–7.39 (20H, m). *Anal.* Calcd. for $C_{18}H_{118}NO_{17}F_2P$ (1446.7): C, 67.24; H, 8.22; N, 0.97; F, 2.63; P, 2.14%. Found: C, 67.33; H, 8.52; N, 0.95; F, 2.46; P, 1.97%.

*1-O-[(Benzyloxycarbonyl)acetyl]-6-O-benzyloxycarbonyl-2-deoxy-2-(2,2-difluorotetradecanamido)-4-O-(diphenylphosphoryl)-3-O-[(R)-3-(tetradecanoyloxy)tetradeca-noyl]-α-*D*-glucopyranose* (**14**) *and its β-anomer* (**14′**). To a solution of **10** (250 mg, 0.203 mmol), DMAP (37 mg, 0.305 mmol) and malonic acid monobenzyl ester (51 mg, 0.264 mmol) in CH_2Cl_2 (2 ml) was added DCC (126 mg, 0.610 mmol). After stirring at 24°C for 30 min, the reaction mixture was filtered, and the filtrate was concentrated *in vacuo* to give a mixture, which was diluted with EtOAc. The solution was washed with aq. $NaHCO_3$ and brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give a residue, which was chromatographed in a silica gel column. Elution with cyclohexane–EtOAc (5 : 1) gave α-anomer **14** (108 mg, 38% yield) as a gum and β-anomer **14′** (83 mg, 29% yield). Physical data for **14**. IR ν_{max} ($CHCl_3$): 1750 (shoulder), 1745, 1720 (shoulder), 1700 (shoulder) cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 0.88 (9H, t, J=5.9–7.3 Hz), 1.11–1.58 (62H, m), 1.89–2.18 (4H, m), 2.46 (2H, d, J=5.9 Hz), 3.49, 3.57 (2H, AB-q, J=15.8 Hz), 4.07–4.33 (3H, m), 4.39–4.50 (1H, m), 4.85 (1H, q, J=9.2–9.9 Hz, C4-H), 4.98–5.13 (3H, m), 5.20, 5.26 (2H, AB-q, J=11.9 Hz), 5.45 (1H, dd, J=9.2, 9.9 Hz), 6.30 (1H, d, J=3.3 Hz, C1-H), 7.06 (1H, d, J=9.2 Hz), 7.12–7.43 (20H, m). *Anal.* Calcd. for $C_{78}H_{112}NO_{17}F_2P$ (1404.7): C, 66.69; H, 8.04; N, 1.00; F, 2.70; P, 2.21%. Found: C, 66.53; H, 7.94; N, 1.01; F, 2.68; P, 2.09%. Physical data for **14′**. IR ν_{max} ($CHCl_3$): 1750 (shoulder), 1745, 1735 (shoulder), 1720 (shoulder), 1700 (shoulder) cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 0.88 (9H, t,

J=5.9–7.3 Hz), 1.10–1.58 (62H, m), 1.88–2.23 (4H, m), 2.35–2.46 (2H, d, J=5.9 Hz), 3.43 (2H, s), 3.83–4.00 (2H, m), 4.11–4.83 (2H, m), 4.80 (1H, q, J=9.2 Hz, C4-H), 4.95–5.24 (5H, m), 5.57 (1H, dd, J=9.2, 10.6 Hz), 6.08 (1H, d, J=8.6 Hz, C1-H), 6.89 (1H, d, J=9.0 Hz), 7.10–7.38 (20H, m). *Anal.* Calcd. for $C_{78}H_{112}NO_{17}F_2P$ (1404.7): C, 66.69; H, 8.04; N, 1.00; F, 2.70; P, 2.21%. Found: C, 66.56; H, 8.13; N, 1.22; F, 2.54; P, 2.06%.

1-O-[3-(Benzyloxycarbonyl)propionyl]-6-O-benzyloxycarbonyl-2-deoxy-2-(2,2-difluorotetradecanamido)-4-O-(diphenylphosphoryl)-3-O-[(R)-3-(tetradecanoyloxy)tetradecanoyl]-α-D-glucopyranose (**17**). To a solution of **10** (510 mg, 0.415 mmol), DMAP (76 mg, 0.623 mmol) and succinic acid monobenzyl ester (122 mg, 0.540 mmol) in CH_2Cl_2 (4 ml) was added DCC (257 mg, 1.245 mmol). After stirring at 24°C for 1 h, the reaction mixture was filtered, and the filtrate was concentrated *in vacuo* to give a mixture, which was diluted with EtOAc. The solution was washed with aq. $NaHCO_3$ and brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give a residue, which was chromatographed in a silica gel column. Elution with cyclohexane–EtOAc (6:1) gave **17** (531 mg, 90% yield) as a gum. IR ν_{max} ($CHCl_3$): 2920, 2845, 1750, 1735 (shoulder), 1720 (shoulder), 1590 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 0.88 (9H, t, J=5.9–7.3 Hz), 1.11–1.58 (62H, m), 1.88–2.22 (4H, m), 2.46 (2H, d, J=5.9 Hz), 2.62–2.84 (4H, m), 4.12–4.48 (4H, m), 4.86 (1H, q, J=9.2–9.9 Hz), 4.98–5.20 (5H, m), 5.49 (1H, dd, J=9.2, 11.2 Hz), 6.30 (1H, d, J=3.3 Hz, C1-H), 6.94 (1H, d, J=7.9 Hz, NH), 7.12–7.45 (20H, m). *Anal.* Calcd. for $C_{79}H_{114}NO_{17}F_2P$ (1418.7): C, 66.08; H, 8.10; N, 0.99; F, 2.68; P, 2.19%. Found: C, 66.84; H, 8.32; N, 0.99; F, 2.73; P, 2.05%.

1-O-[5-(Carboxy)pentanoyl]-2-deoxy-2-(2,2-difluorotetradecanamido)-4-O-(diphenylphosphoryl)-3-O-[(R)-3-(tetradecanoyloxy)tetradecanoyl]-α-D-glucopyranose (**12**). To a solution of **11** (167 mg, 0.115 mmol) in THF (10 ml) was added 10% Pd on carbon (40 mg). The suspension was stirred under hydrogen at 25°C for 6 h. The resulting reaction mixture was filtered, and the filtrate was concentrated *in vacuo* to give a residue, which was chromatographed in a silica gel column. Elution with cyclohexane–EtOAc (1:4) and then EtOAc gave amorphous **12** (113 mg, 80% yield). IR ν_{max} ($CHCl_3$): 2920, 2850, 1760, 1730, 1710, 1590 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 0.88 (9H, t, J=5.9–7.3 Hz), 1.01–2.11 (68H, m), 2.20–2.62 (8H, m), 3.57–3.73 (2H, m), 3.85 (1H, d, J=9.2 Hz), 4.45–4.66 (1H, m), 4.82 (1H, q, J=9.9 Hz, C4-H), 5.10–5.22 (1H, m), 5.57 (1H, dd, J=9.9, 11.2 Hz), 6.30 (1H, d, J=4.0 Hz, C1-H), 7.14–7.42 (10H, m), 7.59 (1H, d, J=7.9 Hz, NH). *Anal.* Calcd. for $C_{66}H_{106}NO_{15}F_2P$ (1222.5): C, 64.84; H, 8.74; N, 1.15; F, 3.11; P, 2.53%. Found: C, 64.69; H, 8.86; N, 1.16; F, 3.08; P, 2.32%.

1-O-[3-(Carboxy)propionyl]-2-deoxy-2-(2,2-difluorotetradecanamido)-4-O-(diphenylphosphoryl)-3-O-[(R)-3-(tetradecanoyloxy)tetradecanoyl]-α-D-glucopyranose (**18**). To a solution of **17** (130 mg, 0.092 mmol) in THF (2 ml) was added 10% Pd on carbon (15 mg). The suspension was stirred under hydrogen at 25°C for 6 h. The resulting reaction mixture was filtered, and the filtrate was concentrated *in vacuo* to give a residue, which was chromatographed in a silica gel column. Elution with cyclohexane–EtOAc (1:4) and then EtOAc gave amorphous **18** (84 mg, 77% yield). IR ν_{max} ($CHCl_3$): 2910, 2840, 1750, 1720, 1590 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 0.88 (9H, t, J=5.9–7.3 Hz), 1.11–1.65 (62H, m), 1.91–2.12 (2H, m), 2.14–2.32 (2H, m), 2.41 (2H, d, J=5.9 Hz), 2.61–2.88 (4H, m), 3.35 (1H, broad, OH), 3.58–3.78 (2H, m), 3.83–3.93 (1H, m), 4.43–4.56 (1H, m), 4.84 (1H, q, J=9.9 Hz), 5.12 (1H, quintet, J=5.9 Hz), 5.46 (1H, dd, J=9.9, 10.6 Hz), 6.30 (1H, d, J=3.3 Hz, C1-H), 7.02 (1H, d, J=9.2 Hz), 7.16–7.45 (10H, m). *Anal.* Calcd. for $C_{64}H_{102}NO_{15}F_2P$ (1194.5): C, 64.35; H, 8.61; N, 1.17; F, 3.18; P, 2.59%. Found: C, 64.25; H, 8.27; N, 1.26; F, 3.08; P, 2.48%.

1-O-[5-(Carboxy)pentanoyl]-2-deoxy-2-(2,2-difluorotetradecanamido)-4-O-phosphono-3-O-[(R)-3-(tetradecanoyloxy)tetradecanoyl]-α-D-glucopyranose (**13**). (a) A solution of **12** (73 mg, 0.060 mmol) in THF (3 ml) was stirred for 3 h at 25°C under hydrogen, using PtO_2 (15 mg) as a catalyst. The reaction mixture was then filtered, and the filtrate was concentrated *in vacuo* to give **13** (63 mg, 98% yield) as an amorphous solid. IR ν_{max} (KBr): 3322, 2956, 2922, 2852, 1745, 1726 (shoulder), 1708 (shoulder), 1692 cm^{-1}; ^{1}H-NMR (pyridine-d_5) δ: 0.81–2.61 (83H, m), 3.05 (1H, dd, J=6.4, 16.1 Hz), 3.28 (1H, dd, J=6.4, 16.1 Hz), 4.03–4.18 (1H, m), 4.24–4.41 (1H, m), 4.50–4.65 (1H, m), 5.08–5.38 (2H, m), 5.61–5.74 (1H, m), 6.23 (1H, dd, J=9.3, 10.7 Hz), 6.83 (1H, d, J=3.9 Hz, C1-H), 9.69 (1H, d, J=8.8 Hz, NH). *Anal.* Calcd. for $C_{54}H_{98}NO_{15}F_2P \cdot H_2O$ (1070.3+18.0): C, 59.59; H, 9.26; N, 1.29; F, 3.49; P, 2.85%. Found: C, 59.86; H, 9.18; N, 1.38; F, 3.44; P, 2.77%.

(b) To a solution of **11** (76 mg, 0.062 mmol) in THF (5 ml) was added 10% Pd on carbon (40 mg). The suspension was stirred under hydrogen at 25°C for 6 h. The resulting reaction mixture was filtered, and the filtrate was stirred for 4 h at 25°C under hydrogen, using PtO_2 (15 mg) as a catalyst. This reaction mixture was filtered, and the filtrate was concentrated *in vacuo* to give **13** (63 mg, 95% yield).

Compound **13** (30 mg) was dissolved in $CHCl_3$ (10 ml)–MeOH (20 ml)–0.1 M HCl (8 ml) while stirring at 5–10°C. Additional $CHCl_3$ (10 ml) and 0.1 M HCl (10 ml) were added to this solution to ensure separation of the two phases. The lower chloroform phase was collected and concentrated to give 28 mg of **13**, which was dissolved in 10.3 ml of 0.1% Et_3N (v/v) to measure the biological activities.

The same procedure for compounds **16** and **19** was adopted to prepare each of 5 mM solution.

1-O-[(Carboxy)acetyl]-2-deoxy-2-(2,2-difluorotetradecanamido)-4-O-phosphono-3-O-[(R)-3-(tetradecanoyloxy)tetradecanoyl]-α-D-glucopyranose (**16**). According to procedure (b) just described, **14** (50 mg, 0.036 mmol) in THF (1 ml) was hydrogenolyzed under hydrogen, using 10% Pd on carbon (15 mg) as a catalyst at 24°C for 3 h to give **15**. The solution was filtered, and to this solution was added PtO_2 (12 mg), before the mixture was stirred for 2 h at 24°C and then filtered to give **16** (32 mg, 87% yield). IR ν_{max} (KBr): 3289, 2957, 2920, 2851, 1757, 1738, 1695 cm^{-1}; ^{1}H-NMR (pyridine-d_5) δ: 0.81–0.98 (9H, m), 1.14–1.90 (62H, m), 2.21–2.54 (4H, m), 3.02 (1H, dd, J=6.4, 16.1 Hz), 3.27 (1H, dd, J=6.4, 16.1 Hz), 3.60 (1H, d, J=15.8 Hz, OH), 3.78 (1H, d, J=15.8 Hz, OH), 4.10 (1H, d, J=12.7 Hz), 4.40 (1H, d, J=9.8 Hz), 4.48 (1H, dd, J=10.7, 13.2 Hz), 5.12–5.38 (2H, m), 5.62–5.74 (1H, m), 6.23 (1H, dd, J=9.3, 10.7 Hz), 6.90 (1H, d, J=3.4 Hz, C1-H), 9.22 (1H, d, J=8.9 Hz, NH). *Anal.* Calcd. for $C_{51}H_{92}NO_{15}F_2P \cdot H_2O$ (1028.3+18.0): C, 58.55; H, 8.98; N, 1.34; F, 3.63; P, 2.96%. Found: C, 58.64; H, 9.00; N, 1.45; F, 3.42; P, 3.00%.

1-O-[(Carboxy)acetyl]-2-deoxy-2-(2,2-difluorotetradecanamido)-4-O-phosphono-3-O-[(R)-3-(tetradecanoyloxy)tetradecanoyl]-β-D-glucopyranose (**16′**). Compound **14′** (65 mg, 0.046 mmol) was treated just described to give **16′** (41 mg, 86% yield) *via* corresponding diphenylphosphoryl compound **15′**. IR ν_{max} (KBr): 3304, 2957, 2921, 2852, 1763 (shoulder), 1738, 1690 cm^{-1}; ^{1}H-NMR (pyridine-d_5) δ: 0.80–0.98 (9H, m), 1.06–2.21 (62H, m), 2.26–2.56 (4H, m), 3.05 (1H, dd, J=6.4, 16.1 Hz), 3.33 (1H, dd, J=6.4, 16.1 Hz), 3.78 (1H, d, J=9.3 Hz), 3.84 (2H, s, OH), 4.06 (1H, d, J=13.2 Hz), 4.40 (1H, dd, J=2.4, 13.2 Hz), 4.94 (1H, q, J=9.3–10.3 Hz), 5.24 (1H, q, J=9.8–10.3 Hz), 5.67–5.82 (1H, m), 6.21 (1H, dd, J=9.8, 10.3 Hz), 6.60 (1H, d, J=8.8 Hz, C1-H), 10.51 (1H, d, J=9.3 Hz, NH). *Anal.* Calcd. for $C_{51}H_{92}NO_{15}F_2P \cdot 1/2H_2O$ (1028.3+9.0): C, 59.06; H, 9.04; N, 1.35; F, 3.66; P, 2.84%. Found: C, 58.94; H, 9.01; N, 1.55; F, 3.52; P, 2.84%.

1-O-[3-(Carboxy)propionyl]-2-deoxy-2-(2,2-difluorotetradecanamido)-4-O-phosphono-3-O-[(R)-3-(tetradecanoyloxy)tetradecanoyl]-α-D-glucopyranose (**19**). According to procedure (a) already described for the formation of **13** from **12**, compound **18** (45 mg, 0.038 mmol) in THF (2 ml) was hydrogenolyzed under hydrogen, using PtO_2 (15 mg), and the mixture was stirred for 2 h at 24°C, before being filtered and concentrated to give **19** (38 mg, 98% yield). IR ν_{max} (KBr): 3327, 2957, 2920, 2851, 1743, 1694 cm^{-1}; ^{1}H-NMR (pyridine-d_5) δ: 0.79–0.98 (9H, m), 1.10–1.88 (62H, m), 2.18–2.86 (8H, m), 3.03 (1H, dd, J=6.3, 16.1 Hz), 3.26 (1H, dd, J=6.3, 16.1 Hz), 4.06 (1H, d, J=12.2 Hz), 4.38 (1H, d, J=10.3 Hz), 4.52 (1H, d, J=11.2 Hz), 5.08–5.21 (1H, m), 5.30 (1H, q, J=9.8–10.7 Hz), 5.61–5.74 (1H, m), 6.24 (1H, dd, J=9.3, 10.7 Hz), 6.87 (1H, d, J=3.9 Hz, C1-H), 9.37 (1H, d, J=8.3 Hz, NH). *Anal.* Calcd. for $C_{52}H_{94}NO_{15}F_2P \cdot H_2O$ (1042.3+18.0): C, 58.91; H, 9.13; N, 1.32; F, 3.58; P, 2.92%. Found: C, 59.08; H, 9.12; N, 1.44; F, 3.41; P, 2.81%.

Materials and Methods. Reagents: The sources of materials in this work were lipopolysaccharide (LPS) from *E. coli* serotype 026; B6 and phorbol 12,13-dibutyrate (TPA) from Sigma (St. Louis, MO, U.S.A.); Dulbecco's modified Eagle medium (DMEM) from Gibco (Grand Island, N.Y., U.S.A.); newborn bovine serum (NBBS) from ICN Biomedicals (Costa Mesa, CA, U.S.A.); and mouse TNFα ELISA kit from ENDOGEN (Boston, MA, U.S.A.).

Cell culture: Mouse macrophage-like J774.1 cells (adherent) were maintained in DMEM supplemented with 5% NBBS (growth medium).[9]

Production of TNFα by J774.1 cells: 1.0×10^4 J774.1 cells in Corning 96-well plates containing 200 μl of growth medium were incubated in a humidified atmosphere of 5% CO_2 for 12 h at 37°C. The supernatant of

the culture medium was then aspirated, before the cells were incubated with 200 μl of fresh growth medium containing LPS or a graded concentration of a compound in the presence of 30 ng/ml of TPA in a humidified atmosphere of 5% CO_2 for 6 h at 37°C. After the incubation, the amount of TNFα produced in the culture medium was determined by the ELISA kit. The amount of TNFα produced by 10^5 J774.1 cells under each condition (nanogram order) was calculated by substracting the amount of TNFα produced in the absence of LPS or a compound as the background.

Results. J774.1 cells produced TNFα dose-dependently by stimulating with LPS in the presence of 30 ng/ml of TPA. TNFα production almost peaked and became saturated at a concentration of 10 ng/ml of LPS (data not shown). At this LPS concentration (10 ng/ml), 10^5 J774.1 cells produced 24 nanograms of TNFα (Fig. 3). Compound **13** also dose-dependently induced TNFα-production by J774.1 cells (21 nanograms of TNFα per 10^5 cells at 10 μM), while both compounds **16** and **19** marginally induced TNFα-production (1.1 and 0.88 nanograms of TNFα per 10^5 cells at 10 μM, respectively). (Fig. 3).

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References

1) O. Westphal and O. Luderitz, *Angew. Chem.*, **66**, 407–417 (1954).
2) (a) H. Paulsen and C. Krogmann, *Carbohydr. Res.*, **205**, 31–44 (1990). (b) H. Paulsen and E. C. Hoffgen, *Tetrahedron Lett.*, **32**, 2747–2750 (1991). (c) H. Paulsen and M. Brenken, *Liebigs Ann. Chem.*, 1113–1126 (1991). (d) E. Katzenellenbogen, A. Gamian, E. Romanowska, U. Dabrowski, and J. Dabrowski, *Eur. J. Biochem.*, **196**, 197–201 (1991). (e) M. B. Perry and M. M. MacLeen, *Carbohydr. Res.*, **232**, 143–150 (1992).
3) (a) M. Imoto, H. Yoshimura, N. Sakaguchi, S. Kusumoto, and T. Shiba, *Tetrahedon Lett.*, **26**, 1545–1548 (1985). (b) S. Takahashi, S. Nakamoto, K. Ikeda, and K. Achiwa, *Tetrahedron Lett.*, **27**, 1819–1822 (1986).
4) (a) M. Matsuura, Y. Kojima, J. Y. Homma, Y. Kubota, A. Yamamoto, M. Kiso, and A. Hasegawa, *FEBS Lett.*, **167**, 226–230 (1984). (b) M. Kiso, H. Ishida, and A. Hasegawa, *Agric. Biol. Chem.*, **48**, 251–252 (1984). (c) M. Kiso, S. Tanaka, M. Fujita, Y. Fujishima, Y. Ogawa, H. Ishida, and A. Hasegawa, *Carbohydr. Res.*, **162**, 127–140 (1987). (d) M. Kiso, Y. Ogawa, S. Tanaka, Y. Fujishima, M. Fujita, and A. Hasegawa, *J. Carbohydr. Chem.*, **6**, 625–638 (1987).
5) M. Shiozaki, Y. Kobayashi, M. Arai, T. Watanabe, T. Hiraoka, M. Nishijima, S. Kuge, T. Otsuka, and Y. Akamatsu, *J. Med. Chem.*, **34**, 2644–2646 (1991).
6) (a) M. Shiozaki, Y. Kobayashi, N. Ishida, M. Arai, T. Hiraoka, M. Nishijima, S. Kuge, T. Otsuka, and Y. Akamatsu, *Carbohydr. Res.*, **1991**, 222, 57–68. (b) Y. Kobayashi, N. Ishida, M. Arai, M. Shiozaki, T. Hiraoka, M. Nishijima, S. Kuge, T. Otsuka, and Y. Akamatsu, *Chem. Pharm. Bull.*, **40**, 333–337 (1992).
7) (a) L. M. Haines and E. Singleton, *J. Chem. Soc., Dalton Trans.*, 1891–1896 (1972). (b) J. J. Oltvoort, C. A. A. van Boeckel, J. H. de Koning, and J. H. van Boom, *Synthesis*, **1981**, 305–308.
8) M. A. Nashed and L. Anderson, *J. Chem. Soc., Chem. Commun.*, **1982**, 1274–1276.
9) S. Hara-Kuge, F. Amano, M. Nishijima, and Y. Akamatsu, *J. Biol. Chem.*, **265**, 6606–6610 (1990).