Synthesis of 2-Deoxy-2-[(2*S*,3*R*)-(2-fluoro-3-hydroxytetradecanoyl)amino]-3-*O*-[(3*R*)-3-tetradecanoyloxytetradecanoyl]-D-glucopyranose 4-(Dihydrogen Phosphate) and 2-Deoxy-2-[(2*R*,3*S*)-(2-fluoro-3-hydroxytetradecanoyl)amino]-3-*O*-[(3*R*)-3-tetradecanoyloxytetradecanoyl]-D-glucopyranose 4-(Dihydrogen Phosphate)

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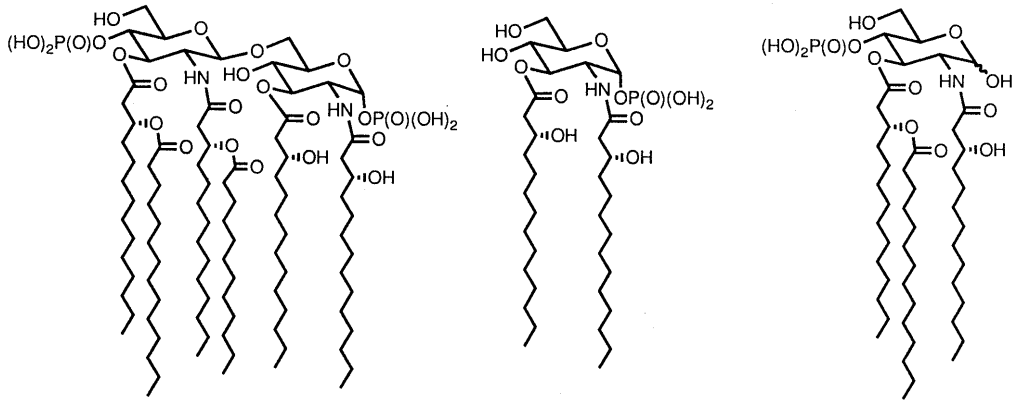
2-Deoxy-2-[(2*S*,3*R*)-(2-fluoro-3-hydroxytetradecanoyl)amino]-3-*O*-[(3*R*)-3-tetradecanoyloxytetradecanoyl]-D-glucopyranose 4-(dihydrogen phosphate) and 2-deoxy-2-[(2*R*,3*S*)-(2-fluoro-3-hydroxytetradecanoyl)amino]-3-*O*-[(3*R*)-3-tetradecanoyloxytetradecanoyl]-D-glucopyranose 4-(dihydrogen phosphate) were synthesized. The (2*S*,3*R*)-compound (9a) was a little more active than GLA-60 in the prostaglandin D_2 releasing test on macrophages, and the (2*R*,3*S*)-compound (9b) was almost inactive.

Keywords lipid A nonreducing monosaccharide; GLA-60 monofluoro analogue; (±)-*threo*-2-fluoro-3-hydroxytetradecanoic acid; (2*S*,3*R*)-3-(benzyloxycarbonyloxy)-2-fluorotetradecanoic acid; (2*R*,3*S*)-3-(benzyloxycarbonyloxy)-2-fluorotetradecanoic acid

Lipopolysaccharide (LPS), an outer membrane component of gram-negative bacterial cells, causes fever and lethal shock in higher animals. This toxic principle is called "endotoxin." Westphal and Luderitz[1] isolated lipid A, which is the lipophilic part of LPS. Lipid A shows most of the endotoxic activities of LPS, and it was first chemically synthesized by Shiba *et al.*[2] Nishijima and Raetz[3] isolated lipid X from a mutant of *Escherichia coli*. Lipid X is the reducing sugar part of lipid A, and is also one of the biosynthetic precursors of lipid A.[4] In a series of investigations on the active center of LPS, Hasegawa *et al.* have demonstrated[5] that nonreducing-sugar subunit analogues of lipid A (namely, 4-*O*-phosphonoglucosamine derivatives)[6] expressed several kinds of biological activities of endotoxin. In particular, GLA-60,[6] one of the 4-phosphonoglucosamine analogues, is a potentially valuable therapeutic agent. We aimed to synthesize compounds that are resistant to metabolic degradation of the tetradecanamide moiety in the glucosamine 2 position of GLA-60, because this property may make the biological activities long-lasting. We thought that a 2-fluorinated tetradecanamide group might be resistant to this type of metabolic degradation. Thus, we synthesized 2-deoxy-2-[(2*S*,3*R* and 2*R*,3*S*)-(2-fluoro-3-hydroxytetradecanoyl)-amino]-3-*O*-[(3*R*)-3-tetradecanoyloxytetradecanoyl]-D-glucopyranose 4-(dihydrogen phosphate) as candidate compounds. The (2*S*,3*R*) compound (**9a**) was a little more active than GLA-60 in the prostaglandin D_2 releasing test on macrophages, but the (2*R*,3*S*) compound (**9b**) was almost inactive. It does not appear that α-monofluorination of the tetradecanamide confers resistance to the metabolic degradation of the amide bond. However, the configuration of the 3-hydroxy group of tetradecanamide part is important for the activity: the 3*R* compound (**9a**) is more active than the 3*S* one (**9b**).

Synthesis The starting allyl 2-amino-2-deoxy-4,6-*O*-isopropylidene-α-D-glucopyranoside (**1**), which was obtained from allyl 2-deoxy-4,6-*O*-isopropylidene-2-trifluoroacetamido-α-D-glucopyranoside,[7] was treated with (±)-*syn*-3-benzyloxycarbonyloxy-2-fluorotetradecanoic acid[8] in dichloromethane using dicyclohexylcarbodiimide (DCC) as a condensing reagent to give two amides, **2a** and **2b**. Alternatively, allyl 2-amino-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranoside (**1′**),[9] obtained from allyl 2-deoxy-4,6-*O*-isopropylidene-2-trifluoroacetamido-β-D-glucopyranoside,[7] was treated with (±)-*syn*-3-benzyloxycarbonyloxy-2-fluorotetradecanoic acid[8] to give both compounds, **2a′** and **2b′**.



lipid A of *E. coli* lipid X GLA-60

Fig. 1

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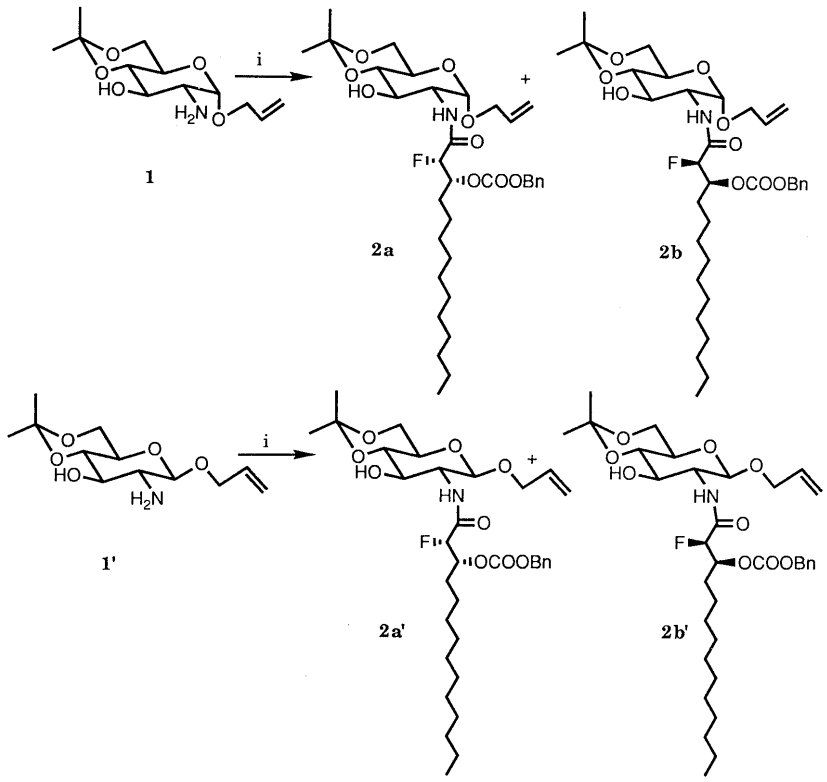
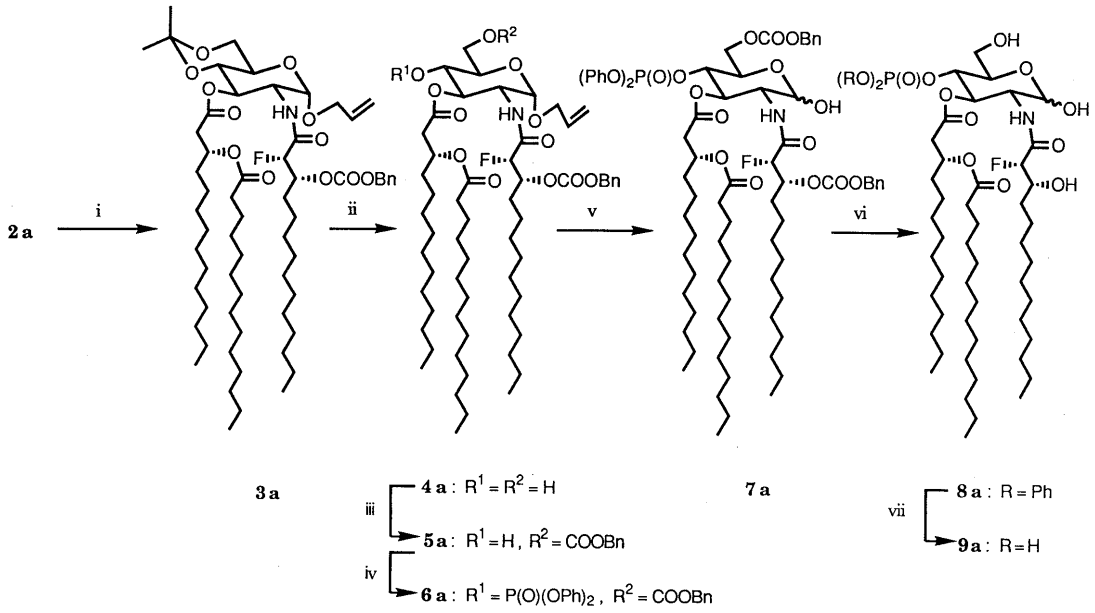


i) (±)-*threo*-3-(benzyloxycarbonyloxy)-2-fluorotetradecanoic acid–DCC

Chart 1



i) (*R*)-3-tetradecanoyloxytetradecanoic acid-DCC-DMAP ; ii) 90% AcOH ; iii) ClCOOBn , DMAP ; iv) $ClP(O)(OPh)_2$, DMAP ; v) $[C_8H_{12}(IrPMePh_2)_2]PF_6$, then H_2O-Py-I_2 ; vi) H_2 , Pd/C ; vii) H_2 , Pt

Chart 2

The configuration of **2a** or **2b** was determined by comparison with authentic **2a** or **2b**, obtained from **1** and (2*S*,3*R*)-*syn*-3-benzyloxycarbonyloxy-2-fluorotetradecanoic acid[10] or (2*R*,3*S*)-*syn*-3-benzyloxycarbonyloxy-2-fluorotetradecanoic acid,[10] respectively. The configuration of **2a′** or **2b′** was determined by comparison with authentic **2a′** or **2b′**, which was also obtained by treatment of **1′** with (2*S*,3*R*)-*syn*-3-benzyloxycarbonyloxy-2-fluorotetradecanoic acid[10] or (2*R*,3*S*)-*syn*-3-benzyloxycarbonyloxy-2-fluorotetradecanoic acid,[10] respectively. Compounds **2a′** and **2b′** were used to synthesize **9a** and **9b** *via* **7a** and **7b**. However, in this paper, we will describe only the route from **2a** and **2b** to **9a** and **9b** *via* **7a** and **7b**, respectively, because the synthetic procedure was almost the same for

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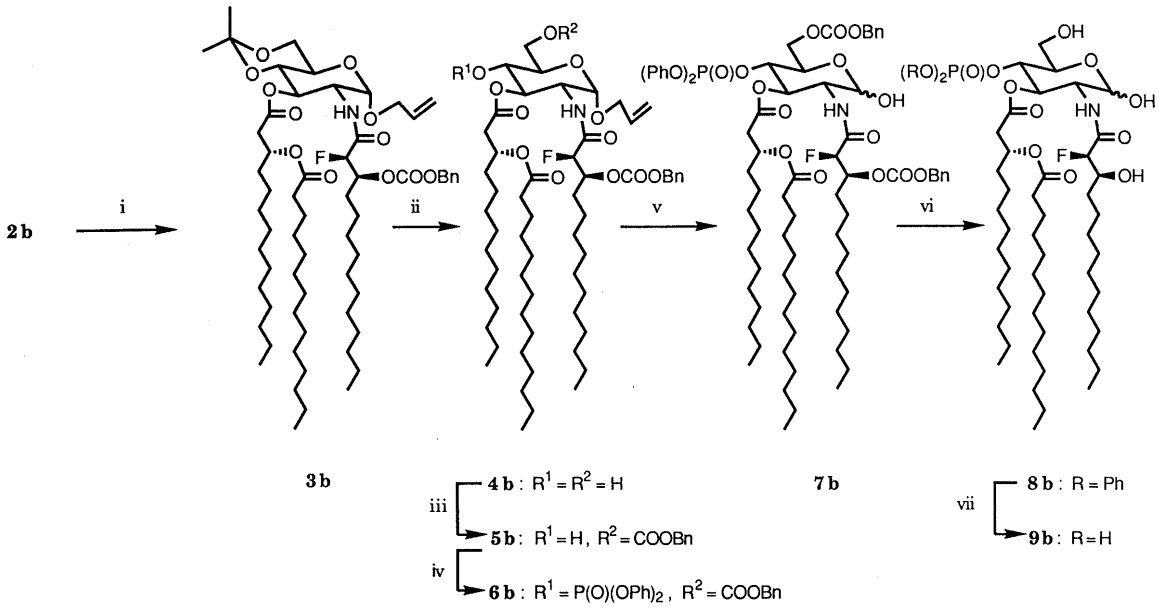

i) (*R*)-3-tetradecanoyloxytetradecanoic acid-DCC-DMAP ; ii) 90% AcOH ; iii) ClCOOBn , DMAP ; iv) $ClP(O)(OPh)_2$, DMAP ; v) $[C_8H_{12}(IrPMePh_2)_2]PF_6$, then H_2O-Py-I_2 ; vi) H_2 , Pd/C ; vii) H_2 , Pt

Chart 3

the series of compounds **2** and **2'**.

Treatment of **2a** or **2b** and (*R*)-3-tetradecanoyloxytetradecanoic acid with DCC and 4-dimethylaminopyridine (DMAP) gave **3a** or **3b**. Treatment of **3a** or **3b** with 90% AcOH at 55—60 °C gave the 4,6-deprotected diol **4a** or **4b**. Protection of the primary alcohol of **4a** or **4b** with benzyl chloroformate using DMAP as a base gave **5a** or **5b**. Phosphorylation of **5a** or **5b** with diphenylphosphoryl chloride and DMAP gave **6a** or **6b**. Deprotection of the allyl group of **6a** or **6b** with 1,5-cyclooctadienebis[methyldiphenylphosphine]iridium hexafluorophosphate ($[C_8H_{12}$-$Ir(PMePh_2)_2]PF_6$)[11] and then pyridine–H_2O–I_2[12] in tetrahydrofuran (THF) was effective to give **7a** or **7b**. Hydrogenolysis of **7a** or **7b** using 10% Pd–C in THF gave **8a** and **8b**. Hydrogenolysis of **8a** or **8b** in THF using Pt as a catalyst gave **9a** or **9b**.

Biological Activity To determine the biological activities of **9a** and **9b**, we examined the effects of those compounds as well as LPS and GLA-60 on the production of prostaglandin D_2 in macrophage-like cell line J774.1, and the stimulation index was determined as described in reference 13. The stimulation indexes of LPS, GLA-60, **9a**, and **9b** (LPS, 1 μg/ml; the others, 10 μM) were 60.0, 17.9, 21.6, and 2.8, respectively. These results indicated that **9a** was a little more active than GLA-60, and **9b** was almost inactive with respect to prostaglandin D_2 production, an indicator of macrophage activation.

Experimental

^{1}H-Nuclear magnetic resonance (^{1}H-NMR) spectra were recorded with a 270 MHz spectrometer in $CDCl_3$ solution using tetramethylsilane as an internal standard. Preparative thin layer chromatography (TLC) was performed on silica gel plates (Merck, Silicagel 60 F_{254}), and column chromatography was carried out on columns packed with Merck Silica gel 60 (230—400 mesh ASTM) using a slightly increased pressure (1.4 atm) for elution. Elemental analyses were performed by the Analytical Center, Analytical and Metabolic Research Laboratories, Sankyo Co., Ltd.

Allyl 2-Amino-2-deoxy-4,6-*O*-isopropylidene-α-D-glucopyranoside (1) A solution of allyl 2-deoxy-4,6-*O*-isopropylidene-2-trifluoroacetamido-α-D-glucopyranoside[6] (49.7 g, 139.9 mmol) in EtOH (840 ml) and 1 M NaOH (420 ml) was refluxed for 1 h. The reaction mixture was concentrated *in vacuo* to 1/3 volume, and extracted with EtOAc (1000 ml, and then 600 ml). The organic layer was washed with H_2O and brine, dried over $MgSO_4$, and concentrated to give 34.5 g (95%) of **1** as a gum, which was employed for the next reaction without purification. IR ($CHCl_3$): 3600—3300 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.45 (3H, s), 1.53 (3H, s), 2.65 (3H, br s, NH_2, OH), 2.93 (1H, dd, J=3.5, 9.8 Hz, C2-H), 3.53—4.40 (7H, m), 4.91 (1H, d, J=3.5 Hz, C1-H), 5.21—5.39 (2H, m), 5.87—6.01 (1H, m). MS *m/z*: 260 (M+1), 244, 218, 202, 201, 186. *Anal.* Calcd for $C_{12}H_{21}NO_5 \cdot 1/3H_2O$ (265.3): C, 54.32; H, 8.17; N, 5.27. Found: C, 54.61; H, 7.86; N, 5.30.

Allyl 2-[(2*S*,3*R*)-(3-Benzyloxycarbonyloxy-2-fluorotetradecanoyl)-amino]-2-deoxy-4,6-*O*-isopropylidene-α-D-glucopyranoside (2a) and Allyl 2-[(2*R*,3*S*)-(3-Benzyloxycarbonyloxy-2-fluorotetradecanoyl)amino]-2-deoxy-4,6-*O*-isopropylidene-α-D-glucopyranoside (2b) A solution of the amine **1** (35.6 g, 137 mmol) and (±)-*threo*-3-(benzyloxycarbonyloxy)-2-fluorotetradecanoic acid (57.1 g, 144 mmol) in CH_2Cl_2 (720 ml) was treated with DCC (33.8 g, 164 mmol) with stirring at 5 °C. The mixture was stirred for 1 h at 25 °C. The precipitated DCC·H_2O was filtered off, and washed with a small amount of CH_2Cl_2. The organic layer was concentrated to give a crude gum, which was dissolved in EtOAc. The solution was washed with saturated $NaHCO_3$ and brine, dried ($MgSO_4$), filtered, and concentrated to give a residue, which was divided into four parts. Each part was chromatographed on a silica gel (1.2 kg) column. Elution with cyclohexane–EtOAc (7 : 3) gave in total 33.9 g (39%) of **2a** (*Rf*=0.168) as a viscous oil and 39.5 g (45%) of **2b** (*Rf*=0.305) as a solid. **2a**: IR (film): 3400, 1740, 1665 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t, J=6.2—7.0 Hz), 1.20—1.42 (18H, m), 1.43 (3H, s), 1.52 (3H, s), 1.68—1.98 (2H, m), 2.67 (1H, d, J=3.3 Hz, OH), 3.54—3.87 (6H, m), 4.00 (1H, m), 4.15 (1H, dt, J=1.5, 3.0 Hz), 4.63 (1H, d, J=3.7 Hz, C1-H), 5.00 (1H, dd, J=2.2, 47.6 Hz), 5.10—5.27 (5H, m), 5.73—5.88 (1H, m), 6.58 (1H, dd, J=3.7, 9.2 Hz, NH), 7.34 (5H, s). MS *m/z*: 637 (M^+), 562, 486, 428, 412. *Anal.* Calcd for $C_{34}H_{52}FNO_9$ (637.8): C, 64.03; H, 8.22; F, 2.98; N, 2.20. Found: C, 63.91; H, 8.35; F, 2.95; N, 2.23.

2b: IR (neat): 3400, 1740, 1670 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t, J=6.4—6.8 Hz), 1.20—1.42 (18H, m), 1.46 (3H, s), 1.52 (3H, s), 1.70—1.90 (2H, m), 3.56—4.00 (7H, m), 4.11—4.25 (2H, m), 4.79 (1H, d, J=3.9 Hz, C1-H), 4.92 (1H, dd, J=2.2, 47.6 Hz), 5.15 (2H, s), 5.18—5.30 (2H, m), 5.79—5.93 (1H, m), 6.65 (1H, dd, J=3.4, 9.3 Hz, NH), 7.31—7.42 (5H, m). MS *m/z*: 637 (M^+), 579, 564, 486, 470, 428, 412, 352. *Anal.* Calcd for $C_{34}H_{52}FNO_9$ (637.8): C, 64.03; H, 8.22; F, 2.98; N, 2.20. Found: C, 63.89; H, 8.32; F, 2.97; N, 2.17.

Allyl 2-[(2*S*,3*R*)-(3-Benzyloxycarbonyloxy-2-fluorotetradecanoyl)-

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amino]-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranoside (2a′) and Allyl 2-[(2*R*,3*S*)-(3-Benzyloxycarbonyloxy-2-fluorotetradecanoyl)amino]-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranoside (2b′) The amine **1′**[9] and (±)-*threo*-3-(benzyloxycarbonyloxy)-2-fluorotetradecanoic acid were treated as described above to give **2a′** (43% yield, *Rf*=0.392, cyclohexane : EtOAc=1 : 1), and **2b′** (42% yield, *Rf*=0.588, cyclohexane : EtOAc=1 : 1). **2a′**: IR ($CHCl_3$): 1750, 1685, 1535 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t, *J*=6.2—6.9 Hz), 1.20—1.40 (16H, m), 1.45 (3H, s), 1.53 (3H, s), 1.53—2.00 (4H, m), 3.27—3.37 (3H, m), 3.55 (1H, t, *J*=9.2—9.5 Hz), 3.80 (1H, t, *J*=10.3—10.6 Hz), 3.91 (1H, dd, *J*=5.5, 11.0 Hz), 4.01—4.14 (2H, m), 4.27 (1H, m), 4.87 (1H, d, *J*=8.5 Hz), 4.91 (1H, dd, *J*=2.6, 48.0 Hz), 5.09—5.29 (5H, m), 5.85 (1H, m), 6.60 (1H, t, *J*=5.1—5.8 Hz, NH), 7.33—7.38 (5H, m). *Anal.* Calcd for $C_{34}H_{52}FNO_9$ (637.8): C, 64.03; H, 8.22; F, 2.98; N, 2.20. Found: C, 63.84; H, 8.33; F, 3.02; N, 2.76.

2b′: IR ($CHCl_3$): 1750, 1685, 1537 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t, *J*=6.4—6.8 Hz), 1.20—1.40 (16H, m), 1.47 (3H, s), 1.53 (3H, s), 1.53—2.00 (4H, m), 3.19 (1H, dt, *J*=5.4, 9.8 Hz), 3.57—4.06 (7H, m), 4.23—4.31 (1H, m), 4.46 (1H, d, *J*=8.3 Hz, Cl-H), 4.94 (1H, dd, *J*=2.4, 47.4 Hz), 5.11—5.29 (4H, m), 5.83 (1H, m), 6.45 (1H, t, *J*=5.4—5.9 Hz, NH), 7.31—7.41 (5H, s). *Anal.* Calcd for $C_{34}H_{52}FNO_9$ (637.8): C, 64.03; H, 8.22; F, 2.98; N, 2.20. Found: C, 63.96; H, 8.44; F, 2.97; N, 2.51.

Allyl 2-[(2*S*,3*R*)-(3-Benzyloxycarbonyloxy-2-fluorotetradecanoyl)-amino]-2-deoxy-4,6-*O*-isopropylidene-3-*O*-[(3*R*)-3-tetradecanoyloxytetradecanoyl]-α-D-glucopyranoside (3a) (*R*)-3-Tetradecanoyloxytetradecanoic acid (25.9 g, 5.70 mmol), DMAP (7.0 g, 5.73 mmol), and DCC (12.8 g, 6.20 mmol) were added to a solution of **2a** (33.1 g, 5.19 mmol) in CH_2Cl_2 (700 ml), in that order, with stirring. After 2 h at 25 °C, the mixture was filtered to remove DCC·H_2O, and the filtrate was concentrated *in vacuo*, diluted with EtOAc, washed with H_2O, saturated $NaHCO_3$, and brine, dried over $MgSO_4$, filtered, and then evaporated to give a residue, which was chromatographed on a silica gel column. Elution with cyclohexane–EtOAc (5 : 1) gave 47.7 g (86%) of **3a** as a viscous oil. IR ($CHCl_3$): 3440, 1745, 1690 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.86—0.90 (9H, m), 1.25—1.77 (68H, m, including two methyl singlets at δ 1.37 and 1.48, respectively), 2.26 (2H, m), 2.49 (1H, dd, *J*=6.3, 15.1 Hz), 2.62 (1H, dd, *J*=6.3, 15.1 Hz), 3.70—3.86 (5H, m), 3.93—3.98 (1H, m), 4.21—4.27 (1H, m), 4.63 (1H, d, *J*=3.9 Hz, Cl-H), 4.90 (1H, dd, *J*=2.4, 47.4 Hz), 5.09—5.21 (7H, m), 5.74—5.84 (1H, m), 6.63 (1H, dd, *J*=3.9, 9.8 Hz, NH), 7.26—7.36 (5H, m). *Anal.* Calcd for $C_{62}H_{104}FNO_{12}$ (1074.5): C, 69.30; H, 9.76; F, 1.77; N, 1.30. Found: C, 69.39; H, 9.86; F, 1.75; N, 1.31.

Allyl 2-[(2*R*,3*S*)-(3-Benzyloxycarbonyloxy-2-fluorotetradecanoyl)-amino]-2-deoxy-4,6-*O*-isopropylidene-3-*O*-[(3*R*)-3-tetradecanoyloxytetradecanoyl]-α-D-glucopyranoside (3b) Compound **2b** (35.6 g, 55.8 mmol) was treated in the same manner as described above to obtain 57.8 g (96%) of **3b**. IR ($CHCl_3$): 3440, 1740, 1685 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.86—0.90 (9H, t, *J*=6.2—7.0 Hz), 1.25 (60H, m), 1.38 (3H, s), 1.50 (3H, s), 1.72 (2H, m), 2.23 (2H, t, *J*=7.3—7.7 Hz), 2.50 (1H, d, *J*=6.6 Hz), 3.71—3.98 (5H, m), 4.12—4.19 (2H, m), 4.79—4.98 (2H, m), 5.10—5.31 (7H, m), 5.77—5.92 (1H, m), 6.81 (1H, dd, *J*=3.3, 8.4 Hz, NH), 7.36 (5H, m). *Anal.* Calcd for $C_{62}H_{104}FNO_{12}$ (1074.5): C, 69.30; H, 9.76; F, 1.77; N, 1.30. Found: C, 68.94; H, 9.58; F, 1.76; N, 1.26.

Allyl 2-[(2*S*,3*R*)-(3-Benzyloxycarbonyloxy-2-fluorotetradecanoyl)-amino]-2-deoxy-3-*O*-[(3*R*)-3-tetradecanoyloxytetradecanoyl]-α-D-glucopyranoside (4a) A suspension of **3a** (46.0 g, 4.28 mmol) in 90% AcOH (900 ml) was stirred for 1 h at 55—60 °C. The mixture became a solution, which was concentrated *in vacuo* to give a residue, which in turn was chromatographed on a silica gel column. Elution with cyclohexane–EtOAc (2 : 1) gave 42.0 g (95%) of **4a** as a gum. IR (KBr): 1741, 1719, 1703, 1670 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.85—0.90 (9H, m), 1.04—1.78 (64H, m), 2.26—2.31 (2H, m), 2.47—2.59 (2H, m), 3.63—3.88 (5H, m), 3.96—4.02 (1H, m), 4.16—4.23 (1H, m), 4.66 (1H, d, *J*=3.7 Hz, Cl-H), 4.89 (1H, dd, *J*=2.2, 47.6 Hz), 5.09—5.21 (7H, m), 5.73—5.87 (1H, m), 6.66 (1H, dd, *J*=3.7, 9.5 Hz), 7.26—7.37 (5H, m). *Anal.* Calcd for $C_{59}H_{100}FNO_{12}$ (1034.4): C, 68.51; H, 9.74; F, 1.84; N, 1.35. Found: C, 68.62; H, 9.70; F, 1.80; N, 1.55.

Allyl 2-[(2*R*,3*S*)-(3-Benzyloxycarbonyloxy-2-fluorotetradecanoyl)-amino]-2-deoxy-3-*O*-[(3*R*)-3-tetradecanoyloxytetradecanoyl]-α-D-glucopyranoside (4b) Compound **3b** (57.6 g) was treated in the same manner as described above to obtain 42.6 g (77%) of **4b**. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (9H, t, *J*=5.9—6.8 Hz), 1.2—1.8 (64H, m), 2.24—2.45 (4H, m), 3.64—3.76 (3H, m), 3.84—3.90 (2H, m), 3.95—4.00 (1H, m), 4.14—4.21 (2H, m), 4.84—5.32 (7H, m), 5.82—5.87 (1H, m), 7.07—7.10 (1H, m, NH), 7.36 (5H, s). *Anal.* Calcd for $C_{59}H_{100}FNO_{12}$ (1034.4): C, 68.51; H, 9.74; F, 1.84; N, 1.35. Found: C, 68.27; H, 9.97; F, 1.92; N, 1.48.

Allyl 6-*O*-Benzyloxycarbonyl-2-[(2*S*,3*R*)-(3-benzyloxycarbonyloxy-2-fluorotetradecanoyl)amino]-2-deoxy-3-*O*-[(3*R*)-3-tetradecanoyloxytetradecanoyl]-α-D-glucopyranoside (5a) Benzyl chloroformate (6.0 ml, 33.5 mmol) was gradually added to a solution of **4a** (23.1 g, 22.3 mmol) and DMAP (4.1 g, 33.5 mmol) in CH_2Cl_2 (600 ml) with stirring at 0—5 °C under nitrogen. Stirring was continued at 3 °C for 1 h, then the reaction temperature was elevated to room temperature, and the mixture was concentrated *in vacuo*, diluted with EtOAc, washed with H_2O and brine, dried over $MgSO_4$, and concentrated to give a residue, which was chromatographed on a silica gel (1 kg) column. Elution with cyclohexane–EtOAc (4 : 1) gave the recovered starting material (6.7 g, 29%), the 4,6-*O*-diprotected compound 7.4 g (29%), and 10.6 g (41%) of **5a** as a gum. IR (KBr): 1747, 1738, 1724, 1712, 1678 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.85—0.90 (9H, t, *J*=6.4—6.8 Hz), 1.02—1.77 (62H, m), 2.25—2.31 (2H, m, $COCH_2$), 2.46—2.59 (2H, m), 3.31 (1H, d, *J*=4.2 Hz), 3.61 (1H, dt, *J*=4.2, 9.3 Hz), 3.76—3.86 (2H, m), 3.93—4.00 (1H, m), 4.16—4.24 (1H, m), 4.38—4.48 (1H, m), 4.88 (1H, dd, *J*=2.2, 47.6 Hz), 5.07—5.19 (9H, m), 5.70—5.85 (1H, m, CH=C), 6.62 (1H, dd, *J*=3.7, 9.5 Hz, NH), 7.26—7.40 (10H, m). *Anal.* Calcd for $C_{67}H_{106}FNO_{14}$ (1168.6): C, 68.86; H, 9.14; F, 1.63; N, 1.42. Found: C, 68.77; H, 9.18; F, 1.64; N, 1.42.

Allyl 6-*O*-Benzyloxycarbonyl-2-[(2*R*,3*S*)-(3-benzyloxycarbonyloxy-2-fluorotetradecanoyl)amino]-2-deoxy-3-*O*-[(3*R*)-3-tetradecanoyloxytetradecanoyl]-α-D-glucopyranoside (5b) Compound **4b** (31.8 g) was treated in the same manner as described above to obtain the recovered starting material (2.9 g, 9%), the 4,6-*O*-diprotected compound 4.7 g (12%), and 27.1 g (75%) of **5b** as a gum. IR (Nujol): 3480, 3300, 1750, 1725, 1720, 1670 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (9H, t, *J*=6.2—6.9 Hz), 1.25—1.76 (62H, m), 2.23—2.45 (4H, m), 3.63—3.65 (2H, m), 3.85—3.98 (2H, m), 4.11—4.19 (2H, m), 4.43—4.48 (2H, m), 4.80—5.30 (11H, m), 5.75—5.95 (1H, m, CH=C), 7.05 (1H, dd, *J*=3.1, 8.3 Hz, NH), 7.35—7.41 (10H, m). *Anal.* Calcd for $C_{67}H_{106}FNO_{14}$ (1168.6): C, 68.86; H, 9.14; F, 1.63; N, 1.20. Found: C, 68.69; H, 9.21; F, 1.63; N, 1.40.

Allyl 6-*O*-Benzyloxycarbonyl-2-[(2*S*,3*R*)-(3-benzyloxycarbonyloxy-2-fluorotetradecanoyl)amino]-2-deoxy-3-*O*-[(3*R*)-3-tetradecanoyloxytetradecanoyl]-α-D-glucopyranoside 4-(Diphenyl Phosphate) (6a) Diphenyl chlorophosphate (7.22 g, 26.9 mmol) and DMAP (3.28 g, 26.9 mmol) were added to a solution of **5a** (10.47 g, 8.96 mmol) in CH_2Cl_2 (300 ml). The mixture was stirred for 6 h at room temperature, concentrated *in vacuo*, diluted with EtOAc, washed with saturated $NaHCO_3$, H_2O and brine, dried over $MgSO_4$, and concentrated to give a residue that was chromatographed on a silica gel (300 g) column. Elution with cyclohexane–EtOAc (5 : 1) gave 11.46 g (91%) of **6a** as a gum. IR (film): 1750, 1690, 1590 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (9H, t, *J*=6.4—6.8 Hz), 1.03—1.80 (62H, m), 2.13—2.19 (2H, m), 2.33 (1H, dd, *J*=7.3, 15.8 Hz), 2.42 (1H, dd, *J*=5.1, 15.8 Hz), 3.73—3.82 (1H, m), 3.89—4.02 (2H, m), 4.17—4.36 (3H, m), 4.64 (1H, d, *J*=3.7 Hz, Cl-H), 4.72 (1H, dd, *J*=9.2, 18.7 Hz), 4.85—5.22 (9H, m), 5.42 (1H, dd, *J*=9.2, 11.0 Hz), 5.70—5.84 (1H, m), 6.56 (1H, dd, *J*=3.7, 9.5 Hz), 7.12—7.65 (20H, m). *Anal.* Calcd for $C_{79}H_{115}FNO_{17}P$ (1400.8): C, 67.74; H, 8.28; F, 1.36; N, 1.00; P, 2.21. Found: C, 67.45; H, 8.23; F, 1.24; N, 1.00; P, 2.14.

Allyl 6-*O*-Benzyloxycarbonyl-2-[(2*R*,3*S*)-(3-benzyloxycarbonyloxy-2-fluorotetradecanoyl)amino]-2-deoxy-3-*O*-[(3*R*)-3-tetradecanoyloxytetradecanoyl]-α-D-glucopyranoside 4-(Diphenyl Phosphate) (6b) Compound **5b** (27.0 g) was treated in the same manner as described above to obtain 31.0 g (96%) of **6b** as a gum. IR (film): 1745, 1690, 1590 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (9H, t, *J*=6.2—7.0 Hz), 1.12—1.72 (62H, m), 2.09 (2H, t, *J*=7.3—8.1 Hz), 2.35 (1H, dd, *J*=7.7, 17.6 Hz), 2.52 (1H, dd, *J*=5.3, 16.7 Hz), 3.90—4.35 (6H, m), 4.68—4.75 (1H, m), 4.78—5.29 (10H, m), 5.47 (1H, dd, *J*=9.2, 11.0 Hz), 5.77—5.84 (1H, m), 6.81 (1H, dd, *J*=3.3, 8.1 Hz, NH), 7.11—7.37 (20H, m). *Anal.* Calcd for $C_{79}H_{115}FNO_{17}P$ (1400.8): C, 67.74; H, 8.28; F, 1.36; N, 1.00; P, 2.21. Found: C, 67.37; H, 8.25; F, 1.31; N, 0.87; P, 2.27.

6-*O*-Benzyloxycarbonyl-2-[(2*S*,3*R*)-(3-benzyloxycarbonyloxy-2-fluorotetradecanoyl)amino]-2-deoxy-3-*O*-[(3*R*)-3-tetradecanoyloxytetradecanoyl]-D-glucopyranose 4-(Diphenyl Phosphate) (7a) $[C_8H_{12}(Ir(PMePh_2)_2]PF_6$ (340 mg) was suspended in a solution of **6a** (11.21 g, 8.0 mmol) in THF (150 ml, freshly distilled over $LiAlH_4$). The air in the reaction flask was completely replaced with nitrogen, and then further replaced with hydrogen to activate the iridium complex. The mixture was stirred for 1 or 2 min, until the red-colored iridium complex formed a colorless solution, and then the hydrogen was completely replaced again with nitrogen. This solution was stirred for 2 h at 20 °C. After checking for the double bond shift to an enol ether (slightly higher *Rf* value on silica gel TLC), pyridine (5 ml), H_2O (50 ml) and iodine (5.0 g) were added to the mixture in this order. The whole was stirred for 30 min at 20—25 °C, and concentrated *in vacuo*. The residue was diluted with EtOAc, and the

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solution was washed with 10% $Na_2S_2O_3$, saturated $NaHCO_3$, water and brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give a residue that was chromatographed on a silica gel column. Elution with cyclohexane–EtOAc (5 : 1) gave 6.80 g (62%) of **7a** as a gum. IR (KBr): 3300, 1739, 1660 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (9H, t, J=6.2—6.9 Hz), 1.18—1.82 (62H, m), 2.12—2.18 (2H, m), 2.32 (1H, dd, J=7.3, 15.8 Hz), 2.41 (1H, dd, J=5.5, 15.8 Hz), 2.70 (1H, dd, J=1.5, 4.8 Hz, OH), 4.09—4.18 (3H, m), 4.29—4.34 (1H, m), 4.67 (1H, dd, J=9.2, 18.7 Hz), 4.87 (1H, m), 4.89 (1H, dd, J=1.8, 47.3 Hz), 5.01—5.25 (6H, m), 6.63 (1H, dd, J=3.3, 8.8 Hz, NH), 7.12—7.38 (20H, m). *Anal.* Calcd for $C_{76}H_{111}FNO_{17}P$ (1360.7): C, 67.09; H, 8.22; F, 1.40; N, 1.03; P, 2.28. Found: C, 67.05; H, 7.97; F, 1.35; N, 1.46; P, 2.15.

6-*O*-Benzyloxycarbonyl-2-[(2*R*,3*S*)-(3-benzyloxycarbonyloxy-2-fluorotetradecanoyl)amino]-2-deoxy-3-*O*-[(3*R*)-3-tetradecanoyloxytetradecanoyl]-D-glucopyranose 4-(Diphenyl Phosphate) (7b) Compound **6b** (15.0 g, 10.7 mmol) was treated in the same manner as described above to obtain 11.6 g (80%) of **7b** as a gum. IR ($CHCl_3$): 3420, 1750, 1690, 1590 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (9H, t, J=6.2—6.9 Hz), 1.14—1.75 (62H, m), 2.08—2.13 (2H, m), 2.33 (1H, dd, J=7.7, 16.9 Hz), 2.51 (1H, dd, J=4.8, 16.9 Hz), 3.63 (1H, dd, J=1.1, 4.3 Hz), 3.97 (1H, m), 4.14—4.36 (3H, m), 4.66—4.77 (1H, m), 4.89 (1H, dd, J=2.7, 47.6 Hz), 5.02—5.20 (6H, m), 5.30 (1H, t, J=3.7 Hz), 5.53 (1H, dd, J=9.2, 11.0 Hz), 6.92 (1H, dd, J=2.9, 7.7 Hz, NH), 7.12—7.34 (20H, m). *Anal.* Calcd for $C_{76}H_{111}FNO_{17}P$ (1360.7): C, 67.09; H, 8.22; F, 1.40; N, 1.03; P, 2.28. Found: C, 67.20; H, 8.29; F, 1.28; N, 0.95; P, 2.21.

2-[(2*S*,3*R*)-(2-Fluoro-3-hydroxytetradecanoyl)amino]-2-deoxy-3-*O*-[(3*R*)-3-tetradecanoyloxytetradecanoyl]-D-glucopyranose 4-(Diphenyl Phosphate) (8a) A 10% Pd on carbon (3.5 g) was added to a solution of **7a** (6.50 g, 4.78 mmol) in THF (100 ml) containing formic acid (50 mg). The mixture was hydrogenolyzed at room temperature with vigorous stirring. After the completion of the reaction (about 3 h), the mixture was filtered and then chromatographed. Development with cyclohexane–EtOAc (1 : 3) gave 4.89 g (94%) of **8a**. IR (KBr): 1735, 1671 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (9H, t, J=6.4—6.8 Hz), 1.18—1.80 (62H, m), 2.13—2.21 (2H, m), 2.37—2.39 (2H, m, $COCH_2$), 3.50—3.61 (4H, m), 3.97—4.06 (2H, m), 4.21—4.28 (1H, m), 4.65—4.83 (2H, m), 5.04—5.13 (1H, m), 5.24—5.28 (2H, m), 5.49—5.57 (1H, m), 6.80—6.85 (1H, m), 7.14—7.38 (10H, m). IR (KBr): 1735, 1671 cm^{-1}. *Anal.* Calcd for $C_{60}H_{99}FNO_{13}P$ (1092.4): C, 65.97; H, 9.13; F, 1.74; N, 1.28; P, 2.84. Found: C, 65.93; H, 9.25; F, 1.63; N, 1.48; P, 2.84.

2-[(2*R*,3*S*)-(2-Fluoro-3-hydroxytetradecanoyl)amino]-2-deoxy-3-*O*-[(3*R*)-3-tetradecanoyloxytetradecanoyl]-D-glucopyranose 4-(Diphenyl Phosphate) (8b) Compound **7b** (11.6 g, 8.53 mmol) was treated in the same manner as described above to obtain 8.14 g (87%) of **8b** as a gum. IR (KBr): 1736, 1661, 1580 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (9H, t, J=6.4—6.8 Hz), 1.19—1.63 (62H, m), 2.15—2.20 (2H, m), 2.37 (1H, dd, J=8.4, 17.2 Hz), 2.68 (1H, d, J=8.8 Hz, OH), 3.38 (1H, m), 3.59—3.62 (2H, m), 4.02—4.05 (3H, m), 4.33—4.40 (1H, m), 4.74 (1H, dd, 1.1, 48.0 Hz), 4.77 (1H, dd, J=9.5, 19.1 Hz), 5.11—5.15 (1H, m), 5.30 (1H, t, J=3.7 Hz), 5.54 (1H, dd, J=9.5, 10.3 Hz), 6.83 (1H, dd, J=3.3, 9.2 Hz), 7.15—7.39 (10H, m). *Anal.* Calcd for $C_{60}H_{99}FNO_{13}P$ (1092.4): C, 65.97; H, 9.13; F, 1.74; N, 1.28; P, 2.84. Found: C, 65.70; H, 8.96; F, 1.66; N, 1.36; P, 2.90.

2-[(2*S*,3*R*)-(2-Fluoro-3-hydroxytetradecanoyl)amino]-2-deoxy-3-*O*-[(3*R*)-3-tetradecanoyloxytetradecanoyl]-D-glucopyranose 4-(Dihydrogen Phosphate) (9a) A solution of **8a** (4.59 g, 4.2 mmol) in THF (100 ml) containing PtO_2 (450 mg) was hydrogenolyzed at room temperature with vigorous stirring. After the completion of the reaction (about 2 h), the mixture was filtered and then concentrated *in vacuo* to give 3.9 g (99%) of **9a**. IR (KBr): 1734, 1661 cm^{-1}. ^{1}H-NMR (C_5D_5N) δ: 0.88 (9H, t, J=6.4—6.8 Hz), 1.03—2.15 (62H, m), 2.43—2.49 (2H, m), 3.08—3.25 (2H, m), 4.09—4.13 (1H, m), 4.52—4.56 (2H, m), 4.62—4.65 (1H, m), 4.99—5.08 (1H, m), 5.21—5.49 (2H, m), 5.63—5.74 (2H, m), 6.24—6.31 (1H, m), 8.03—8.72 (6H, br, OH, NH). *Anal.* Calcd for $C_{48}H_{91}FNO_{13}P$ (940.2): C, 61.32; H, 9.76; F, 2.02; N, 1.49; P, 3.29. Found: C, 60.66; H, 9.87; F, 1.91; N, 1.68; P, 3.10.

2-[(2*R*,3*S*)-(2-Fluoro-3-hydroxytetradecanoyl)amino]-2-deoxy-3-*O*-[(3*R*)-3-tetradecanoyloxytetradecanoyl]-D-glucopyranose 4-(Dihydrogen Phosphate) (9b) Compound **8b** (7.72 g, 7.07 mmol) was treated in the same manner as described above to obtain 6.70 g (quantitatively) of **9b** as a powder. IR (KBr): 1753, 1716, 1657 cm^{-1}. ^{1}H-NMR (C_5D_5N) δ: 0.88 (9H, t, J=6.4—6.8 Hz), 1.25—2.01 (62H, m), 2.38—2.44 (2H, m), 3.19 (2H, d, J=5.9 Hz), 4.11—4.18 (1H, m), 4.43—4.59 (3H, m), 4.99—5.07 (1H, m), 5.15—5.33 (2H, m), 5.74—5.81 (2H, m), 6.28 (1H, t, J=9.8 Hz), 8.02 (1H, dd, J=2.7, 9.8 Hz, NH), 8.61 (5H, br, OH). IR (KBr): 1734, 1661 cm^{-1}. *Anal.* Calcd for $C_{48}H_{91}FNO_{13}P$ (940.2): C, 61.32; H, 9.76; F, 2.02; N, 1.49; P, 3.29. Found: C, 61.04; H, 9.92; F, 1.92; N, 1.60; P, 3.27.

Procedure for Obtaining a Triethylamine Solution of 9a and 9b The powder **9a** or **9b** (15 mg) was suspended in 0.1 M HCl (4 ml) and $CHCl_3$–MeOH (1 : 2, 15 ml) and dissolved by ultrasonication. Additional $CHCl_3$ (5 ml) and 0.1 M HCl (5 ml) were added to this solution to privide two phases. The lower chloroform phase was collected and concentrated to give 13 mg of **9a** or **9b**, which was dissolved in 0.1% triethylamine (v/v) water solution.

Acknowledgment This study was supported in part by the Social Insurance Agency Contract Fund (Japan Health Sciences Foundation).

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13) Assay of released [^{14}C]prostaglandin D_2. Cells were seeded at approximately 5×10^5 cells per well in 12-well dishes containing 1 ml of the culture medium. The cells were cultured overnight and then labeled with 0.1 μCi/ml [^{14}C]arachidonic acid for 18 h. Each well was then washed with 0.5 ml × 3 of the culture medium. After the addition of the stimulants the cells were incubated at 37°C for 12 h. The culture media were collected and centrifuged for 5 min. Prostaglandin D_2 that was released into the medium was extracted with chloroform–ethanol (2 : 1, v/v) after acidification and analyzed by TLC with a solvent system of EtOAc–$CHCl_3$–EtOH–AcOH (20 : 20 : 4 : 1, v/v). The radiolabeled prostaglandin D_2 was localized by autoradiography. The regions corresponding to the radioactivity were scraped from the TLC plates and counted with a scintillation counter.

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